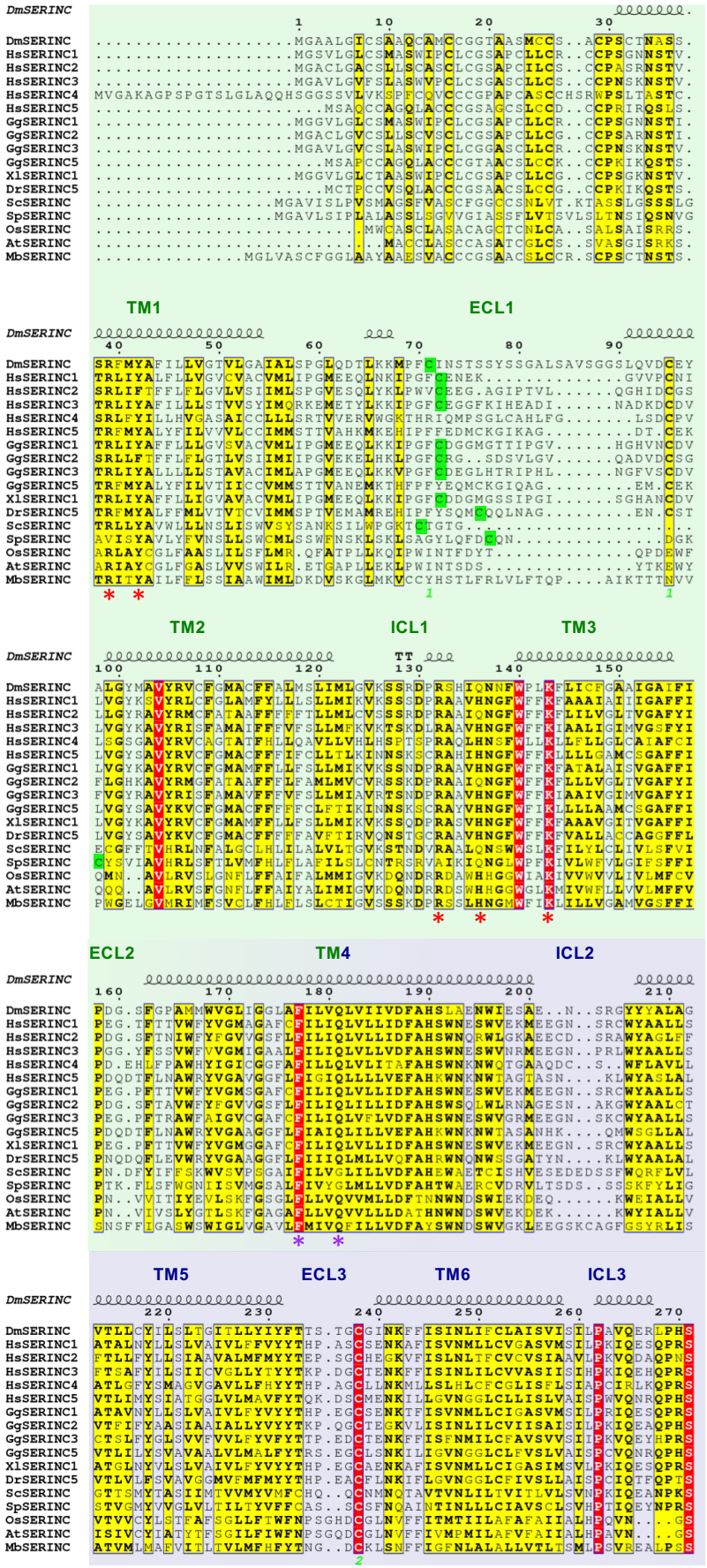
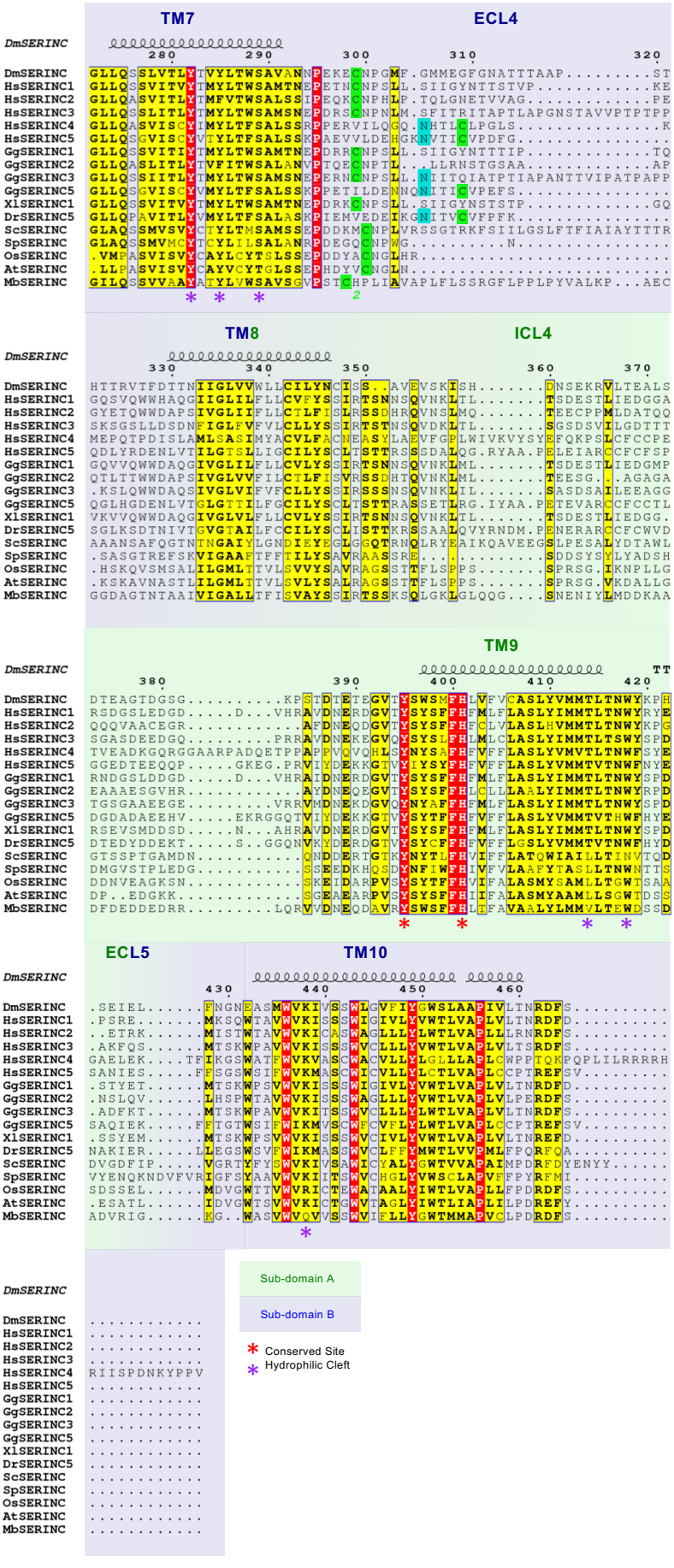
Supplementary Information Table 1 | a, Amino acid sequence alignment of SERINC orthologs: *Drosophila melanogaster* (Dm), *Homo sapiens* (Hs), *Gallus gallus* (Gg), *Xenopus laevis* (Xl), *Danio rerio* (Dr), *Saccharomyces cerevisiae* (Sc), *Saccharomyces pombe* (Sp), *Oryza sativa* (Os), *Arabidopsis thaliana* (At), *Monosiga brevicollis* (Mb). The spans of trans-membrane helices (TM1-10), extracellular loops (ECL1-5) and intracellular loops (ICL1-4) are indicated. Residues residing in subdomains A and B are shaded in green and blue respectively. Disulphide bonds are numbered 1 and 2 in lime green and Cys residues likely involved in the disulphide bonds in variable loops are highlighted in lime green; N-glycosylation sites are highlighted in cyan, alignment made using ESPript[61]. **b**, Amino acid sequence identity matrix. **c**, Phylogenetic tree for SERINC sequences used in alignment.

