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Supplemental Information

Adult Hepatocytes Are Generated by Self-Duplication Rather than Stem Cell Differentiation

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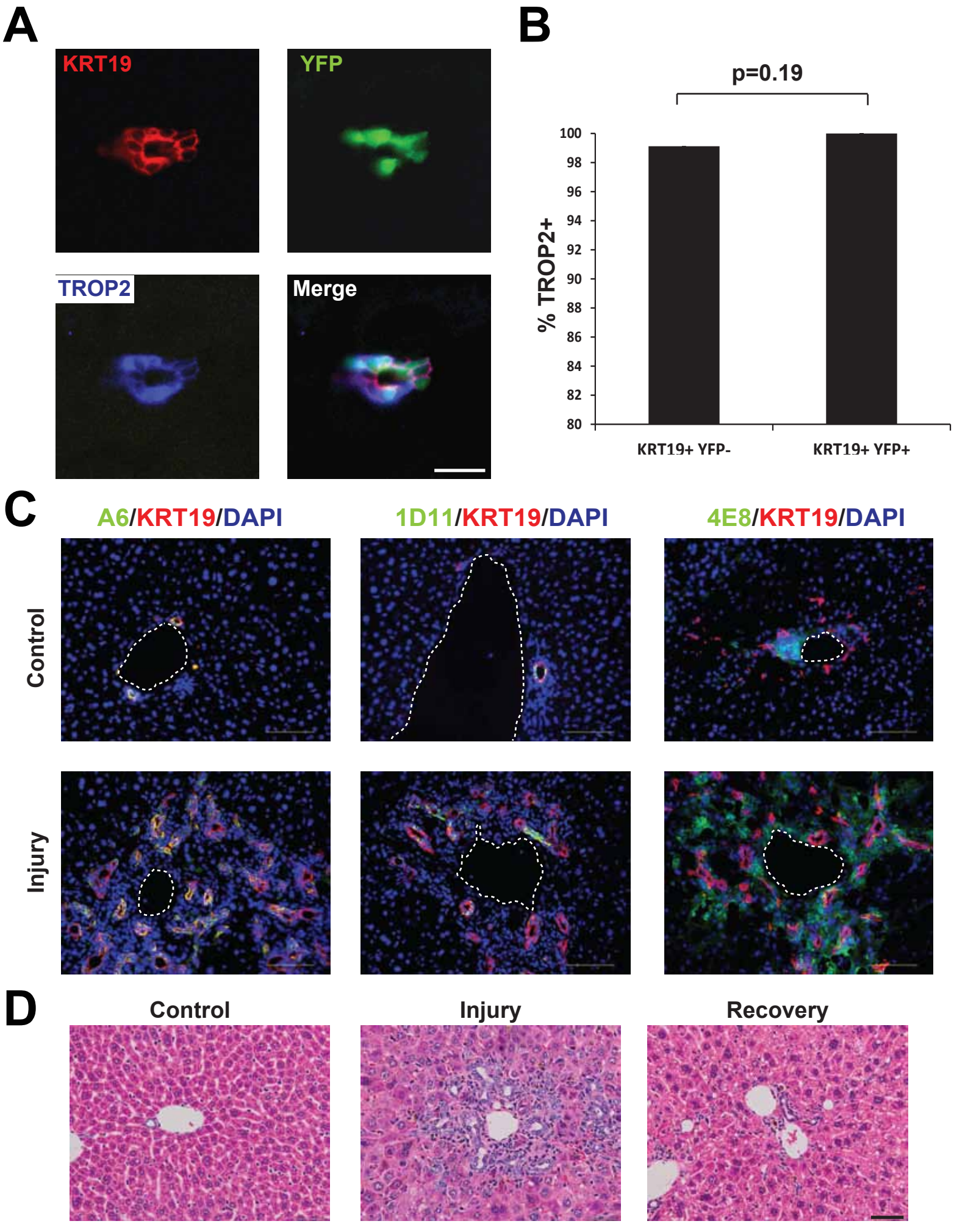

A
KRT19
YFP
TROP2
Merge
B
p=0.19
% TROP2+
100
98
96
94
92
90
88
86
84
82
80
KRT19+ YFP-
KRT19+ YFP+
C
A6/KRT19/DAPI
1D11/KRT19/DAPI
4E8/KRT19/DAPI
Control
Injury
D
Control
Injury
Recovery

Figure S1

Figure S1, Related to Figure 1. TROP2 labeling and example of liver injury resulting in an atypical ductal cell response and cessation after recovery.

