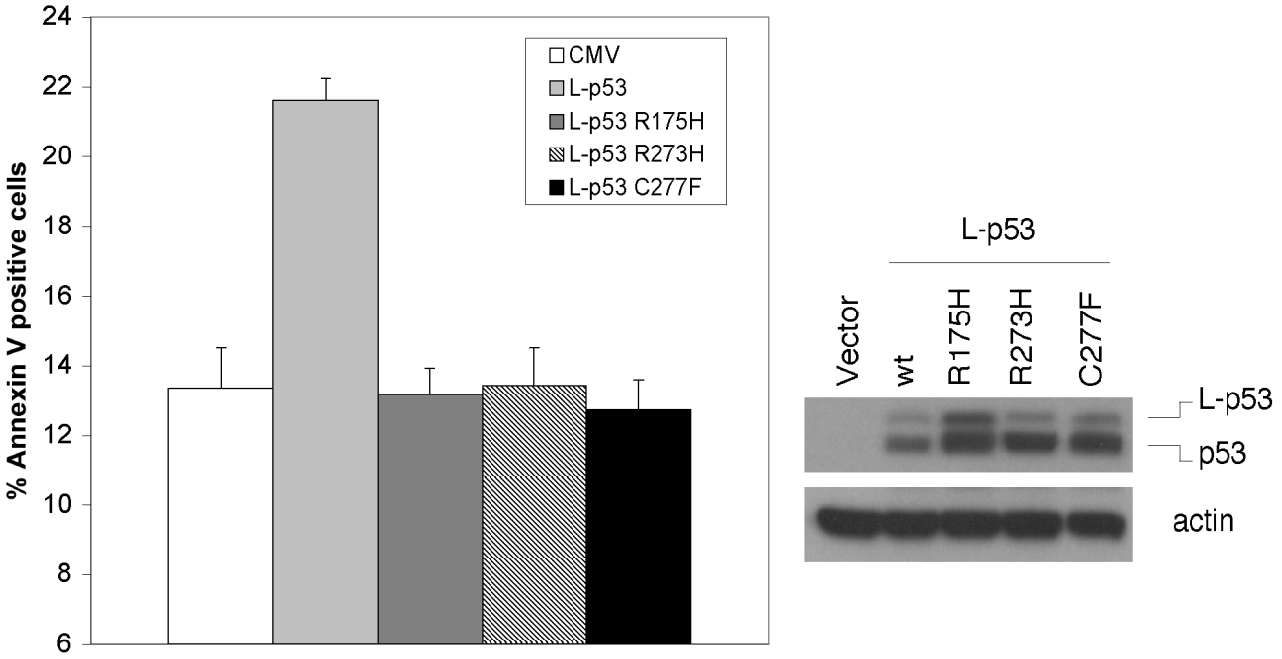
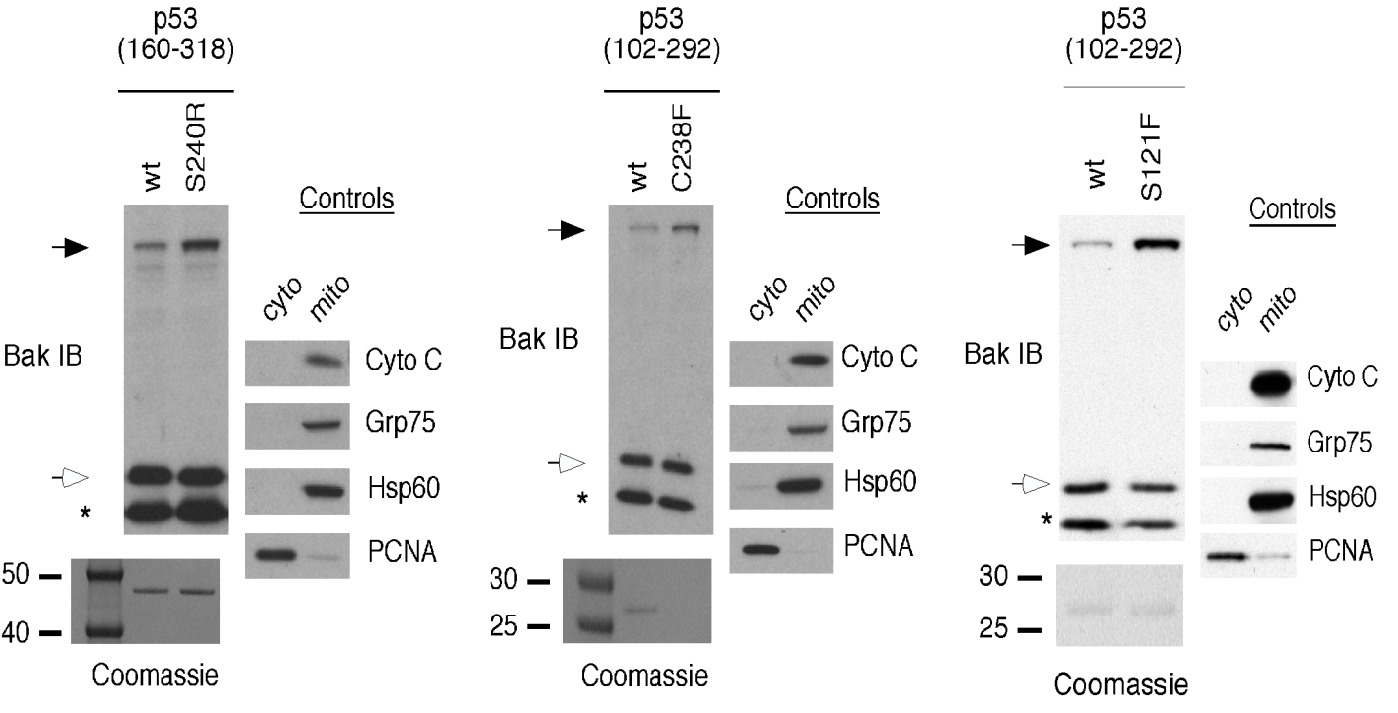


