

The position of the target site for engineered nucleases improves the aberrant mRNA clearance in *in vivo* genome editing

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Supplementary Table 1. Target sequences of engineered nucleases for each target gene

Genes	Type of nucleases	Sequences with deletion mutation at the gene of interest	Direction
<i>p16^{Ink4a}</i>	TALEN	TGCATGACGTGCGGGCACTGNNNNNNNNNNNGTTTCGCCCAACGCCCCGAA	+
<i>p19^{Arf}</i>	TALEN	TTCGTGCGATCCCGGAGACCNNNNNNNNNNNCTGCGCTCTGGCTTTCGTGA	+
<i>p53</i>	TALEN	TATCAGCCTCGAGCTCCCTCNNNNNNNNNNNCATTTTCAGGCTTATGGAAA	+
<i>Cd47</i>	CRISPR	GGAGATGTGGCCCTTGGCGGCGG	+
<i>Ciita</i>	CRISPR	ACTGGATGAAGAGACCCGGGAGG	+
<i>Creb3</i>	CRISPR	GGCTTCCTGCTAGAGGAAAGCGG	+
<i>Il2ry</i>	CRISPR	AGGAGCACTGAGGTGTTTCAGGGG	-
<i>Lepr</i>	CRISPR	AGAAGCCCCCTTCAAAGCCGAGG	-
<i>Lxra</i>	CRISPR	GCACGCTATGTCTGCCACAGCGG	+
<i>Lxrb</i>	CRISPR	AGTGTCCAGAGAACTTGTGGGGG	-
<i>p27^{Kip1}</i>	CRISPR	GCGGATGGACGCCAGACAAGCGG	+
<i>Reep5</i>	CRISPR	CCAGTGGCTGACGTACTGGGTGG	+
<i>Rtp4</i>	CRISPR	TGCAGGCTCCACTTGGCCCCGGG	-

The target sequences of TALENs or gRNAs are indicated in blue, and PAM sequence (NGG) are represented in red.

Supplementary Table 2. Sequences of deletion regions observed in the frameshift mutant mice

Genes	Strains	Sequences with deletion mutation at the gene of interest	Deletion site (Δ)
<i>p16^{Ink4a}</i>	FVB/N	WT GCGGCGGCCAGGGCCGTG TGCATGACGTGCGGGCACTG CTGGAAGCCGGG GTTTCGCCCAACGCCCGAA CTCTTTCGGTCGT A A A Q G R V H D V R A L L E A G V S P N A P N S F G R Mu GCGGCGGCCAGGGCCGTG TGCATGACGTGCGGGCACTG ----- CCCGAACTCTTTCGGTCGT A A A Q G R V H D V R A L ----- <u>P</u> <u>R</u> <u>T</u> <u>L</u> <u>S</u> <u>V</u> <u>V</u>	25
<i>p16^{Ink4a}</i>	B6J	WT GCGGCGGCCAGGGCCGTG TGCATGACGTGCGGGCACTG CTGGAAGCCGGG GTTTCGCCCAACGCCCGAA CTCTTTCGGTCGT A A A Q G R V H D V R A L L E A G V S P N A P N S F G R Mu GCGGCGGCCAGGGCCGTG TGCATGACGTGCGGGCACTG CTG -AAGCCGGG GTTTCGCCCAACGCCCGAACTCTTTCGGTCGT A A A Q G R V H D V R A L L - <u>K</u> <u>P</u> <u>G</u> <u>F</u> <u>R</u> <u>P</u> <u>T</u> <u>P</u> <u>R</u> <u>T</u> <u>L</u> <u>S</u> <u>V</u> <u>V</u>	1
<i>p19^{Arf}</i>	FVB/N	WT GAGAGGGTTTTCTTGGTGAAG TTCGTGCGATCCCGGAGACC CAGGACAGCGAG CTGCGCTCTGGCTTTCGTGA ACATGTTGTTG E R V F L V K F V R S R R P R T A S C A L A F V N M L L Mu GAGAGGGTTTTCTTGGTGAAG TTCGTGCGATCCCGGAGACC CAGGAC -GCGAGCTGCGCTCTGGCTTTCGTGA ACATGTTGTTG E R V F L V K F V R S R R P R T- <u>R</u> <u>A</u> <u>A</u> <u>L</u> <u>W</u> <u>L</u> <u>S</u> <u>*</u>	1
<i>p19^{Arf}</i>	B6J	WT GAGAGGGTTTTCTTGGTGAAG TTCGTGCGATCCCGGAGACC CAGGACAGCGAG CTGCGCTCTGGCTTTCGTGA ACATGTTGTTG E R V F L V K F V R S R R P R T A S C A L A F V N M L L Mu GAGAGGGTTTTCTTGGTGAAG TTCGTGCGATCCCGGAGACC CAGG -----CGAGCTGCGCTCTGGCTTTCGTGA ACATGTTGTTG E R V F L V K F V R S R R P R ----- <u>R</u> <u>A</u> <u>A</u> <u>L</u> <u>W</u> <u>L</u> <u>S</u> <u>*</u>	4
<i>p53</i>	FVB/N	WT ATGGAGGAGTCACAGTCGGAT TATCAGCCTCGAGCTCCCTC TGAGCCAGGAGAC ATTTTCAGGCTTATGGAA ACTACTTCTCCA M E E S Q S D I S L E L P L S Q E T F S G L W K L L P P Mu ATGGAGGAGTCACAGTCGGAT TATCAGCCTCGAGCTCCCTC TGAG -----ACATTTTCAGGCTTATGGAA ACTACTTCTCCA M E E S Q S D I S L E L P L S----- <u>H</u> <u>F</u> <u>Q</u> <u>A</u> <u>Y</u> <u>G</u> <u>N</u> <u>Y</u> <u>F</u> <u>L</u> <u>Q</u>	7
<i>p53</i>	B6J	WT ATGGAGGAGTCACAGTCGGAT TATCAGCCTCGAGCTCCCTC TGAGCCAGGAGAC ATTTTCAGGCTTATGGAA ACTACTTCTCCA M E E S Q S D I S L E L P L S Q E T F S G L W K L L P P Mu ATGGAGGAGTCACAGTCGGAT TATCAGCCTCGAGCTCCCTC TGAG -CAGGAGACATTTTCAGGCTTATGGAA ACTACTTCTCCA M E E S Q S D I S L E L P L S- <u>R</u> <u>R</u> <u>H</u> <u>F</u> <u>Q</u> <u>A</u> <u>Y</u> <u>G</u> <u>N</u> <u>Y</u> <u>F</u> <u>L</u> <u>Q</u>	1

