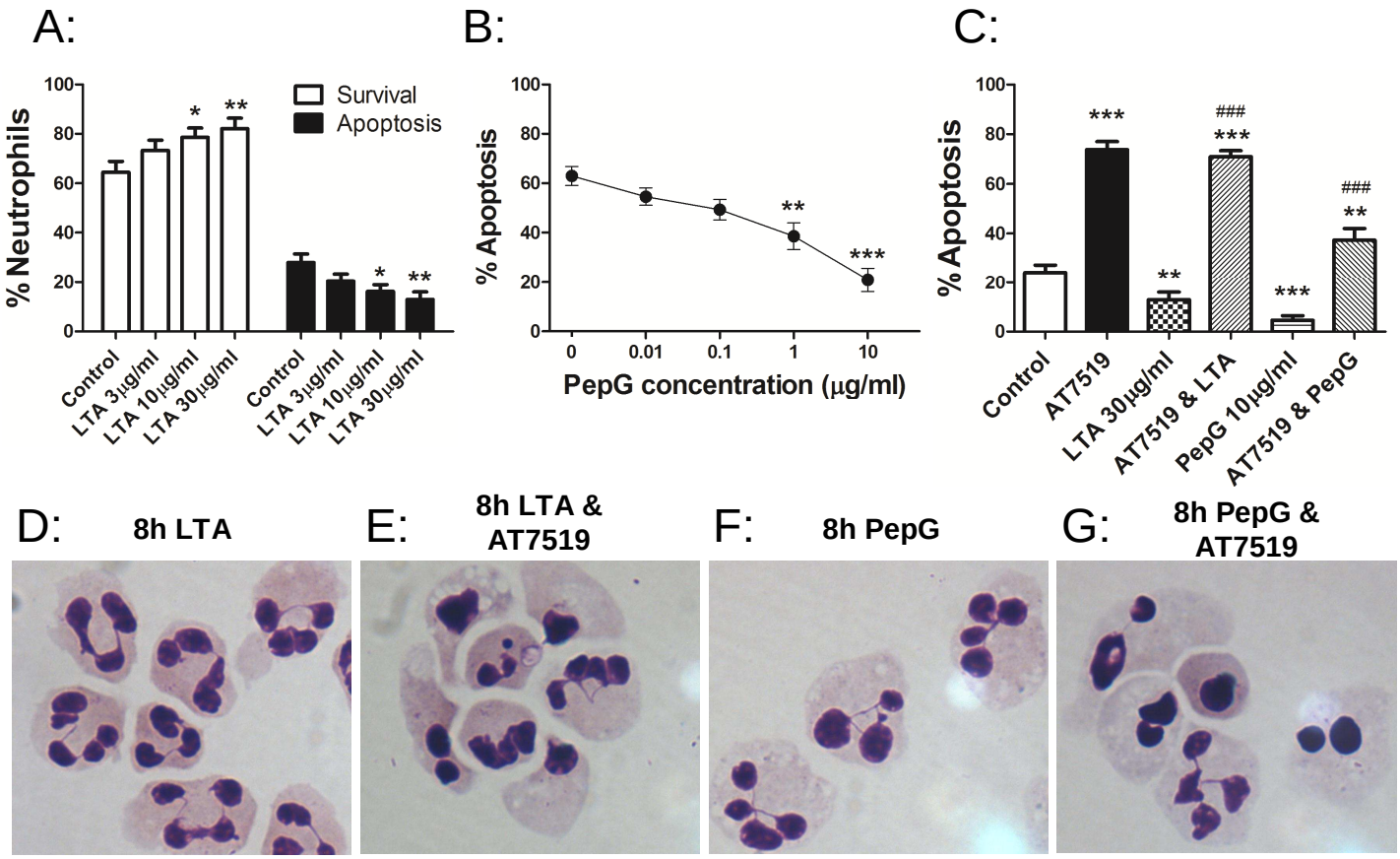


Fig. S1. AT7519 overrides the survival effects of lipoteichoic acid (LTA) and peptidoglycan (PepG). (A) Human neutrophils were cultured with increasing concentrations of lipoteichoic acid (LTA) for 8h prior to determination of viability, apoptosis and necrosis by flow cytometric analysis (AnnV/PI binding) (n=4 separate donors). (B) Neutrophils cultured with increasing concentrations of peptidoglycan (PepG) for 20h prior to assessment of apoptosis (AnnV/PI), (n=4 separate donors). (C) Neutrophils cultured for 8h with control media, AT7519 (1µM), LTA (30µg/ml), PepG (10µg/ml) or combinations prior to assessment of apoptosis (AnnV/PI) (n≥3 separate donors). Representative cyto-centrifuge preparations (x1000 magnification) of (D) LTA, (E) LTA and AT7519, (F) PepG and (G) PepG and AT7519 treated neutrophils after 8h of culture demonstrating enhanced appearance of neutrophils with apoptotic morphology in AT7519 treated groups. *p<0.05, **p<0.01, ***p<0.001 compared to control and ###p<0.001 compared to survival factor (LTA or PepG) alone.

