Supplementary Information Appendix

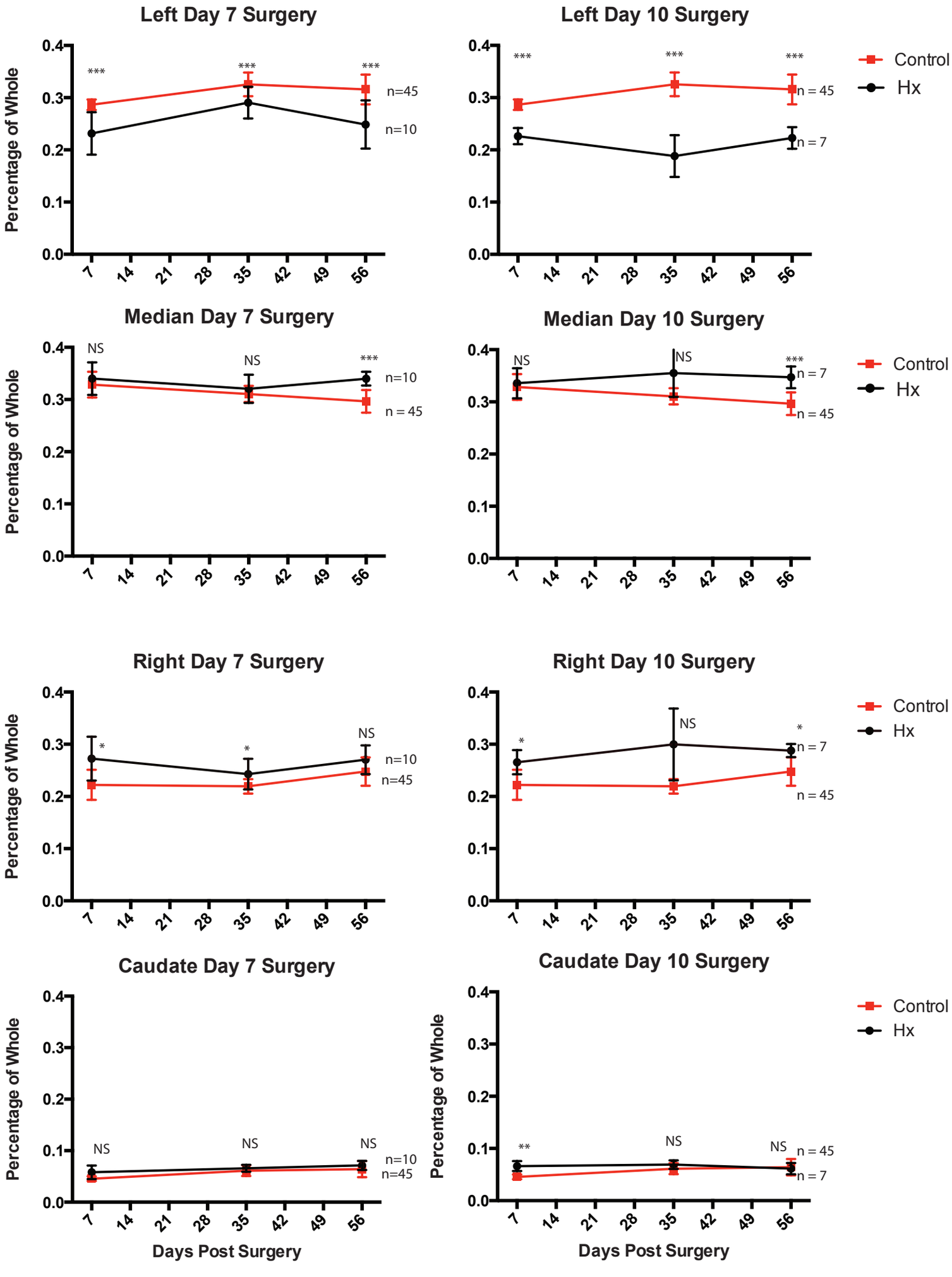
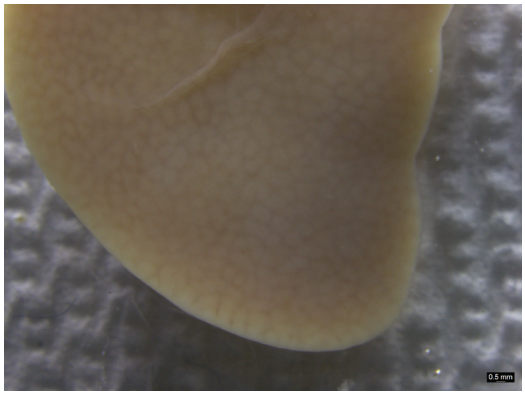

Left Day 7 Surgery
Left Day 10 Surgery
Median Day 7 Surgery
Median Day 10 Surgery
Right Day 7 Surgery
Right Day 10 Surgery
Caudate Day 7 Surgery
Caudate Day 10 Surgery
Percentage of Whole
Days Post Surgery
Control
Hx
n=45
n=10
n = 45
n = 7
***
**
*
NS
0.0
0.1
0.2
0.3
0.4
7
14
21
28
35
42
49
56

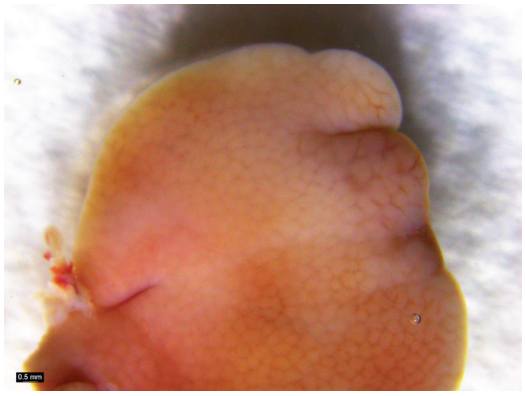
Fig S1 Quantitative regeneration of the left lobe following partial lobular hepatectomy of P7 and P10 mice.

Relative masses of all lobes are presented as percentage of whole liver mass after partial lobular hepatectomy of the left lobe at P7 (A) or P10 (B) after fixation plotted against recovery days post-surgery. Masses of lobes are presented as percentage of whole liver mass after fixation plotted against recovery days post-surgery. The red line indicates uninjured controls. Values are presented as means ± SEM; *$p<0.05$, **$p<0.005$, ***$p<0.0005$; NS not significant.

S0D56



S7D56



S14D56

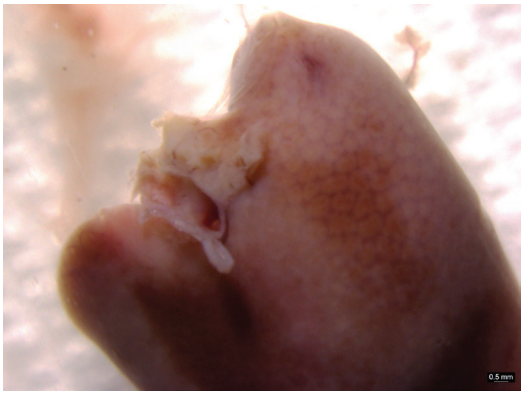


Fig S2 Whole mount representation of regenerated left lobes.

Whole mount images of tips of regenerated left lobes injured at D0, D7, and D14 allowed 56 days recovery are shown. Scale bars are 0.5mm.

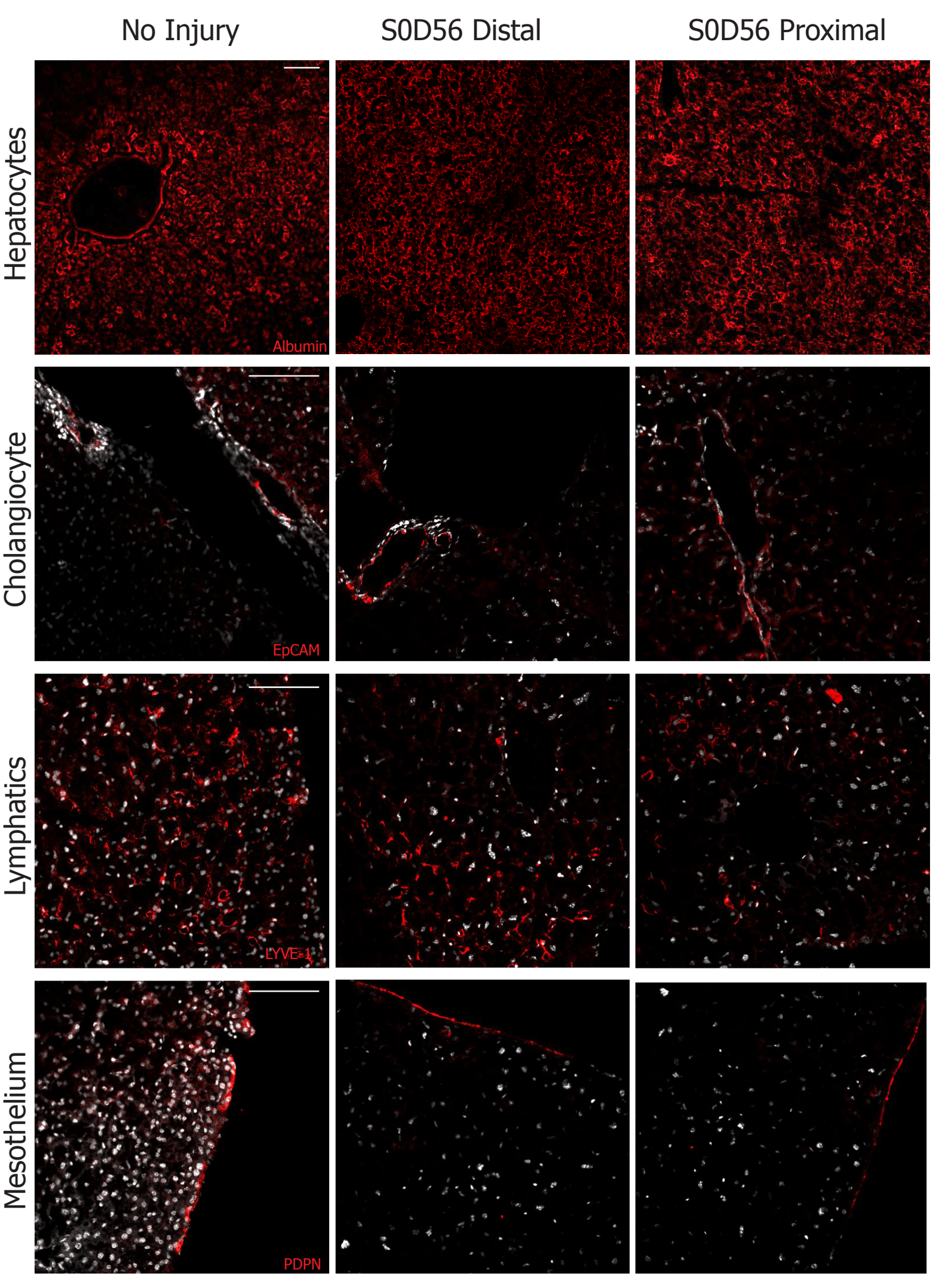
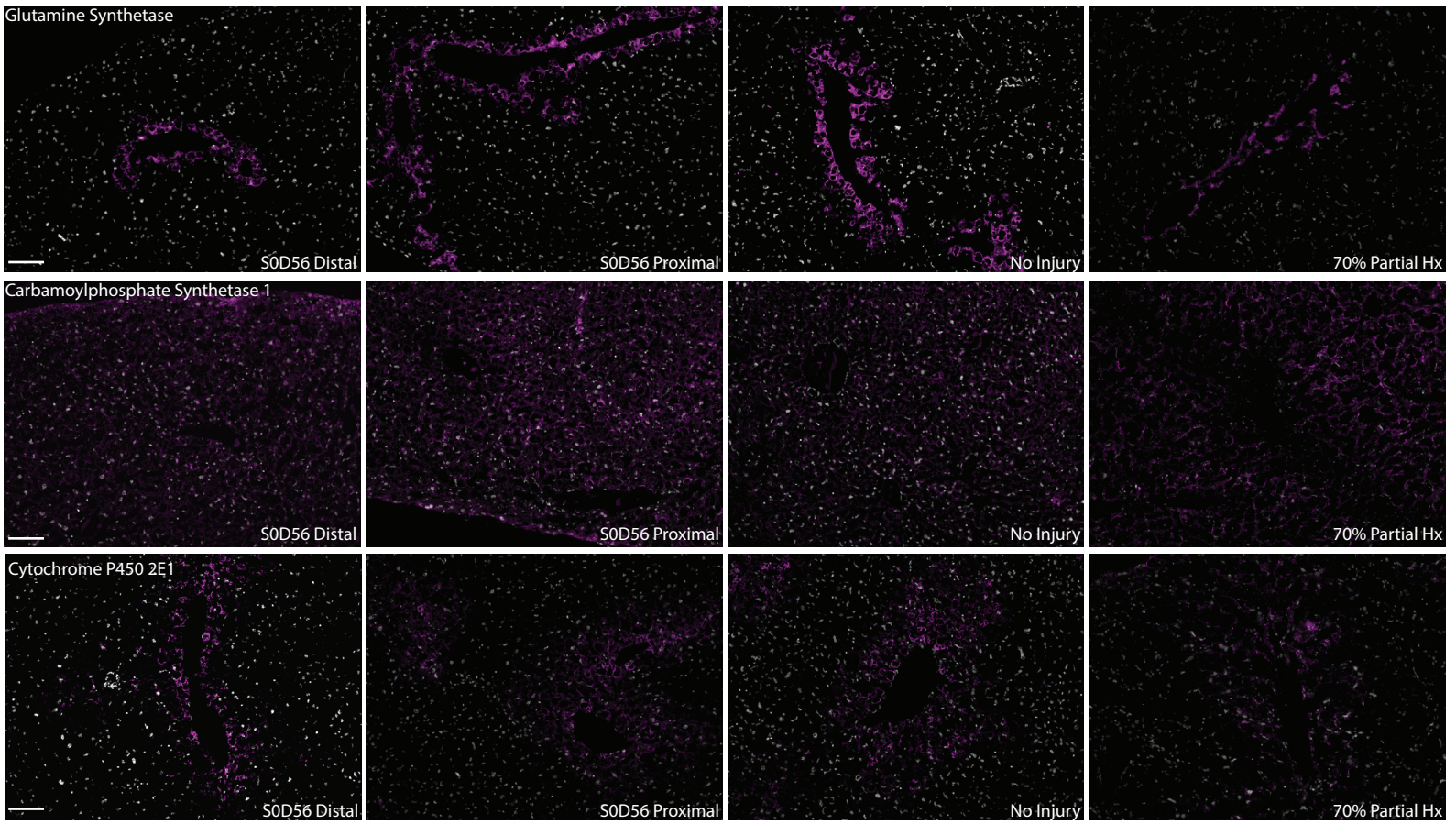

