SUPPLEMENTAL DATA

SUPPLEMENTAL FIGURE LEGENDS

Fig. 1S. Optimization of Gα purification by GST-Ric-8 association required empirical evaluation of independently produced Gα baculovirus stocks. Two-independently produced Gαolf baculovirus preparations were amplified twice for 5 days in exponentially growing suspension *Sf*9 insect cells (1/100 culture vol. each time). Increasing doses of the prepared secondary amplified Gαolf viral stocks (200 µl - 3.5 ml) and 1 ml of the same amplified GST-Ric-8BFL stock were used to infect 50 ml cultures of exponentially growing High-Five insect cells in suspension at 2.0 x 10^6 cells/ml. High-Five cells were recovered 48 h later by centrifugation, and after medium removal, subjected to lysis and glutathione Sepharose isolation of the GST-Ric-8BFL:Gαolf complex as described in the Experimental Procedures. Gαolf was eluted from each column with AlF_4^- buffer and quantified by Bradford assay. Equivalent elution volumes from each column were resolved by reducing SDS-PAGE and proteins visualized with Coomassie Blue. Increasing infection dose with Gαolf baculovirus isolate #1 decreased the amount of recovered Gαolf protein, whereas increasing Gαolf baculovirus isolate #2 infection dose increased Gαolf protein recovery. These experiments demonstrate the need to empirically titrate Gα virus volumes. A viral plaque assay could alternatively be used to quantify Gα virus plaque forming units required to achieve maximal expression, but ultimately, viral infection level required correlation with *functional* Gα recovery from GST-Ric-8/glutathione Sepharose with AlF_4^-. It was found to be simpler to titrate Gα viral stocks empirically by volume used and expression/purification, rather than to also include the additional time-consuming step of conducting plaque-assays.

Fig. 2S. Hi-trap Q anion exchange chromatography of purified GST-Ric-8A and Gαq. Purified GST-Ric-8A (~2 µg) and Gαq (~3 µg) were mixed in 10 ml of Buffer N (20 mM Hepes, pH 8.0, 2 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT, 11 mM CHAPs, and protease inhibitor mixture) and pumped onto a 1 ml Hi-trap Q column at 0.5 ml/min. The column was washed with Buffer N and eluted with a linear gradient to 500 mM NaCl in Buffer N. The column eluate was fractionated. Fractions containing protein were resolved by SDS-PAGE and stained with Coomassie blue. Monomeric Gαq eluted at a lower NaCl concentration than the GST-Ric-8A:Gαq complex, which remained intact during Hi-trap Q chromatography and elution.

Fig. 3S. Expression of GST-Ric-8BFL or GST-Ric-8A reduced endogenous insect cell G protein expression. High-Five insect cells were infected with #1, GST; #2, GST-Ric-8A; or #3 GST-Ric-8BFL baculoviruses. Cells were harvested 48 h post infection and lysed with detergent free lysis buffer (20 mM HEPES-KOH, pH 8.0, 150 mM NaCl, 1 mM DTT, 1 mM EDTA, protease inhibitor mixture) by nitrogen cavitation using a Parr bomb. Lysates were collected and nuclei and unbroken cells were removed by centrifugation at 500 x g (post-nuclear supernatant, PNS). Crude membranes were prepared by centrifuging the PNS at 100,000 x g for 45 min. Washed membranes (Memb.) were solubilized in reducing SDS-Laemmli-sample buffer. PNS (50 µg), or membranes (50 µg) were resolved by SDS-PAGE alongside the indicated purified G protein subunits and Western blotted with, **A and B,** anti-Gαq/11 C-19 antibody (Santa Cruz, Inc.), **C and D,** anti-Gαi/Gαs antiserum, P960[4] and **E and F,** anti-$G\beta_{1-4}$ antiserum, B600[1]. The positions of recombinant (R) and insect cell G protein subunits are indicated. Endogenous insect cell G protein subunit expression was decreased in both the PNS and membrane fractions of GST-Ric-8A and GST-Ric-8B, but not GST expressing cells. A non-specific protein was

detected by the B600 antiserum in the PNSs, but not in the membrane fraction. This non-specific protein did not exhibit a Ric-8-dependent decrease in expression.

