First publ. in: Science Signaling 3 (2010), 136, p. ra65, DOI: 10.1126/scisignal.2000877

Supplementary Materials for

A Direct and Functional Interaction Between G_o and Rab5 During G Protein–Coupled Receptor Signaling

Vladimir Purvanov, Alexey Koval, Vladimir L. Katanaev*

*To whom correspondence should be addressed. E-mail: vladimir.katanaev@uni-konstanz.de

The PDF file includes:

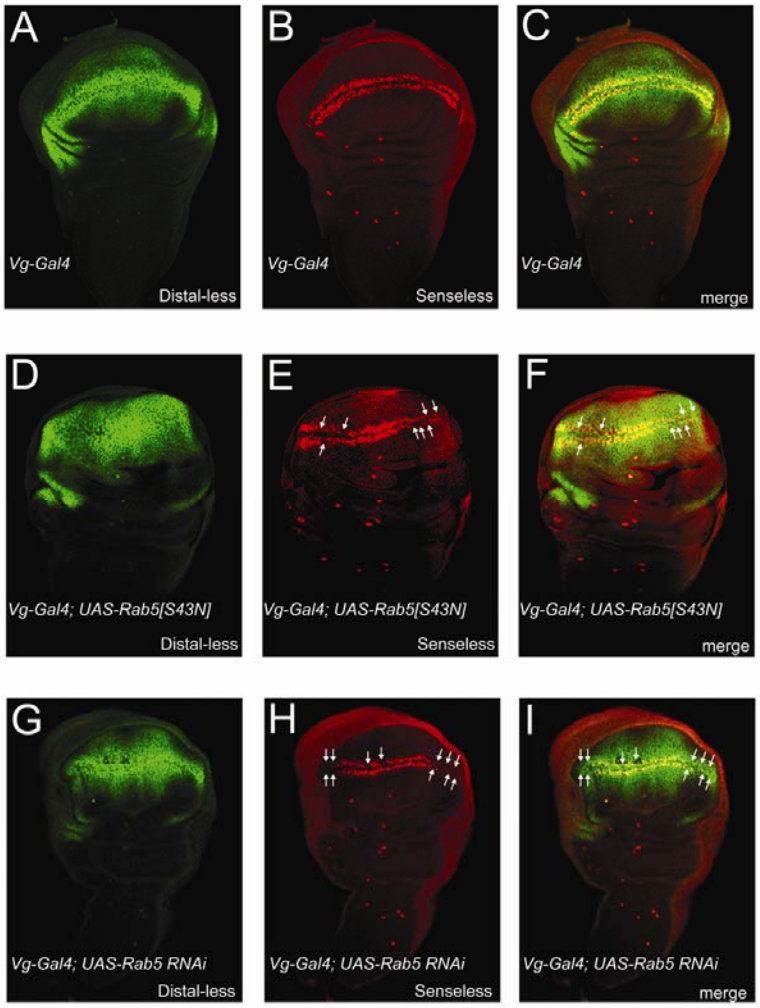

Fig. S1. Reduction in the abundance of Rab5 results in reduced Wg signaling. (**A** to **C**) Wild-type, (**D** to **F**) Rab5[S43N]-expressing, or (**G** to **I**) Rab5-specific RNAi–expressing wing imaginal discs were analyzed for the expression of the Wg long-range target gene *Distal-less* (green) and the Wg short-range target *Senseless* (red). Wing discs with inhibited Rab5 activity reveal gaps in the *Senseless* expression pattern (E and H, arrows), which in the adult wing develop into regions with lost wing margin structures (see Fig. 1, B and C). The expression of *Distal-less* is unaffected (D and G).

