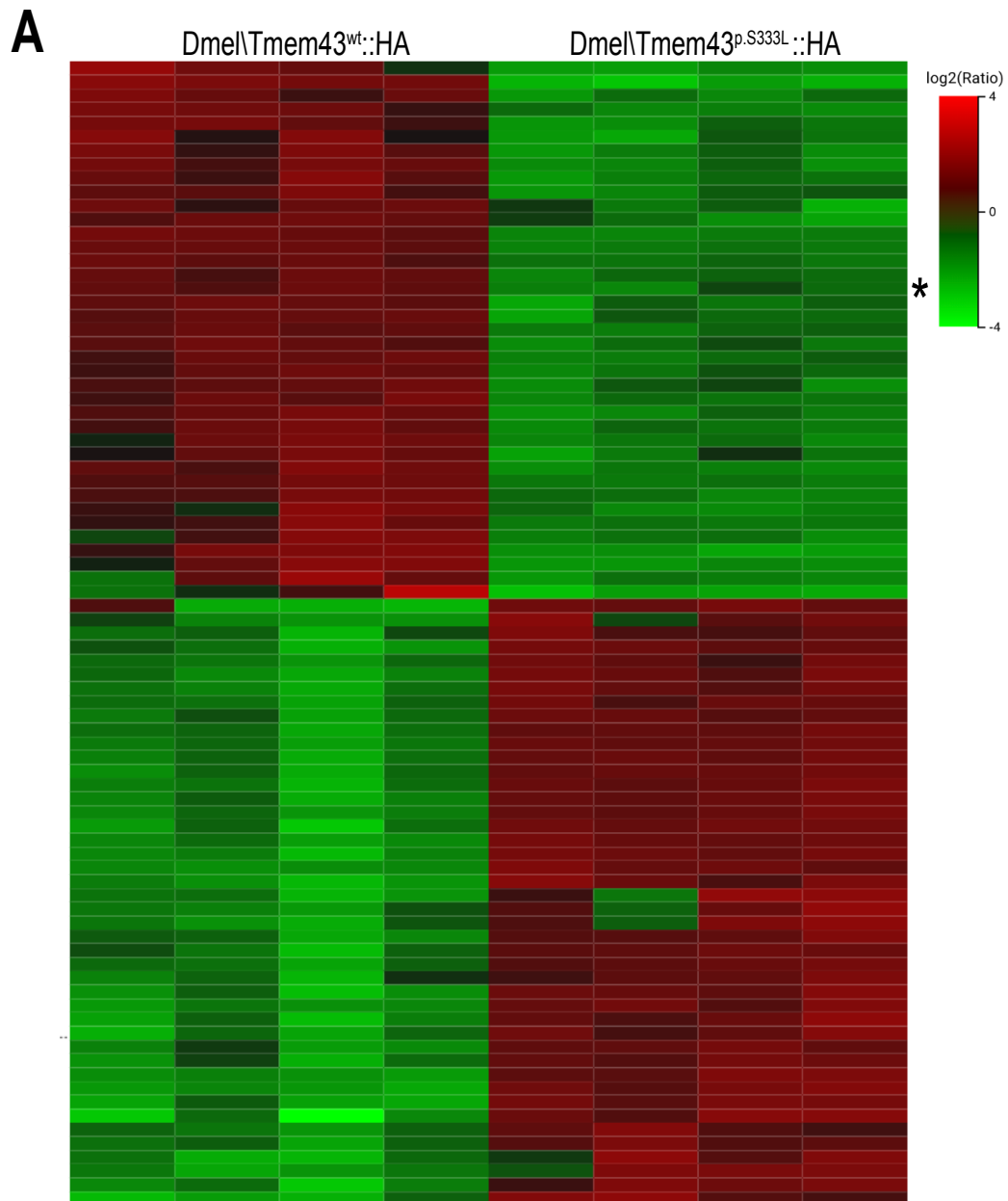
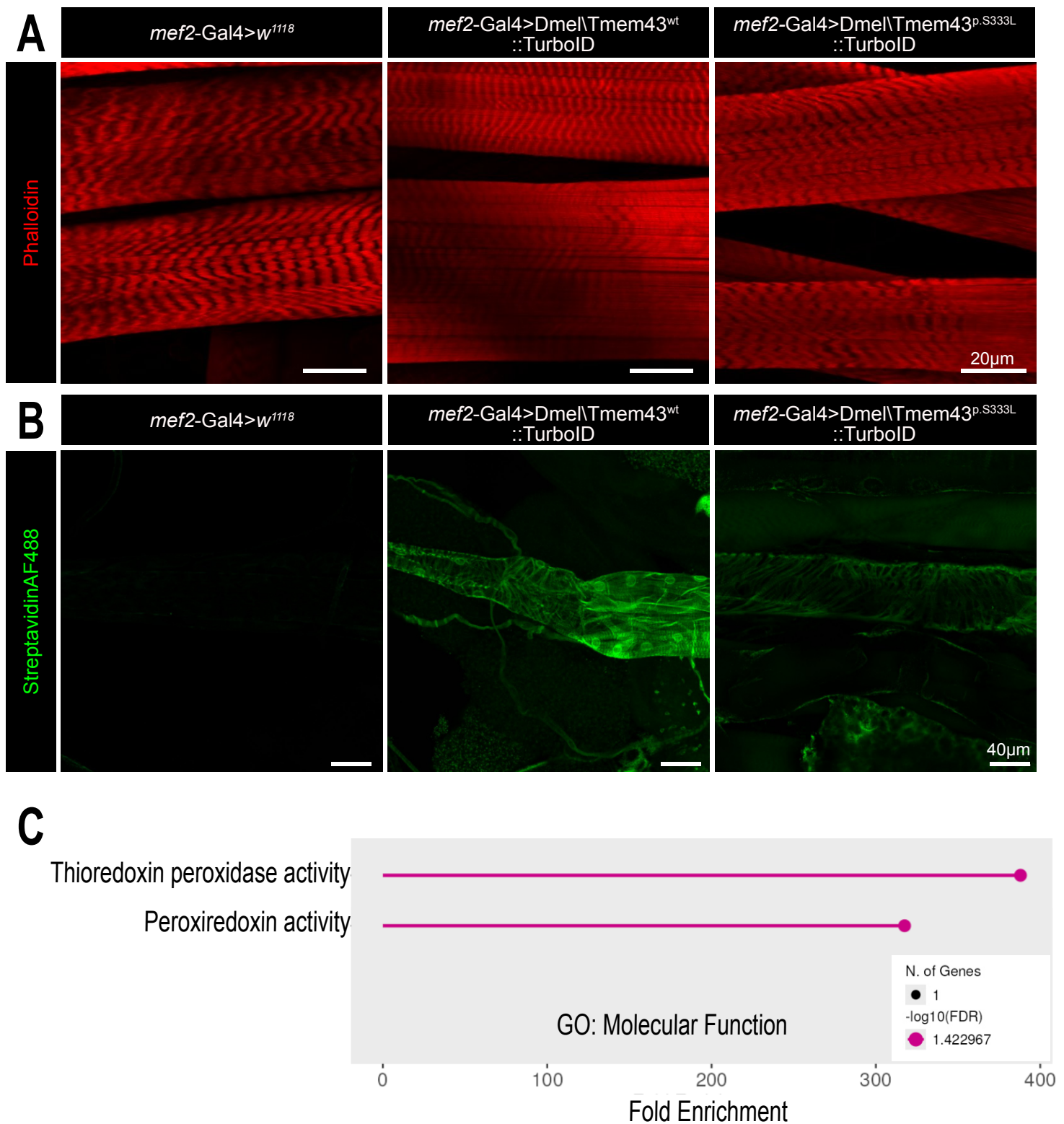
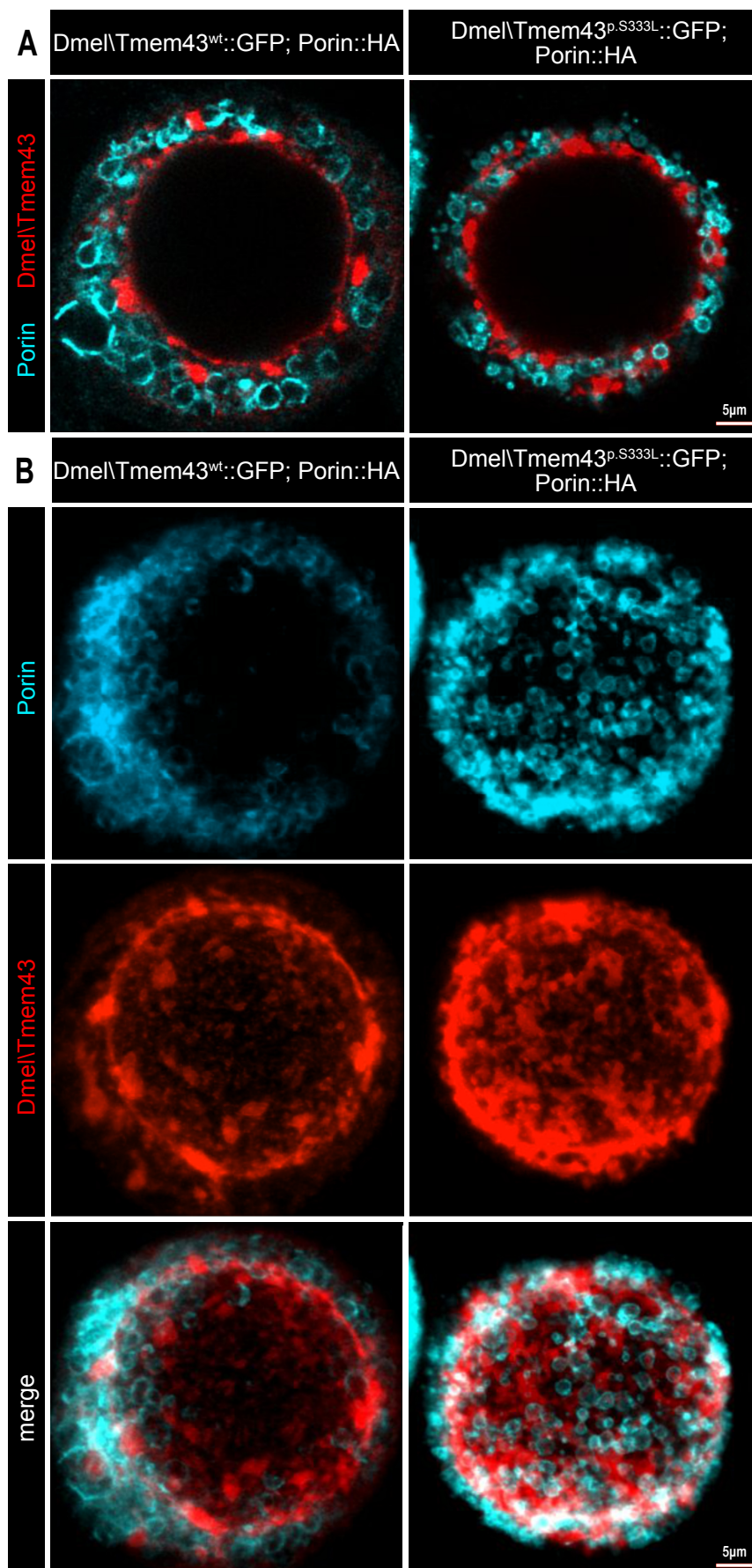




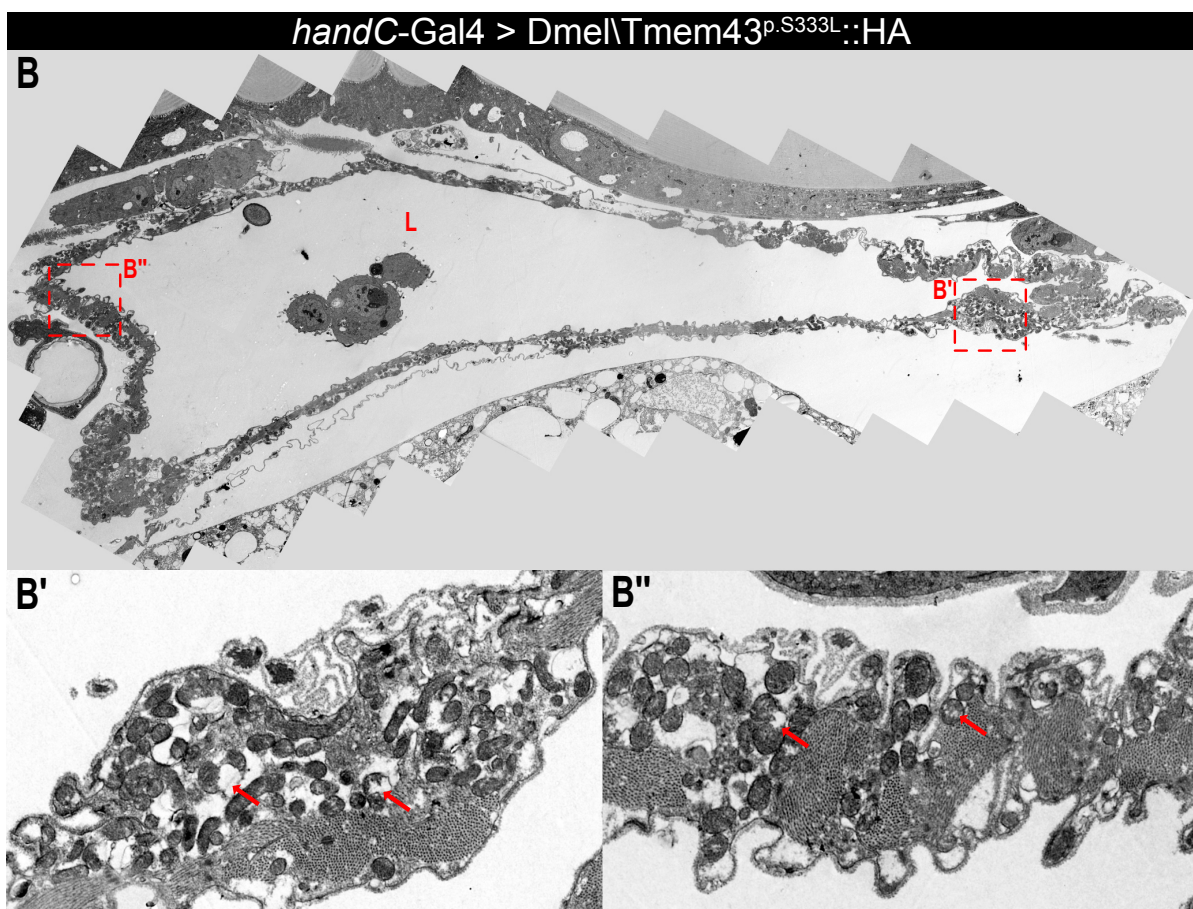
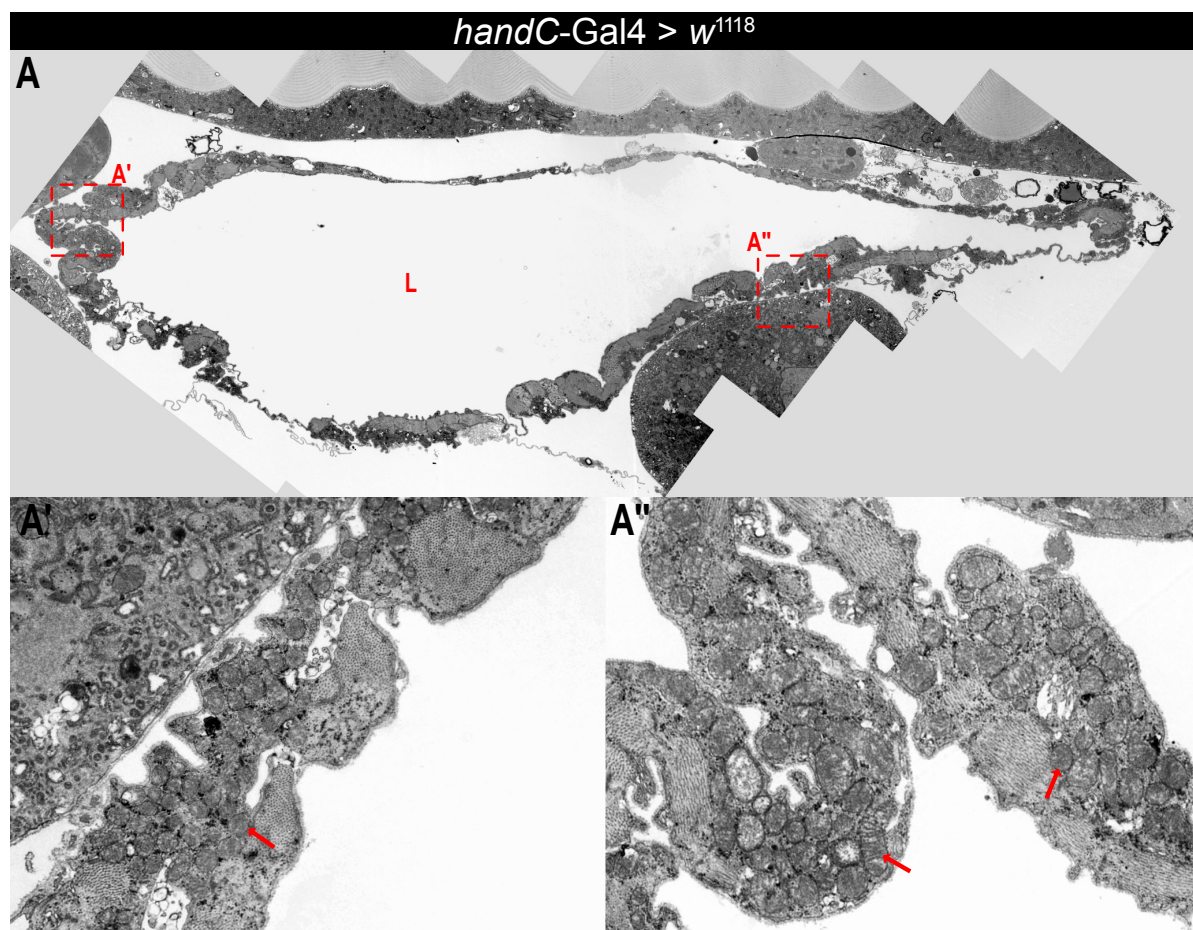
Supplementary Figure 1: Heatmap depicting the results of pull-down assays using Dme\Tmem43^{wt}::HA or Dme\Tmem43^{p.S333L}::HA as bait. Proteins were expressed in 3rd instar larval muscle tissue (*mef2*-Gal4). Porin