Supplemental Information

NvPOU4/*Brain3* Functions as a Terminal

Selector Gene in the Nervous System

of the Cnidarian *Nematostella vectensis*

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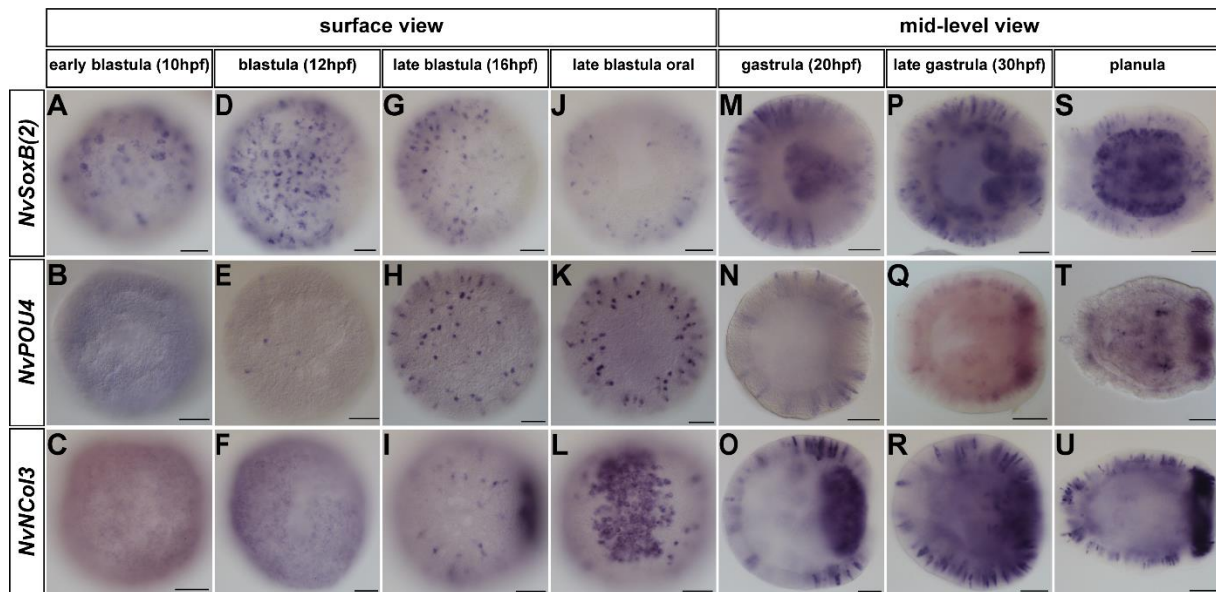
Fig. S1 Relative expression of *NvSoxB(2), NvPOU4* and *NvNcol3* from early blastula stage to late planula stage, related to Fig. 1 *In situ* hybridization with probes indicated on the left side and the developmental stage on top. (G-U) are lateral views with the aboral pole to the left, except for (J-L) 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.
