G-protein (residues)	Sequence (residues 263-289)		
	α3 helix	**α3-β5 Loop**	**β5 sheet**
$G_s\alpha$ (276-282)	NRLQEALNLFKSI	WNNRWLR	TISVILF
$G_t\alpha$	NRMHESLHLFNSI	CNHRYFA	TTSIVLF
$G_s\alpha^{GtL/QL}$ (276-282)	NRLQEALNLFKSI	CNHRYFA	TISVILF

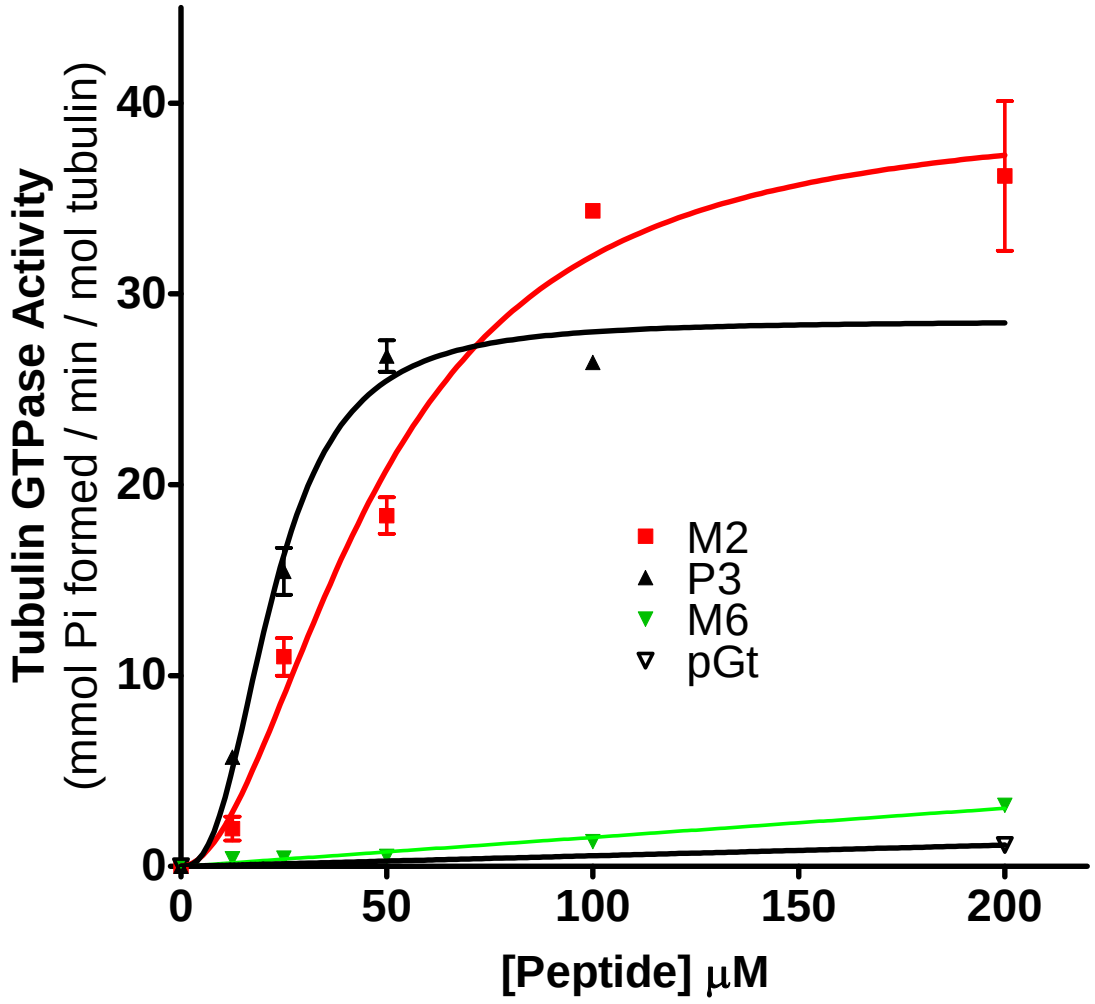
Supplement Table I. Homologous regions on $G_s\alpha$ and $G_t\alpha$ and chimeric protein sequences are shown, and the domains are as indicated above. $G_s\alpha^{GtL/QL}$ is a chimera of $G_s\alpha$ and $G_t\alpha$, and was synthesized as described in "Experimental Procedures" using the forward primer 5' - ctcttcaagagcatctggaatcaccgctacttcgccaccatctctgtgattctg - 3', and its reverse complement as a reverse primer.

