Supplemental Figure 1. Fidelity of GFP reporter expression in the Met^{GFP} transgenic mouse. A and D) Low magnification images of ML-FISH in coronal section of the Met^{GFP} brain prepared at postnatal day 14 (Bregma level -5 mm). RNAscope probes label *Met* (light blue), *GFP* (magenta), and *Tph2* (yellow). B-B") High magnification images of region boxed in panel A that contains 5-HT neurons positioned in the DRC, displayed in merged and split channels. C-C") High magnification images of region boxed in panel A that contains 5-HT neurons located in the MRN. E and F) High magnification image showing regions boxed in panel D with. Orange arrows indicate *GFP* and *Met* double-positive neurons. Green arrows indicate a non-serotonergic (*Tph2*-negative) neuron in the MRN that expresses *Met* and *GFP*. G) Quantification of the percentage of *GFP* and *Tph2* double-positive serotonergic neurons that co-express *Met* transcript. H) Quantification of the percentage of *Met* and *Tph2* double-positive serotonergic neurons that co-express *GFP* transcript. Scale bar = 100um in A and D. Scale bar = 50μm in B-B", C-C", E and F.

Supplemental Figure 2. **Most 5-HT neurons in the DRC express Met.** Immunostaining of an adult Met^{GFP} coronal section containing the DRC (Bregma level – 5 mm). GFP, green; 5-HT, gray; DAPI, blue. Arrows denote the minority populations of 5-HT+ cells in the DRC that do not express GFP.

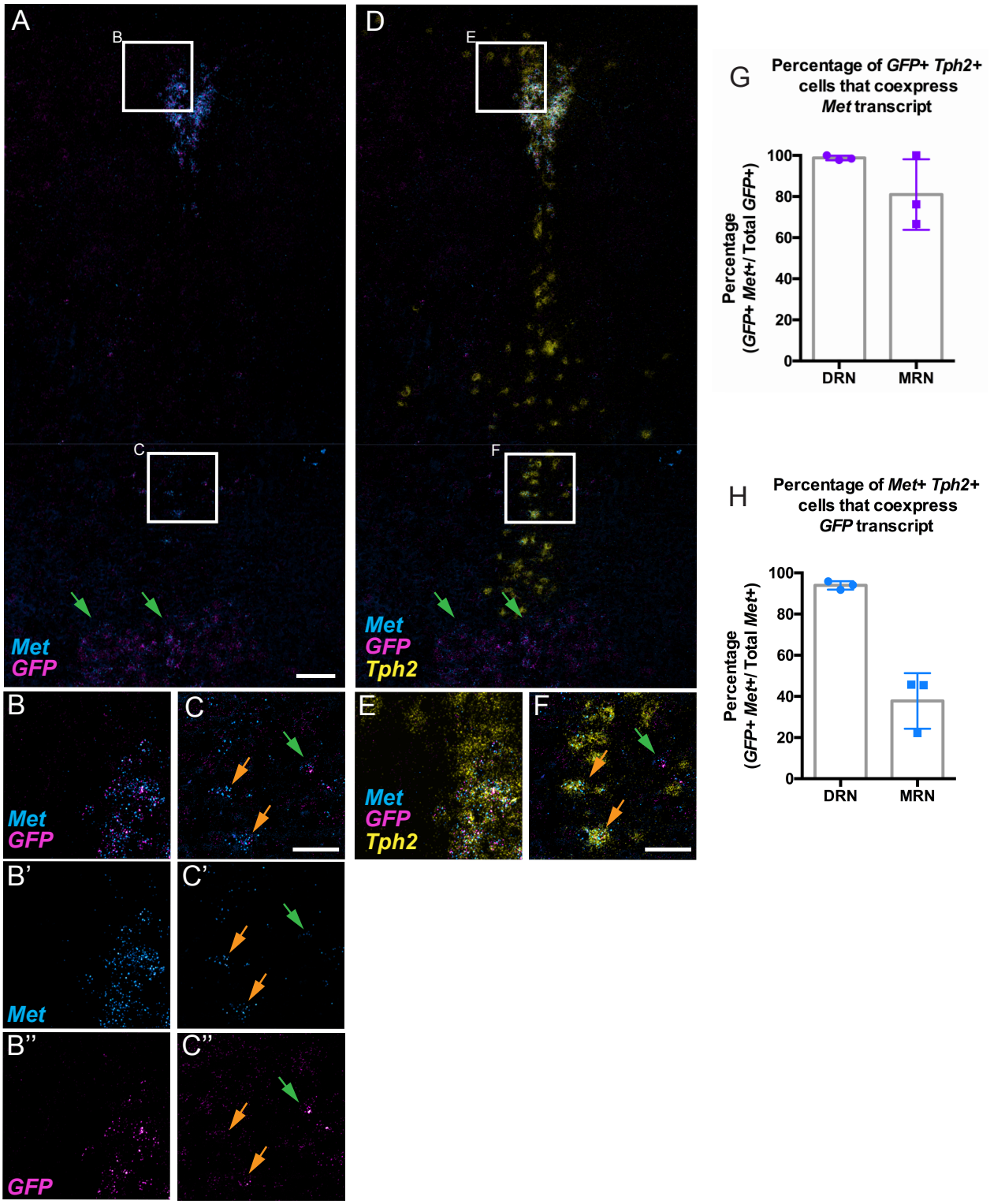
Supplemental Figure 3. Some SVZ projecting raphe neurons located in the rostral DRN and MRN also express MetGFP. Images depict immunolabeling of GFP (green) and colocalization with retrobeads (red), and a complementary set of adjacent sections with 5-HT immunolabeling (White). Cyan arrowheads denote cells co-labeled with GFP and retrobeads. Note the sparseness of the retrobead+ cells and GFP+ cells at this level (Bregma level -4.7 mm). Scale Bar, 100 μm.

