Supplemental Material

Figure S1. Trypsin protection assay of transducin preparations

The sensitivity of αt-GTPγS (lane 2), αt-GDP, (lane 4) and αt-GDP-AlF_4^- (lane 5) to trypsin was examined. In each trypsin digestion reaction, 2 μg of αt was incubated with 0.01 μg of trypsin (Promega) in the buffer containing 20 mM HEPES, pH 7.5, 120 mM NaCl, and 2 mM $MgCl_2$ for 30 min at room temperature. The digestion was terminated by addition of the SDS/DTT containing sample buffer followed by immediate boiling for 5 min. The proteins were resolved on a 15% SDS gel. As shown in the figure, relative to αt-GDP, both αt-GTPγS and αt-GDP-AlF_4^- were resistant to trypsin digestion, indicating conformations of activated αt.

Figure S2. Characterization of the BBM-PDEγ photoprobes

A. Schematic of preparation of the BBM-PDEγ derivatives. BBM is linked to PDEγ at a given position by an S-S bond that is formed through a reaction between the methanethiosulfonate (MTS) group of BBM and the single cysteine on PDEγ. Upon exposure of the mix of BBM-PDEγ and its target(s) to UV light, the ketone in benzophenone is activated into a diradical and reacts with neighboring C-H bonds to form a C-C link with the PDEγ-interacting target protein(s). Following DTT reversal of the S-S bond, PDEγ is released and the biotin label is transferred to the target(s). For more detailed description of the label transfer strategy, please refer to Figure S1 in our previous report (13).

B. Functional activities of BBM-PDEγ derivatives were determined by analyzing their ability to stimulate αt GTPase, as described under Experimental Procedures. The assay was kindly conducted by Dr. Kirill Martemyanov. Data are presented as average ±S.D. (error bar is not seen when it is too small). The dotted line indicates the αt GTP hydrolysis rate with the unmodified wild type PDEγ, which is $0.07632 \pm 0.0027\ S^{-1}$.

C. The BBM-PDEγ photoprobes that were not used for GTPase stimulation assays (see Figure S1B) were determined for their ability to form a complex with αt-GDP-AlF_4^- using the native gel assay. αt-GDP-AlF_4^- (10 μM) was incubated with various BBM-PDEγ derivatives at the indicated molar ratios on ice for 30 min, and then loaded to an 8% native gel. Up-shifted Coomassie-stained bands indicate the complexes formed by GDP-AlF_4^- and BBM-PDEγ derivatives.

Figure S1

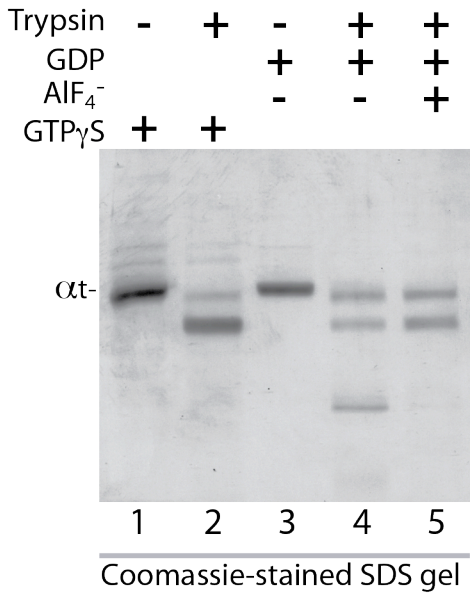
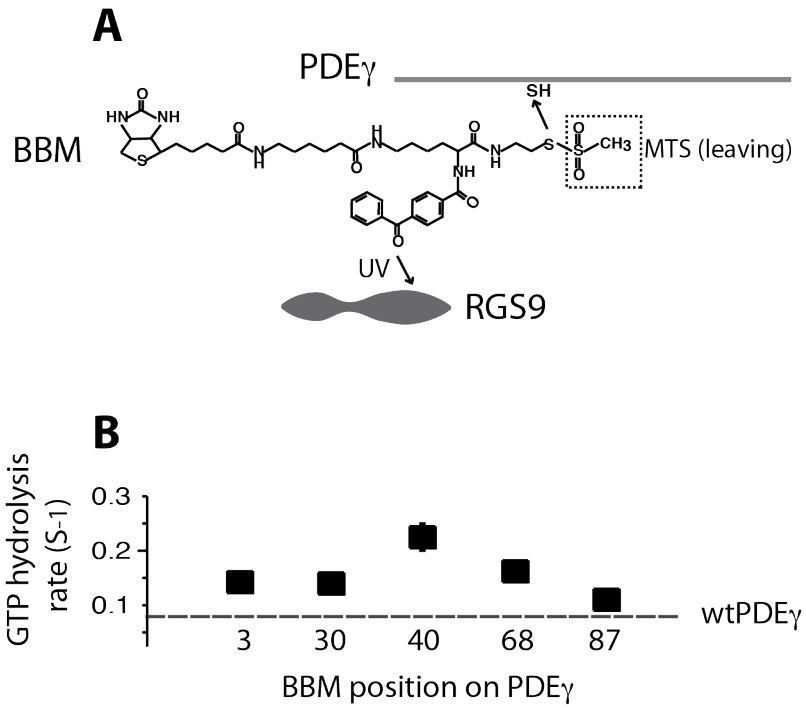


Figure S2


A
PDEγ
BBM
SH
MTS (leaving)
UV
RGS9
B
GTP hydrolysis rate (S-1)
0.3
0.2
0.1
wtPDEγ
3
30
40
68
87
BBM position on PDEγ
C
BBM position
wt
16
38
50
60
73
76
PDEγ/αt
0 1 2 1 2 1 2 1 2 1 2 1 2 1 2
αt:PDEγ–
αt–