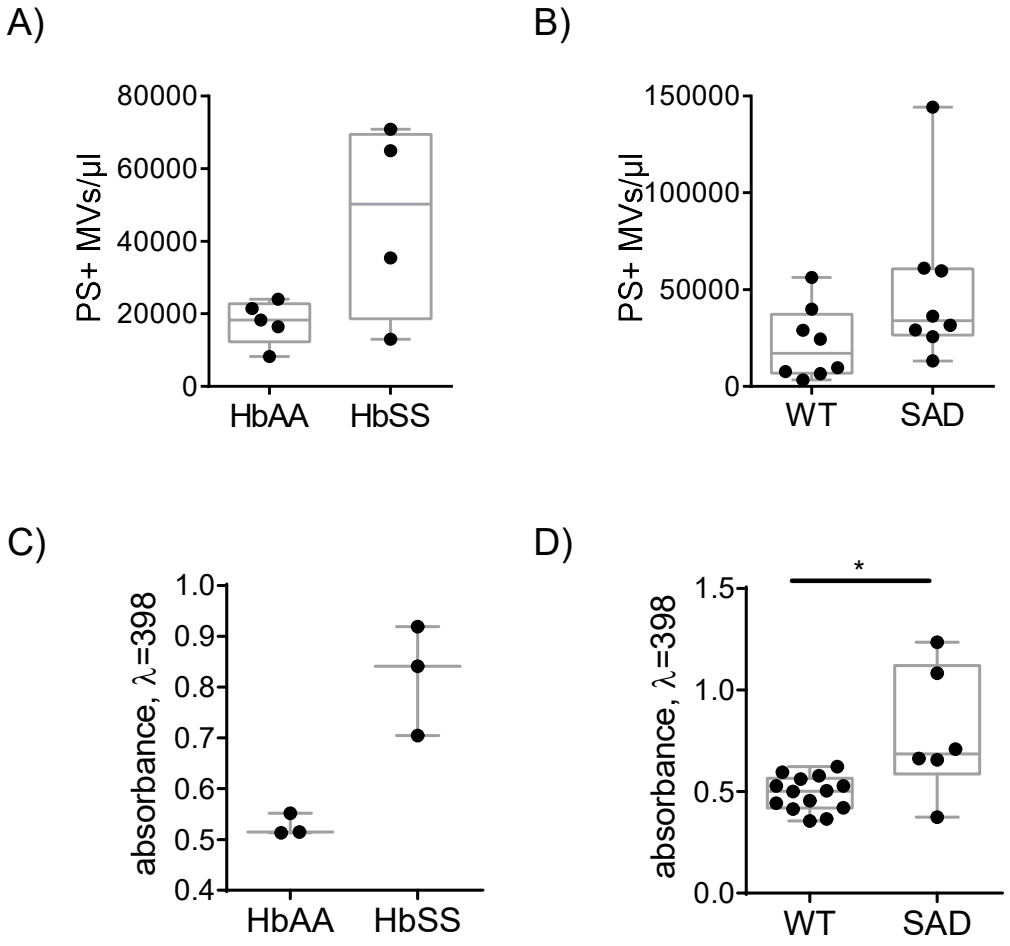
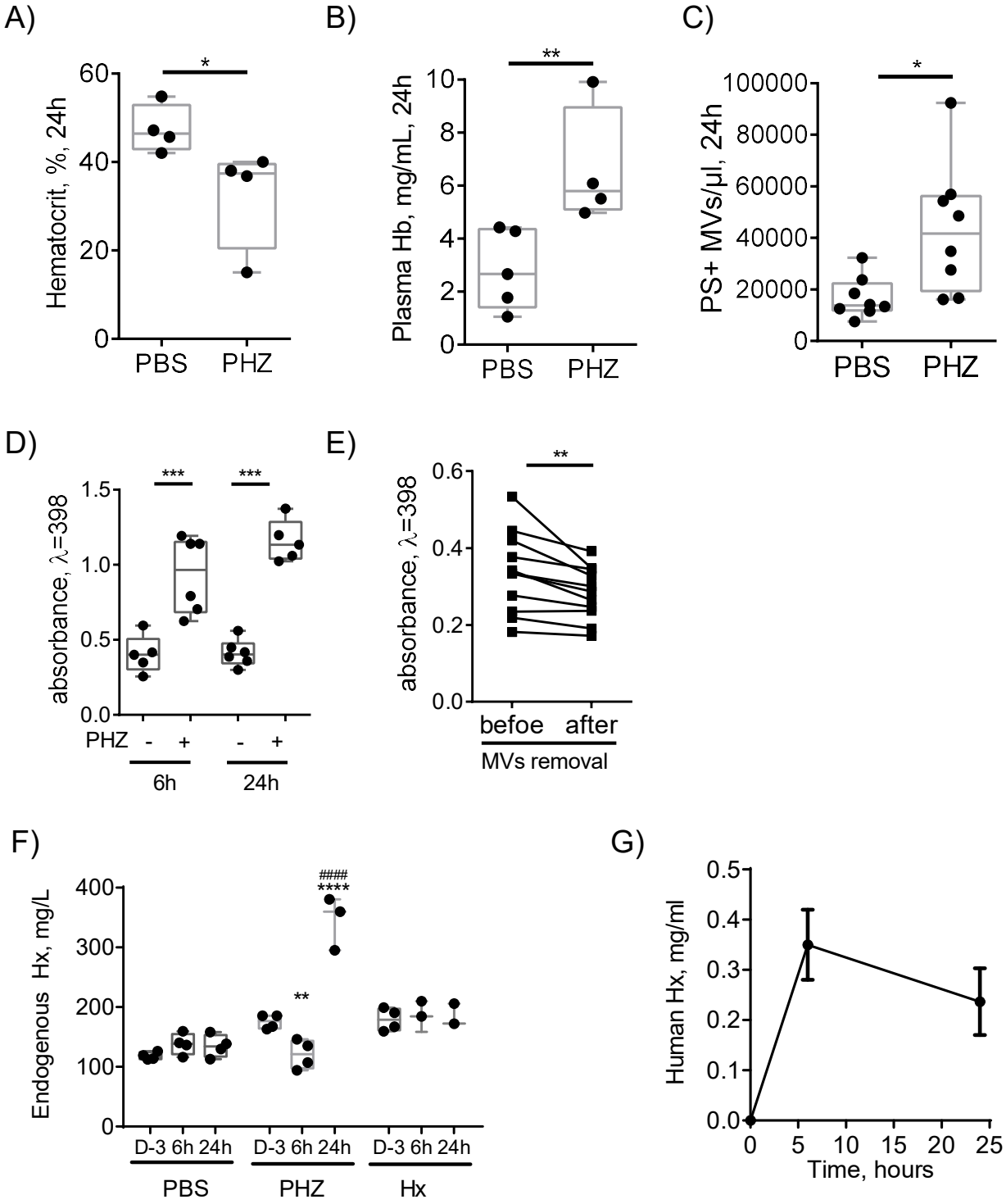
Figure S1



