SUPPLEMENTARY INFORMATION

A central role for Islet1 in sensory neuron development linking sensory and spinal gene regulatory programs

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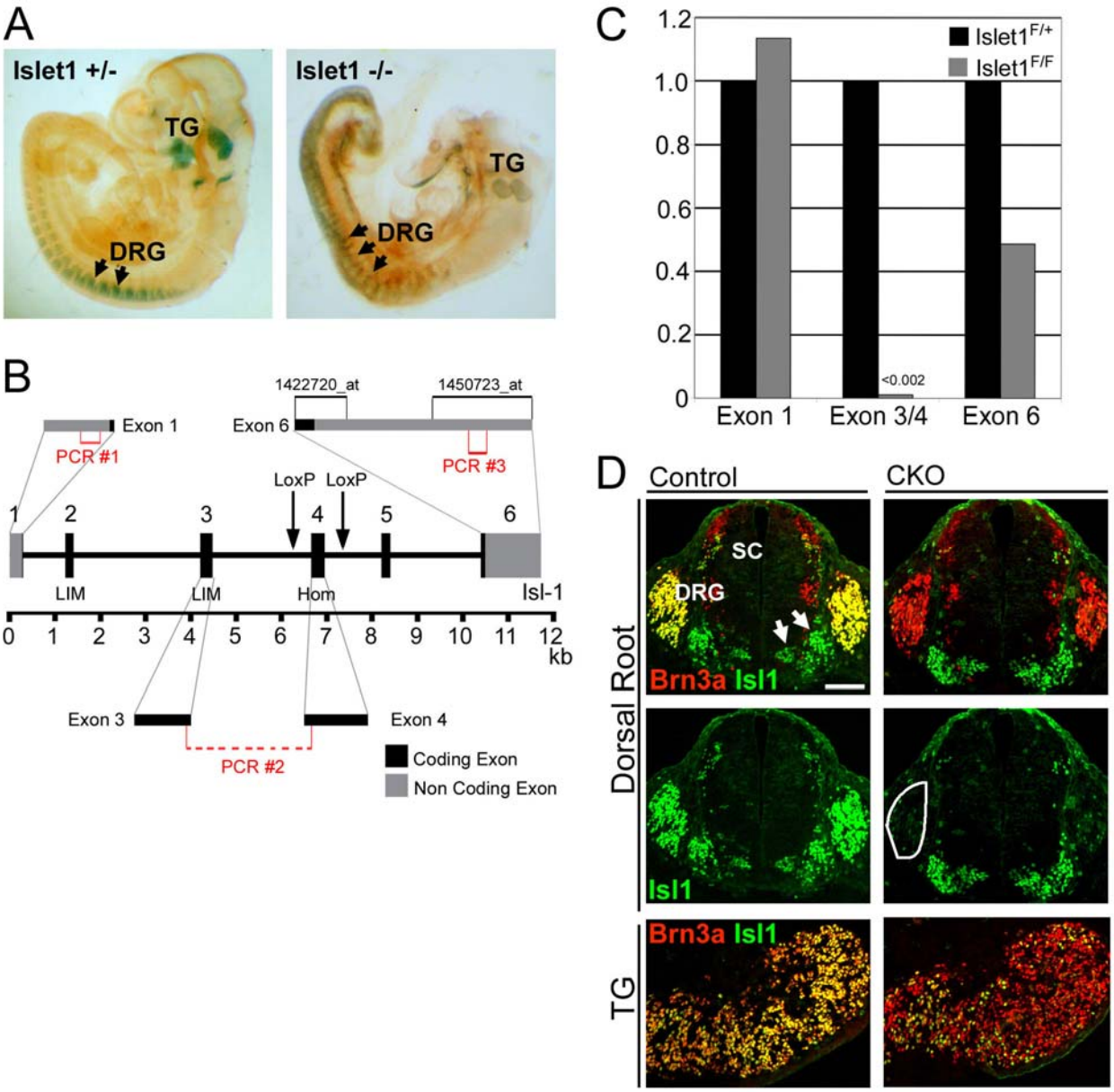
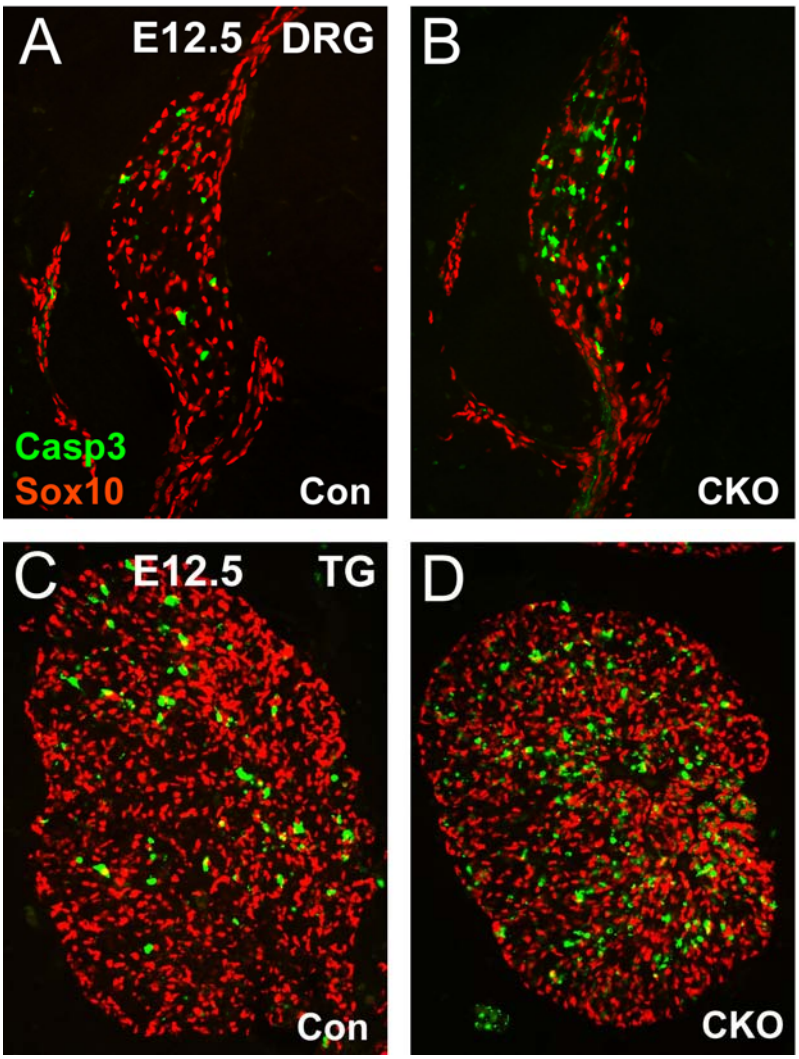
Supplementary Figure 1



