

Fig. S1. Immunostaining of Sox11/Sox4 overexpressed retinal sections by antibodies anti-M-opsin and CARR4. (A) Frozen sectioned adult retina was immunostained with indicated antibodies. Nucleus was stained with DAPI and expressed in gray. (B) Mouse retina at E17 was transfected with Sox11-EGFP, cultured as an explant for 2 weeks and then frozen sectioned. Immunostaining using anti-EGFP, RXR γ , CARR4 and M-opsin was carried out. Nucleus was stained with DAPI and expressed in blue. CARR4 and M-opsin antibodies recognized cells in the border between INL and ONL, confirming that these cells are cone. However, although RXR γ merged with EGFP-positive cells, CARR4 and M-opsin antibodies did not merge with the cells. These results suggest that the cells are of the cone lineage but did not achieve final maturation.

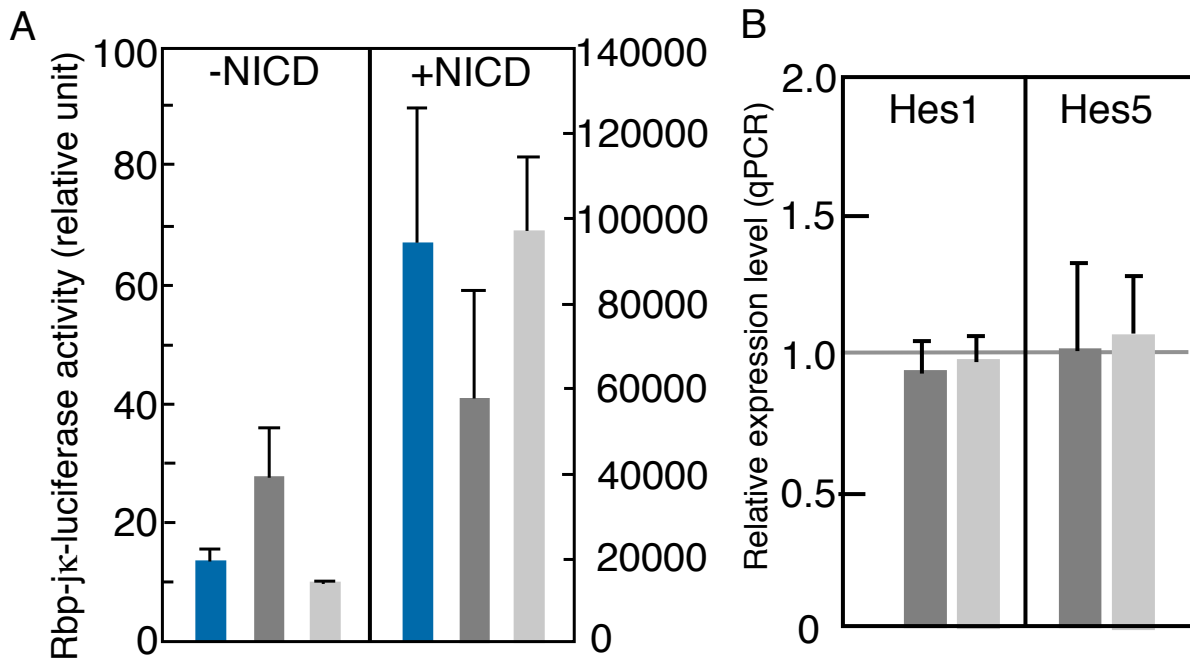


Fig. S2. Effects of Sox11 and Sox4 for Notch signaling. (A) Effects of Sox11 and Sox4 on Notch signaling were examined by luciferase analysis. Y79 cells were transfected with Rbp-jk-luciferase plasmid with pCAG-Sox11, pCAG-Sox4 or control pCAG, and pEF-NICD or control pEF plasmids. After 48 hours, luciferase activities were measured. (B) Retrovirus encoding Sox11-, Sox4-IRES-EGFP or control EGFP was transduced into retinal explants at E16 prepared from ICR mice. On the fourth day, the expression of Hes1 and Hes5 was examined by qPCR. Expression levels relative to values of control plasmid transfected samples are shown.

