"Developmental origins of central norepinephrine neuron diversity."
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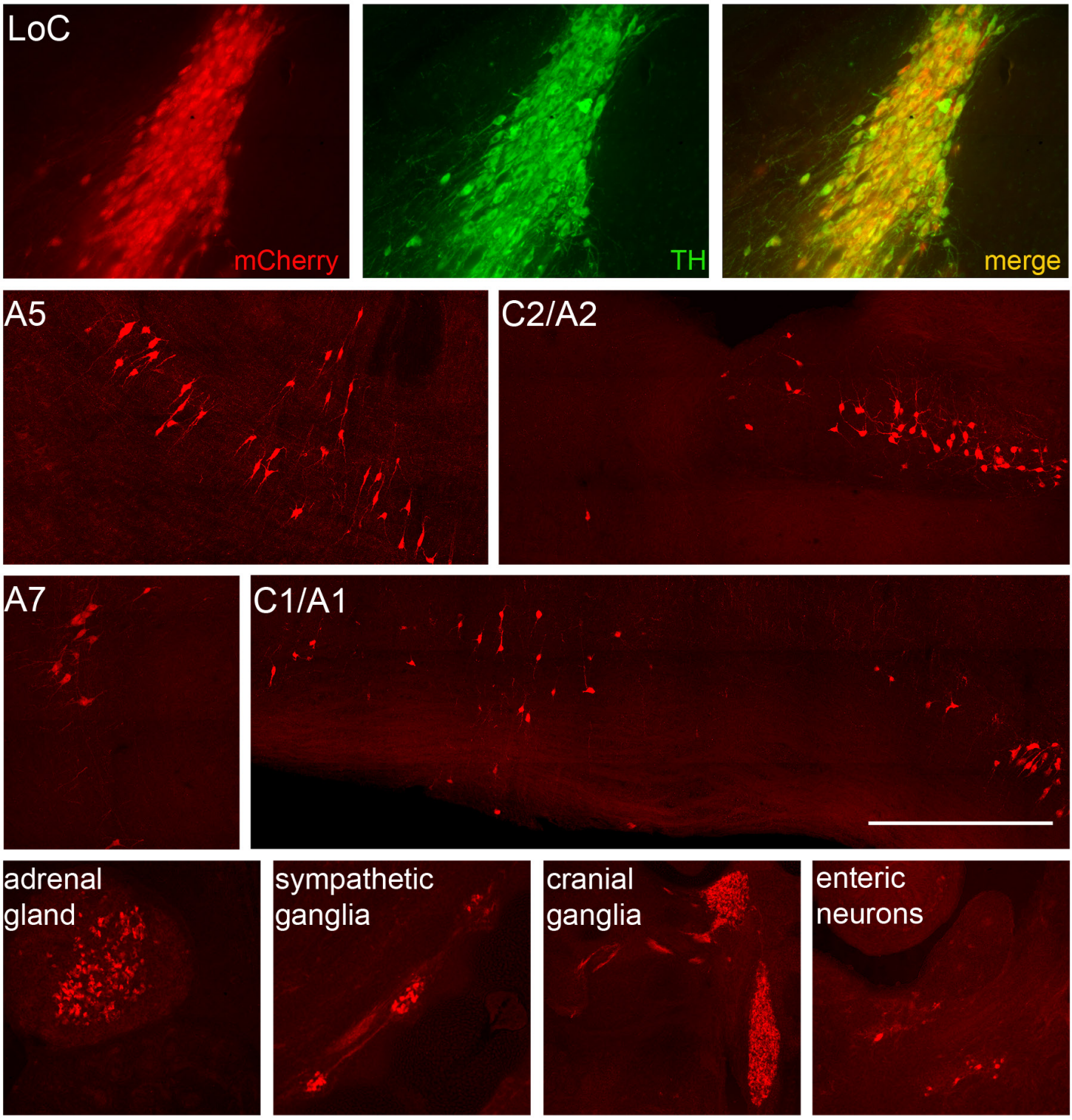
SUPPLEMENTAL DATA

