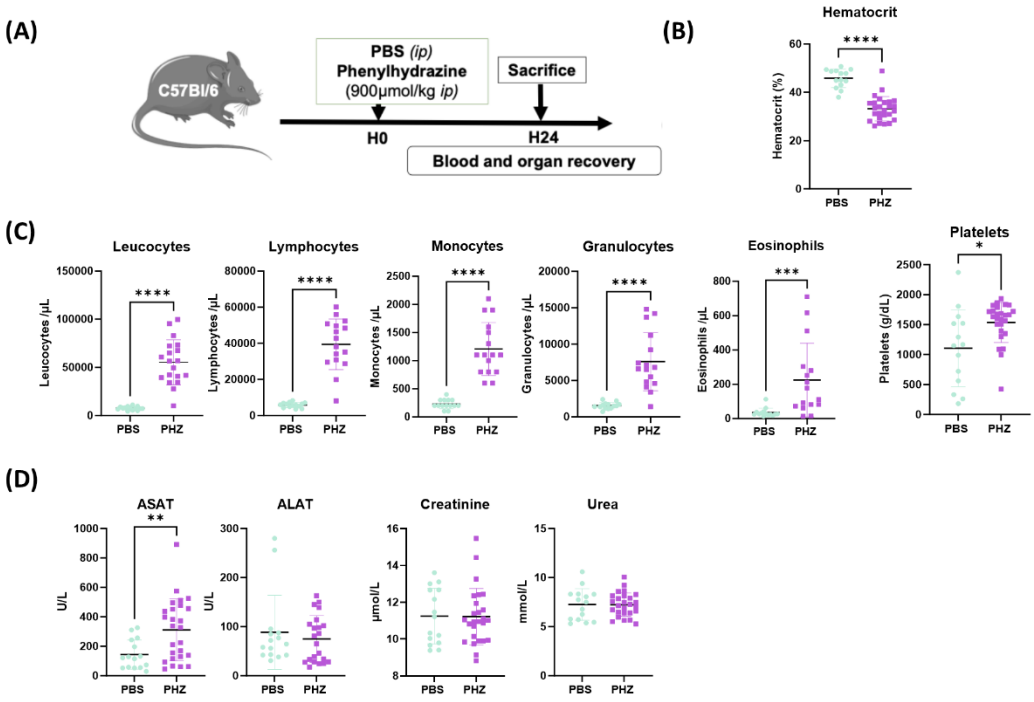
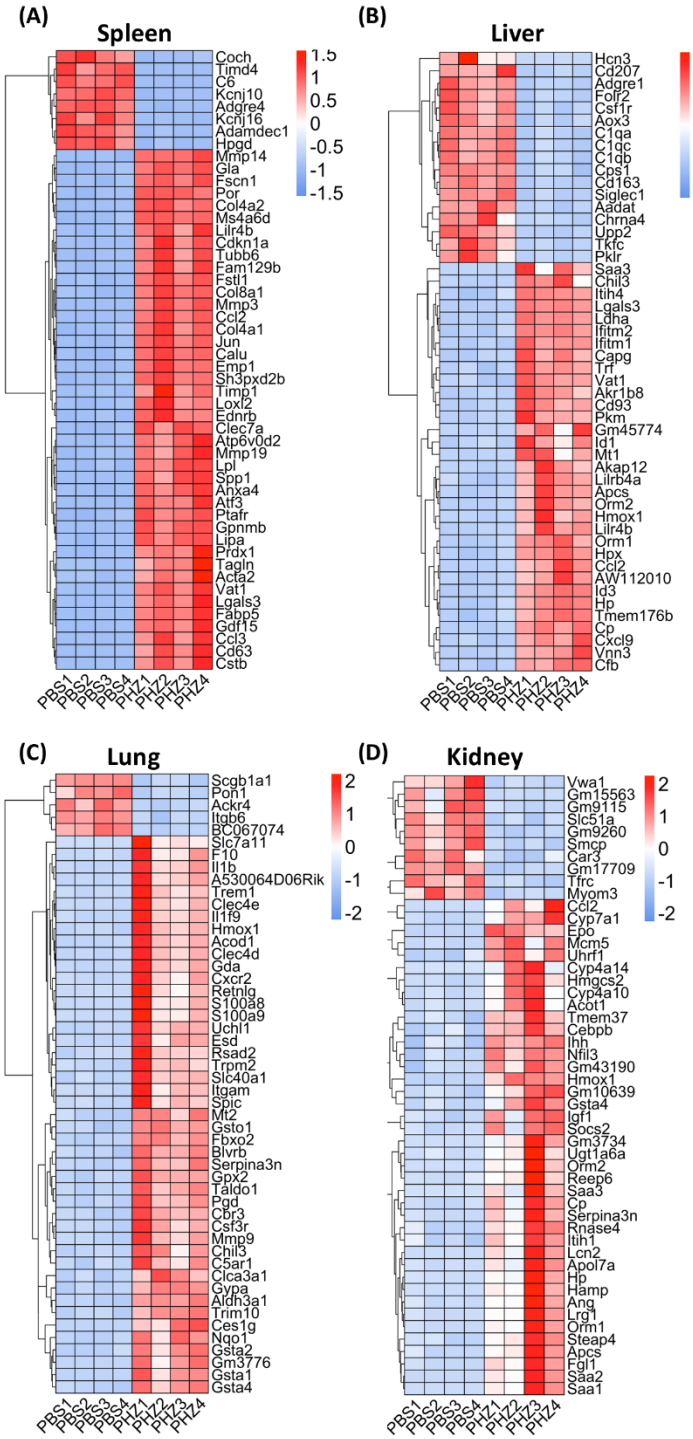
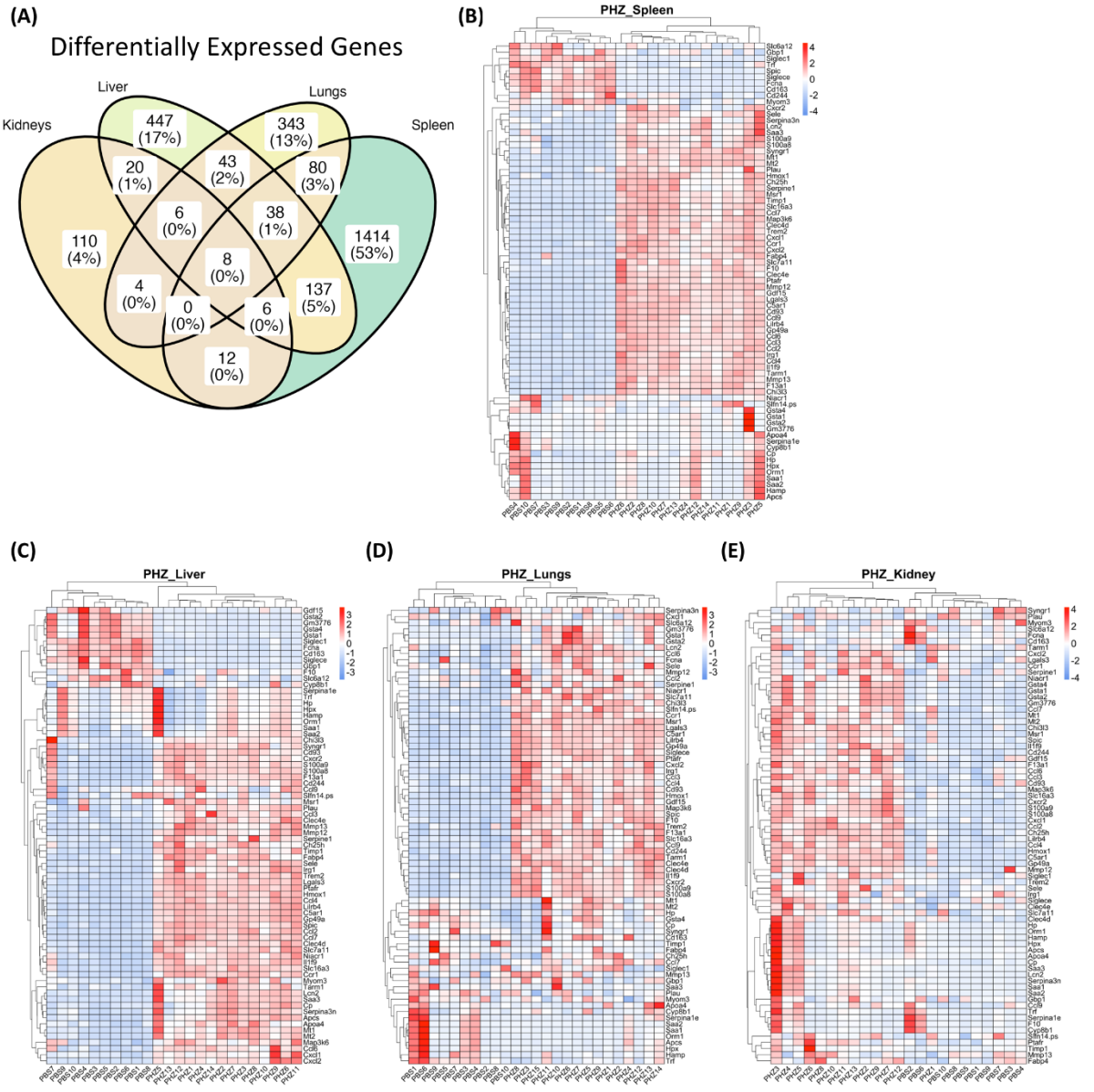


