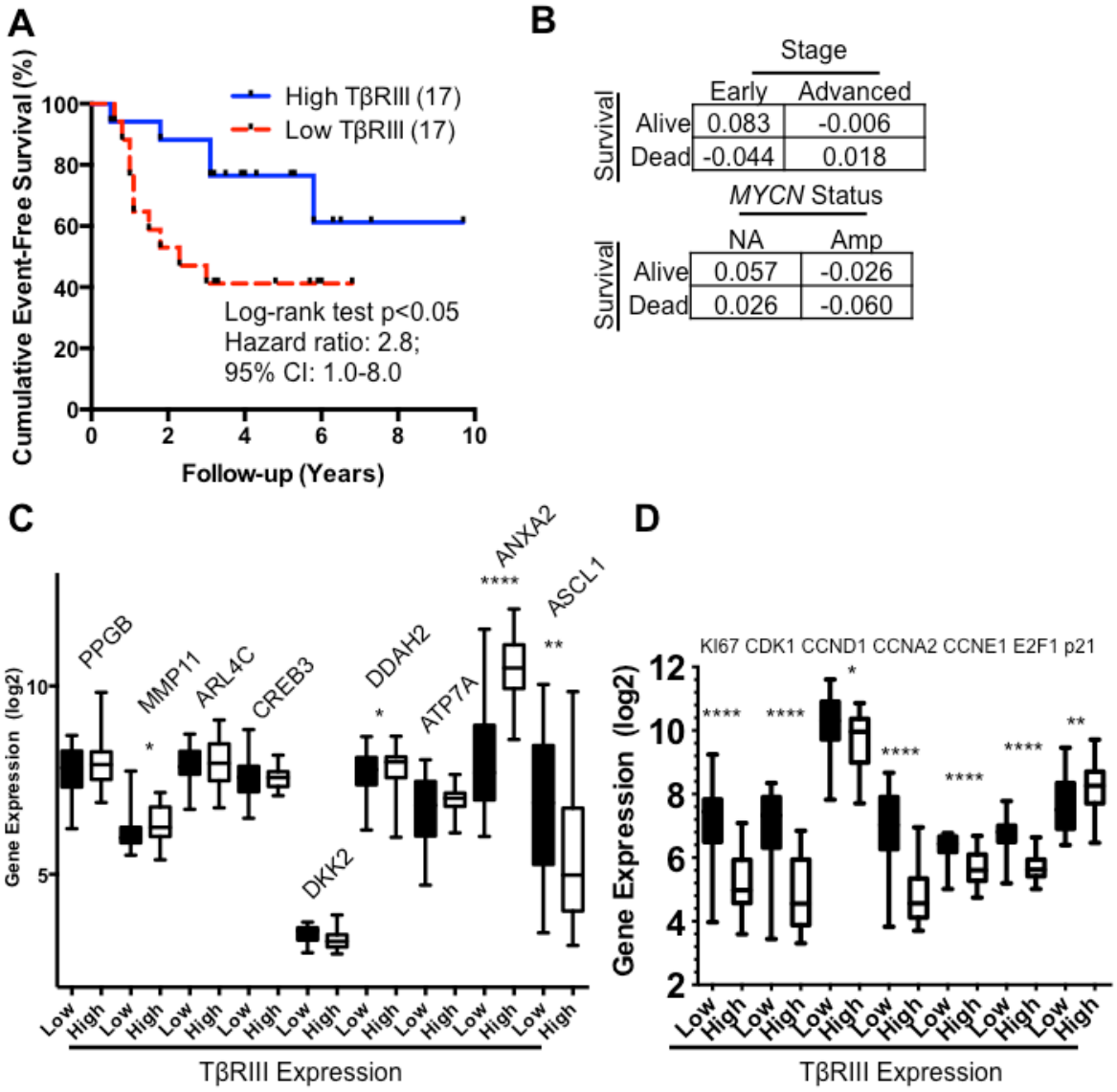
