Supplementary Information

Calcium Ion Induced Structural Changes Promote Dimerization of Secretagogin, Which Is Required for Its Insulin Secretory Function

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Supplementary Figure legends

Supplementary Figure 1 – SCGN is readily dimerized with disulfide linkage following calcium binding.

(A) Recombinant SCGN was incubated with indicated $CaCl_2$ concentration at R.T. for 15 min followed by 0.1 mM H_2O_2 treatment at 37℃ for 1 h. Data are presented as mean ± SD of three experiments (*P < 0.05, Student's t-test). (B) Size exclusion chromatograms of SCGN protein in response to calcium and H_2O_2 treatments. SCGN protein treated with 0, 2 mM $CaCl_2$ for 15 min at room temperature followed by incubation with 0, 1 mM H_2O_2 for 1 h at 37℃. Apo- SCGN without H_2O_2 (black dotted line), Ca^{2+}-bound SCGN (2 mM $CaCl_2$) without H_2O_2 (red dotted line), Apo- SCGN treated with 1 mM H_2O_2 (black line), Ca^{2+}-bound SCGN with 1 mM H_2O_2 (red line). Albumin (66.4 kDa) and α-lactalbumin (14.2 kDa) were used as molecular weight markers. (C) Endogenous SCGN levels were detected in lung fibroblast WI38, cervical cancer cell line HeLa, lung cancer cell line, H358, H157, human embryonic kidney cell line HEK 293T, and mouse insulinoma cell line, NIT-1. The cell lysates were separated on reducing and reducing SDS-PAGE and SCGN was detected by WB. GAPDH was detected for loading control. Non-specific bands were indicated as * (D) SCGN forms dimers and oligomers in HeLa cells in response to H_2O_2. HeLa cells overexpressing SCGN were treated with the indicated H_2O_2 concentration for indicated times. The cell lysates were separated on non-reducing and reducing SDS-PAGE and SCGN was detected by WB. Tubulin was detected for loading control. (E) HeLa cells overexpressing SCGN were treated with the indicated H_2O_2 and ionomycin concentration for 30 min. Proteins were separated under non-reducing and reducing conditions on SDS-PAGE and SCGN n and tubulin were detected with western analysis.

Supplementary Figure 2 - Calcium bound SCGN forms stable dimers through C193-C193 disulfide linkage.

(A) Recombinant SCGN was incubated with 0 or 2 mM $CaCl_2$ at R.T. for 15 min followed by 1 mM H_2O_2 treatment for 1 h. Proteins were separated by non-reducing SDS-PAGE, and each protein bands were analyzed by peptide sequencing with nanoUPLC-ESI-Q-TOF using the DBond disulfide searching algorithm. (B) List of identified disulfide linkages of each SCGN band. (C) Tandem mass spectra of C193-C269, and C253-C269 disulfide linked peptide.

Supplementary Figure 3 - SCGN forms stable dimers via C193-C193 disulfide linkage and has a reactive cysteine readily oxidized by H_2O_2.

(A) Conservation map of cysteine residues in SCGN was obtained from the orthologs present in all of the animal species for which KEGG has complete genomes. Orthologies were manually checked if it is ambiguous. (B) SCGN WT, and Cys mutant (C193S, C253S, C269S) proteins were incubated with/without $CaCl_2$ at R.T. for 15 min followed by H_2O_2 treatment in indicated concentration at 37°C for 1 h. Proteins were separated by non-reducing and reducing SDS-PAGE. (C) Recombinant SCGN was incubated in indicated concentration at 37°C for 1 h. Proteins were incubated with 1 mM NPSB-B at R.T. for 2 h for labeling the reactive Cys residue, followed by separation on reducing SDS-PAGE and detection by streptavidin-HRP. Coomassie staining gel showing amount of gel loaded proteins.

Supplementary Figure 4 - Results of MD simulations.

(A) The RMSDs of backbone Cα atoms during the simulations for apo- SCGN (green) and Ca^{2+}-bound SCGN (magenta). (B) The dimeric Ca^{2+}-bound SCGN constructed with coordinates at 35 ns using HEX 6.3.

Supplementary Figure 5- Celluar localization of SCGN monomer, dimer, and oligomers

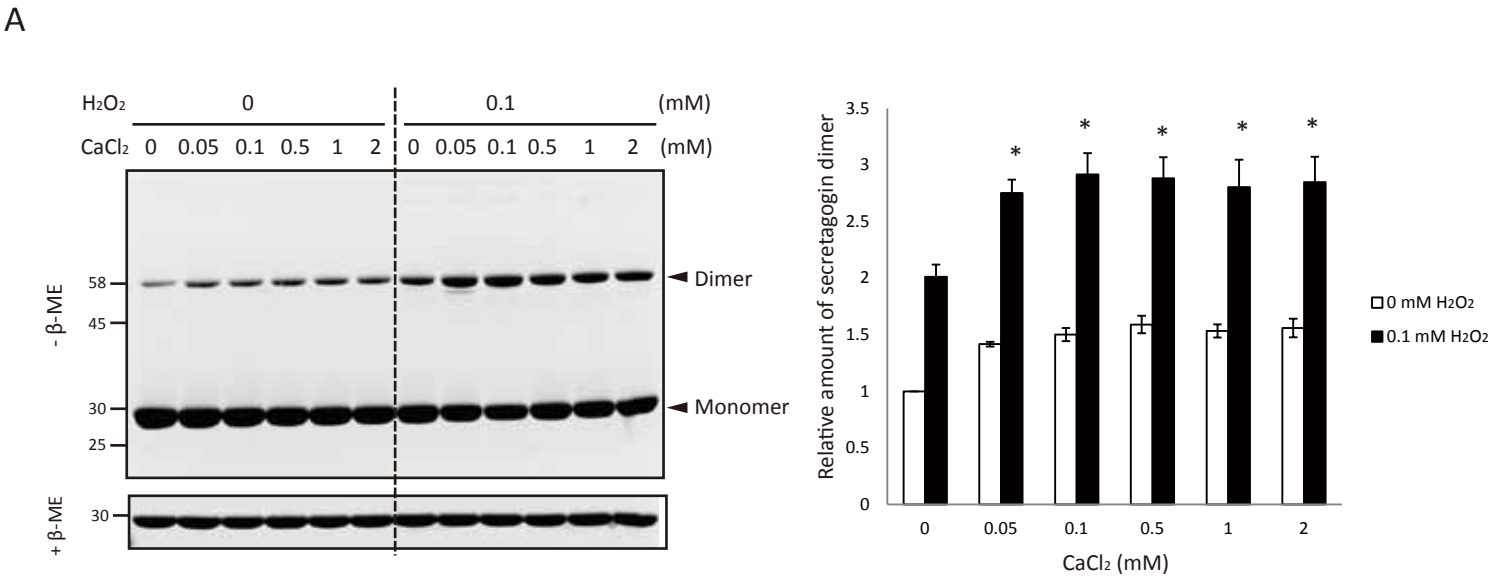
Subcelluar fractionation of Hela cells overexpressing SCGN WT, and Cys mutants (C193S, C253S, C269S) was performed and each fraction was separated on non-reducing and reducing SDS PAGE

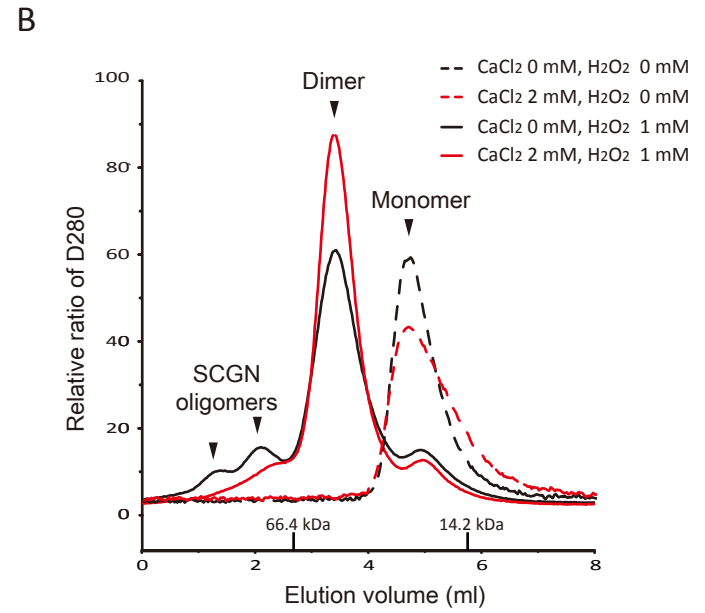
and hSCGN was detected by Western analysis. PRDX6, lamin B, and flotillin, were used as markers for cytosol and nucleus, and membrane respectively.

