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HsNog      195 SKSVHLTVLRWRCQRRGG-----Q
XlNog.L   185 AKSMHLTILRWRCQRRVQ-----Q
XlNog2.L  180 IKSVTKTFLRWYCQGWTR-----QK
ApNL1     192 TSSQYKTLRLRWHCSPNGPSSVLQOY---NSLPGAKSTGSV-----VNQIK
ApNL1-Clip 176 TSSQYKTLRLRWHCSPNGPSSVLQOY---NSLPGAKSTGSV-----VNQIK
ApNL2     172 SSIRVLNLLRWHCWLKRNKRNKRNRERKTMELAFNKKRGHAARGRAAGQRKSKKKDDKF
ApNL2+Clip 175 SSIRVLNLLRWHCWLKRNKRNKRNRERKTMELAFNKKRGHAARGRAAGQRKSKKKDDKF
DmTrk     181 KSEFTIKVLRRTGSCIRIN-----DKLILITAEKFENDY
DmTrk+Clip 182 KSEFTIKVLRRTGSCIRIN-----DKLILITAEKFENDY
ApPTTH    170 AWYSVPVLLKSNTQQVENQ-----DDLQ-----DE-LQQN
DmPTTH    177 LEYKVKVLAQT-S-QSDHPYS-----W-MNK

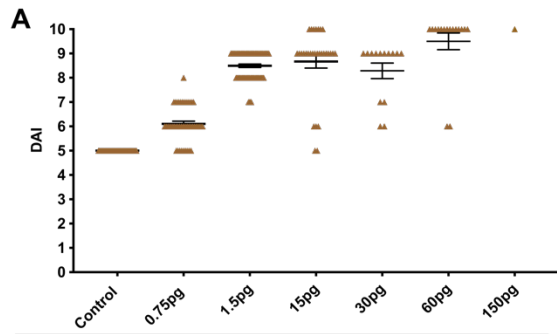
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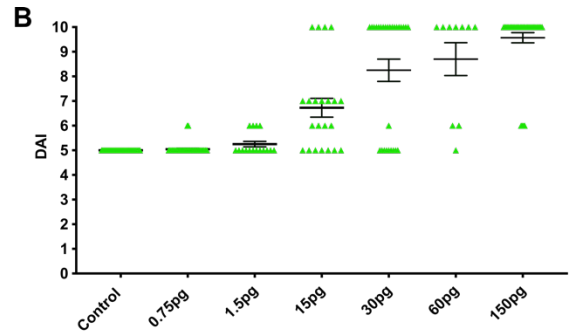
HsNog      214 RCGWIPVQYPIIIECKCSC-----
XlNog.L   204 KCAWITIQYPIIIECKCSC-----
XlNog2.L  200 YCTWIPVQYPIIIECKCSC-----
ApNL1     235 VCGWIKVEYPVVTGCGCGCATDVSE
ApNL1-Clip 219 VCGWIKVEYPVVTGCGCGCATDVSE
ApNL2     232 RCLWIKVPYPVVTGCTCSCCK-PDE
ApNL2+Clip 235 RCLWIKVPYPVVTGCTCSCCK-PDE
DmTrk     215 TQLWIWEEIAVNFCECVML----Y
DmTrk+Clip 216 TQLWIWEEIAVNFCECVML----Y
ApPTTH    199 VNHWKFDVNIIVACYSI-----K
DmPTTH    200 DQPTWQFKTVTVT--AGCFCTK----

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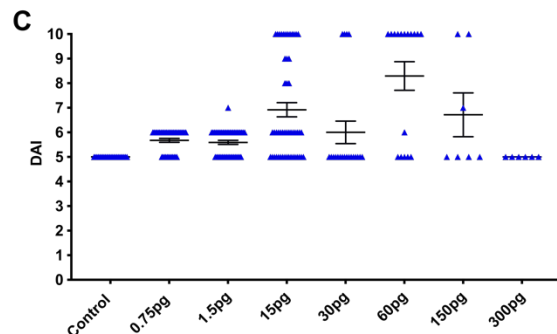
Figure S1: Multiple sequence alignment of Noggin, Noggin-like, Trunk and PTTH proteins with human Noggin. Essential residues in Clip domain of hNoggin - cyan. 15 aa residues of ApNL1- magenta. Deletion and insertion in constructs- red. Hs- *Homo sapiens*, Xl- *Xenopus laevis*, Ap- *Acyrtosiphon pisum*, Dm- *Drosophila melanogaster*. Nog- Noggin, NL- Noggin-like, Trk- Trunk, PTTH- Prothoracicotropic hormone.



