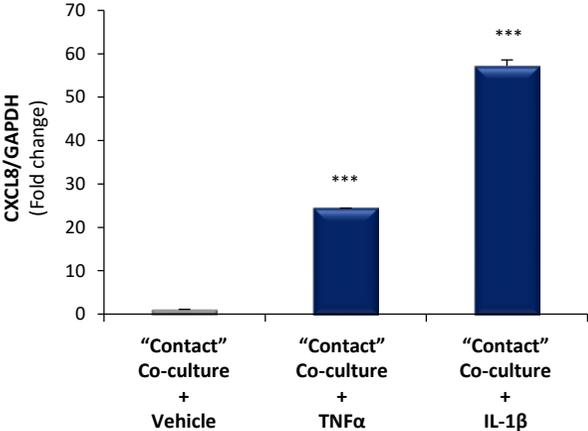


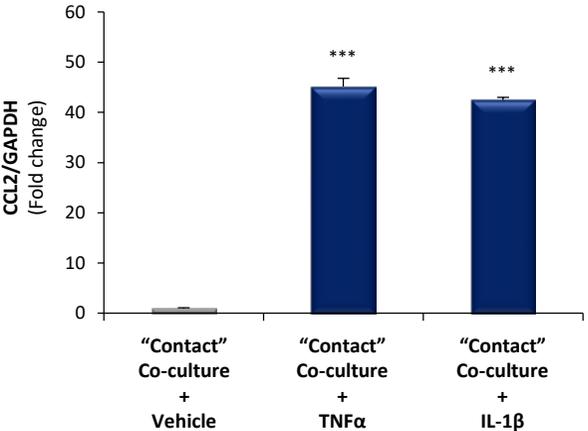
# Supplementary Figure 1

## MDA-MB-231:MSC "Contact" co-cultures

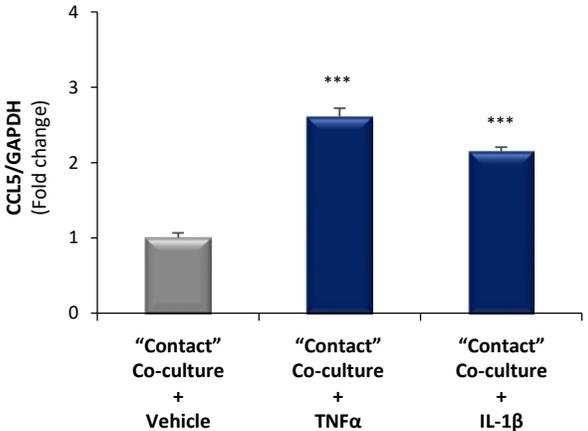
### A. CXCL8 mRNA



### B. CCL2 mRNA



### C. CCL5 mRNA



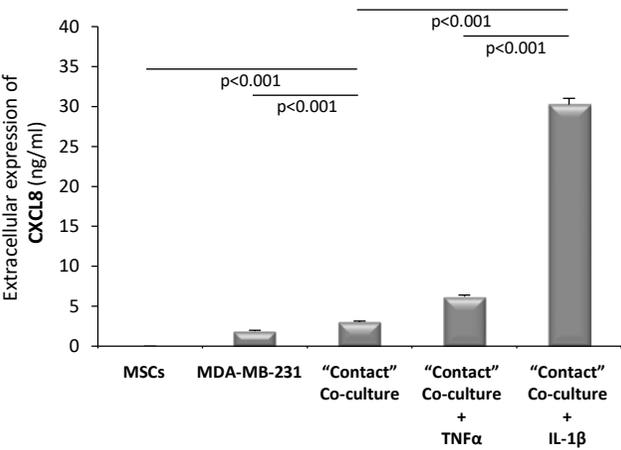
### Supplementary Figure 1

#### In response to TNF $\alpha$ and IL-1 $\beta$ stimulation, CXCL8, CCL2 and CCL5 are up-regulated at the mRNA level in MDA-MB-231:MSC "Contact" co-cultures

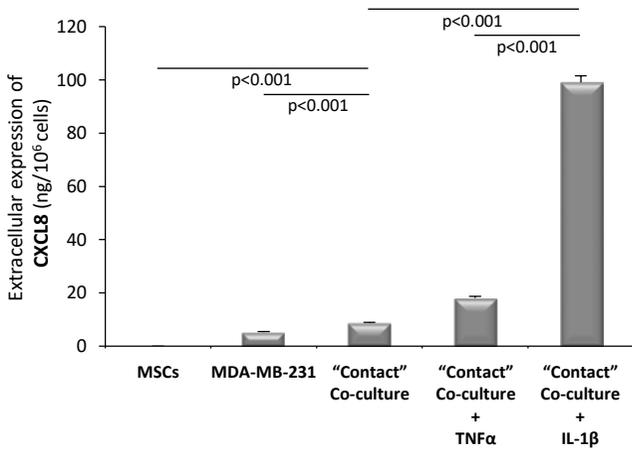
MDA-MB-231:MSC "Contact" co-cultures were stimulated by TNF $\alpha$  (10 ng/ml), IL-1 $\beta$  (350 pg/ml) or vehicle for 7 hrs. The expression levels of CXCL8, CCL2 and CCL5 were determined by qRT-PCR. \*\*\*p<0.001 for differences between cytokine-stimulated and vehicle-treated co-cultures. The results are of a representative experiment of n=3 independent experiments, performed with MSCs of 2 different donors.

