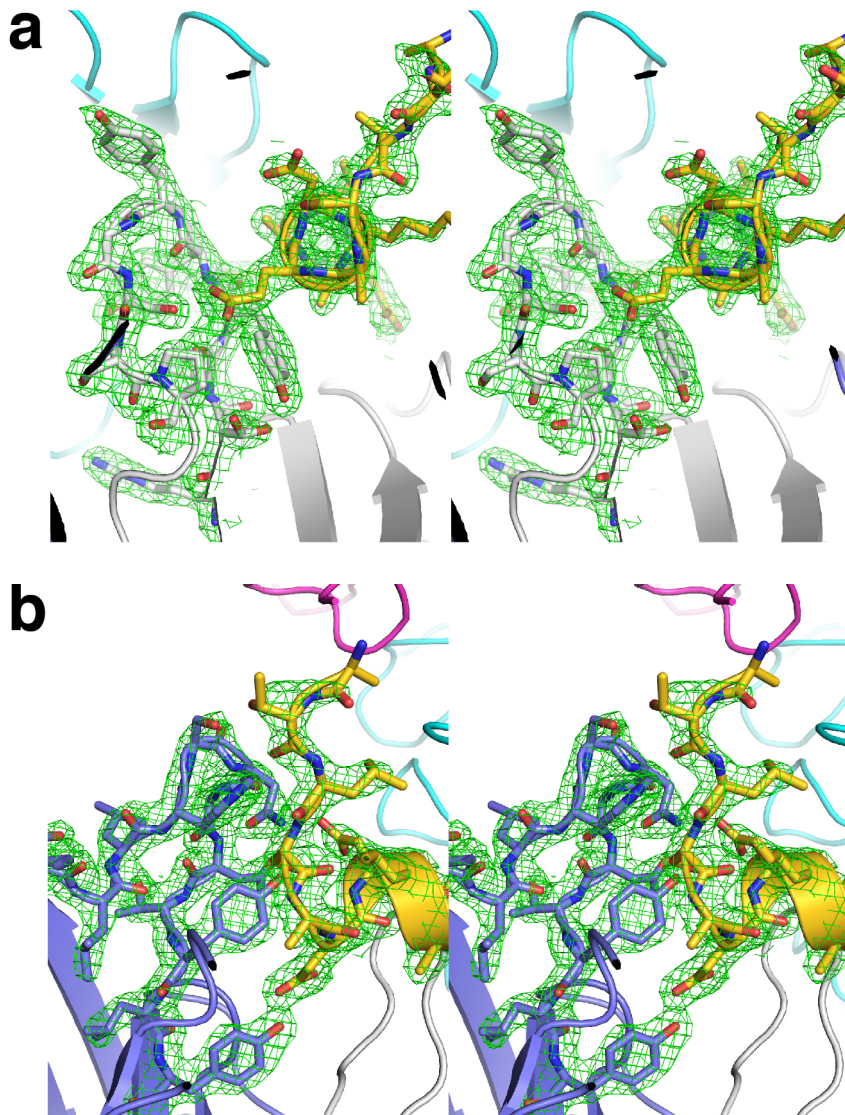


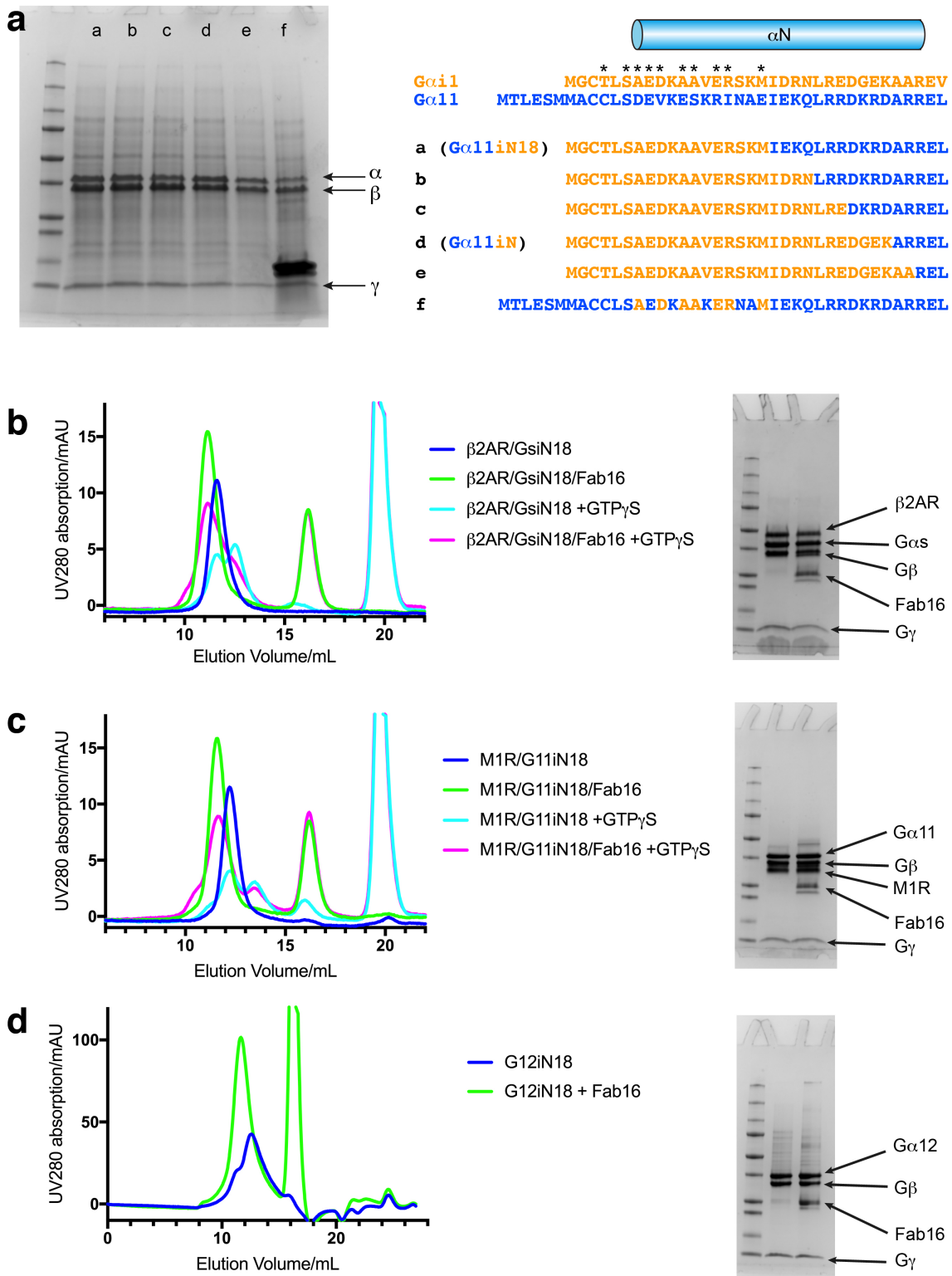
Supplementary Information

Development of an antibody fragment that stabilizes GPCR/G-protein complexes

Maeda et al.



Supplementary Figure 1. Electron density map. Stereo view of the electron density map of **(a)** CDR-H3/ α N helix interface and **(b)** CDR-L1/ α N helix. The electron density is shown as green mesh contoured at 1.5σ . Color scheme is the same as in the main text, and the residues are described as cartoon and sticks in the density map.



Supplementary Figure 2. Minimal chimera constructs for G $_s$, G $_{11}$, and G $_{12}$. (a) Left: Coomassie stained SDS-PAGE gel of chimera constructs co-expressed with G $\beta\gamma$. Proteins were purified from the solubilized lysate by Ni-affinity chromatography. The bands corresponding each subunit are labeled. Right: Sequences of the designed

chimeras corresponding to the labels in the SDS-PAGE. Region of α N helix is shown as a cylinder and the residues interacting with scFv16 are marked with the asterisks. **(b, c)** Analytical SEC of β 2AR/ G_{siN18} and M_1R/G_{11iN18} complexes incubated with GTP γ S in the presence or absence of Fab16. Coomassie-stained SDS-PAGE gels of peak fractions corresponding to the β 2AR/ G_{siN18} or M_1R/G_{11iN18} alone (middle lane), and β 2AR/ G_{siN18} /Fab16 or M_1R/G_{11iN18} /Fab16 (right lane). **(d)** Analytical SEC of G_{12iN18} in the presence or absence of Fab16. Coomassie-stained SDS-PAGE gels of peak fractions corresponding to G_{12iN18} alone (middle lane), and G_{12iN18} /Fab16 (right lane).

