Materials and Methods

Transient coexpression of human TOM complex

The optimized coding DNAs for human TOMM40 (Uniprot: O96008), TOMM22 (Uniprot: Q9NS69), TOMM7 (Uniprot: Q9P0U1), TOMM6 (Uniprot: Q96B49) and TOMM5 (Uniprot: Q8N4H5) were cloned into the pcDNA3.1(-) vector, with tandem twin Strep-tag at the C terminus of TOMM22. The HEK293F cells (Invitrogen) were cultured in SMM 293T-I medium (Sino Biological Inc.) supplemented with 8% CO2 in a ZCZY-CS8 shaker (Zhichu, 120 r.p.m.) at 37°C. When density reached 2 × 10^6 cells per ml, the cells were transfected with the plasmids encoding TOMM70, TOMM40, TOMM22, TOMM20, TOMM7, TOMM6 and TOMM5, incubate with Polyethylenimine (PEI) for 15 minutes at a mass ratio of 1:3. Transfected cells were cultured for 48 hours before harvesting.

Purification of the TOM complex

All procedures are carried out at 4°C. Four liters of transfected cells were harvested, washed with 1X PBS and resuspended in 25 mM Tris pH 7.8, 150 mM NaCl, and 1mM PMSF. The suspension was lysed by sonication for 5 min, then was further centrifuged at 20,000xg for 45 min to obtain the membrane. The pellet was suspended and extracted in 25mM Tris pH 7.8, 150mM NaCl with 1% digitonin. After incubation for 2 hours, the extraction was centrifuged at 20,000xg for 25 min at 4°C and the supernatant was applied to Strep-Tactin Sepharose (IBA) by gravity at 4°C. The resin was washed three times with the W buffer, which contains 25mM Tris pH 7.8, 150mM NaCl, with 0.1% digitonin. The target proteins were eluted with W buffer plus 5mM desthiobiotin (Sigma), concentrated to 100ul by 100 kDa cut-off centrifugal filter (Millipore) and further purified by Superose6 5/150 GL (GE Healthcare) also in the W buffer. The peak fractions were collected for EM sample preparation. The presence of the complex was verified by SDS-PAGE, BN-PAGE and confirmed by mass spectrometry (Supplementary Fig. S1a-c).

Blue native PAGE

Following the Blue native PAGE protocol, the purified TOM core complex was subjected to 3-10% blue native PAGE mini gel (1.5 × 8.3 × 7.3mm) for native electrophoresis at 150V for 4h (Fig. 2g-f, Supplementary Fig. S1c).
Cryo-electron microscopy

4-μL aliquots of digitonin-solubilized TOM-CC at a concentration of 4.5 mg/ml were applied to discharged 400-mesh Quantifoil R1.2/1.3 grids (Quantifoil, Micro Tools GmbH, Germany). Grids were blotted for 3.5 sec and plunged into liquid ethane using an FEI Mark IV Vitrobot operated at 4 °C and 100% humidity. Micrographs were collected on a Titan Krios microscope operated at a voltage of 300 kV with a Gatan K2 Summit direct electron detector. Images were recorded using a 300 kV Titan Krios electron microscope with serialEM software (Thermo Fisher) at a nominal magnification of 130,000 × using super-resolution mode. The pixel size was 1.08 Å/pixel and the defocus range were set from -1.3 μm to -2.3 μm. The total dose rate on the detector was about 50 e/ Å2 with a total exposure time of 8 s. Each micrograph stack contains 32 frames. Four batches of data were collected with two cryo-samples, obtaining 5,908 micrographs in total.

Each micrograph was corrected for sub-region motion correction and dose weighting using UCSF MotionCor22. Gctf was used to determine the contrast transfer function (CTF) parameter and produce the CTF power spectrum on basis of summed micrographs from MotionCor2 for all micrographs3. The 5,443 CTF-corrected cryo-EM images were manually selection. Particles were auto-picked on micrographs with dose-weighting using RELION4. Briefly, about 1,000 particles were manually picked from a subset of images and extracted in a box size of 200 pixels and a mask diameter of 200 Å. Extracted particles were subjected to 2D classification requesting 10 classes, 8 classes of which showed representative views and were selected as templates for automated particle picking. The resulting 2D averages were served as the templates for particle auto-picking, 1,638,577 particles picked from 5,443 images. For the dataset, particle selection, 2D and 3D classifications were performed on a binned dataset with a pixel size of 5.4 Å using RELION, which were then manually inspected to exclude noise and other bad particles. Two rounds of 2D classification requesting 100 classes were resulted in 823,450 particles, and two rounds of 3D classification which contained 408,208 particles and 30,916 dimeric TOM-CC like particles. An initial model was generated from 20,000 best particles using cryoSPARC ab initio reconstruction requesting five classes with C1 symmetry5. A total of 347,796 particles from these 3D classes was re-extracted to the original pixel size of 1.08 Å, and classified into five classes with C1 symmetry using a reference model generated from cryoSPARC, which had been low-pass filters to 20 Å. Two rounds of 3D classification and the most populated class containing 159,369 particles was subjected to further 3D auto-refinement with
C2 symmetry. The refinement resulted in an overall structure at a resolution of 3.91 Å, which allowed initial model building. To further improve the resolution, we performed CTF refinement, which yielded a map at 3.43 Å resolution.

