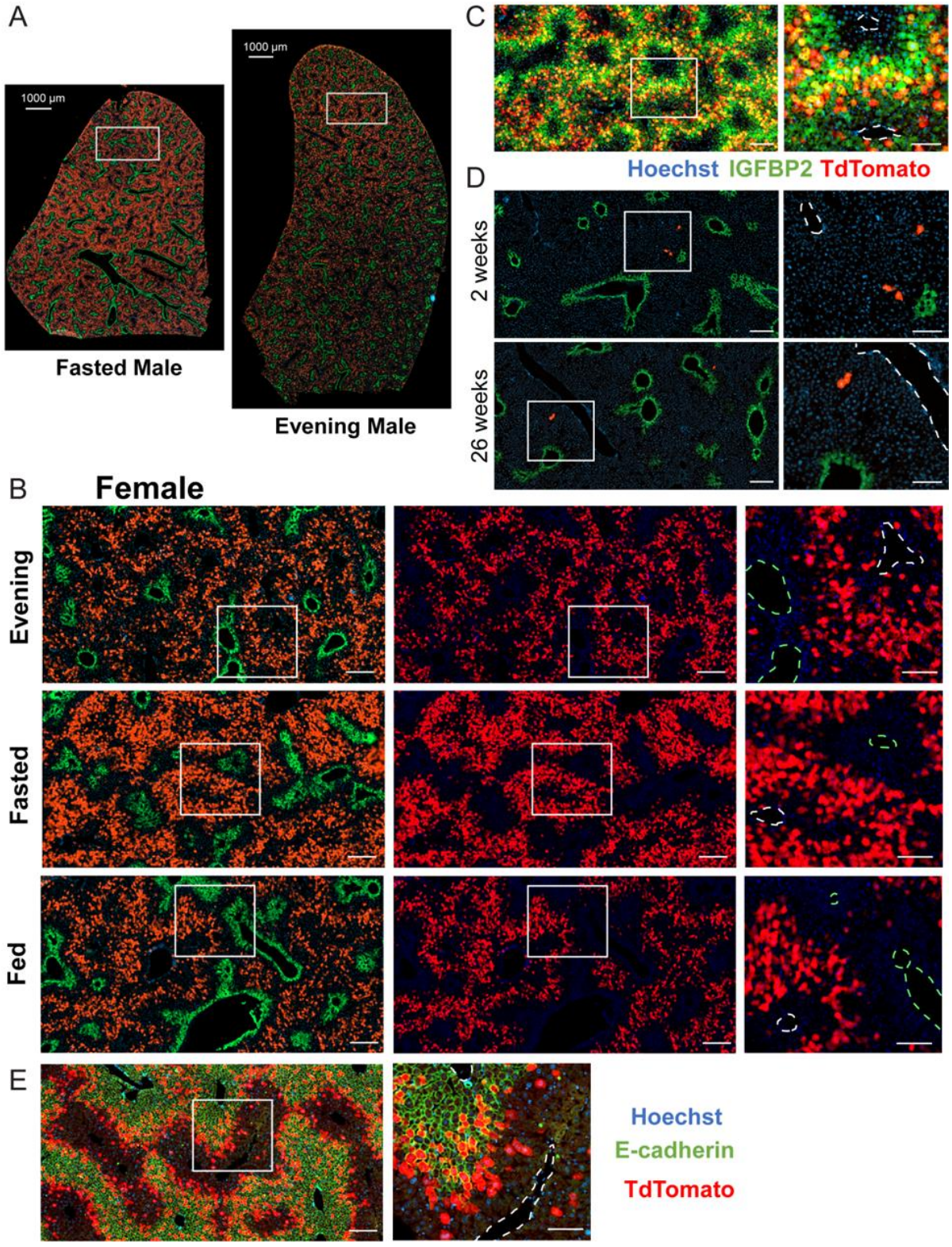
Figure S1.


A
1000 µm
1000 µm
Fasted Male
Evening Male
C
Hoechst IGFBP2 TdTomato
D
2 weeks
26 weeks
B
Female
Evening
Fasted
Fed
E
Hoechst
E-cadherin
TdTomato

Supplemental Figure S1. The *Igfbp2-CreER* strain labels midlobular zone 2 hepatocytes after tamoxifen. Data related to Figure 1.