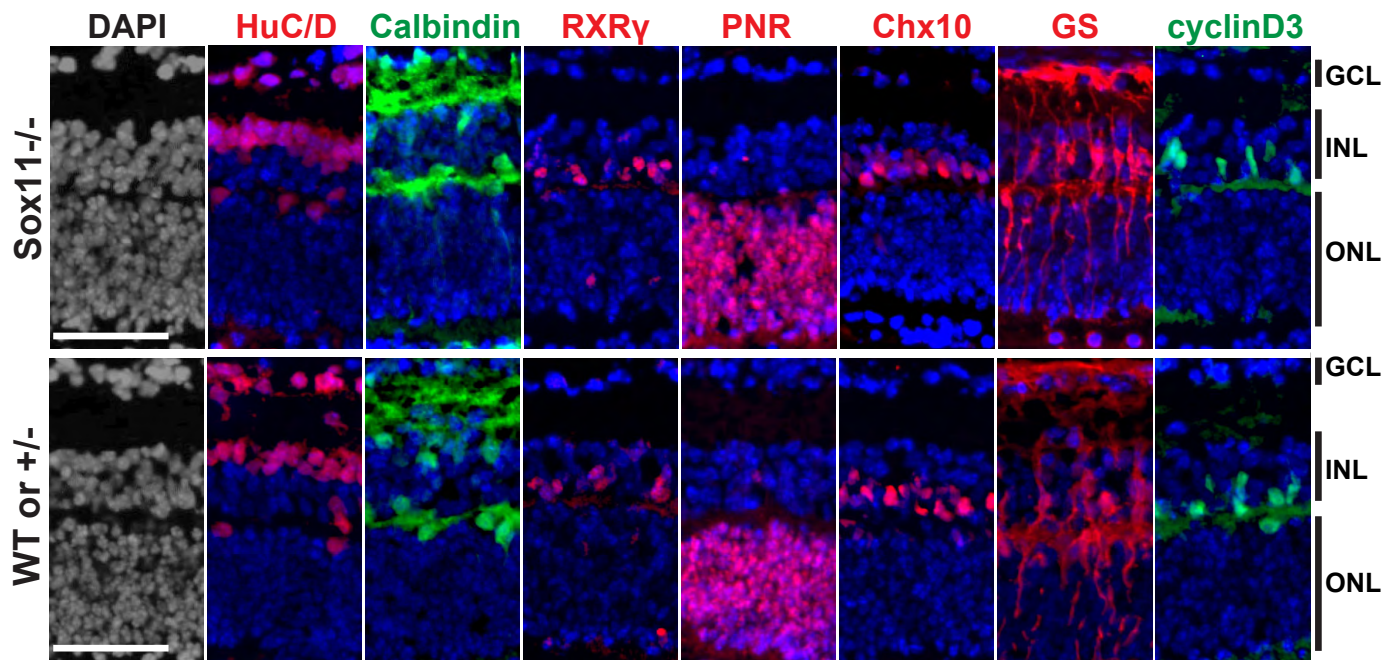


Fig. S3. The late phase of retinal differentiation was correct in Sox11-KO retinas. Retinas of Sox11-KO or control mice were isolated at E18 and cultured as explant cultures for 2 weeks. Retinas were harvested and frozen sectioned. The indicated markers were immunostained. Nuclei were visualized with DAPI. GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer. Scale bars: 50 μ m.

