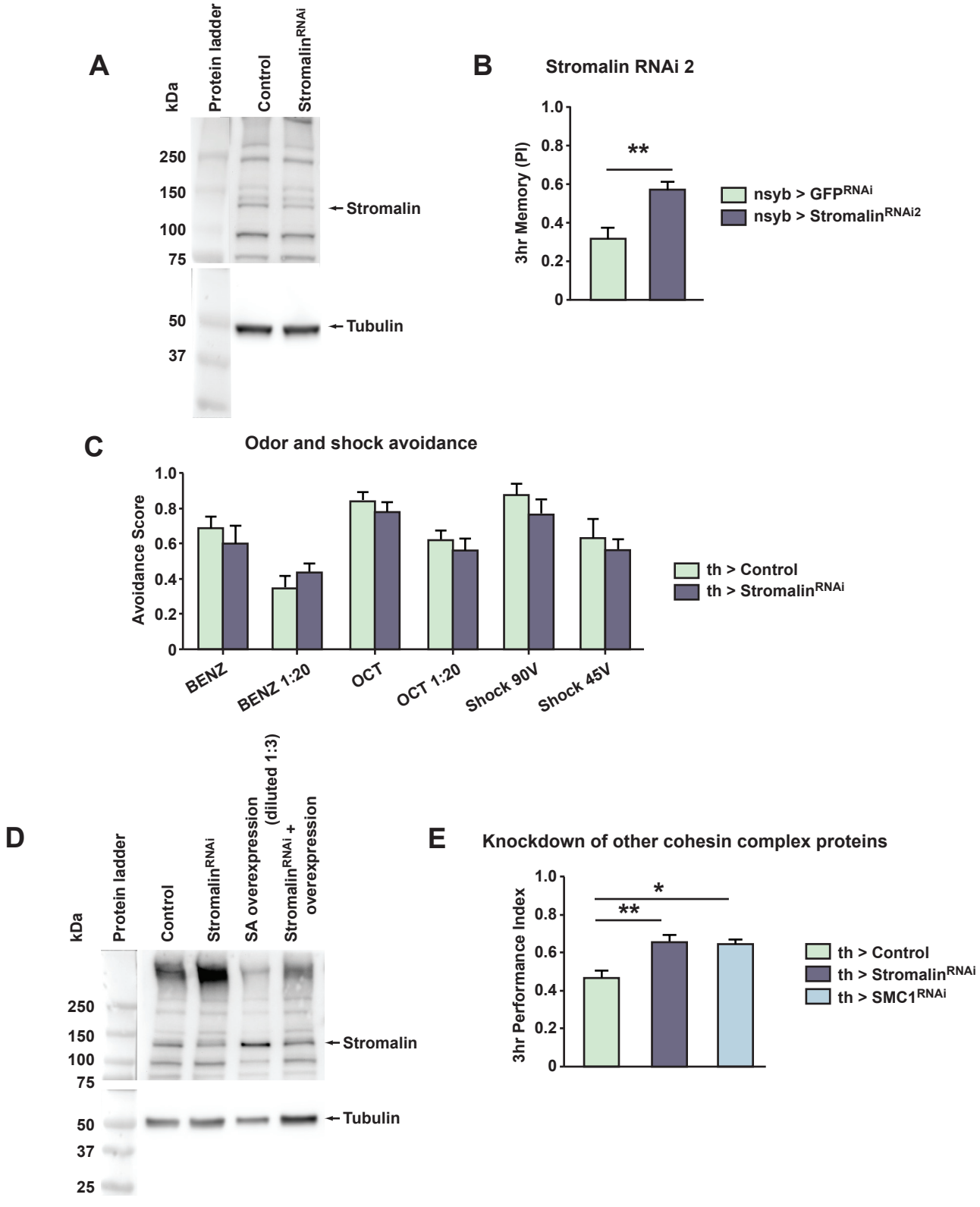


Phan et al., Supplemental Figure 1

Supplemental Figure 1. Knockdown of cohesin complex proteins stromalin and SMC1 enhances 3hr olfactory memory. Related to Figure 1.

(A) A full western blot from Figure 1A. Pan-neuronal *stromalin^{RNAi}* expression reduced stromalin protein levels. The bottom half of the membrane containing the loading control was cut and incubated separately in primary and secondary antibody. Control: *nSyb-GAL4>+, UAS-Dcr2.* Stromalin^{RNAi}: *nSyb-GAL4>UAS-SA^{RNAi}, UAS-Dcr2*.

