

**Characterization of a novel OTX2-driven stem cell program in Group 3 and Group 4
medulloblastoma**

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SUPPLEMENTARY FIGURE AND TABLE LEGENDS:

Supplementary Fig. 1: Knockdown of OTX2 in Group 3 and Group 4 MB decreases tumorsphere formation and self-renewal. (A). Western Blot validation of OTX2 knockdown in tumorspheres from the D341 MB cell line. (B). Representative images of tumorspheres at secondary passage following OTX2 knockdown in D341 cells. (C-D). Quantification of primary (C) and secondary (D) tumorsphere number in D341 tumorspheres following OTX2 knockdown. Error Bars: s.e.m. $p < 0.05^*$, $p < 0.01^{**}$, $p < 0.001^{***}$. (E). Western Blot validation of OTX2 knockdown in tumorspheres from MB3W1 tumorspheres. (F). Representative images of tumorspheres at secondary passage following OTX2 knockdown in MB3W1 cells. (G-H). Quantification of primary (G) and secondary (H) tumorsphere number in MB3W1 tumorspheres following OTX2 knockdown. Error Bars: s.e.m. $p < 0.05^*$, $p < 0.001^{***}$. For all experiments, N=3 biological replicates or independent transfections for each siRNA.

Supplementary Fig. 2: Semaphorin genes are negatively correlated with OTX2 expression in Group 3 and Group 4 MB cells. Expression of select semaphorin pathway genes (*SEMA4D*, *NRP1*, *L1CAM*, *SEMA6A*, *PLXNA2*) in D283 (A) D341 (B) and MB3W1 (C) MB tumorspheres following OTX2 knockdown using 3 siRNAs by qPCR. Error bars: s.e.m. N=3 biological replicates, n=9 technical replicates.

Supplementary Fig. 3: Axon guidance gene expression is upregulated following OTX2 knockdown in Group 3 and Group 4 MB cells. Expression of select axon guidance pathway genes (*SLIT2*, *EFNB2*, *EPHA3*, *EPHA5*, and *DCC*) in D283 (A) and D341 (B) tumorspheres following OTX2 knockdown using 3 siRNAs by qPCR. Error bars: s.e.m. N=3 biological replicates, n=9 technical replicates.

Supplementary Fig. 4: Recombinant semaphorin protein treatment does not significantly affect cell number or viability of D283 tumorspheres. (A-C). Total number of cells (upper) and viability (lower) in primary D283 tumorspheres following 5-day treatment with recombinant L1CAM Fc (A), SEMA4D (B) or NRP1 (C). (D-F) Total number of live cells (upper) and viability (lower) in secondary D283 tumorspheres following 5-day treatment with L1CAM Fc (D) SEMA4D (E) or NRP1 (F). For all experiments, N=4 biological replicates, n=20 L1CAM Fc, n=18 SEMA4D Fc and NRP1 Fc technical replicates. Error bars: s.e.m.

Supplementary Fig. 5: Decreased levels of semaphorin pathway genes in MB3W1 OTX2 KD cells results in a partial rescue of tumorsphere formation and growth. (A) Representative images of MB3W1 tumorspheres following dual OTX2 and either SEMA4D or NRP1 knockdown. Scale bar: 400 μ m. (B-C). Quantification of tumorsphere number following dual OTX2 and either SEMA4D (B) or NRP1 (C) knockdown in MB3W1 tumorspheres over 5 days. Error Bars: s.e.m. $p < 0.001$ ***. N=3 biological replicates, n=12 SEMA4D KD and NRP1 KD technical replicates. (D-E). Quantification of total live cell number following dual OTX2 and either SEMA4D (D) or NRP1 (E) knockdown in MB3W1 tumorspheres over 5 days. Error Bars: s.e.m. $p < 0.05$ *. N=4 biological replicates, n=8 SEMA4D KD and NRP1 KD technical replicates.

Supplementary Fig. 6: Decreased levels of semaphorin pathway genes in D283 and MB3W1 OTX2 KD tumorspheres does not significantly affect viability. (A). Viability in D283 tumorspheres following dual OTX2 and either SEMA4D (left) or NRP1 (right) knockdown over 5 days. Error Bars: s.e.m. N=7 and N=8 biological replicates for SEMA4D and NRP1 respectively. (B). Viability in MB3W1 tumorspheres following dual OTX2 and either SEMA4D (left) or NRP1 (right) knockdown over 5 days. Error Bars: s.e.m. N=4 biological replicates for SEMA4D and NRP1.

Supplementary Table 1: List of antibodies and concentrations used for Western blots

Supplementary Table 2: List of primers sequences used for qPCR

Supplementary Table 3: Neuronal differentiation genes that are significantly and differentially expressed following OTX2 knockdown in D283 tumorspheres. Transcripts differentially expressed at least 2-fold (up- or downregulated) and with a value of $P < 0.05$ were considered significant

Supplementary Table 4: Axon guidance genes that are significantly and differentially expressed following OTX2 knockdown in D283 tumorspheres. Transcripts differentially expressed at least 2-fold (up- or downregulated) and with a value of $P < 0.05$ were considered significant.

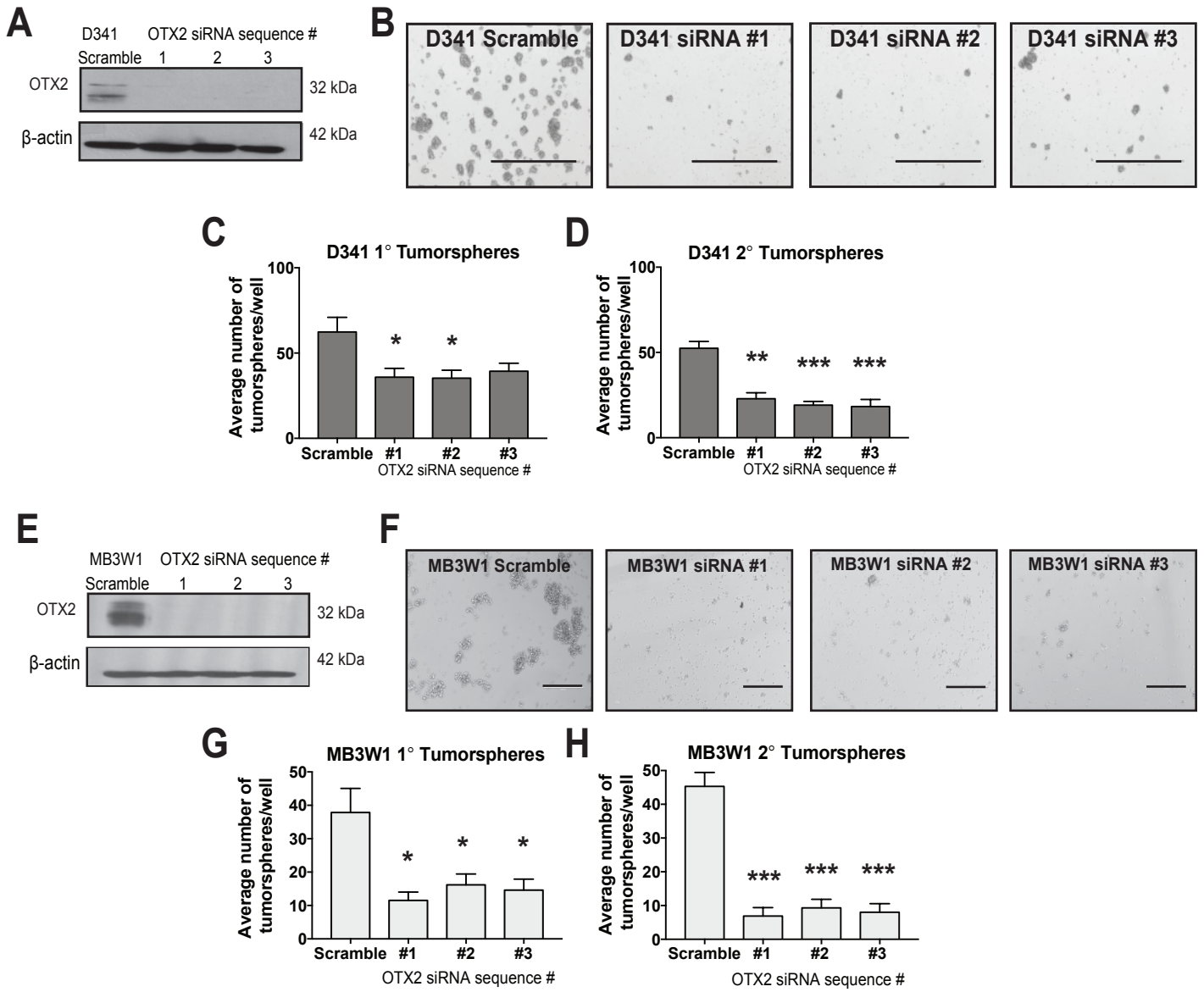
Supplementary Table 5: Axon guidance pathway genes that were significantly and differentially expressed following OTX2 KD and the number of OTX2 binding peaks /overlaps within -5kb+2kb of their transcriptional start sites. Semaphorin genes significantly upregulated following OTX2 KD are highlighted in red.

