Manzi et al., http://www.jcb.org/cgi/content/full/jcb.200811105/DC1

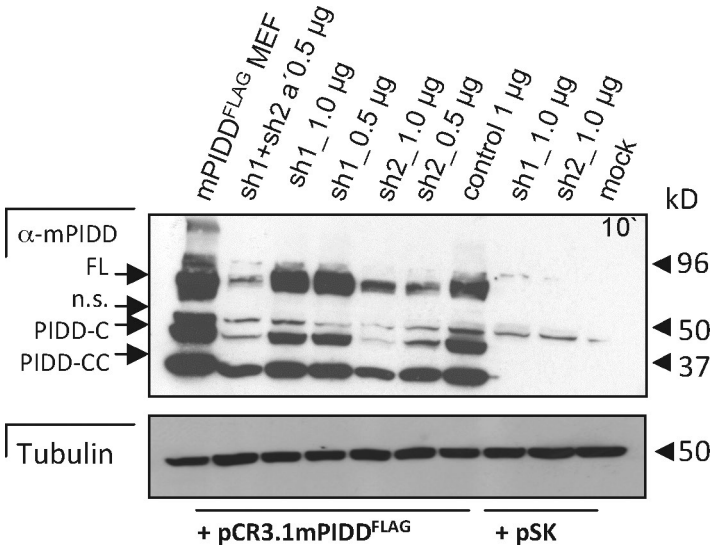

Figure S1. **Specific reactivity of anti-PIDD polyclonal antiserum.** MEFs stably expressing a Flag-tagged mouse version of PIDD show immunoreactivity corresponding to full-length (FL) PIDD (100 kD), the PIDD-C fragment (51 kD), and PIDD-CC (37 kD) with the purified anti-PIDD IgG (first lane). 293T cells were transiently cotransfected with pCR3.1 $mPIDD_{Flag}$ either alone or in combination with one or two different short hairpin RNA–encoding expression plasmids (sh1 or sh2). A partial reduction of anti-PIDD–reactive bands was observed in response to short hairpin RNA 2 by Western blot analysis. Cells transfected with pCR3.1 $mPIDD_{Flag}$ alone or mock-transfected cells were used as controls. Membranes were reprobed using anti–α-tubulin to monitor protein loading. n.s., nonspecific.

