

## Supplemental Information Titles and Legends

### Figure S1. Anatomical characterization of aNs.

A) Digital reconstruction of aNs position along the dorsoventral (top) and rostrocaudal (bottom) axes in the lumbar spinal cord (n = 3 mice).

B and C) Correlation analysis of the positions and dorsoventral and mediolateral distribution aNs.

### Figure S2. Anatomical characterization of dNs.

A) Digital reconstruction of dNs position along the dorsoventral (top) and rostrocaudal (bottom) axes in the cervical spinal cord (n = 3 mice).

B and C) Correlation analysis of the positions and dorsoventral and mediolateral distribution analysis of dNs.

### Figure S3. Validation of the snRNA sequencing dataset.

A) Boxplot representing the number of genes detected per nucleus found in each 96-well plate before quality check analysis. Plates #1-5 contain aNs, while plates #6-10 contain dNs.

B) Number of genes detected per nucleus in each cluster after quality check analysis.

C) Initial number of nuclei sorted, number of nuclei analysed after quality control, and assigned to each cluster following clustering analysis.

D) Schematic showing the local and projection connectivity pattern of *Neurod2*<sup>+</sup> and *Zfhx3*<sup>+</sup> neurons.

E and F) Violin plots of *Zfhx3* and *Neurod2* expression levels (logcounts) in different clusters.

G) Schematic of the viral strategy used to label dNs and representative image of a cervical spinal cord showing rabies<sup>+</sup> dNs and *Zfhx3* expression. The inset shows the magnification of a representative *Zfhx3*<sup>+</sup> dNs. On the right: digital reconstruction of *Zfhx3*<sup>+</sup> dNs position in the cervical spinal cord and percentage of dNs that are *Zfhx3*<sup>+</sup> (n = 2 mice, mean ± SEM).

H) Schematic of the viral strategy used to label aNs and representative image of a lumbar spinal cord showing rabies<sup>+</sup> aNs and *Zfhx3* expression. The inset shows the magnification of a representative *Zfhx3*<sup>+</sup> aNs. On the right: digital reconstruction of *Zfhx3*<sup>+</sup> aNs position in the lumbar spinal cord and percentage of aNs that are *Zfhx3*<sup>+</sup> (n = 3 mice, mean ± SEM).

I) Schematic of the viral strategy used to label aNs and representative image of a lumbar spinal cord showing rabies<sup>+</sup> aNs and *Hoxc10* expression. The inset shows the magnification of a representative *Hoxc10*<sup>+</sup> aNs. On the right: digital reconstruction of *Hoxc10*<sup>+</sup> aNs position in the lumbar spinal cord and percentage of aNs that are *Hoxc10*<sup>+</sup> (n = 3 mice, mean ± SEM).

### Figure S4. Validation of V3-aN s and V0-aNs.

A) Schematic of the viral strategy used to label aNs and representative image of a lumbar spinal cord showing rabies infected aNs (nGFP<sup>+</sup>) and lineage tracing of V3 (tdTomato<sup>+</sup>) neurons in *Sim1*<sup>Cre</sup>;

*Rosa<sup>lsl-tdTomato</sup>* mice. The inset shows the magnification of representative V3-aNs (nGFP<sup>+</sup>; tdTomato<sup>+</sup>) marked by the arrow.

B) Digital reconstruction of V3-aNs (nGFP<sup>+</sup>; tdTomato<sup>+</sup>) position in the lumbar spinal cord (n = 3 mice).

C) Percentage of aNs that present V3 (tdTomato<sup>+</sup>) identity (n = 3 mice, mean  $\pm$  SEM).

D) Percentage of V3 (tdTomato<sup>+</sup>) neurons that are aN (n = 3 mice, mean  $\pm$  SEM).

E) Violin plot showing expression level of *Chat* in different clusters (logcounts).

F) Schematic of the viral strategy used to label aNs and representative image of a lumbar spinal cord showing ChAT expression in rabies infected aNs (nGFP<sup>+</sup>) and lineage traced of Pitx2 V0 (tdTomato<sup>+</sup>) neurons in *Pitx2<sup>Cre</sup>; Rosa<sup>lsl-tdTomato</sup>* mice. The inset shows the magnification of neurons marked by the arrow.

G) Percentage of Pitx2<sup>+</sup> V0-aNs expressing ChAT (n = 2 mice).

