Expanded View Figures

Figure EV1. The observed systemic effects of TDB treatment are largely acute and transient.

A Serum cytokine levels from control-treated (black) and anti-HER2/CD3 TDB-treated (red) mice, 4 days post treatment ($n = 8$ mice/treatment).

B Gating strategy for flow cytometry analysis of circulating immune cells. Singlets are gated based on forward scatter area (FSC-A) versus forward scatter height (FSC-H). $CD45^+$ cells are gated from viable cells, and T cells are sub-divided as $CD8^+$ versus $CD4^+$ T cells from viable cells and $CD45^+$ cells.

C Quantification of $CD4^+$ (top panel) and $CD8^+$ (bottom panel) T cell counts in peripheral blood samples from mice treated with control (black) or anti-HER2/CD3 TDB (red), 4 days post treatment ($n = 8$ mice/treatment).

D Gating strategy for flow cytometry analysis of immune cells in dissociated tissue samples. Singlets are gated based on forward scatter area (FSC-A) versus forward scatter height (FSC-H). $CD45^+$ cells are gated from viable cells and T cells are sub-divided as $CD8^+$ versus $CD4^+$ T cells from viable cells and $CD45^+$ cells.

E, F Representative dual chromogenic IHC for CD8a and pan-cytokeratin (AE1/AE3) staining of Founder5 tumor sections (E) or liver sections (F) from vehicle control-treated and anti-HER2/CD3 TDB-treated mice (top panels). Scale bar = 1,000 μm. Insets show enlarged view of the boxed area. Scale bar of insets = 100 μm. Quantification of $CD8^+$ cell counts in Founder5 whole tumor sections normalized to tumor tissue areas (E) or liver sections normalized to tissue areas (F), 24 h ($n = 9$ mice/treatment) or 4 days post treatment ($n = 8$ mice/treatment) (bottom panels).

G Serum levels of alanine transaminase (ALT, top panel) and aspartate aminotransferase (AST, bottom panel) from control-treated (black) and anti-HER2/CD3 TDB-treated (red) mice, 6 h, 24 h, and 4 days post treatment ($n = 5$–9 mice/treatment).

H, I Tumor (H) or liver (I) tissue cytokine levels normalized to total protein concentration from control-treated (black) and anti-HER2/CD3 TDB-treated (red) mice, 4 days post treatment ($n = 6$/7 mice/treatment).

Data information: Black = control-treated mice. Red = anti-HER2/CD3 TDB-treated mice. Mann–Whitney test was run to determine *P*-values for experiments with two groups, and Kruskal–Wallis one-way ANOVA was used for multiple groups. Data are shown as means ± SEM.
Source data are available online for this figure.

