

Supplemental Materials for:

Developmental patterning of peptide transcription in the central circadian clock in both sexes

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Supplemental Figure Legends

Figure S1. Spatial mapping of SCN *Avp-tdT*⁺ and *Vip-tdT*⁺ neurons. (A) Representative images illustrating *Avp-tdT*⁺ and *Vip-tdT*⁺ neurons across the anteroposterior SCN. Note each slice is color-coded by position and superimposed in rightmost panel using the hyperstack temporal color code. (B) Cells expressing *Avp-tdT* and *Vip-tdT*, divided by anterior, middle, and posterior SCN for representative mice of each sex. (C) Cells expressing *Avp-tdT* and *Vip-tdT* divided by anterior, middle, and posterior SCN for all mice of each sex (top, middle) or all mice collapsed by sex (bottom).

Figure S2. SCN slice atlas over development. (A) Representative slice sets for SCN collected at each age. The slices used for anterior, middle and posterior SCN are color coded by age. (B) DAPI-derived boundaries of the anterior, middle, and posterior SCN across development. (C) SCN area divided by sex across age Cre: $F(1,84) = 8.53, p < 0.005$, Age: $F(6,84) = 59.32, p < 0.0001$, Sex: $F(1,84) = 1.08, p > 0.3$, Cre*Age: $F(6,84) = 1.2, p > 0.3$, Cre*Sex: $F(1,84) = 0.0001, p > 0.9$, Age*Sex: $F(1,84) = 0.66, p > 0.6$, Cre*Age*Sex: $F(6,84) = 0.93, p > 0.4$.

Figure S3. Mapping SCN cell density. (A) Representative cell density maps for an individual image illustrating cell density calculated by counting the number of neighboring cells in a 10, 50, or 100 μm radius. (B) Front and lateral view of *Avp-tdT*⁺ cell density maps illustrating number of cells within a 50 μm radius for each individual sample. (C) Front and lateral view of *Vip-tdT*⁺ cell density maps illustrating number of cells within a 50 μm radius for each individual sample.

Figure S4. SCN *Avp-tdT* and *Vip-tdT* labeling is Cre dependent. (A) Representative images illustrating tdT expression in each genotype. (B-C) Total number of SCN tdT⁺ cells in each genotype (B) and divided by slice position (C). Cre: $F(2,22) = 24, p < 0.0001$, Sex: $F(1,22) = 0.3, p > 0.6$, Position: $F(2,44) = 2.9, p = 0.07$, Cre*Sex: $F(2,22) = 0.6, p > 0.5$, Cre*Position: $F(4,44) = 8.1, p < 0.0001$, Sex*Position: $F(2,44) = 0.2, p > 0.8$, Cre*Sex*Position: $F(4,44) = 0.1, p > 0.9$. (D) tdT expression in the cortex. $t(7) = 8.39, p < 0.0005$. Scale bars = 100 μm .

Figure S5. Evaluation of genetic strategy to label spatial location of SCN peptide classes. (A-B) Anterior, middle, and posterior SCN illustrating tdT and AVP/VIP expression in *Avp-tdT* and *Vip-tdT* samples collected from each sex. (C-D) Number of SCN neurons labeled by tdT or AVP/VIP immunohistochemistry (IHC) in each genotype and sex. **AVP/VIP IHC:** Cre: $F(1,15) = 175, p < 0.0001$; Sex: $F(1,15) = 0.2, p > 0.6$; Cre*Sex: $F(1,15) = 0.3, p > 0.5$. **tdT⁺:** Cre: $F(1,15) = 4.12, p = 0.06$; Sex: $F(1,15) = 2.3, p > 0.1$; Cre*Sex: $F(1,15) = 2.9, p > 0.1$. (E-F) Co-expression of tdT and AVP/VIP labeling does not differ by sex. **%tdT+AVP-IHC-** Sex: $F(1,8) = 1.2, p > 0.3$; Position: $F(2,16) = 2.7, p = 0.09$; Sex*Position: $F(2,16) = 0.4, p > 0.6$. **%tdT+VIP-IHC-** Sex: $F(1,7) = 9.7, p = 0.02$; Position: $F(2,13) = 0.7, p > 0.5$; Sex*Position: $F(2,13) = 1.1, p > 0.3$. **%AVP-IHC+tdT-** Sex: $F(1,8) = 4.2, p = 0.08$; Position: $F(2,16) = 7.9, p < 0.005$; Sex*Position: $F(2,16) = 0.2, p > 0.8$. **%VIP-IHC+tdT-** Sex: $F(1,7) = 1.2, p > 0.3$; Position: $F(2,13) = 2.2, p > 0.1$; Sex*Position: $F(2,13) = 0.3, p > 0.7$. Scale bars = 100 μm .

Figure S6. K means clustering on cellular dispersion in each SCN peptide class. (A) Elbow plots illustrating measures of cellular dispersion without and with normalization to total SCN cells of each sample (i.e., Inertia, Distance), divided by sex. (B) There was greater cellular dispersion in *Avp-tdT*⁺ neurons than *Vip-tdT*⁺ neurons in each sex. **Inertia** – Cre: $F(1,9) = 17.5, p < 0.005$, Sex: $F(1,9) = 4.8, p = 0.06$; Sex*Cre: $F(1,9) = 0.001, p > 0.9$. **Distance**– Cre: $F(1,9) = 99.2, p < 0.0001$, Sex: $F(1,9) = 0.12, p > 0.7$; Sex*Cre: $F(1,9) = 0.02, p > 0.8$.

Figure S7. Cell density maps of *Avp-tdT*⁺ neurons in the adult SCN. (A) Cell density maps illustrating the number of neighboring cells for all mice of each sex. (B) Cell density maps illustrating the number of neighboring cells in each sex relative to the total number of labeled neurons in each sample.

Figure S8. Cell density of *Vip-tdT*⁺ neurons in the adult SCN. (A) Cell density maps illustrating the number of neighboring cells for all mice of each sex. (B) Cell density maps illustrating the number of neighboring cells in each sex relative to the total number of labeled neurons in each sample.

Figure S9. Pregnancy and pup weight does not differ by offspring genotype. (A) Maternal weight gain in females pregnant with pups of each genotype. $F(2,38) = 0.15, p > 0.8$. (B) Litter size in dams bearing pups of each genotype $F(2,38) = 0.1, p > 0.9$. (C) Pup weight over development did not differ by genotype. Genotype: $F(2,18) = 0.76, p > 0.4$; Age: $F(3,18) = 157.3, p < 0.001$; Genotype*Age: $F(6,18) = 0.38, p > 0.8$. D. SCN size in pups of each genotype. Genotype: $F(1,84) = 8.53, p < 0.005$, Age: $F(6,84) = 59.32, p < 0.0001$, Sex: $F(1,84) = 1.08, p > 0.3$, Cre*Age: $F(6,84) = 1.2, p > 0.3$, Cre*Sex: $F(1,84) = 0.0001, p > 0.9$, Age*Sex: $F(6,84) = 0.66, p > 0.6$, Cre*Age*Sex: $F(6,84) = 0.93, p > 0.4$. n.s. not significant. * $p < 0.05$.

