ONLINE METHODS

Animals. Mice were maintained in a 12:12 h dark-light cycle. The animals' care was approved and maintained in accordance with institutional guidelines (IACUC) and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. To dark-adapt, the mice were placed in the dark for 12 hours or more. For light-adaptation, the pupils of the mice were dilated with 1% Tropicamine and the mice were illuminated by 2,000 lux for 25 minutes. Following light-adaptation, mice were returned to the dark for 1.5, 3, 6 or 24 hours. *Unc119*$^{-/-}$ mice were from the colony of GI [12]. *Gnat1*$^{-/-}$ animals were obtained from Dr. Janis Lem.

***C. elegans* Strains.** Strains were maintained using standard methods. Strains were obtained from Caenorhabditis Genetics Center, Morris Maduro, and Piali Sengupta. Stably integrated strains used in this work were *oyIs[P(odr-10)::gfp] V* for examining AWA neurons, *lin-15(n765ts) X kyIs104[str-1::GFP, lin-15(+)] X* for examining AWB neurons[39], and *kyIs140[str-2::GFP + lin-15(+)]*for examining AWC neurons[39]. The AWA, AWB, and AWC neurons expressing the above stably integrated GFP markers were also examined in *unc-119(ed3)* mutants. Double mutant strains were generated using standard methods.

Crystallization of UNC119. Expression and purification of truncated human UNC119 protein (residues 56-240) was carried out as a part of the established high throughput protein production pipeline[40] (Northeast Structural Genomics Consortium (NESG) target HR3066a). The protein was cloned into the pET 14-15C expression vector (Novagen). Selenomethionyl protein was expressed in *Escherichia coli* BL21(DE3) + Magic, purified using Ni-NTA affinity chromatography (Qiagen) and gel filtration (Superdex 75, Amersham/GE Healthcare) in buffer containing 10 mM TrisHCl , 100 mM NaCl, 5 mM DTT, pH 7.5. Protein homogeneity was verified by SDS-PAGE and MALDI-TOF mass spectrometry.

Preliminary crystallization trials were performed using the microbatch crystallization under paraffin oil at 4°C[41]. UNC119 crystals useful for structure determination were grown in a 1:1 ratio with solution containing 40% PEG 4000, 0.1M K acetate, 0.1M Na acetate, pH 5.0 at 4°C. The crystals grew to 0.025 – 0.05 – 0.1 mm after three - four weeks and were transferred to paratone oil and frozen in liquid propane. Single crystals were maintained at 100K and used to collect a SAD data set at beamline X4A at the National Synchrotron Light Source (NSLS) using an ADSC Quantum-4 CCD detector. Data were integrated and scaled using the HKL2000 package[42].

SHELXE/D [43] was used to locate selenium sites and calculate initial phases. Solvent flattening and partial model building were performed using RESOLVE [44]. The remainder of the model was built manually using COOT[45] and was refined with PHENIX [46]. Data collection and refinement statistics are presented in Table S1. The quality of the model was checked using MolProbity[47]. The atomic coordinates and structure factors for UNC119 (PDB ID 3GQQ) have been deposited in the Protein Data Bank.

UNC119/ lauroyl-GAGASAEEKH crystal growth and data collection. UNC119 (Table S1) was co-crystallized with a Tα peptide (lauroyl-GAGASAEEKH). Purified UNC119 and the Tα peptide were dissolved in 10 mM Tris pH 7.4, 100 mM NaCl, and 5 mM DTT. The protein solution was prepared by mixing purified UNC119 and the Tα peptide (1:1.1 molar ratio) for at least one hour at 4 °C. Crystals were grown in drops containing 3 μL UNC119 Tα peptide mixture and 3 μL 40% PEG 5000, 0.1 M sodium acetate, 0.1 M potassium acetate, pH 5.0, and 1.8 μL 30% isopropanol at 4 °C, under 40 μL paraffin oil. Crystals were harvested for data collection after 10 to 14 days.

Crystals were mounted in a nylon loop, briefly immersed in cryoprotection buffer (40% PEG 5000, 0.1 M sodium acetate, 0.1 M potassium acetate, 12% glycerol, pH 5.0) and cooled by plunging into liquid nitrogen. Crystals were maintained at 100 K during data collection. Data were collected at beam line BL9-2 of the Stanford Synchrotron Radiation Lightsource. Data

were integrated and scaled using DENZO and SCALEPACK, respectively[42]. Data were phased by molecular replacement using PHASER[48] using PDB code 3GQQ as the search model. The models were rebuilt using O[49] and refined against a maximum likelihood target function using REFMAC[50]. Structures were checked using MolProbity[47].