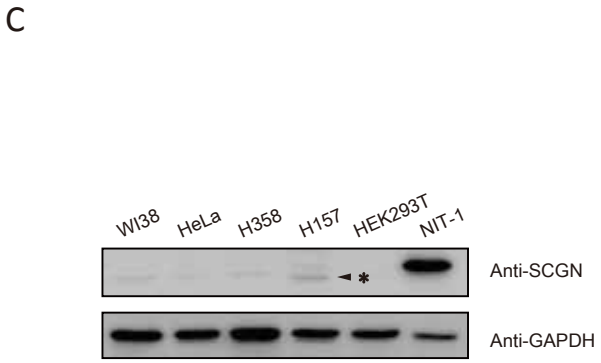
Supplementary Figure 6 - Full size gels used in Figure 1

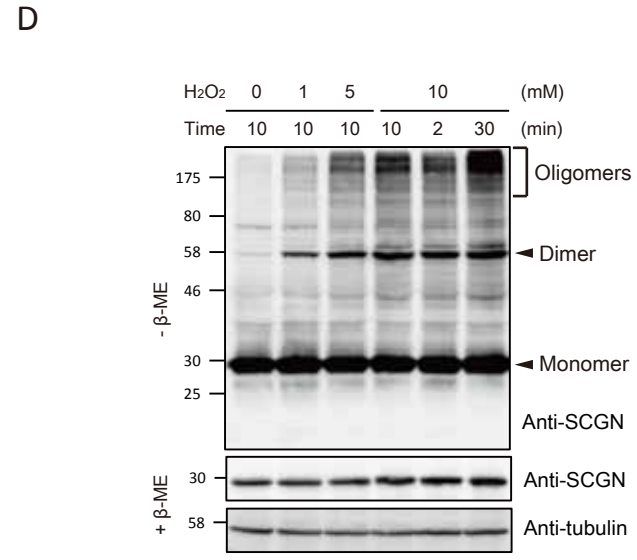
Supplementary Figure 7 - Full size gels used in Figure 4

Supplementary Figure 8 - Full size gels used in Figure 5


A
H2O2 0 0.1 (mM)
CaCl2 0 0.05 0.1 0.5 1 2 0 0.05 0.1 0.5 1 2 (mM)
58
45
30
25
- β-ME
Dimer
Monomer
+ β-ME
30
Relative amount of secretagogin dimer
3.5
3
2.5
2
1.5
1
0.5
0
0 0.05 0.1 0.5 1 2
CaCl2 (mM)
0 mM H2O2
0.1 mM H2O2


B
CaCl2 0 mM, H2O2 0 mM
CaCl2 2 mM, H2O2 0 mM
CaCl2 0 mM, H2O2 1 mM
CaCl2 2 mM, H2O2 1 mM
Dimer
Monomer
SCGN oligomers
66.4 kDa
14.2 kDa
Relative ratio of D280
100
80
60
40
20
0
0 2 4 6 8
Elution volume (ml)


C
WI38 HeLa H358 H157 HEK293T NIT-1
Anti-SCGN
Anti-GAPDH


D
H2O2 0 1 5 10 (mM)
Time 10 10 10 10 2 30 (min)
175
80
58
46
30
25
- β-ME
Oligomers
Dimer
Monomer
Anti-SCGN
+ β-ME
30
58
Anti-SCGN
Anti-tubulin

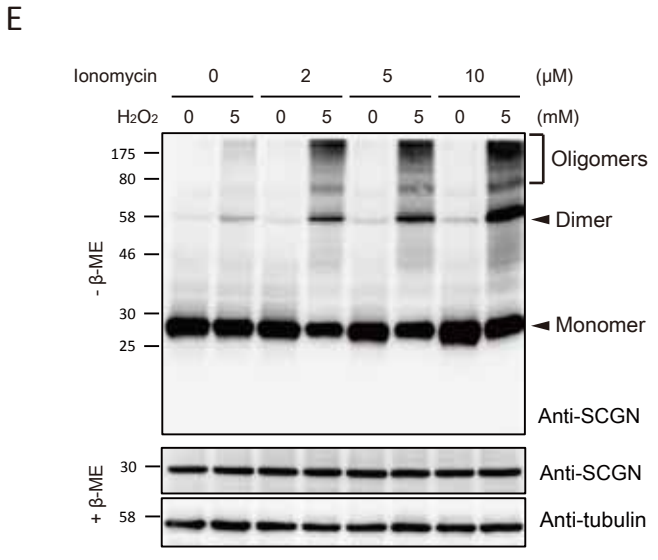
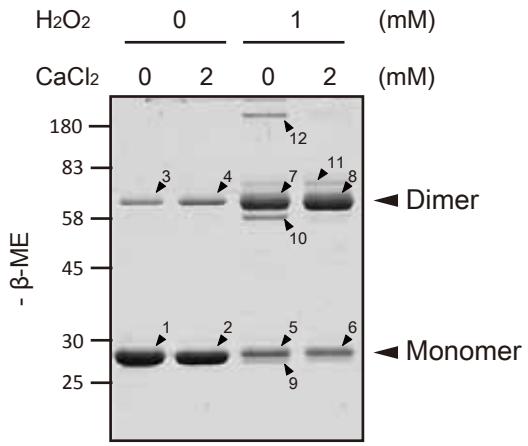

E
Ionomycin 0 2 5 10 (μM)
H2O2 0 5 0 5 0 5 0 5 (mM)
175
80
58
46
30
25
- β-ME
Oligomers
Dimer
Monomer
Anti-SCGN
+ β-ME
30
58
Anti-SCGN
Anti-tubulin

