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

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## Abstract

We have used conditional knockout strategies in mice to determine the developmental events and gene expression program regulated by the LIM-homeodomain factor Islet1 in developing sensory neurons. Early development of the trigeminal and dorsal root ganglia are grossly normal in the absence of Islet1. However, from E12.5 onward, *Islet1* mutant embryos exhibit loss of the nociceptive markers TrkA and Runx1 and a near absence of cutaneous innervation. Proprioceptive neurons characterized by the expression of TrkC/Runx3/Etv1 are relatively spared. Microarray analysis of *Islet1* mutant ganglia reveals prolonged expression of developmental regulators normally restricted to early sensory neurogenesis, and ectopic expression of transcription factors normally found in the CNS but not in sensory ganglia. Later excision of *Islet1* does not reactivate early genes, but results in decreased expression of transcripts related to specific sensory functions. Together these results establish a central role for Islet1 in the transition from sensory neurogenesis to subtype specification.

The peripheral sensory nervous system conveys information about the external world to the CNS, and is organized according to the location and modality of the sensory input. The general somatic senses include pain, touch, temperature and position, and are transduced by sensory neurons innervating the skin and musculoskeletal structures, termed nociceptors, mechanoreceptors, thermoreceptors and proprioceptors, respectively. At spinal levels, general somatic sensation is conveyed by the dorsal root ganglia (DRG), while in the anterior head and face, these sensory modalities are transmitted by the trigeminal ganglia (TG).



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DRG neurons are derived from neural crest, while cranial sensory ganglia are derived from both neural crest and specialized placodes within the embryonic surface ectoderm. At all axial levels, the initial phase of sensory neurogenesis is dependent on the bHLH factors neurogenin1 (Neurog1) and neurogenin2 (Neurog2), with the expression and functional importance of Neurog1 predominating in the TG and DRG and Neurog2 playing a dominant role in sensory ganglia derived from the epibranchial placodes1, 2. In all sensory neurons, the expression of Neurog1 and/or Neurog2 is followed by the bHLH factors Neurod1 and Neurod4 (Math3), which are dependent on the neurogenins2.

The neurogenic phase of sensory development is followed by cell cycle exit, axon growth, and the expression of genes characteristic of neuronal function. Coincident with these events, beginning at E9.5–10.5 in mice, nearly all general sensory neurons co-express the pan-sensory homeodomain transcription factors Islet1 and Brn3a (product of the *pou4f1* gene)3. In mouse embryos lacking Brn3a, the DRG and TG exhibit defective axon growth, show abnormal persistence of early developmental transcription factors, and decreased expression of markers of multiple sensory subtypes4–6. Islet1 has been shown to be critical for early development of motor neurons7, but an essential role for this factor in cardiac development leads to embryonic death at approximately E108–10, and has prevented any significant examination of the role of Islet1 in sensory neurogenesis.

The terminal phase of sensory differentiation is characterized by the expression of developmental regulators that characterize distinct sensory modalities3, 11, 12. The neurotrophin receptors TrkA, TrkB and TrkC (products of the *Ntrk1, Ntrk2* and *Ntrk3* genes) are preferentially expressed in, and essential for the survival of, pain, touch and position-sensing neurons, respectively12. The runt family transcription factor Runx1 is expressed in nociceptors, and is required for the transition of $TrkA^+$ sensory precursors to $Ret^+$ /$TrkA^-$ non-peptidergic neurons14, 15 and for the expression of sensory receptors of the TRP and Mrg families13 In contrast, the related factor Runx3 is required for the expression of proprioceptor markers in the DRG, and for the correct innervation of proprioceptor targets in the spinal cord and periphery16, 17. However, the relationship of the pan-sensory transcription factors to these subsequent events remains largely unknown.

To better understand the transition from sensory neurogenesis to subtype specification, we have used tissue-specific Cre-mediated recombination to excise *Islet1* in developing sensory precursors, and bypass the early embryonic lethality seen in constitutive *Islet1* knockouts. Initial neurogenesis appears normal in conditional *Islet1* knockout (CKO) sensory ganglia, but by embryonic day 12.5, excess apoptosis is observed, and at later stages ganglion size is markedly diminished. *Islet1* CKO embryos exhibit a profound loss of cutaneous innervation, and markers of neurons mediating pain and touch, including TrkA, TrkB, and Runx1, are markedly reduced, while mediators of proprioceptor development, such as TrkC and Runx3, are relatively preserved. Analysis of global gene expression in the DRG of *Islet1* CKO embryos reveals that Islet1 is required to terminate the expression of key regulators of the neurogenic phase of sensory development. Unexpectedly, Islet1 also acts as a repressor of transcription factors normally expressed in the spinal cord and hindbrain but not in sensory neurons. Delayed excision of *Islet1* using a tamoxifen-inducible system demonstrates that the early role of Islet1 in repression of neurogenic factors is separable from its later role as

an activator of functional sensory systems. Together these results define a gene expression program regulated by Islet1 that occupies a pivotal position in the hierarchy of sensory development.

## RESULTS

### Generation of Islet1 conditional knockout mice

To examine the initial formation of the sensory ganglia in mice lacking Islet1, we first interbred mice carrying a null allele of *Islet1*7 with mice carrying a *tauLacZ* reporter integrated into the *pou4f1* locus, *Brn3a*$^{tauLacZ}$, in which βgalactosidase (βgal) is expressed throughout the sensory nervous system18. We then interbred *Islet1*$^{+/-}$, *Brn3a*$^{tauLacZ}$ mice with *Islet1* heterozygotes to yield *Islet1*$^{-/-}$, *Brn3a*$^{tauLacZ}$ embryos. As expected, these embryos were growth arrested from approximately E9.57, and examination of *Islet1*$^{-/-}$ embryos at E10.5 revealed extensive necrosis (Fig. S1A). However, βgal staining showed that the DRG and TG had condensed and differentiated appropriately until this stage.

To overcome this early embryonic lethality, we adopted a conditional knockout strategy. A "floxed" *Islet1* allele (*Islet1*$^{F}$) was generated in which *loxP* sites were inserted into the introns flanking exon 4 of the *Islet1* locus, which encodes the Islet1 homeodomain (Figure S1B). 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 (Methods). Because *Wnt1-cre* activity is present in the neural tube by E8.5, prior to the onset of Islet1 expression in the sensory ganglia, *Islet1*$^{F/F}$, *Wnt1-cre* neurons should never express Islet1. Unless noted, comparisons were made between *Islet1*$^{F/F}$, *Wnt1-cre* conditional knockout (CKO) embryos and *Islet1*$^{F/+}$, *Wnt1-cre* controls. To identify cells in which cre-recombinase is active, and to trace the projections of neurons in which recombination has taken place, a Rosa26-LacZ allele was included in all experimental mice (Methods). In E12.5 DRG of Islet1 CKO embryos expression of the targeted exon was less than 1% of that seen in control ganglia, indicating nearly complete excision, and effective loss of Islet1 protein expression was also confirmed by immunofluorescence (Fig. S1C,D).

Newborn *Islet1* CKO mice appeared normal in gross morphology, but died within a few hours after birth. Body turning and limb movement of knockout mice were essentially indistinguishable from that of control littermates. However, *Islet1* CKO pups presented a reduced or minimal response to a mild noxious stimulus applied to the skin of the trunk or limbs, suggestive of deficits in cutaneous sensation.

Whole-mount βgal staining of *Islet1* CKO and control embryos showed that the DRG and TG condensed normally in mutant embryos, and at E11.5 the sensory ganglia were not obviously different from controls in whole-mount preparations (Fig. 1A). Islet1 and Brn3a are expressed in terminally differentiating sensory neurons, whereas Sox10 is expressed in sensory precursors, and is not normally co-expressed with Islet1 in developing sensory ganglia (Fig. 1B). Thus failure to correctly initiate the terminal differentiation of sensory neurons might lead to persistent expression of Sox10, and/or a failure to initiate expression of Brn3a. However, DRG neurons of CKO embryos initiated expression of Brn3a normally, and did not co-express Sox10 and Brn3a (Fig. 1C).

By E14.5 the DRG of *Islet1* CKO embryos were markedly smaller than those of controls (Fig. 1D,E). Because Brn3a expression is minimally affected in *Islet1* CKO ganglia, we used Brn3a as a marker for counting the differentiated sensory neurons in CKO and control ganglia. Although not significantly different at E11.5, the total neuron population was markedly lower in CKO ganglia by E14.5 (Fig. 1F). Because neurogenesis takes place in the DRG from E10 to E1319, the reduced size of the sensory ganglia in *Islet1* CKO embryos at midgestation could be owing either to reduced generation of late-born sensory neurons or increased cell death. Increased apoptosis was confirmed by immunostaining with antibody to activated caspase-3 (Fig. 1G, Fig. S2), which showed that in the TG cell death is significantly increased at E11.5 and E12.5 in CKO embryos relative to control littermates, and in the DRG cell death is increased at E12.5. At E14.5 caspase3 labeling was comparable in *Islet1* CKO and control DRG, but in the context of diminished cell number in the mutant embryos. Thus it is likely that increased cell death between E12.5–E14.5 is the principal cause of the decreased size of the DRG in *Islet1* CKO embryos, although some contribution from reduced generation of late-born neurons cannot be excluded.

The sensory components of the peripheral nerves were specifically labeled by the expression of βgalactosidase in *Islet1* CKO and control embryos. Overall, the peripheral nerves were diminished in CKO embryos, and the pattern of labeling indicated a modality-specific defect in sensory innervation. At thoracic levels in E14.5 embryos, the spinal nerves could be identified intercostally in CKO mice, but the cutaneous branch of the ventral ramus was absent, consistent with a complete loss of cutaneous sensory fibers subserving pain, touch and temperature (Fig. 1H), which is likely to be due at least in part to the neuronal death observed in the ganglion.

