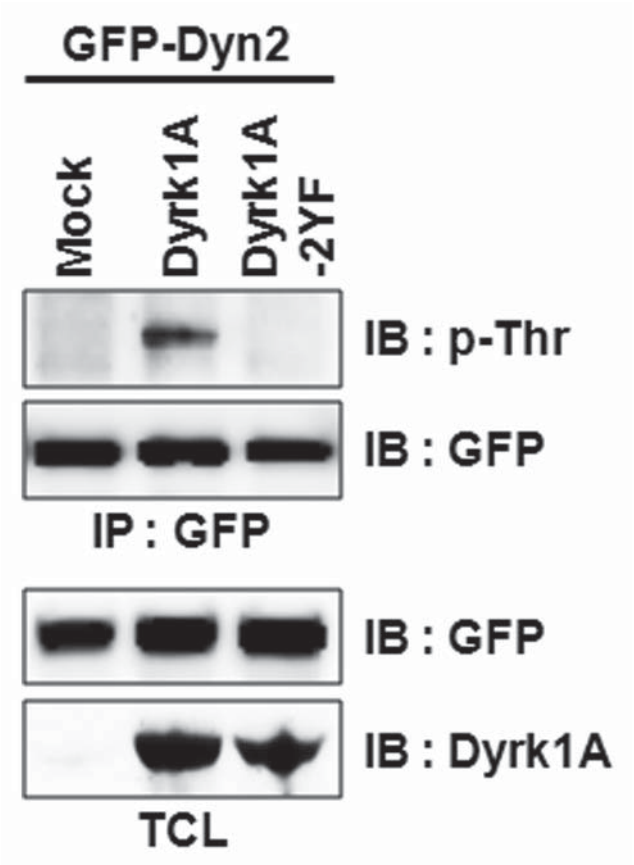
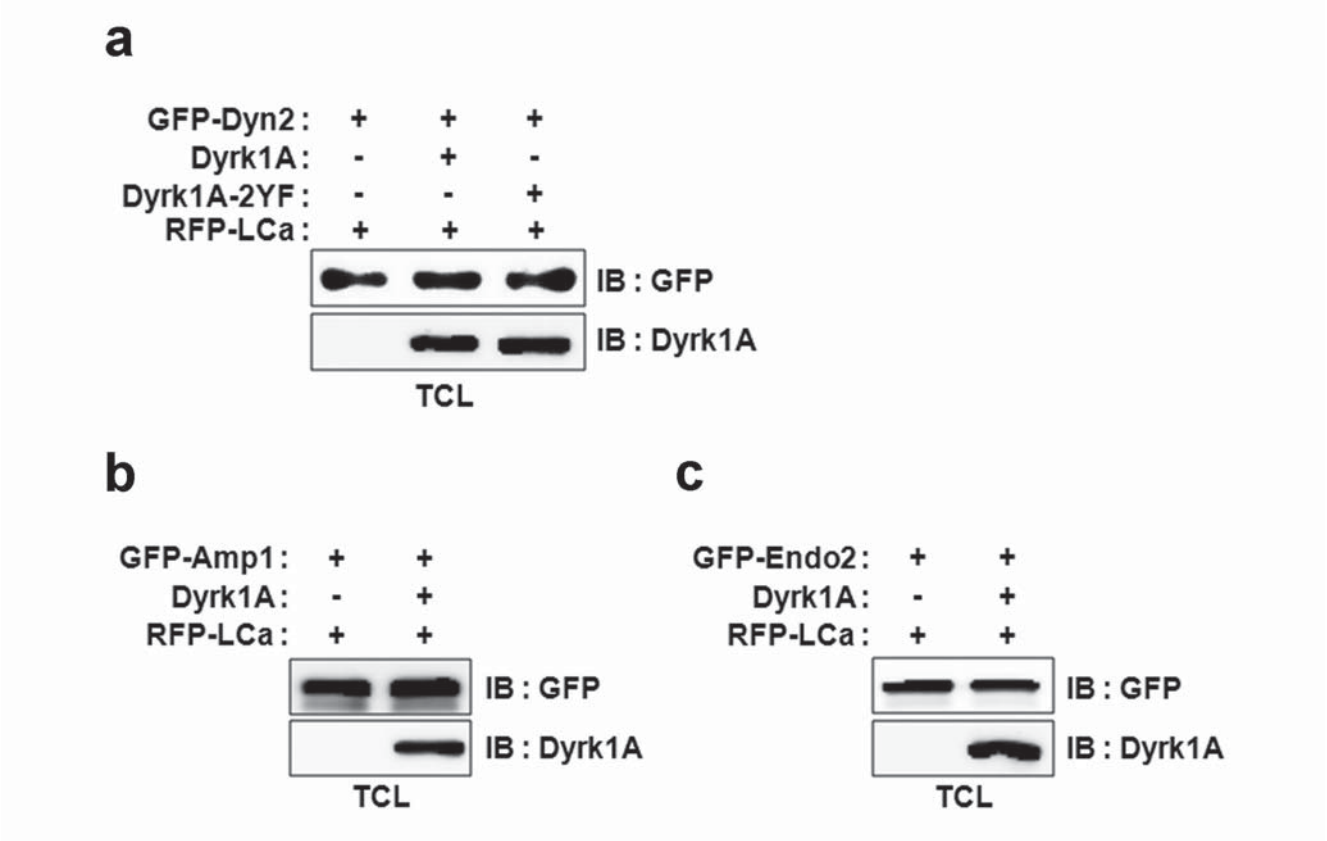
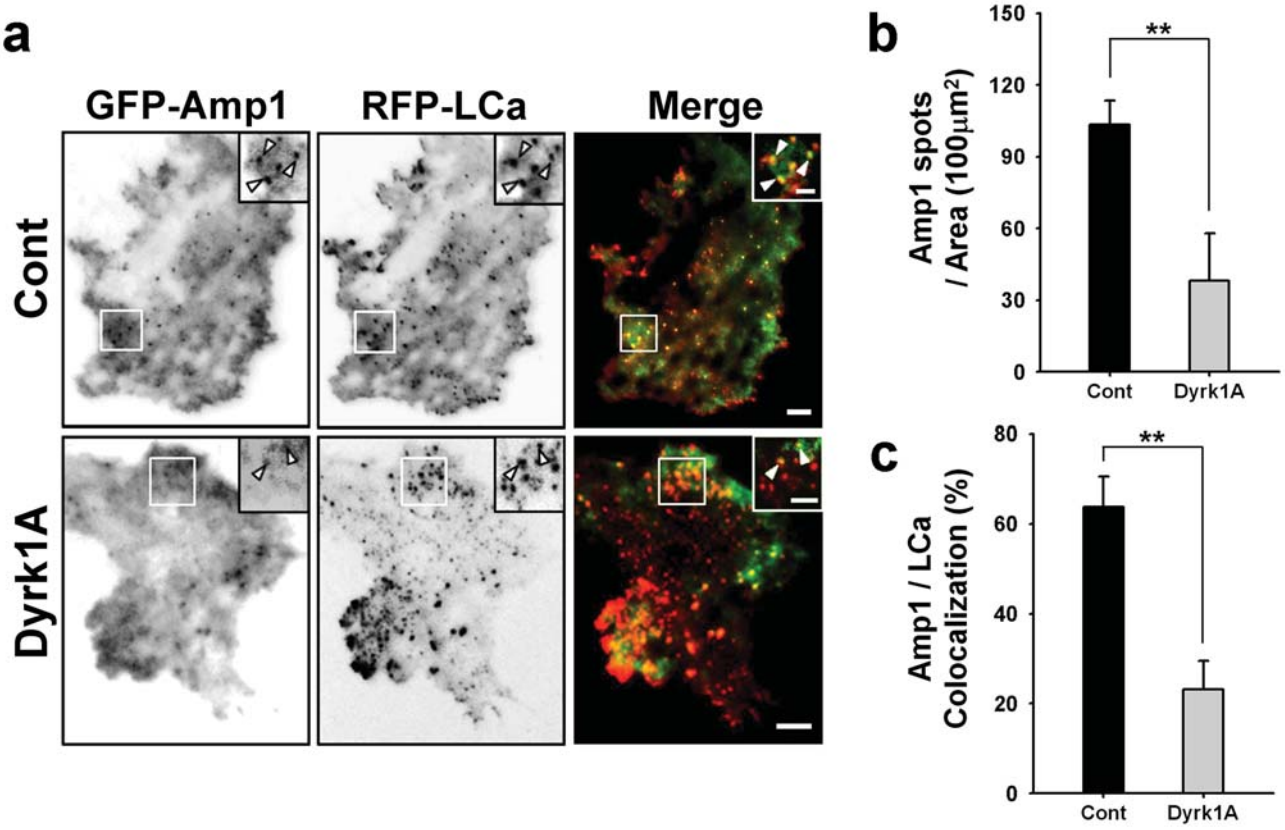


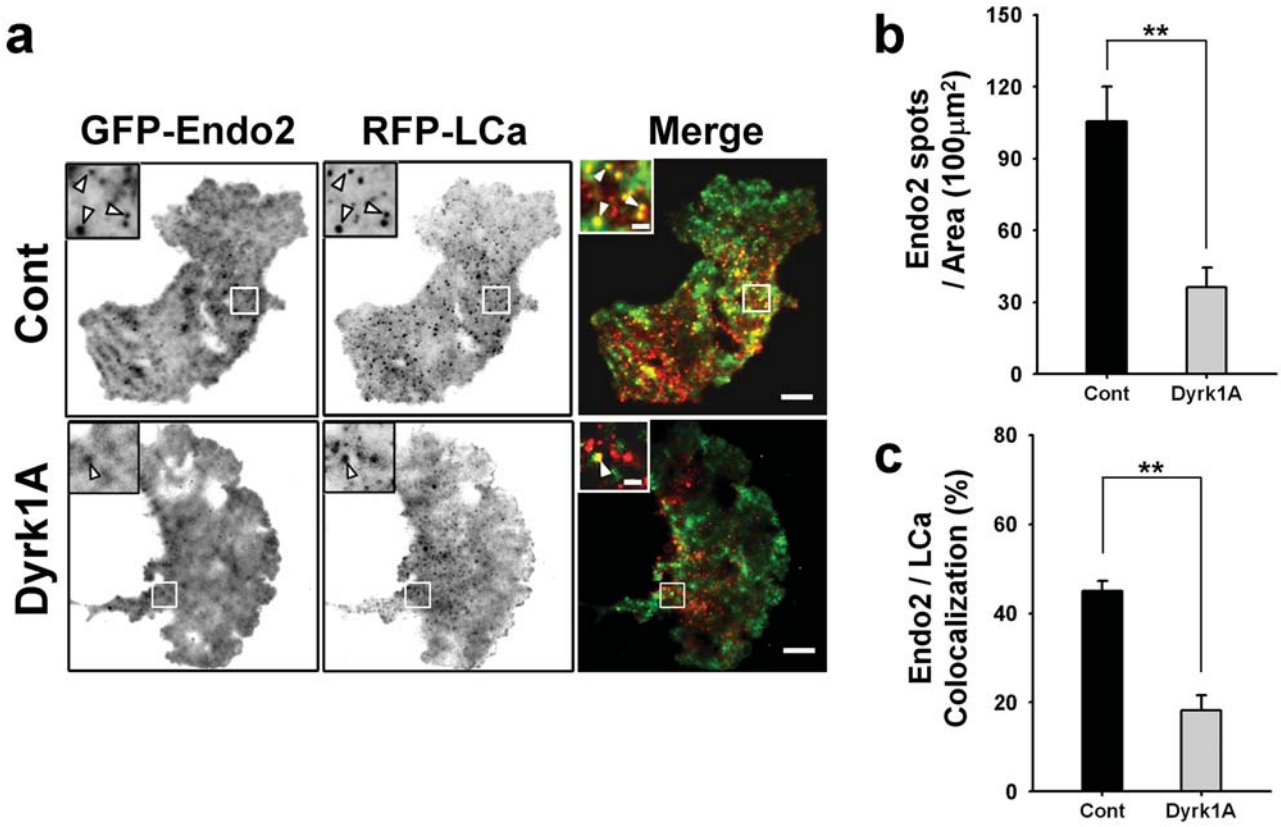
Supplementary Figure 1. Phosphorylation of dynamin 2 by Dyrk1A. HEK293T cells were cotransfected with GFP-dynamin 2 (GFP-Dyn2), and Dyrk1A, Dyrk1A-2YF or pcDNA-His (Cont) vector. 48 h after transfection, the cells were lysed and incubated with a mixture of phosphatase inhibitors for 30 min at 30°C in the presence of 2 mM Mg2+-ATP.. The lysates were immunoprecipitated with anti-GFP antibody and immunoblotted with anti-p-Threonine antibody and anti GFP antibody. TCL: total cell lysate, IP: immunoprecipitation, IB: immunoblot.



