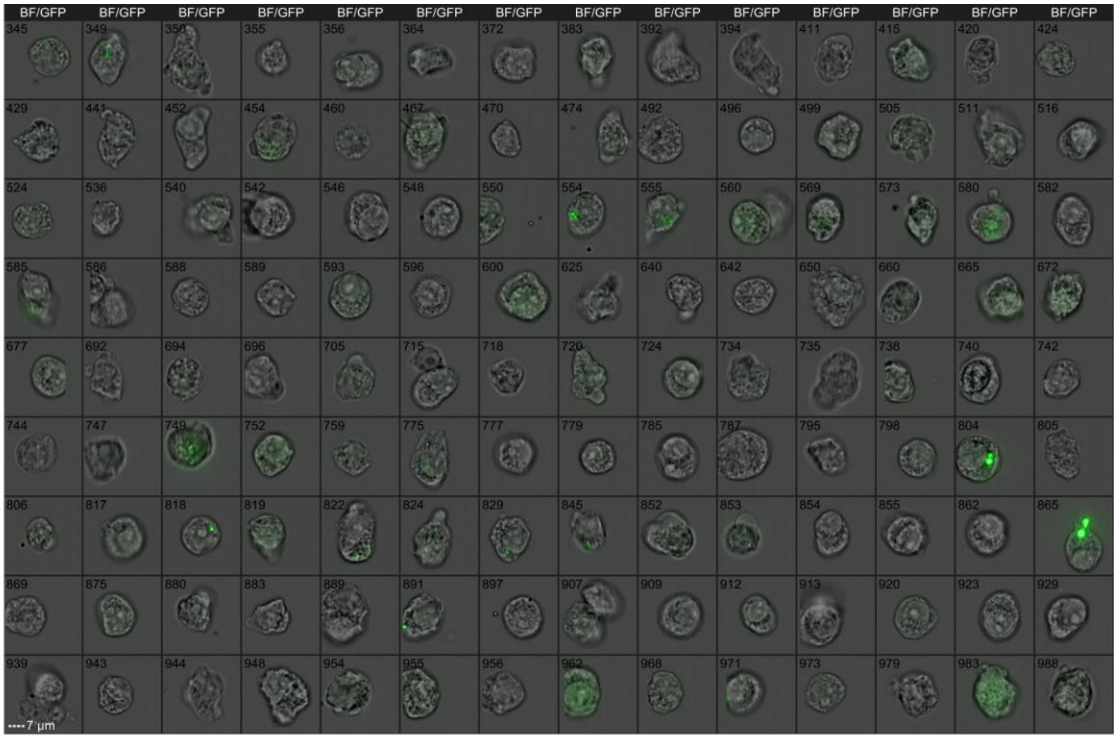

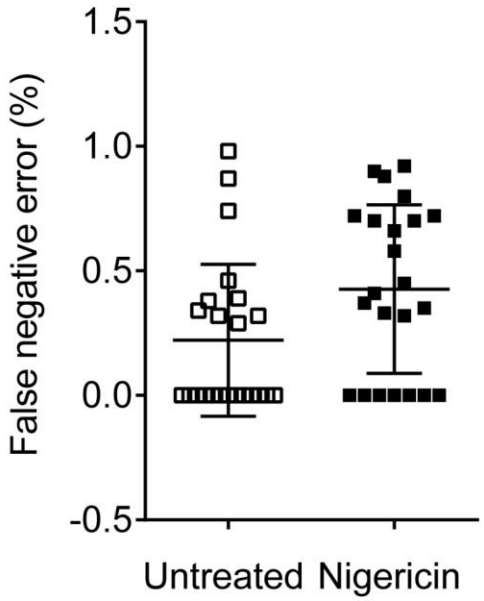

Figure S1

Figure S1: Calculation of false negative error for ICCE. Data from 23 groups of 1000 HEK293T cells were manually evaluated for presence of speck-like structures in speck negative population (Spot count=0). Representative images of single cells (n=126; top). Mean frequency of cells with speck-like structures is shown ± SEM (bottom).

