Supplemental Figures, Tables and Data

Methods

Human RGS protein sequence references (UniProt IDs)

For data shown in Figures and Tables, the following human RGS protein exome sequences were analyzed and presented. The UniProt IDs for each of these are: RGS1: Q08116-1; RGS2: P41220-1; RGS3: P49796-3; RGS4: P49798-3 (longest RGS4-3) and P49798-1 (canonical RGS4-1); RGS5: O15539-3; RGS6: P49758-3; RGS7: P49802-1; RGS8: P57771-2; RGS9: O75916-1; RGS10: O43665-3 (longest RGS10-3) and O43665-1 (canonical RGS10-1); RGS11: O94810-1; RGS12: O14924-1; RGS13: O14921-1; RGS14: O43566-7; RGS16: O15492-1; RGS17: Q9UGC6-1; RGS18: Q9NS28-1; RGS19: P49795-1; RGS20: O76081-1; RGS21: Q2M5E4-1.

Immunoaffinity capture of the RGS4:Gαi1 complex from cells

HEK293 cells were transfected with Venus-RGS4 (WT or R134W) and Gαi1-Luciferase. Cells were washed with ice cold PBS, and then lysed with AlF_4^- lysis buffer (50mM Tris, 150mM NaCl, 1mM EDTA, 2mM DTT, 5mM $MgCl_2$, 1% Triton X-100, protease inhibitors, 10mM NaF, 9mM $MgCl_2$, 30μM $AlCl_3$). Cells rotated end-over-end for 1.5 hours at 4°C. Samples were then centrifuged for 10 minutes at 13,000 RPM at 4°C, and 30μL of cell lysate was collected for input. Protein G sepharose (GE Healthcare) (50μL) was washed 3x with 1mL of ice cold PBS, and then incubated for an hour with 3% BSA (bovine serum albumin) rotating at 4°C. Beads were spun down, 3% BSA was removed, and lysates were added to beads with 2μL anti-GFP (MBL) to rotate for 1.5 hours at 4°C. Beads were then centrifuged for 30 seconds at 3000 RPM at 4°C, washed 4x with 1mL of ice cold wash buffer (0.1% Tween-20 in 1x PBS with AlF_4^-), and boiled in Laemmli buffer for 5 minutes. 13% SDS-PAGE separated samples and nitrocellulose membranes were blotted with anti-Renilla Luciferase (Millipore, 1:1000) and anti-GFP (MBL, 1:1000). Secondary antibodies used were goat anti-mouse (1:5000) and goat anti-rabbit (1:25000), respectively.

Bioluminescence Resonance Energy Transfer (BRET)

For variant and wild type RGS4, we generated RGS4-Luciferase (RGS4-Luc) fusion constructs, a tag suitable for bioluminescence resonance energy transmission (BRET) which emits light at 485nm in the presence of 5 μM coelenterazine H (CTZ) (Brown et al., 2015a). In combination with a YFP-tagged Gαi1 (Gα-YFP), RGS4-Luc will transfer energy to Gα-YFP upon binding, thereby producing a 535nm emission BRET signal. We incubated AlF4- with cells expressing RGS4-Luc and Gα-YFP to mimic the transition state of Gα and recruit RGS4 binding for 30 minutes and then recorded emissions at 485nm and 535nm following CTZ activation. Net BRET was calculated by dividing the emission at 535nm by the emission at 485nm, and then subtracting background signal of 485nm alone.

Missense Tolerance Ratio

We calculated missense tolerance ratio as recently described (Traynelis et al., 2017). Briefly, we used a 31-codon sliding window using the following calculation:

$$MTR = \frac{\sum[missense_{obs}] \,/\, \sum[missense_{obs} + synonymous_{obs}]}{\sum[missense_{expected}] \,/\, \sum[missense_{expected} + synonymous_{expected}]}$$

where observed variants ("obs") were reported variants from the GnomAD database and expected variants were calculated from all possible codon variations.

Post-Translational Modification Alignment Analysis

PTM alignment analysis is based on the SAPH-ire model as described previously (Torres et al., 2016) but was restricted to report only PTM count as described below. All PTMs are experimentally verified and were retrieved from dbPTM and SysPTM (Huang et al., 2016b; Li et al., 2014). To account for the diverse domains observed across the different proteins of the RGS protein family, a domain specific analysis was performed for all domain families observed within the RGS superfamily. Seven domains seen across RGS proteins were analyzed, namely DEP: disheveled, EGL-10, pleckstrin homology domain; DHEX, DEP helical extension (IPR000591), GGL: Gγ subunit-like domain (IPR015898), GPR/GoLoco: G protein regulatory motif (IPR003109), PDZ: domain present in PSD-95, Dlg, and ZO-1/2 (IPR001478), PTB: phospho-tyrosine binding domain (IPR006020), RBD: Raf-like Ras binding domain (IPR003116), and

RGS domain (IPR016137; the focus of this review). The domain sequences for all RGS proteins belonging to each of these domain families were obtained from Interpro (Hunter et al., 2009). The multi-FASTA files generated for each domain family were subsequently aligned using MUSCLE using default parameters (Edgar, 2004). Modified Alignment Positions (MAPs), domain family alignment positions that harbor at least one experimentally verified PTM specific to human RGS proteins, were coalesced and the PTM count within each determined by the sum of human family members for which experimental PTM has been observed and curated in the public domain via dbPTM and Phosphosite Plus (Hornbeck et al., 2015; Huang et al., 2016b). PTMs outside the RGS domain and specific to each given RGS protein are reported as single observations though may, in many cases, coalesce into MAPs of other domain families (data not shown). Non-domain PTMs and RGS domain MAPs were related directly to mutation data based on UniProt ID and native position anchor sequences (Bairoch et al., 2005), and plotted with respect to the MTR value at each native position.

SUPPLEMENTAL DATA

Table S1. RGS protein variants that overlap with post-translational modifications (PTMs). Post translational modifications that overlap with a human variant (GnomAD) for each RGS1-21 are listed. Grey boxes indicate the PTM/Mutation overlap is found within the canonical RGS domain. The type of modification, missense tolerance ratio (MTR), combined annotation dependent depletion (CADD) C-score, and population allele frequency (Prevalence) are listed. Allele frequency is listed in terms of the *most prevalent population*. If multiple populations have high prevalence, then prevalence in listed in terms of global allele frequency. Human variants are described according to GnomAD convention based on the longest splice variant for each human RGS protein, as opposed to the canonical (Uniprot-based) isoform. Thus, we include in parenthesis the canonical residue number in cases where they differ (RGS4, RGS5 and RGS10) from the longest splice variant position.

PTM/Mutation Overlap for All RGS Proteins					
RGS	**Residue Mutation**	**PTM**	**CADD**	**MTR**	**Prevalence**
RGS1	K13E	Methylation	16.31	0.99	0.0065% (South Asian)
	S67Y	Phosphorylation	22.7	0.92	0.0009% (European)
	K164Q	Methylation	7.4	0.95	0.0032% (South Asian)
RGS2	S46N	Phosphorylation	22.5	0.70	0.0232% (East Asian)
	S64G	Phosphorylation	13.77	0.92	0.0009% (European)
	Y116C	Phosphorylation	25.5	0.92	0.0009% (European)
	Y133C	Phosphorylation	27	0.88	0.0009% (European)
	Y178C	Phosphorylation	NA	0.90	0.0182% (Other)
RGS3	S395L	Phosphorylation	29.3	0.97	0.0037% (Global)
	T396I	Phosphorylation	23.3	0.99	0.0033% (South Asian)
	R448H	Methylation	14.5	0.73	0.0210% (Other)
	S496G	Phosphorylation	14.3	1.09	0.0060% (Latino)
	S496N	Phosphorylation	12.11	1.09	0.0065% (African)
	T536M	Phosphorylation	23.9	0.67	0.0089% (Latino)
	S662L	Phosphorylation	27	0.63	0.0455% (South Asian)
	S777L	Phosphorylation	18.2	0.81	0.0009% (European)
	S806F	Phosphorylation	19.4	0.82	0.0060% (Latino)
	T808N	Phosphorylation	15.6	0.84	0.1674% (Global)
	S823F	Phosphorylation	14.8	1.07	0.0018% (European)
	T921M	Phosphorylation	28.8	0.95	0.0390% (South Asian)
	S936L	Phosphorylation	34	0.87	0.1046% (African)
	T941M	Phosphorylation	34	0.90	0.0033% (South Asian)
	S965L	Phosphorylation	13.4	0.75	0.0033% (South Asian)
	S992C	Phosphorylation	25	1.16	0.0067% (European)
	S1007N	Phosphorylation	22	0.88	0.0009% (European)
RGS4	C109(12)R	Palmitoylation	24.9	0.60	0.0083% (African)
RGS5	Y73C	Phosphorylation	17.8	0.88	0.0041% (South Asian)
	Y117C	Phosphorylation	26.5	0.94	0.0167% (African)
	S170 (166) P	Phosphorylation	32	1.13	0.0117% (East Asian)
RGS7	K251Q	Acetylation	24.5	0.93	0.0058% (East Asian)
	S457L	Phosphorylation	21.8	0.69	0.0058% (East Asian)

RGS9	Y153C	Phosphorylation	25.8	0.85	0.0155% (Other)
	Y153S	Phosphorylation	23.5	0.85	0.0030% (Latino)
	Y153D	Phosphorylation	23.4	0.85	0.0016% (European)
	S304R	Phosphorylation	3.7	0.72	0.0016% (European)
	Y413C	Phosphorylation	27.2	0.94	0.0032% (South Asian)
	K419R	Acetylation	17.12	0.93	0.0029% (Latino)
RGS10	S24(16)T	Phosphorylation	7.27	0.78	0.0030% (Latino)
	S27(19)N	Phosphorylation	21.9	0.81	0.0009% (European)
	K148(140)R	Ubiquitination	25	0.81	0.0009% (European)
	Y179(171)N	Phosphorylation	28.5	1.1	0.0009% (European)
RGS12	S16L	Phosphorylation	11.27	0.91	0.0066% (African)
	S16A	Phosphorylation	0.001	0.91	13.0309% (African)
	S88P	Phosphorylation	23.1	0.93	0.0032% (South Asian)
	S107G	Phosphorylation	24	1.00	0.0032% (South Asian)
	S107C	Phosphorylation	23.8	1.00	0.0054% (European)
	S172L	Phosphorylation	26.8	0.99	0.0155% (Other)
	S195C	Phosphorylation	23.2	1.05	0.0009% (European)
	S284N	Phosphorylation	13.8	0.93	0.0009% (European)
	S483I	Phosphorylation	0.64	0.95	0.0010% (European)
	T492N	Phosphorylation	0.001	0.97	0.0028% (European)
	R633W	Methylation	31	1.09	0.0032% (South Asian)
	R633P	Methylation	23.8	1.09	0.0167% (African)
	R633Q	Methylation	23.5	1.09	8.5678% (South Asian)
	R633L	Methylation	21.1	1.09	0.0009% (European)
	R643C	Methylation	29	1.03	0.0682% (South Asian)
	R643S	Methylation	27.8	1.03	0.0009% (European)
	R643H	Methylation	26.4	1.03	0.0365% (Other)
	S649L	Phosphorylation	26.9	0.92	0.0065% (South Asian)
	S649P	Phosphorylation	23.3	0.92	0.0032% (South Asian)
	S649T	Phosphorylation	23.2	0.92	0.0030% (Latino)
	T676M	Phosphorylation	25	0.75	0.1193% (Latino)
	T866M	Phosphorylation	32	0.82	0.0054% (East Asian)
	S934L	Phosphorylation	30	0.78	0.0058% (East Asian)
	S947L	Phosphorylation	17.9	0.56	0.0104% (Ashkenazi)
	T1331M	Phosphorylation	21.6	0.67	0.0092% (Global)