Gβ1	-----MSELDQLRQE	10
Gβ2	-----MSELEQLRQE	10
Gβ3	-----MGEMEQLRQE	10
Gβ4	-----MSELEQLRQE	10
Gβ5	MCDQTFVLVNVFGSCDKCFKQRALRPVFKKSQQLSYCSTCAEIMATEGLHENETLASLKSE	60
Gβ1	AEQLKNQIRDARKACADATLSQITNNIDPVGRIQMRTRRTLRGHLAKIYAMHWGTD S RLL	70
Gβ2	AEQLRNQIRDARKACGDSTLTQITAGLDPVGRIQMRTRRTLRGHLAKIYAMHWGTD S RLL	70
Gβ3	AEQLKKQIADARKACADVTLAELVSGLEVVGRVQMRTRRTLRGHLAKIYAMHWATD S KLL	70
Gβ4	AEQLRNQIQDARKACNDATLVQITSNMDSVGRIQMRTRRTLRGHLAKIYAMHWGYD S RLL	70
Gβ5	AESLKGKLEERAKLHDVELHQVAERVEALGQFVMKTRRTLKGHGKVL C MDWCKDK R RI	120
Gβ1	VSASQDGKLIWDSYTTNK V HAIPLRSSWVMTCAYAPSGNYVACGGLDNIC S IYNLKT R E	130
Gβ2	VSASQDGKLIWDSYTTNK V HAIPLRSSWVMTCAYAPSGNFVACGGLDNIC S IYSLKT R E	130
Gβ3	VSASQDGKLIWDSYTTNK V HAIPLRSSWVMTCAYAPSGNFVACGGLDNM C S IYNLKS R E	130
Gβ4	VSASQDGKLIWDSYTTNK M HAIPLRSSWVMTCAYAPSGNYVACGGLDNIC S IYNLKT R E	130
Gβ5	VSSSQDGKVIWDSFTTN K E H AVTMPCTWVMACAYAPSGCAIACGGLDNK C SVYPL T FD K	180
Gβ1	G N--VRVSRELAGHTGYLSCCRFLD-DNQIVTSSGD T TCALWDIETGQ Q TTTTFTGHTGDV	187
Gβ2	G N--VRVSRELPGHTGYLSCCRFLD-DNQIITSSGD T TCALWDIETGQ Q TVGFAGHSGDV	187
Gβ3	G N--VKVSRELSAHTGYLSCCRFLD-DNNIVTSSGD T TCALWDIETGQ Q KTVFVGHTGDC	187
Gβ4	G N--VRVSRELPGHTGYLSCCRFLD-DSQIVTSSGD T TCALWDIETAQ Q TTTTFTGHSGDV	187
Gβ5	N ENMAAKKKS V AMHTNYLSACSFTNSDMQ I L T ASGDGTCALWDV E SGQL L QSFHGHGADV	240
Gβ1	MSLSLAPD--TRLFVSGACDASAKLWDVREGMC R Q T FTGHESDINAICFFPNGNAFATGS	245
Gβ2	MSLSLAPD--GRTFVSGACDASIKLWDV R DSMC R Q T FTGHESDINAVAFFPNGYAF T TGS	245
Gβ3	MSLAVSPD--FNLFISGACDASAKLWDVREG T CR Q TFTGHESDINAICFFPN G EAI C TGS	245
Gβ4	MSLSLSPD--MRTFVSGACDASSKLWDIRDGMC R Q S FTGHVSDINAVSFFPNGYAFATGS	245
Gβ5	LCLDLAPSETGNTFVSGGCDKKAMVWDMRSG Q C V QAF E THESDINSVRY P SGDAFASGS	300
Gβ1	DDATCRLFDL R ADQ E LMYSHDNIICGITSV S FSKSGRLLLAGYDDFNCNVWDAL K ADRA	305
Gβ2	DDATCRLFDL R ADQ E LLMYSHDNIICGITSV A FSRSGRLLLAGYDDFNCNIWDAM K GDRA	305
Gβ3	DDASCRLFDL R ADQ E LICFSHESIIICGITSV A FSLSGRLLFAGYDDFNCNVWDSM K SERV	305
Gβ4	DDATCRLFDL R ADQ E LLLYSHDNIICGITSV A FSKSGRLLLAGYDDFNCNVWDTL K GDRA	305
Gβ5	DDATCRLYDL R ADREVAI Y SKESII F GASSVDFSLSGRLLFAGYNDY T INVWDVL K GSRV	360
Gβ1	GVLAGHDNRV S CLGVTDDGMAVATGSWDSFLKI W N	340
Gβ2	GVLAGHDNRV S CLGVTDDGMAVATGSWDSFLKI W N	340
Gβ3	GILSGHDNRV S CLGVTADGMAVATGSWDSFLKI W N	340
Gβ4	GVLAGHDNRV S CLGVTDDGMAVATGSWDSFLRI W N	340
Gβ5	SILFGHENRV S TLRVSPDG T AFCSGSWDHTLRV A	395

