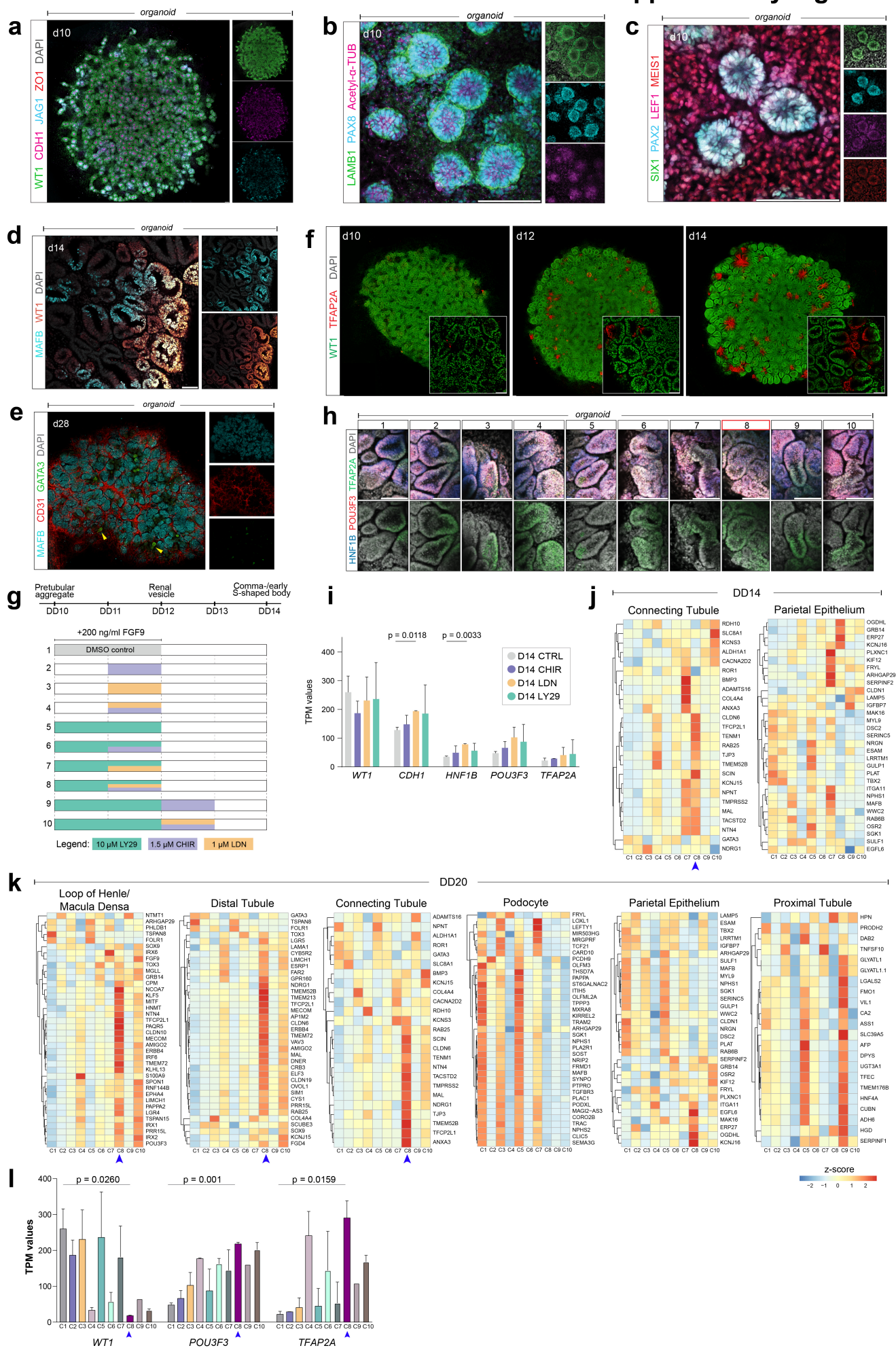


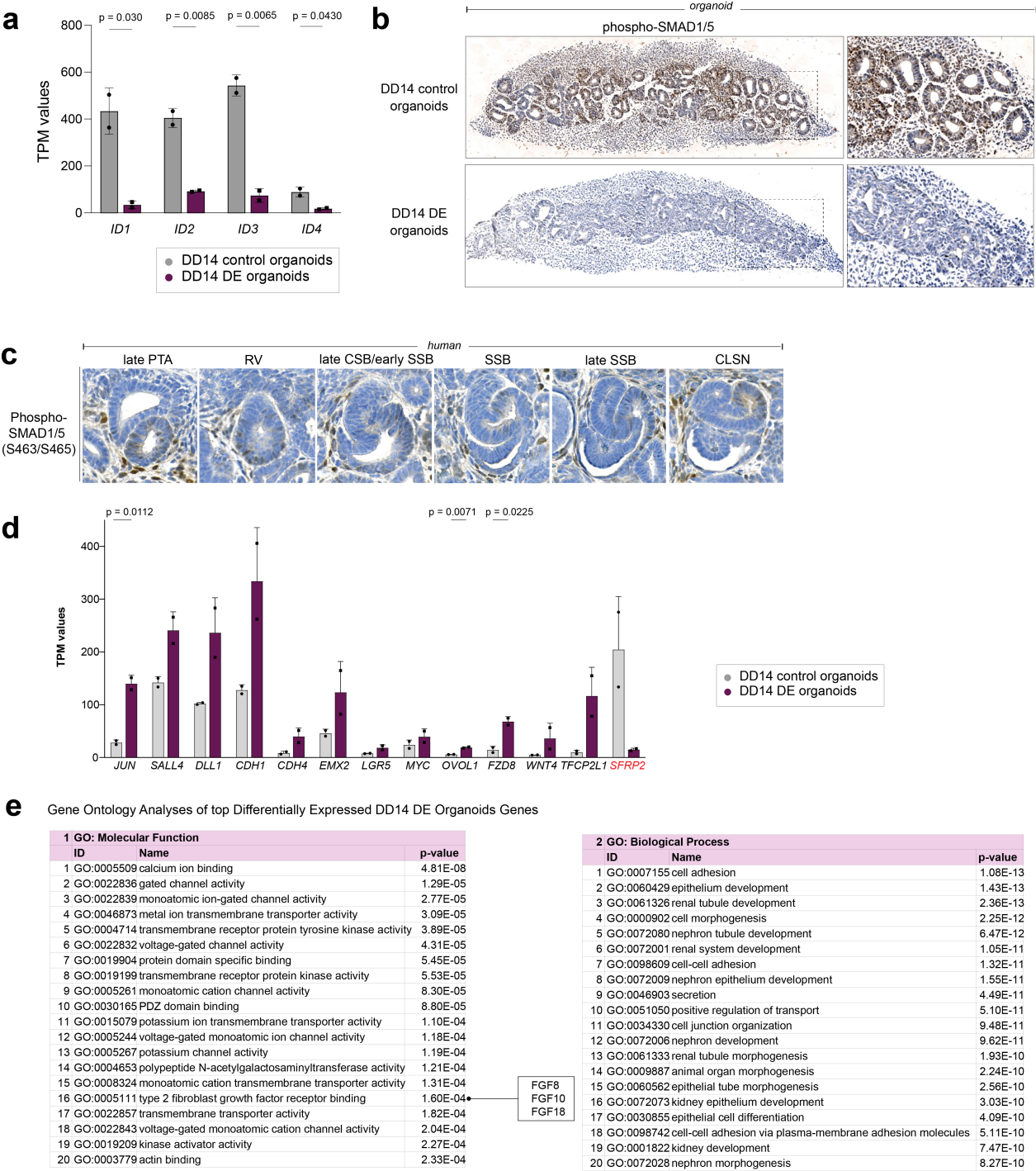
Supplementary Figure 1