RGS13	Y146H	Phosphorylation	27.3	0.99	0.0535% (East Asian)
RGS14	R27M	Methylation	21.4	1.03	0.0191% (Other)
	K86N	Ubiquitination	27.9	0.80	0.0009% (European)
	K86R	Ubiquitination	23.5	0.80	0.0030% (Latino)
	S260F	Phosphorylation	32	0.97	0.0754% (East Asian)
	S288R	Phosphorylation	29.1	1.11	0.0009% (European)
	T292K	Phosphorylation	12.15	1.12	0.1166% (East Asian)
	T292M	Phosphorylation	13.01	1.12	0.0030% (Latino)
	T472A	Phosphorylation	0.371	1.08	0.2729% (South Asian)
	S480G	Phosphorylation	6.092	0.98	0.0067% (European)
	T490S	Phosphorylation	0.004	0.94	0.0060% (Latino)
	T490N	Phosphorylation	12.99	0.94	0.0162% (South Asian)
	S510G	Phosphorylation	23.4	1.06	0.0009% (European)
RGS16	C12	Palmitoylation	33	1.11	0.0115% (African)
	C98Y	Palmitoylation	33	0.96	0.0047% (European)
RGS17	Y171F	Phosphorylation	9.1	0.77	0.0067% (European)
RGS19	Y146C	Phosphorylation	26.3	0.64	0.0009% (European)
	T186A	Phosphorylation	16.4	0.67	0.0009% (European)
	T201I	Phosphorylation	11.5	0.90	0.0511% (Ashkenazi Jewish)
	S211L	Phosphorylation	22.8	1.06	0.0115% (African)
	S213A	Phosphorylation	0.11	1.07	0.0009% (European)
RGS20	S77R	Phosphorylation	23.9	1.13	0.0119% (Latino)
	S77T	Phosphorylation	23	1.13	0.0072% (European)
	T162I	Phosphorylation	15.7	0.90	0.0067% (European)
	S166G	Phosphorylation	13.8	1.15	0.0067% (European)
RGS21	Y116C	Phosphorylation	25.5	0.92	0.0009% (European)
	Y133C	Phosphorylation	27	0.88	0.0009% (European)

Supplemental Figures

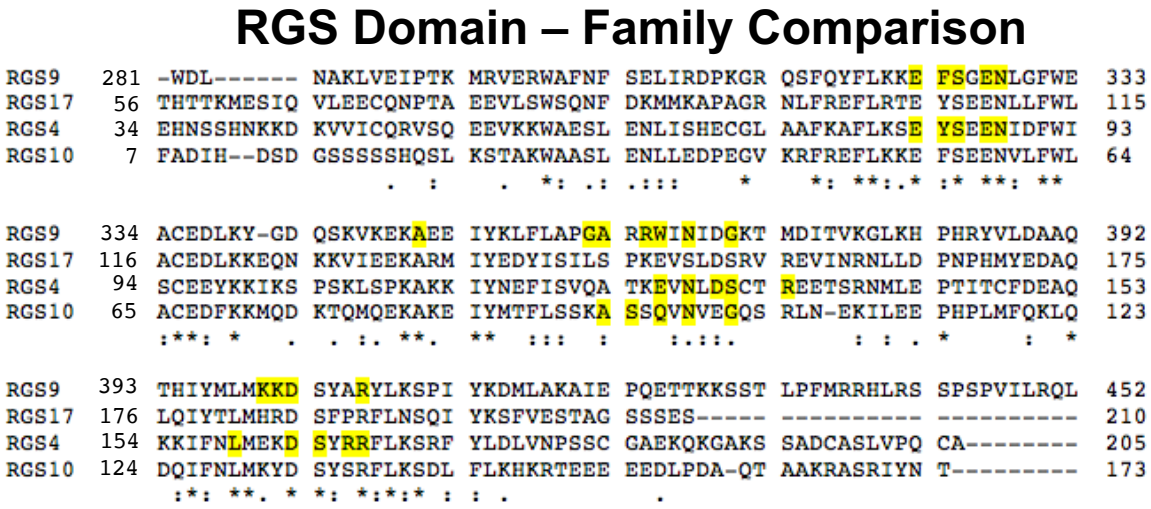


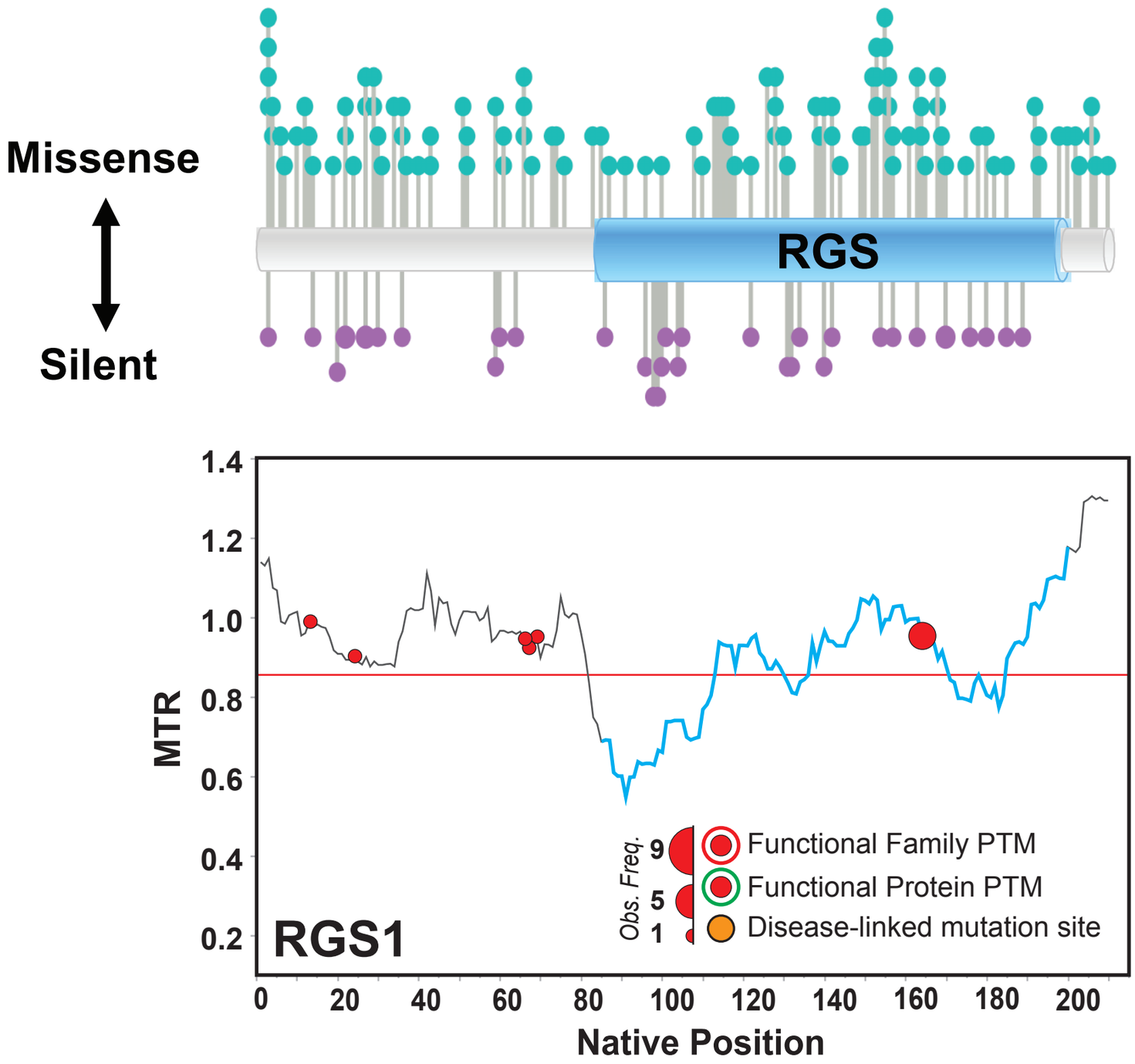
Figure S1. Family alignment of RGS domains. To identify conserved residues critical for Gα interaction, we used Clustal W to align each of the representative subfamily members (RGS4, RGS9, RGS10) with RGS17. We highlighted residues that were noted as important for binding (Figures 2, 4, 5) to compare against RGS17. From this information, we identified residues in RGS17 that are predicted contact points with Gα.

