

Unprovoked Stabilization and Nuclear Accumulation of the Naked Mole-Rat p53 Protein

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Please direct any requests for naked mole-rat related reagents or questions regarding
the animals to rbuffen@calicolabs.com

Abstract Word Count: 192

Main Text Word Count (Excluding References and Figure Legends): 3485

Number of Figures: 4

Supplemental Figure Legends

Supplemental Figure S1

- A) MEF or NEF cells transfected with siRNA targeting *Tp53* were analyzed by immunoblotting with the indicated antisera two hours post-irradiation (12 Gy).

Supplemental Figure S2

- A) Location of CRISPR guide sequences targeting mouse (i) and NMR (ii) p53, as indicated.
- B) Single cell-derived clones of NEF cells generated from pooled populations, transfected with the indicated guide, were analyzed by immunoblotting with the indicated antisera.
- C) Single cell-derived clones of MEF cells generated from pooled populations, transfected with the indicated guide, were analyzed by immunoblotting with the indicated antisera two hours post-irradiation (12 Gy).
- D) p53 KO MEF or NEF cells were treated with the indicated concentration of nutlin 3A for 72 hours prior to before being fixed and stained with crystal violet. crystal violet staining was quantified by solubilizing the fixed dye and assessing the absorbance at 562 nm. Values are normalized to DMSO control and error bars represent standard deviation. Lines indicate non-linear fit model. Unpaired t test; Ns: no significance.

Supplemental Figure S3

- A) NMR and mouse p53 protein sequences were aligned.
- B) NMR and human p53 protein sequences were aligned.

Supplemental Figure S4

- A) Uncropped membranes from images in figure 1A (i), figure 1B (ii), figure 1C (iii), figure 1E (iv), and figure 1F i (v 1) and 1F ii (v 2). Red boxes indicate lanes included in figure. L indicates left blot and R indicates R blot. DO-1 and FL393 indicate antibody used for p53 detection (see supplementary table S1).

- B) Uncropped membranes from images in figure 2A (i), figure 2B (ii) and figure 2C (iii). Red boxes indicate lanes included in figure. DO-1 and FL393 indicate antibody used for p53 detection (see supplementary table S1).
- C) Uncropped membranes from images in figure 3A (i), figure 3B (ii), figure 3E (iii), and figure 3F (iv). Red boxes indicate lanes included in figure. DO-1 and FL393 indicate antibody used for p53 detection (see supplementary table S1).
- D) Uncropped membranes from images in supplementary figure S1. DO-1 and FL393 indicate antibody used for p53 detection (see supplementary table S1).
- E) Uncropped membranes from images in supplementary figure S2A (i) and supplementary figure S2B (ii). DO-1 and FL393 indicate antibody used for p53 detection (see supplementary table S1).

Supplementary Table S1. Antibodies used for immunoblot and immunofluorescence experiments.

Epitope	Company	Clone and Catalog Number	Cell Line Antibody Used In
α -tubulin	Cell Signaling Technology	Clone DM1A; Cat. 3873	Mouse and NMR
β -actin	Cell Signaling Technology	Clone 8H10D10; Cat. 3700	Mouse and NMR
β -actin	Cell Signaling Technology	Clone 13E5; Cat. 4970	Mouse and NMR
c-MYC	Cell Signaling Technology	Clone D3N8F; Cat. 13987	Mouse and NMR
CREB	Cell Signaling Technology	Clone 48H2; Cat. 9197	Mouse and NMR
FLAG	Sigma Aldrich	Clone M2; Cat. F1804	Mouse and NMR
GAPDH	Cell Signaling Technology	Clone D16HL; Cat. 5174	Mouse and NMR
p21	BD Biosciences	Clone SXM30; Cat. 556431	Mouse and NMR
p53	Santa Cruz	Clone D-O1; Cat. SC-126	NMR
p53	Santa Cruz	Clone FL-393; Cat. SC-6243	Mouse (immunoblots)
p53	Leica Biosystems	Clone CM5; Cat. P53-CM5P-L	Mouse (immunofluorescence)
Phospho- γ H2A.X Ser139	Abcam	Clone 9F3; Cat. 26350	Mouse and NMR
Phospho- γ H2A.X Ser139	Cell Signaling Technology	Clone 20E3; Cat. 9718	Mouse and NMR
Ubiquitin	Cell Signaling Technology	Cat. 3933	Mouse and NMR