(A) Co-staining for TROP2 and Krt19 in YFP^+ and YFP^- cells after tamoxifen-mediated labeling of BECs with *Krt19-CreER*.

(B) Quantification of staining from (A) presented as percentage of $Krt19^+YFP^+$ or $Krt19^+YFP^-$ cells that co-stained for TROP2 (273 cells counted; n=2).

(C) Representative images showing that DDC treatment is associated with an expansion of cells expressing a variety of previously-described oval cell/ADC markers, including A6, 1D11 and 4E8. Note that cells which stain positively for these markers are also present in the normal liver (top panels).

(D) The injury-recovery protocol (2 weeks of DDC followed by 5 weeks of normal diet) is associated with an expansion and regression of the ADCs within the parenchyma.

Scale: white bar=12.5 μm; black bar=100 μm; yellow bar=50 μm.

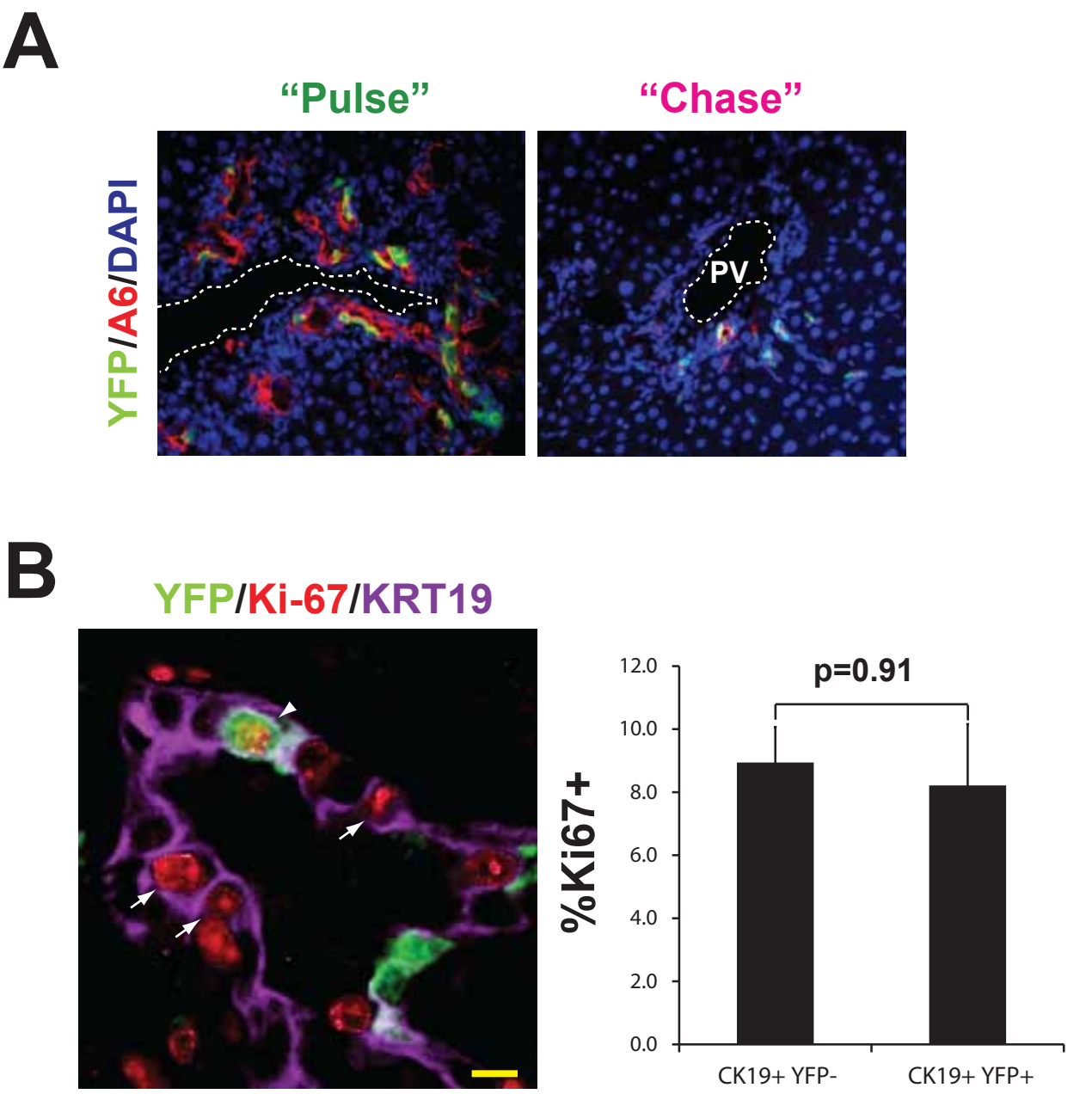
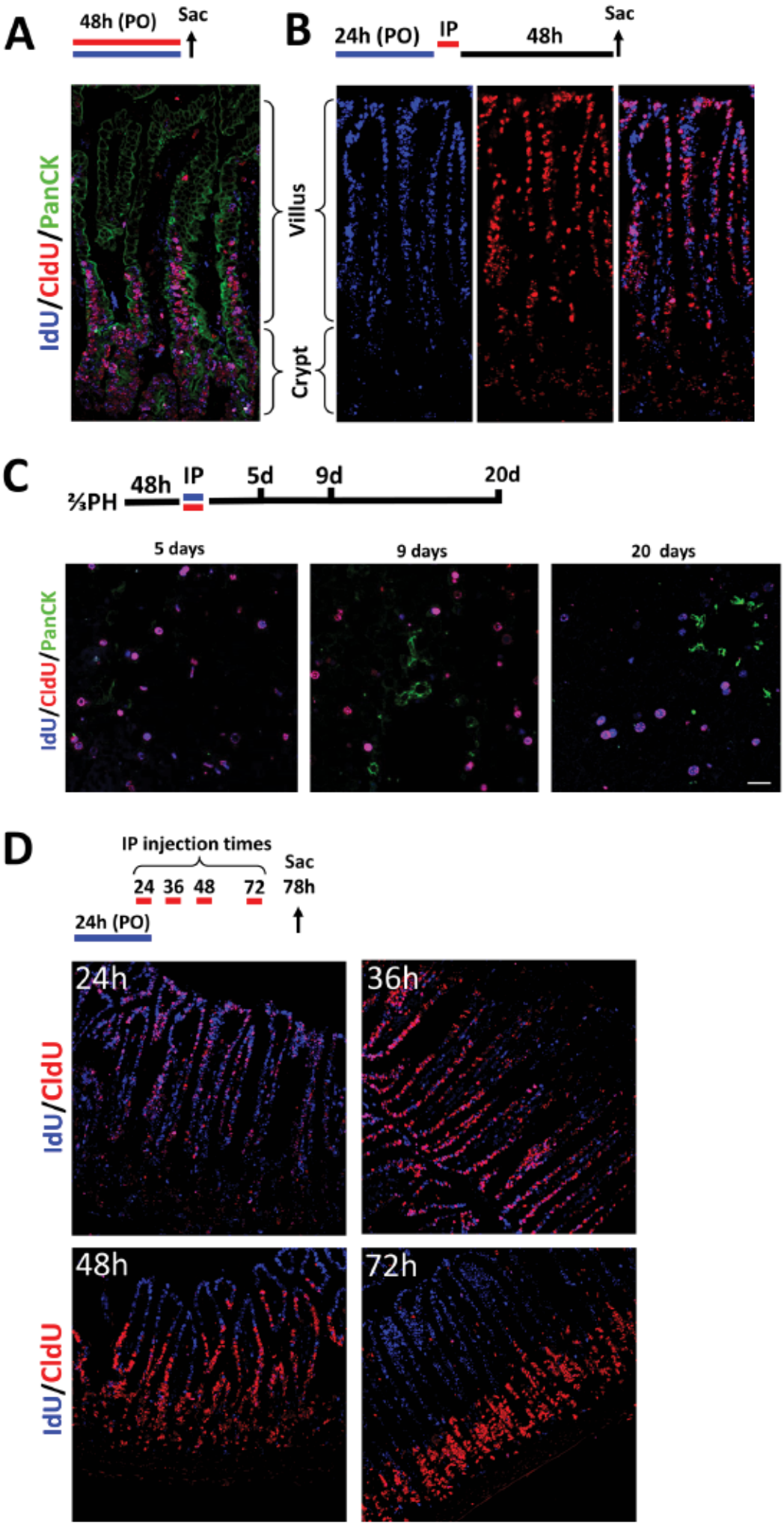

