SUPPLEMENTARY INFORMATION

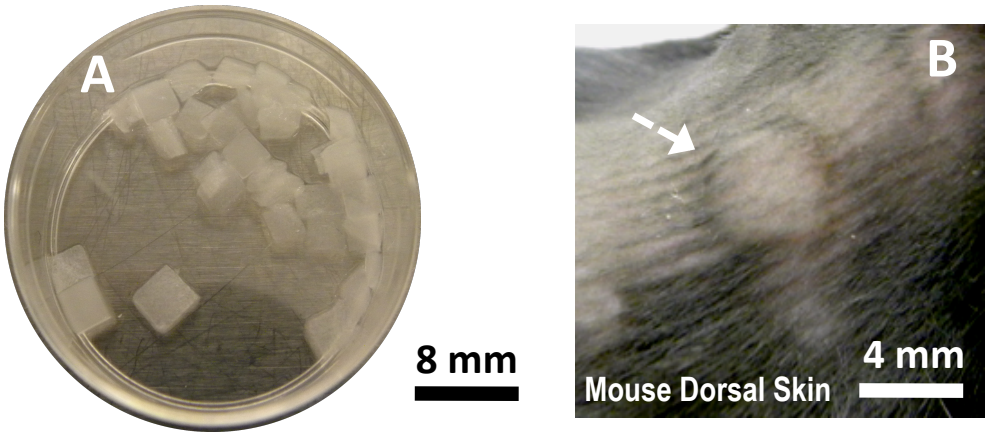


Figure 1. Injectable square-shaped alginate sponge-like cryogels with shape-memory properties. Photographs depicting cryogel (4 × 4 × 1 mm) before (**A**) and after (**B**) syringe injection in the subcutaneous tissue of a mouse.

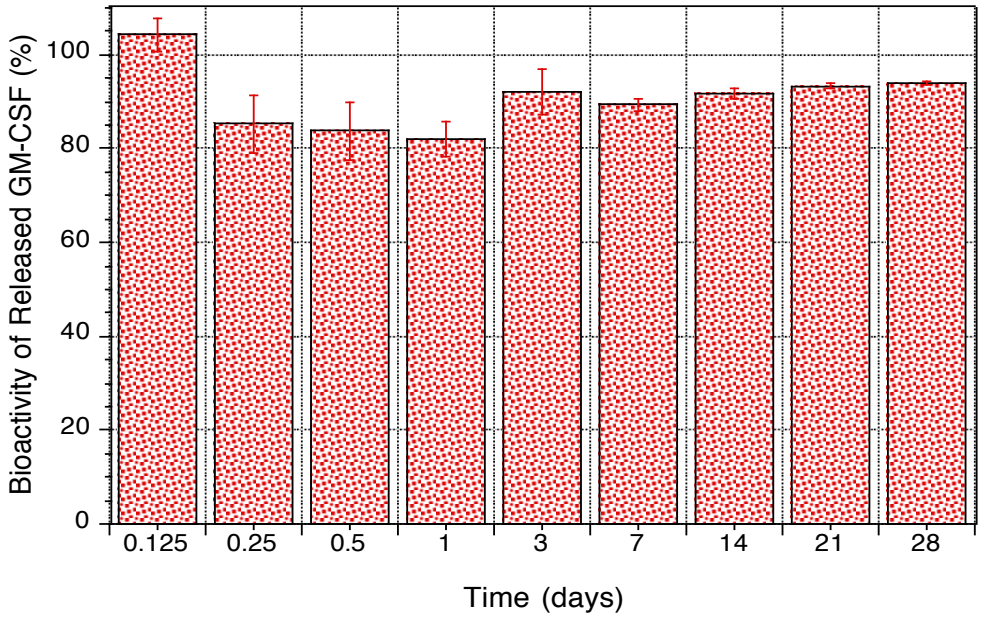


Figure 2. A high fraction of released GM-CSF from the cryogels remains bioactive. The quantification of bioactive GM-CSF was assessed by an *in vitro* bioactivity assay as indicated in the methods section of the main manuscript.

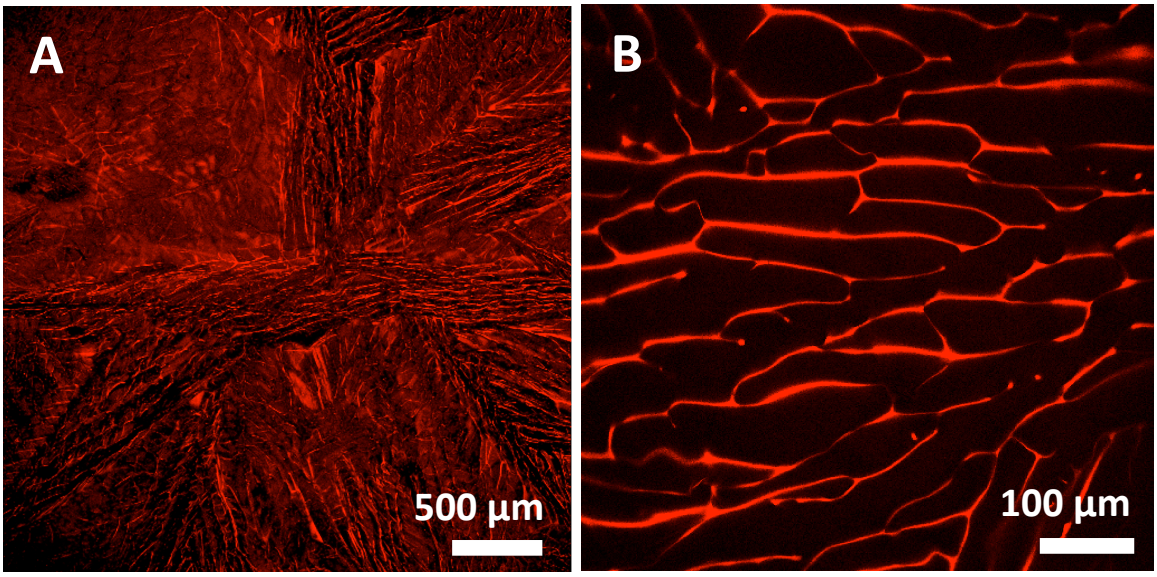


Figure 3. Homogeneous distribution of physically entrapped CpG throughout the gel network. TAMRA-labeled CpG (IDT, Coralville, IA) was loaded in RGD-containing alginate cryogels (50µg/gel) prepared by cryogelation at -20 °C. The gels were subsequently washed and examined by confocal microscopy. High-resolution image stacks with 2D projection (A) and 2D image from a single focal plane (B). 2-D confocal micrographs show homogenous immobilization and distribution of TAMRA-labeled CpG throughout the macroporous gel matrix network after gel formation**.**

