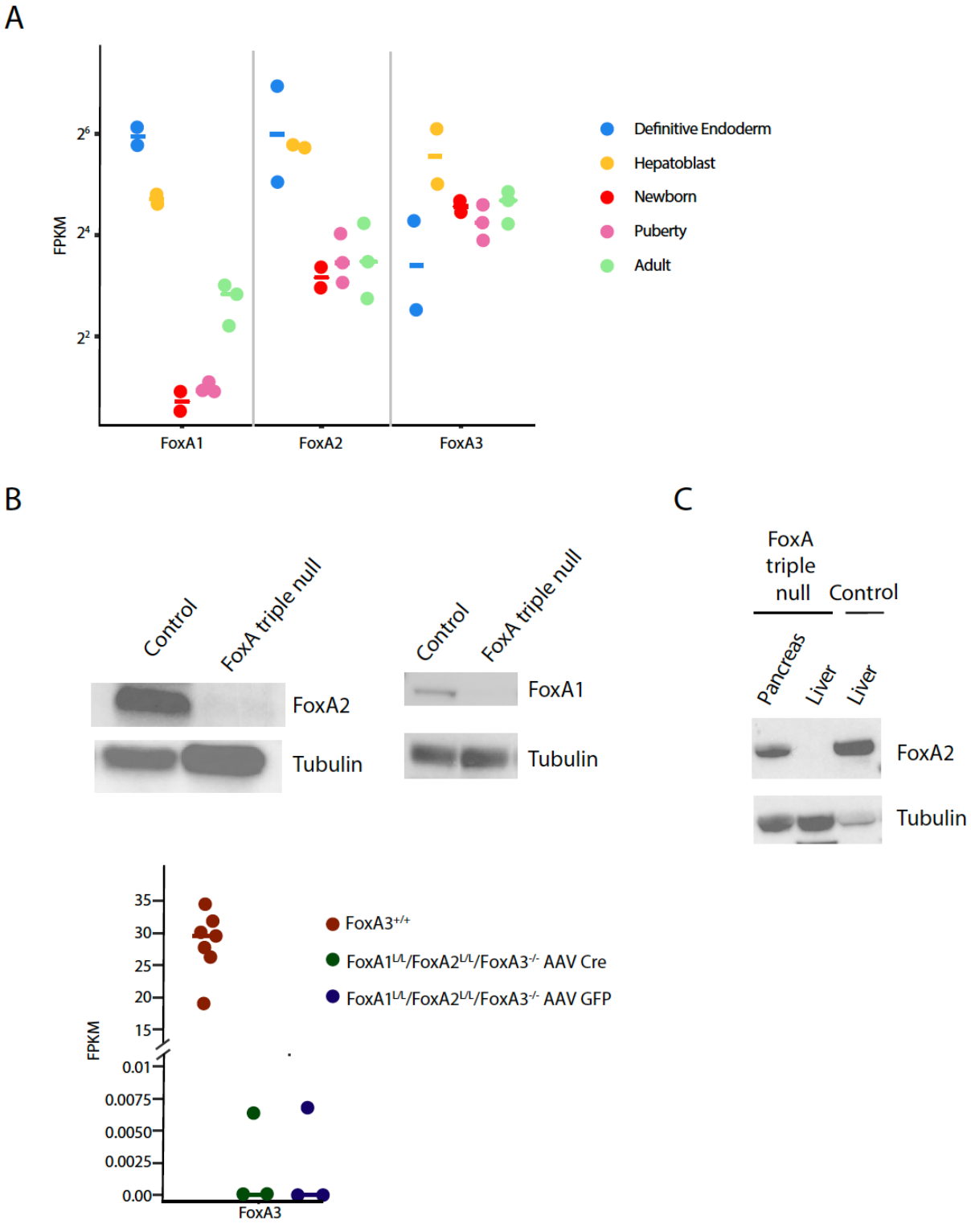
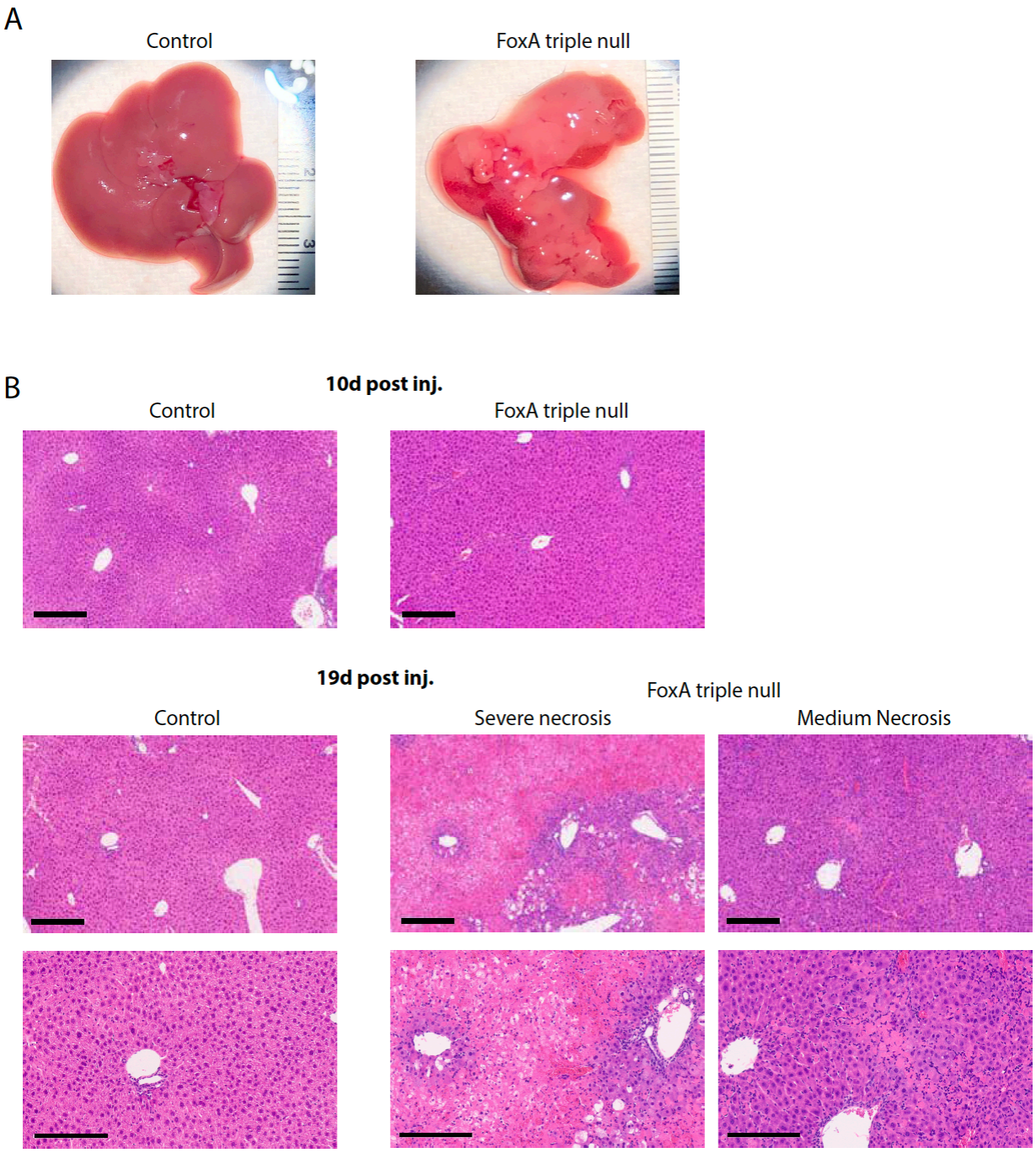
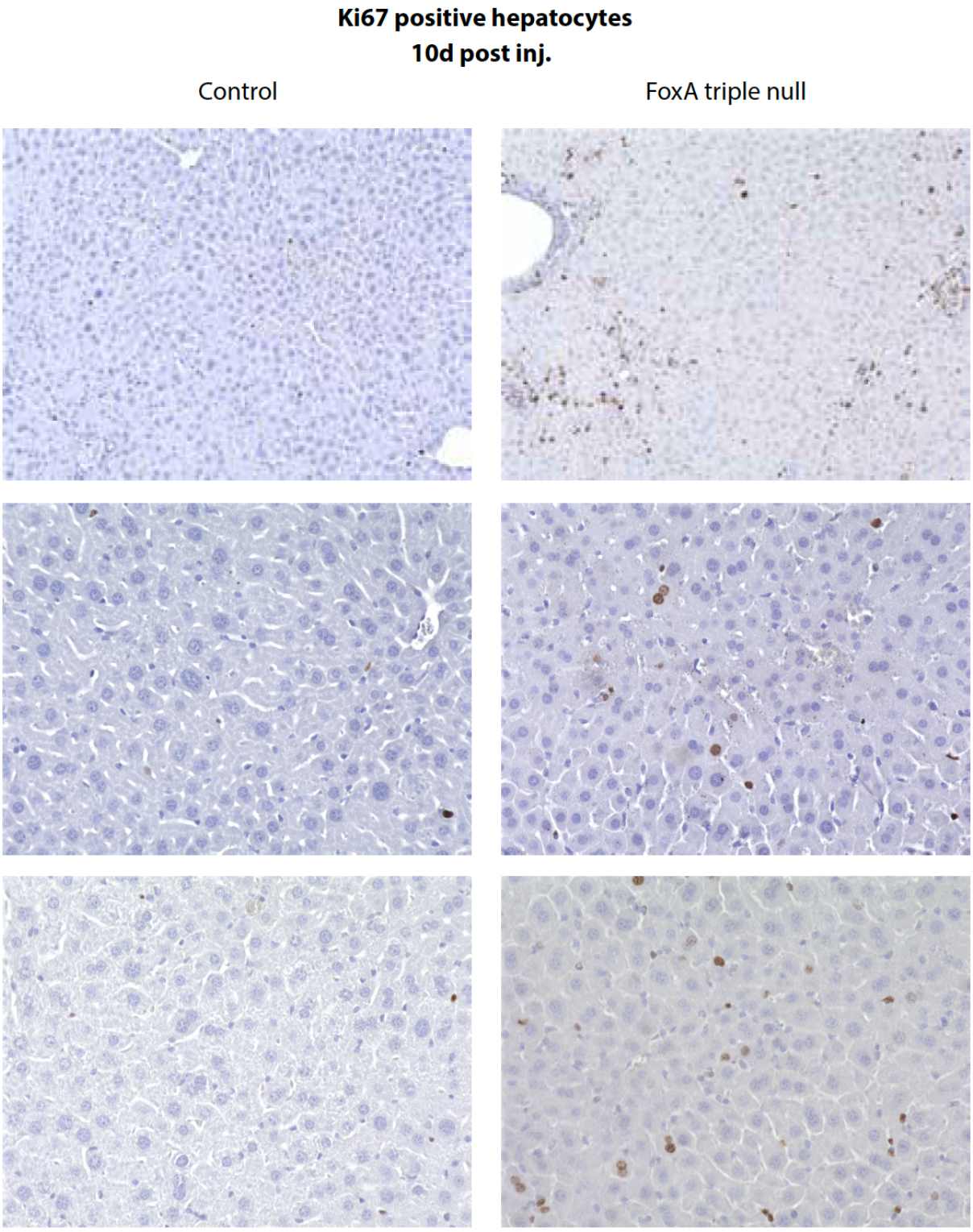
Supplemental Information



