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Supplemental information

Dynamics of hepatocyte-cholangiocyte cell-fate decisions during liver development and regeneration

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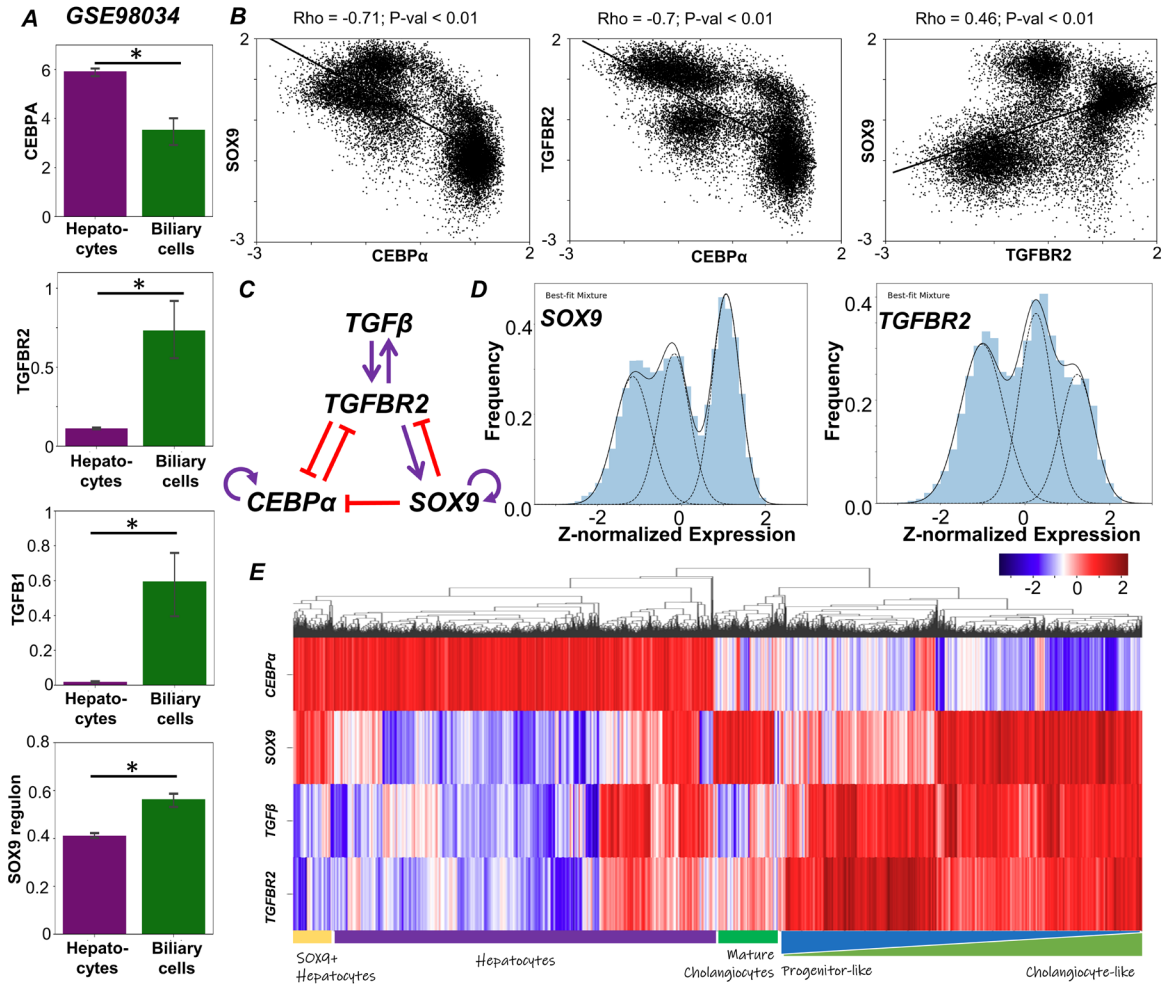

Figure S1: **Data analysis and simulation results showing different phenotypes enabled by the considered gene regulatory network, related to Figure 1 A)** Gene expression levels of c/EBPα, TGFBR2, TGFβ1 and SOX9 regulon activity in hepatocytes and biliary cells (GSE98034). * represents a statistically significant difference in the proportion of cases resulting in a particular phenotype compared to control case (Students' two tailed t-test; p-value < 0.05) **B)** Scatter plots showing pairwise correlation between steady state values obtained via simulations for the given gene regulatory network in Fig 1A. Spearman correlation coefficient (Rho) and p-value (p-val) are given. **C)** A variant gene regulatory network showing TGFβ ligand explicitly (instead of it being lumped together with TGFBR2 as a single node). **D)** Multimodal gene expression levels of SOX9 and TGFBR2 for the simulated variant circuit. **E)** Cluster map showing the steady state solutions of the four nodes. The possible biological mapping of 3 distinct phenotypes have been labelled – hepatocytes, SOX9^{+} hepatocytes, and mature cholangiocytes. A possible continuum of progenitor-like cells to cholangiocyte-like cells is also shown. Red represents higher expression levels while blue denotes lower expression levels.

