Supplementary Figure 1. A. Full gel images of a dephosphorylation assay of CaBP4 by retinal phosphatase, purified PP2A or recombinant PP1 and its dose-dependent inhibition by fostriecin as shown in main figure 1E. Recombinant CaBP4 was first phosphorylated with recombinant PKCζ in the presence of [γ-32P] ATP. After inactivation of PKCζ, radioactively labeled CaBP4 was incubated with mouse retina extract, rPP1 or purified PP2A without or with fostriecin as indicated and the incubation was carried on for 45 min at 30°C. The reactions were loaded on SDS-PAGE gels followed by transfer onto immobilon membrane. In the autoradiograph, the lower major band contains cleaved CaBP4 (~37 kDa) coming from the cleavage of the GST tag. Thus for clarity, we choose to display only the GST-tagged CaBP4 band in the main figures. B. Full gel images of a dephosphorylation assay of CaBP4 by retinal phosphatase in the presence or absence of Ca2+ as shown in main figure 1B. CaBP4 dephosphorylation was analyzed in retinal extracts as described in A without or with OA (5 μM), 5 mM CaCl2, 5 mM EGTA or 5 mM EDTA as indicated.

Supplementary Figure 2. A and B. Dephosphorylation of CaBP4 by purified PP2A (A) or by recombinant PP1 (B) and its inhibition by OA. 32P –labeled CaBP4 was added to 0.1 U of purified PP2A or 0.1U of recombinant PP1 with or without OA as indicated and the incubation was carried on for 45 min at 30°C. Right lane: Reaction stopped with SDS-PAGE sample buffer at T=0 min. Phosphorylation level was analyzed by autoradiography (upper panel) and equivalent input and transfer of CaBP4 in all reactions was confirmed by Ponceau staining (lower panel). The arrow indicates the position of recombinant CaBP4. C. Purification of active recombinant NIPP-1 and protein phosphatase inhibitor 2 expressed in E. coli. Coomassie staining of various known amounts of BSA and various amounts of purified NIPP-1 and
inhibitor-2 (see Methods). Inhibitor concentration was determined by quantification of the corresponding band relative to bovine serum albumin D. Dephosphorylation of CaBP4 by recombinant PPI is inhibited by recombinant NIPP-1 or inhibitor 2. $^{32}$P–labeled CaBP4 was added to 0.1U of recombinant PPI without or with indicated concentrations of recombinant NIPP-1 and inhibitor 2. The incubation was carried on for 45 min at 30°C and the reactions loaded on SDS-PAGE.
Supplementary Figure 1

A

CaBP4

Retina

Purified PP2A

Autoradiograph                  Ponceau

Recombinant PP1

Fostriecin (µM) 0 0.1 1 3 10 30 0 0.1 1 3 10 30

CaBP4

100 75 50 37 25

KDa

Calcium buffer

CaBP4

B

CaBP4

 Autoradiograph                  Ponceau

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Supplementary Figure 2

A

Purified PP2A + OA (nM) 0 10 100 1000 5000 T=0

Autoradiograph

Ponceau

B

Recombinant PP1 + OA (nM) 0 10 100 1000 5000 T=0

Autoradiograph

Ponceau

C

Recombinant PP1 + Inhibitor 2 (nM) 0 10 100 1000

Autoradiograph

Ponceau

D

Recombinant PP1 + NIPP-1 (nM) 0 10 100 1000

Autoradiograph

Ponceau