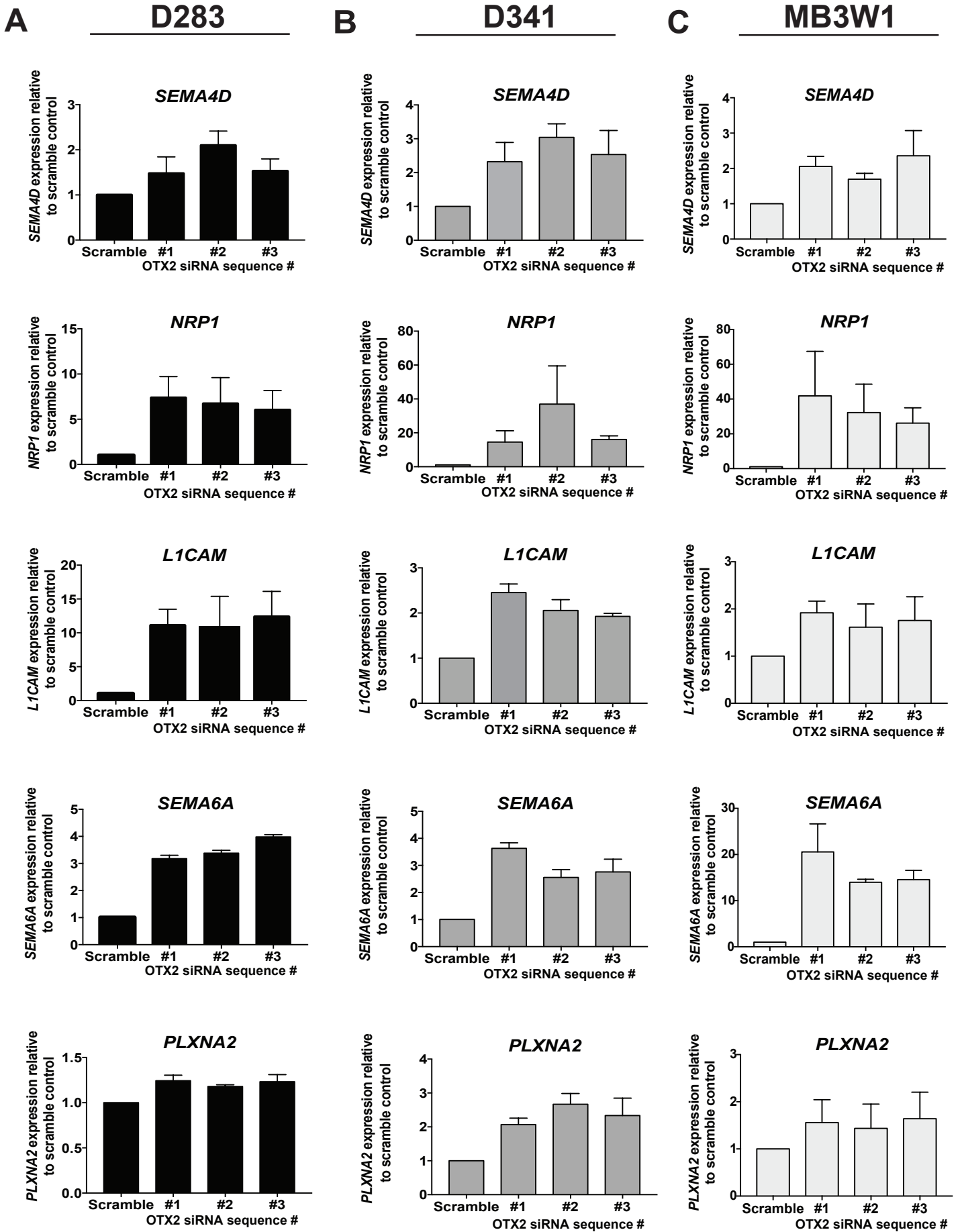
Supplementary Table 6a: Univariable cox regression analysis of survival by increasing *SEMA4D* expression across Group 3 and 4 medulloblastoma.

Supplementary Table 6b: Statistical analyses of survival in patients exhibiting high *SEMA6A*, *NRP1*, *NRP2*, or *LICAM* gene expression relative to patients with low *SEMA6A*, *NRP1*, *NRP2* or *LICAM* gene expression. $P < 0.05$ was considered significant.

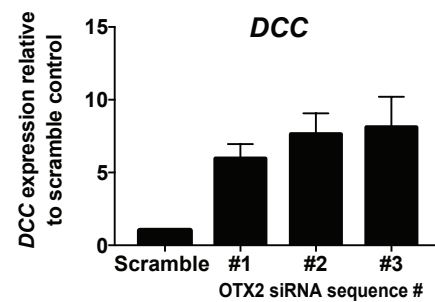
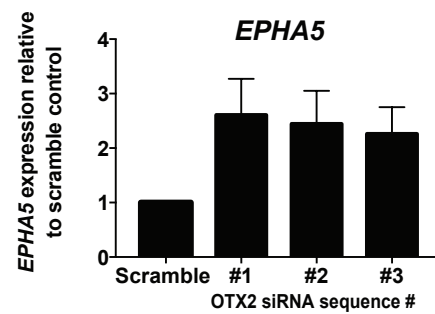
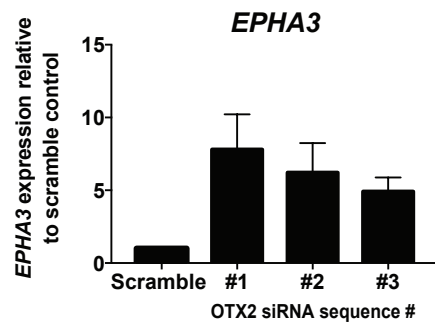
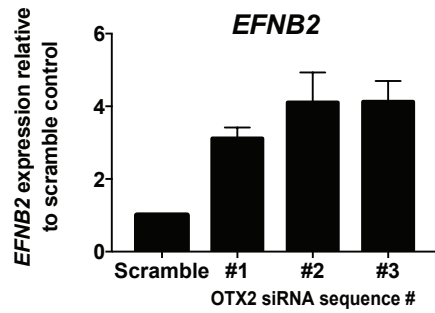
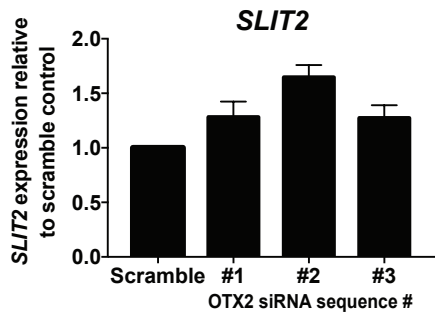
Supplementary Table 7: Gene Set Enrichment Analysis (GSEA) results for Reactome and KEGG databases identified pathways significantly enriched in gene sets that were downregulated in D283 Scramble relative to OTX2 KD tumorspheres.

