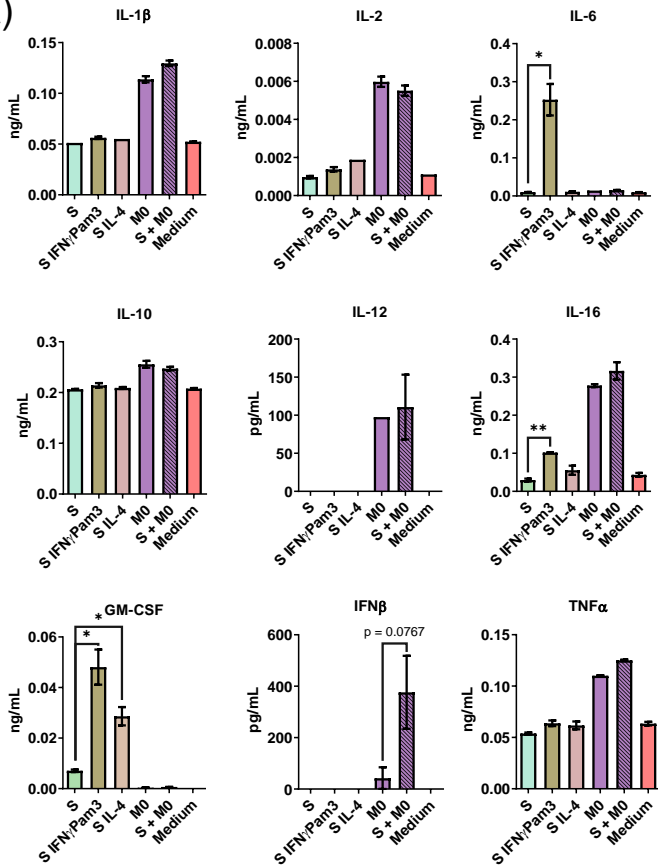
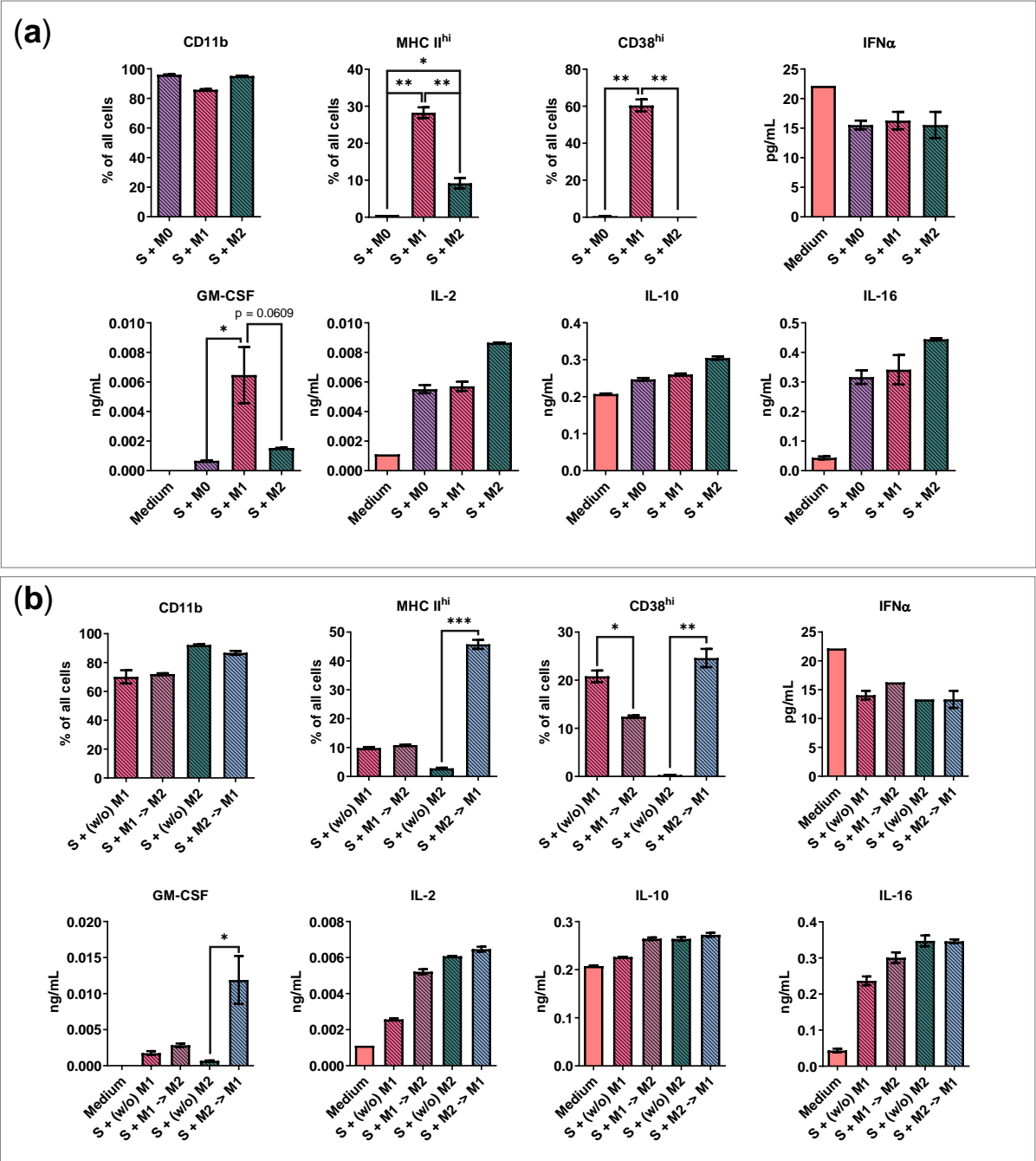


**Figure S1.** BMDMs programming and reprogramming in free-floating 3D conditions. BMDMs were plated in 96-well Black Round Bottom Ultra-Low Attachment plate in a medium containing programming factors: 50 ng/mL recombinant mouse IFN $\gamma$  and 100 ng/mL Pam3SCK4 to achieve M1 phenotype or with 20 ng/mL IL-4 to achieve M2 phenotype. **(a)** Cell number, flow cytometry determined marker expression and levels of cytokines (ELISA/Luminex) of 3D polarized macrophages 72 h after activation. Data are presented as % of all cells. **(b)** Cell number, flow cytometry determined marker expression and levels of cytokines (ELISA/Luminex) of 3D reprogrammed macrophages 72 h after reprogramming. Data are presented as % of all cells. Data are shown as the mean of two experiments  $\pm$  SEM ( $n = 2$ , each experiment is a pool of 4 biological replicates).

