Supplemental Materials

Molecular Biology of the Cell

Takano et al.

Supplemental Figure Legends:

Supplemntal Figure S1. Knockdown of LMTK1 by micro-RNA (miRNA) in cortical neurons. (A) Immunoblots to show the knockdown of LMTK1 by miRNA. Cortical neurons were transfected with miRNA-LMTK1#3, miRNA-LMTK1#4 or scrambled sequence (SC) at 0 DIV. Cell lysates were prepared at 7 DIV and immunoblotted with anti-LMTK1. Actin was used as the loading control. (B) Immunostaining to show the knockdown of LMTK1 by miRNA. Cortical neurons were transfected with miRNA-SC, miRNA-LMTK1#3 or miRNA-LMTK1#4 at 0 DIV. At 7 DIV, neurons were immunostained with anti-LMTK1 (right panels). EGFP is shown in left panels. Bar, 20 μm. (C) and (D) Cortical neurons were transfected with miRNA-SC or miRNA-LMTK1#3 at 0 DIV and at 7 DIV neurons were subjected to immunostaining with anti-PSD95 (C) or anti-GluR2 (D), which are localized in postsynaptic regions. Merges are shown in right panels. Bar, 20 μm.

Supplemental Figure S2. Downregulation of LMTK1 induced many short protrusions along dendrites of cortical neurons. (A) Protrusions formed by downregulation of LMTK1. Cortical neurons were transfected with miRNA-SC, miRNA-LMTK1#3 or miRNA-LMTK1#4 at 0 DIV, and were observed at 7 DIV. Bar, 5 μm. (B) The number of protrusions was counted and expressed as the number/25 μm along dendrites in miRNA-expressing neurons (n= 30 for each of neurons transfected with miRNA-SC, miRNA-LMTK1#3 and miRNA-LMTK1#4). Data represent the means ± SD of three independent experiments (*$P < 0.01$, Student's *t*-test).

Supplemental Figure S3. Colocalization of LMTK1 with Rab5A or Rab7 in the cell body and dendrites of cortical neurons. LMTK1 was cotransfected into primary cortical neurons with EGFP-Rab5A or EGFP-Rab7. LMTK1 was visualized by immunostaining with anti-LMTK1 7 days after transfection (left panels). Right side panels are dendrites, in which a portion of LMTK1 was colocalized with Rab5A (arrows). Bar, 10 μm in the cell body and 5 μm in dendrite.

Supplenmental Figure S4. Expression of LMTK1 and phospho-LMTK1 in cultured neurons.

The lysates of mouse cortical neurons were collected from 3 to 13 DIV in culture and immunoblotted with antibodies against LMTK1, phospho-Ser34 of LMTK1 (pS34), p35, Cdk5, Rab11A, GluR2 and PSD95. Actin was used as the loading control.

Supplemental Videos:

Video 1. Transport of Rab11A-positive endosomes in the cell body of LMTK1$^{+/+}$ neurons. EGFP-Rab11A was transfected into cortical neurons at 6 DIV. Movements of Rab11A-positive endosomes were recorded by real time imaging at 7 DIV in the cell body. Frames were taken every 1 s with a Zeiss Confocal microscope LSM 710 (Carl Zeiss).

Video 2. Transport of Rab11A-positive endosomes in the cell body of LMTK1$^{-/-}$ neurons. EGFP-Rab11A was transfected into LMTK1$^{-/-}$ neurons at 6 DIV. Movements of Rab11A-positive endosomes were recorded by real time imaging at 7 DIV in the cell body. Frames were taken every 1 s with a Zeiss Confocal microscope LSM 710 (Carl Zeiss).

Video 3. Transport of Rab11A-positive endosomes in dendrites of LMTK1$^{+/+}$ neurons. EGFP-Rab11A was transfected into cortical neurons at 6 DIV. Movements of Rab11A-positive endosomes were recorded by real time imaging at 7 DIV in secondary dendrites. Frames were taken every 1 s with a Zeiss Confocal microscope LSM 710 (Carl Zeiss).

Video 4. Transport of Rab11A-positive endosomes in dendrites of LMTK1$^{-/-}$ neurons. EGFP-Rab11A was transfected into LMTK1$^{-/-}$ neurons at 6 DIV. Movements of Rab11A-positive endosomes were recorded by real time imaging at 7 DIV in secondary dendrites. Frames were taken every 1 s with a Zeiss Confocal microscope LSM 710 (Carl Zeiss).

