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Haberman et al., http://www.jcb.org/cgi/content/full/jcb.201108088/DC1

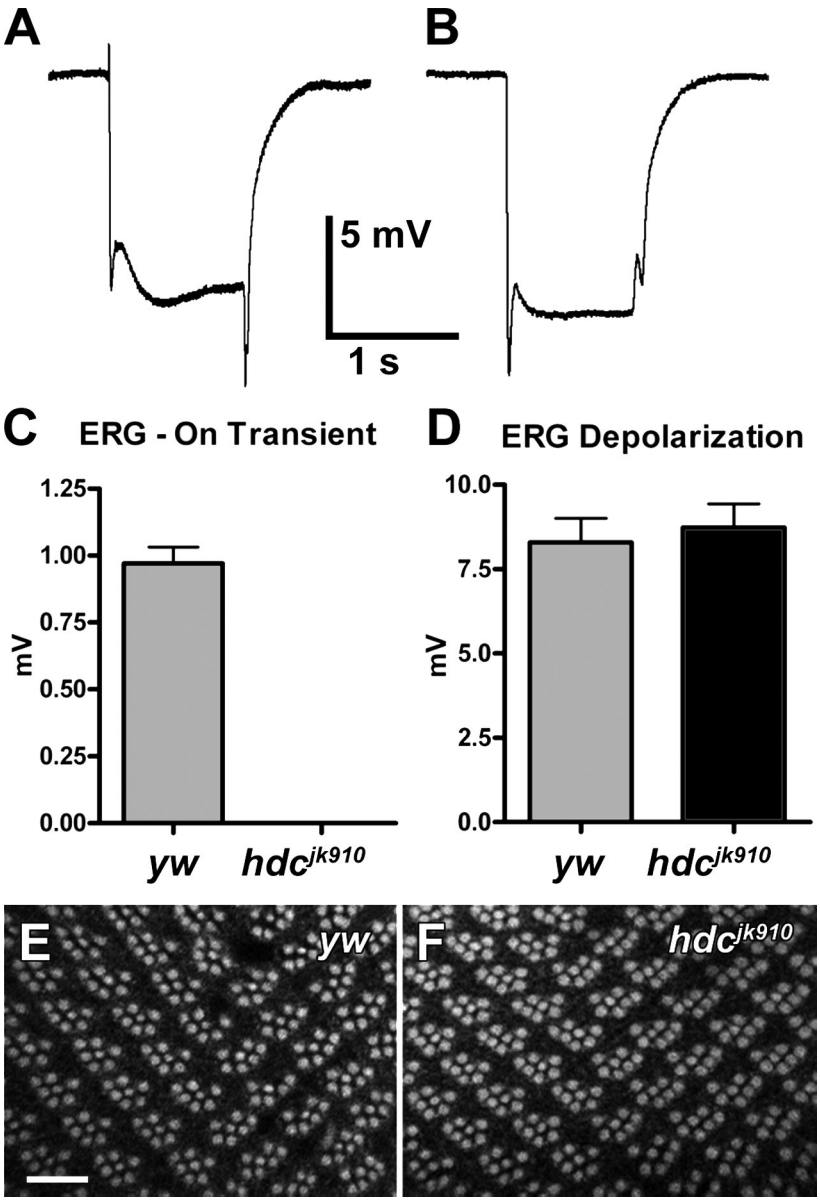

Figure S1. **Loss of neurotransmitter release in *Drosophila* photoreceptors in a mutant for histamine synthesis does not cause degeneration.** (A and B) Sample electroretinogram (ERG) traces for 3-wk-old control (A) and *hdc*^{jk910} (B). (C) Quantification of "on" transient indicates complete loss of neurotransmission. (D) Unaltered ERG response amplitude indicates no degeneration. (E and F) Phalloidin-labeled cross sections through 3 -wk-old eyes reveal no degeneration. Bar, 10 µm. Error bars are SEM.

