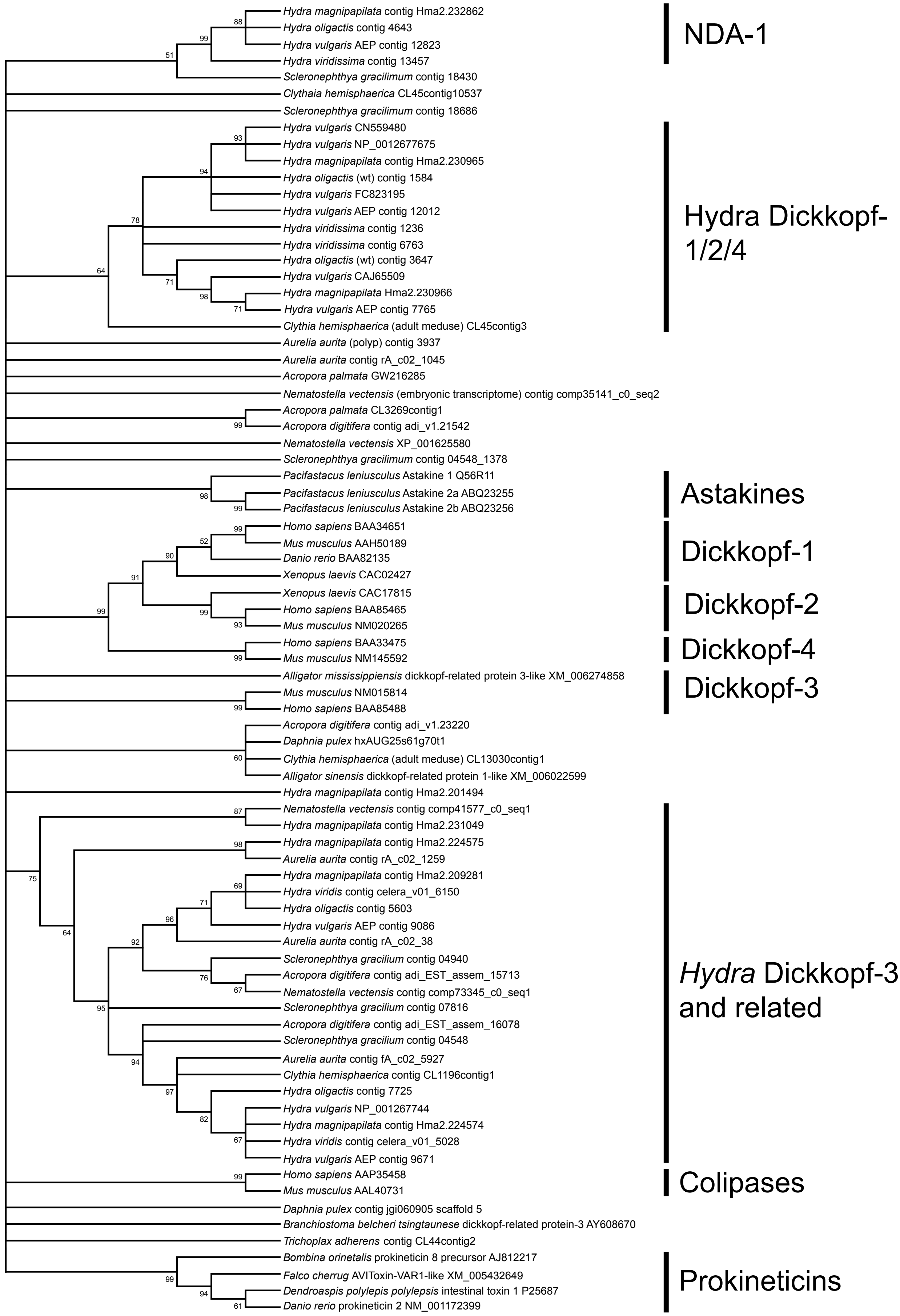
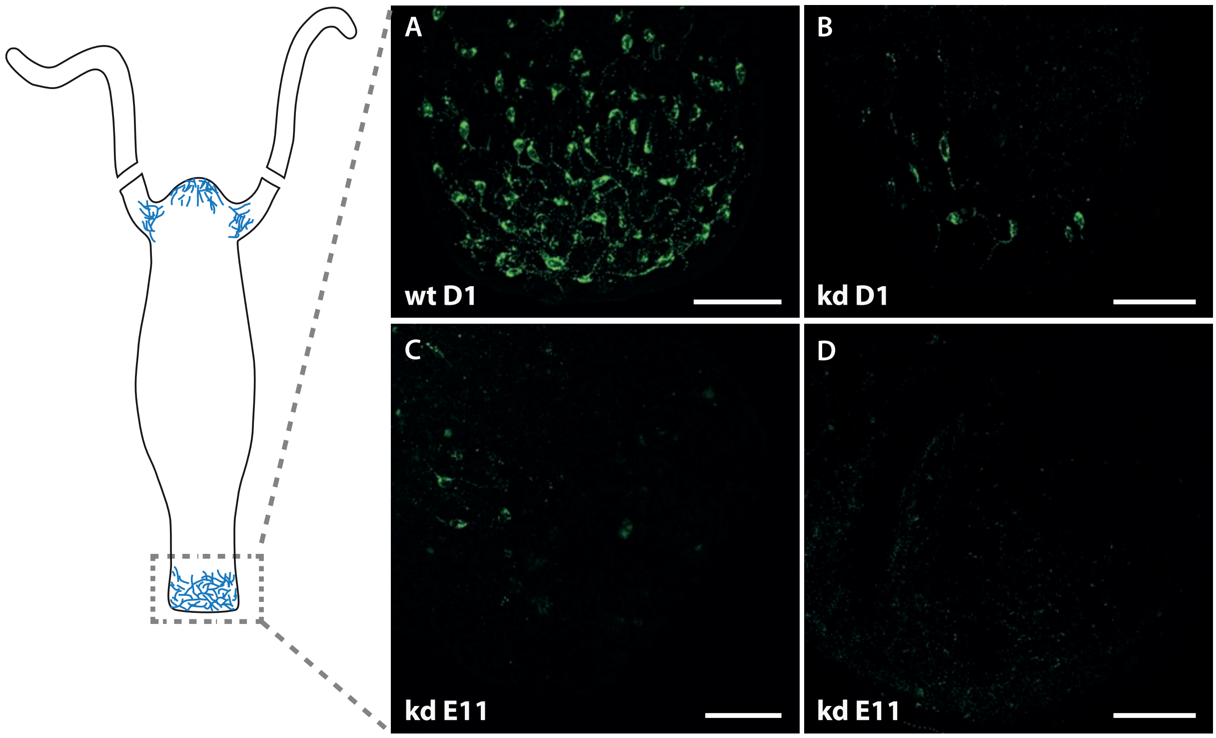
