Supplementary Figures

TCDD dysregulation of lncRNA expression, liver zonation and intercellular communication across the liver lobule

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Fig. S1. A. Mouse liver cell subpopulations and cell-type specific markers. A. UMAP displaying clusters of liver nuclei clusters based on 15,573 single-nuclei transcriptomes integrated from all four snRNA-seq samples, two control liver and two TCDD liver snRNA-seq samples (biological replicates). Cell count values for each cell type and for each condition (C: control; T: TCDD) are indicated in square brackets. **B.** Dot plot showing average expression values for marker genes (shown on X-axis) across the eight hepatic cell clusters (identified on y-axis) for liver nuclei aggregated from all 4 samples -- control and TCDD.

Fig. S2. **Gene induction and gene repression responses to TCDD**. Shown are bar plots indicating the total number of up regulated (green) and down regulated genes (red). (A), all genes, (B) protein-coding genes, and (C) lncRNA genes, in each of the 8 indicated liver cell types, at |fold-change| >4 and FDR <0.05. Many fewer genes were up regulated than were down regulated in several of the NPC clusters. (D) Pie charts showing numbers of lncRNAs and protein-coding genes that are differentially expressed in TCDD-exposed vs control liver in hepatocytes only, in non-parenchymal cells (NPCs) only, or is both hepatocytes and NPCs based on data shown in Table S1, columns AO and AP.

Fig S3. **Violin plots and feature plots showing expression data for select TCDD-responsive genes**. **A.** Violin plots for Cyp1a1, a classic TCDD/Ahr response gene that is strongly induced by TCDD in multiple liver cell types, and for two lncRNAs whose expression is highly inducible in cholangiocytes but not in other liver cell types. **B.** Feature plots showing expression data for two lncRNAs that are highly induced by TCDD in hepatocytes but not in other cell clusters. Expression data is presented superimposed on the UMAP of cell clusters shown in Fig. S1A, where the individual cell clusters are identified. **C.** Expression data for lncRNAs repressed by TCDD. **Top** panel of C, violin plots showing combined average expression values for 13 lncRNAs repressed by TCDD across multiple liver cell types (Table S1C; lnc35408, lnc10883_Serpina3h, lnc12772_AC118710.3, lnc17117_C730036E19Rik, lnc19880, lnc19881, lnc19883, lnc23635, lnc3297_Gm12909, lnc34452, lnc38016, lnc7809_Gm47465, lnc9497). **Middle** panel of C, violin plots for 2 of the 13 lncRNAs, lnc10883 and lnc17117, which reveals these lncRNAs are most highly expressed in hepatocytes and in Kupffer cells. Repression also occurs in the other cell types, but the level of expression in the other cell types is too low for visualization on the scale of these violin plots. **Bottom** panel of C, feature plots, as described in panel B, for the same two lncRNAs shown in the middle panel of C.

Figure S4. **Transcription factor (TF) superfamilies dysregulated by TCDD exposure.** Bar plot showing 72 TF families comprising a total of 238 differentially expressed TFs (one TF, Nr4a1, was excluded as it did meet significance in the analysis of separated hepatocyte populations presented in Table S3C; see note in that table legend). TF family classification was based on the DNA-binding domain. *Red* bars mark TF families that were significantly enriched compared to a background set of TFs from the full AnimalTFDB list (Table S3B) that are not differentially expressed at $p < 0.05$, based on Fisher's exact test. Numbers above each bar, numbers of TFs in the family whose expression was altered by TCDD. The total number of TFs in each family is shown in parentheses along the Y-axis. The THR-like family includes 20 of the 49 NRs shown in Fig. 3.

