

1-16-2015

## Memory T Cells Specific for Murine Cytomegalovirus Re-Emerge after Multiple Challenges and Recapitulate Immunity in Various Adoptive Transfer Scenarios.

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### Recommended Citation

Quinn, Michael; Turula, Holly; Tandon, Mayank; Deslouches, Berthony; Moghbeli, Toktam; and Snyder, Christopher M., "Memory T Cells Specific for Murine Cytomegalovirus Re-Emerge after Multiple Challenges and Recapitulate Immunity in Various Adoptive Transfer Scenarios." (2015). *Department of Microbiology and Immunology Faculty Papers*. Paper 69.

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1 Quinn *et. al.* Memory T cells recapitulate MCMV immunity after transfer

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3 **Memory T cells specific for murine CMV reemerge after multiple challenges and**  
4 **recapitulate immunity in various adoptive transfer scenarios**

5

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16 **Running Title:** Memory T cells recapitulate MCMV immunity after transfer

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26 **Abstract**

27 Reconstitution of CMV-specific immunity following transplant remains a primary clinical  
28 objective to prevent CMV disease, and adoptive immunotherapy of CMV-specific T cells can be  
29 an effective therapeutic approach. Due to viral persistence, most CMV-specific CD8<sup>pos</sup> T cells  
30 become terminally differentiated effector cells (T<sub>EFF</sub>). A minor subset retains a memory-like  
31 phenotype (T<sub>M</sub>), but it is unknown whether these cells retain memory function or persist over  
32 time. Interestingly, recent studies suggest that CMV-specific CD8<sup>pos</sup> T cells with different  
33 phenotypes have different abilities to reconstitute sustained immunity following transfer. The  
34 immunology of human CMV (HCMV) infections is reflected in the murine model (MCMV). We  
35 found that HCMV- and MCMV-specific T cells displayed shared genetic programs, validating  
36 the MCMV model for studies of CMV-specific T cells *in vivo*. The MCMV-specific T<sub>M</sub>  
37 population was stable over time and retained a proliferative capacity that was vastly superior to  
38 T<sub>EFF</sub> cells. Strikingly, after transfer, T<sub>M</sub> cells established sustained and diverse T cell populations  
39 even after multiple challenges. Although both T<sub>EFF</sub> and T<sub>M</sub> cells could protect Rag<sup>-/-</sup> mice, only  
40 T<sub>M</sub> cells persisted after transfer into immune replete, latently-infected recipients and responded if  
41 recipient immunity was lost. Interestingly, transferred T<sub>M</sub> cells did not expand until recipient  
42 immunity was lost, supporting that competition limits the antigen stimulation of T<sub>M</sub> cells.  
43 Ultimately, these data show that CMV-specific T<sub>M</sub> cells retain memory function during MCMV  
44 infection and can reestablish CMV immunity when necessary. Thus, T<sub>M</sub> cells may be a critical  
45 component for consistent, long-term adoptive immunotherapy success.

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## 51 **Introduction**

52 Latent Cytomegalovirus (CMV) is present within a large percentage of the population but is  
53 effectively controlled by the immune system (1-6). However, in transplant patients, immune  
54 suppression can allow CMV reactivations to progress to disease and increase mortality. Despite  
55 the advancements of anti-viral medications, long-term prevention of CMV disease is dependent  
56 on the reconstitution of CMV-specific immunity, which can be achieved through adoptive  
57 immunotherapy (5-18).

58

59 In adoptive immunotherapy, healthy CMV-seropositive donors provide CMV-specific T cells to  
60 an immune suppressed recipient. Due to the persistent nature of CMV infection, CMV-  
61 seropositive donors accumulate large numbers of CMV-specific CD8<sup>pos</sup> T cells (approximately  
62 5-10% of the total CD8<sup>pos</sup> T cells), a process termed “memory inflation,” (19-28). Studies in  
63 humans and the well-characterized mouse model (MCMV) have shown that the majority of  
64 inflationary populations are composed of terminally differentiated effector phenotype (T<sub>EFF</sub>) T  
65 cells that presumably develop as a result of repeated antigen stimulation and may not possess the  
66 proliferative or survival capacity necessary for long-term maintenance of CMV immunity (22,  
67 27, 29-34). Interestingly however, a fraction of these inflationary T cells retain a memory-like  
68 (T<sub>M</sub>) phenotype, despite sharing epitope specificity and T cell receptor sequences with the T<sub>EFF</sub>  
69 subset (23, 25, 35-37). Studies with other infection models have shown that such a memory  
70 phenotype can identify cells that have “stem-cell like” characteristics (38, 39). If this model  
71 holds true for CMV immunity, the CMV-specific T<sub>M</sub> cells would be ideal to use in an adoptive  
72 immunotherapy setting. Recent evidence supports this hypothesis. In a non-human primate  
73 model, CMV-specific effector T cells that were expanded in vitro from sorted T<sub>M</sub> cell had a  
74 superior ability to survive after adoptive transfer (40). Moreover, a human study showed a  
75 positive correlation between the presence of CMV-specific T<sub>M</sub> cells in a donor transfer and the

76 long-term maintenance of donor derived cells (41).

77

78 The goal of our study was to utilize the mouse model (MCMV) to directly address the capacity

79 of the CMV-specific  $T_M$  population to restore long-term CMV-specific immunity after transfer.

80 Importantly, we found that the MCMV-specific  $T_M$  cells share a common genetic program with

81 their human CMV-specific counterparts and that these cells could repeatedly restore long-term

82 CMV-specific immunity under a spectrum of transfer scenarios. Our data suggest that adoptive

83 immunotherapy with CMV-specific  $T_M$  cells will improve consistency and clinical outcomes in

84 patients at-risk for developing CMV disease.

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## 101 **Materials and Methods**

### 102 Mice

103 Unless otherwise indicated, C57BL/6 mice, CD45.1 mice (B6.SJL-Ptprc<sup>a</sup> Pepc<sup>b</sup>/BoyJ), Thy1.1  
104 mice (B6.PL-Thy1<sup>a</sup>/CyJ) and Rag<sup>-/-</sup> mice (B6.129S7-Rag1<tm1Mom>J) were purchased from  
105 Jackson Laboratory. OT-I transgenic mice (C57BL/6-Tg(TcraTcrb)1100Mjb/J) also purchased  
106 from Jackson, were bred with CD45.1 mice to produce double positive (CD45.2<sup>pos</sup>/CD45.1<sup>pos</sup>)  
107 OT-I mice. All protocols were approved by the Thomas Jefferson University Institutional  
108 Animal Care and Use Committee.

109

### 110 Infections

111 Unless otherwise indicated, mice were infected intraperitoneally (i.p.) with  $2 \times 10^5$  pfu of  
112 MCMV strain MW97.01 (42). Mice were considered latently-infected at 8 weeks post-infection.  
113 Rag<sup>-/-</sup> mice were infected with  $5 \times 10^4$  pfu of MCMV-TK virus (43). OT-I T cell transfer  
114 recipients were challenged with  $2 \times 10^5$  pfu MCMV-SL8, which expresses the SIINFEKL  
115 peptide (44, 45).

116

### 117 Tetramer Staining, Antibodies and FACS Analysis

118 MHC-tetramers were provided by the NIH Tetramer Core Facility  
119 (<http://tetramer.yerkes.emory.edu/>) and have been described previously (27). Staining was  
120 performed as described previously (27) with tetramers and the following antibodies: [CD8(53-  
121 6.7); CD44(IM7); CD27(LG.3A10); CD127(A7R34); KLRG1(2F1); CD62L(MEL-14);  
122 CD45.1(A20); CD45.1(104); Thy1.1(OX-7); Thy1.2(30-H12); IFN- $\gamma$ (XMG1.2); TNF- $\alpha$ (MP6-  
123 XT22); CD107a(1D4B)]. In all cases, samples were collected on an LSR II and analyzed with  
124 FlowJo software (Tree Star). The gating strategy for phenotypic characterization of tetramer<sup>pos</sup>  
125 CD8<sup>pos</sup> T cells involved first gating lymphocytes and then singlets. CD8<sup>pos</sup> cells were gated as

126 frequency of singlets. Tetramer<sup>pos</sup> cells were identified as a frequency of CD8<sup>pos</sup> cells. A B8R  
127 tetramer (specific for the B8R peptide from Vaccinia), was used as a negative tetramer control.  
128 Tetramer<sup>pos</sup> cells were phenotypically defined by they expression of KLRG1, CD27, CD127 or  
129 CD62L.

130

### 131 Adoptive Transfers

132 CD8<sup>pos</sup> splenocytes from latently-infected donors were enriched using EasySep Biotin selection  
133 kit (StemCell Technologies) and biotinylated antibodies against RBCs(Ter<sup>119</sup>), CD4(GK1.5) and  
134 CD19(6D5) according to the recommended protocol. Enriched cells were stained to determine  
135 the frequency of tetramer<sup>pos</sup> cells within the enriched fraction and then sorted on either a MoFlo  
136 (Dako Cytomation) or an ARIA II (BD Biosciences) cell sorter. Sorted cells were counted, and 5  
137 x 10<sup>4</sup> cells were transferred via the retro-orbital sinus. Sort purity was analyzed on an LSR II.  
138 The number of transferred tetramer-binding CD8<sup>pos</sup> T cells was estimated using the tetramer  
139 frequency within the enriched CD8<sup>pos</sup> population and the post-sort purity analysis. Fold change  
140 was calculated as the number of tetramer-binding T cells in the spleen 7 days post-challenge over  
141 the total number of tetramer<sup>pos</sup> cells transferred (assuming 100% engraftment). The gating  
142 strategy for analyzing donor cells in the recipients was identical to that described above with  
143 antibodies specific for the relevant congenic marker (CD45.1 or Thy1.2).

144

145 For OT-I adoptive transfers, splenocytes from naive mice containing 600 OT-I T cells were  
146 transferred. Recipients were challenged with MCMV-SL8. To establish secondary and tertiary  
147 populations, OT-I T<sub>M</sub> CD8<sup>pos</sup> T cells were FACS sorted and transferred as described in  
148 Supplemental Figure 1 and the legend for Figure 5. Following challenge with MCMV-SL8, the  
149 frequencies of donor OT-Is were determined in the blood of recipients using the strategy  
150 described above except that singlets were not identified and OT-I donors were identified by



151 expression CD45.1 and V $\alpha$ 2.

152

### 153 Intracellular Stimulation (ICS)

154 ICS and staining was performed as previously described (27, 45), with minor modifications.

155 Specifically, cells were incubated with 1  $\mu$ g/mL peptide (Genemed Synthesis), 1  $\mu$ g/mL

156 brefeldin A (GolgiPlug, BD Biosciences) and CD107a-specific antibody for 3 hours.

157

### 158 CD70 Blocking Antibody Treatment

159 CD70 antibody blockade was performed as previously described (46), with minor modifications.

160 Briefly, mice received either 150  $\mu$ g of anti-CD70(FR70) or control rat IgG2b (both from

161 BioXCell) via the i.p. route. Injections were administered at days -1, 0 and 3 post-infection.

162

### 163 Antibody Depletions

164 Antibody depletions were performed with Thy1.1(19E12), CD4(GK1.5) and NK1.1(PK136)

165 antibodies. 300  $\mu$ g of each antibody were administered i.p. in PBS. Three subsequent injections

166 of 100  $\mu$ g of each antibody were given at 7 day intervals.

167

### 168 Microarray

169 Splenocytes from latently-infected mice were co-stained with tetramers loaded with the antigenic

170 peptides from M38, m139 and IE3 (25) and sorted on a MoFlo (Dako Cytomation) cell sorter.

171 MCMV-specific T cells were identified as CD8<sup>pos</sup>, CD44<sup>hi</sup> and tetramer binding and then further

172 segregated into T<sub>M</sub> and T<sub>EFF</sub> cells subsets by expression of KLRG1 and CD127. Naïve CD8<sup>pos</sup>

173 cells were CD44<sup>lo</sup>. Total RNA was isolated using the Qiagen RNeasy Plus Kit (Qiagen),

174 quantified on a NanoDrop 2000c Spectrophotometer (Thermo Scientific) and processed at the

175 Microarray Core Facility at Thomas Jefferson University. Briefly, 2.5  $\mu$ g fragmented and

176 biotinylated cDNA was hybridized to Mouse gene 1.0 ST array (Affymetrix). Chips were  
177 scanned on an Affymetrix Gene Chip Scanner 3000 and data were analyzed using the R  
178 programming language and various packages from Bioconductor (47). The oligo package (48)  
179 was used to extract expression data from the Affymetrix CEL files and perform background and  
180 RMA normalization (49). Annotation information was added using the  
181 `mogene10stranscriptcluster.db` (50) package. Probes without valid annotations (7,196 of 35,556  
182 probes) were removed before differential expression analysis using the `limma` package's (51)  
183 linear modeling and Bayes methods (52). Genes showing up- or down-regulation of at least two-  
184 fold and  $p$ -value  $< 0.05$  in each of three contrasts ( $T_{\text{EFF}}$  vs. Naïve,  $T_{\text{M}}$  vs. Naïve, and  $T_{\text{EFF}}$  vs.  $T_{\text{M}}$ )  
185 were considered for gene set enrichment analysis (GSEA). Microarray data have been deposited  
186 in the Gene Expression Omnibus (GEO) database (53) (accession number: GSE61927)

187

### 188 Gene Set Enrichment Analysis

189 Human data for series GSE24151 (54) was retrieved from NCBI's GEO database (53), extracted  
190 using Partek<sup>®</sup> Genomics Suite<sup>®</sup> software, version 6.6 (Partek Inc., St. Louis, MO) and curated  
191 for input into GSEA software (55) (<http://www.broadinstitute.org/gsea/>). Since the data for  
192 GSE24151 have been deposited in GEO as  $\log_{10}$  ratios of the reference pool to sample, each  
193 value was inverted by multiplying by -1. Gene names in the six mouse gene lists (up- or down-  
194 regulated in each of the three contrasts described above) were converted to human names using  
195 data from NCBI's Homologene database, Release 68  
196 (<http://www.ncbi.nlm.nih.gov/homologene>). The converted gene lists along with genes specific  
197 to the liver and the TCR receptor pathway from the Molecular Signature Database (MSigDB)  
198 (55) were analyzed for enrichment in the human data using recommended settings for the GSEA  
199 command-line interface.

200

## 201 **Results**

### 202 **MCMV-specific inflationary T<sub>M</sub> populations are stable and share a common** 203 **transcriptional program with HCMV-specific CD8<sup>pos</sup> T cells in humans**

204 In the mouse model, MCMV infection of C57BL/6 (B6) mice results in inflation of select  
205 MCMV-specific CD8<sup>pos</sup> T cells specific for peptides from the viral proteins M38, m139 and IE3  
206 (Fig. 1A and (25, 27, 28)). As in humans infected with human CMV (HCMV), the majority of  
207 MCMV-specific inflationary T cells express a T<sub>EFF</sub> phenotype (often defined as T<sub>EMRA</sub> in  
208 humans), while only a small fraction express a T<sub>M</sub>-like phenotype, defined here as  
209 KLRG1<sup>lo</sup>/CD27<sup>hi</sup> and further sub-divided into central memory (T<sub>CM</sub> – CD127<sup>hi</sup>/CD62L<sup>hi</sup>) and  
210 effector memory (T<sub>EM</sub> – CD127<sup>hi</sup>/CD62L<sup>lo</sup>) subsets (Fig. 1A and (22, 23, 27, 29-33, 56)). In  
211 contrast, “non-inflationary” MCMV-specific CD8<sup>pos</sup> T cell responses, represented by the  
212 response against the viral protein M45, contract after acute infection and are thought to be  
213 maintained by homeostatic mechanisms thereafter (Fig. 1A and (25, 27, 57)). As expected, non-  
214 inflators express a predominately memory (T<sub>M</sub>) phenotype, which also includes both T<sub>CM</sub> and  
215 T<sub>EM</sub> subsets (Fig. 1A and (23, 27)).

216  
217 It remains unknown whether the constant immune stimulation needed to maintain memory  
218 inflation causes a decline of the T<sub>M</sub> subset within inflationary populations over time. Using  
219 infection-matched cohorts, we found that the numbers of T<sub>M</sub> cells that were specific for  
220 inflationary antigens were stable over time and remarkably similar to the numbers of non-  
221 inflationary T<sub>M</sub> cells, despite great differences between the numbers of inflationary and non-  
222 inflationary T<sub>EFF</sub> cells (Fig. 1B, 1C). Thus, although continuous antigen stimulation maintains  
223 memory inflation, the inflationary T<sub>M</sub> population remains stable.

224  
225 The MCMV model is well characterized and the T cell responses clearly recapitulate those seen

226 in HCMV-infected people. To determine whether MCMV-specific  $T_M$  and  $T_{EFF}$  cells share a  
227 common transcriptional program with their human counterparts, we sorted MCMV-specific  $T_M$   
228 ( $CD44^{hi}/CD127^{hi}/KLRG1^{lo}$ ) and  $T_{EFF}$  ( $CD44^{hi}/CD127^{lo}/KLRG1^{hi}$ ) cells specific for the M38,  
229 m139 and IE3 antigens. Microarray analyses were performed on these cells. Genes that were  
230 significantly up- or down-regulated in  $T_M$  and  $T_{EFF}$  subsets relative to each other or to naïve  
231 ( $CD44^{low}$ ) T cells, were mapped to the corresponding human genes and compared with the  
232 profiles of HCMV-specific T cells, previously defined by the van Lier group as  
233  $CD27^{hi}/CD45RA^{lo}$  ( $T_M$ ) or  $CD27^{lo}/CD45RA^{hi}$  ( $T_{EFF}$ ) (54). The CD27 and CD127 (IL-7R $\alpha$ )  
234 molecules both mark CMV-specific T cells with a memory phenotype in mice and humans (27,  
235 29, 32, 58, 59) and nearly all MCMV-specific  $KLRG1^{lo}/CD27^{hi}$  cells ( $T_M$ ) co-expressed CD127  
236 (either  $T_{CM}$  or  $T_{EM}$ , Figure 1A). Gene set enrichment analyses (GSEA) were used to measure the  
237 overall correlation between the mouse and human gene expression data. As shown in Fig. 2A,  
238 genes that distinguished mouse  $T_{EFF}$  and  $T_M$  cells from each other were highly enriched within  
239 the corresponding human data set. That is: genes up-regulated specifically in mouse  $T_M$  cells  
240 relative to mouse  $T_{EFF}$  cells were highly enriched within the genes that distinguish human  $T_M$   
241 cells from human  $T_{EFF}$  and vice versa. Moreover, relative to naïve T cells, mouse genes that were  
242 up and down-regulated by  $T_{EFF}$  or  $T_M$  cells were highly enriched within genes that distinguished  
243 their human counterparts from human naïve T cells (Fig. 2B). The analyzed mouse genes and the  
244 core enrichment profiles of each comparison are listed in Supplemental Table 1. Importantly,  
245 several of these genes corresponded to our sorting parameters and the known phenotypes of  $T_M$   
246 and  $T_{EFF}$  populations. As controls, identical analyses were performed with genes associated with  
247 the T cell receptor signaling pathway or liver and the data exhibited expected patterns (Fig. 2B).  
248  
249 Overall, these data show that MCMV-specific and HCMV-specific T cells share a common  
250 genetic program, validating the use of the MCMV model to investigate the function of HCMV-

251 specific T cells. To our knowledge, this is the first direct comparison of MCMV-specific and  
252 HCMV-specific T cell gene expression profiles.

253

### 254 **The inflationary T<sub>M</sub> population retains proliferative capacity**

255 To test the proliferative capacity of the T<sub>M</sub> and T<sub>EFF</sub> cells, both populations were sorted from  
256 spleens of latently-infected B6 mice (>3 months post-infection) using their differential  
257 expression of KLRG1 and CD27. Sorted cells were transferred into naive congenic recipients  
258 and re-challenged. The M45- and M38-specific T<sub>M</sub> cells proliferated robustly within 7 days after  
259 challenge, each expanding almost 1000-fold in the spleen alone, assuming 100% engraftment of  
260 the donor cells (Fig. 3A, 3B). In contrast, the M38-specific T<sub>EFF</sub> population expanded less than  
261 10-fold in the same time period. Importantly, while the T<sub>EFF</sub> donor cells remained exclusively  
262 KLRG1<sup>hi</sup>, the T<sub>M</sub> donor cells produced large numbers of both T<sub>EFF</sub> and T<sub>M</sub> progeny (Fig. 3C). In  
263 fact, donor M45- and M38-specific T<sub>M</sub> phenotype cells were present in the spleen 7 days after  
264 challenge at numbers that were approximately 50 to 100-fold higher than had been transferred  
265 (dotted line, Fig. 3D), indicating expansion of this subset without terminal differentiation. These  
266 data show that MCMV-specific T<sub>M</sub> cells retain robust proliferative capacity and can produce  
267 phenotypically diverse progeny including new T<sub>M</sub>-phenotype cells.

268

269 Recent work has shown that interaction between CD27 and its ligand CD70 plays a functional  
270 role in the proliferation of MCMV-specific inflationary T cells (46). To test the contribution of  
271 this interaction specifically within the T<sub>M</sub> population, we sorted and transferred T<sub>M</sub> cells as above  
272 and blocked the CD27-CD70 interaction as described in the Methods. Blocking the CD27-CD70  
273 interaction significantly decreased the expansion of the M38- and M45-specific T<sub>M</sub> cells 7 days  
274 post-challenge by approximately 4- to 6-fold (Fig. 3E), which is in line with the impact of CD70  
275 blockade on unsorted (i.e. combined T<sub>M</sub> and T<sub>EFF</sub> populations) inflationary T cells (46). These

276 data further suggest that the majority of proliferative potential of inflationary T cells is contained  
277 within the minor T<sub>M</sub> subset. It should be noted that even in the presence of CD70 blockade, the  
278 T<sub>M</sub> population retained a proliferative capacity that was greater than the T<sub>EFF</sub> population,  
279 suggesting that additional pathways contribute to the total proliferative potential of these cells  
280 (Fig. 3B, 3E and unpublished observations).

281

### 282 **The inflationary T<sub>M</sub> population persists and can repeatedly recapitulate memory inflation**

283 To determine the ability of the T<sub>M</sub> donor cells to persist long-term, we tracked the progeny from  
284 T<sub>M</sub> donor cells in the blood after re-challenge. M38-specific T cells from T<sub>M</sub>-sorted donors  
285 persisted at high frequencies in recipients, while the M45-specific donor cells contracted after  
286 their initial expansion in the same mice (Fig. 4A, 4B). Despite their initial T<sub>M</sub> phenotype, the  
287 donor M38-specific T cells largely expressed a T<sub>EFF</sub> phenotype after challenge (Fig. 4C, 4D),  
288 consistent with a typical “inflationary” population. The population as a whole retained its ability  
289 produce IFN- $\gamma$ , TNF- $\alpha$  and expose CD107a (Fig. 4E, 4F). Importantly, a small portion of donor  
290 T cells retained their T<sub>M</sub> phenotype even after this secondary challenge (Fig. 4C, 4D).

