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Supplemental information

Drosophila Mpv17 forms an ion channel and regulates energy metabolism

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Supplemental information

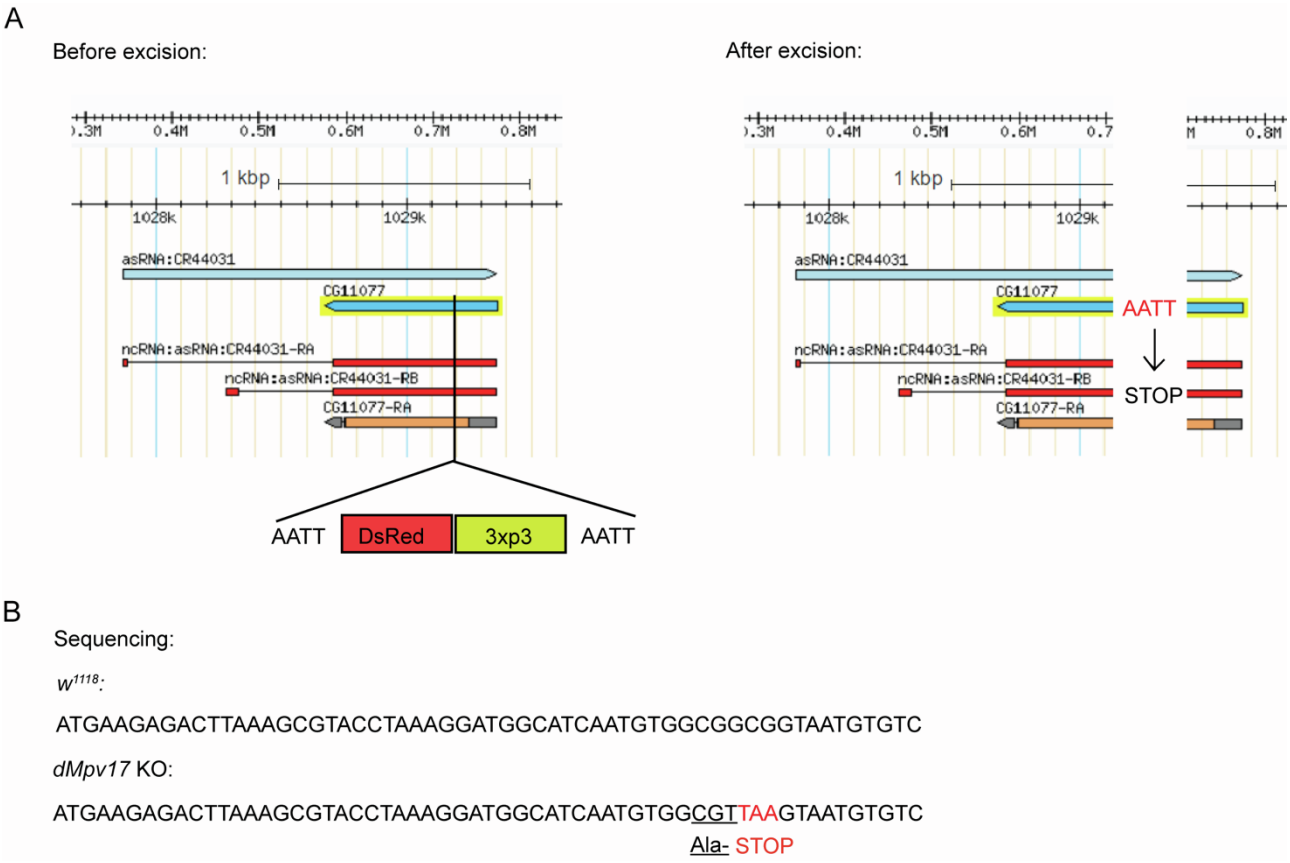

Figure S1. Strategy for Drosophila dMpv17 KO strain generation, Related to Figure 2.