Supplementary Table 8: GSEA revealed that genes associated with SEMA4D signaling were enriched in gene sets that were downregulated in D283 scramble relative to OTX2 KD tumorspheres. All transcripts in the core enrichment were significantly different with a $P < 0.05$.

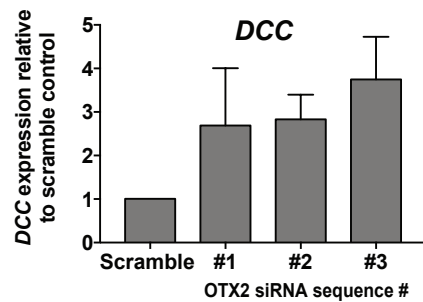
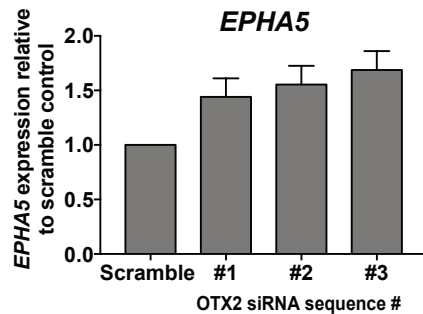
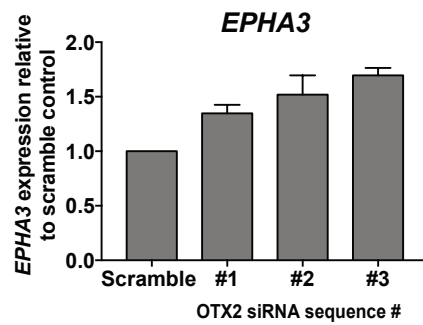
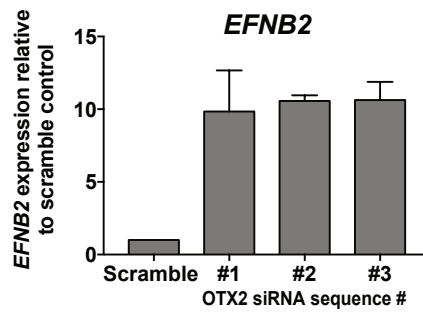
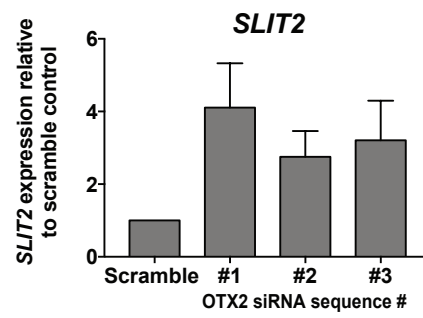