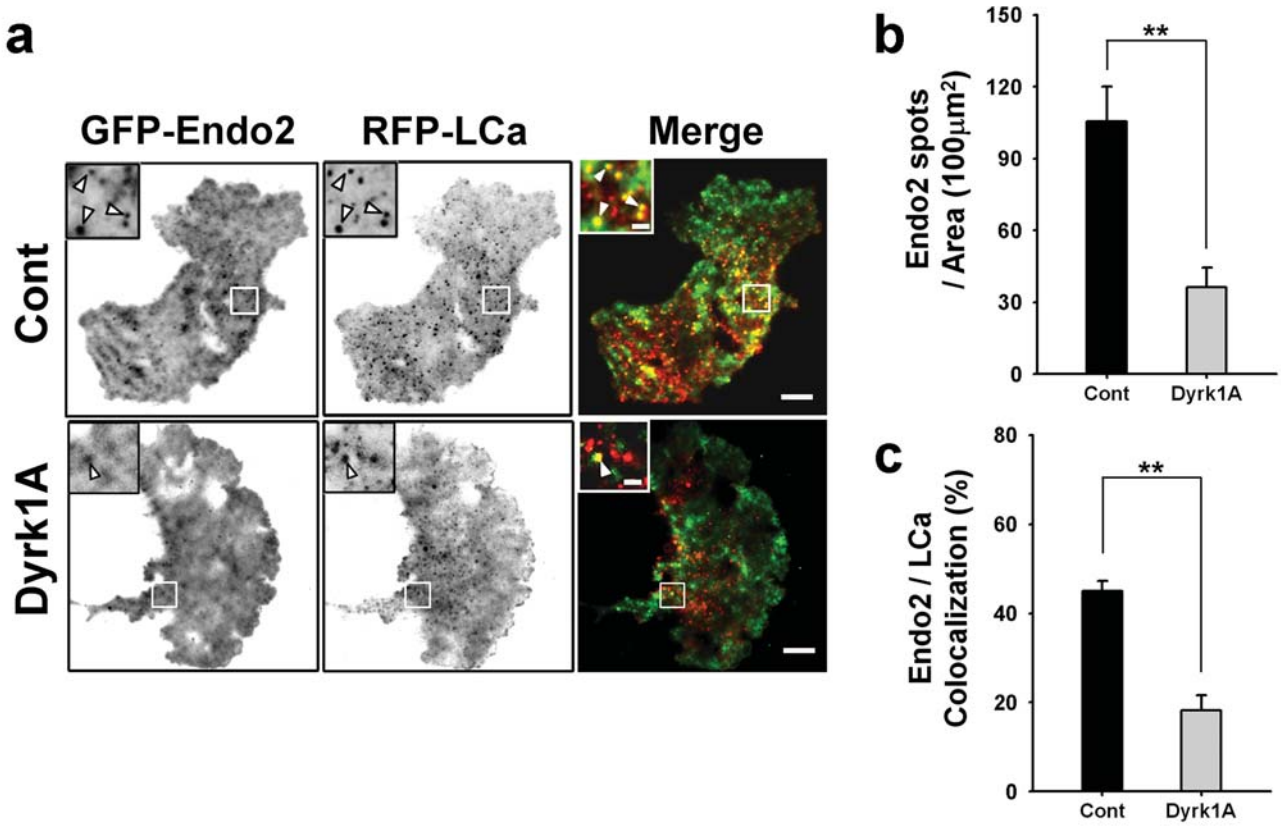
Supplementary Figure 2. The expression levels of transfected endocytic proteins are not affected by Dyrk1A overexpression. (a) COS-7 cells were triply transfected with mRFP-clathrin-light chain (RFP-LCa), GFP-Dyn2, and Dyrk1A, Dyrk1A-2YF or pcDNA-His (Cont) vector. 48 h after transfection, the cells were lysed and immunoblotted with anti-Dyrk1A antibody and anti-GFP antibody. There is no difference in protein levels regardless of presence of Dyrk1A. (b, c) Cells were triply transfected with RFP-LCa, GFP-amphiphysin 1(GFP-Amp1, b) or -endophilin 2 (Endo2, c), and Dyrk1A or Cont vector. 48 h after transfection, the cells were lysed and immunoblotted with anti-Dyrk1A antibody and anti-GFP antibody. There is no difference in protein levels regardless of presence of Dyrk1A.