A) Genome editing map before and after pBac excision. Organization of the *dMpv17* locus and position of the insertion (left). Mutation after excision of the cassette (right). DsRed: discosoma red fluorescent protein; 3xP3: artificial promoter containing 3 Mmus\Pax6 homodimer binding sites. B) Comparison between wildtype and mutated *dMpv17* sequence.

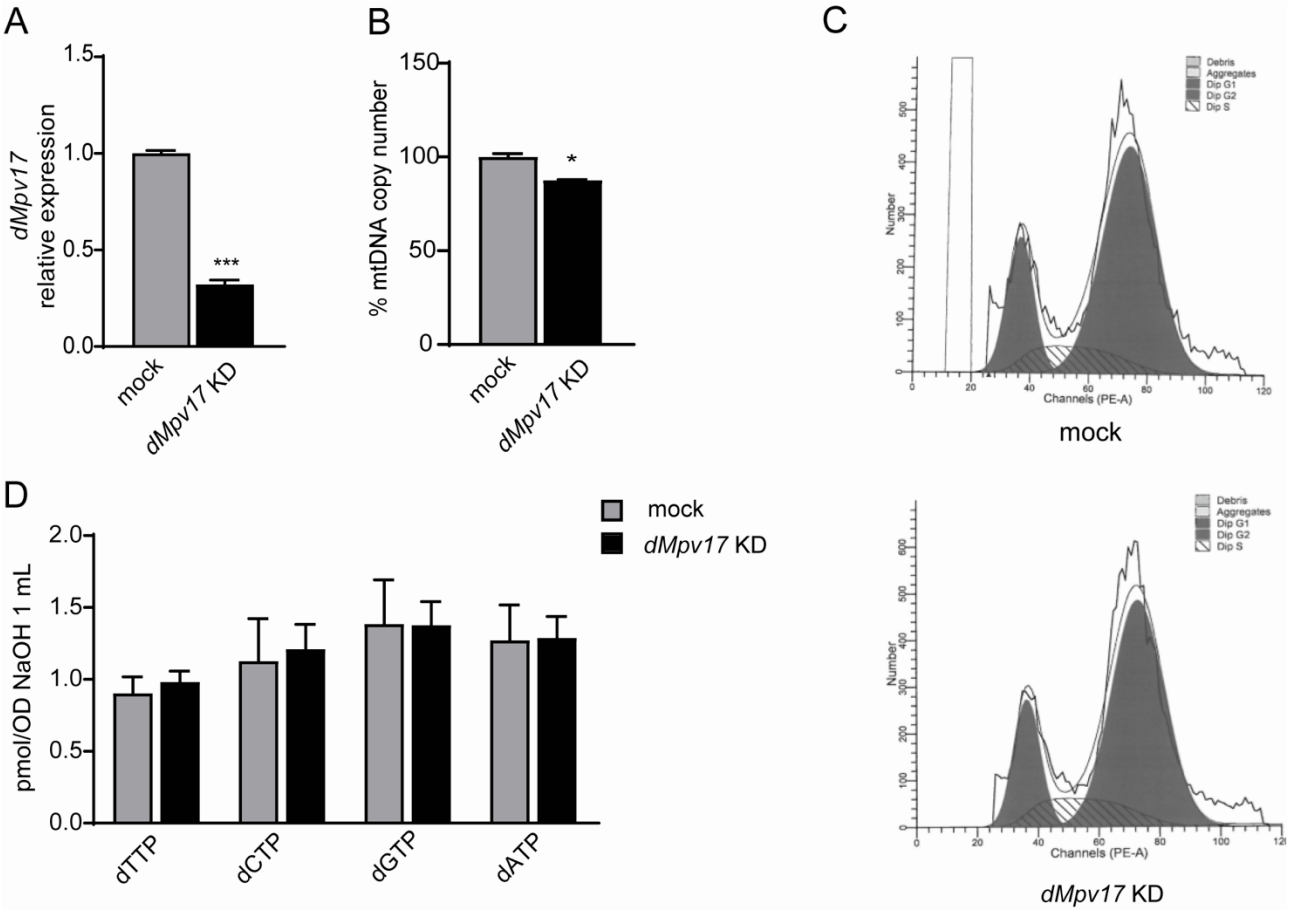

Figure S2. Characterization of *dMpv17* KD in S2R+ cells, Related to Figure 2.