Supplemental Figure 4 Injection sites for retrograde tracing performed in the Met^{GFP} mouse. A) Lateral ventricle injection. B) CA1 of dorsal hippocampusl Injection. C) Lateral entorhinal cortex injection. Scale bar = 1 mm.

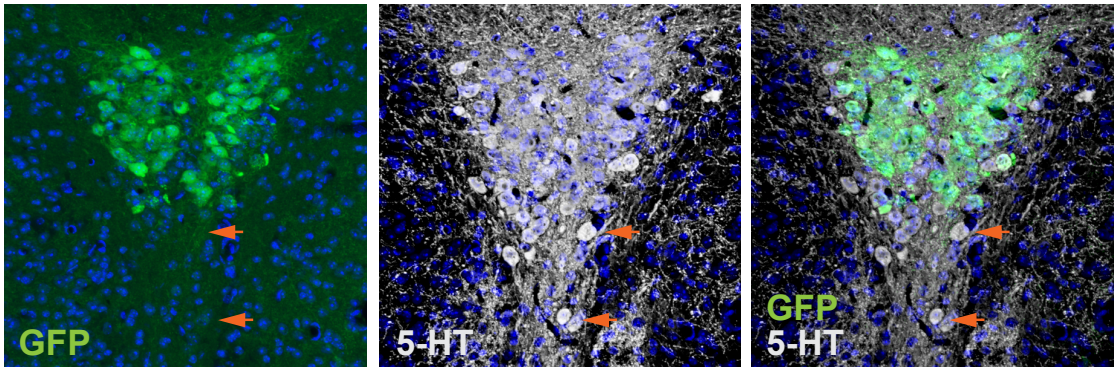
Supplemental Figure 5. Genes enriched in 5-HT^{MET+} neurons in the DRC. A) *In situ* hybridization reveals *Oprm1* transcript enrichment in the DRC. Sagittal section of the postnatal day 14 (P14) mouse brain from the Allen Mouse Brain Atlas[41]. B) *In situ* hybridization displaying *Chrm2* transcript enrichment in the DRC. Sagittal section of postnatal day 14 (P14) mouse brain from the Developing Mouse Brain Atlas. C) *In situ* hybridization displaying *Chrna7* transcript enrichment in the DRC. Sagittal section of P4 mouse brain from the Developing Mouse Brain Atlas. A'-C') Coronal section through the DRC and DRI (Bregma level -5 mm) of P14 mouse brain. ML-FISH performed using RNAscope probes toward *Tph2* (orange), *Met* (light blue), and *Oprm1* (A'), *Chrm2* (B'), or *Chrna7* (C') (magenta); DAPI, white. Green arrows denote the expression of candidate genes by non-5-HT (*Tph2*-negative) cells surrounding the DRC. A"-C") Coronal section through the DRD and DRV (Bregma level -4.9 mm, A" and B"; Bregma level -4.7mm) of P14 mouse brain. ML-FISH performed as in A', B' and C'. Note the reduced expression of the candidate genes (magenta) in the *Tph2*-positive neurons of these more rostral sections. Scale bar in low mag (C') images = 100 μm. Scale bar in high magnification insets, 25 μm.

