Supporting Information for:

The mammalian cholesterol synthesis enzyme squalene monooxygenase is proteasomally truncated to a constitutively active form

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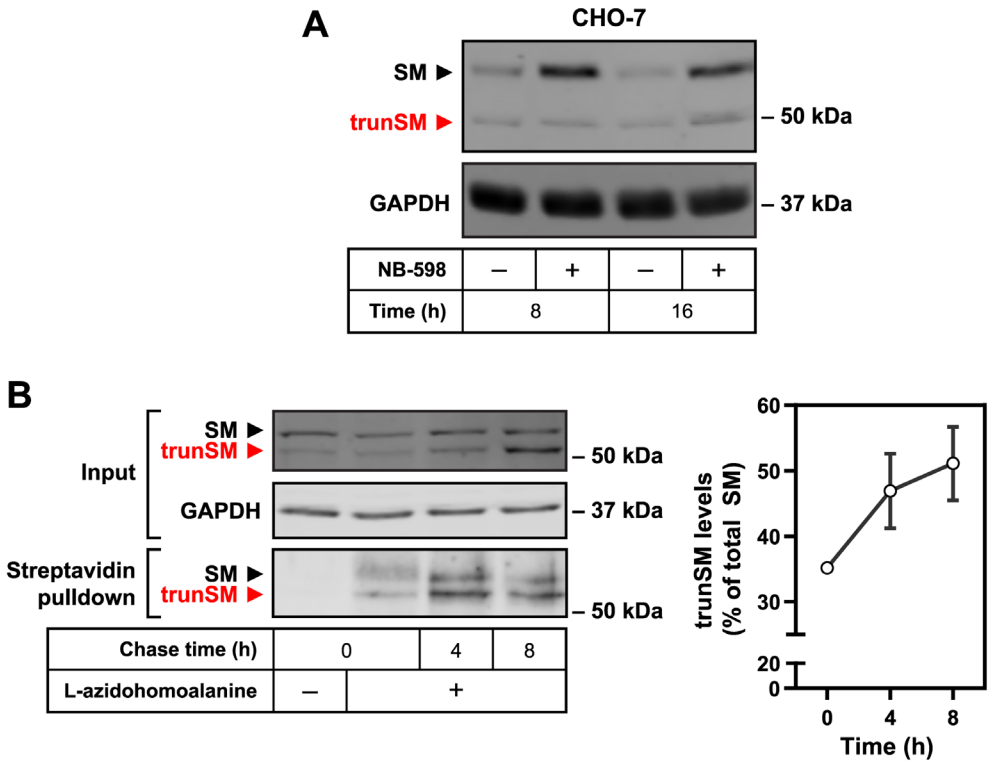


Figure S1. Related to Fig. 1

(A) CHO-7 cells express a trunSM-like protein. CHO-7 cells were treated in the presence or absence of 1 µM NB-598 for the indicated times, and immunoblotting was performed for SM and truncated SM (trunSM, red). Immunoblot is representative of $n = 2$ independent experiments.

(B) trunSM is derived from full-length SM. HEK293T cells were pre-treated in sterol-depletion medium for 16 h and starved in methionine- and sterol-depletion medium for 1 h. Newly synthesized proteins were labelled by treating cells in the presence or absence of 50 µM L-azidohomoalanine in methionine- and sterol-depletion medium for 4 h. Cells were then incubated in maintenance medium containing 2 mM L-methionine and 1 µM NB-598 for the indicated times. Labelled proteins were conjugated with biotin and isolated by streptavidin pulldown. Graph depicts trunSM levels as a proportion of total SM levels at each timepoint. Data presented as mean ± half-range from $n = 2$ independent experiments.

