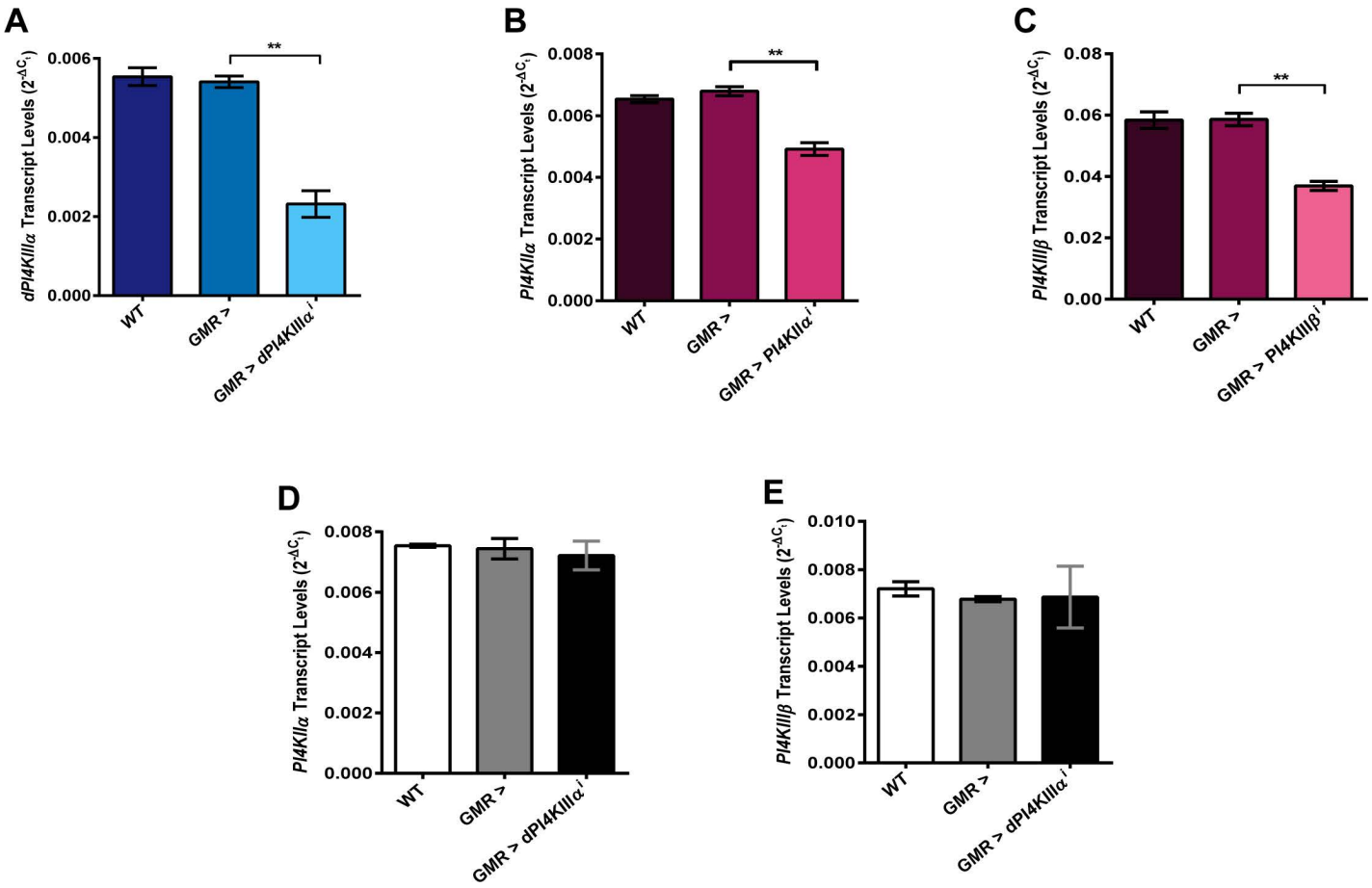

Figure S1: Q-PCR analysis of transcript depletion in transgenic RNAi manipulations

A) - E) Quantitative PCR (Q-PCR) from retinal tissue of genotypes indicated. Detected transcript, normalized to the house keeping transcript RP49, is indicated on y-axis. n=75retinae per sample(** - $p<0.01$, two-tailed unpaired t-test).The experiment has been repeated two times, one of the trials is shown here.

