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Supporting Information

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Tolerance induction towards cardiac allografts under costimulation blockade is impaired in CCR7-deficient animals but can be restored by adoptive transfer of syngeneic plasmacytoid dendritic cells

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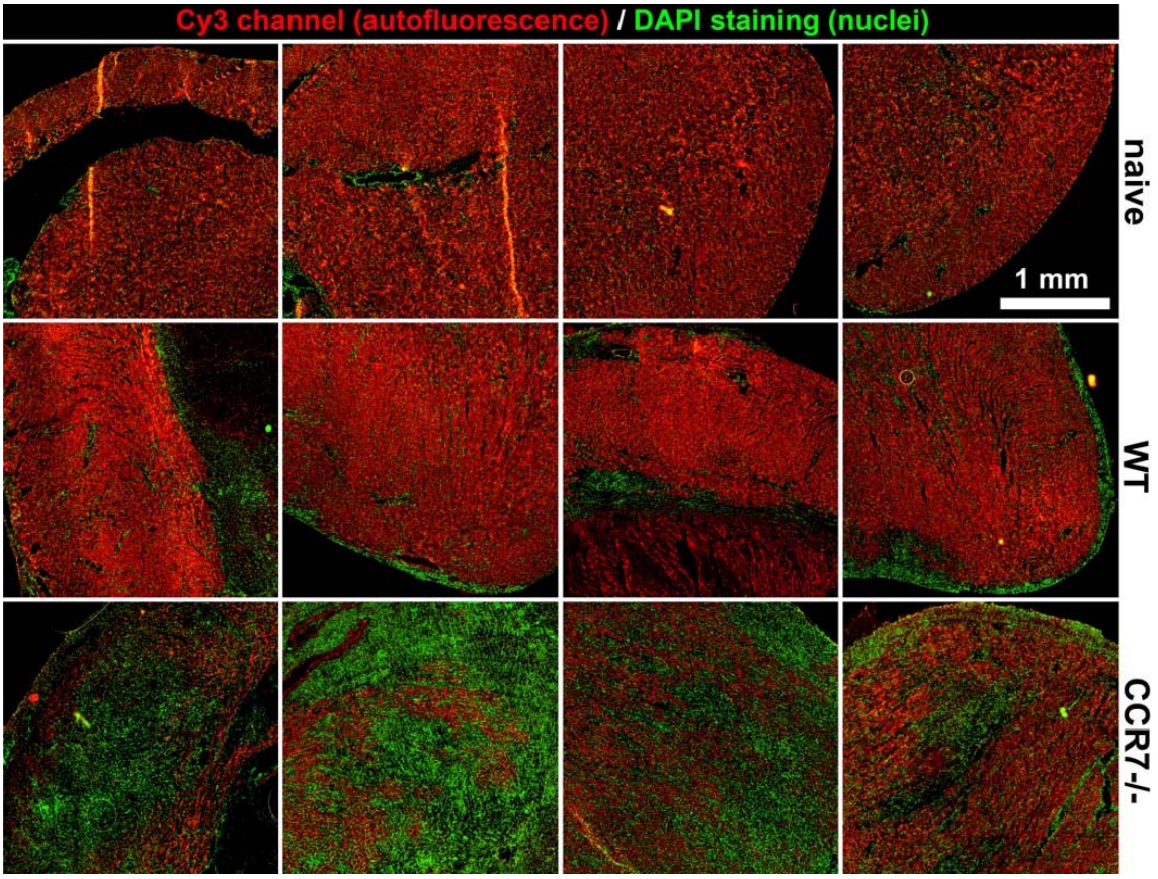
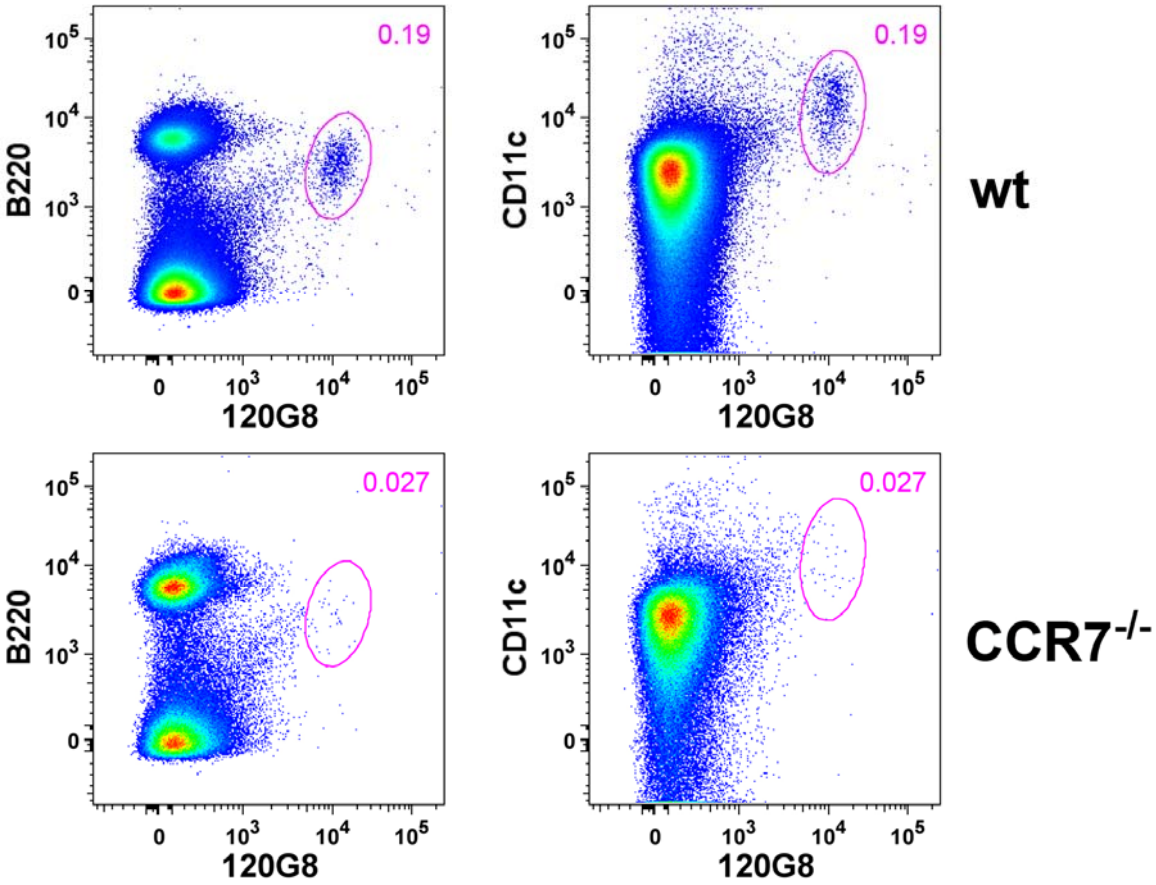
Supplementary Figure S1