The 30,916 tetrameric TOM complex like particles performed two rounds 2D classification and one round 3D classification which obtained 11,184 good particles. After re-extracted to the original pixel size of 1.08 Å, 11,184 particles were subjected to further 3D auto-refinement with C2 symmetry. The global resolution for tetrameric TOM complex was 8.53 Å. All reported resolutions are based on the gold-standard Fourier Shell Correlation (FSC) = 0.143 criteria, and the final FSC curve was corrected for the effect of a soft mask using high-resolution noise substitution. Final density maps were sharpened by B-factors calculated with the RELION post-processing program. All reported resolutions are based on the gold-standard FSC = 0.143 criteria, and the final FSC curve was corrected for the effect of a soft mask using high-resolution noise substitution. The local resolution map was calculated using ResMap (Supplementary Fig. S1d-e, 2, Table S1).

Quantification and Statistical Analyses
After refinement, map CC between model and EM map was 0.66, indicative of a reasonable fit at the present resolution. The resulting model was also used to calculate a model-map FSC curve, which agreed well with the gold-standard FSCs generated during the RELION refinement. The final model has good stereochemistry, as evaluated using MolProbity.

Model Building, Refinement and Validation
Atomic models of the TOM-CC subunits were predicted and modeled using the webserver (http://www.sbg.bio.ic.ac.uk/phyre2) of Phyre2. We assigned homology models of the TOM-CC subunits, Tom40, Tom22, Tom7, Tom6 and Tom5 into our density maps. The models were further optimized by Coot. For cross-validation against overfitting, we randomly displaced the atom positions of the final model by up to a maximum of 0.5 Å, and refined against the half map generated by RELION 3D auto-refine procedure, resulting a model named Test. Then we calculated the FSC curve of both half map against the model Test and compared with the FSC curve of the final model against the summed map generated by RELION 3D auto-refine procedure. To build the human tetrameric TOM complex models, we fitted the density maps of TOM-CC into the 8.53 Å tetramer
map and combined these part maps into a whole map in UCSF Chimera. Based on the combined map, the human tetramer TOM complex model was built. And then, the model with the ligands were subjected to global refinement and minimization in real_space_refinement using PHENIX. All ligands and phospholipids models were generated using elbow\textsuperscript{11} module in PHENIX\textsuperscript{12} by their geometric constraints. The phospholipid was docked into densities and refined in COOT. All the figures were created in PyMOL\textsuperscript{13}, Chimera and UCSF Chimera(X)\textsuperscript{14}, quantitative electrostatics were calculated using APBS\textsuperscript{15}, and conservation levels were generated with ConSurf\textsuperscript{16}, sequences alignment of Tom subunits were generated by the ESPript\textsuperscript{17}, the structure predictions of human & yeast Tom subunits are based on the Predict Secondary Structure (PSIPRED)\textsuperscript{18}.

**Cell Culture and Transient knockdown of Tom subunits**

Hela (Thermo Fisher Scientific) cells were grown in DMEM media (Corning) supplemented with 10% fetal bovine serum (Thermo Fisher Scientific) and cultured in a 37°C, 5% CO\textsubscript{2} incubator. 4.5x10\textsuperscript{5} Hela cells were incubated with corresponding short hairpin RNA (siRNA) at a final concentration of 66nM, and cultured in a 37°C, 5% CO\textsubscript{2} incubator for 48h. RNA was extracted by RNeasy Plus Mini Kit (Qiagen), and cDNA conversion was performed by TransScript OneStep gDNA Removal and cDNA Synthesis SuperMix (Trans) at 42°C for 20min. Quantitative real-time polymerase chain reaction (RT-qPCR) was conducted by the PerfectStart Green qPCR SuperMix (Trans) as the manual recommended. The mRNA expression of Tom5, Tom6, Tom7, Tom20, Tom22, Tom40 and Tom70 were normalized to GAPDH. And the sequences used were as follows:

Tom5, forward: GACCCGGAGGAGATGAAA
Tom5, reverse: AGATAAATGGAGTGACTCGC
Tom6, forward: CTGCTGGCTCGGCTAATGAAA
Tom6, reverse: ATCAGTGGCAAAGCGGTAGAC
Tom7, forward: CATTCGCTGGGGCTTTATCC
Tom7, reverse: TCTGCACCCCTCTTAAATCCC
Tom20, forward: GGTACTGCATCTACTTCGACCG
Tom20, reverse: TGGTCTACGCCCTTCTCATATTC
Tom22, forward: CAGTCCCCGGACGAATTGC
Tom22, reverse: CGACAGGGTCTCATCTAGCTC
Western Blotting
Harvested Hela cells were lysed by 1% digitonin (1% digitonin, 25mM Tris pH=7.4, 150mM NaCl) at 4°C for 30min. After centrifugation at 13,000xg, 4°C for 15min, the supernatant was collected for Blue native PAGE (BN-PAGE) with the acrylamide concentration from 3% to 12%. The separated proteins were electro-transferred to PVDF membranes (Millipore) at 300 mA for 1h. The membranes were incubated with acetic acid for 8min, followed with incubation with methyl alcohol for 5min for 3 times. Tom40 was detected with the antibodies against Tom40 (Proteintech) (Supplementary Fig. S2g-f).

