Supplementary Figures

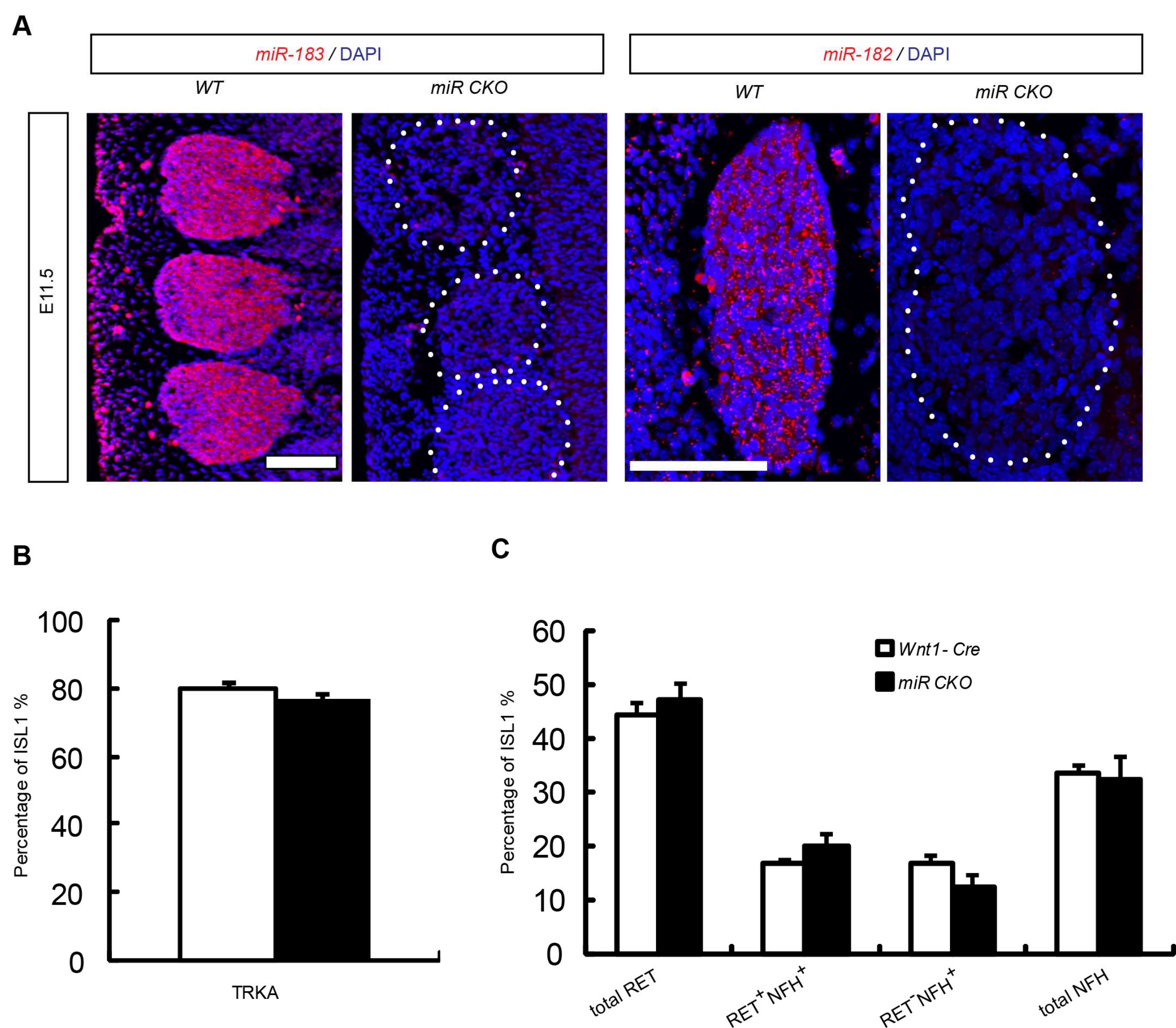

Fig. S1 (Related to Fig.1). Depletion of miR-183 cluster in the *miR*^{CKO} mice and the numbers of TRKA, RET and NFH DRG neurons were not changed in P0 *miR*^{CKO} mice compared to *Wnt1-cre* control mice.

(A) Expression of miR-183 cluster was depleted in the *miR*^{CKO} mice. Images were collected from E11.5 mouse embryos. DRG are circled by dashed line. Scale bar =100µM.

(B) Quantification of the expressing neurons as percent of all neurons (n=5, 4; P=0.23), in L3-L5 ganglia.

(C) Quantification of RET and NFH expressing neurons as percent of all neurons (n=3, 3, p>0.05), in L3-L5 ganglia.

Data shown as mean±s.e.m. and analyzed by t-test.