A
"Pulse"
"Chase"
YFP/A6/DAPI
PV
B
YFP/Ki-67/KRT19
%Ki67+
p=0.91
12.0
10.0
8.0
6.0
4.0
2.0
0.0
CK19+ YFP-
CK19+ YFP+

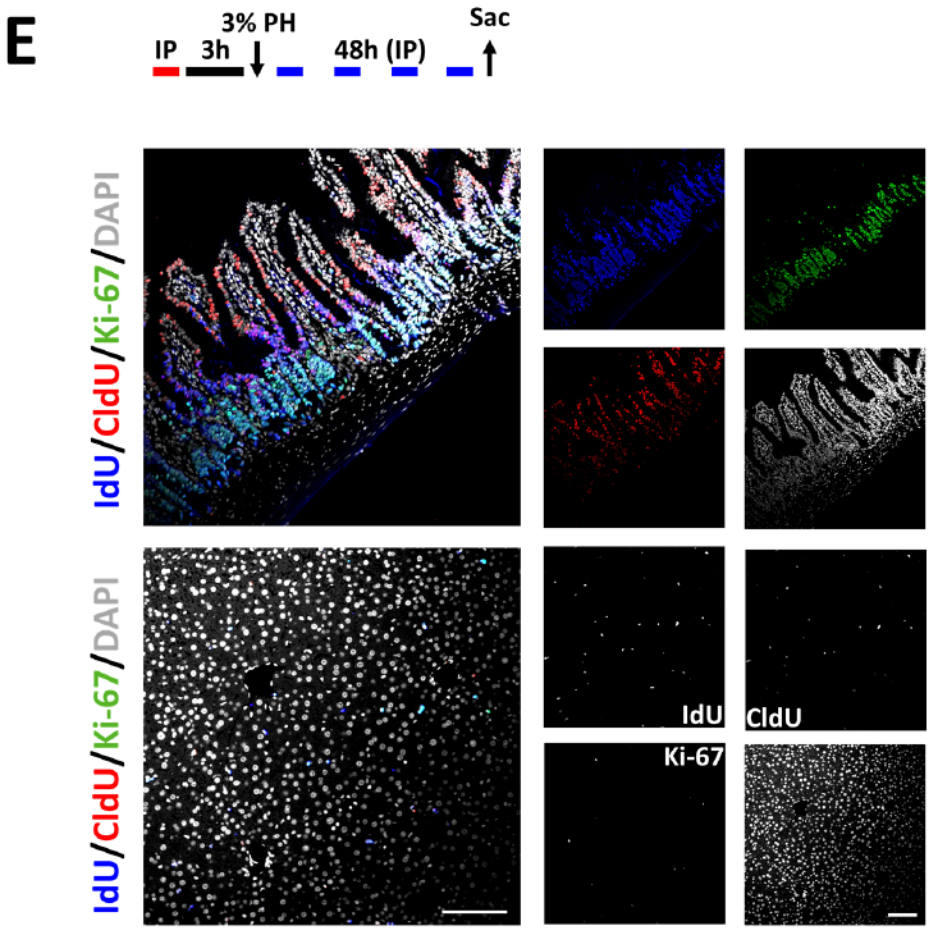
Figure S2

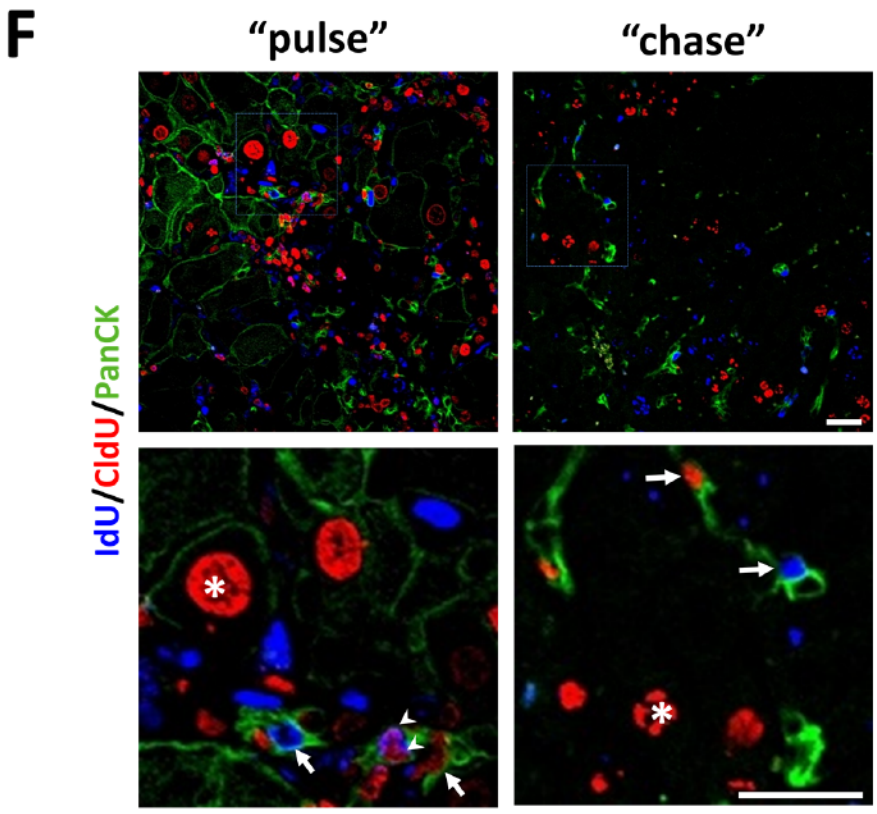
Figure S2, Related to Figure 2. Tamoxifen administration to *Krt19-CreER;Rosa*^{YFP} mice labels A6^{+} ADCs and results in equal labeling of proliferating and non-proliferating ADCs.

(A) A6^{+} ADCs were labelled during DDC injury.

(B) Co-immunofluorescence for Ki-67, Krt19, and YFP was performed in livers from *Krt19-CreER;Rosa*^{YFP} mice following tamoxifen pulse, and the fraction of proliferating cells in both YFP^{-} (arrows) and YFP^{+} (arrowheads) populations was