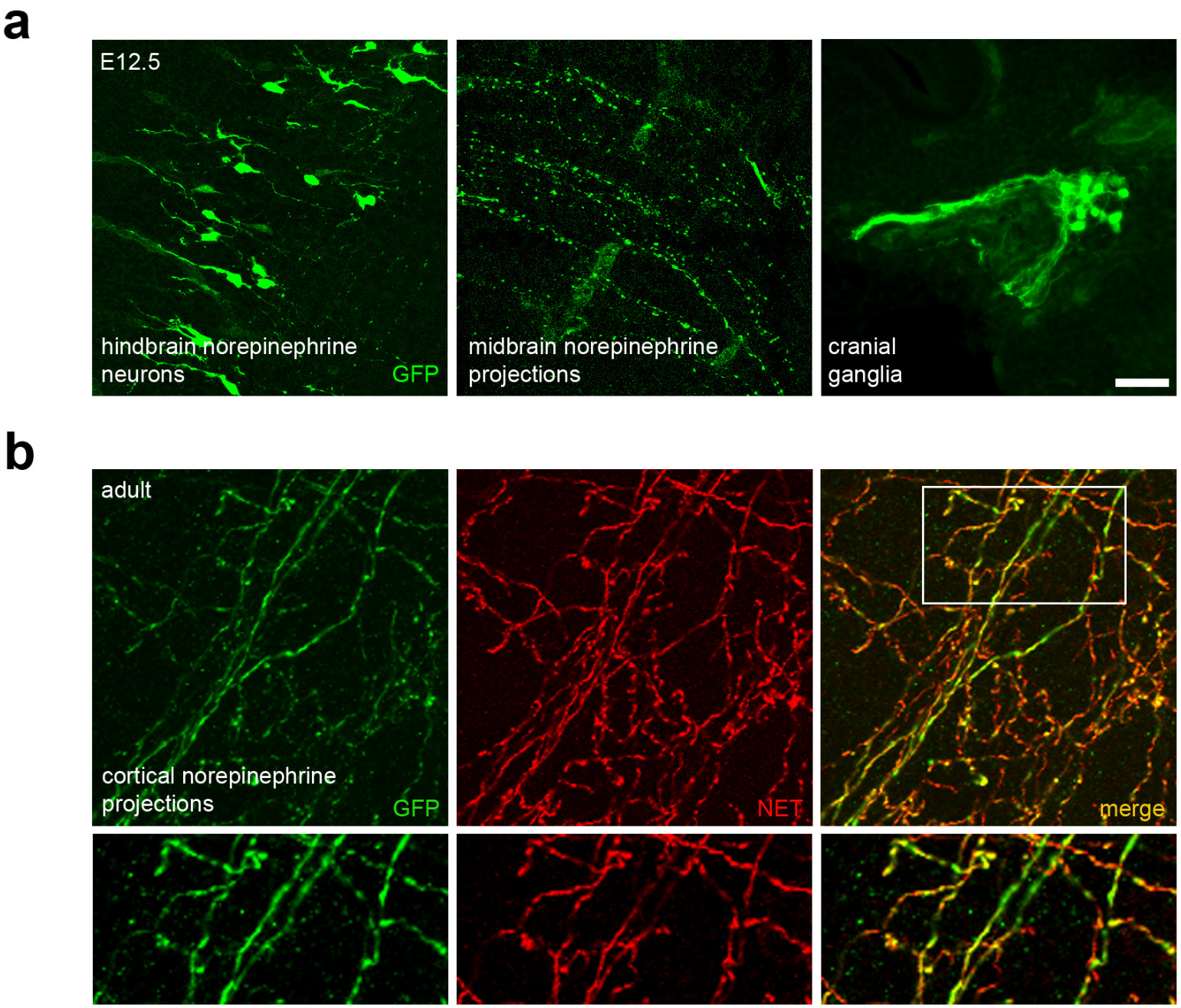
Supplementary Figure 1



Supplementary Fig. 1. The *Dbh^{Flpo}* allele expresses Flpo recombinase in all norepinephrine and epinephrine neurons.