291

292 To understand whether these persistent T<sub>M</sub> phenotype donors continued to be functional, we  
293 turned to the OT-I transgenic system to facilitate sorting and avoid the possible selection of  
294 different T cell clones (Fig. 5A). As shown previously, transferred naive OT-Is undergo inflation  
295 and produce both T<sub>M</sub> and T<sub>EFF</sub> progeny after primary challenge with SIINFEKL-expressing  
296 MCMV-SL8 (45). We sorted the T<sub>M</sub> phenotype OT-I cells that formed after primary challenge,  
297 transferred these cells and challenged the recipients to establish secondary populations  
298 (Supplemental Fig. 1A). As with non-transgenic T cells (Fig. 4), secondary challenge of T<sub>M</sub> OT-  
299 Is induced inflation and T<sub>EFF</sub> formation as well as a persistent KLRG1<sup>lo</sup> population  
300 (Supplemental Fig. 1B). These secondary T<sub>M</sub> cells were again sorted (Supplemental Fig. 1C),

301 transferred into a 3<sup>rd</sup> set of naïve recipients and re-challenged. Incredibly, the donor secondary  
302 T<sub>M</sub> population inflated and produced both KLRG1<sup>hi</sup> and KLRG1<sup>lo</sup> progeny following this tertiary  
303 challenge (Fig. 5B-5E).

304  
305 Repeated acute viral challenges of small numbers of T cells in naïve mice drives T<sub>EFF</sub>  
306 differentiation (60-63) and indeed the overall frequency of tertiary inflationary cells that retained  
307 a T<sub>M</sub> phenotype was reduced (Fig. 5E and Supplemental Fig. 1B). However, these tertiary  
308 stimulated OT-Is remained functional, producing both IFN- $\gamma$  and TNF- $\alpha$ , as well as exposing  
309 CD107a (Fig. 5F, 5G). These data show that T<sub>M</sub> phenotype T cells specific for inflationary  
310 antigens can repeatedly recapitulate memory inflation upon viral challenge and produce  
311 functional T<sub>EFF</sub> and T<sub>M</sub> progeny.

312

### 313 **Memory and Effector Subsets protect Rag<sup>-/-</sup> mice**

314 To test the ability of transferred T<sub>M</sub> cells to protect against a lethal MCMV challenge, T<sub>M</sub> and  
315 T<sub>EFF</sub> populations were sorted from latently-infected B6 mice as above and transferred into Rag<sup>-/-</sup>  
316 recipients. One day later, the Rag<sup>-/-</sup> recipients were challenged with MCMV-TK, which lacks  
317 the m157 gene and is therefore resistant to NK-mediated control (43). Both transferred T<sub>M</sub> or  
318 T<sub>EFF</sub> cells expanded following the challenge and were sufficient to protect the recipients (Fig.  
319 6A, 6B). In contrast, Rag<sup>-/-</sup> mice that received no T cell therapy became moribund in 2-4 weeks  
320 and had to be sacrificed (Fig. 6B). Notably, the T<sub>EFF</sub> population, which proliferated very poorly  
321 in immune replete mice (Fig. 3), expanded and persisted in immune deficient hosts for at least 11  
322 weeks post-challenge (Fig. 6A, 6B). However, the T<sub>EFF</sub> responses lacked M45-specific, non-  
323 inflationary T cells (Fig. 6A). These data show that MCMV-specific T<sub>M</sub> cells are capable of  
324 protecting immune deficient mice and producing immune responses with broad specificities.

325

**326 The T<sub>M</sub> population can persist long-term and respond when necessary**

327 Patients undergoing hematopoietic stem cell transplantation (HSCT) are most susceptible to late-  
328 onset (>100 days) reactivating CMV, as opposed to an acute CMV infection ((12, 64-66) and  
329 reviewed in (67)). Furthermore, transferred CMV-specific T cells will need to compete with host  
330 immunity. Therefore, we developed a model to test whether T<sub>M</sub> and T<sub>EFF</sub> subsets could respond  
331 to viral reactivation after a long delay. To this end, T<sub>M</sub> and T<sub>EFF</sub> cells were sorted from latently-  
332 infected mice (> 3 months post-infection) and transferred into immune replete, infection matched  
333 or naive, congenic recipients differing at the Thy1 locus (Fig. 7A). Following the transfer, the  
334 latently-infected recipients were rested as described in the figure legend. Donor T cells did not  
335 expand dramatically in any animal following transfer (Supplemental Fig. 2A) supporting our  
336 previous conclusion that competition between T cells dictates MCMV-specific T cell expansion  
337 (45). Recipient T cells and NK cells were then eliminated in all mice using a cocktail of  
338 depletion antibodies that targeted the host cells (Thy1.1<sup>pos</sup>), but left the donor cells (Thy1.2<sup>pos</sup>)  
339 intact (Fig. 7B and Supplemental Fig. 2B). This depletion protocol did not induce detectible viral  
340 transcription in any animal as assessed by nested RT-PCR (unpublished observations), likely due  
341 to the presence of anti-viral antibodies (68). Despite the 9-12 week rest period, MCMV-specific  
342 donor T<sub>M</sub> cells responded robustly in all infected recipients after host depletion (Fig. 7C and 7D).  
343 Importantly, donor T<sub>M</sub> cells did not expand to detectible levels in depleted naive recipients  
344 (Supplemental Fig. 2C). However, viral challenge of naïve mice that received T<sub>M</sub> donor cells 12  
345 weeks previously induced a robust donor response in 3 of the 4 animals, indicating that the T<sub>M</sub>  
346 cells persisted in these mice, even without any antigen (Supplemental Fig. 2C). Thus, antigen  
347 rather than homeostatic mechanisms accounts for the donor T<sub>M</sub> response in infected recipients.  
348

349 In marked contrast, after depletion, donor T cells were only detectible in 2 animals that had  
350 received T<sub>EFF</sub> cells and then only at very low frequencies (Fig. 7C, 7D). Control experiments



351 (Supplemental Fig. 3A-3C) supported previous work (69) suggesting that the KLRG1-specific  
352 antibody did not induce depletion of the transferred T<sub>EFF</sub> subset. Thus, the failure of T<sub>EFF</sub> cells to  
353 expand in this setting is not a sorting artifact, but rather the inability to persist and/or expand in  
354 response to low amounts of viral antigen.

355

356 After expansion, all infected mice that received T<sub>M</sub> cells had a donor population specific for  
357 multiple epitopes and the progeny had differentiated to form new T<sub>EFF</sub> populations (Fig. 7E and  
358 unpublished observations). Furthermore, the four tetramers used only stained ~60% of the total  
359 donor population in each animal (Fig. 7E), suggesting that the remaining 40% of each donor  
360 population contained cells specific for additional MCMV antigens. In contrast, in the two  
361 animals in which T<sub>EFF</sub> donors expanded to detectable levels, each was skewed substantially  
362 towards a single inflationary epitope (Fig. 7E). Since these sorted T<sub>EFF</sub> populations included  
363 large numbers of T cells specific for M38, m139 and IE3, this “hit-or-miss” expansion of donor  
364 T cells with select specificities implies that a very small number of non-T<sub>EFF</sub> cells may have  
365 contaminated the transfer.

366

367 In the mice that received T<sub>M</sub> donor cells, their diverse progeny persisted in recipients for more  
368 than 11 weeks after termination of the depletion regimen, even though host immunity had  
369 returned (Fig. 7F). These data suggest that T<sub>M</sub> cells with inflationary specificities are capable of  
370 surviving in an environment with very little or no antigen stimulation and then responding as  
371 needed during a period in which the host is immune compromised and viral antigen becomes  
372 available.

373

374 In total, these data show that protective MCMV-specific T<sub>M</sub> cells persist throughout infection,  
375 retain superior proliferative function, and can respond to viral antigen as needed, in contrast to

376 the numerically dominant T<sub>EFF</sub> cells. Since MCMV-specific T<sub>M</sub> cells share a transcriptional  
377 program with HCMV-specific T<sub>M</sub> cells, our data suggest that T<sub>M</sub> cells may be ideal candidates to  
378 restore functional immune surveillance in patients at risk for CMV reactivation.

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## 401 Discussion

402 Adoptive immunotherapy using CMV-specific CD8<sup>pos</sup> T cells can be a successful therapeutic  
403 strategy for combating CMV reactivations (5-18). However, the majority of CMV-specific  
404 CD8<sup>pos</sup> T cells isolated from healthy donors will express an effector-differentiated phenotype  
405 (CD27<sup>lo</sup>/CD127<sup>lo</sup>/CD45RA<sup>hi</sup>/KLRG-1<sup>hi</sup>/CD57<sup>hi</sup> - reviewed in (70)), and in vitro expansion of  
406 CMV-specific T cells drives their differentiation towards an effector phenotype (40). We used  
407 the MCMV model to show that the ability to restore MCMV-immunity is contained almost  
408 entirely within the minor T<sub>M</sub> subset that retains CD27. Although both T<sub>M</sub> and T<sub>EFF</sub> cells protected  
409 Rag<sup>-/-</sup> mice (Fig. 6), humans are unlikely to remain completely immune depleted like Rag<sup>-/-</sup>  
410 mice, and bolus CMV infections are of lesser concern than reactivation following  
411 transplantation. The inability of the T<sub>EFF</sub> population to consistently expand after immune  
412 depletion in latently-infected hosts, suggests that these cells will only be protective under limited  
413 conditions. These data support a previous study in humans that correlated the transfer of CD27<sup>hi</sup>  
414 CMV-specific T cells with an increased likelihood of T cell persistence and expansion (41).

415  
416 To validate the use of the MCMV model, we compared human and mouse MCMV-specific T  
417 cells and show for the first time, to our knowledge, that T<sub>M</sub> and T<sub>EFF</sub> populations in mice and  
418 humans share a common transcriptional profile. The power of the GSEA analysis used for this  
419 comparison is that it identifies significant correlations across the entire transcriptional profile,  
420 rather than comparing individual genes. Nevertheless, we expect that future studies examining  
421 conserved and divergent genetic pathways will reveal significant and relevant information about  
422 CMV-specific immunity in mouse and man. These results highlight the usefulness of the MCMV  
423 model to: 1) perform CMV-specific CD8<sup>pos</sup> T cell functional studies that are difficult or  
424 impossible to perform in humans and 2) provide translational insights into novel or improved  
425 therapeutic strategies.

426  
427 Understanding how CMV-specific T cell immunity is maintained is critical for the improvement  
428 of CMV adoptive immunotherapy. Persistent antigen stimulation from CMV reactivations results  
429 in the majority of inflationary CD8<sup>pos</sup> T cells developing a T<sub>EFF</sub> phenotype and function.  
430 However, our previous work showed that unsorted inflationary CD8<sup>pos</sup> T cells, containing  
431 primarily T<sub>EFF</sub> cells, declined after transfer into congenic, latently-infected recipients (27). These  
432 data suggest that MCMV-specific T<sub>EFF</sub> cells are unable to sustain themselves in an immune  
433 replete environment, even in the presence of antigen. Thus, we proposed that the accumulation of  
434 T<sub>EFF</sub> cells is the result of continual antigen stimulation of the T<sub>M</sub> population. Our data show that a  
435 small, stable MCMV-specific T<sub>M</sub> population has strong functional similarities to classical  
436 memory T cells that develop after acute infections. For example, the ability to proliferate in  
437 response to antigen without terminal differentiation is hallmark of functional memory T cells  
438 (71). In addition to producing differentiated progeny that accumulated after MCMV challenge  
439 (Figure 4D), donor T<sub>M</sub> cells also produced T<sub>M</sub> phenotype progeny that outnumbered the cells  
440 transferred (Fig. 3C and D) and persisted throughout our observation period (Fig. 4C and D).  
441 These data suggest that MCMV-specific T<sub>M</sub> cells have the ability to replace themselves even  
442 while producing differentiated progeny in response to antigen. Importantly, this was true through  
443 at least three rounds of stimulation using sorted splenic T<sub>M</sub> cells (Figure 5). Thus, MCMV-  
444 specific T<sub>M</sub> cells have the capacity to respond repeatedly to viral antigen during this persistent  
445 infection and can recapitulate memory inflation.

446  
447 It is interesting that transferred T<sub>M</sub> cells failed to expand in immune-replete, latently-infected  
448 hosts. Detectable numbers of donor T cells were only evident in one out of six mice prior to  
449 immune depletion (Supplemental Fig. 2A). In this case, the donors were not positive for any of  
450 the tetramers used in the analyses and made up less than 1% of the total CD8<sup>pos</sup> population.

451 However, loss of the host T cell populations led to rapid and robust expansion of donor T cells  
452 with diverse specificities and phenotypes in all T<sub>M</sub> cell recipients (Figure 7). The failure of  
453 transferred T<sub>M</sub> cells to expand in the presence of host MCMV-specific immunity may reflect the  
454 relative lack of available antigen during the latent phase of MCMV infection. Indeed, viral  
455 reactivations occur in only a fraction of latently-infected cells at any given time, and only rarely  
456 produce infectious viral particles (72, 73). Moreover, we have found that competition between T  
457 cells for access to this limited antigen regulates the expansion of individual T cell clones (45).  
458 Thus, the combination of low antigen and large numbers of MCMV-specific T cells in the  
459 recipients may have "shielded" the majority of the donor T<sub>M</sub> cells from the ongoing infection –  
460 an idea we have proposed previously (45, 74). Importantly, MCMV antigen is not required for  
461 MCMV-specific T<sub>M</sub> cell survival. We have previously shown that MCMV-specific T<sub>M</sub> cells  
462 divide at a consistent rate with or without antigen (28) and our new data (Supplemental Fig. 2C)  
463 show that inflationary T<sub>M</sub> cells can survive in naïve mice without any antigen. Thus, homeostatic  
464 mechanisms can support the inflationary T<sub>M</sub> population when it does not have access to antigen,  
465 which may partially explain the preservation of memory function within the T<sub>M</sub> subset. Taken  
466 together, these data suggest that the highly functional T<sub>M</sub> population, which can persist without  
467 access to antigen, proliferates robustly and produces new T<sub>M</sub> cells as well as more differentiated  
468 progeny upon antigen stimulation.

469  
470 Overall, our data further support the model that the burden of maintaining memory inflation falls  
471 on the functional T<sub>M</sub> population, which can provide a stable and consistent source of new T<sub>EFF</sub>  
472 progeny whenever needed, over for prolonged periods of time. However, T cell competition for  
473 limited antigen appears to prevent the continuous stimulation of most T<sub>M</sub> cells. Nonetheless, the  
474 T<sub>M</sub> population is capable of robustly responding if T cell competition is lost - a conclusion with  
475 important clinical implications for adoptive immunotherapy. Variations in transplant protocols,

476 patients and anti-viral therapy responses make it difficult to predict and standardize CMV  
477 prevention therapies. Our data suggest that the plasticity of the T<sub>M</sub> population, transferred before  
478 any disease develops, may offer a “personalized” therapy, where the treatment adapts to the  
479 conditions of the patient and responds if and when antigen becomes available. Future studies will  
480 be needed to explore whether the addition of homeostatic cytokines (e.g. IL-15) or  
481 pharmacotherapeutics (e.g. rapamycin (75)) preserves the T<sub>M</sub> phenotype either *in vivo* or during  
482 *in vitro* expansion.

483

#### 484 **Acknowledgments**

485 We thank the Kimmel Cancer Center Flow Cytometry Facility and Animal Facility at Thomas  
486 Jefferson University.

487

#### 488 **Conflict-of-Interest**

489 The authors declare no conflict-of-interests.

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501 **References**

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776 **Footnotes**

- 777 1. H.T. and M.T. contributed equally to this work
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- 781 5. This work was supported by a faculty start-up package from Thomas Jefferson University  
782 and grants from the National Institutes of Health (K22-AI081866 and RO1AI106810  
783 grants awarded to CMS).
- 784 6. Experiments conceived and designed by MQ and CMS. Experiments conducted by MQ,  
785 HT, BD and TM. Data analyzed by MQ, HT, MT and CMS. Manuscript written by MQ  
786 and CMS.
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788 Philadelphia, PA 19107; christopher.snyder@jefferson.edu; Phone: 215-503-2543; Fax:  
789 215-923-9248
- 790 8. Abbreviations: CMV, Cytomegalovirus; HCMV, Human Cytomegalovirus; MCMV,  
791 Murine Cytomegalovirus; T<sub>M</sub>, memory phenotype CD8<sup>+</sup> T cells; T<sub>EFF</sub>, terminal  
792 differentiated effector phenotype CD8<sup>+</sup> T cells; T<sub>CM</sub>, central memory CD8<sup>+</sup> T cells; T<sub>EM</sub>,  
793 effector memory CD8<sup>+</sup> T cells; T<sub>EMRA</sub>, terminal differentiated CD8<sup>+</sup> T cell phenotype in  
794 humans; i.p., intraperitoneal route; GSEA, gene set enrichment analysis; GEO, gene  
795 expression omnibus; SEM, standard error of the mean

796

797 **Figure Legends**798 **Figure 1: The non-inflationary and inflationary CD8<sup>pos</sup> T cell populations retain similar**  
799 **numbers of T<sub>M</sub> phenotype cells.**

800 Cohorts of age-matched B6 female mice were infected with MCMV and sacrificed at the  
801 indicated time points ( $n = 4$  per time point). Tetramer staining and phenotypic analyses were  
802 performed on blood and splenocytes. (A) Frequency of tetramer-binding CD8<sup>pos</sup> T cells in the  
803 blood at indicated time points. The phenotypic analysis shown was performed at d326 post-  
804 infection. T<sub>M</sub> cells were identified as CD27<sup>hi</sup>/KLRG1<sup>lo</sup>. T<sub>CM</sub> and T<sub>EM</sub> were further identified as  
805 CD127<sup>hi</sup> and either CD62L<sup>hi</sup> or CD62L<sup>lo</sup>, respectively. (B) Absolute numbers of KLRG1<sup>hi</sup>  
806 tetramer-binding CD8<sup>pos</sup> T cells in the spleen. (C) Absolute numbers of T<sub>CM</sub> and T<sub>EM</sub> tetramer-  
807 binding CD8<sup>pos</sup> T cells. Data are displayed as mean  $\pm$  SEM and represent two independent  
808 experiments.

809

810 **Figure 2: Gene Set Enrichment Analyses reveal significant overlap between the**  
811 **transcriptional profile of CMV-specific T cells in humans and mice. (A) Gene set enrichment**

812 was performed as described in the Methods. Shown are the enrichment plots for mouse genes  
813 that differed in a T<sub>EFF</sub> vs. T<sub>M</sub> comparison, plotted relative to human T<sub>EFF</sub> and T<sub>M</sub> cells. Values  
814 represent the normalized enrichment score (NES) and Family Wise Error Rate (FWER), which  
815 estimates the probability of a false positive NES. (B) Lists of significantly altered mouse genes  
816 (2-fold up or down and  $P < .05$ ) were generated for T<sub>EFF</sub> and T<sub>M</sub> cells relative to each other and  
817 relative to naive (CD44<sup>low</sup>) T cells. GSEA analyses were performed with these mouse gene sets  
818 relative to each of the indicated human data sets, rank ordered by expression (see methods). Stars  
819 indicate FWER corrected significance to control for multiple testing (\*  $P < .05$ , \*\*  $P < .01$ , \*\*\*  
820  $P < .001$ ).

821

822 **Figure 3: T<sub>M</sub> cells dramatically expand 7 days post-challenge and produce both T<sub>M</sub> and**  
823 **T<sub>EFF</sub> progeny.**

824 Age matched B6 mice received either T<sub>M</sub> or T<sub>EFF</sub> cells and were challenged with MCMV as  
825 described in the Methods. Spleens were collected 7 days later for analysis. (A) Representative  
826 FACS plots of tetramer<sup>pos</sup> donors in the spleen 7 days post-challenge. Frequencies in the corner  
827 are relative to total CD8<sup>pos</sup> cells. (B) Fold change of donor cells in the spleen, calculated as  
828 described in the Methods, 7 days after challenge. As antigen-specific T cells were not sorted,  
829 approximately equal numbers of M38- and M45-specific T<sub>M</sub> cells were transferred but ~10-fold  
830 more M38-specific T<sub>EFF</sub> cells were transferred compared with the T<sub>M</sub> cells. Due to the extremely  
831 low number of M45-specific T<sub>EFF</sub> transferred and the minimal expansion at day 7, it was not  
832 possible to calculate a comparable fold change value for the M45-specific T<sub>EFF</sub> population. Data  
833 were collected from two independent experiments (T<sub>M</sub>: *n* = 6 total; T<sub>EFF</sub>: *n* = 5 total) are shown.  
834 Statistical significance was determined by a Student's t-test (\*\*\*) *P* < .001; \*\*\*\* *P* < .0001). (C)  
835 Representative FACS plots of M38-specific CD8<sup>pos</sup> T cell progeny from either T<sub>M</sub> or T<sub>EFF</sub> donors  
836 in the spleen at 7 days post-challenge. Frequencies in the corner are relative to M38-specific  
837 CD8<sup>pos</sup> cells. (D) Absolute number of T<sub>M</sub> and T<sub>EFF</sub> phenotypic progeny that were produced from  
838 T<sub>M</sub> donors. Data are from the same experiments described in (B). (E) Fold change of donor cells  
839 in the spleen following treatment with either isotype control or anti-CD70 antibody. Data were  
840 collected 7 days post-challenge and represent two independent experiments (*n* = 6 total).  
841 Statistical significance was determined by a Student's t-test (\*\*\*) *P* < .001; \*\*\*\* *P* < .0001). All  
842 graphical data are displayed as mean ± SEM.

843

844 **Figure 4: T<sub>M</sub> cells re-inflate following re-challenge and retain function.**

845 Age matched B6 mice received T<sub>M</sub> cells and were challenged with MCMV as in the Methods.  
846 (A) Representative FACS plots of donor-derived T cells in the blood 126 days post-challenge.

847 (B) Frequencies of tetramer-binding T cells in the blood over time. Data were collected from  
848 three independent experiments ( $n = 17$  total). (C) Representative FACS plot of the phenotype of  
849 donor-derived M38-specific T cells in the blood 126 days post-challenge. (D) Frequencies of  
850 donor-derived, M38-specific  $T_M$  and  $T_{EFF}$  cells in the blood over time. Data are from the same  
851 experiments described in (B). Each line represents an individual mouse. The square datum point  
852 represents a mouse that appeared to lose the donor T cells after day 7 post-challenge, but  
853 effectors appeared  $\sim 20$  weeks after challenge. (E-F) Intracellular cytokine staining was  
854 performed on splenocytes 221 days post-challenge. Shown are representative FACS plots of  
855 stimulated (with M38 peptide) and unstimulated cells (E) and the frequencies of IFN- $\gamma$  positive  
856 cells that also express TNF- $\alpha$  and/or CD107a (F). Data were collected from a single experiment  
857 ( $n = 5$ ) described above. All graphical data are displayed as mean  $\pm$  SEM.

858

859 **Figure 5:  $T_M$  cells can re-inflate following multiple re-challenges.**

860 (A) Schematic of experimental design. To establish primary OT-I inflationary populations, 600  
861 naïve OT-I T cells expressing CD45.1 were transferred into naïve B6 (CD45.2) recipients  
862 followed by infection with MCMV-SL8 (i.e. primary challenge). Thirteen weeks later, 6,000  $T_M$   
863 phenotype primary OT-Is, isolated by FACS sorting, were transferred into new B6 recipients  
864 followed by MCMV-SL8 challenge (i.e. secondary challenge). This process was repeated a third  
865 time, transferring 3,500  $T_M$  OT-Is into naive mice and challenging with MCMV-SL8 (i.e. tertiary  
866 challenge). (B-C) Representative FACS plot of the donor stain 91 days post tertiary challenge  
867 (B) and frequencies of donor OT-Is (relative to total CD8s) in the blood at the indicated time  
868 points after tertiary challenge (C). Data were collected from two independent experiments ( $n =$   
869 12 total). Each line represents an individual mouse. (D-E) Phenotypic analyses of the mice  
870 described in (B-C). Representative FACS plot of the donor stain 30 weeks post-challenge  
871 Frequencies are relative to donor CD8s. (F-G) Intracellular cytokine staining was performed on

872 splenocytes approximately 20 weeks after the tertiary challenge. Shown are representative FACS  
873 plots of stimulated (with SIINFEKL peptide) and unstimulated cells (F) and the frequencies of  
874 IFN- $\gamma$  positive cells that also express TNF- $\alpha$  and/or CD107a (G). Data were collected from two  
875 independent experiments ( $n = 12$ ). Data are displayed as mean  $\pm$  SEM.

