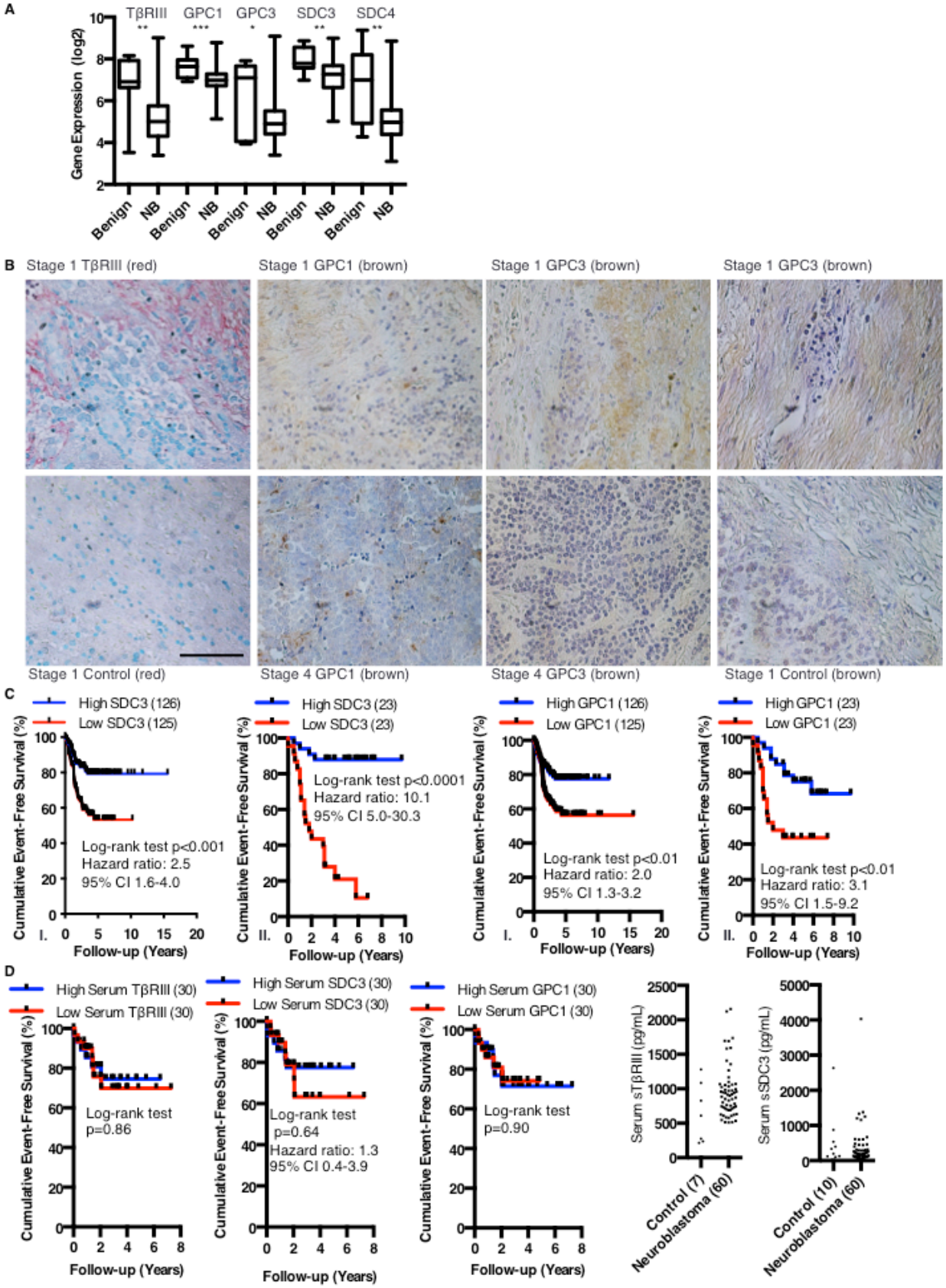
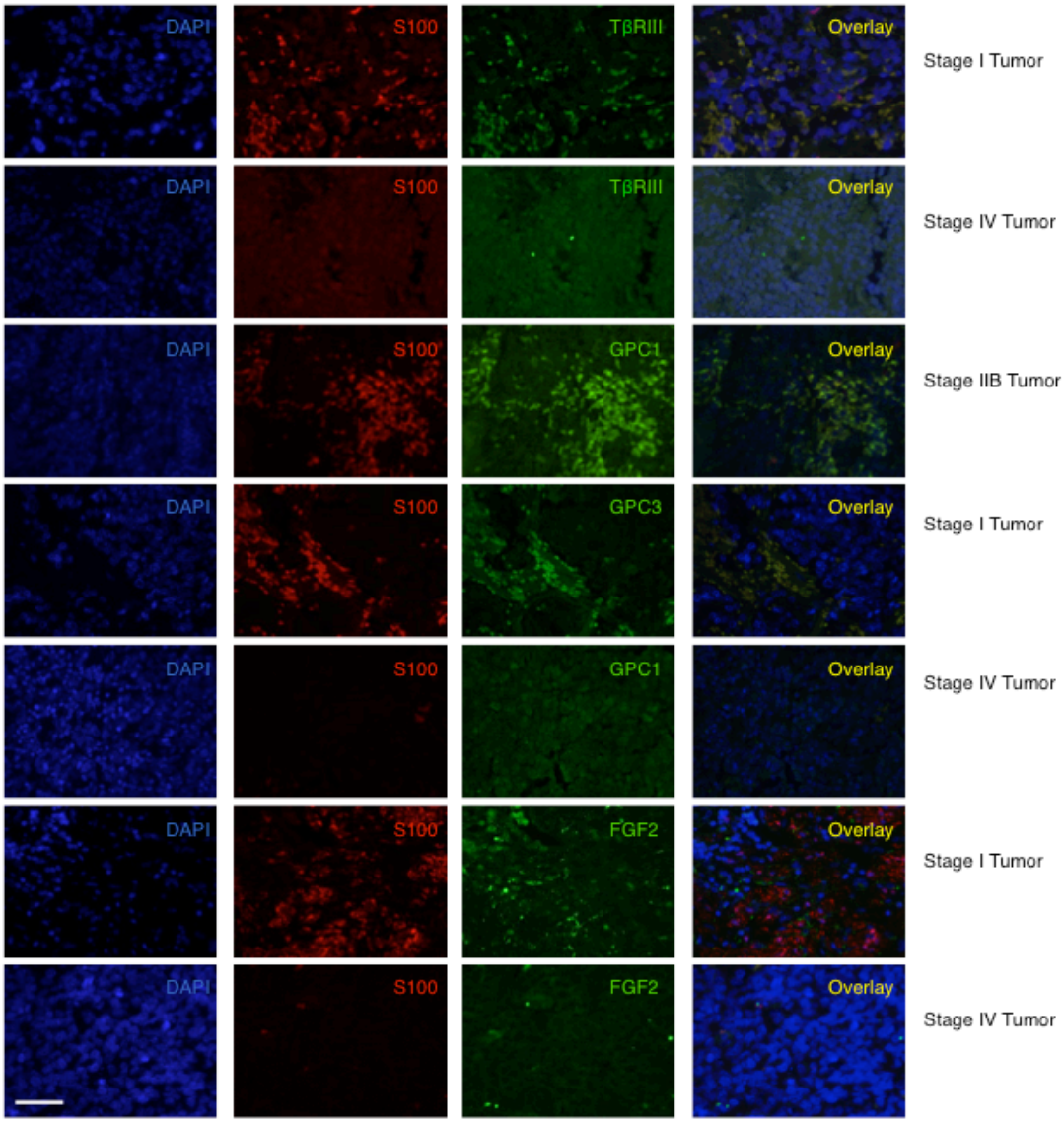
Supplemental Figure 1:



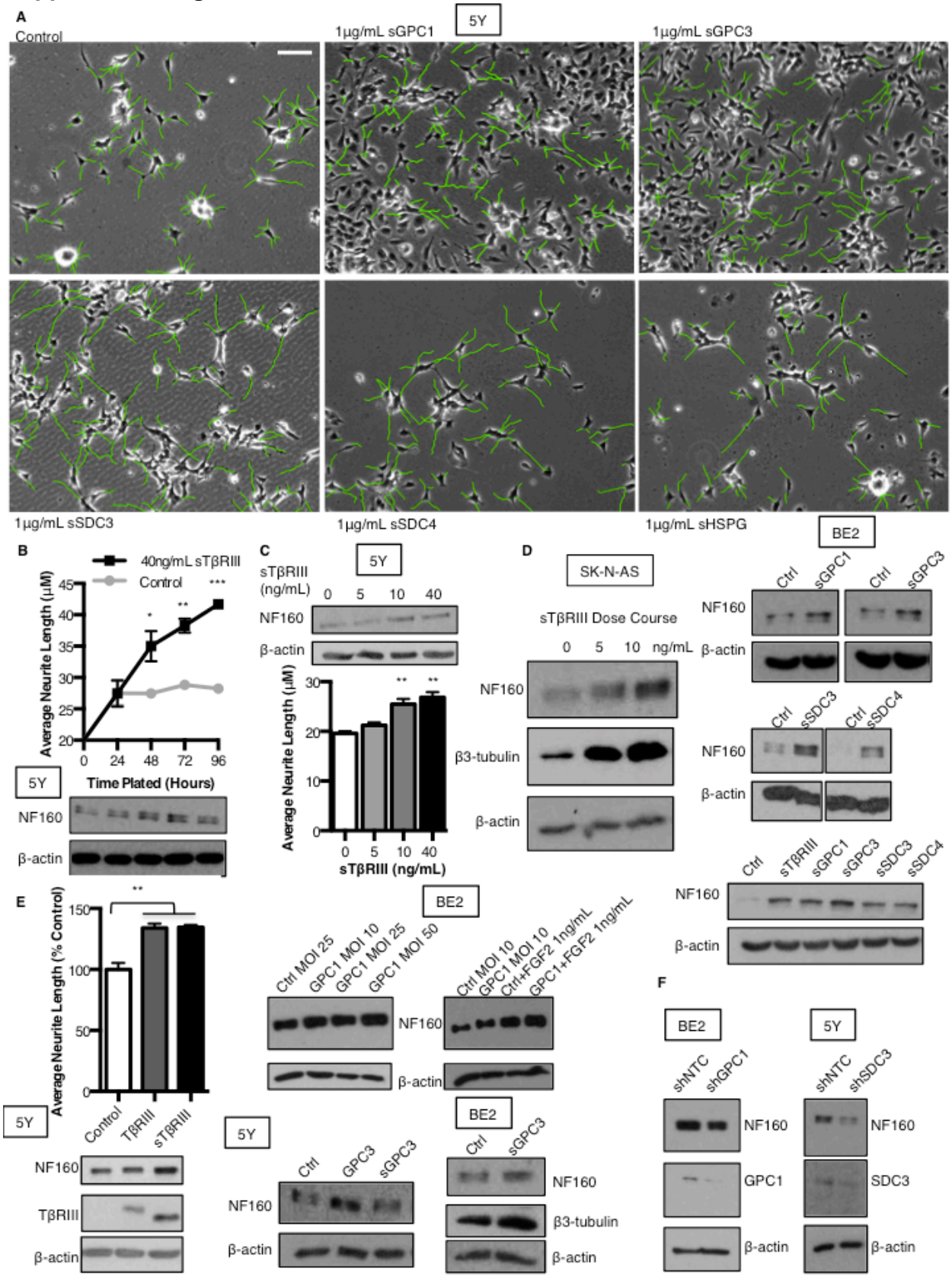
Supplemental Figure 1: HSPG expression is decreased in neuroblastoma, localized to the stroma, and affects prognosis. (A) Microarray meta-dataset analysis of log2 normalized HSPG mRNA expression in benign neuroblastic tumors ganglioneuroma and ganglioneuroblastoma (benign) vs. neuroblastoma (NB). SDC4 data are from the three U133 plus datasets only. Mann-Whitney: ***p<0.001, **p<0.01. *p<0.05. **(B)** Immunohistochemistry in neuroblastoma specimens using TβRIII, GPC1, and GPC3 antibodies and purified pre-bleed control serum. 40x images, scale bar=50μM. **(C)** Analysis of event-free survival in low (bottom 50%; red) and high (top 50%; blue) SDC3- and GPC1-expressing NB using oncogenomics software and the Obertheur (I.) and Neuroblastoma Prognosis (II.) datasets. **(D)** Serum ELISA for TβRIII and SDC3 using neuroblastoma patient samples with survival data (n=60; blue: top 50%, red: bottom 50%). ELISA values compared to remnant pediatric control serum.

Supplemental Figure 2:



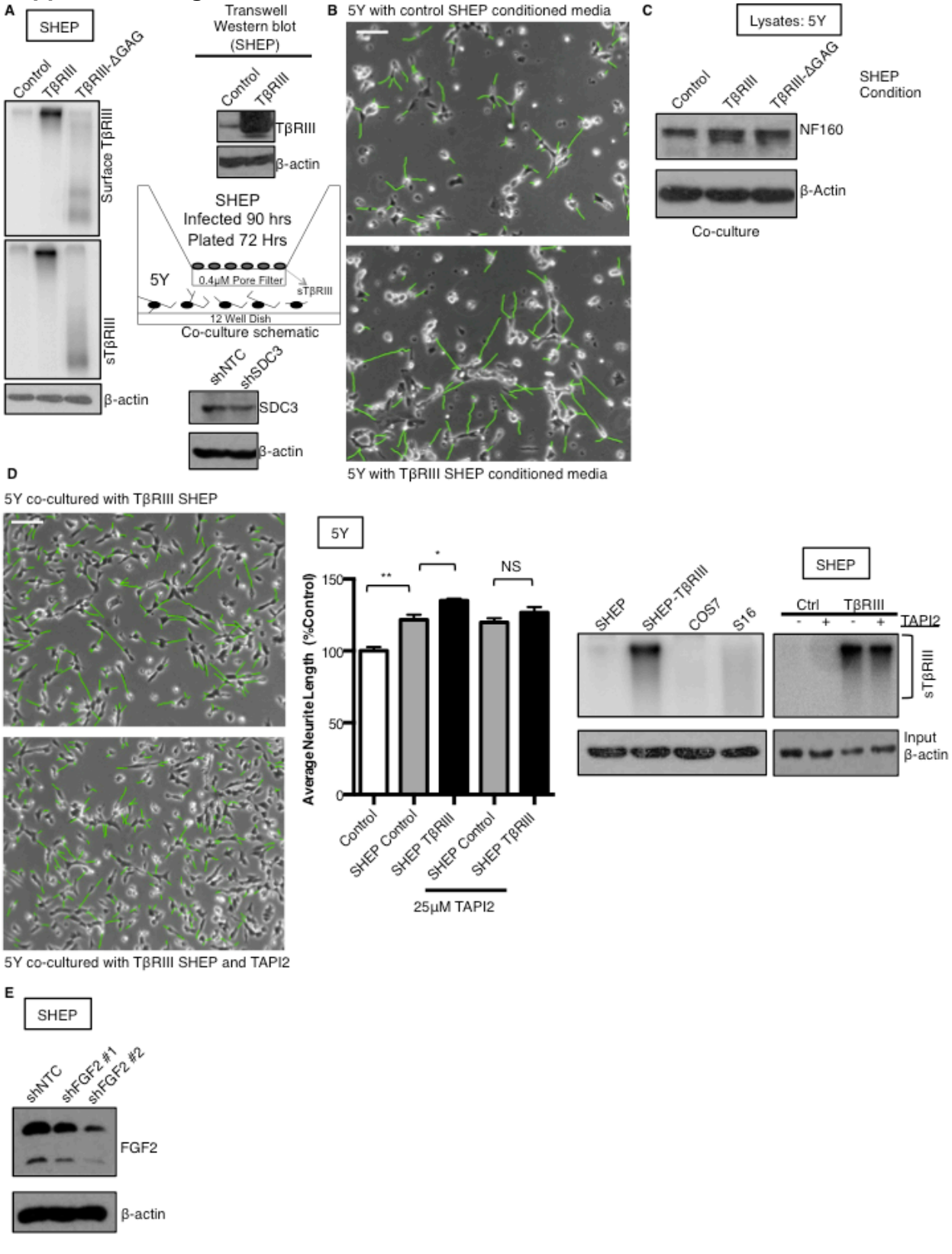
Supplemental Figure 2: HSPGs and FGF2 co-localize with S100 in early-stage neuroblastoma tissue specimens. Immunofluorescence of early-stage/stroma-rich and advanced-stage/stroma-poor neuroblastoma specimens using TβRIII, GPC1, GPC3, and FGF2 antibodies (green) and an S100 Schwannian stroma antibody (red). DAPI nuclear stain in blue. 40x images, scale bar=50μM.

