

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 ($Vps35^{ff}$) 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 ($n = 3\sim 4$ 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.