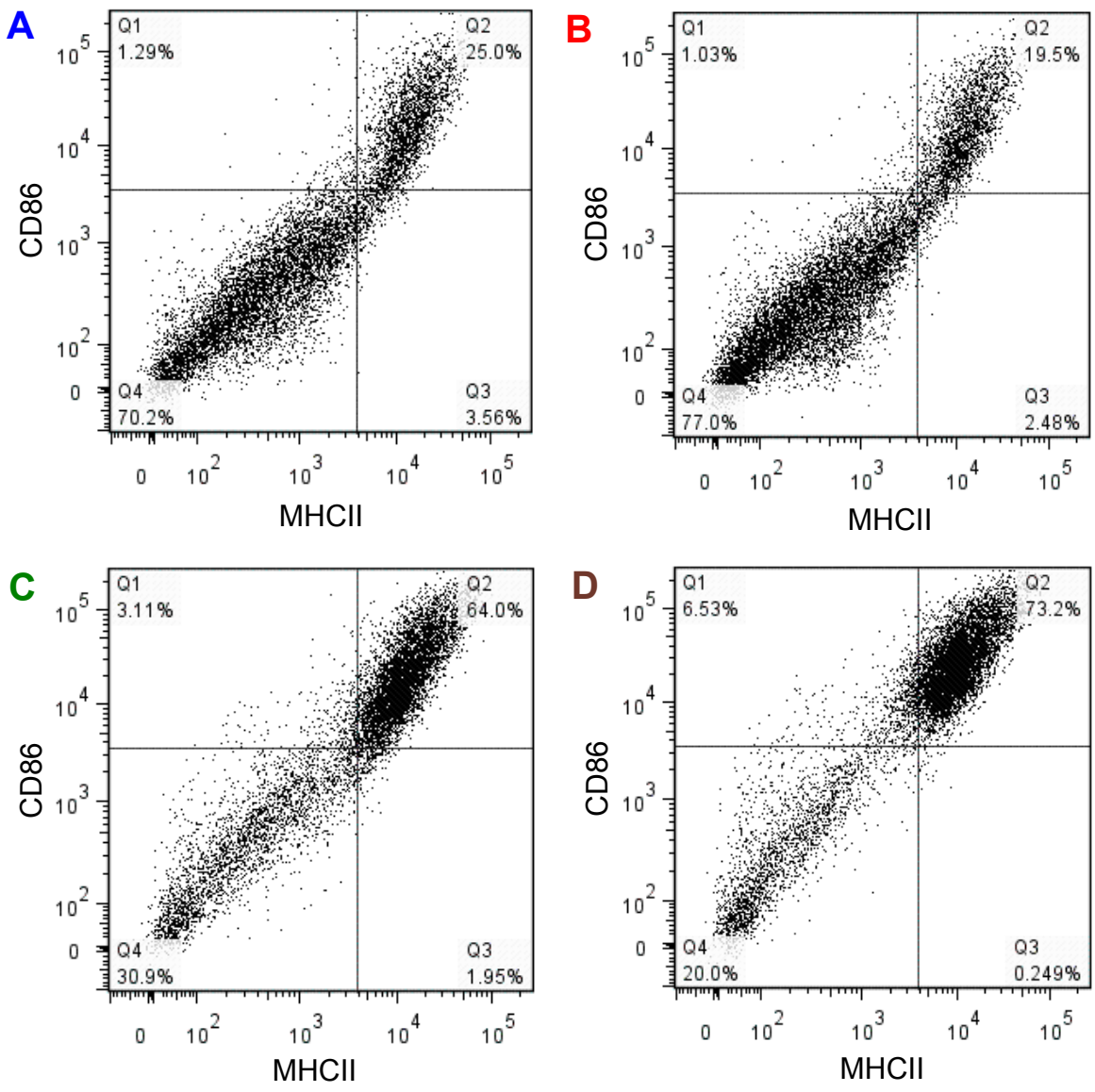
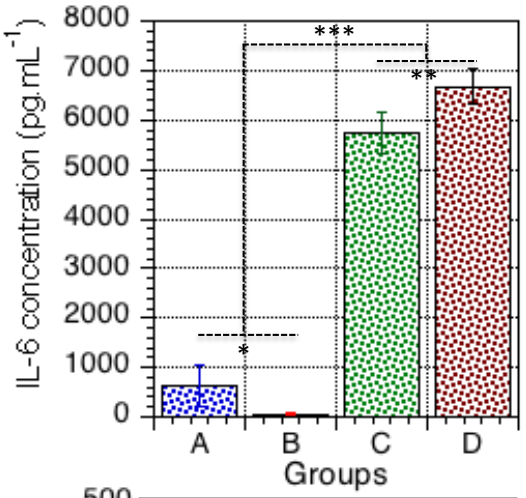
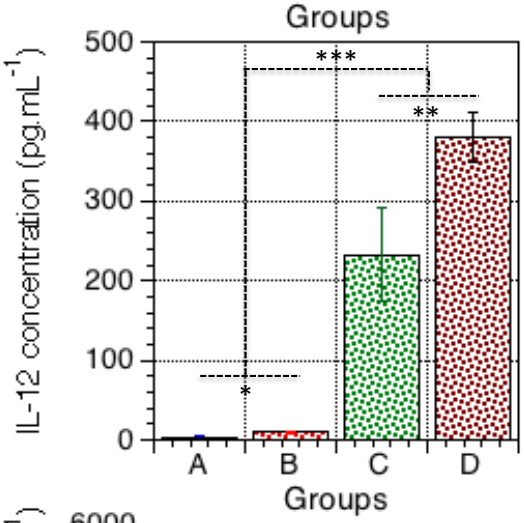
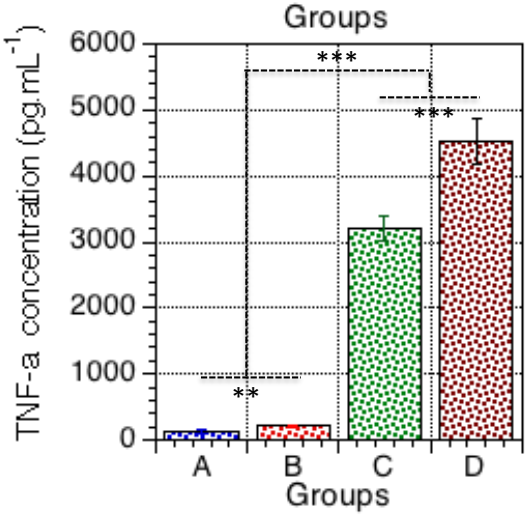