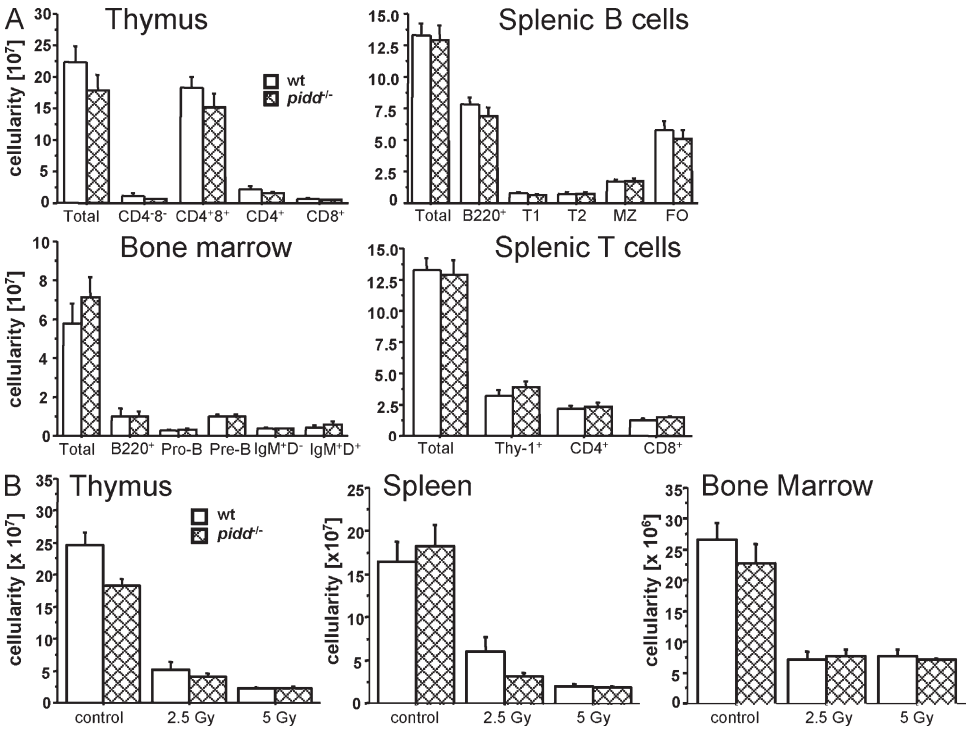

Figure S2. **Loss of PIDD does not interfere with lymphocyte development or cell death induced by γ irradiation in vivo.** (A) The distribution of immature and mature B and T cell subsets in thymus and spleen of wild-type (wt) and *pidd*$^{-/-}$ mice is shown. Single-cell suspensions of bone marrow, thymus, and spleen were stained with subset-selective fluorochrome-labeled cell surface marker–selective antibodies followed by flow cytometric analysis: Pro–B cells (B220^{+}, CD43^{+}, and IgM^{-}) Pre–B cells (B220^{+}, CD43^{-}, and IgM^{-}) T1 B cells (B220^{+}, IgM^{high}, and CD21^{low}), T2 B cells (B220^{+}, IgM^{high}, and CD21^{high}), marginal zone (MZ) B cells (B220^{+}, IgM^{+}, CD21^{high}, and CD23^{low}), and follicular (FO) B cells (B220^{+}, CD21^{+}, and CD23^{+}). Data are presented as means ± SEM of $n \geq 3$ mice per genotype. (B) The impact of one-step whole body irradiation (2.5 and 5 Gy) on cellularity of thymus, spleen, and bone marrow derived from wild-type and *pidd*$^{-/-}$ mice was assessed 20 h after exposure to whole body irradiation. Single-cell suspensions of bone marrow, thymus, and spleen were counted by trypan blue, and aliquots were stained with subset-selective fluorochrome-labeled cell surface marker–specific antibodies followed by flow cytometric analysis. Age-matched mice without radiation treatment were used as controls. Data are presented as means ± SEM of $n \geq 3$ mice per genotype and treatment.