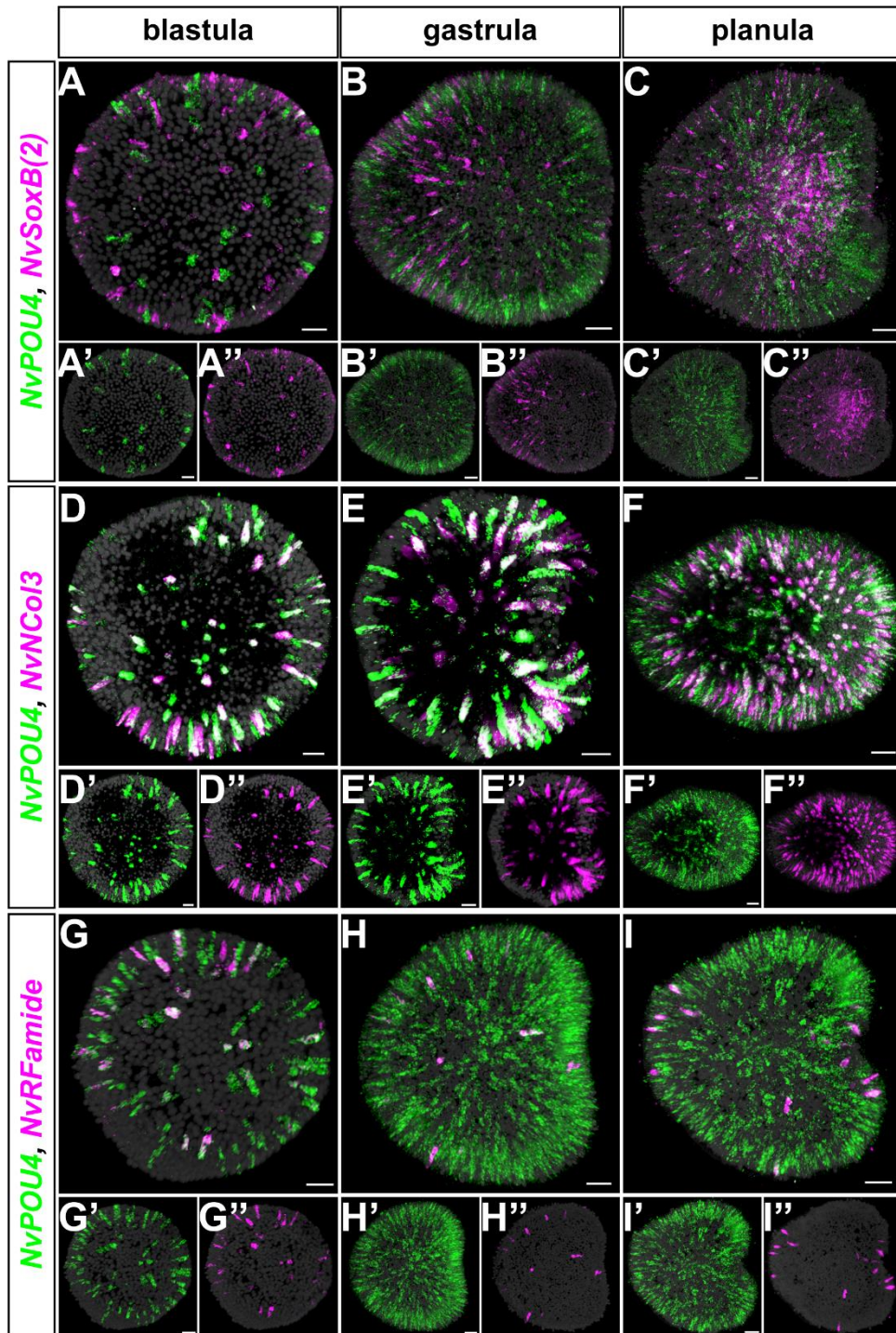
Fig. S2 *NvPOU4* **is expressed in differentiating neural cells from blastula to planula stage, related to Fig. 2** (A-I) Double fluorescent *in situ* hybrization with probes indicated on the left side and the developmental stage on top, Lateral views, for gastrula and planula stages the aboral pole 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.
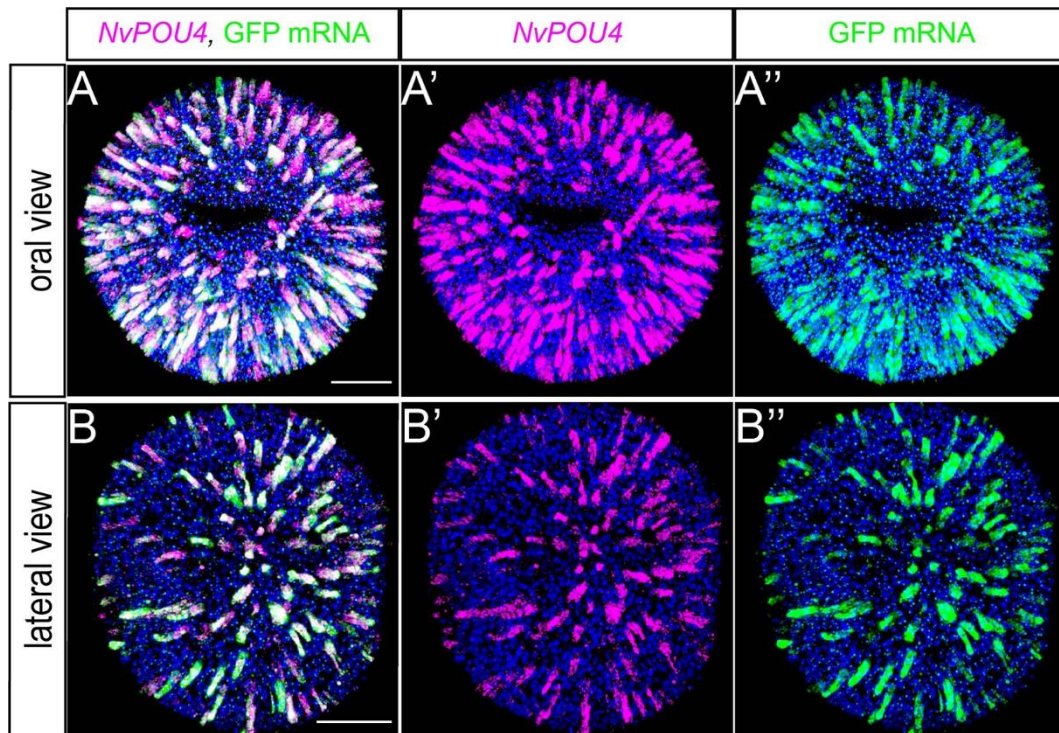
Fig. S3 Co-localization of *NvPOU4* and *memGFP* mRNA in transgenic embryos, related