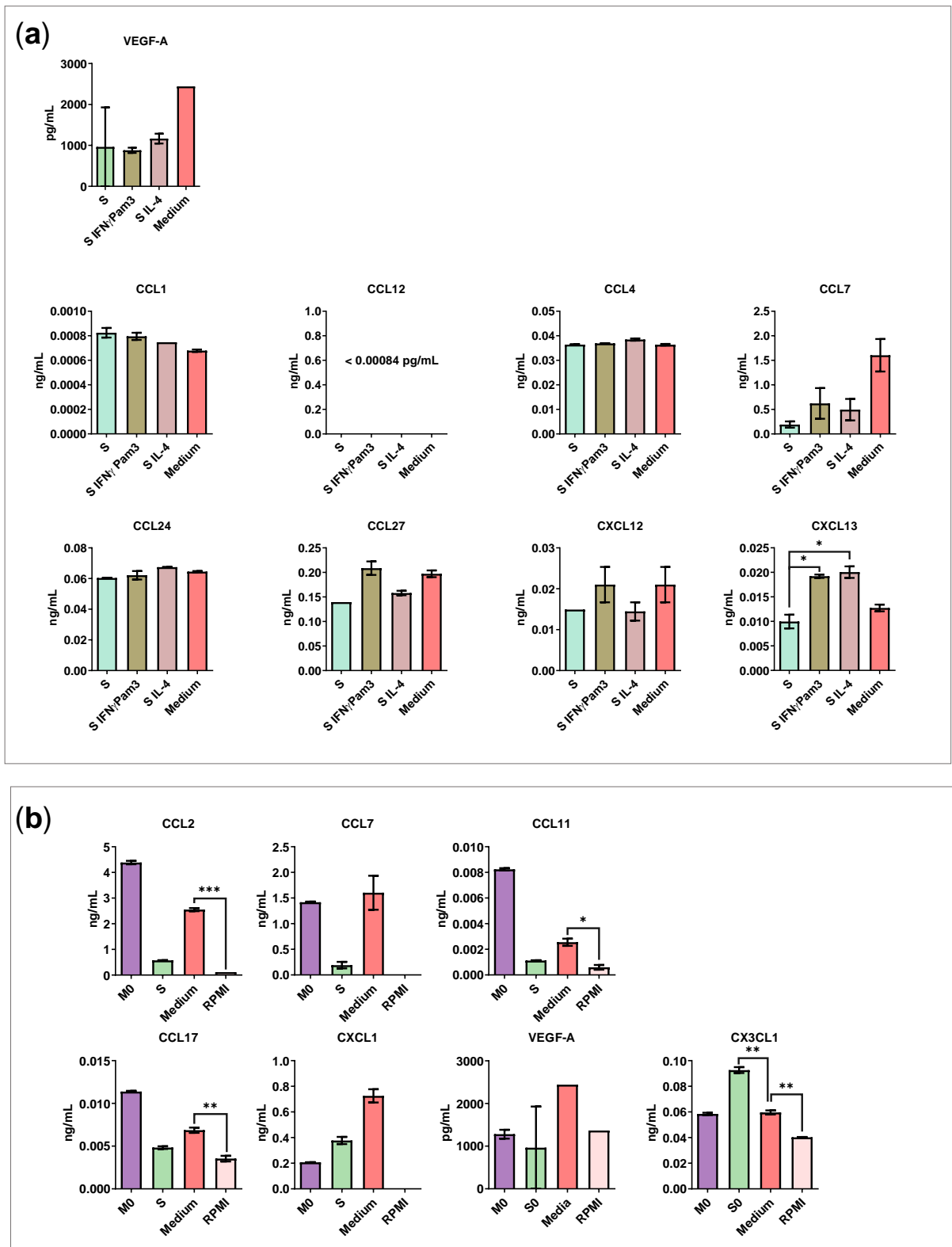
(a)



**Figure S2.** Cytokines produced by 4T1/GFP cancer cell spheroid without stimulation and upon stimulation with IL-4 or IFN $\gamma$  and Pam3SCK4. Cell conditioning media from 3D monocultures and co-cultures were collected 72 h after the addition of IL-4 and IFN $\gamma$ /PAM3SCK4. Then the cytokines were determined using ELISA/Luminex. Data are shown as the mean of two experiments  $\pm$  SEM ( $n = 2$ , each experiment is a pool of 4 biological replicates).

