Supporting Table S1: Primer list

Genotyping Primer name	Version in text	Sequence
mm_Wls_Exon3_fw	Wls	TCTGTACACATTCCCCTCCCT
mm_Wls_Exon3_rv	Wls	ACGTGGTACTCTTTCATGGGC
mm_HMGCR_fw	HMGCR	CTTGTGGAATGCCTTGTGATTG
mm_HMGCR_rv	HMGCR	AGCCGAAGCAGCACATGAT
mm_HMOX1_fw	HMOX1	GGAGATAGAGCGCAACAAGC
mm_HMOX1_rv	HMOX1	CCATACCAGAAGGCCATGTC
mm_CYP7A1_fw	CYP7A1	AGCAACTAAACAACCTGCCAGTACTA
mm_CYP7A1_rv	CYP7A1	GTCCGGATATTCAAGGATGCA
mm_ACTB_fw	β-Actin	GGATGCCACAGGATTCCATAC
mm_ACTB_rv	β-Actin	TCTACGAGGGCTATGCTCTCC
mm_RosaEvi_common_fw	PRE1	GTCGCTCTGAGTTGTTATCAG
mm_RosaEvi_flox_rv	PRE2	GACGACAGTATCGGCCTCAGGAAG
mm_RosaEvi_wt_rv	PRE3	CCAGATGACTACCTATCCTCC
mm_GPR177_fw	PW1	TGTTGGAGGGATTCTTCTGG
mm_GPR177_rv	PW2	ATTGCCGTGTAGGGTACTGC
Cre_iCre (common)_fw	PC1	AAGAACCTGATGGACATGTTCAGG
iCre_rv	PC2	TCTGTCAGAGTTCTCCATCAGGGA
mm_YFP_common_fw	PY1	AAAGTCGCTCTGAGTTGTTAT
mm_YFP_flox_rv	PY2	AAGACCGCGAAGAGTTTGTC
mm_YFP_wt_rv	PY3	GGAGCGGGAGAAATGGATATG

Supporting Table S2: markers of liver damage and liver synthesis parameters in plasma

Plasma parameter (male)	Mean Ctrl (n=5)	SD Ctrl	Mean Wls-HECKO (n=5)	SD Wls-HECKO (n=5)	p-value (t-test)	Pooled or Satterthwaite	Significance (P < 0.05)
ALT in U/L	37	15.86	30	11.20	0.443	Pooled	n.s.
AST in U/L	52.8	20.51	64.8	22.99	0.409	Pooled	n.s.
GLDH in U/L	10.79	3.57	29.41	27.62	0.207	Satterthwaite	n.s.
Protein in g/L	48.8	2.39	46.2	2.68	0.144	Pooled	n.s.
Cholinesterase in U/L	4853.2	946.00	4049.00	846.23	0.194	Pooled	n.s.

Plasma parameter (female)	Mean Ctrl (n=5)	SD Ctrl	Mean Wls-HECKO (n=5)	SD Wls-HECKO (n=5)	p-value (t-test)	Pooled or Satterthwaite	Significance (P < 0.05)
ALT in U/L	22.8	3.56	21	3.54	0.446	Pooled	n.s.
AST in U/L	50.6	7.27	57.6	15.73	0.392	Pooled	n.s.
GLDH in U/L	6.09	1.78	9.87	3.98	0.089	Pooled	n.s.
Protein in g/L	46.6	47.4	47.4	4.51	0.773	Pooled	n.s.
Cholinesterase in U/L	6288.9	667.53	5620.8	488.88	0.109	Pooled	n.s.

Supporting Table S3

A: Plasma and hepatic sterole levels

Plasma parameter (male) in g/dL	Mean Ctrl (n=5)	SD Ctrl	Mean Wls-HECKO (n=5)	SD Wls-HECKO (n=5)	p-value (t-test)	Pooled or Satterthwaite	Significance (Bonferroni P < 0.0055)
8-DHC	9.72	2.09	8.52	3.55	0.533	Pooled	n.s.
Cholestanol	422.32	62.21	277.14	91.30	0.019	Pooled	n.s.
Desmosterol	93.90	23.59	92.98	30.30	0.959	Pooled	n.s.
7-DHC	33.46	14.69	22.30	10.47	0.204	Pooled	n.s.
Lathosterol	18.08	11.52	13.50	4.36	0.423	Pooled	n.s.
b-Sitosterol	471.58	111.08	320.10	113.96	0.066	Pooled	n.s.
Stigmastanol	5.24	2.26	4.60	1.09	0.584	Pooled	n.s.
Campesterol	1558.26	278.97	1072.12	299.22	0.029	Pooled	n.s.
Lanosterol	11.64	3.88	9.74	5.71	0.556	Pooled	n.s.

