**SUPPLEMENTARY MATERIALS**

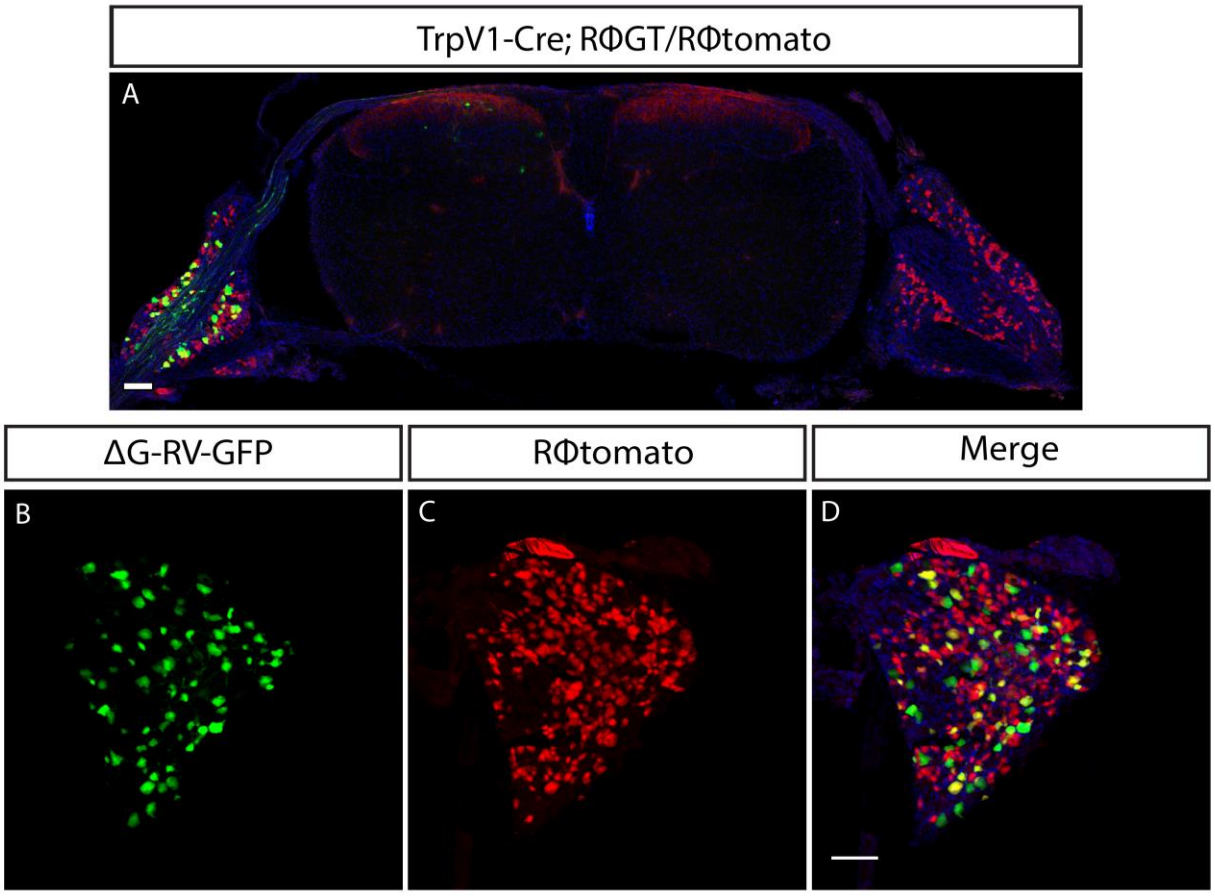
**List of supplementary materials:**

**4 supplementary figures and figure legends**

**2 supplementary movies (submitted as separated files)**

**2 movie legends**

**Figure S1**



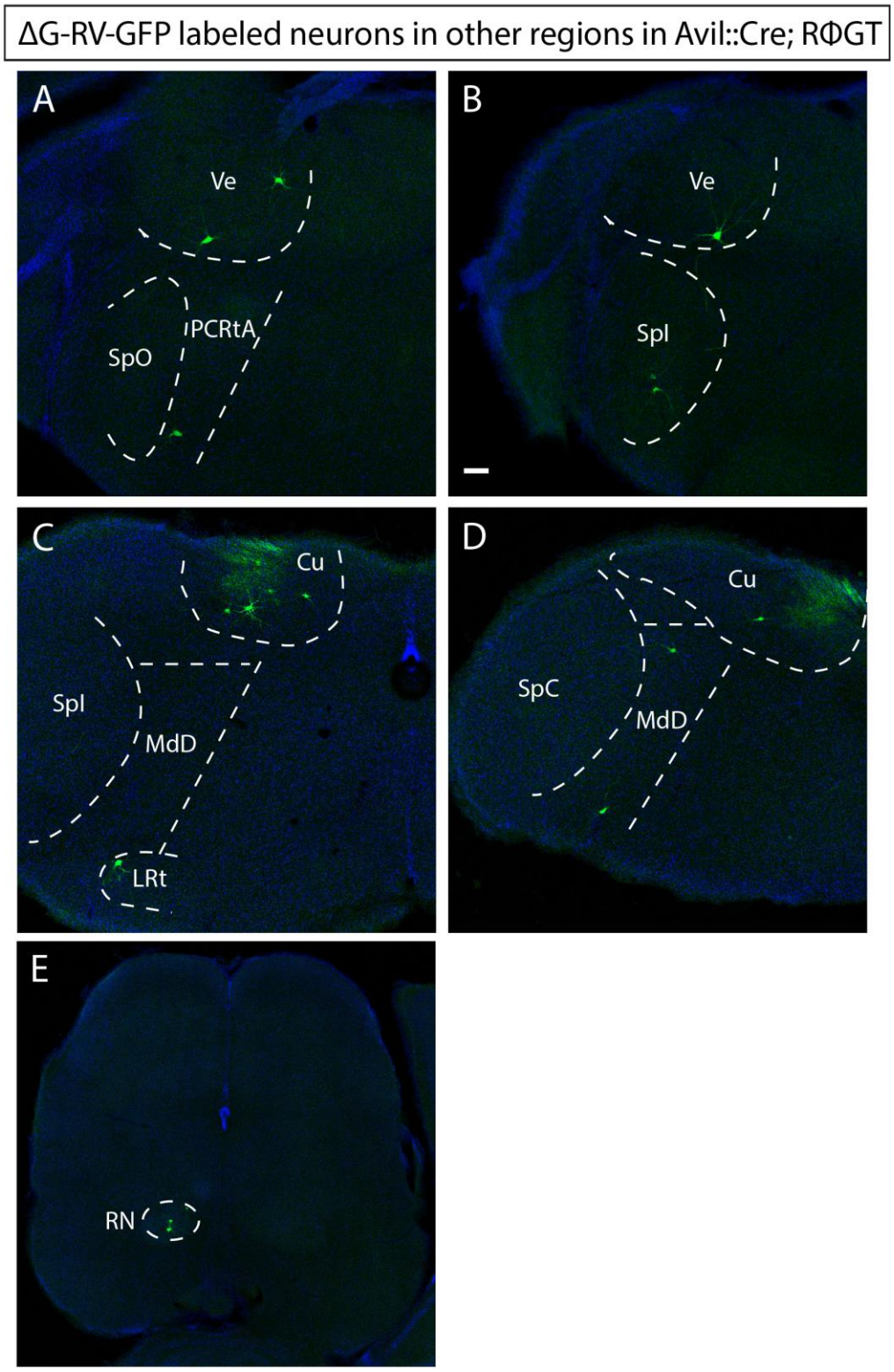

**Supplementary Figure S1 Related to Figure 2.**

**Infection and spreading of ΔG-RV-GFP in the DRG and the spinal cord of TrpV1::Cre; RΦGT/RΦtomato mice.**

(A) Overview of infection and spreading of ΔG-RV-GFP in the DRG and the spinal cord. Scale bar, 100 μm.

