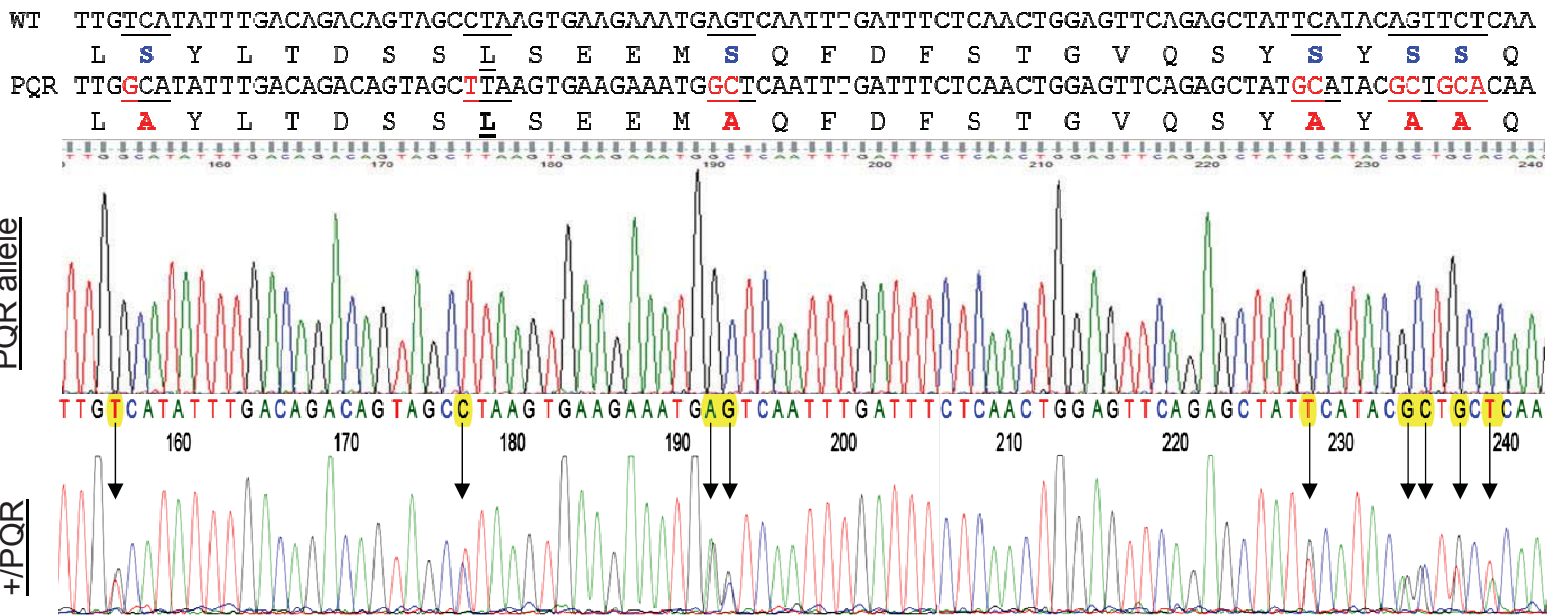


### A. PQR mutation

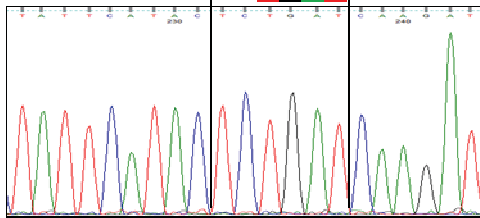
CTA and TTA both encode Leucine.