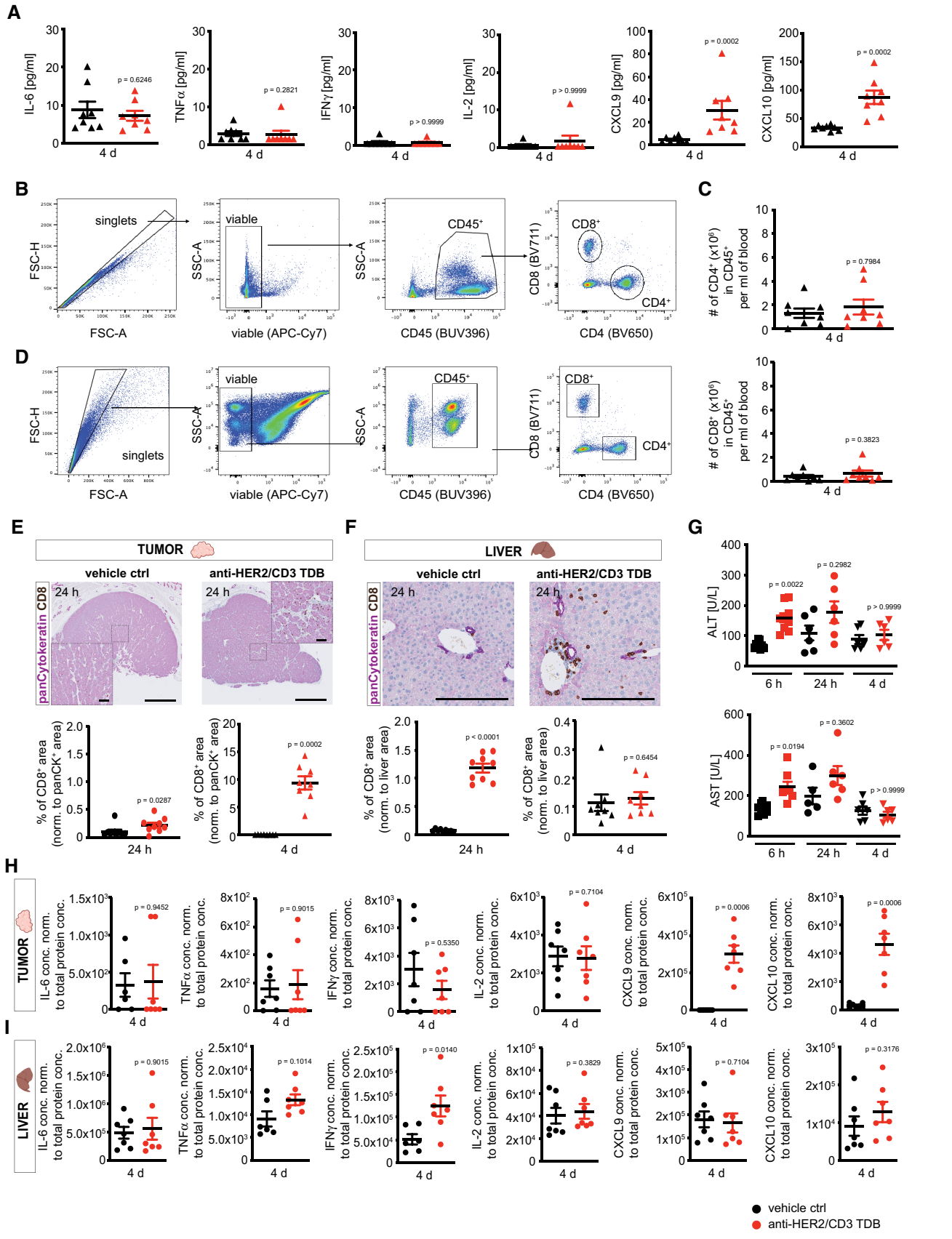

Figure EV1.