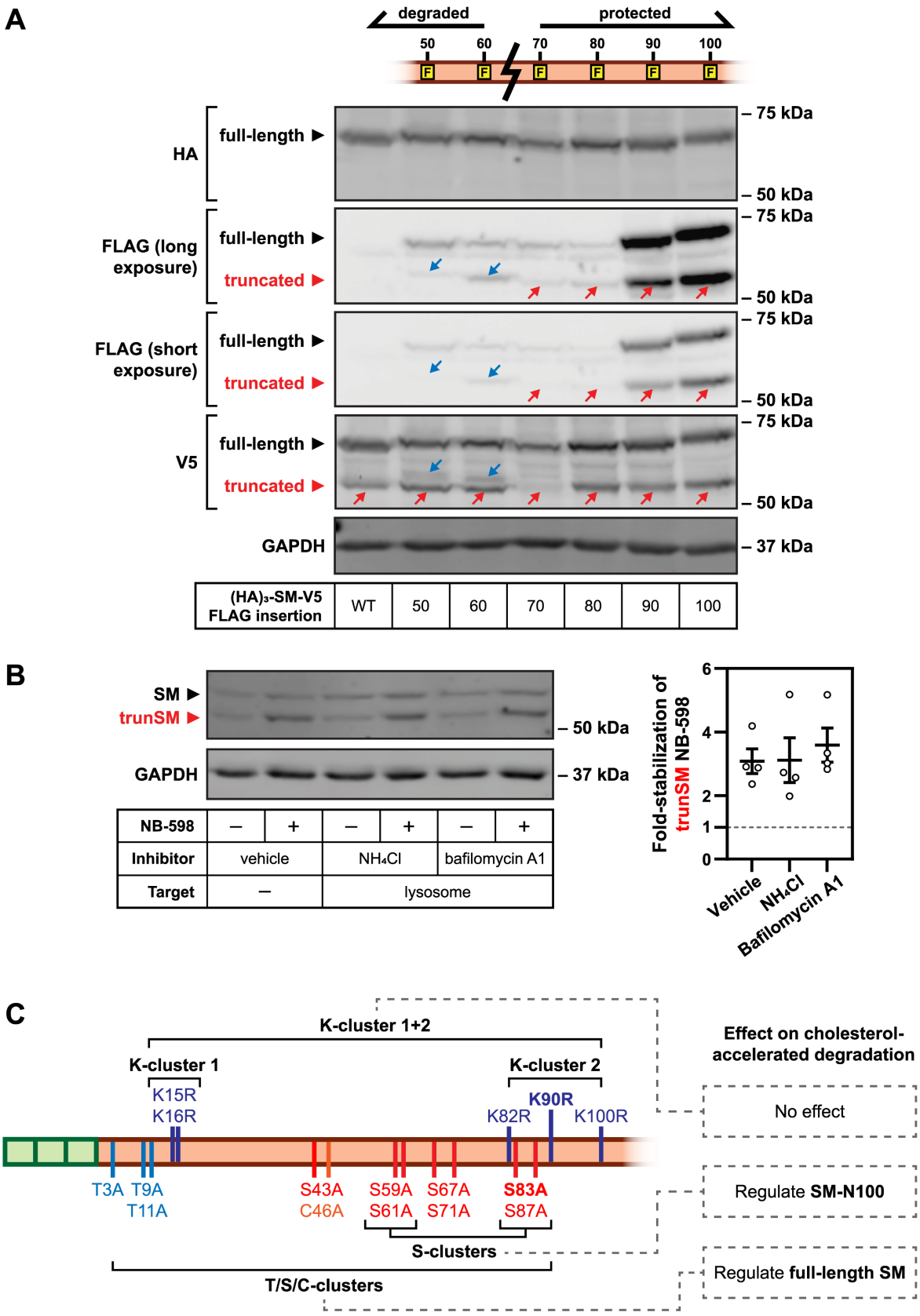
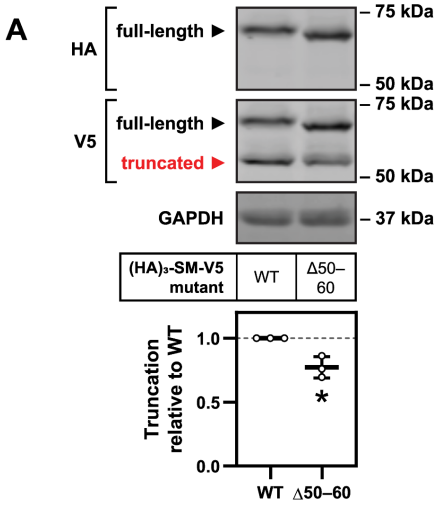

A
degraded
protected
50
60
70
80
90
100
F
HA
full-length ▶
FLAG (long exposure)
truncated ▶
FLAG (short exposure)
V5
– 75 kDa
– 50 kDa
GAPDH
– 37 kDa
(HA)3-SM-V5 FLAG insertion
WT
B
SM ▶
trunSM ▶
NB-598
Inhibitor
Target
vehicle
NH4Cl
bafilomycin A1
lysosome
Fold-stabilization of trunSM NB-598
Vehicle
Bafilomycin A1
C
K-cluster 1+2
K-cluster 1
K-cluster 2
K15R
K16R
K90R
K82R
K100R
T3A
T9A
T11A
S43A
C46A
S59A
S61A
S67A
S71A
S83A
S87A
S-clusters
T/S/C-clusters
Effect on cholesterol-accelerated degradation
No effect
Regulate SM-N100
Regulate full-length SM