876

877 **Figure 6: T<sub>M</sub> and T<sub>EFF</sub> cells protect Rag<sup>-/-</sup> mice following an acute MCMV challenge**

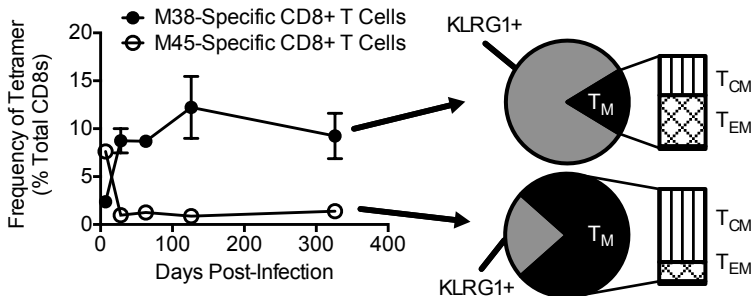
878 Age matched Rag<sup>-/-</sup> mice received either T<sub>M</sub> or T<sub>EFF</sub> cells and were challenged with MCMV-TK  
879 as described in the Methods. Mice were monitored daily for signs of morbidity (lethargy, raised  
880 hair and shaking) and sacrificed if they displayed clear signs of morbidity. Data were collected  
881 from two independent experiments. One experiment was carried out until 77 days post-challenge.  
882 A second experiment was censored at 33 days post-challenge. (A) Representative tetramer  
883 staining of T cells in Rag<sup>-/-</sup> that received either T<sub>M</sub> or T<sub>EFF</sub> transfers. Data were collected 11  
884 weeks post-challenge. Frequencies are relative to total CD8s. (B) Survival curve ( $n = 7$  for  
885 control group;  $n = 7$  for T<sub>M</sub> group;  $n = 6$  for T<sub>EFF</sub> group). Statistical significance was determined  
886 by a log-rank (Mantel-Cox) test (\*\*\*\*  $P < .0001$ ).

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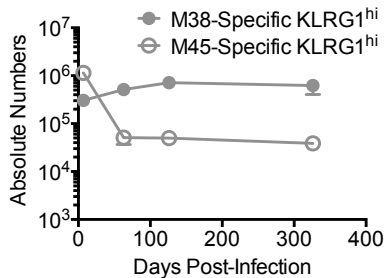
888 **Figure 7: T<sub>M</sub> cells persist in latently-infected, immune replete mice and expand when host  
889 immunity is lost.**

890 (A) Schematic of experimental design. Age matched B6 and Thy1.1 mice were infected with  
891  $1 \times 10^6$  pfu MCMV-Smith. Following the establishment of viral latency (>8 weeks post-infection),  
892 either T<sub>M</sub> or T<sub>EFF</sub> cells from the B6 donors were transferred, as described in the Methods, into the  
893 latently-infected Thy1.1 recipients or into naïve Thy1.1 mice. Latently-infected recipients were  
894 rested for 9-12 weeks, while the naïve recipients were rested for approximately 1.5 weeks. (B)  
895 Antibody depletion schedule. (C-E) The presence of tetramer<sup>pos</sup> donors was analyzed by flow  
896 cytometry immediately following the depletion schedule. Data were collected from two

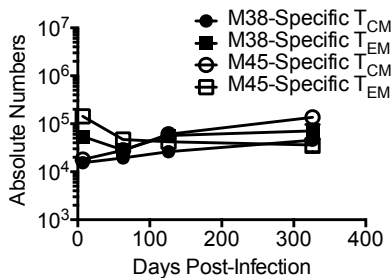
897 independent experiments ( $n = 6$  total). Three mice from each group were depleted 9 weeks after  
898 the transfer; three mice from each group were depleted 12 weeks after transfer. (C) Histograms  
899 of donor T cells within each individual recipient. (D) Representative FACS plots of tetramer<sup>pos</sup>  
900 donors immediately following the depletion regimen. (E) Frequency within each individual  
901 recipient of each analyzed tetramer as a percent of total donor CD8<sup>+</sup> cells. T<sub>EFF</sub> recipients 3-6 are  
902 excluded because they did not have a donor population. (F) Tetramer staining was performed 11  
903 weeks after depletion in one experiment described above ( $n = 3$ ).

**FIGURE 1****A****B**

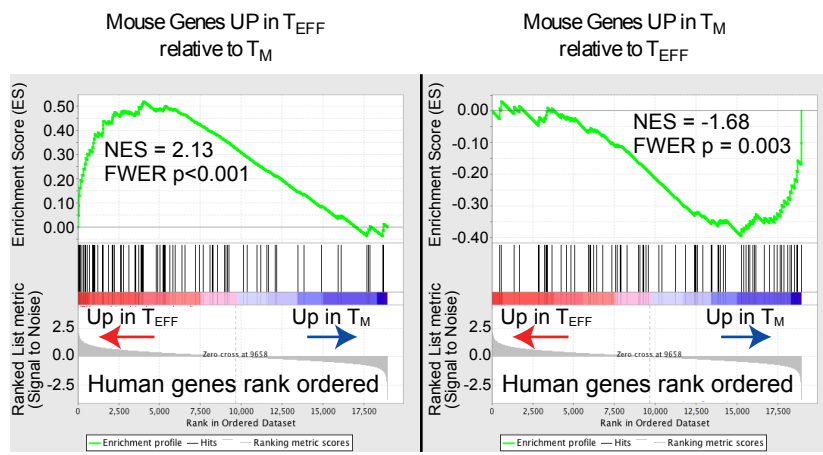
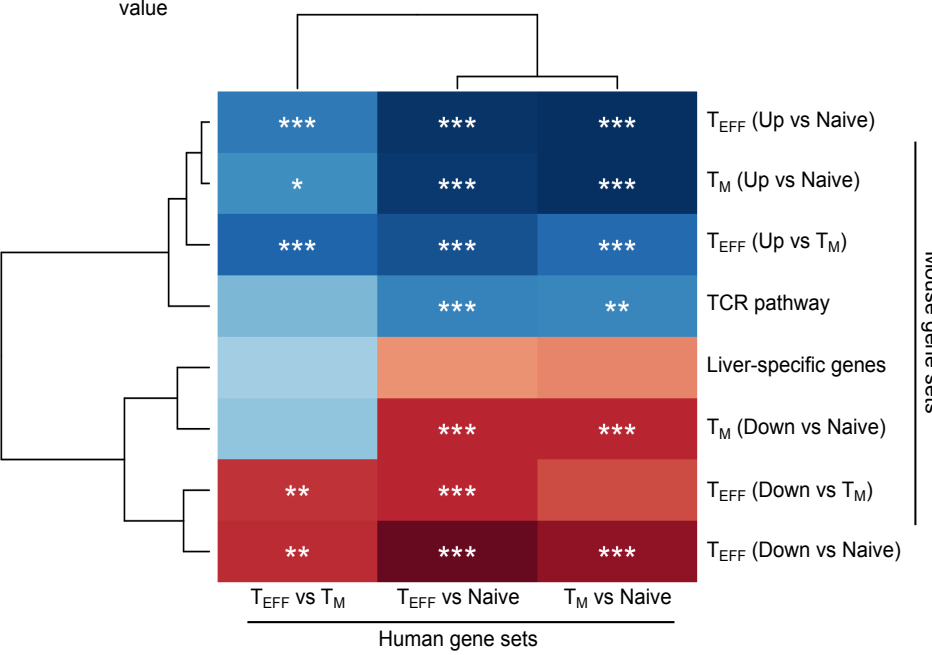
Numbers of Inflationary and Non-Inflationary KLRG1<sup>hi</sup> Subsets

**C**

Numbers of Inflationary and Non-Inflationary  $T_M$  Subsets

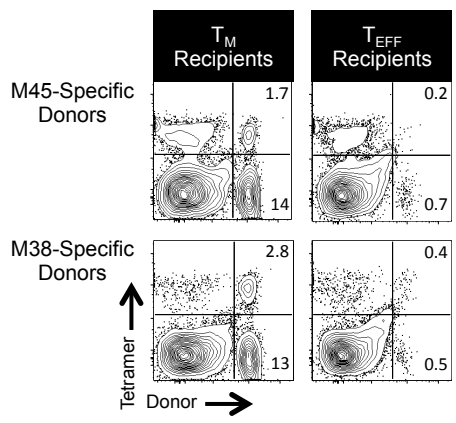




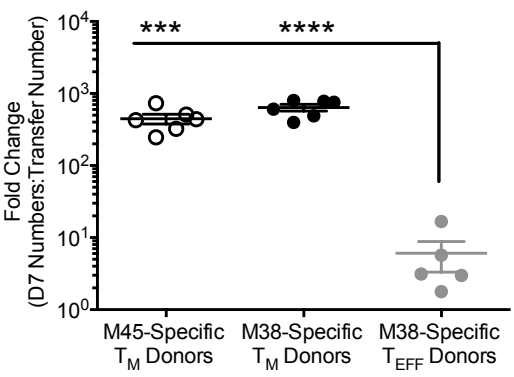
**FIGURE 2****A****Enrichment Plots****B****GSEA Enrichment Scores for Mouse Genes in Human Data**

# FIGURE 3

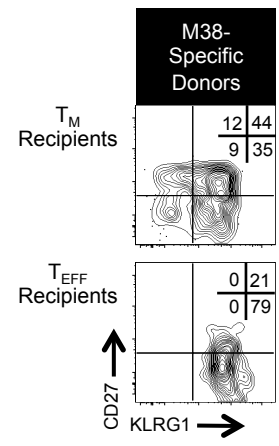
**A**



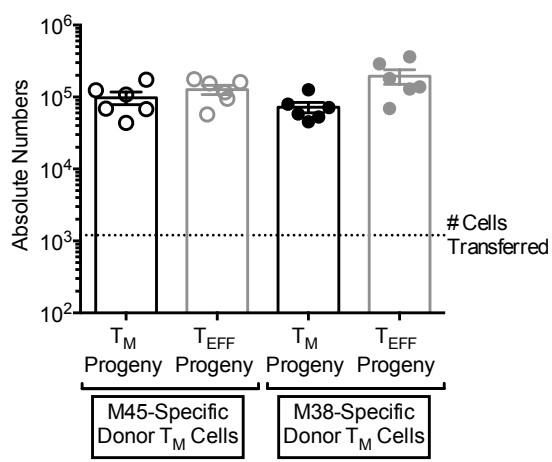
**B**



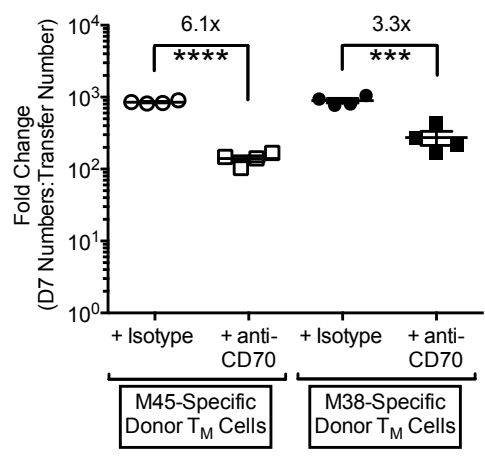
**C**

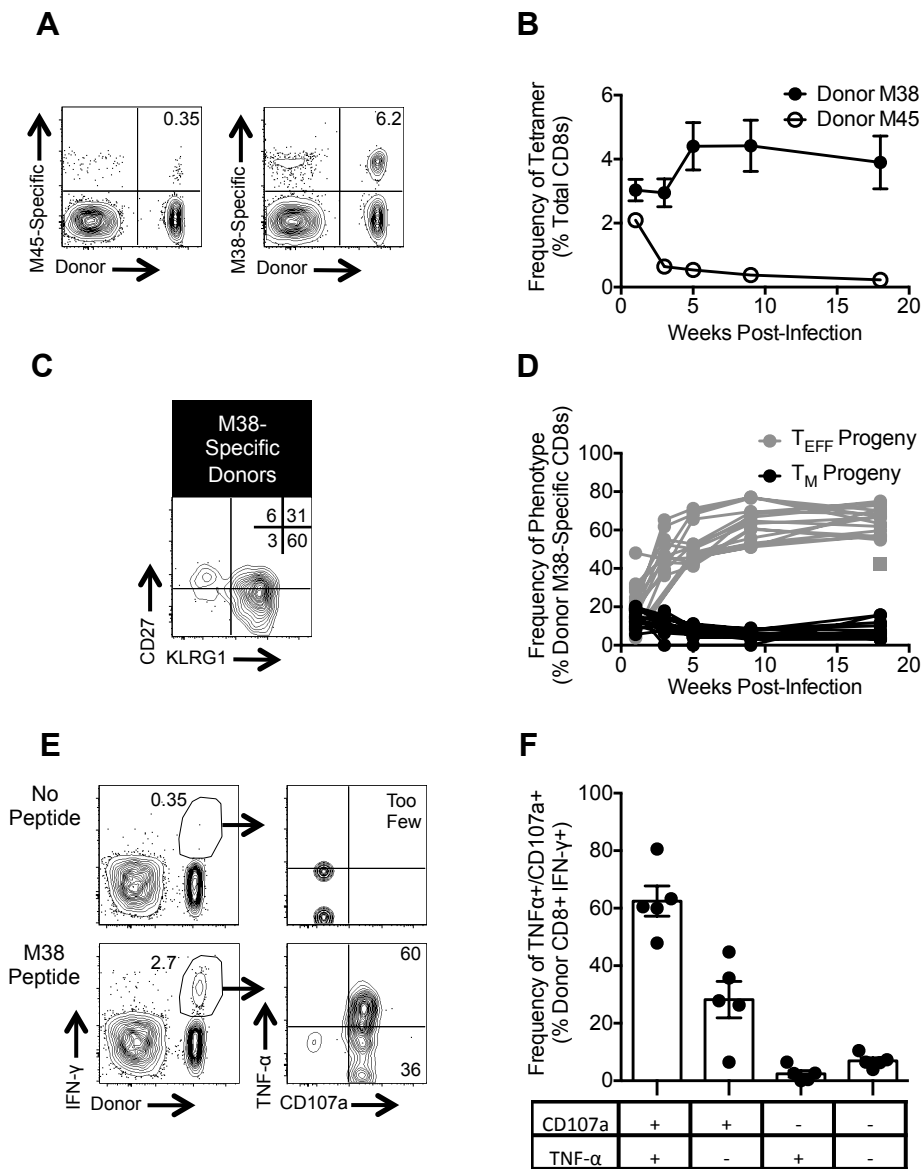


**D**



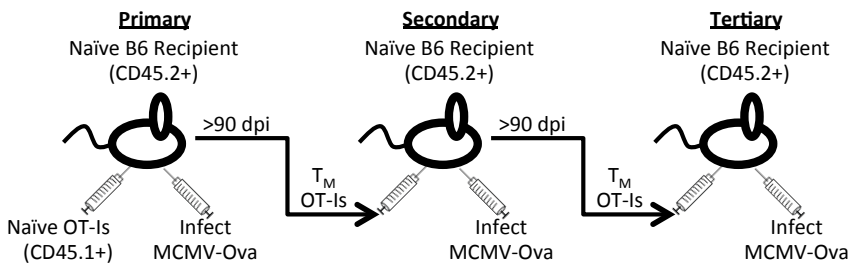
**E**



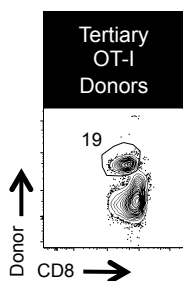
**FIGURE 4**

# FIGURE 5

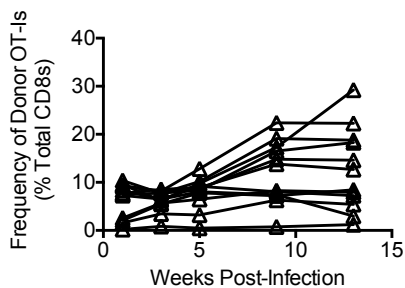
**A**



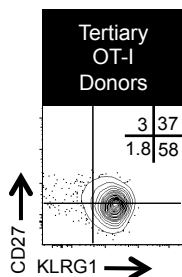
**B**



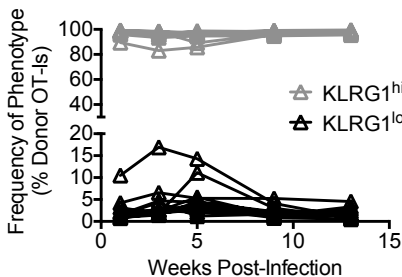
**C**



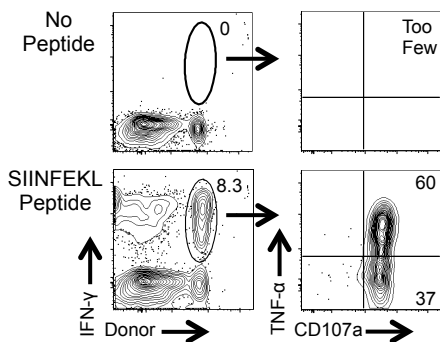
**D**



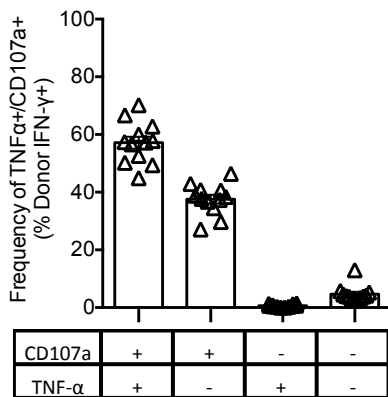
**E**



**F**

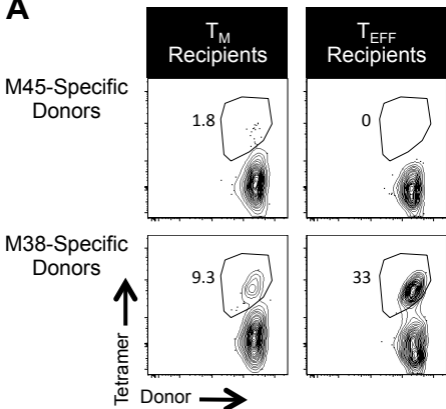


**G**

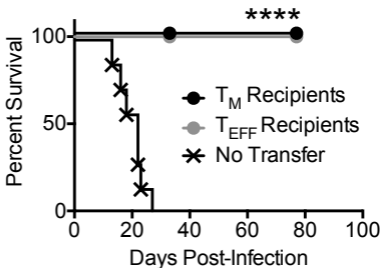


# FIGURE 6

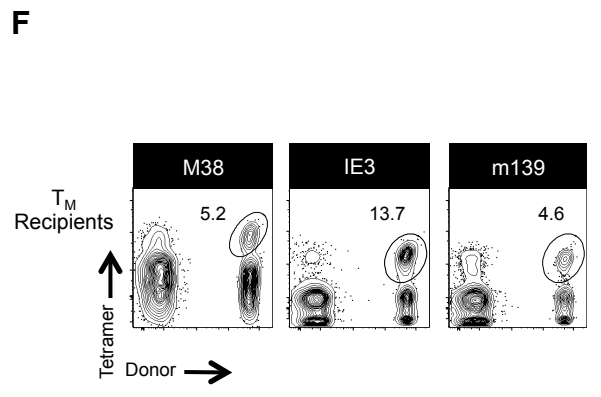
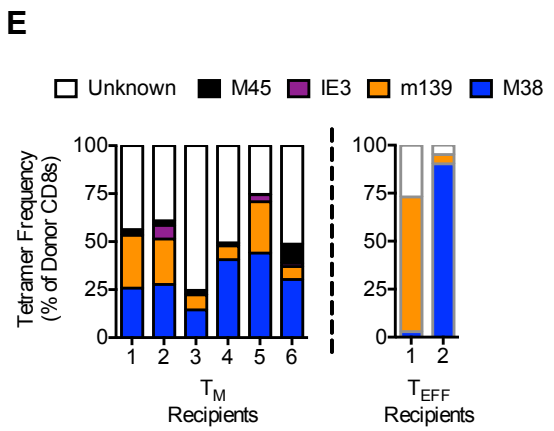
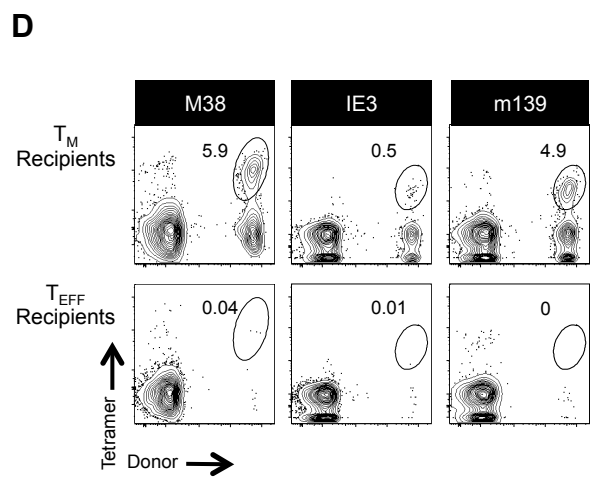
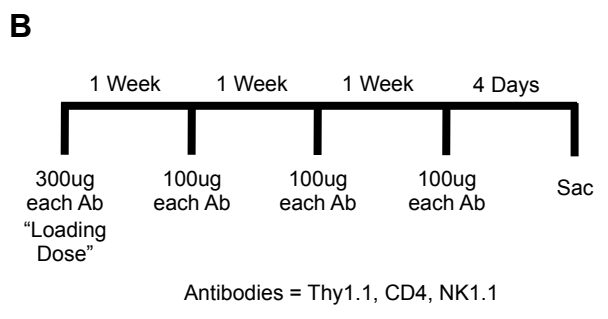
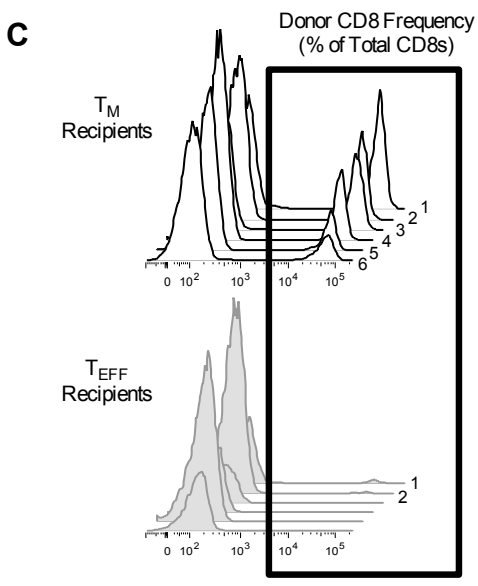
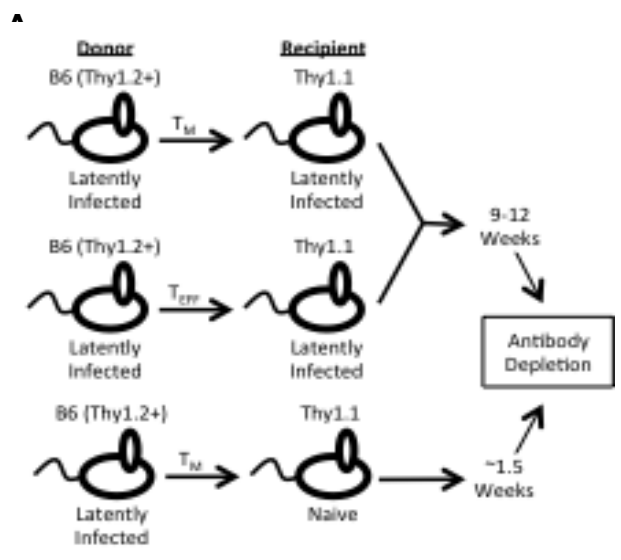
## A