Plasma parameter (female) in g/dL	Mean Ctrl (n=5)	SD Ctrl	Mean Wls-HECKO (n=5)	SD Wls-HECKO (n=5)	p-value (t-test)	Pooled or Satterthwaite	Significance (Bonferroni P < 0.0055)
8-DHC	12.00	3.81	9.26	3.05	0.245	Pooled	n.s.
Cholestanol	176.98	32.54	157.64	60.24	0.545	Pooled	n.s.
Desmosterol	59.76	16.84	44.00	11.06	0.118	Pooled	n.s.
7-DHC	14.68	4.31	9.86	1.37	0.065	Satterthwaite	n.s.
Lathosterol	8.96	2.05	5.36	1.44	0.012	Pooled	n.s.
b-Sitosterol	285.62	72.59	253.72	79.70	0.527	Pooled	n.s.
Stigmastanol	3.22	1.35	3.14	0.85	0.913	Pooled	n.s.
Campesterol	1218.76	227.49	1095.28	380.94	0.551	Pooled	n.s.
Lanosterol	5.94	2.84	3.76	0.67	0.163	Satterthwaite	n.s.

Liver lysate parameter (male) in mg/mg protein	Mean Ctrl (n=5)	SD Ctrl	Mean Wls-HECKO (n=5)	SD Wls-HECKO (n=5)	p-value (t-test)	Pooled or Satterthwaite	Significance (Bonferroni P < 0.0055)
8-DHC	0.79	0.34	0.66	0.15	0.465	Pooled	n.s.
Cholestanol	5.03	2.08	4.00	1.05	0.352	Pooled	n.s.
Desmosterol	1.92	1.02	1.30	0.43	0.251	Pooled	n.s.
7-DHC	1.68	0.57	1.40	0.45	0.431	Pooled	n.s.
Lathosterol	15.66	2.20	13.58	5.20	0.434	Pooled	n.s.
b-Sitosterol	2.01	1.03	1.28	0.52	0.199	Pooled	n.s.
Stigmastanol	92.47	11.29	78.48	29.08	0.345	Pooled	n.s.
Campesterol	0.37	0.11	0.41	0.13	0.574	Pooled	n.s.
Lanosterol	23.25	4.07	21.01	3.11	0.356	Pooled	n.s.

Liver lysate parameter (female) in mg/mg protein	Mean Ctrl (n=5)	SD Ctrl	Mean Wls-HECKO (n=5)	SD Wls-HECKO (n=5)	p-value (t-test)	Pooled or Satterthwaite	Significance (Bonferroni P < 0.0055)
8-DHC	1.39	0.32	1.00	0.10	0.049	Satterthwaite	n.s.
Cholestanol	4.08	1.92	3.79	1.25	0.781	Pooled	n.s.
Desmosterol	1.94	1.01	1.77	0.49	0.749	Pooled	n.s.
7-DHC	2.84	0.95	2.00	0.58	0.133	Pooled	n.s.
Lathosterol	11.32	2.37	14.01	3.86	0.221	Pooled	n.s.
b-Sitosterol	3.13	0.94	2.45	0.85	0.267	Pooled	n.s.
Stigmastanol	105.21	23.82	130.70	34.75	0.213	Satterthwaite	n.s.
Campesterol	0.67	0.55	0.37	0.08	0.293	Pooled	n.s.
Lanosterol	27.42	5.39	28.54	7.26	0.789	Pooled	n.s.

B: Dry blood acylcarnitine and amino acid levels (in nmol/µg protein)

Tested parameters (dry blood cards) (n=47)	Mean Ctrl (n=5)	SD Ctrl	Mean Wls-HECKO (n=5)	SD Wls-HECKO (n=5)	p-value (t-test)	Pooled or Satterthwaite	Significance (Bonferroni P < 0.001)
C0	20.84864	1.622325	20.35648	4.001592	0.805255	Pooled	n.s.
C2	16.60096	1.413986	20.58368	2.527005	0.015221	Pooled	n.s.
C3	0.51456	0.076009	0.6176	0.053642	0.038312	Pooled	n.s.
C4	0.1728	0.0257	0.17024	0.016535	0.856076	Pooled	n.s.
C5_1	0.02048	0.008345	0.02304	0.008283	0.639413	Pooled	n.s.
C5	0.11584	0.02345	0.13248	0.028942	0.347098	Pooled	n.s.
C5OH_HMB	0.09856	0.021154	0.10112	0.016566	0.836617	Pooled	n.s.
C6	0.05888	0.01027	0.08	0.021703	0.084753	Pooled	n.s.
C8_1	0.01728	0.003649	0.02688	0.003649	0.003164	Pooled	n.s.
C8	0.03904	0.009966	0.05504	0.013838	0.069157	Pooled	n.s.
C10:1	0.09664	0.027472	0.11904	0.024621	0.211597	Pooled	n.s.
C10	0.03968	0.008032	0.04096	0.008587	0.813797	Pooled	n.s.
MMA	0.20928	0.035244	0.23552	0.043442	0.324888	Pooled	n.s.
Glut	0.08704	0.021514	0.10048	0.026926	0.408627	Pooled	n.s.
C12	0.03968	0.008937	0.05376	0.010467	0.051469	Pooled	n.s.
MeGlut	0.02368	0.008345	0.03392	0.007707	0.078567	Pooled	n.s.
C14_1	0.0672	0.019855	0.08128	0.01272	0.218562	Pooled	n.s.
C14	0.09664	0.010467	0.12736	0.015742	0.00665	Pooled	n.s.
C14OH	0.04608	0.010757	0.04288	0.012311	0.673189	Pooled	n.s.
C16_1	0.09728	0.020935	0.13632	0.025156	0.028479	Pooled	n.s.
C16	0.7424	0.076566	0.92736	0.057082	0.00251	Pooled	n.s.
C16_1OH	0.03328	0.010757	0.04032	0.004293	0.211168	Pooled	n.s.
C16OH	0.05056	0.00764	0.06016	0.013077	0.194124	Pooled	n.s.