CL-MS analyses and phospholipid identification
Mix BS3 crosslinker solution and the purified protein with a 20-fold excess (20:1 Crosslinker : Protein) so that the final crosslinker concentration is 1.25 mM, 2.50 mM, 3.75 mM, and 5 mM, respectively, incubate for 45 min at room temperature. Quench and unreacted BS3 with 60 mM Tris for 15 min at room temperature. Then, the protein was sampled and separated by SDS-PAGE (Supplementary Fig. S8a). The cross-linked proteins were treated with 5 mM DTT for 60 min and alkylated with 12 mM iodoacetamide for 45 min at room temperature in the dark, followed by trypsin digestion (Promega, #8V5280) at 37°C overnight. The generated peptides were desalted and fractionated by HPLC into 12 fractions at pH 10 and then analyzed by LC-MS/MS. The acquired RAW data files were searched against a database containing Mitochondrial proteins using both XlinkX search engine integrated as a node in Proteome Discoverer software (Version 2.3)\textsuperscript{[19]}(Supplementary Table S2).

To extract the lipids, we add methanol to the purified TOM complex protein solution, shake and centrifuge the mixture to remove the proteins. The supernatant is collected for Matrix-assisted laser desorption/ionization of mass spectrometry (MALDI-MSMS). Data for different types of phospholipids are searched in the Human Metabolome Database (HMDB)\textsuperscript{[20]}(Supplementary Fig. S6).
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Supplementary Fig. S1 | Biochemical characterization of the TOM complex.

a, A representative trace of size-exclusion chromatography of TOM complex by Superose6 5/150 GL column.

b, SDS-PAGE of purified TOM complex. Band of each subunit was indicated, respectively.

c, Comparison of wild type and purified TOM complex, detected by BN-PAGE. Tom40 was detected with the antibodies against Tom40.

d, A representative cryo-EM micrograph of TOM-CC.

e, Power spectrum of the micrograph in (d).
**Supplementary Fig. S2 | Cryo-EM analysis of the TOM-CC.**

**a,** 2D class averages of cryo-EM particles.

**b,** Particle orientation distributions of the TOM-CC in the last iteration of the 3D auto-refinement.

**c,d,** Gold-standard Fourier Shell Correlation (FSC) curve of the final density maps and cross-validation.
with the use of the corresponding maps and models. Dimeric and Tetrameric TOM complex are indicated, respectively.

e. Workflow of 3D classification and refinement of cryo-EM particles. 823,450 particles were kept after 2D classification, and subject to rounds of 3D classification. A final dataset containing 159,396 particles were used for TOM-CC high-resolution refinement, and 11,184 particles were used for tetrameric TOM complex reconstruction (see methods for more details).

Supplementary Fig. S3 | Overall Structure of helical Tom subunits.

Overall structure of Tom5 (a), Tom6 (b), Tom7 (c), Tom22 (d). Distribution and features of each subunit are shown in up panel and models fitted in map are shown in low panel. The cryo-EM densty of each helical Tom subunit is displayed at 4 σ (Tom5), 4 σ (Tom6), 6 σ (Tom7), and 9 σ (Tom22) contour level, respectively.
Supplementary Fig. S4 | Sequence comparison of TOM-CC subunits between different species.

The threshold value of the alignment is 0.7, which means if the similarity score assigned to a column is greater than this value, residues are considered as highly similar and are colored in red and framed in blue. Secondary structure based on human Tom subunits are indicated. The alignments were generated by the ESPript.

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The alignments were generated by the ESPript.
Supplementary Fig. S5 | Distinct features of human Tom40

a, Model of human Tom40 fitted in map. The N- and C- terminal are indicated with dotted cycles.
b, Alignments of human Tom40 model with yeast Tom40 models PDB: 6njf and PDB: 6ucu, respectively. The distinct differences between models are indicated with dotted cycles.
c, Secondary structure prediction of human and yeast Tom40, respectively. The structure predictions are based on the PSIPRED.

Supplementary Fig. S6 | Phospholipid identification

a, Mass spectrum of sample and buffer. The distinct mass spectrum are indicated.
b, List of identified PC molecules with different molecular weight. The data are searched from HMDB.
c, A further identification of each distinct items in (a). The characteristic groups of PC mass spectrum are based on HMDB.
**Supplementary Fig. S7 | Surface conservation analyses of Tom subunits.**

**a**, Surface conservation analyses of the interface between Tom40<sup>L77-K102</sup> and β-strand domains of Tom40. The conserved residue K253 is indicated. Surface representation with residues coloured based on conservation level. The surface conservation analyses are generated by ConSurf, the same below. Shown in IMS view.

**b**, Surface conservation analyses of the interfaces between Tom40 and Tom22 in both sides. The conserved residue P98 is indicated in the right panel. Shown in side views.

**c**, Surface conservation analyses of the interface between Tom40 and Tom5. Shown in side view.

**d**, Surface conservation analyses of the interface between Tom40 and Tom6. Shown in side view.

**e**, Surface conservation analyses of the interface between Tom40 and Tom7. The conserved residue P29 is indicated. Shown in side view.
**Supplementary Fig. S8 | CL-MS analyses and an unusual map between Tom22 and Tom6.**

**a**. The SDS-PAGE of cross-linked TOM complex. The final crosslinker concentration is 1.25 mM, 2.50 mM, 3.75 mM, and 5 mM, respectively. The samples for detection are indicated with dotted box in red.

**b**. Interactions between Tom subunits detected by CL-MS. The crosslinked sites are indicated.

**c**. Diagram for a potential transport pathway of pre-VDAC3 based on CL-MS analyses. The crosslinked sites are indicated.

