

Figure S1. proteomic screening of TMEM175-interacting proteins. Related to Figure 1.

(A) Representative MS spectra of tryptic peptides of LAMP-1 and LAMP-2 identified from the TMEM175-FLAG pull-down samples.

(B) Histogram plots summarizing abundance ratios binned in the logarithm function with base two (\log_2) between pull-down samples and controls. Upper panel: ratios between the protein amounts obtained in the samples after anti-FLAG antibody affinity purification using TMEM175-FLAG transfected cells (TMEM175) and empty-vector transfected cells (Mock control). Lower panel: ratios between the protein amounts obtained in the samples after affinity purification of TMEM175-FLAG transfected cells using anti-FLAG antibodies (FLAG) and un-immunized rabbit IgG antibodies (IgG control). High-confidence threshold for specificity is indicated by the dashed line. Grey bars mark the proteins detected in both the anti-FLAG pull-down sample of TMEM175-FLAG transfected cells and control samples. Red bars are proteins exclusively detected in the pull-down sample or the control samples.

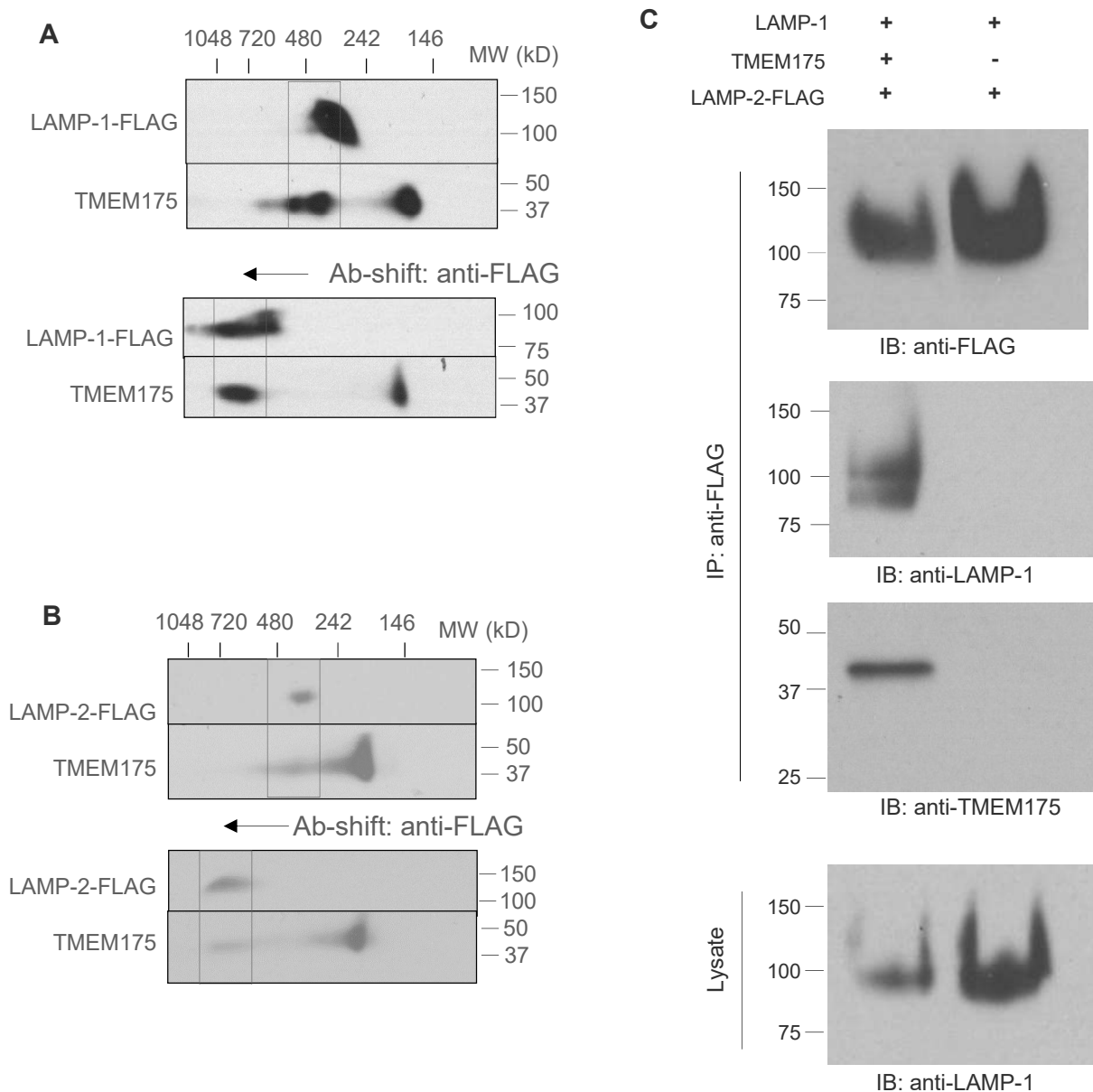


Figure S2. Biochemical analysis of interactions between TMEM175 and LAMP-1 or LAMP-2. Related to Figure 1.

(A) 2-D gel electrophoresis of cell lysate from HEK293 cells co-expressing un-tagged TMEM175 and FLAG-tagged LAMP-1 before and after mobility shift using anti-FLAG antibodies.