### CXCL8 up-regulation

A. ng/ml



B. ng/10<sup>6</sup> cells

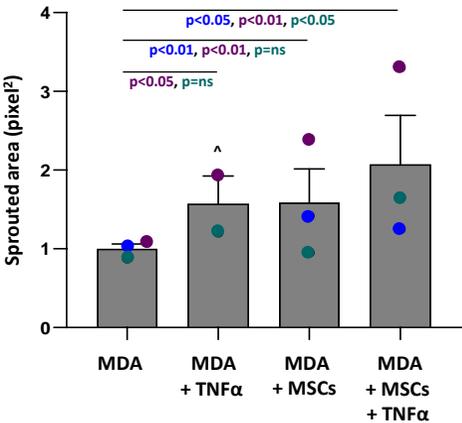


**Supplementary Figure 2**

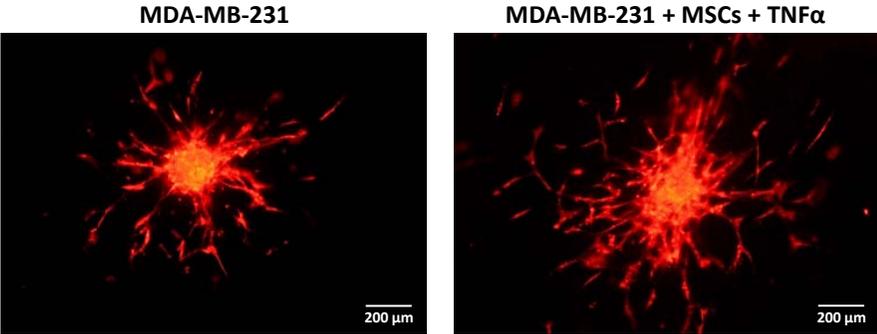
**Elevated CXCL8 levels, induced by TNF $\alpha$ - and IL-1 $\beta$ -stimulation of MDA-MB-231:MSC "Contact" co-cultures, do not result from elevated proliferation of the cells**  
MDA-MB-231 cells and MSCs were grown either alone or in "Contact" co-cultures and were then stimulated by TNF $\alpha$  (10 ng/ml), IL-1 $\beta$  (350 pg/ml) or vehicle for 7 hrs, as described in Figure 3. At the end of the experiment, the cells were counted and in parallel the extracellular expression of CXCL8 in cell supernatants was determined by ELISA. Data are presented as **(A)** CXCL8 levels in values of ng/ml and **(B)** CXCL8 levels in values of ng/10<sup>6</sup> cells. n=1; technical standard deviations of each sample are demonstrated.

