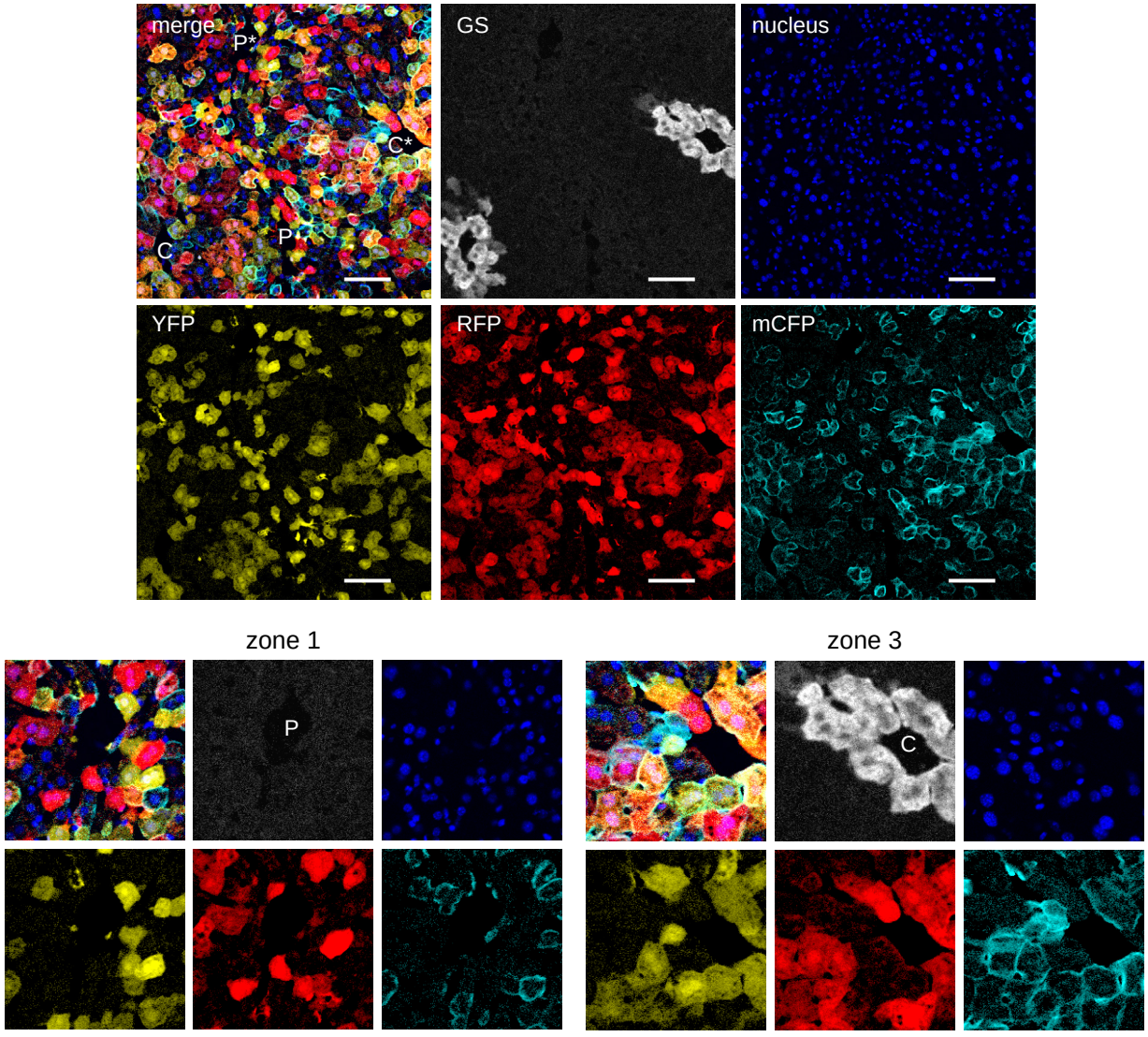
Figure S1

A


merge
P*
C*
C
P
GS
nucleus
YFP
RFP
mCFP
zone 1
zone 3
P
C

B

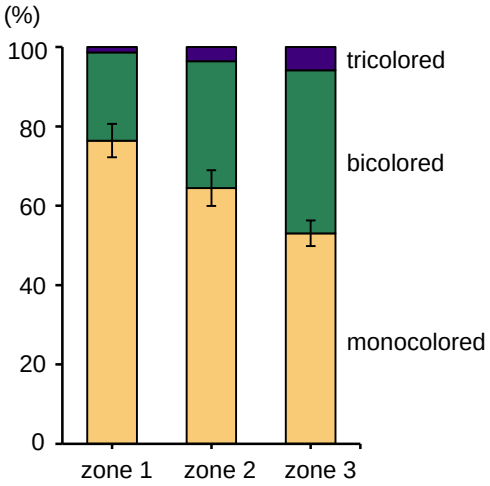

(%)
100
80
60
40
20
0
tricolored
bicolored
monocolored
zone 1
zone 2
zone 3

Figure S1. Zonal distribution of multicolored hepatocytes in Ubc-CreERT2/Rosa-Confetti$^{+/-}$ mice, Related to Figure 1.