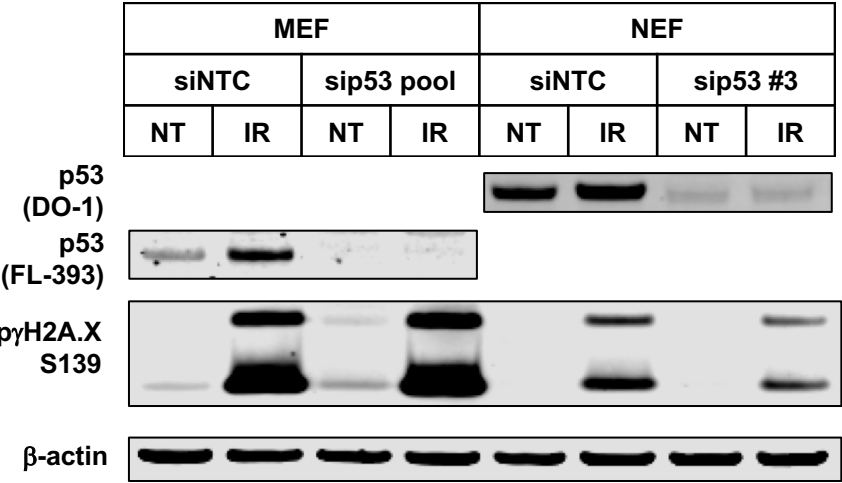
Supplementary Table S2. NMR CRISPR guide sequences

Guide ID	Sequence
sgM1	TGGGAGAGACCGGCGCACAG
sgM3	GTGCGTGTTTGTGCCTGTCC
sgM4	TGGGCTAGTGGGAACCTGCT
sgM5	CTAGTGGGAACCTGCTGGGG

Supplementary Table S3. Pharmacological agents used.

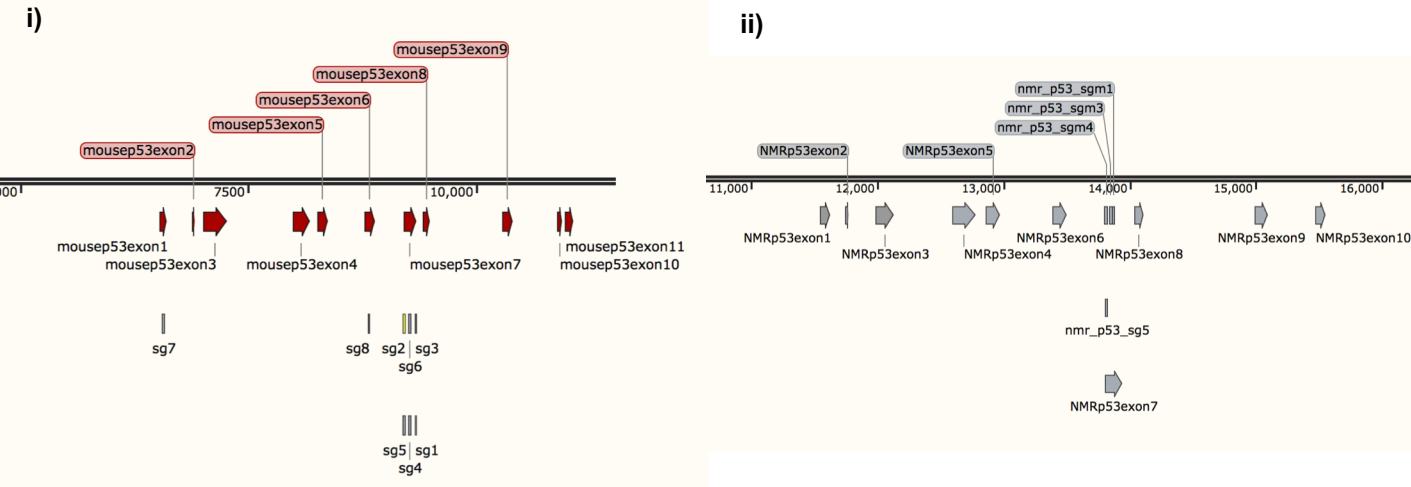
Compound	Company and Catalog Number	Treatment Concentration	Diluent
Bortezomib	Selleck Chemicals; S1013	Varied	DMSO
Cycloheximide	Sigma; 01810	100 µg/mL	Ethanol (200 proof)

