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Supplementary Materials for

The nociceptin receptor inhibits axonal regeneration and recovery from spinal cord injury

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This PDF file includes:

Figure S1

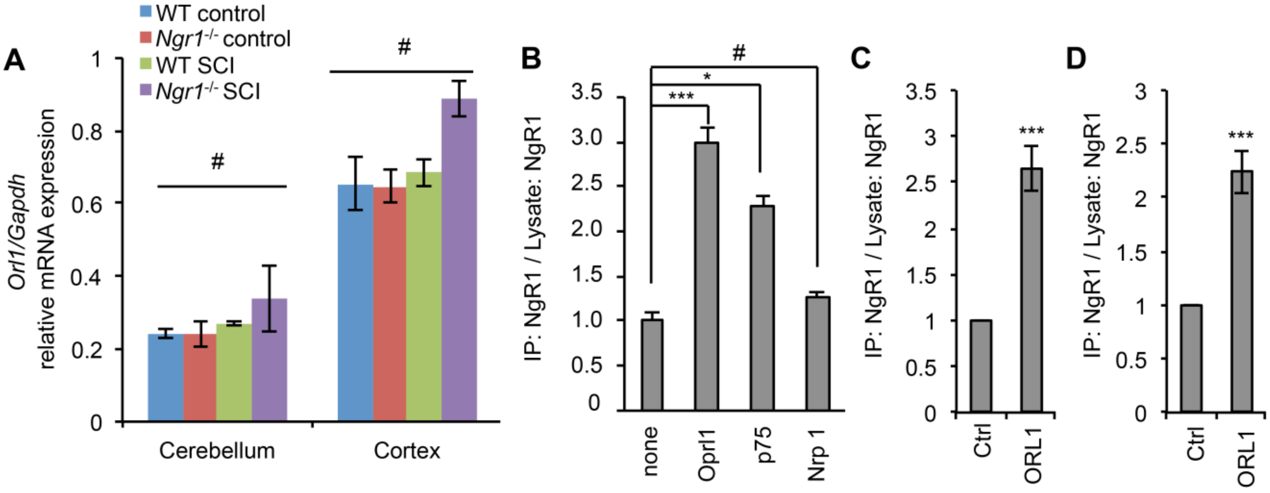


Figure S1. NgR1 interacts with ORL1.

(A) The levels of the *ORL1* mRNA normalized to those of a *GAPDH* internal control in cerebellum and cortex. Tissues were from sham or SCI after 4d in WT or *NgR1*$^{-/-}$ mice (N=3 mice for each group). Mean±SE. #, no significant differences, one-way ANOVA followed by Tukey's test.

(B) Quantification of FLAG-NgR1 protein levels in the immunoprecipitates normalized to total cell lysate in Fig1B from three independent experiments. Mean±SE. *$p<0.05$, ***$p<0.005$, #, not significant, one-way ANOVA followed by Dunnett's test.

(C, D) Quantification of NgR1 protein levels in the immunoprecipitate normalized to total lysate in Fig. 1C and 1D from three independent experiments. Mean±SE. ***$p<0.005$, Student’s two-tailed *t* test.

Figure S2

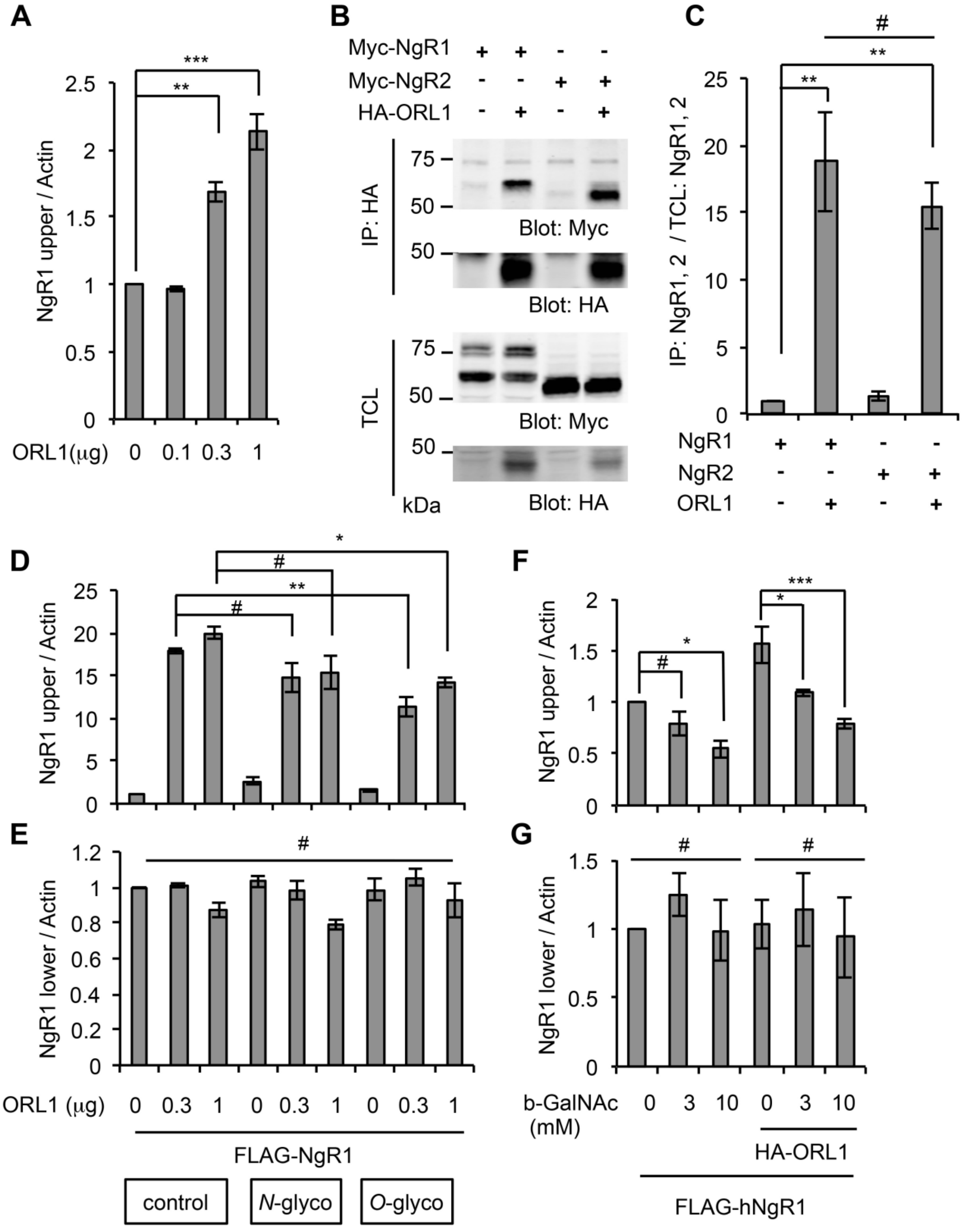


Figure S2. ORL1 interacts with NgR1 and NgR2.

(A) Quantification of NgR1 upper bands normalized to Actin in Fig. 2A from 3 independent experiments. Mean±SE. **p<0.01, ***p<0.005, one-way ANOVA followed by Dunnett's test.