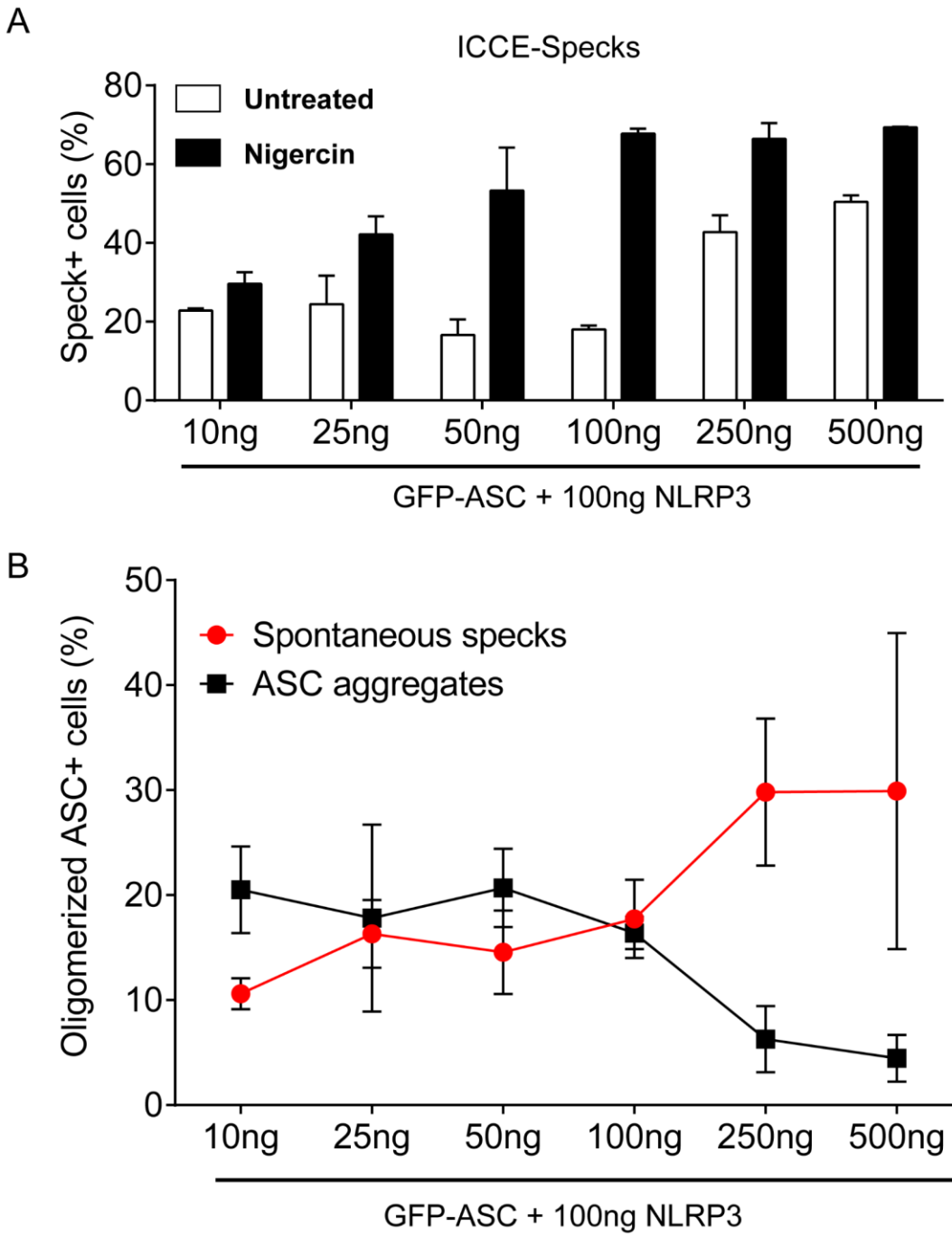

Figure S2

Figure S2: Correlation of increasing ASC concentration with spontaneous ASC speck formation and random ASC aggregates. HEK293T cells were transfected with 100ng NLRP3 and the indicated amounts of GFP-ASC and then stimulated with vehicle or 5μM nigericin. **(A)** Frequency of speck-positive cells (ICCE; ASC mask 14; spot count=1). **(B)** Sample described in A was analyzed for the presence of ASC aggregates (ICCE; ASC mask 8; black boxes) plotted with spontaneous specks (untreated from A; red circles). Mean ± SEM (n=2)