Supplementary Figure 1: PHZ-induced hemolysis is characterized by an increase in circulating immune cells.
(A) Description of the PHZ-induced hemolysis mouse model. (B) Decreased hematocrit as a proof of efficacy of the hemolysis (C) Quantification of leucocytes, lymphocytes, monocytes, granulocytes, eosinophils and platelets in the blood of PBS (light green) and PHZ (pink) C57BL/6 injected mice (D) Quantification of ASAT (marker of hemolysis and liver injury) and ALAT (maker of liver injury) (left) and renal injury markers creatinine and urea (right) in the plasma of PBS (light green) and PHZ (pink) C5BL/6 injected mice (* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001, Mann-Whitney test).

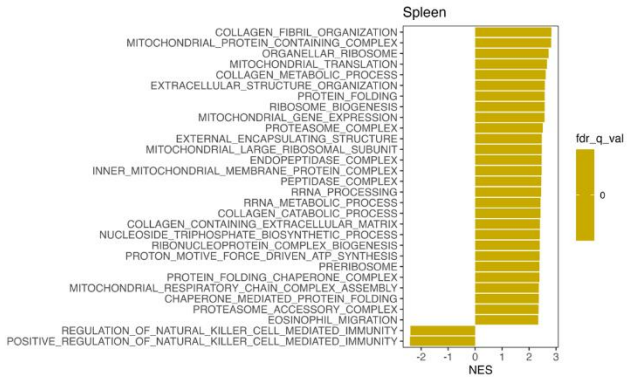


Supplementary Figure 2: Transcriptomic signatures of organ injury in the Phenylhydrazine (PHZ) mouse model. PHZ mice compared to PBS in the spleen (A), liver (B), lung (C) and kidney (D) . The top 50 differentially expressed genes were selected based on adjusted p-value < 0,05; log2 (fold change) ≤ -1 or ≥ 1.

