Expanded View Figures

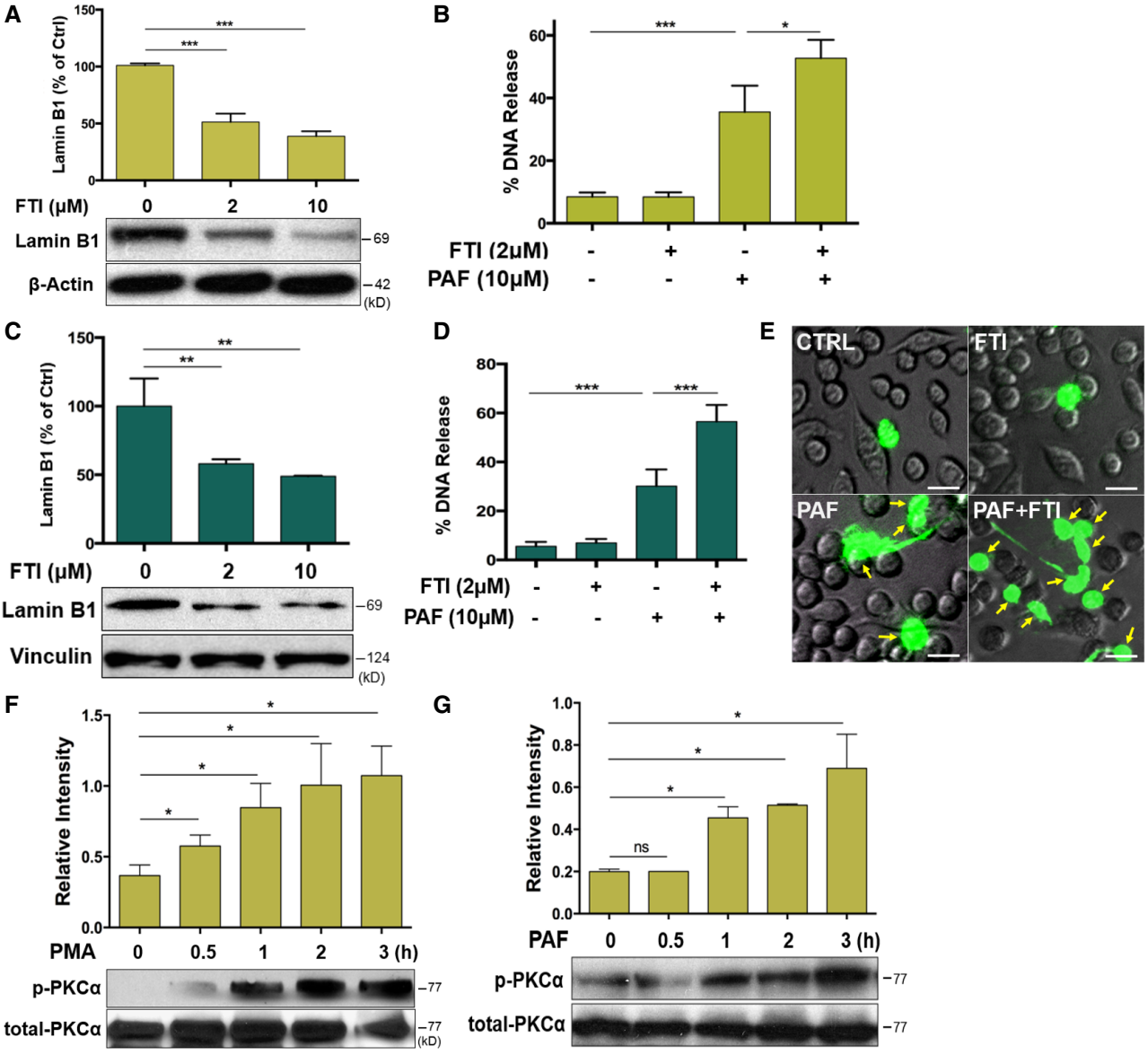

Figure EV1. Decreased lamin B expression enhances extracellular traps formation *in vitro* and time course of PKCα phosphorylation during NET formation (Related to Figs 1 and 3).

A, C Summary and representative immunoblot analysis for the mature lamin B expression in HL-60 dPMNs (A) or RAW264.7 cells (C) that were treated without (0) or with 2 or 10 μM farnesyltransferase inhibitor (FTI) L-744,832 for 48 h.