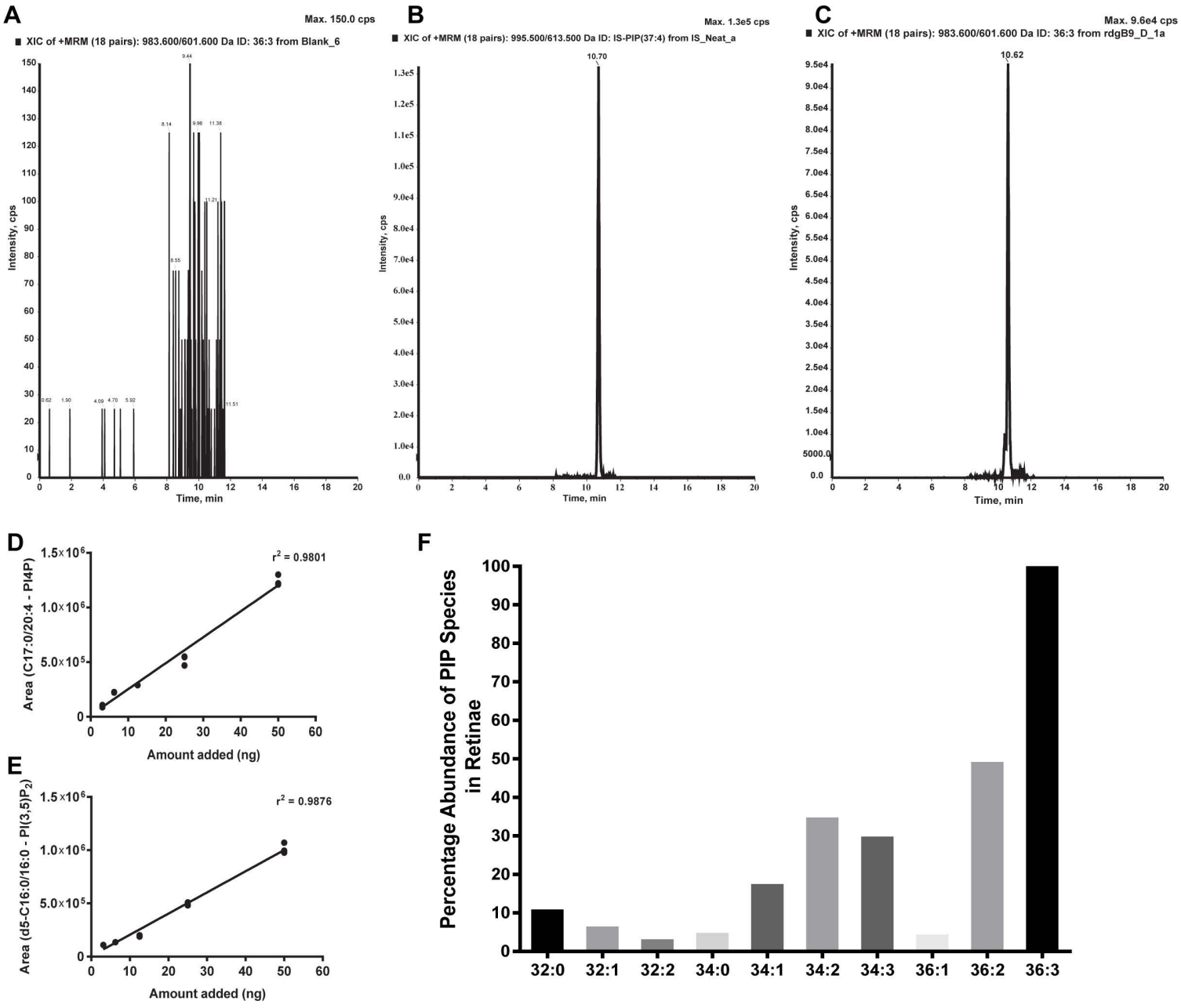

Figure S2: LC-MS analysis of PIP and PIP2 levels in retinal lipid extracts

A) - C) Representative chromatograms of blank, synthetic internal standard (C17:0/20:4-PI4P) and retinal lipid extract (36:3-PIP) respectively. The 36:3 PIP elutes at 10.62 mins, which isnot observed in the chromatograms from blank injections. X-axis is elution time; y-axis is signal in counts per second.