a



Supplementary Information Table 1 |

a cont.

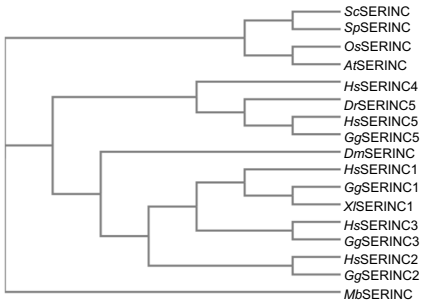


Supplementary Information Table 1 |

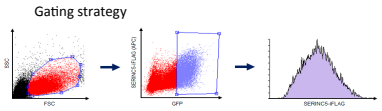
b

	Sc SERINC	*Sp* SERINC	*Os* SERINC	*At* SERINC	*Hs* SERINC4	*Dr* SERINC5	*Hs* SERINC5	*Gg* SERINC5	*Mb* SERINC	*Dm* SERINC	*Hs* SERINC1	*Gg* SERINC1	*Xl* SERINC2	*Hs* SERINC3	*Gg* SERINC3	*Hs* SERINC2	*Gg* SERINC2
***Sc* SERINC**	100	39.53	27.46	27.11	23.65	26.29	26.65	26.34	30.12	27.63	33.09	32.77	31.64	32.37	31.4	27.32	29.17
***Sp* SERINC**	39.53	100	25	24.68	23.22	25.19	25.31	23.83	28.5	28.71	28.5	27.23	28.19	30.12	30.12	26.39	27.6
***Os* SERINC**	27.46	25	100	70.66	22.79	24.69	24.5	25.68	29.17	26.23	28.71	30.58	30.17	31.63	31.14	29.1	27.45
***At* SERINC**	27.11	24.68	70.66	100	23.75	25.69	27.02	25.44	31.75	31	31.02	31.93	32.26	32.51	31.27	28.68	27.75
***Hs* SERINC4**	23.65	23.22	22.79	23.75	100	37.14	41.01	40.04	28.16	31.29	35.31	35.36	35.21	31.03	33.78	35.31	33.56
***Dr* SERINC5**	26.29	25.19	24.69	25.69	37.14	100	61.14	63.83	30.61	33.03	40.05	41.28	40.69	37.5	38.95	38.28	37.94
***Hs* SERINC5**	26.65	25.31	24.5	27.02	41.01	61.14	100	76.14	32.12	36.34	40.23	41.71	41.57	38.13	39.82	37.91	37.79
***Gg* SERINC5**	26.34	23.83	25.68	25.44	40.04	63.83	76.14	100	33.86	36.11	37.59	39.54	39.63	36.67	39.04	36.98	36.15
***Mb* SERINC**	30.12	28.5	29.17	31.75	28.16	30.61	32.12	33.86	100	32.39	38.34	38.33	39.07	37.85	39.66	34.3	35.89
***Dm* SERINC**	27.63	28.71	26.23	31	31.29	33.03	36.34	36.11	32.39	100	43.4	44.62	44.71	43.01	42.89	42.35	44.17
***Hs* SERINC1**	33.09	28.5	28.71	31.02	35.31	40.05	40.23	37.59	38.34	43.4	100	86.28	83.59	60.84	65.85	55.48	55.08
***Gg* SERINC1**	32.77	27.23	30.58	31.93	35.36	41.28	41.71	39.54	38.33	44.62	86.28	100	88.7	62.17	68.63	55.73	56.79
***Xl* SERINC1**	31.64	28.19	30.17	32.26	35.21	40.69	41.57	39.63	39.07	44.71	83.59	88.7	100	63.4	68.56	55.41	55.68
***Hs* SERINC3**	32.37	30.12	31.63	32.51	31.03	37.5	38.13	36.67	37.85	43.01	60.84	62.17	63.4	100	69.28	51.98	56.35
***Gg* SERINC3**	31.4	30.12	31.14	31.27	33.78	38.95	39.82	39.04	39.66	42.89	65.85	68.63	68.56	69.28	100	55.19	55.13
***Hs* SERINC2**	27.32	26.39	29.1	28.68	35.31	38.28	37.91	36.98	34.3	42.35	55.48	55.73	55.41	51.98	55.19	100	66.82
***Gg* SERINC2**	29.17	27.6	27.45	27.75	33.56	37.94	37.79	36.15	35.89	44.17	55.08	56.79	55.68	56.35	55.13	66.82	100

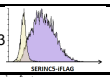
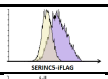
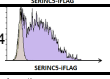
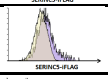
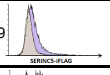
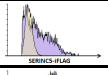
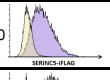
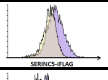
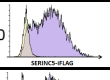
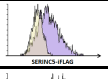
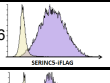
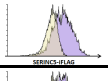
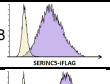
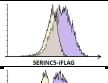
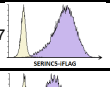
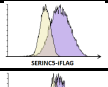
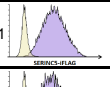
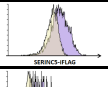
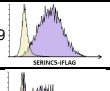
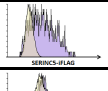
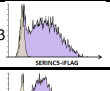
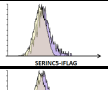
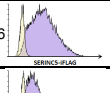
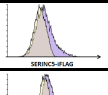
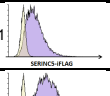
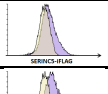
c

