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Table S.1: Additional second site suppressor designs for $G\alpha_{i1}$:GoLoco
Residue numbers above 500 are from GoLoco. The energy terms are described in Table 1 of the main text.

LJ_atr	LJ_rep	Solv	Hb	Elec	Total	dGchn1	dGchn2	mutations							
3.3	-4.2	-3.6	-0.6	0.3	-4.8	-2.7	-2.8	L39M	W211M	I212L	C214A	F215W	V501T	L504F	Q508S
2.1	5.4	-3	-1.1	0.3	3.7			V501T	L504F	Q508S					
6.1	-3.7	-4.1	-0.6	0.3	-2	0.8	-3.5	W211L	L500M	V501A	L504I	Q508S			
1.7	0.8	-2.2	0.4	0	0.9			W211L							
6	-3.8	-2.9	-0.6	-0.2	-1.5	0.4	-2.5	W211L	F215W	L500M	V501A	L504H	Q508S		
2.5	7.8	-1.4	0.4	0	9.4			W211L	F215W						
0.3	0.1	-0.4	0	0	0	-1.3	-2.1	N256W	K257D	W258G	I497G	L500M			
1.2	0	-0.3	0	0	0.9			I497G	L500M						
-0.2	-0.1	-0.3	0	-0.1	-0.8	0.1	-0.1	V72F	E116L	L518D					
1.1	0.1	0.6	0	0.2	1.9			L518D							
-0.2	-0.1	-0.3	0	-0.1	-0.8	0.1	0	V72F	E116L	L518N					
0.9	-0.1	0.9	0	0	1.6			L518N							
0	0.6	-0.3	-0.9	-0.5	-1.1	-2.8	-1	K70R	A71F	V72A	E116L	L518P			
2.4	-0.1	-1.3	0	0	0.9			L518P							
-0.1	-0.2	-0.1	0	0.2	-0.3	-0.5	0.7	S252L	L503V						
1.5	0	0.2	0	0	1.6			L503V							
-1.7	0	-0.2	0	0.2	-1.7	-1.9	0.6	D251E	S252V	L500M					
-1.4	14	-0.6	0	0.2	12.3			D251E	S252V						
5.6	-2.2	-5.5	0.8	0.8	-0.6	-2	-0.7	N76D	Q79S	D523Q					
-0.6	3.7	1.3	-0.1	0.7	4.9			D523Q							
2.9	-2.5	-1.9	-1.2	0.4	-2.3	0.1	-2.2	L39M	W211M	F215W	L500M	V501T	L504Y	Q508S	
4.5	1.5	-2.2	0.4	0	4.2			L39M	W211M	F215W					
0.7	0.6	-0.6	-0.8	-0.8	-0.9	-2.3	0.4	E238L	M240K	R242E	E245V	S246M	V507T		
0.4	0.2	0.8	0	-0.2	1.2			V507T							
6.9	-2.1	-4.3	-1.2	0.4	-0.3	-0.4	-2.8	W211M	F215W	V501T	L504V	Q508S			
1.7	3	-1.5	0.3	0	3.5			W211M	F215W						
3.4	-2.2	-1.7	-0.5	-0.2	-1.2	-1	-3.3	S206V	W211S	I212L	C214A	V501D	Q508M		
2.3	1	-2.3	0	-0.2	0.8			V501D	Q508M						
-5	2.2	4	-1.8	-0.5	-1.1	0.7	0.2	K46D	Q508R						
-2.6	22.6	2.4	0.1	0	22.4			Q508R							
0.3	0.9	-3	-1	0.2	-2.6	-0.4	0.4	E245G	S246M	V507I	Q508M				
-1	3.5	-2.4	0	0.3	0.3			V507I	Q508M						
0.9	1.1	-2.1	0.3	-0.1	0	-0.2	0.9	V72F	E116L	L518F					
0.8	0.3	0.4	0	0.1	1.5			L518F							
-2.5	-0.2	3	-0.7	-0.4	-0.80	1	0.8	K248R	S252M	E502K	L503F				
1.6	-0.2	0.4	0	0	1.80			E502K	L503F						
0.4	-3.9	1.3	0	0	-2.20	-0.4	-1.6	I85L	E528D	F529Y					
0	7.8	1.3	0	0	9.10			I85L							

Table S.2: Additional second site suppressor designs for E6AP:UbcH7
Residue numbers above 600 are from E6AP
The energy terms are described in table 1 of the main text.