Cd47	B6J	<p>WT CGGCGACCCCCGGCGGCGCGGAGATGTGGCCCTTGGCGGGCGGCGCTGTTGCTGGGCTCCTGCTGCTGCGGTTTCAGCTCAACTA M W P L A A A L L L G S C C C G S A Q L</p> <p>Mu CGGCGACCCCCGGCGGCGCGGAGATGTG-----CTGGGCTCCTGCTGCTGCGGTTTCAGCTCAACTA M <u>C</u>----- <u>W</u> <u>A</u> <u>P</u> <u>A</u> <u>A</u> <u>V</u> <u>Q</u> <u>L</u> <u>N</u> <u>Y</u></p>	22
Ciita	B6J	<p>WT AGCAAGCTGTTGCAGGACATGGA<u>ACTGGATGAAGAGACCCGGGAGG</u>CCTATGCCAACATTGCGGAACTGGATCAGTACGTGTTTC S K L L Q D M E L D E E T R E A Y A N I A E L D Q Y V F</p> <p>Mu AGCAAGCTGTTGCAGGACATGGA<u>ACTGGATGAAGAGAC</u>---GGGAGGCCTATGCCAACATTGCGGAACTGGATCAGTACGTGTTTC S K L L Q D M E L D E E T-- <u>G</u> <u>G</u> <u>L</u> <u>C</u> <u>Q</u> <u>H</u> <u>C</u> <u>G</u> <u>T</u> <u>G</u> <u>S</u> <u>V</u> <u>R</u> <u>V</u></p>	2
Creb3	B6J	<p>WT GAGGAAAGCGGAGATTTGTGGGCTGCGACTGAGCCGGACGTGAAGGCTCCGCTGGACTTAGAG//GACTGGGAGGTAGAGGAT E E S G D L W A A T E P D V K A P L D L E--/-D W E V E D</p> <p>Mu GAGGAA-GCGGAGATTTGTGGGCTGCGACTGAGCCGGACGTGAAGGCTCCGCTG-----//---TGGGAGGTAGAGGAT E E -<u>A</u> <u>E</u> <u>I</u> <u>C</u> <u>G</u> <u>L</u> <u>R</u> <u>L</u> <u>S</u> <u>R</u> <u>T</u> <u>*</u></p>	1
Il2ry	B6J	<p>WT GATTTGATCCTGACTTCTACAG<u>CCCCTGAACACCTCAGTGCTCCT</u>ACTCTGCCCCCTCCAGAGGTTTCAGTGCTTTGTGTTCAAC D L I L T S T A P E H L S A P T L P L P E V Q C F V F N</p> <p>Mu GATTTGATCCTGACTTCTACA-----CCTCAGTGCTCCTACTCTGCCCCCTCCAGAGGTTTCAGTGCTTTGTGTTCAAC D L I L T S T ----- <u>P</u> <u>Q</u> <u>C</u> <u>S</u> <u>Y</u> <u>S</u> <u>A</u> <u>P</u> <u>S</u> <u>R</u> <u>G</u> <u>S</u> <u>V</u> <u>L</u> <u>C</u> <u>V</u> <u>Q</u></p>	11
Lepr	B6J	<p>WT CCTGCTGGAGCCCCAAACAATG<u>CCTCGGCTTTGAAGGGGGCTTCTGA</u>AAGCAATTGTTGAAGCTAAATTTAATTCAAGTGGTATC P A G A P N N A S A L K G A S E A I V E A K F N S S G I</p> <p>Mu CCTGCTGGAGCCCCAAACAATGC-----CTTTGAAGGGGGCTTCTGAAGCAATTGTTGAAGCTAAATTTAATTCAAGTGGTATC P A G A P N N A----- <u>F</u> <u>E</u> <u>G</u> <u>G</u> <u>F</u> <u>*</u></p>	5
Lxra	B6J	<p>WT TTCCGCCGAGTGTCATCAAGGGAG<u>GCAGCTATGTCTGCCACAGCGG</u>TGGCCACTGCCCCATGGACACCTACATGCGGATGGGG F R R S V I K G A R Y V C H S G G H C P M D T Y M R R K</p> <p>Mu TTCCGCCGAGTGTCATCAA-----TGGACACCTACATGCGGATGGGG F R R S V I <u>N</u>----- <u>G</u> <u>H</u> <u>L</u> <u>H</u> <u>A</u> <u>D</u> <u>G</u></p>	41
Lxrb	B6J	<p>WT TTCGTGACCCACTATGTCTT<u>CCCCACAAGTTCTCTGGACACT</u>CCCGTGCTGGGAATGGTTCTCCTCAGCCCAGTACCTCCGC M S S P T S S L D T P V P G N G S P Q P S T S A</p> <p>Mu TTCGTGACCCACTATGTCTTCCCCA--AGTTCTCTGGACACTCCCGTGCTGGGAATGGTTCTCCTCAGCCCAGTACCTCCGC M S S P --<u>K</u> <u>F</u> <u>S</u> <u>G</u> <u>H</u> <u>S</u> <u>R</u> <u>A</u> <u>W</u> <u>E</u> <u>W</u> <u>F</u> <u>S</u> <u>S</u> <u>A</u> <u>Q</u> <u>Y</u> <u>L</u> <u>R</u></p>	2

<i>p27^{Kip1}</i>	FVB/N	WT AACGGGAGCCCGAGCCTGGAG GCGGATGGACGCCAGACAAGCG GAGCACCCCAAGCCTTCCGCCTGCAGAAATCTCTCGGCCCG N G S P S L E R M D A R Q A E H P K P S A C R N L F G P Mu AACGGGAGCCCGAGCCTGGA-----CACCCCAAGCCTTCCGCCTGCAGAAATCTCTCGGCCCG N G S P S L <u>D</u> ----- <u>T P S L P P A E I S S A R</u>	25
<i>Reep5</i>	B6J	WT AGTCCCAACAAAGATGATGACACCC AGTGGCTGACGTACTGGGTGG TATATGGTGTGTTTCAGCATTGCCGAATTCTTCTCCGAT S P N K D D D T Q W L T Y W V V Y G V F S I A E F F S D Mu AGTCCCAACAAAGATGATGACACCCAGTGGCTGACGT-----GGTATATGGTGTGTTTCAGCATTGCCGAATTCTTCTCCGAT S P N K D D D T Q W L T ----- <u>W Y M V C S A L P N S S P I</u>	7
<i>Rtp4</i>	B6J	WT GAGCAGACATTTCAAGAACTGATGCAGGAGGAGAAG CCGGGGCCAAGTGGAGCCTGCA TTTGGATAAGAACATTGTACCAGAT E Q T F Q E L M Q E E K P G A K W S L H L D K N I V P D Mu GAGCAG-----CCTGCATTTGGATAAGAACATTGTACCAGAT E Q ----- <u>P A F G *</u>	47
<p>The target sequences of TALENs or gRNAs are indicated in blue, and PAM sequence (NGG) are represented in red in the wild-type nucleotide sequences. The deleted nucleotides and PTCs are denoted by a red dash (-) and asterisk (*), respectively. Mutant amino acid sequences are denoted by underlined fonts. Mu, Mutant</p>			

Supplementary Table 3. Summary of the results of the engineered nucleases-mediated genome editing in mice

Gene name	Strain	Type of nucleases	Distance from ATG to indel (bp)*	Indel size (bp)	Distance in mRNA (bp)			Exon			
					from indel to PTC	from ATG to PTC	from PTC to EJC	with start codon	targeted	with PTC	last exon
<i>Cd47</i>	B6J	CRISPR	5	22	58	63	309	1	1	2	10
<i>Lxrb</i>	B6J	CRISPR	13	2	74	87	65	3	3	4	10
<i>Rtp4</i>	B6J	CRISPR	36	47	15	98	63	1	1	1	2
<i>p53</i>	FVB/N	TALEN	53	7	439	492	51	2	2	5	11
<i>p53</i>	B6J	TALEN	54	1	444	498	51	2	2	5	11
<i>p27^{Kip1}</i>	FVB/N	CRISPR	58	86	59	117	272	1	1	1	3
<i>p16^{Ink4a}</i>	FVB/N	TALEN	69	25	36	105	334	1	1	2	3
<i>p16^{Ink4a}</i>	B6J	TALEN	72	1	57	129	334	1	1	2	3
<i>Creb3</i>	B6J	CRISPR	75	1	36	112	44	1	1	2	9
<i>p19^{arf}</i>	B6J	TALEN	89	22	19	108	60	1	1	1	3
<i>p19^{arf}</i>	FVB/N	TALEN	104	1	25	129	60	1	1	1	3
<i>Il2ry</i>	B6J	CRISPR	135	11	81	216	42	1	2	2	8
<i>Lepr</i>	B6J	CRISPR	170	5	19	189	176	2	3	3	19
<i>Reep5</i>	B6J	CRISPR	265	7	77	349	2	1	3	3	5

<i>Lxra</i>	B6J	CRISPR	376	41	251	627	34	2	4	5	10
<i>Ciita</i>	B6J	CRISPR	503	2	115	618	47	1	3	5	19

*Gene lists were sorted in order of the distance from ATG to indel (bp). Refer to the schematic diagram in Figure S3 to understand each parameter.

