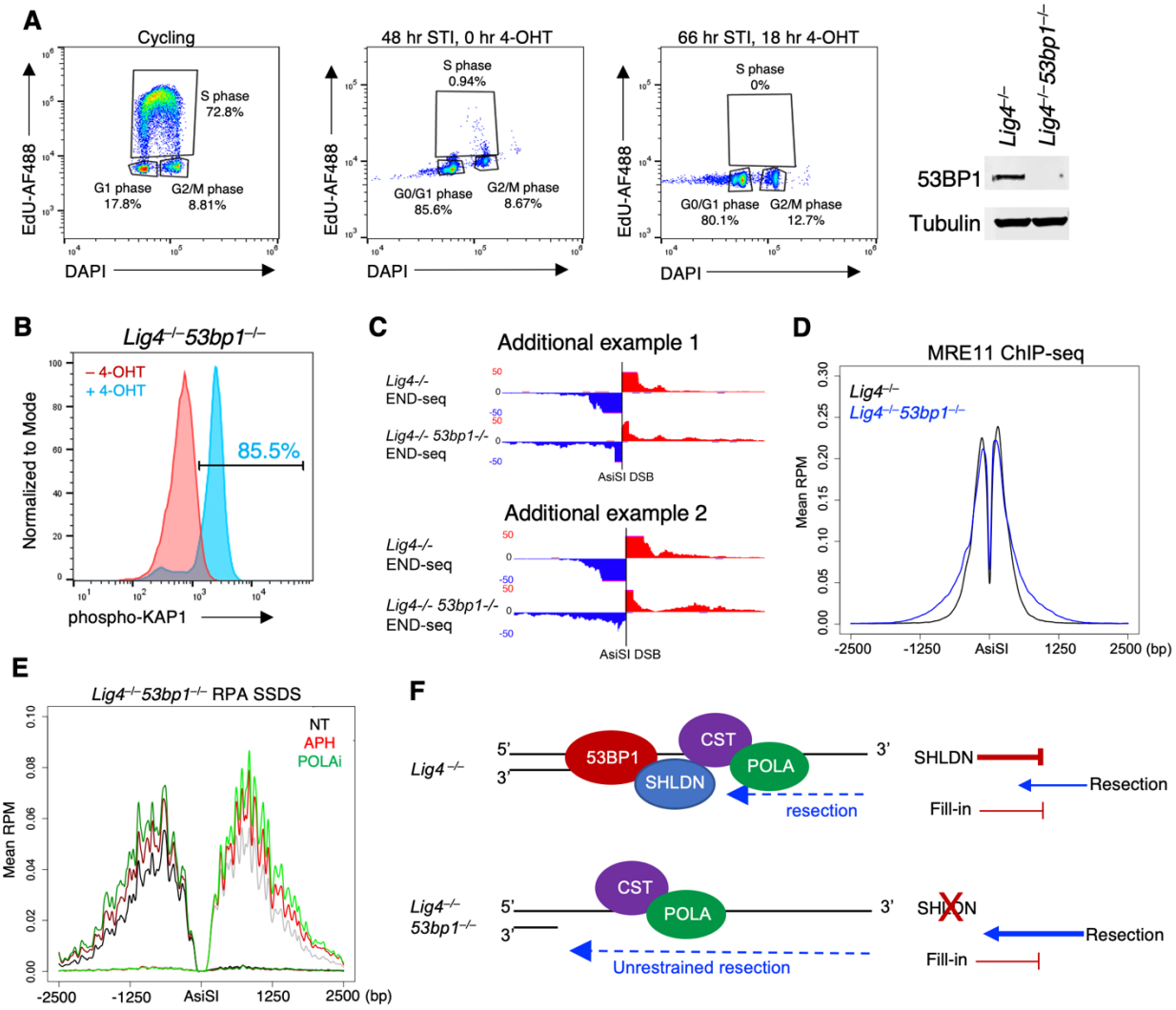
Paiano_Supp_FigS4



Supplemental Fig. S4. 53BP1-deficient cells exhibit normal MRE11 recruitment and are largely unaffected by fill-in synthesis inhibition. A) Cell cycle profiles of *Lig4*$^{-/-}$*53bp1*$^{-/-}$ G0/G1 arrest by EdU and DAPI flow cytometry measurement. While ~70% of cells are in S phase in cycling cultures, >80% of cells are G0/G1 arrested after 48 hr imatinib (STI) treatment and remain arrested 18 hr later after AsiSI induction. Western blot (right) in *Lig4*$^{-/-}$ or *Lig4*$^{-/-}$*53bp1*$^{-/-}$ pre-B cells confirms the absence of 53BP1 protein. **B)** Phosphorylated KAP1 (phospho-KAP1; double-strand break (DSB) marker) FACS histograms of G0/G1-arrested *Lig4*$^{-/-}$*53bp1*$^{-/-}$ cells after 6 hr AsiSI cutting to confirm presence of DSBs. **C)** Genome browser snapshots of two additional examples of END-seq resection at AsiSI DSBs (Example 1, chr2:32236003-32236010; Example 2, chr11:120784162-120784170). **D)** Aggregated reads per million (RPM), averaged across the strongest 200 AsiSI breaks, of MRE11 ChIP-seq signal +/- 2.5 kb around AsiSI recognition sequences. AsiSI was induced for 6 hr in *Lig4*$^{-/-}$ (black) and *Lig4*$^{-/-}$*53bp1*$^{-/-}$ (blue) cells to determine resection initiation by the recruitment of the MRE11/NBS1/RAD50 complex. MRE11 ChIP-seq was performed once. **E)** Aggregated RPA single-strand DNA sequencing (SSDS) in *Lig4*$^{-/-}$*53bp1*$^{-/-}$ cells after 18 hr AsiSI cutting concurrently treated with 5 μM aphidicolin (APH; red), 1 μM polymerase alpha inhibitor (POLAi; green), or no treatment (NT; black). RPA SSDS observations were reproduced with two independent *Lig4*$^{-/-}$*53bp1*$^{-/-}$ clones (see Supplementary Fig. S5). **F)** Model to explain hyper-resection observed after loss of 53BP1. In NHEJ-deficient *Lig4*$^{-/-}$ cells, in which resection frequently occurs, resection is largely limited by 53BP1/Shieldin (SHLDN) exonuclease blockade, while CST/Polα-initiated fill-in

synthesis plays a more minor, yet significant, role in limiting resection tracks. After loss of 53BP1, end protection is abolished, and unrestrained resection occurs. Polα is still recruited to ssDNA in the absence of 53BP1, perhaps through CST, yet subsequent fill-in alone is unable to confine resection.