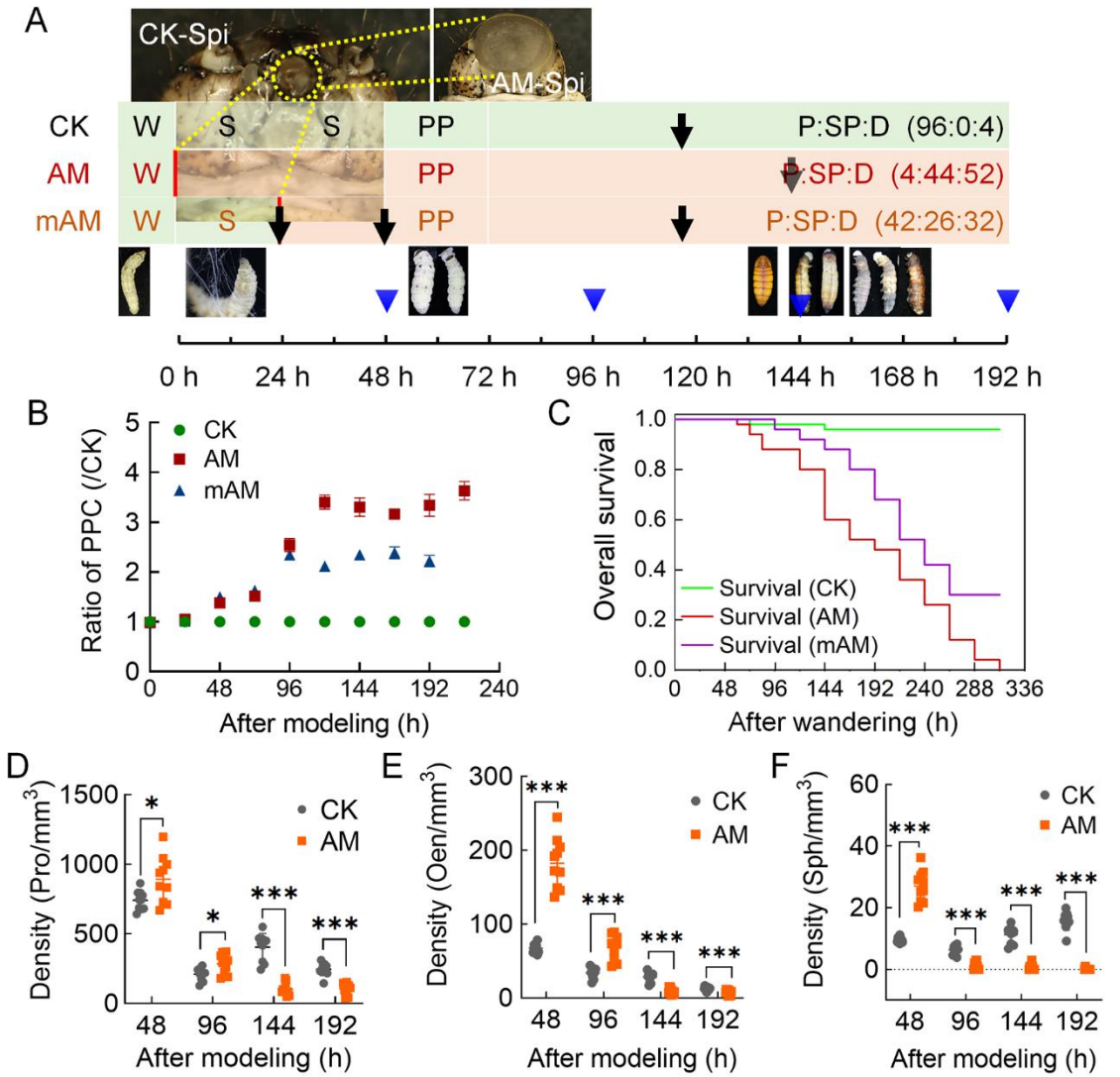
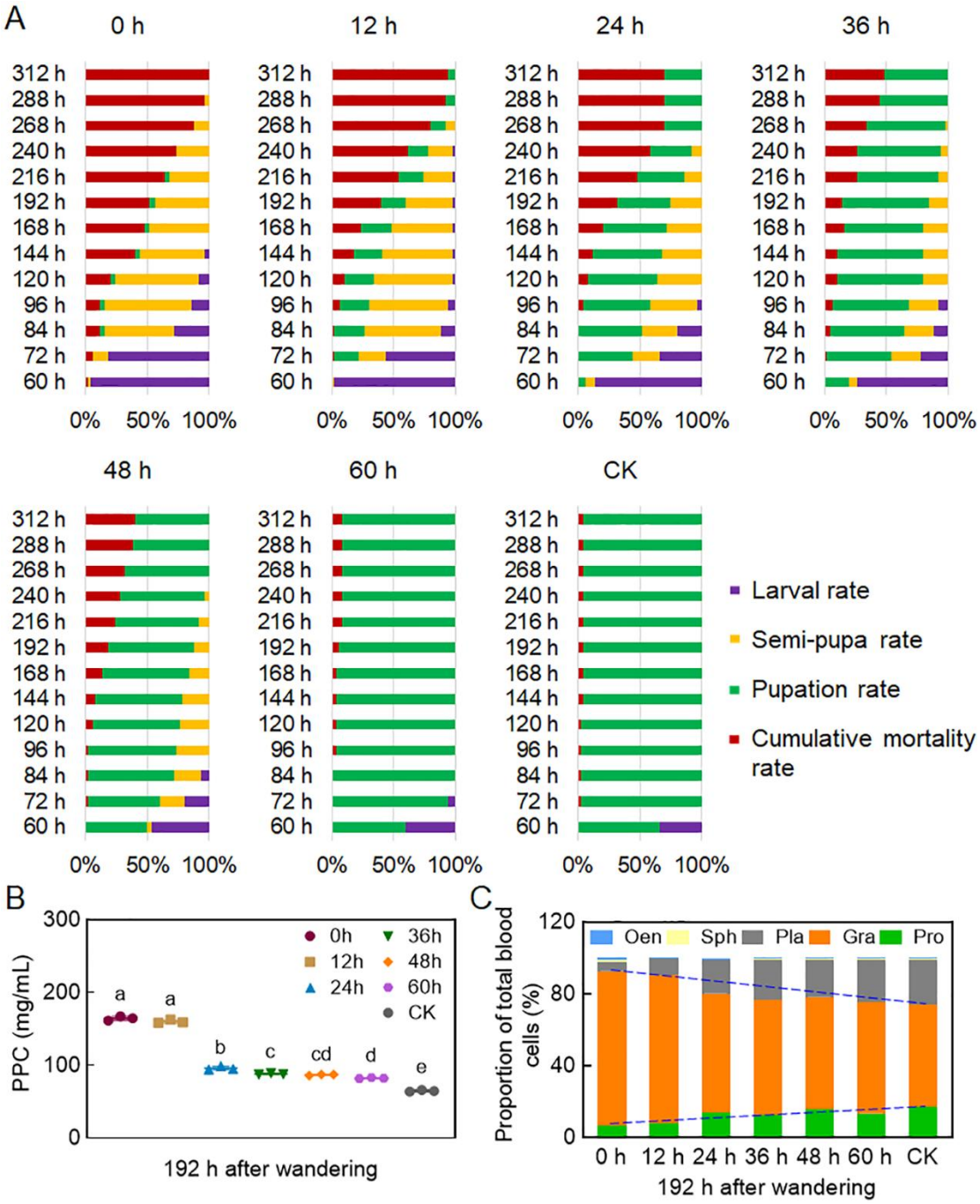
Supplementary Materials



