Trametinib Induces the Stabilization of A Dual *GNAQ* p.Gly48Leu- and *FGFR4* p.Cys172Gly-Mutated Uveal Melanoma. The Role of Molecular Modelling in Personalized Oncology

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Appendix A

FoldX procedure

FoldX is a well-known software for predicting changes in free energy of folding caused by mutations. It permits a rapid estimation of the effect of mutations on the stability of proteins and nucleic acids. Its predictive efficiency has been trained on a large set of mutants covering most of the existing structural environment. The energy terms used in FoldX estimation calculations have been weighted using empirical data obtained from protein engineering experiments [http://foldxsuite.crg.eu/products]. Potapov et al. [1] demonstrated that FoldX_3.0 shown the best correlation (r = 0.96) between experimental and average predicted change in protein stability values (ΔΔG), compare to other software like Rosetta and I-Mutant_2.0.

To predict the potential impact of a mutation in a structure, the FoldX command line *PositionScan* was used. In this procedure, the selected residue is first mutated to alanine and all neighbors residues are identified. Then, FoldX "mutates" the wild-type residue (selected position) to itself. Then, the neighbor residues are "mutated" to themselves; this is followed each time by the "mutation" of the wild-type selected position to itself. This process guarantees that the virtual *real* mutation by FoldX will potentially move only residues that were moved in the wild-type reference. Finally, the selected position is mutated into the desired residue.

Command line example:

```
FoldX --command=PositionScan --pdb=PDB_ID --positions=GA48L
```

In this example, FoldX will mutate Gly48 from the chain A into Leucine.

The output result corresponds to the ΔΔG in kcal/mol between the wild type and the mutant free energy structures ($\Delta\Delta G = \Delta G_{Mutant} - \Delta G_{Wild\text{-}Type}$).

Experimental structure selection

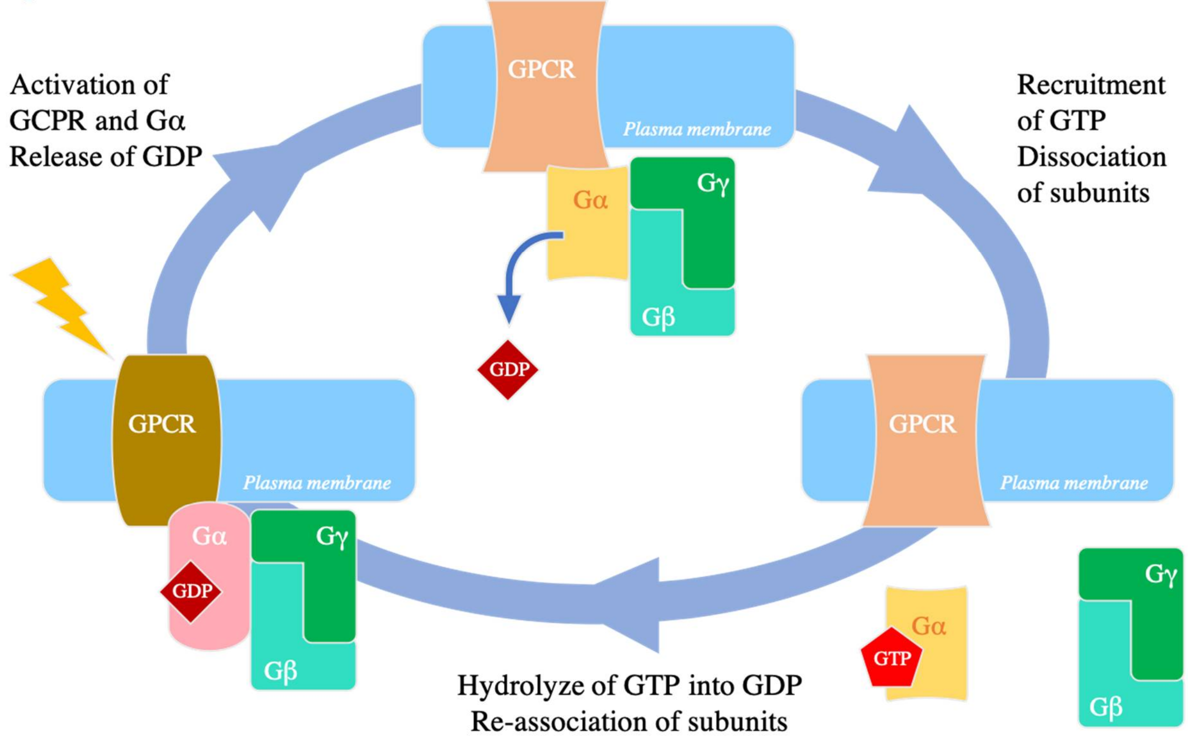
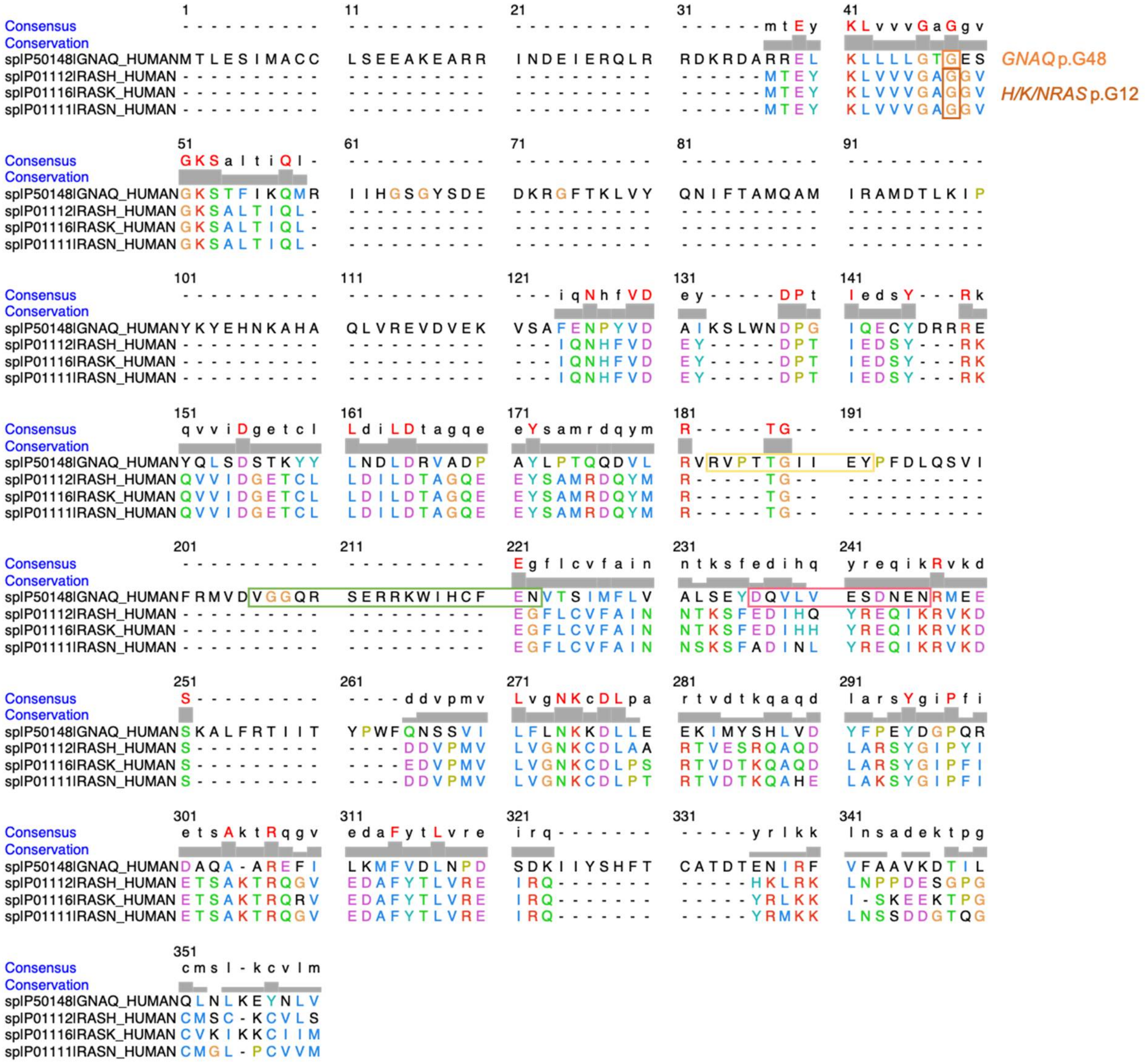


Figure S1. GCPRs and G protein subunits activation cycle.

Figure S2. cBioportal oncoprint of the five mostly mutated genes (*GNAQ, GNAQ11, BAP1, SF3B1, EIF1AX*), plus mutated genes detected in our patient case, NGS results detected in 108 uveal melanoma patients of two cohorts (*n* = 28 [1] and *n* = 80 [2]) [3,4]

Table S1. Sequence identity of FGFR4 with FGFR1,-2 and -3.