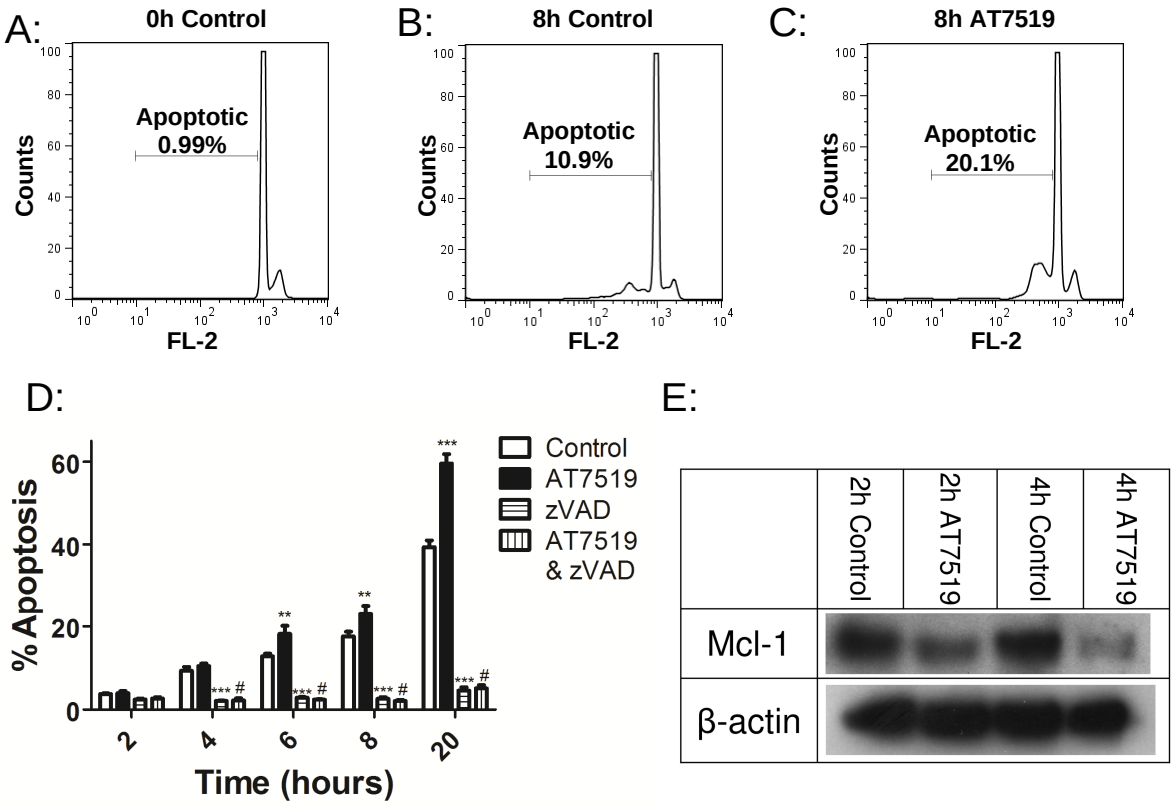
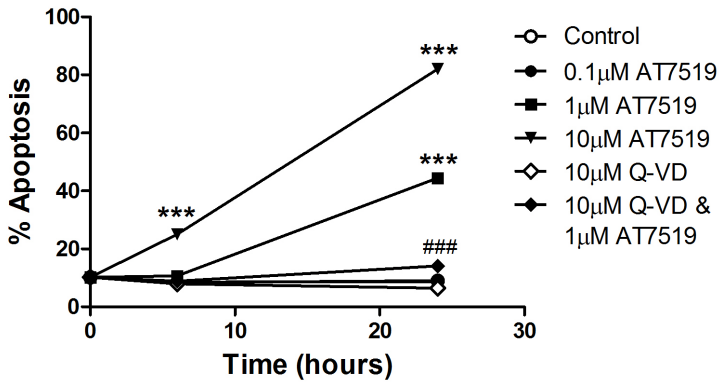
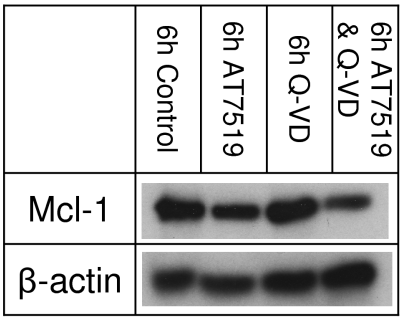


