

Supplementary Figures and Figure Lengends

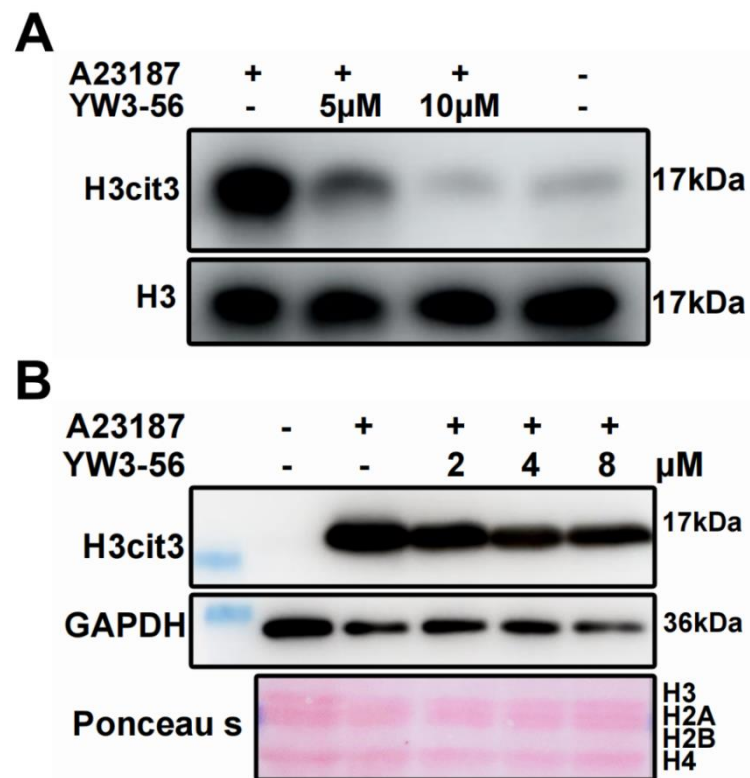


Figure S1. PAD inhibitor YW3-56 inhibits histone citrullination and NET formation of human and mouse neutrophils by Western blot. (A) Upon calcium ionophore A23187 treatment, human peripheral blood neutrophils increased histone citrullination. Histone citrullination were decreased upon YW3-56 treatment. (B) Mouse bone marrow neutrophils showed a dramatic increase in histone citrullination after calcium ionophore treatment. YW3-56 treatment blocked the increase in histone citrullination.

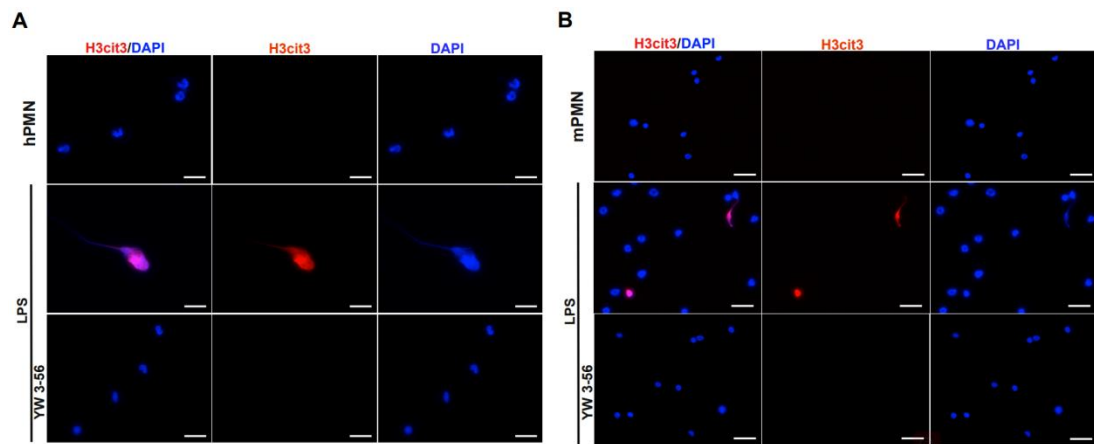


Figure S2. PAD inhibitor YW3-56 inhibits histone citrullination and NET formation of human and mouse neutrophils induced by LPS treatment. (A) Upon LPS treatment, human peripheral blood neutrophils increased histone citrullination and NET formation (middle, panels) detected with immunostaining with a histone H3Cit (Cit2, 8, 17) antibody. DNA dye DAPI staining in blue. NET formation and histone citrullination were decreased upon YW3-56 treatment. (B) Mouse bone marrow neutrophils showed a dramatic increase in histone citrullination after LPS treatment. YW3-56 treatment blocked the increase in histone citrullination. Scale bars represent 20 μm . hPMN, human polymorphonuclear cell; mPMN, mouse polymorphonuclear cell; PAD, peptidylarginine deiminase; NET, neutrophil extracellular trap; LPS, lipopolysaccharides.