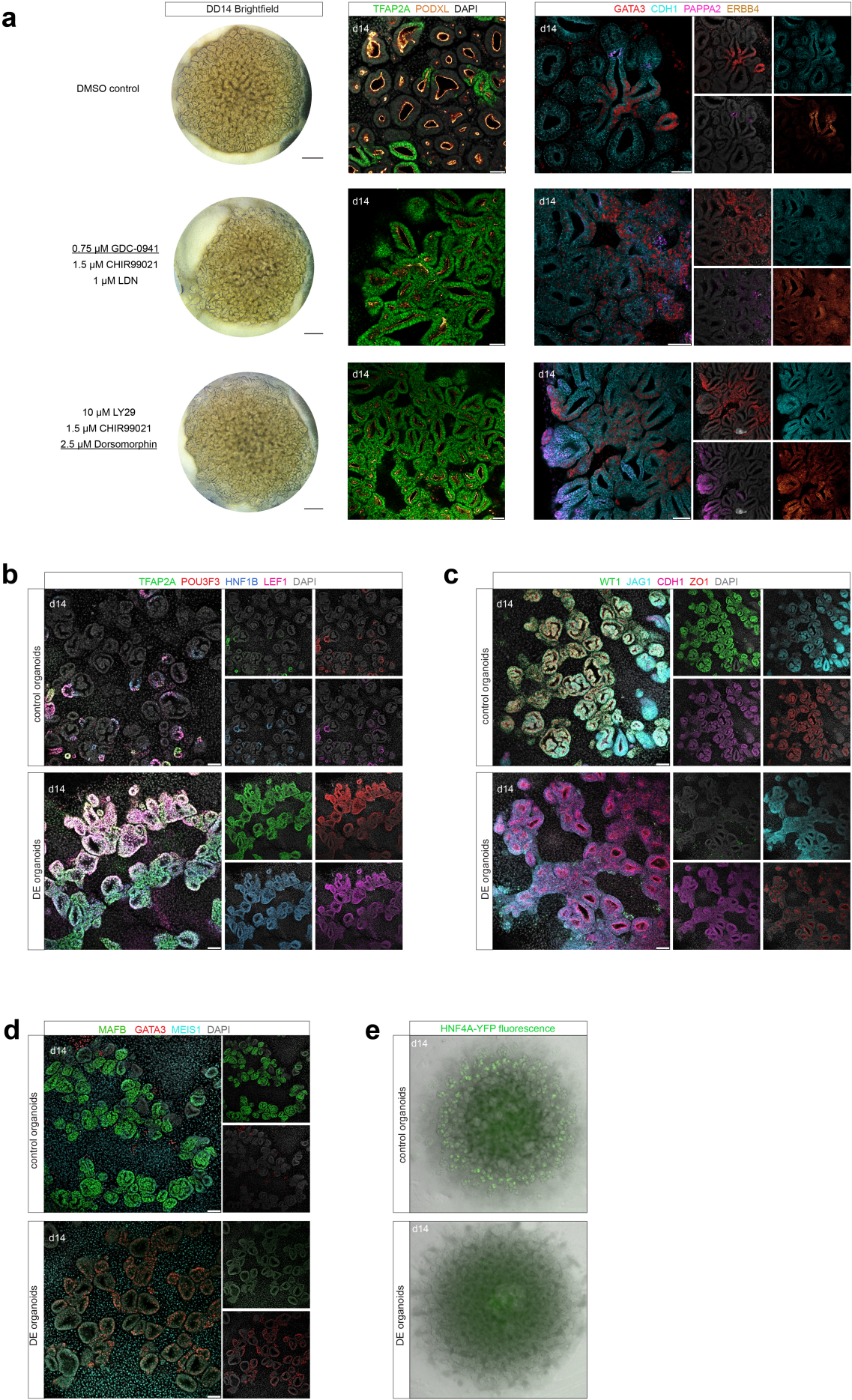
Supplementary Figure 2



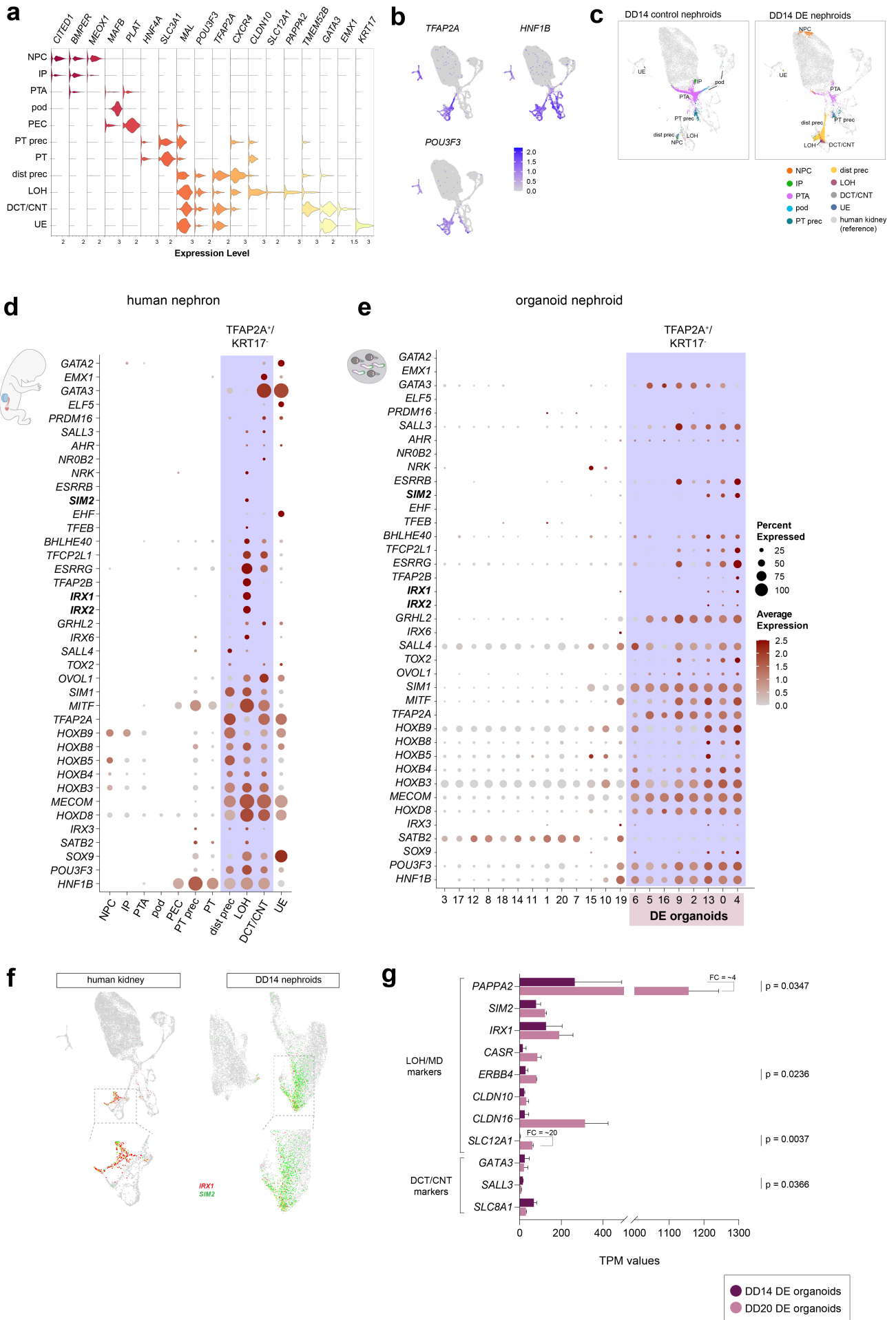
