

# SUPPLEMENTARY MATERIAL

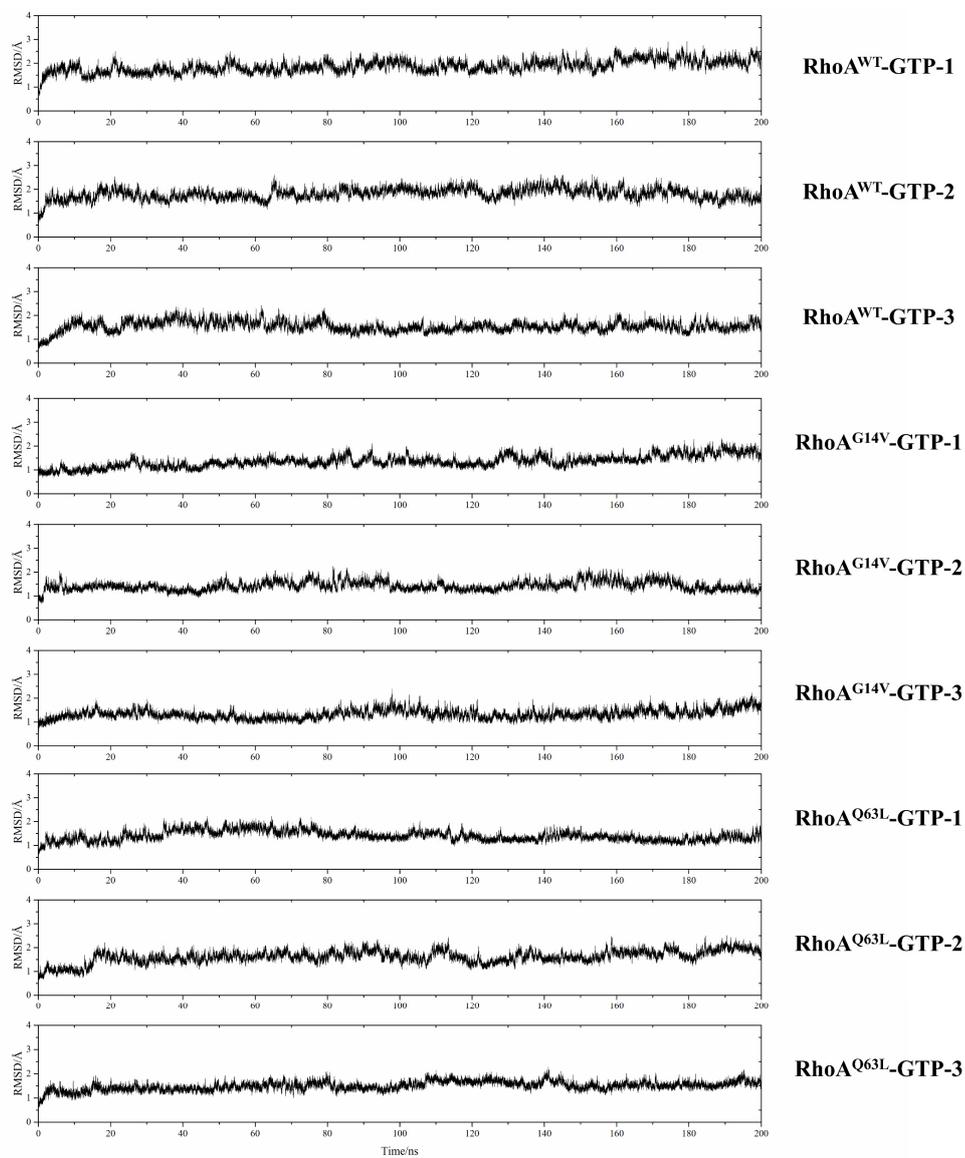
## Activation Mechanism of RhoA Caused by Constitutively Activating Mutations G14V and Q63L