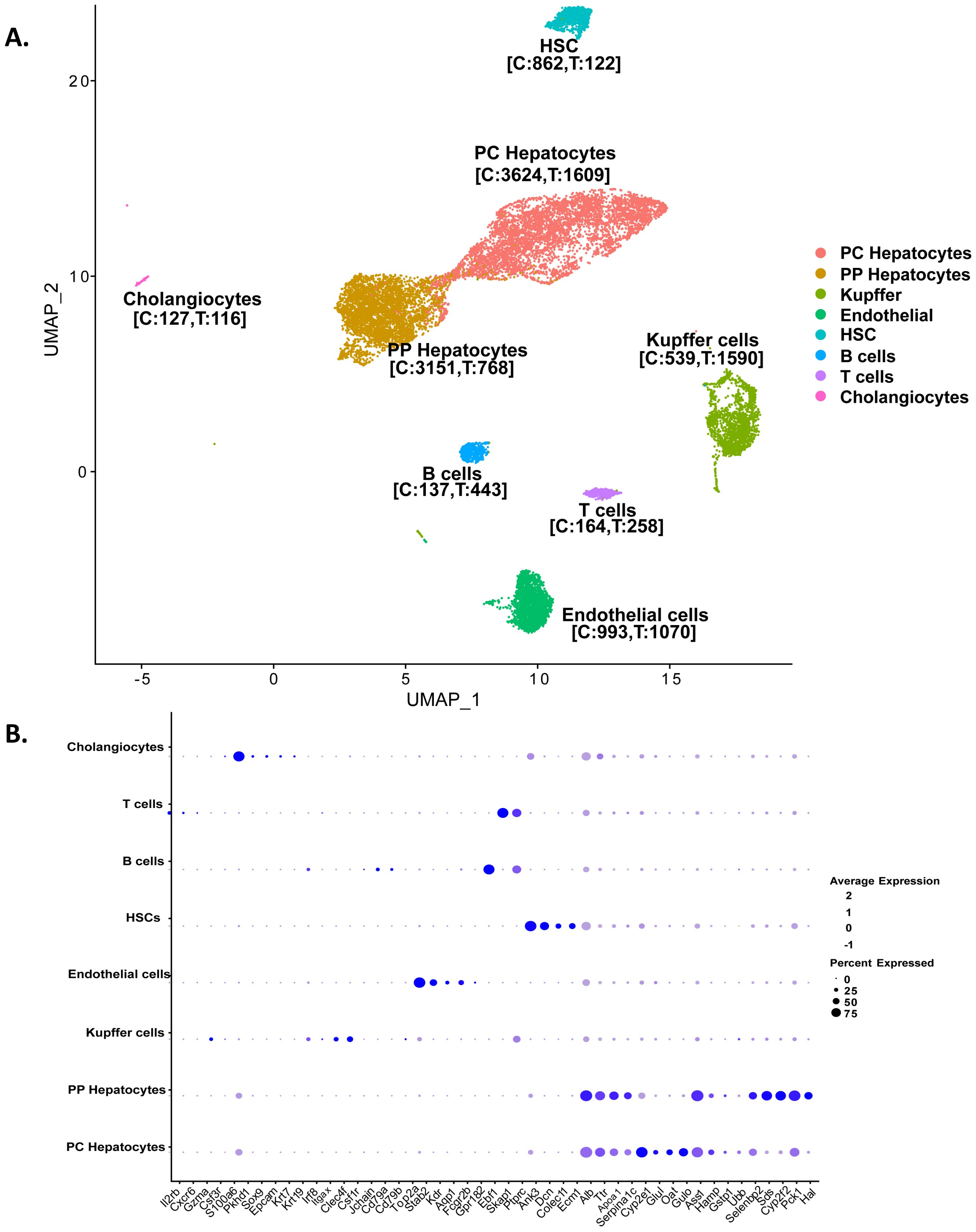
Figure S5. Venn diagram of the number of network-essential genes in control and in TCDD-exposed liver networks. See Table S5B for full listings.

Figure S6. Clustering of 40 network-essential lncRNAs from the control liver gene regulatory network. Protein-coding gene targets that make direct connection with regulatory lncRNAs in the control liver network of Fig. 6A were provided as input to Metascape to obtain top functional enrichment terms based on -log10 (P-value). The color scale represents -log10(P) values, with darker purple coloration indicating more significant p-values. Two-way hierarchical clustering of the functional enrichment heatmap split the 40 lncRNAs into 3 clusters (columns colors) based on their patterns of shared functions. Target genes of lncRNAs from the yellow cluster were enriched for metabolic processes and lipid homeostasis, while the other 2 major functional clusters (blue, green) were primarily enriched in collagen biosynthesis, cell-cell adhesion, signaling and extracellular matrix and morphogenesis, as shown. <u>Bottom</u>: subnetwork of the network shown in Fig. 6A. This subnetwork is comprised of the 40 of the 41 network-essential lncRNAs regulatory lncRNAs and their target genes, which clustered to give gene modules that mirror the clustering results obtained for the same lncRNAs based on their functional group enrichments, shown on top. [Note: the gene targets of 1 of the 41 lncRNA did not yield any enriched pathways; it was therefore excluded from the heatmap]. The subnetwork was derived from the network in Fig. 6A by removing all protein-coding gene regulators and their gene targets, and by retaining regulatory lncRNAs and their direct protein-coding genes connections, which were then used as input for the functional enrichment analysis shown at the top. The regulatory lncRNAs (nodes) at the bottom are color coded to match the lncRNA cluster colors shown in the heat map.

Figure S7. Clustering of 57 network-essential lncRNAs from the TCDD-exposed liver gene regulatory network. Analysis of regulatory lncRNAs from the TCDD network of Fig. 6B, as described in Fig. S6. Two-way hierarchical clustering identified 3 clusters of regulatory lncRNAs, whose target genes were enriched for the pathways and biological processes shown at the right. <u>Bottom</u>: subnetwork of the TCDD network shown in Fig. 6B and obtained as described in Fig. S6.