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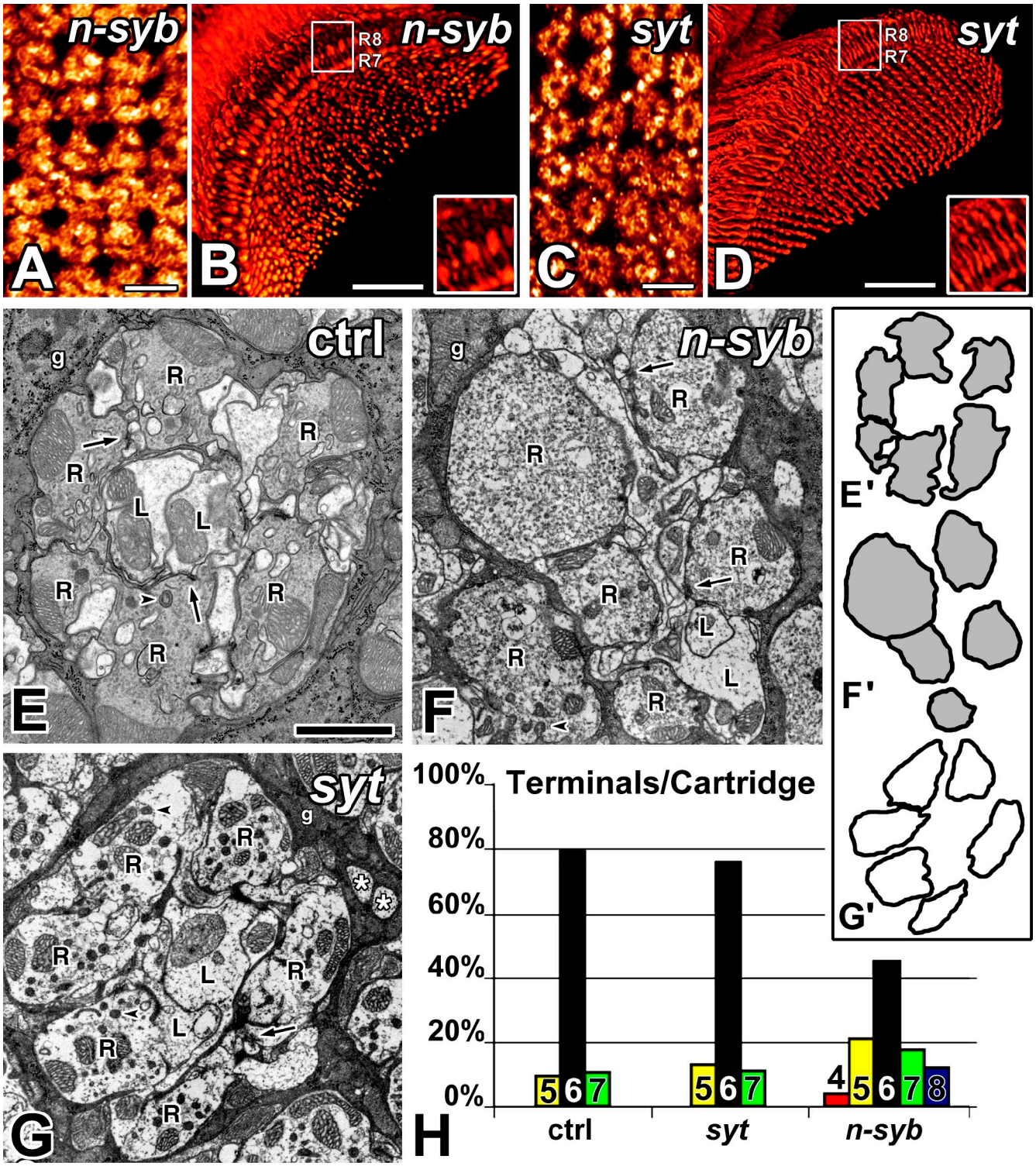

Figure S2. **Lack of *syb*, but not *syt*, in photoreceptors disrupts lamina development.** (A) Lamina cross sections containing *n-syb* mutant photoreceptors visualized from a deconvolved high-resolution confocal image immunolabeled with mAb 24B10 against Chaoptin. The normal number of six R terminals per cartridge is rarely discernible. (B) 3D visualization of *n-syb* R7 and R8 photoreceptor terminals in the medulla (mAb 24B10 labeling). Terminals appear enlarged and disorganized. (inset) Enlarged version of the boxed region. (C) Lamina cross section as in A but containing *syt* mutant photoreceptors. The correct number of six R terminals per cartridge is in most cases recognizable. (D) *syt* mutant R7 and R8 projections in the medulla (mAb 24B10). Note the regular array of R7 terminals. (inset) Enlarged version of the boxed region. (E) EM micrograph of a cartridge cross section with homozygous mutant wild-type photoreceptors. R, photoreceptor terminals; L, lamina monopolar neurons; g, epithelial glia; arrows show tetrad synapses; arrowheads show capitate projections. (F) Cartridge with *n-syb* photoreceptors. (G) Cartridge with syt mutant photoreceptors. Labeling and scale bar for F and G are as in E. R terminal profiles are shown in E′–G′. Asterisks show R7/8 axon profiles. (H) Quantification of R terminals per cartridge for control, *n-syb*, and *syt* mutant photoreceptors. The total number of terminals per cartridge in the available EM data was counted, and the distribution is shown as a percentile. Bars: (A and C) 5 µm; (B and D) 20 µm; (E) 2 µm. ctrl, control.

