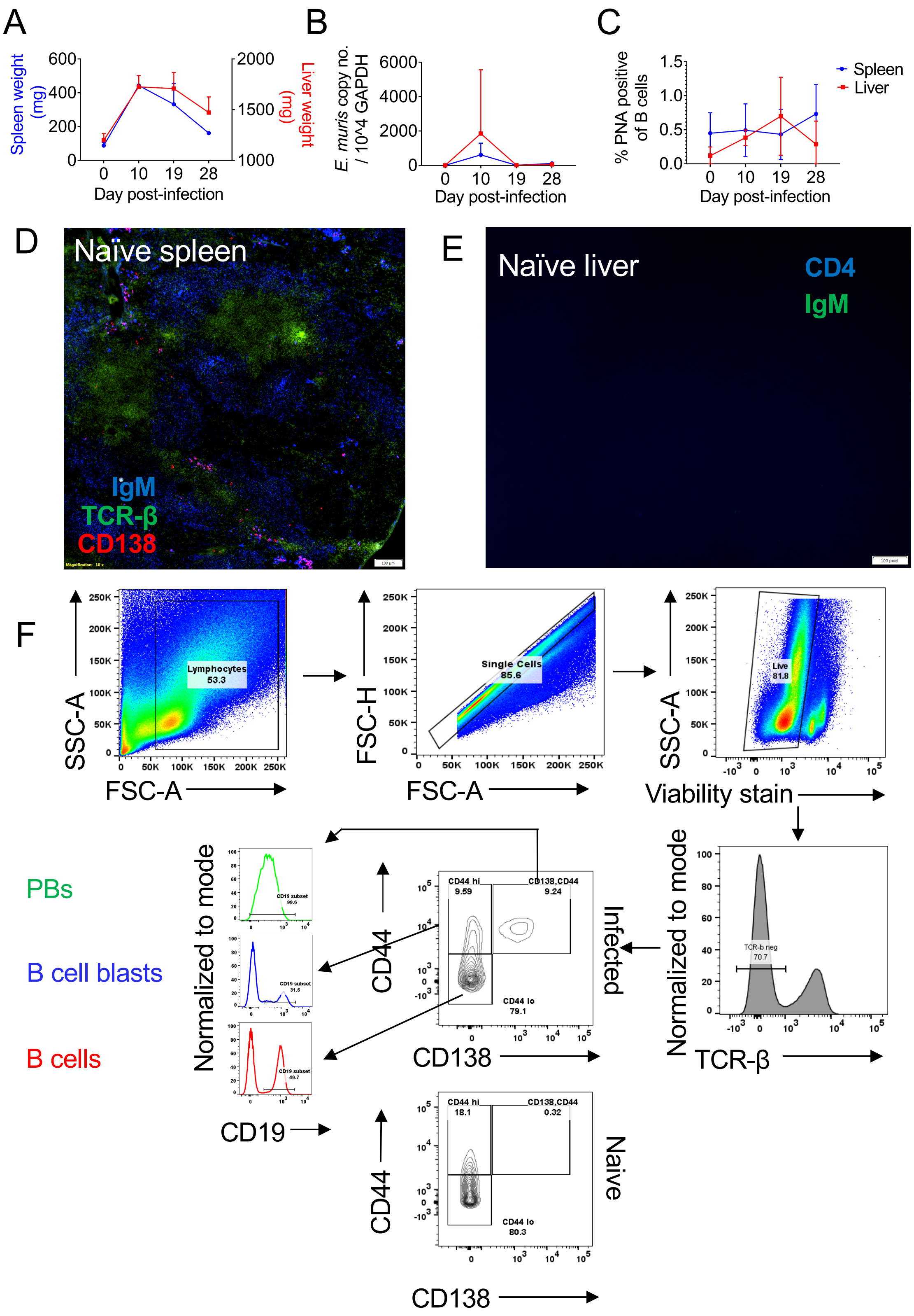
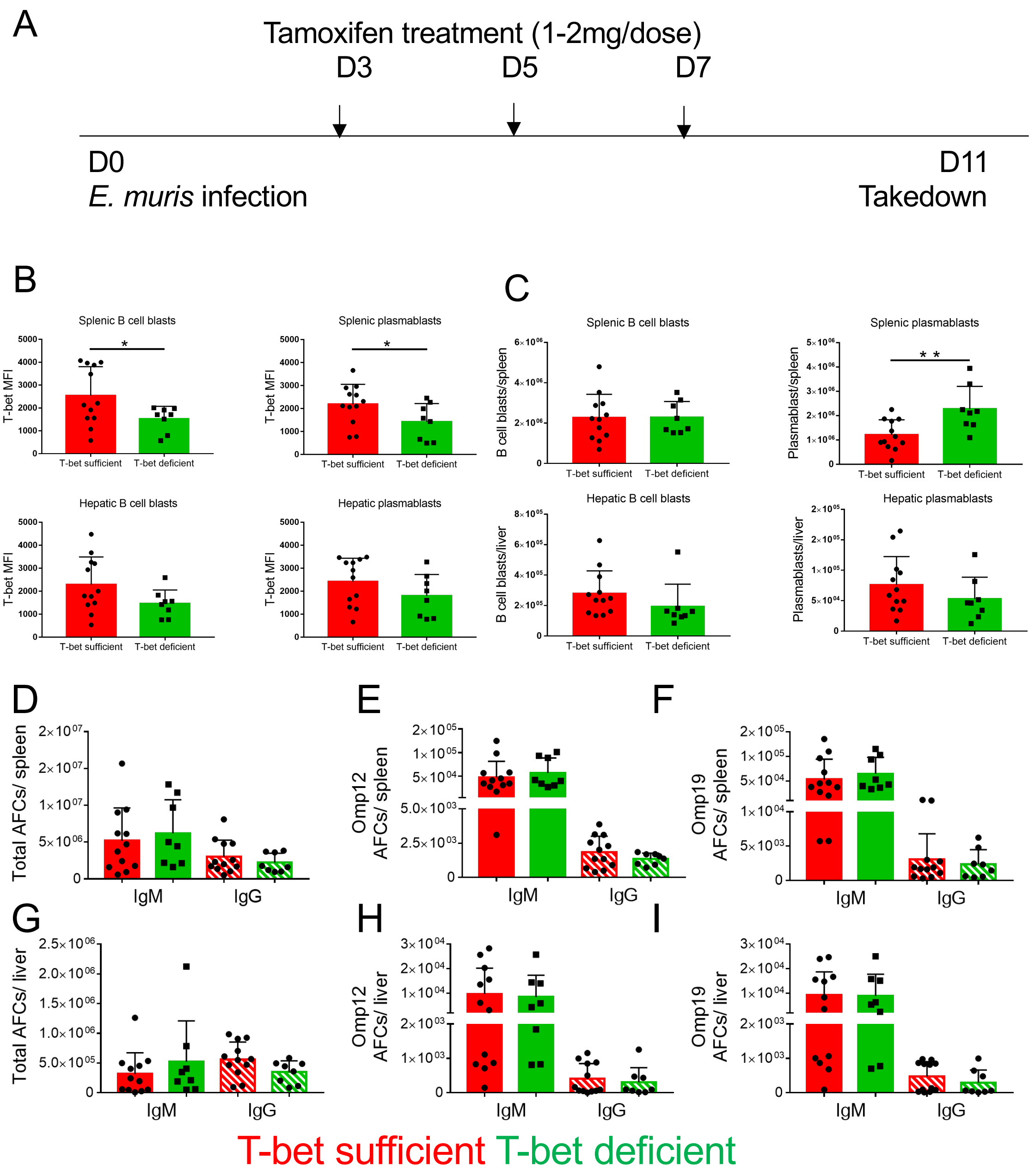
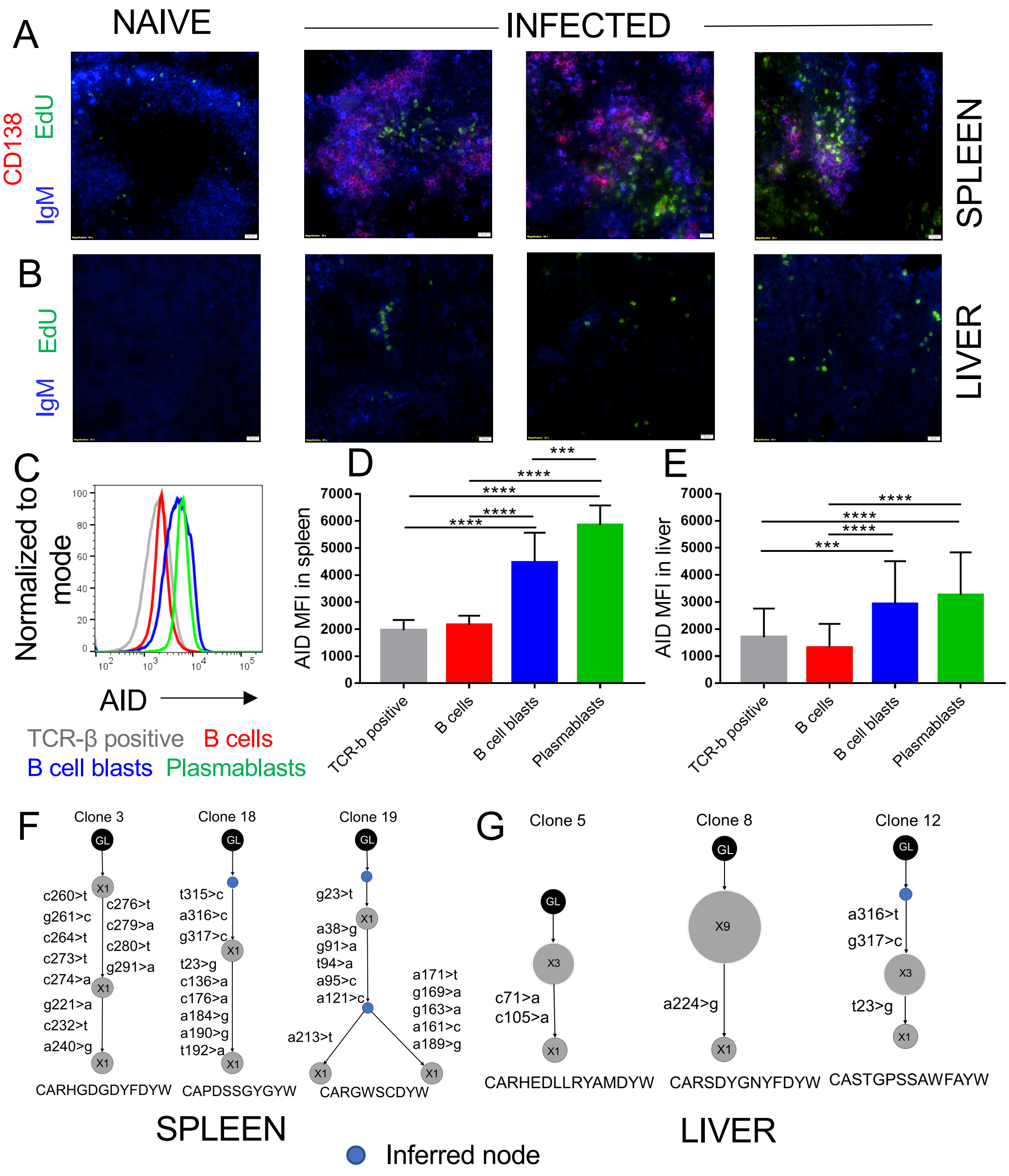


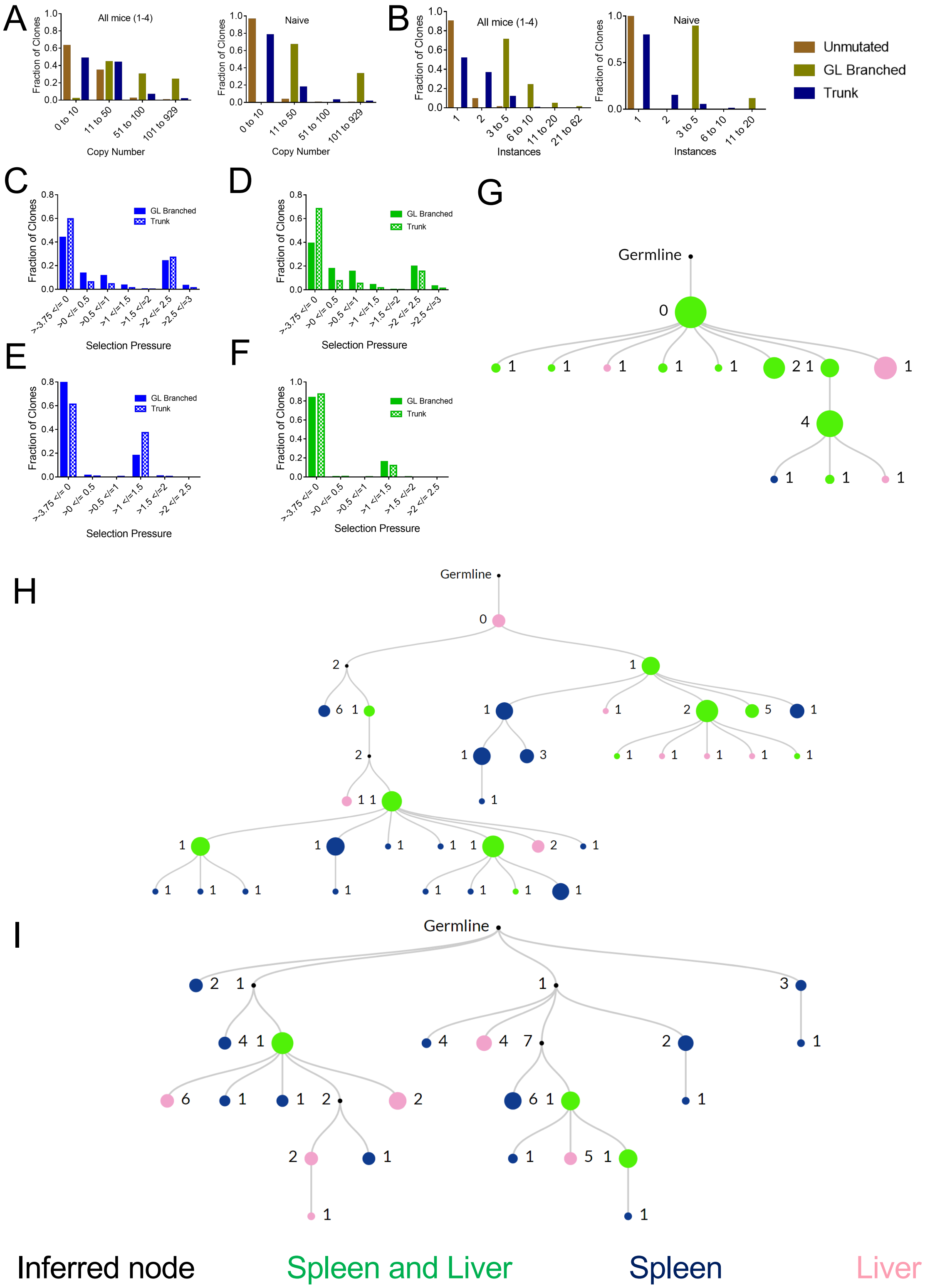
Supplementary figure 1 (related to figure 1). ***E. muris*** **infection induces a robust PB response in the absence of a GC reaction.** (A-C) Weight (A), Bacterial burden (B) and (C) Percentage of PNA$^+$ GC B cells in spleen (blue) and liver (red) over the course of infection. (D-E) Immunofluorescence staining of cryosections from spleen (D) and liver (E) of naïve mice for B cells and T cell markers. Scale bars represent 100µm in D and 100 pixels in E. (F) Gating strategy for B cell blasts and PB. Data are representative of at least two independent experiments and in (A-C) data are represented as mean with SD of groups of at least two mice.



