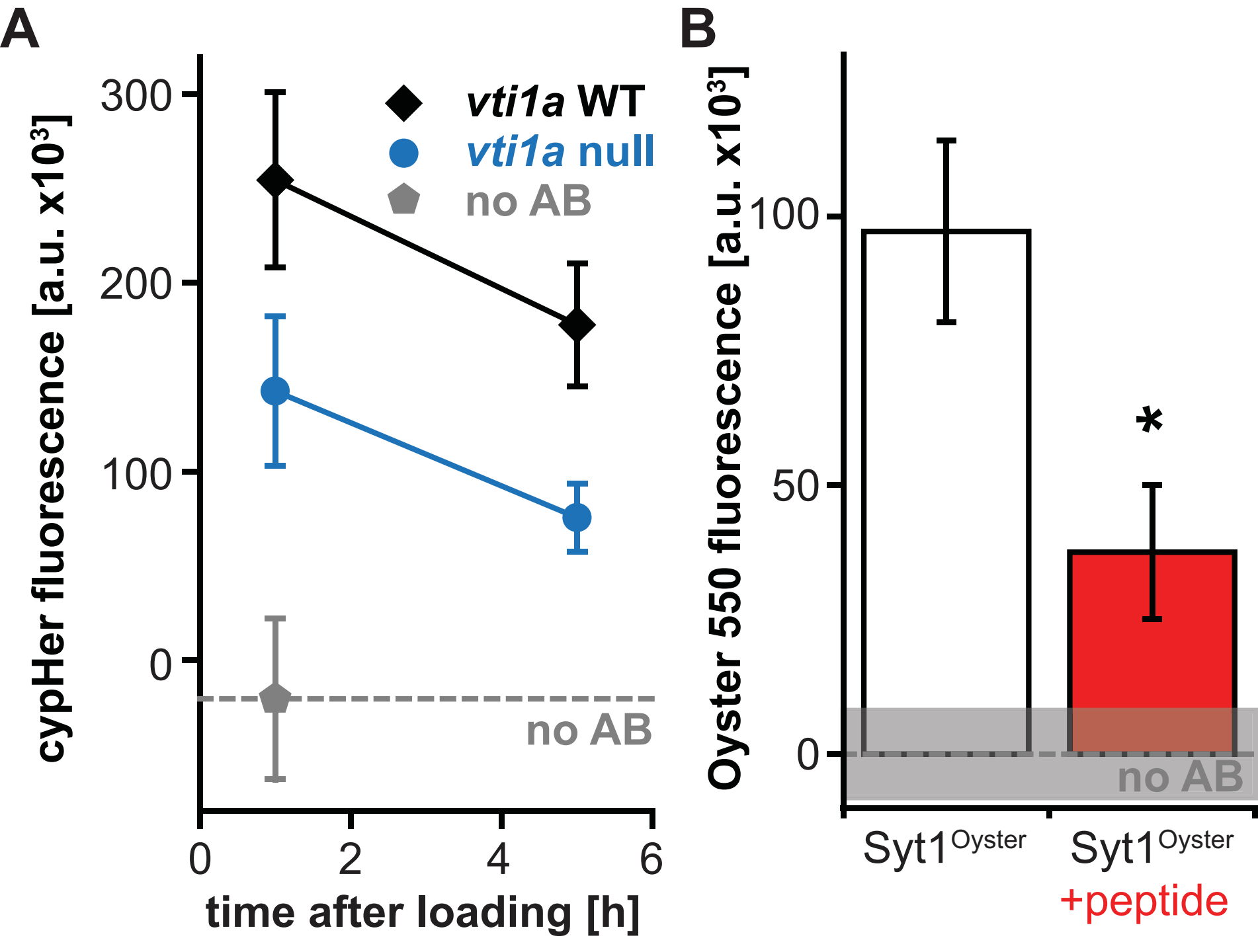
A
B
cypHer fluorescence [a.u. x10³]
300
200
100
0
vti1a WT
vti1a null
no AB
no AB
0
2
4
6
time after loading [h]
Oyster 550 fluorescence [a.u. x10³]
100
50
0
*
no AB
Syt1Oyster
Syt1Oyster
+peptide

Supplementary Figure S4. Loss of vti1a leads to less vesicle cycling, but protein degradation occurs at similar rates as in control cells. A. The total fluorescence of the cypHer-conjugated antibody in wildtype and *vti1a* null cells is shown at two time points (1 and 5 h, the 1h data are the same as in Fig 7). Both conditions show a decrease in fluorescence values at longer intervals, suggesting protein degradation with similar speed (similar slope). In all conditions the total fluorescence was subtracted by the fluorescence upon NH_4Cl-treatment. The grey symbol and dashed line illustrate the fluorescence of wildtype cells stimulated and imaged in the same manner, but without exposure to the antibody (no AB, negative control). B. Antibody uptake occurs by recognition of to the Synaptotagmin-1 epitope. As a specificity test, we investigated whether cypHer uptake into cycling vesicles occurred via the fluid phase (without antibody binding) or required the binding to the Synaptotagmin-1 epitope. Fluorescence was assessed using the same antibody as in panel A (and Fig 7), but labelled with Oyster 550 instead of cypHer. Stimulation of wildtype chromaffin cells in the presence of the antibody led to a robust increase in cellular fluorescence. In contrast, pre-incubation of the antibody with a peptide containing the antibody epitope lead to a partial block of dye uptake, signified by lower fluorescence intensity values. The (unspecific) fluorescence of cells measured in parallel which were stimulated in the absence of the antibody was subtracted from both datasets (baseline fluorescence) indicated by the grey dashed line (the SEM is shown as shaded region in the lower section of the graph). Data are means, error bars are SEM. Number of cells (n) in A at 1 h in the wildtype (control): n=40; *vti1a* null: n=29; no AB: n=19. Number of cells (n) at 5 h in the *vti1a* wildtype: n=10; *vti1a* null: n=10. Number of cells (n) in B in the *vti1a* wildtype: n=110; vti1a null: n=64; no AB: n=33.