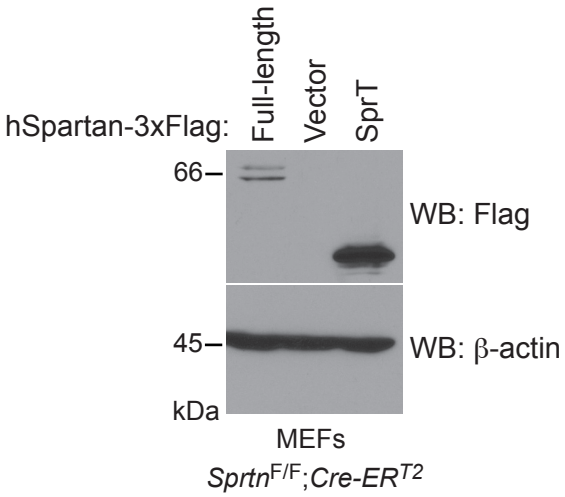
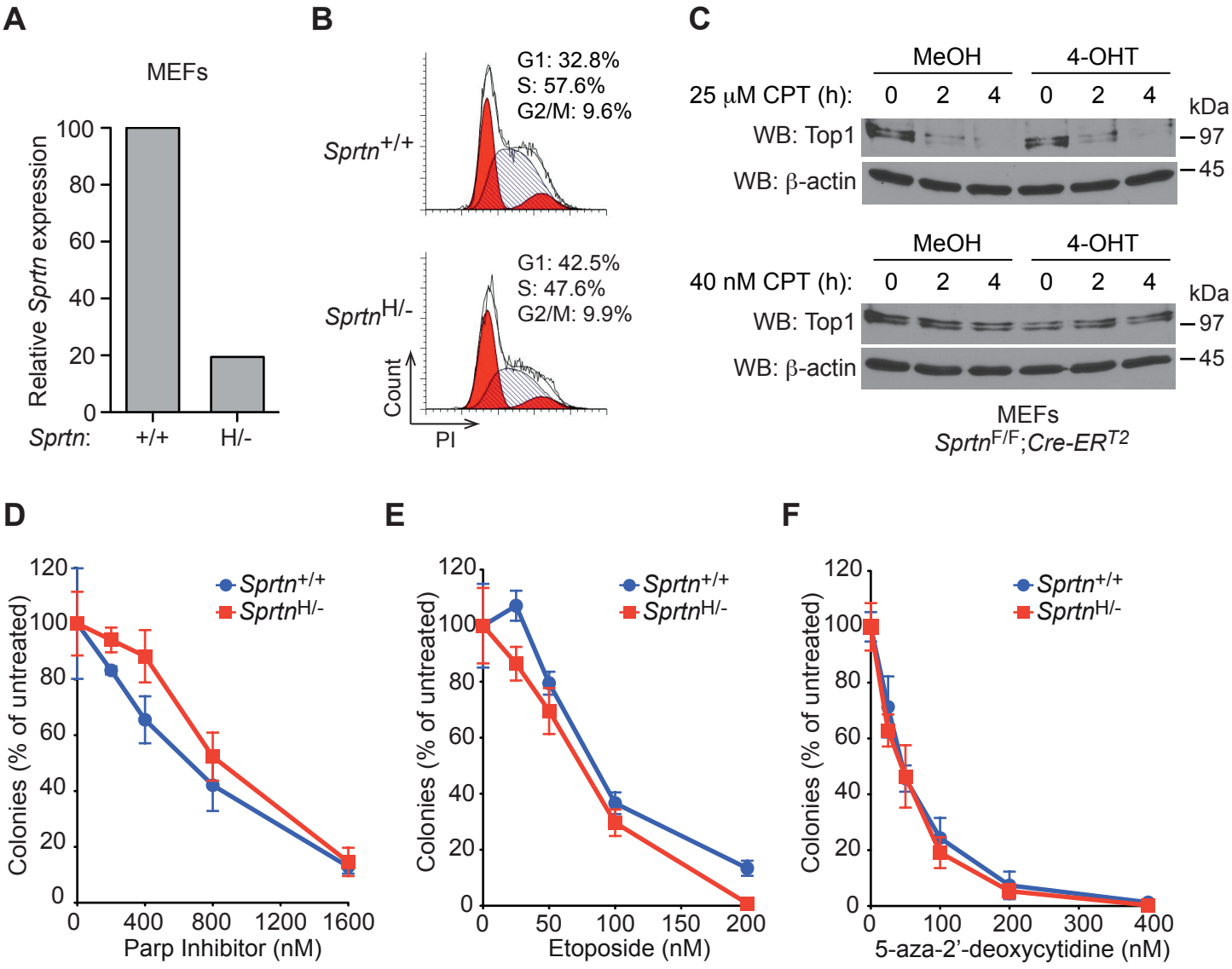
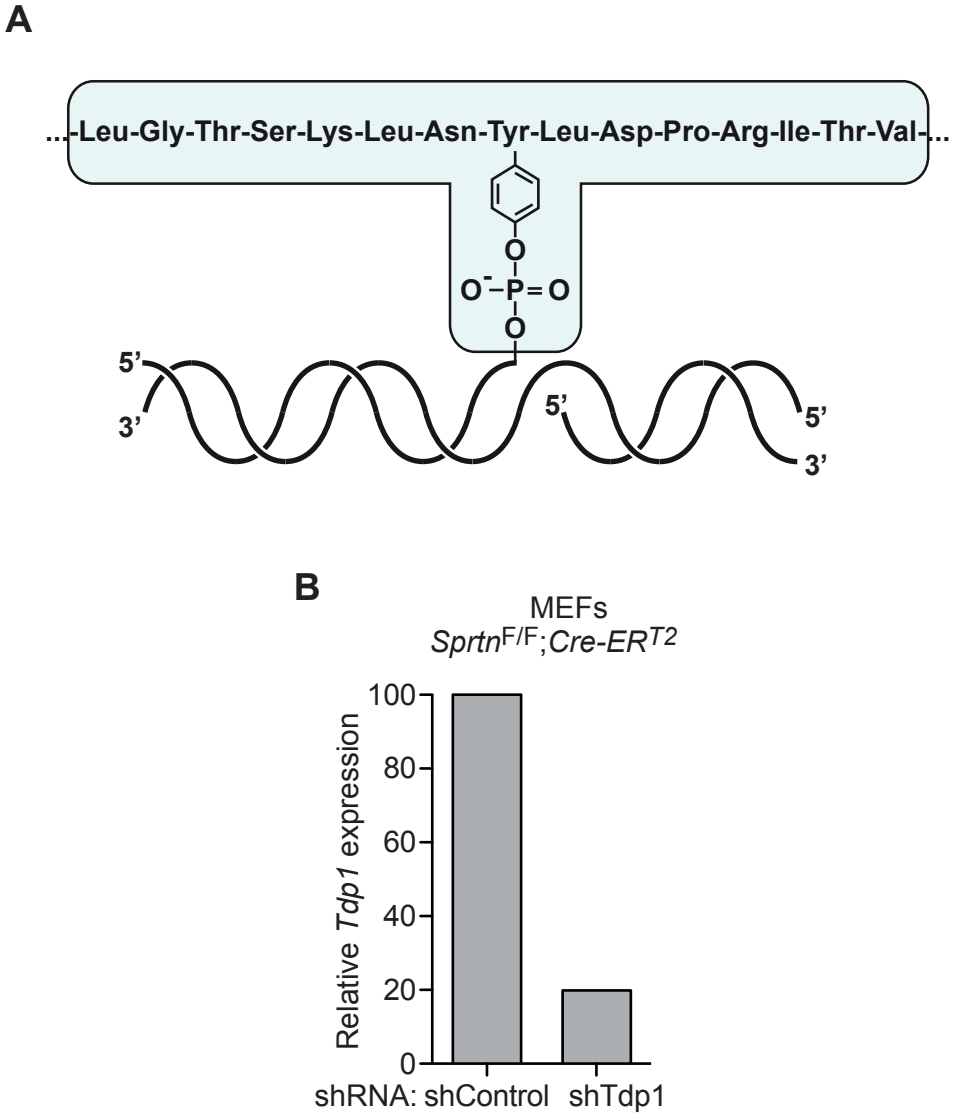


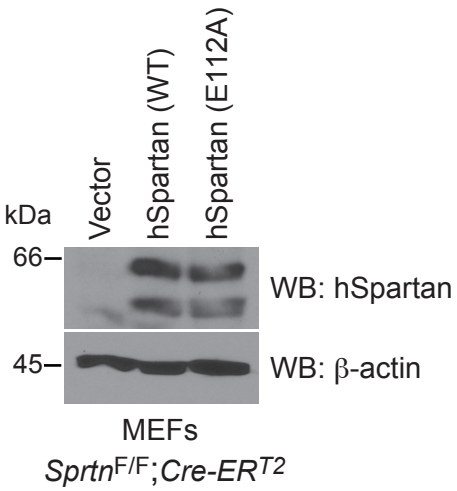
Supplemental Figure S1. Exogenous expression of human Spartan proteins in *Sprtn*$^{F/F}$; *Cre-ER*^{T2} MEFs. Full-length and the SprT domain only (amino acids 1-219) were expressed with a C-terminal 3xFlag tag using retrovirus vectors. Two SV40 nuclear localization signals were added to the C-terminus of the SprT protein to ensure nuclear localization. Expression was assessed by Western blotting using anti-Flag antibodies. Full-length Spartan protein is detected as two major bands due to its mono-ubiquitination. β-actin is shown as a loading control.



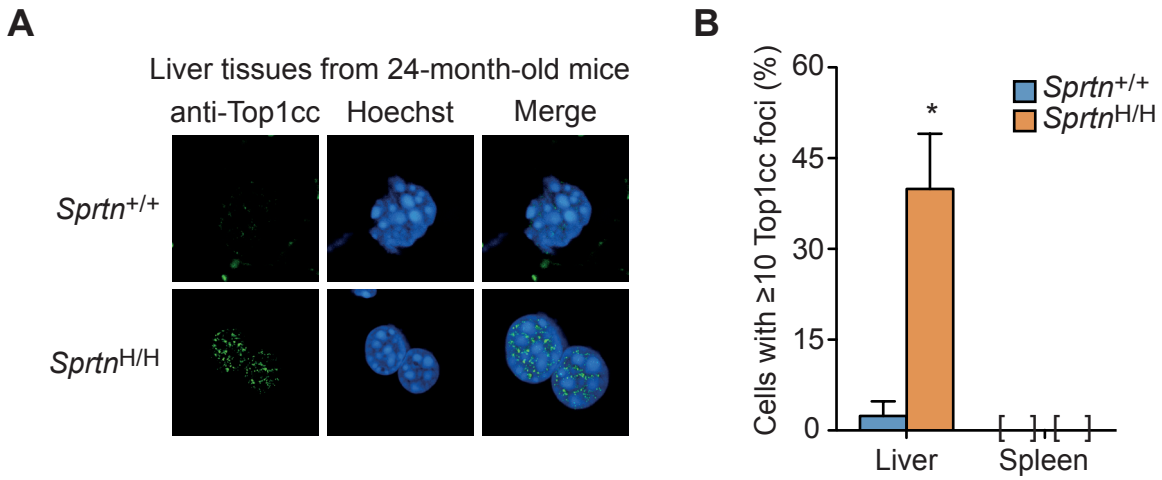
Supplemental Figure S2. Sensitivity of *Sprtn*$^{H/-}$ MEFs to various DPC-inducing drugs. (**A**) qPCR analyses of *Sprtn* mRNA levels in *Sprtn*$^{+/+}$ and *Sprtn*$^{H/-}$ MEFs. Total RNA was isolated from cells and *Sprtn* expression was measured by RT-PCR. Values