No Injury
S0D56 Distal
S0D56 Proximal
Hepatocytes
Albumin
Cholangiocyte
EpCAM
Lymphatics
LYVE-1
Mesothelium
PDPN

Fig S3 The neonatal mouse liver regenerates hepatocyte and cholangiocyte lineages. Amputated P0 mice allowed to recover 56 days post partial hepatectomy were stained for (**A**) albumin (**B**) EpCAM, (**C**) Lyve-1 and (**D**) podoplanin, to show regeneration of the major lineages in the liver. Sections distal and proximal to the injured area were compared to uninjured livers and adult livers undergoing adult 70% partial hepatectomy. All scale bars are 100um.

A


Glutamine Synthetase
S0D56 Distal
S0D56 Proximal
No Injury
70% Partial Hx
Carbamoylphosphate Synthetase 1
S0D56 Distal
S0D56 Proximal
No Injury
70% Partial Hx
Cytochrome P450 2E1
S0D56 Distal
S0D56 Proximal
No Injury
70% Partial Hx

B

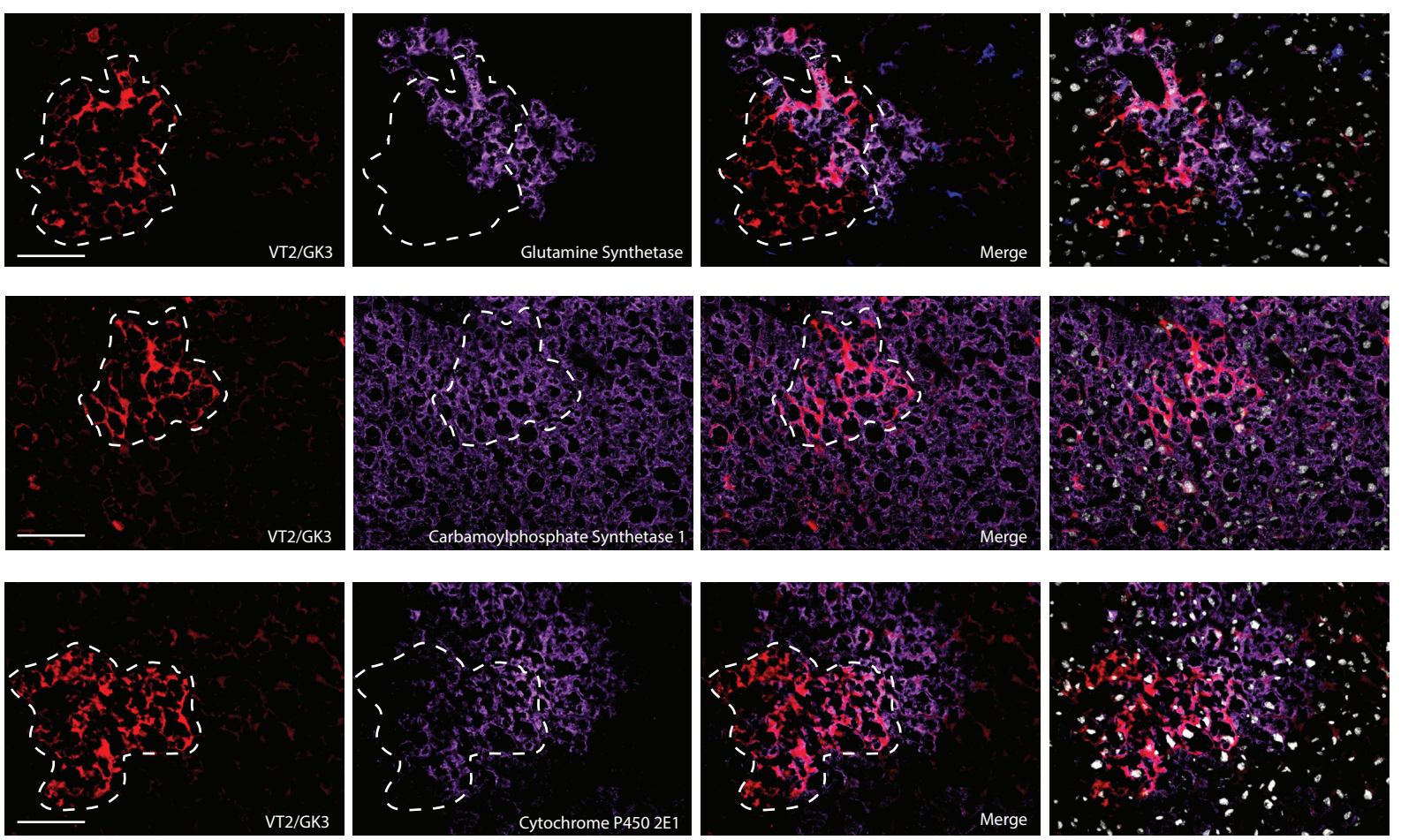
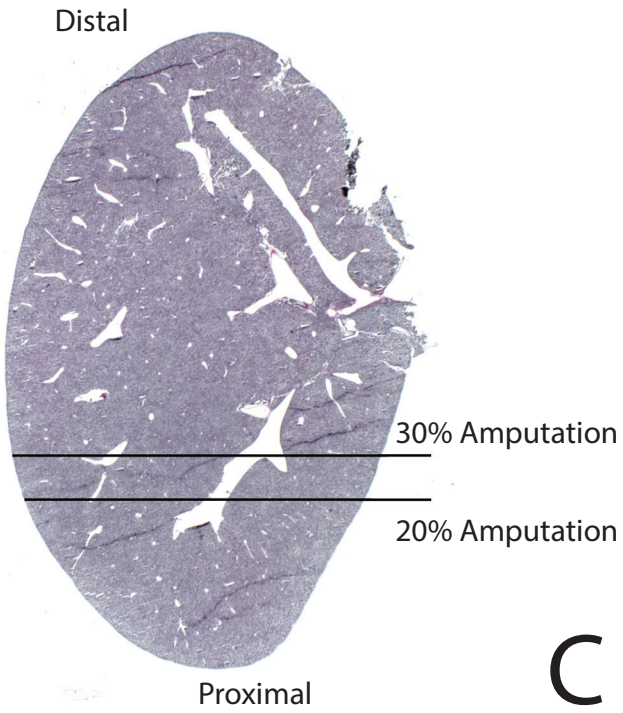

VT2/GK3
Glutamine Synthetase
Merge
VT2/GK3
Carbamoylphosphate Synthetase 1
Merge
VT2/GK3
Cytochrome P450 2E1
Merge

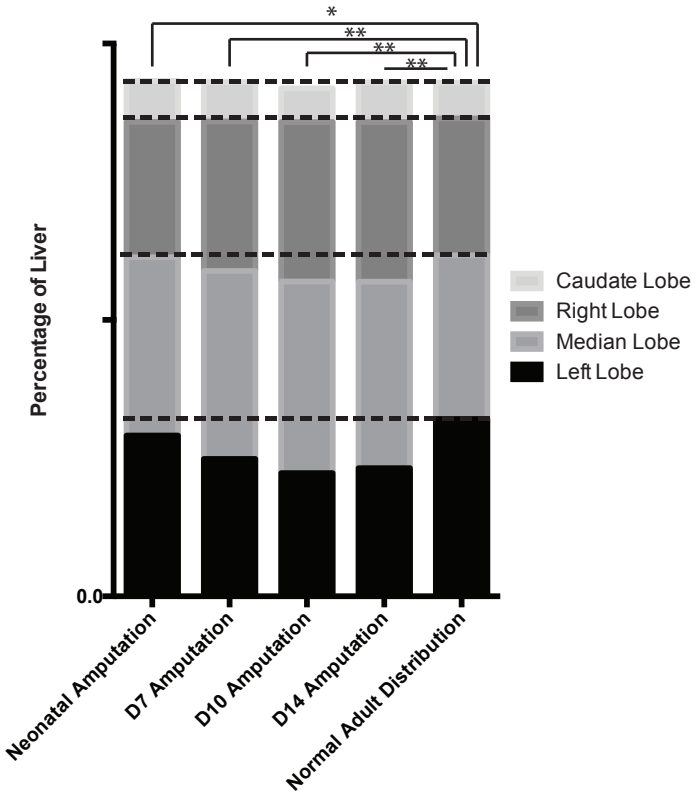
Fig S4 Liver function is normal 35 and 56 days post partial lobular hepatectomy.

(**A**) Recovered lobes from S0D56 mice were sectioned and stained for the key liver enyzmes glutamine synthetase, carbamoylphosphate synthethase 1, and cytochrome P450 2E1. (**B**) Recovered lobes from S0D56 *Actin*^{CreER}; *R26*$^{VT2/GK3}$ were stained for glutamine synthetase, carbamoylphosphate synthetase 1, and cytochrome P450 2E1. Clones in the regenerating lobe were analyzed in conjunction with functional stains. All scale bars are 100um.