Fig. S2. AT7519 induces caspase-dependent apoptosis and down-regulates the key survival protein Mcl-1 in mouse bone marrow-derived neutrophils. (A-D) Bone marrow-derived mouse neutrophils were incubated for 2, 4, 6, 8 or 20h in either control media, AT7519 (1μM), zVAD (100μM) or combined AT7519 and zVAD prior to assessment of apoptosis by flow cytometric analysis of DNA fragmentation (hypodiploid peak). Representative flow cytometry plots (FL-2 histogram) are shown for (A) freshly isolated, (B) 8h control and (C) 8h AT7519 treated (1μM) bone marrow-derived neutrophils with cumulative data (n=4 separate animals) shown in (D). (E) Mouse bone marrow-derived neutrophils were aged for either 2h or 4h with and without AT7519 (1μM), prior to lysis and western blotting for Mcl-1 (40kDa) and β-actin (42kDa). Blot representative of two separate experiments. **p<0.01, ***p<0.001 compared to control, #p<0.001 compared to AT7519 alone.

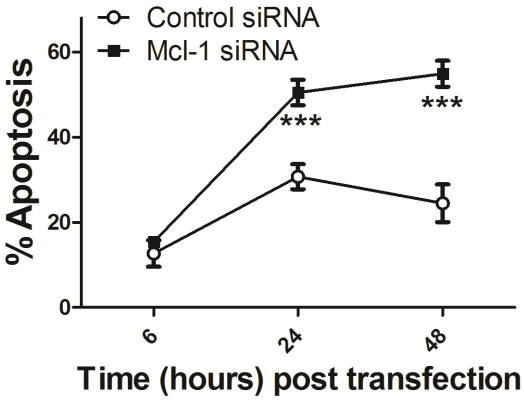
A:


% Apoptosis
Time (hours)
Control
0.1μM AT7519
1μM AT7519
10μM AT7519
10μM Q-VD
10μM Q-VD & 1μM AT7519
***
***
***
###

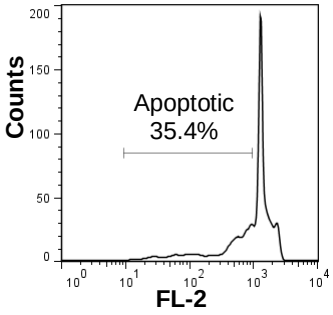
B:


6h Control
6h AT7519
6h Q-VD
6h AT7519 & Q-VD
Mcl-1
β-actin

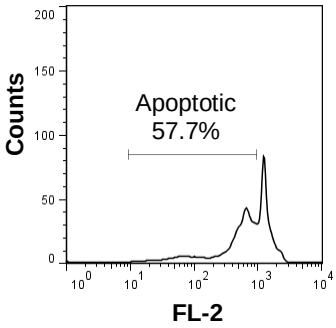
C:


Control siRNA
Mcl-1 siRNA
% Apoptosis
Time (hours) post transfection
***
***

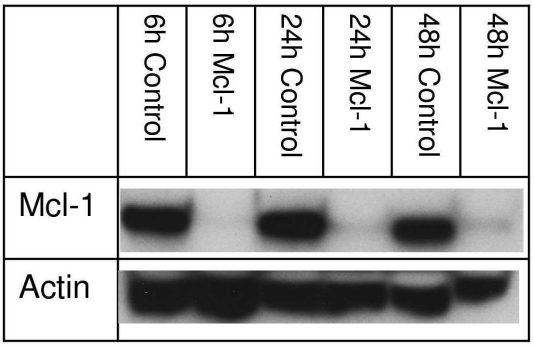
D:


24h Control siRNA
Apoptotic
35.4%
Counts
FL-2

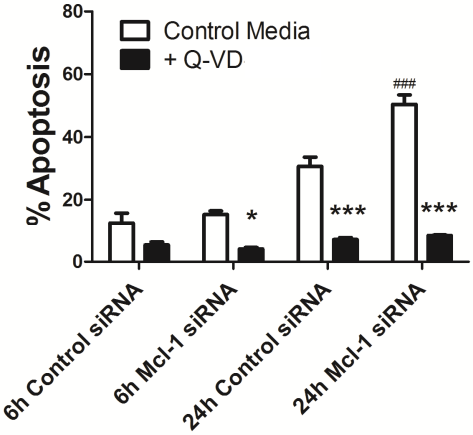
E:


24h Mcl-1 siRNA
Apoptotic
57.7%
Counts
FL-2

F:


6h Control
6h Mcl-1
24h Control
24h Mcl-1
48h Control
48h Mcl-1
Mcl-1
Actin

G:


Control Media
+ Q-VD
% Apoptosis
*
***
###
***
6h Control siRNA
6h Mcl-1 siRNA
24h Control siRNA
24h Mcl-1 siRNA

H:

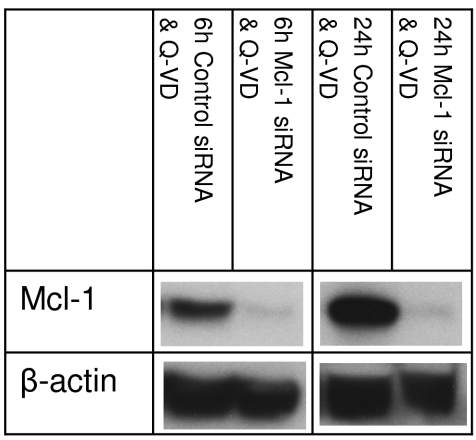

6h Control siRNA & Q-VD
6h Mcl-1 siRNA & Q-VD
24h Control siRNA & Q-VD
24h Mcl-1 siRNA & Q-VD
Mcl-1
β-actin

Fig. S3. Mcl-1 down-regulation by either AT7519 or siRNA drives death by caspase-dependent apoptosis in HL-60 cells. (A) HL-60 cells were cultured with increasing concentrations of AT7519 (0.1μM-10μM) with or without the broad spectrum caspase inhibitor Q-VD (10μM) for 6 and 24h prior to apoptosis assessment by flow cytometry (hypodiploid peak; n=4), demonstrating time, concentration and caspase-dependent induction of apoptosis. ***p<0.001 compared to control and ### p<0.001 compared to 1μM AT7519 treated sample. (B) HL-60 cells were cultured for 6h in control media, AT7519 (1μM), Q-VD (10μM) or combined AT7519 and Q-VD prior to lysis and western blotting for Mcl-1 (40kDa) and β-actin (42kDa). (C) Apoptosis assessed by hypodiploid peak at 6, 24 and 48h post transfection by nucleofector with either control or Mcl-1 siRNA (n=5) with representative flow cytometry plots (FL-2 histogram) at 24h post transfection shown for (D) control and (E) Mcl-1 siRNA transfected cells, with apoptotic nuclei appearing as a broad hypodiploid DNA peak below the narrow peak of nuclei with a normal (diploid) DNA content. (F) Confirmation of Mcl-1 knockdown preceding apoptotic cell death by western blot. *p<0.05, **p<0.01, ***p<0.001 compared to control siRNA transfected cells. (G-H) Transfection of HL-60 cells by nucleofector with either control or Mcl-1 siRNA followed by culture in either control media or media containing 10μM of the caspase inhibitor Q-VD prior to assessment of apoptosis (G) (n=4) and Mcl-1 levels (H) at 6h and 24h. *p<0.05, **p<0.01, ***p<0.001 compared to control media, ###p<0.001 compared to control siRNA in control media.