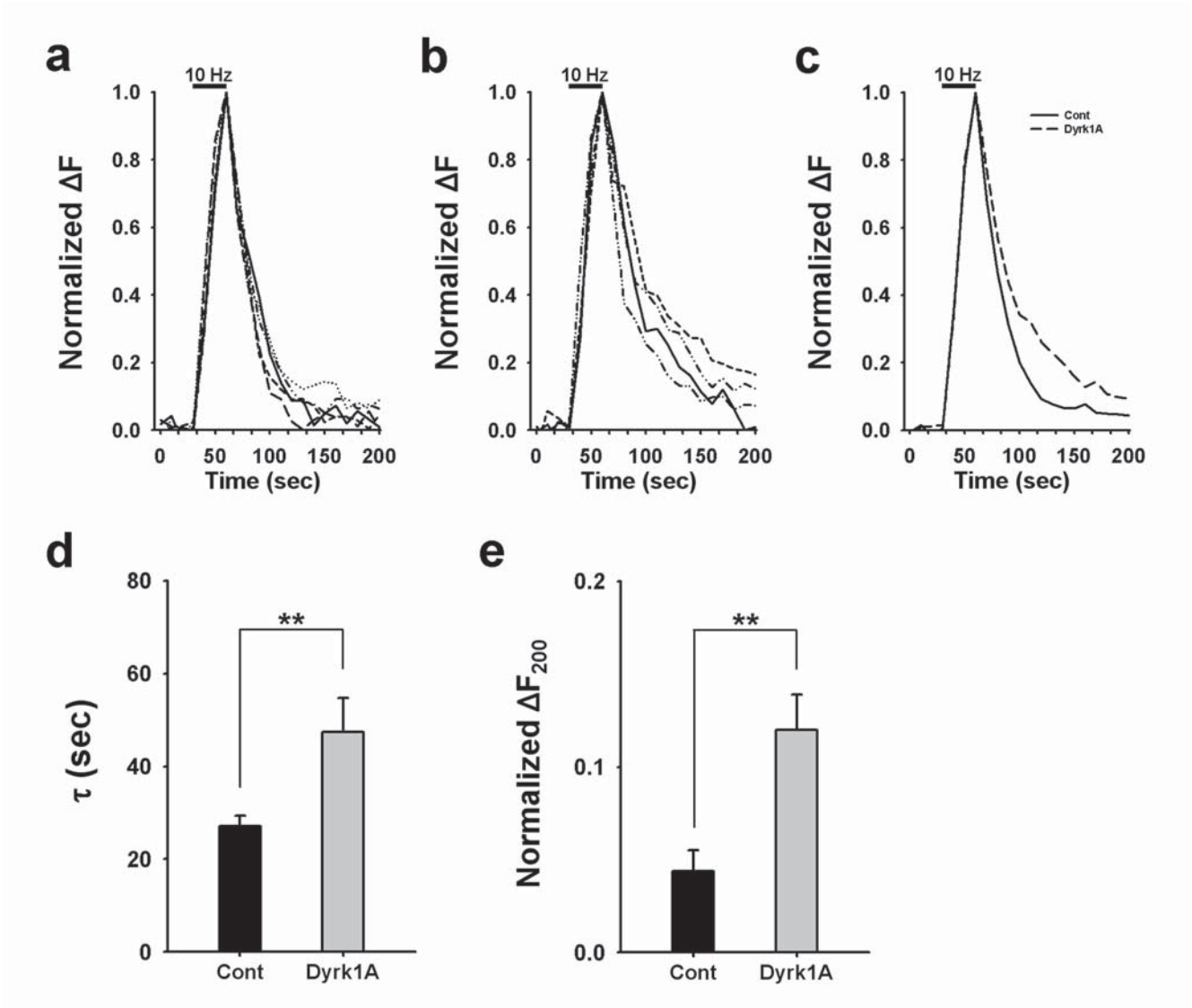
Supplementary Figure 3. The recruitment of amphiphysin 1 to the sites of clathrin-coated assembly is reduced by Dyrk1A. (a) TIRFM images of COS-7 cells which were triply transfected with RFP-LCa, GFP-Amp1, and Dyrk1A or pcDNA-His (Cont) vector. Arrowheads indicate the colocalization between Amp1 and LCa. Scale bars: low magnification, 10 μm; high magnification, 5 μm. (b) The number of Amp1 spots within 100 μm2 of the plasma membrane. (c) The degree of colocalization between Amp1 and LCa was reduced by Dyrk1A overexpression (n=5 for Cont, n=6 for Dyrk1A). 6 to 10 cells were selected in each independent experiment and were analyzed. Data are presented as means ± s.e. ** $p < 0.01$ (Student's t-test).