Supplemental Figure 3:



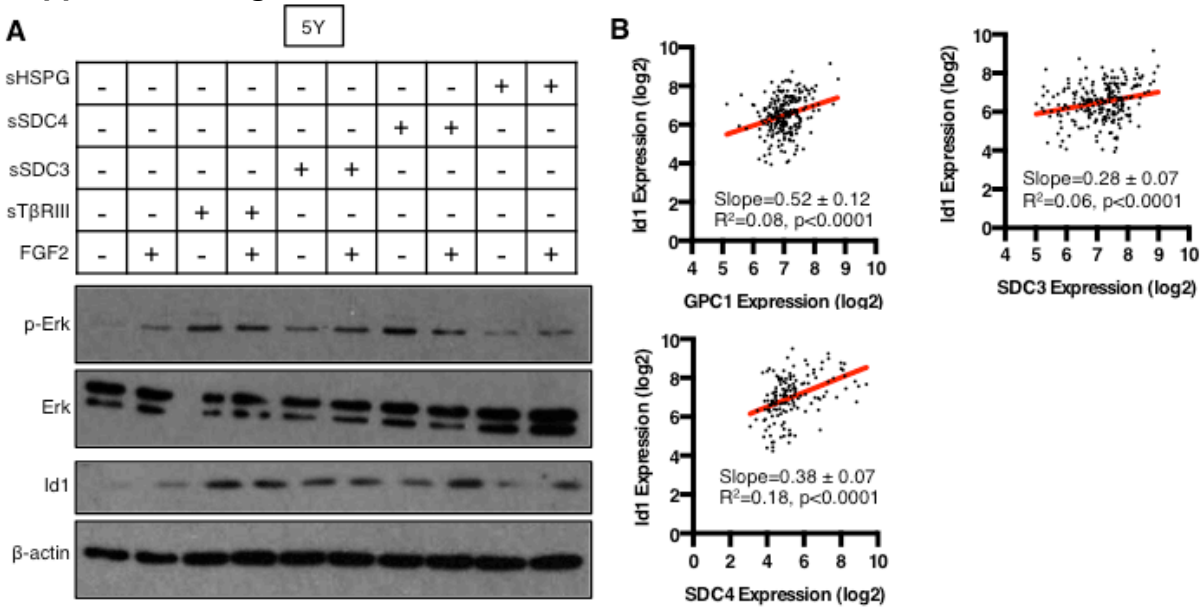
Supplemental Figure 3: Soluble HSPGs promote differentiation in neuroblastoma cell lines. (A) Phase contrast images of 5Y cells after 96 hours of treatment with sGPC1, sGPC3, SDC3, sSDC4, or sHSPG (1μg/mL). Neurites traced in green using NeuronJ. 10x images; scale bar=100μm. **(B)** Time course of neurite outgrowth in 5Y measured using NeuronJ. Data are presented as mean of three fields ± SEM. Two-tailed t-test: *p<0.05, **p<0.01, ***p<0.001. **(C)** sTβRIII dose-course in 5Y treated for 72 hours. Western blot for the differentiation marker NF160. Neurite length measured using NeuronJ. Data are presented as mean of three fields ± SEM. One-way ANOVA: p<0.001. Two-tailed t-test: **p<0.01. **(D)** Western blots for differentiation markers in SK-N-AS and BE2 cells treated with sTβRIII (10ng/mL), sGPC1 or sGPC3 (100ng/mL), or sSDC3 or sSDC4 (1μg/mL) for 72 hours. **(E)** Western blots for differentiation markers in 5Y and BE2 transiently transduced with surface and soluble TβRIII, GPC1 and GPC3 constructs. Neurite length after 72 hours measured using NeuronJ. Data are presented as mean of three fields ± SEM. One-way ANOVA: p<0.01. Bonferroni corrected t-test: **p<0.01. **(F)** Western blot for NF160 in 5Y and BE2 transiently expressing shRNA to GPC1 and SDC3.

Supplemental Figure 4:



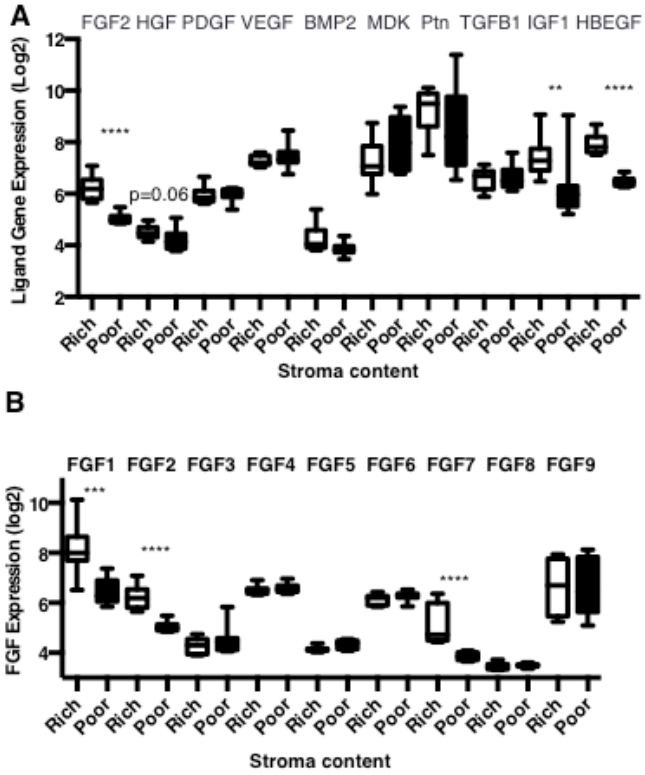
Supplemental Figure 4: Inhibition of soluble TβRIII shedding prevents its differentiating effects. (A) TGF-β binding and crosslinking with TβRIII pulldown for surface and soluble TβRIII to confirm expression in SHEP cells expressing full-length or GAG mutant TβRIII (TβRIII-ΔGAG). Western blot for TβRIII in SHEP lysates collected from the co-culture transwell. Schematic of the co-culture system. Western blot to confirm SDC3 knockdown in SHEP. **(B)** Phase contrast images of 5Y cells after 96 hours of treatment with conditioned media, with neurites traced in green using NeuronJ. 10x images; scale bar=100μm. **(C)** Western blot for the differentiation marker NF160 in 5Y after 72 hours of co-culture with SHEP expressing full-length or GAG mutant TβRIII (TβRIII-ΔGAG). **(D)** Phase contrast images of 5Y cells after 72 hours of co-culture with SHEP expressing TβRIII and treated with 25 μM TAPI2 to decrease shedding. Neurites traced in green using NeuronJ. 10x images; scale bar=100μM. Quantification of neurite length from three fields using NeuronJ. Data are presented as mean of three fields ± SEM. One-sample t-test: *p<0.05, **p<0.01. TGF-β binding and crosslinking with TβRIII pulldown for soluble TβRIII to confirm expression in SHEP cells expressing TβRIII compared to COS7 negative control and S16 positive control. Right panel: SHEP treated with 25 μM TAPI2 to decrease shedding. This experiment is a control for co-culture treatment with TAPI2 in Figure 3A. **(E)** Western blot for FGF2 in antibiotic-selected SHEP stable cell lines expressing a non-targeted control shRNA (shNTC), or two different shRNAs against FGF2 (shFGF2#1, #2).

Supplemental Figure 5:



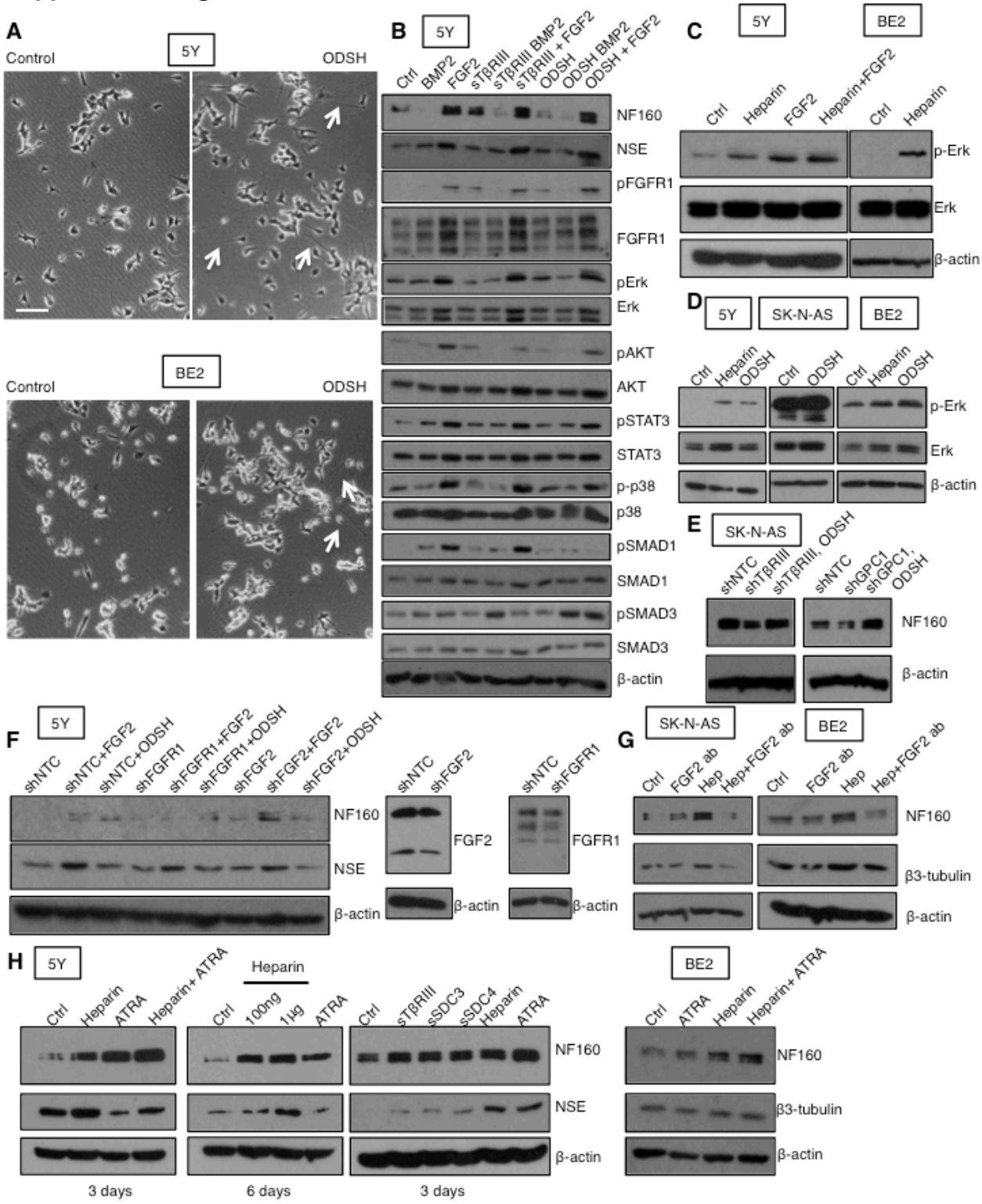
Supplemental Figure 5: Soluble HSPGs enhance FGF2 signaling in neuroblastoma cells to promote differentiation via Id1. (A) Western blot for phospho-Erk (p-Erk), total Erk, and Id1 in 5Y treated for 96 hours with FGF2 (1ng/mL), sTβRIII (10ng/mL), sSDC3, sSDC4, or sHSPG (1µg/mL). **(B)** Microarray meta-dataset analysis for GPC1, SDC3 or SDC4 and Id1 expression linear correlation.

Supplemental Figure 6:



Supplemental Figure 6: Expression of growth factors in neuroblastoma patient samples based on stroma content. (A) Microarray dataset analysis (GSE7529) for ligand expression in neuroblastic tumors based on stroma status (N=8 stroma rich, N=11 stroma poor). Data are presented as median and inter-quartile range, Mann-Whitney: *p<0.05, ****p<0.0001. **(B)** Microarray dataset analysis (GSE7529) for FGF expression. Data are presented as median and inter-quartile range, Mann-Whitney: ***p<0.001, ****p<0.0001.

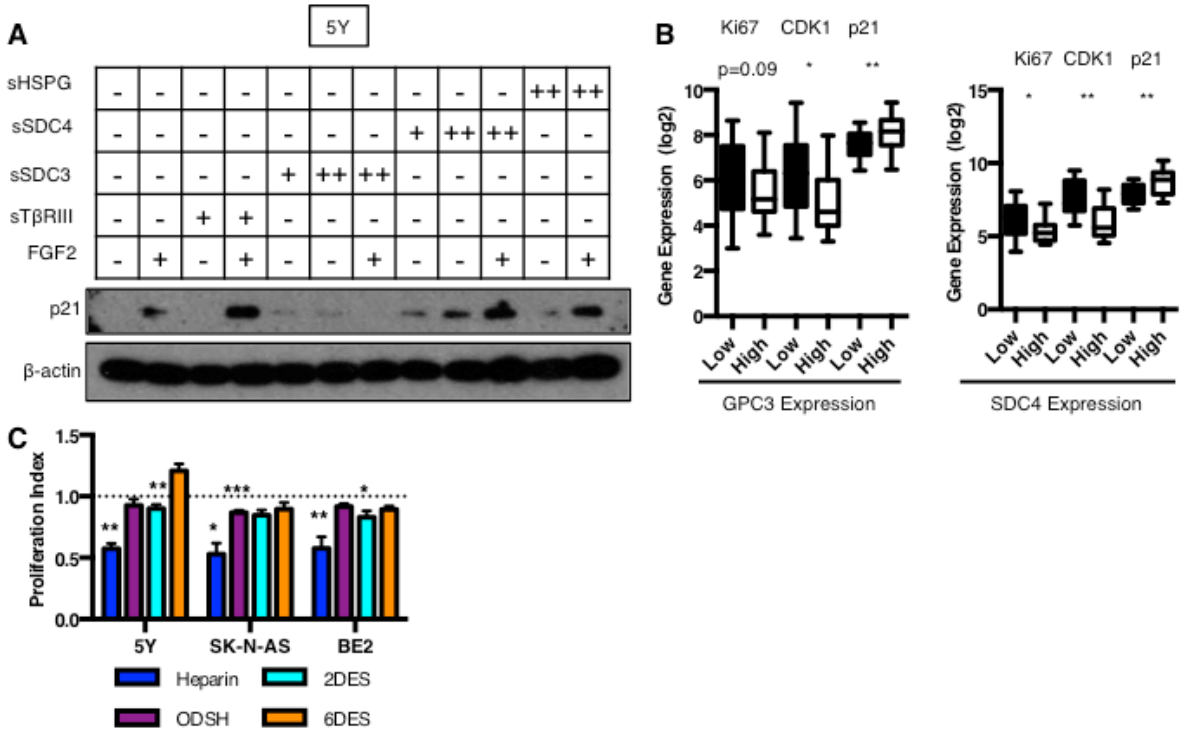
Supplemental Figure 7:



