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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

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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; “x” 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)
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	TCAAAAGAAGCTGCGGCAAGT	TCAATGCGGCCATGAAGAAG	32	2.0
2	1,461–3,143 bp	1,683	GGTTTGGCCGTTTCTTGCTC	AGAGCCAGGACCATGACAAT	32	2.0
3	2,219–3,143 bp	925	TCCTTCTTTTCTGGGCTTG	AGAGCCAGGACCATGACAAT	32	1.5
4	2,986–4,305 bp	1,320	CCCAATGGCTACCCCTTCT	TCAGTTGGAGCTGCTCCCCCA	32	1.5
<i>Genomic</i>						
Ex3	Exon 3	395	CGTTGCTAGTTTGTCCCAAGT	GTGGCAGCGCTGAGAATTAT	30	1.5
Ex4	Exon 4	279	CGGCTTTCCTTGCCAAAA	TTGAACACTTTCCTCTCTG	30	1.5
Ex9	Exon 9	499	TATCAGCAGACGGTTGGAGG	GGGAAGGGGAGGACACAAAA	30	1.5
Ex10	Exon 10	258	CCTTCCCTCTCTCCGATTCC	GAAAGGTGTGTTGGCCAGAG	30	1.5
Ex16	Exon 16	448	TCCTGGGCTTTCGTTCTTA	TACTGTCAGCCCTATTCC	30	1.5
Ex17	Exon 17	496	TATCTTGTGGGACTGGGGC	ACACACCCTCATCTGTCTCC	30	1.5
Ex18	Exon 18	392	GTCCCCAGCTTACCTCACTT	AAACAAAATTCCCGGCTGC	30	1.5
Ex19	Exon 19	265	AGGCCACATCGAGACTTTGA	TCTGTCAGTGTGAGCCAGTT	30	1.5
Ex21	Exon 21	416	GCAGGGTGTGGAGGAATCTA	GTTGACCGAAAAGCCCAGAC	30	1.5
Ex22	Exon 22	600	TGCCAGAATCGTGAGTGTCT	TTCTGTGATTGACCCCG	32	1.5
Ex21,22	Exon 21,22	1,044	TTGACTTCTCAGCCCAGCAG	TTCTTGCACCCCATCTGTCT	32	1.5
Ex16–18	Exons 16–18	4,163	ACTGGGAGGATCATGCCAAA	GCAGGCTGATGTATTGCTCC	30	2.0
Ex20–23	Exons 20–23	6,167	CACTCTGCTGGGTGTACTGA	GACAAGGAGCCAGAGTCCAG	30	1.5

Supplementary Table S3. Primers for sequencing

Target	Primer ID	Start site (bp)	Primer sequence (5' to 3')	Direction
cDNA 240–1,651 ^a	B6F	1,045 (B6)	AGCATACCTCCTCGCTCAC	F
	B6R	1,063 (B6)	GTGAGCGAGGAGGTATGC	R
	C3HF	713 (C3H)	CTCCTTTACGGTGGACAAACT	F
	C3HR	986 (C3H)	ACAATCAACTCCTCCTGCCA	R
gDNA Exons 16–18 ^b	1F	38,995	TTGAGTGTAGGTGTGGGCAG	F
	2F	39,511	AGATATCATTTCCTCTCCTGGGG	F
	3F	40,012	ACACAGGTCATCAGAGGCTG	F
	4F	40,524	GCATCATTAATCCGAGCGCT	F
	5F	41,002	TCGCTAGGGATTGTGTGTTT	F
	1R	39,387	TTGCACGCCACTATAAAGCA	R
	2R	39,889	GTAGGTCTTGTGGGCTTTGC	R
	3R	40,389	AATACCCATGCCTCCACAGT	R
	4R	40,888	AACGCTCACTTCTCAACGTG	R
	5R	41,893	AGCCACCTCTTGAGCCTTT	R
gDNA Exons 20–23 ^b	6F	48,025	TGTGTTTGGGTCCCTTCTGA	F
	7F	48,597	TTTCTGAGTTCAAGGCCAGC	F
	8F	49,192	AATATGCTCCCTCATCATCTGG	F
	9F	50,402	TGGGAACGCAGAACTCACT	F
	10F	50,988	CACAGGCAGGAGATTTTCATCA	F
	11F	51,589	CGATGTCTGGCTTCTCTGGT	F
	12F	52,222	AATCACAGGAAACCACCAGC	F
	13F	52,815	GTGTATTCCAGTCCCAGGCT	F
	14F	53,387	GTTGAGATCTATGGGCAGTGG	F
	6R	47,781	GTCCCCTCCACAGTTCTGAA	R
	7R	48,396	CAATGCTACCAAGGACAGCC	R
	8R	49,015	TTCACTTATTCCATGCATTCCGG	R
	9R	49,590	ACCACAAAGCAGAGATGGGT	R
	10R	50,209	AAGAGAGAGGGACGGCTAGA	R
	11R	50,797	AGGCAGCTAATCCACTGTGA	R
12R	51,383	TGACCTCAGGACACGGTC	R	
13R	51,993	CCCTGCTGTGCTTCGTATTG	R	
14R	52,604	GCCACAAAGCTACGGGAAAG	R	
15R	53,194	GAAGCAAATGTACTGTAAGAGCA	R	

