SUPPLEMENTARY INFORMATION

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Developmental neuropathologies in animal model of FMCD

(**a**) Schematic illustration of *in utero* electroporation. Plasmid constructs were injected into one side of the developing cortex at E14.5 followed by electric pulses. The expression of GFP allowed visualizing cells expressing the DNA constructs. Cortical sections at postnatal or E18.5 brains were stained for layer markers, SATB2 and CTIP2 and brain regions were subdivided as marginal zone (MZ), upper cortical plate (uCP), middle cortical plate (mCP), lower cortical plate (loCP) and intermediate/subventricular zone (IZ/SVZ) to quantify neuronal migration. (**b**) Representative images of cortical sections at E18.5 stained for layer markers SATB2 and CTIP2. (**c**) Localization of GFP^+ neurons was quantified in indicated cortical regions. (**d**) GFP^+ neurons were quantified for layer specific markers (SATB2 or CTIP2). (**e**) $SATB2^+$ cells in IZ/SVZ were quantified for GFP expression (n = 3, 3, 5 for each condition). FOV: field-of-view. (**f**) Brains were electroporated with indicated plasmids at E14.5 and isolated at E16.5 or E18.5. Brain sections were immunostained for differentiating neuronal marker, MAP2. MAP2 positivity of electroporated cells below subplate (SP) is shown (see insets). CP: cortical plate. Values: mean ± s.d. n.s., not significant; *, $P < 0.05$; ***, $P < 0.001$, Student's t-test (**e**); G-test of goodness-of-fit (**c**, **d**). Scale bars: 100 μm (**a**, **b**, **f**); 10 μm (**f** inset)

Supplementary Figure 2. Kinase activity of AKT3 required for aberrant migration

Developing brains were electroporated with indicated plasmid constructs at E14.5 and cortical sections from E18.5 brains were imaged. (**a–d**) Representative images of cortical sections (**a**, **c**). Migration of GFP^+ neurons were quantified in indicated cortical regions (n = 3, 3, 3, 4 in **b**; n = 3, 3, 3, 6 in **d**). (**e, f**) Soma size was quantified from z-stacked confocal microscopic images. For each condition, three non-consecutive images were taken from each of three brains. 20 cells per images were quantified. Values: mean ± s.d. n.s., not significant; ***, $P < 0.001$, G-test of goodness-of-fit (**b, d**); Student's t-test (**f**). Scale bars: 100 μm.

Supplementary Figure 3. Pharmacological rescue of $AKT3^{E17K}$-induced phenotypes

(**a–c**) Developing brains were electroporated with indicated plasmids at E14.5. Rapamycin (3 μg/gram-body-weight/day) was maternally delivered for 4 days before isolation of brain at E18.5. (**a**) Experimental scheme. (**b, c**) Cortical sections were immunostained for phospho-S6 (pS6) and length of $pS6^+$ layer were quantified. Values: mean ± s.d. (n = 3, 3, 3, 5, 3, 5). n.s., not significant; ***, $P < 0.001$ (Student's t-test). Scale bar: 100 μm.

Supplementary Figure 4. Non-cell autonomous effect on neuronal migration

(**a–c**) Developing brains were electroporated with indicated plasmids followed by 5-bromo-2'-deoxyuridine (BrdU) injection 6 hours later. Brains sections at E18.5 were stained for BrdU. GFP^+BrdU^+ cells or GFP^-BrdU^+ cells were quantified in indicated

region. Values: mean ± s.d. (n = 3, 3, 5 for each condition) *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, G-test of goodness-of-fit (**b**); Student's t-test (**c**). Scale bars: 100 μm.

Supplementary Figure 5. Genetic recombination of AKT3^{E17K} defines reversibility of FMCD networks

(**a**) hNPCs were transduced by pBOB/Switch or pBOB/Switch-AKT3^{E17K} vector construct-containing lentivirus. RFP^{+}GFP^{-} hNPCs were collected by fluorescence-activated cell sorting (FACS). Virally transduced hNPCs were also treated with Cre-expressing adenovirus followed by sorting of RFP^{-}GFP^{+} cells. Total RNA from three independent cultures were sequenced for mRNA (RNA-seq). (**b**) Analysis from RNAseq with highly interconnected networks of genes in four major categories (Neuronal development, Signaling and homeostasis, Cell cycle and Migration), which correlate with major FMCD phenotypes. Light nodes: restored or reversed changes upon Cre recombination. Dark nodes: persisted changes upon Cre induction. Node sizes correlates with number of links. Note in the Migration category, *RELN* is represented by a light circle, meaning that it was changed (upregulated) and then reversed upon Cre recombination. Interactions of components between categories are not shown for simplicity. (**c**) pBOB/Switch or pBOB/Switch-AKT3^{E17K} vectors were transfected to NIH3T3 cells. Cells were treated with Cre-expressing adenovirus (AdCre) followed by immunocytochemistry of AKT. AKT immunofluorescence was taken by short exposure to image overexpression. Scale bar: 20 μm.

