

Supplementary Information

Figures S1 to S5 and Tables S1 to S5

***NBS1* I171V variant underlies individual differences in chromosomal radiosensitivity within human populations**

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a

WT allele

5'...ACTATATGCACTCATTGTGGACGTCCAATTATAAAACCAGAATACTTTTCTGAATTTCTCAAAGCAG...3'
I171 (c.511A) PAM Cas9 recognition sequence

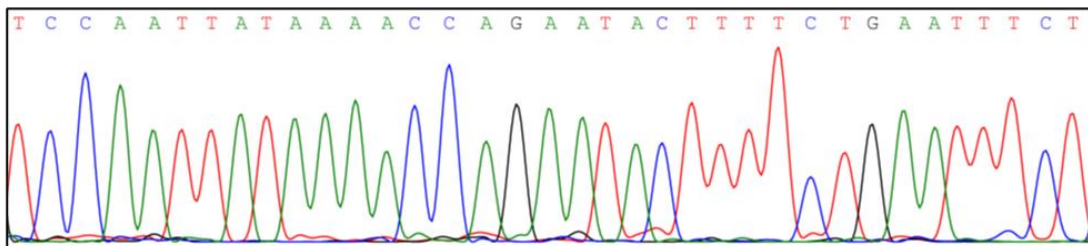
▼ Presumptive DSB site

↓ CRISPR/Cas9-mediated editing

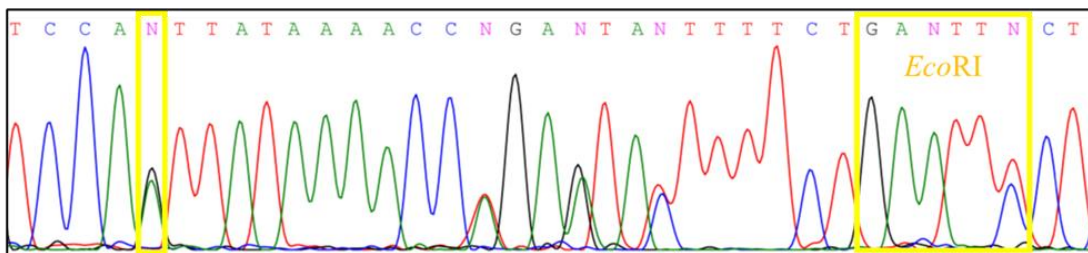
ssODN(100 mer) / Targeted allele

5'...ACTATATGCACTCATTGTGGACGTCCAGTTATAAAACCTGAGTATTTTCTGAATTCCTCAAAGCAG...3'
V171 (c.511G) EcoRI

b *Nbs1*^{+/+} clone 1



c *Nbs1*^{I171V/+} clone 1



d *Nbs1*^{I171V/I171V} clone 1

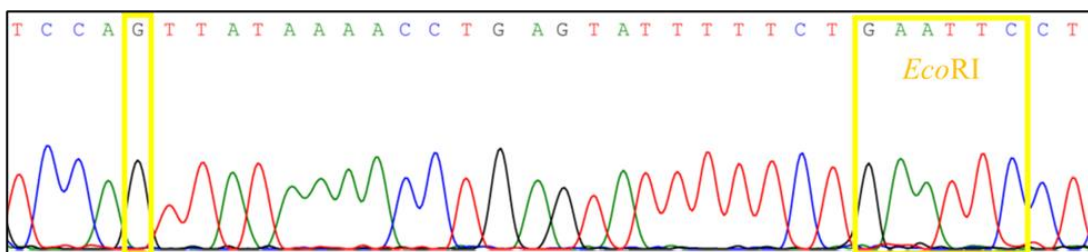


Figure S1. Generation of *Nbs1* I171V knock-in mice.

(a) Targeting strategy for *Nbs1* I171V knock-in using the CRISPR/Cas9 system. Blue bases indicate silent mutations.

