Immunity, Volume *29*

Supplemental Data

Plasmacytoid Dendritic Cells Mediate Oral Tolerance

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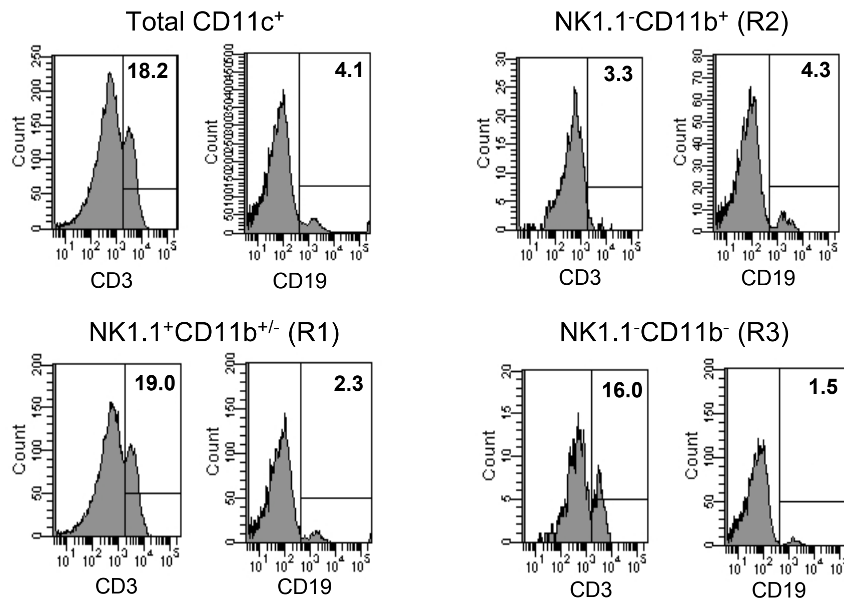
Please find Figures S1-S4 below.

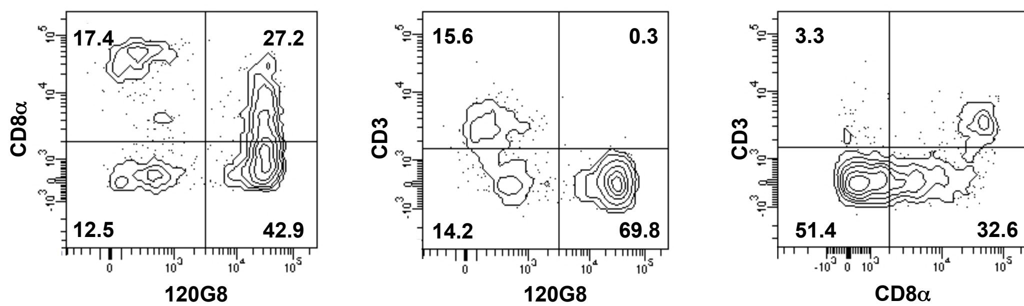
A

Phenotype of $CD11c^+$ liver cell subsets



B

Phenotype of $CD11c^+CD11b^-NK1.1^-$ liver cells



C

Phenotype of $120G8^+$ liver cells

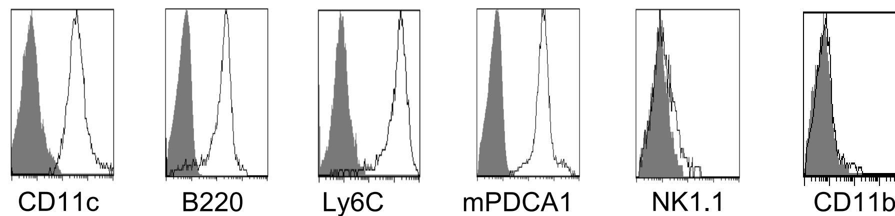


Figure S1: Phenotype of liver $CD11c^+$ subsets

(A,B) Liver leukocytes were stained for 6C flow cytometry with anti-BST2-Alexa488 (120G8 mAb), anti-NK1.1-PE, anti-CD11b-PerCP-Cy5.5, anti-CD11c-APC, anti-CD8-PE-Cy7 and either biotinylated anti-CD3 or anti-CD19 mAb followed by incubation with APC-Cy7-labeled-streptavidin. Cells were analyzed with a FACSCanto using the DIVA software (BD Biosciences). (A) Frequency of T and B cells in the various subsets of $CD11c^+$ liver cells depicted in Fig. 3B. (B) Frequency of BST-2^+ pDC in liver $CD11c^+NK1.1^-CD11b^-$ cells. (C) $120G8^+$ liver cells were analyzed by flow cytometry for the expression of CD11c, CD11b, B220, Ly6C, BST2 (mPDCA1 mAb) and NK1.1 using specific mAbs (white histograms). Staining with isotype-matched irrelevant antibodies was used as a negative control (grey histograms).These data are representative of 2 (A,B) to 4 experiments (C).

