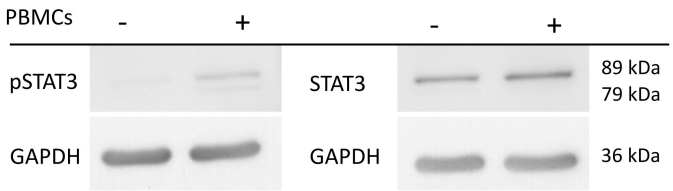
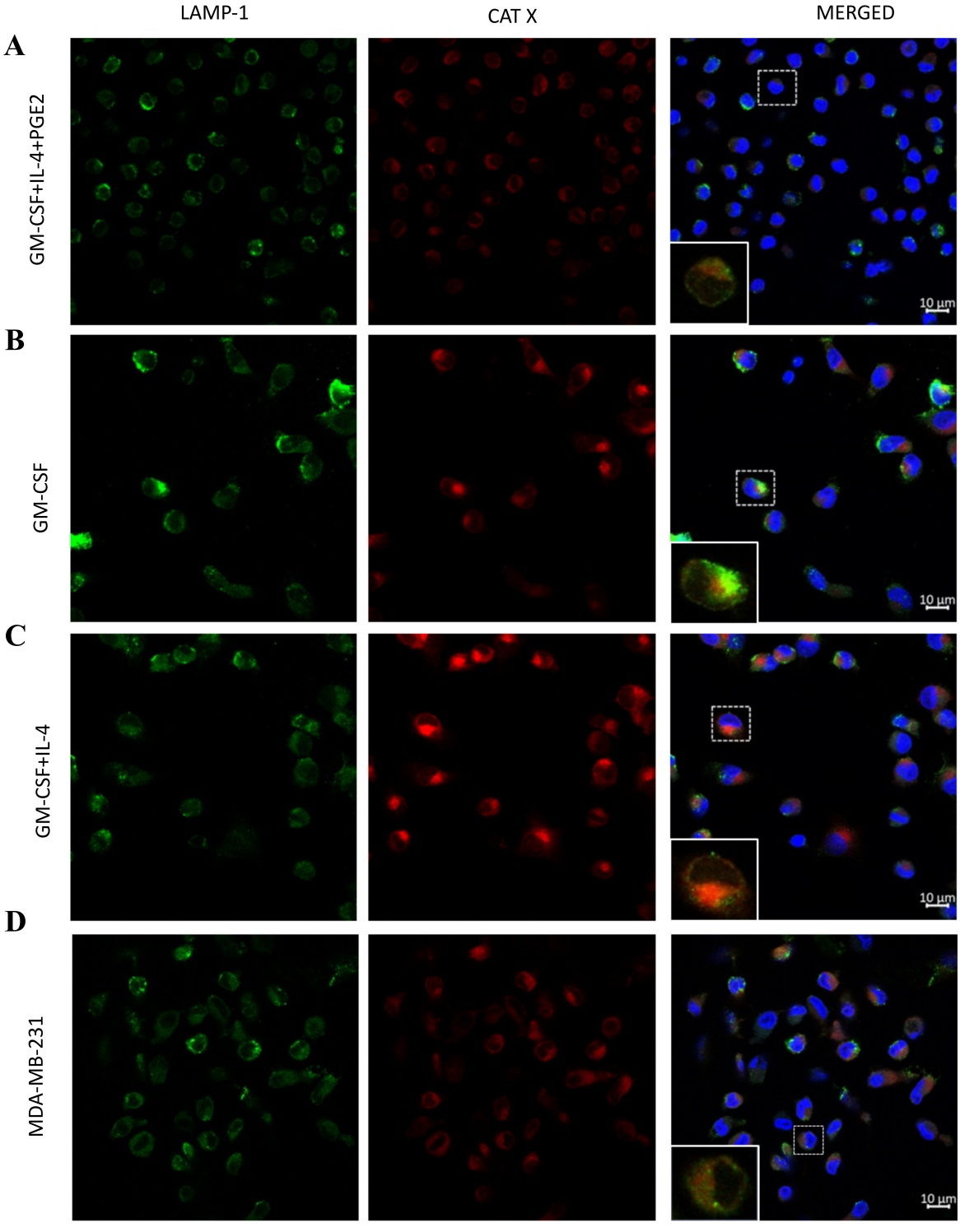
Cancer Immunology, Immunotherapy (submitted in 2020) - Tanja Jakoš et al.