Tested parameters (dry blood cards) (n=47)	Mean Ctrl (n=5)	SD Ctrl	Mean Wls-HECKO (n=5)	SD Wls-HECKO (n=5)	p-value (t-test)	Pooled or Satterthwaite	Significance (Bonferroni P < 0.001)
C18_1	0.21632	0.044834	0.25664	0.0477	0.205742	Pooled	n.s.
C18	0.19392	0.028586	0.24	0.048425	0.104261	Pooled	n.s.
C18_1OH	0.04864	0.018985	0.07168	0.024117	0.131765	Pooled	n.s.
C18OH	0.02496	0.007968	0.0288	0.013194	0.592708	Pooled	n.s.
C18_2	0.10944	0.025938	0.1248	0.026581	0.382124	Pooled	n.s.
3HMG	0.00704	0.002677	0.01024	0.005258	0.259845	Pooled	n.s.
Ala	121.5072	22.17187	103.136	19.89833	0.205251	Pooled	n.s.
Val	59.29408	10.51636	60.75968	11.50202	0.838707	Pooled	n.s.
Met	22.70656	11.25498	22.97152	10.80883	0.970644	Pooled	n.s.
Phe	22.1984	4.290788	27.2096	5.432534	0.144183	Pooled	n.s.
Tyr	32.5856	8.423213	46.48704	11.21141	0.05748	Pooled	n.s.
Asp	36.1504	5.099941	38.4608	9.214838	0.636932	Pooled	n.s.
Glu	129.8547	10.60818	143.9373	21.00347	0.217601	Pooled	n.s.
Pyg	2.23616	1.203043	1.2288	0.321723	0.135767	Satterthwaite	n.s.
Trp	20.97408	1.95106	22.9792	3.876071	0.331721	Pooled	n.s.
Pro	211.8848	73.36309	225.0912	52.8388	0.752333	Pooled	n.s.
His	1127.94	1500.006	778.1069	938.7162	0.670145	Pooled	n.s.
Thr	67.3088	9.105629	72.89792	13.92564	0.474086	Pooled	n.s.
Gly	193.7158	38.08381	207.593	27.99063	0.529909	Pooled	n.s.
Orn	26.0864	5.547613	41.10848	22.62555	0.215409	Satterthwaite	n.s.
Arg	57.89248	7.476778	64.58368	15.13921	0.401404	Pooled	n.s.
Cit	22.1536	3.335288	27.9136	3.226173	0.024086	Pooled	n.s.
Asa	0.2336	0.066318	0.27392	0.080275	0.411779	Pooled	n.s.
Hci	0.92224	0.183957	0.8832	0.156064	0.726824	Pooled	n.s.

C: Plasma and hepatic totale bile acid levels

Plasma total bile acid in µM	Mean Ctrl (n=5)	SD Ctrl	Mean Wls-HECKO (n=5)	SD Wls-HECKO (n=5)	p-value (t-test)	Pooled or Satterthwaite	Significance (P < 0.05)
Male	12.73	12.55	31.36	28.46	0.217	Pooled	n.s.
Female	5.44	1.50	22.57	24.36	0.191	Satterthwaite	n.s.

Liver total bile acid in µM /mg protein	Mean Ctrl (n=5)	SD Ctrl	Mean Wls-HECKO (n=5)	SD Wls-HECKO (n=5)	p-value (t-test)	Pooled or Satterthwaite	Significance (P < 0.05)
Male	0.68	0.30	1.08	0.31	0.0723	Pooled	n.s.
Female	1.06	0.43	1.52	0.98	0.368	Pooled	n.s.

Supporting Figure legends

Supporting Fig. S1. Stab2-Cre recombinase activity in the portal area of the hepatic lobule. Co-immunofluorescence (63x objective) of HEC in Wls-HECKO;eYFP liver show Stab2-Cre mediated cytoplasmatic eYFP (green) expression (arrows) along with membranous ICAM1 (blue) expression.

* marks a hepatic artery, # marks periportal LSEC area, PV = Portal Vein branch, Scale bar = 50μm, n=5.

Supporting Fig. S2. Stab2-Cre recombinase activity in the pericentral area of the hepatic lobule. Co-immunofluorescence (63x objective) of HEC in Wls-HECKO;eYFP liver show Stab2-Cre mediated eYFP (green) expression (arrows) along with membranous ICAM1 (blue) expression.

§ marks pericentral LSEC area, CV = Central Vein, Scale bar = 50μm, n=5.

Supporting Fig. S3. Stab2-Cre recombinase activity in the hepatic lobule. Co-immunofluorescence (63x objective) of HEC in Wls-HECKO;eYFP liver show Stab2-Cre mediated eYFP (green) expression (arrows) in pericentral, EMCN (red) positive HEC and midzonal LYVE1 (blue) positive HEC.