Fig. 4S. Prepared Gαi/q/13 subunits do not contain detectable insect cell G protein contaminants, while Gαs-class preparations may contain trace levels of a contaminating insect cell Gαq-like protein. Gα subunits (100 ng ea.) produced by the GST-Ric-8 association method were resolved alongside 50 µg of washed insect cell membranes by SDS-PAGE. The gels were Western blotted with G protein subunit antibodies described in the legend to Fig. 2S (anti-Gαq/11, anti-$G\beta_{1\text{-}4}$, anti-Gαi/Gαs). **A,** The Santa Cruz Gαq/11 antibody C-19 strongly detected purified recombinant Gαq, and a Gαq-like protein in insect cell membranes (●). This antibody also weakly detected doublet protein bands in lanes containing pure $G\alpha s_s$ and Gαolf. The lower molecular weight species (●) migrated at a distance on the gel that was nearly coincident with recombinant and insect cell Gαq. The higher molecular weight species migrated at positions where recombinant $G\alpha s_s$ (*) and Gαolf (■) migrated. One possibility is that the lower molecular weight band is a trace level of contaminating insect cell Gαq present in the $G\alpha s_s$ and Gαolf preparations. Given the intensity of the recombinant Gαq (100 ng) signal detected by this antibody, these potential Gαq contaminants represent <1% mol Gαq / mol $G\alpha s_s$ or Gαolf in each preparation. **B,** Common Gα antiserum, P960 detected insect cell Gαi, and a band of higher apparent molecular weight (~47-48 kDa) that might be an insect cell Gαs-like variant. Neither insect cell Gαi, nor the higher molecular weight protein (Gαs) were detectable contaminants in any recombinant Gα preparation. **C,** Common $G\beta_{1\text{-}4}$ antiserum, B600 detected insect cell Gβ on membranes. Gβ was not a detectable contaminant in any Gα preparation.

Fig. 5S. GST-Ric-8A co-expression did not significantly alter the yield of $G\beta_1\gamma_2$ prepared by the traditional $G\alpha i_1$-hexahistidine-tag association method[3,5]. High-five insect cells (1L) grown to a density of 2.0 x 10^6 cells/ml were infected with Hexahistine-tagged-$G\alpha i_1$, $G\beta_1$, $G\gamma_2$, and GST, or GST-Ric-8A recombinant baculoviruses. Cells were harvested 48 h post infection and lysed by nitrogen cavitation in a detergent free buffer (20 mM HEPES-KOH, pH 8.0, 100 mM NaCl, 1 mM EDTA, 2 mM $MgCl_2$, 10 mM βME, 10 µM GDP, protease inhibitor mixture) using a Parr bomb. Unbroken cells and nuclei were removed by centrifugation at 500 x g, and membranes were prepared by centrifugation at 100,000 x g. Membranes (5.5 mg/ml) were extracted with 1% w/v sodium cholate buffer. Detergent insoluble material was removed by centrifugation at 100,000 x g. The detergent extract was diluted four-fold with C12E10 buffer (20 mM HEPES-KOH, pH 8.0, 100 mM NaCl, 1 mM $MgCl_2$, 10 mM βME, 10 µM GDP, 0.5% C12E10, and protease inhibitor mixture) and loaded onto pre-equilibrated 2 ml bed volume Ni-NTA (Qiagen) columns. The columns were washed and $G\beta_1\gamma_2$ and His_6-$G\alpha i_1$ were sequentially isolated using the published method[3,5]. **A,** Ni-column Mg-GDP-AlF_4 eluates (fractions 2-4 are shown) contained recombinant $G\beta_1$ (and γ_2, not shown), and minor contaminants. Subsequent Ni-column elution with 150 mM imidazole released His_6-$G\alpha i_1$ (fractions 1-3 are shown). GST-Ric-8A was detected by Western blot as a co-eluate with the His_6-$G\alpha i_1$. **B,** The Ni-column Mg-GDP-AlF_4 eluate fractions were then pooled and $G\beta_1\gamma_2$ was purified using the method of Kozasa and Gilman with a HiTrap Q anion exchange column (GE Biosciences) used in place of the standard Mono Q anion exchange column[3,5]. The purity of $G\beta_1\gamma_2$ obtained whether GST or GST-Ric-8A was expressed was the same (nearly homogenous). Slightly more $G\beta_1\gamma_2$ (16%) was obtained per mass of membrane input into the purification procedure when GST-Ric-8A was expressed (0.78 µg $G\beta_1\gamma_2$/mg membrane protein) versus GST (0.67 µg $G\beta_1\gamma_2$/mg membrane protein). However, this owed to the fact that less GST-Ric-8A membranes were recovered from 1L of culture (770 mg) than GST membranes (910 mg), resulting in nearly equivalent yields from both preparations (GST, 0.61 mg $G\beta_1\gamma_2$) and GST-Ric-8A (0.6 mg $G\beta_1\gamma_2$).

