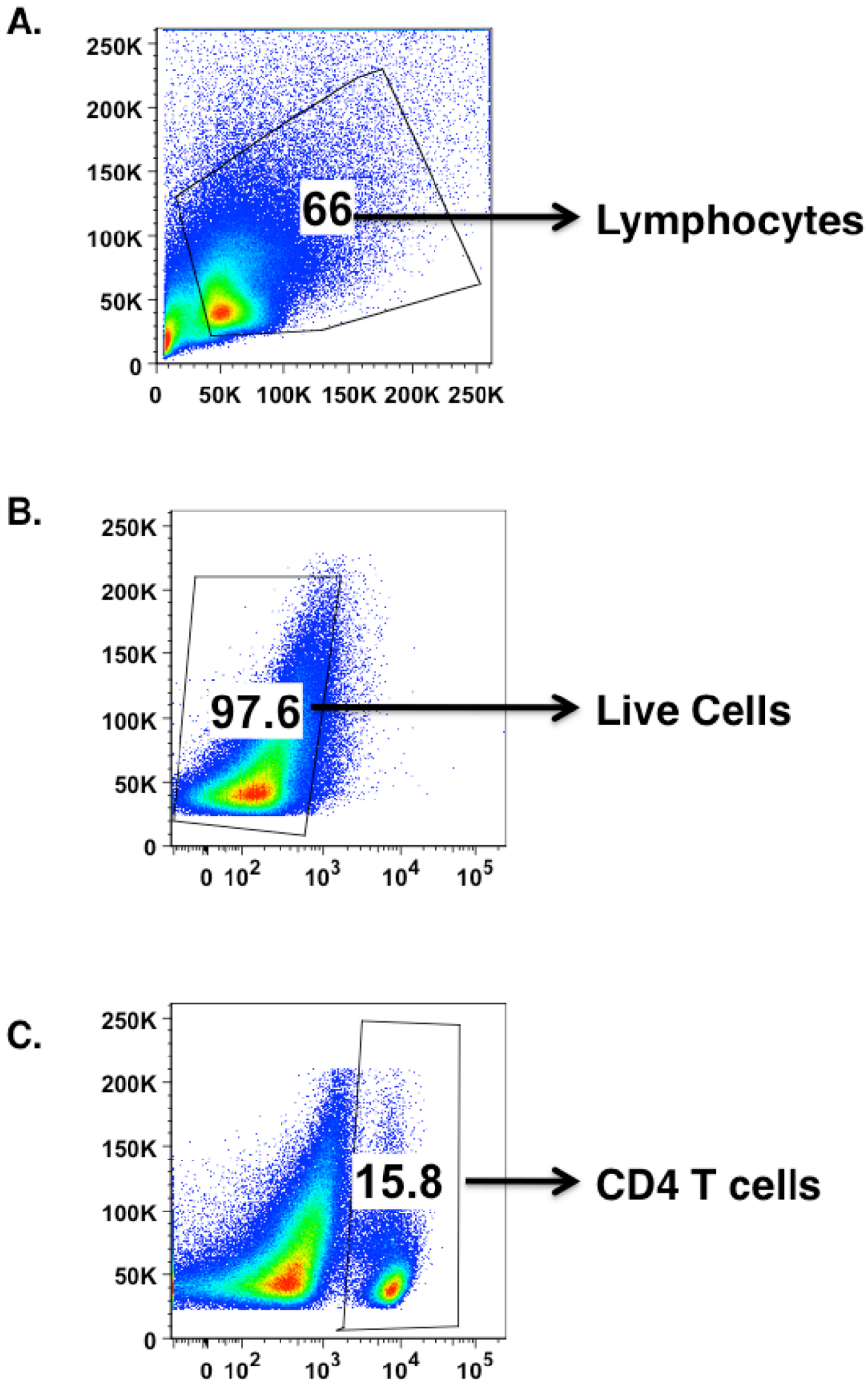
SUPPLEMENTARY DATA

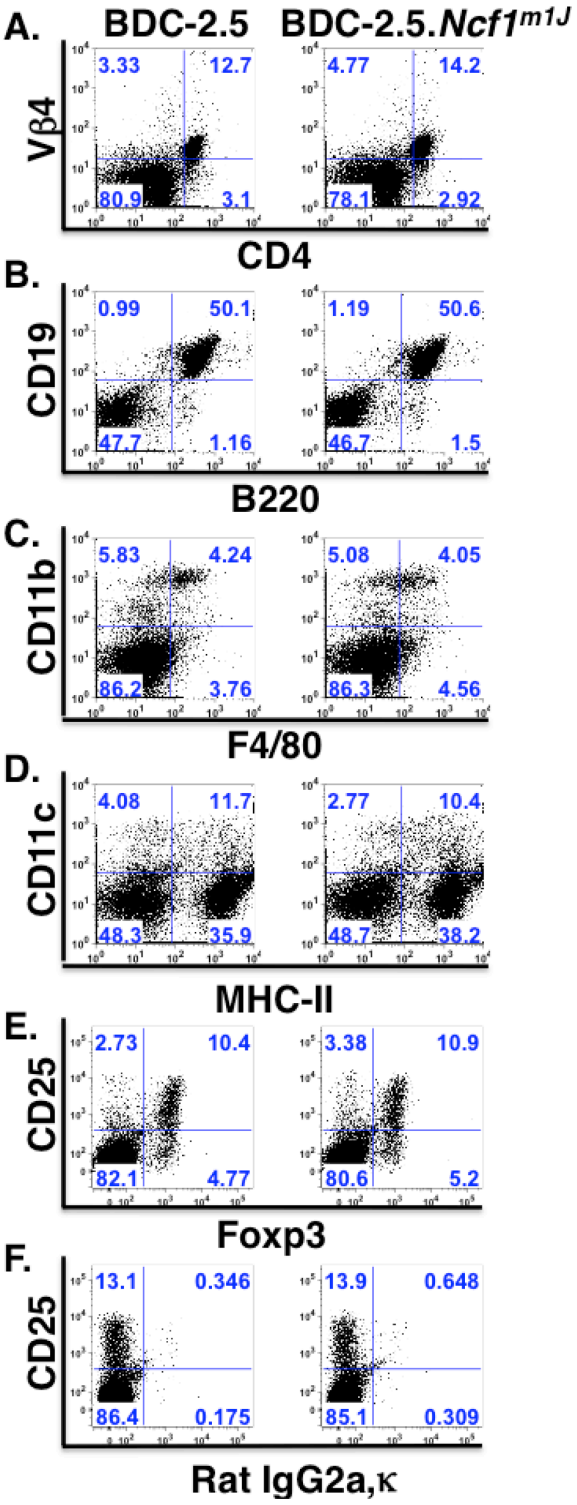
Supplementary Figure 1. Gating strategy for T lymphocyte, B lymphocyte, macrophages, dendritic cells, and T regulatory cells within BDC-2.5 and BDC-2.5.*Ncf1*^{m1J} splenocytes. Lymphocytes were gated using FSC/SSC profile (**A**). Subsequently live cells were gated (**B**), and expression of CD25 by Foxp3/rat Ig2a,κ was shown by live CD4^{+} T cells (**C**).



