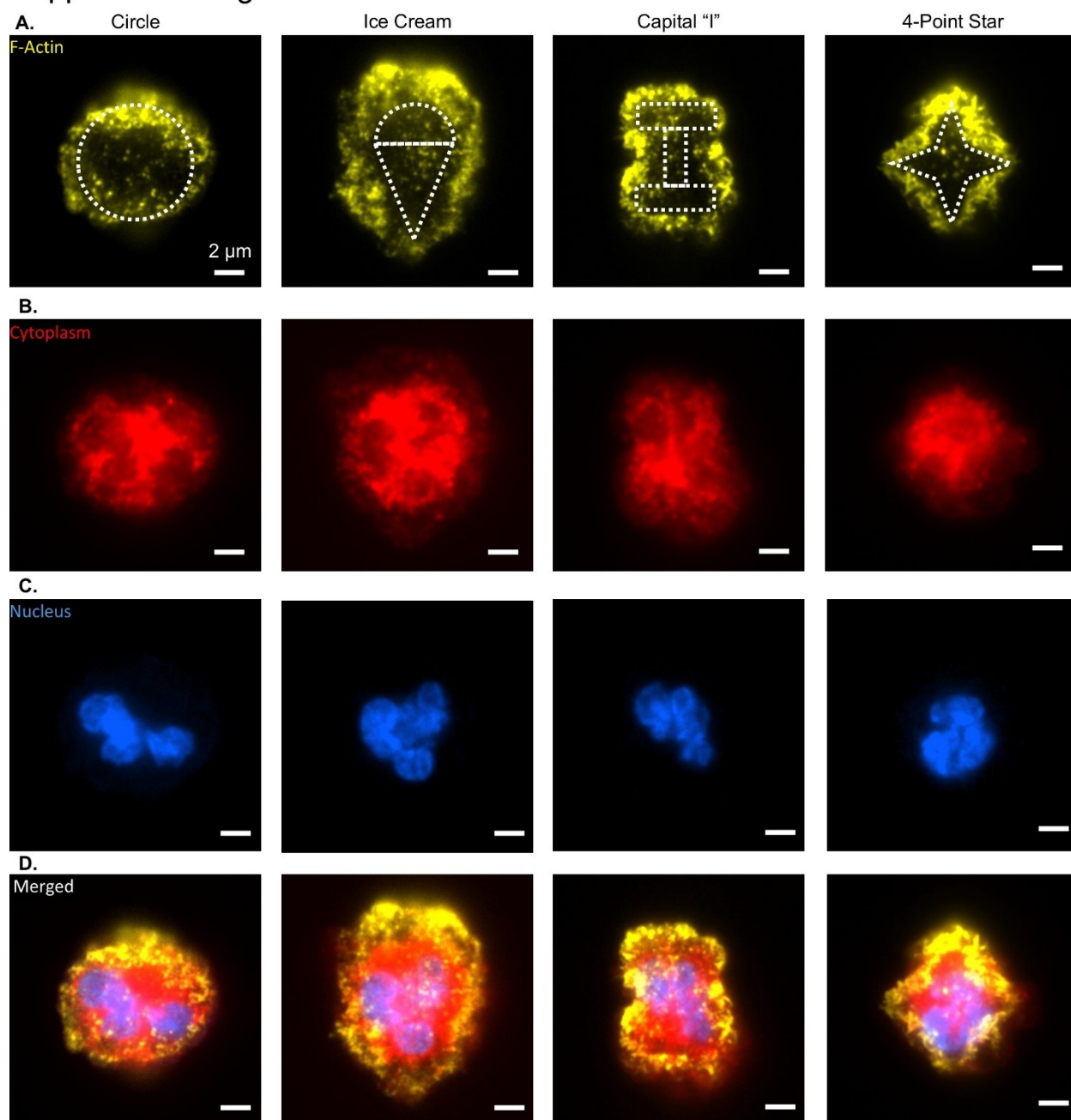
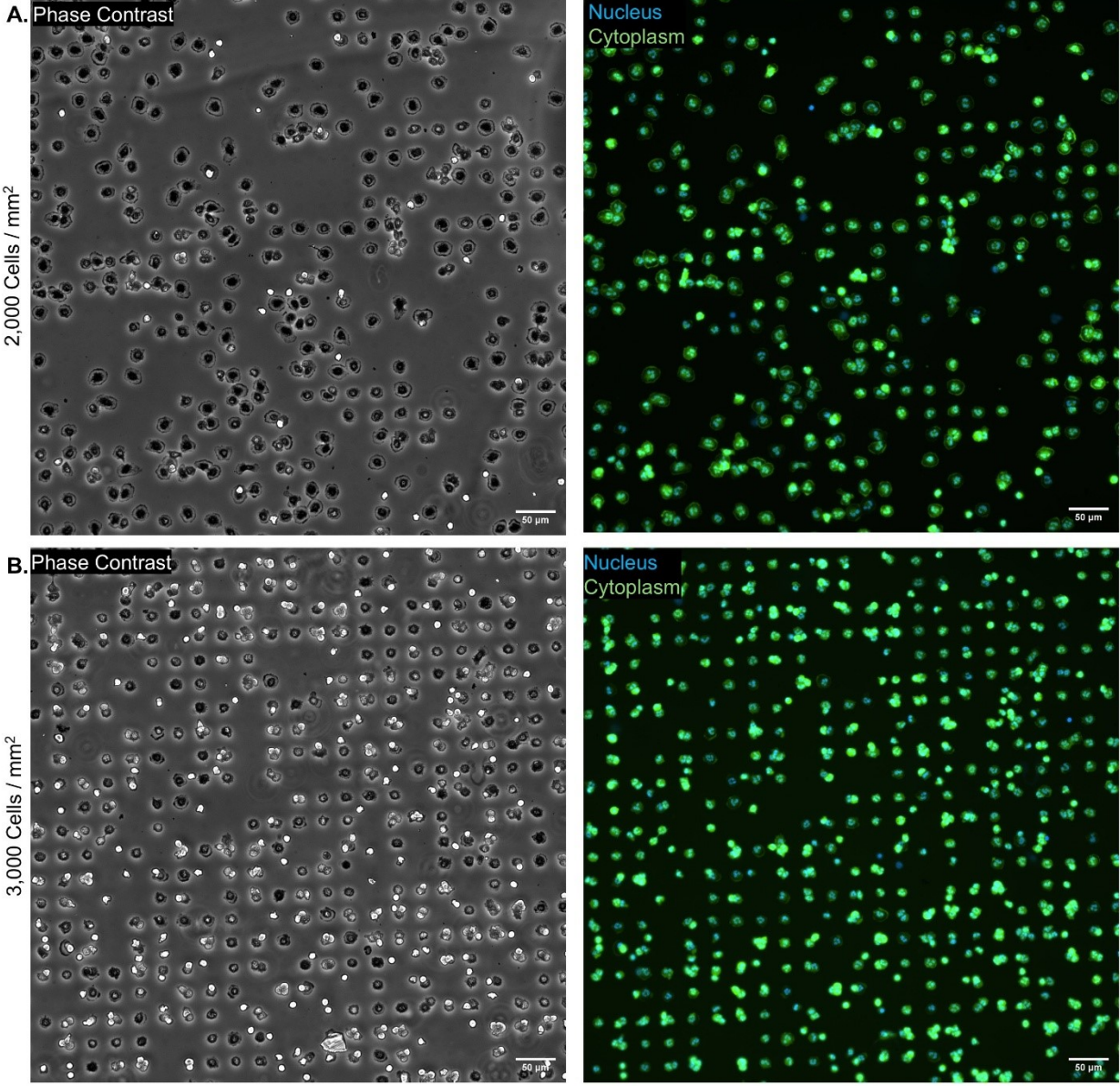