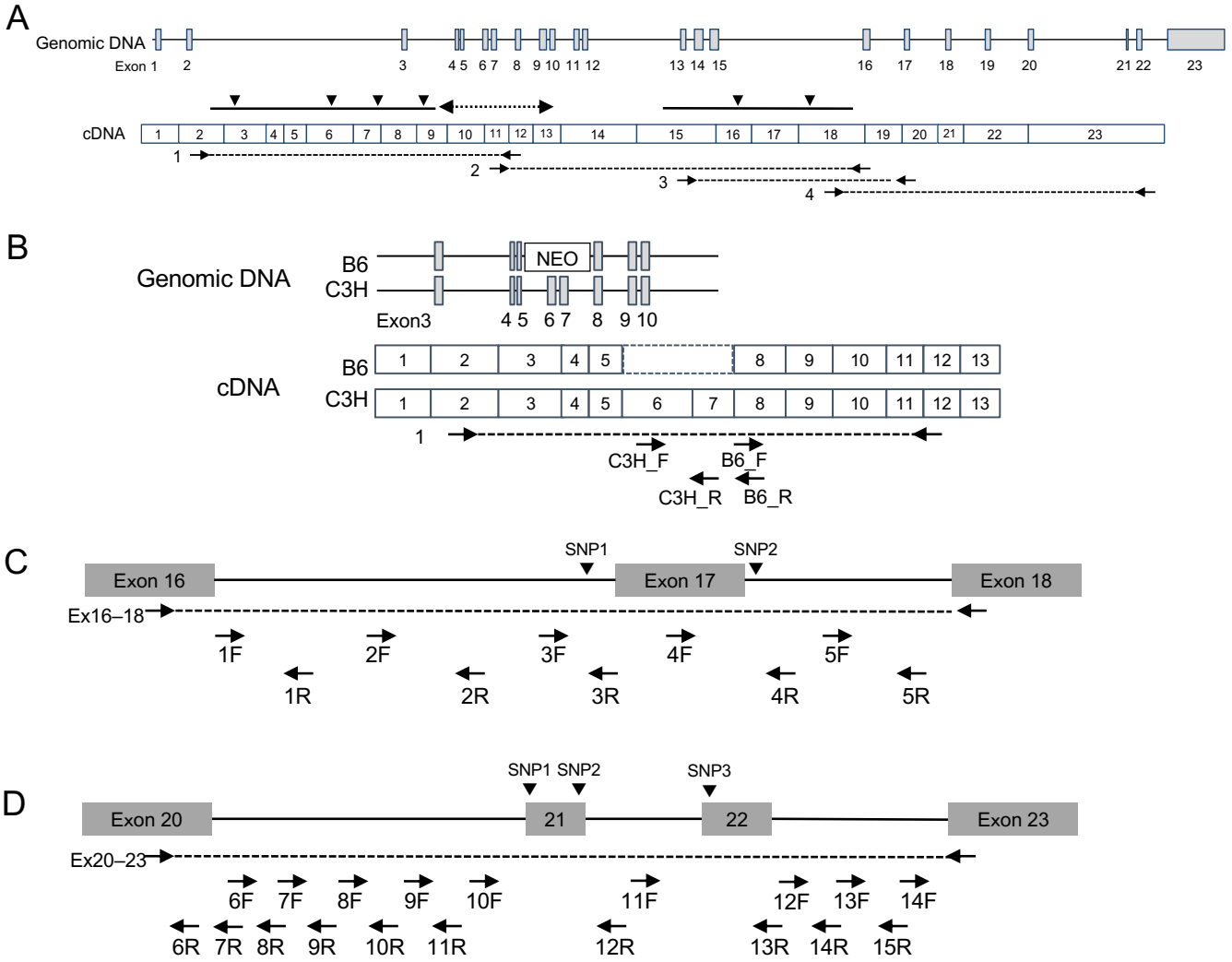
^a Primers for sequencing the strain-specific allele (B6 and C3H, as indicated) in the PCR product corresponding to position 240–1,651 of the cDNA.

