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Supplemental Information

Synapse Formation Activates a Transcriptional Program for Persistent Enhancement in the Bi-directional Transport of Mitochondria

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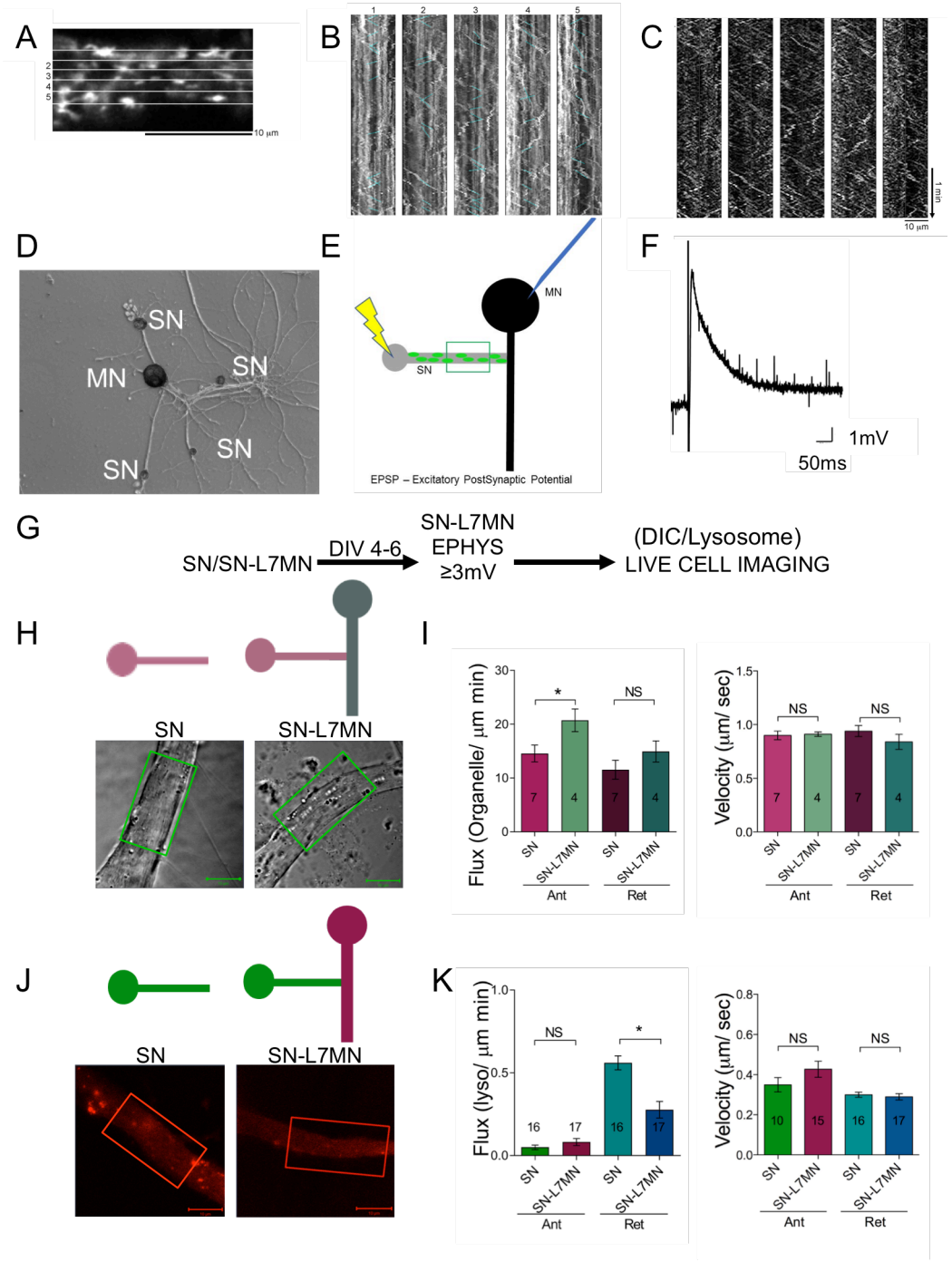
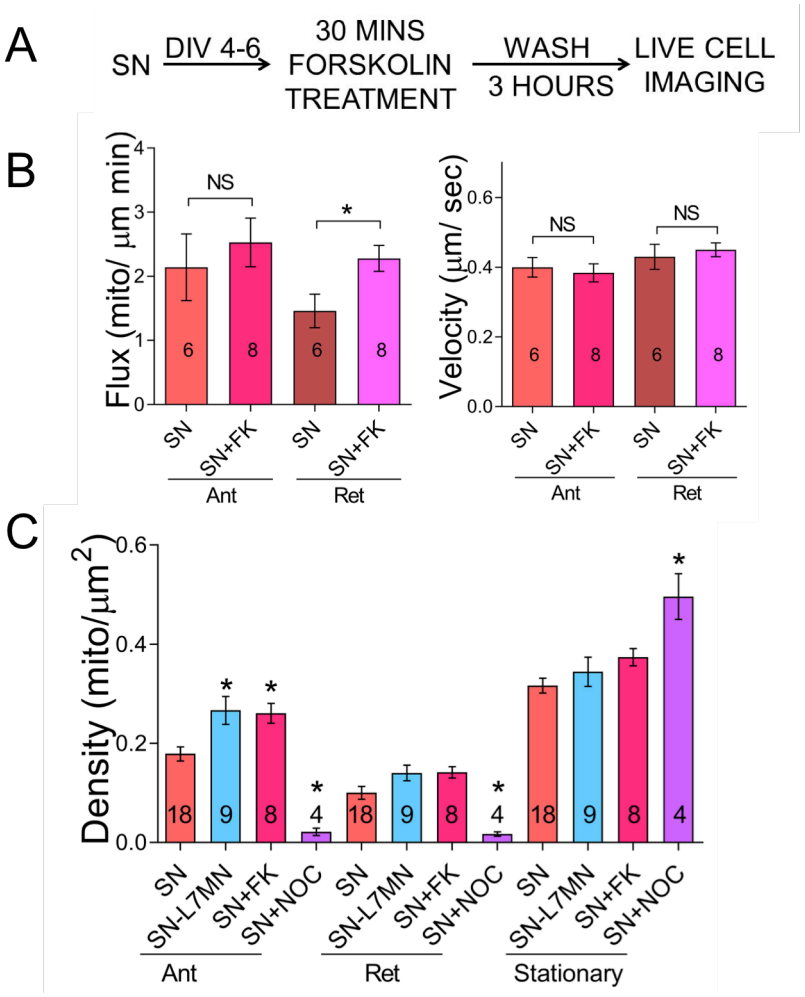
Supplementary Figures