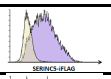
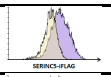
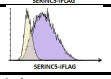
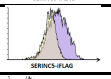
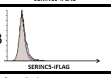
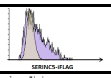
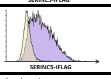
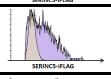
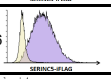
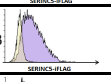
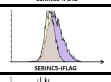
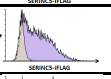
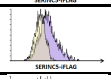
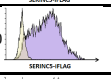
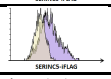
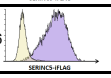
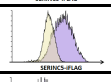
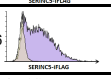
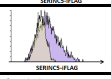
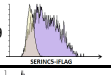
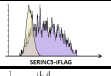
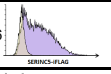
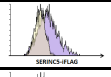
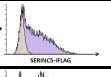
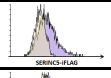


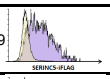
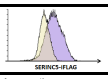
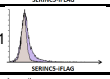
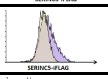
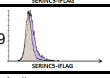
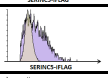
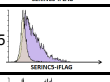
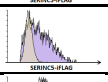
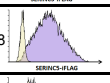
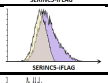
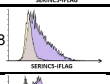
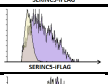
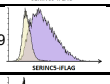
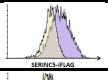
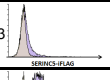
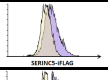
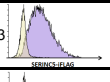
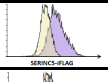
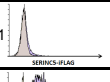
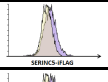
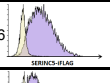
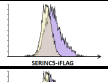
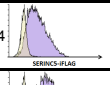
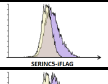
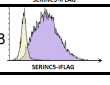
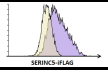
Supplementary Information Table 2 | Phenotypic characterisation of SERINC5-iFLAG-HA variants. Residual restriction activity, total and surface expression were investigated as described in Methods. Residual restriction activity: the average values of n replicates are shown as number and bars (t.r.: technical replicates; b.r.: biological replicates, bars represent s.d.). Residual surface and total expression: shown are representative histograms of HEK293T cells transfected with the different SERINC5-iFLAG-HA variants and overlaid on their respective negative controls. Numbers represent residual expression considering the expression of wild type SERINC5-iFLAG-HA in each experiment as 100%. The gating strategy for fluorescence acquisition is illustrated on the inset to the top right of the Table.



SERINC5-iFLAG-HA variant	Residual restriction (relative to WT)	Surface expression (relative to WT)	Total expression (relative to WT)	Additional information on the mutants		
Mutation abbreviation	**PBJ6-SERINC5-iFLAG-HA (500 ng)**	**pcDNA-SERINC5-iFLAG-HA (50 ng)**		**Original amino acid sequence**	**Mutant amino acid sequence**	**Comments**
WT	1.00 n=4, t.r.	1.00	1.00			
ΔN/mCAT	0.04	0.38	0.51	^{1}MSAQCCAGQLACCC GSAGCSLCCDCCPRIR^{3}	MGCKNLLGLGQQML RRKVVDCSREESRLSR CLNTYD	SERINC5/mCAT chimera
CC13LL	0.53 n=4, t.r.	1.07	0.99	^{13}CC^{14}	LL	
C22L/C23L	0.35 n=4, t.r.	0.43	0.40	^{22}CC^{23}	LL	
CC25LL	0.38 n=4, t.r.	0.69	0.29	^{25}CC^{26}	LL	
R36Q	0.26 n=4, t.r.	0.54	0.23			
Y39F	0.28 n=4, t.r.	0.38	0.60			
C73A	0.00 n=4, t.r.	0.42	0.56			
R119A	0.19 n=4, t.r.	0.35	0.35			
N124A	0.02 n=4, t.r.	0.08	0.18			
F128A	0.77 n=4, t.r.	0.78	0.97			
W127I	0.01 n=4, t.r.	0.06	0.11			
K130A	0.03 n=5, b.r.	0.03	0.28			
G162S	0.50 n=4, t.r.	1.30	1.44			
F165A	0.01 n=4, t.r.	0.06	0.19			
Q169A	0.06 n=3, b.r.	0.51	0.42			
E175A	0.33 n=4, t.r.	0.83	0.88			
Y178A	0.12 n=4, t.r.	0.23	0.95			
K179S	0.81 n=4, t.r.	0.79	0.37			

