Supplementary Figures and legends

Progressive recruitment of mesenchymal progenitors reveals a time-dependent process of cell fate acquisition in mouse and human nephrogenesis

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Supplementary figure legends

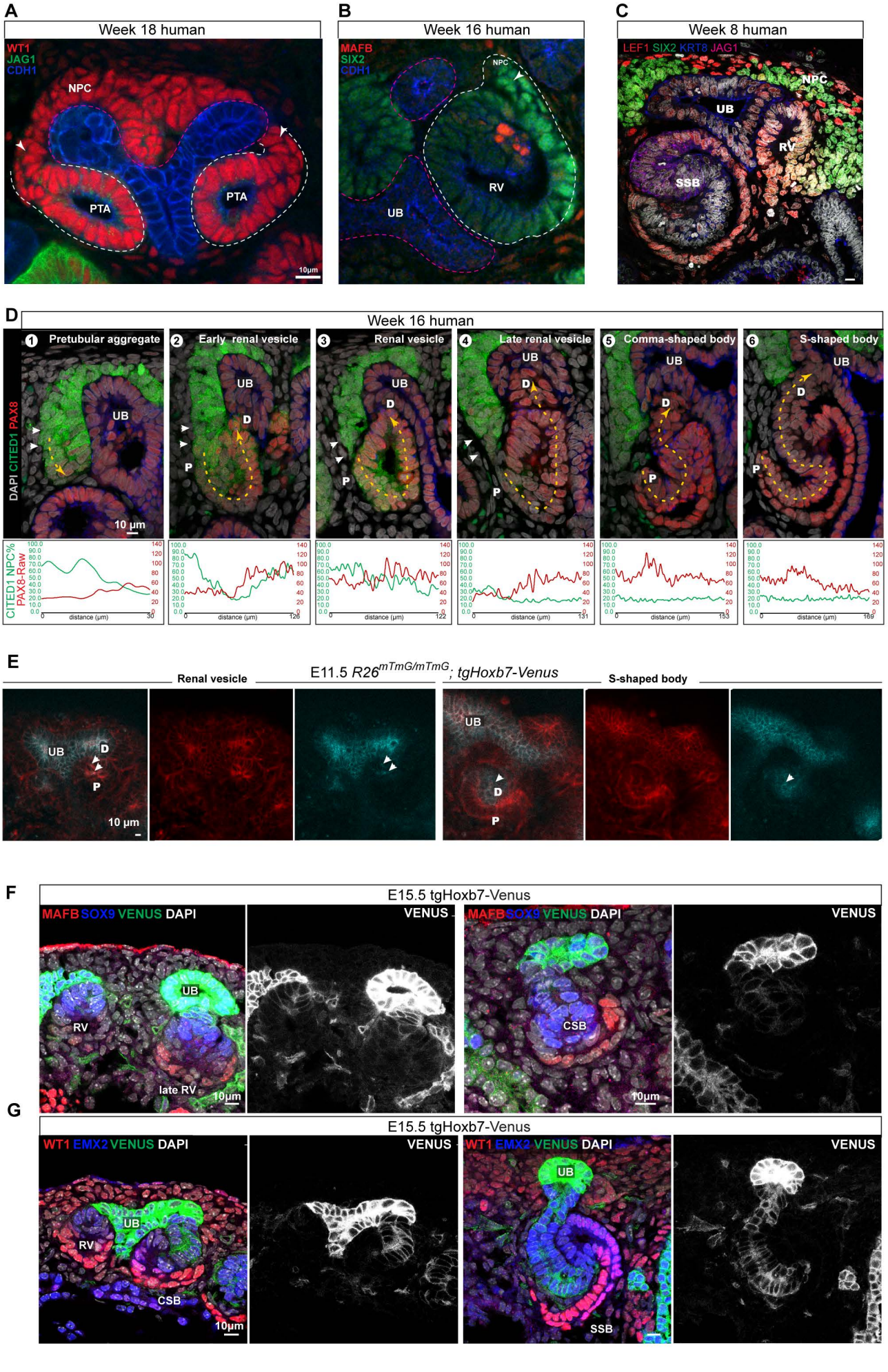
Supplementary fig.1 Cellular connection from NPCs to nephron is prevalent during human kidney organogenesis – as relating to Fig.1. (A-B) Immunofluorescent detection of whole nephrogenic niches showing single optical sections. Arrowheads mark the cellular connection. Dashed and white magenta lines indicate UB and nascent nephron, respectively. (C) Cellular connection in week 8 human kidneys. (D) Immunofluorescent stain (top) showing developmental progression from PTA to SSB and quantification (bottom) of CITED1 and PAX8 signals. Dashed yellow lines indicates where intensity measurements were made and corresponds to x-axis for graph. (E) Live Cdh1-CFP protein localized in distal RVs and SSBs on a membrane-Tomato background. (F-G) Immunofluorescent detection of distal (SOX9, EMX2) and proximal (MAFB, WT1) markers on sectioned *in vivo* tgHoxb7-Venus E15.5 kidney sections. NPC: nephron progenitor cell, RV: renal vesicle, PTA: pretubular aggregate, SSB: s-shaped nephron. UB: ureteric bud, D: distal, P: proximal.

