

Supplementary Information

Allosteric communication pathways routed by $\text{Ca}^{2+}/\text{Mg}^{2+}$ exchange in GCAP1 selectively switch target regulation modes

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Supplementary Tables

Supplementary Table T1. Hubs for the PSN of each mGCAP1 state generated by PyIntergraph.

Residues associated with cone, cone-rod and macular dystrophies are underlined and in bold

Deg	$\text{EF2}^{\text{Ca}}\text{EF3}^{\text{Ca}}\text{EF4}^{\text{Ca}}$	EF2^{Mg}	$\text{EF2}^{\text{Mg}}\text{EF3}^{\text{Mg}}$
8	D64 <u>D100</u>	W21 D64	D64 A78 <u>D100</u> D175
7	W21 R40 F96 D144 V160	R40 A78 F96 <u>D100</u> D175	W21 R40 D72 F96 I116 I122 V139 L166 L176
6	A52 F60 D72 E111 I119 <u>I143</u> D163 L166 D175	A52 F60 I116 I119 F135 V139 D144 V160 D163 L176	V10 A52 F60 E75 A79 I119 F135 D144 V160 D163 L183
5	V10 F25 L45 V56 M59 F73 E75 A78 A79 K91 L92 Y95 I122 V139 L170 L176 V180	V10 F25 V56 M59 E61 D72 F73 E75 A79 <u>L84</u> K91 L92 R93 Y95 R109 I122 S126 F156 L166 T171	F25 V56 M59 E61 F73 L92 W94 Y95 <u>Y99</u> D108 R109 E111 I12L I115 R120 A121 S126 F156 L170 R178 I179 V180 R181
4	L13 C29 F43 S53 Y55 E57 D68 L82 <u>L84</u> R93 W94 <u>Y99</u> D108 R109 T114 I115 I116 R120 A121 S126 F135 F140 D148 <u>E155</u> F156 I157 D168 T171 I179 R182 L183 Ca1 Ca2	E11 L13 E17 Y22 C29 L36 F39 F43 L45 S53 Y55 E57 F63 F65 D68 L80 L82 V83 W94 T114 A121 A132 F140 L153 I157 D168 L170 R172 R178 R181 L183	M5 E6 L13 Y22 C29 E38 F43 L45 S51 S53 Y55 E57 F63 F65 N66 D68 V77 L80 L82 V83 <u>L84</u> K91 R93 <u>N104</u> <u>I107</u> T114 M130 A132 F140 <u>I143</u> I157 D168 T171 R172 R182 Mg1 Mg2

Supplementary Table T2. Hubs for the PSN of each nmGCAP1 state generated by PyInteraph.

Residues associated with cone, cone-rod and macular dystrophies are underlined and in bold