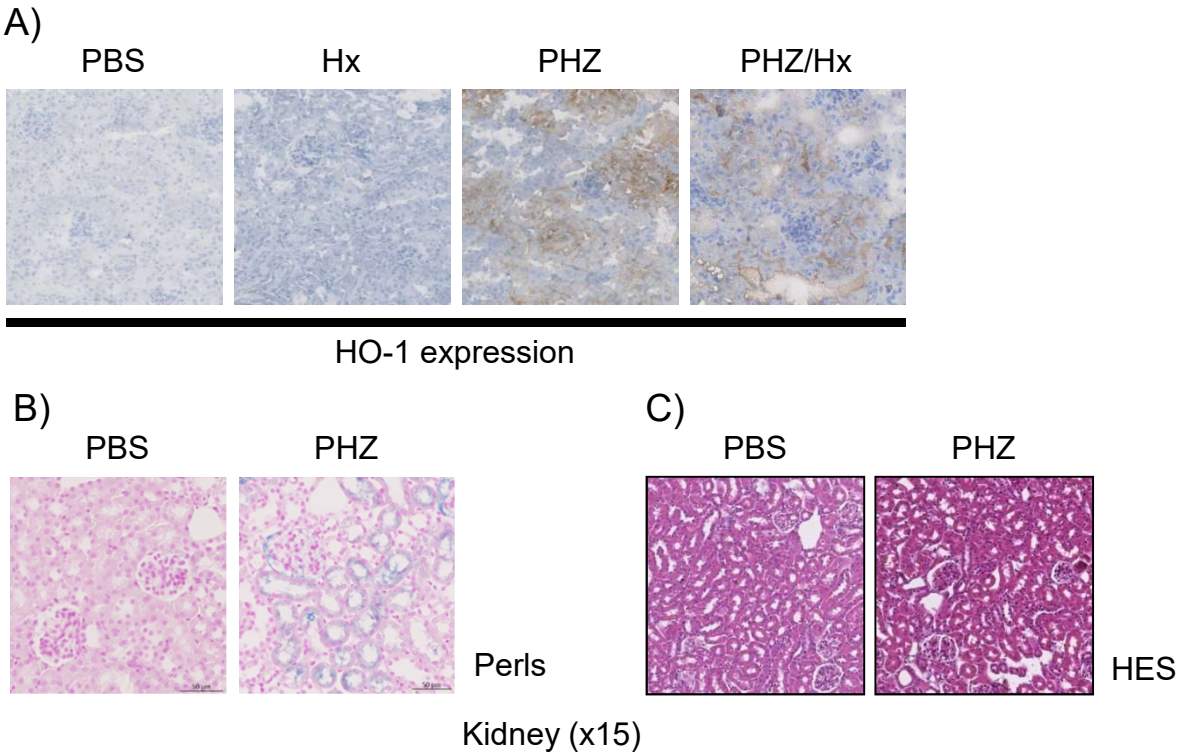
Supplementary figure 1: Characterization of hemolysis is SCD mouse models. Plasma phosphatidylserine positive (PS+) MVs (mostly of RBC origin) of A) HbAA (n=3) versus HbSS (n=4) mice or B) WT (n=8) versus SAD (n=8). D) Evaluation of the total plasma heme by absorbance at λ=398nm in C) HbAA (n=3) versus HbSS Townes (n=3) and D) WT (n=14) versus SAD (n=6) mice. *: p<0,05 performed by Mann Whitney test.

