

**Neuronal Connectivity between Habenular Glutamate-Kisspeptin1 Co-expressing Neurons  
and the Raphe 5-HT System**

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**SUPPORTING INFORMATION**

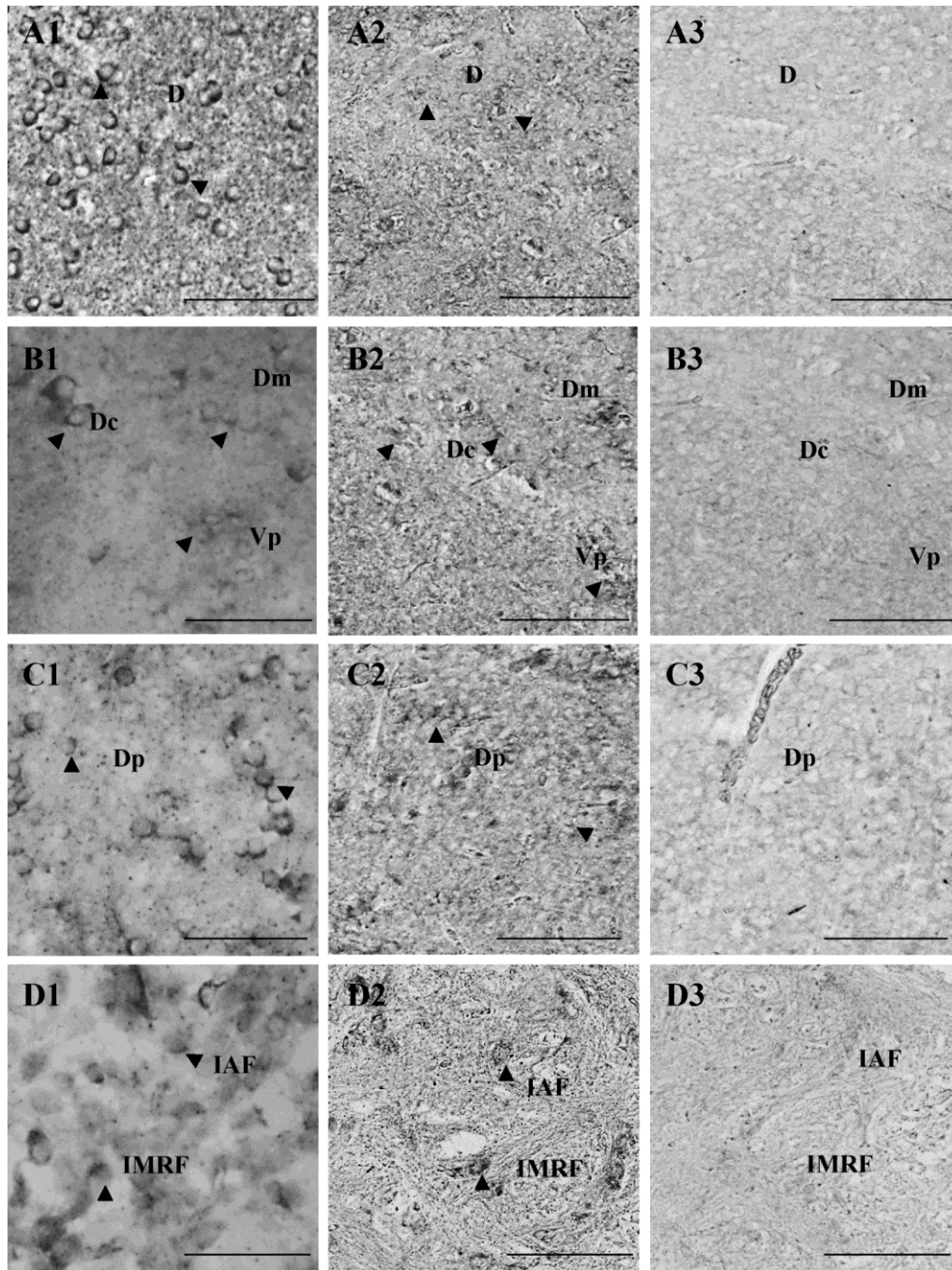