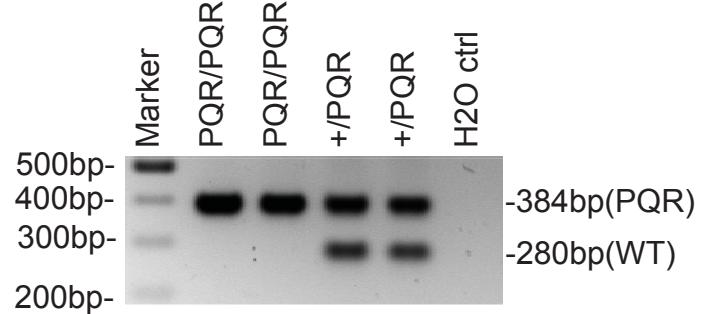
### B. s2053D mutation

WT-TATTCATACAGTTCTCAAGAT  
 Y S Y S S Q D

SD-TATTCATAC**TCTGAT**CAAGAT  
 Y S Y S **D** Q D  
 TATTCATAC**TCTGAT**CAAGAT



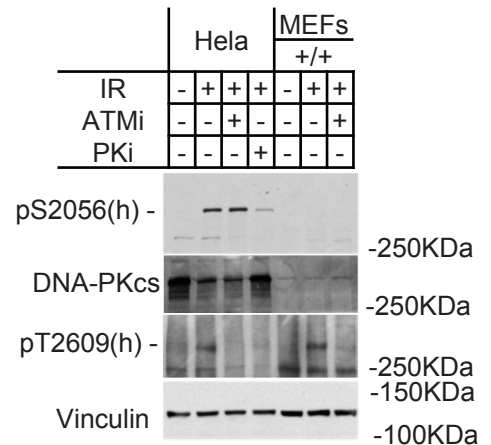
### C.



### D.

DNA-PKcs Genotype	n=	Weight(g)
+/+	9	22.3±3.0
PQR/PQR	9	25.3±3.9
PQR/-	2	22.9±1.4
SD/SD	9	21.5±3.4
+/SD	3	22.2±1.9

### E.



**Supplementary Figure 1:** The alignment of genomic and predicted protein sequence of the *DNA-PKcs*<sup>PQR</sup> (A) and *DNA-PKcs*<sup>SD</sup> (B) allele from homozygous *DNA-PKcs*<sup>PQR/PQR</sup>, *DNA-PKcs*<sup>SD/SD</sup> mice. The synonymous substitution (CTA→TTA) generates a new restriction site (CTTAAG) for Afill, which was used for screening. (C) A set of representative genotyping results from crosses between *DNA-PKcs*<sup>PQR/+</sup> parents. The PCR products corresponding to the WT allele is 280bp, to PQR is 387bp. (D) The body weight of adult *DNA-PKcs*<sup>PQR/PQR</sup>, *DNA-PKcs*<sup>PQR/-</sup> (compound heterozygous with null), *DNA-PKcs*<sup>+/SD</sup> *DNA-PKcs*<sup>SD/SD</sup> mice (average± standard error). Based on student t test, there is no significant difference between any two genotype groups. (E) Western blotting using commercial antibodies developed against pS2056 of human DNA-PKcs (Bethyl Laboratories Inc., BL2423, 1:500)

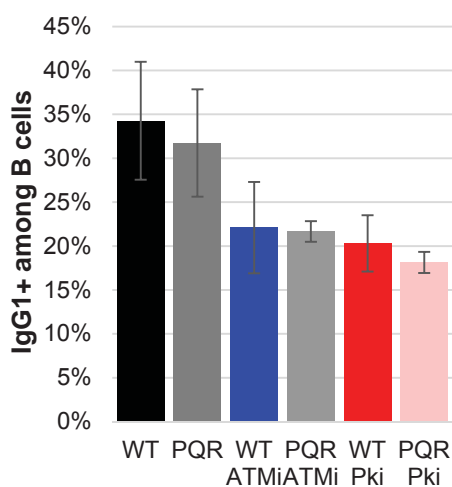
A.

At 8-10 week	Weight (mg)		Count ( $10^6$ )	
	Thymus	Spleen	Thymus	Spleen
WT (n=3)	38.90±11.24	95.93±3.56	68.5±33.2	64.8±18.8
PQR/PQR(n=3)	34.33±9.72	80.07±13.15	58.57±11.9	52.2±14.9
PQR/- (n=3)	41.25±6.45	92.50±35.6	61.5±11.3	64.5±35.6

B.

