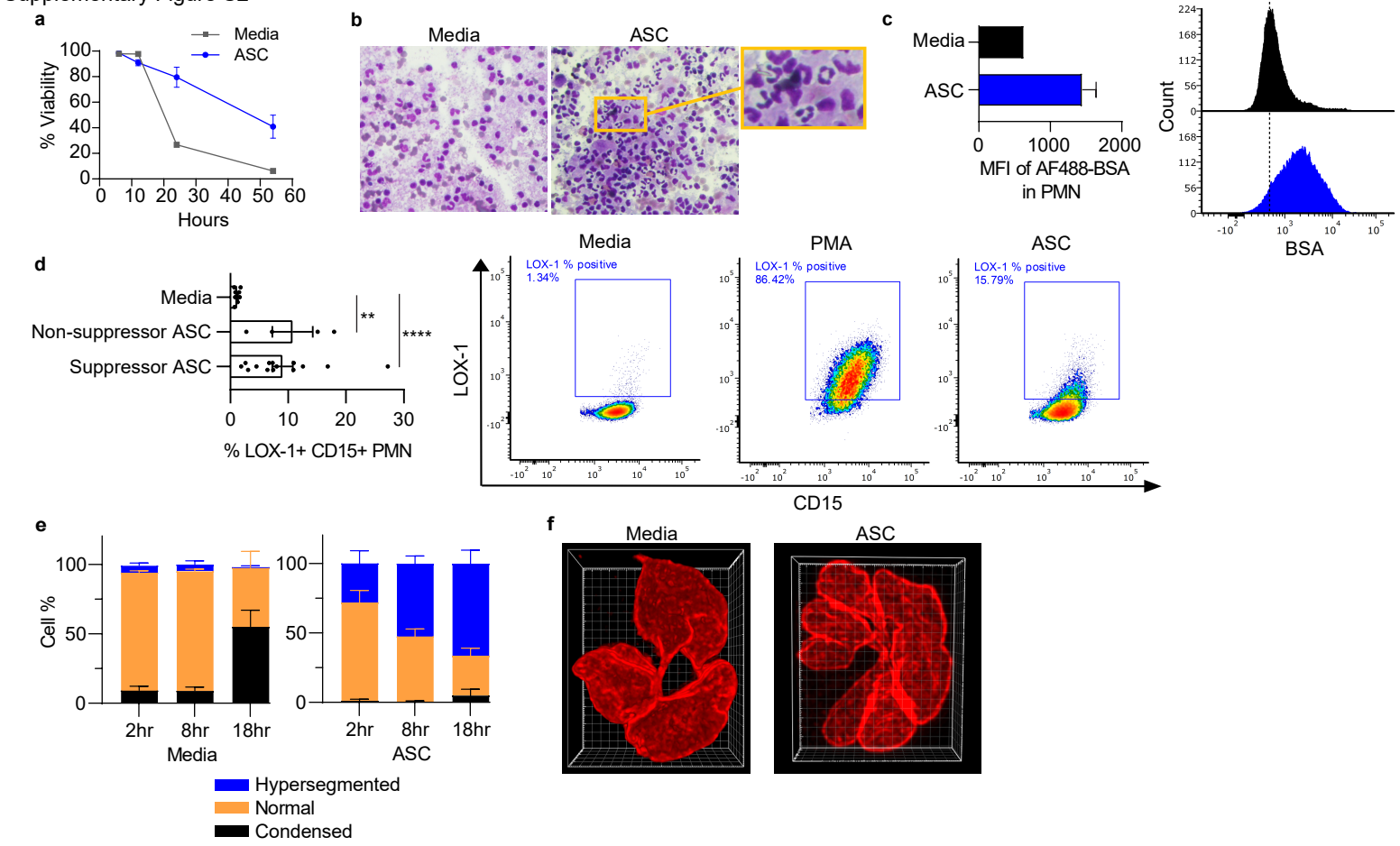
Supplementary Figure S2



Supplementary Figure S2: OC ascites fluid exposure increases neutrophil lifespan, increases the proportion of low-density neutrophils, increases LOX-1 expression, and induces neutrophil nuclear hypersegmentation. (**a**) Ascites fluid increased neutrophil lifespan. Normal donor neutrophils incubated in ASC (n=4) or media were assessed for viability by PI and annexin V staining at the indicated times. Mean neutrophil viability at 24hrs was 79.7% vs 26.7% following incubation in ASC or media, respectively. Data representative of two independent experiments and bars represent ± SEM. (**b**) ASC exposure increased the proportion of low-density neutrophils (LDN). Representative Wright-Giemsa stained slides show abundance of LDN in the PBMC monolayer following ASC exposure. Buffy coat cells from normal donor blood were incubated in ASC or media for 1hr, then subjected to gradient centrifugation. The PBMC monolayer consisted of 34.5% vs. 10.5% LDN following exposure to ASC or media, respectively. Data representative of two independent experiments. (**c**) ASC enhanced extracellular protein uptake. Neutrophils were incubated in ASC or media overnight with AF488-BSA. AF488-BSA uptake by cells was quantified by flow cytometry. Data representative of two independent experiments and bars represent ± SEM. Representative flow cytometry is shown. (**d**) Exposure to suppressor and non-suppressor ASC increased LOX-1 expression on neutrophils. Normal donor neutrophils were incubated in media, ASC (n=14 suppressors, n=4 non-suppressors), or 20nM PMA (positive control) for 1.5 hours, then analyzed by flow cytometry. Representative gating shows effects of media, PMA, and ASC on LOX-1 expression. Symbols represent individual samples (n) and bars represent ± SEM. Data were analyzed by Mann-Whitney (**p<0.01, ****p<0.0001). (**e**) ASC increased neutrophil nuclear hypersegmentation. Neutrophils were incubated in ASC or media for 18hrs, and nuclear morphology was scored as normal, hypersegmented (≥ 5 lobes), or condensed (characteristic of apoptosis). Data representative of three independent experiments and bars represent ± SEM. (**f**) Stimulated emission depletion (STED) images from super-resolution microscopy show representative nuclear hypersegmentation in ASC-activated neutrophils.