Supplemental Figure 7: Heparin and ODSH promote neuroblast differentiation via Erk and Id1. (A) Phase contrast images of 5Y and BE2 cells after 72 hours of ODSH

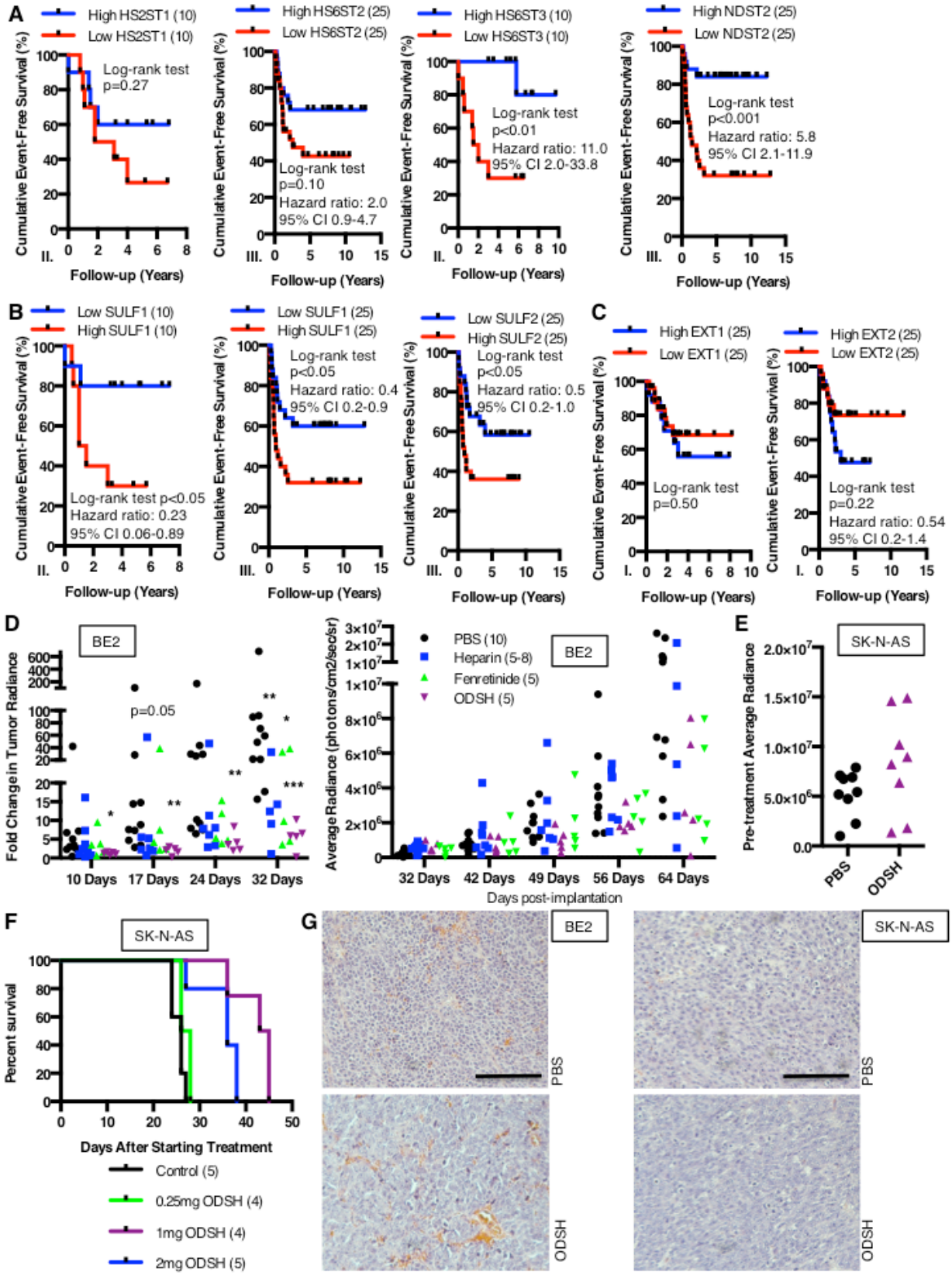
treatment (1µg/mL). 10x images; scale bar=100µm. Arrows identify abnormally long neurites. **(B)** Western blots for signaling markers in 5Y treated 96 hours with BMP2 (10nM), FGF2 (1ng/mL), sTβRIII (10ng/mL), or ODSH (1 µg/mL). **(C)** Western blots for phosphorylated (p-Erk) and total Erk in 5Y and BE2 treated 72 hours with 1µg/mL heparin and/or 1ng/mL FGF2. **(D)** Western blots for phosphorylated (p-Erk) and total Erk in cells treated 72 hours with heparin or ODSH (1 µg/mL). **(E)** Western blots for NF160 in cells stably expressing TβRIII shRNA or infected with adenoviral shRNA for GPC1 and treated 72 hours with 1µg/mL ODSH. **(F)** Western blots for differentiation markers in 5Y expressing knockdown constructs against FGFR1 (shFGFR1) or FGF2 (shFGF2), or a non-targeted control shRNA construct (shNTC) for 72 hours during treatment with FGF2 (1ng/mL) or ODSH (1 µg/mL). Construct expression confirmed by western blot (right panels). **(G)** Western blots for differentiation markers in SK-N-AS and BE2 treated for 72 hours with 1µg/mL heparin (hep) and/or 5µg/mL FGF2 neutralizing antibody. **(H)** Western blot for differentiation markers in 5Y and BE2 treated 3 or 6 days with 10ng/mL sTβRIII, 1µg/mL heparin, sSDC3, or sSDC4, and/or 10µM ATRA.

Supplemental Figure 8:



Supplemental Figure 8: Soluble HSPGs and heparin suppress neuroblast proliferation. (A) Western blot for p21 in 5Y treated for 96 hours with FGF2 (1ng/mL), sTβRIII (10ng/mL), sSDC3, sSDC4, or sHSPG (100ng/mL or 1µg/mL). **(B)** Microarray meta-dataset expression of cell-cycle genes in low GPC3 or SDC4 (bottom 10%; shaded) vs. high GPC3 or SDC4 (top 10%; unshaded) neuroblastoma. Data are presented as median and inter-quartile range. Mann Whitney test: *p<0.05, **p<0.01. **(C)** Proliferation index for neuroblastoma cell lines after 24 hour treatment with heparin or de-sulfated heparins (1µg/mL). One sample t-test: *p<0.05, **p<0.01.

Supplemental Figure 9:



Supplemental Figure 9: Sulfyltransferase expression correlates with survival in neuroblastoma patients and supports the use of ODSH as a differentiating agent. **(A)** Analysis of event-free survival split by epimerase and sulfyltransferase expression in the Obertheur dataset (denoted I. blue=top 10%, red=bottom 10%), NB prognosis dataset (denoted II. blue=top 20%, red=bottom 20%), and Seeger dataset (denoted III. blue=top 25%, red=bottom 25%) using oncogenomics software. **(B)** Analysis of event-free survival split by sulfatase expression in the NB prognosis datasets. **(C)** Analysis of event-free survival split by extension enzyme expression in the Obertheur dataset. **(D)** BE2 orthotopic xenograft. Tumor radiance was measured before treatment after 32 days of tumor growth using luciferase in vivo imaging (photons/s/cm^2/steradian), and again after 10, 17, 24, and 32 days of treatment. Fold change in tumor radiance was calculated (shown, along with average radiance). **(E)** SK-N-AS orthotopic xenograft. Tumor radiance was measured before treatment after 28 days of tumor growth using luciferase in vivo imaging (photons/s/cm^2/steradian). **(F)** Dose course of ODSH treatment. Survival until humane endpoints is shown as percent of each condition. **(G)** H+E stain of tumor xenograft sections. 20x images: scale bar=50μM.

Supplemental Tables:

Receptor	Benign	NB Early	NB Late	Change
TβRIII	6.67	**5.46**	**5.01**	↓
GPC1	7.58	**7.06**	**6.96**	↓
GPC2	6.18	**6.64**	**6.63**	↑
GPC3	6.18	**5.05**	**5.09**	↓
GPC4	4.29	3.74	3.65	-
GPC5	3.65	3.4	3.41	-
GPC6	5.69	5.95	5.92	-
SDC1	5.64	5.89	**6.06**	↑
SDC2	6.44	6.79	6.52	-
SDC3	7.91	**7.37**	**6.95**	↓
SDC4	6.81	**5.36**	**5.09**	↓
NRP1	6.78	7.11	6.73	-
NRP2	5.82	5.85	5.78	-
CD44	8.56	8.25	**7.44**	↓

Supplemental Table 1: HSPG expression is decreased in neuroblastoma. Microarray meta-dataset analysis of log2 normalized mRNA expression by stage of disease. Expression of all receptors excepting SDC4 was analyzed in the combined dataset (n=213, combination of five individual datasets). SDC4 data throughout the manuscript are from the three U133 plus datasets only (n=145), as this gene lacked a corresponding probe in the two Illumina datasets. Mann-Whitney tests compared to benign neuroblastic tumors ganglioneuroma and ganglioneuroblastoma: Bold $p<0.05$. Bold and red $p<0.01$. TβRIII, SDC3, and SDC4 demonstrated significant additional decreases from early-stage (NB early) to late-stage (NB late) disease.

Proteoglycan Co-Receptor	Microarray SR/SP Ratio	RT-PCR SR/SP Ratio	p value	Rank
TβRIII	7.8	11.1	<0.0001	31
GPC1	1.5	-	<0.05	-
GPC3	8.1	12.5	<0.0001	28
SDC3	3.0	-	<0.01	-
SDC4	6.8	12.5	<0.001	29
CD44	2.4	-	0.06	-

Supplemental Table 2: HSPG expression is higher in stroma-rich neuroblastoma. HSPGs that demonstrated decreased expression in neuroblastoma compared with benign neuroblastic tumors were investigated in GSE7529, a dataset where Schwannian stroma status was known. Normalized expression values were used to calculate a ratio of expression in stroma rich disease (SR; benign neuroblastic tumors) and stroma poor disease (SP; advanced neuroblastoma). The dataset authors performed RT-PCR to confirm expression ratios at the mRNA level and ranked genes based on the degree of difference from SR to SP tumors.

Gene	**TβRIII**	**N-Myc**	**GPC1**	**GPC3**	**SDC3**	**SDC4**	**CD44**	
Affymetrix Probe ID	204731_at	209757_s_at	202756_s_at	209220_at	202898_at	202071_at	204489_s_at	
Gene	**FGF2**	**Id1**	**p21**	**KI67**	**CDK1**	**SOX10**	**ANZA2**	**ASCL1**
Affymetrix Probe ID	204421_s_at	208937_s_at	202284_s_at	212022_s_at	210559_s_at	209843_s_at	210427_x_at	209988_s_at
Gene	**TβRIII**	**GPC1**	**SDC3**	**GLCE**	**HS6ST1**	**HS6ST2**	**HS6ST3**	
Oncogenomics Probe ID	A_23_P200780	A_23_P209904 (Oberthuer) 812033 (NB Prognosis)	A_23_P74887 (Oberthuer) 2018807 (NB Prognosis)	506281 (NB Prognosis)	A_24_P8220 (Oberthuer); 969769 (NB Prognosis)	858188(NB Prognosis); 230030_at (Seeger)	A_23_P422911 (Oberthuer); 788605 (NB prognosis)	
Gene	**HS2ST1**	**HS3ST1**	**NDST1**	**NDST2**	**SULF1**	**SULF2**	**Ext1**	**Ext2**
Oncogenomics Probe ID	A_24_P242357 (Oberthuer); 1634515 (NB Prognosis)	Hs40968.5 (Oberthuer); 73609 (NB Prognosis)	811827 (NB Prognosis)	769645 (NB Prognosis); 214867_at (Seeger)	2308409 (NB Prognosis); 212353_at (Seeger)	224724_at (Seeger)	A_23_P43273 (Oberthuer); 2568869 (NB Prognosis)	A_23_P13183 (Oberthuer); 302292 (NB Prognosis)

Supplemental Table 3: Affymetrix probe list. All affymetrix probes listed are from the HG-U133 Plus 2.0 platform.

HSPG	Heparan sulfate proteoglycan
sHSPG	Soluble heparan sulfate proteoglycan
TβRIII	Type 3 TGF-β receptor
sTβRIII	Soluble type 3 TGF-β receptor
GPC	Glypican
sGPC	Soluble glypican
SDC	Syndecan
sSDC	Soluble syndecan
NRP	Neuropilin
Id1	Inhibitor of DNA binding 1
FGFR1	Fibroblast growth factor receptor 1
ANXA2	Annexin A2
ASCL1	Achaete-scute homolog 1
HBEGF	Heparin-binding EGF
HS2ST1	2-O sulfyltransferase 1
HS6ST2	6-O sulfyltransferase 2
HS6ST3	6-O sulfyltransferase 3
NDST2	N-deacetylase/N-sulfyltransferase 2
SULF1	Sulfatase 1
SULF2	Sulfatase 2
EXT1	Exostosin 1 extension enzyme
EXT2	Exostosin 2 extension enzyme

TAPI2	A metalloproteinase inhibitor against TACE
SU5402	FGFR kinase inhibitor
PD173074	FGFR kinase inhibitor
U0126	MEK kinase inhibitor
CI1040	MEK kinase inhibitor
2DES	2-O de-sulfated heparin
6DES	6-O de-sulfated heparin
NDES	N de-sulfated heparin
ODSH	2-O, 3-O-de-sulfated heparin
ATRA	All-*trans* retinoic acid

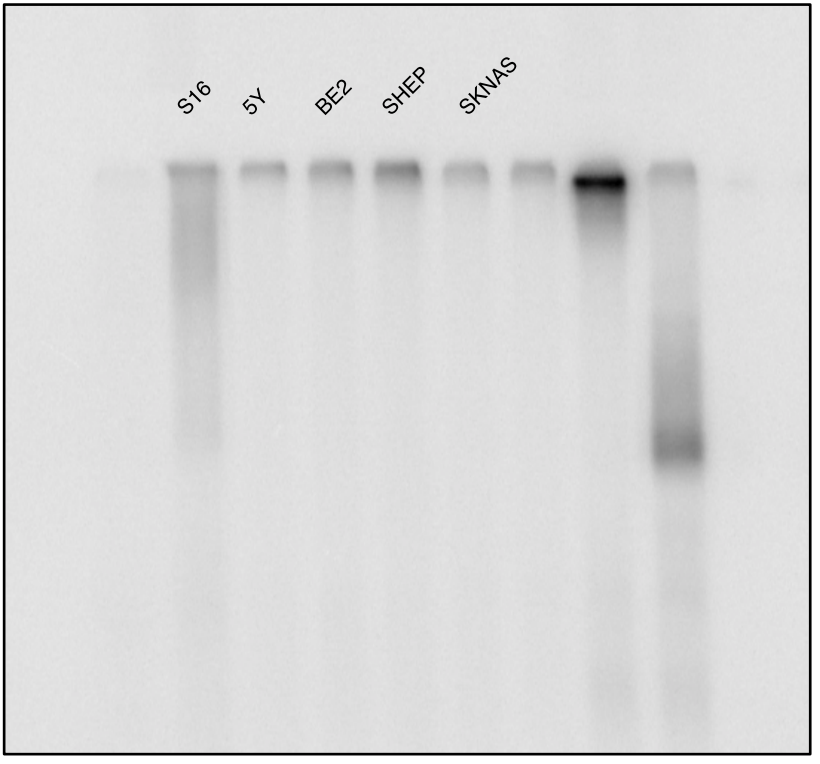
Supplemental Table 4: List of non-standard abbreviations and pharmaceuticals.