Detailed examination of the innervation of the distal limbs at E14.5 confirmed a nearly complete loss of fine cutaneous sensory fibers (Fig. 1I). Innervation of the central part of the forepaw and hindpaw, which contain intrinsic muscles that receive proprioceptive innervation, was preserved. Also preserved was a single sensory branch innervating one side of digits 1,2 and 5 in both the forelimb and hindlimb. To determine the nature of these spared sensory axons, we examined the expression of TrkA and TrkC, which mark nociceptive and proprioceptive sensory neurons, respectively, in digit 5 of control and CKO embryos (Fig. 1J). In controls, the sensory axon bundles in both the medial and lateral aspect of digit 5 were immunoreactive for TrkA and TrkC, indicating that they contain mixed sensory fibers. In CKO animals, the medial branch to digit 5 was absent, and the lateral branch was immunoreactive only for TrkC. These results indicate that the persisting sensory fibers in CKO embryos emanate from a subset of $TrkC^+$ proprioceptors.

## Selective loss of TrkA and TrkB neurons *Islet1* knockout DRG

To assess the role of Islet1 in sensory subtype specification, we examined expression of TrkA, TrkB and TrkC in the DRG of *Islet1* CKO embryos and control littermates across development. In control embryos TrkA was detected from E11.5, and was extensively expressed at subsequent stages (Fig. 2A–D). TrkC was also widely expressed in the DRG at E11.5, and at this stage showed significant overlap with TrkA. However, consistent with

prior studies, from E12.5 onward TrkC was much more restricted in its expression, and the TrkA and TrkC expressing neurons were distinct populations20.

At E11.5, TrkA expression in the DRG of *Islet1* CKO embryos was comparable to controls, but by E12.5 it was significantly reduced (Fig. 2A,B,E). TrkC was not detected until E12.5, a delay of at least two developmental days relative to its normal onset of expression16. By E14.5, the number of $TrkA^+$ neurons in the *Islet1* CKO DRG was reduced to less than one-third of that observed in controls (Fig. 2C,E). $TrkC^+$ neurons were relatively spared, and appeared more dense relative to controls due to loss of ganglion volume (Fig. 2C,F). The few $TrkA^+$ neurons that survived to birth in mutant DRG expressed levels of TrkA similar to controls (Fig. 2D). Furthermore, no significant overlap in the expression of TrkA and TrkC was observed in mutant DRG neurons at later stages (Fig. 2C,D), suggesting that segregation of these two major neuronal subsets proceeds normally in the absence of Islet1.

Many $TrkC^+$ proprioceptors are born in an early wave of Ngn2-dependent neurogenesis, which is later compensated by Ngn1 if Ngn2 is absent1. The delayed expression of TrkC raised the possibility that the first wave of neurogenesis might be defective in *Islet1* CKO ganglia. To determine whether early TrkC neurogenesis takes place in *Islet1* CKO DRG, we injected pregnant mice with bromodeoxyuridine (BrdU) at E10.5, and examined the incorporation of the label in $TrkC^+$ neurons at E15.5 . Total BrdU incorporation and specific labeling of TrkC neurons at E10.5 was not significantly different in *Islet1* CKO ganglia and controls (Fig. S3), indicating that lack of Islet1 results in a delay in TrkC expression *per se*, rather than a delay in neurogenesis.

TrkB expression characterizes subsets of cutaneous mechanoreceptor neurons21. In *Islet1* CKO embryos, $TrkB^+$ neurons were detectable at E12.5, but reduced compared to controls (Fig. 2G). By E14.5, $TrkB^+$ neurons were markedly reduced (Fig. 2H), and similar results were obtained at P1 (data not shown).

TrkA and TrkC immunoreactivity were also used to examine the central projections of DRG neurons in the spinal cord. In control embryos at E14.5, $TrkA^+$ axons projected into the superficial layer (laminae I and II) whereas $TrkC^+$ afferents entered the spinal cord at a more medial position, and projected ventrally (Fig. 2I). In *Islet1* CKO embryos no TrkC expression in the dorsal root was detected at E11.5, consistent with the delay in TrkC expression observed in the ganglion, but $TrkA^+$ central projections were relatively normal (Fig. 2A). At E12.5 and E14.5, $TrkA^+$ central projections were greatly reduced (Fig. 2B,J). The majority of the remaining $TrkA^+$ fibers were appropriately confined to the superficial layers of the spinal cord, although aberrantly located fibers were also detected. In contrast, $TrkC^+$ central projections penetrated into the deep lamina in a manner similar to controls.

## Islet1 regulates subtype-specific transcription factors

We next examined *Islet1* CKO embryos and controls for expression of key transcription factors known to regulate or interact with Trk receptors in sensory subtype specification. Prior work has shown that TrkA expression in the nociceptive population is intimately related to the transcription factor Runx1. In prenatal DRG development, most TrkA neurons express Runx1, and at P1 there is still a high degree of overlap. Postnatally, most $Runx1^+$

sensory neurons downregulate TrkA and instead express Ret. In Runx1 knockouts, expression of TrkA and its co-localization with Ret are expanded13, 15, 22.

Runx1 expression was detected beginning at E12.5 in control DRG, and was dramatically reduced in *Islet1* CKO mice at all developmental stages examined (Fig. 3A–C). As expected from prior studies, at P1 control ganglia had substantial populations of both $Runx1^+/Ret^+$ and $TrkA^+/Ret^+$ neurons. In the DRG of *Islet1* CKO embryos, both populations were markedly diminished, but the extent of co-expression of these markers was not affected (Fig. 3D).

We then examined the expression of Runx3 and the ets-domain transcription factor Etv1, both markers of proprioceptor populations (Fig. 3E–K). Runx3 and Etv1 were co-expressed with Islet1 in a subset of DRG neurons at E11.5 (Fig. 3E,J). In contrast to the delayed expression of TrkC, Runx3 was expressed in *Islet1* CKO ganglia at E11.5 and Runx3 neurons were only slightly reduced compared to controls, indicating that the initiation of Runx3 expression is independent of both Islet1 and TrkC (Fig. 3E–G). By E12.5 nearly all of the early Runx3 neurons co-expressed TrkC in control and CKO ganglia (Fig. 3H). Runx3/TrkC and Etv1/TrkC neurons were spared in CKO mice at E14.5 (Fig. 3I,K), and neurons expressing these markers comprised much of the vestigial ganglion remaining at P1 (Fig. S4).

We next considered possible mechanisms by which Runx3/Etv1/TrkC-expressing proprioceptors might escape Islet1 dependence. These early-born neurons could represent a class of sensory neurons which do not express Islet1, and therefore do not require it, or they could be spared by a redundant function of the closely related factor Islet2, which is also expressed in the DRG. Although at E11.5 nearly all $Etv1^+$ DRG neurons co-expressed Islet1, by E14.5 there was almost no overlap between Islet1 and Runx3 or Etv1 expression (Fig. 3L), indicating that these proprioceptors rapidly downregulate Islet1 as they mature. Islet2 expression follows Islet1 by about one developmental day, and at E12.5, Islet2 was expressed in a significant fraction of $Etv1^+$ neurons (Fig. S4B). However, Etv1 and Islet2 were not co-expressed at E14.5 in control or CKO ganglia (Fig. S4C). Thus $TrkC^+/Runx3^+/Etv1^+$ proprioceptive neurons rapidly downregulate Islet factors as development progresses and subsequently develop by an Islet-independent pathway.

## Global gene regulation by Islet1 in the sensory ganglia

To better understand the program of gene expression regulated by Islet1, we performed microarray analysis to examine changes in transcript levels in the DRG of CKO embryos compared to littermate controls. For these experiments, E12.5 embryos were chosen because it is the last stage at which the DRG do not exhibit a profound reduction in size resulting from apoptosis. Replicate assays of ganglia of the same genotype were highly reproducible, and comparison of *Islet1* control and CKO ganglia exhibited little variation for the large majority of transcripts, but a select number were markedly increased or decreased (Fig. S5).

Among the most-changed transcripts (Table 1 and Table 2), the majority of those with known expression patterns and functions were transcription factors or other kinds of developmental regulators. Other highly changed transcripts encode proteins which mediate

neural transmission, including channels, neuropeptides and receptors, participate in intracellular signal transduction, or play roles in axon, neurite or synapse formation. None of the highly changed transcripts represented “housekeeping” genes mediating general cellular processes, and the expression of most genes which are widely expressed in the nervous system, such as the neurofilaments, were also not significantly changed (Table 1).

The microarray results clearly demonstrate that the DRG of *Islet1* CKO mice fail to correctly execute a gene expression program characteristic of nociceptor differentiation. The nociceptive neuropeptides substance P (Tac1) and CGRP (Calra) were not yet expressed at E12.5. However, several pain-mediating channels and receptors expressed at this stage were among the most decreased transcripts (Table 2), including those encoding the sodium channel Nav1.8 (Scn10a), the neuropeptide galanin, the capsaicin receptor, Trpv111 and the Bv8 receptor/prokineticin receptor-1 (Prokr1), which partners with Trpv123. Microarray analysis also confirmed a marked decrease in TrkA and TrkB expression, while TrkC was not significantly changed (Fig. 4A). In addition, the nociceptive marker Runx1 was profoundly downregulated, while Runx3 was relatively spared. Multiple members of the Ets family, including Etv1, Etv4 (Pea3) and Etv5 (Erm) were also significantly decreased, but not absent, in CKO ganglia.

Microarray and RNA in situ analysis of *Islet1* CKO ganglia also revealed increased expression of transcription factors associated with early sensory neurogenesis (Fig. 4B,C, S6A). These included the neurogenic bHLH factor Neurog1 (Neurogenin1), known to be essential for early steps in sensory neurogenesis1, the related bHLH factors Neurod1, Neurod4 and Neurod6, and the Zn-finger transcription factor insulinoma-associated 1 (Insm1, IA-1)24. Transcripts of multiple genes of the HoxA, B, and C clusters were increased, generally in the range of 1.5–2.5 fold (Fig. S6B). The increased expression of these genes, all of which normally exhibit strong expression in early sensory development and then decline with maturation, clearly results from a failure in their developmental repression. Changes in gene expression in the TG of *Islet1* CKO embryos closely paralleled the DRG for both the decreased and increased transcripts, despite the incomplete excision of the *Islet1* gene observed in the TG (Fig. 4C).