calculated. Ki-67^{+} cells comprised approximately 8% of Krt19^{+} cells in both populations (mean ±SD). Scale bar=10 μm.


A
48h (PO)
Sac
B
24h (PO)
IP
48h
Sac
IdU/CldU/PanCK
Villus
Crypt
C
⅔PH
48h
IP
5d
9d
20d
5 days
9 days
20 days
IdU/CldU/PanCK
D
IP injection times
24
36
48
72
Sac
78h
24h (PO)
24h
36h
48h
72h
IdU/CldU
IdU/CldU


E
IP
3h
3% PH
48h (IP)
Sac
IdU/CldU/Ki-67/DAPI
IdU/CldU/Ki-67/DAPI
IdU
CldU
Ki-67


F
"pulse"
"chase"
IdU/CldU/PanCK

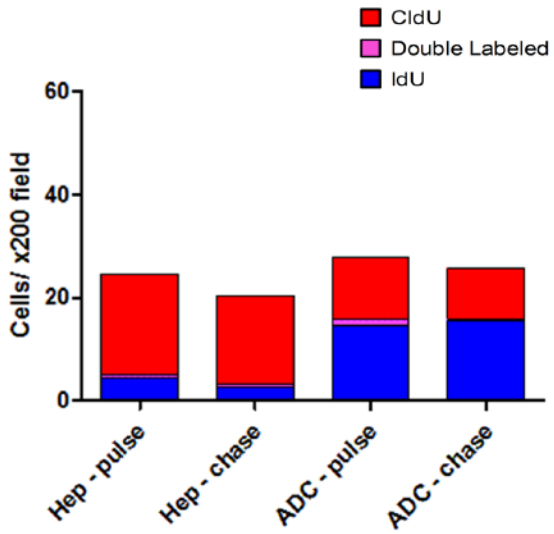

CldU
Double Labeled
IdU
Cells/ x200 field
60
40
20
0
Hep - pulse
Hep - chase
ADC - pulse
ADC - chase

Figure S3

Figure S3, Related to Figure 5. Labeling and tracing transit amplifying (TA) cells *in vivo*.