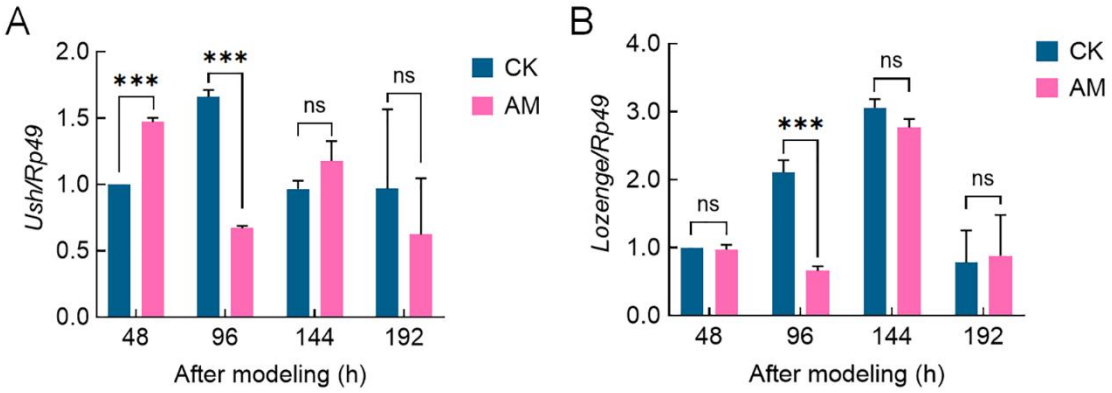
Supplementary Figure S1. Effects of hyperproteinemia on density of different types of hemocytes. A: Timeline. Silkworms (*Dazao*) were reared to mature larvae (wandering, W) in a standardized manner. Spi, spinneret; S, spinning stage; PP, prepupa stage; P, pupa stage; SP, semi-pupa; D, dead individual. CK, control. After 72 h of spinning, 96% entered pupal stage. AM group, HPPC model group. Low-melting point wax (55–60 ℃) was used to block spinning holes at the wandering stage to prevent spinning, and it was difficult to develop to adults. At 192 h, percentage composition ratio of pupa, semi-pupa, and dead individuals was 4:44:52. mAM group, mild HPPC model group. Spinning holes were blocked for modeling after 24 h of spinning, with some individuals able to complete development. At 192 h, percentage composition ratio of pupa, semi-pupa, and dead individuals was 42:26:32. Black arrow indicates individual injection treatment. mAM group was injected with endocrine hormone active substance 20E for rescue at 0 or 24 h after modeling; mAM and CK groups were injected with siRNA-Gcm at 96 h (time axis 120 h) after modeling; AM group was injected with JAK inhibitor AG490 at 144 h after modeling. Blue arrow indicates sampling survey time. B: Ratio of PPC between hyperproteinemia animal model and control groups (n=3). C: Survival curve. At 312 h after modeling, all individuals in AM group died, and 30% of individuals in mAM

group survived and completed generation development. D-F: Densities of different blood cell types (n=10). D, Pro; E, Oen; F, Sph. Ordinate represents number of hemocytes per mm^3 of hemolymph. Data are shown as mean±*SEM*. ns: P>0.05; *: P<0.05; **: P<0.01; ***: P<0.001, Student's t-test.

