Supplemental Figures:

Figure S1. Loss of *jumu* affects circulating hemocyte differentiation. a-c Immunostaining against NimC1 (green) shows that part of the circulating hemocytes of *jumu* heterozygotes do not express NimC1. **d** Agarose gel electrophoresis of RT-PCR products obtained using primer pairs specific for *NimC1-RA* (1138 bp) and *NimC1-RB* (1268 bp). We detected the appropriately sized fragment in all investigated lines. **e-j** The expression levels of eaterGFP and H2 in *jumu* heterozygote circulating hemocytes are similar to those in controls. **k** Immunostaining against NimC1 (red) and phalloidin staining (green) shows that only 50% of the circulating hemocytes of *jumu* double heterozygotes express NimC1, and nearly 60% of the enlarged cells are labeled with an anti-NimC1 antibody (arrows). **l** Immunostaining against L1 (red) and phalloidin staining (green) shows that more than 10% of the lamellocytes are observable in *jumu*

double heterozygotes circulating hemocytes (arrows). **m, n** Immunostaining against L1 (red) shows that nearly 10% of the lamellocytes are observable in *Gcm>jumu RNAi* circulating hemocytes. **o, p** Immunostaining against NimC1 (red) shows that NimC1 expression is reduced in *Gcm>jumu RNAi* circulating hemocytes compared with