B, D, E Summary analysis (B, D) and representative images (E) of extracellular trap release in HL-60 dPMNs (B) RAW264.7 cells (E) that were pretreated without or with 2 μM for 48 h, followed by treatment or not with 10 μM PAF for 3 h and then stained with cell-impermeable SYTOX Green, without fixation. Fluorescent and phase-contrast images were taken by Olympus confocal microscopy. The yellow arrows indicate neutrophils with NET formation. Scale bars, 20 μm (E).

F, G Representative immunoblots and the summary analyses of total and phosphorylated PKCα (p-PKCα), in human dPMNs that were treated either by PMA (F) or PAF (G) for 0, 0.5, 1, 2, 3 h.

Data information: The summary analyses were calculated based on the arbitrary density of immunoblot images (A, C, F, G), or % DNA release index was analyzed by comparison of the fluorescent intensity of the indicated conditions to an assigned value of 100% for the total DNA released by neutrophils lysed by 0.5% (*v/v*) Triton X-100 (B, D) as compared to their untreated controls. Data are given as mean ± SD from at least three independent biological replicates. *$P < 0.05$, **$P < 0.01$, ***$P < 0.01$ between different groups as indicated. Comparisons among three or more groups were performed using ANOVA, followed by Student–Newman–Keuls test.
Source data are available online for this figure.