Expression of *Dbh^{Flpo}* was revealed by mCherry expression from the *RC::FrePe* indicator allele in sagittal sections of *Dbh^{Flpo};RC::FrePe* double heterozygotes. mCherry expression was observed in the anatomically defined norepinephrine and epinephrine nuclei of the brainstem: locus coeruleus (LoC), A5, C2/A2, A7, C1/A1. Identification of norepinephrine neurons in the LoC was confirmed by comparison with tyrosine hydroxylase (TH) immunofluorescence (*top row, center*). An overlay of TH (green) and mCherry (red) immunofluorescence shows concordance between the two domains (*top row, right*). mCherry expression was also observed in regions of the peripheral nervous system known to express *Dbh* (*bottom row*): adrenal medulla, sympathetic ganglia, cranial ganglia, and enteric neurons. Top three rows, adult mouse; bottom row, E14.5 embryo. Scale bar: 435 μM (LoC), 500 μM (A5, C2/A2, A7, C1/A1), 485 μM (adrenal, sympathetic ganglia, enteric neurons), 870 μM (cranial ganglia).

Supplementary Figure 2

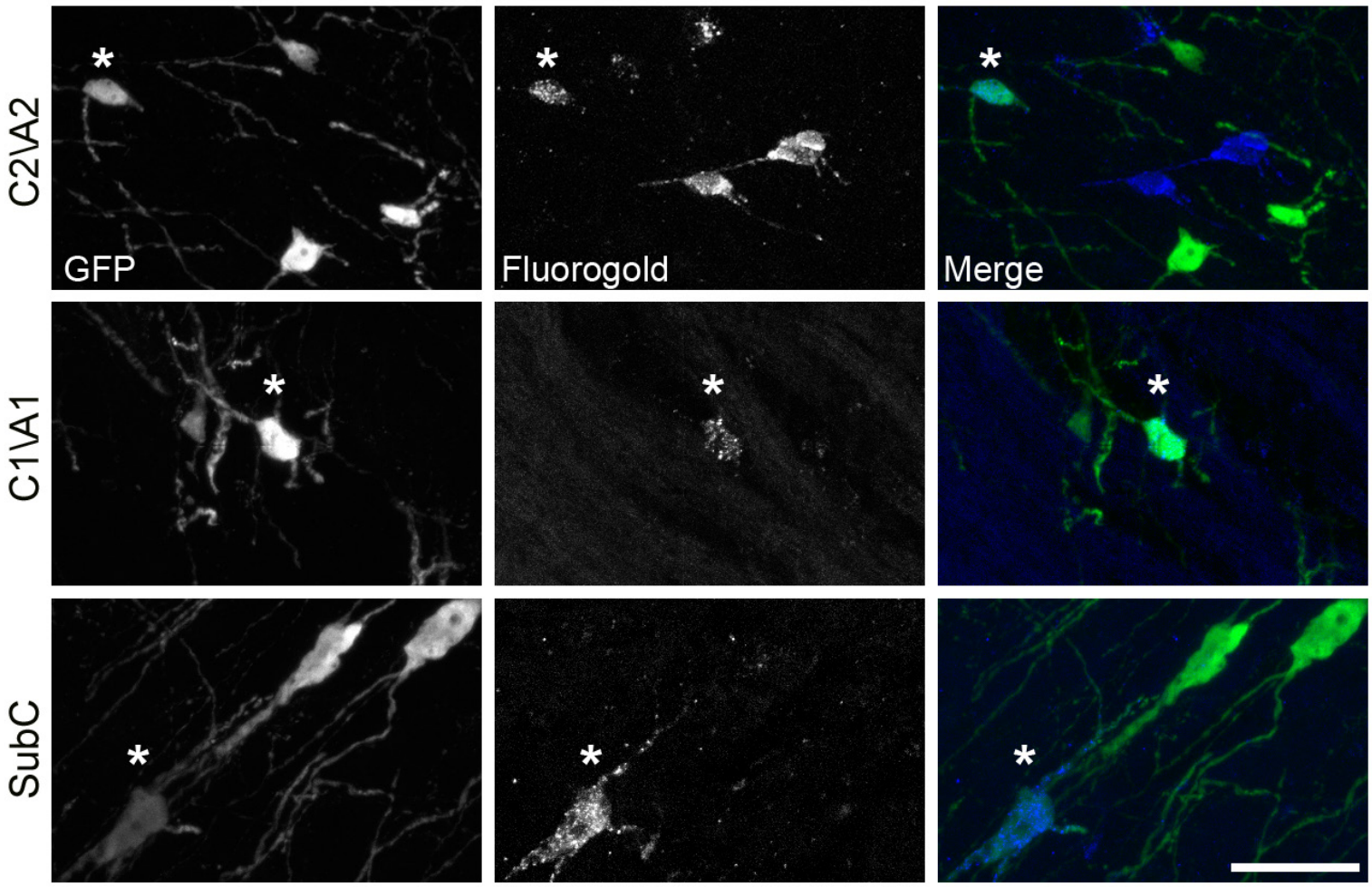


Supplementary Fig. 2. eGFP expression in neurons and axonal projections following *hβactin-cre*- and *Dbh^{Flpo}*-mediated recombination of *RC::FrePe*.

(a) Sagittal sections from *hβactin-cre*;*Dbh^{Flpo}*;*RC::FrePe* triple heterozygous E12.5 embryos. eGFP expression is detected in the cell bodies and axonal projections of

hindbrain norepinephrine neurons and cranial ganglia. (b) In adult animals of the same genotype, co-expression of eGFP and the norepinephrine transporter (NET) in the cortex demonstrates that eGFP fills the axon terminals of hindbrain norepinephrine neurons. Scale bar: 45 μm (E12.5 hindbrain norepinephrine neurons), 32 μm (E12.5 axonal projections), 50 μm (E12.5 cranial ganglia), 12.4 μm (cortical projections, *upper panels*), 9 μm (cortical projections, *lower panels*).