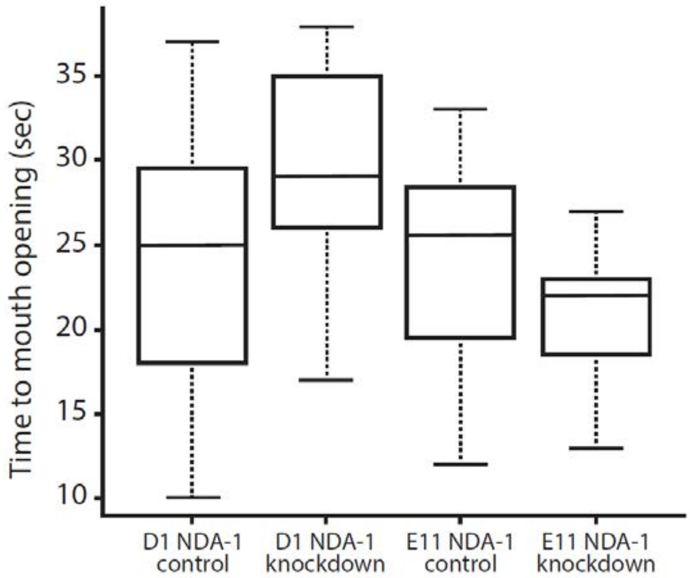


