# Figure S1

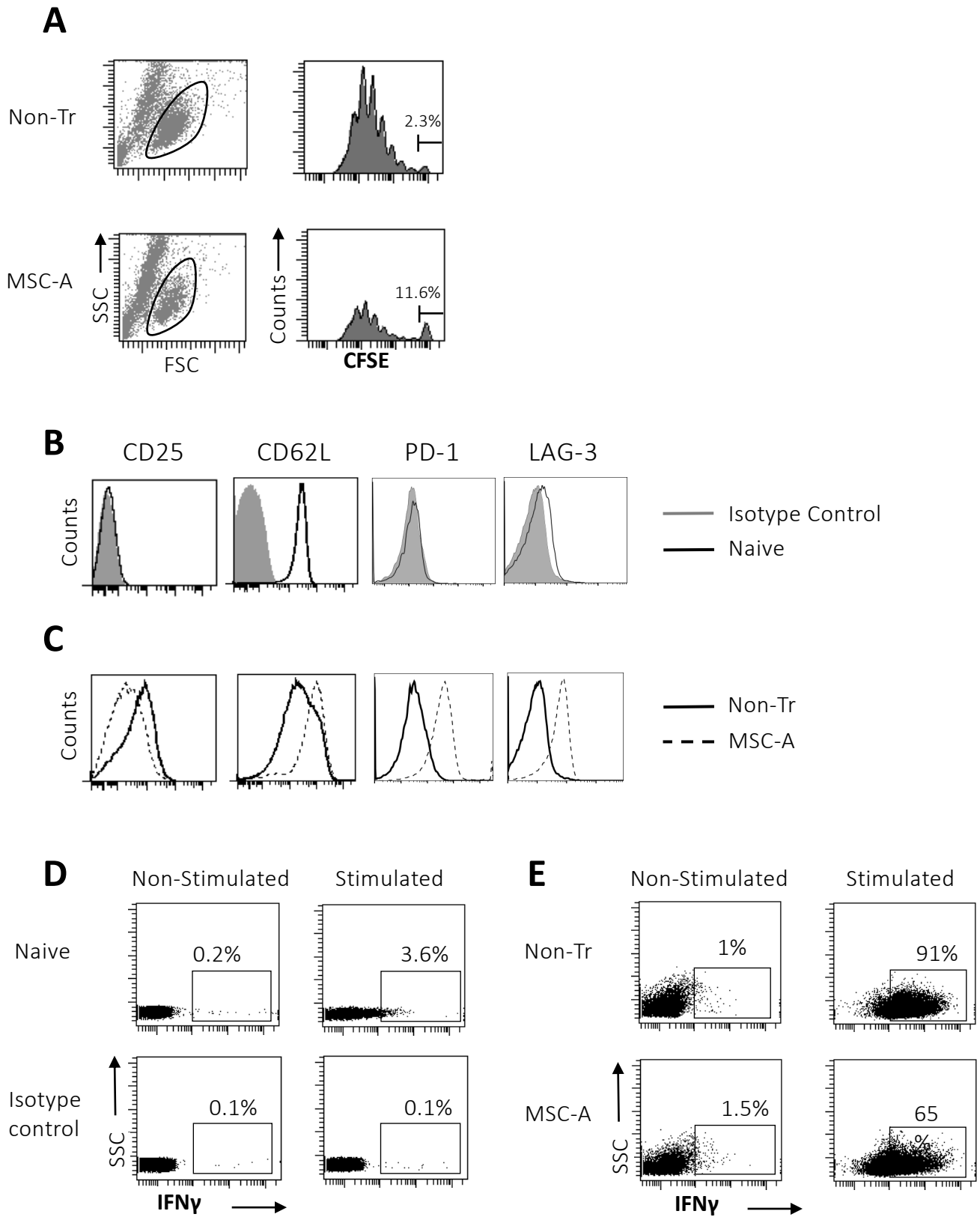
A
Non-Tr
2.3%
MSC-A
SSC
FSC
Counts
11.6%
CFSE
B
CD25
CD62L
PD-1
LAG-3
Counts
Isotype Control
Naive
C
Counts
Non-Tr
MSC-A
D
Non-Stimulated
Stimulated
Naive
0.2%
3.6%
Isotype control
0.1%
0.1%
SSC
IFNγ
E
Non-Stimulated
Stimulated
Non-Tr
1%
91%
MSC-A
1.5%
65 %
SSC
IFNγ