Supplementary Figure 3



Supplementary Fig. 3. r4(*Hoxb1*^{cre})-derived norepinephrine neurons residing in the C2/A2, C1/A1 and SubC nuclei project to the insular cortex.

Sagittal sections from adult *Hoxb1*^{cre}*;Dbh*^{Flpo}*;RC::FrePe* animals injected with FluoroGold into the insular cortex are shown. eGFP-positive r4(*Hoxb1*^{cre})-derived norepinephrine neurons (green) that project to the insular cortex are co-labeled (*) with FluoroGold (blue) in C2/A2 (*top panel*), C1/A1 (*middle panel*), and SubC (*bottom panel*). eGFP-positive r4-derived norepinephrine neurons that are not co-labeled are also shown. Scale bar indicates 50 μm.

Supplementary Table 1

Percent contribution of r-derived norepinephrine neurons to anatomical nuclei				
	r1	r2	r3 and r5	r4
LoC	99.6 ± 0.1%	0.2 ± 0.1%	--	--
SubCD	69.8 ± 2.9%	2.5 ± 0.6%	2.1 ± 1.2%	5.8 ± 1.6%
SubCV	--	0.7 ± 0.4%	17.7 ± 2.6%	77.6 ± 4.1%
A7	52.1 ± 2.9%	3.1 ± 1.2%	--	--
A5	--	1.8 ± 1.0%	22.0 ± 4.6%	64.4 ± 2.9%
C2/A2	--	--	1.5 ± 0.5%	27.6 ± 2.0%
C1/A1	--	--	0.4 ± 0.2%	22.4 ± 3.2%

Supplementary Table 1. Percent contribution of r-derived norepinephrine neurons to anatomical nuclei

The table summarizes the percent contribution of each r-derived norepinephrine subpopulation to the anatomically defined norepinephrine nuclei. From the cell counts reported in (Figs. 2b and 3b), the percentage of eGFP positive r-derived norepinephrine neurons relative to the total number of norepinephrine neurons (eGFP positive plus mCherry positive neurons) is listed.

