cancers

Supplementary Materials: Anthracyclines Suppress both NADPH Oxidase-Dependent and -Independent NETosis in Human Neutrophils

Meraj A. Khan, Adam D'Ovidio, Harvard Tran and Nades Palaniyar

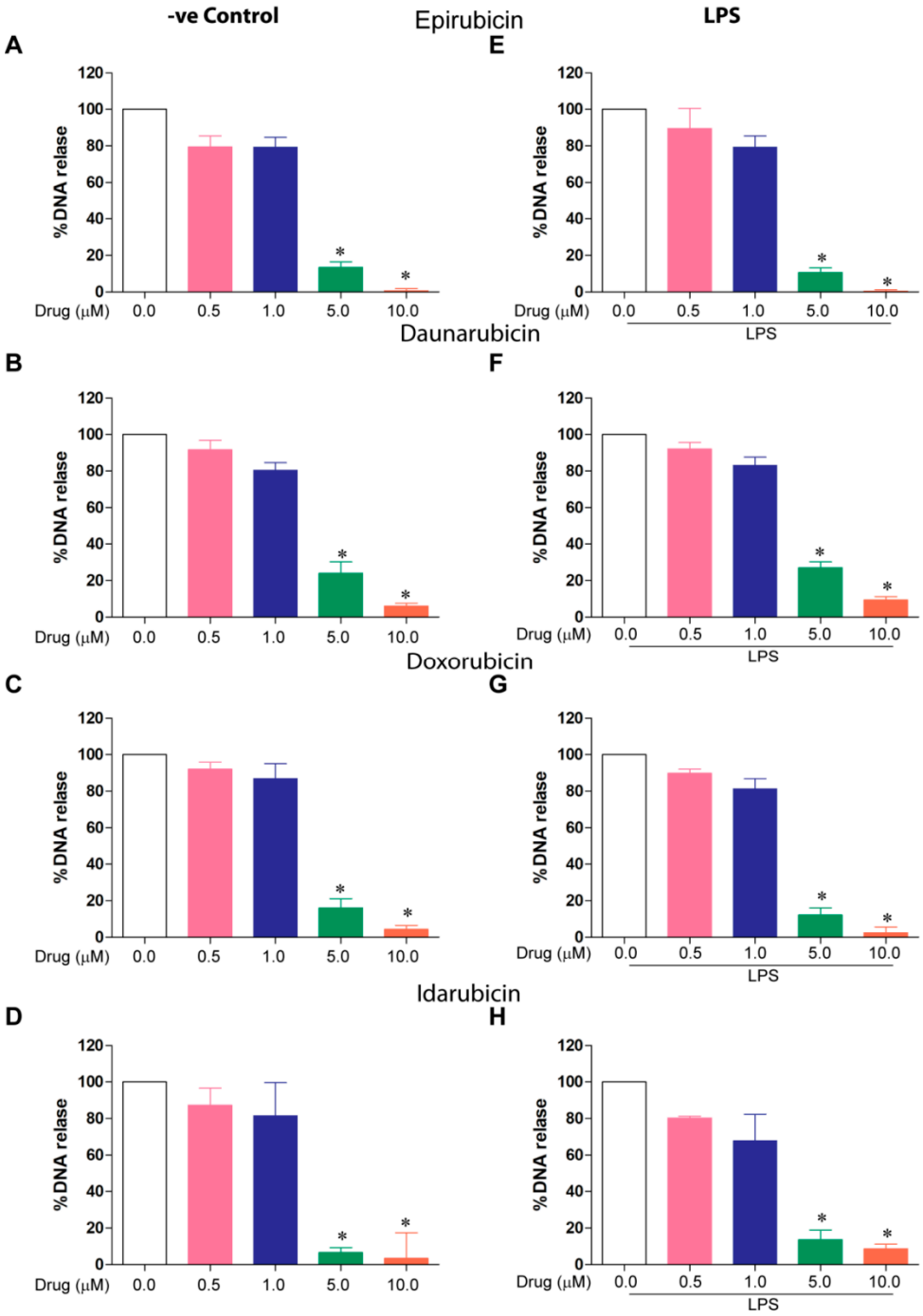

Figure S1. To clarify the inhibitory effect of anthracyclines in the NETosis data of base line (-ve control) and LPS with anthracyclines drug (at 240 min), were further normalized by baseline and LPS-mediated DNA release considering as 100% DNA release respectively. The bar graph shows the inhibitory effect of the drugs in baseline NETosis (**A**) Epirubicin, (**B**) Daunorubicin (**C**) Doxorubicin (**D**) Idarubicin and