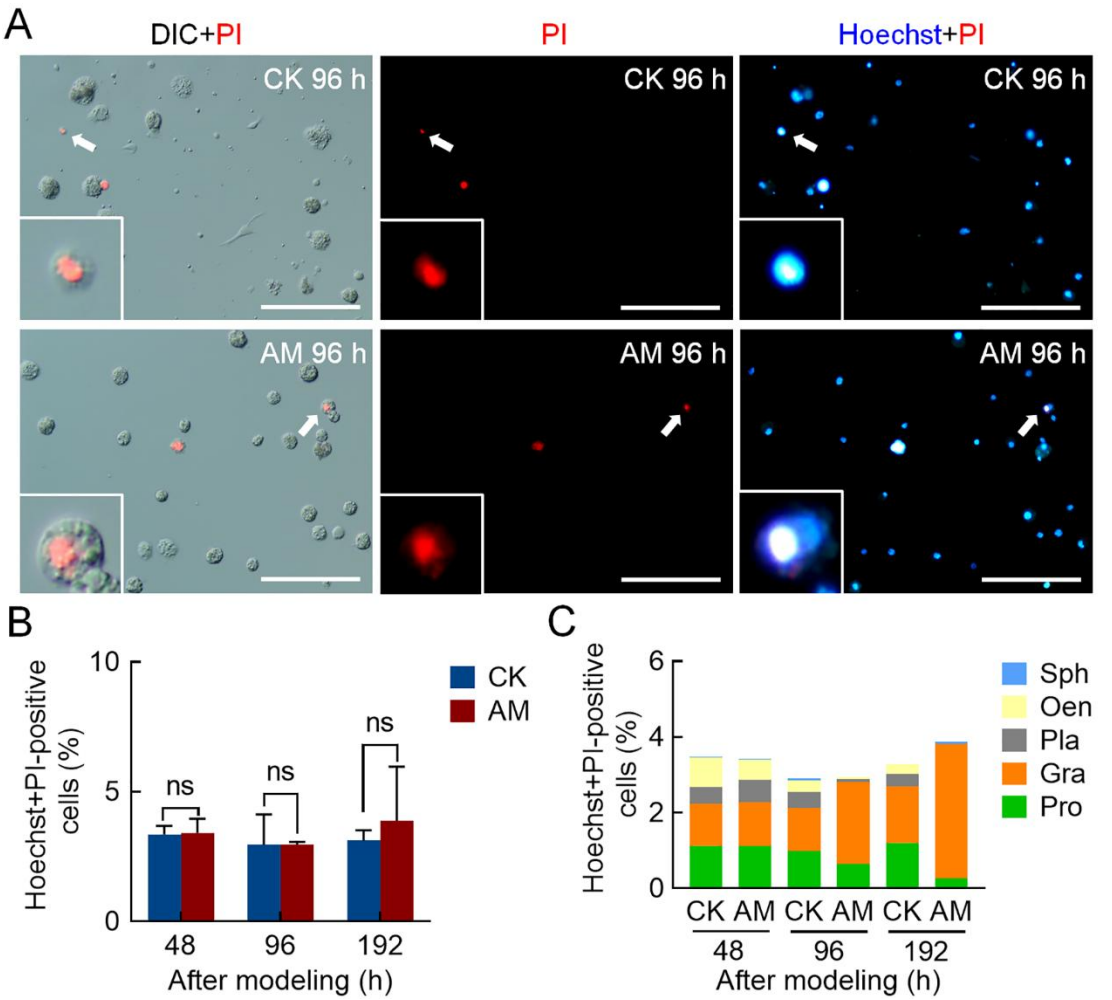


Supplementary Figure S2. Effects of different levels of high PPC (HPPC) on animal development. After spinning for 0, 12, 24, 36, 48, and 60 h, spinning holes of silkworm were blocked with low-melting point paraffin, with 50 individuals in each group. A: Survival and development of HPPC animal model and control group (CK) silkworms. B: Differences in PPC levels between HPPC animal model and CK groups. PPC level in each group was detected and compared at 192 h after spinning (n=3). Significant differences between groups are indicated with different letters (data are mean±*SEM*, P≤0.05, n=3). C: Differences in blood cell classification composition

between HPPC animal model and CK groups. Proportion of different types of blood cells in hemolymph by AO-PI staining at 192 h after spinning. Pro, Prohemocytes; Gra, Granulocytes; Pla, Plasmatocytes; Oen, Oenocytoids; Sph, Spherulocytes.

