Supplemental data:

1. Primers for PCR to amplify hairpin stem-loop precursor mir-145 plus different flanking sequence from human genomic DNA.

Strategy#1: 20nt at both sides:
#1_BglII-Fd primer : 5'-gga agatct CGCTGAAGGCCACTCGCTCC
#1_HindIII-Rs primer : 5'-ccc aagctt GGAGGCAAATCCAGCTGTGA
The resulting clones are called pSuper-hairpin145_20nt (clone #26).

Strategy#2: 40nt at both sides:
#2_BglII-Fd primer : 5'-gga agatct TAGGGACACGGCGGCCTTGG
#2_HindIII-Rs primer : 5'-ccc aagctt GGGCAACTGTGGGGTGGGAA
The resulting clones are called pSuper-hairpin145_40nt (clone #28).

Strategy#3: 80nt at both sides:
#3_BglII-Fd primer : 5'-gga agatct AGAGAACTCCAGCTGGTCCT
#3_HindIII-Rs primer : 5'-ccc aagctt CCAGCCGAGGCCCCATTGGG
The resulting clones are called pSuper-hairpin145_80nt (clone #30).

Strategy#4: predicted endogenous promoter of human miR145 included:
Primers were designed to PCR hairpin pre-mir-145 from human genomic DNA including the predicted promoter at 3' and 160bp at 5' of pre-miR145 sequence.
#4_BglII-Fd primer: 5'-gga agatct ATCTGCCTTCAAATCCATGT
#4_HindIII-Rs primer: 5'-ccc aagctt ATAGACACGATGGAAAGAAA
The resulting clones are called pSuper-hairpin145_160nt (clone #32).

Strategy#5: The mature miR145 (24nt) was also directly cloned into pSuper by annealing the top and bottom strands of the following sequences synthesized by IDT.
pSuper_Top oligo: 5'- gatctGTCCAGTTTT CCCAGGAATCCCTTa
pSuper_Bottom: 5'- agcttAAGGGATTCCTGGGAAAACTGGACa
The resulting clones are called pSuper-mature145_24nt (clone #18).

2. The potential binding sites of miR145 on 3'UTR of human IRS1 were cloned into multi-clonal sites (MCS) of a dual luciferase vector psiCHECK2 (Promega). The top and bottom strands for each binding site (miR145 site #1 or #2) or both (miR145 sites(1+2)) with XhoI and NotI at 5' and 3' end respectively are listed as follows:

miR145 site #1_Sense:
tcgagAGCCAGAGGACCGTCAG**TAGCTCAACTGGAC**ATCACAGCAGAATGAAGACCgc
.
miR145 site#1_AntiSense:
ggccgcGGTCTTCATTCTGCTGTGATGTCCAGTTGAGCTACTGACGGTCCTCTGGCTc

miR145 site#2_Sense:
tcgagTTTACTTTATCCAATCC**TCAGGATTTCATTGACTGAACTGCAC**GTTCTATATTGTGC
CAgc.
miR145 site #2_AS:

ggccgcTGGCACAATATAGAACGTGCAGTTCAGTCAATGAAATCCTGAGGATTGGATAA
AGTAAAc

miR145 site (1+2)_Sense:
tcgagCCAATCC**TCAGGATTTCATTGACTGAACTGCAC**GTTCTATATGTGCCAA**CTCA
ACTGGAC**ATCACc.
miR145 site (1+2)_AS:
ggccgcGTGATGTCCAGTTGAGTTGGCACATATAGAACGTGCAGTTCAGTCAATGAAAT
CCTGAGGATTGc

The synthesized sense and anti-sense oligos were annealed to form double-strand oligos and cloned into psiCHECK2 cut with XhoI and NotI.

The following primers were designed to RT-PCR the 3'UTR of human IRS1 from total RNA extracted from HCT116 cells. This RT-PCR product is about 1kb, which covers the entire 3'UTR of IRS1 mRNA.

XhoI-3UTR primer: ccgCTCG**AG**CTCAACTGGACATCACAGCAG
NotI-3UTR-primer: ttGCGGCCGCTAAAAGATCAACAGTATCTAGTTTA

The corresponding clones were called psiCHECK2-145site#1(clone #81), psiCHECK2-145site#2 (clone #83), psiCHECK2-145site(1+2) (clone #85), and psiCHECK2-entire3UTR_1kb (clone #75).

Additional Supplemental Data
Fig. 1.Phosphorylation of Akt and ERKs is normal in miR145-treated cells. miR neg are HCT116 cells treated with an unrelated oligo, miR145 are cells treated for 48 hrs with miR145 oligos. The time in the figure refers to stimulation with IGF-1 (20 ng/ml). Cells in 10% serum were growing exponentially, when IGF-1 was added. Akt and ERKs activation is normal because IRS-2 is expressed at normal levels (see Fig. 2).