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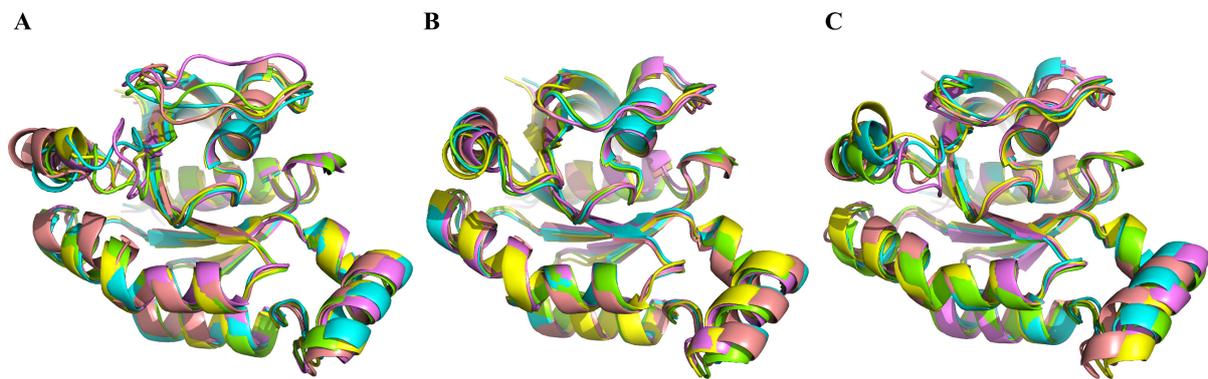
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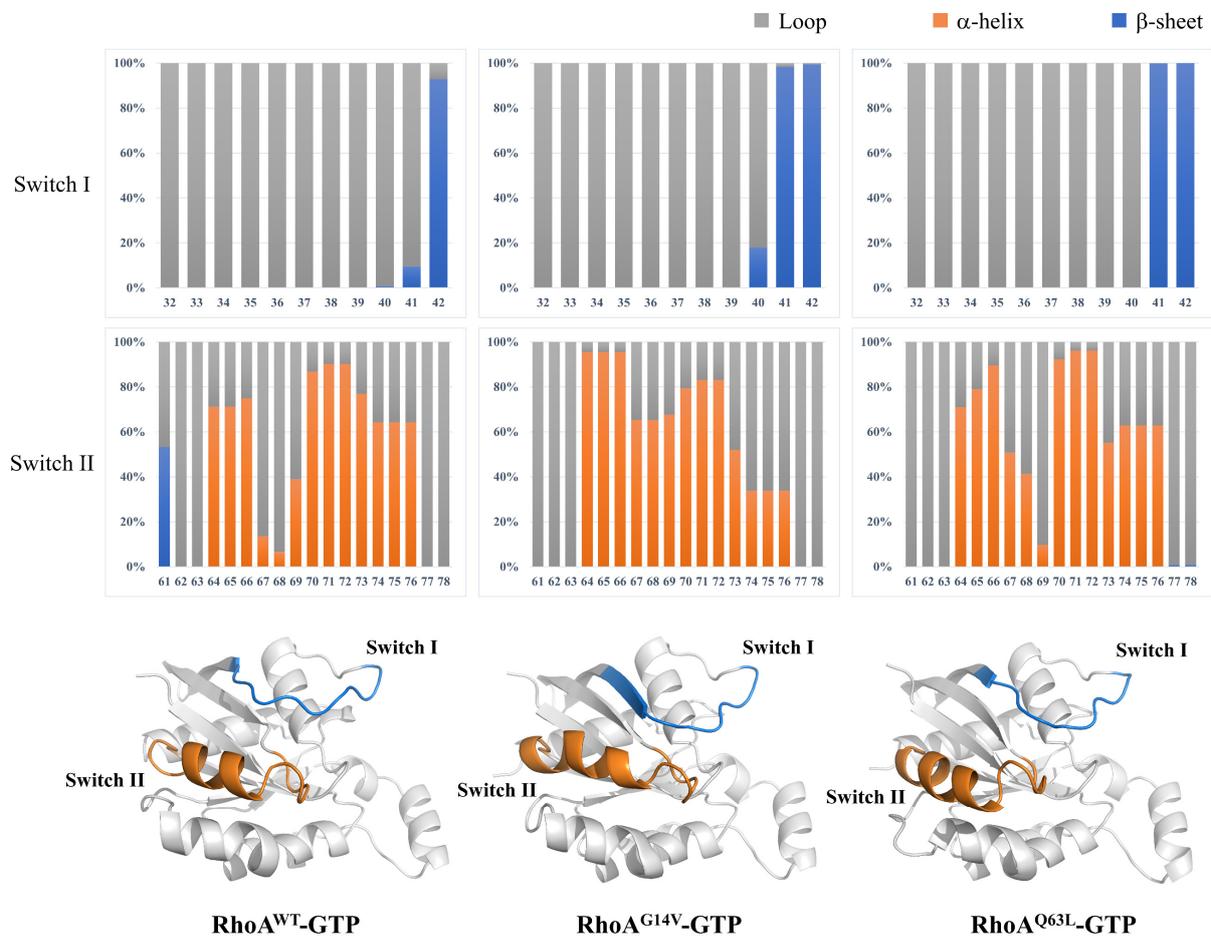
**Figure S1.** Time series of RMSD values of complexes RhoA<sup>WT</sup>-GTP, RhoA<sup>G14V</sup>-GTP, and RhoA<sup>Q63L</sup>-GTP during the triple 200 ns MD simulations.