^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).

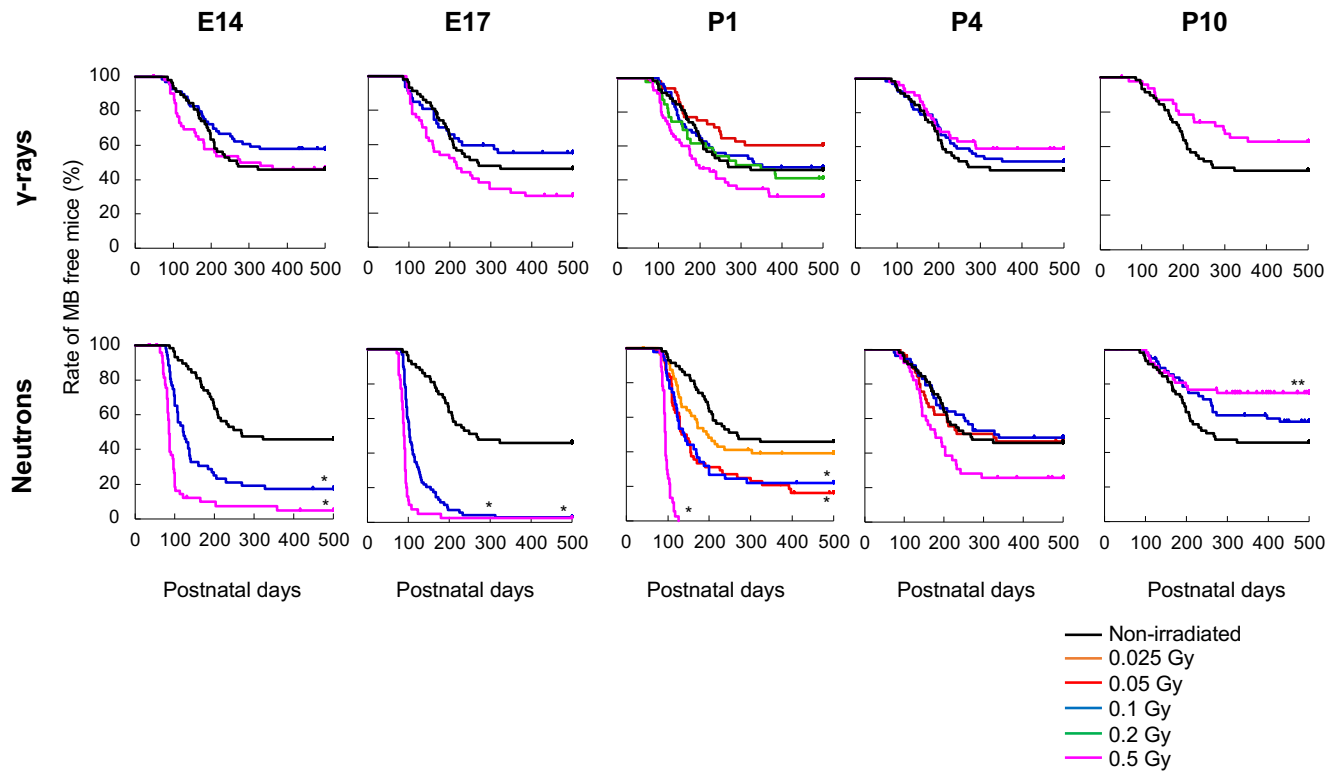
REFERENCE

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

