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Supplemental information

Zinc-dependent histone deacetylases drive neutrophil extracellular trap formation and potentiate local and systemic inflammation

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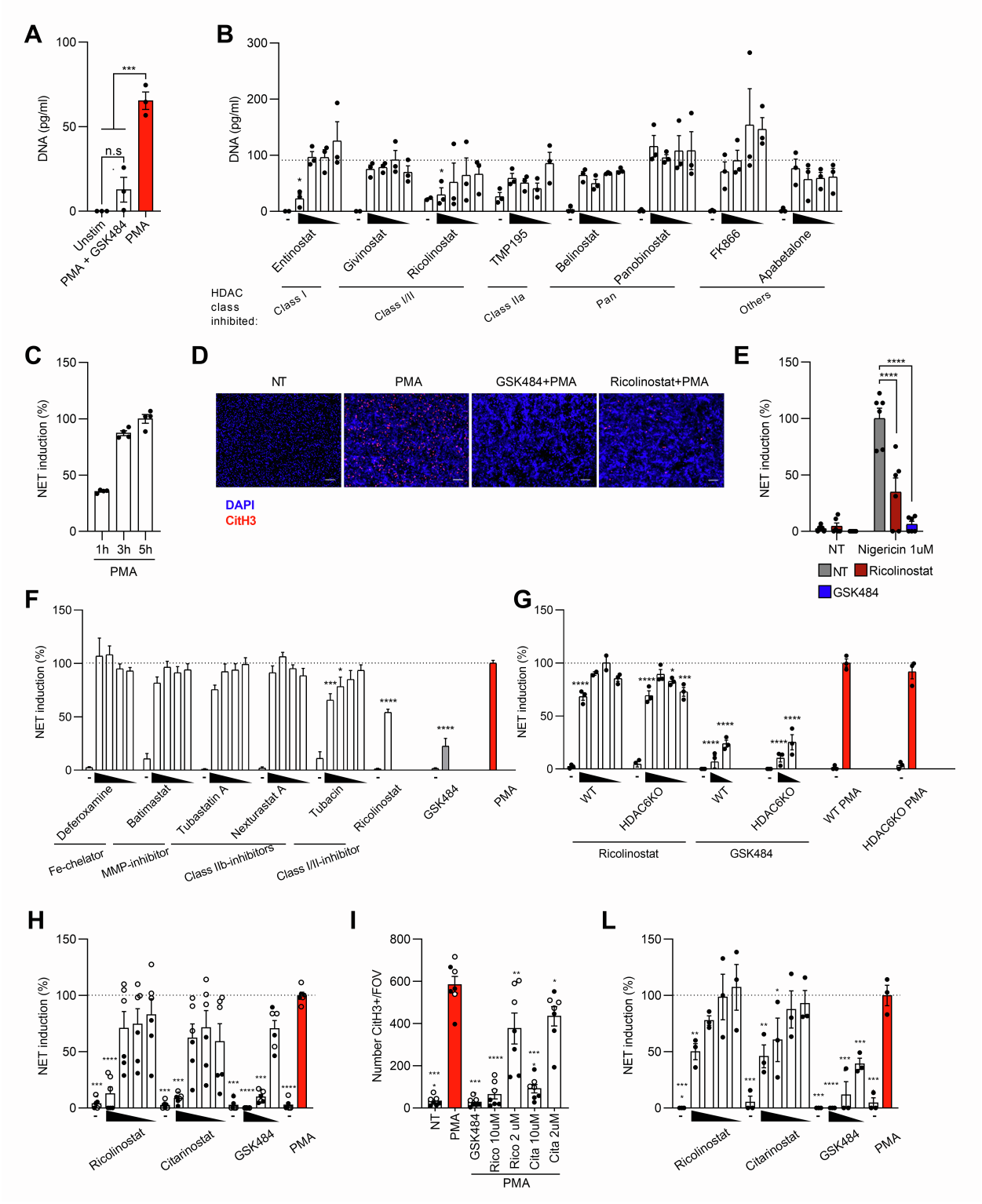
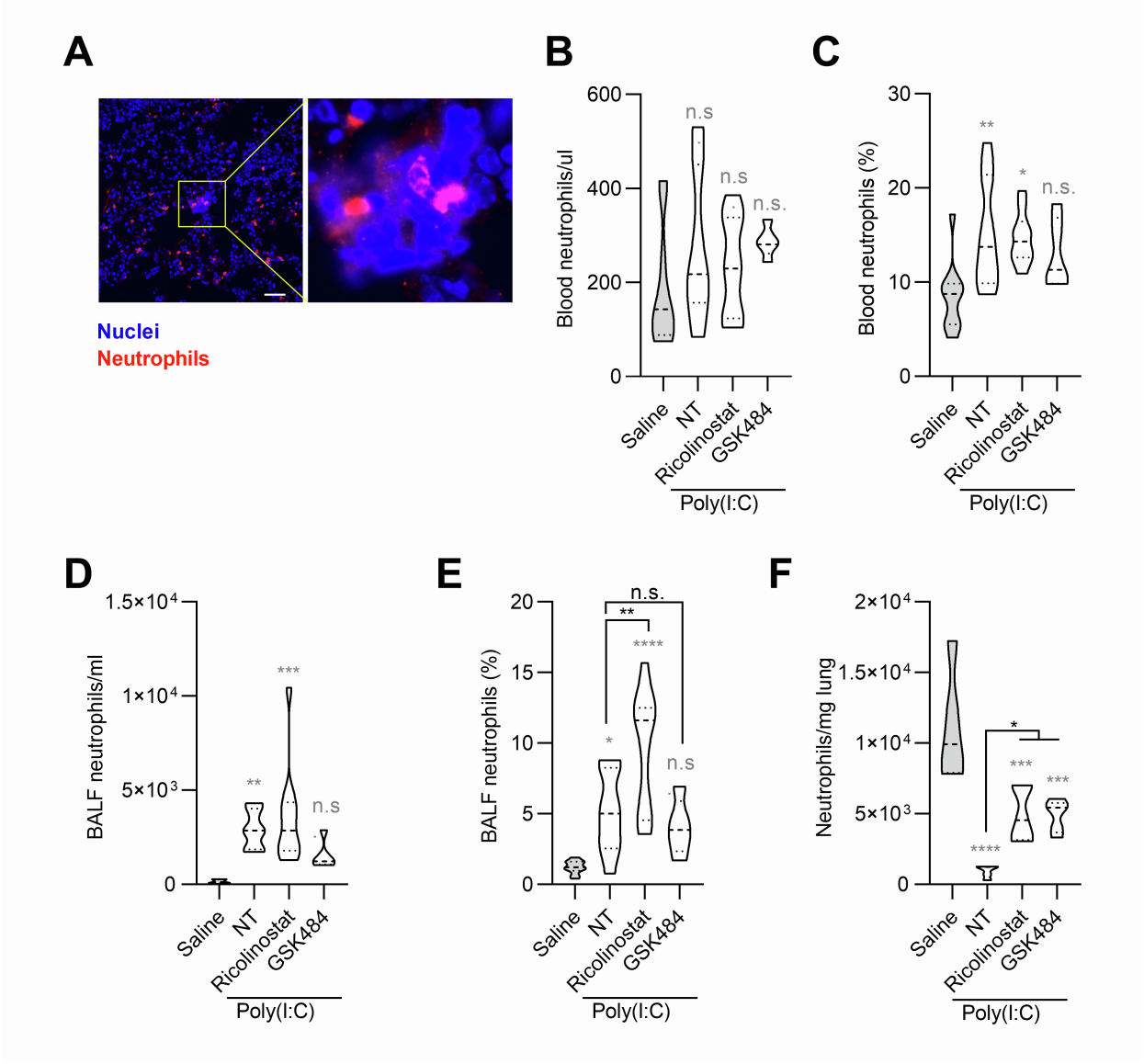
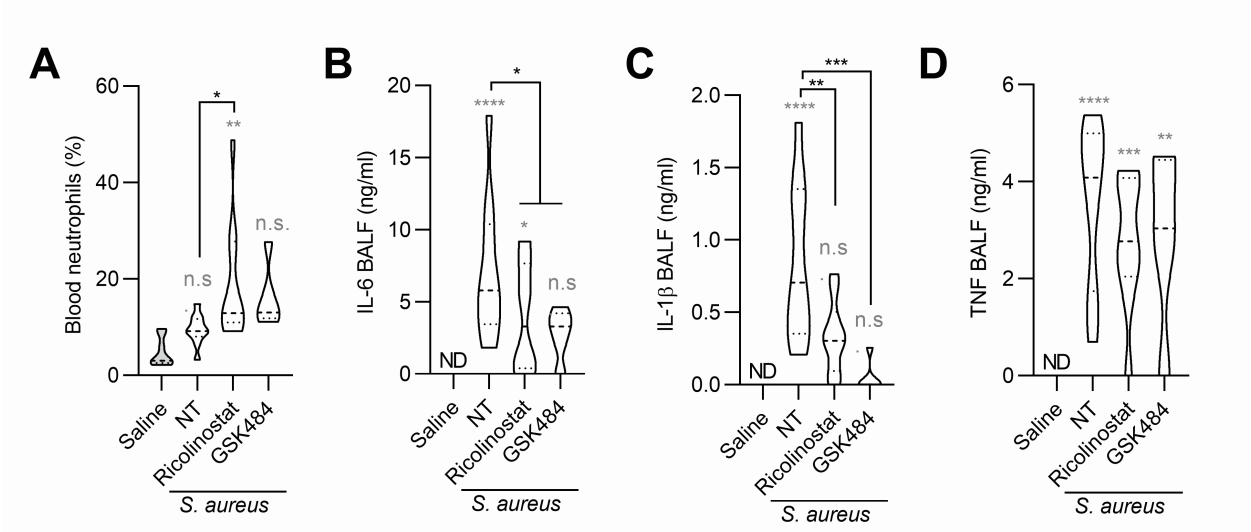


Supplementary Figure 1. Class I/II HDACs drive NET formation. Related to Figure 1. A) *Quantification of extracellular DNA released by neutrophils treated, or not, with 1 μM GSK484 for 1h and then stimulated with 1 μM PMA for 4 hours. Unstimulated, neutrophils not treated with drugs were used as a control.* ***B)*** *Freshly purified human neutrophils were treated with the*

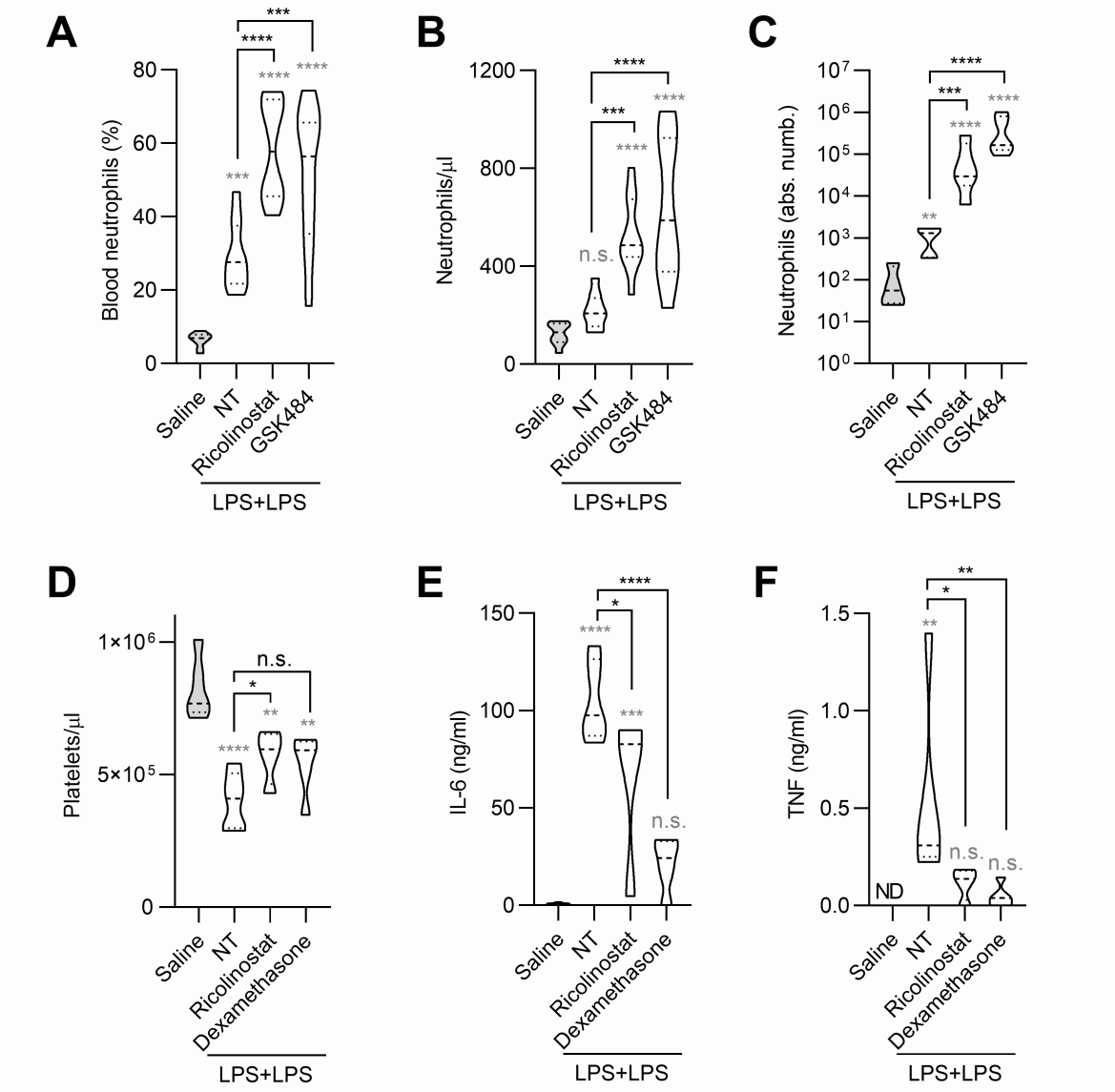
indicated HDAC inhibitors at different doses (0.08, 0.4, 2 and 10 μM) for 1 hour. Extracellular DNA release was assessed in the supernatant 3 hours after PMA stimulation. The dotted line represents the amount of DNA released from neutrophils treated with PMA alone. "-": cells treated with the highest dose of the indicated drug but not activated with PMA. ***C)*** *Freshly purified human neutrophils were stimulated for 1h, 3h or 5 h. Cells were fixed and stained with an anti-CitH3 antibody. NETosis induction was measured as level of anti-CitH3 fluorescent signal. 