that in controls. **q** Real-time PCR analysis of phagocytosis receptor gene levels in circulating hemocytes. Error bars represent the S.E.M of at least 3 independent experiments; *P<0.05; **P<0.01; ***P<0.001 (Student's *t*-test). Scale bars: 20 μm.
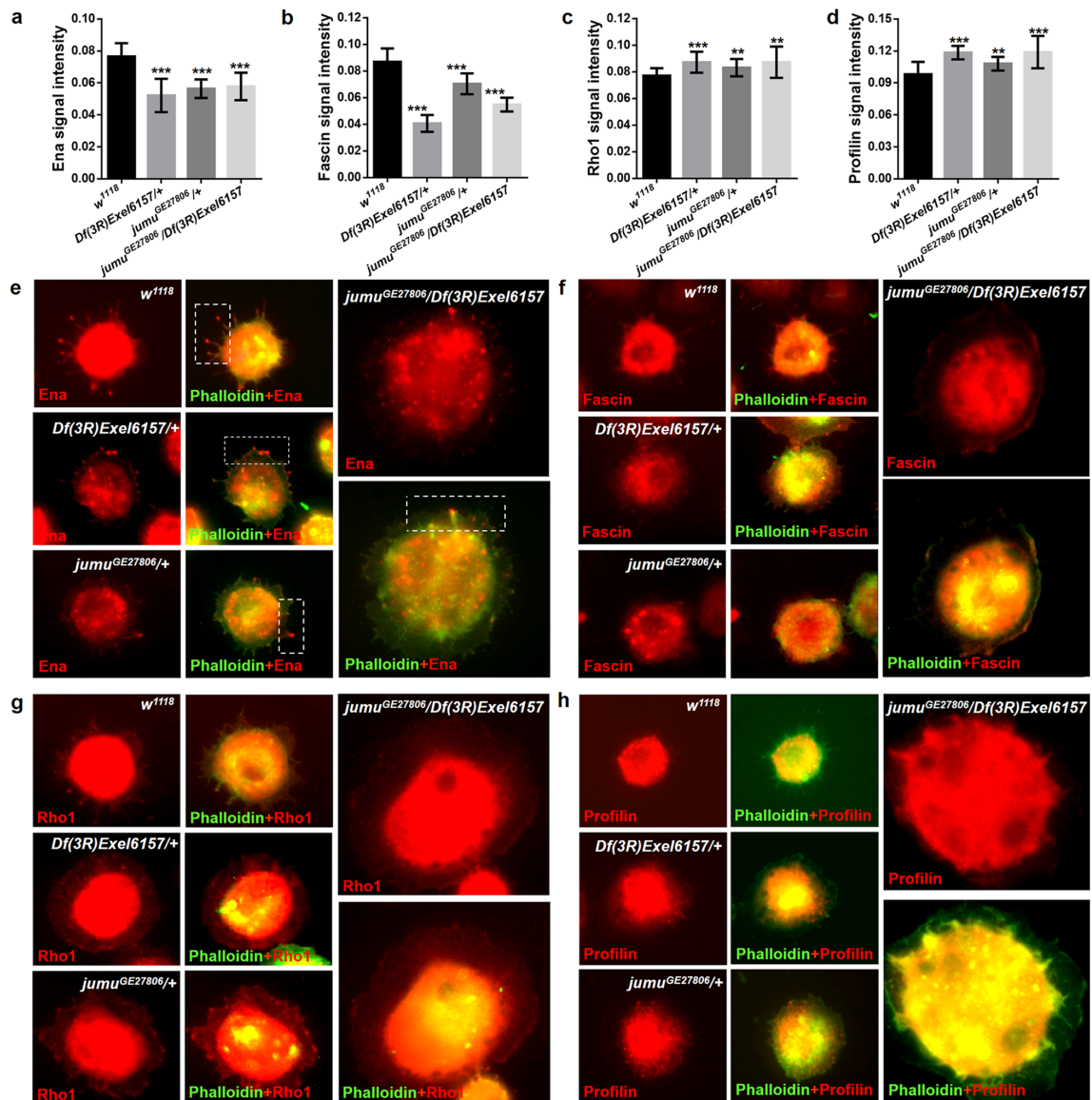
Figure S2. Subcellular localization and expression levels of Ena, Fascin, Rho1 and Profilin in circulating hemocytes. a-d Quantification of signal intensities of Ena, Fascin, Rho1 or Profilin. **e-h** Immunostaining against Ena (red), Fascin (red), Rho1 (red) or Profilin (red) and phalloidin staining (green) shows the subcellular localization of Ena, Fascin, Rho1 and Profilin in control and *jumu* mutants circulating hemocytes. Error bars represent the S.E.M of at least 3 independent experiments; **P<0.01; ***P<0.001 (Student's *t*-test). Scale bars: 10 μm.
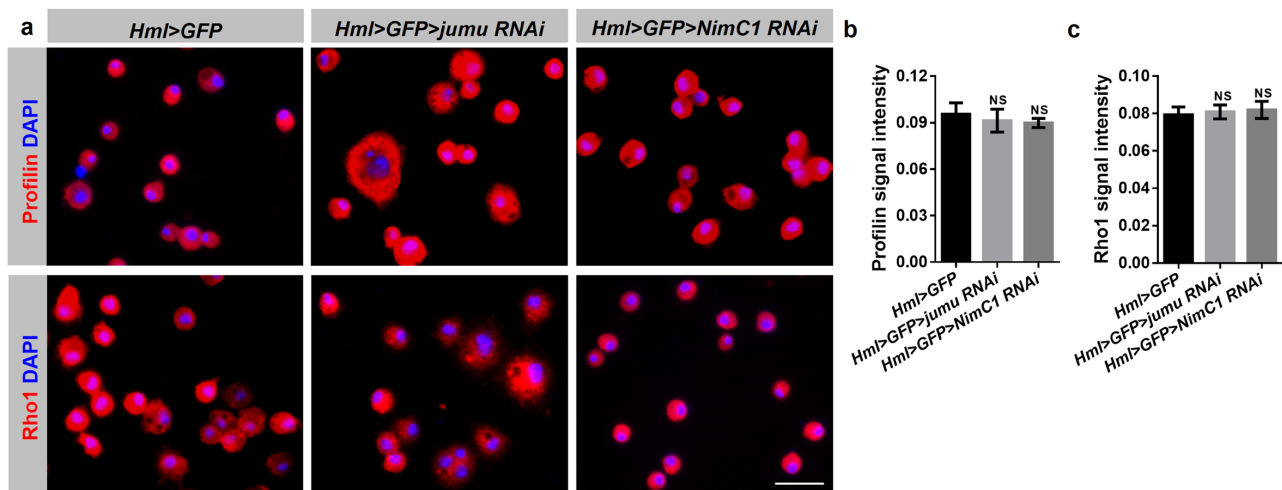

Figure S3. Expression levels of Profilin and Rho1 are unchanged in *jumu* or *NimC1* knockdown hemocytes. a Immunostaining against Profilin (red) and Rho1 (red) in *jumu* or *NimC1* knockdown hemocytes. **b, c** Quantification of signal intensities of Profilin and Rho1. Error bars represent the S.E.M of at least 3 independent experiments; NS, not significant (Student's *t*-test). Scale bars: 20 μm.
