**SUPPLEMENTARY FIGURE LEGENDS**

**Figure S1: Organoid generation protocol and validation of iPSC lines.** A) Schematic of differentiation protocol used to generate kidney organoids. B) Representative karyotype analyses for two reporter iPSC lines, SIX2$^{EGFP}$ (Bi) and SIX2$^{EGFP}$:CITED1$^{mCherry}$ (Bii). Pluripotency was evaluated via immunofluorescence for NANOG (red) of undifferentiated iPSC colonies. Nuclei are highlighted by DAPI (blue). Scale bars represent 100 µm.

**Figure S2: Reporter expression dynamics.** (A) Fluorescence imaging (Ai) and flow cytometry (Aii) of showing endogenous mCherry expression in the in 100% of CITED1$^{mCherry}$ cells at day 7 of differentiation. B) Flow cytometry analysis of cells that do not contain a fluorescent reporter at day 7 of differentiation. C) Flow cytometry of live SIX2$^{EGFP}$ organoids showing endogenous EGFP expression across 3 organoid culture time points, D7+0, D7+3 and D7+18. D) Confocal microscopy of live (Di) and fixed/immunofluorescence-stained (Dii) SIX2$^{Cre/Cre}$:GAPDH$^{dual}$ organoids depicting mCherry$^{+}$ (red) and EGFP$^{+}$ (green) cells (Di), as well as the localisation of mCherry$^{+}$ cells within E-CADHERIN$^{+}$ (green) renal epithelium (Dii).

**Figure S3: Characterisation and validation of MAFB$^{mTagBFP2}$ organoids and GATA3-expressing populations within single and dual GATA3$^{mCherry}$ reporter lines.** A) Confirmation of organoid forming capacity and reporter detection in MAFB$^{mTagBFP2}$ organoids. Immunofluorescence of D7+12 organoids (Ai) confirmed expression of markers for proximal tubules (LTL$^{+}$; blue), distal tubules, (ECAD$^{+}$/LTL$^{-}$; green), ureteric epithelium/connecting segment (ECAD$^{+}$/GATA3$^{+}$; green/red) and podocytes of the glomeruli (NPHS1$^{+}$; grey). Reporter expression was detectable both by live confocal imaging (Aii) and flow cytometry (Aiii) of MAFB$^{mTagBFP2}$ organoids. FACS-isolated mTagBFP2$^{+}$ cells (Aiv; blue bar) from MAFB$^{mTagBFP2}$ ~D7+12 organoids showed enriched endogenous expression for the targeted gene, MAFB, compared to the reporter-negative control population (grey bar) via qRT-PCR. Error bars in Aiv depict SEM and significance was determined using a Student's t test on normalised (ΔCt) values (*P≤0.05, **P≤0.01, ***P≤0.001, ****≤0.0001). Congruence between mTagBFP2 reporter expression (blue) and endogenous MAFB (red) was also confirmed at a protein level via immunofluorescence (Av). Reporter expression onset correlated with endogenous MAFB protein expression (Avi) as determined by flow cytometry (top panels), live imaging (middle panels) and immunofluorescence (bottom panels) across a time course of organoid development (D7+5 - D7+9). Reduced numbers of MAFB$^{mTagBFP2}$ single cells were detected via flow cytometry by D7+9 (top panels, right) owing to the increasing force of cell-cell interactions within the maturing glomeruli which require harsher dissociation. Immunofluorescence (bottom panels) depicts ECAD$^{-}$/LTL$^{+}$ (blue) proximal segments and MAFB$^{+}$/mTagBFP2$^{+}$ podocytes of the glomeruli (grey/red), with the MAFB

(grey) channel removed from insets. B) Evidence of GATA3 protein (left panel) and gene expression (right panel) in non-epithelial populations in D7+18 GATA3$^{mCherry}$ organoids. Left panel depicts immunofluorescence for markers for proximal tubules (LTL$^{+}$; blue), distal tubules, (ECAD$^{+}$/LTL$^{-}$; green), ureteric epithelium/connecting segment (ECAD$^{+}$/GATA3$^{+}$; green and red) and podocytes (NPHS1$^{+}$; grey), with arrows highlighting examples of GATA3$^{+}$ interstitial cells surrounding NPHS1$^{+}$ podocytes of the glomeruli. Right panel depicts single cell RNA sequencing (scRNA seq) showing expression of ECAD in only a subset of *GATA3*-expressing cells. G) Confocal microscopy of a live MAFB$^{mTagBFP2}$:GATA3$^{mCherry}$ D7+6 organoid showing endogenous expression of the mCherry reporter (red) within epithelial structures. Scale bars for A, B and C represent 50µm.