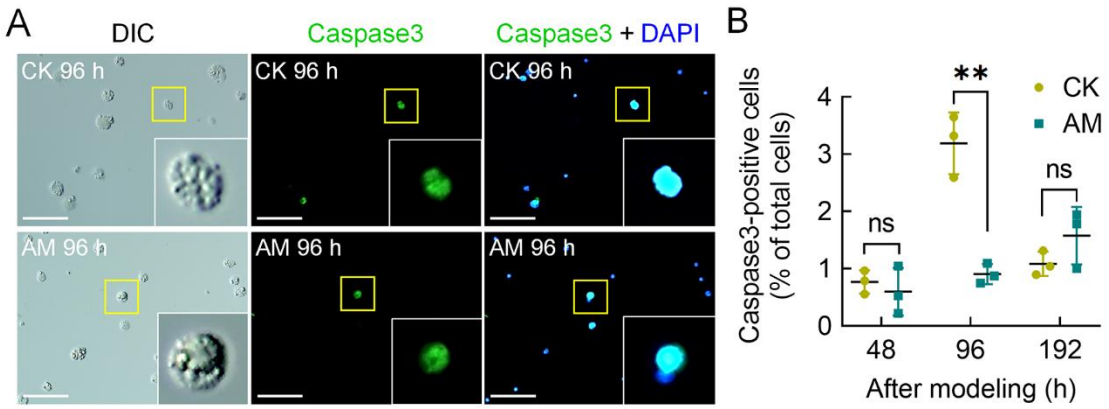


Supplementary Figure S3. Gene transcription levels of hematopoietic-related factors. A: *Ush*. B: *Lozenge*. Internal reference gene was *Bombyx mori Rp49* (*n*=3). Data are mean±*SEM*. ns: *P*>0.05; *: *P*<0.05; **: *P*<0.01; ***: *P*<0.001, Student's *t*-test.