	Sequence identity with FGFR4
FGFR1	57%
FGFR2	58%
FGFR3	61%



```
                     1          11         21         31         41
Consensus            ---------- ---------- ---------- ------mtEy KLvvvGaGgv
Conservation
sp|P50148|GNAQ_HUMAN MTLESIMACC LSEEAKEARR INDEIERQLR RDKRDARREL KLLLLGTGES  GNAQ p.G48
sp|P01112|RASH_HUMAN ---------- ---------- ---------- ------MTEY KLVVVGAGGV
sp|P01116|RASK_HUMAN ---------- ---------- ---------- ------MTEY KLVVVGAGGV  H/K/NRAS p.G12
sp|P01111|RASN_HUMAN ---------- ---------- ---------- ------MTEY KLVVVGAGGV

                     51         61         71         81         91
Consensus            GKSaltiQl- ---------- ---------- ---------- ----------
Conservation
sp|P50148|GNAQ_HUMAN GKSTFIKQMR IIHGSGYSDE DKRGFTKLVY QNIFTAMQAM IRAMDTLKIP
sp|P01112|RASH_HUMAN GKSALTIQL- ---------- ---------- ---------- ----------
sp|P01116|RASK_HUMAN GKSALTIQL- ---------- ---------- ---------- ----------
sp|P01111|RASN_HUMAN GKSALTIQL- ---------- ---------- ---------- ----------

                     101        111        121        131        141
Consensus            ---------- ---------- ---iqNhfVD ey-----DPt IedsY---Rk
Conservation
sp|P50148|GNAQ_HUMAN YKYEHNKAHA QLVREVDVEK VSAFENPYVD AIKSLWNDPG IQECYDRRRE
sp|P01112|RASH_HUMAN ---------- ---------- ---IQNHFVD EY-----DPT IEDSY---RK
sp|P01116|RASK_HUMAN ---------- ---------- ---IQNHFVD EY-----DPT IEDSY---RK
sp|P01111|RASN_HUMAN ---------- ---------- ---IQNHFVD EY-----DPT IEDSY---RK

                     151        161        171        181        191
Consensus            qvviDgetcl LdiLDtagqe eYsamrdqym R-----TG-- ----------
Conservation
sp|P50148|GNAQ_HUMAN YQLSDSTKYY LNDLDRVADP AYLPTQQDVL RVRVPTTGII EYPFDLQSVI
sp|P01112|RASH_HUMAN QVVIDGETCL LDILDTAGQE EYSAMRDQYM R-----TG-- ----------
sp|P01116|RASK_HUMAN QVVIDGETCL LDILDTAGQE EYSAMRDQYM R-----TG-- ----------
sp|P01111|RASN_HUMAN QVVIDGETCL LDILDTAGQE EYSAMRDQYM R-----TG-- ----------

                     201        211        221        231        241
Consensus            ---------- ---------- Egflcvfain ntksfedihq yreqikRvkd
Conservation
sp|P50148|GNAQ_HUMAN FRMVDVGGQR SERRKWIHCF ENVTSIMFLV ALSEYDQVLV ESDNENRMEE
sp|P01112|RASH_HUMAN ---------- ---------- EGFLCVFAIN NTKSFEDIHQ YREQIKRVKD
sp|P01116|RASK_HUMAN ---------- ---------- EGFLCVFAIN NTKSFEDIHH YREQIKRVKD
sp|P01111|RASN_HUMAN ---------- ---------- EGFLCVFAIN NSKSFADINL YREQIKRVKD

                     251        261        271        281        291
Consensus            S--------- ----ddvpmv LvgNKcDLpa rtvdtkqaqd larsYgiPfi
Conservation
sp|P50148|GNAQ_HUMAN SKALFRTIIT YPWFQNSSVI LFLNKKDLLE EKIMYSHLVD YFPEYDGPQR
sp|P01112|RASH_HUMAN S--------- ----DDVPMV LVGNKCDLAA RTVESRQAQD LARSYGIPYI
sp|P01116|RASK_HUMAN S--------- ----EDVPMV LVGNKCDLPS RTVDTKQAQD LARSYGIPFI
sp|P01111|RASN_HUMAN S--------- ----DDVPMV LVGNKCDLPT RTVDTKQAHE LAKSYGIPFI

                     301        311        321        331        341
Consensus            etsAktRqgv edaFytLvre irq------- -----yrlkk lnsadektpg
Conservation
sp|P50148|GNAQ_HUMAN DAQA-AREFI LKMFVDLNPD SDKIIYSHFT CATDTENIRF VFAAVKDTIL
sp|P01112|RASH_HUMAN ETSAKTRQGV EDAFYTLVRE IRQ------- -----HKLRK LNPPDESGPG
sp|P01116|RASK_HUMAN ETSAKTRQRV EDAFYTLVRE IRQ------- -----YRLKK I-SKEEKTPG
sp|P01111|RASN_HUMAN ETSAKTRQGV EDAFYTLVRE IRQ------- -----YRMKK LNSSDDGTQG

                     351
Consensus            cmsl-kcvlm
Conservation
sp|P50148|GNAQ_HUMAN QLNLKEYNLV
sp|P01112|RASH_HUMAN CMSC-KCVLS
sp|P01116|RASK_HUMAN CVKIKKCIIM
sp|P01111|RASN_HUMAN CMGL-PCVVM
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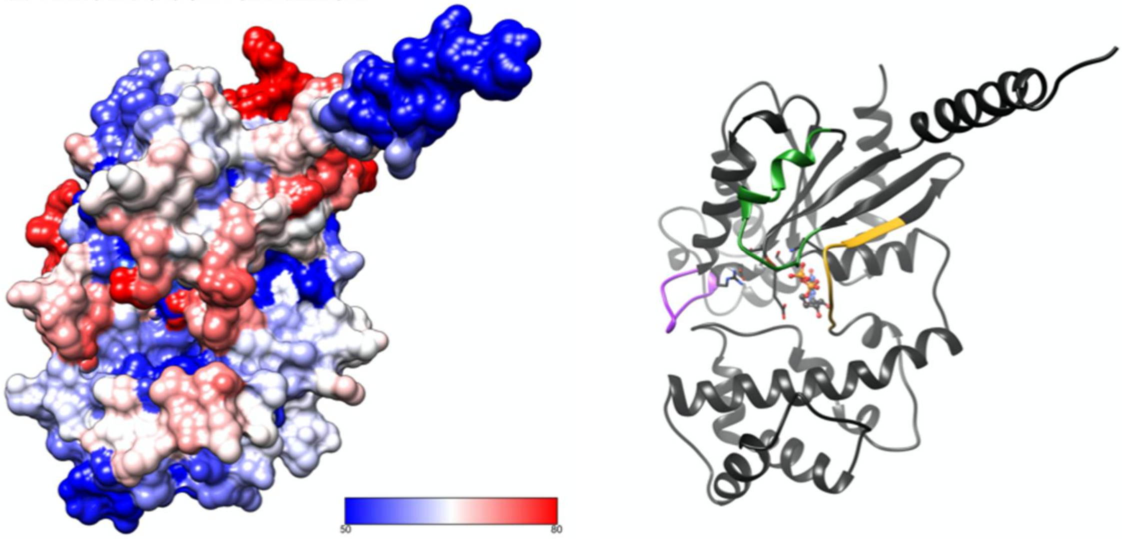
Figure S3. Sequence alignment of human *H*/*K*/*NRAS* and human *GNAQ*. Interestingly, *GNAQ* p.G48 corresponds to *H*/*K*/*NRAS* p.G12 which is a hotspot in RAS. The yellow, green and pink rectangles indicate the switch regions I, II and III in *GNAQ*, respectively.

a. Inactive conformation



b. Active conformation

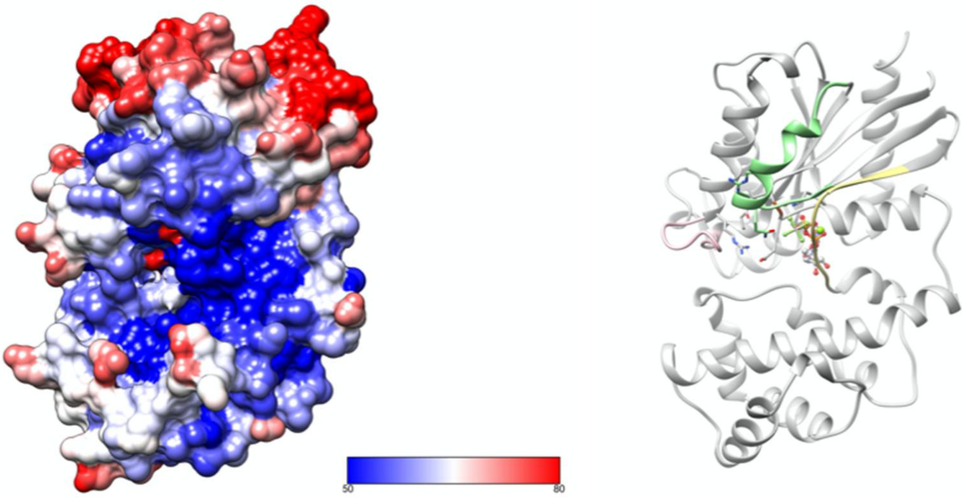
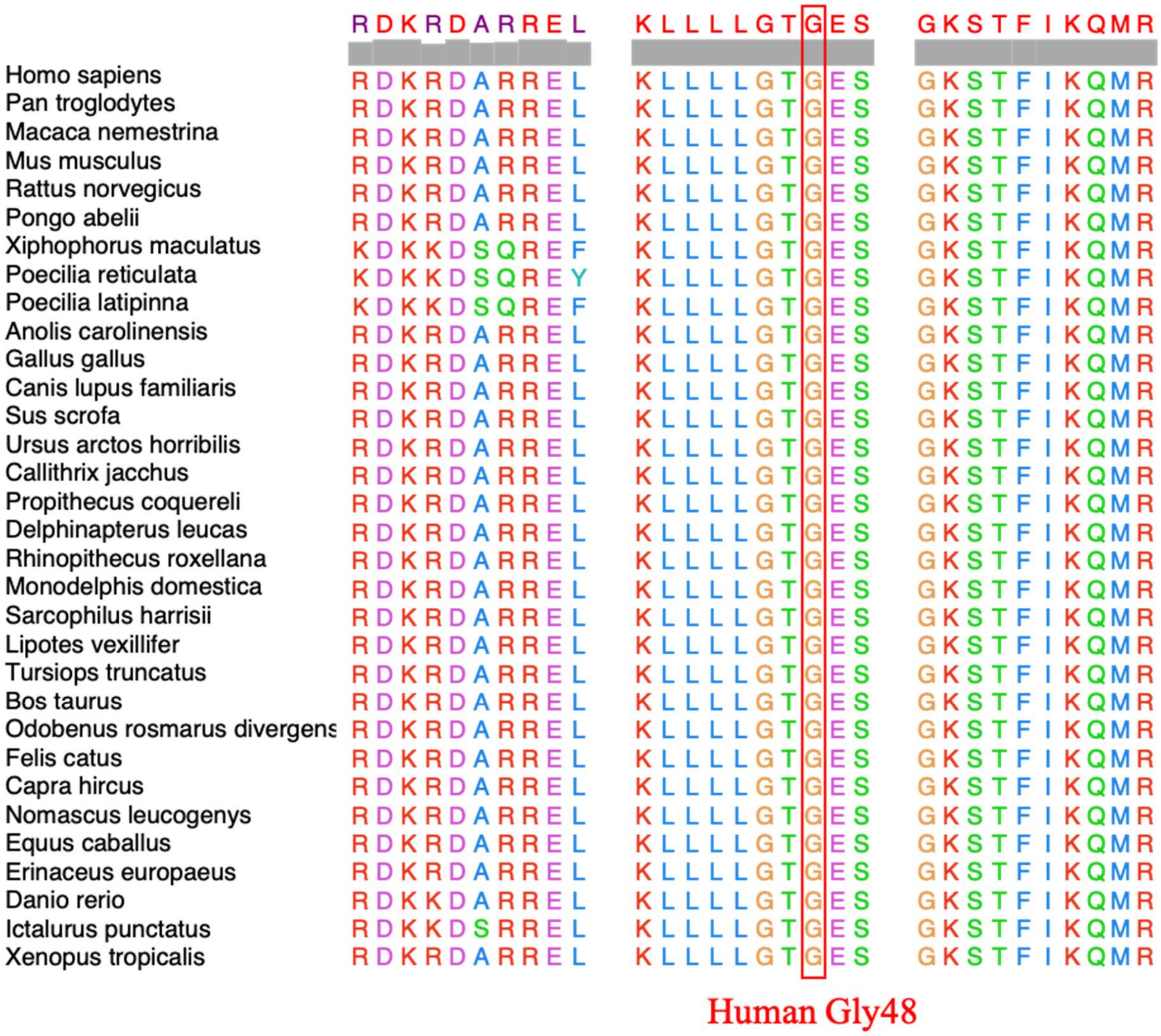
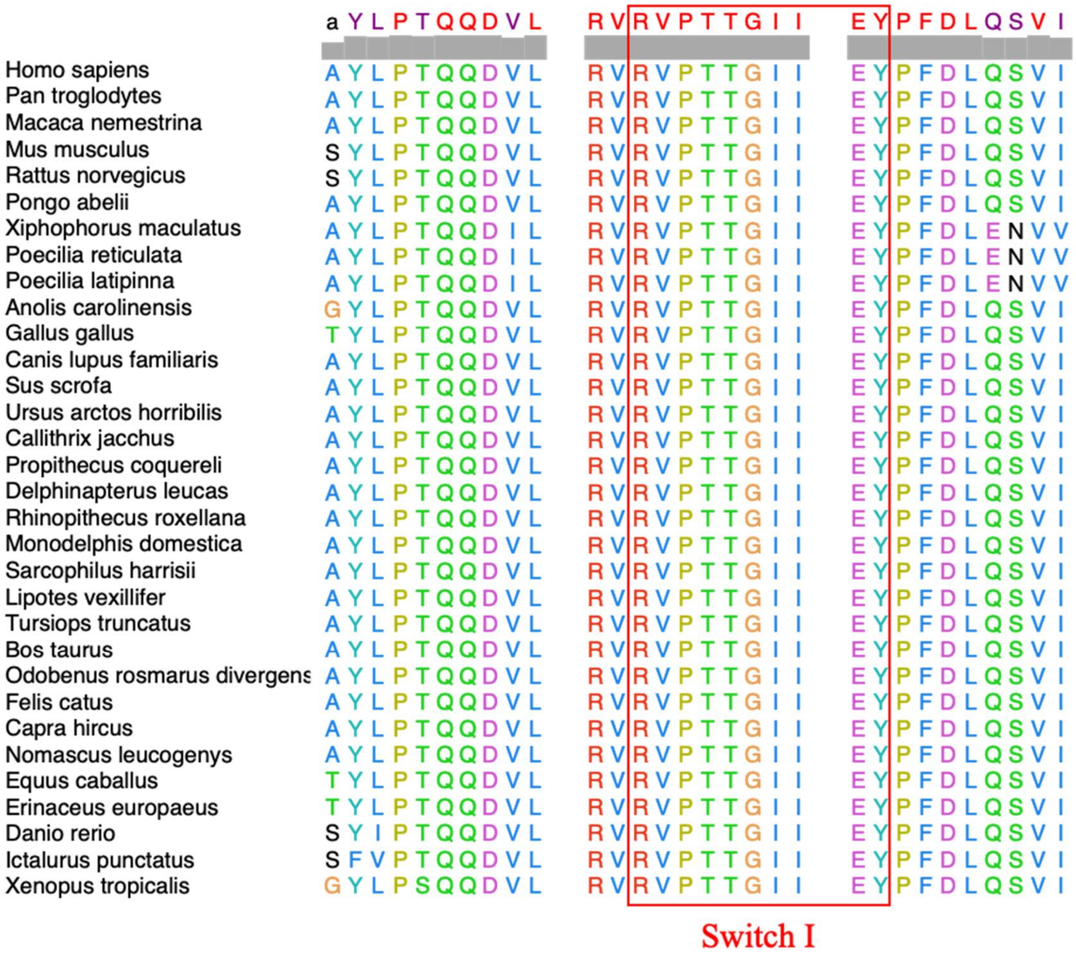


Figure S4. B-factor representation of inactive and active structures. (**a**). The active conformation was obtained as a co-crystal with GDP, AlF_4 and Mg^{2+} (PDB ID 3ohm); (**b**). The inactive conformation as a co-crystal with GDP (PDB ID 3ah8). The gradient corresponding to the B-factor values goes from blue to red, for rigid and flexible regions respectively. GDP and AlF_4 are shown in ball&stick and the Mg^{2+} in sphere representations, respectively. SW-I (183-192), SW-II (206-222) and SW-III (236-246) are respectively colored in gold, green and salmon.



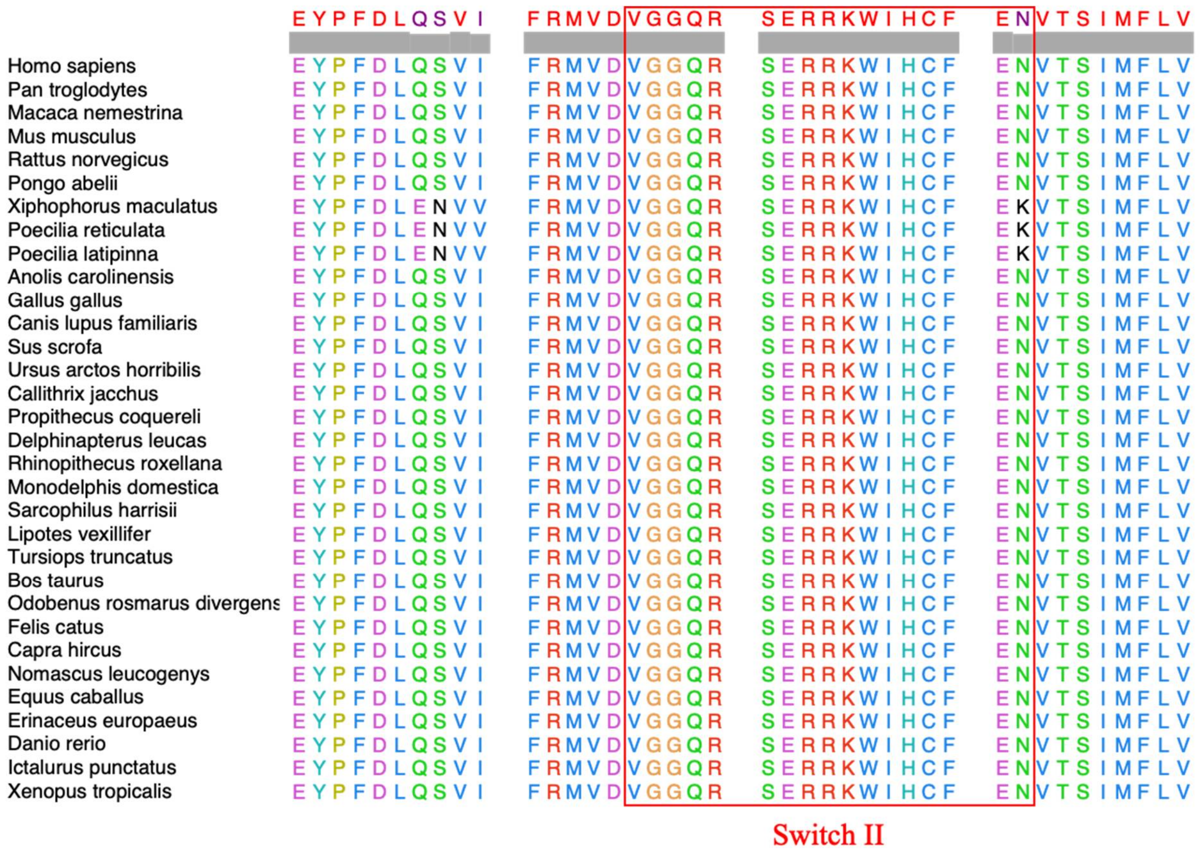
	RDKRDARREL	KLLLLGTGES	GKSTFIKQMR
Homo sapiens	RDKRDARREL	KLLLLGTGES	GKSTFIKQMR
Pan troglodytes	RDKRDARREL	KLLLLGTGES	GKSTFIKQMR
Macaca nemestrina	RDKRDARREL	KLLLLGTGES	GKSTFIKQMR
Mus musculus	RDKRDARREL	KLLLLGTGES	GKSTFIKQMR
Rattus norvegicus	RDKRDARREL	KLLLLGTGES	GKSTFIKQMR
Pongo abelii	RDKRDARREL	KLLLLGTGES	GKSTFIKQMR
Xiphophorus maculatus	KDKKDSQREF	KLLLLGTGES	GKSTFIKQMR
Poecilia reticulata	KDKKDSQREY	KLLLLGTGES	GKSTFIKQMR
Poecilia latipinna	KDKKDSQREF	KLLLLGTGES	GKSTFIKQMR
Anolis carolinensis	RDKRDARREL	KLLLLGTGES	GKSTFIKQMR
Gallus gallus	RDKRDARREL	KLLLLGTGES	GKSTFIKQMR
Canis lupus familiaris	RDKRDARREL	KLLLLGTGES	GKSTFIKQMR
Sus scrofa	RDKRDARREL	KLLLLGTGES	GKSTFIKQMR
Ursus arctos horribilis	RDKRDARREL	KLLLLGTGES	GKSTFIKQMR
Callithrix jacchus	RDKRDARREL	KLLLLGTGES	GKSTFIKQMR
Propithecus coquereli	RDKRDARREL	KLLLLGTGES	GKSTFIKQMR
Delphinapterus leucas	RDKRDARREL	KLLLLGTGES	GKSTFIKQMR
Rhinopithecus roxellana	RDKRDARREL	KLLLLGTGES	GKSTFIKQMR
Monodelphis domestica	RDKRDARREL	KLLLLGTGES	GKSTFIKQMR
Sarcophilus harrisii	RDKRDARREL	KLLLLGTGES	GKSTFIKQMR
Lipotes vexillifer	RDKRDARREL	KLLLLGTGES	GKSTFIKQMR
Tursiops truncatus	RDKRDARREL	KLLLLGTGES	GKSTFIKQMR
Bos taurus	RDKRDARREL	KLLLLGTGES	GKSTFIKQMR
Odobenus rosmarus divergens	RDKRDARREL	KLLLLGTGES	GKSTFIKQMR
Felis catus	RDKRDARREL	KLLLLGTGES	GKSTFIKQMR
Capra hircus	RDKRDARREL	KLLLLGTGES	GKSTFIKQMR
Nomascus leucogenys	RDKRDARREL	KLLLLGTGES	GKSTFIKQMR
Equus caballus	RDKRDARREL	KLLLLGTGES	GKSTFIKQMR
Erinaceus europaeus	RDKRDARREL	KLLLLGTGES	GKSTFIKQMR
Danio rerio	RDKKDARREL	KLLLLGTGES	GKSTFIKQMR
Ictalurus punctatus	RDKKDSRREL	KLLLLGTGES	GKSTFIKQMR
Xenopus tropicalis	RDKRDARREL	KLLLLGTGES	GKSTFIKQMR

Figure S5. Sequence alignment of *GNAQ* orthologs centred on Human Gly48.



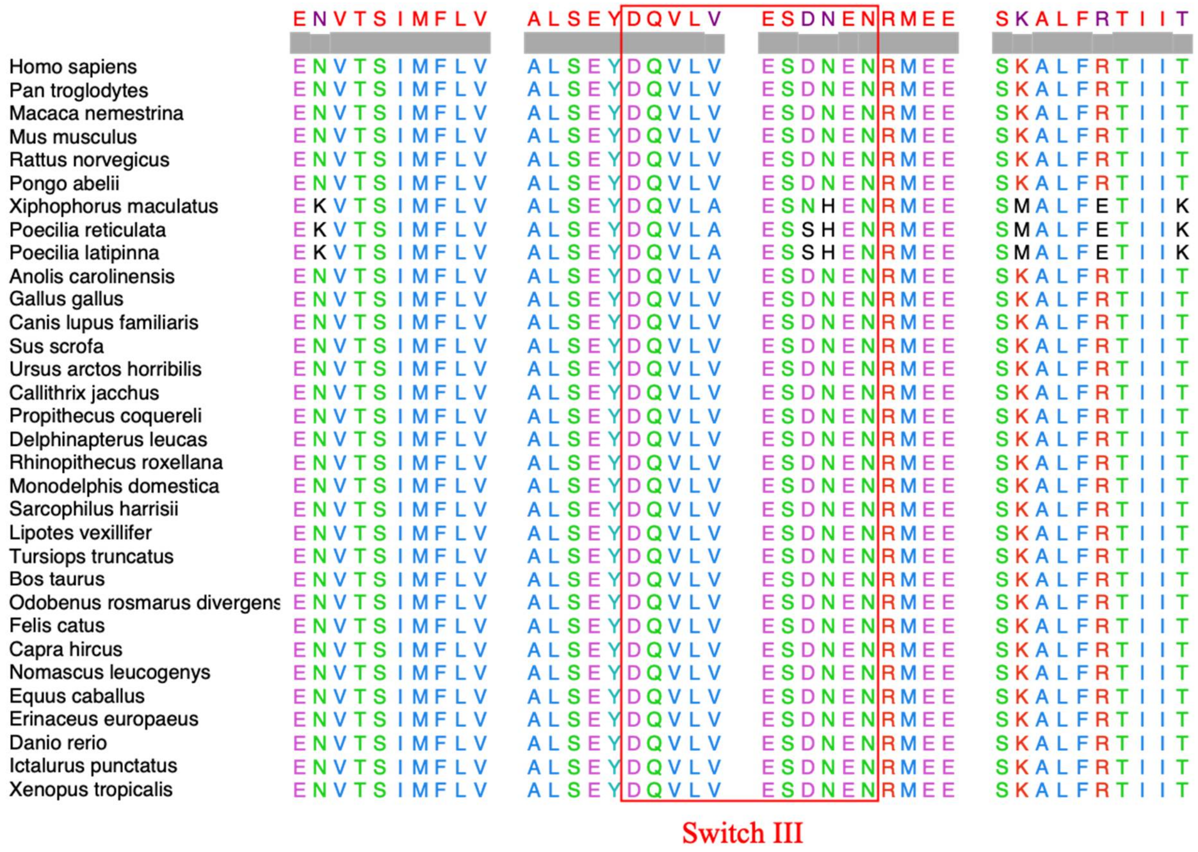
	a Y L P T Q Q D V L	R V R V P T T G I I	E Y P F D L Q S V I
Homo sapiens	A Y L P T Q Q D V L	R V R V P T T G I I	E Y P F D L Q S V I
Pan troglodytes	A Y L P T Q Q D V L	R V R V P T T G I I	E Y P F D L Q S V I
Macaca nemestrina	A Y L P T Q Q D V L	R V R V P T T G I I	E Y P F D L Q S V I