A. Whole-section images of fed tamoxifen and evening tamoxifen males from **Figure 1C**. Scale bar = 1000 μm for cross-sectional images. Slides were stained for GS (green).

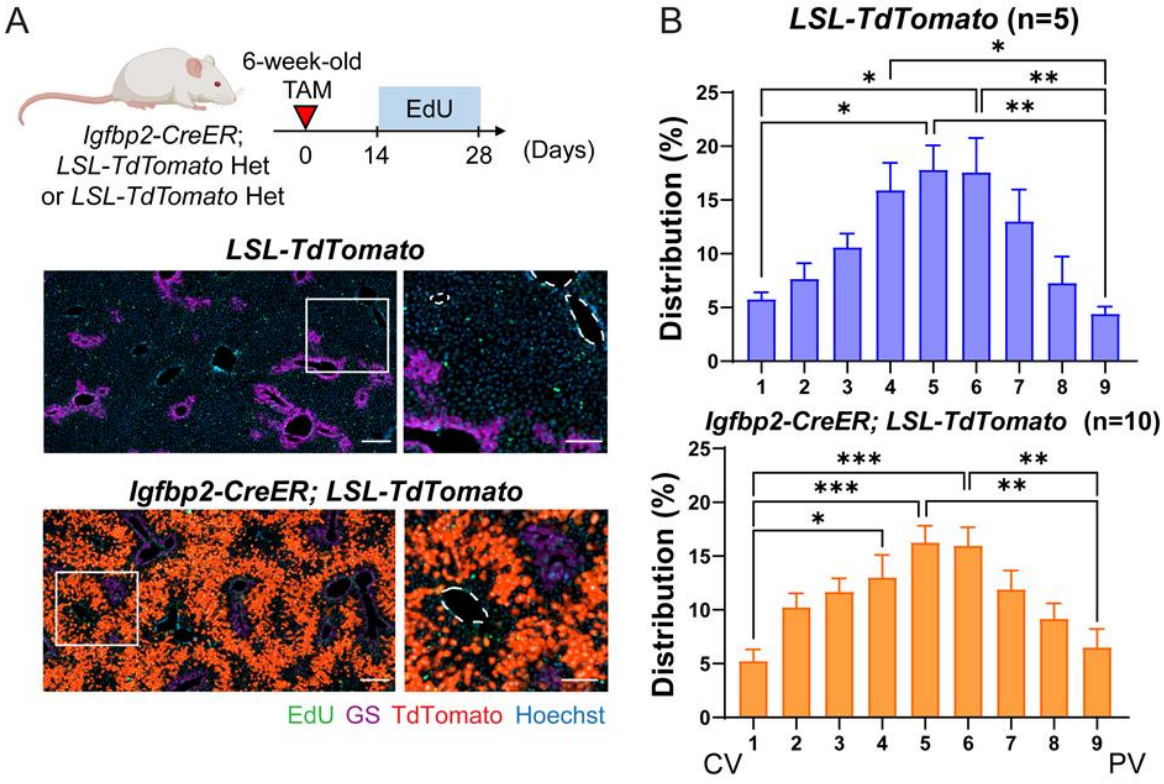
B. Representative images of female *Igfbp2-CreER* mice labeled using three distinct tamoxifen approaches. Scale bar = 200 μm for cropped images and 100 μm for magnified images. Slides were stained for GS (green). The green dashed circles represent CVs (marked by GS), and the white dashed circles represent PVs.

C. Representative images of *Igfbp2-CreER* labeled zone 2 cells (red) co-stained with IGFBP2 (green). Scale bar = 200 μm for cropped images and 100 μm for magnified images. The dashed circles represent veins.

D. Representative images of *Igfbp2-CreER; LSL-tdTomato* livers 2 and 26 weeks after oil administration without tamoxifen. Scale bar = 200 μm for cropped images and 100 μm for magnified images. Slides were stained for GS (green). The white dashed circles represent PVs.

E. Representative images of *Igfbp2-CreER* labeled zone 2 cells co-stained with E-cadherin, a marker of periportal hepatocytes (green). Scale bar = 200 μm for cropped images and 100 μm for magnified images. The dashed circles represent veins.