A


Distal
30% Amputation
20% Amputation
Proximal

B


*
**
**
**
Percentage of Liver
0.0
Caudate Lobe
Right Lobe
Median Lobe
Left Lobe
Neonatal Amputation
D7 Amputation
D10 Amputation
D14 Amputation
Normal Adult Distribution

C

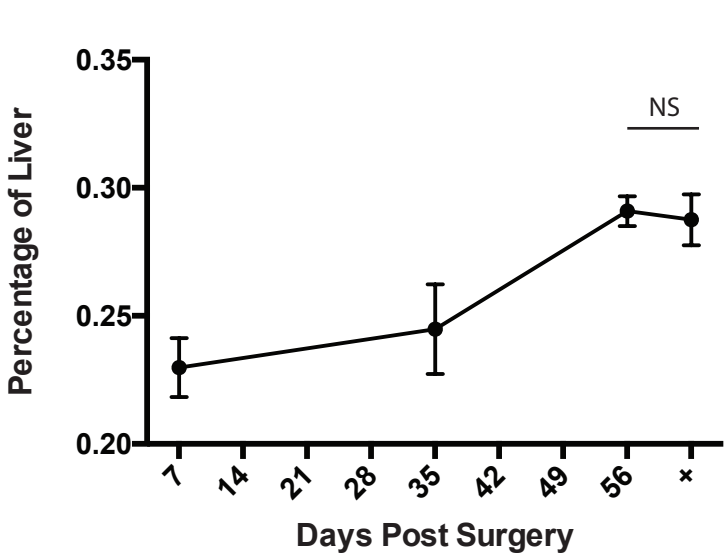
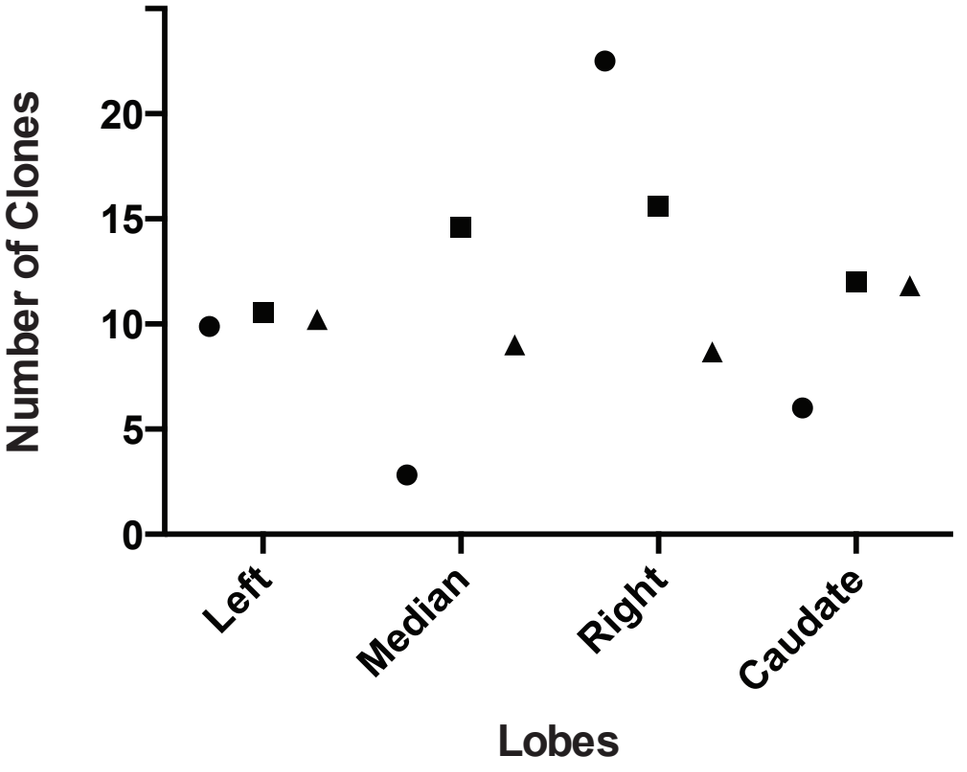

0.35
0.30
0.25
0.20
Percentage of Liver
NS
7
14
21
28
35
42
49
56
+
Days Post Surgery

Fig S5 Lobular contributions to the whole liver after partial lobular hepatectomy

(**A**) Schematic of regenerated left lobe indicating approximate area of amputation (**B**) Percentages of whole liver mass attributed to the left, median, right, and caudate lobes from mice undergoing partial hepatectomy at day 0, 7, 10, and 14 and allowed to recover for 56 days are shown. Masses of lobes are presented as percentage of whole liver mass after fixation. Values are presented as means ± SEM; *p<0.0005, **p<0.0001, NS not significant. (**C**) Relative masses of all lobes are presented as percentage of whole liver mass after partial lobular hepatectomy at P0 after fixation allowed to recover over 56 days plotted against recovery days post-surgery. Values are presented as means ± SEM; *p<0.05, **p<0.005, ***p<0.0005; NS not significant; n = 7 mice allowed to recover over 56 days.

Average Clones Per Field (S0D56)



Average Clones Per Field Control (D56)

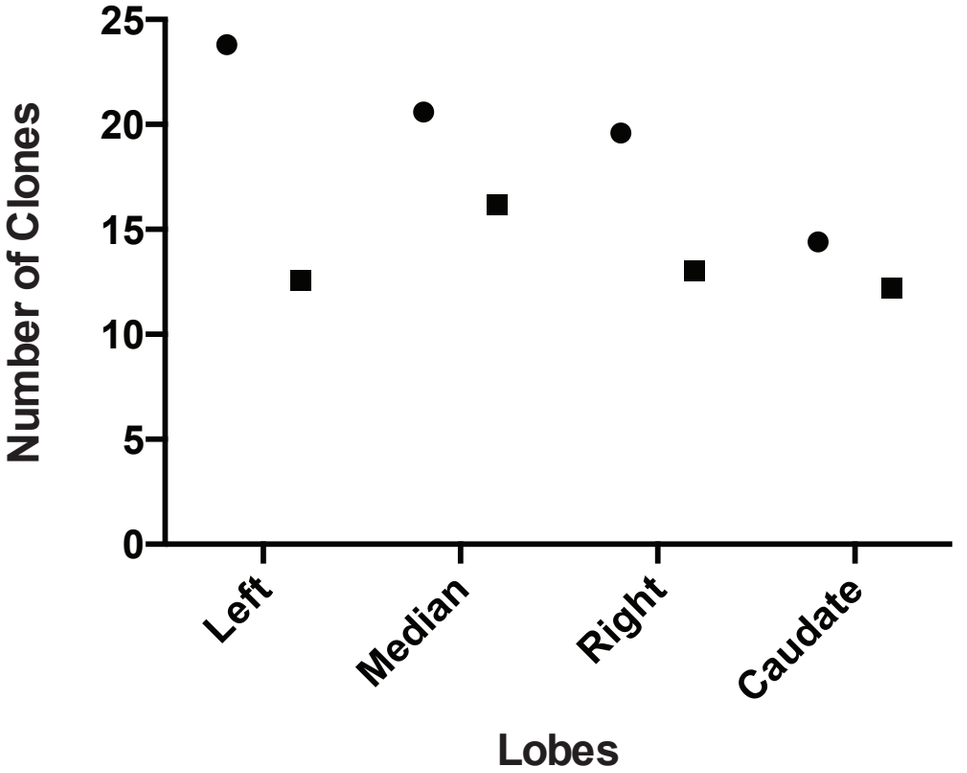
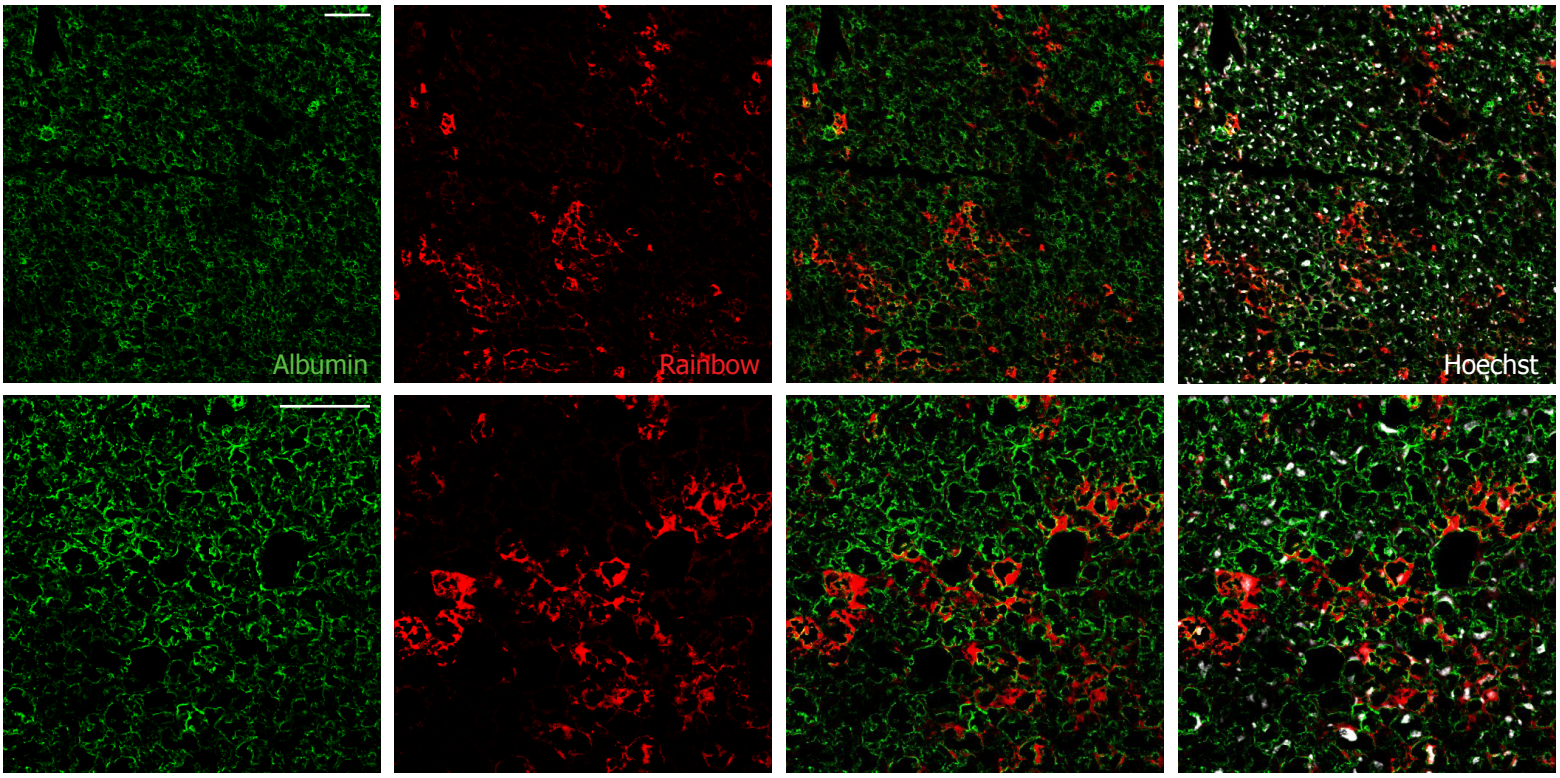


Fig S6 The average number of clones per field per lobe

Average number of clones per field per lobe in mice 56 days post surgery or age matched controls were counted. Number of clones between each lobe of S0, D56 mice were all not significant (NS): Left vs Median (NS; p = 0.7011), Left vs Right (NS; p = 0.25), Left vs Caudate (NS; p = 0.8981), Median vs Right (NS; p=0.2658), Median vs Caudate (NS; p = 0.7873), Right vs Caudate (NS; p = 0.2738). Number of clones between each lobe of D56 control mice were all not significant (NS): Left vs Median (NS; p = 0.9765), Left vs Right (NS; p = 0.7976), Left vs Caudate (NS; p = 0.481), Median vs Right (NS; p=0.6494), Median vs Caudate (NS; p = 0.1739), Right vs Caudate (NS; p = 0.4793).