Mus musculus	S Y L P T Q Q D V L	R V R V P T T G I I	E Y P F D L Q S V I
Rattus norvegicus	S Y L P T Q Q D V L	R V R V P T T G I I	E Y P F D L Q S V I
Pongo abelii	A Y L P T Q Q D V L	R V R V P T T G I I	E Y P F D L Q S V I
Xiphophorus maculatus	A Y L P T Q Q D I L	R V R V P T T G I I	E Y P F D L E N V V
Poecilia reticulata	A Y L P T Q Q D I L	R V R V P T T G I I	E Y P F D L E N V V
Poecilia latipinna	A Y L P T Q Q D I L	R V R V P T T G I I	E Y P F D L E N V V
Anolis carolinensis	G Y L P T Q Q D V L	R V R V P T T G I I	E Y P F D L Q S V I
Gallus gallus	T Y L P T Q Q D V L	R V R V P T T G I I	E Y P F D L Q S V I
Canis lupus familiaris	A Y L P T Q Q D V L	R V R V P T T G I I	E Y P F D L Q S V I
Sus scrofa	A Y L P T Q Q D V L	R V R V P T T G I I	E Y P F D L Q S V I
Ursus arctos horribilis	A Y L P T Q Q D V L	R V R V P T T G I I	E Y P F D L Q S V I
Callithrix jacchus	A Y L P T Q Q D V L	R V R V P T T G I I	E Y P F D L Q S V I
Propithecus coquereli	A Y L P T Q Q D V L	R V R V P T T G I I	E Y P F D L Q S V I
Delphinapterus leucas	A Y L P T Q Q D V L	R V R V P T T G I I	E Y P F D L Q S V I
Rhinopithecus roxellana	A Y L P T Q Q D V L	R V R V P T T G I I	E Y P F D L Q S V I
Monodelphis domestica	A Y L P T Q Q D V L	R V R V P T T G I I	E Y P F D L Q S V I
Sarcophilus harrisii	A Y L P T Q Q D V L	R V R V P T T G I I	E Y P F D L Q S V I
Lipotes vexillifer	A Y L P T Q Q D V L	R V R V P T T G I I	E Y P F D L Q S V I
Tursiops truncatus	A Y L P T Q Q D V L	R V R V P T T G I I	E Y P F D L Q S V I
Bos taurus	A Y L P T Q Q D V L	R V R V P T T G I I	E Y P F D L Q S V I
Odobenus rosmarus divergens	A Y L P T Q Q D V L	R V R V P T T G I I	E Y P F D L Q S V I
Felis catus	A Y L P T Q Q D V L	R V R V P T T G I I	E Y P F D L Q S V I
Capra hircus	A Y L P T Q Q D V L	R V R V P T T G I I	E Y P F D L Q S V I
Nomascus leucogenys	A Y L P T Q Q D V L	R V R V P T T G I I	E Y P F D L Q S V I
Equus caballus	T Y L P T Q Q D V L	R V R V P T T G I I	E Y P F D L Q S V I
Erinaceus europaeus	T Y L P T Q Q D V L	R V R V P T T G I I	E Y P F D L Q S V I
Danio rerio	S Y I P T Q Q D V L	R V R V P T T G I I	E Y P F D L Q S V I
Ictalurus punctatus	S F V P T Q Q D V L	R V R V P T T G I I	E Y P F D L Q S V I
Xenopus tropicalis	G Y L P S Q Q D V L	R V R V P T T G I I	E Y P F D L Q S V I

Figure S6. Sequence alignment of *GNAQ* orthologs centred on the Switch I loop (residues 183-192 based on human sequence).



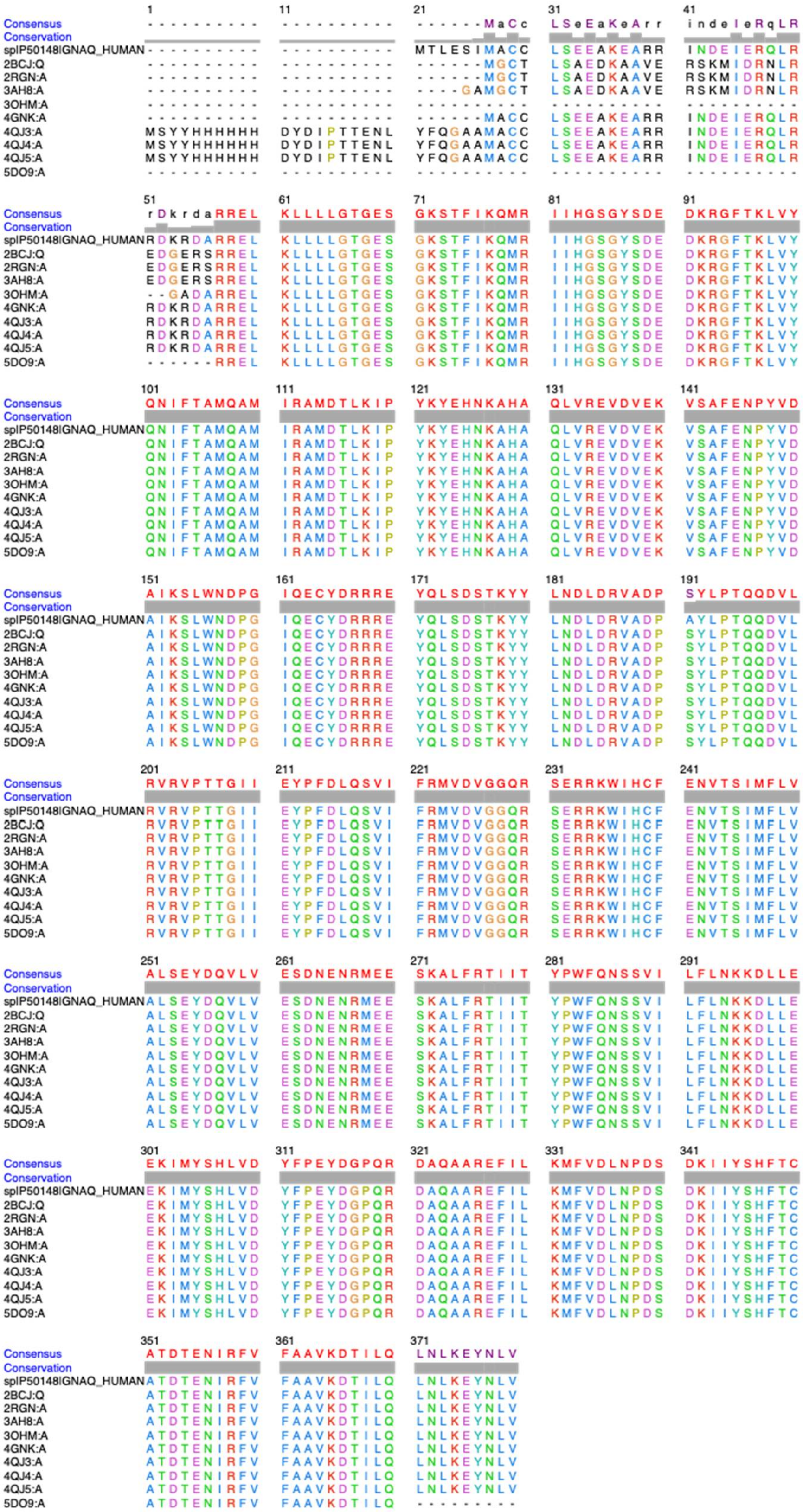
	EYPFDLQSVI	FRMVDVGGQR	SERRKWIHCF	ENVTSIMFLV
Homo sapiens	EYPFDLQSVI	FRMVDVGGQR	SERRKWIHCF	ENVTSIMFLV
Pan troglodytes	EYPFDLQSVI	FRMVDVGGQR	SERRKWIHCF	ENVTSIMFLV
Macaca nemestrina	EYPFDLQSVI	FRMVDVGGQR	SERRKWIHCF	ENVTSIMFLV
Mus musculus	EYPFDLQSVI	FRMVDVGGQR	SERRKWIHCF	ENVTSIMFLV
Rattus norvegicus	EYPFDLQSVI	FRMVDVGGQR	SERRKWIHCF	ENVTSIMFLV
Pongo abelii	EYPFDLQSVI	FRMVDVGGQR	SERRKWIHCF	ENVTSIMFLV
Xiphophorus maculatus	EYPFDLENVV	FRMVDVGGQR	SERRKWIHCF	EKVTSIMFLV
Poecilia reticulata	EYPFDLENVV	FRMVDVGGQR	SERRKWIHCF	EKVTSIMFLV
Poecilia latipinna	EYPFDLENVV	FRMVDVGGQR	SERRKWIHCF	EKVTSIMFLV
Anolis carolinensis	EYPFDLQSVI	FRMVDVGGQR	SERRKWIHCF	ENVTSIMFLV
Gallus gallus	EYPFDLQSVI	FRMVDVGGQR	SERRKWIHCF	ENVTSIMFLV
Canis lupus familiaris	EYPFDLQSVI	FRMVDVGGQR	SERRKWIHCF	ENVTSIMFLV
Sus scrofa	EYPFDLQSVI	FRMVDVGGQR	SERRKWIHCF	ENVTSIMFLV
Ursus arctos horribilis	EYPFDLQSVI	FRMVDVGGQR	SERRKWIHCF	ENVTSIMFLV
Callithrix jacchus	EYPFDLQSVI	FRMVDVGGQR	SERRKWIHCF	ENVTSIMFLV
Propithecus coquereli	EYPFDLQSVI	FRMVDVGGQR	SERRKWIHCF	ENVTSIMFLV
Delphinapterus leucas	EYPFDLQSVI	FRMVDVGGQR	SERRKWIHCF	ENVTSIMFLV
Rhinopithecus roxellana	EYPFDLQSVI	FRMVDVGGQR	SERRKWIHCF	ENVTSIMFLV
Monodelphis domestica	EYPFDLQSVI	FRMVDVGGQR	SERRKWIHCF	ENVTSIMFLV
Sarcophilus harrisii	EYPFDLQSVI	FRMVDVGGQR	SERRKWIHCF	ENVTSIMFLV
Lipotes vexillifer	EYPFDLQSVI	FRMVDVGGQR	SERRKWIHCF	ENVTSIMFLV
Tursiops truncatus	EYPFDLQSVI	FRMVDVGGQR	SERRKWIHCF	ENVTSIMFLV
Bos taurus	EYPFDLQSVI	FRMVDVGGQR	SERRKWIHCF	ENVTSIMFLV
Odobenus rosmarus divergens	EYPFDLQSVI	FRMVDVGGQR	SERRKWIHCF	ENVTSIMFLV
Felis catus	EYPFDLQSVI	FRMVDVGGQR	SERRKWIHCF	ENVTSIMFLV
Capra hircus	EYPFDLQSVI	FRMVDVGGQR	SERRKWIHCF	ENVTSIMFLV
Nomascus leucogenys	EYPFDLQSVI	FRMVDVGGQR	SERRKWIHCF	ENVTSIMFLV
Equus caballus	EYPFDLQSVI	FRMVDVGGQR	SERRKWIHCF	ENVTSIMFLV
Erinaceus europaeus	EYPFDLQSVI	FRMVDVGGQR	SERRKWIHCF	ENVTSIMFLV
Danio rerio	EYPFDLQSVI	FRMVDVGGQR	SERRKWIHCF	ENVTSIMFLV
Ictalurus punctatus	EYPFDLQSVI	FRMVDVGGQR	SERRKWIHCF	ENVTSIMFLV
Xenopus tropicalis	EYPFDLQSVI	FRMVDVGGQR	SERRKWIHCF	ENVTSIMFLV

Figure S7. Sequence alignment of *GNAQ* orthologs centred on the Switch II loop (206-222 based on human sequence)



	ENVTSIMFLV	ALSEYDQVLV	ESDNENRMEE	SKALFRTIIT
Homo sapiens	ENVTSIMFLV	ALSEYDQVLV	ESDNENRMEE	SKALFRTIIT
Pan troglodytes	ENVTSIMFLV	ALSEYDQVLV	ESDNENRMEE	SKALFRTIIT
Macaca nemestrina	ENVTSIMFLV	ALSEYDQVLV	ESDNENRMEE	SKALFRTIIT
Mus musculus	ENVTSIMFLV	ALSEYDQVLV	ESDNENRMEE	SKALFRTIIT
Rattus norvegicus	ENVTSIMFLV	ALSEYDQVLV	ESDNENRMEE	SKALFRTIIT
Pongo abelii	ENVTSIMFLV	ALSEYDQVLV	ESDNENRMEE	SKALFRTIIT
Xiphophorus maculatus	EKVTSIMFLV	ALSEYDQVLA	ESNHENRMEE	SMALFETIIK
Poecilia reticulata	EKVTSIMFLV	ALSEYDQVLA	ESSHENRMEE	SMALFETIIK
Poecilia latipinna	EKVTSIMFLV	ALSEYDQVLA	ESSHENRMEE	SMALFETIIK
Anolis carolinensis	ENVTSIMFLV	ALSEYDQVLV	ESDNENRMEE	SKALFRTIIT
Gallus gallus	ENVTSIMFLV	ALSEYDQVLV	ESDNENRMEE	SKALFRTIIT
Canis lupus familiaris	ENVTSIMFLV	ALSEYDQVLV	ESDNENRMEE	SKALFRTIIT
Sus scrofa	ENVTSIMFLV	ALSEYDQVLV	ESDNENRMEE	SKALFRTIIT
Ursus arctos horribilis	ENVTSIMFLV	ALSEYDQVLV	ESDNENRMEE	SKALFRTIIT
Callithrix jacchus	ENVTSIMFLV	ALSEYDQVLV	ESDNENRMEE	SKALFRTIIT
Propithecus coquereli	ENVTSIMFLV	ALSEYDQVLV	ESDNENRMEE	SKALFRTIIT
Delphinapterus leucas	ENVTSIMFLV	ALSEYDQVLV	ESDNENRMEE	SKALFRTIIT
Rhinopithecus roxellana	ENVTSIMFLV	ALSEYDQVLV	ESDNENRMEE	SKALFRTIIT
Monodelphis domestica	ENVTSIMFLV	ALSEYDQVLV	ESDNENRMEE	SKALFRTIIT
Sarcophilus harrisii	ENVTSIMFLV	ALSEYDQVLV	ESDNENRMEE	SKALFRTIIT
Lipotes vexillifer	ENVTSIMFLV	ALSEYDQVLV	ESDNENRMEE	SKALFRTIIT
Tursiops truncatus	ENVTSIMFLV	ALSEYDQVLV	ESDNENRMEE	SKALFRTIIT
Bos taurus	ENVTSIMFLV	ALSEYDQVLV	ESDNENRMEE	SKALFRTIIT
Odobenus rosmarus divergens	ENVTSIMFLV	ALSEYDQVLV	ESDNENRMEE	SKALFRTIIT
Felis catus	ENVTSIMFLV	ALSEYDQVLV	ESDNENRMEE	SKALFRTIIT
Capra hircus	ENVTSIMFLV	ALSEYDQVLV	ESDNENRMEE	SKALFRTIIT
Nomascus leucogenys	ENVTSIMFLV	ALSEYDQVLV	ESDNENRMEE	SKALFRTIIT
Equus caballus	ENVTSIMFLV	ALSEYDQVLV	ESDNENRMEE	SKALFRTIIT
Erinaceus europaeus	ENVTSIMFLV	ALSEYDQVLV	ESDNENRMEE	SKALFRTIIT
Danio rerio	ENVTSIMFLV	ALSEYDQVLV	ESDNENRMEE	SKALFRTIIT
Ictalurus punctatus	ENVTSIMFLV	ALSEYDQVLV	ESDNENRMEE	SKALFRTIIT
Xenopus tropicalis	ENVTSIMFLV	ALSEYDQVLV	ESDNENRMEE	SKALFRTIIT

Figure S8. Sequence alignment of *GNAQ* orthologs centred on the Switch III loop (236-246 based on human sequence)



