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Structure and topography of the synaptic V-ATPase–synaptophysin complex

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Summary

Synaptic vesicles are organelles with a precisely defined protein and lipid composition, yet the molecular mechanisms for the biogenesis of synaptic vesicles are mainly unknown. Here, we discovered a well-defined interface between the synaptic vesicle V-ATPase and synaptophysin by in situ cryo-electron tomography and single particle cryo-electron microscopy of functional synaptic vesicles isolated from mouse brains. The synaptic vesicle V-ATPase is an ATP-dependent proton pump that establishes the protein gradient across the synaptic vesicle, which in turn drives the uptake of neurotransmitters. Synaptophysin and its paralogs synaptoporin and synaptogyrin belong to a family of abundant synaptic vesicle proteins whose function is still unclear.

We performed structural and functional studies of synaptophysin knockout mice, confirming the identity of synaptophysin as an interaction partner with the V-ATPase. Although there is little change in the conformation of the V-ATPase upon interaction with synaptophysin, the presence of synaptophysin in synaptic vesicles profoundly affects the copy number of V-ATPases. This effect on the topography of synaptic vesicles suggests that synaptophysin assists in their biogenesis. In support of this model, we observed that synaptophysin knockout mice exhibit severe seizure susceptibility, suggesting an imbalance of neurotransmitter release as a physiological consequence of the absence of synaptophysin.

Keywords:

Synaptic vesicle; synapse; neurotransmitter release; V-ATPase; synaptophysin; cryo-electron tomography
Introduction

Synaptic neurotransmission involves the fusion of neurotransmitter-filled synaptic vesicles with the presynaptic plasma membrane upon Ca\(^{2+}\) influx into the presynaptic terminal. Synaptic vesicles are small organelles with an average diameter of ~40 nm and a specific composition of proteins and lipids\(^1,2\). The structure and function of some presynaptic proteins are known\(^9,10\), along with a putative interaction map of synaptic vesicle proteins based on cross-linking mass-spectrometry\(^11\). However, the molecular architecture of the whole synaptic vesicle is largely unknown, hindering the molecular mechanistic understanding of neurotransmitter release and its regulation.

In situ maps of the V-ATPase

As a first step to decipher the molecular architecture of synaptic vesicles, we isolated and purified glutamatergic synaptic vesicles (ISV) from mouse brains\(^1,2,12\). The ISVs are functional as assessed by a Ca\(^{2+}\)-triggered vesicle–vesicle fusion assay\(^3\). We first imaged the ISVs with cryo-electron tomography (cryo-ET) (Fig. 1a, b). Although cryo-ET can potentially resolve protein structures at near-atomic resolution by sub-tomogram averaging\(^13,14\), the success depends on both the size of the molecule and the ability to localize it within a membrane environment. Here, we focused on the synaptic vesicle vacuolar (H\(+\))-ATPase (V-ATPase) since it has a large cytoplasmic domain readily observable in cryo-ET reconstructions (Fig. 1b).

V-ATPases constitute a highly conserved family of ATP-dependent proton pumps that are widely expressed in eukaryotic cells\(^15\). V-ATPases establish proton gradients critical for enabling organelle-specific functions, including membrane trafficking, endocytosis, lysosomal degradation, and neurotransmitter release\(^5\). In addition to its role as a proton pump on synaptic vesicles, the synaptic vesicle V-ATPase has also been implicated in modulating neuronal exocytosis\(^4,16,17\), although it is not directly involved in membrane fusion.

V-ATPases consist of an integral membrane V0 domain that functions as the proton pump and a cytosolic V1 domain that catalyzes ATP hydrolysis. The V1 domain
comprises three pairs of subunits responsible for ATP binding and hydrolysis, and its activity is coupled to the rotation of a central stalk in the V0 domain, mediating the translocation of protons across the membrane. The V1 domain reversibly disassembles from the V0 domain in a luminal pH-dependent fashion. Several V-ATPase subunits have multiple isoforms with distinct tissue expression, suggesting tissue-specific roles.

In our cryo-ET reconstructions of ISVs, we found both “intact” (i.e., V0 and V1) and V0-domain-only V-ATPase assemblies in the ISVs (Fig. 1b), demonstrating that a mixture of intact and V0-only V-ATPases exists in functional ISVs, consistent with observations of V1 domain dissociation. We then performed subtomogram averaging and classification (Fig. 1c-d and Extended Data Fig. 1), yielding three well-defined states of the intact V-ATPase at ~17 Å resolution corresponding to about 120-degree rotations of the rotor subcomplex between the states. Our data also produced one state for the V0-only assembly, consistent with previous work. Surprisingly, both the intact and V0-only V-ATPase maps revealed an extra density large enough to fit an ~20 kDa protein (Fig. 1c, d), which had not been observed in any structures of purified V-ATPases, implying that the extra density comprises at least one specific binding partner that exists only in the context of synaptic vesicles.

**Identification of the binding partner**

To achieve higher resolution maps and to resolve the binding partner better, we collected a large set of cryo-EM projection images and performed single-particle cryo-EM analysis (SPA) of the V-ATPase (Fig. 2a, b and Extended Data Fig. 2). Refinements resulted in maps of the intact V-ATPase assembly, again consisting of three states all at 4.3 Å resolution and a map of the V0-only V-ATPase assembly at 3.8 Å resolution. We note that the resolution of our V0-only map exceeds that of a prior mammalian V0-only V-ATPase structure, and is comparable to that of a recently reported V0 structure in non-specifically isolated synaptic vesicles. All these maps clearly show the density of the binding partner.
To identify the binding partner, we generated density maps from models predicted by AlphaFold2 for all synaptic proteins detected in our ISVs by high-resolution mass spectrometry (MS) (Supplementary Table 1). The predicted density maps were docked as rigid bodies into the densities of the binding partner for the intact (State 3) and V0-only V-ATPase assemblies using CoLoRes/Situs. The fitted models were sorted by their map cross-correlation coefficient (Supplementary Table 1). Candidates that were not membrane proteins or had no membrane domain matching the binding partner density in the membrane region were ignored for further analysis. Models with the highest cross-correlation coefficient were checked and adjusted by inspecting the models and maps, and the map outlier percentage was calculated (see Methods). Synaptophysin (also called synaptophysin-1, ref. 6) exhibited the least outliers, followed by its paralogs synaptoporin (also called synaptophysin-2, ref 7) and synaptogyrin-1 & -3 (ref. 8) (Fig. 2c). We compared the AlphaFold2 prediction of synaptogyrin-1 with the recently determined NMR structure of synaptogyrin-1 (PDB ID 8A6M, ref. 32), and found the experimental structure to be similar to the predicted model (RMSD = 1.4 Å).

Consistent with our fitting results, a cross-linking mass spectrometry study of ISVs had identified a possible interaction between synaptophysin and the synaptic V-ATPase. However, this cross-linking study employed crosslinkers that act on the cytoplasmic site of the ISV, potentially limiting the detection of luminal interactions. Moreover, further supporting our findings, the V0 domain of the synaptic vesicle V-ATPase was associated with synaptophysin in synaptosome preparations. Taken together, synaptophysin is the most likely candidate for the binding partner to the synaptic vesicle V-ATPases that we discovered in our in situ cryo-ET and single particle cryo-EM studies of ISVs.

V-ATPase–synaptophysin validation

To further confirm the identity of the binding partner, we used synaptophysin knockout (Syp−/−) mice (see Methods), and purified the Syp−/− ISVs following the same protocol as for wild-type (WT) mice. As anticipated, essentially no synaptophysin was detected in the Syp−/− ISVs (Fig. 2d and Extended Data Fig. 3a, b) as judged by western blot. The vesicular glutamate transporter-1 (VGLUT1) and synaptotagmin-1 (SYT1) were present
at similar levels in Syp$^{+}$ ISVs and wild-type ISVs (Fig. 2d and Extended Data Fig. 3c).

Using VGLUT1 (Extended Data Fig. 3d) or SYT1 (Extended Data Fig. 3e) for band
density normalization, synaptoporin-1 (SYNPR1) was present at a higher level, and
synaptogyrin-1 (SYG1) was present at a similar level in the Syp$^{+}$ ISVs. In contrast,
synaptobrevin-2 (SYB2) was present at a lower level in Syp$^{+}$ ISVs compared with the
wild-type ISVs, consistent with a role of synaptophysin for synaptobrevin-2 sorting into
synaptic vesicles$^{34-36}$. The observed V-ATPase level, either targeting subunit A (V1A) or
subunit a (V0a) of the V-ATPase, was higher in Syp$^{+}$ ISVs.

The hydrodynamic size distributions of wild-type and Syp$^{+}$ ISVs were very similar in
solution as judged by dynamic light scattering (DLS, Fig. 2e), consistent with size
measurements by cryo-EM (Fig. 2f, representative images in Extended Data Fig. 2a and
4a). Finally, the binding partner density was absent in Syp$^{+}$ ISV SPA maps for both
intact and V0-only V-ATPase assemblies (Fig. 2g, h and Extended Data Fig. 4b-f),
validating the identity of the binding partner density as synaptophysin.

V-ATPase–synaptophysin structure

Starting with published V-ATPase structures and the AlphaFold2-predicted
synaptophysin model, we refined atomic models of both the intact and V0-only V-
ATPase assemblies in complex with synaptophysin using the wild-type ISV SPA average
maps (Fig. 2a-b and Fig. 3a-b, Extended Data Table 2, see Methods). Generally, there is
good agreement between the models and maps (Extended Data Fig. 5a-c). We observed
the glycosylated mammalian-specific ATP6AP1 binding partner in our maps (Extended
Data Fig. 5d). In addition to six previously reported glycosylation sites$^{26}$, a putative
glycosylation site was also observed for asparagine 406.

While both e1 (ATP6V0E1) and e2 (ATP6V0E2) isoforms have been reported for
subunit e, our high-resolution V0-only V-ATPase map suggests e2 as the subunit present
in our sample (Extended Data Fig. 6a). This finding was further supported by MS
experiments, which only detected unique peptides specific to ATP6V0E2 (Supplementary
Table 2). The luminal parts of subunits e2 and a form an interface with synaptophysin (Fig. 3c), and electrostatic interactions are involved in this interface.

Synaptoporin shares function and primary sequence similarities with synaptophysin, and it is present at a slightly higher level in Syp−/− ISVs (Extended Data Fig. 4c-e), potentially to compensate for the loss of synaptophysin function. However, it is improbable that it interacts quantitatively with the V-ATPase since there is no density for it in the Syp−/− ISV SPA maps (Fig. 2g-h). The absence of an interaction likely arises due to primary sequence differences between synaptophysin and synaptoporin at and near the V-ATPase–synaptophysin interface (Extended Data Fig. 6b-d), suggesting the specificity of this interaction.

At the current resolution, we do not observe substantial differences in maps and models between the V-ATPases in wild-type and Syp−/− ISVs (Extended Data Fig. 7). Overall, our V-ATPase models are similar to structures of mammalian V-ATPases from purified samples (PDB IDs 6WM3, 7U4T, 6VQG, 7UNF, refs. 18,23,26) (Supplementary Table 3), and recently reported structures from non-specifically isolated synaptic vesicles28,37.