Goubier et al. Supplementary Fig. 1

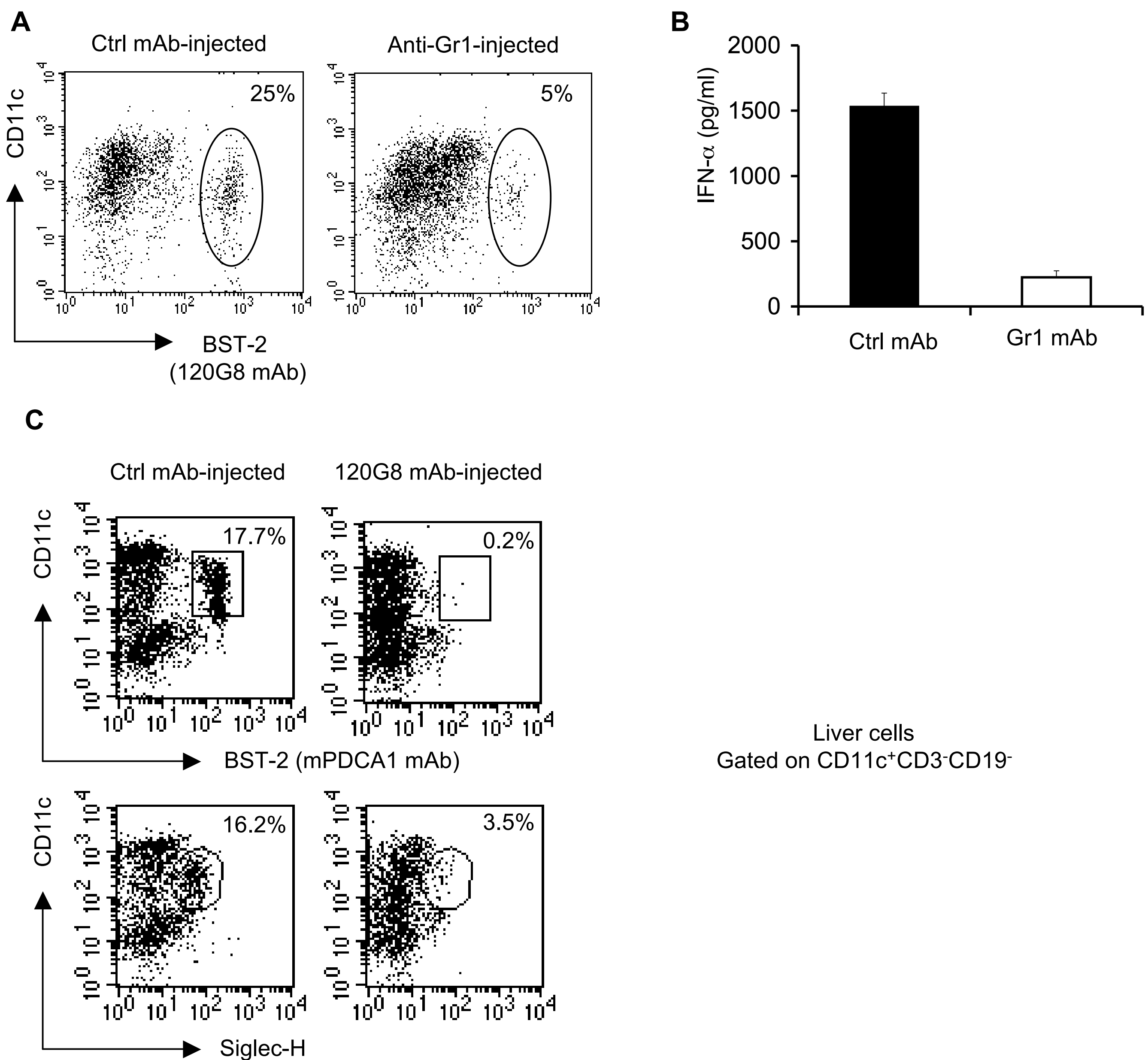
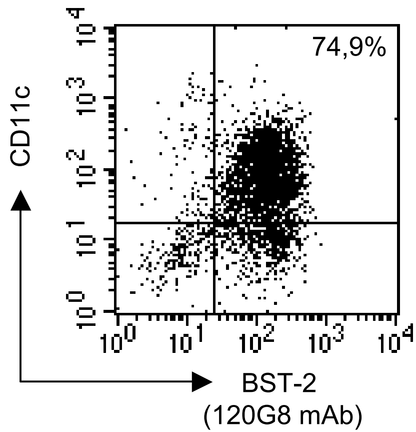