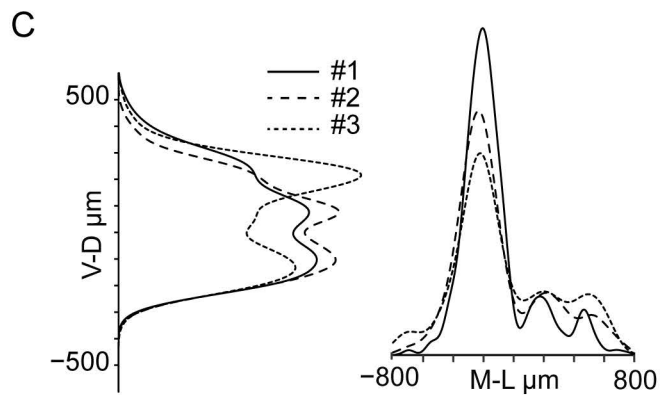
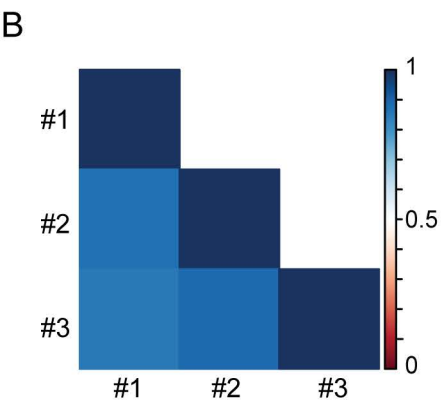
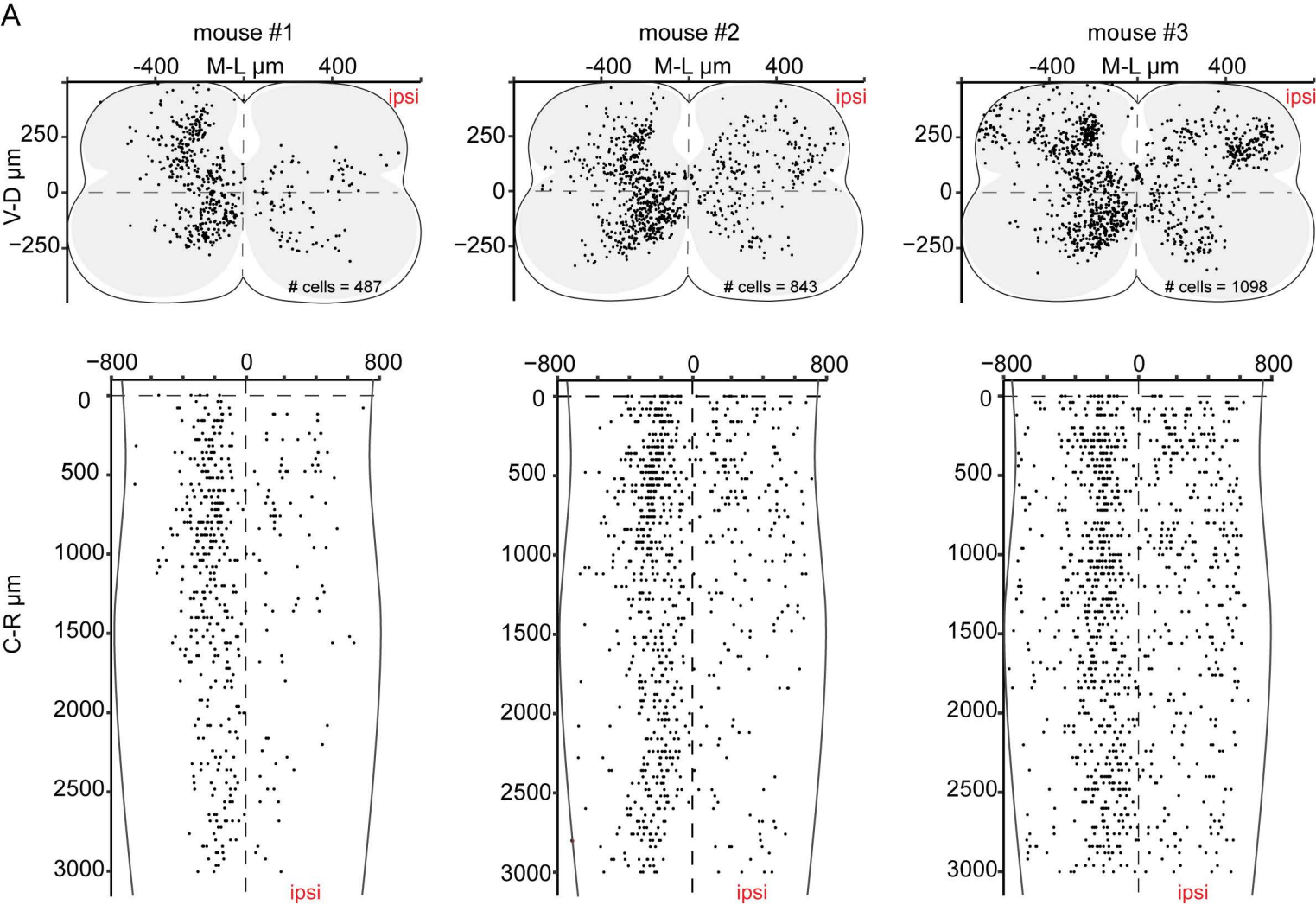
#### **Figure S5. Input and output connectivity of V0g-aNs.**

