Supplemental information

The clavesin family: neuron-specific lipid- and clathrin-binding Sec14 proteins regulating lysosomal morphology

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Running title: Clavesin family of Sec14 proteins

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SUPPLEMENTAL FIGURE LEGENDS

Supplemental Fig. 1. The clavesin polyclonal antibody recognizes clavesin 1 and 2. HEK-293 cells were transfected with plasmids encoding FLAG-tagged clavesin 1, clavesin 2 or the C-terminal region of enthoprotin/epsinR. Cell lysates were then processed for Western blot with antibody against the FLAG epitope tag or clavesin.

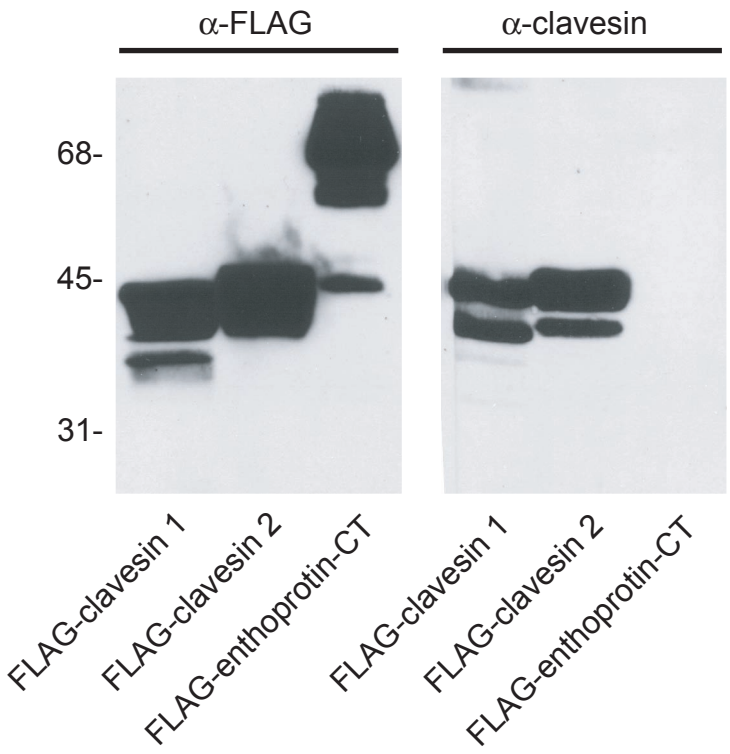
Supplemental Fig. 2. Clavesins are not detected in various cell lines. The level of clavesin 1 and 2 in the indicated tissue and cell lines was determined by Western blot.

Supplemental Fig. 3. Clavesins are expressed in neurons. Hippocampal cultures at 21 DIV were processed by indirect immunofluorescence with a rabbit polyclonal antibody against clavesin (red), a mouse monoclonal antibody against GFAP (green) and a chicken polyclonal antibody against MAP2. Bar is 10 μm.

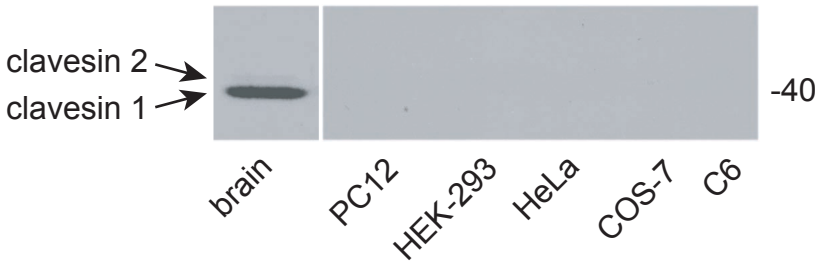
Supplemental Fig. 4. Membrane localization of clavesin 1 is PtdIns and clathrin binding independent. Neurons were transfected with plasmids encoding GFP-clavesin 1-Sec14 wt or PtdIns binding mutant (RKF/AAA). The cells were subsequently processed by indirect immunofluorescence with antibody against AP-1 (red). Bar is 10 μm.

Supplemental Fig. 5. Knock down of clavesins does not alter the localization of mannose-6-phosphate receptors. Hippocampal neurons at 7 DIV were transduced with lentivirus encoding a non-targeting miRNA or clavesin 1-1 and 2-1 miRNAs. At 14 DIV cells were processed for indirect immunofluorescence with antibodies against the 46 kDa form of the mannose-6-phosphate receptor (MPR46) or the 300 kDa form of the receptor (MPR300) as well as antibodies against AP-1.