Supplementary Figure 3



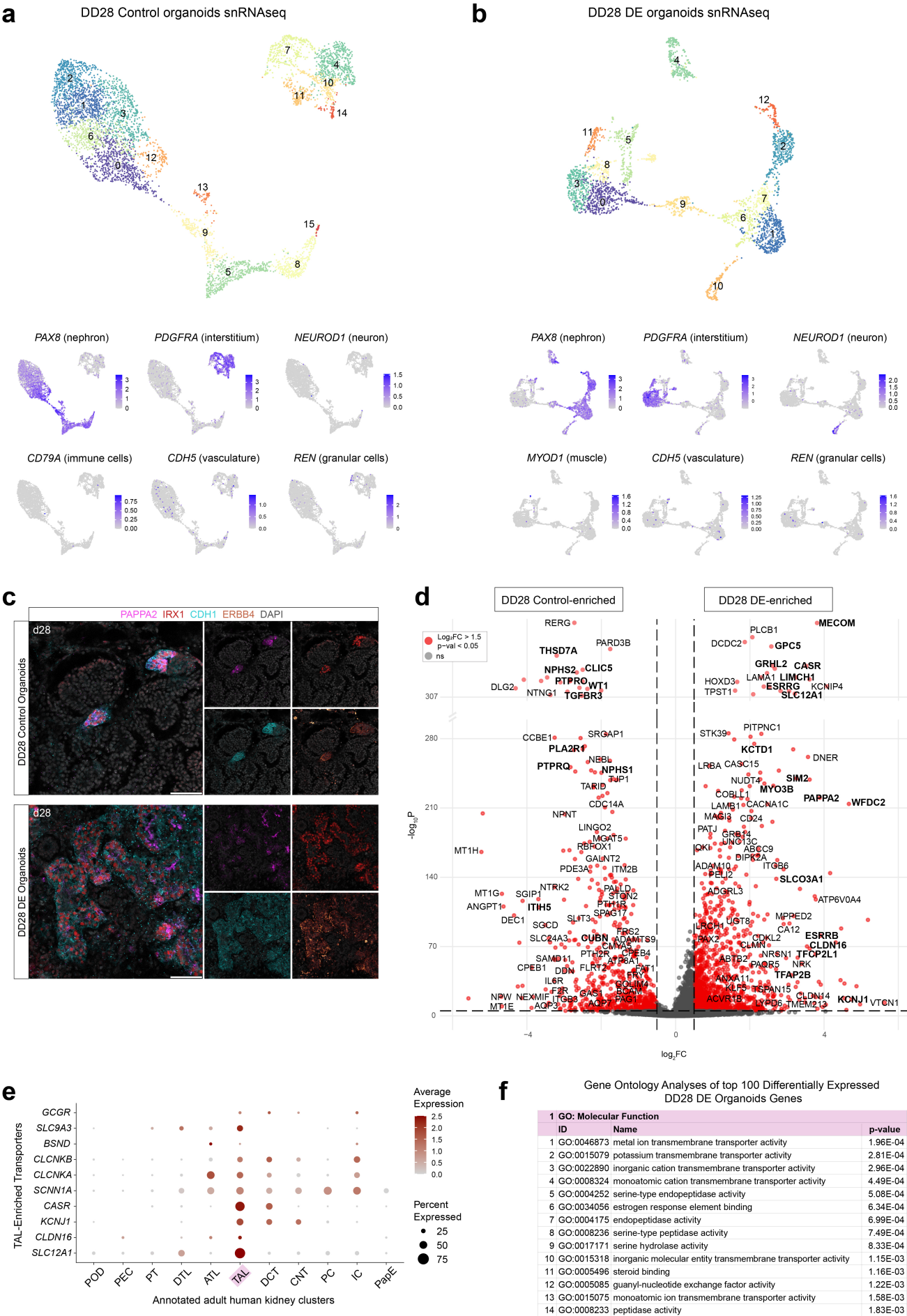
Supplementary Figure 4