©2015 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db15-0546/-/DC1

SUPPLEMENTARY DATA

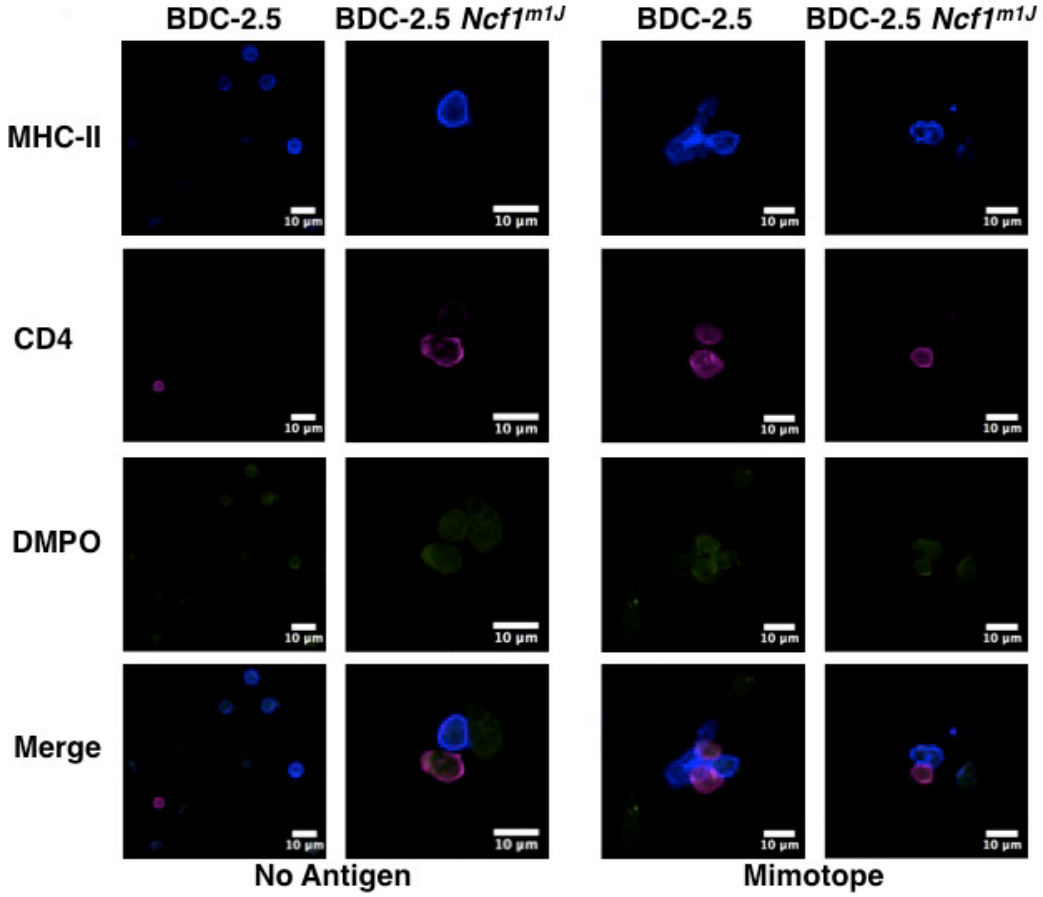
Supplementary Figure 2. BDC-2.5 and BDC-2.5.*Ncf1*^{m1J} mice displayed similar percentages of Vβ4-expressing CD4 T lymphocytes, B lymphocytes, macrophages, dendritic cells, and regulatory CD4 T cells. Flow cytometric analysis of (**A**) Vβ4, CD4 T lymphocytes, (**B**) B220, CD19 B lymphocytes, (**C**) CD11b, F4/80 macrophages, (**D**) I-A^{g7}, CD11c dendritic cells, (**E**) CD4, CD25, Foxp3 regulatory CD4 T cells, and (**F**) isotype control (rat IgG2a,κ) staining to verify Foxp3 specificity from BDC-2.5 and BDC-2.5.*Ncf1*^{m1J} splenocytes.