Legend Supplemental Figure 1

Transient transfection of mitochondrially targeted wild type p53, but not mutant p53, elicits an apoptotic response. Saos-2 cells were transiently transfected for 24 hours with vector control or expression vectors for mitochondrially targeted wild type p53 or tumor derived p53 mutant proteins (L-p53 wt, L-p53 R175H, L-p53 R273H, and L-p53 C277F) using Fugene 6. After 24 hours of tansfection cells were washed with phosphate buffered saline, growth media was added back to cells, and cells were grown for 48 hours. At the end of the incubation period, apoptosis induction in the transfected cells was assessed by Annexin V staining (Guava Nexin Assay Kit; GUAVA Technologies). The results presented are the mean percent of apoptotic cells ± standard deviation acquired from three independent experiments on a GUAVA Personal Cytometer (GUAVA Technologies) according to the manufacturer's instructions. To control for equal transfection efficiency of the various p53 expression plasmids used, parallel transfected cells were assayed for expression of p53 levels by Western blot analysis. The p53 Western blot shows L-p53 and p53 which has been cleaved off the mitochondrial leader peptide of ornithine transcarbamylase by mitochondrial endopeptidases (see reference 16)



Legend Supplemental Figure 2

Mutation of the L3 loop amino acid residues S240 and C238 and the L1 loop amino acid residue S121 within p53 results in an increased ability of p53 to oligomerize BAK. 20 ug of mitochondria isolated from H1299 cells were incubated with 0.5 ug of wildtype p53 and the L3 loop mutants S240R (left panel) and C238F (middle panel) and the L1 loop mutant S121F (right panel). The S240R mutant was generated in the p53 (160-318) background. The C238F and S121F mutants were generated in the backbone of the core DNA binding domain of p53 (102-292). Following incubation and cross-linking with BMH, BAK monomers and oligomers were detected by immunoblotting using a BAK specific antibody. Arrows indicate BAK oligomers; open arrowheads indicate BAK monomers; asterisk indicates a common intra-molecular crosslink in BAK. To assess the integrity of purified mitochondria, levels of the mitochondrial proteins cytochrome C, Grp75, Hsp60 and the nuclear protein PCNA were assessed by immunoblotting as described in the Materials and Methods section. To ensure equal input of recombinant proteins, 0.5 ug of protein were subjected to SDS-PAGE and Coomassie staining (bottom panel).