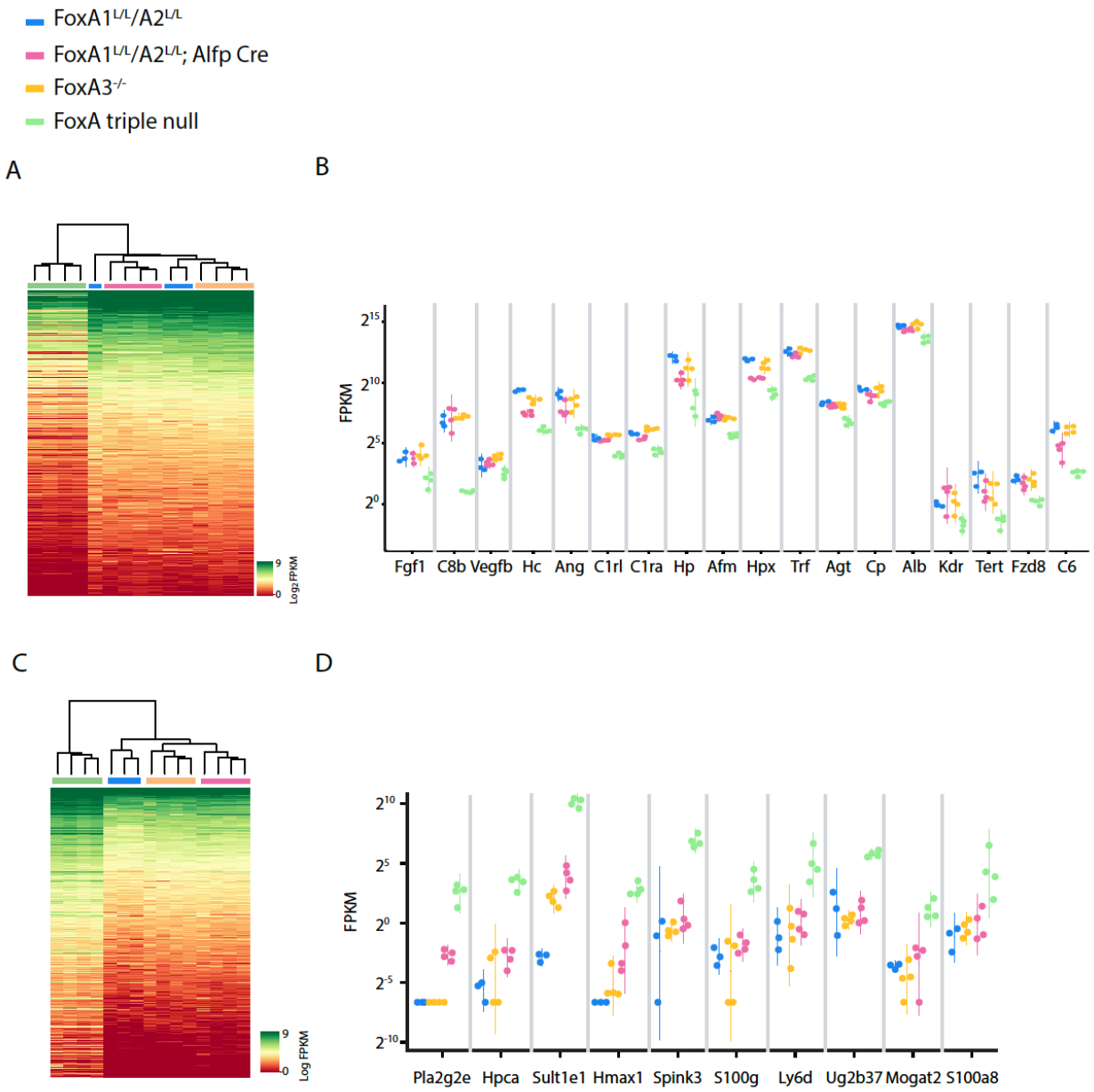
Supplemental Figure 1. FoxA expression in development and validation of FoxA depletion. A. FoxA1, FoxA2 and FoxA3 expression levels at different developmental stages. FoxA1 and FoxA2 in the adult are statistically significant lower compared with hepatoblast ($p < 0.05$, T-test). B. Western blots on FoxA1 and FoxA2 and mRNA expression levels of FoxA3 in the triple null compared with controls, Cre or GFP were induced under the control of the hepatocyte-specific thyroid-binding globulin (Tbg) promoter. The FoxA3 null allele used in these studies is a germline deletion of the gene as described in (Kaestner et al. 1998; Shen et al. 2001). This mutant allele replaced the entire FoxA3 coding sequence with LacZ, and thus does not express any FoxA3. C. Western blot showing no depletion of FoxA2 in the pancreas in our liver specific FoxA triple null model.



