



Supplemental Fig. S5. Verification of 53BP1-null findings in an independently generated 53bp1-KO clone. A) Heatmaps of END-seq signal +/- 3 kb around the top 200 AsiSI breaks in *Lig4*^{-/-} and *Lig4*^{-/-}*53bp1*^{-/-} clone #2 pre-B cells after 18 hr AsiSI cutting. **B)** Genome browser snapshot of DNA strand-separated END-seq in *Lig4*^{-/-} and *Lig4*^{-/-}*53bp1*^{-/-} clone #2 cells at a single AsiSI break (chr12:111,039,088-111,039,096). **C)** Boxplots of maximum resection lengths per AsiSI site (left) and ratio of resected breaks versus total breaks (right) as detected by END-seq after AsiSI induction with either no additional treatment (NT), 5 μM aphidicolin (APH), or 1 μM DNA Polα inhibitor (POLAi) concurrent treatment. **D)** Boxplots of RPA single-strand DNA sequencing (SSDS) intensity in *Lig4*^{-/-}*53bp1*^{-/-} clone #2 cells after 18 hr AsiSI cutting with either no additional treatment (NT), 5 μM APH, or 1 μM POLAi concurrent treatment. **E)** Genome browser snapshot of SAR-seq signal at an individual AsiSI DSB with END-seq resection as reference (chr5: 115,630,000-115-635,000). **F)** Heatmaps of SAR-seq signal +/- 3 kb around the top 200 AsiSI sites in *Lig4*^{-/-} and *Lig4*^{-/-}*53bp1*^{-/-} clone #2 cells after 18 hr break induction. **G)** Pearson's correlation of normalized read intensity at each AsiSI break location between *Lig4*^{-/-} and *Lig4*^{-/-}*53bp1*^{-/-} clone #2 SAR-seq from "F". **H)** Boxplots of SAR-seq intensity in *Lig4*^{-/-}*53bp1*^{-/-} clone #2 cells after 18 hr AsiSI cutting with either no additional treatment (NT), 5 μM APH, or 1 μM POLAi concurrent treatment. All panels show datasets from one experiment and serve as an independent biological replicate to findings in Fig. 5.