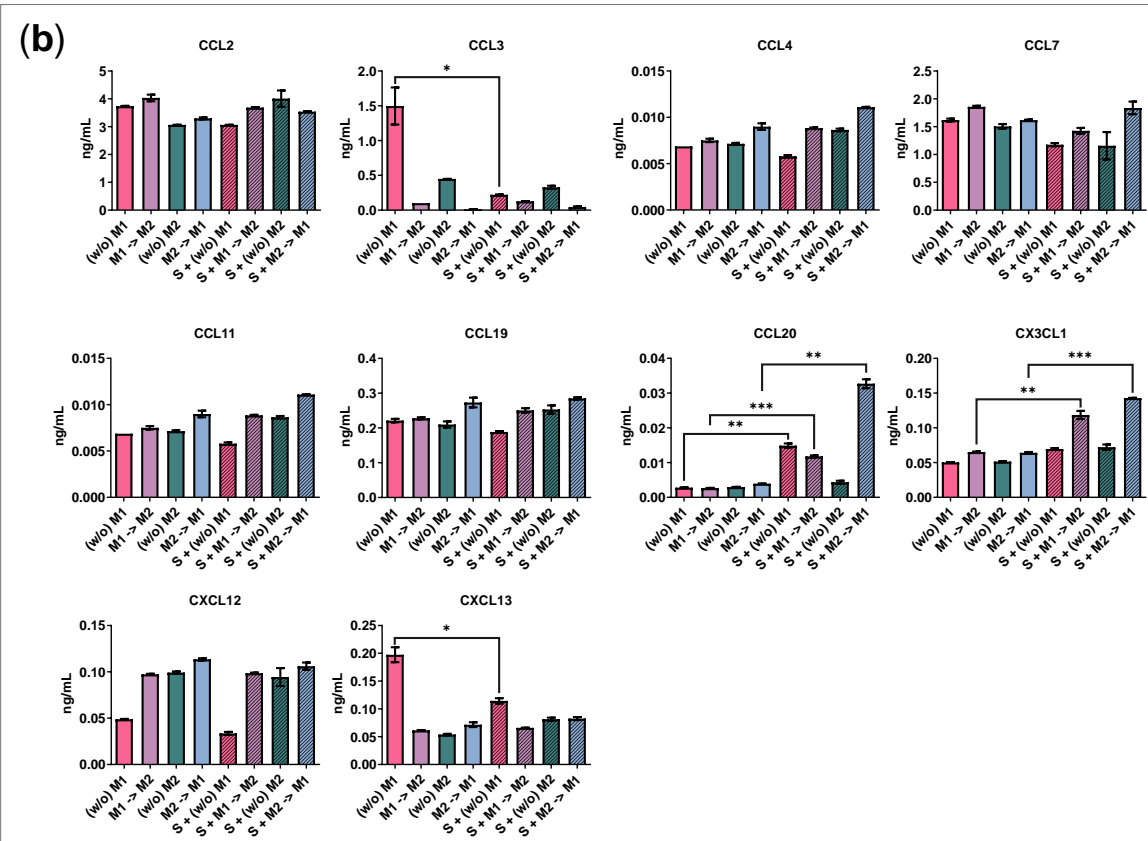
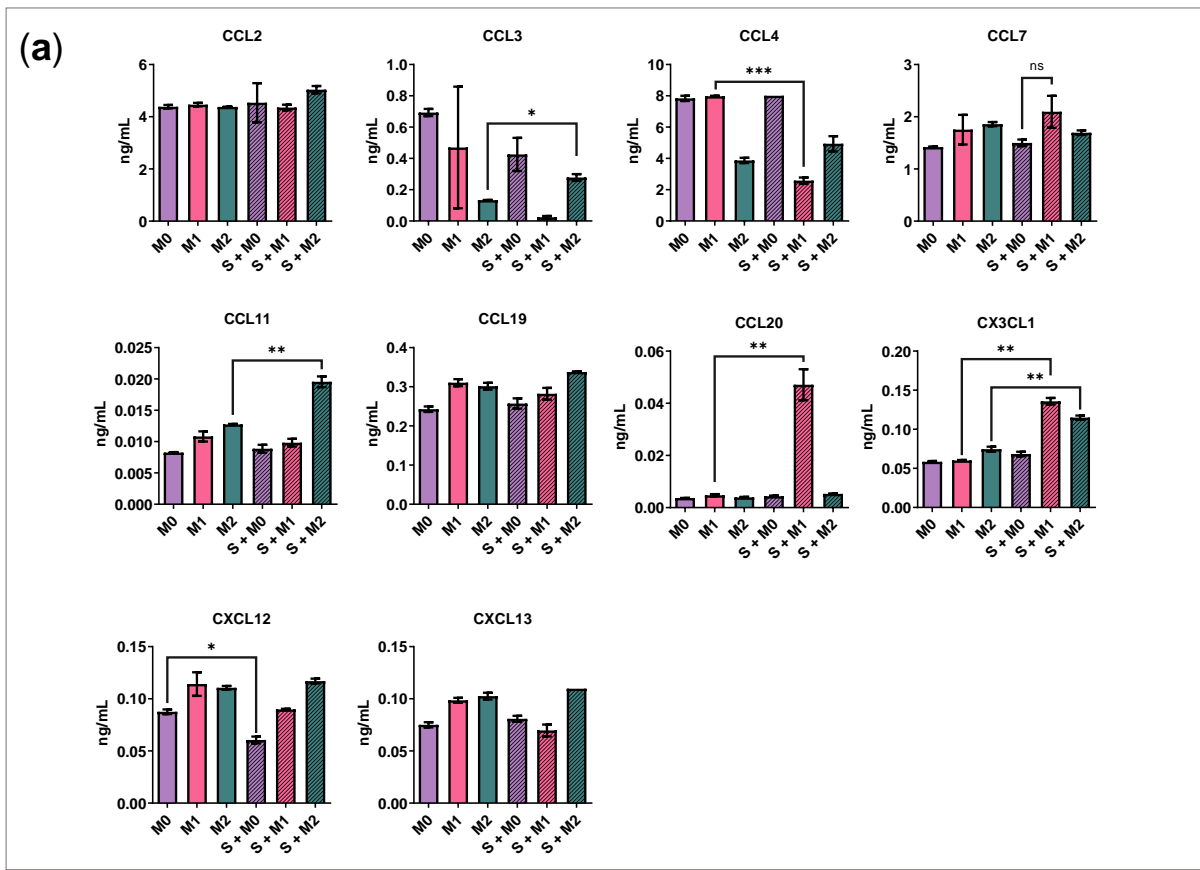


**Figure S3.** BMDMs programming and reprogramming in free-floating 3D conditions in the presence of 4T1 cancer cell spheroid. (a) Flow cytometry determined marker expression and levels of cytokines (ELISA/Luminex) of 3D macrophages polarized in the presence of cancer cell spheroid 72 h after activation. Data are presented as % of all cells. (b) Flow cytometry determined marker expression and levels of cytokines (ELISA/Luminex) of 3D macrophages reprogrammed in the presence of cancer cell spheroid 72 h after reprogramming. Data are presented as % of all cells. Data are shown as the mean of two experiments  $\pm$  SEM ( $n = 2$ , each experiment is a pool of 4 biological replicates).