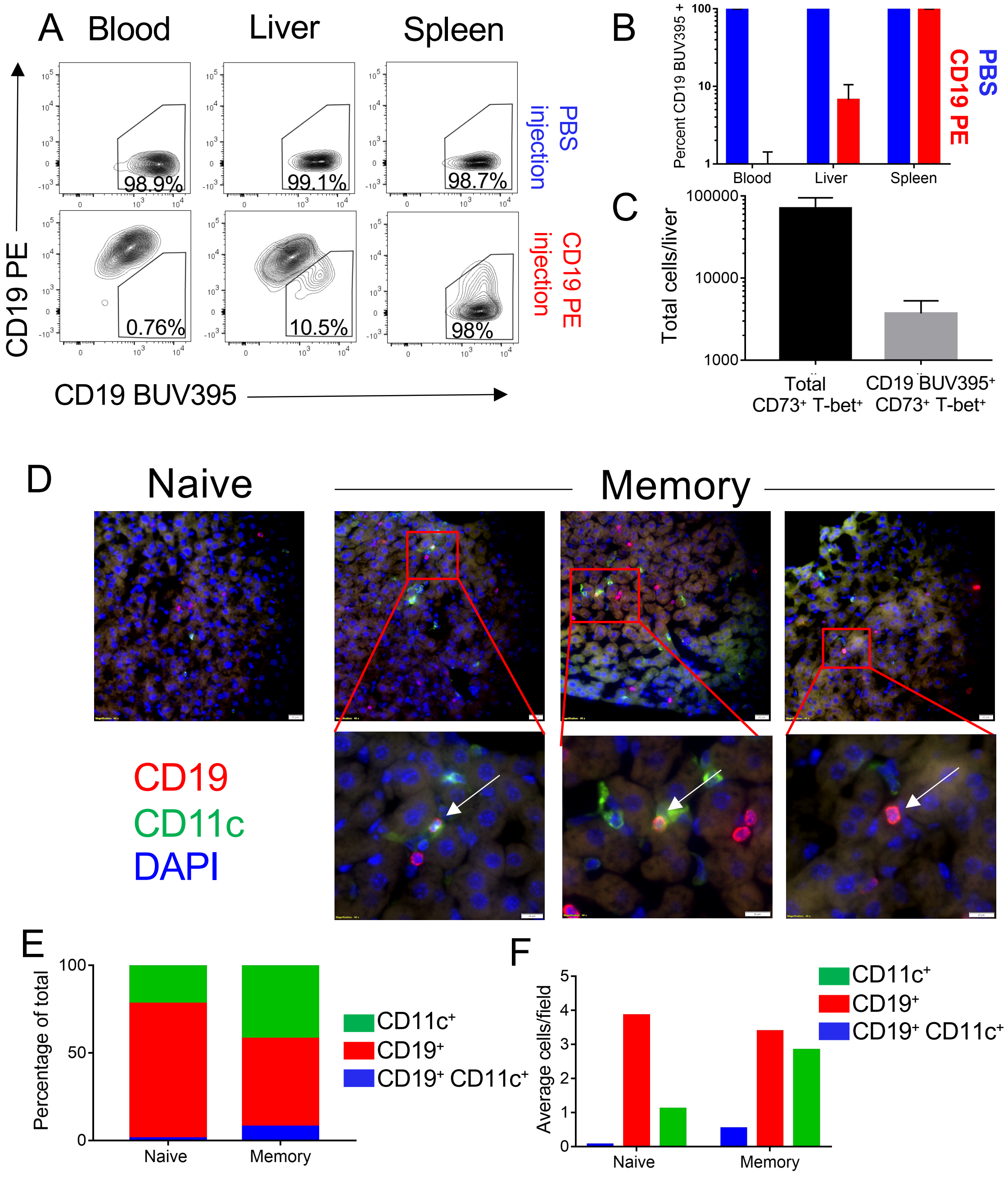
Supplementary figure 2 (related to figure 1): Tamoxifen-inducible B cell-specific T-bet deletion increases PB in the spleen. (A) Schematic diagram for T-bet deletion. The mice were infected with *E. muris* and treated with 1-2 mg dose of tamoxifen 3, 5 and 7 days after infection. (B) MFI of T-bet in B cell blasts and PB of T-bet sufficient (*huCD20.TamCre* or *Tbx21fl/fl*) and T-bet deficient mice (*huCD20 TamCre Tbx21 fl/fl*). (C) Total number of PB and B cell blasts measured by flow cytometry in the spleen and liver of T-bet sufficient and T-bet deficient