D) and E) Response linearity curves for extracted internal standards C17:0/20:4-PI4P and d5-C16:0/16:0-PI(3,5)P_2, respectively. X-axis represents amount added before extraction; y-axis represents area under curve for the peak being quantified.

F) Representation of the acyl chain length and desaturation of PIP species extracted and detected from *Drosophila* retinae. X-axis represents the species detected from wild type *Drosophila* retinae in the format a:b where a is the combined acyl chain length of *sn-1* and *sn-2* and b is the number of double bonds in both acyl chains. Y-axis represents the relative abundance of each species. The PIP species 36:3 is most abundant with 36:2, the next most abundant species, being the about 50% of the levels of 36:3.

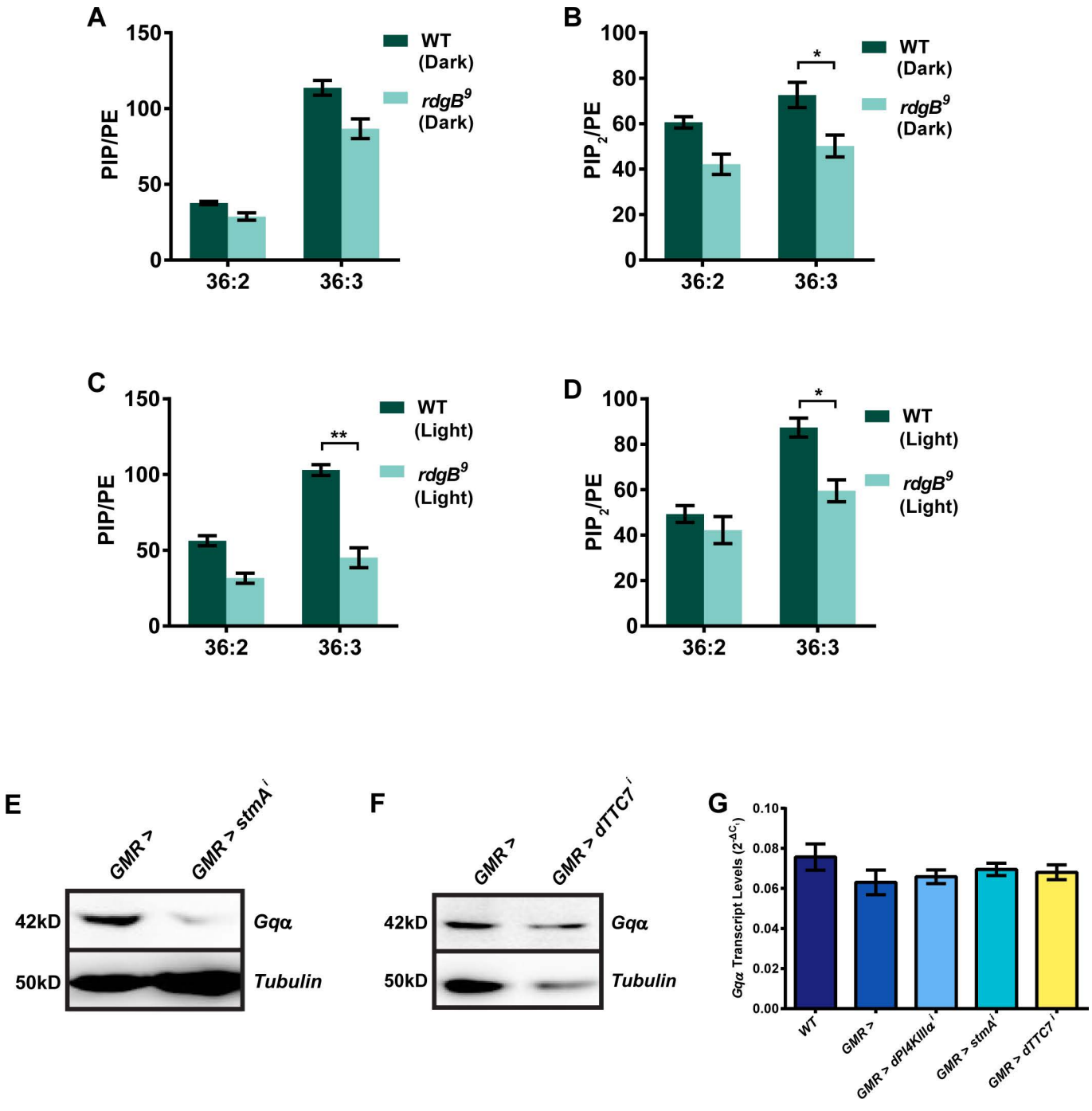

Figure S3: LC-MS measurements of lipids from *rdgB*^{9} flies

A) and B) LC-MS measurement of total PIP and PIP_2 levels, respectively, from retinae of one day old WT and *rdgB*^{9} flies. Flies were reared and processed completely in dark. Levels for the two most abundant molecular species of PIP and PIP_2 [36:2 and 36:3] are shown. Y-axis represents PIP and PIP_2 levels normalized to phosphatidylethanolamine (PE). Values are mean ± s.e.m., n=25 retinae per sample (* - $p < 0.05$, ANOVA followed by Tukey's multiple comparison test). The experiment has been repeated three times, one of the trials is shown here.

C) and D) LC-MS measurement of total PIP and PIP_2 levels, respectively, from retinae of one day oldWT and *rdgB*^{9}flies. Flies were reared in dark and exposed to one minute of bright illumination before processing. Y-axis represents PIP and PIP_2 levels normalized to phosphatidylethanolamine (PE). Levels for the two most abundant molecular species of PIP and PIP_2 [36:2 and 36:3] are shown. Values are mean ± s.e.m., n=25 retinae per sample (* - $p < 0.05$, ** - $p < 0.01$, ANOVA followed by Tukey's multiple comparison test). The experiment has been repeated three times, one of the trials is shown here.

E) and F) Western blot of head extracts made from flies of the indicated genotypes. Tubulin is used as a loading control, genotypes are indicated above.

G) Quantitative PCR (Q-PCR) from retinal tissue of genotypes indicated. Detected transcript normalized to the house keeping transcript RP49 is indicated on y-axis. n=75 retinae per sample.