Figure S10. Anteroposterior differences in the appearance of SCN *Avp-tdT*⁺ and *Vip-tdT*⁺ cells over postnatal development. (A-B) Representative coronal images of labeled cells in each SCN peptide class across postnatal ages, summed across the anterior, middle, or posterior SCN.

Figure S11. Developmental appearance of total labeled cells (A) and measures of cell dispersion (B) divided by sex. (A) Cre: $F(1,99) = 89.6, p < 0.001$, Age: $F(7,99) = 411.9, p < 0.0001$, Sex: $F(1,99) = 0.7, p > 0.4$, Cre*Age: $F(7,99) = 11.25, p < 0.0001$, Cre*Sex: $F(1,99) = 0.01, p > 0.9$, Age*Sex: $F(7,99) = 5.2, p < 0.0001$, Cre*Age*Sex: $F(7,99) = 0.8, p > 0.5$. (B) **Inertia**: Cre: $F(1,88) = 34.4, p < 0.001$, Age: $F(6,88) = 124.7, p < 0.0001$, Sex: $F(1,88) = 0.2, p > 0.6$, Cre*Age: $F(6,88) = 4.0, p < 0.005$, Cre*Sex: $F(1,88) = 0.1, p > 0.7$, Age*Sex: $F(6,88) = 1.9, p = 0.08$, Cre*Age*Sex: $F(6,88) = 0.3, p > 0.9$. **Distance**: Cre: $F(1,88) = 408.4, p < 0.001$, Age: $F(6,88) = 33.1, p < 0.0001$, Sex: $F(1,88) = 2.1, p > 0.1$, Cre*Age: $F(6,88) = 6.7, p < 0.001$, Cre*Sex: $F(1,88) = 1.0, p > 0.3$, Age*Sex: $F(6,88) = 0.3, p > 0.9$, Cre*Age*Sex: $F(6,88) = 0.6, p > 0.7$.

Supplementary Videos

Video S1. *Avp-tdT*⁺ cell density in adult P84 males. Rotating view of adult male SCN. *Avp-tdT*⁺ neurons are collapsed across samples. Color represents number of neighboring cells in a 50 μ m radius.

Video S2. *Vip-tdT*⁺ cell density in adult P84 males. Conventions as in Video S1.

Video S3. *Avp-tdT*⁺ cell density in adult P84 females. Conventions as in Video S1.

Video S4. *Vip-tdT*⁺ cell density in adult P84 females. Conventions as in Video S1.

Video S5. *Avp-tdT*⁺ cell density in P01 males. Conventions as in Video S1.

Video S6. *Avp-tdT*⁺ cell density in P05 males. Conventions as in Video S1.

Video S7. *Avp-tdT*⁺ cell density in P01 females. Conventions as in Video S1.

Video S8. *Avp-tdT*⁺ cell density in P05 females. Conventions as in Video S1.

Video S9. *Vip-tdT*⁺ cell density in P01 males. Conventions as in Video S1.

Video S10. *Vip-tdT*⁺ cell density in P05 males. Conventions as in Video S1.

Video S11. *Vip-tdT*⁺ cell density in P01 females. Conventions as in Video S1.

Video S12. *Vip-tdT*⁺ cell density in P05 females. Conventions as in Video S1.