Supplemental Raw Data Files

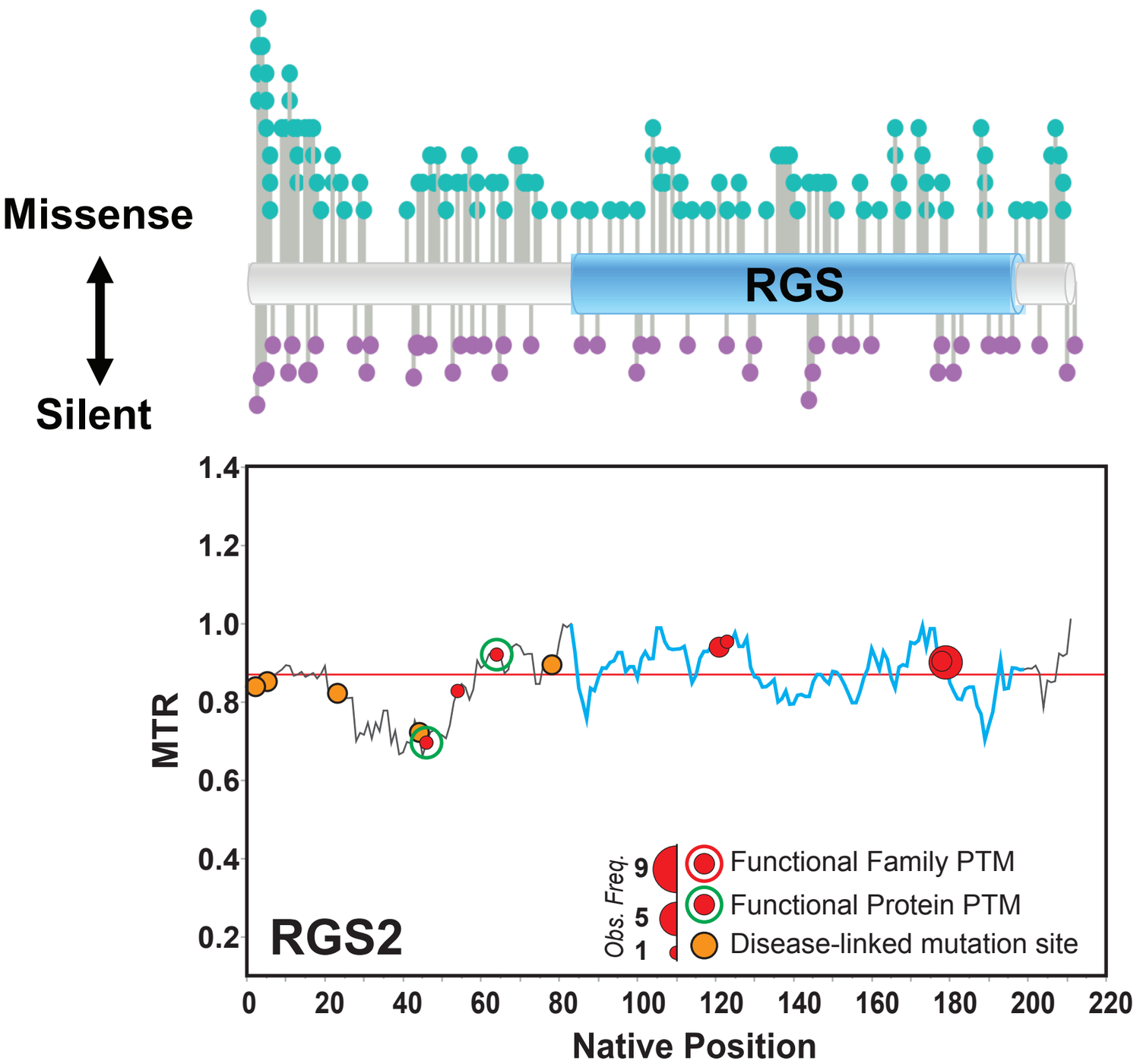
We include MTR plots with overlayed PTMs for the remaining RGS proteins that were not highlighted in the body of the text. We also include our comprehensive curated RGS PTM/Mutation database with all reported human variants for all canonical human RGS proteins from the GnomAD consortium (version 2.0). These are available as downloadable and expandable MS Excel Files (XLSX). We include only variants that were not filtered out by the Broad Institute to ensure quality source data. Human variants are described according to GnomAD convention based on the longest splice variant for each human RGS protein, as opposed to the canonical (UniProt-based) reference isoform. Thus, we include in parenthesis the canonical residue number in cases where they differ from the longest splice variant position.

Figure S2. Analysis of rare variants and PTMs found within human RGS proteins. Data is presented for all human RGS proteins that were not highlighted in the body of the text. Human variants correspond to GnomAD convention based on the longest splice variant for each human RGS protein (see Methods for UniProt IDs). **(For each RGS, TOP)** Rare human variants of RGS proteins are plotted along the protein sequence using Lollipops (https://github.com/pbnjay/lollipops). Missense (amino acid change) variants are displayed on top in teal, while silent (no amino acid change) variants are displayed on the bottom in purple. **(For each RGS, BOTTOM)** Missense Tolerance Ratio (MTR) and Post-Translational Modification (PTM) cluster analysis plot for each RGS (see text for details). If the RGS domain (highlighted in blue) or other protein region is under selective pressure, the MTR will fall below the median (red line). The location of functional or disease-linked post-translational modifications (PTMs) are plotted as colored circles on the line. The PTM identities and amino acid location, as well as the MTR data for each RGS are available in Table S1 and in the downloadable and expandable MS Excel Files (XLSX).

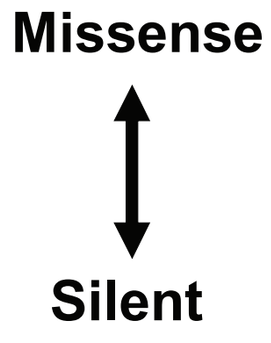
RGS1

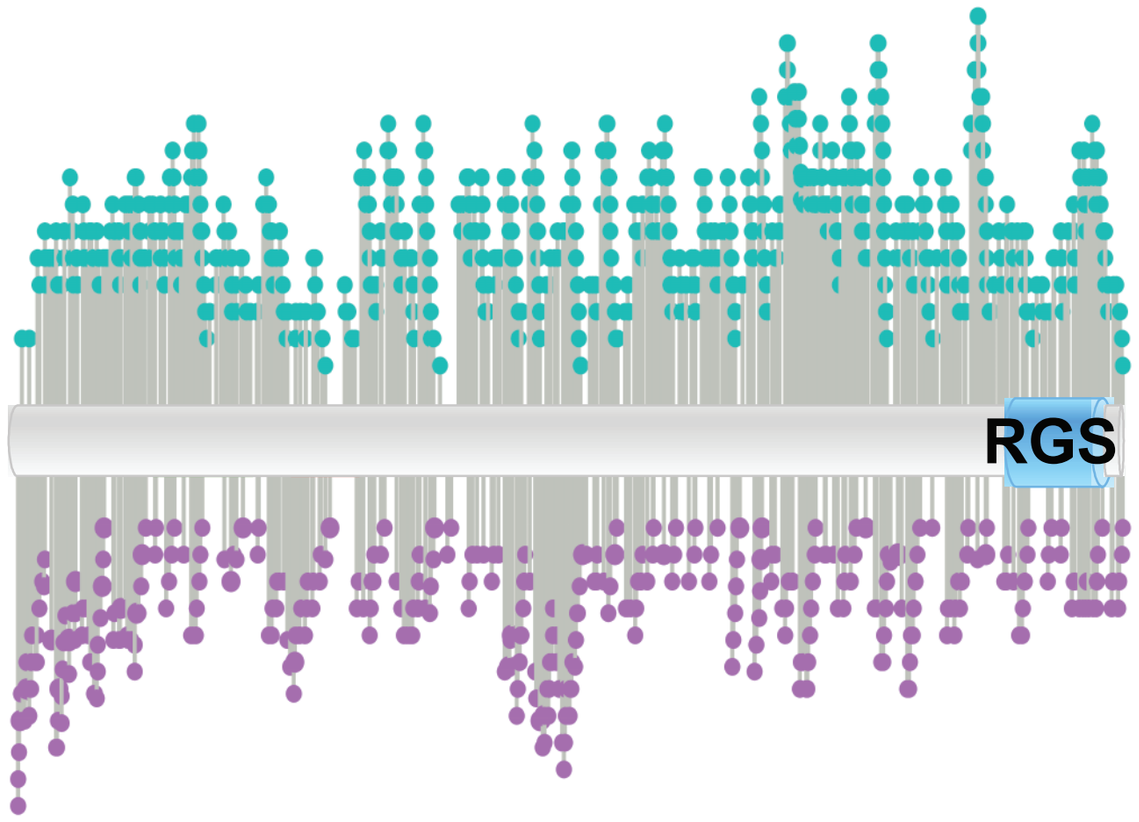

Missense
Silent
RGS
1.4
1.2
1.0
0.8
0.6
0.4
0.2
MTR
RGS1
Obs. Freq.
9
5
1
Functional Family PTM
Functional Protein PTM
Disease-linked mutation site
0
20
40
60
80
100
120
140
160
180
200
Native Position

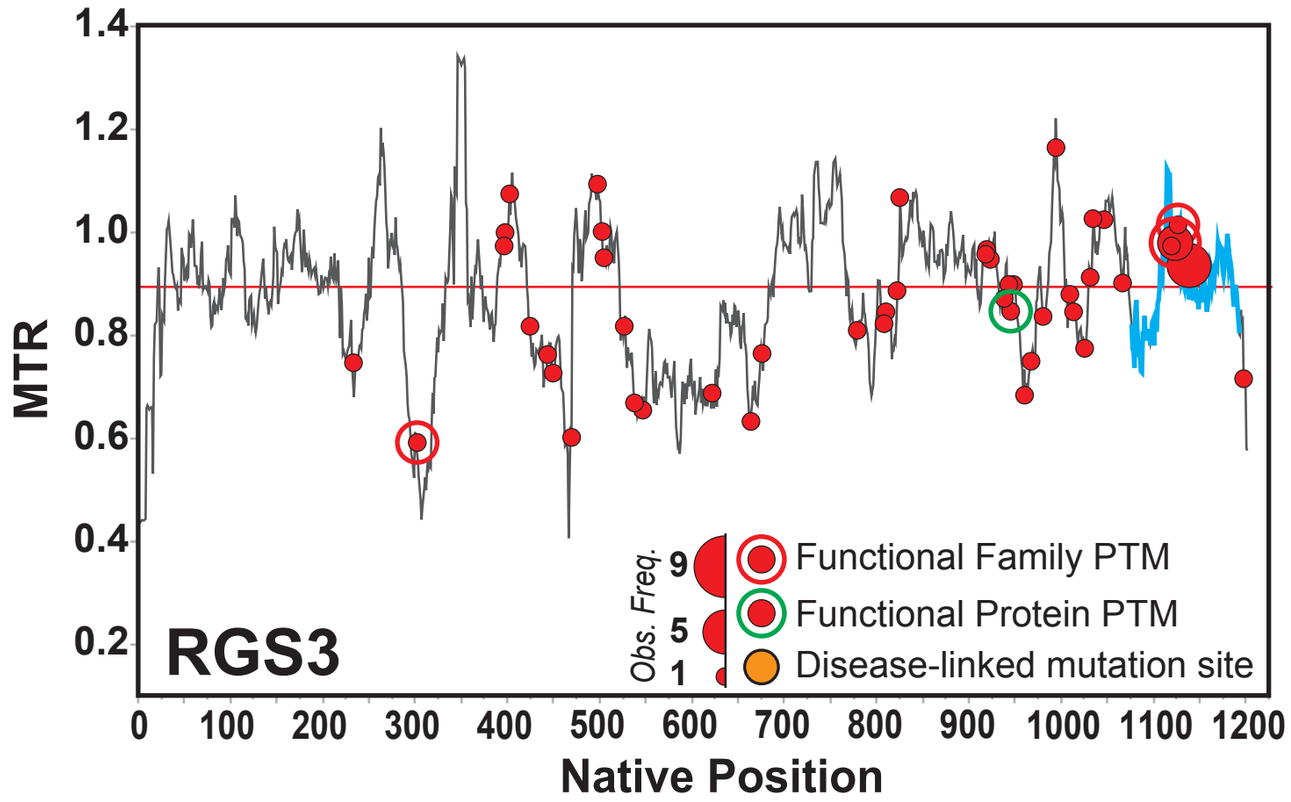
RGS2


Missense
Silent
RGS
1.4
1.2
1.0
0.8
0.6
0.4
0.2
MTR
RGS2
Obs. Freq.
9
5
1
Functional Family PTM
Functional Protein PTM
Disease-linked mutation site
0
20
40
60
80
100
120
140
160
180
200
220
Native Position