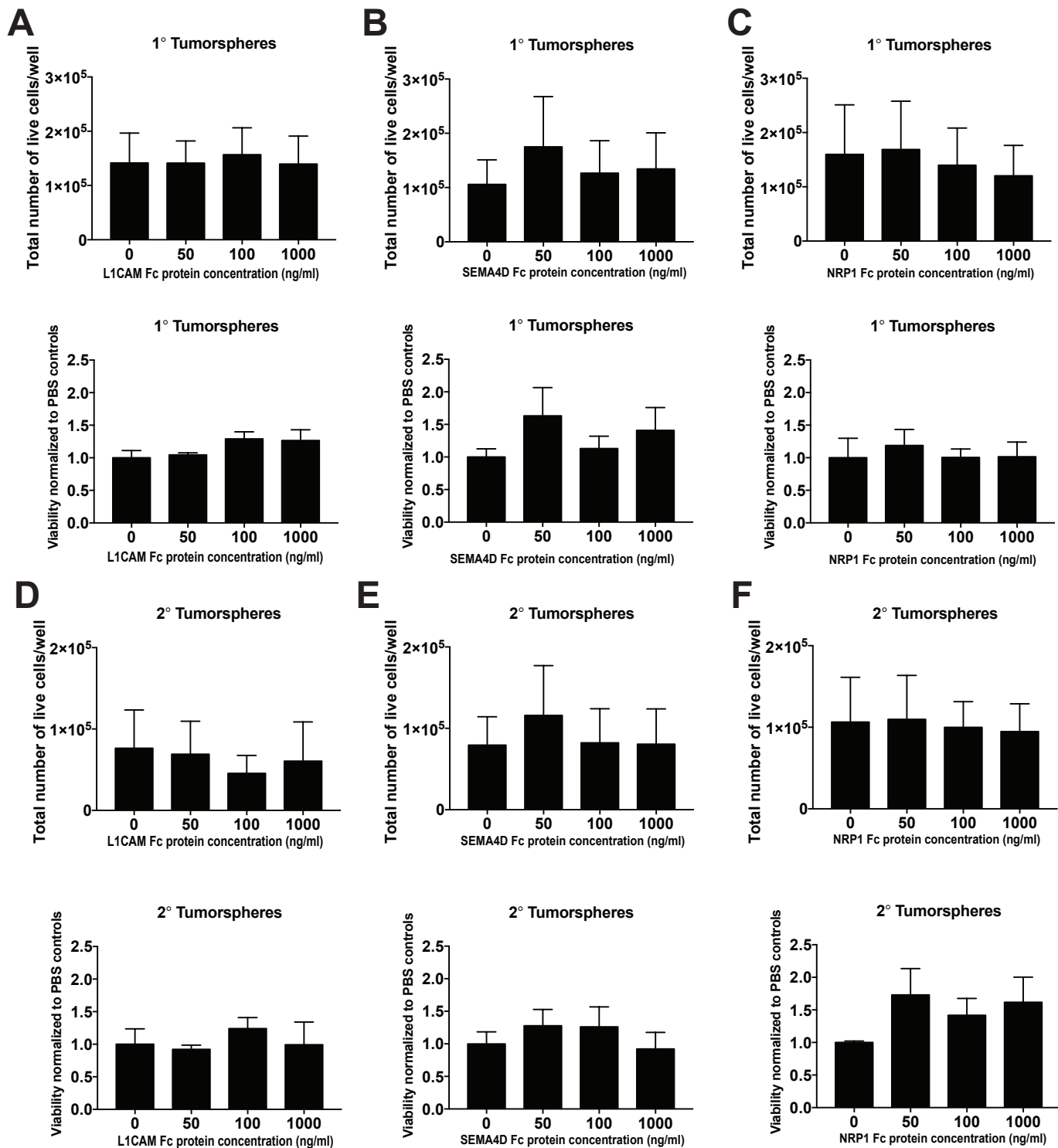


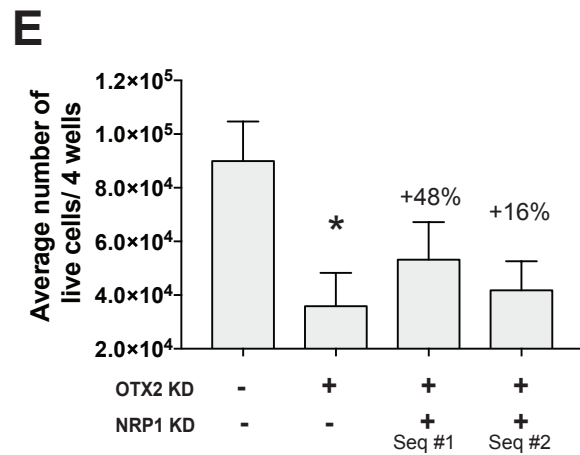
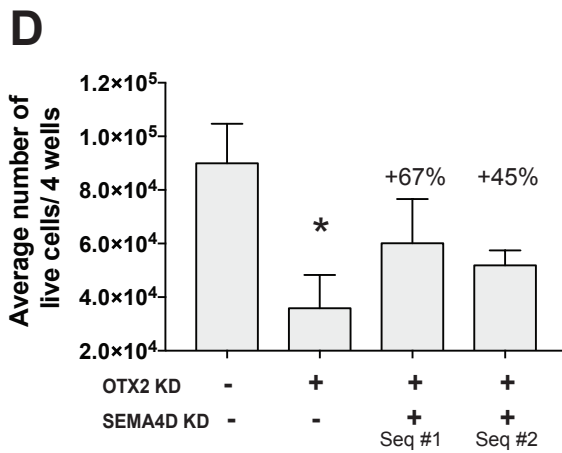
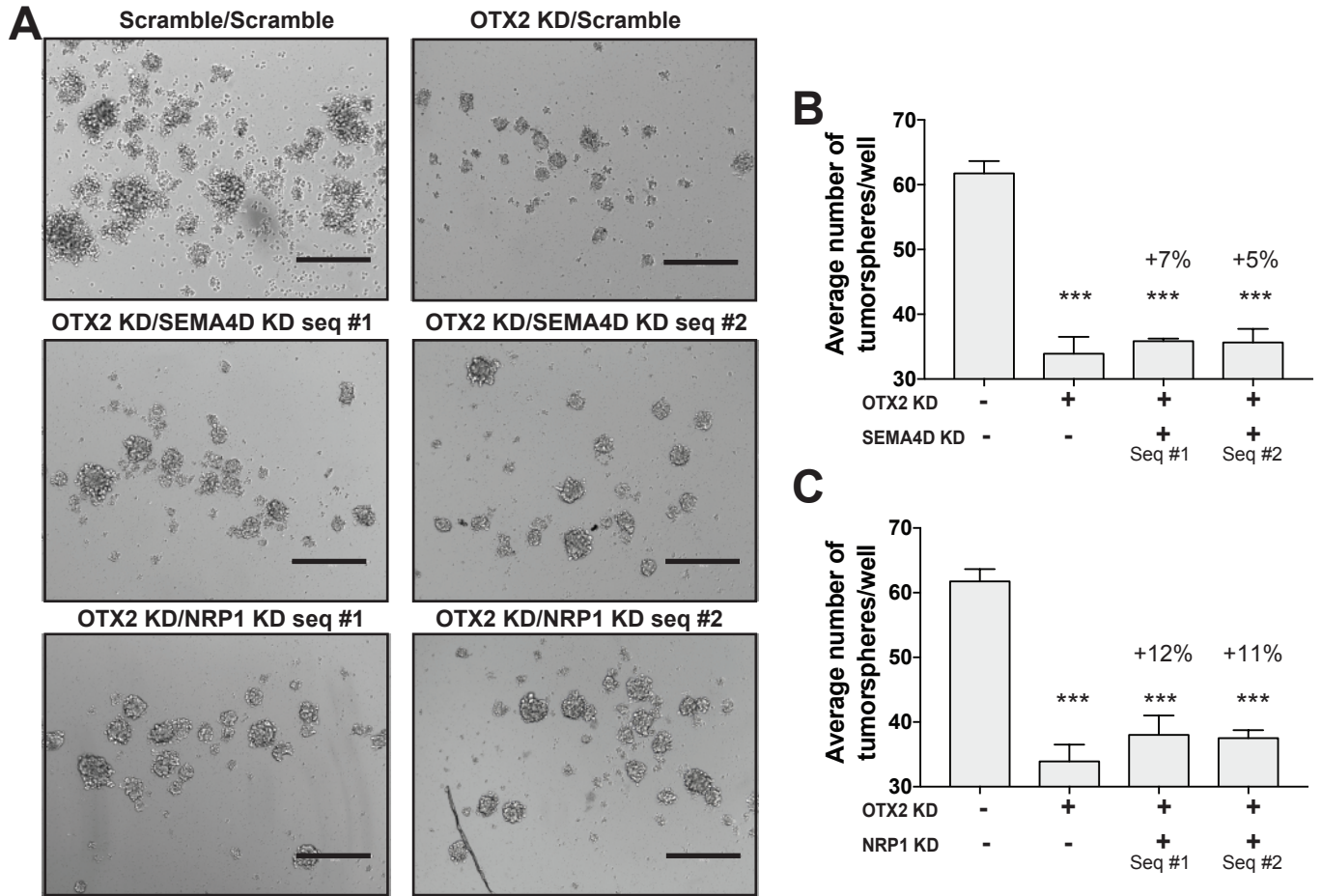
A **D283**

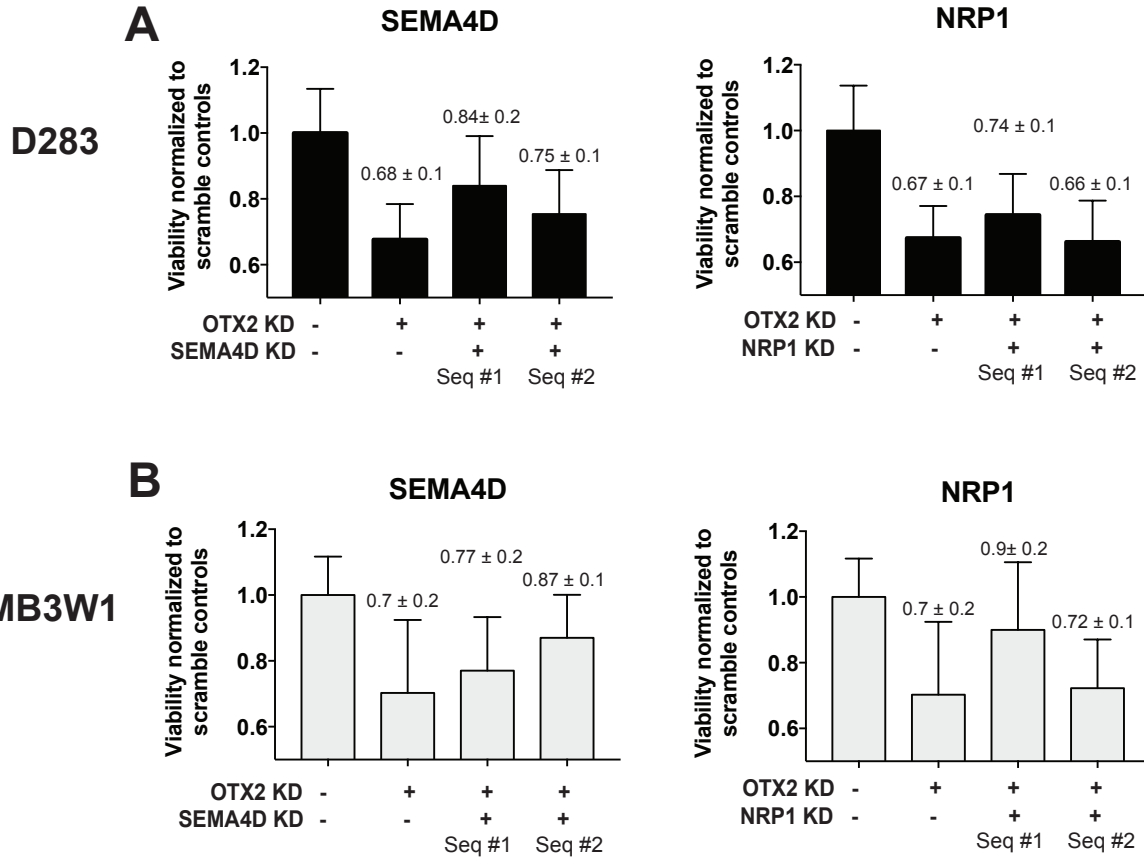


B **D341**









Supplementary Table 1: List of antibodies and concentrations used for Western blots