Supplementary Table 2

Brain region	r1	r2	r3 and r5	r4
Cerebral Cortex				
Auditory Ctx	+++	–	–	–
Insular Ctx	+++	–	–	++
Limbic Ctx (prelimbic, cingulate, infralimbic)	+++	–	–	–
Motor Ctx	+++	–	–	–
Piriform Ctx	+++	–	–	–
Orbital Ctx	+++	–	–	+
Rhinal Ctx	+++	–	–	–
Somatosensory Ctx	+++	+	–	–
Visual Ctx	+++	–	–	–
Olfactory Bulb	++	–	–	+
Septum				
Lateral Septal Nucleus	+	–	–	+
Medial Septal Nucleus	+++	–	–	+
Basal Ganglia				
Caudate putamen	+/–	–	–	+/–
Accumbens nucleus core	+	–	–	+
Accumbens nucleus shell	++	–	–	+
Globus pallidus	++	–	–	+
Ventral pallidum	+	–	–	+
Bed nucleus of the stria terminalis				
BNST, dorsal medial	+	–	–	++
BNST, dorsal lateral	+	–	–	+++
BNST, ventral medial	++	–	–	++++
BNST, ventral lateral	+++	–	–	++++
Hypothalamus				
Medial Preoptic area	+	–	–	+++
Lateral preoptic area	+	–	–	+++
Paraventricular hypothalamic nucleus	+	–	–	++++
Mammillary nucleus	+	–	–	+++
Dorsomedial hypothalamic nucleus	+	–	–	++++
Arcuate nucleus	+	–	–	+
Lateral hypothalamic area	+	–	+/–	+++
Posterior hypothalamic area	+	–	–	+++
Suprachiasmatic nucleus	+	–	–	+
Hippocampus	+++	–	–	+/–
Amygdala				
Central Amygdaloid nucleus	+	–	–	+++
Basolateral amygdaloid nucleus	+++	–	–	++++
Basomedial amygdaloid nucleus	+++	+/–	–	++

Supplementary Table 2 continued

Brain region	r1	r2	r3 and r5	r4
Epithalamus,thalamus, and subthalamus				
Medial habenular nucleus	+	–	–	–
Lateral habenular nucleus	++	–	–	–
Reticular thalamic nucleus	+++	–	–	+/–
Parafascicular thalamic nucleus	+++	–	–	+/–
Central medial thalamic nucleus	++	–	–	+++
Mediodorsal thalamic nucleus	+++	–	–	+/–
Paratenial thalamic nucleus	+++	–	–	+/–
Anterodorsal thalamic nucleus	+++	–	–	–
Anteromedial thalamic nucleus	+++	–	–	–
Anteroventral thalamic nucleus	+++	–	–	–
Laterodorsal thalamic nucleus	+++	–	–	–
Lateral posterior thalamic nucleus	+++	–	–	–
Ventrolateral thalamic nucleus	+++	–	–	–
Ventromedial thalamic nucleus	+++	–	–	–
Ventral posterolateral thalamic nucleus	+++	–	–	–
Ventral posteromedial thalamic nucleus	+++	–	–	–
Dorsal lateral geniculate nucleus	+++	–	–	–
Paraventricular thalamic nucleus	++	–	–	+++
Substantia innominata	+++	–	–	+
Zona incerta	+++	–	–	+
Subthalamic nucleus	+++	–	–	++
Brainstem				
Substantia Nigra, compact	+	–	–	+++
Substantia Nigra, reticular	+	–	–	+
Ventral Tegmental Area	++	–	+/–	+++
Retrorubral Field	++	–	–	++++
Dorsal raphe nucleus	+	+/–	–	++++
Median raphe nucleus	+++	–	+/–	+
Locus Coeruleus	*in*	+	+	++++
Parabrachial Nucleus	++	+/–	+	++++
Parvicellular reticular nucleus	++	+	++	+++
Intermediate reticular nucleus	++	+	++	++++
Medial reticular nuclei (PNc, Gi, MdV)	+++	–	+/–	+
Solitary nucleus	+	+/–	+++	++++
Cerebellum	++	+	+	+
Inferior Colliculi	+++	+/–	+/–	+

Supplementary Table 2. Genetically defined subpopulations of norepinephrine neurons project to unique sets of targets.

The relative density of norepinephrine innervation from r1, r2, r3&5, and r4-derived norepinephrine neurons across various regions of the brain is shown from coronal sections immunostained with eGFP. For each fate map and specific brain region, the density of eGFP immunolabeling is graded as one of the following: undetectable in all animals (–), presence of rare fiber and not seen in all animals (+/–), sparse but consistent fibers in all animals (+), moderate presence with consistent fibers observed in all animals in multiple sections (++), strong innervation (+++), dense and heavy innervation (++++), indistinguishable due to presence of eGFP positive neurons (*in*).