Supplementary Fig. 1
A)



Supplementary Fig. 2

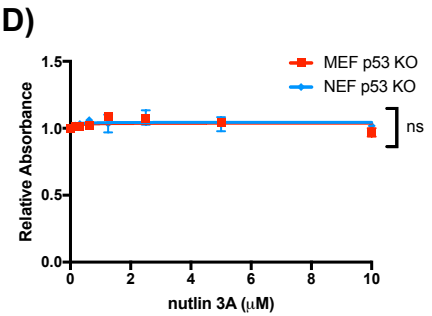
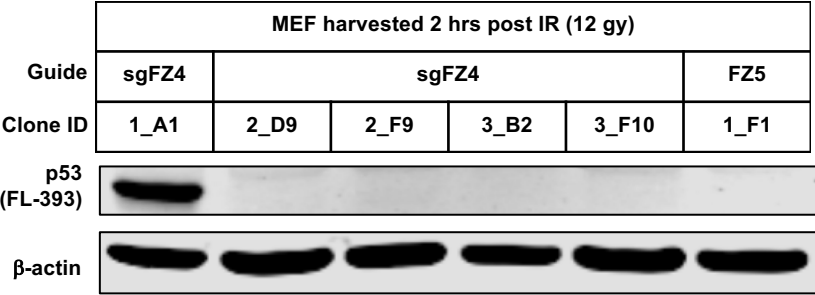
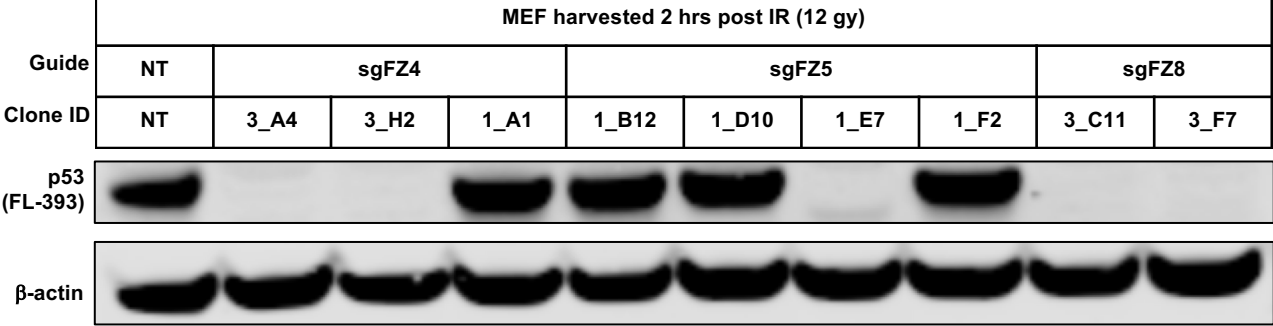
A)



B)



C)



Supplementary Fig. 3

A)

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cellular tumor antigen p53 isoform a [Mus musculus]
Sequence ID: [NP_035770.2](#) Length: 390 Number of Matches: 1
[▶ See 9 more title\(s\)](#)