Supplemental Table 1. Retrograde tracing sites and corresponding stereotaxic coordinates

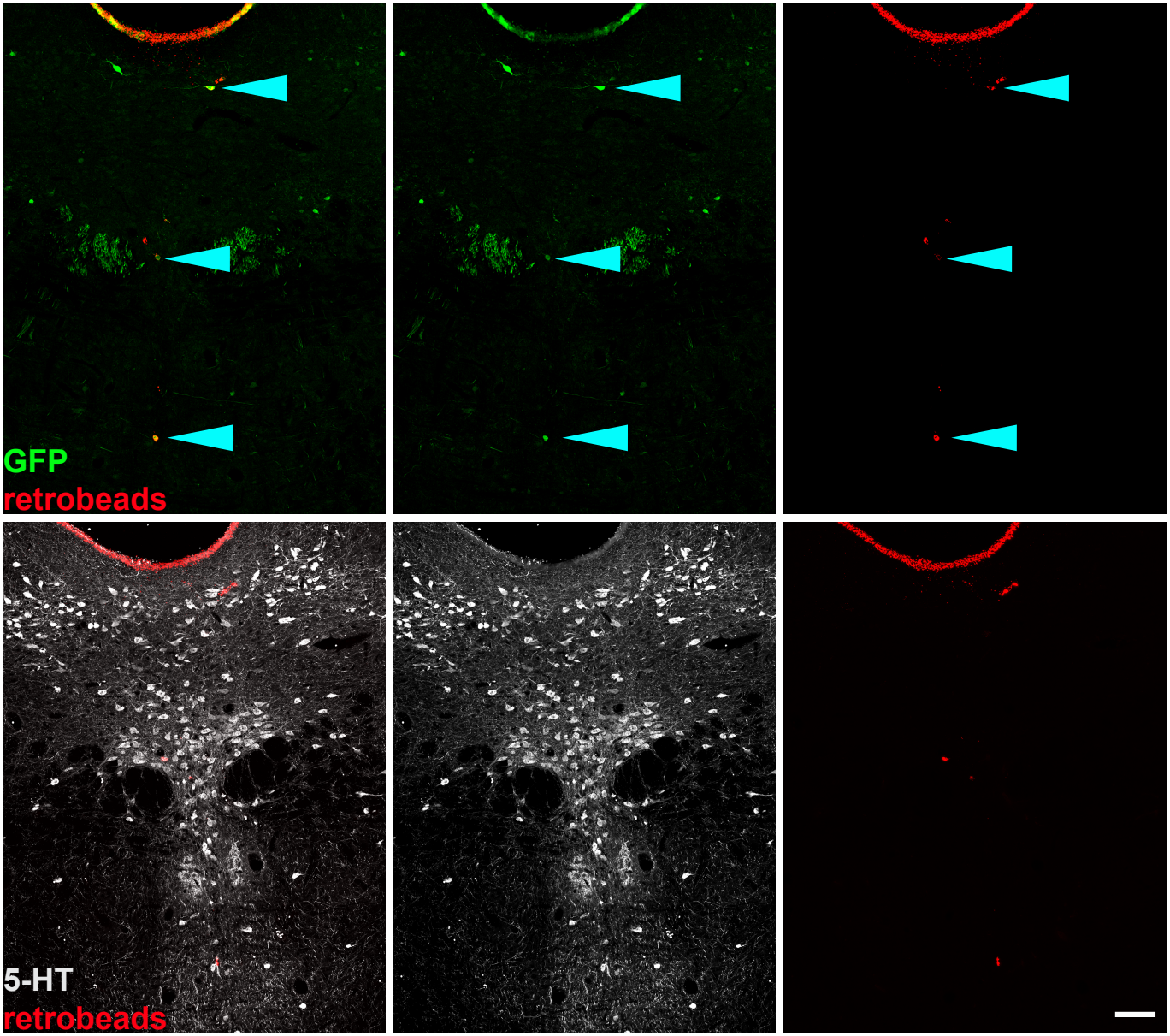
Supplemental Table 2. Links to Allen Brain Institute *in situ* hybridization data showing DRC enriched gene expression from the Developing Mouse Brain Atlas.