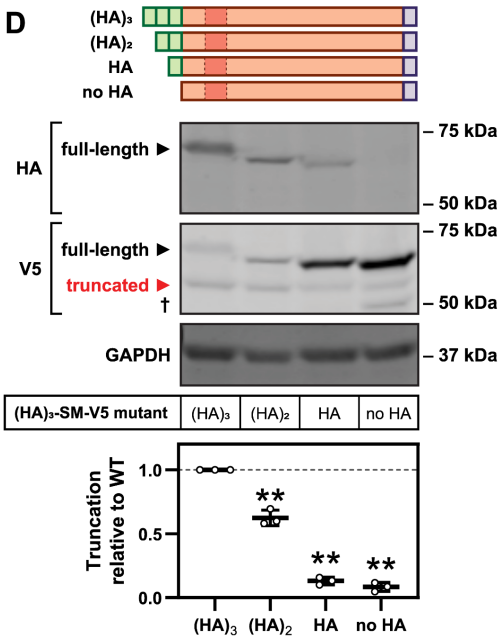
Figure S2. Related to Fig. 3

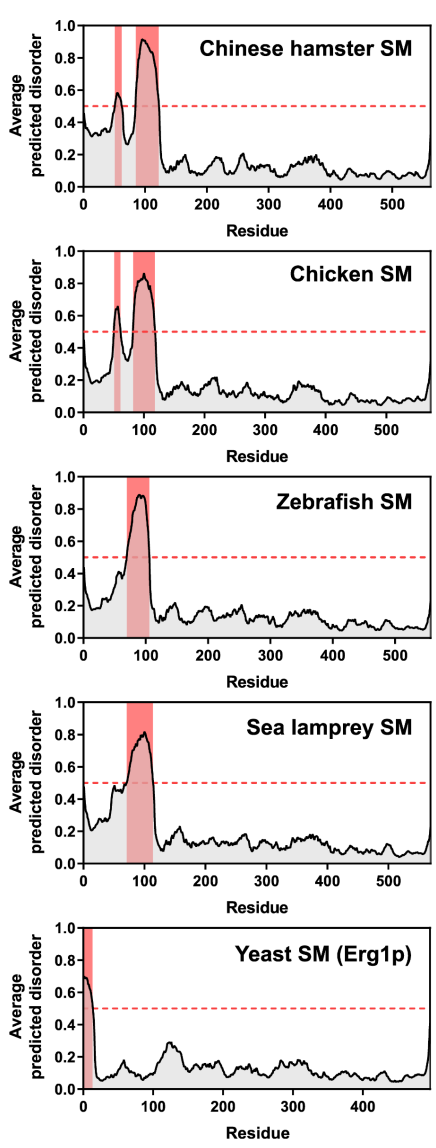
(A) The estimated SM truncation site is between residues 60 and 70. HEK293T cells were transfected with the indicated constructs for 24 h, treated with 1 μM NB-598 for 16 h, and then treated with 20 μM MG132 for 8 h. Immunoblot is representative of $n \geq 2$ independent experiments. Red arrows indicate fragments corresponding to trunSM, and blue arrows indicate additional FLAG-tagged fragments that do not correspond to trunSM.

(B) SM truncation does not depend on the lysosome. HEK293T cells were treated with 20 mM ammonium chloride (NH_4Cl) or 10 nM bafilomycin A1, in the presence or absence of 1 μM NB-598, for 8 h. Graph depicts densitometric quantification of trunSM stabilization by NB-598. Data presented as mean ± SEM from $n = 4$ independent experiments.

(C) Schematic of putative ubiquitination sites within the SM-N100 domain. Lysine residues are not required for cholesterol-induced degradation of SM or SM-N100 [5, 20]. Serine residues are required for maximal cholesterol-induced degradation of SM-N100 [20], while clusters of threonine, cysteine and serine residues are required for maximal cholesterol-induced degradation of full-length SM [20]. Bolded residues indicate known ubiquitination sites [20, 27].


A
HA
full-length ▶
– 75 kDa
– 50 kDa
V5
full-length ▶
truncated ▶
– 75 kDa
– 50 kDa
GAPDH
– 37 kDa
(HA)3-SM-V5 mutant
WT
Δ50–60
Truncation relative to WT
1.0
0.5
0.0
*
WT Δ50–60


D
(HA)3
(HA)2
HA
no HA
HA
full-length ▶
– 75 kDa
– 50 kDa
V5
full-length ▶
truncated ▶
†
– 75 kDa
– 50 kDa
GAPDH
– 37 kDa
(HA)3-SM-V5 mutant
(HA)3
(HA)2
HA
no HA
Truncation relative to WT
1.0
0.5
0.0
**
**
**
(HA)3 (HA)2 HA no HA


B
Chinese hamster SM
Chicken SM
Zebrafish SM
Sea lamprey SM
Yeast SM (Erg1p)
Average predicted disorder
1.0
0.8
0.6
0.4
0.2
0.0
0
100
200
300
400
500
Residue