**Figure supplementary 1. MSCs suppress $CD8^+$ T cell responses.** **A**. CFSE-labeled purified naïve Clone $CD8^+$ T cells were activated with anti-CD3 and anti-CD28 mAbs and cultured in the presence of C57Bl/6 bone marrow derived TNFα and IFNγ-treated MSCs (MSC-A) or left untreated without MSCs (Non-Tr). After 3 days, Clone 4 $CD8^+$ T cells were harvested and their morphology and CFSE fluorescence were analyzed by FACS. Values represent percentage of undivided cells. Data from one representative experiment out of 3, described in Fig. 1, is presented. **B.** Expression of CD25, CD62L, PD-1 and LAG-3 in purified naïve Clone 4 $CD8^+$T cells was analyzed by FACS. Shaded histograms represent isotype control-matched controls. **C**. Day 3 activated Clone 4 $CD8^+$T cells cultured in the presence or absence of MSC-A were harvested and expression of CD25, CD62L, PD-1 and LAG-3 was assessed by FACS. Data from one representative experiment out of 3, described in Fig. 1, is presented. **D**. Naïve Clone 4 $CD8^+$ T cells were restimulated with PMA and ionomycin in the presence of brefeldin A and production of intracellular IFNγ was assessed by FACS. **E**. Day 3 activated Clone 4 $CD8^+$T cells cultured with or without MSC-A were restimulated with PMA and ionomycin in the presence of brefeldin A and production of intracellular IFNγ was assessed by FACS. Percentage of cytokine producing T cells is indicated. Non-stimulated controls are shown. Data from one representative experiment out of 3, described in Fig. 1, is presented.

## Figure S2

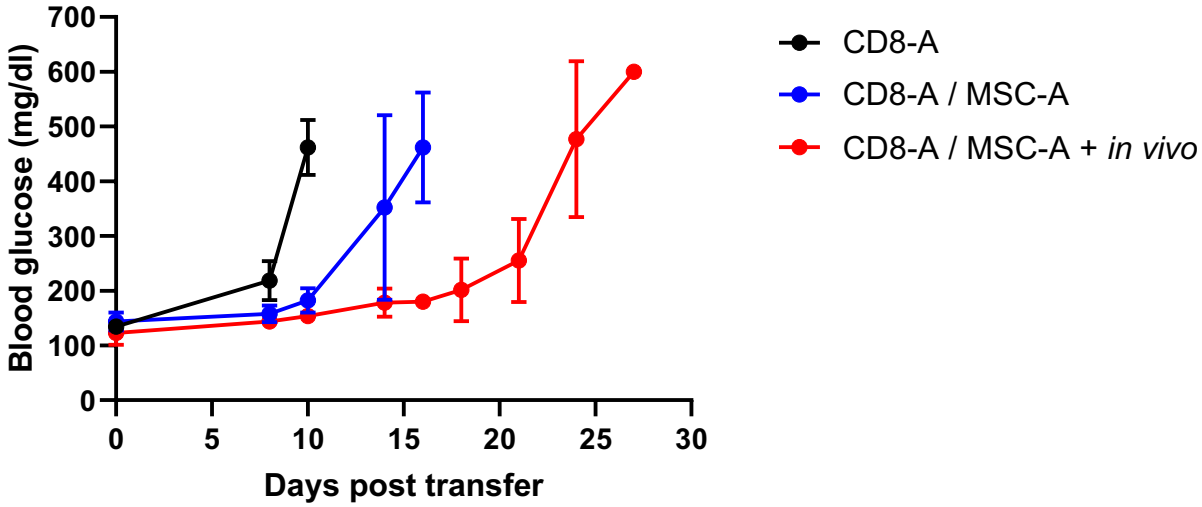


**Figure supplementary 2. MSCs delay the onset of T cell mediated autoimmune diabetes.** Sublethaly irradiated InsHA mice were adoptively transferred with $3x10^6$ naïve HNT $CD4^+$ T cells along with $3x10^6$ day 3 activated Clone 4 $CD8^+$ T (CD8-A) or $3x10^6$ day 3 activated Clone 4 $CD8^+$ T cells co-cultured with TNFα and IFNγ-treated MSCs (CD8-A/MSC-A). Some CD8-A/MSC-A adoptively transferred mice were also injected with $10^6$ MSCs on days 0 and 5 after transfer (CD8-A/MSC-A + MSC in vivo) (n=4 mice per group). Mice were monitored for the onset of autoimmune diabetes by measuring blood glucose levels.