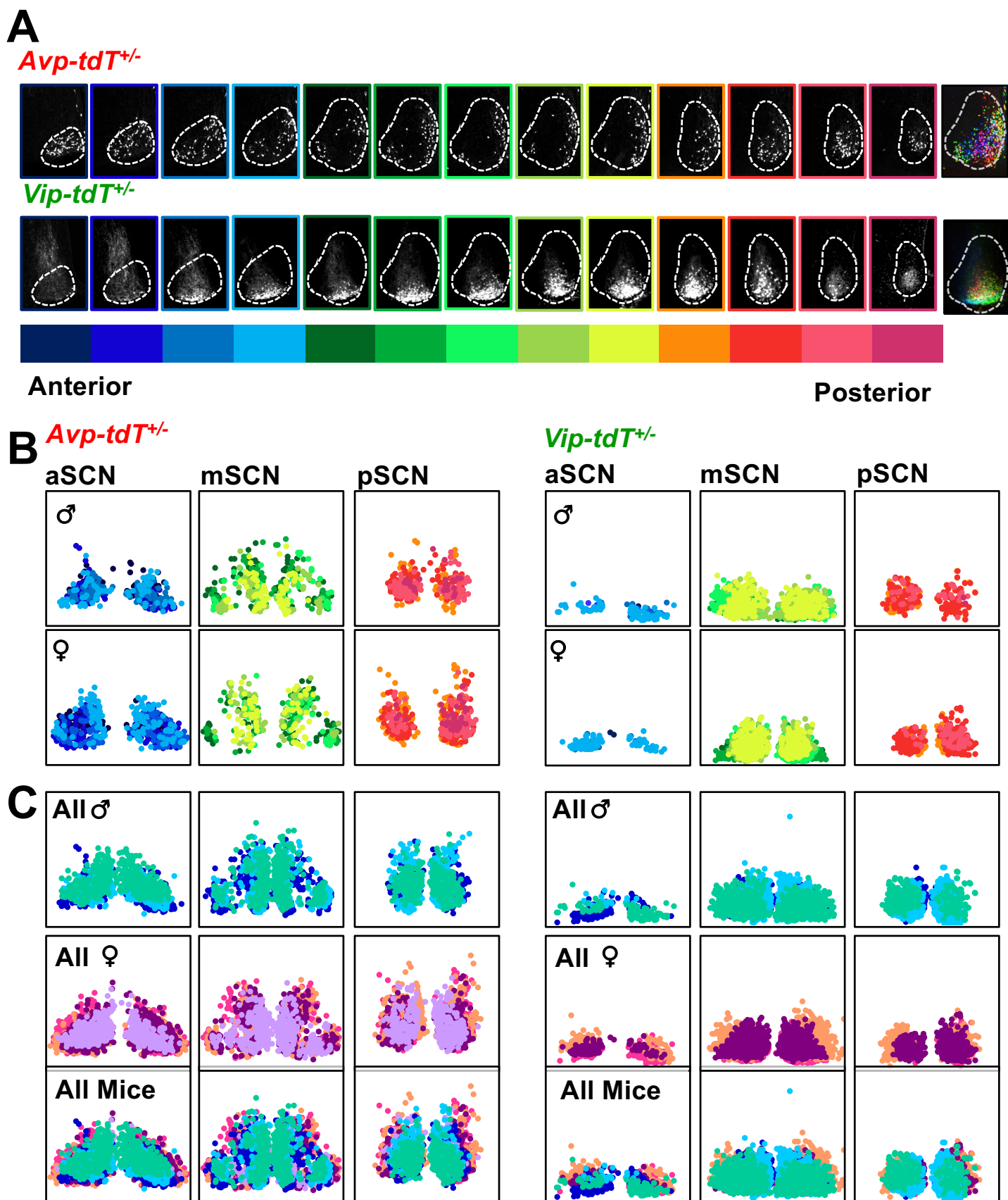


Figure S1

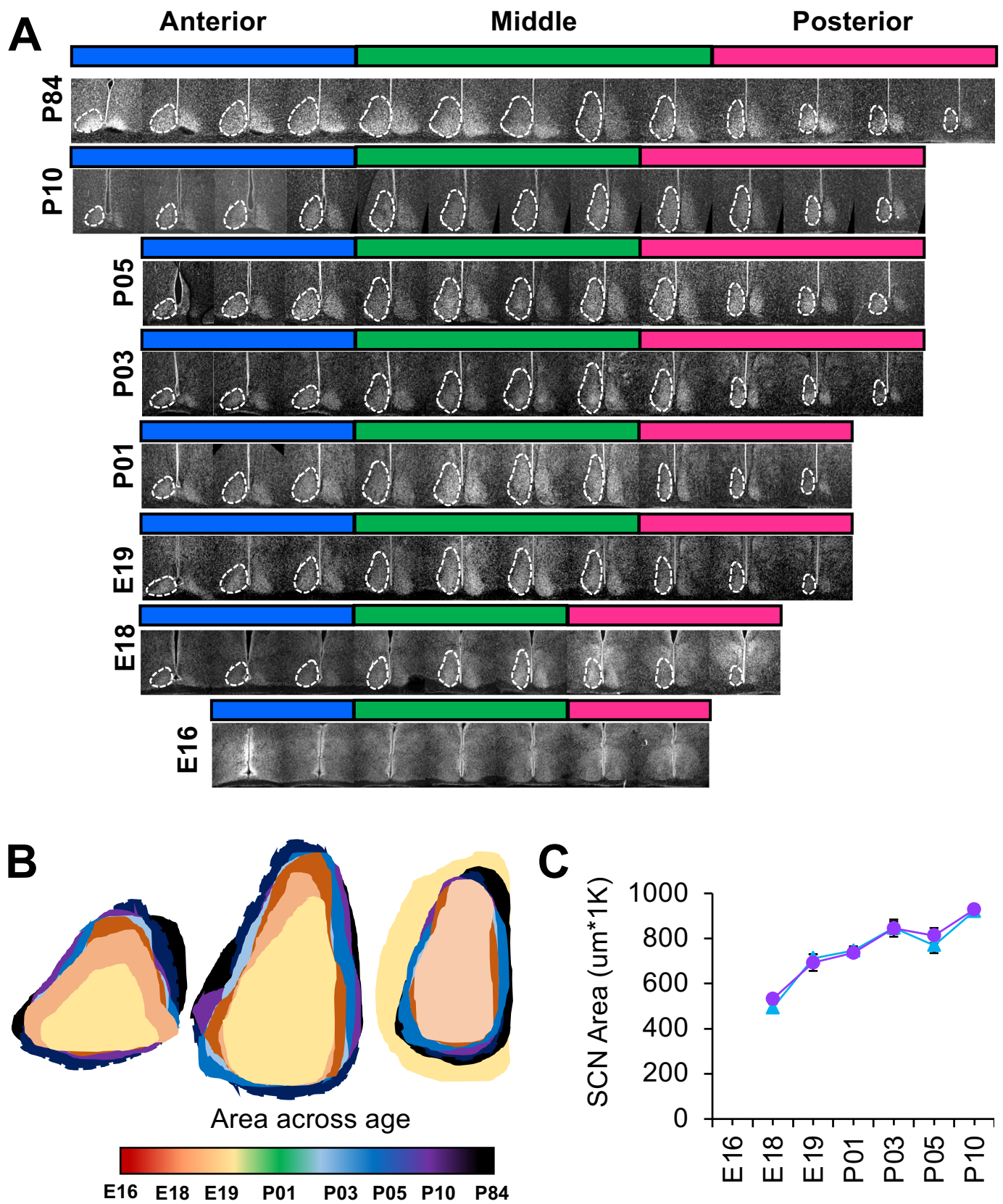
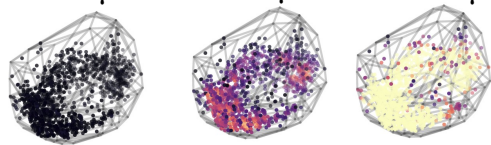


Figure S2

A *Avp-tdT*

$r = 10\mu\text{m}$ $r = 50\mu\text{m}$ $r = 100\mu\text{m}$

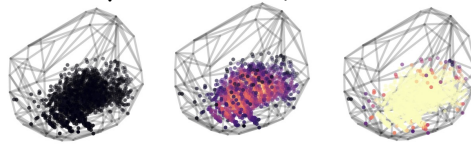


Neighbors in radius r

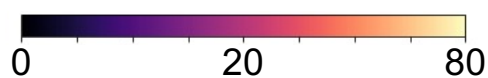


Vip-tdT

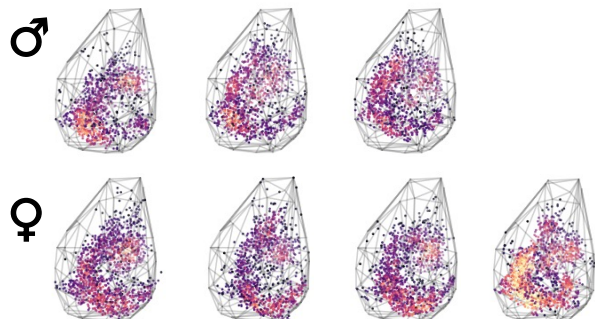
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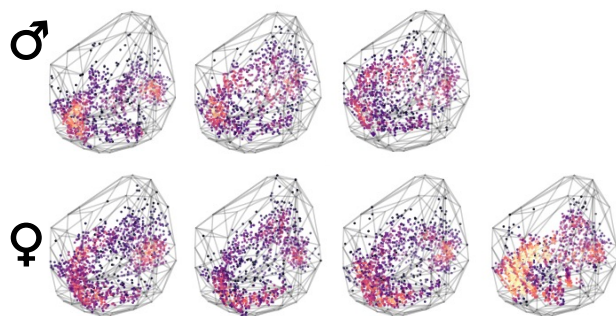
Neighbors in radius r