A) Quantification of *dMpv17* mRNA. Error bars: SEM; ***p<0.001 on unpaired, two-tailed Student's t test. B) Quantification of mtDNA. Error bars: SEM; *p<0.05 on unpaired, two-tailed Student's t test. C) Results of FACS analysis showing no difference in the percentage of cells in S-phase in *dMpv17* KD vs mock control cells. D)Quantification of mitochondrial nucleotide pools. Pool sizes are expressed as picomoles of dNTP/OD NaOH 1ml (mean±SEM from four independent experiments).

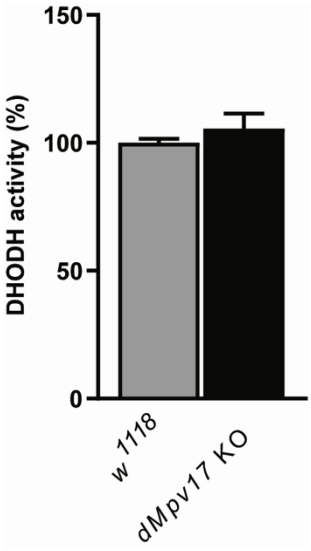

Figure S3. DHODH activity upon dMpv17 deletion, Related to Figure 2.

Percentage DHODH activity in mitocohondria isolated from whole fly homogenates in *dMpv17* KO and control flies (mean±SEM).

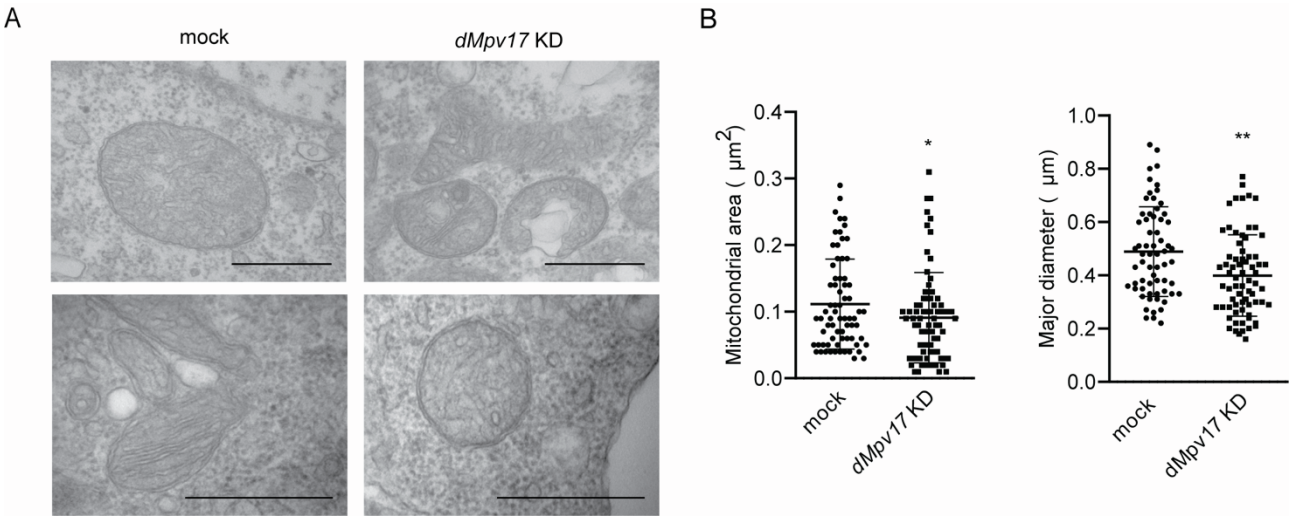

Figure S4. Electron microscopy analysis in *dMpv17* KD S2R+ cells, Related to Figure 3.