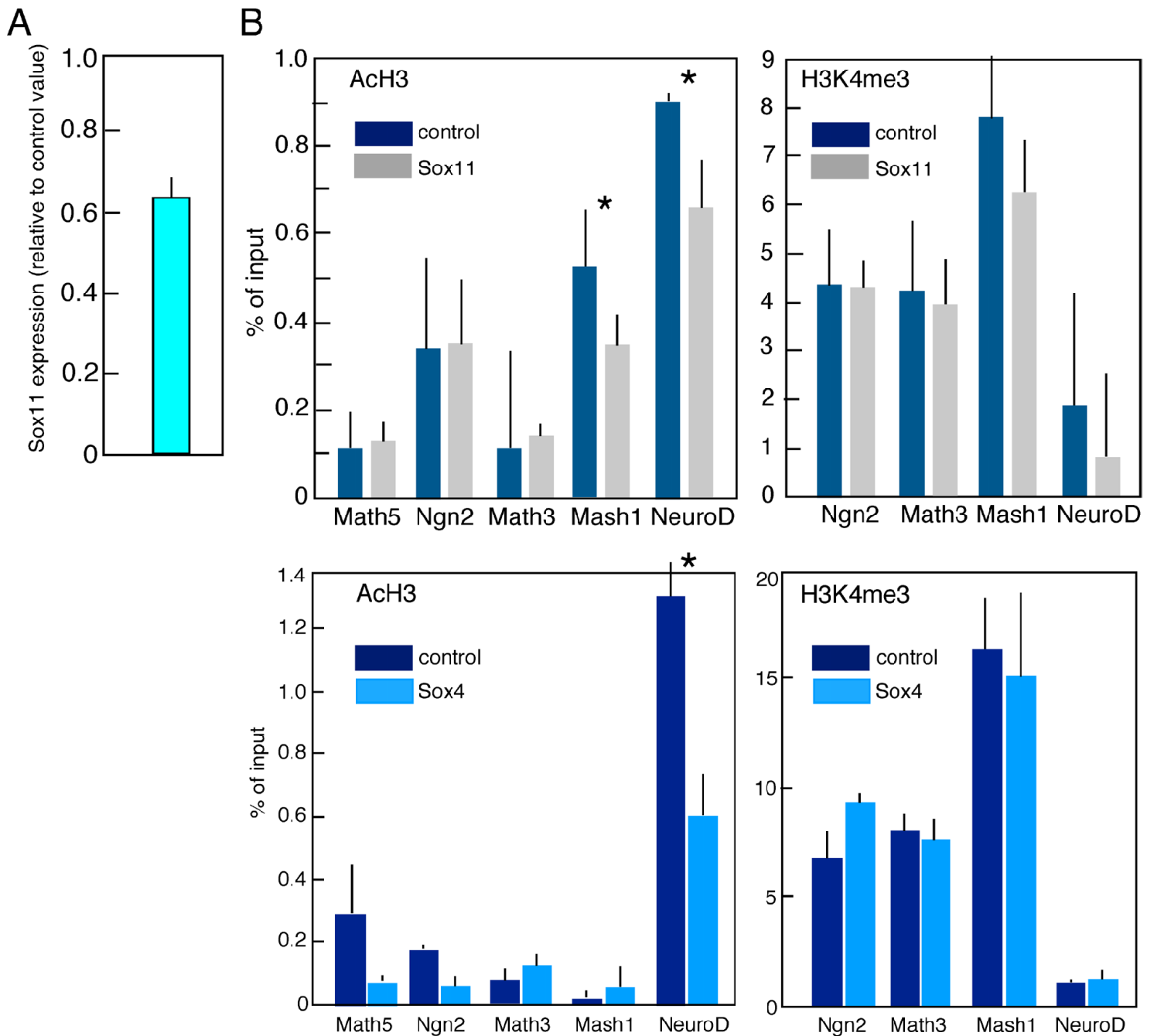


Fig. S4. Effects of Sox11 or Sox4 overexpression for histone modification of genes. (A) Effects of sh-Sox11 on expression levels of Sox11 in retina. Electroporation of sh-Sox11 was carried out using retinas at E15, and the retina was cultured for 3 days. Expression levels of Sox11 were examined by qPCR. Results are average of four independent samples \pm s.d. (B) Effects of Sox11 or Sox4 overexpression on the histone modification of genes. Expression plasmid of Sox11-EGFP or Sox4-EGFP was introduced into retina (\sim 20 retinas for one sample) at E15 by electroporation, and retinas were cultured for 2 days as explants. Retinas were then treated with trypsin to dissociate into single cells, and EGFP-positive cells were purified using a cell sorter and $\sim 1 \times 10^6$ purified cells were served for each ChIP sample. Modification of AcH3 and H3K4me3 levels in Math5, Ngn2, Math3, Mash1 and NeuroD enhancer region was analyzed by ChIP analysis. We repeated the same design of experiments at least three times, but some of results were not stable. By repeated experiments, reproducible results are shown in this figure. * $P < 0.05$.