B *Avp-tdT* – Front view



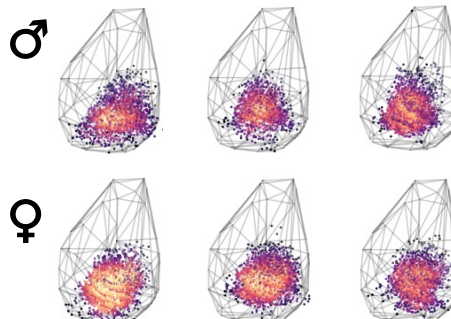
Avp-tdT – Lateral view



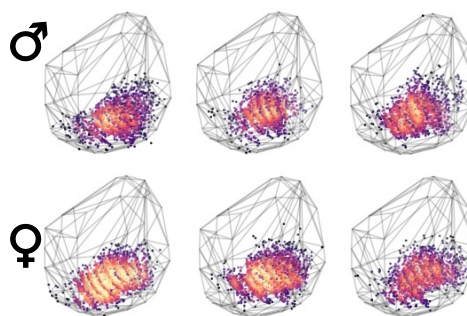
Neighbors in $50\mu\text{m}$ radius



C *Vip-tdT*⁺ – Front view



Vip-tdT⁺ – Lateral view



Neighbors in $50\mu\text{m}$ radius



Figure S3

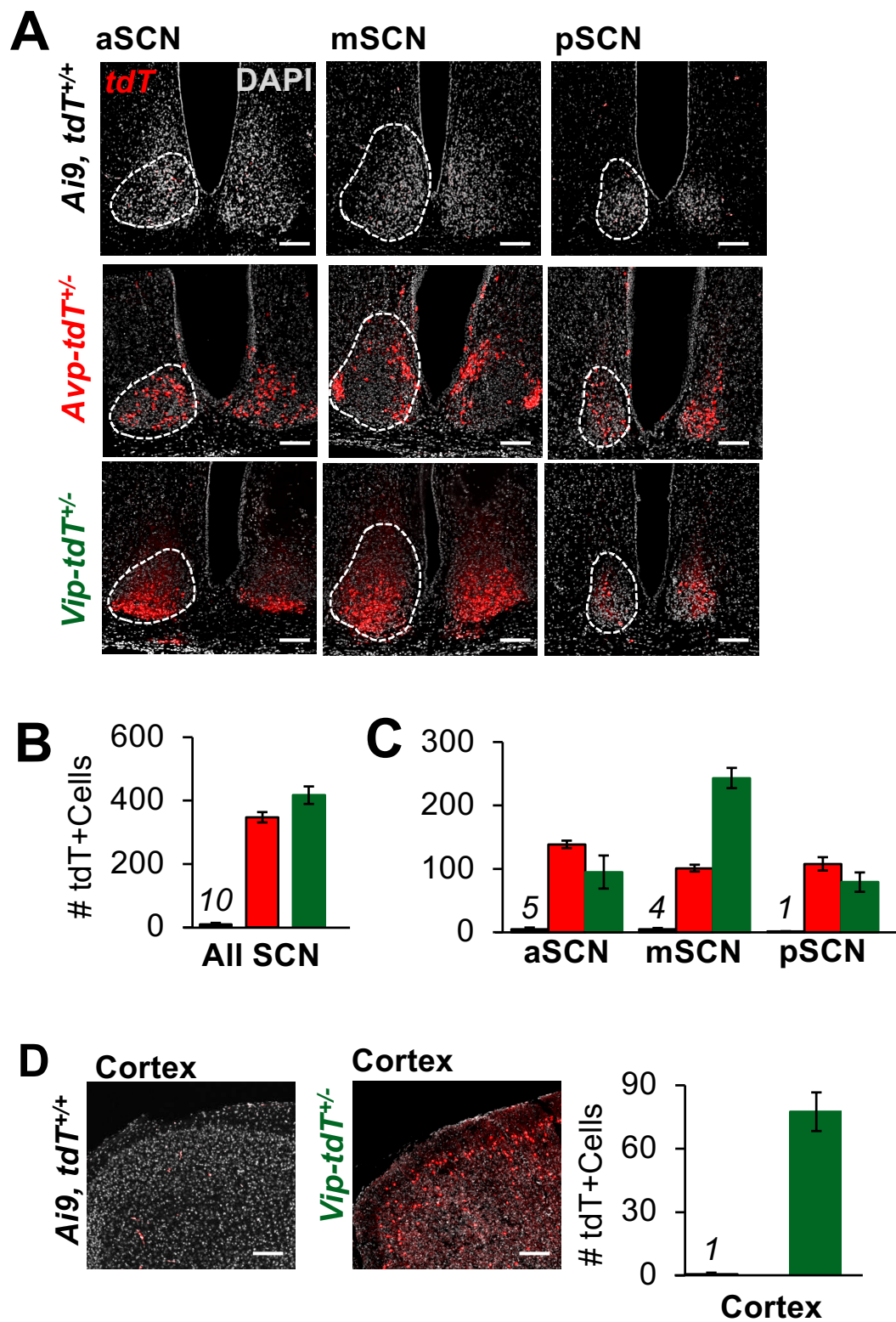


Figure S4

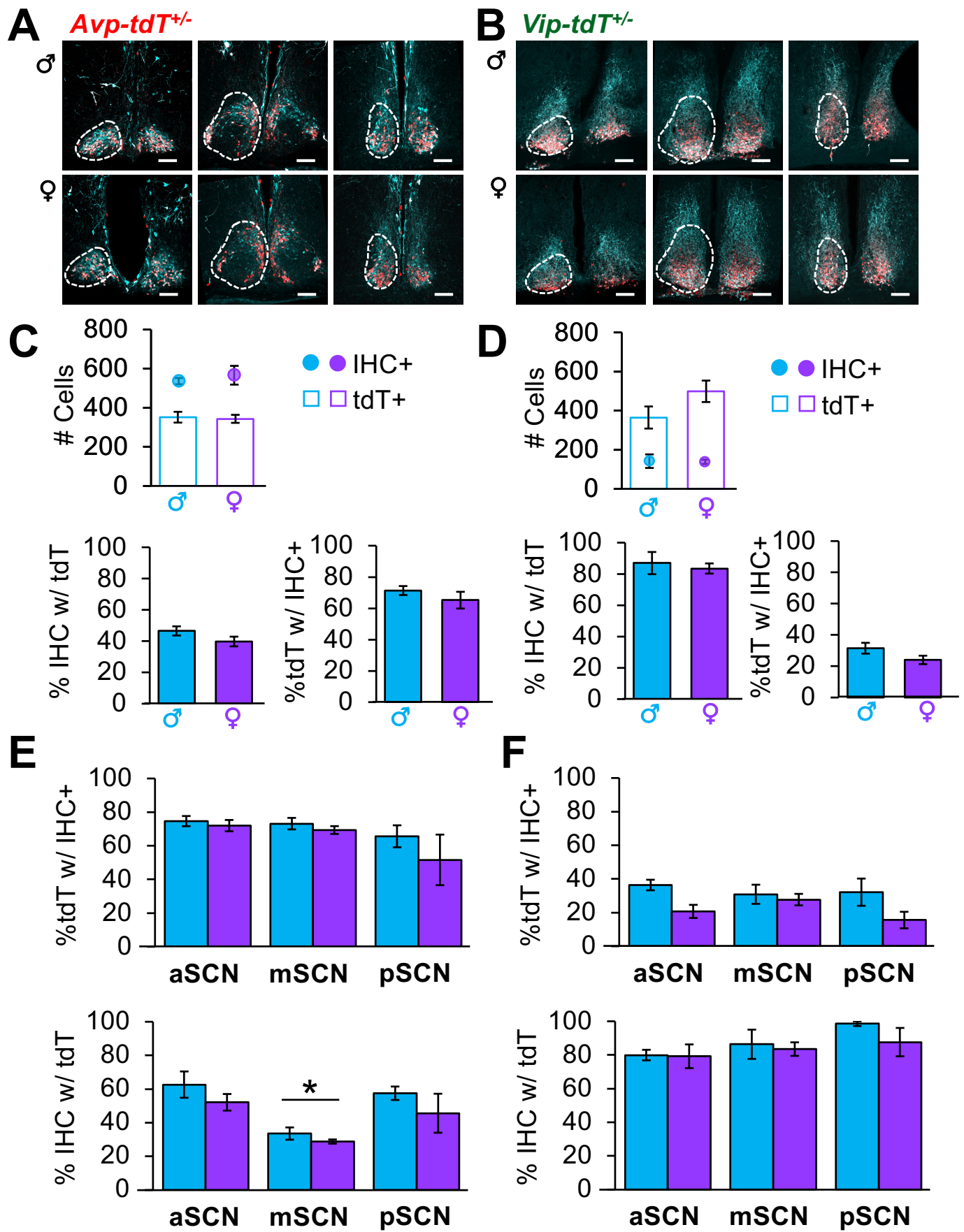


Figure S5