N181A	0.44 n=4, t.r.		1.03		1.66				
K182Q	0.81 n=4, t.r.		0.64		0.46				
W184I	0.53 n=4, t.r.		0.19		0.11				
ASN189EECDS	0.66 n=4, t.r.		0.70		0.65		^{89}ASN^{191}	EECDS	
Y195I	0.62 n=4, t.r.		0.80		0.79				
QK222EP	0.28 n=4, t.r.		1.96		2.35		^{222}QK^{223}	EP	
DS224SG	0.27 n=4, t.r.		1.38		2.10		^{224}DS^{225}	SG	
C226A	0.41 n=4, t.r.		4.57		1.84				
MEN227HEG	0.75 n=4, t.r.		1.81		1.36		^{227}MEN^{229}	HEG	
N229G	0.12 n=4, t.r.		0.69		0.39				
K230A	0.73 n=4, t.r.		0.83		0.39				
S249L	1.18 n=4, t.r.		0.86		0.46				
P250A	0.19 n=4, t.r.		0.21		0.31				
W251A	0.63 n=4, t.r.		1.01		0.87				
W251K	0.51 n=4, t.r.		0.98		0.97				
V252A	0.47 n=4, t.r.		0.88		1.56				
Q253A	0.41 n=6, b.r.		1.14		1.39				
N254D	0.44 n=4, t.r.		0.63		0.59				
R255A	1.06 n=4, t.r.		0.98		0.48				
Q256A	0.44 n=4, t.r.		0.94		0.54				
P257A	0.51 n=4, t.r.		1.11		1.20				
H258A	0.37 n=4, t.r.		1.24		0.78				
H258N	0.78 n=4, t.r.		1.08		0.72				
S259A	0.42 n=4, t.r.		1.61		1.19				
Q263A	0.40 n=7, b.r.		1.42		1.17				
Q253/H178	0.07 n=4, b.r.		1.55		1.21				

Q253/Q263A	0.18 n=4, t.r.		0.81		1.46			
Q253/S259A	0.11 n=4, t.r.		0.80		0.85			
Y270I	0.04 n=4, t.r.		0.03		0.10			
Y273F	0.28 n=4, t.r.		0.41		0.20			
S277A	0.18 n=4, t.r.		0.76		0.90			
G317A	0.41 n=4, t.r.		0.43		0.47			
C324M	0.70 n=4, t.r.		0.47		0.50			
Y327I	0.54 n=4, t.r.		0.70		0.65			
S328I	0.19 n=4, t.r.		1.36		0.93			
Y345I	1.07 n=3, b.r.		0.56		0.44			
K373R	0.63 n=4, t.r.		0.49		0.63			
Y380I	1.66 n=4, t.r.		0.56		0.52			
KK383RR	1.52 n=4, t.r.		0.37		0.42		^{383}KK^{384}	RR
K384D	0.67 n=4, t.r.		1.13		0.69			
Y388A	0.01 n=3, b.r.		0.04		0.09			
H394A	0.01 n=3, b.r.		0.04		0.26			
V396C	0.14 n=3, b.r.		1.29		1.44			
F397L	0.07 n=3, b.r.		0.89		2.32			
Y403F	0.38 n=4, t.r.		0.47		0.84			
W411A	0.00 n=4, t.r.		0.03		0.11			
W411F	0.01 n=4, t.r.		0.09		0.27			
NY413KP	0.10 n=3, b.r.		1.41		4.15		^{413}NY^{414}	KP
ES415GE	0.23 n=3, b.r.		1.15		5.06		^{415}ES^{416}	GE
AN417TR	0.22 n=4, t.r.		1.62		6.68		^{417}AN^{418}	TR
IE419KM	0.16 n=3, b.r.		2.35		6.52		^{419}IE^{420}	KM
E420M	0.22 n=4, t.r.		0.77		1.55			

W431I	0.17 n=4, t.r.		0.89		1.15				
K433A	0.05 n=3, b.r.		0.01		0.52				
W438I	0.02 n=4, t.r.		0.09		0.15				
Y444A	0.06 n=3, b.r.		0.25		0.22				
C446W	0.87 n=4, t.r.		1.58		0.80				
P451A	0.14 n=4, t.r.		0.58		0.53				
CC453LL	0.46 n=4, t.r.		0.79		1.49		^{453}CC^{454}	LL	
ECL1A	0.02 n=3, b.r.		0.03		0.49		^{54}MSTTVAHKMKEHIP FFEDMCKGIKAG^{79}	LSPGVESQLYKLP WVCEEGAGIPTVL QGH	ECL1 swap with SERINC2
ECL3A	0.16 n=6, b.r.		0.83		1.81		^{222}QKDSCMEN^{229}	EPSGCHEG	ECL3 swap with SERINC2
ECL4A	0.06 n=3, b.r.		0.01		0.07		^{282}KPAEVVLDEHGKNV TICVPDFGQDLYRDEN LVT^{314}	IPEDYKDDDDKDQ KCNPHLPTQLGNE TVVAGPEGYETQW WDAP	ECL4 swap with SERINC2
ECL4B	0.25 n=3, b.r.		0.96		0.62		^{282}KPAEVVLDEHGKNV TICVPDFGQDLYRDEN LVT^{314}	IPEQKCNPHLPTQL GNEDYKDDDDKDT VVAGPEGYETQW WDAPS	ECL4 swap with SERINC2
ECL5A	0.02 n=3, b.r.		0.44		0.53		^{412}FNYESANIESFFSGS WSIF^{430}	YKPGETRKMISTW TAV	ECL5 swap with SERINC2
ECL5B	0.03 n=6, b.r.		1.28		1.72		^{413}NYESANIE^{420}	KPGETRKM	ECL5 swap with SERINC2