**d**. The potential short peptide is indicated with dotted cycles. Tom subunits are indicated. Shown in side view.
Supplementary Fig. S9 | Surface electrostatics of TOM-CC.

a, Surface electrostatic potential of helical Tom subunits. Side views are shown.

b, Surface electrostatic potential of Tom40. The distinct potential regions are indicated with dotted cycles, corresponding regions of copy units in model are also indicated, respectively. Shown are cutaway side views.
Supplementary Fig. S10 | Tetrameric TOM complex

a, Dimeric TOM complex maps fitted in the tetramer map. Two dimeric units are indicated by a and b. Cytosol views are shown.

b, Fitted model of tetrameric TOM complex. The tetrameric arrangement blurs the boundary between the two proposed exit sites of the TOM complex, which is indicated with dotted cycles. IMS views are shown.
Supplementary Table S1 | Cryo-EM data collection, refinement and validation statistics

| Data collection and processing |  |
|-------------------------------|--|
| Microscope                    | Tian Krios TEM (Thermo Fisher) |
| Camera                        | Gatan K2 Summit direct electron detector |
| Energy filter                 | Gatan GIF Quantum, 20eV slit |
| Magnification (calibrated)    | 130,000 X |
| Voltage                       | 300 kV |
| Defocus range (μm)            | -1.3 - 2.3 |
| Total electron exposure (e-/Å²) | 50 |
| Exposure rate (e-/pixel/sec)  | 10 |
| Number of frames collected    | 32 |
| Pixel size (Å)                | 1.08 |

| Reconstruction                |  |
|-------------------------------|--|
| Software                      | cryoSPARC and RELION |
| Micrographs used              | 5,443 |
| Total extracted particles     | 1,636,577 |
| Total refined particles       | 159,369 11,184 |
| Symmetry applied              | C2 |
| Resolution of unmasked/masked reconstructions at 0.5 FSC (Å) | 3.68/3.48 12.17/9.59 |
| Resolution of unmasked/masked reconstructions at 0.143 FSC (Å) | 3.36/3.43 9.12/8.53 |
| Local resolution range (Å)    | 2.4-4.0 |

| Real space refinement         |  |
|-------------------------------|--|
| Software                      | phenix_real_spac_refine in the PHENIX |
| Resolution (Å)                | 3.43 8.53 |
| Model composition             |  |
| Number of protein atoms       | 7,696 15,392 |
| Number of ligand atoms        | 1 2 |
| CC (box)                      | 0.6 0.48 |
| B factor of protein residues (mean) | 102.83 222.80 |
| B factor of protein ligand (mean) | 21.3 35.4 |
| R.M.S deviations              |  |
| Bond lengths (Å)              | 0.006 0.006 |
| Bond angles (Å)               | 0.838 1.047 |

| Validation                    |  |
|-------------------------------|--|
| Molprobitity Score            | 2.09 3.31 |
| Clashscore                    | 9.19 35.87 |
| Rotamer outliers (%)          | 1.25 7.75 |
| C-beta deviations             | 0 0 |
| CaBLAM outliers (%)           | 5.24 5.24 |
| Ramachandran plot statics     |  |
| Favored (%)                   | 90.76 88.09 |
| Allowed (%)                   | 9.14 11.60 |
| Outliers (%)                  | 0.1 0.31 |
### Cross-linked peptides of Tom subunits.

| Subunits | Cross-linked sequences | Lys-Lysα |
|----------|------------------------|-----------|
| Tom40-Tom40 | E[K]KLPLLPLTL-GYQLDLPI[K]ANL | 300-309 |
| Tom40-Tom22 | GYQLDLPI[K]ANL-PSLPG[K]I | 309-141 |
| Tom40-Tom20 | VGT[K]QI-L-A[K]ERAGLSKL | 139-49 |
| Tom40-Tom70 | H[K]ASDL-NPKVY[K]AL | 277-188 |
| Tom40-Tom70 | LNHR[K]NKF-EVEI[K]K | 349-470 |
| Tom40-Tom70 | GPGLRS[K]MAIQTTQSKF-AD[K]VLKL | 175-230 |
| Tom40-Tom70 | TVN[K]GL-AD[K]VLKL | 107-230 |
| Tom20-Tom5 | A[K]ERAGL-[K]KLDL | 49-46 |
| Tom20-Tom20 | [K]DAEAVQF-S[K]LDPL | 61-56 |
| Tom22-Tom20 | SAAGATFDLSLFVAQ[K]MYR-AGLS[K]LDPL | 76-56 |
| Tom22-Tom20 | SAAGATFDLSLFVAQ[K]MYR-LPDLK[K]DAEAVQK | 76-61 |
| Tom22-Tom22 | VQA[K]MY-VQA[K]MY | 76-76 |
| Tom22-Tom70 | PVFETE[K]E[LV]E[K]ENSYL | 105-239 |
| Tom22-Tom70 | VQA[K]MYR-AD[K]VL | 76-230 |
| Tom22-Tom70 | [K]EANVKRANAL-D[K]VLISL | 359-354 |
| Tom70-Tom5 | D[K]NKL-[E-K]KLDLS | 204-46 |
| Tom70-Tom70 | D[K]KVLISL-AD[K]VL | 354-230 |
| Tom70-Tom7 | IGNANAA[K]PD[L]-S[K]EAKQRLQQL | 349-6 |
| Tom22-Tom70 | PKGDAE[K]PEEEL-[K]RGADPMGPEPTVLSL | 22-37 |