(B) 2-D gel electrophoresis of cell lysate from HEK293 cells co-expressing un-tagged TMEM175 and FLAG-tagged LAMP-2 before and after mobility shift using anti-FLAG antibodies.

(C) Co-IP of TMEM175 and LAMP proteins in HEK293 cells co-expressing untagged TMEM175 and LAMP-1, and FLAG-tagged LAMP-2. Overexpressed LAMP-2 was immunoprecipitated by anti-FLAG antibody and both TMEM175 and LAMP-1 were detected in the IP samples. In the control cells without TMEM175 co-expression, neither TMEM175 or LAMP-1 were detected in the IP samples. This result demonstrates that the dimeric TMEM175 can simultaneously interact with both LAMP-1 and 2, one on each side, but LAMP-1 and 2 do not interact with each other. This experiment is discussed in the **Limitations of the study**.

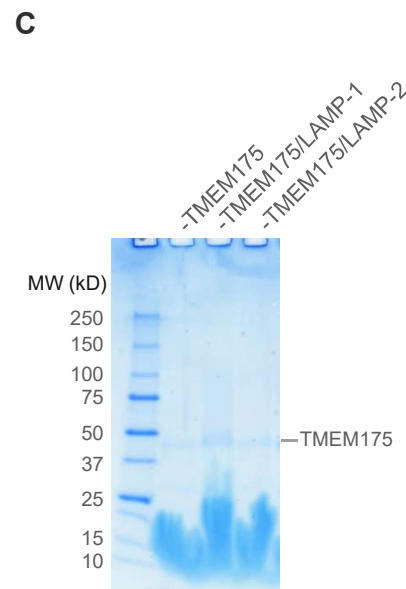
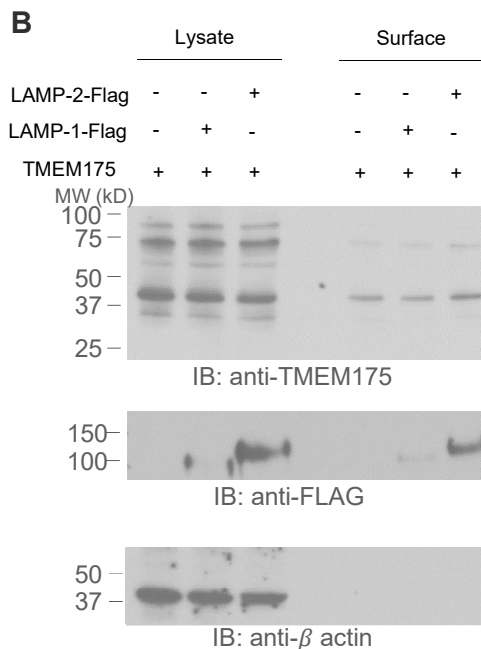
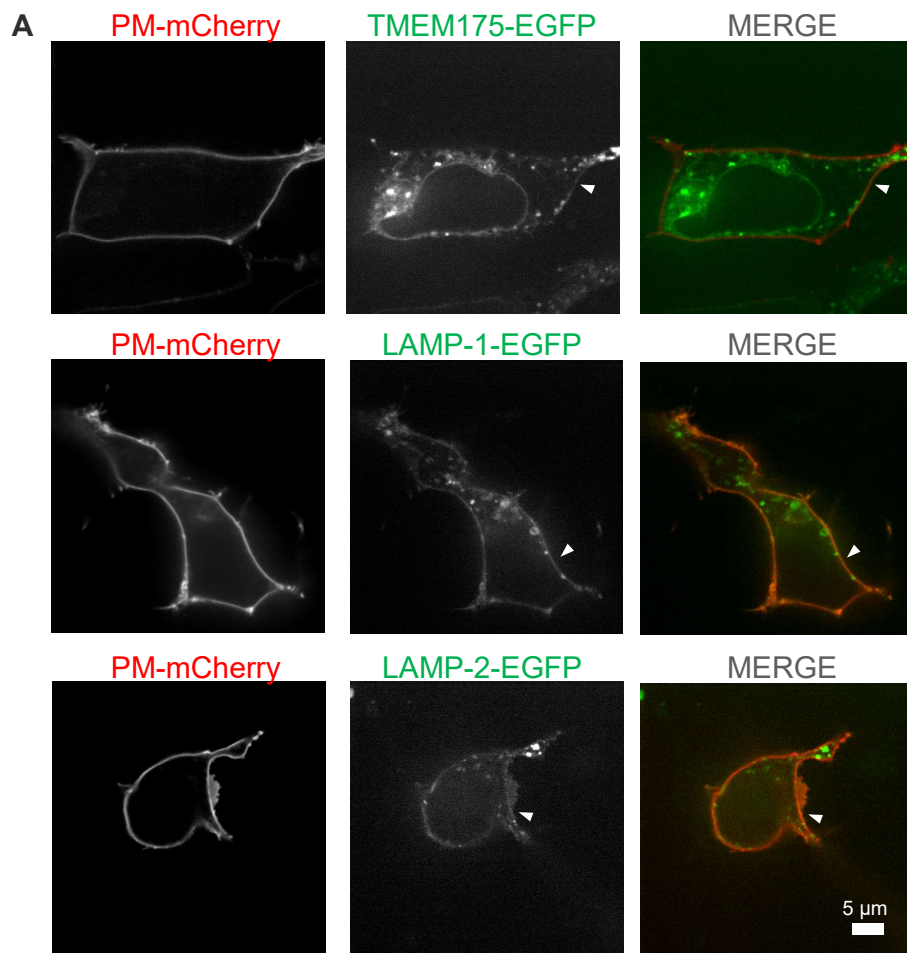


Figure S3. Plasma membrane localization of TMEM175, LAMP-1, and LAMP-2 when overexpressed in HEK293 and the amount of TMEM175 in proteoliposomes. Related to Figure 2.