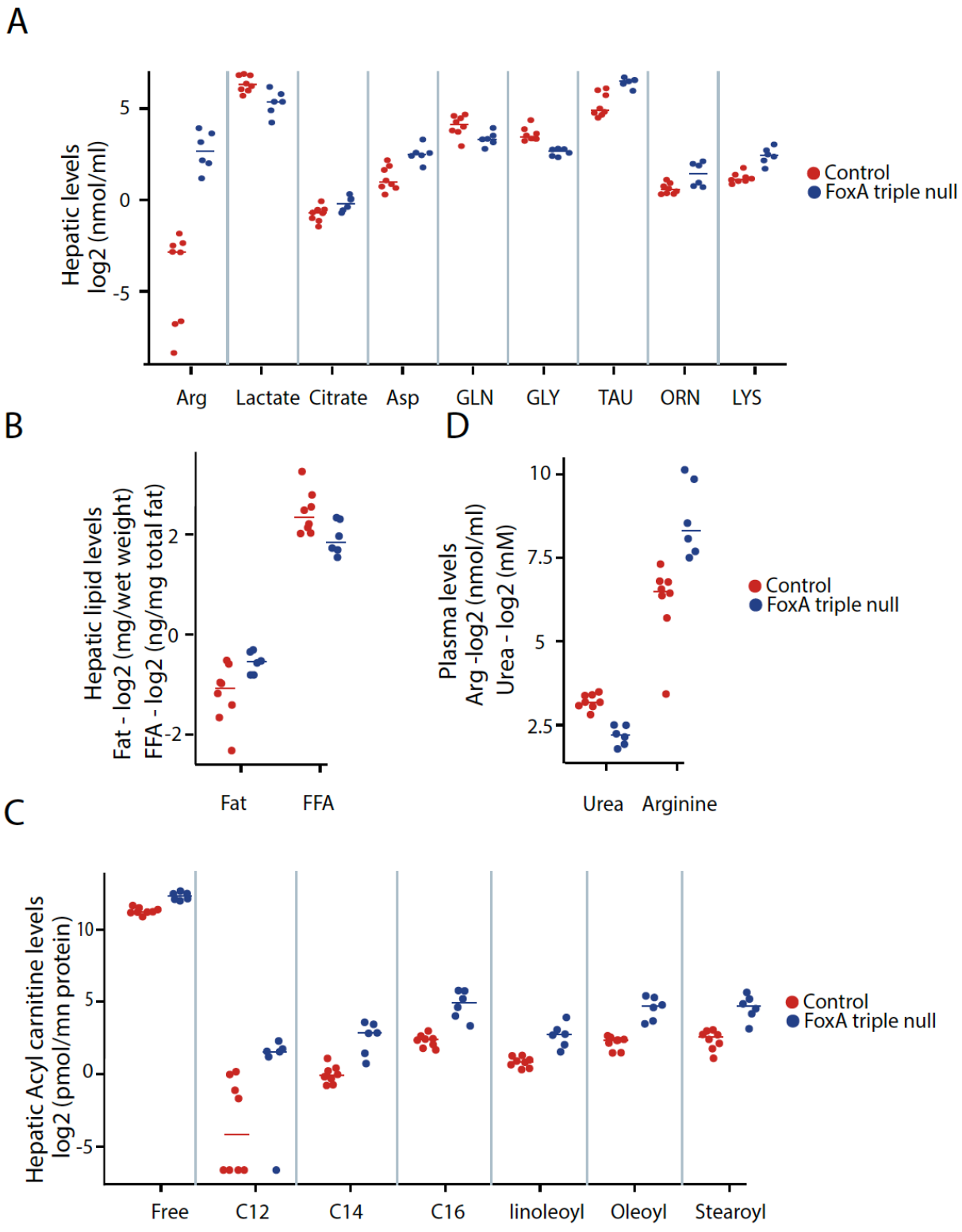
Supplemental Figure 2. Liver phenotype of FoxA triple null. A. Liver anatomy of FoxA triple nulls and controls. B. Hematoxylin and Eosin staining for FoxA triple nulls and controls, 10 and 19 days after Cre or GFP induction, including an example clearly showing a midzonal effect of FoxA complete depletion. Scale is 200 μm.