©2015 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db15-0546/-/DC1

SUPPLEMENTARY DATA

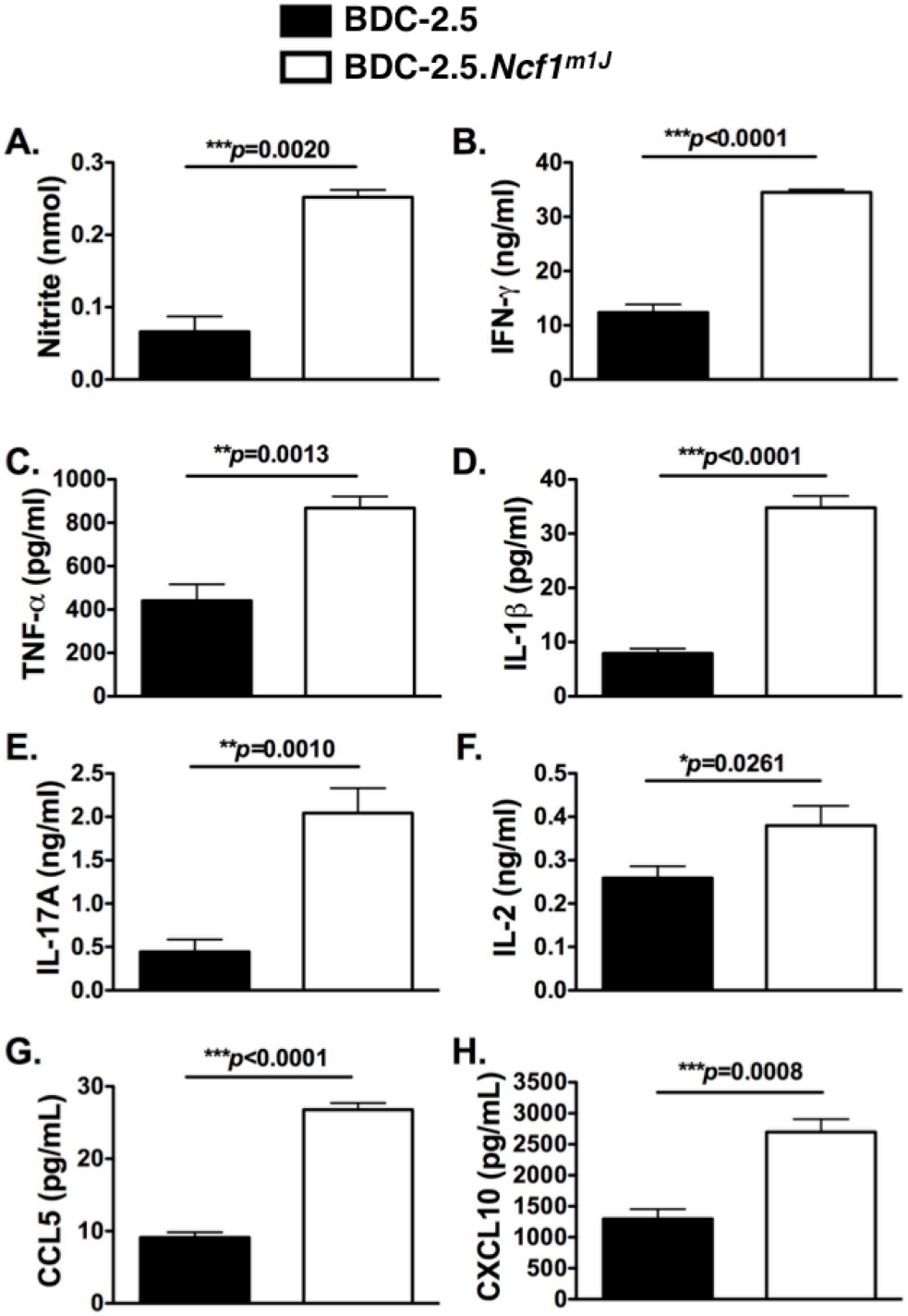
Supplementary Figure 3. BDC-2.5 and BDC-2.5.*Ncf1*^{m1J} splenocytes exhibited minimal self-oxidation upon mimotope stimulation. Immunofluorescence identification of CD4 T cells (Alexa Fluor 647), MHC-II (OX-6; PE), and DMPO adducts (FITC) within BDC-2.5 and BDC-2.5.*Ncf1*^{m1J} splenocytes stimulated with 1µM BDC-2.5 mimotope for 12 hours in the absence of DMPO addition.