Figure 4. FACS analysis of activated CD86$^+$ MHCII$^+$ BMDCs in response to CpG ODN-loaded cryogels. BMDCs were cultured for 24h in the following conditions: medium (A, negative control), blank cryogels/medium (B), CpG ODN loaded cryogels/medium (C), or soluble CpG ODN/medium (D, positive control). Live cells were gated based upon FSC and SSC characteristics.

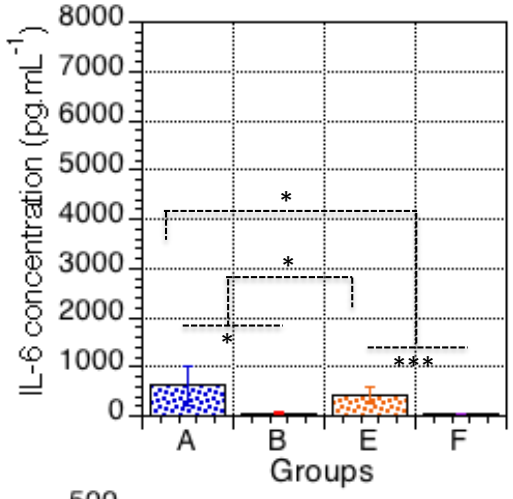
- CpG-ODN loaded Cryogels

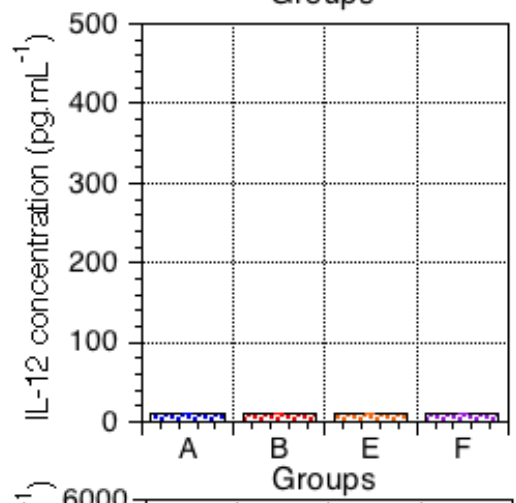






- GM-CSF loaded Cryogels





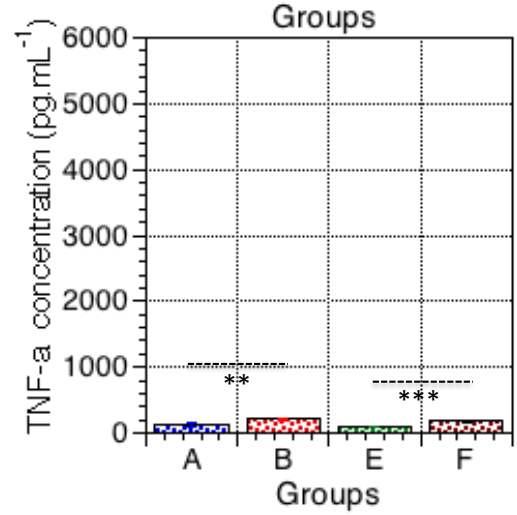


Figure 5. *In vitro* activation of BMDCs in response to CpG ODN- and GM-CSF-loaded cryogels. BMDCs were cultured for 24h in the following conditions: medium (A, negative control), blank cryogels/medium (B), CpG ODN loaded cryogels/medium (C), soluble CpG ODN/medium (D, positive control), GM-CSF loaded cryogels/medium (E), soluble GM-CSF/medium (F, positive control). Production of IL-6, IL-12 and TNF-α (or TNF-a) in culture media in response to DCs stimulated by exposure to CpG ODN-loaded cryogels (C) and GM-CSF-loaded cryogels (E) and other various conditions was analyzed. Concentrations of IL-6, IL-12 and TNF-α in the cell-culture supernatant were evaluated with ELISA (R&D systems), according to the manufacturer's instructions. Values represent mean and SD (n= 5). Data were analyzed using Student's t test and one-way analysis of variance (ANOVA). Differences between groups were statistically significant (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