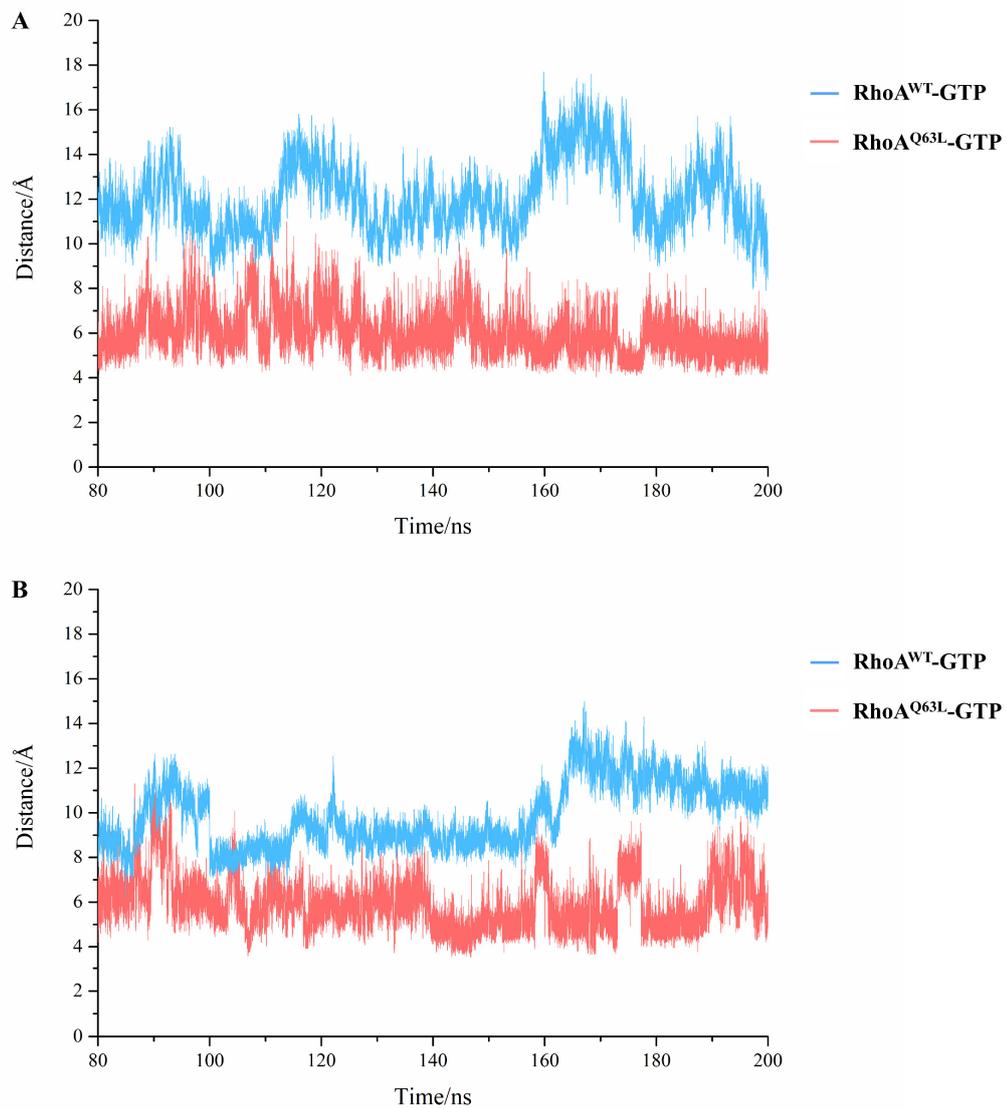
**D**

Cluster	Color	Fraction (%)		
		RhoA <sup>WT</sup> -GTP	RhoA <sup>G14V</sup> -GTP	RhoA <sup>Q63L</sup> -GTP
1	Green	46.06	36.61	34.77
2	Cyan	24.06	34.94	28.92
3	Violet	17.75	19.35	24.97
4	Yellow	8.02	6.94	5.86
5	Salmon	4.10	2.16	5.47