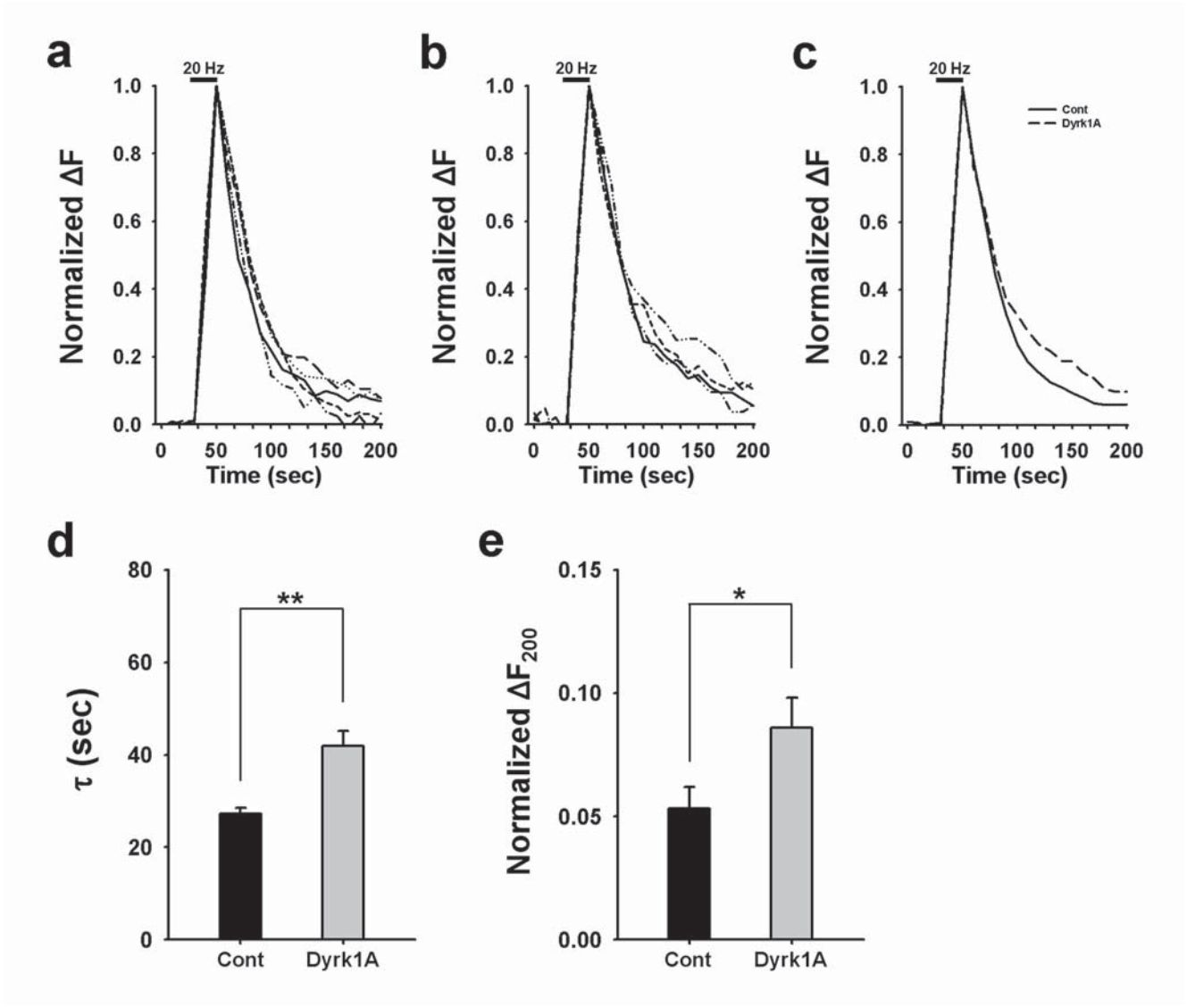
Supplementary Figure 4. The recruitment of endophilin 2 to the sites of clathrin-coated assembly is reduced by Dyrk1A. (a) TIRFM images of COS-7 cells which were triply transfected with RFP-LCa, GFP-Endo2, and Dyrk1A or pcDNA-His (Cont) vector. Arrowheads indicate the colocalization between Endo2 and LCa. Scale bars: low magnification, 10 μm; high magnification, 2 μm. (b) The number of Endo2 spots within 100 μm2 of the plasma membrane (c) The degree of colocalization between Endo2 and LCa was reduced by Dyrk1A overexpression (n=5 for Cont, n=4 for Dyrk1A). 6 to 10 cells were selected in each independent experiment and were analyzed. Data are presented as means ± s.e. ** $p < 0.01$ (Student's t-test).



