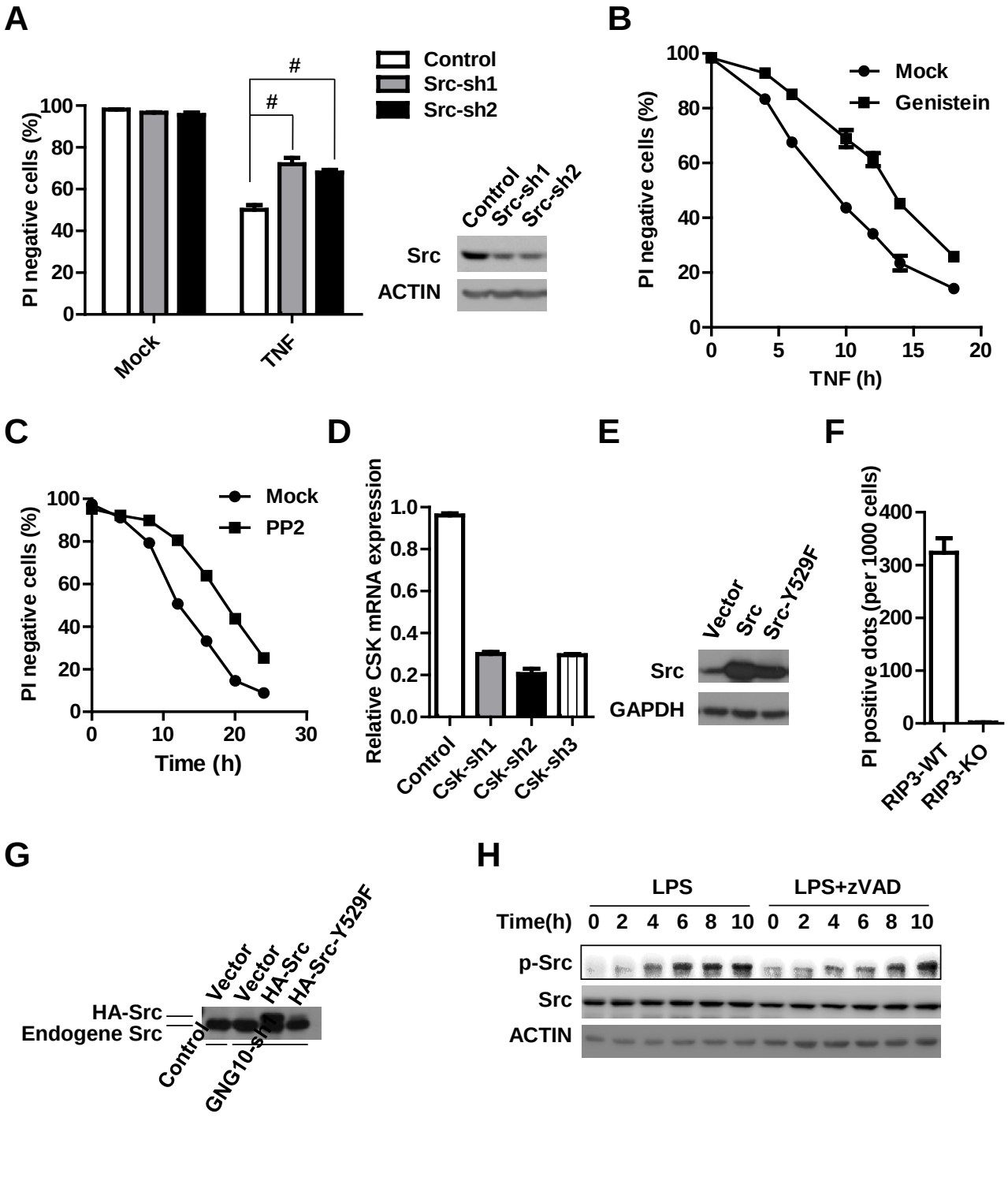
A
Control
Src-sh1
Src-sh2
#
#
PI negative cells (%)
100
80
60
40
20
0
Mock
TNF
Control
Src-sh1
Src-sh2
Src
ACTIN
B
Mock
Genistein
PI negative cells (%)
100
80
60
40
20
0
0
5
10
15
20
TNF (h)
C
Mock
PP2
PI negative cells (%)
100
80
60
40
20
0
0
10
20
30
Time (h)
D
Relative CSK mRNA expression
1.0
0.8
0.6
0.4
0.2
0.0
Control
Csk-sh1
Csk-sh2
Csk-sh3
E
Vector
Src
Src-Y529F
Src
GAPDH
F
PI positive dots (per 1000 cells)
400
300
200
100
0
RIP3-WT
RIP3-KO
G
Vector
Vector
HA-Src
HA-Src-Y529F
HA-Src
Endogene Src
Control
GNG10-sh
H
LPS
LPS+zVAD
Time(h)
0
2
4
6
8
10
0
2
4
6
8
10
p-Src
Src
ACTIN

Supplementary information, Figure S5. Src kinase is involved in necroptosis, related to Figure 6. (A) L929 cells were infected with lentivirus encoding control or *Src* shRNA. After 48 hours, knockdown efficiency of *Src* was determined by Western blot. Control and *Src* knockdown cells were then treated with TNF (10 ng/ml) for 10 hours. Cell viability was measured using PI exclusion. #: $p<0.01$, t test. (B) and (C) Cells were pretreated with Genistein (100 μM) for 2 hours or PP2 (2 μM) for 6 hours and then were treated with TNF (10 ng/ml) for different time periods. Cell viability was measured. (D) L929 cells were infected with lentivirus expressing *Csk* shRNA or control shRNA. After 48 hours, knockdown efficiency of *Csk* was determined using qRT-PCR. Analyses of these samples are shown in Figure 6C. (E) The same samples shown in Figure 6D were analyzed for Src and Src-Y529F expression. (F) Control or *RIP3* knockout L929 cells were infected with constitutively active mutant Y529F Src for 48 hours. The average numbers of PI positive dots (dead cells) per 1,000 cells were determined and shown. (G) The same samples shown in Figure 6E were analyzed for HA-Src and HA-Src-Y529F expression by Western blot. (H) Peritoneal macrophages were stimulated with LPS alone or LPS plus zVAD. Phosphorylation levels of Src were measured as Figure 6F. Cell viability was measured. Data in (B)-(D), and (F) depict mean ± SEM of one representative experiment of three or more.