Full Unedited Gels for Figure 1C

TβRIII
(binding and crosslinking)

Full Unedited Gels for Figure 1C (continued)

sTβRIII
(binding and crosslinking)



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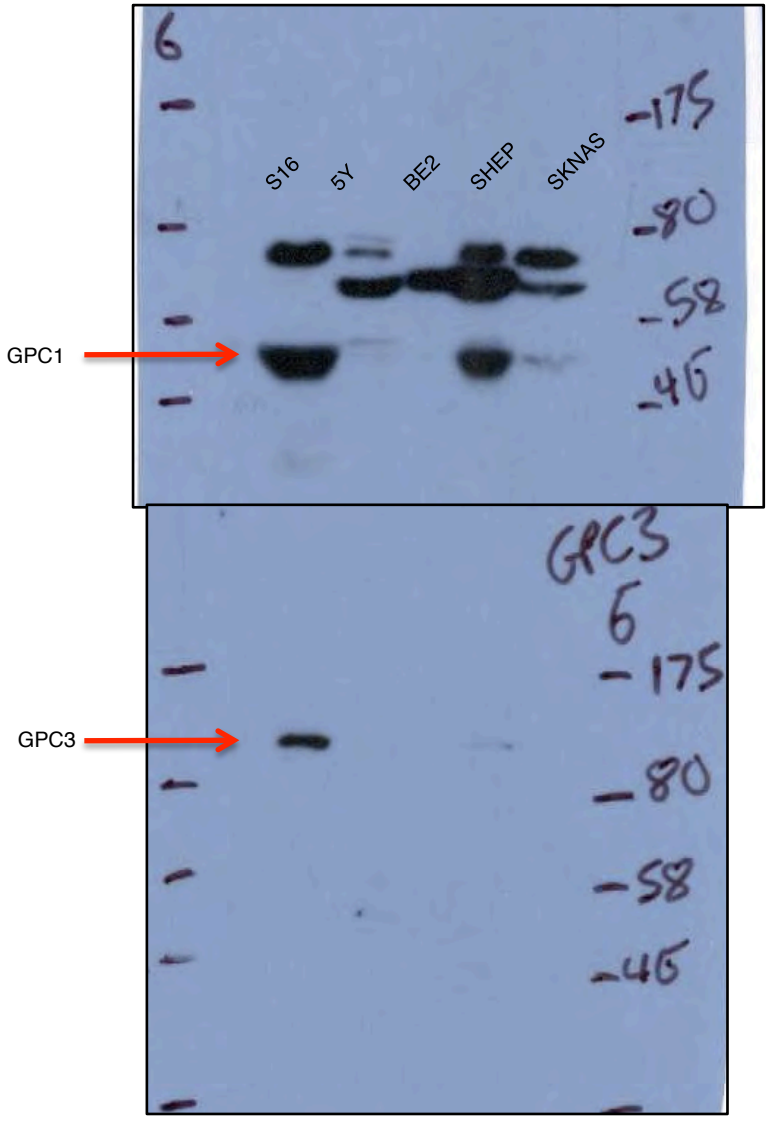

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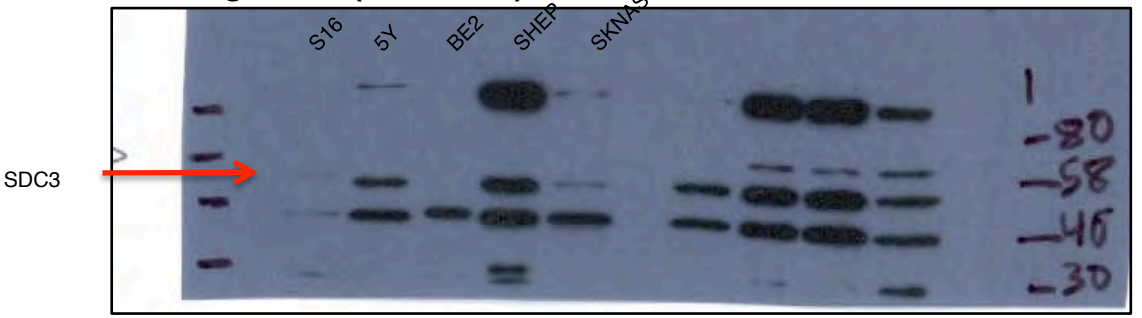

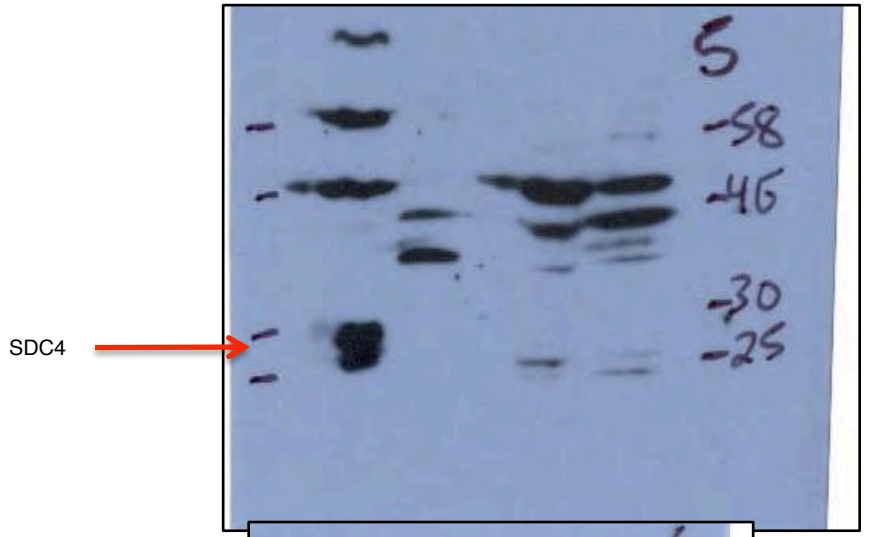

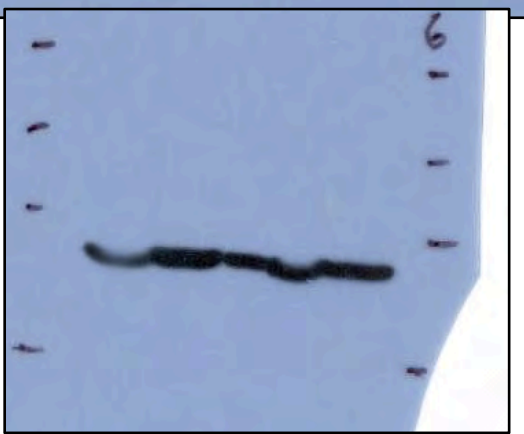

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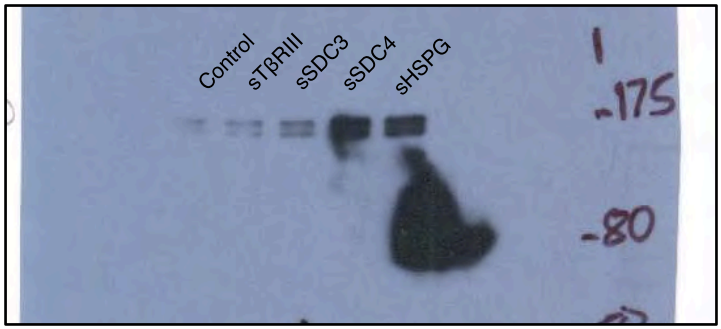

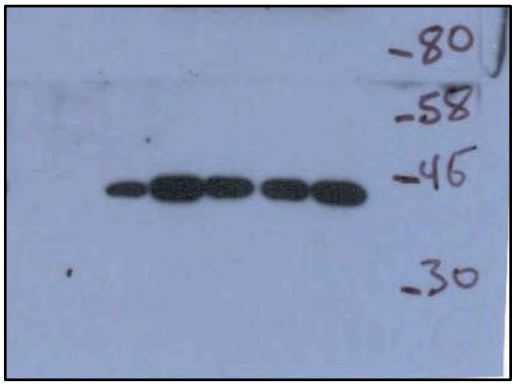

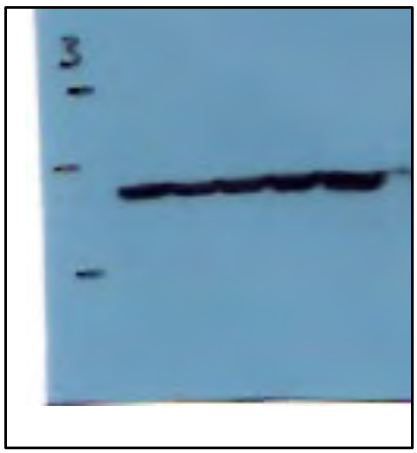

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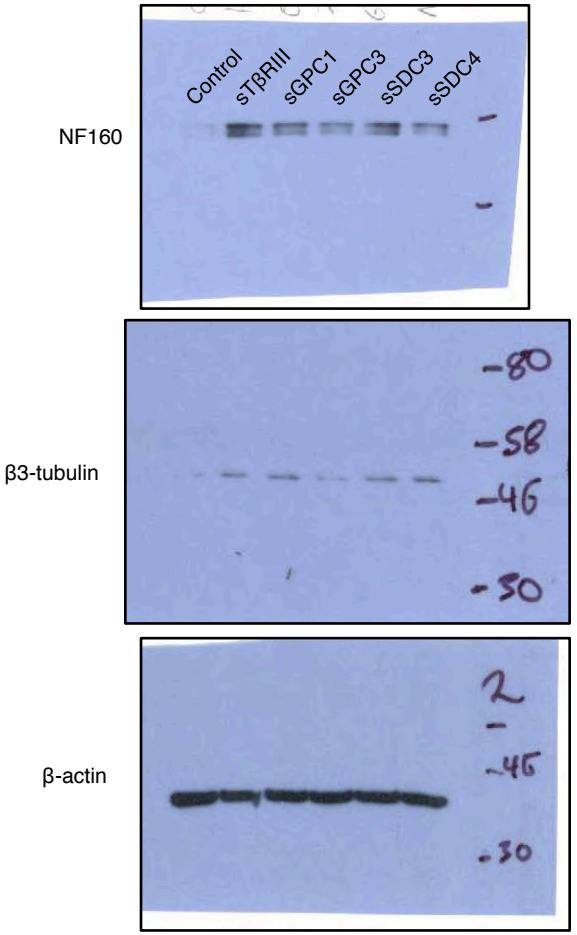

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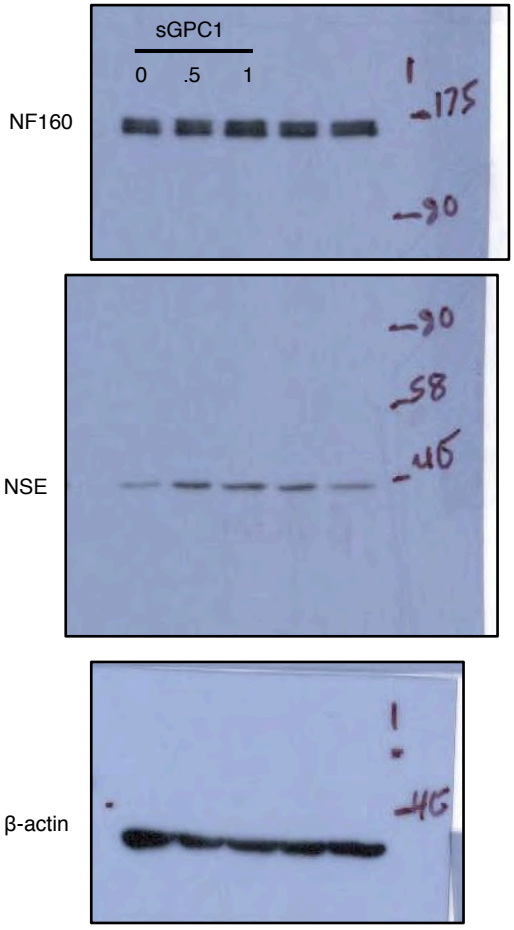

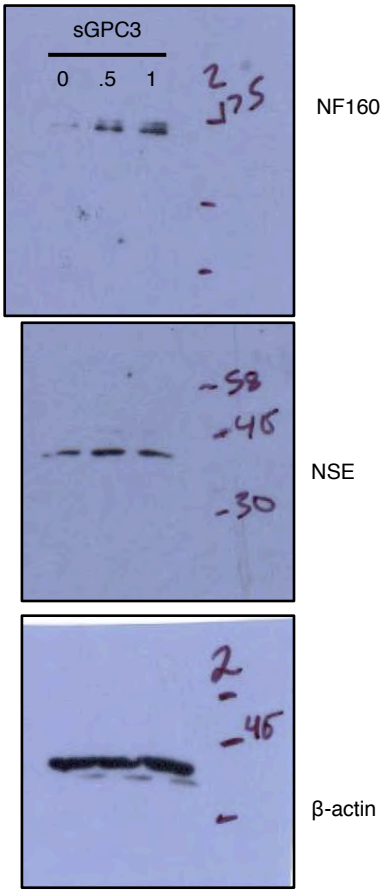

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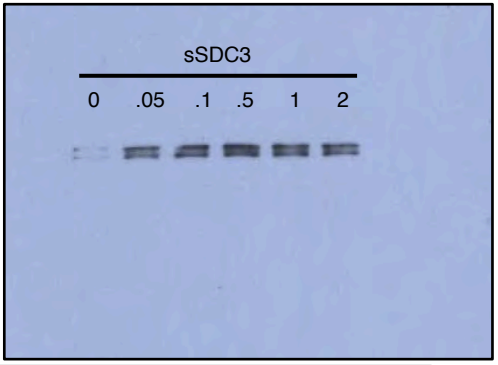
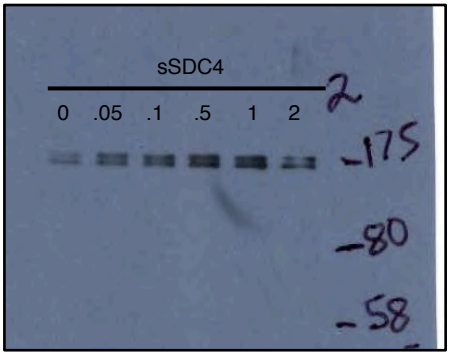
NF160





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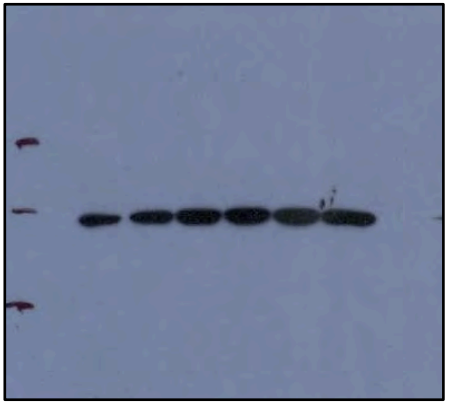
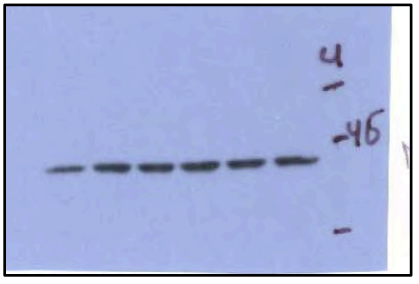
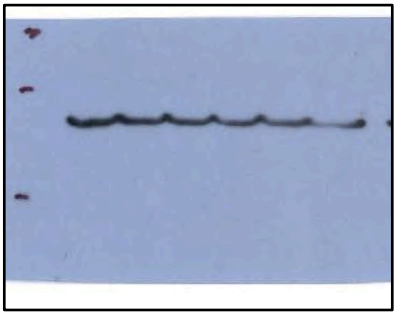
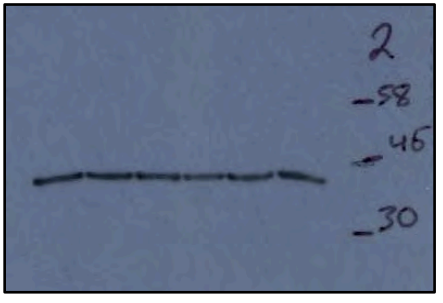