### Supplemental Figure 1



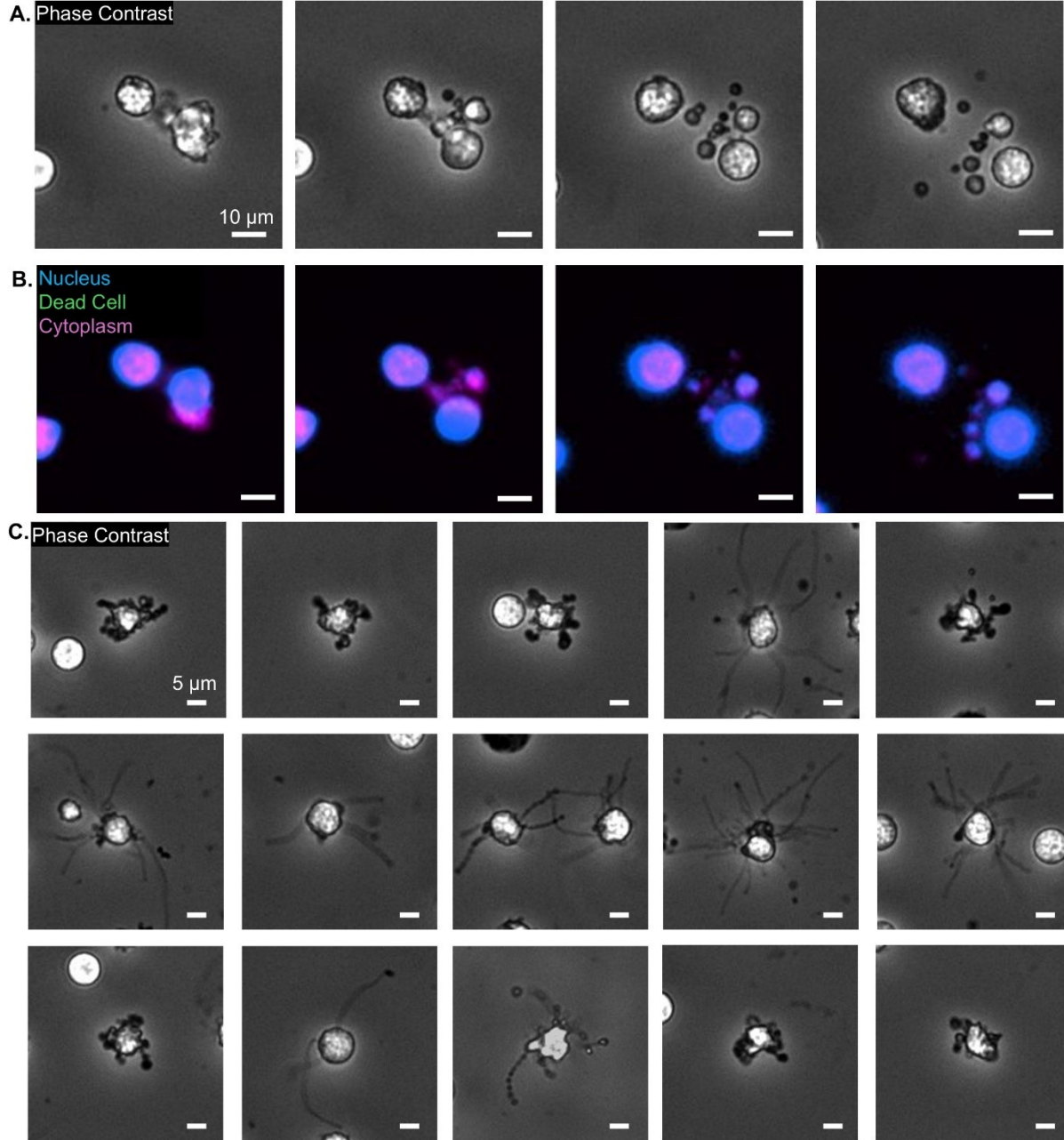
Supplemental Figure 1. Total internal reflective fluorescence microscopy images of (A) F-Actin, (B) cytoplasm, (C) nucleus and (D) merged staining of neutrophils around the circle, ice cream, capital I and 4-point star patterns (left to right, scale bar = 2 $\mu\text{m}$ ).