Figure S8. Combined clustering of 97 network-essential lncRNAs from control and TCDD-exposed liver networks. Shown is a merged functional enrichment heatmap showing the top enriched terms (as rows) for genes targets of all regulatory lncRNAs (as columns) from the control and TCDD-exposed liver networks combined and analyzed as described in Fig. S6. This figure indicates that the target genes of the regulatory lncRNAs of the control and TCDD liver networks largely have different enriched functional terms.

Fig. S1


A.
HSC
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PC Hepatocytes
[C:3624,T:1609]
Cholangiocytes
[C:127,T:116]
PP Hepatocytes
[C:3151,T:768]
Kupffer cells
[C:539,T:1590]
B cells
[C:137,T:443]
T cells
[C:164,T:258]
Endothelial cells
[C:993,T:1070]
20
10
0
-5
0
5
10
15
UMAP_1
UMAP_2
PC Hepatocytes
PP Hepatocytes
Kupffer
Endothelial
HSC
B cells
T cells
Cholangiocytes
B.
Cholangiocytes
T cells
B cells
HSCs
Endothelial cells
Kupffer cells
PP Hepatocytes
PC Hepatocytes
Il2rb
Cxcr6
Gzma
Csf3r
S100a6
Pkhd1
Sox9
Epcam
Krt7
Krt19
Irf8
Itgax
Clec4f
Csf1r
Jchain
Cd79a
Cd79b
Top2a
Stab2
Kdr
Aqp1
Fcgr2b
Gpr182
Ebf1
Skap1
Ptprc
Ank3
Dcn
Colec11
Ecm1
Alb
Ttr
Apoa1
Serpina1c
Cyp2e1
Glul
Oat
Gulo
Ass1
Hamp
Gstp1
Ubb
Selenbp2
Sds
Cyp2f2
Pck1
Hal
Average Expression
2
1
0
-1
Percent Expressed
0
25
50
75