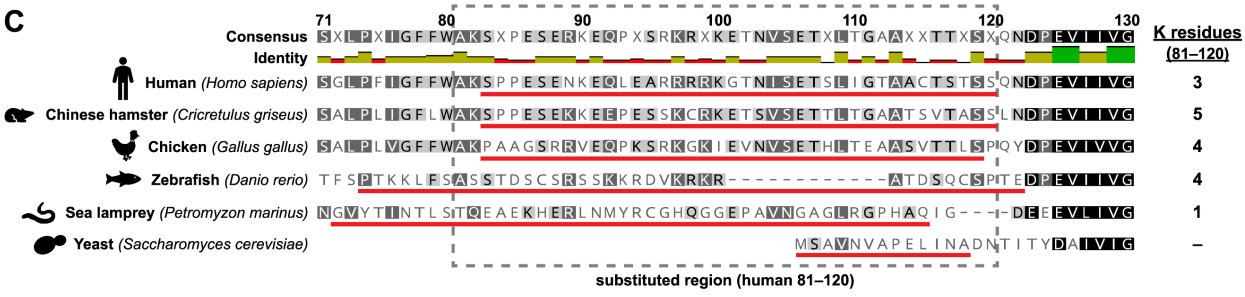

C
71
80
90
100
110
120
130
Consensus
Identity
Human (Homo sapiens)
Chinese hamster (Cricretulus griseus)
Chicken (Gallus gallus)
Zebrafish (Danio rerio)
Sea lamprey (Petromyzon marinus)
Yeast (Saccharomyces cerevisiae)
K residues (81–120)
3
5
4
4
1
–
substituted region (human 81–120)

Figure S3. Related to Fig. 4

(A) A low-complexity sequence within the SM-N100 domain has a small effect on SM truncation. HEK293T cells were transfected with the indicated constructs for 24 h and refreshed in maintenance medium for 24 h. Graph depicts densitometric quantification of truncation normalized to the wild-type (WT) construct, which was set to 1 (dotted line). Data presented as mean ± SEM from $n = 3$ independent experiments (*, $p \leq 0.05$, two-tailed paired t-test vs. WT).

(B) The intrinsic disorder of the SM 81–120 region is highly conserved amongst SM orthologues. Intrinsically disordered regions (red) are indicated for SM orthologues from Chinese hamster (*Cricetulus griseus*), chicken (*Gallus gallus*), zebrafish (*Danio rerio*), sea lamprey (*Petromyzon marinus*) and yeast (*Saccharomyces cerevisiae*).

(C) The sequence of the SM 81–120 region is poorly conserved amongst SM orthologues. Alignment of human SM residues 71–130 with SM orthologues from the indicated species. Red bars indicate regions of intrinsic disorder, and grey dashed box indicates regions that were substituted into SM constructs in Fig. 4C.

(D) Removal of HA tags from the SM N-terminus affects truncation. HEK293T cells were transfected with the indicated constructs for 24 h and refreshed in maintenance medium for 24 h. Graph depicts densitometric quantification of truncation normalized to the wild-type (WT) construct, which was set to 1 (dotted line). Data presented as mean ± SEM from $n = 3$ independent experiments (**, $p \leq 0.01$, two-tailed paired t-test vs. WT). Dagger indicates a non-trunSM fragment.

