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A biomaterial-based vaccine eliciting durable tumour-specific responses against acute myeloid leukaemia

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Contents

Supplementary Figure 1. Extended characterization of cryogel vaccine. In vitro release of encapsulated granulocyte macrophage colony stimulating factor (GM-CSF)

Supplementary Figure 2. Extended characterization of cryogel vaccine

Supplementary Figure 3. Extended characterization of cryogel vaccine. In vitro release of encapsulated antigen consisting of either cell lysates or WT-1¹²⁶⁻¹³⁴ antigen.

Supplementary Figure 4. Injection site dynamics following vaccination.

Supplementary Figure 5. Expression of the WT-1 gene in AML cells relative to that in the lineage depleted bone marrow cells from wild type C57Bl/6 mice.

Supplementary Figure 6. T cell priming in AML-exposed mice.

Supplementary Figure 7. Comparison of AML progression between mice that were naïve and non irradiated, sublethally irradiated, and sublethally irradiated with bone marrow transplant.

Supplementary Figure 8. Survival rate in AML models and expression of a subset of AML associated genes.

Supplementary Figure 9. Therapeutic efficacy of single-factor, antigen-free cryogel vaccines.

Supplementary Figure 10. Localization of AML bioluminescence signal at cryogels.

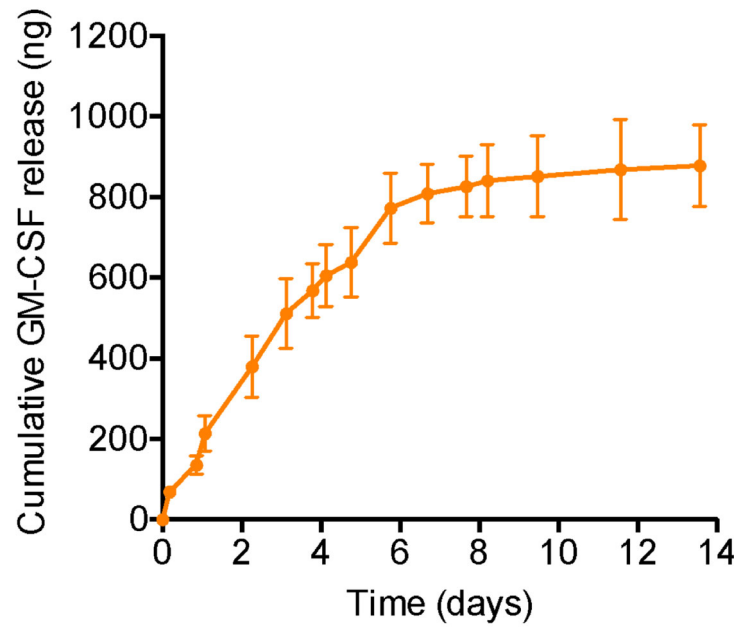
Supplementary Figure 11. Dendritic cell recruitment and activation in cryogels delivered to mice with established AML untreated or given chemotherapy.

Supplementary Figure 12. AML burden in bone marrow and spleen.

Supplementary Figure 13. Representative gating strategies.

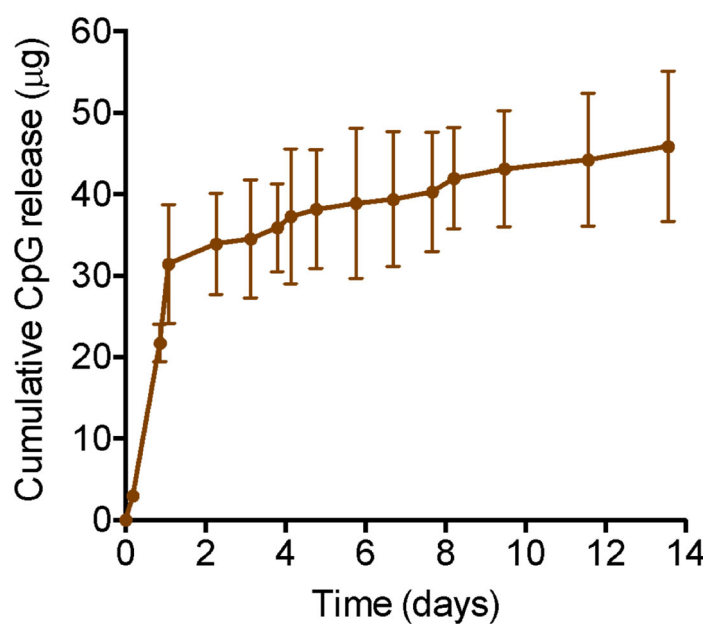
Supplementary Figure 14. WT-1-specific T cell responses comparing full versus antigen-free cryogel vaccines.