NSE

β-actin





β-actin

Full Unedited Gels for Figure 2E (continued)

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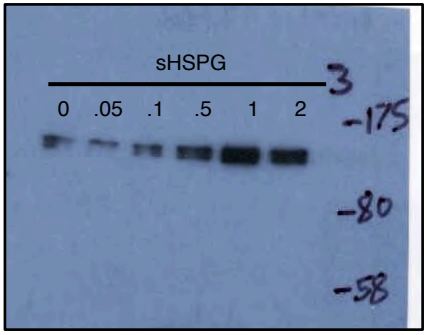

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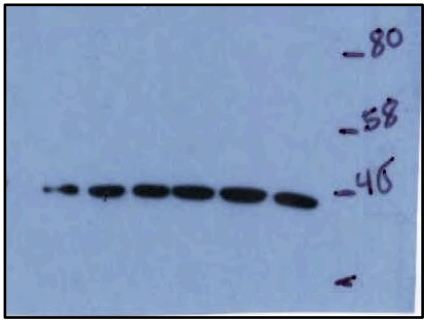
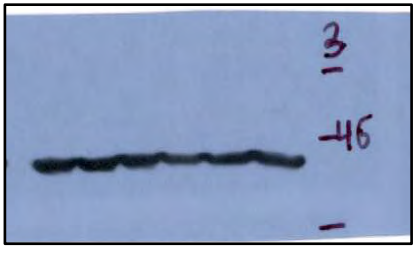
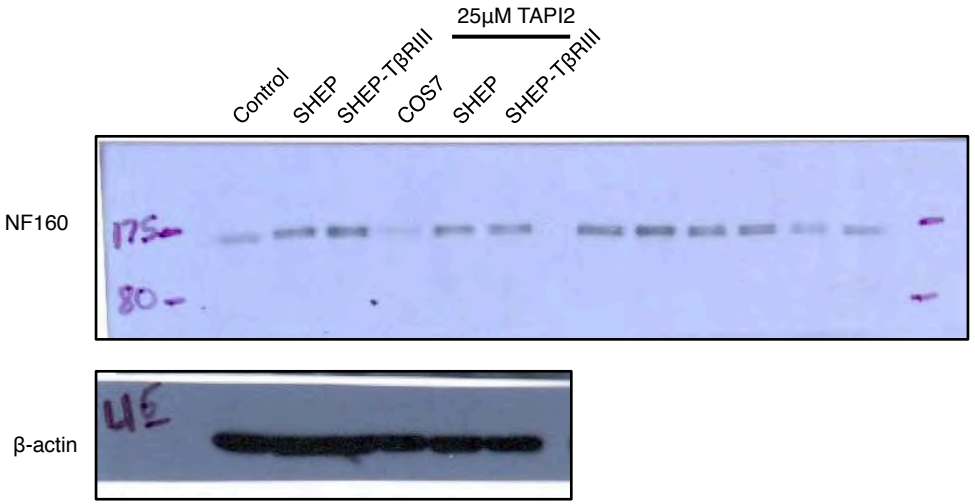