```
                     1           11          21          31          41
Consensus            ----------  ----------  ------MaCc  LSeEaKeArr  indeIeRqLR
Conservation
spIP50148IGNAQ_HUMAN ----------  ----------  MTLESIMACC  LSEEAKEARR  INDEIERQLR
2BCJ:Q               ----------  ----------  ------MGCT  LSAEDKAAVE  RSKMIDRNLR
2RGN:A               ----------  ----------  ------MGCT  LSAEDKAAVE  RSKMIDRNLR
3AH8:A               ----------  ----------  ----GAMGCT  LSAEDKAAVE  RSKMIDRNLR
3OHM:A               ----------  ----------  ----------  ----------  ----------
4GNK:A               ----------  ----------  ------MACC  LSEEAKEARR  INDEIERQLR
4QJ3:A               MSYYHHHHHH  DYDIPTTENL  YFQGAAMACC  LSEEAKEARR  INDEIERQLR
4QJ4:A               MSYYHHHHHH  DYDIPTTENL  YFQGAAMACC  LSEEAKEARR  INDEIERQLR
4QJ5:A               MSYYHHHHHH  DYDIPTTENL  YFQGAAMACC  LSEEAKEARR  INDEIERQLR
5DO9:A               ----------  ----------  ----------  ----------  ----------