**Syp−/− Increases V-ATPase copy numbers**

Considering that the interaction between synaptophysin and the V-ATPase does not appear to affect the V-ATPase structure, we asked what other roles this interaction might play. We collected a cryo-ET data set (Fig. 4a, Extended Data Fig. 8a) for Syp−/− ISVs using the same procedures as for the cryo-ET data set for wild-type ISVs (Extended Data Figure 8b). We inspected the cryo-ET reconstructions and identified intact and V0-only V-ATPase assemblies in both wild-type and Syp−/− ISV datasets, which allowed us to analyze the V-ATPase copy numbers (see Methods, Extended Data Fig. 8a, b). There is little correlation between the V-ATPase copy numbers and ISV diameters for both wild-type and Syp−/− ISVs (Extended Data Fig. 8c).
Compared to wild-type ISVs, we observed a substantial increase in the average copy number of V-ATPases in Syp\(^{+/−}\) ISVs (Fig. 4b, c, Extended Data Fig. 8d, e, and Supplementary Tables 4-5). Not only are there more ISVs with more than two V-ATPases, but we also observed ISVs with up to eight V-ATPases on a single ISV. To estimate the average copy number of V-ATPases per ISV, we fitted Poisson distributions to the observed copy numbers (Extended Data Figure 8f-i). However, since it is difficult to determine the true copy number of ISVs without any V-ATPase assembly due to the missing wedge effect of tomographic reconstructions, we only fitted the Poisson distributions to copy numbers \(\geq 1\). The Poisson distributions could be well fit to the copy numbers \(\geq 1\) (Extended Data Figs. 8f-i), suggesting that the incorporation of a V-ATPase is a Poisson process, i.e., independent of the presence of other V-ATPases in the same synaptic vesicle. We, therefore, used the \(\lambda\) parameters of the fitted Poisson distributions to estimate the average copy numbers. For Syp\(^{+/−}\) ISVs, there is a 2.1-fold increase in the average copy number of intact V-ATPase assemblies and a 1.7-fold increase in the average copy number of V0-only assemblies.

For wild-type ISVs, the majority (71.3% of wild-type ISVs with visible V-ATPase densities) contained only one intact V-ATPase assembly (Fig. 4b and Supplementary Table 4). The remaining ISVs (21.9%) primarily contained two intact V-ATPase assemblies, along with a few cases with more than two copies. We calculated the average combined copy number of intact and V0-only V-ATPases from the Poisson fits to be 1.04 (sum of 0.62 and 0.42, respectively). These numbers are in close agreement with the previously estimated copy number of 0.7 intact V-ATPases per ISV using quantitative western blotting against the V1B subunit of the V-ATPase\(^1\) and an estimate of the combined copy number of intact and V0-only V-ATPases per ISV of 1.27 using fluorescent imaging with antibody labeling\(^{38}\).

**Syp\(^{+/−}\) causes severe seizures in mice**

Given the profound dysregulation of V-ATPase copy number observed in Syp\(^{+/−}\) ISVs, we examined what physiological consequences this may have. While Syp\(^{+/−}\) mice exhibit no large differences in neurotransmitter release probability compared to wild-type mice\(^{39,40}\).
we observed a striking susceptibility to kainic acid stress-induced seizures (Fig. 4d).

Compared to wild-type controls displaying minimal susceptibility, Syp<sup>−/−</sup> mice are highly susceptible to seizure and subsequent death. This dramatic phenotype highlights a significant role for synaptophysin in synaptic function and in the regulation of synaptic vesicle properties. While there may be other roles of synaptophysin at the synapse, the severe seizure susceptibility phenotype and the regulation of V-ATPase copy number highlight a previously unrecognized and critical role for synaptophysin in synaptic vesicle function.

**Discussion**

We performed *in situ* cryo-ET reconstructions, obtained SPA maps, and determined structures of synaptic vesicle V-ATPases in functional, glutamatergic mouse ISVs. We observed both intact and V0-only V-ATPase assemblies (Fig. 1 and 2a, b), consistent with the notion that V-ATPases reversibly dissociate the V1 domain upon acidification of the synaptic vesicle<sup>4</sup>. Unexpectedly, the cryo-ET reconstructions and SPA maps revealed that synaptophysin is bound to the synaptic vesicle V-ATPase. This interaction was not previously observed in structural studies of reconstituted V-ATPases<sup>18,22,23,25,26,41</sup>, illustrating the power of *in situ* structural studies by cryo-ET and single-particle cryo-EM. The confined membrane environment of a synaptic vesicle, including vesicle curvature, likely contributes to stabilizing the relatively small V-ATPase–synaptophysin interface (Fig. 3c). The interaction is identical for both intact and V0-only V-ATPase assemblies (Fig. 2a, b), suggesting that this interaction does not appear to directly regulate the hydrolysis mechanism of the V-ATPase.

SPA maps of Syp<sup>−/−</sup> ISVs validated the V-ATPase–synaptophysin interaction and demonstrated the specificity of this interaction for synaptophysin, but not for the paralogs synaptoporin and synaptogyrin (Fig. 2d, g, h, Extended Data Fig. 6c, d). Comparison of the structures shows that the V-ATPase structure is not substantially affected by the interaction with synaptophysin (Extended Data Fig. 7 and Supplementary Table 3).
At the gross morphological level, Syp\textsuperscript{-} mice form normal synapses and synaptic vesicles\textsuperscript{42}, as also corroborated by our cryo-ET reconstructions of Syp\textsuperscript{-} ISVs (Fig. 4a).

Syp\textsuperscript{-} mice exhibit normal EPSCs and mEPSC release probabilities, though a small but significant increase in EPSC quantal amplitude was previously observed\textsuperscript{40}. More profound changes were observed with double genetic deletion of both synaptophysin and synaptogyrin, resulting in reduced short-term and long-term plasticity in neurons\textsuperscript{39}.

Quadruple knockout studies produced an even larger increase in quantal amplitude\textsuperscript{43}.

Importantly, we observed that Syp\textsuperscript{-} mice exhibit a severe seizure phenotype under pharmacological treatment with a glutamate agonist (Fig. 4d), which is concordant with the increase in quantal size observed for both Syp\textsuperscript{-} and quadruple knockout\textsuperscript{40,43}.

In addition to the interaction with the V-ATPase, synaptophysin also interacts with synaptobrevin\textsuperscript{44–46}. Synaptophysin is a highly abundant synaptic vesicle protein with an estimated 30 copies in synaptic vesicles\textsuperscript{1}. Consistent with the lower amount of synaptobrevin in Syp\textsuperscript{-} ISVs (Fig. 2d), synaptophysin plays a role in synaptobrevin sorting into synaptic vesicles\textsuperscript{34–36} and endocytosis\textsuperscript{47}, and presumably this sorting mechanism is assisted by the synaptophysin–synaptobrevin interactions. Despite the lower synaptobrevin amount in the Syp\textsuperscript{-} ISVs, the transmitter release probability is normal\textsuperscript{40}, consistent with the observation that two synaptobrevin molecules are sufficient for evoked synaptic vesicle fusion\textsuperscript{48} compared with the much larger copy number of synaptobrevin molecules in wild-type ISVs (~70 per vesicle, ref. \textsuperscript{1}).

The copy number of V-ATPases roughly doubled in Syp\textsuperscript{-} ISVs (Fig. 4b, c and Extended Data Fig. 8d-i). A possible explanation is that in wild-type ISVs, the V-ATPase–synaptophysin interaction effectively increases the lateral membrane cross-section of the combined molecular assembly. This increased membrane cross-section will limit the available area in the synaptic vesicle membrane due to molecular crowding by increasing the excluded surface area\textsuperscript{49}, resulting in a lower V-ATPase copy number in wild-type ISVs compared to Syp\textsuperscript{-} ISVs. Moreover, synaptophysin can form hexameric assemblies\textsuperscript{50} and interact with synaptobrevin\textsuperscript{11,46}. Since we did not observe densities for synaptobrevin or synaptophysin oligomers in our cryo-ET and SPA maps (Fig. 1c, d and Fig. 2a, b), it is possible that these interactions, if they exist, are heterogeneous and
averaged out in the EM maps. Nevertheless, these additional interactions would further increase the membrane cross-section of the combined assembly.

The increased V-ATPase copy number in Syp\(^{-}\) ISVs offers a possible explanation for the observed seizure phenotype under pharmacological treatment with a glutamate agonist (Fig. 4d), which is concordant with the increase in quantal size observed in Syp\(^{-}\) knockout studies\(^{40,43}\). This increased quantal size could potentially be different in excitatory and inhibitory synapses, as suggested by different synaptophysin copy numbers in glutamatergic and GABAergic wild-type ISVs\(^{2}\). Moreover, the increased copy number could exacerbate the leakiness of ISVs due to ultraslow mode switching\(^{51}\), further affecting this imbalance. Multiple studies have described seizure phenotypes due to mutations in the V-ATPase\(^{52-55}\), and some of which are close to the V-ATPase—synaptophysin interface. The phenotypic similarity between these mutations and synaptophysin knockout, in conjunction with our data, suggests that the increased seizure susceptibility has a similar etiology in both cases. Our observation of a substantially altered V-ATPase copy number per SV is a plausible contributor to the observed phenotype. In summary, we showed that synaptophysin is a factor that assists in establishing the proper copy number of V-ATPases in synaptic vesicles, and loss of synaptophysin causes severe stress-induced seizure phenotype likely due to an imbalance in neurotransmitter uptake and release.
Supplementary Information is available in the online version of the paper.

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References


Leitz, J. *et al.* Observing isolated synaptic vesicle association and fusion *ex vivo*. *Nat Protoc*, 10.1038/s41596-024-01014-x (2024).


Figure Captions

Figure 1 | Cryo-electron tomography of glutamatergic ISVs.

a, Schematic overview of glutamatergic synaptic vesicle isolation and cryo-EM grid preparation (see Methods). LP2: second lysis pellet. ISV: isolated synaptic vesicle.

b, A representative tomogram (one of 52 tomograms) (cryoCARE denoised) of ISVs is shown as a tomographic slice (left, thickness 1 nm) with a corresponding three-dimensional rendering (see Methods) (right). The ISV membrane, intact, and V0-only V-ATPase assemblies are colored in grey, yellow, and cyan, respectively. Three representative ISVs are boxed and annotated with the copy numbers of intact and V0-only V-ATPase assemblies specified within each.

c & d, Subtomogram averaging maps (see Methods and Extended Data Figure 1) of intact (State 3) and V0-only V-ATPase assemblies (transparent surfaces). The extra binding partner density (not part of the V-ATPase assembly) is colored orange and denoted with an asterisk.

Figure 2 | Maps of V-ATPases imaged in wild-type and Syp−/− ISVs.

a&b, SPA maps of intact and V0-only V-ATPase assemblies imaged in wild-type (WT) ISVs: (a) intact V-ATPase (State 3), (b) V0-only assemblies. The extra binding partner density is colored orange. The background grey arc represents the ISV membrane density.

c, Docking of models into the binding partner density (orange) extracted from the map shown in (a): solution NMR structure of synaptogyrin-1 (PDB:8A6M) and AlphaFold2 predicted atomic models of synaptogyrin-3, synaptoporin, and synaptophysin. The atomic models are shown in cartoon representation and colored yellow and blue to indicate if the model is in the interior or exterior of the map, respectively. For clarity, only the transmembrane and luminal parts are displayed, omitting the cytosolic regions that are predicted to be unstructured.
d. Representative western blots (one of nine independent measurements) of synaptic proteins in LP2 and ISV samples from wild-type and Syp\textsuperscript{−/−} ISVs (Extended Data Fig. 4).

