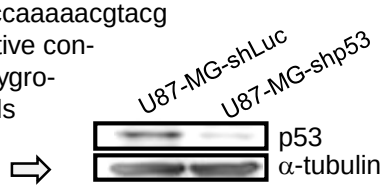
Supplemental Materials & Methods

Note: Chemicals and reagents were purchased from Sigma Aldrich if not otherwise specified.

Generation of p53-knockdown cell line. As previously detailed, U87-MG-shp53 cells were generated with an shRNA-mediated stable knockdown of p53 [20]. Here, an shRNA (shp53) according to Brummelkamp et al. [22] (upper strand: 5‘-gatccccgactccagtggtaatctacttcaagagagtagattaccactggagtc tttttggc−3‘, bottom strand: 5‘-tcgagccaaaaagactccagtggtaatctactctcttgaagtagattaccactggagtcggg-3‘) ligated into the self-inactivating retroviral Moloney murine leukemia virus (MoMuLV) backbone vector pRVH-1-Hygro containing an H1 polymerase III promoter for expression of shRNA was applied. A previously described RNA targeting sequence for luciferase mRNA (shLuc: upper strand: 5‘-gatcccccgtacgcggaa tacttcgattcaagagatcgaagtattccgcgtacgtttttggc-3‘, bottom strand: 5´-tcgagccaaaaacgtacg cggaatacttcgatctcttgaatcgaagtattccgcgtacgggg-3´) was included as negative control [21]. Transduced cells were selected and cultured using 300 µg/ml Hygromycin B (Life Technologies) for the generation of stable p53-deficient cells (shp53) and isogenic U87-MG cells with an shRNA targeting firefly luciferase (shLuc). Western blotting revealed efficient knockdown.



3-D spheroid culturing & handling. Single cell suspensions from exponentially growing monolayer cultures were prepared and $2x10^3$ (U87-MG-shLuc, U87-MG-shp53), $9x10^3$ (U138-MG) or $1.2x10^4$ (U251-MG) cells in 200 µl were seeded per well into 1.5% agarose-coated 96-well plates to obtain spheroids with a standard size of 370 - 400 µm after an initiation interval of 4 days. Spheroids were cultured under routine conditions in liquid overlay with the supernatant being refreshed every 2-3 days by exchanging 50% of the medium. For the exposure to arginine-deprived conditions, spheroids were transferred onto 96-well plates coated with 1.5% agarose dissolved in arginine-free MEM instead of standard medium (both without FCS). Spheroid dissociation for cell counting was achieved by exposure to trypsin/EDTA (0.05 %/0.02 % in PBS) for 10 - 30 min at room temperature and mild mechanic means.

Colony formation assays. *Note:* Two of the initial cell lines could be systematically studied in clonogenic survival assays; U138-MG cells did not form countable colonies.

Primary and secondary antibodies used in Western blot analyses.

Primary antibody	Manufacturer‘s data	Dilution
anti-ASS polyclonal rabbit IgG	Sigma Aldrich conc.: 0.1 g/l	1: 500
anti-ASL polyclonal rabbit IgG	Sigma Aldrich conc.: 0.2 g/l	1: 1000
anti-a-Tubulin, clone DM1A monoclonal mouse IgG1	Merck Millipore conc.: 1 g/l	1: 10000
anti-p53 polyclonal goat IgG	R&D systems conc.: 1.25 g/l	1: 5000

Secondary antibody	Manufacturer‘s data	Dilution
HRP-conjugated, polyclonal swine anti-rabbit IgG	Dako conc.: 0.34 g/l	1: 3000
HRP-conjugated, polyclonal rabbit-anti-mouse-IgG	Dako conc.: 1.3 g/l	1: 5000