5h-PMA treatment represents 100% NET induction.* ***D)*** *Representative images of freshly isolated human neutrophils treated or not with 10 μM of the indicated drugs and stimulated with 1 μM PMA for 3 hours. Cells were fixed and then stained for CitH3 (red) and nuclei (blue). Scale bar = 100 μm.* ***E)*** *Murine neutrophils were treated with ricolinostat or GSK484 and then stimulated with 1 μM nigericin for 3 hours. NET induction was measured as in (C). Nigericin alone represents 100% NET induction.* ***F)*** *Freshly purified human neutrophils were treated with the indicated drugs at different doses (10, 2, 0.4, 0.08 μM), ricolinostat or with GSK484, for 1 hour and then stimulated with 1 μM PMA for 3 hours. Cells were fixed and stained with an anti-CitH3 antibody. NETosis induction was measured as level of anti-CitH3 fluorescent signal. PMA alone represents 100% of NET induction. "-": cells treated with the highest dose of the indicated drug but not activated with PMA.* ***G)*** *Neutrophils purified from wild-type or HDAC6KO mice were treated with different concentration of ricolinostat (10, 2, 0.4, 0.08 μM) or GSK484 (10, 2 μM) and then stimulated with PMA. NET induction was measured as in (F).* ***H, I)*** *Freshly isolated neutrophils from two different donors were treated with different doses of ricolinostat, citarinostat (10, 2, 0.4, 0.08 μM) or GSK484 (10, 5, 2 μM) for 1 h and then stimulated with 1 μM PMA. NET induction was measured as level of anti-CitH3 fluorescent signal (H) and by microscopy as number of cells positive for CitH3 signal per field of view (FOV) (I). Black dots represent donor 1, white dots donor 2. n = 7 images representing different areas within the well (I).* ***L)*** *Murine neutrophils were treated as in G). NET induction was measured as level of anti-CitH3 fluorescent signal. PMA alone represents 100% of NET induction. "-": cells treated with the highest dose of the indicated drug but not activated with PMA. Graph shows mean ± SEM. Statistics were calculated using One-way ANOVA (A, E, I) and Two-way ANOVA (B, , F, G, H, L). (*P<0.05; **P<0.01; ***P<0.001 ****P < 0.0001. n.s., not significant). Statistic refers to the comparison with neutrophils stimulated with PMA alone.*



Supplementary Figure 2. Inhibition of class I/IIb HDACs protects against NETosis and ARDS in a mouse model of RNA viral lung infection. Related to Figure 2. A) *Representative image of lung section of mice challenged with poly(I:C). Nuclei are stained with DAPI (blue), and anti-neutrophils antibody (red). Scale bar = 100 µm.