Abs: antibodies; SYP1: synaptophysin-1; VGLUT1: vesicular glutamate transporter-1; SYT1: synaptotagmin-1; SYB2: synaptobrevin-2; SYNPR1: synaptoporin-1; SYG1: synaptogyrin-1; V1A: subunit A of V-ATPase V1 assemblies; V0a: subunit a of V-ATPase V0 assemblies.

e. DLS measurements of wild-type and Syp\textsuperscript{−/−} ISVs. The means and standard deviations of the ISV diameters were calculated from three independent ISV preparations. P-values are calculated by the Student’s t-test; n.s.: not statistically significant.

f. Size distribution analysis of wild-type and Syp\textsuperscript{−/−} ISVs by inspection of cryo-EM images. The means and standard deviations of the wild-type and Syp\textsuperscript{−/−} ISV diameters were calculated from 326 wild-type ISVs and 362 Syp\textsuperscript{−/−} ISVs, respectively. P-values are calculated by the Student’s t-test; n.s.: not statistically significant.

g&h, SPA maps of intact and V0-only V-ATPase assemblies imaged in Syp\textsuperscript{−/−} ISVs: (g) intact V-ATPase (State 3) and (h) V0-only assemblies. The V-ATPase subunits are colored as in panels a and b. The corresponding location of the extra binding partner density identified in wild-type ISVs is indicated as a dashed orange silhouette for comparison. The background grey arc represents the ISV membrane density.

Source numerical data are available in Source Data SourceDataFig2.xlsx
Figure 3 | Structures of V-ATPases imaged in wild-type ISVs.

a&b, Atomic models of the V-ATPase–synaptophysin complex imaged in wild-type ISVs: (a) intact V-ATPase (State 3) and (b) V0-only V-ATPase assembly. The V-ATPase subunits are colored as indicated, and synaptophysin is colored orange. The background grey arc represents the ISV membrane density. (a) and (b) use the same scale bar.

c, Interface (black circle) between V-ATPase subunits e2, a, and synaptophysin (SYP) (structure of the V0-only V-ATPase assembly of wild-type ISVs). The electrostatic surface potential of this interface is also shown along with an “Open book” view. The interface area is ~350 Å^2.

Figure 4 | Increase of V-ATPase copy numbers and seizures in Syp^-/- mice

a, A representative tomogram (one of 78 tomograms) (cryoCARE denoised) of ISVs from Syp^-/- mouse brains is shown as a tomographic slice (left, thickness 35.5 nm) with a corresponding three-dimensional rendering (right). The ISV membrane, the intact, and V0-only V-ATPase assemblies are colored in grey, yellow, and cyan, respectively. Five representative ISVs are boxed and labeled with the copy number of intact and V0-only V-ATPase assemblies identified within each. More examples and views are shown in Extended Data Figure 8a,b.

b&c, Distribution of intact (b) and V0-only (c) V-ATPase copy number per wild-type ISV or Syp^-/- ISV. Statistical significance tests are shown in Extended Data Figure 8d and e, and numerical data are available in Supplementary Tables 4-5.

d, Mice of age 4-6 months of two genotypes (wild-type (n=9), Syp^-/- (n=5)) were injected with kainic acid (25mg/kg intraperitoneal (i.p.)) and observed for 60 minutes with video recording. Seizure severity was scored blind by two observers using a modified Racine scale and the latency survival to R6 or R8 seizure severity is reported (see Methods). **p=0.0013, ***p<0.0001 (one-sided Gehan-Breslow-Wilcoxon test).

Source numerical data are available in Source Data SourceDataFig4.xlsx
Methods

Wild-type and Syp<sup>−/−</sup> mice

Male wild-type CD1 mice (23-26 days old, purchased from Charles River) and C57BL6 mice (23-26 days old, purchased from Jackson Laboratory) were used for synaptic vesicle preparations. Syp<sup>−/−</sup> mice<sup>42</sup> were a gift of R. Leube (RWTH Aachen University). Male Syp<sup>−/−</sup> mice (23-26 days old) were used for synaptic vesicle preparations. Both sexes of 4-6 months-old wild-type Black 6 (B6NTac, purchased from Jackson Laboratory) and Syp<sup>−/−</sup> mice were used in animal behavior experiments. Mice were maintained in a colony room with a 12 h light/dark cycle (lights on at 7:00 h) with ad libitum access to food and water.

Syp<sup>−/−</sup> mice genotyping

A one-millimeter mouse ear notch was placed into a 1.5 mL microcentrifuge tube and treated with 200 μL of 50 mmol NaOH. Samples were heated to 95°C in a heating block for 10 min. The tubes were cooled on ice for 10 minutes, and then 20 μL of Tris-HCl, pH 8.0, was added to neutralize the sample. The samples were centrifuged to pellet the tissue debris and the supernatant was used immediately for PCR using the following primers, 5’-ACTTCCATCCCTATTTCCCACACC-3’, 5’-TTCCACCCACCAGTTCAGTAGGA-3’, 5’-TCGCCCTTCTTGACGAGTTCTTCTG-3’ and the following cycle parameters: 3 min 95°C, 35 cycles of (30 sec 95°C, 30 sec 58°C, 40 sec 72°C), 3 min 72°C, hold 4°C. PCR products were analyzed by agarose gel electrophoresis using a 1KB ladder (NEB, N3231S). The wild-type fragment appears at ~280 bp (256 expected), and the Syp<sup>−/−</sup> mutant fragment at ~650 bp (500 expected).

Isolation of glutamatergic synaptic vesicles

Wild-type and Syp<sup>−/−</sup> glutamatergic ISVs were prepared and purified as described<sup>1,2,12</sup>. Eight wild-type mice (CD1 for WT TOMO data<sup>1</sup> only, specified below; all the other wild-type data are from C57BL6) and two Syp<sup>−/−</sup> mice were sacrificed to purify the
respective ISVs. Mouse brains were pooled in homogenizing buffer (HB: 50 mL 4 mM Na-HEPES, pH 7.4, and 320 mM sucrose; 9 mL for one brain) with protease inhibitors and homogenized in a 40 mL-Dounce homogenizer four times with the "A" pestle and three times with the "B" pestle. The homogenate was centrifuged at 880 × g for 10 min. The supernatant (S1) was collected and centrifuged at 12,074 × g for 15 min. For the wild-type P2 pellet, the supernatant (S2) was removed, the P2 pellet was resuspended with 25 mL HB with protease inhibitors, the dark center of the pellet was discarded, and the resuspension was transferred to a new tube and centrifuged at 27,167 g for 15 min. However, the centrifugation step was skipped for the Syp−/− P2 pellet since the P2 sample size was small, following another previously published protocol. The resulting wild-type pellet (P3) and Syp−/−-P2, both of which contain synaptosomes, were resuspended with 5 mL or 1 mL HB and then hypo-osmotically lysed by 40 mL or 9 mL H2O, respectively, followed by adding 1 M Na-HEPES, pH 7.4 and protease inhibitors to achieve a final concentration of 5 mM Na-HEPES. This solution was homogenized in a 40- or 25 mL-Dounce homogenizer three times and then centrifuged at 43,589 × g for 20 min. The supernatant (LS1) was ultra-centrifuged at 256,631 × g for 2 hours. The resulting pellet (LP2) containing different types of SVs was then homogenized in a 2 mL-Dounce homogenizer in 2 mL vesicle buffer (VB, 20 mM Na-HEPES, pH 7.4 and 90 mM NaCl). The homogenized SVs were further mechanically sheered through a 27-gauge needle. SV concentration was determined by BCA, aliquoted, flash frozen in liquid nitrogen, and stored at −80°C until use.

To isolate the glutamatergic synaptic vesicles, 500 μg of LP2 fractions were thawed on ice and diluted by the blocking buffer (VB+ 0.5% (w/v) BSA) with 5 μg mouse VGLUT1 monoclonal antibody (SySy, Cat.# 135303) to achieve a final volume of 1 mL. This solution was incubated overnight at 4 °C, and then added to 50 μL Dynabeads (Thermo Fisher Scientific, Cat.# 10004D) incubated for another 2 hours at 4 °C. The Dynabeads were washed once in the blocking buffer and twice in VB alone. Immuno-enriched VGLUT1-bound synaptic vesicles were then eluted three times by adding 25 μL of 40 mg/mL (for the WT TOMO data only, see below) or 1.1 mg/mL (for all the other wild-type and Syp−/− ISV tomography and SPA data collections) VGLUT1 epitope peptide. We tested the function of similar preparations of ISVs by a single-vesicle Ca2+-triggered
fusion assay\(^3\). The size and concentration of freshly prepared ISVs were determined via dynamic light scattering (DLS) using a DynaPro NanoStar (Wyatt Technologies).

**SDS PAGE and western blotting**

The wild-type LP2, wild-type ISV, Syp\(^{-}\) LP2, and Syp\(^{-}\) ISV samples were diluted with Laemmli sample buffer (BioRad, Cat.# 1610737) containing β-mercaptoethanol. The samples were loaded in TGX gels (BioRad, Cat.# 4569036) and separated by electrophoresis with a standard SDS running buffer. For western blot analysis, samples in the gel were transferred to PVDF membranes by the iBlot1 (Invitrogen). To verify the SYP depletion in the Syp\(^{-}\) samples, the membrane was incubated with primary anti-rabbit-synaptophysin-1 (1:1,000; SySy, Cat.# 101008) and secondary goat anti-rabbit-IRDye800CW (1: 3,000; LI-COR, Cat.# 926-32211) for fluorescent bands detection by iBright1500 (Invitrogen). To detect the other synaptic proteins on this membrane for a fair comparison, the secondary antibody was stripped by Restore fluorescent western blot stripping buffer (Thermo Scientific, Cat.# 62300) and recovered by the EveryBlot blocking buffer (BioRad, Cat.# 12010020). The primary and secondary antibodies were stripped from the western blot membranes following the manufacturer’s instructions. We incubated the probed blot in the 1X stripping buffer for 15 minutes and washed it 3 times for 5 minutes each in TBST washing buffer, then incubated the membrane in EveryBlot blocking buffer for 5 minutes until re-probing. The membrane was incubated with primary anti-mouse-synaptobrevin-2 (1:1,000; SySy, Cat.# 104211) and secondary goat anti-mouse-HRP (1: 10,000; Abcam, Cat.# ab6789) for chemiluminescent bands detection by iBright1500 (Invitrogen). For one replicate (Extended Data Fig. 4a), the stripped membrane was cut at 37 kDa, and the upper membrane was incubated with primary anti-mouse-synaptotagmin-1 (1:1,000; SySy, Cat.# 105011) and secondary goat anti-mouse-HRP (1: 10,000; Abcam, Cat.# ab6789) for chemiluminescent bands detection by iBright1500 (Invitrogen).

