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Supplemental Information

Adult Mouse Liver Contains Two Distinct Populations of Cholangiocytes

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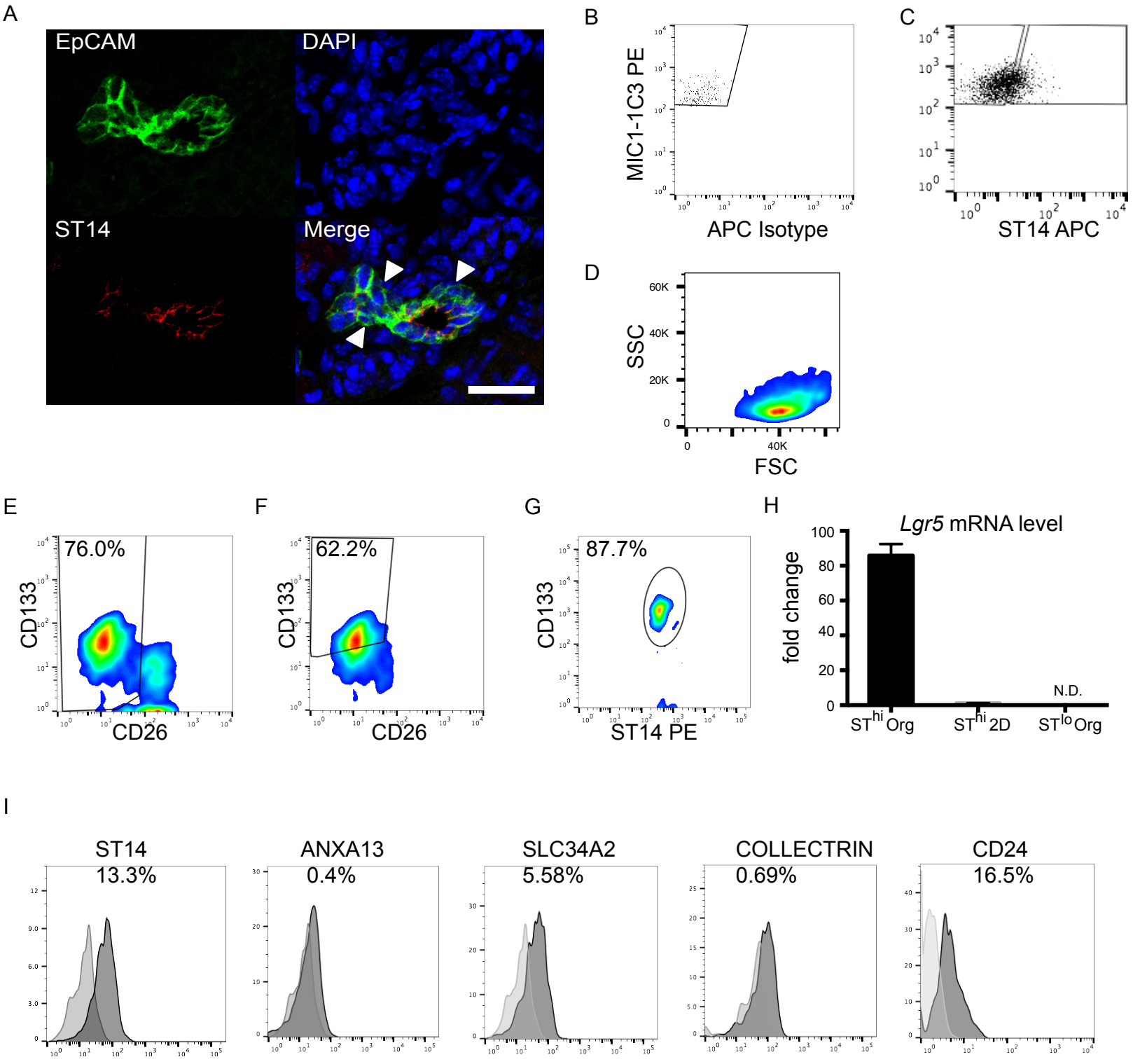