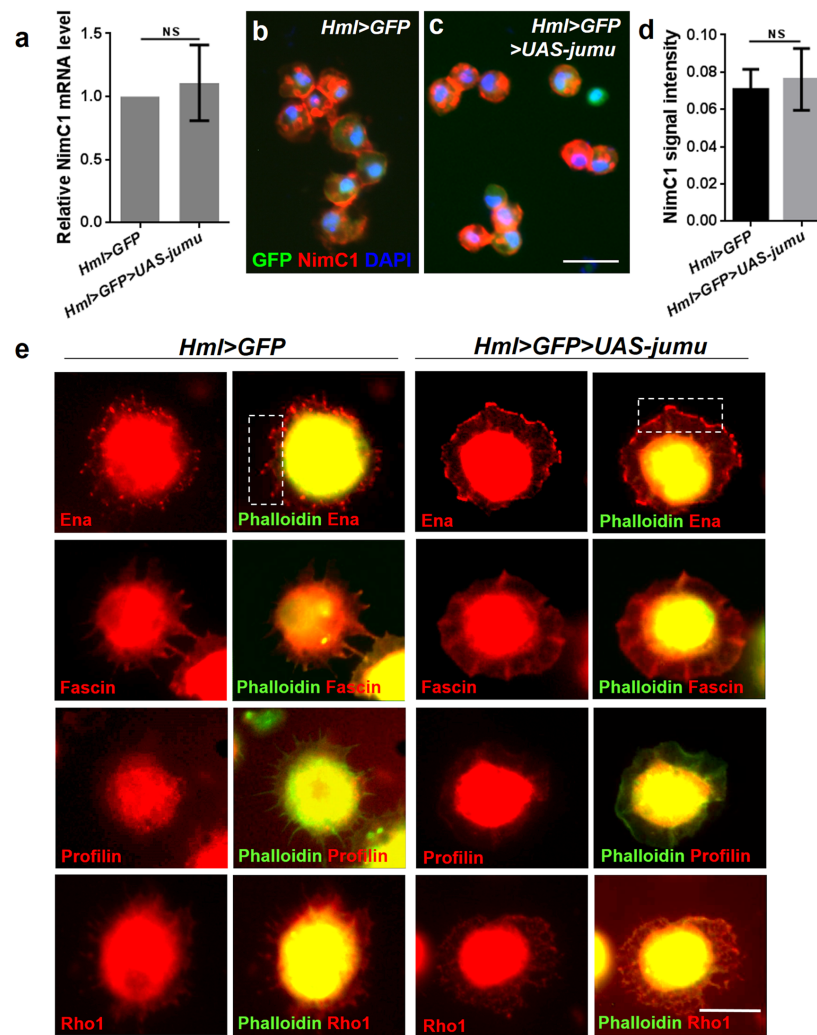
Figure S4. The expression of NimC1 and the subcellular localization of Ena, Fascin, Rho1 or Profilin. a Real-time PCR analysis of *NimC1* levels in circulating hemocytes. **b, c** Immunostaining against NimC1 (red) shows that NimC1 expression is unchanged in *Hml>GFP>UAS-jumu* circulating hemocytes compared with that in the controls. **d** Quantification of the signal intensities of NimC1. **e** Immunostaining against Ena, Fascin, Rho1 or Profilin and phalloidin staining (green) show the subcellular localization of Ena, Fascin, Rho1 and Profilin in control and *Hml>GFP>UAS-jumu* circulating hemocytes. Error bars represent the S.E.M of at least 3 independent experiments; NS, not significant (Student's *t*-test). Scale bars: 20 μm (**b, c**); 10 μm (**e**).
