NINJ1 plays a vital role in the release of neutrophil extracellular traps in acute lung injury

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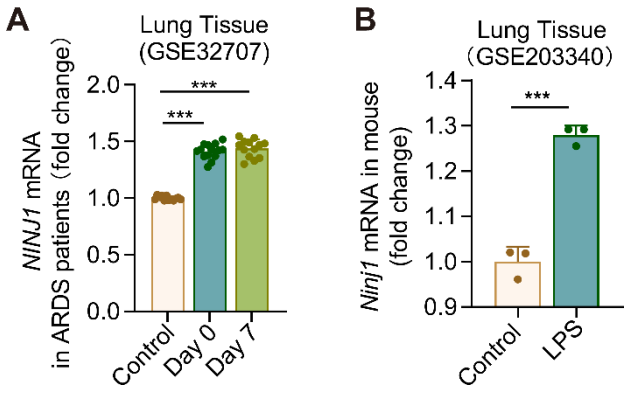

Figure S1. NINJ1 expression is significantly increased in ALI/ARDS.

A) Human *NINJ1* mRNA expression in the lungs from ARDS patients was determined by microarray (GSE32707) (n=14). Blood samples were collected from enrolled ARDS patients at the time of hospital admission (Day 0) and on Day 7 thereafter. B) *Ninj1* mRNA expression in the lungs from LPS-induced ALI mice (GSE203340) (n=3). ***$P < 0.001$.