To compare different synaptic protein expression levels within a western blot, the membrane was cut into slices corresponding to 20 kDa, ~45 kDa, and 100 kDa ladder positions. The <20 kDa membrane slice was incubated with primary anti-mouse-
synaptobrevin-2 (1:1,000) and secondary goat anti-mouse-IRDye800CW (1: 3,000; LI-COR, Cat.# 926-32210). The 20-45 kDa membrane slice was incubated with primary anti-rabbit-synaptoporin-1 (1:500; SySy, Cat.# 102002) and secondary goat anti-rabbit-IRDye800CW (1: 3,000). The 45-100 kDa membrane slice was incubated with primary anti-rabbit-ATP6V1A (1:500; CST, Cat.# 39517) and secondary goat anti-rabbit-IRDye800CW (1: 3,000). The >100 kDa membrane slice was incubated with primary anti-rabbit-ATP6V1A1 (1:1,000; NovusBio, Cat.# NBP1-89342) and secondary goat anti-rabbit-IRDye800CW (1: 3,000). The fluorescent bands in the membrane slices were imaged by an iBright1500 imaging system (Thermo Fisher Scientific). To analyze other synaptic proteins on the same membrane, the secondary antibodies of the 20-45 kDa and 45-100 kDa membrane slices were then stripped and recovered. The primary and secondary antibodies were stripped from the western blot membranes following the manufacturer’s instructions (Thermo Fisher, Cat.# 62300). We incubated the probed blot in the 1X stripping buffer for 15 minutes and washed it 3 times for 5 minutes each in TBST washing buffer, then incubated the membrane in 5% BSA blocking buffer for 30 minutes until re-probing. The 20-45 kDa membrane slice was incubated with primary anti-rabbit-synaptotygrin-1 (1:500; SySy, Cat.# 103002) and secondary goat anti-rabbit-IRDye800CW (1: 3,000) and fluorescent bands were imaged by the iBright1500 imaging system. The stripped 45-100 kDa membrane slice was incubated with a mixture of primary anti-rabbit-VGLUT1 (1:1,000; SySy, Cat.# 135303) and anti-mouse-synaptotagmin-1 (1:1,000). The fluorescent band of VGLUT1 was imaged after incubation of the secondary goat anti-rabbit-IRDye800CW (1: 3,000); the chemiluminescent band of synaptotagmin-1 was imaged after incubation of the secondary goat anti-mouse-HRP (1: 10,000).

Densitometry and normalization were performed with the iBright Analysis Software (Thermo Fisher Scientific).

Cryo-EM grid preparation

The freshly isolated wild-type ISVs or Syp⁻/⁻ ISVs were mixed with or without pellets of the AURION 10 nm-BSA Gold Tracer (EMS, Cat.# 25487) solution (v/v=5/2) for the
cryo-EM grids preparation. For the wild-type samples, Quantifoil (R2/4, 300 mesh) gold grids with a continuous layer of graphene (Graphenea) were plasma cleaned for 30~40 seconds by using a Fischione Nanoclean 1070 plasma cleaner (30~34 W forward power, <<2 W reverse power, 55%~76% hydrogen delivered at 20 sccm). For the Syp⁺⁻ ISV samples, Quantifoil (R2/1, 300 mesh) gold holey-carbon grids (EMS, Cat.# Q3100AR1) were used without pre-treatment. Grids were then placed on parafilm in a custom humidity chamber with approximately 2 mL of MilliQ water added to the periphery of the chamber. 3.5~4 μL of ISVs were applied to the grids and incubated for 8 to 15 minutes. Grids were then directly loaded onto a Vitrobot Mark I (FEI, Field Electron and Ion Company) and an additional 3 μL of ISVs were applied. Grids were then back-blotted by replacing the sample side blot paper with a custom-cut Teflon sheet or Parafilm with a blot force of 5, a wait time of 1-10 sec at 22 ℃, and 95% relative humidity. For each round of freezing, we froze grids with varying blotting times of 1-4 seconds. Grids were plunged into pure ethane and stored in liquid nitrogen until use.

Tomographic data collection & reconstruction

For the first cryo-ET data set of wild-type ISVs, grids were loaded onto a Titan Krios transmission cryo-electron microscopy (TEM) at the Max Planck Institute of Biochemistry operated at 300 kV and equipped with a field emission gun and a Gatan post-column energy filter. Images were recorded by a Gatan K2 Summit direct detector using dose fractionation mode (6.09 e⁻/pixel/s dose rate, 0.33 s exposure time per frame). Tilt series were collected using SerialEM⁵⁷ at a nominal magnification of 53 000×, resulting in a pixel size of 2.6 Å/pixel, with defocus range between 4.0 to 5.0 μm. 74 tomographic tilt series were collected using a dose-symmetric tilt scheme⁵⁸ between ±60° with an increment of 3°. The same exposure time (3.3 s exposure time and 10 frames per image) was used for each tilt angle, resulting in a total dose of around 120 e⁻/Å² per tilt series. We refer to this data set as WT TOMO data1 in Extended Data Table 1, and it was used for the experiments shown in Fig. 1b-d and Extended Data Fig. 1. Movie frames were aligned using Motioncor2 (ref. ⁵⁹), and motion-corrected tilt series were then aligned using fiducial tracking with the IMOD software package⁶⁰.
A second cryo-ET data set of wild-type ISVs (used in Figure 4b-c and Extended Data Fig. 8b, we refer to this data set as WT TOMO data2 in Extended Data Table 1) and the cryo-ET data set of Syp\textsuperscript{\textdagger} ISVs (used in Figure 4a\&b-c and Extended Data Fig. 8a, we refer to this data set as Syp\textsuperscript{\textdagger} TOMO data1 in Extended Data Table 1) were collected on a Titan Krios TEM at the Center of Electron Microscopy (cEMc) at Stanford operated at 300 kV and equipped with a field emission gun and a Gatan post-column energy filter. Images of wild-type ISVs and Syp\textsuperscript{\textdagger} ISVs were recorded by a Gatan K3 Summit direct detector using dose fractionation mode with SerialEM\textsuperscript{57} using the PACEtomo script and a built-in dose-symmetric tilt scheme, respectively. 203 wild-type ISVs and 109 Syp\textsuperscript{\textdagger} ISVs tomographic tilt series data were collected using SerialEM in low-dose mode at a physical pixel size of 1.1 Å/pixel. The energy filter slit-width was set to 20 eV. Tilt series were collected using a dose-symmetric tilt scheme\textsuperscript{58} between ±60˚ with an increment of 3˚. The defocus was set to be 1.0 to 4.0 μm with a step of 0.3 μm per tilt. The dose per tilt was 3.4 e/Å\textsuperscript{2} with a per-frame dose of 0.34 e/Å\textsuperscript{2}, resulting in a total dose of 139.4 e/Å\textsuperscript{2} over 41 tilts. Movie frames were aligned using Motioncor2\textsuperscript{59}, and the motion-corrected tilt series were then aligned using the patch-based local motion alignment with the AreTomo software package\textsuperscript{61}.

For all three datasets, the tomograms were reconstructed from aligned tilt-series using the weighted back projection approach in the IMOD software package\textsuperscript{60}. After manual inspection, a subset of 52 tomograms from the WT TOMO data1 dataset, 156 tomograms from the WT TOMO data2 dataset, and 78 tomograms from the Syp\textsuperscript{\textdagger} TOMO data1 dataset were kept for further analysis.

Subtomogram averaging

For the analysis of WT TOMO data1 experiments shown in Figure 1 and Extended Data Fig. 1, the MATLAB (Mathworks) TOM toolbox\textsuperscript{62} was used as a general platform for image processing. All the tomograms were binned (binning factor = 4, 10.4 Å/pixel) for processing. Six of them were used to generate the initial model. The template-free detection method PySeg\textsuperscript{63} was employed to identify membrane-bound complexes. In brief, all the synaptic vesicle membranes were segmented out by a template-matching
approach, using hollow spheres with different diameters as templates. A discrete Morse theory-based algorithm was used to trace all the densities associated with synaptic vesicles. Subtomogram candidates were selected based on the direction and size of these densities. For each subtomogram, the normal vector of the attaching membrane was determined, and a rotational average was generated around this direction. The averages were classified by affinity propagation method\textsuperscript{63}. Classes with clear membrane attaching densities were combined for constrained alignment and classification using RELION\textsuperscript{64}. The resulting structure clearly showed a V-ATPase (Extended Data Fig. 1a).

The resulting structure was then used as a new template to perform a new round of template matching using PyTom\textsuperscript{65}, but this time using all the binned tomograms. The resulting hits were manually sorted to produce the final dataset. In total, 1860 V-ATPases were identified from 52 tomograms. Subsequently, the unbinned (full-size) tomograms were used for classification and averaging. Subtomograms of 192×192×192 pixel volume were extracted with Warp\textsuperscript{66}, with which 3D-CTF models were generated for each subtomogram. The classification was performed using the 3D tomography workflow of RELION\textsuperscript{64}, with a soft mask applied in the cytosolic domain. Three different conformations of the cytosolic domain were identified. A soft mask, including the entire molecular assembly, was used for the final refinement. Another round of multi-particle refinement was applied to the final maps using Warp-M\textsuperscript{67}, further refining the tilt series alignment parameters. For each subtomogram, only the first 15 subtilts (45 e\textsuperscript{-}/Å\textsuperscript{2} accumulative dose) were included for the final reconstruction and resolution estimation. The resolution was determined using the 0.143 criteria according to the gold-standard Fourier Shell Correlation (FSC) (ref. \textsuperscript{68}).

Tomogram segmentation and 3D rendering

To visualize the ISV membranes, we first employed an automated tracing method based on tensor voting\textsuperscript{69}. Subsequently, any necessary manual corrections were made using Amira software (Thermo Fisher Scientific). V-ATPase assemblies were positioned in their native locations and orientations. This positioning utilized low-pass filtered reconstructed maps and the Euler angle information obtained from either subtomogram.
averaging (Fig. 1b) or template matching (Fig. 4a). ChimeraX was used to generate the final renderings shown in Fig. 1b (right) and Fig. 4a (right).

V-ATPase copy number analysis

The V-ATPase maps from WT TOMO data 1 were filtered to 30 Å and used as templates for V-ATPase assemblies identification in the WT TOMO data2 and Syp\(^+\) TOMO data1 tomograms. Template matching was performed on the binned tomograms (binning factor =8, 8.8 Å/pixel) with PyTom\(^65\). Tomograms were then Wiener-filtered using PyCresta (examples are in Extended Data Fig. 8a-b). The Wiener filtering was performed with a defocus setting of 3.0 μm, and a signal-to-noise ratio falloff of 0.9. In addition, the tomograms were denoised by cryoCARE\(^38\) without Wiener-filtering (an example is in Fig. 4a). The template matching results were manually checked in both the Wiener-filtered and cryoCARE denoised maps.

Two individuals independently examined two half sets of the tomograms and counted the copy numbers of intact and V0-only V-ATPase assemblies per ISV (Supplementary Table 4-5). For the intact V-ATPase, we examined 1,326 wild-type ISVs and 1,453 Syp\(^+\) ISVs. For the V0-only V-ATPase, we examined a smaller number of ISVs since it is much more time-consuming to identify the smaller V0-only assemblies (106 wild-type ISVs and 188 Syp\(^+\) ISVs, respectively).