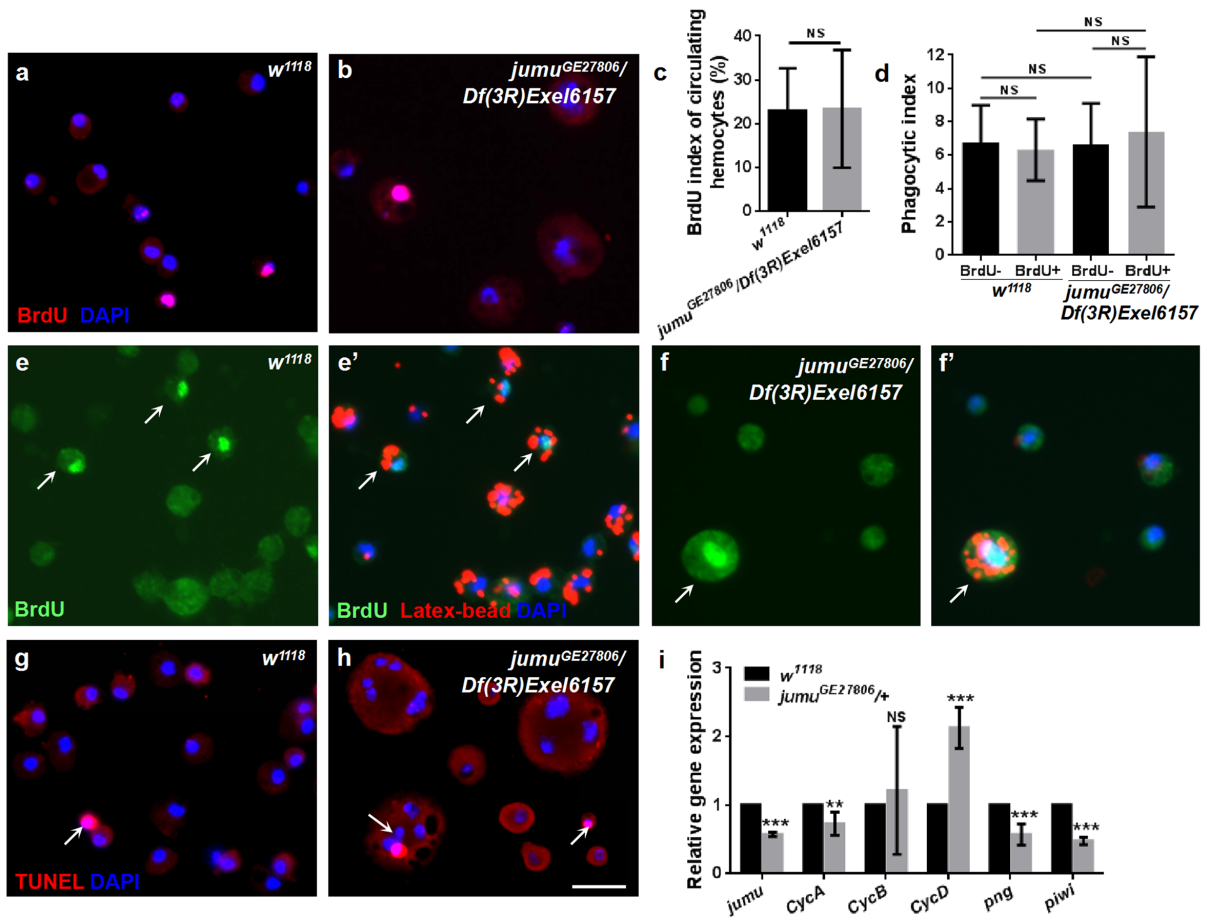
Figure S5. Loss of *jumu* does not cause DNA overreplication or cell apoptosis. a, b
Cells in the S phase from the third-instar larval circulating hemocytes are analyzed using BrdU incorporation (red); the amount of BrdU incorporation is not increased in the *jumu* double heterozygotes compared with that in the controls. **c** Quantification of the BrdU index. **d** Quantification of phagocytic indexes of latex beads. **e-f'** Immunostaining against BrdU (green) in circulating hemocytes isolated from third-instar larvae injected with latex beads (red). **g, h** Apoptosis is analyzed through TUNEL assays in circulating cells. **i** Real-time PCR analysis of genes associated with the cell cycle and division levels of *jumu* mutant hemocytes. Error bars represent the S.E.M of at least 3 independent experiments; NS, not significant; **P<0.01; ***P<0.001 (Student's *t*-test in **c**, I; one-way ANOVA in **d**). Scale bars: 20 μm.
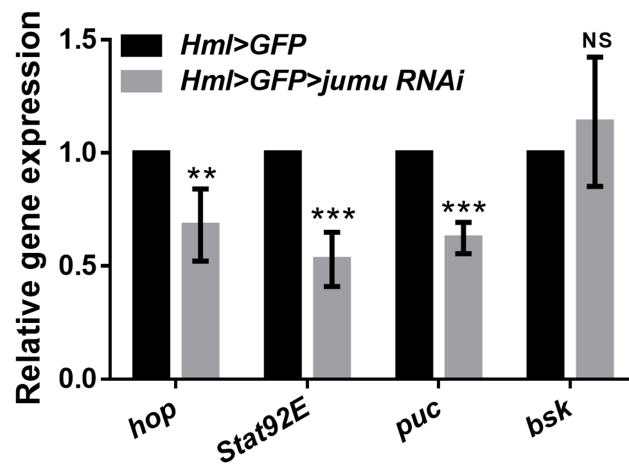
Figure S6. Analysis of the activation of JAK/STAT or JNK signaling pathways.

Real-time PCR analysis of target genes of JAK/STAT or JNK levels from *jumu* knockdown hemocytes. Error bars represent ±S.E.M for at least 3 independent experiments; NS, not significant; **P<0.01; ***P<0.001 (Student's *t*-test).