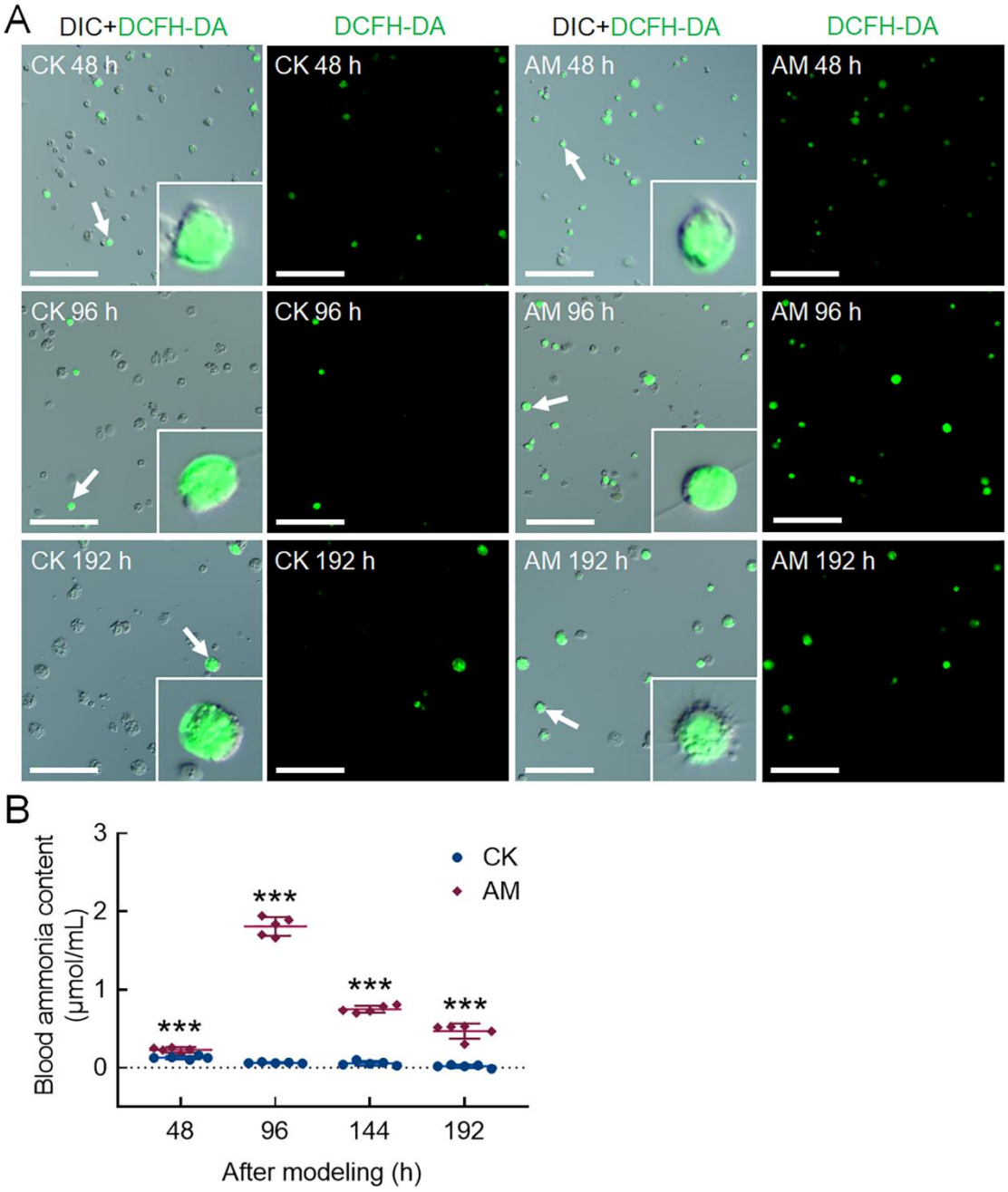


Supplementary Figure S4. Blood cell necrosis levels. A: Hoechst+ propidium iodide (PI)-stained fluorescence image at 96 h after modeling. Scale bars, 100 μm. Apoptosis and Necrosis Assay Kit (C1056, Beyotime, China) staining was used to detect

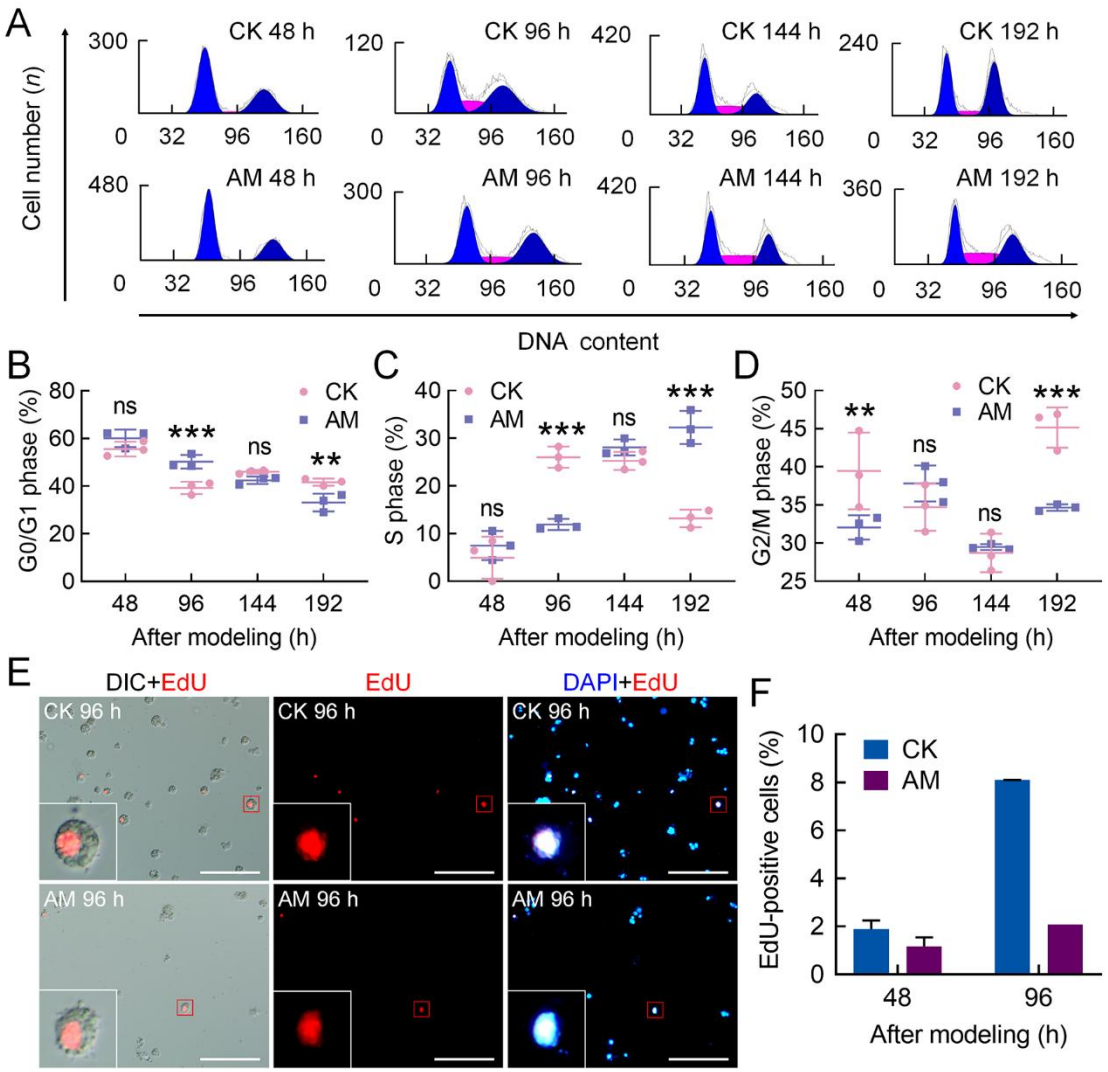
hemocyte necrosis. Blood samples (60 μL) were diluted 5–10 times (v/v) with HBSS, with slide climbing for 5 min. After washing with PBS three times, 1 000 μL of cell staining working solution was added, followed by incubation for 30 min in an ice bath in the dark. After washing with PBS three times in the dark, blue fluorescence (352/461 nm) and red fluorescence (488/630 nm) were observed. Hoechst+PI staining indicates necrotic hemocytes in hemolymph at 48, 96, and 192 h after modeling. DIC means cells in bright field, and Hoechst+PI double positive means necrotic cells. B: Hoechst+PI staining positive rate of hemocytes (n=3). Positive rate (%)=(Hoechst+PI double positive cell number/total cell number)×100. C: Hoechst+PI staining positive rate of different types of hemocytes. Data are mean±*SEM*. ns: P>0.05; *: P<0.05; **: P<0.01; ***: P<0.001, Student's t-test.