To conduct statistical significance tests, we employed a bootstrapping statistical procedure by a random re-sampling with replacement of the observed copy numbers for each ISV for the four groups (Extended Data Fig. 8d-e). This process was repeated 10,000 times for each group, leading to the generation of 10,000 simulated distributions of V-ATPase copy numbers for each group. The mean and standard deviations were calculated for each copy number and group. Statistical significance was assessed using the Student’s t-test. All the analyses were performed using the R software.

To fit Poisson distributions to the copy numbers for each of the four groups, ISVs with at least one intact or V0-only V-ATPase assemblies were used (Extended Data Fig 8. f-i). Scale factors between the observed copy number and the Poisson distributions, as well as
the lambda parameters of the Poisson distributions, were estimated using a least squares
method implemented in a Python script. For the case of intact V-ATPase assemblies, the
Poisson fits predict the number of ISVs without intact V-ATPases and suggest that our
observations overestimated the number of ISVs without intact V-ATPases. This degree of
overestimation is largely due to the missing wedge effect. On the other hand, the missing
wedge effect also implies that we underestimated the number of intact V-ATPases for
copy numbers ≥1. However, the λ parameter of the fitted Poisson distribution should be
independent of the missed fraction of V-ATPases, so it represents the true average copy
number of intact V-ATPases per ISV. For the case of V0 assemblies, similar arguments
apply. However, there is no overestimation of the number of ISVs without V0 assembly
in wild-type ISVs, possibly due to slight uncertainties in counting V0 assemblies. All
calculations were performed using the SciPy module from Python (v3.8).

Single-particle cryo-EM

For SPA data collection, cryo-EM grids with wild-type ISVs and Syp+/− ISVs were
imaged using a Titan Krios electron microscope (Thermo Fisher Scientific) equipped
with a K3 camera (Gatan) at the Stanford cEMc using the Serial-EM automation
software57. The nominal magnification was 81,000×, resulting in a physical pixel size of
1.1 Å. At each stage position, a group of 4 or 9 holes was imaged using the multiple
record setup, and each hole contained 3 or 4 imaging spots. A 50-frame movie stack was
collected at each imaging spot with a total exposure time of 4.0 s. The dose rate was ~
15.5 e−/pixel/s with a 0.08 s exposure time per frame; the total dose of one movie stack
was 50 e−/Å².

All images of wild-type ISVs were preprocessed in RELION v3.1 (ref. 70). In total,
21,577 movies were collected and aligned with Motioncor2 (ref. 59) and CTF parameters
were estimated from the average of aligned frames with CTFFIND471. We used Topaz72
for particle picking. 524 particles of apparent intact V-ATPases were manually picked
from 108 micrographs for the training set. Using this training set, Topaz picked 33,094
particles from 4,404 micrographs. We then performed several rounds of 2D and 3D
classification with RELION, which returned 4,461 intact V-ATPase candidates. The
template for 3D classification was obtained from previous work.\textsuperscript{41} Topaz was retrained using these particles and returned 321,087 particles from the entire dataset. After several rounds of 2D and 3D classification, 77,779 particles were kept for further analysis. To classify these particles, we adopted a soft mask of the cytoplasmic region of the intact V-ATPase assembly, which resulted in three classes, representing the three States of the V-ATPase. The map quality was further improved by several rounds of 3D refinement, CTF refinement, postprocessing, and Bayesian polishing in RELION. Next, these particles were exported into cryoSPARC v3.2 (ref. \textsuperscript{73}), followed by non-uniform refinement, which resulted in overall resolutions of 4.3 Å for all three states. For the V0-only V-ATPase assemblies, a re-scaled V0-only focused-2D-class from the intact V-ATPase 2D classes was used as a template for particle picking in 13,036 micrographs using Topaz. A total of 4218 particles were selected by several rounds of 2D classification to re-train the Topaz picker. Ultimately, 699,268 particles were picked, followed by a series of sequential steps in RELION: 2D classification, 3D classification, 3D refinement, CTF refinement, postprocessing, and Bayesian polishing. The refined particles were subsequently exported into cryoSPARC. After non-uniform refinement, the overall resolution was 3.8 Å.

All images of Syp\textsuperscript{−/−} ISVs were preprocessed in cryoSPARC v4.4 (ref. \textsuperscript{73}). In total, 20,027 movies were collected and aligned with patch motion correction and CTF parameters were estimated from the average of aligned frames with patch CTF estimation. Again, we used Topaz\textsuperscript{22} for V-ATPase particle picking. 179 particles of apparent intact V-ATPase assemblies were manually picked from 327 micrographs for the training set. We used this training set to pick 47,880 particles from 3,500 micrographs, followed by several rounds of 2D classification in cryoSPARC, which returned a total of 10,860 particles. Topaz was re-trained using these particles, and it picked 423,510 particles from the entire dataset. After several rounds of 2D classification, 137,987 particles were exported into RELION v4.0 (ref. \textsuperscript{74}) for further 3D classification. To classify these particles with no bias, we first used a soft mask that was derived from the map of the intact V-ATPase assembly in wild-type ISVs. The resulting 84,686 particles were further classified using a soft mask of the cytoplasmic region of the V-ATPase, resulting in three classes representing three States of the V-ATPase. The map quality was further improved by several rounds of 3D
refinement, postprocessing, and CTF refinement in RELION, which resulted in three
maps with resolutions of 4.5, 4.4, and 4.5 Å for States 1, 2, and 3, respectively. For the
V0-only V-ATPase assemblies in Syp⁻/⁻ ISVs, we used the V0-only Topaz model of the
wild-type ISVs dataset and performed particle picking from 13036 micrographs. A total
of 639,377 particles were picked, followed by two rounds of 2D-classification. To further
classify these particles, we used the 2D-classification-accepted 91,542 particles for ab
initio reconstruction of four classes, and one decoy class consisting of 2D-classification-
declined particles. Then, we applied these 5 classes as hetero-refinement templates of
91,542 particles, which returned 56,885 particles for further processing in cryoSPARC:
non-uniform refinement, global CTF refinement, local CTF refinement, local refinement,
and postprocessing. The final refined map of V0-only V-ATPase assemblies in Syp⁻/⁻
ISVs had a resolution of 3.6 Å.

All FSC curves were calculated with independently refined half-maps, and the resolution
was assessed using the 0.143 criterion with a correction for the masking effects. The local
resolutions of all the wild-type maps and V0-only V-ATPase maps of Syp⁻/⁻ ISVs were
estimated by the “Local Resolution Estimation” method in cryoSPARC. The local
resolutions of the intact V-ATPase maps for Syp⁻/⁻ ISVs were estimated using the “Local
Resolution” method in RELION. The visualization of the local resolution maps
(Extended Data Fig. 2f and 4f) was performed with ChimeraX. Orientational sampling
was assessed for the final maps of the intact and V0-only V-ATPase assemblies
(Extended Data Fig. 2g and 4g) using the Euler angles from RELION 3D refinement and
cryoSPARC non-uniform refinement results.

**Measurement of ISV diameters**

Diameters of ISVs were estimated from micrographs collected for SPA and from
tomograms. For each tomogram, a two-dimensional projection was generated by
summing intensities along the Z-axis. For each ISV containing at least one detected intact
or V0-only V-ATPase, three points on its membrane edge were manually selected. Then,
a circle was fit based on these points to calculate the diameter of the ISV.
Model building and refinement

Initial models of the V-ATPase and synaptophysin complexes for our wild-type (mouse) ISV SPA data were generated based on the deposited rat (PDB IDs: 6VQ6, 6VQ7, 6VQ8, 6VQH) and human (PDB IDs: 6WM2, 6WM3, 6WM4, 6WLW) V-ATPase structures and a mouse synaptophysin model predicted by AlphaFold2. After mutation of the model according to the mouse sequence with Coot, the models were fitted as rigid bodies into cryo-EM maps using ChimeraX. These models were manually adjusted in Coot before being imported into ISOLDE within ChimeraX to adjust sidechain rotamers. The final models were evaluated through multiple rounds of refinement using Coot and Phenix and validated with EMRinger. The atomic models for the remaining regions of the V-ATPase assemblies were modeled based on previous work, followed by mutation according to the mouse sequence using Coot. The resulting models were then rigid-body fitted into the focused maps by ChimeraX, and then refined with Coot and Phenix. For illustration purposes, composite models of three rotational states were generated and followed the same strategy in previous work.

The structures of the intact and V0-only V-ATPase assemblies in Syp−/− ISVs were modeled similarly to our wild-type structures, which involves automatic remodeling by Rosetta, and iterative manual adjustment by Coot, real space refinement, and ADP refinement with Phenix.

Structural analysis

We used the ChimeraX electrostatic function to calculate the electrostatic potential of the interface region of the V-ATPase–synaptophysin complex.

To gain insights into structural variations, we computed the root-mean-square differences (RMSDs) for Ca atoms between all the wild-type and Syp−/− models of both intact and V0-only V-ATPases (Supplementary Table 3). We used State 2 of the intact V-ATPase for PDB IDs 6WM3, 7U4T, 6VQG, and 7UNF, since the 7UNF deposition only includes State 2. These models, along with the Syp−/− ones, were globally aligned to the wild-type
models, and the RMSD values were subsequently calculated using a custom Python
script. The RMSDs were visualized in PyMol with the ColorbyRMSD function.

**Proteomics of wild-type ISV**

To obtain a comprehensive list of proteins from the ISV sample, we used the high-
resolution MS sample preparation and liquid chromatography (LC)-MS/MS method.
Briefly, add 100 μL 6M GdmCl, 10 mM TCEP, 40 mM CAA, and 100 mM Tris pH 8.5
buffer to three different ISVs preparations (26 μg each). Lysates were incubated at 95 °C
for 5 min and briefly vortexed. The protein concentration was measured using the
Bicinchoninic Acid Assay (BCA) method. Samples were then digested by trypsin
overnight at 37 °C with a protein-to-enzyme ratio of 50:1. Digestion was stopped by
adding 1% Trifluoroacetic Acid (TFA) and samples were cleaned up using Oasis HLB
cartridge (1cc/10mg, Waters). Digested peptide samples were dried by speed vac and re-
dissolved in 100 mM Triethylammonium Bicarbonate (TEAB), and were labeled with
TMT 10plex reagent (Thermo Fisher Scientific) as instructed by the vendor and
subsequently combined at equal amounts.

Waters 2D LC (Waters MClass 2DnLC) was used for peptide separation. Peptides were
separated by reverse phase chromatography at high pH in the first dimension, followed
by an orthogonal separation at low pH in the second dimension. In the first dimension,
the mobile phases were buffer A: 20 mM ammonium formate at pH10 and buffer B:
Acetonitrile. Peptides were separated on a BEH 300 μM × 5 cm C18 5.0 μM column
(Waters) using 12 discontinuous step gradients at 2 μL/min. In the second dimension,
peptides were loaded to an in-house packed 75 μM ID/10μM tip ID × 28 cm C18-AQ 1.8
μM resin column with buffer A (0.1% formic acid in water). Peptides were separated
with a gradient from 5% to 40% buffer B (0.1% formic acid in acetonitrile) at a flow rate
of 300 nL/min in 180 min. The LC system was directly coupled in-line with an Orbitrap

The source was operated at 1.8-2.2 kV to optimize the nanospray with the ion transfer
tube at 275 °C. The mass spectrometer was run in a data-dependent mode. A full MS
scan was acquired in the Orbitrap mass analyzer from 400-1500 m/z with a resolution of 120,000. Precursors were isolated with an isolation window of 0.7 m/z and fragmented using CID at 35% energy in the ion trap in rapid mode. MS1 AGC is $4 \times 10^4$; MS2 AGC is $10^4$. The maximum injection time was 100 ms. Subsequently, 8 fragment ions were selected for MS3 analysis, isolated with an m/z window of 1.6, and fragmented with HCD at 65% energy. Resulting fragments were detected in the Orbitrap at 60,000 resolution, with a maximum injection time of 150 ms or until the MS3 AGC target value of $10^5$ was reached.

