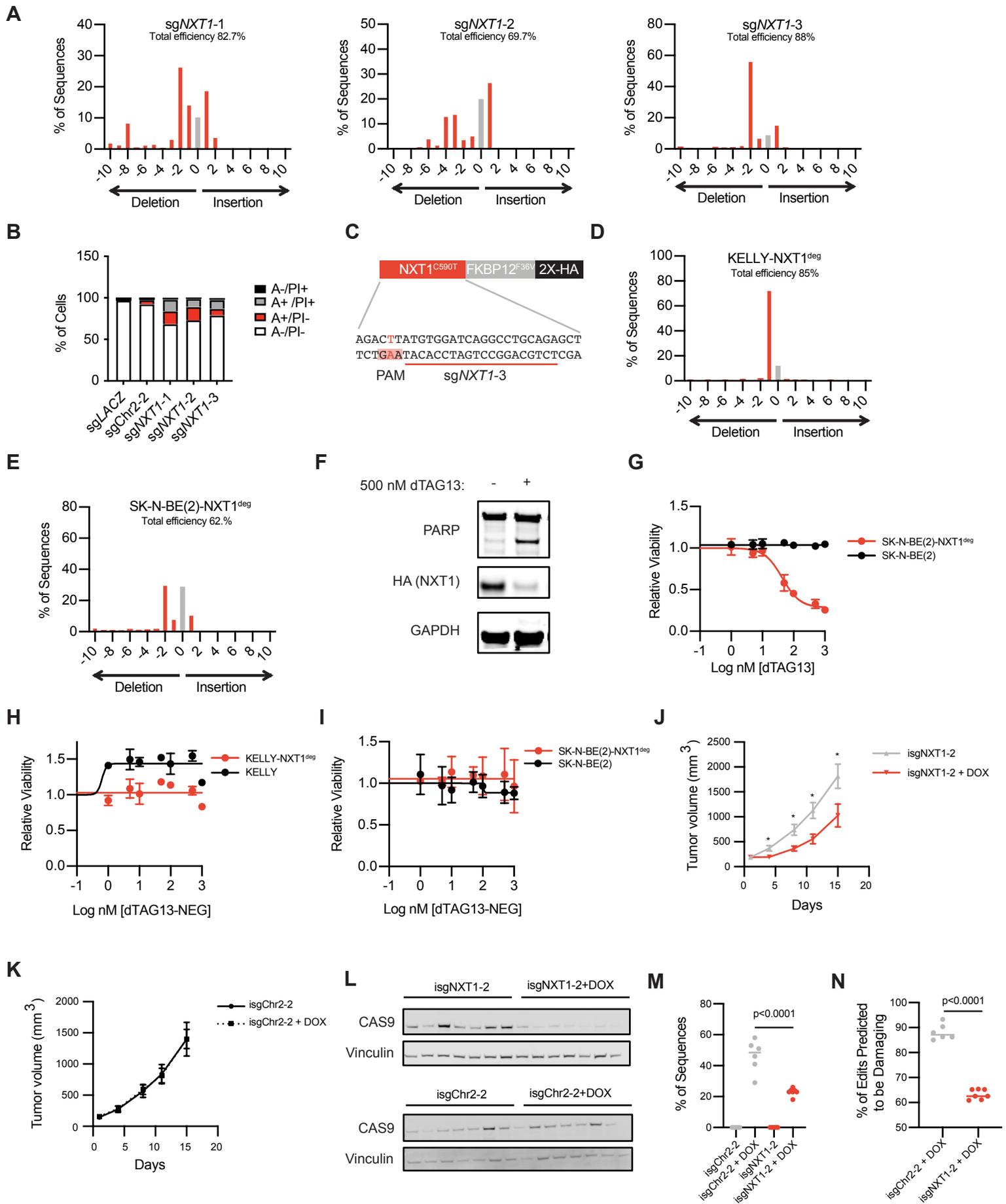# Supplemental Figure S2

Supplemental Figure S2. *NXT1* **is a selective and lethal dependency in neuroblastoma**

