Supplementary Figures

Short-Term Pulmonary Toxicity Assessment of Pre- and Post-Incinerated Organomodified Nanoclay in Mice

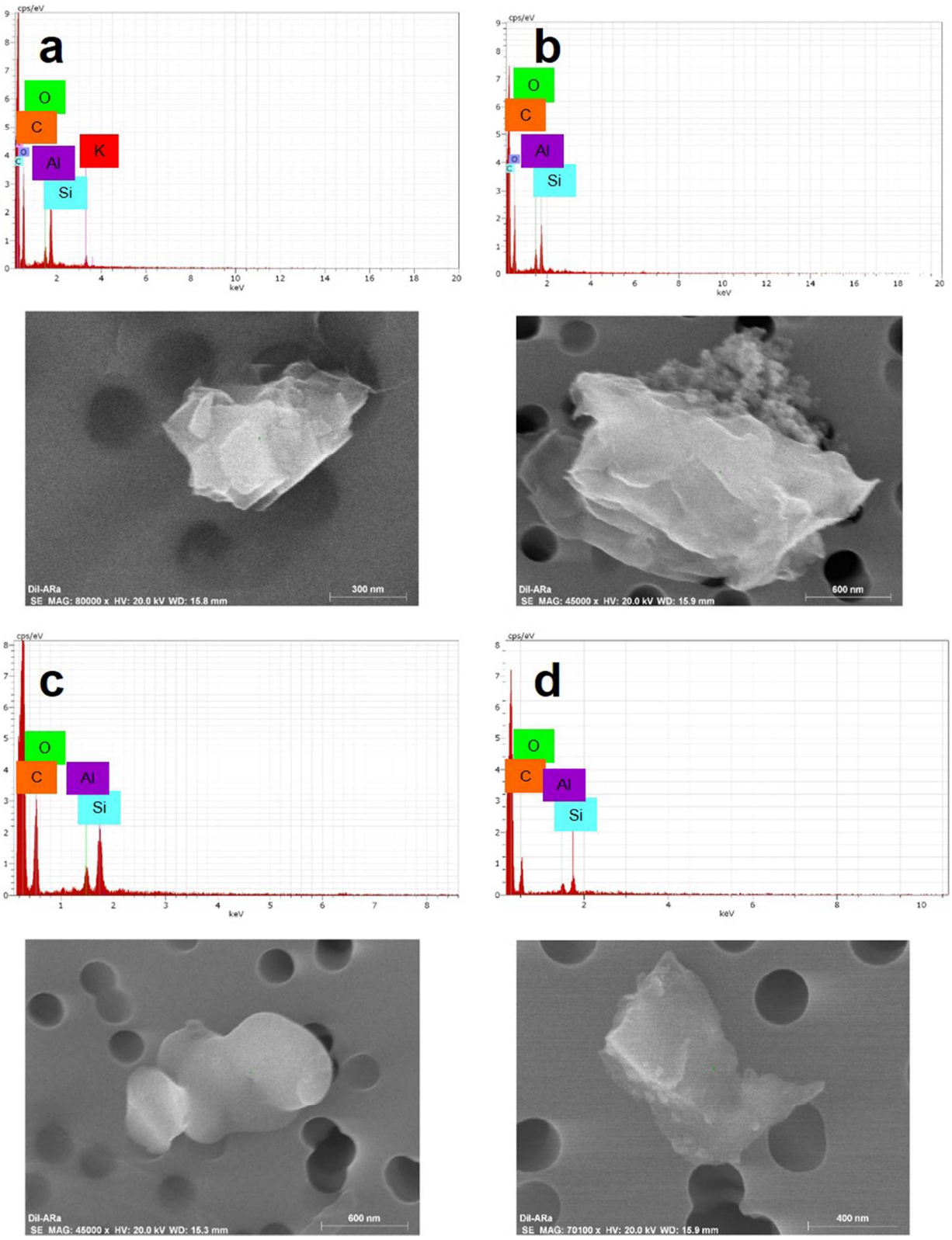
Todd A. Stueckle[1], Donna C. Davidson[1], Ray Derk[1], Tiffany G. Kornberg[1,2], Lori Battelli[1], Sherri Friend[1], Marlene Orandle[1], Alixandra Wagner[3], Cerasela Zoica Dinu[3], Konstantinos A. Sierros[4], Sushant Agarwal[3], Rakesh K. Gupta[3], Yon Rojanasakul[2], Dale W. Porter[1], Liying Rojansakul[1]*

