Supplement 1 - Sequences used in this study

Murine μOR

MKTIIALSYIFCLVFADYKDDDDAMGPGNISDCSDPLAPASCSPAPGSWLNLSHVDGNQSDPCGPNRTGLG**E**
NLYFQGSHSLCPQTGSPSMVTAITIMALYSIVCVVGLFGNFLVMYVIVRYTKMKTATNIYIFNLALADALATST
LPFQSVNYLMGTWPFGNILCKIVISIDYYNMFTSIFTLCTMSVDRYIAVCHPVKALDFRTPRNAKIVNVCNWIL
SSAIGLPVMFMATTKYRQGSIDCTLTFSHPTWYWENLLKICVFIFAFIMPVLIITVCYGLMILRLKSVRMLSGSK
EKDRNLRRITRMVLVVVAVFIVCWTPIHIYVIIKALITIPETTFQTVSWHFCIALGYTNSCLNPVLYAFLDENFKR
CFREFCIPTSSTI**LEVLFQ**GPEQQNSARIRQNTREHPSTANTVDRTNHQLENLEAETAPLPDIHHHHHH

TEV and 3C sites, respectively, labelled in bold. Receptor was cleaved prior to imaging.

Human $G\alpha_{i1}$

MGCTLSAEDKAAVERSKMIDRNLREDGEKAAREVKLLLLGAGESGKSTIVKQMKIIHEAGYSEE
ECKQYKAVVYSNTIQSIIAIIRAMGRLKIDFGDSARADDARQLFVLAGAAEEGFMTAELAGVIKRL
WKDSGVQACFNRSREYQLNDSAAYYLNDLDRIAQPNYIPTQQDVLRTRVKTTGIVETHFTFKDL
HFKMFDVGGQRSERKKWIHCFEGVTAIIFCVALSDYDLVLAEDEEMNRMHESMKLFDSICNNK
WFTDTSIILFLNKKDLFEEKIKKSPLTICYPEYAGSNTYEEAAAYIQCQFEDLNKRKDTKEIYTHFT
CATDTKNVQFVFDAVTDVIIKNNLKDCGLF

Human $G\beta_1$

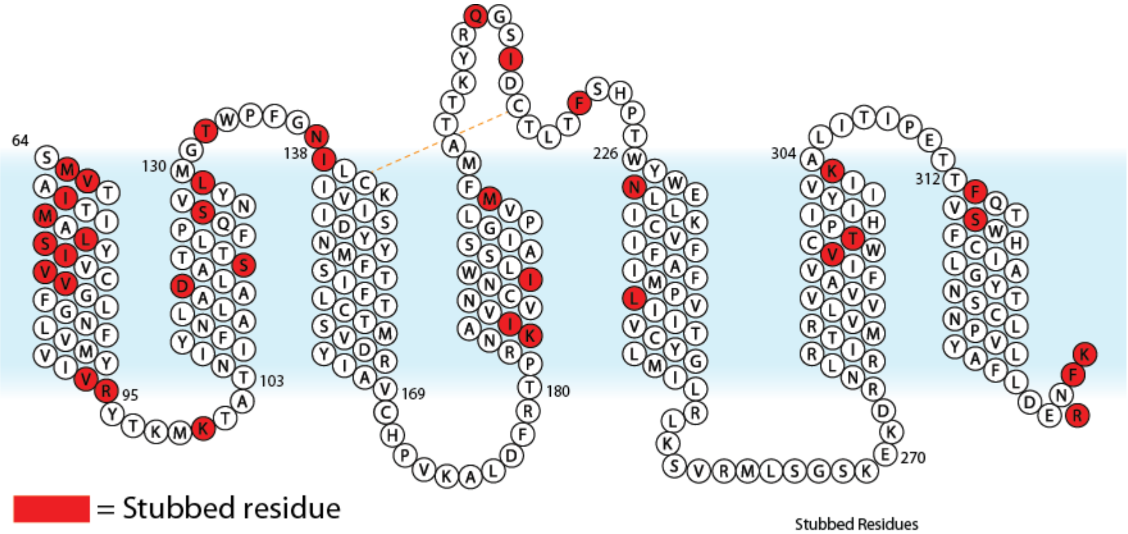
PGSSGSELDQLRQEAEQLKNQIRDARKACADATLSQITNNIDPVGRIQMRTRRTLRGHLAKIYA
MHWGTDSRLLVSASQDGKLIIWDSYTTNKVHAIPLRSSWVMTCAYAPSGNYVACGGLDNICSI
YNLKTREGNVRVSRELAGHTGYLSCCRFLDDNQIVTSSGDTTCALWDIETGQQTTTFTGHTGD
VMSLSLAPDTRLFVSGACDASAKLWDVREGMCRQTFTGHESDINAICFFPNGNAFATGSDDAT
CRLFDLRADQELMTYSHDNIICGITSVSFSKSGRLLLAGYDDFNCNVWDALKADRAGVLAGHD
NRVSCLGVTDDGMAVATGSWDSFLKIWN