©2015 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db15-0546/-/DC1

SUPPLEMENTARY DATA

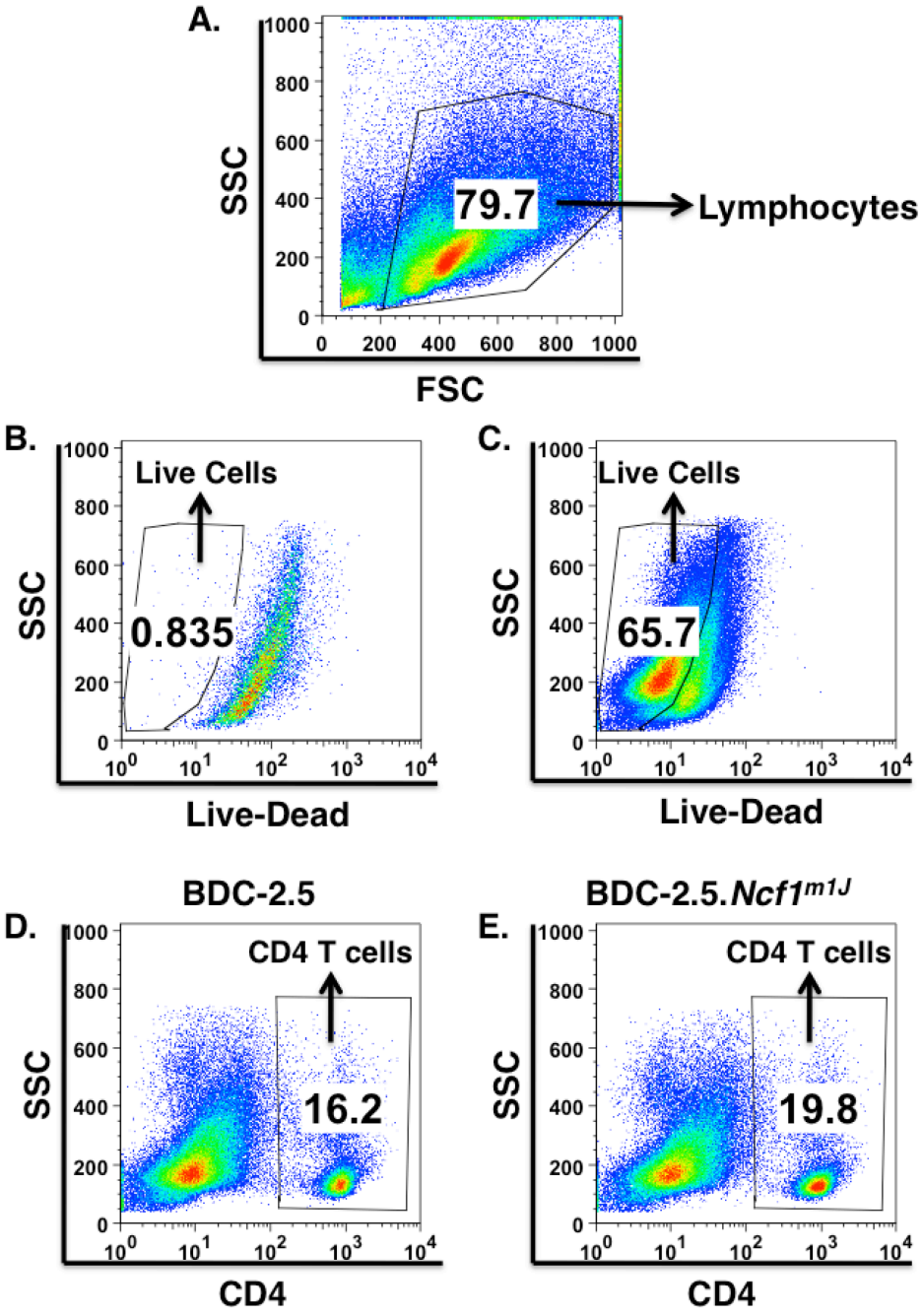
Supplementary Figure 4. BDC-2.5.*Ncf1*^{m1J} CD4 T cells displayed enhanced production of nitrite, pro-inflammatory cytokines and chemokines. Supernatants from BDC-2.5 and BDC-2.5.*Ncf1*^{m1J} CD4 T cells stimulated for 48 hours with plate bound anti-CD3ε (0.1μg/mL), anti-CD28 (1μg/mL) were assayed for production of (**A**) nitrite, (**B**) IFN-γ, (**C**) TNF-α, (**D**) IL-1β, (**E**) IL-17A, (**F**) IL-2, (**G**) CCL5, and (**H**) CXCL10. Data shown represent average of 3 experiments performed in triplicate. **p*<0.05; ***p*<0.01; ****p*<0.0001.



©2015 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db15-0546/-/DC1

SUPPLEMENTARY DATA

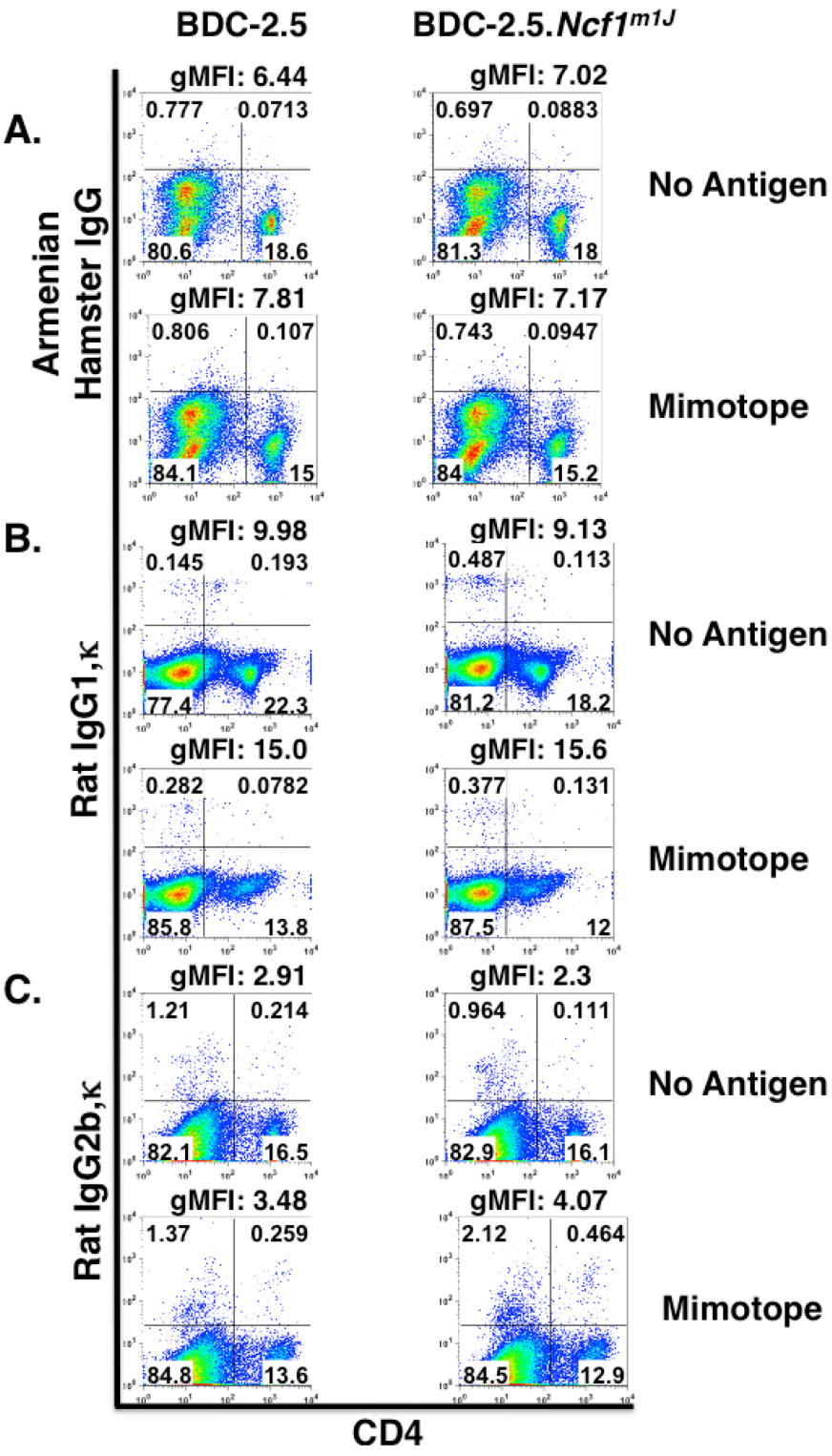
Supplementary Figure 5. Gating strategy for CD4 T cell activation markers by BDC-2.5 and BDC-2.5.*Ncf1*^{m1J}. Lymphocytes were gated using FSC/SSC profile (**A**). Live cells were gated using a fixable live-dead dye with a positive control of heat-killed cells (**B**), and representative live cell gating (**C**). Expression of T cell activation markers via geometric MFI (gMFI) was calculated on BDC-2.5 (**D**) and BDC-2.5.*Ncf1*^{m1J} (**E**) CD4 T cells.