At 4 week	Count ( $10^6$ )	
	Thymus	Spleen
WT (n=4)	173.4±34.3	65.9±9.8
SD/SD(n=11)	127.8±46.9	59.8±19.7
+/SD (n=3)	160.8±35.4	56.9±19.9

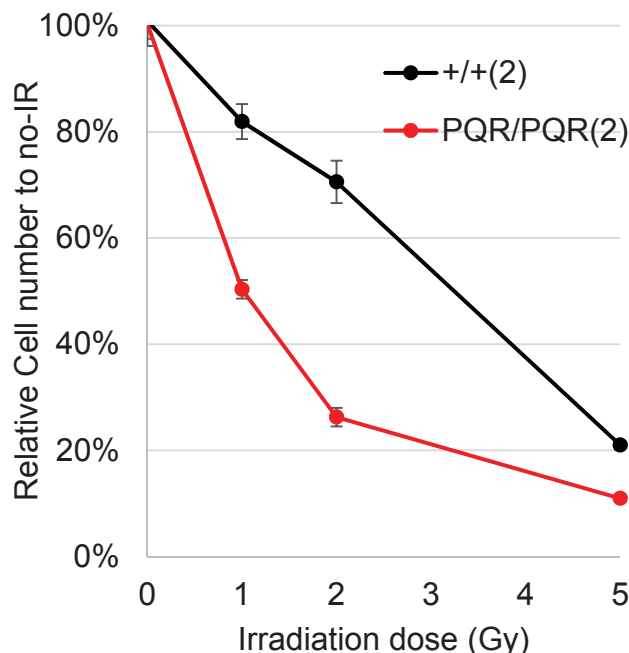
C.



D.

ATM		-/-	+/-	+/+	p
PKCs	SD/SD	5	11	6	>0.9
	+/+	26	57	32	

E.



### Supplementary Figure 2

(A) Thymus and Spleen weight (g) and total cellularity in *DNA-PKCs<sup>PQR/PQR</sup>* and control mice. (B) Total cell count of thymus and spleen from *DNA-PKCs<sup>SD/SD</sup>* and *DNA-PKCs<sup>+/+</sup>* littermates. The data represents the average and standard deviation of >3 mice of each genotype. (C) The percentage of IgG1+ cells among all B220+ B cells at day 4 of class switch. The data represents the average and standard deviation of 3 independent experiments. ATM inhibitor KU55933 was used at final concentration of 7.5µM and DNA-PKCs inhibitor NU7441 was used at a final concentration of 2.5 µM. (D) The distribution of ATM genotyping from breeding between *DNA-PKCs<sup>SD/SD</sup>ATM<sup>+/-</sup>* parents. The breeding between *ATM<sup>+/-</sup>* mice were used as control. P was calculated using the Fisher exact test. (E) The independent repeats of radiation sensitivity of B cells activated for class switch recombination. The activated B cells were irradiated on the day of purification and cytokine stimulation (anti-CD40 and IL4) and the cell counts were determined 4 days later.