[1] Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown WV, 26505

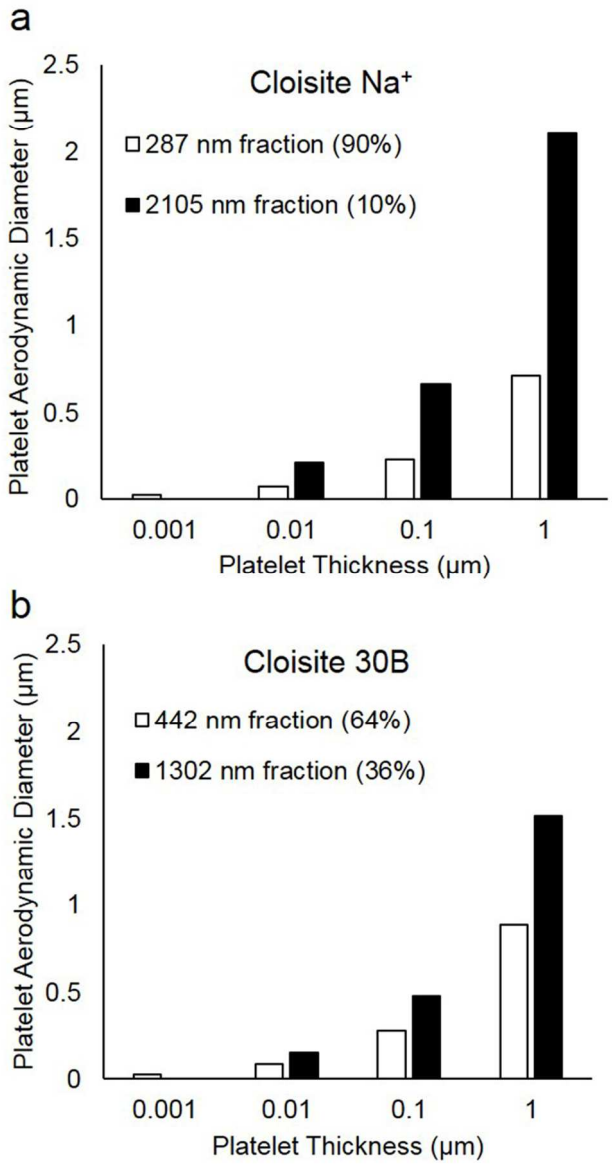
[2] Department of Basic Pharmaceutical Sciences, West Virginia University, Morgantown, WV 26506

[3] Department of Chemical and Biomedical Engineering, West Virginia University, Morgantown, WV, 26506