### MDA-MB-231:MSCs – HPMEC sprouting in response to CM

#### A. Quantification



#### B. Representative images

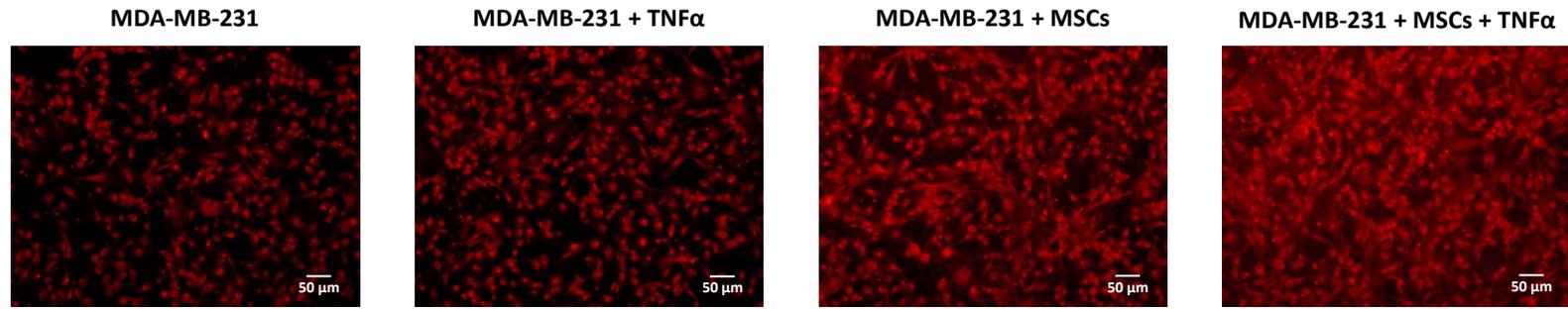


### Supplementary Figure 3

#### The tumor-stroma-inflammation network in TNBC promotes endothelial cell sprouting

Studies of HPMEC sprouting out of 3D spheroids, in response to TNF $\alpha$ -free CM derived from TNF $\alpha$ -stimulated “Contact” co-cultures of MDA-MB-231 cells (“MDA”) and MSCs (TNF $\alpha$ : 10 ng/ml) or in response to TNF $\alpha$ -free CM of tumor cells alone (treated by vehicle), of tumor cells treated by TNF $\alpha$  (10 ng/ml) or of tumor cells co-cultured with MSCs. **(A)** Averages and standard errors of sprout quantification (in multiple photos in each experiment) in n=3, by ImageJ (^ In this groups, n=2). The Figure provides p values of each of the three experimental repeats, comparing sprouting when HPMEC were treated with TNF $\alpha$ -free CM of the different conditions vs. CM of MDA-MB-231 cells alone. **(B)** Representative photos of HPMEC sprouting in the two groups that were significantly different in each of the three experimental repeats : HPMEC treated with TNF $\alpha$ -free CM derived from “Contact” co-cultures of MDA-MB-231 cells vs. CM derived from MDA-MB-231 cells. Photos and their quantifications were derived from studies performed with MSCs of 2 different donors. Bar, 200  $\mu$ m.

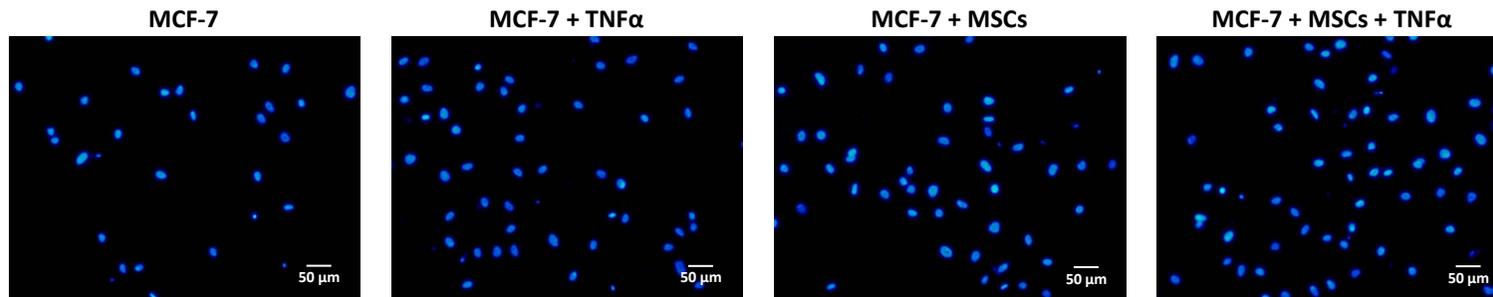
Representative photos of migrating tumor cells



**Supplementary Figure 4**

**TNBC cells acquire elevated migratory capacity upon co-culturing with MSCs in the presence of TNF $\alpha$**

The Figure demonstrates representative photos of migrating tumor cells, whose quantitative analysis was provided in Figure 9A2, in which the migration of mCherry-MDA-MB-231 cells was determined under different conditions. Tumor cells were identified by Abs to RFP/mCherry, followed by DyLight 550-conjugated secondary Abs (preliminary studies using DyLight550 signals and Hemacolor staining further indicated that  $\sim$ 100% of migrating cells were the tumor cells). Photos are representatives of many fields photographed in a representative experiment of n=3 independent experiments, performed with MSCs of 3 different donors. Bar, 50  $\mu$ m.



**Supplementary Figure 5**

**Migration of MCF-7 cells upon co-culturing with MSCs in the presence of TNF $\alpha$**

This Figure is complementary to Figure 9B2, and it demonstrates representative photos of migrating cells in co-cultures of Hoechst-MCF-7 cells and MSCs, stimulated by TNF $\alpha$  (10 ng/ml) (Bar, 50  $\mu\text{m}$ ). Photos are representatives of many fields photographed in a representative experiment of  $n > 3$  independent experiments, performed with MSCs of 3 different donors.

## Supplementary Table 1

<b>Gene</b>	<b>Forward primer (5'-3')</b>	<b>Reverse primer (5'-3')</b>
CXCL8	TTCTGCAGCTCTGTGTGAAG	CAGTGTGGTCCACTCTCAAT
CCL2	AGTCTCTGCCGCCCTTCT	GTGACTGGGGCATTGATTG
CCL5	ACCCTGCTGCTTTGCCTACA	ACACACTTGCCGGTTCTTTC
GAPDH	CCACATCGCTCAGACACCAT	CAACAATATCCACTTTACCAGAGTTAA

### Supplementary Table 1

Primers used in qRT-PCR analyses. For detailed description of qRT-PCR procedures, please see "Materials and methods".