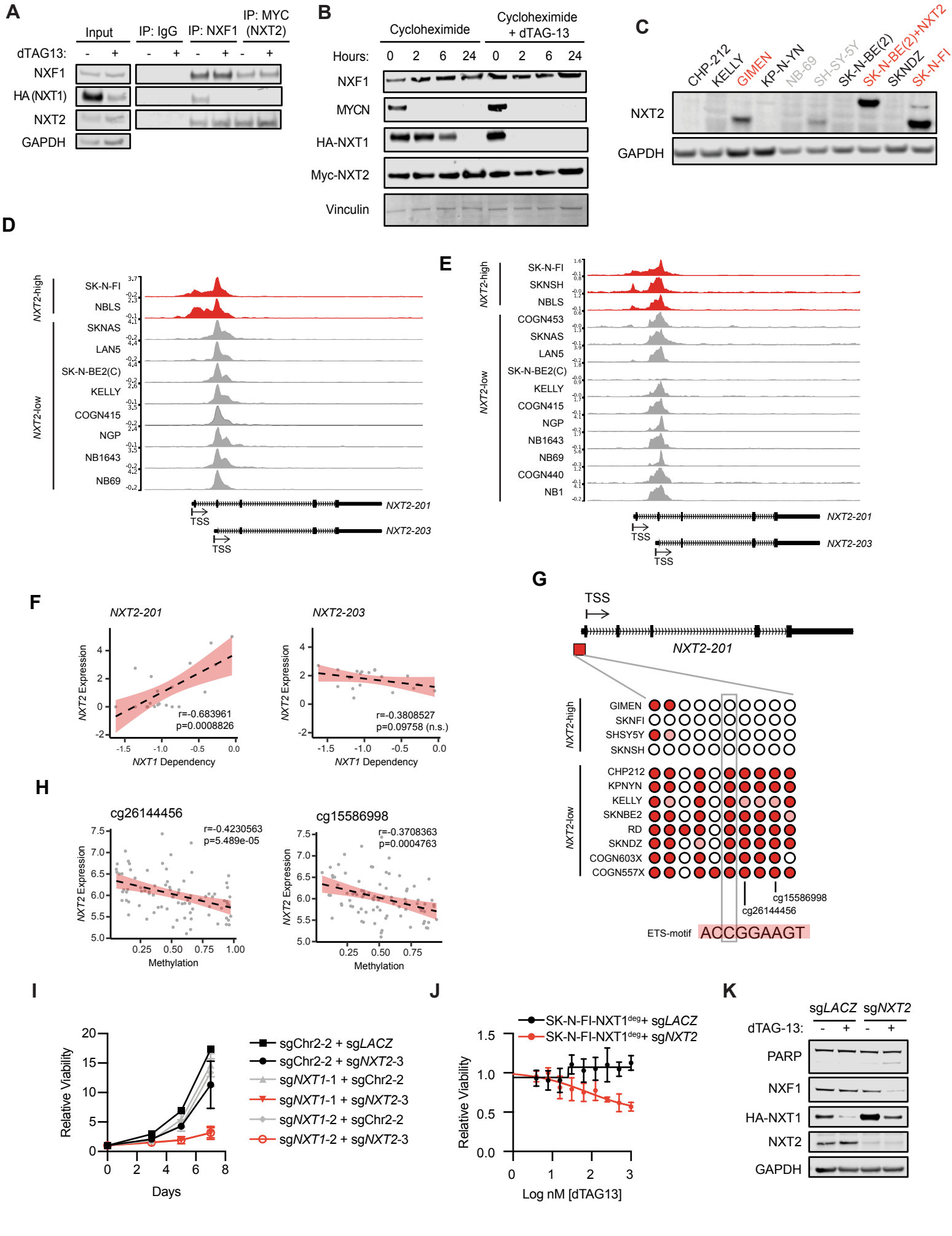
Supplemental Figure S5



Supplemental Figure S5. Differential *NXT2* expression in neuroblastoma cell lines