Hepatocytes



Cholangiocyte

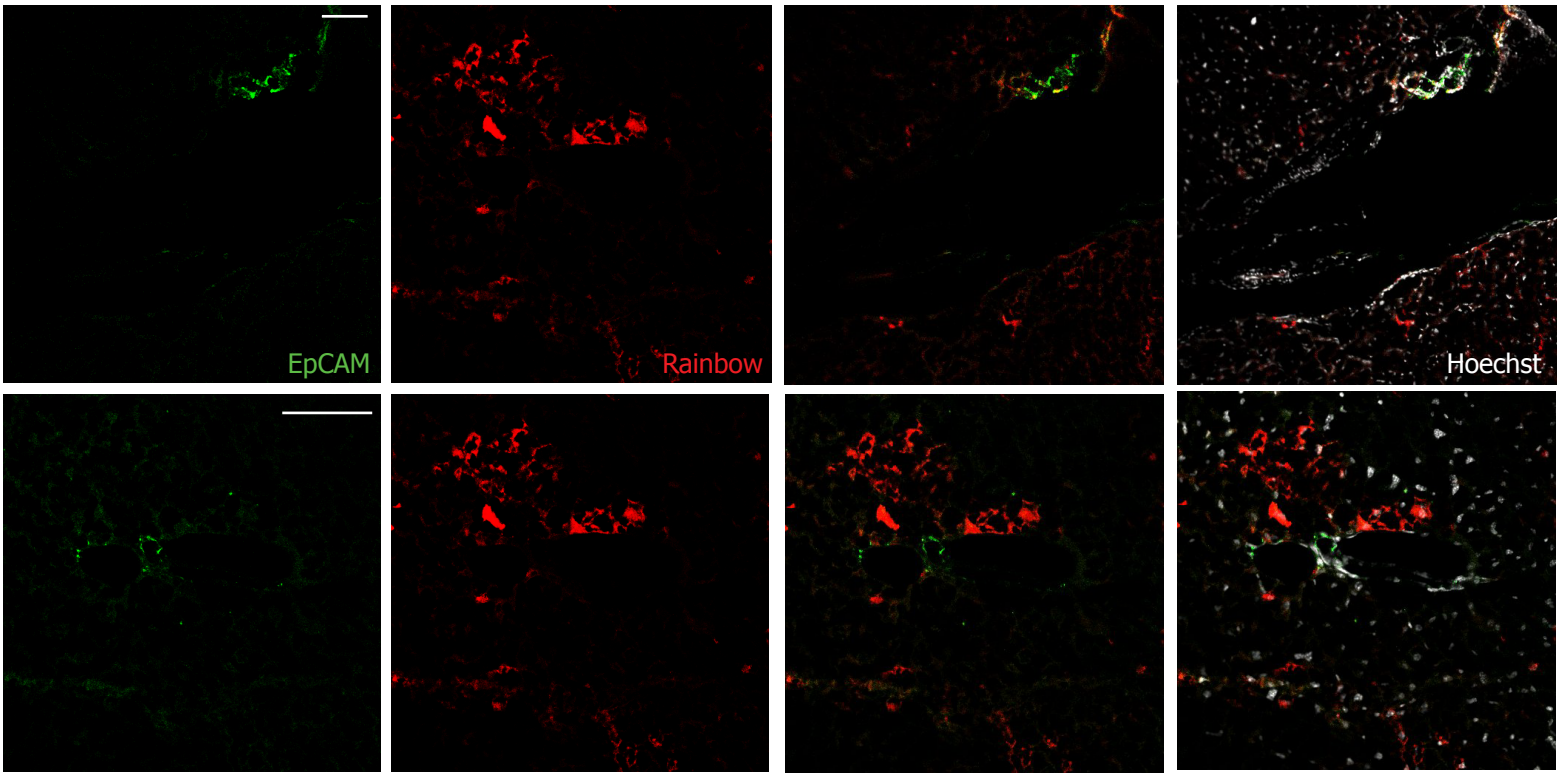
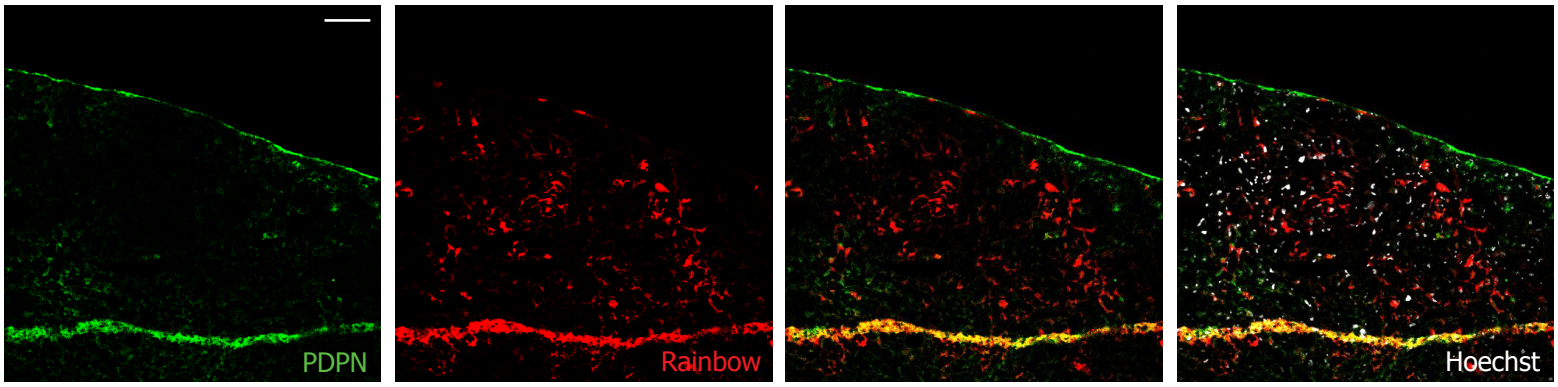


Fig S7 *Actin*^{CreERT2}*R26*$^{VT2/GK3}$ clones are predominantly hepatocytes

Representative images of *Actin*^{CreERT2}*R26*$^{VT2/GK3}$ clones from S0D56 mice co-stained with albumin or EpCAM. Scale bars are 100um.

Mesothelium



Lymphatics

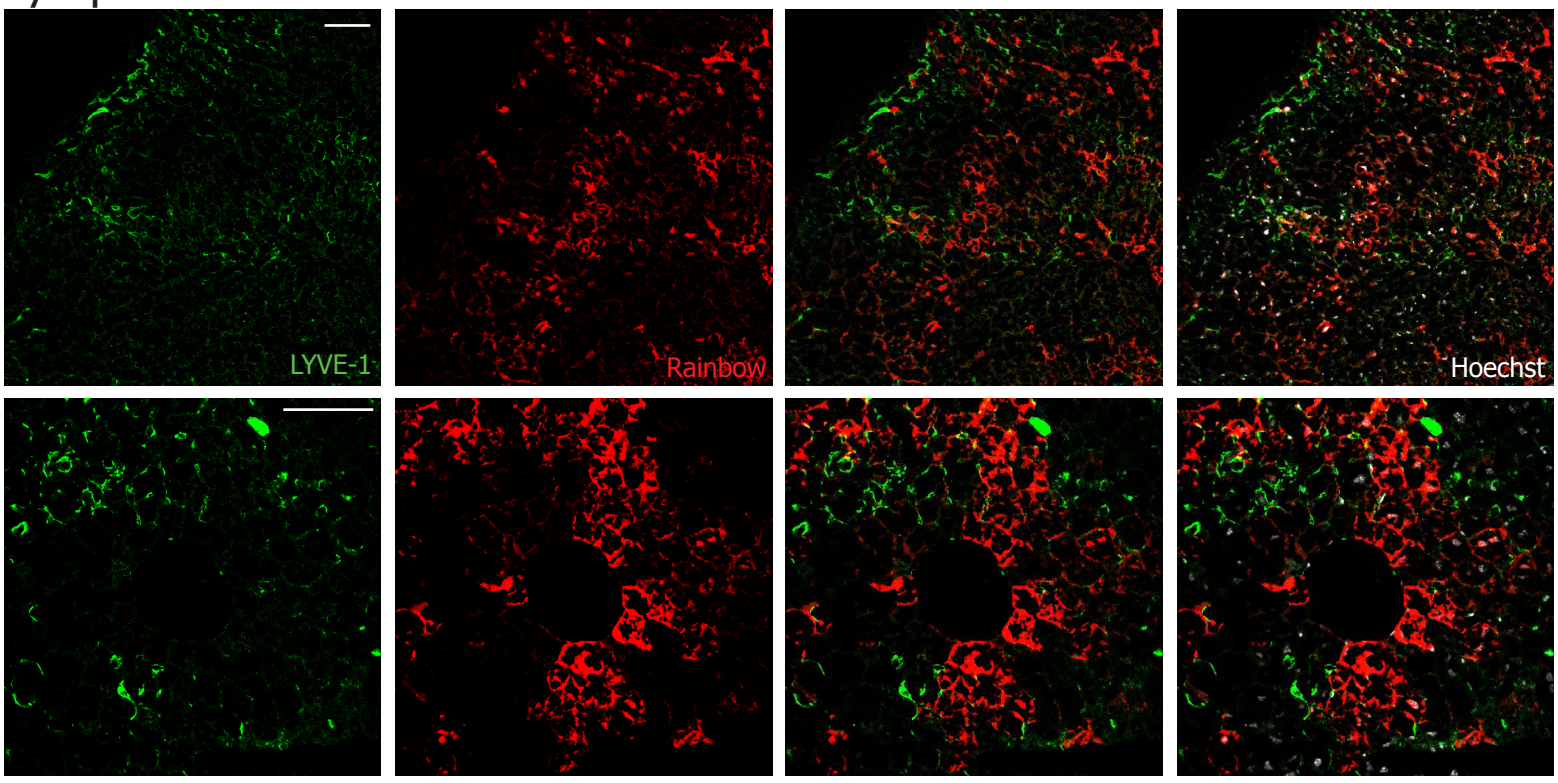
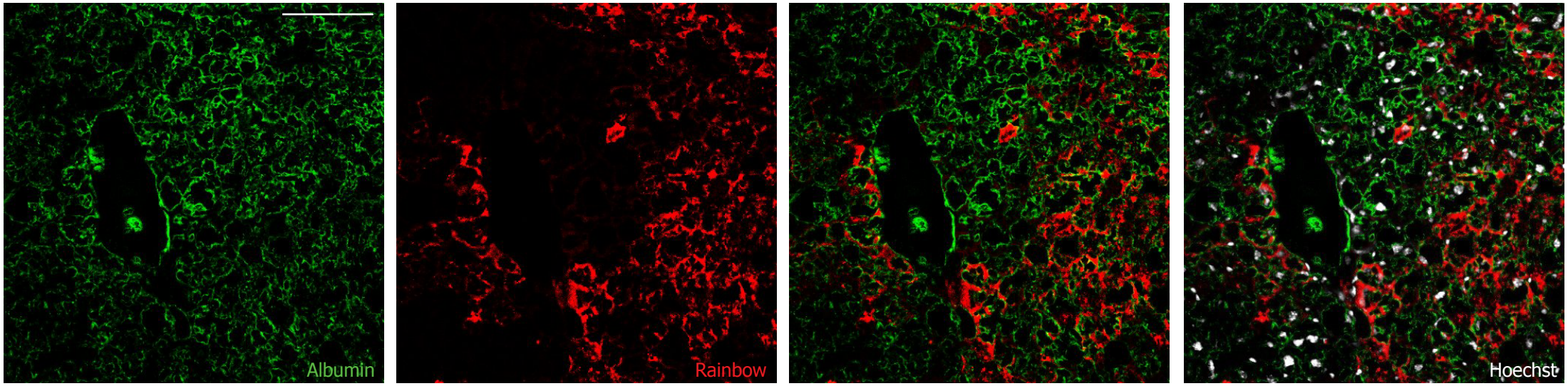


Fig S8 *Actin*^{CreERT2}*R26*$^{VT2/GK3}$ clones are predominantly hepatocytes

Representative images of *Actin*^{CreERT2}*R26*$^{VT2/GK3}$ clones from S0D56 mice co-stained with PDPN or LYVE1. Scale bars are 100um.

Regenerating Area



Non-Regenerating Area

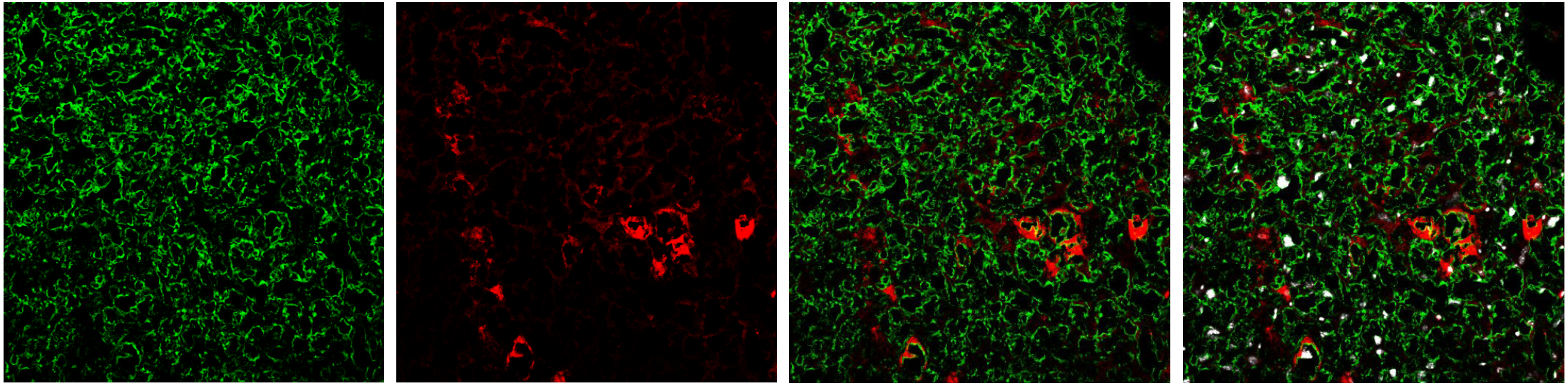
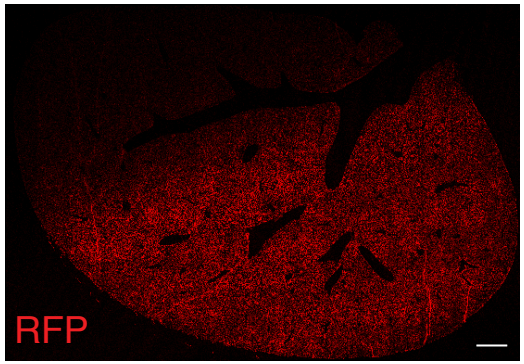


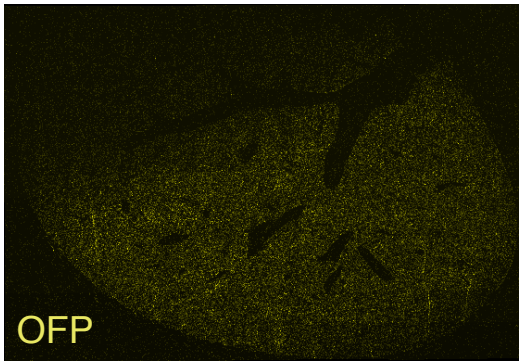
Fig S9 *Actin*^{CreERT2}*R26*$^{VT2/GK3}$ are heterogeneous throughout liver lobes

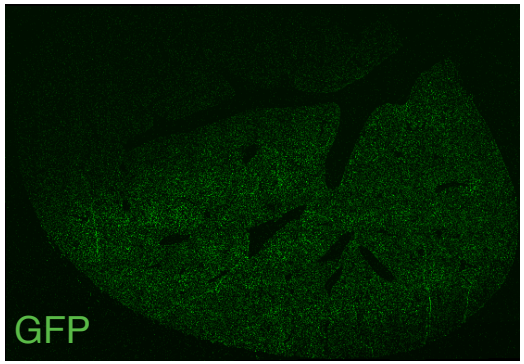
Images of *Actin*^{CreERT2}*R26*$^{VT2/GK3}$ clones from the regenerating (lower tip) or non-regenerating area (central or upper tip) of the left lobe S0D56 mice co-stained with albumin. Scale bars are 100um.