β-actin

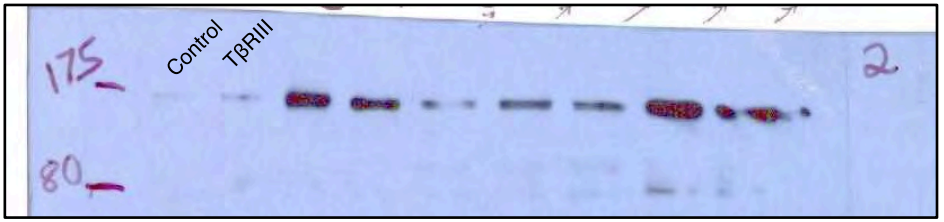


Full Unedited Gels for Figure 3A

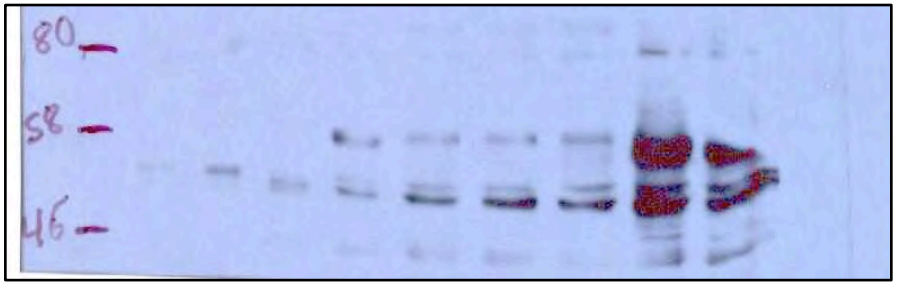


Full Unedited Gels for Figure 3C

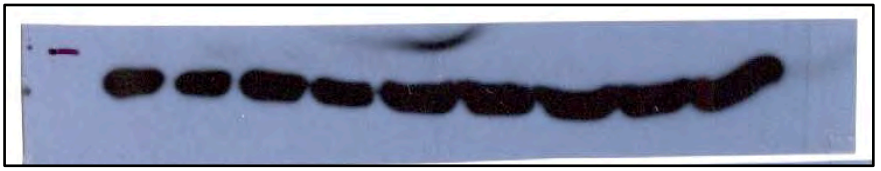
NF160



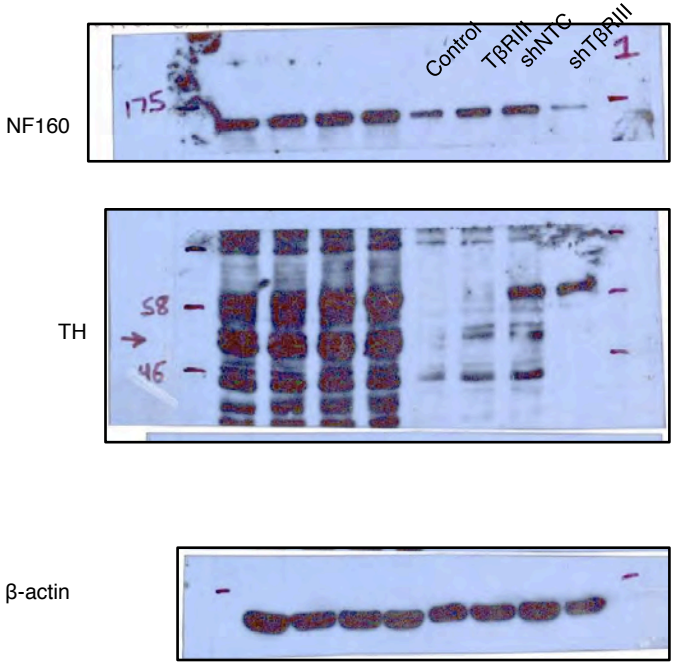
TH



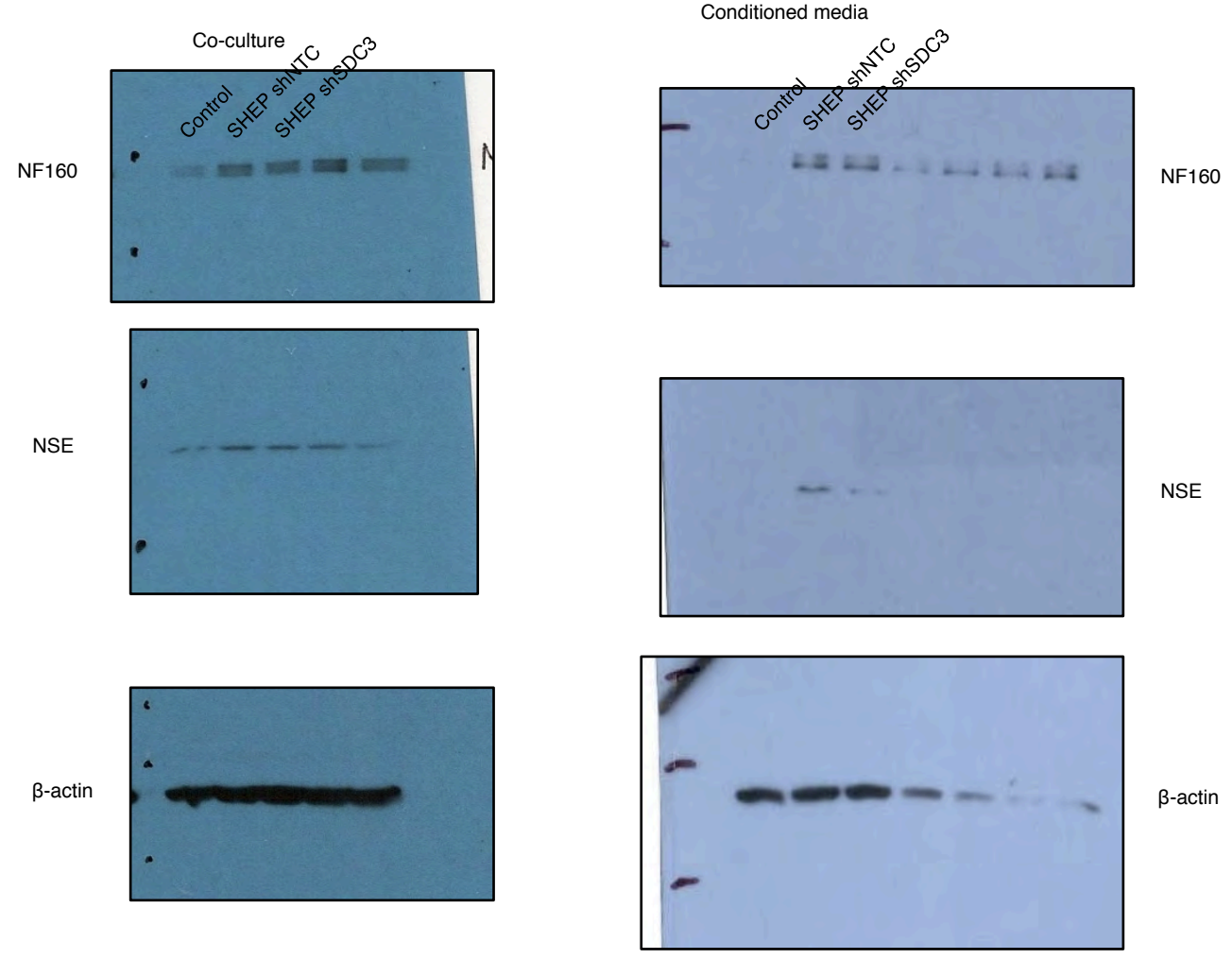
β-actin



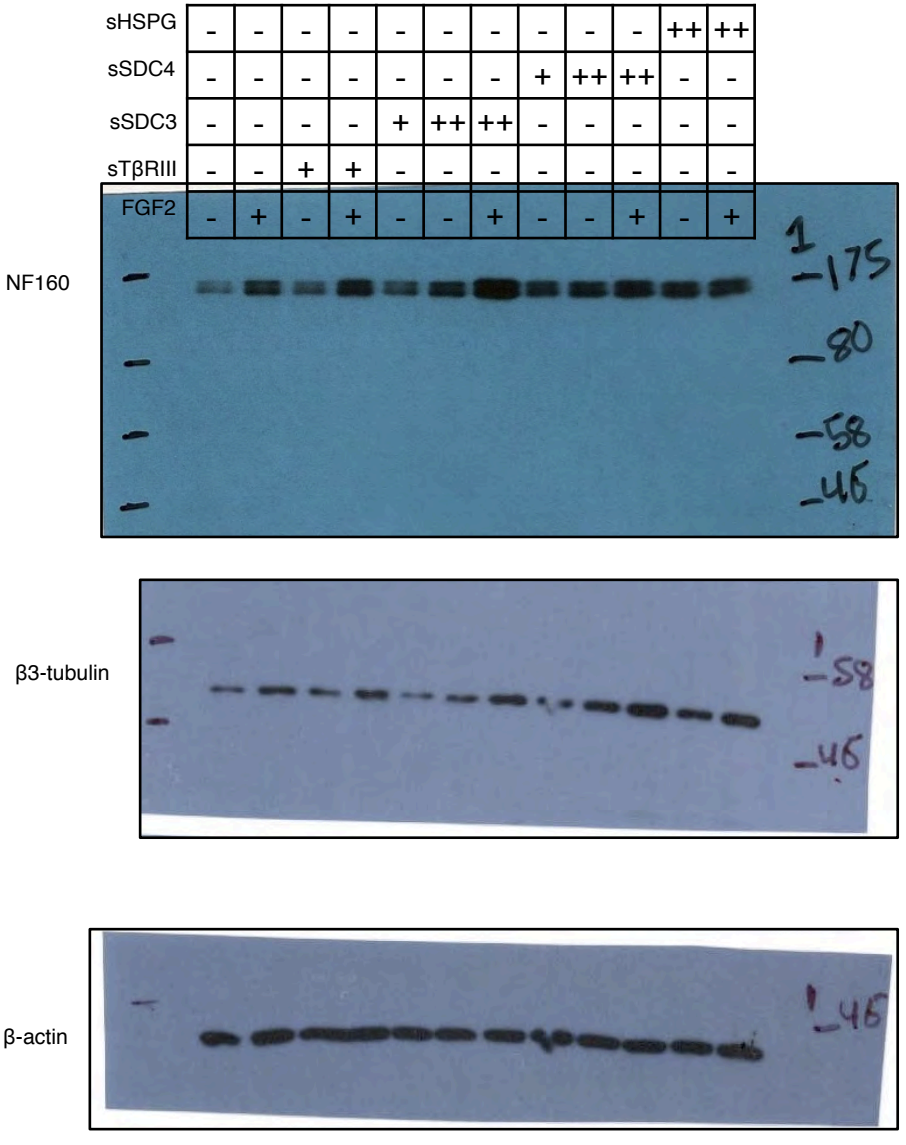
Full Unedited Gels for Figure 3D



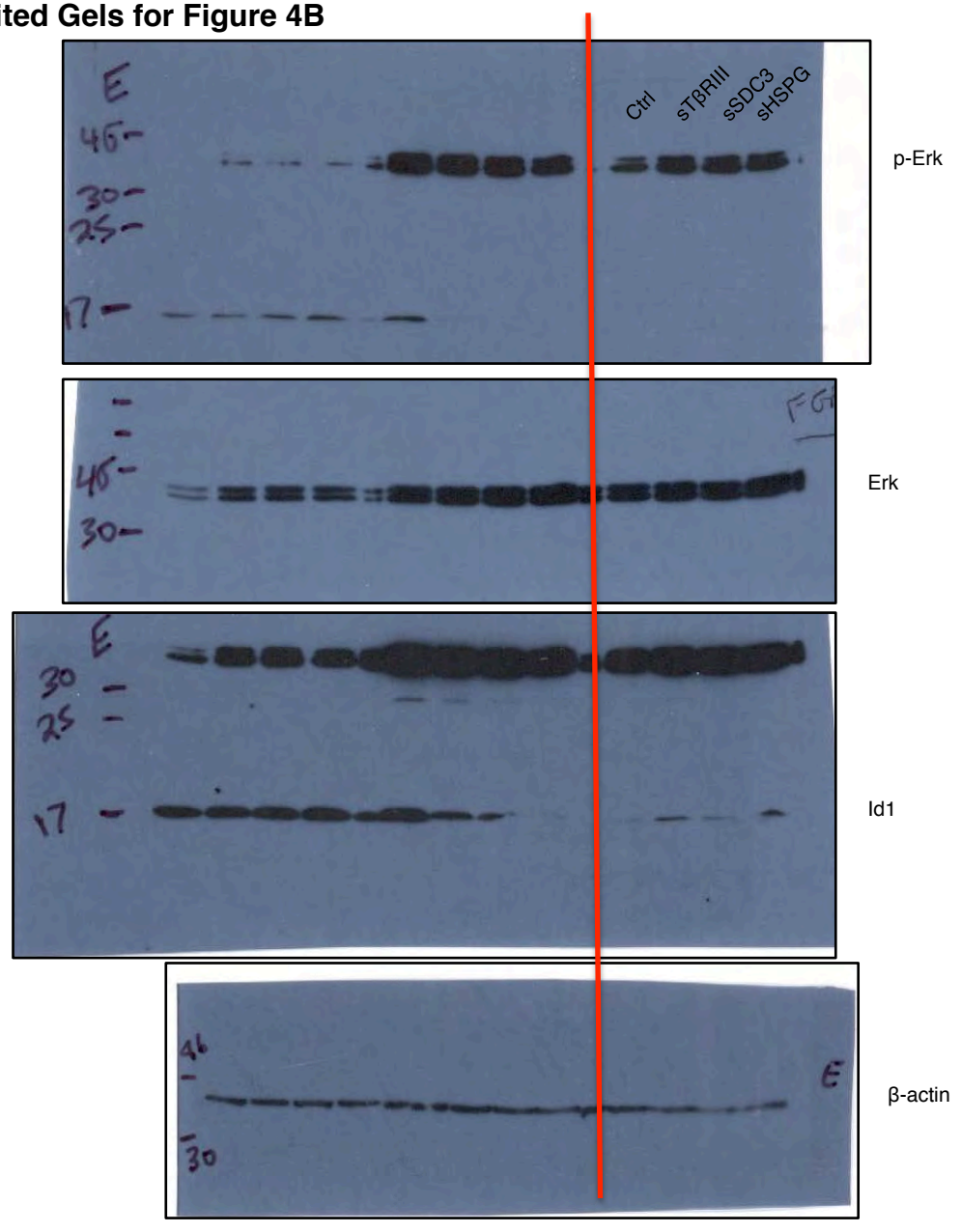
Full Unedited Gels for Figure 3E



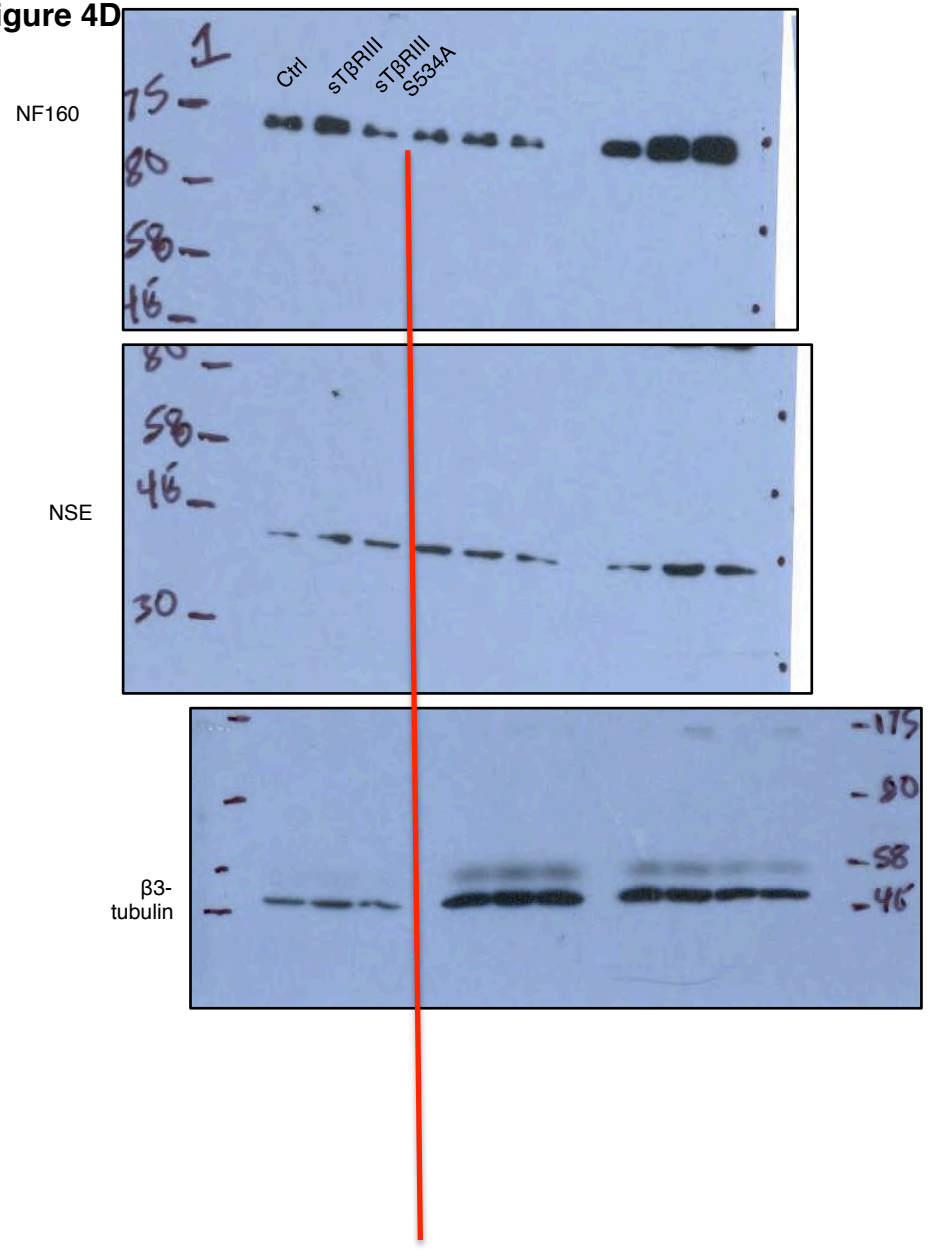
Full Unedited Gels for Figure 4A



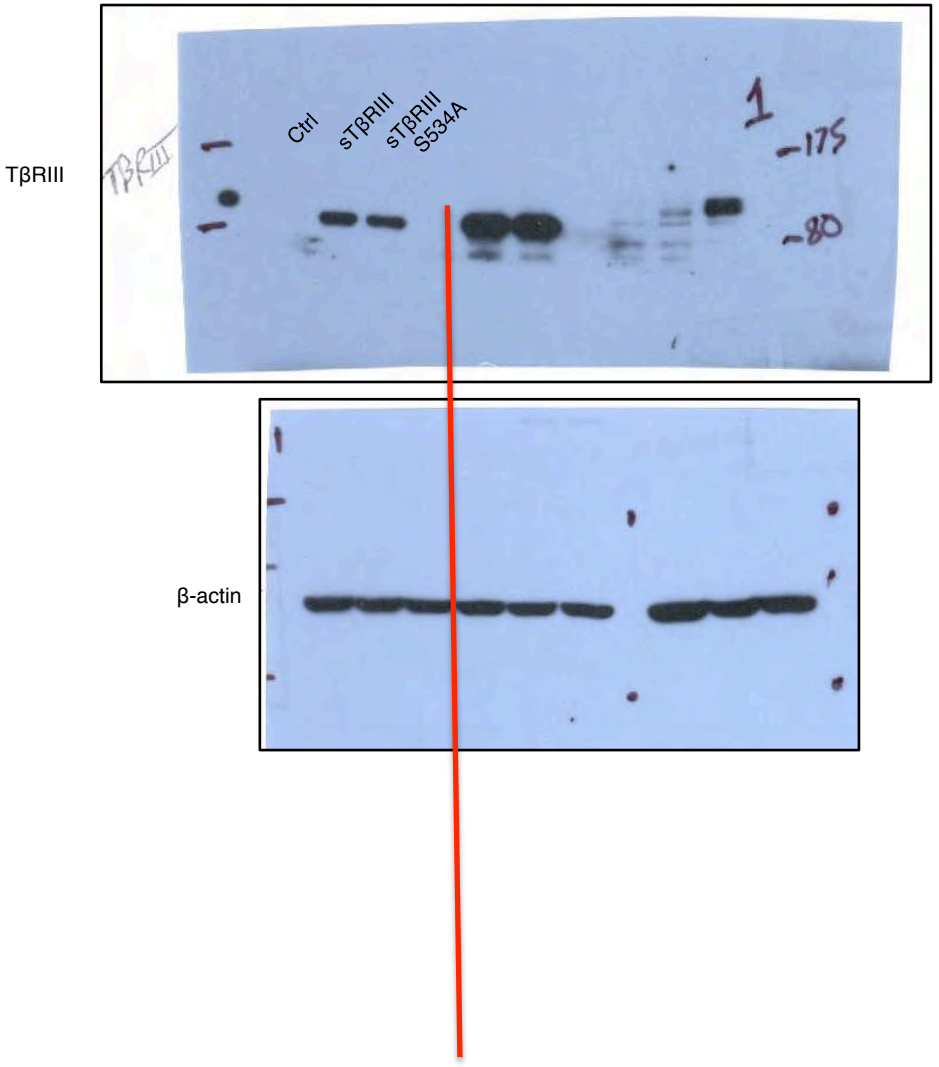
Full Unedited Gels for Figure 4B



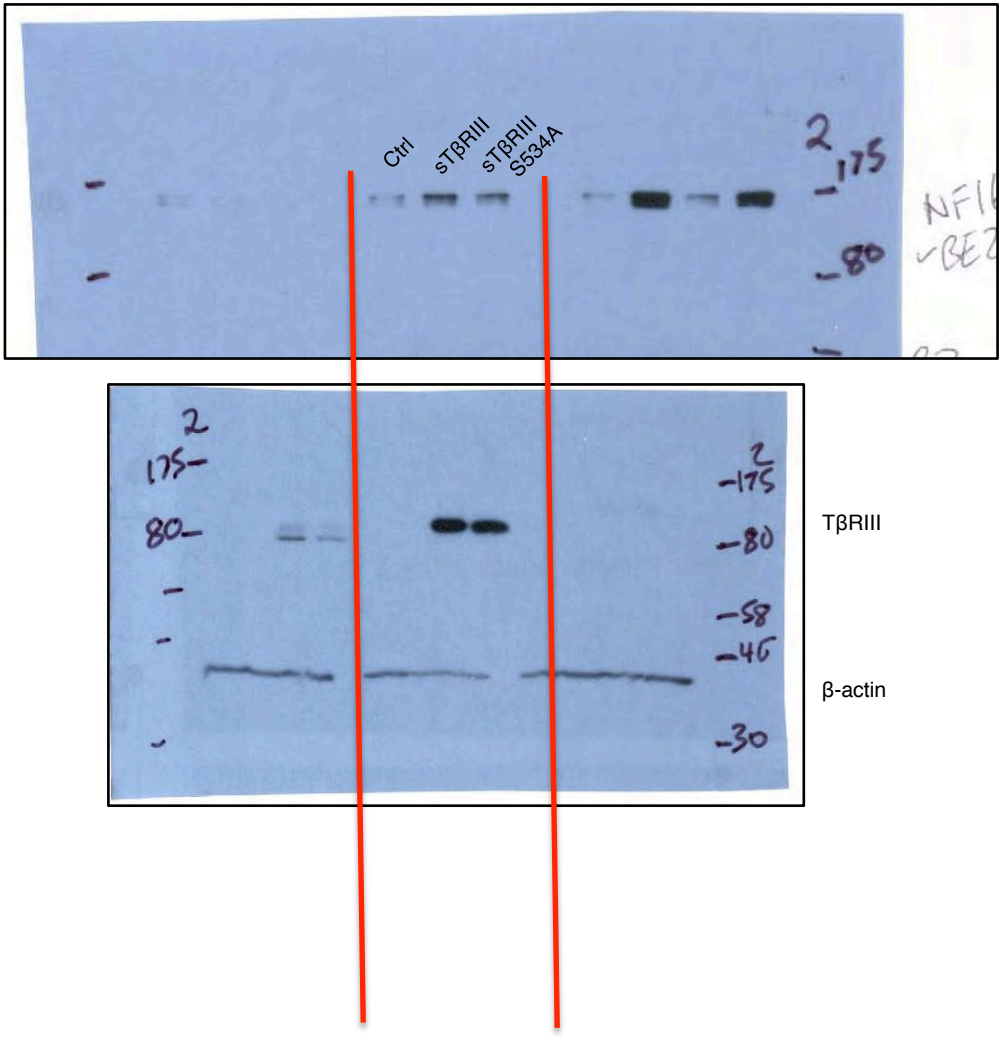
Full Unedited Gels for Figure 4D



Full Unedited Gels for Figure 4D (continued)



Full Unedited Gels for Figure 4D (continued)



Full Unedited Gels for Figure 4E

dnFGFR1	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+
CI1040	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-
U0126	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-
SU5402	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-
PD173074	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
sSDC3	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+
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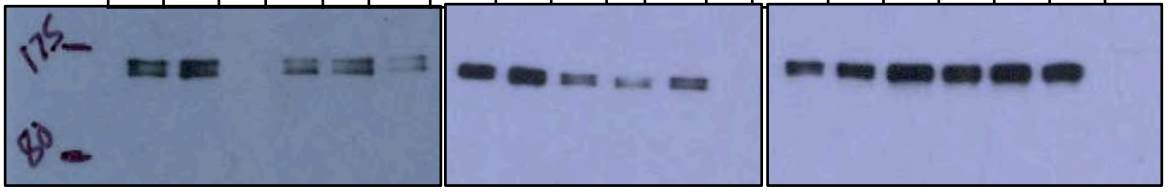
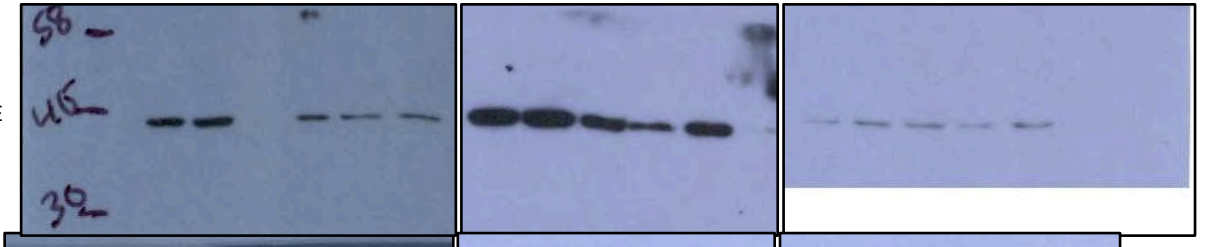
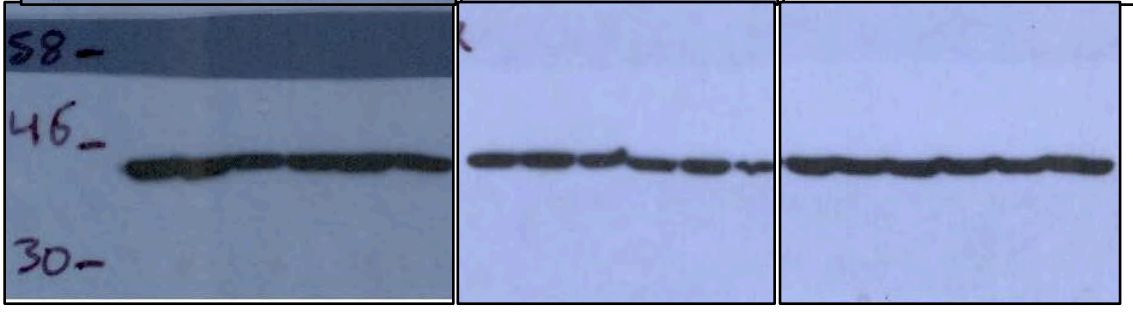
NF160