©2015 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db15-0546/-/DC1

SUPPLEMENTARY DATA

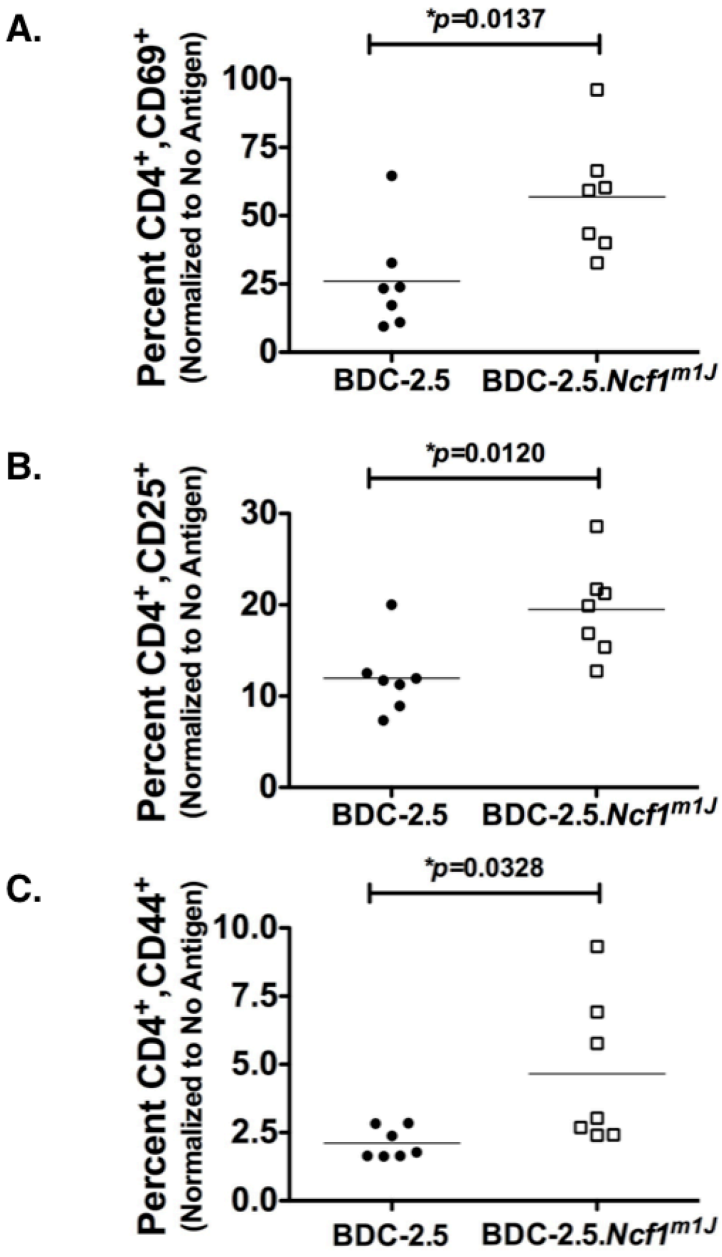
Supplementary Figure 6. BDC-2.5 and BDC-2.5.*Ncf1*^{m1J} splenocytes exhibited minimal expression with isotype control staining. Flow cytometric analysis of Armenian hamster IgG (**A**), rat IgG1,κ (**B**), and rat IgG2b,κ (**C**) by live CD4 T cells within BDC-2.5 and BDC-2.5.*Ncf1*^{m1J} splenocytes at 24 hours post stimulation with mimotope. Upper panels represent no antigen, while lower panels represent mimotope stimulation.