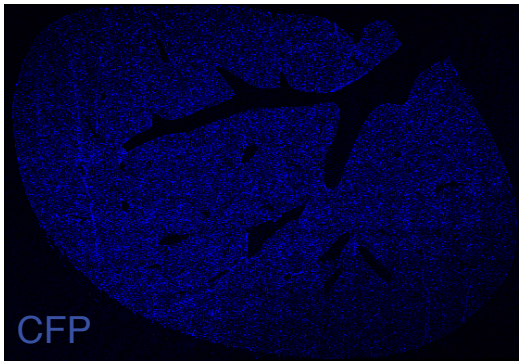
S0D56

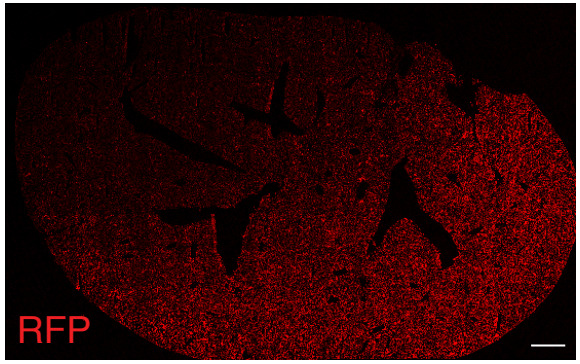
D56

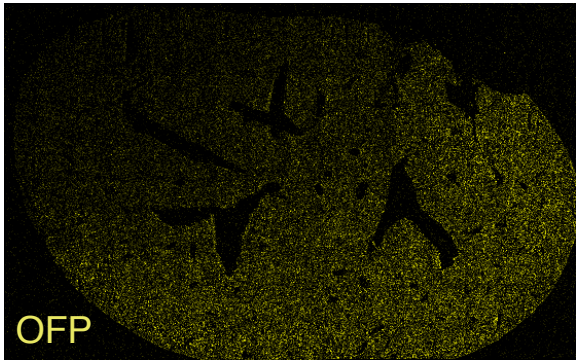


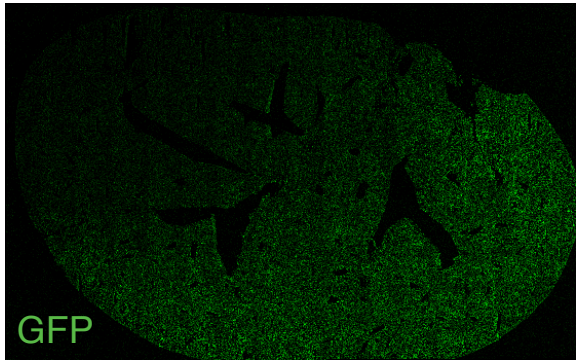


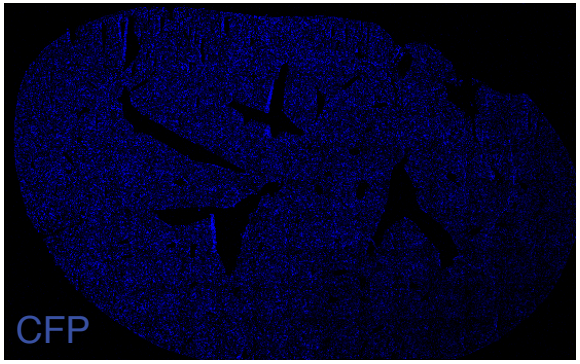


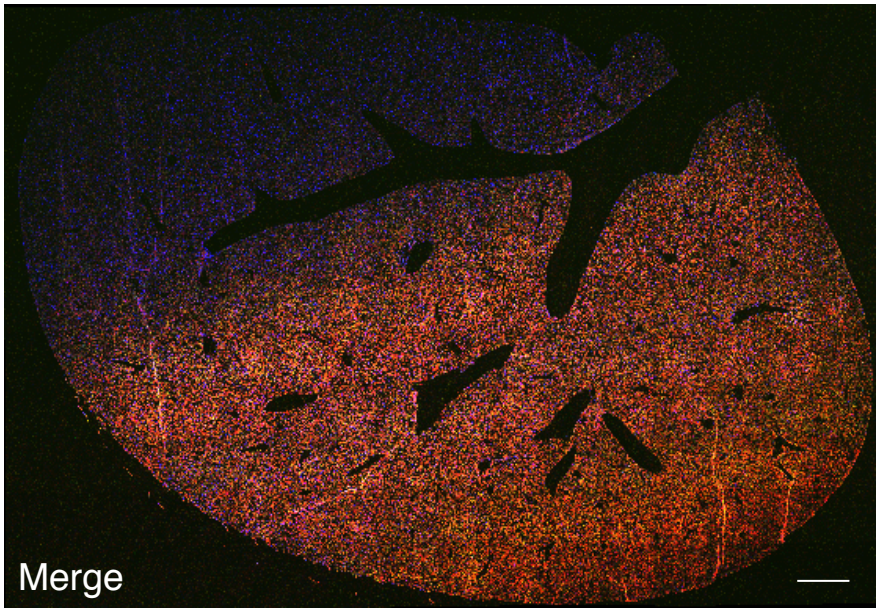












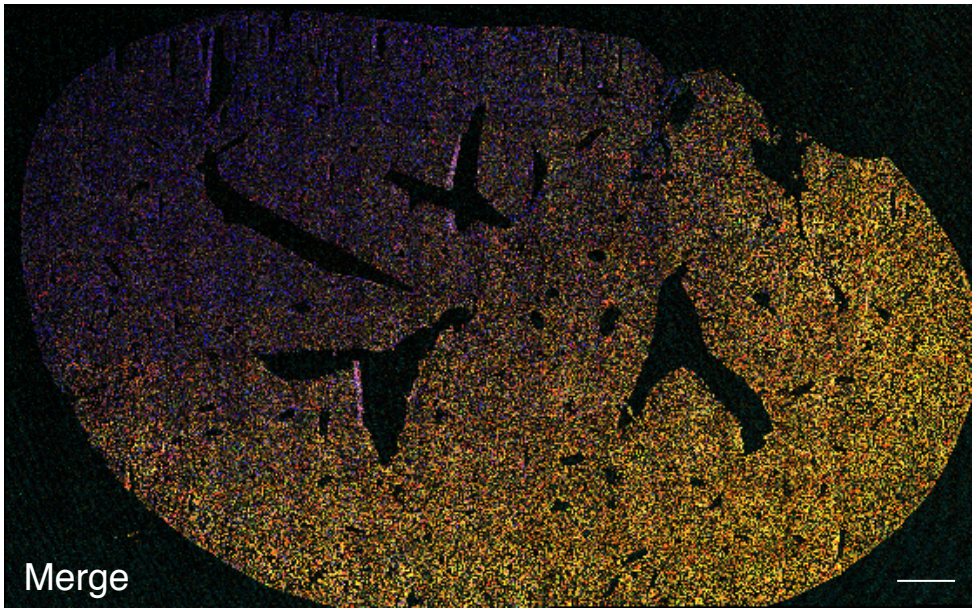
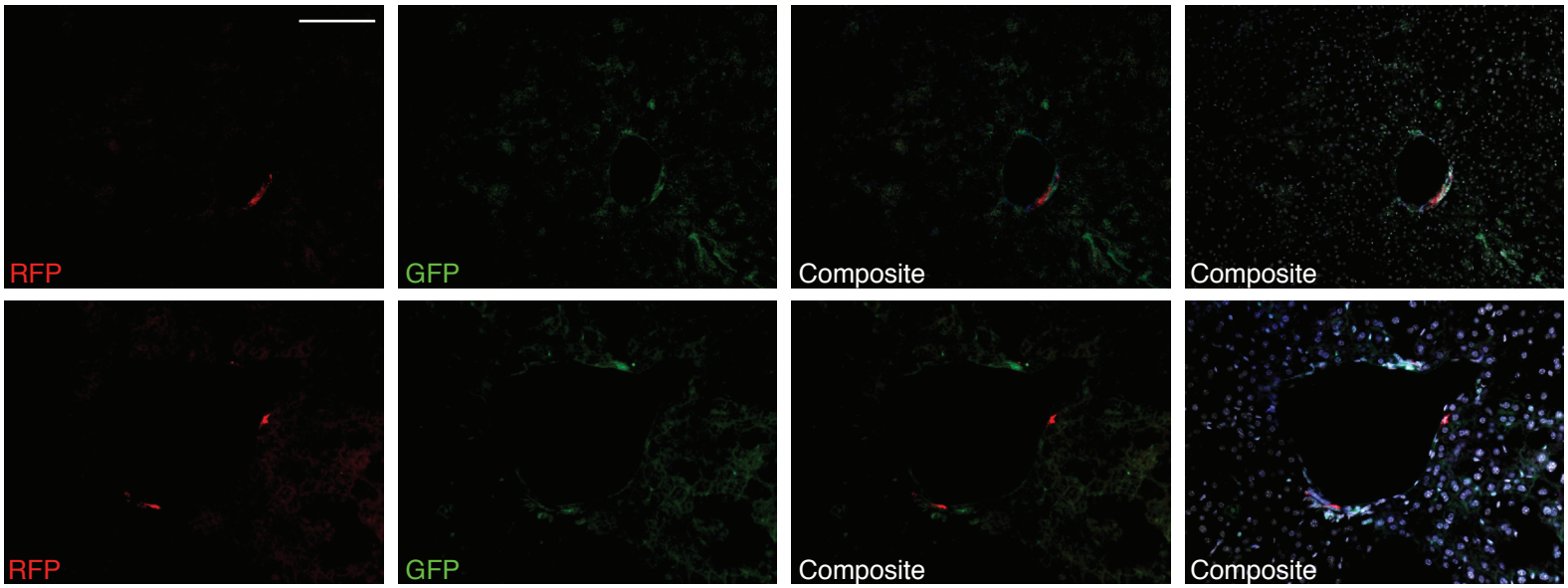


Fig S10 Clonal distributions across whole lobes

Tiled images of whole left lobes from S0D56 and uninjured D56 *Actin*^{CreERT2}*R26*$^{VT2/GK3}$ mice are shown. Images are of individual channels (RFP = red fluorescent protein, CFP = cyan fluorescent protein, GFP = green fluorescent protein, OFP = orange fluorescent proteins) and merged.