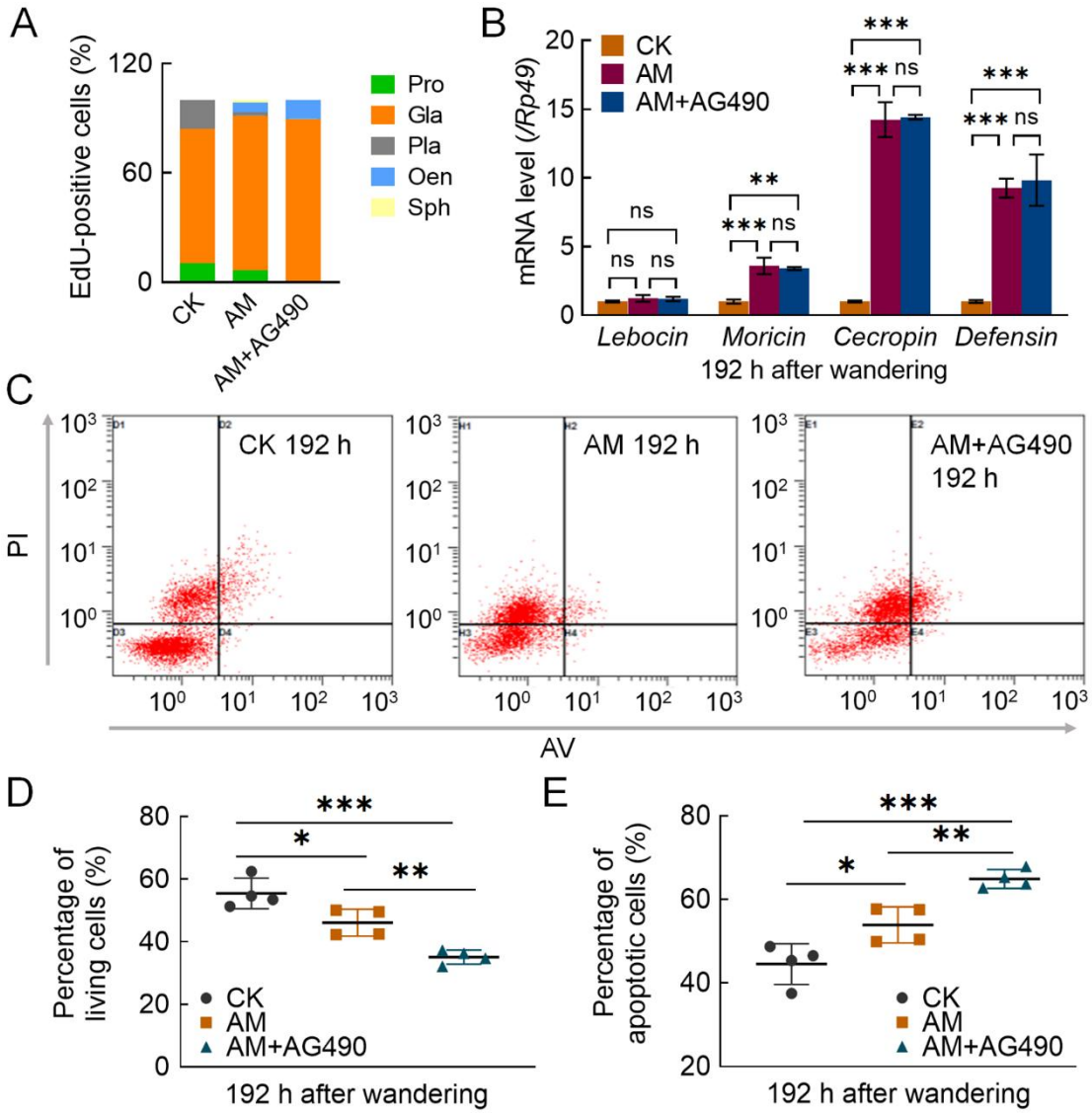
Supplementary Figure S5. Expression level of caspase-3 in blood cells detected by immunofluorescence after modeling. Caspase-3 immunofluorescence staining indicates hemocytes expressing caspase-3 protein in hemolymph at 48, 96, and 192 h after modeling, and DAPI staining indicates all cell nuclei (n=3). A: Caspase-3-immunostained fluorescence image at 96 h after modeling. Scale bars, 50 μm. B: Caspase-3 immunofluorescence staining positive rate of hemocytes. Positive rate (%)=(caspase-3 immunofluorescence positive cell number/total cell number)×100. Data are mean±*SEM*. ns: P>0.05; *: P<0.05; **: P<0.01; ***: P<0.001, Student's t-test.