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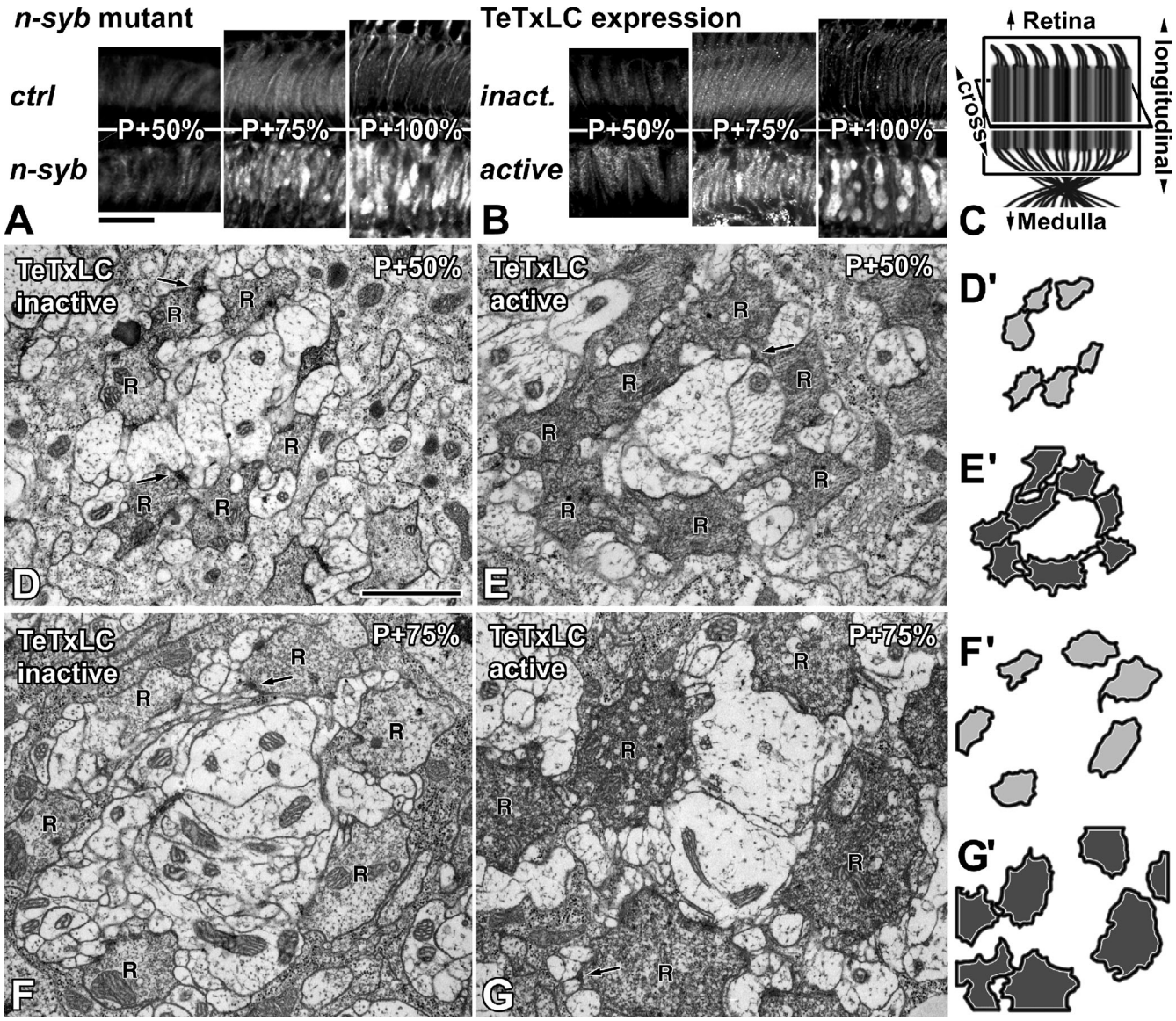