Supplementary Figure 1. A strategy for tissue-specific deletion of Islet1 in the sensory system.

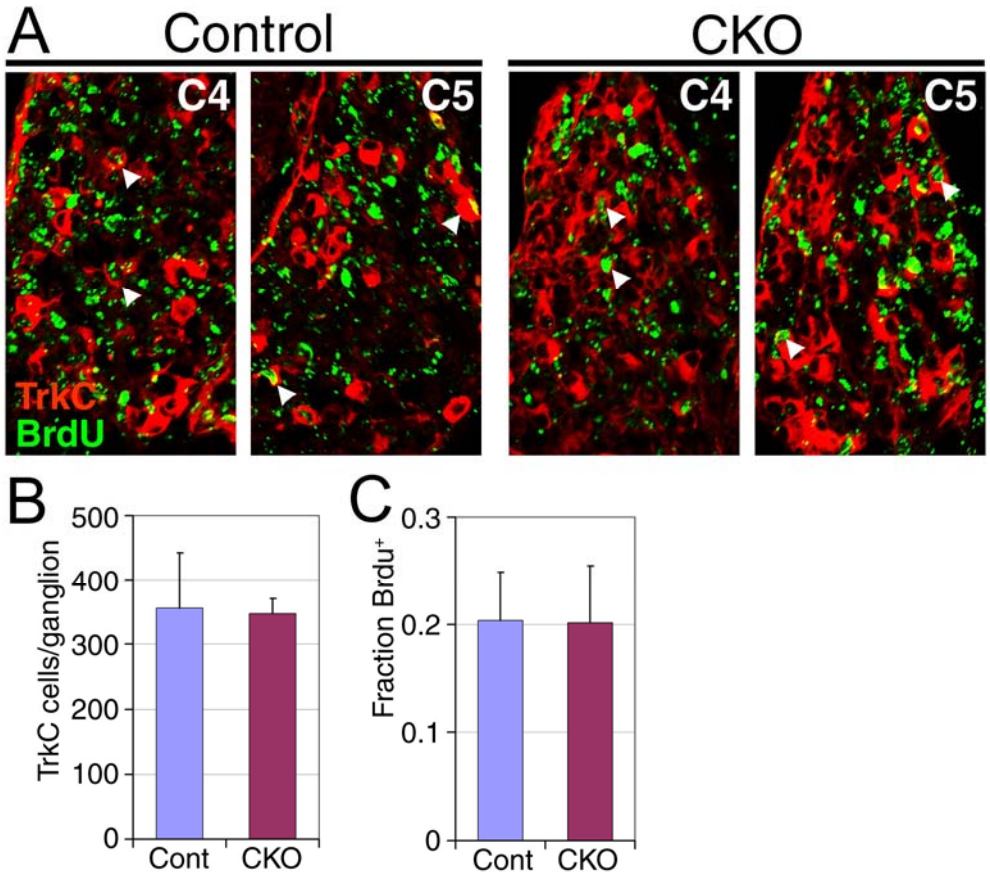
(**A**) Development of the sensory nervous system in a conventional *Islet1* null embryo and heterozygous control, at E10.5. The *Islet1*$^{-/-}$ embryo is no longer viable, and the dorsal forebrain-midbrain has been lost due to necrosis. However, initial formation of the DRG and TG at the appropriate locations can be visualized by Xgal staining due to the presence of a *Brn3a*^{tauLacZ} reporter. (**B**) Strategy for the conditional deletion of the *Islet1* homeodomain. Exon 4, encoding the homeodomain, is flanked by *loxP* sites. The location of sequences assayed by Affymetrix probe sets from the mouse genome 430 array and RT-PCR primers are shown. Details of the generation of floxed Islet1 mice will be published elsewhere. (**C**) RT-PCR primers targeting Exon 1, Exon 3-4 and Exon 6 were used to assay Islet1 mRNA in the E12.5 DRG of *Islet1*$^{F/+}$ (control) and *Islet1*$^{F/F}$ (CKO) mice in the presence of a *Wnt1-Cre* allele. Exon 1 levels in CKO ganglia were equal to controls, indicating that the recombined locus was expressed at normal levels. Exon 4 sequences were detected at less than 1% of normal levels, demonstrating highly efficient excision by Wnt1-Cre. Exon 6 sequences were present at reduced levels, indicating that the mRNA expressed from the targeted allele was spliced but with lower efficiency than the native mRNA. (**D**) Immunofluorescence staining for the expression of Islet1 protein in horizontal sections of E12.5 of control and CKO DRG and TG. Brn3a is normally co-expressed with Islet1 in a large majority of sensory neurons, is not significantly changed in the knockout ganglia, and is used here as a marker for the sensory population. Islet1 protein was nearly absent from E11.5 DRG, but it continued to be expressed in the TG in a subset of sensory neurons which may be derived from the trigeminal placodes rather than the neural crest, and thus do not express the *Wnt1-cre* transgene. Expression of Islet1 in motor neurons (arrows) is not affected. Scale (D) 200μm.