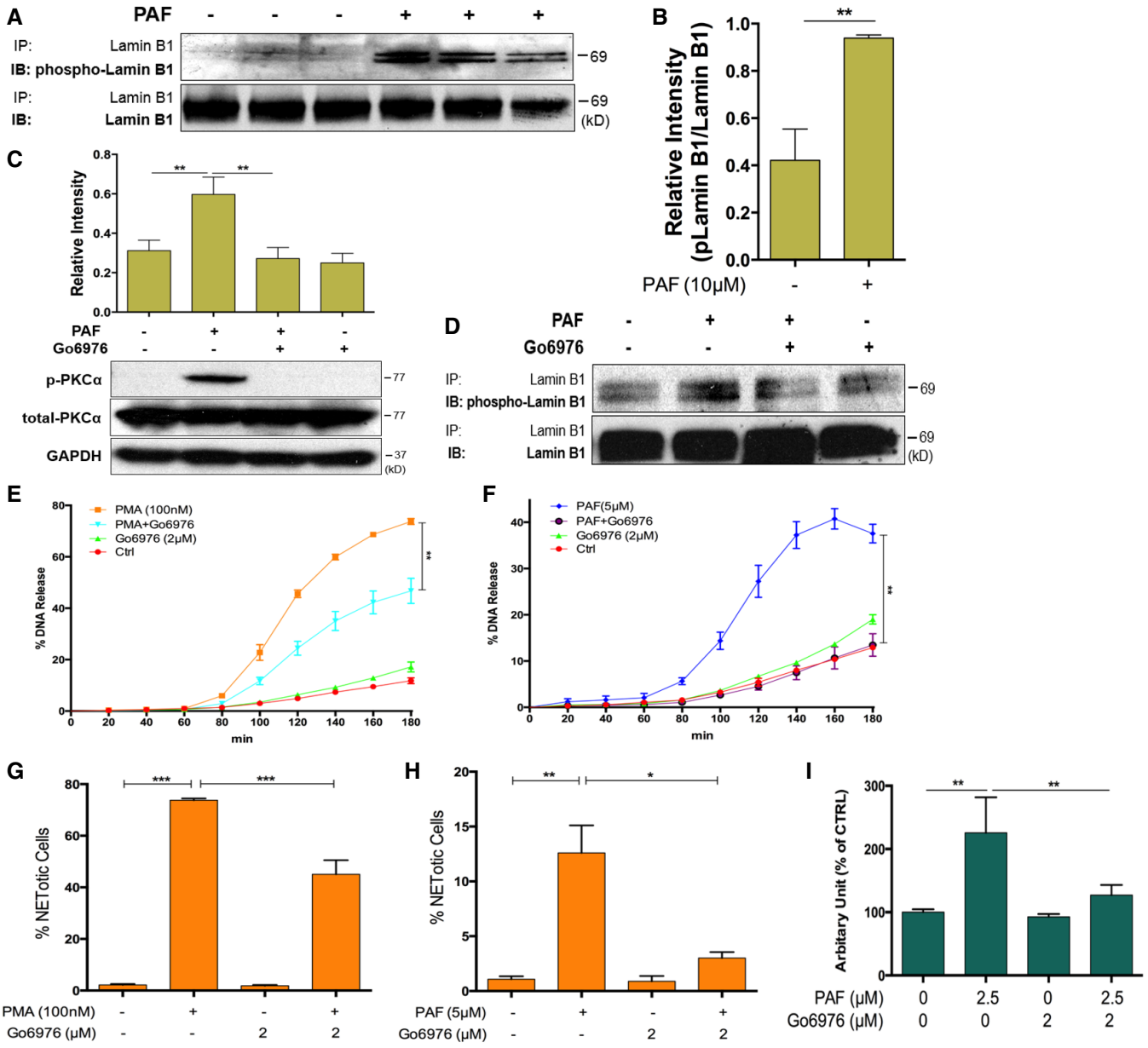

Figure EV2.

Figure EV2. Inhibition of PKCα phosphorylation attenuated Lamin B1 phosphorylation and alleviated extracellular trap formation. (Related to Figs 4 and 5).

A, B Representative and summary immunoblot (IB) detection of phosphor-lamin B and total lamin B with the lamin B protein purified by immunoprecipitation (IP) with anti-lamin B from human dPMNs that were treated either by PAF (A, B) for 0 or 3 h.

C Summary and representative immunoblots of the total PKCα and p-PKCα in dPMNs that were pretreated without or with PKC inhibitor Go6976 for 1 h and then treated without or with PAF (B) for 3 h.

D Representative immunoblot (IB) detection of the phospho-lamin B and total lamin B with lamin B protein purified by immunoprecipitation (IP) with anti-lamin B from human dPMNs that were pretreated without or with PKC inhibitor Go6976 for 1 h and then treated either by PAF (D) for 3 h.

E, F The kinetic analysis of NET-DNA release index was determined by coincubation of primary human pPMNs that were pretreated without or with PKCα inhibitor Go6976 for 1 h and then treated without (control) or with 100 nM PMA (E) or 5 μM PAF (F) in medium containing 1 μM SYTOX Green dye for 3 h with recording by a microplate reader for every 20 min. The NET-DNA release index was reported in comparison with an assigned value of 100% for the total DNA released by neutrophils lysed by 0.5% (*v*/*v*) Triton X-100.

G, H Summary analysis of PMA (G)- or PAF (H)-induced NET formation in pPMNs that were stimulated without or with 100 nM PMA or 5 μM PAF for 3 h and stained with both cell-permeable SYTO Red and cell-impermeable SYTOX Green, without fixation. Images were taken by Olympus confocal microscopy, followed by automated quantification of NETs on 5–6 non-overlapping area per well using ImageJ for calculation of % NETotic cells.

F Summary analyses of extracellular trap formation in RAW264.7 cells that were pretreated without or with PKCα inhibitor Go6976 for 1 h and then treated without or with PAF (I) for 3 h. Then, the plates were analyzed with microplate reader for fluorometric NET quantification.

Data information: Comparison between two groups was analyzed by the Student *t* test for panel (B) from 3 independent experiments. The summary analyses of panels (C, E–I) were calculated based on the arbitrary unit (E), or NET-DNA release index (G, H), or % NETotic cell (E, F), or arbitrary fluorescent unit (I) from at least 3 independent biological replicates. Data are given as mean ± SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ between groups as indicated. Comparisons among three or more groups were analyzed using ANOVA, followed by Student–Newman–Keuls test.
Source data are available online for this figure.

