Suppl. Figure 1. Absence of STAT1 in donor lymphocytes leads to significantly less morbidity in mHA-mismatched setting.

Induction of GVHD in the MHC-matched mHA-mismatched 129[$H2^b$] to B6[$H2^b$] strain combination. Lethally irradiated (1075cGy) B6 mice received $5x10^6$ BMC and $4x10^7$ splenocytes from either 129.$STAT1^{-/-}$ (△) or 129.$STAT1^{+/+}$ (▲) mice. Morbidity was assessed by weight changes in B6 recipients of 129.$STAT1^{+/+}$ or 129.$STAT1^{-/-}$ spleen cells. # $p<0.01$ at all indicated time points. One representative experiment with 6-7 animals per group from a total 3 is shown.

Suppl. Figure 2. Absence of STAT1 signaling in donor grafts does not impair engraftment.

GVHD was induced in BALB/c mice following fully MHC-mismatched BMT using 129.$STAT1^{+/+}$ or 129.$STAT1^{-/-}$ splenocytes. On day+6 post-BMT, donor chimerism was assessed in myeloid cells and B and T lymphocytes from bone marrow or spleen. 5 animals were studied per group. Data represent mean ± SEM.

Suppl. Figure 3. Absence of STAT1 in donor lymphocytes leads to delayed GVHD in MHC-mismatched BMT setting.

A) Lethally irradiated (800cGy) BALB/c mice received $5x10^6$ TCD BMC and $3x10^6$ purified $CD4^+$ cells from either $STAT1^{+/+}$ or $STAT1^{-/-}$ mice. Clinical GVHD score was monitored over time. **B**) GVHD-associated tissue damages were assessed in small intestine, colon, and liver from recipients of syngeneic (SYN) and $STAT1^{-/-}$ TCD BMCs plus $STAT1^{-/-}$ $CD4^+$ cells on day+23 post-BMT. **C**) GVHD was induced in the fully MHC-mismatched [129Sv($H2^b$) to BALB/c ($H2^d$)] strain combination using 129.$STAT1^{-/-}$ or 129.$STAT1^{+/+}$ splenocytes. CD44 and CD62L

expression on donor $CD4^+$ cells were studied on day+14 post-BMT. Data represent mean ± SEM with 3 animals per group. **D**) Anti-host reactivity was analyzed by MLR assay by studying the proliferation of CFSE-labeled splenocytes (SPC) from recipients of $STAT1^{-/-}$ grafts (BMT $STAT1^{-/-}$) on day+14 post BMT versus SPC from naïve $STAT1^{-/-}$ mice ($nSTAT1^{-/-}$). Responder cells were stimulated for 3 days in the absence (upper row) or presence of irradiated BALB/c SPC. Upper panel shows proliferative response of a day+14 post-BMT animal against medium. Middle panel shows proliferative response of naïve STAT1 responder against BALB/c stimulators. Lower panel shows proliferative response of a day+14 post-BMT $STAT1^{-/-}$ animal against BALB/c stimulators. Numbers in the histogram represent the percentages of proliferating $CFSE^{lo}$ cells.

Suppl. Figure 4. Activation and in vivo expansion of STAT1-deficient T cells in MHC-mismatched allogeneic BMT.

GVHD was induced in the fully MHC-mismatched [129Sv($H2^b$) to BALB/c ($H2^d$)] strain combination using $129.STAT1^{-/-}$ or $129.STAT1^{+/+}$ pan-T cells labeled with 5μM CFSE. On day +6 post-BMT, animals (3-4 in each group) were sacrificed, and splenocytes were analyzed by FCM. **A**) The absolute cell numbers of donor-derived $CD4^+$ and $CD8^+$ cells in host spleens were calculated. **B**) In vivo proliferation of donor $CD4^+$ or $CD8^+$ T cells was studied by CFSE-dilution. Percentages of slowly dividing cells ($CFSE^{hi}$) in donor $CD4^+$ and $CD8^+$ cells are shown. **C**) Summary of CD25 expression in donor $CD4^+$ or $CD8^+$ cells. **D**) Representative dot plots show CD25 expression and CFSE dilution for assessment of in vivo proliferation of donor $CD4^+$ or $CD8^+$ T cells. Numbers represent the percentages of cells present in the given quadrant. **E**) Percentage of $CD44^+CD62L^-$ in donor $CD4^+$ cells. **F**) Percentage of apoptotic cells in donor

$CD4^+CD25^+$ cells assessed by Annexin V staining. Representative results from one of 5 independent experiments are shown. Data represent mean ± SEM.