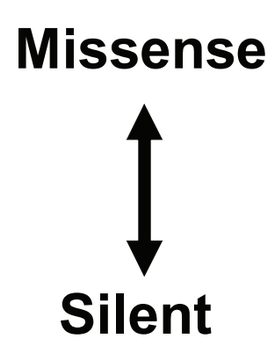
RGS3

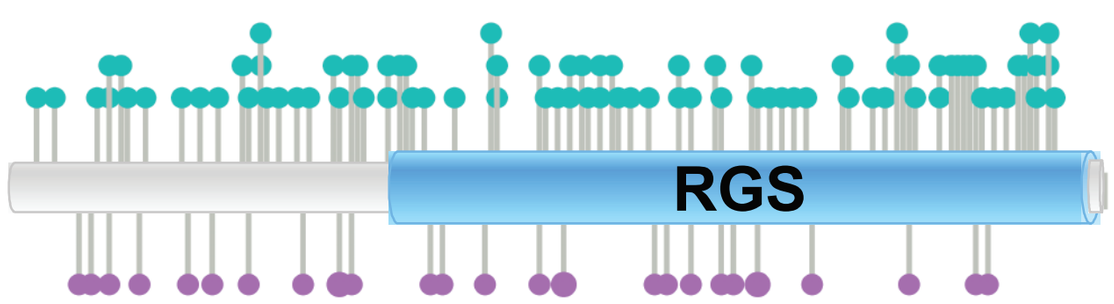

Missense
Silent

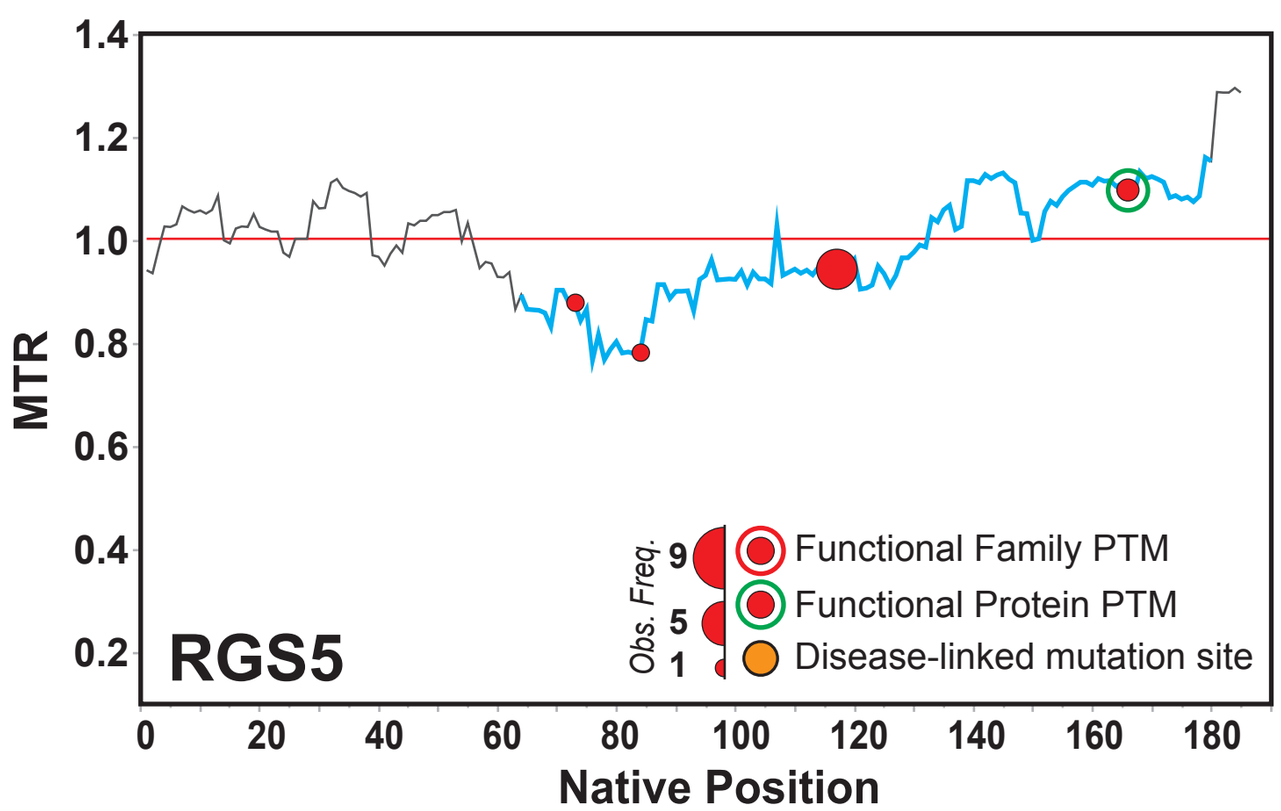

RGS


MTR
1.4
1.2
1.0
0.8
0.6
0.4
0.2
RGS3
Obs. Freq.
9
5
1
Functional Family PTM
Functional Protein PTM
Disease-linked mutation site
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
Native Position

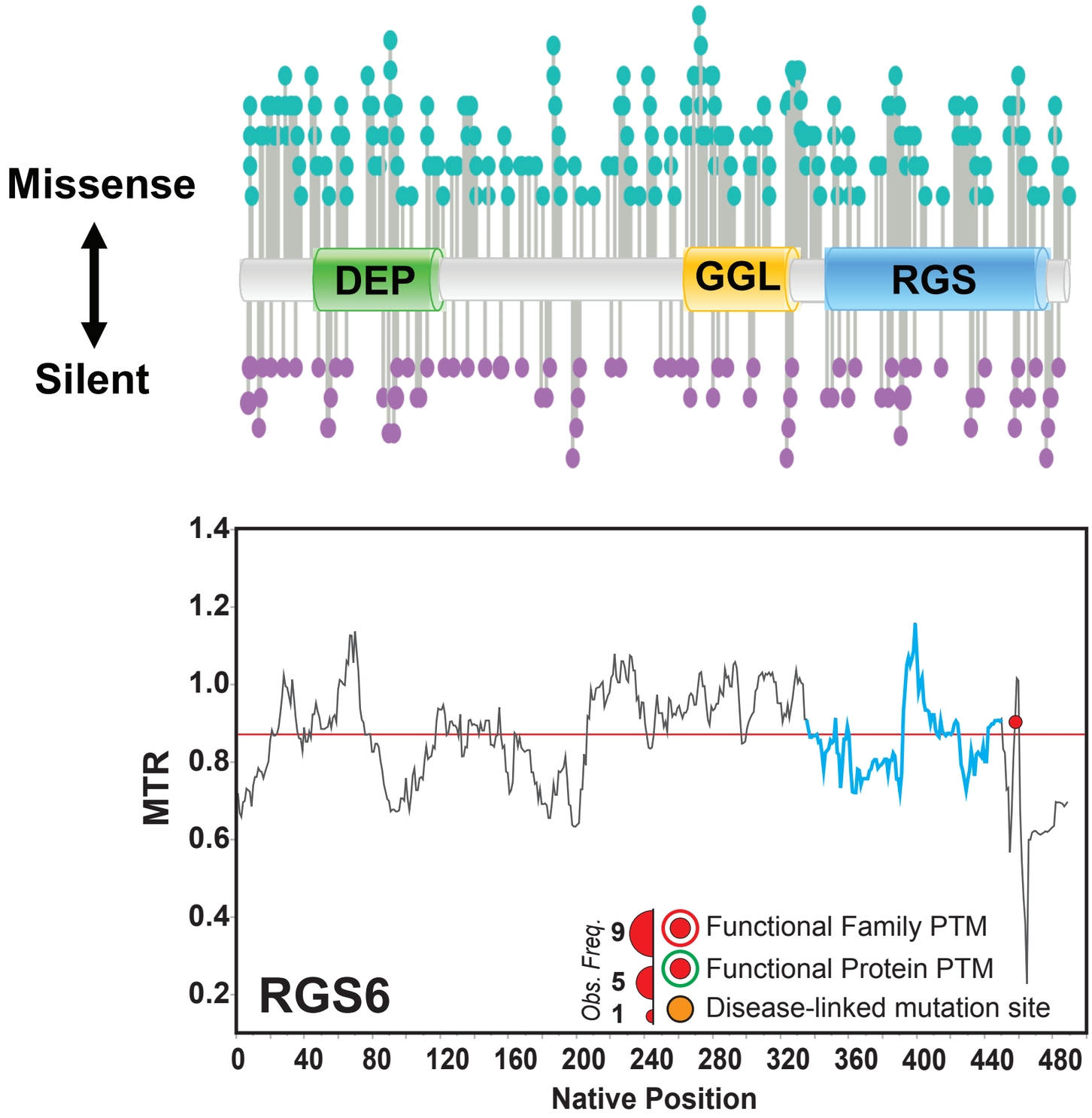
RGS5


Missense
Silent

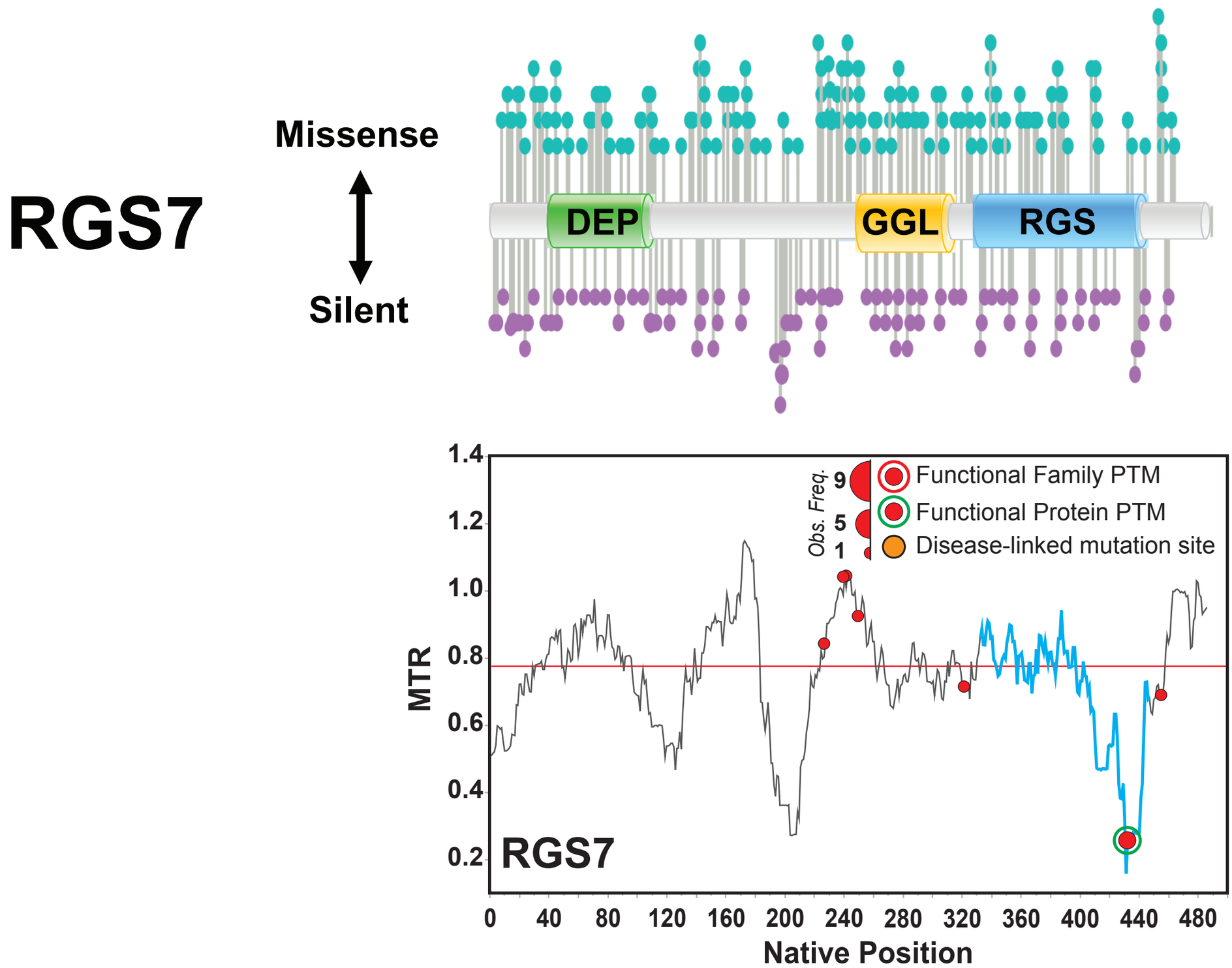

RGS


MTR
1.4
1.2
1.0
0.8
0.6
0.4
0.2
RGS5
Obs. Freq.
9
5
1
Functional Family PTM
Functional Protein PTM
Disease-linked mutation site
0
20
40
60
80
100
120
140
160
180
Native Position