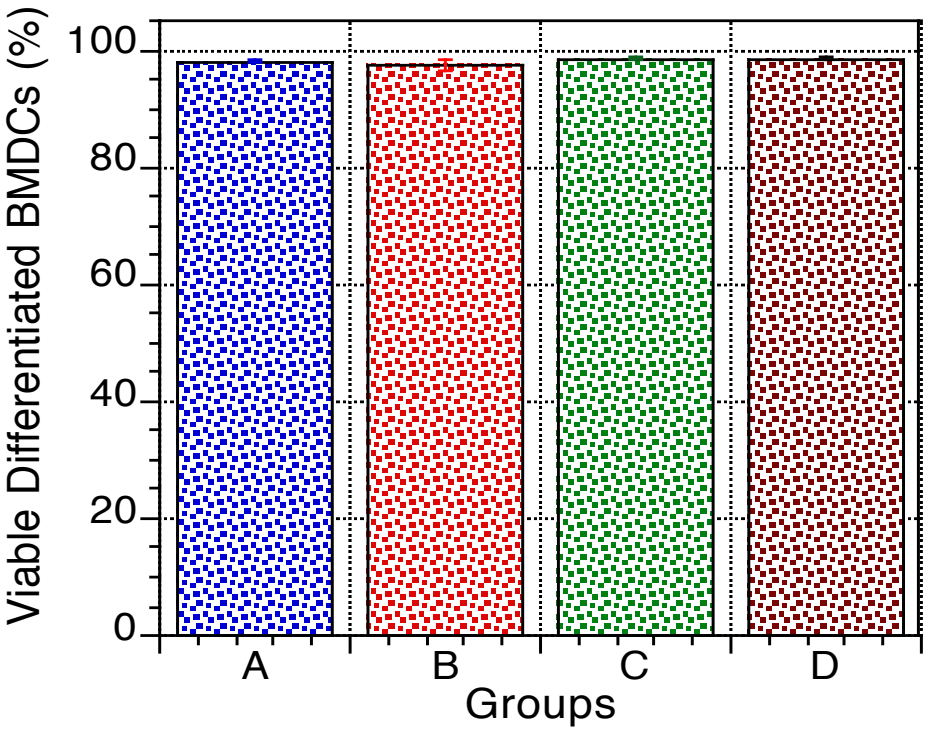


Figure 6. Viability of BMDCs cultured with cryogels. Differentiated bone marrow-derived dendritic cells (BMDCs) were cultured for 24h in contact with: medium (A, negative control), blank cryogels/medium (B), CpG ODN loaded cryogels/medium (C), CpG ODN/medium (D, positive control).

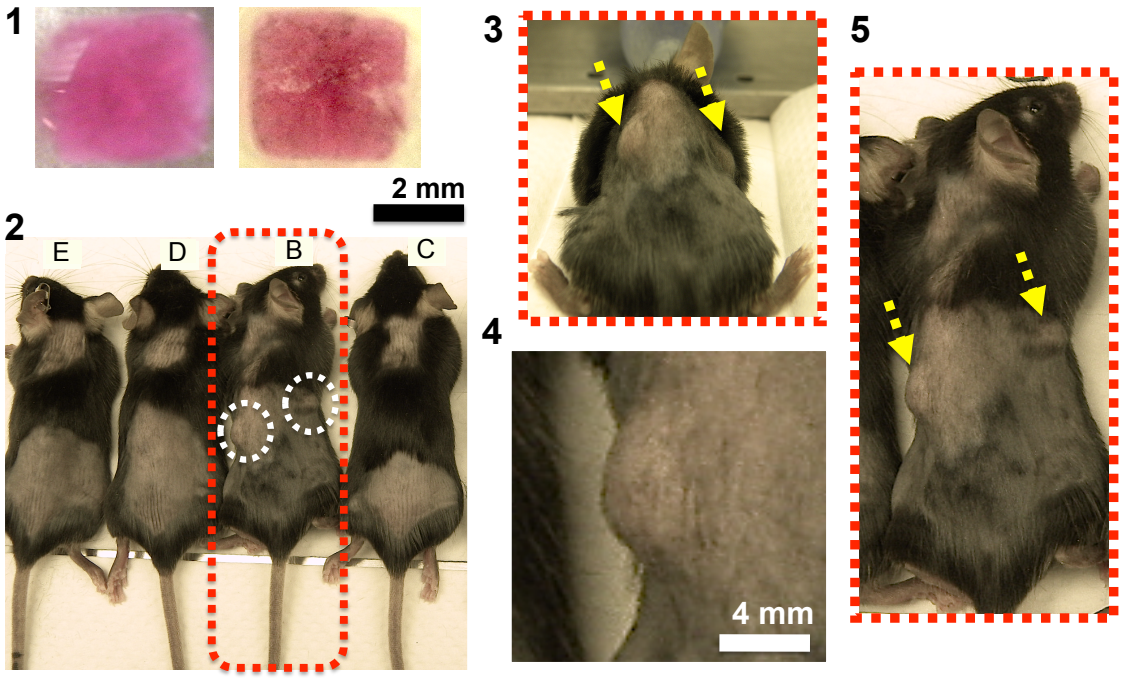


Figure 7. Induration at the vaccination site. Photographs showing a typical cryogel vaccine before (left) and after (right) seeding with irradiated B16-F10 cells (**1**). Photograph showing a group of mice vaccinated with formulations indicated in Fig. 6 (**2**). Significant swelling was depicted at day 13 only for mice vaccinated with cryogel vaccines (B: 2 x 2 x 10^5 irradiated B16-F10 cells + 2 x 1.5 µg GM-CSF + 2 x 50 µg CpG-ODN), as shown in photographs: **2**, **3**, **4**, and **5**.