REFERENCES

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FIG 1S The amounts of Gα baculovirus used to optimize expression and purification of functional Gα protein required empirical evaluation.

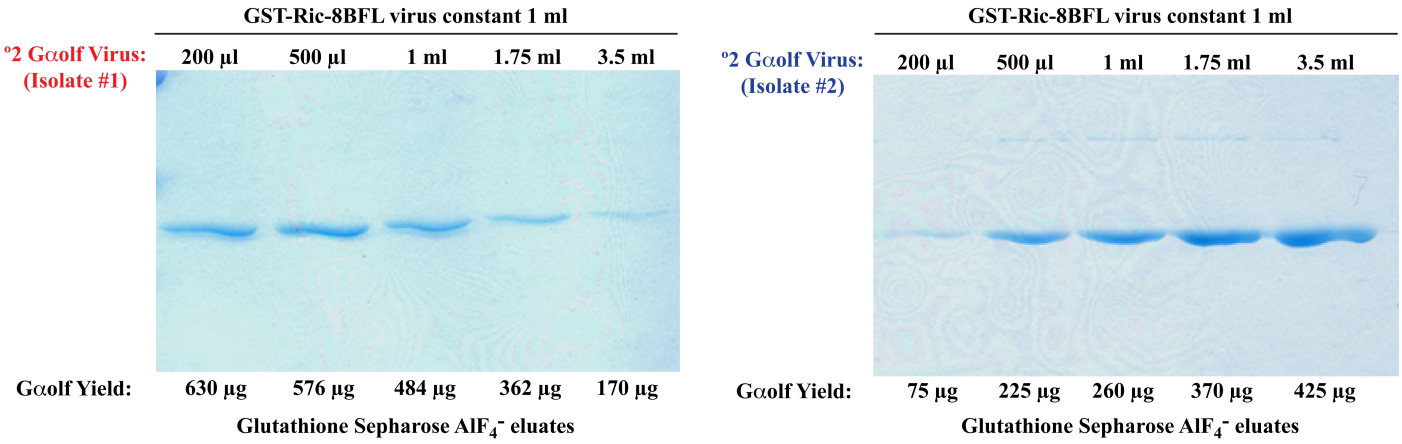


Fig. 2S Anion Exchange Separation of the GST-Ric-8A:Gαq complex from Gαq.

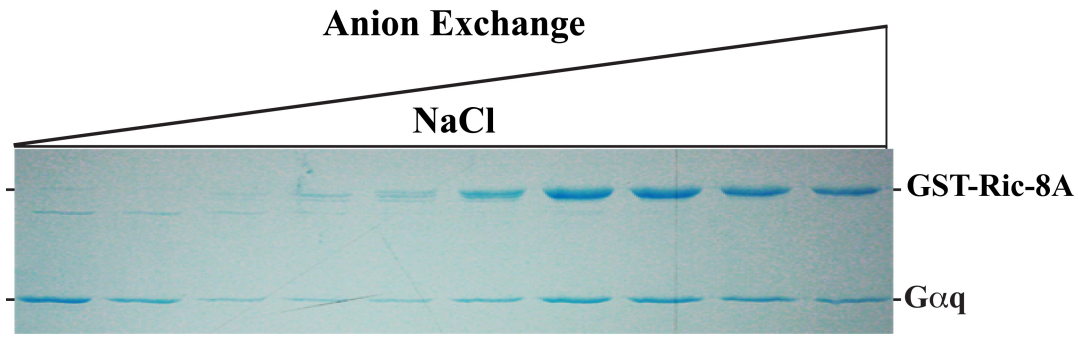


Fig. 3S **Analysis of Whole Cell and Membrane Expressed Endogenous Insect Cell G proteins after GST, GST-Ric-8A, of GST-Ric-8B expression.**