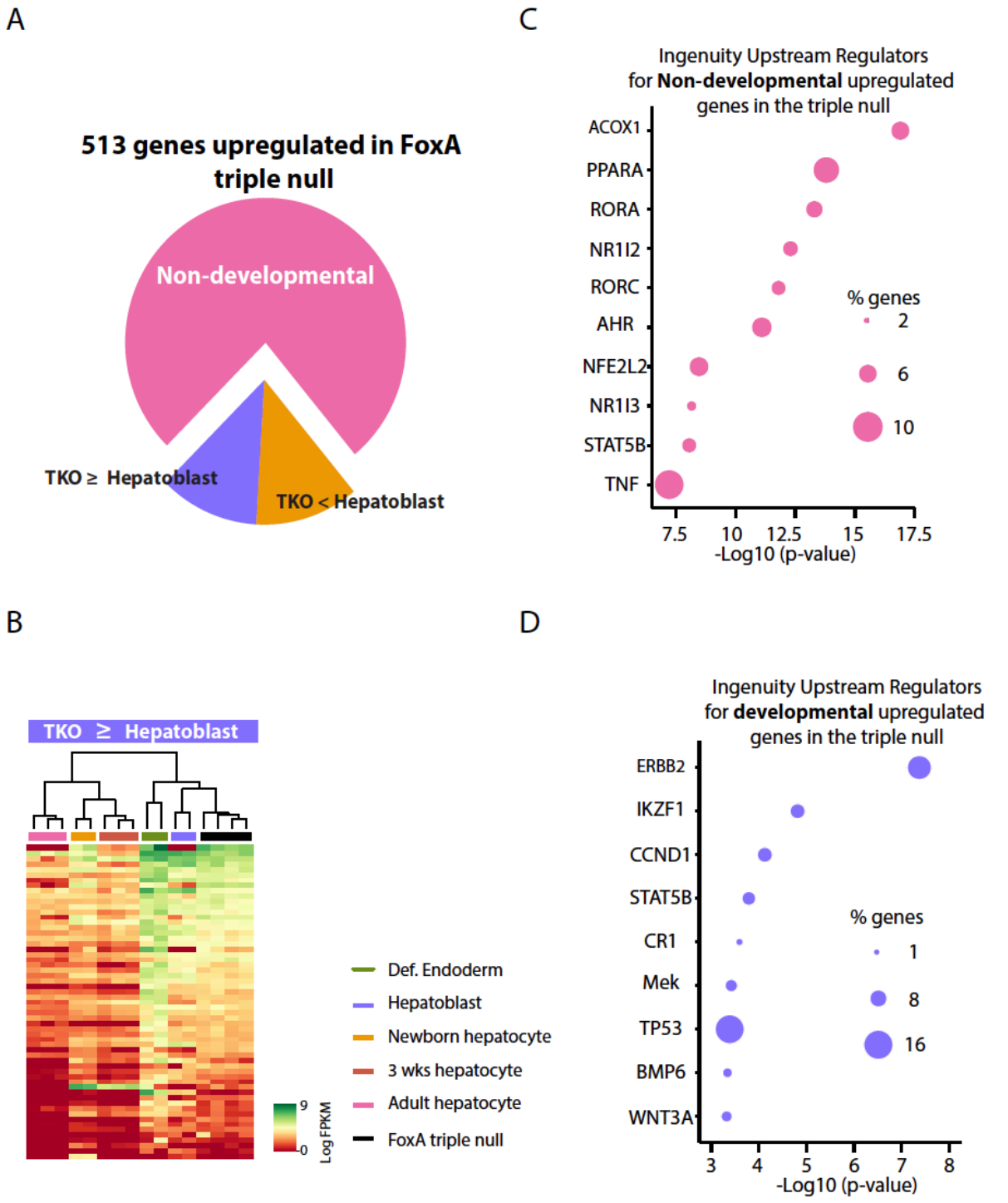
Supplemental Figure 3. Higher proliferation rates in FoxA triple nulls. Immunohistochemistry for Ki67 in FoxA triple nulls and controls, 10 days after Cre induction.