©2015 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db15-0546/-/DC1

SUPPLEMENTARY DATA

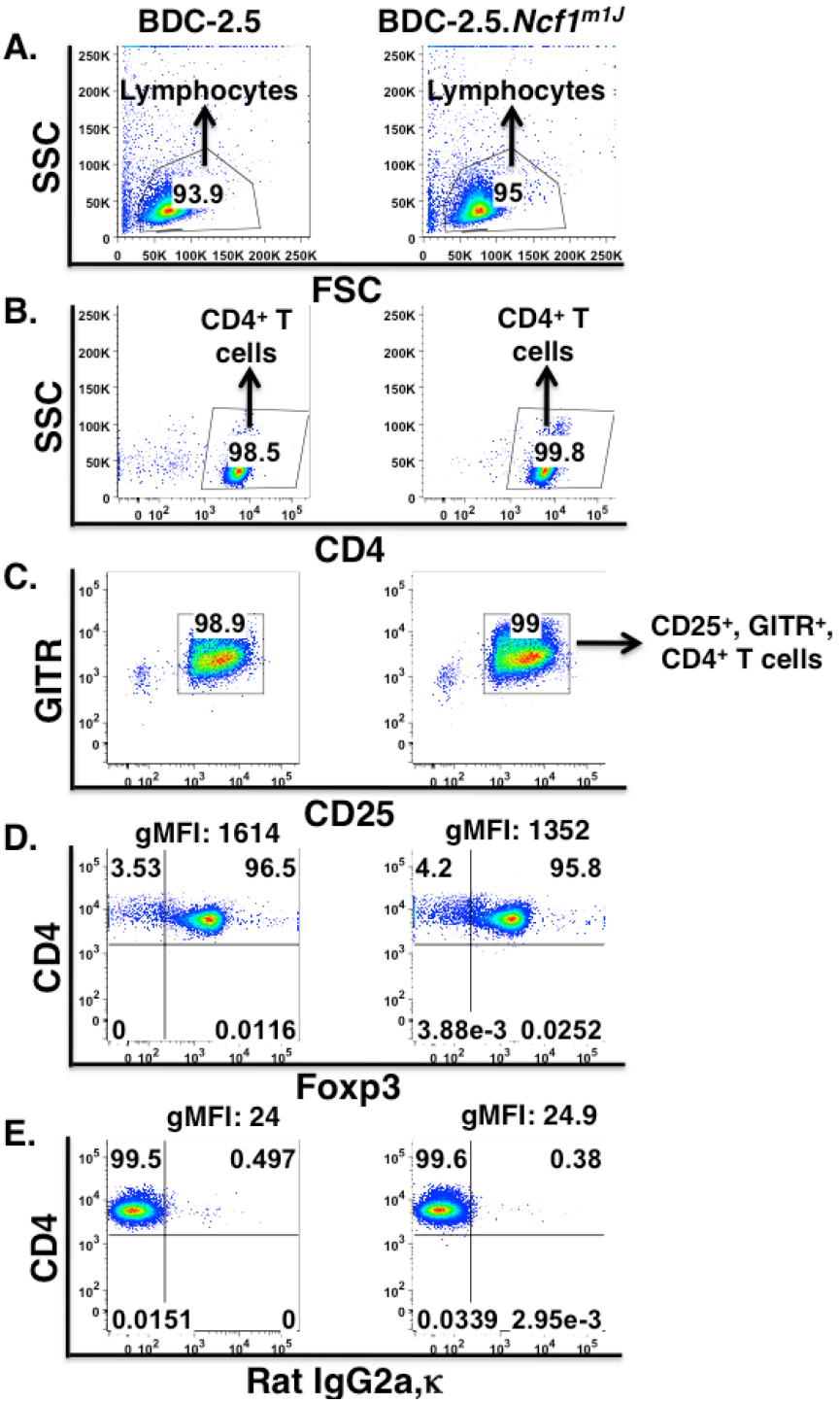
Supplementary Figure 7. BDC-2.5.*Ncf1*^{m1J}. CD4 T cells displayed enhanced CD69, CD25, and CD44 upon mimotope stimulation from pooled experiments. Frequency of CD69^{+}, CD4^{+} (**A**), CD25^{+}, CD4^{+} (**B**), and CD44^{+}, CD4^{+} (**C**) T cells of mimotope-stimulated BDC-2.5 and BDC-2.5.*Ncf1*^{m1J} splenocytes. Values were calculated by normalizing to no antigen. Data shown represent pooled mice from 3 independent experiments.