Supplementary Table 4. Publications for the genome editing in mice using engineered nucleases

Gene name	Type of nuclease	Targeting strategy	Distance in mRNA (bp)			Exon				Method to detect expression	Accession No.	Ref
			from ATG to indel	Indel size	from indel to PTC	with ATG	Target	with PTC	last exon			
<i>Sepw1</i>	TALEN	first coding exon	0	2~266	0	1	1	0	6	WB	NM_009156	1
<i>Hprt</i>	Cas9	first coding exon	3	124	37	1	1	3	9	NT	NM_013556	2
<i>Pate1</i>	Cas9	first coding exon	3	+1	71	1	1	2	5	NT	NM_001199953	3
<i>Slx2</i>	Cas9	first coding exon	4	8	15	2	2	2	9	WB, ICC	NM_001200013	4
<i>Cetn1</i>	Cas9	first coding exon	17	8	38	1	1	1*	1	NT	NM_007593	5
<i>P11</i>	Cas9	first coding exon	21	31	48	2	2	2	3	RT, WB	NM_009112	6
<i>Prm1</i>	Cas9	first coding exon	25	5	114	1	1	1*	1	NT	NM_013637	5
<i>Wdr63</i>	Cas9	first coding exon	27	73	34	2	2	3	23	NT	NM_172864	7
<i>Ttc36</i>	TALEN	first coding exon	27	2	19	1	1	1	3	WB, RT	NM_138951	8
<i>Pate3</i>	Cas9	first coding exon	35	1	26	1	1	2	3	NT	NM_001167592	3
<i>Dmrt1</i>	Cas9	first coding exon	35	+1	412	1	1	2	5	NT	NM_015826	9
<i>Fam83h</i>	Cas9	first coding exon	39	26	42	2	2	2	5	RT, WB	NM_001168253	10
<i>Fabp7</i>	Cas9n	first coding exon	42	28	37	1	1	2	4	WB	NM_021272	11
<i>Mikl</i>	TALEN	first coding exon	54	2	1	2	2	2	11	WB	NM_001310613	12
<i>Psd2</i>	Cas9	first coding exon	65	112	5	1	1	1	4	WB, IHC	NM_001289602	13
<i>Mikl</i>	Cas9	first coding exon	101	1	102	2	2	2	11	NT	NM_001310613	14
<i>Pibf1</i>	TALEN	first coding exon	110	2~14	1	2	2	2	18	NT	NM_029320	1

<i>Dmrt3</i>	Cas9	first coding exon	121	8	333	1	1	1	2	NT	NM_177360	9
<i>Fam83h</i>	Cas9	first coding exon	133	26	42	2	2	2	5	RT, WB	XM_006520236	10
<i>FATS</i>	TALEN	first coding exon	154	151	159	2	2	2	8	WB	NM_001081331	15
<i>Tyr</i>	Cas9	first coding exon	230	1~32	20	1	1	1	5	NT	NM_011661	16
<i>Tet2</i>	Cas9	first coding exon	3008	8	104	3	3	3	11	NT	NM_001040400	17
<i>Ptch1</i>	Cas9	proximity to ATG	34	4~251	1	1	2	2	24	NT	NM_001328514	18
<i>Ccdc63</i>	Cas9	proximity to ATG	50	+1	154	2	3	4	12	NT	NM_001289809	7
<i>Notch1</i>	Cas9	proximity to ATG	77	5	47	1	2	2	34	IHC	NM_008714	19
<i>Jag1</i>	ZFN	proximity to ATG	84	2	19	1	2	2	26	NT	NM_013822	20
<i>Ccdc63</i>	Cas9	proximity to ATG	112	+1	154	3	4	5	13	NT	NM_183307	7
<i>Pate2</i>	Cas9	proximity to ATG	153	4	79	1	3	4	4	NT	NM_001033421	3
<i>Prss55</i>	Cas9	proximity to ATG	136	17	12	1	2	2	5	NT	NM_001081063	21
<i>Nxt2</i>	Cas9	proximity to ATG	140	101	5	1	3	3	4	NT	NM_172782	21
<i>Lyzl1</i>	Cas9	proximity to ATG	187	50	68	2	3	3	5	NT	NM_026092	21
<i>Plcd1</i>	Cas9	proximity to ATG	180	2~45	66	1	2	2	15	RT, IHC	NM_001293648	22
<i>Notch3</i>	ZFN	proximity to ATG	208	1	270	1	3	4	34	NT	NM_008716	20
32 cases			Ave: 76									

RT, RT-PCR; WB, Western blotting; IHC, Immunohistochemistry; ICC, Immunocytochemistry; NT, Not tested; Ave, Average;

*PTCs are present in the last exon.

Supplementary Table 5. Tumor spectrum of homozygous p53 mutant mice

Tissue	Diagnosis	Incidence	
Brain	Astrocytoma	2	3/21
	Neoplastic Perivascular cuffing	1	
Liver	Hypertrophy	1	13/21
	Inflammation	2	
	Leukemia	2	
	Lymphocytic leukemia	3	
	Necrosis	1	
	Histiocytic sarcoma	3	
	Malignant lymphoma	1	
	Focal lymphocytic infiltration	1	
kidney	Interstitial Nephritis	2	6/21
	Lymphocytic leukemia	3	
	Metaplasia	1	
spleen	Lymphoma	1	9/21
	Lymphocytic leukemia	2	
	Histiocytic sarcoma	2	
	Lympho sarcoma	1	
	Metaplasia	1	
	Hyperplasia	1	
	Malignant lymphoma	1	

Pancreas	Necrosis	1	1/21
Heart	Leukemia	1	2/21
	Lymphocytic leukemia	1	
Thymus	Thymoma Malignant	1	5/21
Lung	Hemangio sarcoma	1	9/21
	Leukemia	1	
	Lymphocytic leukemia	3	
	Metaplasia	1	
	Histiocytic sarcoma	2	
	Malignant lymphoma	1	
Lymph node	Leukemia	1	4/21
	Hemangio sarcoma	1	
	Lympho sarcoma	1	
	Histiocytic sarcoma	1	
	Lymphoma	1	
Small intestine	Leukemia	1	3/21
	Lymphocytic leukemia	1	
	Histiocytic sarcoma	1	
Colon	Lymphocytic leukemia	1	1/21
Ovary	Hemangio sarcoma	1	1/21
Bladder	Hypertrophy	1	3/21
	Hyperplasia	1	
	Lymphocytic leukemia	1	
Utreus	Histiocytic sarcoma	1	1/21