Supplementary Figure 2



Supplementary Figure 2. Increased cell death in the sensory ganglia of Islet1 CKO embryos. Immunofluoresecence for Caspase-3 was used to identify cells undergoing apoptotic cell death in E12.5 DRG and TG from Islet1 control and CKO embryos. Caspase-3 rarely localized with Sox10, indicating that cell death occurs primarily in differentiating neurons rather than precursors. (**A,B**) Apoptosis in DRG. (**C,D**) Apoptosis in TG. Analysis of cell counts appears in Figure 1.

Supplementary Figure 3

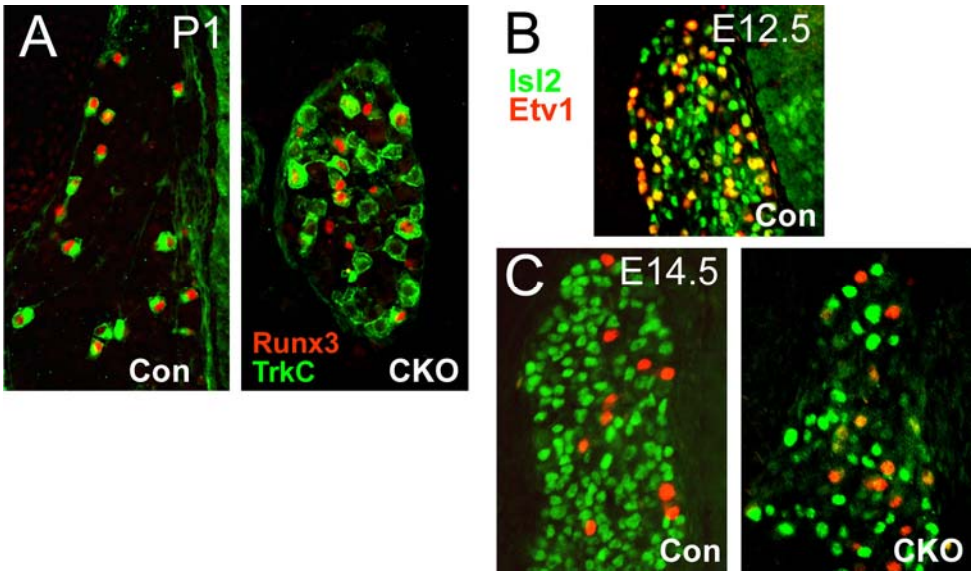


Supplementary Figure 3. Birthdating TrkC neurons in Islet1 CKO and control DRG. Pregnant dams were injected with BrdU at E10.5, and embryos were harvested at E15.5. Immunofluorescence was performed for TrkC and BrdU in all sections from C3 to C6 in littermate embryos. (A) Immunofluorescence for TrkC and BrdU. C4, C5 indicate axial level of DRG shown. Arrows indicate examples of labeled nuclei in TrkC immunoreactive cells. (B,C) TrkC and BrdU expressing cell counts for cervical ganglia C3 to C6. CKO ganglia were markedly diminished in size but contained similar numbers of TrkC neurons, and a similar fraction of TrkC neurons were strongly labeled at E10.5.

Methods for Supplementary Figure 3: Birthdating of DRG neurons was performed by injecting timed-pregant dams at noon and 4PM of E10.5 with 500µl of BrdU (proprietary