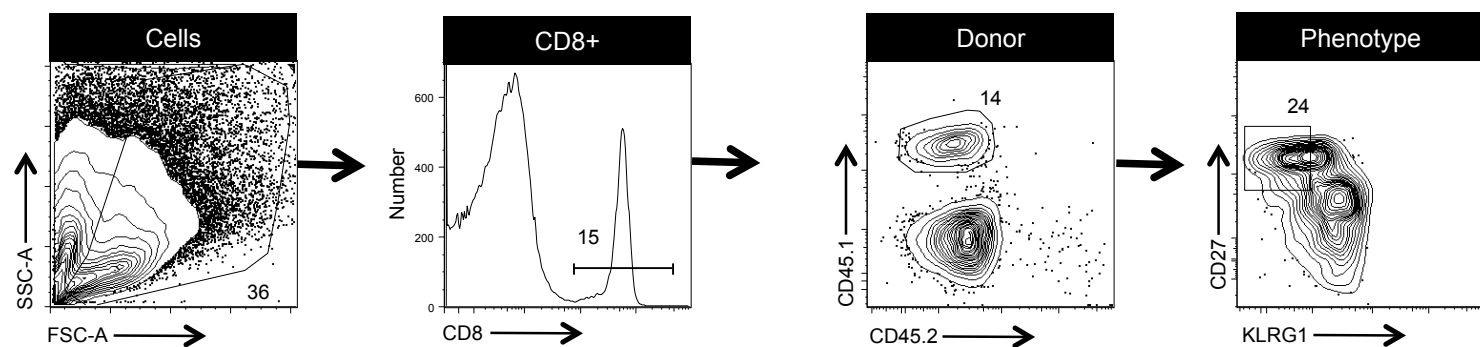
## B



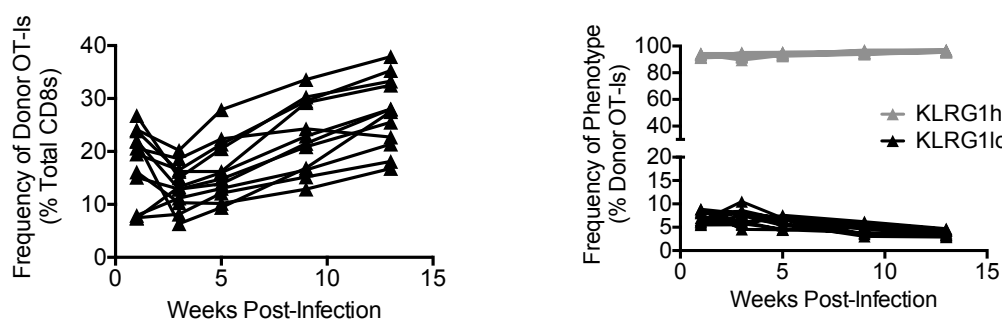
**FIGURE 7**



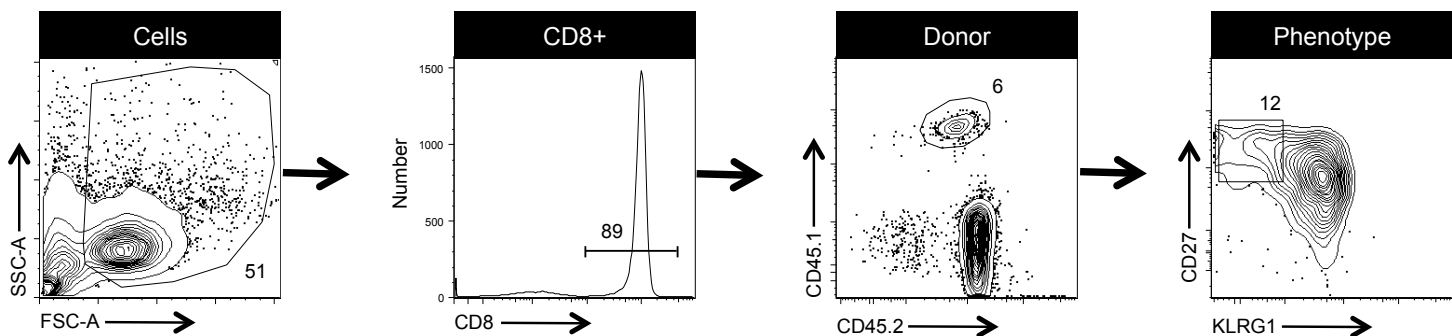
A



B



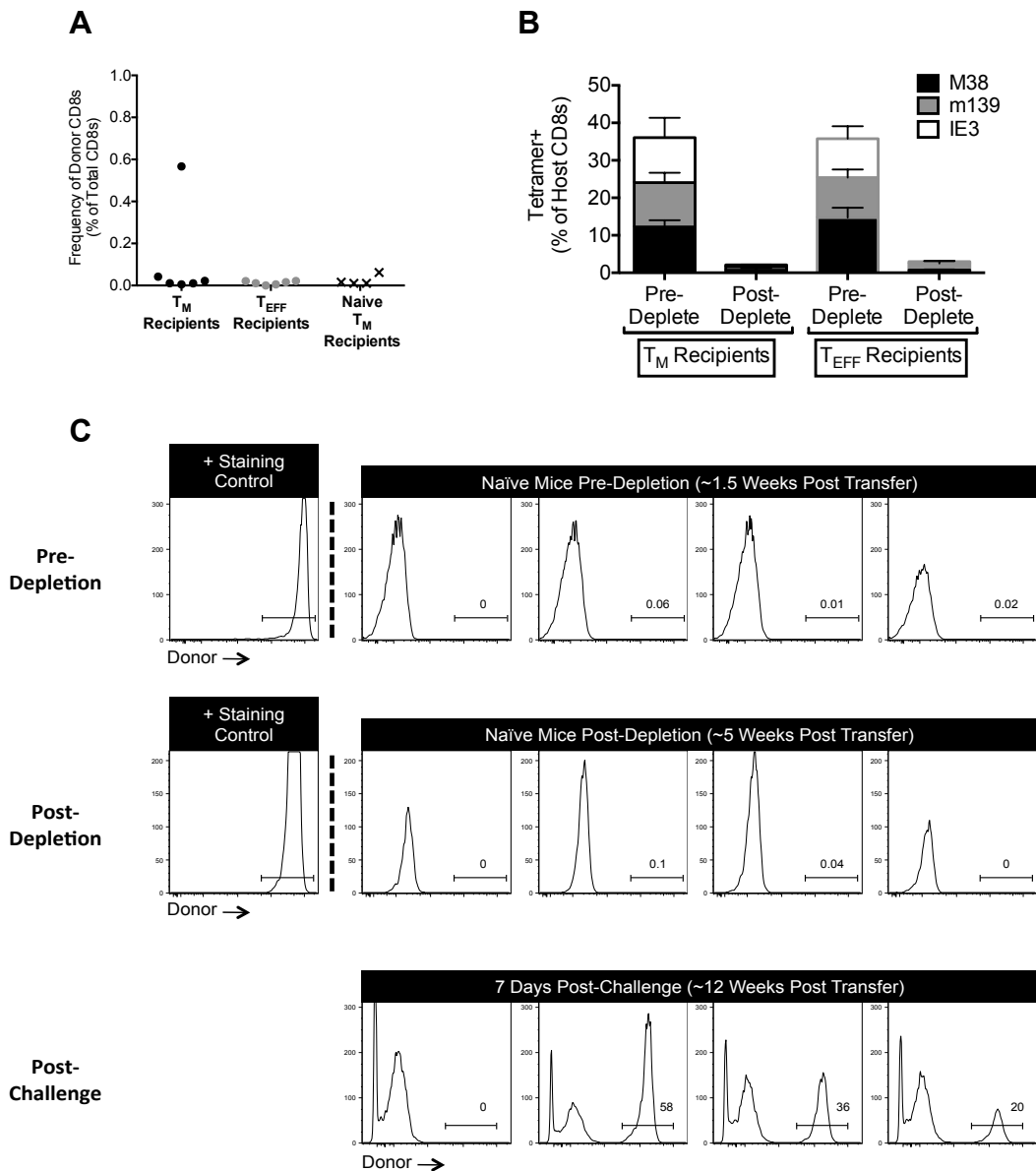
C



### Supplemental Figure 1: Representative plots for repeat transfers and data from secondary stimulated OT-I-s

(A) Sorting strategy for isolating OT-I T<sub>M</sub> T cells after primary challenge. Briefly, 600 naïve OT-I T cells expressing CD45.1 were transferred into naïve B6 (CD45.2) recipients and challenged MCMV-SL8, (i.e. primary challenge). Thirteen weeks after the primary challenge, donor OT-I T cells were sorted using the CD45.1 marker and the T<sub>M</sub> subset was identified as CD27<sup>hi</sup> and KLRG1<sup>lo</sup>. (B) 6,000 sorted T<sub>M</sub> OT-I T cells were transferred into naïve B6 recipients and challenged with MCMV-SL8 (i.e. secondary challenge). Shown is the frequency and phenotype of blood-localized OT-I-s over time, relative to total CD8<sup>pos</sup> T cells. Data was collected from two independent experiments (n=7 total). (C) Sorting strategy for isolating OT-I T<sub>M</sub> T cells after secondary challenge. Briefly, thirteen weeks after the secondary challenge, donor OT-I T cells were sorted as described in (A).

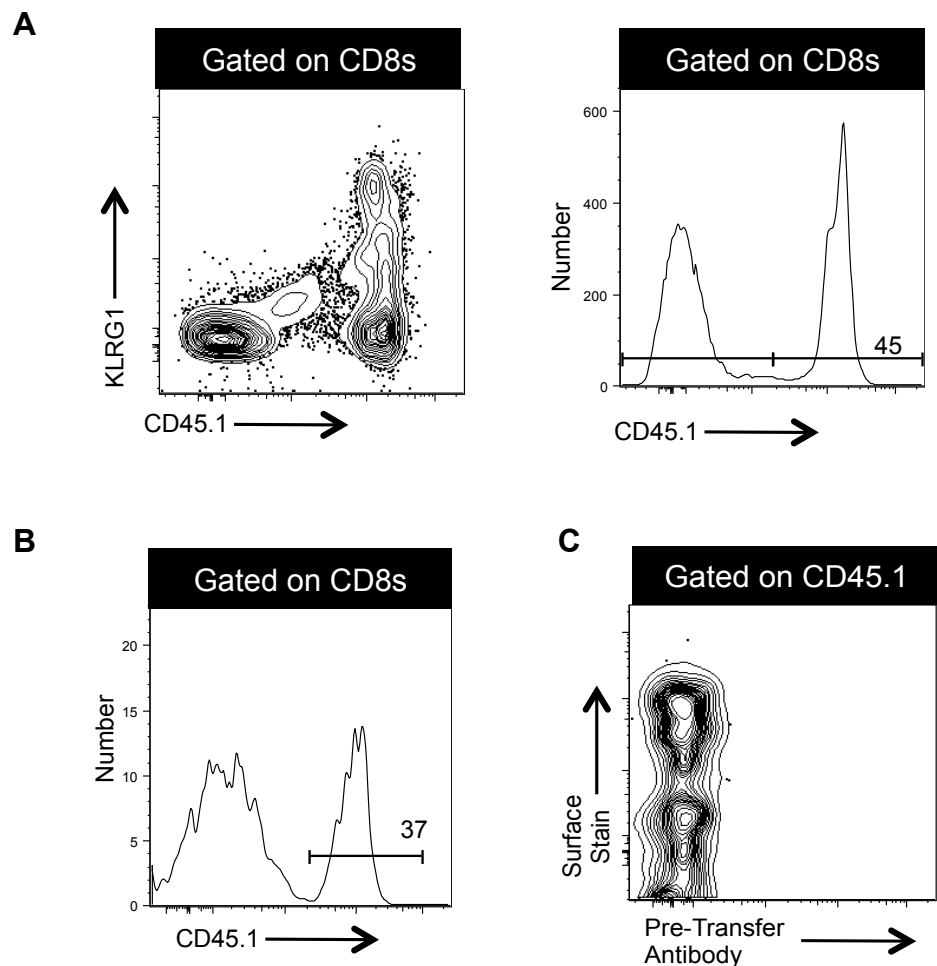
## Supplemental Fig. 2



### Supplemental Figure 2: Antibody depletion does not induce $T_M$ proliferation in naïve recipients.

(A) Immediately prior to antibody depletion, the presence of donors in the blood was determined by flow cytometry. For  $T_M$  and  $T_{EFF}$  recipients, data was collected from two independent experiments ( $n=6$  total). For naïve recipients, data was collected from one experiment ( $n=4$  total) (B) Frequencies of total tetramer-binding  $CD8^{pos}$  cells were determined in the blood of the chronically-infected Thy1.1 recipient mice prior to and immediately following the depletion scheduled in Figure 7B. Data was collected from two independent experiments ( $n=6$  total) and is displayed as mean  $\pm$  SEM. (C) Age matched naïve Thy1.1 recipients received  $T_M$  cells from a MCMV chronically-infected donor. Recipients were bled “pre-depletion” at 1.5 weeks post-transfer. Mice were started on the antibody depletion regimen immediately following the “pre-depletion” bleed and were bled again “post-depletion” at approximately 5 weeks post-transfer. A B6 mouse (Thy1.2<sup>pos</sup>) was used as the positive staining control. Naïve mice were challenged with MCMV approximately 12 weeks after the adoptive transfer. Data was collected from one experiment ( $n=4$  total).





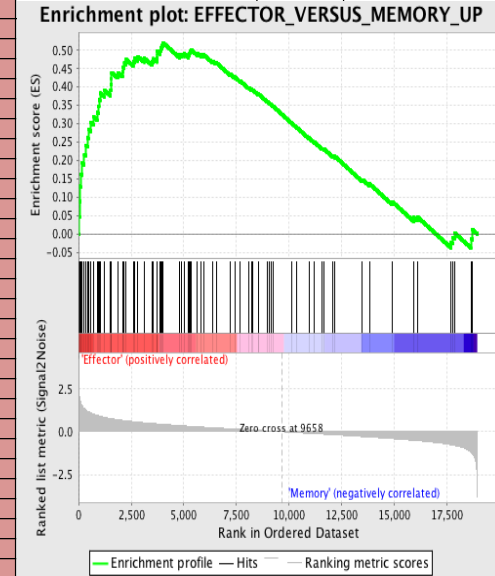
**Supplemental Figure 3: The KLRG1-specific antibody used for sorting does not deplete transferred cells.**

(A) CD45.1 splenocytes were stained with the anti-KLRG1 antibody (clone 2F1), mixed with unstained CD45.2 splenocytes in a 1:1 ratio and transferred into B6.CB17-*Prkdc*<sup>scid</sup> (SCID) mice from Jackson. The representative FACS plots show the KLRG1 stain of CD45.1<sup>pos</sup>, but not CD45.2<sup>pos</sup> cells (left) and the proportion of transferred cells that were CD45.1<sup>pos</sup> (right). (B) Six days after transfer, SCID recipients were bled and analyzed by flow cytometry. CD45.1<sup>pos</sup> cells were still a similar proportion of the transferred T cells (compare to A), suggesting that the large CD45.1<sup>pos</sup> KLRG1-stained population was not depleted. (C) Anti-KLRG1 staining six days after the transfer showed that there were still KLRG1<sup>hi</sup> CD45.1<sup>pos</sup> cells. As KLRG1 expression has been shown to be dependent on antigen stimulation, it is unlikely that the KLRG1<sup>hi</sup> cells were newly formed. Importantly, the CD45.1<sup>pos</sup> cells detected 6 days after transfer were no longer positive for the KLRG1 antibody used prior to the transfer (not shown), suggesting that the cells have not been depleted and have cleared the antibody off their surface. Data was collected from a single experiment ( $n=3$  total).

**Supplemental Table 1A: Transcripts UP in T<sub>EFF</sub> relative to T<sub>M</sub>**

NAME	PROBE	GENE SYMBOL	GENE TITLE	RANK IN GENE LIST	RANK METRIC SCORE	RUNNING ES	CORE ENRICHMENT
row_0	ANXA6	ANXA6	annexin A6	29	2.201579809	0.045963388	Yes
row_1	HMGB2	HMGB2	high-mobility group box 2	48	2.035401106	0.088924415	Yes
row_2	ANXA4	ANXA4	annexin A4	59	1.94921422	0.13044995	Yes
row_3	GZMB	GZMB	granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1)	123	1.62263608	0.16212006	Yes
row_4	CD244	CD244	CD244 molecule, natural killer cell receptor 2B4	153	1.540912032	0.19382909	Yes
row_5	CAPN2	CAPN2	calpain 2, (m/II) large subunit	271	1.351189613	0.21678028	Yes
row_6	AP1S2	AP1S2	adaptor-related protein complex 1, sigma 2 subunit	332	1.288847923	0.24140769	Yes
row_7	LGALS1	LGALS1	lectin, galactoside-binding, soluble, 1 (galectin 1)	430	1.199626088	0.26214892	Yes
row_8	EBP	EBP	emopamil binding protein (sterol isomerase)	465	1.172553301	0.28564534	Yes
row_9	IKZF3	IKZF3	IKAROS family zinc finger 3 (Aiolos)	582	1.106175661	0.30336317	Yes
row_10	CA5B	CA5B	carbonic anhydrase VB, mitochondrial	710	1.037609816	0.3190186	Yes
row_11	SPN	SPN	sialophorin (leukosialin, CD43)	881	0.962003529	0.33076355	Yes
row_12	CX3CR1	CX3CR1	chemokine (C-X3-C motif) receptor 1	939	0.938199341	0.34798452	Yes
row_13	SEP11	SEP11	septin 11	970	0.926925778	0.3663934	Yes
row_14	STMN1	STMN1	stathmin 1/oncoprotein 18	1020	0.913609505	0.38350785	Yes
row_15	PYCARD	PYCARD	PYD and CARD domain containing	1208	0.852552652	0.39199024	Yes
row_16	NGK7	NGK7	natural killer cell group 7 sequence	1518	0.775441468	0.39234224	Yes
row_17	NSMAF	NSMAF	neutral sphingomyelinase (N-SMase) activation associated factor	1534	0.772185385	0.40820757	Yes
row_18	SYTL2	SYTL2	synaptotagmin-like 2	1560	0.765723646	0.4234034	Yes
row_19	ZEB2	null	null	1566	0.764536202	0.43963376	Yes
row_20	KLRG1	KLRG1	killer cell lectin-like receptor subfamily G, member 1	1857	0.708328545	0.43954486	Yes
row_21	CASP1	CASP1	caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)	2087	0.667113006	0.44180003	Yes
row_22	CRIPT	CRIPT	cysteine-rich PDZ-binding protein	2120	0.659288228	0.45432845	Yes
row_23	S1PR5	null	null	2173	0.652283788	0.4656456	Yes
row_24	RAP2A	RAP2A	RAP2A, member of RAS oncogene family	2263	0.638532639	0.4747049	Yes
row_25	SUOX	SUOX	sulfite oxidase	2596	0.594230175	0.469928	Yes
row_26	NUF2	null	null	2677	0.583921313	0.47828606	Yes
row_27	HIST1H2BN	HIST1H2BN	histone cluster 1, H2bn	2817	0.562394679	0.48305234	Yes
row_28	RAB27A	RAB27A	RAB27A, member RAS oncogene family	3105	0.530466557	0.47928497	Yes
row_29	GZMA	GZMA	granzyme A (granzyme 1, cytotoxic T-lymphocyte-associated serine esterase 3)	3488	0.489474744	0.46959764	Yes
row_30	REEP5	REEP5	receptor accessory protein 5	3539	0.485018671	0.477412	Yes
row_31	SYT11	SYT11	synaptotagmin XI	3719	0.466942877	0.4779986	Yes
row_32	GABARAPL2	GABARAPL2	GABA(A) receptor-associated protein-like 2	3737	0.46474728	0.48712474	Yes
row_33	S100A4	S100A4	S100 calcium binding protein A4	3739	0.464631855	0.49709648	Yes
row_34	SLAMF7	SLAMF7	SLAM family member 7	3882	0.447826445	0.49923187	Yes
row_35	KLRC1	KLRC1	killer cell lectin-like receptor subfamily C, member 1	3929	0.443369746	0.50635964	Yes
row_36	DOCK5	DOCK5	dedicator of cytokinesis 5	3976	0.438772231	0.51338816	Yes
row_37	CD48	CD48	CD48 molecule	4013	0.435482979	0.5208758	Yes
row_38	GIMAP7	GIMAP7	GTPase, IMAP family member 7	4790	0.363893896	0.48759487	No
row_39	FUT11	FUT11	fucosyltransferase 11 (alpha (1,3) fucosyltransferase)	4895	0.355715871	0.48975712	No
row_40	CENPE	CENPE	centromere protein E, 312kDa	5014	0.345196247	0.49095032	No
row_41	KLRD1	KLRD1	killer cell lectin-like receptor subfamily D, member 1	5222	0.32846266	0.487065	No
row_42	RAP1GAP2	null	null	5275	0.322605729	0.49126914	No
row_43	CYSLTR2	CYSLTR2	cysteinyl leukotriene receptor 2	5287	0.321255773	0.4976174	No
row_44	ESM1	ESM1	endothelial cell-specific molecule 1	5351	0.316171378	0.50109965	No
row_45	FGL2	FGL2	fibrinogen-like 2	5635	0.294212759	0.49244696	No
row_46	PLEK	PLEK	pleckstrin	5828	0.278107136	0.48827025	No
row_47	KCNJ8	KCNJ8	potassium inwardly-rectifying channel, subfamily J, member 8	5934	0.270331919	0.48853728	No
row_48	NCAPG	NCAPG	non-SMC condensin I complex, subunit G	6372	0.237775877	0.4705041	No
row_49	ST3GAL4	ST3GAL4	ST3 beta-galactoside alpha-2,3-sialyltransferase 4	6538	0.227307007	0.4666625	No
row_50	INSL6	INSL6	insulin-like 6	7220	0.179024309	0.4344284	No
row_51	MYADM	MYADM	myeloid-associated differentiation marker	7421	0.162519217	0.42733377	No
row_52	KIAA0101	KIAA0101	KIAA0101	7670	0.143709034	0.41728905	No
row_53	KLRK1	KLRK1	killer cell lectin-like receptor subfamily K, member 1	8076	0.112943329	0.3982587	No
row_54	RUNX1	RUNX1	runt-related transcription factor 1 (acute myeloid leukemia 1; aml1 oncogene)	8205	0.104097836	0.39371997	No
row_55	CSPP1	CSPP1	centrosome and spindle pole associated protein 1	8271	0.099030837	0.3924113	No