Figure S1

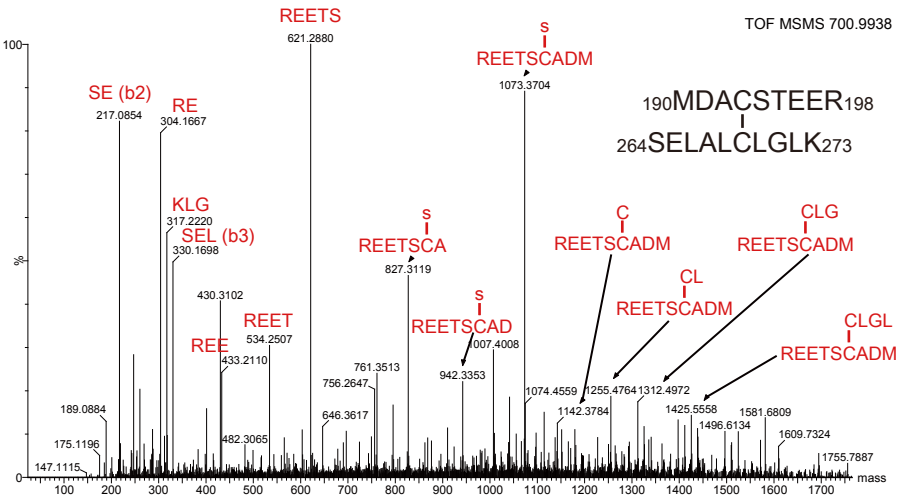
A



B List of identified disulfide linkages of each SCGN band

Band No.	Description			Disulfide linkage	Observed mw (CS)	Calculated mw	DeltaM	Dbond Score
		H_2O_2	Ca^{2+}					
1	Monomer	-	-	No ID				
2			+	Cys253 - Cys269	586.9968(3+)	1757.8804	0.0882	33.9
3	Dimer	-	-	Cys253 - Cys269	586.9984(3+)	1757.8804	0.093	7.3
4			+	Cys253 - Cys269	586.9981(3+)	1757.8804	0.0921	2.8
5	Monomer	+	-	No ID				
6			+	Cys253 - Cys269	586.9966(3+)	1757.8804	0.0876	36.4
7	Dimer	+	-	Cys193 - Cys193	520.6976(4+)	2078.7649	-0.0036	159.3
8			+	Cys193 - Cys193	520.6973(4+)	2078.7649	-0.0048	223
9	Lower shifted Monomer	+	-	Cys193 - Cys269	525.9949(4+)	2099.9537	-0.0032	17.2
10	Lower shifted Dimer	+	-	Cys193 - Cys269	525.9955(4+)	2099.9537	-8.0E-4	45.6
11	Upper shifted Dimer	+	+	Cys193 - Cys269	700.9935(3+)	2099.9537	0.005	7
				Cys253 - Cys269	586.9656(3+)	1757.8804	-0.0054	9.2
12	Upper shifted Dimer	-	+	Cys193 - Cys269	700.9938(3+)	2099.9537	0.0059	18.9

C



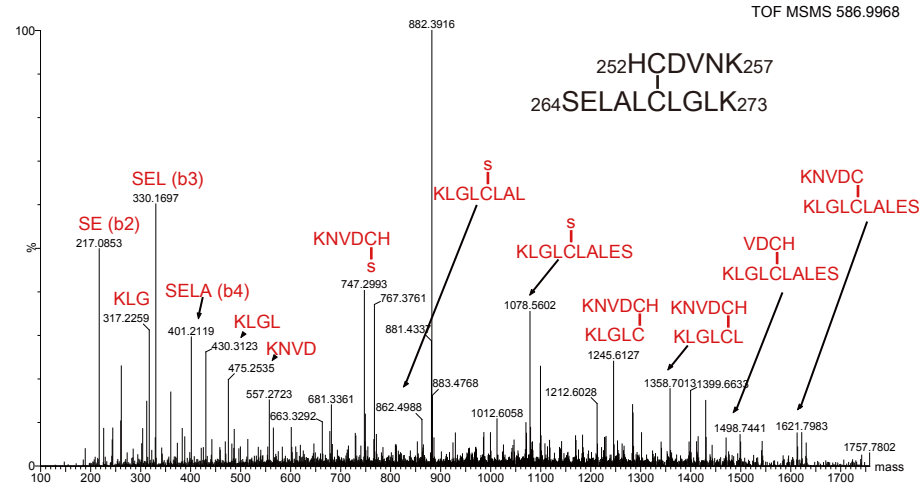
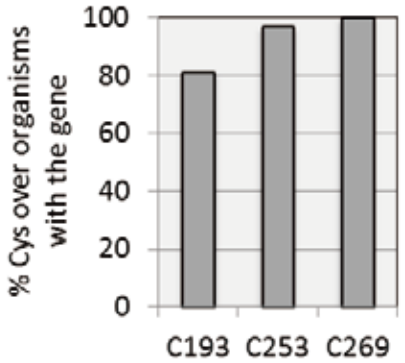


Figure S2

A

	Species	C193	C253	C269
MAMMALS	Humans	C	C	C
	Pan troglodytes (chimpanzee)	C	C	C
	Pan paniscus (bonobo)	C	C	C
	Gorilla gorilla gorilla (western lowland gorilla)	C	C	C
	Pongo abelii (Sumatran orangutan)	C	C	C
	Nomascus leucogenys (northern white-cheeked gibbon)	C	C	C
	Macaca mulatta (rhesus monkey)	C	C	C
	Macaca fascicularis (crab-eating macaque)	C	C	C
	Callithrix jacchus (white-tufted-ear marmoset)	C	C	C
	Mus musculus (mouse)	S	C	C
	Rattus norvegicus (rat)	S	C	C
	Cricetulus griseus (Chinese hamster)	S	C	C
	Nannospalax galili (Upper Galilee mountains blind mole rat)	C	C	C
	Heterocephalus glaber (naked mole rat)	S	C	C
	Oryctolagus cuniculus (rabbit)	C	C	C
	Tupaia chinensis (Chinese tree shrew)	C	C	C
	Canis familiaris (dog)	C	C	C
	Ailuropoda melanoleuca (giant panda)	C	C	C
	Ursus maritimus (polar bear)	C	C	C
	Felis catus (domestic cat)	C	C	C
	Panthera tigris altaica (Amur tiger)	C	C	C
	Bos taurus (cow)	C	C	C
	Bos mutus (wild yak)	C	C	C
	Pantholops hodgsonii (chiru)	C	C	C
	Capra hircus (goat)	C	C	C
	Ovis aries (sheep)	C	C	C
	Sus scrofa (pig)	C	C	C
	Camelus ferus (Wild Bactrian camel)	C	C	C
	Balaenoptera acutorostrata scammoni (minke whale)	C	C	C
	Lipotes vexillifer (Yangtze River dolphin)	C	C	C
	Equus caballus (horse)	C	C	C
	Myotis brandtii (Brandt's bat)	NG	NG	NG
	Myotis davidii	NG	NG	NG
	Pteropus alecto (black flying fox)	C	C	C
	Monodelphis domestica (opossum)	C	C	C
	Sarcophilus harrisii (Tasmanian devil)	C	C	C
	Ornithorhynchus anatinus (platypus)	C	C	C
BIRDS	Gallus gallus (chicken)	C	C	C
	Meleagris gallopavo (turkey)	C	C	C
	Anas platyrhynchos (mallard)	C	C	C
	Taeniopygia guttata (zebra finch)	C	C	C
	Geospiza fortis (medium ground-finch)	S	C	C
	Ficedula albicollis (collared flycatcher)	C	C	C
	Pseudopodoces humilis (Tibetan ground-tit)	C	C	C
	Corvus cornix (hooded crow)	C	C	C
	Falco peregrinus (peregrine falcon)	C	C	C
	Falco cherrug (Saker falcon)	C	C	C
	Columba livia (rock pigeon)	C	C	C
REPTILES	Alligator sinensis (Chinese alligator)	C	C	C
	Alligator mississippiensis (American alligator)	C	C	C
	Pelodiscus sinensis (Chinese soft-shelled turtle)	C	C	C
	Chelonia mydas (green sea turtle)	C	C	C
	Anolis carolinensis (green anole)	C	C	C
	Python bivittatus (Burmese python)	S	C	C
AMPHIBIANS	Xenopus laevis (African clawed frog)	S	C	C
	Xenopus tropicalis (western clawed frog)	S	C	C
FISHES	Danio rerio (zebrafish)	S	C	C
	Takifugu rubripes (torafugu)	C	C	C
	Maylandia zebra (zebra mbuna)	C	C	C
	Oryzias latipes (Japanese medaka)	C	C	C
	Xiphophorus maculatus (southern platyfish)	C	C	C
	Latimeria chalumnae (coelacanth)	C	C	C
CARTILAGINOUS FISHES	Callorhinchus milii (elephant shark)	C	C	C
LANCELETS	Branchiostoma floridae (Florida lancelet)	K	V	C
ASCIDIANS	Ciona intestinalis (sea squirt)	G	C	C
ECHINODERMS	Strongylocentrotus purpuratus (purple sea urchin)	M	F	C