(B-D) High-magnification images of the infected DRG, showing about 60% GFP+ sensory neurons are also tomato+ (i.e. TrpV1::Cre positive neurons). Scale bar, 100 μm.

**Figure S2**


ΔG-RV-GFP labeled neurons in other regions in Avil::Cre; RΦGT
A
Ve
PCRtA
SpO
B
Ve
SpI
C
Cu
SpI
MdD
LRt
D
Cu
SpC
MdD
E
RN

**Supplementary Figure S2**

**ΔG-RV-GFP also labeled a few scattered neurons in several central brain regions in Avil::Cre; RΦGT mice.**

(A) Labeled neurons in ipsilateral vestibular and trigeminal spinal oralis nucleus.

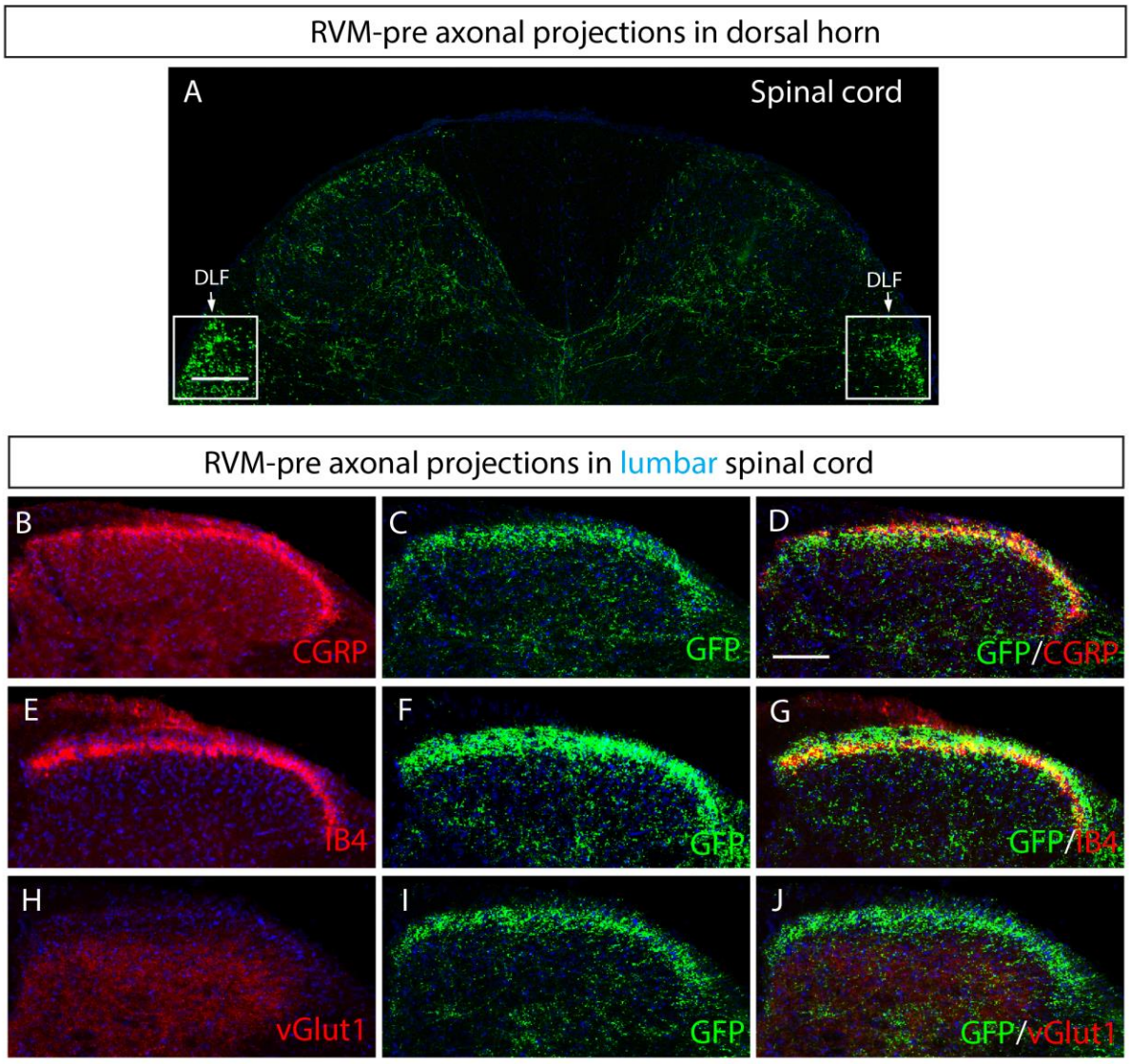
(B) Labeled neurons in ipsilateral vestibular and trigeminal spinal interpolaris nucleus.