**Figure S1**

**Figure S2**

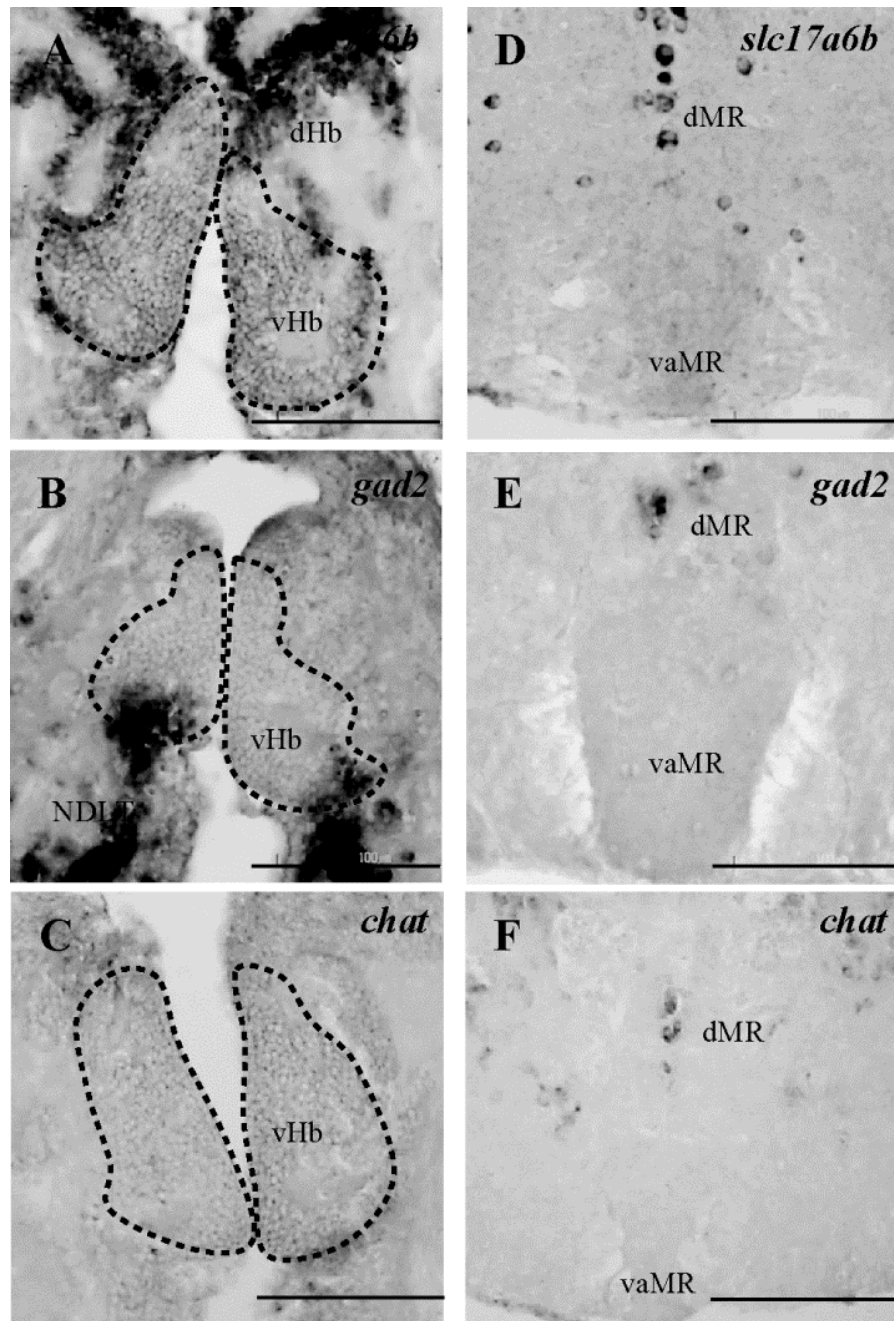
**Figure S3**

**Figure S4**

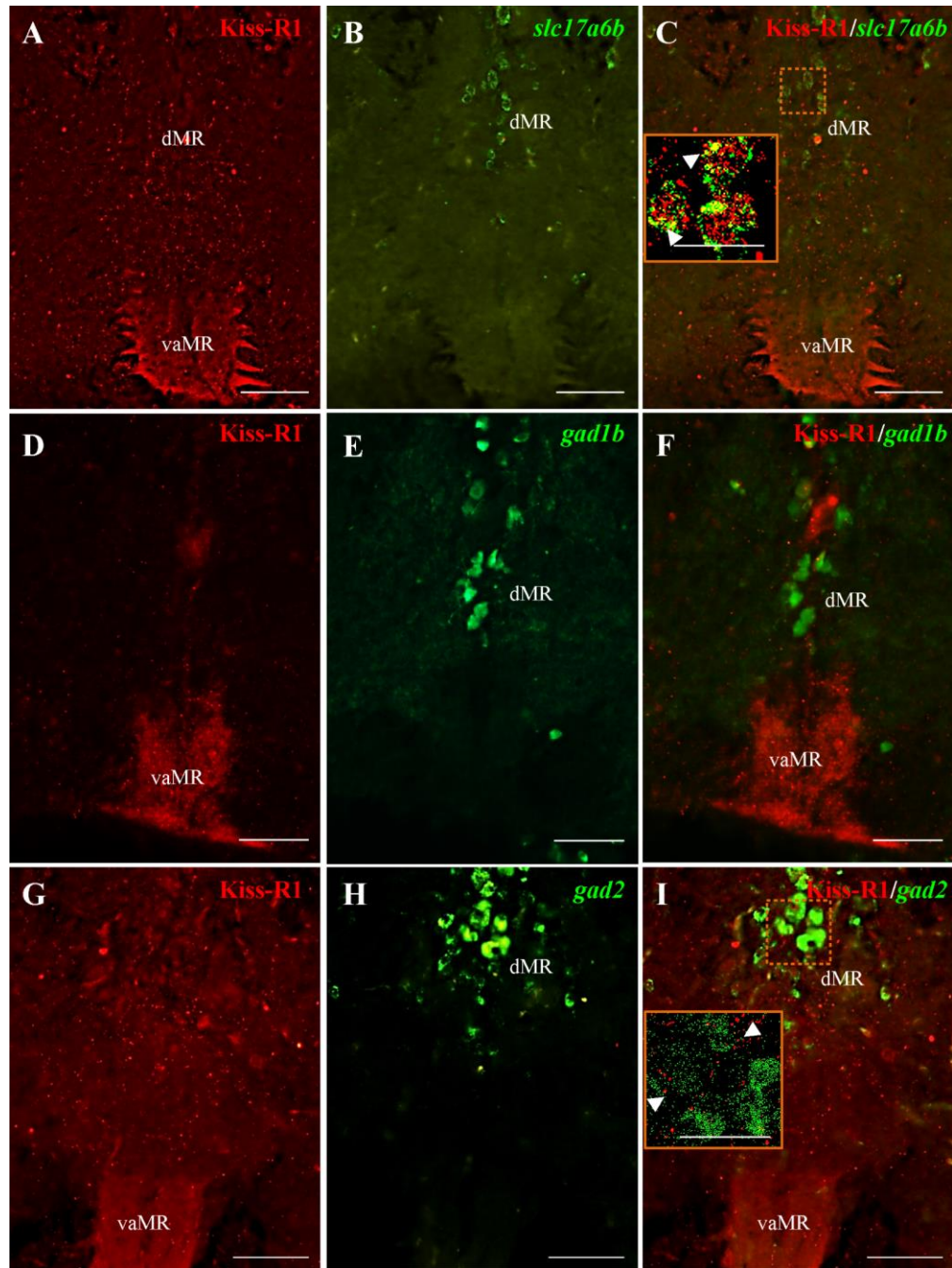
**Table S1**



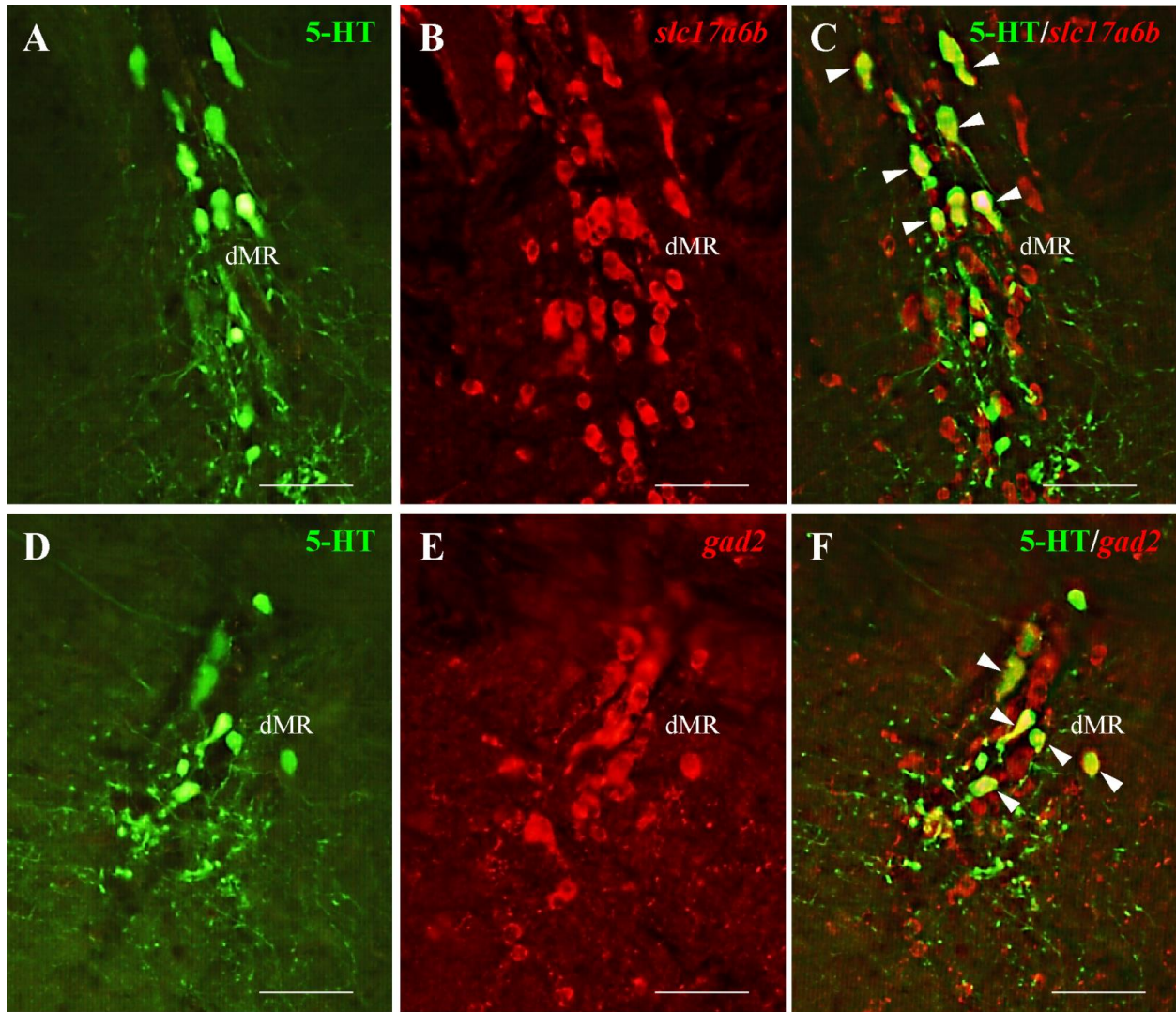
**Figure S1: Kiss-R1-ir localization and *KRBDP2* expression in other regions of the zebrafish brain.** A1-D1, Kiss-R1-immunoreactive (-ir) cell somata observed outside of the habenula such as the dorsal telencephalic area (A1), central, medial (B1) and posterior (C1) zone of dorsal telencephalic area, postcommissural nucleus of ventral of the ventral telencephalic area (B1), inner arcuate nucleus and intermediate reticular formation (D1). A2-D2, *KRBDP2* mRNA expression was also observed in same regions as Kiss-R1-ir cells. A3-D3, The sense riboprobe showed no expression of *KRBDP2* mRNA. For abbreviations, see Table 2. Scale bars, 100  $\mu$ m.