significantly co-precipitates with Dme\Tmem43^{wt}::HA but not with Dme\Tmem43^{p.S333L}::HA (asterisk). Data are based on four individual biological replicates.



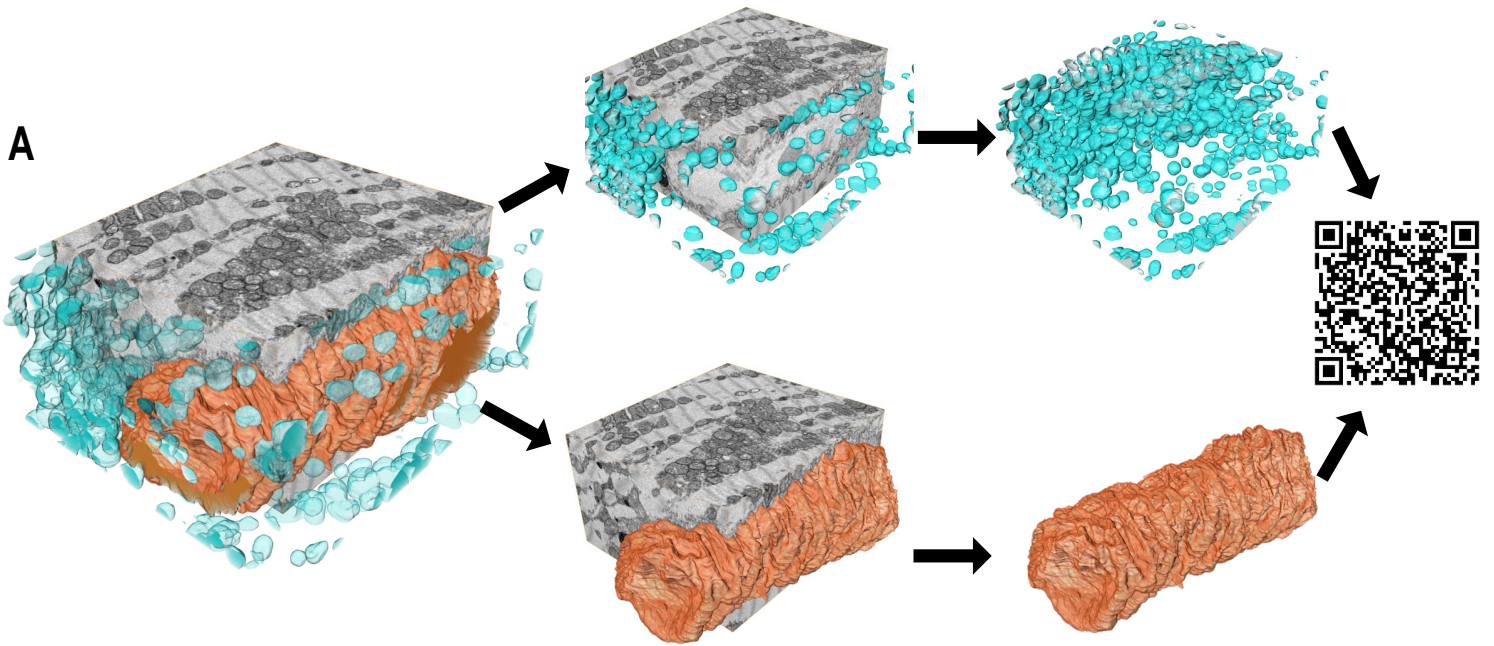
Supplementary Figure 2: (A) Phalloidin staining of 3rd instar larval body wall muscles expressing either wild-type Tmem43 (Dmel\Tmem43wt::TurboID) or mutated Tmem43 (Dmel\Tmem43p.S333L::TurboID). The