SERINC5-iFLAG-HA variant	PBJ5-SERINC5-iFLAG-HA (100 ng)			Original amino acid sequence	Mutant amino acid sequence
WT	1.00 n=4, t.r.		1.00		1.00			
Y414P	0.55 n=4, t.r.		1.50		1.52			
Y414F	0.71 n=4, t.r.		1.42		1.58			
E415G	0.26 n=4, t.r.		1.17		0.33			
E415Q	0.49 n=4, t.r.		1.05		0.15			
Δ419I	0.36 n=4, t.r.		1.76		1.73		^{419}NIE^{420}	NE
Δ419IE	0.26 n=4, t.r.		1.98		2.62		^{419}NIES^{421}	NS
Δ421S	0.24 n=4, t.r.		1.38		1.32		^{420}ESF^{422}	EF

Supplementary Note 1

Extended Methods

Recombinant yeast strains and human cell lines for protein expression. DNA fragment encoding *Dm*SERINC (UniProt accession code Q9U6P4) codon-optimised for expression in budding yeast were cloned into integrative yeast expression vector pGC014 under control of the GAL promoter. Linearized constructs were introduced into *S. cerevisiae* JF1 cells[59] using lithium acetate, and stable transformants were selected on tryptophan-dropout media. A DNA fragment encoding human SERINC5 (UniProt accession code Q86VE9-4) was inserted into pQCXIP retroviral vector (Clontech) under control of the cytomegalovirus (CMV) promoter. Retroviral particles, produced by co-transfection of human embryonic kidney (HEK293T) cells with the resulting plasmid along with pCG-GAGPOL and pCG-VSVG[60], were used to infect a fresh culture of HEK293T cells. Stably transduced cells were selected with 1 μg/ml puromycin and then adapted to growth in suspension in EX CELL 293 medium (Sigma-Aldrich). All constructs used for recombinant protein expression were designed to produce full-length wild type proteins with a C-terminal extension (GSSGLEVLFQGPGSGGSAWSHPQFEKGGGSGGGSGGSAWSHPQFEK) containing human rhinovirus 14 3C protease recognition site (underlined) followed by a TwinStrep tag.

Cell lines used. Human embryonic kidney T cells (HEK293T) originate from the American Type Culture Collection (ATCC CRL-3216). TZM-bl-GFP cells were generated in house [2] and originate from TZM-bl, NIH AIDS Reagent Program cat. 8129. All cell lines were negative for mycoplasma infection, tested at in-house facilities, and were not authenticated.

***Dm*SERINC expression and purification.** Starter cultures of selected yeast strain were grown in YP medium containing 10 g/l yeast extract (Melford) and 20 g/l bactopeptone (Oxoid) in the presence of 2% (w/v) raffinose (Carbosynth) and 50 μg/l kanamycin (Melford) at 32°C, 250 rpm for 20 h. Next day, cells were diluted 1:125 in YP supplemented with 2% (w/v) galactose (Acros organics) and 50 μg/l kanamycin and grown in shake flasks at 32°C, 250 rpm for 24-30 h. Cells, harvested by centrifugation, were washed with phosphate buffered saline (PBS) and stored frozen at -80°C. Cell pellets, thawed and resuspended in core buffer (40 mM NaCl, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) - NaOH, pH 7.5, 0.5 mM Tris(2-carboxyethyl)phosphine (TCEP), 0.5 mM dithiothreitol (DTT)), and EDTA-free protease inhibitor mix (Pierce), were disrupted by passing through an Emulsiflex C5 (Avestin) three times at 30,000 psi. Lysates, supplemented with 1.5% n-dodecyl-β-D-maltoside (DDM, Anatrace), were gently mixed at 4°C for 4-6 h. Extracts were pre-cleared by centrifugation at 100,000 *g* for 1 h at 4°C, and supernatants were incubated with glutathione Sepharose (GE Healthcare) for 30 min at 4°C to absorb non-specific binding proteins. After gentle centrifugation, supernatants were incubated with StrepTactin Sepharose (IBA) overnight at 4°C. Following morning, the resin was extensively washed with core buffer supplemented with 0.05% lauryl maltose neopentyl glycol (LMNG, Anatrace) and protein eluted in the presence of 1 mg/ml *d*-Desthiobiotin (Sigma-Aldrich). Fractions containing *Dm*SERINC were pooled and concentrated by ultrafiltration using a 100-kDa cut-off membrane (Satorius). The protein was further purified by size exclusion chromatography through a Superdex 200 10/300 GL (GE Healthcare) column in core buffer plus 0.0003% LMNG. Fractions from the first peak, with elution volume 9.8

