SUPPLEMENTAL INFORMATION

Supplemental Figure Legends

Figure S1. Phylogenetic analysis of the putative Gα-binding motif found in Calnuc and NUCB2. Rat Calnuc and NUCB2 sequences were used to identify homologous protein sequences by BLAST-Protein search. The sequences corresponding to the Gα-binding motif in the identified orthologues were aligned using CLUSTAL W. Conserved residues are shaded in black; similar residues in grey. The result of the alignment showed that Calnuc and NUCB2 Gα-binding motif is evolutionarily conserved from invertebrates to humans.

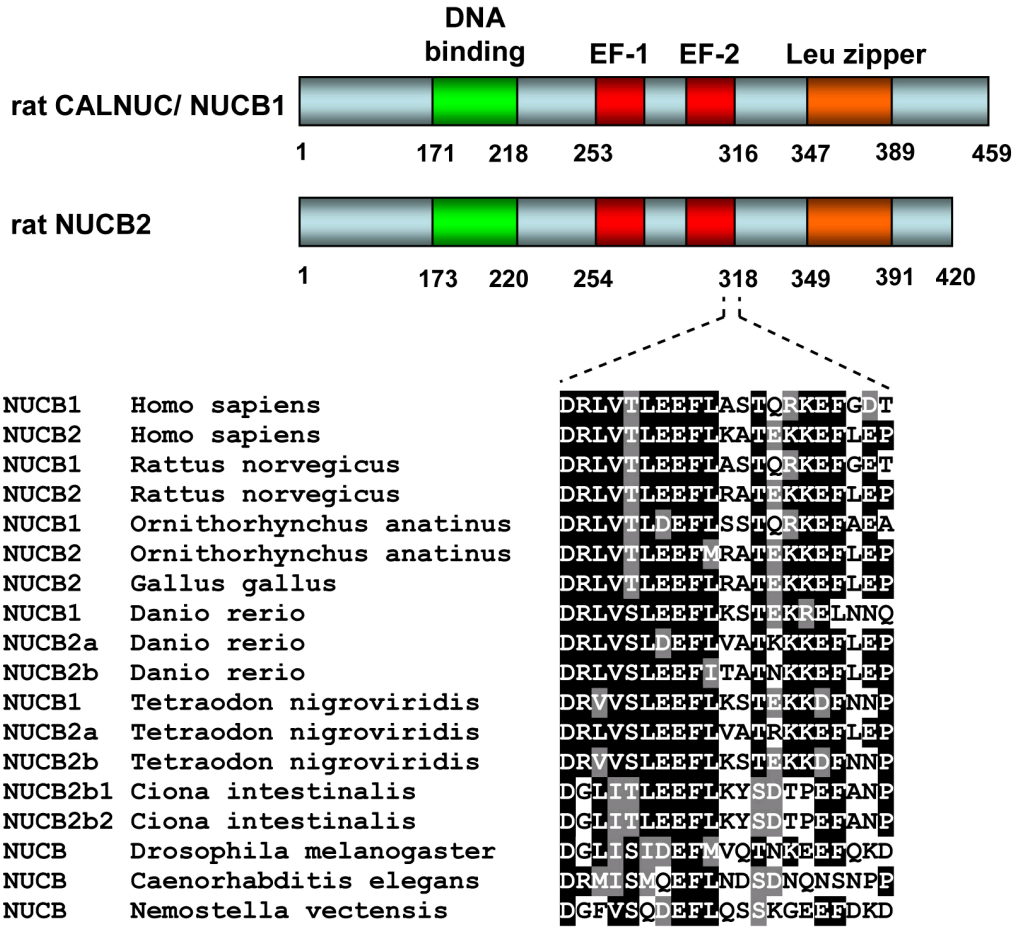

Figure S1

Figure S2. The interaction between Gαi3 and Calnuc is stabilized by an electrostatic contact between Gαi3 aa 248 and Calnuc aa 314. **(A)** K248 of Gαi3 is predicted to be located in close proximity to E314 of Calnuc, suggesting that they might establish a molecular point of interaction by electrostatic attraction. The coordinates of Calnuc's putative Gα-binding sequence (aa 309-320, red) were extracted for the Protein Data Bank [PDB#: 1SNL] and threaded over the structure of KB-752 (not shown) in complex with Gαi1 (blue) [PDB#: 1Y3A] as described in **Fig.1**. **(B)** Mutation of Calnuc's E314 to K restores the defective binding of Calnuc WT to Gαi3 K248E. His-CalnucΔN wild-type (lane 4) but not His-CalnucΔN E314K (lane 5) binding to GST-Gαi3 K248E·GDP (Gαi3 KE) is dramatically impaired compared to wild-type GST-Gαi3·GDP (Gαi3 WT, lane 3). No binding of His-CalnucΔN WT (lane 2) or His-CalnucΔN E314K (lane 5) to GST is detected. 10 µg His-CalnucΔN WT (lanes 2-4) or His-CalnucΔN E314K (lanes 5-6) were incubated with purified GST (lanes 2 and 6), wild-type GST-Gαi3 (lane 3) or GST-Gαi3 K248M (lanes 4 and 5) pre-loaded with GDP immobilized on glutathione beads and analyzed as in **Fig. 2A.** Input: 1 µg His-CalnucΔN WT (lane 1) or His-CalnucΔN E314K (lane 7).