(A) Representative images of immunofluorescence for a zone 3 marker, GS. Lower panels show high-magnification images of periportal and pericentral areas, which are indicated by asterisks. C, central veins; P, portal veins. Scale bars: 100 μm. (B) Frequencies of mono-, bi-, and tri-colored hepatocytes among labeled cells in each zone. More than 2000 cells in 4 mice were analyzed.

Figure S2

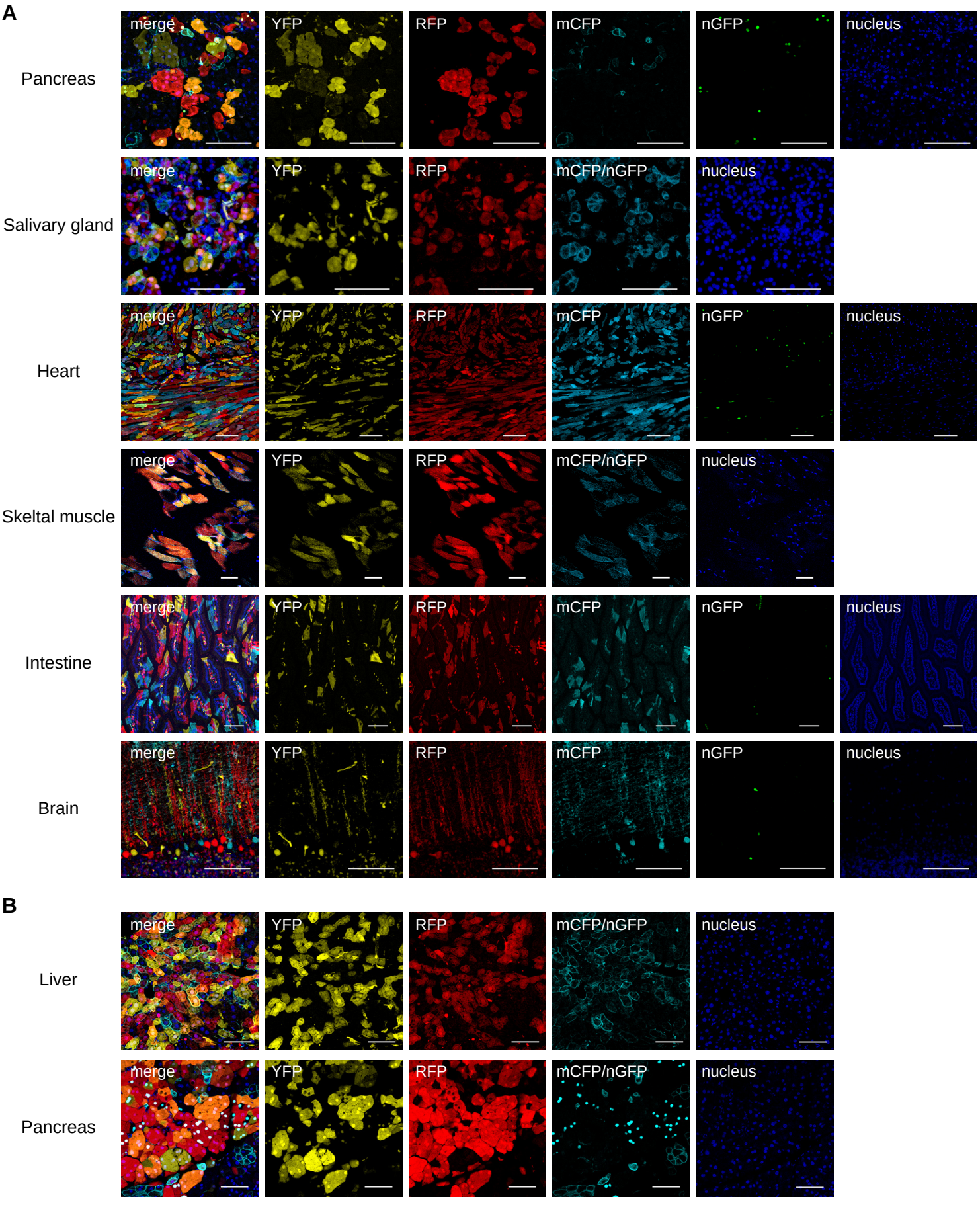

A
Pancreas
merge
YFP
RFP
mCFP
nGFP
nucleus
Salivary gland
merge
YFP
RFP
mCFP/nGFP
nucleus
Heart
merge
YFP
RFP
mCFP
nGFP
nucleus
Skeltal muscle
merge
YFP
RFP
mCFP/nGFP
nucleus
Intestine
merge
YFP
RFP
mCFP
nGFP
nucleus
Brain
merge
YFP
RFP
mCFP
nGFP
nucleus
B
Liver
merge
YFP
RFP
mCFP/nGFP
nucleus
Pancreas
merge
YFP
RFP
mCFP/nGFP
nucleus