A, Genomic editing at the guide target site was assessed by sequencing and the TIDE algorithm was used to deconvolute sequencing (see methods). Representative editing in a cell line (HT-29) that is not dependent on *NXT1* is shown for each of the three sgRNAs used for low-throughput experiments. The y-axis indicates the percent of reads that are predicted to have an insertion or deletion of the indicated number of bases (x-axis) around the predicted cut site in *NXT1.* In gray, the percent of sequences that are predicted to be unedited is shown, with insertions shown in red to the right, and deletions shown in red to the left. At the top, the overall predicted editing efficiency is shown. **B,** SK-N-BE(2) cells were infected with a vector containing Cas9 and the indicated sgRNAs. Nine days after infection cells were collected and stained with Annexin-V (A) and Propidium Iodide (PI). The percent of cells that were double negative (white), Annexin-V positive (red), PI positive (black), or double positive (gray) is shown. **C,** Diagram of the exogenous *NXT1*^{C590T} (red) with c-terminal *FKBP12*^{F36V} (gray) and HA-tags (black). The C590T alteration mutates the PAM site of sg*NXT1*-3, as shown below, rendering the exogenous construct resistant to this sgRNA. The guide sequence of sg*NXT1*-3 is underlined in red, and the PAM site is highlighted in red with the C→T alteration indicated in red. **D,** Stable, polyclonal KELLY cells with endogenous editing of *NXT1* with sg*NXT1*-3 and exogenous expression of the resistant construct described in **C** were generated (KELLY-NXT1^{deg}) and editing of the endogenous *NXT1* locus was assessed as in **A.** The bar graph indicates the estimated percent of sequences with insertions or deletions as indicated, and the overall estimation of editing efficiency is shown at the top. **E,** SK-N-BE(2) -NXT1^{deg} cells were generated as in **D,** and editing was assessed as in **A**. **F,** Western blot of PARP, HA-NXT1, and GAPDH after 24 hours of treatment with DMSO or 500 nM dTAG-13 in SK-N-BE(2)-NXT1^{deg} cells. **G,** Relative viability for parental SK-N-BE(2) (black) and SK-N-BE(2)-NXT1^{deg} (red) after 72 hours of treatment with the indicated concentrations of dTAG-13 is shown. The y-axis depicts the relative viability, the x-axis indicates the log of the nM concentration of dTAG-13. Each data point is the mean +/- stdev of six replicates. **H,** Relative viability for

parental KELLY (black) and KELLY-NXT1^{deg} (red) after 72 hours of treatment with the indicated concentration of an inactive form of dTAG-13 which cannot recruit cereblon is shown. The y-axis depicts the relative viability, the x-axis indicates the log of the nM dose of dTAG-13. Each data point is the mean +/- stdev of six technical replicates. **I,** Dose response curves as in **H** for parental SK-N-BE(2) (black) or SK-N-BE(2)-NXT1^{deg} (red). **J,** Tumor volume for tumors with inducible sg*NXT1*-2 with (red) and without (grey) doxycycline treatment. *indicates p<0.05. Error bars are standard error. **K,** As in **J,** Tumor volume for tumors with inducible sgChr2-2 is shown with (dotted line) or without (solid line) doxycycline treatment. No time points are statistically significantly different across groups. Error bars are standard error. **L,** Western blot of tumors at the endpoint of the experiment shows that tumors that grow out with doxycycline induction of sg*NXT1*-2 do not express Cas9. Below, endpoint tumors expressing an inducible sgChr2-2 show no change in Cas9 expression between treatment groups. **M,** Dot plot indicates the percent of sequences that have been edited in endpoint tumors in inducible sgChr2-2 (gray) or sgNXT1-2 (red) with or without doxycycline induction as indicated. Editing predictions were made using the ICE v2.0 algorithm. **N,** The ICE v2.0 algorithm was used to predict which mutations produce out of frame indels and would be expected to disrupt gene function. Percent of total edits that are predicted to knockout gene function in the same tumors as shown in **M**.