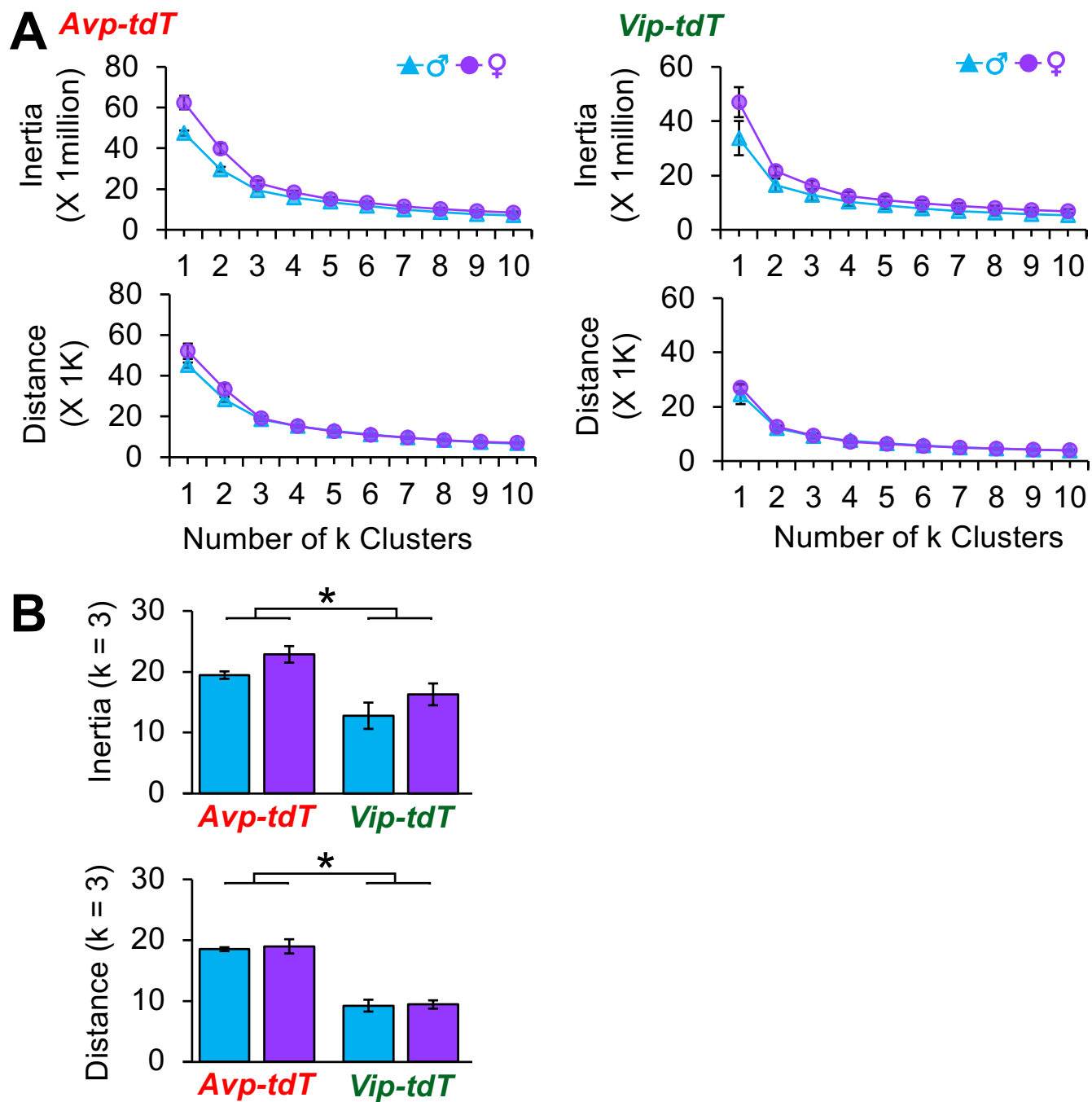


Figure S6

A *Avp-tdT*

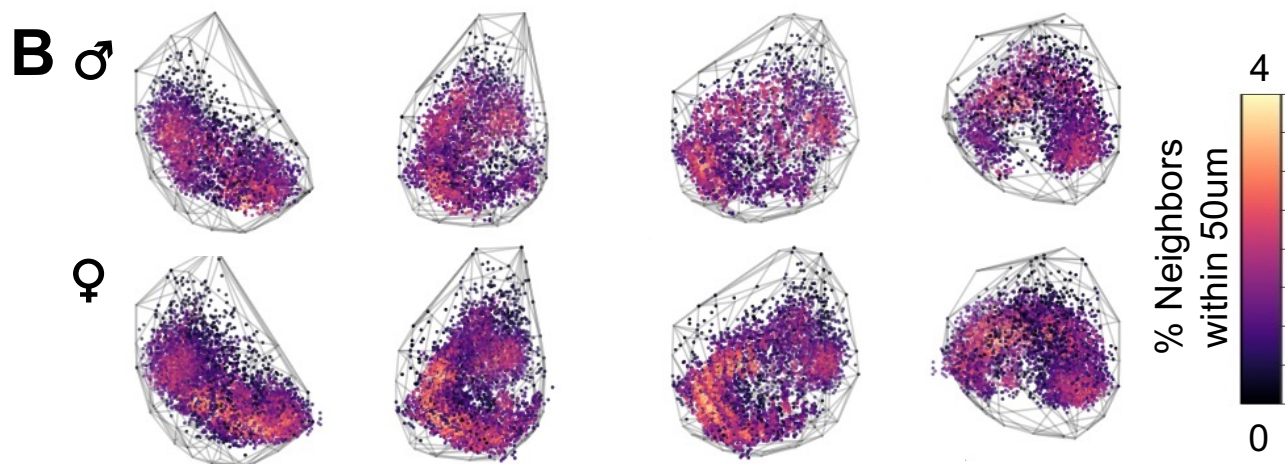
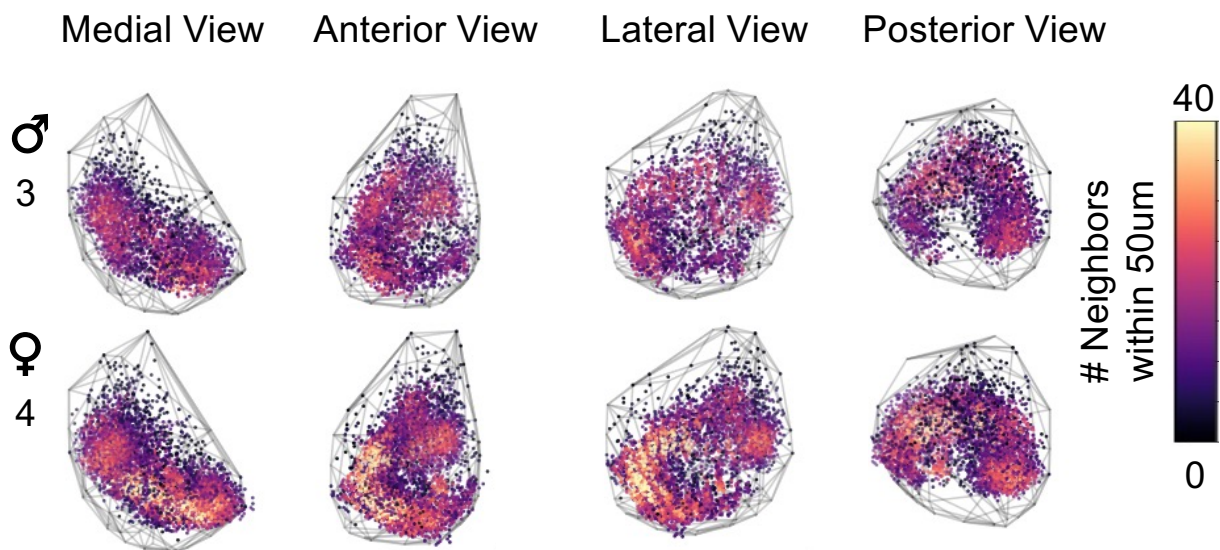
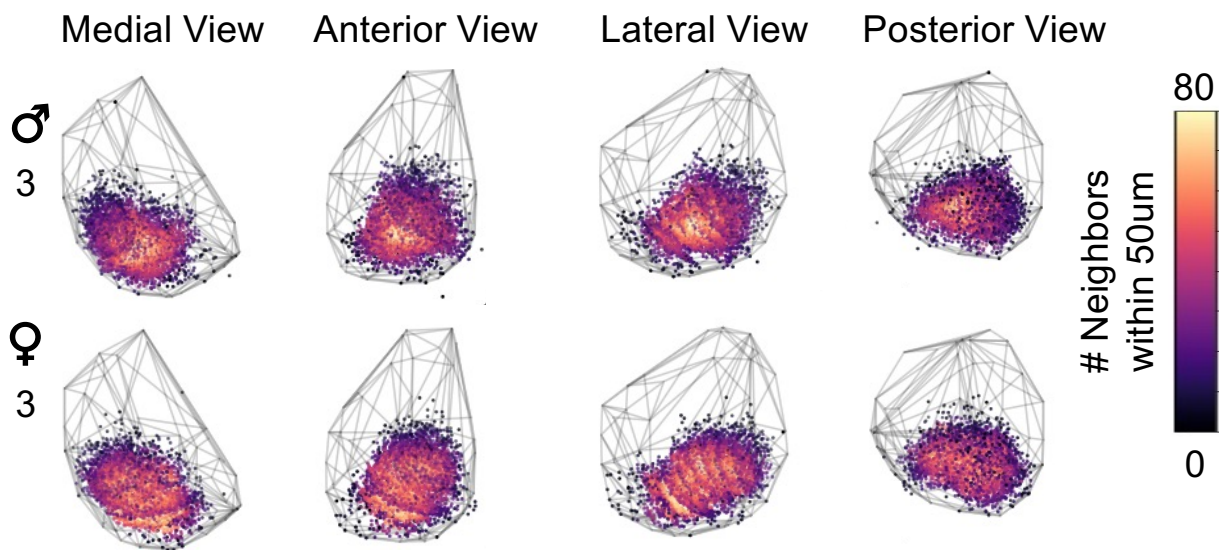