T-EFF biased	
Normalized Enrichment Score (NES)	2.1315544
Nominal p-value	0
FDR q-value	0
FWER p-Value	0



**Supplemental Table 1A: Comparing transcripts UP in CMV-specific T<sub>EFF</sub> relative to CMV-specific T<sub>M</sub> cells from mice and humans.**

List shows significantly altered mouse genes (2-fold up or down and  $p < .05$ ). The table also includes a GSEA analysis of these mouse gene sets relative to each of the indicated human data sets, rank ordered by expression (see methods) and FWER significance to control for multiple testing.

row_56	TAF9B	TAF9B	TAF9B RNA polymerase II, TATA box binding protein (TBP)-associated factor, 31kDa	8535	0.080059439	0.38019818	No
row_57	NUSAP1	NUSAP1	nucleolar and spindle associated protein 1	8559	0.078409061	0.3806708	No
row_58	ANXA1	ANXA1	annexin A1	8977	0.046614461	0.35957327	No
row_59	GPX8	null	null	9050	0.042167794	0.35666668	No
row_60	ANXA2	ANXA2	annexin A2	9161	0.033796653	0.35156527	No
row_61	KIF11	KIF11	kinesin family member 11	9239	0.028000101	0.348088	No
row_62	CASC5	CASC5	cancer susceptibility candidate 5	10134	-0.028975241	0.3013263	No
row_63	HIST1H2BI	HIST1H2BI	histone cluster 1, H2bi	10379	-0.047193091	0.28941122	No
row_64	PRDM1	PRDM1	PR domain containing 1, with ZNF domain	10943	-0.087488279	0.2614568	No
row_65	RAB5B	RAB5B	RAB5B, member RAS oncogene family	11206	-0.106175564	0.24986018	No
row_66	SPC25	null	null	11582	-0.130460307	0.23279792	No
row_67	S100A10	S100A10	S100 calcium binding protein A10	11678	-0.136860937	0.23071527	No
row_68	SYTL3	SYTL3	synaptotagmin-like 3	12076	-0.165555894	0.21324411	No
row_69	AS3MT	AS3MT	arsenic (+3 oxidation state) methyltransferase	12183	-0.173790261	0.21137518	No
row_70	APOBEC2	APOBEC2	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 2	13491	-0.275097936	0.14803253	No
row_71	RPA3	RPA3	replication protein A3, 14kDa	13828	-0.302330554	0.13674569	No
row_72	OSBPL3	OSBPL3	oxysterol binding protein-like 3	14911	-0.398935348	0.08800114	No
row_73	MKI67	MKI67	antigen identified by monoclonal antibody Ki-67	15923	-0.497105926	0.04513806	No
row_74	MXI1	MXI1	MAX interactor 1	16098	-0.51361376	0.04699668	No
row_75	IL18RAP	IL18RAP	interleukin 18 receptor accessory protein	17706	-0.753345549	-0.02192908	No
row_76	PRC1	PRC1	protein regulator of cytokinesis 1	17790	-0.772486866	-0.009661613	No
row_77	CCNA2	CCNA2	cyclin A2	17875	-0.79208225	0.002975634	No
row_78	GZMK	GZMK	granzyme K (granzyme 3; tryptase II)	18643	-1.195715308	-0.011881162	No
row_79	TOP2A	TOP2A	topoisomerase (DNA) II alpha 170kDa	18720	-1.290148854	0.011926243	No

T-MEM biased

**Supplemental Table 1A: Comparing transcripts UP in CMV-specific T<sub>EFF</sub> relative to CMV-specific T<sub>M</sub> cells from mice and humans.**

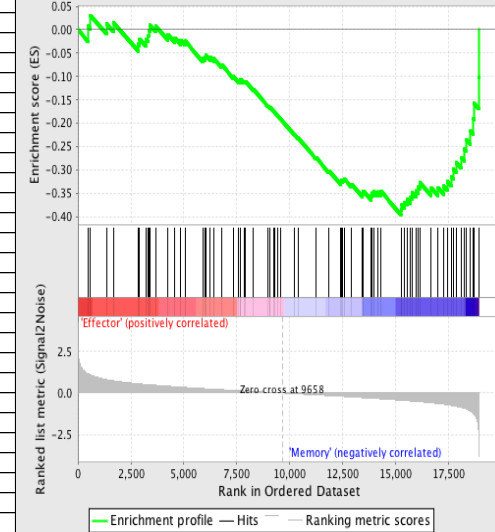
List shows significantly altered mouse genes (2-fold up or down and  $p < .05$ ). The table also includes a GSEA analysis of these mouse gene sets relative to each of the indicated human data sets, rank ordered by expression (see methods) and FWER significance to control for multiple testing.

**Supplemental Table 1B: Transcripts UP in T<sub>M</sub> relative to T<sub>EFF</sub>**

NAME	PROBE	GENE SYMBOL	GENE TITLE	RANK IN GENE LIST	RANK METRIC SCORE	RUNNING ES	CORE ENRICHMENT
row_0	PHPT1	PHPT1	phosphohistidine phosphatase 1	37	2.124774694	0.026149271	No
row_1	HSPB11	null	null	290	1.328898907	0.030335644	No
row_2	LSM7	LSM7	LSM7 homolog, U6 small nuclear RNA associated (S. cerevisiae)	329	1.291045427	0.045399252	No
row_3	IFI27L2	null	null	592	1.101828933	0.04604919	No
row_4	RTP4	RTP4	receptor transporter protein 4	600	1.096092939	0.060181282	No
row_5	MED4	MED4	mediator of RNA polymerase II transcription, subunit 4 homolog (S. cerevisiae)	842	0.978564978	0.06031665	No
row_6	RPS29	RPS29	ribosomal protein S29	901	0.955645084	0.06987862	No
row_7	RAPGEF4	RAPGEF4	Rap guanine nucleotide exchange factor (GEF) 4	1484	0.782799721	0.049292903	No
row_8	CYB5A	CYB5A	cytochrome b5 type A (microsomal)	1502	0.778091848	0.05868529	No
row_9	MRPS5	MRPS5	mitochondrial ribosomal protein S5	2032	0.676983833	0.03951729	No
row_10	PIP4K2A	null	null	2133	0.656981051	0.04289404	No
row_11	APEX1	APEX1	APEX nuclease (multifunctional DNA repair enzyme) 1	2262	0.638559878	0.0445383	No
row_12	QSER1	QSER1	glutamine and serine rich 1	2270	0.637303054	0.052599363	No
row_13	SH3BP5	SH3BP5	SH3-domain binding protein 5 (BTK-associated)	2576	0.596591115	0.044277374	No
row_14	PDK1	PDK1	pyruvate dehydrogenase kinase, isozyme 1	2753	0.571303666	0.042479545	No
row_15	SEPP1	SEPP1	selenoprotein P, plasma, 1	2844	0.559307992	0.045095507	No
row_16	STAMBPL1	STAMBPL1	STAM binding protein-like 1	3096	0.531519473	0.03878356	No
row_17	ITGAE	ITGAE	integrin, alpha E (antigen CD103, human mucosal lymphocyte antigen 1; alpha polypeptide)	3106	0.530423522	0.04532398	No
row_18	TLR1	TLR1	toll-like receptor 1	3210	0.518464327	0.046708275	No
row_19	ZNF761	ZNF761	zinc finger protein 761	3302	0.50811398	0.04859363	No
row_20	F2RL1	F2RL1	coagulation factor II (thrombin) receptor-like 1	3342	0.504218102	0.053192213	No
row_21	NAT1	NAT1	N-acetyltransferase 1 (arylamine N-acetyltransferase)	3507	0.488090605	0.05093128	No
row_22	DAPL1	DAPL1	death associated protein-like 1	3673	0.47194767	0.04840356	No
row_23	HINT3	HINT3	histidine triad nucleotide binding protein 3	3834	0.453408092	0.04589636	No
row_24	LTV1	LTV1	LTV1 homolog (S. cerevisiae)	4081	0.428189248	0.03848292	No
row_25	IMPDH2	IMPDH2	IMP (inosine monophosphate) dehydrogenase 2	4847	0.359094143	0.002560527	No
row_26	DUSP10	DUSP10	dual specificity phosphatase 10	4855	0.35811159	0.00692713	No
row_27	RRP15	null	null	4880	0.356415987	0.010367425	No
row_28	XCL1	XCL1	chemokine (C motif) ligand 1	5056	0.342206746	0.005591196	No
row_29	BCL9	BCL9	B-cell CLL/lymphoma 9	5238	0.326921731	2.94E-04	No
row_30	PRDX6	PRDX6	peroxiredoxin 6	5248	0.325534701	0.004122876	No
row_31	CD3D	CD3D	CD3d molecule, delta (CD3-TCR complex)	5438	0.309563071	-0.001829683	No
row_32	TOX	TOX	-	5553	0.300573617	-0.003913531	No
row_33	DKC1	DKC1	dyskeratosis congenita 1, dyskerin	5581	0.298592925	-0.001397898	No
row_34	RPS19	RPS19	ribosomal protein S19	5641	0.293431848	-6.52E-04	No
row_35	RPLP1	RPLP1	ribosomal protein, large, P1	5781	0.281639785	-0.004315579	No
row_36	CCDC91	CCDC91	coiled-coil domain containing 91	5822	0.278348088	-0.002759035	No
row_37	SLFN5	SLFN5	schlafen family member 5	5899	0.273217082	-0.003184468	No
row_38	FBL	FBL	fibrillarin	5910	0.27217257	-1.15E-04	No
row_39	TMLHE	TMLHE	trimethyllysine hydroxylase, epsilon	6244	0.246418536	-0.014559025	No
row_40	IFT80	IFT80	intraflagellar transport 80 homolog (Chlamydomonas)	6313	0.242544681	-0.014964985	No
row_41	LY6E	LY6E	lymphocyte antigen 6 complex, locus E	6424	0.234440193	-0.017711282	No
row_42	ATP1B1	ATP1B1	ATPase, Na <sup>+</sup> /K <sup>+</sup> transporting, beta 1 polypeptide	6548	0.226953819	-0.02124784	No
row_43	TTC27	TTC27	tetratricopeptide repeat domain 27	7498	0.156670496	-0.06963192	No
row_44	USP28	USP28	ubiquitin specific peptidase 28	7576	0.150735766	-0.07173128	No
row_45	ACTN1	ACTN1	actinin, alpha 1	7597	0.148684606	-0.07082716	No
row_46	SSBP2	SSBP2	single-stranded DNA binding protein 2	7678	0.14299418	-0.07318846	No
row_47	HRSP12	HRSP12	heat-responsive protein 12	7680	0.142702043	-0.0713533	No
row_48	SLAMF6	SLAMF6	SLAM family member 6	7685	0.142558053	-0.06967954	No
row_49	RPL29	RPL29	ribosomal protein L29	7738	0.138756722	-0.0706082	No
row_50	RPL14	RPL14	ribosomal protein L14	7785	0.135675624	-0.07125861	No
row_51	RRAS2	RRAS2	related RAS viral (r-ras) oncogene homolog 2	7850	0.130808711	-0.07293046	No
row_52	KBTBD11	KBTBD11	kelch repeat and BTB (POZ) domain containing 11	7905	0.126582831	-0.07412655	No
row_53	RPL10A	RPL10A	ribosomal protein L10a	7936	0.123617478	-0.074085824	No
row_54	LZTF1	LZTF1	leucine zipper transcription factor-like 1	8371	0.092378221	-0.0959387	No
row_55	TTC3	TTC3	tetratricopeptide repeat domain 3	8384	0.091630973	-0.0953642	No

T-EFF biased	Normalized Enrichment Score (NES)
No	-1.6801751
No	0.00777202
No	8.90E-04
No	0.003

**Enrichment plot: EFFECTOR\_VERSUS\_MEMORY\_DOWN**



**Supplemental Table 1B: Comparing transcripts UP in CMV-specific T<sub>M</sub> relative to CMV-specific T<sub>EFF</sub> cells from mice and humans.**

List shows significantly altered mouse genes (2-fold up or down and  $p < .05$ ). The table also includes a GSEA analysis of these mouse gene sets relative to each of the indicated human data sets, rank ordered by expression (see methods) and FWER significance to control for multiple testing.

row_56	RPS26	RPS26	ribosomal protein S26	8636	0.072893247	-0.10774501	No
row_57	KLHDC1	KLHDC1	kelch domain containing 1	8877	0.055129666	-0.11977602	No
row_58	HMP19	HMP19	-	9065	0.040402696	-0.12918396	No
row_59	BZW2	BZW2	basic leucine zipper and W2 domains 2	9068	0.040138628	-0.12875916	No
row_60	GID4	null	null	9096	0.038265821	-0.12968835	No
row_61	CHURC1	CHURC1	churchill domain containing 1	9274	0.025683668	-0.13875937	No
row_62	KIAA1033	KIAA1033	KIAA1033	9314	0.022567095	-0.14053434	No
row_63	MRPL23	MRPL23	mitochondrial ribosomal protein L23	9397	0.016983615	-0.14466944	No
row_64	EGR2	EGR2	early growth response 2 (Krox-20 homolog, Drosophila)	10037	-0.020757651	-0.17836967	No
row_65	LPHN2	LPHN2	latrophilin 2	10097	-0.025689999	-0.1811667	No
row_66	XKRX	XKRX	XK, Kell blood group complex subunit-related, X-linked	10256	-0.038267016	-0.18906099	No
row_67	LCLAT1	null	null	10373	-0.04668713	-0.19461079	No
row_68	INSR	INSR	insulin receptor	10396	-0.048602778	-0.19513735	No
row_69	RPL8	RPL8	ribosomal protein L8	10560	-0.059909303	-0.20301111	No
row_70	GPATCH4	null	null	10752	-0.074455388	-0.21218112	No
row_71	TRIB2	TRIB2	tribbles homolog 2 (Drosophila)	11027	-0.093001895	-0.22551872	No
row_72	RPL18	RPL18	ribosomal protein L18	11234	-0.108678244	-0.23503341	No
row_73	TRIM13	TRIM13	tripartite motif-containing 13	11801	-0.14649047	-0.2631885	No
row_74	LYPD6B	null	null	11911	-0.15424104	-0.2669429	No
row_75	MGST2	MGST2	microsomal glutathione S-transferase 2	11950	-0.156489357	-0.26689252	No
row_76	ACSL3	ACSL3	acyl-CoA synthetase long-chain family member 3	12373	-0.187292844	-0.2868514	No
row_77	DPP4	DPP4	dipeptidyl-peptidase 4 (CD26, adenosine deaminase complexing protein 2)	12434	-0.19199796	-0.2875009	No
row_78	ZBTB10	ZBTB10	zinc finger and BTB domain containing 10	12514	-0.198208988	-0.28907838	No
row_79	NEDD4L	NEDD4L	neural precursor cell expressed, developmentally down-regulated 4-like	12830	-0.22265242	-0.3028803	No
row_80	EPHX1	EPHX1	epoxide hydrolase 1, microsomal (xenobiotic)	12871	-0.225831643	-0.30201867	No
row_81	MYC	MYC	v-myc myelocytomatosis viral oncogene homolog (avian)	12915	-0.229761392	-0.30126455	No
row_82	TSPAN13	TSPAN13	tetraspanin 13	12940	-0.232248098	-0.29946736	No
row_83	EEF1B2	EEF1B2	eukaryotic translation elongation factor 1 beta 2	13130	-0.247839764	-0.30623668	No
row_84	TFRC	TFRC	transferrin receptor (p90, CD71)	13593	-0.283705652	-0.32704648	No
row_85	P2RY10	P2RY10	purinergic receptor P2Y, G-protein coupled, 10	13647	-0.288299233	-0.32604948	No
row_86	LEF1	LEF1	lymphoid enhancer-binding factor 1	13701	-0.291425049	-0.32501107	No
row_87	IFNGR2	IFNGR2	interferon gamma receptor 2 (interferon gamma transducer 1)	13751	-0.295711279	-0.3237033	No
row_88	SPRED2	SPRED2	sprouty-related, EVH1 domain containing 2	13872	-0.30734697	-0.32601652	No
row_89	TNFSF8	TNFSF8	tumor necrosis factor (ligand) superfamily, member 8	13970	-0.315796912	-0.32699504	No
row_90	CMSS1	null	null	13996	-0.318091691	-0.32411507	No
row_91	PECAM1	PECAM1	platelet/endothelial cell adhesion molecule (CD31 antigen)	14002	-0.318354994	-0.3201682	No
row_92	MYB	MYB	v-myb myeloblastosis viral oncogene homolog (avian)	14205	-0.335532546	-0.32646832	No
row_93	JUN	JUN	jun oncogene	14239	-0.338070452	-0.3237493	No
row_94	RPL13	RPL13	ribosomal protein L13	14346	-0.346955091	-0.32479405	No
row_95	RPS2	RPS2	ribosomal protein S2	14452	-0.356475323	-0.32565963	No
row_96	PCGF5	PCGF5	polycomb group ring finger 5	14522	-0.362784624	-0.32452768	No
row_97	CNGA1	CNGA1	cyclic nucleotide gated channel alpha 1	14723	-0.381109834	-0.33011833	No
row_98	GRIA3	GRIA3	glutamate receptor, ionotropic, AMPA 3	14748	-0.382496178	-0.32633293	No
row_99	DPH5	DPH5	DPH5 homolog (S. cerevisiae)	14846	-0.391592532	-0.32630846	No
row_100	RG510	RG510	regulator of G-protein signalling 10	15259	-0.433039695	-0.34248376	No
row_101	EMB	EMB	embigin homolog (mouse)	15436	-0.449893624	-0.34588817	No
row_102	SELL	SELL	selectin L (lymphocyte adhesion molecule 1)	15556	-0.460011512	-0.34612808	No
row_103	RPS5	RPS5	ribosomal protein S5	15705	-0.475598007	-0.3477036	No
row_104	RPL15	RPL15	ribosomal protein L15	15770	-0.480787158	-0.3447443	No
row_105	INPP4B	INPP4B	inositol polyphosphate-4-phosphatase, type II, 105kDa	15848	-0.489236385	-0.34236437	No
row_106	BTF3L4	BTF3L4	basic transcription factor 3-like 4	15895	-0.494006366	-0.3382731	No
row_107	IL6ST	IL6ST	interleukin 6 signal transducer (gp130, oncostatin M receptor)	16084	-0.512494206	-0.34148717	No
row_108	WDR12	WDR12	WD repeat domain 12	16295	-0.535118759	-0.34557158	No
row_109	RPL28	RPL28	ribosomal protein L28	16305	-0.537068784	-0.3389432	No
row_110	ST6GAL1	ST6GAL1	ST6 beta-galactosamide alpha-2,6-sialyltransferase 1	16420	-0.552493393	-0.33769348	No
row_111	ITGA6	ITGA6	integrin, alpha 6	16596	-0.574453831	-0.33939645	No
row_112	RPLP0	RPLP0	ribosomal protein, large, P0	16715	-0.589519203	-0.33786944	No
row_113	PELL1	PELL1	pellino homolog 1 (Drosophila)	17138	-0.650897205	-0.35169357	No
row_114	SATB1	SATB1	special AT-rich sequence binding protein 1 (binds to nuclear matrix/scaffold-associating DNA's)	17264	-0.669767857	-0.34947684	No
row_115	RPSA	RPSA	ribosomal protein SA	17448	-0.702700913	-0.3499081	No
row_116	PPIC	PPIC	peptidylprolyl isomerase C (cyclophilin C)	17721	-0.756398022	-0.35436085	Yes
row_117	DGKA	DGKA	diacylglycerol kinase, alpha 80kDa	17727	-0.758206606	-0.34459355	Yes

T-MEM biased

### Supplemental Table 1B: Comparing transcripts UP in CMV-specific T<sub>M</sub> relative to CMV-specific T<sub>EFF</sub> cells from mice and humans.

List shows significantly altered mouse genes (2-fold up or down and  $p < .05$ ). The table also includes a GSEA analysis of these mouse gene sets relative to each of the indicated human data sets, rank ordered by expression (see methods) and FWER significance to control for multiple testing.

row_118	CD69	CD69	CD69 molecule	17870	-0.790673494	-0.3416808	Yes
row_119	ST8SIA1	ST8SIA1	ST8 alpha-N-acetyl-neuraminidase alpha-2,8-sialyltransferase 1	17913	-0.802390575	-0.3332961	Yes
row_120	CCR9	CCR9	chemokine (C-C motif) receptor 9	18031	-0.836443365	-0.32844844	Yes
row_121	HOOK1	HOOK1	hook homolog 1 (Drosophila)	18237	-0.906686187	-0.32735014	Yes
row_122	RAB3IP	RAB3IP	RAB3A interacting protein (rabin3)	18248	-0.911961794	-0.3158141	Yes
row_123	TCP11L2	TCP11L2	t-complex 11 (mouse) like 2	18438	-1.008909702	-0.31251243	Yes
row_124	PDE3B	PDE3B	phosphodiesterase 3B, cGMP-inhibited	18484	-1.036287308	-0.30119213	Yes
row_125	CD2AP	CD2AP	CD2-associated protein	18520	-1.068836212	-0.28890944	Yes
row_126	ICOS	ICOS	inducible T-cell co-stimulator	18674	-1.228775859	-0.28078425	Yes
row_127	CD27	null	null	18716	-1.286845565	-0.26593572	Yes
row_128	ZEB1	null	null	18771	-1.377133369	-0.25058365	Yes
row_129	TCF7	TCF7	transcription factor 7 (T-cell specific, HMG-box)	18790	-1.420588374	-0.23274246	Yes
row_130	SH3PXD2A	SH3PXD2A	SH3 and PX domains 2A	18808	-1.47138989	-0.21417587	Yes
row_131	RP59	RP59	ribosomal protein S9	18857	-1.63666749	-0.19507043	Yes
row_132	LTB	LTB	lymphotoxin beta (TNF superfamily, member 3)	18891	-1.829720855	-0.17261285	Yes
row_133	SESN3	SESN3	sestrin 3	18900	-1.950626612	-0.14722613	Yes
row_134	CCR7	CCR7	chemokine (C-C motif) receptor 7	18917	-2.157334805	-0.11952945	Yes
row_135	IL7R	IL7R	interleukin 7 receptor	18927	-2.443236351	-0.087677345	Yes
row_136	TRAT1	TRAT1	T cell receptor associated transmembrane adaptor 1	18943	-2.915472031	-0.04989529	Yes
row_137	GPR183	null	null	18945	-3.77461338	-1.15E-07	Yes

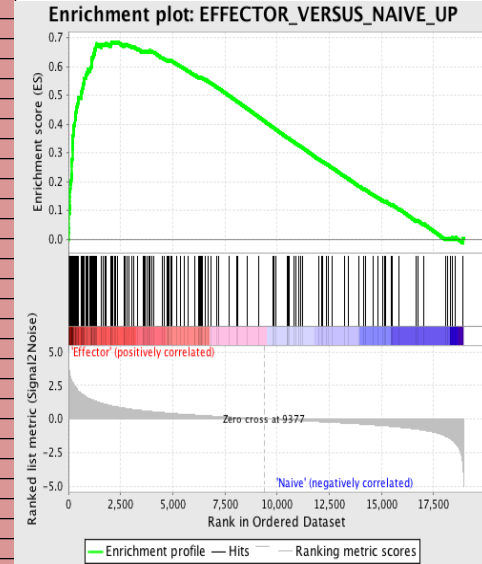
**Supplemental Table 1B: Comparing transcripts UP in CMV-specific T<sub>M</sub> relative to CMV-specific T<sub>EFF</sub> cells from mice and humans.**

List shows significantly altered mouse genes (2-fold up or down and  $p < .05$ ). The table also includes a GSEA analysis of these mouse gene sets relative to each of the indicated human data sets, rank ordered by expression (see methods) and FWER significance to control for multiple testing.

