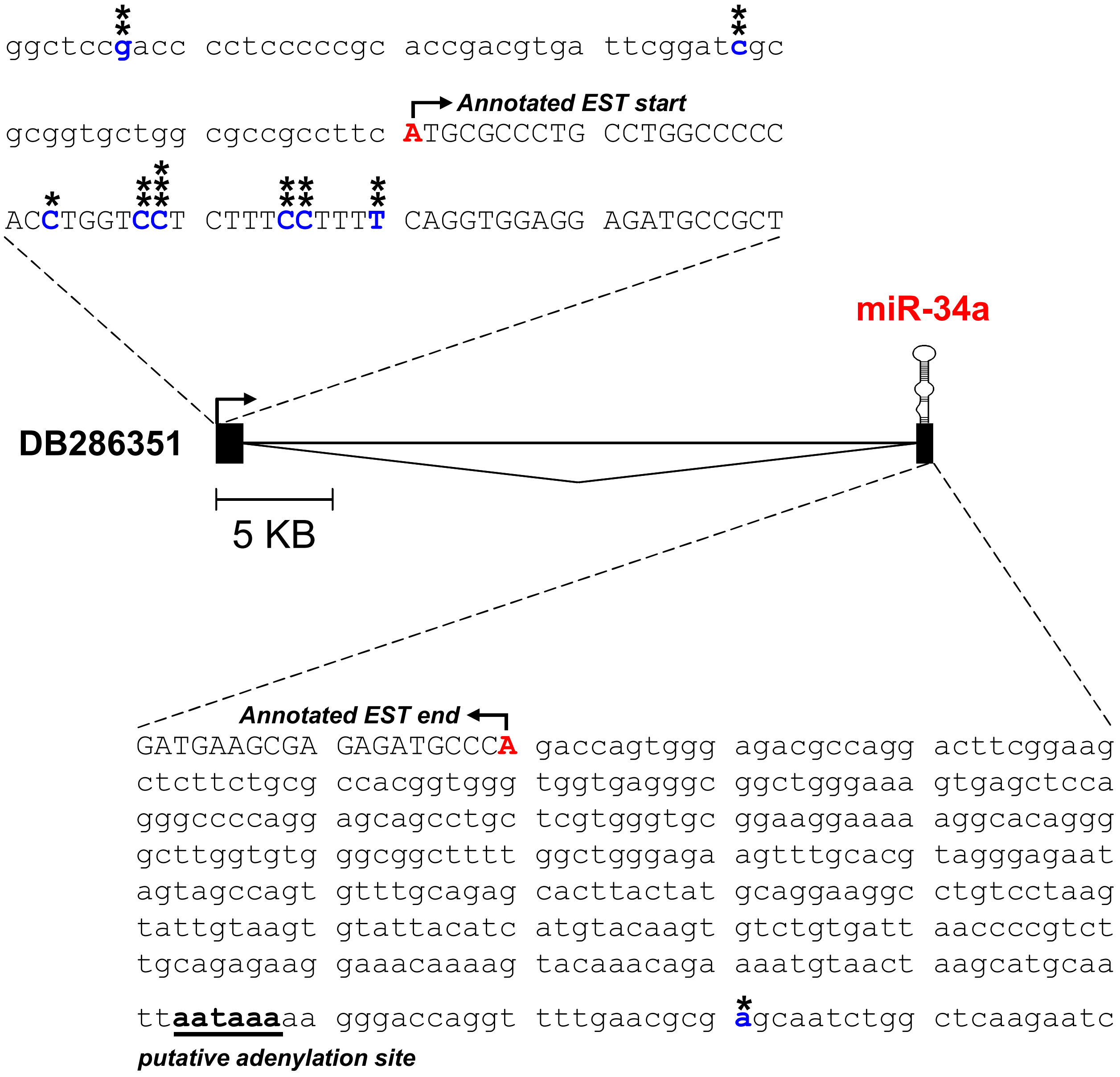
Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis

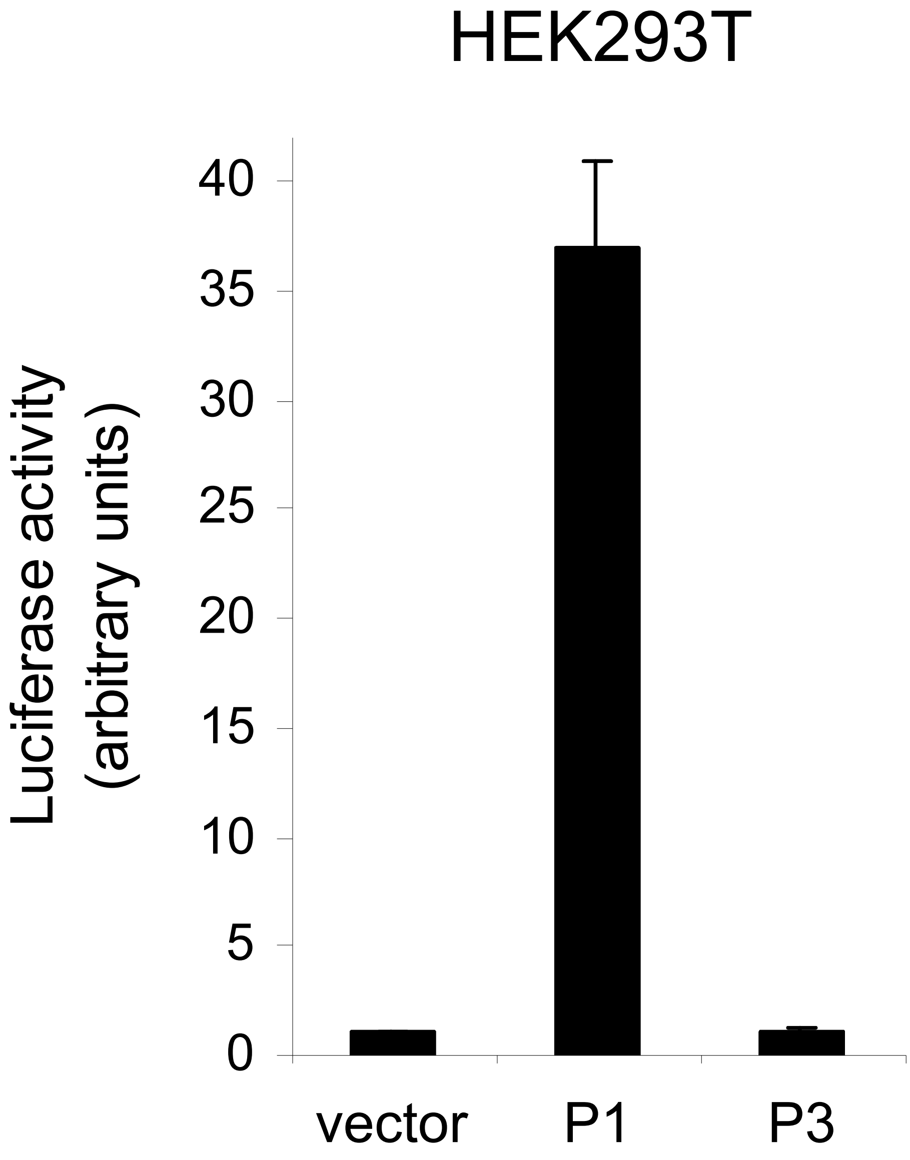
Supplemental Material

Tsung-Cheng Chang[1,5], Erik A. Wentzel[1,5], Oliver A. Kent[1], Kalyani Ramachandran[1], Michael Mullendore[2], Kwang Hyuck Lee[2], Georg Feldmann[2], Munekazu Yamakuchi[4], Marcella Ferlito[4], Charles J. Lowenstein[3,4], Dan E. Arking[1], Michael A. Beer[1], Anirban Maitra[1,2,3], and Joshua T. Mendell[1*]