Figure S7

A *Vip-tdT*



B

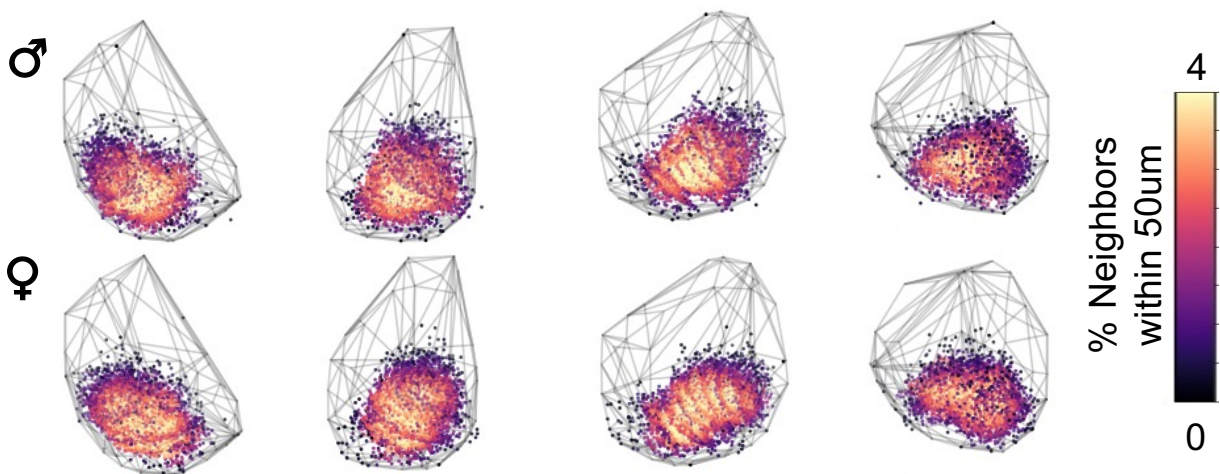


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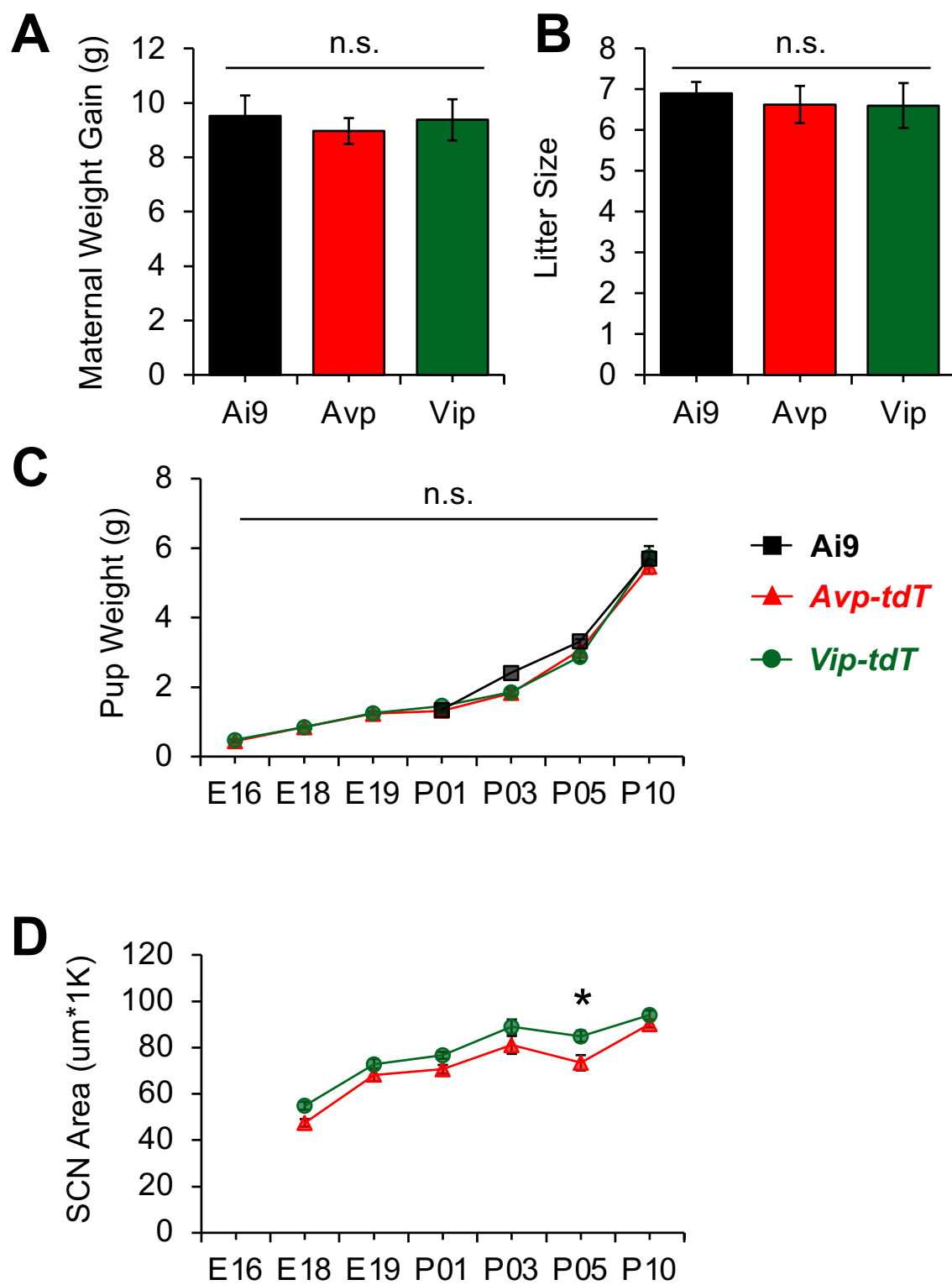