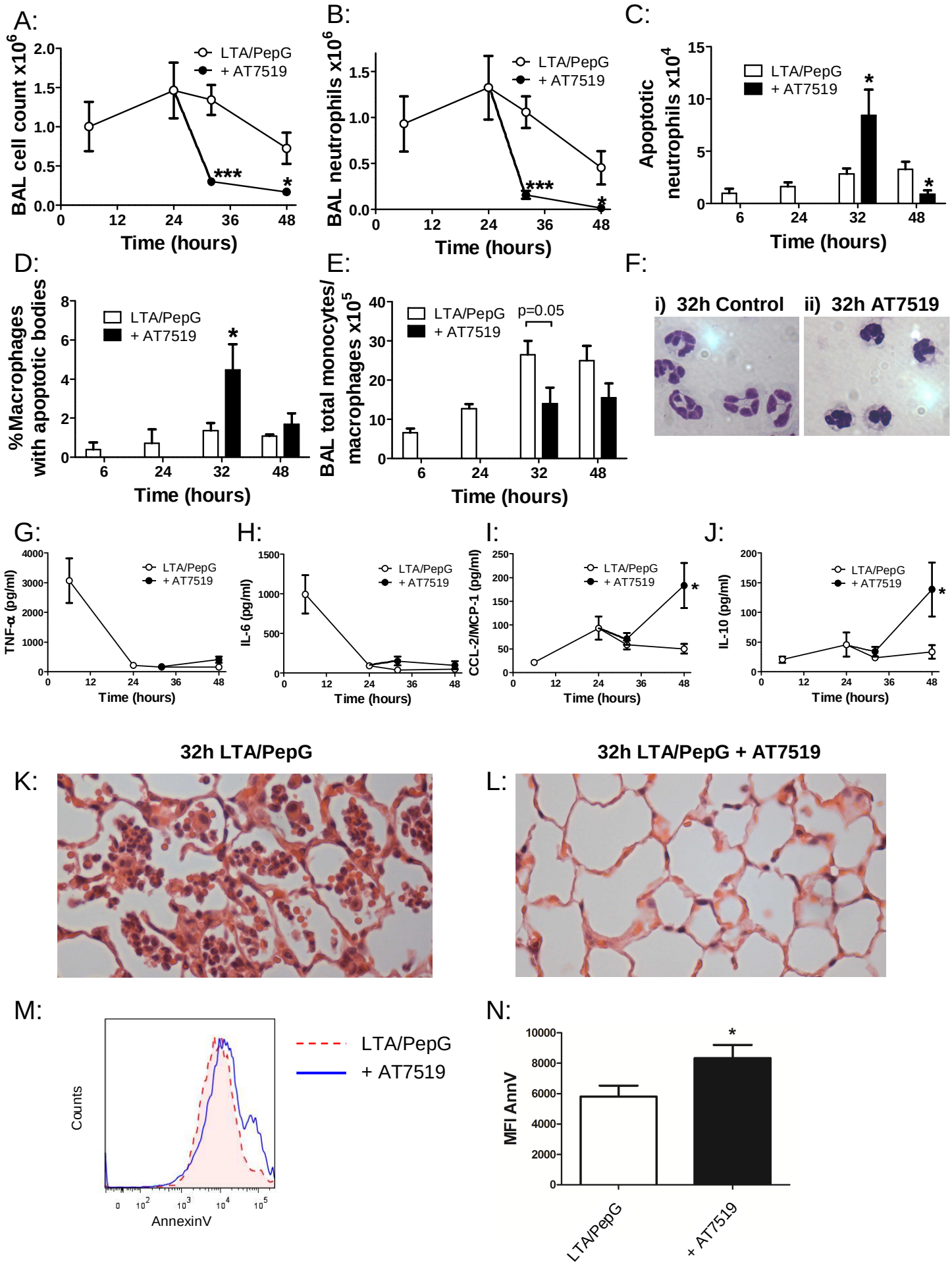

A:
BAL cell count x10^6
LTA/PepG
+ AT7519
Time (hours)
B:
BAL neutrophils x10^6
C:
Apoptotic neutrophils x10^4
D:
%Macrophages with apoptotic bodies
E:
BAL total monocytes/ macrophages x10^5
p=0.05
F:
i) 32h Control
ii) 32h AT7519
G:
TNF-α (pg/ml)
H:
IL-6 (pg/ml)
I:
CCL-2/MCP-1 (pg/ml)
J:
IL-10 (pg/ml)
32h LTA/PepG
32h LTA/PepG + AT7519
K:
L:
M:
Counts
AnnexinV
N:
MFI AnnV

Fig. S4. AT7519 accelerates resolution of established lipoteichoic acid/peptidoglycan (LTA/PepG) mediated lung inflammation. 24h after i.t. administration of combined LTA (150µg) and PepG (50µg) mice received AT7519 (30mg/kg i.p.) or vehicle control. (A) BAL inflammatory cells, (B) BAL total neutrophils, (C) apoptotic neutrophils, (D) macrophages containing apoptotic bodies and (E) total monocytes/macrophages at each time point is shown. (F) Representative morphology of BAL neutrophils at 32h post LPS (x1000 magnification) show (i) normal neutrophil morphology in the LTA/PepG alone group and (ii) neutrophils with apoptotic morphology following AT7519 treatment. BAL fluid (G) TNF-α, (H) IL-6, (I) CCl-2/MCP-1 and (J) IL-10 are shown. Lung sections are shown at 32h after LTA/PepG in (K) control and (L) AT7519 treated animals (x400 magnification). (M-N) Analysis of BAL fluid cells with apoptosis of neutrophils ($Ly6G^{+ve}/F4/80^{-ve}$ cells) analysed by Annexin V (AnnV) binding. (M) Representative AnnV histogram for neutrophils from control and AT7519 treated animals and (N) cumulative data of AnnV mean fluorescence intensity (MFI) of neutrophils. n=5 mice per group at each time point, *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared to control.