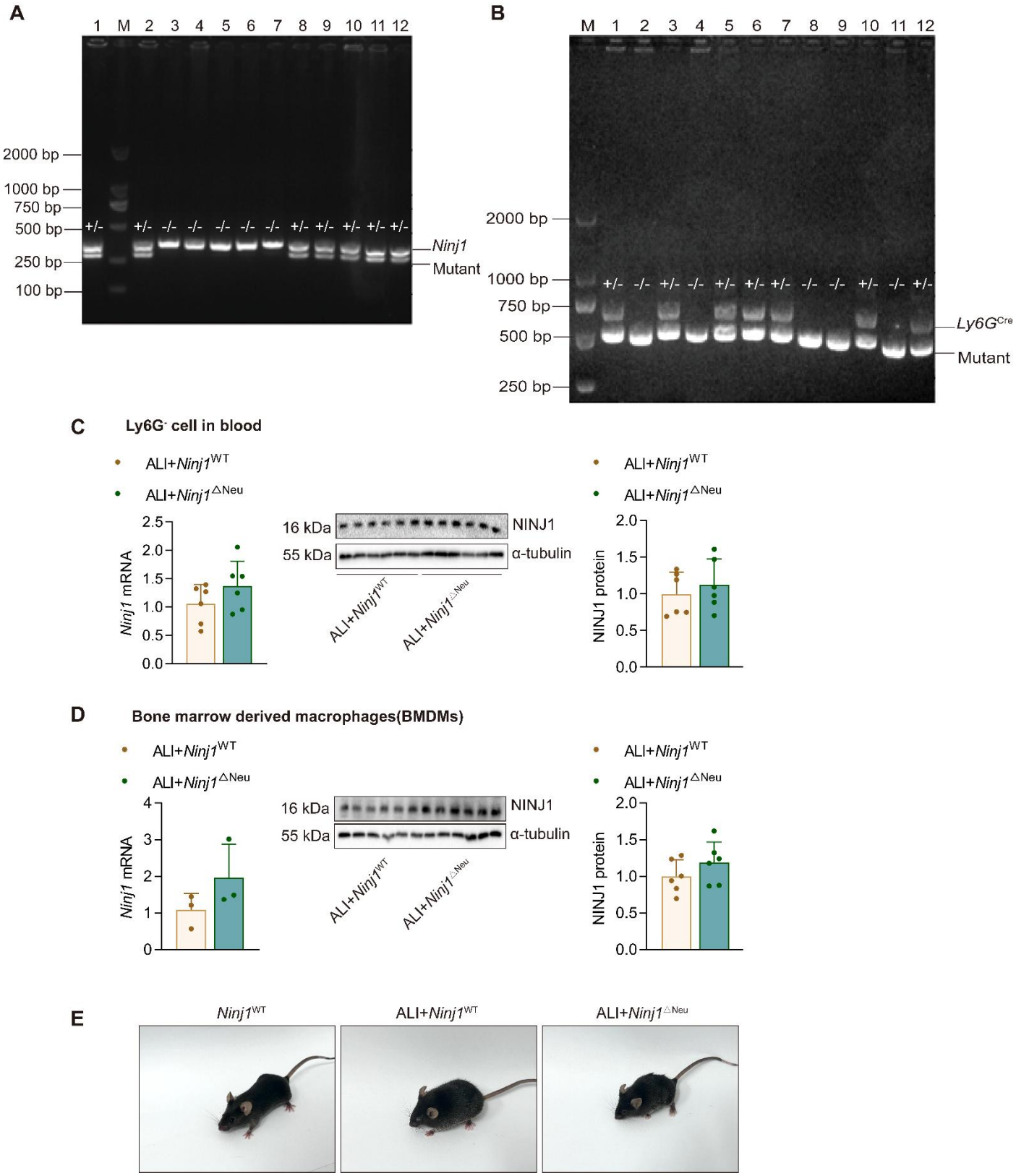

Figure S2. Generation of neutrophil-specific conditional *Ninj1*-null mice.

A) Targeted disruption of the *Ninj1* gene was verified by PCR of genomic DNA isolated from candidate mice. B) Targeted disruption of the $Ly6G^{Cre}$ gene was verified by PCR of genomic DNA isolated from candidate mice. $Ninj1^{WT}$ and $Ninj1^{\Delta Neu}$ mice were intratracheally administered with LPS (5 mg/kg). C) Real-time PCR and Western blot were employed to detect NINJ1 expression in $Ly6G^-$ cells isolated from bone marrow from $Ninj1^{WT}$ and $Ninj1^{\Delta Neu}$ mice. D) Detection of NINJ1 expression by Real-time PCR and Western blot in BMDMs from $Ninj1^{WT}$ and $Ninj1^{\Delta Neu}$ mice. E) The physical appearance of $Ninj1^{WT}$ and $Ninj1^{\Delta Neu}$ mice

administered with LPS. $n = 6$ mice/group, $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

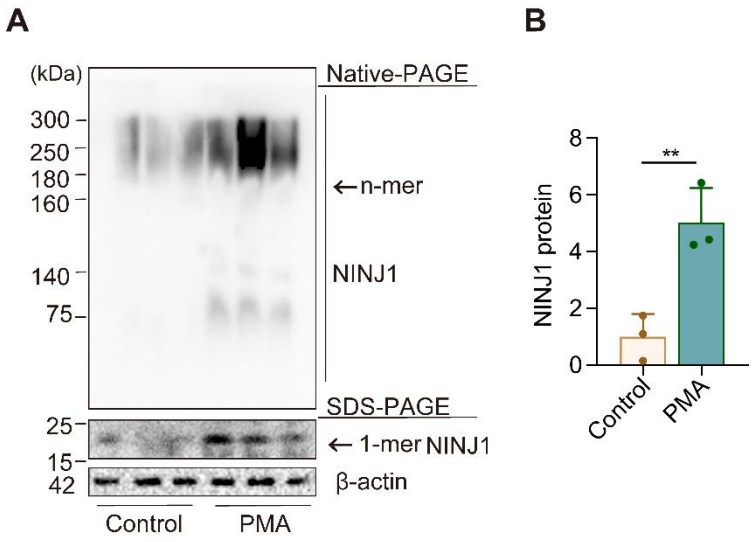

Figure S3. NINJ1 oligomerization is elevated in PMA-induced neutrophils.

Bone marrow neutrophils in mice were treated with PMA (10 nM) for 4 h. A) Native-PAGE analysis of NINJ1 oligomerization in PMA-treated neutrophils. SDS-PAGE analysis of NINJ1 monomer and β-actin was used as the internal control. B) Quantification of NINJ1 monomer protein levels, n =3, $^{**}P < 0.01$.

