

Supplementary Table 2: Constructs used in this study

Constructs:

Construct descriptions	Internal ref.	Figure
Rhodopsin constructs		
TST-Rho _{ext} (TMDs 1-2) glycosylatable	pPL-TST-VHP-glyc-linker- <i>Bos Taurus</i> Rho 1+2 TMD-F56C cyt.Sec61β (N36Q, C39S)	MK16 (gblock) 1b; ED1c-f
TST-Rho _{ext} (TMDs 1-2) nonglycosylatable	pPL-TST-VHP-linker- <i>Bos Taurus</i> Rho 1+2 TMD-F56C cyt.Sec61β (N36Q, C39S)	MK15 (gblock) ED1c-d.
FLAG-Rho _{ext} (TMDs 1-2) glycosylatable	pPL-3xFLAG-VHP-glyc-linker- <i>Bos Taurus</i> Rho 1+2 TMD-F56C cyt.Sec61β (N36Q, C39S)	MK18 (gblock) 1b-c; ED1b, ED1e-f; ED7g
FLAG-Rho _{ext} (TMDs 1-2) glycosylatable [used in Chitwood & Hegde, (2020), <i>Nature</i> , 584 :630-634]	1xFlag glyc <i>Bos Taurus</i> Rho-1+2 TMD-F56C cyt.Sec61β (N36Q, C39S)-6xHis	PC250 (plasmid) ED1b
Matched TST-Rho _{ext} (TMDs 1-2) nonglycosylatable constructs with wildtype or mutant TMD1.	pPL-TST-VHP-linker- <i>Bos Taurus</i> Rho 1-2 TMD-F56C cyt.Sec61β (N36Q, C39S)	MK22 (gblock) 1c
	pPL-TST-VHP-linker- <i>Bos Taurus</i> Rho 1-2 TMD-N55L, F56C cyt.Sec61β (N36Q, C39S)	MK26 (gblock) 1c
	pPL-TST-VHP-linker- <i>Bos Taurus</i> Rho 1-2 TMD-L47R, N55L, F56C cyt.Sec61β (N36Q, C39S)	MK27 (gblock) 1c
	pPL-TST-VHP-linker- <i>Bos Taurus</i> Rho 1-2 TMD-G51L, N55L, F56C, T58L cyt.Sec61β (N36Q, C39S)	MK28 (gblock) 1c
Rho (TMDs 1-2) TMD1 contains cysteine; <i>can be inserted post-translationally</i>	pSP64 HA-glyc <i>Bos Taurus</i> Rho-1+2 TMD-F56C cyt.Sec61β (N36Q, C39S)-6xHis	PC54 (plasmid) 2d; ED5c, ED5e
Rho (TMDs 1-2) photocrosslinkable through TMD1; <i>can be inserted post-translationally</i>	HA-glyc- <i>Bos Taurus</i> Rho F56Amb 1-2 TMD-cyt.Sec61β (N36Q, C39S)-6His	LSG39 (gblock) 3b
Matched constructs for monitoring Sec61 lateral gate engagement after insertion of transmembrane domains	HA-glyc- <i>Bos Taurus</i> Rho 1-3TMD-ERYV-pPL	LS1074 3d
	HA-glyc-L ₂₂ +C-ERYV-pPL	LS1075 3d
TST-Rho _{ext} (TMDs 1-7), glycosylatable	pPL-TST-VHP-glyc-linker-FL <i>Bos Taurus</i> Rho-F56C cyt.Sec61β (N36Q, C39S)	MK25 (gblock) MK1009 (plasmid) 4a-b, 4d (plasmid); ED7b (gblock), ED7d-f (plasmid)
Rho _{ext} N-terminus exchanged with HA-glyc N-terminus for Sec61-independent insertion of TMD1	HA-glyc FL <i>Bos Taurus</i> Rho-F56C cyt.Sec61β (N36Q, C39S)	LS1035 (plasmid) 4e; ED9b-d
Matched Rho 3 TMD constructs with either F56C Rho TMD1 or L ₂₂ +C as a control,	HA-glyc- <i>Bos Taurus</i> Rho-F56C, C110S, TMD1-3 cyt.Sec61β (N36Q, C39S)	LS1073 (plasmid) ED7h
	HA-glyc-22L+C- <i>Bos Taurus</i> Rho-TMD2-3 C110S, cyt.Sec61β (N36Q, C39S)	LS1038 (plasmid) ED7h