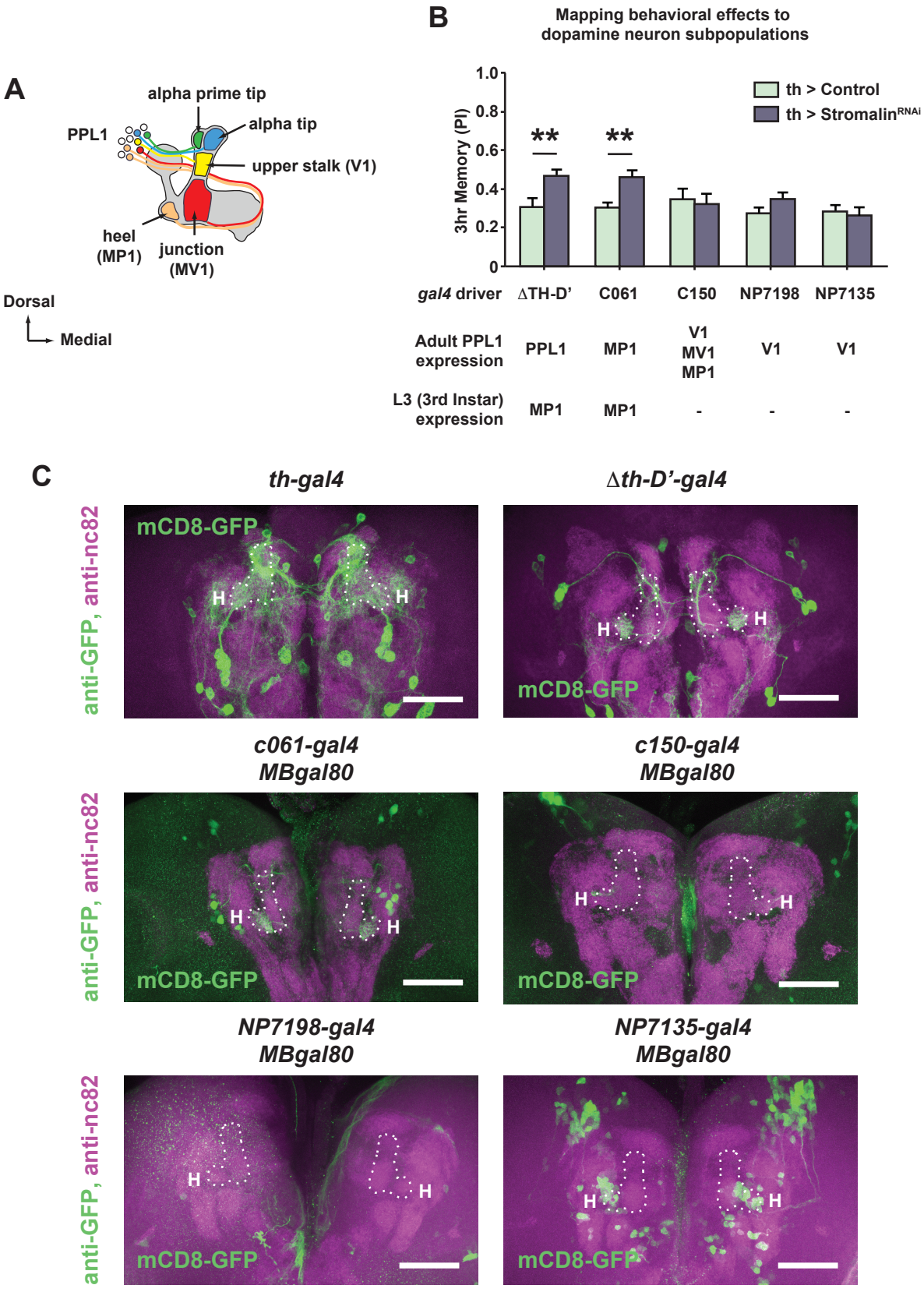
(B) Pan-neuronal expression of a second, independently derived RNAi (SA^{RNAi}; NIG library) targeted against *stromalin* exons 1-3 increased 3hr aversive olfactory memory. The *stromalin^{RNAi}* used throughout most of the paper is targeted against *stromalin* exon 5. A GFP^{RNAi} (NIG library) was used as a control. Control: *nSyb-GAL4>UAS-GFP^{RNAi}, UAS-Dcr2.* Stromalin^{RNAi}: *nSyb-GAL4>UAS-SA^{RNAi}, UAS-Dcr2*. Student's t-test, **p<0.01. n=10. Mean±SEM.

(C) Knockdown of Stromalin in DAn did not significantly alter the naïve odor and shock avoidance behavior of the flies. BENZ and OCT concentrations are those used for behavioral experiments. These behavioral concentrations were also diluted (1:20) to test odor avoidance. Control: *TH-GAL4>+, UAS-Dcr2.* Stromalin^{RNAi}: *TH-GAL4>UAS-SA^{RNAi}, UAS-Dcr2*. n=7-8. Mean±SEM.

