Targeted delivery of immune therapeutics to lymph nodes prolongs cardiac allograft survival

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Supplementary Data

Supplementary Figure 1:

(A) Immunofluorescence staining of HEV in naïve LN vs. DLN (upper panel), iDISCO HEV imaging in the naïve LN vs. DLN (lower panel). (B) Quantification of the HEV in naïve LN vs DLN using ImageJ software (n=4-7/group, 9 sections per each LN per mouse, student *t*-test, ***$p < 0.001$). (C) Color Doppler echography of DLN showed markedly increased blood supply (62.9 ml/s) following transplantation, as compared to naïve LN (2.9 ml/s).

Supplementary Figure 2:

(A) Immunofluorescence staining of DLN at 24 hours post-IV administration of IgM-IR800-NPs reveals minimal accumulation of NPs. (B) Fluorescent image of IR800-NPs (red) endocytosed by a DC *in vitro*.

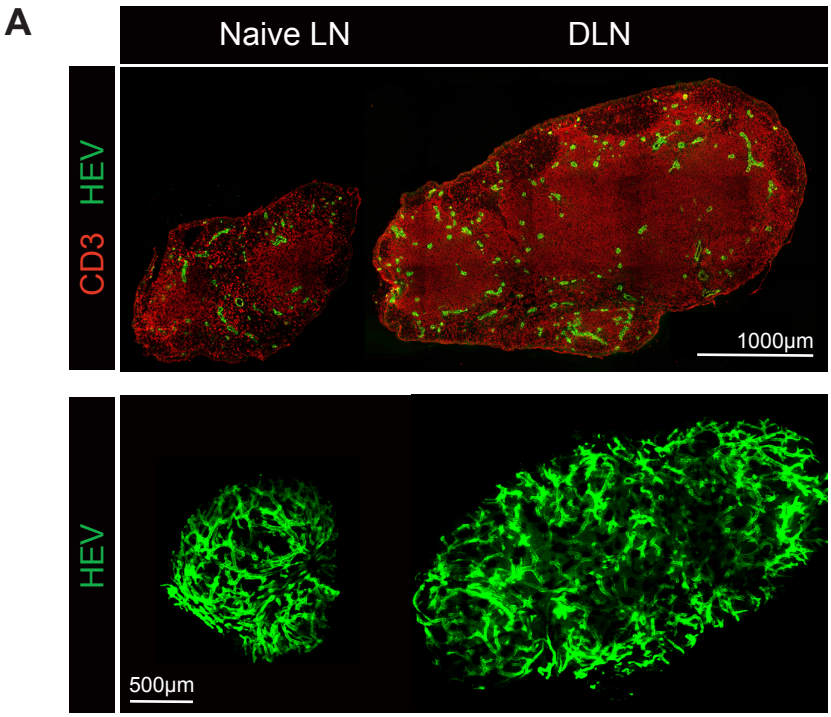
Supplementary Figure 3:

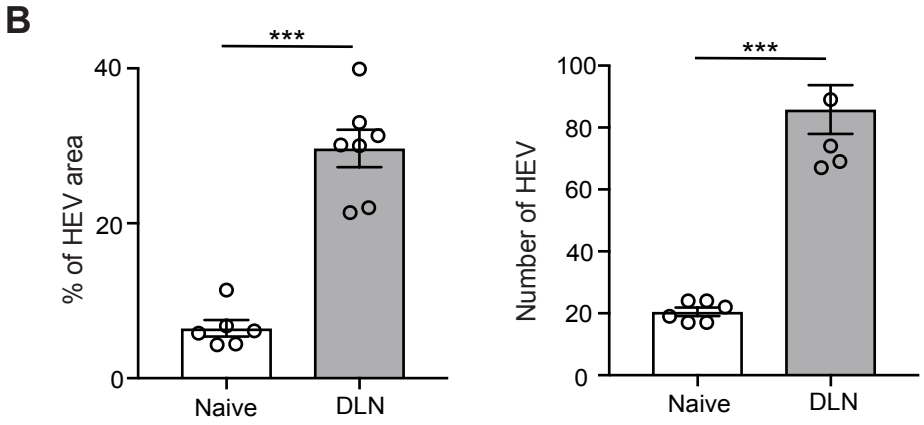
(A) T cell proliferation assay comparing level of proliferation following treatment with free anti-CD3 (10μg) and anti-CD3-NPs (10μg). The bar graph represents the percentage of T cell proliferation in comparison to the negative control (no stimulation) (free anti-CD3 and anti-CD3-NP vs negative control, student *t*-test, ***$p<0.001$, n=3 mice/group). (B) Luminex assay of supernatant of DLN T cells stimulated with irradiated donor cells showed significantly lower production of IFNγ, TNFα and IL-6 following treatment with free anti-CD3 or MECA79-anti-CD3-NP as compared to untreated control group. No differences observed between the two treated groups. (ANOVA test). (C) Histological examination of heart allografts treated with