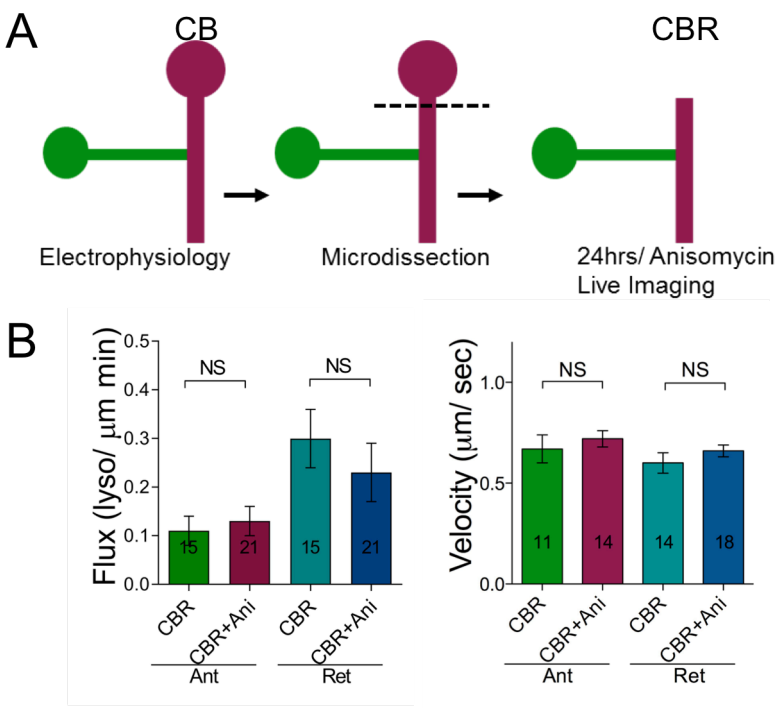


Supplementary Figure S1 Related to Figure 1. A: First frame of a time-lapse movie showing mitochondrial transport. The cell body is towards the left and the movie is split into 1.3 μm regions shown by the white horizontal lines (labeled 1 - 5). **B:** Each region is made into a kymograph. **C:** To enhance the visualization of transport through regions