(b–d) Sanger sequencing of *Nbs1*^{+/+}-MEF clone 1 (b), *Nbs1*^{I171V/+}-MEF clone 1 (c), and *Nbs1*^{I171V/I171V}-MEF clone 1

(d). A single base substitution of the *Nbs1* I171V variant and a silent *EcoRI* site are indicated by yellow boxes.

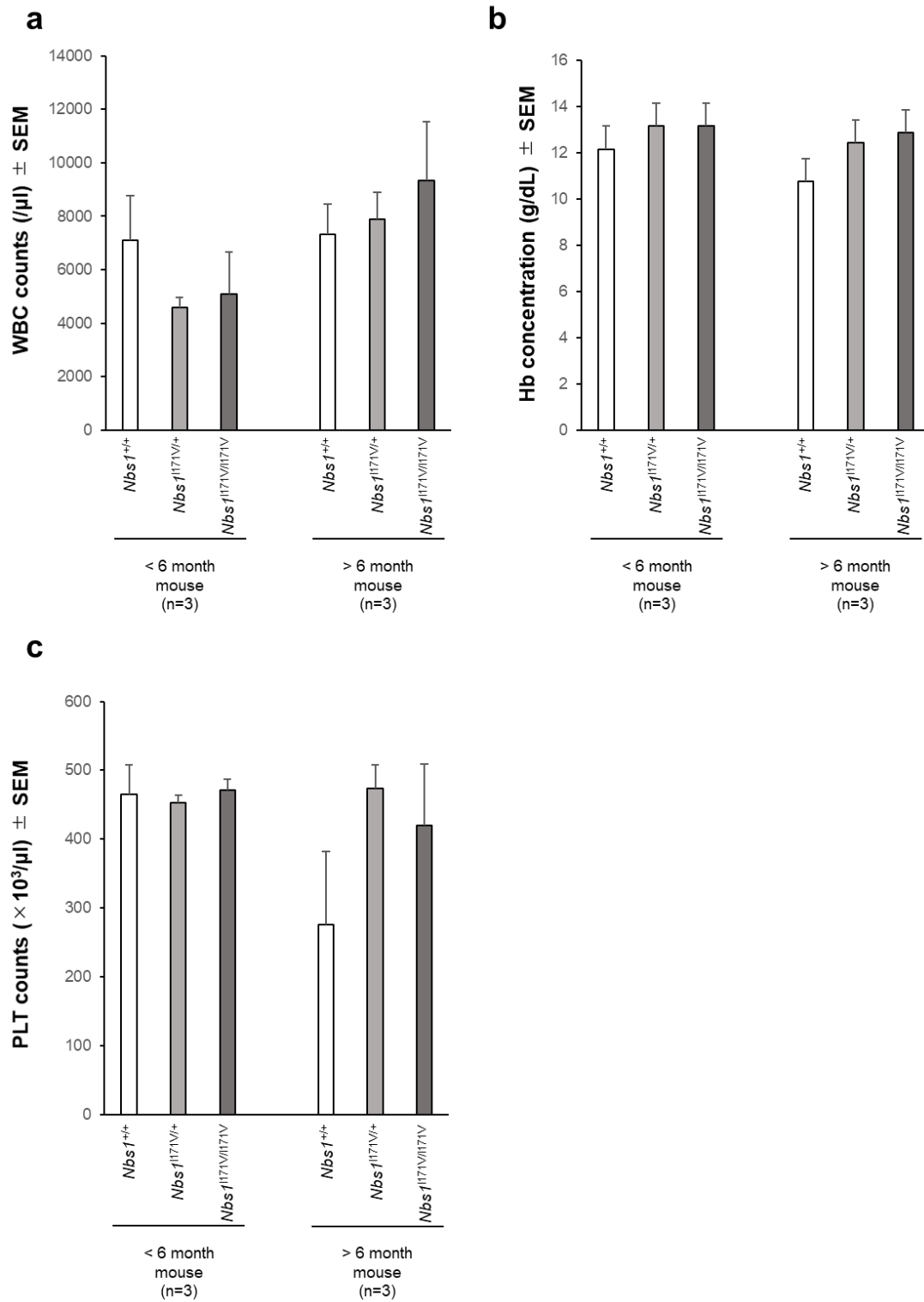


Figure S2. Complete blood counts in the *Nbs1* I171V knock-in mice.