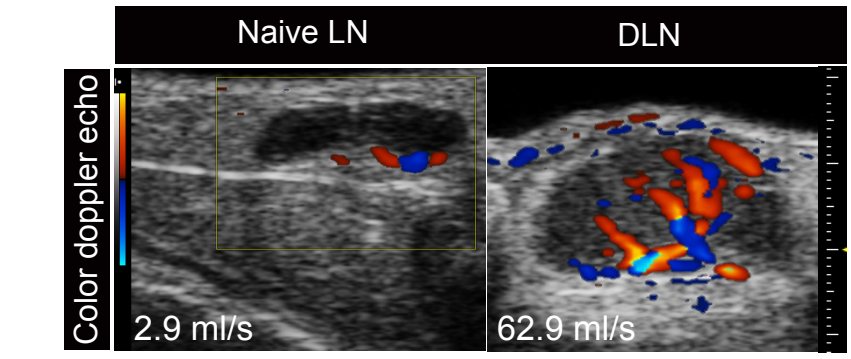
MECA79-anti-CD3-NPs showed myocyte necrosis, fibrosis, and cellular infiltration (H&E) at 125 days post-transplantation.

Supplementary Figure 4:

(A) BALB/c hearts were transplanted into C57BL/6 recipients, treated with either MECA79-anti-CD3-NPs or free anti-CD3. DLNs were harvested at 17 days post-transplantation. Bar graphs represent the number of $CD3^+$ cells, the percentage of $CD4^+$ $CD69^+$, $CD4^+$ $CD44^+$ $CD62L^{low}$ T cells, Tregs and IFNγ-producing $CD4^+$ T cells in the DLN (free anti-CD3 vs. MECA79-anti-CD3-NP, 14.6±2.6 vs. 15.6±1.8%, mean ± SEM, student *t*-test, *p*=ns for $CD4^+$ $CD44^+$ $CD62L^{low}$ cells, 12.4±1.9 vs. 12.1±1.1, mean ± SEM, student *t*-test, *p*=ns for Tregs, and 4.5±0.9 vs. 3.2±0.3%, mean ± SEM, student *t*-test, *p*=ns for IFNγ-producing $CD4^+$ cells, n=4 mice/group). (B) Plasma cytokine measurement shows higher levels of IFNγ and IL-2 in the anti-CD3-NP-treated mice, as compared to MECA79-anti-CD3-NP (control vs. free anti-CD3 vs. MECA79-anti-CD3-NP, 3.5±0.4 vs. 304.7±54.7 vs. 112.8±31.5 $x10^4$ for IFNγ, 3.7±0.9 vs. 70.5±16.5 vs. 24.5±4.2 for IL-2, mean ± SEM, ANOVA test, *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$, n=4 mice/group, duplicate samples).

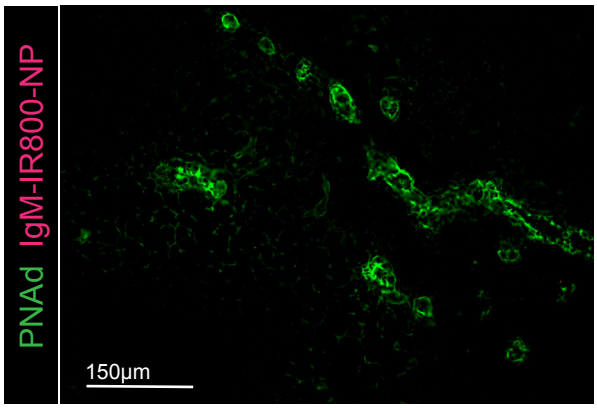






Bahmani et al. Supplementary Figure 1.