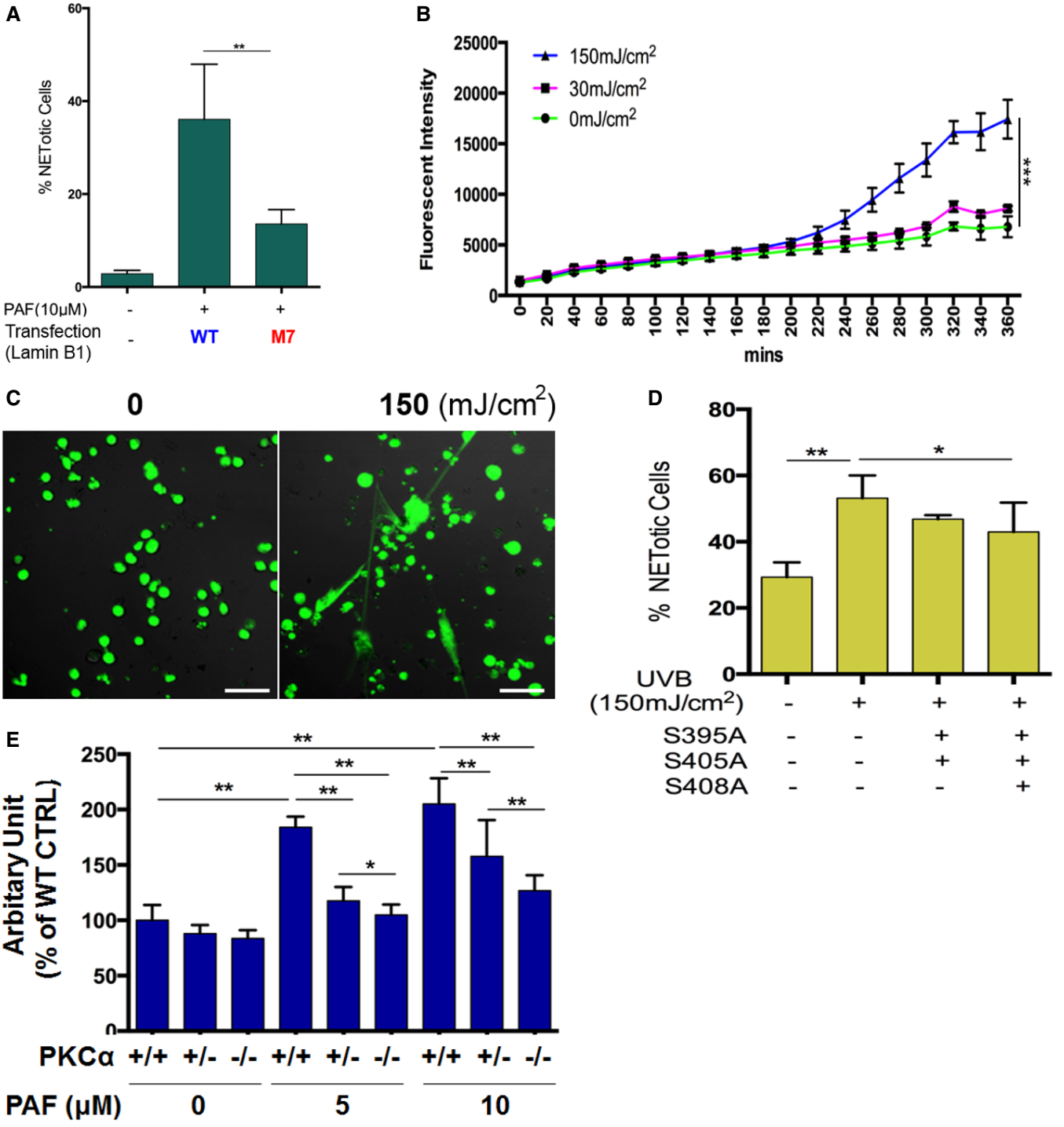
A
% NETotic Cells
PAF(10μM)
Transfection (Lamin B1)
WT
M7
B
Fluorescent Intensity
150mJ/cm2
30mJ/cm2
0mJ/cm2
mins
C
0
150 (mJ/cm2)
D
% NETotic Cells
UVB (150mJ/cm2)
S395A
S405A
S408A
E
Arbitary Unit (% of WT CTRL)
PKCα
PAF (μM)

Figure EV3.

Figure EV3. Mutation of PKCα phosphorylation site in lamin B or PKCα deficiency impaired extracellular trap formation-induced by PAF or UVB irradiation (Related to Figs 5 and 6).

A Summary analysis of extracellular trap formation in the RAW264.7 cells that were transfected with plasmids of either wild-type lamin B (WT control), or the M7 mutant of PKCα-consensus phosphorylation sites (S395A/S405A/S408A) of lamin B, and treated by 10 μM PAF for 3 h. The cells were stained by cell-impermeable dye SYTOX Green (500 nM), and the total cells were detected by staining with cell-permeable dye SYTO Red (500 nM). Images were taken by Olympus confocal microscopy, followed by automated quantification of NETs using ImageJ for quantification of % cells with extracellular trap release. RAW264.7 cells without transfection and without PAF treatment were served as basal control.