Suppl. Figure 5. Lack of STAT1 in lymphocytes leads to enhanced in vitro proliferation.

Freshly spleen cells (**A**) or $CD4^+$ T cells (**B**) from $STAT1^{+/+}$ or $STAT1^{-/-}$ mice were stimulated with irradiated maturated BALB/C BM-derived dendritic cells at the indicated DC/Responder ratios for 5 days. Proliferation of responder cells was assessed by ^3H-incorporation. Results are given as mean ± SEM; $*p<0.01$. Representative results from one of 3 independent experiments are shown.

Suppl. Figure 6. nT_{reg} in spleen and thymus of $STAT1^{+/+}$ and $STAT1^{-/-}$ mice.

A-B) $CD4^+CD25^+Foxp3^+$ T_{reg} cells were enumerated in the spleens and thymi of $129.STAT1^{+/+}$ and $129.STAT1^{-/-}$ mice. Results are given as mean ± SEM; $*p<0.05$. One experiment with 3 animals per group is shown.

Suppl. Figure 7. Characterization of T_{reg} cells.

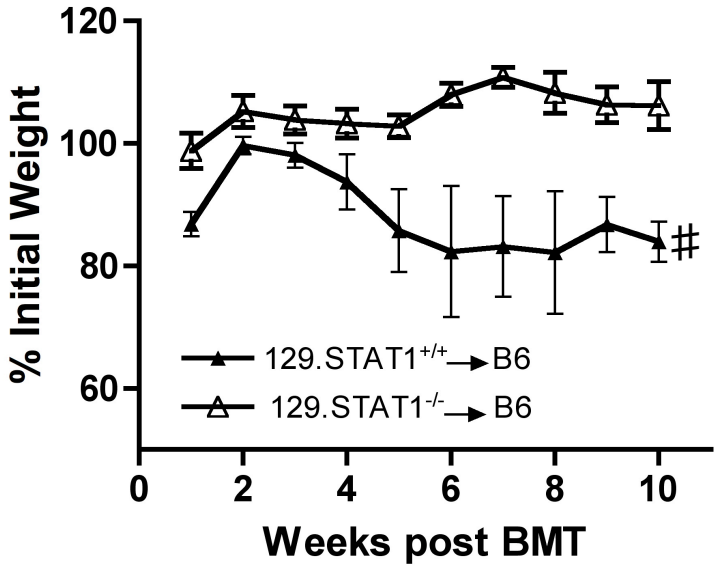
A-B) GVHD was induced in the fully MHC-mismatched [129Sv($H2^b$) to BALB/c ($H2^d$)] strain combination, and splenocytes were harvested on day+6 following BMT and examined by FCM for expression of CTLA4 and GITR in donor $CD4^+CD25^+Foxp3^+$ T_{reg} cells. Relative proportion (**A**) and absolute numbers (**B**) are shown. Data from one out of 3 independent experiments are shown. **C**) Freshly purified $STAT1^{+/+}$ or $STAT1^{-/-}$ $CD4^+CD25^+$ cells were cultured with α-

CD3/α-CD28 antibodies in the presence of IL-2 for 3 days, and supernatant was studied for TGF-β1 secretion by ELISA. Representative results from 2 independent experiments are shown.