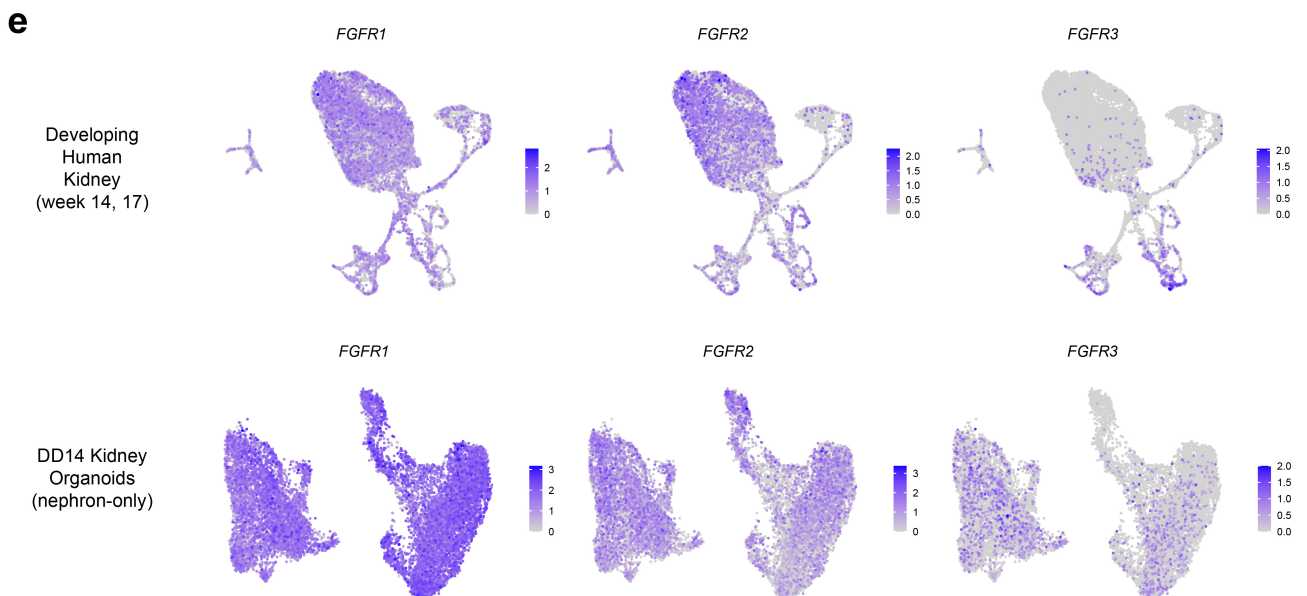
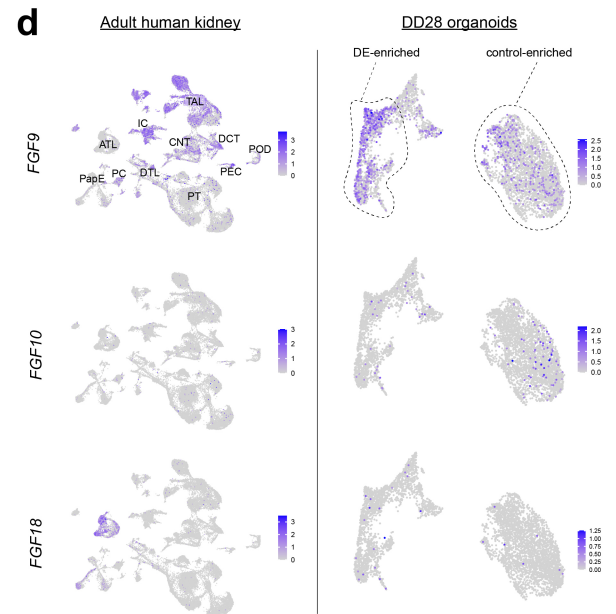
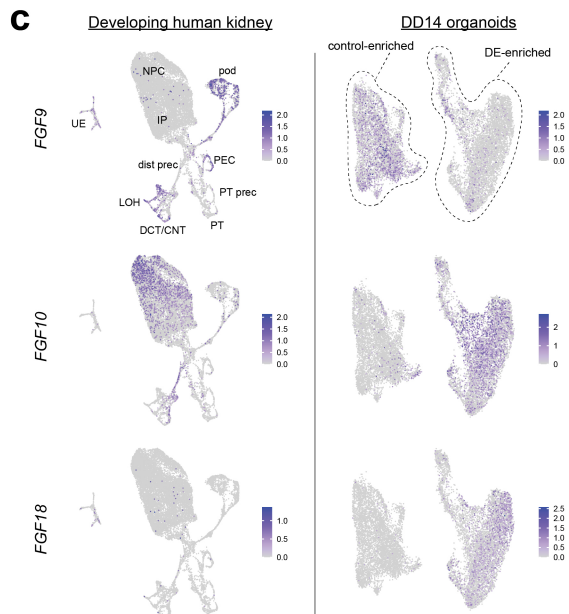