Table S1. Antibodies

Primary antibodies

Mouse monoclonal antibodies

β III tubulin (Covance)
PNR (ppmx)
glutamine synthetase (GS, Chemicon)
HuC/D (Molecular Probes)
Ki67 (BD Bioscience)
BrdU (Roche)
kip1/p27(BD Bioscience)
NF160 (Sigma Aldrich)
Cyclin D3 (Santa Cruz)
 β -galactosidase (DSHB)
Islet-a (DSHB)

Rabbit polyclonal antibodies

GFP (Clontech)
 β -galactosidase (Cappel)
Sox4 (Abcam)
Calbindin D28K (Millipore)
Phospho histone H3 (Upstate)
RXR γ (Santa Cruz Biotechnology)
Active Caspase 3 (Promega)
Cone Arrestin 4 (Dr. Cheryl Craft, USC)
M-opsin (Dr. Cheryl Craft, USC)
Histone H3 (AcH3) antibody (Millipore)
Histone H3 tri-methyl Lys4 (H3K4me3) antibody (Activemotif)
rabbit control IgG (Santa Cruz)

Rat monoclonal antibody

GFP (Nakarai)

Sheep polyclonal antibody

Chx10 (Exalpha)

Goat polyclonal antibody

Brn3b (Santa Cruz Biotechnology)

Chicken polyclonal antibody

GFP (abcam)

Secondary antibodies

Alexa Fluor 488 goat anti-chicken IgG (H+L) (Invitrogen Molecular Probes)
Alexa Fluor 680 goat anti-mouse IgG (H+L) (Invitrogen Molecular Probes)
Alexa Fluor 568 goat anti-rabbit IgG (H+L) (Invitrogen Molecular Probes)

Table S2. Primers

RT-PCR

G3PDH: 5'-tgaccacagtcctatgccatc-3' and 5'-cataccaggaaatgagcttgac-3'
Sox11: 5'-tgaaactcgggtgcccaacag-3' and 5'-atcgcggcttaaaggtccc-3'
Sox4: 5'-gagcttaggggagcattggc-3' and 5'-gctgcaaggacaaggggaaa-3'
 β III tubulin: 5'-gctgtccgcctgcctttt-3' and 5'-gacctcccagaacttgccc-3'
Math5: 5'-caggacaagaagctg-3' and 5'-gggtctacctggagcc-3'
Hes1: 5'-atccgcagcaaactgcaaga-3' and 5'-ggacactgtgtgggacctta-3'
Hes5: 5'-cttctgcgaagtctctggtc-3' and 5'-atgtggacctgaggtgagg-3'
Math3: 5'-atcagggtcctgaagagtc-3' and 5'-gttccttgccagtcgaagag-3'
Mash1: 5'-aacaaccagacagccaacc-3' and 5'-aggaacctctgtgattcg-3'
NeuroD: 5'-caaagccacggatcaatctt-3' and 5'-cccgggaatagtgaactga-3'
Ngn2: 5'-tcgccagggactgtatct-3' and 5'-ctgtgaagtggagtcg-3'

