SUPPLEMENTAL EXPERIMENTAL PROCEDURE

Limited Proteolysis of Atp8a2−Hypotonically lysed ROS were prepared as described (1). The ROS were resuspended at 4mg/ml in buffer (20mM Tris-HCl pH 8, 150mM NaCl, 0.5mM EDTA, 10% sucrose). Hypotonically lysed ROS or reconstituted Atp8a2 were then mixed 1:1 with trypsin in the same buffer (final trypsin concentration 4μg/ml) and incubated on ice for the indicated time. To stop digestion, soybean trypsin inhibitor was added (final concentration 1.67mg/ml) in buffer E and the samples were loaded onto an SDS-PAGE gel.

SUPPLEMENTAL TABLE

Table 1. **The concentrations of various antibodies used in this study for Western blot.**

Antibody	Dilution
Atp6C11	1:25
Atp2F6	1:10
Rho 1D4	1:500
Rim 3F4	1:25
PMc 1D1	1:25

SUPPLEMENTAL REFERENCE

1. Bungert, S., Molday, L. L., and Molday, R. S. (2001) *J Biol Chem* **276**(26), 23539-23546

ABBREVIATIONS

PS, phosphatidylserine; PE, phosphatidylethanolamine; PC, phosphatidylcholine; DOPS, 1,2-dioleoyl-sn-glycero-3-phosphoserine; C6 NBD-PS, 1-oleoyl-2-{6-[7-nitro-2-1,3-benzoxadiazol-4-yl)]hexanoyl}-sn-glycero-3-phosphoserine; C12 NBD-PS , 1-oleoyl-2-{12-[7-nitro-2-1,3-benzoxadiazol-4-yl)]dodecanoyl}-sn-glycero-3-phosphoserine; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid; ROS, rod outer segment; NEM, N-ethylmaleimide.

SUPPLEMENTAL FIGURES

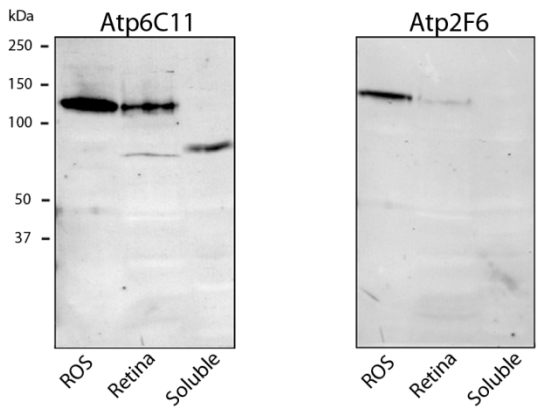

Fig. 1. **Western blot analysis of different sucrose gradient fractions from bovine retina.** Approximately, 30µg of protein from rod outer segments (ROS), retina membranes (Retina), and soluble retina proteins (Soluble) were labelled with **A.** Atp6C11 and **B.** Atp2F6. The Atp6C11 antibody non-specifically labels a 75 kDa soluble retina protein which is not present in ROS and retina membranes and is therefore this antibody is not suitable for immunocytochemical studies.