                     51          61          71          81          91
Consensus            rDkrdaRREL  KLLLLGTGES  GKSTFIKQMR  IIHGSGYSDE  DKRGFTKLVY
Conservation
spIP50148IGNAQ_HUMAN RDKRDARREL  KLLLLGTGES  GKSTFIKQMR  IIHGSGYSDE  DKRGFTKLVY
2BCJ:Q               EDGERSRREL  KLLLLGTGES  GKSTFIKQMR  IIHGSGYSDE  DKRGFTKLVY
2RGN:A               EDGERSRREL  KLLLLGTGES  GKSTFIKQMR  IIHGSGYSDE  DKRGFTKLVY
3AH8:A               EDGERSRREL  KLLLLGTGES  GKSTFIKQMR  IIHGSGYSDE  DKRGFTKLVY
3OHM:A               --GADARREL  KLLLLGTGES  GKSTFIKQMR  IIHGSGYSDE  DKRGFTKLVY
4GNK:A               RDKRDARREL  KLLLLGTGES  GKSTFIKQMR  IIHGSGYSDE  DKRGFTKLVY
4QJ3:A               RDKRDARREL  KLLLLGTGES  GKSTFIKQMR  IIHGSGYSDE  DKRGFTKLVY
4QJ4:A               RDKRDARREL  KLLLLGTGES  GKSTFIKQMR  IIHGSGYSDE  DKRGFTKLVY
4QJ5:A               RDKRDARREL  KLLLLGTGES  GKSTFIKQMR  IIHGSGYSDE  DKRGFTKLVY
5DO9:A               ------RREL  KLLLLGTGES  GKSTFIKQMR  IIHGSGYSDE  DKRGFTKLVY