The raw data acquired were processed with the Proteome Discoverer (Thermo Fisher Scientific). A mass tolerance of 10 ppm was used for precursor ions and 0.6 Dalton for fragment ions for the UniProt Mus musculus proteins database search. The search included cysteine carbamidomethylation as a fixed modification. Acetylation at the protein N-terminus, methionine oxidation, and TMT at the peptide N-terminus and Lysine were used as variable modifications. Up to two missed cleavages were allowed for trypsin digestion. Only unique peptides with a minimum 6 amino acid length were considered for protein identification. The peptide false discovery rate (FDR) was set as less than 1%. Data was searched against the Mouse database from UniProt. Spectra with more than 50% interference were excluded for subsequent quantitative analysis. The final protein lists (2,687 protein candidates in total, Supplementary Table 1) used two filters: 1. a protein with more than two unique peptides; 2. it is marked as a master protein.

**Identification of the e subunit**

To identify the isoform of V-ATPase subunit e in ISVs, we used chymotrypsin in the mass-spectrometry sample preparation. 5 μg of each of three different ISV preparations were transferred to a new tube and normalized to 50 μL with 10% sodium dodecyl sulfate (SDS)/100mM triethylammonium bicarbonate (TEAB). The samples were then reduced with 10 mM dithiothreitol (DTT) for 20 min at 55 °C, cooled to room temperature, and then alkylated with 30 mM acrylamide for 30 min. They were then acidified to a pH ~1 with 2.6 μL of 27% phosphoric acid and in 165 μL of S-trap loading buffer (90% methanol/10% 1M TEAB) and loaded onto S-trap microcolumns (Protifi, USA). After
loading, the samples were washed sequentially with 150 μL increments of 90% methanol/10% 100mM TEAB, 90% methanol/10% 20 mM TEAB, and 90% methanol/10% 5mM TEAB solutions, respectively. Samples were digested at 47 °C for two hours with 600 ng of mass spectrometry-grade chymotrypsin (Promega, USA). The digested peptides were then eluted with two 35 μL increments of 0.2% formic acid in water and two more 40 μL increments of 80% acetonitrile with 0.2% formic acid in water. The four eluents were consolidated in 1.5 mL S-trap recovery tubes and dried via SpeedVac (Thermo Fisher Scientific). Finally, the dried peptides were reconstituted in 2% acetonitrile with 0.1% formic acid in water for LC-MS analysis.

Proteolytically digested peptides were separated using an in-house pulled and packed reversed-phase analytical column (~25 cm long, 100 μm of I.D.), with Dr. Maisch 1.9 μm C18 beads as the stationary phase. Separation was performed with an 80-minute reverse-phase gradient (2-45% B, followed by a high-B wash) on an Acquity M-Class UPLC system (Waters Corporation, Milford, MA) at a flow rate of 300 nL/min. Mobile Phase A was 0.2% formic acid in water, while Mobile Phase B was 0.2% formic acid in acetonitrile. Ions were formed by electrospray ionization and analyzed by an Orbitrap Exploris 480 mass spectrometer (Thermo Fisher Scientific). The mass spectrometer was operated in a data-dependent mode using HCD fragmentation for MS/MS spectra generation.

The raw data were analyzed using Byonic v5.1.1 (Protein Metrics, Cupertino, CA) to identify peptides and infer proteins. Concatenated FASTA files containing UniProt mus musculus proteins and other likely contaminants and impurities were used to generate an in silico peptide library. Proteolysis with chymotrypsin was assumed to be semi-specific, allowing for N-ragged cleavage with up to two missed cleavage sites. The precursor and fragment ion tolerances were set to 12 ppm. Cysteine modified with propionamide was set as a fixed modification in the search. Variable modifications included oxidation on methionine, deoxidation on tryptophan, glutamine and glutamic acid cyclization, and N-terminal acetylation. Proteins were held to a false discovery rate of 1% using the standard reverse-decoy technique\textsuperscript{82}. The final identified protein lists (Supplementary Table 2) used
two filters: 1. The identified peptide is unique; 2. The spectrum score is larger than 300 according to Byonic's scoring algorithm\textsuperscript{83}.

**Unbiased matching of the binding partner density**

The binding partner densities were manually extracted from both the SPA maps of the intact (State 3) or V0-only V-ATPase assembly of wild-type ISVs using UCSF Chimera. The extracted maps were placed in a 100 pixel\(^3\) box with a pixel size of 1.1 Å. The AlphaFold\textsuperscript{29} predicted atomic models corresponding to the high-resolution-MS-detected ISV proteins were downloaded. The unbiased matching was performed using the CoLoRes program in the Situs package\textsuperscript{30,31} with a resolution setting of 6 Å, a searching degree of 15, and a map contour cut-off of 0.01 or 0.004 for the intact or V0-only segmented extra density map, respectively. The matching was scored and ranked by unnormalized CoLoRes cross-correlation scores (CCS) (rows 2-2641 of Supplementary Table 1). There are 49 other proteins (rows 2642-2694 of Supplementary Table 1) without an AlphaFold\textsuperscript{2} prediction, but they are either too large in size or not known to be synaptic membrane proteins. Moreover, some large structures that led to unrealistically high CCS values (>1) were checked but ignored. The top 200 hits with reasonable CCS values (< 1) were all inspected individually with the target densities in ChimeraX. Candidates that were not membrane proteins or had no membrane domain matching the observed binding partner density in the membrane region were ignored for further analysis. Among those that appeared reasonable, we rigid-body adjusted the fit using ChimeraX. We then calculated the percentage of outliers using ChimeraX by normalizing the number of C\(\alpha\) atoms residing outside the binding partner density against the total number of C\(\alpha\) atoms. Using the percentage of outliers as a criterion, the final set of top candidates was synaptogyrin-1, synatogyrin-3, synatoporin, and synaptophysin.
Pharmacological seizure susceptibility

Mice of each genotype were assayed between 4-6 months of age. All mice were weighed on the day of the experiment and were administered 25 mg/kg kainic acid intraperitoneal (i.p.) from a freshly prepared 5mg/mL PBS solution (n=9 wild-type and n=6 Syp−/− mice). Mice were immediately placed in a cylindrical observation chamber and monitored and scored in real-time. The observational behavior was scored blind according to the modified Racine scale 6 or 8 as defined in ref. 84. The latency curves were compared using a survival Log-rank (Mantel-Cox) test in GraphPad Prism.

Software and code

We used AlphaFold229, Amira v2020.2 (Thermo Fisher Scientific), AreTomo v1.3.4 (ref. 61), Byonic by Protein Metrics v5.1.1 (ref. 83), Chimera v1.16 & ChimeraX v1.3&1.7 (ref. 76), cryoSPARC v4.4 (ref. 73), CTFFIND471, iBright1500 Analysis Software (v5.2.2, Thermo Fisher Scientific), EMRinger v1.0.0 (ref. 79), IMOD v 4.11.3 (ref. 60), ISOLDE v1.3 (ref. 77), Motioncor2 (ref. 59), Phenix v1.21 (ref. 78), Prism v10.2.2 (GraphPad Software), Proteome Discoverer v2.1 (Thermo Fisher Scientific), PyCrESTA (https://github.com/brungerlab/pycresta), PyMol v2.3.2 (Schrödinger, LLC), Python v3.8, PySeg v1.0.0 (ref. 63), PyTom v0.98a1&1.1(ref. 65), R v3.4.2, RELION v3.1&4.0 (ref. 70), Rosetta80, Scientific Xcalibur (v4.1, Thermo Fisher Scientific), SerialEM v4.0&4.1 (ref. 57), Situs v3.251, Topaz v0.2.572, Warp v1.0.9(ref. 66). Several script files are available at https://github.com/brungerlab/ISV_scripts.

Animal statement

All animal procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Stanford Administrative Panel on Laboratory Animal Care (APLAC) institutional guidelines (protocol #29981) and by the University of Colorado Boulder Institutional Animal Care and Use Committee (IACUC) (protocol #1106.02).

Statistics and reproducibility
No statistical method was used to predetermine the sample size, but experiments described in this study were performed with at least three to nine samples for each group. The bootstrapping statistical procedures were done by randomly sampling repeats with replacement (Extended Data Fig. 8d, e). Two individuals independently examined two halves of the tomograms and counted the copy numbers of V-ATPase per ISV (Fig. 4b, c). The mice observational behavior was scored blind by two individuals (Fig. 4d).

Data availability
The subtomogram averaging maps (wild-type V0-only: 44858, wild-type State 1: 44855, wild-type State 2: 44856, wild-type State 3: 44857), the SPA maps (wild-type V0-only: 44846, wild-type State 1: 44843, wild-type State 2: 44839, wild-type State 3: 44840, SypΔ V0-only: 44845, SypΔ State 1: 44844, SypΔ State 2: 44842, SypΔ State 3: 44841) and representative binned tomograms (SypΔ ISV:44847, wild-type ISV:44848) have been deposited in the EMDB, and the atomic coordinates have been deposited in the PDB (wild-type V0-only: 9BRZ, wild-type State 1: 9BRT, wild-type State 2: 9BRA, wild-type State 3: 9BRQ, SypΔ V0-only: 9BRY, SypΔ State 1: 9BRU, SypΔ State 2: 9BRS, SypΔ State 3: 9BRR).

Methods references


Extended Data Figure Captions

Extended Data Figure 1 | Workflow of subtomogram averaging of V-ATPase assemblies in wildtype ISVs (related to Figure 1).

a, Workflow of particle picking, subtomogram averaging, and classification of intact and V0-only V-ATPase assemblies (see Methods).

b, Gold-standard Fourier shell correlation (FSC) curves of the resulting subtomogram average maps.

Extended Data Figure 2 | Single-particle averaging workflow of V-ATPase assemblies in wild-type ISVs (related to Figure 3).

a, Representative micrograph (one of 21,577 micrographs) of ISVs and V-ATPase assemblies 2D classification averages. The micrograph was Topaz-denoised for better visualization.

b&c, Cryo-EM image processing workflow of (b) intact V-ATPase assemblies in three rotational states and (c) V0-only V-ATPase assemblies (see Methods).

d&e, Gold-standard FSC curves of the intact V-ATPase assembly in three rotational states and of the V0-only V-ATPase assembly.

f, Refined maps of intact V-ATPase State 3 and V0-only V-ATPase assemblies, colored according to local resolution estimated in cryoSPARC (see Methods).

g, Particle orientation distributions of intact V-ATPase assemblies in three rotational states and V0-only V-ATPase assemblies (see Methods). The orientational distributions should adequately sample Fourier space.