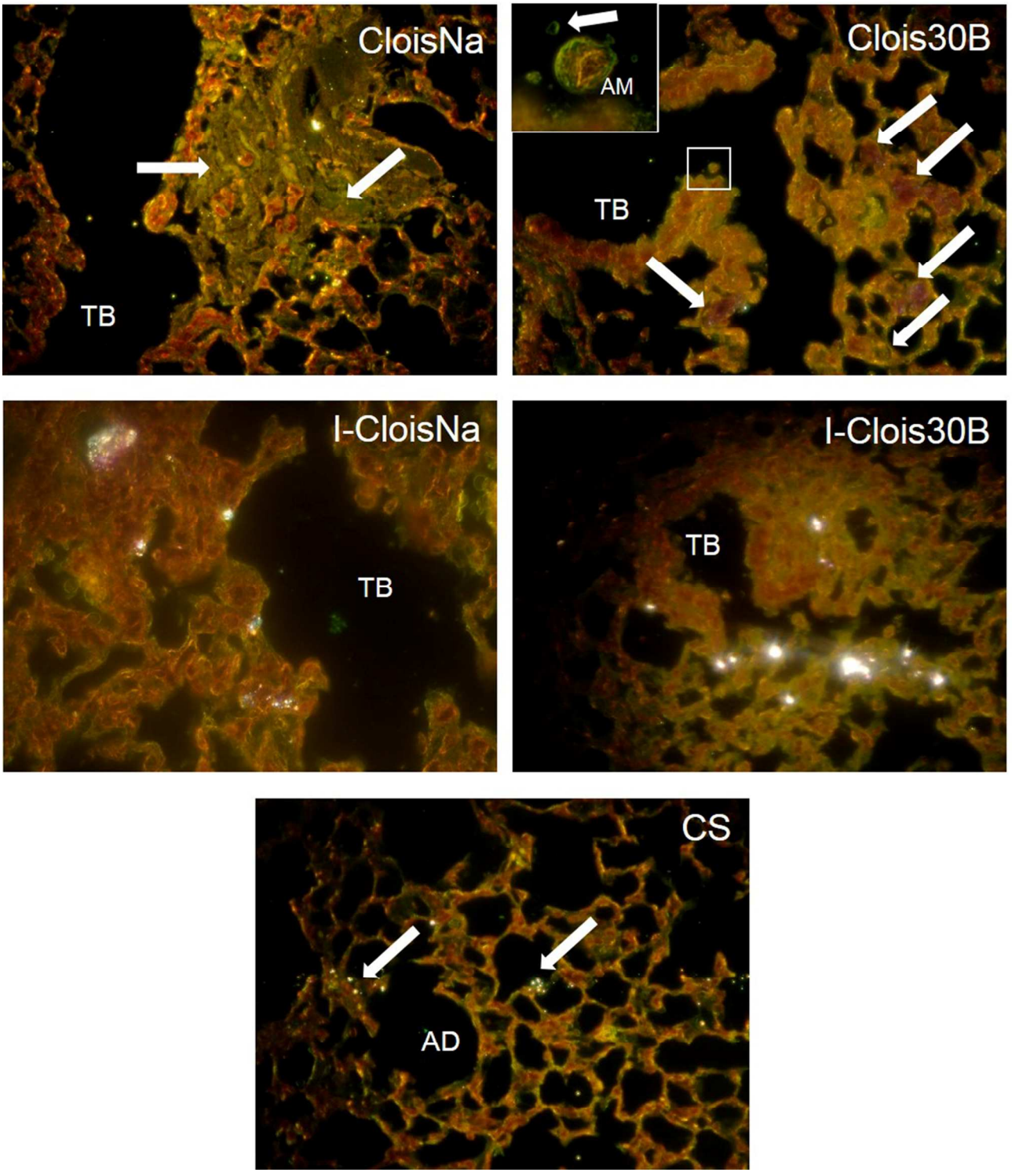
[4] Department of Mechanical and Aerospace Engineering, West Virginia University, Morgantown, WV 26506