Prostate	Histiocytic sarcoma	1	1/21
Tumor	Hemangio sarcoma	1	5/21
	Fibro sarcoma	2	
	Histiocytic sarcoma	1	
	Malignant thymoma	1	

Supplementary Table 6. List of primers used for PAGE- or PCR-based genotyping

Target gene	Forward primer	Reverse primer	Size of PCR product	
			WT	Mutant
<i>Cd47</i>	GGTCGGTCGTTTCCCTTGAA	GATCCCCGAGCCACTCAC	147	125
<i>Ciita</i>	GCTGGAGAAAAAGCACTGGC	CATAGGCCTCCCGGGTCT (WT)	241	-
	ATGGAACTGGATGAAGAGACGG (Mutant)	CCCTCCCTTCTAGCCCTAGTT	-	157
<i>Creb3</i>	GTTGTGTTGGAAGCTGAGGA	CAGAGCAGTATGGGGGAT	788	649
<i>Il2rg</i>	CTCTCCCAGCTAACCTCCCT	GACTTCTACAGCCCCCTGA (WT)	200	-
	GTCCTCATGTCCAGTGCGAA	GGAGCACTGAGGTGTAG (Mutant)	-	259
<i>Lepr</i>	CTGCTGGAGCCCCAAACAATGC	TTCAACAATTGCTTCAGAAGCC	59	54
<i>Lxra</i>	GGCTACATATTATGAATCAG	GAAGTTTTAATCCCACTCA	514	473
<i>Lxrb</i>	AGCCTAGAGTAGTGTGTTCC	GAGAGATAGCGACAAAGAGA	655	653
<i>p16^{link4a} (FVB)</i>	GAGGAGAGCCATCTGGAG	CCTTGCCTACCTGAATCG	158	133
<i>p16^{link4a} (B6)</i>	GTTTAATGGGTGGCTCCGGT	CGTGCGGGCACTGCTGGAAGCCGG (WT)	220	-
	GGTGTTAGCGTGGGTAGCAG	TTGGGCGAAACCCAGC (Mutant)	-	455
<i>p19^{Arf} (FVB)</i>	GCTTCTCACCTCGCTTGTC	AGAGCGCAGCTCGCTG (WT)	177	-
	GCTTCTCACCTCGCTTGTC	GAGCGCAGCTCGCG (Mutant)	-	176

p19^{Arf} (B6)	TCCTCTCTAGCCTCAACAAC	AGGGTTTTCTTGGTGAAGTTC	94	90
p27^{Kip1} (FVB)	GATGTCAAACGTGAGAGTGT	CACTTGCCTGACTCGCT	180	155
p53 (FVB)	ATTTCCCTACTGGATGTCCCACC	TTTCCATAAGCCTGAAAATGTCTCCTGG (WT)	212	-
	GAGCTCCCTCTGAGACATT (Mutant)	TTCTCTCAGGCAAGGGGAGGATA	-	107
p53 (B6)	ATTTCCCTACTGGATGTCCCACC	TTTCCATAAGCCTGAAAATGTCTCCTGG (WT)	212	-
	TCGAGCTCCCTCTGAGCA (Mutant)	TTCTCTCAGGCAAGGGGAGGATA	-	115
Reep5	CAGATGTAGGAGCTGTGGCA	AATGACTCCGCTTCAGCACT	533	526
Rtp4	CTCCTTTATTTCAATCCCCACAC	GATGGTTAGGGTCGCTTGTA	648	601

Mutant, specifically binds to the site of mutation in mutant alleles but not WT alleles; WT, specifically binds to the WT alleles but not to mutant alleles. Blue fonts represent the sequences downstream of deletion site in mutant alleles. Red fonts indicate the sequences that are only in the WT alleles because they were deleted in the mutant alleles.

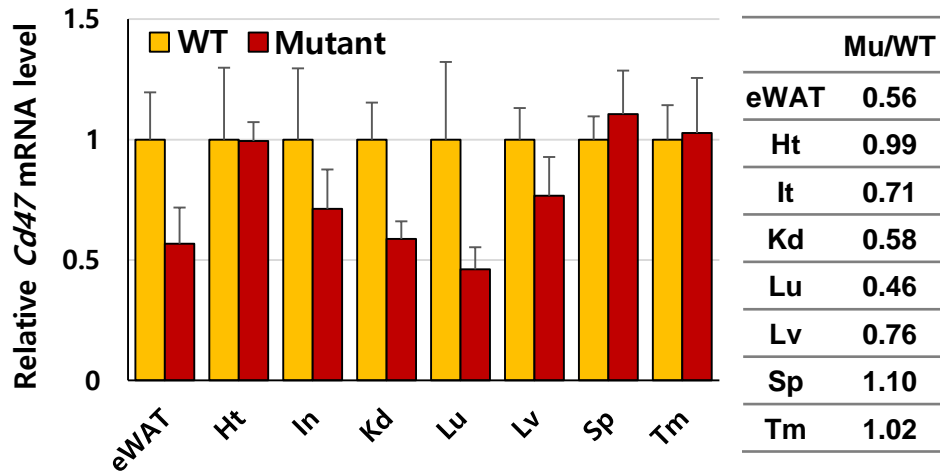
Supplementary Table 7. List of primers used for RT-qPCR reactions

Target gene	Forward primer	Reverse primer	bp	Reference sequence
<i>Actin</i>	CATTGCTGACAGGATGCAGAAGG	TGCTGGAAGGTGGACAGTGAGG	138	NM_007393
<i>Cd47</i>	GGCGCAAAGCACCGAAGAAATGTT	CCATGGCATCGCGCTTATCCATTT	179	NM_010581
<i>Ciita</i>	GATCAGTACGTGTTCCAGGATACC	AGTTCAGTGAGGTCCTAGAG	208	NM_007575
<i>Creb3</i>	TGTTGCACCGCAAGCTTCGTG	AAGATCCCAGAGTGGCCAGTG	206	NM_013497
<i>Gapdh</i>	AGGTCGGTGTGAACGGATTTG	GGGGTCGTTGATGGCAACA	95	NM_008084
<i>Hprt</i>	CTGGTGAAAAGGACCTCTCGAAG	CCAGTTTCACTAATGACACAAACG	146	NM_013556
<i>Ii2rg</i>	AATTCCCCCATCAAGAATC	GTAGTCTGGCTGCAGACTCTCA	109	NM_001308535
<i>Lepr</i>	ACTGTGCAGCTGAGGTATCAC	GGCTGGACTGCTCCAATTACT	507	NM_001122899
<i>Lxra</i>	ATGCTACGCAAGGCTCTCTCC	GCAAGTGTTTGCCCTTCGCC	203	NM_013839
<i>Lxrb</i>	ATCTGAGCCAGCAGCCAGCA	TGACTTTGGGCTGGTCGGAGAA	188	NM_009473
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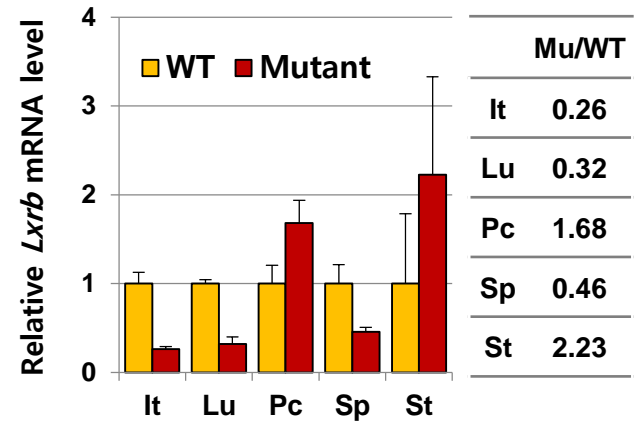
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<i>p27^{Kip1}</i>	GCGGTGCCTTTAATTGGGTCT	GGCTTCTTGGGCGTCTGCT	230	NM_009875
<i>p53</i>	CCCCTGTCATCTTTTGTCCCT	TTTCCTTCCACCCGGATAAG	326	NM_011640
<i>Reep5</i>	GAGATGTAGGCTGGGTATCCGAA	GGTTCCTGCACGAGAAGAACTG	181	NM_007874
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Supplementary Figure 1