**Figure S2: Expression of *slc17a6b*, *gad2* and *chat* mRNAs in the habenula and raphe regions.** A and D, *slc17a6b* mRNA was noted in some population of cells in the vHb and in the dMR and sparsely in the DR. B and E, *gad2* mRNA expressing cells was observed as a minute population in the ventral region of the vHb and in the dMR. C and F, *chat* mRNA was weakly present in the vHb with some cells in the dMR and DR. Scale bars, 100  $\mu$ m.



**Figure S3: Dual-fluorescence labeling of Kiss-R1 (red) in glutamatergic and GABAergic neurons (green) in raphe nuclei.** A-C: Kiss-R1-immunoreactivity co-expressed with *slc17a6b*-expressing cells as denoted by the confocal image (inset C; 79.8X; N.A. = 1.4; z-step 0.15  $\mu\text{m}$ ). D-F: GFP-labeled *Gad1b* neurons were only observed in the dMR with no close associations observed with Kiss-R1 fibers. G-I: There was no co-localization of Kiss-R1 with *gad2*-expressing cells, but Kiss-R1-ir fibers were noted within close proximity in the dMR. Presence of actual space of at least 0.15  $\mu\text{m}$  noted between fibers and cells (inset I). Scale bars, A-F: 100  $\mu\text{m}$  and inset C and I: 50  $\mu\text{m}$ .



**Figure S4: Co-expression of *slc17a6b* and *gad2* (red) with 5-HT (green).** In the raphe nuclei, some 5-HT neurons co-express both *slc17a6b* (A-C) and *gad2* (D-F). Scale bars, 100 μm.

**Table S1: Gene abbreviations, the primer sequences, probe size and GenBank accession numbers for probes for DIG-*in situ* hybridization**

<b>Gene abbreviation</b>	<b>Gene name</b>	<b>Nucleotide sequence (5' - 3') of primers used for probe synthesis</b>	<b>Probe size (nt)</b>	<b>DDBJ/EMBL/Gen Bank Accession Number</b>
kissr1	Kisspeptin receptor1	Forward: GTCTGATGGCAGAACTAAC Reverse: CAGAGGTCAGGATGAAAGAA	1106	EU 047918
KRBDP2	<i>kissr1b</i> -derived protein 2	Forward: TATGTGTGCGCTGGTCAATTATC Reverse: TCAGGATGAAAGAAAGTTCATCTC	720	HQ 222885
slc17a6b	solute carrier family 17 sodium-dependent inorganic phosphate cotransporter member 6b transcript variant 2, also known as vesicular glutamate transporter 2 ( <i>vglut2</i> )	Forward: TGTCGGCTTCAGTGGATTTG Reverse: ACTCCTCTCGTTTATCCCATCCT	437	NM_001005398.1
gad1b	glutamic acid decarboxylase 1b	Forward: TGTGAACCATCCTCCGTGTG Reverse: AGTAGATCTCGCGCGAACAG	405	NM_194419.1
gad2	glutamic acid decarboxylase 2	Forward: TACCTGCACTGGACGGAGAC Reverse: GGTGTTTCCGGGACATTAGC	840	NM_001017708.2
chat	choline acetyltransferase	Forward: TGTTTGTGTCTGGTGTGTTT Reverse: AGCATCAGGGCTCATTTTCT	450	NM_001130719.1