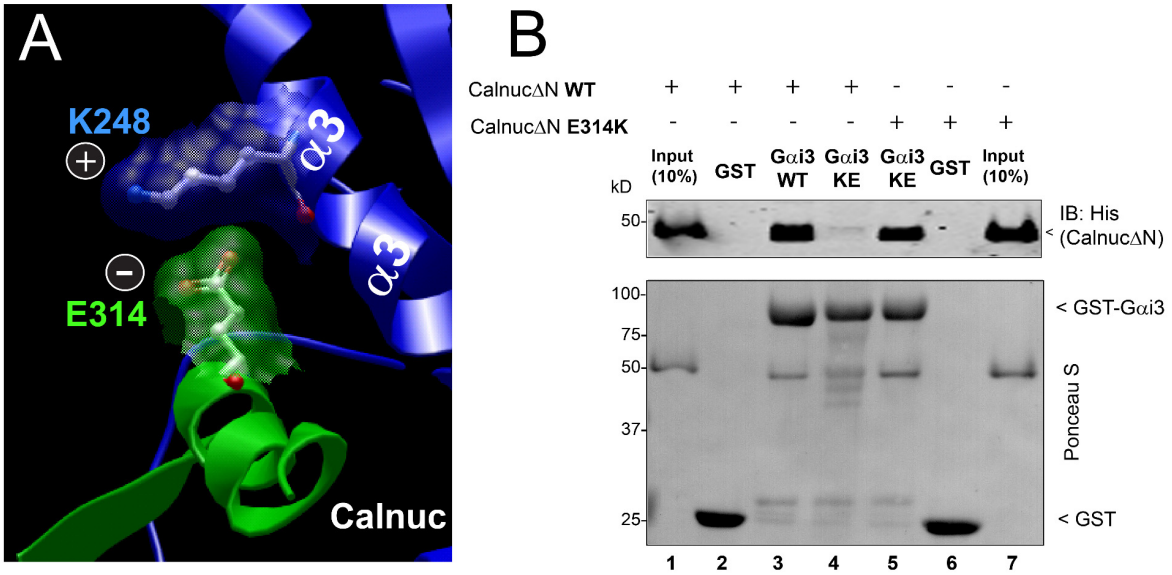

Figure S2

Figure S3. Mutation of W211 or F215 to A in Gαi3 abolishes Calnuc binding (A) W211 and F215 in the α3/ SwII cleft of Gαi are predicted to mediate the hydrophobic interaction with L313, F316 and L317 in Calnuc's Gα-binding motif. The coordinates of Calnuc's Gα-binding sequence (aa 309-320, green) were extracted for the Protein Data Bank [PDB#: 1SNL] and threaded over the structure of KB-752 (not shown) in complex with Gαi1 (blue) [PDB#: 1Y3A] as described in **Fig.1**. Gαi's W211 and F215 are in close proximity to Calnuc's L313, F316 and L317. **(B)** His-CalnucΔN binding to GST-Gαi3 W211A·GDP (lane 4) or GST-Gαi3 F215A·GDP is virtually abolished compared to wild-type GST-Gαi3·GDP (Gαi3 WT, lane 3). No binding of His-CalnucΔN WT to GST is detected (lane 2). 10 µg His-CalnucΔN WT were incubated with purified GST (lanes 2), wild-type GST-Gαi3 (lane 3), GST-Gαi3 W211A (lanes 4) or GST-Gαi3 F215A (lane 5) pre-loaded with GDP immobilized on glutathione beads and analyzed as in **Fig. 2A.** Input (lane 1): 1 µg His-CalnucΔN.