§ marks a pericentral LSEC, CV = Central Vein, PV = Portal Vein branch, Scale bar = 50μm, n=5.

Supporting Fig. S4. Stab2-Cre recombinase activity during different developmental stages. A: Co-immunofluorescence (63x objective) of HEC in 6 week old eYFP-reporter mouse liver shows Stab2-Cre mediated eYFP (green) in pericentral (left) and periportal (right) areas of the lobule. Pericentral HEC marker EMCN (red) and membranous ICAM1 (blue) expression was used for orientation. B: Co-immunofluorescence (63x objective and zoom) of HEC in the fetal liver (E11.5). eYFP (green), LYVE1 (blue) and Stab2 (red) triple-positive cells represent developing HEC.

CV = Central Vein area, PF = Portal Field area, Scale bar = 50μm (A), 20μm (B), n=3.

Single eYFP+ non-endothelial cells with a round morphology in the fetal liver were negative for Stab2 and Lyve1. Previously it was shown that approximately 10% of these cells are Kit+ and few of them are Ter119+, indicating that they correspond to hematopoietic progenitor cells or hematopoietic stem cells in the fetal liver (1) with previous Stab2-expression or Stab2-promoter activation. No corresponding non-endothelial cells were found in adult livers of 6 or 12 week old mice.

Supporting Fig. S5. Ultratructure of LSEC and the Space Of Disse in Wls-HECKO and Ctrl livers in Transmission Electron Microscopy.

Black arrows indicte fenestrations, n=3, 8 week old female mice were used.

Black spots in HC correspond to glycogen which has a high density due to the the fixation process with osmium-tetroxide.

Supporting Fig. S6 Hepatic HMGCR and CYP7A1 expression shown by qRT-PCR. Fold change relative to β-Actin is shown, Ctrl was set to 1. Bars represent SEM. Mean fold induction in Wls-HECKO did not reach statistical significance.

Mice used were 12-week old males, n=7.

Supporting Fig. S7. Ultrastructure of bile canaliculi in Wls-HECKO and Ctrl livers in Transmission Electron Microscopy.

White arrows indicte tight junctions, n=3, 8 week old female mice were used.

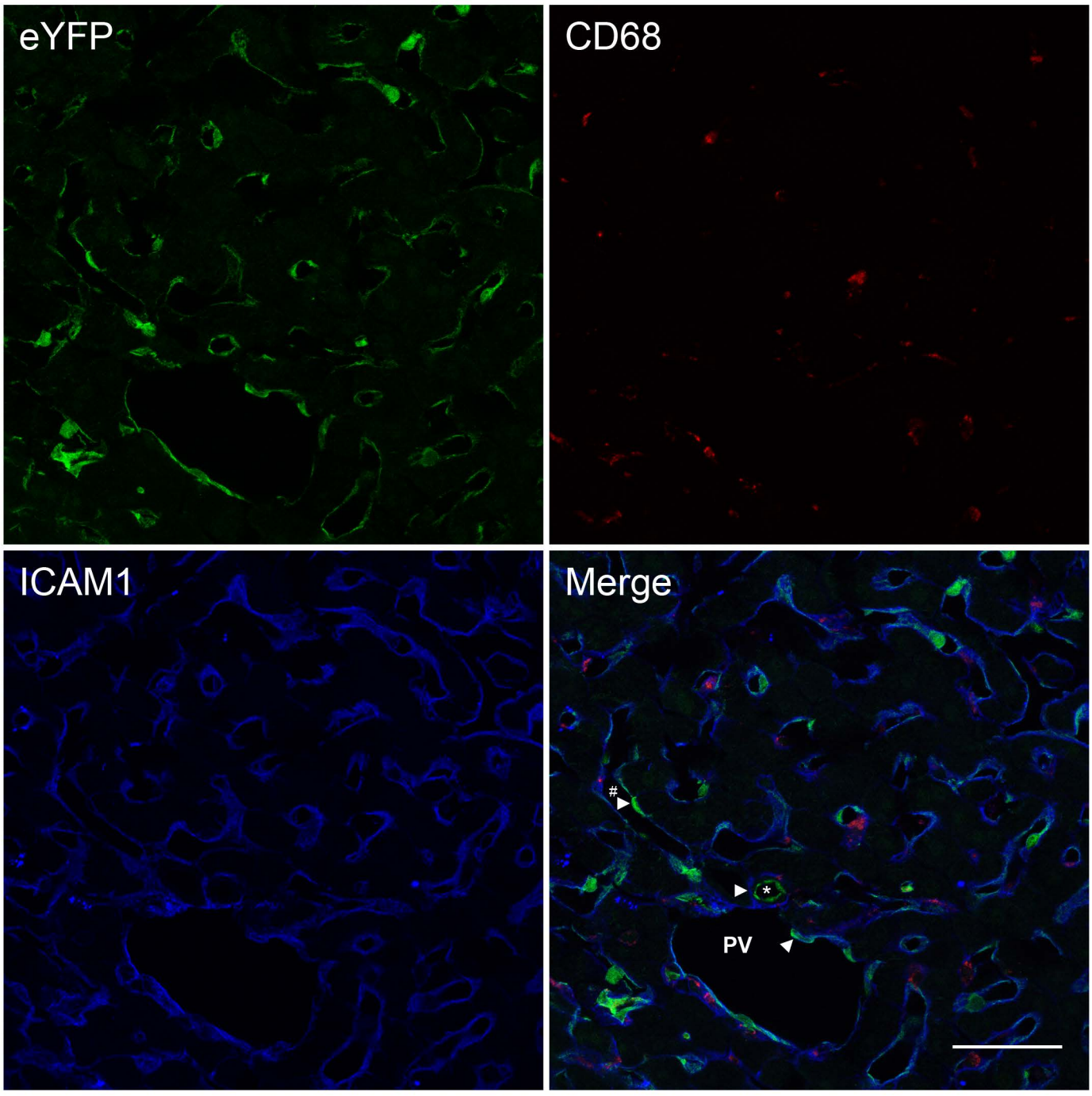
White spots in HC correspond to glycogen removed during the fixation process with osmium-ferrocyanide.