(B) HEK293T cells were transfected with Myc-NgR1or Myc-NgR2 with or without HA-ORL1. After 36h, cells were lysed. HA immunoprecipitates were immunblotted for Myc and HA. N=3 independent experiments.

(C) Quantification of Myc-NgR1 or Myc-NgR 2 protein levels in the immunoprecipitates normalized to total cell lysate (TCL) from three independent experiments. Mean±SE. **p<0.01, #, no significant difference, one-way ANOVA followed by Tukey's test.

(D, E) Quantification of NgR1 upper (D) or lower (E) bands normalized to Actin from three independent experiments in Fig. 2B. Mean±SE.. *p<0.05, **p<0.001, #, no significant differences, one-way ANOVA followed by Tukey's test.

(F, G) Quantification of NgR1 upper (F) or lower (G) bands normalized to Actin from four independent experiments in Fig. 2C. Mean±SE. *p<0.05, ***p<0.005, #, no significant differences, one-way ANOVA followed by Tukey's test.

Figure S3

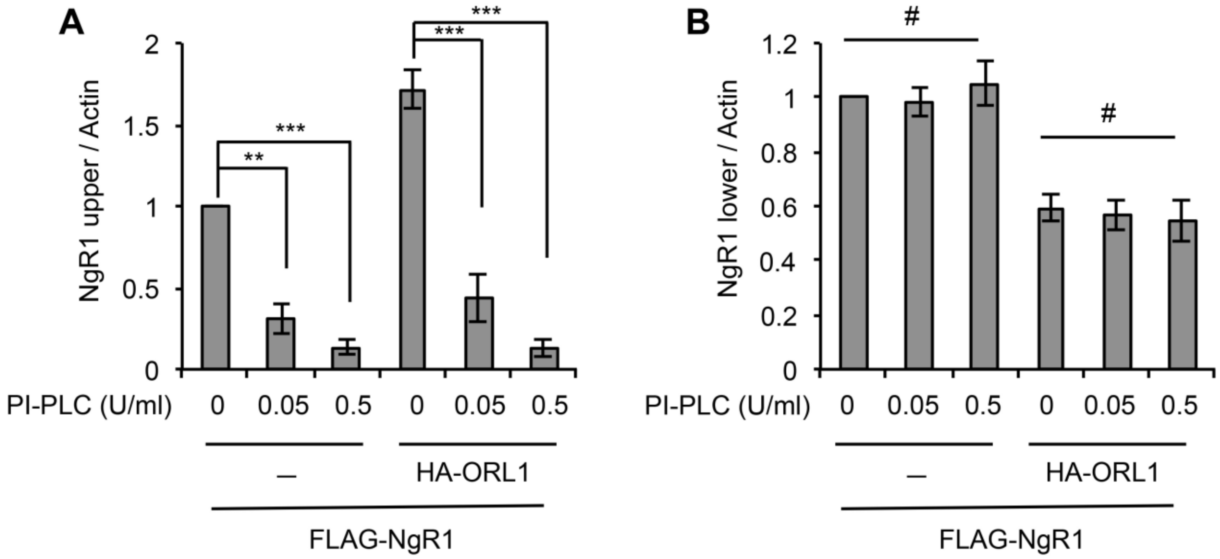


Figure S3. Quantification of NgR1 protein in PI-PLC–treated HEK293T cells.
(A, B) Quantification of NgR1 upper (A) or lower (B) bands normalized to Actin in Fig. 3A from three independent experiments. Mean±SE. **p<0.01, ***p<0.005, #, no significant differences, one-way ANOVA followed by Tukey's test.

Figure S4

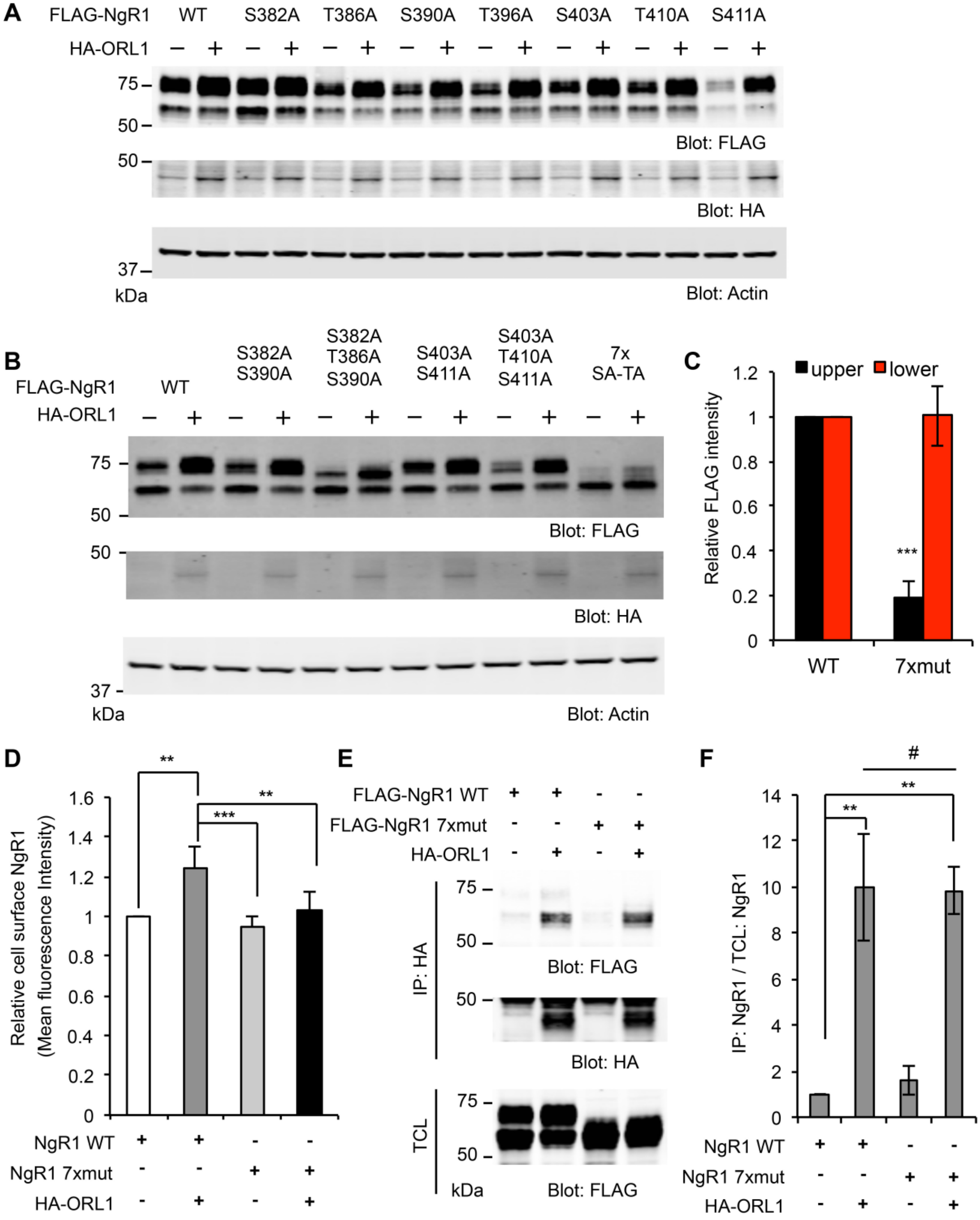


Figure S4. Identification of O-glycosylation sites in NgR1.