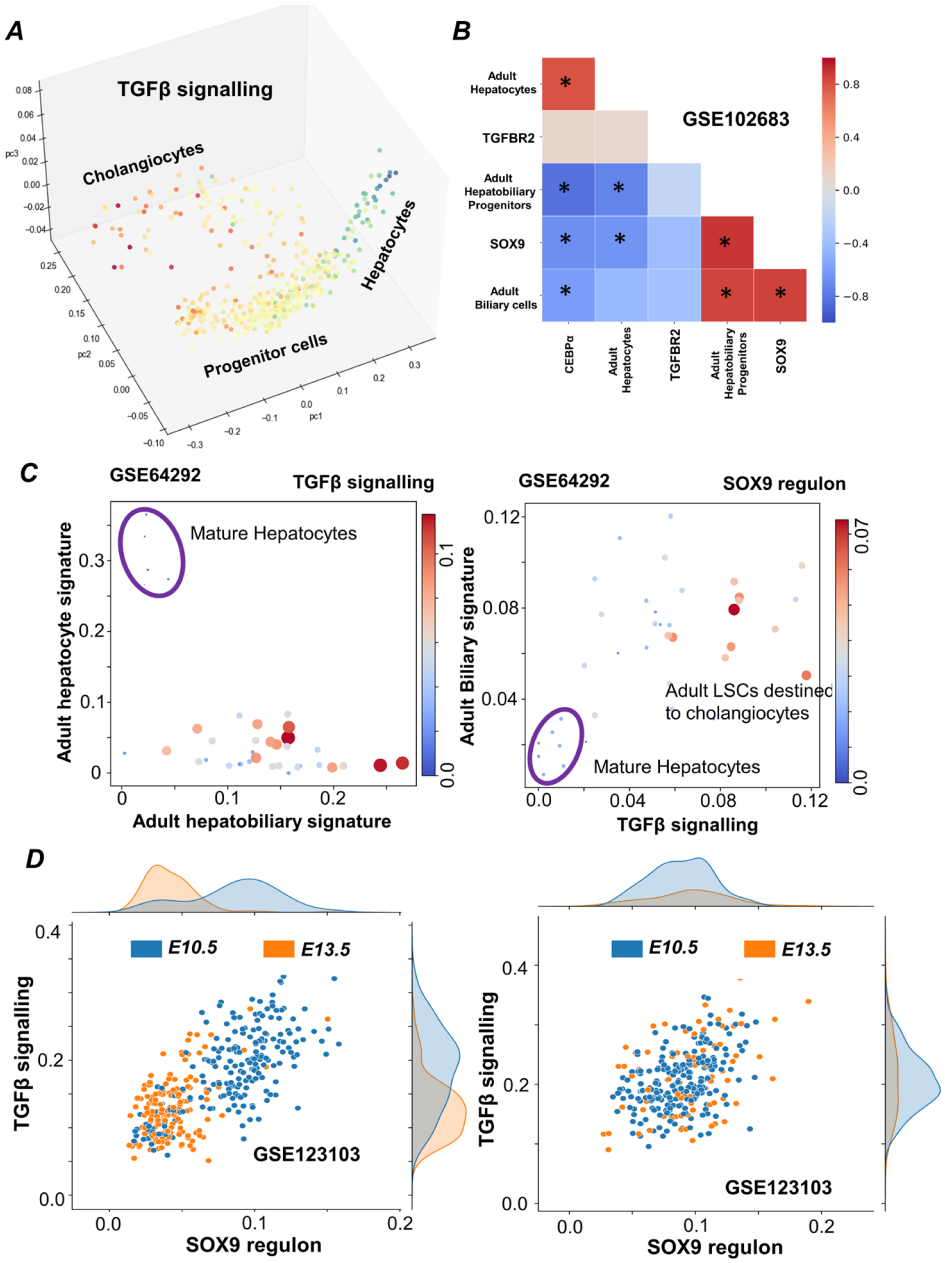

Figure S2: Single cell and bulk tissue RNA-Seq data analysis, related Figure 2 A) Trajectory analysis of hepatocytes and cholangiocytes as they are formed from progenitor cells (coloured by TGFβ signalling activity). Note the relatively high/medium levels of TGFβ signalling activity in the cholangiocyte and the progenitor cell branch and its low expression in the hepatocyte branch. **B)** Diagonal correlation matrix between expression levels (c/EBPα, SOX9 and TGFBR2) and the gene expression signatures (adult hepatocyte, adult hepatobiliary progenitors and adult cholangiocytes) in bulk liver tissue samples (GSE102683). **C)** Scatter plot (left) showing the TGFβ signalling activity and SOX9 regulon activity in a population of all hepatoblasts (LGR5+ or otherwise) at two distinct developmental stages. Note the relative drop in the values of both the quantities at E13.5. Scatter plot (right) showing the TGFβ signalling activity and SOX9 regulon activity in a