References

1. **Geraud C, Koch PS, Zierow J,** Klapproth K, Busch K, Olsavszky V, Leibing T, et al. GATA4-dependent organ-specific endothelial differentiation controls liver development and embryonic hematopoiesis. J Clin Invest 2017.

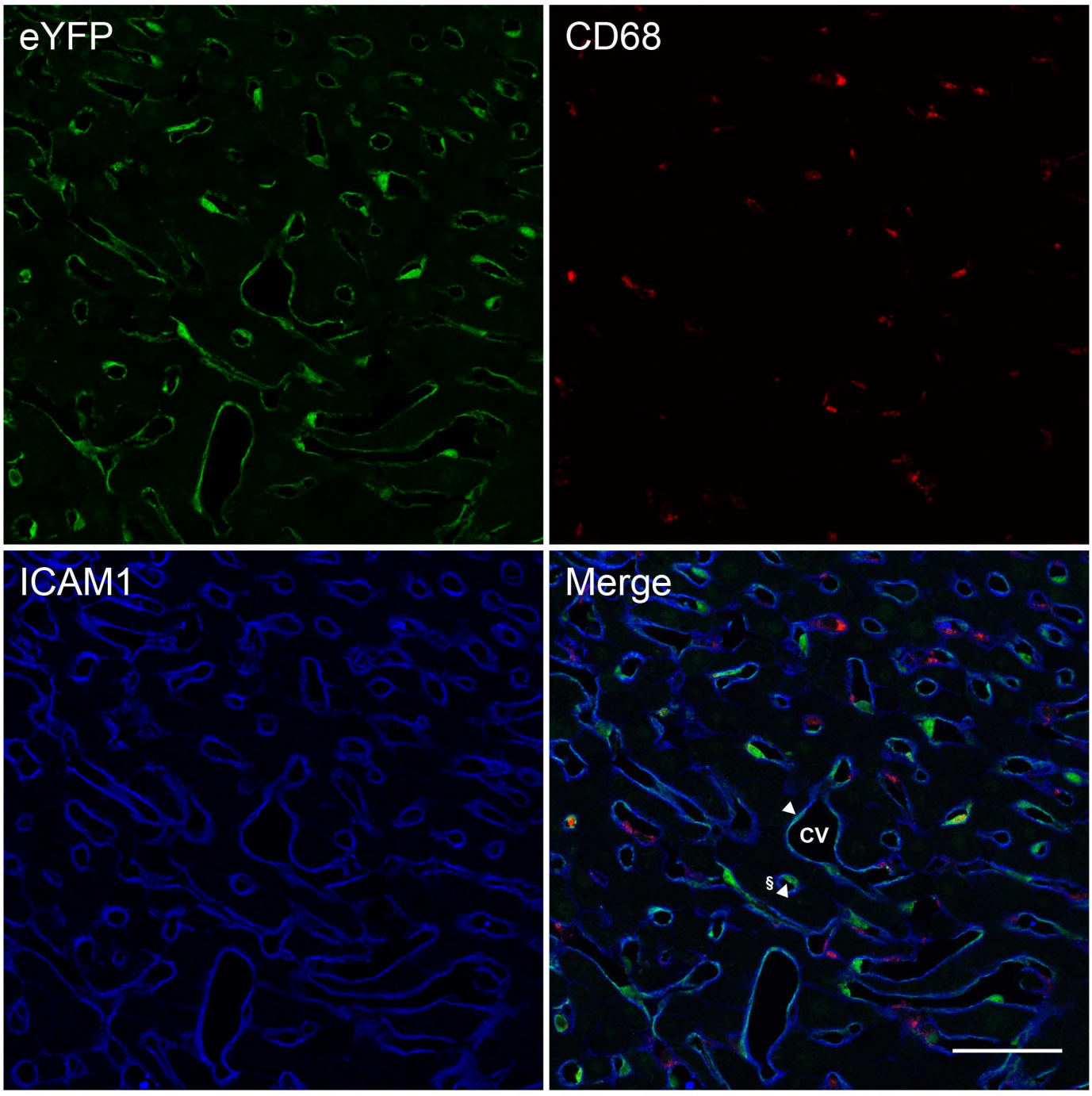
Author names in bold designate shared co-first authorship