in LPS mediated NETosis suppression by drugs (**E**) Epirubicin, (**F**) Daunorubicin (**G**) Doxorubicin (**H**) Idarubicin (* p-value < 0.05; One-sample t test, compares to hypothetical value 100).

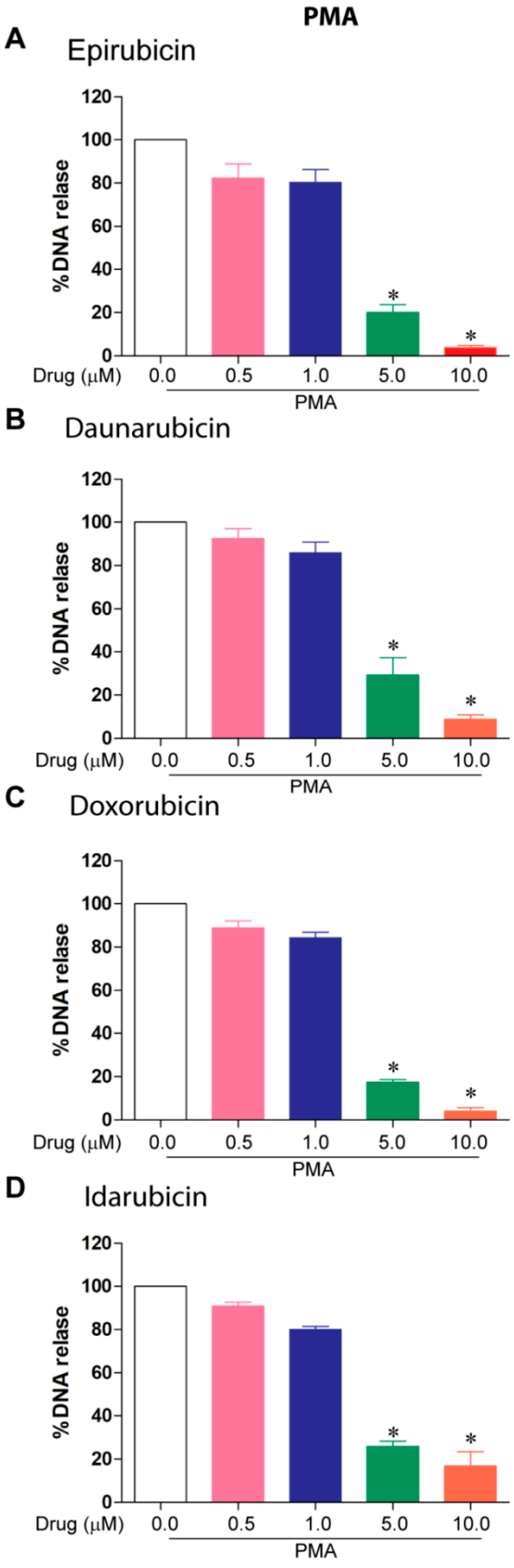

Figure S2. To clarify the anthracyclines' inhibitory effect in the NETosis data of PMA with anthracyclines drug (at 240 min; last time point), were further normalized by PMA-mediated DNA

release considering as 100% DNA release. The bar graph shows the inhibitory effect of the drugs in PMA mediated NETosis (**A**) Epirubicin, (**B**) Daunorubicin (**C**) Doxorubicin (**D**) Idarubicin (* $p < 0.05$; One-sample *t* test compared to hypothetical value 100).