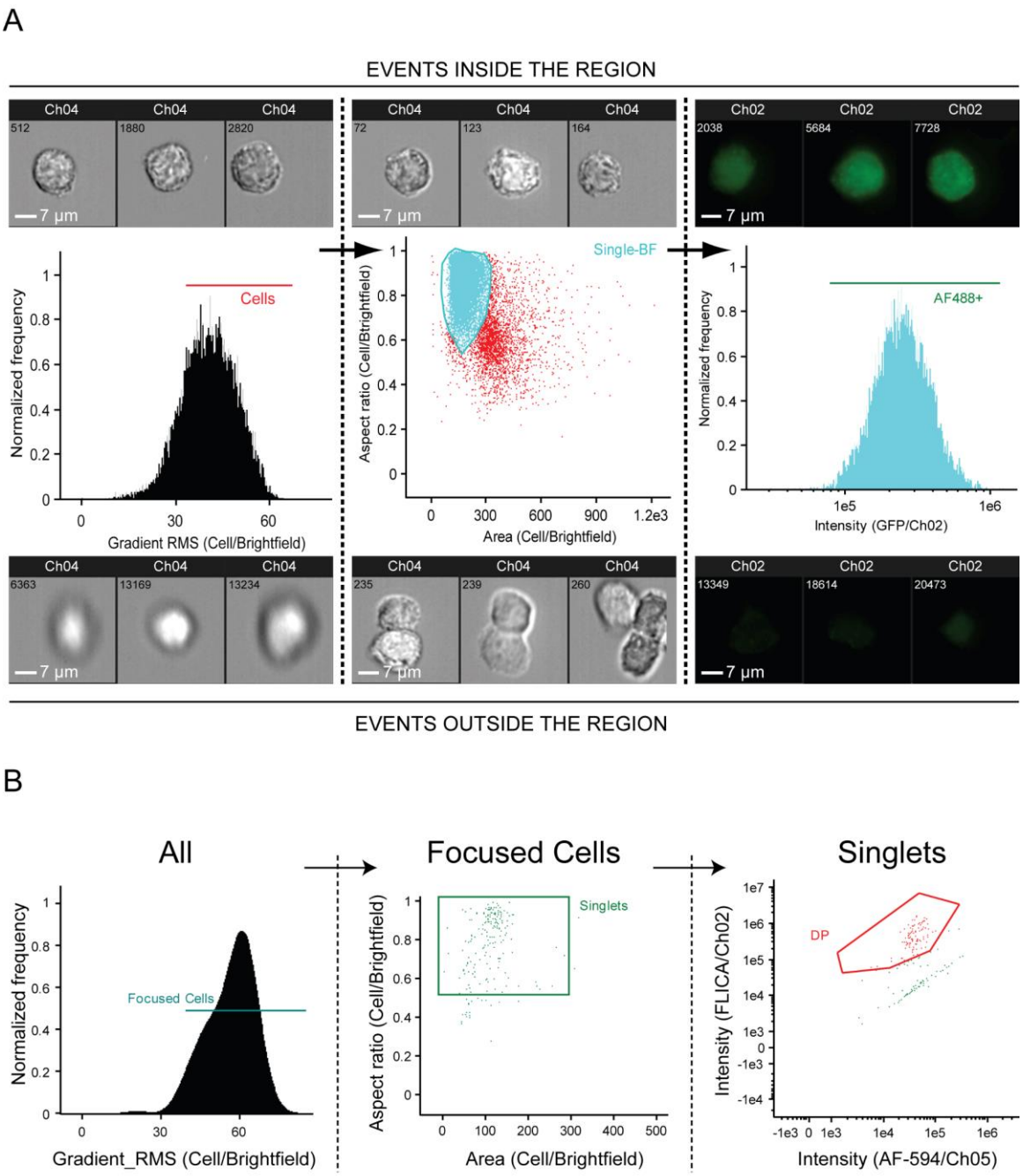

Figure S3

Figure S3: Gating strategy to identify ASC positive cells in THP1 and primary human monocytes. A. *For THP-1:* From left to right: histogram of Gradient_RMS of Focused cell mask with a region set to return focused cells (cells; red), dotplot of Area vs. the Aspect Ratio of the Focused cell mask with a region set to exclude non-single cell events (singlets; cyan), histogram showing intensity of Alexa Fluor 488 staining with the region set to exclude dim events (AF488+; green). **B.** *For primary human monocytes:* From left to right: histogram of Gradient_RMS of Focused cell mask with a region set to return focused cells (cells; blue), dotplot of Area vs. the Aspect Ratio of the Focused cell mask with a region set to exclude non-single cell events (singlets; green), dotplot showing intensity of FAM-FLICA vs Alexa Fluor 594 staining with the region set to select cells positive for ASC staining and active caspase-1 (DP; red).