Gene	Concentration	Source and catalogue number
β-actin	1:1000	Abcam Inc., (ab8226)
SEMA4D	1:500	Abcam Inc., (ab134128)
L1CAM	1:500	Gene Tex, (GTX23200)
NRP1	1:500	Abcam Inc., (ab81321)
βIII Tubulin	1:1000	R&D Systems, (MAB1195)
OTX2	1:500	Abcam Inc., (ab21990)
RHO	1:500	Thermo Fisher, (1862332)
Peroxidase AffiniPure Donkey Anti-Rabbit IgG (H+L)	1:5000	Jackson ImmunoResearch Laboratories Inc., (711-035-152)
Goat Anti-Mouse IgG H&L (HRP)	1:5000	Abcam Inc., (ab6789)

Supplementary Table 2: List of primer sequences used for qPCR

Gene	Forward sequence	Reverse sequence
<i>GAPDH</i>	3'-GAAATCCCATCACCATCTTCCAGG-5'	3'-GCAAATGAGCCCCAGCCT TCTC-5'
<i>OTX2</i>	5'-GAGGTGGCACTGAAAATCAAC-3'	5'-TCTTCTTTTTGGCAGGTCTCA-3'
<i>TUJ1</i>	5'-GGCCTTTGGACATCTCTTCA-3'	5'-TCGCAGTTTTCACACTCCTTC-3'
<i>MAP2</i>	5'-ATTCTGGCAGCAGTTCTCAA-3'	5'-TGCTTCCTCGGTTAGAGACAA-3'
<i>SEMA4D</i>	5'-CGTCATGGTTGATGGAGAACT-3'	5'-AGCCAAGGGATTGCATATTCT-3'
<i>L1CAM</i>	5'-AAATGGCTGTGAAGACCAATG-3'	5'-GATGAAGCAGAGGATGAGCAG-3'
<i>NRP1</i>	5'-CGATTTGGAGGACAGAGACTG-3'	5'-GGGGCTATCTTTCCACAGAAC-3'
<i>SEMA6A</i>	5'-TTTGTTCAAGCCGTGGATTAC-3'	5'-GCCACTCTTGGGAAAACCTACC-3'
<i>PLXNA2</i>	5'-ACCATCACACAGGTCAAGGAG-3'	5'-CACTCCAAGTCCATGTCCACT-3'
<i>SLIT2</i>	5'-TGAATTTACCGTGTGGAAGC-3'	5'-CAGATGCTCCTTCAAATGCTC-3'
<i>DCC</i>	5'-TACTGGACCACCTTCCAACCTG-3'	5'-CCTCACATGAAGAGAGCTTGG-3'
<i>EPHA3</i>	5'-GGAAGAGATCAGTGGTGTGGA-3'	5'-TTTTGACTGTGGTCCATGACA-3'
<i>EPHA5</i>	5'-ACAAAGGAAGCCAAATCACCT-3'	5'-GGTAGAAACCCAAAGGCAGAC-3'
<i>EPHB2</i>	5'-GACTCCACTACAGAGACTGCT-3'	5'-TCTCATCGTAGCCACTCACCT-3'

Supplementary Table 3: Neuronal differentiation genes that are significantly and differentially expressed following OTX2 knockdown in D283 tumorspheres

Gene Assignment	Predictive effect on neuronal differentiation (based on measurement direction and literature compiled in Ingenuity® Knowledge Base)	Fold change
<i>DPYSL2</i>	Increased	2.284692494
<i>NEUROD2</i>	Increased	2.470837274
<i>NTRK3</i>	Increased	3.721798631
<i>HMGB2</i>	Increased	0.334250124
<i>EFNA3</i>	Increased	3.294364069
<i>GAP43</i>	Increased	10.4468783
<i>MID1</i>	Increased	0.398320048
<i>NRXN1</i>	Increased	7.459091891
<i>NSG1</i>	Increased	2.187070915
<i>CNR1</i>	Increased	9.787904329
<i>EPHA4</i>	Increased	2.713208655
<i>LRP1</i>	Increased	2.435132037
<i>NR2F1</i>	Increased	3.140512475
<i>KLF7</i>	Increased	2.797232165
<i>DLG5</i>	Increased	2.100889088
<i>ACTL6B</i>	Increased	2.239225777
<i>CNTNAP2</i>	Increased	9.493894381
<i>NCAM2</i>	Increased	13.78473856
<i>TSC1</i>	Increased	2.037782393
<i>PAX6</i>	Increased	3.031433133
<i>RELN</i>	Increased	4.00277355
<i>SEMA6A</i>	Increased	5.544279543
<i>NUMBL</i>	Increased	2.0265138
<i>SDK1</i>	Increased	2.396618043
<i>CRMP1</i>	Increased	8.190775203
<i>DPYSL3</i>	Increased	2.261061134
<i>SNCA</i>	Increased	5.95045505
<i>DPYSL5</i>	Increased	2.223758315
<i>CIT</i>	Increased	0.377356492
<i>KIDINS220</i>	Increased	2.979354926
<i>SLIT2</i>	Increased	3.365917929
<i>KIF3A</i>	Increased	2.844154821
<i>GRIA1</i>	Increased	6.520578659
<i>BCL2</i>	Increased	2.63170905
<i>FNBP1</i>	Increased	2.488023307
<i>KIF1A</i>	Increased	3.38932974
<i>PDGFRA</i>	Increased	4.982949662
<i>RIMS1</i>	Increased	4.161201066
<i>APP</i>	Increased	2.360348687
<i>KALRN</i>	Increased	4.806544198
<i>ELAVL4</i>	Increased	26.6857934
<i>FYN</i>	Increased	3.280691645

<i>mir-181</i>	Increased	2.987626914
<i>CAMK4</i>	Increased	3.431882122
<i>TRIP10</i>	Increased	0.219912269
<i>CAMK1G</i>	Increased	3.020945171
<i>NEUROD1</i>	Increased	2.193143177
<i>CDK5R1</i>	Increased	2.756810306
<i>ADGRB2</i>	Increased	2.494931144
<i>MAPT</i>	Increased	4.17564771
<i>NEUROG2</i>	Increased	2.797232165
<i>SEMA4D</i>	Increased	3.45575275
<i>HES1</i>	Increased	0.400257405
<i>ID1</i>	Increased	0.4181232
<i>NEFH</i>	Increased	0.291587342
<i>RYR2</i>	Increased	11.03488737
<i>TNFRSF21</i>	Increased	0.494142826
<i>PRKCE</i>	Increased	3.396384986
<i>TBR1</i>	Increased	2.698205069
<i>CHRNA1</i>	Increased	0.410940094
<i>NEUROD6</i>	Increased	2.946495372
<i>SV2A</i>	Increased	2.352182501
<i>MAP6</i>	Increased	12.92417244
<i>ADGRL3</i>	Increased	10.70342044
<i>PCYT1B</i>	Increased	2.200757219
<i>LRRTM2</i>	Increased	3.415270858
<i>ASCL1</i>	Increased	3.640155296
<i>ADGRB3</i>	Increased	2.040609318
<i>CLSTN2</i>	Increased	6.315951094
<i>SOX11</i>	Increased	2.380063393
<i>NLGN3</i>	Increased	2.136130816
<i>DAGLA</i>	Increased	2.319799309
<i>SLITRK5</i>	Increased	2.265767771
<i>CAPRN2</i>	Increased	2.926142441
<i>TENM4</i>	Increased	8.24774655
<i>LRRN3</i>	Increased	6.050271989
<i>FLRT1</i>	Increased	4.23393758
<i>CLSTN3</i>	Increased	2.233025924
<i>LRRTM1</i>	Increased	4.334903867
<i>CBLN2</i>	Increased	2.11696879
<i>NEUROD4</i>	Increased	4.401514437
<i>RIMS3</i>	Increased	2.713208655
<i>EPHB2</i>	Increased	2.72829567
<i>SEPT4</i>	Decreased	0.276241121
<i>ERBB2</i>	Decreased	0.438910899
<i>SULF1</i>	Decreased	0.458819941
<i>MUSK</i>	Decreased	0.368311921
<i>BMP7</i>	Decreased	0.414659773
<i>EEF2K</i>	Decreased	0.281654807
<i>PPP3CA</i>	Decreased	2.488023307
<i>RIT1</i>	Decreased	2.051956291
<i>DOCK10</i>	Decreased	0.403880389

