

lrh, TSURUOKA ET AL.

rrh, FAST NEUTRONS AND MEDULLOBLASTOMA IN *Ptch1^{+/−}* MICE

SUPPLEMENTARY INFORMATION

High Relative Biological Effectiveness of 2 MeV Fast Neutrons for Induction of Medulloblastoma in *Ptch1^{+/−}*

Mice with Radiation-specific Deletion on Chromosome 13

Chizuru Tsuruoka,^a Mutsumi Kaminishi,^a Mayumi Shinagawa,^a Yi Shang,^a Yoshiko Amasaki,^a Yoshiya

Shimada^b and Shizuko Kakinuma^{a,1}

^aDepartment of Radiation Effects Research, National Institute of Radiological Sciences, National Institutes for

Quantum and Radiological Science and Technology, Chiba, Japan; and ^bInstitute for Environmental Science,

Rokkasho-mura, Japan

SUPPLEMENTARY INFORMATION

Table S1. Experimental groups and results of MBs development.

Table S2. *Ptch1* primers and PCR conditions.

Table S3. Primers for sequencing.

Fig. S1. Structure of mouse *Ptch1* and location of primers used for mutation analyses. Panel A: Genomic DNA (upper) and cDNA (lower) of wild-type *Ptch1*. Topological domains (thick lines), which include glycosylation sites (triangles) and the sterol-sensing domain (dashed double-headed arrows), are indicated above the cDNA. The cDNA was divided into four segments (dashed lines with numbers) in the sequence analysis. Arrows flanking each segment indicate the primers listed in Supplementary Table S2. Panel B: Relevant exons of knockout (B6) and wild-type (C3H) alleles of *Ptch1*. Exons 6 and 7 in the genome are replaced by a neomycin resistance cassette. Dashed line reproduces segment 1 in panel A. Primers “B6_F” and “B6_R” were designed to sequence the B6 allele, whereas primers “C3H_F” and “C3H_R” are for the C3H allele. Primers “B6_R” and “C3H_R” were designed to analyze exons 3 and 4 of cases no. 4 and 12 (see Table 1), and primers “B6_F” and “C3H_F” were designed to analyze exons 9 and 10 of cases no. 8, 13 and 25 (see Table 1). Panel C: Primers for genomic sequencing to identify the cause of exon 17 deletion in cDNA from case no. 1. A 4,163-bp region between exons 16 and 18 (dashed line) was first amplified and then sequenced using primers referred to by the bracketed numbers. Alleles were identified using the two single-nucleotide polymorphisms (SNPs, triangles). Panel D: Primers for genomic sequencing to identify the cause of deletion of exons 21 and 22 in cDNA from case no. 21. A 6,167-bp region from exons 20 to exon 23 (dashed line) was first amplified and then sequenced using primers referred to by the bracketed numbers. Alleles were identified using the three SNPs (triangles).

Fig. S2. MB-free Kaplan-Meier plots after γ rays and neutron irradiation. * $P < 0.01$, ** $P < 0.05$ vs. nonirradiated group by log-rank test.

Fig. S3. Loss of heterozygosity (LOH) of chromosome 13 in medulloblastomas from nonirradiated and neutron- and γ -ray irradiated groups. Panel A: Nonirradiated and neutron-irradiated groups. Panel B: γ -ray

irradiated group. Chromosomal regions and positions of polymorphic markers are shown on the upper left side in the schematic in panel A. Open circles indicate loss of the C3H allele, and filled circles indicate retention of both the B6 and C3H alleles. Results obtained for consecutive markers for individual MBs are aligned vertically; “ \times ” indicates unavailable results. S-type or S = LOH in all consecutive markers distal to *Ptch1*; R-type or R = LOH confined to interstitial markers (big circles) or a polymorphism at position 4,016 in exon 23 of *Ptch1* (small circles); N-type or N = no evidence of LOH; “No” indicates tumors that were unavailable for LOH analysis. The numbers at the top of each column indicate the number of MBs with each LOH pattern. *These MBs showed multiple stretches of LOH.

Fig. S4. Mutations detected in *Ptch1* of medulloblastomas. Panels A–C: Characterization of mutations in MBs from nonirradiated (panel A), neutron-irradiated (panel B), and γ -ray irradiated (panel C) mice. Sequences of the wild-type allele (upper) and identified mutations (lower) are indicated along with schematics for the relevant region of the cDNA (right-side bottom), in which solid boxes indicate exons, dashed boxes indicate deleted exons, and arrowheads indicate mutation sites. Numbers in the upper left side of each panel indicate the case number (see Table 1). Upper- and lowercase letters represent exonic and intronic sequences, respectively. Letters in blue = insertions; in green = duplications; in orange = base substitutions; in red = microhomology presumably used in end joining, which generated the deletion.