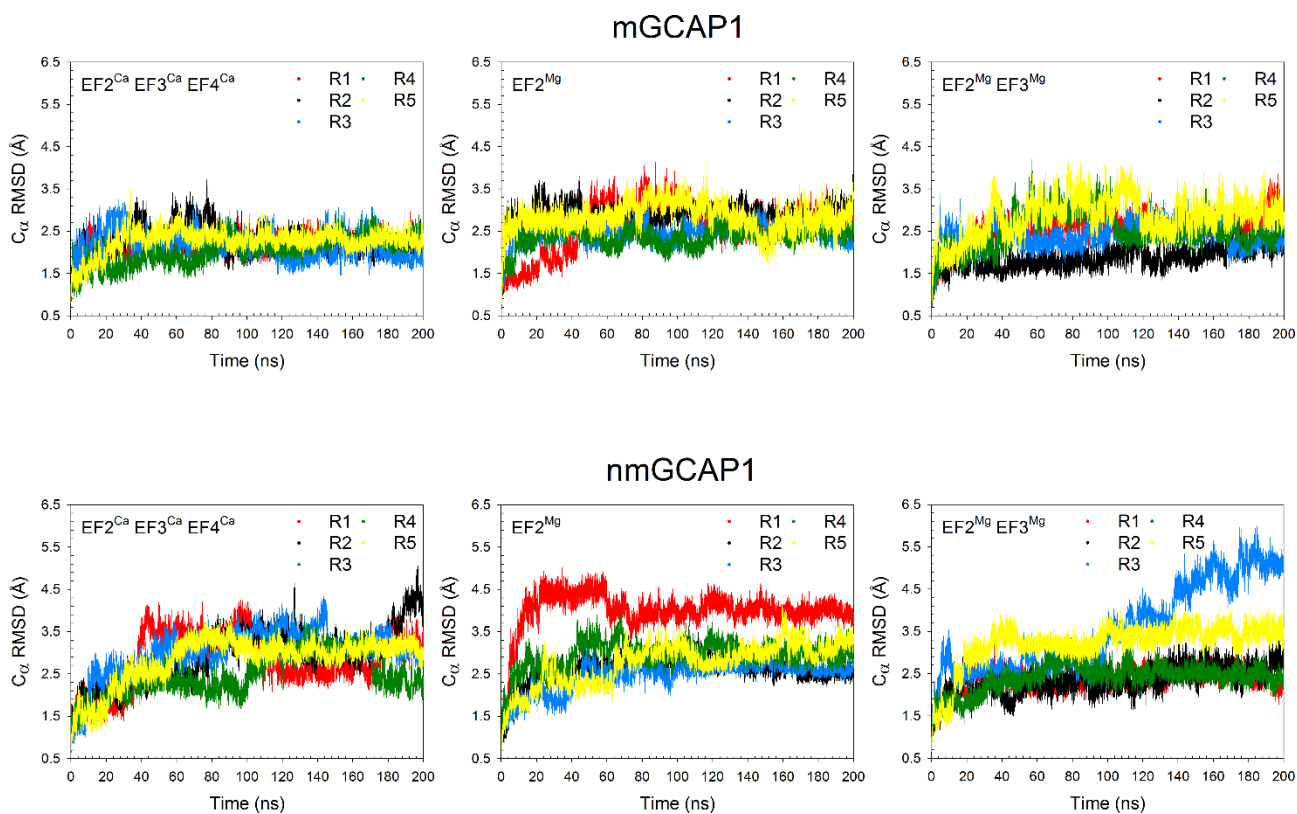
Deg	EF2 ^{Ca} EF3 ^{Ca} EF4 ^{Ca}	EF2 ^{Mg}	EF2 ^{Mg} EF3 ^{Mg}
8	W21 D64 A78 <u>D100</u> L183	W21 D64	W21 D64
7	F96 I122 <u>I143</u> D144 V160 L166	A78 A79 <u>D100</u>	A78 <u>D100</u> V139
6	V10 F39 A52 F60 D72 E75 K91 L92 Y95 E111 I119 F156 D163 D175	A52 F60 E75 F96 I116 I119 I122 V160 D163	V10 R40 A52 M59 F60 E75 A79 I119 I122 V160
5	M5 F25 R40 Y55 V56 M59 E61 A79 L82 S126 V139 <u>E155</u> L170 L176 I179 V180 R182	V10 E11 F25 M59 D72 L92 R109 S126 F135 V139 D144 L166 D175 R178 I179 R181	V56 D72 F96 R109 E111 S126 F140 D144 D163 L170 D175 R178 V180 L183
4	E6 E11 L13 C29 L36 F42 F43 S49 D68 F73 V77 L80 V83 K85 R93 W94 D108 R109 T114 I115 I116 A121 R120 F135 F140 D148 <u>L151</u> I157 D168 T171 R172 S173 R178 R181 Q184 Ca1 Ca2	E17 L36 F39 R40 V56 E61 F63 F65 D68 F73 L80 V83 <u>L84</u> K91 W94 Y95 T114 R120 A132 F156 I157 L170 R172 L174 V180 L183 Mg1	T16 E17 F25 S53 Y55 N66 D68 F73 Y76 <u>L84</u> K85 K91 L92 <u>I107</u> D108 T114 I115 I116 A121 F135 F156 I157 L166 D168 R172 I179 R181 Mg1

Supplementary Table T3. Hubs for the PSN of each nmGCAP1 state generated by WEBPSN.