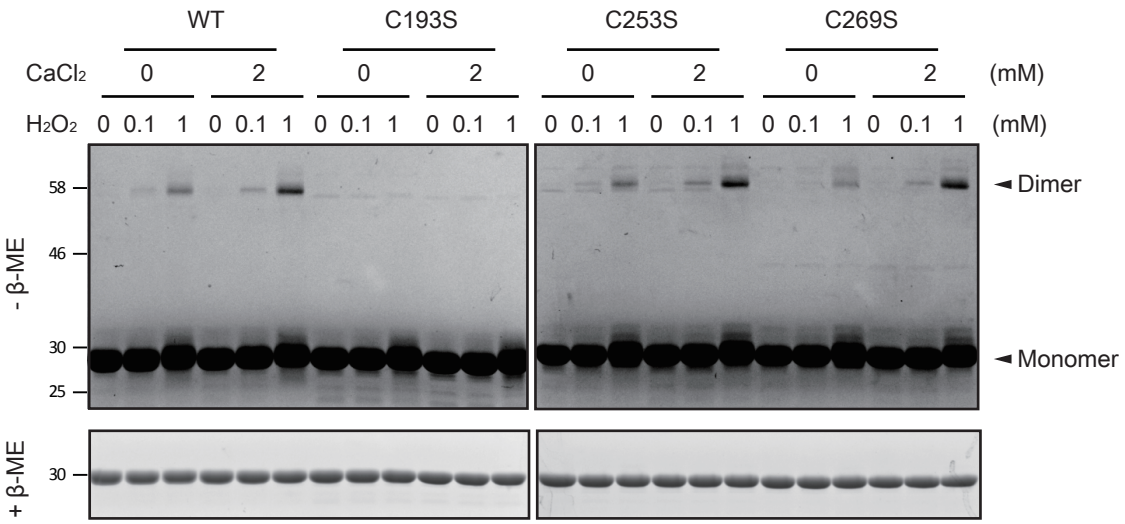
	Species	C193	C253	C269
INSECTS	Drosophila melanogaster (fruit fly)	NG	NG	NG
	Drosophila pseudoobscura pseudoobscura	NG	NG	NG
	Drosophila ananassae	NG	NG	NG
	Drosophila erecta	NG	NG	NG
	Drosophila persimilis	NG	NG	NG
	Drosophila sechellia	NG	NG	NG
	Drosophila simulans	NG	NG	NG
	Drosophila willistoni	NG	NG	NG
	Drosophila yakuba	NG	NG	NG
	Drosophila grimshawi	NG	NG	NG
	Drosophila mojavensis	NG	NG	NG
	Drosophila virilis	NG	NG	NG
	Musca domestica (house fly)	NG	NG	NG
	Anopheles gambiae (mosquito)	NG	NG	NG
	Aedes aegypti (yellow fever mosquito)	NG	NG	NG
	Culex quinquefasciatus (southern house mosquito)	NG	NG	NG
	Apis mellifera (honey bee)	NG	NG	NG
	Solenopsis invicta (red fire ant)	NG	NG	NG
	Acromyrmex echinatior (Panamanian leafcutter ant)	NG	NG	NG
	Harpegnathos saltator (Jerdon's jumping ant)	NG	NG	NG
	Camponotus floridanus (Florida carpenter ant)	NG	NG	NG
	Nasonia vitripennis (jewel wasp)	NG	NG	NG
	Tribolium castaneum (red flour beetle)	NG	NG	NG
	Bombyx mori (domestic silkworm)	NG	NG	NG
	Plutella xylostella (diamondback moth)	NG	NG	NG
	Acyrthosiphon pisum (pea aphid)	NG	NG	NG
	Pediculus humanus corporis (human body louse)	NG	NG	NG
MITES AND TICKS	Ixodes scapularis (black-legged tick)	NG	NG	NG
NEMATODES	Caenorhabditis elegans (nematode)	NG	NG	NG
	Caenorhabditis briggsae	NG	NG	NG
	Brugia malayi (filaria)	NG	NG	NG
	Loa loa (eye worm)	NG	NG	NG
	Trichinella spiralis	NG	NG	NG
ANNELIDS	Helobdella robusta	NG	NG	NG
MOLLUSKS	Lottia gigantea (owl limpet)	NG	NG	NG
	Crassostrea gigas (Pacific oyster)	NG	NG	NG
FLATWORMS	Schistosoma mansoni	NG	NG	NG
CNIDARIANS	Nematostella vectensis (sea anemone)	NG	NG	NG
	Hydra vulgaris	NG	NG	NG
PLACOZOANS	Trichoplax adhaerens	NG	NG	NG
PORIFERANS	Amphimedon queenslandica (sponge)	NG	NG	NG
		HIGH	HIGH	HIGH



"NG" means there is no true ortholog (no gene) in that species

Figure S3

B



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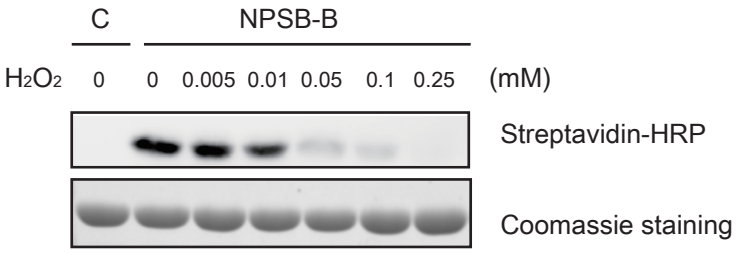


Figure S3 (continued)

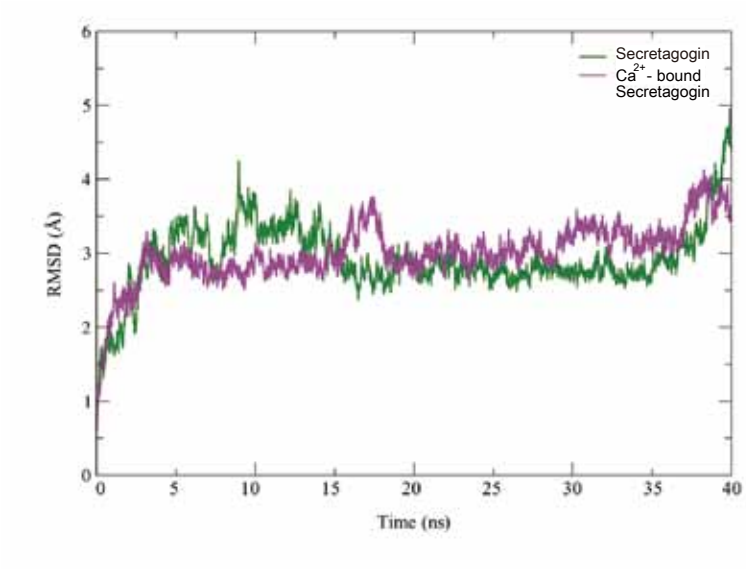
Table S1 - Differential deuterium exchange rates of identified SCGN peptides in HDX-MS experiment.