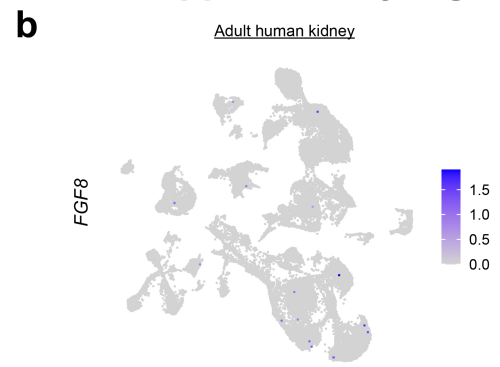
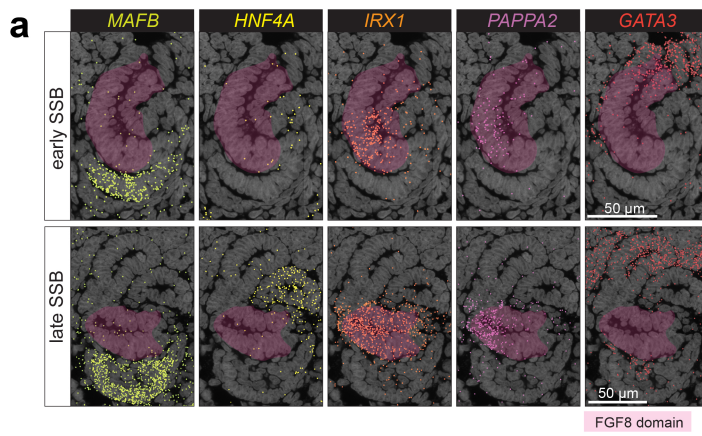
Supplementary Figure 5