(D) Full western blot from Figure 1G. Pan-neuronal *stromalin^{RNAi}* expression and Stromalin overexpression resulted in lower and higher levels of Stromalin protein relative to the control, respectively. The bottom half of the membrane containing the loading control was cut and incubated separately in primary and secondary antibody. Stromalin^{RNAi} + stromalin overexpression in neurons resulted in Stromalin protein levels similar to the control genotype, and higher than Stromalin knockdown samples. Control:

nSyb-GAL4>+, UAS-Dcr2. Stromalin^{RNAi}: *nSyb-GAL4>UAS-SA^{RNAi}, UAS-Dcr2*. Stromalin overexpression: *nSyb-GAL4>+, UAS-Dcr2, UAS-SA.* Stromalin^{RNAi} + Overexpression: *nSyb-GAL4>UAS-SA^{RNAi}, UAS-Dcr2, UAS-SA*.

(E) Knockdown of two components of the cohesin complex, Stromalin and SMC1, in DAn enhanced 3hr aversive olfactory memory. Control: *TH-GAL4>+, UAS-Dcr2*. Stromalin^{RNAi}: *TH-GAL4>UAS-SA^{RNAi}, UAS-Dcr2.* SMC1^{RNAi}: *TH-GAL4>UAS-SMC1^{RNAi}, UAS-Dcr2*. One-way ANOVA, Bonferroni *post hoc*, *p<0.05, **p<01. n=12. Mean±SEM.



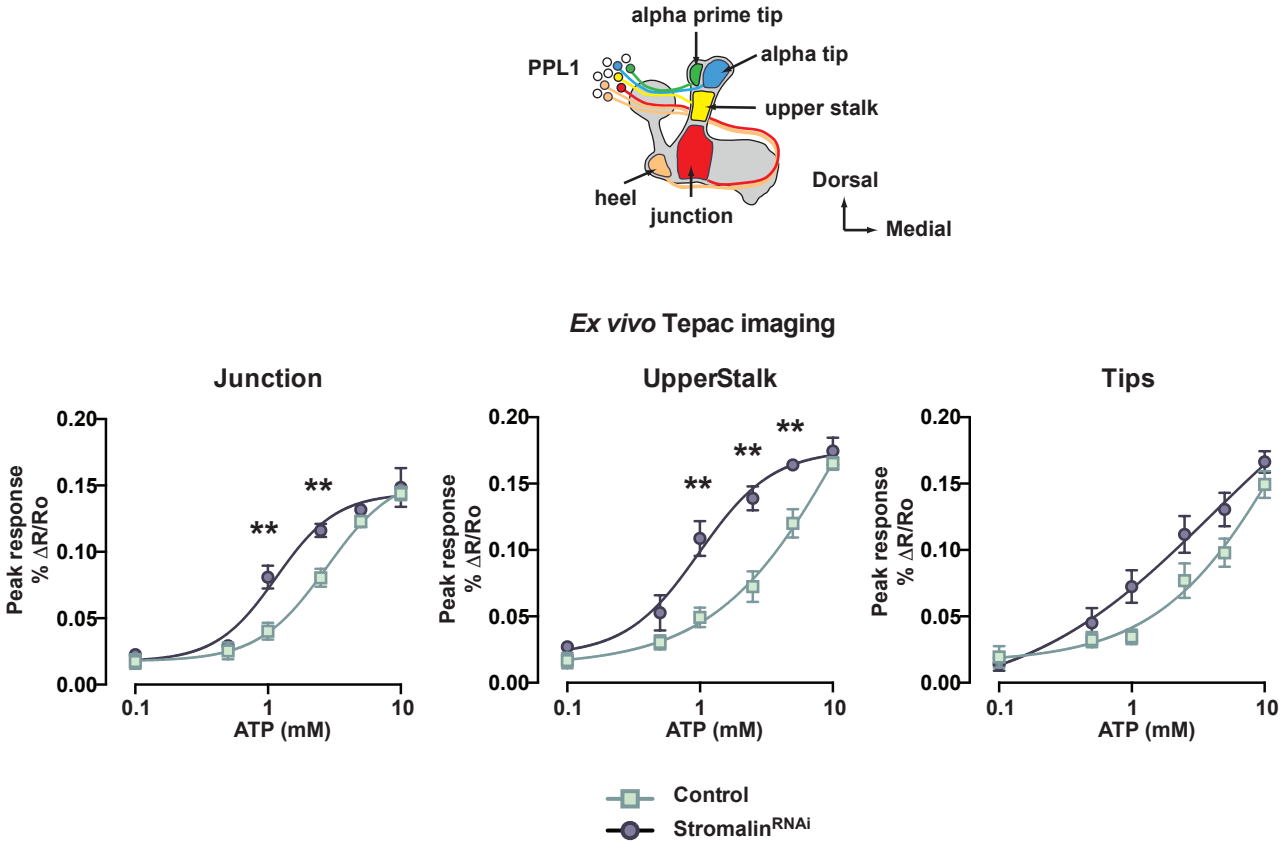
Phan et al., Supplemental Figure 2

Supplemental Figure 2. Mapping behavioral effects to subpopulations of PPL1 neurons and developmental *GAL4* driver expression. Related to Figure 2.

