

Supplemental information

**Presynaptic activity and protein turnover
are correlated at the single-synapse level**

Sebastian Jähne, Fabian Mikulasch, Helge G.H. Heuer, Sven Truckenbrodt, Paola Agüi-Gonzalez, Katharina Grewe, Angela Vogts, Silvio O. Rizzoli, and Viola Priesemann

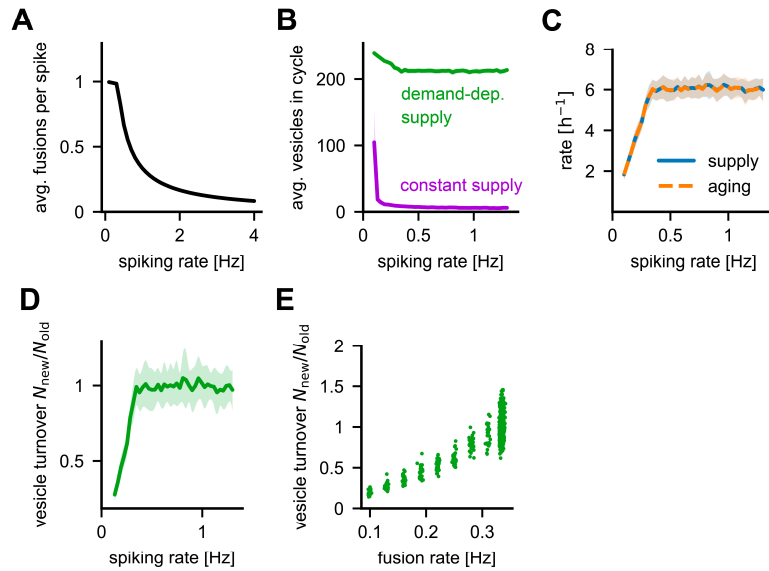


Figure. S1. The sizes of vesicle pools have no critical impact on vesicle dynamics. Related to Figure 1. We simulated the synaptic vesicle dynamics with 250 vesicle slots in the synapse. All pools were scaled up by a factor of 2.5. Results are qualitatively similar to a model synapse with 100 vesicles (Fig. 1). **(A)** The average number of fusions per spike (when fixing the number of vesicles to 100) is higher for slow spiking rates than in the smaller synapse mainly due to a larger primed pool. For higher spiking rates the number of fusions per spike is similar for both models, since here the rate of endocytosis becomes the limiting factor (compare with Fig. 1B). **(B)** For constant vesicle supply the pools are almost empty for most stimulation rates, due to the high fusion rates. For demand dependent supply the number of vesicles is stable. **(C)** For demand dependent supply the supply and aging rate match for all stimulation rates. **(D,E)** The turnover for demand dependent supply is still dependent on spike rate and fusion rate. However, turnover is much smaller since the individual vesicles are less likely to be fused. The shaded areas in panels C and D represent the standard deviations of the respective simulations (30 experiments conducted).