ChIP analysis

Primers for ChIP were designed around transcription start site

Math5: 5'-atccgcagcaaactgcaaga-3', 5'-ggacactgtgtgggacctta-3'
Ngn2: 5'-gattgtttcttggtggtatataagg-3', 5'-gactccaaggcactccagttaaa-3'
Math3: 5'-aggaggtgtgtctttaggct-3', 5'-cagctcctgtgatctgactg-3'
Sox11: 5'-tgaggcccggtggttaataac-3', 5'-tgagggtctctctcaactcg-3'
Sox4: 5'-cttctcattgcacgcggaga-3', 5'-cagccgctgtaactaacgct-3'
Mash1: 5'-cagcagtctctcacttctgg-3', 5'-tgagcctgcttctgggatta-3'
NeuroD: 5'-acagatgggccactttcttc-3', 5'-atatggtcttcccgggtccag-3'
Fbxo: 5'-ggaggaggaaagctccgata-3', 5'-aggaggacatatggagtcgg-3'
Adi1: 5'-gccttctctctctgaatt-3', 5'-ttccactaactggctggaag-3'

Table S3. Comparison of expression level of members of SoxC group in different subsets of retinal progenitor cells

Gene	Probe No	Unigene No.	SSEA1+(E14)	c-kit-(P1)	ckit+(P1)
Sox11	1426790_a_at	Mm.41702	715.0	309.2	229.5
Sox4	1419155_a_at	Mm.240627	307.3	1471	1061.8
Sox12	1456882_at	Mm.28424	10.1	6.8	5.0

Table S4. Expression levels of members of SoxC group in retinal explant cultures

Gene	Probe No	Unigene No.	E15	5 days cultured
Sox11	1426790_a_at	Mm.41702	486.1	246.7
Sox4	1419155_a_at	Mm.240627	1082.7	1607.3
Sox12	1456882_at	Mm.28424	2.9	8.0

Table S5. Genes downregulated and upregulated in Sox11 knockout retina compared with littermate wild-type retina at E16
Genes are listed from smallest (Table 5A) and largest (Table 5B) fold change; the top 30 genes are shown.

A

Probe-set	Accession No.	Unigene	Gene	WT signal	KO signal	Fold change
1450813_a_at	NM_021467	Mm.44379	troponin I, skeletal, slow 1	33.4	2	0.06
1423232_at	X63190	Mm.5025	ets variant gene 4 (E1A enhancer binding protein, E1AF)	22.3	2.1	0.08
1431225_at	BB656631	Mm.466344	SRY-box containing gene 11	3205.7	252.4	0.08
1438648_x_at	AV069898	Mm.46387	gastrokine 3	29	1.8	0.11
1455869_at	BG862223	Mm.458283	calcium/calmodulin- dependent protein kinase II, beta	95.4	8	0.13
1419025_at	NM_009118	Mm.1276	retinal S-antigen	246.7	42.8	0.16
1438758_at	AU046270	Mm.291504	acireductone dioxygenase 1	129.4	20.1	0.16
1435197_at	BE993443	Mm.477577	POU domain, class 3, transcription factor 3	31.1	5.4	0.18
1451255_at	BC004672	Mm.4067	lipolysis stimulated lipoprotein receptor	33.8	12	0.19
1439143_at	BB312992	Mm.285300	RIKEN cDNA A930018M24 gene	28	3.7	0.19
1437121_at	BB168293	Mm.284447	DENN/MADD domain containing 1C	40.1	9	0.23
1449578_at	AW536705	Mm.479197	suppressor of Ty 16 homolog (S. cerevisiae)	157.7	41.5	0.27
1435205_at	C88013	Mm.331881	transcription factor AP-2, epsilon	61.4	14.6	0.27
1418726_a_at	NM_011619	Mm.247470	troponin T2, cardiac	47.5	15.6	0.33
1419740_at	NM_008806	Mm.1372	phosphodiesterase 6B, cGMP, rod receptor, beta	36.5	10.8	0.38
1423631_at	BB540797	Mm.103641	nuclear receptor subfamily 2, group E, member 3	179.3	76.3	0.38
1434369_a_at	AV016515	Mm.178	crystallin, alpha B	183.1	72.6	0.38
1429024_at	AK003783	Mm.479259	RNA binding motif protein 20	96.7	50.8	0.38
1425900_at	BC016235	Mm.213213	hexokinase domain containing 1	277.4	149.7	0.41
1427536_at	AI615965	Mm.442631	predicted gene 10330 /// zinc finger protein 125	49.8	12.9	0.41
1441899_x_at	BB335613	Mm.4598	brevican	57.9	13.9	0.41
1425892_a_at	D50055	Mm.16347	prepronociceptin	58.9	28.7	0.44
1431581_at	AK013705	---	RIKEN cDNA 4922502B01 gene	99.6	45.9	0.44
1441107_at	BB292639	Mm.32825	doublesex and mab-3 related TF like family A2	22	14.9	0.44
1444687_at	BB804635	Mm.337409	complement component 1, q subcomponent-like 2	56.4	26.3	0.44
1424865_at	BC010821	Mm.46248	peptide YY	35	12.4	0.47
1425288_at	BC026991	Mm.477583	sterile alpha motif domain containing 11	85.8	43	0.47
1427004_at	BB311718	Mm.262287	F-box protein 2	26.8	11.9	0.47
1450680_at	NM_009019	Mm.828	recombination activating gene 1	44.8	20.1	0.47
1450946_at	BG298773	Mm.20422	neural retina leucine zipper gene	632.8	289	0.47
1429945_at	AK013012	Mm.153585	kelch-like 35 (Drosophila)	27.9	15.1	0.47
1442120_at	AA153229	---	RIKEN cDNA G730007D18 gene	45.6	19.2	0.47