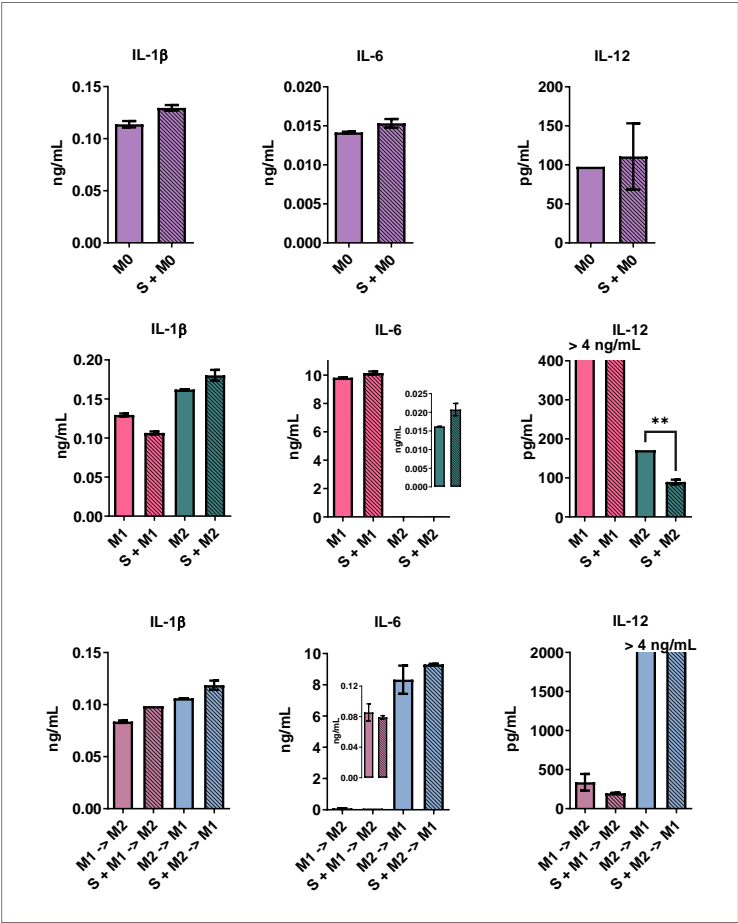
**Figure S4.** Chemokines produced by macrophages and cancer cell spheroid in 3D conditions. Cell conditioning media from monocultures and co-cultures were collected 72 h after the addition of IL-4 and IFN $\gamma$ /PAM3SCK4. Then the chemokines were determined using ELISA/Luminex. **(a)** Chemokines produced by the 4T1/GFP cancer cell spheroid and upon stimulation with IL-4 or IFN $\gamma$ /Pam3SCK4. **(b)** Chemokines found in the BMDM cultivation medium containing L929-CM in comparison with the medium of non-polarized macrophages (M0), cancer cell spheroid (S), and RPMI medium without L929-CM (RPMI).

Data are shown as the mean of two experiments  $\pm$  SEM ( $n = 2$ , each experiment is a pool of 4 biological replicates).

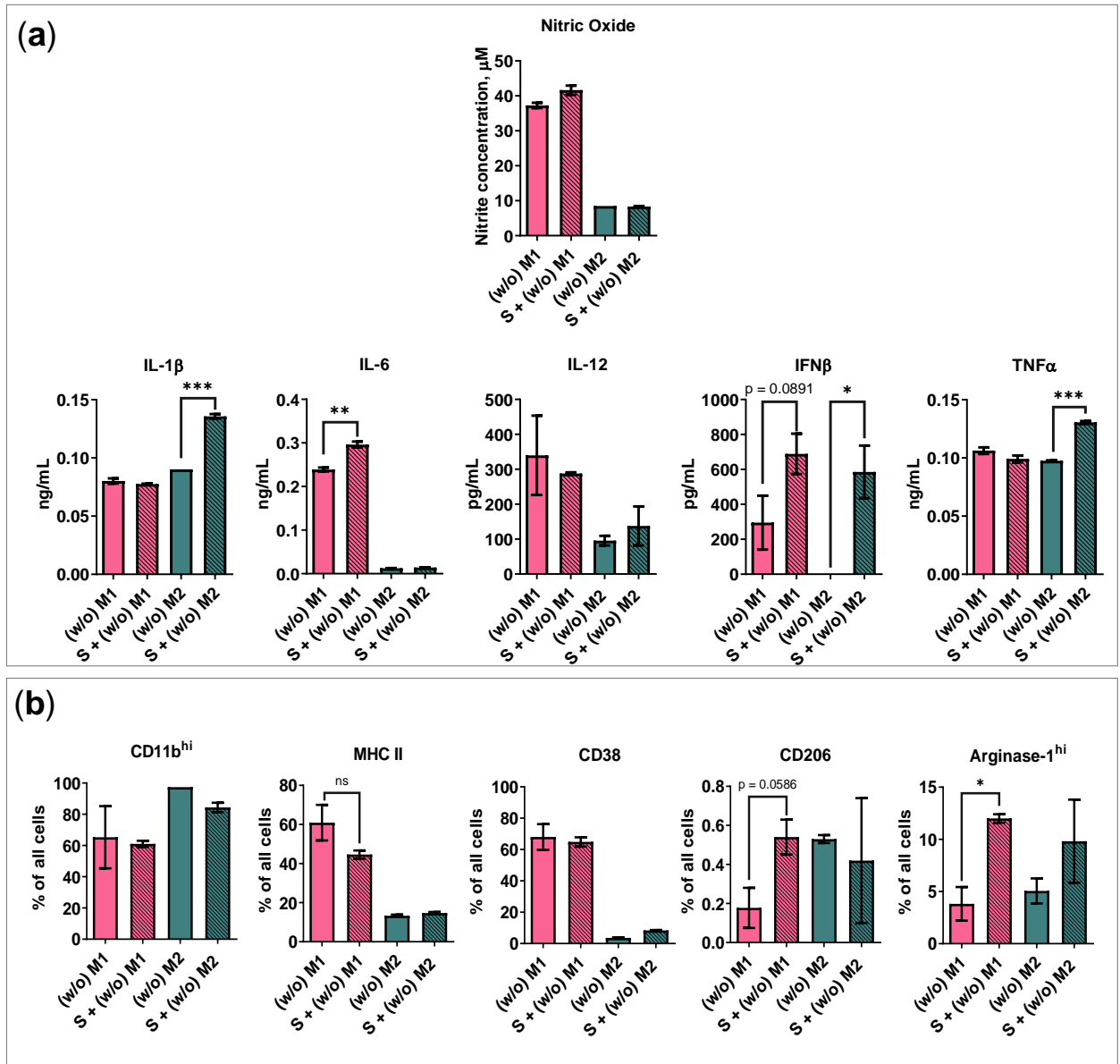


**Figure S5.** Chemokines produced by programmed/reprogrammed macrophages and cancer cell spheroid in 3D conditions. Cell conditioning media from monocultures and co-cultures were collected 72 h after the addition of IL-4 and IFN $\gamma$ /PAM3SCK4. Then the chemokines were determined using ELISA/Luminex. **(a)** Chemokines found in the medium of programmed macrophages in the presence (S + M) and in the absence (M) of cancer cell spheroid. **(b)** Chemokines found in the medium of reprogrammed macrophages in the presence and in the absence of cancer cell spheroids.

Data are shown as the mean of two experiments  $\pm$  SEM ( $n = 2$ , each experiment is a pool of 4 biological replicates).



**Figure S6.** Cytokines produced by macrophages in the presence (S + M) and absence (M) of 4T1/GFP cancer cell spheroid. Cell conditioning media from 3D monocultures and co-cultures were collected 72 h after the addition of IL-4 and IFN $\gamma$ /PAM3SCK4. Then the cytokines were determined using ELISA/Luminex. Data are shown as the mean of two experiments  $\pm$  SEM ( $n = 2$ , each experiment is a pool of 4 biological replicates).



**Figure S7.** 4T1 breast cancer cell spheroid influences macrophages. Macrophages were co-cultivated with breast cancer cell spheroid 24 h before programming and 72 h after. **(a)** Changes in the levels of secreted cytokines of macrophages cultivated in the presence (S + M) and absence (M) of cancer cell spheroid. **(b)** Changes in cell surface marker levels of macrophages cultivated in the presence (S + M) and the absence (M) of cancer cell spheroid. Cells have been analyzed using flow cytometry. Data are shown as the mean of two experiments  $\pm$  SEM ( $n = 2$ , each experiment is a pool of 4 biological replicates).