**Supplemental Table 1C: Transcripts UP in T<sub>EFF</sub> relative to Naive**

NAME	PROBE	GENE SYMBOL	GENE TITLE	RANK IN GENE LIST	RANK METRIC SCORE	RUNNING ES	CORE ENRICHMENT
row_0	ITGB1	ITGB1	integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12)	5	4.547115803	0.019984426	Yes
row_1	TBX21	TBX21	T-box 21	12	4.094614983	0.037900336	Yes
row_2	S1PR5	null	null	22	3.814461708	0.054408733	Yes
row_3	GZMB	GZMB	granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1)	23	3.791385651	0.07129387	Yes
row_4	SAMD3	SAMD3	sterile alpha motif domain containing 3	25	3.75134635	0.08794741	Yes
row_5	GNPTAB	GNPTAB	N-acetylglucosamine-1-phosphate transferase, alpha and beta subunits	45	3.55580759	0.10277109	Yes
row_6	GOLIM4	null	null	57	3.421685934	0.117423676	Yes
row_7	FASLG	FASLG	Fas ligand (TNF superfamily, member 6)	63	3.370791435	0.13216928	Yes
row_8	CHSY1	CHSY1	carbohydrate (chondroitin) synthase 1	64	3.352074385	0.14709792	Yes
row_9	GZMA	GZMA	granzyme A (granzyme 1, cytotoxic T-lymphocyte-associated serine esterase 3)	67	3.331688643	0.16182922	Yes
row_10	CAMK2N1	CAMK2N1	calcium/calmodulin-dependent protein kinase II inhibitor 1	84	3.213935375	0.17529018	Yes
row_11	MYO1F	MYO1F	myosin IF	88	3.202200413	0.18939151	Yes
row_12	RAB8B	RAB8B	RAB8B, member RAS oncogene family	105	3.118102789	0.20242569	Yes
row_13	LGALS1	LGALS1	lectin, galactoside-binding, soluble, 1 (galectin 1)	134	2.969976187	0.2141608	Yes
row_14	CCL5	CCL5	chemokine (C-C motif) ligand 5	152	2.908002853	0.226206	Yes
row_15	BHLHE40	null	null	161	2.877945662	0.23859686	Yes
row_16	ITGAL	ITGAL	integrin, alpha L (antigen CD11A (p180), lymphocyte function-associated antigen 1; alpha polypeptide)	162	2.873387337	0.25139365	Yes
row_17	FAM49A	FAM49A	family with sequence similarity 49, member A	164	2.851606131	0.26404014	Yes
row_18	ITGB2	ITGB2	integrin, beta 2 (complement component 3 receptor 3 and 4 subunit)	169	2.844150066	0.2764936	Yes
row_19	CHST11	CHST11	carbohydrate (chondroitin 4) sulfotransferase 11	186	2.792424679	0.28807735	Yes
row_20	CD244	CD244	CD244 molecule, natural killer cell receptor 2B4	187	2.791054487	0.30050746	Yes
row_21	ZEB2	null	null	188	2.790454865	0.3129349	Yes
row_22	CASP4	CASP4	caspase 4, apoptosis-related cysteine peptidase	197	2.750204802	0.32475683	Yes
row_23	CCL4	CCL4	chemokine (C-C motif) ligand 4	203	2.743010283	0.33670658	Yes
row_24	F2R	F2R	coagulation factor II (thrombin) receptor	209	2.721811771	0.3485619	Yes
row_25	IQGAP2	IQGAP2	IQ motif containing GTPase activating protein 2	213	2.718414068	0.36050868	Yes
row_26	CX3CR1	CX3CR1	chemokine (C-X3-C motif) receptor 1	227	2.690318346	0.37179753	Yes
row_27	FYN	FYN	FYN oncogene related to SRC, FGR, YES	232	2.68144393	0.38352638	Yes
row_28	PRDM1	PRDM1	PR domain containing 1, with ZNF domain	246	2.635905027	0.39457288	Yes
row_29	KLRG1	KLRG1	killer cell lectin-like receptor subfamily G, member 1	284	2.517981768	0.4038155	Yes
row_30	NGK7	NGK7	natural killer cell group 7 sequence	315	2.45875144	0.41316733	Yes
row_31	ANXA2	ANXA2	annexin A2	326	2.432535887	0.42346796	Yes
row_32	HOPX	null	null	344	2.38733077	0.4331943	Yes
row_33	GOLM1	null	null	357	2.348069429	0.4430122	Yes
row_34	IFNG	IFNG	interferon, gamma	394	2.25906682	0.45115504	Yes
row_35	CXCR3	CXCR3	chemokine (C-X-C motif) receptor 3	404	2.241060257	0.4606562	Yes
row_36	IL18RAP	IL18RAP	interleukin 18 receptor accessory protein	430	2.17173934	0.46899617	Yes
row_37	GGH	GGH	gamma-glutamyl hydrolase (conjugase, folylpolyglutamyl hydrolase)	453	2.1221416	0.4772751	Yes
row_38	IL10RA	IL10RA	interleukin 10 receptor, alpha	484	2.0806427	0.48494297	Yes
row_39	S100A4	S100A4	S100 calcium binding protein A4	493	2.068409681	0.49372852	Yes
row_40	ATP2B4	ATP2B4	ATPase, Ca++ transporting, plasma membrane 4	599	1.917457938	0.49667367	Yes
row_41	ANXA4	ANXA4	annexin A4	605	1.91564846	0.5049387	Yes
row_42	CMKLR1	CMKLR1	chemokine-like receptor 1	621	1.891701698	0.51256436	Yes
row_43	SYT11	SYT11	synaptotagmin XI	665	1.837343931	0.51845604	Yes
row_44	HMGB2	HMGB2	high-mobility group box 2	679	1.822000504	0.5258778	Yes
row_45	DSTN	DSTN	destrin (actin depolymerizing factor)	697	1.800909758	0.5329925	Yes
row_46	CDC42EP3	CDC42EP3	CDC42 effector protein (Rho GTPase binding) 3	707	1.787336588	0.540473	Yes
row_47	SLAMF7	SLAMF7	SLAM family member 7	736	1.758211851	0.5468114	Yes
row_48	SLC20A1	SLC20A1	solute carrier family 20 (phosphate transporter), member 1	747	1.752829194	0.55408496	Yes
row_49	CAPN2	CAPN2	calpain 2, (m/I) large subunit	760	1.739896894	0.5611943	Yes
row_50	KLRC1	KLRC1	killer cell lectin-like receptor subfamily C, member 1	829	1.675436616	0.56503296	Yes
row_51	SNX10	SNX10	sorting nexin 10	831	1.672691703	0.5724291	Yes
row_52	CASP1	CASP1	caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)	870	1.62736547	0.57765204	Yes
row_53	SYTL2	SYTL2	synaptotagmin-like 2	912	1.590363145	0.5825504	Yes
row_54	IL18R1	IL18R1	interleukin 18 receptor 1	914	1.5899297	0.5895779	Yes
row_55	TNF	TNF	tumor necrosis factor (TNF superfamily, member 2)	1030	1.50204432	0.5901402	Yes

T-EFF biased	
Normalized Enrichment Score (NES)	2.6822367
Nominal p-value	0
FDR q-value	0
FWER p-Value	0



**Supplemental Table 1C: Comparing transcripts UP in CMV-specific T<sub>EFF</sub> relative to Naive T cells from mice and humans.**

List shows significantly altered mouse genes (2-fold up or down and  $p < .05$ ). The table also includes a GSEA analysis of these mouse gene sets relative to each of the indicated human data sets, rank ordered by expression (see methods) and FWER significance to control for multiple testing.



row_56	REEP5	REEP5	receptor accessory protein 5	1047	1.485551	0.59590375	Yes
row_57	AHNAK	AHNAK	AHNAK nucleoprotein (desmoyokin)	1089	1.449422479	0.60017437	Yes
row_58	PLEK	PLEK	pleckstrin	1103	1.443816662	0.60591185	Yes
row_59	KLRD1	KLRD1	killer cell lectin-like receptor subfamily D, member 1	1105	1.442571759	0.6122832	Yes
row_60	S100A6	S100A6	S100 calcium binding protein A6	1113	1.434784532	0.6183001	Yes
row_61	SYTL3	SYTL3	synaptotagmin-like 3	1125	1.42879796	0.62407726	Yes
row_62	IKZF3	IKZF3	IKAROS family zinc finger 3 (Aiolos)	1128	1.427701592	0.630329	Yes
row_63	SLC4A7	SLC4A7	solute carrier family 4, sodium bicarbonate cotransporter, member 7	1178	1.401299	0.6339591	Yes
row_64	PTPRJ	PTPRJ	protein tyrosine phosphatase, receptor type, J	1214	1.369297743	0.6381926	Yes
row_65	ITGA4	ITGA4	integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor)	1215	1.368969679	0.6442894	Yes
row_66	NSMAF	NSMAF	neutral sphingomyelinase (N-5Mase) activation associated factor	1243	1.353946567	0.64888066	Yes
row_67	PTMS	PTMS	parathyromosin	1245	1.353373647	0.6548547	Yes
row_68	LMNB1	LMNB1	lamin B1	1263	1.344273448	0.6599358	Yes
row_69	SLC25A24	SLC25A24	solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 24	1309	1.323735595	0.66343355	Yes
row_70	FAM3B	FAM3B	family with sequence similarity 3, member B	1320	1.320166111	0.66878015	Yes
row_71	ATP2B1	ATP2B1	ATPase, Ca++ transporting, plasma membrane 1	1338	1.308222771	0.67370063	Yes
row_72	RAP2A	RAP2A	RAP2A, member of RAS oncogene family	1345	1.304151654	0.6791891	Yes
row_73	CENPE	CENPE	centromere protein E, 312kDa	1574	1.186092257	0.6723237	Yes
row_74	FCHO2	FCHO2	FCH domain only 2	1591	1.179958582	0.6767263	Yes
row_75	ANXA1	ANXA1	annexin A1	1668	1.140519023	0.67775637	Yes
row_76	PPP1R12A	PPP1R12A	protein phosphatase 1, regulatory (inhibitor) subunit 12A	1761	1.099994302	0.67775357	Yes
row_77	SPN	SPN	sialophorin (leukosialin, CD43)	1801	1.082511306	0.6804967	Yes
row_78	AiM1	AiM1	absent in melanoma 1	2012	1.000916958	0.67376566	Yes
row_79	CTSD	CTSD	cathepsin D (lysosomal aspartyl peptidase)	2037	0.994105399	0.6769143	Yes
row_80	ID2	ID2	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein	2052	0.98960793	0.68057567	Yes
row_81	DOCK5	DOCK5	dedicator of cytokinesis 5	2061	0.986442983	0.6845426	Yes
row_82	RORA	RORA	RAR-related orphan receptor A	2125	0.963672876	0.68547773	Yes
row_83	ARSB	ARSB	arylsulfatase B	2225	0.930148363	0.68434554	Yes
row_84	RAP1GAP2	null	null	2273	0.917509854	0.6859276	Yes
row_85	OSBPL3	OSBPL3	oxysterol binding protein-like 3	2337	0.898226559	0.6865713	Yes
row_86	SEP11	SEP11	septin 11	2657	0.81385839	0.6731998	No
row_87	SLC9A7	SLC9A7	solute carrier family 9 (sodium/hydrogen exchanger), member 7	2683	0.806290448	0.67545867	No
row_88	S100A10	S100A10	S100 calcium binding protein A10	2710	0.799574256	0.6776343	No
row_89	PRR13	PRR13	proline rich 13	2811	0.772674739	0.6757475	No
row_90	ITGAX	ITGAX	integrin, alpha X (complement component 3 receptor 4 subunit)	2898	0.752966523	0.6745189	No
row_91	IER3	IER3	immediate early response 3	3054	0.718982935	0.6694626	No
row_92	PRC1	PRC1	protein regulator of cytokinesis 1	3147	0.702128828	0.6676879	No
row_93	ZDHHC2	ZDHHC2	zinc finger, DHHC-type containing 2	3316	0.668632925	0.66171473	No
row_94	KCNJ8	KCNJ8	potassium inwardly-rectifying channel, subfamily J, member 8	3565	0.622722626	0.6512748	No
row_95	PRR11	PRR11	proline rich 11	3576	0.62104547	0.6535079	No
row_96	GZMK	GZMK	granzyme K (granzyme 3; tryptase II)	3622	0.614801168	0.65384835	No
row_97	NABP1	null	null	3696	0.603634	0.65264726	No
row_98	ARHGAP26	ARHGAP26	Rho GTPase activating protein 26	3752	0.59414047	0.65236294	No
row_99	NOD1	null	null	3788	0.589279234	0.65312254	No
row_100	CCR5	CCR5	chemokine (C-C motif) receptor 5	3858	0.57842803	0.65202236	No
row_101	MAPRE2	MAPRE2	microtubule-associated protein, RP/EB family, member 2	3870	0.577029943	0.6540061	No
row_102	FAM204A	null	null	3886	0.574480057	0.6557654	No
row_103	ELL2	ELL2	elongation factor, RNA polymerase II, 2	3978	0.556759	0.65339655	No
row_104	DAPK2	DAPK2	death-associated protein kinase 2	4080	0.540438652	0.6504222	No
row_105	CD38	CD38	CD38 molecule	4498	0.478245825	0.6303346	No
row_106	FGL2	FGL2	fibrinogen-like 2	4605	0.466220081	0.62676334	No
row_107	SERPINB9	SERPINB9	serpin peptidase inhibitor, clade B (ovalbumin), member 9	4733	0.447111845	0.62198806	No
row_108	RUNX1	RUNX1	runt-related transcription factor 1 (acute myeloid leukemia 1; am1 oncogene)	4775	0.441229612	0.62176865	No
row_109	PDE8A	PDE8A	phosphodiesterase 8A	4779	0.440728962	0.62357163	No
row_110	CPEB2	CPEB2	cytoplasmic polyadenylation element binding protein 2	4840	0.432008088	0.62229884	No
row_111	CCNB2	CCNB2	cyclin B2	4902	0.424213678	0.62093806	No
row_112	RNF216	null	null	4949	0.418337315	0.6203503	No
row_113	KLRK1	KLRK1	killer cell lectin-like receptor subfamily K, member 1	5195	0.387992203	0.6090248	No
row_114	PRUNE	PRUNE	prune homolog (Drosophila)	5385	0.366202533	0.60058594	No
row_115	CYSLTR2	CYSLTR2	cysteinyl leukotriene receptor 2	5555	0.350188047	0.5931413	No
row_116	METTL7A	METTL7A	methyltransferase like 7A	5745	0.330821484	0.58454484	No
row_117	LAMC1	LAMC1	laminin, gamma 1 (formerly LAMB2)	6032	0.300099283	0.5706434	No

### Supplemental Table 1C: Comparing transcripts UP in CMV-specific T<sub>EFF</sub> relative to Naive T cells from mice and humans.

List shows significantly altered mouse genes (2-fold up or down and  $p < .05$ ). The table also includes a GSEA analysis of these mouse gene sets relative to each of the indicated human data sets, rank ordered by expression (see methods) and FWER significance to control for multiple testing.



row_118	IL12RB2	IL12RB2	interleukin 12 receptor, beta 2	6066	0.29641816	0.57020533	No
row_119	NRP1	NRP1	neuropilin 1	6243	0.279611409	0.56207347	No
row_120	CHPT1	CHPT1	choline phosphotransferase 1	6252	0.279017806	0.5628898	No
row_121	RPA2	RPA2	replication protein A2, 32kDa	6281	0.275597423	0.5626254	No
row_122	NUDT4	NUDT4	nudix (nucleoside diphosphate linked moiety X)-type motif 4	6342	0.268916696	0.56062627	No
row_123	TM6SF1	TM6SF1	transmembrane 6 superfamily member 1	6361	0.266775548	0.5608553	No
row_124	F2RL2	F2RL2	coagulation factor II (thrombin) receptor-like 2	6367	0.266414434	0.56177545	No
row_125	NCAPG	NCAPG	non-SMC condensin I complex, subunit G	6414	0.2617791	0.5604904	No
row_126	CASC5	CASC5	cancer susceptibility candidate 5	6573	0.245987505	0.5531678	No
row_127	PLOD2	PLOD2	procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2	6580	0.24478434	0.5539383	No
row_128	GBP4	GBP4	guanylate binding protein 4	6681	0.235348612	0.5496585	No
row_129	ESM1	ESM1	endothelial cell-specific molecule 1	6827	0.220466107	0.54291487	No
row_130	STMN1	STMN1	stathmin 1/oncoprotein 18	7111	0.19096157	0.52868724	No
row_131	ATF6	ATF6	activating transcription factor 6	7209	0.181822732	0.52432895	No
row_132	CLIC4	CLIC4	chloride intracellular channel 4	7692	0.13941212	0.49926916	No
row_133	CXCR6	CXCR6	chemokine (C-X-C motif) receptor 6	8054	0.106374994	0.48050907	No
row_134	CD44	CD44	CD44 molecule (Indian blood group)	8103	0.102266327	0.4784071	No
row_135	EEA1	EEA1	early endosome antigen 1, 162kD	8584	0.063421227	0.45311546	No
row_136	VIM	VIM	vimentin	9105	0.021833856	0.42550746	No
row_137	MYADM	MYADM	myeloid-associated differentiation marker	9817	-0.020807154	0.3877185	No
row_138	MKI67	MKI67	antigen identified by monoclonal antibody Ki-67	9850	-0.023376763	0.38611767	No
row_139	KIAA0101	KIAA0101	KIAA0101	9923	-0.029381931	0.3824124	No
row_140	ITGA1	ITGA1	integrin, alpha 1	10477	-0.07660681	0.3532901	No
row_141	ST3GAL6	ST3GAL6	ST3 beta-galactoside alpha-2,3-sialyltransferase 6	10483	-0.076973282	0.35336652	No
row_142	CROT	CROT	carnitine O-octanoyltransferase	10549	-0.082258396	0.3502697	No
row_143	NUSAP1	NUSAP1	nucleolar and spindle associated protein 1	10569	-0.083916672	0.34963113	No
row_144	NDNF	null	null	10795	-0.101919957	0.33809718	No
row_145	AS3MT	AS3MT	arsenic (+3 oxidation state) methyltransferase	10916	-0.110689312	0.33219662	No
row_146	USP48	USP48	ubiquitin specific peptidase 48	11039	-0.121618956	0.3262382	No
row_147	TTC39B	null	null	11153	-0.130319729	0.32079798	No
row_148	INSL6	INSL6	insulin-like 6	11231	-0.136164784	0.3173019	No
row_149	LAIR1	LAIR1	leukocyte-associated immunoglobulin-like receptor 1	11995	-0.199535102	0.27753842	No
row_150	APOBEC2	APOBEC2	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 2	12148	-0.213587001	0.27039117	No
row_151	CA5B	CA5B	carbonic anhydrase VB, mitochondrial	12176	-0.216359437	0.2699162	No
row_152	GPX8	null	null	12358	-0.229924336	0.2612966	No
row_153	TTC39C	null	null	12451	-0.236867934	0.2574498	No
row_154	S100A9	S100A9	S100 calcium binding protein A9	12649	-0.252709955	0.24807924	No
row_155	PLSCR1	PLSCR1	phospholipid scramblase 1	13247	-0.305294454	0.21763112	No
row_156	LGALS1	null	null	13438	-0.32125023	0.20893875	No
row_157	GIMAP7	GIMAP7	GTPase, IMAP family member 7	13941	-0.372332722	0.18385072	No
row_158	KIF11	KIF11	kinesin family member 11	14172	-0.395769566	0.17335905	No
row_159	PFKP	PFKP	phosphofructokinase, platelet	14258	-0.406878978	0.17064238	No
row_160	TOP2A	TOP2A	topoisomerase (DNA) II alpha 170kDa	14614	-0.448333681	0.15372488	No
row_161	ABCB1	ABCB1	ATP-binding cassette, sub-family B (MDR/TAP), member 1	14816	-0.4721964	0.14511868	No
row_162	ENTPD1	ENTPD1	ectonucleoside triphosphate diphosphohydrolase 1	15062	-0.5	0.13429202	No
row_163	DDX28	DDX28	DEAD (Asp-Glu-Ala-Asp) box polypeptide 28	15159	-0.51004833	0.13144873	No
row_164	SPC25	null	null	15182	-0.512632906	0.13255963	No
row_165	EMP1	EMP1	epithelial membrane protein 1	15494	-0.558685958	0.11847789	No
row_166	PTGER4	PTGER4	prostaglandin E receptor 4 (subtype EP4)	15502	-0.559961319	0.120598756	No
row_167	TRPS1	TRPS1	trichorhinophalangeal syndrome I	15864	-0.615894198	0.10410783	No
row_168	PRKAR2B	PRKAR2B	protein kinase, cAMP-dependent, regulatory, type II, beta	16668	-0.758772969	0.064703755	No
row_169	GSTM3	GSTM3	glutathione S-transferase M3 (brain)	16749	-0.77436173	0.06389007	No
row_170	BCL2A1	BCL2A1	BCL2-related protein A1	17032	-0.847740293	0.052640747	No
row_171	DENND4A	DENND4A	DENN/MADD domain containing 4A	18063	-1.284453034	0.003483407	No
row_172	EID3	EID3	-	18196	-1.37605083	0.00257885	No
row_173	SYPL1	SYPL1	synaptophysin-like 1	18299	-1.472677708	0.003703007	No
row_174	CD48	CD48	CD48 molecule	18401	-1.591840267	0.005411139	No
row_175	CCNA2	CCNA2	cyclin A2	18565	-1.803973913	0.0047607	No
row_176	DENND5A	null	null	18912	-3.465185165	0.001758436	No

T-Naive biased

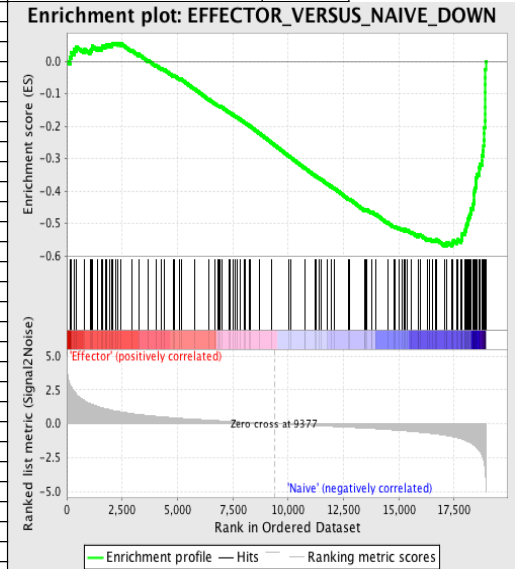
### Supplemental Table 1C: Comparing transcripts UP in CMV-specific T<sub>EFF</sub> relative to Naive T cells from mice and humans.

List shows significantly altered mouse genes (2-fold up or down and  $p < .05$ ). The table also includes a GSEA analysis of these mouse gene sets relative to each of the indicated human data sets, rank ordered by expression (see methods) and FWER significance to control for multiple testing.