(a) White blood cell (WBC) counts, (b) hemoglobin (Hb) concentrations, and (c) platelet (PLT) counts of *Nbs1*^{+/+}, *Nbs1*^{I171V/+}, and *Nbs1*^{I171V/I171V} mice (mean \pm SEM; no significant change in each t-test parameter; n=3).

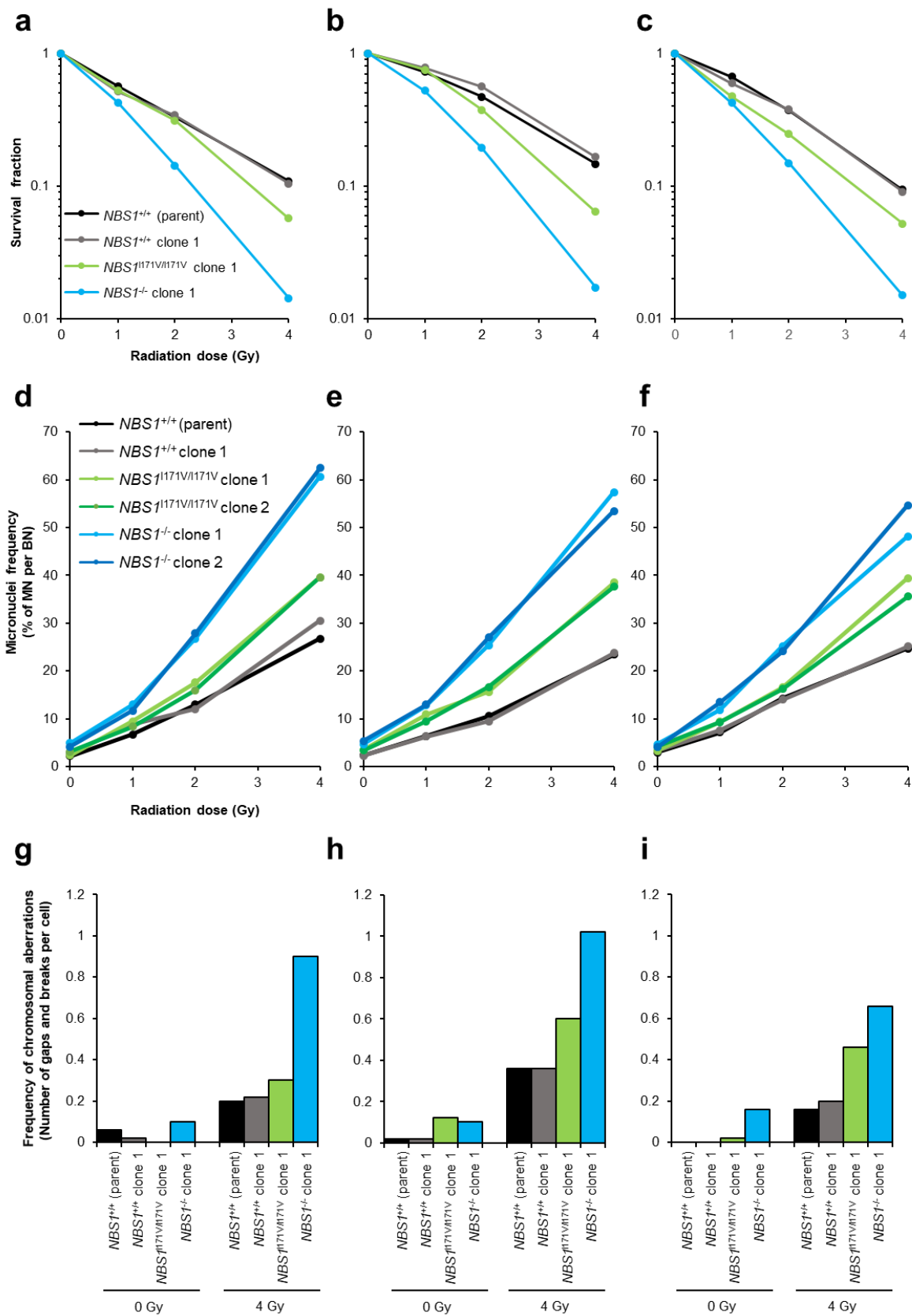


Figure S3. Three independent colony survival, CBMN, and