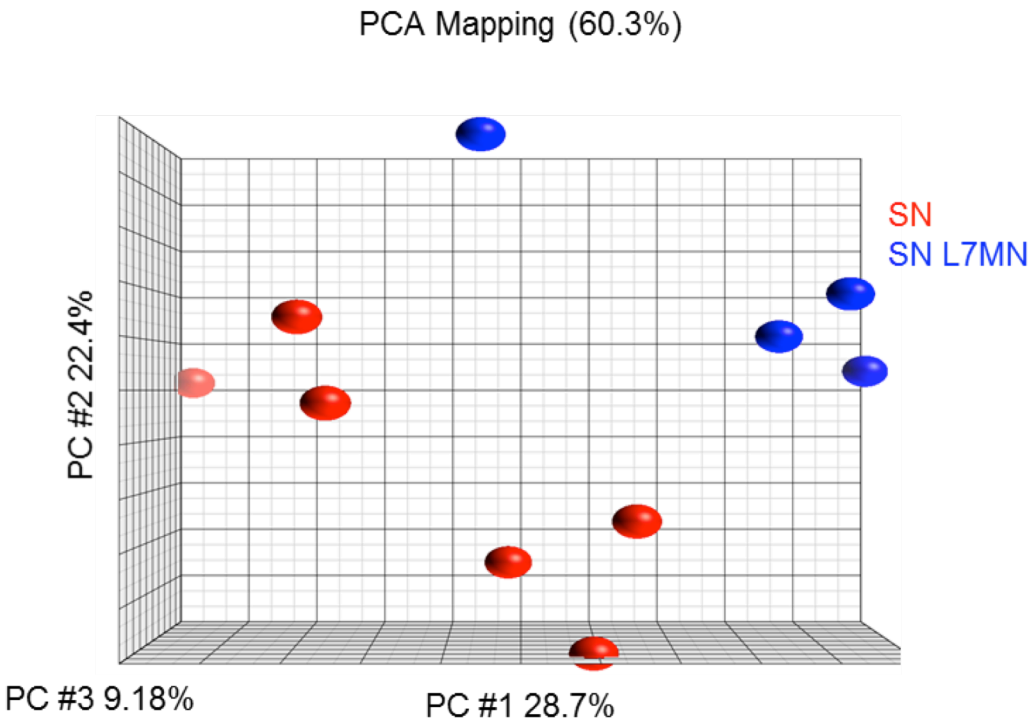
where mitochondria are stationary, the movie is processed using a background subtraction algorithm and a second set of kymographs are constructed for reference. Lines, shown in light blue, are traced over mitochondria that clearly cross the center of the axon. Flux in each direction is calculated by counting the number of mitochondria that cross the center line and dividing by the time of the movie and width of the analyzed region (i.e. 1.3 μm). Velocity is calculated from the line slopes; bars = 10 μm, time arrow = 1 min. **D:** *Aplysia* neuronal cell culture with labeled MN (Motor neurons) and SNs (Sensory Neurons). **E:** Cartoon schematic of electrophysiology recording: EPSP – excitatory post-synaptic potential by stimulating SN and intracellular recording form MN. **F.** Trace of an EPSP recording from SN-L7MN co-culture. **G:** Experimental design schematic. **H.** Representative snapshots of transport in DIC live imaging in SN or SN-L7MN. Scale bar 10 μm. **I.** Bar graphs showing measurements of flux and velocity of anterograde (Ant) and retrograde (Ret) transport in SN or SN-L7MN. NS: non-significant. Error bars are SEM. "*" is $p < 0.05$; Student's t test. Numbers of neurons used indicated in bar graphs. **J.** Representative snapshots of transport in lysosome live imaging in SN or SN-L7MN. Scale bar 10 μm. **K.** Bar graphs showing measurements of flux and velocity of transport in SN or SN-L7MN. NS: non-significant. Error bars are SEM. Values are shown in Supplementary Table S5. Numbers of neurons used indicated in bar graphs "*" is $p < 0.05$; Student's unpaired t test. See also Table S1E.



Supplementary Figure S2 Related to Figure 2. A: Experimental design schematics. **B:** Bar graphs showing measurements of flux and velocity of anterograde (Ant) and retrograde (Ret) transport after 3 hr after forskolin (FK) exposure. NS: non-significant. Numbers of neurons used are indicated in bar graphs. "*" is $p < 0.05$; Student's unpaired t test. **C.** Bar graph showing the density of mitochondria moving in the anterograde direction, retrograde direction or that are stationary. Values are shown in Supplementary Table S6. "*" is $p < 0.05$; One-way ANOVA and with post-hoc Tukey test. See also Table S1F.



Supplementary Figure S3 Related to Figure 4. A. Experimental design schematics. **B.** Bar graphs showing measurements of flux and velocity of anterograde (Ant) and retrograde (Ret) transport of lysosomes in control and anisomycin (Ani) treated SN-L7MN where the cell body was removed (CBR) or with intact cell body (CB). Numbers of neurons used are indicated in bar graphs. NS: non-significant, Values are shown in Supplementary Table S7. "*" is $p < 0.05$; Student's unpaired t test. See also Table S1G.



Supplementary Figure S4 Related to Figure 4. Principal Component Analysis (PCA) of microarray data showing the gene expression profiles in the SN cell body for neurons grown alone (n=4) or in the presence of L7MN (n=6). The figure shows the first two principal components of microarray analysis data (PC1, PC2, and PC3) in X, Y, and Z respectively.