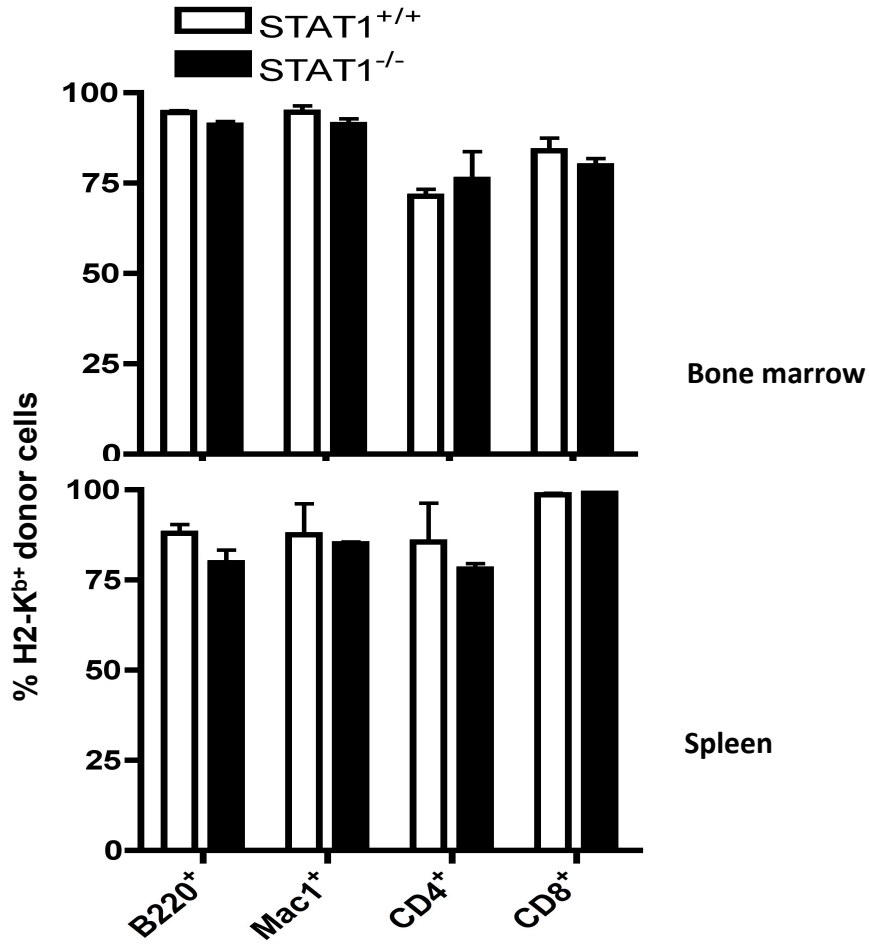
Suppl. Figure 8. In vivo suppressive function of T_{reg} cells.

Lethally irradiated BALB/c mice were reconstituted with $5x10^6$ $STAT1^{+/+}$ TCD BMC plus $5x10^5$ $STAT1^{+/+}$ pan-T cells for induction of GVHD. In vitro expanded $STAT1^{+/+}$ or $STAT1^{-/-}$ $CD4^+CD25^+$ nT_{reg} cells were added at 1:1 ratio. Morbidity was assessed by weight changes in BALB/c recipients of $STAT1^{+/+}$ or $STAT1^{-/-}$ nT_{reg} cells. *$p<0.05$ at all indicated time points. Representative experiment is shown with 5-6 animals per group.