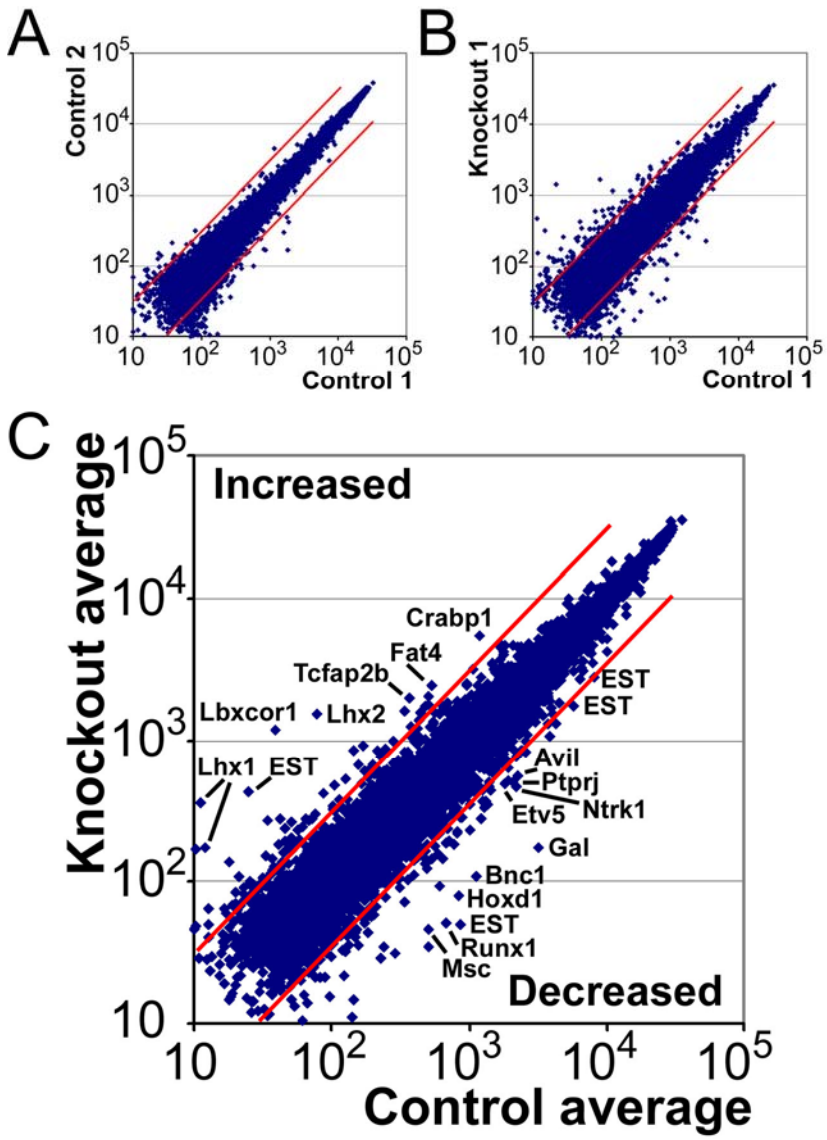
mixture, Ambion). Embryos were harvested at E15.5 and the vertebral columns with attached DRGs were fixed in 4% PFA at RT for 3 hours and processed for cryosectioning. For coimmunostainning of BrdU and TrkC, TrkC staining was performed first as described above and the slides were then dehydrated in graded ethanol solutions, air dried, treated with 4N HCL for 20 min, washed extensively in PBS and stained with antibody to BrdU (Abcam, Rat anti-mouse, 1:100) followed by appropriate secondary antibodies. Cells immunoreactive for TrkC and BrdU, which displayed a clear nuclear outline in the plane of section and strong labeling for BrdU indicative of cell cycle exit during the period of labeling, were counted in every fourth section throughout the cervical DRG in matched sections of control and CKO embryos.

Supplementary Figure 4



Supplementary Figure 4. Subtype specific transcription factor expression in Islet1 CKO DRG. (**A**) Co-expression of Runx3 and TrkC in control ganglion vestigial CKO ganglion at P1. (**B,C**) Expression of Islet2 and Etv1. At E12.5 these markers are co-expressed in a significant fraction of DRG neurons, but at E14.5 Etv1 neurons no longer express Islet2 in both the control and CKO DRG.

