

cells is significantly lower in *Hand2*^{DBHCre} ganglia as compared to *Hand2*^{w^t/fl} controls (P<0,001). Double-labeling reveals that all TH-positive cells co-express TuJ1 in both control and *Hand2*-knockout animals (right graph). (E) Number of Ki67- and Hu-positive cells/section of E14.5 *Hand2*^{w^t/fl} (black bars) and *Hand2*^{DBHCre} (white bars) embryos. Double-labeling reveals that none of residual Ki67-positive cells co-express Hu in *Hand2*-knockout animals whereas many Hu-positive neurons are Ki67-positive in control animals (right graph). *** significantly different from control P<0.001. Data shown are the mean ± s.e.m. (n=3),

Supplementary Figure 1

Rescue of *Hand2* knockdown by forced expression of *mHand2* and *zHand2*. E12 sympathetic neurons were co-transfected with control *NP25* siRNA, *GFP* and *zHand2* (A, a-c), with *Hand2* siRNA1 and *GFP* (A, d-f) or with *Hand2* siRNA1, *GFP* and *zHand2* (A, g-i) and stained for TH (red) and GFP (green). The proportion of transfected GFP-positive neurons expressing TH is quantified in (B). Overexpression of *mHand2* and *zHand2* increases the proportion of TH-expressing cells in control transfections (*NP25* siRNA) (black bars). The reduction of TH-expressing cells induced by *Hand2* siRNA1 is rescued by *mHand2* and *zHand2* overexpression (white bars). In (C) *Hand2* protein expression upon transfection of E12 sympathetic ganglion cells with pCAGGS-*Hand2* is revealed by immunostaining. Please note the selective *Hand2* expression in sympathetic neurons in control transfection (arrows in C, a-b) in contrast to nuclear *Hand2* staining of both neurons (arrows) and non-neuronal cells (arrowheads) upon transfection with pCAGGS-*zHand2* (arrows in C, c-d). Data shown in (B) represent mean ± s.e.m. (n=4), *P<0,05; **P<0,01; ***P<0,001 as referred to *NP25* siRNA control.

Supplementary Figure 2

Hand2 knockdown selectively affects TH but not TuJ1 expression. E12 sympathetic neurons were transfected with *Hand2* siRNA1 and either stained for TH (green) and *Hand2* (red) (A,B) or for TuJ1 (green) and *Hand2* (red) (C,D). Two neurons negative for *Hand2* and TH are indicated by arrows in (A,B). A *Hand2*-negative neuron expressing

TuJ1 is indicated by arrows in (C,D). The proportion of siRNA-transfected neurons expressing TH or TuJ1 has been quantified for Hand2-negative neurons (E) and Hand2-positive neurons (F). Please note the selective loss of TH but not TuJ1 in Hand2-negative neurons (E). Data shown in (E, F) represent mean \pm s.e.m. (n=3).