(A, B) HEK293T cells were transfected with FLAG-NgR1 WT, a series of FLAG-NgR1 mutants or HA-ORL1. After 36h, cells were immunoblotted for FLAG or HA. N=3 independent experiments.

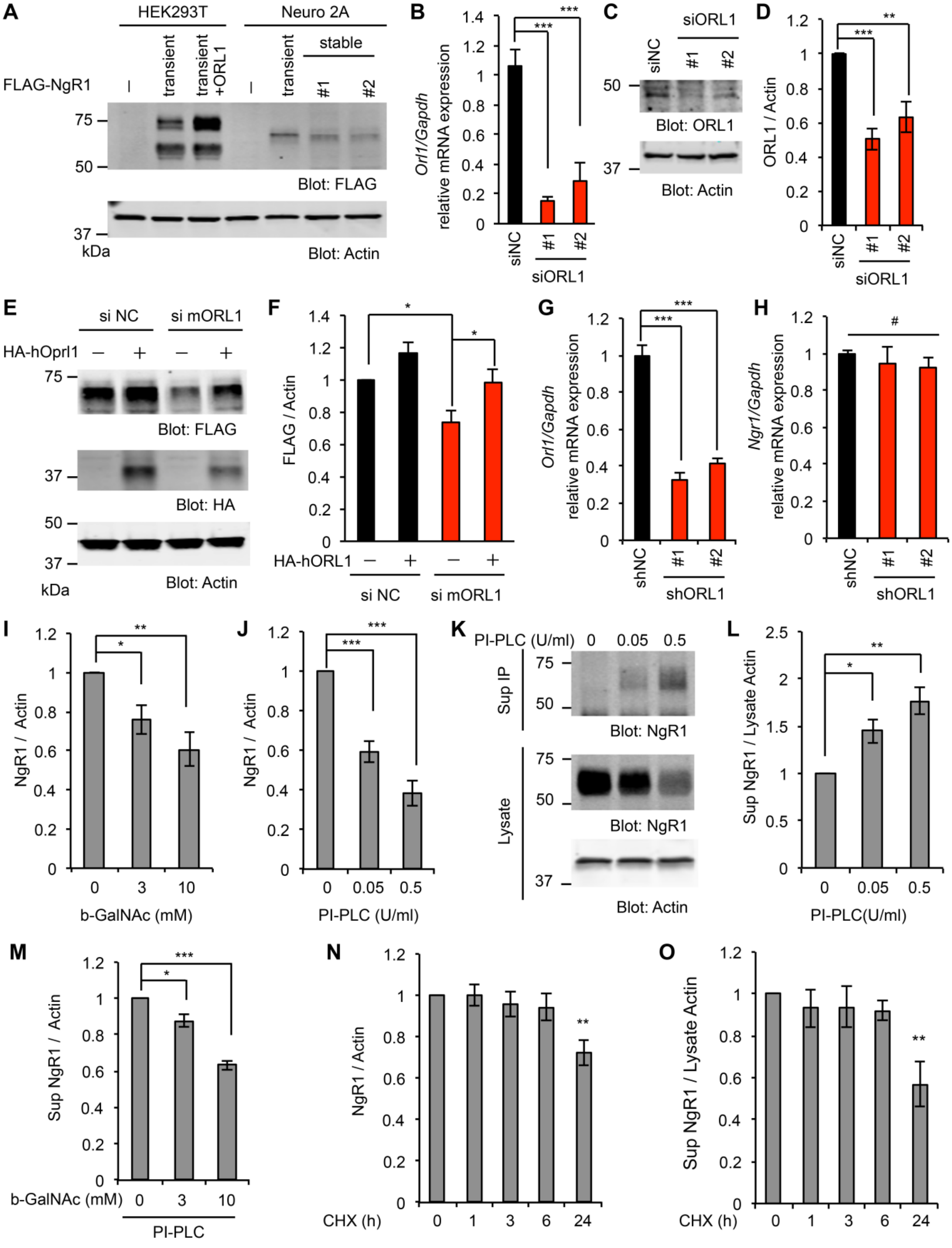
(C) Quantification of NgR1 upper or lower bands normalized to Actin in FigS4A WT and 7x SA-TA mutant. Mean±SE. N=4 ***$p<0.005$, Student's two-tailed *t* test.

(D) HEK293T cells were transfected with FLAG-NgR1 WT, a series of FLAG-NgR1 mutants and/or HA-ORL1. After 36h, cells were stained for FLAG and analyzed by flow cytometry. The data are mean fluorescence intensity from 3 independent experiments ±SE. ***$p<0.005$, **$p<0.01$, one-way ANOVA followed by Tukey's test.

(E) HEK293T cells were transfected with FLAG-NgR1 WT, FLAG-NgR1 7xmut and/or HA-ORL1. HA immunoprecipitates were blotted for FLAG and HA. N=3 independent experiments.

(F) Quantification of FLAG-NgR1 protein levels in the immunoprecipitates normalized to total cell lysate from 3 independent experiments. Mean±SE. **$p<0.01$, #, no significant difference, one-way ANOVA followed by Tukey's test.

Figure S5


A
HEK293T
Neuro 2A
FLAG-NgR1
–
transient
transient +ORL1
–
transient
stable
#1
#2
75
50
37
kDa
Blot: FLAG
Blot: Actin
B
Orl1/Gapdh relative mRNA expression
1.4
1.2
1
0.8
0.6
0.4
0.2
0
***
***
siNC
#1
#2
siORL1
C
siORL1
siNC
#1
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50
37
Blot: ORL1
Blot: Actin
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ORL1 / Actin
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0.2
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siORL1
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si NC
si mORL1
HA-hOprl1
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+
75
Blot: FLAG
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Blot: HA
50
37
kDa
Blot: Actin
F
FLAG / Actin
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1.2
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0.6
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HA-hORL1
–
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si NC
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G
Orl1/Gapdh relative mRNA expression
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0.8
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0.4
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shNC
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H
Ngr1/Gapdh relative mRNA expression
1.2
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0.8
0.6
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#
shNC
#1
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I
NgR1 / Actin
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0.8
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0.2
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*
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3
10
b-GalNAc (mM)
J
NgR1 / Actin
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1
0.8
0.6
0.4
0.2
0
***
***
0
0.05
0.5
PI-PLC (U/ml)
K
PI-PLC (U/ml)
0
0.05
0.5
Sup IP
75
50
Blot: NgR1
Lysate
75
50
Blot: NgR1
37
Blot: Actin
L
Sup NgR1 / Lysate Actin
2.5
2
1.5
1
0.5
0
**
*
0
0.05
0.5
PI-PLC(U/ml)
M
Sup NgR1 / Actin
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0.4
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*
b-GalNAc (mM)
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PI-PLC
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NgR1 / Actin
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0.8
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0.2
0
**
CHX (h)
0
1
3
6
24
O
Sup NgR1 / Lysate Actin
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0.8
0.6
0.4
0.2
0
**
CHX (h)
0
1
3
6
24

Figure S5. Reduction of ORL1 decreases NgR1 expression in Neuro2A and neurons.
(A) Cell lysate were collected from following cells: not transfected, transfected with FLAG-NgR1, or co-transfected with FLAG-NgR1 and HA-ORL1 in HEK293T cells; not transfected, transiently transfected with FLAG-NgR1, stably transfected FLAG-NgR1 clone #1 or clone #2 in Neuro2A cells. Lysates were immunoblotted for FLAG and Actin. N=3 independent experiments.