Figure S2. Merged and single color images of Ubc-CreERT2/Rosa-Confetti$^{+/-}$ mice, Related to Figure 1.

(A) Single color images of Figure 1G. (B) Single color images of Figure 1H. Note that a part of nGFP signal leaks into the YFP channel when nGFP is expressed. To enhance the sensitivity for mCFP detection, mCFP was detected in combination with nGFP detection in some organs. Signals of mCFP and nGFP are distinguishable according to their membrane- and nuclear-localized expressions, respectively. Scale bars: 100 μm.

Figure S3

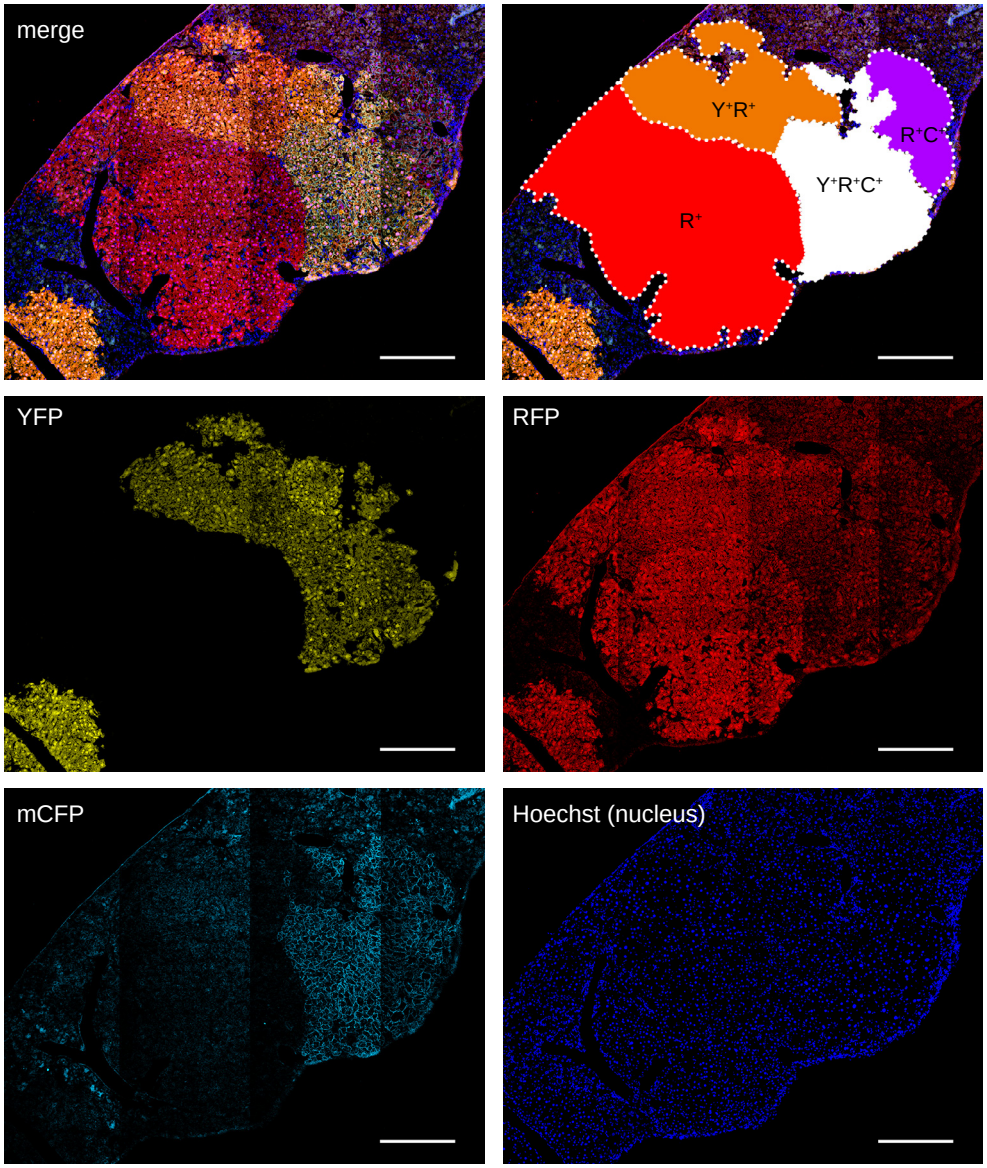


Figure S3. Representative microscopic images of the recipient liver repopulated with YFP^+RFP^+ 8c donor-derived cells, Related to Figure 2.