Expression and Purification of Recombinant Bovine UNC119 Protein. The *UNC119* cDNA was amplified by PCR from a bovine retina cDNA library and cloned into the BamHI/EcoRI sites of a pGEX-2T vector (Amersham/GE Healthcare). A His-tag (6 histidines) was placed immediately following the ATG of the *UNC119* cDNA. The construct was transformed into an expression E. coli strain ER2556 (New England Biolab). Expression of the recombinant protein was induced by 0.1 mM IPTG for 5 hours at 37 °C. The protein was purified by a Histrap (Amersham/GE Healthcare) column followed by a GSTrap column (Amersham/GE Healthcare) according to the manufacturer's protocol and reduced GSH was removed by a Microcon centrifuge filter with an exclusion size of 30 kD (Millipore).

Expression and Purification of Recombinant Human UNC119. *Unc119* cDNA was amplified by PCR from a human retina cDNA library and directionally cloned into a pET151/D-TOPO vector (Invitrogen). The 6x His-tag in the vector was converted to a 12x His-tag using a QuickChange site-directed mutagenesis kit (Stratagene) and verified by DNA sequencing. The construct was expressed in BL21 Codon+ *E. coli* cells (Stratagene) in ZY autoinduction media for 6 hours at 37^{o} C and then overnight at 19^{o} C. Cells were harvested by centrifugation, resuspended, and lysed in 10 mg/mL lysozyme in lysis buffer (20 mM imidazole, 700 mM NaCl, 50 mM Tris pH 7.4, 1 mM DTT) and protease inhibitors (PMSF, aprotinin, leupeptin, pepstatin) for 1 hour at 4^{o} C, followed by sonication. The lysate was clarified by centrifugation (45 min, 15000 rpm) and soluble hUNC119 protein was bound to a Ni^{2+} sepharose column (Amersham/GE Healthcare), washed with 10 column volumes of lysis buffer and eluted with 300 mM imidazole in 700 mM NaCl, 50 mM Tris pH 7.4, and 1 mM DTT. Fractions were assayed by SDS-PAGE, pooled, and the 12x His-tag was removed by

incubation with TEV protease (~1 mg/100mg protein, 20 h at 25^{o} C.) in 2 L of 500 mM NaCl, 50 mM Tris pH 7.4, and 1 mM DTT. Unprocessed protein and TEV were removed by Ni^{2+} sepharose chromatography, cleaved protein was collected in the flow through, concentrated, and purified to homogeneity by anion exchange (HiTrap Q FF, GE Life Sciences, 20-1000 mM NaCl gradient in 25 mM Tris pH 7.4, 1 mM DTT) and gel filtration (SD200, Amersham/GE Healthcare), 100mM NaCl, 25 mM Tris pH 7.4, 1 mM DTT) chromatography.

Pulldown Assays. For protein sequencing, a bovine retina was homogenized by brief sonication in 1 ml PBS buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4. 1.76 mM KH_2PO_4 pH 7.4) with 1mM DTT and protease inhibitor cocktail (Roche). The insoluble debris was removed by centrifugation. The retina lysate was mixed with 10-80 µg of purified GST-UNC119 or 40 µg of GST followed by overnight incubation at 4°C. GST-UNC119 and its interacting proteins were pulled down by glutathione beads and the bound proteins were eluted with SDS gel loading buffer. The proteins were resolved by 12.5% SDS-PAGE. The protein sequencing by LC-MS/MS was carried out in the Mass Spectrometry Core Facility at the University of Utah.

For the competitive binding assay (**Fig. 3A**), 400 µl of retina lysate was incubated with 10 µg GST-UNC119 in the presence of 150 µg lauroyl-GAGASAEEKH and GAGASAEEKH peptides, respectively. All peptides were synthesized by the Utah Peptide Core facility.

For pull downs with light- and dark-adapted retinas, mouse retinas were homogenized in 1xPBS buffer containing 40 µM GTP (light) or hypotonic buffer (dark) (10 mM TrisHCl pH 7.4, 1 mM EDTA, 0.1 mM DTT). The insoluble fraction was removed by centrifugation. The supernatant was used for pulldown assays using GST and GST-UNC119. Solubilized transducin was reconstituted in 1xPBS buffer, pulled down with GST-UNC119 and identified by western blot using anti-Tα antibody (UUTA).

Immunoblot. Proteins were separated by 12.5% (for detection of Tα and GCAP1) or 15% (for detection of Tα and Tγ) SDS-PAGE and transferred to a nitrocellulose membrane. Membranes were processed as described [24]. The dilutions of the primary antibodies were 1:10,000 for SC389 (anti-rod Tα, Santa Cruz Biotechnology), 1:6,000 UW101 (anti-GCAP1), 1:10,000 for UUTA (anti-rod Tα) and 1:5000 for GN2 (anti-rod Tγ). UUTA and GN2 were obtained from Dr. CK Chen (VCU).