Supplemental Figure 4. Gene expression profile of FoxA triple nulls. A. Heatmap of 663 downregulated in FoxA triple null livers seven days after Cre induction compared to the partially FoxA depleted models indicated (adjusted p <0.05, fold change > 1.5). B. Gene expression levels of key liver genes downregulated in the FoxA triple nulls compared to partially depleted FoxA models (adjusted p value < 0.05, fold change > 1.5). C. Heatmap of 513 induced genes in FoxA triple nulls compared with partially depleted FoxA models (adjusted p value < 0.05, fold change > 1.5). D. Gene expression levels of liver genes upregulated in the FoxA triple nulls compared to partially depleted FoxA models (adjusted p value < 10^{-5}, fold change > 20).

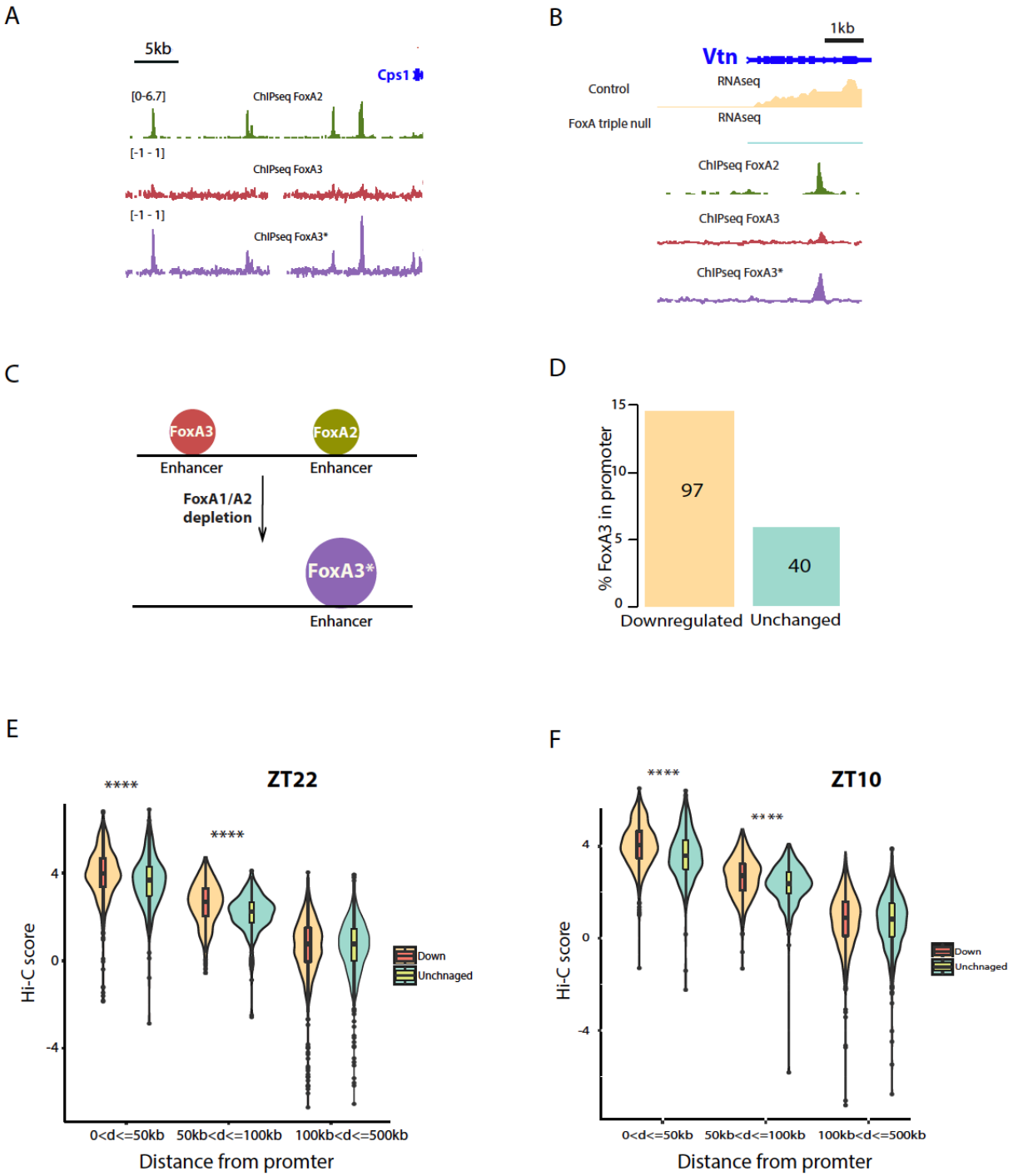

A
Hepatic levels
log2 (nmol/ml)
5
0
-5
Arg
Lactate
Citrate
Asp
GLN
GLY
TAU
ORN
LYS
Control
FoxA triple null
B
Hepatic lipid levels
Fat - log2 (mg/wet weight)
FFA - log2 (ng/mg total fat)
2
0
-2
Fat
FFA
D
Plasma levels
Arg -log2 (nmol/ml)
Urea - log2 (mM)
10
7.5
5
2.5
Urea
Arginine
Control
FoxA triple null
C
Hepatic Acyl carnitine levels
log2 (pmol/mn protein)
10
5
0
-5
Free
C12
C14
C16
linoleoyl
Oleoyl
Stearoyl
Control
FoxA triple null

