

Supplementary Information

Title: Localization and function of neurosecretory protein GM, a novel small secretory protein, in the chicken hypothalamus

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Supplemental Figure Legends

Supplemental Figure 1. Specificity of anti-NPGM and anti-NPGL antibody. (a, b)

Competitive ELISA with anti-NPGM (**a**) and anti-NPGL (**b**) antibody (Ab) against synthetic NPGM and NPGL, respectively.

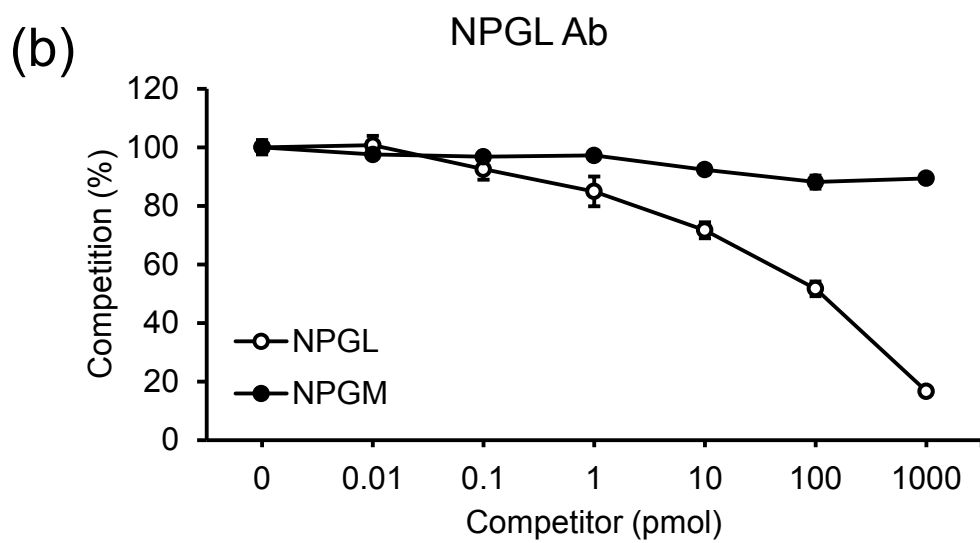
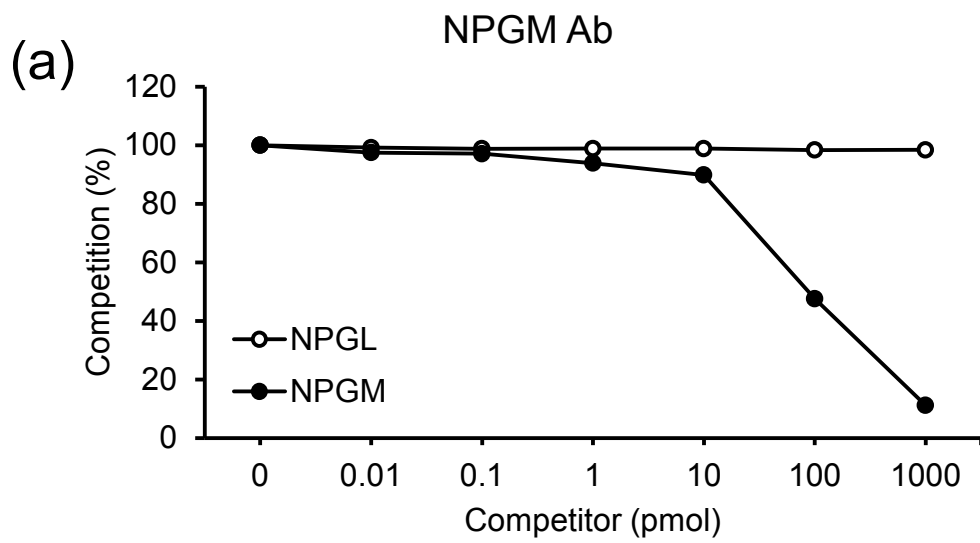
Supplemental Figure 2. Co-localization of NPGM and NPGL neurons. (a–c)

Merged images of NPGM and NPGL neurons in 1-day-old (**a**) and 15-day-old (**b, c**) chicks in the medial mammillary nucleus (MM) (**a, b**) and the infundibular nucleus (IN) (**c**). Arrow-heads indicate NPGM- and NPGL-coexpressing neurons. Scale bar = 25 μ m.

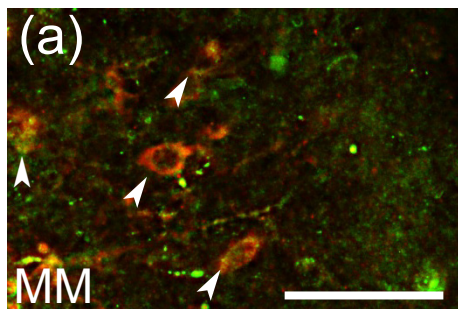
Supplemental Figure 3. Expression level of *HDC* mRNA during post-hatch

development. The expression level of *HDC* mRNA in the hypothalamic infundibulum in 1-, 8-, or 15-day-old chicks. Data are expressed as the mean $\pm$ SEM (n = 5–6).