LJ_atr	LJ_rep	Solv	Hb	Elec	Total	dGchn1	dGchn2	
-0.2	-1.2	-2.2	0.1	1.7	-1.9	-1.6	-6	Q637A D641Y K96R T99W
-1.7	18.1	-2.1	0.1	1.1	15.5	-1.3	0	Q637W D641Y
-6	-7.5	1	0.6	1	-10.9	-7.6	-2	S638A D641Y M653S R6K F63W P97K A98I
-6.3	86.4	6.4	0.1	-0.5	86.2	0	-2.4	R6K F63W P97K A98I
-2.1	1.2	0.4	0.4	0	-0.1	-4.6	0.7	L628M S638A L639D S695A F63W
-2.4	2.2	1.4	0	0	1.2	0	0.7	F63W
-0.2	-1.3	-2.2	0.1	1.4	-2.2	-1.7	-1.9	Q637W D641F K96R
-1.6	10.3	-2.1	0.1	1.1	7.8	-1	0	Q637W D641F K96R
-1.7	-12.1	0.6	0.2	0.3	-12.7	-6.5	0.1	Y645D M653A A98W
-3.2	27.5	0.8	0	0	25.2	0	-0.4	A98W
-3.3	-12	1.5	0.1	-0.1	-13.8	1.7	4.3	Y645W M653T A98K
-1.1	28.3	0.9	0	0	28.1	0	0.8	A98K
-7.6	3.5	3.7	0.3	-0.4	-0.5	-8.3	-5.7	L642V Y645W M653E I655W R6K A98W
-3.9	27.5	1.6	0.1	-0.1	25.2	0	-5.9	R6K A98W
-0.4	-0.5	-1	0	-0.6	-2.6	-10.8	-1.9	M654A P681A R5Y
-0.8	3	-1	0	0	1.2	0	-1.9	R5Y
1	2.5	-3.3	0.2	-0.8	-0.4	-6.7	-3.1	M654C P681K R5Y K9E
1	2.8	-3.6	0.2	0.4	0.8	0	-3.1	R5Y K9E
-1.9	-0.3	0.1	0.3	0.1	-1.6	-8.2	0	Q658E M670L D672H D678R S4W
-0.7	2.5	0.1	0.5	0.2	2.6	0	-0.7	S4W
-1.3	-0.3	0.7	-0.4	-0.2	-1.3	-2.8	-1.3	Q658S M670W S4T
-0.4	111.1	0.1	0	0	110.8	0	-1.3	S4T
-3.8	2.7	0.5	-0.1	0.5	-0.3	-15.8	-29.9	I659V Q661H Y694T S695A I698G E60W
-5.2	400.3	4.4	0.1	0.4	400	0	-27.7	E60W

Figure S.1. Binding titration of GoLoco V507M with wild type $G\alpha_{i1}$.

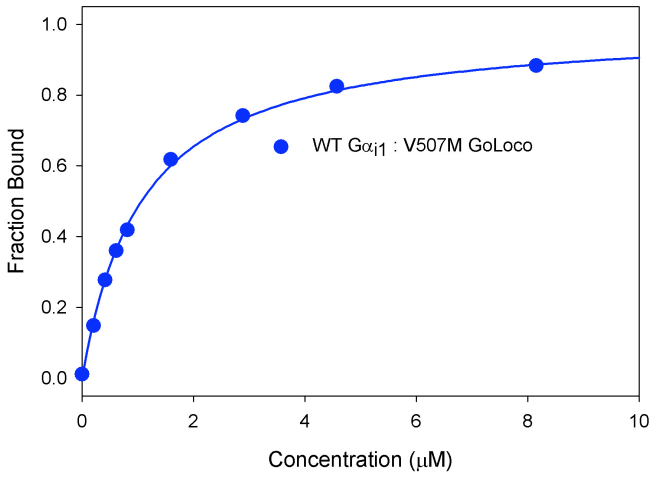


Figure S.2 Binding titrations for GoLoco L503K and $G\alpha_{i1}$ K248E, S252L.