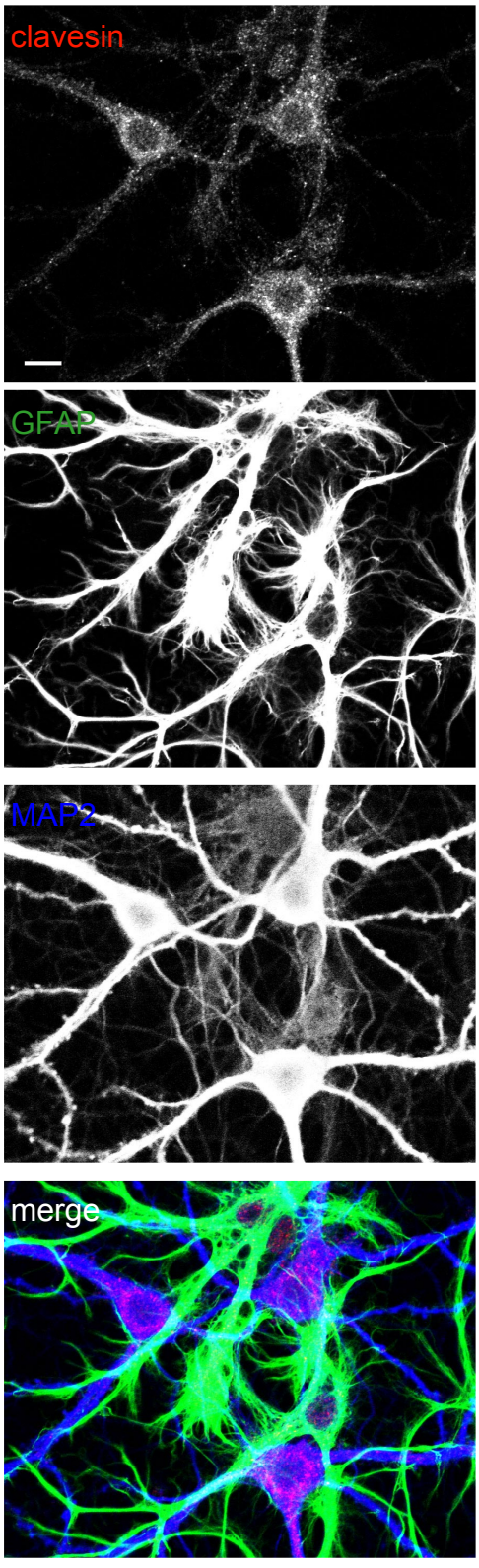
Supplemental Fig. 6. Knock down of clavesins does not alter the TGN localization of CHC or AP-1. Hippocampal neurons at 7 DIV were transduced with lentivirus encoding a non-targeting miRNA or clavesin 1-1 and 2-1 miRNAs. At 14 DIV cells were processed for indirect immunofluorescence with antibodies against CHC or AP-1. The total fluorescence intensity of these proteins on the TGN was then quantified.