A) Representative TEM images of *dMpv17* KD and mock control S2R+ cells. Scale bar: 500 nm. B) Quantification of the mitochondrial area (mock:71, *dMpv17* KD: 76; error bars: ±SEM, *p<0.05 on on unpaired, two-tailed Student's t-test) and major diameter (error bars: ±SEM, **p<0.01 on unpaired, two-tailed Student's t-test).

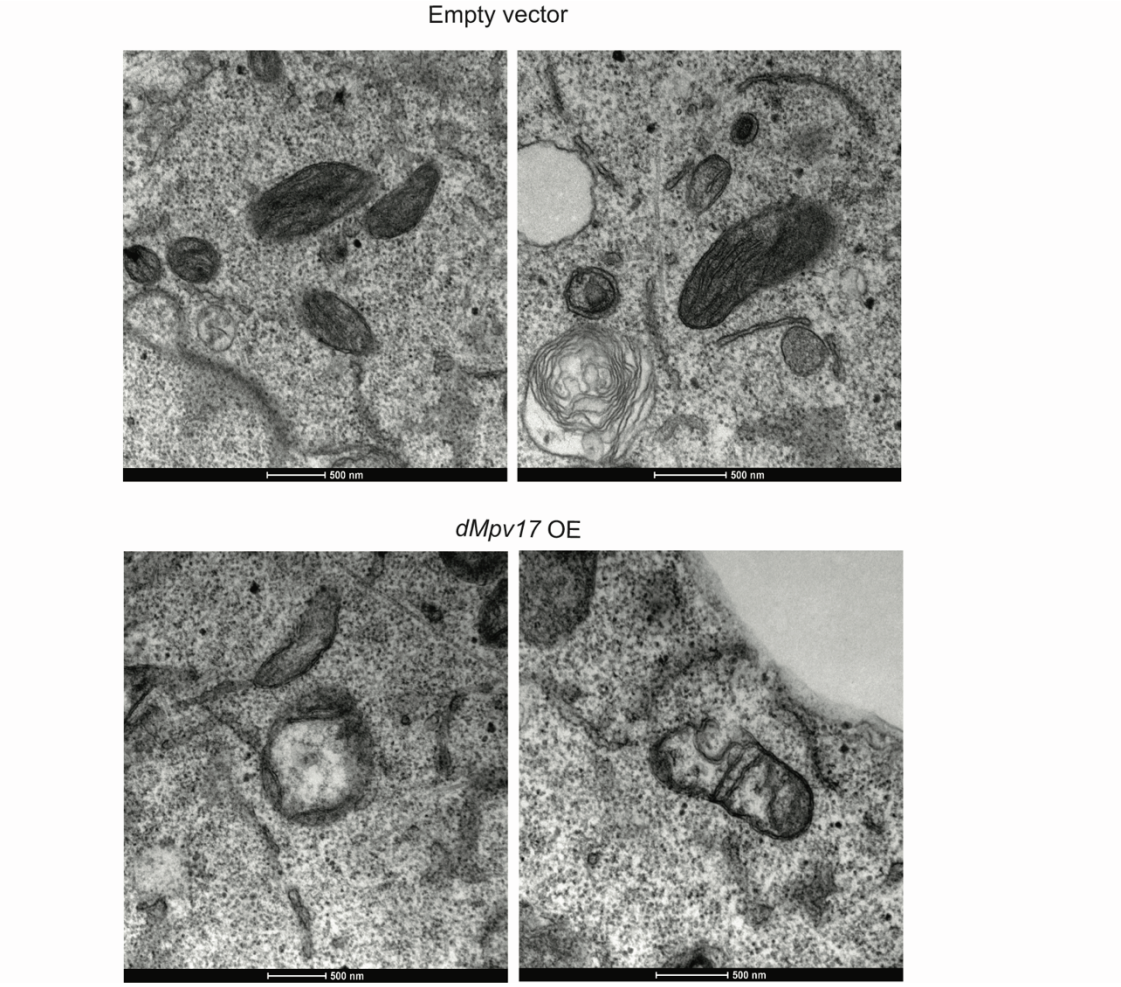

Figure S5. Mitochondrial ultrastructure, Related to Figure 3.