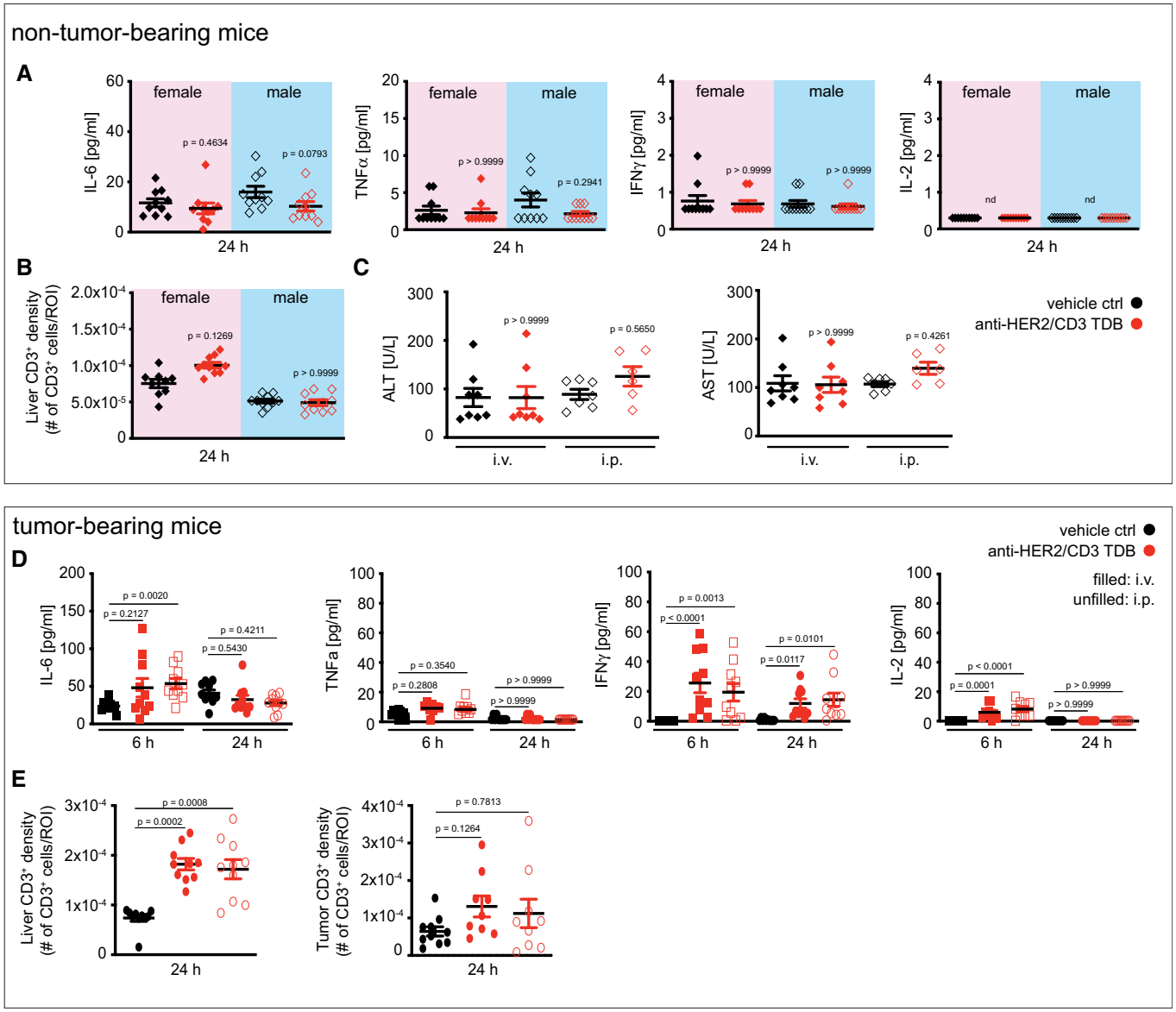

Figure EV2. The observed effects of TDB treatment are dependent on the engagement of TDB with T cells and tumor antigen.

A Serum cytokine levels from control-treated (black) and anti-HER2/CD3 TDB-treated (red) non-tumor-bearing mice, 24 h post treatment ($n = 10$ mice/treatment) in female (left, rose-shaded) and male (right, blue-shaded). Kruskal–Wallis one-way ANOVA analysis showed no difference between groups. nd, not detected.

B Quantification of $CD3^+$ cell counts in liver sections normalized to tissue areas from control-treated (black) and anti-HER2/CD3 TDB-treated (red) non-tumor-bearing mice, 24 h post treatment ($n = 9/10$ mice/treatment). Kruskal–Wallis one-way ANOVA analysis showed no difference between groups.

C Serum levels of alanine transaminase (ALT, left) and aspartate aminotransferase (AST, right) from control-treated (black) and anti-HER2/CD3 TDB-treated (red) non-tumor-bearing mice, 24 h post treatment ($n = 6–8$ mice/treatment), comparing intravenous (i.v., filled symbols) and intraperitoneal (i.p., unfilled symbols) injections.

D Serum cytokine levels from control-treated (black) and anti-HER2/CD3 TDB-treated (red) tumor-bearing mice, 6 h and 24 h post treatment ($n = 9/10$ mice/treatment), comparing intravenous (i.v., filled symbols) and intraperitoneal (i.p., unfilled symbols) injections. Kruskal–Wallis one-way ANOVA analysis showed no difference between groups.