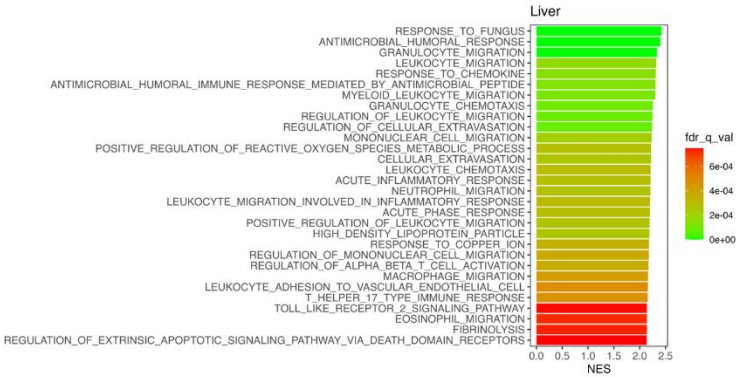


Supplementary Figure 3: Common differentially expressed genes (DEG) upon PHZ treatment. (A) Venn diagram plot showing the percentage of shared DEG (*adjusted p-value < 0,05; log2 (fold change) ≤ -1 or ≥ 1)* across spleen, liver, lung and kidney in PHZ- vs PBS-treated mice. (B-E) Heatmap of a Quantigene panel performed on spleen (B), liver (C), lungs (D) and kidneys (E) of PBS- and PHZ- treated mice. It comprised DEG that are common in two or more organs.

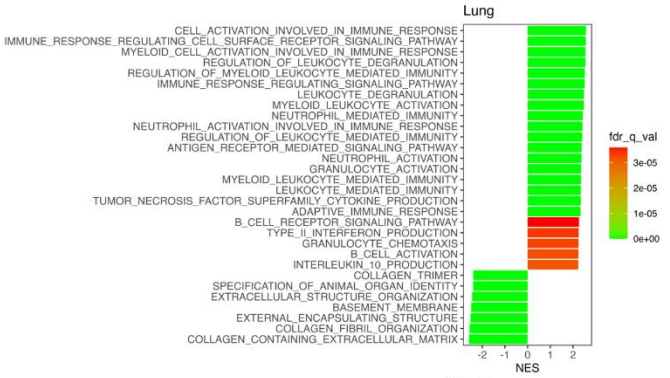
(A)



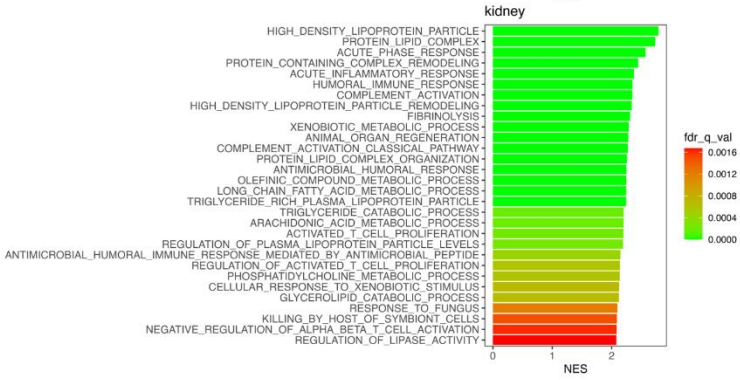
(B)



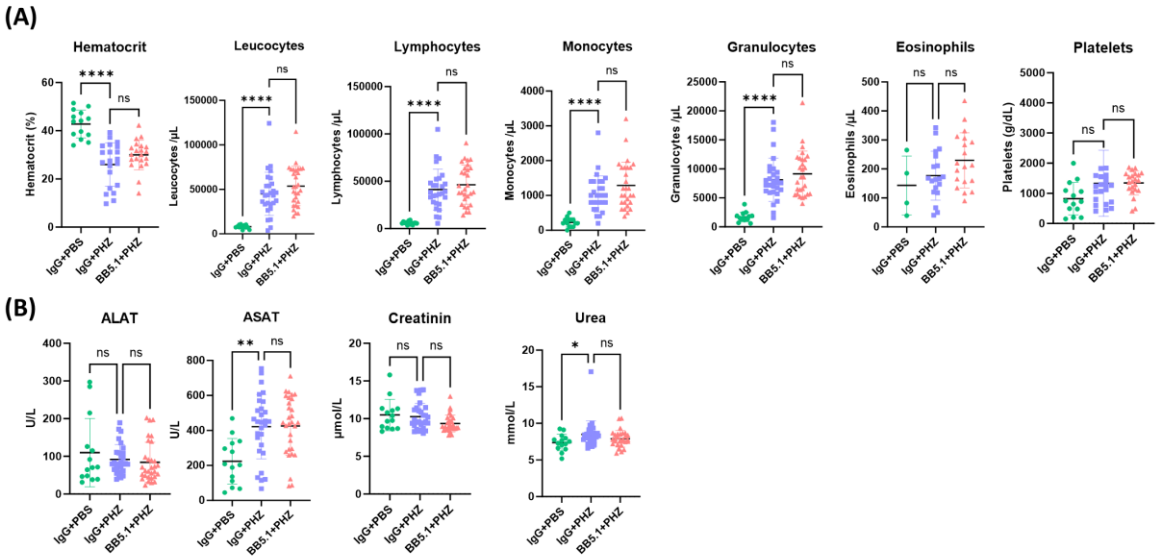
(C)