Supplemental Figure 2



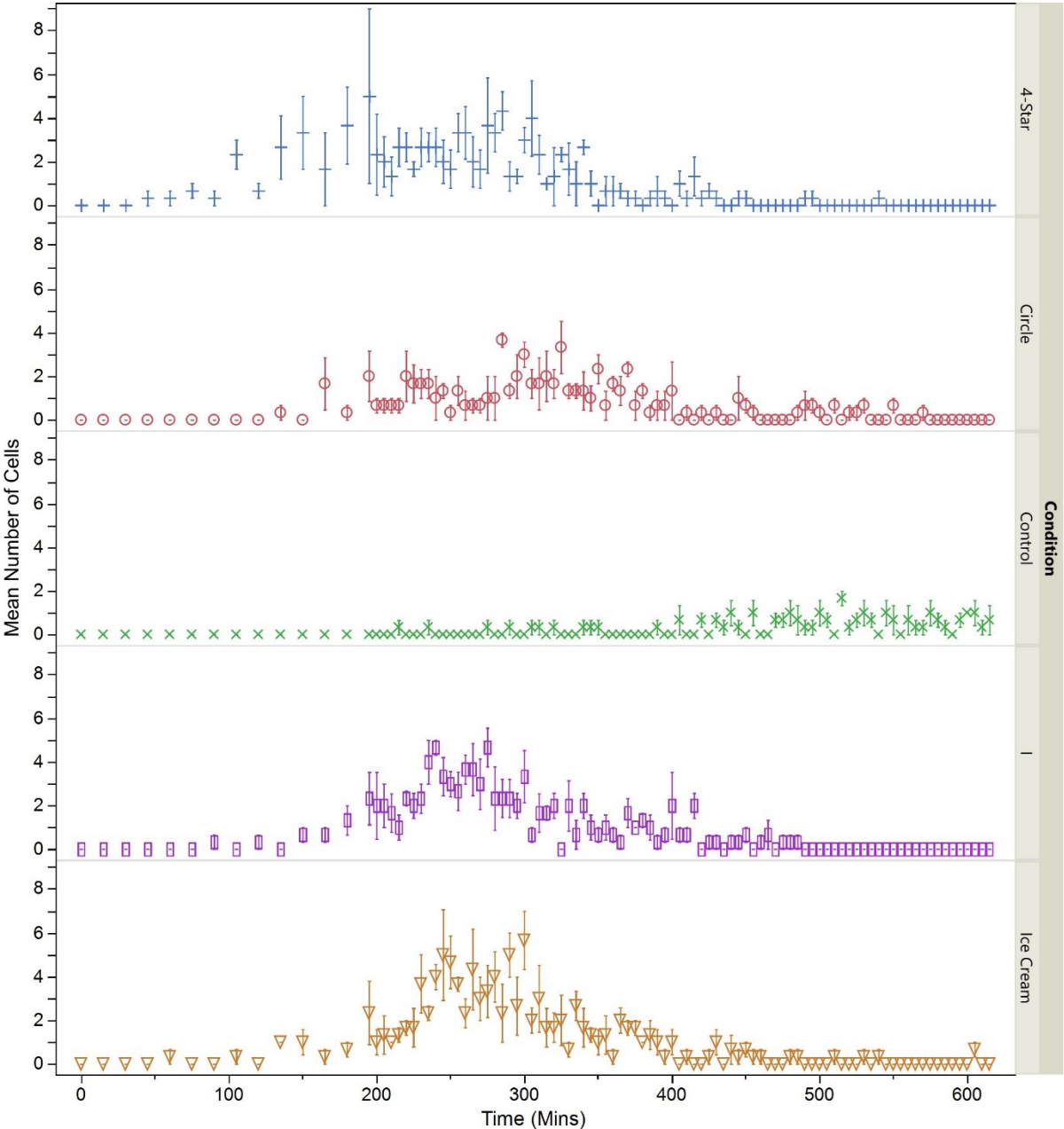
Supplemental Figure 2. Phase contrast image (left) and Nucleus (blue) + Cytoplasm (green) (right) for (A) 2,000 Cells / mm<sup>2</sup> and (B) 3,000 Cells / mm<sup>2</sup> (scale bar = 50μm).