B, C Summary (B) and representative (C) analysis of NET formation in dPMNs that were irradiated without or with UVB at 30 or 150 mJ/cm^2, in medium containing 1 μM SYTOX Green dye for 6 h with recording every 20 min by a microplate reader for up to 6 h. Then, the plates were analyzed with microplate reader for fluorometric NET quantification. Scale bar, 50 μm.

D Summary analyses of NET formation in the dPMNs that were transfected with plasmids of either wild-type lamin B (WT control for the left two columns), or the M4 or M7 mutants and then were irradiated without or with UVB at 150 mJ/cm^2. Then, the staining and quantification of % NETotic cells were conducted as described in panel (A).

E Summary analyses of NET formation in mouse bone marrow mPMNs, from WT, heterozygous, or homozygous PKCα-deficient mice, which were treated without or with either 5 or 10 μM of PAF for 3 h, following by fluorometric NET quantification analysis. Data information: The panels (A, B, D) were calculated based on at least 3 independent biological replicates. Panel (E) is a summary analysis that was calculated based on the arbitrary fluorescent unit from 3 independent biological replicates. Data are given as mean ± SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ between groups as indicated. Comparisons among three or more groups were analyzed using ANOVA, followed by Student–Newman–Keuls test.

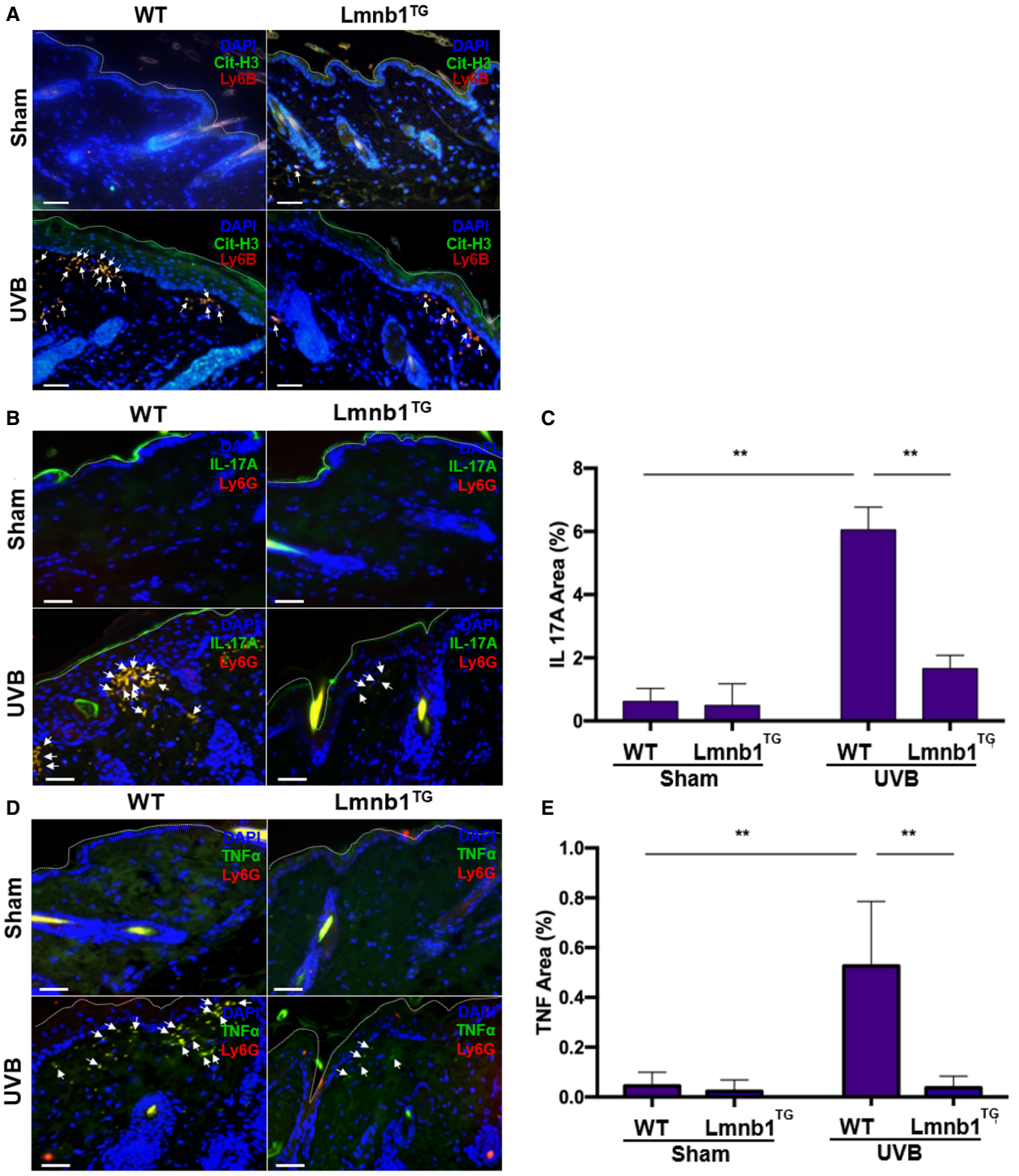
A
WT
Lmnb1TG
Sham
UVB
DAPI
Cit-H3
Ly6B
B
IL-17A
Ly6G
C
IL 17A Area (%)
D
TNFα
E
TNF Area (%)
**
WT
Lmnb1TG
Sham
UVB