which does not engage the MPT			
Rho _{ext} variants with amber codons positioned at TMDs 1-3	pSP64 pPL-TST-VHP-glyc-linker-FL <i>Bos Taurus</i> Rho F56Amb-cyt.Sec61β (N36Q, C39S)	LS1030 (plasmid)	4d; ED7d
	pSP64 pPL-TST-VHP-glyc-linker-FL <i>Bos Taurus</i> Rho F85Amb-cyt.Sec61β (N36Q, C39S)	LS1070 (plasmid)	4d; ED7e
	pSP64 pPL-TST-VHP-glyc-linker-FL <i>Bos Taurus</i> Rho F88Amb-cyt.Sec61β (N36Q, C39S)	LS1071 (plasmid)	4d; ED7e
	pSP64 pPL-TST-VHP-glyc-linker-FL <i>Bos Taurus</i> Rho F115Amb-cyt.Sec61β (N36Q, C39S)	LS1026 (plasmid)	4d; ED7d
	pSP64 pPL-TST-VHP-glyc-linker-FL <i>Bos Taurus</i> Rho W126Amb-cyt.Sec61β (N36Q, C39S)	LS1029 (plasmid)	4d; ED7d
	pSP64 pPL-TST-VHP-glyc-linker-FL <i>Bos Taurus</i> Rho F116Amb-cyt.Sec61β (N36Q, C39S)	LS1027 (plasmid)	ED7d
	pSP64 pPL-TST-VHP-glyc-linker-FL <i>Bos Taurus</i> Rho L119Amb-cyt.Sec61β (N36Q, C39S)	LS1028 (plasmid)	ED7d
	pSP64 pPL-TST-VHP-glyc-linker-FL <i>Bos Taurus</i> Rho F56Amb F115Amb-cyt.Sec61β (N36Q, C39S)	LS1031 (plasmid)	ED7d
	pSP64 pPL-TST-VHP-glyc-linker-FL <i>Bos Taurus</i> Rho F56Amb F116Amb-cyt.Sec61β (N36Q, C39S)	LS1032 (plasmid)	ED7d
	pSP64 pPL-TST-VHP-glyc-linker-FL <i>Bos Taurus</i> Rho F56Amb L119Amb-cyt.Sec61β (N36Q, C39S)	LS1033 (plasmid)	ED7d
	pSP64 pPL-TST-VHP-glyc-linker-FL <i>Bos Taurus</i> Rho F56Amb W126Amb-cyt.Sec61β (N36Q, C39S)	LS1034 (plasmid)	ED7d
	pSP64 pPL-TST-VHP-glyc-linker-FL <i>Bos Taurus</i> Rho V81Amb-cyt.Sec61β (N36Q, C39S)	LS1067 (plasmid)	ED7e
	pSP64 pPL-TST-VHP-glyc-linker-FL <i>Bos Taurus</i> Rho A82Amb-cyt.Sec61β (N36Q, C39S)	LS1068 (plasmid)	ED7e
	pSP64 pPL-TST-VHP-glyc-linker-FL <i>Bos Taurus</i> Rho L84Amb-cyt.Sec61β (N36Q, C39S)	LS1069 (plasmid)	ED7e
	pSP64 pPL-TST-VHP-glyc-linker-FL <i>Bos Taurus</i> Rho F91Amb-cyt.Sec61β (N36Q, C39S)	LS1072 (plasmid)	ED7e
Misc. substrates			
Pre-prolactin (pPL)	TST-pPL	MK29 (gblock)	ED1f
Full length human TRAM2 (Model substrate of N _{cyt} topology)	pSP64 3xFLAG-huTRAM2-pPL	M39 (plasmid)	ED1g; ED7a, ED7c
Tagged MPT components			
Human Asterix-FLAG variants (C-term. tagged)	Wt	LSG001 (gblock)	2c-e; ED5b-f
	F44Amb	LSG002 (gblock)	2d-e; ED5c-d
	W58Amb	LSG003 (gblock)	2e; ED5d-e
	V59Amb	LSG004 (gblock)	2e; ED5d-e
	Y62Amb	LSG005 (gblock)	2c-e; ED5c-e
	A69Amb	LSG006 (gblock)	2e; ED5d-e
	F84Amb	LSG007 (gblock)	2e; ED5d-e
	Y95Amb	LSG008 (gblock)	2e; ED5d-e
	Y36Amb	LSG009 (gblock)	2d-e; ED5d
	M42Amb	LSG010 (gblock)	2c-e; ED5c-e
	M46Amb	LSG011 (gblock)	2d-e; ED5d