Supplementary Figure 1



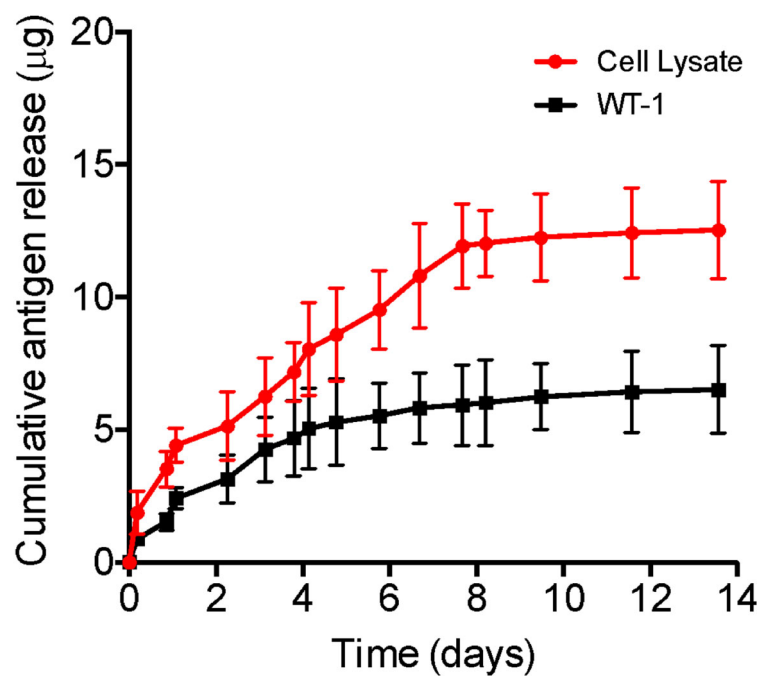
Supplementary Figure 1. Extended characterization of cryogel vaccine. In vitro release of encapsulated granulocyte macrophage colony stimulating factor (GM-CSF). Results shown from one experiment; n = 5 independent samples per time point; means depicted; error bars, s.d.