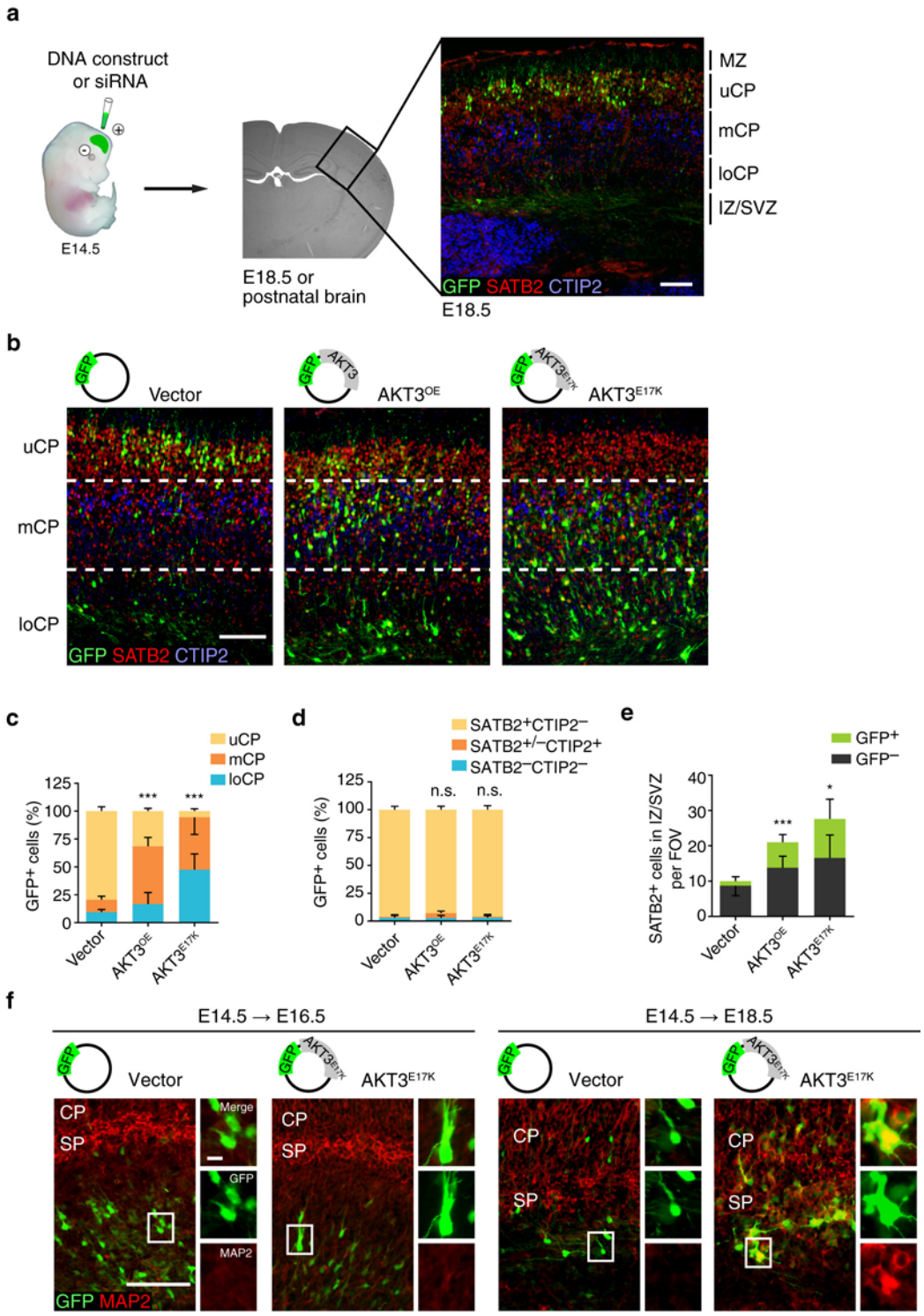
Supplementary Figure 6. AKT3-mediated inactivation of FOXG1 resulting reelin misexpression in developing neurons