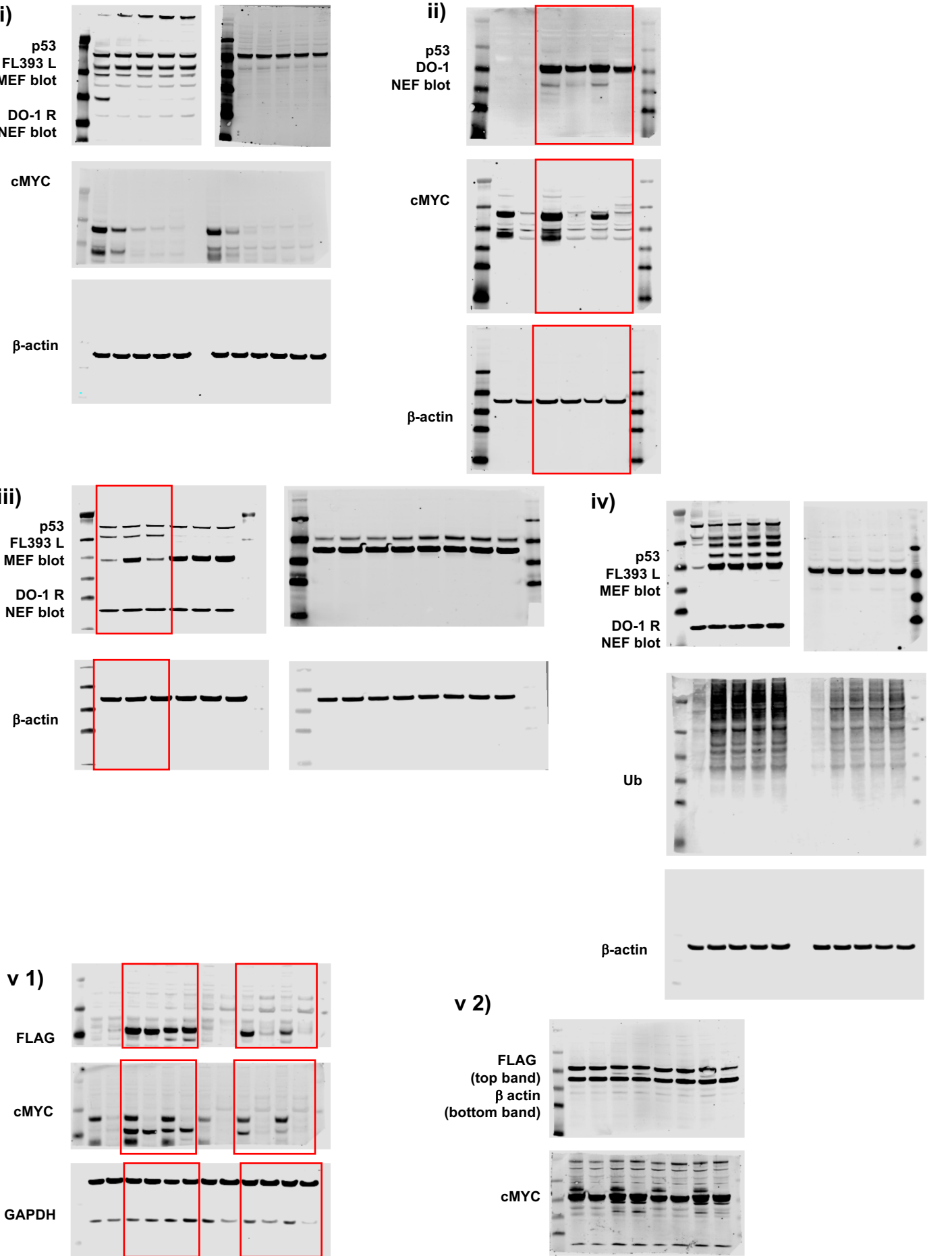
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		MEE QSD+S+E PLSQETFS LWKLLP ++L S MDDL L P+DV + G P E				
Sbjct 4		MESQSDISLELPLSQETFSGLWKLLPPEDILPSPHC--MDDL L -PQDV EEF FEG -PSE				59
Query 61		DVQVSAAPVPEPPTPVAPAPAAPAPATSWPLSSSVPSHKTYQGNYGFHLGFLQSGTAKSV				120
		++VS AP + P P P APAPAT WPLSS VPS KTYQGN YGFHLGFLQSGTAKSV				
Sbjct 60		ALRVSGAPAAQDPVTETPGPVAPAPATPWPLSSFVPSQKTYQGNYGFHLGFLQSGTAKSV				119
Query 121		TCTYSPVLNKLFCQLAKTCTPVQVWVESPPPPGTRVRAMAIYKKSQHMTEVVRRCPHHERC				180
		CTYSP LNKLF CQLAKTCTPVQ+WV + PP G+RVRAMAIYKKSQHMTEVVRRCPHHERC				
Sbjct 120		MCTYSPPLNKLFCQLAKTCTPVQLWVSATPPAGSRVRAMAIYKKSQHMTEVVRRCPHHERC				179
Query 181		SDSDGLAPPQHLIRVEGNLRAEYLDRTTFRHSVVVPYDLPEVGSDCTTIHYNMNCSSC				240
		SD DGLAPPQHLIRVEGNL EYL+DR TFRHSVVVPY+ PE GS+ TTIHY YMCNSSC				
Sbjct 180		SDGDGLAPPQHLIRVEGNLYPEYLEDRTQTFRHSVVVPYEPPEAGSEYTTIHYKYMNCSSC				239
Query 241		MGMNRRPILTIITLEDSSGNLLGRNSFEVRVCACPGDRDRTEENFHKKGGSCEPTPG				300
		MGMNRRPILTIITLEDSSGNLLGR+SFEVRVCACPGDRDRTEENF KK CPE PG				
Sbjct 240		MGMNRRPILTIITLEDSSGNLLGRDSFEVRVCACPGDRDRTEENFRKKEVLCPELPPG				299
Query 301		SIKRALPTGTNSSPQKKKPLDGEYFTLKIRGRERFEMFRELNEALELKDAQTEKEPGES				360
		S KRALP T++SP KKKPLDGEYFTLKIRGR+RFEMFRELNEALELKDA +E G+S				
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Query 361		RPHSSYLKSKKGQSTSCHKKLMFKKEGPDSD 391				
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B)