Images of a mouse which is different from that in Figure 2H is shown. Images were stitched to generate large composite images. Each clonally repopulated area is indicated by dotted lines in the schematic diagram. Y^+, YFP^+; R^+, RFP^+; C+, $mCFP^+$. Scale bars = 500 μm.

Figure S4

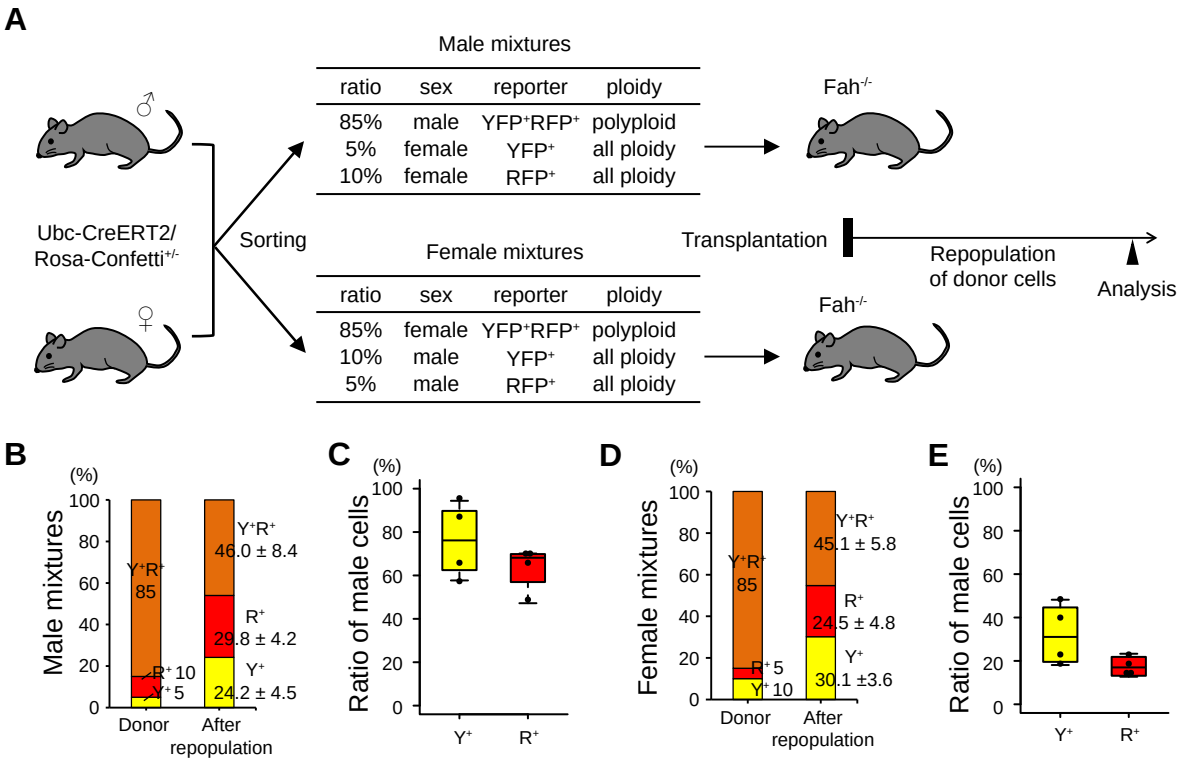
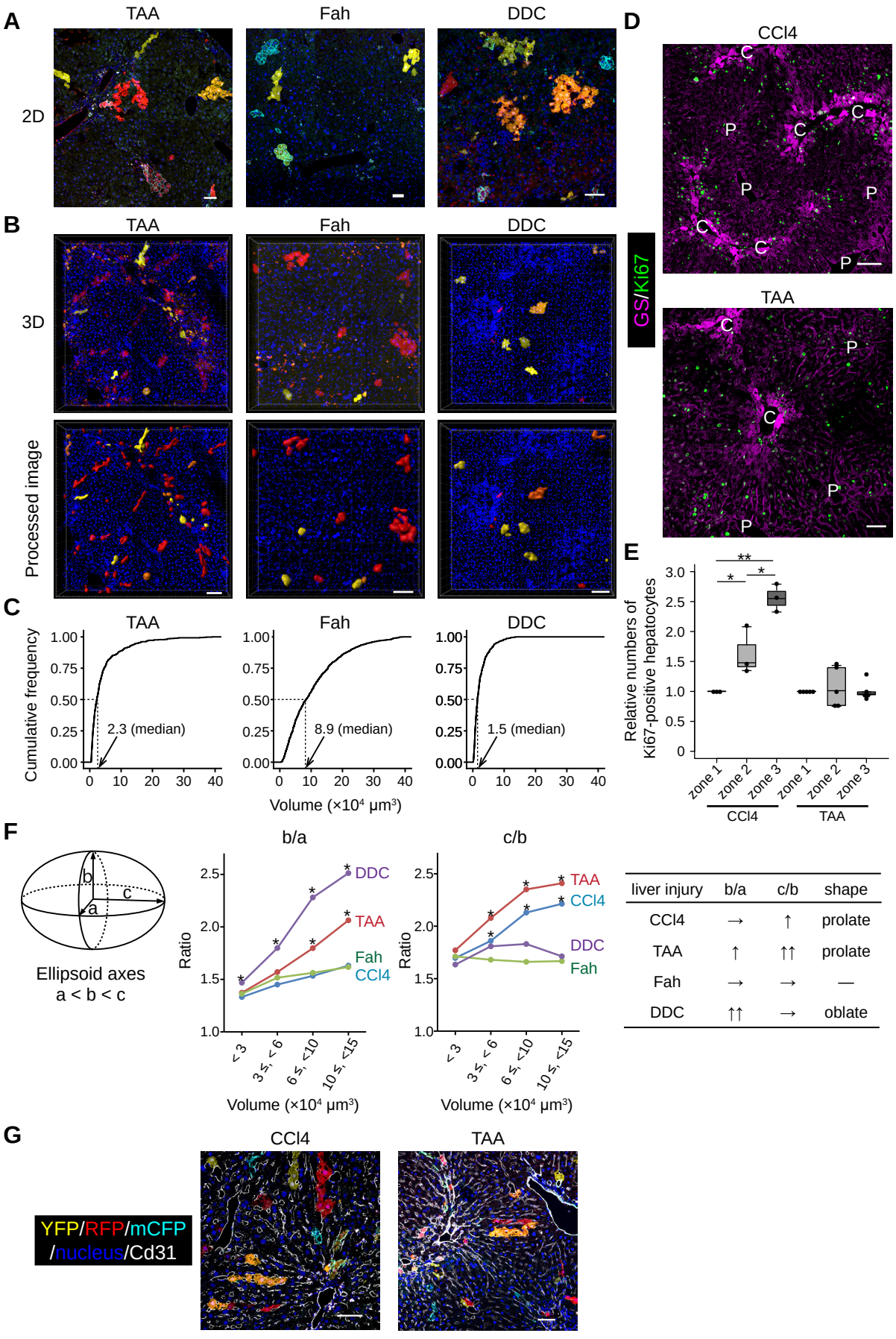