Supplemental Figure 3



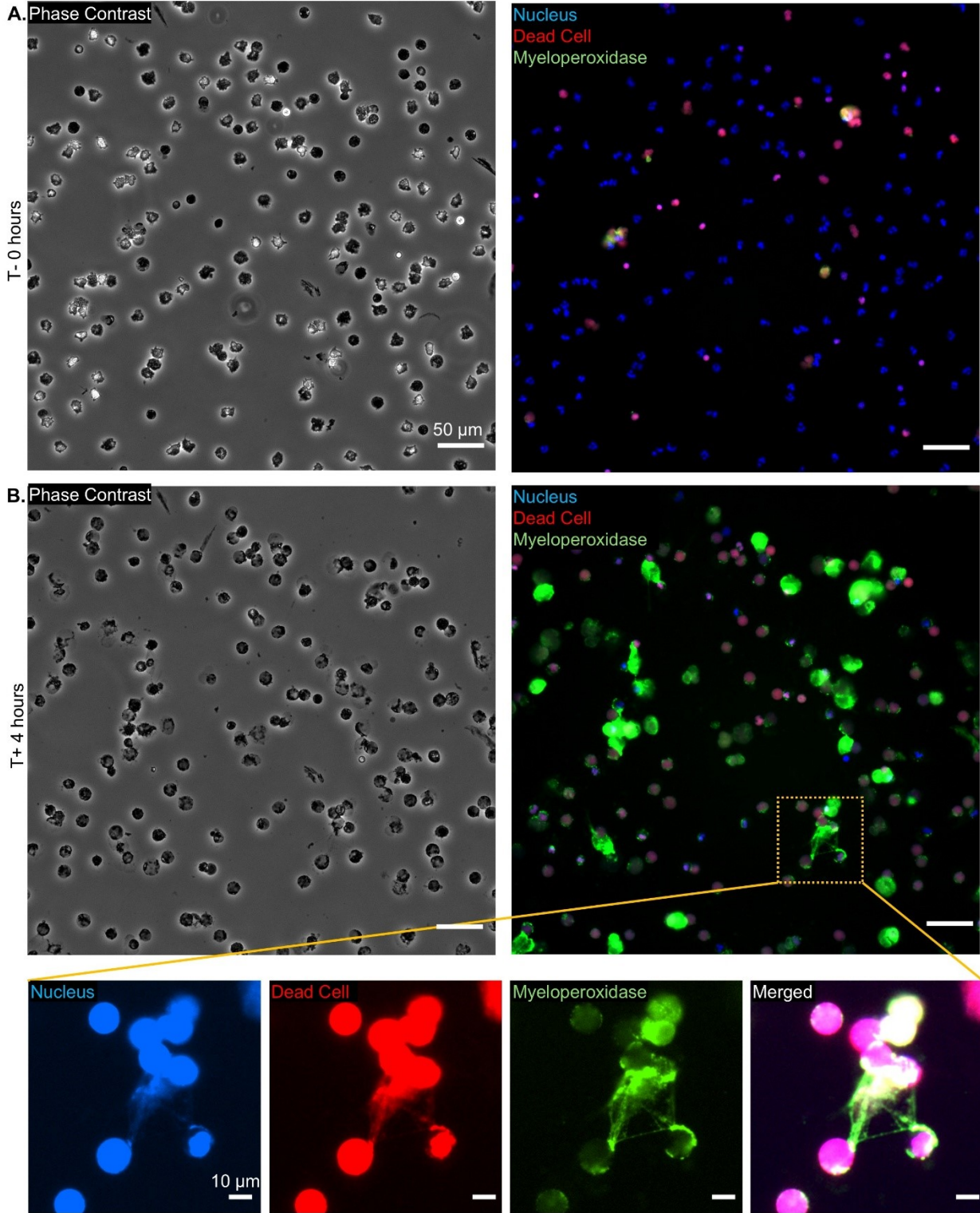
Supplemental Figure 3. (A) Phase contrast and (B) nucleus (blue), dead cell (green) and cytoplasm (purple) staining (scale bar = 10μm). (C) Vital NETosis heterogeneity phase contrast (scale bar = 5μm).