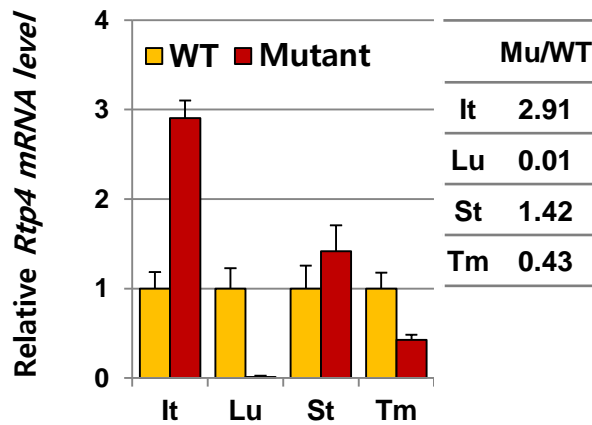
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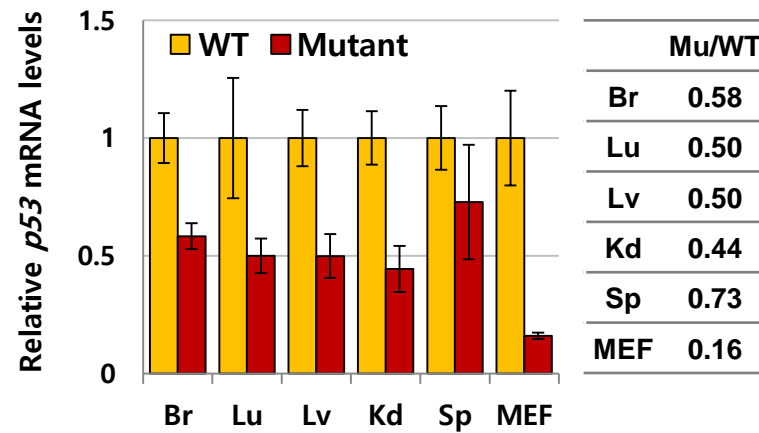
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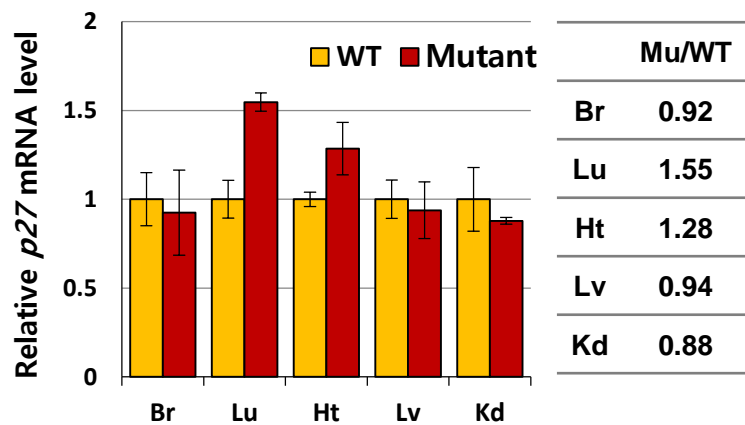


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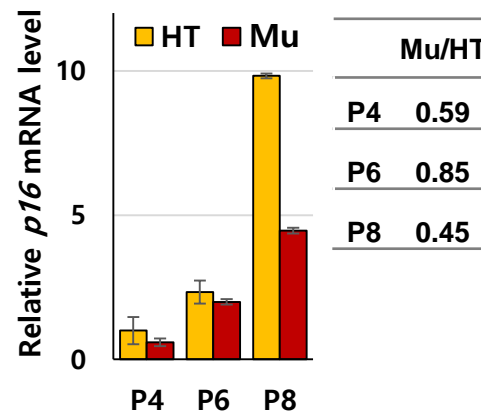


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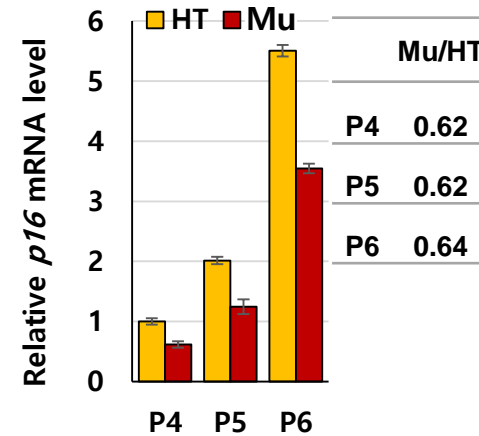
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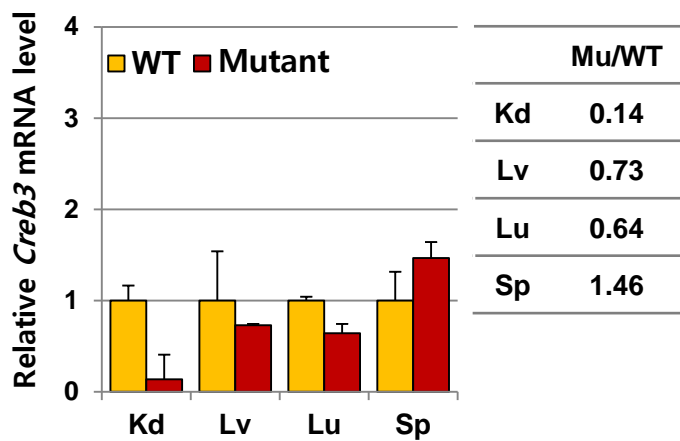
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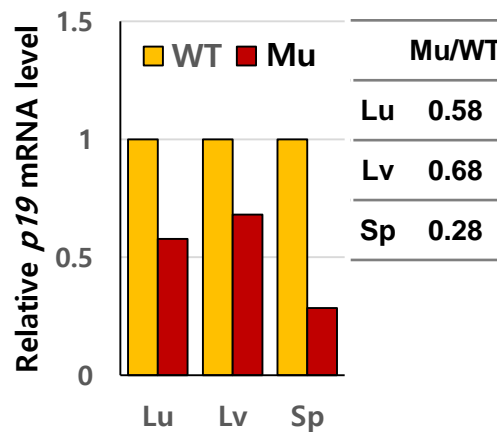
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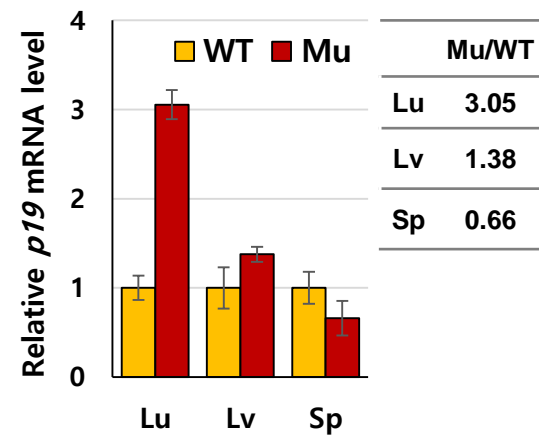
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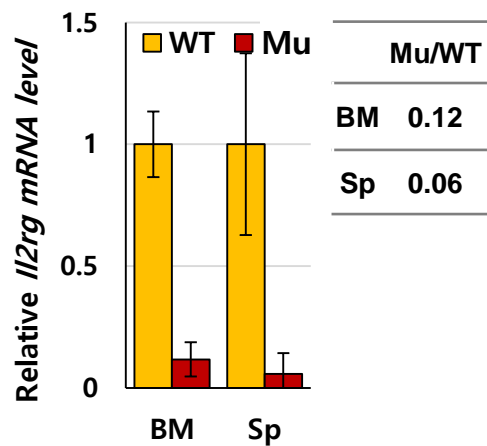