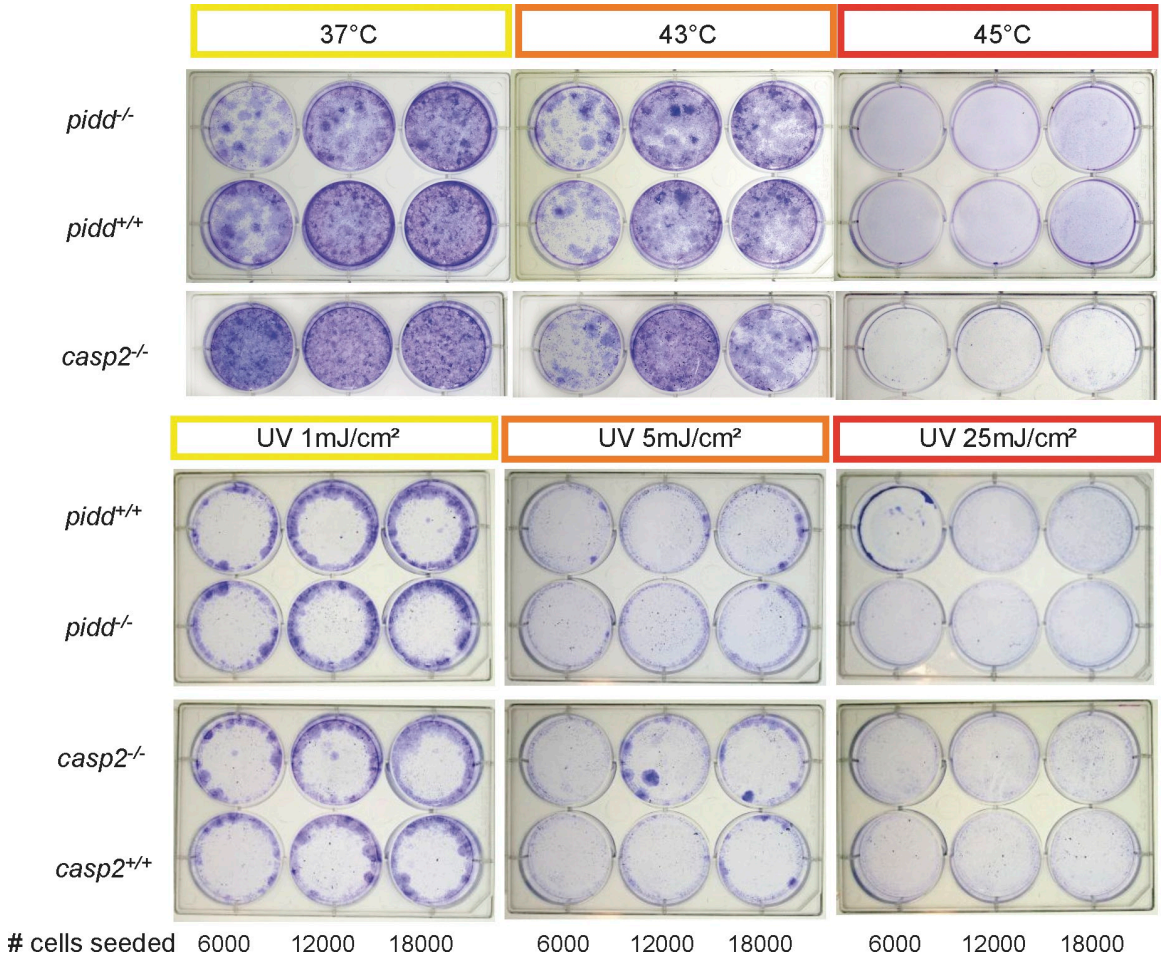

Figure S3. **Colony formation of primary MEFs in the absence of PIDD.** Colony formation of primary MEFs was assessed by seeding an increasing number of cells per well (6,000, 12,000, and 18,000 cells) 24 h before DNA damage or exposure to heat shock. To induce heat shock, cells were incubated for 60 min in a tissue culture incubator set to 43°C or 45°C and then grown under standard conditions. One out of two independent experiments yielding comparable results is shown.

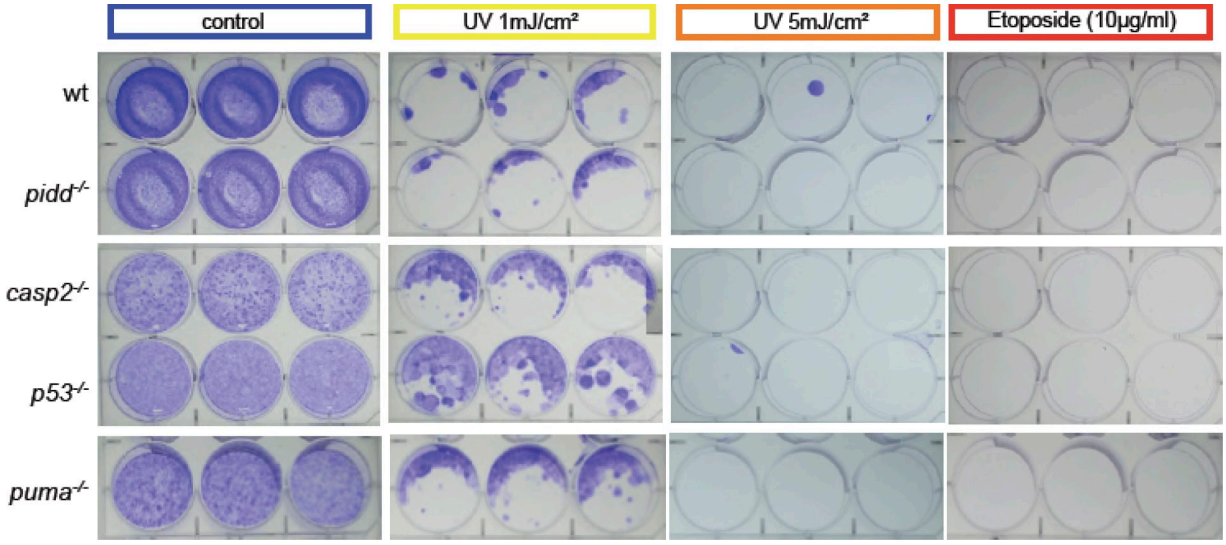

Figure S4. **Colony formation of SV40-immortalized MEFs in the absence of PIDD.** Colony formation of SV40-MEFs was assessed by seeding 12,000 cells/well in triplicate 24 h before DNA damage. Etoposide was removed after 24 h of incubation by changing the media. Surviving cells were stained 2 wk later using crystal violet. wt, wild type.

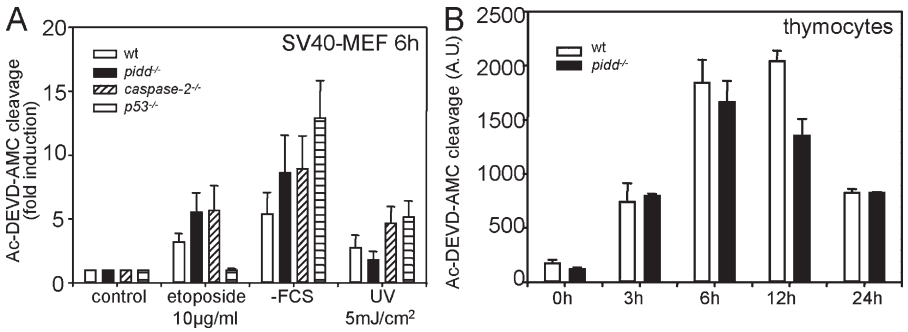

Figure S5. **Quantification of caspase activity in the absence of PIDD.** (A) Caspase-3/7–like (Ac-DEVD-AMC) activity in SV40-immortalized MEFs of the given genotypes 6 h after drug treatment. Error bars indicated SD. (B) Caspase-3/7–like activity in thymocytes of the indicated genotypes treated with 10 µg/ml etoposide over indicated time points are given in means ± SEM of $n = 3$. A.U., arbitrary unit; wt, wild type.