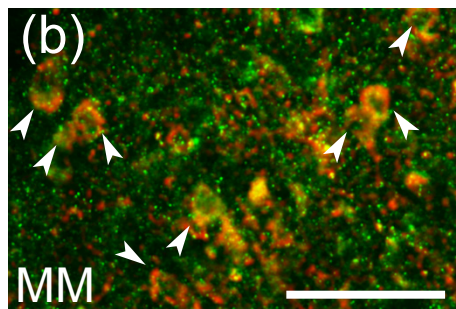
Asterisk indicates statistically significant difference versus vehicle (One-way ANOVA with Tukey's test as a post-hoc test: *P < 0.05, ***P < 0.005 vs. D1).



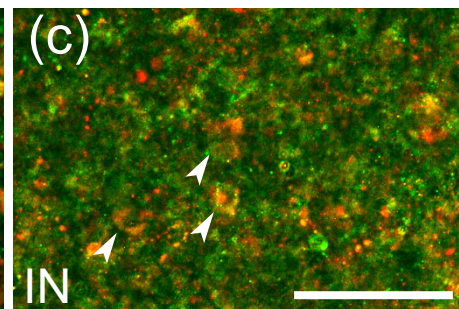
1-day-old

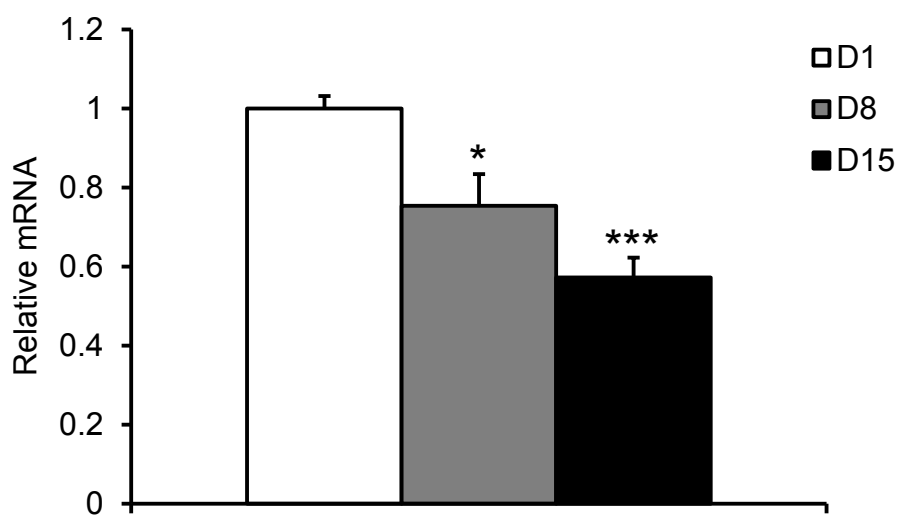


15-day-old



15-day-old





Supplemental Table 1-1. Sequences of oligonucleotide primers for real-time RT-PCR

gene	Forward primer	Reverse primer	Accession no.
<i>NPGM</i>	GTACGTAGACTGCGTGCTCT (Nucleotide no. 285 to 304)	GTGAAAGCAGACCCTTGTA (Nucleotide no. 395 to 376)	XM_429770.2
<i>NPGL</i>	CTAGGAAAAAGACAGCTTGC (Nucleotide no. 334 to 353)	CTTTCTTCGTCAGAACTGGT (Nucleotide no. 454 to 435)	AB909129
<i>HDC</i>	GTAAGAAGTGATCCGCTGTT (Nucleotide no. 1226 to 1245)	TGCTCAGTTCTTTCAGGAGT (Nucleotide no. 1337 to 1318)	AB900795
<i>ACTB</i>	AGCCAACAGAGAGAAGATGA (Nucleotide no. 339 to 358)	CCAGAGTCCATCACAATACC (Nucleotide no. 467 to 448)	NM_205518.1

Supplemental Table 1-2. Sequences of oligonucleotide primers for *in situ* hybridization

gene	Forward primer	Reverse primer
<i>NPGM</i>	ATGGAATTCATGTGGAAGAG (Nucleotide no. 1 to 20)	AGCATCTACAGTAAATGCTG (Nucleotide no. 493 to 474)
<i>NPGL</i>	GGAGCGCCGAGTGAC (Nucleotide no. -18 to -3)	CTTTCTTCGTCAGAACTGGT (Nucleotide no. 472 to 453)
<i>HDC</i>	TGGAGATATGCTGGCTGATG (Nucleotide no. 270 to 289)	ATGGGCACTAGGCCTTTCTT (Nucleotide no. 713 to 694)