### Cross-linked peptides of Tom subunits and VDAC1-3.

| Subunits | Cross-linked sequences | Lys-Lysα |
|----------|------------------------|-----------|
| Tom40-VDAC1 | VGT[K]QI-QL-SALLDGKNVNA[Q]H[K]I | 139-274 |
| Tom40-VDAC2 | AG[K]YTL-NNWL-TD[K]VTDL | 253-74 |
| Tom40-VDAC3 | P[K]ANLLF-G[K]AAKDVF | 309-12 |
| Tom40-VDAC3 | P[K]ANLLF-GKAA[K]DVF | 309-15 |
| Tom40-VDAC3 | EECHRKC[K]EL-EN[K]LAEGL | 91-90 |
| Tom22-VDAC3 | VLPVFETE[K]E[LV]-[K]GYGFMVKIDL | 105-20 |
| Tom22-VDAC1 | PKGDAE[K]P[GEEL]-ARGL[K]L | 27-96 |
| Tom70-VDAC1 | VH[K]GL-AD[K]VL | 516-32 |
| Tom20-VDAC3 | QMLLT[K]-SALIDG[K]NF | 125-266 |

### Cross-linked peptides of Tom subunits and Tim subunits.

| Subunits | Cross-linked sequences | Lys-Lysα |
|----------|------------------------|-----------|
| Tom40-Tim44 | AG[K]YTLNNWL-TD[K]VTDL | 253-282 |
| Tom40-Tim29 | P[K]ANLLF-QE[K]KDRNAL | 309-242 |
| Tom40-Tim9 | DLP[K]ANLL-DCV[K]KDF | 309-134 |
| Tom40-Tim14 | VGT[K]QI-QL-YRGGFEP[K]-MTKREAAL | 139-57 |
| Tom40-Tim44 | TVN[K]GL-DNV[K]QEL | 107-68 |
| Tom40-Tim44 | RS[K]MIAQTTQSKF-TD[K]-VTDLGLG | 175-282 |
| Tom40-Tim14 | AG[K]YTL-SSGYYRRGFEP[K]-MTKREAALIL | 253-57 |
| Tom40-Tim44 | D[K]-[K]QDLDRGLELQ-MSELEEL | 524-252 |
| Tom70-Tim29 | AD[K]V[L]-[K]PVVL | 230-218 |
| Tom22-Tim9 | ET[KE]-KL-AAA[K]AGL | 105-81 |
| Tom70-Tim50 | [K]NREPL-EKQ[K]-KEVLAD | 245-457 |
| Tom40-DNACJ7 | IVGATLEK[K]-[Q]-KAYL | 331-329 |
| Tom70-DNACJ7 | D[K]-[K]-AEACLAML | 354-182 |
| Tom70-DNACJ7 | [K]-QDLDRGLELQ-MSELEEL | 524-252 |
| Tom70-Tim29 | [K]-QDLDRGLELQ-MSELEEL | 524-239 |
| Tom70-MIC60 | AQA[K]-CFAL-K-[L]-KEVL | 441-299 |
| Tom70-Tim50 | [K]-AKQY-[K]-EHCYY | 300-265 |
| Tom70-Tim50 | NP[K]-YVKAL-[S]-[K]-AEKL | 185-247 |
| Tom70-Tim16 | LA[K]-VL-M-[K]-AYL | 230-3 |
| Tom70-Tim23 | VH[K]-GL-Y-[K]-CTGGL | 516-169 |
| Tom70-Tim23 | AD[K]-VL-EHM-[K]-KGSLS | 230-201 |
| Tom70-MIC60 | D[K]-[K]-VISL-[K]-GMSVSLADKSLTTDDLSL | 354-385 |
| Tom70-MIC60 | D[K]-[K]-VISL-SLAVE[L]-[K]-EY | 354-577 |
| Tom20-MIC60 | S[K]-LPDL-[K]-PPPEL | 56-675 |
| Tom70-SAM50 | EQLQ[K]-W-F-[K]-PRPGNF | 168-178 |
Supplementary Table S3 | Model completeness of Tom subunits

| Subunit | Mature protein | Chain ID | Modelled residues | Assigned residues |
|---------|----------------|----------|-------------------|-------------------|
| TOM40   | 361aa          | A/B      | 77-360aa          | 77-360aa          |
| TOM22   | 141aa          | C/D      | 49-118aa          | 56-118aa          |
| TOM7    | 55aa           | G/H      | 6-57aa            | 6-54aa            |
| TOM6    | 73aa           | E/F      | 23-74aa           | 23-66aa           |
| TOM5    | 51aa           | I/J      | 12-49aa           | 12-49aa           |
Fig. 2f