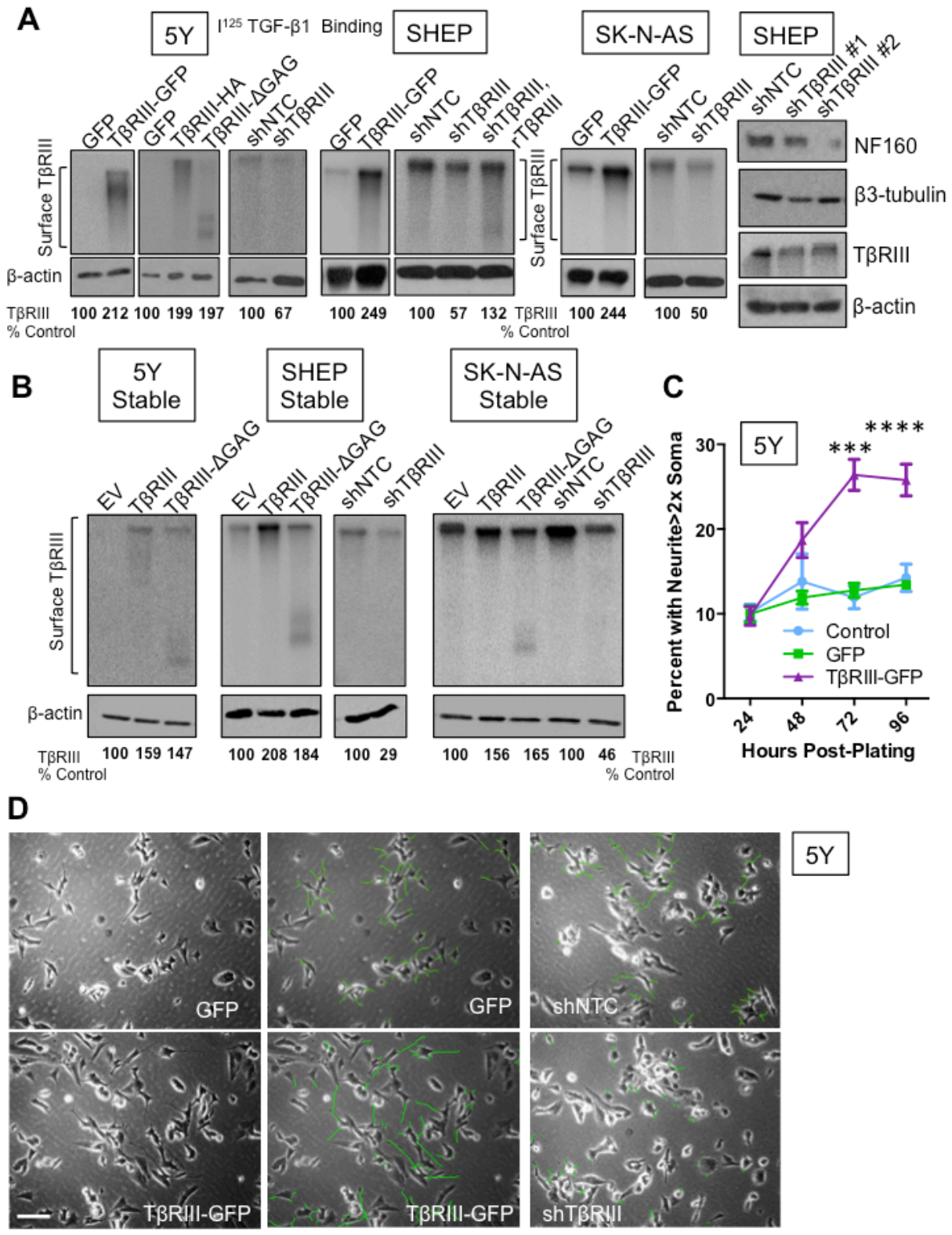
Supplemental Data:

