

## Supplementary Figures

### “Biochemical and Structural Characterization of the rod cGMP Phosphodiesterase 6”

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**Figure S1. Two-step purification of PrBP/ $\delta$ -PDE6.** All procedures were performed under dim red light illumination. A. Extraction of proteins from bovine ROS membranes. Soluble proteins were first removed with isotonic buffer. PDE6-PrBP/ $\delta$  was extracted by incubating ROS membranes at RT with PrBP/ $\delta$ -GST. W1, W2, W3 - washes of ROS membranes after incubation with PrBP/ $\delta$ -GST; Mem - final wash of ROS membranes; P- pooled fractions W1, W2, and W3. B. PDE6-PrBP/ $\delta$ -GST purification on GSTrap column; fractions W1, W2, and W3 were loaded onto a GSTrap column and eluted with 50 mM Tris-HCl, pH 8.0, buffer containing 10 mM glutathione and 1 mM DTT; FT - flow through; W - last wash of proteins loaded onto the GSTrap column. C. Gel filtration of pooled concentrated GSTrap fractions digested overnight with thrombin before injection.

**Figure S2. Data quality and completeness.** According to the 50% criterion, the Fourier shell correlation (FSC) function documents a resolution of 18 Å, with additional information above the theoretical three-sigma level out to 13 Å. We bandwidth limited the data to 13 Å as inclusion of the data out to 13 Å results in a better map, but choose to use the more conservative 50% criterion for the FSC as an estimate of the resolution.

**Figure S3. Comparison of PDE6 and PDE2A structure.** A. 18 Å map of PDE6 represented as a molecular surface. B. Structure of PDE2A represented as surface for one monomer and cartoon for other monomer. C. Superposition of PDE map with PDE2A structure. PDE2a is much longer and thinner than the map for PDE6.

**Figure S4. Location of PrBP/ $\delta$  and PDE6 $\gamma$  binding.** Close examination of micrographs of PDE6-PrBP/ $\delta$  reveals the location of PrBP/ $\delta$  binding on PDE6 (Figure 2 B and C). PrBP/ $\delta$  (denoted in cyan) from the Arl-2/ PrBP/ $\delta$  complex (PDB ID: 1KSH) structure was placed in the approximate location of binding. Unmodeled density present after the fitting of our homology model (denoted in red) represents the likely location of the central portion of PDE6 $\gamma$ .