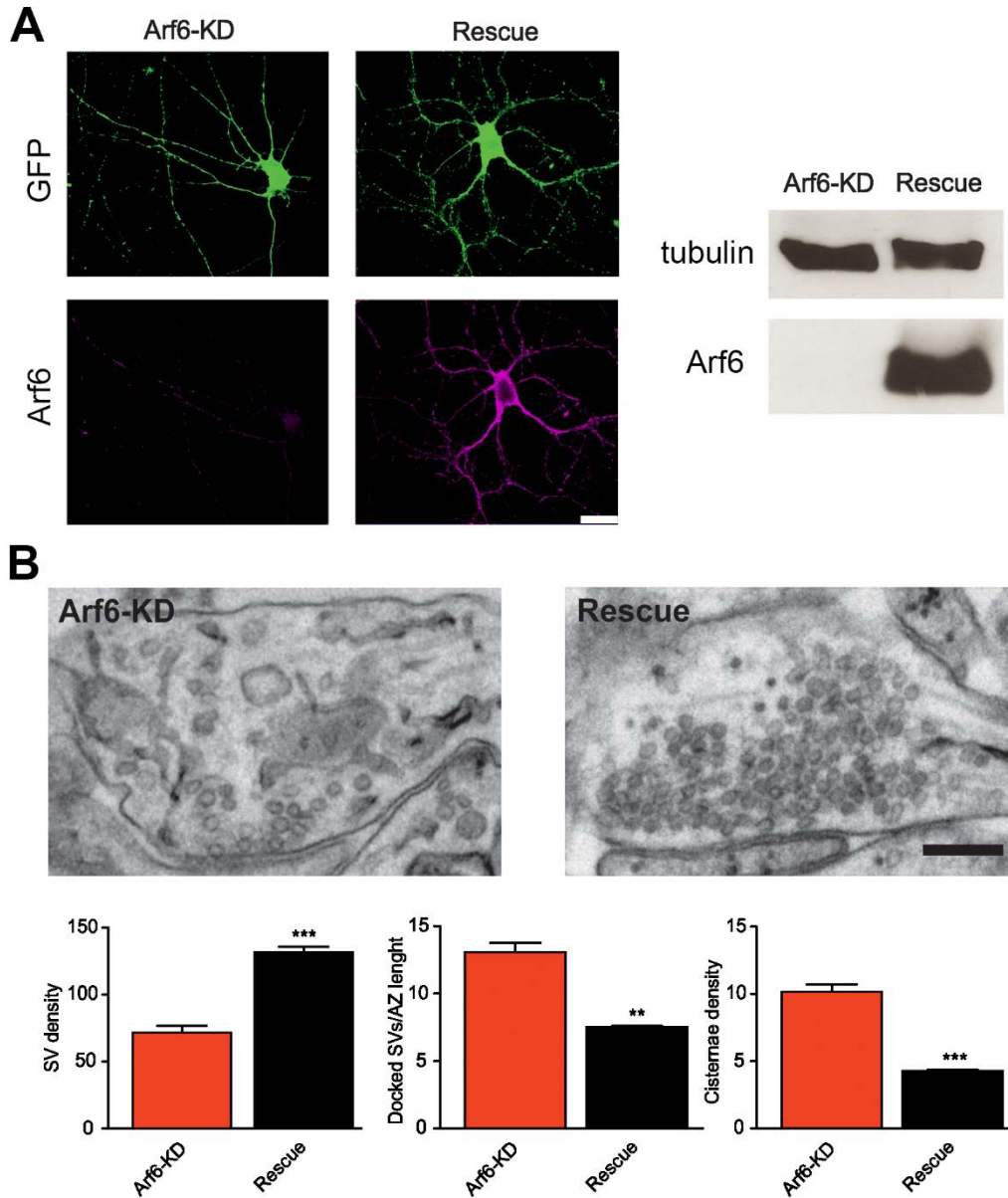


**Arf6 regulates the cycling and the readily releasable pool of synaptic vesicles at hippocampal synapse.**

**Figure 1: figure supplement 3.** Rescue of the ultrastructural phenotype.



**A, left panels,** representative images of neurons transduced at 12 DIV with lentiviruses expressing an Arf6 shRNA together with empty vector (Arf6-KD) or with Arf6 resistant isoform (rescue) and labelled at 17 DIV with anti-Arf6 primary antibody and Alexa-647 secondary antibody (Arf6). Scale bar 25  $\mu$ m. **Right panel,** WB analysis from lysates of rat hippocampal neurons (17 DIV) transduced as above and decorated with anti-Arf6 and anti-tubulin

antibodies. Both immunocytochemistry and western blotting confirmed the expression of Arf6 resistant to shRNA#1. **B**, *upper panels*, representative electron micrographs of synaptic terminals from cultured hippocampal neurons (17DIV) transduced as above. *Lower panels*, morphometric analysis of the density of SVs, docked SVs and cisternae in Arf6-KD (red) and rescued (black) synapses. Data are means  $\pm$  SEM of from 3 independent preparations (n=100 synapses for Arf6-KD and Rescue).