(B) The levels of the *ORL1* mRNA normalized to those of a *Gapdh* internal control in siRNA transfected Neuro2A cells. Mean ±SE. N=3 independent experiments. ***p<0.005, one-way ANOVA followed by Tukey's test.

(C, D) Neuro2A cells stably expressing FLAG-NgR1 were transfected with control, ORL1#1 or #2 siRNA. Cell lysates were immunoblotted for ORL1 and Actin (C). Quantification of ORL1 protein levels in the lysates normalized to Actin from 3 independent experiments (D). Mean ±SE. **p<0.01, ***p<0.005, one-way ANOVA followed by Tukey's test.

(E, F) Neuro2A cells stably expressing FLAG-NgR1 were transfected with control or ORL1 siRNA, and with or without HA-ORL1 plasmid. After 36h, cells were immunoblotted for FLAG and Actin (E). Quantification of FLAG-NgR1 protein levels in the lysates normalized to Actin from three independent experiments (F). Mean ±SE. *p<0.05, one-way ANOVA followed by Tukey's test. N=6 independent experiments.

(G, H) The levels of *ORL1* (G) and *Ngr1* (H) mRNA normalized to those of a GAPDH internal control in neurons transduced with lentiviral vector for control, ORL1 shRNA#1 or #2 at 3 div for 7d. Mean ±SE. ***p<0.005, #, no significant differences, one-way ANOVA followed by Tukey's test**.** N=3 independent experiments.

(I, J) Quantification of NgR1 normalized to Actin from 3 independent experiments in Fig. 4I, 4J. Mean ±SE. *p<0.05, **p<0.01, ***p<0.005, one-way ANOVA followed by Tukey's test.

(K, L) Culture medium of cortical neurons at 10 div was replaced with PI-PLC containing medium for 1 h. NgR1 immunoprecipitates from culture medium and cell lysates were immunoblotted for NgR1 and Actin (K). Quantification of NgR1 in medium normalized to Actin from 3 independent experiments in (L). Mean±SE. *p<0.05, **p<0.01, one-way ANOVA followed by Tukey's test.

(M-O) Quantification of NgR1 normalized to Actin from three independent experiments in Fig4K-4M. Mean±SE. *p<0.05, **p<0.01, ***p<0.005, one-way ANOVA followed by Tukey's test.

Figure S6

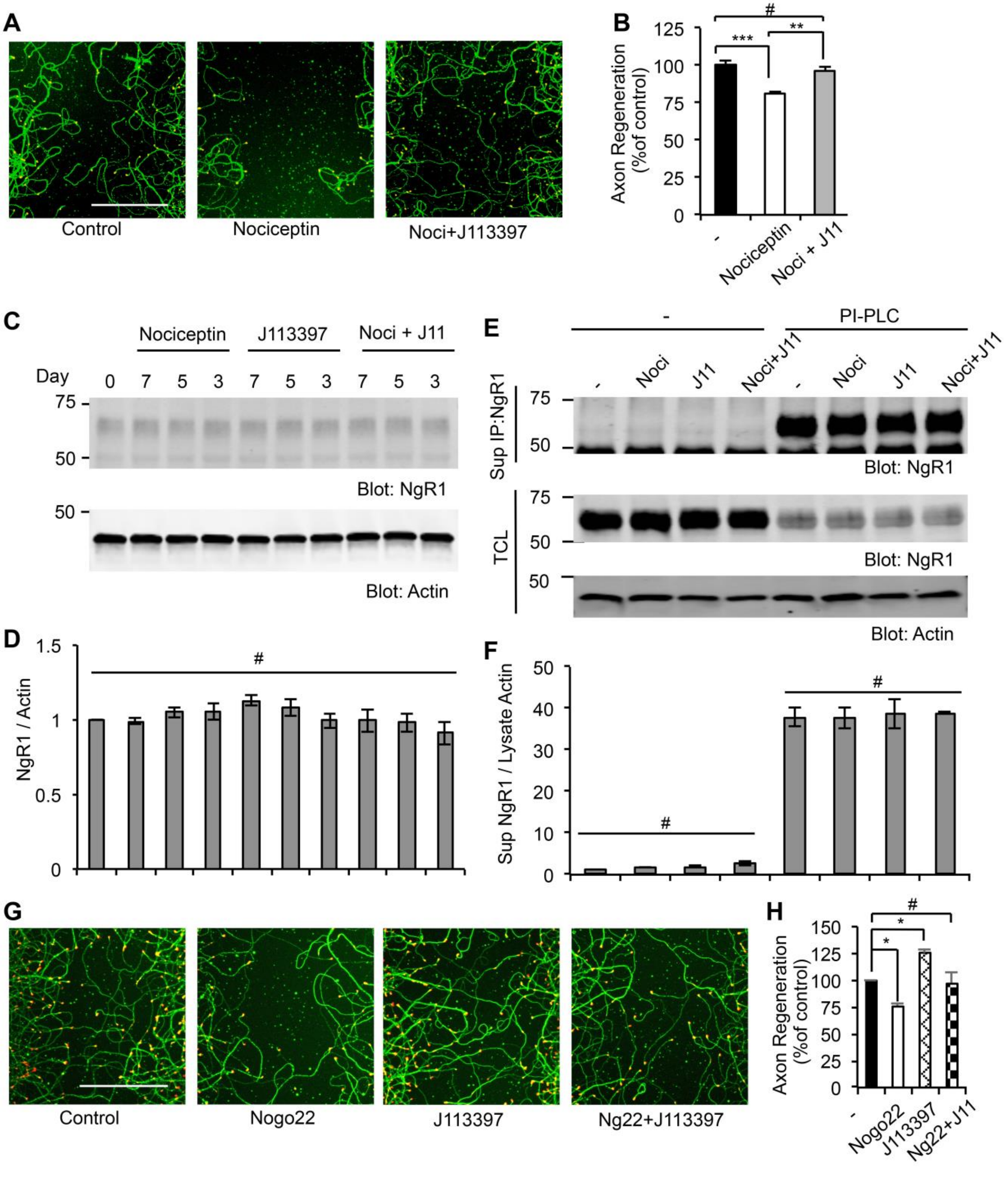

Figure S6. Effect of J113397 on nociceptin- and Nogo-mediated inhibition of regeneration. (A, B) Cortical neurons were scraped and treated with vehicle, nociceptin (300 nM) or nociceptin (300 nM) + J113397 (10 nM) at 8 div for 3 days. The microphotographs show ßIII tubulin of axon (green) and phalloidin of F-actin (red) to illustrate the growth cones of cortical neurons in the middle of scraped area (A). The graph shows quantification of axon regeneration. Mean±SE. N=5 cultures for each group, **p<0.01, ***p<0.005, #, no significant difference, one-way ANOVA followed by Tukey's test (B).