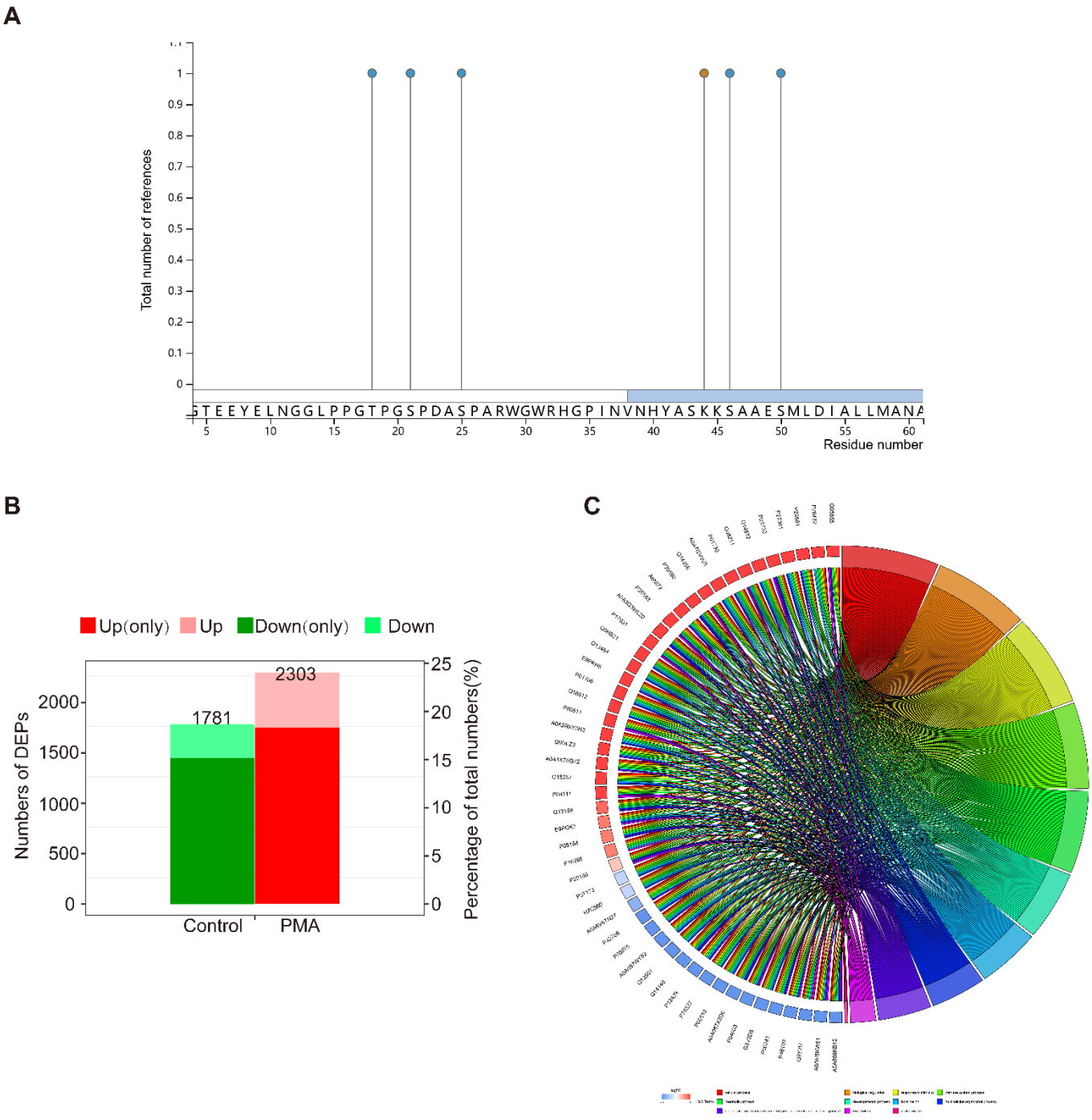

Figure S4. Lack of upregulated NINJ1 phosphorylation upon PMA stimulation.

A) To predict potential phosphorylation sites, Bioinformatic analysis with CSSPalm suggested that NINJ1 contains several putative phosphorylation sites, notably at Thr18, Ser25, Ser46, and Ser50. B) Phosphoproteomic profiling of PMA-treated neutrophils identified 2303 upregulated phosphoproteins, none of which corresponded to NINJ1. C) Phosphoproteomic profiling of PMA-treated neutrophils did not detect any upregulated phosphorylation at the predicted sites on NINJ1. $n = 3$.