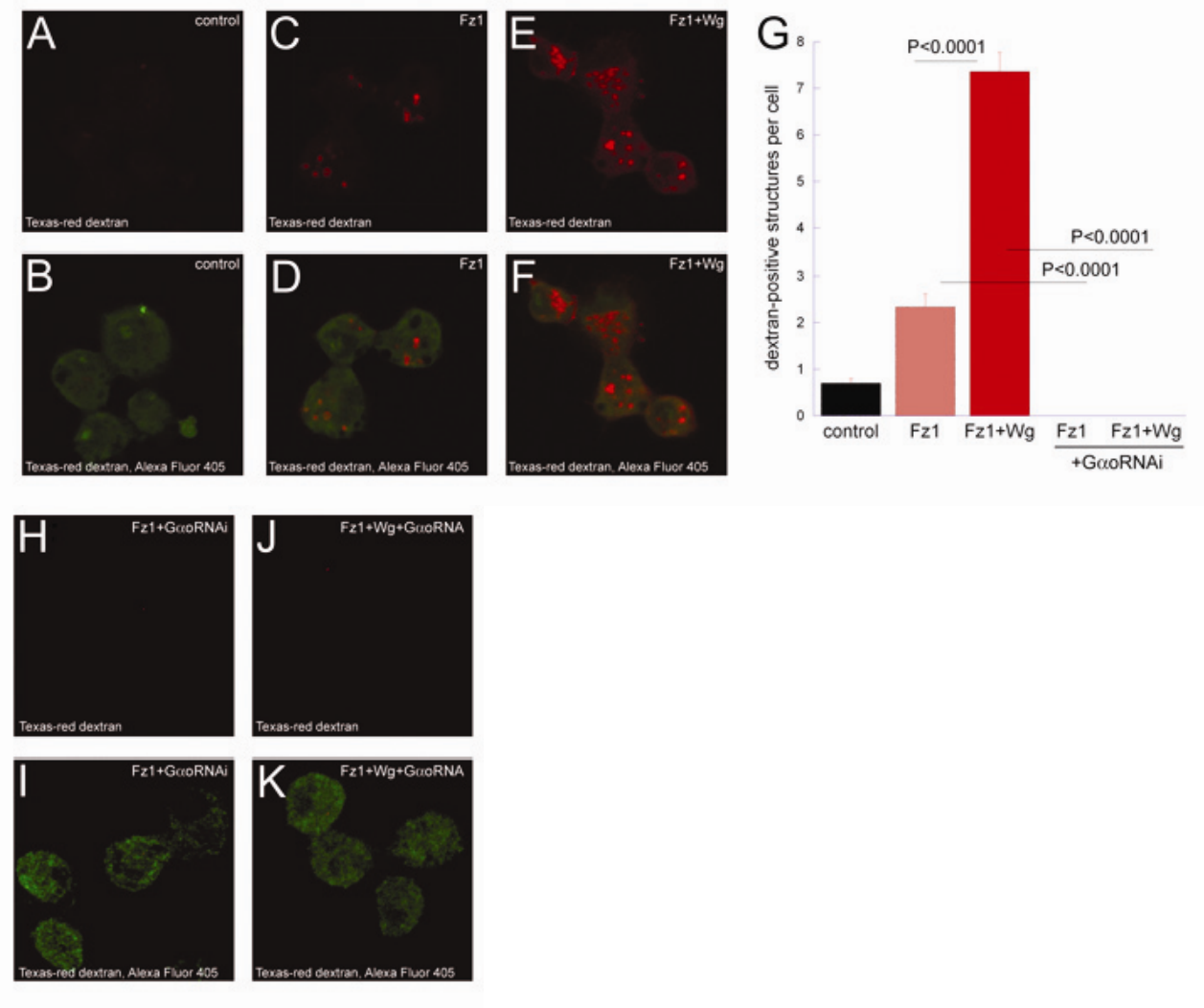

Fig. S2. Fz1 simulates endocytosis in a Wg- and $G\alpha_o$-dependent manner. Texas-red dextran was used to monitor endocytosis in control hemocytes (**A** and **B**) or hemocytes overexpressing Fz1 (**C** to **F**). Fzl-expressing cells displayed stimulated endocytosis (C and D), which was further enhanced upon addition of Wg (E and F). Quantification of dextran uptake by different hemocytes (**G**). Sample size was 30 to 100 cells; data are shown as described for Fig. 2C. (**H** to **K**) Fzl-induced endocytosis, both in the absence and presence of Wg, was prevented by knockdown of $G\alpha_o$. Nonspecific staining with Alexa Fluor 405–conjugated secondary antibodies against mouse antibody was used to visualize the outlines of the cells.

