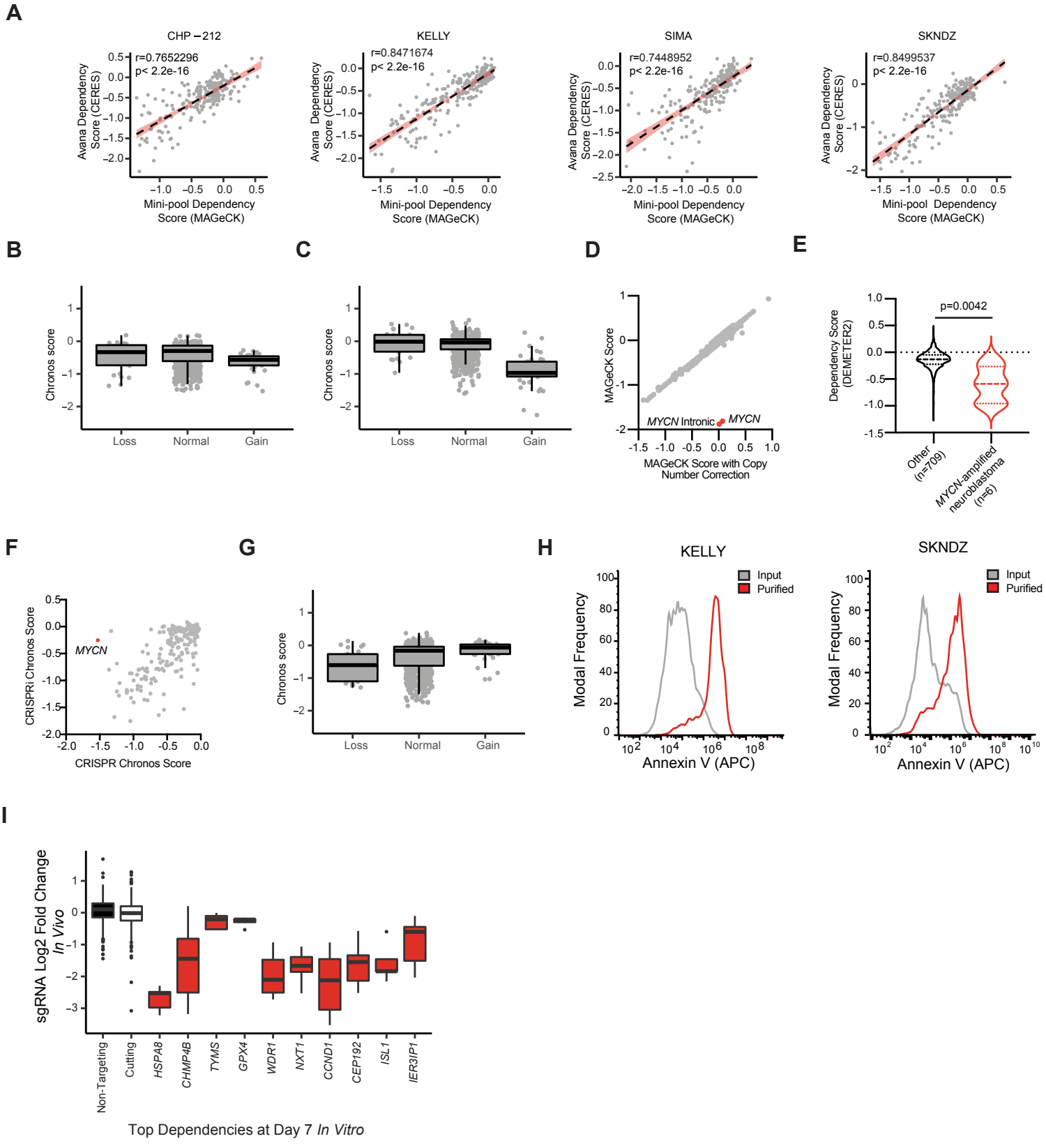
Supplemental Figure S1



Supplemental Figure S1. CRISPR screens prioritize rapidly lethal *in vivo* relevant dependencies

A, The correlation between gene effects in DepMap and in our secondary CRISPR screen is shown for four neuroblastoma cell lines. On the x-axis are gene effect scores from day 21 using the sgRNA library described in **Fig1A,** collapsed to gene level using the MAGeCK algorithm. On the y-axis the gene effect score collapsed to gene level using the CERES algorithm from the DepMap dataset using the Avana library, also collected at day 21, is shown. Each dot represents an individual gene. The best fit line is plotted as a dashed line, the standard deviation of the line is in red, and the Pearson correlation coefficient and *p*-value for each cell line is shown. **B,** Gene effect sizes for CRISPR cutting guides for the three time points in Cas9+ cell lines were collapsed to a single gene effect score using the Chronos algorithm (see methods). Genes were binned as copy gained, neutral, or lost for each of the four cell lines as indicated on the x-axis (see methods). On the y-axis the gene effect size calculated in Chronos is plotted. Each dot represents an individual gene's effect in a single cell line. The box and whisker plots show the median gene effect size for genes in that copy number category. **C,** As in **B**, genes were divided by copy number status in each of the four cell lines. The three intronic guides for each gene across three time points were collapsed to a single score using the Chronos algorithm. The effect size for these intronic guides in Cas9+ cell lines is shown on the y-axis. Each dot indicates a single gene in a single cell line. The box and whisker plots show the median gene effect size for genes in that copy number category. **D,** MAGeCK scores with (x-axis) and without (y-axis) computational copy number correction for intronic and gene targeting guides in KELLY Cas9 at day 21. In red, the gene targeting and intronic guide scores of *MYCN* are highlighted. **E,** The DEMETER2 dependency score for *MYCN* is shown on the y-axis for cell lines in the Novartis Project DRIVE shRNA screen. Violin plots depict the dependency on *MYCN* for *MYCN*-amplified neuroblastoma cell lines in red at right, and all other cell lines in black at left. The *p*-value for an unpaired two-

tailed t-test with Welch's correction is shown. **F,** The data in **Fig 1E** for the KELLY cell line are replotted with *MYCN* highlighted in red, as it depletes strongly in CRISPR but not CRISPRi. **G,** As in **B** and **C**, genes were divided by copy number status. The gene effect score across all three time points was calculated in Chronos for CRISPRi sgRNAs screened in the dCas9-KRAB cell lines, and this is shown on the y-axis. Each dot indicates a single gene in a single cell line. The box and whisker plots show the median gene effect size for genes in that copy number category. **H,** Seven days after infection with the sgRNA library, cells were Annexin-V purified using a magnetic column (see **Fig 1E** and methods). To confirm enrichment of Annexin-V positivity in purified cells, Annexin V staining and flow cytometry were performed on the input population (black) and the purified population (red). **I,** KELLY cells expressing Cas9 were infected with the sgRNA library described in **Fig 1A**, selected, and implanted into the flanks of NSG mice three days after selection, and tumors were harvested after 26 days. Guide level depletions for negative controls *in vivo* are shown in black (non-targeting n=600) and in white (cutting controls n=600). In red, guide level depletions (n=5) *in vivo* for each of the top 10 dependencies (without copy number correction) at day 7 in the *in vitro* screen are shown, with increasing *in vitro* dependency from left to right.