## Figure S3

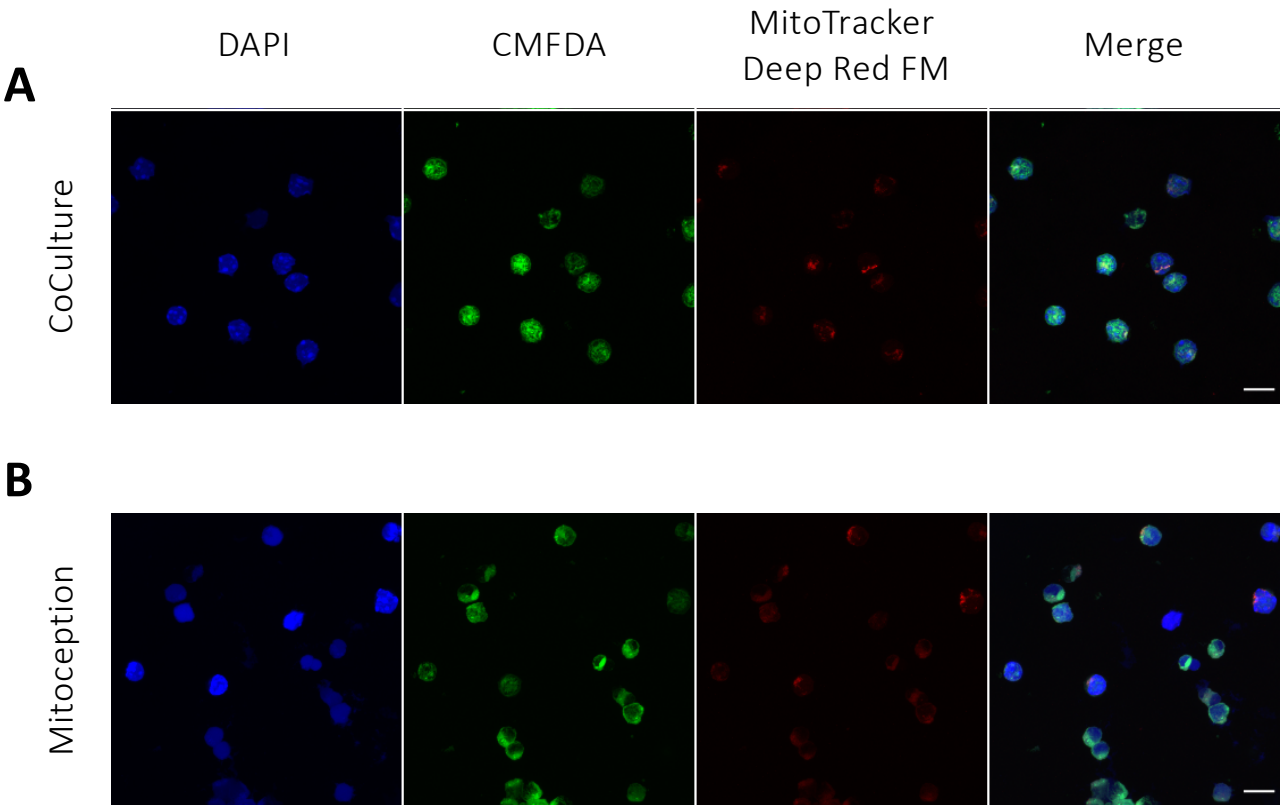


**Figure supplementary 3**. **Subcellular localization of transferred mitochondria in CD8$^+$ T cells. A.** Clone 4 CD8$^+$ T cells were stained with green cell tracker CMFDA, activated and cultured with Mitotracker Deep Red labeled MSC-A. 12 h later T cells were harvested and processed for microscopy. Maximum intensity projection of the frame presented in Fig 1F. **B**. Clone 4 CD8$^+$ T cells were stained with green cell tracker CMFDA and mitocepted with isolated mitochondria from Mitotracker Deep Red labeled MSC-A or mock mitocepted. 12 h later T cells were harvested and processed for microscopy. Maximum intensity projection of the frame presented in Fig 2B. Nuclei appear in blue, cell membrane and cytoplasm in green and mitochondria in red. The right panels correspond to the merge of three channels. Scale bar 10 μm.