driver line (*mef2-Gal4*) crossed to *w1118* served as a control. (B) Streptavidin AlexaFluor488 staining of 3rd instar larval hearts expressing either wild-type Tmem43 (Dmel\Tmem43wt::TurboID) or mutated Tmem43 (Dmel\Tmem43p.S333L::TurboID). The driver line (*mef2-Gal4*) crossed to *w1118* served as a control. (C) Gene Ontology (Molecular Function) analysis of the proteins enriched in Dmel\Tmem43p.S333L::TurboID compared to Dmel\Tmem43wt::TurboID.



Supplementary Figure 3: Representative images of *Sf21* cells co-expressing Dmel\Tmem43^{wt}::GFP or Dmel\Tmem43^{p.S333L}::GFP with Porin::HA. (A) Optical slices stained for Dmel\Tmem43^{wt}::GFP (red channel) and Porin::HA (cyan channel). (B) Average intensity projections of the same cells with individual and merged channels.



Supplementary Figure 4: (A) Representative transmission electron micrograph of a cross-sectioned heart from a 3rd instar larva expressing *w¹¹¹⁸* in a heart-specific manner (*handC-Gal4*). *A'* and *A''* show high-magnification images of cardiomyocytes with intact mitochondria (arrows). (B) Representative transmission electron micrograph of a cross-sectioned heart from a 3rd instar larva expressing *Dmel\Tmem43^{p.S333L::HA}* in a heart-specific manner (*handC-Gal4*). *B'* and *B''* show high-magnification images of cardiomyocytes with numerous damaged mitochondria (arrows).



Supplementary Figure 5: (A) The figure illustrates the segmentation of mitochondria and nuclei based on serial block-face SEM datasets. Mitochondria are coloured in blue, and the nucleus is coloured in orange. The QR code links to movies that depict the individual three-dimensional datasets.