Sox9CreER Rainbow



Axin2CreER mTmG

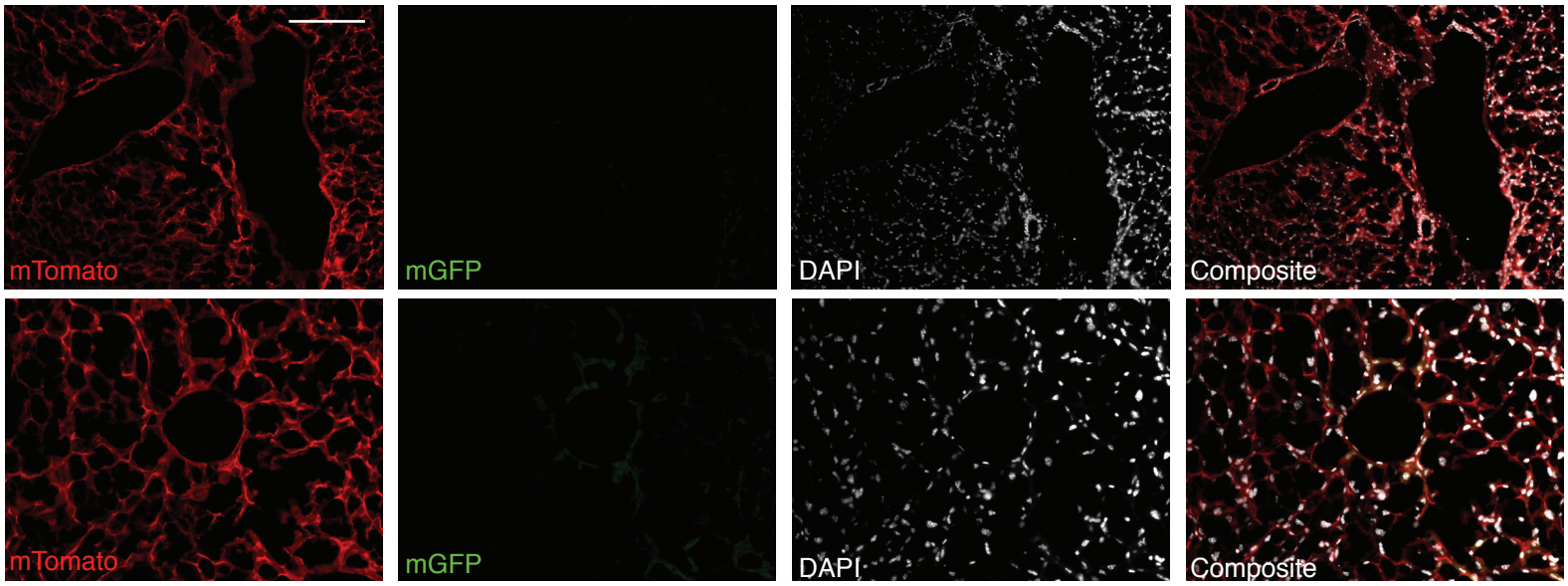
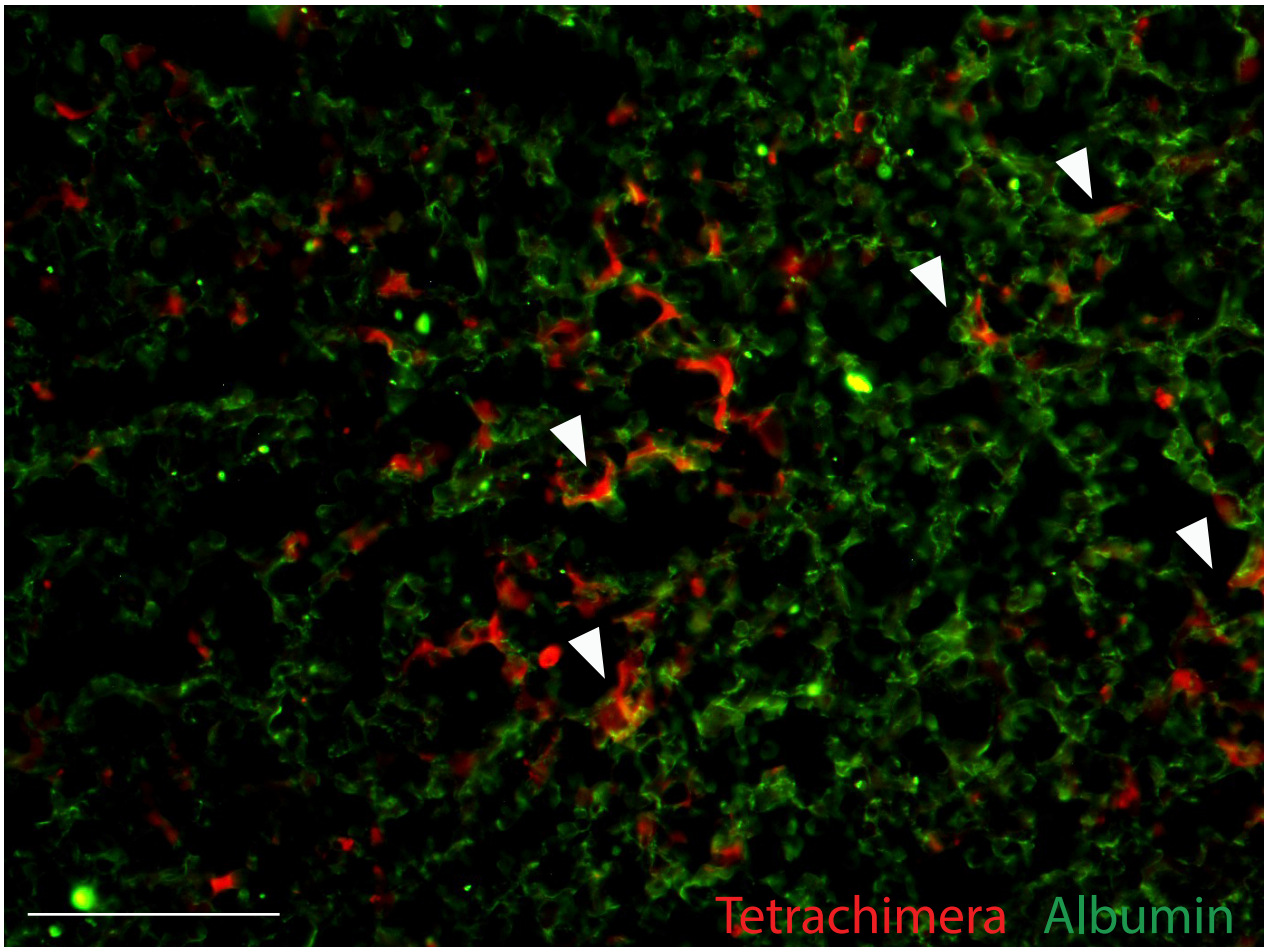


Fig S11 Axin2 and Sox9 are not markers of neonatal regeneration

Representative images across two channels (GFP, RFP) showing few small color clones in a 56 day post partial hepatectomy day 0.5 mouse merged with Hoechst 33342 in *Sox9*^{CreERT2}*R26*$^{VT2/GK3}$ or *Axin2*^{CreERT2}*R26*$^{mT/mG}$ mice. All scale bars are 100um.

E15



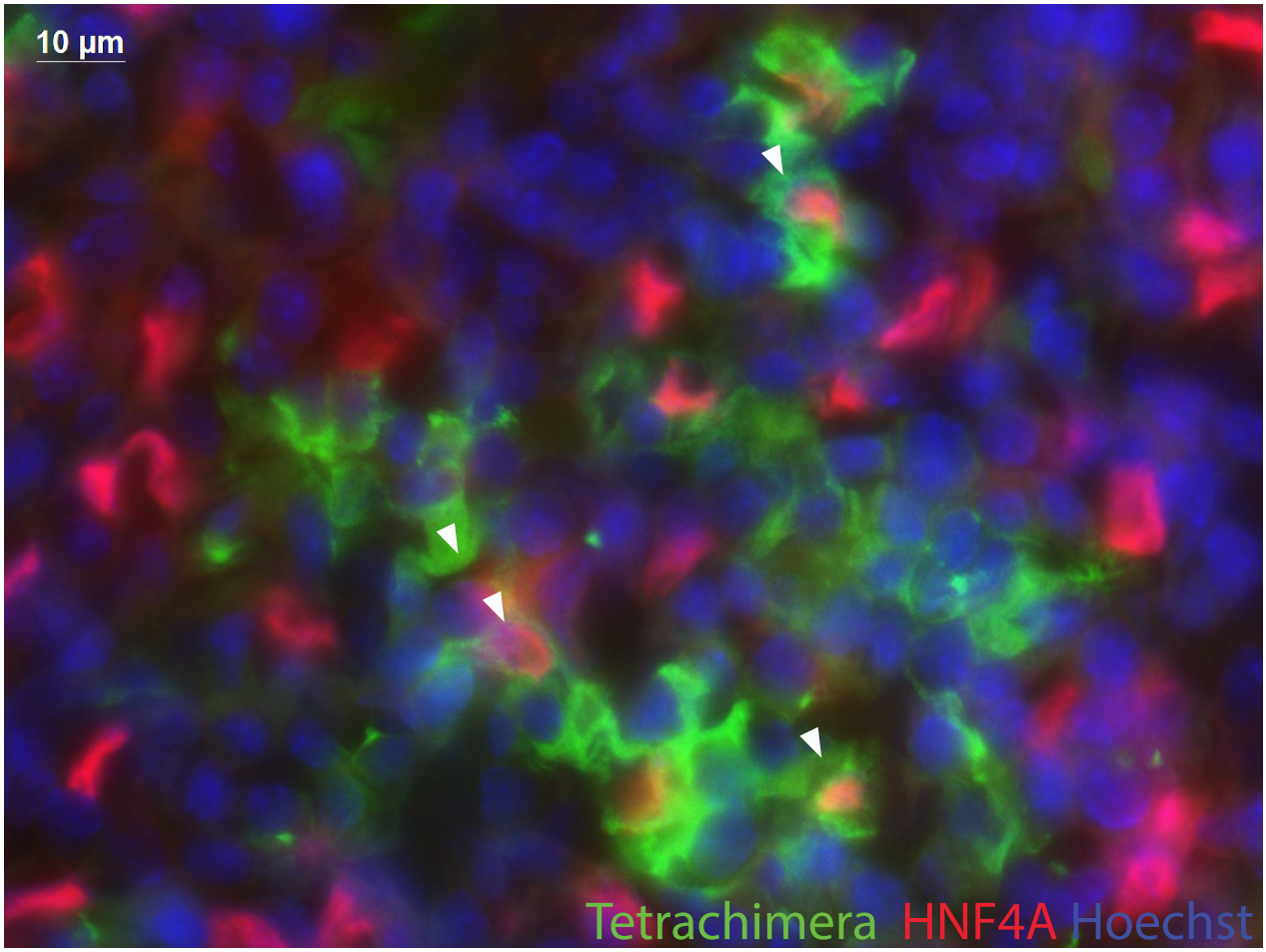


Fig S12 Embryonic and P0 tetrachimeric clones

Representative images of clones of embryonic day 15 (e15) tetrachimeric mice, co-stained with albumin or HNF4A. All scale bars are 100um.

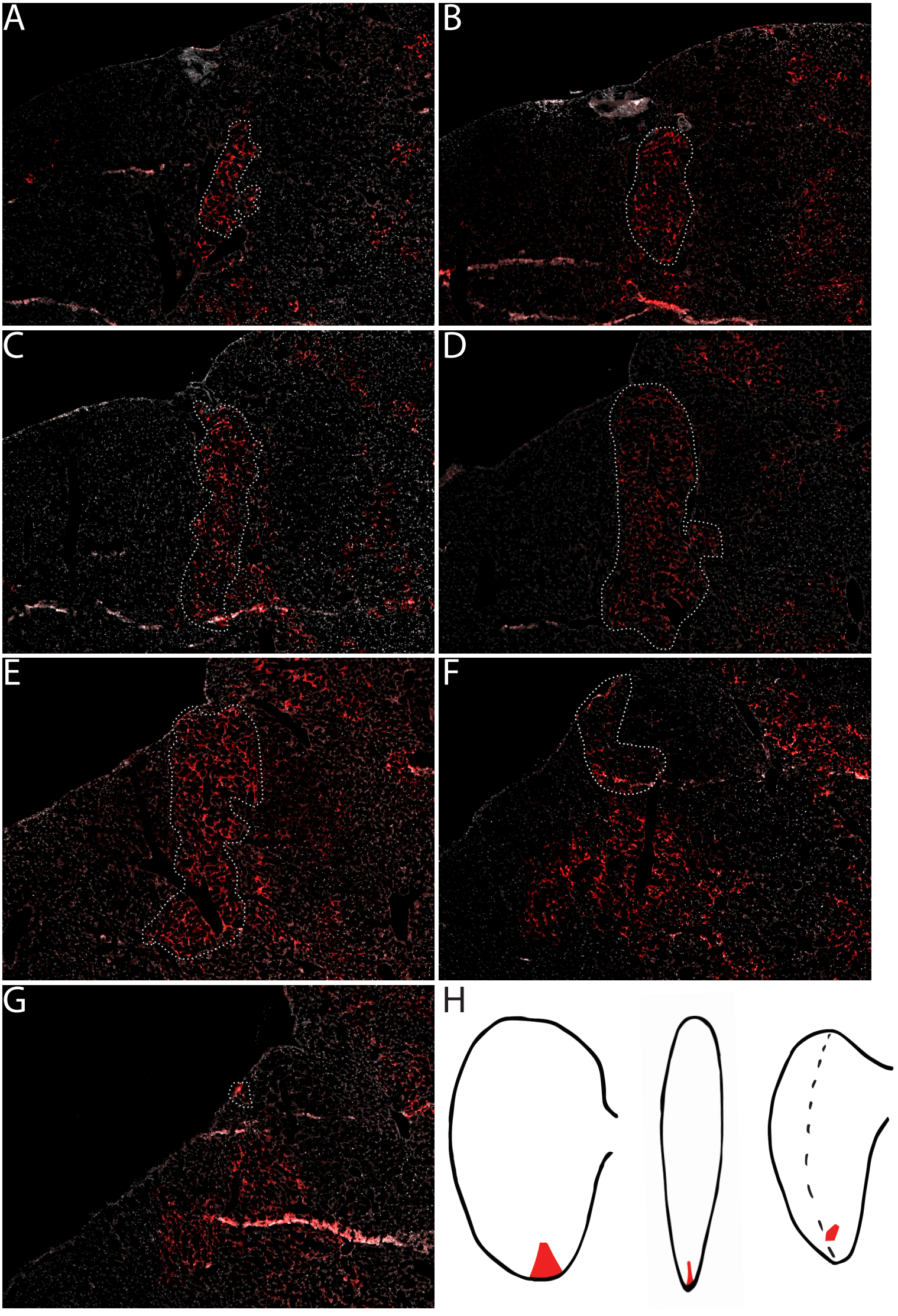

A
B
C
D
E
F
G
H

Fig S13 Adult tetrachimeric clones in the left lobe

A representative image of tetrachimeric clones in the regenerated region of the left lobe of a S0,D56 mouse. A-E are each 110um apart. E is 200um apart from F. F is 80um from G. H is a schematic indicating the location of the clone shown in A-G (portrayed in red) in the left lobe from different angles. All scale bars are 100um.