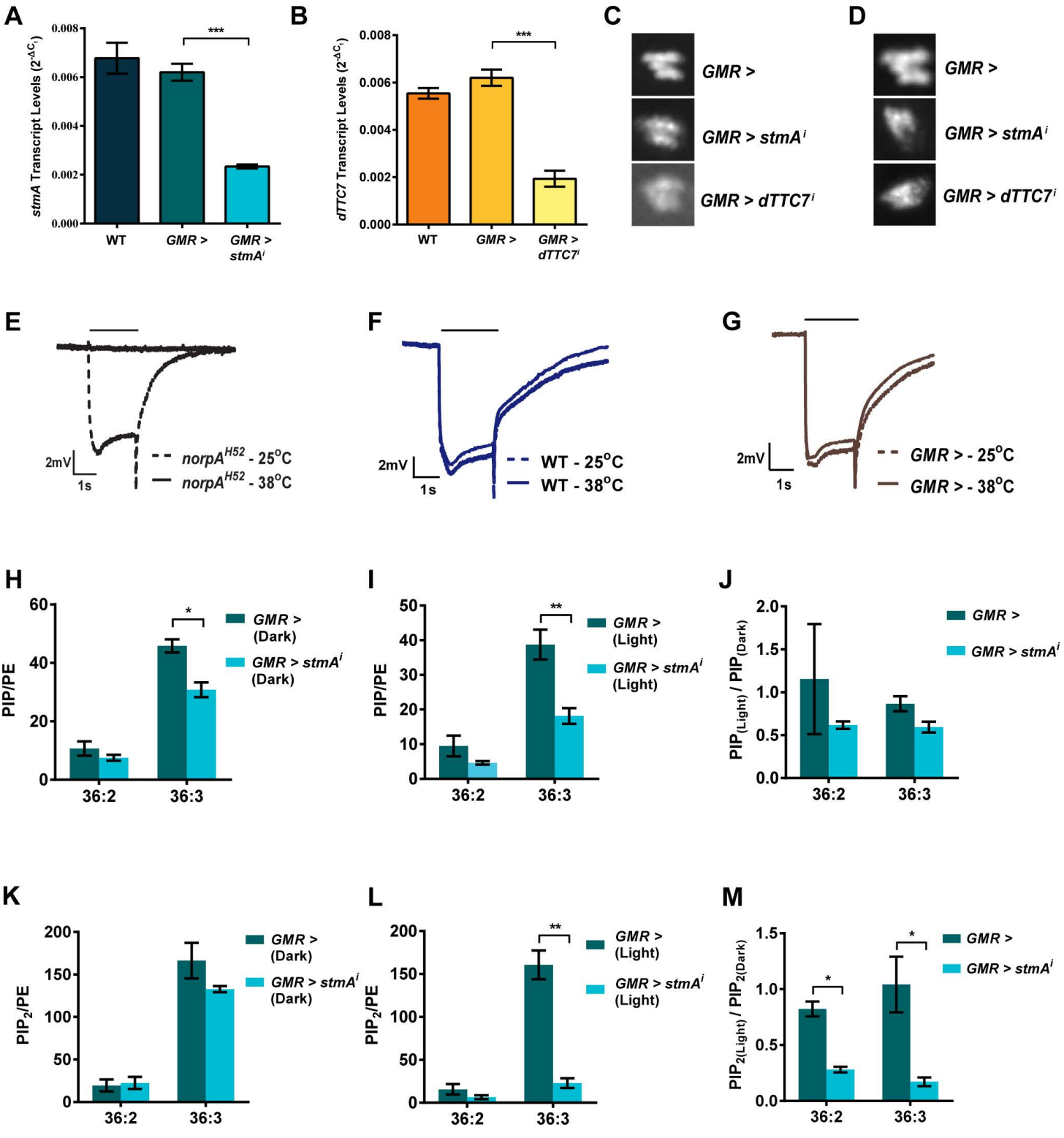

Figure S4: *stmA* is required for maintenance of PIP and PIP2 levels in photoreceptors

A) - B) Quantitative PCR (Q-PCR) from retinal tissue of genotypes indicated. Detected transcript, normalized to the house keeping transcript RP49, is indicated on y-axis. n=75 retinae per sample (*** - p<0.001, two-tailed unpaired t-test).

C) Representative images of fluorescent deep pseudopupil from one day old flies expressing the P4M::GFP probe, genotype as indicated.

D) Representative images of fluorescent deep pseudopupil from flies expressing the PLCδPH::GFP probe, genotype as indicated.

E) – G)Representative electroretinogram (ERG) trace of one day old flies, genotype as indicated. X-axis is time in (s); y-axis is amplitude in mV. Black bar above traces indicates duration of the light stimulus. The experiment has been repeated two times, one of the trials is shown here.

H) and K) LC-MS measurement of total PIP and PIP_2 levels, respectively, from retinae of one day old flies, genotypes as indicated. Flies were reared and processed completely in dark. Levels for the two most abundant molecular species of PIP and PIP_2 [36:2 and 36:3] are shown. Y-axis represents PIP and PIP_2 levels normalized to phosphatidylethanolamine (PE). Values are mean ±s.e.m., n=25 retinae per sample (* - $p < 0.05$, ANOVA followed by Tukey's multiple comparison test).