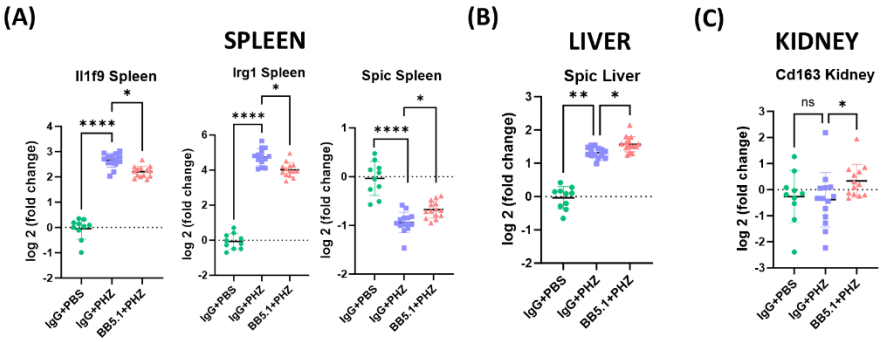
(D)



<u>Supplementary Figure 4:</u> Gene set enrichment analysis (GSEA) of the GENE ONTOLOGY (GO) genesets affected in the 4 organs in PHZ mice compared to PBS in the spleen (E), liver (F), lung (G) and kidney (H) (FDR q value ≤ 0.05). FDR: false discovery rate, PBS: phosphate buffer saline, PHZ : phenylhydrazine



<u>Supplementary Figure 5</u>: Complement inhibition does not affect the biological parameters of the PHZ mice: (A) Quantification of hematocrit, leucocytes, lymphocytes, monocytes, granulocytes, eosinophils,, and platelets in the blood of C57BL/6 mice injected by phenylhydrazine (PHZ) or PBS (i.p.), preventively treated by a C5 inhibitor, BB5.1, or a control IgG (i.p., H-2) : IgG+PBS (dark green), IgG+PHZ (purple) and BB5.1+PHZ (coral) (B) Quantification of ALAT, ASAT, creatinin and urea in the plasma of IgG + PBS (dark green), IgG + PHZ (purple) and BB5.1 + PHZ (coral) C5BL/6 injected mice. (* $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$, Kruskal-Wallis test with Dunn's test for multiple pairwise comparisons).



Supplementary Figure 6. Genes significantly impacted by complement inhibition in (A) spleen, (B) liver and (C) kidney, measured by Quantigene. * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001, Kruskal-Wallis test with Dunn's multiple comparisons test).

Supplementary Table S1: Panel Quantigen 80 plex. In coral are the housekeeping genes.

Actb	**Cxcr2**	**Mt2**
Gapdh	**Cyp8b1**	**Myom3**
Gusb	**F10**	**Niacr1**
Polr2a	**F13a1**	**Orm1**
Rps18	**Fabp4**	**Plau**
Rps3	**Fcna**	**Ptafr**
	Gbp1	**S100a8**
Apcs	**Gdf15**	**S100a9**
Apoa4	**Gm3776**	**Saa1**
C5ar1	**Gp49a**	**Saa2**
Ccl2	**Gsta1**	**Saa3**
Ccl3	**Gsta2**	**Sele**
Ccl4	**Gsta4**	**Serpina1e**
Ccl6	**Hamp**	**Serpina3n**
Ccl7	**Hmox1**	**Serpine1**
Ccl9	**Hp**	**Siglec1**
Ccr1	**Hpx**	**Siglece**
Cd163	**Il1f9**	**Slc16a3**
Cd244	**Irg1**	**Slc6a12**
Cd93	**Lcn2**	**Slc7a11**
Ch25h	**Lgals3**	**Slfn14-ps**
Chi3l3	**Lilrb4**	**Spic**
Clec4d	**Map3k6**	**Syngr1**
Clec4e	**Mmp12**	**Tarm1**
Cp	**Mmp13**	**Timp1**
Cxcl1	**Msr1**	**Trem2**
Cxcl2	**Mt1**	**Trf**

Supplementary Table 2. mMCP counter genes

Cell type	Gene	Accession number
Eosinophils	Inpp5j	ENSMUSG00000034570
Fibroblasts	Kcne4	ENSMUSG00000047330
	Agtr1a	ENSMUSG00000049115
	Cpxm1	ENSMUSG00000027408
	Ccl19	ENSMUSG00000071005
	Pde3a	ENSMUSG00000041741
	Rarres2	ENSMUSG00000009281
	Abcc9	ENSMUSG00000030249
Granulocytes	Mrgpra2b	ENSMUSG00000096719
Lymphatics	Nts	ENSMUSG00000019890
	Coch	ENSMUSG00000020953
	Mmrn1	ENSMUSG00000054641
	Fxyd6	ENSMUSG00000066705
Mast cells	Cma2	ENSMUSG00000068289
	Mcpt9	ENSMUSG00000068289
	Tph1	ENSMUSG00000040046
	Mrgpra4	ENSMUSG00000067173
	Mrgprb1	ENSMUSG00000070547
	Mrgprx2	ENSMUSG00000074109
	Mrgprb2	ENSMUSG00000050425
Memory B cells	Fcrl5	ENSMUSG00000048031
Monocytes / macrophages	Fcgr1	ENSMUSG00000015947
Neutrophils	Ceacam10	ENSMUSG00000054169
NK cells	Klra1	ENSMUSG00000067599
	Klra9	ENSMUSG00000072717
	Klra10	ENSMUSG00000072718
T cells	Cd6	ENSMUSG00000024670
	Cd3d	ENSMUSG00000032094
	Cd3e	ENSMUSG00000032093
Vessels	Mmrn2	ENSMUSG00000041445
	Shank3	ENSMUSG00000022623
	Emcn	ENSMUSG00000054690
	Tie1	ENSMUSG00000033191
	Ldb2	ENSMUSG00000039706
	Fgd5	ENSMUSG00000034037
	Cdh5	ENSMUSG00000031871

Supplementary Table 3. Differentially expressed genes in 2 or more organs. In yellow are the ones selected for the Quantigene panel to validate the RNAseq data and to evaluate the effect of complement inhibition.