Supplementary Figure 3. Sequence alignment of G-protein β-subunits. Multiple sequence alignment of Gβ subunit from human. The residues interacting with scFv16 in the crystal structure were shown with the black background.

Heavy chain

AB16_HC MDSRLNLVFL VLTLKGVQCD VQLVESGGGL VQPGGSRKLS CSASGFAFSS FGMHWRQAP 60
--leader sequence--- CDR1

AB16_HC EKGLEWVAYI SSGSGTIYYA DTVKGRFTIS RDDPKNTLFL QMTSLRSEDY AMYYCVRSIY 120
CDR2

AB16_HC YYGSSPFDY WQGTTLTVS SAKTTPPSVY PLAPGCGDIT GSSVTLGCLV KGYFPESVTV 179
CDR3 ---constant region-----

AB16_HC SGPISTINPC PPKCECHKCP APNLEGGPSV FIFPPNIKDV LMISLTPKVT CVVVDVSEDD 299
-----constant region-----

AB16_HC PDVQISWVFN NVEVHTAQTQ THREDYNSTI RVVSTLPIQH QDWMSGKEFK CKVNNKDLPS 359
-----constant region-----

AB16_HC PIERTISKIK GLVRAPQVYI LPPPAEQLSR KDVSLTCLVV GFNPGDISVE WTSNGHTEEN 419
-----constant region-----

AB16_HC YKDTAPVLDS DGSYFIYSKL NMKTSKWEKT DSFSCNVRHE GLKNYYLKKT ISRSPGK 476
-----constant region-----

Light chain

AB16_LC MRCLAEFLGL LVLWIPGAIG DIVMTQATSS VPVTPGESVS ISCRSSKSL HSNGNTYLY 59
---leader sequence--- -----CDR1-----

AB16_LC WFLQRPGQSP QLLIYRMSNL ASGVPDRFSG SSGGTAFTLT ISRLEAEDVG VYYCMOHLEY 119
--CDR2-- --CDR3

AB16_LC PLTFGAGTKL ELKRADAAPT VSIFPPSSEQ LTSGGASVVC FLNNFYPKDI NVKWKIDGSE 179
CDR3 -----constant region-----

AB16_LC RQNGVLNSWT DQDSKDSTYS MSSTLTLTKD EYERHNSYTC EATHKTSTSP IVKSFNRNEC 239
-----constant region-----

Supplementary Note 1. Amino-acid sequence of mAb16. Sequence of Heavy- and light-chain of mAb16 is provided. Leader sequence, CDRs, constant region is indicated under the amino-acid sequence.

Supplementary Note 2 Amino acid sequences of the constructs used in the study

HA signal sequence highlighted in purple

FLAG-tag highlighted in cyan

His-tag highlighted in green

ICL3 highlighted in grey

Sequences from G α i1 colored in orange

M1R

MKTIIALSYIFCLVFA^{HA}DYKDDDD^{FLAG}AAAQTSAPPAVSPQITVLAPGKGPWQVAFIGI
TTGLLSLATVTGNLLVLISFKVNTTELKTVNNYFLLSLACADLIIGTFSMNLYTTY
LLMGHWALGTLACDLWLALDYVASQASVMNLLLISFDRYFSVTRPLSYRAKR
TPRRAALMIGLAWLVSFVLWAPAILFWQYLVGERTVLAGQCYIQFLSQPIITFG
TAMAAFYLPVTVMCTLYWRIYRETENRARELAALQGSETPGGKEQLAKRKTFS
LVKEKKAARTLSAILLAFILTWTPYNIMVLVSTFCKDCVPETLWELGYWLCYV
NSTINPMCYALCNKAFRDTFRLLLLCRWDKRRWRKIPKRPGSVHRTPSRQC^{His}
^{His}HHHH*