A) Digital reconstruction of starter cells (Rabies<sup>+</sup>; nGFP<sup>+</sup>) and presynaptic neurons (Rabies<sup>+</sup>; nGFP<sup>+</sup>) positions in the lumbar spinal cords of four mice. Number of labelled neurons in the bottom right corner of each map.

B) Schematic of the viral strategy used to label V0g-aNs membrane (mGFP) and presynaptic boutons (sRuby) in *Pitx2<sup>Cre</sup>* mice and representative image of a lumbar (left) and cervical (right) spinal cords showing ChAT expression. The insets show the magnification of a V0g-aN and a V0c.

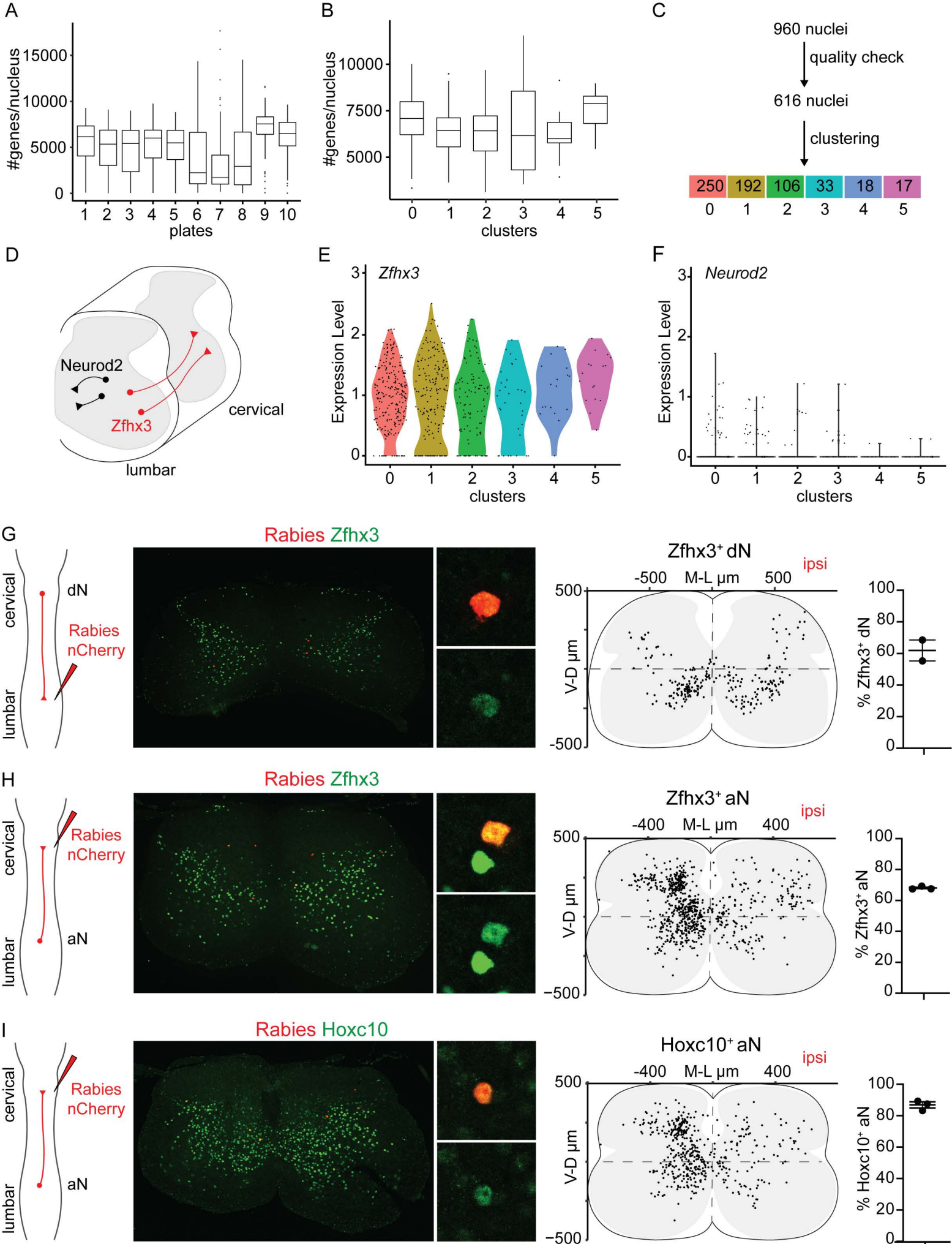
#### **Figure S6. Elimination of V0g-aNs does not perturb kinematic parameters.**