Figure S3. **Developmental analysis of synaptic terminals in the lamina.** (A) Longitudinal sections of laminae with *n-syb* mutant R terminals and controls at 50, 75, and 100% of pupal development, visualized by mAb 24B10 immunolabeling. Slight disturbances at P+50% become gradually stronger during the second half of pupal life. (B) Longitudinal sections of laminae with inactive (inact.) and active tetanus toxin light chain (TeTxLC) expression (mAb 24B10 immunolabels). No differences from *n-syb* mutant photoreceptors were observed. Scale bar as in A. (C) Schematic diagram of longitudinal sections and cross sections through the lamina. (D) Transmission EM micrograph of a lamina cross section with photoreceptor-specific inactive TeTxLC expression (control) at P+50%. R terminals (R) are already sorted around the axons of lamina cells. R terminal profiles are depicted in D′. Arrows show nascent synapses. (E) Active TeTxLC expression at P+50%. Note the aberrant number of R terminals in this nonequatorial cartridge. R terminal profiles are depicted in E′. (F) Photoreceptor-specific inactive TeTxLC expression (control) at P+75%. Numerous profiles of dendritic spines are visible between the R terminals. (G) Active TeTxLC expression at P+75%. Many profiles of dendritic spines are visible, but R terminals appear darker and enlarged in this missorted cartridge. Labels and scale bar for E–G are as in D. Bars: (A) 20 µm; (D) 2 µm. ctrl, control.

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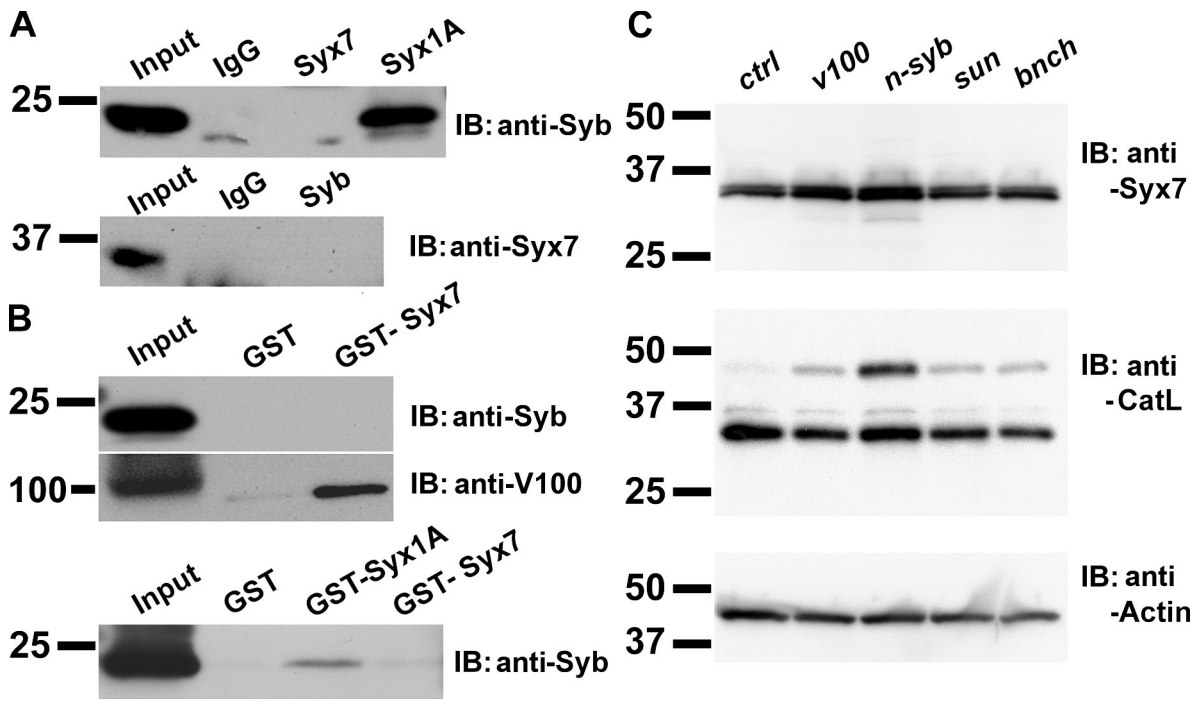

Figure S4. **n-Syb does not directly interact with the early endosomal Syntaxin Syx7, and loss of *n-syb* causes more severe pro-Cathepsin accumulations than lysosomal degradation mutants.** (A) Coimmunoprecipitations from fly head protein extracts. (top) Anti-Syb coimmunoprecipitates Syx1A but not Syx7; (bottom) conversely, anti-Syx7 does not coimmunoprecipitate Syb. (B) GST pull-downs with GST-tagged Syntaxins from fly head protein extracts. (top) GST-Syx7 pulls down V100 but not n-Syb. (bottom) GST-Syx1A, but not GST-Syx7, pulls down n-Syb. (C) Western blots from whole fly head protein extracts, probed with anti-Syx7, anti–Cathepsin L (CatL), and anti-Actin. Molecular markers are given in kilodaltons. IB, immunoblot; ctrl, control; bnch, benchwarmer.