Fig. S5. Kaplan-Meier plots for the incidence of S- and R-type medulloblastomas. Separate graphs are shown for γ -ray irradiated and neutron irradiated groups. Plotted are the ages at death or moribundity of mice with MBs that were retrospectively determined to be S- or R-type; N-type tumors were treated as S-type. Data for mice irradiated with γ rays at E14 (0.1 and 0.5 Gy) and E17 (0.1 and 0.5 Gy) and P1 (0.1 and 0.5 Gy) and P4 (0.1 and 0.5 Gy) and for the nonirradiated group were reproduced from a previously reported study (29). Plots for the P1 group are the same as in Fig. 3. * $P < 0.01$, ** $P < 0.05$ vs. nonirradiated group by log-rank test.

Supplementary Table S1. Experimental groups and results of MBs development

Exposure	Age at irradiation	Dose (Gy)	No. of mice	No. of mice reaching moribundity (%)	No. of mice with MBs (%)	Time of moribundity (days), mean [range]
Non-irradiated ^a		0	58	32 (55)	31 (53)	179.3 [86 - 323]
Neutrons	E14	0.1	52	44 (85)	43 (83)	127.0 [77 - 328]
		0.5	53	52 (98)	46 (87)	95.3 [62 - 358]
	E17	0.1	69	67 (97)	67 (97)	118.5 [82 - 312]
		0.5	40	39 (98)	39 (98)	91.3 [71 - 179]
	P1	0.025	57	38 (67)	34 (60)	147.4 [78 - 303]
		0.05	48	43 (90)	40 (83)	148.9 [71 - 397]
		0.1	43	35 (81)	33 (77)	136.3 [65 - 291]
		0.5	37	37 (100)	37 (100)	96.4 [80 - 126]
	P4	0.05	46	26 (57)	24 (52)	168.3 [90 - 332]
		0.1	53	29 (55)	27 (51)	183.9 [74 - 338]
		0.5	39	33 (85)	29 (74)	161.8 [83 - 294]
	P10	0.1	58	33 (57)	23 (40)	207.8 [99 - 429]
		0.5	52	35 (67)	13 (25)	152.9 [102 - 175]
γ -rays	E14	0.1 ^a	69	33 (48)	29 (42)	176.3 [70 - 328]
		0.5 ^a	53	31 (58)	28 (53)	155.4 [77 - 362]
	E17	0.1 ^a	47	25 (53)	21 (47)	163.4 [85 - 317]
		0.5 ^a	50	37 (74)	35 (70)	171.6 [93 - 382]
	P1	0.05	48	19 (40)	19 (40)	183.6 [71 - 310]
		0.1 ^a	60	33 (55)	31 (52)	183.9 [99 - 352]
		0.2	39	24 (62)	23 (59)	187.4 [69 - 385]
		0.5 ^a	56	43 (77)	37 (66)	163.0 [64 - 369]
	P4	0.1 ^a	66	33 (50)	32 (48)	183.8 [73 - 351]
		0.5 ^a	51	27 (43)	21 (41)	179.3 [81 - 289]
	P10	0.5	47	20 (43)	17 (36)	199.6 [68 - 355]

E, embryonic; P, postnatal day.

^a Previous data (1).

Supplementary Table S2. *Ptch1* primers and PCR conditions

Primer name	Position	Product (bp)	Forward primer (5' to 3')	Reverse primer (5' to 3')	Cycles	MgCl ₂ (mM)
<i>cDNA</i>						
1	240–1,651 bp	1,412	TCAAAAGAACTGCGGCAAGT	TCAATGCGCCATGAAGAAG	32	2.0
2	1,461–3,143 bp	1,683	GGTTTGCCTTCTTGCTC	AGAGCCAGGACCATGACAAT	32	2.0
3	2,219–3,143 bp	925	TCCTCTTTCTGGGCTTG	AGAGCCAGGACCATGACAAT	32	1.5
4	2,986–4,305 bp	1,320	CCCAATGGCTACCCCTCCT	TCAGTTGGAGCTGCTCCCCA	32	1.5
<i>Genomic</i>						
Ex3	Exon 3	395	CGTTGCTAGTTGTCCAAGT	GTGGCAGCGCTGAGAATTAT	30	1.5
Ex4	Exon 4	279	CGGCTTCTCCTTGGCAAAA	TTGAACACTTCCCCTCTCTG	30	1.5
Ex9	Exon 9	499	TATCAGCAGACGGTTGGAGG	GGGAAGGGAGGACACAAAAA	30	1.5
Ex10	Exon 10	258	CCTTCCCTCTCTCCGATTCC	GAAAGGTGTGTTGGCCAGAG	30	1.5
Ex16	Exon 16	448	TCCTGGCTTCTGGGACTGGGC	TACACTGCCAGCCCTATTCC	30	1.5
Ex17	Exon 17	496	TATTCTGTGGGACTGGGC	ACACACCCTCATCTGTCTCC	30	1.5
Ex18	Exon 18	392	GTCCCCAGCTTACCTCACTT	AAACAAAACCTCCGGCTGC	30	1.5
Ex19	Exon 19	265	AGGCCACATCGAGACTTG	TCTGTCAGTGTGAGCCAGTT	30	1.5
Ex21	Exon 21	416	GCAGGGTGTGGAGGAATCTA	GTTGACCGAAAAGCCCAGAC	30	1.5
Ex22	Exon 22	600	TGCCAGAACATCGTGAGTGTCT	TTTCCTGTGATTGACCCCG	32	1.5
Ex21,22	Exon 21,22	1,044	TTGACTTCTCAGCCCAGCAG	TTCTTGACCCCCATCTGTCT	32	1.5
Ex16–18	Exons 16–18	4,163	ACTGGGAGGATCATGCCAAA	GCAGGCTGATGTATTGCTCC	30	2.0
Ex20–23	Exons 20–23	6,167	CACTCTGCTGGGTGTACTGA	GACAAGGAGGCCAGAGTCCAG	30	1.5