Supplemental Figure 4



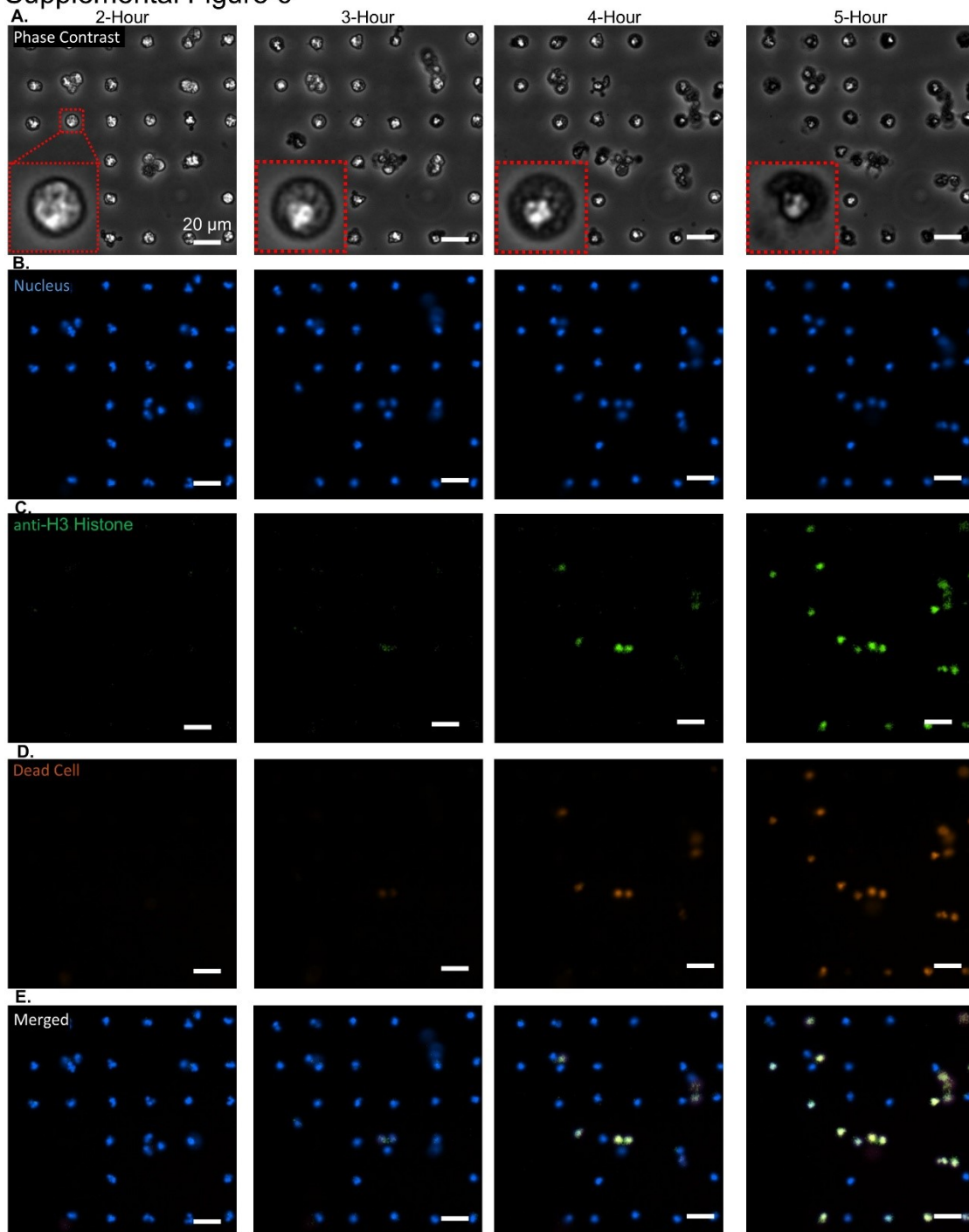
Supplemental Figure 4. (A) Individual cell time to NETosis graphs for 4-point star, circle, control, capital “I” and ice cream (top to bottom).