Fig. S2

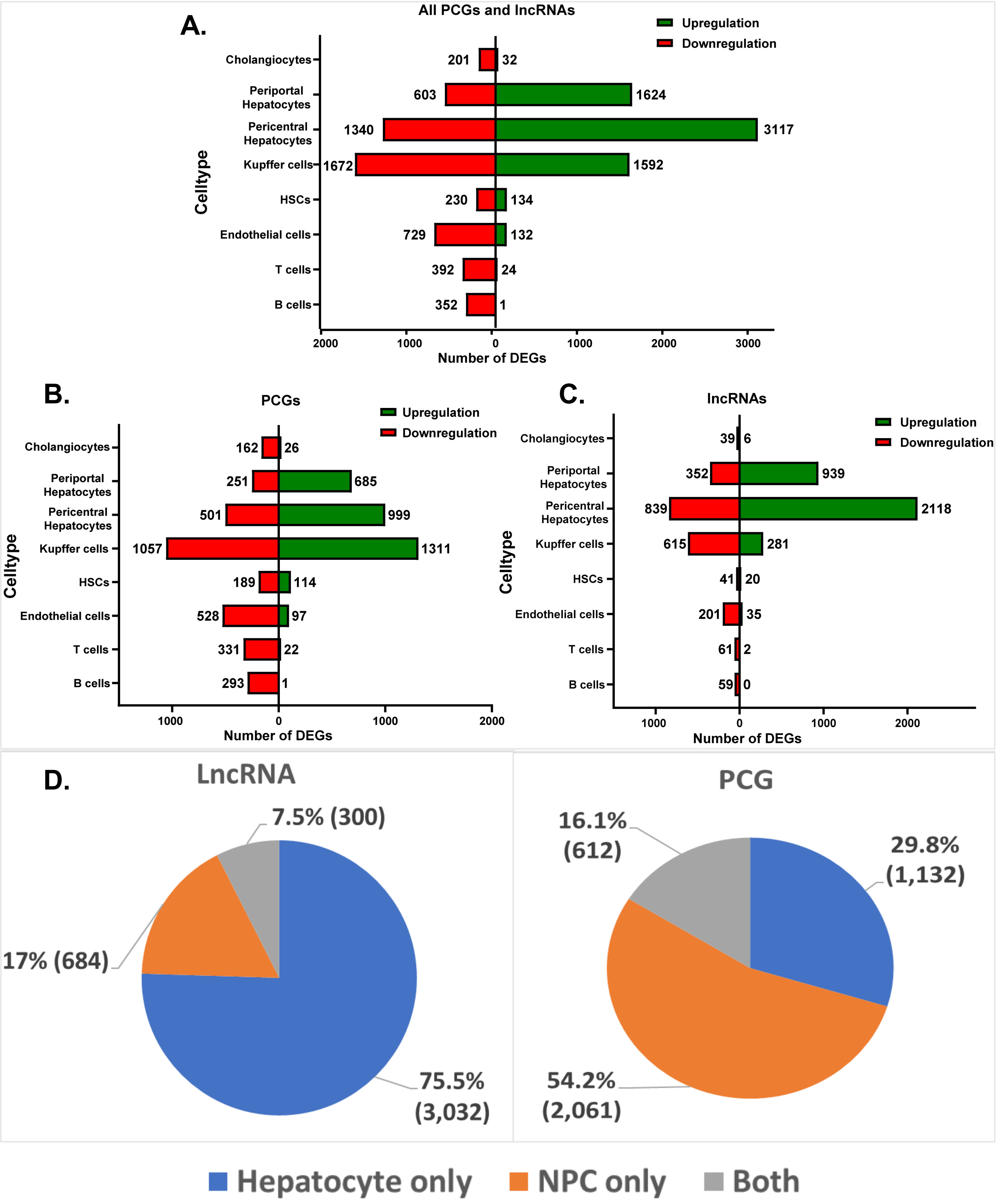
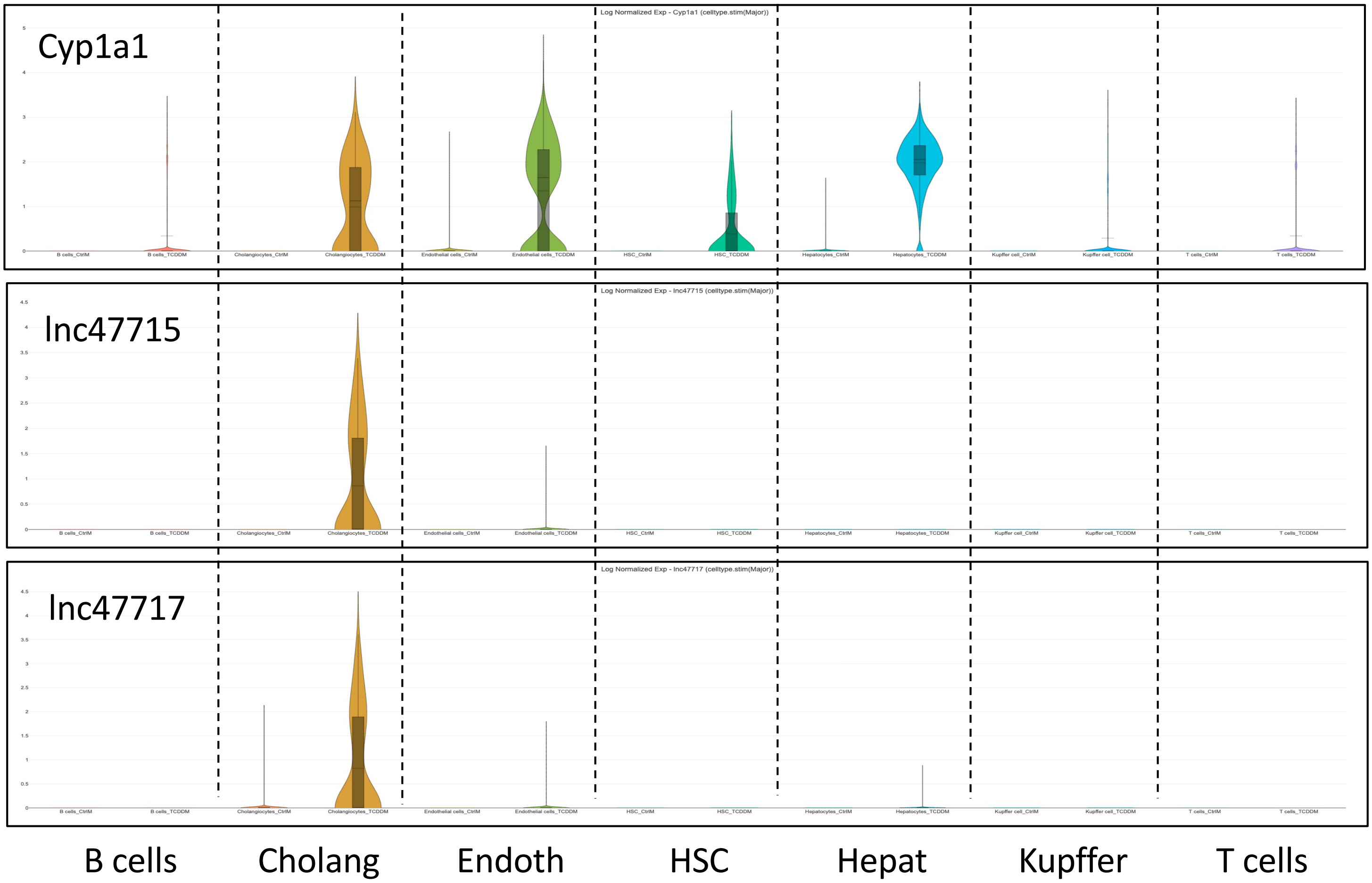
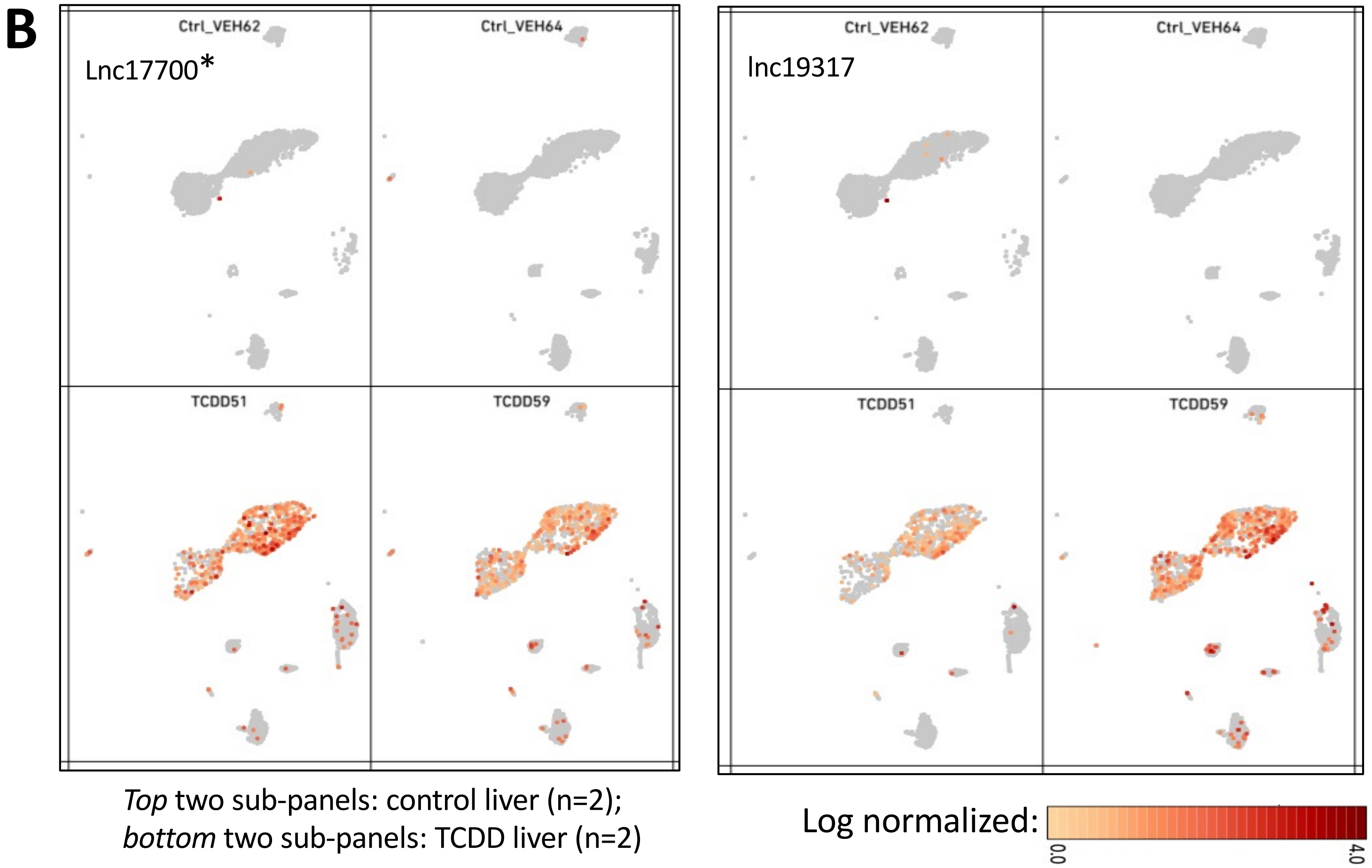