I) and L) LC-MS measurement of total PIP and PIP_2 levels, respectively, from retinae of one day old flies, genotypes as indicated. Flies were reared in dark and exposed to one minute of bright illumination before processing. Y-axis represents PIP and PIP_2 levels normalized to phosphatidylethanolamine (PE). Levels for the two most abundant molecular species of PIP and PIP_2 [36:2 and 36:3] are shown. Values are mean ±s.e.m., n=25 retinae per sample (** - $p < 0.01$, ANOVA followed by Tukey's multiple comparison test).

J) and M) LC-MS measurement of total PIP and PIP_2 levels, respectively, from retinae of one day old flies, genotypes as indicated. The y-axis represents a ratio of lipid levels, $[PIP_{(light)}/PIP_{(dark)}]$ and $[PIP_{2(light)}/PIP_{2(dark)}]$. Ratios for the two most abundant molecular species of PIP and PIP_2 [36:2 and 36:3] are shown. A reduction in the ratio indicates a drop in the levels of PIP and PIP_2. Values are mean ± s.e.m. (* - $p < 0.05$, **- $p < 0.01$, ANOVA followed by Tukey's multiple comparison test).

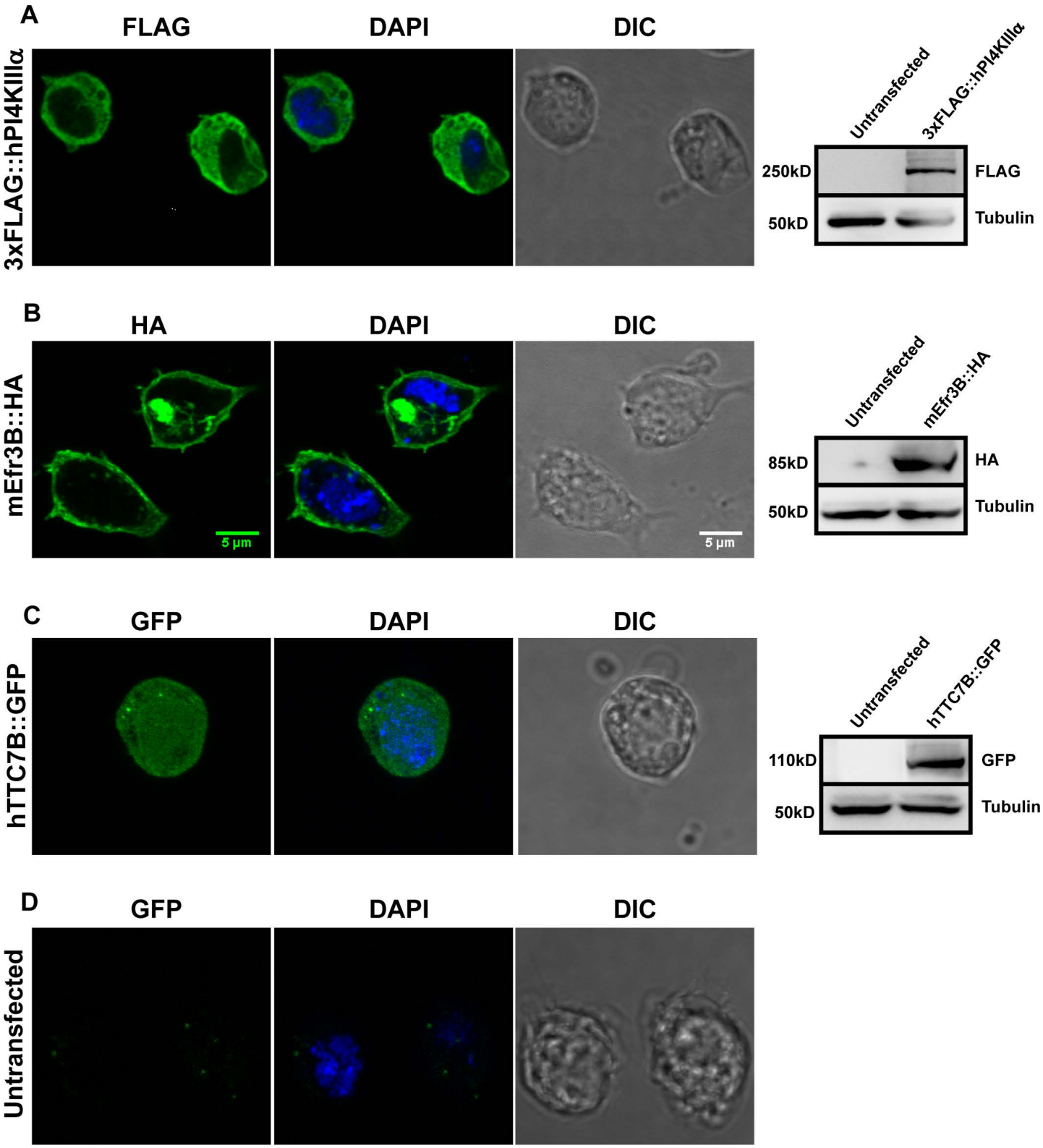

Figure S5: Localization of mammalian PI4KIIIα, Efr3 and TTC7 in S2R+ cells

A) Confocal images of S2R+ cellstransfected with pUAST-3xFLAG::hPI4KIIIα, followed by staining with DAPI and α-FLAG. Blue represents DAPI, which marks the nucleus, and green represents FLAG. Western blot indicates the presence of the FLAG-tagged protein in transfected cells. Scale bar - 5µm. The experiment has been repeated two times, one of the trials is shown here.