* ***B-F)*** *Neutrophils numbers and percentage were calculated in blood (B, C), BALFs (D, E), and lung tissues (F) of mice challenged, or not, with poly(I:C). The saline group represents mice that received saline instead of poly (I:C). NT group represent mice that were challenged with poly(I:C) without receiving any drug. Ricolinostat (30 mg/kg/dose) or GSK484 (25 mg/kg/dose) were administered intraperitoneally at day 2, day 4 and 3h prior the endpoint. Violin plots represent median (dashed line) with quartiles (dotted line). Statistics were calculated using One-way ANOVA (*P<0.05; **P<0.01; ***P<0.001; ****P < 0.0001; n.s., not significant). Grey stars represent comparison of all experimental groups with the saline control. Black stars represent comparison of drug treated groups with NT group.*



Supplementary Figure 3. Class I/IIb HDACs increase NETosis and ARDS in a mouse model of bacterial pneumonia. Related to Figure 3. A) *Neutrophil percentage in mice challenged, or not, with S. aureus was determined by cytofluorimetry. Saline group represent healthy mice. NT group represents mice that were challenged with S. aureus and did not received any drug. Ricolinostat (30 mg/kg/dose) or GSK484 (25 mg/kg/dose) were administered intraperitoneally at -24 hours and -3 hours prior challenge.