Supplemental Figure 1:



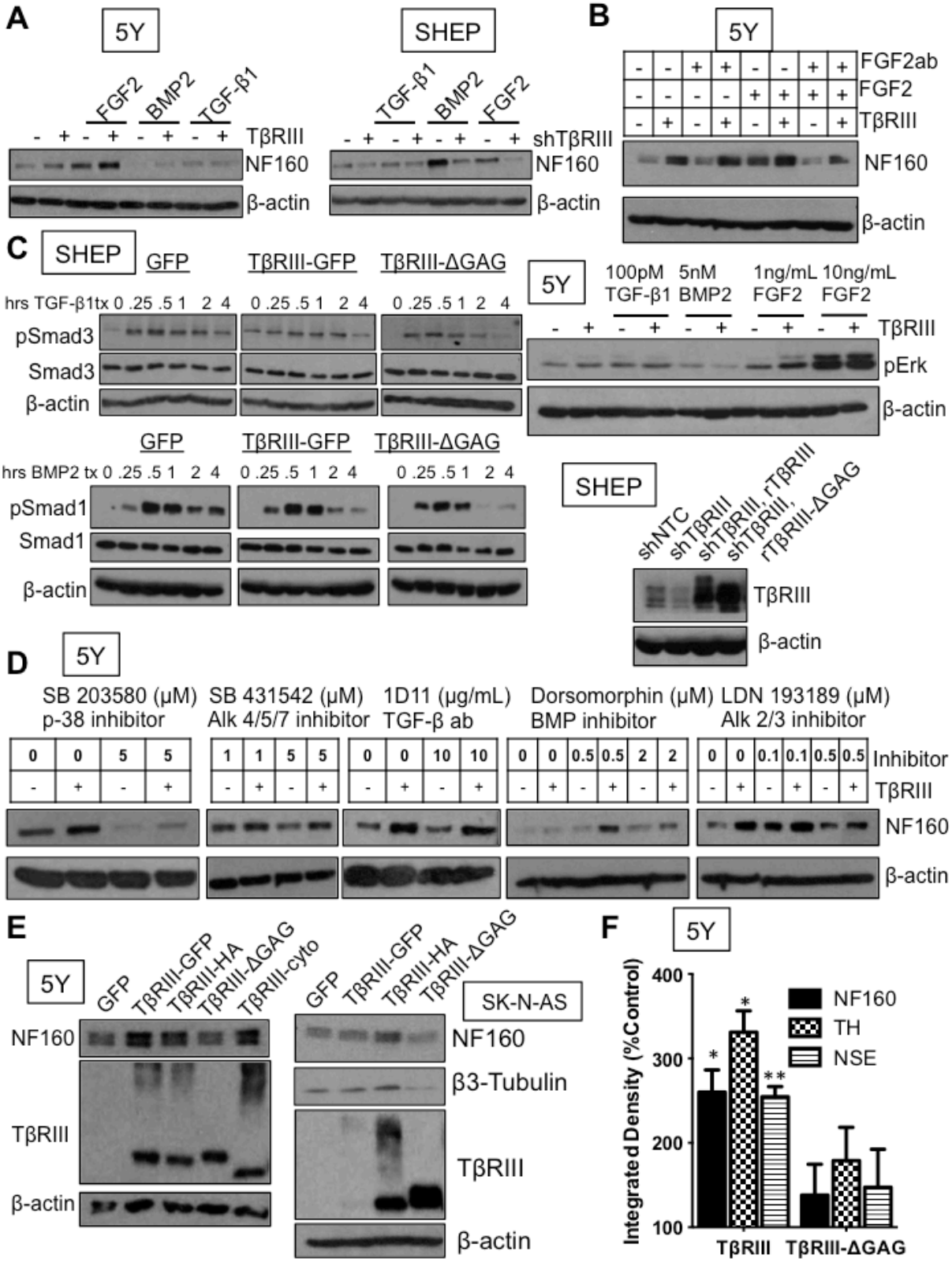
Supplemental Fig. 1: Neuroblastoma microarray dataset analysis. (A) Analysis of event-free survival in low (bottom 30%; red) and high (top 30%; blue) TβRIII-expressing NB using oncogenomics software and the neuroblastoma prognosis dataset. **(B)** Tables of TβRIII expression from the Obertheur dataset. **(C)** Microarray meta-dataset expression of differentiation markers identified by Hahn et al in low TβRIII (bottom 10%) vs. high TβRIII (top 10%) NB. ASCL1 represents a negative marker of differentiation. Data are presented as median and inter-quartile range. Mann Whitney test: *p<0.05, **p<0.01, ****p<0.0001. **(D)** Microarray meta-dataset expression of cell-cycle genes in low TβRIII (bottom 10%) vs. high TβRIII (top 10%) NB (median and inter-quartile range). Mann-Whitney test: *p<0.05, **p<0.01, ****p<0.0001.

