



**Supplementary Figure 1. WRN dependency validation experiments in dMMR CRC preclinical models.** **A**, Alterations of *KRAS*, *BRAF*, *NTRK1*, *ALK* and *RSPO3* genes in 105 dMMR/MSI CRC samples (MSKCC, Cancer Cell 2018). **B**, WRN essentiality values from whole-genome CRISPR-cas9 dropout screening in two dMMR (red bar) and one MMR-proficient (blue bar) CRC organoids. Fold changes are the average of three technical replicates. **C**, Validation of WRN dependency in HCM-SANG-0273-C18 organoid by CRISPR-based co-competition assay. Data are mean  $\pm$  SD of three independent experiments. A co-competition score of less than 1 indicates a relative

reduction in BFP-positive cells, resulting from targeting of a loss-of-fitness gene. **D**, Normalized viability in dMMR organoids upon transduction of WRN sgRNAs. Non-essential (sgNon) and PLK1 (sgPLK1) sgRNAs were used as negative and positive controls, respectively. Data represent the mean and SD of 3 independent experiments with 10 technical replicates each. Statistical significance was evaluated comparing WRN sgRNA versus non essential gene sgRNA (sgNon): ns, not significant;  $P < 0.05$ ;  $P < 0.01$ ;  $P < 0.001$  (two-tailed Student's *t* test). **E**, siRNA-mediated depletion of WRN verified by Western blot (images are representative of two independent experiments). siRNA non-targeting controls (siNTC), siRNA targeting WRN (siWRN). **F**, WRN knockout with WRN-targeting sgRNAs confirmed by Western blot (images are representative of two independent experiments). **G**, chromatid breaks in SW620 (MSS), SNU175 and HCT15 (MSI WRN independent) and SW48 (MSI WRN dependent) cell line 96h after WRN depletion ( $\geq 20$  metaphase spreads analysed). **H**, Representative images of metaphases affected by chromatid breaks (red arrows) after 96h of transduction with sgWRN2.