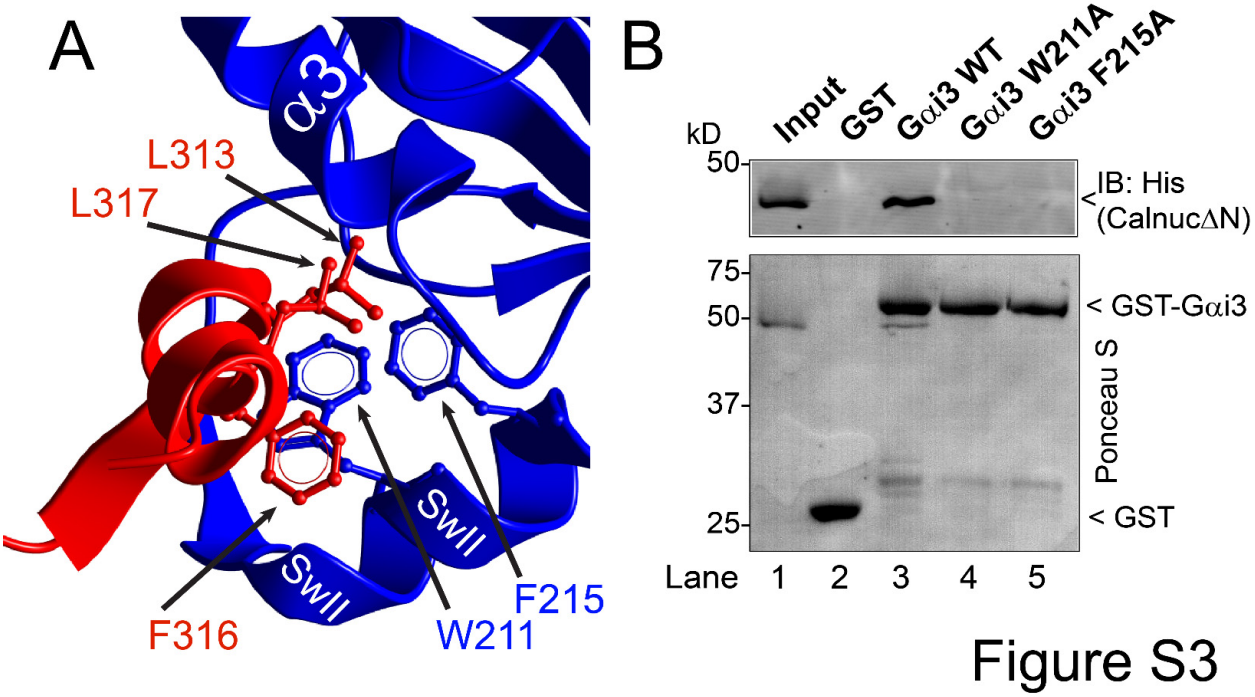

Figure S3

Figure S4. Mutation of F316 and F318 to A in Calnuc and NUCB2, respectively, impair activation of Gαi3. (A) His-CalnucΔN increases the steady-state GTPase activity of His-Gαi3 in a dose-dependent manner, and this effect is impaired (~70% reduction) by mutation of His-CalnucΔN's F316 to A (F316A). The steady-state GTPase activity of purified His-Gαi3 (50 nM) was determined in the presence of the indicated amounts (0, 6, 16, 30 and 60 μM) of purified wild-type His-CalnucΔN (closed circles) or His-CalnucΔN F316A mutant (open circles) by quantification of the amount of [γ-^{32}P]GTP (0.5 μM, ~50 c.p.m/ fmol) hydrolyzed in 10 min. Data expressed as % of GTP hydrolyzed by the G protein alone (0 μM) were fitted to a non-linear, one-site hyperbola (solid lines) using Prism 4.0. Results are shown as mean ± S.D of one representative experiment out of three performed in duplicate. The milder effect of this mutant (~70% reduction) compared to the double mutant L313A/ L317A (~100% reduction, **Fig 7A**) is consistent with their respective binding to Gαi3 (**Fig 6A**) **(B)** Mutation of F318 to A (F318A) abolishes GST-NUCB2 (173-333) activation of Gαi3 steady-state GTPase hydrolysis. The steady-state GTPase activity of purified His-Gαi3 (100 nM) was determined exactly as described in **A** but using 0, 5, 12.5, 25 and 37.5 μM NUCB2. The complete lack of effect of this mutant on the GTPase activity of Gαi3 is consistent with its lack of binding to the G protein (**Fig 6B**)