Figure S2.



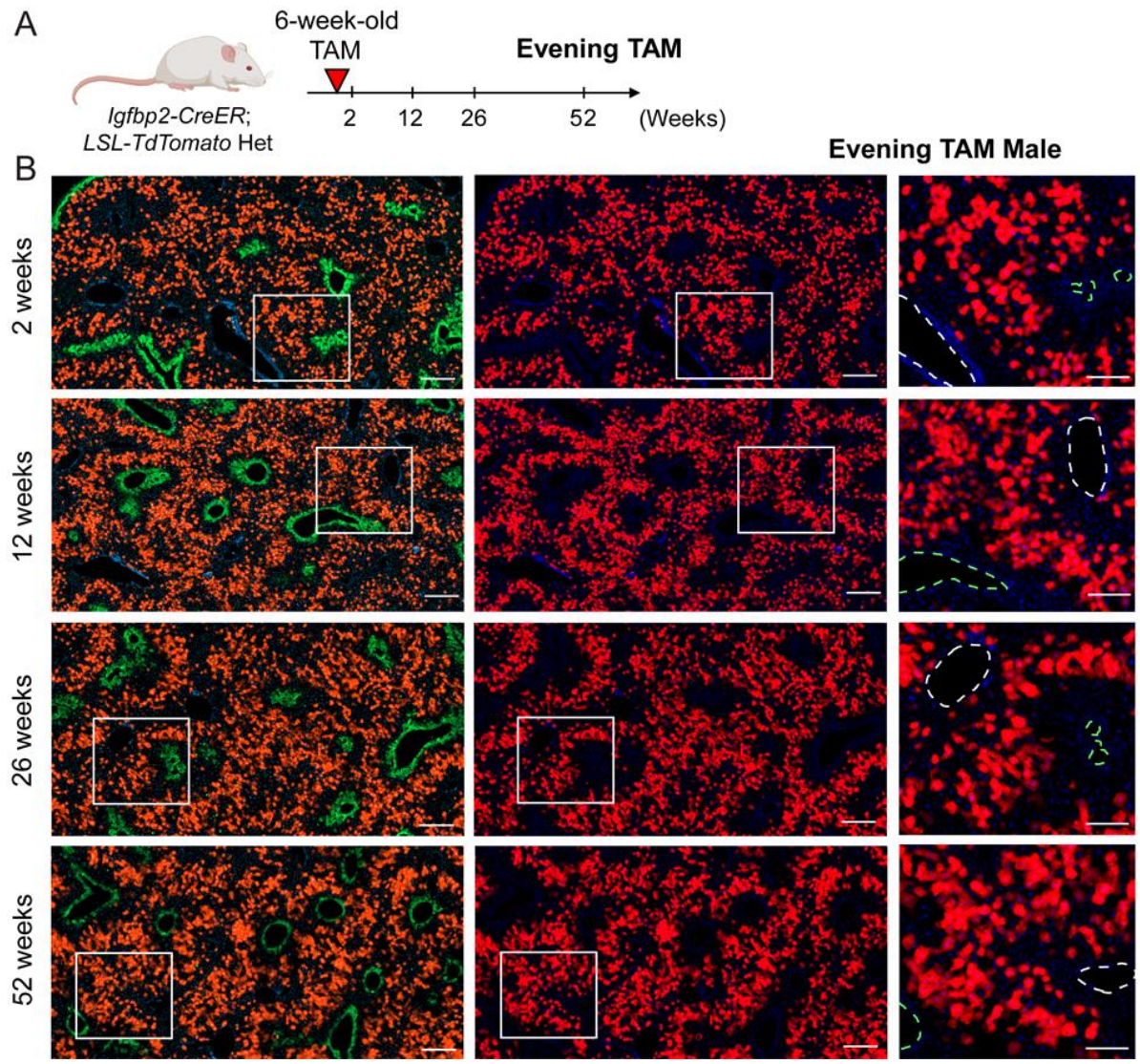
Supplemental Figure S2. The *Igfbp2-CreER* transgene does not alter the distribution of zonal proliferation when compared to WT littermates. Data related to Figure 1.