<i>SMAD3</i>	Decreased	0.44844408
<i>TGFB1</i>	Decreased	0.279709275
<i>FGF2</i>	Decreased	0.281069731
<i>FGFR2</i>	Decreased	0.248445273
<i>SDC2</i>	Decreased	0.492433221
<i>COL25A1</i>	Decreased	0.5
<i>GDI1</i>	Decreased	2.09216988
<i>KIF23</i>	Decreased	0.146807746
<i>DCX</i>	Decreased	5.241573615
<i>SLC12A2</i>	Decreased	0.334713814
<i>SERPINF1</i>	Decreased	0.195467411
<i>SYNGAP1</i>	Decreased	2.522754818
<i>RTN4</i>	Decreased	2.057653416
<i>CBLN1</i>	Decreased	0.342695701
<i>GAS7</i>	Decreased	0.185436867
<i>RAPGEF2</i>	Decreased	3.655325801
<i>NTF3</i>	Decreased	0.321078952
<i>DCLK1</i>	Decreased	4
<i>RYR1</i>	Decreased	0.483973513
<i>CHRNA7</i>	Decreased	0.295452887
<i>GLI3</i>	Decreased	0.243163737
<i>KLF9</i>	Decreased	0.219912269
<i>NGF</i>	Decreased	0.220064753
<i>GEM</i>	Decreased	0.268501118
<i>L1CAM</i>	Decreased	5.105315075
<i>MYO5A</i>	Decreased	2.324628215
<i>IGF1</i>	Decreased	0.467163673
<i>DOCK1</i>	Decreased	0.464902471
<i>MAPK8IP1</i>	Decreased	2.67585511
<i>PARD3</i>	Decreased	0.431071773
<i>PLPPR5</i>	Decreased	0.336108749
<i>RAB17</i>	Decreased	0.16747295
<i>REM2</i>	Decreased	2.270484204
<i>ROBO2</i>	Decreased	2.406606052
<i>MDGA1</i>	Decreased	2.236123702
<i>BARHL2</i>	Affected	2.202283196
<i>LRP4</i>	Affected	0.400812665
<i>NYAP2</i>	Affected	9.553305497
<i>CELSR2</i>	Affected	4.597979392
<i>CPT1C</i>	Affected	2.236123702
<i>PCDHB9</i>	Affected	2.020902893
<i>RNF165</i>	Affected	3.073750363
<i>PRICKLE2</i>	Affected	0.267757706
<i>BRSK1</i>	Affected	2.043440165
<i>PCDHB14</i>	Affected	3.673104649
<i>ATL1</i>	Affected	2.37676621
<i>PDZRN3</i>	Affected	0.321747312
<i>GPM6A</i>	Affected	2.430073584
<i>LRRC7</i>	Affected	22.7059737
<i>RUFY3</i>	Affected	2.496661098

<i>SHTN1</i>	Affected	4.717426369
<i>NSMF</i>	Affected	4.319906239
<i>CCDC88A</i>	Affected	2.069095163
<i>BBS4</i>	Affected	0.395842933
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<i>SEZ6</i>	Affected	2.944453724
<i>TULP1</i>	Affected	0.385552706
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<i>CACNA1F</i>	Affected	0.406126198
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<i>ATCAY</i>	Affected	11.08855909
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<i>PLXND1</i>	Affected	2.284692494
<i>LRRC4C</i>	Affected	3.69609029
<i>CASP6</i>	Affected	0.480630464
<i>GABRA5</i>	Affected	0.252437824
<i>GFRA1</i>	Affected	2.615342697
<i>SPR</i>	Affected	0.272815969
<i>CACNA2D2</i>	Affected	8.299359493
<i>HSPB1</i>	Affected	0.407253782
<i>SYN1</i>	Affected	2.599078125
<i>LZTS1</i>	Affected	2.350552657
<i>MAP1B</i>	Affected	2.711328654
<i>NPTXR</i>	Affected	22.54913208
<i>SLC1A3</i>	Affected	0.257563488
<i>NCAN</i>	Affected	4.422922613
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<i>NRG1</i>	Affected	3.60500185
<i>PCDHB13</i>	Affected	4.456774603
<i>CXCR4</i>	Affected	0.35013908
<i>EPB41L3</i>	Affected	3.977880675
<i>DNER</i>	Affected	4.707626949
<i>NR4A2</i>	Affected	3.906834116
<i>CTNNA2</i>	Affected	2.327853069

<i>CAST</i>	Affected	0.41065535
<i>TP73</i>	Affected	5.045509635
<i>SPTBN4</i>	Affected	2.507066041
<i>NRP2</i>	Affected	5.392670927
<i>PLXNA3</i>	Affected	3.431882122
<i>ONECUT1</i>	Affected	4.352969767
<i>THRA</i>	Affected	3.928558381
<i>RAB3A</i>	Affected	2.353813474
<i>ERBB3</i>	Affected	0.416099367
<i>ARHGEF28</i>	Affected	2.170458744
<i>MYOD1</i>	Affected	0.172898829
<i>LOX</i>	Affected	0.35404386
<i>RDX</i>	Affected	0.498615626
<i>CADM1</i>	Affected	2.096524951
<i>C3</i>	Affected	0.190650207
<i>UNC5A</i>	Affected	3.647732662
<i>UGT8</i>	Affected	0.335875856
<i>FZD5</i>	Affected	0.456599125
<i>VIM</i>	Affected	0.355765865
<i>OPTN</i>	Affected	3.518596304
<i>WNT7B</i>	Affected	3.035638506
<i>VLDLR</i>	Affected	0.280097304
<i>DISC1</i>	Affected	0.278548413
<i>COL4A1</i>	Affected	0.240148716
<i>PLPPR4</i>	Affected	0.409802304
<i>DCC</i>	Affected	19.91798777
<i>NRP1</i>	Affected	4.525257851
<i>GABRG2</i>	Affected	5.172995728
<i>ARHGAP33</i>	Affected	3.786854525
<i>CLU</i>	Affected	0.393926943
<i>CACNA1S</i>	Affected	0.47204621
<i>RAB29</i>	Affected	0.206183387
<i>MAP2</i>	Affected	17.31559431
<i>TOP2B</i>	Affected	2.046274939
<i>PRDM1</i>	Affected	0.285982743
<i>CDC20</i>	Affected	0.178376813
<i>LIF</i>	Affected	0.414659773
<i>SRCIN1</i>	Affected	2.633533844
<i>SRGAP2</i>	Affected	2.106722072
<i>SIPA1L1</i>	Affected	2.049113646
<i>HERC1</i>	Affected	2.724516069
<i>PDLIM5</i>	Affected	0.454074209
<i>EFNB2</i>	Affected	8.456144324
<i>WEE1</i>	Affected	0.340800652
<i>SIX4</i>	Affected	0.346517471
<i>BMP2</i>	Affected	0.166200889
<i>CACNA1A</i>	Affected	14.89751712
<i>LYN</i>	Affected	0.185436867
<i>PITPNA</i>	Affected	0.47237352
<i>NR2F6</i>	Affected	0.469435874

<i>NR1D1</i>	Affected	2.30697121
<i>KIF20B</i>	Affected	0.33985706
<i>PAK3</i>	Affected	5.540437872
<i>FNBP1L</i>	Affected	2.648177821
<i>PCDHB8</i>	Affected	2.049113646
<i>PCDHB10</i>	Affected	4.23393758
<i>PCDHB11</i>	Affected	2.873880353
<i>EPHA3</i>	Affected	35.97692556
<i>LAMB2</i>	Affected	0.404160434
<i>LINGO1</i>	Affected	2.153972752

**Note: 85 of 252 differentially expressed genes have measurement direction consistent with an increase in neuronal development or differentiation, Z-score: 3.2