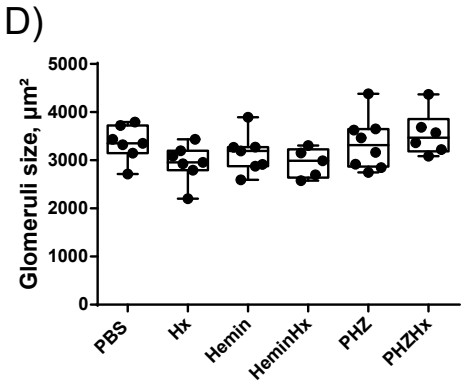
Figure S2



Supplementary figure 2: PHZ injection triggered hemolysis and modulated endogenous Hx expression. A) Hematocrit percentage, B) Plasma Hb and C) Plasma PS+ (mostly RBC) MVs of PBS and PHZ-treated mice at 24h (n=5 mice per group). D) Evaluation of the total plasma heme by absorbance at λ=398nm in PBS or PHZ-injected mice at 6h and 24h after treatment. *: p<0,05, **: p<0.01, performed by Mann-Whitney test. E) Quantification of the total heme by absorbance at λ=398nm in the plasma of PHZ-injected mice (n=11/group) before and after untracentrifugation (20500g/4h) to remove the MVs. The decrease in the absorbance is proportional to the quantity of total heme, contained in the RBC MVs. **: p<0.01, performed by paired t-test F) Quantification of endogenous Hx from plasma of PBS, PHZ and Hx-treated mice (n=4 mice per group). **: p<0,005; ****: p<0,0001 compared to D-3; ####: p<0,0001 compared to 6H; performed by Two-Way ANOVA with Tukey's test for multiple comparisons. G) Quantification of human Hx in plasma of Hx-treated mouse 6 hours and 24 hours after injection (n=4). Values are mean +/- SD.