Figure S1 Related to Figure1. Evaluation of duct-subdividing antibody candidates.
(A) Representative immunohistochemistry of ST14 (red) and EpCAM (green) colocalization in mouse liver duct cells. DAPI: blue. Scale bar = 50 μm. (B-D) Flow cytometry analyses of M+26-CD45/31/11b-PI- cells stained with (B) secondary APC isotype control.only and (C) the ST14 primary antibody. (D) Size and scatter properties of fully gated ST14loM+ cells. (E-G) Flow cytometry analyses showing the extent of CD133 and ST14 coexpression in duct cells. (E) 76% of M+CD45/31/11b-PI- cells are CD26 negative. (F) in the M+CD26-CD45/31/11b-PI- cells from (E) 62.2% are CD133 expressing. (G) 87.7% of the M+ST14hi cells are CD133 positive. (H) *Lgr5* mRNA expression was activated by the organoid culture conditions in the ST14hi but not in the ST14lo organoids. (I) Antigen screening for ST14, ANXA13, SLC34A2, COLLECTRIN and CD24 in the CD26-M+ duct cells. Lighter grey delineates the isotype control.

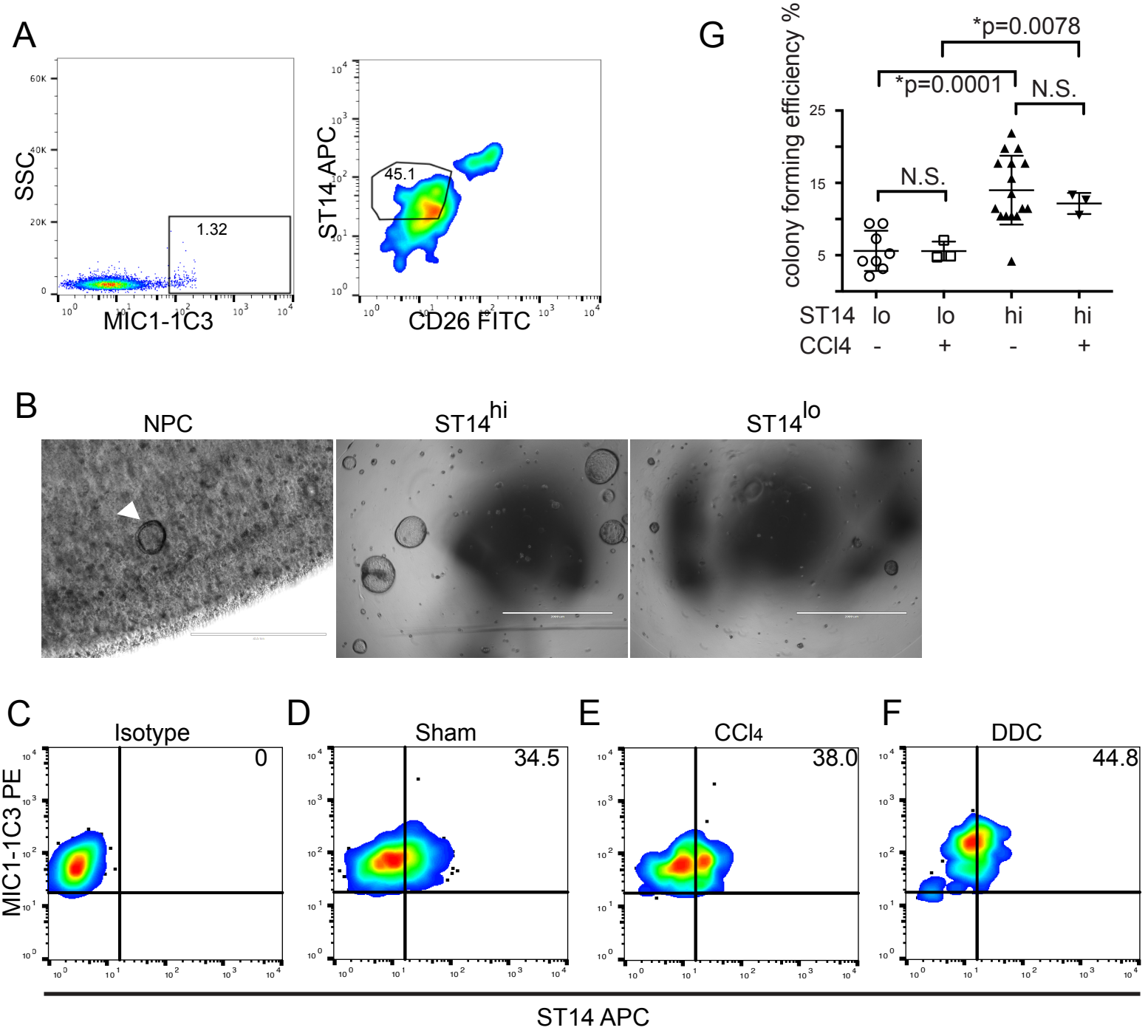

Figure S2 Related to Figure 2. Clonogenic duct cells survival during ischemia and in the injured liver
Flow cytometry analyses for MIC1-1C3+ and ST14. 45% of MIC1-1C3 duct cells were ST14hi after 24hrs of warm ischemia. After warm ischemia organoid formation was strictly limited to only ST14hi ductal cells. Far left panel: unsorted liver NPCs in organoid cell culture. FACS sorted ST14hi (middle panel) and ST14lo (far right panel) cells were embedded in Matrigel droplets at a density of 2,000 per 24 well. Culture date 14. No organoids formed from the ST14lo population. (C-G) ST14 expression in M+ liver duct cells in uninjured controls (D) and CCl4 induced liver injury day 6 (E) and DDC treated liver day 6 (F). CCl_4 was diluted in corn oil and administrated via I.P. injection at single dose of 1ml/kg body weight. Sham: corn oil only. Isotype control was used as negative gating. (G) NPCs from livers injured by CCl_4 were sorted into 96 well dishes for single cell organoid analysis. There were no significant injury-induced differences in the organoid forming ability of either population. ST14hi cells were superior to ST14lo duct cells under both conditions. Statistical analyses: Mann-Whitney U test for normal vs treated, paired t test for ST14hi vs ST14lo. Independent mice t reated with CCl_4 n=3; Independent mice treated with sham n=4.

