SUPPLEMENTAL INFORMATION

SUPPLEMENTAL DATA

Table 1: Nuclear proteins with the same NLS sequence as nuc-ErbB3

Protein	Topology	Function
SFR2	Nucleus	Splicing factor, RNA binding
SFR5	Nucleus	Splicing factor, RNA binding
SFR6	Nucleus	Splicing factor, RNA binding
SFR7	Nucleus	Splicing factor, RNA binding, Zinc Finger
SFR8	Nucleus	Splicing factor, RNA binding, Transcription regulation
SFRB	Nucleus	Splicing factor, RNA binding
U2AG	Nucleus	Splicing factor, RNA binding, Zinc Finger
U2R2	Nucleus	Small ribonucleoprotein, RNA binding
RU17	Nucleus	Small ribonucleoprotein, RNA binding
SON	Nucleus	RNA binding, repressor
SRA4	Nucleus	Links transcription and pre-mRNA processing
TR2A	Nucleus	RNA binding, mRNA processing
Z265	Nucleus	Zinc finger transcription factor
GGC1	Nucleus	Unknown

Table 2: Schwann cell genes with promoters that interact with nuc-ErbB3

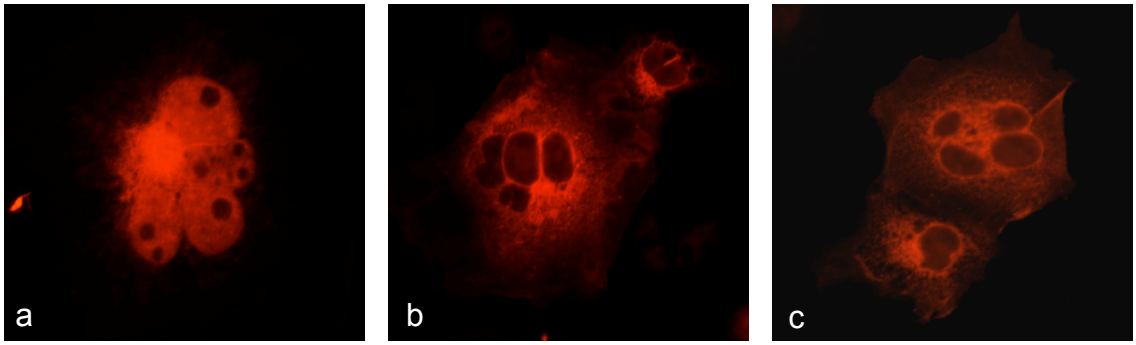
Accession number	Gene Name	Cluster
NM_012983	myosin ID (Myo1d)	IO,A
BC088108	protein kinase inhibitor, gamma	IO,N,IMO
NM_021579	nuclear RNA export factor 1 (Nxf1)	IO,N,IMO
XM_340861	dual specificity phosphatase 14	
XM_213679	CGG triplet repeat binding protein 1	
NM_031579	protein tyrosine phosphatase 4a1 (Ptp4a1)	IO,N,IMO
NM_017353	solute carrier family 7, member 5 (Slc7a5)	PH
NM_022962	latrophilin 1 (Lphn1)	PH
XM_214340	NIMA (never in mitosis gene a)-related expressed kinase 1 (Nek1)	IO,N,IMO
BC107657	adducin 1 (alpha)	IO,A,PH
NM_001017486	glioblastoma amplified sequence (Gbas)	
NM_173838	frizzled homolog 5 (Fzd5)	DEV,DIF
BC061858	polypyrimidine tract binding protein 1	IO,N,TR,PH,IMO
NM_001011925	nucleoporin 93kDa (Nup93)	IO,N,IMO
NM_001024248	alpha2u globulin	
XM_001071407	similar to Nuclear transport factor 2 (NTF-2)	
NM_001007711	general transcription factor IIF, polypeptide 1 (Gtf2f1)	IO,IMO