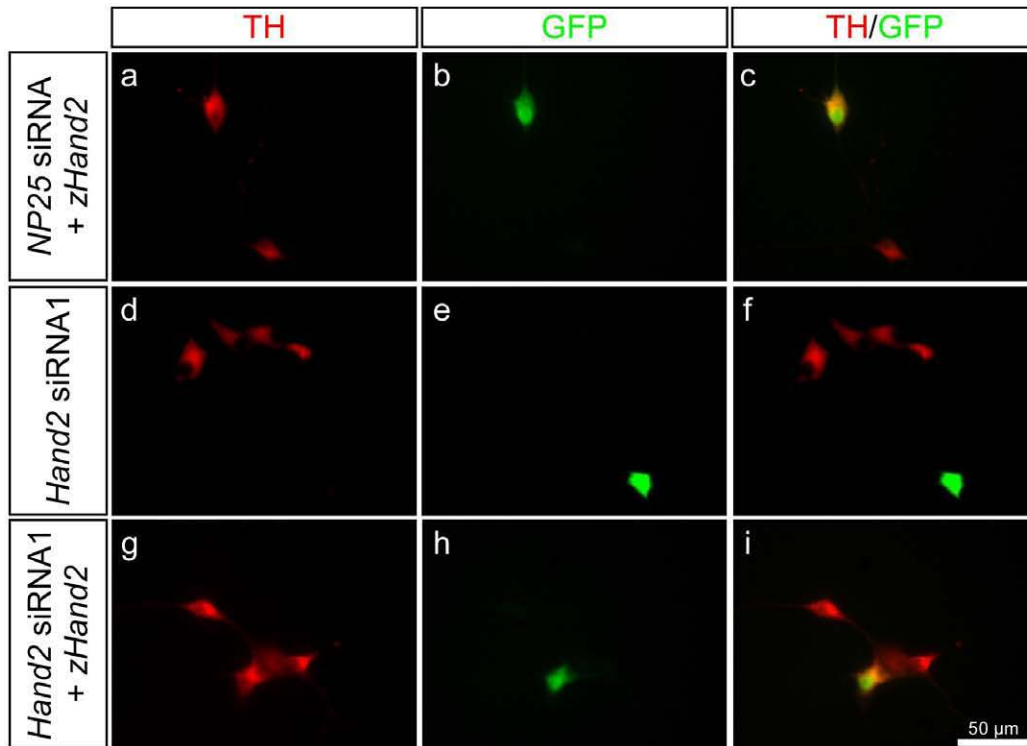
Supplementary Figure 3

Effect of *Hand2* knockdown on the expression of *Gata2* in cultured sympathetic neurons. E12 sympathetic neurons were transfected with control *GFP* siRNA, *NP25* siRNA or *Hand2* siRNAs and analyzed after 2 days (A) or 4 days (B) in culture for *Gata2* mRNA expression by in situ hybridization as described for *TH* in Fig. 3. In cultures treated with *Hand2* siRNA the proportion of *Gata2*-expressing sympathetic neurons decreased from 89 \pm 2 and 92 \pm 2% in control transfections to 80 \pm 5 and 79 \pm 6% in cultures transfected with *Hand2* siRNA after 2 days in culture (A). At 4 days in culture no significant change in *Gata2* expression was evident (B).

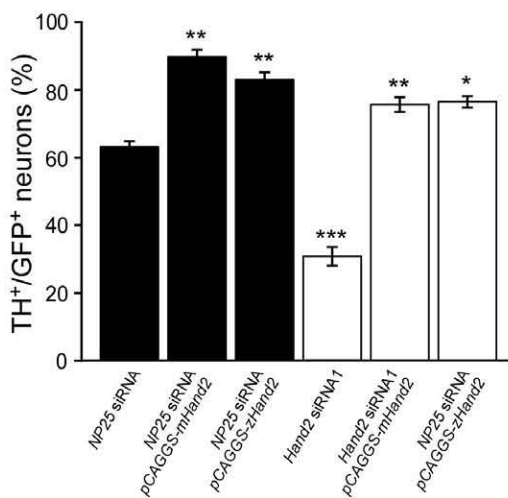
Supplementary Figure 4

Co-expression of *Hand2*, *VACHT* and *SCG10* in E20 chick lumbar sympathetic ganglia, analysed by double-in situ hybridisation. Frozen sections from E20 chick lumbar region were first hybridized for *Hand2* (A) or *SCG10* (B) using red color reaction. After documentation the sections were re-hybridized for *VACHT* (C) and *Hand2* (D) using blue color reaction. Please note that *VACHT*-positive neurons are characterized by low level expression for *Hand2* (A, C), whereas the signal intensities for *SCG10* and *Hand2* seem to be comparable (B,D).