A-C) Stance duration, B) swing duration, and C) cadence in mice locomoting on a treadmill at different locomotor speeds before and after DT and PBS treatment. Data are mean  $\pm$  SEM. Letters reflect post-hoc analysis results for all pair-wise comparisons. Boxplots sharing the same letter are not to be considered significantly different.

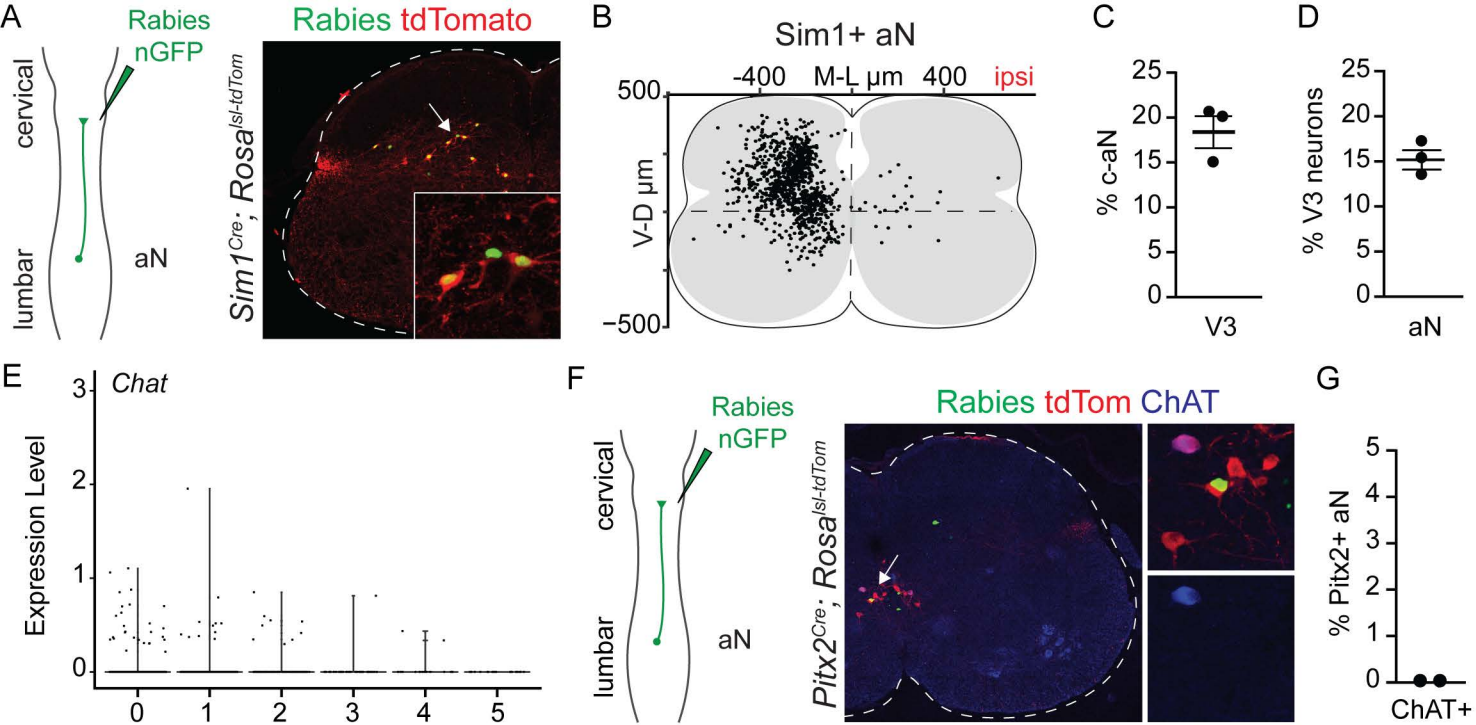