We also identified a second class of increased transcripts which are not normally expressed in the sensory ganglia at any phase of development (Fig. 5). These genes include the LIM-homeodomain factors Lhx1 (Lim1) and Lhx2 (LH2A), the transcriptional co-regulator Lbxcor1, and the bHLH factors Olig1 and Olig2. Although these factors are not expressed in the DRG, each has a known role in spinal cord development (Discussion). The transcription factor Tcafp2b (Ap2β, Fig. 5B) has characteristics of both classes of increased transcripts, in that it is normally expressed in early sensory development as are the bHLH genes, and is also expressed in postmitotic spinal neurons at E12.5. Lbxcor1 and Tcafp2b were strongly expressed only in a subset of DRG neurons in CKO embryos (Fig. 5B, enlarged views), perhaps owing to the activity of redundant repressive factors in the neurons which do not upregulate these genes.

Changes in gene expression in the TG closely followed those observed in the DRG for this class of increased genes. Lhx2 and Lbxcor1 were not expressed in the TG of E12.5 control

embryos (Fig. 5C), but were activated in the *Islet1* CKO TG, and were also expressed in the adjacent hindbrain. Tcafp2b also showed increased expression in *Islet1* CKO TG from low basal levels in control ganglia (Fig. 5C). Thus in the absence of Islet1, developing sensory neurons de-repress a gene expression program common to the spinal cord and hindbrain.

## Distinct early and late roles for Islet1

The changes in gene expression observed in sensory neurons lacking Islet1 from the onset of development imply a role as a repressor of neurogenic genes, and also as an activator of sensory-specific phenotypes. However, the loss of sensory specific markers at later stages could be due in part to the death of subsets of neurons. In order to better distinguish early and late roles of Islet1, and to discriminate between specific gene regulation and the effects of cell loss, we adopted an delayed excision strategy using a tamoxifen-inducible MerCreMer recombinase targeted to the *Islet1* locus (*Islet1*$^{MCM}$)25. *Islet1*$^{MCM/+}$ mice were interbred with *Islet1*$^{F/F}$ mice to produce *Islet1*$^{MCM/F}$ induced knockout (IKO) and *Islet1*$^{F/+}$ control ganglia. Cre-mediated excision was induced at E11.5, approximately two days after the onset of Islet1 expression in the TG and cervical DRG. Like *Islet1* CKO mice, the IKO mice died in the perinatal period. The DRG and TG of the induced knockout embryos were analyzed at E14.5 and at E18.5, just prior to birth.

Immunofluorescence for Islet1 protein (Fig. 6A–C) in the DRG and qPCR analysis of the targeted exon (Fig. 6F) in the TG indicated generally efficient excision of the Islet1 homeodomain, with some residual Islet1$^{+}$ cells. In contrast, Islet2 expression was preserved in the IKO DRG (Fig. 6D,E). Microarray analyses of the TG of E14.5 IKO and control embryos revealed that the early neurogenic genes (Neurog1, Neurod1, Neurod4) and mediators of spinal cord development (Lhx1, Lhx2, Lbxcor1, Olig1) which were markedly increased in the *Wnt1-Cre*-mediated CKO sensory ganglia were not increased in E14.5 IKO ganglia (Table S1), demonstrating that 2–3 days of Islet1 expression at the onset of neurogenesis is sufficient to permanently repress these genes.

Transcripts which show decreased expression in *Islet1* CKO ganglia had a mixed expression pattern in the IKO TG at E14.5 (Table S2). Expression of TrkA and TrkB was maintained normally after delayed excision. Transcripts for several genes which define sensory phenotypes were significantly decreased, including, galanin, tyrosine hydroxylase, receptor channel TrpV1 and serotonin receptor 5HTR3a ($p>0.998$, Fig. 6G, Table S2). Analysis of the full set of transcripts changed at E14.5 revealed Islet1 dependence of several other neural genes which had not been detected at E12.5 (Fig. 6H), including serotonin receptor 5HTR3b (Htr3b), synaptoporin (Synpr), sensory-specific sodium channel NaV1.9 (Scn11a), metabotropic glutamate receptor mGluR7 (Grm7), and the nociceptor-associated carboxypeptidase inhibitor latexin (Lxn).

In order to assess whether ongoing Islet1 expression is necessary to maintain sensory survival, we also examined *Islet1* IKO DRG at E18.5 (Figure 7). Neuron number did not differ significantly between *Islet1* IKO ganglia and controls at this stage, Runx1 and TrkA were expressed in a pattern similar to controls, and the expression of Drg11 was maintained Fig. 7E–J). However, the numbers of neurons expressing TrpV1 and the menthol receptor TrpM8 were markedly decreased (Fig. 7K–N). Taken together, the induced knockout results

show that a set of Islet1 functions, including the repression of neurogenic and CNS-specific transcription factors, as well as maintenance of TrkA, TrkB, and cell survival in the nociceptor lineage, are unique to the early phase of Islet1 expression. In contrast, continued expression of Islet1 is required to maintain the expression of numerous genes that mediate specific sensory functions.

## DISCUSSION

### Pan-sensory homeodomain factors and the gene regulatory program of sensory development

Islet1 is expressed at the transition from neurogenesis to terminal differentiation in sensory neurons at all levels of the neural axis, including the trigeminal ganglion, mesencephalic trigeminal, hindbrain sensory ganglia and DRG. In the present study we have used a conditional *Islet1* knockout model to bypass cardiac lethality and show that mice lacking Islet1 have profound deficits in the sensory innervation of the CNS and periphery, extensive changes in sensory gene expression, and markedly increased sensory apoptosis, with relative sparing of proprioceptor neurons.

Throughout the sensory system, Islet1 is co-expressed with another pan-sensory factor, the POU-homeodomain factor Brn3a3, 26. These factors share a common role in the repression of early sensory transcription factors, but their effects on the repression of CNS gene expression programs and on specific downstream sensory phenotypes are quite distinct. Furthermore, the common functions of Brn3a and Islet1 do not result from cross-regulation of these genes. Brn3a expression is unaltered in the DRG of *Islet1* CKO mice, and Islet1 expression does not significantly change in the *Brn3a* null DRG and TG5, 6. Thus Brn3a and Islet1 have independent roles which intersect at the target gene level for a subset of their regulatory functions.

The principal common role of both factors is to terminate gene expression programs characteristic of early sensory precursors. At E12.5–E13.5, Neurod1 and NeuroD4 exhibit increased expression in both *Islet1* and *Brn3a* null mice, due to a failure in the normal developmental downregulation of these genes, which normally decline by midgestation5, 6, 27. In *Islet1* knockouts, expression of Neurog1, which precedes and is required for expression of the NeuroD class, also persists abnormally. Clearly related to this pathway is the increased expression of the Zn-finger factor Insm1 in both knockouts, which is known to interact with Neurod128. In the DRG, *Islet1* and *Brn3a* mutants also both fail to developmentally downregulate multiple members of the Hox A, B and C classes6.

### Islet1 and the differentiation of sensory subtypes

Although Islet1 and Brn3a have similar functions in the repression of early neurogenic factors, their roles in the development of sensory subtypes are distinct, with nociceptors and proprioceptors having greater dependence on Islet1 and Brn3a, respectively. Because Islet1 and Brn3a are initially expressed in all sensory precursors, they are unlikely to act as a selective signals for subtype differentiation. Instead, it is likely that Islet1 and Brn3a are permissive for the differentiation, or required for the survival, of specific subtypes.

In the nociceptive lineage of *Islet1* CKO mice, TrkA expression is initiated normally, but by E12.5 the number of $TrkA^+$ neurons and TrkA expression levels are markedly reduced, and few $Runx1^+$ neurons are detected at any stage. Because Runx1 is not dependent on TrkA for its initial expression15, the loss of Runx1 expression is probably not mediated solely by reduced levels of TrkA. Conversely, although misexpression of Runx1 can induce TrkA expression in sensory precursors29, TrkA is not dependent on Runx113, suggesting that Islet1 is upstream of both of these factors. TrkB expression levels are also markedly reduced in *Islet1* CKO ganglia, indicating a loss of cutaneous mechanoreceptors21.

In contrast to TrkA, *Islet1* CKO ganglia show selective sparing of $TrkC^+$ neurons, and most of the surviving $TrkC^+$ neurons also express Runx3 and Etv1, markers characteristic of early-born Ia proprioceptors1, 12. These neurons initially express Islet1 and Islet2, but rapidly downregulate Islet expression, and appear to subsequently develop by an Islet-independent pathway. These subtype-specific deficits in *Islet1* knockout mice are clearly distinct from those seen in Brn3a null embryos, in which sensory ganglia exhibit early loss of TrkC30, 31 and Runx35, 6 expression and loss of proprioceptive innervation of central and peripheral targets31, 32, and in which changes in the expression of TrkA, TrkB and Runx1 are relatively late and of smaller magnitude5, 30.

Islet1 is also required for the normal expression of all members of the “Pea3 group” of Ets-family transcription factors in the DRG, which in addition to Etv1 includes Etv4 (Pea3) and Etv5 (ERM). Etv1 expression is maintained in $TrkC^+/Runx3^+$ proprioceptors in the DRG of *Islet1* DKO mice, but overall Etv1 mRNA expression is reduced to ~40% of normal. In addition to the large Ia proprioceptors, Etv1 is expressed in small, later-developing neurons which co-express Islet26, and the decrease in Etv1 expression probably represents the loss of this population. Expression of Etv4 and Etv5 has been described in the neural crest33 and sensory ganglia34, and Etv4 has a known role in motor neuron development, but the specific functions of these factors in sensory development are not known. There is some evidence for redundancy of function in this gene class35, and understanding the specific roles of Etv4, Etv5, and the late expression of Etv1 may require analysis of compound loss-of-function embryos.