Table S1. Plasmids used for transfection

Plasmid	Description
totalSQLE qRT-PCR standard	pGL3-Basic vector containing a *totalSQLE* (NM_003129.4, exon 7) qRT-PCR amplicon sequence.
fullSQLE qRT-PCR standard	pGL3-Basic vector containing a *fullSQLE* (NM_003129.4, exon 1) qRT-PCR amplicon sequence.
trunSQLE1 qRT-PCR standard	pGL3-Basic vector containing a *trunSQLE1* (ENST00000523430.5, exon 1) qRT-PCR amplicon sequence.
trunSQLE2 qRT-PCR standard	pGL3-Basic vector containing a *trunSQLE2* (XM_011517246.2, exon 1) qRT-PCR amplicon sequence.
pCMV-$(HA)_3$-SM-V5	pcDNA3.1/V5-His TOPO vector containing the coding sequence of human SM (NM_003129.4) fused with three N-terminal HA epitope tags and C-terminal V5 and 6×His epitope tags, under the transcriptional control of a constitutive CMV promoter. Generated previously in our laboratory by Dr Julian Stevenson.
pCMV-$(HA)_3$-SM-V5 FLAG50	pCMV-$(HA)_3$-SM-V5 containing a FLAG epitope tag insertion after SM residue 50.
pCMV-$(HA)_3$-SM-V5 FLAG60	pCMV-$(HA)_3$-SM-V5 containing a FLAG epitope tag insertion after SM residue 60.
pCMV-$(HA)_3$-SM-V5 FLAG70	pCMV-$(HA)_3$-SM-V5 containing a FLAG epitope tag insertion after SM residue 70.
pCMV-$(HA)_3$-SM-V5 FLAG80	pCMV-$(HA)_3$-SM-V5 containing a FLAG epitope tag insertion after SM residue 80.
pCMV-$(HA)_3$-SM-V5 FLAG90	pCMV-$(HA)_3$-SM-V5 containing a FLAG epitope tag insertion after SM residue 90.
pCMV-$(HA)_3$-SM-V5 FLAG100	pCMV-$(HA)_3$-SM-V5 containing a FLAG epitope tag insertion after SM residue 100.
pTK-SM-V5 ΔN60	pcDNA3.1/V5-His TOPO vector containing the coding sequence of human SM fused with C-terminal V5 and 6×His epitope tags, under the transcriptional control of a TK promoter (pTK-SM-V5, generated previously in our laboratory [5]) and lacking the N-terminal 60 amino acids of SM.
pTK-SM-V5 ΔN65	pTK-SM-V5 lacking the N-terminal 65 amino acids of SM.
pTK-SM-V5 ΔN70	pTK-SM-V5 lacking the N-terminal 70 amino acids of SM.
pCMV-$(HA)_3$-SM-V5 K15R	pCMV-$(HA)_3$-SM-V5 containing an SM K15R substitution.
pCMV-$(HA)_3$-SM-V5 K16R	pCMV-$(HA)_3$-SM-V5 containing an SM K16R substitution.
pCMV-$(HA)_3$-SM-V5 K82R	pCMV-$(HA)_3$-SM-V5 containing an SM K82R substitution.

Plasmid	Description
pCMV-$(HA)_3$-SM-V5 K90R	pCMV-$(HA)_3$-SM-V5 containing an SM K90R substitution.
pCMV-$(HA)_3$-SM-V5 K100R	pCMV-$(HA)_3$-SM-V5 containing an SM K100R substitution.
pCMV-$(HA)_3$-SM-V5 K-cluster 1	pCMV-$(HA)_3$-SM-V5 containing SM K15R and K16R substitutions.
pCMV-$(HA)_3$-SM-V5 K-cluster 2	pCMV-$(HA)_3$-SM-V5 containing SM K82R, K90R and K100R substitutions.
pCMV-$(HA)_3$-SM-V5 K-cluster 1/2	pCMV-$(HA)_3$-SM-V5 containing SM K15R, K16R, K82R, K90R and K100R substitutions.
pCMV-$(HA)_3$-SM-V5 S-clusters	pCMV-$(HA)_3$-SM-V5 containing SM S59A, S61A, S83A and S97A substitutions.
pCMV-$(HA)_3$-SM-V5 T/S/C-clusters	pCMV-$(HA)_3$-SM-V5 containing SM T3A, T9A, T11A, S43A, C46A, S59A, S61A, S67A, S71A, S83A and S87A substitutions.
pCMV-$(HA)_3$-SM-V5 Δ50–60	pCMV-$(HA)_3$-SM-V5 containing a deletion of SM residues 50–60.
pCMV-$(HA)_3$-SM-V5 Δ81–120	pCMV-$(HA)_3$-SM-V5 containing a deletion of SM residues 81–120.
pCMV-$(HA)_3$-SM-V5 Δ91–110	pCMV-$(HA)_3$-SM-V5 containing a deletion of SM residues 91–110.
pCMV-$(HA)_3$-SM-V5 dup81–120	pCMV-$(HA)_3$-SM-V5 containing a tandem duplication of SM residues 81–120.
pCMV-$(HA)_3$-SM-V5 (Chinese hamster)	pCMV-$(HA)_3$-SM-V5 containing a substitution of SM residues 81–120 with *Cricetulus griseus* SM residues 83–122.
pCMV-$(HA)_3$-SM-V5 (chicken)	pCMV-$(HA)_3$-SM-V5 containing a substitution of SM residues 81–120 with *Gallus gallus* SM residues 80–119.
pCMV-$(HA)_3$-SM-V5 (zebrafish)	pCMV-$(HA)_3$-SM-V5 containing a substitution of SM residues 81–120 with *Danio rerio* SM residues 77–104.
pCMV-$(HA)_3$-SM-V5 (sea lamprey)	pCMV-$(HA)_3$-SM-V5 containing a substitution of SM residues 81–120 with *Petromyzon marinus* SM residues 80–116.
pCMV-$(HA)_3$-GAr-SM-V5	pCMV-$(HA)_3$-SM-V5 containing an insertion of a glycine-alanine repeat sequence (AGAGGGAGAGGAGGAGGAGAGGAGAGGGAG) from the Epstein-Barr virus nuclear antigen-1.
pCMV-$(HA)_3$-$(GAr)_2$-SM-V5	pCMV-$(HA)_3$-SM-V5 containing an insertion of two glycine-alanine repeat sequences from the Epstein-Barr virus nuclear antigen-1.
pCMV-$(HA)_3$-DHFR-SM-V5	pCMV-$(HA)_3$-SM-V5 containing an insertion of the coding sequence of human dihydrofolate reductase (DHFR, NM_000791.4).
pCMV-$(HA)_2$-SM-V5	pCMV-$(HA)_3$-SM-V5 containing a deletion of one HA epitope tag.