(A) Diagram of the PPL1 DAn innervation pattern of MB lobe neuropil.

(B) Stromalin KD in DAn innervating the heel of the MB (MP1) during the 3rd instar larval stage resulted in an enhanced memory phenotype in adult flies. We found that the expression pattern for most dopaminergic *GAL4* drivers during the 3rd instar larval stage differs from their adult expression patterns. Control: indicated *GAL4>+, UAS-Dcr2*. Stromalin^{RNAi}: indicated *GAL4>UAS-SA^{RNAi}*, *UAS-Dcr2*. Student's t-test, **p<0.01. n=8-12. Mean±SEM.

(C) Characterization of known adult PPL1 dopaminergic *GAL4* driver expression patterns during the 3rd instar larval stage, visualized using membrane-localized GFP and counterstained with NC82 (maximum projection images). Dotted lines indicate the MB heel, junction and upper stalk regions based on NC82 staining, while the 'H' indicates the MB heel region. The green areas within the dotted lines in the NP7135-GAL4 brain occur above/below the MB plane. Genotypes: indicated *GAL4>UAS-mCD8:GFP*. Scale bar 50μm.

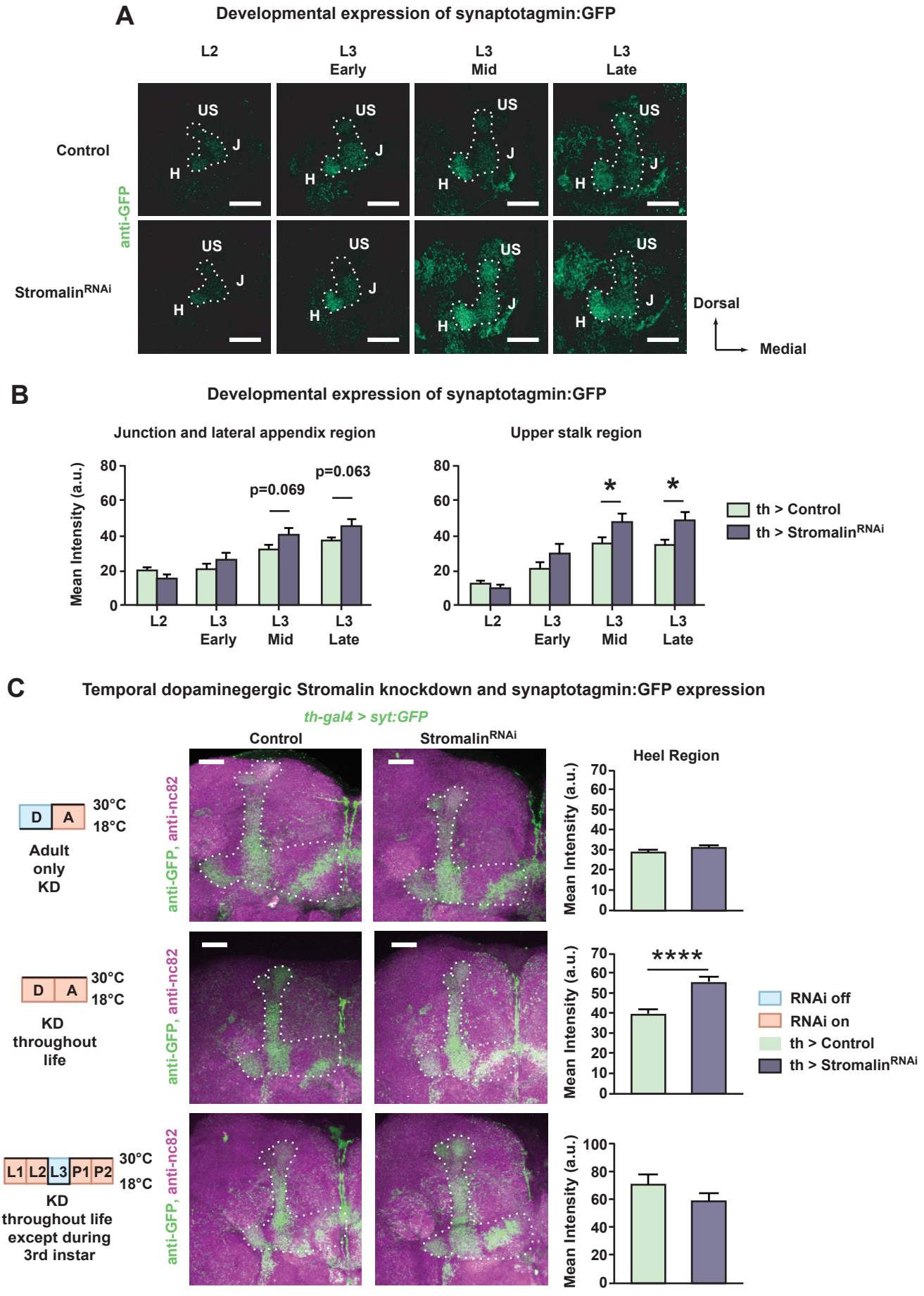


Phan et al., Supplemental Figure 3

Supplemental Figure 3. Stromalin knockdown in dopamine neurons increases the cAMP signal generated in the MB neuropil upon dopamine neuron stimulation.