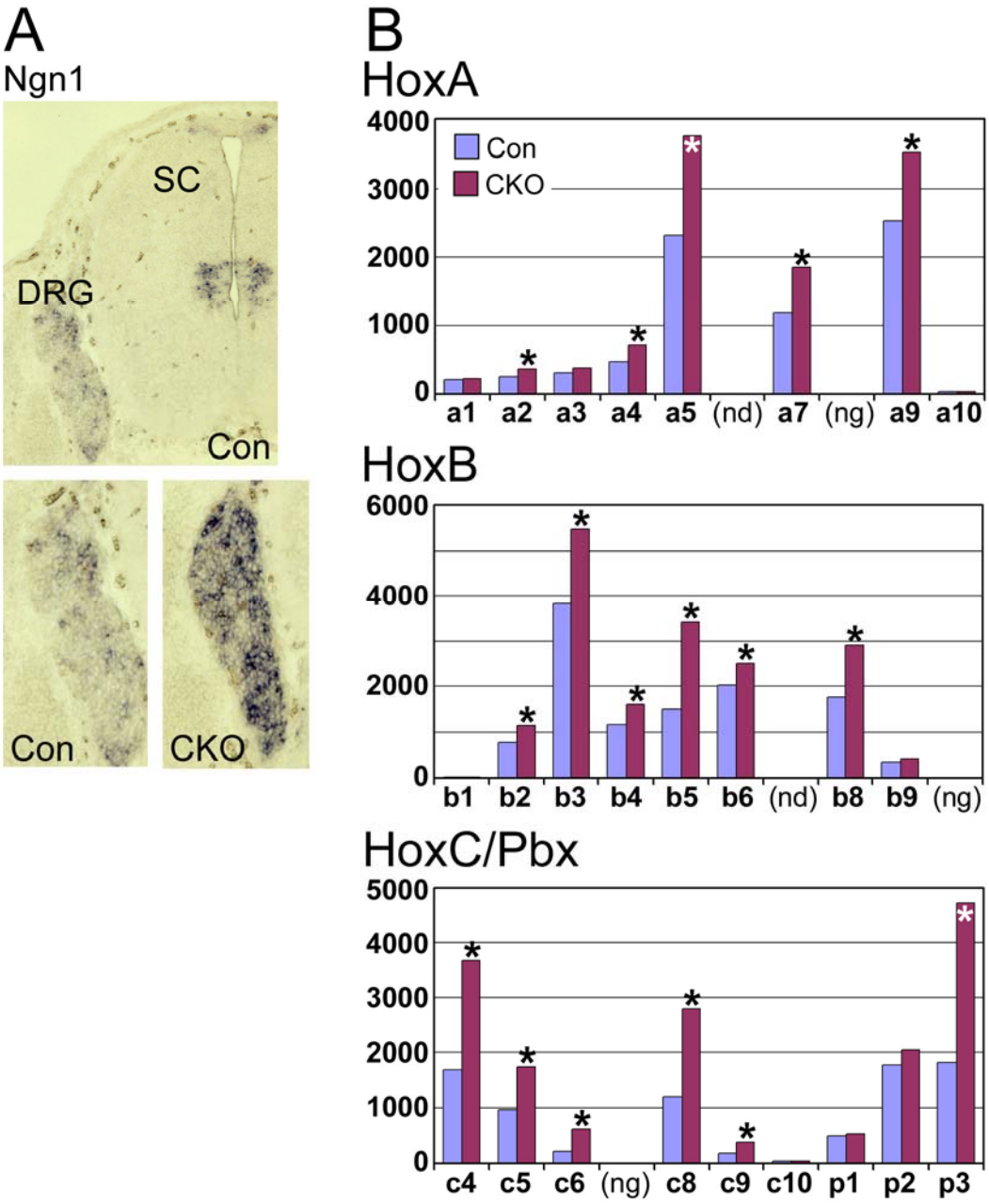
Supplementary Figure 5



Supplementary Figure 5. Global comparison of gene expression in Islet1 knockout and control DRG. Cervical/upper thoracic DRG were harvested from E12.5 embryos with the genotypes *Islet1*$^{F/+}$, *Wnt1-Cre* (control) and *Islet1*$^{F/F}$, *Wnt1-Cre* (knockout) as described in Methods. Data from two independent experiments were analyzed using MAS 5.0, and expression levels for all transcripts present in at least one condition were compared. Parallel red lines indicate three-fold change. (**A**) Replicate assays of control ganglia were highly correlated. The small number of differentially expressed genes could in several cases be identified as sex chromosome-linked transcripts related to embryo gender. (**B,C**) Comparison of transcript levels

in control and knockout DRG showed significant and highly reproducible changes in gene expression for a small number of transcripts.

Supplementary Figure 6



Supplementary Figure 6. Regulation of Neurogenin1, Hox and Pbx genes by Islet1. (A) Ngn1 (Neurog1) expression in the cervical DRG of E12.5 Islet1 control and CKO embryos. Ngn1 is also expressed in a subset of spinal cord neuron precursors. Views are of littermate embryos processed together on the same slide. **(B)** Multiple transcripts of the *HoxA*, *B*, and *C* clusters are increased in the DRG of *Islet1* CKO embryos. Asterisks indicate significant increase or decrease (change $p < 0.002$ or > 0.998) in two concordant comparisons. Nd, not determined; ng, no gene at the corresponding position in cluster.

Supplementary Table 1.

		Replicate 1			Replicate 2			Mean values		
Wnt1 -Cre fold	Symbol	Con1	KO1	Chg P1	Con2	KO2	Chg P2	Con avg	KO avg	Con/ KO fold
32.3	Lhx1	(12)	(6)	n.s.	(12)	(9)	n.s.	n.s.	n.s.	n.s.
30.4	Sall3	(10)	(3)	n.s.	(15)	(15)	n.s.	n.s.	n.s.	n.s.
30.2	Lbxcor1	(12)	(4)	n.s.	(72)	(26)	n.s.	n.s.	n.s.	n.s.
19.5	Lhx2	103	162	n.s.	(157)	200	n.s.	130	181	1.4
7.6	Olig2	(25)	(3)	n.s.	(13)	(12)	n.s.	n.s.	n.s.	n.s.
5.8	Stxbp6	173	124	n.s.	253	251	n.s.	213	187	0.9
5.4	Tcfap2b	119	(109)	n.s.	(77)	(100)	n.s.	n.s.	n.s.	n.s.
5.1	Daam1	228	239	n.s.	262	259	n.s.	245	249	1.0
4.8	Nova1	325	302	n.s.	169	256	n.s.	247	279	1.1
4.6	Fat4	268	253	n.s.	270	279	n.s.	269	266	1.0
4.5	Crabp1	5598	6328	n.s.	7047	6966	n.s.	6322	6647	1.0
4.2	Mpeg1	373	280	n.s.	(61)	(159)	n.s.	217	220	1.0
4.0	Gpm6a	1355	1482	n.s.	1038	1303	n.s.	1197	1393	1.2
4.0	Glra2	206	202	n.s.	222	(120)	n.s.	214	161	0.8
3.6	Pcdh20	(24)	(36)	n.s.	(97)	(32)	n.s.	n.s.	n.s.	n.s.
3.5	Elmod1	77	96	n.s.	(128)	127	n.s.	102	111	1.1
3.5	Olig1	(17)	(5)	n.s.	(13)	(15)	n.s.	n.s.	n.s.	n.s.
3.5	Ctss	654	527	n.s.	468	459	n.s.	561	493	0.9
3.4	Pcdh9	970	1028	n.s.	779	781	n.s.	875	904	1.0
3.4	Pira2	(22)	(24)	n.s.	(29)	(149)	n.s.	n.s.	n.s.	n.s.
3.3	Dab1	95	129	n.s.	(159)	104	n.s.	127	116	0.9
3.3	Dach2	141	155	n.s.	(110)	(188)	n.s.	n.s.	n.s.	n.s.
3.2	Eya1	116	143	n.s.	245)	171	n.s.	180	157	0.9
3.0	Neurod4	(2)	(4)	n.s.	(14)	(16)	n.s.	n.s.	n.s.	n.s.
2.9	Kcnd2	187	186	n.s.	(145)	158	n.s.	166	172	1.0
2.9	Shox2	225	129	n.s.	132	193	n.s.	178	161	0.9
2.8	Ror1	203	209	n.s.	239	241	n.s.	221	225	1.0
2.8	Hoxc6	(44)	(77)	n.s.	(35)	(29)	n.s.	n.s.	n.s.	n.s.
2.7	Insm1	353	351	n.s.	455	480	n.s.	404	416	1.0
2.7	Neurog1	(25)	(8)	n.s.	(25)	(30)	n.s.	n.s.	n.s.	n.s.