(**a**) *In utero* electroporation of $AKT3^{E17K}$ construct at E14.5, immunostained for GFP and reelin at P20. Arrowheads indicate $AKT3^{E17K}$-expressing (GFP^+) neurons with no strong reelin immunofluorescence. Asterisks indicate reelin-positive inhibitory neurons negative for GFP. (**b**) $pS6^+$ or $pS6^-$ cells were laser-captured microdissected from brain sections of FMCD patient with somatic mosaic *AKT3* c.49G>A (p.E17K) mutation, and extracted for total RNA followed by cDNA synthesis. *RELN* expression was detected by qPCR. (**c**) Schematic representation of 5′-upstream region of *RELN*. Chromatin immunoprecipitation was performed with hNPCs using primers for four putative FOX-binding sites (R1–R4) and negative region with no FOX-binding site (n = 3 cultures). (**d**) hNPCs expressing vector or $AKT3^{E17K}$ were immunostained and imaged for FOXG1 expression. Arrowheads indicate examples of FOXG1 localization in nucleus (left) or cytoplasm (right). (**e**) Localization of FOXG1 staining was quantified in nucleus and cytoplasm. Three non-overlapping images per culture were taken from three cultures for quantification. (**f, g**) GFP fusion of Foxg1 constructs were co-transfected with vector or $AKT3^{E17K}$ constructs into NIH3T3 cells. After 72 h, fluorescence images were taken. Arrowheads indicate cytoplasmic GFP. To quantify cells having cytoplasmic GFP, five non-overlapping images were taken from three cultures each. (**h**) hNPCs were electroporated with GFP or $Foxg1^{T271A}$ construct. After 48 h, *RELN* mRNA expression was measured by qRT-PCR and normalized by electroporation efficiency. Three independent experiments were quantified in triplicate. Values: mean ± s.d. n.s., not

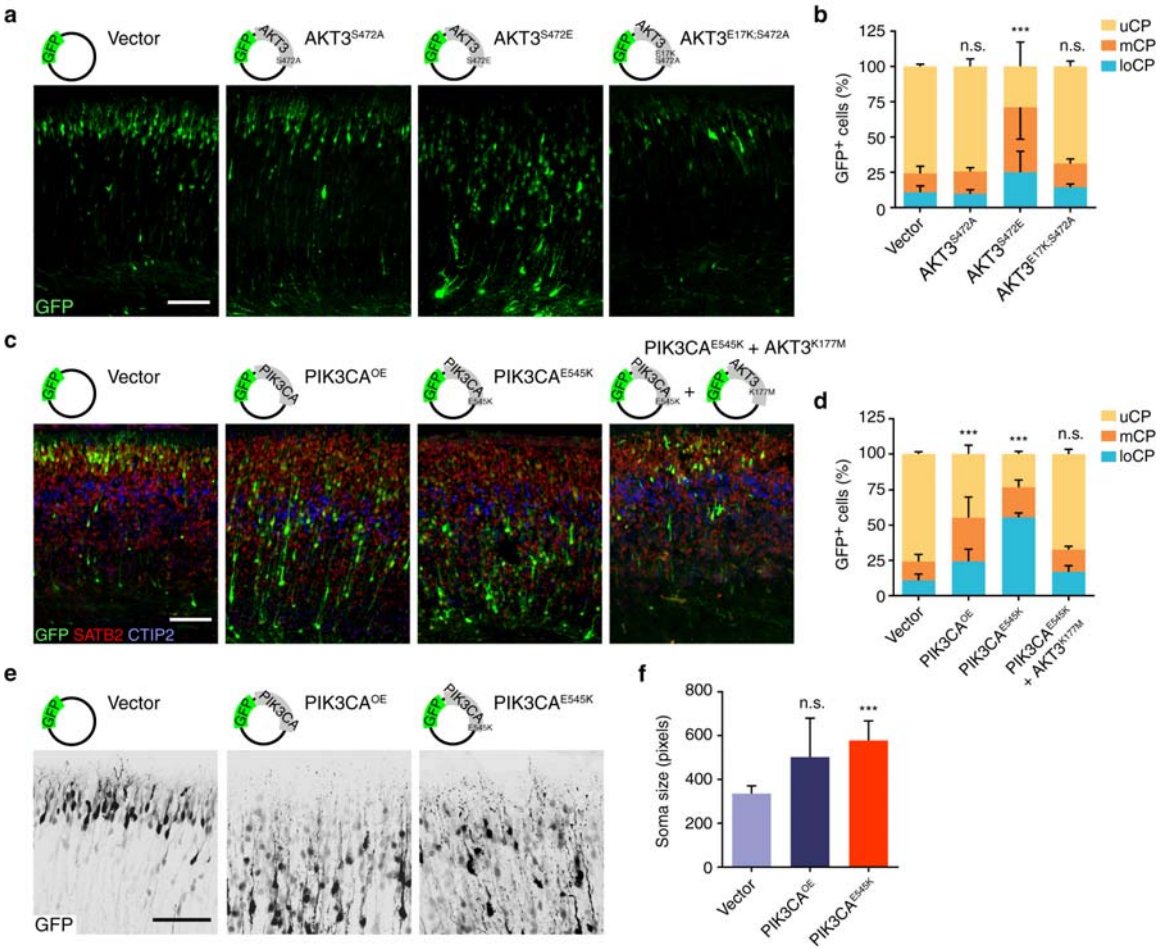
significant; *, $P < 0.05$, ***, $P < 0.001$, Student's *t*-test (**c, g, h**); G-test of goodness-of-fit (**e**). Scale bars: 100 μm (**a**, **d**); 20 μm (**f**).