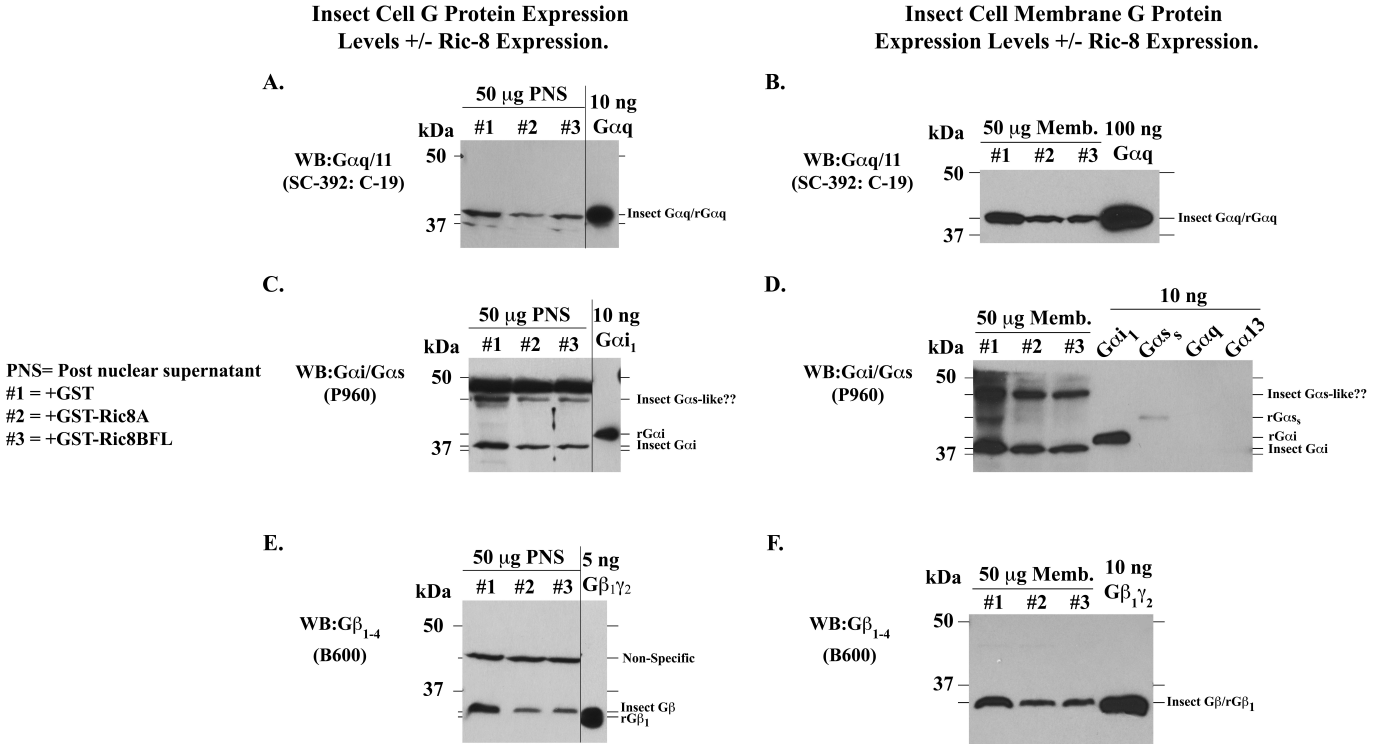


Fig. 4S

Analysis of Insect Cell G Protein Contaminants in Recombinant Gα Preparations.