Islet1 does not appear to affect TrkA expression via regulation of the Kruppel-like factor 7 (Klf7), which is expressed in the DRG and TG from the time of ganglion condensation and has been shown to have synergistic effects with Brn3a in the regulation of TrkA36. Klf7 expression is only modestly changed in the mid-gestation DRG of *Islet1* CKO and *Brn3a* KO mice (10–30% decrease, data not shown), and Brn3a expression also appears to be normal in *Klf7* knockout mice36. However, the expression of Islet1 in *Klf7* knockout mice has not been reported, and given the profound effects of Islet1 on TrkA expression, it may interact with or mediate the effects of Klf7.

Islet1 is also required for the normal expression of Drg11 (*Prrxl1* gene product) a homeodomain protein expressed in peptidergic and nonpeptidergic nociceptors of the TG and DRG, and in their primary targets in the CNS, the trigeminal nucleus and spinal cord dorsal horn37, 38. Drg11 null mice exhibit delayed and defective nociceptive innervation of

the dorsal spinal cord37, and loss of Drg11 expression may thus cause some of the defects in CNS innervation observed in *Islet1* null mice.

## Islet1 function links sensory and spinal gene expression programs

*Islet1* CKO mice exhibit derepression of multiple genes which have known roles in spinal cord development, but are not normally expressed in sensory ganglia, including Lhx1, Lhx2, Lbxcor1, Olig1 and Olig2. The relationship between Islet1 and Lhx1 is especially instructive. In the spinal cord, Islet1 has an essential role in the early survival of motor neurons7. Islet1 is initially expressed in all neurons of the lateral motor column (LMC), which innervates the limb musculature. However, as motor differentiation progresses, motor neurons of the lateral subdivision of the LMC, which innervates dorsal muscle groups, activate the expression of Lhx1, and downregulate Islet139. Islet1 and Lhx1 are not co-expressed, and misexpression studies of Islet1 and Lhx1 in this context have shown them to have a mutually repressive interaction40.

The corepressor Lbxcor1 is expressed extensively in the dorsal cord, where it is co-expressed with Lhx1/5 and Brn3a, and in scattered neurons in the ventral spinal cord. In both the dorsal and ventral spinal cord, Lbxcor1$^+$ neurons are interspersed with Islet1$^+$ cells, but their expression is mutually exclusive41. Furthermore, the transcriptional partner of Lbxcor1, Lbx1, represses Islet1 in the dorsal spinal cord41–43. Olig1 and Olig2 are expressed in the spinal motor neuron progenitor (pMN) domain, and are necessary for the generation of motor neurons and oligodendrocytes from this region44. Although Olig2 is required for motor neuron differentiation and the initiation of Islet1 expression, it is rapidly downregulated as motor neurons differentiate45, and forced constitutive expression of Olig2 inhibits Islet1 expression46. It has not been determined whether Islet1 plays an active role in terminating Olig expression in differentiating motor neurons, but the derepression of Olig1/2 in sensory neurons in the absence of Islet1 suggests that this is likely. Together these findings define a common set of targets for Islet1 repression in spinal and sensory neurons.

## Distinct early and late roles of Islet1

Delayed induction of Islet1 excision demonstrates that 2–3 days of early Islet1 expression are sufficient to rescue many but not all aspects of the *Islet1* knockout phenotype. Specifically, transient expression is sufficient to permit cell survival, developmental repression of neurogenic genes, and normal levels of TrkA, TrkB and DRG11. Transient expression of Islet1 also permanently represses the spinal cord/hindbrain gene expression program. However, several genes mediating specific sensory functions are Islet1-dependent in late gestation, including the neuropeptide galanin, receptors including Htr3a, Htr3b, Grm7 and Trpv1, the sodium channel Scn11a, and tyrosine hydroxylase. Thus the neurogenic repressor functions of Islet1 appear to be confined to the early stages of sensory differentiation, but a subset of its activator functions persist.

Multiple mechanisms may contribute to the persisting repression of Islet1 targets in the delayed knockout ganglia. Partial compensation by Islet2 may contribute, because in *Wnt1-cre* mediated *Islet1* knockout ganglia, Islet2 is markedly decreased, but in the late *Islet1* knockout, Islet2 is expressed at normal levels. However, the persistent repression of

neurogenic and CNS gene expression programs in the delayed knockout also suggests that transient expression of Islet1 may induce lasting modifications of chromatin at these loci which maintain a repressed state47.

### Relationship of the role of Islet1 in the nervous system to endocrine and cardiac development

In addition to its key roles in sensory and motor neuron development, Islet1 is required for the development of pancreatic islet cells48 and the cardiac progenitors which contribute a majority of cells to the developing heart8. One of the central questions regarding developmental transcription factors such as Islet1, which show cell-specific expression in diverse tissues, is whether they regulate similar or distinct programs of gene expression in different cell types. Two of the principal regulatory targets of Islet1 in the sensory ganglia, Neurod1 and Insm1, also play key roles in the pancreas, suggesting a conserved core gene regulatory network in sensory and islet cell development.

Neurod1 null mice exhibit developmental apoptosis of β-islet cells, severe diabetes, and neonatal death49. In mice lacking Insm1, development of the pancreatic islet cells is arrested, and the principal products of the α- and β-islet cells, glucagon and insulin, are markedly decreased24. Islet1, Neurod1 and Insm1 are all expressed in the pancreatic primordia from E9.524, 48, 49, and in cell transfection studies, cross regulation of Neurod1 and Insm1 has been described28, 50, but the full regulatory relationship between these factors has not been determined. It will be interesting to see whether similar regulatory relationships pertain in neural and endocrine tissues, and also in cardiac progenitors, in which the downstream targets of Islet1 are yet to be identified.

## METHODS

### Transgenic mice

Mice bearing a constitutive null allele of *Islet1* were a gift of Sam Pfaff 7. The *Brn3a*$^{tauLacZ}$ mouse line has been previously described18. Generation of *Islet1*$^{MCM}$ (*MerCreMer*) mice has also been reported25. Details of the generation of *Islet1*$^{F}$ mice will be reported elsewhere. Briefly, a genomic fragment of encompassing exon 4 of mouse *Isl1* gene was cloned and a Neo-selectable targeting construct was generated in which this fragment is flanked by *loxP* sites (Fig. S1). Embryonic stem (ES) cells were electroporated with this construct and neomycin-resistant ES cell clones were screened for correct targeting of the *Isl1* locus by Southern analysis. Two recombinant clones were used for the blastocyst injection and chimeric mice were crossed to C57BL/6J females to generate heterozygous mice (Islet$^{F/+}$). The Neomycin resistance gene was removed by crossing Islet$^{F/+}$ mice to a FLPeR deleter strain (Supplementary Methods online). *Islet1*$^{F/+}$ mice were intercrossed to generate homozygous floxed *Islet*1 mice (*Islet1*$^{F/F}$).

Methods for genetic crosses, tamoxifen-induced *Islet1* excision, tissue fixation, Xgal staining, immunostaining, in situ hybridization, microarray and Q-PCR analysis and in situ hybridization appear in the Supplementary Methods online. Primers for conventional and real-time genotyping of the floxed Isl1 and *Wnt1-cre* alleles appear in Table S3.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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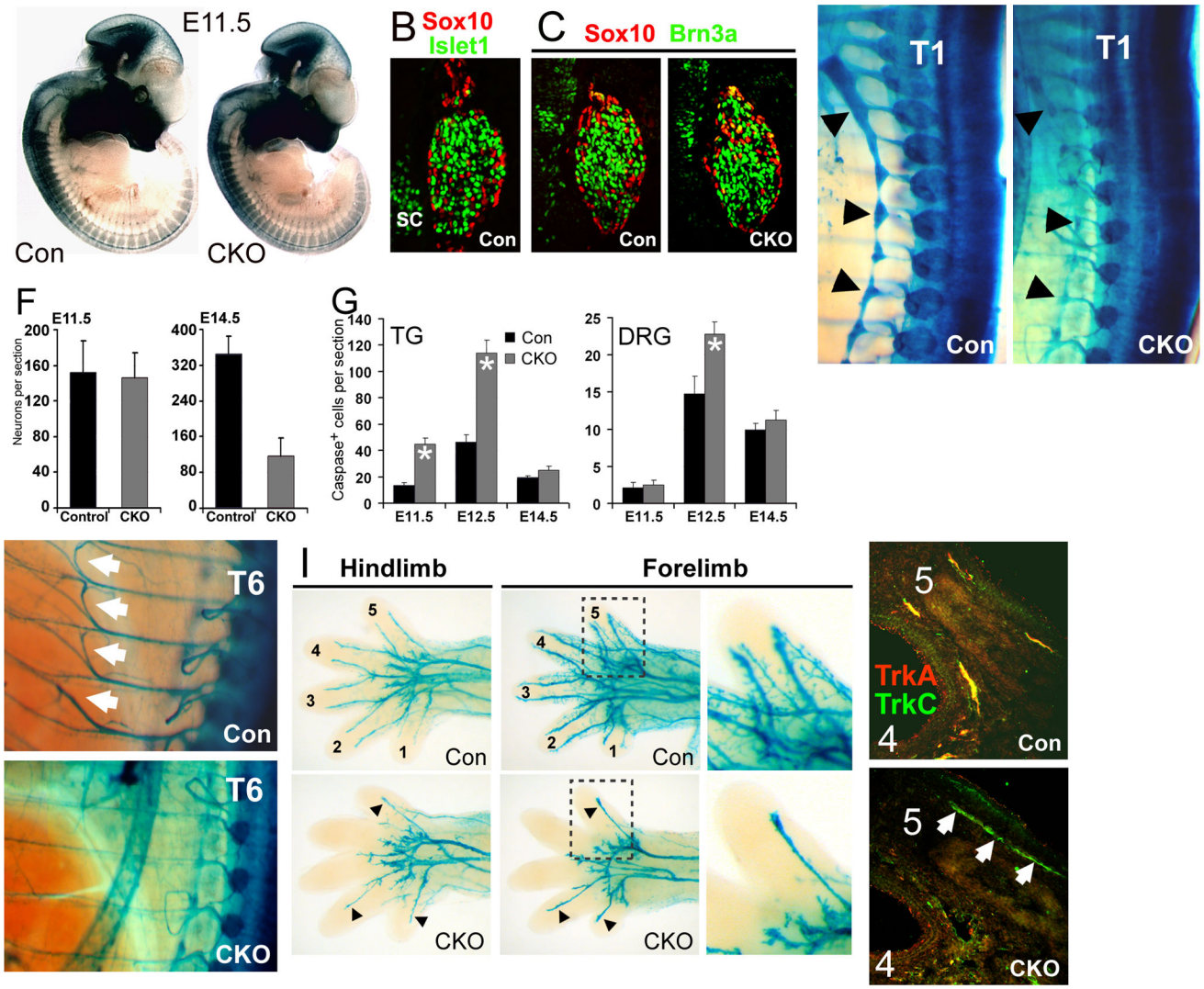