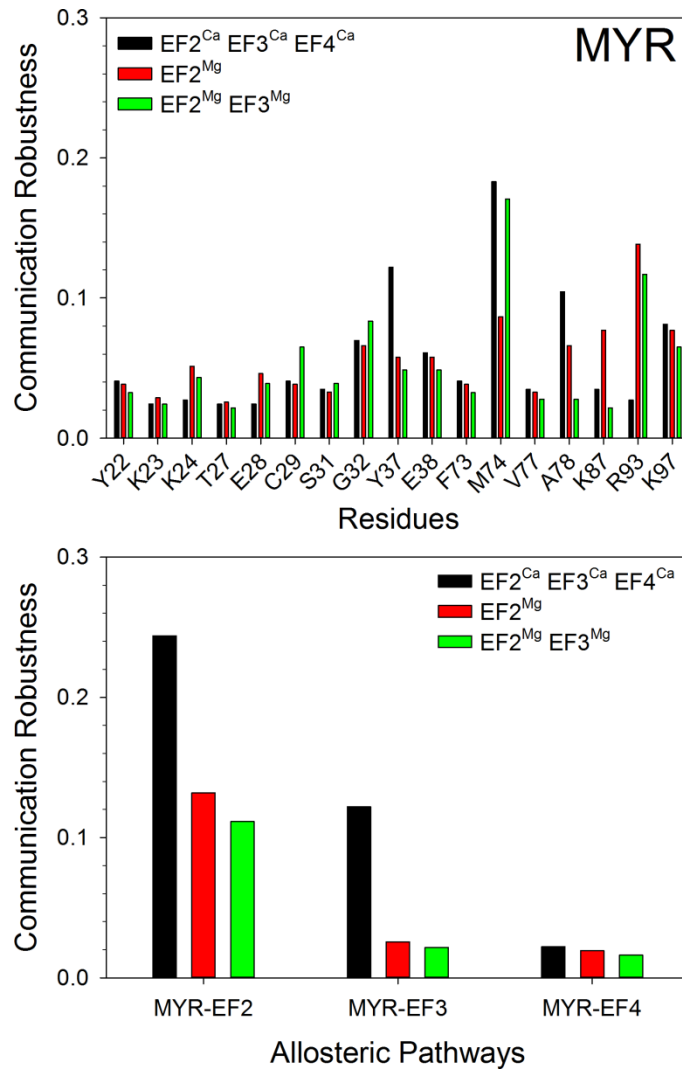
Residues associated with cone, cone-rod and macular dystrophies are underlined and in bold

mGCAP1															
Deg	EF2 ^{Ca} EF3 ^{Ca} EF4 ^{Ca}					EF2 ^{Mg}					EF2 ^{Mg} EF3 ^{Mg}				
6	E111					E75					E75				
5	F73	E75	<u>E155</u>			F25	D68	F73	L80	Y95	W21	N66	Y76	<u>I107</u>	E111
4	W21	Y22	F43	D64	N66	<u>Y99</u>	Y22	F39	F63	D64	F42	F43	T62	D64	<u>N104</u>
	D68	W94	I122	<u>N104</u>	<u>I107</u>	N66	Y76	R109	F135	Mg1	V139				
	D148	<u>L151</u>	Ca1	Ca2											
nmGCAP1															
Deg	EF2 ^{Ca} EF3 ^{Ca} EF4 ^{Ca}					EF2 ^{Mg}					EF2 ^{Mg} EF3 ^{Mg}				
8						F42									
7						Y76									
6						W21	F39	F63	L92	L166	E111				
5	W21	E111	<u>E155</u>			F25	<u>L84</u>	F135	I179	N66 E75 F135					
4	F25	F39	F42	D68	E75	L34	F43	Y55	T62	D64	L34	D64	D68	I71	V83
	Y76	Y95	<u>I143</u>	D148	V160	D68	E75	L80	L82	V83	Y95	<u>Y99</u>	<u>N104</u>	L112	F140
	Ca1						R109	L153	I157	V160	L170	L174	Mg1		