Sequence	Start	End	Domain	Structure (Residue no.)	Differential deuterium exchange rate (%)					Mean
					10 sec	60 sec	300 sec	1800 sec	10800 sec	
DSSREPTLGRL	2	12			0.00	0.00	0.00	0.00	0.00	0.00
DSSREPTLGRLDAAGF	2	17	Domain I		6.25	3.13	0.00	0.00	0.00	1.88
WQVWQRFDADE	18	28		EF 1 (25-36)	18.18	18.18	18.18	9.09	4.46	13.62
WQVWQRFDADEKGYIEE	18	34			17.65	11.76	17.65	0.00	0.00	9.41
YIEEKELDAFF	31	41			9.09	9.09	9.09	18.18	0.00	9.09
FLHMLMKLGTDD	41	52			0.00	0.00	0.00	0.00	0.00	0.00
FLHMLMKLGTDDTVM	41	55			0.00	0.00	0.00	0.00	0.00	0.00
LMKLGTDDTVM	45	55			0.00	0.00	0.00	0.00	0.00	0.00
LMKLGTDDTVMKANL	45	59			0.00	0.00	0.00	0.00	0.00	0.00
KANLHKVKQQF	56	66			9.09	0.00	-9.09	0.00	0.00	0.00
MTTQDASKDGRIRMKELAGMF	67	87		EF 2 (71-82)	4.76	7.14	4.76	4.76	4.76	5.24
KELAGMF	81	87			14.29	7.14	14.29	0.00	0.00	7.14
LLFRRENPLDSSVEF	96	110	Linker 1		10.00	6.67	6.67	0.00	0.00	4.67
LFRRENPLDSSVEF	97	110			3.57	10.71	3.57	0.00	0.00	3.57
FRRENPLDSSVEF	98	110			0.00	0.00	0.00	0.00	0.00	0.00
MQIWRKYDADSSG	111	123	Domain II	EF 3 (118-129)	-7.69	-3.85	0.00	0.00	0.00	-2.31
WRKYDADSSGF	114	124			-9.09	-9.09	0.00	0.00	-18.18	-7.27
ISAAELRNFLRD	125	136			0.00	0.00	0.00	0.00	0.00	0.00
AELRNFLRDLFLHHKKAISE	128	147			-2.50	-2.50	-5.00	0.00	-7.50	-3.50
LRNFLRDLFLHHKKAISE	130	147			-5.56	0.00	0.00	0.00	-2.78	-1.67
AKLEEYTGTM	148	157			0.00	-15.00	-10.00	-10.00	0.00	-7.00
EEYTGTM	151	157		EF 4 (162-173)	-14.29	0.00	7.14	-14.29	0.00	-4.29
MKIFDRNKDGRLDLNDL	158	174			0.00	0.00	0.00	-8.82	-5.88	-2.94
DLNDLARILALQENF	170	184			3.33	6.67	0.00	0.00	0.00	2.00
ARILALQENFLL	175	186			-8.33	-4.17	4.17	4.17	4.17	0.00
QENFLLQFKMDACST	185	195	Linker 2		9.09	9.09	9.09	0.00	0.00	5.45
EERKRDFEKIFA	196	207	Domain III		0.00	0.00	-8.33	0.00	0.00	-1.67
AYYDVSKTGALEGPEVDG	207	224		EF 5 (210-221)	0.00	-5.56	-16.67	-5.56	0.00	-5.56
AYYDVSKTGALEGPEVDGF	207	225			-7.89	-10.53	-21.05	-10.53	0.00	-10.00
YYDVSKTGALEGPEVDG	208	224			-5.88	-5.88	-17.65	-5.88	0.00	-7.06
YDVSKTGALEGPEVDG	209	224			-6.25	-6.25	-18.75	-6.25	0.00	-7.50
ELVQPSISGVDLDKF	231	245			-13.33	0.00	0.00	0.00	0.00	-2.67
ELVQPSISGVDLDKFREIL	231	249			-5.26	0.00	-10.53	-5.26	0.00	-4.21
LVQPSISGVDLDKF	232	245			-10.71	0.00	-7.14	0.00	0.00	-3.57
PSISGVDLDKFREIL	235	249			-6.67	0.00	-6.67	-6.67	-6.67	-5.33
LRHCDVNKDGKIQKSEL	250	266		EF 6 (254-265)	-11.76	-11.76	-17.65	-17.65	-11.76	-14.12
CLGLKIN	269	276			0.00	12.50	0.00	0.00	0.00	2.50

Table S2 - Amino acids sequences of the six EF-hand loops of SCGN.
The amino acids with low occurrence (less than 5%) at each position in known EF-loops are underlined (Gifford, Walsh et al., 2007).

EF-loop position	1	2	3	4	5	6	7	8	9	10	11	12
	+X (D)		**+Y (D,N)**		**+Z (D,S,N)**				**-X (D,S,T,E ,N,G,Q)**			**-Z (E,D)**
EF1 (D25-E36)	D	A	D	E	K	G	Y	I	E	E	K	E
EF2 (D71-E82)	D	A	S	K	D	G	R	I	R	M	K	E
EF3 (D118-E129)	D	A	D	S	S	G	F	I	S	A	A	E
EF4 (D162-D173)	D	R	N	K	D	G	R	L	D	L	N	D
EF5 (D210-E221)	D	V	S	K	T	G	A	L	E	G	P	E
EF6 (D254-E265)	D	V	N	K	D	G	K	I	Q	K	S	E

A


Secretagogin
Ca2+ - bound Secretagogin
RMSD (Å)
Time (ns)
0
1
2
3
4
5
6
0
5
10
15
20
25
30
35
40