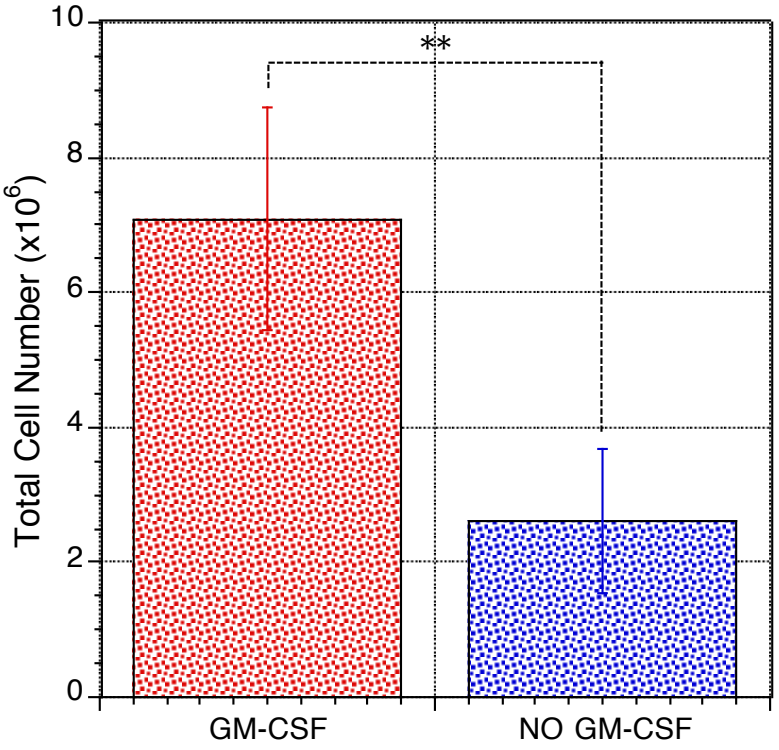
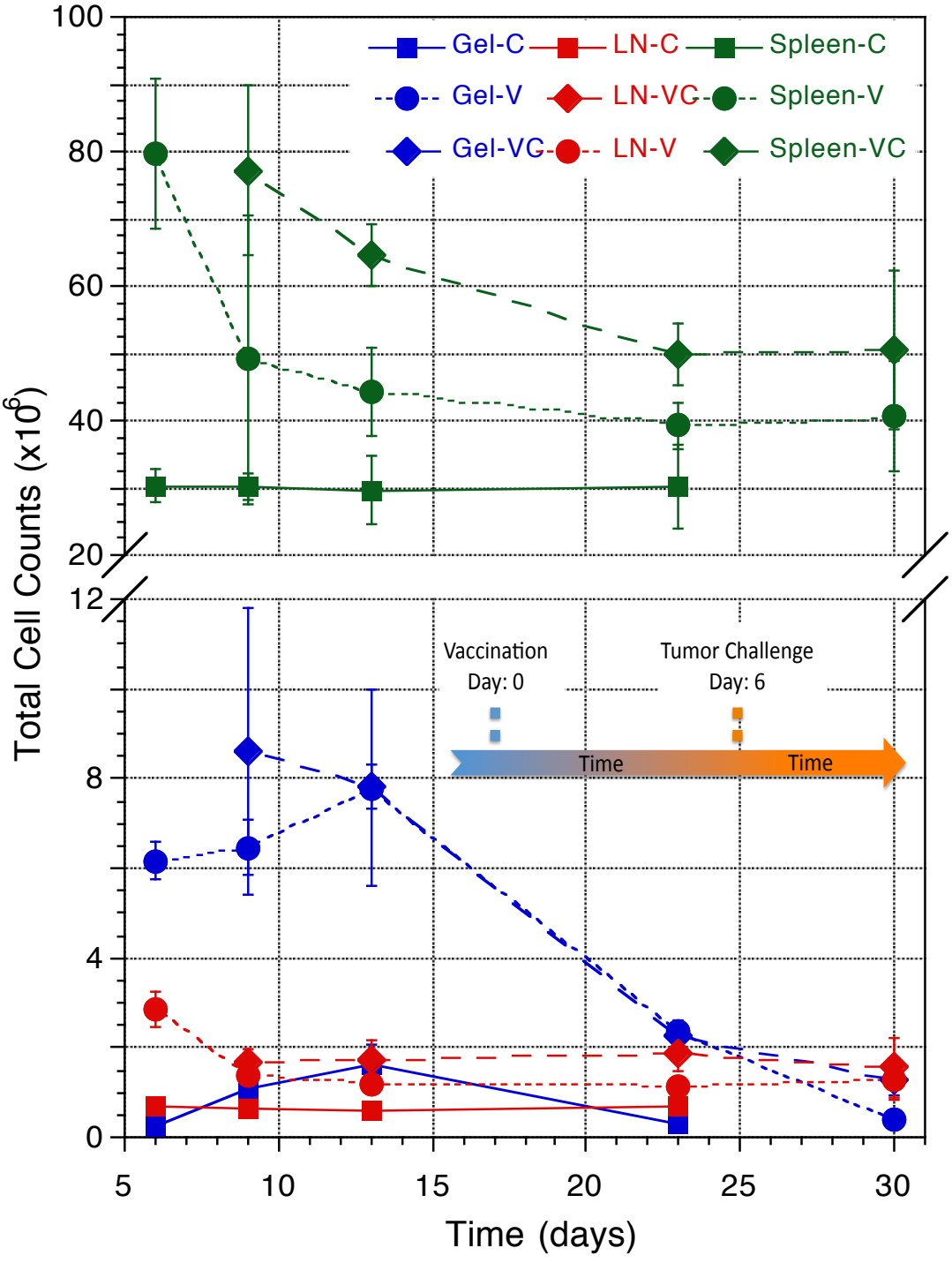


Figure 8. Total cell infiltration at the cryogel sites. Quantification of the total number of cells infiltrating cryogels loaded with GM-CSF or blank (NO GM-CSF) cryogels 4 days post-injection. Values represent mean and SD (n= 5). Data were analyzed using Student's t test. Differences between the 2 groups were statistically significant (**P < 0.01).

A


Gel-C
LN-C
Spleen-C
Gel-V
LN-VC
Spleen-V
Gel-VC
LN-V
Spleen-VC
Vaccination
Day: 0
Tumor Challenge
Day: 6
Time
Time
Total Cell Counts (x10^6)
Time (days)
100
80
60
40
20
12
8
4
0
5
10
15
20
25
30