ml (*Dm*SERINC hexamer) were pooled, concentrated and re-injected into the column. Fractions from the resulting peak were used to prepare cryo-EM grids, or concentrated for all other experiments. Fractions from the second major peak with elution volume 12.5 ml (*Dm*SERINC monomer) were pooled and concentrated (Extended Data Fig. 3 a,b). After potential disulphide bonds were identified in the cryo-EM structure, the reducing agents TCEP and DTT were omitted from the core buffer for all subsequent experiments.

Production of SERINC5 in human cells. Continuous culture of HEK293T cells over-expressing human SERINC5 with a C-terminal TwinStrep tag was maintained in serum-free EX-CELL 293 media supplemented with 1 μg/ml puromycin in stirrer flasks at a density of 1-3x10^6 cells/ml. Cells, harvested by gentle centrifugation and washed in PBS, were stored frozen at -80°C. For purification, cell pellet from 1 L suspension culture was thawed and resuspended in 25 ml core buffer containing 100 mM NaCl, 10 mM HEPES-NaOH, pH 7.5, supplemented with 1% DDM and EDTA-free protease inhibitor mix. Cell lysates were gently mixed at 4°C for 2 h. After detergent-extraction, SERINC5 was purified following the protocol described above for *Dm*SERINC in the absence of reducing agents. For cryo-EM, SERINC5 was supplemented with 0.1 mM 1,2-dipalmitoyl-*sn*-glycero-3-phosphoserine (DPPS; Echelon Biosciences) at the affinity chromatography wash and elution stage, the protein was further purified by size exclusion chromatography through a Superdex 200 Increase 3.2/300 GL (GE Healthcare) column in core buffer supplemented with 0.003% LMNG and 0.001 mM DPPS.

Generation of the monoclonal antibody and the Fab to SERINC5. Mouse immunization was done according to protocols approved by the Biological Services Division at the National Institute for Biological Standards and Control and within the remit of a UK Home Office Project License (PPL 80/2537). The 5-week old female BALB/c mouse was purchased from Charles River Laboratories (Kent, UK). The mouse was immunised and boosted subcutaneously with 5 μg recombinant SERINC5 using the Sigma Adjuvant System (Sigma-Aldrich) as per manufacturer's instruction; boosting was done at days 28 and 56 thereafter. The mouse was culled at day 59 and the spleen recovered for isolation of hybridomas. Cells extracted from the whole spleen were fused to $5x10^7$ murine myeloma cell line SP2/0 using polyethylene glycol 1,500 (Sigma-Aldrich) and seeded into 96-well plates in RPMI medium supplemented with 10% FCS, 80 pg/ml interleukin 6 (Sigma-Aldrich), and selective agent supplement (5 mM sodium hypoxanthine, 20 μM aminopterin, 0.8 mM thymidine) (ThermoFisher Scientific). Hybridomas were screened for anti-SERINC5 antibody production by ELISA. Maxisorp plates (Nunc), coated with recombinant SERINC5 protein at 1 μg/ml in PBS (50 μl/well) overnight at 4°C, were washed with PBS/0.05%Tween-20 (PBS-T, Sigma-Aldrich) and blocked with 5% nonfat dried milk powder (Marvel) in PBS-T for 1 h at room temperature. After 3 washes, 50 μl hybridoma supernatant was added to each well and incubated for 1 h at room temperature. Secondary anti-mouse IgG HRP-conjugated antibody diluted 1:5,000 in blocking buffer was added for 1 h at room temperature. After 3 washes, 50 μl of Enhanced K-Blue Substrate (3,3',5,5'-tetramethylbenzidine/hydrogen peroxide, Neogen) was added and reactions stopped with the addition of equal volume of 1N sulfuric acid. Plates were read at the wavelength 450 nM using FluorStar Omega (BMG Labtech). Hybridomas producing SERINC5-specific antibodies were expanded and cloned by limiting dilution twice to

ensure clonality of the final hybridoma line. Three single clone hybridomas were tested positive against SERINC5 by ELISA as previously described, of which one (EVG_S5.2) was selected for further use. The epitope was established by the failure of the antibody to recognize the SERINC5 with the FLAG tag insertion within ECL4. The antibody was isolated from supernatants of hybridoma cells grown in stirrer flasks in RPMI medium supplemented with 3% foetal calf serum under 5% CO_2 atmosphere by absorption to protein A Sepharose (GE Healthcare). The Fab fragment was produced by digestion of the antibody with papain agarose (Pierce). Following depletion of the Fc fragment using a Nab protein A spin column, Fab was purified by size exclusion chromatography through a Superdex-200 10/30 column.