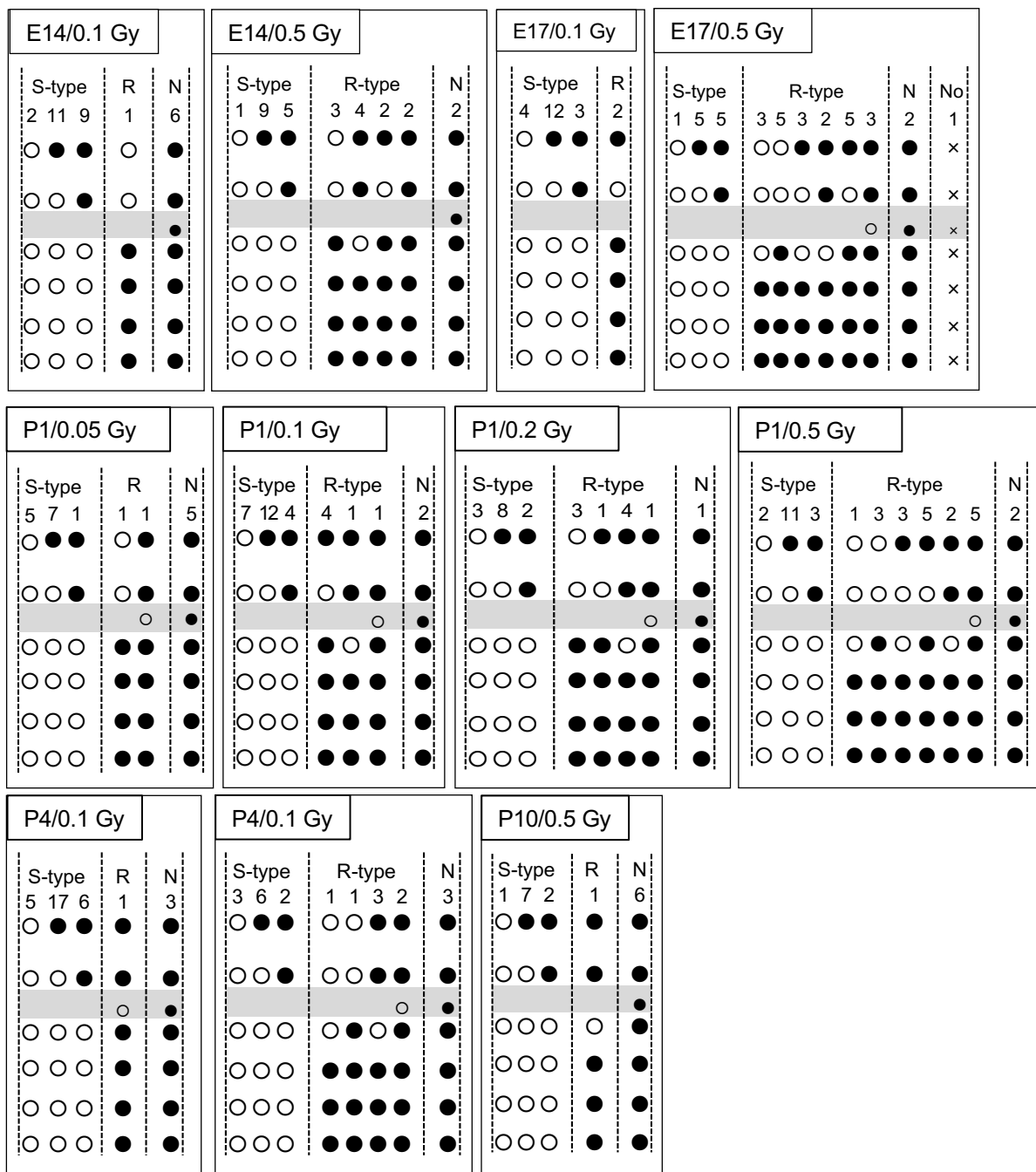


Supplementary Figure S2



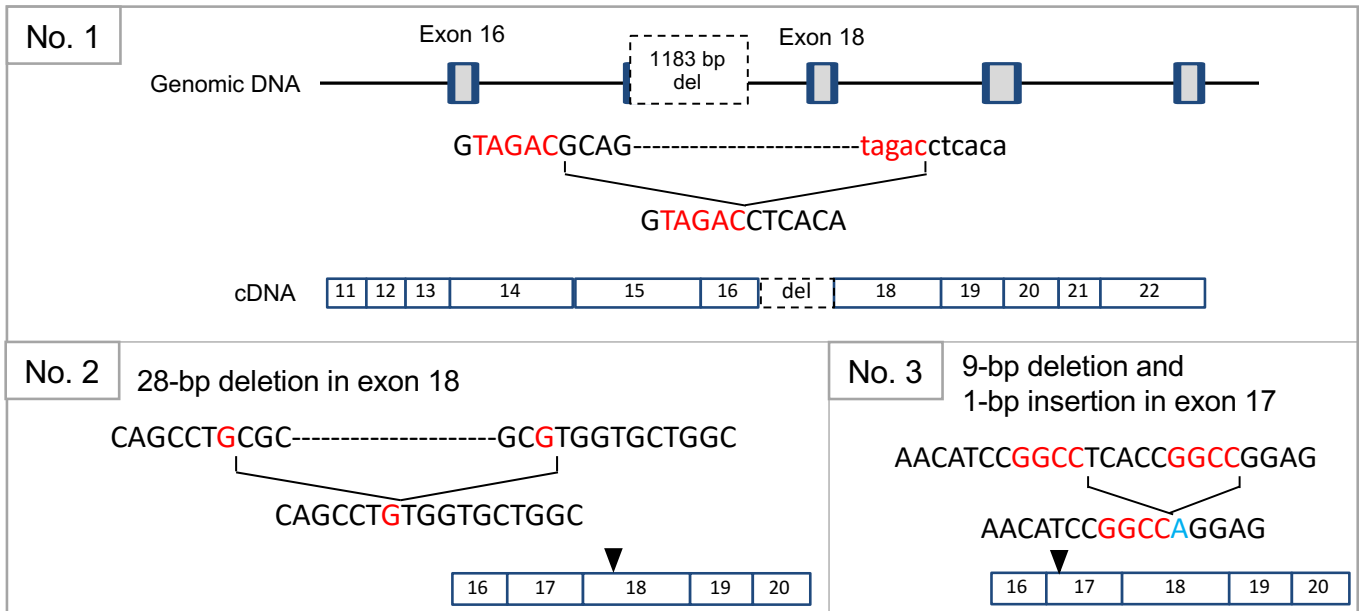
Supplementary Figure S3 (continued)

