Supplementary Figure 1: β2-AR activation increases inflammatory cytokine levels. (A and B) The levels of inflammatory cytokines in the plasma of mice housed at ST (22°C) or TT (30°C) bearing 4T1 or AT-3 tumors respectively, as determined by multiplex. (C) Inflammatory cytokine levels in WT and β2-AR−/− mice bearing 4T1 tumor cells. Data are presented as mean ± SEM from 5 mice per group from two independent replicates, and the students T test was used to analyze statistical significance between two groups. In all panels * P<0.05, ** P<0.01 and *** P<0.001. A P value less than 0.05 was considered significant.

Supplementary Figure 2: β2-AR activation increases tumor growth and metastasis. (A) Tumor mass of mice housed at ST (22°C) or TT (30°C) bearing 4T1 and AT-3 tumors. Tumor mass is presented as mean ± SEM from 5 mice per group from two replicates. (B) Tumor mass in WT and β2-AR−/− mice bearing 4T1 tumors. Tumor mass is presented as mean ± SEM from groups of 5 mice from three replicate studies. (C) WT and β2-AR−/− mice were injected with 4T1 cells. On day 25, lung tissue was collected, stained with hematoxylin and eosin, and tumor metastatic nodules were counted. These data are presented as median ± min to max from 5 mice per group from two replicates, and the students T test was used to analyze statistical significance between two groups. In all panels * P<0.05. A P value less than 0.05 was considered significant.

Supplementary Figure 3: β2-AR activation during chronic stress increases the accumulation of MDSCs in blood, lymph node and metastatic lung. (A and B) Percentage of G-MDSCs and M-MDSCs in blood (A) and lymph node (B) of healthy or tumor bearing mice (4T1 or AT-3) day 15 after tumor injection housed in standard temperature (ST) or thermoneutral temperature (TT). (C) On day 25, lung tissue was collected, crushed, disassociated with a tumor dissociation kit, and single cell suspensions were prepared. CD11b+ Gr-1+ cells were gated from live CD45+ cells. These data are presented as median ± min to max from 5 mice per group from two replicates, and the students T test was used to analyze statistical significance between two groups. In all panels * P<0.05 and ** P<0.01. A P value less than 0.05 was considered significant.

Supplementary Figure 4: β2-AR activation increases the immunosuppressive function of MDSCs. (A) Arginase I and PDL-1 expression by intratumoral MDSCs of 4T1 tumor bearing mice measured by flow cytometry 25 days after tumor injection (n=5). (B) T cells co-cultured with MDSCs sorted by MDSCs isolation kit from 4T1 tumors of WT or β2-AR−/− mice (n=3). These data are presented as median ± min to max, and the students T test was used to analyze statistical significance between two groups. In all panels * p<0.05 and ** p<0.01. A P value less than 0.05 was considered significant.

Supplementary Figure 5: Direct β2-AR activation by salbutamol increases tumor growth and accumulation of MDSC in the spleen and tumor tissue. (A) Tumor growth kinetics of 4T1 tumors orthotopically injected into WT mice. Mice were treated with PBS or salbutamol (i.p. daily injection) (n=10). (B and C) Absolute number of MDSCs in spleen (B) and tumor (C) of WT mice treated with PBS or salbutamol (n=5). Two-way ANOVA was used to analyze statistical significance between tumor growth in different groups. These data are presented as mean ± SEM. Other data are presented as median ± min to max, and the students T test was used to analyze statistical significance between two groups. In all panels * P<0.05 and **** P<0.0001. A P value less than 0.05 was considered significant.
Supplementary Figure 1
Supplementary Figure 2

A

4T1 (BALB/c)

Tumor mass (mg)

ST    TT

0     200

400   600

800

AT3 (C57BL/6)

Tumor mass (mg)

ST    TT

0     50

100   150

200   250

200

4T1 (BALB/c)

Tumor mass (mg)

WT  β2AR⁻/⁻

0     800

400   600

800  1000

B

C

WT  β2AR⁻/⁻
Supplementary Figure 3

A

CD11b^+Ly6G^+ (% of live cells)

B

CD11b^+Ly6G^+ (% of live cells)

C

CD11b^+Ly6C^+

Tumor-free 4T1 AT3

Tumor-free 4T1 AT3

Tumor-free 4T1 AT3

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns

ns
Supplementary Figure 5

A

4T1 (BALB/c)

Tumor volume (mm³)

Days

PBS Control
β2-AR agonist (Salbutamol)

****

B

PBS Control
β2-AR agonist (Salbutamol)

Cell number per mg of spleen

G-MDSC
M-MDSC

C

PBS Control
β2-AR agonist (Salbutamol)

Cell number per mg of tumor

G-MDSC
M-MDSC

* ns