B

Probe-set	Accession No.	Unigene	Gene	WT signal	KO signal	Fold change
1439113_at	BB379753	Mm.327413	RIKEN cDNA 2410018L13 gene	24.2	91.1	4.9
1427820_at	BC021831	Mm.466733	---	47.4	159.7	4.0
1415801_at	M63801	Mm.378921	gap junction protein, alpha 1	39.2	132.6	3.4
1422674_s_at	NM_007775	Mm.440053	crystallin, gamma B /// crystallin, gamma C	24.2	88.3	3.0
1436115_at	BB829749	Mm.81916	predicted gene 266	28.6	87.3	3.0
1437726_x_at	BB111335	Mm.2570	complement component 1, q subcomponent, beta	35.7	96	3.0
1429948_x_at	AK013953	Mm.275362	crystallin, gamma F	20.7	95.6	3.0
1436127_at	AI854101	Mm.316614	corticotropin releasing hormone binding protein	156.9	474.7	3.0
1422652_at	NM_007774	Mm.26904	crystallin, gamma A	25.8	93.7	2.8
1449401_at	NM_007574	Mm.439732	complement component 1, q subcomponent, C chain	26.5	67.3	2.8
1426314_at	BB770914	Mm.229532	endothelin receptor type B	53.5	157.7	2.8
1450796_at	NM_016864	Mm.228661	atonal homolog 7 (Drosophila)	179.6	419.2	2.6
1417381_at	NM_007572	Mm.439957	complement component 1, q subcomponent, alpha	33.8	85.6	2.6
1446950_at	BM124834	Mm.87051	thymocyte selection-associated HMG box gene	23.8	60	2.6
1456659_at	BM116906	---	hypothetical LOC552902 non-catalytic region of tyrosine kinase adaptor protein 2	27.2	69.2	2.6
1458432_at	BE948993	Mm.447891	2	21.5	57.6	2.6
1423754_at	BC010291	Mm.141021	interferon induced transmembrane protein 3	25.2	65.7	2.6
1443337_at	BB531021	Mm.448265	glutamate receptor interacting protein 1	22.5	46.5	2.6
1445874_at	BB470735	Mm.439301	---	72.1	208	2.6
1438754_at	AV372127	---	---	26.8	69.1	2.6
1431335_a_at	AK018575	Mm.87599	WAP four-disulfide core domain 1	44.2	120.6	2.6
1460208_at	NM_007993	Mm.271644	fibrillin 1	44.4	82.1	2.4
1445561_at	BM218868	Mm.413457	---	25.6	56.4	2.4
1421141_a_at	BG962849	Mm.234965	forkhead box P1	22.2	60.4	2.4
1427183_at	BC023060	Mm.44176	EGF-containing fibulin-like extracellular matrix protein 1	85.6	253.9	2.4
1441577_at	BB419140	---	RIKEN cDNA C530014P21 gene	22.2	29.9	2.4
1437165_a_at	BB250811	Mm.262345	procollagen C-endopeptidase enhancer protein	24.8	64.9	2.4
1436905_x_at	BB218107	Mm.271868	lysosomal-associated protein transmembrane 5	26.7	64.7	2.4
1452892_at	AK014819	Mm.389950	serine/threonine kinase 33	23.3	52	2.4
1453145_at	AK007420	Mm.247625	phosphatidylserine decarboxylase, pseudogene 3	47	112.2	2.4
1417643_at	NM_025290	Mm.12743	radial spoke head 1 homolog (Chlamydomonas)	92.7	215.2	2.3
1448591_at	NM_021281	Mm.3619	cathepsin S	113.8	196.2	2.3
1421375_a_at	NM_011313	Mm.100144	S100 calcium binding protein A6 (calcyclin)	33.6	68.7	2.3
1449470_at	NM_010053	Mm.475101	distal-less homeobox 1	32.2	62.2	2.3
1458721_at	BG073332	Mm.247203	Protocadherin gamma cluster	24.3	50.3	2.3
1426607_at	BG068672	Mm.46679	predicted gene 7120	86.4	171.7	2.3