Supplementary Table S3. Primers for sequencing

Target	Primer ID	Start site (bp)	Primer sequence (5' to 3')	Direction
cDNA	B6F	1,045 (B6)	AGCATAACCTCCTCGCTCAC	F
240–1, 651 ^a	B6R	1,063 (B6)	GTGAGCGAGGAGGTATGC	R
	C3HF	713 (C3H)	CTCCTTACGGTGGACAAACT	F
	C3HR	986 (C3H)	ACAATCAACTCCTCGCCA	R
gDNA	1F	38,995	TTGAGTGTAGGTGTGGCAG	F
Exons 16–18 ^b	2F	39,511	AGATATCATTCCTCTCCTGGGG	F
	3F	40,012	ACACAGGTCATCAGAGGCTG	F
	4F	40,524	GCATCATTAATCCGAGCGCT	F
	5F	41,002	TCGCTAGGGATTGTGTGTTT	F
	1R	39,387	TTGCACGCCACTATAAAGCA	R
	2R	39,889	GTAGGTCTGTGGCTTGC	R
	3R	40,389	AATACCCATGCCTCACAGT	R
	4R	40,888	AACGCTCACTCTAACGTG	R
	5R	41,893	AGCCACCTCTTGAGCCTTT	R
gDNA	6F	48,025	TGTGTTGGGTCCCTCTGA	F
Exons 20–23 ^b	7F	48,597	TTTCTGAGTTCAAGGCCAGC	F
	8F	49,192	AATATGCTCCCTCATCATCTGG	F
	9F	50,402	TGGGAACGCAGAACTCACT	F
	10F	50,988	CACAGGCAGGAGATTTCATCA	F
	11F	51,589	CGATGTCTGGCTCTCTGGT	F
	12F	52,222	AATCACAGGAAACCACCAAGC	F
	13F	52,815	GTGTATTCCAGTCCCAGGCT	F
	14F	53,387	GTTGAGATCTATGGGCAGTGG	F
	6R	47,781	GTCCCCCTCACAGTTCTGAA	R
	7R	48,396	CAATGCTACCAAGGACAGCC	R
	8R	49,015	TTCACTTATTCCATGCATTCGG	R
	9R	49,590	ACCACAAAGCAGAGATGGGT	R
	10R	50,209	AAGAGAGAGGGACGGCTAGA	R
	11R	50,797	AGGCAGCTAACCAACTGTGA	R
	12R	51,383	TGACCTCAGGACACGGTC	R
	13R	51,993	CCCTGCTGTGCTTCGTATTG	R
	14R	52,604	GCCACAAAGCTACGGAAAG	R
	15R	53,194	GAAGCAAATGTACTGTAAGAGCA	R

^a Primers for sequencing the strain-specific allele (B6 and C3H, as indicated) in the PCR product corresponding to position 240–1,651 of the cDNA.

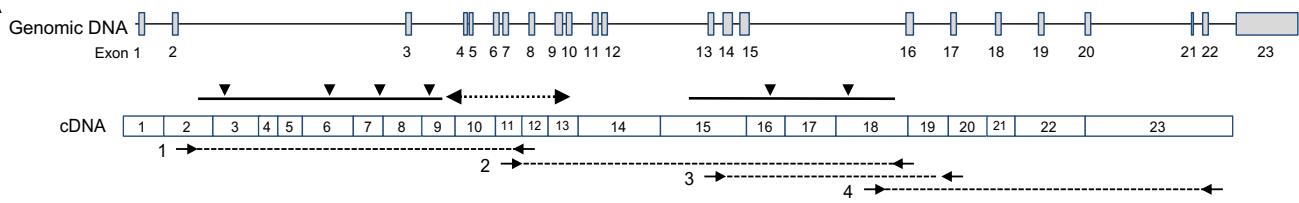
^b Primers for sequencing the regions encompassing the deletion mutation in the PCR products of exons 16–18 or exons 20–23 of the genomic DNA (gDNA).