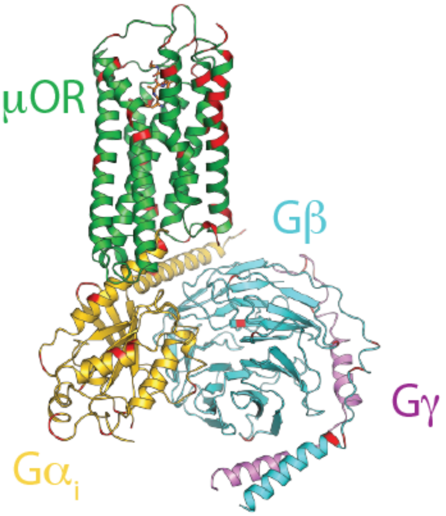
Human $G\gamma_2$

MASNNTASIAQARKLVEQLKMEANIDRIKVSKAAADLMAYCEAHAKEDPLLTPVPASENPFREK
KFFCAIL

scFv16

DVQLVESGGGLVQPGGSRKLSCSASGFAFSSFGMHWVRQAPEKGLEWVAYISSGSGTIYYADTVKGRFTISR
DDPKNTLFLQMTSLRSEDTAMYYCVRSIYYYGSSPFDFWGQGTTLTVSSGGGGSGGGGSGGGGSDIVMTQ
ATSSVPVTPGESVSISCRSSKSLLHSNGNTYLYWFLQRPGQSPQLLIYRMSNLASGVPDRFSGSGSGTAFTLTIS
RLEAEDVGVYYCMQHLEYPLTFGAGTKLELKAAAHHHHHHHH





Stubbed Residues

μOR (Chain R)

TM1	Met 65$^{1.29}$	Val 66$^{1.30}$	Ile 69$^{1.33}$	Met 72$^{1.36}$	Leu 74$^{1.38}$	Ser 76$^{1.40}$
	Ile 77$^{1.41}$	Val 80$^{1.44}$	Val 81$^{1.45}$	Val 94$^{1.58}$	Arg 95$^{1.59}$	
ICL1	Lys 100^{12x51}					
TM2	Asp 114$^{2.50}$	Ser 119$^{2.55}$	Ser 125$^{2.61}$	Leu 129$^{2.65}$		
ECL1	Thr 132^{23x49}					
TM3	Asn 137$^{3.22}$	Ile 138$^{3.23}$				
TM4	Lys 185$^{4.43}$	Ile 186$^{4.44}$	Ile 193$^{4.51}$	Met 203$^{4.61}$		
ECL2	Gln 212	Ile 215	Phe 221			
TM5	Asn 230$^{5.36}$	Leu 246$^{5.52}$				
TM6	Val 291$^{6.46}$	Thr 294$^{6.49}$	Lys 303$^{6.58}$			
TM7	Phe 313$^{7.30}$	Ser 317$^{7.34}$				
Helix 8	Phe 343$^{8.50}$	Lys 344$^{8.51}$	Arg 345$^{8.52}$			

Gα_i (Chain A)

Leu 5	Glu 28	Glu 43	Cys 192	Asp 193	Glu 207	Asn 241
Lys 270	Glu 275	Lys 279	Lys 280	Ile 285	Cys 305	Glu 308
Thr 327	Asp 337	Asp 350				

Gβ (Chain B)

Lys 23	Cys 25	Ser 31	Glu 130	Met 217	Cys 271	Lys 301
Asp 312	Cys 317					

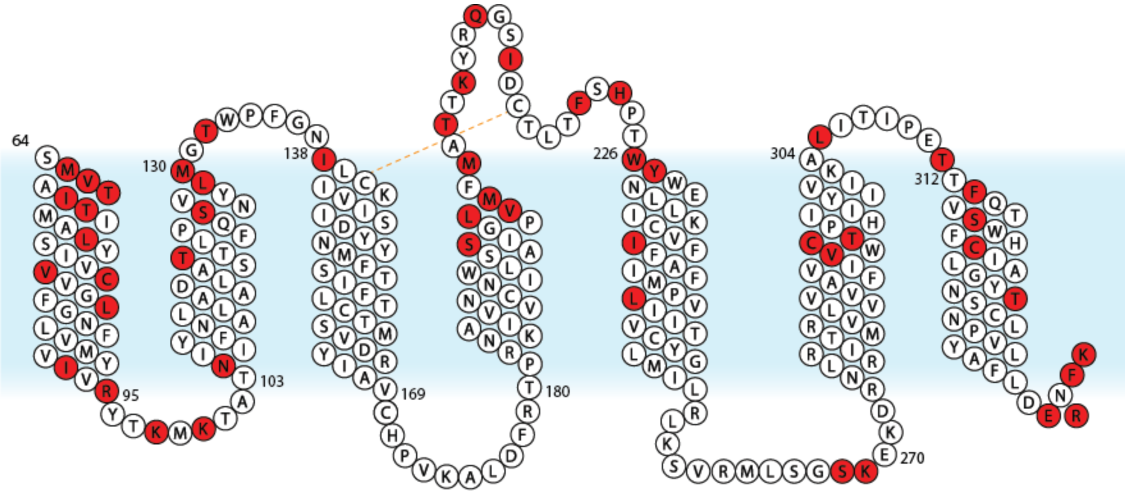
Gγ (Chain C)