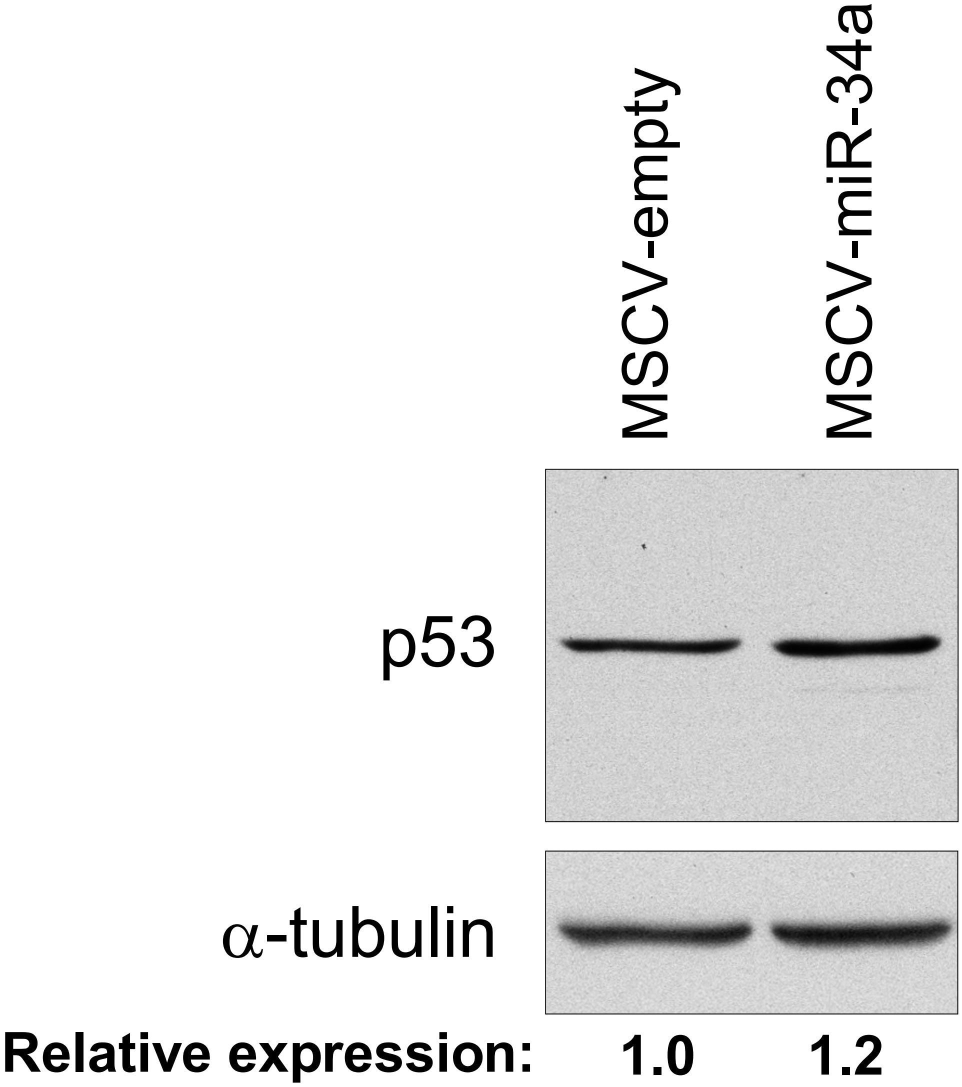
Contents:



Supplemental Figure 1. Characterization of the miR-34a primary transcript RACE was performed to map the 5' and 3' ends of human EST DB286351, which contains miR-34a. 5' RACE results are shown in the upper panel with each asterisk indicating the position of the 5' end of an individual RACE clone. 3' RACE results are depicted in the lower panel. All transcripts terminated at the nucleotide indicated with an asterisk.



Supplemental Figure 2. Activity of promoter constructs in HEK293T cells
Error bars represent standard deviations from three independent transfections each measured in triplicate.



Supplemental Figure 3. Western blot showing p53 levels in retrovirally-infected cell populations.