to Fig. 3 Double fluorescent *in situ*, using probes for *NvPOU4* (magenta), *memGFP* (green) and DAPI (blue), demonstrates that the reporter gene expression mimics endogenous *NvPOU4* expression at blastula stage in embryos from the *NvPOU4*::memGFP transgenic line. (A-A") oral views; (B-B") lateral views with the aboral pole to the left. Scale bars represent 50 µm
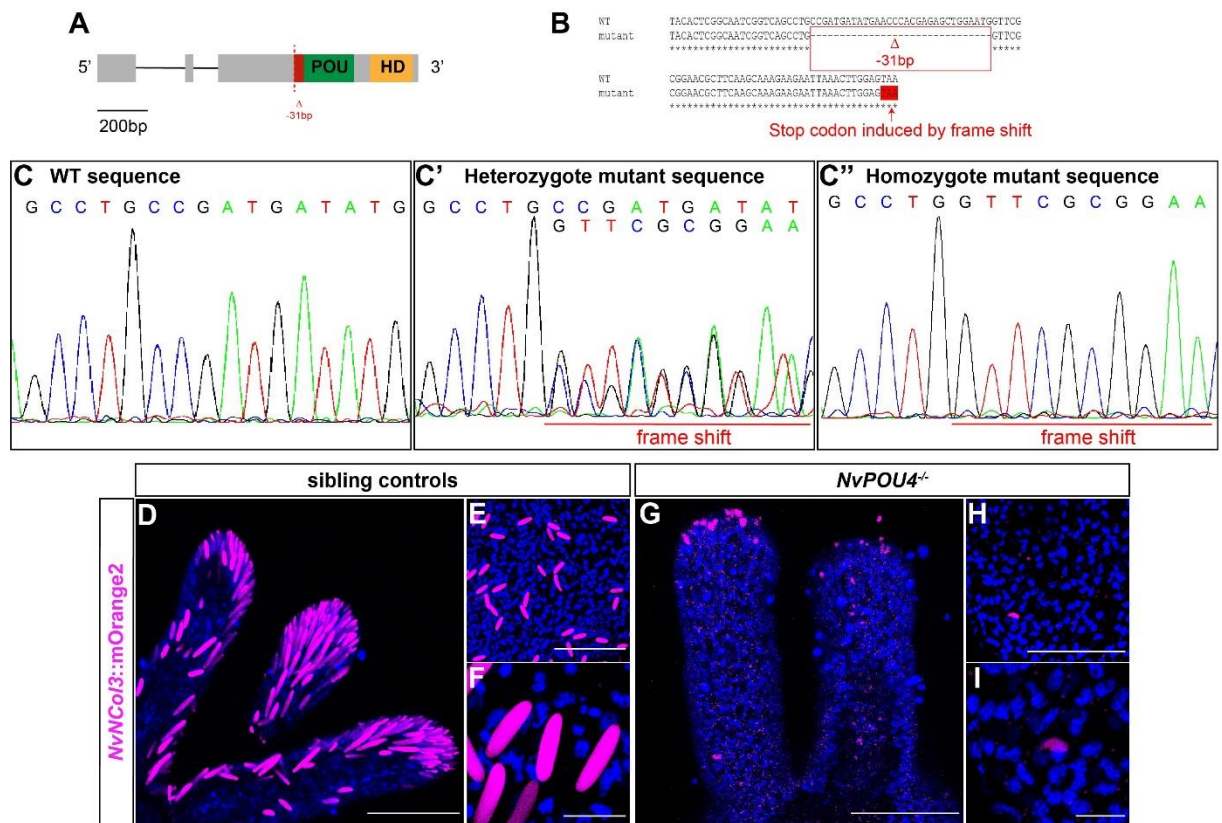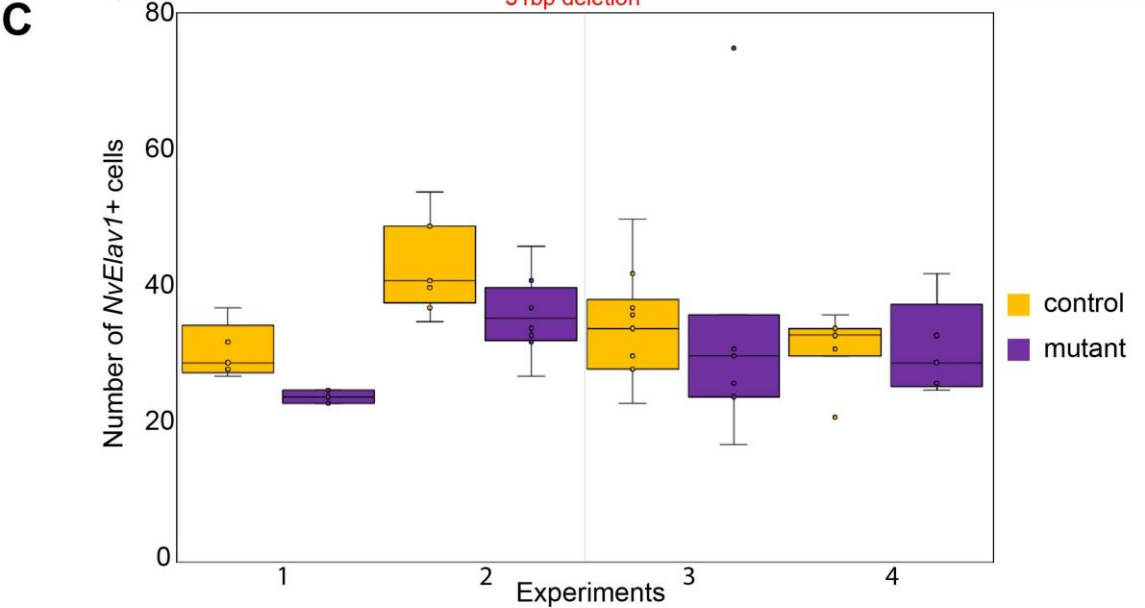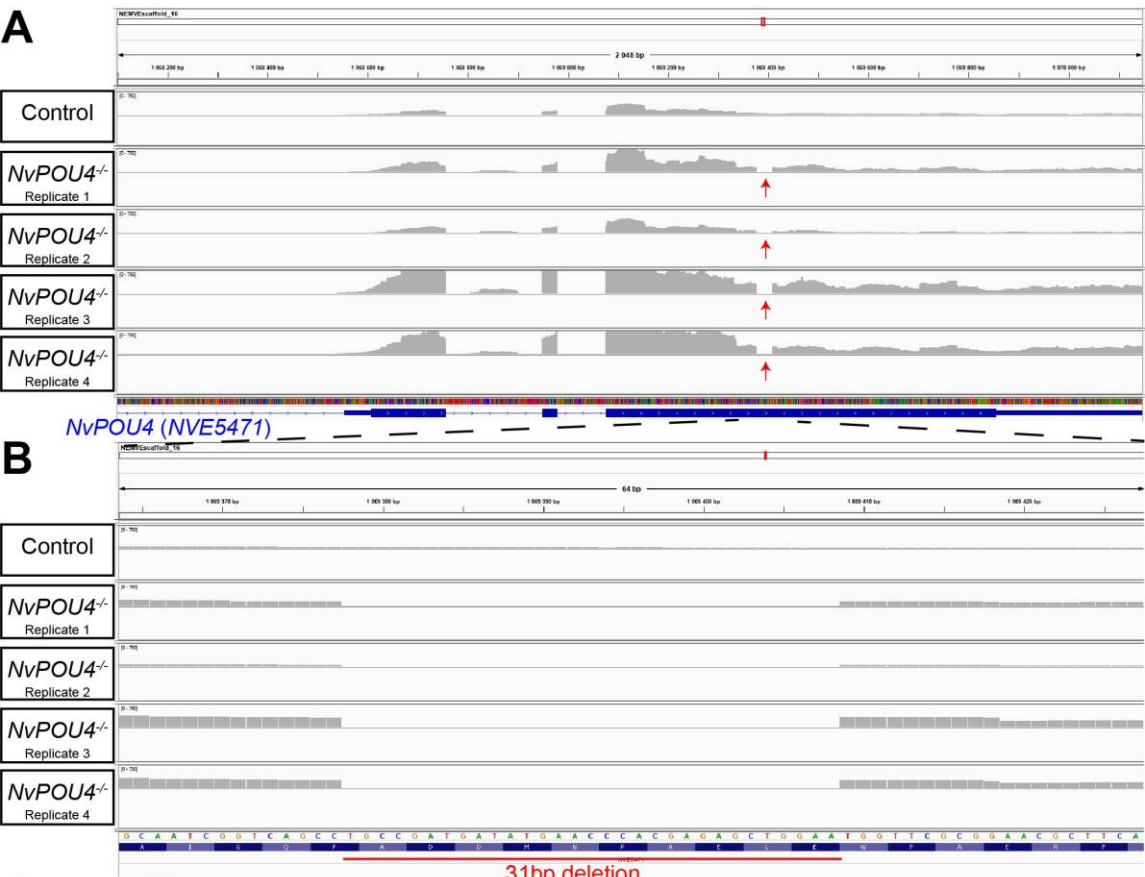
Fig. S4 Generation of *NvPOU4⁻/⁻* via CRISPR/Cas9 and analysis of the cnidocyte phenotype in *NvNCol3*::mOrange2 transgenics, related to Fig. 5 (A) Schematic of the knock out targeting strategy. Exons are in grey boxes, the POU domain is shown as a green and the homeodomain as a yellow box. The gRNA 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⁺/⁺* or *NvPOU4⁺/⁻*, *NvNcol3*::mOrange2⁺/⁻) and (G - I) *NvPOU4⁻/⁻*, *NvNcol3*::mOrange2⁺/⁻ primary polyps. (D, G) tentacle tips, (E, H) body column, (F, I) higher magnification pictures of the cnidocyte capsules. *NvPOU4⁻/⁻* 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.

