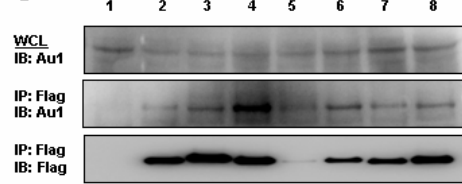


Figure S8. TM domain amino acids contributing to lipid raft association, identified by alanine scan mutagenesis. (A) Sequences of wild type Tip, and its mutants carrying alanine scan mutations in their TM domain. (B) 293T cells transiently expressing wild type Tip or its mutants were lysed and processed for lipid raft fractionation. Proteins from each fraction of the sucrose gradient were subjected to immunoblotting with an anti-Flag antibody to detect Tip or its mutants. CTB-HRP was used to confirm the localization and integrity of lipid rafts. The degree of lipid raft association was estimated by densitometry analysis and indicated as a percentage, as shown on the right side of each panel. (C) 293T cells were transfected with plasmids encoding AU1-tagged wild type Tip and Flag-tagged wild type Tip or its mutants. Cell lysates were analyzed by immunoprecipitation with an anti-Flag antibody followed by SDS-PAGE and immunoblotting with an anti-AU1 antibody or an anti-Flag antibody.

A

	TM
Tip wt	ANERNIVKDLKRLKINVIICLVVIVLAVLLLVTVLSILHIGMKS
Tip TM1	ANERNIVKDLKRLKINVIAAAALVVVILAVLLLVTVLSILHIGMKS
Tip TM2	ANERNIVKDLKRLKINVIICAAAALAVLLLVTVLSILHIGMKS
Tip TM3	ANERNIVKDLKRLKINVIICLVVIAAAALLLVTVLSILHIGMKS
Tip TM4	ANERNIVKDLKRLKINVIICLVVIVLAVAAAIVTVLSILHIGMKS
Tip TM5	ANERNIVKDLKRLKINVIICLVVIVLAVLLLVAAAIVTVLSILHIGMKS
Tip TM6	ANERNIVKDLKRLKINVIICLVVIVLAVLLLVTVLSAAAGMKS

C



1: Au1-Tip, vector
 2: Au1-Tip, Flag-Tip wt
 3: Au1-Tip, Flag-Tip TM1
 4: Au1-Tip, Flag-Tip TM2
 5: Au1-Tip, Flag-Tip TM3
 6: Au1-Tip, Flag-Tip TM4
 7: Au1-Tip, Flag-Tip TM5
 8: Au1-Tip, Flag-Tip TM6

B