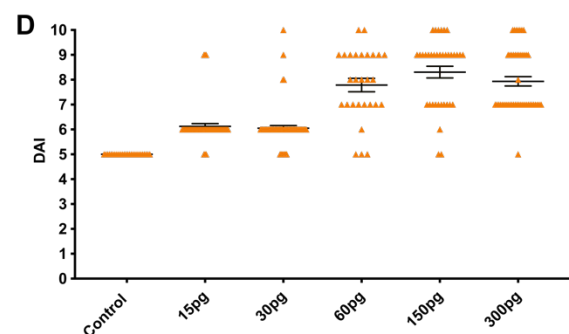
	Control	0.75pgg	1.5pgg	15pgg	30pgg	60pgg	150pgg
N	38	48	77	30	14	16	1
Mean	5	6.104	8.494	8.667	8.286	9.5	10
SD	0	0.7506	0.5988	1.47	1.204	1.366	0
SEM	0	0.1083	0.06823	0.2684	0.3219	0.3416	0
ANOVA	--	****	****	****	****	****	****



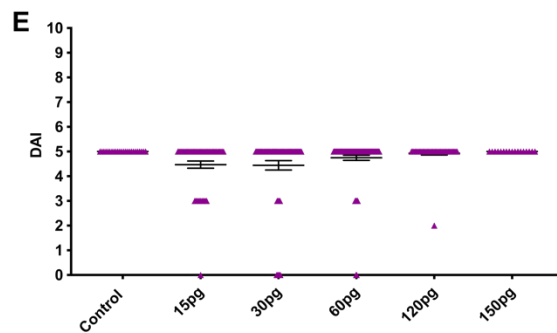
	Control	0.75pgg	1.5pgg	15pgg	30pgg	60pgg	150pgg
N	38	43	16	22	28	10	37
Mean	5	5.047	5.25	6.727	8.25	8.7	9.568
SD	0	0.2131	0.4472	1.778	2.398	2.111	1.259
SEM	0	0.03249	0.1118	0.379	0.4532	0.6675	0.207
ANOVA	--	ns	ns	****	****	****	****



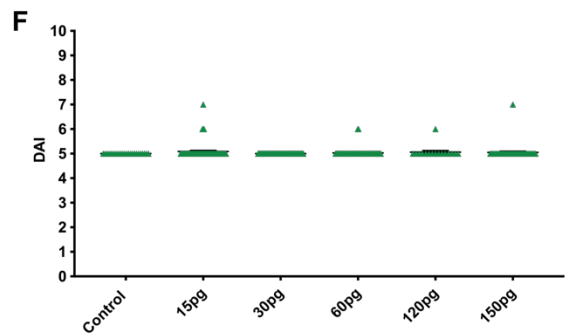
	Control	0.75pgg	1.5pgg	15pgg	30pgg	60pgg	150pgg	300pgg
N	24	37	44	50	20	17	7	6
Mean	5	5.676	5.591	6.92	6	8.294	6.714	5
SD	0	0.4746	0.5421	2.049	2.052	2.392	2.36	0
SEM	0	0.07802	0.08172	0.2897	0.4588	0.5801	0.8921	0
ANOVA	--	ns	ns	****	ns	****	*	ns



