Supporting information

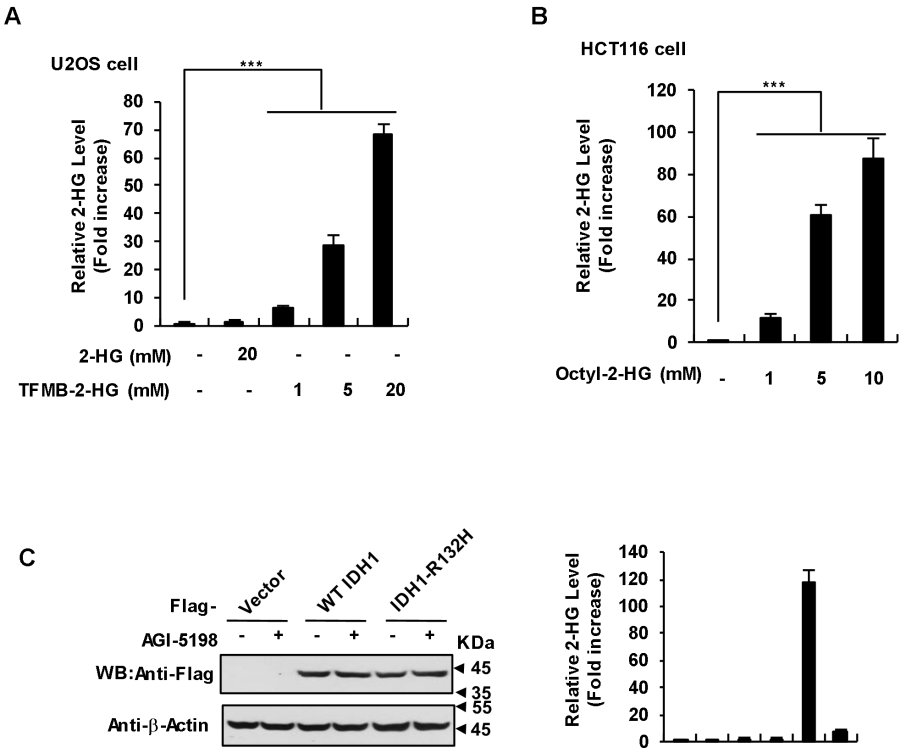

Figure S1. LC-MS analysis of intracellular 2-HG level. (A) U2OS cells were treated with various concentrations of TFMB-2-HG indicated for 8h. Cells were washed with PBS three times, the cell extracts were subjected to LC-MS for analysis of relative 2-HG levels. Error bars show the standard deviation of three independent experiments (***p<0.001, unpaired Student's t test). **(B)** HCT116 cells were treated with various concentrations of Octyl-2-HG indicated for 8h. Results were presented as in (A). **(C)** HCT116 cells were infected with control lentivirus or lentivirus expressing Flag-WT IDH1 or IDH1-R132H. At 48 hr post-infection, cells were treated with or without 1.5 μM AGI-5198 for 2 days, followed by measurement of 2-HG levels by LC-MS. Left: WB to detect the indicated proteins. Right: Quantitation of 2-HG levels using LC-MS. Data are shown as means ± S.D. (n=3).