E Quantification of $CD3^+$ cell counts in Founder5 liver (left) and tumor (right) sections normalized to tissue areas from control-treated (black) and anti-HER2/CD3 TDB-treated (red) mice, 24 h post treatment ($n = 9/10$ mice/treatment), comparing intravenous (i.v., filled symbols) and intraperitoneal (i.p., unfilled symbols) injections. Kruskal–Wallis one-way ANOVA analysis showed no difference between groups.

Data information: Black = control-treated mice. Red = anti-HER2/CD3 TDB-treated mice. Kruskal–Wallis one-way ANOVA was used for multiple groups. Data are shown as means ± SEM.
Source data are available online for this figure.

Figure EV3. TDB treatment effects are independent of tumor model and TDB used.

A Serum cytokine levels from control-treated (black) and anti-LYPD1/CD3 TDB-treated (purple) tumor-bearing mice from the ID8/LYPD1X4 ovarian cancer model, 6 h, 24 h, and 4 days post treatment ($n = 8$ mice/treatment).

B Quantification of $CD4^+$ (top panel) and $CD8^+$ (bottom panel) T cell counts in peripheral blood samples from mice treated with control (black, $n = 8$) or anti-LYPD1/CD3 TDB (purple, $n = 8$), 24 h post treatment.

C, D Representative images of ID8/LYPD1X4 tumor sections (C, left) or liver sections (D, left) stained with CD3 (red), Endomucin (white), and DAPI (blue) 24 h post treatment (top panels). Scale bar = 200 μm. Quantification of $CD3^+$ cell counts in ID8/LYPD1X4 whole tumor sections (C) or liver sections (D) normalized to tissue areas 24 h and 4 days post treatment ($n = 8$ mice/treatment) (bottom panels).

E Serum levels of alanine transaminase (ALT, top panel) and aspartate aminotransferase (AST, bottom panel) from control-treated (black) and anti-LYPD1/CD3 TDB-treated (purple) mice, 24 h and 4 days post treatment ($n = 7$–8 mice/treatment).

F, G Quantification of vascular ICAM-1^+ (ICAM-1^+ Endomucin$^+$) and vascular VCAM-1^+ (VCAM-1^+ Endomucin$^+$) areas in tumor sections (F) and liver sections (G) normalized to tissue areas from control-treated (black) and anti-LYPD1/CD3 TDB-treated (purple) mice, 24 h post treatment ($n = 6$–8 mice/treatment).

Data information: Black = control-treated mice. Purple = anti-LYPD1/CD3 TDB-treated mice. Mann–Whitney test was run to determine *P*-values for experiments with two groups; Kruskal–Wallis one-way ANOVA was used for multiple groups. Data are shown as means ± SEM. ROI, region of interest.
Source data are available online for this figure.