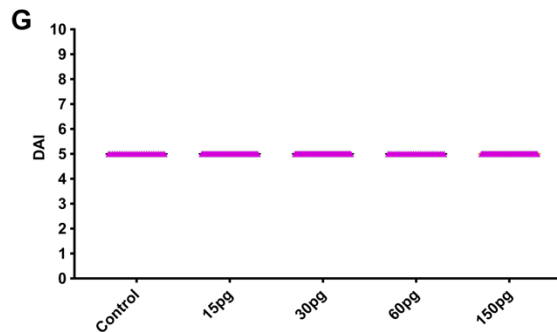
	Control	15pgg	30pgg	60pgg	150pgg	300pgg
N	26	49	61	28	36	47
Mean	5	6.122	6.049	7.786	8.306	7.936
SD	0	0.7808	0.8252	1.424	1.411	1.275
SEM	0	0.1115	0.1056	0.269	0.2351	0.186
ANOVA	--	****	***	****	****	****



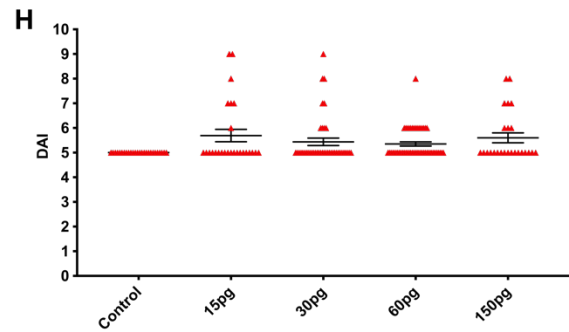
	Control	15pgg	30pgg	60pgg	120pgg	150pgg
N	24	51	59	79	42	17
Mean	5	4.471	4.441	4.747	4.929	5
SD	0	1.046	1.454	0.9124	0.4629	0
SEM	0	0.1465	0.1892	0.1027	0.07143	0
ANOVA	--	ns	ns	ns	ns	ns



	Control	15pgg	30pgg	60pgg	120pgg	150pgg
N	24	59	70	71	16	41
Mean	5	5.085	5	5.028	5.063	5.049
SD	0	0.3367	0	0.1666	0.25	0.3123
SEM	0	0.04384	0	0.01978	0.0625	0.04878
ANOVA	--	ns	ns	ns	ns	ns



	Control	15pgg	30pgg	60pgg	150pgg
N	32	51	61	34	60
Mean	5	5	5	5	5
SD	0	0	0	0	0
SEM	0	0	0	0	0
ANOVA	--	ns	ns	ns	ns



	Control	15pgg	30pgg	60pgg	150pgg
N	26	26	41	51	25
Mean	5	5.692	5.439	5.353	5.6
SD	0	1.289	0.9759	0.5941	1
SEM	0	0.2528	0.1524	0.08319	0.2
ANOVA	--	*	ns	ns	*

Figure S2: DAI scores for the microinjections done in this study. A- *Xlnog.L*, B- *Xlnog2.L*, C- *ApNL1*, D- *ApNL1ΔClip*, E- *ApNL2*, F- *ApNL2+Clip*, G- *DmTrk*, H- *DmTrk+Clip*. Controls were injected with dH₂O. Oneway ANOVA was performed comparing means with control injections. N- survived embryos with DAI phenotypes at stage 32. SD- standard deviation. SEM- standard error of mean. Error bars denote SEM. ns - >0.05, * - $P \leq 0.05$, ** - $P \leq 0.01$, *** - $P \leq 0.001$, **** - $P \leq 0.0001$.