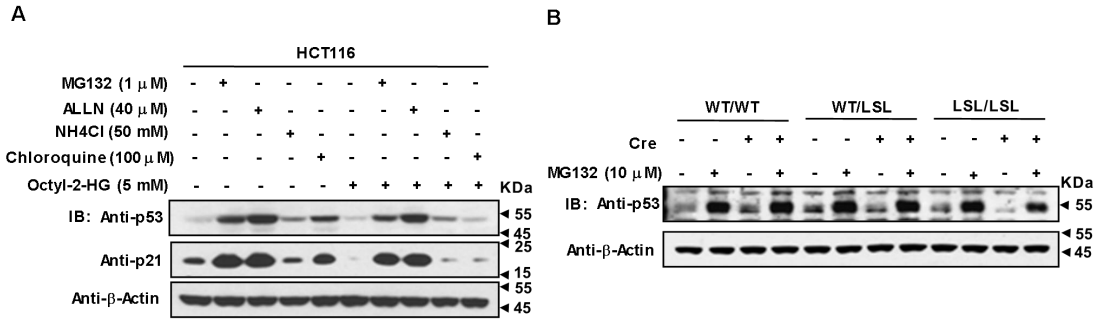

Figure S2. Effect of protease inhibitors on p53 protein level. (A) Western blot to detect p53 in HCT116 cells that were treated with Octyl-2-HG alone or in combination with the proteosome inhibitor MG132, the calpain inhibitor ALLN, or the lysosome inhibitors NH4Cl and chloroquine for 8 hr. **(B)** WB determination of p53 protein level in MEF treated with or without proteosome inhibitor MG132 for 8 hr.

A

miRNA ID	log2Ratio (WT/R132Q)	Up-Down Regulation (WT/R132Q)	P-value
miR-125b-1	-0.014165224	Down	0.595268
miR-504	-0.514573173	Down	0.740976
miR-25	0.506959989	Up	0.26365
miR-30d	0.000852898	Up	0.913272
miR-380-5p	-1.563223842	Down	6.93E-13

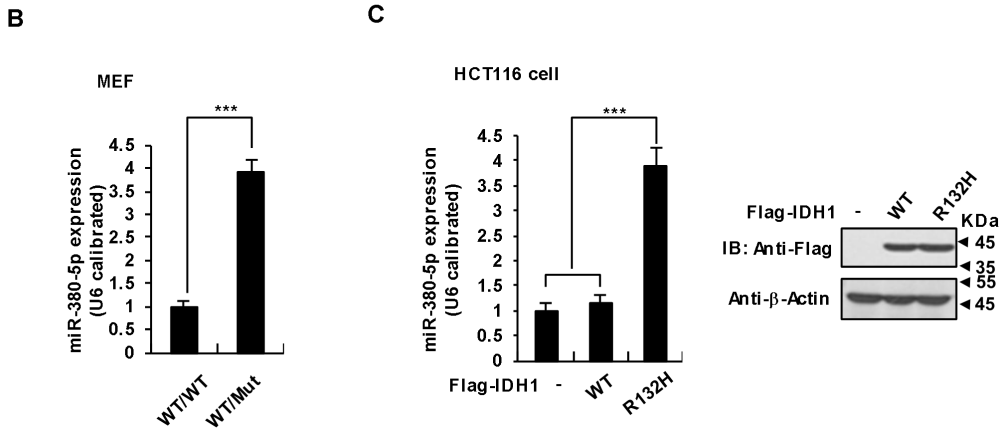

Figure S3. miR-380-5p were dramatically upregulated in IDH1 mutant cells. (A) The expression of multiple miRNAs in liver samples from IDH1 WT and mutant mice determined by a sequencing-based RNA profiling analysis. **(B)** The expression of miR-380-5p in MEF with indicated genotypes were analyzed with qRT-PCR and normalized to U6, mean±SD of three independent experiments is shown (***p<0.001, unpaired Student's *t* test). **(C)** HCT116 cells were transfected with Flag-tagged wildtype (WT) IDH1 or its R132H mutant. The expression of miR-380-5p is shown as in (B).