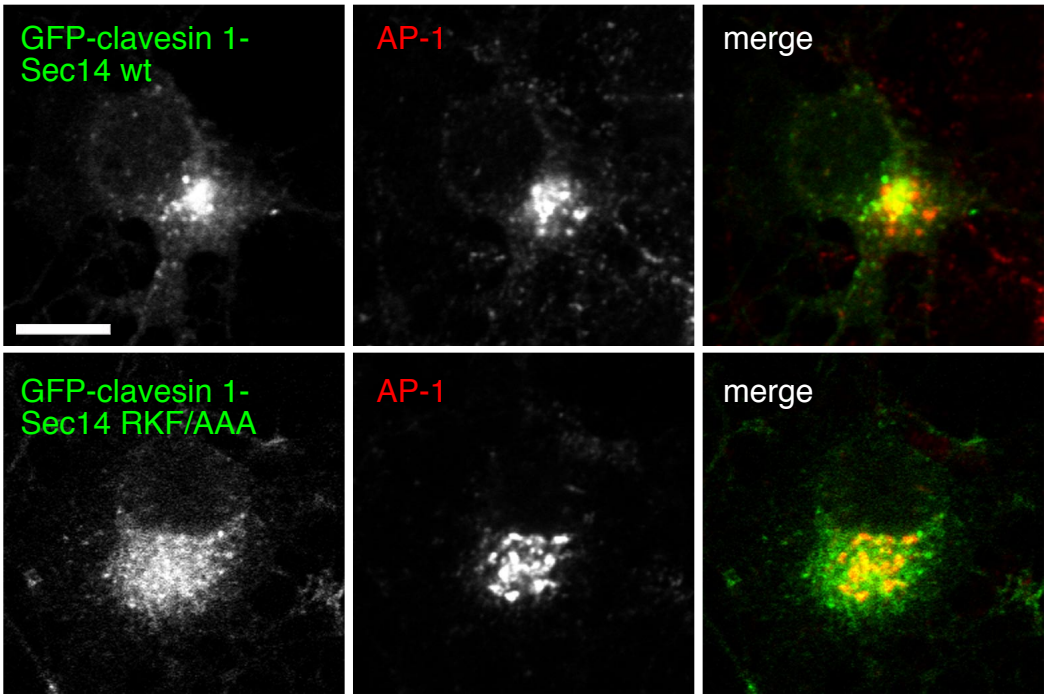
Katoh et al., supplemental figure 1



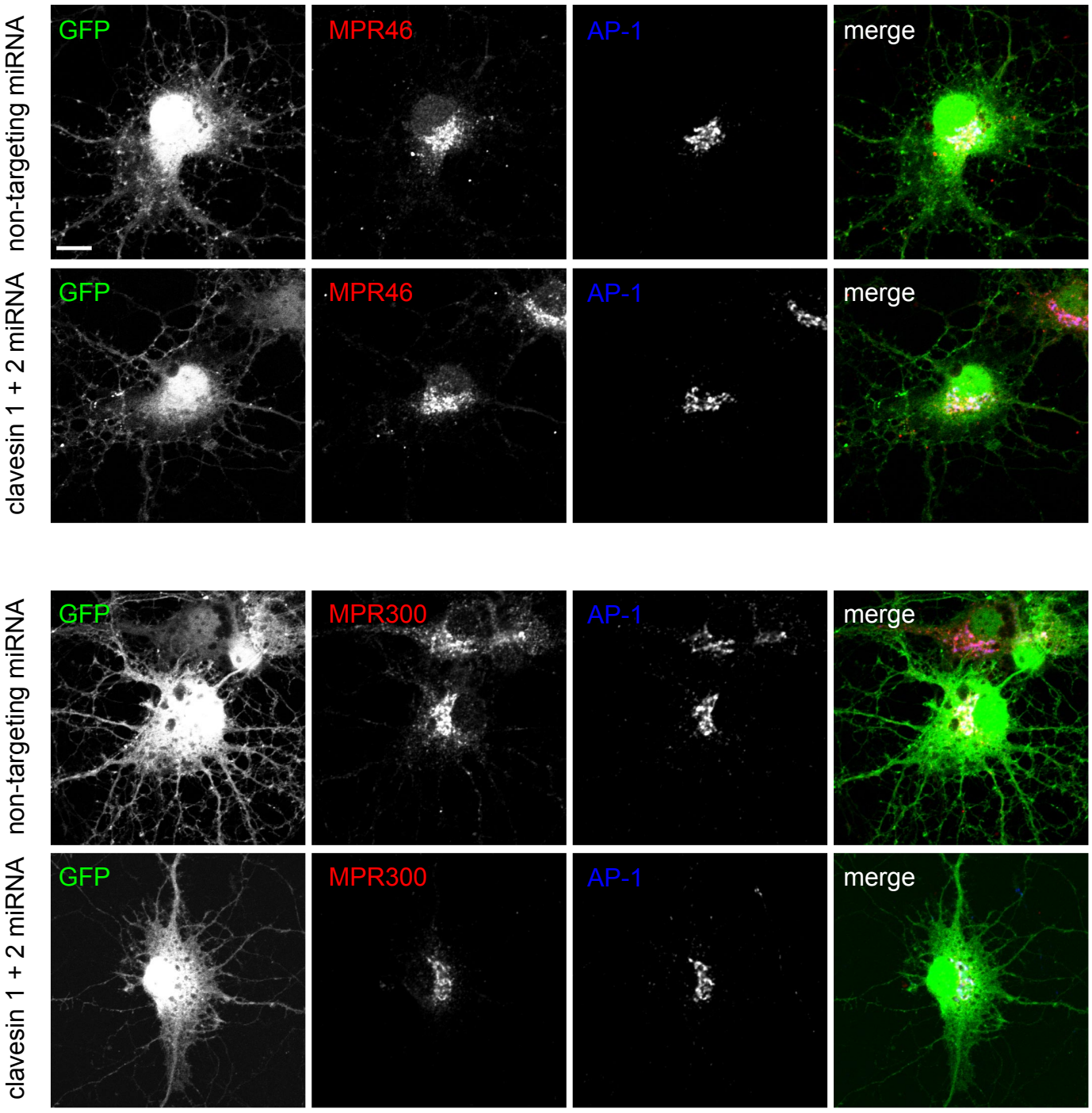
Katoh et al., supplemental figure 2