	kidney & lungs	kidney & liver	kidney & spleen	lungs & liver	lungs & spleen	liver & spleen	kidney & lungs & liver	kidney & lungs & spleen	kidney & liver & spleen	lungs & liver & spleen	kidney & lungs & liver & spleen
Combination	kidney & lungs	kidney & liver	kidney & spleen	lungs & liver	lungs & spleen	liver & spleen	kidney & lungs & liver	kidney & lungs & spleen	kidney & liver & spleen	lungs & liver & spleen	kidney & lungs & liver & spleen
Genes	Hmox1; Saa2; Lcn2; Saa3; Fgl1; Saa1; Cp; Orm2; Myom3; Lrg1; Steap4; Gsta4; Car3; Hp; Hamp; Gm3734; Orm1; Ccl2; Serpina3n; Apcs; Ppp1r3b; Mt2; S100a9; Gm13502; Trf; Chil3; Saa4; Mt1; C5ar1; S100a8; Gm29966; Lilr4b; Vnn3; Hpx; Gm3776; Gm12840; Itih4; Bcl6; Gsta1; Slc16a3	Hmox1; Saa3; Gm10639; Gsta4; Ccl2; Serpina3n; Mt2; S100a9; A2ml1; Chil3; C5ar1; S100a8; Lilr4b; Gm3776; Xlr3a; Gsta1; Slc16a3; Abcb4	Hmox1; Saa3; Cp; Myom3; Lrg1; Cebpb; Ccl2; Serpina3n; Socs2; Mt2; Cox6a2; Abca6; Trf; Mt1; C5ar1; Ftl1.ps2; Lilr4b; Mmp19; Cyp8b1; Vnn3; Pemt; Cd1d1; Serpina1e; Slc16a3; Slc6a12; Impg2	Lgals3; Hmox1; Ccl2; Lilr4b; Saa3; Gm45774; Chil3; Cd207; Lilrb4a; Serpina3m; Trem2; Ptafr; Cfp; Serpina3n; Plek; Atf3; Fpr1; S100a4; Fcna; Serpine1; Slc16a3; Alox5ap; A530064D06Rik; S100a8; Clec4d; Mt2; F13a1; Clec4n; Spic; Gsta2; Clec4e; AI662270; Siglece; Myo1f; Ccl4; Ccl3; Gm36161; Gsta4; Cd300lb; Gpnmb; Tlr13; Cxcl2; Itgam; Gm21188; Fkbp5; Map3k6; Cdkn1a; Cd300a; Pla2g7; Acod1; Rgs1; Gm49339; Gm3776; Slc7a11; Retnlg; Il1f9; Mmp8; C5ar1; Lrp8; Ccr1; Gfra2; Trem3; S100a9; Ackr4; Cd244a; Gpr35; Marco; Hr; Fcgr1; Apln; Gm14548; Ms4a4c; Trpm2; Gm19951; Ces2b; Cd300lf; Dhrs9; Hcar2; Noxa1; Atp1a3; Ms4a4a; Gsta1; Lilra6; Tlr8; Hspa1a; Pilrb2; Slc2a6; Mefv; F630028O10Rik; Fndc1; Pram1; NA.; Gpr141; Ddias; Cxcr2	Lgals3; Cp; Hmox1; Vnn3; Cd93; Ccl2; Lilr4b; Saa3; Ifitm1; Ifitm2; Cd163; Cxcl9; Pkm; Akr1b8; Adgre1; AW112010; C1qa; Gm45774; Csf1r; Siglec1; Cps1; C1qc; Trf; Lilrb4a; Vat1; Aox3; Mt1; Ccl7; Trem2; Ptafr; Fabp4; Apold1; Col4a2; Adam8; Olfr1034; Serpina3n; P2ry13; Gadd45b; Atf3; Gbp2b; Fpr1; Adgre4; S100a4; Kcna2; Fcna; Lrg1; Col4a1; Chp2; Adamts4; P2ry12; Serpine1; Sele; Bcl6b; Slc16a3; A530064D06Rik; Timd4; Bank1; Clec4d; Mt2; Clec4n; Adamdec1; Gprin3; Ubd; Il1rn; Slc1a4; Lilra5; Kcnj10; Clec4e; Plau; Crybb3; Emp1; Siglece; Armcx4; Fscn1; Abca9; Klra2; Gla; Ccl4; Ddah1; Ccl3; Cd300lb; Rgs16; Igfbp2; Ckap4; Basp1; Gpnmb; Clec12a; Gm20708; Tlr13; Lgals1; Gm1966; Cd63; Cxcl2; Inhbb; Serpinh1; Kcnj16; Map3k6; Cdkn1a; Csf1; Pla2g7; Ccl6; Plaur; Dbn1; Acod1; Gbp8; C1qtnf6; Gm49339; Slc7a11; Clec7a; Frzb; Mmp12; Loxl2; Ecscr; Adam15; Il1f9; Nt5e; Cd200r1; Igfbp3; Vim; Ier3; C5ar1; Ccr1; Gfra2; Cxcl1; Trem3; Ackr4; Septin5; Sh3pxd2b; Galnt18; Gm2814; Dok2; Cerkl; Klrk1; Timp1; Apln; Gm44220; Ppp1r9a; Ddah2; Mest; Ch25h; Gpr137b.ps; Bmper; Smyd1; Raet1d; Adrb1; Fblim1; Raet1e; Dpep2; Apoa4; Procr; Kcne4; Zfp9; Mmp13; Car13; Ms4a4a; Anxa2; Dntt; Creb3l1; Pcdhgc4; Loxl4; Plet1; Hspa1a; Upp1; Uap1l1; Pilrb2; Rab7b; Fam71e1; Pcolce2; Myom3; Fxyd2; Cx3cr1; Arl11; H1f3; Syngr1; Cd300e; Fndc1; Btbd11; NA.; Cib3; Asb2; Gm19426; Chst11; Col20a1; Slit1; X0610009E02Rik; Siglech; Gm6560; Gfpt2; Gm33280	Clec4d; Uchl1; Clec4e; Hmox1; Serpina3n; Acod1; Mt2; Gypa; Pgd; A530064D06Rik; Ackr4; Slc7a11; Esd; Il1f9; Trim10; F10; C5ar1; Ccl9; Cxcl2; B430306N03Rik; Trem3; Ptafr; Ccr1; Cd300lb; Lilrb4a; Gpnmb; Nrcam; Snca; Cd68; Gfra2; Kdelr3; Siglece; Wfdc17; Gm10252; Fpr1; Hspa1b; Col12a1; Gm49339; Mrgpra2b; Spp1; Srxn1; Gdf15; Apln; Gsr; Esm1; NA.; Clec4n; Pla2g7; AI506816; Hspa1a; Slc16a3; Mrc2; Stfa2l1; S100a4; Pilrb2; Asprv1; Slfn14; Col6a2; Mrgpra2a; Col1a2; X2500002B13Rik; Lilr4b; Il1r2; Ipcef1; Trem2; Tnc; Nr2f1; Msr1; Tarm1; Serpine1; Gm11346; Cstdc4; Slc7a8; Ugt1a7c; Fndc1; Gm32051; Tcf23; Shisa2; Tmem119; Slc4a1; Col5a3; Epb42; Ms4a6d; Saa3; Fam107a; Lgals3; Cxcr1; Atf3; Tlr13; Cdkn1a; Gm45540; Pla1a; Ccl3; Nxpe5; Col3a1; Apoc2; Crlf1; Vcan; Tgfbi; Sycp2; Mthfd2; X1600010M07Rik; Prok2; Gm20658; Fcamr; Ms4a4a; Lrrn2; Prss34; Ypel4; Gm4294; H2bu2; X9030404E10Rik; F930017D23Rik; Map3k6; Ccl2; Slc25a34; Col6a3; Gm9973; Gpr88; Fcna; Itgb2l; Ccl4; Fosl1; Gm45774; Prtn3; Gm16364	Hmox1; Saa3; Gsta4; Ccl2; Serpina3n; Mt2; S100a9; Chil3; C5ar1; S100a8; Lilr4b; Gm3776; Gsta1; Slc16a3	Hmox1; Saa3; Cp; Myom3; Lrg1; Ccl2; Serpina3n; Mt2; Trf; Mt1; C5ar1; Lilr4b; Vnn3; Slc16a3	Hmox1; Saa3; Ccl2; Serpina3n; Mt2; C5ar1; Lilr4b; Slc16a3	Lgals3; Hmox1; Ccl2; Lilr4b; Saa3; Gm45774; Lilrb4a; Trem2; Ptafr; Serpina3n; Atf3; Fpr1; S100a4; Fcna; Serpine1; Slc16a3; A530064D06Rik; Clec4d; Mt2; Clec4n; Clec4e; Siglece; Ccl4; Ccl3; Cd300lb; Gpnmb; Tlr13; Cxcl2; Map3k6; Cdkn1a; Pla2g7; Acod1; Gm49339; Slc7a11; Il1f9; C5ar1; Ccr1; Gfra2; Trem3; Ackr4; Apln; Ms4a4a; Hspa1a; Pilrb2; Fndc1; NA.	Hmox1; Saa3; Ccl2; Serpina3n; Gm45774; Chil3; Cd207; Lilrb4a; Serpina3m; Trem2; Ptafr; Cfp; Serpina3n; Plek; Atf3; Fpr1; S100a4; Fcna; Serpine1; Slc16a3; Alox5ap; A530064D06Rik; S100a8; Clec4d; Mt2; F13a1; Clec4n; Spic; Gsta2; Clec4e; AI662270; Siglece; Myo1f; Ccl4; Ccl3; Gm36161; Gsta4; Cd300lb; Gpnmb; Tlr13; Cxcl2; Itgam; Gm21188; Fkbp5; Map3k6; Cdkn1a; Cd300a; Pla2g7; Acod1; Rgs1; Gm49339; Gm3776; Slc7a11; Retnlg; Il1f9; Mmp8; C5ar1; Lrp8; Ccr1; Gfra2; Trem3; S100a9; Ackr4; Cd244a; Gpr35; Marco; Hr; Fcgr1; Apln; Gm14548; Ms4a4c; Trpm2; Gm19951; Ces2b; Cd300lf; Dhrs9; Hcar2; Noxa1; Atp1a3; Ms4a4a; Gsta1; Lilra6; Tlr8; Hspa1a; Pilrb2; Slc2a6; Mefv; F630028O10Rik; Fndc1; Pram1; NA.; Gpr141; Ddias; Cxcr2
Count	40	18	26	95	189	126	14	14	8	46	8