mice. (D-I) Total AFC (D,G), Omp12-specific AFC (E,H) and Omp19-specific AFC (F,I) measured by ELISpot in the spleen and liver of T-bet sufficient and T-bet deficient mice. Data are representative of at least two independent experiments and are represented as mean with SD of groups of at least three mice. **p<0.01, *p<0.05. Statistics for (B) were done by Mann-Whitney test. Statistics for panels C-I were done by unpaired t-test.



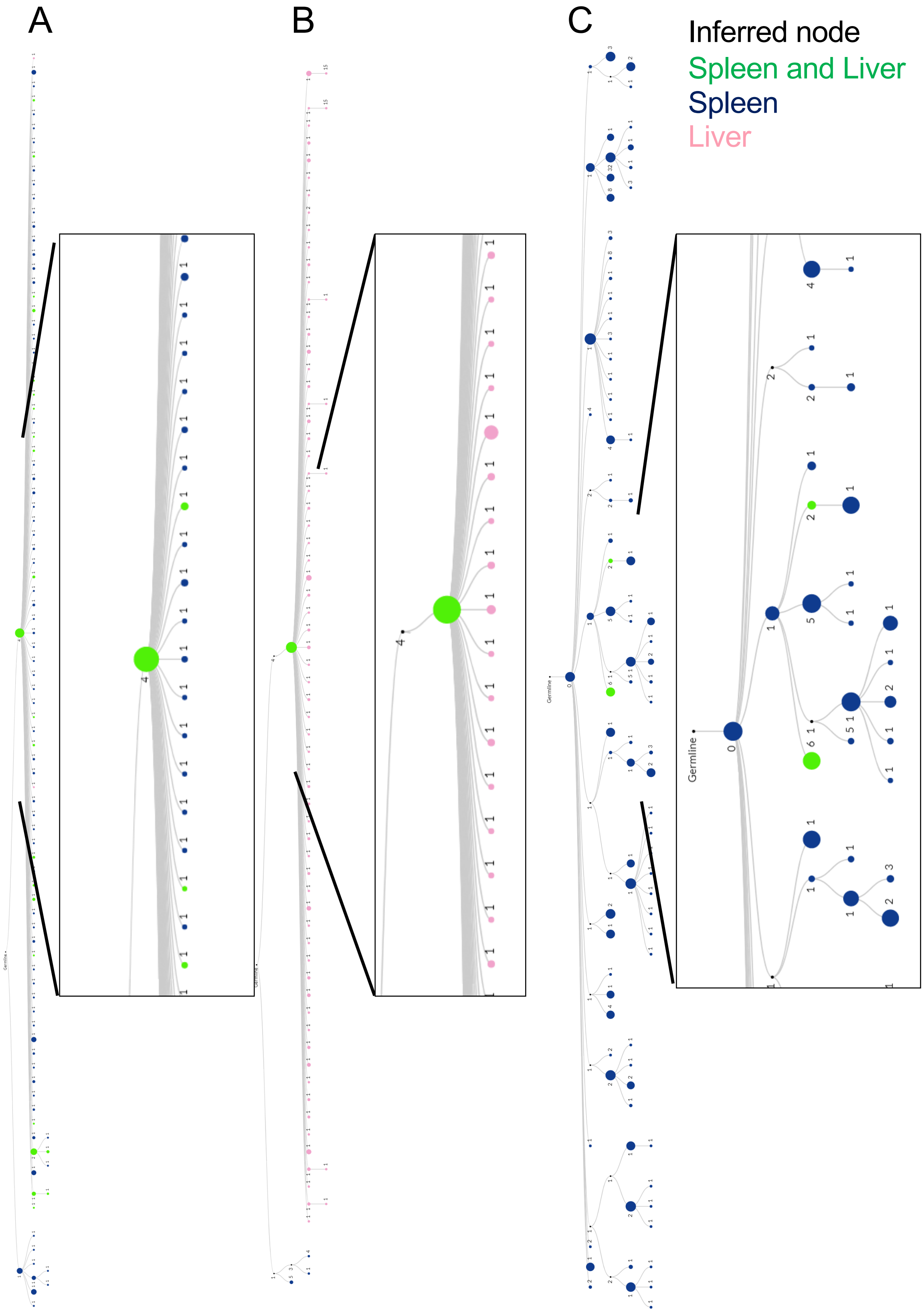
Supplementary figure 3 (related to figure 3): *E. muris* infection induces proliferation, AID expression, and SHM in splenic and hepatic B cells. (A) 40X images demonstrating EdU-positive IgM PB in spleen at D0 and D10 post infection. (B) 40X images demonstrating EdU positive B cells in the liver parenchyma at D0 and D10 post infection. Scale bars represent 20µm. (C-E) Histogram (C) and quantification of AID in T cells, B cells, B cell blasts and PB in spleen (D) and liver (E). (F-G) Genealogical trees derived from V region sequences of B cell clones obtained by laser micro-dissected sections from spleen (F) (clones 3,18,19) and liver (G) (clones 5,8,12). Diagrams F-G are organized as described in the legend to figure 3. Data are representative of at least two independent experiments and in (D,E) data are represented as mean with SD of groups of at least three mice. . ***p<0.001, ****p<0.0001. Statistics for panels D and E were done two-way anova.