1460133_at	BF607205	Mm.450032	ephrin A5	21.8	61.4	2.3
1459731_at	BE996194	---	---	25.3	63.7	2.3
1446708_at	BB486740	Mm.135110	hypoxia inducible factor 3, alpha subunit	29.2	63.8	2.3
1443175_at	BB466434	---	RIKEN cDNA A830010M09 gene	22.9	66.3	2.3
1444345_at	AW123227	Mm.463057	---	23	55.9	2.3
1457111_at	AV318727	---	expressed sequence AA415038	26.9	55.9	2.3
1421011_at	NM_053262	Mm.46019	hydroxysteroid (17-beta) dehydrogenase 11	27	63.7	2.1
1418455_at	NM_019877	Mm.22144	coatamer protein complex, subunit zeta 2	27.6	55.9	2.1
1419665_a_at	NM_019738	Mm.18742	nuclear protein 1	49.2	120.1	2.1
1422903_at	NM_010745	Mm.2639	lymphocyte antigen 86	38	71.1	2.1
1417962_s_at	NM_010284	Mm.3986	growth hormone receptor	63.3	169	2.1
1448877_at	NM_010054	Mm.3896	distal-less homeobox 2	25.1	47.1	2.1
1421385_a_at	NM_008663	Mm.1403	myosin VIIA	38.4	79.2	2.1
1417346_at	BG084230	Mm.24163	PYD and CARD domain containing	37.9	76.2	2.1
1444349_at	BG071091	---	---	33.6	72.7	2.1
1452114_s_at	BF225802	Mm.405761	insulin-like growth factor binding protein 5	157.7	331.4	2.1
1442109_at	BE989344	Mm.445031	hypoxia inducible factor 3, alpha subunit	31.7	61.1	2.1
1422882_at	BE333485	Mm.246304	synaptophysin-like protein	24.4	58	2.1
1417625_s_at	BC015254	Mm.6522	chemokine (C-X-C motif) receptor 7	46.1	104.1	2.1
1450020_at	BC012653	Mm.44065	chemokine (C-X3-C) receptor 1	26.3	39.5	2.1
1452141_a_at	BC001991	Mm.392203	selenoprotein P, plasma, 1	212.5	484.9	2.1
1436098_at	BB667452	Mm.250719	butyrylcholinesterase	21.1	47.4	2.1
1436714_at	BB297498	Mm.209385	LIM domain containing preferred translocation partner in lipoma	43.2	82.9	2.1
1458408_at	BB160137	Mm.102765	sterile alpha motif domain containing 8	28.9	59.8	2.1
1435888_at	AV369812	Mm.420648	epidermal growth factor receptor	62.2	125.8	2.1
1456312_x_at	AV224521	Mm.21109	gelsolin	23.8	72.2	2.1