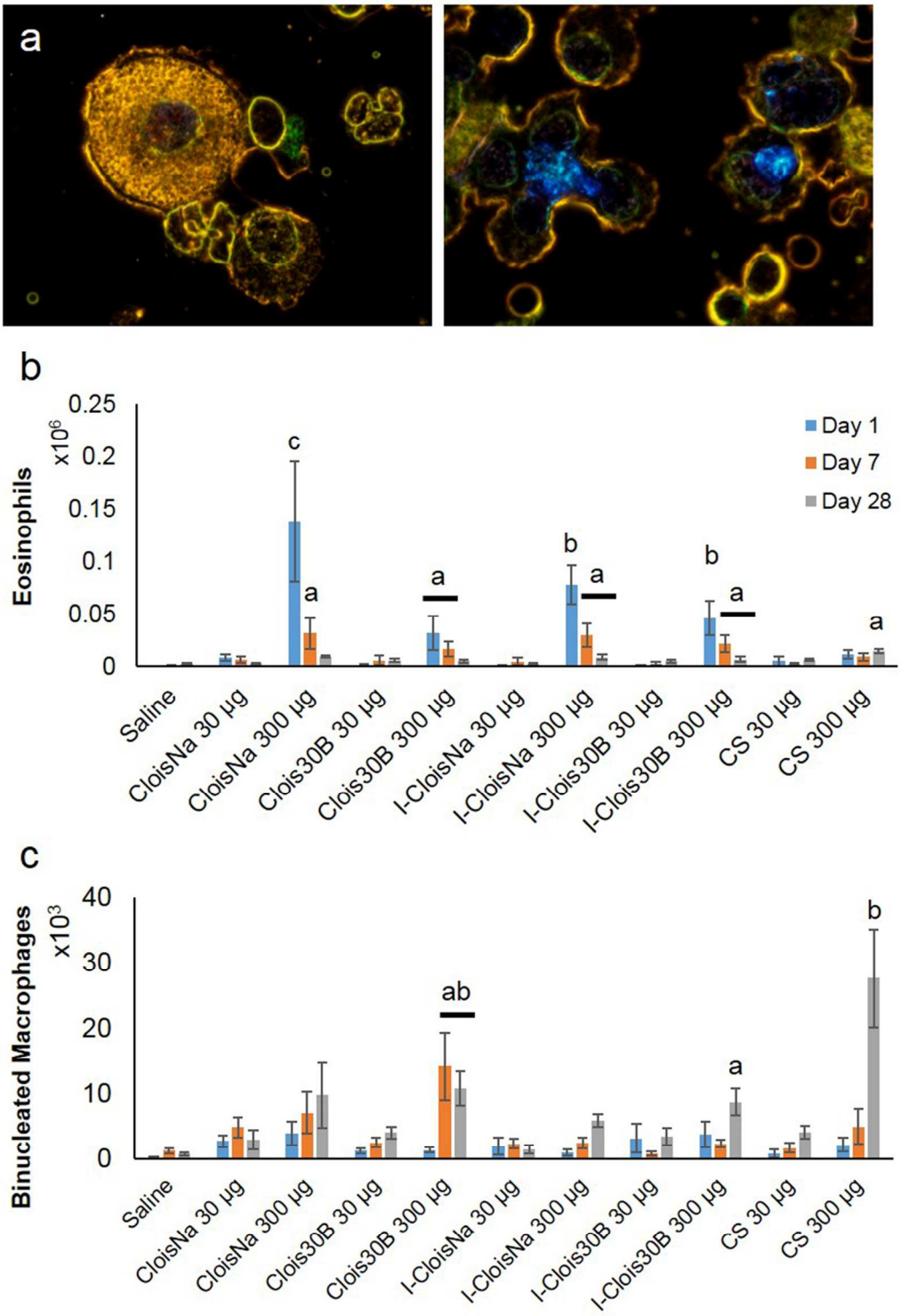
Supplemental Figure 1. EDX analysis of dispersed pre- and post-incinerated organomodified nanoclays. A) CloisNa, b) Clois30B, c) I-CloisNa, and d) I-Clois30B.



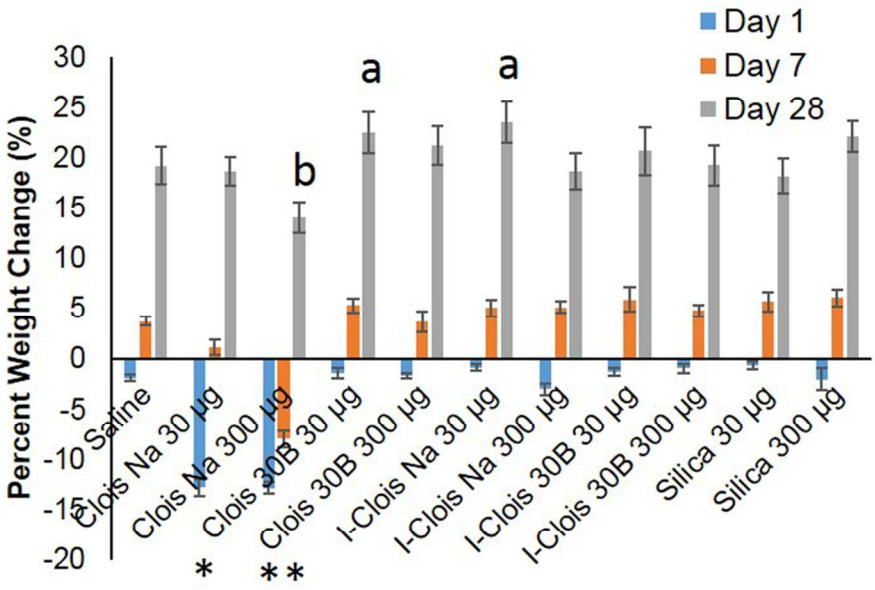
Supplemental Figure 2. Modeled estimates of nanoclay platelet aerodynamic diameter for a) CloisNa and b) Clois30B based on platelet thickness and particle density. Based on [44]. Dispersed particulate in vehicle showed bimodal size fraction for each particle. DLS measurements provided data for projected particle diameter and percent fraction (in parentheses).