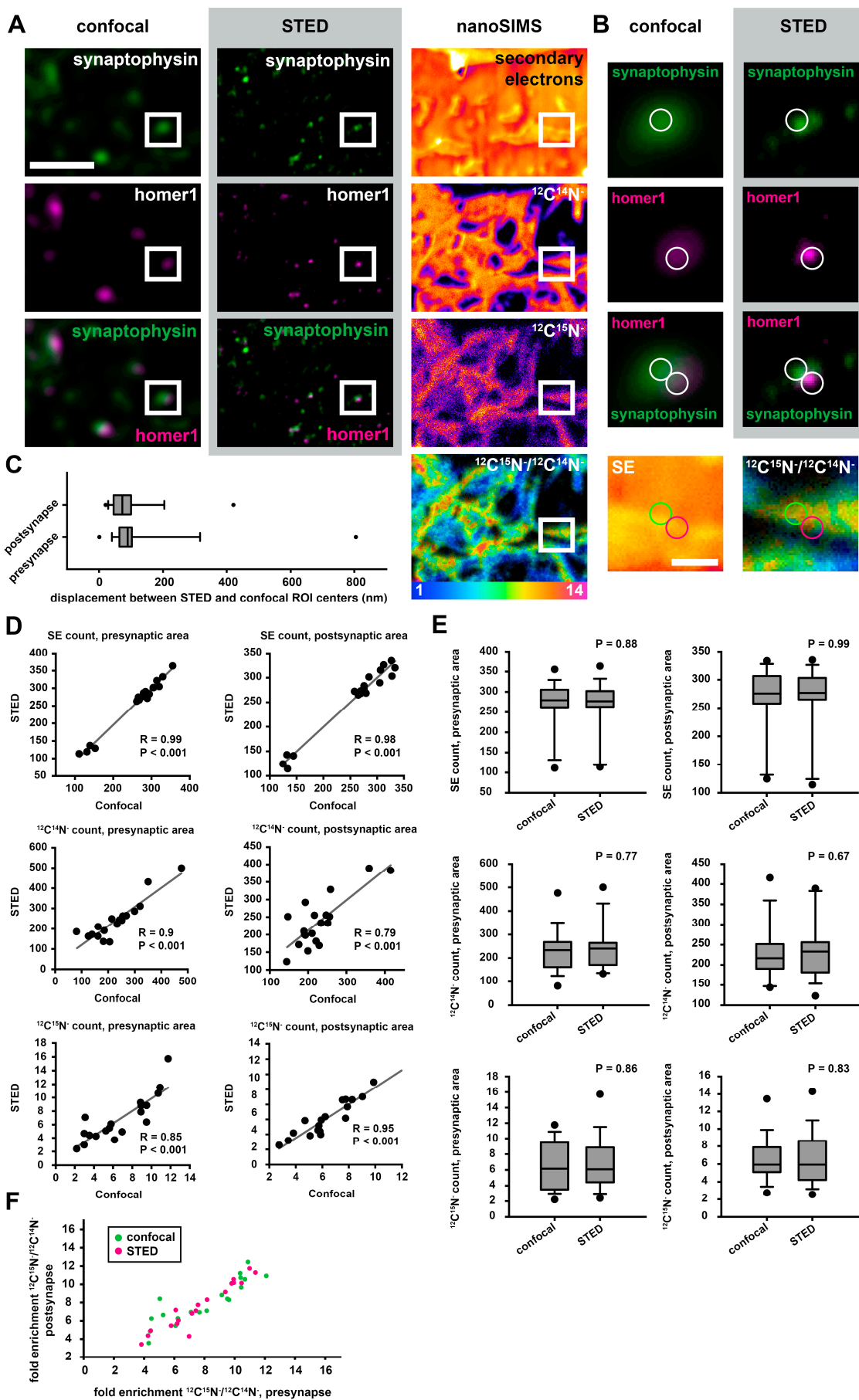


Figure S2. STED imaging does not change the measurement precision. Related to Figure 2. (A) Newly made proteins were labeled with ^{15}N -leucine for 24 hours, followed by fixation and immunostaining against synaptophysin and homer1. The samples were then imaged in confocal or STED microscopy, and were finally analyzed in nanoSIMS, measuring the secondary electrons (SE), $^{12}\text{C}^{14}\text{N}^-$ ions and $^{12}\text{C}^{15}\text{N}^-$ ions. Exemplary images are shown. Example pre- and postsynapses and regions of interest (ROIs, 200 nm in diameter) are shown by the squares and circles, respectively. Scale bar = 2 μm **(B)** Close-up of a typical synapse. Scale bar = 400 nm **(C)** Signals were then analyzed in circular regions of interest, 200 nm in diameter, whose centers were selected by an expert. The positions of the selected spots differed little, when they were analyzed in confocal or STED microscopy. The displacement for the pre- and postsynaptic centers are analyzed (medians around 70-80 nm). **(D)** We analyzed the different nanoSIMS measurements in the regions of interest selected in STED or confocal. The measurements correlated well in all cases (see R and P values). **(E)** The same measurements, shown as box plots. No significant differences could be found (Mann-Whitney Ranksum tests). **(F)** Scatter plots of ^{15}N enrichment in the pre- and postsynapse, based on regions of interest selected in STED or confocal imaging. The two graphs are virtually identical. 19 synapses were analyzed, from 4 STED/confocal sets.

