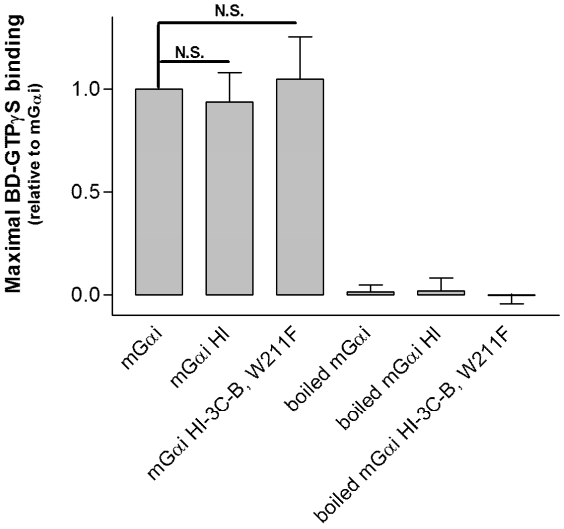
Supplementary Figures

Fig. S1: Pepsin cleavage map of $G\alpha_i$. Peptides indicated by solid arrows indicate peptides used for H/D-exchange analysis due to their abundance, signal-to noise, and isotopic envelopes. Peptides were separated by reverse-phase HPLC, and eluted peptides were unambiguously identified by high-resolution MS/MS fragmentation by collision-induced dissociation as described in methods. Dotted arrows indicate mapped peptides which were not well resolved in Gα H/D exchange experiments. Grey bar and asterisk denotes hexahistadine tag.

Fig. S2: A. Control demonstrating that bimane labeled $G\alpha_i$ HI protein m3C-B, W211F is competent to exchange GDP for BD-GTPγS. Shown are the maximal levels of BD-GTPγS fluorescence (ex/em 485/515 nm) for myr $G\alpha_i$ HI and $G\alpha_i$ HI m3C-B, W211F as compared to maximum level of binding for wild-type myr $G\alpha_i$, left bar, set to 1.0. Boiling abrogated the nucleotide binding properties of all three proteins. BD-GTPγS binding

is measured as increase in BD-GTPγS fluorescence 1 hr after addition of GαGDP as described in methods. Data are the average of 3 independent experiments; results are mean ± SEM. B. Tryptic digests performed as described in methods on $G\alpha_i$ proteins in the inactive (GDP) versus active (GDP-AlF_4) states confirms that the bimane-labeled W211F myr$G\alpha_i$ HI-3C protein (upper panel) retains the ability to undergo activation-dependent changes similar to unlabeled wild-type $G\alpha_i$ protein (bottom panel). U, untreated samples; 5 and 30 refer to bimane labeled samples treated for 5 and 30 minutes, respectively, with wild type $G\alpha_i$ samples digested for periods ranging from 15 minutes to 1.5 hours.

Fig. S2A



S2B.