Supporting Figure S1



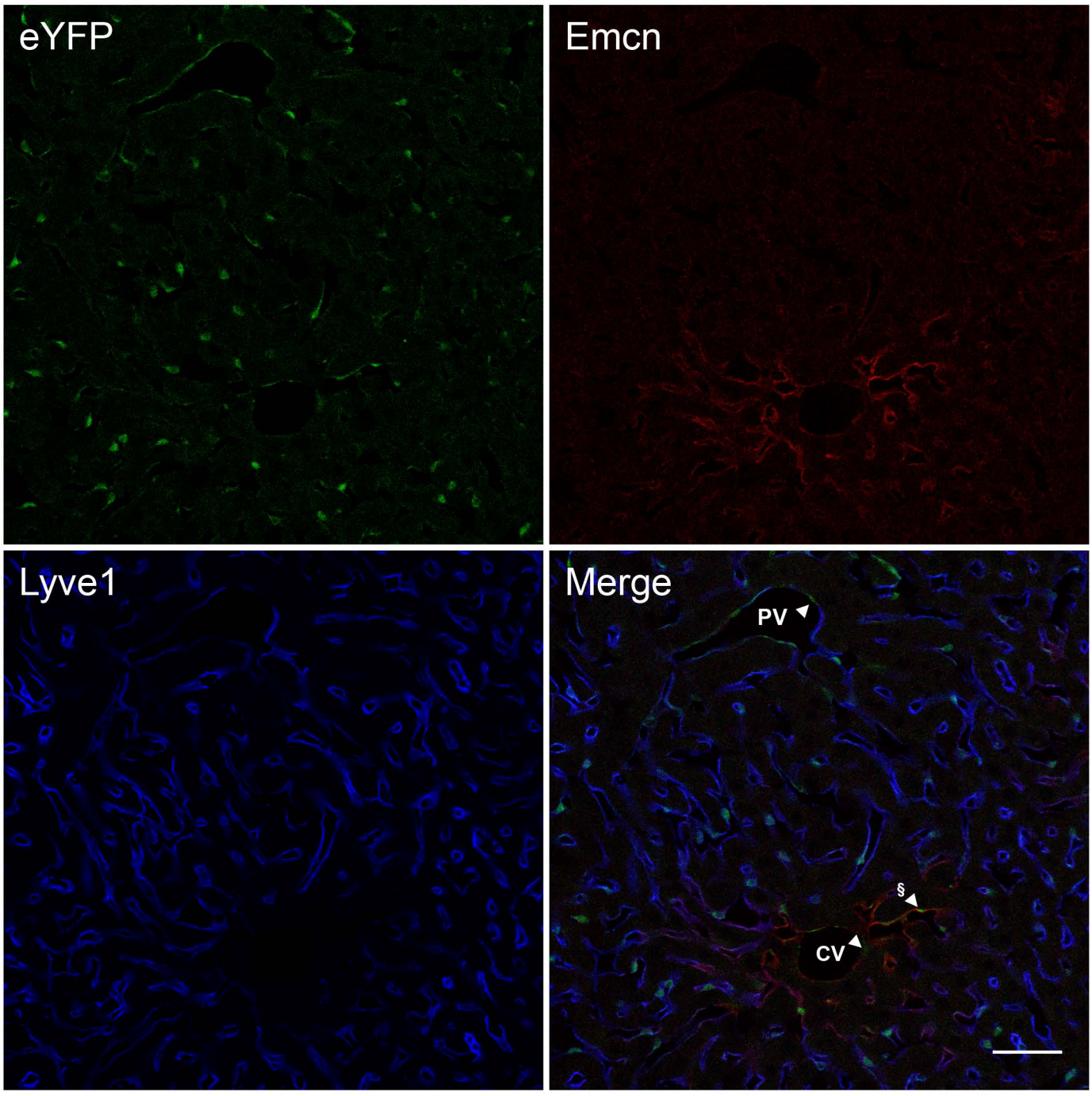
Wls-HECKO;eYFP

Supporting Figure S2



Wls-HECKO;eYFP

Supporting Figure S3