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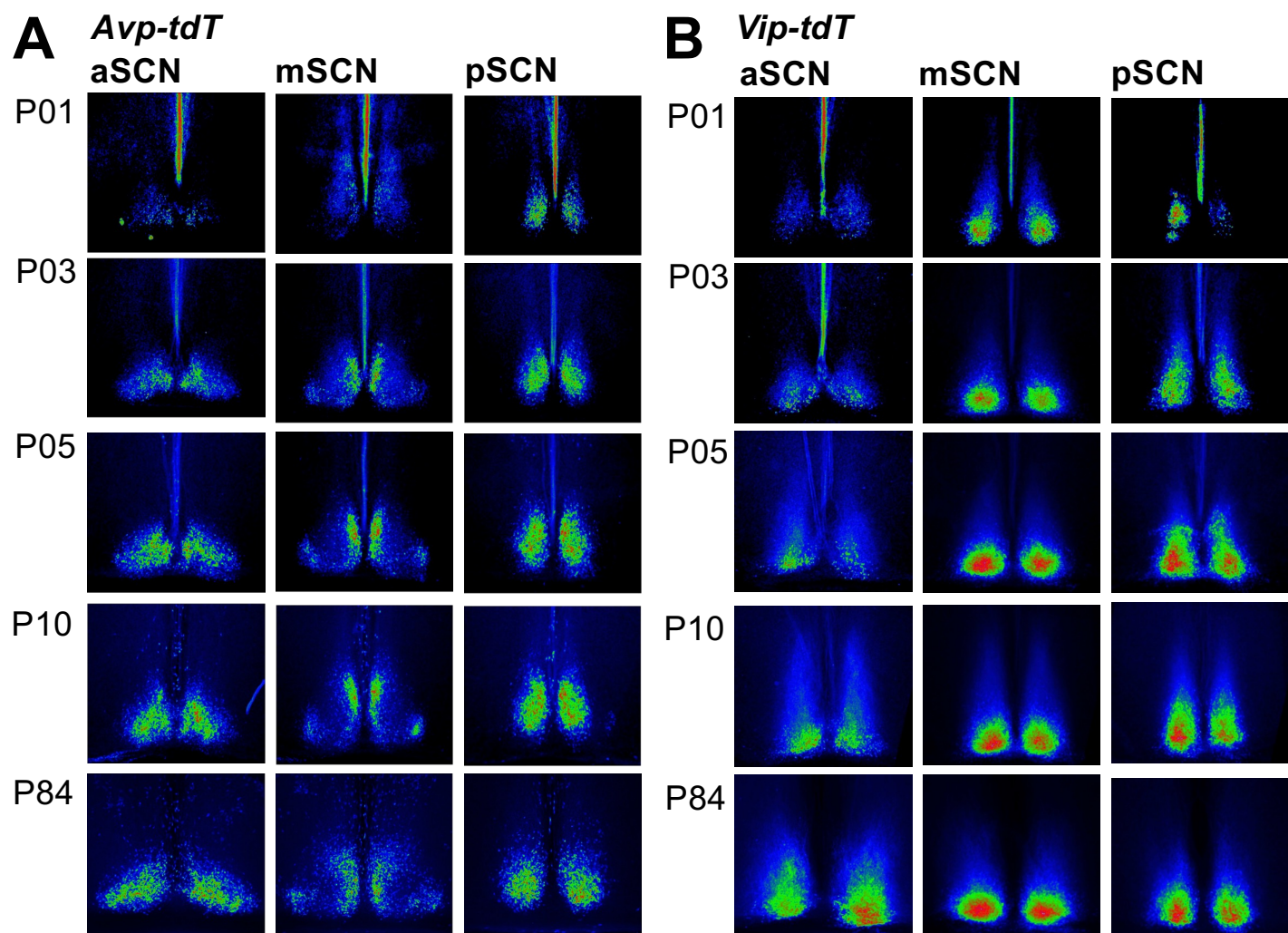


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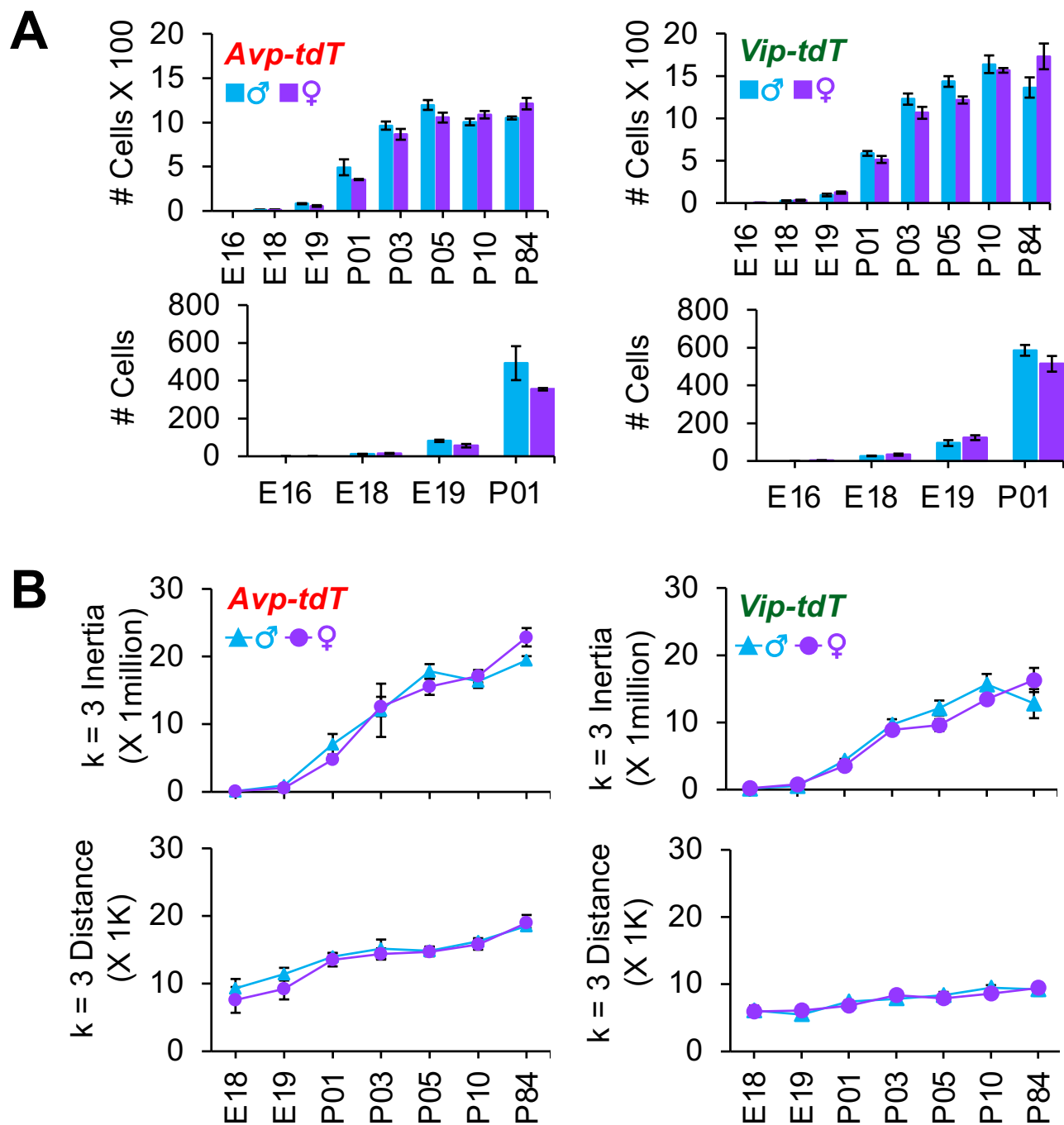


Figure S11