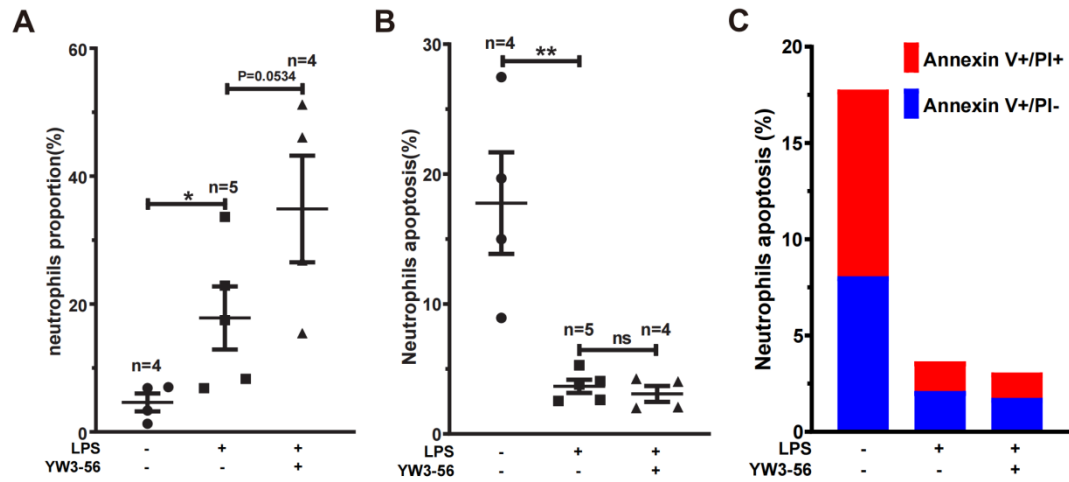


Figure S3. The proportion and apoptosis of neutrophils in blood at different treatment groups. (A) The proportion of neutrophils in leukocytes significantly increased after LPS treatment, and YW3-56 further enhanced it ($n = 4, 5, 4$), which was consistent with that the proportion of neutrophils in peritoneal lavage fluid increased after LPS treatment and YW3-56 further enhanced it (Figure 3F). (B) The apoptosis of neutrophils significantly decreased in peripheral blood of mice after LPS treatment, and YW3-56 didn't reverse this decrease ($n = 4, 5, 4$). (C) Neutrophils apoptosis was identified by Annexin V and PI. All data in figures were presented as mean \pm SEM. $*P < 0.05$; $**P < 0.01$; ns, no significance. LPS, lipopolysaccharides.

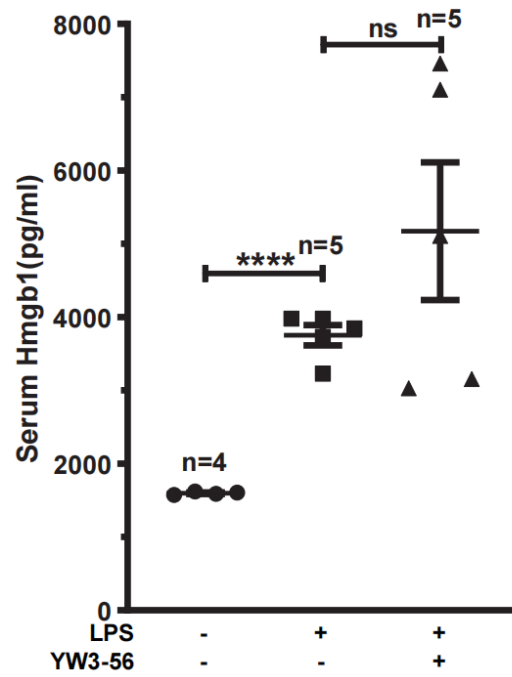


Figure S4. Serum HMGB1 levels were much increased after LPS treatment, while PAD inhibitor YW3-56 treatment didn't decrease HMGB1 levels (n = 4, 5, 5). All data in figures were presented as mean \pm SEM. **** $P < 0.0001$. HMGB1, high mobility group protein B1.