(C, D) Lysates from div21 cortical neurons treated with nociceptin (300 nM), J113397 (10 nM), or nociceptin (300 nM) + J113397 (10 nM) for indicated period were immunoblotted for NgR1 and Actin. N=3 independent experiments. Quantification of NgR1 normalized to Actin from 3 independent experiments. Mean±SE. #, no significant differences, one-way ANOVA followed by Tukey's test.

(E, F) Div21 cortical neurons treated with nociceptin (300 nM), J113397 (10 nM), or nociceptin (300 nM) + J113397 (10 nM) for 3d were washed with culture medium and then treated with PI-PLC (1 U/ml) for 1 h. PI-PLC treated neuron culture medium was collected and immunoprecipitated with anti-NgR1 antibody and blotted for NgR1. Neurons were also harvested and lysed, then immunoblotted for NgR1 and Actin. Quantification of NgR1 in culture medium normalized to Actin from 3 independent experiments. Mean±SE. #, no significant differences, one-way ANOVA followed by Tukey's test.

(G, H) Cortical neurons were scraped and treated with vehicle, Nogo22 (100 nM), nociceptin (300 nM) or Nogo22 (100 nM) + nociceptin (300 nM) at 8 div for 3 days. The microphotographs show ßIII tubulin of axon (green) and phalloidin of F-actin (red) to illustrate the growth cones of cortical neurons in the middle of scraped area (G). The graph shows quantification of axon regeneration. Mean±SE. N=3 cultures for each group, *p<0.05, #, no significant difference, one-way ANOVA followed by Tukey's test (H).

Figure S7

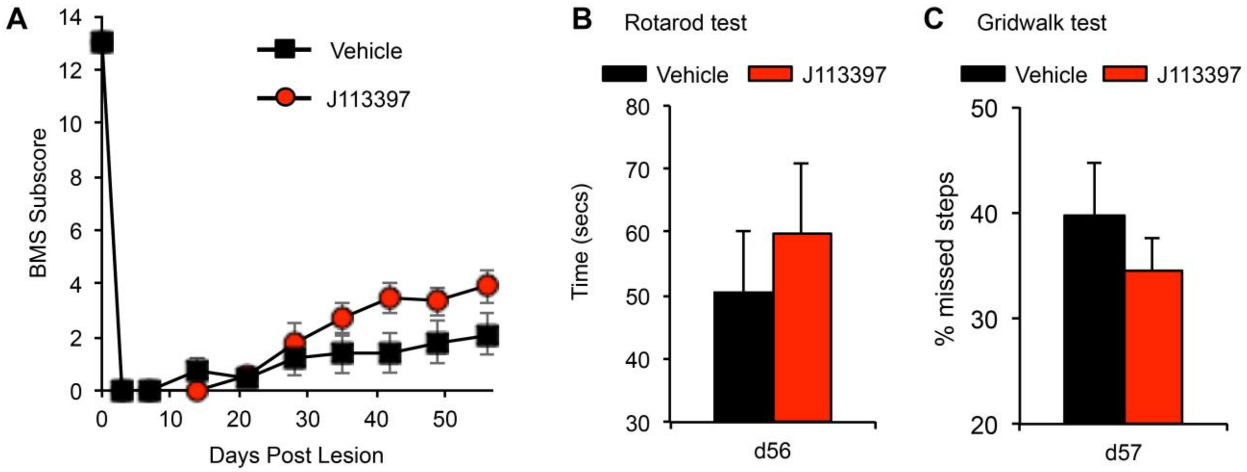

Figure S7. Motor performance after SCI and J113397 treatment.

(A to C) Open-field locomotion performance as assessed by the BMS subscore (A) of vehicle and J113397 injected mice. Mean±SE. N=10 vehicle-injected mice and n=9 J113397-injected mice. RotaRod performance (B) and gridwalk test (C) of vehicle and J113397 treated animals on the indicated day after injury.

Figure S8

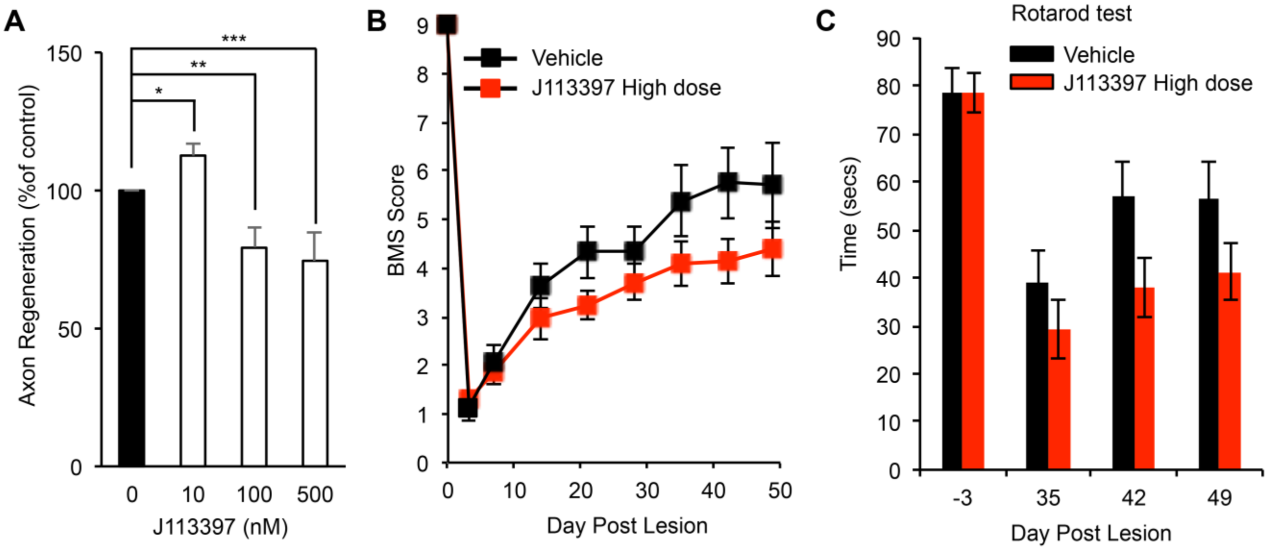

Figure S8. Effect of high-dose J113397.
(A) Cortical neurons were scraped and treated with indicated amount of J113397 starting at 8 div for 3 days. The graph reports the extent of axon regeneration. Mean±SE. N=4 biological replicates, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.005$, one-way ANOVA followed by Dunnett's test.
(B) Open-field locomotion performance is assessed by the BMS for WT mice after T7 dorsal over-hemisection and treatment with vehicle or high dose J113397 (2 mg/kg/day). Mean±SE. N=12 mice in each group. No significant difference between groups by repeated measures one-way ANOVA.
(C) Rotarod test at the indicated days after T7 dorsal over-hemisection for the same group as in B. Mean±SE. N=12 mice in each group. No significant differences between treatment groups, Student’s two-tailed *t* test at each time point.