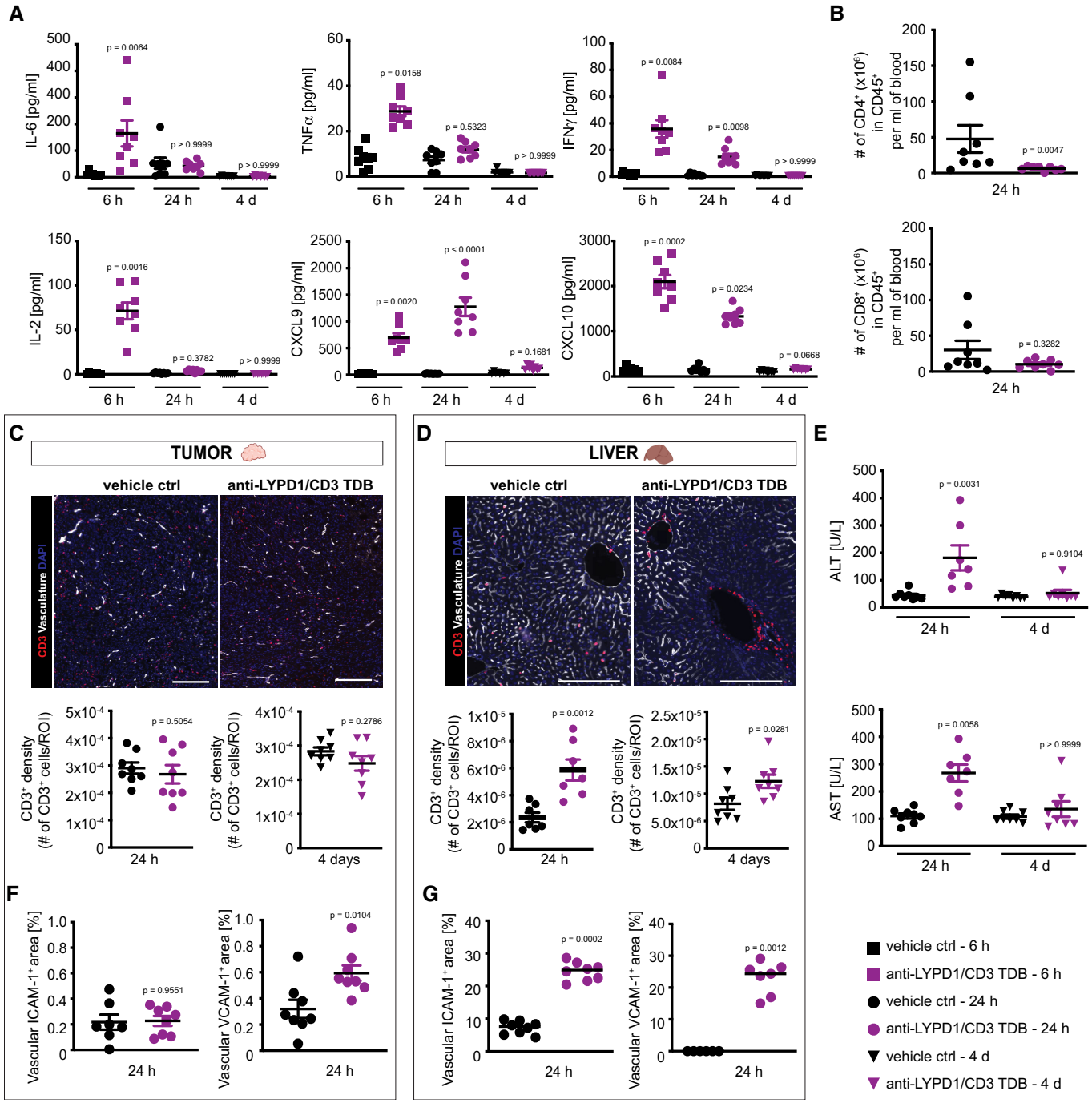

Figure EV3.

Figure EV4. Anti-HER2/CD3 TDB treatment induces dramatic transcriptional changes in the liver but does not change EC subpopulation identities.

A t-SNE plot with color-coded clusters of liver cells shown per replicate (left). Right panel shows relative abundance of detected cell types per sample. No batch effect was detected.

B t-SNE plot highlighting expression levels of selected cell type markers in the liver.

C Heatmap of selected established cell type markers.

D Calculated DoubletScore levels are shown as t-SNE plot (left) or as box plot (right). Boxes represent interquartile ranges (IQR); medians are lines in the boxes. Whiskers above and below the boxes are the lowest and highest values within 1.5 times IQR. Outliers are shown as circle beyond the whiskers.

E UMAP plot of endothelial cells color-coded for mouse organs/tissues based on reference dataset (Data ref: Kalucka *et al*, 2020b) and annotation (left) and our data mapped into the same UMAP space (right, # indicates the number of cells that mapped into the indicated organ transcriptional profile), confirming the origin of our endothelial cells being liver.

Source data are available online for this figure.