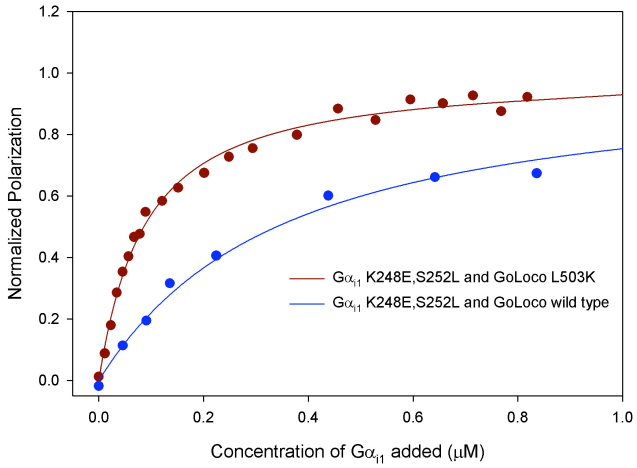


Figure S.3 Binding titration of GoLoco L519T with $G\alpha_{i1}$ I78S, A111Q

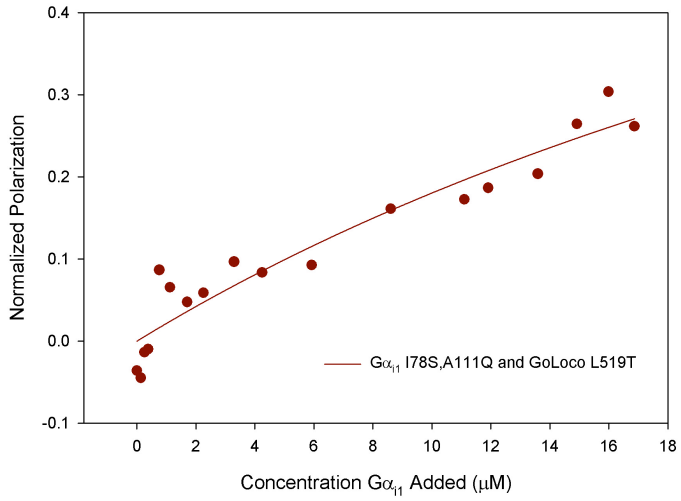


Figure S.4 Binding titrations of UbcH7 K64E and E6AP V634R

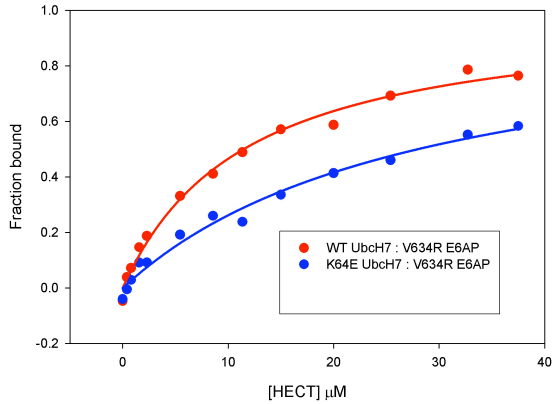


Figure S.5 Binding titrations of UbcH7 E60L and E6AP T662F Q661W. E6AP T662F Q661W is only soluble below a concentration of 10 μM.

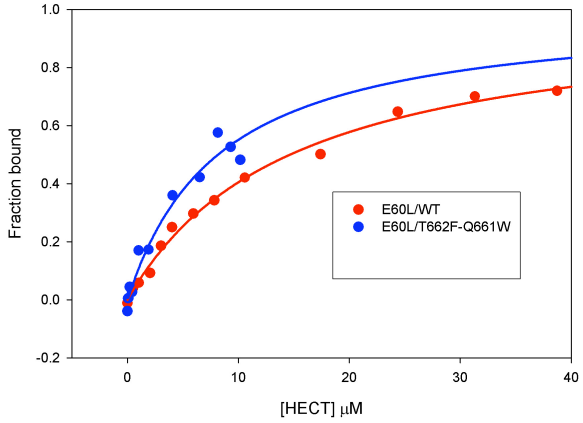
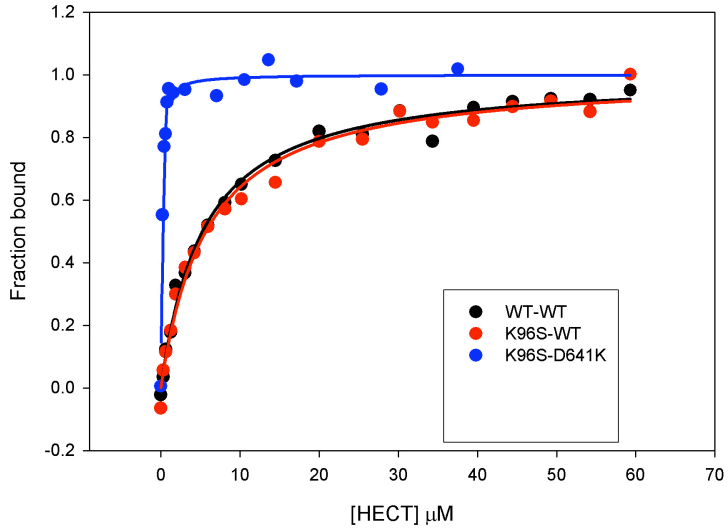


Figure S.6 Binding titrations of UbcH7 K96S, UbcH7 K96S, F63H, A98W and E6AP D641K



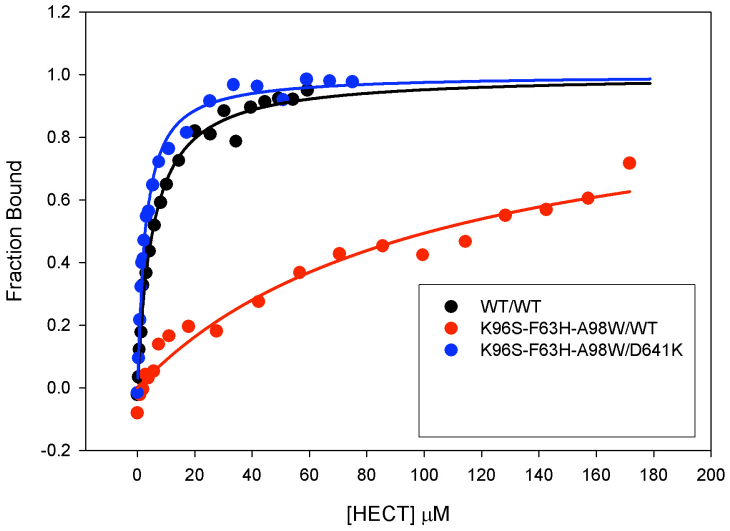


Figure S.7 Plots of calculated versus experimental $\Delta\Delta G^0$ of binding. The top plot shows all the data from table 1, the bottom plot excludes the two points with large positive calculated values (from the repulsive energy score).