## Figure S4

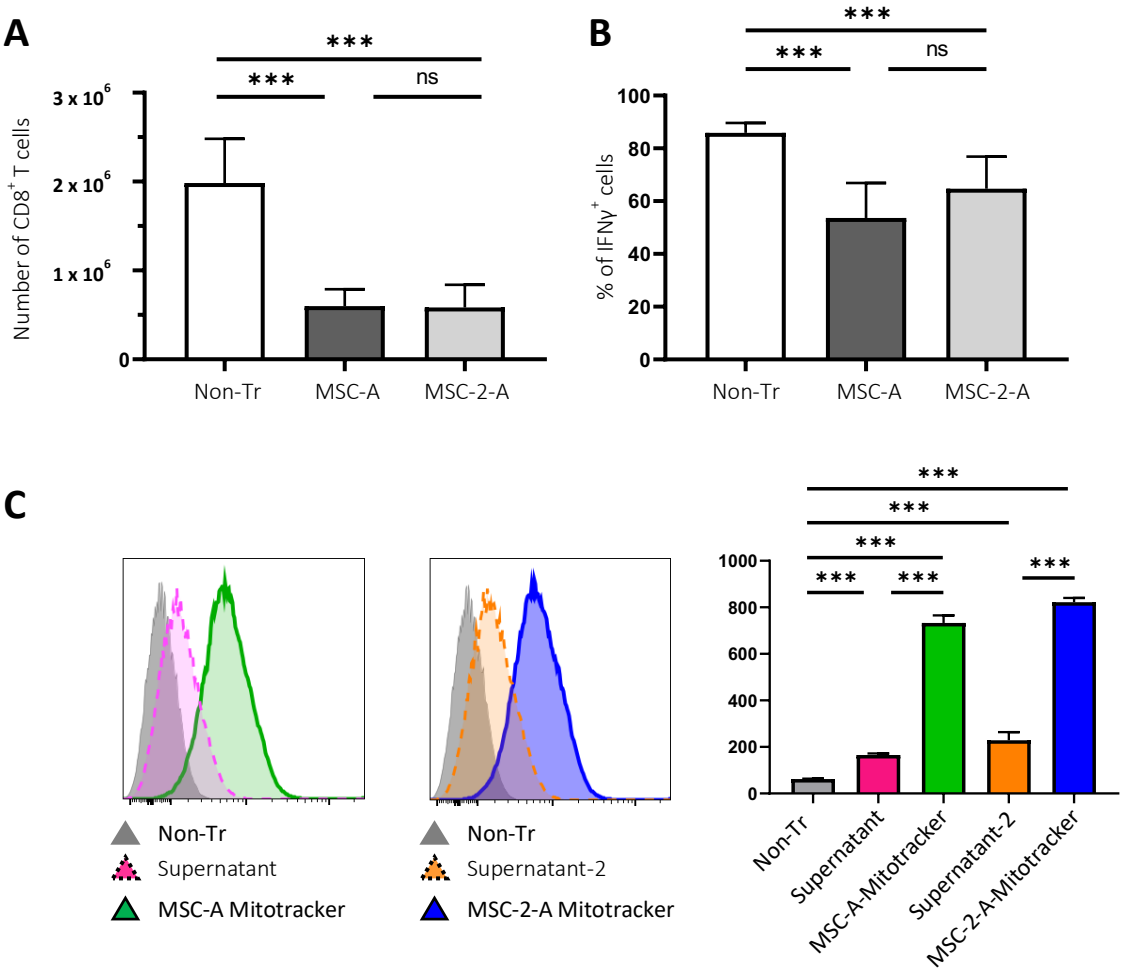


**Figure supplementary 4**. **Different MSC preparations transfer mitochondria to CD8⁺ T cells. A.** Purified Clone 4 CD8⁺ T cells were activated with anti-CD3 and anti-CD28 mAbs and cultured with two different C57Bl/6 bone marrow-derived TNFα and IFNγ-treated MSCs (MSC-A or MSC-2-A) or left untreated in the absence of MSCs (Non-Tr). After 3 days, CD8⁺ T cells were harvested and enumerated. Absolute numbers of Clone 4 CD8⁺ T cells are shown. **B**. Day 3 activated Clone 4 CD8⁺ T cells were restimulated with PMA and ionomycin in the presence of brefeldin A and intracellular IFNγ was assessed by FACS. Percentage of cytokine producing CD8⁺ T cells is indicated. **C**. Clone 4 CD8⁺ T cells were activated and cultured with Mitotracker Deep Red labeled MSC-A, Mitotracker Deep Red labeled MSC-2-A or left untreated. As control for dye leakage, Clone 4 CD8⁺ T cells were activated and cultured with the supernatant of Mitotracker Deep Red labeled MSC-A or MSC-2-A that underwent the same process of labeling, washing and culturing time. After 12 h, T cells were analyzed by FACS. Data from 3 independent experiments is presented and values are represented as mean ± SEM.

