**Figure S1.** (A) Green/Red tract ratios obtained from the distribution of CldU and IdU tract length of Pds5A and Pds5B siRNA depleted RPE-1 cells. Bars represent the mean. Out of 2 repeats; \( n \geq 300 \) tracts scored for each data set. Statistics: Mann-Whitney; ****, \( P < 0.0001 \). (B) Green/Red tract ratios obtained from the distribution of CldU and IdU tract length of Pds5A, Pds5B, and Pds5A/Pds5B siRNA depleted U-2 OS cells. Bars represent the mean. Out of 2 repeats; \( n \geq 300 \) tracts scored for each data set. Statistics: Mann-Whitney; ****, \( P < 0.0001 \). (C) Green/Red tract ratios obtained from the distribution of CldU and IdU tracts lengths in Pds5A, Pds5B KO MEF cells. Bars represent the mean. Out of 2 repeats; \( n \geq 300 \) tracts scored for each data set. Statistics: Mann-Whitney; ****, \( P < 0.0001 \). (D) Expression of Pds5B and BRCA2 in Pds5B, BRCA2, and Pds5B/BRCA2 siRNA depleted U-2 OS cells.
Figure S2. Endogenous BRCA2 interacts with BirA-Pds5B. (A) After induction of BirA-Pds5B with doxycycline, TetON BirA-Pds5B HEK293T cells were treated with 50μM biotin and 4 mM HU for 5h. Complexes were pulled-down with streptavidin beads and analyzed by western blotting with the indicated antibodies. BRCA2 detection was also performed on whole cell extract after BRCA2 depletion to confirm the signal specificity in the pull down. (B) 48h after transfection with GFP-Pds5B, HEK293T cells were treated with 4mM HU for 4h and whole cell extracts were subjected to GFP-trap pulldowns. Bound proteins were analyzed by western blotting with the indicated antibodies.
Figure S3. (A) Single-molecule DNA fiber-labeling scheme (top). Size distribution of IdU tract length in Pds5B siRNA depleted RPE-1 cells treated with 50 µM Mirin. Bars represent the mean. Out of 2 repeats; n ≥ 300 tracts scored for each data set. Statistics: Mann-Whitney; ****, P < 0.0001. (B) Size distribution of IdU tract length in MRE11 shRNA depleted Pds5B knockout MEF cells. Bars represent the mean. Out of 2 repeats; n ≥ 300 tracts scored for each data set. Statistics: Mann-Whitney; ****, P < 0.0001. (C) Size distribution of IdU and CldU tract length in Pds5A, Pds5B, Nbs1, Pds5A/Nbs1 and Pds5B/Nbs1 siRNA depleted U-2 OS cells. Bars represent the mean. Out of 2 repeats; n ≥ 300 tracts scored for each data set. Statistics: Mann-Whitney; ****, P < 0.0001. (D) Size distribution of IdU tract lengths in Pds5A, Pds5B, Rad50, Pds5A/Rad50 and Pds5B/Rad50 siRNA depleted U-2 OS cells. Bars represent the mean. Out of 2 repeats; n ≥ 300 tracts scored for each data set. Statistics: Mann-Whitney; ****, P < 0.0001. (E) Expression of MRE11, Nbs1, and Rad50 in Pds5A siRNA depleted U-2 OS cells. (F) Expression of MRE11, Nbs1, and Rad50 in Pds5B siRNA depleted U-2 OS cells. (G) Expression of Rad51 and Smarcal1 in Pds5A siRNA depleted U-2 OS cells. (H) Expression of Rad51 and Smarcal1 in Pds5B siRNA depleted U-2 OS cells.
Figure S4. (A) Expression of Pds5A, Pds5B, and Wapl in Pds5A, Pds5B, Wapl, Pds5A/Wapl, and Pds5B/Wapl siRNA depleted U-2 OS cells. (B) Expression of Pds5A and Rad21 in Pds5A, Rad21, and Pds5A/Rad21 siRNA depleted U-2 OS cells. (C) Expression of Pds5B and Rad21 in Pds5B, Rad21, and Pds5B/Rad21 siRNA depleted U-2 OS cells. (D) Expression of Wapl and Rad21 in Wapl, Rad21, and Wapl/Rad21 siRNA depleted U-2 OS cells. (E) Neutral comet assay to measure the accumulation of DSBs in Pds5A and Pds5B knockout MEF cells treated with vehicle or with 2 mM HU for 18 hours. The y-axis the Olive Moment (a.u.). Out of 3 repeats; n ≥ 100 comets scored for each data set. Data are represented as mean ± SEM. (F) Brightfield imaging assessing cell morphology changes in Pds5A, Pds5B, Wapl, Rad21, Pds5A/Rad21, Pds5B/Rad21 and Wapl/Rad21 siRNA depleted U-2 OS cells.
Figure S5. (A) Western blot analysis Chk1-S345, Chk1, RPA-S33, γ-H2AX, H2AX, and Pds5B in PdsB depleted U-2 OS cells in the presence and absence of 4 mM HU for 2 hours. (B) Western blot analysis Chk1-S345, Chk1, γ-H2AX, and Pds5B in Pds5B knockout MEFs in the presence and absence of 4 mM HU for 2 hours. (C) Left, representative immunofluorescence images showing the accumulation of 53BP1 bodies upon Pds5A and Pds5B depletion in U-2 OS cells. CENP-F was used as a marker for cells in G2. Right, quantification of the number of 53BP1 bodies per cell. At least 100 cells were scored for each data set. n = 2. Data are represented as mean ± SEM. Statistics: two tailed paired t-test.