Representative TEM images of control (empty vector) and *dMpv17* over-expressing (*dMpv17* OE) S2R+ cells. *dMpv17* overexpression induced a severe alteration of mitochondrial morphology with organelle swelling and disappearance of cristae. Scale bar: 500 nm.

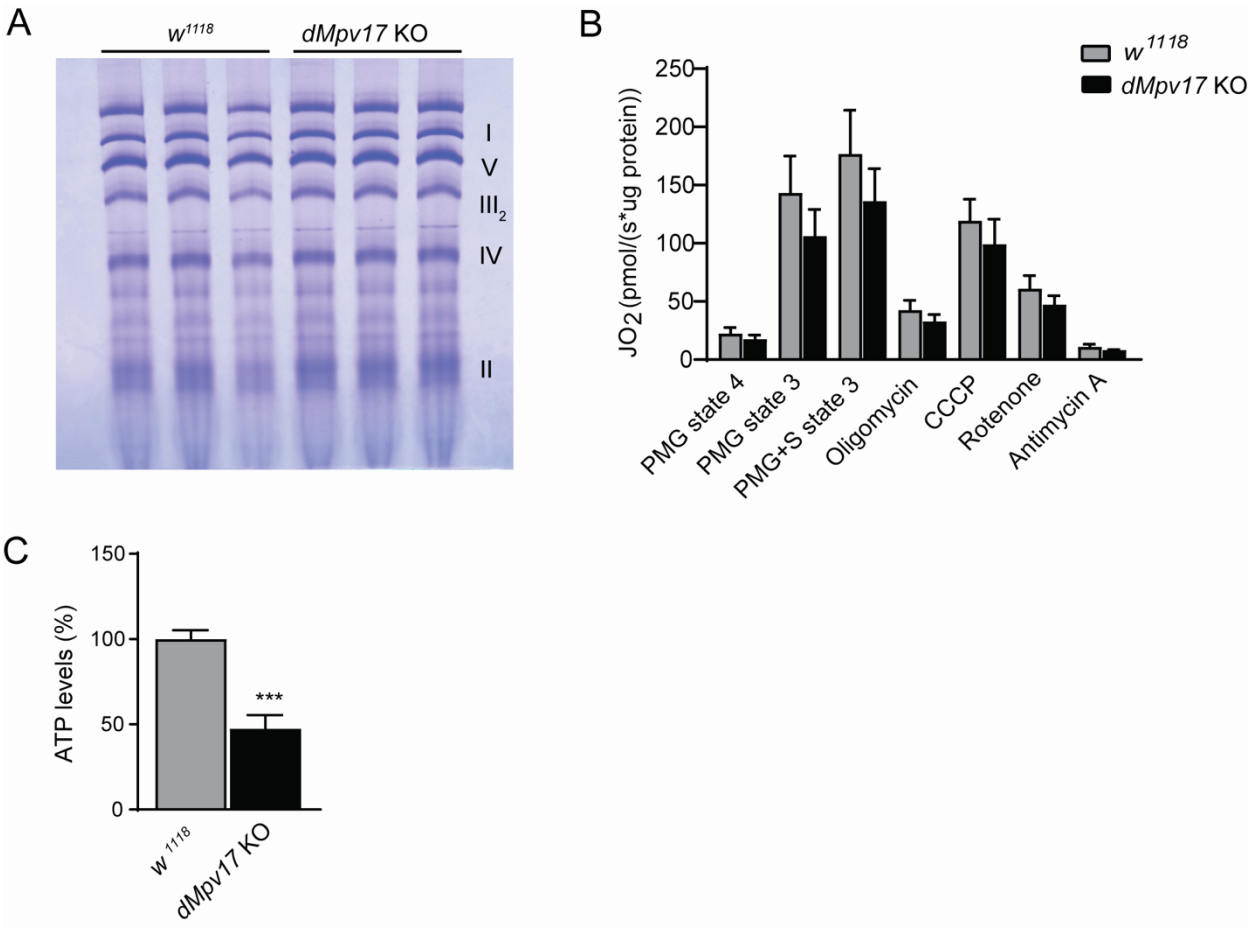

Figure S6. Bioenergetic analysis of *dMpv*17 KO flies, Related to Figure 4.