Extended Data Figure 3 | Western blots of synaptic proteins in wild-type and Syp⁻/⁻ samples (related to Figure 2).

a&b, Western blots of (a) synaptophysin in LP2 and ISV samples from wild-type and Syp⁻/⁻ mouse brains, and (b) quantification. Blotting was performed with antibodies (Abs) for synaptophysin-1 (SYP1) (for Syp⁻/⁻ validation), synaptobrevin-2 (SYB2), and
synaptotagmin-1 (SYT1). (b) For each antibody and blot repeat, the band densities for Syp<sup>−/−</sup> LP2 and Syp<sup>−/−</sup> ISVs were first normalized to the respective band densities of wild-type LP2 and wild-type ISV, respectively. The synaptophysin-1 density of Syp<sup>−/−</sup> LP2 and Syp<sup>−/−</sup> ISV was then normalized to the synaptobrevin-2 density of wild-type LP2 and wild-type ISV, respectively. Data are presented as mean ± SEM; error bars (black) represent the SEM of nine independent measurements (biological and technical repeat numbers are specified in the figure); p-values are indicated in the figure and calculated by Student’s t-test (n = 9), *** p < 0.001.

c&d, Western blots of (c) synaptic proteins in LP2 and ISV samples from wild-type and Syp<sup>−/−</sup> ISVs mouse brains, followed by quantification (d, e). Blotting was performed with antibodies (Abs) for synaptotagmin-1 (SYT1), vesicular glutamate transporter-1 (VGLUT1), synaptoporin-1 (SYNPR1), synaptogyrin-1 (SYG1), subunit A of the V-ATPase V1 assembly (V1A), and subunit a of the V-ATPase V0 assembly (V0a). (e) For each antibody and blot repeat, the band densities for Syp<sup>−/−</sup> ISV were first normalized to the respective band densities of wild-type ISVs. The synaptic protein densities of Syp<sup>−/−</sup> ISVs were then normalized to the SYT1 (d) or VGLUT1 (e) densities in wild-type ISVs, respectively. Data are presented as mean ± SEM; error bars (black) represent the SEM of nine independent measurements (biological and technical repeat numbers are specified in the figure); p-values are indicated in the figure and calculated by Student’s t-test (n = 9), *p < 0.05, ** p < 0.01.

Unprocessed blots and source numerical data are available in Source Data

SourceDataExtendedDataFig3.xlsx

Extended Data Figure 4 | Single-particle averaging workflow of V-ATPase assemblies in Syp<sup>−/−</sup> ISVs.

a, Representative micrograph (one of 20,027 micrographs) of Syp<sup>−/−</sup> ISVs and V-ATPase 2D classification averages. The micrograph was Topaz-denoised for better visualization.

b&c, Cryo-EM image processing workflow of (b) intact V-ATPase assemblies in three rotational states and (c) V0-only V-ATPase assemblies in Syp<sup>−/−</sup> ISVs (see Methods).
d&e, Gold-standard FSC curves of intact V-ATPase assemblies in three rotational states and V0-only V-ATPase assemblies in Syp⁻/⁻ ISVs.

f, Refined maps of intact V-ATPase State 3 and V0-only V-ATPase assemblies colored according to local resolution estimated in RELION and cryoSPARC (see Methods).

g, Orientation distributions for the particles of intact V-ATPase assemblies in three rotational states and V0-only V-ATPase assemblies in Syp⁻/⁻ ISVs (see Methods).

Extended Data Figure 5 | Examples of map quality and atomic model fits (related to Figure 3).
a, Models of subunits A, B2, G2, D, and E1 superimposed on the wild-type intact V-ATPase map.

b, Models of subunits c’ and d superimposed on the wild-type intact V-ATPase map.

c, Models of subunits ATP6AP1/AC45 and ATP6AP2/PRR superimposed on the wild-type intact V-ATPase map.

d, Model of the ATP6AP1 luminal domain superimposed on the wild-type V0-only V-ATPase map. Potential glycosylated sites are shown and annotated using a stick model.

Extended Data Figure 6 | Analysis of the V-ATPase–synaptophysin interface (related to Figure 3).
a, Upper: primary sequence alignment of V-ATPase subunit e1 (ATP6V0E1) and e2 (ATP6V0E2). Representative residues of V-ATPase subunit e2 that differ from e1 are highlighted. Lower: Superposition of the structure and the map for the V0-only V-ATPase assembly in Syp⁻/⁻ ISVs (we used the Syp⁻/⁻ data since it produced the highest resolution for the V0 domain).

b, SPA map of subunits e2 (blue), a (green) and synaptophysin (orange) for the V0-only V-ATPase assembly of wild-type ISVs. The inset shows a close-up view.
c, The atomic model of synaptophysin fitted into the V0-only V-ATPase map of wild-type ISVs (see Methods) is colored according to the primary sequence conservation score compared with synaptoporin. The inset shows a close-up view.

d, Primary sequence alignment of synaptophysin and synaptoporin. Red-shaded regions indicate strictly conserved residues, and blue boxes indicate conserved residues. Thick blue and pink stripes indicate the transmembrane and luminal domains of synaptophysin and synaptoporin, respectively. The V-ATPase–synaptophysin interface region is annotated with a green box.

Extended Data Figure 7 | Comparison of V-ATPase structures in wild-type and Syp\textsuperscript{-/-} ISVs (related to Figure 3).

a, Superposition of wild-type and Syp\textsuperscript{-/-} intact State 1, State 2, State 3, and V0-only V-ATPase assemblies (from left to right). Models were aligned globally. Most of the larger differences are in regions that are poorly determined in the maps.

Extended Data Figure 8 | V-ATPase copy numbers from wild-type and Syp\textsuperscript{-/-} mouse brains (related to Figure 4).

a&b, Representative tomographic slices of Syp\textsuperscript{-/-} ISVs (one of 78 tomograms) (a, upper) or wild-type ISVs (one of 156 tomograms) (b, bottom) (thickness = 0.9 nm), respectively (obtained from Wiener filtered tomograms). Four representative ISVs are selected (colored boxed), and a series of higher magnification tomographic Wiener-filtered planes of each ISV are shown below and annotated with yellow and green arrows for intact and V0-only V-ATPase assemblies, respectively. Scale bars: 50 nm.

c, Scatter plot of the ISV diameters and V-ATPase copy numbers (including both intact and V0-only assemblies) for wild-type and Syp\textsuperscript{-/-} ISVs from 6 randomly selected tomograms, respectively.

d&e, Bootstrapping statistical significance tests (see Methods) for intact (c) or V0-only (d) V-ATPase copy number per ISV from wild-type and Syp\textsuperscript{-/-} mouse brains. The data were resampled 10,000 times with replacement to estimate the mean and standard
deviation of each intact or V0-only V-ATPase assembly copy number group. For the intact V-ATPase assembly (Supplementary Table 4), the wild-type ISV and Syp\(^{-}\) ISV sample pool comprises 1,326 ISVs and 1,453 ISVs, respectively. For the V0-only V-ATPase assembly (Supplementary Table 5), the wild-type ISV and Syp\(^{-}\) ISV sample pool comprises 106 ISVs and 188 ISVs, respectively. Data are presented as mean ± SD; error bars (black) represent the SD of 10,000 statistically independent sampling; *** p < 0.001 by Student’s t-test (n = 10,000).

**f, g, h & i,** Fitted Poisson distribution for the copy numbers of intact V-ATPase assemblies for ISVs from wild-type (**f**) and Syp\(^{-}\) mouse brains (**g**), and to the copy numbers of the V0-only V-ATPase assemblies for ISVs from wild-type (**h**) and Syp\(^{-}\) mouse brains (**i**). The Poisson distributions were fitted to the observed copy numbers ≥ 1, i.e., the copy numbers of ISVs without any V-ATPase were not used for the fit (see Methods). The least-squares residuals shown in the figure legends refer to copy numbers ≥ 1.

Source numerical data are in Source Data SourceDataExtendedDataFig8.xlsx
Extended Data Fig. 1
Extended Data Fig. 2
Extended Data Fig. 3
Extended Data Fig. 4
Extended Data Fig. 5
Extended Data Fig. 6

ATP6V0E1: MAYHGLTVPLIVMPSVFWGTFVGLLVVFPIPKGPNGRVIITMLVSCYLTCSVCCYLFWLIALQLNLPFGQQLKNETIWYLKHWP

ATP6V0E2: MTAHSFAPLVIIITTFNGEGIGLGFVFPGNPNGRVIITMLVACCCYLFMLIALQLNLPFGQQLKNETIWYVRYFLWE

V-ATPase
Synaptophysin

subunit e2
subunit a

V-ATPase interface

Synaptophysin

0.0 0.5 1.0
conservation score

V-ATPase interface

Synaptophysin


V-ATPase interface

Synaptophysin

transmembrane domain
same similar meaning
lumenal domain

250 260 270 280 290 300
Extended Data Fig. 7
Extended Data Fig. 8
## Extended Data Table 1 | CryoET data and map statistics

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(FSC = 0.143, Å)
## Extended Data Table 2 | Cryo-EM data collection, refinement, and validation statistics

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### Data collection and processing

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### Refinement

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### Model composition

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### R.m.s. deviations

| Bond lengths (Å) | 0.004 | 0.004 | 0.004 | 0.004 | 0.004 | 0.004 | 0.004 | 0.004 |
| Bond angles (°)  | 0.519 | 0.519 | 0.519 | 0.519 | 0.519 | 0.519 | 0.519 | 0.519 |

### Validation

| MolProbity score | 2.33 | 2.33 | 2.33 | 2.33 | 2.33 | 2.33 | 2.33 | 2.33 |
| Poor rotamers (%)| 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |

### Ramachandran plot

| Favored (%)   | 95.72 | 95.72 | 95.72 | 95.72 | 95.72 | 95.72 | 95.72 | 95.72 |
| Allowed (%)   | 2.85  | 2.85  | 2.85  | 2.85  | 2.85  | 2.85  | 2.85  | 2.85  |
| Disallowed (%)| 0.50  | 0.50  | 0.50  | 0.50  | 0.50  | 0.50  | 0.50  | 0.50  |
Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
  - Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  - Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

<table>
<thead>
<tr>
<th>Data collection</th>
<th>iBright1500 imaging system, SerialEM v4.0.4&amp;v4.1, Thermo Scientific Xcalibur v4.1</th>
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<tbody>
<tr>
<td>Data analysis</td>
<td>AlphaFold2, Amira v2020.2, AreTomo v1.3.4, Byonic by ProteinMetrics v5.1.1, Chimera v1.16 &amp; ChimeraX v1.3&amp;1.7, cryoSPARC v4.4, CTFFIND4, iBright1500 Analysis Software, IMOD v 4.11.3, ISOLDE v1.3, Motioncor2, Phenix v1.21, EMRinger v1.0.0, Prism v10.2.2 (GraphPad Software), Proteome Discoverer v2.1 [Thermo Fisher Scientific], PyCRESTA [<a href="https://github.com/brungerlab/pycrest">https://github.com/brungerlab/pycrest</a>], PyMol v2.3.2 (Schrödinger, LLC), Python v3.8, PySeg v1.0.0, PyTom v0.98a1&amp;1.1, R v3.4.2, RELION v3.1&amp;4.0, Rosetta 2, Scientific Xcalibur (v4.1, Thermo Fisher Scientific), SerialEM v4.0&amp;4.1, Situs v3.2, Topaz v0.2.5, Warp v1.0.9. Several script files are available at <a href="https://github.com/brungerlab/ISV_scripts">https://github.com/brungerlab/ISV_scripts</a>.</td>
</tr>
</tbody>
</table>

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. Github). See the Nature Portfolio guidelines for submitting code & software for further information.
Data

Policy information about availability of data. All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The subtomogram averaging maps (wild-type V0 only: 44858, wild-type State 1: 44855, wild-type State 2: 44856, wild-type State 3: 44857), the SPA maps (wild-type V0-only: 44846, wild-type State 1: 44843, wild-type State 2: 44839, wild-type State 3: 44840, Syv/-: VO-only: 44845, Syv/-: State 1: 44844, Syv/-: State 2: 44842, Syv/-: State 3: 44841) and representative binned tomograms (Syv/-: ISV-44847, wild-type ISV-44848) have been deposited in the EMDB, and the atomic coordinates have been deposited in the PDB (wild-type VO-only: 989Z, wild-type State 1: 989T, wild-type State 2: 989A, wild-type State 3: 989Q, Syv/-: VO-only: 989Y, Syv/-: State 1: 989RU, Syv/-: State 2: 989RS, Syv/-: State 3: 989R).