**Video S1:** Time-lapse imaging of the D7+9 MAFB$^{mTagBFP2}$:GATA3$^{mCherry}$ organoid from Video 1 at increased magnification demonstrating endogenous mCherry (red) and mTagBFP2 (blue) expression across a 35 hour period.

## SUPPLEMENTARY FIGURES

### Figure S1

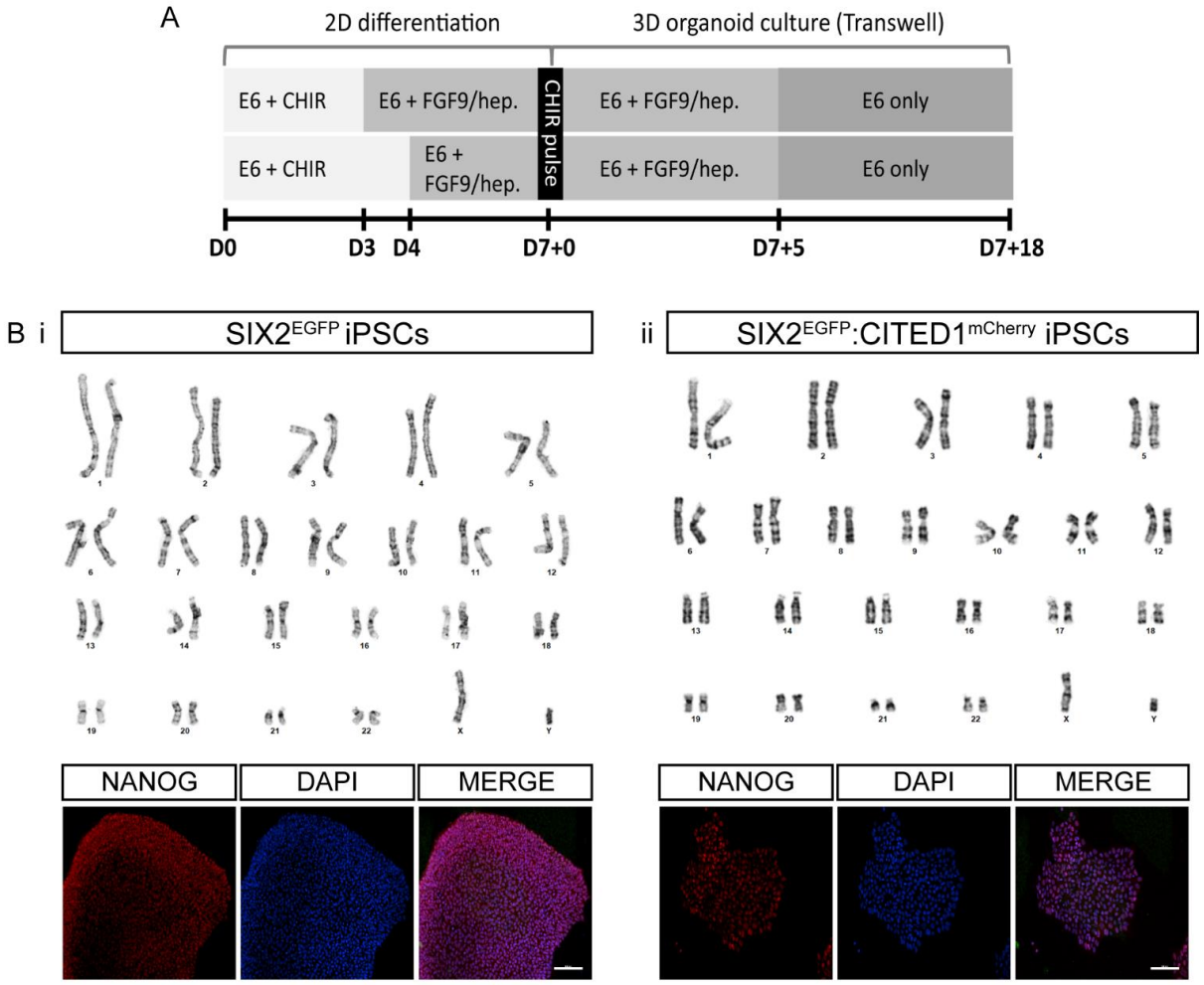


**Figure S2**

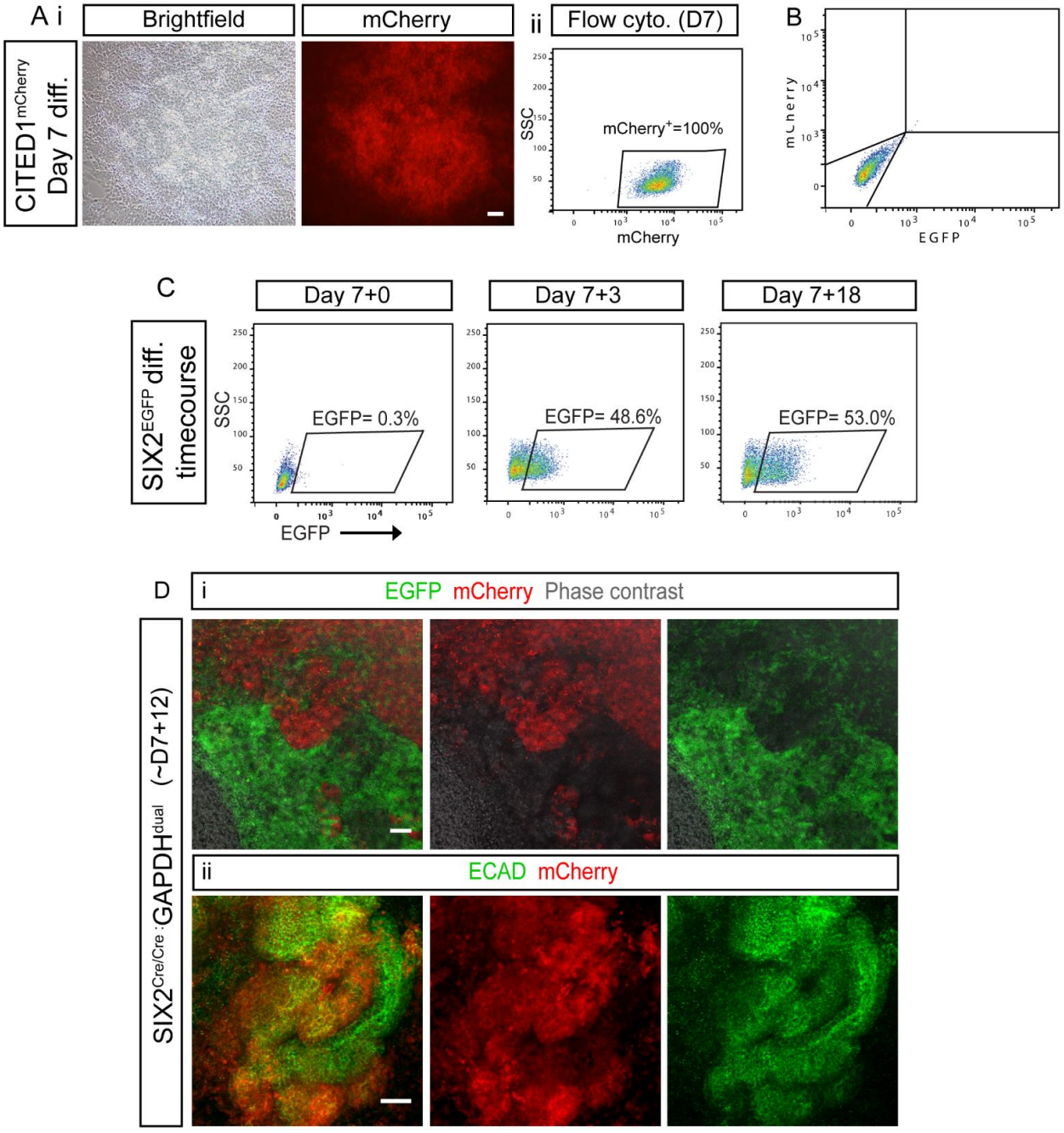
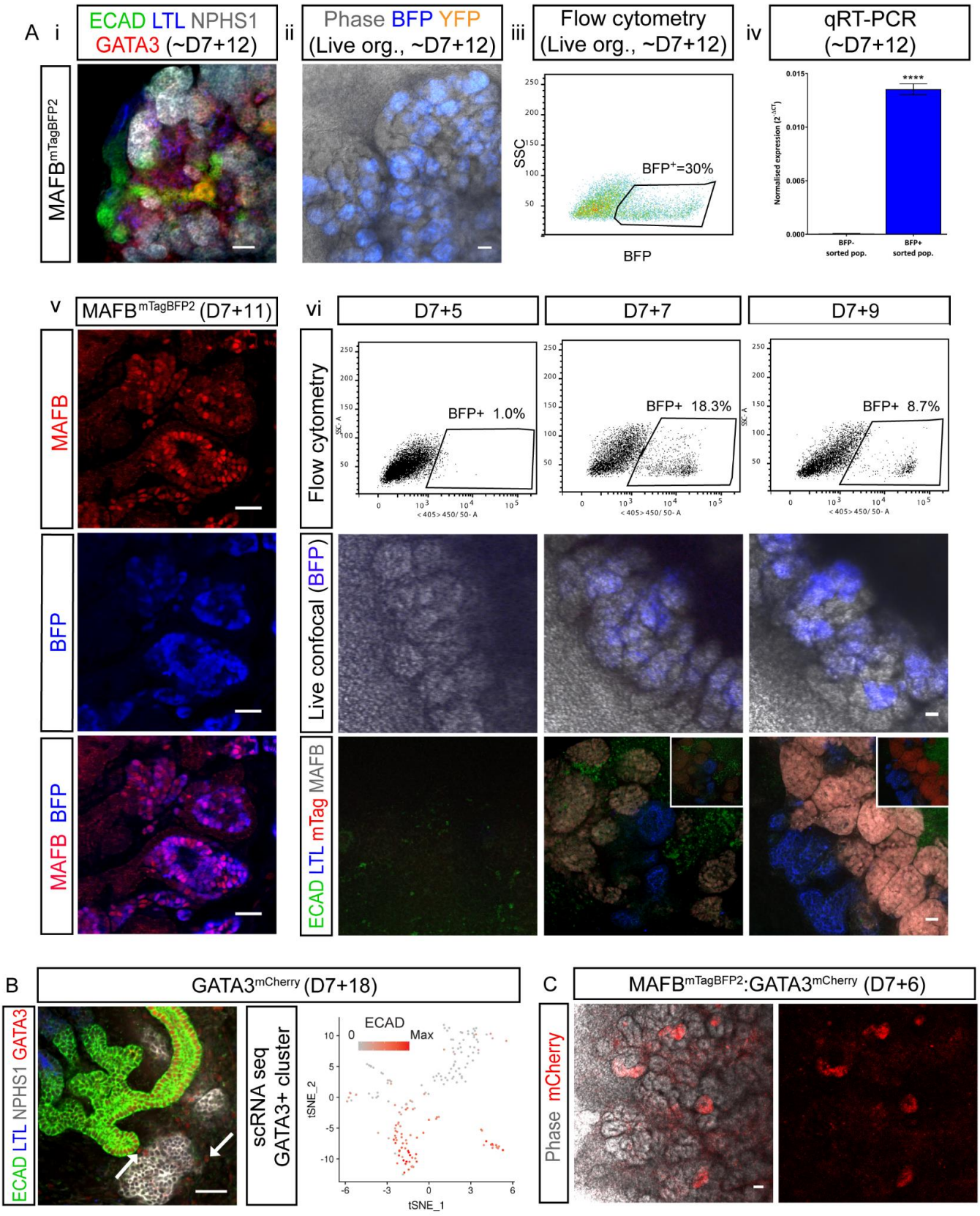