(A) Confocal images of HEK293 cells co-expressing EGFP-tagged TMEM175, LAMP-1, or LAMP-2 with the plasma membrane marker KRas tail tagged with mcherry.

(B) Surface protein pulldown assay of HEK293 cells used for electrophysiology. The anti-FLAG antibodies were used to detect LAMP-1 and 2 in the total lysate and biotinylated surface proteins.

(C) SDS-PAGE of proteoliposomes containing TMEM175 alone, TMEM175/LAMP-1, or TMEM175/LAMP-2 indicating a comparable amount of TMEM175 in all samples

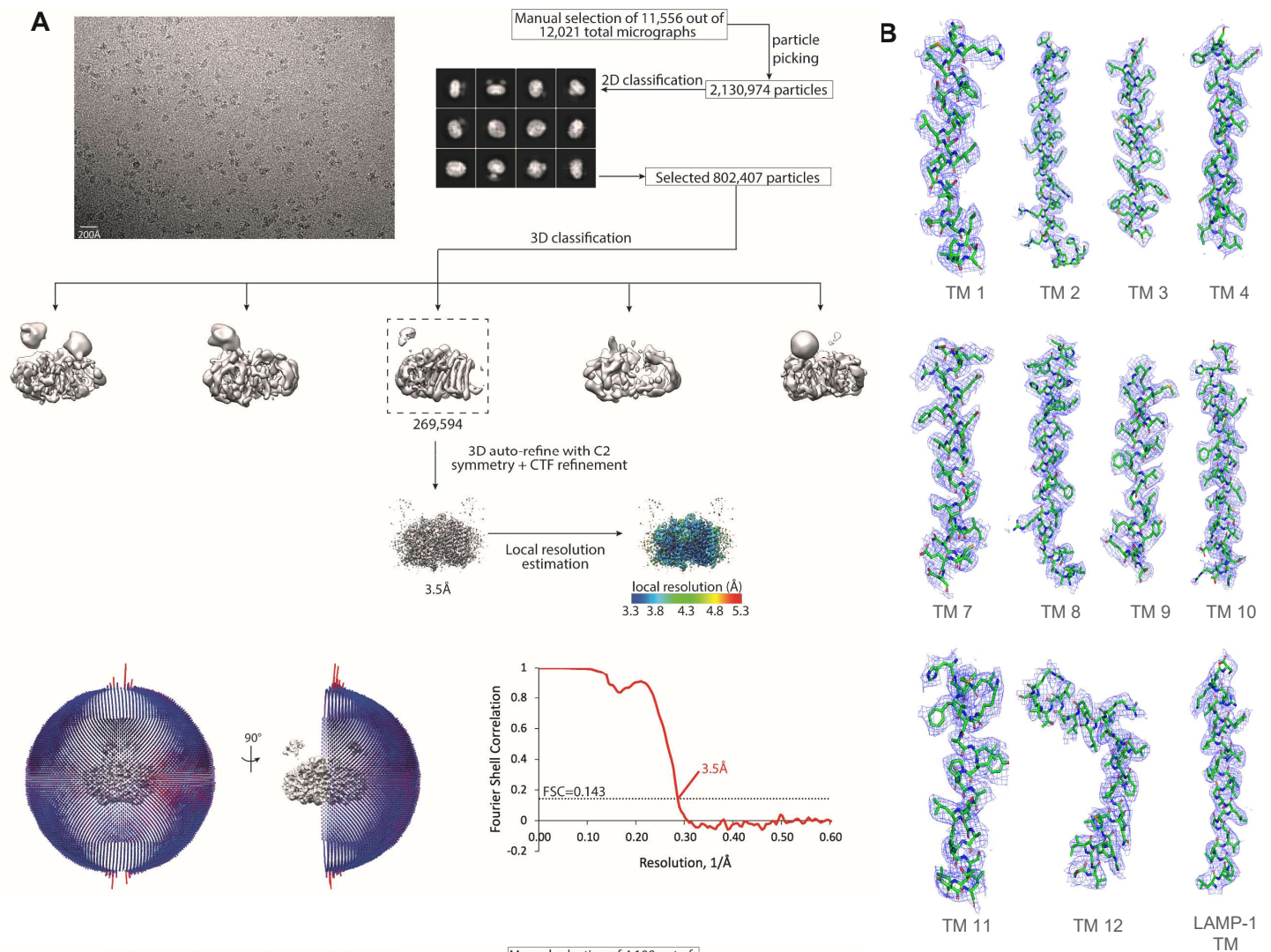


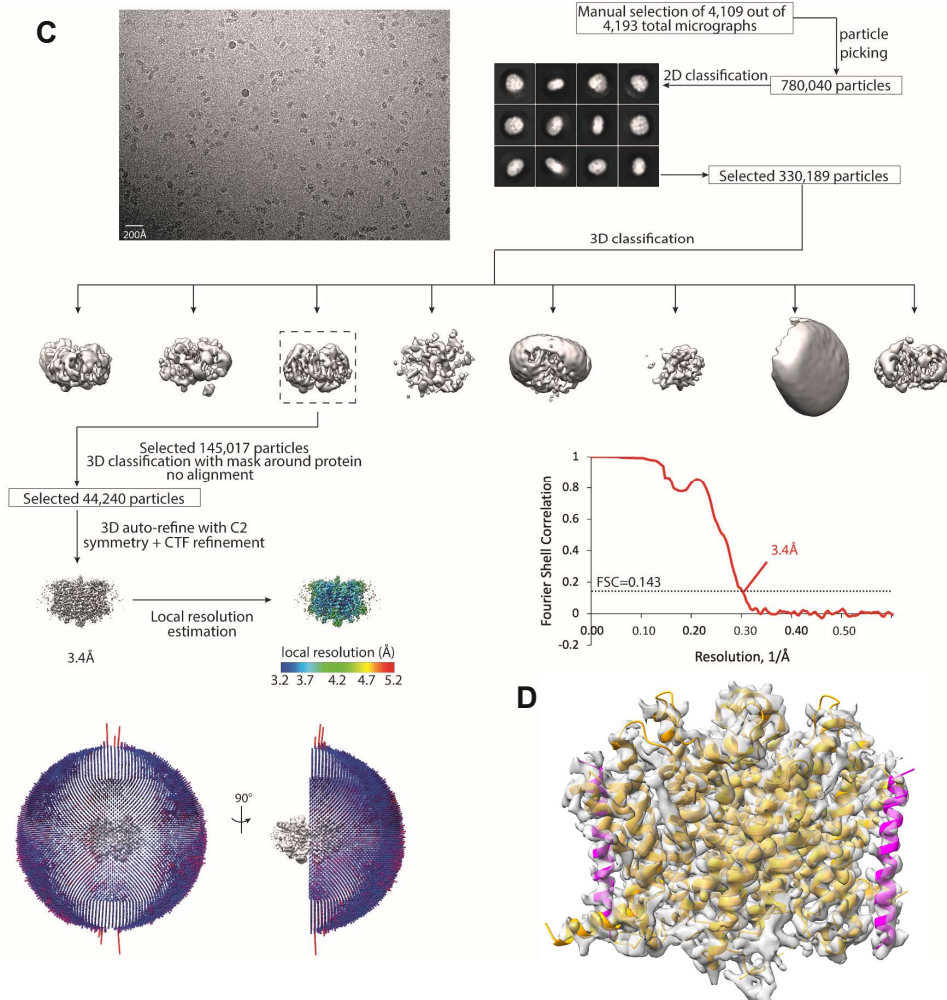
Figure S4. Structural determination of TMEM175 in complex with LAMP-1 and LAMP-1-TM. Related to Figure 3.

(A) Cryo-EM data processing scheme of TMEM175/LAMP-1 complex with Euler angle distribution of particles used in the final three-dimensional reconstruction. A representative micrograph and selected 2D class averages are shown. The scale bar is at 20 nm. Fourier Shell Correlation curve shows the overall resolution of 3.5Å at FSC=0.143.

(B) Sample density maps of human TMEM175/LAMP-1 contoured at 4σ. The luminal domain of LAMP-1 is disordered.

(C) Cryo-EM data processing scheme of TMEM175/LAMP-1-TM with the same information as that in (A). The final overall resolution is 3.4Å.

(D) EM map (grey, contour at 4σ) with the structural models of TMEM175 dimer (orange) and LAMP-1 TM domains (magenta).