chromosomal aberration assays in *NBS1* I171V knock-in HCT116 cell clones.

Three graphs showing the results of three independent experiments: survival fractions (a–c), IR-induced MN formation (d–f), and IR-induced chromosomal aberration (g–i).

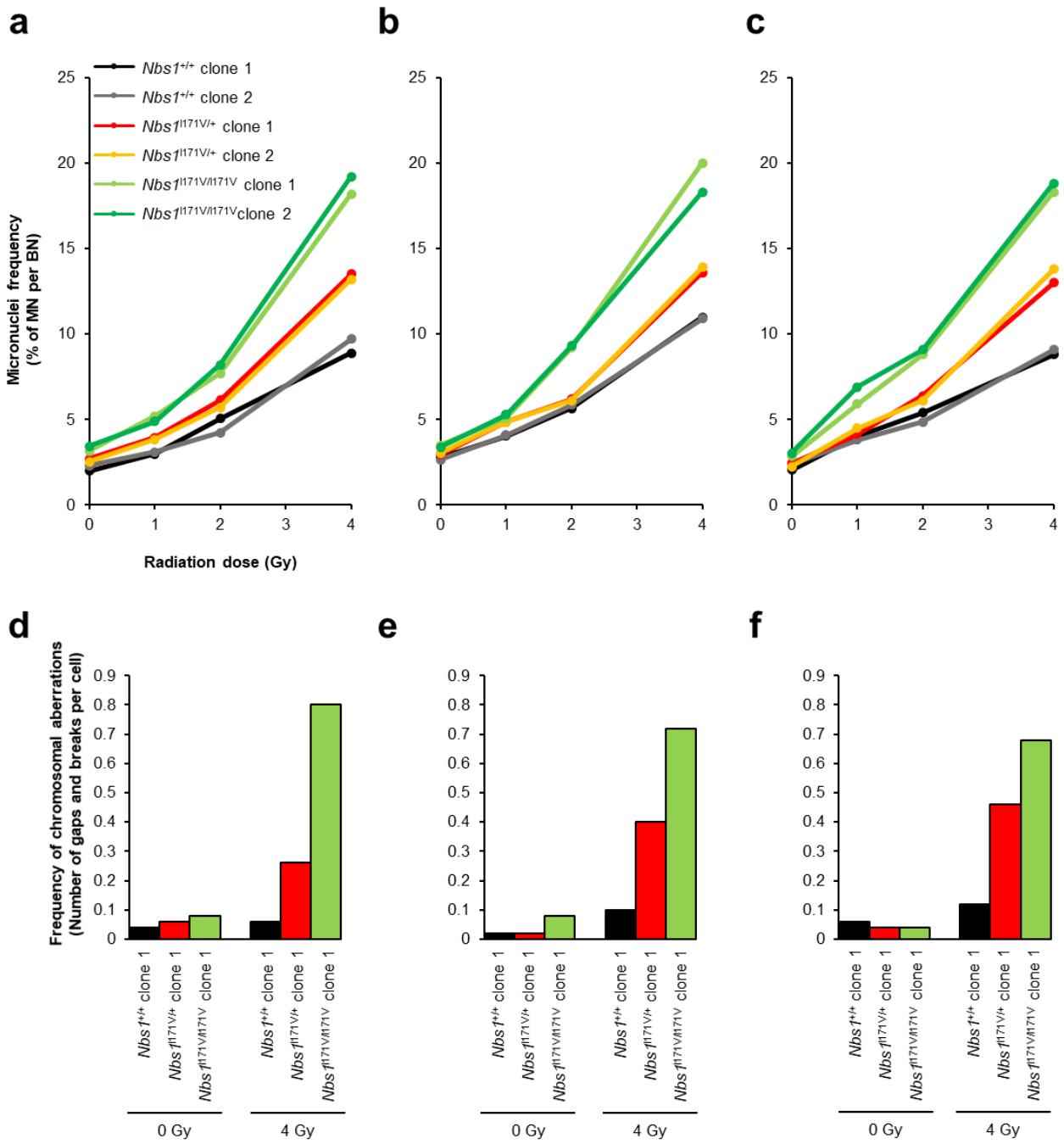
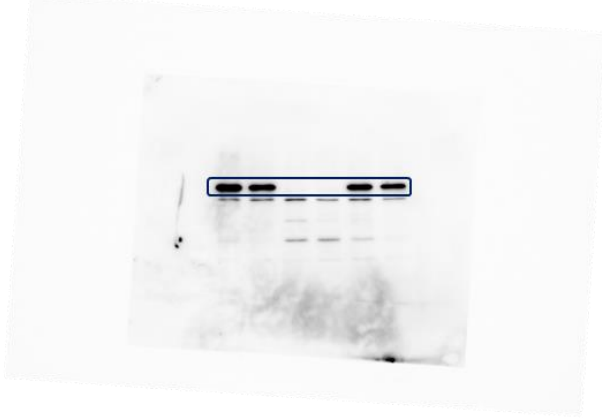