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

<table>
<thead>
<tr>
<th>Reporting on sex and gender</th>
<th>N/A</th>
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<tr>
<td>Reporting on race, ethnicity, or other socially relevant groupings</td>
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</tr>
<tr>
<td>Population characteristics</td>
<td>N/A</td>
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<td>Recruitment</td>
<td>N/A</td>
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<td>Ethics oversight</td>
<td>N/A</td>
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</table>

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- [x] Life sciences
- [ ] Behavioural & social sciences
- [ ] Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/rr-reporting-summary-list.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

<table>
<thead>
<tr>
<th>Sample size</th>
<th>Experiments described in this study were performed with at least 3-9 samples for each group, which is consistent with sample sizes commonly used in similar studies in the field. And the sample sizes provided adequate power to detect significant effects.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data exclusions</td>
<td>Since it is difficult to determine the true number of ISVs without any V-ATPase assembly due to the missing wedge effect of tomographic reconstructions, we fitted the Poisson distributions to copy numbers ≥ 1.</td>
</tr>
<tr>
<td>Replication</td>
<td>All successful data were generated over multiple attempts and in at least three replicates (replicates number are specified in the manuscript)</td>
</tr>
<tr>
<td>Randomization</td>
<td>Experiments randomization was not necessary, but in all experiments, control and experimental samples were analyzed in parallel.</td>
</tr>
<tr>
<td>Blinding</td>
<td>Two individuals independently examined two halves of the tomograms and counted the copy numbers of V-ATPase per ISV. The two individuals knew the samples of the dataset, but didn’t know each other’s counting results. The mice observational behavior was scored blind by two individuals.</td>
</tr>
</tbody>
</table>

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.
### Materials & experimental systems

<table>
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<tr>
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<td>☒ Antibodies</td>
<td></td>
</tr>
<tr>
<td>☒ Eukaryotic cell lines</td>
<td></td>
</tr>
<tr>
<td>☒ Palaeontology and archaeology</td>
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<td>☒ Animals and other organisms</td>
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<td>☒ Clinical data</td>
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<td>☒ Dual use research of concern</td>
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<td>☒ Plants</td>
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### Methods

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<td>☒ ChiP-seq</td>
<td></td>
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<td>☒ Flow cytometry</td>
<td></td>
</tr>
<tr>
<td>☒ MRI-based neuroimaging</td>
<td></td>
</tr>
</tbody>
</table>

### Antibodies

**Antibodies used**

- mouse-VGLUT1[1:200; SySy, Cat.# 135303];
- rabbit-synaptophysin-1[1:1,000; SySy, Cat.# 101008];
- rabbit-IRDye800CW[1:3,000; LI-COR, Cat.# 926-32211];
- mouse-synaptobrevin-2[1:1,000; SySy, Cat.# 104211];
- mouse-HRP[1:10,000; Abcam, Cat.# ab6789];
- mouse-synaptotagmin-1[1:1,000; SySy, Cat.# 150511];
- mouse-IRDye800CW[1:3,000; LI-COR, Cat.# 926-32210];
- rabbit-synaptotagmin-1[1:500; SySy, Cat.# 102002];
- rabbit-ATP6V1A1[1:1,000; NovusBio, Cat.# NBP1-89342];
- rabbit-synaptogyrin-1[1:500; SySy, Cat.# 103002];
- rabbit-VGLUT1[1:1,000; SySy, Cat.# 135303];

**Validation**


### Animals and other research organisms

**Policy information about studies involving animals:** ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research.

**Laboratory animals**

- Male wild-type CD1 mice (23-26 days old) and C57BL6 mice (23-26 days old) were used for synaptic vesicle preparations. Male Syp-/- mice (23-26 days old) were used for synaptic vesicle preparations. Both sexes of 4-6 months-old wild-type Black 6 [B6NTac] and Syp-/- mice were used in animal behavior experiments.

**Wild animals**

- N/A

**Reporting on sex**

- Sexes were reported above

**Field-collected samples**

- N/A

**Ethics oversight**

- All animal procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Stanford Administrative Panel on Laboratory Animal Care (APLAC) Institutional guidelines [protocol #29981] and by the University of Colorado Boulder Institutional Animal Care and Use Committee (IACUC) [protocol #1106.02]. No field collected samples were used in the study.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

### Plants

**Seed stocks**

- N/A

**Novel plant genotypes**

- N/A

**Authentication**

- N/A
Supplementary Information

Structure and Topography of the Synaptic V-ATPase–Synaptophysin Complex

Chuchu Wang$^{1-5}$†, Wenhong Jiang$^{6}$†, Jeremy Leitz$^{1-5}$†, Kailu Yang$^{1-5}$, Luis Esquivies$^{1-5}$, Xing Wang$^{6}$, Xiaotao Shen$^{7,8}$, Richard Held$^{1-5}$, Daniel J. Adams$^{9}$, Tamara Basta$^{9}$, Lucas Hampton$^{9}$, Ruiqi Jian$^{7,8}$, Lihua Jiang$^{7,8}$, Michael H.B. Stowell$^{9}$, Wolfgang Baumeister$^{10}$, Qiang Guo$^{6}$$^*$, Axel T. Brunger$^{1-5}$$^*$

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$^2$Department of Neurology and Neurological Sciences, Stanford University, Stanford, United States
$^3$Department of Structural Biology, Stanford University, Stanford, United States
$^4$Department of Photon Science, Stanford University, Stanford, United States
$^5$Howard Hughes Medical Institute, Stanford University, Stanford, United States
$^6$State Key Laboratory of Protein and Plant Gene Research, School of Life Sciences and Peking-Tsinghua Center for Life Sciences, Peking University, Beijing 100871, China
$^7$Department of Genetics, Stanford University, Stanford, United States.
$^8$Stanford Center for Genomics and Personalized Medicine, Stanford University, Stanford, United States.
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†These authors contributed equally
$^*$Correspondence: brunger@stanford.edu, guo.qiang@pku.edu.cn
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**Supplementary Table 1** – List of wild-type ISV proteins identified by high-resolution mass-spectrometry with map cross-correlation scores. Separate Excel file (SupplementaryTable1.xlsx)

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**Supplementary Table 3** – Root-mean square differences between V-ATPase structures. 3

**Supplementary Table 4** – Copy numbers of intact V-ATPase assemblies per ISV. 3

**Supplementary Table 5** – Copy numbers of V0-only V-ATPase assemblies per ISV. 4
**Supplementary Table 2.** Identification of unique peptides of the V-ATPase subunit e2 from wild-type ISV samples

<table>
<thead>
<tr>
<th>Identified protein</th>
<th>Biological replicate</th>
<th>Score</th>
<th>Unique peptide$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP6V0E2</td>
<td>ISV-1</td>
<td>307.7</td>
<td>Y.VRFLWE.</td>
</tr>
<tr>
<td></td>
<td>ISV-2</td>
<td>308.8</td>
<td>F.TTF.I</td>
</tr>
<tr>
<td></td>
<td>ISV-3</td>
<td>303.7</td>
<td>F.TTF.W</td>
</tr>
</tbody>
</table>

$^1$The spectrum score uses Byonic's scoring algorithm.

**Supplementary Table 3.** Root-mean-square differences between V-ATPase structures

<table>
<thead>
<tr>
<th>RMSD (Å)</th>
<th>V-ATPase</th>
<th>In wild-type ISVs</th>
<th>State 1</th>
<th>State 2</th>
<th>State 3</th>
<th>V0-only</th>
</tr>
</thead>
<tbody>
<tr>
<td>In Syp+/− ISVs</td>
<td>State 1</td>
<td>2.3</td>
<td>2.1</td>
<td>1</td>
<td>1.7</td>
<td></td>
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<tr>
<td></td>
<td>State 2</td>
<td>2.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>State 3</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>V0-only</td>
<td>1.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDB IDs of other State 2</td>
<td>6WM3</td>
<td>3.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7U4T</td>
<td>3.8</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>6VQG</td>
<td>4</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>7UNF</td>
<td>4.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Structures were globally aligned by superimposing Cα atoms and root-mean-square differences (RMSDs) calculated for Cα atoms. State 2 was used for comparisons of wild-type intact V-ATPase with the other mammalian structures (PDB IDs: 6WM3, 7U4T, 6VQG, 7UNF) since the 7UNF deposition only includes State 2.

**Supplementary Table 4.** Copy numbers of intact V-ATPase assemblies

<table>
<thead>
<tr>
<th>Intact V-ATPase copy number</th>
<th>Number of ISVs</th>
<th>Percentage$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>wild-type</td>
<td>Syp+/−</td>
</tr>
<tr>
<td>0</td>
<td>2228</td>
<td>927</td>
</tr>
<tr>
<td>1</td>
<td>945</td>
<td>688</td>
</tr>
<tr>
<td>2</td>
<td>290</td>
<td>419</td>
</tr>
<tr>
<td>3</td>
<td>72</td>
<td>218</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>79</td>
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<tr>
<td>5</td>
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<td>6</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

| Total examined number of ISVs | 3554 | 2380 |
| Number of ISVs with at least one intact V-ATPase | 1326 | 1453 |

$^1$Normalized to the number ISVs with at least one intact V-ATPase
**Supplementary Table 5.** Copy numbers of V0-only V-ATPase assemblies

<table>
<thead>
<tr>
<th>V0-only V-ATPase copy number</th>
<th>Number of ISVs</th>
<th>Percentage$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>wildtype</td>
<td>Syp$^{IC}$</td>
</tr>
<tr>
<td>0</td>
<td>197</td>
<td>233</td>
</tr>
<tr>
<td>1</td>
<td>86</td>
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<td>3</td>
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<td>11</td>
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<tr>
<td>4</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total examined number of ISVs</td>
<td>303</td>
<td>421</td>
</tr>
<tr>
<td>Number of ISVs with at least one V0-only V-ATPase</td>
<td>106</td>
<td>188</td>
</tr>
</tbody>
</table>

$^1$Normalized to the number ISVs with at least one V0-only V-ATPase.