Supplemental Figure 1

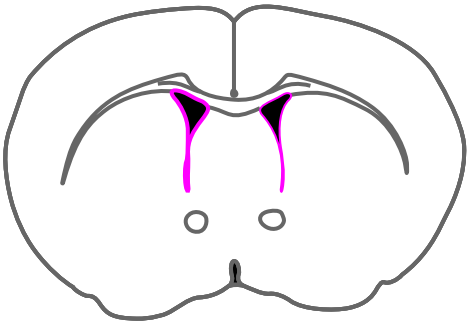


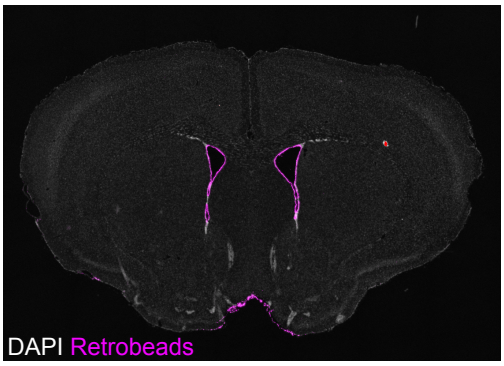
Supplemntal Figure 2



Supplemental Figure 3

A





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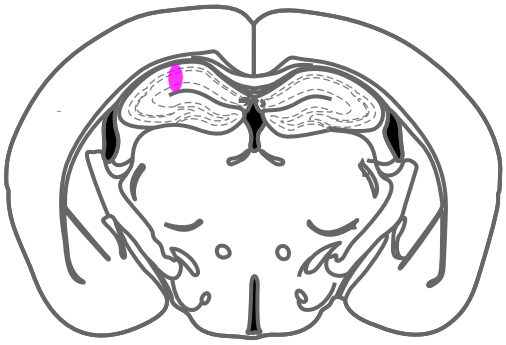

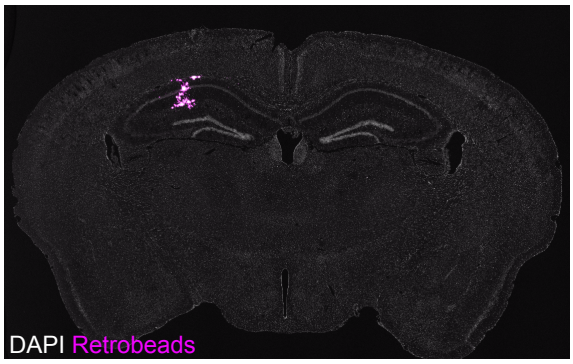

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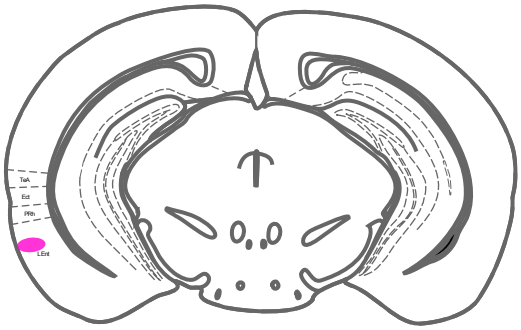
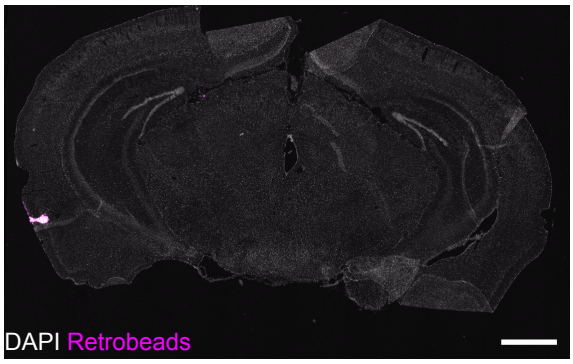
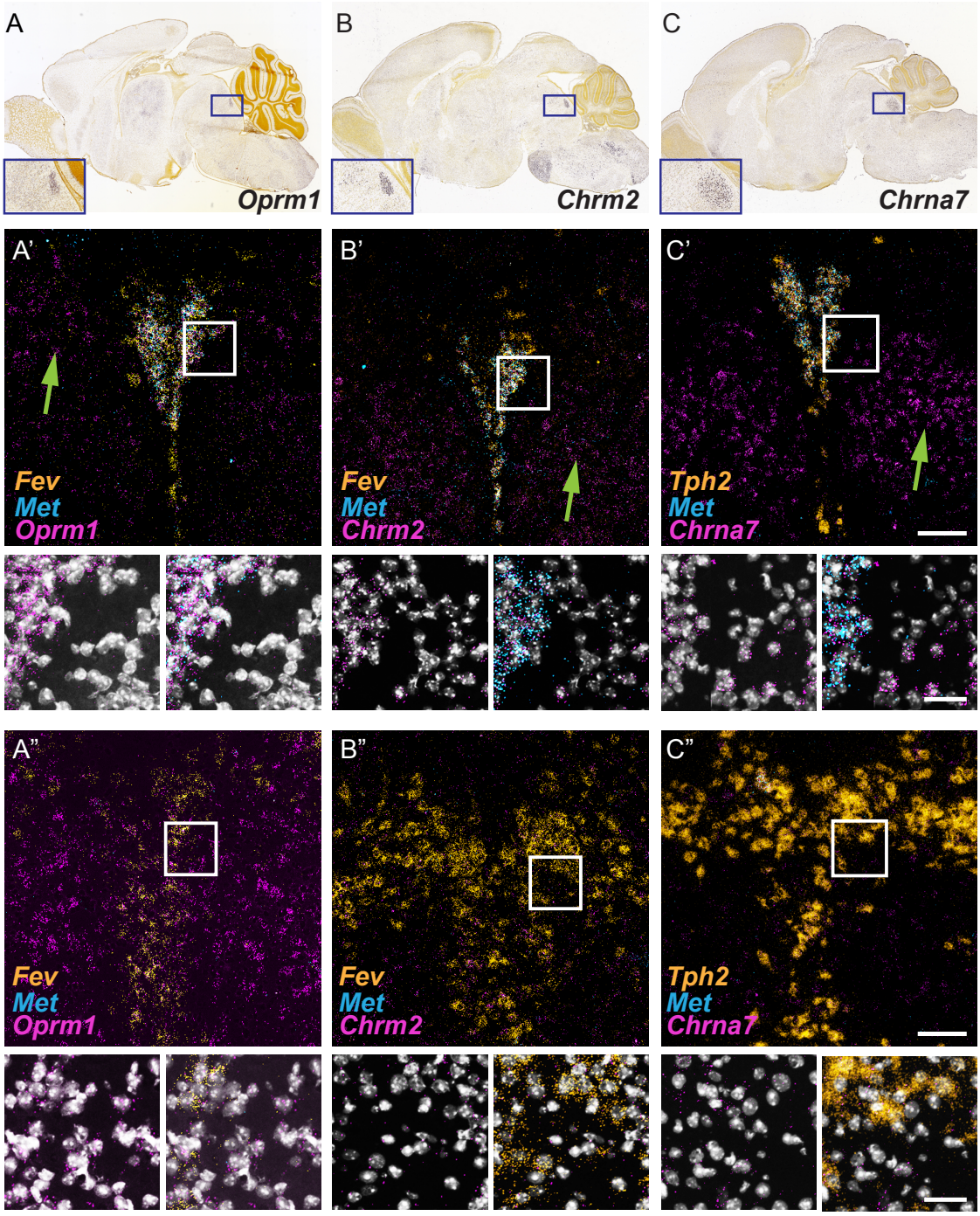