Video 5. Movement of a Rab11A-positive endosome in axon of LMTK1$^{+/+}$ neurons at 7 DIV. EGFP-Rab11A was transfected into cortical neurons at 6 DIV. Movements of Rab11A-positive endosomes in axon were recorded by real time imaging at 7 DIV. Frames were taken every 1 s with a

Zeiss Confocal microscope LSM 710 (Carl Zeiss).

Video 6. Movement of a Rab11A-positive endosome in axon of LMTK1$^{-/-}$ neurons at 7 DIV. EGFP-Rab11A was transfected into LMTK1$^{-/-}$ neurons at 6 DIV. Movements of Rab11A-positive endosomes in axon was recorded by real time imaging at 7 DIV. Frames were taken every 1 s with a Zeiss confocal microscope LSM 710 (Carl Zeiss).

Video 7. Movement of a constitutive active Rab11A-Q70L-postive endosomes in LMTK1$^{+/+}$ neurons. EGFP-Rab11A-Q70L was transfected into cortical neurons at 6 DIV. Movements of Rab11A-Q70L-positive endosomes in dendrites were recorded by real time imaging at 7 DIV. Frames were taken every 1 s with a Zeiss Confocal microscope LSM 710 (Carl Zeiss).

Video 8. Movement of a dominant negative Rab11A-S25N-postive endosome in LMTK1$^{+/+}$ neurons. EGFP-Rab11A-S25N was transfected into cortical neurons at 6 DIV. Movements of Rab11A-S25N-positive endosomes were recorded by real time imaging at 7 DIV in dendrites. Frames were taken every 1 s with a Zeiss Confocal microscope LSM 710 (Carl Zeiss).

Video 9. Movement of Rab11A-positive endosomes in growth cone of LMTK1$^{+/+}$ neurons. EGFP-Rab11A was cotransfected with DsRed into cortical neurons at 6 DIV. Movements of Rab11A-positive endosomes in growth cone were recorded by real time imaging at 7 DIV. Frames were taken every 1.6 s with a Zeiss Confocal microscope LSM 710 (Carl Zeiss).

Video 10. Movement of Rab11A-positive endosomes in growth cone of LMTK1$^{-/-}$ neurons. EGFP-Rab11A was cotransfected with DsRed into LMTK1$^{-/-}$ neurons at 6 DIV. Movements of Rab11A-positive endosomes in growth cone were recorded by real time imaging at 7 DIV. Frames were taken every 1.6 s with a Zeiss Confocal microscope LSM 710 (Carl Zeiss).