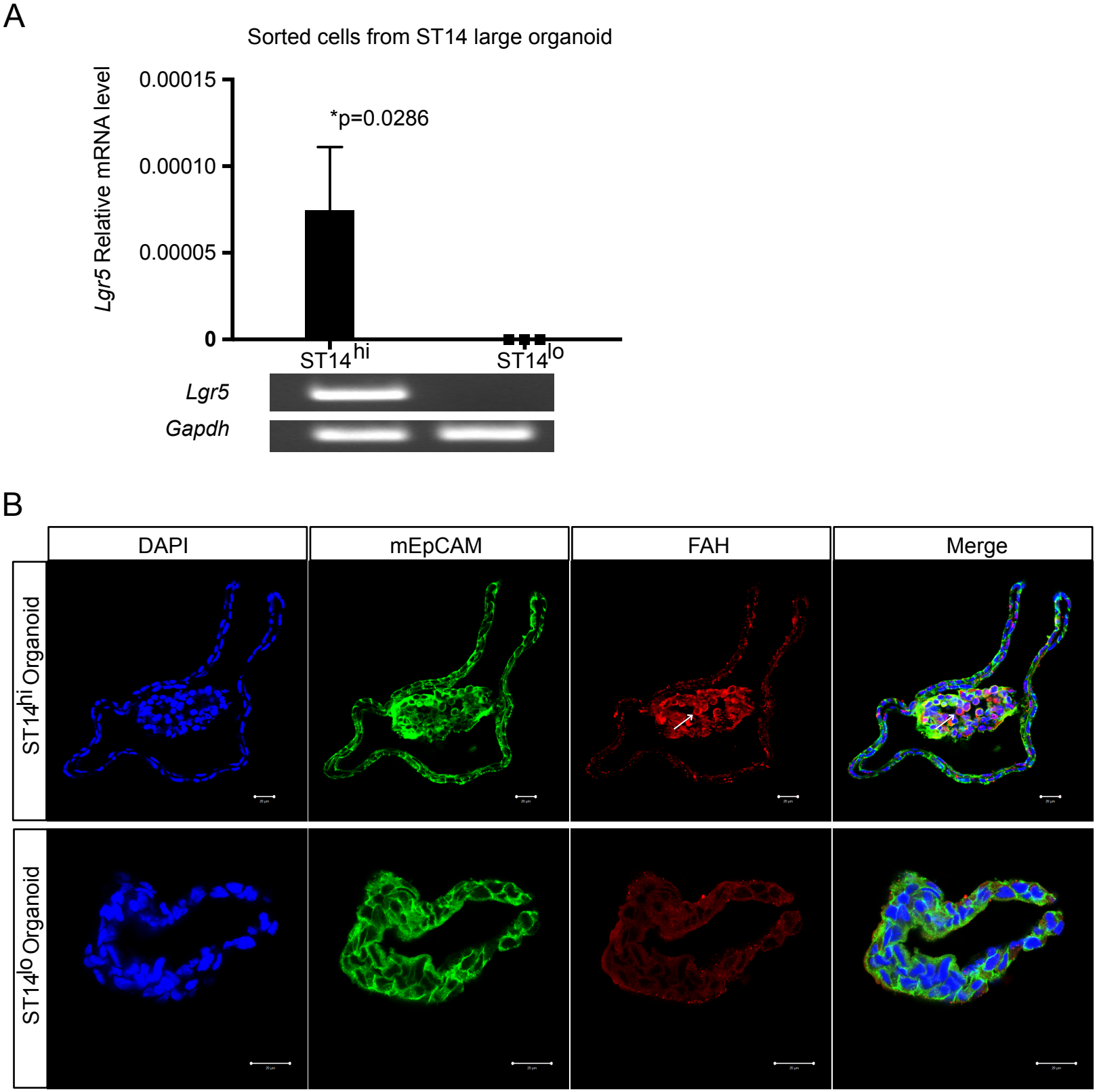

Figure S3. Related to Figure 3. Characterization of ST14hi derived organoids in vitro.
ST14hi derived organoids at passage 1 were dissociated and resorted using surface maker ST14 as in Figure 2I. (A) Relative *Lgr5* mRNA level from the ST14hi and ST14lo population detected by qPCR. Relative *Lgr5* mRNA expression was normalized to house keeping gene *Gapdh*. Single bands for the *Lgr5* and *Gapdh* PCR products were confirmed by agarose gel. Quantities were calculated with –ΔΔCt. Independent experiments n=3, upaired t test. *P=0.0286. (B) Immunofluorence staining showed Fah expression in cultured ST14hi and ST14lo derived organoids. Arrow shows FAH+ but EpCAM- cells. Scale bar = 20 μm.