Suppl. Figure 1



Suppl. Figure 2



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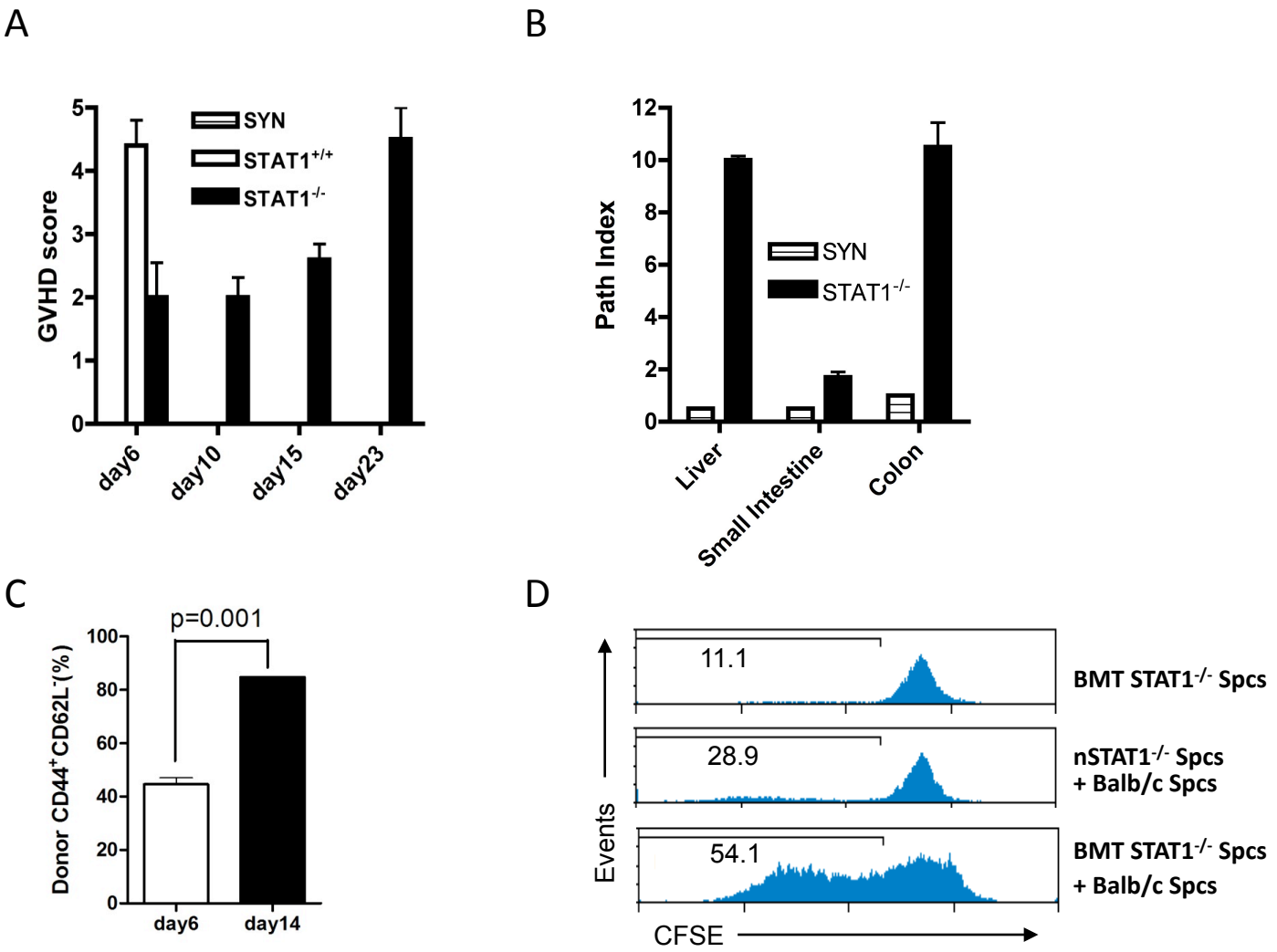