                     101         111         121         131         141
Consensus            QNIFTAMQAM  IRAMDTLKIP  YKYEHNKAHA  QLVREVDVEK  VSAFENPYVD
Conservation
spIP50148IGNAQ_HUMAN QNIFTAMQAM  IRAMDTLKIP  YKYEHNKAHA  QLVREVDVEK  VSAFENPYVD
2BCJ:Q               QNIFTAMQAM  IRAMDTLKIP  YKYEHNKAHA  QLVREVDVEK  VSAFENPYVD
2RGN:A               QNIFTAMQAM  IRAMDTLKIP  YKYEHNKAHA  QLVREVDVEK  VSAFENPYVD
3AH8:A               QNIFTAMQAM  IRAMDTLKIP  YKYEHNKAHA  QLVREVDVEK  VSAFENPYVD
3OHM:A               QNIFTAMQAM  IRAMDTLKIP  YKYEHNKAHA  QLVREVDVEK  VSAFENPYVD
4GNK:A               QNIFTAMQAM  IRAMDTLKIP  YKYEHNKAHA  QLVREVDVEK  VSAFENPYVD
4QJ3:A               QNIFTAMQAM  IRAMDTLKIP  YKYEHNKAHA  QLVREVDVEK  VSAFENPYVD
4QJ4:A               QNIFTAMQAM  IRAMDTLKIP  YKYEHNKAHA  QLVREVDVEK  VSAFENPYVD
4QJ5:A               QNIFTAMQAM  IRAMDTLKIP  YKYEHNKAHA  QLVREVDVEK  VSAFENPYVD
5DO9:A               QNIFTAMQAM  IRAMDTLKIP  YKYEHNKAHA  QLVREVDVEK  VSAFENPYVD

                     151         161         171         181         191
Consensus            AIKSLWNDPG  IQECYDRRRE  YQLSDSTKYY  LNDLDRVADP  SYLPTQQDVL
Conservation
spIP50148IGNAQ_HUMAN AIKSLWNDPG  IQECYDRRRE  YQLSDSTKYY  LNDLDRVADP  AYLPTQQDVL
2BCJ:Q               AIKSLWNDPG  IQECYDRRRE  YQLSDSTKYY  LNDLDRVADP  SYLPTQQDVL
2RGN:A               AIKSLWNDPG  IQECYDRRRE  YQLSDSTKYY  LNDLDRVADP  SYLPTQQDVL
3AH8:A               AIKSLWNDPG  IQECYDRRRE  YQLSDSTKYY  LNDLDRVADP  SYLPTQQDVL
3OHM:A               AIKSLWNDPG  IQECYDRRRE  YQLSDSTKYY  LNDLDRVADP  SYLPTQQDVL
4GNK:A               AIKSLWNDPG  IQECYDRRRE  YQLSDSTKYY  LNDLDRVADP  SYLPTQQDVL
4QJ3:A               AIKSLWNDPG  IQECYDRRRE  YQLSDSTKYY  LNDLDRVADP  SYLPTQQDVL
4QJ4:A               AIKSLWNDPG  IQECYDRRRE  YQLSDSTKYY  LNDLDRVADP  SYLPTQQDVL
4QJ5:A               AIKSLWNDPG  IQECYDRRRE  YQLSDSTKYY  LNDLDRVADP  SYLPTQQDVL
5DO9:A               AIKSLWNDPG  IQECYDRRRE  YQLSDSTKYY  LNDLDRVADP  SYLPTQQDVL