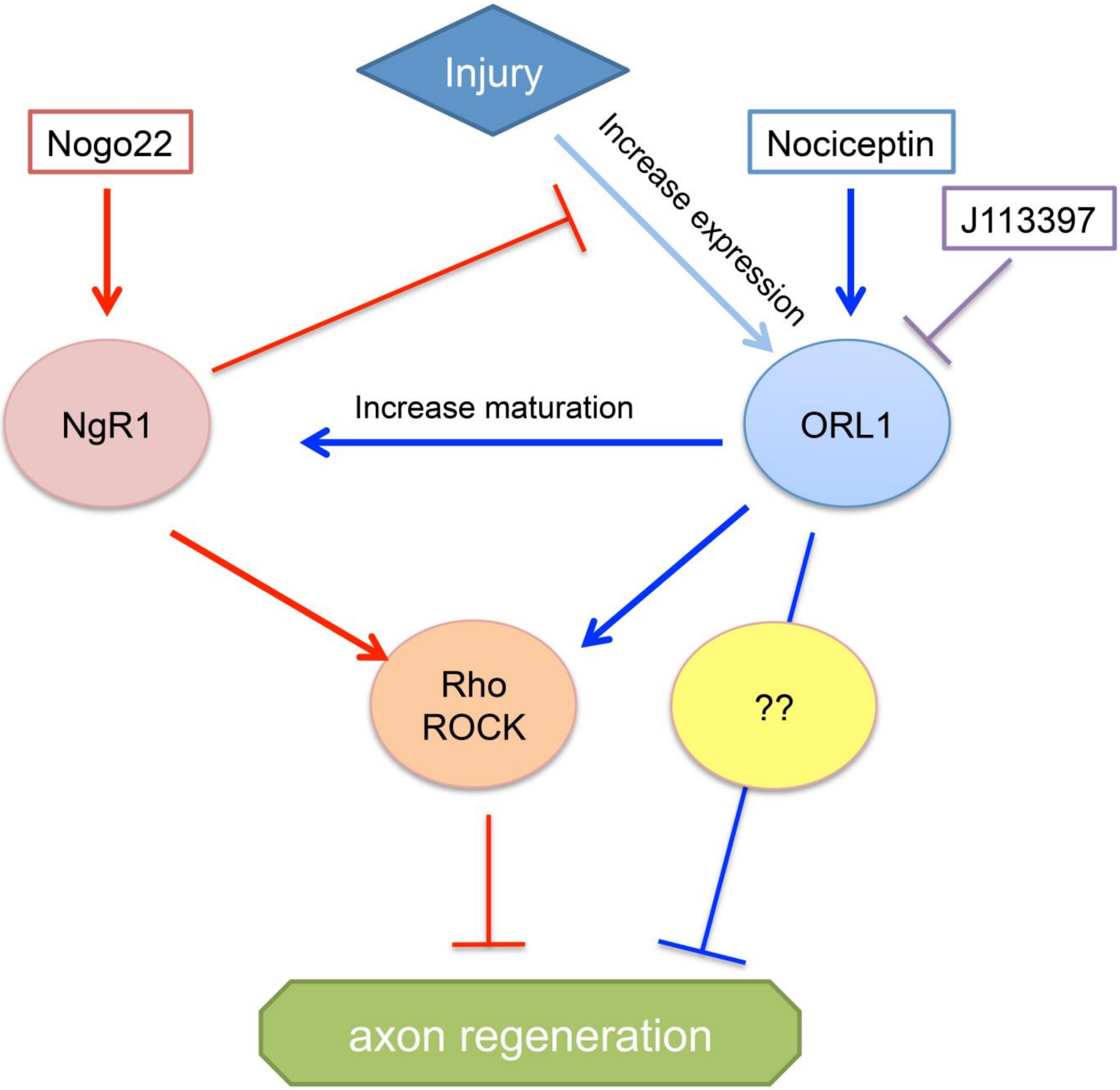

Figure S9. Model for nociception/ORL1-mediated regulation of neural repair in relation to NgR1. After spinal cord injury *ORL1* mRNA is increased in the background of *Ngr1* deficiency. NgR1 maturation is increased by ORL1 through O-linked glycosylation. ORL1 enhances O-linked glycosylation of NgR1 that results in increased NgR1 surface expression. Nogo/NgR1 and Nociceptin/ORL1 signaling through Rho/ROCk pathway suppress axonal regeneration. The ORL1 antagonist J113397 inhibits Nociceptin/ORL1-mediated suppression of axon regeneration.

Table S1. Gene expression in brainstem after SCI in *Ngr1*$^{–/–}$ and *Ngr1*$^{+/–}$ mice.
Brain stem tissue was collected 4 days after mid-thoracic spinal dorsal hemisection injury of *Ngr1*$^{-/-}$ and *Ngr1*$^{+/-}$ adult mice. Mean gene expression level was determined by hybridization of biotinylated cRNA prepared from Trizol-extracted total RNA to Affymetrix genechips for 2 mice of each genotype. Mean expression values for each genotype were filtered for those values greater than 200. Differences between genotypes were filtered for those values greater than 2-fold and p<0.05 by Student's two-tailed *t* test between genotypes without correction for multiple gene tests. The mean expression level for 87 genes with increased expression and 97 genes with decreased expressionmeeting these criteria are listed. The full data set is available at the Mendeley website (https://data.mendeley.com, doi:10.17632/d5cz46zv8z.1).

Genes with increased expression in *Ngr1*$^{-/-}$ mice

Affy ID	Description	*Ngr1*$^{-/-}$	*Ngr1*$^{+/-}$	Fold change
168328_i_at	opioid receptor-like	5726	706	8.11
164205_at	RIKEN cDNA 1110014F12 gene	1329	208	6.38
104949_at	RIKEN cDNA E230026N22 gene	1978	409	4.83
109353_at	RIKEN cDNA 2310043K02 gene	1601	332	4.82
163272_at	tripartite motif protein 12	996	212	4.70
166509_r_at	neoplastic progression 3	17895	4397	4.07
109933_at	RIKEN cDNA 1110028C15 gene	1021	252	4.05
113334_at	C lectin-related protein A	893	222	4.02
112658_at	heparanase	1047	263	3.98
133062_r_at		1052	270	3.90
108871_at	RIKEN cDNA 6330549H03 gene	1290	346	3.73
116181_at	RIKEN cDNA 5730512J02 gene	1407	401	3.51
117227_at	Mus musculus transcribed sequences	1737	502	3.46
167688_f_at	malonyl-CoA decarboxylase	2249	666	3.38
168350_f_at	RIKEN cDNA 5930403L14 gene	838	249	3.37
107767_at	peroxiredoxin 1	870	262	3.32
166185_at	Mus musculus transcribed sequence with moderate similarity to protein ref:NP_080533.1 (M.musculus) RIKEN cDNA 4930506L07 [Mus musculus]	1284	388	3.31
135786_at	Mus musculus transcribed sequences	862	261	3.30
115304_at	Mus musculus transcribed sequences	1152	357	3.23
115205_at	Mus musculus 0 day neonate thymus cDNA, RIKEN full-length enriched library, clone:A430093F15 product:hypothetical protein, full insert sequence.	840	260	3.23
171517_at		3090	979	3.16
107869_at	DNA segment, Chr 4, ERATO Doi 681, expressed	3508	1156	3.03
105145_at	RIKEN cDNA A530058N18 gene	688	227	3.03

