Double strand break repair deficiency in NONO knockout murine embryonic fibroblasts and compensation by spontaneous up-regulation of the PSPC1 paralog

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SUPPLEMENTARY FIGURES

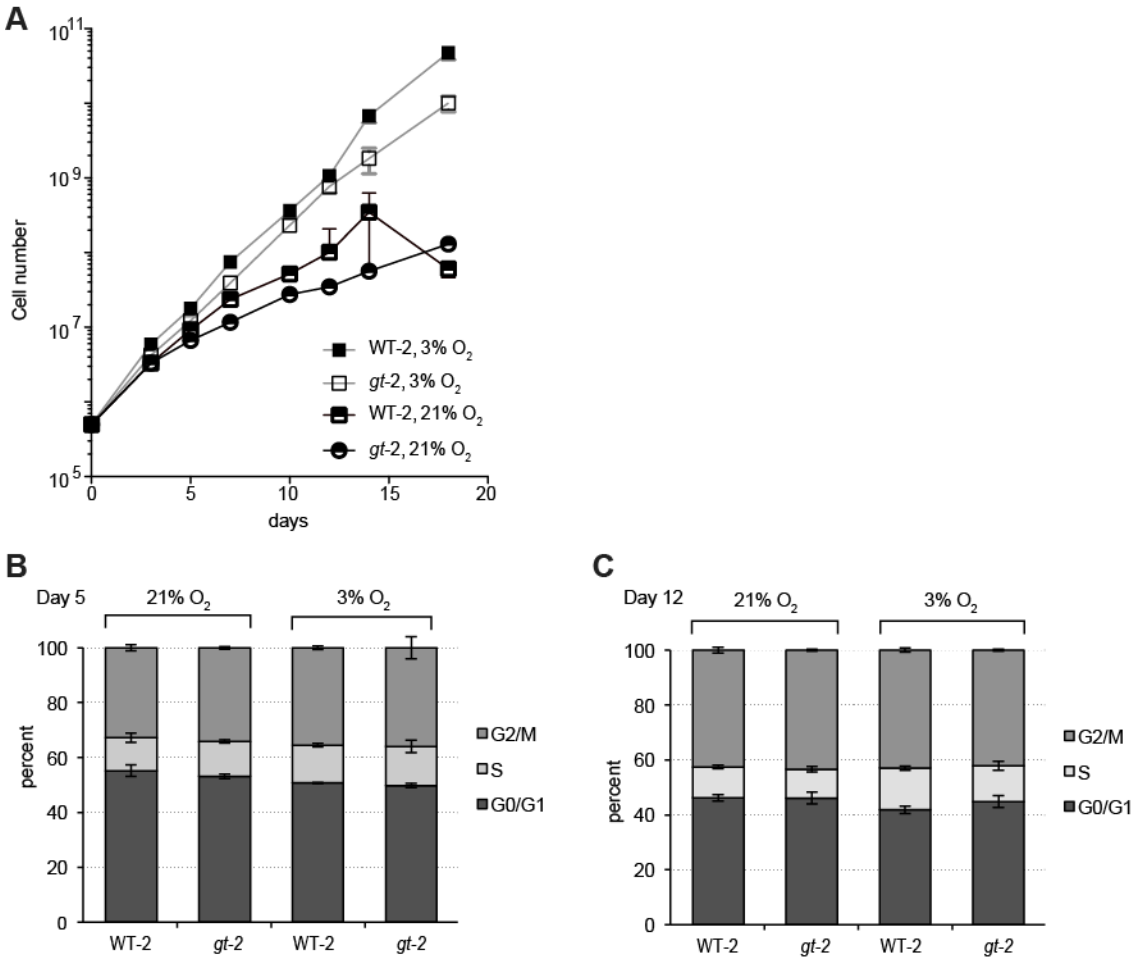

Figure S1. Effect of NONO deficiency on cell growth under 3% O_2 and atmospheric (21%) O_2. Cells were thawed and allowed to recover in a 3% O_2/5% CO_2 atmosphere. Replicate sub-cultures were prepared and one set of duplicates was returned for growth in 3% O_2/5% CO_2 and the other was transferred to a standard air/5% CO_2 atmosphere. A. Growth curves. Cells were harvested, counted, and re-plated every 2-4 days as indicated. Each point represents the mean of two or more replicate cultures. Error bars denote standard deviation. B. C. Cell cycle distributions were determined by flow cytometry using propidium iodide staining as described for Figure 2 of the main text. Cells were analyzed on day 5 (panel B) or day 12 (panel C).

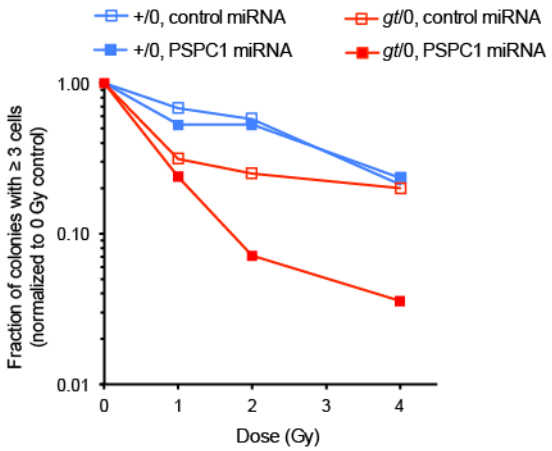

Figure S2. Green cell microcolony formation assay. Same experiment as Figure 4 of the main text except that colonies of ≥ 3 cells were scored. Data are expressed as a proportion of colonies of ≥ 3 cells relative to total colonies, normalized to non-irradiated control group. For each genotype and radiation dose, 200 green microcolonies were scored.