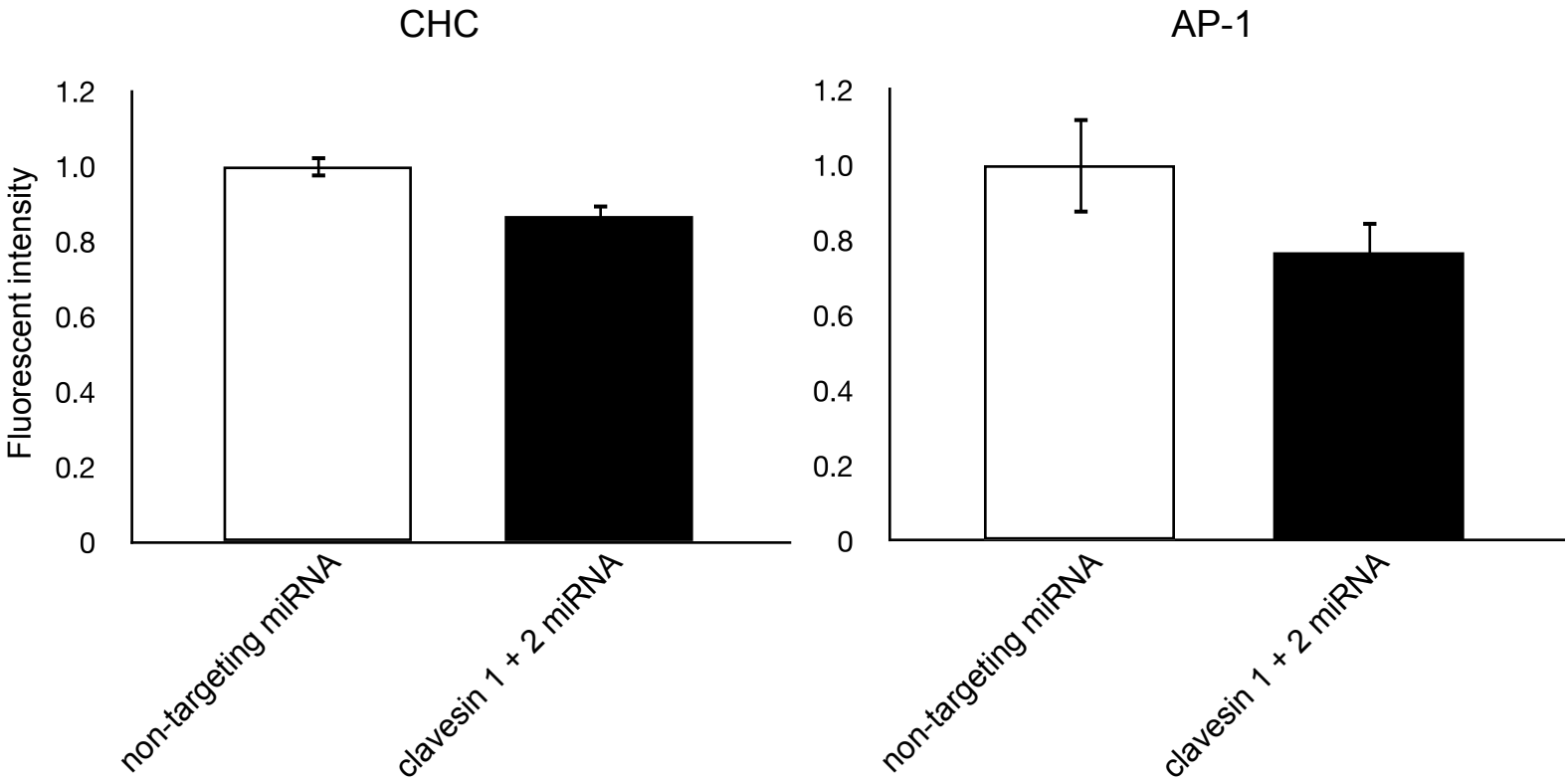
Katoh et al., supplemental figure 3



Katoh et al., supplemental figure 4



Katoh et al., supplemental figure 5



Katoh et al., supplemental figure 6