A. Schema and representative images of *LSL-tdTomato* and *Igfbp2-CreER; LSL-tdTomato* livers after fasting tamoxifen followed by 14 days of EdU incorporation. Scale bar = 200 μm for cropped images and 100 μm for magnified images. Slides were stained for EdU (green) and GS (purple). The white dashed circles represent PVs.

B. Quantification of spatial distribution of EdU+ cells from **A**.

All data in this figure are presented as mean ± SEM. Significance is displayed as $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$(***), and $p < 0.0001$ (****).

Figure S3.

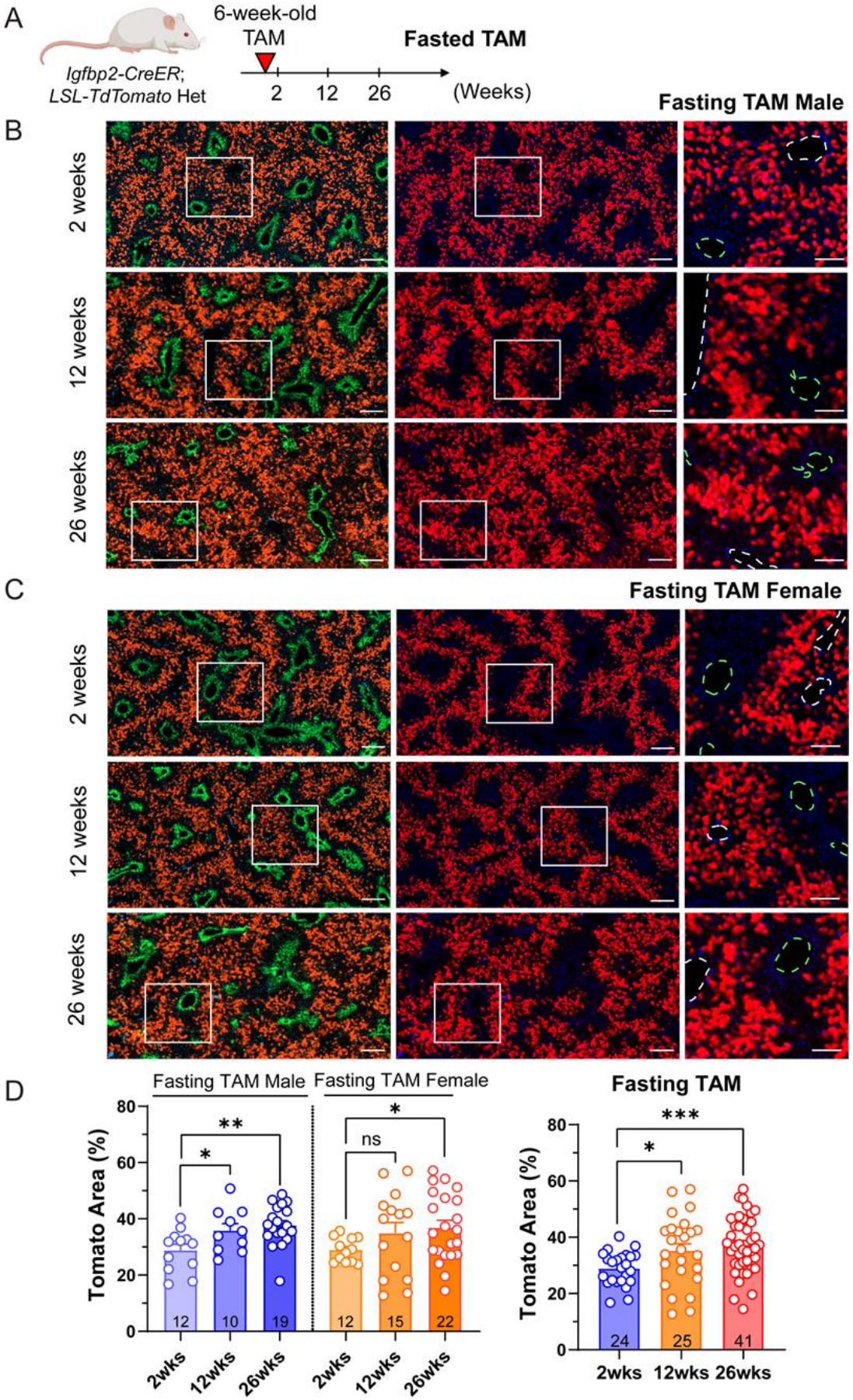


Supplemental Figure S3. Zone 2 cells labeled by the evening tamoxifen approach expand during homeostasis in female mice. Data related to Figure 2.