Related to Figure 3.

Stromalin KD in DAn increased cAMP responses in the junction and upper stalk regions of the MB lobe neuropil upon DAn activation using the ATP-gated $P2X_2$ ion channel in an *ex vivo* preparation. R=ratio of CFP/YFP fluorescence intensity of cAMP reporter $Tepac^{vv}$. Control: *MB>Tepac^{vv}, TH-GAL4>+, UAS-Dcr2, UAS-P2X$_2$.* Stromalin^{RNAi}: *MB>Tepac^{vv}, TH-GAL4>UAS-SA^{RNAi}, UAS-Dcr2, UAS-P2X$_2$.* Mann-Whitney U with Bonferroni correction for multiple comparisons, **p<0.01. n=7-9. Mean±SEM.



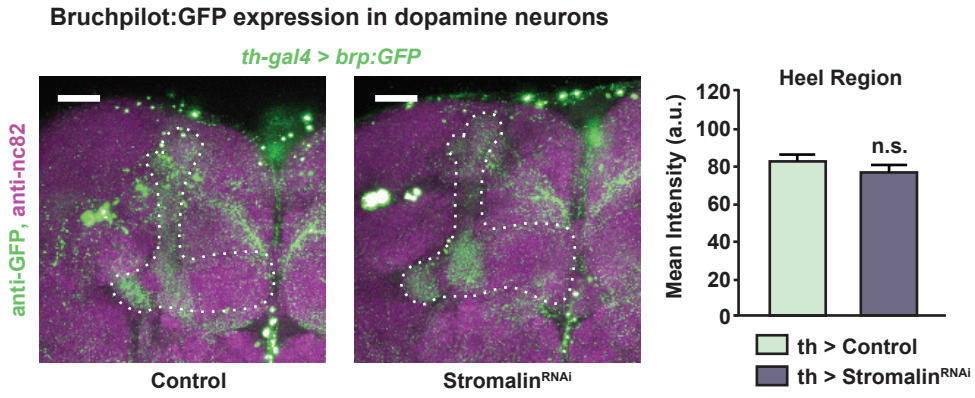
Phan et al., Supplemental Figure 4

Supplemental Figure 4. Stromalin knockdown in dopaminergic neurons increases synaptotagmin:GFP levels during the 3rd instar larval stage. Related to Figure 4.

(A) Images of syt:GFP expression in DAn during specific developmental stages. The dotted lines indicate the DAn innervation pattern of MB lobes. 'H' indicates the heel, 'J' the junction, and 'US' the upper stalk regions of DAn innervation of the MB. We could not clearly distinguish DAn innervation of the MB tips. Scale bar 20μm.

(B) Similar to what was found in the heel region (Figure 4E), Stromalin KD in DAn also increased syt:GFP labeling in the upper stalk region during the 3rd instar larval period. Syt:GFP measures in the junction region were trending towards significance, suggesting that the junction region may undergo further synaptic changes during the pupal stage. Control: *TH-GAL4>+*, *UAS-Dcr2, UAS-syt:GFP, UAS-myr-tdTom.* Stromalin^{RNAi}: *TH-GAL4>UAS-SA^{RNAi}*, *UAS-Dcr2, UAS-syt:GFP, UAS-myr-tdTom*. Mann-Whitney U, *p<0.05. n=11-15. Mean±SEM.