Figure S1. Takano et al

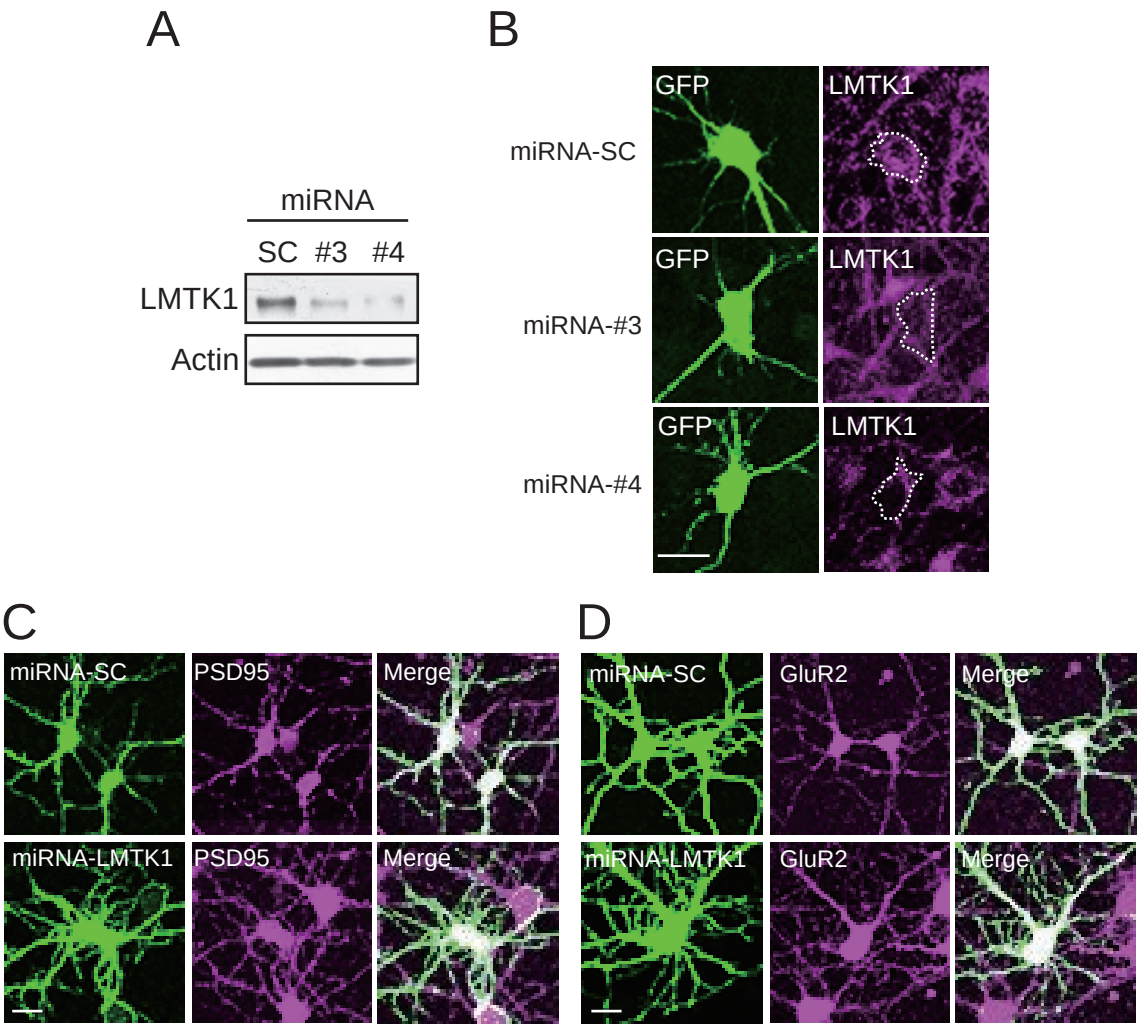

Figure S2. Takano et al

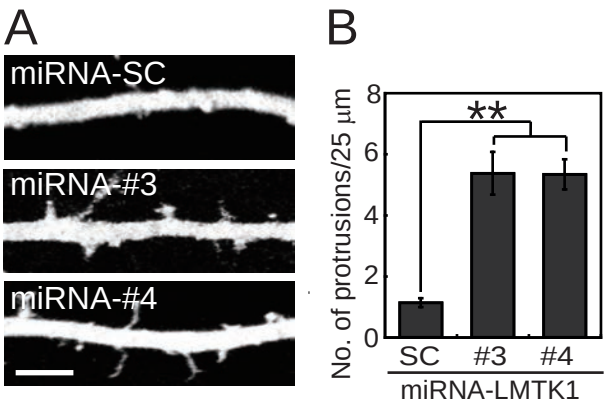

Figure S3. Takano et al

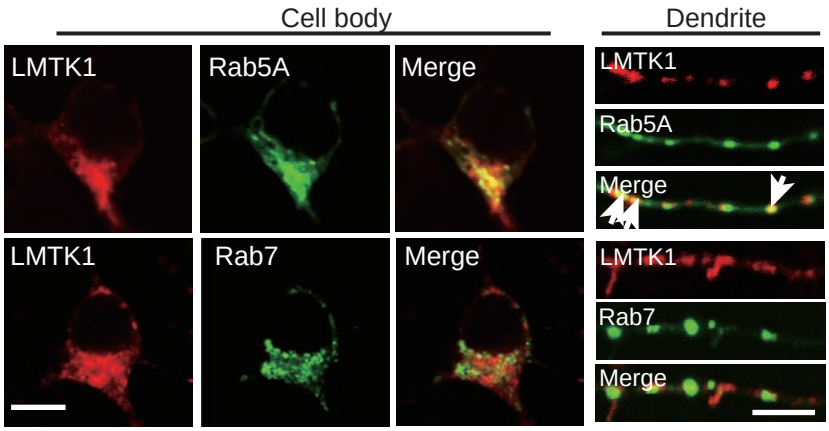

Figure S4. Takano et al