REFERENCE

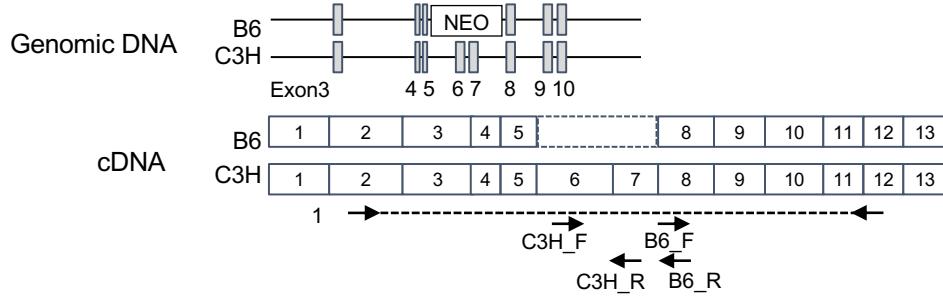
1. Tsuruoka C, Blyth BJ, Morioka T, Kaminishi M, Shinagawa M, Shimada Y, et al. Sensitive detection of radiation-induced medulloblastomas after acute or protracted gamma-ray exposures in Ptch1 heterozygous mice using a radiation-specific molecular signature. Radiat Res 2016; 186:407-14.

