S7: Additional material and methods

Extraction from tumor cells, frozen tumor tissue and formalin fixed paraffin embedded (FFPE) tumor tissue

DNA was extracted from cell lines and frozen tumor tissue using the DNA Mini Kit (Qiagen, Courtaboeuf, France) according to the manufacturer's instructions. Tumor DNA from FFPE samples was extracted from 10 sections of 20 µm thickness using the iPrep ChargeSwitch® Forensic Kit (Life Technologies). Extracted DNA was quantified using Nanodrop® spectrophotometer and qualified by migration on 1% agarose gel.

***MDM2* gene copy number and *TP53* mutational statuses**

MDM2 status was determined using three different platforms. For FFPE samples, genomic DNA was first fragmented and labeled by random priming, Cy3 for germline DNA and Cy5 for tumor DNA. Labeled DNA was hybridized on Agilent 4x180K DNA microarrays. For frozen samples and cell lines, DNA was analyzed on SNP-array outsourced to Integragen (Evry, France). Two hundred and fifty nanograms of DNA were run upon Infinium Illumina Human 610-Quad SNP array (Illumina, Essex, UK). Extracted data using Feature Extraction software were imported and analyzed using Nexus 6.0 (Biodiscovery, Hawthorne, USA). Two different Taqman copy number assays (Applied Biosystem, Life Technology, Saint-Aubin, France) were also used to quantify regions within *MDM2* introns 3 and 9. Gene copy number data was normalized over that of RNase P reference assay.

TP53 sequencing was performed on tumor and blood DNA by outsourcing to Integragen (Evry, France). Target enrichment was performed using Capture Agilent SureSelect All Exon v5 (targeting coding regions only) and UTR 70Mb (targeting coding and UTR regions). Three microgram of DNA was processed for paired-end sequencing on Illumina HiSeq2000 platform which generated 2 x 75 base pair reads. Analysis was performed using CASAVA 1.8 software.

Cell RNA extraction and Reverse Transcriptase-quantitative Polymerase Chain Reaction (RT-qPCR)

Total RNA extraction was performed using RNeasy Lipid Tissue Mini kit (Qiagen) according to the manufacturer's instructions. RNA quality was assessed on Bioanalyzer System (Agilent Technologies, Paolo Alto, USA) using RNA Nano Chips.

First Strand cDNA Synthesis Kit (Fisher Scientific, Illkirch, France) was used to generate cDNA. Gene expression was measured by quantitative PCR using

LightCycler® 480 Probes Master and RealTime Ready RT-qPCR kits for MDM2, MDM4, TP53, and PPIA (Roche, La Rochelle, France), which contains primers and Universal Probe Library™ probes specific to each genes. Real time-qPCR cycling conditions consisted of an initial denaturation step at 95°C for 10 min; 45 cycles of 95°C for 10s, 60°C for 30s and 72°C for 1s; and one cycle of 40°C for 30s. The reactions were carried out using a LightCycler® 480 (Roche). Gene expression was normalized over PPIA (human peptidylprolyl isomerase A) expression. The $2^{-\Delta CT}$ method was used to compare the relative expression of MDM2, MDM4, and TP53, mRNA among the cell lines, where $\Delta CT = CT_{\text{target gene}} - CT_{PPIA}$.