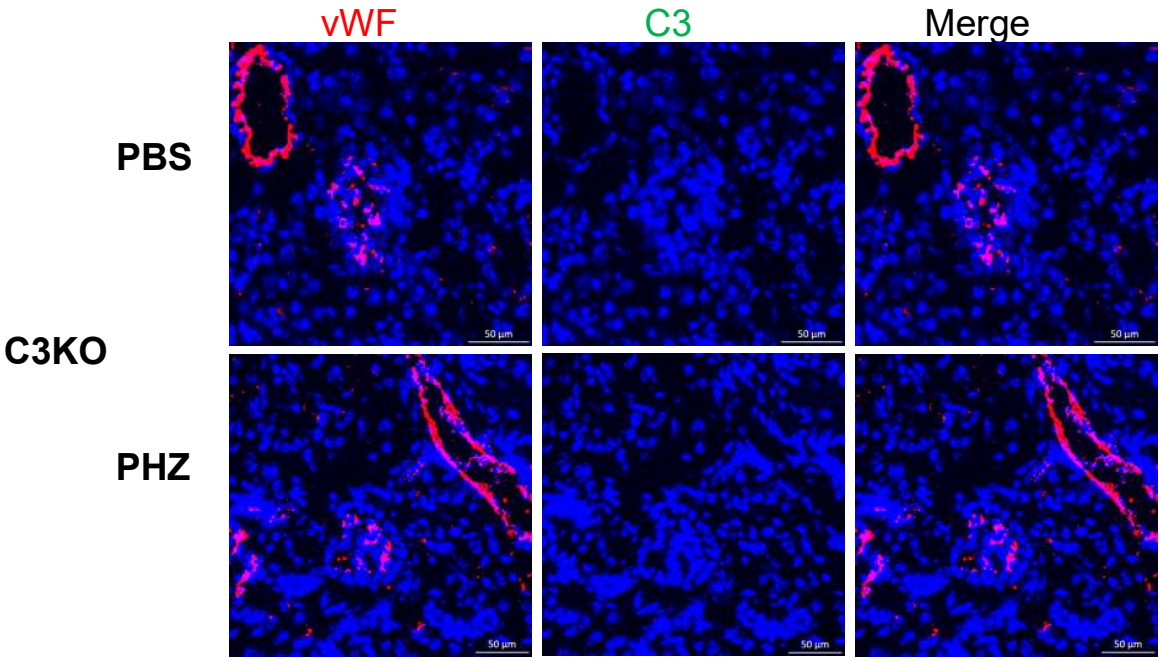
Figure S3





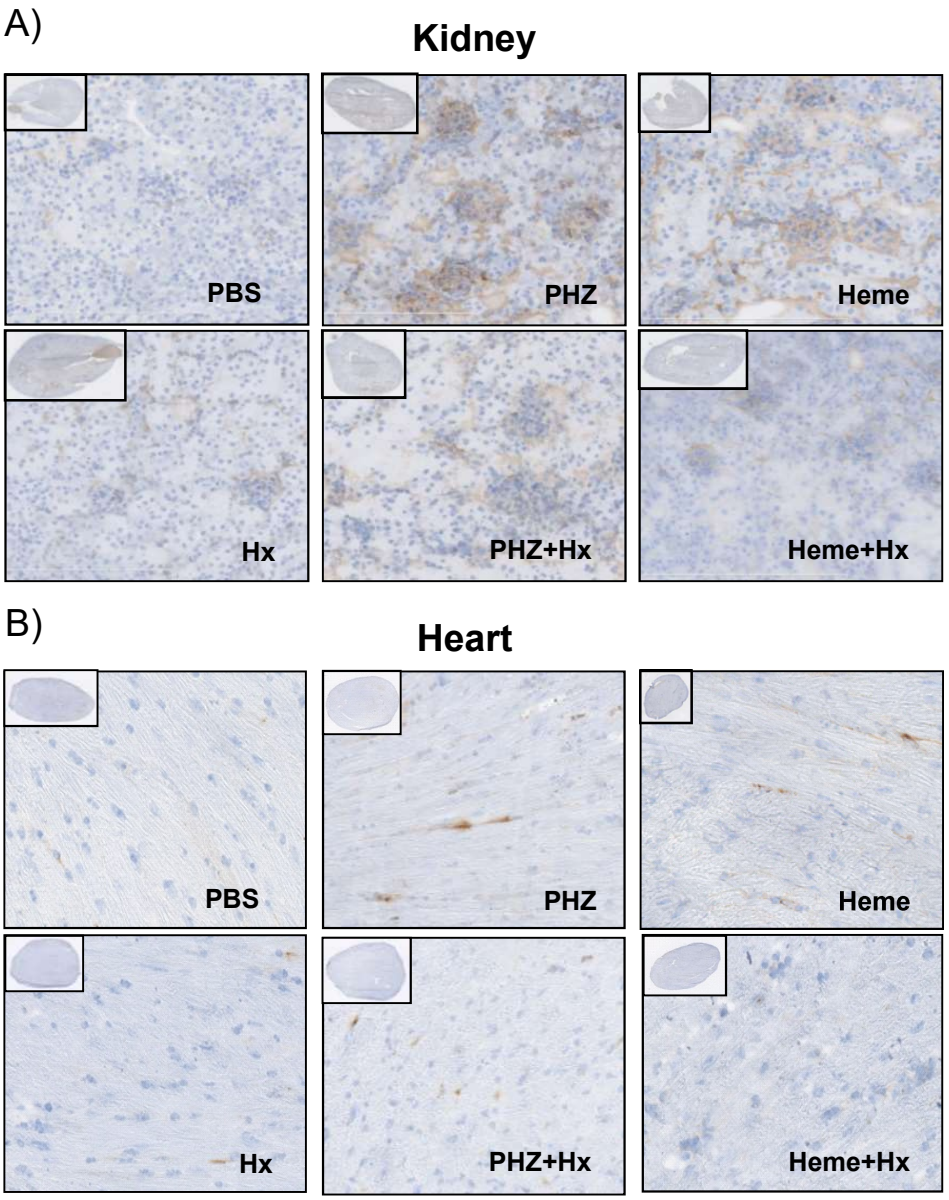
Supplementary figure 3: Renal histology of PHZ-injected mice. A) Immunohistochemistry analysis of frozen kidney sections of mice (x20), injected with PHZ or with the vehicle only, with or without Hx at 24h. The staining for HO-1 appears in brown and nuclei in blue. Representative images from one out of three or five mice per group. Fixed and paraffin-embedded kidneys were cut at 5 µm and stained with Perls Prussian Blue (B) and hematoxylin-eosin (C). D) Quantitative analysis of glomerular size, each point represents the average size of glomeruli in one mouse (n=7).