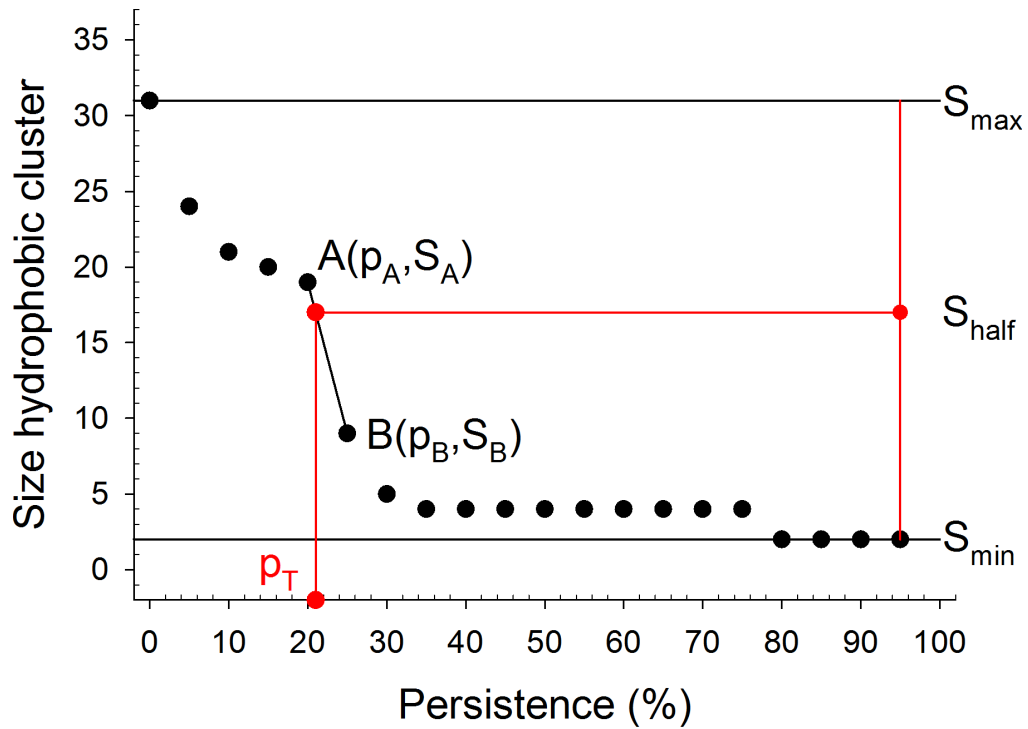
Supplementary Figures



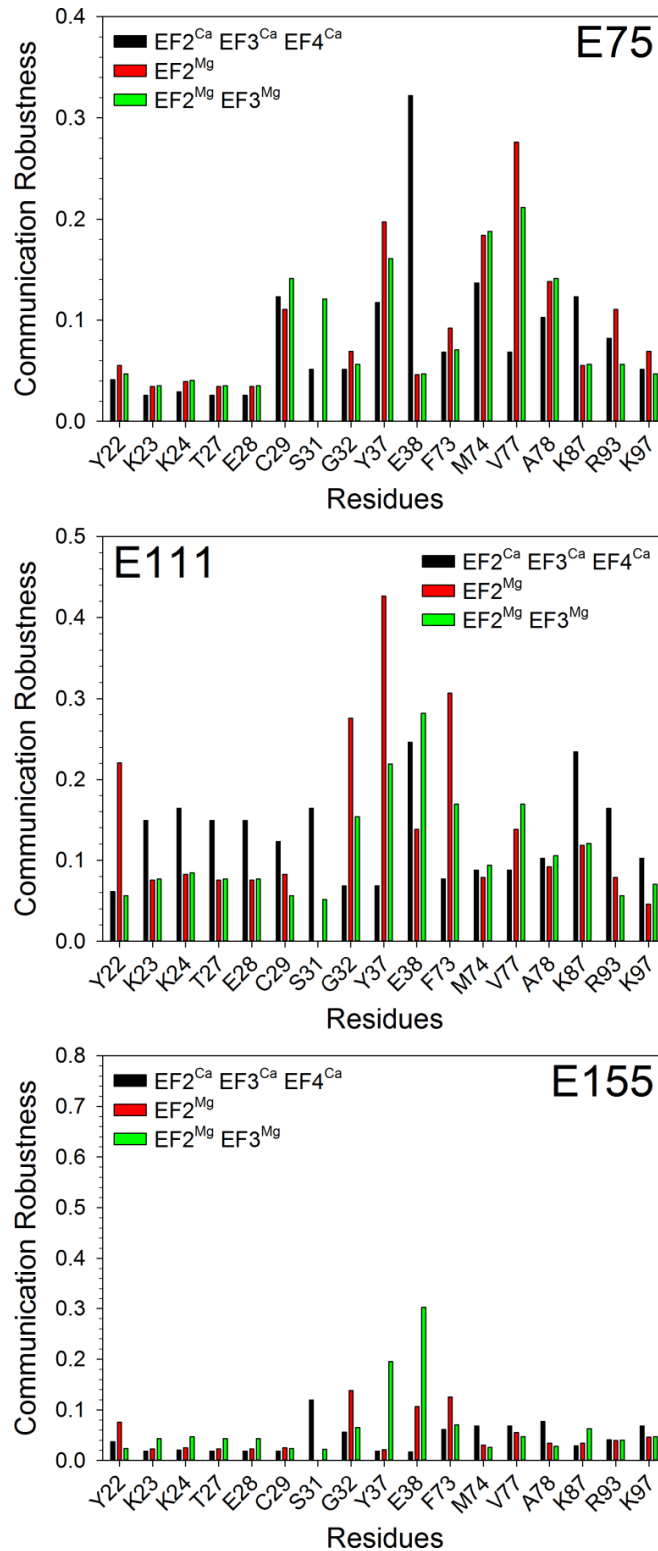
Supplementary Figure S1. Time evolution of the C_{α} RMSD of the five 200 ns MD simulation replicas (R1 to R5) calculated with respect to the relative equilibrated structure of mGCAP1 (top panels) and nmGCAP1 (bottom panels) in their EF2^{Ca}EF3^{Ca}EF4^{Ca} (left), EF2^{Mg} (center) and EF2^{Mg}EF3^{Mg} (right) forms. R1 RMSD is represented in red, R2 RMSD is black, R3 RMSD is blue, R4 RMSD is green, R5 RMSD is yellow.