**Figure 1. Defective development of the DRG and spinal nerves in *Islet1* conditional knockout mice**

The sensory ganglia in control and CKO embryos were compared at E11.5 (A–C) and E14.5 (D,E, H–J). Presence of a conditional Rosa26-LacZ allele, activated by *Wnt1-cre*, allowed staining of sensory and autonomic ganglia and their axons in whole embryo preparations. In cranial regions, extensive staining was also seen in other neural crest-derived tissues. (**A**) LacZ staining of E11.5 embryos showing grossly normal morphology and normal-sized DRG in *Islet1* CKO embryos at this stage. (**B**) Islet1 expression in differentiating neurons of E11.5 control ganglia. Islet1$^{+}$ neurons no longer express Sox10, a marker of multipotent precursors and glia. (**C**) Normal initiation of Brn3a expression in E11.5 CKO embryos. Sox10 is characteristic of DRG precursors and is not co-expressed with Brn3a in either genotype. Occasional appearance of co-labeling is due to nuclear overlap. (**D,E**) Thoracic region of whole-mount Xgal stained, hemisected E14.5 embryos, showing marked reduction of the DRG in the CKO specimen. There is also profound reduction of the sympathetic chain

ganglia (arrowheads). (**F**) Brn3a immunoreactivity as a measure of neuronal number in E11.5 and E14.5 DRG. Asterisks indicate statistical significance (t-test) for a given comparison. E11.5, p=0.38; E14.5, p <0.001. **(G)** Increased cell death in the TG and DRG of CKO embryos. Caspase-3 immunoreactive cells were counted in 6–10 slides for each sample. E11.5 TG, p<0.001; E12.5 TG, p<0.001; E12.5 DRG p=0.02. (**H**) The mid-thoracic body wall of a hemisected E14.5 embryo, showing βgalactosidase expression activated by *Wnt1-cre* in the sensory but not the motor component of the intercostal nerves. In CKO embryos, labeling of the intercostal nerves is diminished but detectable, however the cutaneous branches (arrows in top panel) are absent. The esophagus is prominent in the lower panel but is out of the plane of focus in the top panel. (**I**) Innervation of the distal forelimb and hindlimb at E14.5. In CKO embryos fine cutaneous sensory branches are lost throughout the limb (inset views). In the digits, labeled sensory axons persist only in a single fiber bundle in a corresponding position in digits 1, 2, and 5 of both the forelimb and hindlimb (arrowheads). (**J**) Immunofluorescence for TrkA and TrkC in sensory fiber bundles innervating digit 5. In control embryos, sensory fibers are immunoreactive for both TrkA and TrkC. In CKO embryos, the medial axon bundle adjacent to digit 4 is not labeled, and the persisting fiber bundle is immunoreactive for TrkC only (arrows). Legend: 1–5, digits 1–5; SC, spinal cord; T1, T6, thoracic dorsal root ganglion 1, 6. Error bars in all figures indicate mean +/− S.D.

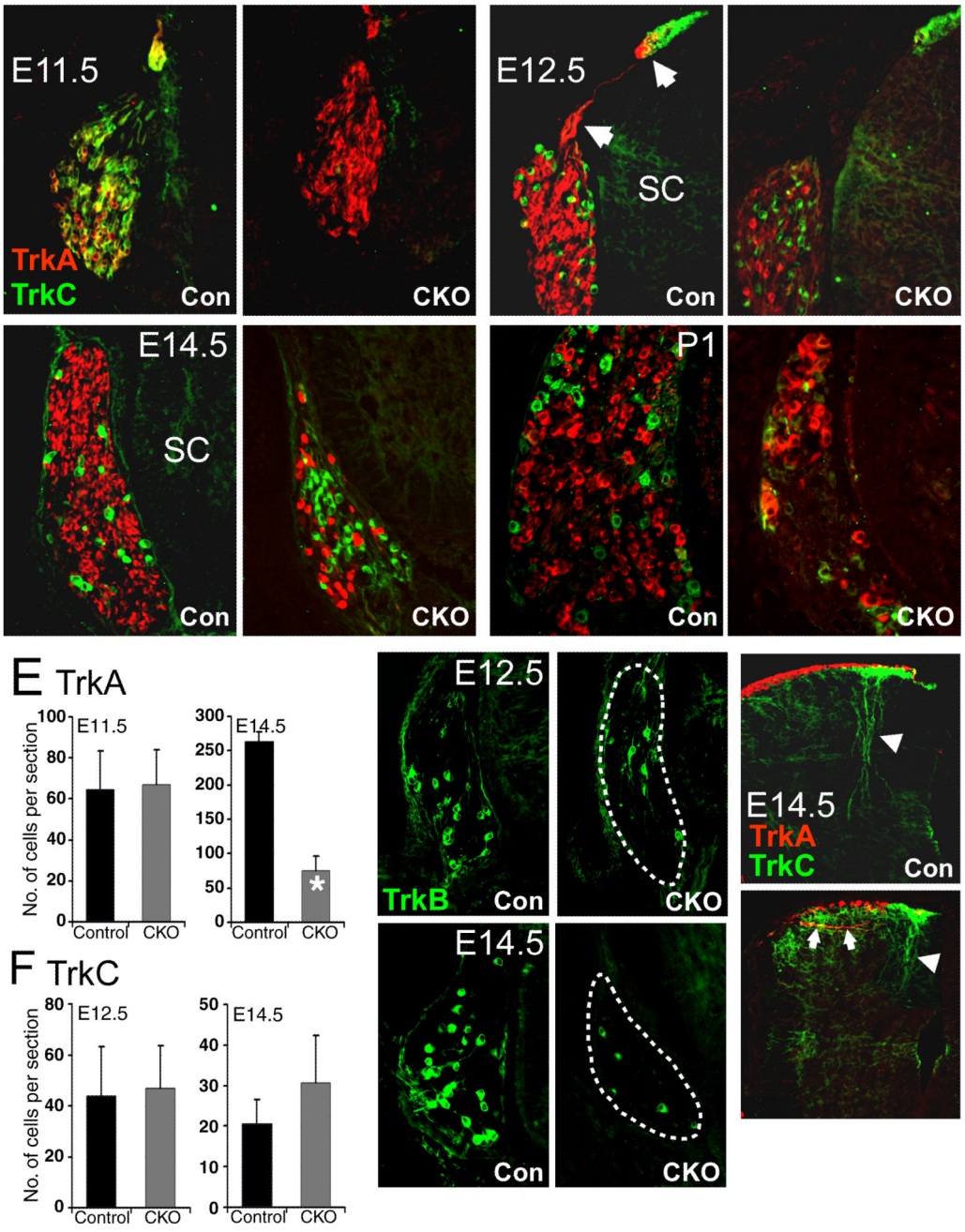


**Figure 2. Neurotrophin receptor expression in sensory ganglia lacking Islet1**

(**A–D**) Expression of TrkA and TrkC in the brachial level DRG of control and CKO embryos of the specified developmental stages. TrkC expression is delayed until E12.5 in *Islet1* CKO ganglia. However, there is a subsequent loss of $TrkA^+$ neurons and relative sparing of $TrkC^+$ cells at later stages. TrkA immunoreactivity is also diminished in sensory fibers in the dorsal root (arrows, B). (**E–F**) Cell counts for TrkA and TrkC immunoreactive neurons. For control versus CKO: TrkA E11.5 p=0.39; E14.5 p=0.0002; TrkC E12.5 p=0.24; E14.5 p=0.0002 (increase). (**G–H**) TrkB expression is markedly diminished from

E12.5 onward in the DRG of *Islet1* CKO embryos. (**I–J**) TrkA and TrkC expression in the spinal projections of sensory neurons at E14.5. Innervation of the superficial layers of the spinal cord by TrkA fibers is markedly reduced, and ectopic fibers are observed (arrows, J). TrkC immunoreactive fibers appear undiminished, and project ventrally in both control and mutant specimens (arrowheads, I, J). SC, spinal cord.