Figure S4



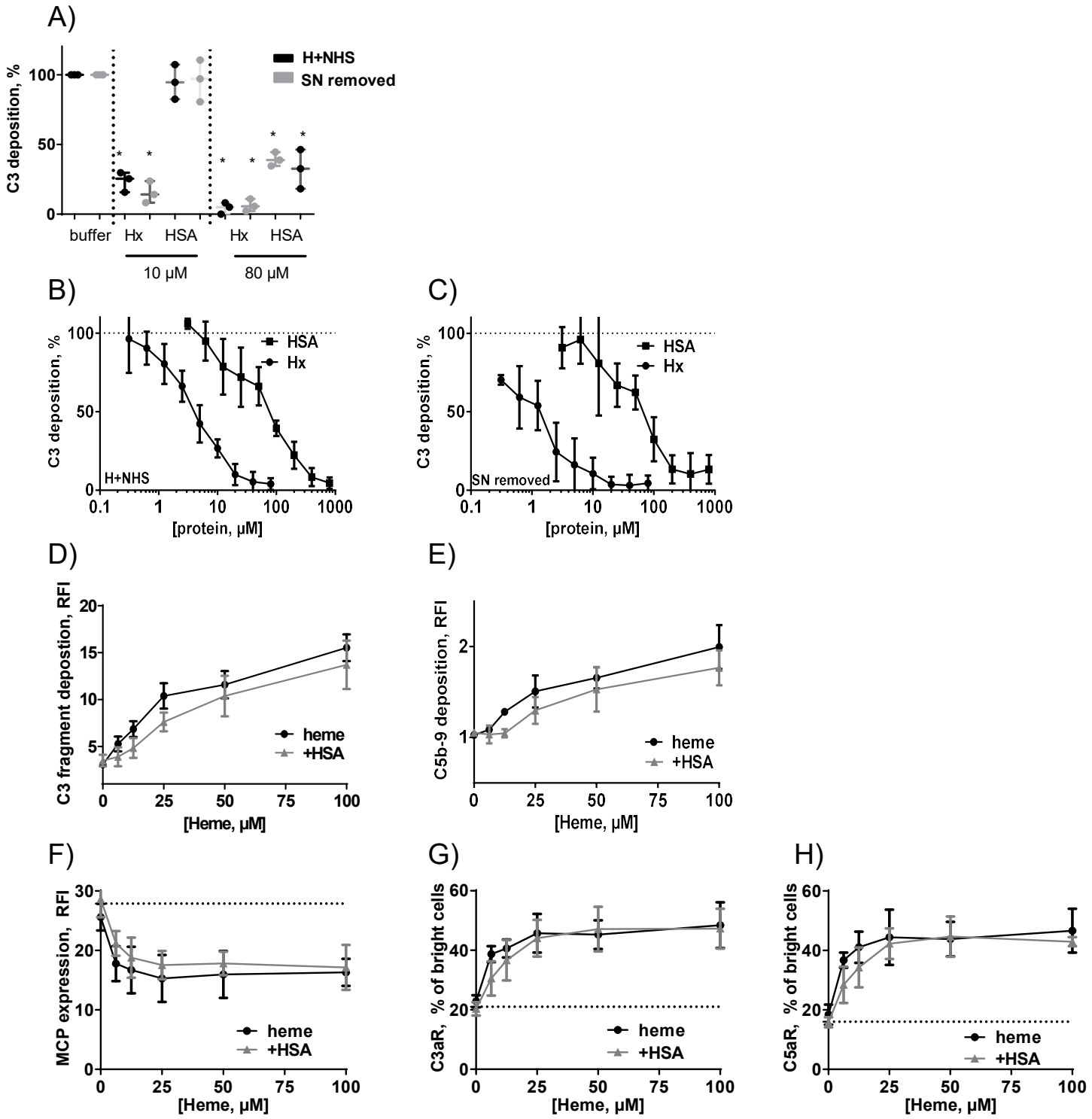
Supplementary figure 4: Absence of C3 activation fragments deposits in C3-/- mice. C3b/iC3b (false green color) staining of frozen kidney sections of C3-/-mice, injected with PBS or PHZ and sacrificed at 24h. Double staining with endothelial marker vWF (false color red).