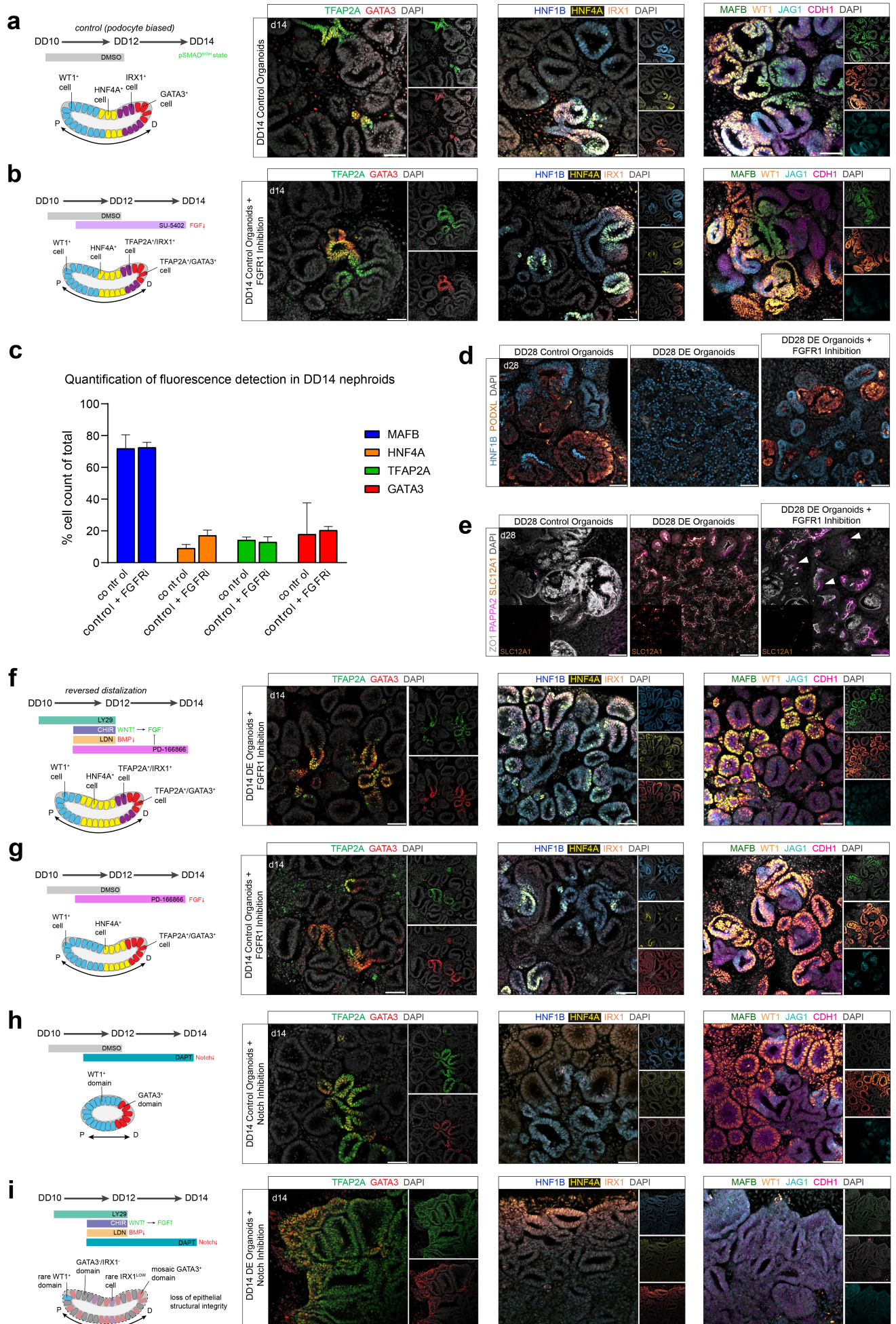
Supplementary Figure 6



Supplementary Figure 7



Supplementary Figure 8



SUPPLEMENTARY DATA LEGENDS

Supplementary Figure 1. Characterization of developmental stages of kidney organoid differentiation. Related to Figure 1. (a-c) Immunofluorescence stains of DD10 organoids illustrating expression of select induced nephron progenitor markers. Scale bar: 50 microns. (d) Immunofluorescence stain of DD14 control organoids illustrating emergence of MAFB⁺ podocyte precursors. (e) Immunofluorescence stain of DD28 control organoids showing MAFB⁺ glomerular-like structures and surrounding vasculature-like structures and sporadic GATA3⁺ tubules (yellow arrows). Scale bar: 50 microns. (f) Immunofluorescence stain of DD10, DD12 and DD14 control organoids showing symmetry breaking of TFAP2A emergence in WT1⁺ domains. Whole organoid tile-scans consist of 4 merged Z-stacks while insets feature a single plane. Scale bar: 50 microns. (g) Schematic of SMI treatment screening to optimize nephron distalization. Top bar shows timeline for SMI treatment for each condition. (h) Immunofluorescence stains of DD14 organoids generated from the 10 condition-screen showing distal transcription factor. Red box denotes condition 8. Scale bars: 50 microns. (i) Bar graph of TPM values show expression levels of *WT1*, *CDH1*, *HNF1B*, *POU3F3* and *TFAP2A* in DD14 organoids (n = 2) treated with different SMIs. Error bars show standard deviation, and top bars show statistical significance between each SMI treatment against control based on non-parametric t test (* - p<0.05, ** - p<0.01). (j-k) Heatmaps of TPM value z-scores of nephron cell type markers at the SSB stage, expressed across 10 differentiation conditions in (j) DD14 and (k) DD20 organoids (n = 2). (l) Bar graph of TPM values show expression levels of *WT1*, *POU3F3* and *TFAP2A* in DD14 organoids (n = 2) from all 10 conditions. Data are represented as mean ± SD with statistical significance based on non-parametric t test between condition 8 and DMSO control (* - p<0.05, *** - p<0.001).

