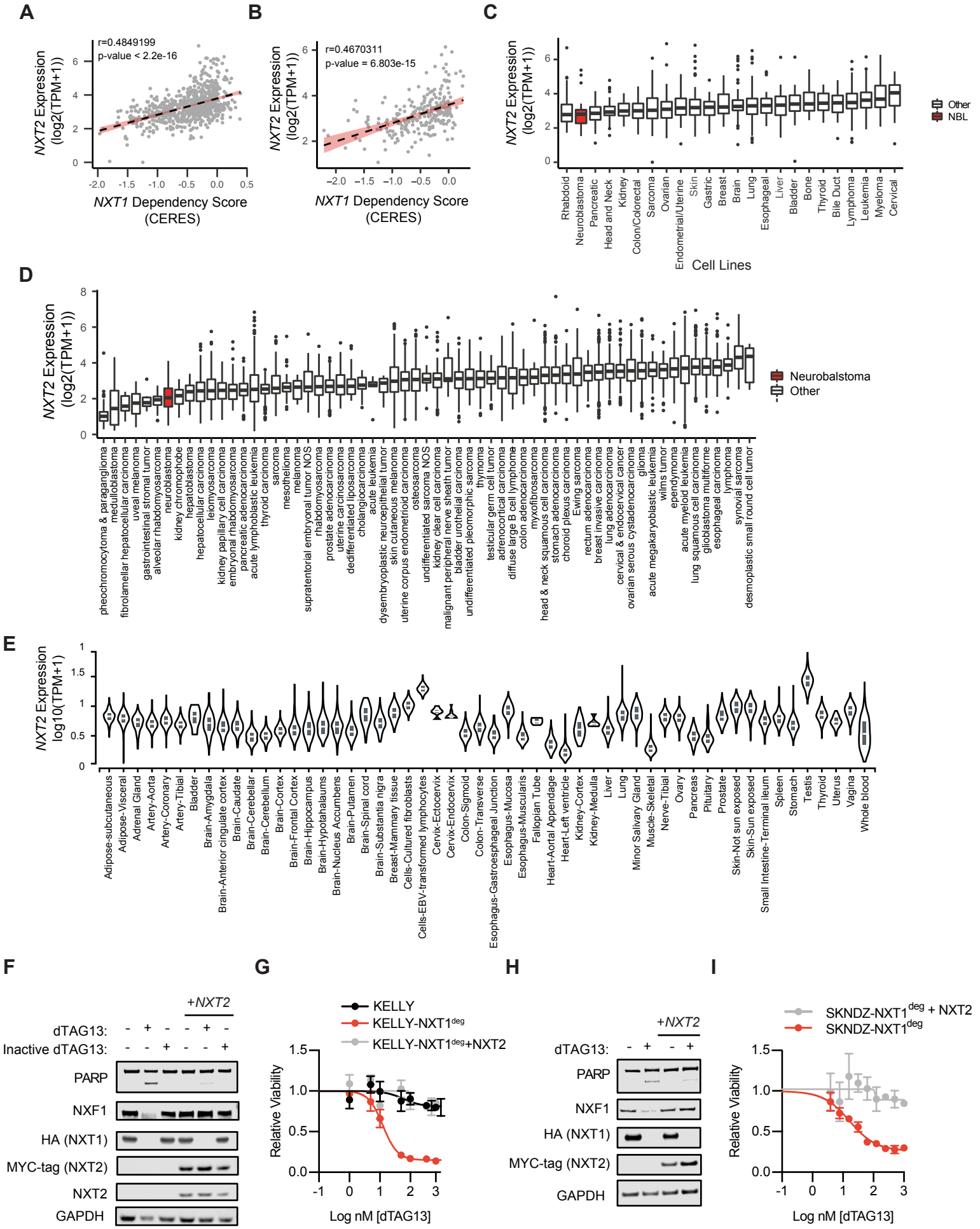
Supplemental Figure S4



Supplemental Figure S4. *NXT2* expression is associated with NXT1 dependency

A, Correlation between *NXT2* expression (y-axis, $\log_2$ of TPM +1) and *NXT1* gene dependency CERES score (x-axis) for all 739 cell lines in DepMap. The Pearson correlation coefficient and *p*-value are shown at the top. The linear regression is shown. **B,** The correlation between *NXT2* expression (y-axis, $\log_2$ of TPM +1) and *NXT1* dependency CERES score (x-axis) from the Sanger Cancer Dependency Map dataset for all 249 cell lines with dependency and expression data. The Pearson correlation coefficient and *p*-value are shown at the top. The best-fit line is plotted as a dotted line. **C,** A box and whisker plot of *NXT2* expression for cancer cell lines in CCLE ($\log_2$(TPM+1), grouped by histology on the x-axis. Histologies with fewer than 10 cell lines were excluded. Neuroblastoma is highlighted in red. Sample sizes are as follows: Bile duct (n=32), Bladder (n=36), Bone (n=36), Brain (n=78), Breast (n=56), Cervical (n=12), Colon/Colorectal (n=66), Endometrial/Uterine (n=32), Gastric (n=41), Head and Neck (n=41), Kidney (n=33), Leukemia (n=94), Liver (n=24), Lung (n=206), Lymphoma (n=76), Myeloma (n=30), Neuroblastoma (n=26), Ovarian (n=55), Pancreatic (n=50), Rhabdoid (n=22), Sarcoma (n=35), Skin (n=68), Thyroid (n=12). **D,** *NXT2* expression was assessed in the Treehouse database of human tumor samples (Tumor Compendium Version 11). *NXT2* expression $\log_2$ (TPM+1) is shown on the y-axis. Disease subtypes with fewer than 6 samples were excluded. Sample sizes are as follows: gastrointestinal stromal tumor (n=7), melanoma (n=7), desmoplastic small round cell tumor (n=8), fibrolamellar hepatocellular carcinoma (n=8), malignant peripheral nerve sheath tumor (n=9), acute leukemia (n=10), undifferentiated sarcoma NOS (n=13), dysembryoplastic neuroepithelial tumor (n=14), myxofibrosarcoma (n=17), hepatoblastoma (n=19), supratentorial embryonal tumor NOS (n=19), choroid plexus carcinoma (n=26), cholangiocarcinoma (n=37), synovial sarcoma (n=41), diffuse large B-cell lymphoma (n=47), undifferentiated pleomorphic sarcoma (n=47), alveolar rhabdomyosarcoma (n=49), dedifferentiated liposarcoma (n=50), lymphoma (n=50), rhabdomyosarcoma (n=57),