Supplementary Figure S1

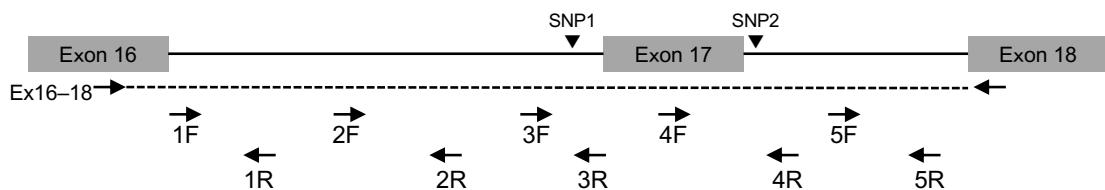
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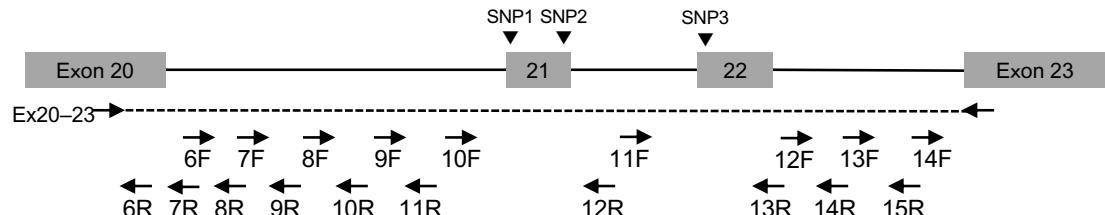