## Figure S5

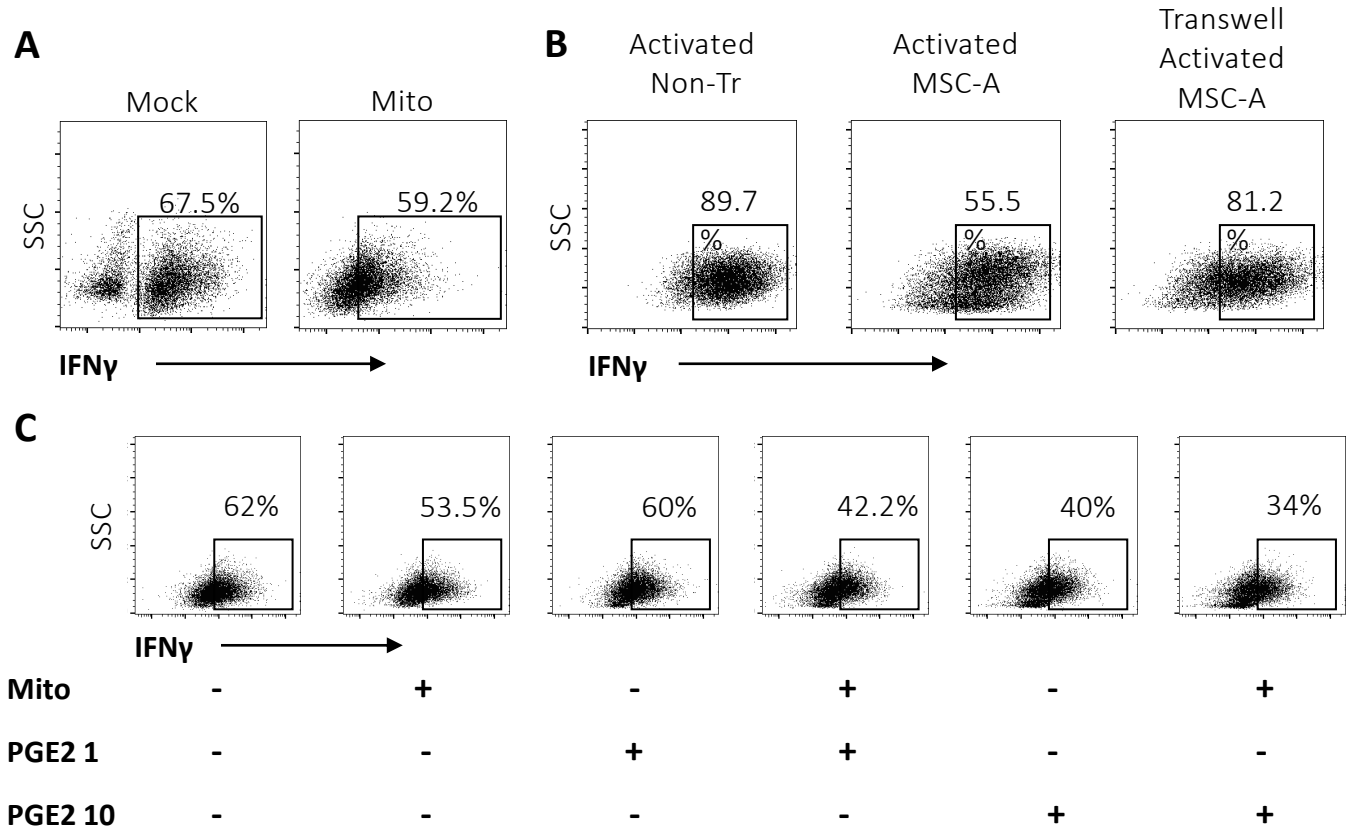


**Figure supplementary 5. MSC mitochondrial transfer to CD8$^+$ T cells inhibit IFNγ production.** Day 3 activated Clone 4 CD8$^+$T cells were restimulated with PMA and ionomycin in the presence of brefeldin A and production of intracellular IFNγ was assessed by FACS. Percentage of cytokine producing T cells is indicated. **A.** Data from one representative experiment described in Fig. 2D is presented. **B.