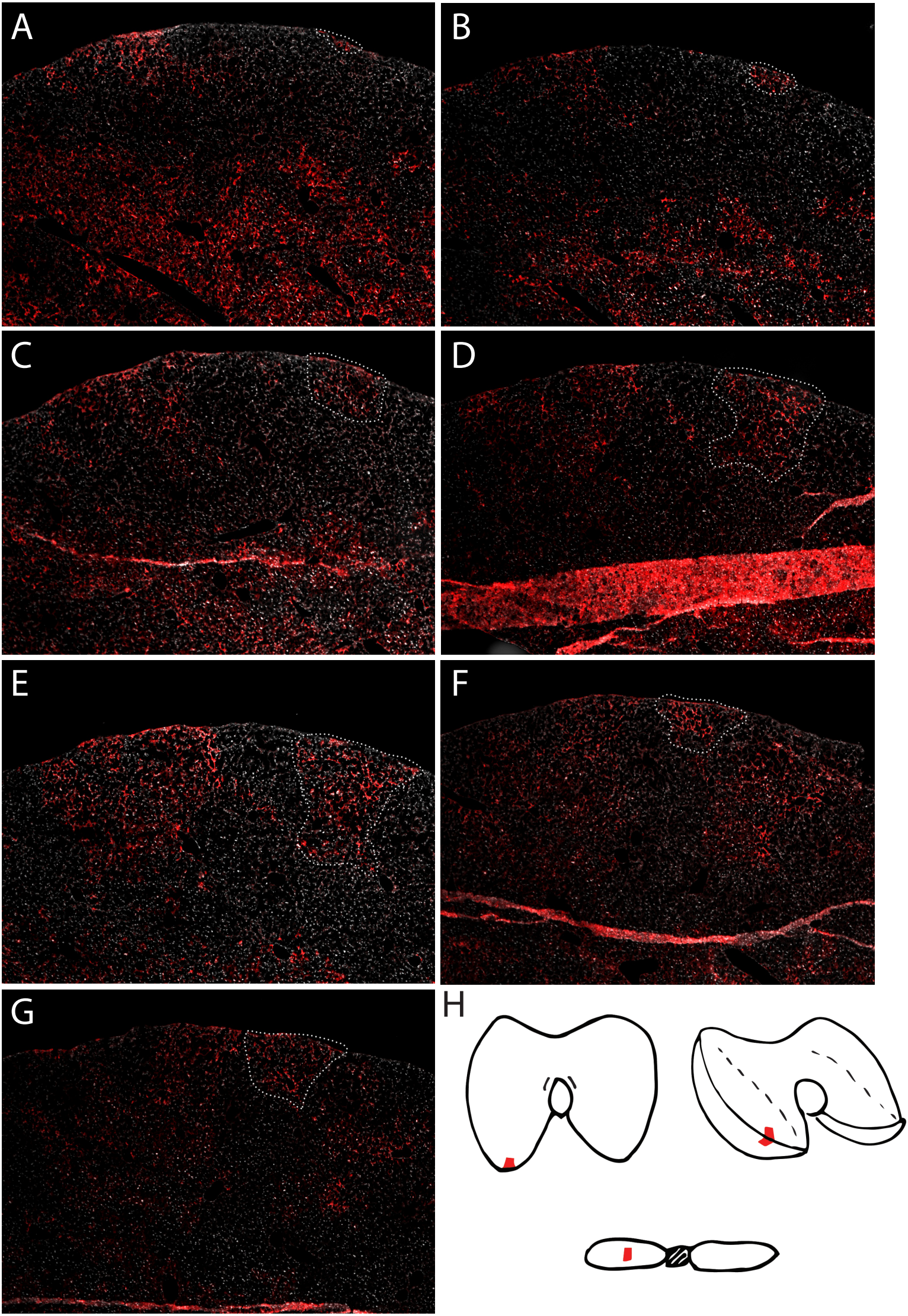

A
B
C
D
E
F
G
H

Fig S14 Adult tetrachimeric clones in the median lobe

A representative image of tetrachimeric clones in the regenerated region of the left lobe of a S0,D56 mouse. A is 170um from B. B-G are each 50um apart. H is a schematic indicating the location of the clone shown in A-G (portrayed in red) in the median lobe from different angles. All scale bars are 100um.

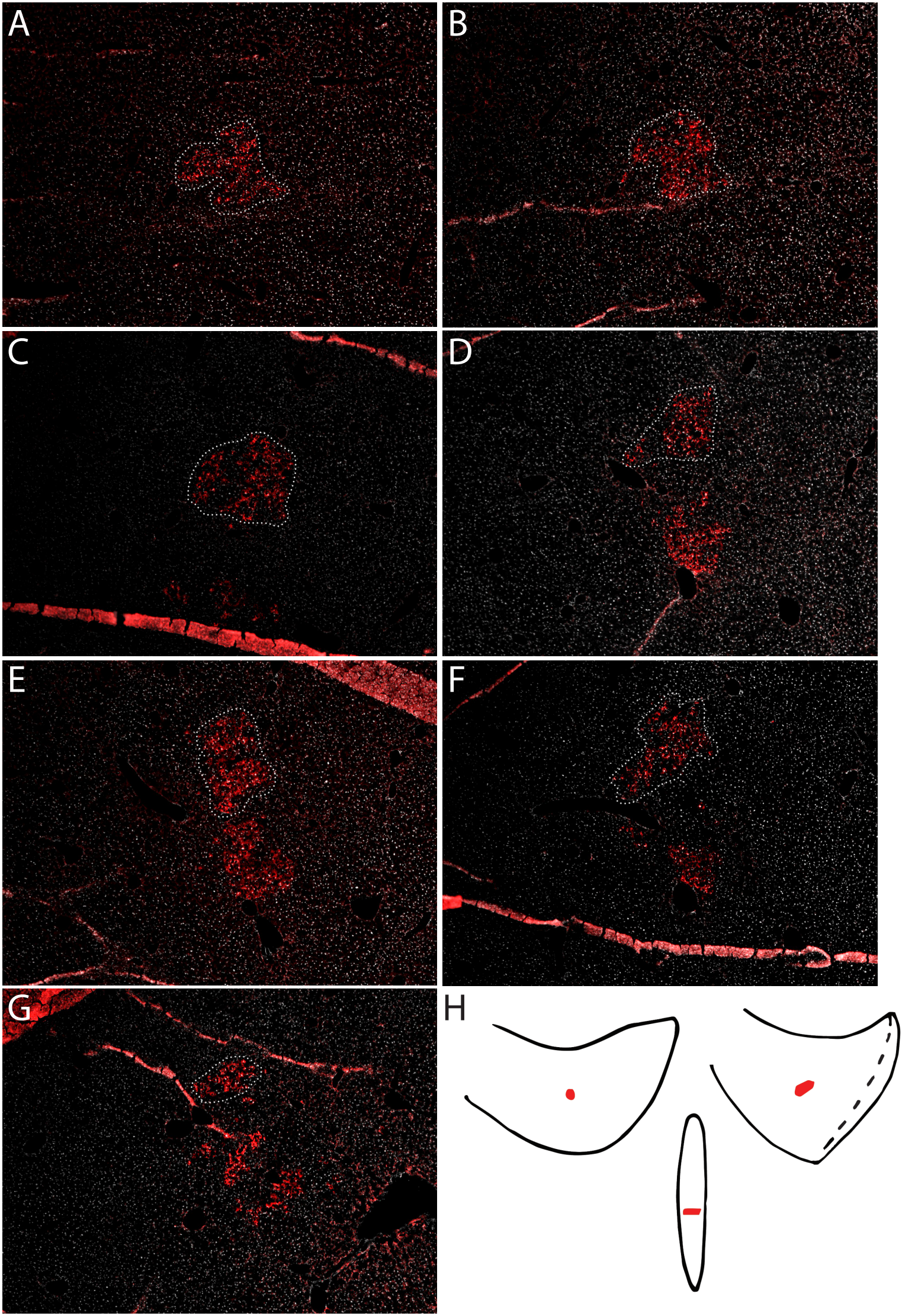

A
B
C
D
E
F
G
H

Fig S15 Adult tetrachimeric clones in the right lobe

A representative image of tetrachimeric clones in the regenerated region of the left lobe of a S0,D56 mouse. A-G are 50um apart each. H is a schematic indicating the location of the clone shown in A-G (portrayed in red) in the right lobe from different angles. All scale bars are 100um.