XM_243390	fizzy/cell division cycle 20 related 1 (Drosophila)	
XM_001057132	SKI-like, transcript variant 2 (Skil)	
XM_346305	similar to ribosomal protein S27a (predicted)	IO
NM_031770	guanine nucleotide binding protein (G protein), beta 5 (Gnb5)	
Y10370	GABA-BR1b receptor	DEV,DIF,PH
XM_341241	Max dimerization protein 4 (Mxd4)	IMO
NM_054004	cullin-associated and neddylation-dissociated 1 (Cand1)	IO,DEV,DIF,N,PRE,TR,IMO
NM_031143	diacylglycerol kinase zeta (Dgkz)	IO,N,PH,IMO
XM_213574	polymerase (RNA) II (DNA directed) polypeptide H (Polr2h)	IO,N,IMO
XM_578547	mitogen-activated protein kinase kinase kinase kinase 5 isoform 2	
BC060518	valosin-containing protein	IO,DEV,DIF,N,PH,IMO
XM_236210	poliovirus receptor-related 1 (Pvrl1)	
NM_001009268	ARP2 actin-related protein 2 homolog (yeast) (Actr2)	IO,A
BC060597	tubulin, beta 2c	IO,PH
XM_343119	jagged 2 (Jag2)	DEV,DIF
NM_031037	guanine nucleotide binding protein	
XM_341998	zinc and double PHD fingers family 2 (Dpf2_predicted)	DEV,DIF
NM_019357	ezrin (Ezr)	IO,DEV,DIF,A,PH
XM_001067052	tousled-like kinase 2 (Arabidopsis)(Tlk2_predicted)	
XM_341145	TIP41, TOR signalling pathway regulator-like (S. cerevisiae)	
XM_215466	general transcription factor II H, polypeptide 2 (predicted) (Gtf2h2)	IO,N,PRE,TR,PH,IMO
XM_214476	similar to E2f3 protein	
NM_212495	bromodomain containing 2 (Brd2)	IO,DEV,DIF,N,PH,IMO
NM_013079	asparagine synthetase (Asns)	
BC105826	protein-tyrosine sulfotransferase 1	IO,IMO
BC062225	protein-tyrosine sulfotransferase 1	IO,N,TR,IMO
NM_139113	nuclear receptor subfamily 2, group F, member 6 (Nr2f6)	IO,DEV,DIF,N,IMO
NM_080778	nuclear receptor subfamily 2, group F, member 2 (Nr2f2)	IO,DEV,DIF,N,TR,PH,IMO
BC099822	v-raf murine sarcoma 3611 viral oncogene homolog	IO,PH,IMO
XM_225512	WD repeat domain 37 (Wdr37)	
NM_017348	solute carrier family 6 member 8 (Slc6a8)	
XM_001075055	High mobility group protein 1 (HMG-1)	
NM_053996	solute carrier family 6 member 7 (Slc6a7)	PH
XM_001054666	Mediator of RNA polymerase II transcription subunit 12	
XM_213569	eukaryotic translation initiation factor 4 gamma, 1 (Eif4g1)	
NM_001024793	cell division cycle 27 homolog (cdc27)	IO,N,IMO
XM_215371	ubiquitin-conjugating enzyme E2G 2	IO,IMO
NM_001007005	Rho GDP dissociation inhibitor (GDI) alpha (Arhgdia)	IO,DEV,DIF

XM_236687	oxidative-stress responsive 1	IO,DEV,DIF,N,TR,PH,IMO
NM_134353	poly(A) binding protein, cytoplasmic 1 (Pabpc1)	IO,N,PH,IMO
NM_175843	sequestosome 1 (Sqstm1	
NM_001008281.	proteasome 26S subunit, non-ATPase, 3 (Psmd3)	
BC098831	FUS interacting protein (serine-arginine rich) 1	IO,N,IMO
XM_213421	vascular endothelial zinc finger 1 (Vezf1_predicted)	IO,DEV,DIF,N,TR,IMO
XM_574173	solute carrier family 25, member 5	IO,IMO
XM_343776	plastin 3 (T-isoform) (Pls3)	IO,A,PH

IO: Intracellular organelle, N: Nucleus, DIF: Cell differentiation
DEV: Cellular development, PH: Phosphoprotein, A: Actin cytoskeleton
PRE: Transcriptional preinitiation complex assembly,
TR: Regulation of transcription from RNA polymerase II promoter,
IMO: Intracellular membrane bound organelle

Supplementary Figure 1: Cos-7 cells were transfected with STREP-ErbB4-FL construct, which expresses the full-length ErbB4 receptor. After addition of 10nM beta1-Heregulin for 30min, ErbB4 was localized in the nucleus of Cos-7 cells as detected with an ErbB4 specific antibody (Figure S1a). Addition of gamma-secretase inhibitor or MMP inhibitor during the treatment with heregulin completely blocked the cleavage and subsequent nuclear localization of ErbB4 (Figures S1b & 1c respectively) showing that the protease inhibitors were functionally active.