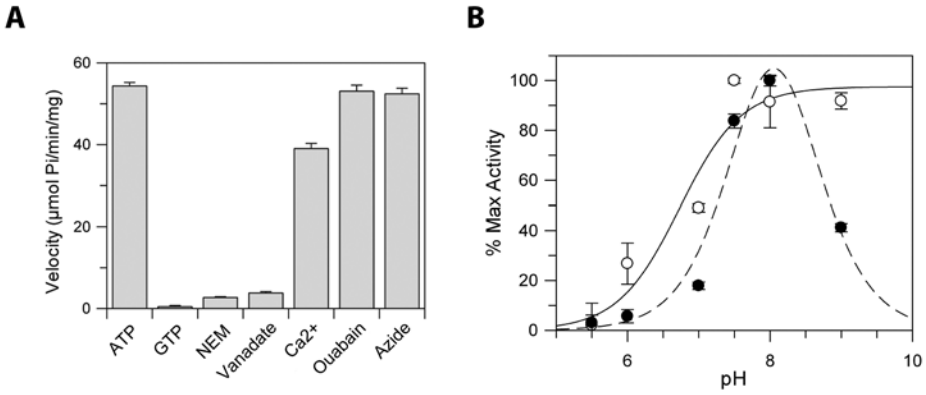

Fig. 2. **Effect of different nucleotides, inhibitors, and pH on Atp8a2 activity. A.** The ATPase inhibitors used were NEM, N-ethylmaleidemide at 1mM; Vanadate at 0.1mM; Ca^{2+}, calcium at 0.5mM; Ouabain at 0.2mM, and Azide at 1mM. Inhibitors were incubated with purified protein for 30min prior to assay. **B.** The pH dependence of ATPase activation in PS (open circles, solid line) and PE (closed circles, dotted line).

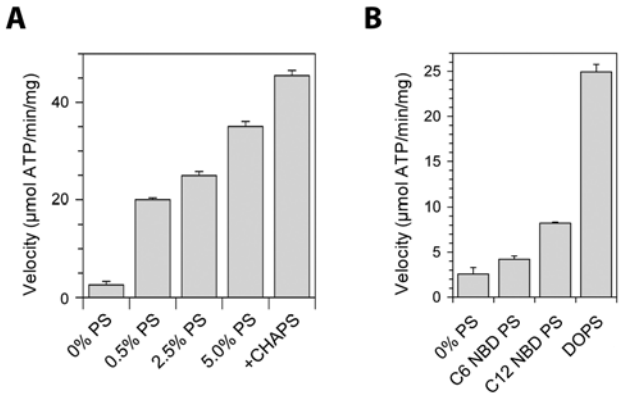

Fig. 3. **The effect of PS concentration on the ATPase activity of Atp8a2 reconstituted into liposomes. A.** Purified Atp8a2 was reconstituted into liposomes by detergent dialysis in the presence of increasing mole % of PS in the presence of PC. In one sample (+CHAPS), the reconstituted Atp8a2 was solubilized in CHAPS detergent (10 mM) for comparison with unsolubilized liposomes. The ATPase activity for reconstituted Atp8a2 in the presence and absence of CHAPS is similar. **B.** The ATPase activity of reconstituted Atp8a2 at 5 mM ATP prepared in the presence of zero PS (0% PS) and 2.5% C6-NBD-PS, C12-NBD-PS, and DOPS.

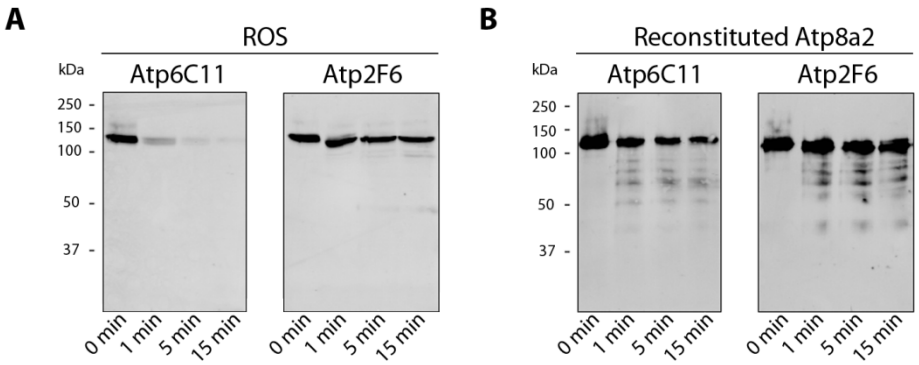

Fig. 4. **Orientation of Atp8a2 in PC liposomes as measured by trypsin accessibility.** Rod outer segments (ROS) and reconstituted liposomes containing Atp8a2 were digested with trypsin (final concentration of 4µg/ml) for the indicated times. **A.** ROS (30µg) and **B.** Reconstituted Atp8a2 (100ng) labelled with Atp6C11 and Atp2F6 antibodies. The Atp6C11 epitope was rapidly degraded in ROS, but not reconstituted vesicles indicating that as much as 70% of the epitope of Atp8a2 in reconstituted vesicles was oriented toward the inside of vesicles.