Supplementary methods

Materials & Methods

Animal experimentation

C57BL/6J male mice were purchased from Charles River Laboratory. The experiments with the HbAA and HbSS Townes mice were described previously [1] and the data is reused here. Mice were housed in specific pathogen-free rooms to limit infections and kept on a 12-hour light/dark cycle at 21°C and were used for experimentation at 8-10 weeks of age. Experimental protocols were approved by the Charles Darwin ethical committee (Paris, France) and by the French Ministry of Agriculture (Paris, France) number APAFIS#2148 2019091015099240v1. All experiments were conducted in accordance with their recommendations for care and use of laboratory animals. Only male mice were used, as female mice have weaker complement activation capacity, have a more heterogeneous phenotype in the PHZ model and develop less severe kidney injury. If mice in any experiment had to be excluded from analysis, it was because of technical issues with sample collection or handling. For PBS or PHZ or treatment injection mice were randomized by randomly injecting the mice with each treatment and placing mice injected with both treatments in the same cage to avoid cage effect. One person prepared each mouse and the injection syringe and another person, blinded for the content of the syringe, injected the mice. The experiment was performed in the morning and the animals were observed at midday and in the late afternoon for the 24h experiments. Mice were kept in groups of 2 to 5 and housed in appropriate Type IIL cages offering sufficient living space, on racks with individual thermoregulated ventilation (22°C, +/-2°C), 12-hour lighting (daytime phase from 7 a.m. to 7 p.m.) and automatic watering. An enriched environment suitable for housing mice is provided. Human endpoint included moribund/unresponsive, non-ambulatory, or unable to reach food/water and recumbency. The choice of the experimental mouse strain and the methodology is standard [2].