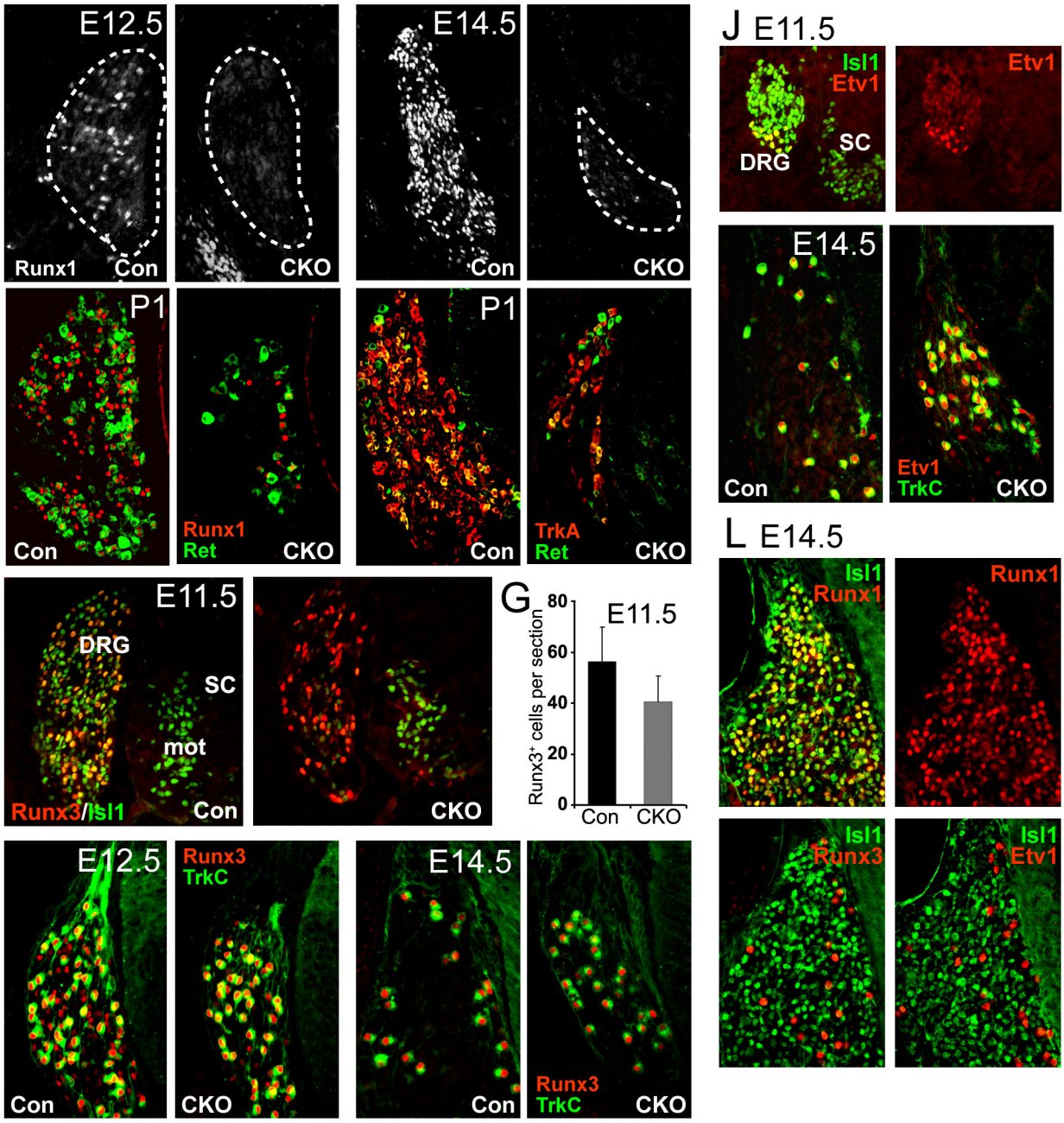


**Figure 3. Expression of transcription factors regulating sensory subtype specification is altered in the DRG of *Islet1* CKO embryos**

(**A,B**) Runx1 expression in the brachial level DRG at E12.5 and E14.5, showing markedly diminished expression in CKO ganglia. (**C,D**). Runx1, Ret and TrkA expression in P1 DRG. Subsets of neurons expressing all combinations of these markers appear markedly diminished in CKO DRG. (**E–G**) Runx3 and Islet1 expression in the DRG of control and CKO embryos at E11.5. In control embryos, Runx3 immunoreactive neurons are a subset of Islet1$^{+}$ cells. However, in the absence of Islet1, Runx3 expression is largely preserved. In G the number of Runx3 cells shows a modest decrease in CKO ganglia which did not reach

statistical significance. (**H,I**) Runx3 and TrkC expression at E12.5 and E14.5. Neurons expressing these proprioceptive markers are relatively spared in *Islet1* CKO ganglia, and account for an increasing fraction of the remaining DRG neurons as development progresses. (**J,K**) At E11.5, Islet1 is co-expressed with Etv1 in a subset of DRG neurons. At E14.5, $Etv1^+$ neurons are relatively spared, and nearly all co-express TrkC, indicating that they are highly overlapping with the Runx3/TrkC population. (**L**) At 14.5, Islet1 is extensively co-expressed with Runx1 but is no longer co-expressed with Runx3 or Etv1. Mot, motor neurons; SC, spinal cord.

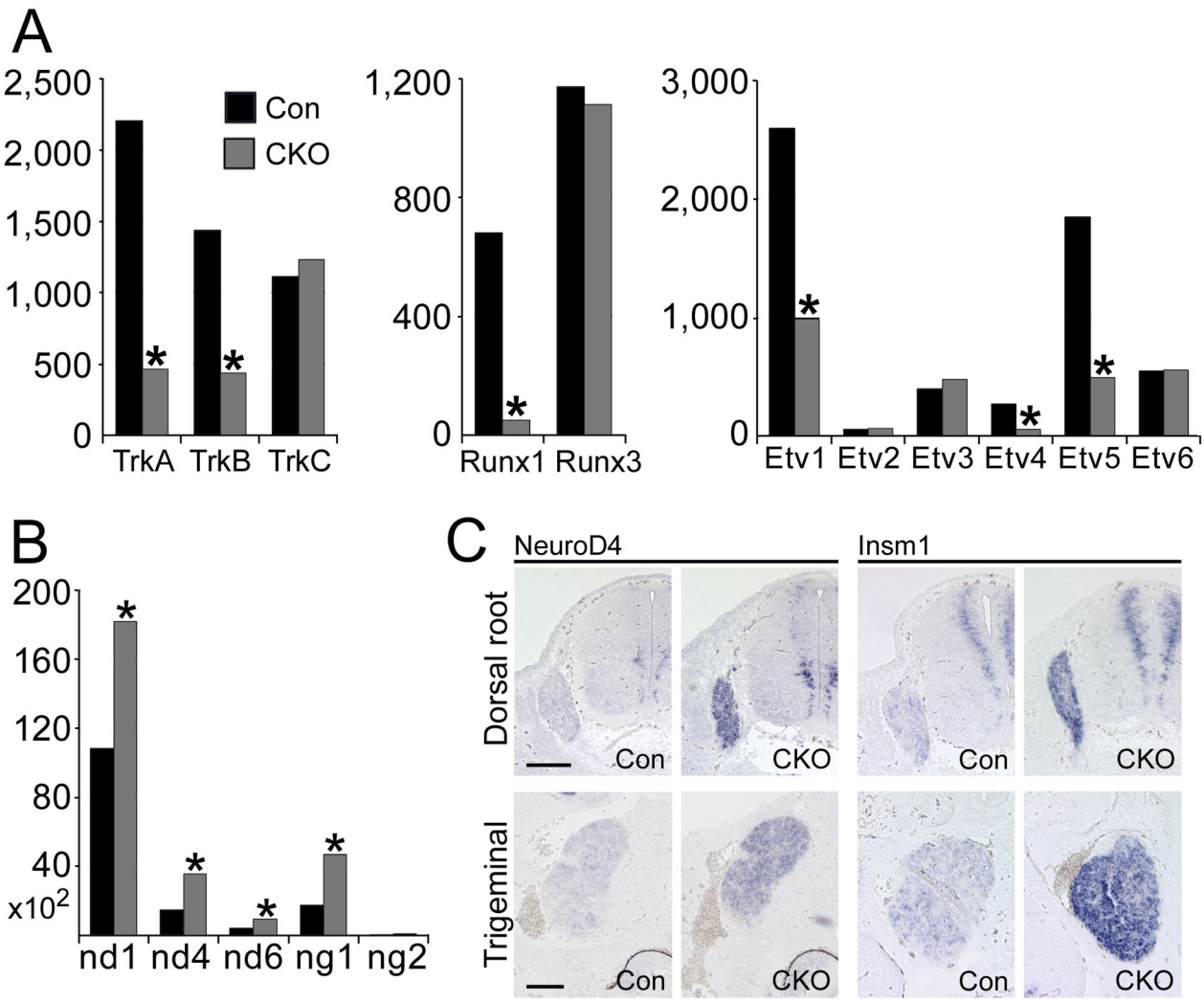


**Figure 4. Islet1 regulates early and late programs of sensory gene expression**

Microarray analysis of E12.5 DRG reveals coordinated changes in expression of gene families regulating specific phases of sensory development. (**A**) Factors associated with sensory subtype specification, including members of the Trk, Runx and Ets families, show profound decreases for genes associated with nociceptive neurons and little or no change for markers of proprioceptive neurons. (**B**) Neurogenic genes of the bHLH family, associated with early steps in sensory differentiation, are increased in *Islet1* CKO DRG. (**C**) Gene expression changes for NeuroD4 and Insm1 are concordant in the DRG and TG. Asterisks indicate significant increase or decrease (change $p < 0.002$ or $> 0.998$) in two independent comparisons. Scale: 200μm.