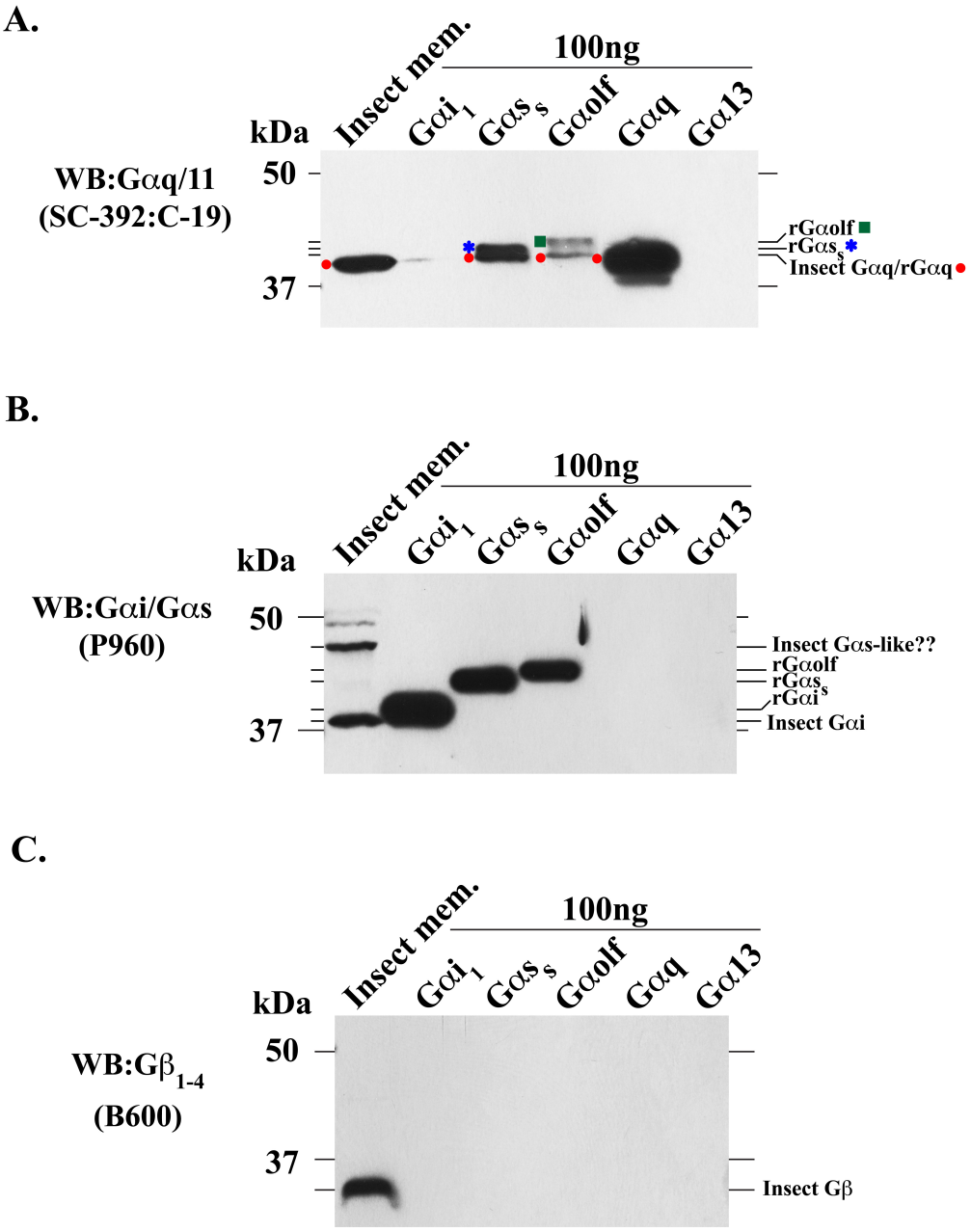
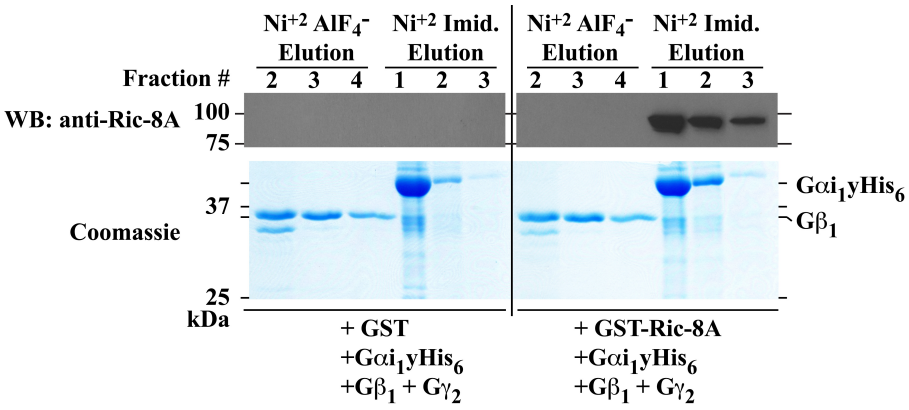
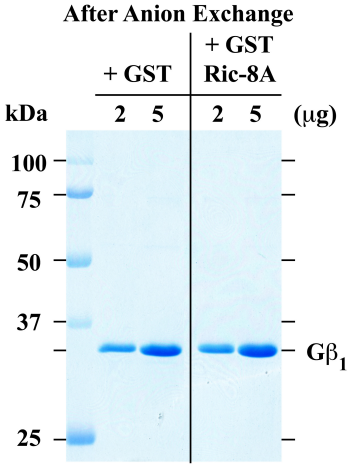


Fig. 5S **Traditional Purification of $G\beta_1\gamma_2$ from GST-Ric-8A- and GST-Expressing Insect Cells.**

A.



B.



After Anion Exchange

	Yield $G\beta_1\gamma_2$	$G\beta_1\gamma_2$ per mg membrane
+ GST	0.61 mg	0.67 µg/mg
+ GST-Ric-8A	0.60 mg	0.78 µg/mg