Extraction of Transducin from Mouse Retinal Membrane by UNC119. Mouse retinas were prepared from wild-type mice undergoing 20 min light-adaptation followed by 30 min dark-adaptation. The retinas were homogenized in 200 µl PBS with 1 mM DTT and protease inhibitors. The soluble components were removed by centrifugation at 4 °C for 10 min. The pellet was washed once with PBS, resuspended in 400 µl PBS, and aliquoted in four microcentrifuge tubes. In these four tubes, 9 µg GST, 9 µg GST and 40 µM GTP (final concentration), 18 µg GST-UNC119, and 18 µg GST-UNC119 and 40 µM GTP (final concentration) were added, respectively. After overnight incubation at 4°C, the membrane was pelleted and the supernatant was removed. The pellet was resuspended in 100 µl PBS. 10 µl of the supernatant from each tubes and their corresponding pellets were subjected to western blot using anti-Tα antibody (UUTA).

***In vitro* Expression of Tα and Tα(G2A).** *Gnat1* cDNA was amplified from a bovine cDNA library and cloned into the XhoI and NotI sites of pEGFP-N2 vector to replace the eGFP gene. The G2A mutant construct was generated by site-directed mutagenesis kit (Stratagene). Hek293 cells were transfected using Lipofectamine 2000 (Invitrogen) following the manufacturer's instructions. The cells were harvested 48 hours after transfection.

Immunocytochemistry of Retina Sections. Immunocytochemistry was peformed as described [5]. Dilutions for the primary antibodies were 1:1000 for UUTA (anti-rod Tα), 1:1000 for G8 (anti-GRK1). FITC-conjugated secondary antibodies were diluted 1:300.

Isothermal Titration Calorimetry. The transducin N-terminal peptides lauroyl-, myristoyl-GAGASAEEKH or GAGASAEEKH, and recombinant human UNC119 protein samples were suspended in 25 mM Tris (pH 7.4), 100 mM NaCl, and 1 mM DTT. Peptide and hUNC119 concentrations were varied as a result of the varying solubility of the peptides in the above-described buffer. ITC measurements were done on a MicroCal ITC-200 MicroCalorimeter at 25 ^{o}C. The peptide was injected into the hUNC119 samples at 180 sec intervals. Data obtained from the peptide injections into the buffer blanks were subtracted from the experimental data for analysis using the MicroCal Origin Software.

GTPase Assay. Transducin and depleted ROS membranes were purified from bovine retinas as previously described [27].The GTPase assay mix (20 µl) contained 4 pmoles (0.17 µg) rhodopsin (in depleted ROS membranes), 54 pmoles transducin (4.5 µg), 61 µM GTP spiked with γ-P^{32}-GTP, 20 mM Tris HCl pH 7.5, 5 mM $MgCl_2$, 1 mM DTT, and 0.1 mM EDTA. Some assays included 133 pmoles UNC119 (2.27 µg) or 130 pmoles of bovine serum albumin (BSA). Assays were incubated at 35^{o}C, 2 ul aliquots were withdrawn at 0, 5,10,15,20 minutes and spotted on PEI cellulose (Brinkmann). The PEI plates were washed in 0.12 M LiCl (30 min) and quantitated using a Typhoon Trio (GE Health Sciences) and Image Quant software.

Immunocytochemistry of *C. elegans.* Animals were permeabilized, fixed, and stained following standard methods. The images were acquired using an FV1000 Olympus confocal microscope. Polyclonal ODR-3 and GPA-13 antibodies were gifts from Dr. Gert Jansen (Center for Biomedical Genetics, Rotterdam, The Netherlands).

Cell-specific Rescue of *C. elegans unc-119* Mutant. The DNA used for microinjection was generated by fusion PCR. Briefly, the GFP gene was amplified by PCR from pPD104.53 (provided by Andrew Fire, Stanford) and cloned into P#MM016, in which the GFP cDNA was fused in frame to the 5' of *unc-119* genomic DNA. The gfp-*unc119* fusion gene including the flanking transcription termination sequence was amplified by PCR. A 2.7 kb promoter

sequence of *gpa-13* was amplified from *C. elegans* genomic DNA. The 3’ end of the *gpa-13* promoter was fused to the 5’ end of the *gfp-unc119* fusion gene by nested-PCR. The PCR product from the fusion PCR was microinjected into the germline.