A.
All PCGs and lncRNAs
Upregulation
Downregulation
Celltype
Cholangiocytes
Periportal Hepatocytes
Pericentral Hepatocytes
Kupffer cells
HSCs
Endothelial cells
T cells
B cells
201
32
603
1624
1340
3117
1672
1592
230
134
729
132
392
24
352
1
2000
1000
0
1000
2000
3000
Number of DEGs
B.
PCGs
Upregulation
Downregulation
Celltype
Cholangiocytes
Periportal Hepatocytes
Pericentral Hepatocytes
Kupffer cells
HSCs
Endothelial cells
T cells
B cells
162
26
251
685
501
999
1057
1311
189
114
528
97
331
22
293
1
1000
0
1000
2000
Number of DEGs
C.
lncRNAs
Upregulation
Downregulation
Celltype
Cholangiocytes
Periportal Hepatocytes
Pericentral Hepatocytes
Kupffer cells
HSCs
Endothelial cells
T cells
B cells
39
6
352
939
839
2118
615
281
41
20
201
35
61
2
59
0
1000
0
1000
2000
Number of DEGs
D.
LncRNA
7.5% (300)
17% (684)
75.5% (3,032)
PCG
16.1% (612)
29.8% (1,132)
54.2% (2,061)
Hepatocyte only
NPC only
Both