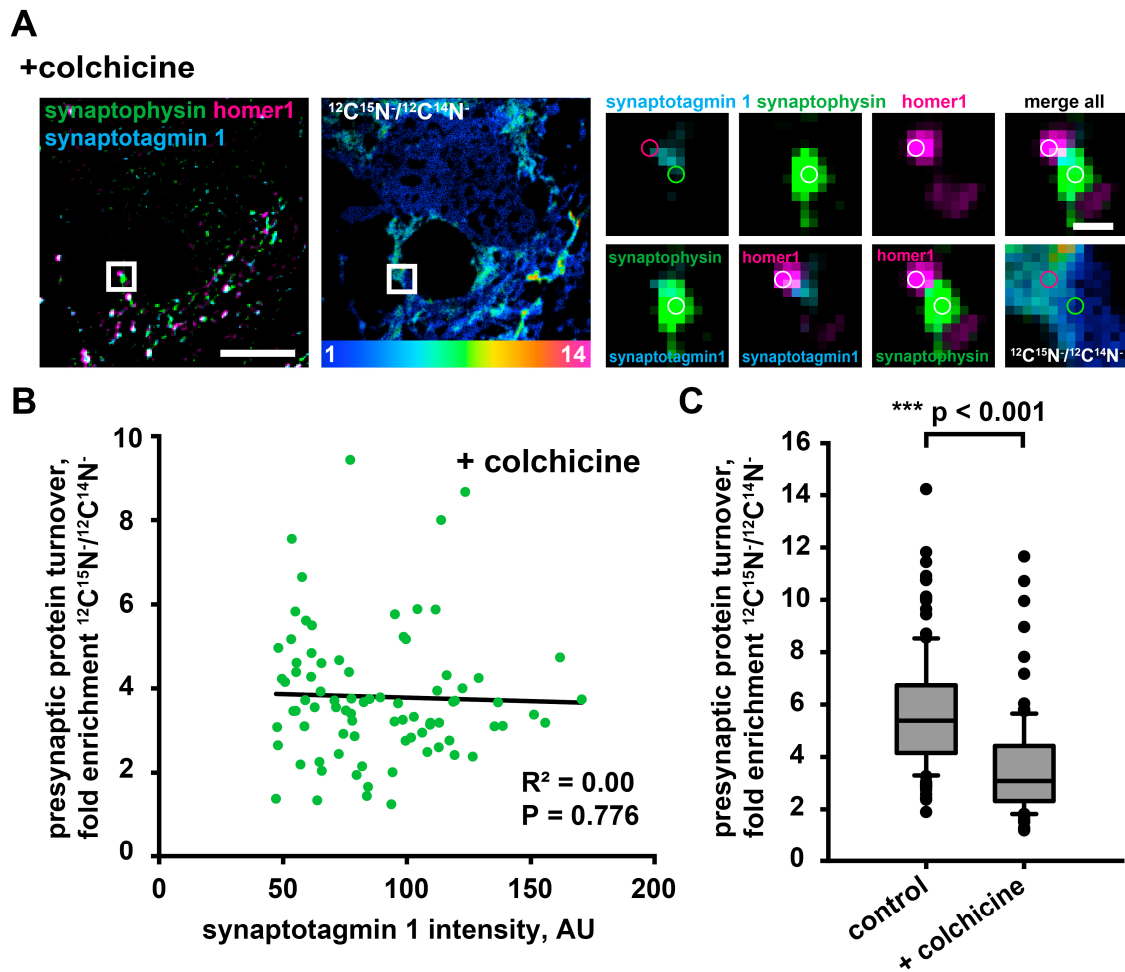


Figure S3. Colchicine reduces presynaptic turnover. Related to Figure 3. (A) Newly made proteins were labeled with ^{15}N -leucine for 24 hours, while the neurons were treated with the microtubule disruptor colchicine (10 μM). During the last hour of labeling, exocytosing synaptic vesicles were tagged with a fluorophore-conjugated antibody against synaptotagmin 1, as in Fig. 3, followed by fixation and immunostaining against synaptophysin and homer1. Exemplary images are shown, as in Fig. 2. Scale bar = 5 μm . Scale bar close-up = 500 nm. (B) Presynaptic protein turnover is not correlated to synaptic activity. 83 synapses from three independent experiments were analyzed. (C) The ^{15}N enrichment was reduced substantially after the colchicine treatment. 133 (control) and 101 (colchicine) synapses from three independent experiments were analyzed. (Mann-Whitney ranksum test).