M2R

MKTIIALSYIFCLVFA^{HA}DYKDDDD^{FLAG}ASTDSSDNSLALTSPYLEKTFEVVFIVLVAGS
LSLVTHIGNILVMVSIKVNRLHLQTVNNYFLFSLACADLIIGVFSMNLYTLYTVIGY
WPLGPVCDLWLALDYVVSNASVMNLLIISFDRYFCVTKPLTYPVKRTTKMAG
MMIAAAWVLSFILWAPAILFWQFIVGVRTVEDGECYIQFFSNAAVTFGTAAIAAF
YLPVIIMTVLYWHISRASKSRIKKDKKEPVANQDPVSIVARKIVKMTKQPAKKK
PPPSREKKVTRTILAILLAFIITWAPYNMVLINTFCAPCIPNTVWTIGYWLCYIN
STINPACYALCNATFKKTFKLLMCHYKNIGATRPAG^{His}HHHHHHHH*

G α iN

MGCTLSAEDKAA^{G α i1}VERSKMIDRN^{G α i1}REDGEKAA^{G α i1}ATHRLLLLGAGESGKSTIVKQ
MRILHVNGFNGDSEKATKVQDIKNNLKEAIETIVAAMSNLVPPVELANPENQFR
VDYILSVMNVPDFDFPPEFYEHAKALWEDEGVRACYERSNEYQLIDCAQYFLD
KIDVIKQADYVPSDQDLLRCRVLTSGIFETKFQVDKVNFMFDVGGQRDERRK
WIQCFNDVTAIIFV^{G α i1}ASSYNMVIREDNQTNRLQEALNLFKSIWNNRWLRTISVI

LFLNKQDLLAEKVLGKSKIEDYFPEFARYTTPEDATPEPGEDPRVTRAKYFIRD
EFLRISTASGDGRHYCYPHFTCAVDTENIRRVFNDCRDIIQRMHLRQYELL*

G α iN18

MGCTLSAEDKAAVERSKMIEKQLQKDKQVYRATHRLLLLGAGESGKSTIVKQ
MRILHVNGFNVDSEKATKVDIKNNLKEAIETIVAAMSNLVPPVELANPENQFR
VDYILSVMNVPDFDFPPEFYEHAKALWEDEGVRACYERSNEYQLIDCAQYFLD
KIDVIKQADYVPSDQDLLRCRVLTSGIFETKFQVDKVNFMFDVGGQRDERRK
WIQCFNDVTAIIFVVASSYNMVIREDNQTNRLQEALNLFKSIWNNRWLRTISVI
LFLNKQDLLAEKVLGKSKIEDYFPEFARYTTPEDATPEPGEDPRVTRAKYFIRD
EFLRISTASGDGRHYCYPHFTCAVDTENIRRVFNDCRDIIQRMHLRQYELL*

G α 11iN18 (Construct **a** in Supplementary Figure 1a)

MGCTLSAEDKAAVERSKMIEKQLRRDKRDARRELKLLLLGTGESGKSTFIKQM
RIIHGAGYSEEDKRGFTKL VYQNIFTAMQAMIRAMETLKILYKYEQNKANALLI
REVDVEKVTTFEHQYVSAIKTLWEDPGIQECYDRRREYQLSDSAKYYLTDVDR
IATLGYLPTQQDVL RVRVPTTGII EYPFDLENIIFRMVDVGGQRSERRKWIHCFE
NVTSIMFLV ALSEYDQVLVESDNENRMEESKALFR TIITYPWFQNSSVILFLNKK
DLLEDKILYSHLVDYFPEFDGPQRDAQAAREFILKMFVDLNPDSDKIIYSHFTCA
TDTENIRFVFAAVKDTILQLNLKEYNLV*