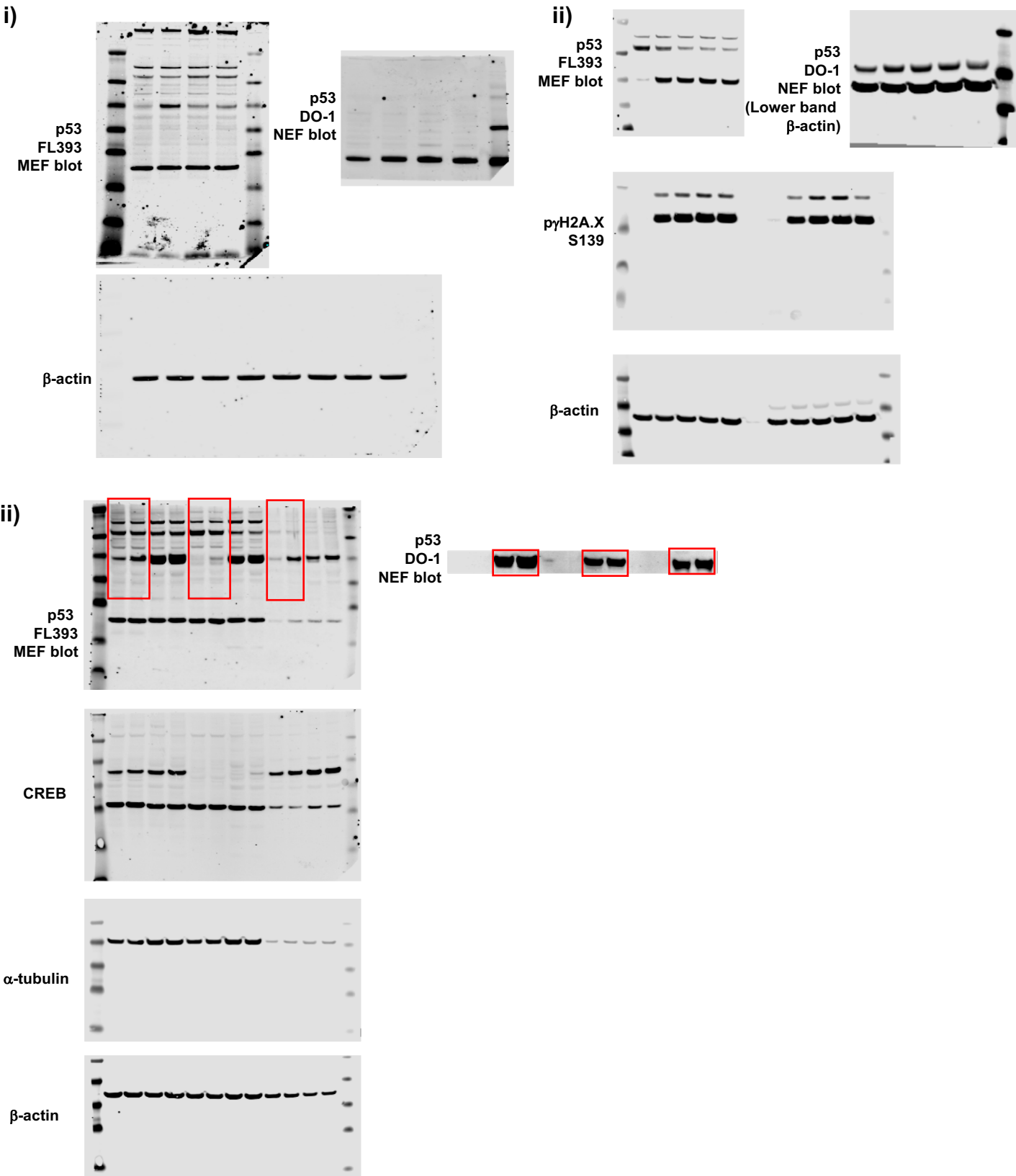
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Tumor protein p53 [Homo sapiens]
Sequence ID: [AAH03596.1](#) Length: 393 Number of Matches: 1
[▶ See 2 more title\(s\)](#)