Fig. 2g
Supplementary Fig. S1b
Supplementary Fig. S1c
Supplementary Fig. S8a
| Checked | Confidenc | Max. Xlink | Sequence | Accession | Position A | Sequence | Accession | Position B |
|---------|-----------|-----------|----------|-----------|------------|----------|-----------|------------|
| FALSE   | High      | 59.82     | A[K]ERAGL| Q15388    | 49 [K]KLDSI| Q8N4H5  | 46        |
| FALSE   | High      | 55.39     | RS[K]MAIQ| O96008    | 175 ARGL[K]LTf| P21796 | 96        |
| FALSE   | High      | 53.17     | [K]EANKVL| O94826    | 359 D[K]VISL| O94826  | 354       |
| FALSE   | High      | 50.16     | [K]DAEVAG| Q15388    | 61 S[K]LPDL | Q15388  | 56        |
| FALSE   | High      | 47.04     | GYQLDLP[K]| O96008  | 309 PSLPG[K]I| Q9NS69  | 141       |
| FALSE   | High      | 40.04     | DN[K]EKLE| O94826    | 204 [K]KLDSI| Q8N4H5  | 46        |
| FALSE   | High      | 30.72     | [K]AECLAM| Q96615    | 182 D[K]VISL| O94826  | 354       |
| FALSE   | High      | 30.72     | [K]TKSENG| P21796    | 32 VH[K]GL  | O94826  | 516       |
| FALSE   | High      | 29.35     | E[K]KLPLP| O96008    | 330 GYQLDLP[K]| O96008 | 309       |
| FALSE   | High      | 29.32     | D[K]VISL| O94826    | 354 AD[K]VL| O94826  | 230       |
| FALSE   | High      | 28.42     | AG[K]YTLN| O96008    | 253 TD[K]VTDL| O43615  | 282       |
| FALSE   | High      | 28.09     | VAQ[K]MYQ| O9NS69    | 76 VAQ[K]MY| Q9NS69  | 76        |
| FALSE   | High      | 27.1      | N[K]GYGFG| Q9Y277    | 20 VLPPVFET| Q9NS69  | 105       |
| FALSE   | High      | 26.6      | A[K]ERAGL| Q15388    | 49 VGT[K]QL | O96008  | 139       |
| FALSE   | High      | 26.6      | Q[K]QKEDR| O94826    | 239 [K]QDDLDR| O94826 | 524       |
| FALSE   | High      | 26.6      | Q[K]KEKDR| O94826    | 242 P[K]ANLL| O96008  | 309       |
| FALSE   | High      | 25.83     | AQAQ[K]CF| O94826    | 441 LKA[K]EEL | Q16891  | 299       |
| FALSE   | High      | 25.17     | DLP[K]ANL| O96008    | 309 LDCV[K]DF| Q9Y5J7  | 34        |
| FALSE   | High      | 25.17     | G[K]AAKDVR| Q9Y277    | 12 P[K]ANLLF| O96008  | 309       |
| FALSE   | High      | 25.17     | GKAA[K]DV| Q9Y277    | 15 P[K]ANLLF| O96008  | 309       |
| FALSE   | High      | 24.64     | VAQ[K]MYQ| O9NS69    | 76 [K]PPTL | O94826  | 604       |
| FALSE   | High      | 24.3      | SALLDG[K]| P21796    | 266 VGT[K]QL| O96008  | 139       |
| FALSE   | High      | 23.6      | [K]RGADPG| Q9P0U1    | 37 P[K]GDAEKR| Q9NS69 | 22        |
| FALSE   | High      | 23.56     | PVVFETE[K]| Q9NS69  | 105 EV[K]ENSG| O94826  | 293       |
| FALSE   | High      | 23.48     | [K]EHCVY| Q02776    | 265 [K]AKQY | O94826  | 300       |
| FALSE   | High      | 23.48     | [K]GATLE[K]| O96008  | 331 [I]JAYL| Q9L9515  | 329       |
| FALSE   | High      | 22.86     | EKQ[K]EVD| Q02776    | 457 [K]NREPL| O94826  | 245       |
| FALSE   | High      | 22.86     | SALLDGKN| P21796    | 274 VGT[K]QL| O96008  | 139       |
| FALSE   | High      | 22.86     | LAD[K]VL| O94826    | 230 MA[K]YL| Q9Y3D7  | 3         |
| FALSE   | High      | 22.86     | LQLQW[K]| O94826    | 524 AEGL[K]L| Q9Y777  | 96        |
| FALSE   | High      | 22.86     | YRGGFEP[K]| Q96DA6   | 57 VGT[K]QL| O96008  | 139       |
| FALSE   | High      | 22.1      | Y[K]CTGGL| O14925    | 169 VH[K]GL| O94826  | 516       |
| FALSE   | High      | 22.1      | NPKYV[K]A| O94826    | 188 H[K]ASDQL| O96008  | 277       |
| FALSE   | High      | 21.53     | S[K]EAKQR| Q9P0U1    | 6 IGNANAA[K]| O94826  | 349       |
| FALSE   | High      | 21.4      | [K]WCEYGL| P45880    | 74 AG[K]YTL| O96008  | 253       |
| FALSE   | High      | 21.4      | DNV[K]QEQL| O43615  | 68 TVN[K]GL| O96008  | 107       |
| FALSE   | High      | 21.4      | SALIDG[K]| Q9Y277    | 266 QMLLT[K]L| Q15388  | 125       |
| FALSE   | High      | 21.4      | EHM[K]GSL| O14925    | 201 AD[K]VL| O94826  | 230       |
| FALSE   | High      | 21.4      | LNHRE[K]NK| O96008   | 349 EEV[K]KF| O94826  | 470       |
| FALSE   | High      | 21.23     | GMSVS[K]| Q16891    | 385 D[K]VISL| O94826  | 354       |
| FALSE   | High      | 20.9      | RS[K]MAIQ| O96008    | 175 TD[K]VTDL| O43615  | 282       |
| FALSE   | High      | 20.81     | PKGDAE[K]| Q9NS69    | 27 ARG[L][K]L| P21796  | 96        |
| FALSE   | High      | 20.81     | [K]PPPEL| Q16891    | 675 S[K]LPDL| Q15388  | 56        |
| FALSE   | High      | 20.81     | F[K]PRPGN| Q9Y512    | 178 EQLQ[K]W| O94826  | 168       |
| FALSE   | High      | 20.79     | [K]PVVL | Q9BSF4    | 218 AD[K]VL| O94826  | 230       |
| FALSE   | High      | 20.78     | AA[K]AGL | Q9Y5J7    | 81 ETE[K]L| Q9NS69  | 105       |
| FALSE   | High      | 20.38     | SGGYRGGQ| Q96DA6    | 57 AG[K]YTL| O96008  | 253       |
| FALSE   | High      | 20.28     | S[K]EAKQR| Q9P0U1    | 6 VGT[K]QL| O96008  | 139       |
