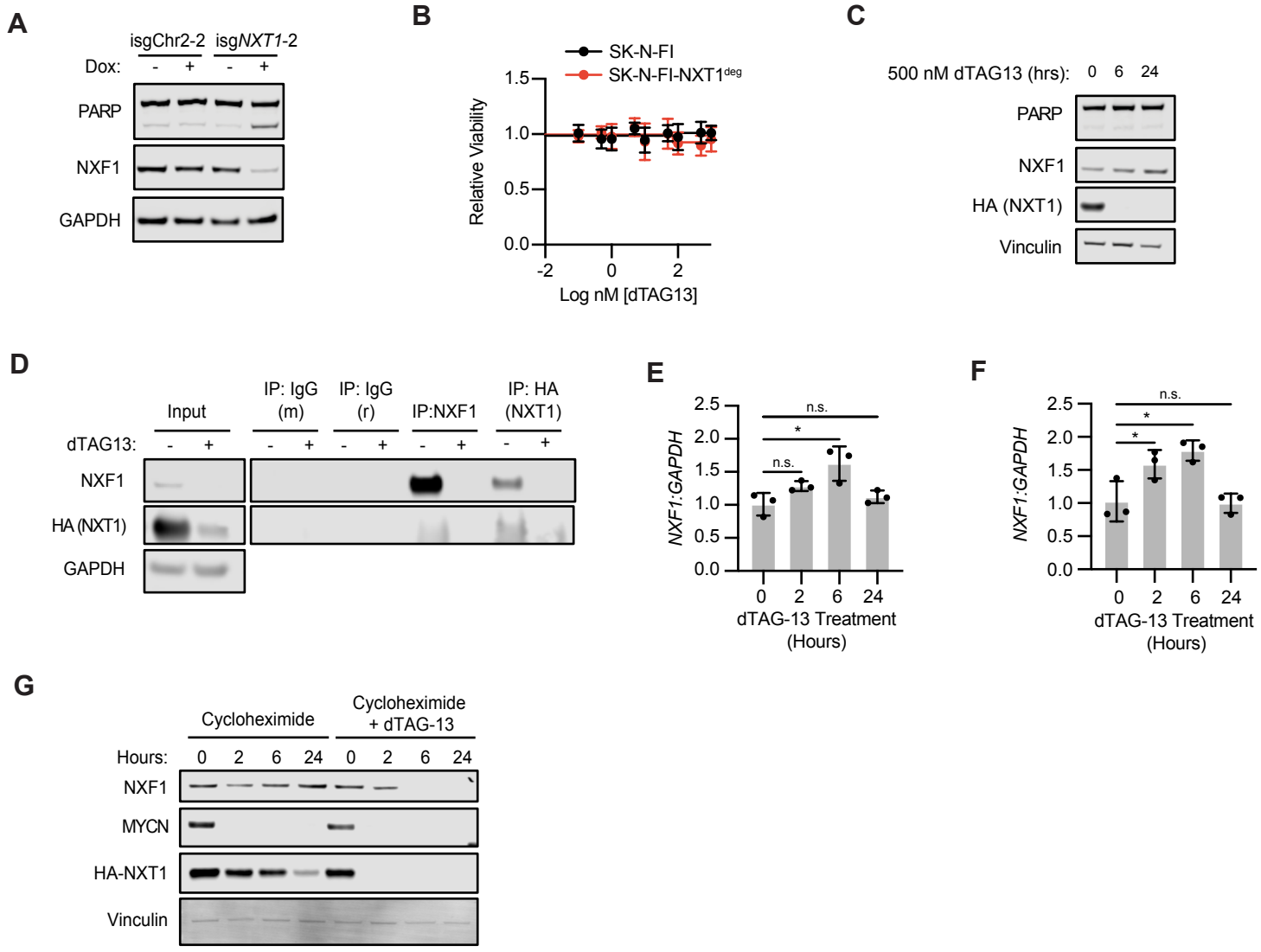
Supplemental Figure S3



Supplemental Figure S3. NXT2 loss leads to loss of NXF1

A, KELLY-Cas9 cells were infected with doxycycline inducible sgRNAs as indicated (see methods). After 72 hours of 1 μg/mL doxycycline (Dox) cells were harvested and protein levels of NXF1, PARP, and GAPDH were assessed by western blot. **B,** Dose response curve of SK-N-FI cells with deletion of endogenous *NXT1* and expression of an sgRNA resistant degron-tagged *NXT1* (SK-N-FI-NXT1^{deg}) for dTAG-13 after 72 hours of treatment. 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 replicates. **C**, SK-N-FI-NXT1^{deg} were treated with 500 nM dTAG-13 for the indicated number of hours (hrs) and PARP, NXF1, HA-NXT1, and GAPDH levels were assessed by western blot. **D,** Immunoprecipitations were performed using antibodies targeting mouse and rabbit IgG, NXF1 (mouse) and HA (rabbit) six hours after DMSO or 500 nM dTAG13 treatment, as indicated, in the SK-N-BE(2)-NXT1^{deg} cell line. NXF1 and HA levels are shown detecting endogenous NXF1 and HA-tagged NXT1 respectively. Input lysate is shown to the left with NXF1, HA, and GAPDH. **E,** Quantitative RT-PCR for was performed after indicated number of hours of 500 nM dTAG-13 treatment in KELLY-NXT1^{deg} cells. Mean of three biological replicates +/- stdev of relative *NXF1:GAPDH* expression is shown on the y-axis, with all samples normalized to the 0 hour sample. * indicates $p < 0.05$ and n.s. indicates not significant in a two-way ANOVA with Dunnett's multiple comparisons test. **F,** Quantitative RT-PCR was performed after indicated number of hours of 500 nM dTAG-13 treatment in SK-N-BE(2)-NXT1^{deg} cells. Mean of three biological replicates +/- stdev of relative *NXF1:GAPDH* expression is shown on the y-axis, with all samples normalized to the 0 hour sample. * indicates $p < 0.05$ and n.s. indicates not significant in a two-way ANOVA with Dunnett's multiple comparisons test. **G**, KELLY-NXT^{deg} cells were treated with 50 μg/mL cycloheximide plus DMSO or 500 nM dTAG-13 as indicated. Cells were lysed the indicated amount of time after treatment, and the western blot depicts NXF1,

MYCN, HA-NXT1, and vinculin levels. MYCN is a short half-life protein used as a control for cycloheximide treatment.