Supplemental Figure 5



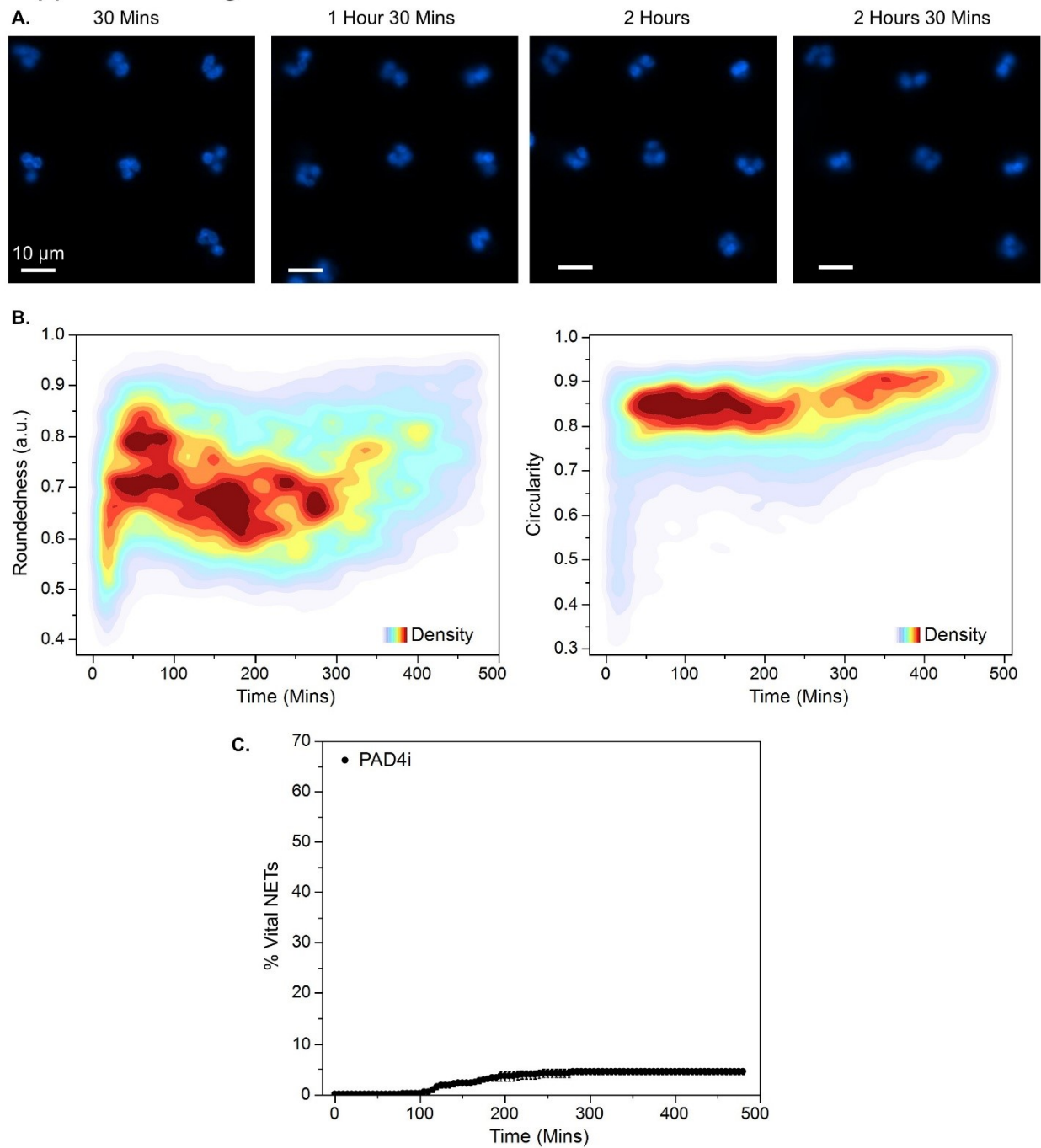
Supplemental Figure 5. Phase contrast (left) and nucleus (blue), dead cell (pink) and myeloperoxidase (green, right) at (A) time equal 0 hours and (B) time equal 4 hours post 4nM PMA stimulation (scale bars = 50µm) with subset showcasing a group of cells with delineated NETs (scale bars = 10µm).