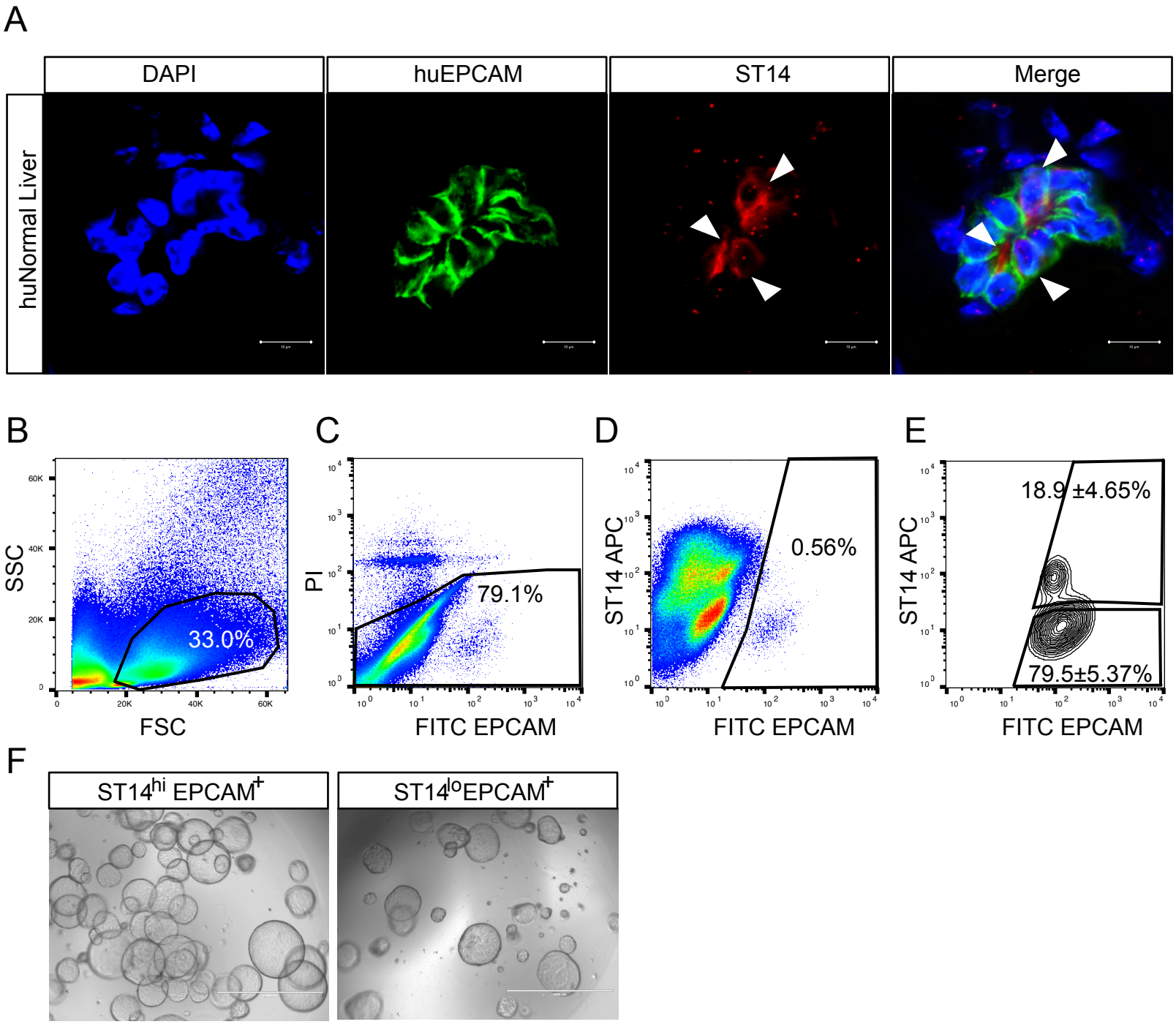

Figure S4. Related to Figure 5. ST14 protein expression in human liver.
(A) Immunofluorence showed the ST14(red) expression in the EPCAM(green) expressing duct cells in the human liver. Nuclei stained with DAPI as blue. Arrow heads show the ST14 expressing cells. Scale bar = 20 μm. (B-E) Primary human liver NPCs were FACS analyzed after staining with EPCAM and ST14. In the EPCAM+ subpopulation, 18.9±4.65% cells were ST14hi (Independent patients n=3, unpaired t test, *p=0.001) (E). (F) Sorted cells were put into matrigel organoid culture at a density of 2000 cells/well in the 24 well suspension culture plate. Scale bar = 2 mm.