**Figure S1**



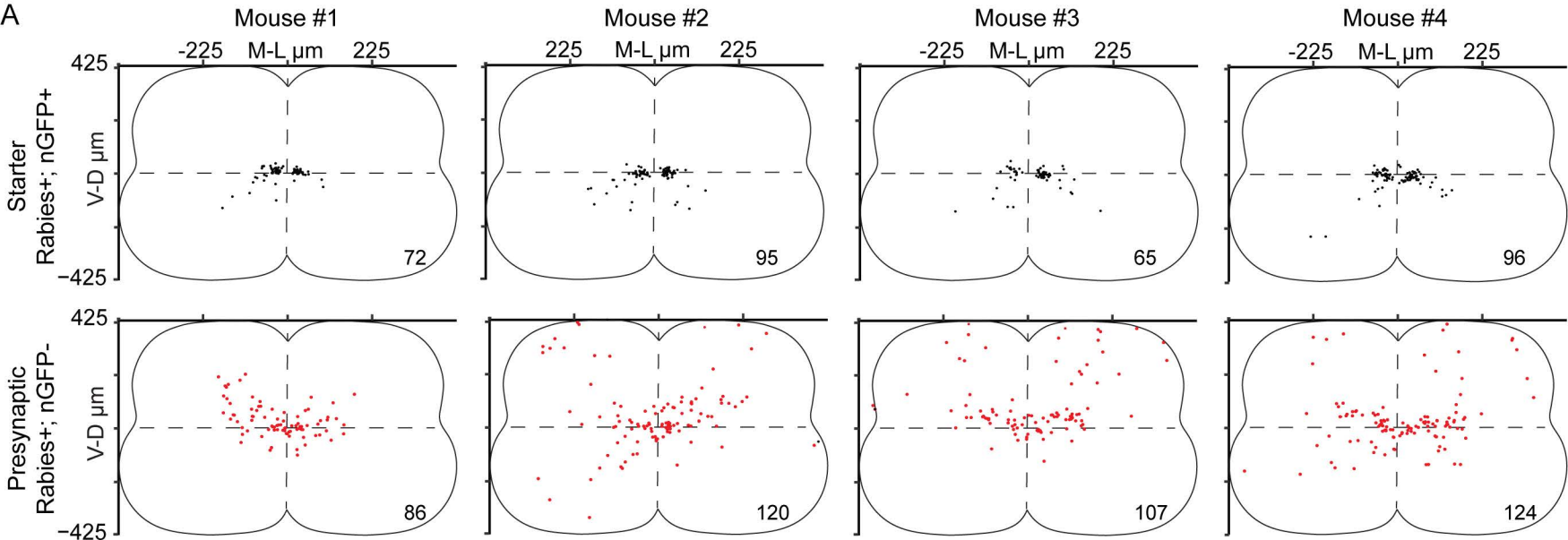


**Figure S3**

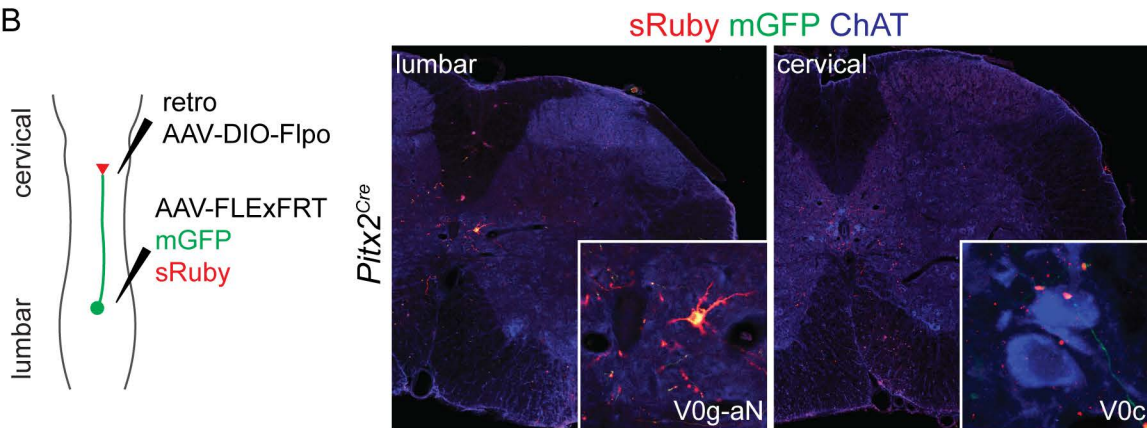


**Figure S4**

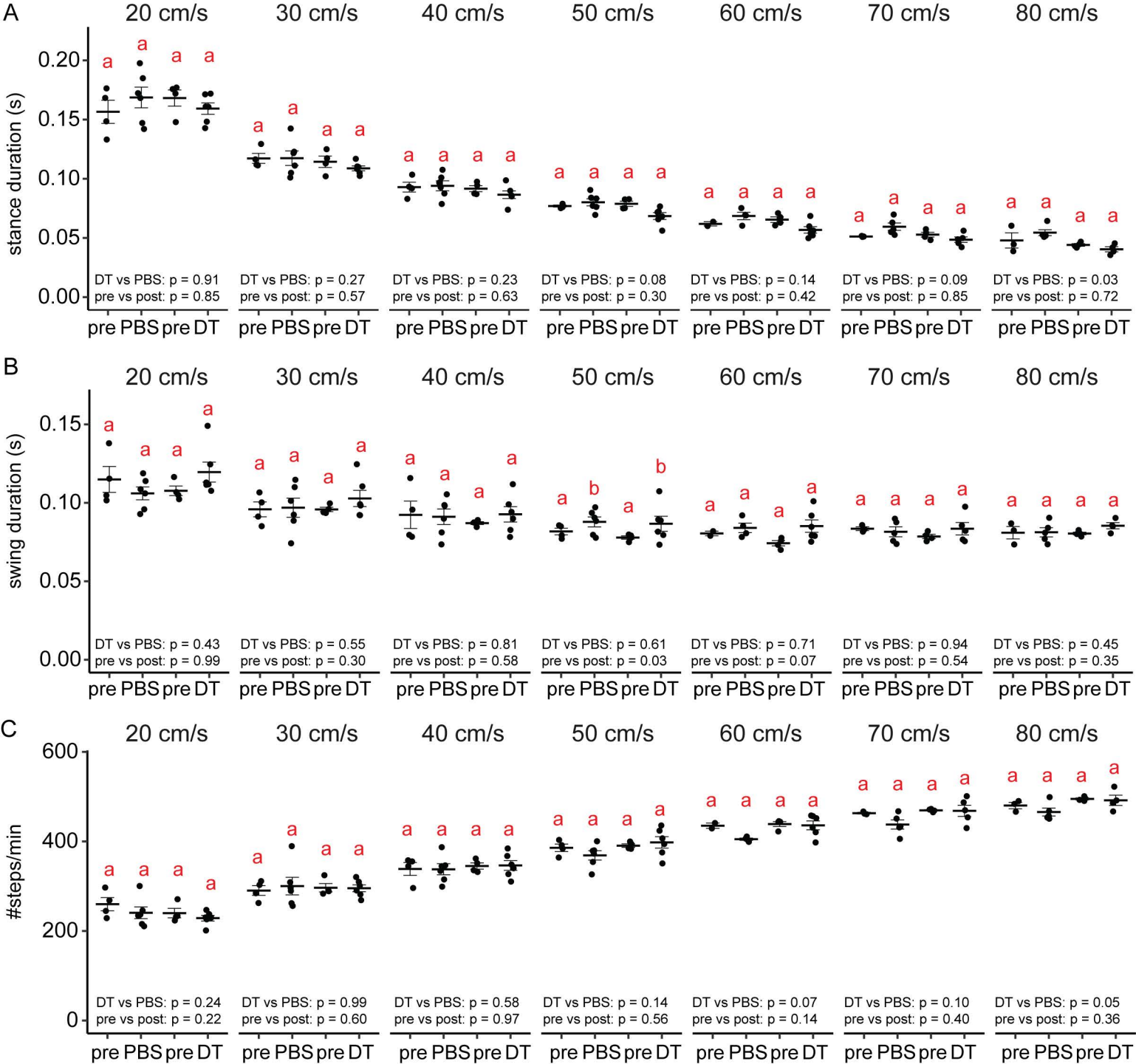




**B**



**Figure S5**



**Figure S6**