were normalized to *Gapdh* and presented relative to *Sprtn*$^{+/+}$ MEFs. (**B**) Cell-cycle profiling of *Sprtn*$^{+/+}$ and *Sprtn*$^{H/-}$ MEFs. Cells were stained with PI and analyzed by flow cytometry. (**C**) CPT-induced Top1 degradation assay. *Sprtn*$^{F/F}$; *Cre-ER*^{T2} MEFs were treated with MeOH or 4-OHT for 48 h followed by treatment with CPT, 25 μM (upper panel) or 40 nM (lower panel) for 0, 2 or 4 h at 37^{o}C. Subsequently, cells were incubated in CPT-free medium for another 30 min to reverse Top1cc (for visualizing total Top1 levels). Cells were lysed using alkaline lysis procedure followed by S7 nuclease treatment. Top1 levels were assessed by Western blotting using anti-Top1 antibodies. β -actin is shown as a loading control. (**D-F**) Clonogenic survival assays. *Sprtn*$^{+/+}$ and *Sprtn*$^{H/-}$ MEFs were continuously treated for 6 days with the PARP inhibitor MK-4827 (**D**), Top2 poison etoposide (**E**), and DNA methyltransferase inhibitor 5-aza-2'-deoxycytidine (**F**). Culture medium with drug