Table S1 Selected hepatic biliary and progenitor markers in $ST14^{hi}$ vs $ST14^{lo}$ populations

Gene		Transcript	$ST14^{hi}$	$ST14^{lo}$	FC	Pval	FDR
St14	Suppression of tumorigenicity 14 (colon)	NM_011176	100	100	1.1	1.09	0.35
Krt19	Keratin 19	NM_008471	617.75	428.5	-1.44	0.32	0.54
Sox9	SRY (sex determining region Y)-box 9	NM_011448	114.3	119.8	-1.05	0.56	0.65
Epcam	Epithelial cell adhesion molecule	NM_008532	334.3	346.8	-1.04	0.67	0.69
Hnf1b	Transcription factor 2,transcription factor 2,	NM_009330	195.75	207.25	1.06	0.55	0.65
Cftr	Cystic fibrosis transmembrane conductance	NM_021050	16.25	20.5	1.26	0.07	0.32
Prom1	Prominin 1 (CD133 antigen)	NM_008935	181.75	187.75	1.03	0.83	0.73
Lgr5	Leucine rich repeat containing G protein coupled receptor 5	NM_010195	0.25	0	2.5	0.02	0.17
Thy1	Thymus cell antigen 1, theta	NM_009382	3	1.25	2.4	0.11	0.37
Icam1	Intercellular adhesion molecule 1	NM_010493	59.5	36.25	1.64	1	1
Bmi1	Bmi1 polycomb ring finger oncogene	NM_007552	32.75	34.75	-1.06	0.7	0.7
Cd47	CD47 antigen	NM_010581	52.25	67.5	-1.29	0.06	0.3

Tag numbers are given in RPKM for the $ST14^{hi}$ and $ST14^{lo}$ populations; FC = fold change. None of the genes listed were differentially expressed between progenitor and non-progenitor ducts.

Table S2 Selected gene sets significantly enriched in the clonogenic ST14^{hi} population

NAME	ES	NOM p-val	FDR q-val
BOQUEST_STEM_CELL_UP	0.53	0.00	0.00
ANASTASSIOU_CANCER_MESENCHYMAL_TRANSITION_SIGNATURE	0.64	0.00	0.00
LIM_MAMMARY_STEM_CELL_UP	0.46	0.00	0.00
LIEN_BREAST_CARCINOMA_METAPLASTIC	0.67	0.00	0.00
VECCHI_GASTRIC_CANCER_ADVANCED_VS_EARLY_UP	0.47	0.00	0.00
ONDER_CDH1_SIGNALING_VIA_CTNNB1	0.53	0.00	0.00
SERVITJA_ISLET_HNF1A_TARGETS_UP	0.47	0.00	0.00
MIKKELSEN_IPS_ICP_WITH_H3K4ME3_AND_H327ME3	0.50	0.00	0.00
STAMBOLSKY_TARGETS_OF_MUTATED_TP53_UP	0.56	0.00	0.01
EBAUER_TARGETS_OF_PAX3_FOXO1_FUSION_UP	0.43	0.00	0.01
ACEVEDO_LIVER_CANCER_WITH_H3K27ME3_DN	0.44	0.00	0.01
KEGG_HEDGEHOG_SIGNALING_PATHWAY	0.50	0.00	0.02
PLASARI_TGFB1_TARGETS_10HR_UP	0.38	0.00	0.02
BOQUEST_STEM_CELL_CULTURED_VS_FRESH_UP	0.35	0.00	0.02
SWEET_KRAS_TARGETS_UP	0.44	0.00	0.02
WIEDERSCHAIN_TARGETS_OF_BMI1_AND_PCGF2	0.49	0.00	0.02
DASU_IL6_SIGNALING_SCAR_UP	0.52	0.00	0.03
VERRECCHIA_RESPONSE_TO_TGFB1_C2	0.54	0.00	0.03
BOQUEST_STEM_CELL_CULTURED_VS_FRESH_DN	0.55	0.00	0.03
TURASHVILI_BREAST_DUCTAL_CARCINOMA_VS_DUCTAL_NORMAL_DN	0.39	0.00	0.03
SCHUETZ_BREAST_CANCER_DUCTAL_INVASIVE_UP	0.35	0.00	0.03
JECHLINGER_EPITHELIAL_TO_MESENCHYMAL_TRANSITION_UP	0.44	0.00	0.03
PID_HEDGEHOG_2PATHWAY	0.55	0.01	0.03
GUO_HEX_TARGETS_UP	0.43	0.00	0.04
LEE_LIVER_CANCER_HEPATOBLAST	0.62	0.01	0.04
IVANOVA_HEMATOPOIESIS_STEM_CELL_LONG_TERM	0.35	0.00	0.04
CHIANG_LIVER_CANCER_SUBCLASS_CTNNB1_DN	0.38	0.00	0.04
WHITFIELD_CELL_CYCLE_G1_S	0.37	0.00	0.06
CONRAD_STEM_CELL	0.50	0.02	0.06
SAKAI_CHRONIC_HEPATITIS_VS_LIVER_CANCER_DN	0.49	0.02	0.06
PLASARI_TGFB1_TARGETS_10HR_DN	0.33	0.00	0.08
ELVIDGE_HIF1A_AND_HIF2A_TARGETS_DN	0.37	0.00	0.08
ZHANG_GATA6_TARGETS_DN	0.39	0.01	0.09
PID_BETA_CATENIN_DEG_PATHWAY	0.54	0.05	0.09
HAN_JNK_SINGALING_DN	0.45	0.02	0.09
HOSHIDA_LIVER_CANCER_SURVIVAL_UP	0.38	0.01	0.09