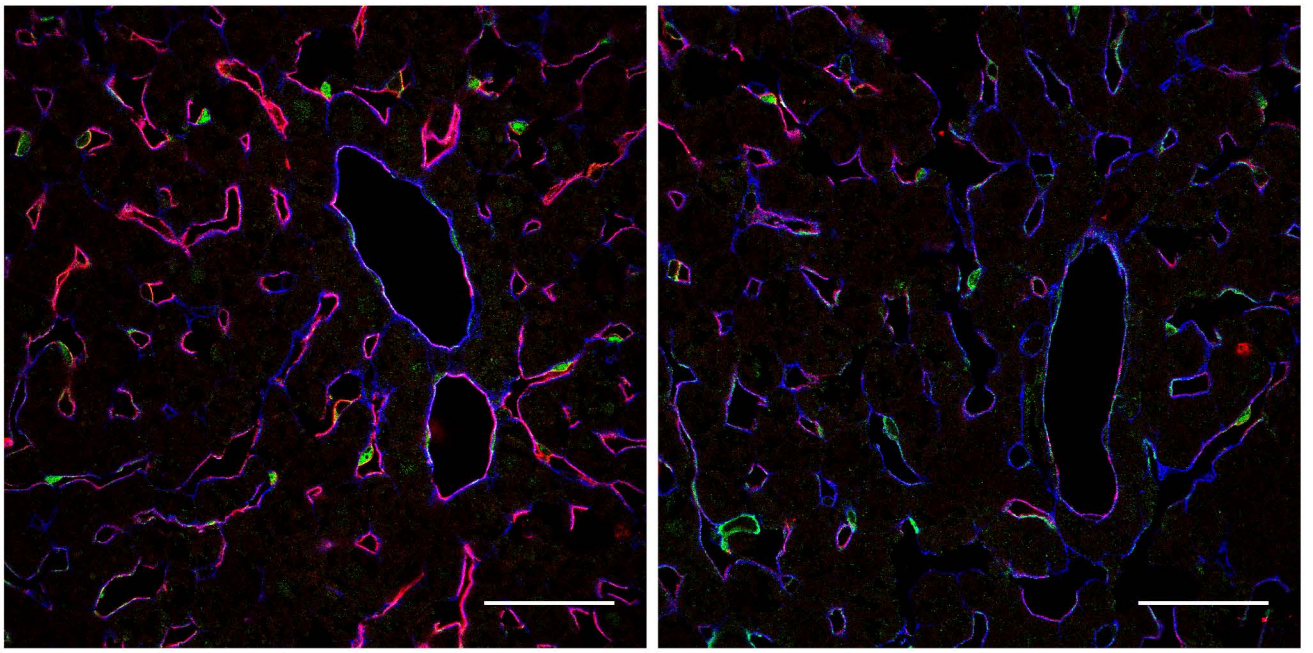
Wls-HECKO;eYFP

Supporting Figure S4

A

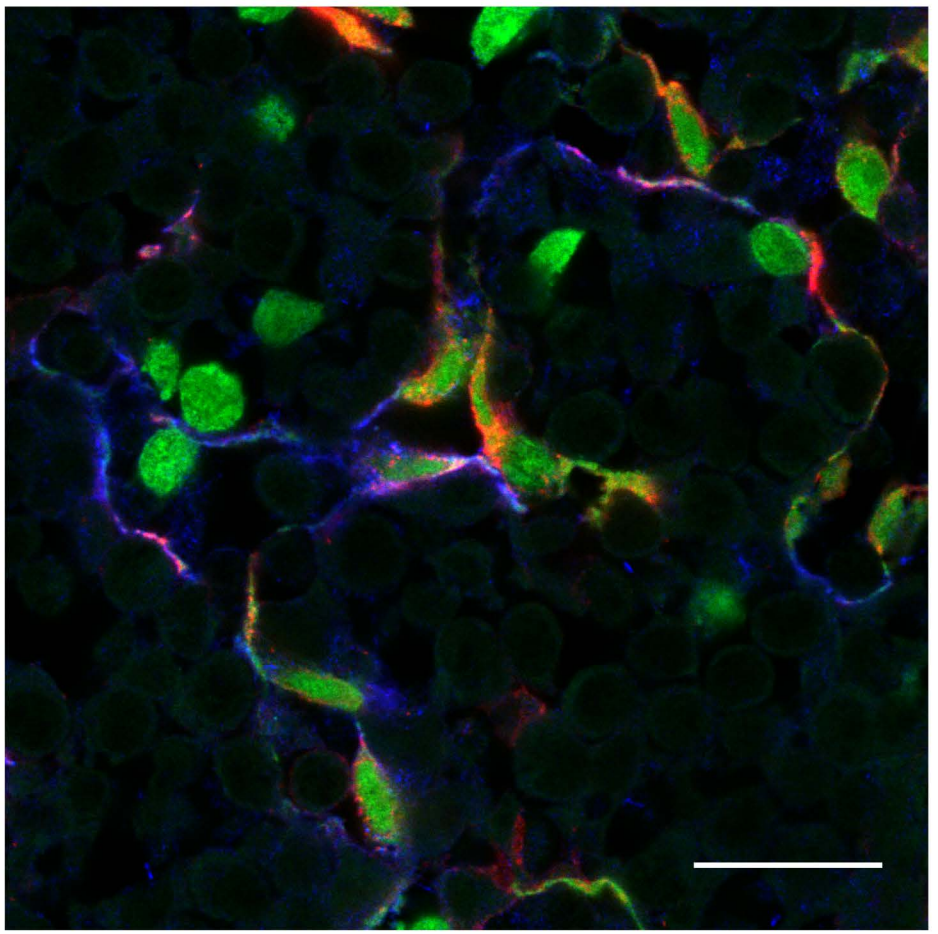
CV
6 wk Stab2-Cre$^{tg/wt}$;eYFP$^{fl/wt}$