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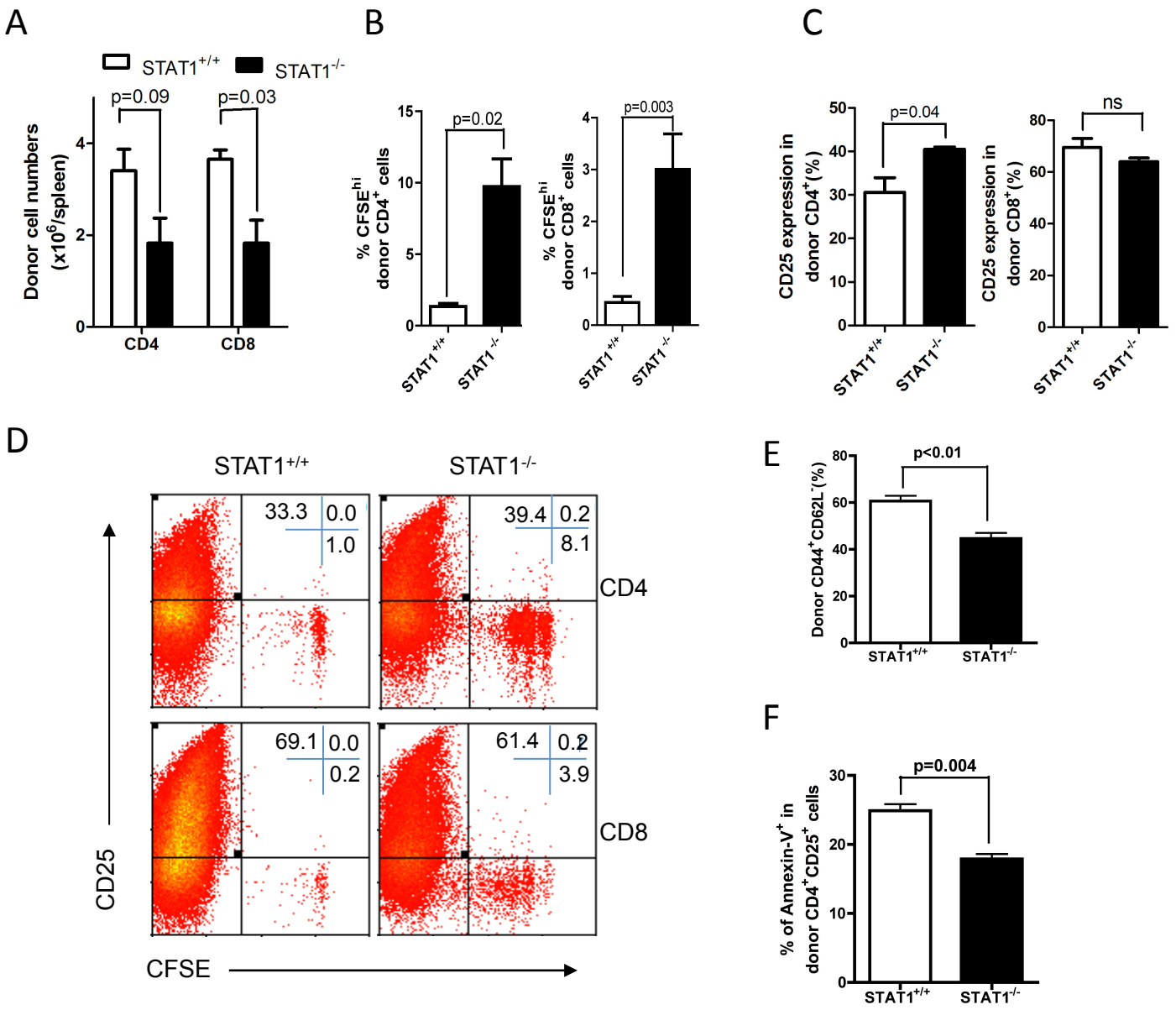