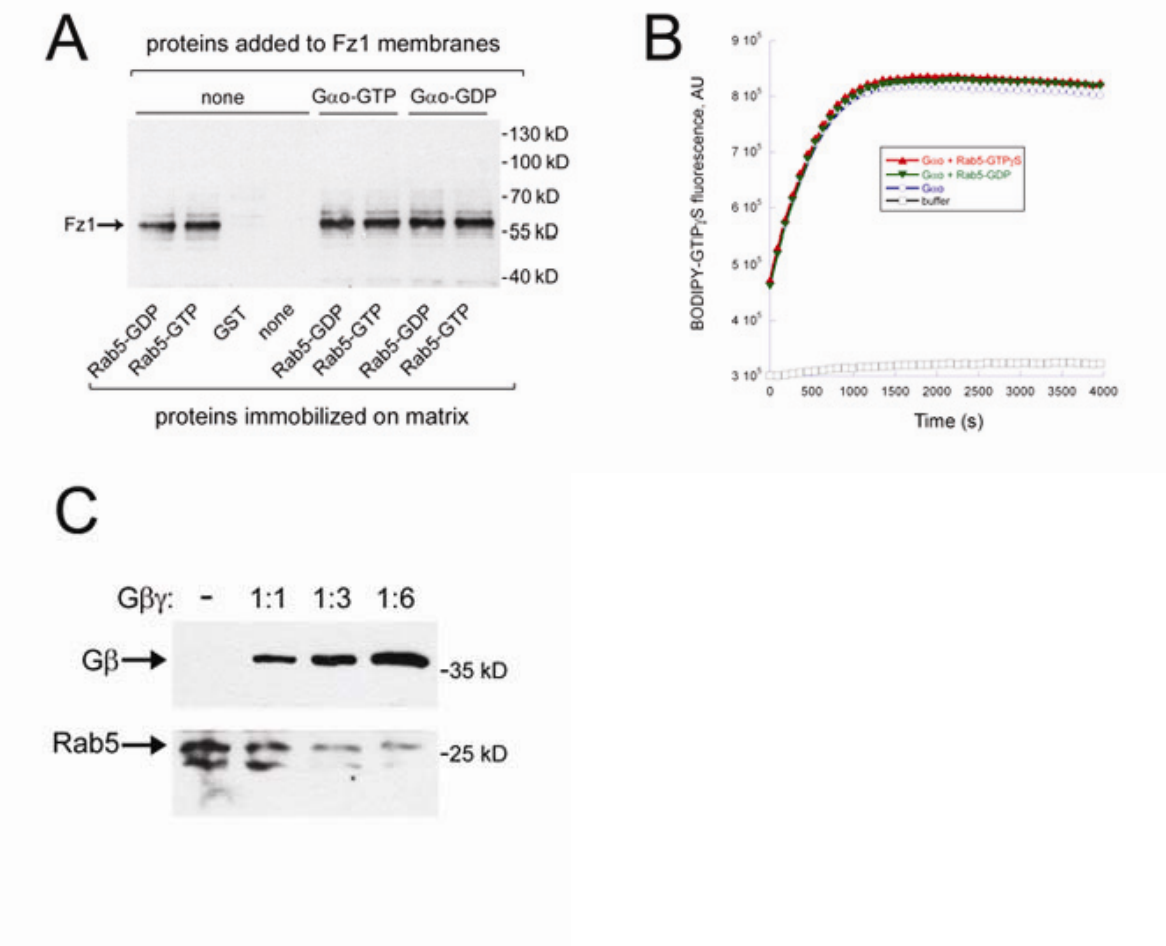

Fig. S3. Biochemical interactions of Rab5, G α_o, G $\beta\gamma$, and Fz1. (**A**) His6-tagged Rab5 immobilized on CNBr-sepharose and preloaded with GDP or GTP γS was used to pull-down MBP-Fz1 in the absence or presence of His6-tagged G α_o (added in amounts equimolar to Rab5) preloaded with GTPγS or GDP. Fz1 Specifically bound to Rab5 but not control matrixes (GST-linked or empty sepharose), as detected with antibodies against MBP. The binding efficiency was unaffected by G α_o. (**B**) G α_o or equimolar amounts of Rab5 preloaded with different nucleotides was probed with BODIPY-GTPγS in the presence of 20 mM $MgCl_2$. No stimulation of G α_o by Rab5 could be observed. Although Rab5 does not efficiently bind GTP in this condition, excess amounts of Rab5 started to compete with G α_o for the fluorescent nucleotide. (**C**) G$\beta\gamma$ efficiently outcompetes Rab5 from binding to Gα_o-GDP. CNBr-sepharose–immobilized Gα_o was preloaded with GDP and incubated with equimolar Rab5-GDP with increasing amounts of G$\beta\gamma$ (proportions to Rab5 are indicated on top). Efficient binding of G $\beta\gamma$ to G α_o (top panel) was accompanied by the reduced binding of Rab5 (bottom panel). Each panel shows a representative image of at least three independent experiments.