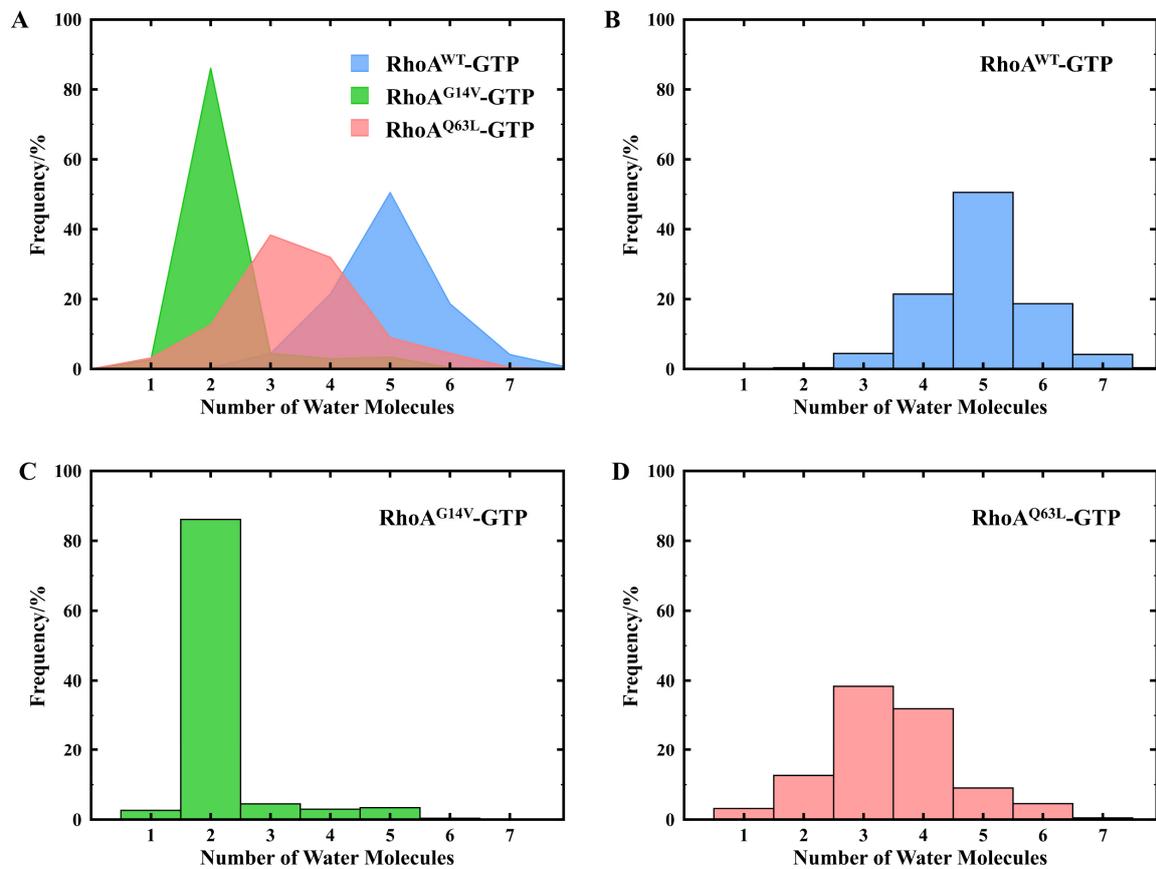
**Figure S2.** Representative structures of the five clusters of RhoA in systems RhoA<sup>WT</sup>-GTP (A), RhoA<sup>G14V</sup>-GTP (B), and RhoA<sup>Q63L</sup>-GTP (C) obtained from clustering. The colors and fractions of each cluster are listed in (D).



**Figure S3.** The overall secondary structure assignment for residues located on switch I and II regions in GTP-bound RhoA<sup>WT</sup>, RhoA<sup>G14V</sup>, and RhoA<sup>Q63L</sup> throughout the MD simulations. The representative structure for each protein was shown as cartoon, with the two switch regions highlighted and labeled.