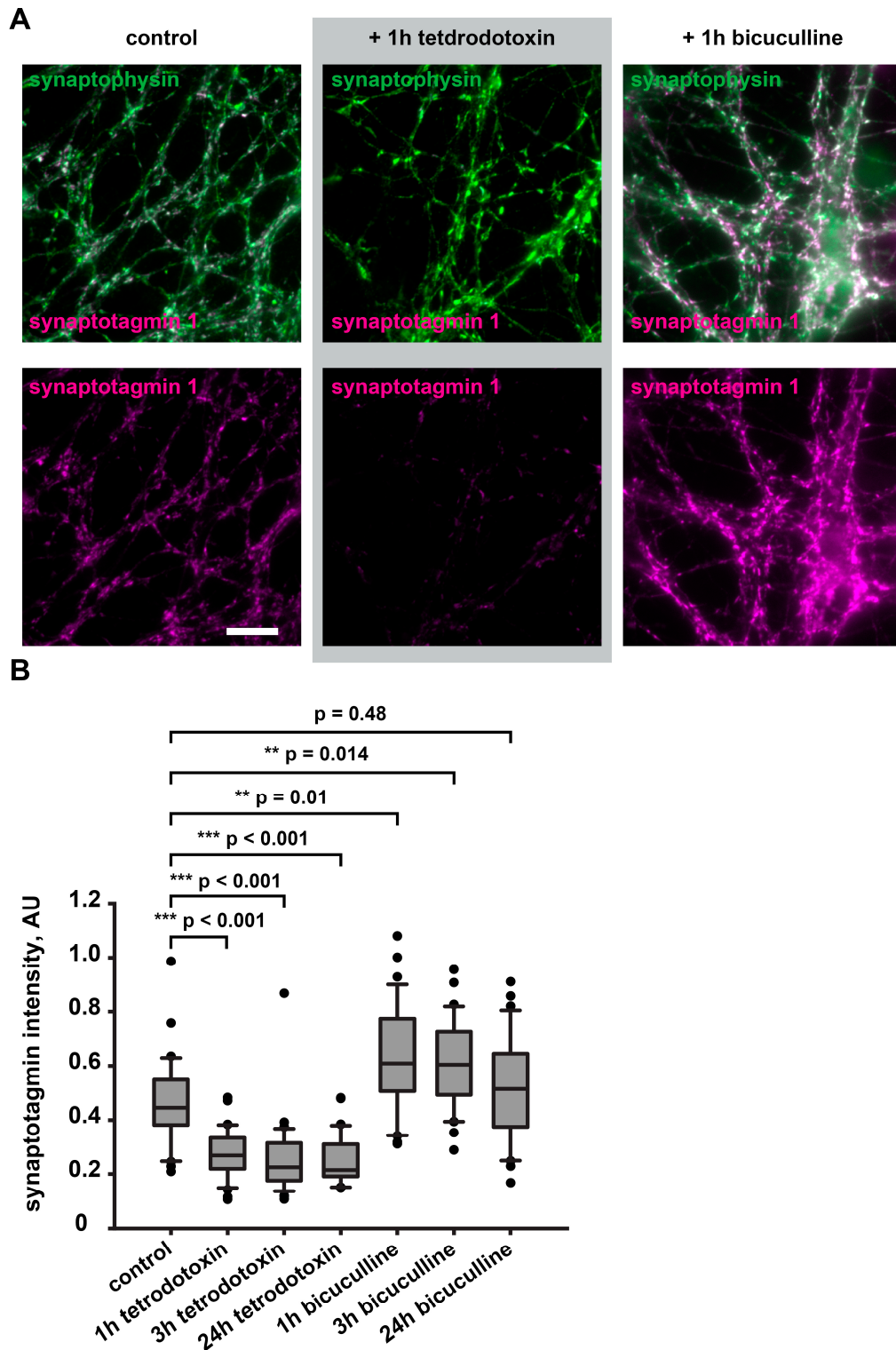


Figure S4. TTX and bicuculline effects on synapse activity. Related to Figure 4. (A) Neurons were treated with TTX or bicuculline for 1, 3 or 24 hours before recycling synaptic vesicles were tagged for one additional hour with a fluorophore-conjugated antibody against synaptotagmin 1, as in Fig. 3. Overlays of synaptotagmin 1 (magenta) and synaptophysin (green) are shown in the top row. The bottom row shows the synaptotagmin 1 images alone. Scale bar = 10 μ m **(B)** An analysis of the signal intensity is shown. TTX reduces the synaptotagmin 1 staining substantially (essentially reducing to the surface pool of synaptotagmin 1; Truckenbrodt et al., 2018), while BIC increases it, albeit by 24 hours the levels of activity have been brought by homeostatic effects almost to baseline (Kruskal-Wallis tests, followed by Fisher post hoc tests; 3 independent experiments with at least 10 imaging fields analyzed per experiment).