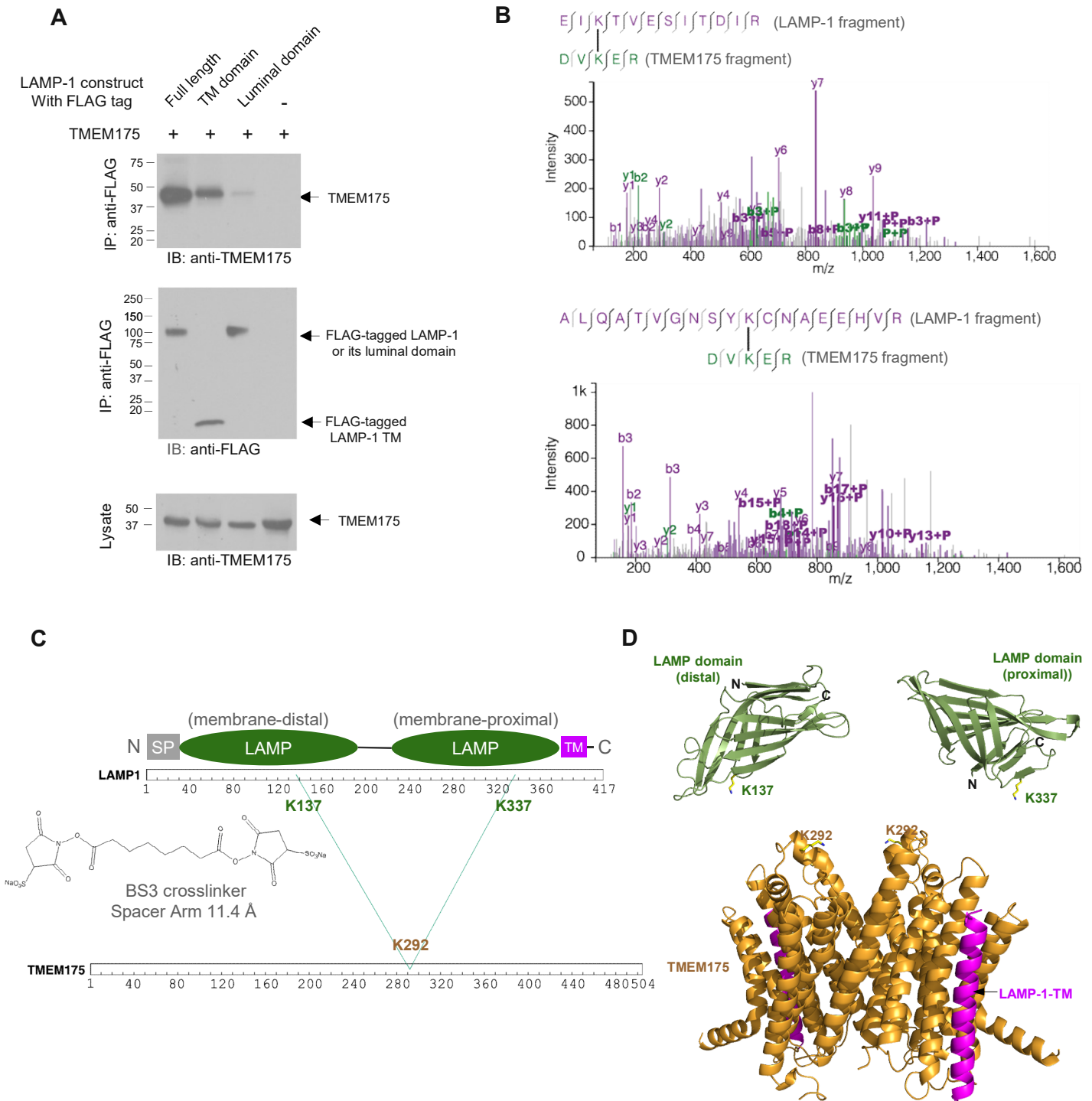


Figure S5. Transient interactions between the LAMP-1 luminal domain and TMEM175. Related to Figure 4.

(A) Co-IP of TMEM175 with full-length LAMP-1, LAMP-1 TM or LAMP-1 luminal domain in HEK293 cells co-expressing untagged TMEM175 and FLAG-tagged LAMP-1, LAMP-1 TM or LAMP-1 luminal domain. Overexpressed LAMP-1 or its domains were immunoprecipitated by anti-FLAG antibodies. TMEM175 interacts strongly with the LAMP-1 TM domain but weakly with the LAMP-1 luminal domain.

(B) MS spectra of the crosslinked tryptic peptides from LAMP-1 and TMEM175, respectively.

(C) Mapping the crosslinking lysine residues onto the 1-D sequence of LAMP-1 (K137 and K337) and TMEM175 (K292).

(D) Mapping the crosslinking residues onto the 3-D structures of LAMP domains and TMEM175. AlphaFold was used to predict the two LAMP domain structures which are similar to the crystal structure of the membrane-proximal LAMP domain of human DC-LAMP (PDB code: 4AKM).