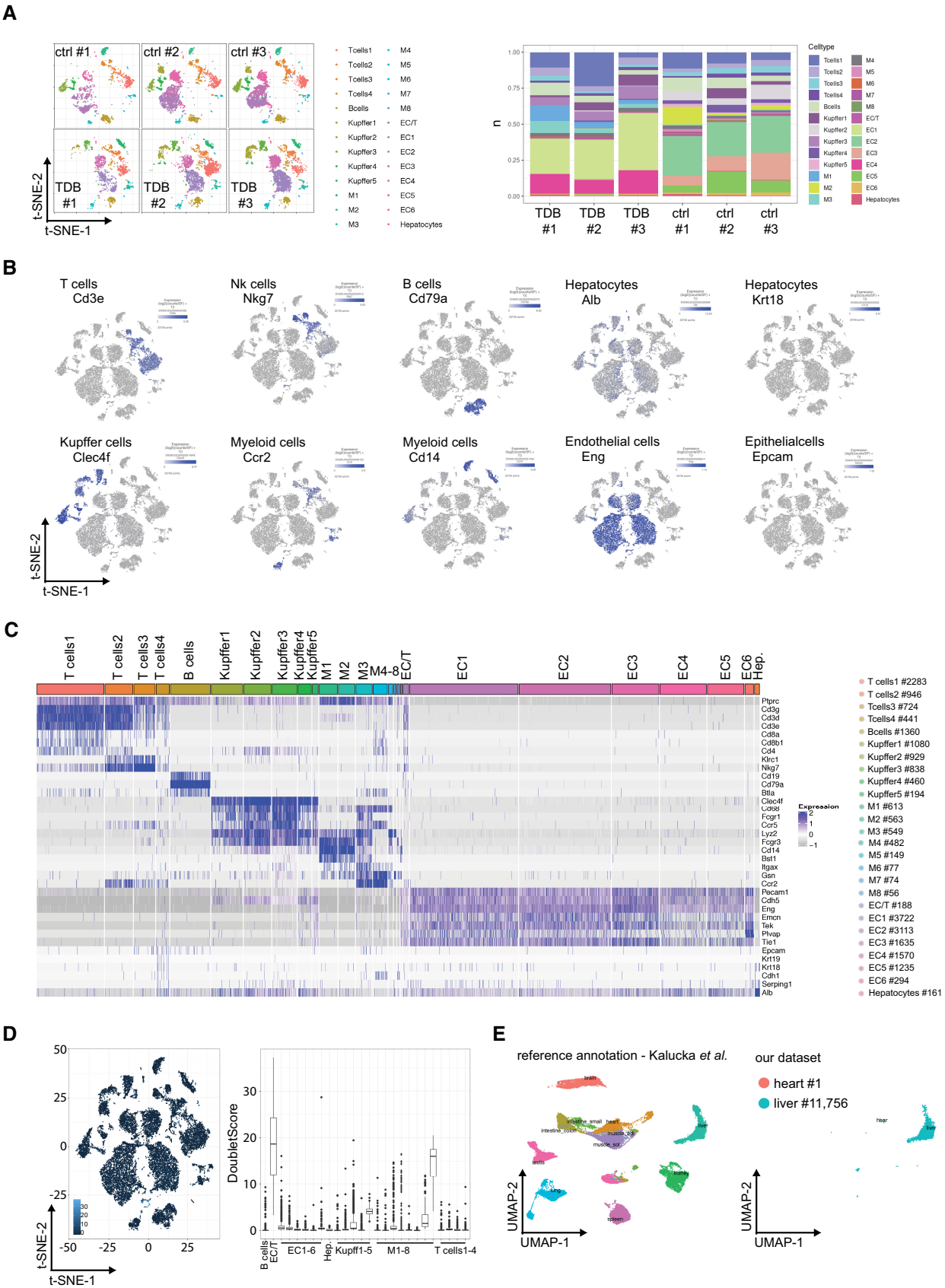

Figure EV4.

Figure EV5. Topic modeling captures the transcriptional dynamics within the different EC subtypes with and without TDB treatment.

A UMAP plot showing the relative expression levels for the nine topics based on topic modeling.

B Box plots showing the relative expression levels for the nine topics based on topic modeling across the previous identified 12 EC sub-clusters. Boxes represent interquartile ranges (IQR); medians are lines in the boxes. Whiskers above and below the boxes are the lowest and highest values within 1.5 times IQR.