A



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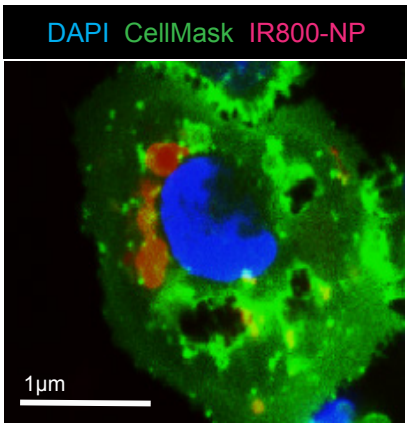
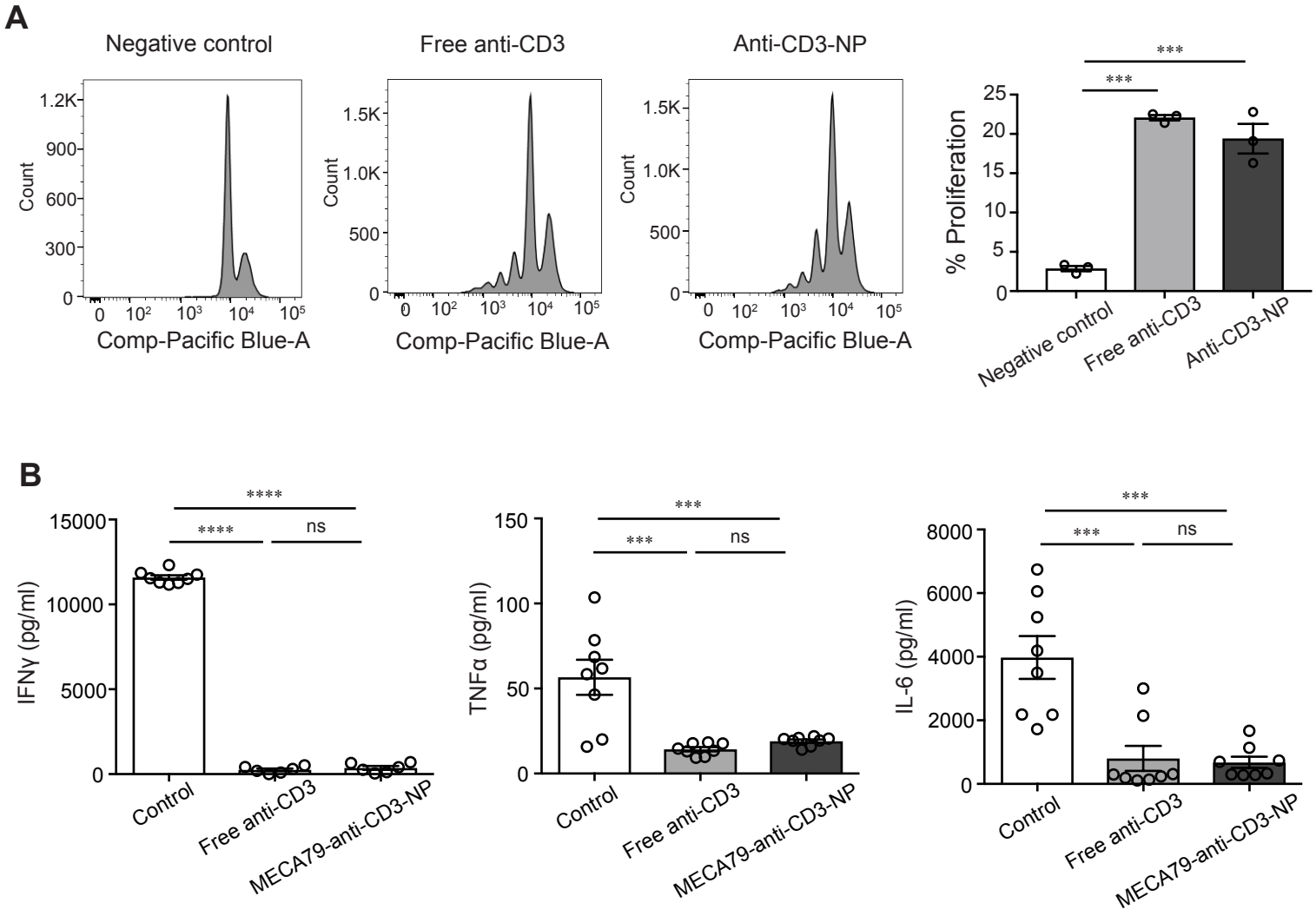