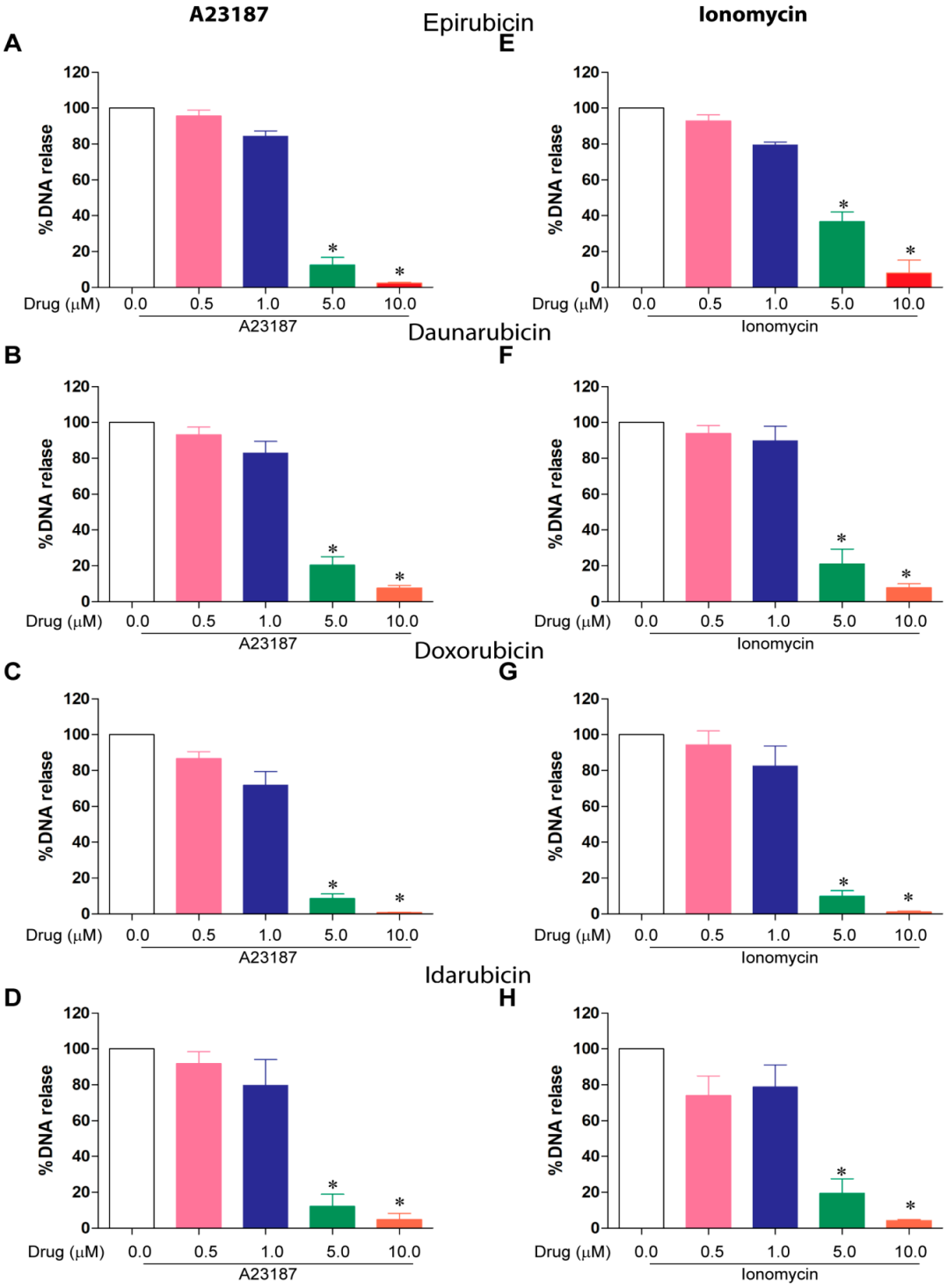

Figure S3. To clarify the anthracyclines' inhibitory effect in the NETosis data of A23187 and ionomycin with anthracyclines drug (at 240 min; last time point), were further normalized by A23187 and ionomycin-mediated DNA release considering as 100% DNA release respectively. The bar graph shows the inhibitory effect of the drugs in A23187-mediated NETosis (**A**) Epirubicin, (**B**) Daunorubicin (**C**) Doxorubicin (**D**) Idarubicin and in ionomycin mediated NETosis suppression by drugs (**E**) Epirubicin, (**F**) Daunorubicin (**G**) Doxorubicin (**H**) Idarubicin (* $p < 0.05$; One-sample *t* test, compared to hypothetical value 100).