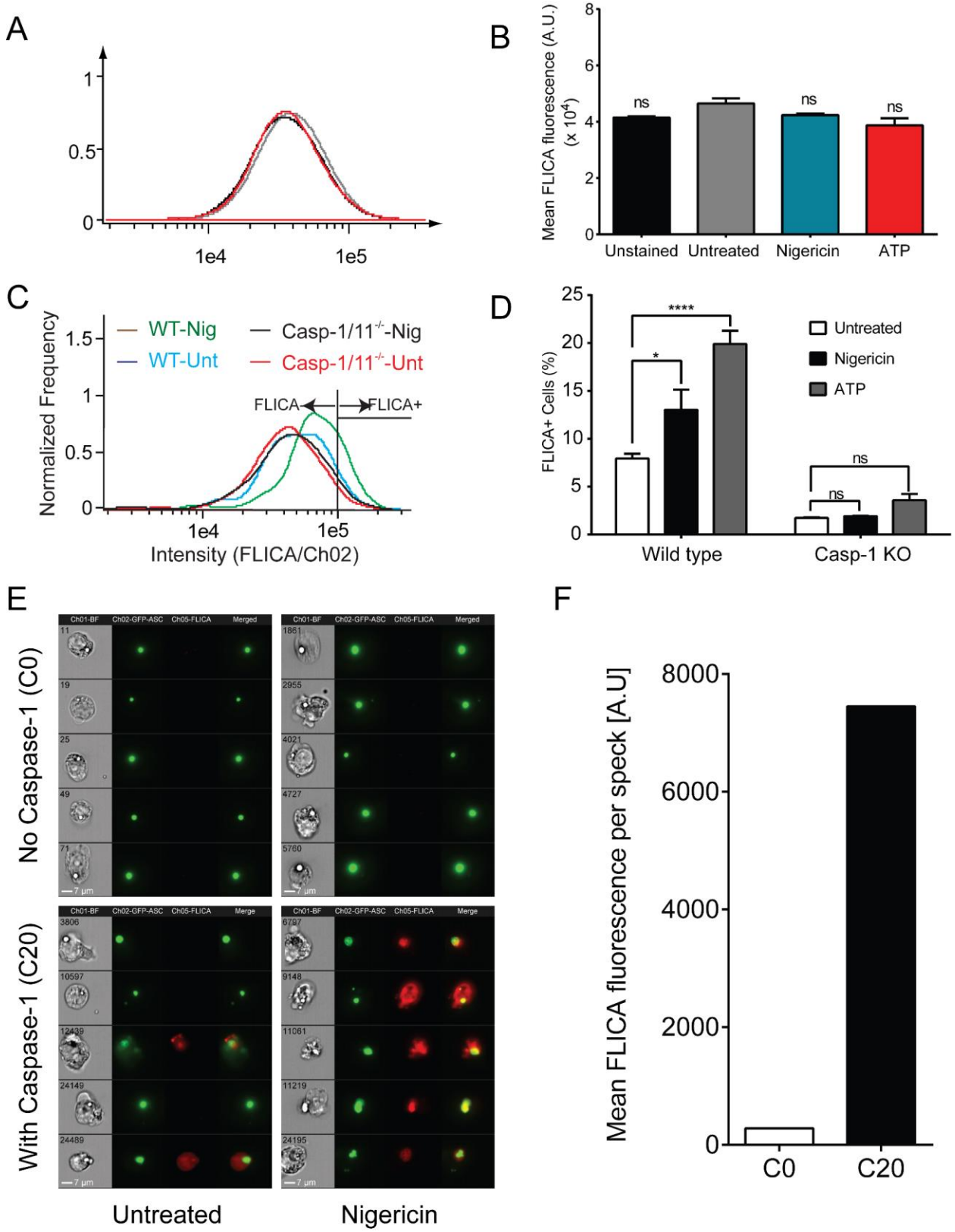

Figure S4

Figure S4: Evaluating the specificity of FLICA staining. (A) Normalized frequency and FLICA mean fluorescence intensity (MFI; intensity) of immortalized BMDMs (Casp-1/11$^{-/-}$) primed with 100ng/ml LPS for 3-4 hrs and stimulated without or with ATP or nigericin for 30 min. **(B)** Quantitation of the MFI values from (A). Mean ± SEM, **** $p<0.001$, One-way ANOVA followed by Tukey's multiple comparison test. **(C)** Normalized frequency and FLICA mean fluorescence intensity (MFI; intensity) of immortalized BMDMs (WT and Casp-1/11$^{-/-}$) primed with 100ng/ml LPS for 3-4 hrs and stimulated without or with nigericin for 30 min. **(D)** Frequency of FLICA-positive cells from the data in A. Mean ± SEM (n=2). **(E)** Representative images of ASC specks showing coincident FLICA staining in cells transfected without/with caspase-1 and treated with nigericin or vehicle. **(F)** FLICA intensity at ASC specks. Cells with specks were counted and FLICA MFI determined without threshold application. The calculated average FLICA intensity of cells with detectable FLICA is shown.