Supplementary Table 4: Axon guidance genes that are significantly and differentially expressed following OTX2 knockdown in D283 tumorspheres

Gene Assignment	Entrez Gene Name	Fold change
<i>ABLIM3</i>	actin binding LIM protein family, member 3	0.358488812
<i>ADAM11</i>	ADAM metallopeptidase domain 11	4.629960868
<i>ADAM23</i>	ADAM metallopeptidase domain 23	2.298989696
<i>ADAMTS7</i>	ADAM metallopeptidase with thrombospondin type 1 motif, 7	2.484576564
<i>ARPC1B</i>	actin related protein 2/3 complex, subunit 1B, 41kDa	0.467163673
<i>BMP1</i>	bone morphogenetic protein 1	2.38998241
<i>BMP2</i>	bone morphogenetic protein 2	0.166200889
<i>BMP7</i>	bone morphogenetic protein 7	0.414659773
<i>C9orf3</i>	chromosome 9 open reading frame 3	0.428390977
<i>CXCR4</i>	chemokine (C-X-C motif) receptor 4	0.35013908
<i>DCC</i>	DCC netrin 1 receptor	19.91798777
<i>DOCK1</i>	dedicator of cytokinesis 1	0.464902471
<i>DPYSL2</i>	dihydropyrimidinase-like 2	2.284692494
<i>DPYSL5</i>	dihydropyrimidinase-like 5	2.223758315
<i>EFNA3</i>	ephrin-A3	3.294364069
<i>EFNA4</i>	ephrin-A4	0.178624267
<i>EFNB2</i>	ephrin-B2	8.456144324
<i>EPHA2</i>	EPH receptor A2	0.18595172
<i>EPHA3</i>	EPH receptor A3	35.97692556
<i>EPHA4</i>	EPH receptor A4	2.713208655
<i>EPHA5</i>	EPH receptor A5	29.79503423
<i>EPHB2</i>	EPH receptor B2	2.72829567
<i>EPHB4</i>	EPH receptor B4	0.273763118
<i>ERBB2</i>	erb-b2 receptor tyrosine kinase 2	0.438910899
<i>FYN</i>	FYN proto-oncogene, Src family tyrosine kinase	3.280691645
<i>FZD1</i>	frizzled class receptor 1	0.411510173
<i>FZD5</i>	frizzled class receptor 5	0.456599125
<i>FZD7</i>	frizzled class receptor 7	0.08207007
<i>GLI3</i>	GLI family zinc finger 3	0.243163737
<i>GNAO1</i>	guanine nucleotide binding protein (G protein), alpha activating activity polypeptide O	5.747760706
<i>GNB3</i>	guanine nucleotide binding protein (G protein), beta polypeptide 3	0.257206677
<i>GNB4</i>	guanine nucleotide binding protein (G protein), beta polypeptide 4	0.294226684
<i>GNB5</i>	guanine nucleotide binding protein (G protein), beta 5	2.131693472
<i>GNG2</i>	guanine nucleotide binding protein (G protein), gamma 2	3.020945171
<i>GNG3</i>	guanine nucleotide binding protein (G protein), gamma 3	6.143241079
<i>GNG5</i>	guanine nucleotide binding protein (G protein), gamma 5	0.40584479
<i>GNG11</i>	guanine nucleotide binding protein (G protein), gamma 11	0.367801686
<i>GNG12</i>	guanine nucleotide binding protein (G protein), gamma 12	0.300200857
<i>IGF1</i>	insulin-like growth factor 1 (somatomedin C)	0.467163673
<i>ITGA4</i>	integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor)	2.25792881
<i>KALRN</i>	kalirin, RhoGEF kinase	4.806544198
<i>KLC1</i>	kinesin light chain 1	2.164449289

<i>L1CAM</i>	L1 cell adhesion molecule	5.105315075
<i>LINGO1</i>	leucine rich repeat and Ig domain containing 1	2.153972752
<i>LRRC4C</i>	leucine rich repeat containing 4C	3.69609029
<i>MICAL1</i>	microtubule associated monooxygenase, calponin and LIM domain containing 1	2.425025638
<i>MYL4</i>	myosin, light chain 4, alkali; atrial, embryonic	0.357000995
<i>NGF</i>	nerve growth factor (beta polypeptide)	0.220064753
<i>NRP1</i>	neuropilin 1	4.525257851
<i>NRP2</i>	neuropilin 2	5.392670927
<i>NTF3</i>	neurotrophin 3	0.321078952
<i>NTRK3</i>	neurotrophic tyrosine kinase, receptor, type 3	3.721798631
<i>PAK3</i>	p21 protein (Cdc42/Rac)-activated kinase 3	5.540437872
<i>PAK7</i>	p21 protein (Cdc42/Rac)-activated kinase 7	4.688089135
<i>PAPPA2</i>	pappalysin 2	0.147624083
<i>PLCB4</i>	phospholipase C, beta 4	9.428315262
<i>PLCD3</i>	phospholipase C, delta 3	0.392292049
<i>PLCD4</i>	phospholipase C, delta 4	3.267075964
<i>PLCE1</i>	phospholipase C, epsilon 1	0.341036959
<i>PLCG1</i>	phospholipase C, gamma 1	2.403272099
<i>PLCL1</i>	phospholipase C-like 1	4.500233939
<i>PLXNA2</i>	plexin A2	3.43664302
<i>PLXNA3</i>	plexin A3	3.431882122
<i>PLXND1</i>	plexin D1	2.284692494
<i>PPP3CA</i>	protein phosphatase 3, catalytic subunit, alpha isozyme	2.488023307
<i>PRKAR2B</i>	protein kinase, cAMP-dependent, regulatory, type II, beta	2.486299338
<i>PRKCE</i>	protein kinase C, epsilon	3.396384986
<i>PRKD3</i>	protein kinase D3	0.459456442
<i>PTCH2</i>	patched 2	3.164549205
<i>ROBO2</i>	roundabout guidance receptor 2	2.406606052
<i>RRAS</i>	related RAS viral (r-ras) oncogene homolog	0.323985241
<i>RTN4</i>	reticulon 4	2.057653416
<i>SDC2</i>	syndecan 2	0.492433221
<i>SEMA4D</i>	sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4D	3.45575275
<i>SEMA6A</i>	sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6A	5.544279543
<i>SEMA6C</i>	sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6C	2.8108374
<i>SHC1</i>	SHC (Src homology 2 domain containing) transforming protein 1	0.486327474
<i>SLIT1</i>	slit guidance ligand 1	13.04115732
<i>SLIT2</i>	slit guidance ligand 2	3.365917929
<i>SMO</i>	smoothened, frizzled class receptor	0.422786144
<i>SOS2</i>	son of sevenless homolog 2 (Drosophila)	2.033549347
<i>SRGAP2</i>	SLIT-ROBO Rho GTPase activating protein 2	2.106722072
<i>TUBB6</i>	tubulin, beta 6 class V	0.284204243
<i>TUBB2A</i>	tubulin, beta 2A class IIa	2.358713185
<i>TUBB2B</i>	tubulin, beta 2B class IIb	2.00416321
<i>TUBB4A</i>	tubulin, beta 4A class IVa	2.124318373
<i>UNC5A</i>	unc-5 netrin receptor A	3.647732662
<i>UNC5B</i>	unc-5 netrin receptor B	2.599078125
<i>UNC5D</i>	unc-5 netrin receptor D	8.094825608
<i>WNT7B</i>	wingless-type MMTV integration site family, member 7B	3.035638506

Supplementary Table 5: Axon guidance pathway genes that were significantly and differentially expressed following OTX2 KD and the number of OTX2 binding peaks /overlaps within -5kb-+2kb of their transcriptional start sites