A) 1D-BNGE analysis of mitochondria isolated from whole flies. B) OCR (mean±SEM) measured in homogenates of *dMpv17* KO (dark grey) and control (light grey) flies; no significant differences were detected. State 3 respiration was stimulated by the addition of Proline, Malate and Glutamate (PMG) and PMG+succinate (PMG+S), in the presence ofADP. LEAK-respiration was measured through the inhibition of ATP synthase with oligomycin. Maximal respiration was measured adding the uncoupler CCCP. C) ATP content in 20 day-old flies. Error bars: SEM; **p<0.01 on unpaired, two-tailed Student's t-test.

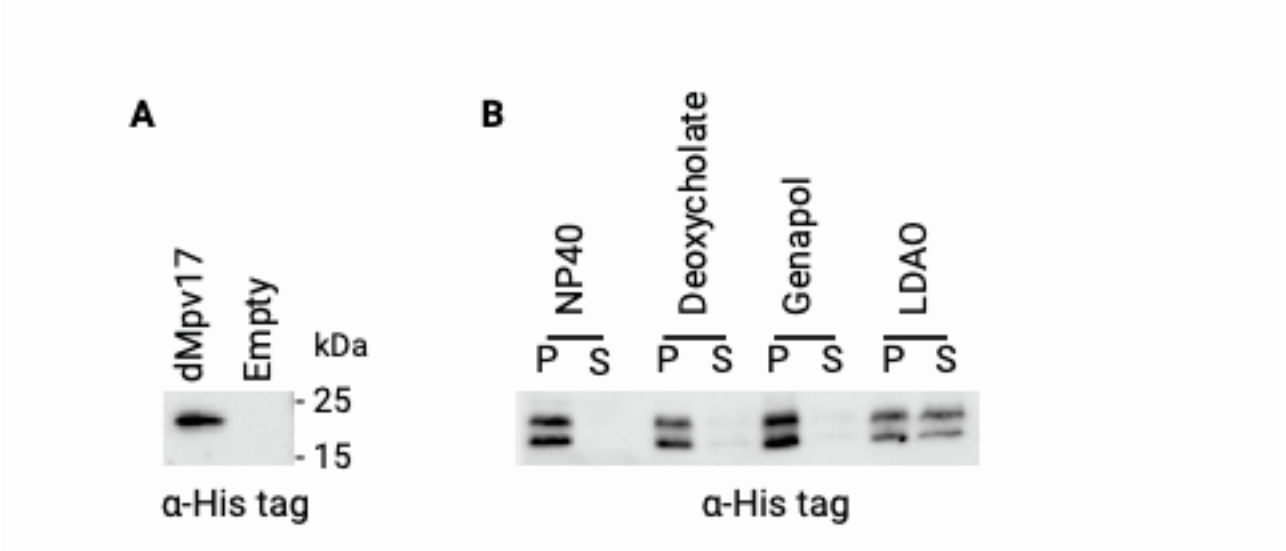

Figure S7 Analysis of *dMpv17-his* expression, Related to Figure 5.