uterine carcinosarcoma (n=57), embryonal rhabdomyosarcoma (n=61), sarcoma (n=61), kidney chromophobe (n=66), leiomyosarcoma (n=78), uveal melanoma (n=79), Ewing sarcoma (n=85), mesothelioma (n=87), acute megakaryoblastic leukemia (n=93), rectum adenocarcinoma (n=93), adrenocortical carcinoma (n=96), ependymoma (n=102), thymoma (n=119), medulloblastoma (n=125), wilms tumor (n=137), testicular germ cell tumor (n=155), pancreatic adenocarcinoma (n=179), uterine corpus endometrioid carcinoma (n=181), esophageal carcinoma (n=182), pheochromocytoma & paraganglioma (n=184), osteosarcoma (n=189), glioblastoma multiforme (n=200), neuroblastoma (n=201), kidney papillary cell carcinoma (n=298) , colon adenocarcinoma (n=293), cervical & endocervical cancer (n=306), hepatocellular carcinoma (n=375), bladder urothelial carcinoma (n=407), stomach adenocarcinoma (n=414), ovarian serous cystadenocarcinoma (n=427), skin cutaneous melanoma (n=469), prostate adenocarcinoma (n=496), lung squamous cell carcinoma (n=498), lung adenocarcinoma (n=516), thyroid carcinoma (n=516), head & neck squamous cell carcinoma (n=520), kidney clear cell carcinoma (n=532), acute myeloid leukemia (n=538), glioma (n=738), acute lymphoblastic leukemia (n=878), breast invasive carcinoma (n=1099). **E,** *NXT2* expression ($\log_{10}$(TPM+1)) is shown on the y-axis for normal tissues in the Genotype-Tissue Expression (GTEX) project. **F,** Western blot depicting PARP, NXF1, HA-NXT1, MYC-tagged NXT2, and GAPDH levels after 24 hours of treatment with DMSO, 500 nM dTAG13, or 500 nM of an inactive form of dTAG13, as indicated at the top. At left is KELLY-NXT1^{deg}, at right KELLY-NXT1^{deg}+NXT2. **G**, Dose response curve to dTAG-13 for KELLY parental (black), KELLY cells with deletion of endogenous *NXT1* and expression of degron-tagged exogenous *NXT1* (KELLY-NXT1^{deg}, red), and KELLY cells with deletion of exogenous *NXT1*, expression of degron-tagged exogenous *NXT1* and expression of exogenous *NXT2* (KELLY-NXT1^{deg}+NXT2, gray). The x-axis indicates the log of the nM concentration of dTAG-13, and the y-axis indicates viability assessed by CellTiter-Glo relative to DMSO. Each data point is mean +/- stdev. **H,** SKNDZ cells with deletion of endogenous *NXT1* and overexpression of degron-tagged *NXT1* (SKNDZ-NXT1^{deg}) with and without *NXT2*

overexpression, as indicated, were treated with 500 nM dTAG13 or DMSO for 24 hours. Western blot depicts PARP, NXF1, HA (NXT1), Myc(NXT2) and GAPDH. **I,** Dose response curve to dTAG-13 for SKNDZ cells with deletion of endogenous *NXT1* and expression of degron-tagged exogenous *NXT1* (SKNDZ-NXT1^{deg}, red), and SKNDZ cells with deletion of exogenous *NXT1*, expression of degron-tagged exogenous *NXT1* and expression of exogenous non-degradable *NXT2* (SKNDZ-NXT1^{deg}+NXT2, gray). The x-axis indicates the log of the nM concentration of dTAG-13, and the y-axis indicates viability assessed by CellTiter-Glo relative to DMSO. Each data point is mean +/- stdev.