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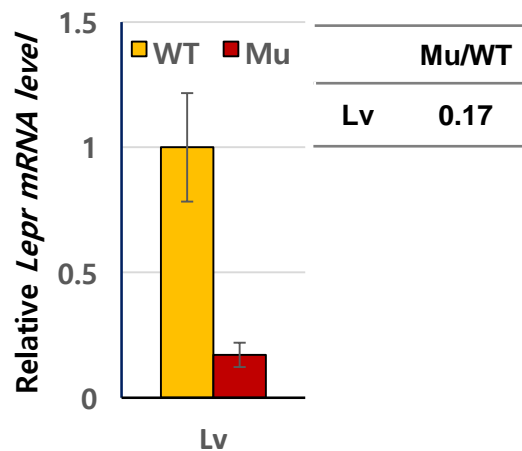


Supplementary Figure 1 (continued)

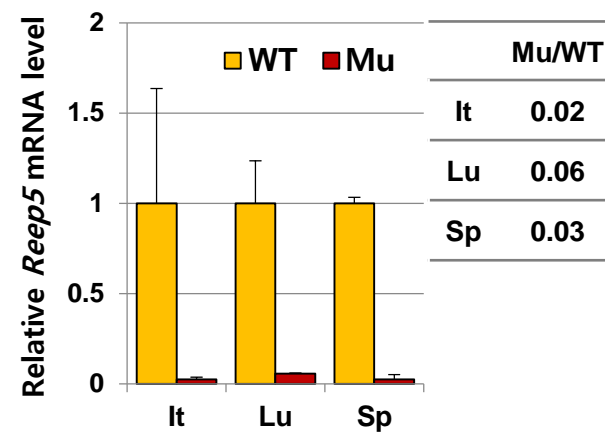
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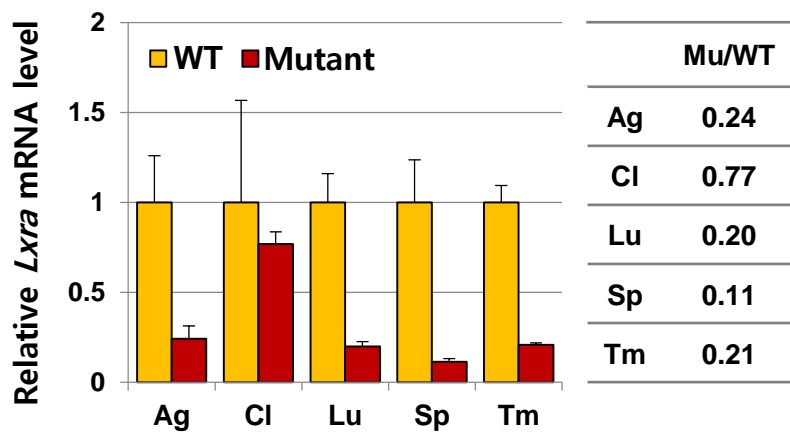
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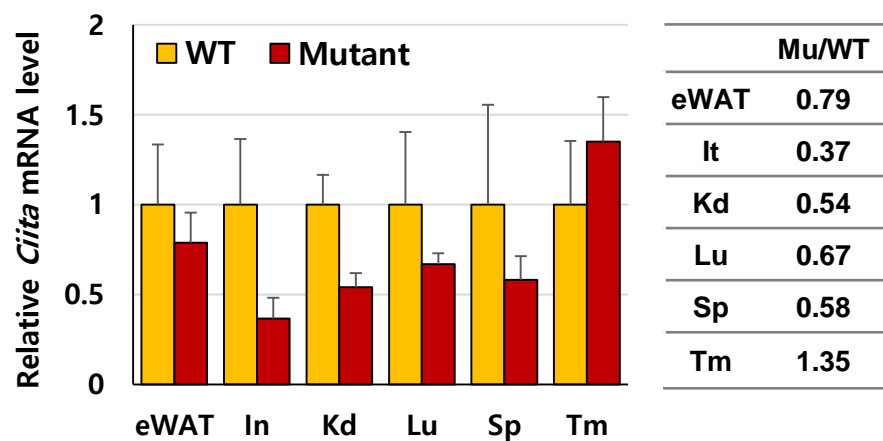
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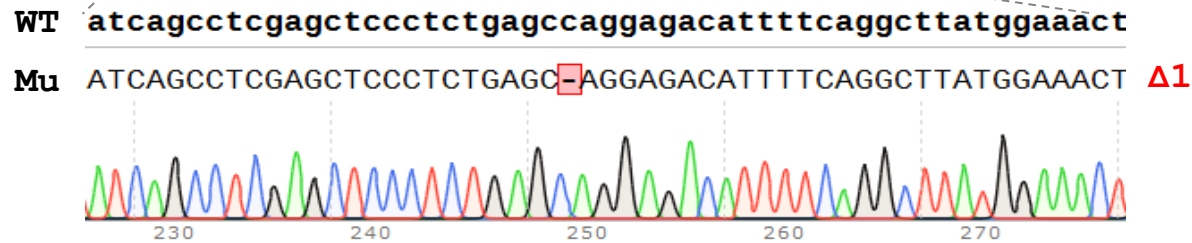
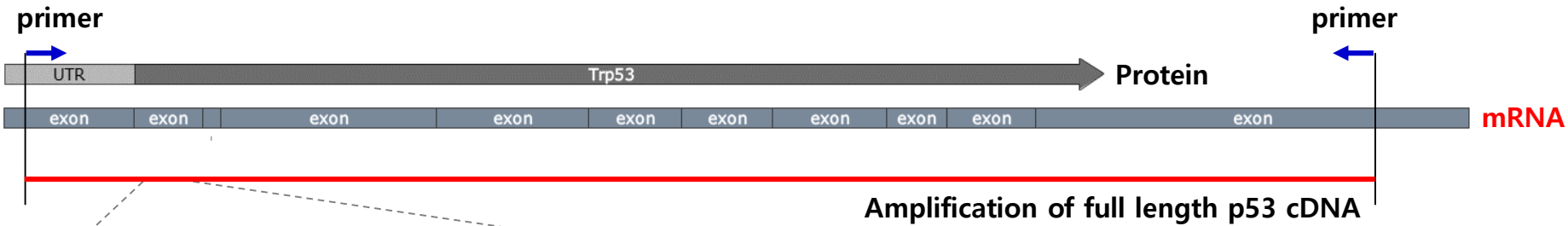
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Supplementary Figure 2