Supplementary Figure S6. HPPC increased ROS levels in circulating hemocytes. A: Fluorescence images of hemocytes by DCFH-DA staining at 48, 96, and 192 h after modeling. Scale bars, 100 μm. B: Changes in blood ammonia levels in hemolymph between 48 and 192 h after modeling (n=5). Data are mean±*SEM*. ns: $P>0.05$; *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$, Student's t-test.



Supplementary Figure S7. HPPC affected cycle phase distribution of circulating hemocytes. Circulating hemocytes were investigated at 48, 96, 144, and 192 h after modeling. Cell cycle was divided into G0/G1, S, and G2/M phases. A: Phase distribution of blood cell cycle was detected by PI-FCM (n=3). B-D: Percentage of blood cells in each phase (n=3). B, G0/G1 phase, showing cells before DNA replication, at which time cells did not proliferate; C, S phase, showing cells during DNA replication; D, G2/M phase, showing cell division after DNA replication. Data are mean±*SEM*. ns: $P>0.05$; *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$, Student's t-test. E, F: Second experiment of EdU staining. E, Fluorescence of EdU staining at 96 h after modeling. Scale bars, 100 μm. F, EdU-positivity rate in cells at 48 and 96 h after modeling.



Supplementary Figure S8. Effects of JAK inhibitor injection on blood cell apoptosis. AM+AG490, AM group injected with JAK inhibitor AG490 (per individual, 50 μmol/10 μL) at 144 h after modeling, with hemolymph collected 48 h after injection. A: Composition of EdU-positive blood cells. Positive rate (%)=(number of EdU-positive blood cells of same type/total number of EdU-positive cells)×100. B: mRNA levels of antimicrobial peptide genes in circulating hemocytes, detected by qPCR (n=3). Reference gene was *Bombyx mori Rp49*. C: Annexin V-FITC/PI-FCM results of blood cells. Negative AV and PI indicates living cells, while others are apoptotic cells. Hemocytes in AM group showed promotion of apoptosis after injection of AG490. D, E: Annexin V-FITC/PI staining statistics (n=4). D, Percentage of living cells. E, Percentage of apoptotic cells. Data are mean±*SEM*. ns: P>0.05; *: P<0.05; **: P<0.01; ***: P<0.001, Student's t-test.

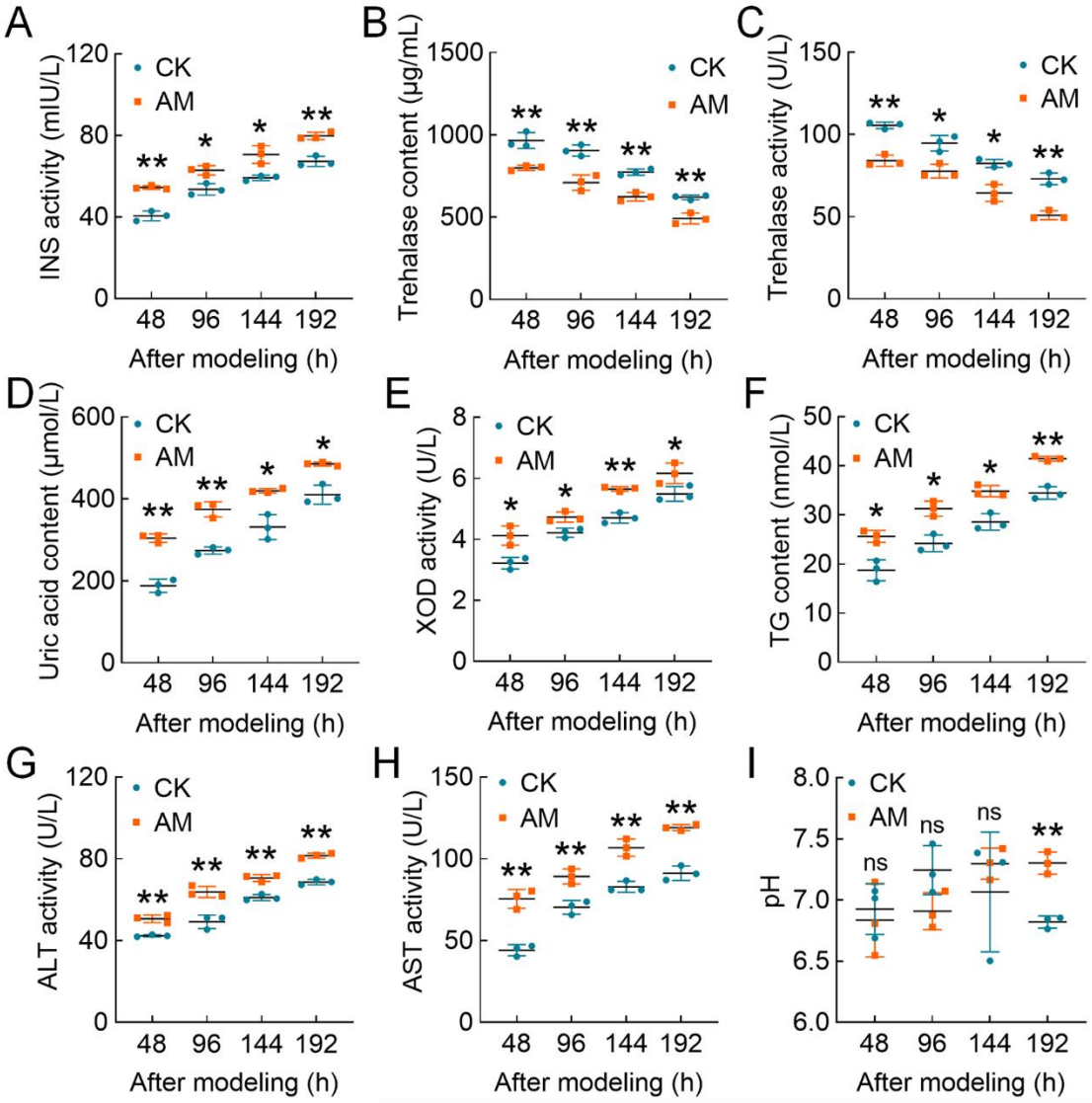
CI	TP (g/L)	AB	MV ($\times 10^9$/L)	RV ($\times 10^9$/L)	Up /down
KTS1 (M)	76.5	None			
KTS2 (F)	69.9	None			
KTS3 (F)	70.9	None			
PE (M)	72.4	None			
MM1 (M)	110.7	Eosinophil count	0.54	0.02-0.52	up
		Basophil count	0.07	0.00-0.06	up
		Erythrocyte count /RBC	3140	4300-5800	down
MM2 (M)	90.9	Leukocyte count /WBC	3.05	3.50-9.50	down
		Neutrophil count	1.21	1.80-6.30	down
		Erythrocyte count /RBC	2640	4300-5800	down
		platelet count /PLT	78	125-350	down
HI1 (M)	90.4	Leukocyte count /WBC	3.30	3.50-9.50	down
		Erythrocyte count /RBC	3280	4300-5800	down
		platelet count /PLT	73	125-350	down
HI2 (F)	96.9	platelet count /PLT	69	125-350	down
TP refervence value (g/L) : 65-85					