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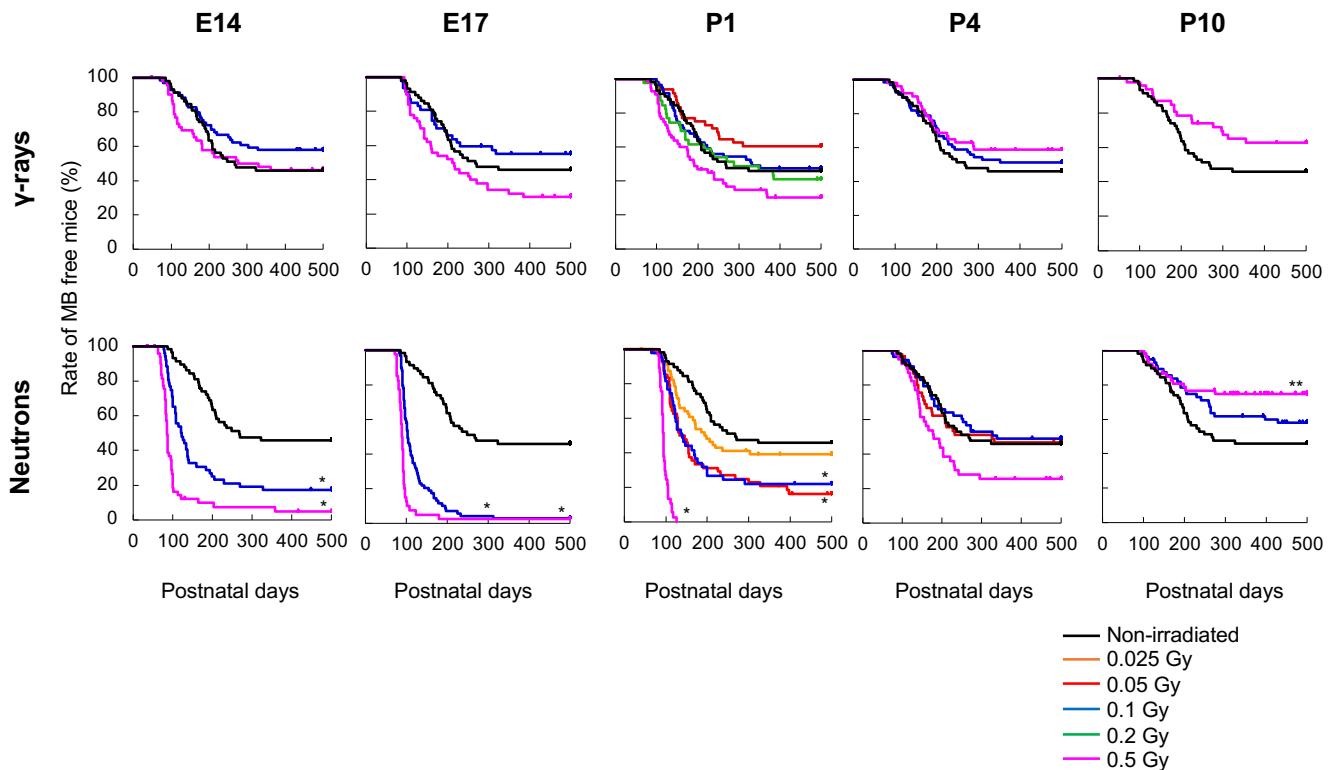
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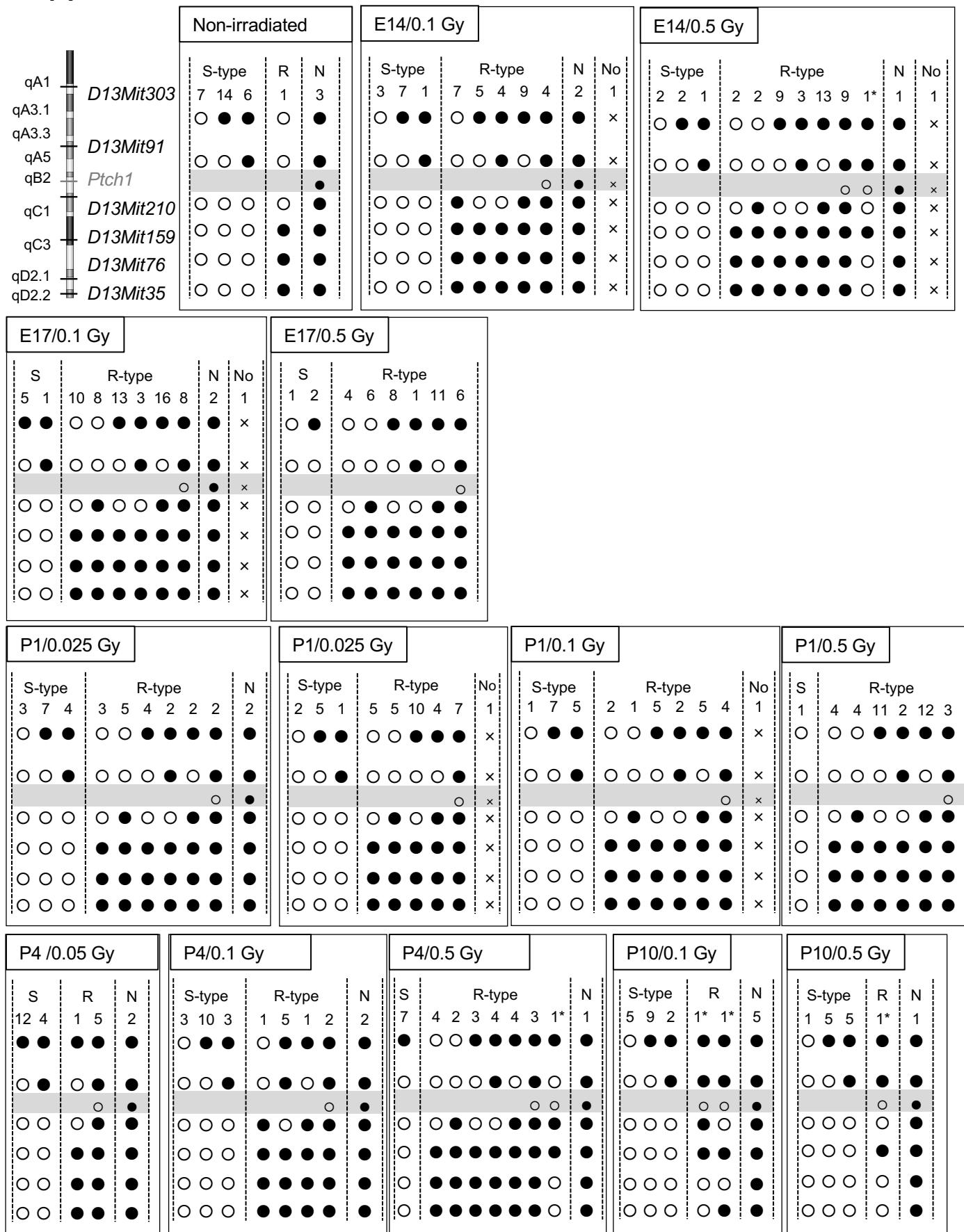