Figure S2: efficacy of pDC depletion in vivo

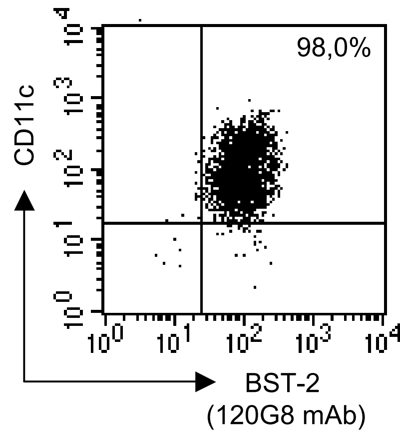
(A, B) Efficiency of pDC depletion following anti-Gr1 mAb treatment was analyzed on MACS-sorted liver $CD11c^+$ leukocytes 48 hours after the last mAb injection by flow cytometry analysis of CD11c and BST-2 (120G8 mAb) expression (A) or ability to produce IFN-α upon virus exposure (B). Briefly, cells (10^6 cells/ml) were cultured with 10^4 hemagglutinin units/ml of formaldehyde-inactivated human influenza virus (strain New Caledonia, NK/TM/138/00, From Aventis Pasteur, Val de Reuil, France). IFN-α production in 18-h culture supernatant was titrated using an ELISA kit (PBL Biomedical laboratories, Newbrunswick, NJ). (C) Efficacy of pDC depletion following 120G8 treatment was checked by analysis of CD11c and BST-2 (mPDCA1 mAb) and CD11c and Siglec-H (440c mAb) expression on liver $CD3^-CD19^-$ leukocytes 48 hours after the last mAb injection .

Goubier et al. Supplementary Fig. 2

A MACS-enriched pDC



B FACSsorted pDC



C Phenotype of CD11c^{int}BST2^{+} liver cells

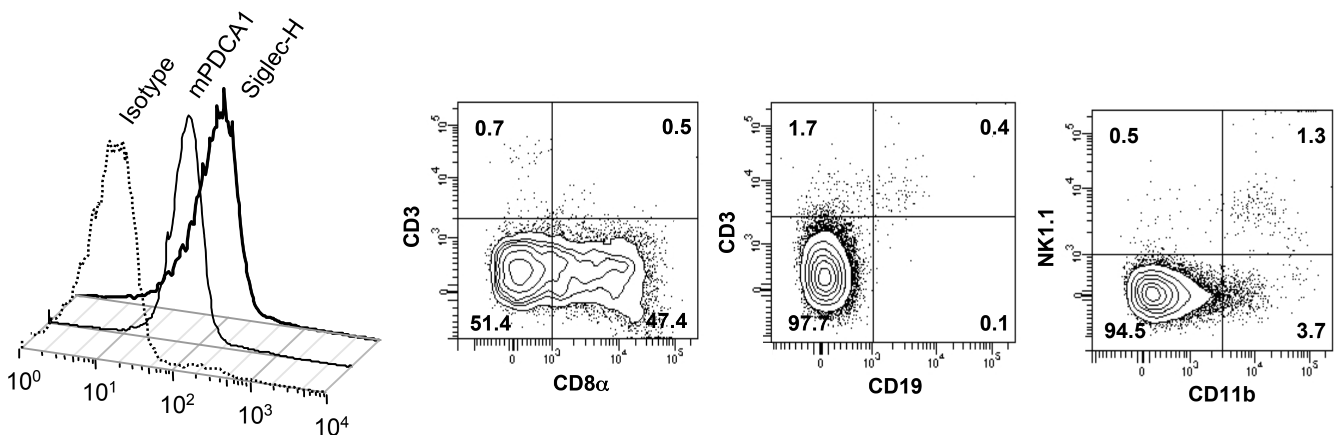


Figure S3: Purity of liver pDC used for transfer experiments

(A) pDC were enriched by MACSsorting from leukocytes using mPDCA1-coated microbeads and analyzed for the expression of CD11c and BST-2 (120G8 mAb). MACS-enriched pDC contained 70-80% CD11c^{int}BST-2^{+} cells (mean: 76.8%).

(B) For certain adoptive transfer experiments MACS-enriched pDC were further stained with Alexa488-conjugated anti-BST2 (120G8 mAb) and PE-anti-CD11c and FACSsorted into CD11c^{int}BST-2^{+} cells (>97% purity).

(C) CD11c^{int}BST-2^{+} cells were stained with biotynilated anti-Siglec-H (440c) or anti-BST2 (mPDCA1) mAb and streptavidin-PerCP-Cy5.5, or combination of anti-CD8α-PE and -CD3-PerCP-Cy5.5, anti-CD3-PE and -CD19-PerCP-Cy5.5, anti-NK1.1-PE and -CD11b-PerCP-Cy5.5, and analyzed by 4C flow cytometry using a FACSCanto (BD Biosciences). .