Histological analysis of leukocyte infiltrations within naïve hearts and cardiac allograft transplants at day 12 post-Tx.

Large areas of representative tissue sections of naïve C57BL/6 wt hearts (naïve, top row) as well as cardiac allografts (BALB/c wt) transplanted into C57BL/6 wt (WT, middle row) or CCR7$^{-/-}$ (CCR7$^{-/-}$, bottom row) recipients (receiving a single dose of 500µg MR-1 (anti-CD154-mAb) together with 5x10^{6} donor-specific (BALB/c wt) splenocytes at the day of transplantation) were imaged at day 12 post-Tx by fluorescence microscopy using automated composite image generation (Zeiss Axiovison software). DAPI staining (visualization of cell nuclei, green) illustrates the presence of massive leukocyte infiltration clusters within cardiac allografts of CCR7$^{-/-}$ recipients, in some cases resulting in almost complete destruction of cardiac muscle tissue (autofluorescence signal in the Cy3-channel, red). In contrast, in cardiac allografts transplanted into wt recipients, concentrations of leukocytes are almost exclusively observed directly below the endocard layer, rarely infiltrating in higher numbers between the muscle cells of the myocard. At least four individual organs were analyzed per group. Scale bar, 1 mm.

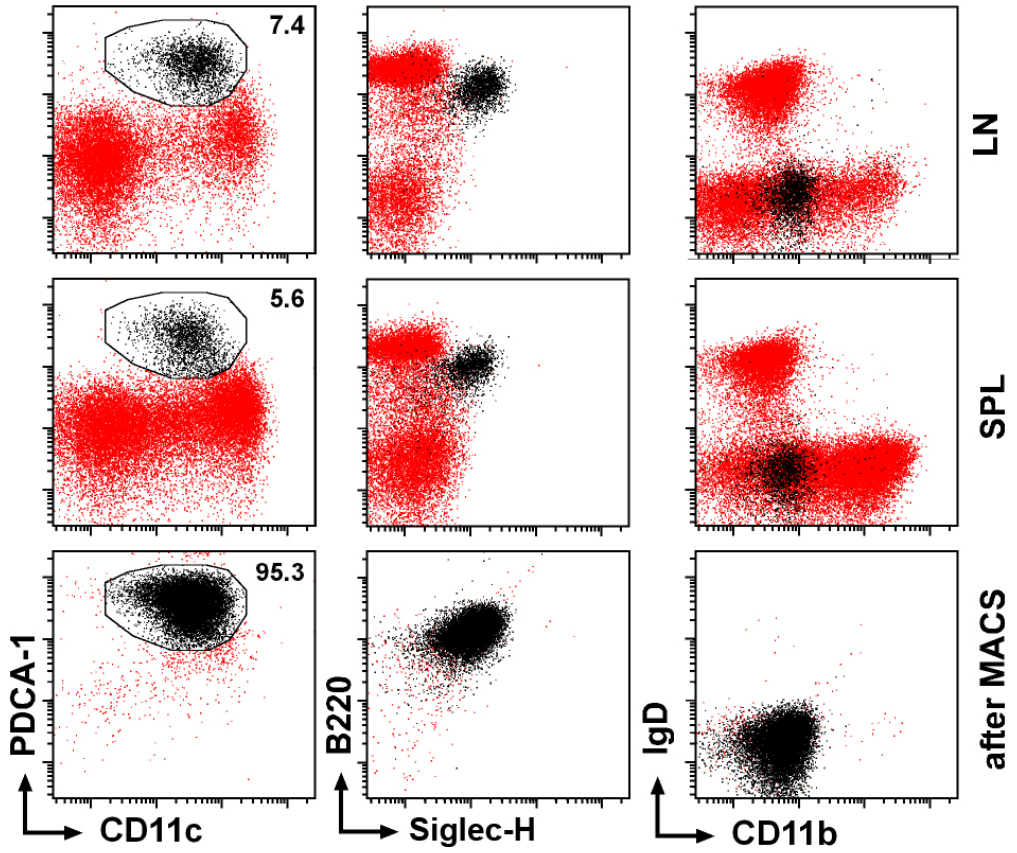
Supplementary Figure S2



FACS analysis of pDC within pLN of wt and CCR7$^{-/-}$ mice.

Representative FACS plots illustrating the analysis of pDC frequencies within pLN of untreated C57BL/6 wt (upper row) and CCR7$^{-/-}$ (lower row) animals. Numbers indicate percentage of cells within gates relative to the parent population (FSC/SSC-gated DAPI^{-} living leukocytes). pDC were readily identified as 120G8^{+}B220^{int}CD11c^{int} cells. At least three individual animals were analyzed per group.

