Post-Translational Regulation of p53 Function Through 20S Proteasome-Mediated Cleavage

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Supplementary Information

Running title: p53 functional regulation through 20S proteasome cleavage

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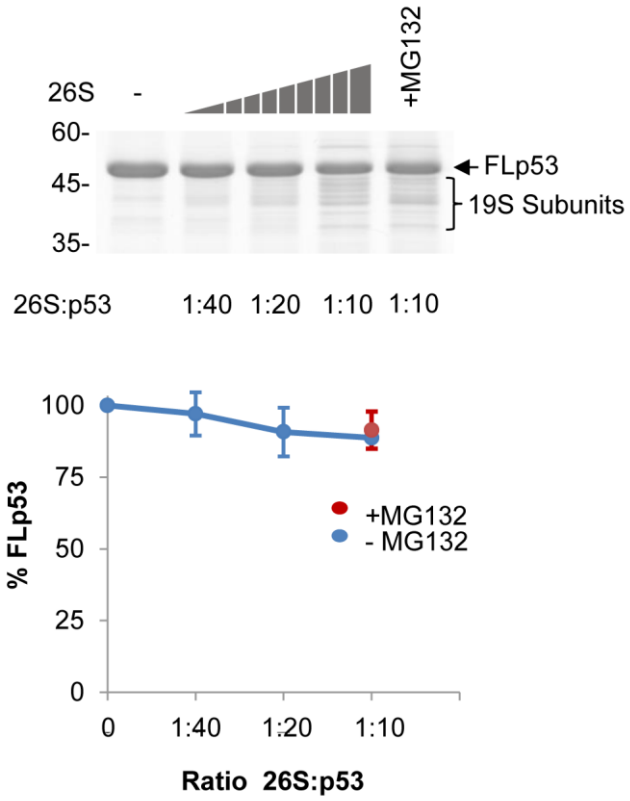
Table S1. Primer sequences used for quantitative real-time PCR of human genes

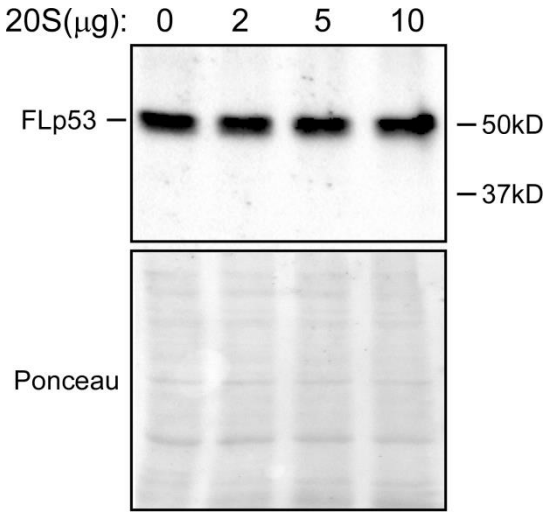
Gene ID	Forward Primer	Reverse Primer
p21	GGCAGACCAGCATGACAGATT	GCGGATTAGGGCTTCCTCTT
BTG2	AGGCACTCACAGAGCACTACAAAC	GCCCTTGGACGGCTTTTC
GAPDH	ACCCACTCCTCCACCTTTGA	CTGTTGCTGTAGCCAAATTCGT