Range 1: 1 to 393 GenPept Graphics						▼ Next Match ▲ Previous Match
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Sbjct 1		MEEPQSDSPSEPPPLSQETFSDLWKLLPENNVLSPLPSQAMDDLMLSPDDIEQWFTEDPGP				60
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		DE ++ A P P AP PAAPAPA SWPLSSSVPS KTYQG+YGF LGFL SGTA				
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Query 119		SVTCTYSPVLNKLFCQLAKTCTPVQVWVESPPPPGTRVRAMAIYKKSQHMTEVVRRCPHHE				178
		SVTCTYSP LNK+FCQLAKTCTPVQ+WV+S PPPGTRVRAMAIYK+SQHMTEVVRRCPHHE				
Sbjct 121		SVTCTYSPALNKMFCQLAKTCTPVQLWVDSTPPPPTGTRVRAMAIYKQSQHMTEVVRRCPHHE				180
Query 179		RCSDSDGLAPPQHLIRVEGNLRAEYLDRTTFRHSVVVPYDLPEVGSDCTTIHYNMNCNS				238
		RCSDSDGLAPPQHLIRVEGNLR EYLDDR TFRHSVVVPY+ PEVGSDCTTIHYNMNCNS				
Sbjct 181		RCSDSDGLAPPQHLIRVEGNLRVEYLDRTTFRHSVVVPYEPPEVGSDCTTIHYNMNCNS				240
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		SCMGMNRRPILTIITLEDSSGNLLGRNSFEVRVCAC GRDRRTEEN KKG E				
Sbjct 241		SCMGMNRRPILTIITLEDSSGNLLGRNSFEVRVCACAGDRDRTEENLRKKGEPHHELP				300
Query 299		PGSIKRALPTGTNSSPQKKKPLDGEYFTLKIRGRERFEMFRELNEALELKDAQTEKEPG				358
		PGS KRALP T+SSPQKKKPLDGEYFTL+IRGRERFEMFRELNEALELKDAQ KE				
Sbjct 301		PGSTKRALPNNTSSSPQKKKPLDGEYFTLQIRGRERFEMFRELNEALELKDAQAGKEPG				360
Query 359		ESRPHSSYLKSKKGQSTSCHKKLMFKKEGPDSD 391				
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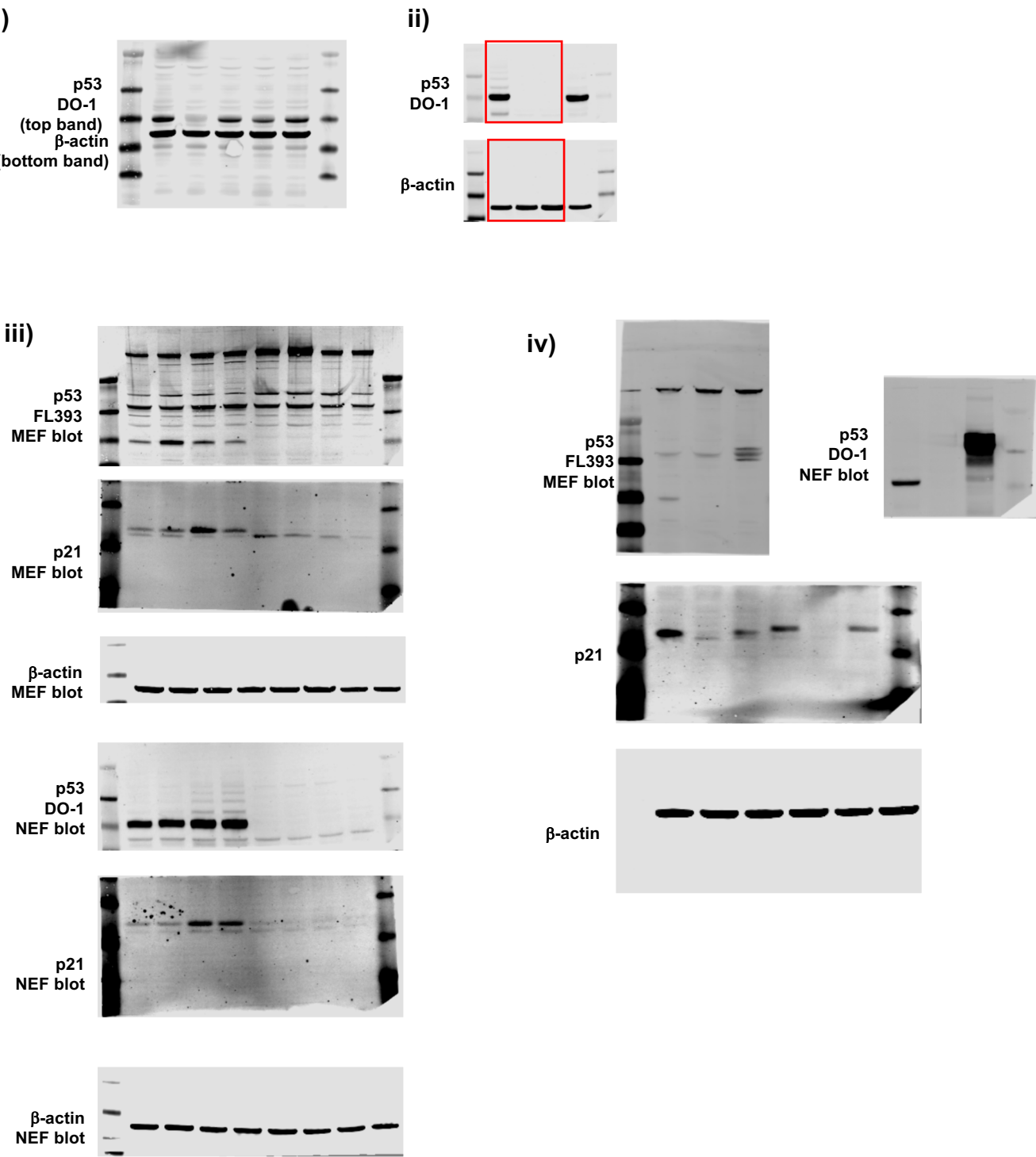
Supplementary Fig. S4A: Uncropped membranes from Fig. 1