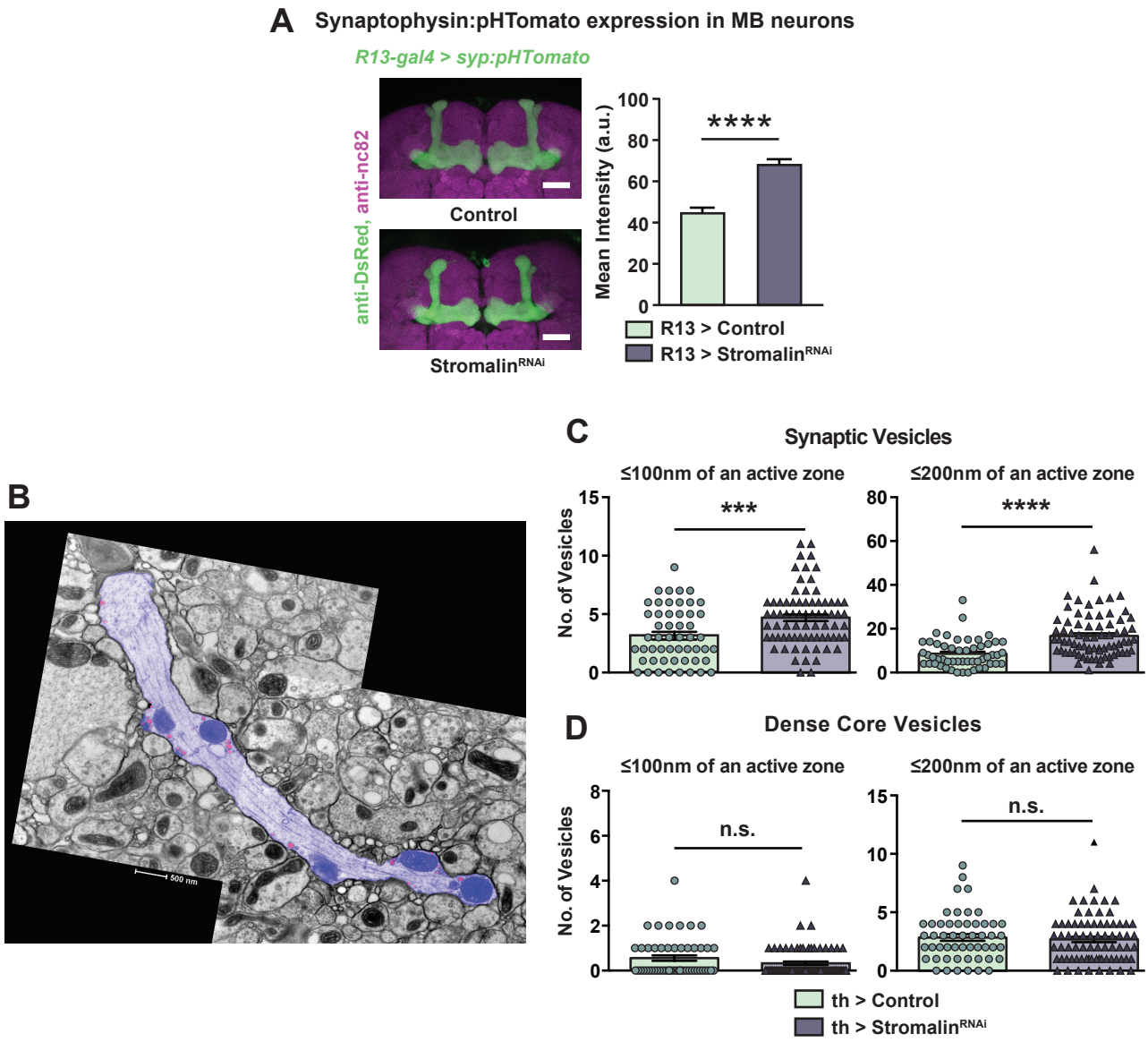
(C) Developmental suppression of DAn *stromalin^{RNAi}* using the TARGET system did not alter the syt:GFP signal in the MB heel (top row of images and graph). Stromalin KD throughout development and adulthood significantly increased the syt:GFP signal (middle row of images and graph). Expression of *stromalin^{RNAi}* throughout development and adulthood, except during the critical 3rd instar period, failed to increase the MB heel syt:GFP signal (bottom row of images and graph). These syt:GFP results parallel the behavioral data using the TARGET system (Figure 2D-E). Control*: tub>gal80^{ts}*, *TH-GAL4>+*, *UAS-Dcr2, UAS-syt:GFP.* Stromalin^{RNAi}: *tub>gal80^{ts}*, *TH-GAL4>UAS-SA^{RNAi}*, *UAS-Dcr2, UAS-syt:GFP*. Mann-Whitney U, ****p<0.0001. n=10-13. Mean±SEM. Scale bar 20μm.



Phan et al., Supplemental Figure 5

Supplemental Figure 5. The active zone marker, bruchpilot:GFP, is not affected by Stromalin knockdown in DAn. Related to Figure 5.

Expression of *stromalin*^{RNAi} using *TH-GAL4* did not alter levels of the active zone marker brp:GFP in DAn axons, consistent with the GRASP and SIM data that demonstrated no observable differences in the numbers of DAn synapses (Figure 5). Control: *TH-GAL4>+*, *UAS-Dcr2, UAS-brp:GFP.* Stromalin^{RNAi}: *TH-GAL4>UAS-SA*^{RNAi}, *UAS-Dcr2, UAS-brp:GFP*. n=13-15. Mean±SEM. Scale bar 20μm.