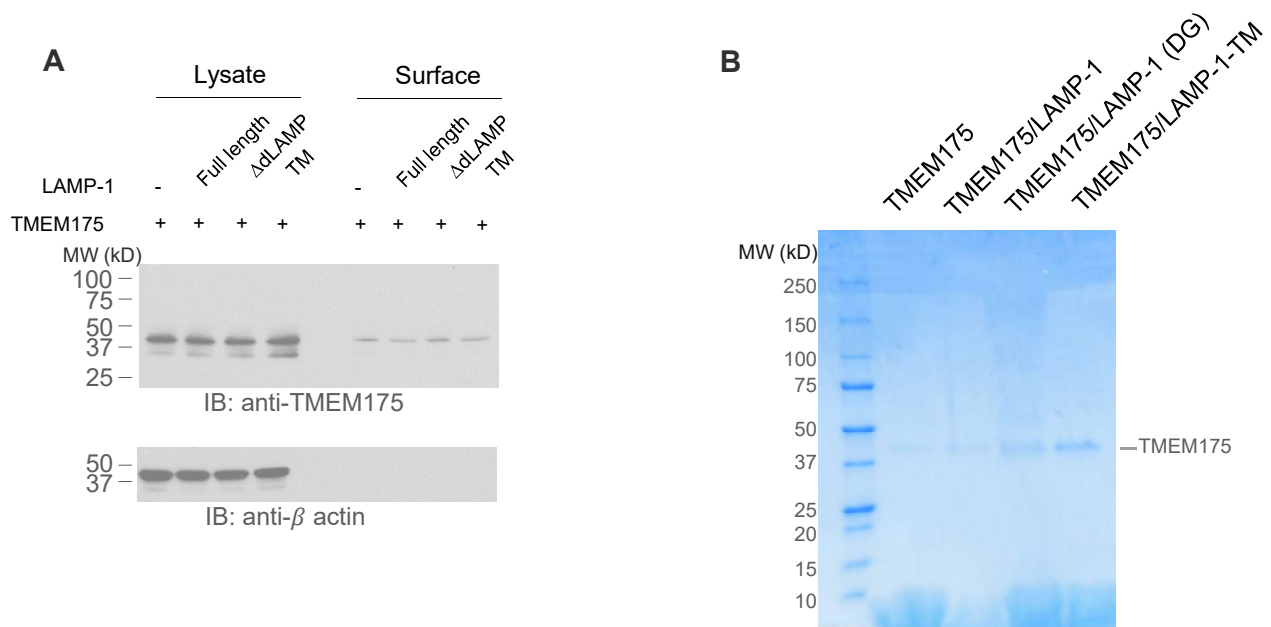


Figure S6. The protein expression in cells and the amount of TMEM175 in proteoliposomes. Related to Figure 4.

(A) Expression of TMEM175 when co-expressed with WT LAMP-1 or its two deletion mutants (LAMP-1- Δ dLAMP and LAMP-1-TM) in HEK293 cells used for electrophysiology.

(B) SDS-PAGE of proteoliposomes containing TMEM175 alone, TMEM175/LAMP-1, TMEM175/LAMP-1 after de-glycosylation (DG), or TMEM175/LAMP-1-TM indicating a comparable amount of TMEM175 in all liposome samples..

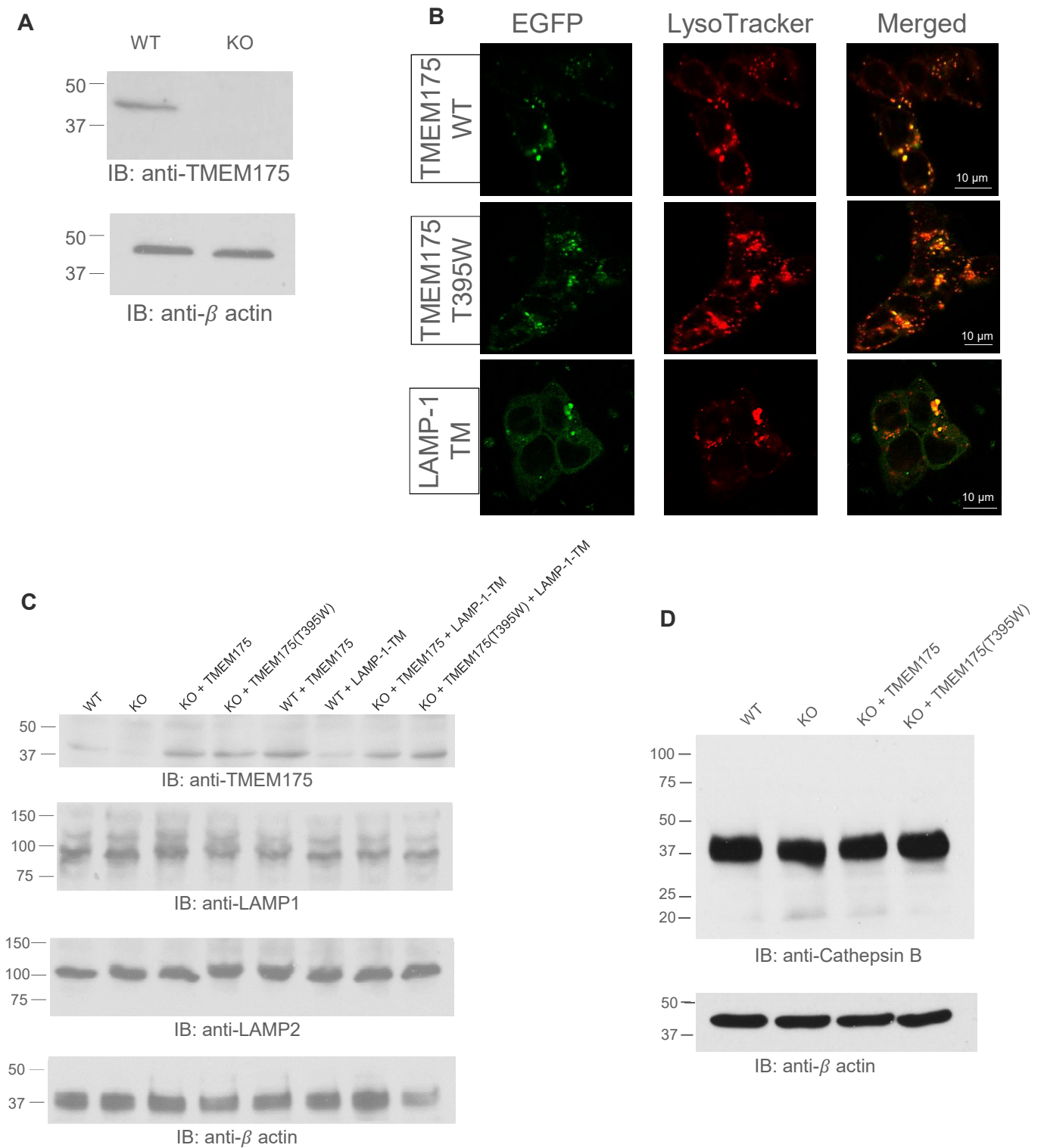


Figure S7. Protein expression in native and TMEM175-knockout HAP1 cells used for imaging. Related to Figure 6.