                     201         211         221         231         241
Consensus            RVRVPTTGII  EYPFDLQSVI  FRMVDVGGQR  SERRKWIHCF  ENVTSIMFLV
Conservation
spIP50148IGNAQ_HUMAN RVRVPTTGII  EYPFDLQSVI  FRMVDVGGQR  SERRKWIHCF  ENVTSIMFLV
2BCJ:Q               RVRVPTTGII  EYPFDLQSVI  FRMVDVGGQR  SERRKWIHCF  ENVTSIMFLV
2RGN:A               RVRVPTTGII  EYPFDLQSVI  FRMVDVGGQR  SERRKWIHCF  ENVTSIMFLV
3AH8:A               RVRVPTTGII  EYPFDLQSVI  FRMVDVGGQR  SERRKWIHCF  ENVTSIMFLV
3OHM:A               RVRVPTTGII  EYPFDLQSVI  FRMVDVGGQR  SERRKWIHCF  ENVTSIMFLV
4GNK:A               RVRVPTTGII  EYPFDLQSVI  FRMVDVGGQR  SERRKWIHCF  ENVTSIMFLV
4QJ3:A               RVRVPTTGII  EYPFDLQSVI  FRMVDVGGQR  SERRKWIHCF  ENVTSIMFLV
4QJ4:A               RVRVPTTGII  EYPFDLQSVI  FRMVDVGGQR  SERRKWIHCF  ENVTSIMFLV
4QJ5:A               RVRVPTTGII  EYPFDLQSVI  FRMVDVGGQR  SERRKWIHCF  ENVTSIMFLV
5DO9:A               RVRVPTTGII  EYPFDLQSVI  FRMVDVGGQR  SERRKWIHCF  ENVTSIMFLV

                     251         261         271         281         291
Consensus            ALSEYDQVLV  ESDNENRMEE  SKALFRTIIT  YPWFQNSSVI  LFLNKKDLLE
Conservation
spIP50148IGNAQ_HUMAN ALSEYDQVLV  ESDNENRMEE  SKALFRTIIT  YPWFQNSSVI  LFLNKKDLLE
2BCJ:Q               ALSEYDQVLV  ESDNENRMEE  SKALFRTIIT  YPWFQNSSVI  LFLNKKDLLE
2RGN:A               ALSEYDQVLV  ESDNENRMEE  SKALFRTIIT  YPWFQNSSVI  LFLNKKDLLE
3AH8:A               ALSEYDQVLV  ESDNENRMEE  SKALFRTIIT  YPWFQNSSVI  LFLNKKDLLE
3OHM:A               ALSEYDQVLV  ESDNENRMEE  SKALFRTIIT  YPWFQNSSVI  LFLNKKDLLE
4GNK:A               ALSEYDQVLV  ESDNENRMEE  SKALFRTIIT  YPWFQNSSVI  LFLNKKDLLE
4QJ3:A               ALSEYDQVLV  ESDNENRMEE  SKALFRTIIT  YPWFQNSSVI  LFLNKKDLLE
4QJ4:A               ALSEYDQVLV  ESDNENRMEE  SKALFRTIIT  YPWFQNSSVI  LFLNKKDLLE
4QJ5:A               ALSEYDQVLV  ESDNENRMEE  SKALFRTIIT  YPWFQNSSVI  LFLNKKDLLE
5DO9:A               ALSEYDQVLV  ESDNENRMEE  SKALFRTIIT  YPWFQNSSVI  LFLNKKDLLE

                     301         311         321         331         341
Consensus            EKIMYSHLVD  YFPEYDGPQR  DAQAAREFIL  KMFVDLNPDS  DKIIYSHFTC
Conservation
spIP50148IGNAQ_HUMAN EKIMYSHLVD  YFPEYDGPQR  DAQAAREFIL  KMFVDLNPDS  DKIIYSHFTC
2BCJ:Q               EKIMYSHLVD  YFPEYDGPQR  DAQAAREFIL  KMFVDLNPDS  DKIIYSHFTC
2RGN:A               EKIMYSHLVD  YFPEYDGPQR  DAQAAREFIL  KMFVDLNPDS  DKIIYSHFTC
3AH8:A               EKIMYSHLVD  YFPEYDGPQR  DAQAAREFIL  KMFVDLNPDS  DKIIYSHFTC
3OHM:A               EKIMYSHLVD  YFPEYDGPQR  DAQAAREFIL  KMFVDLNPDS  DKIIYSHFTC
4GNK:A               EKIMYSHLVD  YFPEYDGPQR  DAQAAREFIL  KMFVDLNPDS  DKIIYSHFTC
4QJ3:A               EKIMYSHLVD  YFPEYDGPQR  DAQAAREFIL  KMFVDLNPDS  DKIIYSHFTC
4QJ4:A               EKIMYSHLVD  YFPEYDGPQR  DAQAAREFIL  KMFVDLNPDS  DKIIYSHFTC
4QJ5:A               EKIMYSHLVD  YFPEYDGPQR  DAQAAREFIL  KMFVDLNPDS  DKIIYSHFTC
5DO9:A               EKIMYSHLVD  YFPEYDGPQR  DAQAAREFIL  KMFVDLNPDS  DKIIYSHFTC