A. Schema of evening tamoxifen lineage tracing experiment under normal homeostasis.

B. Representative images of male evening tamoxifen tracing over 2, 12, 26 and 52 weeks. Scale bar = 200 μm for cropped images and 100 μm for magnified images. Slides were stained for GS (green). The green dashed circles represent CVs (marked by GS), and the white dashed circles represent PVs.

Figure S4.


A
6-week-old
TAM
Fasted TAM
Igfbp2-CreER;
LSL-TdTomato Het
2
12
26
(Weeks)
B
Fasting TAM Male
2 weeks
12 weeks
26 weeks
C
Fasting TAM Female
2 weeks
12 weeks
26 weeks
D
Fasting TAM Male
Fasting TAM Female
Tomato Area (%)
**
*
*
ns
12
10
19
12
15
22
2wks
12wks
26wks
2wks
12wks
26wks
Fasting TAM
***
*
Tomato Area (%)
24
25
41
2wks
12wks
26wks

Supplemental Figure S4. Zone 2 cells expand during homeostasis. Data related to Figures 2.

A. Schema of fasting tamoxifen lineage tracing experiment under normal homeostasis.

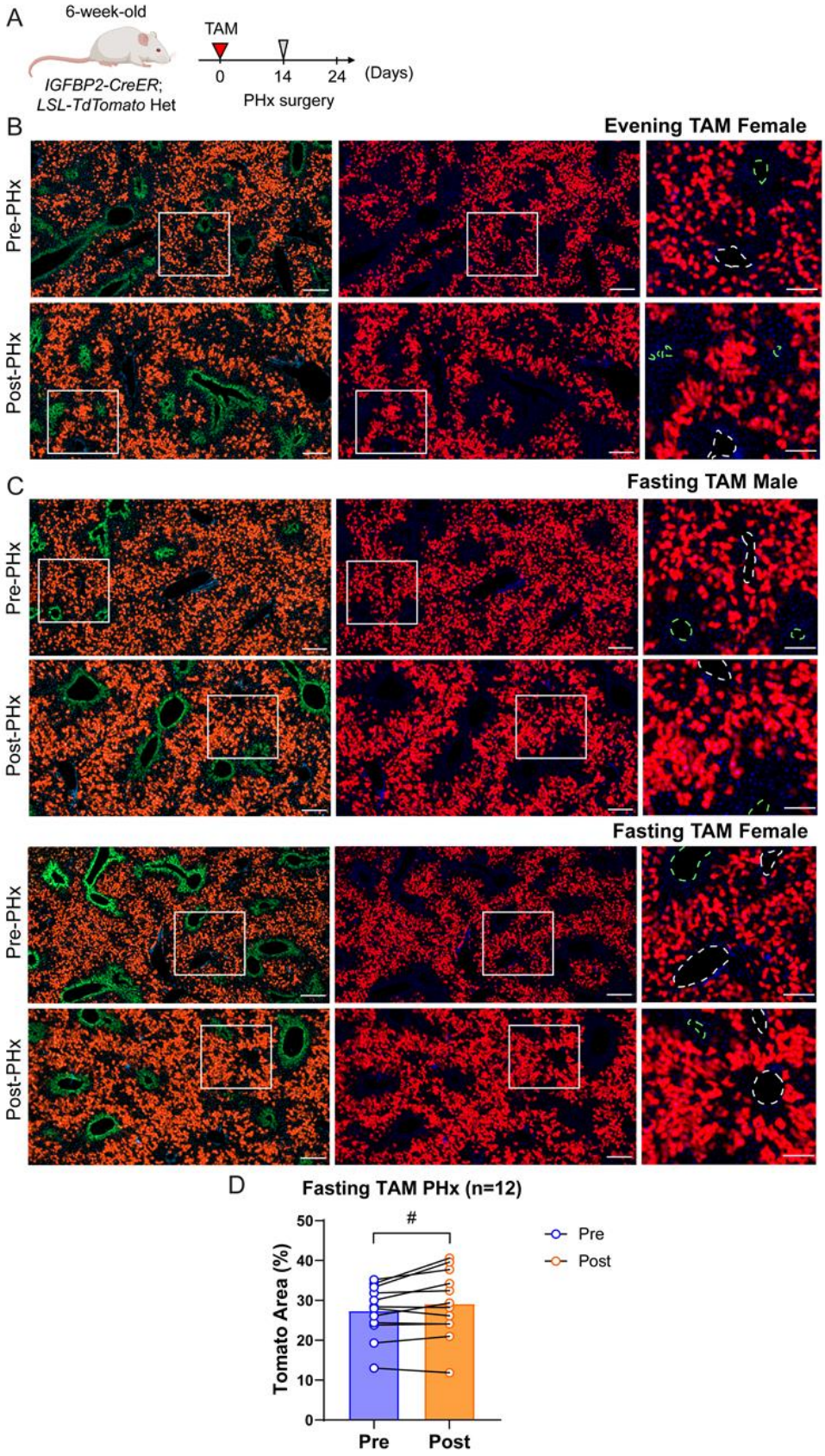
B. Representative images of male fasting tamoxifen tracing over 2, 12 and 26 weeks. Scale bar = 200 μm for cropped images and 100 μm for magnified images. Slides were stained for GS (green). The green dashed circles represent CVs (marked by GS), and the white dashed circles represent PVs.