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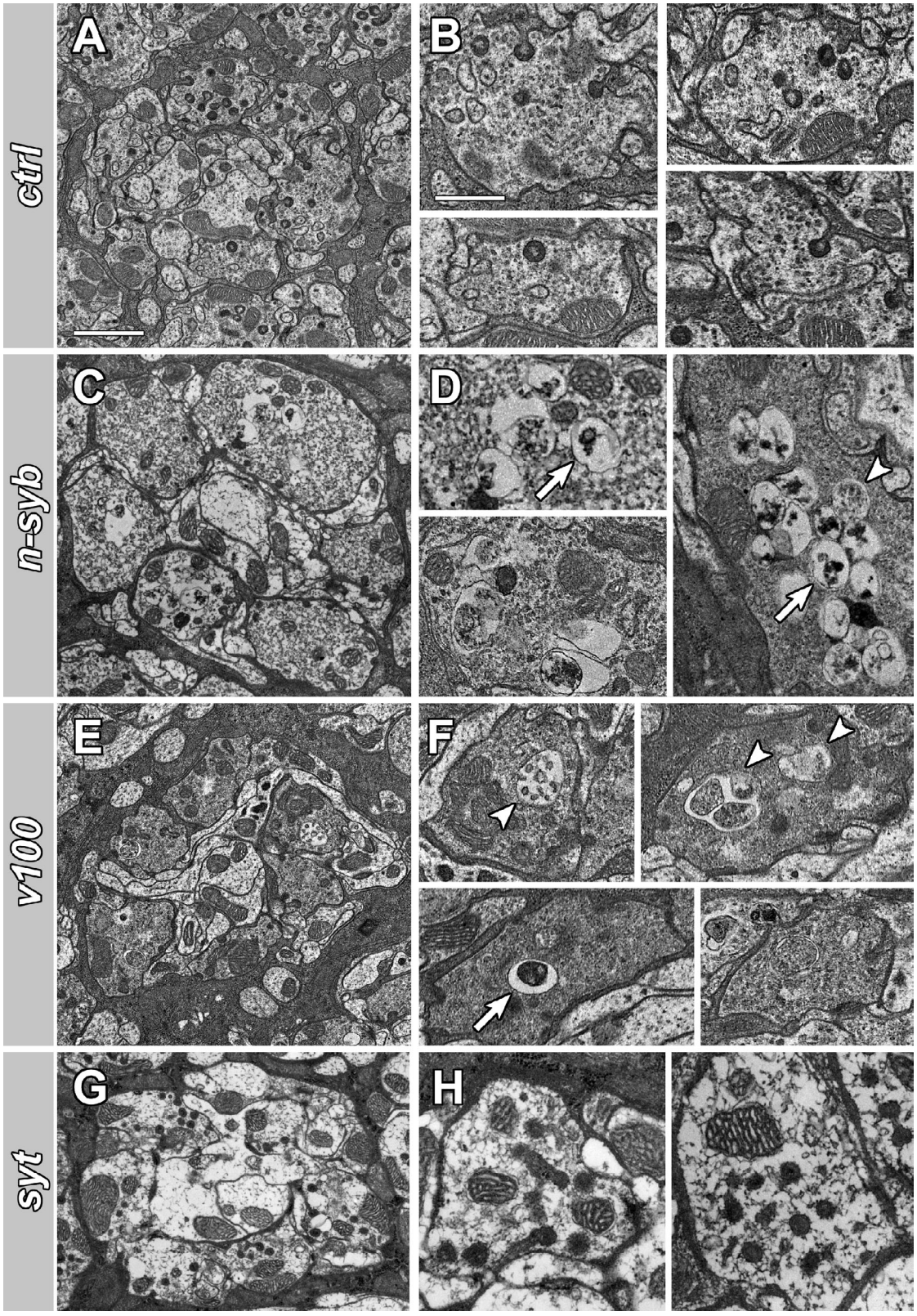

Figure S5. ***n-syb* mutant synaptic terminals contain many late autophagosomal compartments.** (A–H) Representative examples of EM micrographs of complete cartridges (A, C, E, and F) and individual synaptic terminals (B, D, F, and H) for control (A and B), *n-syb* (C and D), *v100* (E and F), and *syt* (G and H). Arrowheads show early autophagosome (AVIs), and arrows show late autophagosomes (AVDs). Bars: (A, also for C, E, and G) 1 µm; (B, also for D, F, and H) 500 nm. ctrl, control.