Supplemental Figure 5. Metabolomics profiling of FoxA triple null. FoxA triple null metabolomics profiling of hepatic amino acids, lipids, and Acyl carnitine (A-C) as well as plasma levels of Urea and Arginine (D). FoxA triple nulls levels are statistically significant different from controls for all the metabolites presented in this figure. $p < 0.05$ Mann-Whitney-Wilcox test.



Supplemental Figure 6. Adult ablation of FoxA factors induces an indirect upregulation of genes associated mainly with the activation of nuclear receptors. A. Only a minority of the upregulated genes in the triple null are repressed during liver development, and an even smaller portion are reactivated in the FoxA triple nulls back to total hepatoblast levels, probably

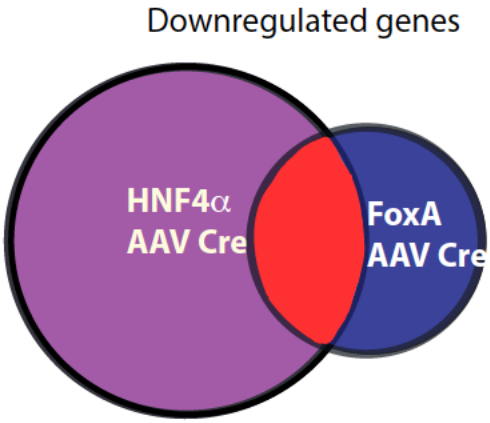
demonstrating an indirect effect of FoxA depletion on the expression of these genes. B. Heatmap of the few upregulated genes whose expression is elevated back to prehepatic stage. C-D. Upstream regulators of developmental and non-developmental upregulated genes in the FoxA triple nulls. Non-developmental genes are mainly characterized by upstream regulators associated with nuclear receptors, and developmental genes are mainly associated with proliferation.



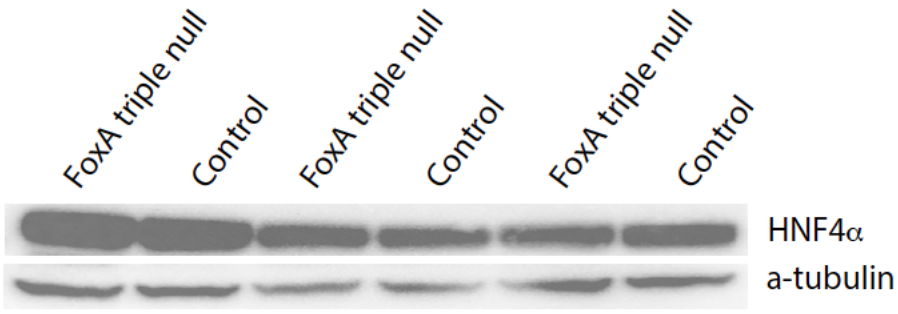
Supplemental Figure 7. FoxA3* binding sites are associated with down-regulated genes in FoxA triple null. A-C. Two examples of enhancer switching of FoxA3 in FoxA1/A2 double mutants and a model showing that FoxA3 doubles its hepatic binding sites in FoxA1/A2 double

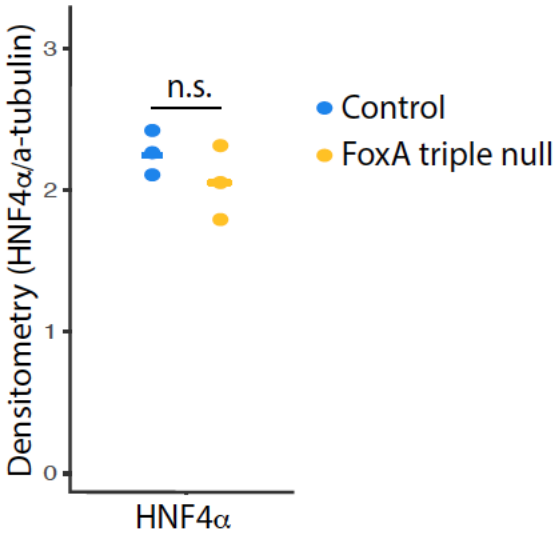
mutants. Most of these sites overlap with FoxA1/A2 binding sites. B. also serves as an example of FoxA3* binding next to Vtn promoter which is differentially expressed. D. Percentage of FoxA3* binding promoters in downregulated genes in FoxA triple null compared with controls and genes that are unchanged in FoxA triple null ($p < 0.0001$, proportional test, numbers in bars represent number of genes). E-F. Violin plots showing higher HiC contacts between FoxA3* binding sites and promoters of downregulated genes in FoxA triple null compared with promoters of unchanged genes. (**** means $p<0.00001$ one sided Wilcoxon test, shown are two different HiC data sets). E and F represent two different circadian time points. Data in E is based on livers isolated at 5 am (ZT22), data in F is based on livers isolated at 5 pm (ZT10). The similar association between FoxA3* binding and gene expression at these two data sets indicates the FoxA3* binding is not affected by circadian changes.