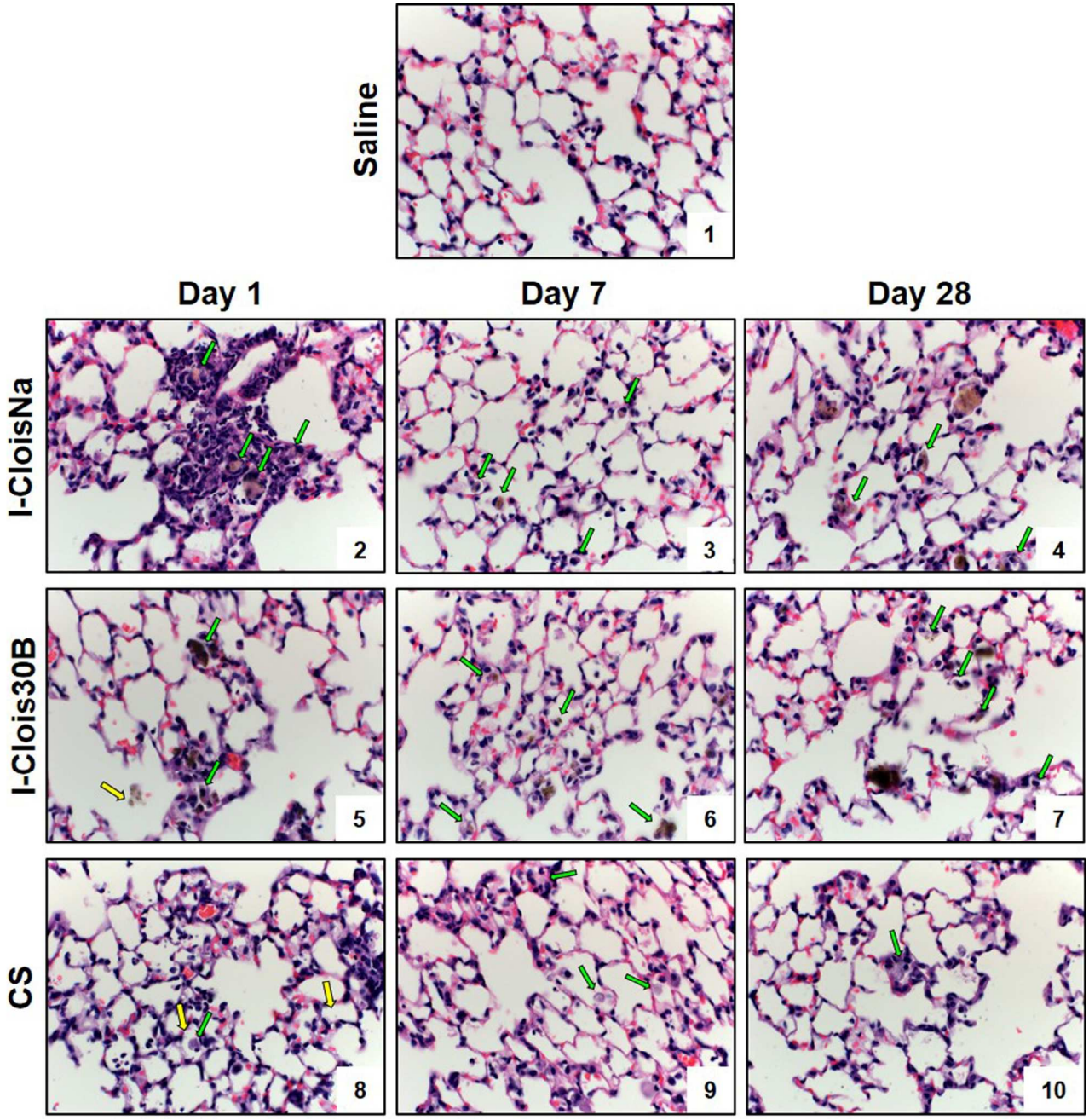
Supplemental Figure 3. Enhanced darkfield microscopy of deposited pre- and post-incinerated nanoclay particles at Day 1 post-exposure in mouse lung following oropharyngeal aspiration. Both CloisNa and Clois30B showed weak scattered light ability. Inset depicts nano-sized Clois30B particle (white arrow) next to an alveolar macrophage (AM). TB and AD indicate terminal bronchiole and alveolar duct, respectively. White arrows indicate areas of particle deposition. 200x magnification.