(A) Immunoblotting of TMEM175 in native and KO HAP1 cells.

(B) Co-localization of LysoTracker and GFP-tagged TMEM175, its T395W mutant, and LMAP-1-TM.

(C) TMEM175 expression and endogenous LAMP-1 and LAMP-2 in WT and KO cells used for lysosomal pH measurement.

(D) Cathepsin B expression in all cell samples used for its activity measurement.

HEK293:																
Protein names	Accession	# of peptides	# of MS/MS spectra	MW (kDa)	Sequence length	Average abundance	Rep 1		Rep 2		Rep 3		Rep 4		Rep 5	
							TMEM175/Mock	FLAG/IgG	TMEM175/Mock	FLAG/IgG	TMEM175/Mock	FLAG/IgG	TMEM175/Mock	FLAG/IgG	TMEM175/Mock	FLAG/IgG
Beta-1,4-glucuronyltransferase 1	B4GAT1	2	9	47.119	415	52239.725	578.8	578.8	316.41	316.41	65.795	65.795 NA	0.00234299	4.264204124	172.47	172.47
E3 ubiquitin-protein ligase MYCBP2	MYCBP2	3	15	513.63	4678	1279915.025	4.930247009	7934 NA	NA	NA	8408	8408	493795	493795	50475	50475
Lysosome-associated membrane glycoprotein 1	LAMP1	5	16	44.882	417	1350403.8	2.189317187	913	3367.45	3367.45	1121.1	1121.1	87345	87345	52.71826346	9362.5
Lysosome-associated membrane glycoprotein 2	LAMP2	6	22	44.96	410	4866691.275	306.68	306.68	0.050391796 NA	NA	NA	NA	200.5825876	215185	18101.5	18101.5
Cornifin-B;Cornifin-A	SPRR1B;SPRR1A	4	4	9.8875	89	36659.333	28.431	28.431	86.685	86.685 NA	NA	NA	NA	NA	76.975	76.975
Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial	SDHA	8	23	72.691	664	40645.7 NA	0.002856368	374.735	374.735	49.8075	49.8075	0.470967742	0.24377313	512.95	512.95	512.95
Voltage-dependent anion-selective channel protein 2	VDAC2	11	61	31.566	294	957754	22.86546931	60.32308224	11.58085521	4313	574.6	574.6	19450.5	1.080103287	3.941155305	30.0718057
Spectrin beta chain, non-erythrocytic 1	SPTBN1	16	32	274.61	2364	1223.203	67.955	67.955	285.365	285.365	129.615	129.615	1.296279253	1.618434012	78.99	78.99
Peroxiredoxin-4	PRDX4	2	3	30.54	271	16292.033 NA	NA	NA	44.8465	44.8465 NA	NA	NA	397.88	397.88	61.405	61.405
Butyrophilin subfamily 2 member A1	BTN2A1	3	4	59.632	527	36022.733	17.564	17.564	246.745	246.745 NA	NA	NA	492.765	492.765 NA	NA	NA
Staphylococcal nuclease domain-containing protein 1	SND1	8	11	102	910	17697.333	129.125	129.125	92.175	92.175 NA	NA	NA	4678.75	4678.75	0.002946246 NA	NA
ATPase family AAA domain-containing protein 1	ATAD1	4	5	40.744	361	64372.667 NA	NA	NA	112.195	112.195 NA	NA	NA	1155.2	1155.2	301.375	301.375
Vitamin K epoxide reductase complex subunit 1	VKORC1	3	7	18.234	163	190831	204.53	204.53	214.685	214.685	69.825	69.825 NA	NA	NA	272.675	272.675
Transmembrane protein 175	TMEM175	20	585	55.614	504	132651987.2	13.90872394	784.3308477	53.18446417	278660	42.54335909	113695	157.573336	277.3486491	1401.47459	313.7120066
Extended synaptotagmin-1	ESYT1	9	15	122.85	1104	10818.367	88.13	88.13	144.525	144.525 NA	NA	NA	3.7737995	0.95093189	133.955	133.955
Transmembrane protein 43	TMEM43	4	5	44.875	400	55600.5	201.805	201.805	65.02	65.02 NA	NA	NA	1847.7	1847.7	177.835	177.835
Centrosome-associated protein CEP250	CEP250	2	2	281.13	2442	96069.4	60.145	60.145 NA	NA	NA	NA	NA	15719.5	15719.5	24.0695135	5180
Thioredoxin-related transmembrane protein 1	TMX1	3	5	31.791	280	48629 NA	NA	NA	143.635	143.635	92.805	92.805 NA	NA	NA	145.085	145.085
Reticulon-4	RTN4	5	12	129.93	1192	47529	319.25	319.25	185.54	185.54 NA	NA	NA	2734.2	2734.2	0.002899433 NA	NA
Transducin beta-like protein 2	TBL2	3	4	49.797	447	9099.525	190.345	190.345	28.3715	28.3715	25.768	25.768 NA	NA	NA	161.585	161.585