RGS6


Missense
Silent
DEP
GGL
RGS
1.4
1.2
1.0
0.8
0.6
0.4
0.2
MTR
RGS6
Obs. Freq.
9
5
1
Functional Family PTM
Functional Protein PTM
Disease-linked mutation site
0
40
80
120
160
200
240
280
320
360
400
440
480
Native Position

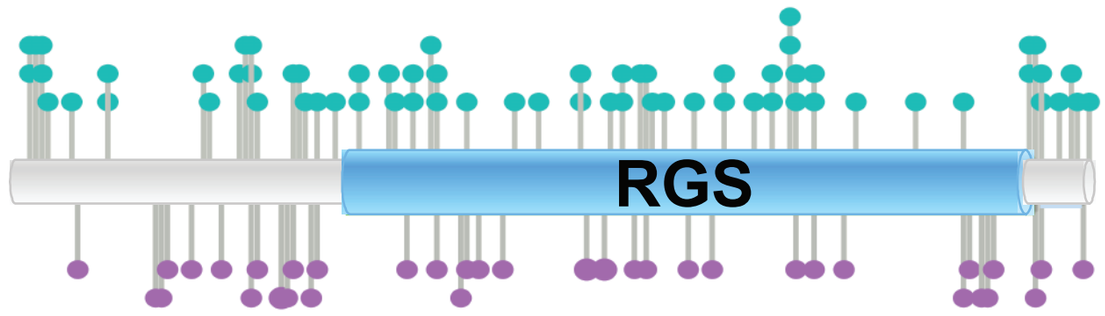
RGS7

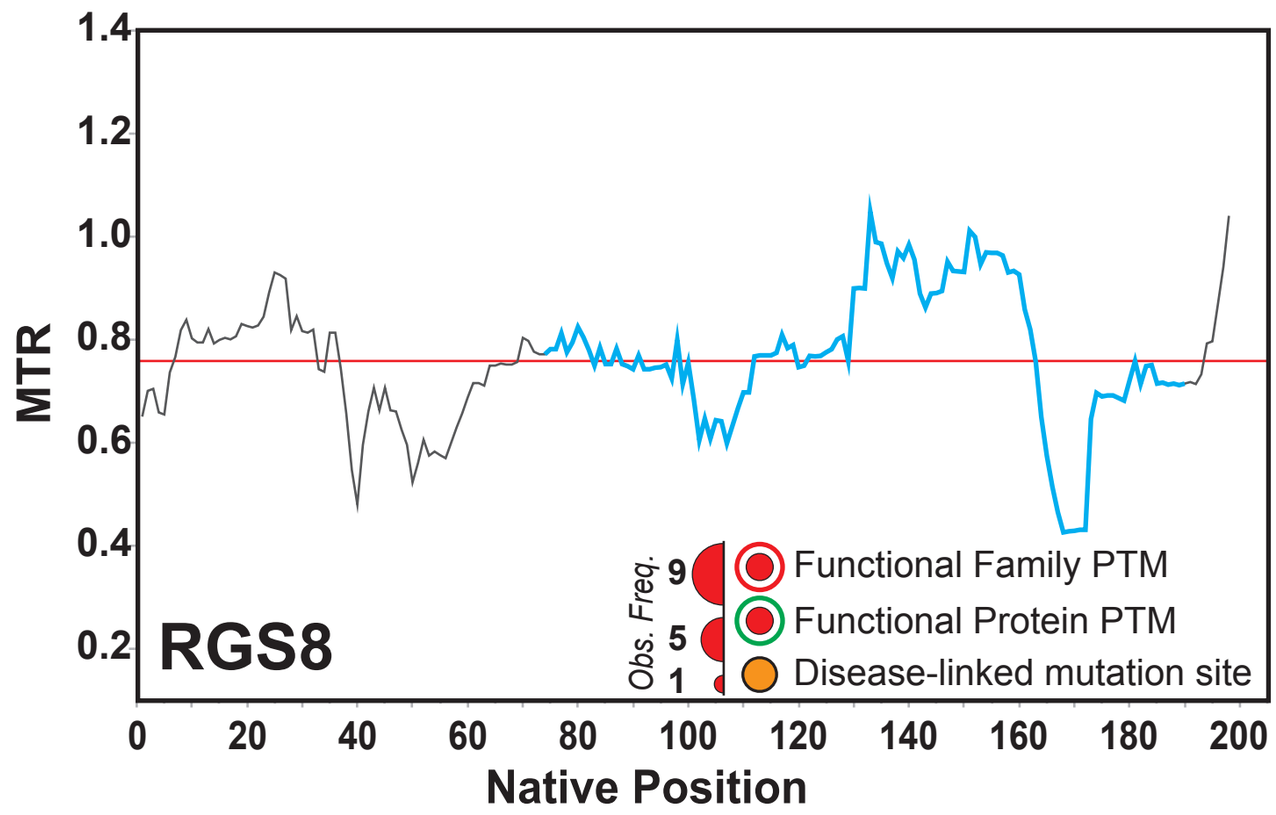

Missense
Silent
DEP
GGL
RGS
1.4
1.2
1.0
0.8
0.6
0.4
0.2
MTR
RGS7
Obs. Freq.
9
5
1
Functional Family PTM
Functional Protein PTM
Disease-linked mutation site
0
40
80
120
160
200
240
280
320
360
400
440
480
Native Position