Supplementary Figure 2. Single cell RNA sequencing of DD14 organoids. Related to Figure 2. (a-b) (Top) UMAPs of single cell sequencing data of DD14 control and DE organoids. (Bottom) Feature plots of select cell type markers in DD14 control and DE organoids. (c-e) Feature plots of DD14 organoid scRNAseq data showing detection of *WT1*, *TFAP2A*, *PAX2*, *PAX8* and *LHX1*. (f) Violin plots split by condition showing total expression of top 16 DEGs in DD14 control (top 2 rows) and DE (bottom 2 rows) organoids from scRNAseq dataset. Colors behind gene names correspond to cell types indicated in Figure 2b.

Supplementary Figure 3. Combined β -catenin activation and BMP inhibition is sufficient to bias organoids towards a distal fate. Related to Figure 2. (a) Bar graph of TPM values of DD14 control and DE organoids of BMP target genes. Error bars show standard deviation, and horizontal bars show statistical significance based on non-parametric t test (* - $p < 0.05$, ** - $p < 0.01$). (b) (top) DD14 control and (bottom) DE organoid cross-sections showing nuclear phospho-SMAD1/5 detection. (c) IHC staining of week 16 human kidneys showing nuclear dual phospho-SMAD1/4 detection. (d) Bar graph of TPM values of DD14 control and DE organoids ($n = 2$) of canonical WNT target genes. Data are represented as mean $\pm$ SD with statistical significance based on non-parametric t test. (e) GO term analyses of molecular function and biological process predicted from top DEGs of DD14 DE organoids ($\log_2FC > 1.5$).

Supplementary Figure 4. Secondary validation of distal-enrichment protocol. Related to Figure 2. (a) Brightfield images and immunofluorescence stains on DD14 control and organoids treated with dosages indicated (left) showing detection of select distal nephron markers. (b-d) Immunofluorescence stains on DD14 control and DE protocol-treated organoids cultured from HNF4A-YFP iPSCs. Immunostains show detection of select podocyte and distal nephron markers. Scale bar: 50 microns. (e) Live imaging of YFP fluorescence in DD14 control and DE protocol-treated organoids cultured from HNF4A-YFP iPSCs.