Figure S5



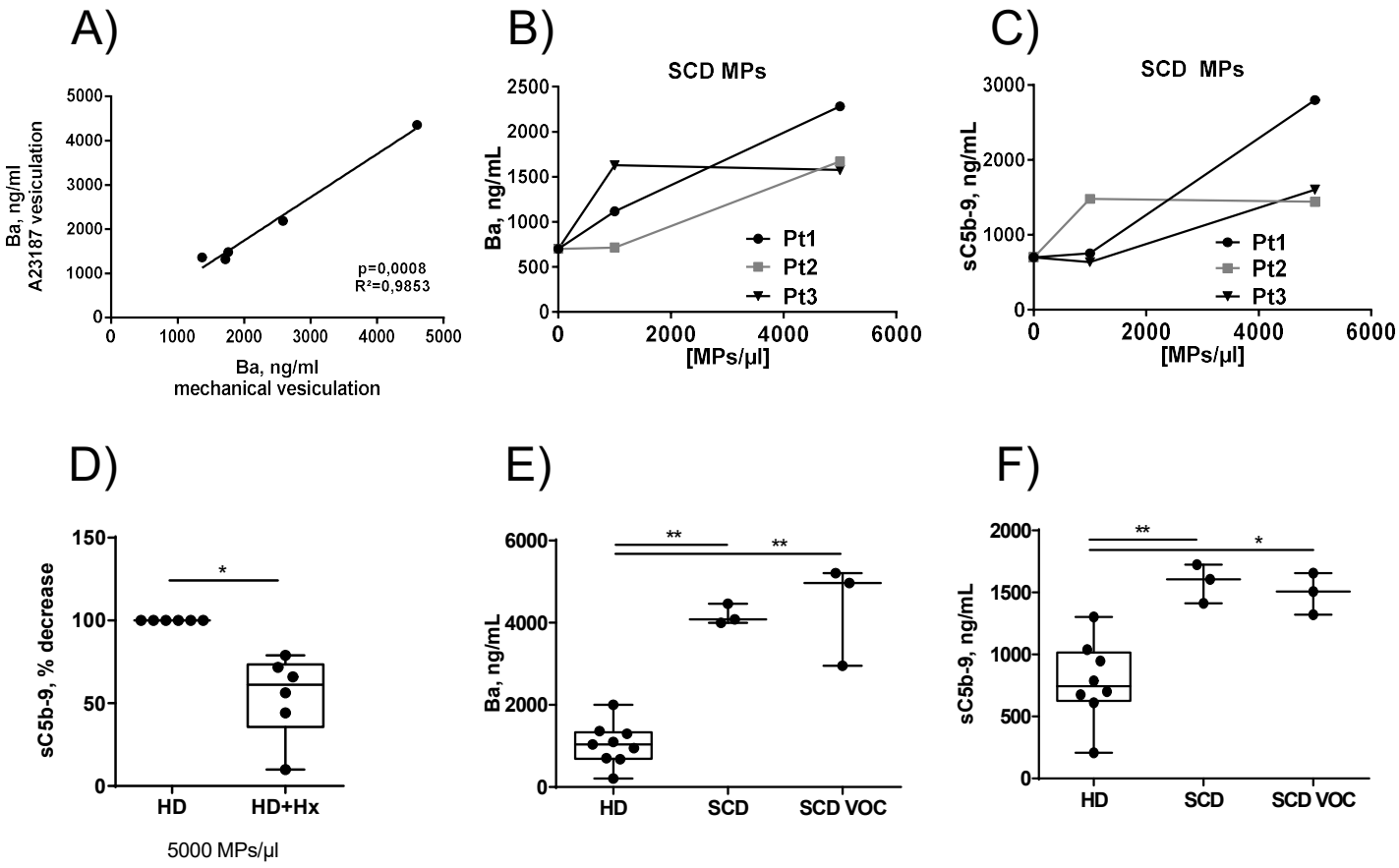
Supplementary figure 5: Complement activation is induced *in vivo* under hemolytic conditions and differentially affects kidneys and heart. Immunohistochemistry analysis of (A) kidney (x20) and (B) heart (x10) sections of mice, injected with PHZ, heme, or with the vehicle only, with or without Hx. The staining for C3 activation fragments deposition appears in brown and nuclei in blue. Representative images from one out of five mice per group (n=5, N=3).