PN	KQLQKDKQVYRATHR
PG_tN	EDAEKDARVYRATVK
P3	LNLFKSIWNNRWLRT
PG_t3	L<u>H</u>LF<u>N</u>SI<u>C</u>N<u>H</u>R<u>YFA</u>T
M1	L<u>H</u>LF<u>N</u>SIWNNRWLRT
M2	LNLFKSI<u>C</u>N<u>H</u>RWLRT
M3	LNLFKSIWNNR<u>YFA</u>T
M5	L<u>H</u>LF<u>N</u>SIWNNR<u>YFA</u>T
M6	LNLFKSI<u>C</u>N<u>H</u>R<u>YFA</u>T

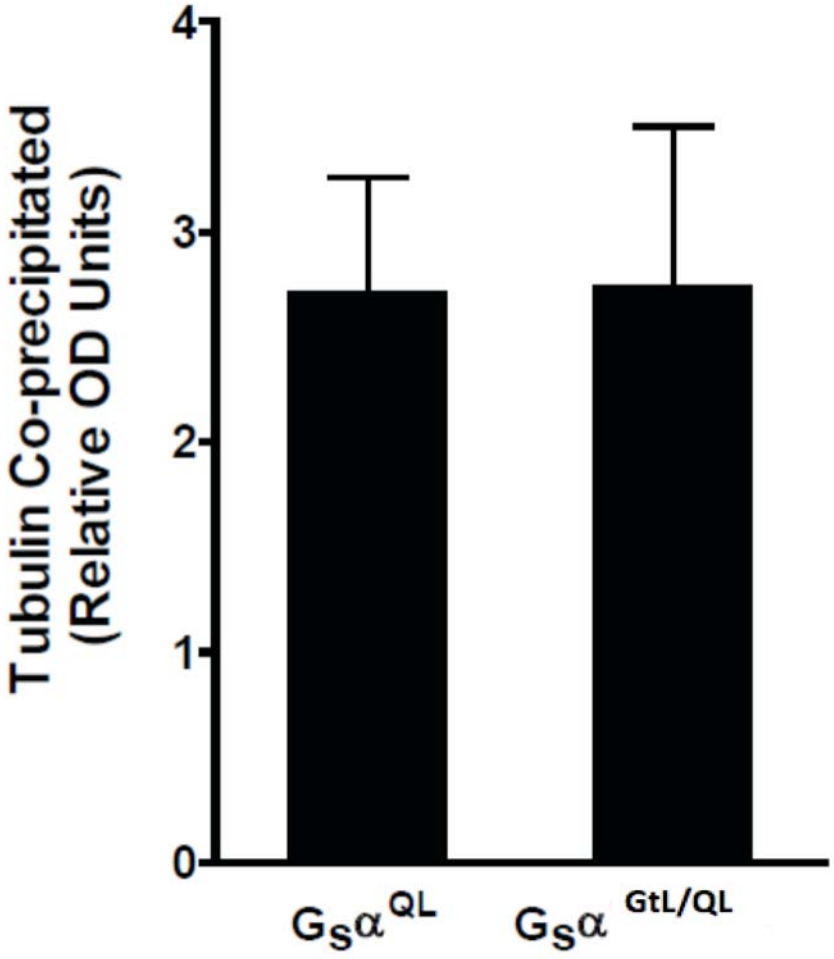
Supplement Table II. Peptide sequences. PN and PG_tN are derived from a region near N-terminal (residues 28-42) of $G_s\alpha$ and $G_t\alpha$, respectively. P3 and peptide G_t3 are derived from α3-β5 region (269-283) of $G_s\alpha$ and $G_t\alpha$, respectively. Peptide M1, M2, M3, M5 and M6 are derivative of P3 in which some residues were replaced by their $G_t\alpha$ homologues (underlined).

Dynamic Instability Parameters	**Control**	**$G_s\alpha^{WT}$ (4 μM)**
Growing Rate (μm/min)	1.6 ± 0.1	1.6 ± 0.2
Shortening Rate (μm/min)	8.9 ± 0.7	9 ± 0.8
Time Growing (%)	39	30
Time Shortening (%)	11	12
Time Attenuated (%)	50	58
Catastrophe Freq (per min)	0.26 ± 0.02	0.28 ± 0.06
Rescue Freq (per min)	1.42 ± 0.2	1.40 ± 0.1
Dynamicity	1.44	1.40

Supplement Table III. Effect of $G_s\alpha^{wt}$ on microtubule dynamic instability parameters. Microtubule dynamic instability parameters were determined in the absence or in the presence of 4 μM $G_s\alpha^{wt}$. $G_s\alpha^{wt}$ did not significantly alter any of the dynamics parameters. Ten microtubules were measured.



Supplement Figure 1. Effect of variant peptides on tubulin GTPase. Variations of peptide P3 were incubated with tubulin-GTP, and tubulin GTPase was determined. Peptides P3, M2 and M6 all bound tubulin with similar affinities. However, only peptides P3 and M2 (not M6) significantly stimulated tubulin GTPase.



Supplement Figure 2. The amount of tubulin co-precipitated with either His-$G_s\alpha^{QL}$ or His-$G_s\alpha^{GtL/QL}$ was quantified on a Coomassie-stained gel, and normalized to the amount of $G_s\alpha$ precipitated. There is no significant difference between parental $G_s\alpha$ and the chimeric $G_s\alpha$, suggesting that the two proteins bind tubulin to a similar extent.