Supplementary Figure S2

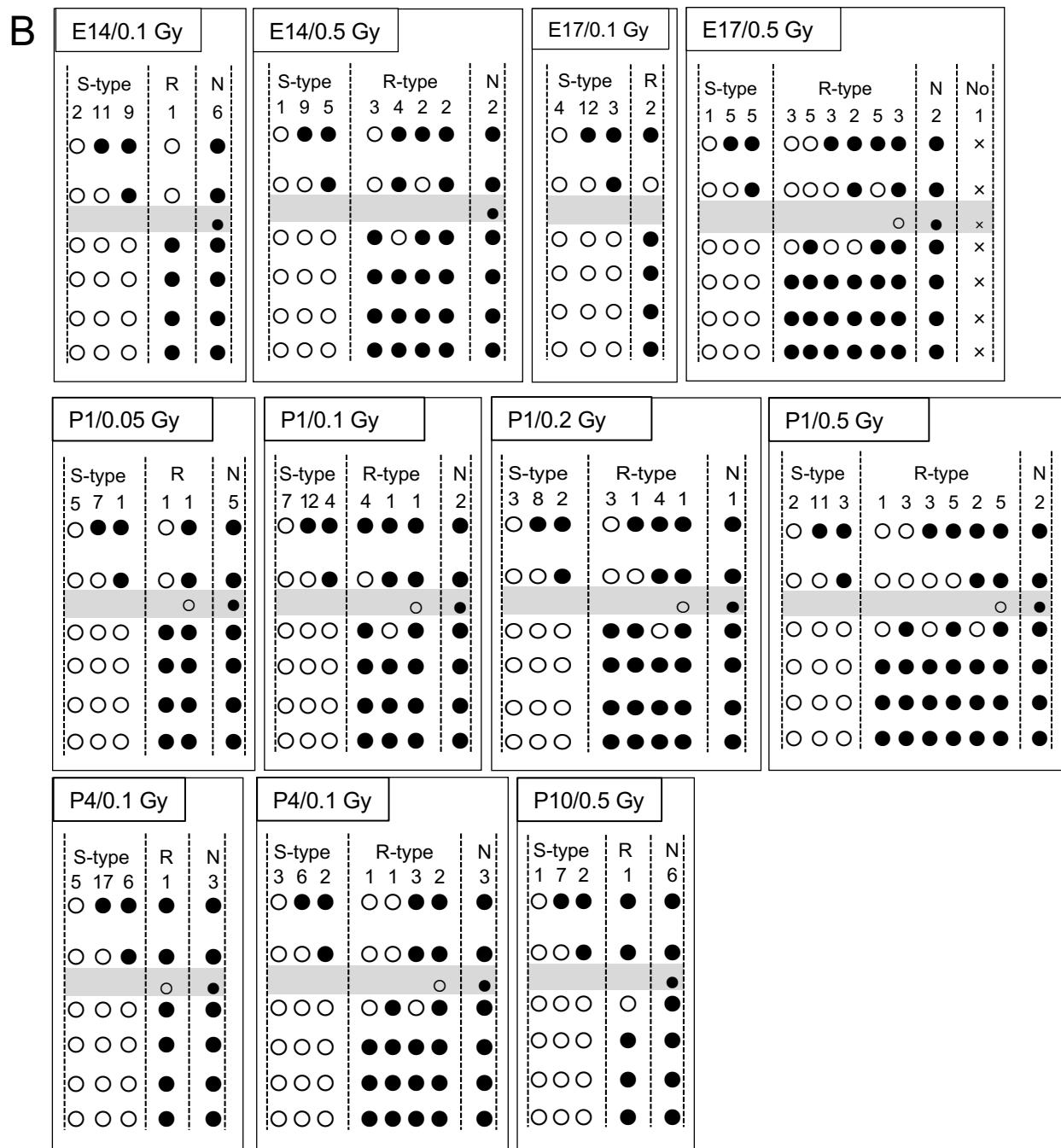


Supplementary Figure S3

A

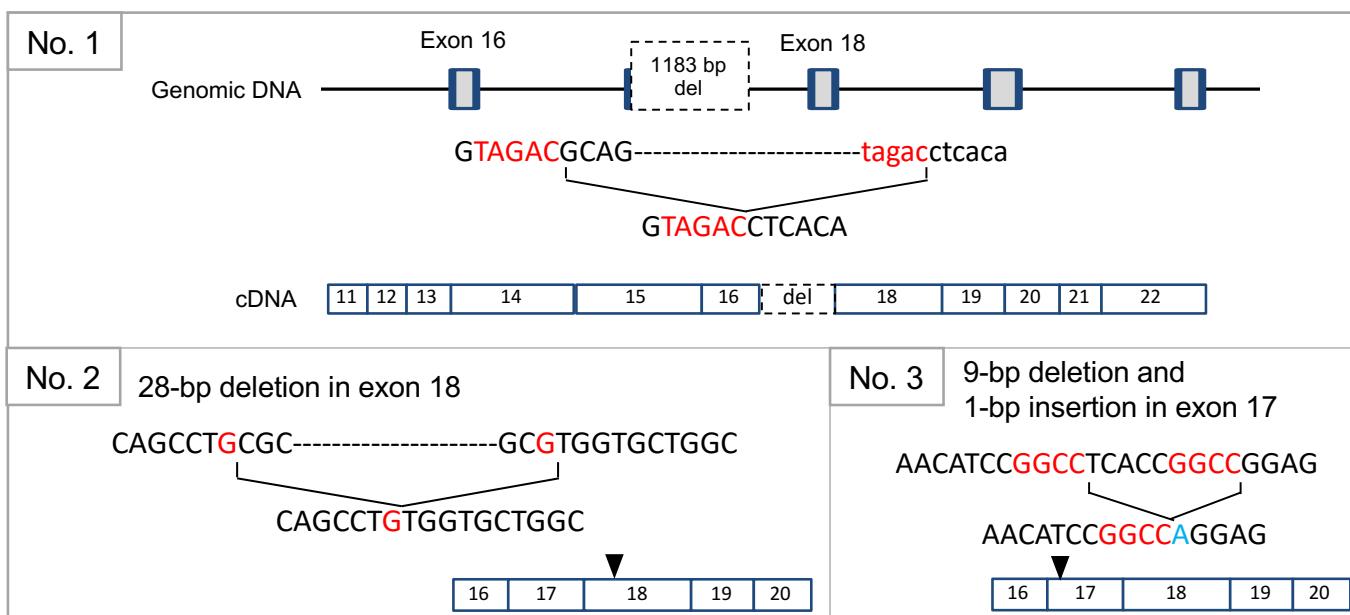


Supplementary Figure S3 (continued)