B

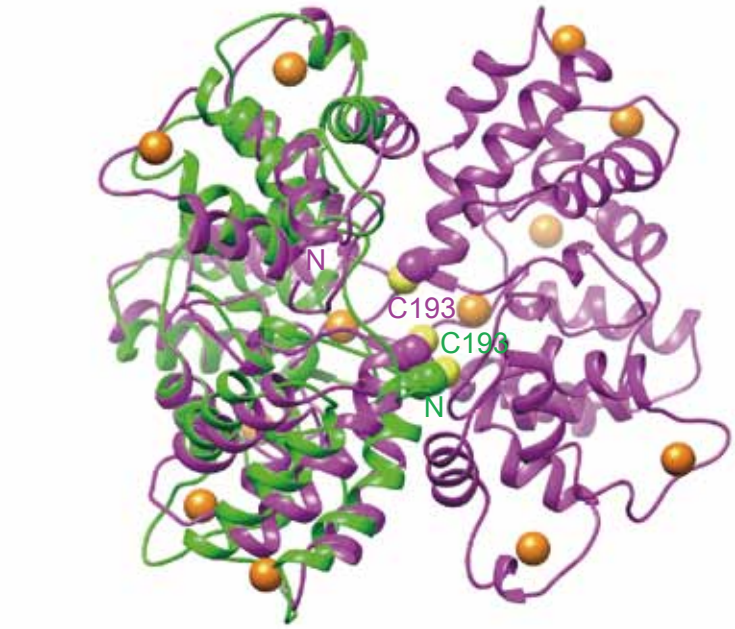

N
C193
C193
N

Figure S4

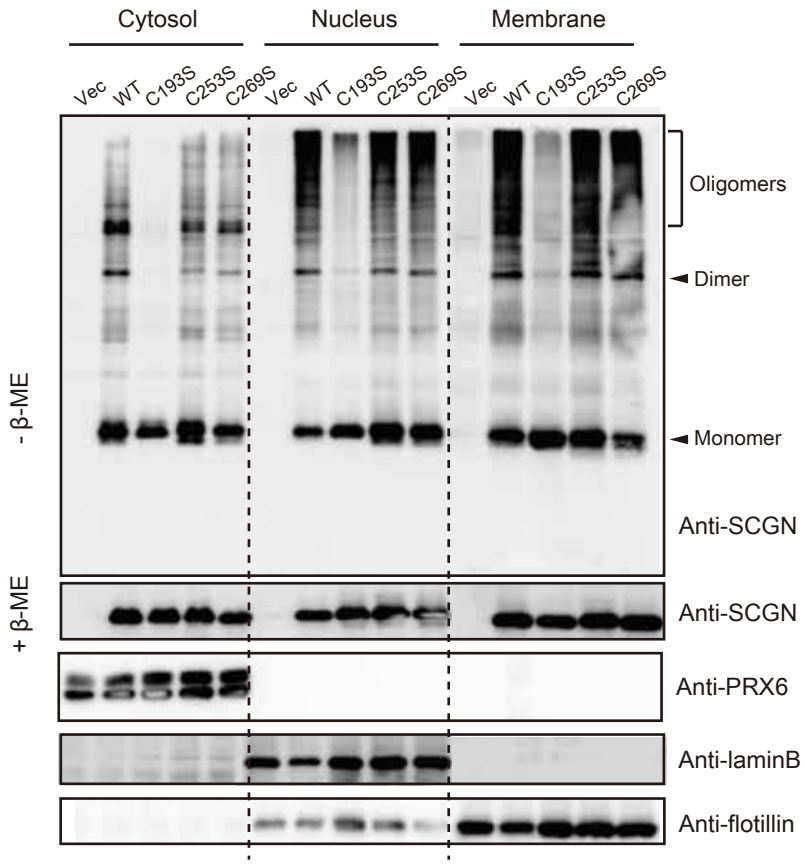

Cytosol
Nucleus
Membrane
Vec
WT
C193S
C253S
C269S
Oligomers
Dimer
Monomer
- β-ME
+ β-ME
Anti-SCGN
Anti-SCGN
Anti-PRX6
Anti-laminB
Anti-flotillin