Supplemental Figure 4



Supplemental Figure 5

Supplemental Table 1 Retrograde tracing sites and corresponding stereotaxic coordinates

	Stereotaxic Coordinates (Measured from Bregma)		
Forebrain Target	AP	ML	Depth
Lateral Ventricle	+0.38mm	0.8mm	2.2mm
CA1 Dorsal Hippocampus	-2.1mm	2.0mm	1.4mm
Entorhinal Cortex	-3.5mm	4.5mm[A]	0.5mm

A) For entorhinal cortex injections, prior to coordinate measurements, the stereotaxic frame was rotated 90˚ to allow the injection needle to enter the cortex perpendicular to the pial surface. This prevented contamination of more dorsally located cortical regions. Thus, the mediolateral (ML) distance is measured following the 90˚ rotation, and relative to the dorsal surface of the cranium at the sagittal suture, 3.5mm posterior to Bregma.

Supplemental Table 2 Links to Allen Brain Institute *in situ* hybridization data showing DRC enriched gene expression from the Developing Mouse Brain Atlas.

Transcript	Figure	Allen Institute URL
Kcna4	6 A	http://mouse.brain-map.org/experiment/siv?id=71529965&imageId=71331730&initImage=ish&coordSystem=pixel&x=7024.5&y=4064.5&z=1
Kcnd2	6 B	http://developingmouse.brain-map.org/experiment/siv?id=100056149&imageId=101106019&initImage=ish&x=5888&y=3480&z=3
Tacr3	6 C	http://developingmouse.brain-map.org/experiment/siv?id=100073401&imageId=101225732&initImage=ish&x=2768&y=2696&z=0
Oprm1	S5 A	http://developingmouse.brain-map.org/experiment/siv?id=100015413&imageId=100550787&initImage=ish&x=4848&y=3440&z=3
Chrm2	S5 B	http://developingmouse.brain-map.org/experiment/siv?id=100056132&imageId=101098974&initImage=ish&x=6144&y=3328&z=3
Chrna7	S5 C	http://developingmouse.brain-map.org/experiment/siv?id=100056114&imageId=101098652&initImage=ish&x=8082&y=3218&z=6

*** "Figure" corresponds to the figure in the current study that contains images of associated transcript.*