Fig. S3AB

A *Control, 1st violin in each cell cluster; TCDD, 2nd violin in each cluster*



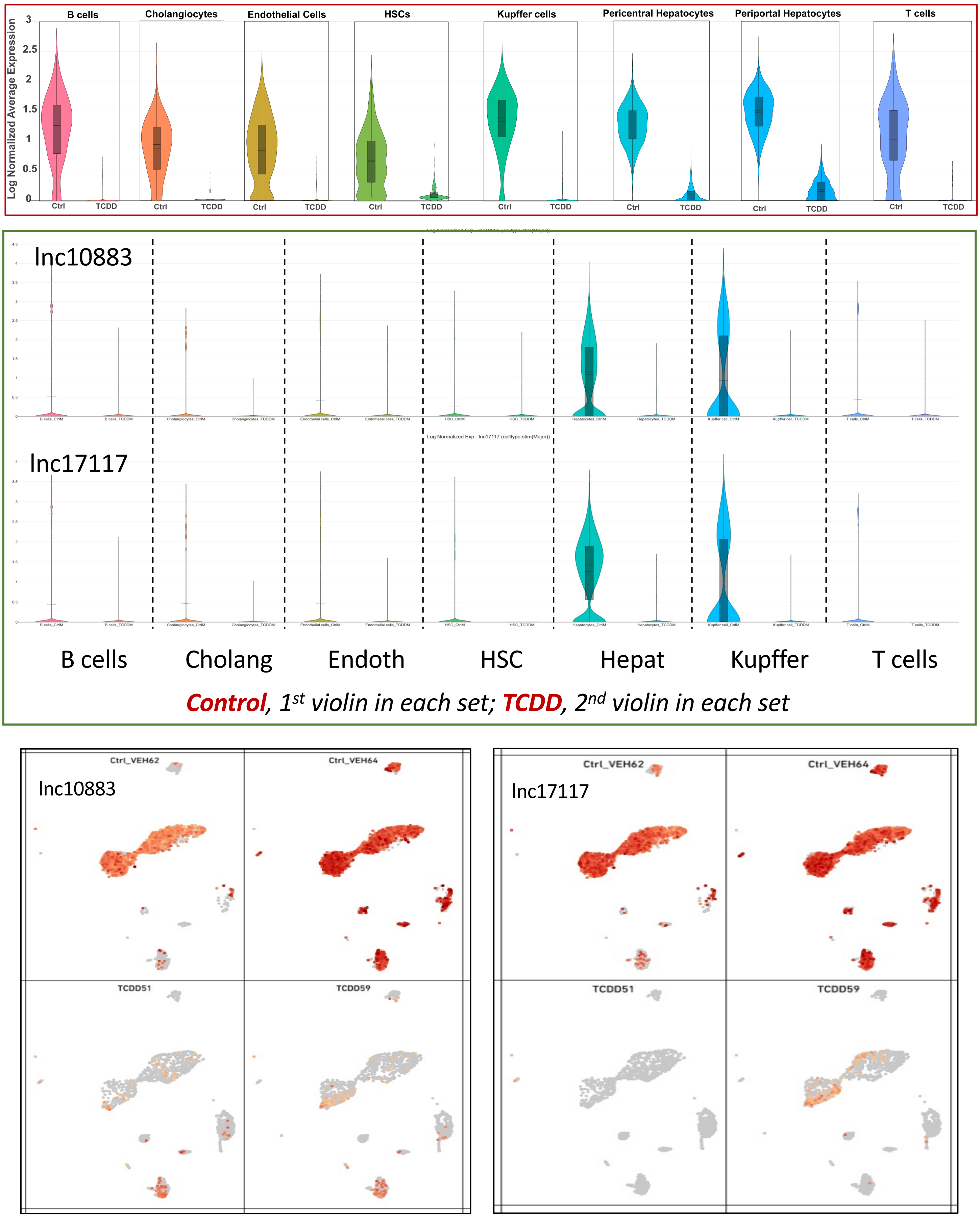
B



Top two sub-panels: control liver (n=2); *bottom* two sub-panels: TCDD liver (n=2)