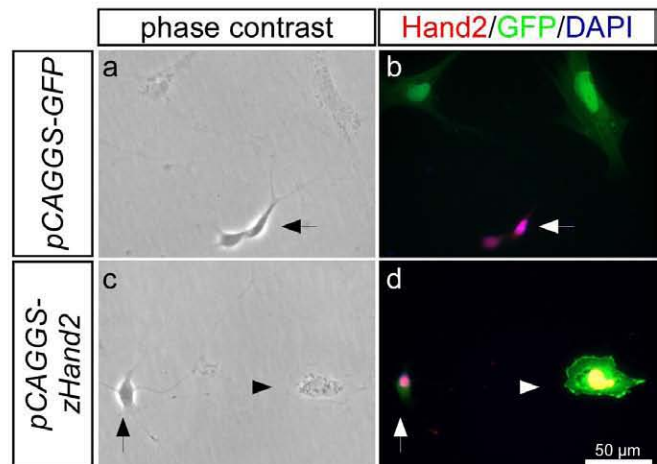
A



B

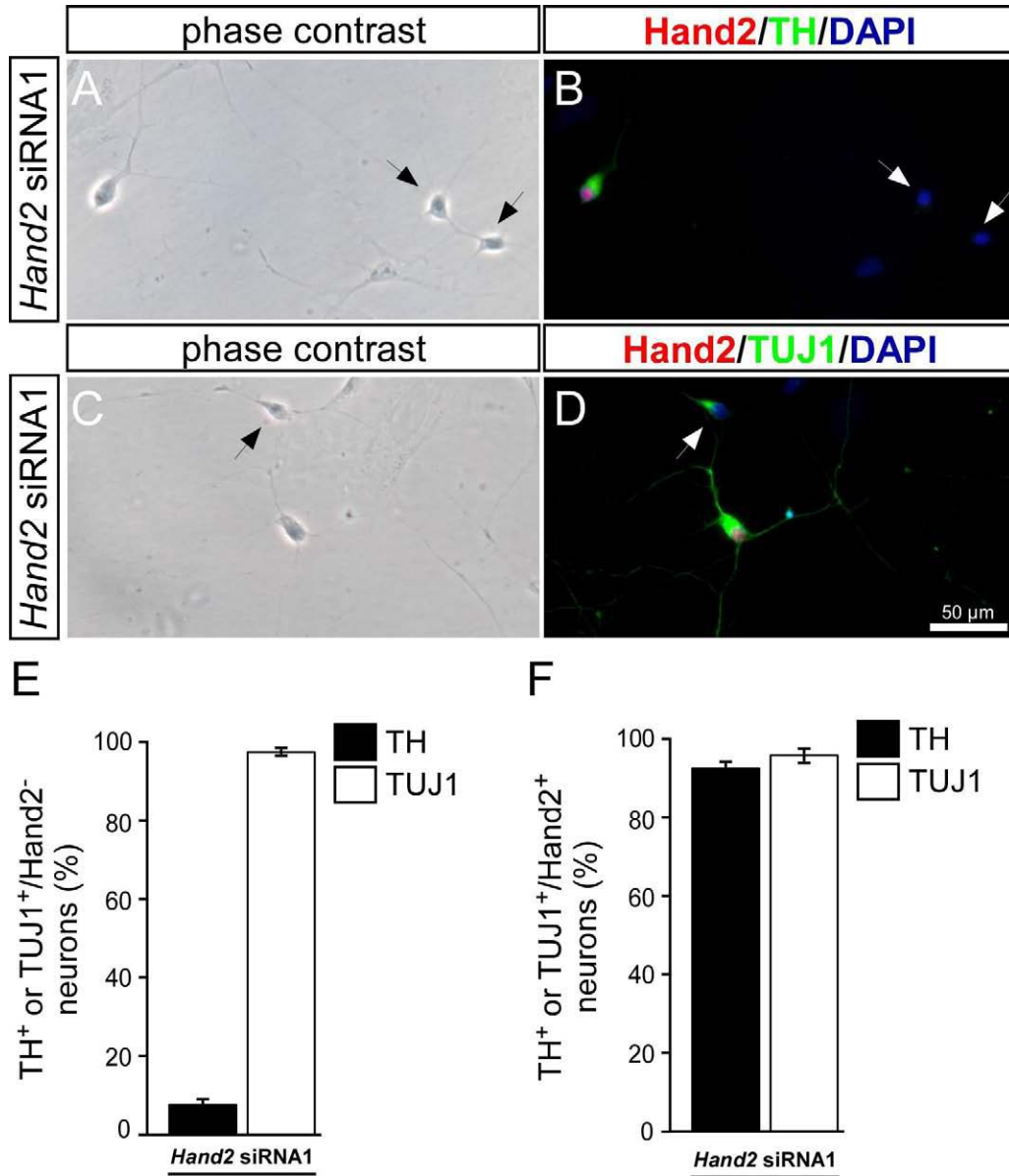


C

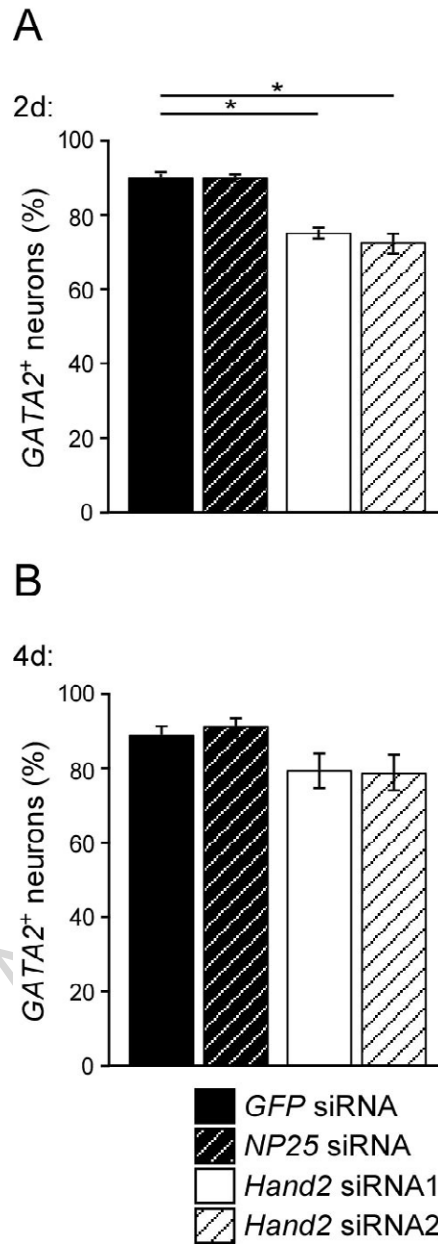


Supplementary Figure 1

Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4