Figure S5

Figure 1A

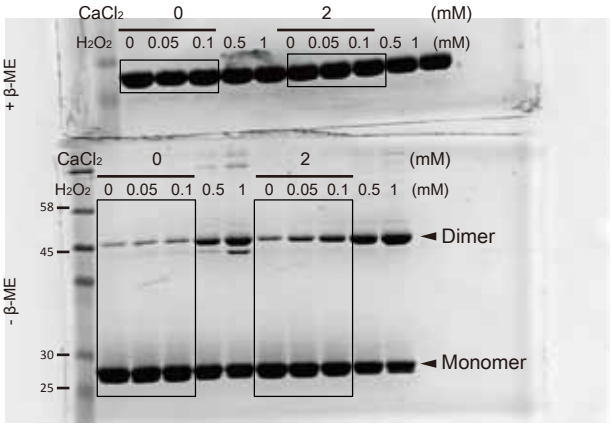


Figure 1B

Figure 1D

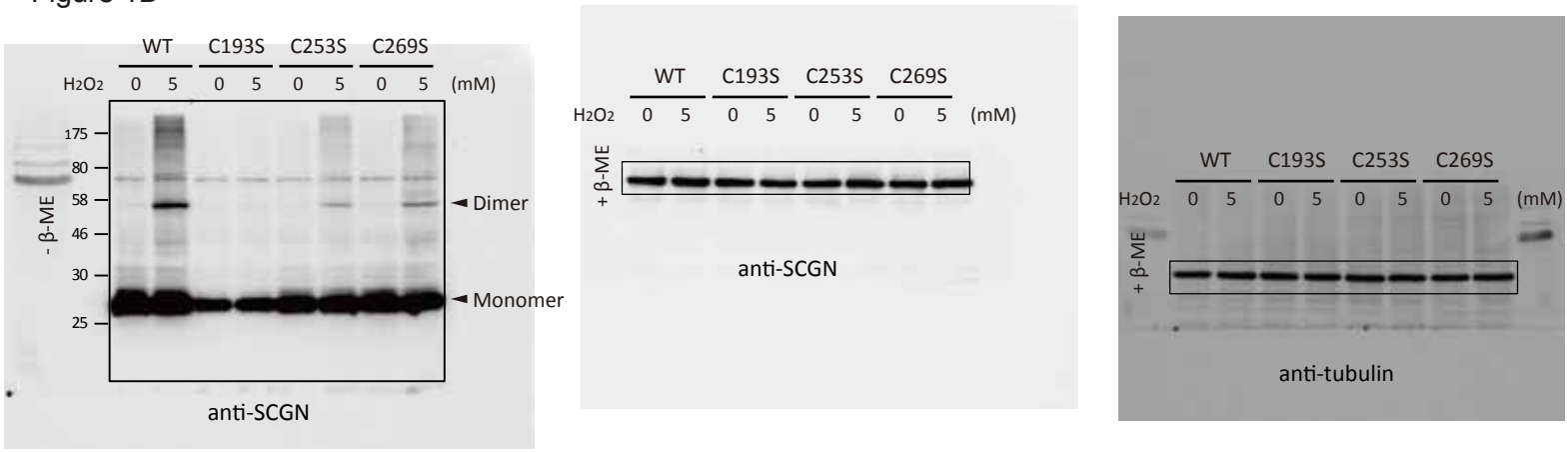


Figure 1E

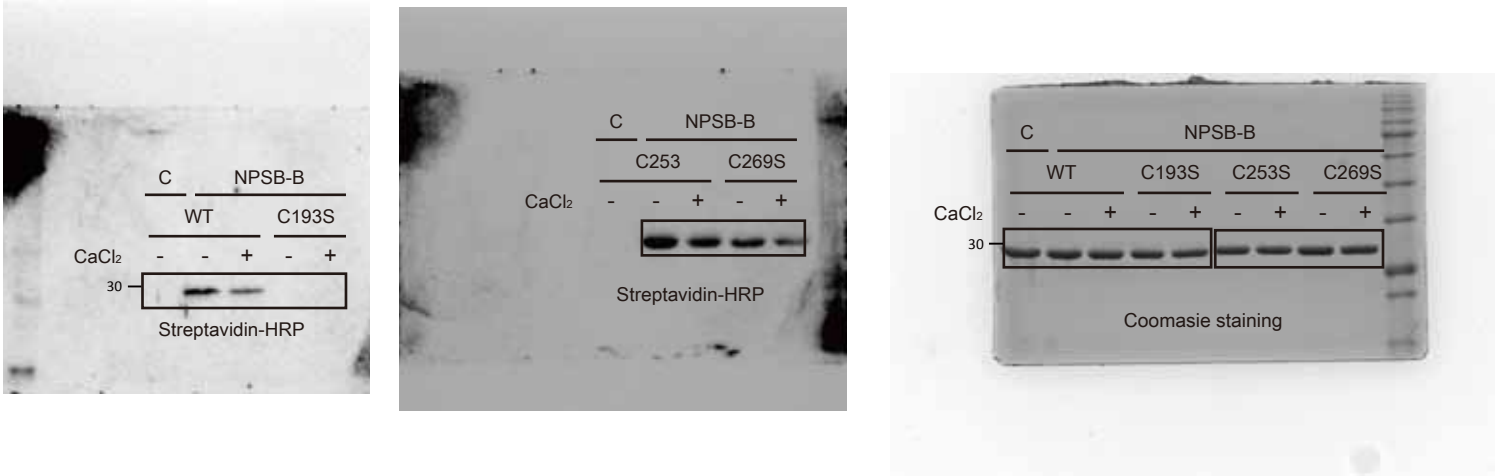


Figure S6

Figure 4A

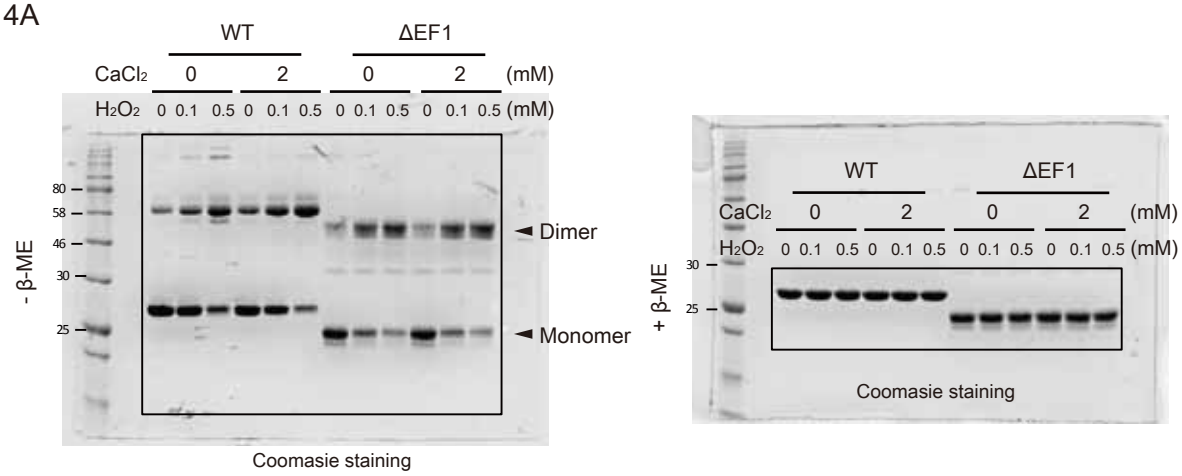


Figure 4B

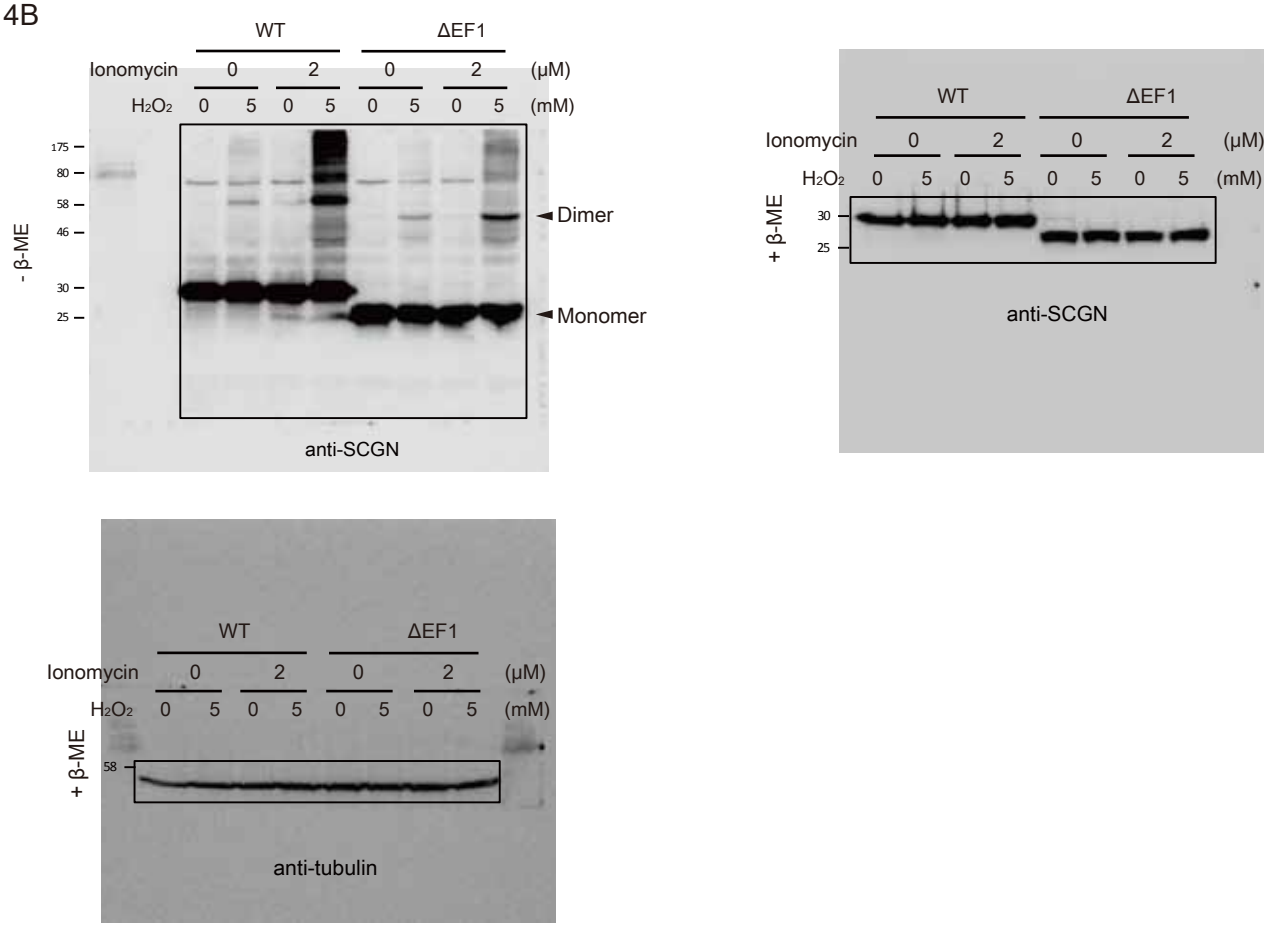