Plasmid	Description
pCMV-HA-SM-V5	pCMV-$(HA)_3$-SM-V5 containing a deletion of two HA epitope tags.
pCMV-SM-V5	pcDNA3.1/V5-His TOPO vector containing the coding sequence of human SM (NM_003129.4) fused with C-terminal V5 and 6×His epitope tags, under the transcriptional control of a constitutive CMV promoter. Generated previously in our laboratory [5].
pCMV-$(HA)_3$-SM-V5 Y195F	pCMV-$(HA)_3$-SM-V5 containing an SM Y195F substitution.
pCMV-$(HA)_3$-SM-V5 Q207A	pCMV-$(HA)_3$-SM-V5 containing an SM Q207A substitution.
pCMV-$(HA)_3$-SM-V5 F224A	pCMV-$(HA)_3$-SM-V5 containing an SM F224A substitution.
pCMV-$(HA)_3$-SM-V5 D272A	pCMV-$(HA)_3$-SM-V5 containing an SM D272A substitution.
pCMV-$(HA)_3$-SM-V5 Y335F	pCMV-$(HA)_3$-SM-V5 containing an SM Y335F substitution.
pCMV-$(HA)_3$-SM-V5 Y365A	pCMV-$(HA)_3$-SM-V5 containing an SM Y365A substitution.
pCMV-$(HA)_3$-SM-V5 T417S	pCMV-$(HA)_3$-SM-V5 containing an SM T417S substitution.
pTK-SM-N100-GFP-V5	pcDNA3.1/V5-His TOPO vector containing the coding sequence of human SM-N100 (NM_003129.4) fused with green fluorescent protein (GFP) and C-terminal V5 and 6×His epitope tags, under the transcriptional control of a constitutive TK promoter. Generated previously by our laboratory [5].
pCMV-SM-V5 ΔN65	pCMV-SM-V5 lacking the N-terminal 65 amino acids of SM.

Table S2. Primers used for qRT-PCR and DNA cloning

Non-annealing nucleotides for DNA insertions, deletions and substitutions are indicated in lowercase. Abbreviations for cloning methods: OEC (overlap extension cloning) [70] PIPE (polymerase incomplete primer extension cloning) [68]; SLIC (sequence- and ligation-independent cloning) [69].

DNA cloning primer pair		Primer sequence (5′–3′)	Method
totalSQLE qRT-PCR standard	Forward	cattccggtactgttggtaaagccaccGCTTCCTTCCTCCTTCATCAGTG	OEC (amplified from HEK293T cDNA and used to extend the pGL3-Basic plasmid)
	Reverse	ggccggccgccccgactctagaaGCAACAGTCATTCCTCCACCA	
fullSQLE qRT-PCR standard	Forward	cattccggtactgttggtaaagccaccCCAGTTCGCCCTCTTCTCGG	
	Reverse	ggccggccgccccgactctagaaATTGGTTCCTTTTCTGCGCCTC	
trunSQLE1 qRT-PCR standard	Forward	cattccggtactgttggtaaagccaccCCCGCGAGGGATGCTGCG	
	Reverse	ggccggccgccccgactctagaaCTTCTGGGTCATTCTGAGAAGATG	
trunSQLE2 qRT-PCR standard	Forward	cattccggtactgttggtaaagccaccGGGTAAGGATTGGATTTGTGCC	
	Reverse	ggccggccgccccgactctagaaTGGGTCATTCTGAGAAGATGTTGA	
FLAG50 insertion	Forward	gactacaaagacgatgacgacaagGGGGGTCTCCTCGGGC	PIPE
	Reverse	cttgtcgtcatcgtctttgtagtcGTTTCGGTGGCGACAGC	
FLAG60 insertion	Forward	gactacaaagacgatgacgacaagTCCCAGTTCGCCCTCTTCTCG	
	Reverse	cttgtcgtcatcgtctttgtagtcGCCGCTCTGCTGGCGCC	
FLAG70 insertion	Forward	gactacaaagacgatgacgacaagTCAGGCCTGCCTTTCATTGGC	
	Reverse	cttgtcgtcatcgtctttgtagtcGAGAATATCCGAGAAGAGGGCGAAC	
FLAG80 insertion	Forward	gactacaaagacgatgacgacaagGCCAAATCCCCCCCTGAATC	
	Reverse	cttgtcgtcatcgtctttgtagtcCCAGAAGAAGCCAATGAAAGGC	
FLAG90 insertion	Forward	gactacaaagacgatgacgacaagGAGCAGCTCGAGGCCAGGAG	
	Reverse	cttgtcgtcatcgtctttgtagtcCTTATTTTCTGATTCAGGGGGGGATTTG	
FLAG100 insertion	Forward	gactacaaagacgatgacgacaagGGAACCAATATTTCAGAAACAAGCTTAATAGG	
	Reverse	cttgtcgtcatcgtctttgtagtcTTTTCTGCGCCTCCTGGCC	
ΔN60	Forward	ggaattgcccttatgTCCCAGTTCGCCCTCTTCTCG	PIPE
	Reverse	AAGGGCAATTCCACCACACTGGACTAGTGGATC	
ΔN65	Forward	ggaattgcccttatgTTCTCGGATATTCTCTCAGGCCTGC	
	Reverse	AAGGGCAATTCCACCACACTGGACTAGTGGATC	
ΔN70	Forward	ggaattgcccttatgTCAGGCCTGCCTTTCATTGGC	
	Reverse	AAGGGCAATTCCACCACACTGGACTAGTGGATC	
BGHR2	Reverse	GCGATGCAATTTCCTCATTT	OEC (generic reverse primer for point mutations)
K15R	Forward	GCCACTTTCACCTATTTTTATAgGAAGTTCGGGGACTTCATCAC	OEC (with BGHR2 reverse)

