Bellono et al., http://www.jgp.org/cgi/content/full/jgp.201311094/DC1

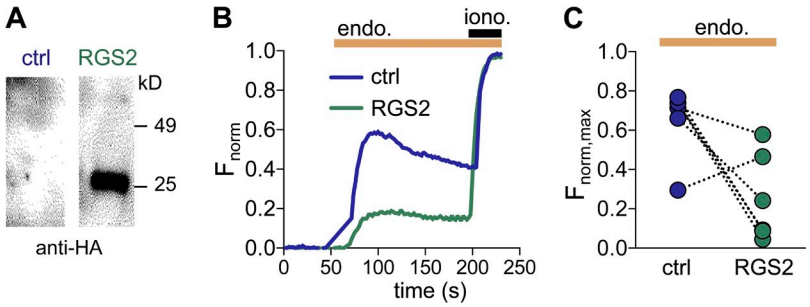

Figure S1. RGS2 expression in HEMs reduces Ca^{2+} responses to endothelin. (A) Representative Western blot of lysates from mock-transfected (ctrl) or RGS2-HA–expressing (RGS2) HEMs probed with anti-HA antibody stained a band of the molecular weight corresponding to RGS2-HA only in RGS2-transfected cells. $n = 3$ independent experiments. (B) Ca^{2+} responses (F_{norm}) elicited by treatment of HEMs with endothelin (endo.; 6 nM) were reduced in cells expressing RGS2 compared with control (ctrl) cells. Ca^{2+} responses to endothelin were normalized to ionomycin (iono.; 1 µM). $n = 3$–10 cells per condition from one experiment. (C) Mean peak endothelin-induced Ca^{2+} responses ($F_{norm,max}$) measured in paired experiments were reduced in HEMs expressing RGS2 compared with control cells. $n = 6$ experiments per condition.

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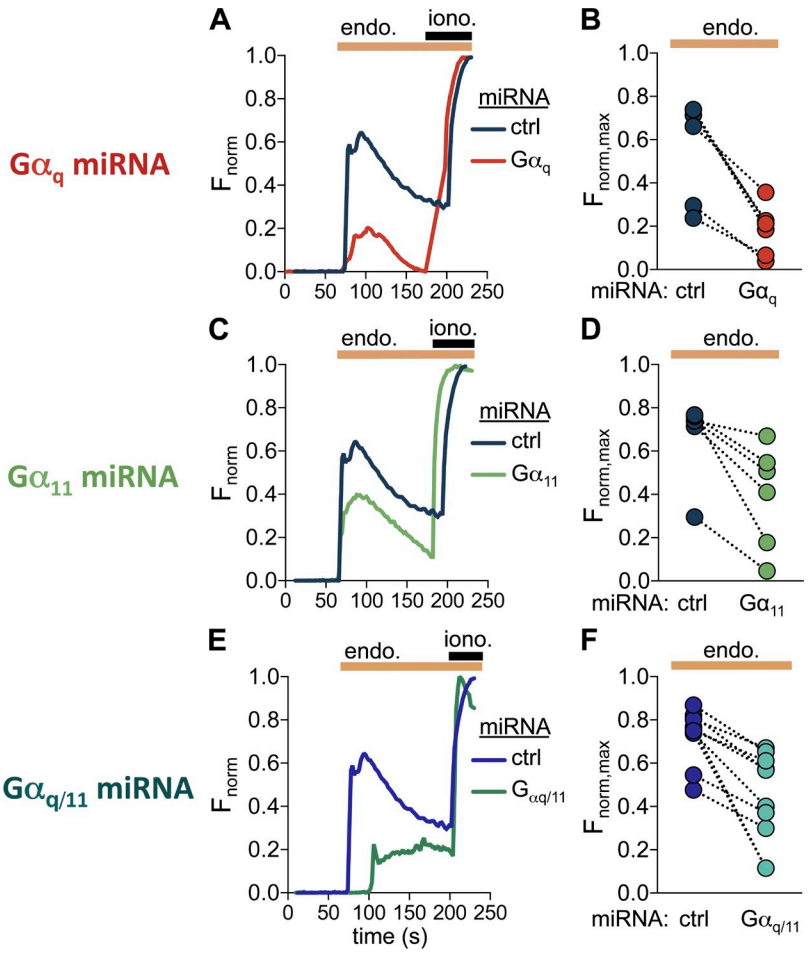

Figure S2. HEMs expressing $G\alpha_q$-, $G\alpha_{11}$-, or $G\alpha_{q/11}$-targeted miRNA have reduced Ca^{2+} responses to endothelin. (A) Ca^{2+} responses (F_{norm}) elicited by treatment with endothelin (endo.; 6 nM) were reduced in HEMs expressing $G\alpha_q$ targeted relative to control (ctrl) miRNA. n = 2–10 cells per condition from one experiment. (B) Mean peak endothelin-induced Ca^{2+} responses ($F_{norm,max}$) measured in paired experiments were reduced in HEMs expressing $G\alpha_q$-targeted versus control miRNA. n = 6 experiments per condition, $P < 0.005$. (C) Ca^{2+} responses (F_{norm}) elicited by treatment with 6 nM endothelin were reduced in HEMs expressing $G\alpha_{11}$-targeted compared with control miRNA. n = 3–10 cells per condition from one experiment. (D) Mean peak endothelin-induced Ca^{2+} responses ($F_{norm,max}$) measured in paired experiments were reduced in HEMs expressing $G\alpha_{11}$-targeted miRNA relative to control miRNA. n = 6 experiments per condition, $P < 0.05$. (E) Peak Ca^{2+} responses (F_{norm}) elicited by 6 nM endothelin were reduced in HEMs expressing $G\alpha_{q/11}$-targeted miRNA relative to control miRNA-expressing cells. n = 2–10 cells per condition from one experiment. (F) HEMs expressing $G\alpha_{q/11}$-targeted miRNA had decreased mean peak Ca^{2+} responses to endothelin compared with control miRNA expressing cells. n = 10 experiments per condition.