Figure 4C

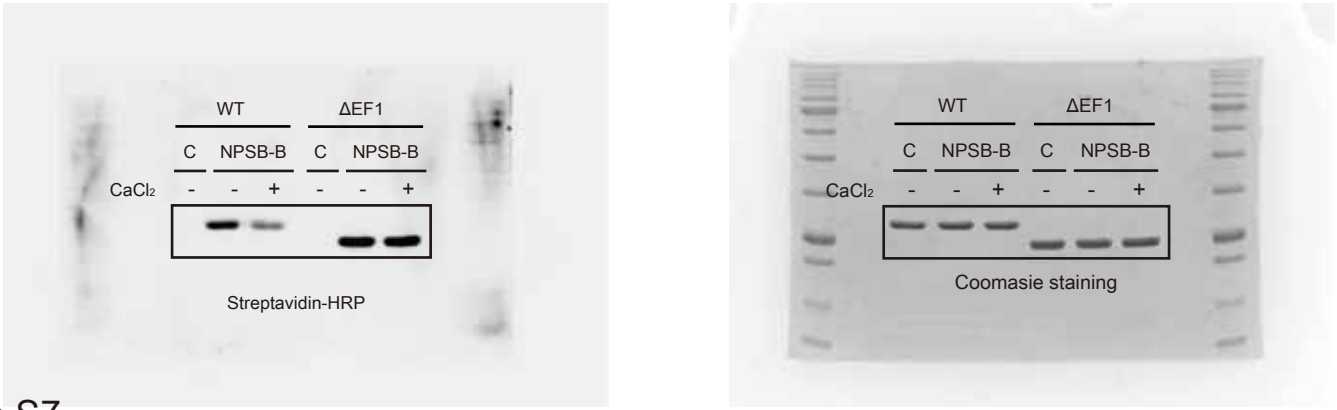


Figure S7

Figure 5A

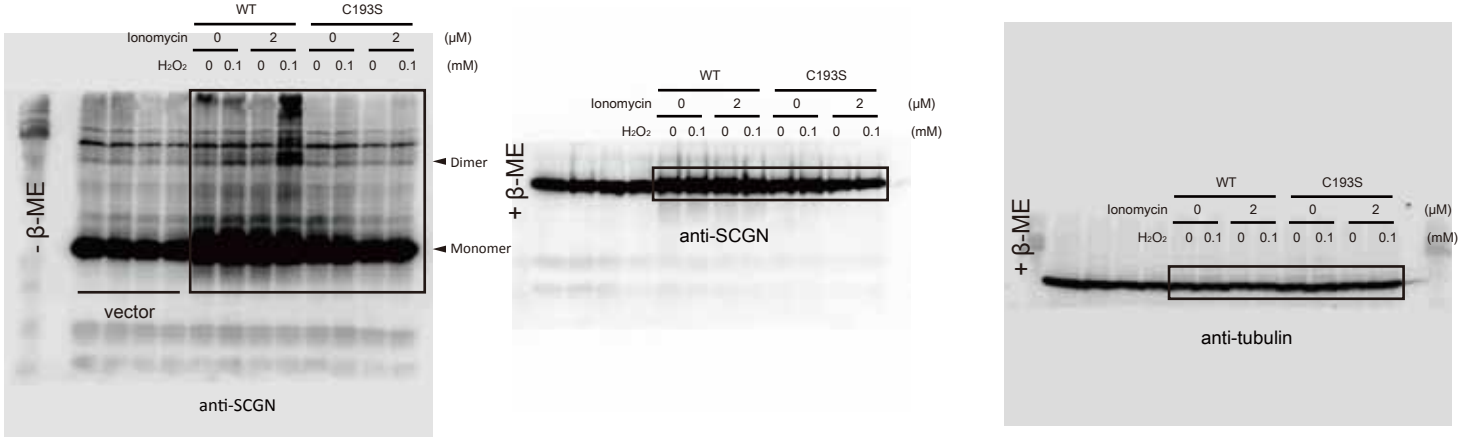


Figure 5B

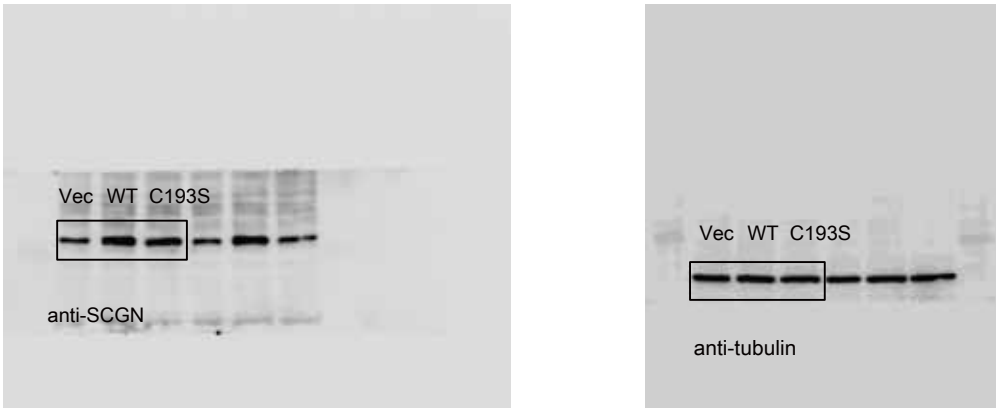
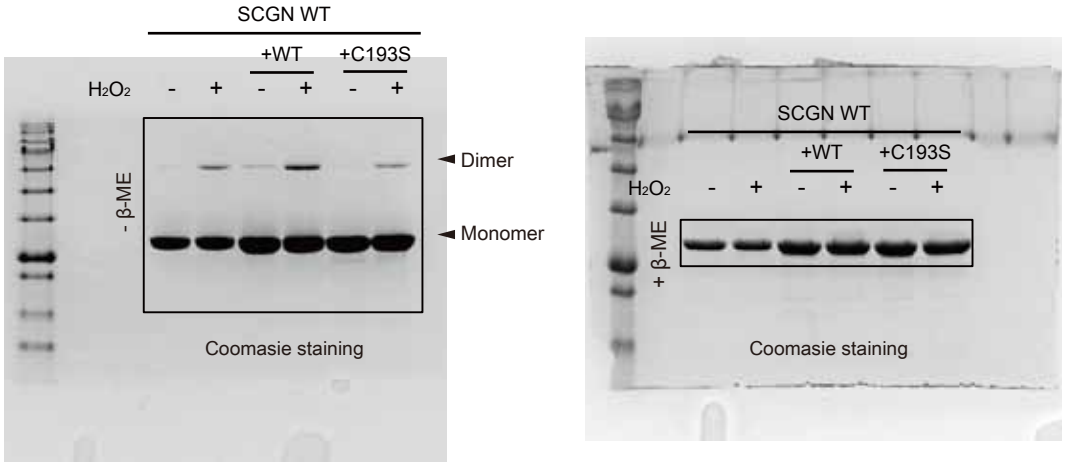


Figure 5C



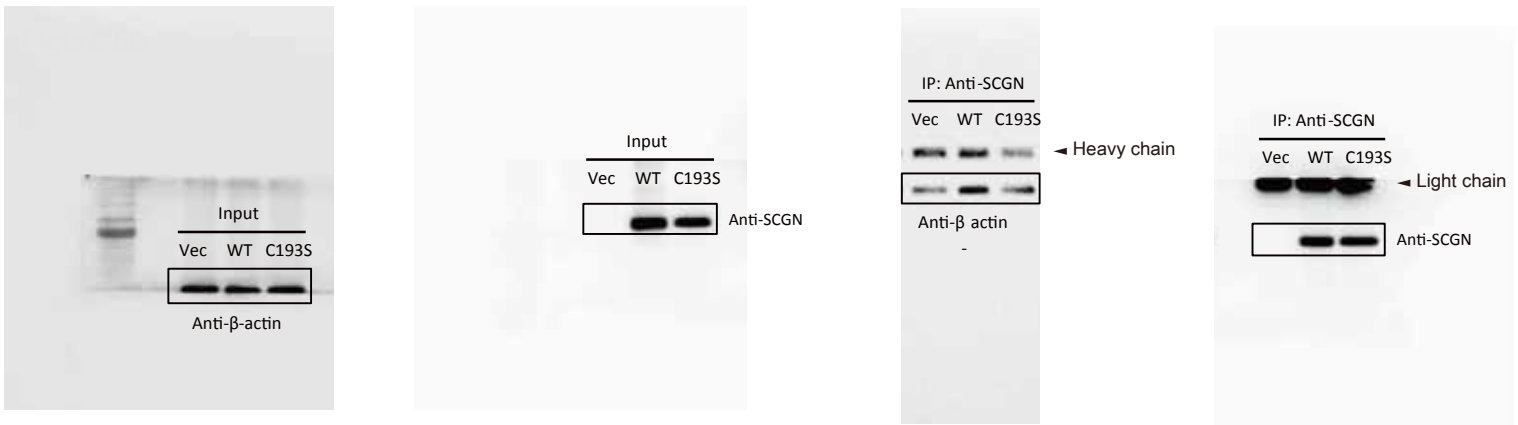


Figure S8