112384_at	RIKEN cDNA 9830169G11 gene	2097	703	2.98
95896_at	cytochrome c oxidase, subunit VIIc	701	239	2.94
108884_at	Mus musculus, Similar to RIKEN cDNA 1700067C01 gene, clone MGC:31405 IMAGE:4456622, mRNA, complete cds	660	226	2.92
163222_at	RIKEN cDNA 1300019C06 gene	618	215	2.88
136558_at	RIKEN cDNA 1810026B05 gene	4030	1403	2.87
128811_at	SNF related kinase	667	237	2.82
102021_at	interleukin 4 receptor, alpha	782	277	2.82
95184_f_at		1607	576	2.79
164760_f_at	RIKEN cDNA 2510003D18 gene	791	284	2.78
168053_f_at	RIKEN cDNA 3110052F15 gene	4072	1465	2.78
107291_at	zinc finger protein 131	1022	372	2.75
170233_at	HLA-B associated transcript 4	1749	639	2.74
167230_f_at	annexin A4	2050	762	2.69
163269_at	SMC (structural maintenance of chromosomes 1)-like 1 (S. cerevisiae)	1577	593	2.66
116177_at	regulator of G-protein signaling 12	1607	608	2.64
99664_at	pericentrin 2	607	230	2.64
102069_at	metal response element binding transcription factor 2	2871	1089	2.64
162786_r_at	secretory carrier membrane protein 4	1047	397	2.63
166483_at	Mus musculus transcribed sequence with strong similarity to protein ref:NP_071338.1 (H.sapiens) potassium channel, subfamily K, member 12; tandem pore domain potassium channel THIK-2 [Homo sapiens]	7174	2737	2.62
170608_at	hypothetical protein MGC27683	1609	618	2.60
114517_at	glutamate receptor interacting protein 1	1150	447	2.57
111466_at	Mus musculus transcribed sequence with moderate similarity to protein pir:T17319 (H.sapiens) T17319 hypothetical protein DKFZp434N178.1 - human (fragment)	596	232	2.57
111119_at	salvador homolog 1 (Drosophila)	736	290	2.54
95395_at	RIKEN cDNA 9130022A11 gene	828	328	2.52
113788_at	cysteine-rich motor neuron 1	2439	967	2.52
116114_at	DNA segment, Chr 11, ERATO Doi 498, expressed	530	211	2.51
134226_r_at	RIKEN cDNA 2900024O10 gene	636	257	2.47
168457_i_at	natriuretic peptide precursor type C	1446	587	2.46
166930_at	CTF18, chromosome transmission fidelity factor 18 homolog (S. cerevisiae)	22705	9359	2.43
160613_at	lipocalin 7	927	384	2.41
133717_at		2360	980	2.41

100980_at	Rho-associated coiled-coil forming kinase 1	1024	425	2.41
115231_at	Casitas B-lineage lymphoma-like 1	960	405	2.37
129165_at	Mus musculus 18-day embryo whole body cDNA, RIKEN full-length enriched library, clone:1110066J06 product:unclassifiable, full insert sequence.	882	372	2.37
139034_at	RIKEN cDNA 1110033E03 gene	7363	3134	2.35
106142_at	adenylate cyclase 5	507	226	2.24
100412_g_at	AE binding protein 1	1225	546	2.24
134067_r_at	inhibitor of growth family, member 3	1283	574	2.23
116357_at		2129	964	2.21
102307_at	doublecortin	1091	494	2.21
168311_f_at	eukaryotic translation initiation factor 2B, subunit 4 delta	40480	18536	2.18
166258_at	RIKEN cDNA 2900084M21 gene	7900	3627	2.18
94192_at	ganglioside-induced differentiation-associated-protein 10	1118	515	2.17
104327_at	RIKEN cDNA 9030612M13 gene	3398	1569	2.17
109351_r_at	T-cell activation kelch repeat protein	533	247	2.16
167449_f_at	DnaJ (Hsp40) homolog, subfamily B, member 10	17097	7961	2.15
138511_at	Mus musculus adult male diencephalon cDNA, RIKEN full-length enriched library, clone:9330161H13 product:TRANSCRIPTION FACTOR XLMO1 homolog [Xenopus laevis], full insert sequence.	1861	867	2.15
102132_i_at	alkaline phosphatase 5	919	428	2.15
130718_at	tissue inhibitor of metalloproteinase 4	53790	25440	2.11
100751_at	a disintegrin and metalloprotease domain 10	1615	766	2.11
133864_r_at	oxysterol binding protein-like 6	909	434	2.10
93853_at	RIKEN cDNA 2010306G19 gene	1855	885	2.10
109344_at	cDNA sequence BC031748	3167	1523	2.08
116629_at	RIKEN cDNA 4832412D13 gene	3754	1814	2.07
137180_at	RIKEN cDNA 1500009C09 gene	15861	7719	2.05
100706_f_at	Scm-related gene containing four mbt domain 2	472	230	2.05
166469_f_at	sepiapterin reductase	37714	18385	2.05
105657_at	Mus musculus adult male hypothalamus cDNA, RIKEN full-length enriched library, clone:A230095J21 product:unknown EST, full insert sequence.	1720	844	2.04
99874_at	RAP2B, member of RAS oncogene family	490	241	2.03
114481_at	alkylglycerone phosphate synthase	1842	906	2.03
167421_at	ectonucleotide pyrophosphatase/phosphodiesterase 5	24101	11866	2.03
104125_at	Hoxa1 regulated gene	2059	1016	2.03
105102_at	RIKEN cDNA 2410085M17 gene	1441	712	2.02

161457_at	cell division cycle 2 homolog (S. pombe)-like 2	404	202	2.00

Genes with decreased expression in *Ngr1*$^{-/-}$ mice

Affy ID	Description	Rtn4r-/-	Rtn4r+/-	Fold change
163780_at	RIKEN cDNA 2610511E22 gene	241	1848	0.13
132030_at	Mus musculus transcribed sequence with weak similarity to protein sp:O00268 (H.sapiens) T2D3_HUMAN Transcription initiation factor TFIID 135 kDa subunit (TAFII-135) (TAFII135) (TAFII-130) (TAFII130)	377	2564	0.15
130656_at	Mus musculus transcribed sequences	257	1441	0.18
115791_at	RIKEN cDNA 4932702F08 gene	824	4479	0.18
101741_at		3424	18481	0.19
166167_f_at	serine/threonine protein kinase SSTK	205	1091	0.19
170471_i_at	ferritin, heavy polypeptide-like 17	216	1113	0.19
138551_at	Mus musculus transcribed sequences	449	2088	0.22
129004_at	Mus musculus transcribed sequences	250	1126	0.22
133032_at	Mus musculus transcribed sequences	558	2436	0.23
171281_at	COP9 (constitutive photomorphogenic) homolog, subunit 7a (Arabidopsis thaliana)	234	999	0.23
137544_at	Mus musculus transcribed sequences	232	973	0.24
130564_at	Mus musculus transcribed sequences	232	953	0.24
171246_at	Mus musculus adult male testis cDNA, RIKEN full-length enriched library, clone:4930405O22 product:unknown EST, full insert sequence.	626	2521	0.25
115389_at	RIKEN cDNA 2310012I10 gene	498	1992	0.25
167341_r_at	aspartate-beta-hydroxylase	218	869	0.25
114867_at	RIKEN cDNA 3632431M01 gene	386	1489	0.26
160868_at	RAB3B, member RAS oncogene family	457	1743	0.26
138115_at	Mus musculus transcribed sequences	202	770	0.26
135289_at	Mus musculus transcribed sequence	586	2212	0.26
169155_f_at	RIKEN cDNA 4930403L05 gene	312	1166	0.27
171445_i_at	proliferin related protein	561	2056	0.27
114793_at	RIKEN cDNA B830029I03 gene	1213	4342	0.28
103651_r_at	Mus musculus RIKEN cDNA 1110031C13 gene, mRNA (cDNA clone MGC:18965 IMAGE:3986466), complete cds	651	2220	0.29
112397_at	Mus musculus nuclear cap binding protein subunit 1, 80kDa, mRNA (cDNA clone IMAGE:6402922)	660	2232	0.30
164183_at	RIKEN cDNA 2610318O14 gene	346	1170	0.30