Gene Assignment	Fold change	Overlaps/OTX2-binding peaks
<i>UNC5B</i>	2.599856508	5
<i>SLIT2</i>	3.365945926	4
<i>EFNB2</i>	8.458995374	3
<i>SEMA6A</i>	5.542465942	3
<i>PLXNA2</i>	3.436757362	3
<i>LRRC4C</i>	3.696844429	3
<i>KLC1</i>	2.163857258	3
<i>IGF1</i>	0.467094383	3
<i>SHC1</i>	0.486217368	3
<i>FZD5</i>	0.456667281	3
<i>GNG12</i>	0.30012554	3
<i>GNG3</i>	6.14369672	2
<i>NRP1</i>	4.525936467	2
<i>SEMA6C</i>	2.811277756	2
<i>FZD1</i>	0.411479558	2
<i>EPHA4</i>	2.713058833	2
<i>PLCE1</i>	0.340932018	2
<i>PLCD3</i>	0.392306551	2
<i>SRGAP2</i>	2.107118816	2
<i>NRP2</i>	5.392071649	2
<i>PLCL1</i>	4.499103846	2
<i>RRAS</i>	0.324050897	2
<i>GNG11</i>	0.367793953	2
<i>EPHA5</i>	29.7964111	1
<i>DCC</i>	19.9143847	1
<i>FZD7</i>	0.082085886	1
<i>EPHA3</i>	35.96546453	1
<i>UNC5D</i>	8.093918561	1
<i>PLCB4</i>	9.425849634	1
<i>SLIT1</i>	13.04052457	1
<i>GNB3</i>	0.257122066	1
<i>KALRN</i>	4.807760401	1
<i>EPHA2</i>	0.185952064	1
<i>BMP2</i>	0.166236374	1
<i>CXCR4</i>	0.350026972	1

GNB4	0.294242592	1
EFNA3	3.293833584	1
PPP3CA	2.488226239	1
NGF	0.220082042	1
PTCH2	3.165072031	1
UNC5A	3.648270412	1
MYL4	0.356958518	1
WNT7B	3.035941518	1
DPYSL5	2.223862104	1
TUBB6	0.284219872	1
EFNA4	0.178674171	1
PLCD4	3.266598176	1
SDC2	0.492339251	1
SOS2	2.033516927	1
BMP7	0.414541181	1
MICAL1	2.42543301	1
GNG5	0.405713159	1
PRKD3	0.45951462	1
GNB5	2.131834339	1
ERBB2	0.438769353	1
ARPC1B	0.46707323	1
L1CAM	5.106598619	0
PAPPA2	0.14758633	0
GNAO1	5.746351854	0
PLXNA3	3.432025645	0
PAK3	5.539728734	0
PAK7	4.687338552	0
SEMA4D	3.456588028	0
ADAM23	2.299033785	0
EPHB2	2.728741378	0
SMO	0.422761137	0
BMP1	2.389216627	0
ADAM11	4.631441635	0
DPYSL2	2.284545748	0
LINGO1	2.15434803	0
ADAMTS7	2.484673007	0
EPHB4	0.273709611	0
TUBB4A	2.125011035	0
RTN4	2.057498911	0
PLXND1	2.284524635	0
DOCK1	0.465017957	0

<i>PRKAR2B</i>	2.486517642	0
<i>TUBB2B</i>	2.004743045	0
<i>ABLIM3</i>	0.358447897	0
<i>GLI3</i>	0.243223242	0
<i>ROBO2</i>	2.405882747	0
<i>MIR23B</i>	0.428406121	0
<i>ITGA4</i>	2.258284631	0

Supplementary Table 6a: Univariable cox regression analysis of survival by increasing *SEMA4D* expression across Group 3 and 4 medulloblastoma

Group	HR	95% CI	p-value
Group 3 and 4 (n=377)	0.5987	0.4538-0.7898	0.000283
Group 3 (n=113)	0.8996	0.58-1.395	0.636
Group 4 (n=264)	0.4622	0.2644-0.808	0.00676

Supplementary Table 6b: Statistical analyses of survival in patients exhibiting high *SEMA6A*, *NRP1*, *NRP2*, or *L1CAM* gene expression relative to patients with low *SEMA6A*, *NRP1*, *NRP2* or *L1CAM* gene expression

Gene Assignment	Survival_Pvalue	Survival_Qvalue
<i>SEMA6A</i>	0.107	0.433
<i>NRP1</i>	0.935	0.973
<i>NRP2</i>	0.419	0.723
<i>L1CAM</i>	0.961	0.981

Supplementary Table 7: Gene Set Enrichment Analysis (GSEA) results for Reactome and KEGG databases identified pathways significantly enriched in gene sets that were downregulated in D283 Scramble relative to OTX2 KD tumorspheres

NAME (Reactome Database)	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val
SEMAPHORIN INTERACTIONS	63	-0.65	-1.96	0	0.0411	0.047
AXON GUIDANCE	237	-0.52	-1.88	0	0.0640	0.139
DEVELOPMENTAL BIOLOGY	377	-0.49	-1.84	0	0.0780	0.237
INTERACTION BETWEEN L1 AND ANKYRINS	21	-0.74	-1.84	0.0026	0.0591	0.240
SIGNALING BY ROBO RECEPTOR	28	-0.71	-1.81	0	0.0661	0.315
L1CAM INTERACTIONS	82	-0.52	-1.65	0.0030	0.3009	0.894
NETRIN1 SIGNALING	37	-0.58	-1.63	0.0078	0.3097	0.926
BOTULINUM NEUROTOXICITY	17	-0.67	-1.57	0.0300	0.4194	0.982
INSULIN SYNTHESIS AND PROCESSING	19	-0.65	-1.54	0.0389	0.4923	0.995
SEMA4D IN SEMAPHORIN SIGNALING	29	-0.59	-1.53	0.0354	0.4746	0.995
NAME (KEGG Database)	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val
AXON GUIDANCE	126	-0.66	-2.21	0	0	0
ERBB SIGNALING PATHWAY	86	-0.51	-1.57	0.0185	0.5618	0.641
VASOPRESSIN REGULATED WATER REABSORPTION	43	-0.53	-1.48	0.0256	0.7283	0.873
CELL ADHESION MOLECULES CAMS	125	-0.43	-1.45	0.0101	0.6949	0.926
MAPK SIGNALING PATHWAY	260	-0.40	-1.43	0.0046	0.6085	0.938
TYPE I DIABETES MELLITUS	36	-0.52	-1.41	0.0635	0.5669	0.956
LONG TERM DEPRESSION	66	-0.46	-1.40	0.0483	0.5385	0.969
LONG TERM POTENTIATION	70	-0.46	-1.38	0.0718	0.5132	0.979
TYPE II DIABETES MELLITUS	46	-0.48	-1.37	0.0507	0.5142	0.987
T CELL RECEPTOR SIGNALING PATHWAY	105	-0.42	-1.36	0.0401	0.4860	0.988

Supplementary Table 8: GSEA revealed that genes associated with SEMA4D signaling were enriched in gene sets that were downregulated in D283 Scramble vs. OTX2 KD tumorspheres

GENE SYMBOL	GENE_TITLE	RUNNING ES
ARHGEF12	Rho guanine nucleotide exchange factor (GEF) 12	-0.5662338
ROCK2	Rho-associated, coiled-coil containing protein kinase 2	-0.54986334
ROCK1	Rho-associated, coiled-coil containing protein kinase 1	-0.55364746
MYL6	myosin, light chain 6, alkali, smooth muscle and non-muscle	-0.54413474
MYL12B	null	-0.5143689
MYH10	myosin, heavy chain 10, non-muscle	-0.48504782
PLXNB1	plexin B1	-0.4504612
MYH9	myosin, heavy chain 9, non-muscle	-0.40517303
MYL9	myosin, light chain 9, regulatory	-0.3621211
ARHGEF11	Rho guanine nucleotide exchange factor (GEF) 11	-0.29258606
SEMA4D	sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4D	-0.19715172
RHOB	ras homolog gene family, member B	-0.098245345
ARHGAP35	null	0.011831163