Fig. 2. IRS-1 and IRS-2 levels in miR145-treated HCT116 cells. The levels of the 2 IRS proteins were determined 96 hrs after treatment with miR145 oligo, or an unrelated oligo (miR neg). Western blots with specific antibodies. Actin monitors the amount of protein in each lane. IRS-2 levels are not decreased by miR145. The database in fact indicates that IRS-2 is not among the targets of miR145.

Fig. 3. c-myc levels are decreased in HCT116 cells treated with miR145 oligos. Stimulation with IGF-1 induces c-myc expression within 30-60 min in mouse embryo fibroblasts (Reiss et al. 1998). Lysates of cells in 10% serum or in SFM 60 min after IGF-1 stimulation (20 ng/ml). Western blots with anti-c-myc antibody. IGF-1 increases the expression of c-myc in miR neg cells, but not in cells treated with miR145 oligos. These experiments indicate that IRS-2 can replace IRS-1 in the activation of ERKs and Akt, but not in the expression of c-myc, which is the first step in cell cycle progression.

Fig. 1

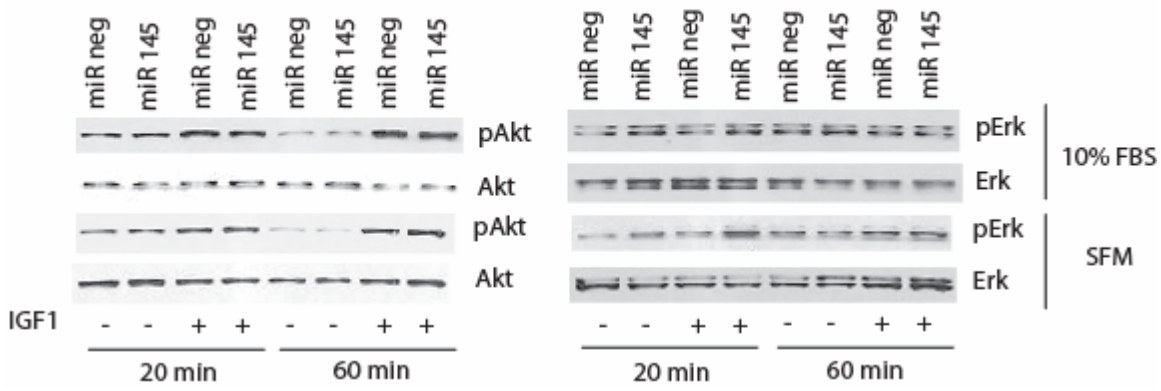

miR neg
miR 145
miR neg
miR 145
miR neg
miR 145
miR neg
miR 145
pAkt
Akt
pAkt
Akt
IGF1
- - + + - - + +
20 min
60 min
miR neg
miR 145
miR neg
miR 145
miR neg
miR 145
miR neg
miR 145
pErk
Erk
pErk
Erk
10% FBS
SFM
- - + + - - + +
20 min
60 min

Fig. 2

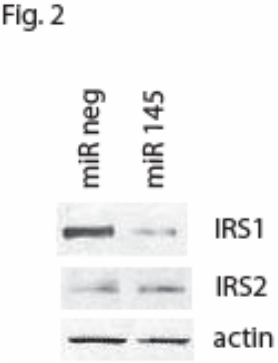
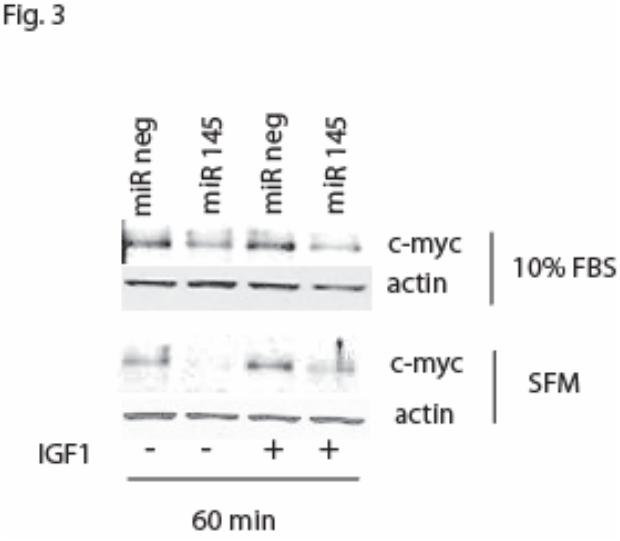


Fig. 3


miR neg
miR 145
miR neg
miR 145
c-myc
actin
10% FBS
c-myc
actin
SFM
IGF1
-
-
+
+
60 min