Supplementary Figure S2. Communication Robustness between the myristoyl group and residues belonging to the GC binding interface (top) and bidentate Glu residues (bottom) of EF2, EF3 and EF4 of mGCAP1 in its EF2^{Ca}EF3^{Ca}EF4^{Ca} (black), EF2^{Mg} (red) and EF2^{Mg}EF3^{Mg} (green) forms.



Supplementary Figure S3. Quantitative assessment of the persistence threshold p_T for PSN analyses. S_{\max} and S_{\min} are, respectively, the maximum and the minimum size of the largest hydrophobic cluster detected over 5% persistence intervals. S_{half} is half the difference between S_{\max} and S_{\min} and p_T is the calculated x value corresponding to S_{half} , derived from the linear interpolation between $A(p_A, S_A)$ and $B(p_B, S_B)$, where $[S_A, S_B]$ is the smallest 5% persistence interval containing S_{half} .



Supplementary Figure S4. Communication Robustness between bidentate Glu residues of EF2 (top), EF3 (middle), EF4 (bottom) and residues belonging to the GC binding interface of nmGCAP1 in its EF2^{Ca}EF3^{Ca}EF4^{Ca} (black), EF2^{Mg} (red) and EF2^{Mg}EF3^{Mg} (green) forms.

Supplementary Videos

Supplementary Video V1: Paths constituted by nodes with the highest selective betweenness connecting bidentate Glu residues of EF2 and the residues belonging to the GC binding interface with the highest CR. Secondary structure is represented in grey cartoons, Ca^{2+} ions are shown as red spheres, the myristoyl group is represented as teal sticks, $\text{C}\alpha$ of the residues belonging to the paths are represented as spheres, edges are represented by sticks, the side chains of the extremes of the paths are represented as sticks. Nodes and edges specific of the $\text{EF2}^{\text{Ca}}\text{EF3}^{\text{Ca}}\text{EF4}^{\text{Ca}}$ form are represented in yellow, those specific of the EF2^{Mg} form are represented in blue, those in common are represented in green.

Supplementary Video V2: Paths constituted by nodes with the highest selective betweenness connecting bidentate Glu residues of EF3 and the residues belonging to the GC binding interface with the highest CR. Secondary structure is represented in grey cartoons, Ca^{2+} ions are shown as red spheres, the myristoyl group is represented as teal sticks, $\text{C}\alpha$ of the residues belonging to the paths are represented as spheres, edges are represented by sticks, the side chains of the extremes of the paths are represented as sticks. Nodes and edges specific of the $\text{EF2}^{\text{Ca}}\text{EF3}^{\text{Ca}}\text{EF4}^{\text{Ca}}$ form are represented in yellow, those specific of the EF2^{Mg} form are represented in blue, those in common are represented in green.

Supplementary Video V3: Paths constituted by nodes with the highest selective betweenness connecting bidentate Glu residues of EF4 and the residues belonging to the GC binding interface with the highest CR. Secondary structure is represented in grey cartoons, Ca^{2+} ions are shown as red spheres, the myristoyl group is represented as teal sticks, $\text{C}\alpha$ of the residues belonging to the paths are represented as spheres, edges are represented by sticks, the side chains of the extremes of the

paths are represented as sticks. Nodes and edges specific of the $EF2^{Ca}EF3^{Ca}EF4^{Ca}$ form are represented in yellow, those specific of the $EF2^{Mg}$ form are represented in blue, those in common are represented in green, residues whose mutations are associated with Cone, Cone-Rod or Macular Dystrophies are represented in orange.