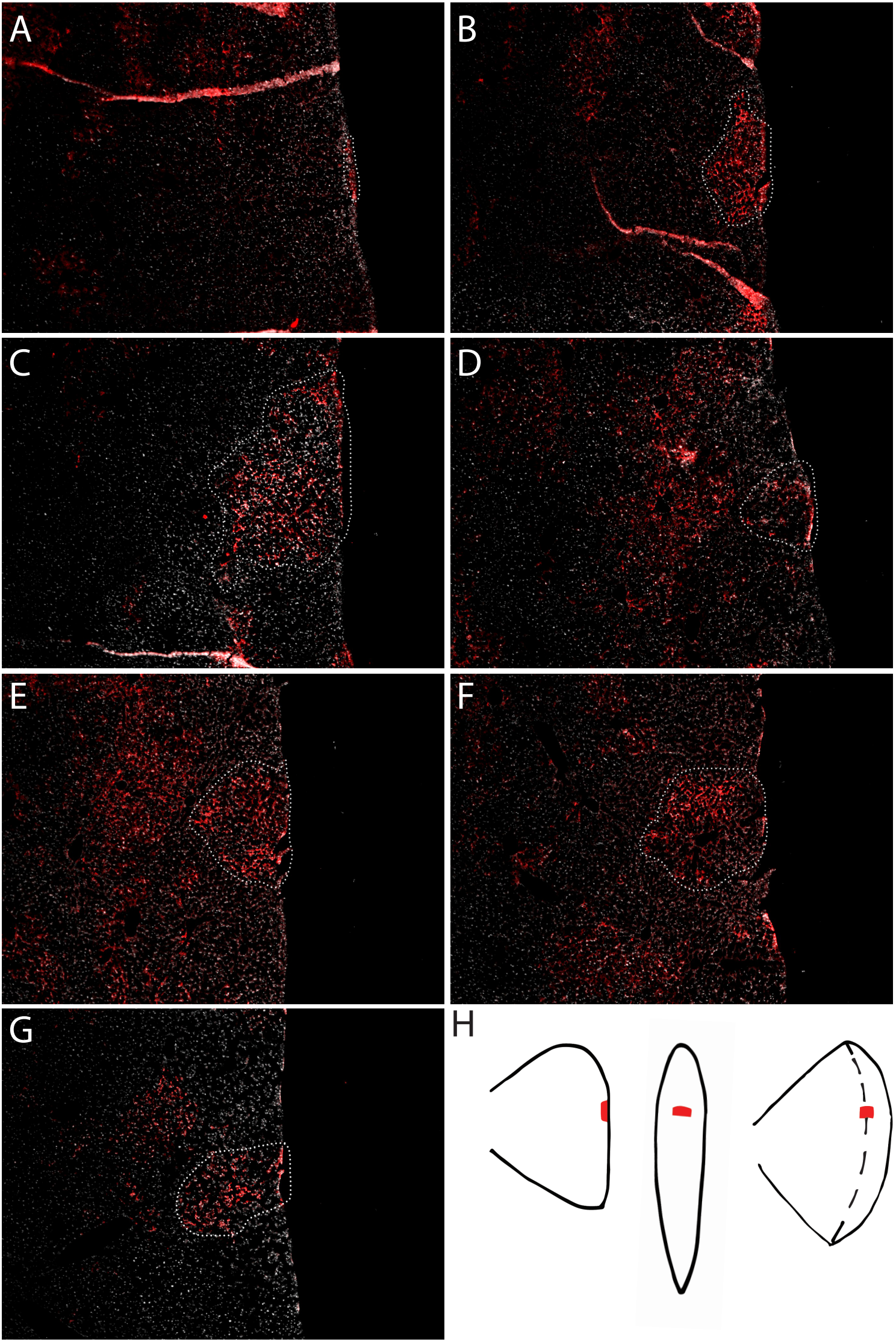
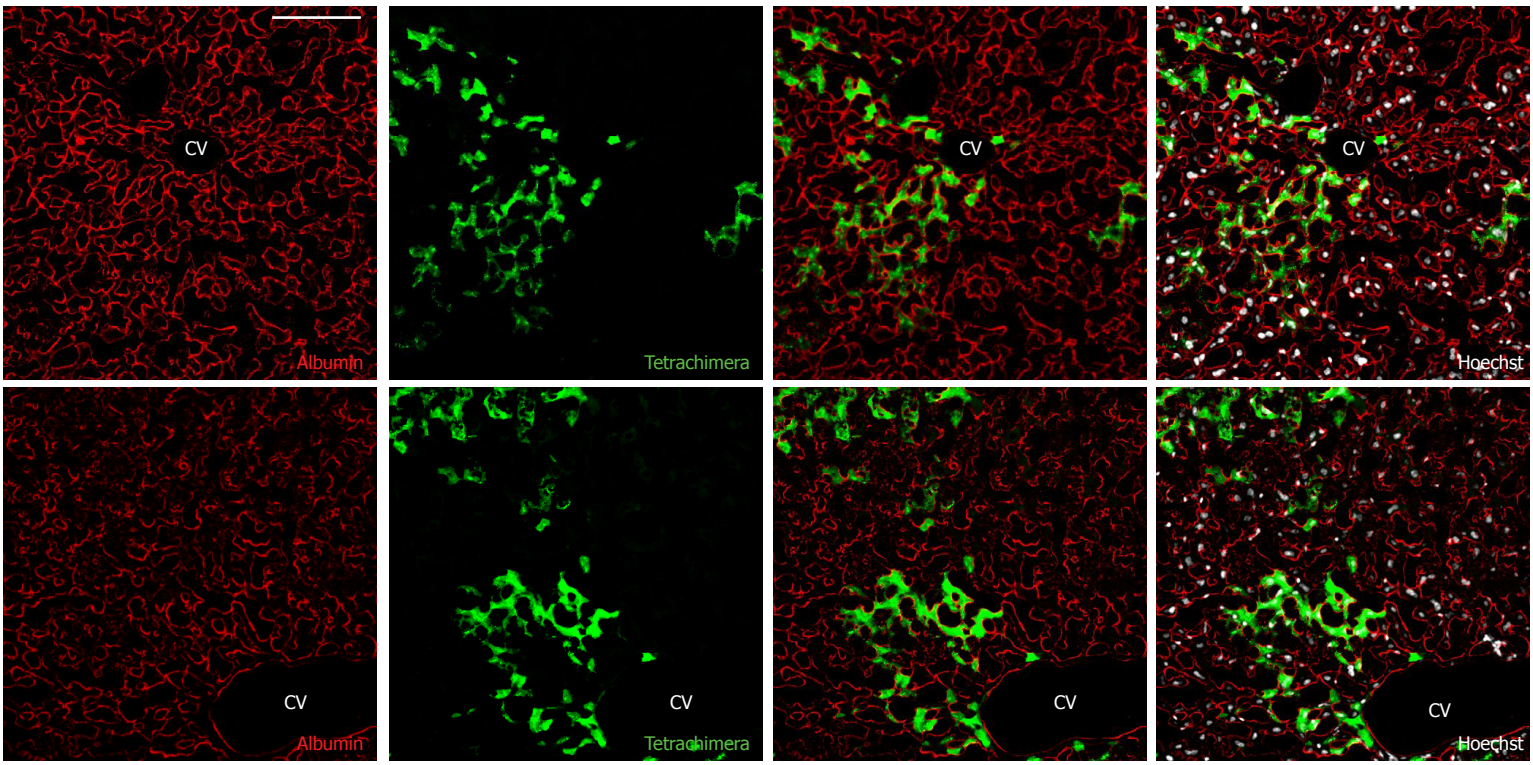
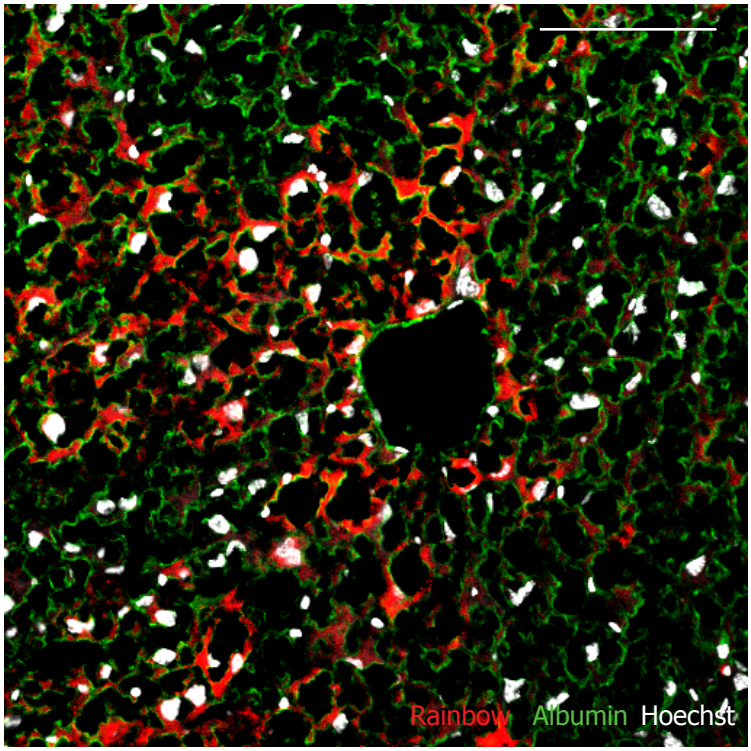

A
B
C
D
E
F
G
H

Fig S16 Adult tetrachimeric clones in the caudate lobe

A representative image of tetrachimeric clones in the regenerated region of the left lobe of a S0,D56 mouse. A is 80um from B. C-F are 50um apart each. F is 230um apart from G. H is a schematic indicating the location of the clone shown in A-G (portrayed in red) in the caudate lobe from different angles. All scale bars are 100um.


CV
Albumin
Tetrachimera
Hoechst


Rainbow Albumin Hoechst

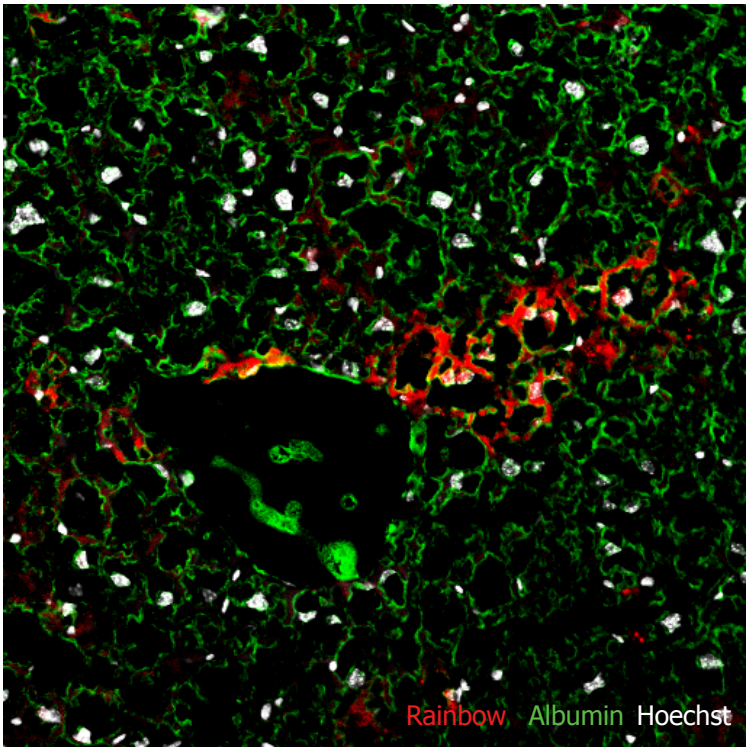

Rainbow Albumin Hoechst

Fig S17 Tetrachimeric clones are vasculature associated

Representative images of tetrachimeric (A) and Rainbow (B) clones associated with central veins. All scale bars are 100um.

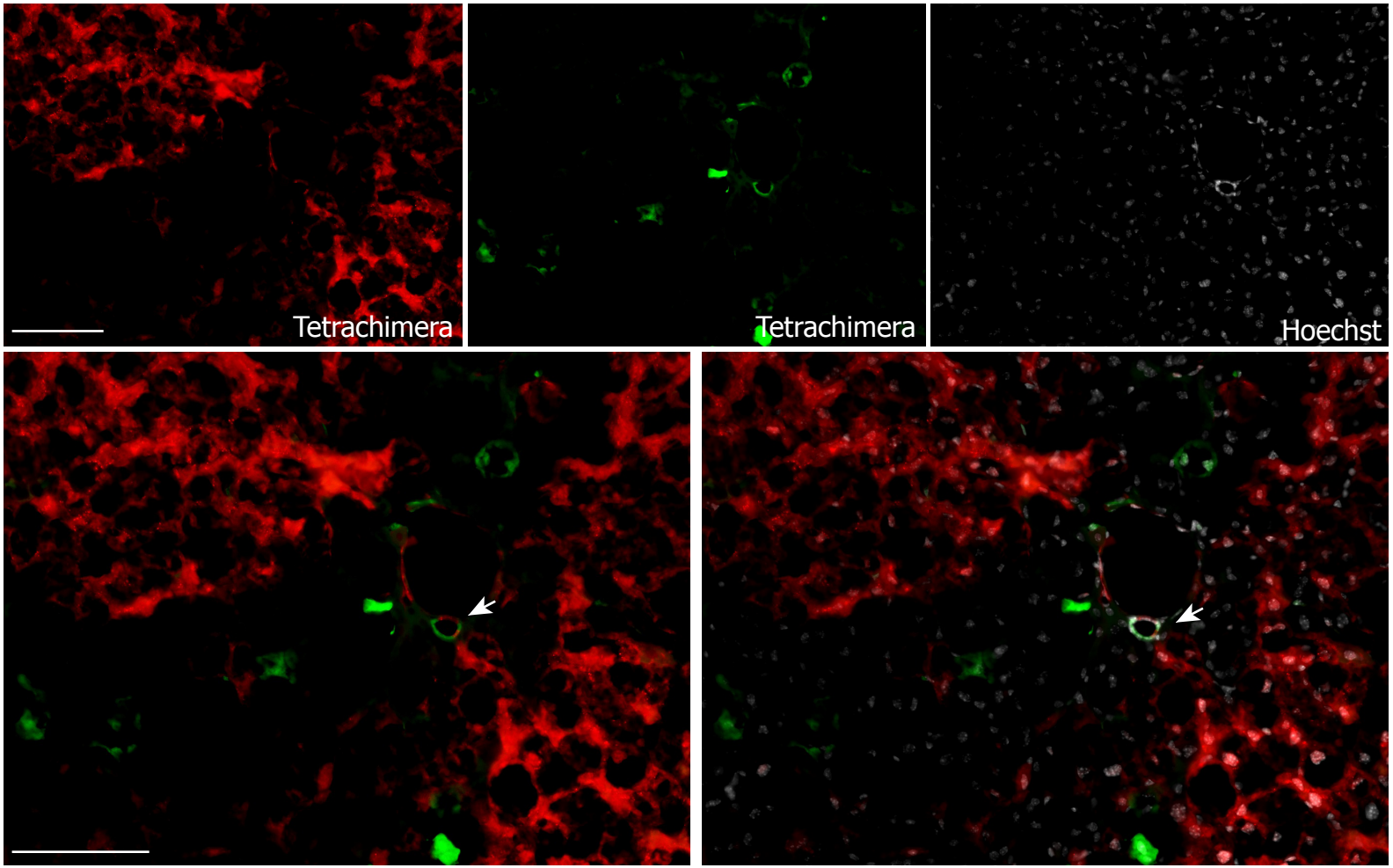

Tetrachimera
Tetrachimera
Hoechst

Fig S18 Tetrachimeric analyses indicate that bipotent progenitors are a rare population

A representative image of bile duct epithelium (as denoted by arrow) and surrounding clones in a tetrachimera liver. All scale bars are 100um.