Fig. S3C

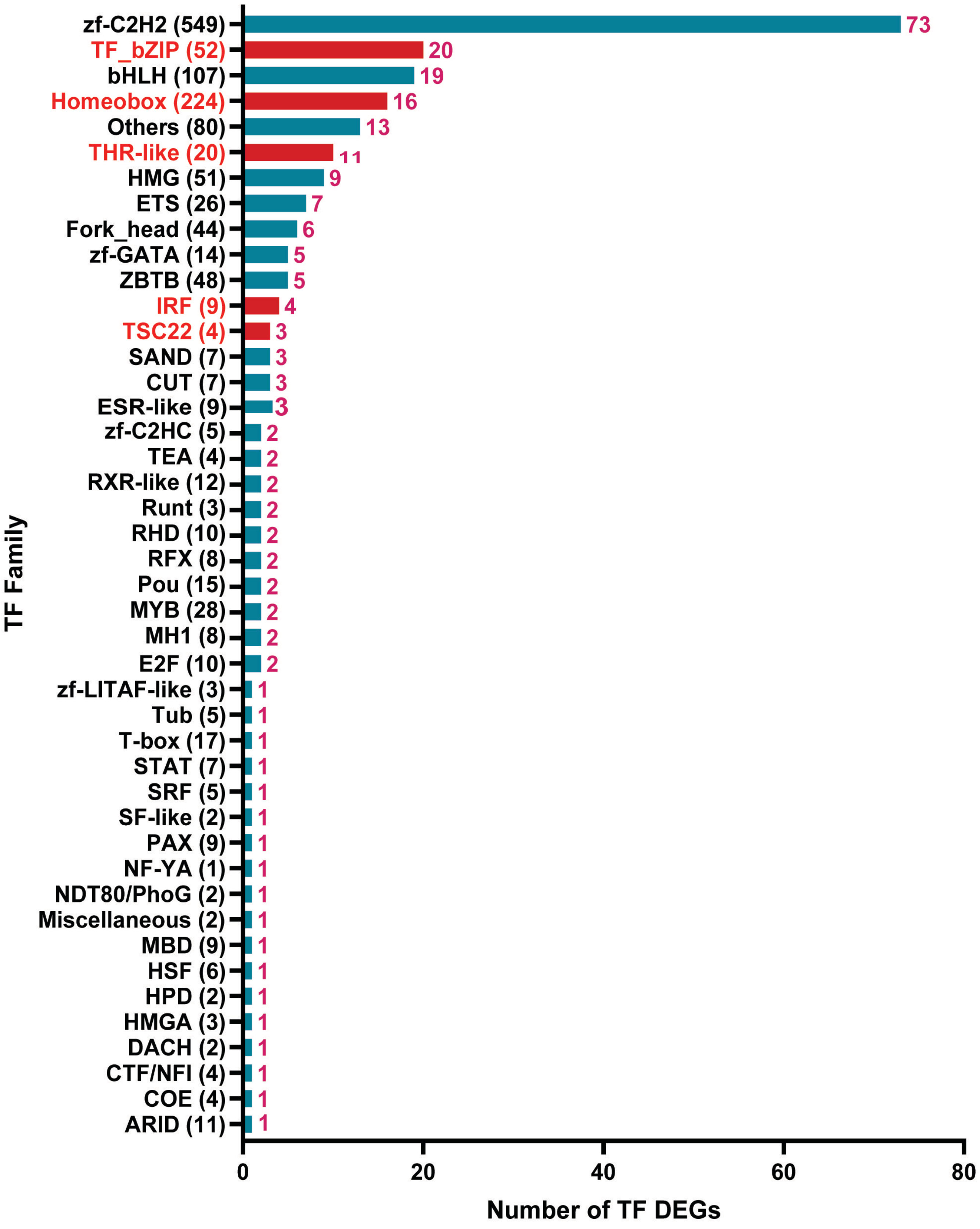
C



Control, *1st violin in each set;* ***TCDD***, *2nd violin in each set*

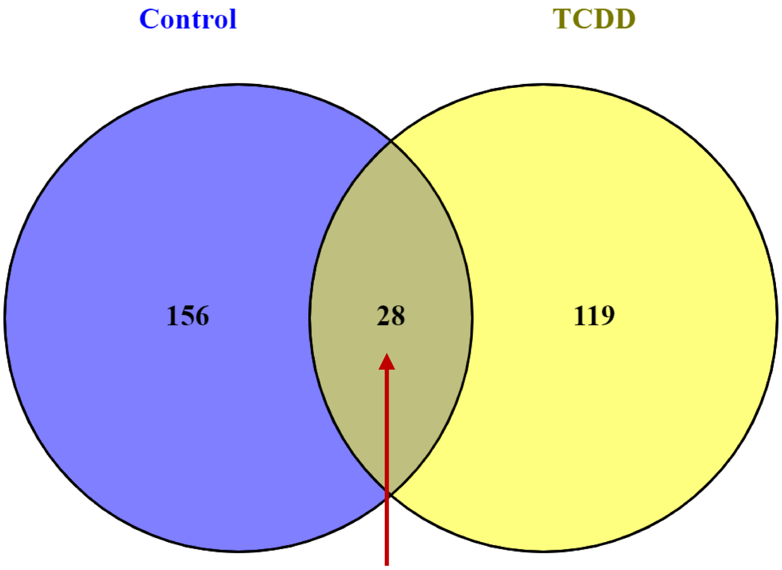
Top two sub-panels: control liver (n=2); bottom two sub-panels: TCDD liver (n=2)