Lipid stocks for thermostability and HDX. Lipids were dissolved in an appropriate chloroform/methanol solution (as per manufacturers' guidelines) and dried under a nitrogen stream. Liposomes were made by resuspending desiccated lipids in water to achieve 4.5 mM lipid concentration and homogenised in a sonicating water bath at room temperature for 1 h. The resulting suspensions were used to supplement purified proteins. The following lipids and fatty acids were used: from Echelon Biosciences - 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphocholine (PC), 1,2-dioleoyl-sn-glycero-3-phosphoethanol (DOPEth), 1,2-dipalmitoyl-sn-glycero-3-phosphoserine, sodium salt (DPPS), 1-palmitoyl-2-hydroxy-sn-glycero-3-phospho-L-serine (16:0, LysoPS), phosphatidylinositol 4-phosphate diC16 (PI(4)P diC16), phosphatidylinositol 3,4-bisphosphate diC4 (PI(3,4)P2 diC4), phosphatidylinositol 3,4-bisphosphate diC8 (PI(3,4)P2 diC8), phosphatidylinositol 3,4,5-trisphosphate diC16 (PI(3,4,5)P3 diC16) , phosphatidylinositol 3,4,5-trisphosphate diC4 (PI(3,4,5)P3 diC4), phosphatidic acid (PA), D-erythro-Sphingosine, sphingosine 1-phosphate (S1P), D-

(+)-sn-1-O-oleoyl-glyceryl-3-phosphate (Oleoyl LPA, 18:1 LPA); from Avanti Polar Lipids - 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine (sodium salt) 16:0-18:1 PS (POPS), brain cerebrosides, 1',3'-bis[1,2-dioleoyl-sn-glycero-3-phospho]-glycerol (sodium salt) 18:1 (cardiolipin), sn-(3-oleoyl-2-hydroxy)-glycerol-1-phospho-sn-3'-(1',2'-dioleoyl)-glycerol (S,R isomer; ammonium salt) 18:1 (Hemi-BMP), bis(monooleoylglycero)phosphate (S,R isomer; ammonium salt) 18:1 (BMP), sn-[2,3-dioleoyl]-glycerol-1-phospho-sn-1'-[2',3'-dioleoyl]-glycerol (S isomer; ammonium salt) (BDP); from Matreya LLC – sulfatides, N-(R)-*alpha*-Hydroxytetracosanoyl-D-*erythro*-sphingosine, N-Octanoyl-*beta*-D-galactosylceramide; from Sigma-Aldrich – cholesterol (as a water-soluble complex with methyl-β-cyclodextrin), *N*-acyl-D-sphingosine-1-phosphocholine (Sphingomyelin), palmitic acid, and myristic acid.

Amino acid sequence of anti-SERINC5 monoclonal antibodies (EVG_S5.2 and EVG_S5.3). Primary structures of the heavy and light chains were deduced based on cDNA sequencing. Briefly, cDNA, produced using mRNA isolated from hybridoma cells and random hexamers was fragmented and subjected to paired-end sequencing on the Illumina platform (Absolute Antibody). Two hybridomas producing SERINC5-specific antibodies, EVG_S5.2 and EVG_S5.3, were analysed, and the antibodies were predicted to share identical heavy and light chains. The deduced amino acid sequences were as follows (signal peptides are underlined, and variable regions are shown in bold type). Heavy chain (IgG1):

MNFVLSLIFLALILKGVHC**EVQLVESGGGLVKAGGSLKLSCAASGFTFSTY AMSWVRQTPEKRLEWVATISSGGGYTYYPDSVKGRFTISRDNAKNTLYL QMSSLRSEDTAMYYCARHLTTIVEGFAYWGQGTLVTVSA**AKTTPPSVYPL APGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSDLYT

LSSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIVPRDCGCKPCICTVPEVSSV

FIFPPKPKDVLTITLTPKVTCVVVDISKDDPEVQFSWFVDDVEVHTAQTQPRE

EQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPKAP

QVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIM

DTDGSYFVYSKLNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSPGK

Light chain (Kappa):

MKLPVRLLVLMFWIPASSS**DVLMTQIPLSLPVSLGDQASISCRSSQSIVHSN**

GNTYLEWYLQKPGQSPKLLMYKVSNRFSGVPDRFSGSGSGTDFTLKISR

VEAEDLGVYYCFQDSHVPFTFGSGTKLEMKRADAAPTVSIFPPSSEQLTSG

GASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLT

LTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC

Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.