B) Confocal images of S2R+ cells transfected with pUAST-mEfr3B::HA followed by staining with DAPI and α-HA. Blue represents DAPI, which marks the nucleus, and green represents HA. Western blot indicates the presence of the HA-tagged protein in transfected cells. Scale bar - 5μm. The experiment has been repeated two times, one of the trials is shown here.

C) Confocal images of S2R+ cells transfected with pUAST-hTTC7B::GFP, followed by staining with DAPI. Blue represents DAPI, which marks the nucleus, and green represents GFP. Western blot indicates the presence of the GFP-tagged protein in transfected cells. Scale bar - 5μm. The experiment has been repeated two times, one of the trials is shown here.

D) Confocal images of untransfected S2R+ cells followed by staining with DAPI. Blue represents DAPI, which marks the nucleus, and green represents GFP. Scale bar - 5μm.

Table S1: Conditions for LC separation Solvent A: Water + 0.1% Formic Acid; Solvent B: Acetonitrile. **Gradient:**

Time (min)	Flow Rate	%A	%B
0	0.1	55	45
5	0.1	55	45
10	0.1	0	100
15	0.1	0	100
16	0.1	55	45
20	0.1	55	45

Table S2: MRM details

MRMs:

Polarity: Positive; Ion Source: Turbo Spray Ion Drive; Resolution Q1: Unit; Resolution Q3: Unit

Q1 Mass (Da)	Q3 Mass (Da)	Dwell Time (ms)	Species
809.4	535.4	60	IS – PI (17:0/14:1)
823.5	549.5	60	PI - 32:2
849.5	575.5	60	PI - 34:2
851.6	577.6	60	PI - 34:3
877.5	603.6	60	PI - 36:2
879.6	605.6	60	PI - 36:3
938.5	556.4	60	IS - PI4P (17:0/20:4)
957.5	575.5	60	PIP - 34:2
959.5	577.5	60	PIP - 34:3
985.6	603.6	60	PIP - 36:2
987.6	605.6	60	PIP - 36:3
1046.5	556.4	60	IS – d5-PI(3,5)P_2 (16:0/16:0)
1065.5	575.5	60	PIP_2 - 34:2
1067.5	577.5	60	PIP_2 - 34:3
1093.6	603.6	60	PIP_2 - 36:2
1095.6	605.6	60	PIP_2 - 36:3
690.5	535.5	60	IS – PE (17:0/14:1)
754.4	601.4	60	PE - 34:2
752.5	603.5	60	PE - 34:3
732.5	577.5	60	PE - 36:2
730.4	575.4	60	PE - 36:3