Supplementary Figure 2



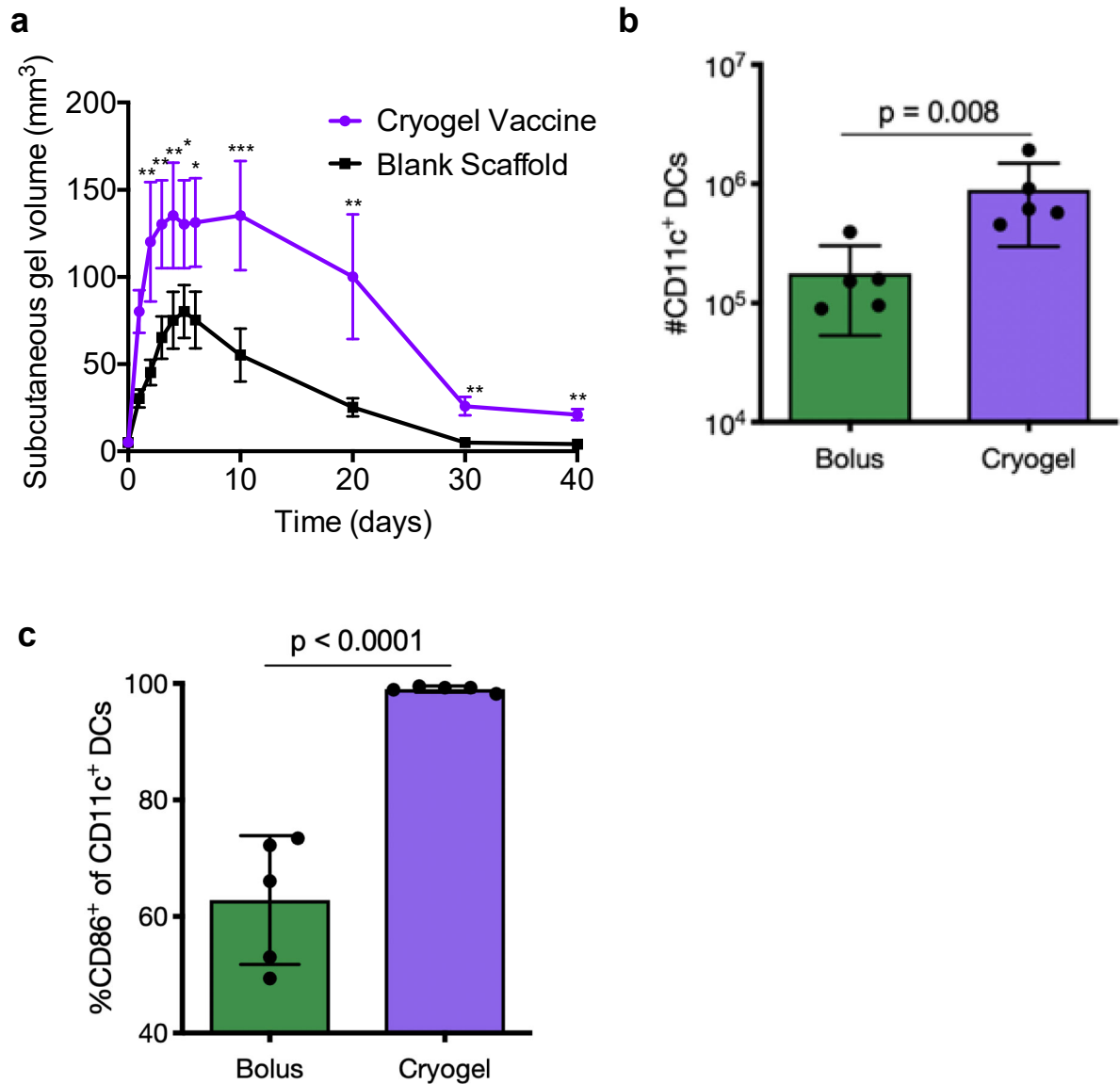
Supplementary Figure 2. Extended characterization of cryogel vaccine. In vitro release of encapsulated CpG-ODN. Results shown from one experiment; $n = 5$ independent samples per time point; means depicted; error bars, s.d.