Figure S6



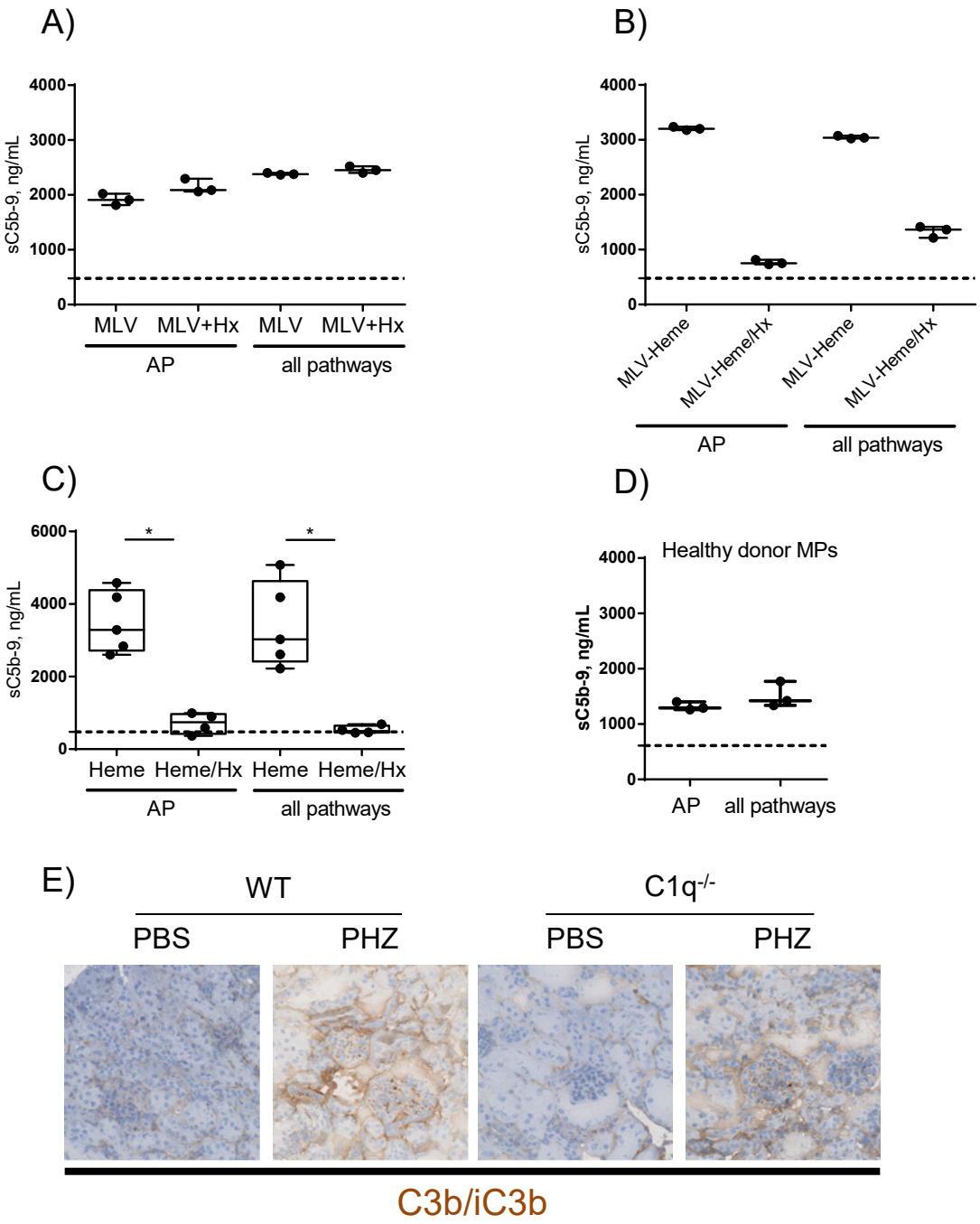
Supplementary figure 6: Albumin was less efficient than Hx to prevent heme effects. A, B, C) HUVEC were treated with increased concentration of albumin or Hx and 50 µM of heme, diluted in M199 FCS-free medium for 30 mins at 37°C. HUVEC were exposed to NHS (33% final concentration diluted in M199 FCS-free medium) after removing the supernatant (SN removed) (B), or not (H+NHS) (C), for 30 mins at 37°C. Cells were detached and stained for C3 deposition by flow cytometry (n=4). D, E) HUVEC were treated with increased concentration of heme with or without 5 µM of albumin following the same protocol. Cells were detached and stained for (D) C3 deposition or (E) C5b-9 formation by flow cytometry. F) HUVEC were treated with increased concentration of heme with equimolar concentration of albumin, diluted in FCS-free medium for 30 mins at 37°C. HUVEC were exposed to NHS (33% final concentration diluted in M199 FCS-free medium) after removing the supernatant. Cells were detached and stained for C3 deposition by flow cytometry (n=4). G, H, I) HUVEC were treated with increased concentration of heme with or without 5 µM of Hx for 30 mins at 37°C. Cells were detached and stained for (G) MCP, (H) C3aR or (I) C5aR expression. Detection was done by flow cytometry (for each measure, n≥3). *: p<0,05, ***: p<0,001, ****: p<0.0001, performed by Two-Way ANOVA with Sidak's test for multiple comparisons.