Supplementary Figure 1



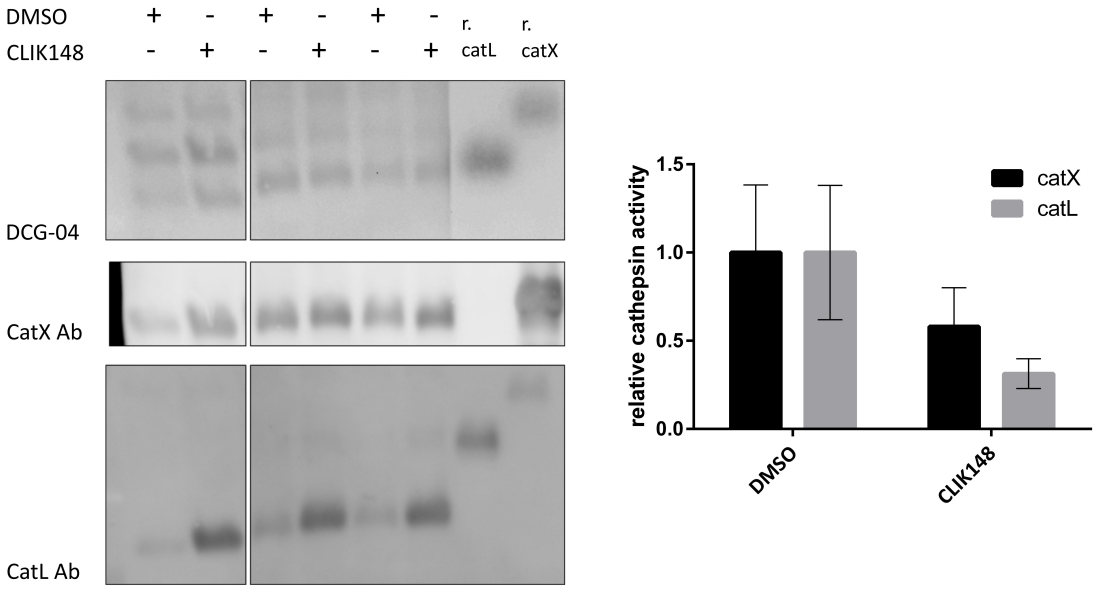
Supplementary Figure 1 STAT3 activation in the MDA-MB-231 cells upon exposure to the peripheral blood mononuclear cells (PBMCs). MDA-MB-231 cells were grown alone and together with PBMCs in co-culture, then lysed and assayed for expression of pSTAT3 (left) and STAT3 (right). Significant up-regulation of activated STAT3 (pSTAT3) was seen in the MDA-MB-231 cells after 72 h of PBMC co-culture