Supplemental Figure 2:



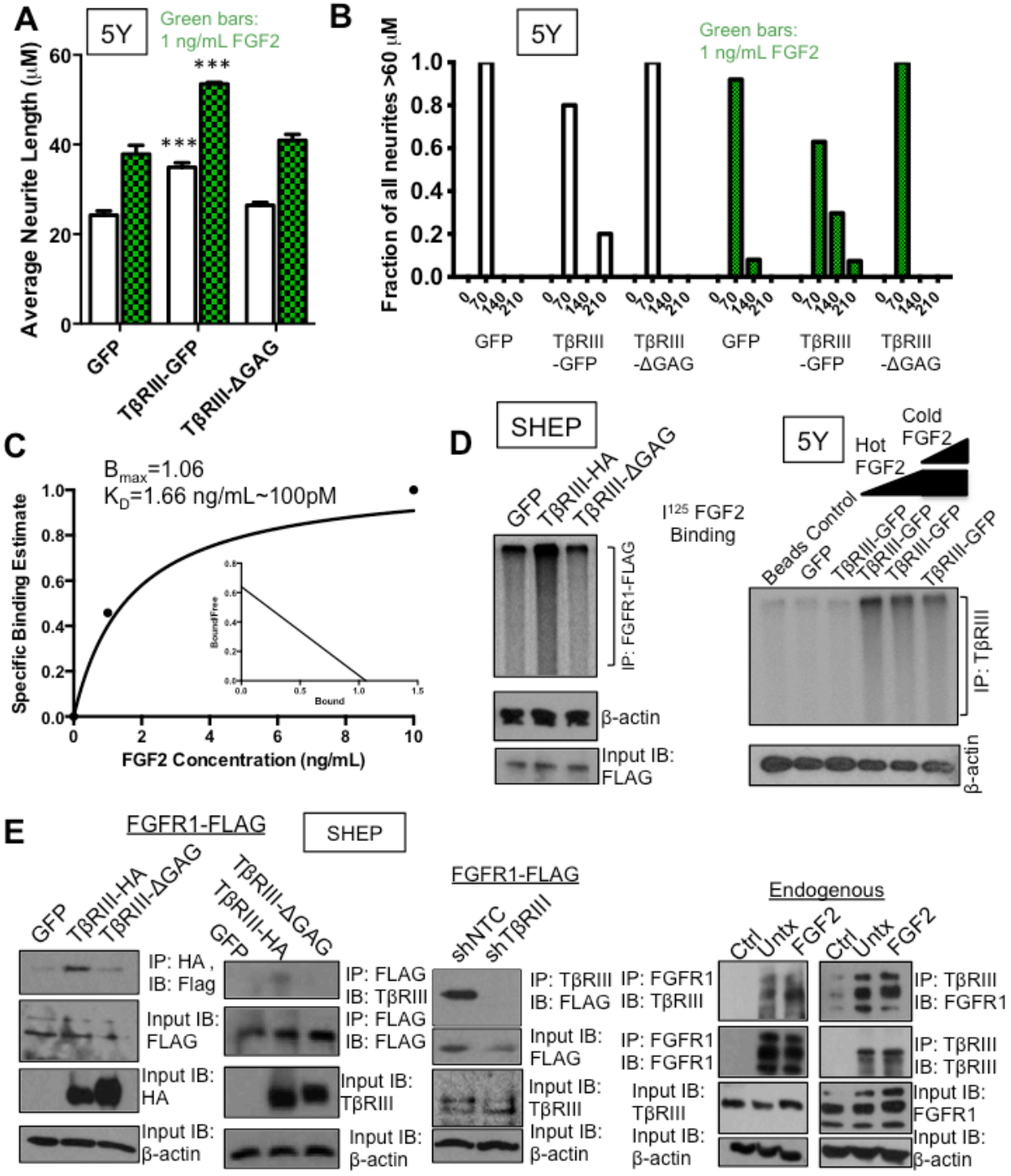
Supplemental Fig. 2: TβRIII expression promotes neuritogenesis and neuronal differentiation. (A) I^{125} TGF-β binding and crosslinking with TβRIII pull-down in 5Y, SHEP and SK-N-AS transduced with TβRIII and shRNA adenoviral constructs. Densitometry for TβRIII normalized to β-actin. **(B)** I^{125} TGF-β binding and crosslinking with TβRIII pull-down in lentivirus-generated antibiotic-selected stable cell lines. Densitometry for TβRIII normalized to β-actin. **(C)** Neurite quantification over time in adenovirus-transduced 5Y by counting cells with at least one neurite greater than twice the length of the cell body (soma). Data are presented as mean of three fields ± SEM. Two-way ANOVA $p<0.0001$ for main effects of time and receptor expression; interaction $p<0.001$; Bonferonni post-hoc comparisons to control: ***$p<0.001$ ****$p<0.0001$. **(D)** Phase contrast microscopy of 5Y after 48 hours of adenoviral transduction with neurites traced in green using NeuronJ in the middle and far right panels. 10x objective; scale bar=100μM.