* ***B-D)*** *Absolute amounts of serum cytokines were measured by ELISA. Violin plots represent median (dashed line) with quartiles (dotted line). Statistics were calculated using One-way ANOVA (*P<0.05; **P<0.01; ***P<0.001; ****P < 0.0001; n.s., not significant). Grey stars represent comparison of all experimental groups with the saline control. Black stars represent comparison of drug treated groups with NT group.*



Supplementary Figure 4. Class I/IIb HDAC inhibition protects against systemic inflammation. Related to Figure 4. A-C) *Neutrophils numbers and percentage were calculated in blood (A, B) and peritoneal lavage (C) of mice challenged, or not, with LPS. Saline group represent healthy mice. NT group represent mice that were challenged with LPS without receiving any drug. Ricolinostat (30 mg/kg/dose) or GSK484 (25 mg/kg/dose) were administered intraperitoneally at day -1, and 2 hour prior LPS priming.* ***D)*** *Concentration of platelets in the blood was assessed by cytofluorimetry 2 hours post LPS-challenge.* ***E, F)*** *Absolute amounts of serum cytokines were measured by ELISA. Violin plots represent median (dashed line) with quartiles (dotted line). Statistics were calculated using One-way ANOVA (*P<0.05; **P<0.01; ***P<0.001; ****P < 0.0001; n.s., not significant). Grey stars represent comparison of all experimental groups with the saline control. Black stars represent comparison of drug treated groups with NT group.*