A) Western blot immunovisualization of dMpv17-His with an antibody against the His tag; B) Solubilization analysis of dMpv17-His using different detergents. S: supernatant, P: pellet

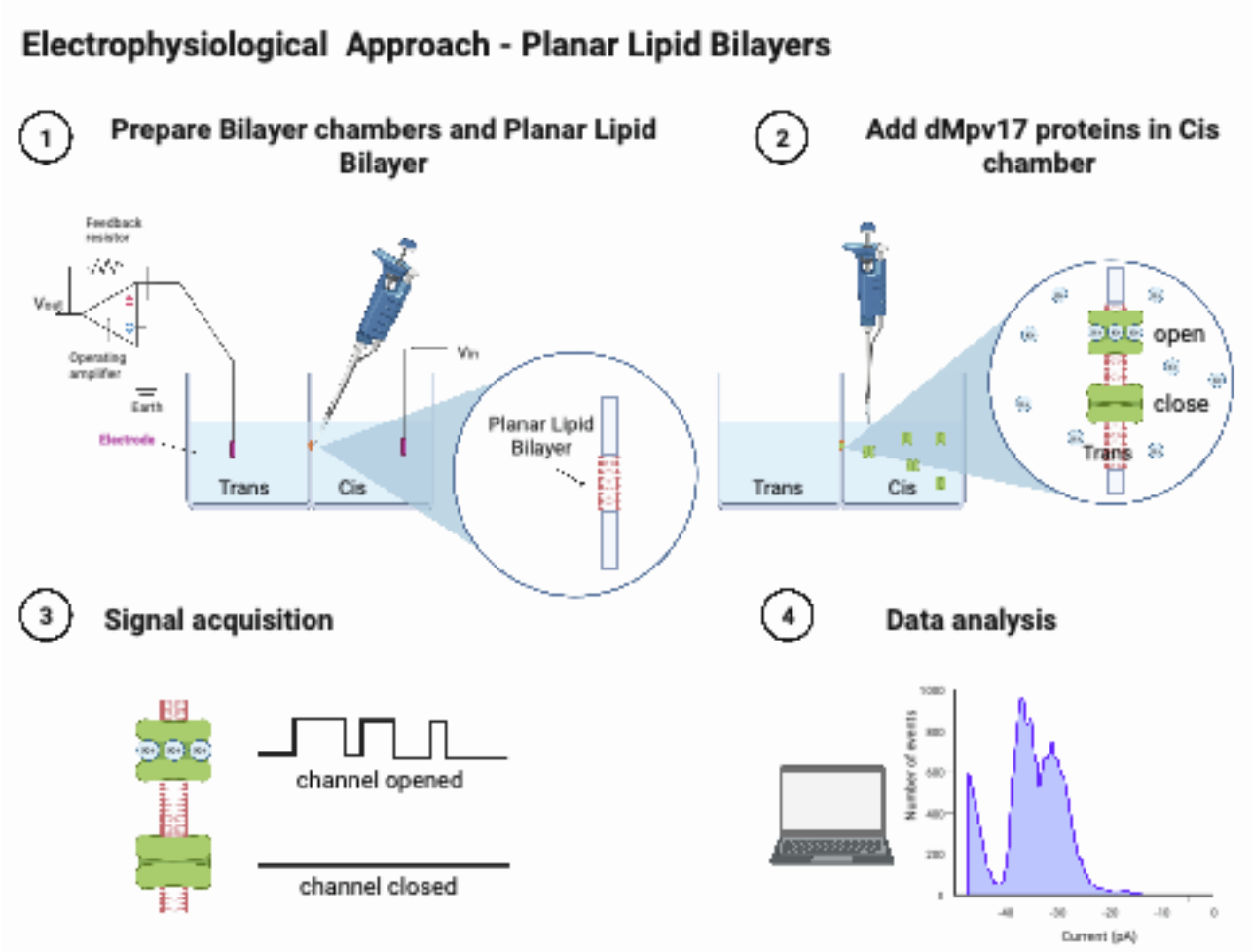

Figure S8. Schematic representation of the planar lipid bilayer technique, Related to Figure 5.

1) The bilayer chambers: lipid bilayers are painted on the hole in the middle of the cuvette. Silver-silver chloride electrodes are connected to each compartment (cis and trans) to obtain the ionic current that is amplified using a voltage-clamp amplifier; 2) Protein addition to the cis compartment, and representation of the incorporated ion channels; 3) The ion channel activity; 4) Data analyses.