population of LGR5+ hepatoblasts only at the two developmental stages. **D)** Single cell RNA-seq data (GSE64292) on the hepatocyte – hepatobiliary signature plane coloured by TGFβ signalling activity (left) and SOX9 regulon activity (right).

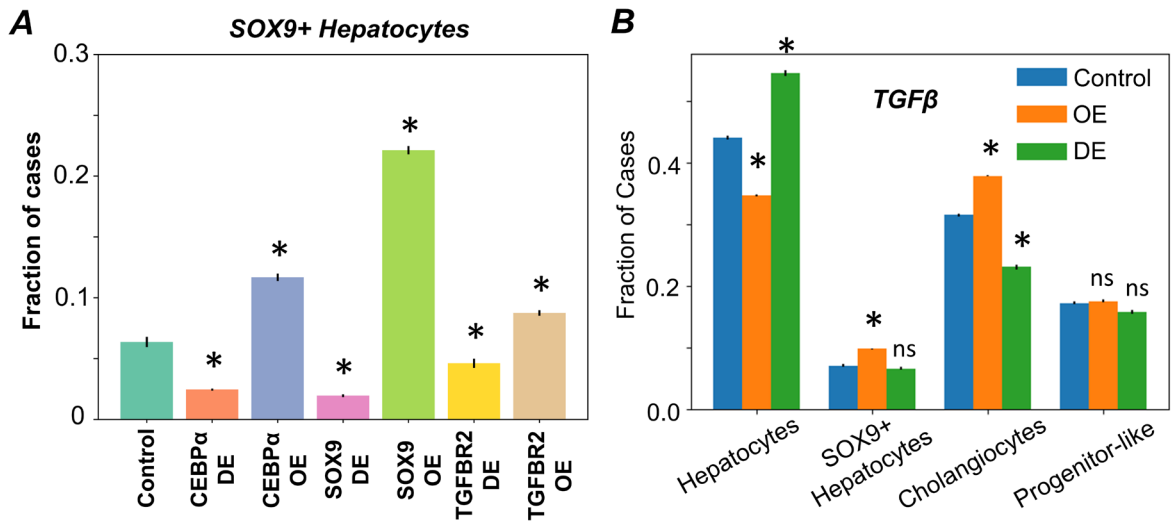

Figure S3: Perturbation in-silico experiments on the base and the extended networks, related to Figure 4 A) Simulation results quantifying the levels of SOX9^{+} hepatocytes under various perturbations to the gene regulatory network. * represents a significant difference in the proportion of cases resulting in a particular phenotype compared to the control case (p-value < 0.05) **B)** Simulation results quantifying the effects of perturbations to TGFβ in the variant circuit and the corresponding effects on the four phenotypes. * represents a significant difference in the proportion of cases resulting in a particular phenotype compared to the control case (p-value < 0.05); ns signifies a non-significant difference.