Goubier et al. Supplementary Fig. 3

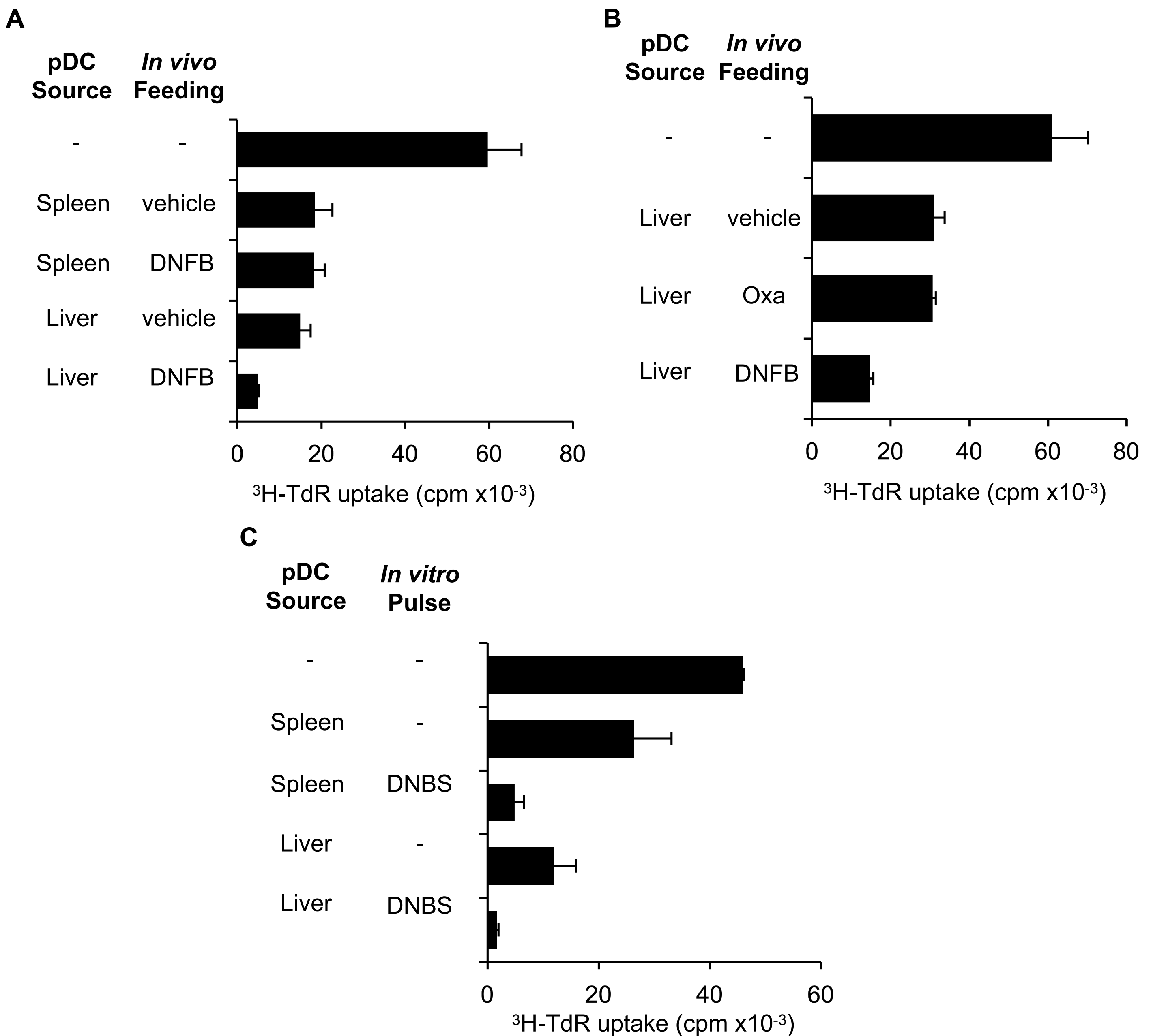


Figure S4: Liver pDC suppress the proliferation of hapten-specific CD8+ T cells in vitro
Hapten-specific CD8+ T cells purified from DNFB-sensitized mice were cultured for 3 days with DNBS-pulsed BM-DC in the presence or absence of *ex vivo* purified pDC. (A-B) pDC were MACS-enriched from the liver or spleen of mice that were fed 18 hours earliear with DNFB, OXA or vehicle as control. (C) pDC were MACS-enriched from the liver of naïve mice and pulsed with DNBS *in vitro* before culture with CD8+ T cells. T cell proliferation was determined by [^{3}H]thymidine uptake during the last 8 h of culture. Results are expressed as mean of cpm ± SD of triplicate wells. No cpm above background were detected in cultures with un-pulsed BM-DC (not shown). Data are representative of 1 out of 2 experiments.