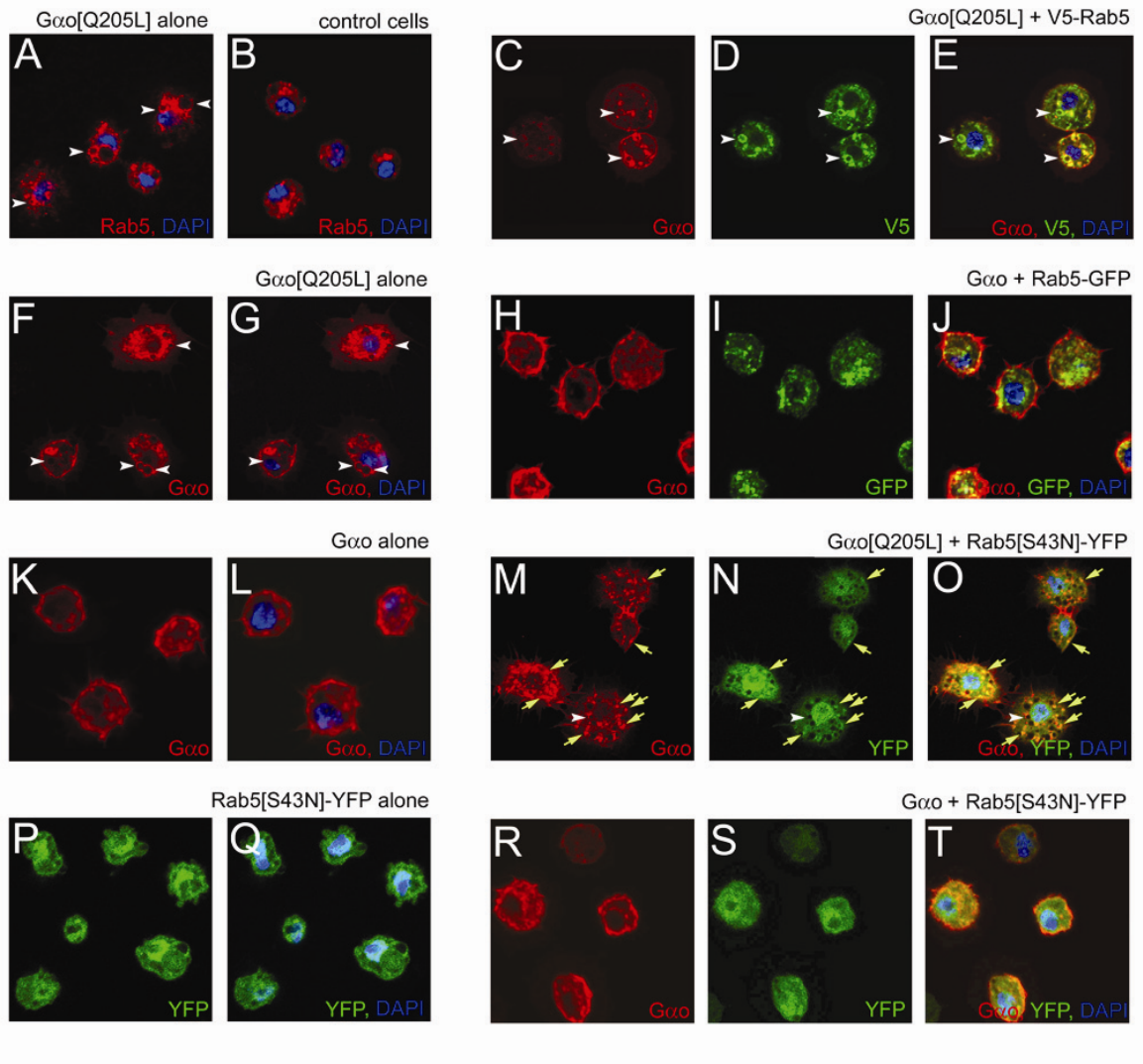

Fig. S4. Analysis of the localization of $G\alpha_o$ and Rab5 in hemocytes from flies of different genotypes. (**A** and **B**) Endogenous Rab5 can be seen in giant endosomes upon expression of $G\alpha_o$[Q205L] (A), but not in control hemocytes (B). (**C** to **G**) Expression of $G\alpha_o$[Q205L], either with V5-Rab5 (C to E) or alone (F and G), induced the formation of giant endosomes, as seen with antibodies against $G\alpha_o$ (C and F) and the V5 tag (D). In contrast, overexpression of wild-type $G\alpha_o$ with Rab5-GFP (**H** to **J**) or alone (**K** and **L**) failed to induce the formation of giant endosomes detectable with antibody against $G\alpha_o$ (H and K) or with Rab5-GFP (I). (**M** to **T**) Expression of Rab5[S43N]-YFP results in nuclear and diffuse cytoplasmic localization (P