Figure EV4.

Figure EV4. Lamin B overexpression decreased NET release and alleviated exhibition of NET-associated proinflammatory cytokines in skin of lamin B1 transgenic mice after UVB irradiation (Related to Fig 7).

A–E Fluorescent staining of the skin tissues from $Lmnb1^{TG}$ mice and their WT littermates that were irradiated or not (sham) by UVB. (A) The citrullinated histone H3 was probed by rabbit anti-mouse citrullinated histone H3, following stained by Alexa Fluor-488-labeled donkey anti-rabbit secondary antibody, while neutrophil marker was probed by rat anti-mouse Ly6B Ab following stained by Alexa Fluor-647 conjugated goat anti-rat secondary antibody. (B–E) The representative (B, D) or summary (C, E) analysis of neutrophil surface marker Ly6G was stained by PE-conjugated rat anti-mouse Ly6G Ab. FITC-labeled rat anti-mouse IL-17A antibody was used to detect IL-17A. FITC-labeled rat anti-mouse TNF-α antibody was used to detect TNF-α. DNA was stained by DAPI for or panel (A–E).

Data information: White arrows indicate neutrophils with NET formation (A) or NETs with IL-17A (B, C) or TNF-α (D, E) display in skin. Scale bar, 100 μm. The panels (C, E) were calculated based on 3–4 individual mice (n = 3–4 biological replicates). Data are given as mean ± SD. $^{**}P < 0.01$, between groups as indicated. Comparisons among three or more groups were analyzed using ANOVA, followed by Student–Newman–Keuls test.