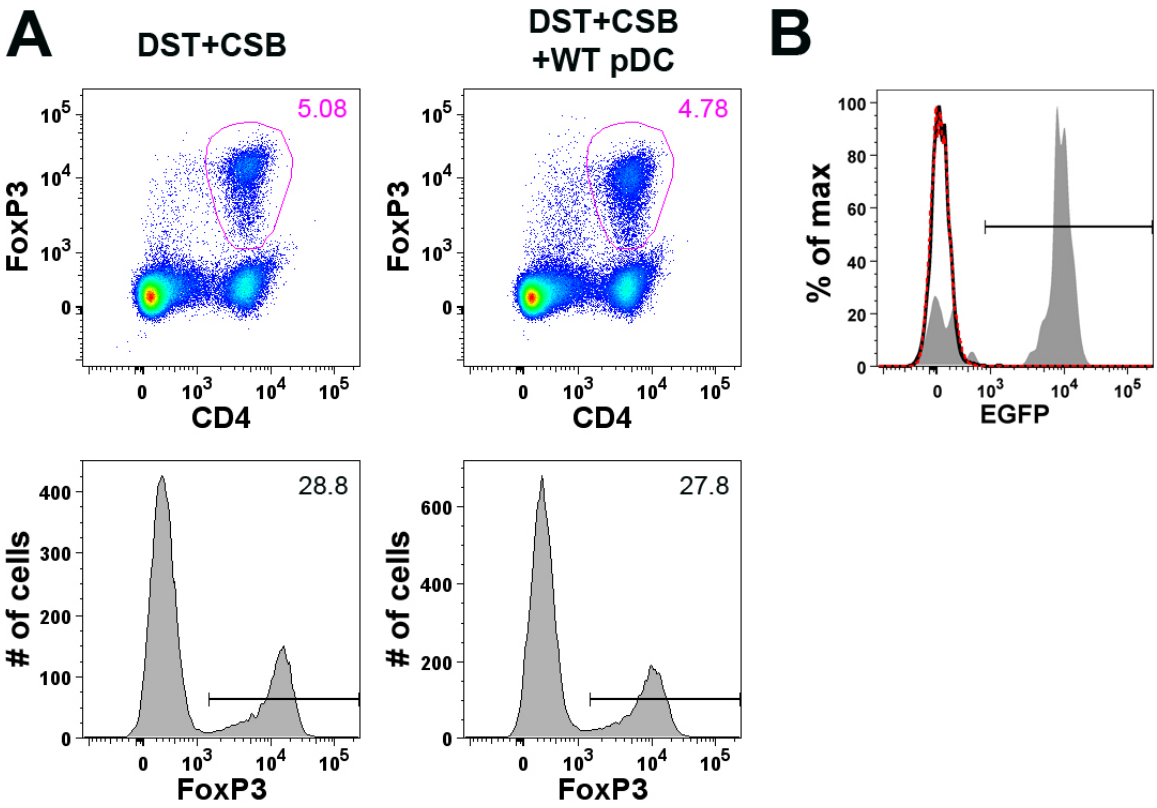
Supplementary Figure S3



MACS-purification of *in vivo*-expanded pDC.

To generate large numbers of pDC *in vivo*, C57BL/6 wt mice were s.c. injected with $5x10^5$ to $1x10^6$ Flt3L-producing B16-FL tumor cells into both flanks. After 10-14 days, pooled LN (upper row) and SPL (middle row) were harvested, and pDC were isolated by MACS depletion using the Plasmacytoid Dendritic Cell Isolation Kit II (see M&M). The purity of the PDCA-1^+B220^{int}CD$11c^{int}$ pDC target fraction (black) was 87-95% after MACS separation (lower row). Numbers indicate percentage of cells within gates relative to the parent population (FSC/SSC-gated $DAPI^-$ living leukocytes).

Supplementary Figure S4



Analysis of Treg within pLN of DST+CSB-treated CCR7$^{-/-}$ allograft recipients.

(**A**) Treg frequencies at day 12 post-Tx within pLN of CCR7$^{-/-}$ cardiac allograft recipients, receiving four doses of 250µg MR-1 (anti-CD154-mAb) at day -7 (together with 1x10^7 donor-specific (BALB/c wt) splenocytes), -4, 0 and +4 relative to transplantation (DST+CSB, left column), or additionally being transfused with 3-5x10^6 syngeneic C57BL/6 wt pDC at day -7 (DST+CSB+WT pDC, right column). The frequency of CD4$^+$FoxP3$^+$ cells within total lymphocytes (upper row), as well as the frequency of FoxP3$^+$ cells within the CD4$^+$ T cell population (lower row) did not increase following the additional transfer of syngeneic wt pDC. At least three individual animals were analyzed per group.

(**B**) Representative FACS plot illustrating the analysis of transgenic FoxP3EGFP reporter T cells within pLN of DST+CSB-treated CCR7$^{-/-}$ mice at day 0. At day -7, 8x10^6 FACS-sorted EGFP$^-$CD4$^+$ T cells (purity >99%) from transgenic FoxP3EGFP donors [27] were transferred as reporter cells into CCR7$^{-/-}$ recipients together with 250µg MR-1 (anti-CD154-mAb) and 1x10^7 donor-specific (BALB/c wt) splenocytes, with (red dashed line), or without (black line,) the additional co-transfer of 8x10^6 syngeneic wt pDC at the same time point. An additional dose of 250µg MR-1 was applied at day -4, and FACS analysis of pLN, MLN and SPL was performed at day 0. Due to their expression of Thy1.1, the reporter T cell population could be easily identified by FACS within the recipients' LN and SPL at day 0 (data not shown), but no increase of the EGFP$^+$ fraction was detected in

any of the SLO analyzed, of the co-transfer of syngeneic wt pDC at day -7 (with pDC: red dashed line, 0.24% are $EGFP^+$; without pDC: black line, 0.42% $EGFP^+$). As a control, $4x10^6$ FACS-sorted $EGFP^+CD4^+$ T cells (purity >95%) from FoxP3EGFP donors were transferred at day -7 into $CCR7^{-/-}$ recipients that received the same DST+CSB-treatment, but no pDC (gray shade, 73.7% in gate). Although some of the transferred FoxP3EGFP cells obviously loose their EGFP-expression, a large fraction of $EGFP^+CD4^+$ reporter T cells can readily be detected within pLN of these control mice at day 0. Percentage values indicate the proportion of $EGFP^+$ cells (in gate) within the parent population of $Thy1.1^+CD4^+$ reporter T cells present in pLN. At least two individual animals were analyzed per group.