**Supplemental Table 1D: Transcripts DOWN in T<sub>EFF</sub> relative to Naïve**

NAME	PROBE	GENE SYMBOL	GENE TITLE	RANK IN GENE LIST	RANK METRIC SCORE	RUNNING ES	CORE ENRICHMENT
row_0	DUSP10	DUSP10	dual specificity phosphatase 10	141	2.939956188	0.011949813	No
row_1	PIP4K2A	null	null	199	2.746984005	0.027089376	No
row_2	XCL1	XCL1	chemokine (C motif) ligand 1	329	2.424889326	0.036270257	No
row_3	SH3BP5	SH3BP5	SH3-domain binding protein 5 (BTK-associated)	434	2.161664009	0.04503923	No
row_4	HSPB11	null	null	794	1.710548043	0.03726619	No
row_5	TOX	TOX	-	1049	1.482677102	0.03356861	No
row_6	MRPS5	MRPS5	mitochondrial ribosomal protein S5	1126	1.428400517	0.038976066	No
row_7	SLAMF6	SLAMF6	SLAM family member 6	1145	1.419834614	0.04741066	No
row_8	IFI27L2	null	null	1403	1.269733906	0.042145036	No
row_9	LZTFL1	LZTFL1	leucine zipper transcription factor-like 1	1557	1.191928625	0.041894328	No
row_10	LY6E	LY6E	lymphocyte antigen 6 complex, locus E	1603	1.174182415	0.04726847	No
row_11	EMB	EMB	embigin homolog (mouse)	1737	1.11072886	0.047544036	No
row_12	ST8SIA1	ST8SIA1	ST8 alpha-N-acetyl-neuraminidase alpha-2,8-sialyltransferase 1	1791	1.085347176	0.05190522	No
row_13	STAMBP1	STAMBP1	STAM binding protein-like 1	1935	1.02846241	0.051104933	No
row_14	MED4	MED4	mediator of RNA polymerase II transcription, subunit 4 homolog (S. cerevisiae)	2034	0.994327545	0.05247146	No
row_15	RTP4	RTP4	receptor transporter protein 4	2082	0.977881908	0.056440815	No
row_16	PHPT1	PHPT1	phosphohistidine phosphatase 1	2204	0.936563373	0.05620237	No
row_17	EGR2	EGR2	early growth response 2 (Krox-20 homolog, Drosophila)	2325	0.900809228	0.055780597	No
row_18	SPRED2	SPRED2	sprouty-related, EVH1 domain containing 2	2460	0.864051044	0.05437132	No
row_19	RPLP1	RPLP1	ribosomal protein, large, P1	2933	0.746564031	0.034213834	No
row_20	ATP1B1	ATP1B1	ATPase, Na+/K+ transporting, beta 1 polypeptide	3246	0.683011651	0.022142995	No
row_21	NAT1	NAT1	N-acetyltransferase 1 (arylamine N-acetyltransferase)	3671	0.606945157	0.003614093	No
row_22	HINT3	HINT3	histidine triad nucleotide binding protein 3	4043	0.546061218	-0.012499581	No
row_23	ITGAE	ITGAE	integrin, alpha E (antigen CD103, human mucosal lymphocyte antigen 1; alpha polypeptide)	4278	0.5118379	-0.021555495	No
row_24	USP28	USP28	ubiquitin specific peptidase 28	4424	0.48942703	-0.026027618	No
row_25	GID4	null	null	4838	0.432452857	-0.045125857	No
row_26	HMP19	HMP19	-	4885	0.425784498	-0.04475523	No
row_27	JUN	JUN	jun oncogene	5091	0.399972111	-0.053009197	No
row_28	ACSL3	ACSL3	acyl-CoA synthetase long-chain family member 3	5165	0.391422004	-0.054301426	No
row_29	SLFN5	SLFN5	schlafen family member 5	5772	0.32738024	-0.08435627	No
row_30	BCL9	BCL9	B-cell CLL/lymphoma 9	6430	0.260354906	-0.11756607	No
row_31	CCR9	CCR9	chemokine (C-C motif) receptor 9	6700	0.233178288	-0.1303261	No
row_32	TTC27	TTC27	tetratricopeptide repeat domain 27	6854	0.218153343	-0.13701795	No
row_33	CD3D	CD3D	CD3d molecule, delta (CD3-TCR complex)	6890	0.215069339	-0.13745627	No
row_34	LPHN2	LPHN2	latrophilin 2	6942	0.210390016	-0.13877623	No
row_35	SEPP1	SEPP1	selenoprotein P, plasma, 1	7007	0.202965602	-0.1408365	No
row_36	HRS12	HRS12	heat-responsive protein 12	7324	0.171926096	-0.15650065	No
row_37	PPIC	PPIC	peptidylprolyl isomerase C (cyclophilin C)	7382	0.1669918	-0.15842669	No
row_38	EPHX1	EPHX1	epoxide hydrolase 1, microsomal (xenobiotic)	7497	0.156613216	-0.163452	No
row_39	IJT80	IJT80	intraflagellar transport 80 homolog (Chlamydomonas)	7613	0.146471366	-0.16859758	No
row_40	PRDX6	PRDX6	peroxiredoxin 6	7705	0.138377637	-0.17252062	No
row_41	RPS26	RPS26	ribosomal protein S26	7821	0.12824057	-0.17778678	No
row_42	TLR1	TLR1	toll-like receptor 1	8020	0.109741025	-0.18758833	No
row_43	TMLHE	TMLHE	trimethyllysine hydroxylase, epsilon	8072	0.104750194	-0.18960707	No
row_44	TRIM13	TRIM13	tripartite motif-containing 13	8261	0.088052206	-0.19902039	No
row_45	NEDD4L	NEDD4L	neural precursor cell expressed, developmentally down-regulated 4-like	8274	0.0869992	-0.19908294	No
row_46	DAPL1	DAPL1	death associated protein-like 1	8730	0.05147025	-0.22293432	No
row_47	QSER1	QSER1	glutamine and serine rich 1	9237	0.011975013	-0.24975856	No
row_48	GRIA3	GRIA3	glutamate receptor, ionotropic, AMPA 3	10043	-0.039689511	-0.29229698	No
row_49	DKC1	DKC1	dyskeratosis congenita 1, dyskerin	10139	-0.049450032	-0.2970209	No
row_50	RRAS2	RRAS2	related RAS viral (r-ras) oncogene homolog 2	10748	-0.098168731	-0.32869825	No
row_51	LYPD6B	null	null	10756	-0.098722182	-0.32841742	No
row_52	F2RL1	F2RL1	coagulation factor II (thrombin) receptor-like 1	11236	-0.136392936	-0.35298312	No
row_53	DPP4	DPP4	dipeptidyl-peptidase 4 (CD26, adenosine deaminase complexing protein 2)	11283	-0.140664518	-0.35449845	No
row_54	RAPGEF4	RAPGEF4	Rap guanine nucleotide exchange factor (GEF) 4	11391	-0.149277762	-0.3592001	No
row_55	TFRC	TFRC	transferrin receptor (p90, CD71)	11483	-0.15601249	-0.3630065	No

T-EFF biased	
Normalized Enrichment Score (NES)	-2.4599857
Nominal p-value	0
FDR q-value	0
FWER p-Value	0



T-Naive biased

**Supplemental Table 1D: Comparing transcripts DOWN in CMV-specific T<sub>EFF</sub> relative to Naïve T cells from mice and humans.**

List shows significantly altered mouse genes (2-fold up or down and  $p < .05$ ). The table also includes a GSEA analysis of these mouse gene sets relative to each of the indicated human data sets, rank ordered by expression (see methods) and FWER significance to control for multiple testing.

row_56	KIAA1033	KIAA1033	KIAA1033	11781	-0.183877766	-0.3775814	No
row_57	ICOS	ICOS	inducible T-cell co-stimulator	11945	-0.196053505	-0.38495108	No
row_58	RRP15	null	null	12080	-0.207625285	-0.39070237	No
row_59	INSR	INSR	insulin receptor	12743	-0.260480523	-0.42417717	No
row_60	CD2AP	CD2AP	CD2-associated protein	12789	-0.264684618	-0.424819	No
row_61	TSPAN13	TSPAN13	tetraspanin 13	13458	-0.323553056	-0.45819563	No
row_62	MGST2	MGST2	microsomal glutathione S-transferase 2	13478	-0.325819671	-0.45705065	No
row_63	KKRX	KKRX	XK, Kell blood group complex subunit-related, X-linked	13496	-0.328198284	-0.45578364	No
row_64	ZNF761	ZNF761	zinc finger protein 761	13578	-0.335966825	-0.45786804	No
row_65	RPS29	RPS29	ribosomal protein S29	13767	-0.35352698	-0.46552533	No
row_66	CHURC1	CHURC1	churchill domain containing 1	13799	-0.356838018	-0.46481323	No
row_67	RPL8	RPL8	ribosomal protein L8	13990	-0.376885176	-0.47242236	No
row_68	LCLAT1	null	null	14502	-0.434282362	-0.49671906	No
row_69	CNGA1	CNGA1	cyclic nucleotide gated channel alpha 1	14777	-0.467894018	-0.5081924	No
row_70	RPSA	RPSA	ribosomal protein SA	14858	-0.476895005	-0.5092914	No
row_71	WDR12	WDR12	WD repeat domain 12	15015	-0.496033192	-0.5143047	No
row_72	KLHDC1	KLHDC1	kelch domain containing 1	15155	-0.509133816	-0.5183275	No
row_73	ZBTB10	ZBTB10	zinc finger and BTB domain containing 10	15248	-0.523607731	-0.51975554	No
row_74	TTC3	TTC3	tetratricopeptide repeat domain 3	15281	-0.528237998	-0.5179629	No
row_75	PCGF5	PCGF5	polycomb group ring finger 5	15374	-0.540068984	-0.5192821	No
row_76	MYC	MYC	v-myc myelocytomatosis viral oncogene homolog (avian)	15574	-0.571345031	-0.52608347	No
row_77	RPL14	RPL14	ribosomal protein L14	15877	-0.618567109	-0.5380489	No
row_78	TRAT1	TRAT1	T cell receptor associated transmembrane adaptor 1	15885	-0.619778156	-0.5343215	No
row_79	RPLP0	RPLP0	ribosomal protein, large, P0	15971	-0.63402319	-0.53464705	No
row_80	CCDC91	CCDC91	coiled-coil domain containing 91	16303	-0.687809348	-0.54769635	No
row_81	MRPL23	MRPL23	mitochondrial ribosomal protein L23	16385	-0.704515219	-0.54734296	No
row_82	KBTBD11	KBTBD11	kelch repeat and BTB (POZ) domain containing 11	16543	-0.733644545	-0.5508377	No
row_83	RPS2	RPS2	ribosomal protein S2	16655	-0.757363677	-0.5517298	No
row_84	RPS19	RPS19	ribosomal protein S19	16716	-0.768323898	-0.54983777	No
row_85	RPL10A	RPL10A	ribosomal protein L10a	17070	-0.856434166	-0.56294143	No
row_86	CYB5A	CYB5A	cytochrome b5 type A (microsomal)	17166	-0.881539941	-0.56216145	No
row_87	BTF3L4	BTF3L4	basic transcription factor 3-like 4	17188	-0.886925817	-0.5574113	No
row_88	IMPDH2	IMPDH2	IIMP (inosine monophosphate) dehydrogenase 2	17415	-0.955320895	-0.56310844	Yes
row_89	CMSS1	null	null	17453	-0.969784021	-0.5586609	Yes
row_90	P2RY10	P2RY10	purinergic receptor P2Y, G-protein coupled, 10	17467	-0.974344432	-0.5529072	Yes
row_91	LTV1	LTV1	LTV1 homolog (S. cerevisiae)	17636	-1.036688805	-0.549823	Yes
row_92	TNFSF8	TNFSF8	tumor necrosis factor (ligand) superfamily, member 8	17663	-1.047024012	-0.5494391	Yes
row_93	ST6GAL1	ST6GAL1	ST6 beta-galactosamide alpha-2,6-sialyltransferase 1	17827	-1.123234868	-0.55067587	Yes
row_94	CD69	CD69	CD69 molecule	17857	-1.142017961	-0.5446638	Yes
row_95	LSM7	LSM7	LSM7 homolog, U6 small nuclear RNA associated (S. cerevisiae)	17871	-1.145340919	-0.537779	Yes
row_96	RAB3IP	RAB3IP	RAB3A interacting protein (rab3)	17878	-1.147305012	-0.53050905	Yes
row_97	HOOK1	HOOK1	hook homolog 1 (Drosophila)	17979	-1.21851027	-0.527766	Yes
row_98	SATB1	SATB1	special AT-rich sequence binding protein 1 (binds to nuclear matrix/scaffold-associating DNA's)	17993	-1.225864649	-0.52034855	Yes
row_99	FBL	FBL	fibrillarin	18055	-1.275766611	-0.51515317	Yes
row_100	SESN3	SESN3	sestrin 3	18061	-1.280887604	-0.5069465	Yes
row_101	PDE3B	PDE3B	phosphodiesterase 3B, cGMP-inhibited	18103	-1.315524936	-0.50042474	Yes
row_102	PELL1	PELL1	pellino homolog 1 (Drosophila)	18124	-1.324875116	-0.4927246	Yes
row_103	SSBP2	SSBP2	single-stranded DNA binding protein 2	18126	-1.326386809	-0.48400426	Yes
row_104	RPL13	RPL13	ribosomal protein L13	18189	-1.370167017	-0.47823763	Yes
row_105	APEX1	APEX1	APEX nuclease (multifunctional DNA repair enzyme) 1	18231	-1.406233907	-0.4711159	Yes
row_106	INPP4B	INPP4B	inositol polyphosphate-4-phosphatase, type II, 105kDa	18255	-1.430721879	-0.46287513	Yes
row_107	RPL29	RPL29	ribosomal protein L29	18282	-1.452724934	-0.45464832	Yes
row_108	RPL18	RPL18	ribosomal protein L18	18347	-1.518245578	-0.44800857	Yes
row_109	BZW2	BZW2	basic leucine zipper and W2 domains 2	18379	-1.561169744	-0.43933028	Yes
row_110	CD27	null	null	18403	-1.594195485	-0.4300082	Yes
row_111	GPATCH4	null	null	18411	-1.60322082	-0.41977572	Yes
row_112	GPR183	null	null	18412	-1.608658433	-0.4091351	Yes
row_113	EEF1B2	EEF1B2	eukaryotic translation elongation factor 1 beta 2	18428	-1.626211882	-0.3991759	Yes
row_114	RPL28	RPL28	ribosomal protein L28	18463	-1.667272091	-0.3899553	Yes
row_115	TRIB2	TRIB2	tribbles homolog 2 (Drosophila)	18496	-1.701058149	-0.38040492	Yes
row_116	PDK1	PDK1	pyruvate dehydrogenase kinase, isozyme 1	18502	-1.709740758	-0.36936152	Yes
row_117	DGKA	DGKA	diacylglycerol kinase, alpha 80kDa	18512	-1.720613718	-0.35845888	Yes

### Supplemental Table 1D: Comparing transcripts DOWN in CMV-specific T<sub>EFF</sub> relative to Naïve T cells from mice and humans.

List shows significantly altered mouse genes (2-fold up or down and  $p < .05$ ). The table also includes a GSEA analysis of these mouse gene sets relative to each of the indicated human data sets, rank ordered by expression (see methods) and FWER significance to control for multiple testing.

row_118	TCP11L2	TCP11L2	t-complex 11 (mouse) like 2	18552	-1.774849534	-0.34879252	Yes
row_119	RPS9	RPS9	ribosomal protein S9	18631	-1.944758415	-0.3400759	Yes
row_120	RPL15	RPL15	ribosomal protein L15	18634	-1.947595	-0.3272997	Yes
row_121	MYB	MYB	v-myb myeloblastosis viral oncogene homolog (avian)	18678	-2.026391506	-0.3161822	Yes
row_122	ITGA6	ITGA6	integrin, alpha 6	18768	-2.315096617	-0.3056008	Yes
row_123	SELL	SELL	selectin L (lymphocyte adhesion molecule 1)	18773	-2.32630682	-0.2904259	Yes
row_124	IL7R	IL7R	interleukin 7 receptor	18808	-2.507570744	-0.27564707	Yes
row_125	ZEB1	null	null	18839	-2.68319726	-0.2594939	Yes
row_126	SH3PXD2A	SH3PXD2A	SH3 and PX domains 2A	18846	-2.73724103	-0.24170715	Yes
row_127	TCF7	TCF7	transcription factor 7 (T-cell specific, HMG-box)	18862	-2.879874945	-0.22345547	Yes
row_128	LTB	LTB	lymphotoxin beta (TNF superfamily, member 3)	18866	-2.933085918	-0.2042138	Yes
row_129	IL6ST	IL6ST	interleukin 6 signal transducer (gp130, oncostatin M receptor)	18893	-3.206775904	-0.18438466	Yes
row_130	IFNGR2	IFNGR2	interferon gamma receptor 2 (interferon gamma transducer 1)	18901	-3.34031558	-0.162662	Yes
row_131	PECAM1	PECAM1	platelet/endothelial cell adhesion molecule (CD31 antigen)	18903	-3.406530619	-0.14018235	Yes
row_132	CCR7	CCR7	chemokine (C-C motif) receptor 7	18906	-3.42228055	-0.117651686	Yes
row_133	DPH5	DPH5	DPH5 homolog (S. cerevisiae)	18907	-3.440943003	-0.094891235	Yes
row_134	RG510	RG510	regulator of G-protein signalling 10	18910	-3.448899031	-0.0721845	Yes
row_135	RPS5	RPS5	ribosomal protein S5	18913	-3.483943701	-0.049245965	Yes
row_136	ACTN1	ACTN1	actinin, alpha 1	18917	-3.560284615	-0.025855627	Yes
row_137	LEF1	LEF1	lymphoid enhancer-binding factor 1	18933	-4.125883102	6.38E-04	Yes

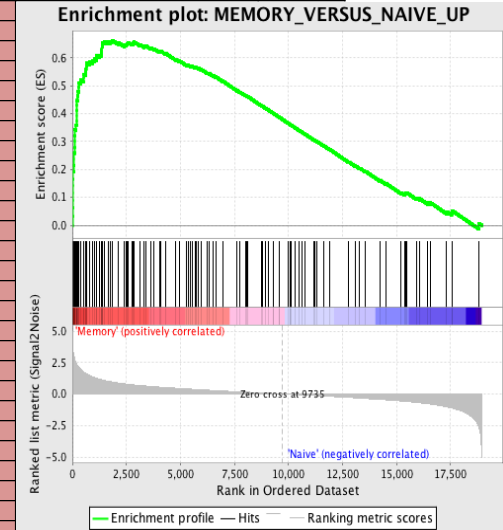
**Supplemental Table 1D: Comparing transcripts DOWN in CMV-specific T<sub>EFF</sub> relative to Naïve T cells from mice and humans.**

List shows significantly altered mouse genes (2-fold up or down and  $p < .05$ ). The table also includes a GSEA analysis of these mouse gene sets relative to each of the indicated human data sets, rank ordered by expression (see methods) and FWER significance to control for multiple testing.

**Supplemental Table 1E: Transcripts UP in T<sub>M</sub> relative to Naïve**

NAME	PROBE	GENE SYMBOL	GENE TITLE	RANK IN GENE LIST	RANK METRIC SCORE	RUNNING ES	CORE ENRICHMENT
row_0	ITGB1	ITGB1	integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12)	3	4.403458118	0.033498626	Yes
row_1	TBX21	TBX21	T-box 21	9	3.787709236	0.06218451	Yes
row_2	IER3	IER3	immediate early response 3	27	3.474953413	0.087842345	Yes
row_3	ANXA2	ANXA2	annexin A2	29	3.467990398	0.114296935	Yes
row_4	IL18R1	IL18R1	interleukin 18 receptor 1	30	3.436789513	0.14056617	Yes
row_5	PRDM1	PRDM1	PR domain containing 1, with ZNF domain	32	3.428418159	0.16671829	Yes
row_6	TNF	TNF	tumor necrosis factor (TNF superfamily, member 2)	39	3.323193789	0.1918005	Yes
row_7	GZMA	GZMA	granzyme A (granzyme 1, cytotoxic T-lymphocyte-associated serine esterase 3)	63	3.111444473	0.2143611	Yes
row_8	BHLHE40	null	null	83	2.946973085	0.23587705	Yes
row_9	CXCR3	CXCR3	chemokine (C-X-C motif) receptor 3	84	2.945517063	0.25839123	Yes
row_10	MYO1F	MYO1F	myosin IF	96	2.830258846	0.27944005	Yes
row_11	GZMK	GZMK	granzyme K (granzyme 3; tryptase II)	101	2.814285755	0.30073866	Yes
row_12	CCL5	CCL5	chemokine (C-C motif) ligand 5	103	2.810801268	0.32217	Yes
row_13	SLAMF7	SLAMF7	SLAM family member 7	112	2.729997635	0.34261185	Yes
row_14	IFNG	IFNG	interferon, gamma	168	2.514009953	0.35890597	Yes
row_15	ZEB2	null	null	203	2.384581566	0.37532642	Yes
row_16	IL18RAP	IL18RAP	interleukin 18 receptor accessory protein	205	2.380190134	0.39346638	Yes
row_17	CAMK2N1	CAMK2N1	calcium/calmodulin-dependent protein kinase II inhibitor 1	206	2.379069328	0.41165087	Yes
row_18	PTPRJ	PTPRJ	protein tyrosine phosphatase, receptor type, J	210	2.366587877	0.4295806	Yes
row_19	S1PR5	null	null	213	2.358679295	0.44750303	Yes
row_20	XCL1	XCL1	chemokine (C motif) ligand 1	246	2.268402576	0.46314168	Yes
row_21	LGALS1	LGALS1	lectin, galactoside-binding, soluble, 1 (galectin 1)	262	2.228570938	0.479379	Yes
row_22	FCHO2	FCHO2	FCH domain only 2	299	2.135591984	0.49379003	Yes
row_23	RGS1	RGS1	regulator of G-protein signalling 1	305	2.109815836	0.5096509	Yes
row_24	CCL4	CCL4	chemokine (C-C motif) ligand 4	368	1.997584224	0.5216258	Yes
row_25	CD44	CD44	CD44 molecule (Indian blood group)	508	1.743003964	0.52756435	Yes
row_26	HOPX	null	null	511	1.739763975	0.54075605	Yes
row_27	IL2RB	IL2RB	interleukin 2 receptor, beta	605	1.639035821	0.54834354	Yes
row_28	CCR5	CCR5	chemokine (C-C motif) receptor 5	627	1.613669157	0.5595621	Yes
row_29	S100A4	S100A4	S100 calcium binding protein A4	637	1.605283499	0.57135403	Yes
row_30	CHSY1	CHSY1	carbohydrate (chondroitin) synthase 1	656	1.586069226	0.582521	Yes
row_31	ITGA4	ITGA4	integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor)	783	1.489005804	0.5872087	Yes
row_32	AHNAK	AHNAK	AHNAK nucleoprotein (desmoyokin)	925	1.377583385	0.59024787	Yes
row_33	TYROBP	TYROBP	TYRO protein tyrosine kinase binding protein	1012	1.307673335	0.59567446	Yes
row_34	FASLG	FASLG	Fas ligand (TNF superfamily, member 6)	1108	1.245491982	0.60014766	Yes
row_35	PTPN3	PTPN3	protein tyrosine phosphatase, non-receptor type 3	1121	1.241511583	0.6089997	Yes
row_36	ATP2B1	ATP2B1	ATPase, Ca++ transporting, plasma membrane 1	1266	1.171560645	0.61030483	Yes
row_37	CTSD	CTSD	cathepsin D (lysosomal aspartyl peptidase)	1342	1.137916207	0.61501825	Yes
row_38	ID2	ID2	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein	1353	1.132620811	0.6231442	Yes
row_39	CASP4	CASP4	caspase 4, apoptosis-related cysteine peptidase	1364	1.127617121	0.63123196	Yes
row_40	ANXA1	ANXA1	annexin A1	1367	1.12607944	0.63973296	Yes
row_41	GZMB	GZMB	granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1)	1387	1.118411899	0.6472722	Yes
row_42	PLEK	PLEK	pleckstrin	1396	1.115830541	0.65537614	Yes
row_43	KLRG1	KLRG1	killer cell lectin-like receptor subfamily G, member 1	1485	1.075441837	0.6589214	Yes
row_44	SAMD3	SAMD3	sterile alpha motif domain containing 3	1649	1.020359516	0.65806144	Yes
row_45	P2RY13	P2RY13	purinergic receptor P2Y, G-protein coupled, 13	1768	0.977391183	0.65926355	Yes
row_46	HMOX1	HMOX1	heme oxygenase (decycling) 1	1836	0.954161108	0.6629974	Yes
row_47	VCAM1	VCAM1	vascular cell adhesion molecule 1	2118	0.876853645	0.654772	No
row_48	CDC42EP3	CDC42EP3	CDC42 effector protein (Rho GTPase binding) 3	2405	0.811948538	0.64578474	No
row_49	RORA	RORA	RAR-related orphan receptor A	2483	0.79405731	0.6477636	No
row_50	SLC25A24	SLC25A24	solute carrier family 25 (mitochondrial carrier, phosphate carrier), member 24	2523	0.785699964	0.65169734	No
row_51	CAPN2	CAPN2	calpain 2, (mII) large subunit	2575	0.775719941	0.6549173	No
row_52	DSTN	DSTN	destrin (actin depolymerizing factor)	2742	0.739924014	0.6517544	No
row_53	CD38	CD38	CD38 molecule	2824	0.724371314	0.65298814	No
row_54	S100A6	S100A6	S100 calcium binding protein A6	2842	0.721523046	0.6576	No
row_55	MITF	MITF	microphthalmia-associated transcription factor	3146	0.671954572	0.64663965	No

