Supporting Information

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SI Text

Cell Culture. HeLa and HEK293 cells (American Type Culture Collection, Manassas, VA) were cultured in DMEM media (Invitrogen) supplemented with 10% FBS. HCT116 (p53 wt and p53 knockout) cells (Bert Vogelstein, The Johns Hopkins University School of Medicine) were cultured in McCoy’s 5A media supplement with 10% FBS.

Northern blot analysis. Total RNA were extracted from cells by using TRIzol (Invitrogen). Human tissue RNAs were obtained from Applied Biosystems. For Northern blot analysis of miRNA, 10 μg of each RNA were loaded onto 15% TBU-gel (Invitrogen), transferred to a nitrocellulose membrane, and hybridized with 32P-end-labeled probes specific for miR-34a or scrambled oligonucleotide at 42°C for 16 h. The miR-34a probe, 5′-AACAACCAGCTAAGACTGCCA-3′ was synthesized by Integrated DNA Technologies. All other reagents were purchased from Applied Biosystems.

Western blot analysis. Antibodies to acetylated p53 were from Cell Signaling Technology. All other primary antibodies and appropriate secondary antibodies were from Santa Cruz.
miR-34a

Total RNA

Brain  Lung  Liver  Kidney  Testis  Sk.Muscle  Thymus  Heart  Spleen  Bladder  Ovary  Adipose

Fig. S1. Expression of miR-34a in human tissues by Northern blot analysis.
miR-34a → SIRT1
p53
p53-Ac

DNA damage

Gene transcription,
Cell cycle arrest,
Apoptosis

Fig. S2. Schematic of proposed mechanism for miR-34a regulation of SIRT1 and p53 signaling.