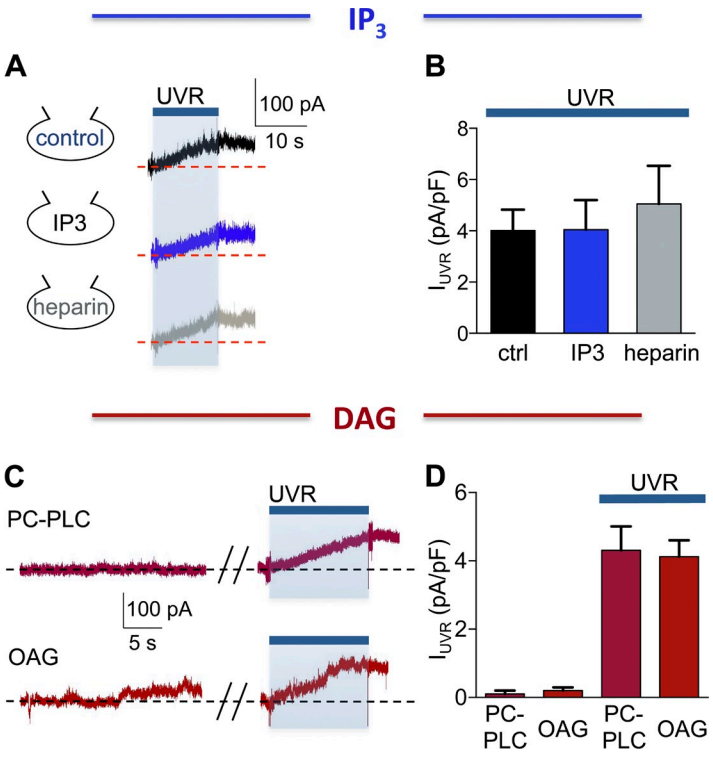

Figure S3. Neither IP_3-mediated Ca^{2+} release nor DAG activate the UVR photocurrent. (A) Representative HEM whole-cell currents measured at +80 mV in response to 240 mJ/cm2 UVR were similar in cells dialyzed with pipette solution that allowed for a UVR-induced increase in intracellular Ca^{2+} (control), or in cells dialyzed with IP_3 (100 µM in control solution) or heparin (1 mg/ml in control solution), used to block IP_3-mediated Ca^{2+} release in response to UVR. (B) The mean amplitude of HEM whole-cell current density evoked by UVR in cells dialyzed with control solution, IP_3, or heparin was not significantly different. n = 6–7 cells per condition, ±SEM (error bars). (C) Bath application of PC-PLC (10 U/ml), which generates DAG, or the DAG analogue OAG (100 µM) did not elicit a significant whole-cell current in representative HEMs and did not affect the retinal-dependent photocurrents measured 5 min after application in response to 240 mJ/cm^2 UVR. The broken horizontal lines represent the baseline current for each recording. (D) The mean amplitude of current density at +80 mV measured in HEMs preincubated with retinal was not affected by treatment with PC-PLC or OAG before or after stimulation with 240 mJ/cm^2 UVR. n = 4–6 cells per condition, ±SEM (error bars).

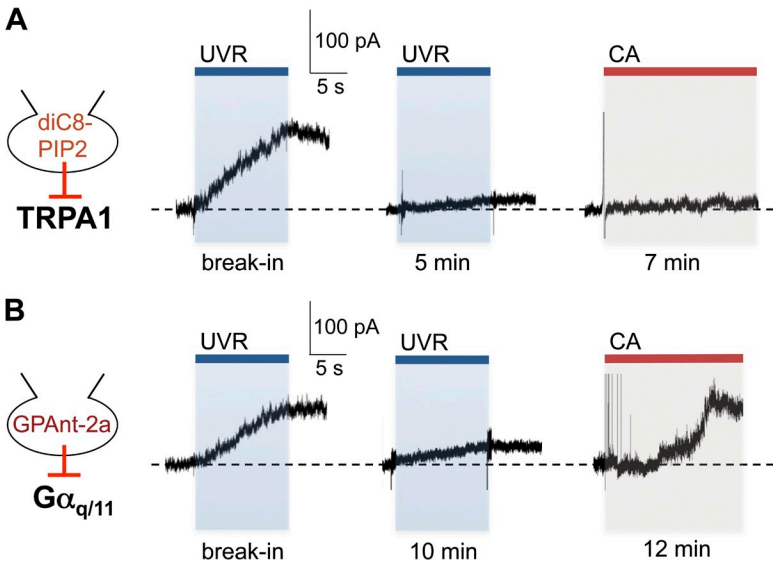

Figure S4. Selective inhibition of UVR phototransduction signaling components versus ion channels by cellular dialysis. (A) In a representative HEM dialyzed with diC8-PIP_2, 240 mJ/cm^2 UVR induced an increase in whole-cell current measured at +80 mV immediately after break-in, but this current was significantly reduced after 5 min of patch pipette dialysis. In the same cell, the TRPA1 agonist CA (500 µM) failed to elicit a significant change in whole-cell current. (B) In a representative HEM dialyzed with the $G\alpha_{q/11}$ signaling inhibitor peptide GPAnt-2a, 240 mJ/cm^2 UVR induced a significant current measured at +80 mV immediately after break-in, but this current was markedly reduced after 10 min of dialysis. In the same cell, CA (500 µM) elicited a significant increase in whole-cell current. The broken horizontal lines represent the baseline current for each recording.