(C) Labeled neurons in ipsilateral cuneate and medullary reticular nucleus.

(D) Labeled neurons in ipsilateral cuneate, intermediate and medullary reticular nucleus.

(E) Labeled neurons in red nucleus. Scale bar, 100 μm.

**Figure S3**


RVM-pre axonal projections in dorsal horn
A
Spinal cord
DLF
DLF
RVM-pre axonal projections in lumbar spinal cord
B
CGRP
C
GFP
D
GFP/CGRP
E
IB4
F
GFP
G
GFP/IB4
H
vGlut1
I
GFP
J
GFP/vGlut1

**Supplementary Figure S3**

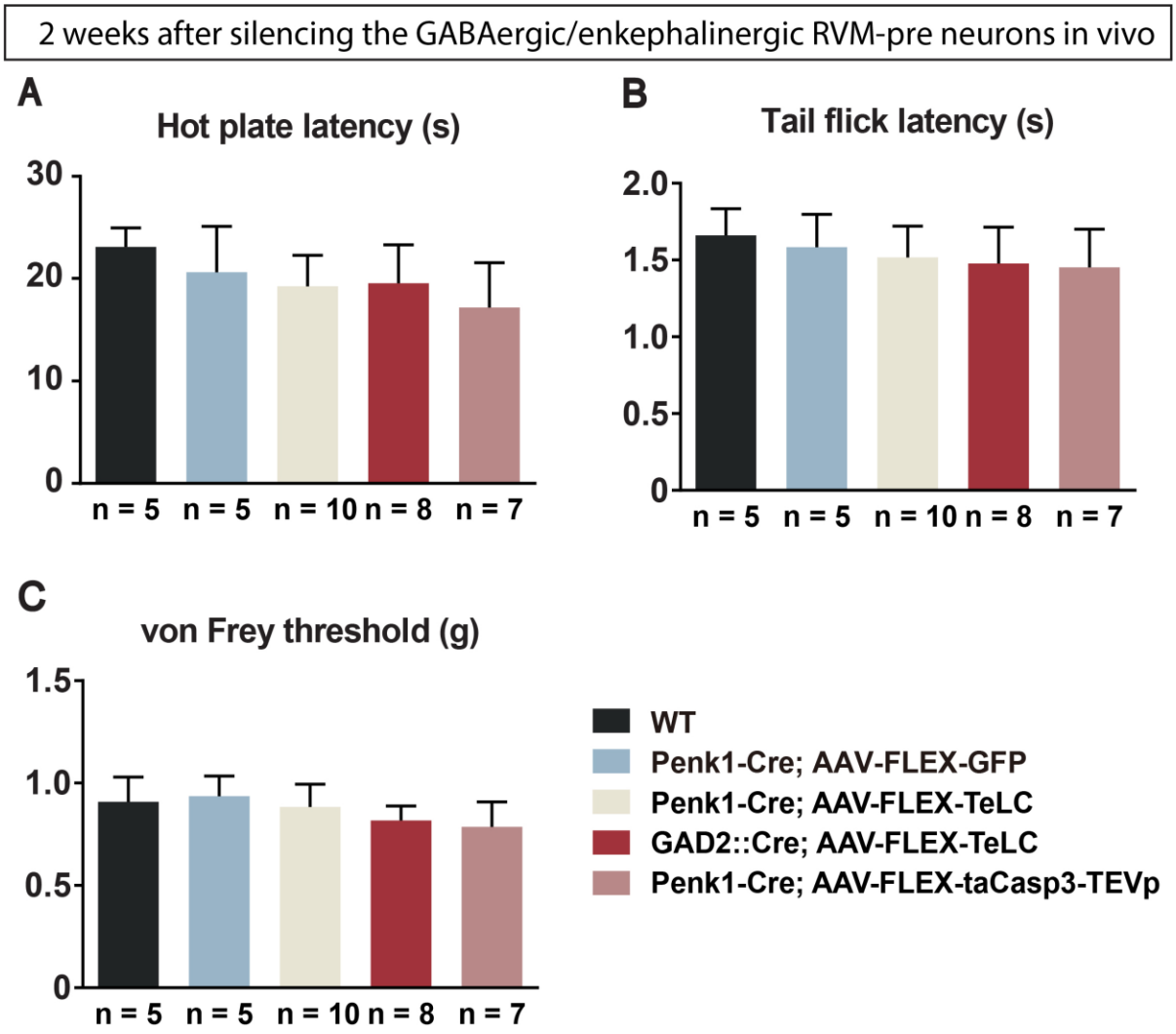
**Lenti-Penk1-Cre and AAV-FLEX-GFP labeled RVM-pre neurons project along the entire length of the spinal cord, including the lumbar spinal cord.**