A, Immunoprecipitations were performed using antibodies targeting mouse IgG, NXF1 and MYC (NXT2) six hours after DMSO or 500 nM dTAG13 treatment, as indicated in the SK-N-BE(2)-NXT1^{deg} cell line overexpressing *NXT2*. NXF1, NXT2 and HA (NXT1) levels are shown. Input lysate is shown to the left with NXF1, NXT2, HA (NXT1), and GAPDH. **B,** KELLY-NXT^{deg} cells overexpressing *NXT2* were treated with 50 μg/mL cycloheximide plus DMSO or 500 nM dTAG-13 as indicated. Cells were lysed at the indicated time after treatment, and the western blot depicts NXF1, MYCN, HA-NXT1, myc-NXT2, and vinculin levels. MYCN is a short half-life protein used as a control for cycloheximide treatment. **C,** Western blot of NXT2 levels across a panel of neuroblastoma cell lines. In black are neuroblastoma lines that are dependent on *NXT1* in the DepMap dataset. In red are neuroblastoma cell lines that are not dependent on *NXT1* in DepMap or in low-throughput experiments. Gray cell lines have not been assessed for *NXT1* dependency. **D,** H3K4me3 ChIP seq tracks for *NXT2* in neuroblastoma cell lines. Cell lines are ordered based on *NXT2* expression, with SK-N-FI the highest and NB69 the lowest. Cell lines with high expression are colored in red, others are in grey. Two main isoforms of *NXT2* are shown below. **E,** ATAC-seq tracks for *NXT2* in neuroblastoma cell lines. Cell lines are ordered and colored by *NXT2* expression as in **D.** Two main isoforms of *NXT2* are shown below. **F,** Transcript level expression for two major isoforms of *NXT2* is shown. At left, the expression of the full-length *NXT2-201* isoform is shown on the y-axis, and *NXT1* dependency CERES score is shown on the x-axis for 19 neuroblastoma cell lines. The best fit line is shown as a dotted line, and the *p*-value and Pearson correlation coefficient are shown at bottom right. At right, the same data is depicted for the shorter *NXT2-203* isoform with an alternative start site that results in a different first exon. **G,** Bisulfite sequencing for a stretch of 10 CpGs upstream of the TSS for the full length *NXT2* (indicated by red box) described by Sung et al. (29) was performed for neuroblastoma cell lines with high or low *NXT2* expression. Each circle represents a CpG in that region: white circles are

unmethylated, red circles are methylated, and pink circles are partially methylated. A CpG that falls in a consensus ETS-family recognition sequence is indicated with a box. CpGs included in the Illumina 450k methylation array are labeled with their array ID. **H**, Correlation of DNA methylation with *NXT2* expression in neuroblastoma tumors in the TARGET dataset. The y-axis indicates the Affymetrix array expression of *NXT2*, and the x-axis indicates the methylation beta value for the indicated CpG in the Illumina 450K array, where 0 is unmethylated and 1 is fully methylated. The CpG array ID is shown at top, and corresponds with the CpGs labeled in **G.** The best fit line is shown with a dotted line, and Pearson correlation coefficient and *p*-values are shown at top right. **I,** GIMEN cells were stably transduced with sgRNAs targeting Chr2-2 (negative control) or *NXT1* as indicated. They were then transduced with sgRNAs targeting *LACZ* or *NXT2*. Cells with knockout of both *NXT1* and *NXT2* are shown in red. Viability relative to day 0 was assessed by CellTiter-Glo. Relative viability is graphed on the y-axis, and days are shown on the x-axis. Each data point represents the mean +/- stdev of replicates. **J,** Dose response curve for dTAG-13 after 48 hours in SK-N-FI-NXT1^{deg} cells infected with sg*LACZ* (black) or sg*NXT2* (red). Viability (relative to DMSO) is shown on the y-axis, and the log of the nM concentration of dTAG-13 is shown on the x-axis. Data points indicate the mean +/- stdev of technical replicates. **K**, SK-N-FI-NXT1^{deg} infected with sg*LACZ* or sg*NXT2*, were treated with 500 nM dTAG-13 for 24 hours and PARP, NXF1, HA-NXT1, NXT2, and GAPDH levels were assessed by western blot.