Asp 48	Val 54	Ser 57	Glu 58

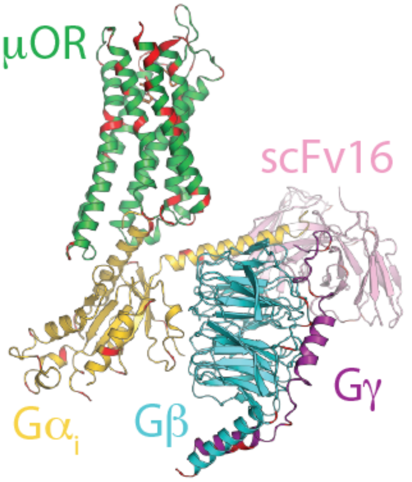
Supplemental Figure 2

Residues of the μOR-G$_i$ complex without scFv16 (scFv16 subtracted) for which we could not observe density were stubbed at the Cβ position. Top: snake diagram of the μOR showing that the majority of stubbed residues were in the extracellular ends of the transmembrane helices, as well as much of TM1.

Bottom left: model of the μOR-G$_i$ complex with stubbed residues colored red. Bottom right: Full list of all residues that we could not model correctly.



🟥 = Stubbed residue



	Stubbed Residues					
uOR (Chain R)						
TM1	Met 65$^{1.29}$	Val 66$^{1.30}$	Thr 67$^{1.31}$	Ile 69$^{1.33}$	Thr 70$^{1.32}$	Leu 74$^{1.38}$
	Cys 79$^{1.43}$	Val 80$^{1.44}$	Leu 83$^{1.47}$	Ile 93$^{1.57}$	Arg 95$^{1.59}$	
ICL1	Lys 98$^{12.49}$	Lys 100$^{12.51}$				
TM2	Asn 104$^{2.40}$	Thr 118$^{2.54}$	Ser 125$^{2.61}$	Leu 129$^{2.65}$	Met 130$^{2.66}$	
ECL1	Thr 132$^{23.49}$					
TM3	Ile 138$^{3.23}$					
TM4	Ser 196$^{4.54}$	Leu 200$^{4.58}$	Val 202$^{4.60}$	Met 203$^{4.61}$	Met 205$^{4.63}$	
ECL2	Thr 207	Lys 209	Ile 215	Phe 221	His 223	
TM5	Trp 226$^{5.32}$	Tyr 227$^{5.33}$	Ile 238$^{5.44}$	Leu 246$^{5.52}$		
ICL3	Ser 268					
TM6	Lys 269$^{6.24}$	Val 291$^{6.46}$	Cys 292$^{6.47}$	Thr 294$^{6.49}$	Leu 305$^{6.60}$	
TM7	Thr 311$^{7.28}$	Phe 313$^{7.30}$	Ser 317$^{7.34}$	Cys 321$^{7.38}$	Thr 327$^{7.44}$	
Helix 8	Glu 341$^{8.48}$	Phe 343$^{8.50}$	Lys 344$^{8.51}$	Arg 345$^{8.52}$		
Gai (Chain A)						
Asp 26	Glu 28	Glu 43	Lys 46	Glu 186	Aps 193	Glu 207
Asp 229	Leu 232	Val 233	Lys 248	Lys 257	Asp 272	Lys 280
Glu 289	Glu 297	Thr 327	Asp 328	Asp 350		
Gb (Chain B)						
Asp 5	Gln 6	Cys 25	Gln 32	Asn 36	Asp 38	Glu 130
Met 217						
Gg (Chain C)						
Gln 11	Lys 14	Glu 17	Lys 20	Asp 26	Asp 48	Glu 58
scFv16 (Chain E)						
Ser 17	Glu 42	Ser 52	Ser 53	Asp 73	Glu 89	Met 180
Asp 189	Thr 198	Glu 210	Glu 234			

Supplemental Figure 3

Residues of the μOR-G$_i$ complex with scFv16 for which we could not observe density were stubbed at the Cβ position. Top: snake diagram of the μOR showing that the majority of stubbed residues were in the extracellular ends of the transmembrane helices, as well as much of TM1.

Bottom left: model of the μOR-G$_i$ complex with stubbed residues colored red. Bottom right: Full list of all residues that we could not model correctly.