Supplemental Figure 3:



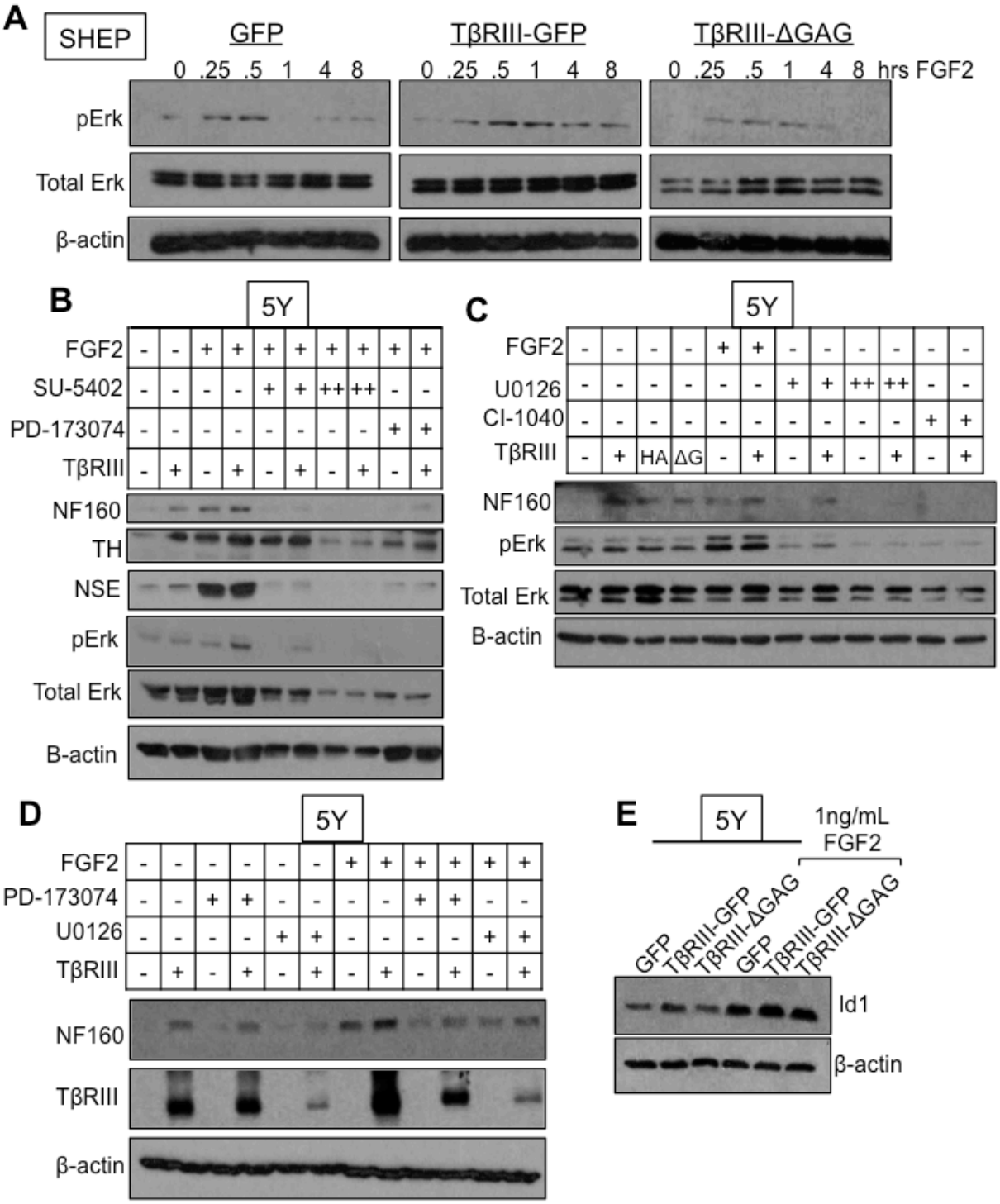
Supplemental Fig. 3: The differentiating effects of TβRIII are not via canonical TGF-β signaling. (A) Western blot for NF160 after 72 hour plating and ligand treatment (10ng/mL FGF2, 5nM BMP2, 100pM TGF-β1). 5Y were transduced with TβRIII-GFP (+) or GFP control (-). SHEP were transduced with shTβRIII (+) or non-targeted control shRNA (-). **(B)** Western blot for NF160 after 72 hour plating, transduction, and ligand treatment (1ng/mL FGF2, 5μg/mL FGF2 neutralizing antibody). **(C)** Western blot for phosphorylated and total SMAD1 and SMAD3 in SHEP after 72 hour transduction and treatment with 20 pM TGF-β1 or 10 nM BMP2 for the times indicated. Western blot for phosphorylated erk after 96 hrs of adenoviral transduction with TβRIII or GFP control and treatment with TGF-β1 (100pM), BMP2 (5nM) or FGF2 at the concentrations shown. TβRIII western in SHEP after 72 hr knockdown and rescue with shRNA resistant rodent TβRIII constructs (rTβRIII, rTβRIII-ΔGAG). **(D)** Western blot for NF160 in 5Y transduced with TβRIII-GFP or GFP control for 72 hours and treated with various signaling inhibitors at the concentrations shown. **(E)** Western blot for differentiation markers in 5Y and SK-N-AS transduced with TβRIII constructs or GFP control for 96 hours. **(F)** 5Y transiently transduced with TβRIII-GFP, TβRIII-ΔGAG, or GFP control for 96 hours. Quantification of differentiation markers from three independent experiments normalized to β-actin, presented as mean percent GFP control ± SEM. One-sample t-test *$p<0.05$ **$p<0.01$.