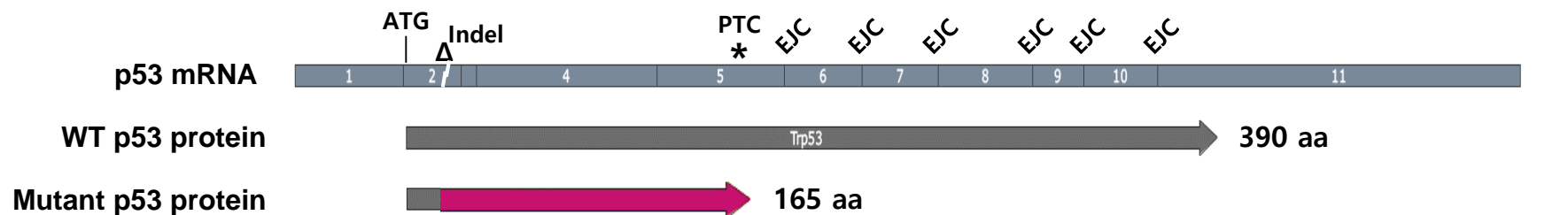


> Mutant p53 cDNA sequence (c.54_54del1)

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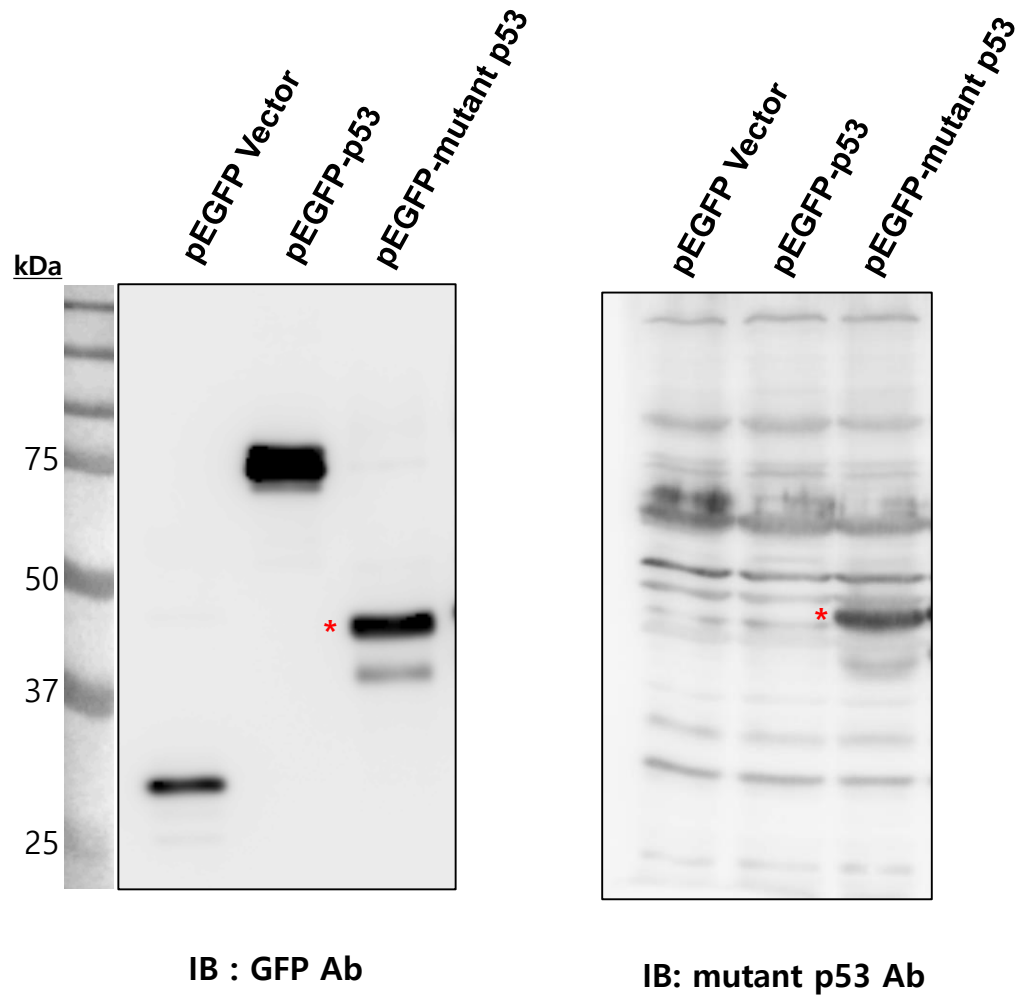
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Supplementary Figure 3



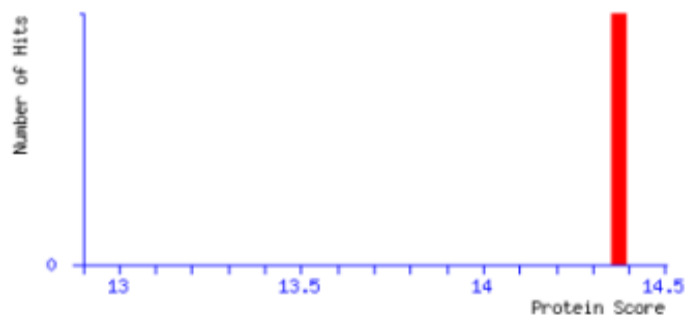
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WT	61	LRVSGAPAAQ	DPVTETPGPV	APAPATPWPL	SSFVPSQKTY	QGNYG FHLGF	LQSGTAKSVM	120
Mu	61	SECQELLQHR	TLSRPLGQW	PLPQPLHGPC	HLLSLLK KLT	RATMASTWAS	CSLGQPSLLC	120
WT	121	CTYSPPLNKL	FCQLAKTCPV	QLWVSATPPA	GSRVRAMAIY	KKSQHMT EVV	RRCPHHERCS	180
Mu	121	ARTLLPSISY	SASWRRRALC	SCGSAPHLQL	GAVSAPWPST	RSNST*	180	
WT	181	DGDGLAPPQH	LIRVEGNLYP	EYLED RQTFR	HSV VVPYEPP	EAGSEYTTIH	YKYM CNSSCM	240
WT	241	GGMNRRPILT	IITLEDSSGN	LLGRDSFEVR	VCACPGRDRR	TEEENFRKKE	VLCPELPPGS	300
WT	301	AKRALPTCTS	ASPPQKKKPL	DGEYFTLKIR	GRKRFEMFRE	LNEALELKDA	HATEESGDSR	360
WT	361	AHSSYLKTKK	GQSTSRHKKT	MVKKVGPDS*				