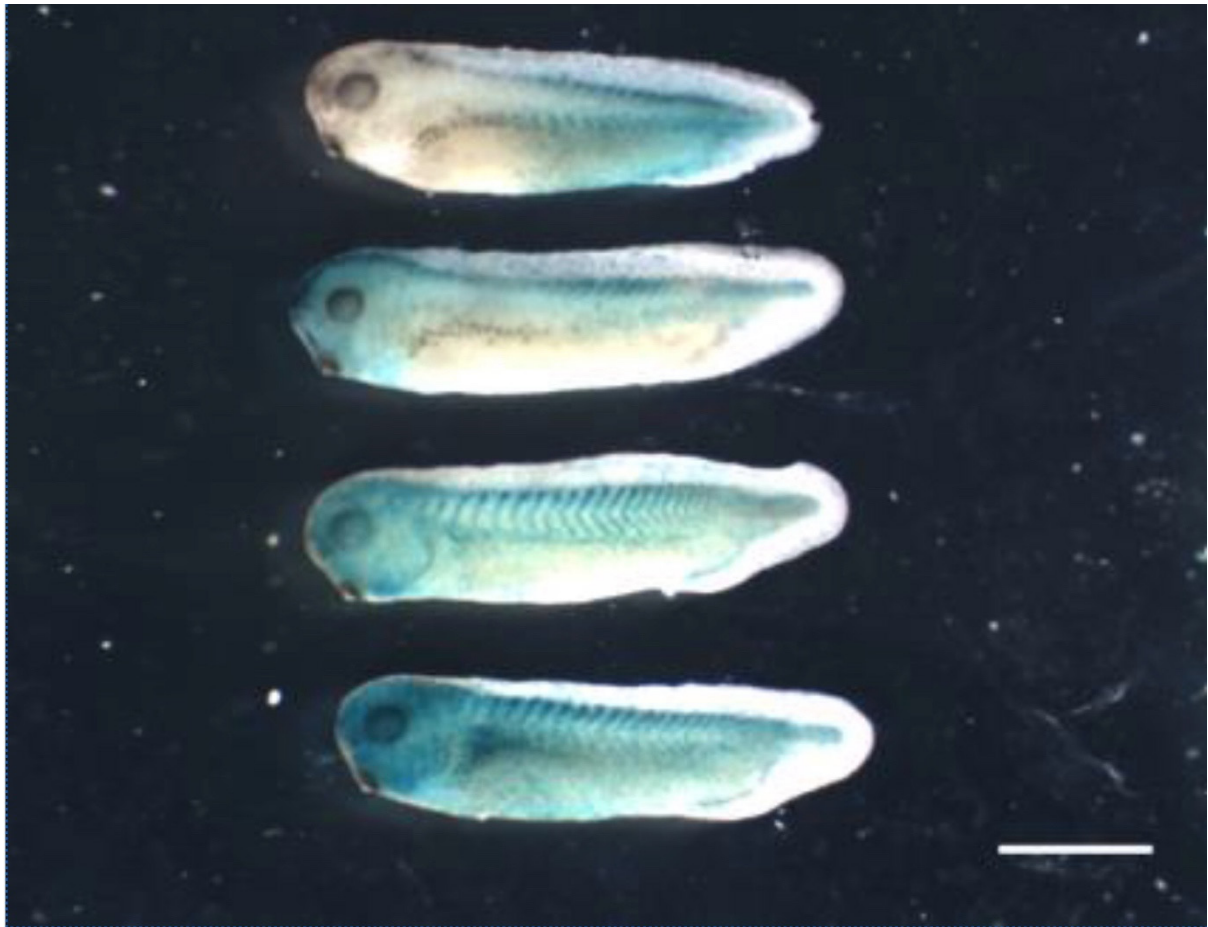


Figure S3: Stage 35 *X. laevis* embryos (DAI5) which have been injected with 230 pg of β-galactosidase mRNA, and stained for β-gal expression.

Table S1: NCBI accession numbers for the genes used in this study

Gene	NCBI accession number	
	mRNA	Protein
<i>A. pisum NL1</i>	XM_029486464.1	XP_029342324.1
<i>A. pisum NL2</i>	XM_003241931.4	XP_003241979.1
<i>A. pisum PTTH</i>	KY305418.1	ARM65502.1
<i>D. melanogaster Trk</i>	NM_057419.3	NP_476767.2
<i>D. melanogaster PTTH</i>	NM_001298621.1	NP_001285550.1
<i>X.laevis Nog.L</i>	NM_001085644.1	NP_001079113.1
<i>X.laevis Nog2.L</i>	NM_001095556.1	NP_001089025.1
<i>H. sapiens Nog</i>	NM_005450.6	NP_005441.1