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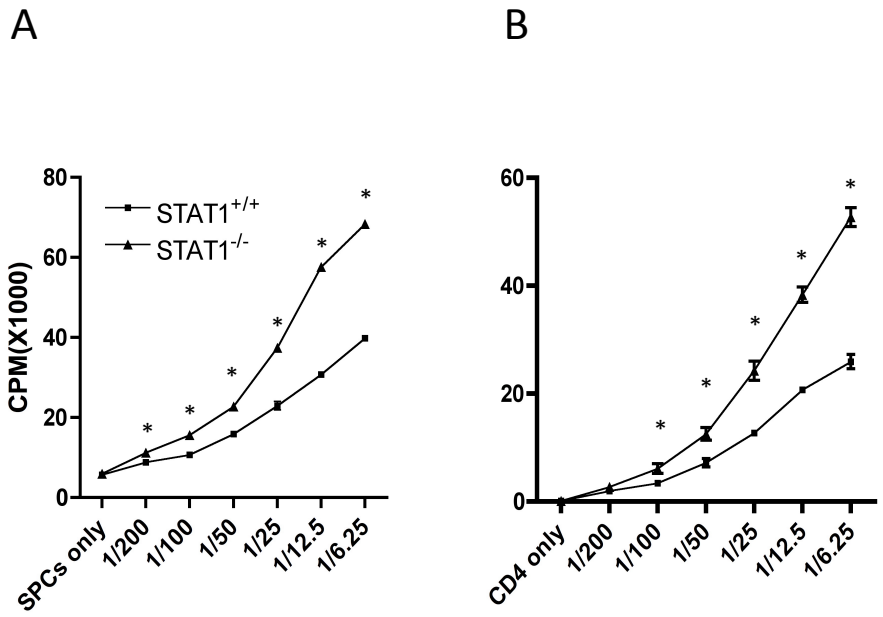

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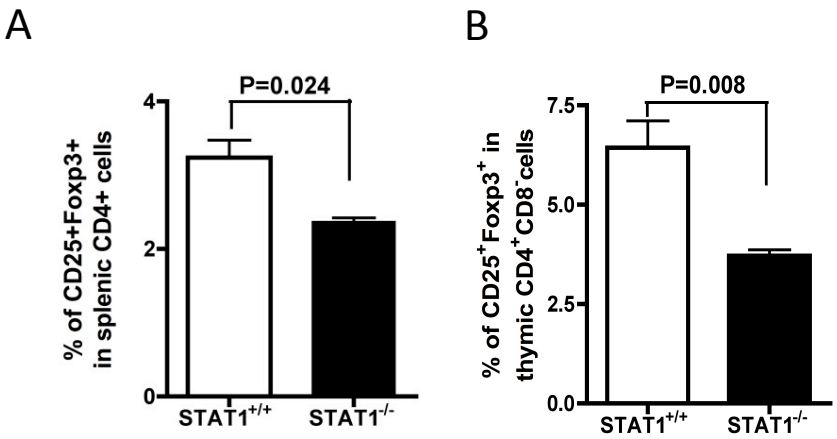

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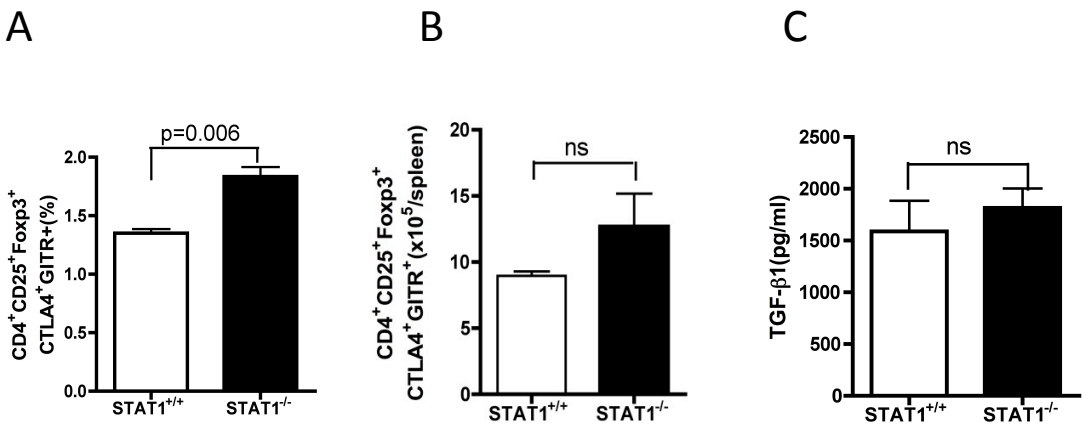

Suppl. Figure 8