C. Representative images of female fasting tamoxifen tracing over 2, 12 and 26 weeks.

D. Quantification of the Tomato area from **B** and **C**. The right panel combines all of the data from the two sexes shown in the left panel. The 2-week data points are the same data as the fasting timepoints in **Figure 1E** (n = 24, 25, 41 mice for 2, 12, 26 weeks).

All data in this figure are presented as mean ± SEM. Significance is displayed as $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$(***), and $p < 0.0001$ (****).

Figure S5.


A
6-week-old
IGFBP2-CreER;
LSL-TdTomato Het
TAM
0
14
24
(Days)
PHx surgery
B
Evening TAM Female
Pre-PHx
Post-PHx
C
Fasting TAM Male
Pre-PHx
Post-PHx
Fasting TAM Female
Pre-PHx
Post-PHx
D
Fasting TAM PHx (n=12)
#
Pre
Post
Tomato Area (%)
50
40
30
20
10
0
Pre
Post

Supplemental Figure S5. Zone 2 hepatocytes labeled in *Igfbp2-CreER* mice expand after PHx. Data related to Figure 4.

A. Schema of the *Igfbp2-CreER* lineage tracing experiment in the context of PHx.

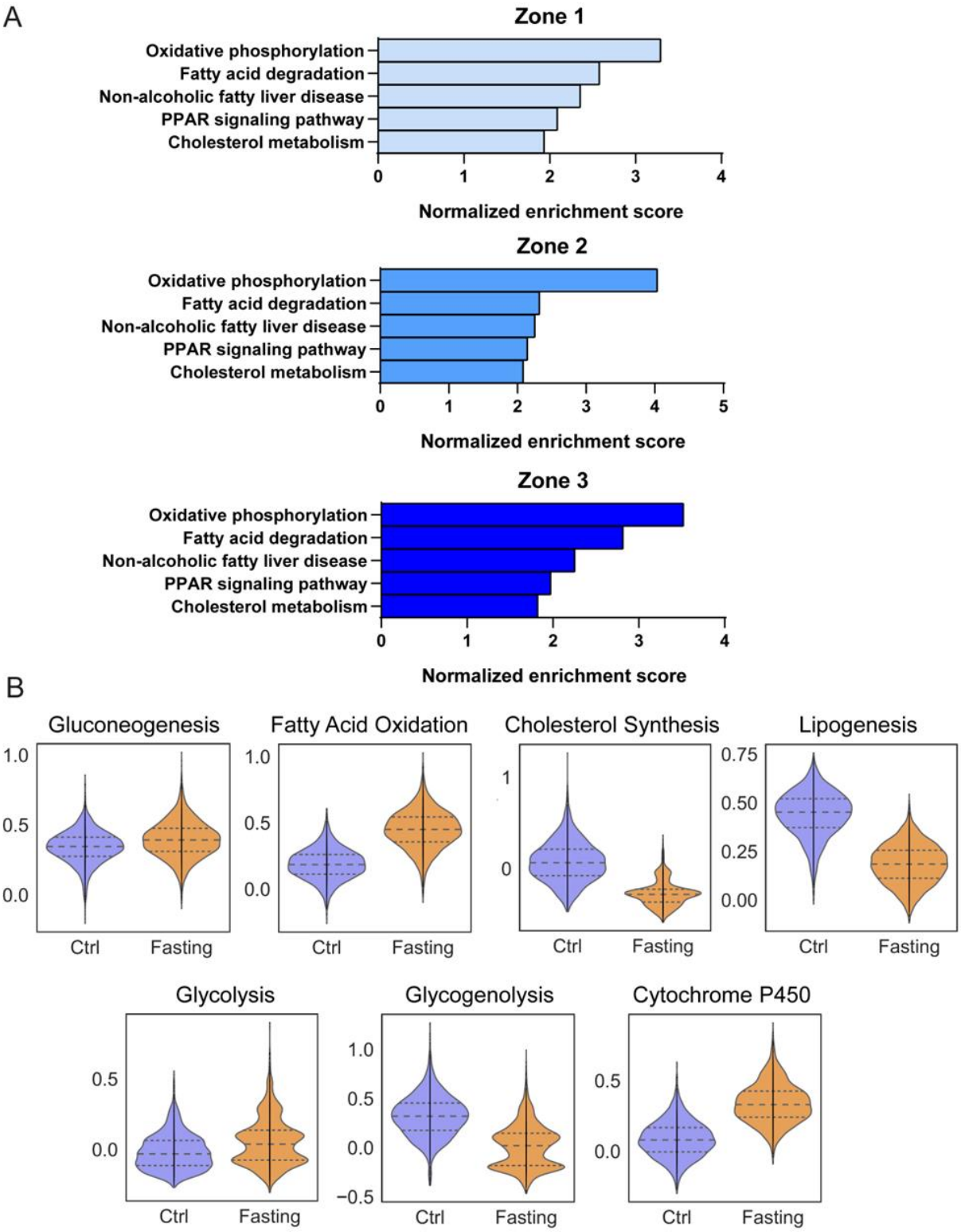
B. Representative images of evening tamoxifen labeled females, pre- and post-PHx. Scale bar = 200 μm for cropped images and 100 μm for magnified images. Slides were stained for GS (green). The green dashed circles represent CVs (marked by GS), and the white dashed circles represent PVs.