| FALSE   | High      | 20.28     | VAQ[K]MYIQ| Q9NS69  | 76 AD[K]VL| O94826  | 230       |
| FALSE | High  | 20.28 | MPSPQF|KO94826 | 258 | L[K]NAQLEI|Q99615 | 368 |
|FALSE | High  | 20.16 | GPG|LR[K]|O96008 | 175 | AD[K]VLKL|O94826 | 230 |
|FALSE | High  | 20.16 | EECHR|KC|O96008 | 91 | EN[K]LAEG |Q9Y277 | 90 |
|FALSE | High  | 20.16 | AD[K]VLKL|O94826 | 230 | TVN[K]GL |O96008 | 107 |
|FALSE | High  | 20.16 | SD[K]IAEKL|Q02776 | 247 | NP[K]YVKA |O94826 | 185 |
| Crosslinker | Crosslink T | # CSMs | Protein Descriptions A | Protein Descriptions B |
|------------|-------------|--------|------------------------|------------------------|
| DSS        | Inter       | 1      | Q15388                 | Q8N4H5                 |
| DSS        | Inter       | 7      | 096008                 | P21796                 |
| DSS        | Intra       | 4      | O94826                 | 094826                 |
| DSS        | Intra       | 6      | Q15388                 | Q15388                 |
| DSS        | Inter       | 6      | 096008                 | Q9NS69                 |
| DSS        | Inter       | 10     | O94826                 | Q8N4H5                 |
| DSS        | Inter       | 9      | Q99615                 | 094826                 |
| DSS        | Inter       | 2      | P21796                 | 094826                 |
| DSS        | Intra       | 1      | 096008                 | 096008                 |
| DSS        | Intra       | 1      | O94826                 | 094826                 |
| DSS        | Intra       | 1      | 096008                 | O43615                 |
| DSS        | Intra       | 1      | Q9NS69                 | Q9NS69                 |
| DSS        | Inter       | 1      | Q9Y277                 | Q9NS69                 |
| DSS        | Inter       | 2      | Q15388                 | O96008                 |
| DSS        | Inter       | 1      | Q9BSF4                 | 094826                 |
| DSS        | Inter       | 1      | Q9BSF4                 | 096008                 |
| DSS        | Inter       | 1      | O94826                 | Q16891                 |
| DSS        | Inter       | 4      | 096008                 | Q9Y5J7                 |
| DSS        | Inter       | 1      | Q9Y277                 | O96008                 |
| DSS        | Inter       | 1      | Q9Y277                 | O96008                 |
| DSS        | Inter       | 1      | Q9NS69                 | O94826                 |
| DSS        | Inter       | 2      | P21796                 | O96008                 |
| DSS        | Inter       | 1      | Q9P0U1                 | Q9NS69                 |
| DSS        | Inter       | 1      | Q9NS69                 | O94826                 |
| DSS        | Inter       | 1      | Q27776                 | O94826                 |
| DSS        | Inter       | 1      | O96008                 | Q99615                 |
| DSS        | Inter       | 1      | Q27776                 | O94826                 |
| DSS        | Inter       | 1      | P21796                 | O96008                 |
| DSS        | Inter       | 1      | O94826                 | Q9Y3D7                 |
| DSS        | Inter       | 1      | O94826                 | Q9Y277                 |
| DSS        | Inter       | 1      | Q96DA6                 | O96008                 |
| DSS        | Inter       | 1      | O14925                 | O94826                 |
| DSS        | Inter       | 1      | O94826                 | O96008                 |
| DSS        | Inter       | 1      | Q9P0U1                 | O94826                 |
| DSS        | Inter       | 2      | P45880                 | O96008                 |
| DSS        | Inter       | 1      | O43615                 | O96008                 |
| DSS        | Inter       | 1      | Q9Y277                 | Q15388                 |
| DSS        | Inter       | 1      | O14925                 | O94826                 |
| DSS        | Inter       | 1      | O96008                 | O94826                 |
| DSS        | Inter       | 1      | Q16891                 | Q96008                 |
| DSS        | Inter       | 1      | O96008                 | O43615                 |
| DSS        | Inter       | 1      | Q9NS69                 | P21796                 |
| DSS        | Inter       | 1      | Q16891                 | Q15388                 |
| DSS        | Inter       | 1      | Q9Y512                 | O94826                 |
| DSS        | Inter       | 1      | Q9BSF4                 | 094826                 |
| DSS        | Inter       | 1      | Q9Y5J7                 | Q9NS69                 |
| DSS        | Inter       | 1      | Q96DA6                 | O96008                 |
| DSS        | Inter       | 1      | Q9P0U1                 | O96008                 |
