USP28 is recruited to sites of DNA damage by the tandem BRCT domains of 53BP1 but plays a minor role in double-strand break metabolism

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Supplemental Methods and Data: 5 Figures

Supplemental Materials and Methods

Cell growth and checkpoint assays

For the 3T3 assay, 300,000 primary MEFs (p0) were plated and cells were trypsinized, counted and 300,000 reseeded every third day. Data plotted is combined from 3 pairs of *Usp28*$^{+/+}$ and *Usp28*$^{T/T}$ cultures.

Genotyping and monitoring of *Usp28* deficient animals

Zygosity was determined by the presence or absence of the genetrap using the primers g10-F2 (5'-ACAGCTTTGTGGAGGCATGAGAGT) and B32-reverse (5'-CAAGGCGATTAAGTTGGGTAACG) and the uninterrupted wild type locus using the forward primer g10-F2 and reverse primer g10-R1(5'-TGGCTGTCCTGGAACTCACTTTGT). These generate a 1025 bp and 200 bp product respectively. Subsequent PCR genotyping was performed using real time PCR primers designed by Transnetyx (www.transnetyx.com, details available upon request). Mice were monitored 3 times weekly over 18 months for the appearance of tumors or other pathology and any animals with signs of distress were sacrificed. All animals were handled in accordance with European community guidelines (86/609/EEC) in the facilities of the Barcelona Science Park. Protocols were approved by the Barcelona Science

Park (CEEA-PCB) animal care and use committee (IACUC) in accordance with appropriate legislation (Law 5/1995/GC; Order 214/1997/GC; Law 1201/2005/SG). All efforts were made to minimize use and suffering.

Supplemental figures

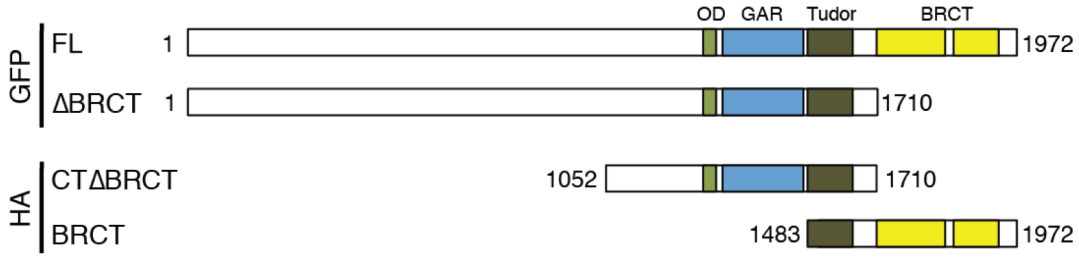

Figure S1: 53BP1 expression constructs. Schematic indicating the regions of 53BP1 included in HA or GFP tagged mammalian expression constructs.