©2015 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db15-0546/-/DC1

SUPPLEMENTARY DATA

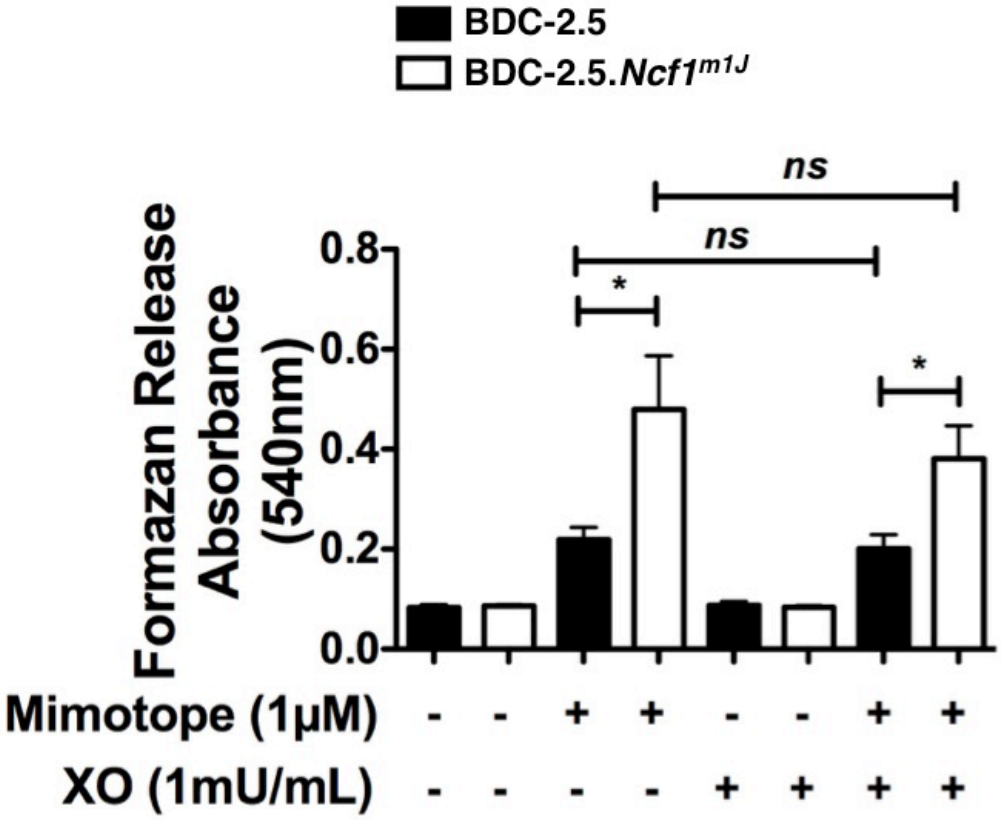
Supplementary Figure 8. Gating strategy reveals similar Foxp3 expression by FACS-sorted BDC-2.5 and BDC-2.5.*Ncf1*^{m1J} Tregs. Lymphocytes within FACS-sorted CD4$^+$, CD25$^+$, and GITR$^+$ BDC-2.5 and BDC-2.5.*Ncf1*^{m1J} T regulatory cells were gated using FSC/SSC profile (**A**). CD4 T cells were gated via SSC (**B**) and CD25$^+$, GITR$^+$ T cells (**C**) were subsequently selected for Foxp3 analysis by intracellular staining (**D**). Specificity of intracellular stain was verified by isotype control staining (**E**). Geometric MFI was calculated from CD4$^+$, CD25$^+$, and GITR$^+$ T cells.



©2015 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db15-0546/-/DC1

SUPPLEMENTARY DATA

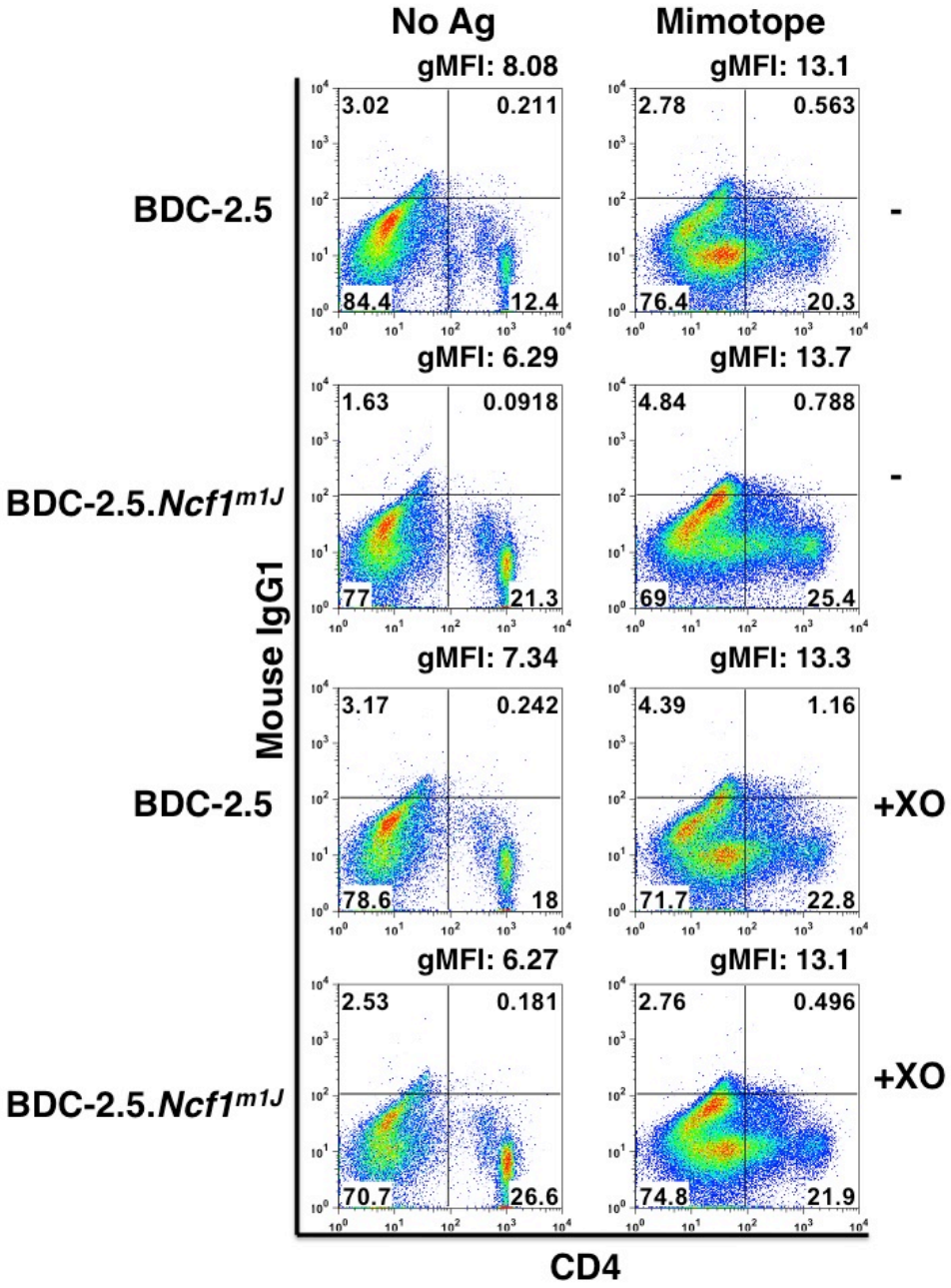
Supplementary Figure 9. BDC-2.5.*Ncf1*^{m1J} splenocytes have an increased proliferative potential in contrast to BDC-2.5 splenocytes. T cell proliferation and formazan release was determined by an MTT assay of NOD.BDC-2.5 and NOD.BDC-2.5.*Ncf1*^{m1J} splenocytes after stimulation with 1μM BDC-2.5 mimotope for 24 hours in the presence or absence of XO (1mU/mL). Data shown represent average of 3 experiments performed in triplicate. *p<0.05.