(A) IdU and CldU were administered together in the drinking water for 48h, resulting in efficient double labeling of TA cells. CldU administration is depicted as a red bar and IdU administration is depicted as a blue bar.

(B) IdU was administered in the drinking water for 24h followed by a single injection of CldU and mice were sacrificed 48h afterwards. Double labeled cells (TA cell progeny) can be seen at the villus tips.

(C) Liver cells were labeled by single injection of IdU + CldU 48h after 70% partial hepatectomy. Liver sections were examined for signal discrepancy between IdU and CldU at the indicated post-operative days. No preferential signal loss was detected.

(D) Kinetics of IdU and CldU labeling were examined to prevent overlap in labeling. IdU was administered in drinking water for 24h and then CldU was injected at 24h, 36h, 48h and 72h after IdU initiation. All mice were sacrificed at 78h post initiation of IdU. The time when the second analog (CldU) was injected appears at the top left corner of each photomicrograph. At 72 hours no overlap between the two analogs was detected.

(E) 3% partial hepatectomy (PH) does not cause compensatory proliferation. Thymidine analogs were administered by injection 3h before and 4 injections during the 48h after liver biopsy. 48h post PH liver (bottom) and intestine (top) were stained with anti- IdU, CldU and Ki-67. No compensatory proliferation was detected in the liver.

(F) Dual nucleoside labeling of cells in CDE-injured livers before and after a recovery period. Left - representative immunofluorescence images showing detection of labeled cells in livers from “pulse” (left) and “chase” (right) of CDE treated mice followed by an injury

free chase (4 week recovery period). Middle panels are magnifications of the indicated areas in the top panels. Right - quantification of labeled cells in livers: 5.4±3.5% of cycling BECs were dual labeled at the “chase”. The proportion of dual labeled hepatocytes did not changed from “pulse” (2.4±1.7%) to “chase” (1.9±1.9%; ≥6 x200 fields per mice, n=5). Asterisk – single-labeled hepatocyte, arrow – single labeled BEC, arrowhead – double labeled BEC; TA, transit amplifying; ADC, atypical ductal cells; BEC, biliary epithelial cell; IdU, Iododeoxyuridine; CldU, Chlorodeoxyuridine; PanCK, Pancytokeratin; PO, per os; Sac, sacrifice. Scale bar=50μm.