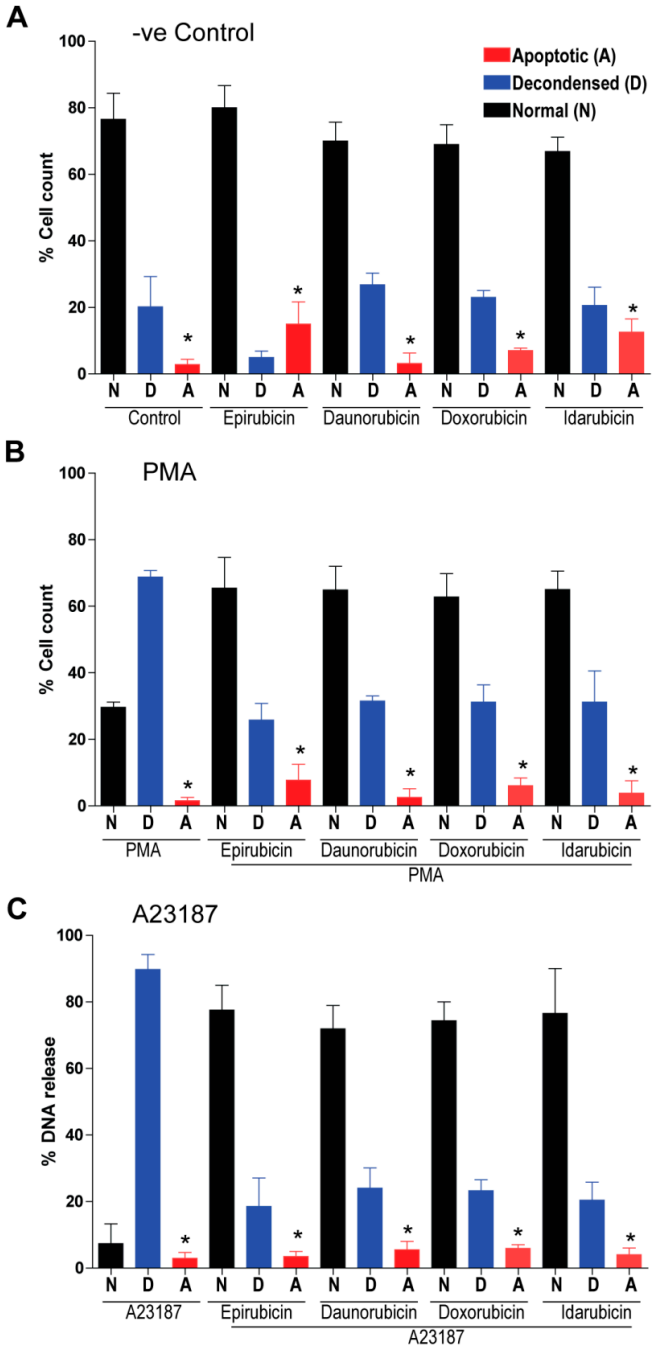

Figure S4. Anthracycline-mediated NETosis suppression does not induce apoptosis. Neutrophils were incubated with anthracyclines and then induced to produce NETs using either media only (-ve control), PMA (25 nM) or A23187 (4 μM) and then fixed by 4% paraformaldehyde in 8-chamber slides. cCasp-3 and DNA were stained with anti-cleaved cCasp-3 (red) and DAPI (blue). Based on the immunostaining and colocalization, cell and NETs counting data, show significantly reduced apoptosis in agonists and drug conditions compared to media control (n = 3, * p-value < 0.05; One-way ANOVA with Tukey's post-test compared to apoptotic cells).