Supplementary Figure 3

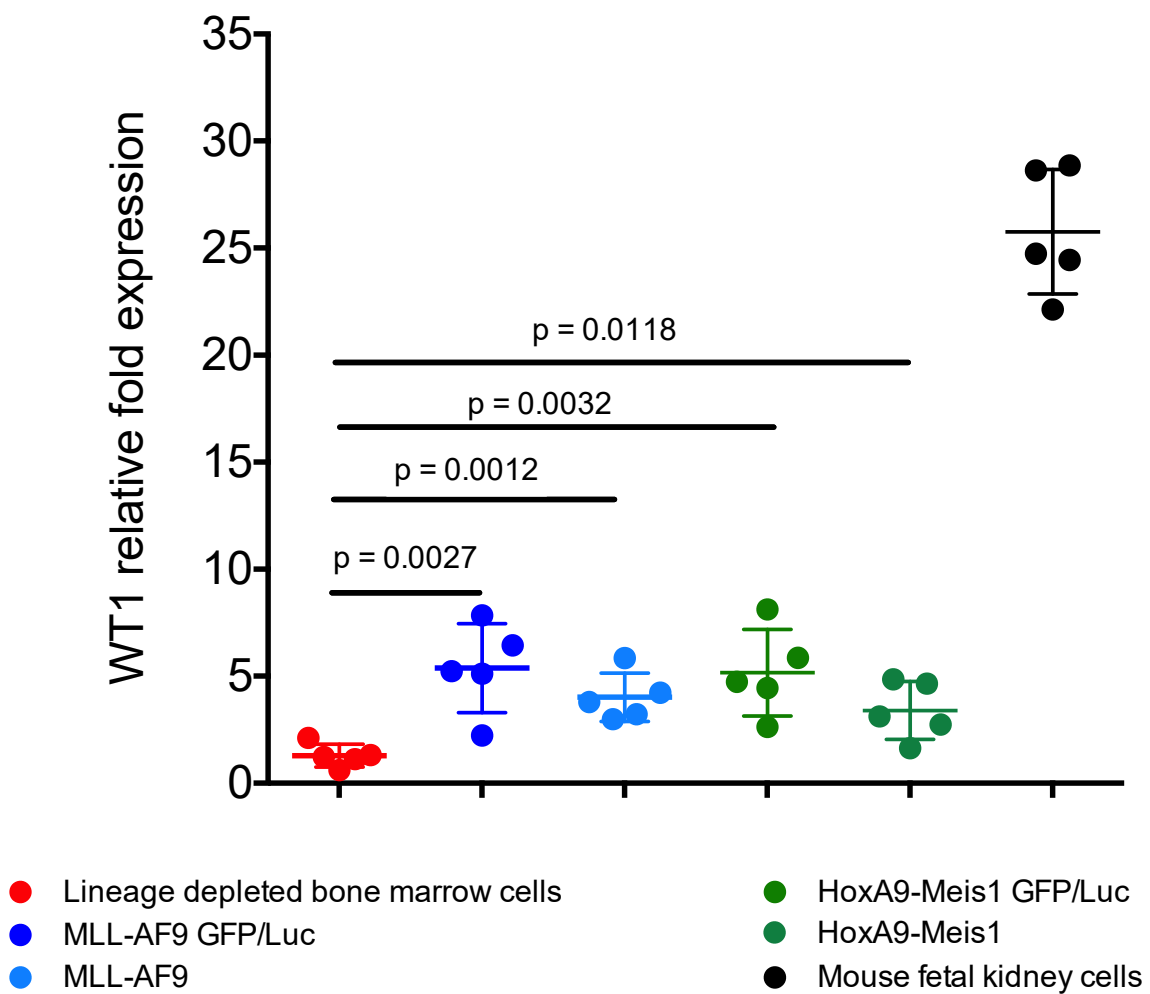


Supplementary Figure 3. Extended characterization of cryogel vaccine. In vitro release of encapsulated antigen consisting of either cell lysates or WT-1₁₂₆₋₁₃₄ antigen. Results shown from one experiment; n = 5 independent samples per time point; means depicted; error bars, s.d.

Supplementary Figure 4

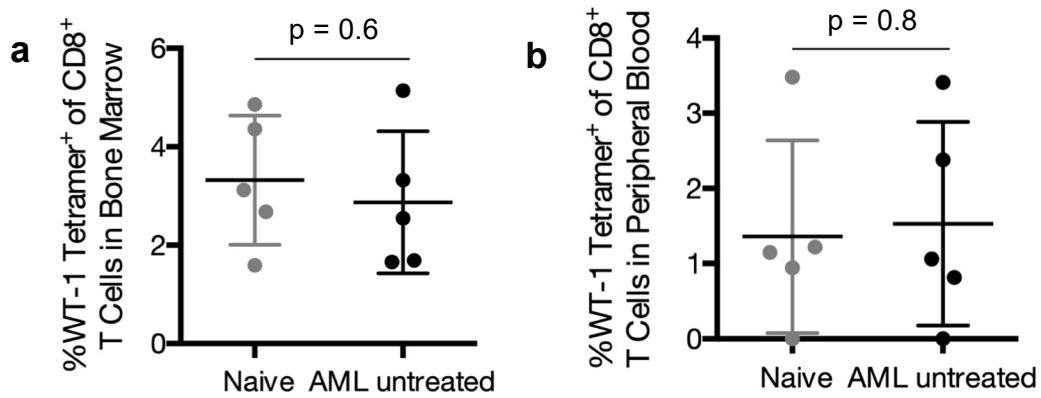


Supplementary Figure 4. Injection site dynamics following vaccination. (a) Measurement of the hydrogel injection site volume after subcutaneous injection over a period of 2 weeks (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, n.s., not significant ($P > 0.05$), analysis of variance (ANOVA) with a Tukey post hoc test; $n = 5$ biologically independent animals per group per time point; means depicted; error bars, s.d.). (b) CD11c⁺ DC recruitment to a cryogel vaccine containing only GM-CSF and CpG compared to subcutaneous skin in bolus-injected mice (two-tailed Mann-Whitney test). (c) Percent of CD11c⁺ DCs expressing CD86 (two-tailed t test). Results shown from one experiment; $n = 5$ biologically independent animals per group; means depicted; error bars, s.d. (b-c).



