Crystal structure of multifunctional G β 5–RGS9: an R7family RGS protein complex

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Supplementary Figure 1



Supplementary Figure 1 R7-RGS and Gβ secondary-structure and sequence alignments. Secondary-structure elements from the Gβ5–RGS9 complex are indicated above sequence alignments as cylinders (α-helices) and arrows (β-strands) and are colored as in **Figure 1**. Residue numbers above sequences are for mouse RGS9 and mouse Gβ5. Intra- and intermolecular contacts of RGS9 as discussed above or in ref. 1 (Gα contacts) are boxed and colored according to the inset legend. An SST2 segment with no homology to R7-RGS proteins is boxed with magenta. Residues boxed on the Gβ5 sequence represent contacts with RGS9. Residues boxed on Gβ1-4 sequences are Gβ subunit consensus contacts with Gα and Gγ subunits²⁻⁴. Sequentially and/or functionally divergent Gβ residues are indicated as red text. Additional contacts or modifications as discussed in the text are indicated and described in the figure. GenBank accession numbers for the proteins from top to bottom are BAE23565, NP_001074538, Q9Z2H2, AAH51133, P49809, NP_740905, P11972, NP 619733, P62874, P62880, Q61011 and P29387.

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Supplementary Figure 2

Gly7 O	Asn154 ND2
GIn8 NE2	Asn154 OD1
Gin8	Leu125
	Tyr155
	Lys219
	Ser240
Arg13	Asp241
Arg13 NH1	Asp241 OD1
\rightarrow	Cys217 O
Ala15	Asp259
Ala15 N	Asp259 OD1
Phe16	Ile283 O
Lys19	Asp260 O
lle20	🗕 lle283
Arg62 NH1	Glu280 OE2
Leu63	Glu280
lle65	Ser281
Leu68	lle320
Glu69	Phe322
Glu69 CA	Ser281 OG
Glu69 OE1	Arg317 NH1
Glu69 OE2	Arg317 NH2
Asn72	Asp304
Asn72 ND2	Asp304 O
Leu73	• Thr306
Phe76	> Phe284
Tyr80	• Tyr305
Asp202 OD2	Lys65 NZ
Asp202 O	Arg327 NH1
Tyr203 C	🗕 Trp345
Gly204 CA	🗕 Trp107
Leu205	Met109
Arg207	Cys217
Arg207 CA	• Leu199
Arg207 NH1	Asp241 OD2
Val208 N	Asp197 OD2
	Asn154
Val208	• Gly173 O
Thr209 N	Asp197 OD1
Thr209	Ala196

0		
	Val219	Leu12
	Val222	Leu15
HIS192 O	Lys224 NZ	
	Glu225	US16 CA
GIN233 CA		Ala19
Ala234 N	Tyr228 OH	Ala19 0
	Tyr228	Leuzz
	Tyr229	Lysz3
Cysz310	GIN231 NEZ	
Val232 CA	Ala222.0	
Val232 CA	Ala232 0	Argso NH2
	A10232	
	Arg225.0	
Val222	Arg235 0	Argso NHT
Vaizoz	Thr227	Arg20
Arg269.0	Thr227 CA	Aigsu
Alg2090	Val238 N	
	Val230 N	
	Val230 C	Leuss
/	Vai230	
Arc:269	Eys239	Lou22 0
Alg269	Ser240 N	
Ala270	Ser240 OG	His34 CA
Asp207	Ser240	HIS34 NDT
Asp267 001	Ser241 N	Asp35 OD1
	Ser241 OG	Asp35 ODZ
Alp252	Ser241 CA	vaise
	Sor242 OG	Glu27 C
Ser250		
Tyr248 OH	Gly245 CA	Leuso CA
Pro249		
Gly295 C	lle247	Val41
Ser294 0	Val248	Ala42
Ser294 N	Tyr250	Val45
Ser294	Ser251 OG	
Ser294 OG	Ser251 N	
Leu293	Ser251	
Ser292 O	Glu252	
Ser292	Phe254	
Leu297	Asn257	
Phe299	Asp258 OD1	
Arg296	Asp258	
Gly337 0 🖈	Ala259	
Thr338	lle260	Met53
Thr338 O	lle260 N	
Thr338 OG1	Met261	Leu48
Ser334 OG	Asn268	i <u> </u>
Asp336 OD1	Asn268 ND2	
Lys75 0 4 -	Pro269	
Trp71	Trp270 NE1	
Ser92	Trp270 O	Arg56
Phe93	Trp270	Arg57
Ala339	lle271	
Val351	Asp273	
Ala353	Asp273 OD1	Arg57 NH2
Asp336	• Thr275	
Asp336 OD2	Phe277	
Lys75	Trp278 NE1	
Arg76	Trp278	
Lys75 NZ	Asn281 O	-

RGS domain

Pro205 0	Arg305 NH1
Ser206 O	Arg305 NH2
Ser206 C	• Arg305
GIn167 NE2	Lys336 O
Ser164 OG	Tyr337 O
Ash163 ND2	Tyr337 OH
Gly209 0	Tyr337
1111162 App162	Tyr337 C
Asii103	Gly338 O
Gin167	Asp339 CA
	Asp339
Asp181	Gin340
Asp181 OD2	GIn340 N
Glu183	• His381
Leu188 •	• Arg382
Met226	• Tyr383
Arg227	Asp386 OD2
Asn210 ND2	Tyr392
Glu207 OE2	- 1yr393
Glu207	Lys397 NZ
Glu207 OE1	Ly3001 NZ

Hydrogen bond Salt-bridge van der Waals contact Water-mediate H-bond N-terminal region DHEX-GGL linker DEP domain GGL domain RGS domain Gβ5 **Supplementary Figure 2** Specific contacts between residues of RGS9 and G β 5 residues. Diagram illustrating specific intermolecular contacts between RGS9 and G β 5 (blue) residues. The RGS9 contact residues are colored based on their positions in the N-terminal tail (light-grey), DHEX-GGL linker (dark-grey), DEP domain (orange), GGL domain (red) and RGS domain (green).

Supplementary Figure 3



Supplementary Figure 3 The electrostatic potential of G β 5–RGS9 supports the membrane orientation model. Electrostatic potential maps, calculated at ± 2 *kT*, and depicted as positive (blue) and negative (red) contours, are superposed on the G β 5–RGS9 structure backbone trace colored as in **Figure 1**. **Top**, Oriented as in **Figure 6a** relative to the membrane. **Bottom**, The structure is rotated 90° around the X-axis with respect to the **top** image.