B

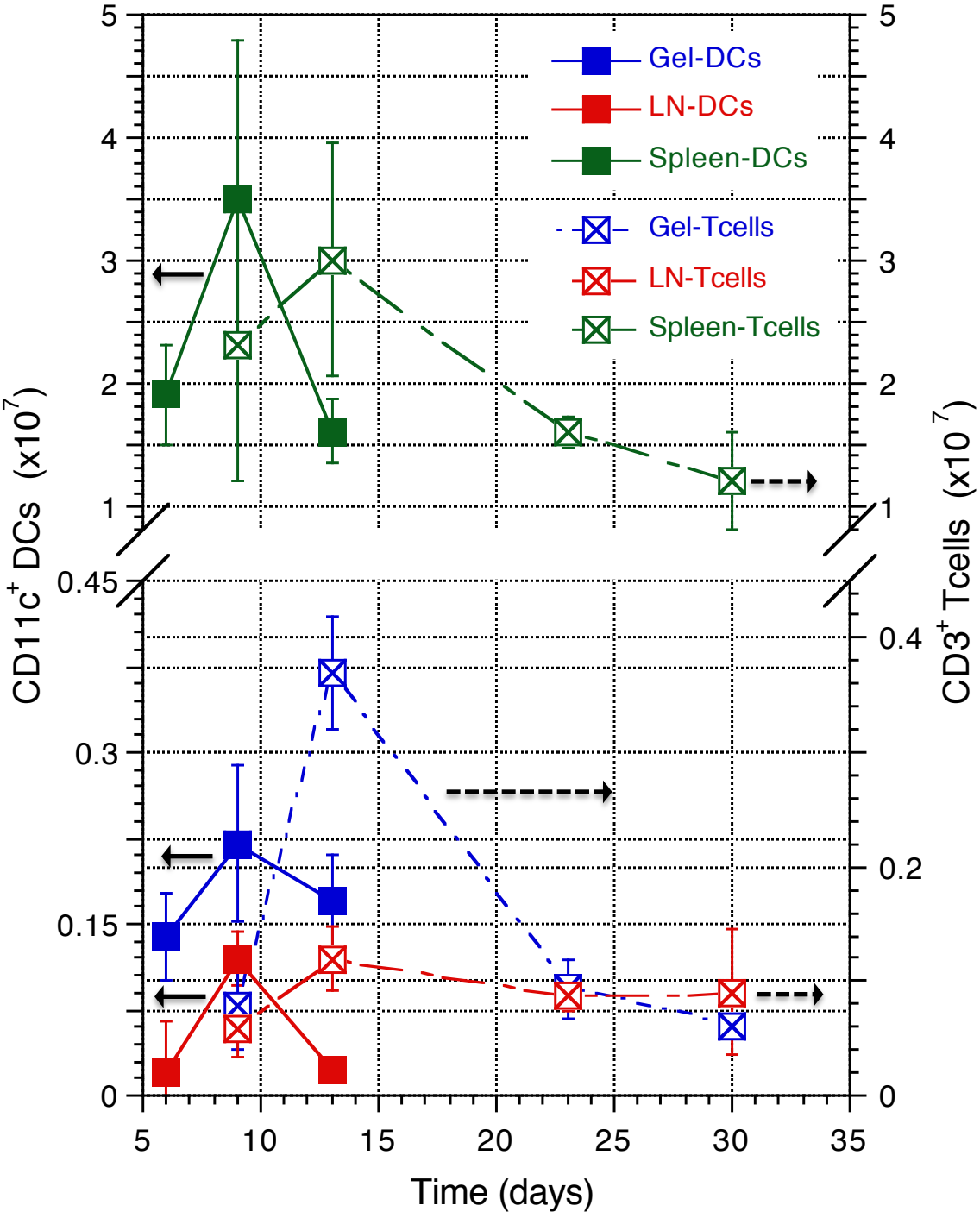

Gel-DCs
LN-DCs
Spleen-DCs
Gel-Tcells
LN-Tcells
Spleen-Tcells
CD11c+ DCs (x10^7)
CD3+ Tcells (x10^7)
Time (days)
5
4
3
2
1
0.45
0.3
0.15
0
0.4
0.2
10
15
20
25
30
35

Figure 9. Local delivery of the cryogel vaccine promotes recruitment of CD11c$^+$ DC and CD3$^+$ T cells. A. Cell recruitment and expansion at the injection site and secondary lymphoid organs (LN, spleen) in response to cryogel vaccination. The inset shows the chronological order of the immunization and tumor challenge. C, V, and VC groups correspond to mice injected with blank cryogels at day 0 (C), mice immunized with cryogel vaccines at day 0 (V), and mice immunized with cryogel vaccines at day 0 + tumor challenged at day 6 (VC), respectively. **B**. Cryogel vaccines co-delivering GM-CSF (2 x 1.5μg), CpG ODN (2 x 50μg), and presenting attenuated B16F10 melanoma cells (2 x 2 x 10^5) enhance local and systemic CD11c$^+$ DC and CD3$^+$ T cell numbers in secondary lymphoid organs (LN, spleen) as well as the cryogel scaffolds. Mice were vaccinated but not tumor challenged. Values in A and B represent mean and standard deviation (n=5).

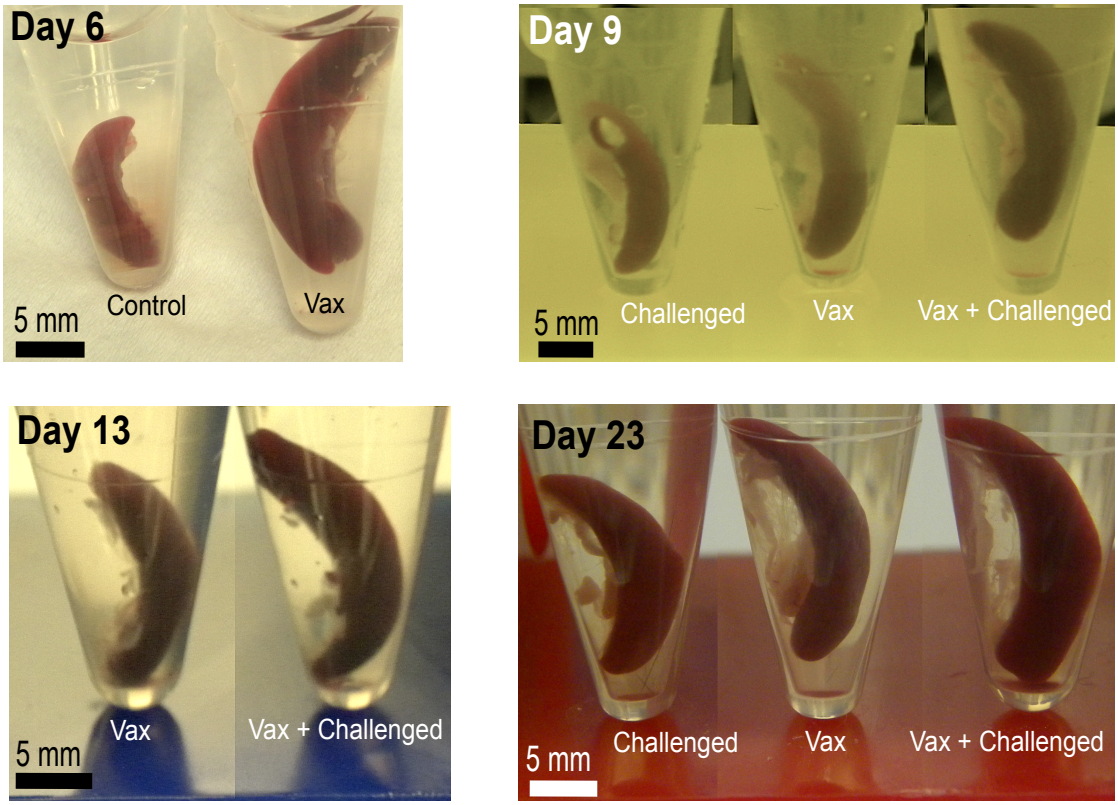


Figure 10. The magnitude of the immune response with the cryogel vaccines could be appreciated grossly, as the spleens of vaccinated mice were markedly enlarged. Mice were vaccinated at day 0, and spleens explanted at different time points. Control (naïve mice), Challenged (naïve mice challenged at day 6), Vax (naïve mice vaccinated at day 0), Vax + Challenged (naïve mice vaccinated at day 0 and subsequently challenged at day 6).