                     351         361         371
Consensus            ATDTENIRFV  FAAVKDTILQ  LNLKEYNLV
Conservation
spIP50148IGNAQ_HUMAN ATDTENIRFV  FAAVKDTILQ  LNLKEYNLV
2BCJ:Q               ATDTENIRFV  FAAVKDTILQ  LNLKEYNLV
2RGN:A               ATDTENIRFV  FAAVKDTILQ  LNLKEYNLV
3AH8:A               ATDTENIRFV  FAAVKDTILQ  LNLKEYNLV
3OHM:A               ATDTENIRFV  FAAVKDTILQ  LNLKEYNLV
4GNK:A               ATDTENIRFV  FAAVKDTILQ  LNLKEYNLV
4QJ3:A               ATDTENIRFV  FAAVKDTILQ  LNLKEYNLV
4QJ4:A               ATDTENIRFV  FAAVKDTILQ  LNLKEYNLV
4QJ5:A               ATDTENIRFV  FAAVKDTILQ  LNLKEYNLV
5DO9:A               ATDTENIRFV  FAAVKDTILQ  ---------
```

Figure S9. Sequence alignment of the PDB sequences of the chains of interest and the Human sequence of *GNAQ*, retrieved from UniProt

For our analysis, we decided to analyse experimental structures from *Mus musculus* Gα_q and *Mus Musculus x Rattus norvegicus* Gα_i/Gα_q chimera, as they share high sequence identities with human

$G\alpha_q$. Structures were retrieved from the PDB [2]: 3ah8 [3] (sole inactive conformation), 2bcj [4], 2rgn [4], 3ohm [5], 4gnk [6], 4qj3 [7], 4qj4 [7], 4qj5 [7], 5do9 [8]. Two other structures of *Mus musculus* $G\alpha_q$ protein are available in the PDB (i.e., 4ekd, 4ekc). As they contain mutations, we chose not to include them in our study. A description of the structure information is presented in Table .

Table S2. 3D structures information. A is for active conformation and I for inactive conformation. * Sequence identities between the human and the chain sequences were calculated using Chimera.

PDB_id	Organism	Protein	Chain	Identity with human $G\alpha_q$*	Resolution (Å)	R-value Free	R-value work	pH	Temperature (K)
3ah8 (I)	*Rattus norvegicus, Mus musculus* (chimeric)	$G\alpha_i/G\alpha_q$	A	94.1%	2.9	0.315	0.259	5.1	293
2rgn (A)	*Rattus norvegicus, Mus musculus* (chimeric)	$G\alpha_i/G\alpha_q$	A	94.6%	3.5	0.299	0.243	6.5	277
2bcj (A)	*Rattus norvegicus, Mus musculus* (chimeric)	$G\alpha_i/G\alpha_q$	Q	94.6%	3.06	0.292	0.243	6.5	277
4gnk (A)	*Mus musculus*	$G\alpha_q$	A	99.7%	4.00	0.255	0.214	6.75	277.15
5do9 (A)	*Mus musculus*	$G\alpha_q$	A	99.7%	2.60	0.226	0.178	5.5	277.15
3ohm (A)	*Mus musculus*	$G\alpha_q$	A	99.1%	2.70	0.275	0.204	6	291
4qj3 (A)	*Mus musculus*	$G\alpha_q$	A	98.1%	3.00	0.240	0.195	6	277.15
4qj4 (A)	*Mus musculus*	$G\alpha_q$	A	98.1%	3.30	0.267	0.208	6.5	277.15
4qj5 (A)	*Mus musculus*	$G\alpha_q$	A	98.1%	3.41	0.277	0.212	6.25	277.15

All structures were obtained with high resolution and the chains of interest have high sequence identities with the human $G\alpha_q$. The pH range of crystals preparation is comprised between 5.1 for 3ah8 and 6.25 for 5do9, and the temperature ranges from 277K for 2grn and 2bcj, to 291K for 3ah8.

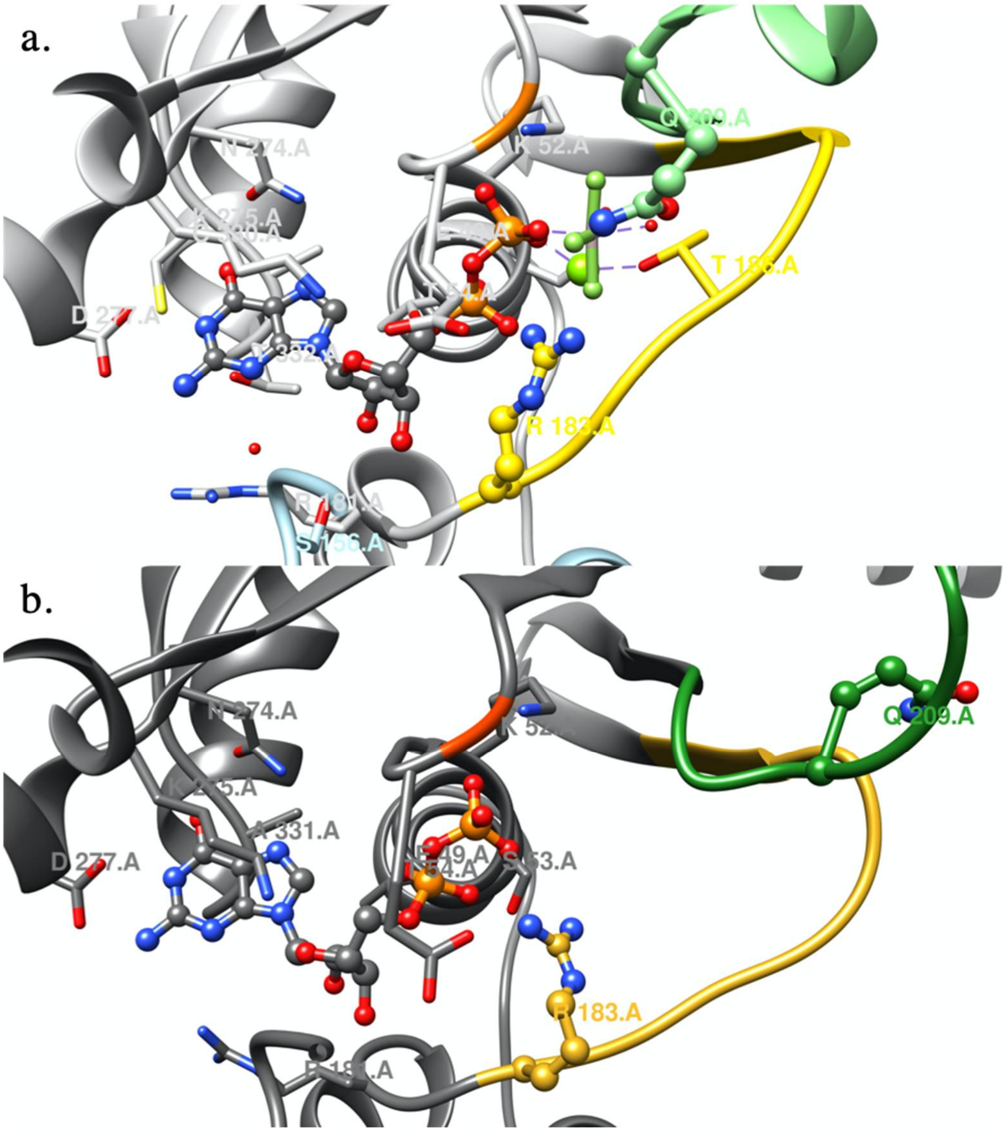
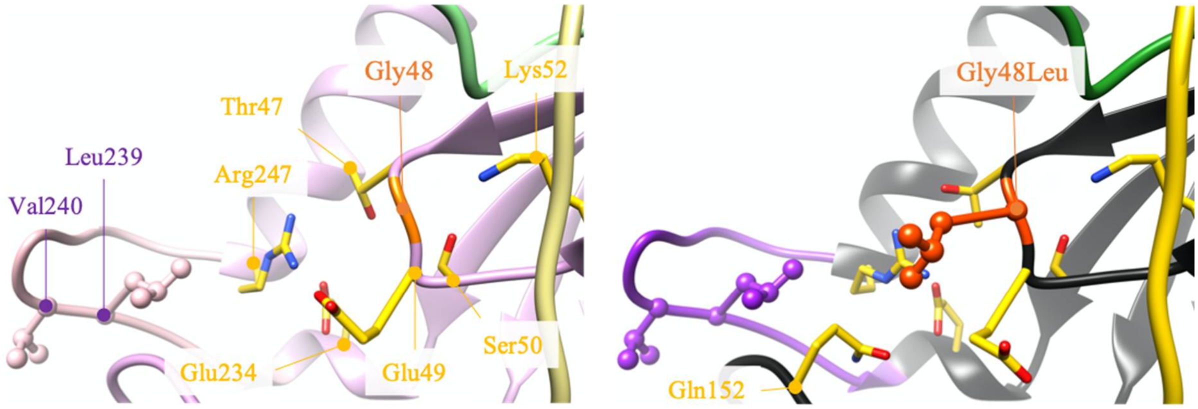


Figure S10. Residues in contact with ligands. (**a**). Active conformation crystallized in the presence of GDP, AlF_4 and Mg^{2+}(PDB ID3ohm); (**b**) Inactive conformation crystallized in the presence of GDP (PDB ID3ah8). Hotspots Arg183 and Gln209 and GDP and AlF_4 are in ball&stick, Mg^{2+} is in sphere, and the residues in contact with ligands are in sticks. SW-I (183-192) is in gold and SW-II (206-222 in green. SW-III is cut from the pictures to make them readable.

Table S3. FoldX energy analysis. Gibbs energies (ΔG) of each complex calculated by FoldX in kcal/mol.

PDB-ID	conformation	ΔG – WT (kcal/mol)	ΔG – MUT (kcal/mol)	ΔΔG (kcal/mol)
3ah8	Inactive	686.08	685.7	0.38
2rgn	Active	623.772	622.804	0.968
4gnk	Active	770.213	769.101	1.112
5do9	Active	107.297	106.156	1.141
3ohm	Active	787.41	785.7	1.71
4qj4	Active	458.572	456.77	1.802
4qj5	Active	651.392	649.573	1.819
2bcj	Active	697.385	695.083	2.302
4qj3	Active	334.318	331.149	3.169

a. Inactive conformation



b. Active conformation

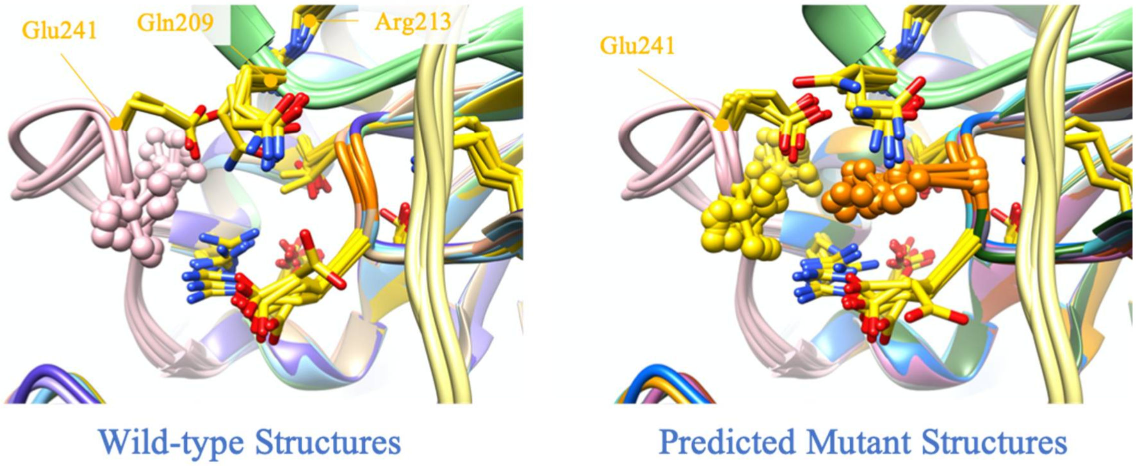
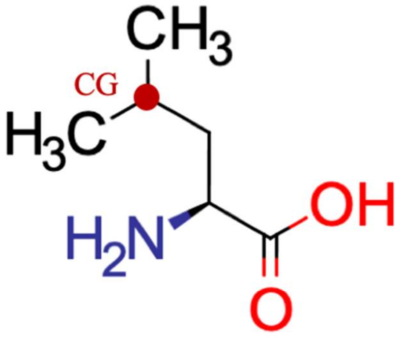
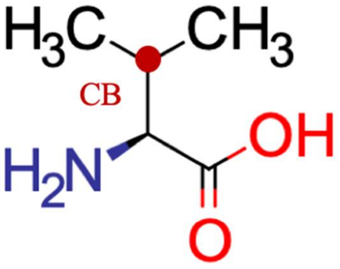


Figure S11. Wild-type and Mutated residue's neighbors. (**a**). Inactive structures (3ah8); (**b**). Active structures (2bcj, 2rgn, 3ohm, 4gnk, 4qj3, 4qj4, 4qj5, 5do9). Gly48Leu, Leu239 and Val240 are in ball&stick, residues that are at a distance inferior to 5Å to position 48 are in golden sticks. SW-I, SW-II and SW-III are in yellow, green and pink, respectively. Position 48 is colored in orange. For clarity, residues that are already mentioned in (a)-wild-type are not mentioned in the other pictures. Left pictures represent the wild-type structures and the right one the Gly48Leu predicted structures generate with FoldX.



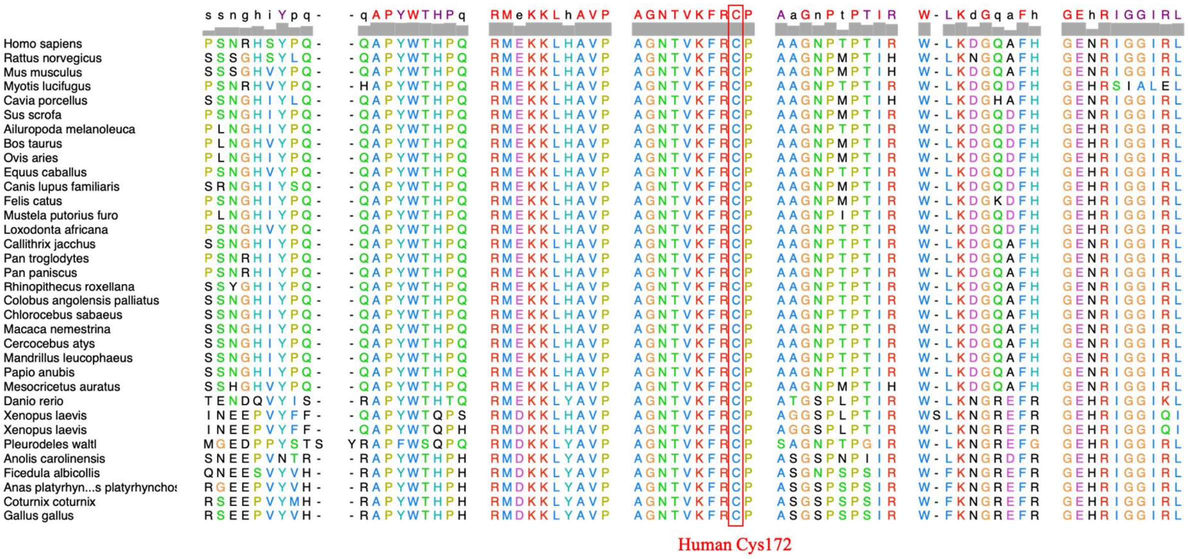
Leucine



Valine

PDB-ID	Conformation	Distance (Å)	
		V240_CB-G48L_CG	L239_CG-G48L_CG
3ah8	Inactive	8.769	15.823
2rgn	Active	5.447	6.971
4gnk	Active	5.256	7.048
5do9	Active	5.44	6.858
3ohm	Active	5.234	6.274
4qj4	Active	5.315	6.037
4qj5	Active	5.393	7.178
2bcj	Active	5.435	6.626
4qj3	Active	5.274	6.481

Figure S12. Structural analysis. Distances measured between atoms L239 C and G48L C , and V240 C and G48L C .



	ssnghiYpq-	-qAPYWTHPq	RMeKKLhAVP	AGNTVKFRCP	AaGnPtPTIR	W-LKdGqaFh	GEhRIGGIRL
Homo sapiens	PSNRHSYPQ-	-QAPYWTHPQ	RMEKKLHAVP	AGNTVKFRCP	AAGNPTPTIR	W-LKDGQAFH	GENRIGGIRL
Rattus norvegicus	SSSGHSYLQ-	-QAPYWTHPQ	RMEKKLHAVP	AGNTVKFRCP	AAGNPMPTIH	W-LKNGQAFH	GENRIGGIRL
Mus musculus	SSSGHVYPQ-	-QAPYWTHPQ	RMEKKLHAVP	AGNTVKFRCP	AAGNPMPTIH	W-LKDGQAFH	GENRIGGIRL
Myotis lucifugus	PSNRHVYPQ-	-HAPYWTHPQ	RMEKKLHAVP	AGNTVKFRCP	AAGNPTPTIR	W-LKDGQDFH	GEHRSIALEL
Cavia porcellus	SSNGHIYLQ-	-QAPYWTHPQ	RMEKKLHAVP	AGNTVKFRCP	AAGNPMPTIH	W-LKDGHAFH	GENRIGGIRL
Sus scrofa	PSNGHIYPQ-	-QAPYWTHPQ	RMEKKLHAVP	AGNTVKFRCP	AAGNPMPTIR	W-LKDGQDFH	GENRIGGIRL
Ailuropoda melanoleuca	PLNGHIYPQ-	-QAPYWTHPQ	RMEKKLHAVP	AGNTVKFRCP	AAGNPTPTIR	W-LKDGQDFH	GEHRIGGIRL
Bos taurus	PLNGHVYPQ-	-QAPYWTHPQ	RMEKKLHAVP	AGNTVKFRCP	AAGNPMPTIR	W-LKDGQDFH	GEHRIGGIRL
Ovis aries	PLNGHIYPQ-	-QAPYWTHPQ	RMEKKLHAVP	AGNTVKFRCP	AAGNPMPTIR	W-LKDGQDFH	GEHRIGGIRL
Equus caballus	PSNGHVYPQ-	-QAPYWTHPQ	RMEKKLHAVP	AGNTVKFRCP	AAGNPTPTIR	W-LKDGQDFH	GEHRIGGIRL
Canis lupus familiaris	SRNGHIYSQ-	-QAPYWTHPQ	RMEKKLHAVP	AGNTVKFRCP	AAGNPMPTIR	W-LKDGQDFH	GEHRIGGIRL
Felis catus	PSNGHIYPQ-	-QAPYWTHPQ	RMEKKLHAVP	AGNTVKFRCP	AAGNPMPTIR	W-LKDGKDFH	GEHRIGGIRL
Mustela putorius furo	PLNGHIYPQ-	-QAPYWTHPQ	RMEKKLHAVP	AGNTVKFRCP	AAGNPIPTIR	W-LKDGQDFH	GEHRIGGIRL
Loxodonta africana	PSNGHVYPQ-	-QAPYWTHPQ	RMEKKLHAVP	AGNTVKFRCP	AAGNPTPTIR	W-LKDGQDFH	GEHRIGGIRL
Callithrix jacchus	SSNGHIYPQ-	-QAPYWTHPQ	RMEKKLHAVP	AGNTVKFRCP	AAGNPTPTIR	W-LKDGQAFH	GENRIGGIRL
Pan troglodytes	PSNRHIYPQ-	-QAPYWTHPQ	RMEKKLHAVP	AGNTVKFRCP	AAGNPTPTIR	W-LKDGQAFH	GENRIGGIRL
Pan paniscus	PSNRHIYPQ-	-QAPYWTHPQ	RMEKKLHAVP	AGNTVKFRCP	AAGNPTPTIR	W-LKDGQAFH	GENRIGGIRL
Rhinopithecus roxellana	SSYGHIYPQ-	-QAPYWTHPQ	RMEKKLHAVP	AGNTVKFRCP	AAGNPTPTIR	W-LKDGQAFH	GENRIGGIRL
Colobus angolensis palliatus	SSNGHIYPQ-	-QAPYWTHPQ	RMEKKLHAVP	AGNTVKFRCP	AAGNPTPTIR	W-LKDGQAFH	GENRIGGIRL
Chlorocebus sabaeus	SSNGHIYPQ-	-QAPYWTHPQ	RMEKKLHAVP	AGNTVKFRCP	AAGNPTPTIR	W-LKDGQAFH	GENRIGGIRL
Macaca nemestrina	SSNGHIYPQ-	-QAPYWTHPQ	RMEKKLHAVP	AGNTVKFRCP	AAGNPTPTIR	W-LKDGQAFH	GENRIGGIRL
Cercocebus atys	SSNGHIYPQ-	-QAPYWTHPQ	RMEKKLHAVP	AGNTVKFRCP	AAGNPTPTIR	W-LKDGQAFH	GENRIGGIRL
Mandrillus leucophaeus	SSNGHIYPQ-	-QAPYWTHPQ	RMEKKLHAVP	AGNTVKFRCP	AAGNPTPTIR	W-LKDGQAFH	GENRIGGIRL
Papio anubis	SSNGHIYPQ-	-QAPYWTHPQ	RMEKKLHAVP	AGNTVKFRCP	AAGNPTPTIR	W-LKDGQAFH	GENRIGGIRL
Mesocricetus auratus	SSHGHVYPQ-	-QAPYWTHPQ	RMEKKLHAVP	AGNTVKFRCP	AAGNPMPTIH	W-LKDGQAFH	GENRIGGIRL
Danio rerio	TENDQVYIS-	-RAPYWTHTQ	RMEKKLYAVP	AGNTVKFRCP	ATGSPLPTIR	W-LKNGREFR	GEHRIGGIKL
Xenopus laevis	INEEPVYFF-	-QAPYWTQPS	RMDKKLHAVP	AGNTVKFRCP	AGGSPLPTIR	WSLKNGREFR	GEHRIGGIQI
Xenopus laevis	INEEPVYFF-	-QAPYWTQPH	RMDKKLHAVP	AGNTVKFRCP	AGGSPLPTIR	W-LKNGREFR	GEHRIGGIQI
Pleurodeles waltl	MGEDPPYSTS	YRAPFWSQPQ	RMDKKLYAVP	AGNTVKFRCP	SAGNPTPGIR	W-LKNGREFG	GEHRIGGIRL
Anolis carolinensis	SNEEPVNTR-	-RAPYWTHPH	RMDKKLYAVP	AGNTVKFRCP	ASGSPNPIIR	W-LKNGRDFR	GEHRIGGIRL
Ficedula albicollis	QNEESVYVH-	-RAPYWTHPH	RMDKKLYAVP	AGNTVKFRCP	ASGNPSPSIR	W-FKNGREFR	GEHRIGGIRL
Anas platyrhyn...s platyrhynchos	RGEEPVYVH-	-RAPYWTHPH	RMDKKLYAVP	AGNTVKFRCP	ASGSPSPSIR	W-FKNGREFR	GEHRIGGIRL
Coturnix coturnix	RSEEPVYMH-	-RAPYWTHPH	RMDKKLYAVP	AGNTVKFRCP	ASGSPSPSIR	W-FKNGREFR	GEHRIGGIRL
Gallus gallus	RSEEPVYVH-	-RAPYWTHPH	RMDKKLYAVP	AGNTVKFRCP	ASGSPSPSIR	W-FKNGREFR	GEHRIGGIRL

Figure S13. *FGFR4* Multiple sequence alignment centered on the Homo sapiens Cys172. Sequence retrieved from UniProt and alignment done with MUSCLE.

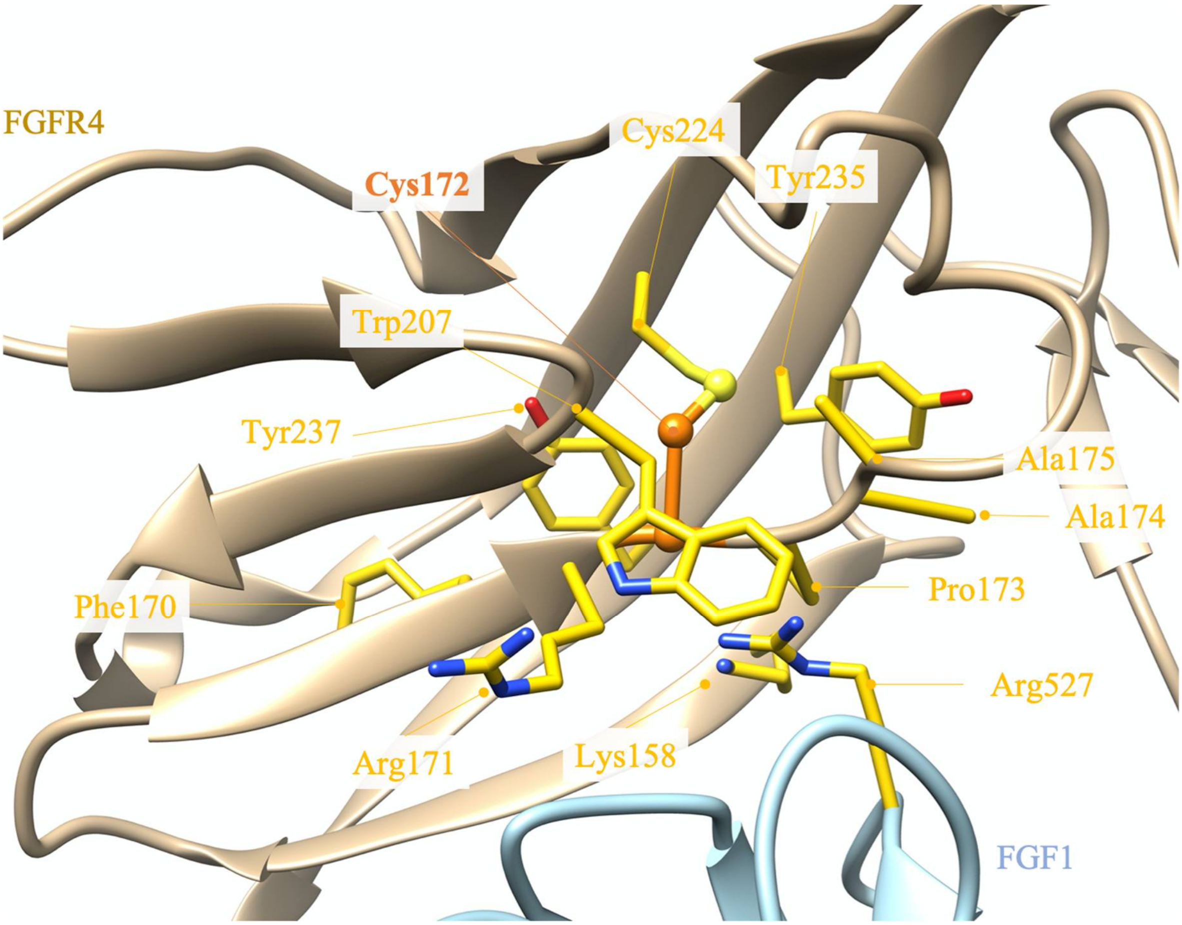


Figure S14. *FGFR4* p.Cys174 environment. Residues that are within 5Å from the *FGFR4* p.Cys174 are represented in golden sticks. Cys172 is in orange ball&stick. FGFR4 is represented in tan ribbon and FGF1 in light blue ribbon (PDB ID 1qct) [9]

Table S4. The results of the in-house NGS panel. BAP1 presented both with a mutation and a loss of one allele leading to a loss of heterozygosity (LOH)

Gene ID	Alterations	Allele frequency or level of CNV
GNAQ	c.142_143delinsTT (p.Gly48Leu)	41%
FGFR4	c.514T>G (p.Cys271Gly)	47%
BAP1	c.68-4_84delinsGA and LOH of 3p	82% (due to LOH)
NRAS	Deletion	1x copy left
3p	Loss	LOH
6q	Loss	LOH
6p	Gain	3x

References

[1] Potapov, V.; Cohen, M.; Schreiber, G. Assessing computational methods for predicting protein stability upon mutation: Good on average but not in the details. *Protein Eng. Des. Sel.* **2009**, *22*, 553–560. https://doi.org/10.1093/protein/gzp030.

[2] Moorhouse, M.; Barry, P. The Protein Databank. *Bioinforma. Biocomput. Perl*, **2005.** https://doi.org/10.1002/0470020571.ch10.

[3] Nishimura, A.; Kitano, K.; Takasaki, J.; Taniguchi, M.; Mizuno, N.; Tago, K.; et al. Structural basis for the specific inhibition of heterotrimeric G q protein by a small molecule. *Proc. Natl. Acad. Sci.* **2010**, *107*, 13666–13671. https://doi.org/10.1073/pnas.1003553107.

[4] Tesmer, V.M.; Kawano, T.; Shankaranarayanan, A.; Kozasa, T.; Tesmer, JJG. Snapshot of Activated G Proteins at the Membrane: The Gαq-GRK2-Gßγ Complex. *Science* (80-) **2005**.

[5] Waldo, G.L.; Ricks, T.K.; Hicks, S.N.; Cheever, M.L.; Kawano, T.; Tsuboi, K.; et al. Kinetic scaffolding mediated by a phospholipase C-β and Gq signaling complex. *Science* (80-) **2010**, https://doi.org/10.1126/science.1193438.

[6] Lyon, A.M.; Dutta, S.; Boguth, C.A.; Skiniotis, G.; Tesmer, JJG. Full-length Gαq-phospholipase C-β3 structure reveals interfaces of the C-terminal coiled-coil domain. *Nat. Struct. Mol. Biol.* **2013**, https://doi.org/10.1038/nsmb.2497.

[7] Lyon, A.M.; Tesmer, JJG. Structural insights into phospholipase C-β function. *Mol. Pharmacol.* **2013**, https://doi.org/10.1124/mol.113.087403.

[8] Taylor, V.G.; Bommarito, P.A., Tesmer, JJG. Structure of the Regulator of G Protein Signaling 8 (RGS8)-Gα q Complex. *J. Biol. Chem.* **2016**, https://doi.org/10.1074/jbc.m115.712075.

[9] Huhtala, M.T.; Pentikainen, O.T.; Johnson, M.S. A dimeric ternary complex of FGFR1, heparin and FGF-1 leads to an "electrostatic sandwich" model for heparin binding. *Structure* **1999**, *7*, 699–709, https://doi.org/10.1016/S0969-2126(99)80129-4.