Construct **b** in Supplementary Figure 1a

MGCTLSAEDKAAVERSKMIDRNLRRDKRDARRELKLLLLGTGESGKSTFIKQM
RIIHGAGYSEEDKRGFTKL VYQNIFTAMQAMIRAMETLKILYKYEQNKANALLI
REVDVEKVTTFEHQYVSAIKTLWEDPGIQECYDRRREYQLSDSAKYYLTDVDR
IATLGYLPTQQDVL RVRVPTTGII EYPFDLENIIFRMVDVGGQRSERRKWIHCFE
NVTSIMFLV ALSEYDQVLVESDNENRMEESKALFR TIITYPWFQNSSVILFLNKK
DLLEDKILYSHLVDYFPEFDGPQRDAQAAREFILKMFVDLNPDSDKIIYSHFTCA
TDTENIRFVFAAVKDTILQLNLKEYNLV*

Construct **c** in Supplementary Figure 1a

MGCTLSAEDKAAVERSKMIDRNLEDKRDARRELKLLLLGTGESGKSTFIKQM
RIIHGAGYSEEDKRGFTKL VYQNIFTAMQAMIRAMETLKILYKYEQNKANALLI

REVDVEKVTTFEHQYVSAIKTLWEDPGIQECYDRRREYQLSDSAKYLLTDVDR
IATLGYLPTQQDVLRLVRVPTTGIIIEYFPDLENIIFRMVDVGGQRSERRKWIHCFE
NVTSIMFLValseyDQVLVESDNENRMEESKALFRTIITYPWFQNSSVILFLNKK
DLLEDKILYSHLVDYFPEFDGPQRDAQAAREFILKMFVDLNPDSDKIIYSHFTCA
TDTENIRFVFAAVKDTILQLNLKEYNLV*

G α 11iN (Construct **d** in Supplementary Figure 1a)

MGCTLSAEDKAAVERSKMIDRNLEDGEKARRELKLLLLGTGESGKSTFIKQM
RIIHGAGYSEEDKRGFTKL VYQNIFTAMQAMIRAMETLKILYKYEQNKANALLI
REVDVEKVTTFEHQYVSAIKTLWEDPGIQECYDRRREYQLSDSAKYLLTDVDR
IATLGYLPTQQDVLRLVRVPTTGIIIEYFPDLENIIFRMVDVGGQRSERRKWIHCFE
NVTSIMFLValseyDQVLVESDNENRMEESKALFRTIITYPWFQNSSVILFLNKK
DLLEDKILYSHLVDYFPEFDGPQRDAQAAREFILKMFVDLNPDSDKIIYSHFTCA
TDTENIRFVFAAVKDTILQLNLKEYNLV*

Construct **e** in Supplementary Figure 1a

MGCTLSAEDKAAVERSKMIDRNLEDGEKAARELKLLLLGTGESGKSTFIKQM
RIIHGAGYSEEDKRGFTKL VYQNIFTAMQAMIRAMETLKILYKYEQNKANALLI
REVDVEKVTTFEHQYVSAIKTLWEDPGIQECYDRRREYQLSDSAKYLLTDVDR
IATLGYLPTQQDVLRLVRVPTTGIIIEYFPDLENIIFRMVDVGGQRSERRKWIHCFE
NVTSIMFLValseyDQVLVESDNENRMEESKALFRTIITYPWFQNSSVILFLNKK
DLLEDKILYSHLVDYFPEFDGPQRDAQAAREFILKMFVDLNPDSDKIIYSHFTCA
TDTENIRFVFAAVKDTILQLNLKEYNLV*

Construct **f** in Supplementary Figure 1a

MTLESMMACCLS**AEDKAAKER**NAMIEKQLRRDKRDARRELKLLLLGTGESGK
STFIKQMRIIHGAGYSEEDKRGFTKL VYQNIFTAMQAMIRAMETLKILYKYEQN
KANALLIREVDVEKVTTFEHQYVSAIKTLWEDPGIQECYDRRREYQLSDSAKY
YLLTDVDRIATLGYLPTQQDVLRLVRVPTTGIIIEYFPDLENIIFRMVDVGGQRSERR
KWIHCFENVTSIMFLValseyDQVLVESDNENRMEESKALFRTIITYPWFQNSS
VILFLNKKDLLEDKILYSHLVDYFPEFDGPQRDAQAAREFILKMFVDLNPDSDKI
IYSHFTCATDTENIRFVFAAVKDTILQLNLKEYNLV*