## Supplemental Figure 6



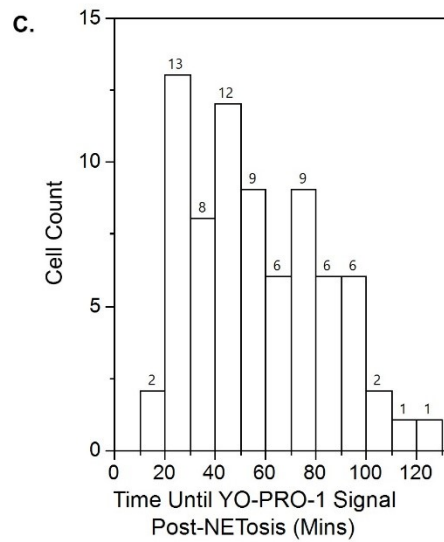
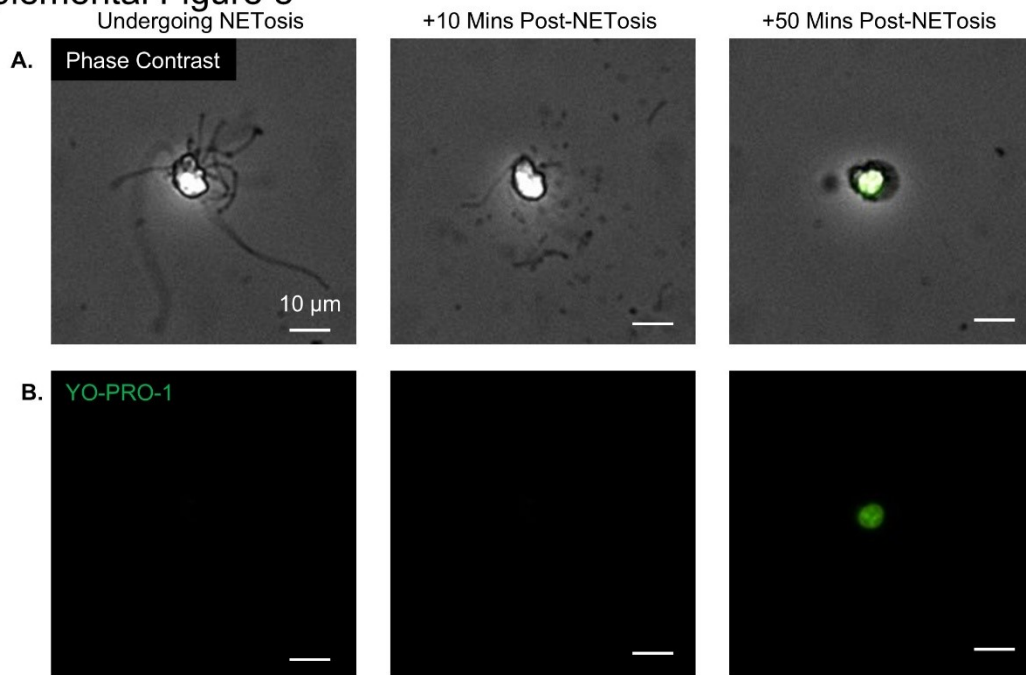
Supplemental Figure 6. (A) Phase contrast, (B) nucleus, (C) anti-H3 histone, (D) dead cells and (E) merged images of neutrophils at 2-, 3-, 4- and 5-hour post 4nM PMA stimulation (left to right, scale bar = 20μm).