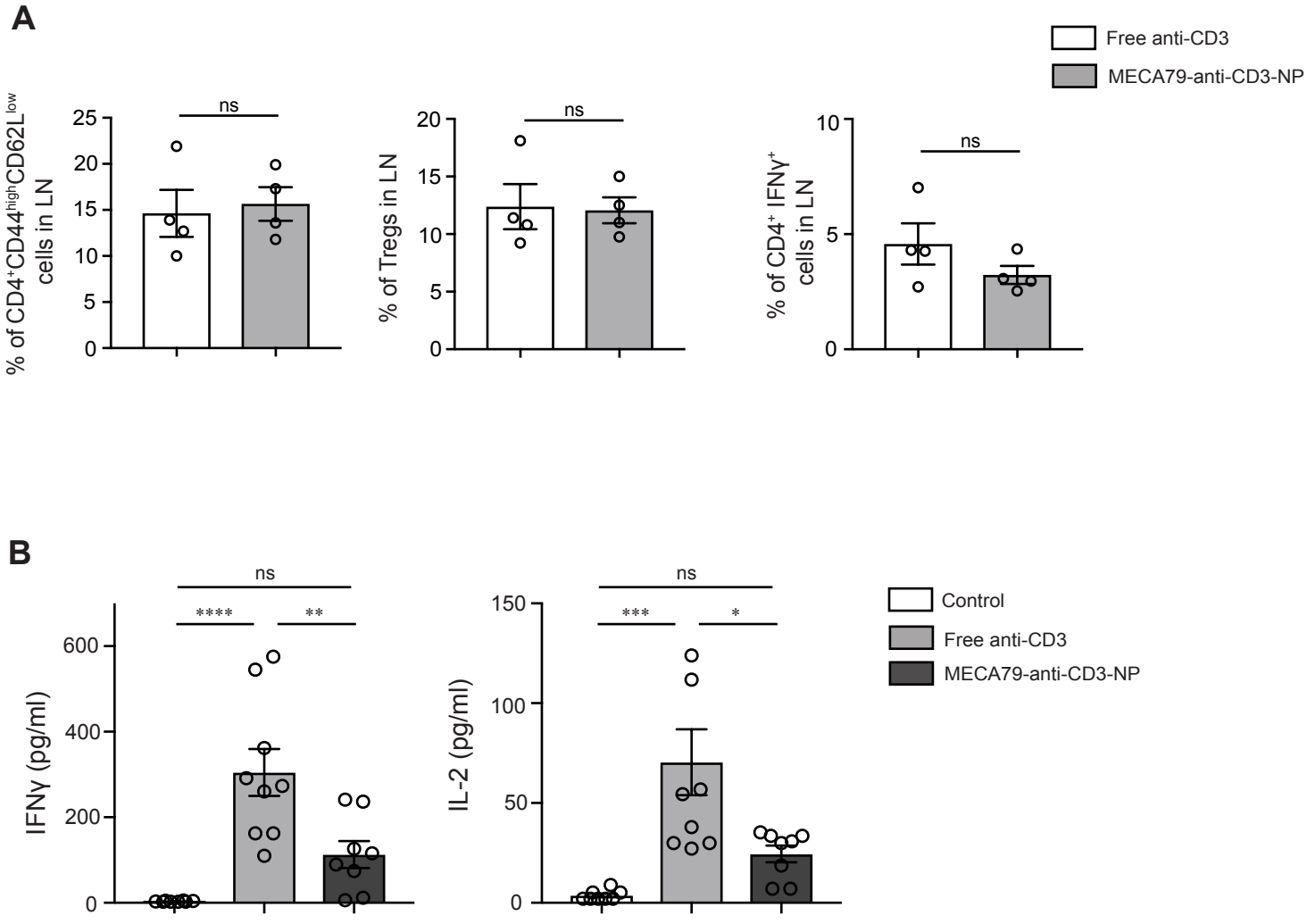


Bahmani et al. Supplementary Figure 2.



Bahmani et al. Supplementary Figure 3.



Bahmani et al. Supplementary Figure 4.