A

NEMVEscaffold_16

2 048 bp

1 063 200 bp | 1 063 400 bp | 1 063 600 bp | 1 063 800 bp | 1 069 000 bp | 1 069 200 bp | 1 069 400 bp | 1 069 600 bp | 1 069 800 bp | 1 070 000 bp

Control

NvPOU4⁻/⁻ Replicate 1

NvPOU4⁻/⁻ Replicate 2

NvPOU4⁻/⁻ Replicate 3

NvPOU4⁻/⁻ Replicate 4

NvPOU4 (NVE5471)

B

NEMVEscaffold_16

64 bp

1 069 370 bp | 1 069 380 bp | 1 069 390 bp | 1 069 400 bp | 1 069 410 bp | 1 069 420 bp

Control

NvPOU4⁻/⁻ Replicate 1

NvPOU4⁻/⁻ Replicate 2

NvPOU4⁻/⁻ Replicate 3

NvPOU4⁻/⁻ Replicate 4

G C A A T C G G T C A G C C T G C C G A T G A T A T G A A C C C A C G A G A G C T G G A A T G G T T C G C G G A A C G C T T C A

31bp deletion

C

Number of *NvElav1*+ cells

control (yellow)
mutant (purple)

Experiments: 1, 2, 3, 4

D

	Experiment 1		Experiment 2		Experiment 3		Experiment 4	
	control 1	mutants 1	control 2	mutants 2	control 3	mutants 3	control 4	mutant 4
	32	23	54	33	42	36	30	29
	37	23	41	37	30	75	36	33
	29	24	35	46	34	17	21	42
	27	25	49	32	28	24	33	25
	28	25	37	41	37	26	34	26
			40	27	50	30	34	
			41	37	36	31	31	
			49	34	34			
					23			
					28			
median	29	24	41	35.5	34	30	33	29
average	30.3	24	43.3	35.9	34.2	34.1	31.29	31
standard dev	4.0	1	6.6	5.8	7.76	19.0	4.96	6.89
T Test		0.01		0.03		0.98		0.93
U test						0.4		

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.