DNA cloning primer pair		Primer sequence (5′–3′)	Method
K16R	Forward	GCCACTTTCACCTATTTTTATAAGAgGTTCGGGGACT TCATCAC	OEC (with BGHR2 reverse)
K82R	Forward	GGCTTCTTCTGGGCCAggTCCCCCCCTGAATCAG	
K90R	Forward	CAGAAAATAgGGAGCAGCTC	
K100R	Forward	CCAGGAGGCGCAGAAggGGAACCAATATTTCAGAAA CAAG	
K15R / K16R	Forward	Appropriate mutagenic primer/s	
K82R / K90R / K100R			
K15R / K16R / K82R / K90R / K100R			
S-clusters	Vector: Forward Reverse	GGAACCAATATTTCAGAAACAAGCTTAATAG AGCGTAATCTGGAACGTCATATG	PIPE (insert amplified from SM-N100 plasmids generated in [20])
T/S/C-clusters	Insert: Forward Reverse	gttccagattacgctTGGACTTTTCTGGGCATTGCC TGAAATATTGGTTCCTTTTCTGCGC	
Δ50–60	Forward Reverse	TACCGCTGTCGCCACCGATCCCAGTTCGCCCTCTTCT CG TCGGTGGCGACAGCGGTAGGAGAGCAC	PIPE (primers designed in [17])
Δ81–120	Forward Reverse	attggcttcttctggCAGAATGACCCAGAAGTTATCATCG ttctgggtcattctgCCAGAAGAAGCCAATGAAAGGCAG	PIPE
Δ91–110	Forward Reverse	gaatcagaaaataagGGAACAGCTGCCTGTACATCAAC acaggcagctgttcCCTTATTTTCTGATTCAGGGGGG	
dup81–120	Vector: Forward Reverse Insert: Forward Reverse	AAATCCCCCCCTGAATCAGAAAATAAGGAG GGCCCAGAAGAAGCCAATGAAAGG ggcttcttctgggccAAATCCC ttcagggggggatttCTGAGAAGATGTTGATGTACAGGCAGC	SLIC (insert amplified from pCMV-HA_3-SM-V5)
81–120 substitution (Chinese hamster)	Forward Reverse	cctgcctttcattggcttcttctggGCCAAGTCACCCCCTGAG cacgatgataacttctgggtcattctgAGAAGATGCTGTTACTGAGG TAGC	OEC (amplified from SM-N100-GFP-V5 plasmids generated in [17] and used to extend the pCMV-HA3-SM-V5 plasmid)
81–120 (chicken)	Forward Reverse	cctgcctttcattggcttcttctggGCCAAGCCCGCCGC cacgatgataacttctgggtcattctgcggagacaatgtcgttacagaagccgcctcag tgaggtgggtttcggacacgttcacCTCGATCTTGCCCTTCCTG C	
81–120 substitution (zebrafish)	Forward Reverse	cctgcctttcattggcttcttctggGCCTCTAGCACCGATAGCTG cacgatgataacttctgggtcattctgtgggctacattgGGAATCTGTGGCT CTCTTCCG	
81–120 substitution (sea lamprey)	Forward Reverse	cctgcctttcattggcttcttctggACCCAGGAAGCAGAAAAACAC G cacgatgataacttctgggtcattctgaccaatttgggcgtgaggcccccggaggccc gcaccattgacggccggCTCTCCCCCCTGGTGC	