Western blot

Tumor cells were harvested and total protein extraction was done using RIPA buffer (Pierce, Brebieres, France) supplemented with EDTA and Phosphatase and Protease Inhibitor cocktail. Equal amounts of protein (20-30 µg) were run on 4-12% Bis-Tris gels (Life Technologies) at 150 volts. Proteins were transferred on nitrocellulose membranes (Sigma) by liquid transfer at 300 mAmp or using BioRad semi dry transferring device at 110 V for 1 hour. Fixation sites were blocked overnight with SuperBlock Blocking Buffer in TBS (Pierce) or 5% milk/PBS and the blots were labeled using the following primary antibodies: anti-MDM2 (#ab16895, Abcam, Paris, France; dilution 1:200 or #sc-965, Santa Cruz, Dallas, USA; dilution 1:1000), anti-p53 (#2524, Ozyme, Montigny-le-Bretonneux, France; dilution 1:1000 or #MA5-14516, Thermo Scientific; dilution 1:100 or #9282, Cell Signaling, Danvers, USA; dilution 1:1000), anti-β-actin (#ab8226, Abcam; dilution 1:5000 or #A2228, Sigma; dilution 1:1000), anti-p21 (#2947, Ozyme; dilution 1:1000) diluted in SuperBlock Blocking Buffer in TBS (Pierce) or 5% milk/PBS. The secondary antibodies used were Odyssey IRDye goat anti-rabbit or mouse secondary antibodies (Science-Tec, Courtaboeuf, France) diluted 1:5000 in SuperBlock Blocking Buffer in TBS (Pierce), or goat anti rabbit-HRP 1:10000 (Zymed, South San Francisco, USA) and rabbit anti Mouse-HRP 1:2000 (EnVision™ kit, Dako, Carpinteria, USA) diluted in 5% milk/PBS. Blots were scanned and quantified on the Odyssey CLx (Science-Tec) or revealed using HyGlo solution (Denville Scientific, South Plainfield, USA). Quantification values were normalized to the corresponding β-actin band. Each western blot figure is representative of three independent experiments and the numbers below each band represent the average of quantification values +/- SEM of all three experiments.

Immunostaining in tumor or tissue cells

Cells were plated on poly-D-lysine and laminin pre-coated culture slides (BD bioscience, Le Pont de Claix, France). The day after, cells were fixed with paraformaldehyde 4% (Euromedex, Strasbourg, France). Fixation sites were blocked with SuperBlock Blocking Buffer in TBS (Pierce) and cells were stained with anti-p53 (#2524, Ozyme; dilution 1:2000) and anti-p21 (#2947, Ozyme; dilution 1:400) primary antibodies, and Alexa Fluor® Goat anti-rabbit 488 and Goat anti-mouse 594 diluted 1:200 in SuperBlock Blocking Buffer in TBS (Pierce). Cells were counterstained with Dapi (Thermo Scientific, Villebon sur Yvette, France). Quantification was done using ImageJ 1.49g (National Institute Health, USA) by determining the area of positive pixels over the total number of nuclei.

For all immunofluorescence imaging, the brain of terminated mice was harvested, sectioned, fixed by acetone/methanol 1:1 and stained as described above using the following primary antibodies: anti-p21 (#MA5-14949, Thermo Scientific; dilution 1:200), anti-p53 (#MA5-14516, Thermo Scientific; dilution 1:100) and anti-MDM2 (#PA527209, Thermo Scientific; dilution 1:200). Sections were counterstained with Dapi (Thermo Scientific), or propidium iodide (Sigma) for Hoechst-stained sections only.

For immunohistochemistry on intracranial tumors, brains were harvested, frozen and sectioned. Sections were fixed with cold (-20°C) acetone. For subcutaneous tumors, tissue was harvested, fixed in 4% paraformaldehyde (Affymetrix, Santa Clara, USA) and embedded in paraffin (Fisher). Deparaffinized sections were subjected to antigen retrieval with 1 mM Na citrate (Sigma). Blocking was done using peroxidase blocking reagent (Dako, Carpinteria, USA) and normal goat serum (Jackson Immunoresearch, West Grove, USA). The following primary antibodies were incubated on sections overnight at 4°C: anti-Ki67 (# Ki67-MM1-CE, Leica; dilution 1:200), anti-MDM2 (# OP46, EMD Millipore ; dilution 1 :50), anti-p21 (# 556431, BD Biosciences; dilution 1:300), anti-p53 (# MA5-12571, Thermo Fisher; dilution 1:200), anti-cleaved caspase 3 (# 9661S, Cell Signalling; dilution 1:150). Rabbit and mouse secondary antibodies (K4001 and K4003, Dako) were incubated on sections for 1 h at room temperature. Staining was revealed with a DAB+ substrate chromogen kit (Dako), and sections were counterstained with hematoxylin.

For immunostaining on tumor tissue, quantification was done using ImageJ 1.49g and ImmunoRatio plugin (immunohistochemistry) or intensity threshold setting

(immunofluorescence) and by determining the area of positive pixels over the total number of nuclei.