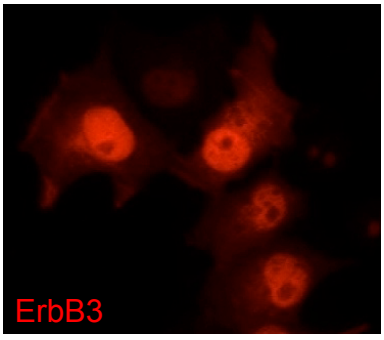


Her + ErbB4 | Her + ErbB4 + gamma secretase inhibitor | Her + ErbB4 + MMP inhibitor

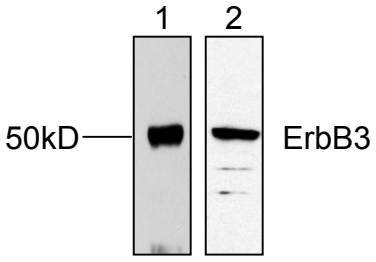
Supplementary figure 2: Cos-7 cells were transfected with the human cDNA clone FLJ43038 (NBRC, Japan), which expresses the human ErbB3 variant AK1205028. 72 hours post-transfection the cells were stained with a c-terminal specific ErbB3 antibody (Santa Cruz) that showed nuclear localization of AK125028 (Figure S2A). Protein lysates from AK125028 transfected Cos-7 cells were blotted with the ErbB3 antibody and compared to Schwann cell nuclear extracts blotted with the same ErbB3 antibody. This showed that the nuclear variant AK125028 runs at 50kD as the endogenous nuc-ErbB3 of rat primary Schwann cells (Figure S2B).

A

Cos-7 + AK125028



B



1: Cos-7 lysates
2: Schwann cell nuclear extracts

Supplementary Figure 3: To examine the role of nuc-ErbB3 during the interaction of Schwann cells with axons we designed two siRNAs that selectively block the expression of the full-length ErbB3 RTK (full length ErbB3 siRNA) without affecting the expression of nuc-ErbB3 or block the expression of both the full-length ErbB3 RTK and the nuc-ErbB3 (nuc-ErbB3 siRNA). We transfected these siRNAs into rat primary Schwann cells using nucleofection and examined the knockdown efficiency by Western blotting. The siRNA targeting the extracellular domain sequence of ErbB3 RTK, downregulated the expression of the full-length receptor by an average of ~50% in comparison to the expression in control (Figure S3A). Similarly, the nuc-ErbB3 siRNA, which is complementary to a sequence in the cytoplasmic domain of ErbB3 showed an average of ~50% downregulation in the expression of full-length ErbB3 as compared to the control (Figure S3A). On the contrary, full length ErbB3 siRNA showed no effect on nuc-ErbB3 expression (Figure S3A nuclear extracts) while nuc-ErbB3 siRNA downregulated the expression of nuc-ErbB3 to an average 80-90% as compared to control expression (Figure S3A nuclear extracts). These experiments show that we can use the combination of these two siRNAs to discriminate between the function of nuc-ErbB3 and the full length ErbB3 since only the nuc-ErbB3 siRNA downregulates the expression of nuc-ErbB3 (by an average of 80-90%) while both siRNAs produce the same effect on the full-length ErbB3 receptor (50% inhibition of expression). In order to reveal the putative function of nuc-ErbB3 on myelination we prepared co-cultures of purified DRG neurons and

siRNA transfected Schwann cells. Treatment of the siRNA transfected Schwann cell-neuron co-cultures with ascorbic acid to initiate myelination resulted in a significant reduction of the amount of myelinated segments in the nuc-ErbB3 siRNA transfected cultures as compared to full-length ErbB3-siRNA or control cultures (Figures S3 B & C).