Supplemental Figure 4:



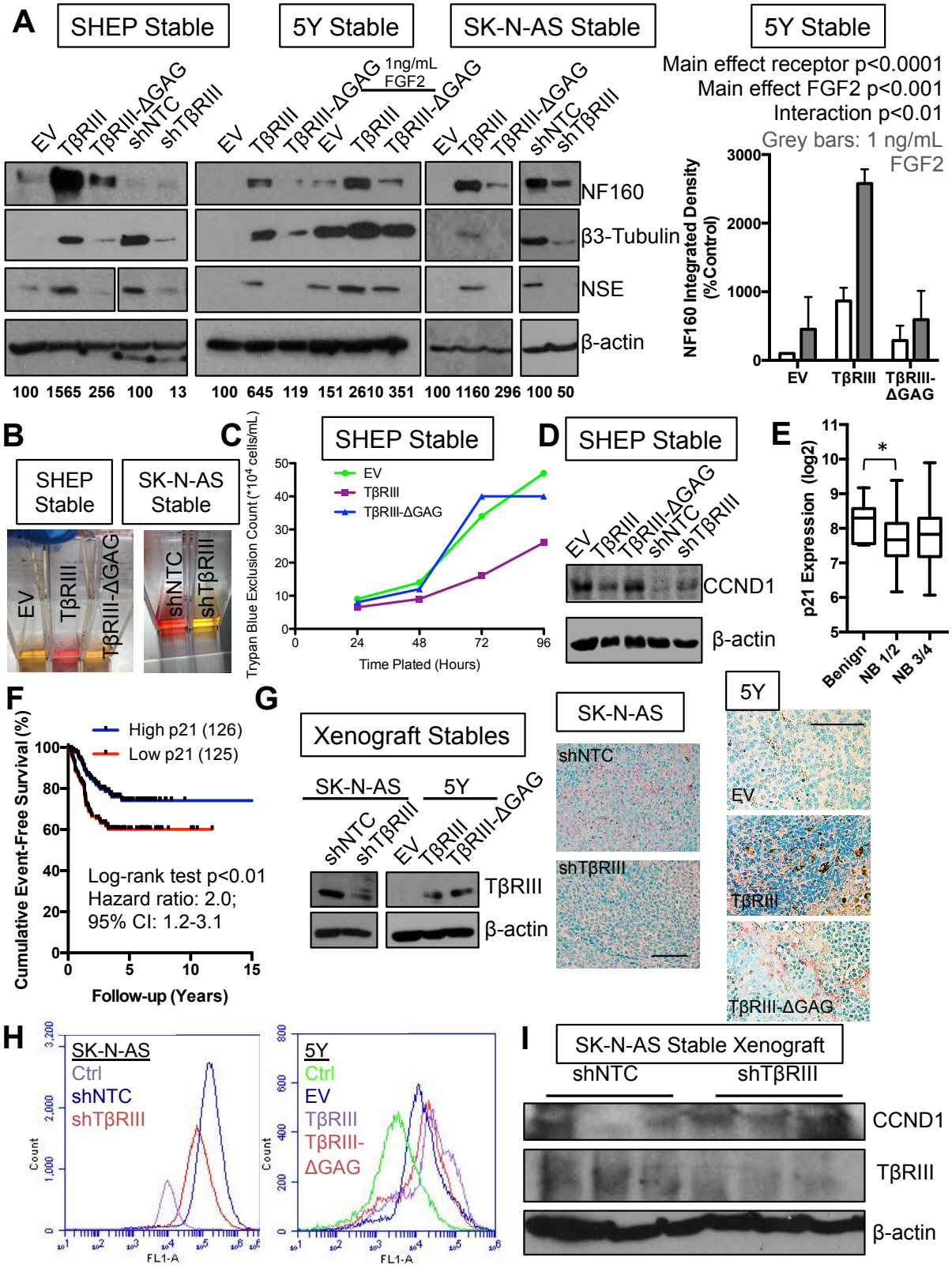
Supplemental Fig. 4: TβRIII enhances neuronal differentiation and FGF2-induced neurite outgrowth. (A) 5Y transiently transduced with TβRIII-GFP, TβRIII-ΔGAG, or GFP control and treated with FGF2 (1 ng/mL) for 48 hrs. Data are presented as mean neurite length from three fields ± SEM. One-way ANOVA for treated (green bars) and untreated $p<0.0001$. Bonferroni post-hoc compared to control and TβRIII-ΔGAG: ***$p<0.001$. **(B)** Fraction of neurites greater than 2x average soma length (60 μM), sorted into bins centered on length indicated on x-axis. **(C)** Scatchard analysis of I^{125} FGF2 binding dose-course data using densitometry for relative binding ratios and assuming 100% binding at 10ng/mL. **(D)** I^{125} FGF2 binding and crosslinking with FGFR1-FLAG pull-down. **(E)** Co-immunoprecipitation of TβRIII-HA and FGFR1-FLAG in SHEP with confirmation of equal FLAG pulldown (panels 1 and 2). Co-immunoprecipitation of TβRIII and FGFR1-FLAG in SHEP with endogenous TβRIII or shRNA knockdown (panel 3). Co-immunoprecipitation of endogenous TβRIII and FGFR1 in SHEP (IP: FGFR1 in panel 4 and IP TβRIII in panel 5). FGF2 treatment: 10ng/mL, 15 min.