SUPPLEMENTARY FIGURES

Supplementary Figure 1



Supplementary Figure 2



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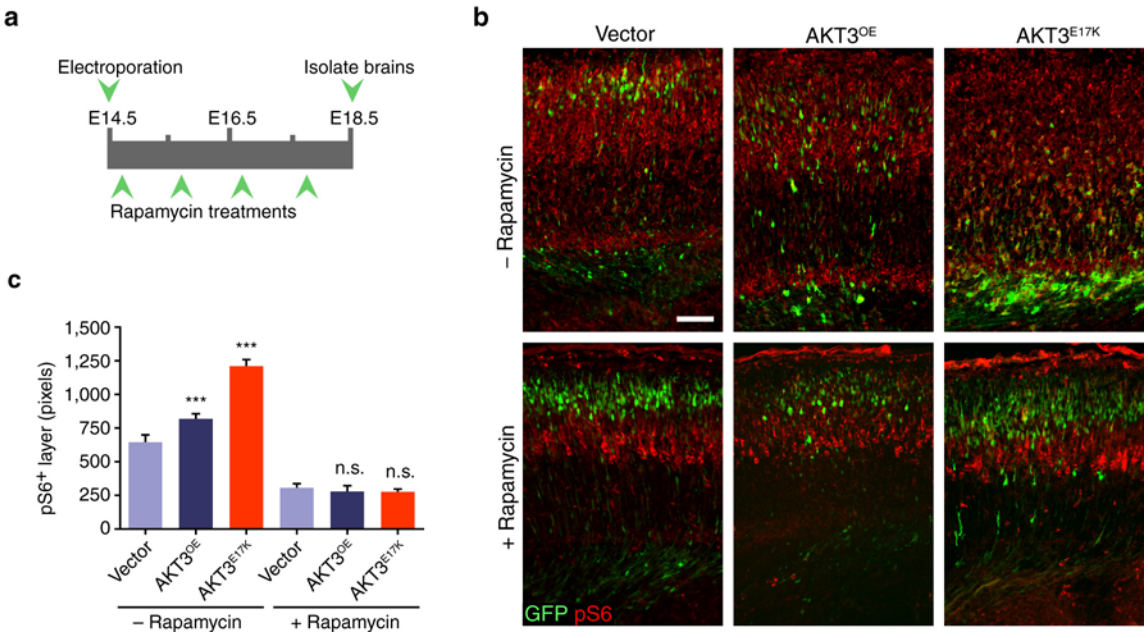