RGS8

Missense

Silent

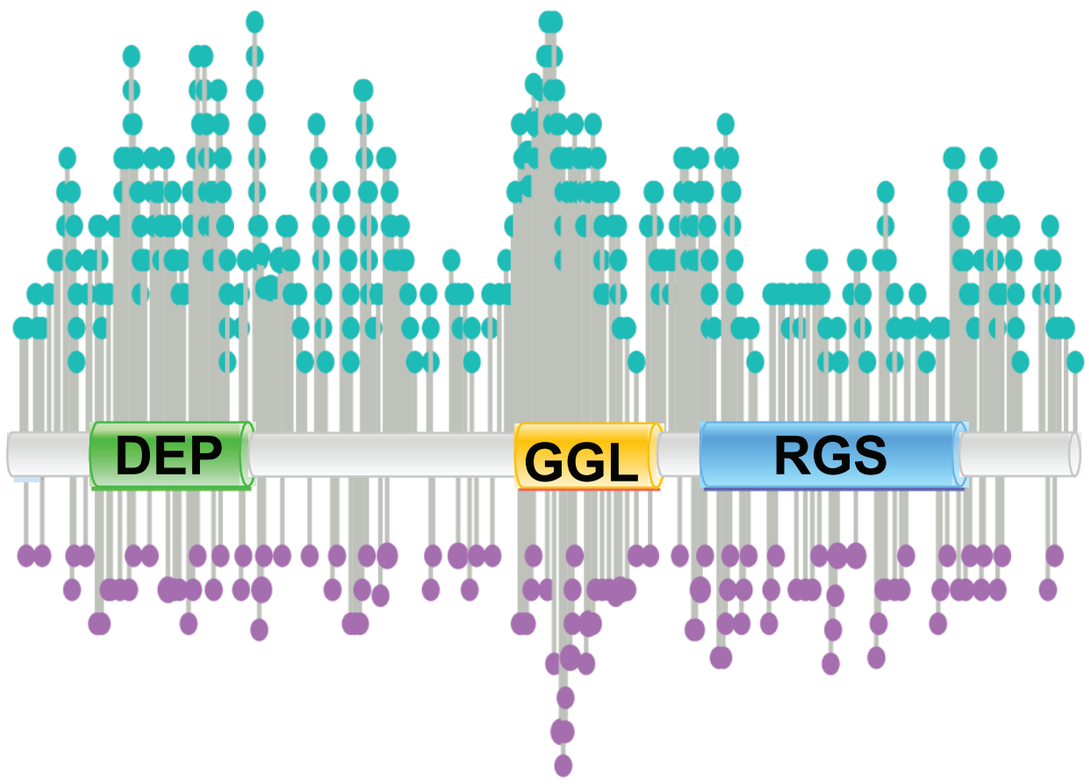


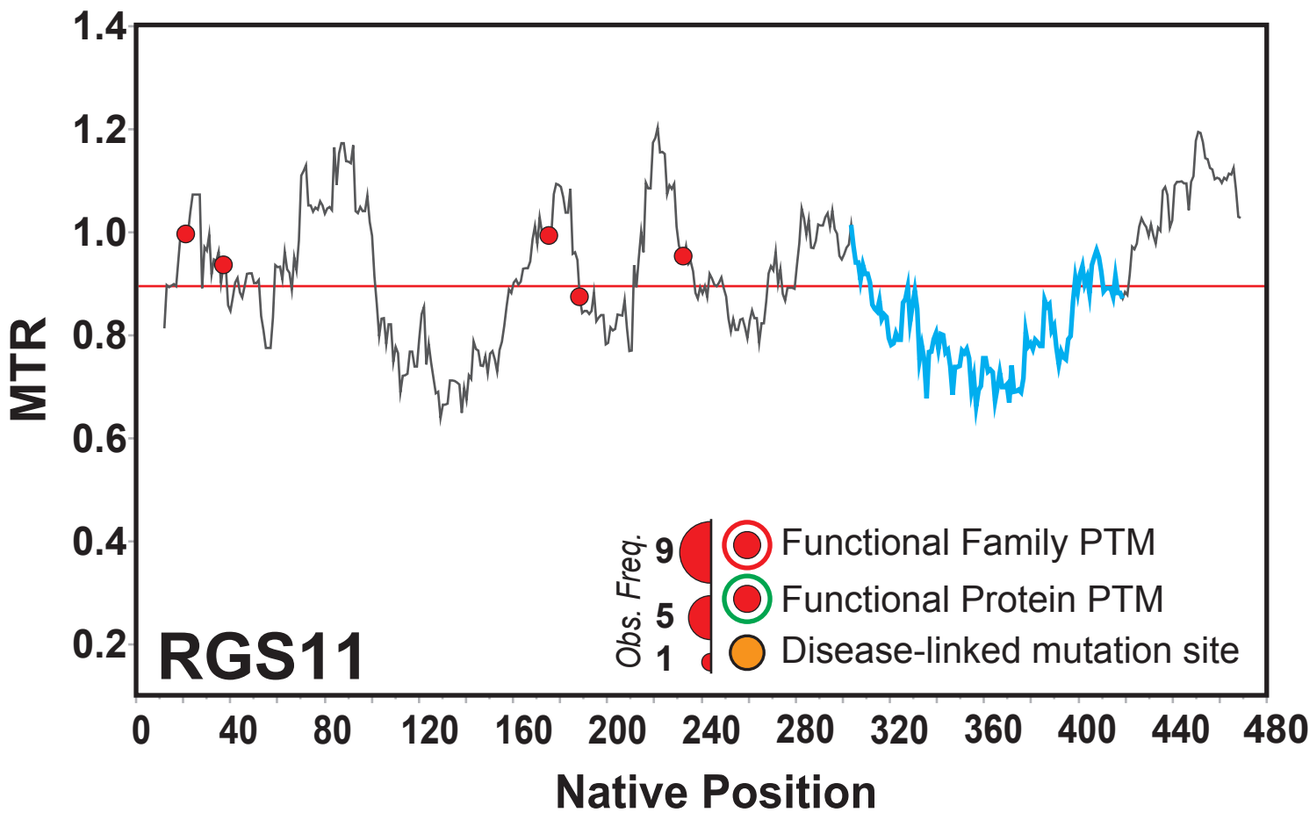


RGS11

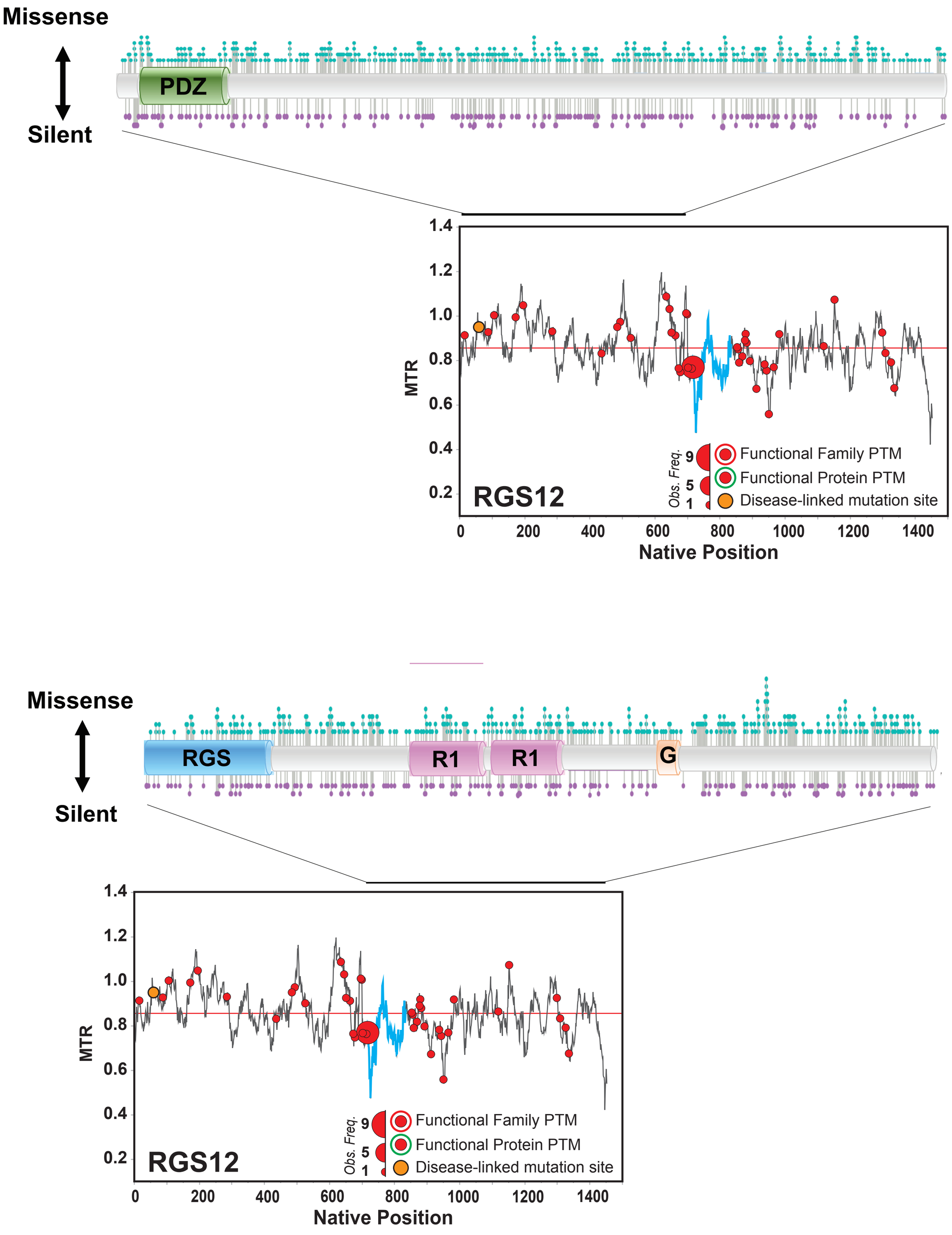
Missense

Silent

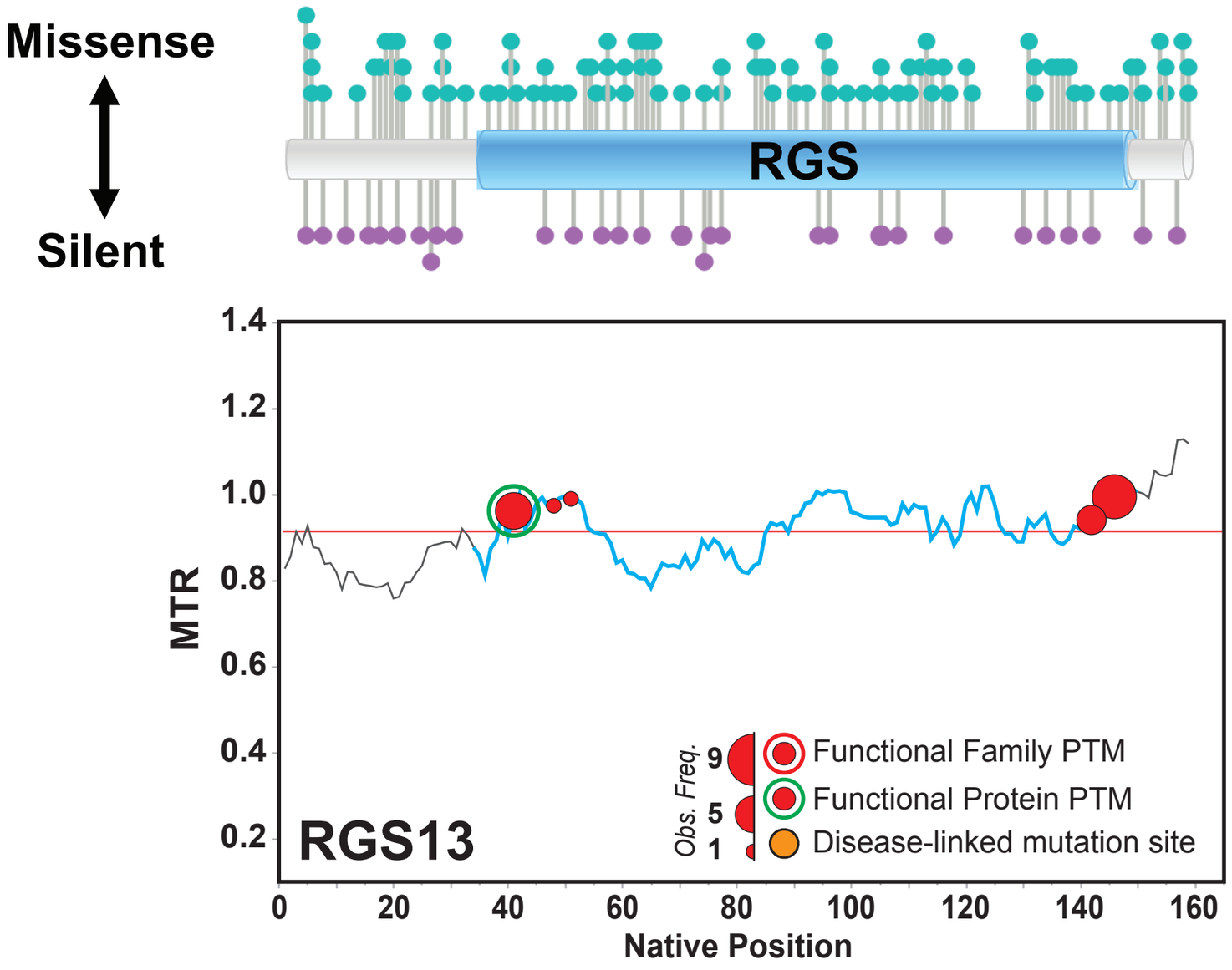




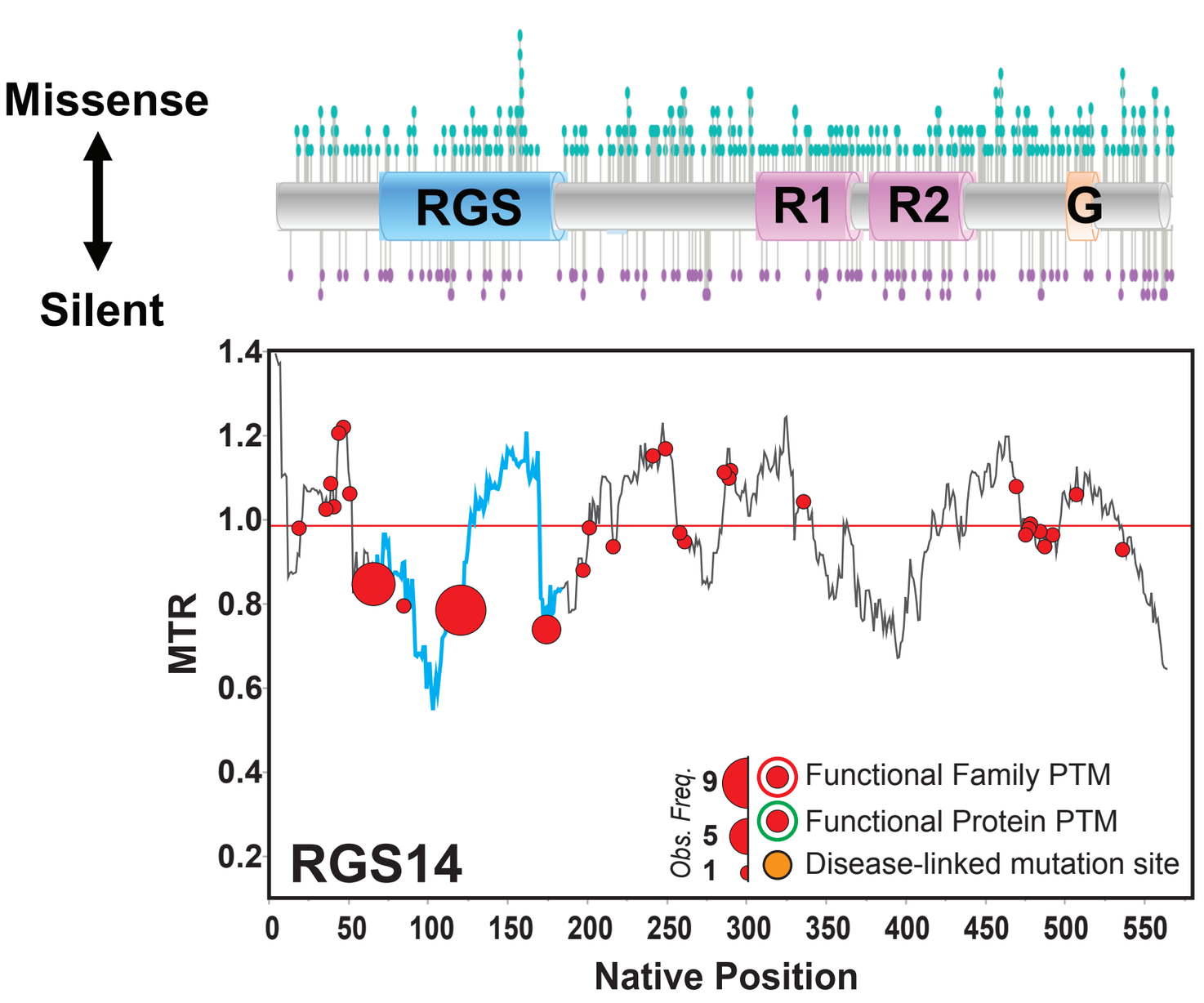
RGS12


Missense
Silent
PDZ
1.4
1.2
1.0
0.8
0.6
0.4
0.2
MTR
RGS12
Obs. Freq.
9
5
1
Functional Family PTM
Functional Protein PTM
Disease-linked mutation site
0
200
400
600
800
1000
1200
1400
Native Position
Missense
Silent
RGS
R1
R1
G
1.4
1.2
1.0
0.8
0.6
0.4
0.2
MTR
RGS12
Obs. Freq.
9
5
1
Functional Family PTM
Functional Protein PTM
