Supplement F1 related to Fig.1 Crotonic acid decreases the level of wild type p53 protein.

Crotonic acid does not reduce the p53 mRNA level in H460 lung cancer cells. H460 cells were treated with different doses of crotonic acid (CA) and harvested at 24 and 48 hrs post treatment for Q-PCR to detect p53 mRNA levels.

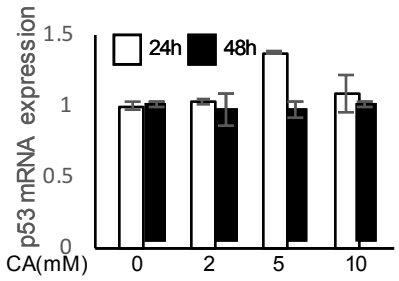
Supplement F2 related to Fig.2

(**A**) The schematic of the functional domains and mutated residues of p53-K24R and p53-4M. (**B**) The schematic of the functional domains and mutated residues of p53-8KR. (**C**) p53-8KR is crotonylated. WT p53 and p53-8KR plasmids were introduced into $HCT116^{p53-/-}$ cells. Cells were harvested for IP-IB with indicated antibodies. (**D**) The blast of p53 family of p53, p63 and p73 of p53 S46 amino acid region. (**E)** Crotonylation reactions were performed with WT and S46A(SA) His p53 beads in the presence and absence of p300 and HEK293 lysate. Crotonylation was detected using Pan Anti-crotonyllysine antibody in the in vitro reaction. (**F)** Serine 46 of p53 is not conserved in species. Serine 46 of p53 only presents in *H.sapiens, P.troglodytes* and *M.mulatta.*

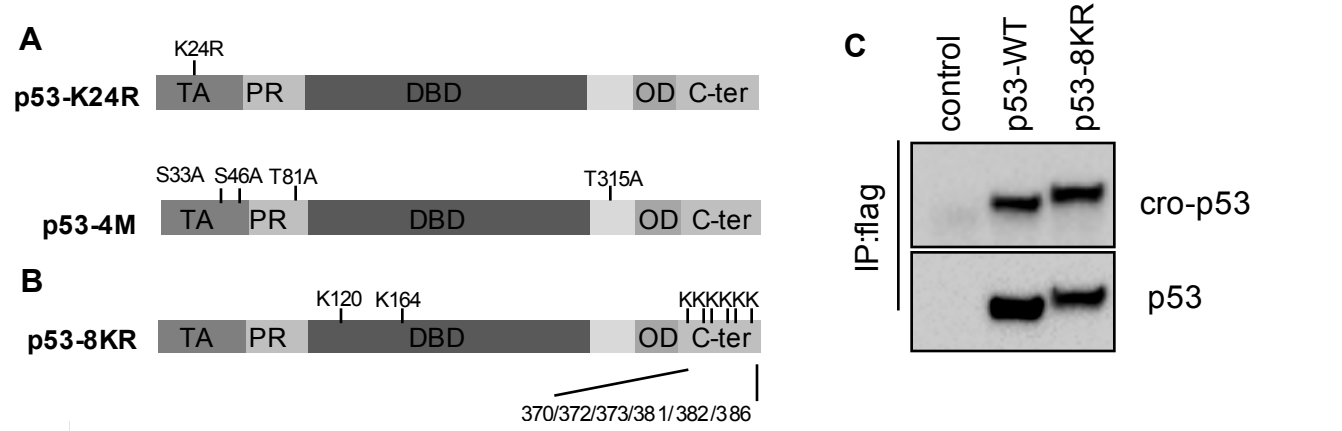
Supplement F3 related to Fig.2

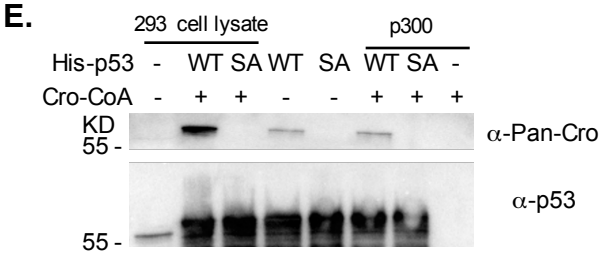
(A) Diagram of serine phosphorylation. **(B)** Diagram of serine Crotonylation. **(C)** CA reduces the p53 protein is independent on MDM2. Human p53 and MDM2 plasmids were introduced into $MEF^{p53-/-\ MDM2-/-}$ cells. Cells were harvested for IP-IB with indicated antibodies. **(D)** CA reduces the p53 protein is independent on MDM2 mediated ubiquitination. Human p53, MDM2 and His-Ub plasmids were introduced into $MEF^{p53-/-\ MDM2-/-}$ cells. Cells were harvested for Ubiquitination and IB assays with indicated antibodies.

Supplement F1 related to Fig. 1



Supplement F2 related to Fig. 2





F

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Organism
H.sapiens              1   ---MEEPQSDPSVEPPLSQETFSDLWKLLPENNVLSPL---PSQAMDDLM   44
P.troglodytes          1   ---MEEPQSDPSVEPPLSQETFSDLWKLLPENNVLSPL---PSQAMDDLM   44
M.mulatta              1   ---MEEPQSDPSIEPPLSQETFSDLWKLLPENNVLSPL---PSQAVDDLM   44
C.lupus                1   ---MEESQSELNIDPPLSQETFSELWNLLPENNVLSSE---LCPAVDELL   44
B.taurus               1   ---MEESQAELNVEPPLSQETFSDLWNLLPENNLLSSE---LSAPVDDLL   44
M.musculus             1   MTAMEESQSDISLELPLSQETFSGLWKLLPPEDILP-----SPHCMDDLL   45
R.norvegicus           1   ---MEDSQSDMSIELPLSQETFSCLWKLLPPDDILPTTATGSPNSMEDLF   47
D.rerio                1   -------------MAQNDSQEFAELWEKNLISIQPP-----GGGSCWDII   32
X.tropicalis           1   ----MEPSSETGMEPPLSQETFEDLWSLLPDPLQ-T-----GTGQMENFA   40

Organism
H.sapiens             45   LSPDDIEQWFTEDPGPDEAPRMPEAAPPVAPAPAAPTPAAPAPAPSWPLS   94
P.troglodytes         45   LSPDDIEQWFTEDPGPDEAPRMPEAAPPVAPAPAAPTPAAPAPAPSWPLS   94
M.mulatta             45   LSPDDLAQWLTEDPGPDEAPRMSEAAPPMAPTPAAPTPAAPAPAPSWPLS   94
C.lupus               45   L-PESVVNWLDED--SDDAPRM----------PATSAPTAPGPAPSWPLS   81
B.taurus              45   P-YTDVATWLDEC--PNEAPQMPEPSA-----PAAPPPATPAPATSWPLS   86
M.musculus            46   L-PQDVEEFFEG---PSEALRVSGAPAAQDPVTETPGPVAPAPATPWPLS   91
R.norvegicus          48   L-PQDVAELLEG---PEEALQVS-APAAQEPGTEAPAPVAPASATPWPLS   92
D.rerio               33   ----NDEEYLPGSFDPNFFENVLEEQPQPSTLP--P-------------T   63
X.tropicalis          41   ----EFSEY---PLAPDM--TVLQEGLMGNTVPTVT-------------S   68
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Supplement F3 related to Fig. 2