Table S2. Primer sequences used for quantitative real-time PCR of mouse genes

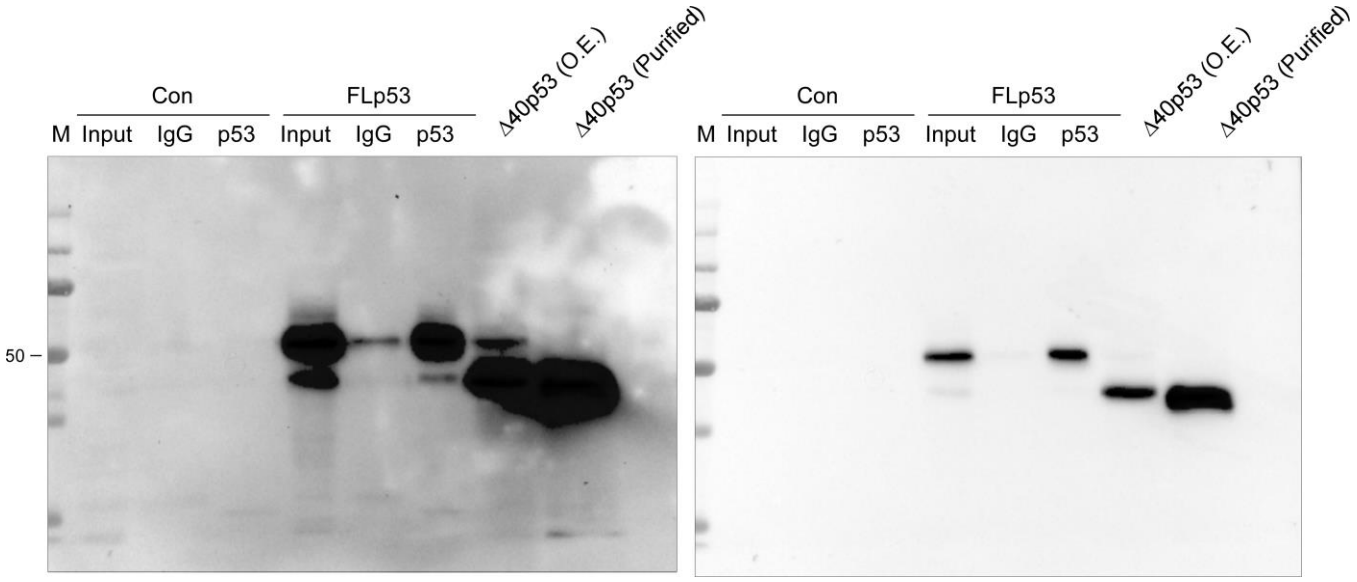
Gene ID	Forward Primer	Reverse Primer
p21	GGCCCGGAACATCTCAGG	AAATCTGTCAGGCTGGTCTGC
MDM2	TGTCTGTGTCTACCGAGGGTG	TCCAACGGACTTTAACAACTTCA
HPRT	GCAGTACAGCCCCAAAATGG	GGTCCTTTTCACCAGCAAGCT



Supplementary Figure S1. p53 degradation is not observed in the presence of purified 26S proteasomes. Degradation assays with purified FLp53 and 26S proteasomes: FLp53 was incubated with serially diluted 26S proteasomes. Aliquots were evaluated by SDS–PAGE, followed by quantitative image analysis (bottom panel). The results indicate that, unlike incubation with the 20S proteasome, p53 levels remained constant in the presence of the 26S proteasome. The positions of the p53 and 19S proteasome subunits are indicated on the right side of the gel. Error bars represent s.d. of three repeats.



Supplementary Figure S2. Cellular FLp53 post-translational processing by the 20S proteasome generates the Δ40p53 isoform. The H1299 non-small cell lung carcinoma cell line, which is p53-deficient, was transiently transfected with pC53-SN3 vector expressing FLp53, followed by cell lysis. Cell extracts were incubated with the indicated elevated concentrations of 20S proteasome for 120 minutes at 37°C. Only the FLp53 isoform was detected by using a monoclonal antibody (DO-1), directed against the p53 N-terminal epitope between amino acid residues 21-25. Thus, unlike the results shown in Fig. 3A wherein a polyclonal anti-p53 antibody was utilized, when using the DO-1 antibody the Δ40p53 isoform is not observed, as it is missing the N-terminal epitope. Ponceau S staining was used as a loading control. The blot is of a representative experiment out of three independent experiments.



Supplementary Figure S3. FLp53 interacts with Δ40p53. H1299 cells were transiently transfected with pC53-SN3 vector expressing FLp53 (FLp53) or with an empty vector as a negative control (Con). After 48 hours cells were lysed and extracts were incubated with either monoclonal antibody against p53 N-terminus (DO-1) that is directed to an epitope that does not appear in the Δ40p53 protein, to immuno-precipitate FLp53, or with a non-specific IgG antibody as a negative control. Immuno-precipitates were loaded on SDS-PAGE gel, and p53 forms were detected using goat polyclonal anti-p53 Horseradish Peroxidase-conjugated antibody (p53HRP). To examine p53 expression, non-immuno-precipitated samples were loaded (Input). As positive controls, cells were transfected with SV-p53Δ40 vector to over-express Δ40p53 (Δ40p53 O.E.) and the purified Δ40p53 protein was used. The long and short exposures of this blot are shown on the left and right panels, respectively. Figure 5C combines the long exposure of the immuno-precipitation results, with the short exposure of the Δ40p53 purified protein. M, protein marker; O.E., over-expression.