Disease-linked mutation site
0
200
400
600
800
1000
1200
1400
Native Position

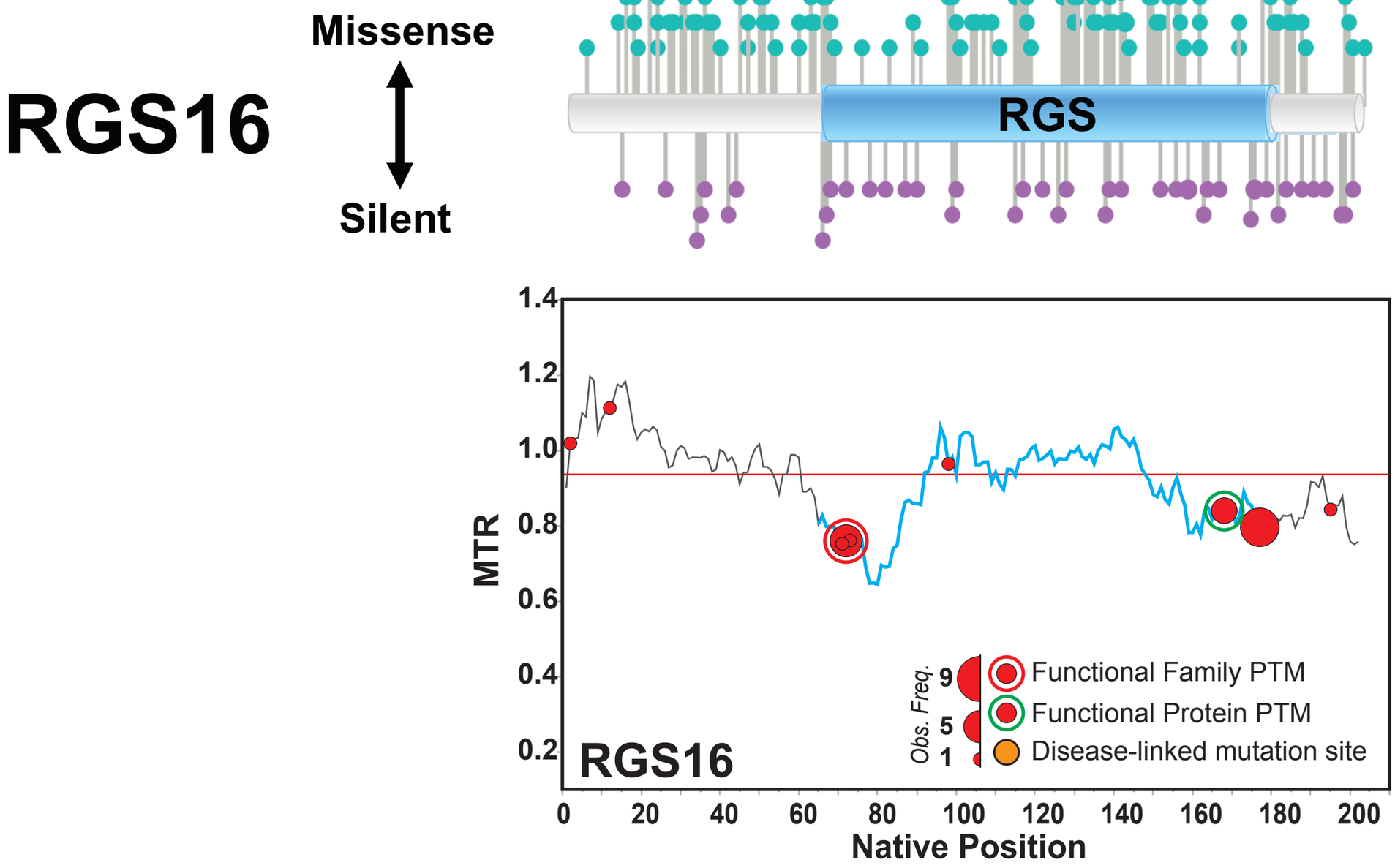
RGS13


Missense
Silent
RGS
1.4
1.2
1.0
0.8
0.6
0.4
0.2
MTR
RGS13
Obs. Freq.
9
5
1
Functional Family PTM
Functional Protein PTM
Disease-linked mutation site
0
20
40
60
80
100
120
140
160
Native Position

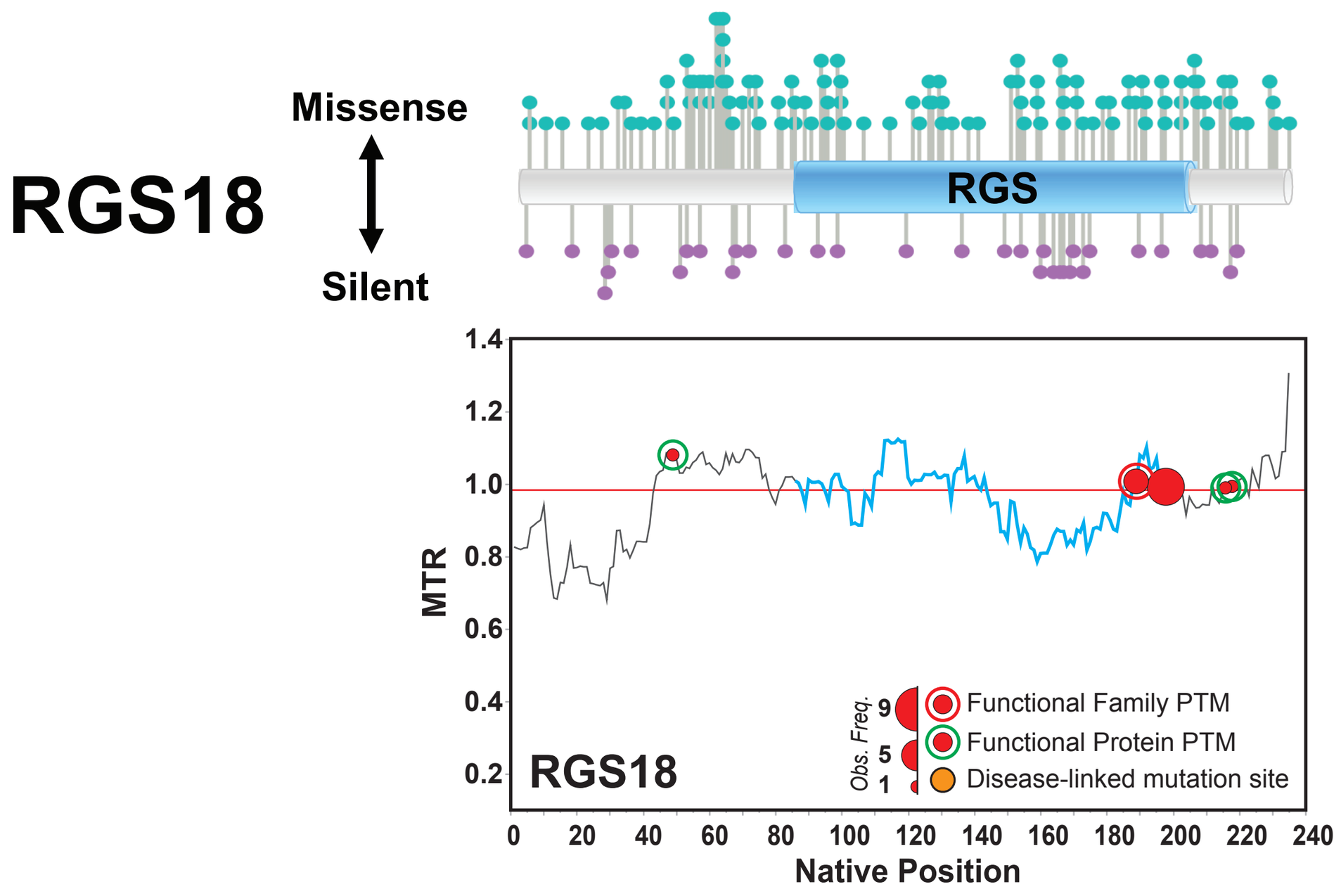
RGS14


Missense
Silent
RGS
R1
R2
G
1.4
1.2
1.0
0.8
0.6
0.4
0.2
MTR
RGS14
Obs. Freq.
9
5
1
Functional Family PTM
Functional Protein PTM
Disease-linked mutation site
0
50
100
150
200
250
300
350
400
450
500
550
Native Position