117333_at	RIKEN cDNA 2810417J12 gene	650	2169	0.30
134146_at		265	880	0.30
169547_at	methyltransferase-like 3	363	1172	0.31
130168_at	Mus musculus transcribed sequence	276	876	0.32
111615_at	inhibitor of kappaB kinase beta	908	2882	0.32
107119_at	FAD-synthetase	1589	4998	0.32
170055_at	replication protein A1	344	1079	0.32
134552_f_at	RIKEN cDNA 1700101O05 gene	277	857	0.32
169292_r_at	Mus musculus, clone IMAGE:4505796, mRNA	381	1173	0.33
169926_at	oxidase assembly 1-like	441	1349	0.33
161648_at	complement receptor related protein	333	1017	0.33
171359_at	RIKEN cDNA 2610209L14 gene	1164	3511	0.33
129340_at		226	680	0.33
133213_at	Mus musculus transcribed sequences	1148	3448	0.33
109062_f_at	PET112-like (yeast)	702	2082	0.34
168055_at	Mus musculus, clone IMAGE:6742657, mRNA	295	857	0.34
113811_at	calcitonin-related polypeptide, beta	375	1076	0.35
134139_r_at	Mus musculus mRNA similar to hypothetical protein FLJ20265 (cDNA clone IMAGE:5010465)	230	655	0.35
165483_at	apoptosis regulator BCL-G	730	2045	0.36
104959_at	Mus musculus zinc finger protein 422, mRNA (cDNA clone MGC:57971 IMAGE:5709349), complete cds	384	1049	0.37
130137_at		445	1205	0.37
169506_r_at	RIKEN cDNA 1110019N10 gene	1334	3609	0.37
116702_at	Mus musculus 12 days embryo spinal ganglion cDNA, RIKEN full-length enriched library, clone:D130092A12 product:unknown EST, full insert sequence.	417	1120	0.37
134285_at	RIKEN cDNA 5930430L01 gene	217	582	0.37
92666_at	SH3-domain binding protein 1	1430	3804	0.38
170867_r_at		211	562	0.38
137106_at	Mus musculus transcribed sequence with moderate similarity to protein pir:S12207 (M.musculus) S12207 hypothetical protein (B2 element) - mouse	451	1193	0.38
169470_at		328	866	0.38
134714_at	Mus musculus transcribed sequences	260	686	0.38
167140_at	RIKEN cDNA 1810035K13 gene	840	2209	0.38
137195_i_at	RIKEN cDNA 4921507I02 gene	453	1188	0.38
129150_at	Mus musculus transcribed sequences	494	1290	0.38

140642_at	Mus musculus transcribed sequences	833	2109	0.39
135737_r_at		240	605	0.40
116393_at	Mus musculus transcribed sequences	757	1861	0.41
130439_at	hypothetical protein MGC49274	1028	2521	0.41
137736_at		847	2072	0.41
164572_s_at	RIKEN cDNA 1190001I08 gene	3448	8394	0.41
111590_at	RIKEN cDNA 1500004O14 gene	768	1867	0.41
102267_at	UDP-Gal:betaGalNAc beta 1,3-galactosyltransferase, polypeptide 4	256	621	0.41
136253_at	Mus musculus transcribed sequences	227	550	0.41
134199_at	Mus musculus 3 days neonate thymus cDNA, RIKEN full-length enriched library, clone:A630031F24 product:unknown EST, full insert sequence.	235	562	0.42
166969_at	Mus musculus transcribed sequences	259	612	0.42
170630_at	apoptotic chromatin condensation inducer in the nucleus	433	1021	0.42
167691_at	Mus musculus transcribed sequence with weak similarity to protein ref:NP_002411.1 (H.sapiens) melastatin 1 [Homo sapiens]	321	748	0.43
117208_at	RIKEN cDNA 1110003O08 gene	5242	12201	0.43
113705_at	DNA segment, Chr 4, ERATO Doi 22, expressed	1271	2889	0.44
113231_at	RIKEN cDNA 1700123O20 gene	1935	4396	0.44
160836_at	sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4D	1053	2389	0.44
130311_at	RIKEN cDNA E130314N14 gene	1026	2310	0.44
169093_i_at	Mus musculus transcribed sequence	756	1693	0.45
163662_at	mitogen-activated protein kinase kinase kinase 7 interacting protein 1	1091	2425	0.45
165738_r_at	RIKEN cDNA 2010007L18 gene	345	766	0.45
137722_at	RIKEN cDNA A930015B13 gene	439	967	0.45
170768_at	enhancer of rudimentary homolog (Drosophila)	580	1273	0.46
170924_f_at	proteasome (prosome, macropain) inhibitor subunit 1	1155	2498	0.46
163015_at	amnionless	1707	3690	0.46
109982_at	fos-like antigen 2	818	1753	0.47
138062_at	neurexin I	676	1447	0.47
116066_at	RIKEN cDNA 4631402G10 gene	864	1833	0.47
132889_at	Mus musculus transcribed sequences	680	1432	0.48
137650_at	Mus musculus transcribed sequence with weak similarity to protein ref:NP_081764.1 (M.musculus) RIKEN cDNA 5730493B19 [Mus musculus]	852	1775	0.48
113596_at	RIKEN cDNA 4931406I20 gene	6104	12710	0.48
167603_at	RIKEN cDNA 1700113B09 gene	403	830	0.49

170533_at	Mus musculus transcribed sequence with weak similarity to protein ref:NP_067680.1 (H.sapiens) KIAA0721 protein [Homo sapiens]	939	1934	0.49
162622_at	RIKEN cDNA 3110004H13 gene	2378	4854	0.49
109330_at	RIKEN cDNA 1810055G02 gene	1243	2530	0.49
166023_f_at	RIKEN cDNA 1700110I01 gene	677	1365	0.50
114274_at	RIKEN cDNA 1810043H04 gene	5653	11273	0.50
113997_at	secreted protein of unknown function	7909	15679	0.50