or diluent was replenished every other day. Cells were stained with Coomassie Blue and colonies with more than 50 cells were counted. Values are mean ± s.d. of triplicate experiments. Results shown are representative of two independent experiments.



Supplemental Figure S3. Antigen used to raise Top1cc antibody and assessment of *Tdp1* mRNA levels in MEFs. (**A**) Top1cc. Peptide shaded in blue was used to raise anti-Top1cc antibody. (**B**) qRT-PCR analyses of *Tdp1* mRNA levels in MEFs. Total RNA was isolated from *Sprtn*$^{F/F}$; *Cre-ER*^{T2} MEFs with or without Tdp1 knockdown, and Tdp1 expression was measured by qRT-PCR. Values were normalized to *Gapdh* and presented relative to shControl cells.



Supplemental Figure S4. Exogenous expression of human Spartan proteins in *Sprtn*$^{F/F}$; *Cre-ER*^{T2} MEFs. Wild-type (WT) human Spartan and the mutant with a point mutation in the metalloprotease active site (E112A) were expressed using retrovirus vectors. Expression was assessed by Western blotting using anti-human Spartan (hSpartan) antibodies. Note that the hSpartan antibody does not recognize mouse Spartan. Spartan proteins are detected as two major bands due to mono-ubiquitination. β-actin is shown as a loading control.



Supplemental Figure S5. The livers of old *Sprtn*$^{H/H}$ mice exhibit increased accumulation of Top1ccs. (**A**) Immunohistochemistry of Top1ccs in the liver of 24-month-old mice. Cryosections of the liver from *Sprtn*$^{+/+}$ and *Sprtn*$^{H/H}$ mice were stained with anti-Top1cc. DNA was stained with Hoechst to visualize nuclei. (**B**) Quantitation of cells containing Top1cc foci in the liver and spleen of 24-month-old mice. At least 100 cells were scored for Top1cc foci in *Sprtn*$^{+/+}$ and *Sprtn*$^{H/H}$ mice. Percentages of cells with 10 or more foci are shown. Values are mean ± s.e.m. (*Sprtn*$^{+/+}$ liver, n=3; *Sprtn*$^{H/H}$ liver, n=4; *Sprtn*$^{+/+}$ spleen, n=3; *Sprtn*$^{H/H}$ spleen, n=3). No cells were scored as positive for Top1cc foci in the spleen. *p <0.05 (two-tailed unpaired t-test).