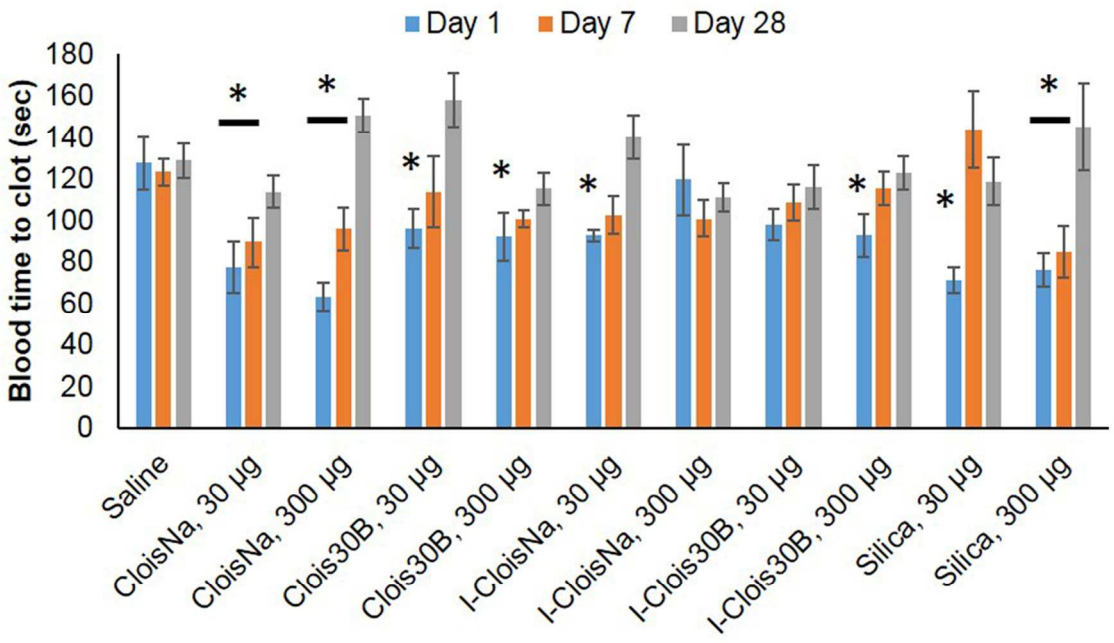
Supplemental Figure 4. Additional BALF characterization following oropharyngeal aspiration of pre- and post-incinerated organomodified nanoclay. a) Enhanced darkfield imaging of particle-laden macrophages and neutrophils for CloisNa- (left panel) and Clois30B-exposed (right panel) in BALF at Day 7 post-exposure. Dose-, particle-, and time-dependent b) lymphocyte infiltrate and c) bi-nucleated macrophage response following aspiration exposure. Different letters indicate those treatments significantly different from each other ($p<0.05$, $n=8$).