and Q). Co-expression of $G\alpha_o$[Q205L] (M to O) led to a marked re-localization of Rab5[S43N]-YFP (N). Rab5[S43N] became visible in cytoplasmic puncta in which $G\alpha_o$ was colocalized (yellow arrows in M to O). When giant endosome-like structures were detected with antibody against $G\alpha_o$, they were devoid of Rab5[S43N]YFP (white arrowhead in M to O). In contrast, coexpression of wild-type $G\alpha_o$ does not change the localization pattern of Rab5[S43N]-YFP (R to T).

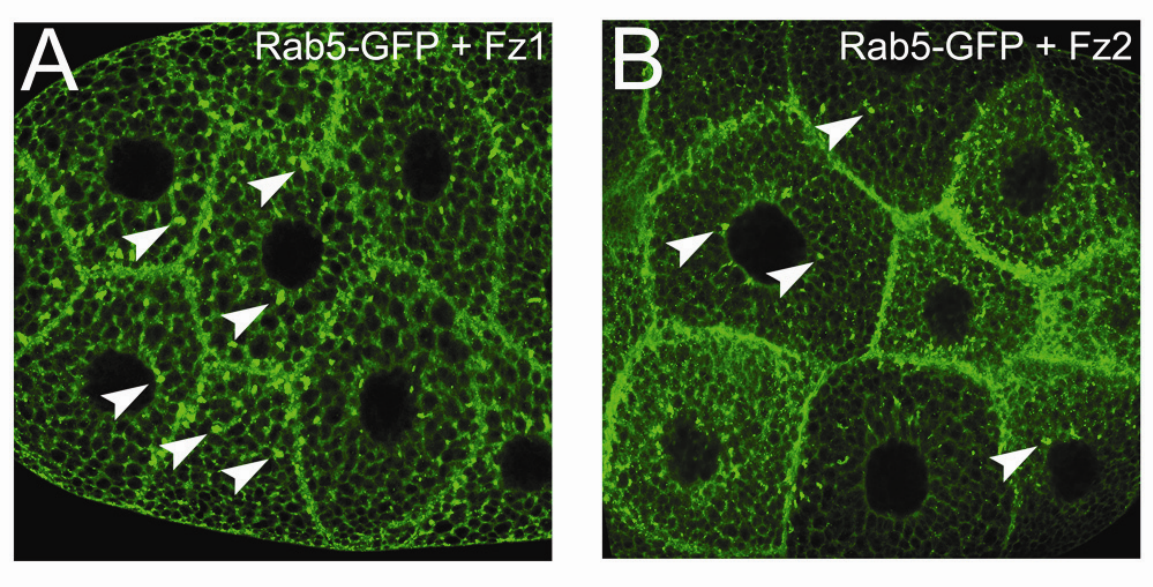

Fig. S5. Fz1 and Fz2 relocalize Rab5 into intrac ellular puncta in salivary glands. Upon expression of Rab5-GFP in salivary glands, coexpression of Fz1 (**A**) or Fz2 (**B**) forces Rab5-GFP to adopt an activa ted pattern (sim ilar to that seen in Fig. 4, B and C) with a bright, intracellular punctate pattern (arrowheads).