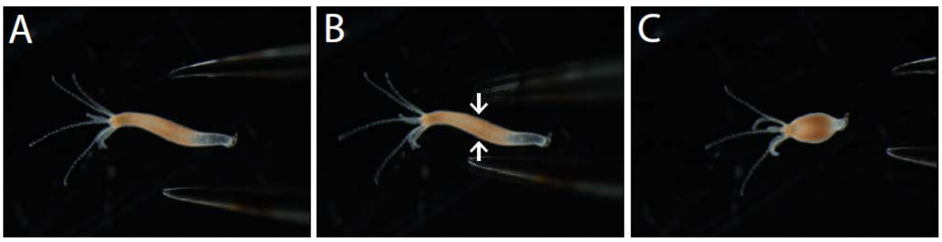

**Supplementary Figure 1.** Neighbour-joining phylogenetic tree of prokineticins, astakines, Dickkopf and colipase gene families showing absence of congruence between NDA-1 (at the top) and the other peptides.




**Supplementary Figure 2.** Confocal laser scanning micrographs of the foot region of Hydra, stained for NDA-1 peptide by means of polyclonal antiserum. Wildtype (panel A) is compared to the transgenic knockdown lines D1 (B) and E11 (C, D), which have lower NDA-1 expression levels. Scale bars: 50 µm.