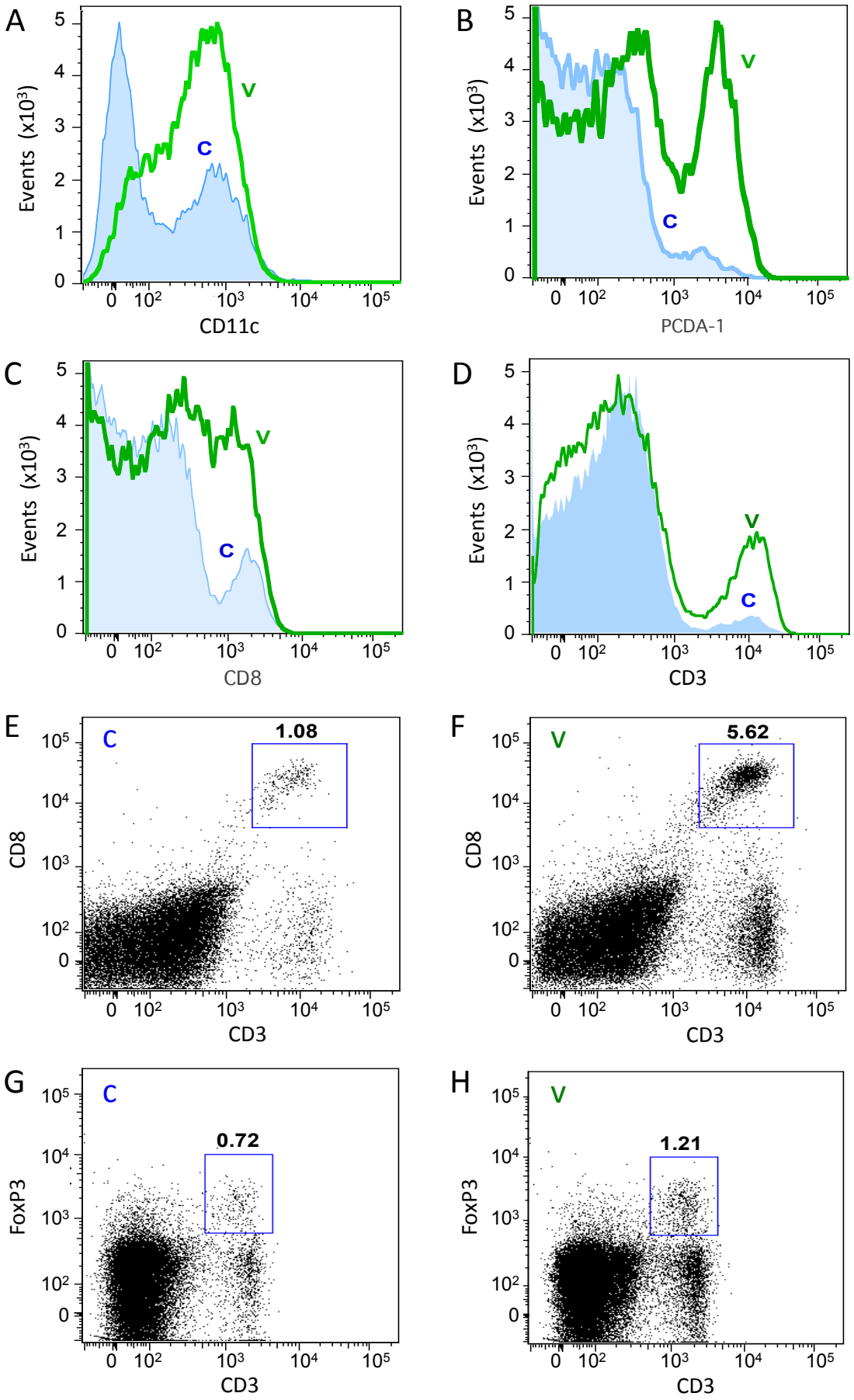

A
Events (x10³)
CD11c
V
C
B
PCDA-1
C
CD8
D
CD3
E
CD8
CD3
1.08
F
5.62
G
FoxP3
0.72
H
1.21

Figure 11. Representative flow cytometry plots for the cell population from explanted cryogel vaccines reported in Fig. 5A-5F. Fractions of $CD11c^+$ DCs (A), $PDCA\text{-}1^+$ $CD11c^+$ DCs (B), and $CD8^+$ $CD11c^+$ DCs (C) at day 9 post-immunization isolated from explanted cryogel vaccines (v) and blank cryogels (c). Cells in (B) and (C) were gated on CD11c positive cells. Fractions of $CD3^+$ T cells (D), $CD8^+$ T cells (E, F), and $FoxP3^+$ T cells (G, H) at day 13 post-immunization isolated from explanted cryogel vaccines (v) and blank cryogels (c). The cells identified as Plasmacytoid DCs are $CD11c^+$ cells that are also positive for PDCA-1. $CD8^+$ DCs are CD11c positive cells that are also positive for CD8.