RGS16


Missense
Silent
RGS
1.4
1.2
1.0
0.8
0.6
0.4
0.2
MTR
RGS16
Obs. Freq.
9
5
1
Functional Family PTM
Functional Protein PTM
Disease-linked mutation site
0
20
40
60
80
100
120
140
160
180
200
Native Position

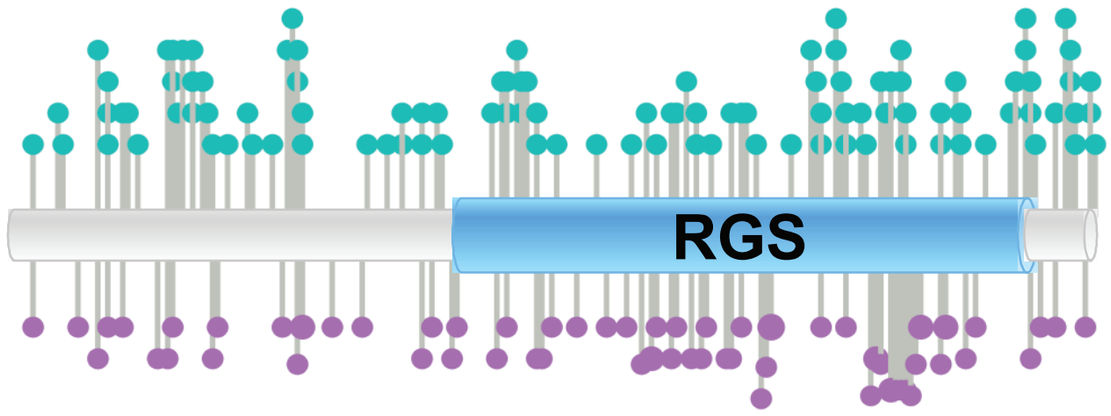
RGS18

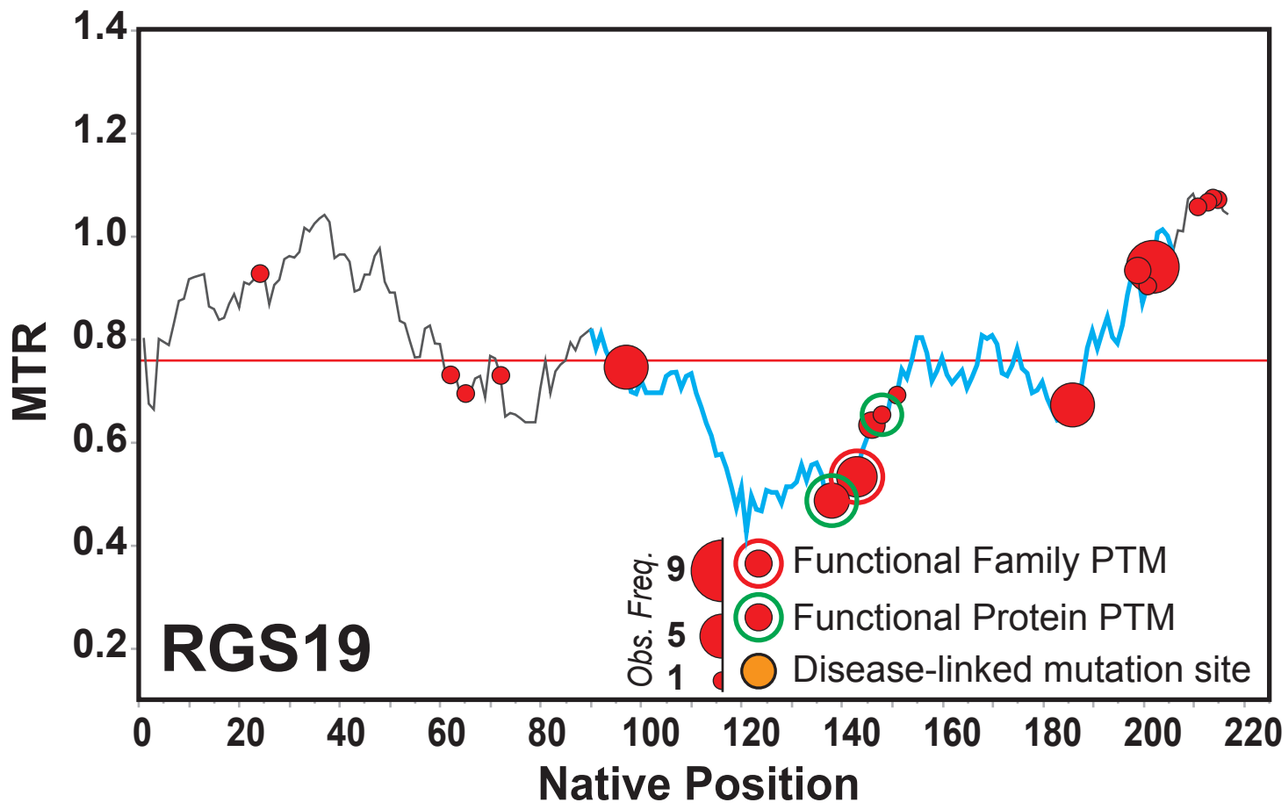

Missense
Silent
RGS
1.4
1.2
1.0
0.8
0.6
0.4
0.2
MTR
RGS18
Obs. Freq.
9
5
1
Functional Family PTM
Functional Protein PTM
Disease-linked mutation site
0
20
40
60
80
100
120
140
160
180
200
220
240
Native Position

RGS19

Missense

Silent

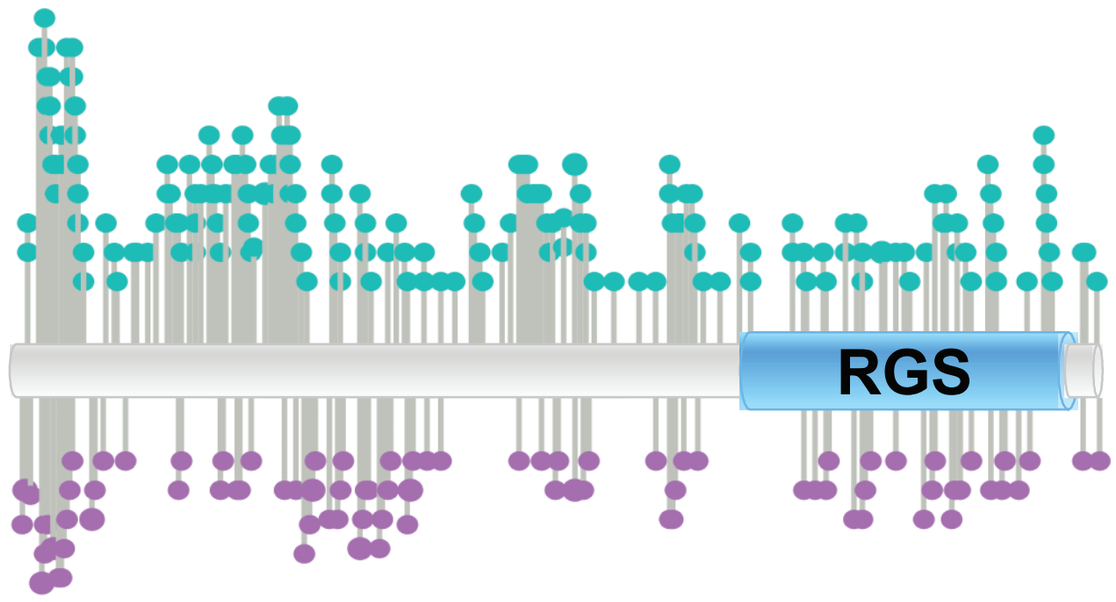


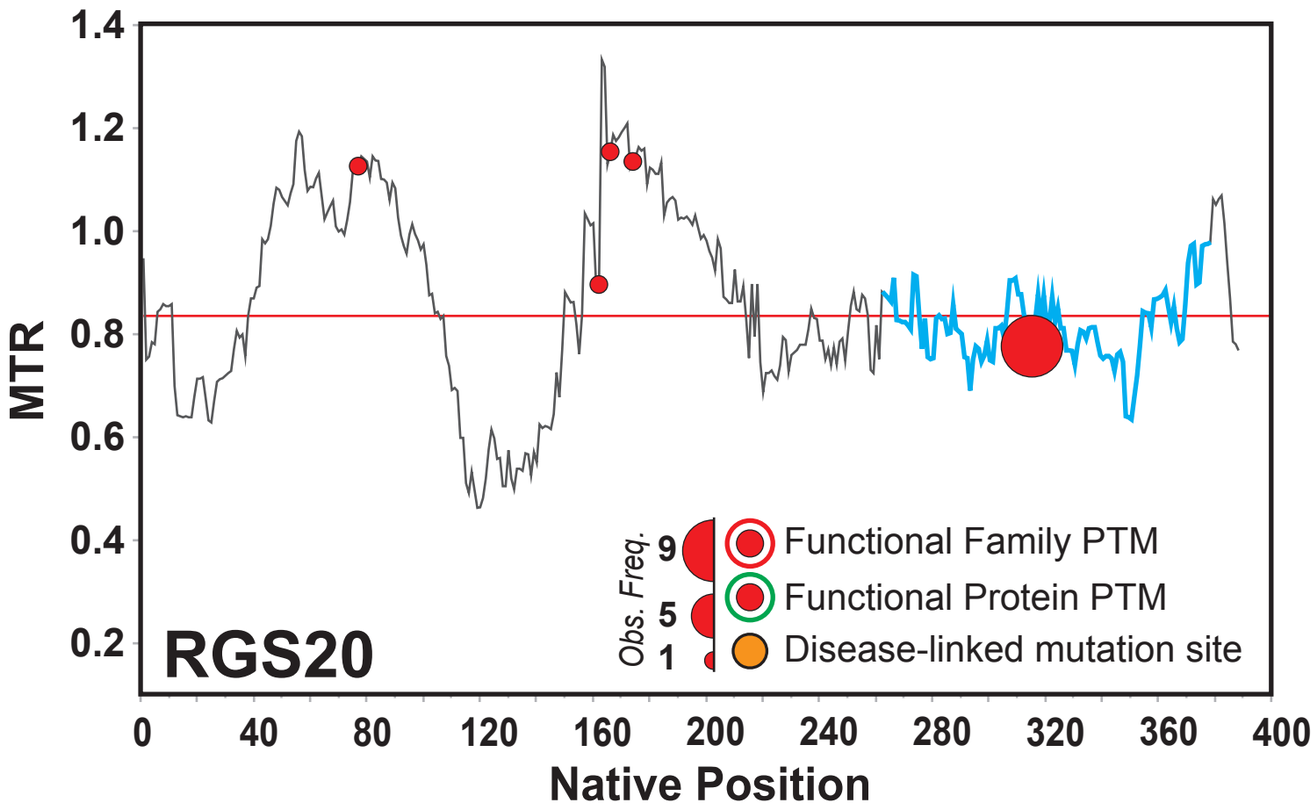


RGS20

Missense

Silent





RGS21

Missense

Silent