B

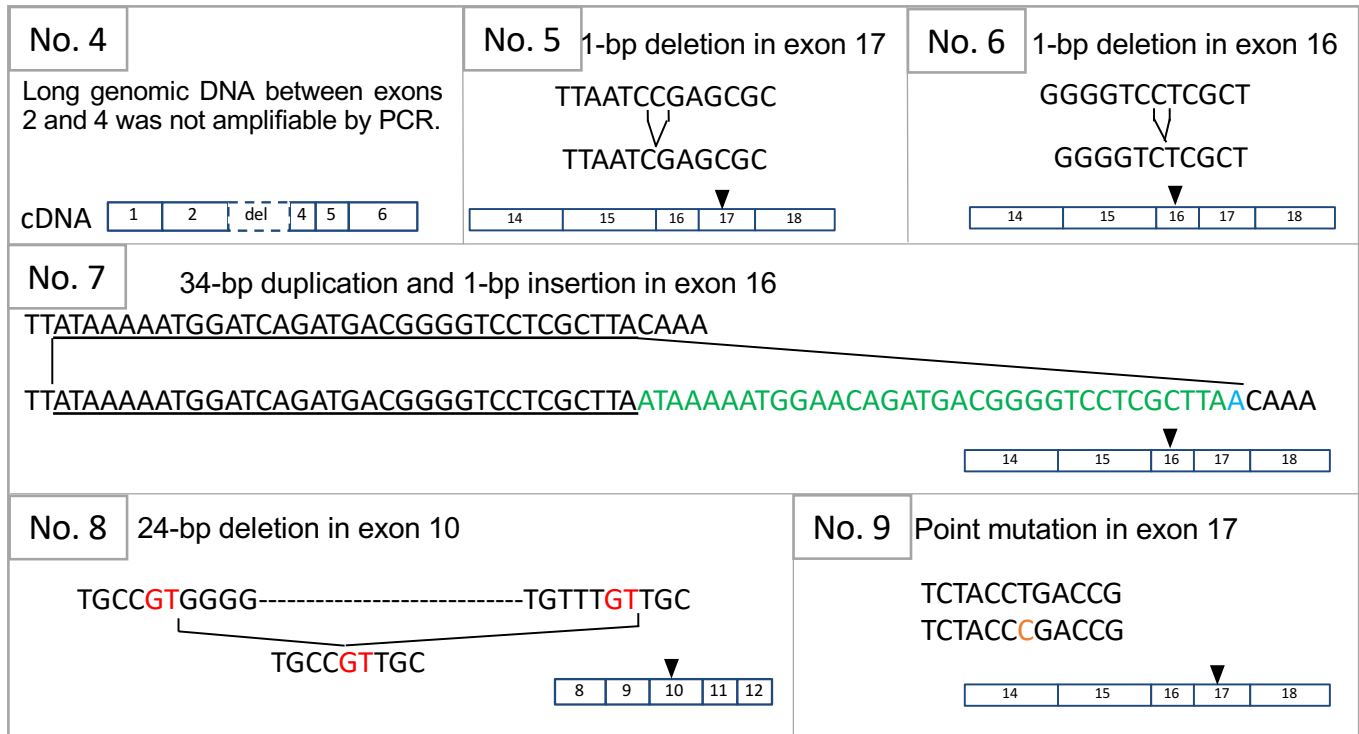


Supplementary Figure S4

A Non-irradiated

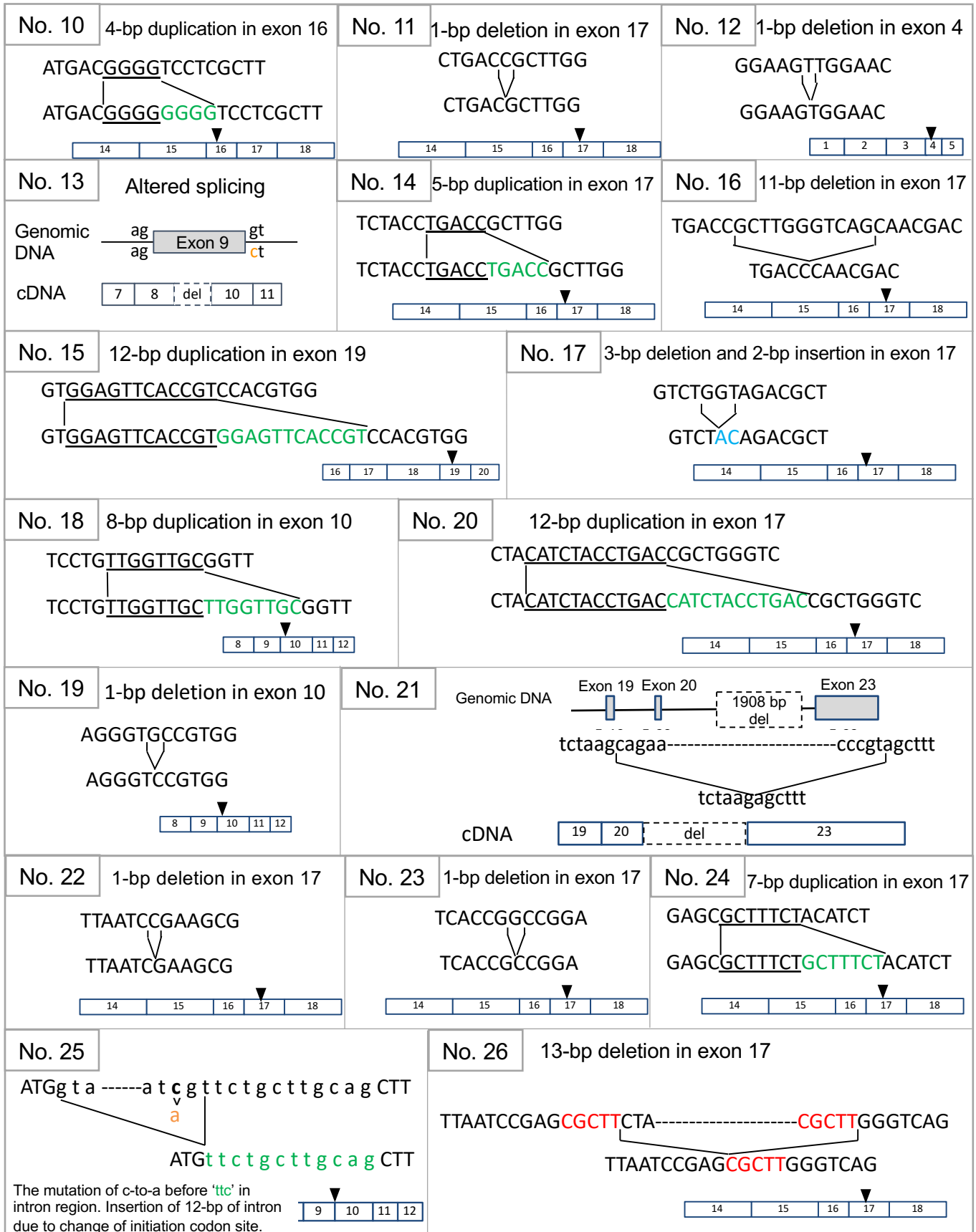


B Neutron-irradiated



Supplementary Figure S4 (continued)

C γ -irradiated



Supplementary Figure S5