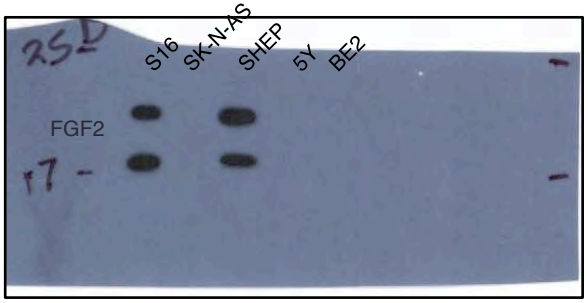
NSE

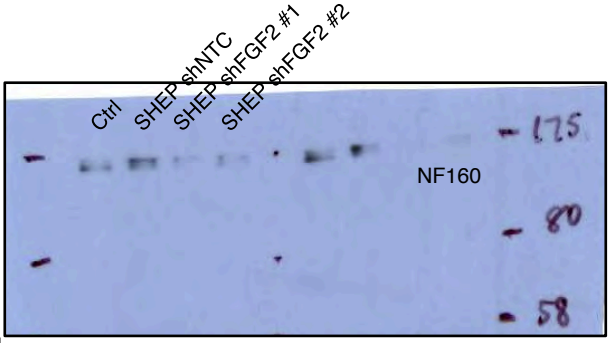


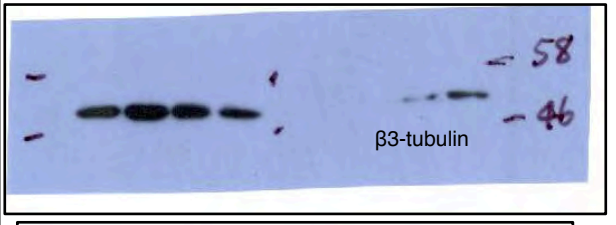
β-actin

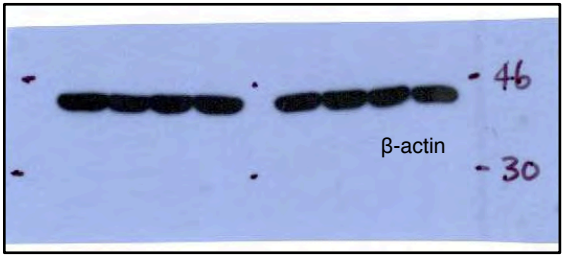


Full Unedited Gels for Figure 5C, D

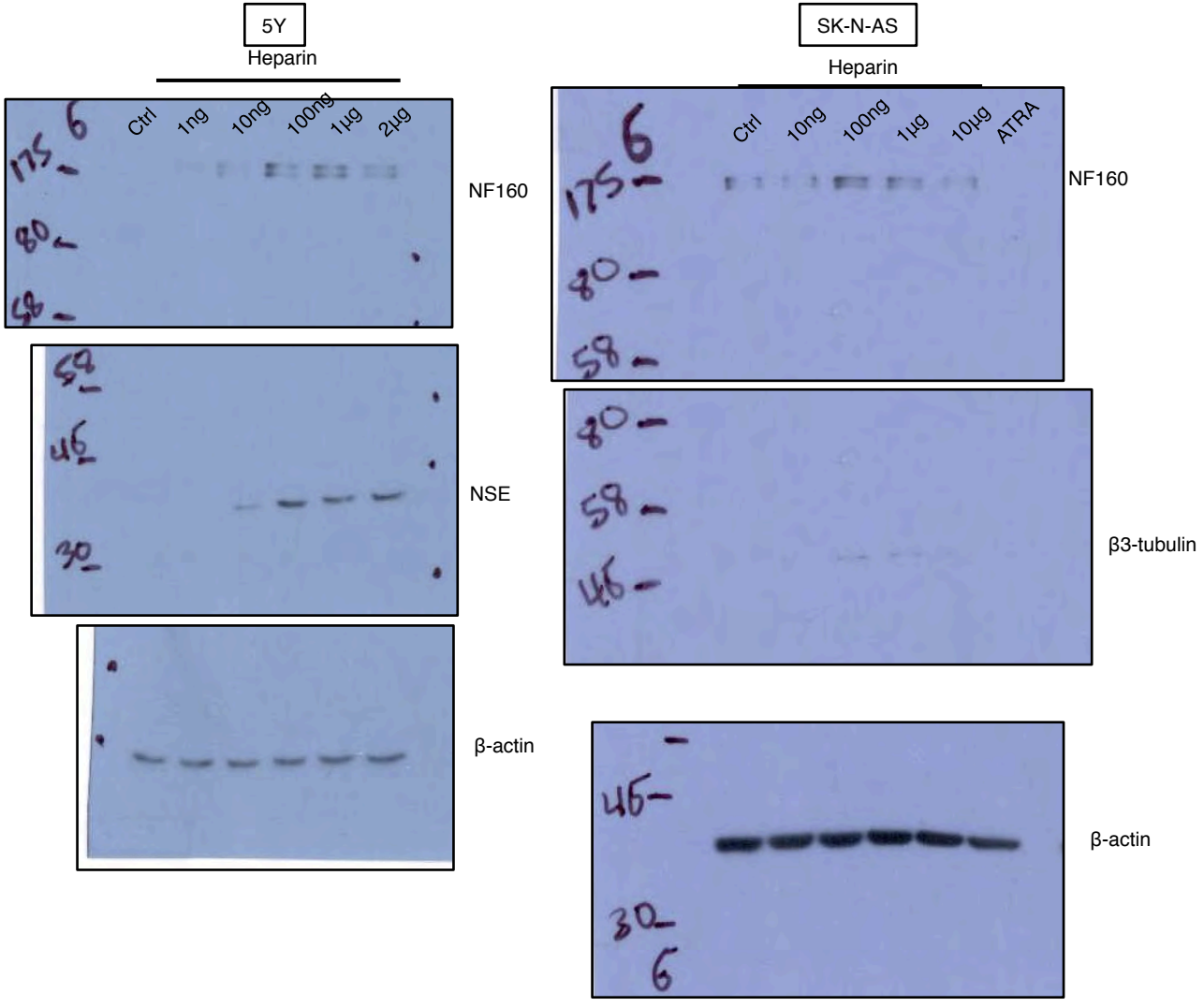




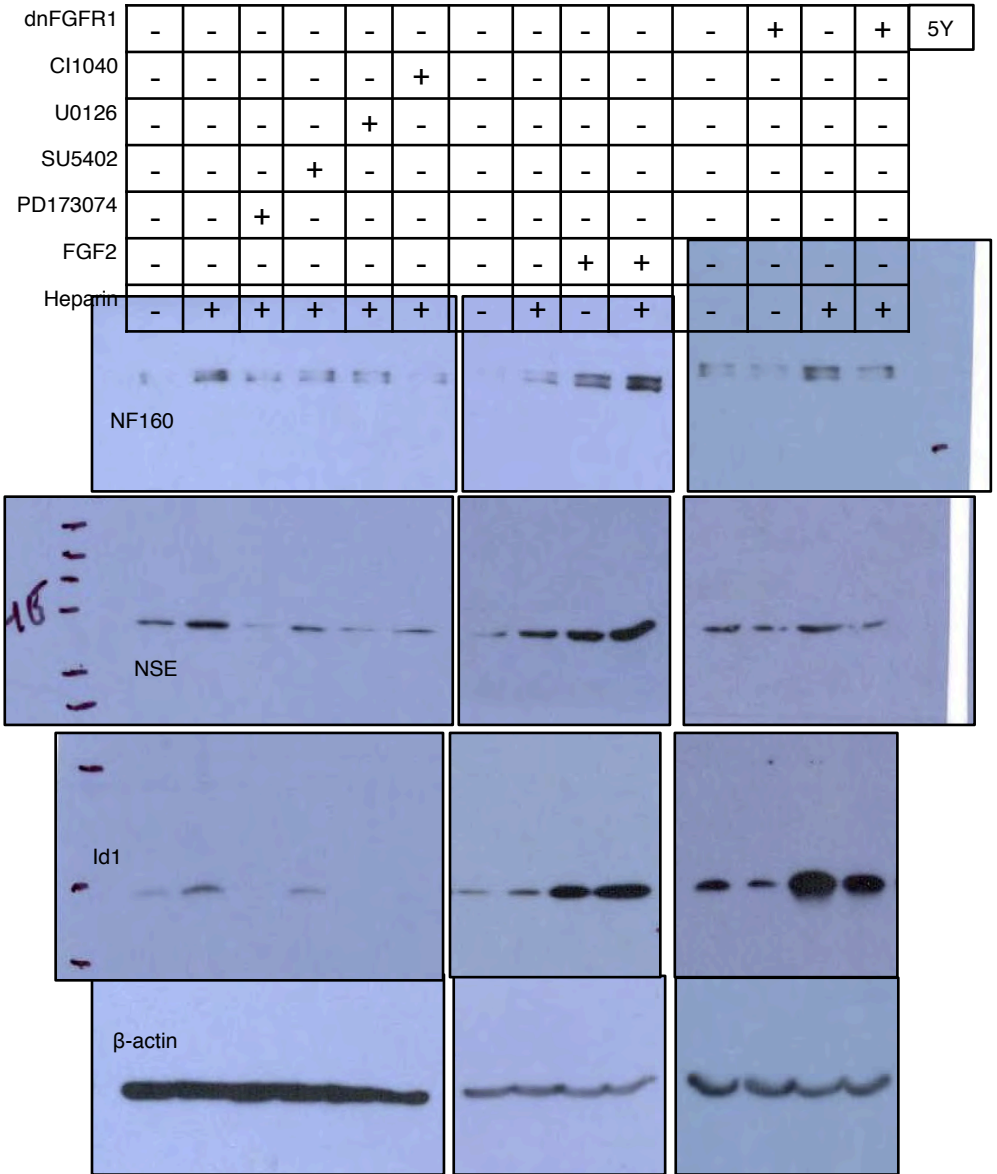




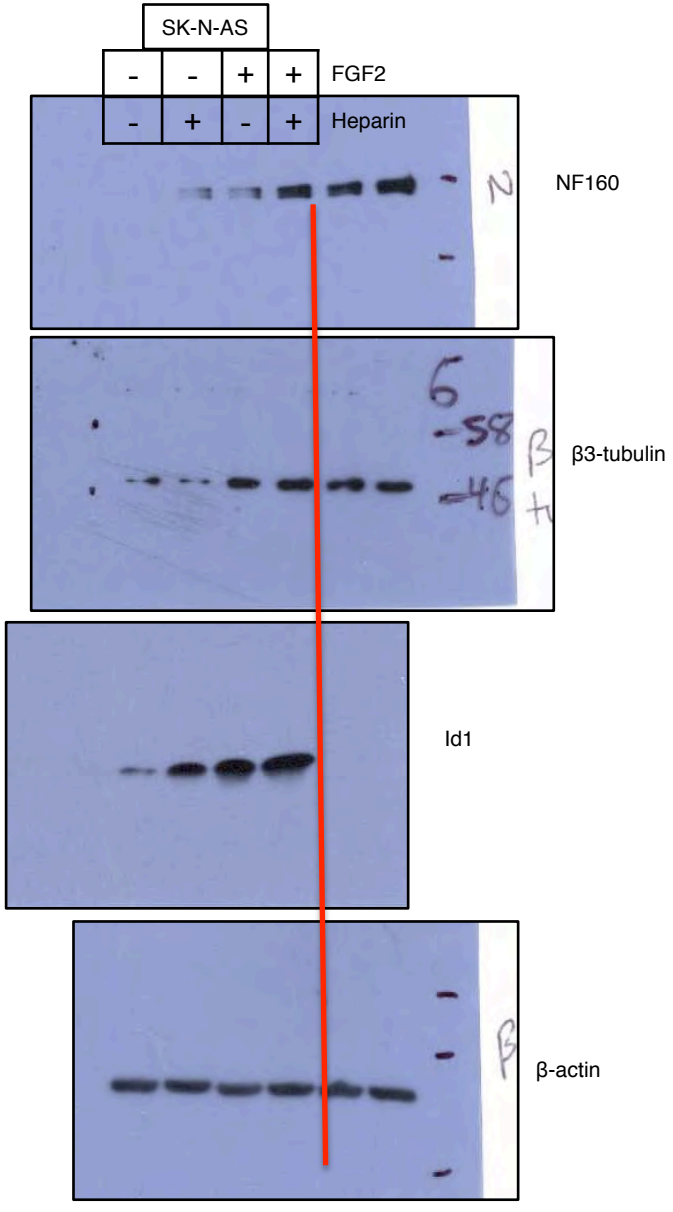
Full Unedited Gels for Figure 6B



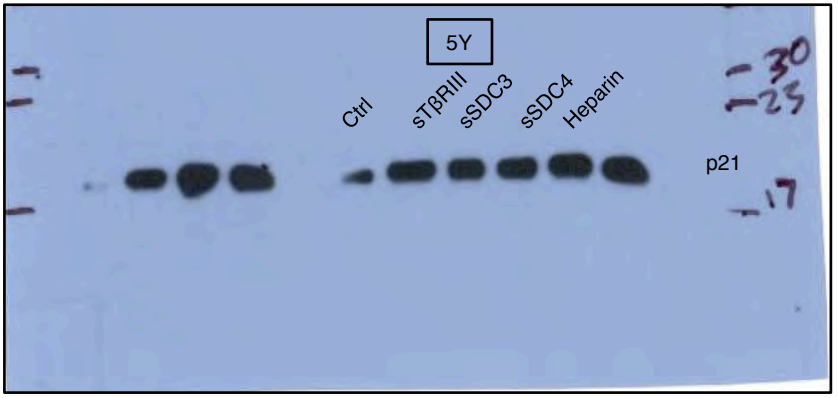
Full Unedited Gels for Figure 6C

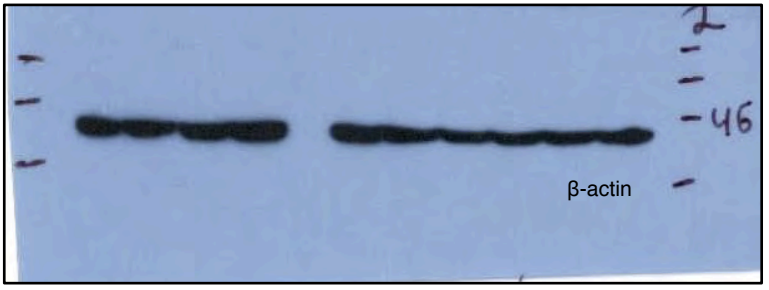


Full Unedited Gels for Figure 6C (continued)