A
Male mixtures
ratio sex reporter ploidy
85% male YFP+RFP+ polyploid
5% female YFP+ all ploidy
10% female RFP+ all ploidy
Fah-/-
Ubc-CreERT2/
Rosa-Confetti+/-
Sorting
Female mixtures
ratio sex reporter ploidy
85% female YFP+RFP+ polyploid
10% male YFP+ all ploidy
5% male RFP+ all ploidy
Fah-/-
Transplantation
Repopulation
of donor cells
Analysis
B
Male mixtures
(%)
Y+R+ 85
R+ 10
Y+ 5
Y+R+ 46.0 ± 8.4
R+ 29.8 ± 4.2
Y+ 24.2 ± 4.5
Donor
After repopulation
C
Ratio of male cells
(%)
Y+
R+
D
Female mixtures
(%)
Y+R+ 85
R+ 5
Y+ 10
Y+R+ 45.1 ± 5.8
R+ 24.5 ± 4.8
Y+ 30.1 ±3.6
Donor
After repopulation
E
Ratio of male cells
(%)
Y+
R+

Figure S4. Intentional contamination experiments to evaluate the impact of contaminated monocolored cells, Related to Figure 2.

(A) Experimental scheme of transplantation using male and female donors. Donor cells were isolated from male and female littermate mice, and mixed as indicated after sorting based on reporter expressions and cellular ploidy (Hoechst). Mixtures of donor cells were transplanted into $Fah^{-/-}$ mice and analyzed after repopulation. (B–E) The composition of donor-derived cells in the livers repopulated with male mixtures (B, C) and female mixtures (D, E). (B, D) The composition of donor-derived cells analyzed by reporter expressions on flow cytometry. Values are shown as means ± SD (n = 4). (C, E) Ratio of male cells among YFP^+ or RFP^+ monocolored cells in the repopulated livers. Monocolored cells were sorted by FACS from the repopulated livers, and extracted DNAs were analyzed by quantitative PCR. The quantified copy number values of Y chromosome (*Sry* gene) were normalized to those of *Fah* gene, and the ratio of male cells was calculated based on standard samples. Box plots show the median and IQR between 25th and 75th percentile, and whiskers show the lowest data within 25th percentile – 1.5 × IQR and the highest data within 75th percentile + 1.5 × IQR. Y^+, YFP^+; R^+, RFP^+.

Figure S5



liver injury	b/a	c/b	shape
CCl4	→	↑	prolate
TAA	↑	↑↑	prolate
Fah	→	→	—
DDC	↑↑	→	oblate

Figure S5. Tracing of single cell-derived clonal expansions in chronically injured livers, Related to Figure 4.

(A and B) Representative images of the livers chronically injured with TAA (left), Fah deficiency (middle), and DDC (right). 2D (A) and 3D (B) images obtained with confocal microscopy are shown. Images were stitched to generate large composite images. Note that autofluorescent signals of deposits are frequently observed in 3D images (B, top panels). Processed images for extraction of YFP^+ and/or RFP^+ cells are also shown (B, bottom panels). (C) Cumulative frequency graphs of the volumes of YFP^+ and/or RFP^+ clonal areas. More than 500 clones in 3 or more mice were analyzed. The median values are indicated. (D) Immunofluorescence for GS and Ki67 of the livers injured with CCl4 or TAA. C, central veins; P, portal veins. (E) Zonal distribution of Ki67-positive hepatocytes in the livers injured with CCl4 or TAA. Numbers of Ki67-positive nuclei per unit area were collected in each zone, and their relative values compared to that of zone 1 were calculated in each mouse. Three and 5 mice were analyzed in CCl4- and TAA-induced injury, respectively. * $p < 0.05$, ** $p < 0.01$. (F) Morphological changes of clonal areas. The lengths of ellipsoid axes of clones were obtained with Imaris, and the ratios of three axes (a, b, and c) were calculated. Asterisks indicate significant difference ($p < 0.05$) from mice injured with Fah deficiency which exhibit constant axis ratios during regeneration. (G) Representative images of immunofluorescence for Cd31. The livers injured with CCl_4 or TAA are shown. The livers were harvested at 4 weeks, 3 months, 5 months, and 3 months after the initiation of injuries in CCl4, TAA, Fah deficiency, and DDC models, respectively. Scale bars: 100 μm.