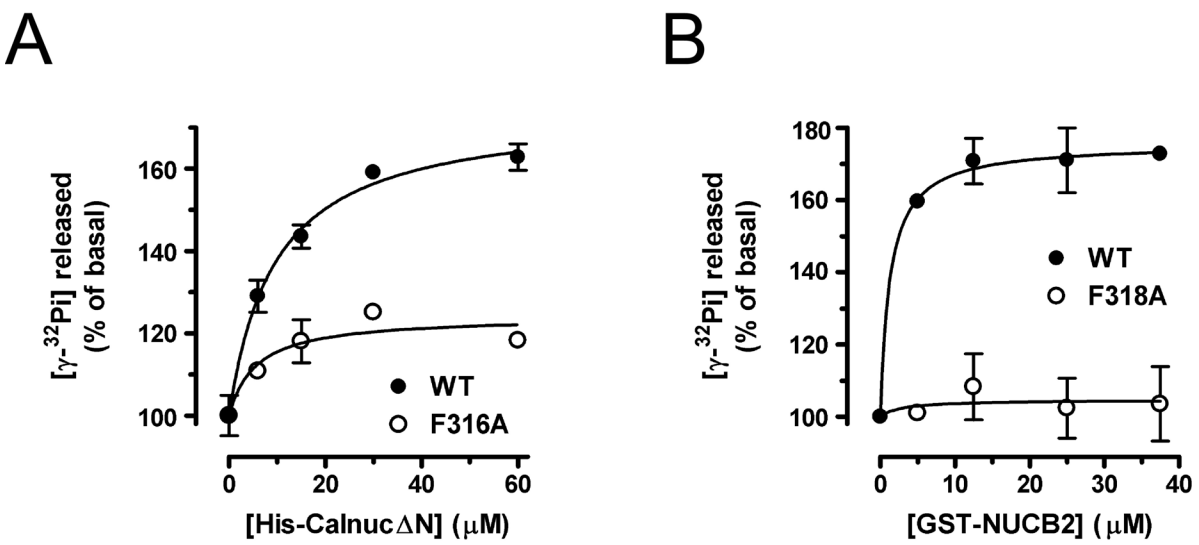

Figure S4

Figure S5. Model illustrating the common and different features of the Gαi interaction with Calnuc or GIV. (A) Alignment of the GBA motif from Calnuc, NUCB2, GIV and the synthetic KB-752 and GSP peptides was performed and analyzed and described in **Fig 1A**. The bar diagram below illustrates the highly conserved "core" of the motif (red) and the N and C-terminal region which are less conserved (blue). The stars (*) represent a non-conserved amino acid in the "core" (corresponding to Calnuc's E314) or several non-conserved amino acids in the C-terminal region; the ψ symbol denotes conserved hydrophobic residues within the motif "core". **(B)** Cartoon illustrating the proposed molecular contacts between Gαi and the GBA motif of Calnuc or GIV. **Left (Calnuc):** Hydrophobic residues of Gαi's SwII (i.e., W211 and F215) interact with hydrophobic residues (conserved) in the "core" of Calnuc's GBA motif (**Fig 6A and Fig S3**). This interaction is further