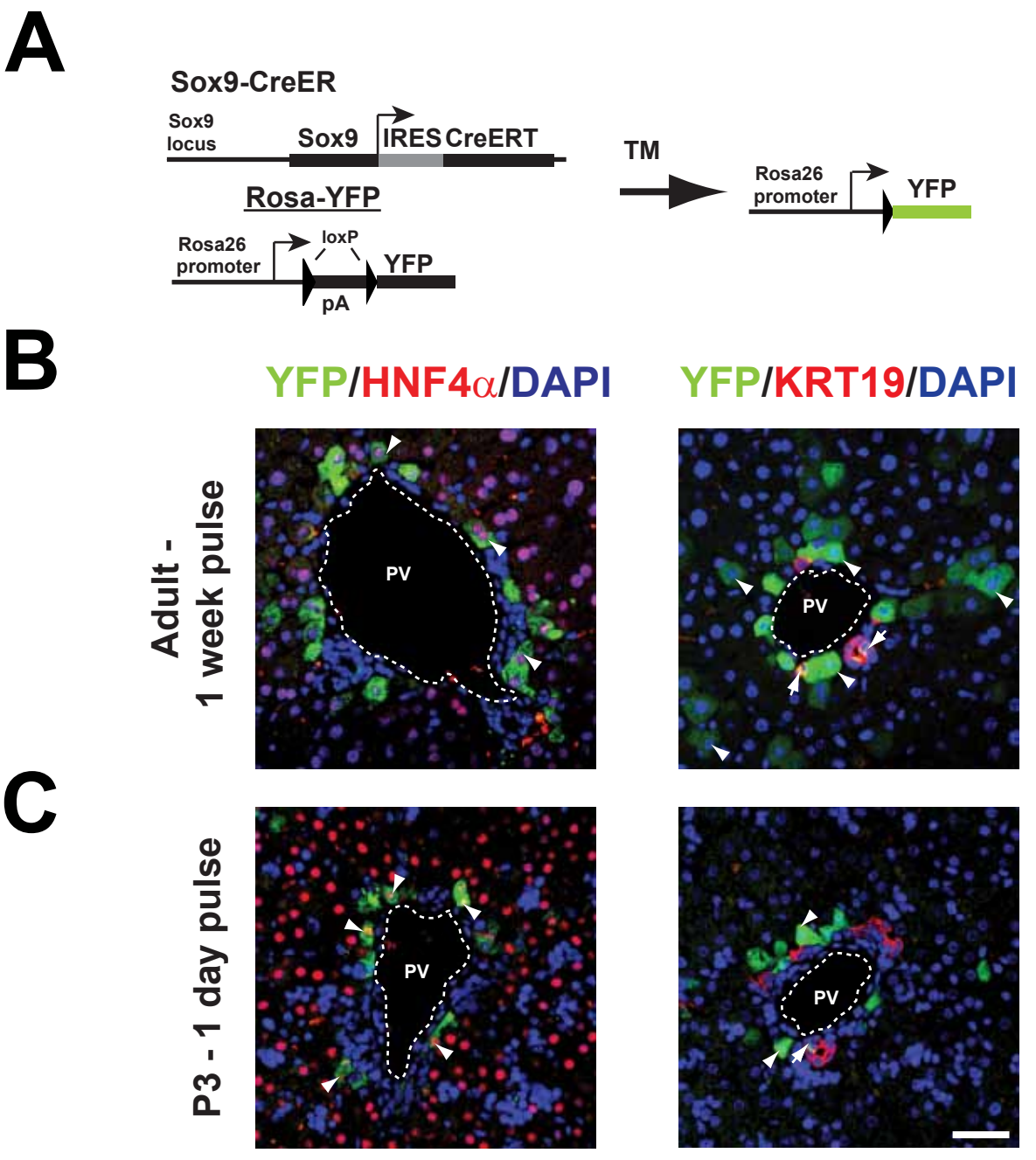

A
Sox9-CreER
Sox9 locus
Sox9
IRES CreERT
Rosa-YFP
Rosa26 promoter
loxP
YFP
pA
TM
Rosa26 promoter
YFP
B
YFP/HNF4α/DAPI
YFP/KRT19/DAPI
Adult - 1 week pulse
PV
PV
C
P3 - 1 day pulse
PV
PV

Figure S4

Figure S4, Related to Discussion. *Sox9-CreER* labels BECs and hepatocytes.

(A) Schematic view of lineage tracing following administration of tamoxifen (TM) to Sox9-*CreER*;*R26*^{YFP} mice.

(B) 6-8 week adults given 40 mg of TM result in labeling of hepatocytes (arrowheads) and inefficient labeling of Krt19^{+} BECs (arrows) after 1 week.

(C) TM administration to a nursing mother and examination 1 day later reveals efficient labeling of hepatocytes (arrowheads) and inefficient labeling BECs (arrows). Scale bar=50μm.

Table S1. List of Antibodies Used

Antibody	Species	Source	Catalog #	Dilution
Cytokeratin 19	Rabbit	D. Melton	NA	1:1000
GFP	Chicken	Abcam	ab13970	1:500
GFP	Goat	Abcam	ab6673	1:500
A6	Rat	V. Factor	NA	1:100
Hnf4α	Goat	Santa Cruz	SC-6556	1:250
Ki-67	Mouse	BD	561165	1:100
1D11	Rat	Novus Biologicals	NBP1-18963	1:100
4E8	Rat	Novus Biologicals	NBP1-18971	1:100
2F3	Rat	Novus Biologicals	NBP1-18964	1:100
3C7	Rat	Novus Biologicals	NBP1-18970	1:100
TROP2	Mouse	R&D	AF1122	1:100
IdU (anti BrdU)	Mouse	BD	347580 (7580)	1:200
CldU (anti BrdU)	Rat	Accurate	OBT0030	1:500
Pancytokeratin	Rabbit	DAKO	Z0622	1:100
Ki-67	Rabbit	Thermo Scientific	MA1-90584	1:100