Supplemental Information

*NvPOU4/Brain3* Functions as a Terminal Selector Gene in the Nervous System of the Cnidarian *Nematostella vectensis*

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Fig. S1 Relative expression of NvSoxB(2), NvPOU4 and NvNcol3 from early blastula stage to late planula stage, related to Fig. 1 In situ hybridization with probes indicated on the left side and the developmental stage on top. (G-U) are lateral views with the aboral pole to the left, except for (J-L) which are oral views. (A-L) From Early blastula to late blastula, the pictures show the ectodermal surface view of the embryo. (M-U) From gastrula to planula stage, the pictures show the middle view of the embryo.

(A-C) NvSoxB(2) is the first of the three gene to be expressed at 10hpf, (D-F) followed at 12hpf by the first NvPOU4 expressing cells. (G-I) NvNcol3 start to be expressed around 16hpf. (J-L) NvNcol3 is strongly expressed at the oral pole before gastrulation, which is not the case for NvPOU4 and NvSoxB(2). (M-U) From gastrula stage to planula stage the expression of NvPOU4 and NvNcol3 becomes more and more similar by being expressed in scattered single cell in the ectoderm and then in the future tentacle buds, whereas NvSoxB(2) is strongly expressed in the pharynx and in the ectoderm. All the embryos used for this experiment came from the same batch. Some images for NvPOU4 are the same as in Fig 1. Scale bars represent 50µm.
Fig. S2 NvPOU4 is expressed in differentiating neural cells from blastula to planula stage, related to Fig. 2 (A-I) Double fluorescent in situ hybrization with probes indicated on the left side and the developmental stage on top. Lateral views, for gastrula and planula stages the aboral pole to the left. NvPOU4 is shown in green, other probes in magenta: (A-C) NvSoxB(2), (D-E) NvNcol3 and (G-I) NvRFamide. All images are projections of stacks of confocal sections. Stacks for blastula, gastrula and planula stages are available as Supplementary movies 1-3, 4-6 and 7-9, respectively. Scale bars represent 20 μm.
Fig. S3 Co-localization of \textit{NvPOU4} and \textit{memGFP} mRNA in transgenic embryos, related to Fig. 3 Double fluorescent \textit{in situ}, using probes for \textit{NvPOU4} (magenta), \textit{memGFP} (green) and DAPI (blue), demonstrates that the reporter gene expression mimics endogenous \textit{NvPOU4} expression at blastula stage in embryos from the \textit{NvPOU4::memGFP} transgenic line. (A-A") oral views; (B-B") lateral views with the aboral pole to the left. Scale bars represent 50 μm
Fig. S4 Generation of *NvPOU4*<sup>−/−</sup> via CRISPR/Cas9 and analysis of the cnidocyte phenotype in *NvNCol3::mOrange2* transgenics, related to Fig. 5 (A) Schematic of the knock out targeting strategy. Exons are in grey boxes, the POU domain is shown as a green and the homeodomain as a yellow box. The gRNA targets the start of the POU domain (red line) and generated a deletion of 31bp (red box) causing a frame shift and the appearance of a premature STOP codon. (B) Sequence alignment between the wild-type and the mutant sequences. The STOP codon is highlighted in red. (C' - C'') DNA chromatograms derived from individual animals with wildtype, heterozygous or homozygous *NvPOU4* mutant genotype. (D - I) Antibody staining of mOrange2 protein (dsRed antibody, in magenta) and DAPI (blue) in (D - F) sibling controls (*NvPOU4*<sup>+/+</sup> or *NvPOU4*<sup>+/−</sup>, *NvNcol3::mOrange2*<sup>+/+</sup>) and (G - I) *NvPOU4*<sup>−/−</sup>, *NvNcol3::mOrange2*<sup>+/−</sup> primary polyps. (D, G) tentacle tips, (E, H) body column, (F, I) higher magnification pictures of the cnidocyte capsules. *NvPOU4*<sup>−/−</sup> animals lack fully differentiated cnidocyte capsules, but still have some NvNCol3. Scale bar represents 50 μm (D, E, G, H) and 10 μm (F, H), respectively.
Fig. S5 Sequencing of *NvPOU4* transcripts and number of *NvElav1*+ neurons in *NvPOU4* mutants, related to Fig. 6 (A) RNA sequencing reads for the control and the four replicates of *NvPOU4*−/−. In the mutants, no reads map to the deleted region (shown via a red arrow), whereas the control sample has reads mapping to it. (B) shows a higher magnification of the deleted region. (C) Graphical representation showing the number of *NvElav1*+ cells counted in a 100 μm x 100 μm square in *NvPOU4*−/− (purple) and in sibling controls (yellow) at primary polyp stage in four independent experiments. Boxes indicate first and third quartile, whiskers indicate 1.5 x interquartile range, and outliers are shown as dots. Plots were generated with the default settings of Excel, quartiles were calculated using Exclusive median. Numbers for the four experiments are shown in (D). A Shapiro-Wilk test was first calculated to test for normal distribution of each data. Then in case of normal distribution a T-test was performed (Experiments 1, 2, 4). In case of abnormal distribution a Mann Whitney test (U-test) was performed (Experiment 3). In all counting experiments there is a general tendency that *NvPOU4*−/− animals have less *NvElav1*+ cells than their siblings controls (shown by the median), but the difference was not statistically significant at p < 0.05 for replicates 3 and 4. This suggests that the loss of *NvPOU4* in *Nematostella* has no or only a very mild effect on the specification of *NvElav1*+ neurons.