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Supplemental Information

Structure of the Vacuolar H⁺-ATPase Rotary

Motor Reveals New Mechanistic Insights

Shaun Rawson, Clair Phillips, Markus Huss, Felix Tiburcy, Helmut Wiczorek, John Trinick, Michael A. Harrison, and Stephen P. Muench

Supplementary Movie 1 (related to Figure 1): cryo-EM analysis of the V-ATPase. The *M. Sexta* V-ATPase reconstruction with subunits fitted and labelled.

Supplementary Movie 2 (related to Figure 2): structural comparison between the *A. hiraе* A₁ and *M. Sexta* V₁ domain. The two structures are morphed starting from the *A. hiraе* structure and finishes with the *M. sexta* structure. Note the straightening of the central axle in the later structure.

Supplementary Figure 1 (related to Figure 2): analysis of the V-ATPase cryo-EM reconstructions from *M. sexta* and Yeast. Comparison between the *M. sexta* (grey surface, top row) and yeast (green surface, bottom row) V-ATPase reconstructions. Each catalytic AB domain is shown with the open site clearly seen between stators S1 and S2 in *M. sexta* and S2 and S3 in yeast. Figure S1 is related to Figure 2.