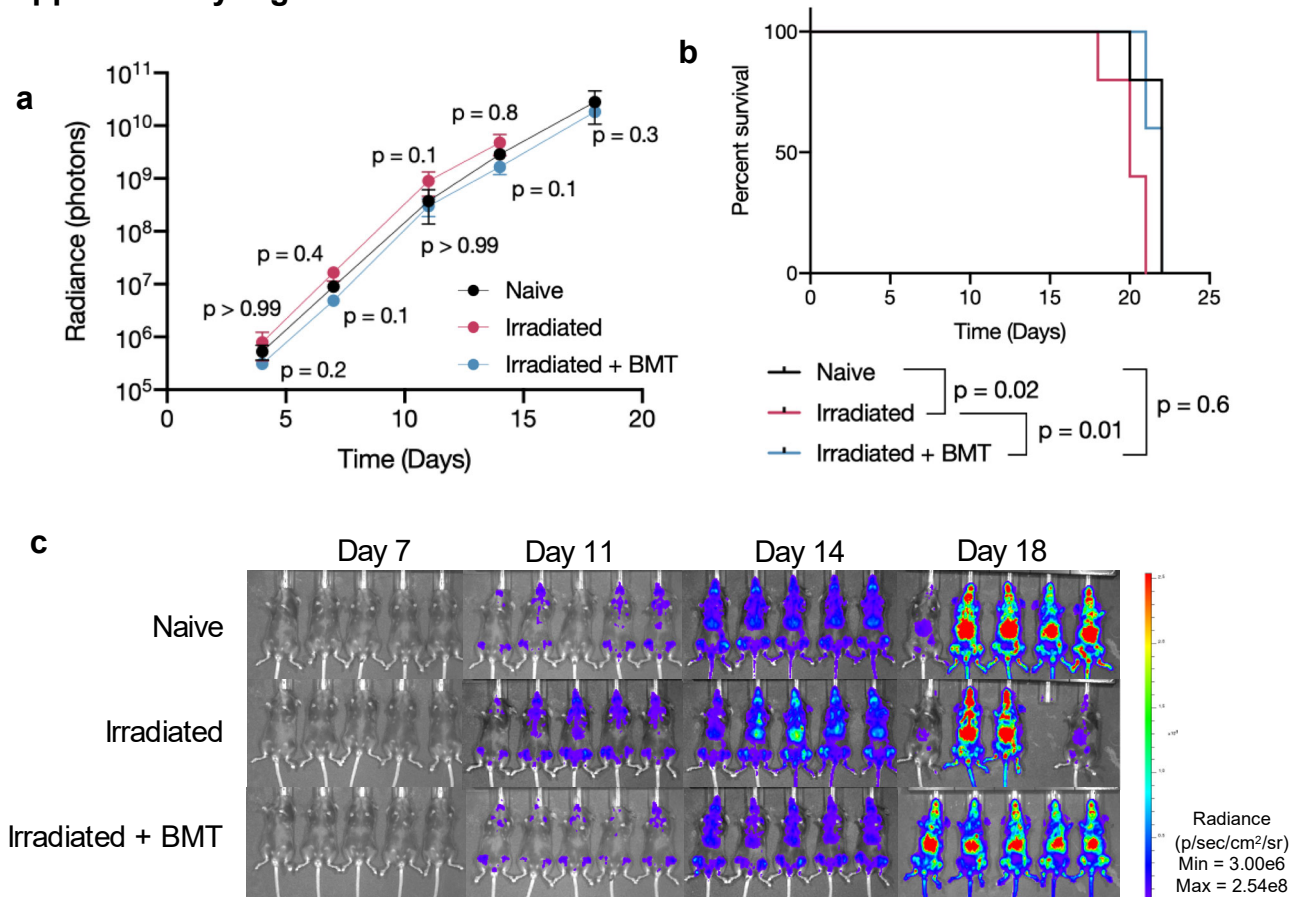
Supplementary Figure 5. Expression of the WT-1 gene in AML cells relative to that in the lineage depleted bone marrow cells from wild type C57Bl/6 mice. Cells isolated from mouse fetal kidney were used as the positive control. Gene expression was normalized to the Gapdh housekeeping gene. (* $P < 0.05$, ** $P < 0.01$, analysis of variance (ANOVA) with a Tukey post hoc test). Results shown from one experiment; $n = 5$ biologically independent animals per group per time point; means depicted; error bars, s.d.