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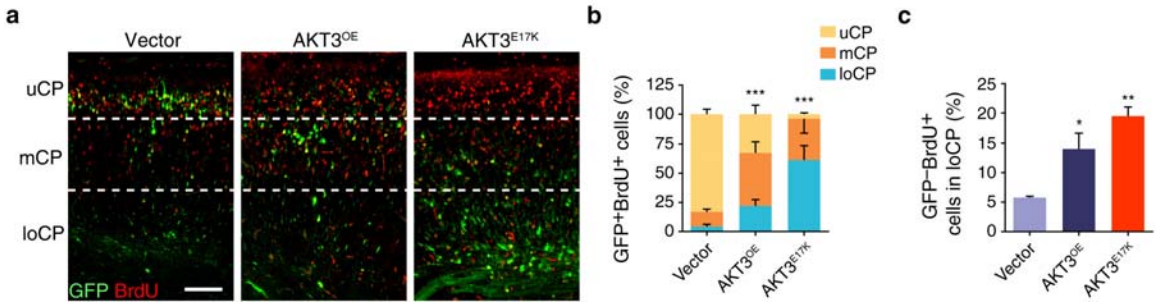
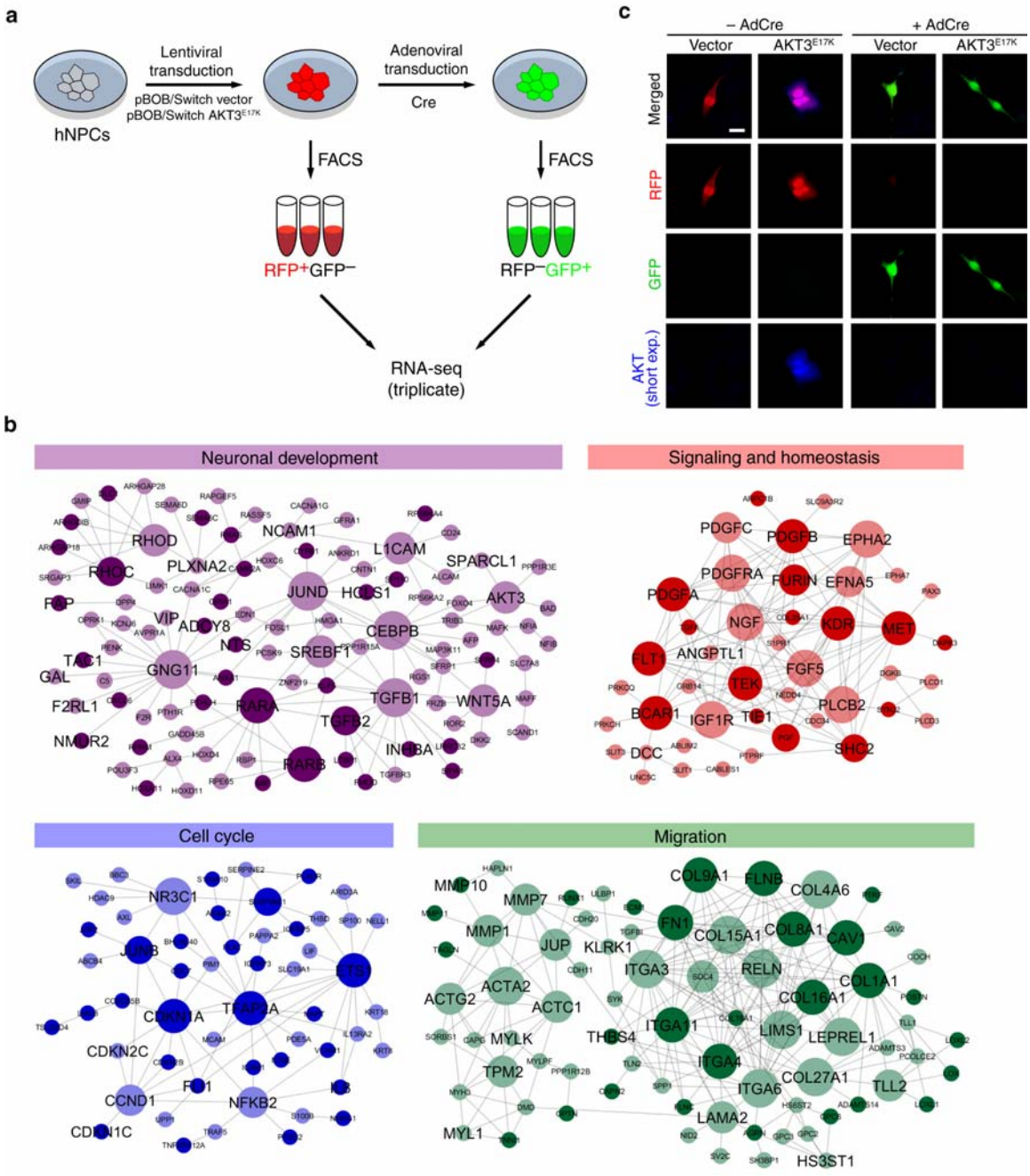


Supplementary Figure 5



Supplementary Figure 6