Supplementary Figure 5. Transcriptional comparison between human and organoid nephron development. Related to Figure 3. (a) Violin plots representing nephron cell type markers used to annotate cell types of single cell RNA sequencing performed on week 14, week 17 human kidney. (b) Feature plots of TFAP2A, HNF1B and POU3F3 detection in human kidney dataset. (c) Annotated mapping of mapped control (left) and DE (right) organoid clusters using the developing human kidney dataset as a reference. (d-e) Dot plot of distal-enriched transcription factor expression in (d) human and (e) organoid scRNA sequencing datasets. Purple box marks nephrogenic *TFAP2A*⁺ clusters. (f) Feature plots showing co-expression of transcription factors *IRX1* (red) and *SIM2* (green) in distalizing cells of human kidney and organoid datasets. (g) Horizontal bar plot showing TPM values of select distal nephron markers in DD14 and DD20 DE organoids ($n = 2$). Data are represented as mean $\pm$ SD with statistical significance based on non-parametric t test. FC = fold change.

Supplementary Figure 6. Characterization of DD28 kidney organoids. Related to Figure 4.

(a-b) (Top) UMAPs of single cell sequencing data of DD28 control and DE organoids. (Bottom) Feature plots of select cell type markers in DD28 control and DE organoids. (c) Immunofluorescence stain of DD28 control and DE organoids showing detection of loop of Henle/macula densa precursor markers. Scale bar: 50 microns. (e) Dot plot of select TAL-enriched ion transporter expression in adult human kidney snRNAseq datasets, based on cell-type annotations (d) Volcano plot showing DESeq analysis of snRNA sequencing data of DD28 control versus DE organoids. Dotted lines show 0.05 p-value cutoff (horizontal) and ± 1.5 -fold change cutoff (vertical). (f) GO term analyses of molecular function predicted from top 100 DEGs of DD28 DE organoids ($\log_2FC > 1.5$).

Supplementary Figure 7. Examining FGF signaling in early and late nephron patterning. Related to Figure 5.

(a) SeqFISH data of week 16 human kidney showing detection of select markers in early and late SSB in relation to *FGF8* domains. (b) Feature plot of *FGF8* detection in adult human kidney snRNAseq data. (c) Side-by-side feature plots of *FGF9*, *FGF10* and *FGF18* expression in the developing human nephron and DD14 organoid Seurat objects. (d) Side-by-side feature plots of *FGF9*, *FGF10* and *FGF18* expression in the adult human kidney and DD28 organoid Seurat objects. (e) Feature plots of FGF receptor expression in the developing human kidney Seurat object (top) and DD14 kidney organoid object (bottom).

Supplementary Figure 8. Examining the effects of FGF and NOTCH inhibition in kidney organoids. Related to Figure 5.

(a-b) Schematics of SMI treatment and nephron segmentation in DD14 organoids (left) and immunofluorescent stains showing detection of DCT/CNT, proximal tubule, loop of Henle/macula densa and podocyte precursor markers (right). Scale bar: 50 microns. (c) Percent quantification of fluorescence detection of podocyte, proximal tubule, and distal nephron markers in select nephroids of DD14 control and control +FGFR1i organoids ($n = 2$). (d-e) Immunofluorescent stains of DD28 control, DE, and DE+FGF-deficient organoids showing (d) podocyte-to-tubule distribution and (e) formation of loop of Henle/macula densa-like precursors. (f-g) Schematics of SMI treatment and nephron segmentation in DD14 organoids (left) and immunofluorescent stains showing detection of DCT/CNT, proximal tubule, loop of

Henle/macula densa and podocyte precursor markers (right). Scale bar: 50 microns. (h-i) Schematics of SMI treatment and nephron segmentation in DD14 DAPT-treated organoids (left) and immunofluorescent stains showing detection of DCT/CNT, proximal tubule, loop of Henle/macula densa and podocyte precursor markers (right). Scale bar: 50 microns.

SUPPLEMENTARY DATA LEGENDS

Supplementary Data 1. DESeq analyses of total mRNA, single cell, and single-nucleus sequencing data from DD14 and DD28 organoids. This table contains sheets of DESeq-derived differentially expressed genes of control vs DE organoids using data from either total mRNA sequencing or single cell RNA sequencing. The “Readme” sheet contains detailed descriptions of each tab. Related to Figure 1, 2 and S6. Wald test p-values are used and corrected by FDR.

Supplementary Data 2. Seurat-based differential gene expression analyses of annotated clusters of DD14 organoid and week 14, 17 human kidney single cell RNA seq data, and DD28 organoid and adult human kidney single-nucleus RNA-seq data. This table contains sheets of differentially expressed genes of all Seurat objects presented in this study. The “Readme” sheet contains detailed descriptions of each tab. Related to Figure 2, 3, 4, S2, S5 and S7.