Supplementary fig. 2 Single-cell RNA-seq analysis of week 17 human nephrogenic niche and SISH validation for single-cell RNA-seq data – as relating to Fig.2. (A) Unbiased clustering analysis of cells isolated from two human week 17 nephrogenic niches merged. (B) Cluster identities and identification and selection of the nephrogenic lineage (dashed line) using nephron-lineage markers e.g. as shown in (C). (D) In situ hybridization for genes from selected clusters and analyses in Fig.3. (E) In situ hybridization for whole kidneys as used for validation in Fig.2 and 3. NPC: nephron progenitor cell, PTA: Pretubular aggregate, RV: renal vesicle, SSB: S-shaped body nephron, UB: ureteric bud, CLN: Capillary loop nephron; CNT: Connecting tubule, PT: Proximal tubule.

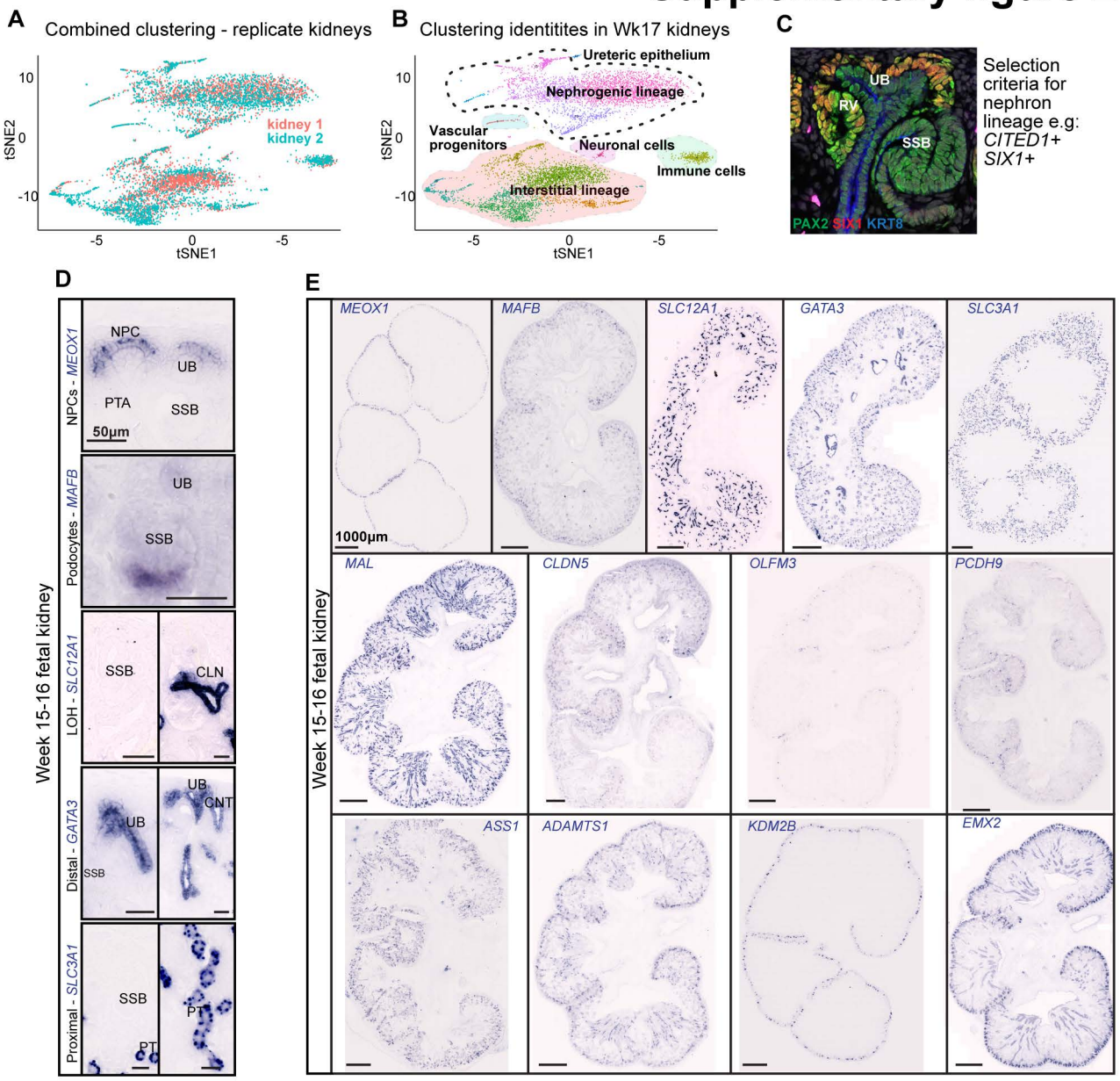
Supplementary fig. 3 Single-cell analyses exploring cluster and single-cell level relationships – as relating to Fig.2. (A) Minimum spanning tree derived from pairwise Bhattacharyya distances between cluster distribution estimates. (B) Distribution of pseudotime values for clusters 1-21, defined as the distance of each single cell to the trajectory start along the learned manifold. (C) Reiterative pseudotime analyses following paths between distinct cell states. Path numbers as used in Fig. 2D. NPC: nephron progenitor cell, PTA: pretubular aggregate, RV: renal vesicle.