Figure S6

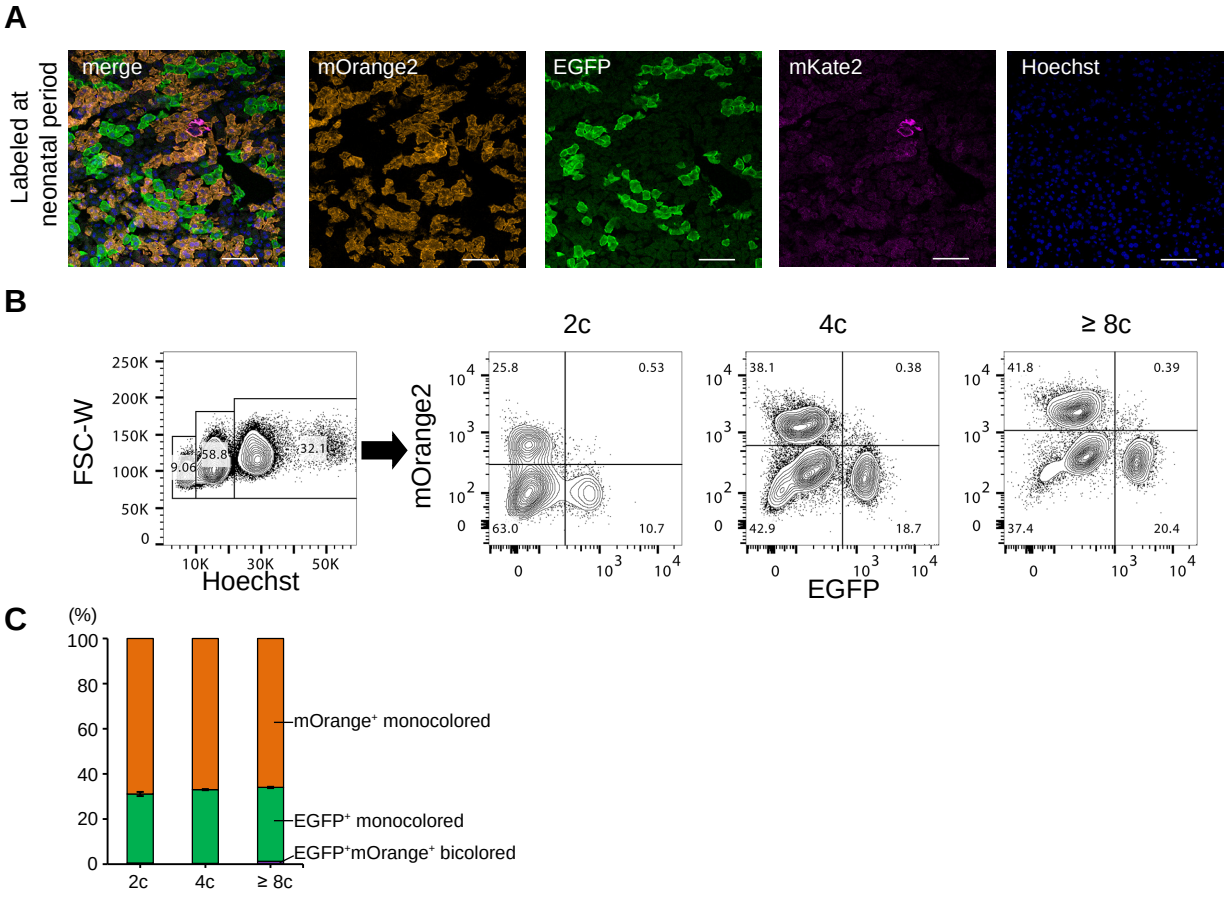

A
Labeled at neonatal period
merge
mOrange2
EGFP
mKate2
Hoechst
B
2c
4c
≥ 8c
FSC-W
Hoechst
mOrange2
EGFP
25.8
0.53
63.0
10.7
38.1
0.38
42.9
18.7
41.8
0.39
37.4
20.4
9.06
58.8
32.1
C
(%)
mOrange+ monocolored
EGFP+ monocolored
EGFP+mOrange+ bicolored
2c
4c
≥ 8c

Figure S6. Rosa-RGBow $^{+/-}$ mice labeled at neonatal period, Related to Figure 5.

(A, B) Representative microscopic images (A) and FACS plots (B) of Rosa-RGBow$^{+/-}$ livers. Mice were labeled with high-dose AAV8-Ttr-Cre on postnatal day1, and analyzed at 8–9 weeks of age. Scale bars: 100 μm. (C) Labeling frequencies of diploid and polyploid hepatocytes estimated by flow cytometry. Only cells positive for mOrange2 or EGFP are analyzed.