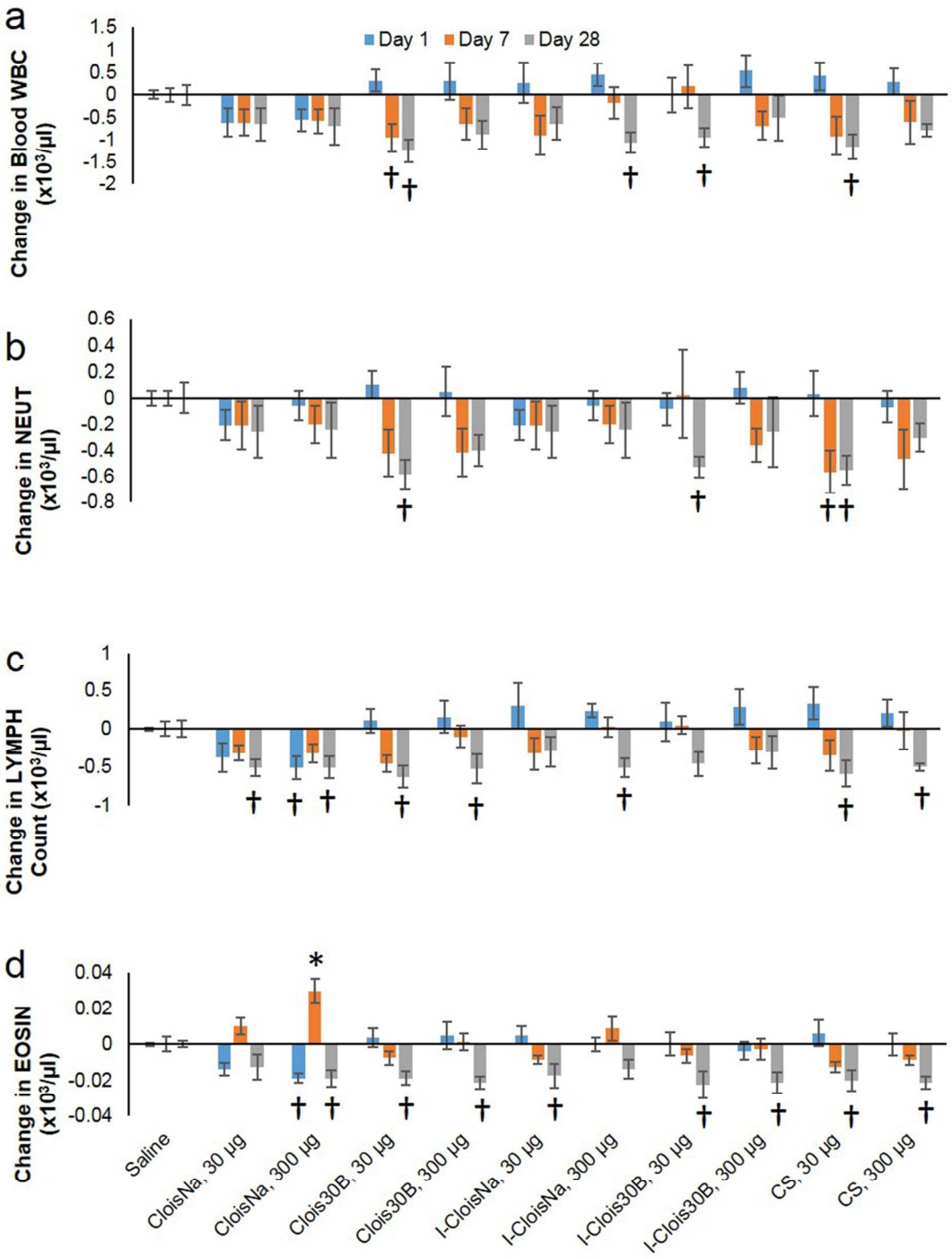
Supplemental Figure 5. Percent weight change following single oropharyngeal aspiration of pre- and post-incinerated organomodified nanoclay. * indicate a significant decrease in body weight from vehicle control. Letters indicate significant differences between vehicle control and other treatments at Day 28 post-exposure ($p<0.05$, $n=12$).



Supplemental Figure 6. Time course comparison of lung histopathology response following aspiration exposure to incinerated pristine (I-CloisNa) or incinerated organomodified nanoclay (I-Clois30B) vs. crystalline silica. I-CloisNa exposure resulted in a robust, transient inflammatory response while I-Clois30B exposure exhibited a minimal, but persistent inflammation with slow clearing particulate surrounded by alveolar macrophages, which compared to crystalline silica-exposed lung. 400X magnification. Yellow and green arrows indicate either free or macrophage engulfed particles, respectively.



Supplemental Figure 7. Differential blood clotting times following aspiration exposure to pre- and post-incinerated organomodified nanoclays. Decreased time to clot indicates platelet activation. * indicate a significant decrease compared to vehicle control ($p<0.05$, $n=12$).



Supplemental Figure 8. Systemic white blood cell differentials following aspiration exposure to pre- and post-incinerated organomodified nanoclays. a) Total white blood cell counts were different on Day 7 and 28 compared to Day 1 for vehicle and both CloisNa doses, while b) neutrophil counts showed a trend for dose-, time- and particle-dependent effects ($p = 0.056$). Letters indicate a significant increase compared to Day 1 animals. Both c) lymphocyte and d) eosinophil systemic counts exhibited significant decreases at Day 28 post-exposure across most treatments. * and † indicate a significant increase or decrease compared to vehicle control, respectively ($p<0.05$, $n=12$).