Phan et al., Supplemental Figure 6

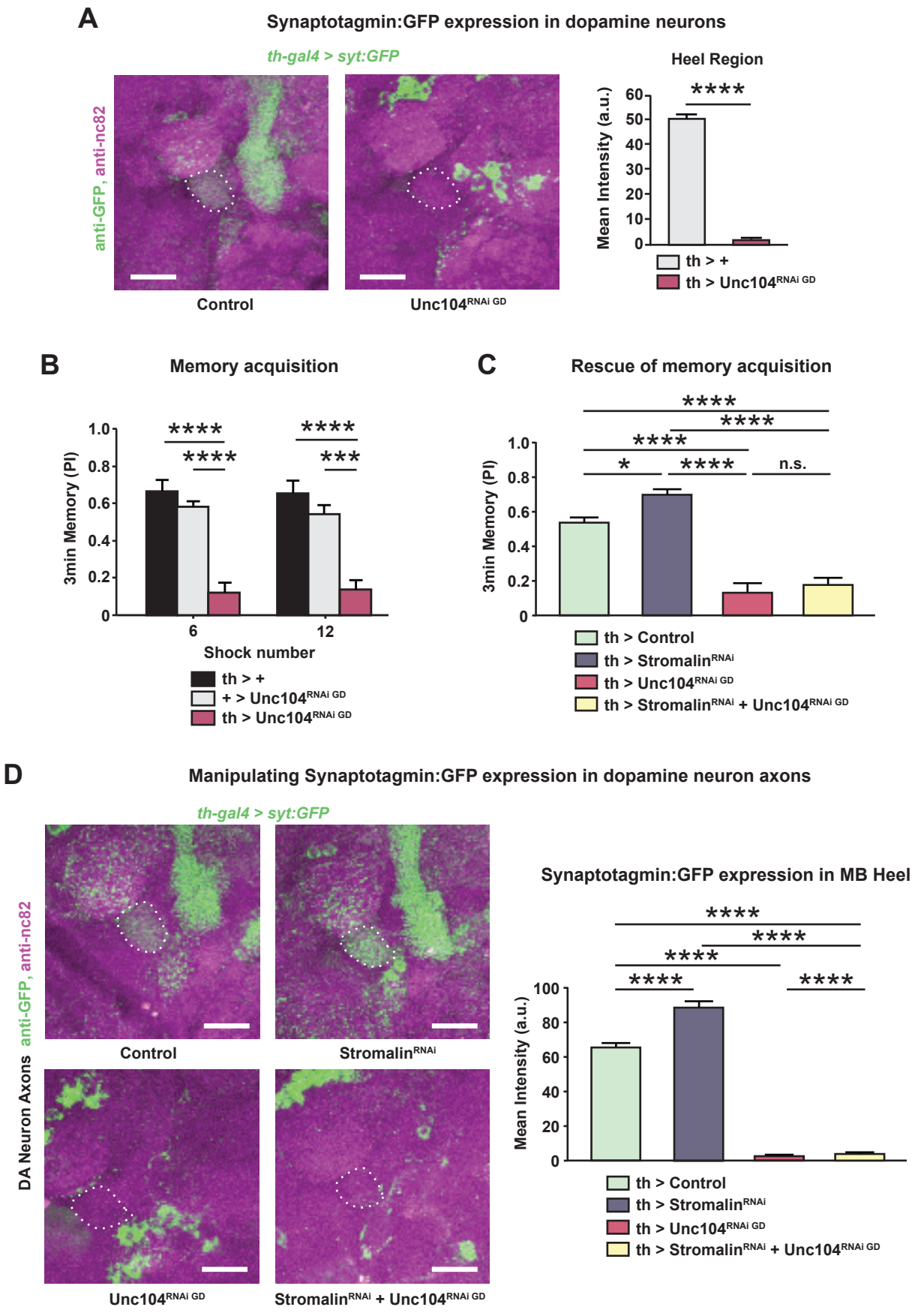
Supplemental Figure 6. Stromalin knockdown increases synaptic vesicles in neurons. Related to Figure 6.

(A) Reducing Stromalin in MBn increased expression of a second synaptic vesicle marker, Synaptophysin:pHTomato, in the adult *Drosophila* brain. Control: *nSyb-GAL4>+, UAS-Dcr2, and UAS-syp:pHTom*. Stromalin^{RNAi}: *nSyb-GAL4>UAS-SA^{RNAi}, UAS-Dcr2, and UAS-syp:pHTom.* Mann-Whitney U, ****p<0.0001. n=13. Mean±SEM. Scale bar 20µm.

(B) Example electron micrograph of control DAn with electron dense membrane labeling (blue) and SVs (red). Some SVs were not labeled in this micrograph, but were labeled in sections before or after this image (in which the vesicles present the largest diameter) to prevent double counting of the same vesicle. Control: *TH-GAL4>+, UAS-Dcr2, UAS-CD4:HRP*.