** Data from one representative experiment described in Fig. 3C is presented. **C.** Data from one representative experiment described in Fig. 4B is presented.

# Figure S6

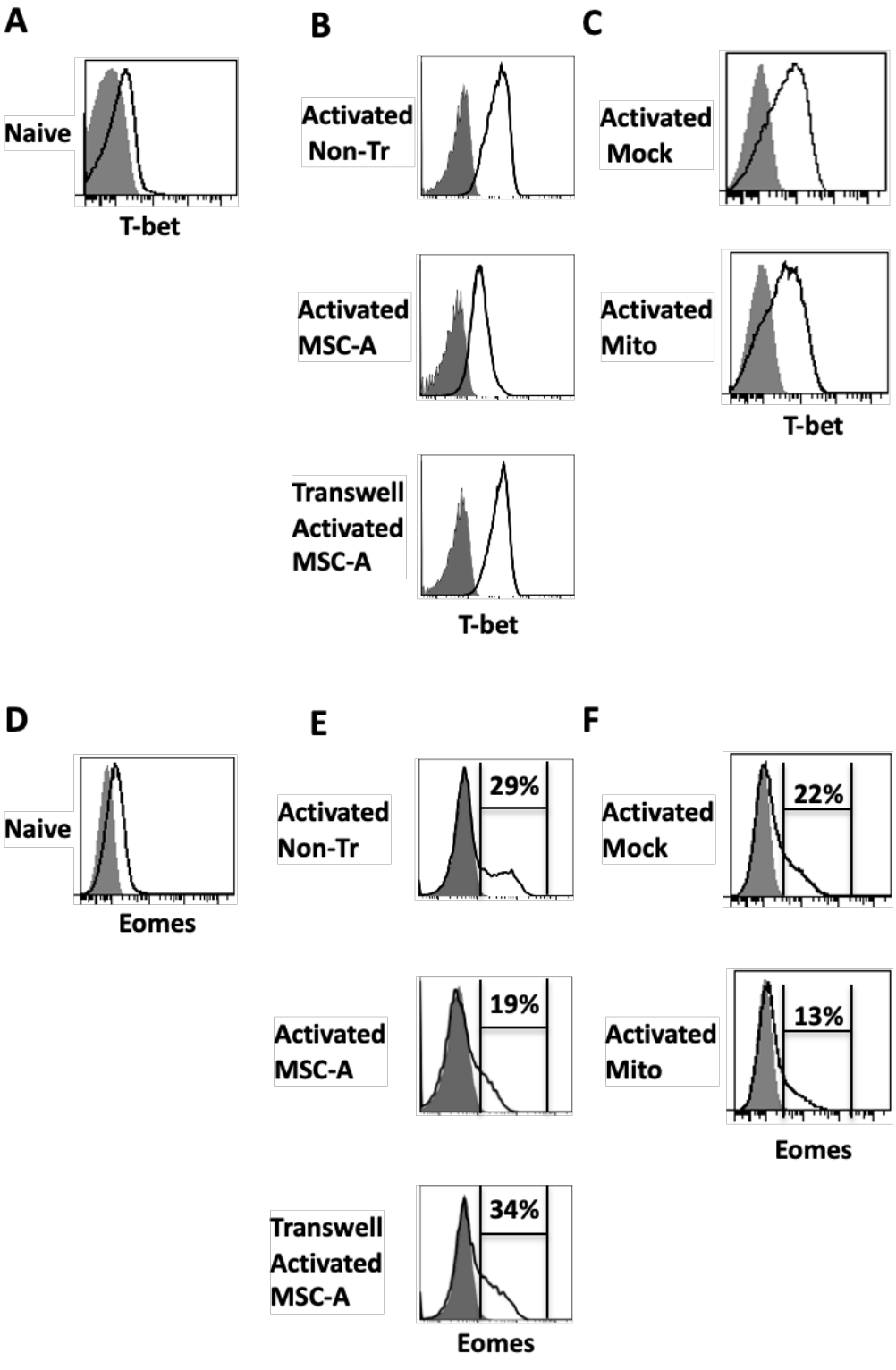
A
Naive
T-bet
B
Activated Non-Tr
Activated MSC-A
Transwell Activated MSC-A
T-bet
C
Activated Mock
Activated Mito
T-bet
D
Naive
Eomes
E
Activated Non-Tr
29%
Activated MSC-A
19%
Transwell Activated MSC-A
34%
Eomes
F
Activated Mock
22%
Activated Mito
13%
Eomes