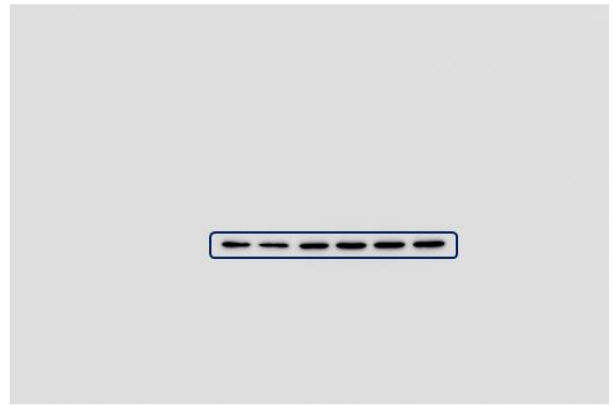
Figure S4. Three independent CBMN and chromosomal aberration assays in the *Nbs1* I171V knock-in MEF clones.

Three graphs showing the results of three independent experiments: percentage of IR-induced MN formation (a–c) and IR-induced chromosomal aberration (d–f).

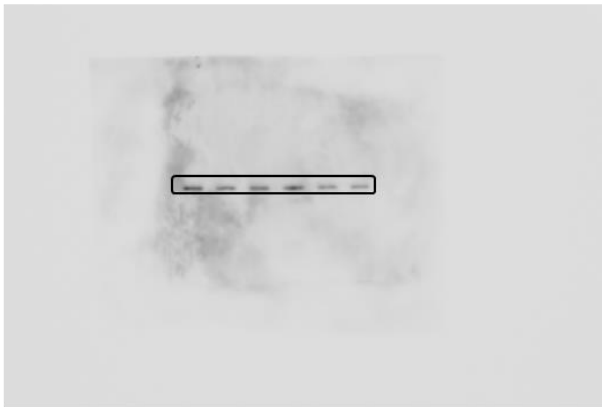
NBS1 knock-in HCT116 cells
NBS1 95 kDa



GAPDH 37 kDa



MRE11 81 kDa



Nbs1 knock-in MEFs
NBS1 95 kDa



GAPDH 37 kDa

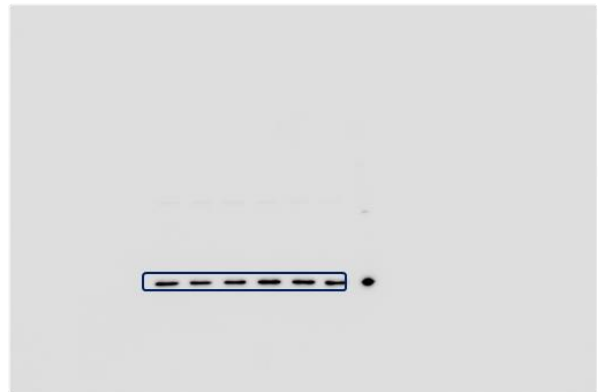


Figure S5. Uncropped images of western blotting data shown in the main text.

		1Gy	2Gy	4Gy
<i>NBSI</i> ^{+/+}	<i>NBSI</i> ^{I171V/I171V}	0.51	0.14	0.013
<i>NBSI</i> ^{+/+}	<i>NBSI</i> ^{-/-}	0.023	0.0027	0.00081
<i>NBSI</i> ^{I171V/I171V}	<i>NBSI</i> ^{-/-}	0.24	0.021	0.00029

Table S1. T-test of survival fraction in *NBSI* I171V knock-in HCT116 cell clones.

		0Gy	1Gy	2Gy	4Gy
<i>NBSI</i> ^{+/+}	<i>NBSI</i> ^{I171V/I171V}	0.061	0.00094	0.00047	0.0000014
<i>NBSI</i> ^{+/+}	<i>NBSI</i> ^{-/-}	0.000022	0.00000045	0.000000055	0.00000016