Source data are available online for this figure.

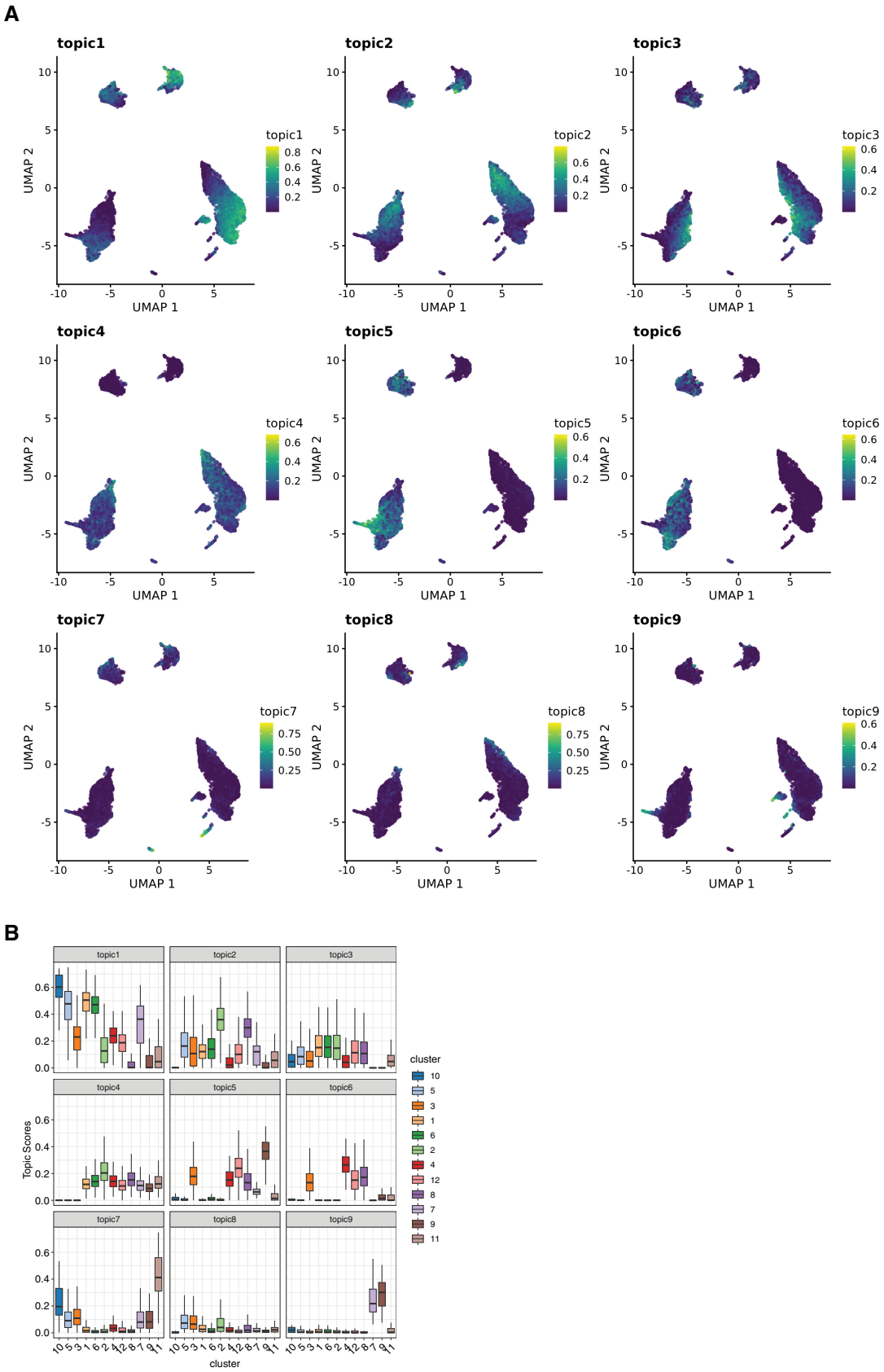

Figure EV5.