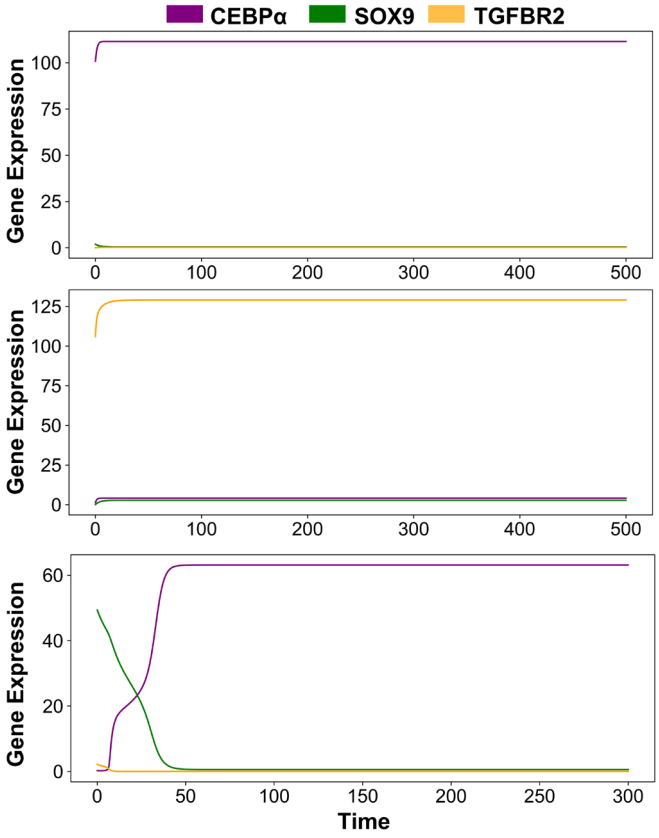

Figure S4: Simulations corresponding to the three representative cases as seen in Figure 4A with the exact same parameter values and initial conditions but noise level set to 0, related to Figure 4.

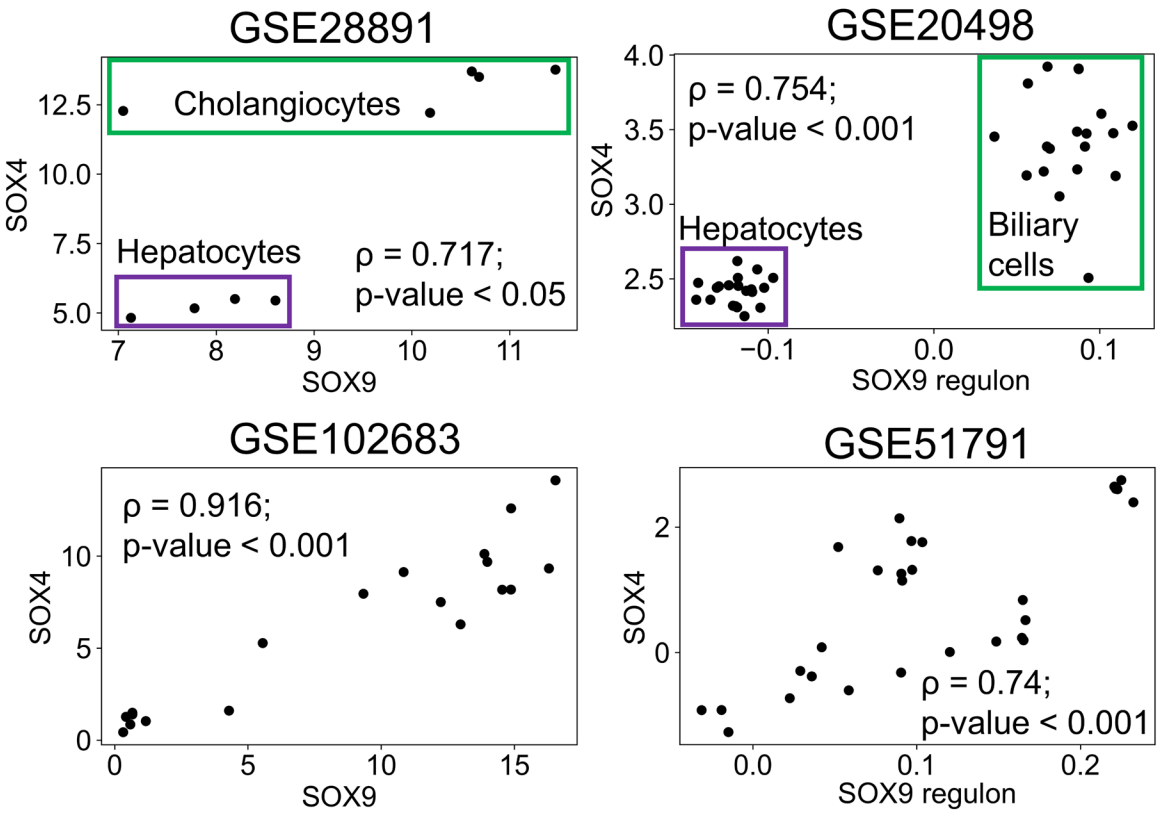

Figure S5: Scatter plots showing the correlations between SOX9/SOX9 regulon activity levels estimated by ssGSEA scores with SOX4 expression levels across 4 representative datasets – GSE28891, GSE20498, GSE102683 and GSE51791. The spearman correlation coefficient (ρ) and the corresponding p-values have been reported. Hepatocyte and cholangiocyte/biliary cell populations are marked wherever applicable, related to the Figure 1 and Discussion section.