(C) Stromalin KD increased the number of dopaminergic synaptic vesicles within 100nm or 200nm of an active zone. Control: *TH-GAL4>+, UAS-Dcr2, UAS-CD4:HRP*. Stromalin^{RNAi}: *TH-GAL4>UAS-SA^{RNAi}, UAS-Dcr2, UAS-CD4:HRP*. Mann-Whitney U, ***p<0.001, ****p<0.0001. n=52 control, n=74 Stromalin^{RNAi}. Mean±SEM.

(D) Stromalin KD did not alter the number of dense core vesicles within 100nm or 200nm of a DAn active zone. Control: *TH-GAL4>+, UAS-Dcr2, UAS-CD4:HRP*. Stromalin^{RNAi}: *TH-GAL4>UAS-SA^{RNAi}, UAS-Dcr2, UAS-CD4:HRP*.



Phan et al., Supplemental Figure 7

Supplemental Figure 7. Strong reduction of synaptic vesicle transport to dopamine neuron axon terminals impairs memory and memory enhancement due to Stromalin knockdown. Related to Figure 7.

(A) Knockdown of the synaptic vesicle anterograde transporter Unc104 using an RNAi line from the GD library (Unc104$^{\text{RNAi GD}}$) severely reduced the syt:GFP levels in the DAn axons innervating the MB heel. Quantitation of GFP fluorescence levels are displayed in the bar graphs. Control: *TH-GAL4>+, UAS-Dcr2, UAS-syt:GFP*. Unc104$^{\text{RNAi GD}}$: *TH-GAL4>UAS-Unc104$^{RNAi\ GD}$, UAS-Dcr2, UAS-syt:GFP*. Mann-Whitney U, ****p<0.0001. n=12-13. Mean±SEM. Scale bar 20μm.

(B) DAn expression of *unc104$^{RNAi\ GD}$* strongly impaired aversive memory acquisition when flies were trained with 6 or 12 shocks. Control: *TH-GAL4>+, UAS-Dcr2.* Unc104$^{\text{RNAi GD}}$: *TH-GAL4>UAS-Unc104$^{RNAi\ GD}$, UAS-Dcr2*. One way ANOVA, Bonferroni *post hoc* ***p<0.001, ****p<0.0001. n=6. Mean±SEM.

(C) *stromalin^{RNAi}* expression in DAn increased, while *unc104$^{RNAi\ GD}$* expression impaired, memory acquisition compared to control flies when trained with 6 shocks. The co-expression of *stromalin^{RNAi}* and *unc104$^{RNAi\ GD}$* in DAn resulted in impaired memory acquisition scores (yellow bar), which were not different from flies expressing *unc104$^{RNAi\ GD}$* alone. Control: *TH-GAL4>+, UAS-Dcr2.* Stromalin$^{\text{RNAi}}$: *TH-GAL4>UAS-SA^{RNAi}, UAS-Dcr2.* Unc104$^{\text{RNAi GD}}$: *TH-GAL4>UAS-Unc104$^{RNAi\ GD}$, UAS-Dcr2*. Stromalin$^{\text{RNAi}}$ + Unc104$^{\text{RNAi GD}}$: *TH-GAL4>UAS-SA^{RNAi}, UAS-Unc104$^{RNAi\ GD}$, UAS-Dcr2.* One-way ANOVA, Bonferroni *post hoc,* *p<0.05, ****p<0.0001. n=8-11. Mean±SEM.

(D) Syt:GFP in DAn from control, *stromalin^{RNAi}*, *unc104$^{RNAi\ GD}$*, and *stromalin^{RNAi}* + *unc104$^{RNAi\ GD}$* fly brains. Mirroring the behavioral results in panel C, *stromalin^{RNAi}*

expression in DAn increased, while *unc104*$^{RNAi\ GD}$ expression severely decreased, syt:GFP levels in the MB heel. When both *stromalin*^{RNAi} and *unc104*$^{RNAi\ GD}$ were expressed within the same DAn (yellow bar, right panel), syt:GFP levels in the MB heel remained severely reduced compared to controls. Control: *TH-GAL4>+, UAS-syt:GFP, UAS-Dcr2.* Stromalin^{RNAi}: *TH-GAL4>UAS-SA*^{RNAi}*, UAS-syt:GFP, UAS-Dcr2.* Unc104$^{RNAi\ GD}$: *TH-GAL4>UAS-Unc104*$^{RNAi\ GD}$*, UAS-syt:GFP, UAS-Dcr2*. Stromalin^{RNAi} + Unc104$^{RNAi\ GD}$: *TH-GAL4>UAS-SA*^{RNAi}*, UAS-Unc104*$^{RNAi\ GD}$*, UAS-syt:GFP, UAS-Dcr2.* Kruskal-Wallis with *a priori* planned comparisons using Mann-Whitney U with Bonferroni correction for multiple comparisons to reduce type I errors. ****p<0.0001. n=14-17. Mean±SEM. Scale bar 20μm.