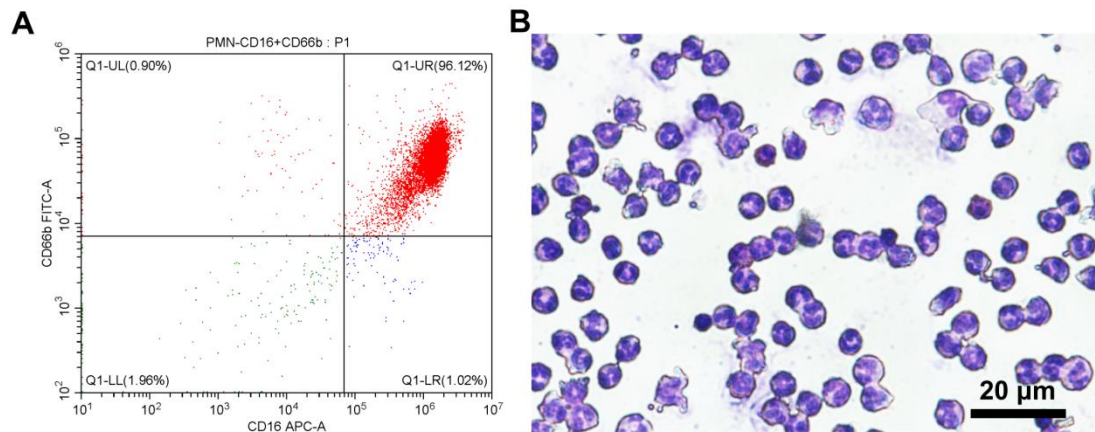


Figure S5. The purity of isolated human peripheral blood neutrophils. (A) The purity of neutrophils labeled as $CD66b^+$ and $CD16^+$ double positive in isolated peripheral blood polymorphonuclear cells had greater than 95%. (B) Isolated peripheral blood polymorphonuclear cells had a typical lobulated nuclear morphology by Wright-Giemsa staining. Scale bars represent 20 µm.

Table S1: Details of the healthy donors

Gender	Age	Number
women	20-30	27
women	31-40	3
women	40-50	1
men	20-30	21
men	31-40	4
men	40-50	2

Table S2: primer index

Genes	Primer Direction	Primer Sequences
<i>Actin</i>	Forward	GTTGGAGCAAACATCCCCCA
<i>Actin</i>	Reverse	ACGCGACCATCCTCCTCTTA
<i>Tnfα</i>	Forward	CCTCTCATCAGTTCTATGGC
<i>Tnfα</i>	Reverse	CACCACTAGTTGGTTGTCTT
<i>Csf3</i>	Forward	ATGGCTCAACTTTCTGCCCAG
<i>Csf3</i>	Reverse	GGGGAAATACCCGATAGAGCC
<i>Il1β</i>	Forward	CTGTGACTCATGGGATGATGATG
<i>Il1β</i>	Reverse	CGGAGCCTGTAGTGCAGTTG
<i>Il6</i>	Forward	CTGCAAGAGACTTCCATCCAG
<i>Il6</i>	Reverse	AGTGGTATAGACAGGTCTGTTGG
<i>Il10</i>	Forward	CTTACTGACTGGCATGAGGATCA
<i>Il10</i>	Reverse	GCAGCTCTAGGAGCATGTGG
<i>Ccl2</i>	Forward	TTAAAAACCTGGATCGGAACCAA
<i>Ccl2</i>	Reverse	GCATTAGCTTCAGATTTACGGGT
<i>Cxcl9</i>	Forward	TCCTTTTGGGCATCATCTTCC
<i>Cxcl9</i>	Reverse	TTTGTAGTGGATCGTGCCTCG
<i>Cxcl10</i>	Forward	CCAAGTGCTGCCGTCATTTTC
<i>Cxcl10</i>	Reverse	GGCTCGCAGGGATGATTTCAA
<i>Nfkb</i>	Forward	AGAGGGGATTTTCGATTCCGC
<i>Nfkb</i>	Reverse	CCTGTGGGTAGGATTTCTTGTTT
<i>Nfkbia</i>	Forward	TGAAGGACGAGGAGTACGAGC
<i>Nfkbia</i>	Reverse	TTCGTGGATGATTGCCAAGTG
<i>Stat3</i>	Forward	CAATACCATTGACCTGCCGAT
<i>Stat3</i>	Reverse	GAGCGACTCAAACCTGCCCT
<i>Padi4</i>	Forward	AAGGGCTACACAACCTTCGG
<i>Padi4</i>	Reverse	GCTGCTTTACCTGTAGGGT
<i>Ly6g</i>	Forward	GACTTCCTGCAACACAACCTACC
<i>Ly6g</i>	Reverse	ACAGCATTACCAGTGATCTCAGT
<i>Mpo</i>	Forward	GACATGCCCACCGAATGACAA
<i>Mpo</i>	Reverse	CAGGCAACCAGCGTACAAAG
<i>Elane</i>	Forward	CAGGAACTTCGTCATGTCAGC
<i>Elane</i>	Reverse	AGCAGTTGTGATGGGTCAAAG