A i
Brightfield
mCherry
CITED1mCherry Day 7 diff.
ii
Flow cyto. (D7)
SSC
mCherry+=100%
mCherry
B
mCherry
EGFP
C
Day 7+0
Day 7+3
Day 7+18
SIX2EGFP diff. timecourse
SSC
EGFP= 0.3%
EGFP= 48.6%
EGFP= 53.0%
EGFP
D
i
EGFP mCherry Phase contrast
SIX2Cre/Cre:GAPDHdual (~D7+12)
ii
ECAD mCherry

**Figure S3**


A
i
ECAD LTL NPHS1
GATA3 (~D7+12)
ii
Phase BFP YFP
(Live org., ~D7+12)
iii
Flow cytometry
(Live org., ~D7+12)
iv
qRT-PCR
(~D7+12)
MAFB mTagBFP2
SSC
BFP+=30%
BFP
Normalised expression (2-ΔCT)
****
BFP- sorted pop.
BFP+ sorted pop.
v
MAFB mTagBFP2 (D7+11)
vi
D7+5
D7+7
D7+9
MAFB
Flow cytometry
BFP+ 1.0%
BFP+ 18.3%
BFP+ 8.7%
BFP
Live confocal (BFP)
MAFB BFP
ECAD LTL mTag MAFB
B
GATA3 mCherry (D7+18)
ECAD LTL NPHS1 GATA3
scRNA seq
GATA3+ cluster
ECAD
0
Max
tSNE_2
tSNE_1
C
MAFB mTagBFP2:GATA3 mCherry (D7+6)
Phase mCherry