Supplementary Figure S9. Clinical blood survey data. NPPC, normal PPC sample; HPPC, high PPC sample; M, male; F, female; CI, clinical impression; TP, total protein, reference value 65–85 g/L; AB, abnormal blood cell count; MV, measured value; RV, reference value; KTS, kidney transplant status; PE, pleural effusion; MM, multiple myeloma; HI, hepatic impairment. RBC, red blood cell count; WBC, white blood cell count.

Effect of HPPC on basic parameters of silkworm hemolymph

To determine whether HPPC caused metabolic disorders, basic metabolic physiological parameters of hemolymph were measured at 48 to 192 h after modeling. The activity levels of insulin in the AM group were significantly higher than that in the CK group (Supplementary Figure S10A), suggesting that an increase in PPC promotes metabolism of blood glucose and protein. It is worth noting that after modeling, the content of trehalose (main component of silkworm blood sugar) and activity of trehalase (its main decomposing enzyme) were significantly lower than that in the CK group (Supplementary Figure S10B, C), suggesting that glucose decomposition affected by the increase in insulin activity in the hemolymph of the AM group was mainly for body energy supply, rather than the synthesis of trehalose energy storage. The end-product of protein and amino acid catabolism, i.e., uric acid, and the key enzyme in the uric acid production pathway, i.e., xanthine oxidase, were significantly higher in the AM group than in the CK group (Supplementary Figure S10D, E) at 48 to 192 h after modeling, indicating that high PPC promotes protein and amino acid catabolism. Increased triacylglycerol in the hemolymph of the AM group implied that protein-to-lipid conversion was activated as PPC levels increased (Supplementary Figure S10F). At the same time, the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which regulate the metabolism of substances in the hemolymph, were significantly higher in the AM group than in the CK group (Supplementary Figure S10G, H), implying that amino acid metabolism in the hemolymph of the AM group was activated. There was no significant difference in pH between the AM and CK groups from 48 to 144 h after modeling. However, at 192 h after modeling, the pH level of the AM group was

significantly higher than that of the CK group (Supplementary Figure S10I), suggesting that individuals in the late stage of modeling tended to collapse, which corresponded to the high mortality rate in the late stage.



Supplementary Figure S10. Determination of basic hemolymph parameters in silkworm HPPC. Basic physiological parameters of hemolymph at 48, 96, 144, and 192 h after modeling were determined by ELISA (n=3). A: INS activity; B: Trehalase; C: Trehalase activity; D: Uric acid content; E: XOD activity; F: TG content; G: ALT activity; H: AST activity; I: pH. Data are mean±*SEM*. Significant differences between AM and CK are marked as: ns: $P>0.05$; *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$, Student's t-test.