SH-SY5Y:															
Protein names	Accession	Number of peptides	# of MS/MS spectra	Molecular weight (kDa)	Sequence length	Average abundance	Rep 1		Rep 2		Rep 3		Rep 4		
							TMEM175/Mock	FLAG/IgG	TMEM175/Mock	FLAG/IgG	TMEM175/Mock	FLAG/IgG	TMEM175/Mock	FLAG/IgG	
Transmembrane protein 175	TMEM175	19	240	55.614	504	38240716.3	99.2335781	123650	29.2219046	2244.729	112.618821	48456.5	50.4092585	840550	
Lysosome-associated membrane glycoprotein 1	LAMP1	6	13	44.882	417	1296123.4	3350.65	3350.65	24.6399758	2855.65	53.7819659	4037.95	49.7116522	87.1181446	
ATP synthase subunit gamma, mitochondrial	ATP5C1	4	13	32.996	298	260939	511.55	511.55	149.165	149.165	1.12681005	0.520561	1.32463583	2.27377351	
Prolactin-inducible protein	PIP	5	8	16.572	146	184630	284.93	284.93 NA	NA	0.008267 NA	NA	NA	3932.7	3932.7	
Dolichyl-diphosphooligosaccharide-protein glycosyltransferase subunit 1	RPN1	8	23	68.569	607	140464.4	850.9	850.9	23.1525	23.1525	0.01506251 NA	NA	1.37480353	1.62651981	
Lysosome-associated membrane glycoprotein 2	LAMP2	3	6	44.96	410	274165	757.2	757.2	22.0166843	1662.7 NA	NA	NA	110.79	110.79	
Very-long-chain (3R)-3-hydroxyacyl-CoA dehydratase 3	HACD3	3	8	43.159	362	53337.6667	112.49	112.49 NA	NA	159.285	159.285	0.70265866	226.365	1101.2	1101.2
Uncharacterized protein KIAA2013	KIAA2013	5	5	69.156	634	307753 NA	NA	NA	127.065	127.065	1.93338514	4.307501	6690	6690	
60S ribosomal protein L27a	RPL27A	3	6	16.561	148	166190.333 NA	NA	NA	159.285	159.285	0.70265866	226.365	1101.2	1101.2	
Serpin H1	SERPINH1	3	9	46.44	418	10952.65	79.07	79.07 NA	NA	NA	182.36	182.36	0.79692359	1.39689897	
ERO1-like protein alpha	ERO1L	3	3	54.392	468	80242.9667	42.3925	42.3925 NA	NA	NA	52.105	52.105	1.36562152	2145.05	
39S ribosomal protein L12, mitochondrial	MRPL12	2	3	21.348	198	82866	613.05	613.05 NA	NA	NA	123.605	123.605 NA	NA	NA	
Superkiller viralicidal activity 2-like 2	SKIVL2	3	6	117.8	1042	7093.35	146.415	146.415	0.46234581	55.285	99.305	99.305	0.90833975	0.82286485	
Cytoskeleton-associated protein 4	CKAP4	2	4	66.022	602	4770 NA	NA	NA	16	16	186.385	186.385	0.00091299	0.00085328	
28S ribosomal protein S22, mitochondrial	MRPS22	2	3	41.28	360	37369.8 NA	NA	NA	NA	NA	246.64	246.64	730.7	730.7	
Neural cell adhesion molecule 1	NCAM1	3	5	94.573	858	2968.55	138.4	138.4	17.3095	17.3095	0.01307873 NA	NA	0.41159932	448.705	
HLA class I histocompatibility antigen, alpha chain E	HLA-E	3	5	40.057	358	33454.5	395.565	395.565 NA	NA	NA	158.41	158.41 NA	NA	NA	
Guanine nucleotide-binding protein-like 3	GNL3	3	3	61.992	549	8657.85	102.7	102.7 NA	NA	NA	134.76	134.76	0.46177028	115.685	
Complement C3	C3	2	4	187.15	1663	17534.55	1764.2	1764.2 NA	NA	NA	148.9	148.9 NA	NA	NA	
Golgi phosphoprotein 3	GOLPH3	2	2	33.81	298	15573.5	169.72	169.72	32.7005	32.7005 NA	NA	NA	NA	NA	
Arachidonate 12-lipoxygenase, 12R-type	ALOX12B	2	2	80.355	701	9991.45	95.965	95.965	0.03058665 NA	NA	NA	NA	352.545	352.545	
Protein PRRC2C	PRRC2C	2	2	316.91	2896	1288.445	95.43	95.43 NA	NA	NA	0.00865988	0.017873	102.69	102.69	

Table S1. Putative TMEM175-interacting proteins from proteomic analysis. Related to Figure 1A and 1B.