Supplementary Figure 4



Supplementary Figure 5

Mascot Score Histogram



Protein sequence coverage: 27%

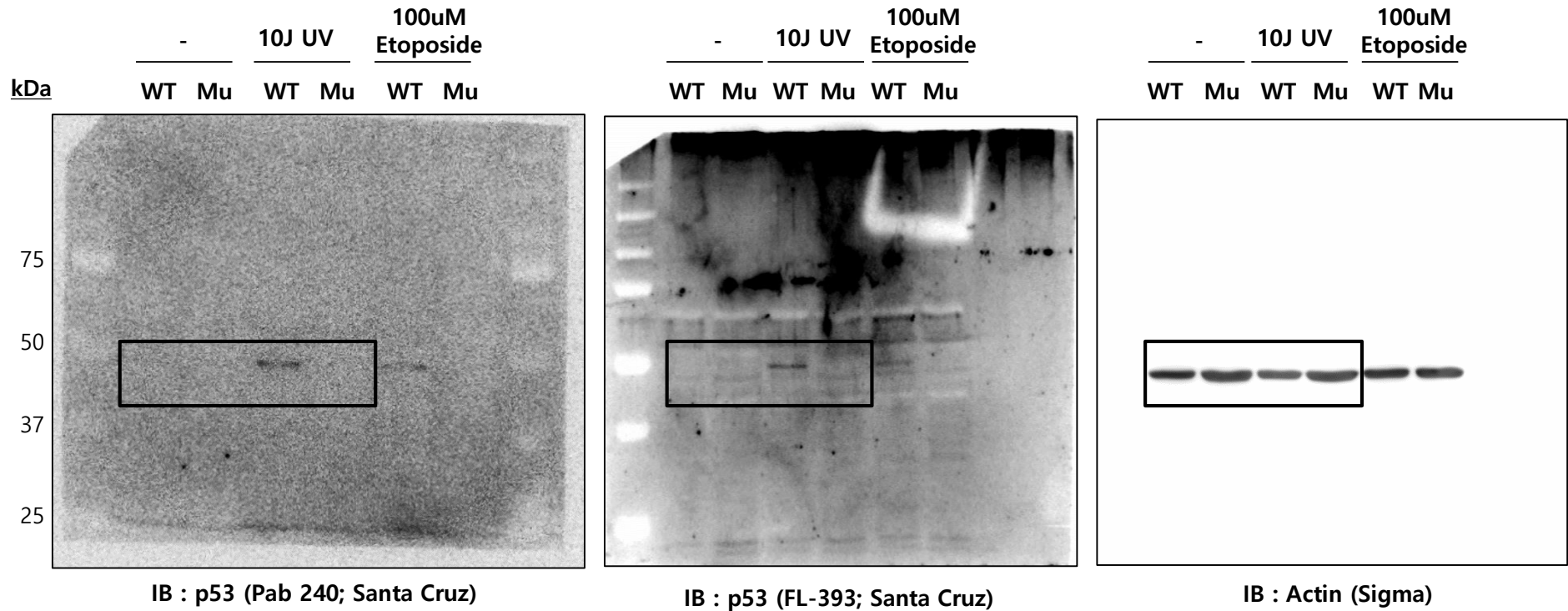
Matched peptides shown in **bold red**.

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Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Unique	Peptide
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<input checked="" type="checkbox"/> 1372	776.2241	2325.6504	2325.0828	0.5676	1	(12)	0.069	1	U	-. <u>MTAMEESQSDISLELPLSRR</u> .H + 2 Oxidation (M)
<input checked="" type="checkbox"/> 1373	776.2247	2325.6522	2325.0828	0.5694	1	(5)	0.34	1	U	-. <u>MTAMEESQSDISLELPLSRR</u> .H + 2 Oxidation (M)
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Supplementary Figure 6



Supplementary Figure Legends

Supplementary Figure 1. Abnormal mRNAs detected in mutant mice generated by engineered nuclease. The mRNA expression levels of each gene of interest were determined by RT-qPCR according to each tissue samples. The genes are listed as follows: *Cd47* (a), *Lxrb* (b), *Rtp4* (c), *p53* (d) in FVB/N mice; *p27^{Kip1}* (e), *p16^{Ink4a}* (f) in C57BL/6J strain; *p16^{Ink4a}* (g) in FVB/N strain; *Creb3* (h), *p19^{Arf}* (i) in C57BL/6J strain; *p19^{Arf}* (j) in FVB/N strain; *Il2rg* (k), *Lepr* (l), *Reep5* (m), *Lxra* (n), and *Ciita* (o). Numerical values of normalized ratios between wild-type (WT) and mutant (Mu) are represented next to each bar graph. The experiments were performed in triplicates, and error bars indicate means \pm SD. P denotes the passage number in MEFs of *p16^{Ink4a}* mutant mice. Ag, adrenal gland; BAT, brown adipose tissue; BM, bone marrow; Br, brain; Cl, colon; eWAT, epididymal white adipose tissue; Ht, heart; In, intestine; iWAT, inguinal white adipose tissue; Kd, kidney; Lu, lung; Lv, liver; MEF, mouse embryonic fibroblasts; Pc, Pancreas; Sp, spleen; St, Stomach; Tm, thymus.

Supplementary Figure 2. The sequence of full-length 1.5 kb *p53* cDNA cloned from the liver of *p53* mutant mice. The schematic diagram represents the p53 protein and mRNA structures. Red line denotes an amplified and cloned PCR product from the first strand of cDNA using the primers represented by blue arrows. The chromatogram shows the sequencing result for the enlarged region of *p53* sequence with deletion mutation. A dash (-) denotes a deleted nucleotide in the mutant *p53* cDNA sequence (c.54_54del1). Red font and red box indicate the start codon and the premature termination codon, respectively. Mu, Mutant; WT, wild-type.

Supplementary Figure 3. The amino acid sequence of mutant *p53* which can be expressed in *p53* mutant mice. The schematic diagram represents the p53 mRNA and its mutant p53 protein sequence compared to WT p53 protein sequence. The red fonts and red box indicate the mutant p53 sequence and the epitope

region of mutant p53 antibody, respectively. Deletion mutations are noted as Δ in the mRNA structure. ATG, translational initiation codon; PTC, premature termination codon; EJC, exon-junction complex; aa, amino acid. Mu, Mutant; WT, wild-type.

Supplementary Figure 4. Western blot analysis of mutant *p53* expression in 293T cells using the mutant p53 antibody we generated. The 293T cells were transfected with the indicated plasmids (pEGFP, pEGFP-p53, and pEGFP-mutant p53). The mutant p53 antibody specifically detected a mutant p53 protein but not WT p53 protein. Left panel shows a Western blot with GFP antibody to confirm the expression of EGFP-tagged p53 and mutant p53 proteins. Asterisks indicate the mutant p53 proteins.

Supplementary Figure 5. Mascot search result for MS/MS measurements of mutant p53 protein. *p53* mutant MEFs treated with 10 J/m² UV were immunoprecipitated using the mutant p53 antibody, and separated by SDS-PAGE on 10% gel. The gel was stained with Coomassie Blue R250. Major protein bands were excised from the gel and identified by MS/MS. The distribution of the Mascot score for MS/MS measurements of mutant p53 protein is shown as histograms. The peptides of mutant p53 protein found in the MS/MS analysis are indicated by red fonts above the full amino acid sequence of the mutant p53 protein. The box in the figure highlights the peptide sequence of the mutant p53 protein.

Supplementary Figure 6. Full-length blots of the experiments shown in Fig. 2b. Uncropped images are boxed in the regions shown in the main text. The etoposide results were excluded from the main text.

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