SupTable 1

	CODINGJOINTs_-LEFT	P	N	P	RIGHT	N+P=	Del=
GL	AAGCGGAGCACCACAGTGCTAACTGGGACC				ACTATGCTATGGACTACTGGGGTCAAGGAA		
1	AAGCGGAGCACCACAGTGCTAACTGG				CTATGGACTACTGGGGTCAAGGAA	0	10
2	AAGCGGAGCACCACAGTGCTAACTGGGA				TGCTATGGACTACTGGGGTCAAGGAA	0	6
3	AAGCGGAGCACCACAGTGCTAACTG				TGCTATGGACTACTGGGGTCAAGGAA	0	9
4	AAGCGGAGCACCACAGTGCTAACTGGG			T	ACTATGCTATGGACTACTGGGGTCAAGGAA	1	3
5	AAGCGGAGCACCACAGTGCTAACTGGGA		CG		TGCTATGGACTACTGGGGTCAAGGAA	2	6
6	AAGCGGAGCACCACAGTGCTAACTGGGACC	GG			ATGCTATGGACTACTGGGGTCAAGGAA	2	3
7	AAGCGGAGCACCACAGTGCTAACTGGGA		GGG		ACTATGCTATGGACTACTGGGGTCAAGGAA	3	2
8	AAGCGGAGCACCACAGTGCT		CCA		ACTATGCTATGGACTACTGGGGTCAAGGAA	3	11
9	AAGCGGAGCACCACAGTGCT		CCG		TATGGACTACTGGGGTCAAGGAA	3	17
10	AAGCGGAGCACCACAGTGCTAACTGGGACC		CC	T	ACTATGCTATGGACTACTGGGGTCAAGGAA	3	0
11	AAGCGGAGCACCACAGTGCTAACTGGGAC	G	GG		TGCTATGGACTACTGGGGTCAAGGAA	3	5
12	AAGCGGAGCACCACAGTGCTAACTG		AGGG		ATGCTATGGACTACTGGGGTCAAGGAA	4	8
13	AAGCGGAGCACCACAGTGCTAACTGGGA		AACG		ATGCTATGGACTACTGGGGTCAAGGAA	4	5
14	AAGCGGAGCACCACAGTGCTAACTG		AGGG		ATGCTATGGACTACTGGGGTCAAGGAA	4	8
15	AAGCGGAGCACCACAGTG		TCTT		TATGGACTACTGGGGTCAAGGAA	4	19
16	AAGCGGAGCACCACAGTGCTAACTGGGA		GTTT		TATGCTATGGACTACTGGGGTCAAGGAA	5	4
17	AAGCGGAGCACCACAGTGCTAACTGGGAC	GT	GGT		CTATGCTATGGACTACTGGGGTCAAGGAA	5	2
18	AAGCGGAGCACCACAGTGCTAACTG		ACCCT	TCC	GGACTACTGGGGTCAAGGAA	8	15
19	AAGCGGAGCACCA		GACGGAGA		CTATGGACTACTGGGGTCAAGGAA	8	23
1	AAGCGGAGCACCACAGTGCTAACTGGGAC		T		TGCTATGGACTACTGGGGTCAAGGAA	1	5
2	AAGCGGAGCACCACAGTGCTAACTG		TT		CTATGCTATGGACTACTGGGGTCAAGGAA	2	6
3	AAGCGGAGCACCACAGTGCTAACTGGGA		AGG		TGCTATGGACTACTGGGGTCAAGGAA	3	6
4	AAGCGGAGCACCACAGTGCTAACTGGG		TC	T	ACTATGCTATGGACTACTGGGGTCAAGGAA	3	3
5	AAGCGGAGCACCACAGTGCTAACTGGGAC	G	GG		GGACTACTGGGGTCAAGGAA	3	11
6	AAGCGGAGCACCACAGTGCTAACTGGGAC	GT	A		ACTATGCTATGGACTACTGGGGTCAAGGAA	3	1
7	AAGCGGAGCACCACAGTGCTAACTGGGAC	G	AA	T	ACTATGCTATGGACTACTGGGGTCAAGGAA	4	1
8	AAGCGGAGCACCACAGTGCTAACTGGGAC	GT		AG	CTATGCTATGGACTACTGGGGTCAAGGAA	4	2
9	AAGCGGAGCACCACAGTGCTAACTGGGAC	G	GGA		CTATGCTATGGACTACTGGGGTCAAGGAA	4	2
10	AAGCGGAGCACCACAGTGCTAACTGGGAC	G	GGG		GCTATGGACTACTGGGGTCAAGGAA	4	6
11	AAGCGGAGCACCACAGTGCTAACT		CTATT		ACTATGCTATGGACTACTGGGGTCAAGGAA	5	6
12	AAGCGGAGCACCACAGTGCTAACTGGGAC	G	GG	GT	ACTATGCTATGGACTACTGGGGTCAAGGAA	5	1
13	AAGCGGAGCACCACAGTGCTAACTGGG		GCCCT		CTATGCTATGGACTACTGGGGTCAAGGAA	5	4
14	AAGCGGAGCACCACAGTGCTAACTGGGAC	GT	AGGG		GCTATGGACTACTGGGGTCAAGGAA	6	6
15	AAGCGGAGCACCACAGTGCTAACTGGGAC	G	ATAT	T	ACTATGCTATGGACTACTGGGGTCAAGGAA	6	1
16	AAGCGGAGCACCACAGTGCTA	T	CGGGAG		GCTATGGACTACTGGGGTCAAGGAA	7	14

**SupTable 1** Endogenous DQ52-JH4 junctions from peripheral B cells in *DNA-PKcs<sup>+/+</sup>* (n=19) and *DNA-PKcs<sup>PQR/PQR</sup>* (n=16) B cells mice. GL= Germline line sequence; P=palindromic (p) nucleotides; N=nontemplate-encoded nucleotides. Del=deletion, from the predicated GL seq.