Gα.12iN18

MGCTLSAEDKAAVERSKMIDALLARERRAVRRLVKILLLGAGESGKSTFLKQM
RIIHGREFDQKALLEFRDTIFDNILKGSRVLVDARDKLGIPWQYSENEKHGMFL
MAFENKAGLPVEPATFQLYVPALSALWRDSGIREAFSRRSEFQLGESVKYFLDN
LDRIGQLNYFPSKQDILLARKATKGIVEHDFVIKKIPFKMVDVGGQRSQRQKWF
QCFDGITSILFMVSSSEYDQVLMEDRRTNRLVESMNIFETIVNNKLFFNVSILFL
NKMDLLVEKVKTVSIKKHFPDFRGDPHRLEDVQRYLVQCFDRKRRNRSKPLFH
HFTTAIDTENVRFVFHAVKDTILQENLKDIMLQ*

Supplementary Table Primer sequences used in this study

M2R ICL3 extension primers	
	TCTTGACGATCTTGCGTGCAACGATGCTAACGGGGTCCTGGTTAGCCACC
	TGACTAAGCAACCTGCTAAGAAGAAGCCCCCTCCATCAAGGGAG
M1R ICL3 extension primers	
	GGGCTGCCAGCTCCCGTGCTCGGTTCTCTGTCTCCCGGTAGATG
	GCCTGGCGTCTCGGAGCCCTGAAGGGCTGCCAGCTCCCGTGCTCGG
	AAGCGGAAGACCTTCTCGCTGGTCAAGGAGAAGAAGGCGGC
	GGAAAGGAGCAGCTGGCCAAGCGGAAGACCTTCTCGCTGGTC
GsiN primers	
	GCTATAACTCGAGGAGCCTGCAGTCTCGAGGCATGCGGTACC
	GGCGGTGCGTGGCCGCCGCTTCTCGCCGTCCTCACGG
	GAAGGCGGCGGCCACGCACCGCCTGCTGCTGCTGGG
	CGAGACTGCAGGCTCCTCGAGTTATAGCAGCTCGTACTGACG
GsiN18 primers	
	AAGGACAAGCAGGTCTACCGGGCCACGCACCGCCTGCTGCTGCTGGG
	CTGCAGCTGCTTCTCGATCATCTTACTCCGCTCCACCG
G11iN primers	
	GGAGTACAACCTGGTCTGACTCGAGTCTAGAGCCTGCAGTCTCG
	GGCTCTAGACTCGAGTCAGACCAGGTTGTACTCCTTGAGGTTGAGC
Construct a	CAGCTGCTTCTCGATCATCTTACTCCGCTCCACCGCC
	GGAGTAAGATGATCGAGAAGCAGCTGCGGCGGGAC
Construct b	GCTTGTCGCGCCGCAGGTTGCGGTCGATCATCTTACTCCGC
	GATCGACCGCAACCTGCGGCGGGACAAGCGCGACG
Construct c	CGTCGCGCTTGTCTCACGGAGGTTGCGGTCGATC
	GCAACCTCCGTGAGGACAAGCGCGACGCCCGGCGCG
Construct d (G11iN18)	GCTCGCGCCGGCCTTCTCGCCGTCCTCACGGAGG
	GGACGGCGAGAAGGCCCGGCGCGAGCTCAAGCTGC
Construct e	GCAGCTTGAGCTCGCGCGCCGCTTCTCGCCGTCCTC
	CGAGAAGGCGGCGCGCGAGCTCAAGCTGCTGCTGC
Construct f	AAGGAGCGCAACGCCATGATCGAGAAGCAGCTGCGGCGGG
	GGCCGCCTTGTCTCTGCGCTCAGGCAACACGCCATCATGG
G12iN primers	
	GCTGCAGTGAAGTCTAGAGCCTGCAGTC
	AGCGCGTCGATCATCTTACTCCGCTCCACCGCC
	GCGGAGTAAGATGATCGACGCGCTGCTGGCCCGCG
	CTAGACTCGAGTCACTGCAGCATGATGTCCTTC