(A) Labeled RVM axons project through the dorsal-lateral funiculus (DLF) (boxed region) and send collaterals to innervate the dorsal horn (this image is from thoracic level).

(B-J) Co-staining with CGRP (marker of lamina I), or with IB4 (marker of lamina II), or with anti-vGlut1 (marker for touch afferents) revealed that labeled RVM axons innervate both lamina I and II, as well as deeper layers (primarily layer V).

Scale bar, 100 μm.

**Figure S4**


2 weeks after silencing the GABAergic/enkephalinergic RVM-pre neurons in vivo
A
Hot plate latency (s)
30
20
10
0
n = 5 n = 5 n = 10 n = 8 n = 7
B
Tail flick latency (s)
2.0
1.5
1.0
0.5
0
n = 5 n = 5 n = 10 n = 8 n = 7
C
von Frey threshold (g)
1.5
1.0
0.5
0
n = 5 n = 5 n = 10 n = 8 n = 7
WT
Penk1-Cre; AAV-FLEX-GFP
Penk1-Cre; AAV-FLEX-TeLC
GAD2::Cre; AAV-FLEX-TeLC
Penk1-Cre; AAV-FLEX-taCasp3-TEVp

**Supplementary Figure S4**

**No behavioral effects at two weeks after viral injections to express TeLC or caspase in RVM-pre neurons.**

(A) Hot plate paw withdraw latency in wild type and various viral injected mice.

(B) Tail flick latency in wild type and various injected mice.

(C) Paw withdraw threshold of von Frey test in wild type and various viral injected mice.

Error bars represent means ± SEM. P-values represent comparison to wild type and control injection values (* $P < 0.05$). Differences were determined by Student's *t* test between two groups, or one-way ANOVA followed by post-hoc Bonferroni test for multiple groups. n=5~10 mice.

**Supplementary Movie S1 Related to Figure 2.**

This is a movie of the serial sections of the spinal cord from a P8 Avil::Cre; RΦGT mouse in which ΔG-RV-GFP rabies virus was injected into the plantar of the right forepaw at P1.

**Supplementary Movie S2 Related to Figure 2.**

This is a movie of serial sections of the spinal cord from a P8 TrpV1::Cre; RΦGT mouse in which ΔG-RV-GFP rabies virus was injected into the plantar of the right forepaw at P1.