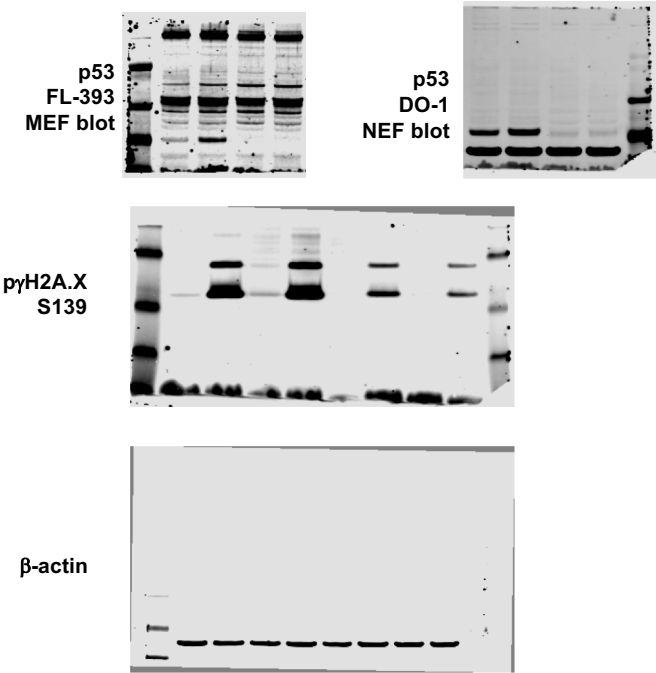
Supplementary Fig. S4B: Uncropped membranes from Fig. 2



Supplementary Fig. S4C: Uncropped membranes from Fig. 3



Supplementary Fig. S4D: Uncropped membranes from Supplementary Fig. S1



Supplementary Fig. S4E: Uncropped membranes from Supplementary Fig. S2