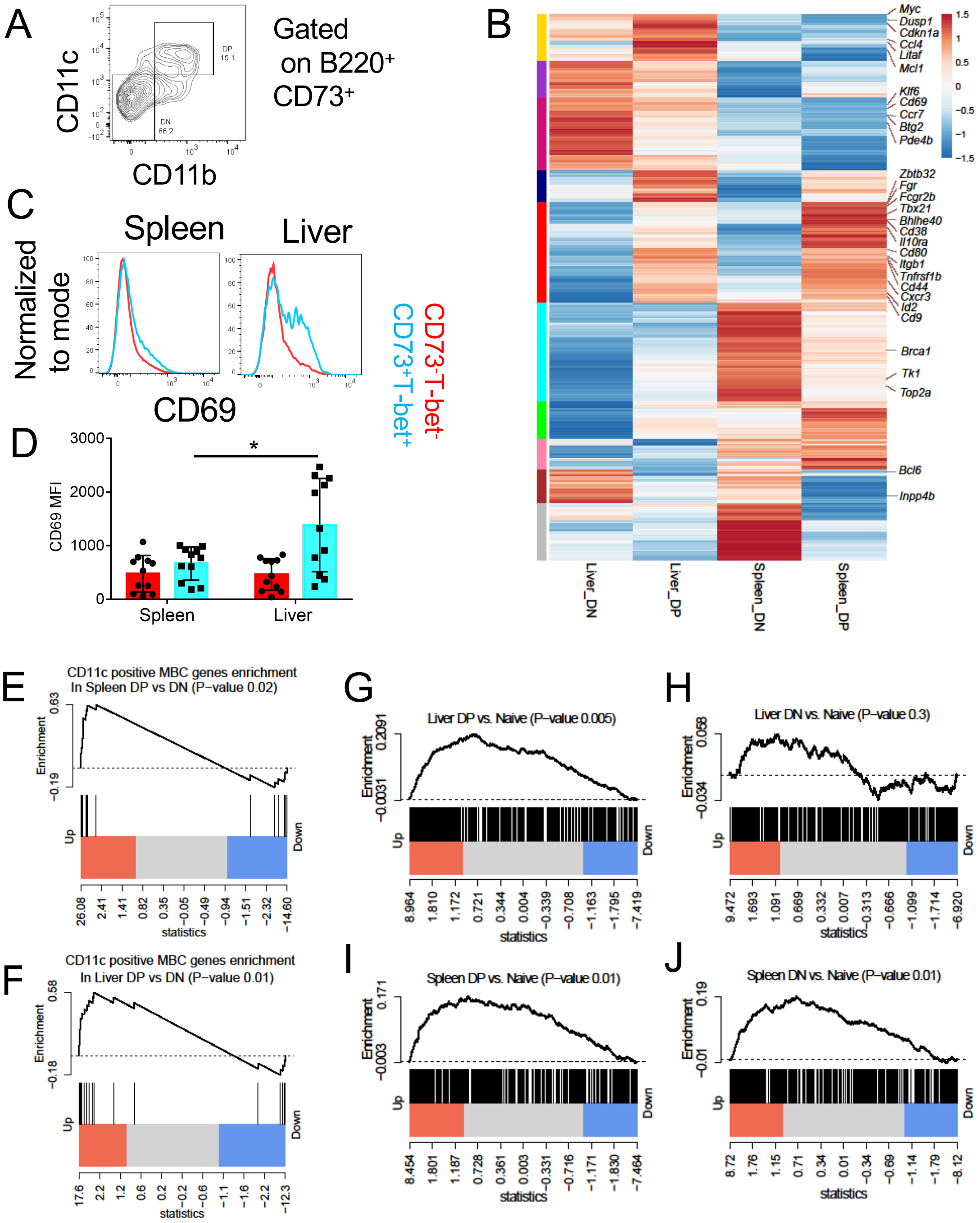
Supplementary figure 4 (related to figure 4): Analysis of the BCR repertoire of splenic and hepatic PB induced by Ehrlichia infection: Analysis of clone size of unmutated, GL branched and trunk populations in *E. muris* infected mice and naïve mice by copy number (A) and instances (B). Distribution of clones with different degrees of selection pressure in CDRs (C,D) and Framework regions (E,F) of B cell blasts (C,E) and PB (D,F). In each scenario, selection pressure in GL branched was significantly different than that in the Trunk clones ($p<0.05$). (G-I) Examples of multi-tiered clonal lineages that were found in both spleen and liver. Nodes are color coded: those found in both organs are green, those found in spleen only are blue, those found in liver only are pink, and inferred nodes are shown in black. The size of a node is proportional to the number of sequences that comprise that node.



Supplementary figure 5 (related to figure 5): Liver localized MBCs after *Ehrlichia* infection. (A) Representative contour plots of CD73$^+$ T-bet$^+$ MBCs unlabeled (top panel) or CD19-PE labeled (bottom panel) in the blood, liver and spleen after either CD19-PE or PBS injection. (B) Percentage of CD19-PE-negative, CD19-BUV395 positive cells in the blood, liver and spleen of mice i.v. injected with PBS or CD19-PE as gated in (A). (C) Number of total CD73$^+$ T-bet$^+$ MBC in the liver (black bar) and number of CD19-BUV395 positive CD19-PE-negative CD73$^+$ T-bet$^+$ MBC (grey bar), as gated in (A). (D) CD19 and CD11c staining in naïve and memory livers at 40X magnification. Scale bars in the first row represent 20µm and in the second row represent 10µm. CD19 and CD11c double positive cells are marked by white arrows. 100 cells (1 CD19$^+$ CD11c$^+$, 64 CD19$^+$, 17 CD11c$^+$) were counted from 20 fields from the liver parenchyma of 3 naïve mice and 371 cells (29 CD19$^+$ CD11c$^+$, 186 CD19$^+$, 156 CD11c$^+$) were counted from 53 fields from the liver parenchyma of 4 memory mice. (E) Percentage of CD11c positive, CD19 positive and CD19 and CD11c double positive cells out of the total number of cells counted in the liver parenchyma of naïve and memory mice. (F) Average cells per each field in the liver parenchyma of naïve and memory mice. Data are represented as mean of groups of at least three mice. Chi-square analysis comparing the numbers of CD19$^+$ and CD19$^+$ CD11c$^+$ B cells from naïve and memory groups had a p-value of <0.0024.