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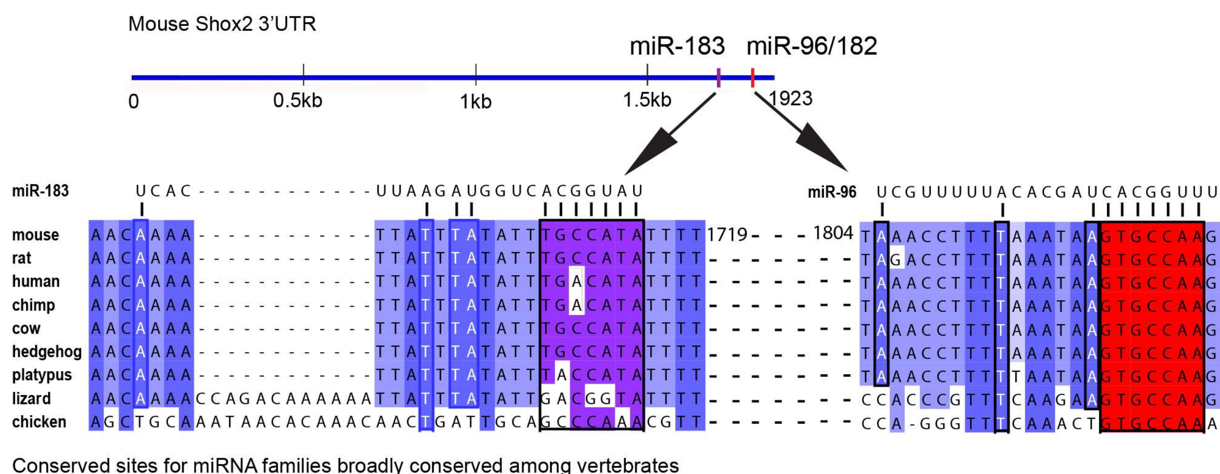

Fig. S2 (Related to Fig.2). Shox2 is a putative conserved target of miR-183 cluster.

Schematic illustration for mouse Shox2 3'UTR carrying two binding sites for miR-183 cluster predicted by TargetScan algorithm (upper panel). These two binding sites are highly conserved among more than 12 species and the sequences matching to the seed sequence of miR-183 and miR-96 are marked in purple or red (Bottom panel). The binding site for miR-96/182 perfectly matches all 7-nucleotides of miR-96's seed sequence and matches 6-nucleotides of miR-182's seed sequence; and this 7-nucleotides sequence is conserved in vertebrates from lizard to human. Due to that chick *Shox2* 3'UTR has not been annotated, TargetScan did not identify any target site for miR-96/182. Nevertheless, as a target site was discovered by TargetScan in lizard, a lower species in the evolutionary tree, so we therefore predicted that it is probably also presented in chicken. We therefore extracted the sequence of chick Shox2 3'UTR from ENSEMBL database and indeed found that the binding site for miR-96 is also conserved on chick Shox2 3'UTR. However, the miR-183 binding site is not as conserved in chicken as the miR-96/182 cluster.

Methods for Fig. S2:

The sequence alignment is based on the alignment from the microRNA prediction server Target Scan Human (Release 6.2) for the Shox2 mouse UTR. All sequences were extracted from ENSEMBL database (release 77，(Flicek et al., 2014)). For the multiple sequence alignment of the orthologs the following parts of the 3´UTR or 3´ flanking sequences of the Shox2 gene were used:

Mouse (*Mus musculus*): ENSMUST00000162098 utr3: bp 1600-1923; Rat (*Rattus norvegicus*): 3' Flanking sequence chromosome:Rnor_5.0:2:183467529:183467829:-1; Human (*Homo sapiens*): ENST00000441443 utr3: bp1700-2016; Chimpanzee (*Pan troglodytes*): 3' Flanking sequence chromosome: CHIMP2.1.4:3:161850370:161850670:-1; Cow (*Bos taurus*): 3' Flanking sequence chromosome: UMD3.1:1:110287694:110287994:1; Hedgehog (*Erinaceus europaeus*): 3' Flanking sequence scaffold: HEDGEHOG:scaffold_376919:35520:35820:-1; Platypus (*Ornithorhynchus anatinus*): 3' Flanking sequence supercontig: OANA5:Contig3858:24433:24733:1; Anole Lizard (*Anolis carolinensis*): 3' Flanking sequence chromosome:AnoCar2.0:3:14459478:14460078:1; Chicken (*Gallus gallus*): 3' Flanking sequence chromosome: Galgal4:9: for predicted miR183 binding: 22087348:22087948:1

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The multiple sequence alignment was performed with the ClustalW2 (Larkin et al., 2007) web server and visualized by using the Jalview software (Waterhouse et al., 2009).