**Figure supplementary 6**. **MSCs and MSC mitochondria down-regulate T-bet and Eomes in activated CD8$^+$ T cells. A** and **D**. Intracellular T-bet and Eomes expression was analyzed in naïve purified Clone 4 CD8$^+$ T cells by FACS. **B** and **E**. Naïve purified Clone 4 CD8$^+$ T cells were activated with anti-CD3 and anti-CD28 mAbs and co-cultured with MSC-A or left untreated without MSCs. After 24h, Clone 4 CD8$^+$ T cells were harvested and the expression of intracellular T-bet and Eomes was analyzed by FACS. **C** and **F**. Naïve Clone 4 CD8$^+$ T cells were mitocepted with isolated mitochondria from MSC-A or mock mitocepted. 12h later, CD8$^+$ T cells were activated with anti-CD3 and anti-CD28 mAbs and cultured for 24 h. Expression of intracellular T-bet and Eomes was analyzed by FACS. Shaded histograms represent isotype-matched antibody controls. Data from one representative experiment described in Fig. 5 is presented.

## Figure S7

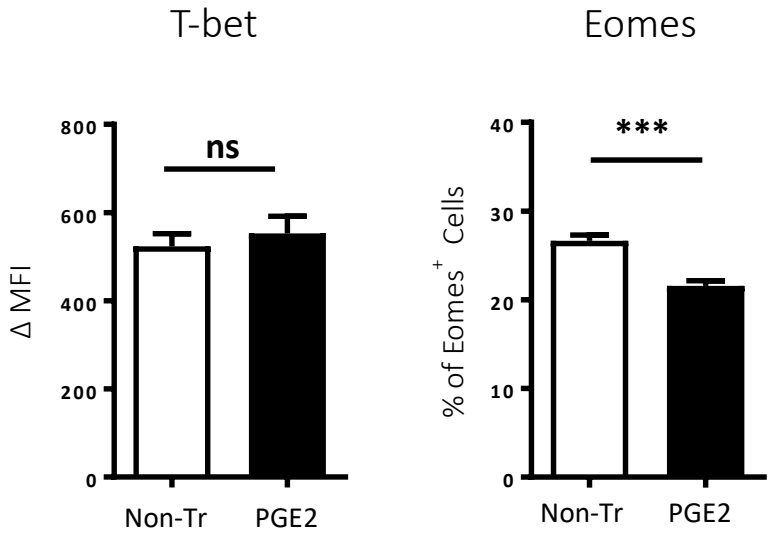


**Figure supplementary 7**. **Modulation of T-bet and Eomes expression by PGE2 in activated CD8$^+$ T cells.** Naïve purified Clone 4 CD8$^+$ T cells were activated with anti-CD3 and anti-CD28 mAbs and cultured in the presence of PGE2 at a final 1 ng/ml concentration or left untreated. After 24h, CD8$^+$ T cells were harvested and intracellular T-bet and Eomes expression was analyzed by FACS. Data from 3 independent experiments is presented. Values are represented as mean ± SEM.