Table S3 List of antibodies used

1st Antibodies

Name	IgG type	Use	Dilution	Company	Lot
ALB	Goat pAB	IF	100	Bethyl	A90-134A-6
ANXA13	Rabbit pAB	IF	100	Sigma-Aldrich	HPA019650
CD11b	Rat mAB	FACS	100	BD biosciences	552850
CD133	Biotin	FACS	100	eBioscience	13-1331-82
CD24	Rat mAB	FACS	100	BD biosciences	561079
CD31	Rat mAB	FACS	100	BD biosciences	561410
CD45	Rat mAB	FACS	100	BD biosciences	552848
CK19	Rabbit pAB	IF	500	Cell Lab Tech	A03189
COLLECTRIN	Sheep pAB	FACS	100	R&D systems	AF4965
EpCAM	Rat mAB	IF	100	BD biosciences	G8.8
EpCAM	Mouse mAB	huIF, FACS	100	DAKO	Ber-EP4
FAH	Rabbit pAB	IF	50	Grompe lab	
HNF4A	Rabbit pAB	IF	100	Santa Cruz Bio	sc-8987
MIC1-1C3	Rat mAB	FACS	20	Grompe lab	
SLC34A2	Rabbit pAB	FACS	100	Abbiotech	251343
ST14	Rabbit pAB	FACS	100	Abcam	ab28266
ST14	Rabbit pAB	huIF, FACS	100	Invitrogen	PA5-29764

2nd Antibodies

Name	IgG type	Use	Dilution	Company	Lot
Anti rat DL649	Mouse	FACS	200	JacksonImmuno	212-496-168
Anti rabbit APC	Donkey	FACS	200	JacksonImmuno	711-056-152
Anti rabbit PE	Donkey	FACS	200	JacksonImmuno	711-116-152
Anti Rat PE	Goat	FACS	200	JacksonImmuno	112-116-143
Anti rabbit Cy3	Donkey	IF	250	JacksonImmuno	711-166-152
Anti sheep AF647	Donkey	FACS	250	Jacksonimmuno	713-606-147
APC/cy7	Streptavidin	FACS	250	Biolegend	405208

Table S4 Human samples information

Use	Gender	Age	Source
IHC	Unknown	Unknown	Gift from Strom lab at University of Pittsburg, U.S.A
FACS	Female	17	Lonza, LLC. U.S.A
FACS	Male	29	Lonza, LLC. U.S.A
FACS, culture	Female	48	Lonza, LLC. U.S.A