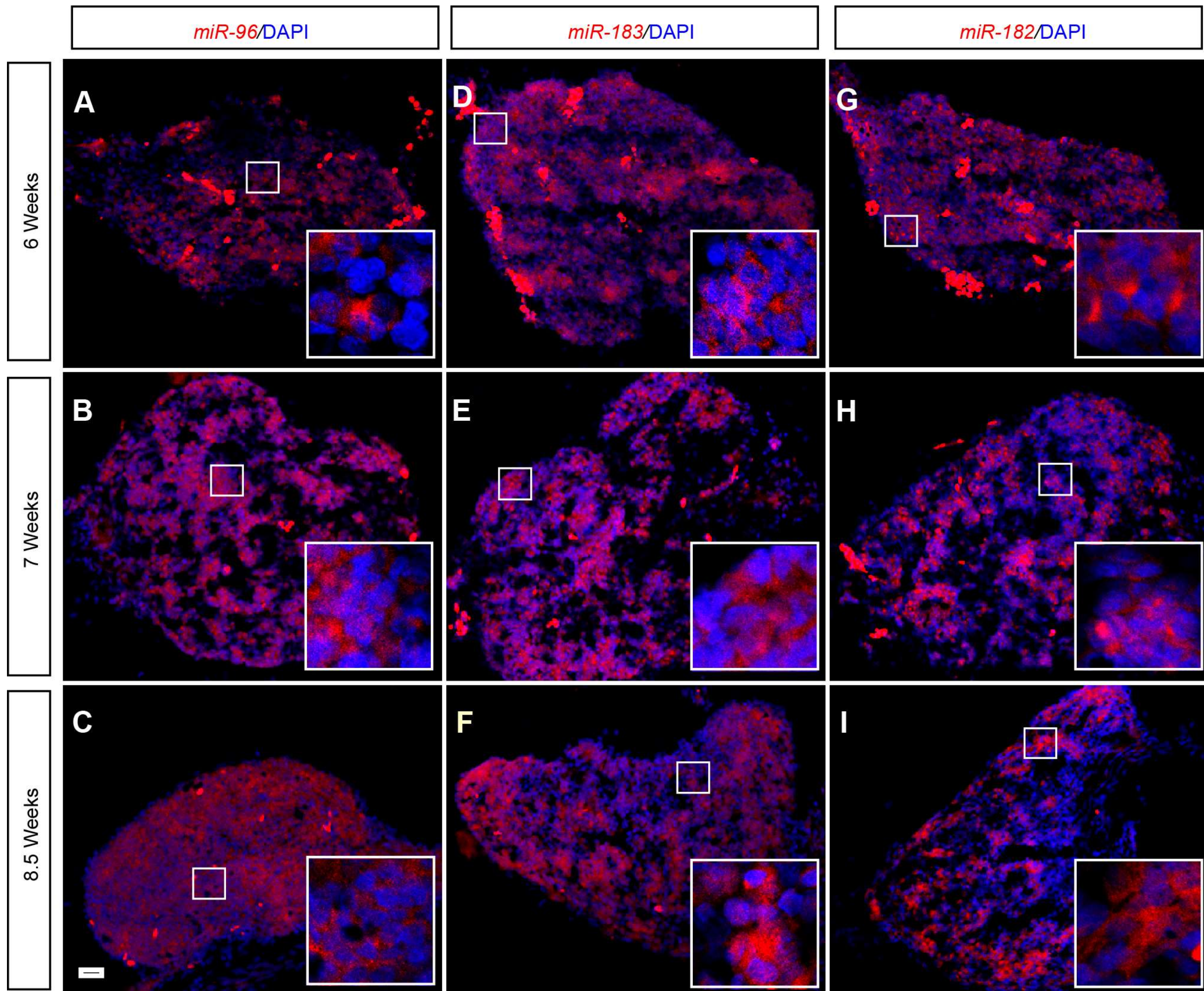

Fig. S3 (Related to Fig.4). Expression of miR-183 cluster in the embryonic human DRG.

In situ hybridization for miR-96 (A-C), miR-183 (D-F) and miR-183 (G-I) shows that miR-183 cluster is broadly expressed in human DRG at 6 weeks (A, D, G), 7 weeks (B, E, K), 8.5 weeks (C, F, I). Scale bar =50μM.