| DSS        | Inter       | 1      | Q9NS69                 | O94826                 |
| DSS | Inter | 1 | 094826 | 099615 |
|-----|-------|---|--------|--------|
| DSS | Inter | 1 | 096008 | 094826 |
| DSS | Inter | 1 | 096008 | 09Y277 |
| DSS | Inter | 1 | 094826 | 096008 |
| DSS | Inter | 1 | 092776 | 094826 |
| Accession | Description | Score |
|-----------|-------------|-------|
| O96008    | Mitochondrial import receptor subunit TOM40 homolog OS=Homo sapiens GN | 17953.35 |
| Q9NS69    | Mitochondrial import receptor subunit TOM22 homolog OS=Homo sapiens GN | 5711.02 |
| Q8N4H5    | Mitochondrial import receptor subunit TOM5 homolog OS=Homo sapiens GN | 1146.37 |
| Q9P0U1    | Mitochondrial import receptor subunit TOM7 homolog OS=Homo sapiens GN | 466.47 |
| Q15388    | Mitochondrial import receptor subunit TOM20 homolog OS=Homo sapiens GN | 463.45 |
| Q96B49    | Mitochondrial import receptor subunit TOM6 homolog OS=Homo sapiens GN | 213.82 |
| Coverage | # Proteins | # Unique Peptides | # Peptides | # PSMs | # AAs |
|----------|------------|------------------|------------|--------|-------|
| 85.60    | 2          | 21               | 38         | 3690   | 361   |
| 100.00   | 2          | 12               | 12         | 1388   | 142   |
| 100.00   | 4          | 16               | 16         | 802    | 51    |
| 83.64    | 3          | 2                | 10         | 246    | 55    |
| 86.90    | 1          | 12               | 12         | 114    | 145   |
| 94.59    | 1          | 6                | 6          | 83     | 74    |
| MW [kDa] | calc. pl |
|---------|----------|
| 37.9    | 7.25     |
| 15.5    | 4.34     |
| 6.0     | 9.70     |
| 6.2     | 10.29    |
| 16.3    | 8.60     |
| 8.0     | 4.89     |
| Accession | Description                                      | Score  | Coverage | # Proteins |
|-----------|---------------------------------------------------|--------|----------|------------|
| O94826    | Mitochondrial import receptor subunit TOM70 OS=Homo sapiens GN=TOM70 | 1695.56 | 78.29    | 1          |
| O96008    | Mitochondrial import receptor subunit TOM40 homolog OS=Homo sapiens | 6138.80 | 73.41    | 3          |
| Q9NS69    | Mitochondrial import receptor subunit TOM22 homolog OS=Homo sapiens | 1946.82 | 97.89    | 1          |
| Q15388    | Mitochondrial import receptor subunit TOM20 homolog OS=Homo sapiens | 987.83  | 87.59    | 1          |
| Q8N4H5    | Mitochondrial import receptor subunit TOM5 homolog OS=Homo sapiens | 181.55  | 64.71    | 4          |
| Q9P0U1    | Mitochondrial import receptor subunit TOM7 homolog OS=Homo sapiens | 34.50   | 70.91    | 2          |
| Q96B49    | Mitochondrial import receptor subunit TOM6 homolog OS=Homo sapiens | 29.17   | 32.43    | 1          |
| Unique Peptide | # Peptides | # PSMs | # AAs | MW [kDa] | calc. pl |
|----------------|------------|--------|-------|----------|---------|
| 55             | 55         | 707    | 608   | 67.4     | 7.12    |
| 30             | 30         | 1686   | 361   | 37.9     | 7.25    |
| 13             | 13         | 736    | 142   | 15.5     | 4.34    |
| 16             | 16         | 396    | 145   | 16.3     | 8.60    |
| 8              | 8          | 95     | 51    | 6.0      | 9.70    |
| 2              | 7          | 20     | 55    | 6.2      | 10.29   |
| 3              | 3          | 14     | 74    | 8.0      | 4.89    |
| Accession | Description                                      | Score  | Coverage |
|-----------|--------------------------------------------------|--------|----------|
| O96008    | Mitochondrial import receptor subunit TOM40 homolog OS=Homo s | 1680.83 | 79.78   |
| Q9NS69    | Mitochondrial import receptor subunit TOM22 homolog OS=Homo s | 766.28  | 78.17   |
| Q15388    | Mitochondrial import receptor subunit TOM20 homolog OS=Homo s | 83.97   | 40.69   |
| Q8N4H5    | Mitochondrial import receptor subunit TOM5 homolog OS=Homo sa  | 72.53   | 86.27   |
| Q96B49    | Mitochondrial import receptor subunit TOM6 homolog OS=Homo sa  | 25.78   | 32.43   |
| Q9P0U1    | Mitochondrial import receptor subunit TOM7 homolog OS=Homo sa  | 5.00    | 43.64   |
| # Proteins | # Unique Peptides | # Peptides | # PSMs | # AAs | MW [kDa] |
|------------|-------------------|------------|--------|------|---------|
| 3          | 22                | 22         | 522    | 361  | 37.9    |
| 2          | 7                 | 7          | 285    | 142  | 15.5    |
| 1          | 5                 | 5          | 38     | 145  | 16.3    |
| 4          | 9                 | 9          | 53     | 51   | 6.0     |
| 1          | 3                 | 3          | 16     | 74   | 8.0     |
| 4          | 3                 | 3          | 5      | 55   | 6.2     |
| calc. pI |
|--------|
| 7.25   |
| 4.34   |
| 8.60   |
| 9.70   |
| 4.89   |
| 10.29  |