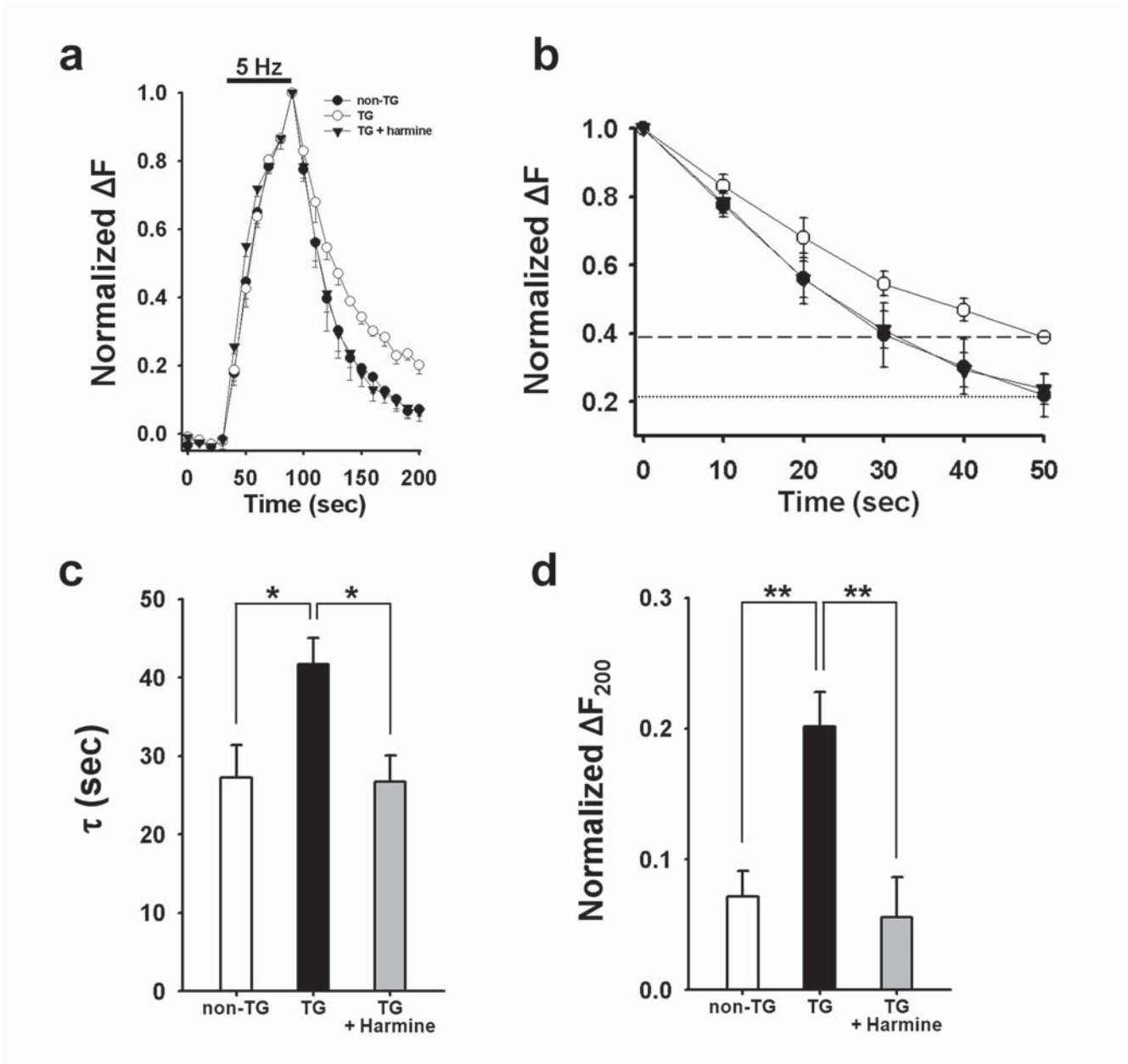
Supplementary Figure 5. The endocytic defects by Dyrk1A overexpression are fully recovered to the control level by shRNA induced knock-down of Dyrk1A in Dyrk1A overexpression background (a) HEK293T cells were cotransfected with Dyrk1A and Dyrk1A-shRNA #1 (sh #1), Dyrk1A-shRNA #2 (sh #2), or control shRNA (sh cont). 48 h after transfection, the cells were lysed and immunoblotted with anti-Dyrk1A antibody and anti-β-tubulin antibody. (b) Hippocampal neurons were triply transfected with vGpH, and Dyrk1A and sh #1, sh #2, or sh cont, or pcDNA-His and sh cont. Average fluorescence intensity profiles were plotted as $\Delta F/F0$ against time, following stimulation with 300 APs at 5 Hz (dark bar). (c) The decay of vGpH fluorescence was fitted by a single exponential with $\tau = 23.7 \pm 1.8$ s (n=4) for sh cont; $\tau = 38.0 \pm 3.4$ s (n=4) for Dyrk1A with sh cont; $\tau = 24.2 \pm 1.9$ s (n=4) for Dyrk1A with sh #1; $\tau = 23.3 \pm 2.0$ s (n=4) for Dyrk1A with sh #2. Data were collected 4 to 5 neurons in each independent experiment, and 10 to 20 boutons from a single neurons were analyzed. (d) The residual vGpH fluorescence intensity at 200 s was normalized to the initial resting state of each presynaptic bouton. Data are presented as means ± s.e. * $p < 0.05$, ** $p < 0.01$ (ANOVA and Turkey's HSD post hoc test).