A

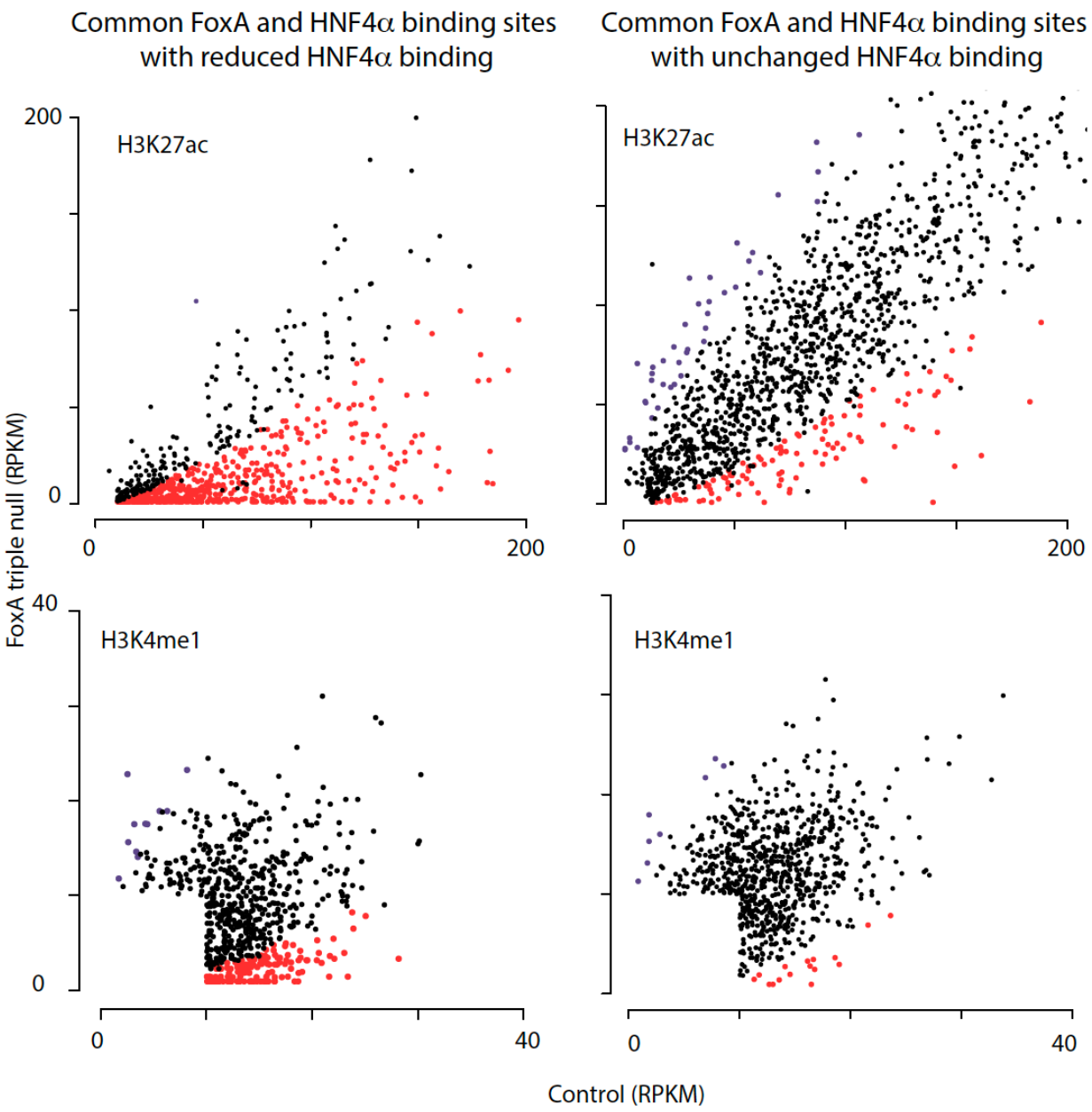

Downregulated genes
HNF4α
AAV Cre
FoxA
AAV Cre

B


FoxA triple null
Control
FoxA triple null
Control
FoxA triple null
Control
HNF4α
a-tubulin

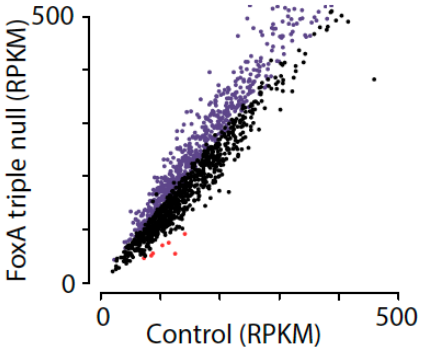

Densitometry (HNF4α/a-tubulin)
3
2
1
0
n.s.
Control
FoxA triple null
HNF4α

Supplemental Figure 8. FoxA triple nulls have no effect on HNF4α protein levels, and many genes are coregulated by FoxA and HNF4α. A. 37% of FoxA triple null repressed genes are also downregulated in the HNF4α deficient liver. B. Western blot showing no change in HNF4α protein levels in FoxA triple null and quantification of the western blot.



Supplemental Figure 9. Co-bound FoxA/HNF4α sites lose enhancer markers in the FoxA triple null. Quantification of H3K27ac and H3K4me1 signal in HNF4α/FoxA3* common sites that show either no loss of HNF4α occupancy or loss of HNF4α binding. Increased or decreased H3K27ac or H3K4me1 is marked in purple or red, respectively. FDR< 0.05. Each group contains 2 samples.