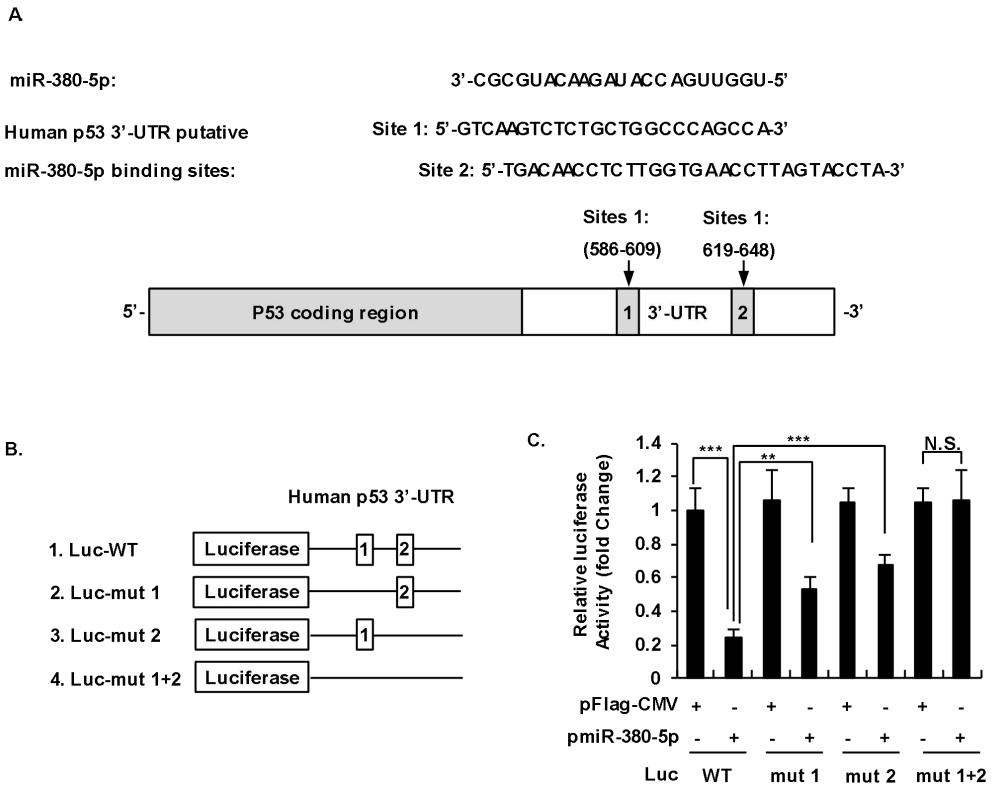

Figure S4. Two miR-380-5p binding sites mediate the downregulation of p53 protein expression. (A) Sequence of miR-380-5p and its putative binding sites in human p53 3′ UTR. **(B)** Luciferase reporter constructs containing wildtype p53 3′ UTR or its mutants with either one or both of the putative binding sites deleted. **(C)**The miR-380-5p binding sites 1 and 2 in human p53 3′ UTR are responsible for its downregulation. HCT116 cells were transfected with luciferase constructs described in (B) together with miR-380-5p expression plasmid or control pFlag-CMV vector. 24 hr after transfection, luciferase activities were measured. Data shown are means±SD of three independent experiments (N.S., not significant, $^{**}p<0.01$, $^{***}p<0.001$, unpaired Student's *t* test).