Supplementary Figure S2



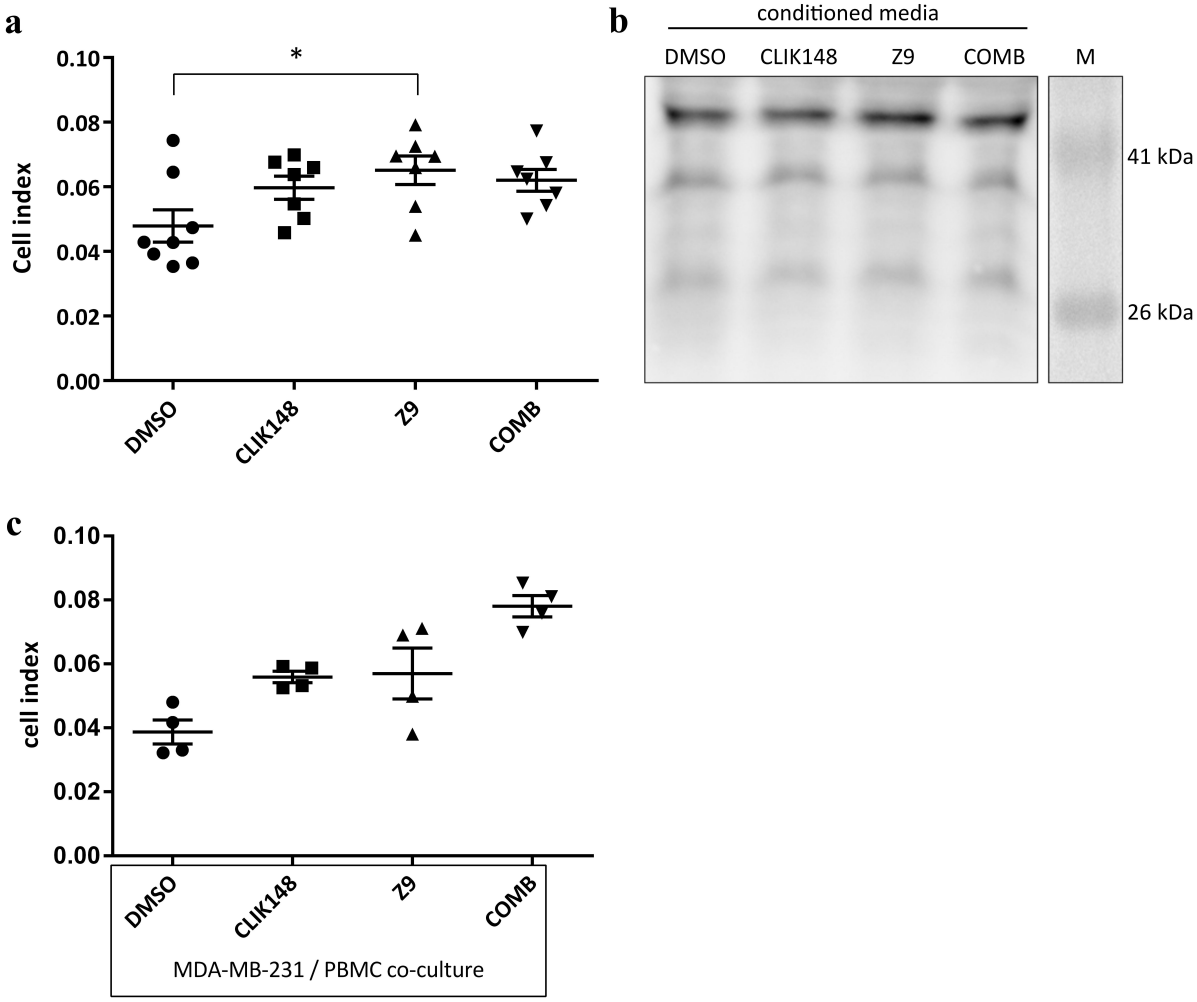
Supplementary Figure 2 Representative confocal microscopy showing the distinct morphological features of the MDA-MB-231–generated myeloid-derived suppressor cells (MDSCs). Representative confocal fluorescent images of CD14+ cells incubated with GM-CSF plus IL-4 plus PGE2 (i.e., cytokine-induced MDSCs) a), with GM-CSF (i.e., macrophages) b), and with GM-CSF plus IL-4 (i.e., dendritic cells) c), and the MDA-MB-231–induced MDSCs d). Green, LAMP-1 lysosomal marker; red, cathepsin X; blue, DAPI-stained nuclei.

Supplementary Figure 3



Supplementary Figure 3 Cathepsin X/L activities and protein levels in lysates of the myeloid-derived suppressor cells (MDSCs) from MDA-MB-231 co-cultures treated with DMSO or the cathepsin L inhibitor. Lysates of MDA-MB-231-cell–generated MDSCs from CD14+ PBMCs from three different donors were incubated with the biotinylated activity-based probe DCG-04, and resolved by SDS-PAGE. Recombinant cathepsins X and L were used as controls. Active cathepsins were probed with horse-radish-peroxidase-conjugated streptavidin. Protein levels of cathepsins L and X were determined using primary antibodies, coupled to detection with fluorochrome-conjugated secondary antibodies Alexa Fluor 488 and 555, respectively. Activity and protein levels were quantified and normalized to DMSO-treated samples.

Supplementary Figure 4



Supplementary Figure 4 a) Invasion of MDA-MB-231 cells pre-treated with cathepsin L/X inhibitors. MDA-MB-231 cells were plated and treated with cathepsin L and/or X inhibitors as in the co-culture experiments. After 72 h, the cells were washed, serum-starved for 4 h, and layered on Matrigel to monitor cell invasion in real-time. Data are means ±standard error of the means for two replicate experiments (n = 8), with no significant differences seen between the inhibitor treatments. *, $p < 0.05$, versus control. b) Activities of the secreted cysteine cathepsins. MDA-MB-231 cells were co-cultured with PBMCs and the cathepsin X/L inhibitors for 72 h. The PBMCs and inhibitors were removed, and the MDA-MB-231 cells were incubated in serum-free medium for an additional 24 h. The conditioned media was collected and concentrated, and the cysteine cathepsin activities were probed with DCG-04 using Western blotting. A representative Western blot is shown. c) Migration of MDA-MB-231 cells from the cathepsin-inhibitor-treated PBMC co-cultures. The migration assay of the tumor cells from the PBMC/MDA-MB-231 cell co-cultures was performed in similar manner to the invasion assay, with the Matrigel layer omitted. Data are means ±standard error of the means for individual experiment (n = 4), with no significant differences seen between the inhibitor treatments.