Phenylhydrazine hydrochloride (PHZ) (114715, Sigma Aldrich) was prepared at 25 mg/mL in sterile Phosphate Buffer Serum (PBS) immediately before use. 900 µmol/kg of PHZ, corresponding to 0.125 mg/g body weight, or PBS was injected intraperitoneally (i.p.) at day 0, and mice were sacrificed by cervical decerebration at day 1 (24h later PHZ injection).

For treatment experiments, mice received a preventive treatment by mouse monoclonal antibody BB5.1 – an inhibitory anti-murin C5 antibody (expressed in house by Roche based on the available sequence [14]) as compared to an IgG isotype (Roche). Both were

diluted in PBS and 10 mg/mL and 1 mg/mouse of BB5.1 or IgG were injected intraperitoneally (i.p.) 2 hours before PBS or PHZ injection.

Organs and blood recovery

Mice were anesthetized with isoflurane 2-3% for injections, blood collection and sacrifice. Whole blood was obtained retro-orbitally with heparinized capillary tubes, and subsequently transferred into microtubes containing 10 µL of EDTA. A centrifugation at 12,000 G for 10 minutes at room temperature enabled to isolate plasma which was promptly frozen at -80°C for storage. Simultaneously, organs (liver, lung, kidney and spleen) were promptly snap-frozen in liquid nitrogen.

Blood analyses

Hematological and biochemical analyses were conducted on whole blood samples using an 18-parameter hematology analyzer/blood counter (scil Vet abc™). Additionally, plasma analyses were carried out using a Konelab Analyzer (Thermo Scientific) by the "Small animal renal physiology platform" from Cordeliers Research Center.

ELISA

Mouse plasma was diluted 1:100 in Reagent diluent (1% BSA in PBS) and the C5a assays were performed using the Mouse Complement Component C5a DuoSet ELISA kit (DY2150, R&D Systems) according to the manufacturer's recommendations. The absorbance was measured at 450 nm with the Infinite® 200 PRO Spectrophotometer (TECAN). The results were analyzed using GraphPad Prism.

Determination of the optimal dose of recombinant BB5.1 to inhibit complement activation in mouse serum *in vitro*

Recombinant BB5.1 expressed as described [3] (Roche) was titrated to normal mouse serum followed by addition of zymosan (B400, Complement Technologies, Tylor, Texas) as activator of complement. 400 µg/ml zymosan caused 100% complement activation in 1/3 diluted mouse serum, as measured by C5a release. Normal mouse serum contains ˜60 µg/ml of C5, being equivalent to ˜0,3µM, therefore 1/3 mouse serum contains 0,1µM C5. Recombinant BB5.1 was titrated with concentrations above and below the equimolar concentration for 30min/37°C. The released C5a was measured by ELISA.

The capacity of this antibody to inhibit complement was confirmed in a zymosan-mediated complement activation assay in mouse serum and the determined effective dose was very similar to the dose used in the literature: 0.75 – 1 mg/mouse [4-7]. Preventive treatment with BB5.1 partially prevented C5a elevation in blood.

RNA extraction and quality evaluation

Thirty-µm-thick frozen sections of kidneys, lungs, spleen and livers were cut with a Cryostat at -20°C (Leica AS-LMD, Leica Biosystem) and homogenized in 300µL of 1-Thioglycerol/Homogenization Solution (Maxwell® 16 LEV simplyRNA Tissue Kit Promega AS1280). Lung RNA was extracted by Macherey Nagel kit (NucleoSpin RNA Mini kit for RNA purification, 740955, Macherey Nagel), according to the manufacturer's recommendations. The quality and quantity of mRNA were evaluated with a 2100 bioanalyzer with TNA 6000 NanoKits (all Agilent Technologies, Palo Alto, CA, USA). RNA Integrity Numbers superior to 7 were eligible for subsequent reverse transcription into cDNA.

Choice of organs for RNAseq:

Spleen and liver were chosen for their role in erythrophagocytosis, lung for its well-known complication during SCD, acute chest syndrome, and kidney as a key organ for free hemoglobin scavenging. All these organs exhibited tissue damage in our SCD atlas. As preliminary RNAseq experiments underlined the heterogeneity of organ-specific response to phenylhydrazine-induced hemolysis (data not shown), 4 homogenous samples by organ (spleen, liver, kidney and lungs) and by condition (PBS and PHZ) were selected using a combination of biochemical, hematological and protein expression data. Therefore, a first QuantiGene analysis for mRNA expression was performed on each sample with a 16-plex panel (*Selp*, *Hmox1*, *Lcn2*, *Gsta1*, *Gusp*, *Cxcl17*, *Cxcl2*, *Havcr1*, *Sox9*, *Fcgr1*, *Ccl2* and 5 housekeeping genes: *Actb*, *Polr2a*, *Gapdh*, *Rps3*, *Rps18*). A hierarchical clustering was performed with pheatmap package (Euclidian clustering method) to select samples while avoiding outliers. The 4 PHZ and 4 PBS samples for each organ were sent for bulk RNA sequencing.