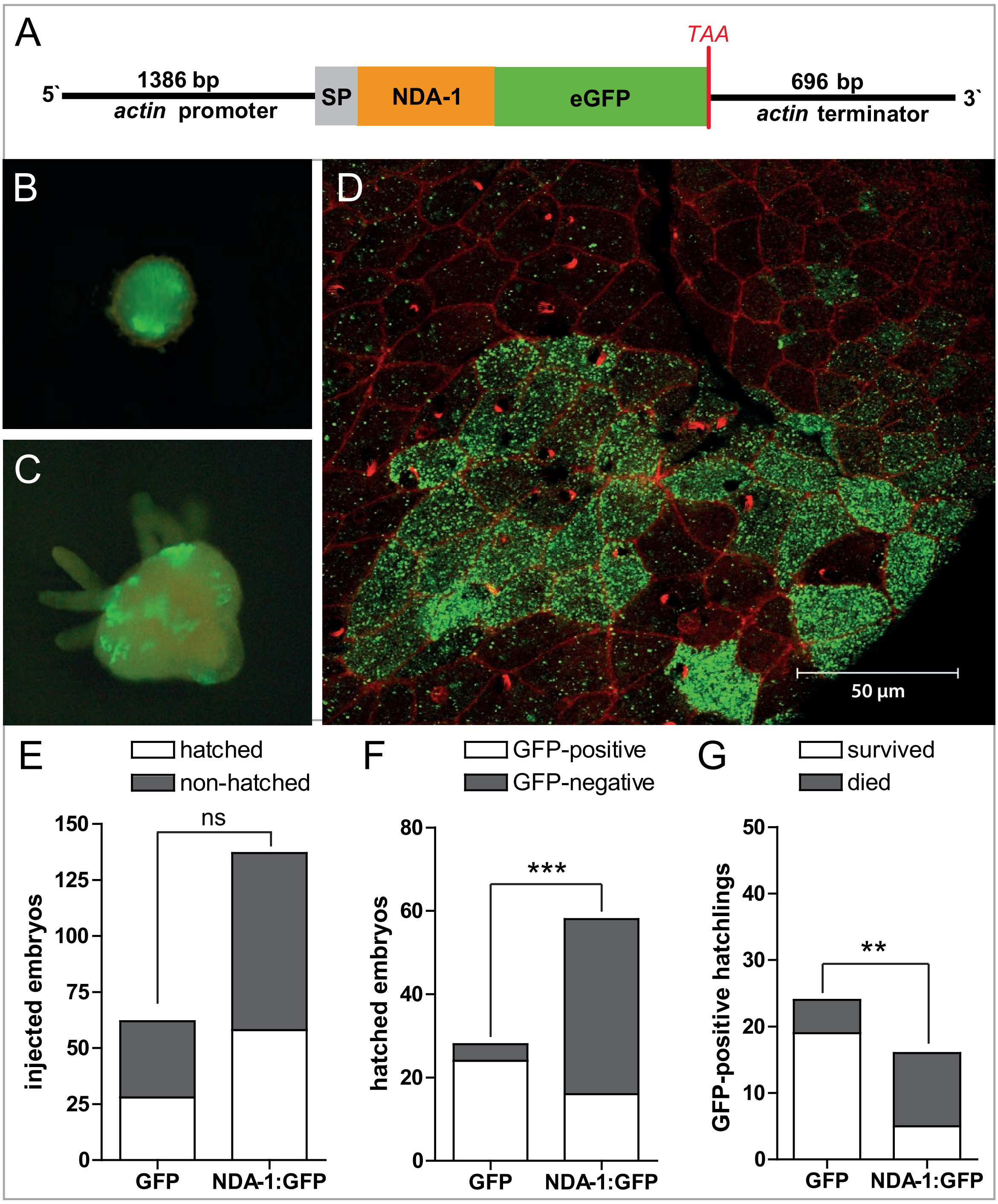

**Supplementary Figure 3. Glutathione (GSH) response of NDA-1 knockdown and control polyps.** The feeding response of *Hydra* can be evoked chemically using micromolar concentrations of γ-glutamil-cysteinyl-glycine (reduced glutathione, GSH), which forces the polyps to open their mouth as they do when capturing prey[1]. This GSH-induced response, like feeding behaviour, is neuron-dependent, since nerve-free animals do not open their mouth[2]. The figure shows the GSH response in 100% of NDA-1 knockdown animals after exposure to 10µM GSH. There was no statistical significant difference in time to mouth opening between NDA-1 knockdown and control animals (t-test: D1: $t = -2$, $df = 20$, $p = 0.1$; NDA-1 control n = 12, NDA-1 knockdown n = 13; E11: $t = 1$, $df = 20$, $p = 0.2$; NDA-1 control n = 12, NDA-1 knockdown n = 12). Boxes in boxplots represent the interquartile range, the line inside the box corresponds to the median, and the wiskers display the span of the data (maximun and minimum values)




D

| | D1 NDA-1 control | D1 NDA-1 knockdown | E11 NDA-1 control | E11 NDA-1 knockdown |
|---|---|---|---|---|
| Number of animals contracting | 20 / 20 | 14 / 14 | 16 / 16 | 19 / 19 |

**Supplementary Figure 4. Contractile behavior induced by mechanical stimulation.**
When *Hydra* polyps are pinched with a pair of forceps, the animals response with rapid contraction of the body column[2]. Here this response to mechanical stimulation is shown. Panel A: no stimulation. Panel B: pincer stimulation as indicated by arrows. Panel C: contraction of body column. Panel D: Response of control and NDA-1 knockdown animals. There was no difference in contractile behaviour.




**Supplementary Figure 5. Ectopic overexpression of NDA-1:eGFP.**
**(A)** Construct used for generation of transgenic hydra overexpressing NDA-1:eGFP. **(B, C)** *In vivo* images of a transgenic *Hydra vulgaris* (AEP) embryo **(B)** and hatchling **(C)** with mosaic expression of NDA-1:eGFP. **(D)** Immunostaining detects the NDA-1:eGFP fusion protein in dense vesicles of ectodermal epithelial cells. Green, eGFP; red, rhodamin-phalloidin for F-actin. **(E)** Embryos injected with the NDA-1:eGFP construct showed no difference in hatching rate compared to embryos injected with a control construct based on the same vector lacking the NDA-1 sequence (eGFP: n=62; NDA-1:eGFP: n=137; p=0.7583, Fisher's exact test). **(F)** NDA-1:eGFP injection resulted in significantly less GFP-positive hatchlings compared to the control construct (eGFP: n=28; NDA-1:eGFP: n= 58; p<0.0001, Fisher's exact test). **(G)** Hatchlings expressing NDA-1:eGFP showed significantly reduced survival compared to hatchlings expressing eGFP only (eGFP: n= 24; NDA-1:eGFP: n=16; p=0.0036, Fisher's exact test). Notably, all surviving NDA-1:eGFP hatchlings lost their GFP-positive cells within 2-3 weeks after hatching and thus, no stable transgenic line was obtained.