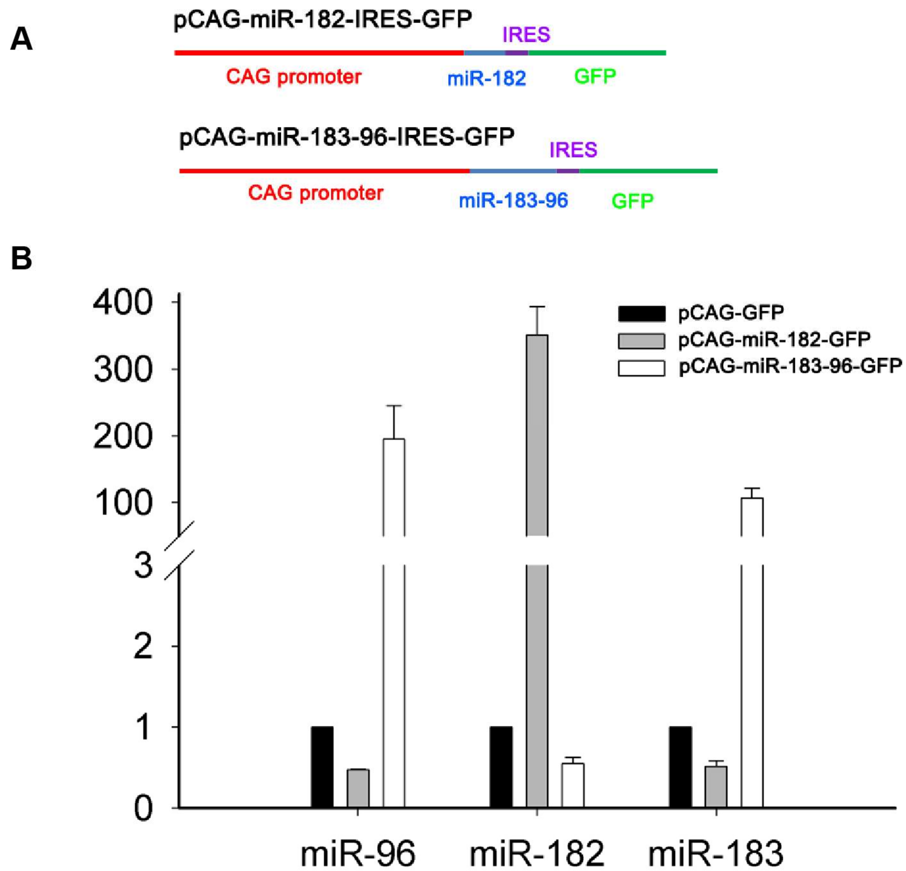

Fig. S4 (Related to Fig.6). Overexpression vectors for miR-182 and miR-183-96 as well as their overexpression level in HEK293 cells after transfection.

(A) Schematic maps of pCAG-miR-182-IRES-GFP and pCAG -miR-183-96-IRES-GFP vectors.

(B) qPCR demonstrated that pCAG-miR-182-IRES-GFP-transfected HEK293 cells had increased expression level of miR-182 (3 bars in middle) compared to the pCAG-GFP-transfected HEK293 cells, and that pCAG-miR-183-96-IRES-GFP-transfected HEK293 cells had increased expression level of both miR-183 (3 bars in right) and miR-96 (3 bars in left) compared to the pCAG-GFP-transfected HEK293 cells. Expression is presented in arbitrary units where control vector is set at 1. Data shown as mean±s.e.m. from three independent experiments.

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Supplementary Table

Table S1^{sheet1}. RNA-seq data (Reads Per Kilobase Million, RPKM) from E12.5 DRG of 3 Controls（2 *Wnt1-Cre* and 1 *WT*） and 3 *Wnt1-Cre; miR$^{fl/fl}$* mice.

Table S1^{sheet2}. Significantly upregulated genes (P<0.05) in DRG of the *miR^{CKO}* mice.

Table S1^{sheet3}. TargetScan algorithm predicted genes targeted by more than one member of miR-183 cluster.

Table S1^{sheet4}. Total 38 genes carrying binding sites of more than one members of miR-183 cluster and upregulated ≥1.3 fold. Among these, two were transcription factors which are marked in red (Shox2 and Zbtb41).

Table S1^{sheet5}. The sequences synthesized and cloned in vectors for overexpression of miR-183-96 and miR-182. Underlined are the precursor sequences for each miRNA.

Table S1^{sheet6}. The list of primers used for cloning mouse *Shox2* 3'UTR for luciferase assay and primers for qRT-PCR.

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Supplementary Reference

Flicek, P., Amode, M. R., Barrell, D., Beal, K., Billis, K., Brent, S., Carvalho-Silva, D., Clapham, P., Coates, G., Fitzgerald, S., et al. (2014). Ensembl 2014. *Nucleic Acids Res* **42**, D749-755.

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., et al. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics* **23**, 2947-2948.

Waterhouse, A. M., Procter, J. B., Martin, D. M., Clamp, M. and Barton, G. J. (2009). Jalview Version 2--a multiple sequence alignment editor and analysis workbench. *Bioinformatics* **25**, 1189-1191.

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