**Figure S4.** Time series of the distances from the centroid of the side-chain heavy atoms of V38 to the centroid of the side-chain heavy atoms of L63 (or Q63 in the wild type) (A) and to the centroid of the side-chain heavy atoms of Y66 (B) in RhoA<sup>WT</sup>-GTP and RhoA<sup>Q63L</sup>-GTP.



**Figure S5.** The probability distributions of the average numbers of water molecules within 3.0 Å around the GTP's  $\gamma$ -phosphate in RhoA<sup>WT</sup>-GTP, RhoA<sup>G14V</sup>-GTP, and RhoA<sup>Q63L</sup>-GTP during the simulation time (A) and the individual frequency histograms for each system (B-D).

**A**

	12	19	32	42	61	78
Human_RhoA	G	D	E	V	A	G
Chimpanzee_RhoA	G	D	E	V	A	G
Mouse_RhoA	G	D	E	V	A	G
Rat_RhoA	G	D	E	V	A	G
Zebrafish_RhoA -A	G	D	E	V	A	G
Zebrafish_RhoA -B	G	D	E	V	A	G
Zebrafish_RhoA -C	G	D	E	V	A	G
Zebrafish_RhoA -D	G	D	E	V	A	G

**B**

Species	Length	Identity	Similarity
Human_RhoA	193	/	/
Chimpanzee_RhoA	193	100.0%	100.0%
Mouse_RhoA	193	99.5%	100.0%
Rat_RhoA	193	99.5%	100.0%
Zebrafish_RhoA-A	193	93.3%	96.4%
Zebrafish_RhoA-B	193	95.9%	96.9%
Zebrafish_RhoA-C	193	96.4%	97.9%
Zebrafish_RhoA-D	193	94.3%	97.4%

**Figure S6.** Comparisons between RhoA encoded in human and other species. (A) Sequence alignment of the P-loop (residues 12-19), switch I (residues 32-42), and switch II (residues 61-78) regions for different RhoA proteins. The two mutation sites G14 and Q63 are colored green and pink, respectively. (B) Sequence similarities between RhoA encoded in human and other species.

**Table S1.** Hydrogen bonds between GTP and RhoA with an average fraction of more than 75% in any of the three systems over the last 120 ns of the simulations.

Molecule (Group)		System (Fraction%)		
GTP	RhoA	RhoA <sup>WT</sup> -GTP	RhoA <sup>G14V</sup> -GTP	RhoA <sup>Q63L</sup> -GTP
GTP@O3G	LYS_18@NZ-HZ	97.23 <sup>†</sup>	99.99 <sup>†</sup>	97.16 <sup>†</sup>
GTP@O2G	PRO_36@CA-HA	9.99	89.02	42.79
GTP@O2G	THR_37@N-H	0.01	98.90	77.77
GTP@O1G	ALA_15@N-H	97.72	99.79	99.05
GTP@O1G	TYR_34@OH-HH	90.47	100.00	99.93
GTP@PB	LYS_18@NZ-HZ	64.52 <sup>†</sup>	81.13 <sup>†</sup>	73.30 <sup>†</sup>
GTP@O2B	THR_19@N-H	95.73	98.73	94.96
GTP@O1B	GLY_17@N-H	97.37	99.39	98.99
GTP@O1B	LYS_18@NZ-HZ	99.98 <sup>†</sup>	99.98 <sup>†</sup>	99.88 <sup>†</sup>
GTP@O1B	LYS_18@CG-HG3	71.09	82.84	77.79
GTP@O1B	LYS_18@N-H	99.98	99.99	99.98
GTP@O3A	GLY_17@N-H	73.81	73.99	75.45
GTP@O1A	CYS_20@N-H	99.92	99.98	99.98
GTP@O1A	CYS_20@SG-HG	98.12	98.43	97.92
GTP@N7	ASN_117@ND2-HD21	95.76	96.88	97.17
GTP@O6	LYS_162@N-H	95.57	96.34	95.06
GTP@O5'	TYR_34@CD1-HD1	40.38	81.99	72.64
GTP@N1-H1	ASP_120@OD1	99.95	97.26	96.40
GTP@N1-H1	ASP_120@OD2	82.34	91.12	89.28
GTP@N2-H21	ASP_120@OD2	99.94	99.88	93.53
<b>Total Fraction</b>		<b>1609.88</b>	<b>1885.63</b>	<b>1779.02</b>

<sup>†</sup>, total fraction of the hydrogen bonds between NZ-HZ1, NZ-HZ2, NZ-HZ3 of Lys18 and O3G, PB, O1B of GTP.