PF
6 wk Stab2-Cre$^{tg/wt}$;eYFP$^{fl/wt}$

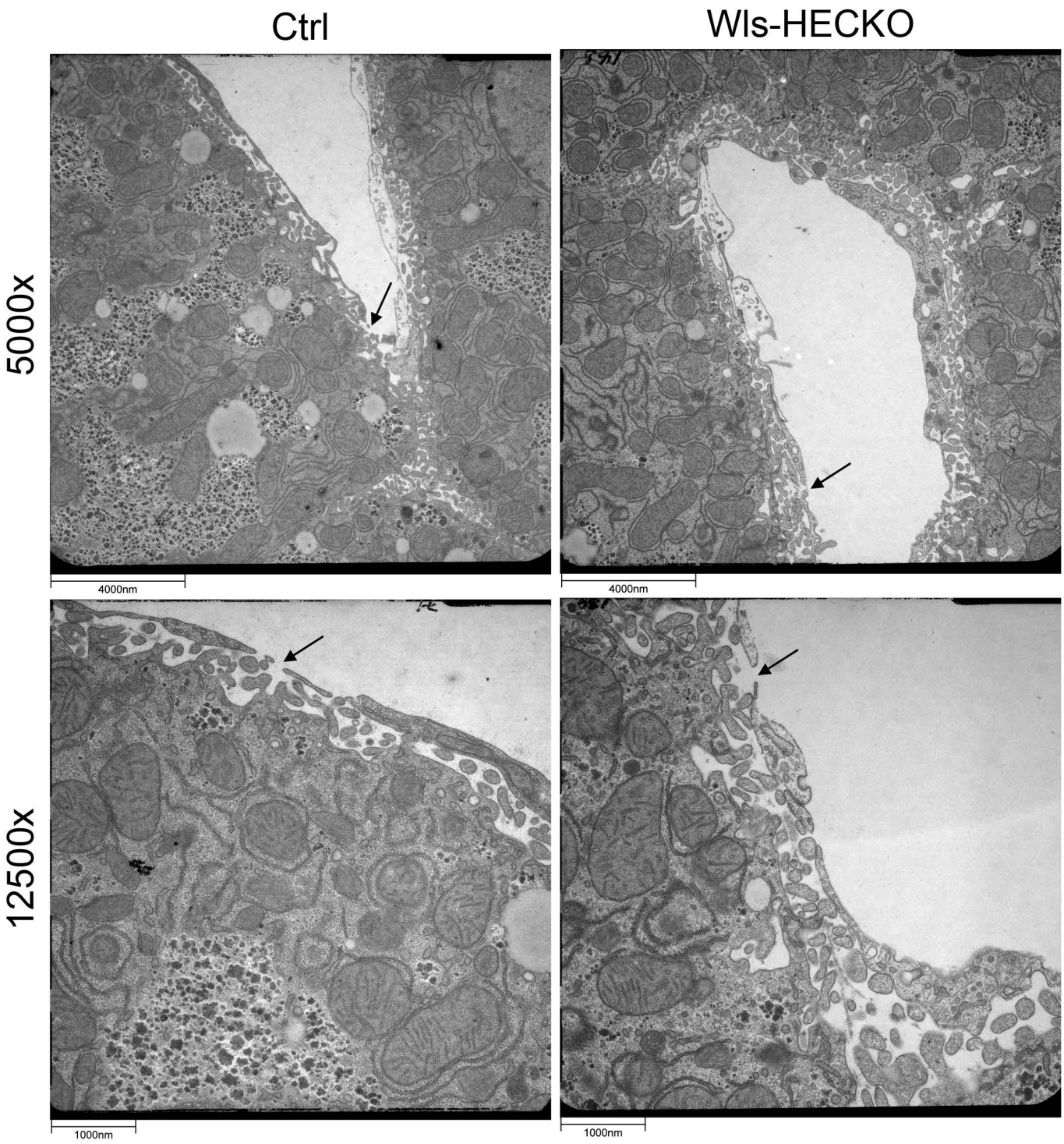


B

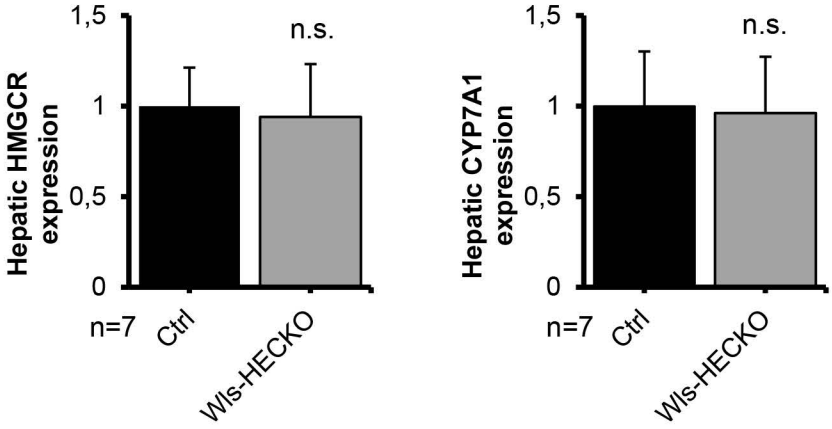
Fetal liver
E11.5 Stab2-Cre$^{tg/wt}$;eYFP$^{fl/wt}$



Supporting Figure S5



Supporting Figure S6



Supporting Figure S7