©2015 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db15-0546/-/DC1

SUPPLEMENTARY DATA

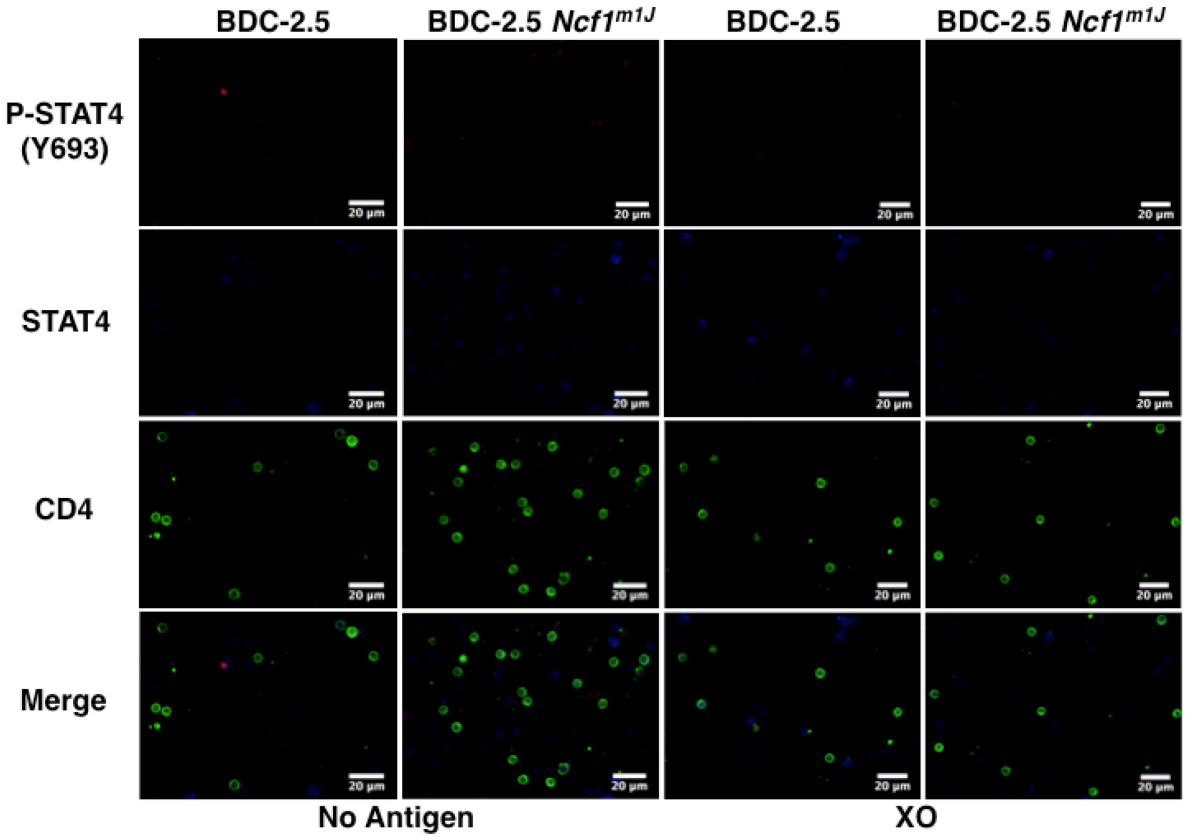
Supplementary Figure 10. Isotype control staining for IL-12Rβ2 by BDC-2.5 and BDC-2.5.*Ncf1*^{m1J} splenocytes. Flow cytometric analysis of mouse IgG1 by live CD4 T cells within BDC-2.5 and BDC-2.5.*Ncf1*^{m1J} splenocytes stimulated with 1μM Mimotope in the presence or absence of XO for 72 hours.



©2015 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db15-0546/-/DC1

SUPPLEMENTARY DATA

Supplementary Figure 11. BDC-2.5 and BDC-2.5.*Ncf1*^{m1J} splenocytes displayed minimal P-STAT4 (Y693) activation with xanthine oxidase treatment alone. Immunofluorescence identification of CD4 T cells (Alexa Fluor 647), STAT4 (Cy3), and P-STAT4 (Y693) (Alexa Fluor 488) by BDC-2.5 and BDC-2.5.*Ncf1*^{m1J} splenocytes in the presence or absence of 1mU/mL XO for 24 hours.



©2015 American Diabetes Association. Published online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db15-0546/-/DC1