Supplementary figure 6 (related to figure 6). Examples of MBC clonal lineages induced by *Ehrlichia* infection (A-C) Examples of a multi-tiered clonal lineages among MBC that were found in: both spleen and liver (A), mostly liver (B) and mostly spleen (C). Nodes are color coded: those found in both organs are green, those found in spleen only are blue, those found in liver only are pink, and inferred nodes are shown in black. The size of a node is proportional to the number of sequences that comprise that node. Boxed areas show enlargement of representative parts of the clones.



Supplementary figure 7 (related to figure 6). Comparison of splenic and hepatic MBC subset gene expression: (A) Gating based on CD11b and CD11c expression on CD73 positive MBC from the spleen that was used to sort DN and DP MBC subsets of cells for RNA-seq analysis. (B) Heat map of 730 differentially expressed genes in splenic and hepatic MBC populations with an FDR of <0.01 and >2-fold difference. (C-D) Representative histogram (C) and quantification (D) of CD69 MFI in splenic and hepatic MBC during acute *Ehrlichia* infection. (E-F) RNA-seq data were used to construct gene set enrichment plots illustrating genes differentially expressed in the DP subset compared with the DN subset (*n* = 3 per group) for spleen (E) and liver (F) with respect to a known set of genes that are differentially expressed in CD11c-positive MBC induced during *Ehrlichia* infection (Winslow et al., 2017). (G-J) RNA-seq data were used to construct gene set enrichment plots illustrating genes differentially expressed in DP or DN subsets compared with naïve B cells from a previously published microarray dataset (Barnett et al., 2016) (*n* = 3 per group) for liver DP (G), liver DN (H), spleen DP (I) and spleen DN (J) with respect to a known set of genes differentially expressed in nitrophenol-induced MBC (data not shown). Data in A, C, and D are representative of at least two independent experiments and are represented as mean with SD of groups of at least three mice. *p<0.05. Statistics for panel D were by two-way ANOVA.

Micro-dissection	Clone #	Organ	CDR3 AA	Vh usage	Number of unique sequences	Total sequences
1	1	Spleen	CAKVPYYFDYW	IGHV1-81*01 F	2	9
1	2	Spleen	CARRSGGAYW	IGHV1-67*01 P	1	4
2	3	Spleen	CARHGDGDYFDYW	IGHV1-81*01 F	3	3
2	4	Spleen	CARGDYDPYWYFDVW	IGHV1-81*01 F	1	7
3	5	Liver	CARHEDLLRYAMDYW	IGHV1-62-2*01 F	2	4
4	6	Liver	CAREGGFAYW	IGHV1-37*01 F	1	14
5	7	Liver	CARGYDGYFDYW	IGHV14-3*01 F	1	9
6	8	Liver	CARSDYGNYFDYW	IGHV1-56*01 F	2	18
7	9	Liver	CARSNWDDRGFDYW	IGHV1-80*01 F	1	16
8	10	Liver	CARREGAQVPLFAYW	IGHV1-81*01 F	3	17
8	11	Liver	CARYYYGRDYFDYW	IGHV1-82*01 F	1	2
9	12	Liver	CASTGPSSAWFAYW	IGHV14-3*01 F	2	4
10	13	Liver	CARYYSNYYAMDYW	IGHV1-9*01 F	1	8
11	14	Liver	CARSGGWLLQAMDYW	IGHV1-42*01 F	1	14
12	15	Spleen	CARGGPYGYHDASYAMDYW	IGHV1-7*01 F	1	4
12	16	Spleen	CARTGTGYYAMDYW	IGHV14-3*01 F	1	3
13	17	Spleen	CARPRAIYYGNSGFAYW	IGHV1-80*01 F	1	2
14	18	Spleen	CAPDSSGYGYW	IGHV14-3*01 F	2	2
14	19	Spleen	CARGWSCDYW	IGHV14-3*01 F	3	3
15	20	Spleen	CARAPSYYGSSHWYFDVW	IGHV1-4*01 F	1	3
16	21	Spleen	CARRGITTVFDYW	IGHV1-47*01 F	1	3

Table S1: LCM clone characteristics (related to figure 3): CDR3 sequence, Vh gene, total number of sequences and number of unique sequences from microdissections of spleen or liver sections from which sequences were obtained.

