

Fig S1. Generation of *TgVps35^{Neurod6}* mice. **(A)** Illustration of generation of LSL-Vps35-mCherry and LSL-Vps35-mCherry; *Neurod6*-Cre mice. **(B)** Western blot analysis of Vps35 and Vps35-mCherry levels in HEK-293 cells transfected with the indicated plasmids. **(C)** Immunostaining of HEK-293 cells transfected with the indicated plasmids. **(D)** Western blot analysis of Vps35-mCherry levels in different brain tissues taken from control (*Vps35^{fl/fl}*) and *TgVps35^{Neurod6}* mice, β -actin was employed as a loading control. **(E)** Relative quantitation of the protein levels reveals the specific expression of mCherry in cortex and hippocampus, not cerebellum. **(F)** Body weight curves of *TgVps35^{Neurod6}* mice and littermate controls. **(G)** Representative Nissl stains of control and *TgVps35^{Neurod6}* mice at indicated age. **(H)** Quantification analysis of Nissl stains that revealed a comparable cortical thickness between control and *TgVps35^{Neurod6}* mice (n = 3~4 animals per genotype, take 8-10 positions for each animal; two-tailed unpaired t test). **(I, J)** Representative images of immunostaining analysis using indicated antibodies in P14 neocortical and hippocampal sections from control and *TgVps35^{Neurod6}* mice. Higher magnification images of the boxed regions were shown in offside and lower panels. **(K, M)** Representative images of immunostaining analysis using indicated antibodies in P14 neocortical sections from *TgVps35^{Neurod6}* mice. **(L, N)** Quantification analysis of co-immunofluorescence from K and M. Scale bars as indicated in each panel. Individual data points were shown as dots with group mean \pm s.e.m; ** $p < 0.01$; *** $p < 0.001$; n.s., not significant.

Fig S2. Terminal differentiation deficits of *TgVps35^{Neurod6}; KO* mice after P14. **(A)** Schematic drawing showing the process of AAV-Syn-mCherry injection. **(B)** Representative images of single neuron labeled with mCherry from hippocampus CA1 and entorhinal cortex. **(C)** Quantification of dendritic complexity and total dendritic length of hippocampal CA1 pyramidal neurons from control, *Vps35^{Neurod6}* and *TgVps35^{Neurod6}; KO* mice at P29 (n=3 neurons from 3 mice per genotype; one-way ANOVA with Tukey's multiple-comparison test). **(D)** Quantification of dendritic complexity and total dendritic length of entorhinal cortex pyramidal neurons from control, *Vps35^{Neurod6}* and *TgVps35^{Neurod6}; KO* mice at P29 (n=3 neurons from 3 mice per

genotype; one-way ANOVA with Tukey's multiple-comparison test). Scale bars as indicated in each panel. Individual data points were shown as dots with group mean \pm s.e.m; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; n.s., not significant.