## Supplemental Figure 7



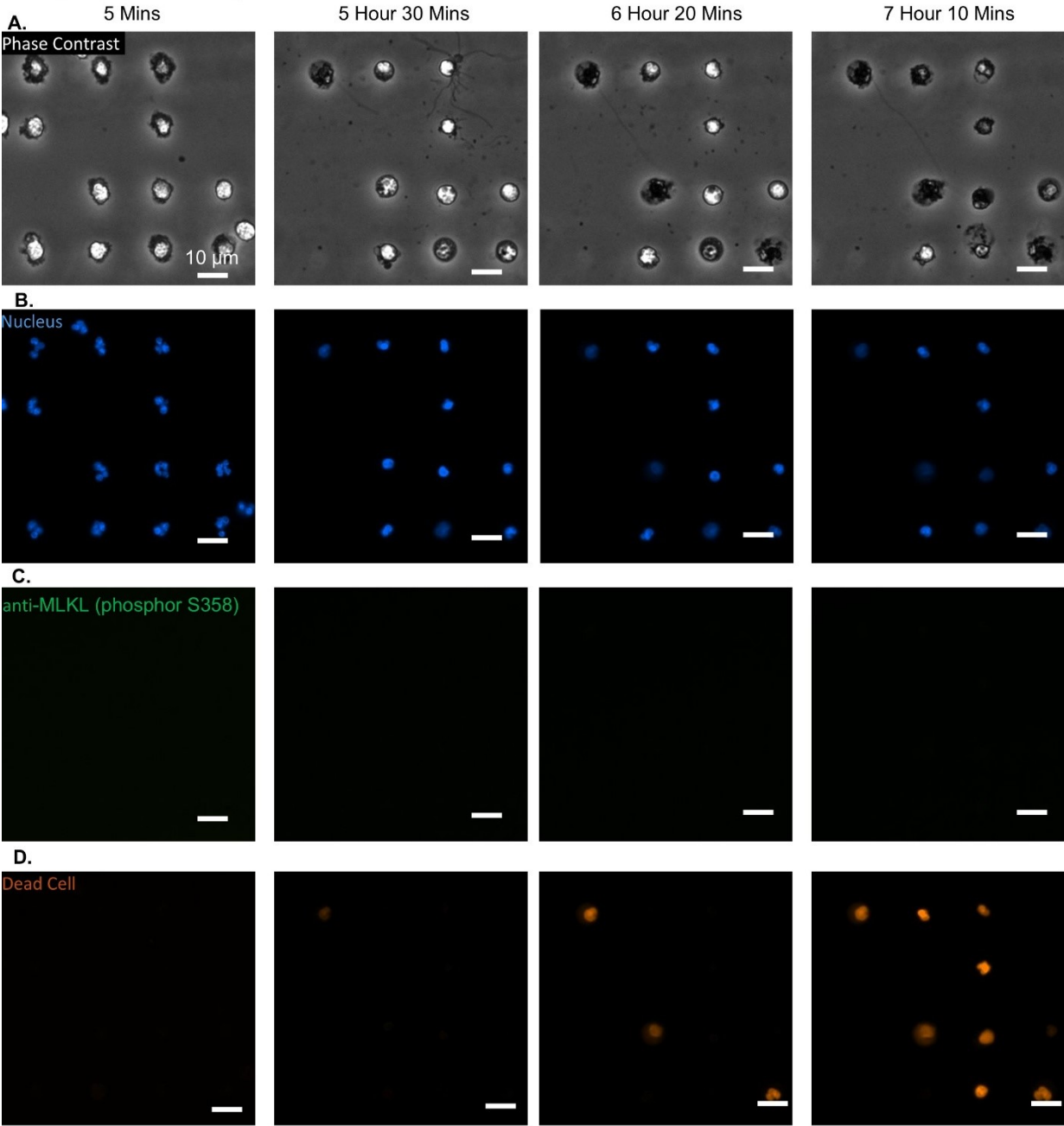
Supplemental Figure 7. (A) Nucleus stain during PAD4 inhibition (10 $\mu$ M GSK484, scale bars = 10 $\mu$ m). (B) Nuclear roundedness (left) and circularity (right) during PAD4 inhibition. (C) Percent vital NETosis during PAD4 inhibition.

# Supplemental Figure 8



Supplemental Figure 8. (A) Phase contrast and (B) YO-PRO-1 staining identifying vital NETosis and delayed onset of YO-PRO-1 staining (scale bars = 10 $\mu$ m). (C) Cell counts for time until YO-PRO-1 signal post vital NETosis.

Supplemental Figure 9



Supplemental Figure 9. (A) Phase contrast, (B) nucleus, (C) anti MLKL (phosphor S358) and (D) dead cell stains at 5 mins, 5 hour 30 mins, 6 hour 20 mins and 7 hour 10 mins (left to right, scale bars = 10µm)