DNA cloning primer pair		Primer sequence (5′–3′)	Method
pCMV-(HA)$_3$-GAr-SM-V5	<u>Vector:</u> Forward Reverse <u>GAr generation:</u> Forward Reverse <u>Insert:</u> Forward Reverse	TGGACTTTTCTGGGCATTGCC AGCGTAATCTGGAACGTCATATG GCTGGAGCAGGCGGTGGAGCAGGTGCTGGAGGTGC AGGTGGAGCAGGCGGTGCAGGAGCA ACCTGCTCCACCTCCAGCACCTGCACCACCTGCTCC TGCACCGCCTGCTCCACCTGCACC tccatatgacgttccagattacgctGCTGGAGCAGGCGGTGGAGC gaaagtggcaatgcccagaaaagtccaACCTGCTCCACCTCCAGCA	SLIC ('GAr generation' primers extended and used as template for insert amplification)
pCMV-(HA)$_3$-(GAr)$_2$-SM-V5			
pCMV-(HA)$_3$-DHFR-SM-V5	<u>Vector:</u> Forward Reverse <u>Insert:</u> Forward Reverse	TGGACTTTTCTGGGCATTGCC AGCGTAATCTGGAACGTCATATG gttccagattacgctGTTGGTTCGCTAAACTGCATCG gcccagaaaagtccAATCATTCTTCTCATATACTTCAAATTT GTAC	PIPE (insert amplified from HEK293T cDNA)
ΔHA	Forward Reverse	cctgactatgcgggCTATCCATATGACGTTCCAGATTAC aacgtcatatggataGCCCGCATAGTCAGGAACAT	PIPE
Δ(HA)$_2$	Forward Reverse	ggaattgcccttatgTATCCATATGACGTTCCAGATTAC aacgtcatatggataCATAAGGGCAATTCCACCACA	PIPE
Q168A	Forward	GTTGGAGAATTCCTGgccCCGGGTGGTTATC	OEC (with BGHR2 reverse)
Y195A	Forward	CCAGGTTGTAAATGGTgcCATGATTCATGATCAGG	
Y195F	Forward	CCAGGTTGTAAATGGTTtCATGATTCATGATCAGG	
Q207A	Forward	AAAGCAAATCAGAGGTTgccATTCCTTACCCTCTGTC	
F224A	Forward	CAGAGTGGAAGAGCTgcCCATCACGGAAGATTC	
D272A	Forward	GGATAAAGAGACTGGAGccATCAAGGAACTCCATGC	
Y335F	Forward	GTCCAGTTCTCATCTtCCAGATTTCATCCAG	
Y365A	Forward	GAATACATGGTTGAAAAAATTgcCCCACAAATACCT GATC	
T417S	Forward	AATATGAGGCATCCACTTtCTGGTGGAGGAATGACT G	

Table S3. siRNA used for transfection

siRNA	Description
SIC001	MISSION® universal negative control #1
SASI_Hs01_00149248	Targets human *SQLE* exon 9 (NM_003129)
SASI_Hs01_00149256	Targets human *SQLE* exon 1 (NM_003129)
SASI_Hs01_00095058	Targets human *UBE2J2* (NM_058167)
SASI_Hs01_00105239	Targets human *MARCHF6* (NM_005885)
SASI_Hs01_00118726	Targets human *VCP* (NM_007126)
CGGCUUUGGCGAC-GGUUCUdTdT (sense)	Targets Chinese hamster (*Cricetulus griseus*) *Sqle* (XM_003515960.5)

Table S4. Primers used for qRT-PCR

All primer pairs were designed during this study.

qRT-PCR primer pair		Primer sequence (5′–3′)
PBGD	Forward	AGGTTGCCATCCTCAGTCGTC
	Reverse	TTGCCACCACACTGTCCGTC
totalSQLE	Forward	GCTTCCTTCCTCCTTCATCAGTG
	Reverse	GCAACAGTCATTCCTCCACCA
fullSQLE	Forward	CCAGTTCGCCCTCTTCTCGG
	Reverse	ATTGGTTCCTTTTCTGCGCCTC
trunSQLE1	Forward	CCCGCGAGGGATGCTGCG
	Reverse	CTTCTGGGTCATTCTGAGAAGATG
trunSQLE2	Forward	GGGTAAGGATTGGATTTGTGCC
	Reverse	TGGGTCATTCTGAGAAGATGTTGA