Figure S7



Supplementary figure 7: RBC macrovesicles (MVs) from stable SCD patients or in vaso-occlusive crisis (VOC) induce equivalent complement activation. NHS was incubated with increased concentration of RBC MVs from healthy donors (HD) or SCD patients, diluted in TBS. After 30 minutes incubation time at 37°C, the level of released Ba and sC5b-9 was measured by ELISA. A) Comparison of Ba release induced by RBC MVs from two different preparations, generated by A23187 ionophore and by mechanical vesiculation. NHS was incubated with 0, 1000 or 5000 MVs/µL of RBC MVs from SCD patients (Pt), diluted in TBS, and (B) Ba and (C) sC5b-9 were measured. D) NHS was incubated with 5000 MVs/µL from HD (n=6) with or without 25 µM of Hx. Release of sC5b-9 was measured. *: p<0,05, performed by Mann-Whitney test. E, F) NHS was incubated with RBC MVs from HD, SCD patients or SCD patients in VOC and release of (E) Ba and (F) sC5b-9 were measured. *: p<0,05, **: p<0,005, performed by Kruskal-Wallis with Dunn's test for multiple comparisons.

Figure S8



Supplementary figure 8: MVs induce alternative pathway activation dependently and independently on heme. sC5b-9 release was analyzed by ELISA kit. (A) Artificial multilayer vesicles (MLV) or (B) heme-loaded MLV (MLV-heme) were incubated during 30 mins at 37°C with 33% NHS, diluted at a final concentration of 5000 MVs/µL with or without 25 µM of Hx. Two dilution buffers were used: complement alternative pathway (AP), TBS with 10 mM EGTA and 4 mM $MgCl_2$ or permissive for activation of all complement pathways (all pathways), TBS with 5 mM $CaCl_2$ and $MgCl_2$. Illustrative results of two independent repetitions. (C) Heme was incubated with 33% NHS for 30 minutes at 37°C with or without 25 µM of Hx, diluted in AP buffer or a buffer allowing activation of all pathways. (D) sC5b-9 release, induced by MVs from healthy donors, incubated at 5000 MVs/µL in 33% NHS in the two different buffers (n=4). (A-D). The dotted line represent the level of sC5b-9 in absence of MVs in serum (vehicle only). E) Immunohistochemistry analysis of frozen kidney sections of WT and $C1q^{-/-}$ mice, injected with PHZ or with the vehicle only. The staining for C3 activation fragments deposition appears in brown and nuclei in blue. Representative images from one out of five mice per group. *: p<0,05, performed by Kruskal-Wallis with Dunn's test for multiple comparisons.