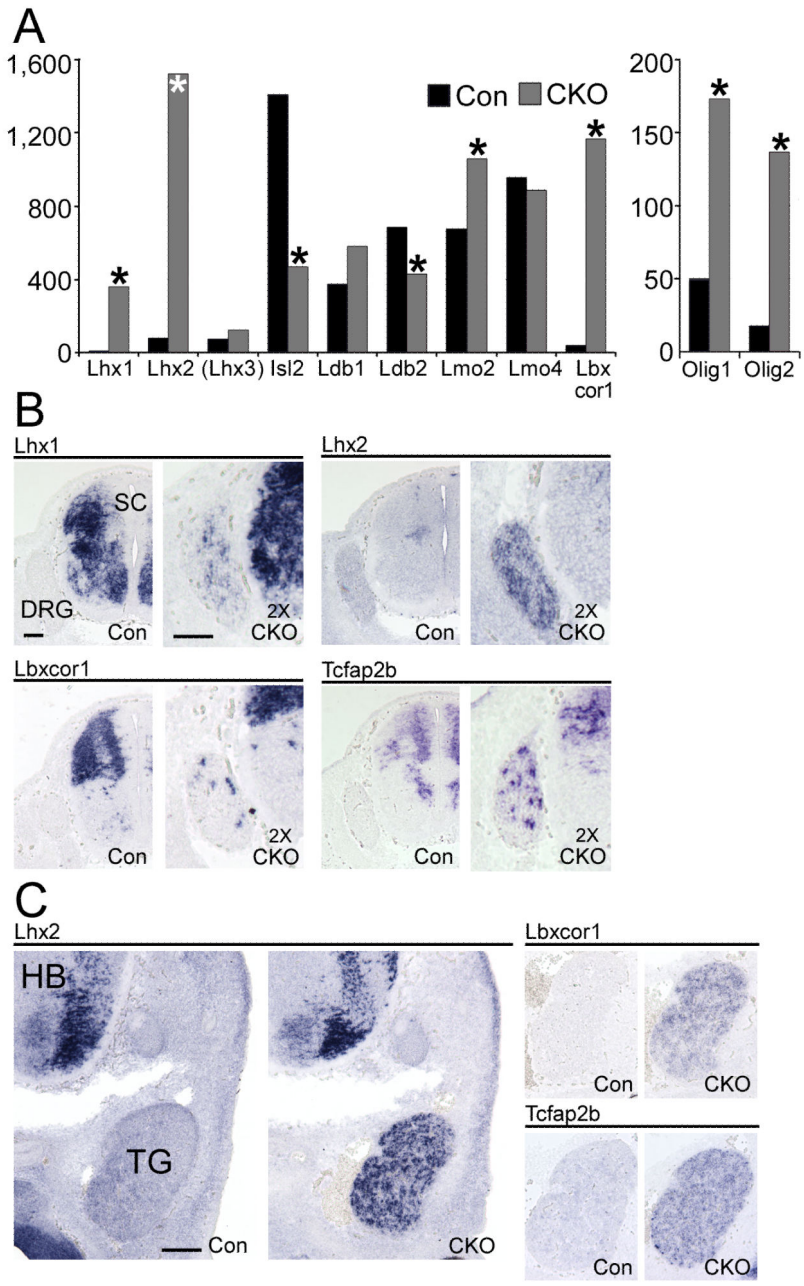


**Figure 5. Ectopic activation of spinal/hindbrain gene expression in *Islet1* CKO sensory ganglia**

(**A**) Microarray analysis of E12.5 DRG reveals abnormal expression of transcription factors usually associated with spinal neuron development (Lhx1, Lhx2, Olig1, Olig2, Lbxcor1), and decreased expression of Islet2. LIM-interacting proteins of the Ldb and LMO families, which are normally expressed in both sensory ganglia and spinal cord, show relatively modest changes. Asterisks indicate significant increase or decrease (change $p < 0.002$ or $> 0.998$) in two concordant comparisons. (**B**) In situ hybridization shows expression of increased transcription factors increased in *Islet1* CKO embryos in the spinal cord and DRG.

*Islet1* CKO views are shown at higher magnification to reveal detail. (**C**) Concordant abnormal expression of CNS transcription factors in the E12.5 trigeminal ganglia. DRG, dorsal root ganglion; HB, hindbrain; SC, spinal cord; TG, trigeminal ganglion. Scale: B, 100μm; C, 200μm.

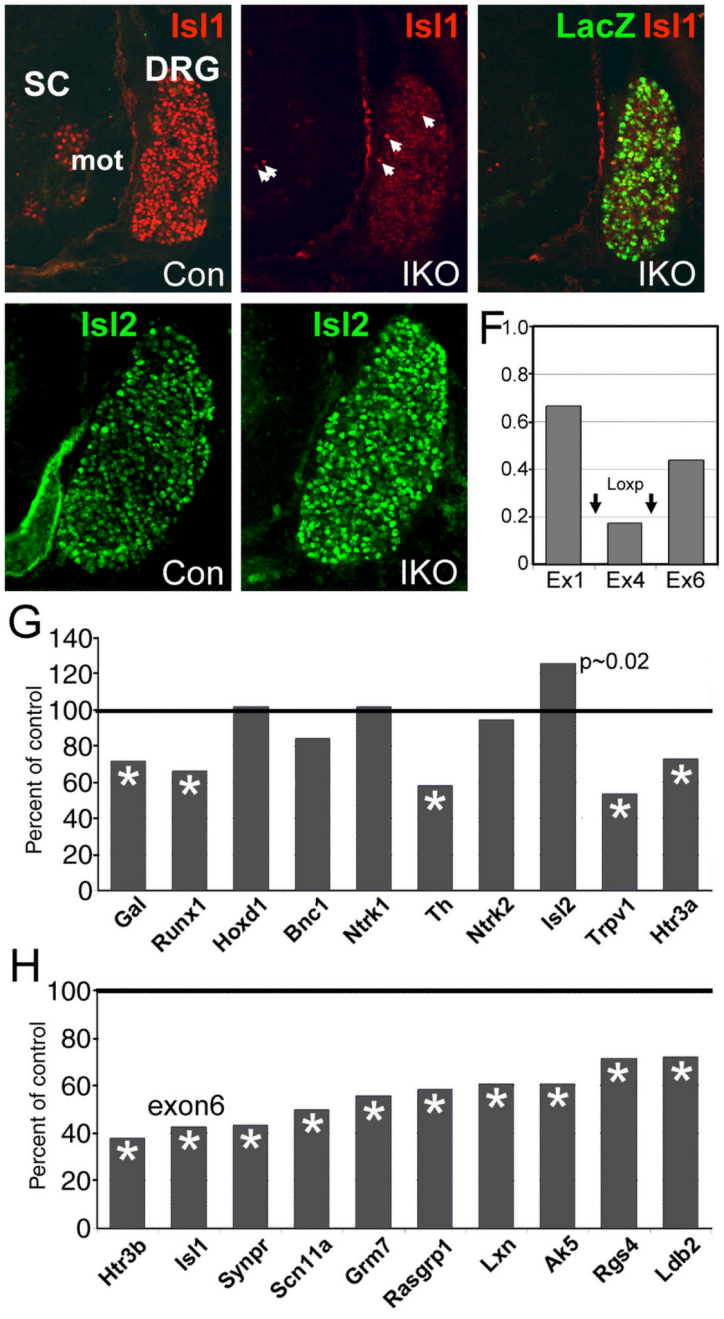


**Figure 6. Late excision of Islet1 supports nociceptor survival but alters downstream gene expression**

*Islet1*$^{F/F}$ and *Islet1*$^{CreER/+}$ mice were interbred to produce *Islet1*$^{F/+}$ control and *Islet1*$^{F/CreER}$ IKO embryos. Tamoxifen was administered to pregnant animals at E11.5 and embryos were examined at E14.5 or E18.5. Because of the need to assess the extent of Islet1 knockdown in each experiment, in E14.5 IKO embryos the DRG were analyzed by immunofluorescence for Islet1 expression, and the TG of the same embryos were used for microarray analysis. (**A–C**) Knock-down of Islet1 protein in cervical-level DRG at E14.5. Note that Islet1

expression is also missing from the motor area of the spinal cord (mot). Arrows in (B) show examples of a small number of neurons in which recombination has not occurred. (A) and (B) were processed on the same slide and photographed at the same exposure. In (C) LacZ expression is activated from a Rosa26-LacZ reporter allele by the induced Cre. (**D,E**) Islet2 expression in control and IKO E14.5 DRG. (**F**) Exon-specific *Islet1* mRNA levels determined by qPCR in DRG of IKO embryo relative to control (1.0). Exon 4 is flanked by *loxP* sites. (**G**) Effect of late *Islet1* excision on gene expression in the E14.5 TG. Genes are displayed in the order of fold decrease in E12.5 DRG (Table 2). Asterisks indicate significant increase or decrease (change $p < 0.002$ or $> 0.998$) in two independent comparisons. (**H**) Additional late Islet1 targets identified in E14.5 IKO TG.