Figure S7

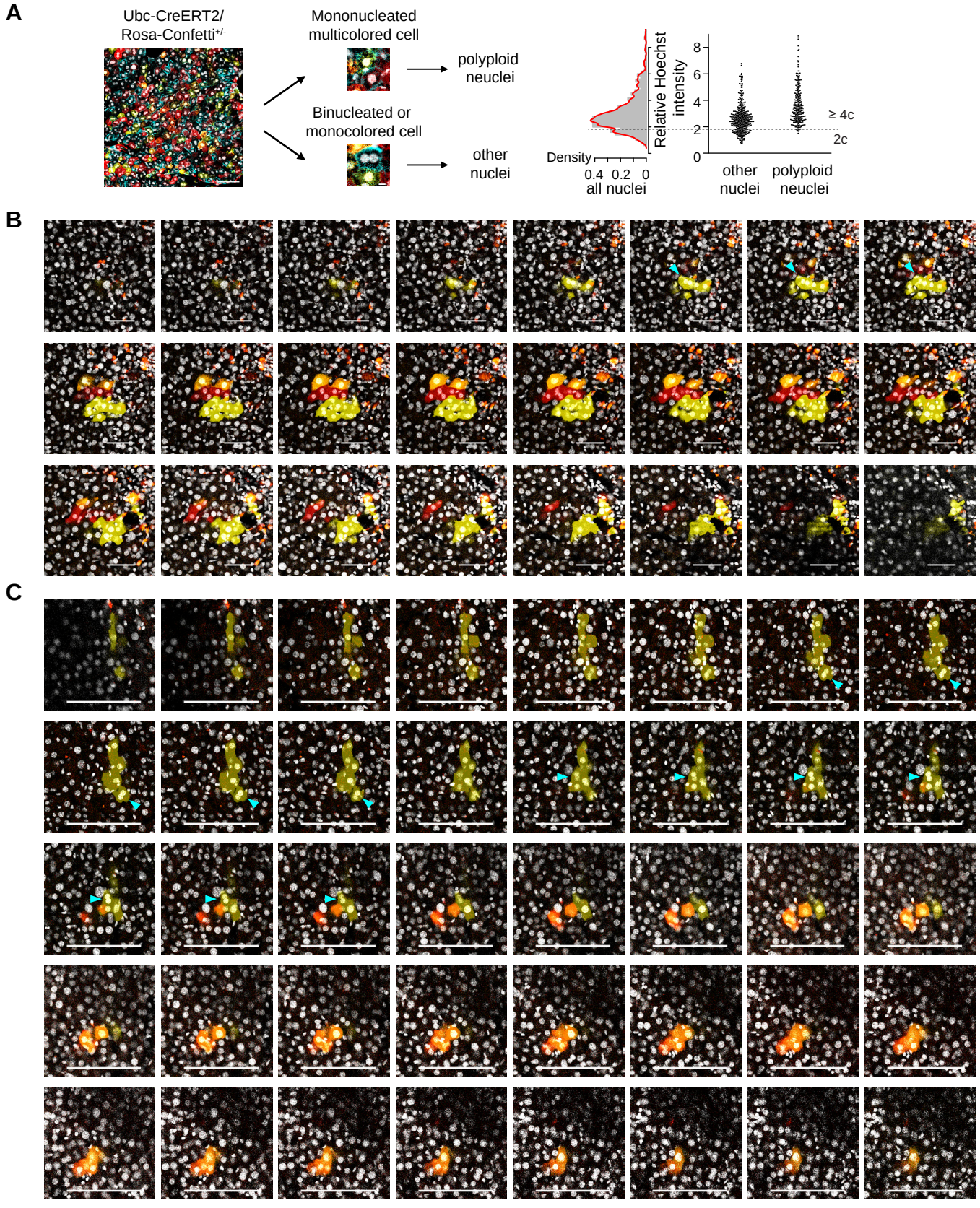

A
Ubc-CreERT2/
Rosa-Confetti+/-
Mononucleated
multicolored cell
polyploid
neuclei
Binucleated or
monocolored cell
other
nuclei
Relative Hoechst
intensity
Density
0.4 0.2 0
all nuclei
8
6
4
2
0
≥ 4c
2c
other
nuclei
polyploid
neuclei
B
C
D
Relative Hoechst
intensity
8
6
4
2
0
≥ 4c
2c
(-)
2 w
4 w
CCl4 injury
E
merge
mCFP
RFP
YFP
Hoechst
≥ 4c
2c
binucleated
(2c + 2c)
YFP/RFP/
mCFP/Hoechst

Figure S7. Binucleated cells with ploidy-reduced nuclei confirmed by microscopic cytometry, Related to Figure 6.

(A) Microscopic cytometry of Ubc-CreERT2/Rosa-Confetti$^{+/-}$ mice. The livers were analyzed after tamoxifen washout for more than 3 weeks. Nuclei of multicolored mononucleated cells were analyzed as polyploid nuclei (n = 283), and those of binucleated or monocolored cells were grouped as other nuclei (n = 452). Normalized Hoechst intensity of each nucleus was plotted as a beeswarm plot, and a histogram of those of all nuclei analyzed was shown in parallel. The threshold which divided polyploid from diploid was determined as a line that was close to the lower edge of polyploid nuclei distribution and passed through the trough point of all nuclei distribution, and indicated by a dotted line. More than 97% of multicolored mononucleated cells were correctly categorized as polyploid by this borderline. Scale bars are 100 μm and 10 μm in an overview image of the liver and enlarged hepatocyte images, respectively. Images were stitched to generate a large composite image. (B, C) Donor-derived clones in wild-type mouse livers transplanted with YFP+RFP+ bicolored polyploid hepatocytes followed by CCl4 injury. Serial z-stack images of clones shown in Figures 6D and 6E are indicated in (B) and (C), respectively. Nuclei stained with Hoechst are visualized by white pseudocolor, and binucleated cells with ploidy-reduced nuclei are indicated by arrowheads. Scale bars: 100 μm. (D) Microscopic cytometry of sparsely-labeled Confetti$^{+/-}$ livers with and without CCl4. Sparsely-labeled livers analyzed in Figure 4 were examined (3 mice in each group). Consistent with Figure 4C, almost all labeled hepatocytes were polyploid before injury. Emergence of labeled diploids in the CCl4-injured livers suggests ploidy reduction. (E) A

regenerative clone suggestive of ploidy reduction and subsequent re-polyploidization. Sparsely-labeled Confetti$^{+/-}$ livers injured with CCl4 for 4 weeks were analyzed. A bicolored polyploid, a monocolored diploid, and a monocolored polyploid cell with two ploidy-reduced (diploid) nuclei are indicated. The binucleated cell with ploidy-reduced nuclei suggests re-polyploidization. Scale bars: 100 μm.