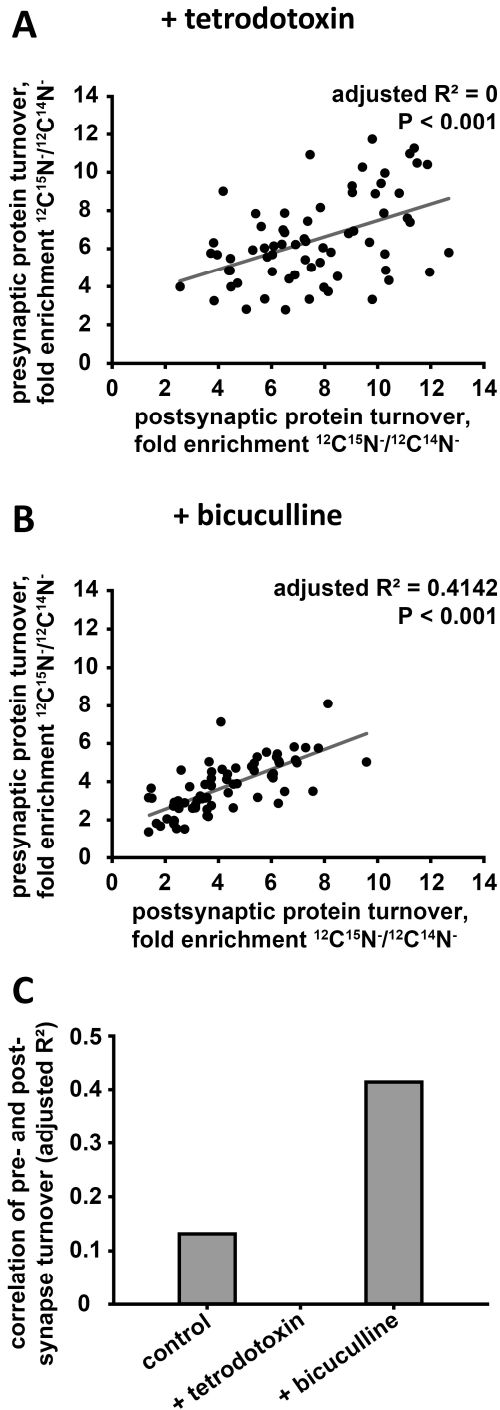


Figure S5. Chronically modulating synaptic activity enhanced or decreased the correlation between pre- and postsynaptic protein turnover. Related to Figure 4. Treatment with tetrodotoxin and bicuculline during ^{15}N -leucine labeling had opposing effects on the correlation between pre- and postsynaptic protein turnover (for the control situation, see figure 1). While the correlation was decreased by the application of TTX (**A,C**), it was strongly increased by bicuculline (**B,C**). The panels report the adjusted R^2 of the graphs; the adjustment was performed by subtracting the R^2 values obtained by performing the same measurement in randomly positioned pre- and postsynaptic areas, throughout the cells. For (**A**), 67 synapses from three independent were analyzed. For (**B**), 68 synapses from three independent experiments were analyzed.

Supplementary Tables

Parameter	Value	Source
r_{sup}	$2h^{-1}$	calculated from Truckenbrodt et al., 2018
τ_{sup}	6.25h	Our estimation
τ_{load}	0.5s	Farsi et al., 2016; Gandhi and Stevens, 2003
τ_{dock}	2s	Betz et al., 1992
τ_{prime}	0.1s	Neher, 2015
p_{fuse}	0.1	Gandhi & Stevens, 2003; Neher, 2015
p_{age}	1/200	Truckenbrodt et al., 2018
r_{reform}	0.33^{-1}	Balaji and Ryan, 2007; Hua et al., 2013; Rizzoli, 2014; Specht and Triller, 2008
$p_{recovery}^{max}$	38	Truckenbrodt et al., 2018; Specht & Triller, 2008
p_{load}^{max}	38	Truckenbrodt et al., 2018; Specht & Triller, 2008
p_{dock}^{max}	20	Schikorski and Stevens, 2001; Truckenbrodt et al., 2018
p_{prime}^{max}	4	calculated from Truckenbrodt et al., 2018
p_{max}	100	Schikorski and Stevens, 1997; Truckenbrodt et al., 2018

Table S1. Related to Figure 1. Parameters as used in the simulation of the vesicle cycle and their respective sources.