	M50Amb	LSG012 (gblock)	2d-e; ED5c-d
	A60Amb	LSG013 (gblock)	2d-e; ED5d
	M85Amb	LSG014 (gblock)	2d-e; ED5d
	M93Amb	LSG015 (gblock)	2d-e; ED5d
	N38L, S45L, Q79L, S82L, S83L, S89L, S94L	LSG029 (gblock)	3b; ED5f
	N38L, S45L, Q79L, S82L, S83L, S89L	LSG030 (gblock)	3b; ED5f
	S45L, Q79L, S82L, S83L, S89L	LSG031 (gblock)	3b; ED5f
	M42V, M46V, M50V, M93V	LSG036 (gblock)	3b; ED5f
	M42V, M46V, M50V	LSG047 (gblock)	3b; ED5f
	M37V, M42V, M46V, M50V	LSG048 (gblock)	3b; ED5f
	M81V, M85V, M93V	LSG049 (gblock)	3b; ED5f
	M42V, M46V, M50V, M81V, M85V, M93V	LSG051 (gblock)	3b; ED5f
	M37V, M42V, M46V, M50V, M80V, M81V, M85V, M93V	LSG052 (gblock)	3b; ED5f
For transient transfection and const. expression of tagged TMCO1	pcDNA5 3xFLAG-TEV-TMCO1	YYP106 (plasmid)	ED7e
Model substrates for monitoring sensitivity towards Sec61 inhibitors			
N _{cyt} signal-anchor protein	pSP64 HA-hu ASGR1-6xHis	HW040 (plasmid)	5e; ED9b-d
N _{exo} multi-pass membrane protein	pSP64 HA-glyc-wt β ₁ AR-3xFLAG	YH102 (plasmid)	ED9b-d
N _{exo} multi-pass membrane protein	pSP64 HA-glyc-ΔCL3 β ₁ AR-3xFLAG	YH103 (plasmid)	ED9b-d
Secretory proteins	pSP64 pPL	374 (plasmid)	ED9b-d
	pSP64 ha-PrP	660 (plasmid)	ED9b-d
N _{exo} signal-anchor protein	HA-LeP FL	4003 (plasmid)	ED9b-d
N _{cyt} signal-anchor protein	Hu-TNFα-1xFLAG	LSG080 (gblock)	ED9b-d
C3AR1 constructs used to show that long extracell. loops engage Sec61 lateral gate for their insertion	HA-glyc-FL-C3AR1	LSG111 (gblock)	ED9e
	HA-glyc-C3AR1 Δ[T166-D326]	LSG112 (gblock)	ED9e
For transient transfection and constitutive expression of fluorescent reporter	SS-T4lysozyme-HA-β ₁ AR-GFP-P2A-RFP	PJC77	ED9h