Supplemental Table 1. Genomic Alterations in Pancreatic Cancer Cell Lines

Cell line	p53 status	1p36 loss	miR-34a expression
BxPc3	bi-allelic loss[a]		undetectable
Panc 04.14	WT[b]	Yes	5-fold reduced
MiaPaCa2	bi-allelic loss[a]		undetectable
Panc1	bi-allelic loss[a]		undetectable
Panc 08.13	unknown	Yes	undetectable
PK8	bi-allelic loss[c]		undetectable
Panc02.03	unknown		3-fold reduced
PK9	bi-allelic loss[c]		2-fold reduced
Panc 02.13	unknown	Yes	undetectable
AsPc1	bi-allelic loss[a]		undetectable
E3LZ10.7	WT[b]		undetectable
CAPAN1	bi-allelic loss[a]		2-fold reduced
Panc 06.03	unknown		10-fold reduced
Su86.86	bi-allelic loss[a]		10-fold reduced
L3.6PL	bi-allelic loss[d]		10-fold reduced

[a] http://pathology2.jhu.edu/pancreas/geneticsweb/Profiles.htm; [b] unpublished observations
[c] Duda et al. (2002). Br. J. Ca. 86:490-496; [d] Keping Xie, personal communication

Supplemental Table 3.

Primer sequences for miR-34a promoter cloning

Amplicon	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
IRES	ATACCGAGATCTGACCCCATGCATCGAATTCC	ATACCGAGATCTTATCATCGTGTTTTTCAAAGG
P1	ATACCGCTCGAGCTTCCCTGTCCTCCTCCTA	ATACCGCTCGAGGAATCCTTTCTCCCCAGAG
P2	ATACCGCTCGAGCTTCCCTGTCCTCCTCCTA	ATACCGCTCGAGAGCAGGTAGTGCAGGCTTC
P3	ATACCGCTCGAGCTTCCCTGTCCTCCTCCTA	ATACCGCTCGAGATGAAGGCGGCGCCAGCA
P4	ATACCGCTCGAGCGCCCTGCCTGGCCCCCAC	ATACCGCTCGAGGAATCCTTTCTCCCCAGAG
P5	ATACCGCTCGAGCGCCCTGCCTGGCCCCCAC	ATACCGCTCGAGAGCAGGTAGTGCAGGCTTC
P6	ATACCGCTCGAGCCCCGTCCCAGCATGCACC	ATACCGCTCGAGGAATCCTTTCTCCCCAGAG
P1^{mut}	GGCTCTGCCTGGGTTTACCTGGGTTTATTCCGAGCCGGGC	GCCCGGCTCGGAATAAACCCAGGTAAACCCAGGCAGAGCC

Primer sequences for characterization of the miR-34a primary transcript

Amplicon	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
5' RACE	CGACTGGAGCACGAGGACACTGA	CGAGCAGGTAGTGCAGGCTTCCAG
5' nested RACE	GGACACTGACATGGACTGAAGGAGTA	CAAAATCTCCAAATGCCCCCGATCT
3' RACE	ACCGGCCAGCTGTGAGTGTTTCTTT	GCTGTCAACGATACGCTACGTAACG
3' nested RACE	TGAGCAATAGTAAGGAAGCAATCAGCAA	CGCTACGTAACGGCATGACAGTG
pri-miR-34a	CCACCTGGTCCTCTTTCCTT	TTCAAAACCTGGTCCCTTTTT

Primer sequences for cloning the miR-34a retrovirus

miRNA	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
miR-34a	ATACCGCTCGAGCCTCCTGCATCCTTTCTTT	ATACCGCTCGAGCCTGTGCCTTTTTCCTTCC

F) Supplemental Table 2 Legend

Supplemental Table 2. Transcripts exhibiting altered expression in miR-34a-expressing cells

Affymetrix gene expression profiling of HCT116 *p53*^{WT} cells infected with empty virus or miR-34a virus was performed as described in Experimental Procedures. The fold change column indicates the level of expression in miR-34a-expressing cells relative to control cells.