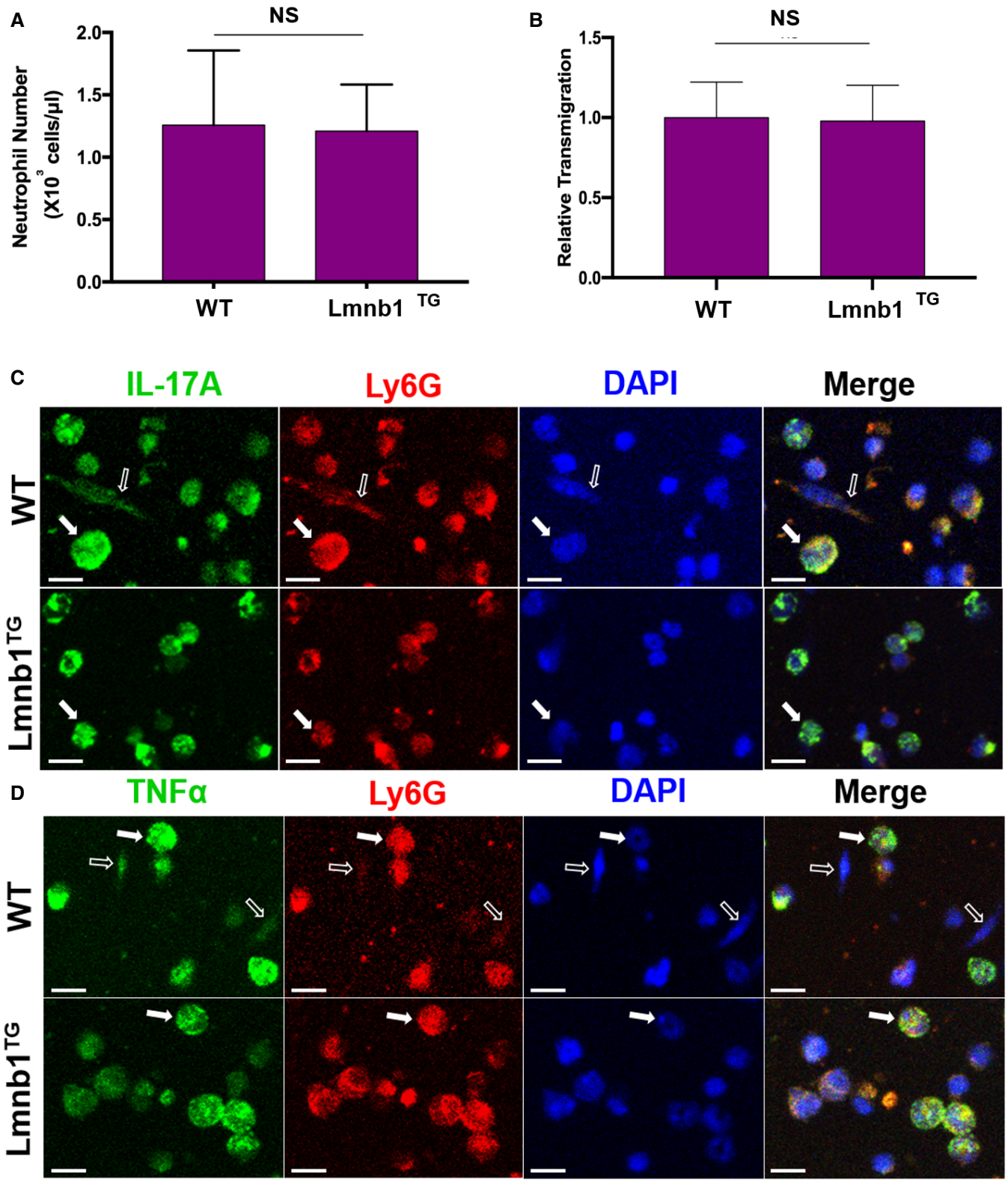
A
NS
Neutrophil Number (X10^3 cells/μl)
2.0
1.5
1.0
0.5
0.0
WT
Lmnb1 TG
B
NS
Relative Transmigration
1.5
1.0
0.5
0.0
WT
Lmnb1 TG
C
IL-17A
Ly6G
DAPI
Merge
WT
Lmnb1TG
D
TNFα
Ly6G
DAPI
Merge
WT
Lmnb1TG

Figure EV5.

Figure EV5. Features of neutrophil counts in peripheral blood, transmigration ability and cytokine expression in neutrophils from Lmnb1^{TG} vs WT mice.

A Neutrophil counts in peripheral blood from Lmnb1^{TG} mice and their WT littermates (n = 4 biological replicates).

B Relative transmigration was measured using Boyden chamber with pore size of 3.0 μm. The pore transmigration ability in neutrophils from the Lmnb1^{TG} mice was expressed as relative transmigration compared to that in neutrophils from WT littermates (n = 3 biological replicates).

C, D The bone marrow neutrophils from Lmnb1^{TG} mice and their WT littermates were treated with 10 μM PAF for 16 h and then fixed with 2% PFA and permeabilized with 0.1% Triton X-100, following immunocytochemistry staining. The neutrophil surface marker Ly6G was stained by PE-conjugated rat anti-mouse Ly6G Ab. FITC-labeled rat anti-mouse IL-17A antibody or FITC-labeled rat anti-mouse TNF-α antibody was used to detect IL-17A or TNF-α. DNA was stained by DAPI for or panels (C, D).

Data information: Panels (A, B) are summary analysis that was calculated based on neutrophil counts of the blood slides with May–Grünwald–Giemsa staining (A), the arbitrary fluorescent unit of SYTOX Green staining of the lysed neutrophils that transmigrated to the bottom chamber of the 24-well transwell plates, from at least 3 independent biological replicates (B). The differences between groups $P > 0.05$ are indicated as no significant difference (NS). Data are given as mean ± SD. Comparisons were calculated by the Student t test.