<b>T-MEM biased</b>	Yes
Normalized Enrichment Score (NES)	2.735306
Nominal p-value	0
FDR q-value	0
FWER p-Value	0



**Supplemental Table 1E: Comparing transcripts UP in CMV-specific T<sub>M</sub> relative to Naïve T cells from mice and humans.**

List shows significantly altered mouse genes (2-fold up or down and  $p < .05$ ). The table also includes a GSEA analysis of these mouse gene sets relative to each of the indicated human data sets, rank ordered by expression (see methods) and FWER significance to control for multiple testing.

row_56	UBD	UBD	ubiquitin D	3333	0.644224048	0.6416828	No
row_57	SLC9A7	SLC9A7	solute carrier family 9 (sodium/hydrogen exchanger), member 7	3405	0.633073628	0.64274997	No
row_58	IGF1	IGF1	insulin-like growth factor 1 (somatomedin C)	3628	0.601582348	0.6355547	No
row_59	CTSB	CTSB	cathepsin B	3760	0.581756353	0.63304216	No
row_60	KLRC1	KLRC1	killer cell lectin-like receptor subfamily C, member 1	4033	0.549523294	0.62279284	No
row_61	SYTL2	SYTL2	synaptotagmin-like 2	4107	0.540566444	0.62304664	No
row_62	ARHGAP26	ARHGAP26	Rho GTPase activating protein 26	4308	0.515345871	0.61636096	No
row_63	PILRA	PILRA	paired immunoglobulin-like type 2 receptor alpha	4785	0.458973765	0.5945823	No
row_64	GOLIM4	null	null	4971	0.43950215	0.5881138	No
row_65	GNPTAB	GNPTAB	N-acetylglucosamine-1-phosphate transferase, alpha and beta subunits	5275	0.404915243	0.5751123	No
row_66	ZDHHC2	ZDHHC2	zinc finger, DHHC-type containing 2	5375	0.395494431	0.57287604	No
row_67	ITGA1	ITGA1	integrin, alpha 1	5481	0.38406226	0.57023364	No
row_68	CASP1	CASP1	caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)	5553	0.378048152	0.5693515	No
row_69	DOCK5	DOCK5	dedicator of cytokinesis 5	5680	0.365191877	0.56544924	No
row_70	C1QB	C1QB	complement component 1, q subcomponent, B chain	5736	0.359394342	0.5652745	No
row_71	CX3CR1	CX3CR1	chemokine (C-X3-C motif) receptor 1	5825	0.350362927	0.5632776	No
row_72	SIRPA	SIRPA	signal-regulatory protein alpha	5967	0.337242603	0.5583649	No
row_73	CEP290	CEP290	centrosomal protein 290kDa	6252	0.308049321	0.54563236	No
row_74	ABCB1	ABCB1	ATP-binding cassette, sub-family B (MDR/TAP), member 1	6346	0.298542351	0.54297376	No
row_75	KCNJ8	KCNJ8	potassium inwardly-rectifying channel, subfamily J, member 8	6525	0.280597478	0.53566253	No
row_76	SIGLEC8	SIGLEC8	sialic acid binding Ig-like lectin 8	6635	0.270513564	0.53193974	No
row_77	ENTPD1	ENTPD1	ectonucleoside triphosphate diphosphohydrolase 1	6960	0.239131674	0.5165555	No
row_78	CHD7	CHD7	chromodomain helicase DNA binding protein 7	7633	0.179831967	0.4822309	No
row_79	MRC1	MRC1	mannose receptor, C type 1	7750	0.169954807	0.4773676	No
row_80	NOD1	null	null	8023	0.147540331	0.4640457	No
row_81	SLC20A1	SLC20A1	solute carrier family 20 (phosphate transporter), member 1	8085	0.141955048	0.46189022	No
row_82	CLEC12A	CLEC12A	C-type lectin domain family 12, member A	8121	0.139091328	0.46109402	No
row_83	KLRK1	KLRK1	killer cell lectin-like receptor subfamily K, member 1	8756	0.083828688	0.42805436	No
row_84	MARCO	MARCO	macrophage receptor with collagenous structure	8799	0.079154007	0.4264282	No
row_85	BCL2A1	BCL2A1	BCL2-related protein A1	8955	0.066597454	0.41870305	No
row_86	C1QC	C1QC	complement component 1, q subcomponent, C chain	9054	0.057710547	0.41393805	No
row_87	FGL2	FGL2	fibrinogen-like 2	9285	0.039783955	0.4020237	No
row_88	CYSLTR2	CYSLTR2	cysteinyl leukotriene receptor 2	9309	0.03815094	0.40109345	No
row_89	CD68	CD68	CD68 molecule	9565	0.014540591	0.38765806	No
row_90	CD5L	CD5L	CD5 molecule-like	9589	0.012351896	0.38653064	No
row_91	SPIC	SPIC	Spi-C transcription factor (Spi-1/PU.1 related)	9974	-0.013060934	0.36623096	No
row_92	KCNJ16	KCNJ16	potassium inwardly-rectifying channel, subfamily J, member 16	10081	-0.021453133	0.36076385	No
row_93	MARCKS	MARCKS	myristoylated alanine-rich protein kinase C substrate	10100	-0.023070196	0.35998395	No
row_94	TF	TF	transferrin	10110	-0.023738414	0.3596873	No
row_95	C1QA	C1QA	complement component 1, q subcomponent, A chain	10307	-0.038720008	0.34957102	No
row_96	DMRTA1	DMRTA1	DMRT-like family A1	10492	-0.053806484	0.34020752	No
row_97	CD302	CD302	CD302 molecule	10626	-0.066097707	0.3336473	No
row_98	PLBD1	null	null	10770	-0.080001555	0.3266621	No
row_99	TM6SF1	TM6SF1	transmembrane 6 superfamily member 1	11164	-0.115543537	0.30666766	No
row_100	CXCL3	CXCL3	chemokine (C-X-C motif) ligand 3	11234	-0.12114767	0.30392814	No
row_101	MYADM	MYADM	myeloid-associated differentiation marker	11320	-0.129930556	0.30040574	No
row_102	EEA1	EEA1	early endosome antigen 1, 162kD	11628	-0.158786342	0.28531048	No
row_103	LGMN	LGMN	legumain	11900	-0.183479294	0.2723164	No
row_104	SERPINB6	SERPINB6	serpin peptidase inhibitor, clade B (ovalbumin), member 6	12789	-0.268000066	0.22719103	No
row_105	ADAMDEC1	ADAMDEC1	ADAM-like, decysin 1	13104	-0.301389694	0.21281388	No
row_106	ATP6VOD2	ATP6VOD2	ATPase, H+ transporting, lysosomal 38kDa, VO subunit d2	13305	-0.322526693	0.2046544	No
row_107	APOE	APOE	apolipoprotein E	13579	-0.353963107	0.19285716	No
row_108	RAB23	RAB23	RAB23, member RAS oncogene family	14232	-0.426638871	0.16148156	No
row_109	CCR3	CCR3	chemokine (C-C motif) receptor 3	14513	-0.457534194	0.1501041	No
row_110	SLC40A1	SLC40A1	solute carrier family 40 (iron-regulated transporter), member 1	15204	-0.549991131	0.11765265	No
row_111	IL18	IL18	interleukin 18 (interferon-gamma-inducing factor)	15396	-0.576450884	0.11191215	No
row_112	EMR1	EMR1	egf-like module containing, mucin-like, hormone receptor-like 1	15417	-0.58118856	0.11529201	No
row_113	TTC39B	null	null	15473	-0.589318216	0.11687469	No
row_114	TRPS1	TRPS1	trichorhinophalangeal syndrome I	15933	-0.662678242	0.09755613	No
row_115	MPEG1	MPEG1	-	16069	-0.689519227	0.0956548	No
row_116	MERTK	MERTK	c-mer proto-oncogene tyrosine kinase	16455	-0.769379318	0.08108297	No
row_117	CD163	CD163	CD163 molecule	16576	-0.799468338	0.08081889	No

T-Naive biased

### Supplemental Table 1E: Comparing transcripts UP in CMV-specific T<sub>M</sub> relative to Naïve T cells from mice and humans.

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row_118	CSF1R	CSF1R	colony stimulating factor 1 receptor, formerly McDonough feline sarcoma viral (v-fms) oncogene homo	17321	-1.016953349	0.049068	No
row_119	DENND4A	DENND4A	DENN/MADD domain containing 4A	17562	-1.106143713	0.044773173	No
row_120	HPGD	HPGD	hydroxyprostaglandin dehydrogenase 15-(NAD)	17591	-1.119443536	0.05184222	No
row_121	AIF1	AIF1	allograft inflammatory factor 1	18811	-2.621035337	0.007118445	No

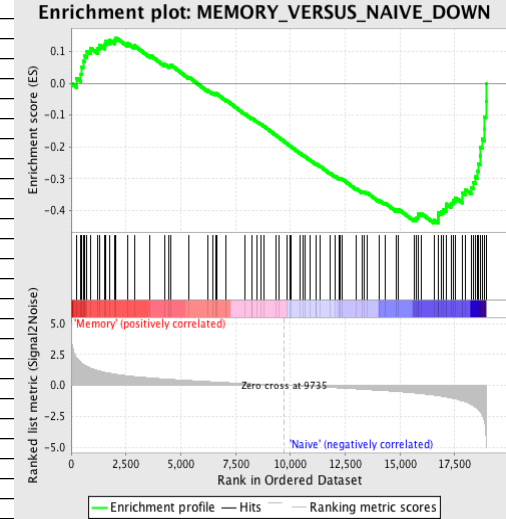
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List shows significantly altered mouse genes (2-fold up or down and  $p < .05$ ). The table also includes a GSEA analysis of these mouse gene sets relative to each of the indicated human data sets, rank ordered by expression (see methods) and FWER significance to control for multiple testing.

**Supplemental Table 1F: Transcripts DOWN in T<sub>M</sub> relative to Naïve**

NAME	PROBE	GENE SYMBOL	GENE TITLE	RANK IN GENE LIST	RANK METRIC SCORE	RUNNING ES	CORE ENRICHMENT
row_0	TRAT1	TRAT1	T cell receptor associated transmembrane adaptor 1	252	2.253664494	0.015806396	No
row_1	IFT52	IFT52	intraflagellar transport 52 homolog (Chlamydomonas)	448	1.839338064	0.029272709	No
row_2	MAP1LC3B	MAP1LC3B	microtubule-associated protein 1 light chain 3 beta	478	1.792060494	0.050929733	No
row_3	KIAA1109	KIAA1109	KIAA1109	544	1.710649014	0.06962404	No
row_4	ST8SIA1	ST8SIA1	ST8 alpha-N-acetyl-neuraminidase alpha-2,8-sialyltransferase 1	611	1.629442453	0.08721425	No
row_5	SMIM7	null	null	722	1.538029432	0.10128806	No
row_6	SMC4	SMC4	structural maintenance of chromosomes 4	885	1.410843372	0.11095824	No
row_7	PPIC	PPIC	peptidylprolyl isomerase C (cyclophilin C)	1210	1.200220346	0.10931178	No
row_8	CCR9	CCR9	chemokine (C-C motif) receptor 9	1284	1.165769219	0.12052943	No
row_9	H3F3B	H3F3B	H3 histone, family 3B (H3.3B)	1519	1.064026237	0.121892706	No
row_10	EGR2	EGR2	early growth response 2 (Krox-20 homolog, Drosophila)	1588	1.039483428	0.13174096	No
row_11	SDHAF1	null	null	1745	0.984709442	0.13621381	No
row_12	USP31	USP31	ubiquitin specific peptidase 31	1970	0.915602505	0.1361863	No
row_13	CRIP1	CRIP1	cysteine-rich PDZ-binding protein	2043	0.8937971	0.14393681	No
row_14	SESN3	SESN3	sestrin 3	2562	0.777974069	0.12653776	No
row_15	CSPP1	CSPP1	centrosome and spindle pole associated protein 1	2878	0.716659307	0.11910977	No
row_16	SLAMF6	SLAMF6	SLAM family member 6	3602	0.604985237	0.088601	No
row_17	ATP1B1	ATP1B1	ATPase, Na <sup>+</sup> /K <sup>+</sup> transporting, beta 1 polypeptide	4260	0.521278799	0.060508642	No
row_18	IFI27L2	null	null	4472	0.495869935	0.055737853	No
row_19	WDR12	WDR12	WD repeat domain 12	4548	0.486908525	0.058062874	No
row_20	GID4	null	null	5386	0.394062251	0.018778916	No
row_21	GRIA3	GRIA3	glutamate receptor, ionotropic, AMPA 3	6236	0.309973687	-0.02222974	No
row_22	SMURF2	SMURF2	SMAD specific E3 ubiquitin protein ligase 2	6467	0.286243409	-0.030721275	No
row_23	TRIM13	TRIM13	tripartite motif-containing 13	6601	0.272644997	-0.03424511	No
row_24	CYB5D1	CYB5D1	cytochrome b5 domain containing 1	6628	0.271089345	-0.032115098	No
row_25	EIF4E2	EIF4E2	eukaryotic translation initiation factor 4E member 2	7077	0.228623614	-0.052912492	No
row_26	DIRC2	DIRC2	disrupted in renal carcinoma 2	7948	0.153479651	-0.097060256	No
row_27	DUSP19	DUSP19	dual specificity phosphatase 19	8244	0.129077777	-0.111032814	No
row_28	TMEM50B	TMEM50B	transmembrane protein 50B	8250	0.128792241	-0.10963099	No
row_29	LYPD6B	null	null	8499	0.108135678	-0.12138229	No
row_30	TFRC	TFRC	transferrin receptor (p90, CD71)	8659	0.092881635	-0.12861155	No
row_31	HSPB11	null	null	8816	0.077869982	-0.13587601	No
row_32	DPP4	DPP4	dipeptidyl-peptidase 4 (CD26, adenosine deaminase complexing protein 2)	9355	0.034692306	-0.163956	No
row_33	HIGD2A	HIGD2A	HIG1 domain family, member 2A	9507	0.019503452	-0.17171077	No
row_34	UFM1	UFM1	ubiquitin-fold modifier 1	9836	-2.79E-04	-0.18910031	No
row_35	HRS12	HRS12	heat-responsive protein 12	9985	-0.014027056	-0.19676688	No
row_36	PSMA5	PSMA5	proteasome (prosome, macropain) subunit, alpha type, 5	10051	-0.019558406	-0.19996054	No
row_37	APOOL	null	null	10453	-0.050357979	-0.22057295	No
row_38	CD69	CD69	CD69 molecule	10576	-0.061757311	-0.22624302	No
row_39	SF3B14	SF3B14	-	10679	-0.070999824	-0.2307329	No
row_40	IFT80	IFT80	intraflagellar transport 80 homolog (Chlamydomonas)	10922	-0.094206497	-0.24234633	No
row_41	LTN1	null	null	11172	-0.116225645	-0.25404596	No
row_42	CNGA1	CNGA1	cyclic nucleotide gated channel alpha 1	11182	-0.117405698	-0.2530036	No
row_43	TXNL4B	TXNL4B	thioredoxin-like 4B	11340	-0.132090256	-0.25961933	No
row_44	PRDX6	PRDX6	peroxiredoxin 6	11840	-0.177881435	-0.28377792	No
row_45	PHPT1	PHPT1	phosphohistidine phosphatase 1	12049	-0.196422234	-0.2922654	No
row_46	MGST2	MGST2	microsomal glutathione S-transferase 2	12218	-0.209586099	-0.29846138	No
row_47	BTF3L4	BTF3L4	basic transcription factor 3-like 4	12266	-0.215067789	-0.29817006	No
row_48	ITGAE	ITGAE	integrin, alpha E (antigen CD103, human mucosal lymphocyte antigen 1; alpha polypeptide)	12369	-0.225916848	-0.30065483	No
row_49	KKRX	KKRX	XK, Kell blood group complex subunit-related, X-linked	13020	-0.292157233	-0.33134153	No
row_50	POLR2H	POLR2H	polymerase (RNA) II (DNA directed) polypeptide H	13272	-0.318979412	-0.34052294	No
row_51	INSIG2	INSIG2	insulin induced gene 2	13368	-0.330971003	-0.3412768	No
row_52	RPS2	RPS2	ribosomal protein S2	13512	-0.347620785	-0.3443605	No
row_53	COP54	COP54	COP9 constitutive photomorphogenic homolog subunit 4 (Arabidopsis)	14072	-0.408545583	-0.36871526	No
row_54	CDC26	CDC26	cell division cycle 26	14324	-0.437779635	-0.37635902	No
row_55	TTC3	TTC3	tetratricopeptide repeat domain 3	14852	-0.500140667	-0.39783135	No

<b>T-MEM biased</b>	No
Normalized Enrichment Score (NES)	-1.7465168
Nominal p-value	0
FDR q-value	5.04E-04
FWER p-Value	0.001



**T-Naive biased**

**Supplemental Table 1F: Comparing transcripts DOWN in CMV-specific T<sub>M</sub> relative to Naïve T cells from mice and humans.**

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row_56	CMSS1	null	null	14939	-0.513141811	-0.39575008	No
row_57	RPL28	RPL28	ribosomal protein L28	15661	-0.616440535	-0.42600456	No
row_58	DAPL1	DAPL1	death associated protein-like 1	15766	-0.633382142	-0.42332152	No
row_59	PRPS2	PRPS2	phosphoribosyl pyrophosphate synthetase 2	15827	-0.644233704	-0.4181648	No
row_60	EEF1E1	EEF1E1	eukaryotic translation elongation factor 1 epsilon 1	15857	-0.648591697	-0.4113078	No
row_61	INPP4B	INPP4B	inositol polyphosphate-4-phosphatase, type II, 105kDa	15986	-0.673618138	-0.40937668	No
row_62	UBE2H	UBE2H	ubiquitin-conjugating enzyme E2H (UBC8 homolog, yeast)	16554	-0.794251204	-0.4291634	Yes
row_63	RAB3IP	RAB3IP	RAB3A interacting protein (rabin3)	16744	-0.841588676	-0.4282929	Yes
row_64	RPS14	RPS14	ribosomal protein S14	16751	-0.84301734	-0.4176998	Yes
row_65	RAPGEF4	RAPGEF4	Rap guanine nucleotide exchange factor (GEF) 4	16777	-0.849250078	-0.40803358	Yes
row_66	TRIB2	TRIB2	tribbles homolog 2 (Drosophila)	16878	-0.874607146	-0.40201622	Yes
row_67	TCP11L2	TCP11L2	t-complex 11 (mouse) like 2	17003	-0.911212921	-0.39679775	Yes
row_68	CCNT1	CCNT1	cyclin T1	17122	-0.946210921	-0.39080814	Yes
row_69	KLHDC2	KLHDC2	kelch domain containing 2	17130	-0.947930574	-0.37891015	Yes
row_70	EIF3H	null	null	17334	-1.021437287	-0.37645426	Yes
row_71	RPL15	RPL15	ribosomal protein L15	17431	-1.054984808	-0.36789015	Yes
row_72	UXT	UXT	ubiquitously-expressed transcript	17544	-1.100017428	-0.35959163	Yes
row_73	EMG1	EMG1	EMG1 nucleolar protein homolog (S. cerevisiae)	17854	-1.251248837	-0.3597822	Yes
row_74	COX4I1	COX4I1	cytochrome c oxidase subunit IV isoform 1	17856	-1.251396775	-0.34363824	Yes
row_75	RANGRF	null	null	17993	-1.332128406	-0.33360818	Yes
row_76	CYB5A	CYB5A	cytochrome b5 type A (microsomal)	18277	-1.537228465	-0.32871854	Yes
row_77	SELL	SELL	selectin L (lymphocyte adhesion molecule 1)	18346	-1.622110724	-0.31132928	Yes
row_78	SATB1	SATB1	special AT-rich sequence binding protein 1 (binds to nuclear matrix/scaffold-associating DNA's)	18441	-1.738813519	-0.29380825	Yes
row_79	CCR7	CCR7	chemokine (C-C motif) receptor 7	18540	-1.883787751	-0.27462292	Yes
row_80	DPH5	DPH5	DPH5 homolog (S. cerevisiae)	18602	-1.983968496	-0.2521789	Yes
row_81	ST13	ST13	suppression of tumorigenicity 13 (colon carcinoma) (Hsp70 interacting protein)	18665	-2.091876268	-0.22839125	Yes
row_82	IFNGR2	IFNGR2	interferon gamma receptor 2 (interferon gamma transducer 1)	18693	-2.151143551	-0.20198052	Yes
row_83	ITGA6	ITGA6	integrin, alpha 6	18759	-2.333361864	-0.17522638	Yes
row_84	PDK1	PDK1	pyruvate dehydrogenase kinase, isozyme 1	18859	-2.859877586	-0.14346042	Yes
row_85	LSM7	LSM7	LSM7 homolog, U6 small nuclear RNA associated (S. cerevisiae)	18862	-2.890331745	-0.1061566	Yes
row_86	LEF1	LEF1	lymphoid enhancer-binding factor 1	18933	-4.036038399	-0.057629675	Yes
row_87	ACTN1	ACTN1	actinin, alpha 1	18942	-4.497610569	1.59E-04	Yes

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