Supplemental Figure 5:



Supplemental Fig. 5: TβRIII enhances FGF2 signaling via Id1 to promote neuronal differentiation. (A) Western blot in SHEP for phosphorylated (pErk) and total Erk after 72 hours of transduction, 60 hours of serum starvation and treatment with 10ng/mL FGF2 for the indicated times. **(B)** Western blots for differentiation markers and Erk signaling following 96 hours of transduction and treatment with FGF2 (10ng/mL), as well as the FGFR inhibitors SU-5402 (10μM, 20μM) and PD-173074 (1 μM). **(C)** Western blots for Erk signaling and the differentiation marker NF160 following 96 hours of transduction and treatment with FGF2 (10ng/mL), as well as the Erk inhibitors U0126 (1μM, 10μM) and CI-1040 (1 μM). **(D)** Western blot for NF160 following 96 hours of transduction and treatment with FGF2 (10ng/mL), PD-173074 (1 μM), and U0126 (10μM). **(E)** Western blot for Id1 in 5Y transduced for 96 hours and treated with FGF2 (1ng/mL).

Supplemental Figure 6:



Supplemental Fig.6: TβRIII promotes differentiation to decrease proliferation in NB stable cell lines and xenografts. (A) Western blot for differentiation markers in stable cell lines. FGF2 treatment (1ng/mL) for 72 hours. Densitometric analysis for β3-Tubulin (SHEP) and NF160 (5Y and SK-N-AS) normalized to β-actin. Quantification of three independent western blots for NF160 in 5Y stable cell lines treated for 72 hours with FGF2 (1ng/mL). Data are presented as mean ± SEM. Two-way ANOVA shown. **(B)** Photograph of stable SHEP and SK-N-AS cell lines plated equally in flasks and grown for 3 days to exhibit media acidification (yellow color). **(C)** Trypan exclusion counting of SHEP stable cells. **(D)** Western blot for cyclin D1 (CCND1) in SHEP stable cells. **(E)** p21 expression in the microarray meta-dataset by stage of disease. Data are presented as median and inter-quartile range. Kruskal-Wallis: $p<0.05$. Inter-group comparisons (Mann-Whitney): $*p<0.05$. **(F)** Analysis of event-free survival in low (bottom 50%; red) and high (top 50%; blue) p21-expressing NB using oncogenomics software and the Obertheur dataset. **(G)** Western blot confirmation of TβRIII expression in stable cell lines immediately prior to xenograft implantation. IHC confirmation of TβRIII knockdown and overexpression in sections of tumors from animals sacrificed at 4 weeks (SK-N-AS) or 7 weeks (5Y); scale bar=50μM. **(H)** TβRIII flow cytometry in SK-N-AS and 5Y stable cell lines. Secondary antibody alone used as control. **(I)** Western blot for cyclin D1 (CCND1) in lysates from SK-N-AS xenografts at 4 weeks.

Supplemental Table 1:

Gene	**TβRIII**	**N-Myc**	**SOX-10**	**Id1**	**KI67**	**CDK1**	**CCND1**	**CCNA2**	**CCNE1**	**E2F1**
Probe ID	204731_at	209757_s_at	209843_s_at	208937_s_at	212022_s_at	210559_s_at	208712_at	203418_at	213523_at	2028_s_at
Gene	**PPGB**	**MMP11**	**ARL4C**	**CREB3**	**DKK2**	**DDAH2**	**ATP7A**	**ANXA2**	**ASCL1**	**p21**
Probe ID	200661_at	203878_s_at	202208_s_at	209432_s_at	224199_at	202262_x_at	205198_s_at	210427_x_at	209988_s_at	202284_s_at

Supplemental Table 1: Affymetrix probe list. All affymetrix probes listed are from the HG-U133 Plus 2.0 platform.

Supplemental Table 2:

Adrenal Macro 4 wk	Adrenal Micro 4 wk	Adrenal Micro End	Lung Micro 4 wk	Lung Micro End	
0/3	0/3	4/5	0/3	1/5	**shNTC**
2/3	3/3	5/5	1/3	5/5	**shTβRIII**

Supplemental Table 2: TβRIII expression suppresses xenograft metastasis. Tabulation of results for metastasis in a cohort from the SK-N-AS xenograft model at 4 weeks and survival to humane endpoints (denoted End in the table).