Bulk RNA sequencing and preprocessing

The RNAseq analysis was conducted at the GenomIC platform of the Cochin Institute INSERM U1016. Each organ (kidney, liver, lungs and spleen) and each condition (PBS vs PHZ) were represented by four samples. For library construction, 1 µg of high-quality total RNA sample (RIN >7) was processed using TruSeq Stranded mRNA kit (Illumina) according to manufacturer instructions. In summary, after purification of poly-A containing mRNA molecules, mRNA molecules underwent fragmentation and reverse-transcription using random primers. The replacement of dTTP by dUTP during the second strand synthesis ensured strand specificity. Addition of a single A base to the cDNA is followed by ligation of Illumina adapters.

Libraries quantification was performed through qPCR using the KAPA Library Quantification Kit for Illumina Libraries (KapaBiosystems, Wilmington, MA) and library profiles were assessed using the DNA High Sensitivity LabChip kit on an Agilent Bioanalyzer. The libraries were then sequenced on an Illumina Nextseq 500 instrument using 75 base-lengths read V2 chemistry in a paired-end mode.

Following sequencing, a primary analysis was executed using AOZAN software (ENS, Paris) for demultiplexing and quality control of the raw data (bases on FastQC modules / version 0.11.5). The resultant fastq files underwent alignment through the STAR algorithm (version 2.5.2b) with subsequent quality control of the alignment realized with Picard tools (version 2.8.1). Read counts were then determined with Featurecount (version Rsubread 1.24.1).

Differential gene expression analysis and related statistical workup

Differential gene expression analysis between the two conditions (PBS vs PHZ) on the preprocessed read counts were performed with the DESeq2 package (version 1.14.1). Volcano plots were designed using the ggplot2 package. Genes with an abs(log2FC) > 1 and an adjusted p-value (Benjamin Hochberg method) < 0.05 are considered differentially expressed between the two groups (differentially expressed genes, DEGs). Heatmaps were visualized using ComplexHeatmap and Pheatmap packages, by performing raw scale normalization and Euclidean clustering. Venn diagrams were visualized using the ggVennDiagram package.

- **Pathway analysis**

Pathway analysis was conducted using Gene Set Enrichment Analysis [8] (GSEA, v4.3.3) on normalized count data with DESeq2 R package. Given the limited sample size (n=4 per condition), the analysis was performed according to GSEA recommendations using the following parameters: permutation type set to "gene_set," enrichment statistic set to "weighted," ranking metric set to "Signal2Noise," and exclusion of gene sets containing fewer than 15 or more than 500 genes. Each run included the following gene set collections together: HALLMARK, GO_CC, GO_BP, REACTOME and BIOCARTA. Results were filtered using a threshold of normalized enrichment score (NES) > 1.5 and false discovery rate (FDR) < 0.25, as recommended by GSEA guidelines for exploratory analyses aimed at identifying enriched signatures with potential biological relevance. Redundancy between gene sets were controlled by hierarchical clustering based on pathway name's similarities using cutree (h = 0.2).
Ingenuity Pathway Analysis software (Qiagen) was used to evaluate the upstream pathways in the tested organs using DEGs as input for analysis. The activation z-score, computed based on gene expression changes (abs(log2FC) > 1 & adj p-val < 0.05), determines whether a pathway is predicted to be activated (positive z-score) or inhibited (negative z-score).

- **Validation of gene signatures by QuantiGene**

A QuantiGene panel was designed to validate the RNAseq signatures shared across multiple organs and to explore the impact of complement inhibition on such signatures. We selected all the genes that were up or down regulated in all or three out of four organs, and a subset of genes that were regulated in two organs and whose functional relevance to hemolysis was determined by literature review. Pseudo-genes or genes of unknown function were removed from the list to achieve an 80-plex (75 genes of interest and 5 housekeeping genes, see Table S1).

Thirty-µm-thick frozen tissue sections of kidneys, spleen, liver and lungs were cut at -20°C using a Cryostat (Leica AS-LMD, Leica Biosystem) and subsequently homogenized in 200 µL of 1-Thioglycerol/Homogenization Solution (QuantiGene®, ThermoFisher). Subsequently, target and housekeeping genes underwent targeted hybridization and signal amplification following the manufacturer's recommendations. The Streptavidin phycoerythrin signals were detected using a Luminex photometer (Luminex Corporation).

To enhance precision, fluorescence from blank wells was subtracted from median fluorescence, and housekeeping genes were validated based on their standard deviation to mean ratio.

- **murine Microenvironment Cell Population counter (mMCP-counter)**

The mMCP-counter method [9] was used to estimate abundance of tissue-infiltrating immune and stromal cell populations in murine samples using normalized gene expression from bulk RNA seq expression data. Additionally, a dedicated QuantiGene panel was designed. Its composition is given in Table S2. mMCP counter genes were included in the panel only if their expression was detected in the RNAseq datasets of the tested organs, as the method was developed originally for tumors. One gene per cell population, if well detected, is enough to determine its proportion in the tested samples.

- **Data sharing statement:** All raw data from the RNAseq is available publicly. Accession numbers as follows:

The normalized matrix for the spleen, lung, kidney and liver from PBS and PHZ treated mice is available at **10.5281/zenodo.15709647**
The normalized matrix of the liver from PBS or PHZ-injected C57Bl/6 mice is also available at: 10.5281/zenodo.14845299[10]
The normalized matrix for the lung, kidney, liver, heart and bone marrow from the HbAA and HbSS mice, injected or not with heme is available at: **10.5281/zenodo.15709821** [1]

Statistical Analyses on biochemical, hematological and QuantiGene data

Biochemical and hematological measurements and QuantiGene data were analyzed using the statistical software GraphPad Prism 10 (La Jolla, USA). Two continuous variables were compared using the Mann-Whitney test. Comparisons between more than 2 groups of mice were performed using Kruskal-Wallis test, followed by Dunn's test. Statistical significance was defined as $p<0.05$, with bilateral tests.

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