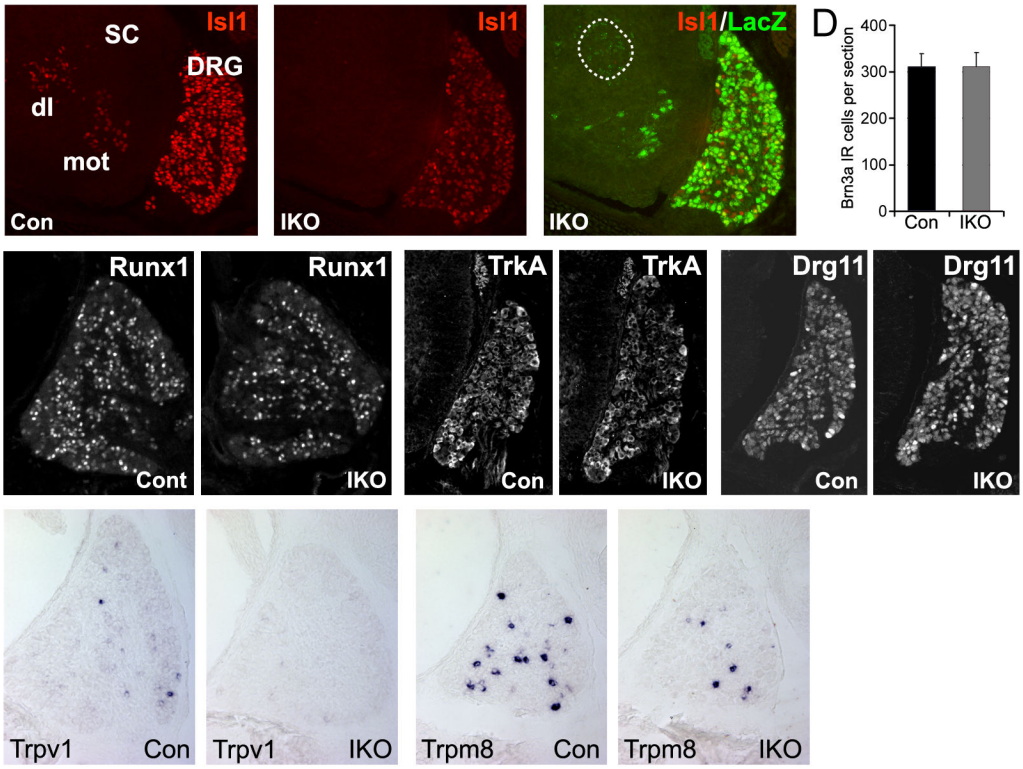


**Figure 7. Analysis of *Islet1* induced knockout DRG at E18.5**

Excision of the *Islet1* homeodomain was induced by tamoxifen injection at E11.5 (Figure 6, Methods), and embryos were analyzed at E18.5. (**A–C**) Knockdown of Islet1 expression assessed by immunofluorescence. Islet1 expression is absent in the dorsal interneuron (dI) and motor neuron (mot) pools, and markedly diminished in the DRG. In (C), co-immunofluorscence for βgal expressed from the Rosa26-LacZ allele reveals a small population of Islet1$^{+}$ neurons in which recombination has not taken place (red). In the spinal cord, βgal immunoreactivity identifies some surviving motor neurons. However, the dI interneurons are represented only by small, dense particles of βgal immunoreactivity, consistent with cellular debris (dashed line), suggesting that the dI population is dependent on Islet1 for survival. Matched sections were processed on the same slide and photographs were taken with the same exposure parameters. (**D**) Brn3a immunoreactivity was used to count neurons in lumbar DRG of Control and IKO embryos. Ten matched sections from control and IKO ganglia were counted, revealing no significant difference in neuronal number, and demonstrating that late expression of Islet1 is not required for DRG viability. Two-tailed T-test for difference between control and IKO, p = 0.93. **(E–F)** Runx1 immunoreactivity was near normal in IKO ganglia, in contrast to the marked decrease observed in CKO mice. **(G–J)** TrkA and Drg11 expression did not appear different in Control and IKO ganglia. **(I–L)** Expression of the nociceptive markers TrpV1 and TrpM8, evaluated by in situ hybridization, was diminished in IKO ganglia. TrpV1 cells/section: Control N=10, mean 5.9 ±3.1, range 2–12; IKO N=10, mean 1.2 ±1.2, range 0–3. T-test for difference between control and IKO, p =0.0003. TrpM8 cells/section: Control N=10, mean

16.8 ±5.3, range 11–23; IKO N=10, mean 5.3 ±2.5, range 2–10. T-test for difference between control and IKO $p = 3 \times 10^{-7}$.

**Table 1**

Transcripts showing increased expression in E12.5 DRG of *Islet1* CKO embryos.

| Gene Title | Symbol | Class | WT | KO | Fold↑ |
|---|---|---|---|---|---|
| LIM homeobox 1 | Lhx1 | TX | 11 | 362 | 32.3 |
| Sal-like 3 | Sall3 | TX | 9 | 269 | 30.4 |
| Ladybird homeobox 1 corepressor 1 | Lbxcor1 | TX | 39 | 1169 | 30.2 |
| LIM homeobox 2 | Lhx2 | TX | 78 | 1522 | 19.5 |
| Oligodendrocyte transcription factor 2 | Olig2 | TX | 18 | 136 | 7.6 |
| Syntaxin binding protein 6 | Stxbp6 | Syn | 146 | 853 | 5.8 |
| Transcription factor AP-2 beta | Tcfap2b | TX | 368 | 1986 | 5.4 |
| Dishevelled associated activator of morphogenesis 1 | Daam1 | ST | 51 | 262 | 5.1 |
| Neuro-oncological ventral antigen 1 | Nova1 | Other | 342 | 1624 | 4.8 |
| FAT tumor suppressor 4 | Fat4 | ST | 537 | 2450 | 4.6 |
| Cellular retinoic acid binding 1 | Crabp1 | ST | 1199 | 5449 | 4.5 |
| Macrophage expressed gene 1 | Mpeg1 | Unk | 127 | 529 | 4.2 |
| Glycoprotein m6a | Gpm6a | AX | 508 | 2026 | 4.0 |
| Glycine receptor, alpha 2 | Glra2 | NT | 51 | 205 | 4.0 |
| Protocadherin 20 | Pcdh20 | AX | 439 | 1572 | 3.6 |
| ELMO domain containing 1 | Elmod1 | ST | 174 | 609 | 3.5 |
| Oligodendrocyte transcription factor 1 | Olig1 | TX | 50 | 173 | 3.5 |
| Cathepsin S | Ctss | Other | 283 | 985 | 3.5 |
| Protocadherin 9 | Pcdh9 | AX | 373 | 1252 | 3.4 |
| Paired-Ig-like receptor A2 | Pira2 | Unk | 202 | 676 | 3.4 |
| Disabled homolog 1 | Dab1 | ST | 102 | 341 | 3.3 |
| Dachshund 2 | Dach2 | TX | 246 | 812 | 3.3 |
| Eyes absent 1 homolog | Eya1 | TX | 196 | 629 | 3.2 |
| Neurogenic differentiation 4 | Neurod4 | TX | 1062 | 3140 | 3.0 |
| **Selected:** | | | | | |
| $K^+$ channel, Shal-related 2 | Kcnd2 | NT | 129 | 378 | 2.9 |
| Short stature homeobox 2 | Shox2 | TX | 403 | 1179 | 2.9 |

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| Gene Title | Symbol | Class | WT | KO | Fold↑ |
|---|---|---|---|---|---|
| Receptor tyrosine kinase-like orphan receptor 1 | Ror1 | ST | 424 | 1207 | 2.8 |
| Homeo box C6 | HoxC6 | TX | 218 | 620 | 2.8 |
| Insulinoma-associated 1 | Insm1 | TX | 1684 | 4579 | 2.7 |
| Neurogenin 1 | Neurog1 | TX | 1749 | 4688 | 2.7 |
| **Unchanged transcripts:** | | | | | |
| Microtubule-associated protein tau | Mapt | AX | 4321 | 3615 | n.s. |
| Neurofilament, light | Nefl | AX | 22510 | 21551 | n.s. |
| Neurofilament, medium | Nef3 | AX | 20951 | 18597 | n.s. |
| Peptidylprolyl isomerase A (cyclophilin A) | Ppia | Other | 25173 | 25452 | n.s. |

AX, axonogenesis; Dev, development; NT, neurotransmission; ST, signal transduction; Syn, synaptogenesis; TX, transcription; Unk, unknown.

**Table 2**

Transcripts showing decreased expression in E12.5 DRG of *Islet1* CKO embryos.

| Gene Title | Symbol | Class | WT | KO | Fold↓ |
|---|---|---|---|---|---|
| $Na_v1.8$ | Scn10a | NT | 148 | 6 | 24.7 |
| Galanin | Gal | NT | 3155 | 172 | 18.4 |
| Runt related txn factor 1 | Runx1 | TX | 684 | 51 | 13.4 |
| Cholecystokinin A receptor | Cckar | NT | 139 | 11 | 12.6 |
| Musculin | Msc | TX | 500 | 46 | 10.8 |
| Homeo box D1 | Hoxd1 | TX | 847 | 79 | 10.7 |
| Basonuclin 1 | Bnc1 | TX | 1125 | 108 | 10.5 |
| Follistatin | Fst | Dev | 606 | 92 | 6.6 |
| Neurotrophic tyrosine kinase, receptor, type 1; TrkA | Ntrk1 | Dev | 2211 | 462 | 4.8 |
| Phospholipase A2, group VII | Pla2g7 | Unk | 776 | 182 | 4.3 |
| Protein tyrosine phosphatase, receptor type, J | Ptprj | ST | 2108 | 505 | 4.2 |
| Advillin | Avil | NT | 2257 | 557 | 4.1 |
| Ig superfamily containing leucine-rich repeat 2 | Islr2 | Unk | 1294 | 333 | 3.9 |
| Tyrosine hydroxylase | Th | NT | 1369 | 352 | 3.9 |
| BMP-binding endothelial regulator | Bmper | Dev | 602 | 155 | 3.9 |
| Protein phosphatase 1, regulatory subunit 2 | Ppp1r2 | Unk | 1902 | 509 | 3.7 |
| Limb expression 1 homolog | Lix1 | Unk | 717 | 193 | 3.7 |
| Ets variant gene 5 | Etv5 | TX | 1847 | 496 | 3.7 |
| Formin 1 | Fmn1 | Unk | 813 | 218 | 3.7 |
| Prokineticin receptor 1 | Prokr1 | NT | 538 | 146 | 3.7 |
| Dedicator of cytokinesis 10 | Dock10 | Unk | 821 | 224 | 3.7 |
| Contactin 4 | Cntn4 | AX | 477 | 133 | 3.6 |
| Protein kinase, cGMP-dep, type II | Prkg2 | ST | 395 | 111 | 3.6 |
| Ser/arg-rich protein specific kinase 3 | Srpk3 | Unk | 356 | 103 | 3.5 |
| Cerebellin 2 precursor protein | Cbln2 | Unk | 1260 | 368 | 3.4 |
| Prostaglandin I receptor | Ptgir | St | 485 | 144 | 3.4 |
| Neurotrophic tyrosine kinase, receptor, type 2; TrkB | Ntrk2 | Dev | 1437 | 440 | 3.3 |
| Doublecortin and CaM kinase-like 3 | Dcamkl3 | Unk | 885 | 286 | 3.1 |

| Gene Title | Symbol | Class | WT | KO | Fold↓ |
|---|---|---|---|---|---|
| BTB domain containing 11 | Btbd11 | Unk | 870 | 283 | 3.1 |
| Insulin related protein 2 | Isl2 | TX | 1408 | 465 | 3.0 |
| Transient receptor potential cation channel, V 1 | Trpv1 | NT | 140 | 47 | 3.0 |
| **Selected:** | | | | | |
| 5-HT receptor 3A | Htr3a | NT | 312 | 107 | 2.9 |
| Regulator of G-protein signalling 10 | Rgs10 | ST | 2299 | 817 | 2.8 |
| H6 homeo box 1 | Hmx1 | TX | 2373 | 852 | 2.8 |
| Ets variant gene 1 | Etv1 | TX | 2604 | 986 | 2.6 |
| Drg11 | Prrxl1 | TX | 3208 | 1620 | 2.0 |