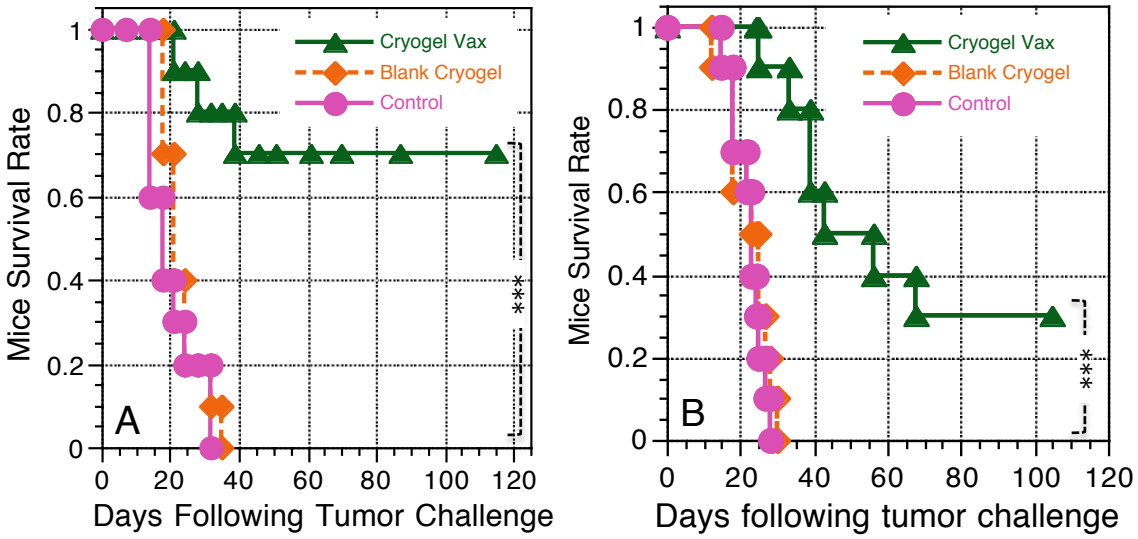


Figure 12. Engineered cryogel vaccines containing irradiated B16-F10 cells, GM-CSF and CpG ODN confer prophylactic (A) and therapeutic efficacy (B). A. A comparison of the survival rate in challenged C57BL/6 mice previously prophylactically vaccinated with (*Cryogel Vax*): Cryogel vaccines (2 x 2 x 10^5 irradiated B16-F10 cells + 2 x 1.5 µg GM-CSF + 2 x 50 µg CpG-ODN); (*Blank Cryogel*): Plain cryogel (control-1); and (*Control*): naïve mice (no immunization, control-2). At day 6 following immunization, C57BL/6J mice (10 mice/group) were challenged with 10^5 B16-F10 tumor cells and monitored for animal survival. **B**. A comparison of the survival of C57BL/6J mice bearing established melanoma tumors (inoculated with 5 x 10^5 B16-F10 cells and allowed to develop for 3 days, tumor area ≥ 10 mm^2, 10 mice/group) and therapeutically treated with (*Cryogel Vax – 2X*): cryogel vaccines (2 x 2 x 10^5 irradiated B16-F10 cells + 2 x 1.5 µg GM-CSF + 2 x 50 µg CpG ODN) at both days 3 and 10; (*Blank Cryogel*): Plain cryogel (control-1); and (*Control*): naïve mice (no immunization, control-2). Data were analyzed using log-rank test (***$P < 0.001$).

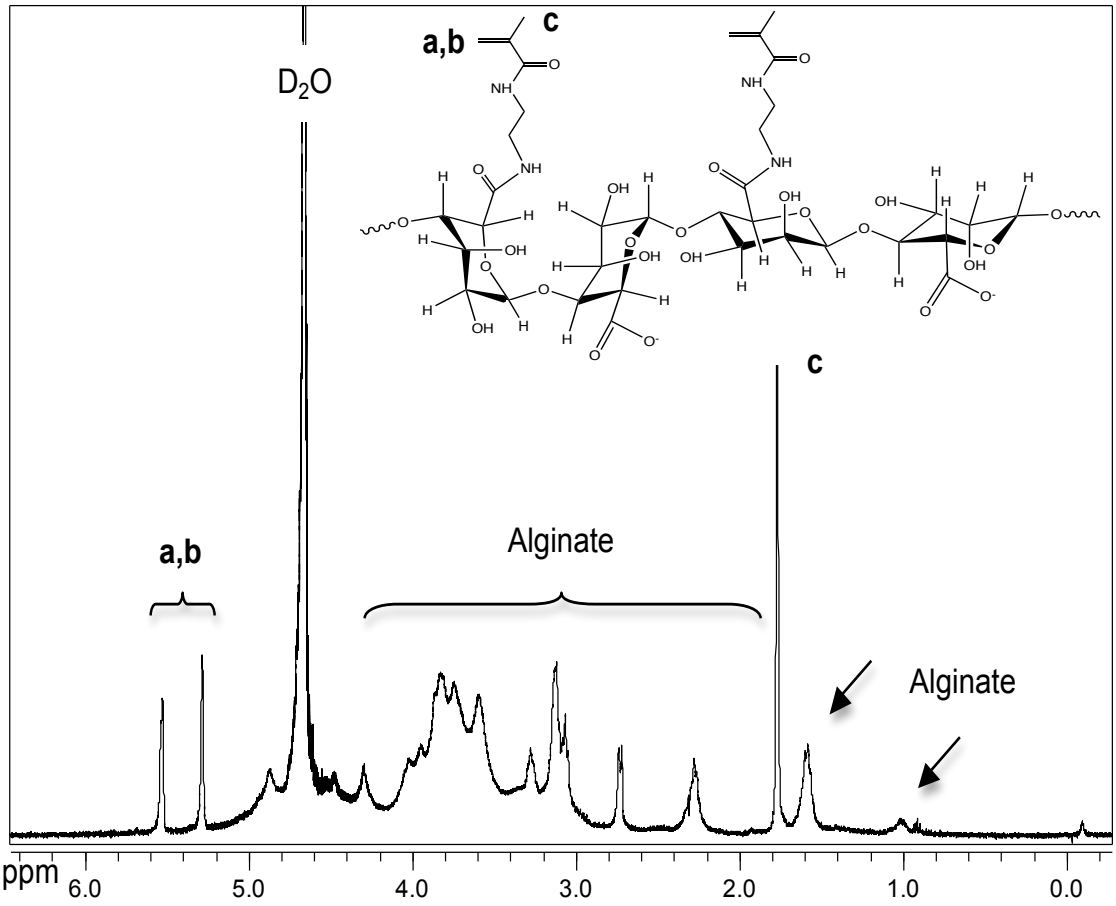


Figure 13. ^{1}H NMR of MA-alginate demonstrating its characteristic vinylic peaks (δ5.3–5.8 ppm). Deuterated water (D_2O) was used as solvent, and the polymer concentration was 1% (wt/vol). The efficiency of alginate methacrylation was calculated based on the ratio of the integrals for alginate protons to the methylene protons of methacrylate. MA-alginate macromonomer was found to have approximately a degree of methacrylation (DM) of 50%.