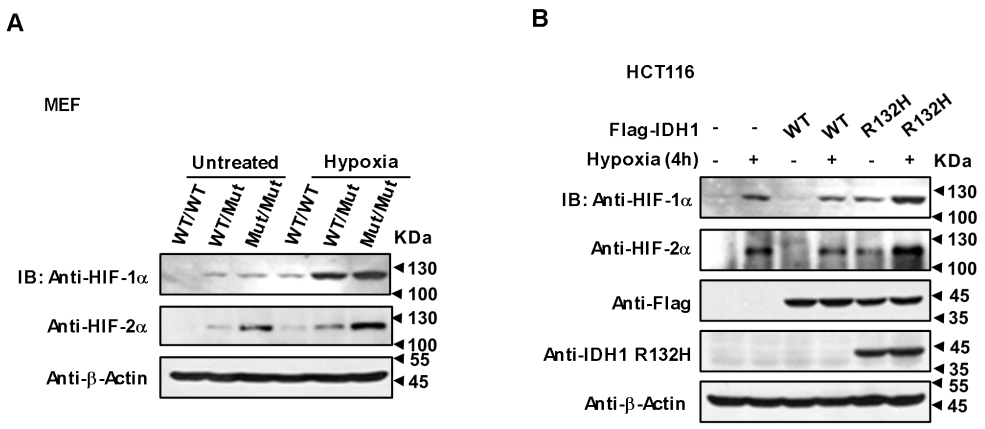

Figure S5. HIFs were dramatically upregulated in IDH1 mutant cells. (A) WB determination of HIF protein levels in MEF of the indicated genotypes under both normoxia and hypoxia condition. **(B)** WB determination of HIF protein levels in HCT116 cells transfected with Flag-tagged WT IDH1 or its R132H mutant under both normoxia and hypoxia condition.