Socs3	*Slc37a2*	*Fuca2*	*Evi2a*	*Spn*	*Sspn*	*Cxcr4*	*Ccl4*
1700026L06Rik	*Sla*	*Hck*	*I830012O16Rik*	*Cd68*	*Unc5cl*	*Zfp169*	*Myadm*
Rassf4	*Efna1*	*Tlr7*	*Aldh3b1*	*Sox5*	*Metrnl*	*Mfsd4*	*Irf2bp2*
Pdlim1	*C1qb*	*Tm6sf1*	*Ptpn22*	*Itgb1*	*Rab31*	*Slc26a10*	*Phyhd1*
Zbtb32	*Il18*	*Tbx21*	*Abcc4*	*Stx3*	*Rps6ka5*	*Ier2*	*S100a10*
Gas7	*Tlr9*	*As3mt*	*Zfp385a*	*Cd44*	*Rgl1*	*Serpinc1*	*Mxi1*
Ahnak	*Spata13*	*Fcgr2b*	*A530032D15Rik*	*Fcer1g*	*Gadd45g*	*Ubc*	*Pim1*
Upb1	*Cd38*	*Serpinb9*	*Mpeg1*	*Fgl2*	*A430078G23Rik*	*Dyrk1b*	*Hilpda*
Crip2	*Tmem101*	*Pld4*	*Csf2rb*	*Padi2*	*Plcxd2*	*Aff3*	*Lilra6*
Pltp	*Ppfibp2*	*Grk5*	*Plscr1*	*Tyrobp*	*Arhgef18*	*Fcgrt*	*Trim3*
Gatsl2	*Hmox1*	*Nod1*	*Nek6*	*Vill*	*Prkab2*	*Nr4a1*	*Pmaip1*
Lilrb3	*2010012O05Rik*	*Itsn1*	*Gm5547*	*Amz1*	*Snord17*	*Tiparp*	*Klf4*
Diras2	*Sesn2*	*Themis2*	*Plbd1*	*Lgals1*	*Sipa1l3*	*Fam65b*	*Sik1*
Ifitm3	*Gns*	*Lrrk1*	*App*	*Tubb6*	*Aldh6a1*	*Psd3*	*Gem*
Rgs1	*Itgb2*	*Slc40a1*	*Pogk*	*Pepd*	*Sik2*	*Gab3*	*Dusp3*
Pira2	*Bhlhe40*	*Zeb2*	*Sirpa*	*Ak8*	*Calcoco1*	*Fry*	*Wfdc17*
Gpcpd1	*Arl4d*	*Fam49a*	*Tubb2b*	*St3gal4*	*Fam199x*	*A630033H20Rik*	*Eps8*
Gucd1	*Gpr65*	*Pion*	*Camkk1*	*Sowahc*	*Rgs2*	*Arhgap5*	*Pstpip2*
Syne3	*Tmcc3*	*Plek*	*Tubb2a*	*Palm*	*Map3k8*	*Mgst1*	*S100a6*
Adora2a	*Adssl1*	*Myo7a*	*Myo1f*	*Gpr137b*	*Arrdc3*	*Plk2*	*Rtn4rl1*
Emr1	*Man2b2*	*Sgpp2*	*Cyth4*	*Pld2*	*Trf*	*Trp53inp2*	*Hmga2-ps1*
Csf1r	*Axl*	*Syk*	*Naaa*	*Tmem51*	*Gstm1*	*Fam46a*	*Bbc3*
Tlr12	*Fgr*	*Smim3*	*Tmem106a*	*Tnfrsf1b*	*Acox1*	*Cdkn1a*	*Dkkl1*
Cldn10	*Armc7*	*Wdr47*	*Nlrp10*	*Rcn3*	*Fam43a*	*Vim*	*Anxa2*
Plod1	*Naip5*	*Acp2*	*Cd80*	*Atrnl1*	*H2-L*	*Smpdl3a*	*Litaf*
Stard10	*Plaur*	*Il10ra*	*Csf2ra*	*Fbxw13*	*Mir3064*	*Ankrd9*	*Gpc1*
Pla2g15	*Vopp1*	*Rin3*	*Capn2*	*Cdc42ep4*	*Add3*	*Nfil3*	*Osm*
B3gnt7	*Stom*	*Tef*	*Mx1*	*Bhlhe41*	*Ssh2*	*Prkar2b*	*Fmnl3*
Slc27a4	*Haus8*	*Abcg1*	*Dnase1l3*	*Lgals3*	*B3gnt5*	*Lmna*	*Bcl7a*
Acss1	*Laptm4b*	*Cmtm3*	*Alox5ap*	*Rap1gap2*	*Xkrx*	*Ccdc114*	*Lax1*
2310044G17Rik	*Gins2*	*Ska2*	*Kpna2*	*Ccnb1*	*Ldha*	*Ms4a4b*	*Zfp239*
Neurl2	*Brca1*	*Cenpw*	*Ezh2*	*Plk1*	*Poc1a*	*Fancd2*	*Spon1*
D930015E06Rik	*Casc5*	*Bid*	*2810417H13Rik*	*Kif11*	*Cdca3*	*Rad54l*	*Ccdc122*
Spic	*Bzw2*	*Mis18bp1*	*Ccnf*	*Kif22*	*Bub1b*	*Tbc1d7*	*BC035044*
Cyb5r3	*Tspan15*	*Cks2*	*Gstt2*	*Ccna2*	*Spc24*	*Tyms*	*Tlr3*
Cd72	*Ffar2*	*Tcf19*	*Rrm2*	*Tacc3*	*Lmnb1*	*Arhgap19*	*Asb2*
Tmem26	*Rrm1*	*Tk1*	*Ncapg2*	*Ccnb2*	*Txn1*	*Rnaseh2b*	*Rnf219*
Edaradd	*Dctpp1*	*Phf19*	*Kif20a*	*Aurka*	*Gtse1*	*Cdca2*	*Smyd2*
Cnr2	*Siva1*	*Fignl1*	*Aurkb*	*Mki67*	*Spc25*	*Dhrs13*	*Nudt19*
Traip	*Chaf1a*	*Rcc1*	*Top2a*	*Cenpe*	*Cks1b*	*Tuba1c*	*Tmem256*

Chaf1b	*Nusap1*	*Ildr1*	*Plp2*	*Esr1*	*Dclk2*	*Fdps*	*Cecr2*
Slc4a8	*Tpx2*	*Snx10*	*Gm14446*	*A930006K02Rik*	*Tifa*	*Ccdc92*	*Marveld2*
Nudt1	*Kif23*	*Cd9*	*Cish*	*Fam84b*	*Rnf144a*	*Pacsin1*	*Impdh1*
Marcks	*Ect2*	*Lilrb4*	*Anxa5*	*Klhl24*	*A430035B10Rik*	*Lrrc56*	*Spint2*
S1pr3	*Lxn*	*Gsg2*	*Slamf9*	*Slc25a23*	*Cd55*	*Cd28*	*Crisp3*
1500011K16Rik	*Ift122*	*Dnaja4*	*Tagln2*	*Clcf1*	*Tgif1*	*Pla2g12a*	*Rapgef4*
Isg15	*Cdc20*	*Hk2*	*Amigo2*	*Prss12*	*Irs2*	*Fgf13*	*Pde2a*