**Supplementary Table 1.** Statistical analysis of the minimum model for tissue and treatment as explanatory variables

**Figure 3A. Abundance of *Curvibacter* sp. at various body parts**
**Side-by-side comparison of tissues**

| Body part | versus | Estimate | Std. | Error | t-value | Pr(>\|t\|) | p adjusted fdr |
|---|---|---|---|---|---|---|---|
| TENTACLE | (Intercept) | 1.9979 | 0.1070 | 18.67 | 4.01e-14 | *** | 5.944444e-12 |
| | FOOT | -2.6821 | 0.1514 | -17.72 | 1.07e-13 | *** | 5.944444e-12 |
| | HEAD | -1.7384 | 0.1514 | -11.48 | 2.94e-10 | *** | 2.041667e-09 |
| | BODY DOWN | -2.0300 | 0.1514 | -13.41 | 1.86e-11 | *** | 3.100000e-10 |
| | BODY UP | -1.9630 | 0.1514 | -12.97 | 3.41e-11 | *** | 4.059524e-10 |
| HEAD | (Intercept) | 0.2595 | 0.1070 | 2.425 | 0.0249 | * | 4.882353e-02 |
| | BODY DOWN | -0.2916 | 0.1514 | -1.926 | 0.0684 | | 1.058824e-01 |
| | BODY UP | -0.2246 | 0.1514 | -1.484 | 0.1534 | | 2.029101e-01 |
| | FOOT | -0.9436 | 0.1514 | -6.234 | 4.35e-06 | *** | 1.647727e-05 |
| | TENTACLE | 1.7384 | 0.1514 | 11.485 | 2.94e-10 | *** | 2.041667e-09 |
| BODY UP | (Intercept) | 0.03491 | 0.10703 | 0.326 | 0.747666 | | 7.675310e-01 |
| | BODY DOWN | -0.06698 | 0.15136 | -0.443 | 0.662845 | | 7.532330e-01 |
| | FOOT | -0.71903 | 0.15136 | -4.750 | 0.000122 | *** | 2.346154e-04 |
| | HEAD | 0.22460 | 0.15136 | 1.484 | 0.153430 | | 1.917875e-01 |
| | TENTACLE | 1.96303 | 0.15136 | 12.969 | 3.41e-11 | *** | 2.131250e-10 |
| BODY DOWN | (Intercept) | -0.03207 | 0.10703 | -0.300 | 0.767531 | | 7.675310e-01 |
| | BODY UP | 0.06698 | 0.15136 | 0.443 | 0.662845 | | 7.584039e-01 |
| | FOOT | -0.65204 | 0.15136 | -4.308 | 0.000343 | *** | 7.656250e-04 |
| | HEAD | 0.29159 | 0.15136 | 1.926 | 0.068383 | | 1.058824e-01 |
| | TENTACLE | 2.03001 | 0.15136 | 13.412 | 1.86e-11 | *** | 3.100000e-10 |
| FOOT | (Intercept) | -0.6841 | 0.1070 | -6.392 | 3.09e-06 | *** | 1.647727e-05 |
| | HEAD | 0.9436 | 0.1514 | 6.234 | 4.35e-06 | *** | 1.647727e-05 |
| | BODY DOWN | 0.6520 | 0.1514 | 4.308 | 0.000343 | *** | 7.656250e-04 |
| | BODY UP | 0.7190 | 0.1514 | 4.750 | 0.000122 | *** | 3.630952e-04 |
| | TENTACLE | 2.6821 | 0.1514 | 17.719 | 1.07e-13 | *** | 5.944444e-12 |

**Figure 3D. NDA-1 expression in hydra knockdown lines D1 and E11**

**General output of the statistical model**

| | F-statistics $F_{1,8}$ | p |
|---|---|---|
| Treatment | 93.006 | 1.11e-05 |
| Line | 6.757 | 0.03164 |
| Line x Treatment | 11.949 | 0.00861 |

**Side-by-side comparison control versus knockdown**

| | diff | lwr | upr | p_adjusted | |
|---|---|---|---|---|---|
| D1 (kd-wt) | -0.8172679 | -14,154,722 | -0.2190636 | 0.0101530 | * |
| E11 (kd-wt) | -17,304,441 | -23,286,483 | -11,322,398 | 0.0000696 | *** |

**Figure 3E. Body site distribution of *Curvibacter* sp. in NDA-1 knockdown lines and controls**

**General output of the statistical model**

| | $\chi^2$ statistics ddl | Deviance | P-value |
|---|---|---|---|
| Tissue | 45 | 9.1787 | 7.507e-09 |
| Treatment | 43 | 1.1432 | 0.02432 |

**Side-by-side comparison control versus knockdown**

| Side-by-side | Estimate | Std.E. | t | value | p adj. fdr |
|---|---|---|---|---|---|
| Head kd-wt | 0.3144 | 0.1354 | 2.312 | 0.025080 | 0.0430 |
| Foot kd-wt | 0.3144 | 0.1354 | 2.312 | 0.0251 | 0.0430 |

## Supplementary References:

[1] Lenhoff, H. M. (1961). Activation of the Feeding Reflex in Hydra littoralis: I. Role played by reduced glutathione, and quantitative assay of the feeding reflex. *J. Gen. Physiol.* **45**, 331–344.

[2] Campbell, R. D., Josephson, R. K., Schwab, W. E. & Rushforth, N. B. *Excitability of nerve-free hydra*. *Nature* **262**, 388–390 (1976).