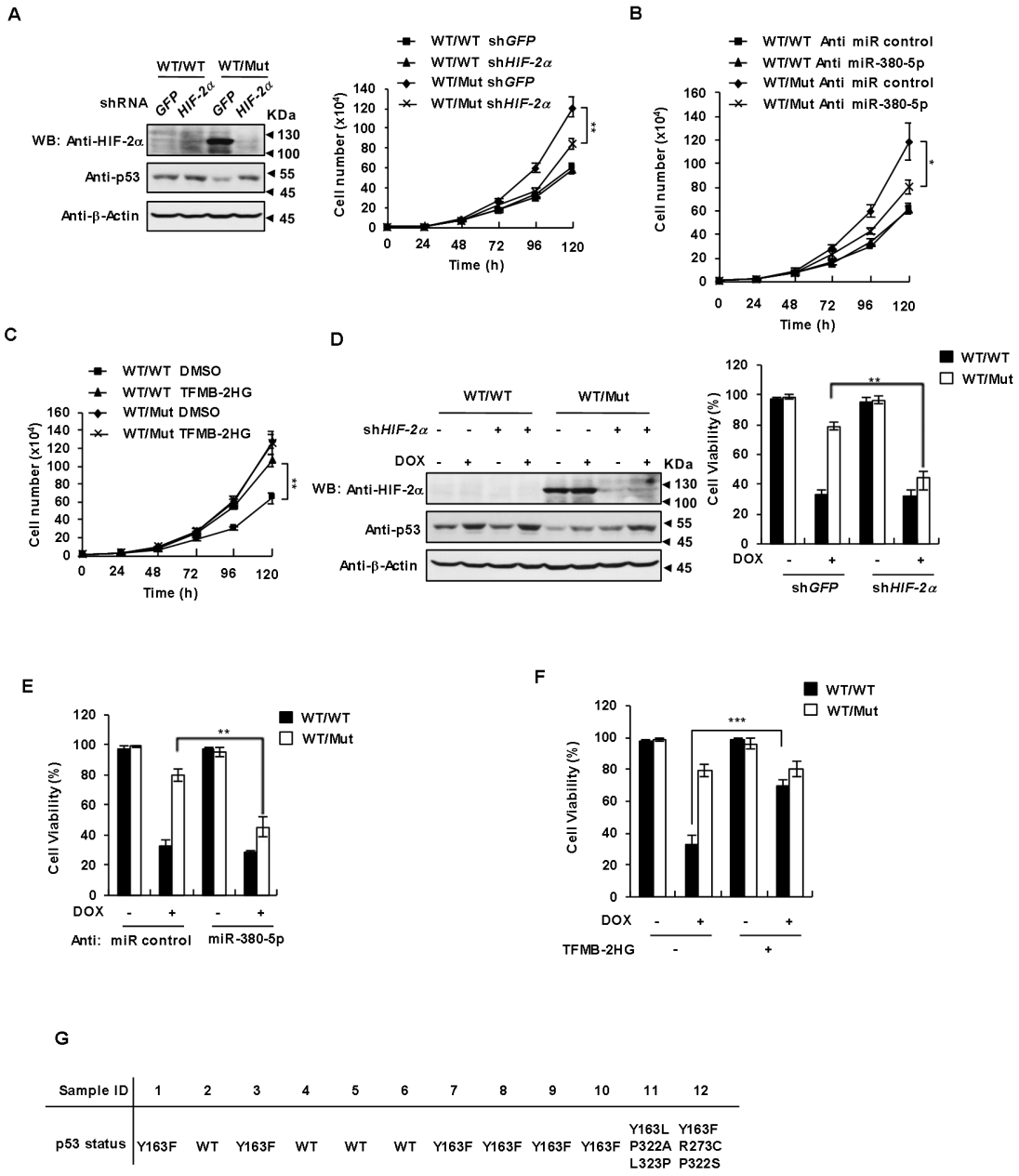

Figure S6. p53 down regulation is involved in IDH1 mutant driven tumorigenesis. (A) Knockdown of *HIF-2α* significantly attenuated proliferation rate of IDH1 R132 mutant cells. Both $IDH1^{WT/WT}$ and $IDH1^{WT/Mut}$ cells were infected with lentiviruses expressing *HIF-2α* shRNA or control shRNA. At 36 hr after infection, proliferation rates were determined by growth curves (Right panel). Means±SD, n=3 independent experiments, are shown (**p<0.01, unpaired Student's t test). Proteins in total cell lysates of the same cell lines were determined by WB (Left panel). **(B)** Inhibition of miR-380-5p markedly suppressed proliferation rate of IDH1 R132 mutant cells. Both $IDH1^{WT/WT}$ and $IDH1^{WT/Mut}$ cells were transfected with chemically synthesized single stranded Anti-miR-380-5p oligonucleotides. 48 hr after transfection, proliferation rates were determined by growth curves (Right panel). Data are presented as in A (*p<0.05, unpaired Student's t test). **(C)** 2-HG dramatically increased proliferation rate of $IDH1^{WT/WT}$ cells but not $IDH1^{WT/Mut}$ cells. $IDH1^{WT/WT}$ and $IDH1^{WT/Mut}$

MEFs were cultured with or without 20 mM TFMB-2-HG. Proliferation rates were determined as in (A) (**p<0.01, unpaired Student's t test). **(D)** *HIF-2α* Knockdown sensitizes IDH1 R132 mutant cells to DOX-induced apoptosis. $IDH1^{WT/WT}$ and $IDH1^{WT/Mut}$ MEF cells were infected with lentiviruses expressing *HIF-2α* shRNA or control shRNA. At 36 hr after infection, cells were treated with or without 2.5 μM DOX for 16 hr. The percentages of surviving cells (Annexin V negative) were determined by a flow cytometer. Data are presented as means±SD of three independent experiments (**p<0.01, unpaired Student's t-test). **(E)** Inhibition of miR-380-5p efficiently antagonizes the insensitivity of $IDH1^{WT/Mut}$ MEFs to DOX induced apoptosis. Both $IDH1^{WT/WT}$ and $IDH1^{WT/Mut}$ cells were transfected with chemically synthesized single stranded Anti-miR-380-5p oligonucleotides. 48 hr after transfection, cells were treated with 2.5 μM DOX for another 16 hr. The percentages of surviving cells were determined as in (D). **(F)** 2-HG desensitized $IDH1^{WT/WT}$ cells rather than $IDH1^{WT/Mut}$ cells to DOX-induced apoptosis. $IDH1^{WT/WT}$ and $IDH1^{WT/Mut}$ MEFs were treated with 2.5 μM DOX together with or without 20 mM TFMB-2-HG. The percentages of surviving cells were determined as in (D) (***p<0.001, unpaired Student's t test). **(G)** Sanger sequencing result showing the p53 genotype in glioma samples used in Figure 7E.