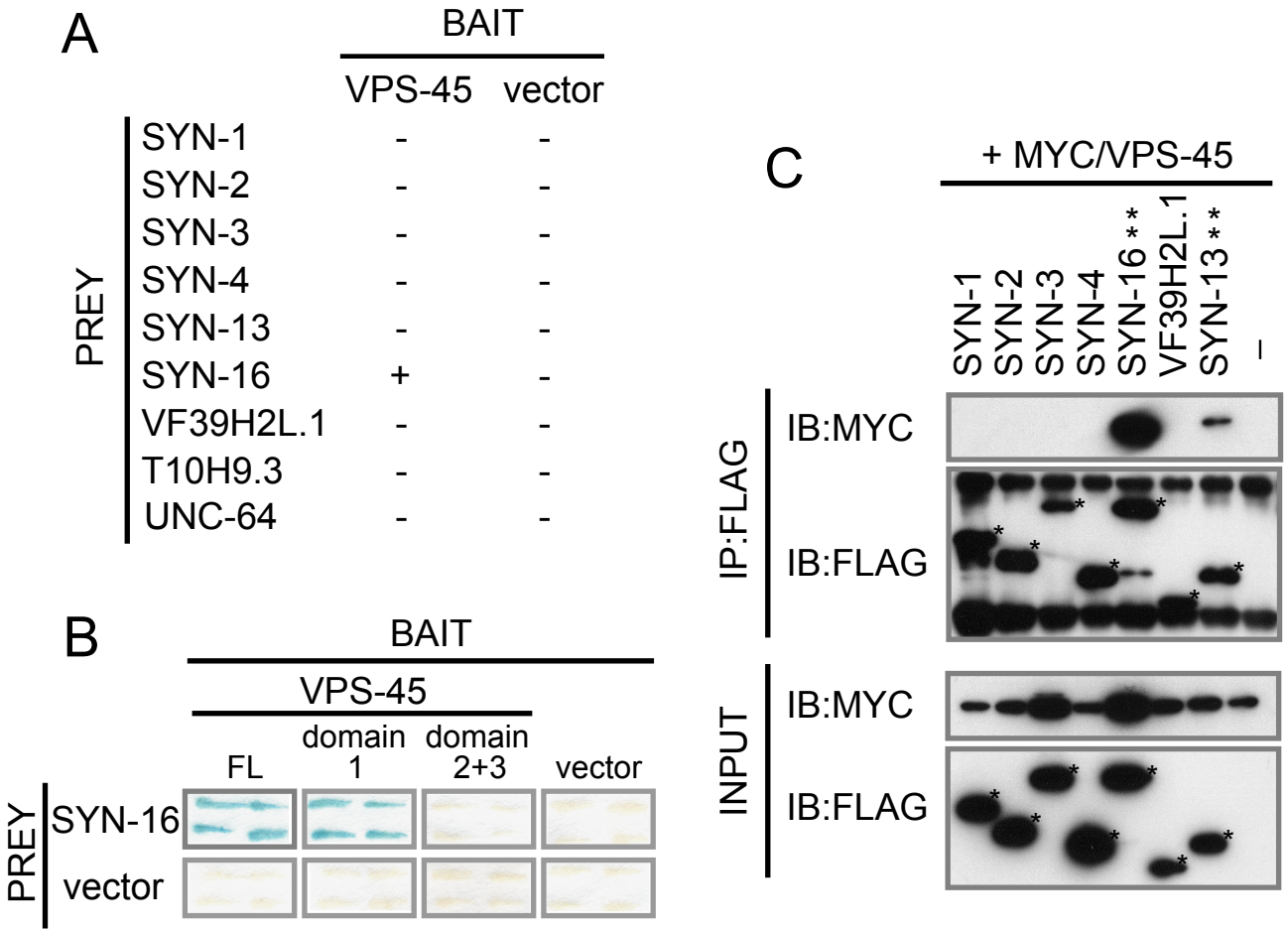


**Supplementary Figure 4**

**Supplementary Figure 4.** VPS-45 interacts with the cognate syntaxins, SYN-13 and SYN-16. (A) Yeast two hybrid assay between *vps-45* and all nine syntaxin genes identified on the *C. elegans* genome. Yeast strains carrying pGBKT7-*vps-45* (bait) and pGADT7 containing indicated syntaxins (prey) were streaked on a filter paper and subjected to the β-galactosidase assay. + means "positive interaction", - means "negative interaction". (B) Yeast two hybrid assay between *vps-45* and *syn-16*. VPS-45 is thought to consist of three domains; domain 1 (1- 127 amino acids), domain 2 (128-228, 452-574 amino acids) and domain 3 (229-451 amino acids). SYN-16 specifically binds to the N-terminal domain 1 of VPS-45 protein. (C) Co-immunoprecipitation analysis between VPS-45 and seven syntaxins. HEK293T cells were cotransfected with Myc-tagged full length of *vps-45* together with either empty vector or Flag-tagged cytoplasmic region of indicated syntaxins. Twenty-two hours after transfection, cells were lysed and the lysates were immunoprecipitated with anti-Flag antibody. Immunoprecipitates (upper) and Input (lower) were analyzed by immunoblotting with anti-Flag or anti-Myc antibodies. Asterisks indicate the positions of Flag/syntaxin fused proteins. In this assay, we found that VPS-45 interacts with not

only SYN-16 but also SYN-13 (**), and the results are consistent with previous reports about VPS-45 orthologs from other species. T10H9.3 did not interact with VPS-45 (data not shown). We did not examine interaction between VPS-45 and UNC-64, since UNC-64 is a neuron-specific syntaxin in *C. elegans*.