Supplementary Table 1. Expression of transcripts in the E14.5 TG of Islet1$^{MCM/F}$ tamoxifen-induced (IKO) embryos, ranked by the order of transcripts most increased in E12.5 CKO ganglia. Expression of the transcripts most increased in E12.5 CKO DRG (Table 1) were examined in IKO trigeminal at E14.5. Pregnant dams were injected with tamoxifen at E11.5 and embryos were harvested at E14.5. Gene expression was compared in replicate samples of *Islet1*$^{MCM/F}$ experimental and *Islet1*$^{F/+}$ littermate embryos. Values in parentheses are absent calls. Average and fold change values are reported as not significant (n.s.) in the case of absent calls. No significant changes (Change P value) were observed in expression of these genes normally repressed by Islet1 at early stages. Corresponding gene names appear in Table 1.

Supplementary Table 2.

		Replicate 1			Replicate 2			Mean values			
Wnt-Cre fold	**Symbol**	**Con1**	**KO1**	**Chg P1**	**Con2**	**KO2**	**Chg P2**	**Con avg**	**KO avg**	**Con KO fold**	**KO % of Con**
24.7	Scn10a	236	158	n.s.	343	288	n.s.	290	223	1.30	77%
18.4	**Gal**	**5678**	**4137**	**0.99998**	**5641**	**3961**	**0.99997**	**5659**	**4049**	**1.40**	**72%**
13.4	**Runx1**	**1215**	**901**	**0.99998**	**1329**	**779**	**0.99983**	**1272**	**840**	**1.51**	**66%**
12.6	Cckar	108	78	0.99965	185	93	n.s.	146	85	1.71	58%
10.8	Msc	166	118	0.99775	249	(200)	n.s.	207	159	1.31	77%
10.7	Hoxd1	3434	3654	n.s.	3473	3386	n.s.	3454	3520	0.98	102%
10.5	Bnc1	4613	3803	n.s.	3387	2921	n.s.	4000	3362	1.19	84%
6.6	Fst	345	250	n.s.	231	166	n.s.	288	208	1.38	72%
4.8	Ntrk1	2757	2826	n.s.	3739	3773	n.s.	3248	3299	0.98	102%
4.3	Pla2g7	2690	2775	n.s.	3255	3138	n.s.	2972	2956	1.01	99%
4.2	Ptprj	564	366	n.s.	606	494	n.s.	585	430	1.36	73%
4.1	Avil	7755	8081	n.s.	7344	7057	n.s.	7550	7569	1.00	100%
3.9	Islr2	2484	2673	n.s.	3908	3797	n.s.	3196	3235	0.99	101%
3.9	**Th**	**2825**	**1694**	**0.99998**	**3495**	**1980**	**0.99998**	**3160**	**1837**	**1.72**	**58%**
3.9	Bmper	1366	1679	n.s.	1422	1493	n.s.	1394	1586	0.88	114%
3.7	Ppp1r2	2865	2665	n.s.	2231	2252	n.s.	2548	2459	1.04	96%
3.7	Lix1	1269	1137	n.s.	1296	1284	n.s.	1282	1211	1.06	94%
3.7	Etv5	1860	1789	n.s.	1532	1490	n.s.	1696	1640	1.03	97%
3.7	Fmn1	531	450	n.s.	432	358	n.s.	481	404	1.19	84%
3.7	Prokr1[1]	763	533	0.99998	565	686	0.99965	664	609	1.09	92%
3.7	Dock10	1089	1009	n.s.	907	843	n.s.	998	926	1.08	93%
3.6	Cntn4	751	889	n.s.	625	553	n.s.	688	721	0.95	105%
3.6	Prkg2	960	926	n.s.	727	848	n.s.	844	887	0.95	105%
3.5	Srpk3	163	187	n.s.	(253)	(213)	n.s.	208	200	1.04	96%
3.4	Cbln2	347	305	n.s.	444	299	n.s.	396	302	1.31	76%
3.4	Ptgir	397	543	n.s.	591	639	n.s.	494	591	0.84	120%
3.3	Ntrk2	1342	1349	n.s.	1288	1141	n.s.	1315	1245	1.06	95%
3.1	Dcamkl3	609	603	n.s.	797	742	n.s.	703	672	1.05	96%
3.1	Btbd11	1277	1173	n.s.	1221	1142	n.s.	1249	1158	1.08	93%
3.0	Isl2	1688	2091	n.s.	1846	2349	n.s.	1767	2220	0.80	126%
3.0	**Trpv1**	**1182**	**620**	**0.99998**	**908**	**504**	**0.99991**	**1045**	**562**	**1.86**	**54%**
2.9	**Htr3a**	**1162**	**995**	**0.99956**	**1020**	**596**	**0.99923**	**1091**	**795**	**1.37**	**73%**
2.8	**Akap7**	**1487**	**1137**	**0.99997**	**1174**	**923**	**0.99987**	**1330**	**1030**	**1.29**	**77%**
2.8	Pde10a	1211	1090	n.s.	828	827	n.s.	1019	958	1.06	94%
2.8	Rgs10	3996	3578	n.s.	3871	3678	n.s.	3934	3628	1.08	92%
2.8	Hmx1	1133	902	0.99665	1232	1003	n.s.	1182	952	1.24	81%
2.6	Etv1	404	237	n.s.	450	234	n.s.	427	235	1.81	55%
2.0	Prrxl1	6662	6057	n.s.	5820	5243	n.s.	6241	5650	1.10	91%

Other decreased transcripts identified in E14.5 TG of Islet1$^{MCM/F}$ embryos:

Abs[2]	Htr3b	308	186	0.99956	483	(113)	0.99989	396	149	2.65	38%
1.4	Isl1[3]	9229	3733	0.99998	8770	3954	0.99998	8999	3844	2.34	43%
0.4	Synpr	1790	723	0.99998	1261	598	0.99997	1526	661	2.31	43%
Abs	Scn11a	537	263	0.99995	500	255	0.99976	519	259	2.00	50%
Abs	Grm7	1208	624	0.99998	822	508	0.99997	1015	566	1.79	56%
Abs	Rasgrp1	376	262	0.99923	372	(173)	0.99993	374	218	1.72	58%
2.1	Lxn	7110	4144	0.99998	5547	3534	0.99998	6329	3839	1.65	61%
2.1	Ak5	1546	907	0.99998	1226	779	0.99998	1386	843	1.64	61%
1.6	Rgs4	2861	1970	0.99998	2650	1965	0.99998	2756	1968	1.40	71%
1.3	Ldb2	2404	1825	0.99998	1741	1164	0.99998	2073	1494	1.39	72%

Supplementary Table 2. Expression of transcripts in the E14.5 TG of *Islet1$^{MCM/F}$* tamoxifen-induced (IKO) embryos, ranked by the order of transcripts most decreased in E12.5 CKO ganglia. Expression of the transcripts most decreased in E12.5 CKO DRG (Table 2) were examined in the IKO trigeminal at E14.5. In addition to all genes exhibiting the highest fold changes in the E12.5 CKO DRG, further decreased transcripts are listed which were detected as highly changed at E14.5, some of which were not yet expressed in the E12.5 DRG. Embryos were induced and harvested as described in Table S1. Values in parentheses are absent calls. Corresponding gene names appear in Table 2.

[1]Probe set appears to have high background.

[2]Absent in E12.5 DRG.

[3]Probe set does not detect Islet1 exon 4 which is flanked by *LoxP* site, and underestimates extent of Islet1 knockdown.

Gene names not shown in Table 2: **Htr3b**, 5-hydroxytryptamine (serotonin) receptor 3B; **Isl1**, ISL1 transcription factor, LIM/homeodomain; **Synpr**, synaptoporin; **Scn11a**, sodium channel, voltage-gated, type XI, alpha; Nav1.9; NaN; **Grm7**, glutamate receptor, metabotropic 7; **Rasgrp1**, RAS guanyl releasing protein 1; **Lxn**, latexin; **Ak5**, adenylate kinase 5; **Rgs4**, regulator of G-protein signaling 4; **Ldb2**, LIM domain binding 2.

Supplementary Table 3

Oligonucleotide primers

Name	Sequence	Amplicon
Conventional genotyping primers		
Islet1^{F} 5’	GGTCTCTGGAACATCCCACAT	202 WT 297 KO
Islet1^{F} 3’	CTGTTCCTACTCCCCATTCACT	
Wnt1-Cre 5’	CCGGGCTGCCACGACCAA	
Wnt1-Cre 3’	GGCGCGGCAACACCATTTTT	
Real-time genotyping primers		All <100
Islet1 constitutive targeted allele, WT 5’	CCCTCTCAGTCCCTTGCATC	
Islet1 WT 3’	TTTCTCAGCCTCTGGGTGGA	
Islet1 KO 5’	CAGAATAAAACGCACGGGTGT	
Islet1 KO 3’	CCTTAGCAGGGCTAGGGAGG	
Cre recombinase 5'	TGGGCCAGCTAAACATGCTT	
Cre recombinase 3'	AACAGCATTGCTGTCACTTGGT	
qRT-PCR primers		All <100
Islet1 Exon1 5’	GGGCCACTATTTGCCACCTA	
Exon1 3’	TGGTTGGACAATTGGTGAATAGC	
Exon3 5’	CCCTCTCAGTCCCTTGCATC	
Exon4 3’	TGGTCTTCTCCGGCTGCTT	
Exon6 5’	TGACTCCTGTCTGTCCAAGAACTT	
Exon6 3’	TTCCTTTTTCCTCTTTATTTCCAGAA	
Cytochrome C oxidase	Proprietary CyC1 primer set (Applied Biosystems Mm00470540_m1)	

Supplementary methods

Transgenic lines. Mice bearing a floxed allele of the *Islet1* gene were generated as described in Methods. The Neomycin resistance cassette was removed from the transgene by crossing *Islet*$^{F/+}$ mice to a *FLPeR* deleter strain[1].

To delete *Islet1* specifically in neural crest cell and its derivatives, we interbred *Islet1*$^{F/F}$ mice with *Wnt1-Cre* mice[2, 3] to generate mice with the genotype *Islet1*$^{F/+}$, *Wnt1-Cre*. Crossing *Islet1*$^{F/+}$, *Wnt1-Cre/Wnt1-Cre* mice with *Islet1*$^{F/F}$ mice yielded equal numbers of *Islet1*$^{F/F}$, *Wnt1-Cre* (CKO) and *Islet1*$^{F/+}$, *Wnt1-Cre* (control) embryos. The cre-deleter strain employed for these studies is a conventional transgenic *Wnt1-cre*, which has been shown to mediate *loxP* recombination in the dorsal neural tube and neural crest[4]. Because Wnt1-cre activity is present in the neural tube by E8.5[2], prior to the onset of Islet1 expression in the sensory ganglia, *Islet1*$^{F/F}$, *Wnt1-cre* neurons should never express Islet1.

For late excision experiments, *Islet1*$^{MCM/+}$ mice were interbred with *Islet1*$^{F/F}$ mice to generated litters with equal numbers of *Islet1*$^{MCM/F}$ inducible knockouts (IKO) and *Islet1*$^{F/+}$ controls. To induce excision in *Islet1*$^{MCM/F}$ embryos, pregnant females were given an oral gavage of 300μl of tamoxifen in sesame oil (10mg/ml) at E11.5 and were harvested at E14.5 or E18.5.