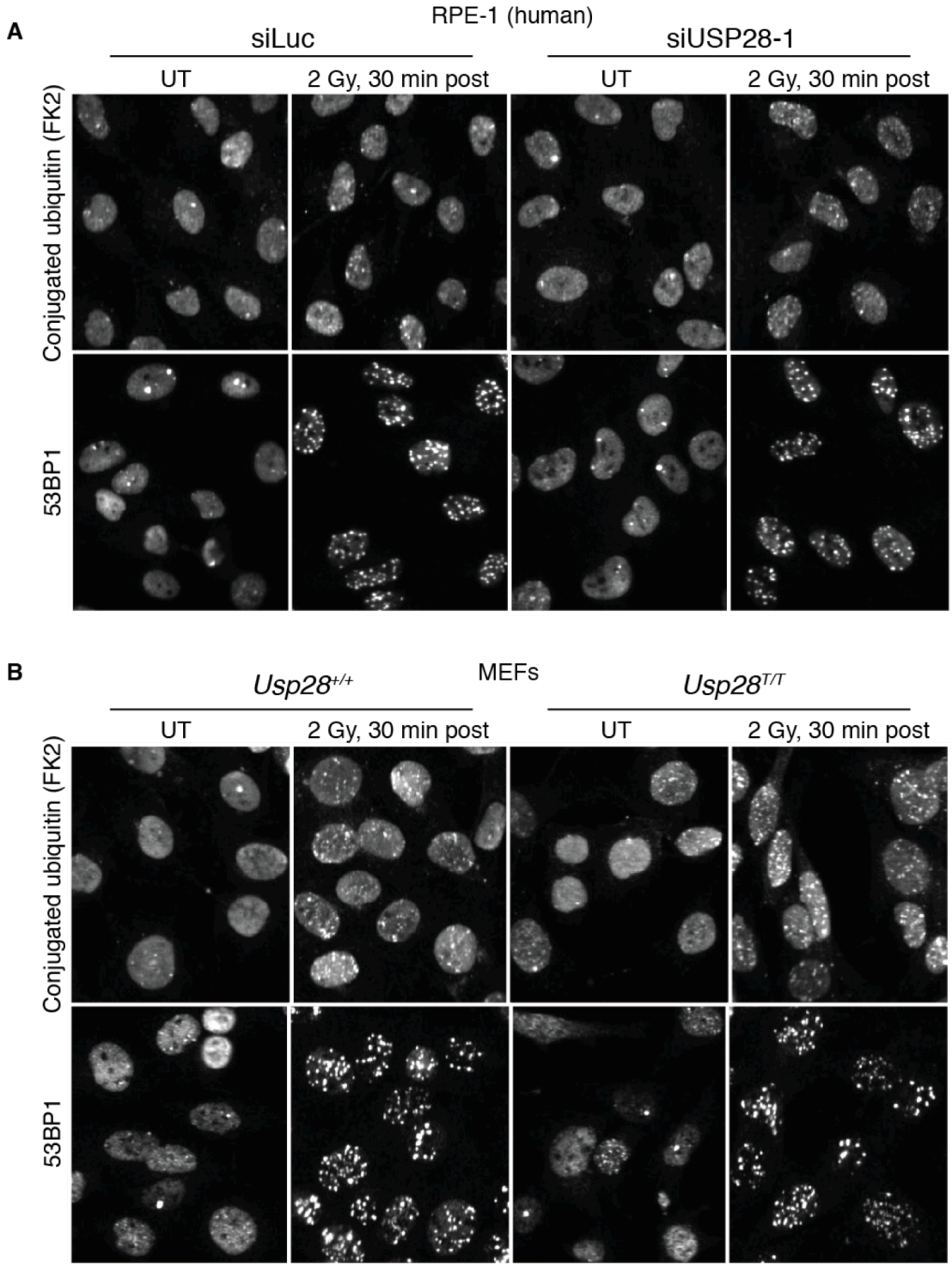

Figure S2: Examples of (A) 53BP1 and (B) conjugated ubiquitin foci stained with the FK2 antibody plotted in Figure 2.

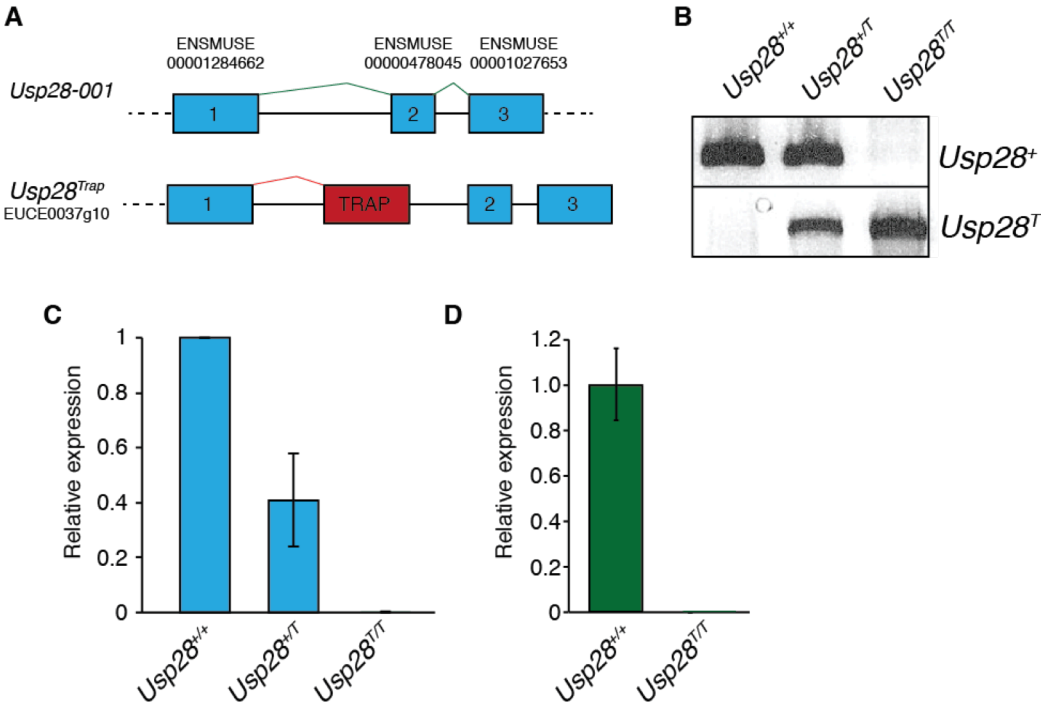

Figure S3: Generation of mice lacking *Usp28* expression. (A) Schematic of the genomic locus of the murine *Usp28* gene with the location of the genetrap indicated (not to scale). (B) Example of PCR genotyping results to identify wild type, heterozygous and homozygous mice. (C) Real time quantitative PCR (ABI, Taqman: Mm00615493_m1) analysis of *Usp28* transcript levels in early passage primary MEFs of the indicated genotype. (D) Real time quantitative PCR analysis of *Usp28* transcript levels in SV40 transformed MEFs of the indicated genotype.