<i>NBSI</i> ^{I171V/I171V}	<i>NBSI</i> ^{-/-}	0.0014	0.000028	0.000000034	0.000011

Table S2. T-test of CBMN assay in *NBSI* I171V knock-in HCT116 cell clones.

		0Gy	4Gy
<i>NBSI</i> ^{+/+}	<i>NBSI</i> ^{I171V/I171V}	0.37	0.034
<i>NBSI</i> ^{+/+}	<i>NBSI</i> ^{-/-}	0.0010	0.00021
<i>NBSI</i> ^{I171V/I171V}	<i>NBSI</i> ^{-/-}	0.16	0.041

Table S3. T-test of Chromosomal aberration in *NBSI* I171V knock-in HCT116 cell clones.

		0Gy	1Gy	2Gy	4Gy
<i>NbsI</i> ^{+/+}	<i>NbsI</i> ^{I171V/+}	0.14	0.030	0.0045	0.0000052
<i>NbsI</i> ^{+/+}	<i>NbsI</i> ^{I171V/I171V}	0.00042	0.00043	0.0000015	0.000000052
<i>NbsI</i> ^{I171V/+}	<i>NbsI</i> ^{I171V/I171V}	0.0054	0.0070	0.0000027	0.000000013

Table S4. T-test of CBMN assay in *NBSI* I171V knock-in MEF clones.

		0Gy	4Gy
<i>NbsI</i> ^{+/+}	<i>NbsI</i> ^{I171V/+}	1	0.011
<i>NbsI</i> ^{+/+}	<i>NbsI</i> ^{I171V/I171V}	0.205106455	0.000084
<i>NbsI</i> ^{I171V/+}	<i>NbsI</i> ^{I171V/I171V}	0.205106455	0.0064

Table S5. T-test of Chromosomal aberration in *NBSI* I171V knock-in MEF clones.