For all experiments, *Islet1*$^{F/F}$ mice were maintained homozygous for a *Rosa26-LacZ* reporter gene. In *ROSA26-LacZ* mice, cre-mediated removal of a transcriptional termination cassette allows constitutive expression of beta-galactosidase, marking all cells in which Cre recombinase has been active, and permitting cell lineage and axon tracing[5].

Animal experiments were conducted under protocols approved by the UCSD Animal Care Program and the Veterinary Medical Unit of the San Diego VA Medical Center.

Mating, fixation, X-gal staining. Following matings, noon of the day of the appearance of a mucous plug was taken to be embryonic day 0.5 (E0.5) of gestation. Embryos were also staged according to the system of Theiler[6]. Embryos harvested at different time points of gestation were fixed for 1-2 hours in 4% PFA, using the longer period for later stage embryos. To optimize tissue fixation and penetration of the X-gal substrate, the chest and abdomen were opened before fixation. At E18.5 and P1 the spinal column and attached DRG were dissected and fixed 3 hours. Embryos were stained several hours to overnight at 37°C in X-gal substrate solution consisting of 5 mM $K_4Fe(CN)_6$, 5 mM $K_3Fe(CN)_6$, 2 mM $MgCl_2$, 0.01% NP-40, 0.1% deoxycholate, and 0.1% X-gal (Roche Molecular, Indianapolis) in PBS.

Immunostaining and axonal labeling. For immunostaining embryos or tissues were fixed for 1-3 hours in 4% PFA, embedded in OCT, and sectioned at 15µm. Immunofluorescence was performed with the following primary antibodies: mouse monoclonal anti-Islet1/2 (DSHB), guinea pig anti-Islet 2 (gift of S. Pfaff), rabbit anti-TrkA (gift of L. Reichardt), goat anti-TrkB and anti-TrkC (R&D Systems), goat anti-cRet (IBL), rabbit anti-Runx1 and anti-Islet1 (gifts of T. Jessell) rabbit anti-Runx3 and rabbit anti-Etv1/Er81 (Gifts of S. Arber), Guinea pig anti Sox 10 (gift of M. Wegner),[7] rabbit anti-Brn3a[8] and guinea pig anti-Brn3a[9]. Following incubation with the primary antibody, sections were washed then incubated with the appropriate secondary antibodies

fluorescently labeled with Alexa 488 or 594 (Invitrogen), and mounted in Vectashield (Vector Laboratories).

Caspase-3 expression, a measure of apoptotic cell death, was measured by immunofluorescence using rabbit anti-caspase-3 (Cell Signal Tech. #9661) Cryostat sections of the DRG and TG of the appropriated developmental stages were cut at 10μm, and every fourth section was stained and the caspase3^{+} cells were counted. At least five sections were analyzed per ganglion, and the mean and SD of the counts determined. A two-tailed T-test was used to determine the significance of differences in apoptotic cell number between control and Islet1 CKO ganglia.

Microarray and qPCR analysis. Microarray analysis was performed using cervical DRG from E12.5 embryos of genotype *Islet1*$^{F/F}$, *Wnt1-Cre* (CKO) and *Islet1*$^{F/F}$, *Wnt1-Cre* (control). The presence of a *Rosa26-LacZ* allele allowed the embryos expressing *Wnt1-Cre* to visually genotyped by rapid Xgal staining, and only the positive embryos were used for dissections. For studies of delayed excision, ganglia were harvested from tamoxfen-induced *Islet1*$^{MCM/F}$ (IKO) and *Islet1*$^{F/+}$ controls at E14.5. Each sample consisted of the pooled cervical/brachial-level DRG from four embryos, and two independent samples for each genotype were generated. Dissected ganglia were placed immediately in RNAlater (Qiagen) and stored overnight at 4C. The following day, RNAlater was removed and the ganglia were frozen at -80C until the completion of genotyping.

The ganglia from individual embryos with the same genotype were pooled at the time of RNA extraction. RNA was prepared using the RNeasy Mini kit (Qiagen) following the manufacturer's instructions. Prior to extraction the ganglia were

homogenized in the provided lysis buffer using an Eppendorf pestle, then processed with a QiaShredder column (Qiagen). The quality of the resulting RNA was verified by the relative intensity of rRNA bands using a Agilent 2100 Bioanalyzer, and RNA was quantified using a Nanodrop ND-1000 spectrophotometer.

For microarray analysis, aRNA was synthesized from 0.5 ±0.1μg of total RNA, using the Message Amp II kit (Ambion), following the manufacturer's instructions for a one-step amplification. Yields of biotin-labeled aRNA ranged from 75-100μg per sample, and 15μg was used for hybridization to Affymetrix Mouse Genome 430 2.0 array. For exon-specific qPCR analysis of Islet1 expression, 0.1μg of the aRNA used for microarray analysis was reverse-transcribed with the Thermoscipt RT-PCR system (Invitrogen) using random hexamer priming following the manufacturer's instructions. Each PCR reaction used 1% of the resulting cDNA samples, and quantitative PCR was performed using SYBR green detection. Primer pairs for exon specific PCR appear in Table S3.

In situ hybridization. Methods used for non-isotopic in situ hybridization and probes for mouse NeuroD4 and Tcfap2b[10], mouse Lhx1 (Lim1) [11], and rat Lhx2 (Lh2a, effective in mouse)[12], and Lbxcor1[13] have been described.

Statistical methods. Statistical analysis of microarray data was performed using Mas 5.0 software (Affymetrix) as previously described[10]. Statistical tests used for cell counts are described in the corrsponding figure legends.

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