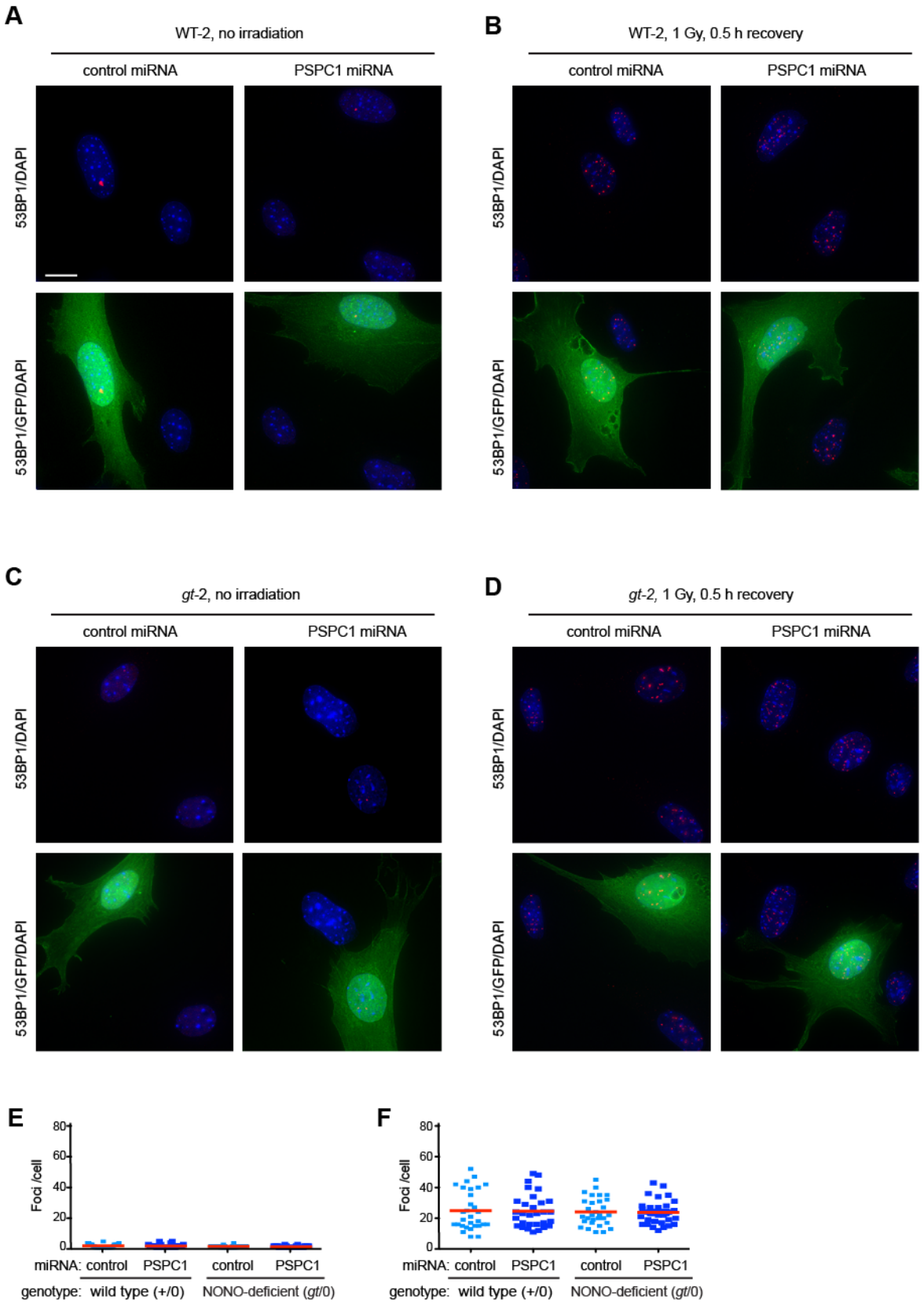
A
WT-2, no irradiation
control miRNA
PSPC1 miRNA
53BP1/DAPI
53BP1/GFP/DAPI
B
WT-2, 1 Gy, 0.5 h recovery
control miRNA
PSPC1 miRNA
53BP1/DAPI
53BP1/GFP/DAPI
C
gt-2, no irradiation
control miRNA
PSPC1 miRNA
53BP1/DAPI
53BP1/GFP/DAPI
D
gt-2, 1 Gy, 0.5 h recovery
control miRNA
PSPC1 miRNA
53BP1/DAPI
53BP1/GFP/DAPI
E
Foci /cell
80
60
40
20
0
miRNA: control PSPC1 control PSPC1
genotype: wild type (+/0) NONO-deficient (gt/0)
F
Foci /cell
80
60
40
20
0
miRNA: control PSPC1 control PSPC1
genotype: wild type (+/0) NONO-deficient (gt/0)

Figure S3. Formation of 53BP1 foci. Vectors encoding control or PSPC1 miRNA were introduced by electroporation into wild type (+/0) or NONO-deficient (*gt*/0) MEFs. Cells were mock irradiated or exposed to 1 Gy of ^{137}Cs γ-rays. At indicated times following irradiation, cells were fixed and analyzed by indirect immunofluorescence using anti-53BP1 primary, red secondary antibody, and DAPI counterstain. A-D. Representative fields showing 53BP1/DAPI channels (top row) or 53BP1/GFP/DAPI merged images for indicated experimental groups. Scale bar denotes 10 μm. E.F. Foci per cell in indicated experimental groups. A total of 30 nuclei were scored per experimental group. Blue symbols depict score for individual cells; red bar depicts mean. Note that 53BP1 foci were equivalently induced in all experimental groups after 1 Gy radiation and 30 min recovery.