Supplementary fig. 4 Single-cell analyses exploring cluster relationships to modules – as relating to Fig.3. Eigengene expression across clusters; with y-axis indicating eigengene and x-axis cluster number. NPC: Nephron progenitor cells.

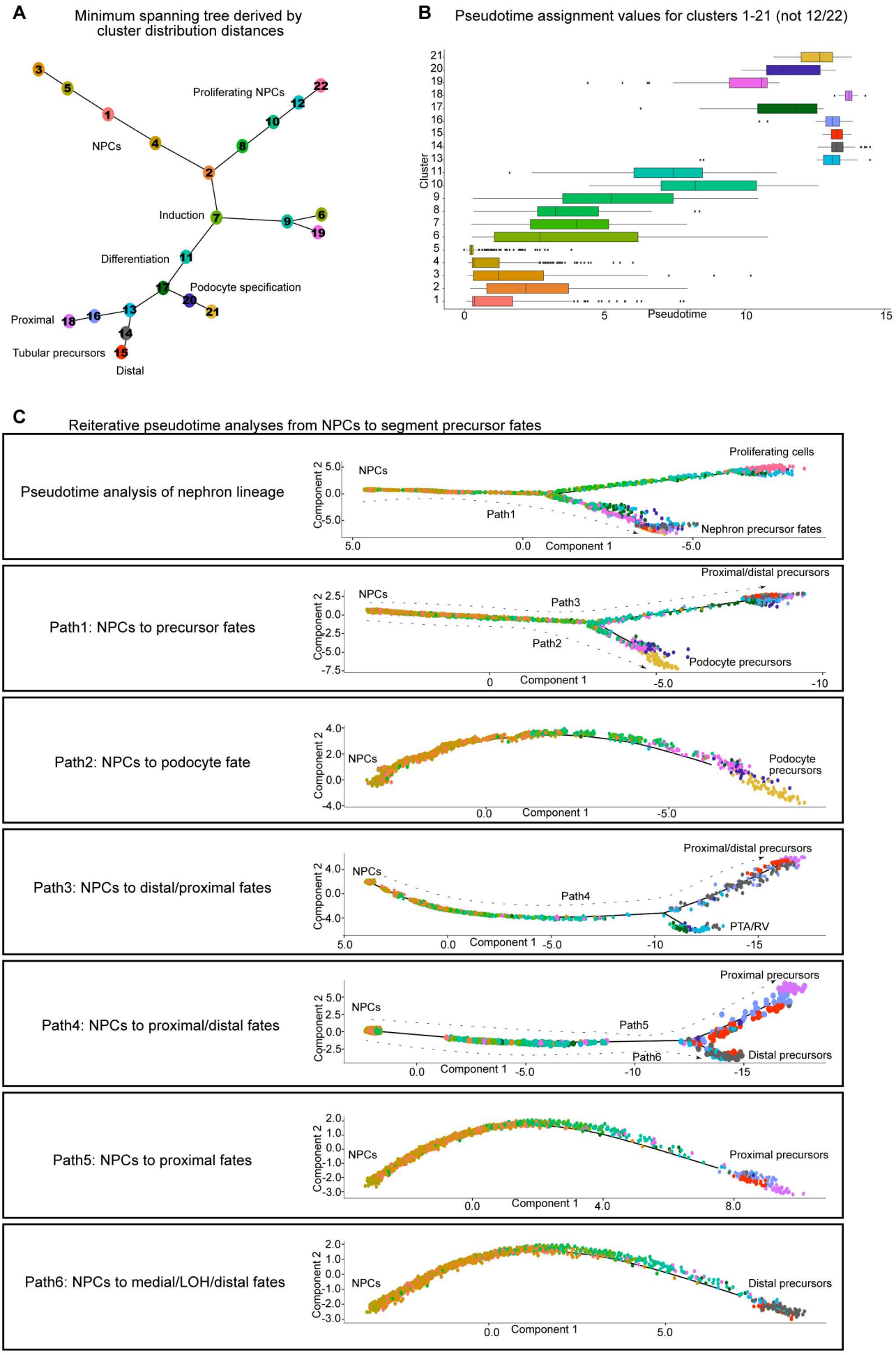
Supplementary figure 1



Supplementary figure 2



Supplementary figure 3



Supplementary figure 4