Fig. S4



zf-C2H2 (549)
73
TF_bZIP (52)
20
bHLH (107)
19
Homeobox (224)
16
Others (80)
13
THR-like (20)
11
HMG (51)
9
ETS (26)
7
Fork_head (44)
6
zf-GATA (14)
5
ZBTB (48)
5
IRF (9)
4
TSC22 (4)
3
SAND (7)
3
CUT (7)
3
ESR-like (9)
3
zf-C2HC (5)
2
TEA (4)
2
RXR-like (12)
2
Runt (3)
2
RHD (10)
2
RFX (8)
2
Pou (15)
2
MYB (28)
2
MH1 (8)
2
E2F (10)
2
zf-LITAF-like (3)
1
Tub (5)
1
T-box (17)
1
STAT (7)
1
SRF (5)
1
SF-like (2)
1
PAX (9)
1
NF-YA (1)
1
NDT80/PhoG (2)
1
Miscellaneous (2)
1
MBD (9)
1
HSF (6)
1
HPD (2)
1
HMGA (3)
1
DACH (2)
1
CTF/NFI (4)
1
COE (4)
1
ARID (11)
1
0
20
40
60
80
Number of TF DEGs
TF Family

Fig. S5



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Gpam,Hgd,Hjv,Immp2l,Inca1,Klf12,Klf15,Ldb2,Ldlr,lnc10476,lnc11040,lnc12776,lnc12905,lnc13247,
lnc1345,lnc1360,lnc1416,lnc14332,lnc16799,lnc17060,lnc1884,lnc22198,lnc2936,lnc31482,lnc40890,
lnc5389,lnc5718,lnc6251,lnc7720,Loxl2,Lpcat3,Lpin2,Mia2,Mlxipl,Msra,Mtarc1,Mthfd1,Nlrp6,Oaf,Optn,
Pbld1,Pcyt2,Pde4dip,Pecr,Pipox,Ppp1r3b,Prickle2,Proc,Prodh2,Prox1,Rapgef4,Rbp4,Rtp3,Sec24a,Sema4g,
Serpinf2,Slc10a1,Slc25a13,Slc25a20,Slc25a21,Slc27a2,Slc39a14,Stard10,Xylb,Ankrd33b,Arhgap31,Arid5b,
Arrb1,Bmp5,Bmp6,Cd38,Cpt1a,Creg1,Ctdspl,Dab2,Dcn,Ddi2,Dennd2a,Elk3,Ets1,Etv1,Fbn1,Haao,Hdac7,
Hebp1,Heyl,Hivep3,Hoxb4,Hsd17b4,Ifi203,Igf1,Igf1r,Igfbp4,Iqgap2,Ksr1,lnc1716,lnc21125,lnc29699,
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Macrod1,Mamld1,Myo1e,Ncoa7,Nsd3,Osbpl1a,Pam,Parp8,Plscr4,Prkag2,Prkch,Rarg,Rnf165,Trerf1,Trip4,Zeb2,Zfpm2

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F12,Hgfac,Hlf,Ikbkg,Larp1b,lnc10151,lnc1018,lnc11590,lnc12046,lnc14604,lnc14605,lnc15262,
lnc1550,lnc2342,lnc33230,lnc41044,lnc41384,lnc43327,lnc4334,lnc4351,lnc4362,lnc43856,
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Map3k13,Otc,Pemt,Pex19,Rcl1,Sec14l4,Sec16b,Selenbp2,Sgk2,Them7,Wdtc1,Aasdh,
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lnc43540,lnc4370,lnc5261,lnc5356,lnc6061,lnc6501,lnc9709,lnc981,lnc987,Lrp4,Mst1,
Ovol1,Pkp2,Slc10a5,Smad9,Sox5,Sox7,Suds3,Tead3,Trpv1,Ugt1a1,Wnt10a,Zfp612

Acsm1,Akap1,Cat,Creb3l3,Echdc2,Erbb3,Gm10768,Hnf4a,Hpn,lnc12772,
lnc1966,lnc23914,lnc6166,lnc8349,lnc979,Mttp,Nr1i2,Pcx,Pex14,Ppara,
Sdc4,Sirt3,Slc25a42,Slc38a3,Tfr2,Timd2,Tmprss6,Upb1

Fig. S6
Control network

Fig. S7
TCDD network

Fig. S8
Control +
TCDD