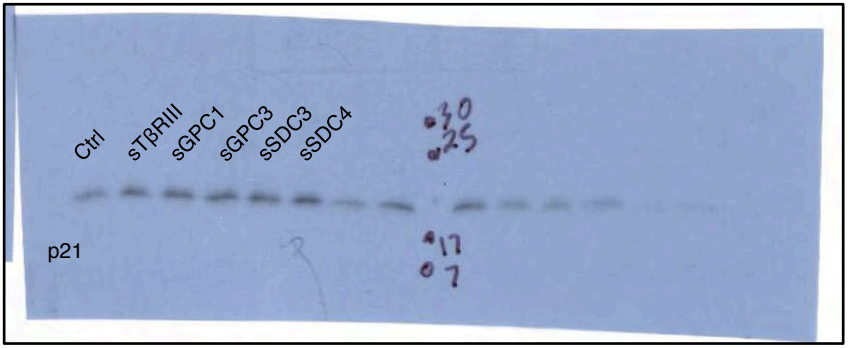


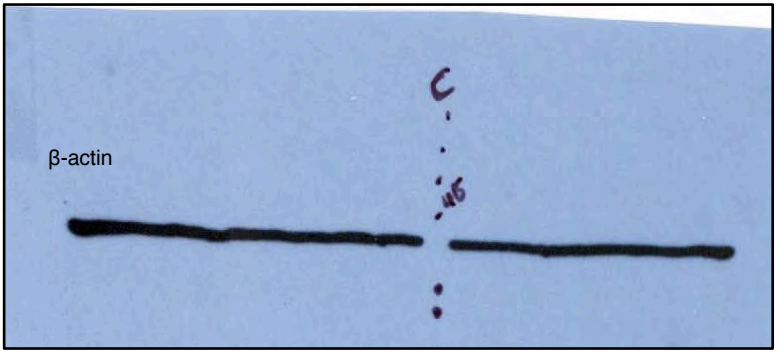
Full Unedited Gels for Figure 7A



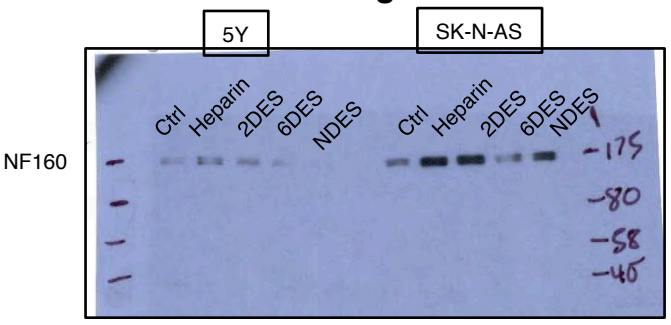


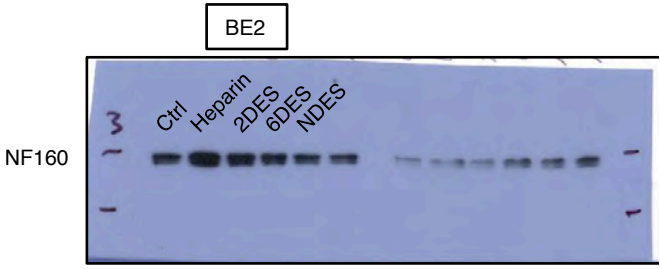
Full Unedited Gels for Figure 7A (continued)

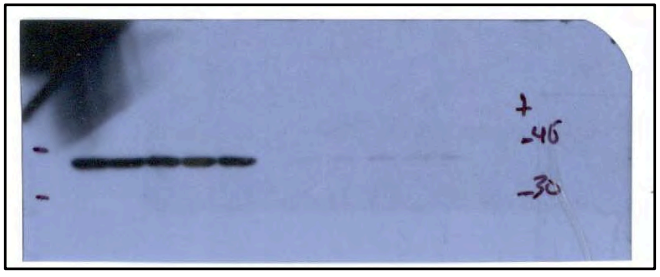


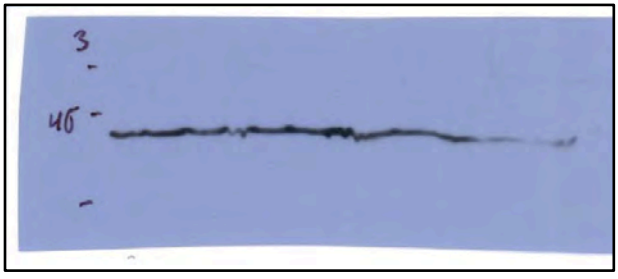


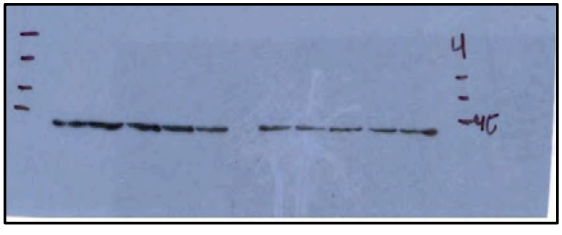
Full Unedited Gels for Figure 8B



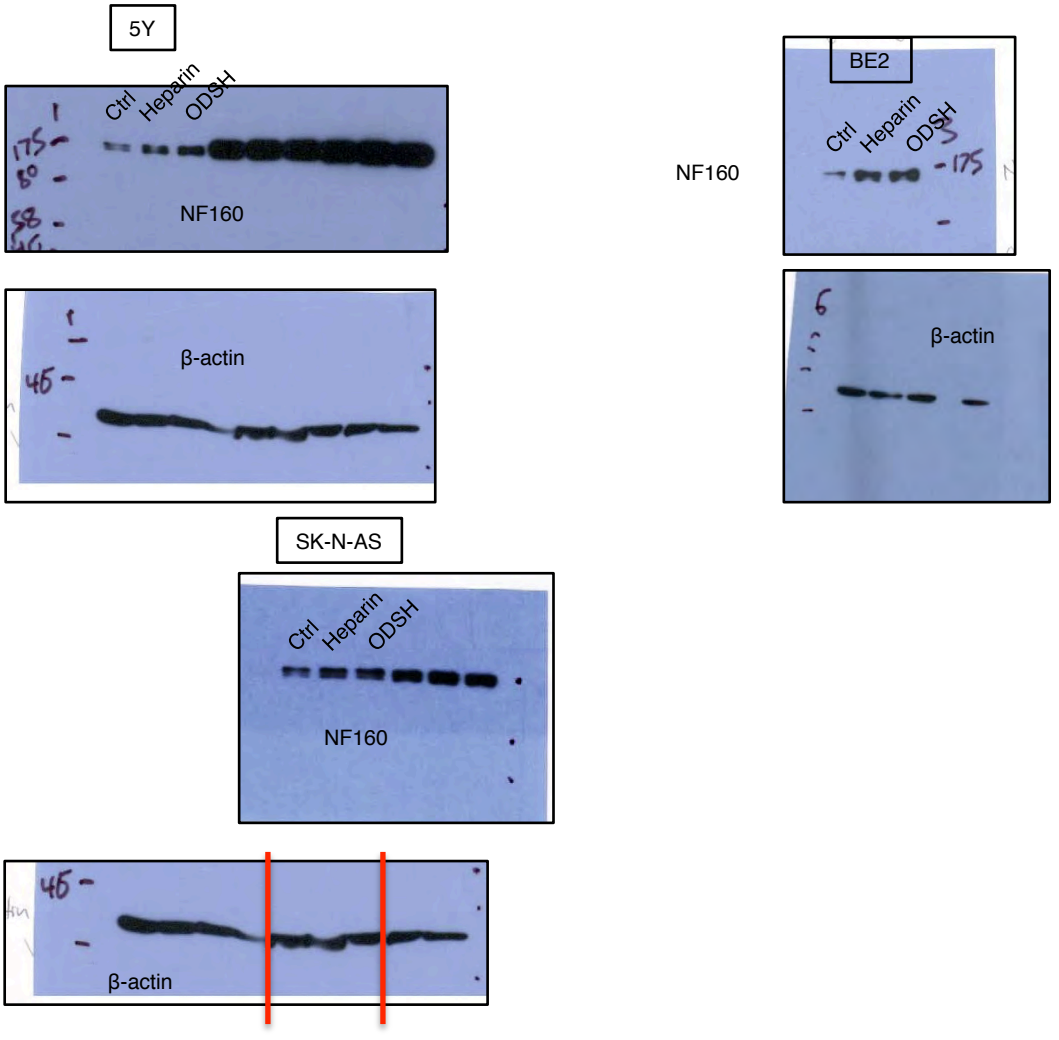




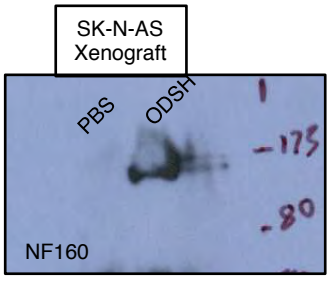


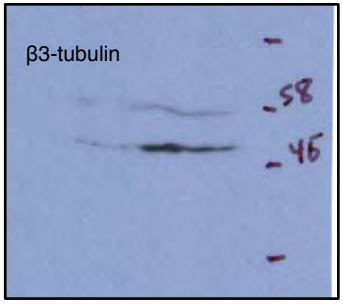


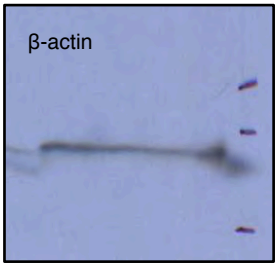
Full Unedited Gels for Figure 8B (continued)

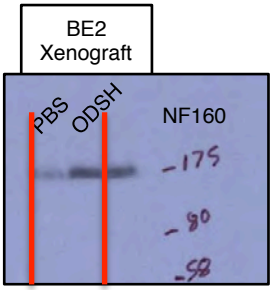


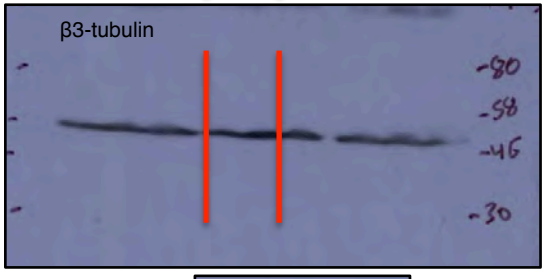
Full Unedited Gels for Figure 9D

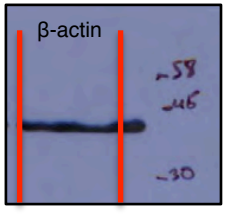


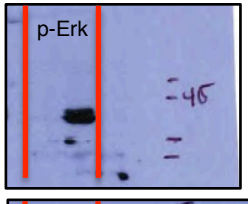


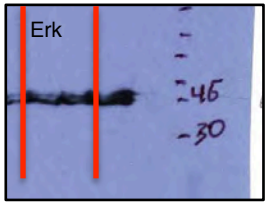


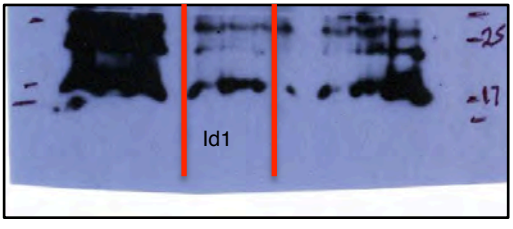






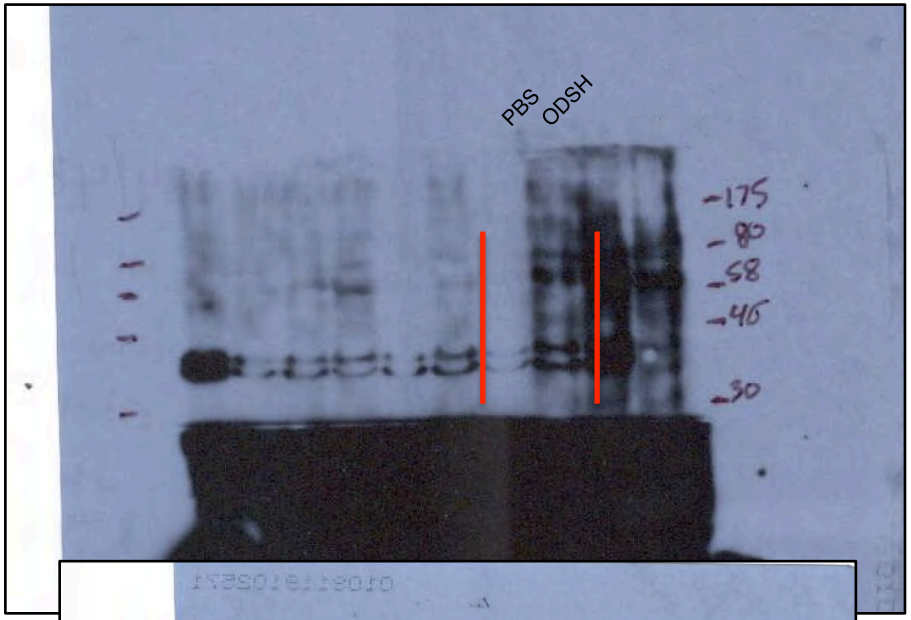




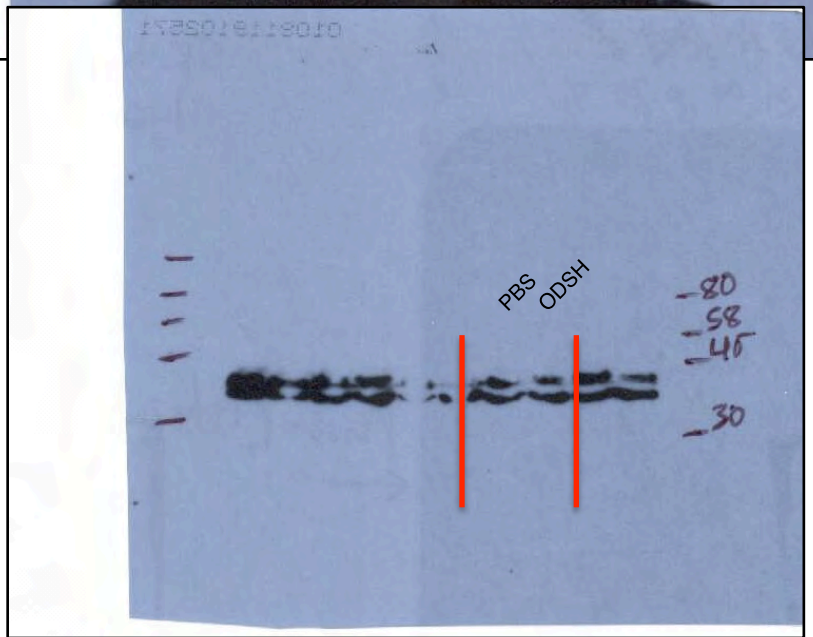


Full Unedited Gels for Figure 9D (continued)

SK-N-AS
Xenograft



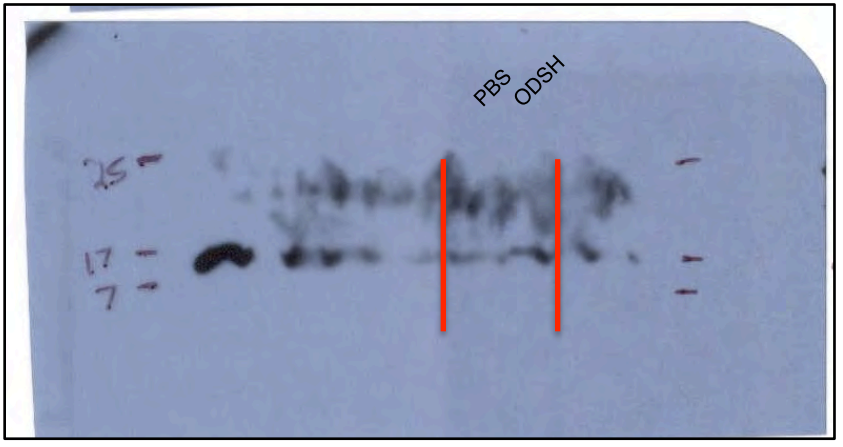
p-Erk



Erk

Full Unedited Gels for Figure 9D (continued)

SK-N-AS
Xenograft



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