SupTable 2

	CODING JOINTs_ - LEFT	P	N	P	RIGHT	N+P=	Del=
GL	AAGCGGAGCACCACAGTGCTAACTGGGACC				ACTATGCTATGGACTACTGGGGTCAAGGAA		
1	AAGCGGAGCACCACAGTGCTAACTGGGACC	GG	G		GCTATGGACTACTGGGGTCAAGGAA	3	5
2	AAGCGGAGCACCACAGTGCTAACTGGGACC				TGCTATGGACTACTGGGGTCAAGGAA	0	5
3	AAGCGGAGCACCACAGTGCTAACTGGGACC				TATGCTATGGACTACTGGGGTCAAGGAA	0	3
4	AAGCGGAGCACCACAGTGCTAACTGGGACC	G	GT		TATGCTATGGACTACTGGGGTCAAGGAA	3	3
5	AAGCGGAGCACCACAGTGCTAACTGGGACC	G	A		GCTATGGACTACTGGGGTCAAGGAA	2	6
6	AAGCGGAGCACCACAGTGCTAACTGGGACC	G		GC	GCTATGGACTACTGGGGTCAAGGAA	3	6
7	AAGCGGAGCACCACAGTGCTAACTGGGACC	G	AGG		GCTATGGACTACTGGGGTCAAGGAA	4	6
8	AAGCGGAGCACCACAGTGCTAACTGGGACC	G	AC		CTATGCTATGGACTACTGGGGTCAAGGAA	3	2
9	AAGCGGAGCACCACAGTGCTAACTGGGACC	G	GGACTTA		ATGGACTACTGGGGTCAAGGAA	8	9
10	AAGCGGAGCACCACAGTGCTAACTGGGACC	G	GGAT	T	ACTATGCTATGGACTACTGGGGTCAAGGAA	6	1
11	AAGCGGAGCACCACAGTGCTAACTGGGA		GGGAGAGT	G	CTATGCTATGGACTACTGGGGTCAAGGAA	9	3
12	AAGCGGAGCACCACAGTGCTAACTGGG		GGGGTGC		TGGACTACTGGGGTCAAGGAA	8	12
13	AAGCGGAGCACCACAGTGCTAACTGGG	CC	TC		CTATGCTATGGACTACTGGGGTCAAGGAA	4	4
14	AAGCGGAGCACCACAGTGCTAACTGGG		TC		ATGCTATGGACTACTGGGGTCAAGGAA	2	6
15	AAGCGGAGCACCACAGTGCTAACTGGG				ACTGGGGTCAAGGAA	0	18
16	AAGCGGAGCACCACAGTGCTAACTGGG			T	ACTATGCTATGGACTACTGGGGTCAAGGAA	1	3
17	AAGCGGAGCACCACAGTGCTAACTGG				TATGCTATGGACTACTGGGGTCAAGGAA	0	6
18	AAGCGGAGCACCACAGTGCTAACTG		T	T	ACTATGCTATGGACTACTGGGGTCAAGGAA	2	5
19	AAGCGGAGCACCACAGTGCTAACT		CCCT	T	ACTATGCTATGGACTACTGGGGTCAAGGAA	5	6
20	AAGCGGAGCACCACAGT	A	TGAGGAT	T	ACTATGCTATGGACTACTGGGGTCAAGGAA	9	13

**Supplementary Table 2**

Endogenous DQ52-JH4 junctions (n=20) from peripheral *DNA-PKcs<sup>SD/SD</sup> B cells*. GL= Germline line sequence; P=palindromic (p) nucleotides; N=non-template-encoded nucleotides. Del=deletion, indicating base pair deletion from the predicated GL seq.