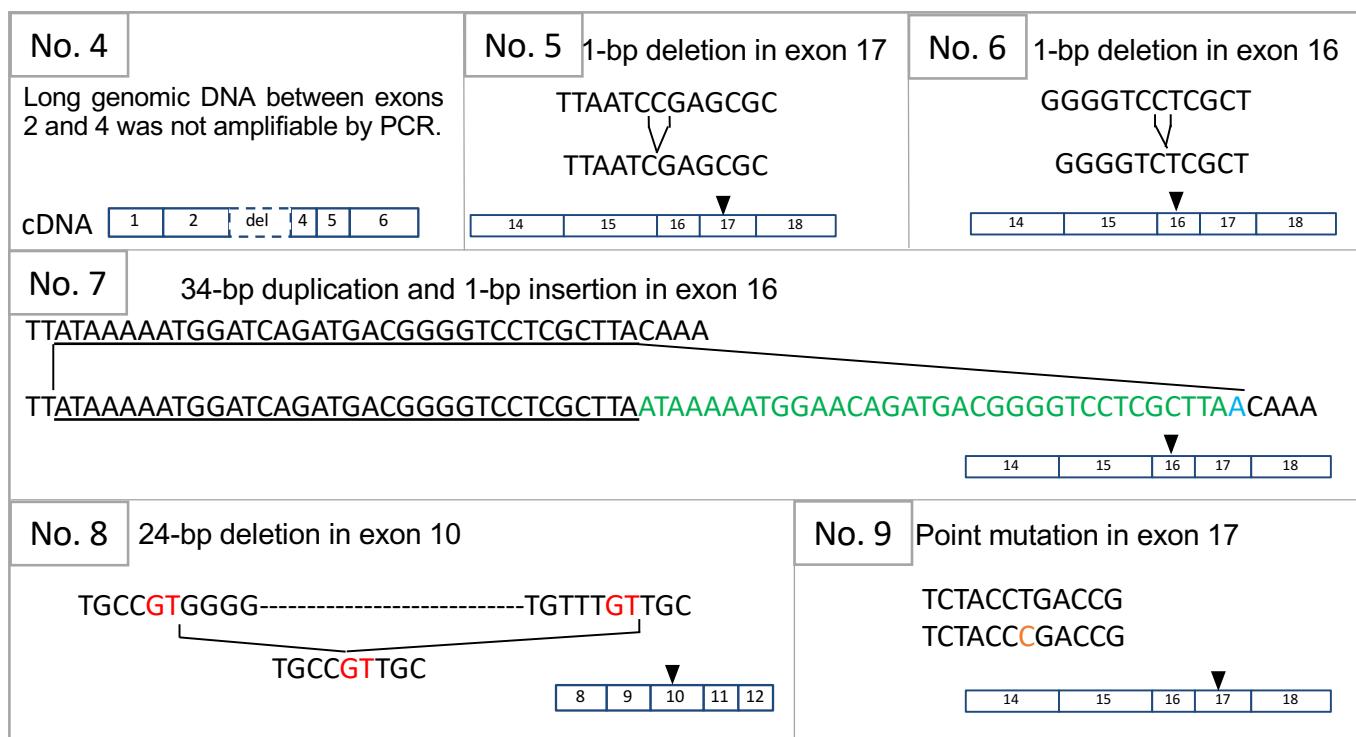


Supplementary Figure S4

A Non-irradiated

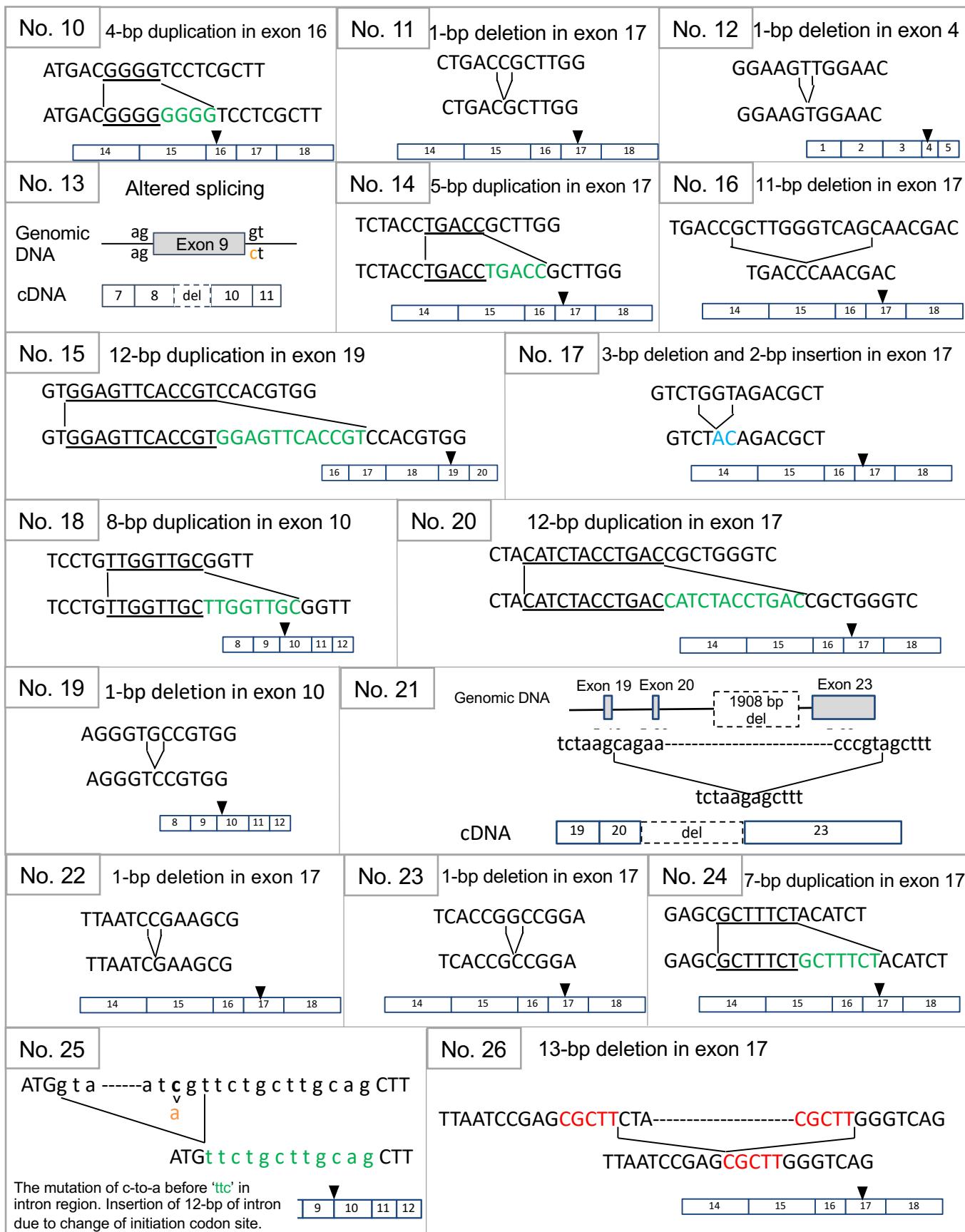


B Neutron-irradiated



Supplementary Figure S4 (continued)

C γ -irradiated



Supplementary Figure S5