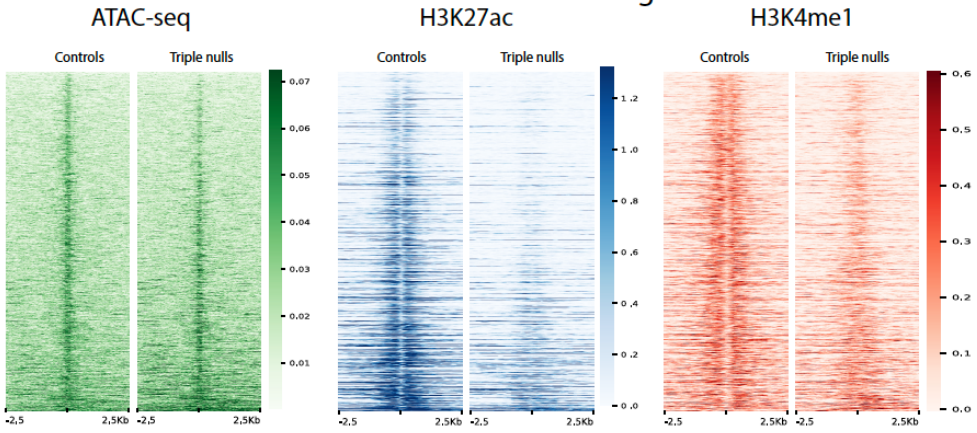
A



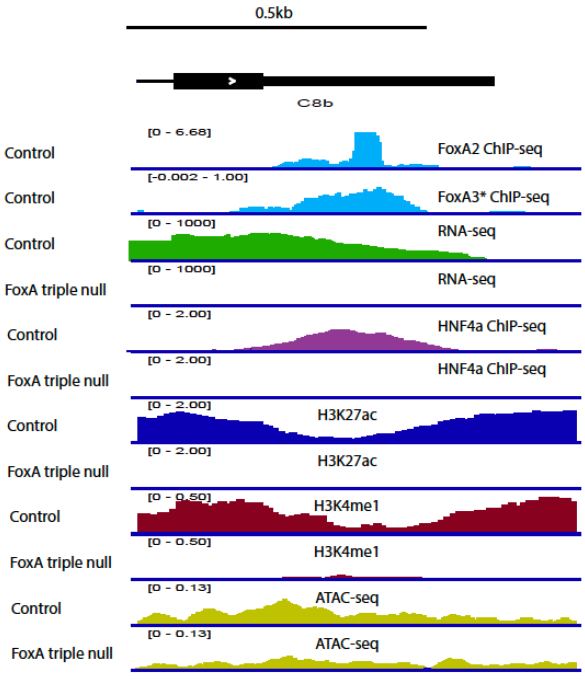
Atac-seq on common FoxA and HNF4α binding sites with unchanged HNF4α binding

B

Common FoxA and HNF4α binding sites with reduced HNF4α binding



C



Supplemental Figure 10. Lower accessibility at HNF4α/FoxA co-bound sites with reduced HNF4α binding. A. Scatter plot of ATAC-seq RPKM values in FoxA triple null livers compared to controls in co-bound HNF4α/FoxA sites that show no loss of HNF4α binding. Decreased accessibility sites are indicated as red dots and increased with purple. FDR< 0.05. Each group contains 3 samples. B. Heatmap of ATAC-seq, H3K27ac and H3K4me1 signal in HNF4α/FoxA common binding sites with loss of HNF4α occupancy, ranked according to the ATAC difference between controls and triple nulls (n=3, for controls or mutants). C. HNF4α/FoxA common site next to the S8 gene in which loss of FoxA binding results in down-regulation of gene expression, loss of HNF4α binding, H3K27ac and H4K4me1 signals and reduced accessibility.

A

Motif analysis on FoxA3* sites with lower or unchanged accessibility in FoxA triple null

Lower accessibility in FoxA triple null

TF	P-value	% traget	% background
FoxA3	1e-224	64.72%	7.3%
HNF6	1e-26	27.77%	10.6%
Cux2	1e-18	21.5%	8.17%
CEBP	1e-11	24.4%	12.6%
HNF4a	1e-7	17.33%	9.34%
RAR	1e-7	62.42%	50.09%
Erra	1e-5	47.39%	37.58%

Unchanged accessibility in FoxA triple null

TF	P-value	% traget	% background
FoxA3	1e-194	61.49%	7.27%
HNF6	1e-24	28.67%	11.0%
Cux2	1e-16	22.1%	8.97%
RARa	1e-12	65.86%	49.26%
HNF4a	1e-11	19.69%	8.93%
CEBP	1e-10	24.29%	12.94%
PPARa	1e-8	30.63%	19.18%

B

Motif analysis on HNF4α/FoxA common site with reduced HNF4α binding and loss of H3K27ac mark in FoxA triple null with or without reduced accessibility

Reduced accessibility

TF	P-value	% traget	% background
FoxA3	1e-63	54.95%	7.23%
HNF4a	1e-6	22.53%	9.53%
NF1	1e-3	13.74%	6.22%
CEBP	1e-3	20.88%	12.26%
Cux1	1e-3	20.8%	12.37%
Erra	1e-3	49.45%	37.92%

Unchanged accessibility

TF	P-value	% traget	% background
FoxA3	1e-57	51.63%	7.11%
HNF4a	1e-11	26.63%	9.28%
PPARa	1e-8	38.59%	19.87%
HNF6	1e-6	22.28%	9.92%
Cux2	1e-5	17.93%	7.96%
RXR	1e-5	33.7%	19.99%

Supplemental Figure 11. Motif analysis in sites with reduced/unchanged accessibility suggest for additional transcription factor binding motifs that are enriched at FoxA regulated enhancers. A-B. Motif analysis using Homer on FoxA3* sites with or without reduced accessibility in the triple nulls (A) or on common HNF4α/FoxA sites that show loss of HNF4α

binding and reduced H3K27ac mark with or without reduced accessibility in the triple nulls (B). Chosen are top liver-specific transcription factors motifs.

Supplemental references

Kaestner KH, Hiemisch H, Schutz G. 1998. Targeted disruption of the gene encoding hepatocyte nuclear factor 3gamma results in reduced transcription of hepatocyte-specific genes. *Mol Cell Biol* **18**: 4245-4251.

Shen W, Scearce LM, Brestelli JE, Sund NJ, Kaestner KH. 2001. Foxa3 (hepatocyte nuclear factor 3gamma) is required for the regulation of hepatic GLUT2 expression and the maintenance of glucose homeostasis during a prolonged fast. *J Biol Chem* **276**: 42812-42817.