Table S2: Primers used in this study

Primer	Sequence	Source
<i>ApNLI-F</i>	ACGAATGAATTCATGACTGTCAAATGG	IDT
<i>ApNLI-R</i>	TGCTTATCTAGATCACTCGGAGACGTC	IDT
<i>ApNL2-F</i>	ACGAATGAATTCATGCGGCGTTCCTTC	IDT
<i>ApNL2-R</i>	TGCTTATCTAGATTATTCGTCAGGCTT	IDT
<i>ApPTTH-F</i>	TAAGCAGAATTCATGAGACCTCAGTAC	IDT
<i>ApPTTH-R</i>	TGCTTATCTAGATTATTTAATAGAACA	IDT
<i>DmTrk-F</i>	ACGAATGAATTCATGTTTCTGCGTATA	IDT
<i>DmTrk-R</i>	TGCTTATCTAGACTAGTATAGCATAAC	IDT
<i>eef1a1.L -F</i>	CCCTGAATCACCCAGGCCAGATTGGTG	Sigma
<i>eef1a1.L -R</i>	GAGGTAGTCTGAGAAGCTCTCCACG	Sigma
<i>actc1.L -F</i>	TCCCTGTACGCTTCTGGTCGTA	Sigma
<i>actc1.L -R</i>	TCTCAAAGTCCAAAGCCACATA	Sigma
<i>tbxt.L -F</i>	AAGAATGGAAGACGAATGTTT	Sigma
<i>tbxt.L -R</i>	TGGGTGAGTCTGGGTGGATA	Sigma
<i>ncam1.L -F</i>	CACAGTTCCACCAAATGC	Sigma
<i>ncam1.L -R</i>	GGAATCAAGCGGTACAGA	Sigma
<i>tubb2b.L -F</i>	ACACGGCATTGATCCTACMG	Sigma
<i>tubb2b.L -R</i>	AGCTCCTTCGGTGTAATGAC	Sigma

Table S3: Fly lines used in this study

Transgenic line	Genotype	Source
<i>nos</i> -GAL4	w ⁻ ; P[w+; GAL4::VP16.nos.UTR]MVB1, P{w+; UASp-GFP65C-alphatub84B}3	Bloomington Drosophila Stock Center
trkΔ9.1/CyO ; <i>nos</i> - GAL4/TM6B	trkΔ9.1/CyO;P[w+;GAL4::VP16.nos.UTR]MVB1, P{w+; UASp-GFP65C-alphatub84B}/TM6B	Johnson Lab, Monash University, Australia
UAS-trk	w ⁻ ; P{w+; UAS-Trk}/TM3, Sb	Johnson Lab, Monash University, Australia
UAS-ApNL1	w ⁻ ; P{w+; UAS- ApNL1}/TM3, Sb	This study
UAS-ApNL2	w ⁻ ; P{w+; UAS- ApNL2}/TM3, Sb	This study
UAS-ApPTTH	w ⁻ ; P{w+; UAS- ApPTTH}/TM3, Sb	This study
UAS-XINog1	w ⁻ ; P{w+; UAS- XINog1}/TM3, Sb	This study
trkΔ9.1/CyO ; UAS- Trk/TM6B	trkΔ9.1/CyO ; P{w+; UAS- Trk}/TM6B	Johnson Lab, Monash University, Australia
trkΔ9.1/CyO ; UAS- ApNL1/TM6B	trkΔ9.1/CyO ; P{w+; UAS- ApNL1}/TM6B	This study
trkΔ9.1/CyO ; UAS- ApNL2/TM6B	trkΔ9.1/CyO ; P{w+; UAS- ApNL2}/TM6B	This study
trkΔ9.1/CyO ; UAS- ApPTTH/TM6B	trkΔ9.1/CyO ; P{w+; UAS- ApPTTH}/TM6B	This study
trkΔ9.1/CyO ; UAS- XINog1/TM6B	trkΔ9.1/CyO ; P{w+; UAS- XINog1}/TM6B	This study