Supplementary Figure 6. The overexpression of Dyrk1A in neurons impairs synaptic vesicle endocytosis when challenged with 300 APs stimulation at 10 Hz. Hippocampal neurons were cotransfected with vGpH and mCherry (Cont) or Dyrk1A. (a and b) Normalized vGpH fluorescence intensity profiles of the boutons from Cont (a) or Dyrk1A (b) overexpressing neurons which were stimulated with 300 APs at 10 Hz. Each line (solid, dotted, etc) represents an average fluorescence trace from a single neuron. 4 to 5 neurons were selected in each independent experiment and 10 to 20 boutons from a single neuron were analyzed. (c) Average fluorescence intensity profiles were plotted as ΔF/F0 against time, following stimulation with 300 APs at 10 Hz (dark bar). Data were collected from 4 to 5 independent experiments for each group. (d) The decay of vGpH fluorescence was fitted by a single exponential with τ = 27.0 ± 2.3 s for control; τ = 47.4 ± 7.4 s for Dyrk1A. (e) The residual vGpH fluorescence intensity at 200 s was normalized to the initial resting state of each presynaptic bouton. Data are presented as means ± s.e. ** $p < 0.01$ (Student's t-test).



Supplementary Figure 7. The overexpression of Dyrk1A in neurons impairs synaptic vesicle endocytosis when challenged with 300 APs stimulation at 20 Hz. Hippocampal neurons were cotransfected with vGpH and mCherry (Cont) or Dyrk1A. (a and b) Normalized vGpH fluorescence intensity profiles of the boutons from Cont (a) or Dyrk1A (b) overexpressing neurons which were stimulated with 300 APs at 20 Hz. Each line (solid, dotted, etc) represents an average fluorescence trace from a single neuron. 4 to 5 neurons were selected in each independent experiment and 10 to 20 boutons from a single neuron were analyzed. (c) Average fluorescence intensity profiles were plotted as ΔF/F0 against time, following stimulation with 300 APs at 20 Hz (dark bar). Data were collected from 4 to 5 independent experiments for each group. (d) The decay of vGpH fluorescence was fitted by a single exponential with $\tau = 27.3 \pm 1.3$ s for control; $\tau = 41.9 \pm 3.2$ s for Dyrk1A. (e) The residual vGpH fluorescence intensity at 200 s was normalized to the initial resting state of each presynaptic bouton. Data are presented as means ± s.e. * < 0.05, ** $p < 0.01$ (Student's t-test).



Supplementary Figure 8. The delay in synaptic vesicle endocytosis observed in Dyrk1A TG mice is recovered to control levels by harmine treatment. Hippocampal neurons from Dyrk1A TG or control littermate were transfected with vGpH and treated with or without 1 μM harmine for 48 h. (a and c) Average fluorescence intensity profiles were plotted as ΔF/F0 against time, following stimulation with 300 APs at 5 Hz (dark bar). The decay of vGpH fluorescence was fitted by a single exponential with $\tau = 27.3 \pm 4.1$ s (n=6) for non-TG littermate; $\tau = 41.7 \pm 3.3$ s (n=4) for Dyrk1A TG; $\tau = 26.7 \pm 3.3$ s (n=5) for Dyrk1A TG with harmine. Data were collected 4 to 5 neurons in each independent experiment, and 10 to 20 boutons from a single neuron were analyzed. (b) Expanded data collected within initial 50 s indicate the marked differences in the initial endocytic kinetics among neurons derived from non-TG mice, Dyrk1A TG mice, and Dyrk1A TG mice with harmine. (d) The residual vGpH fluorescence intensity at 200 s was normalized to the initial resting state of each presynaptic bouton. Data are presented as means ± s.e. * $p < 0.05$, ** $p < 0.01$ (ANOVA and Turkey's HSD post hoc test).