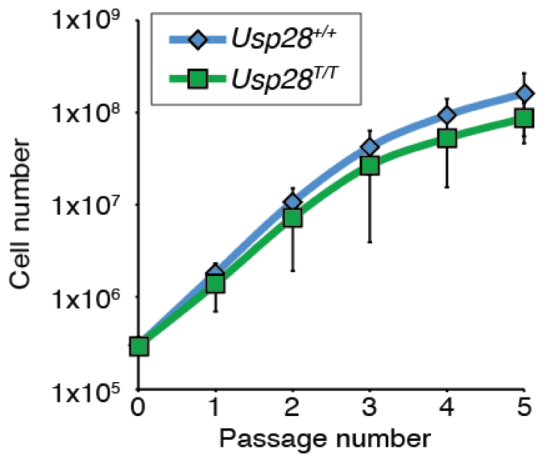

Figure S4: Growth curves of primary MEFs of the indicated genotypes over 5 passages using the 3T3 protocol. The data is combined from 3 independent cultures of each genotype.

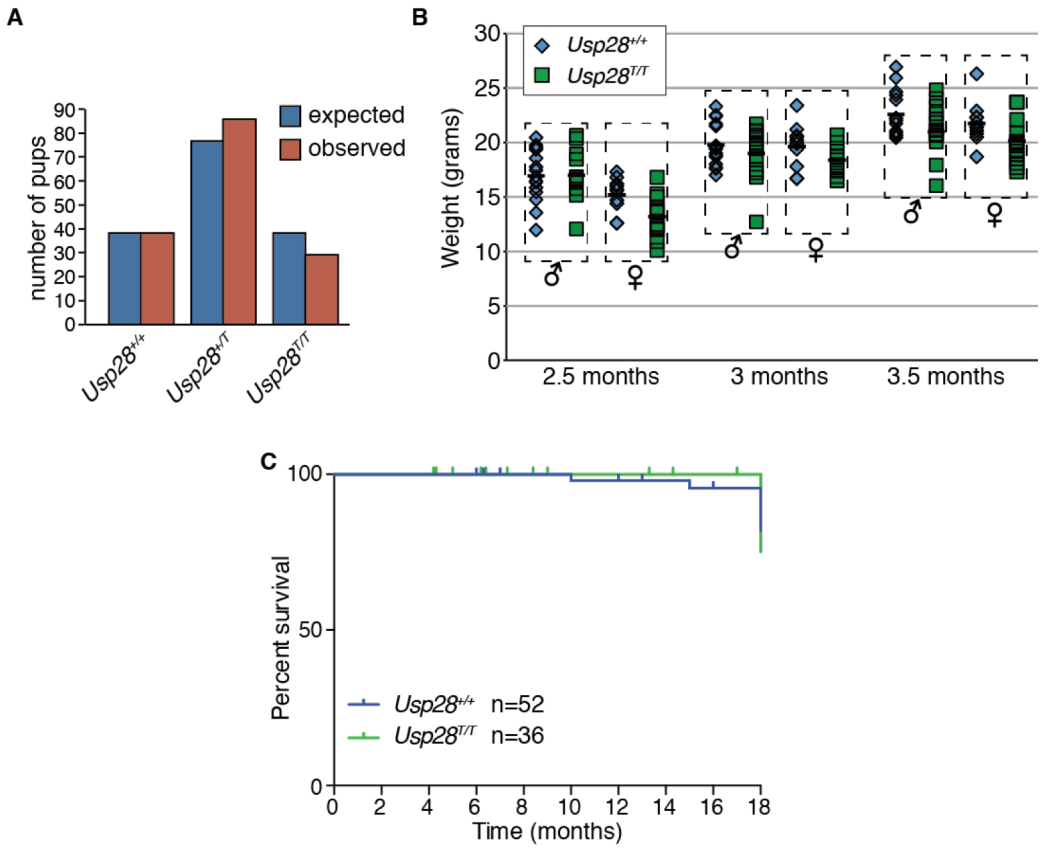

Figure S5: (A) Comparison of the observed numbers of each genotypes from the breeding of heterozygote animals compared to those expected assuming Mendelian inheritance. (B) Weight of animals of the indicated sex and genotype from 2.5-3.5 months of age. (C) Kaplan-Meyer plot of a cohort of *Usp28* deficient animals compared to wild type littermates over 18 months. The total number of animals monitored is indicated.