Cyp39a1	*Cdca8*	*Vps26b*	*Mir150*	*Fchsd2*	*Snx29*	*Treml2*	*Fcer2a*
AW011738	*Fcrl5*	*Prf1*	*Snord35b*	*Scn4a*	*Gpr155*	*Slc29a4*	*Neurl3*
Dtx1	*Traf1*	*Myo10*	*Dusp1*	*Dmxl1*	*Abca1*	*Akap12*	*Mapk11*
Dusp16	*Ccbp2*	*Cfp*	*Stx2*	*Hist1h1d*	*Cacna1i*	*Fam101b*	*Faah*
Abcb1a	*Zc3h12c*	*Slc43a1*	*Rel*	*Lrrk2*	*Ssbp2*	*Zfp318*	*Mapk12*
Nedd4	*Msl3l2*	*Vwa5a*	*Stx17*	*Inpp5a*	*Akt3*	*Zfp608*	*Baiap2*
Ell3	*Dstn*	*Lacc1*	*Dyrk2*	*Fbxl3*	*Slc4a11*	*Pxk*	*Inpp4b*
E2f3	*Ccdc88b*	*Scimp*	*Calcrl*	*Ankrd37*	*Sfn*	*Pcbp3*	*Dhfr*
Pde4d	*Rnf43*	*Phf11a*	*Sgms1*	*Myc*	*Gpr146*	*Il12a*	*Mcm7*
Rangrf	*Akr1e1*	*Nt5e*	*Zfp831*	*Apbb1*	*Hepacam2*	*Chd3*	*Dut*
Rab37	*Rgs3*	*Cd86*	*Pi4k2b*	*Egr2*	*Lca5*	*Frmd6*	*Ada*
Nmral1	*Hs3st1*	*Cd36*	*Foxo3*	*Tspan17*	*L1cam*	*Vpreb3*	*Abcb9*
Jrk	*D8Ertd82e*	*Thyn1*	*Jhdm1d*	*Egr1*	*Gpd1l*	*Tmem108*	*Rgs13*
Lta	*Klrb1f*	*Lst1*	*Hist1h2bc*	*Adamts6*	*Lrrc8a*	*Bcl6*	*Tcf7*
Plxnc1	*Cln8*	*Cxcr3*	*Hist1h1c*	*Brpf3*	*Spata6*	*Ddx25*	*Prkcq*
Gcsh	*9030617O03Rik*	*Trabd2b*	*Ccr7*	*Wdfy1*	*Itga6*	*Rgcc*	*Cd27*
Plxnb2	*Abtb2*	*Gbp8*	*Btg2*	*Sgk1*	*Mcoln2*	*Dck*	*Itk*
Fas	*Extl2*	*Hopx*	*Rhob*	*Jun*	*Il5ra*	*Parp8*	*Tox*
Tmem18	*Ptpn14*	*Pafah1b3*	*Adrb2*	*Cd69*	*Srms*	*Ripk3*	*Ubash3a*
Ralb	*Endod1*	*Crip1*	*4930414L22Rik*	*Klf11*	*E330020D12Rik*	*Dcbld1*	*Rabl5*
Fam92a	*Mettl21a*	*Ldlr*	*Tnfrsf12a*	*Tcp11l2*	*Stac2*	*Sh2d2a*	*Thy1*
Prc1	*Cacnb4*	*Ctla4*	*E230008N13Rik*	*Junb*	*Cmpk2*	*Cd24a*	*Ppp4r2*
Tfdp1	*Tlcd2*	*Ccdc28b*	*Itpkb*	*Slc16a7*	*Cdc25b*	*AI848285*	*Cd4*
Cdca7l	*E2f1*	*Rnf125*	*P2ry10*	*Gdap10*	*Sit1*	*Pcgf5*	*Otub2*
Gatm	*Id2*	*Fxyd5*	*Agpat9*	*Ppp1r15a*	*Uchl3*	*Rbm38*	*Cd3d*
Nlrx1	*Rps6ka1*	*Il6ra*	*Insr*	*Fos*	*Fam216a*	*Acsl3*	*Zap70*
Hmgn3	*Gng12*	*Mcl1*	*Rab6b*	*Ankrd33b*	*Neil1*	*Chchd10*	*Lat*
Plcd3	*Tnfrsf21*	*Glipr2*	*Cd200*	*Tagap*	*Amz2*	*Pdk1*	*Cd6*
Lcp2	*Hpse*	*Dyrk3*	*Zfp36*	*Klf6*	*Icos*	*Syne2*	*Cd5*
Ulbp1	*Dclre1a*	*Aldoc*	*Itih5*	*Tsc22d3*	*Stxbp1*	*Smagp*	*Cd3e*
Slc39a11	*BC147527*	*Itgb7*	*Bnip3*	*Gm11346*	*Emb*	*Epcam*	*Apitd1*
Cdh17	*Cd300lf*	*Kctd14*	*Hist1h2bg*	*Pgm2l1*	*Slfn1*	*Id3*	*Myof*
Rinl	*Ppl*	*Oas3*	*Pde4b*	*Pgap1*	*Sqle*	*Dcaf12*	*Tubg2*

Mef2b	*Ranbp1*	*Icosl*	*8430410A17Rik*	*S1pr2*	*9930012K11Rik*	*Nek2*	*Mcm3*
Itgb3	*Lig1*	*Arvcf*	*Themis*	*H2afx*	*Hat1*	*Pole2*	*Tuba1b*
St14	*Tubb4b*	*9130023H24Rik*	*Sh2b2*	*Cd8b1*	*A930005H10Rik*	*Dlgap5*	*Cd8a*
Klhl6	*Tubb5*	*Pxdc1*	*Il7r*	*Optn*	*Pole*	*Incenp*	*Cst7*
Lef1	*Rfc3*	*Zfp251*	*Cd3g*	*Efnb1*	*Msh6*	*Pfn2*	*Rgs10*
Cd247	*Polh*	*Skp2*	*Myl4*	*Mthfd2*	*Dph5*	*Gmnn*	*C230091D08Rik*
Rassf6	*Mcm2*	*Chek2*	*Cdca7*	*Hmgb2*	*Hmgn2*	*Uhrf1*	
Foxp3	*Orc6*	*B3galt6*	*Tmem48*	*Mcm6*	*Psmc3ip*	*Helq*	
Skap1	*Mcm5*	*2310009B15Rik*	*Rfc4*	*Ncaph*	*Asf1b*	*Mad2l1*	
Gata3	*Snrpd1*	*Cr2*	*Fen1*	*Dbf4*	*Psat1*	*H2afz*	
Lck	*Pola1*	*Stmn1*	*Thop1*	*Serpine2*	*Hells*	*Tipin*	
Cd96	*Prkch*	*Dynlt1a*	*Shmt1*	*Knstrn*	*Rad51*	*Fanci*	

Table S2: Differentially expressed genes in hepatic and splenic MBC subsets (related to figure 6) List of 730 genes that were differentially and significantly expressed in splenic and hepatic MBC subsets with an FDR of <0.01 and >2-fold difference in any pairwise comparison among the four populations.