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Supporting Information

Ruggiero et al. 10.1073/pnas.1121101109

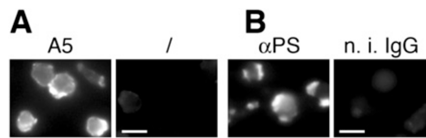

Fig. S1. Detection of surface phosphatidylserine (PS) on purified photoreceptor outer segments (POS) fragments by fluorescence microscopy. (*A*) Fluorescence of unfixed POS particles after labeling for 30 min at 37 °C with FITC-annexin 5 (A5) or with A5 binding buffer alone (/). (*B*) Indirect immunofluorescence labeling of paraformaldehyde-fixed POS particles with αPS or nonimmune IgG (n. i. IgG) as indicated. (Scale bar, 2 μm.) Representative fields are shown of one of three repeated experiments with identical results.

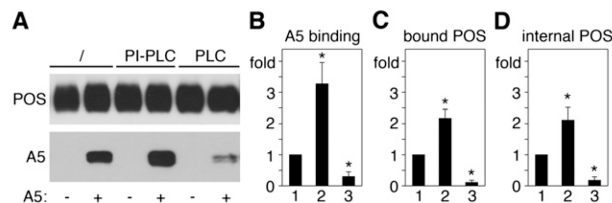

Fig. S2. Altered binding and engulfment of isolated POS fragments with increased or decreased PS exposure. We reasoned that reducing POS particle surface phosphatidylinositol and glycosyl-phosphatidylinositol—anchored proteins by incubating particles with phosphatidylinositol-specific phospholipase C (PI-PLC) may increase accessibility of external leaflet PS. Moreover, incubation with nonspecific PLC may remove external leaflet PS. We therefore directly tested PS exposure after treatment of purified POS fragments for 15 min at 37 °C with 0.1 U/mL purified PI-PLC in PBS or with 0.01 U/mL PLC in PBS (both Sigma), followed by five washes in PBS. Control particles were processed identically but incubated with PBS alone. We then further incubated a fraction of each POS preparation with A5 in A5 binding buffer at 37 °C for 15 min followed by three washes in PBS. (*A*) Immunoblotting analysis of opsin indicative of POS load (*Upper*) and of bound A5 indicative of PS exposure (*Lower*) in POS samples that were untreated (/), PI-PLC treated (PI-PLC), or PLC treated (PLC) and had (lanes A5, +) or had not (lanes A5, -) been incubated with A5. (*B*) Quantification of A5 binding of particles that were untreated (bars 1), PI-PLC-treated (bars 2), or PLC-treated (bars 3). (*C* and *D*) Comparison of RPE-J cell POS particle binding (*C*) and internalization (*D*) after 2.5-h challenge with particles as described in *B*. Bars represent mean ± SD of three independent experiments with duplicate samples and show relative binding and internalization of modified POS particles relative to binding and internalization of untreated particles, which is set as 1 in each panel. *Significant difference from untreated POS particles ($P < 0.05$ by Student *t* test).

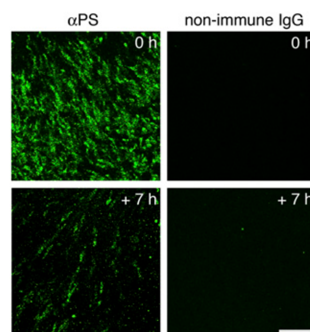

Fig. S3. Increased binding of αPS to WT POS at light onset (0 h) compared with 7 h later. Images show representative maximal projections obtained of immunofluorescence labeling of WT mouse retina with αPS or nonimmune IgG as indicated. (Scale bar, 20 μm.)

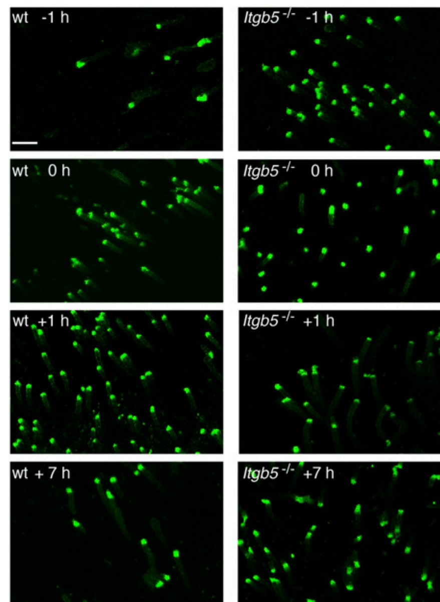

Fig. S4. Diurnal variation in frequency of PS exposing POS tips in WT but not *Itgb5*$^{-/-}$ retina. Fields show maximal projections of retinas from mice at time points as indicated. Representative images are shown of three retinas for each sample type analyzed independently. (Scale bar, 10 μm.)

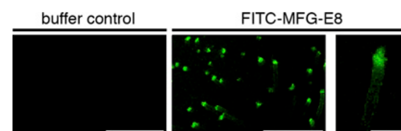

Fig. S5. Specific binding of MFG-E8 to photoreceptor tips. WT mouse retinas were dissected at light onset, incubated for 20 min at 37 °C with 5 μg/mL purified bovine FITC-tagged MFG-E8 (Haematologic Technologies) in PBS, washed twice in PBS, and imaged live. *Center and Right*: Overview of representative area and close-up of individual, labeled photoreceptor, respectively. *Left*: Representative overview of control retina that was incubated with buffer alone. Results shown are representatives of three independent experiments performed. (Scale bars, *Left* and *Center*, 25 μm; *Right*, 5 μm.)