stabilized by an electrostatic interaction between Gαi3's K248 and Calnuc's E314 (**Fig S2**) which is not possible for GIV because this residue is not conserved in its GBA motif (position 4 of the consensus sequence shown in **A**). **Right (GIV):** Hydrophobic residues of Gαi's SwII (i.e., W211 and F215) also interact with hydrophobic residues (conserved) in the "core" of GIV's GBA motif (1). Our published (2) and unpublished data suggest that Gαi's W258 probably makes contact with GIV's residues S1689 and N1690, which is not possible for Calnuc (**Fig 5B**) because these residues in the C-terminal region the GBA motif are not conserved in Calnuc (3 aa downstream of the position 7 of the consensus sequence shown in **A**).

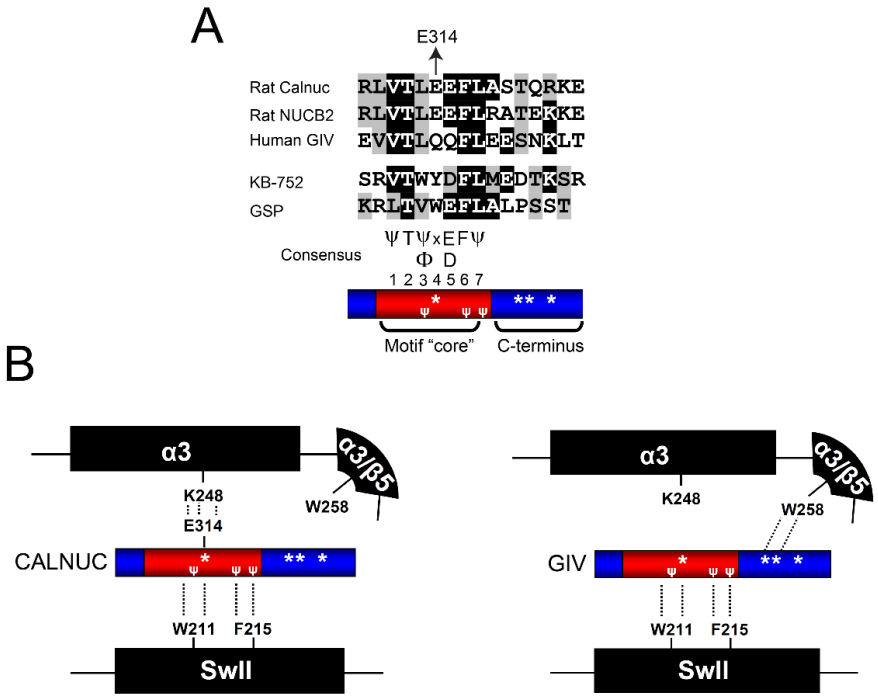

Figure S5

REFERENCES

1. Garcia-Marcos, M., Ghosh, P., and Farquhar, M. G. (2009) *Proc Natl Acad Sci U S A* **106**(9), 3178-3183
2. Garcia-Marcos, M., Ghosh, P., Ear, J., and Farquhar, M. G. (2010) *J Biol Chem* **285**(17), 12765-12777