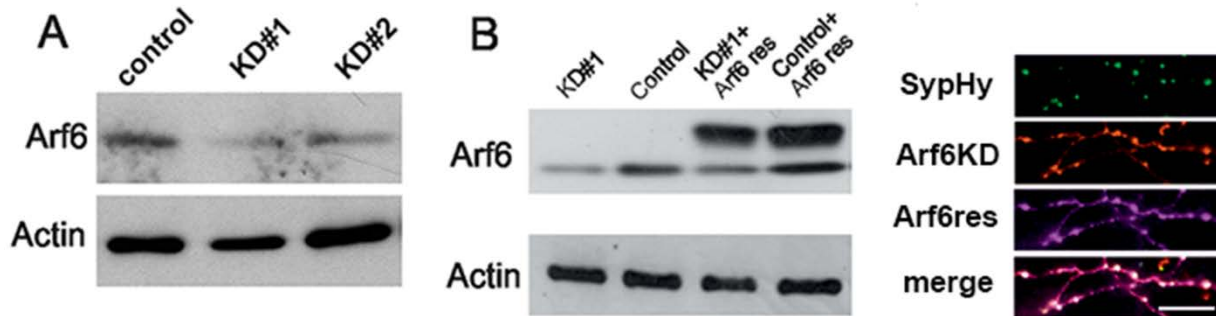


**Arf6 regulates the cycling and the readily releasable pool of synaptic vesicles at hippocampal synapse.**

**Figure 5: figure supplement 1.** Tools for silencing and rescue Arf6 expression by transfection



**A**, Representative WB from lysates of rat hippocampal neurons nucleofected at 0 DIV with either Arf6 shRNA (#1 or #2) or the mismatch control and lysed at 14 DIV. The Arf6 signal is reduced in both shRNA #1 and #2 transfected neurons with respect to control. Actin is shown for normalization. **B. Left panel**, representative WB of lysates of HeLa cells transfected with either shRNA#1 or its mismatch version alone or in combination with V5-Arf6 resistant to shRNA#1 and lysed 48 h after transfection. The lower Arf6 immunopositive band represents endogenous Arf6 that is significantly reduced in Arf6 shRNA#1 transfected cells, whereas the upper band represents the overexpressed V5-Arf6 that is unaffected by Arf6-shRNA#1 silencing. **Right panel**, representative images of neurites from neurons cotransfected with SypHy, RFP-tagged shRNA#1 (Arf6KD) and V5-Arf6 resistant to shRNA#1 (Arf6res). V5-Arf6 is visualized with an anti-V5 antibody followed by Alexa 647 secondary antibody. Merge panel shows colocalization. Scale bar, 10 μm.