C. Representative images of fasting tamoxifen pre- and post-PHx.

D. Quantification of the Tomato area from **C**. Significance was assessed by paired t-tests (n = 12 mice).

All data in this figure are presented as mean ± SEM. The statistical significance is displayed as # ($p < 0.05$) in the paired analysis.

Figure S6.


A
Zone 1
Oxidative phosphorylation
Fatty acid degradation
Non-alcoholic fatty liver disease
PPAR signaling pathway
Cholesterol metabolism
0
1
2
3
4
Normalized enrichment score
Zone 2
Oxidative phosphorylation
Fatty acid degradation
Non-alcoholic fatty liver disease
PPAR signaling pathway
Cholesterol metabolism
0
1
2
3
4
5
Normalized enrichment score
Zone 3
Oxidative phosphorylation
Fatty acid degradation
Non-alcoholic fatty liver disease
PPAR signaling pathway
Cholesterol metabolism
0
1
2
3
4
Normalized enrichment score
B
Gluconeogenesis
Fatty Acid Oxidation
Cholesterol Synthesis
Lipogenesis
Glycolysis
Glycogenolysis
Cytochrome P450
Ctrl
Fasting

Supplemental Figure S6. Metabolic pathways change in the fasted liver. Data related to Figure 6.

A. KEGG pathway enrichment analysis of differentially expressed genes in each zonal population.

B. Metabolic pathway analysis of the snRNA-seq dataset grouped by normal and fasting conditions.