Supplementary Figure 6



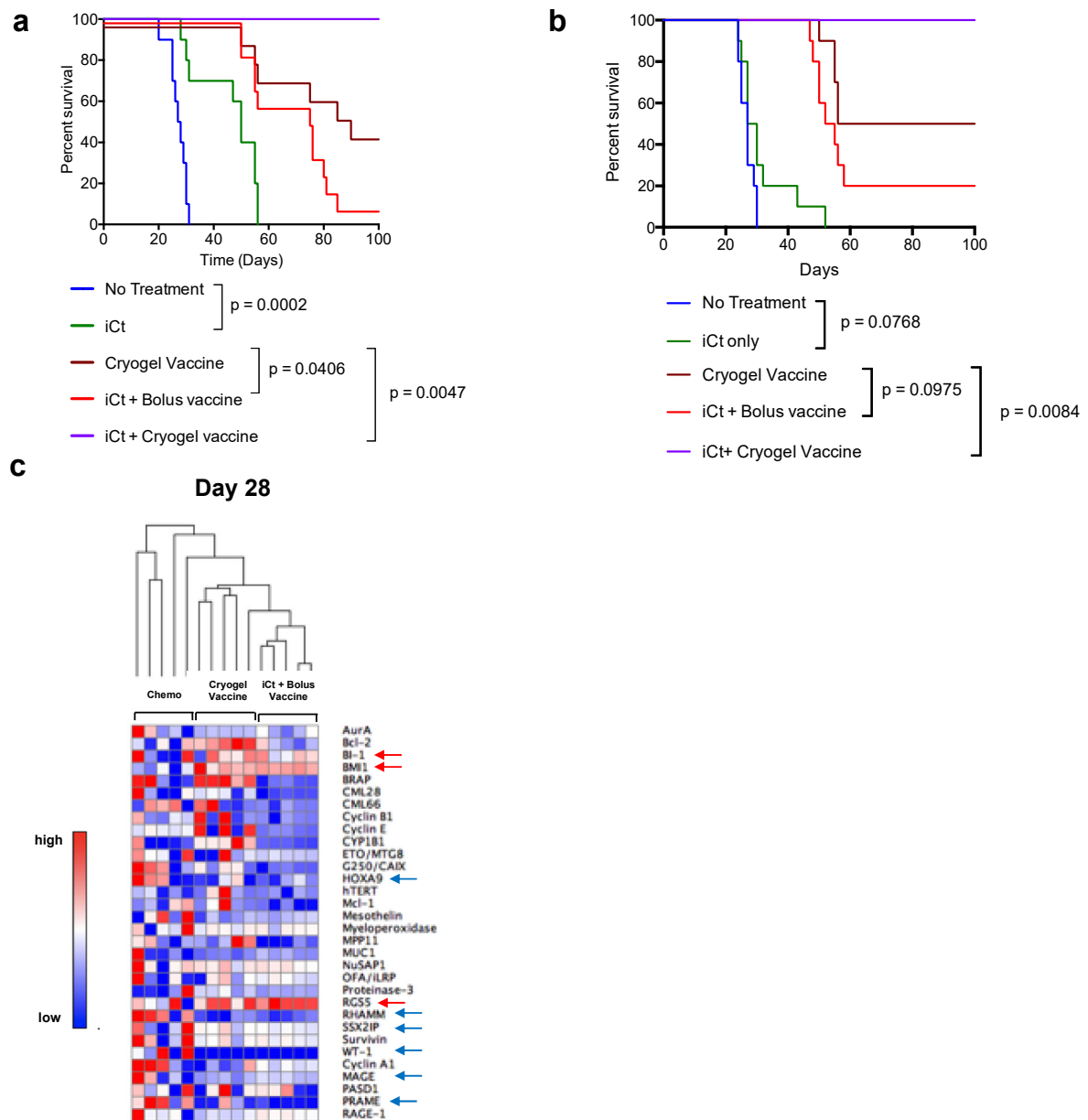
Supplementary Figure 6. T cell priming in AML-exposed mice. Percent of WT-1 tetramer⁺ CTLs in the bone marrow (a) and peripheral blood (b) of naive mice compared to mice inoculated with AML not receiving treatment (two-tailed t test). Results shown from one experiment; n = 5 biologically independent animals per group; means depicted; error bars, s.d.

Supplementary Figure 7



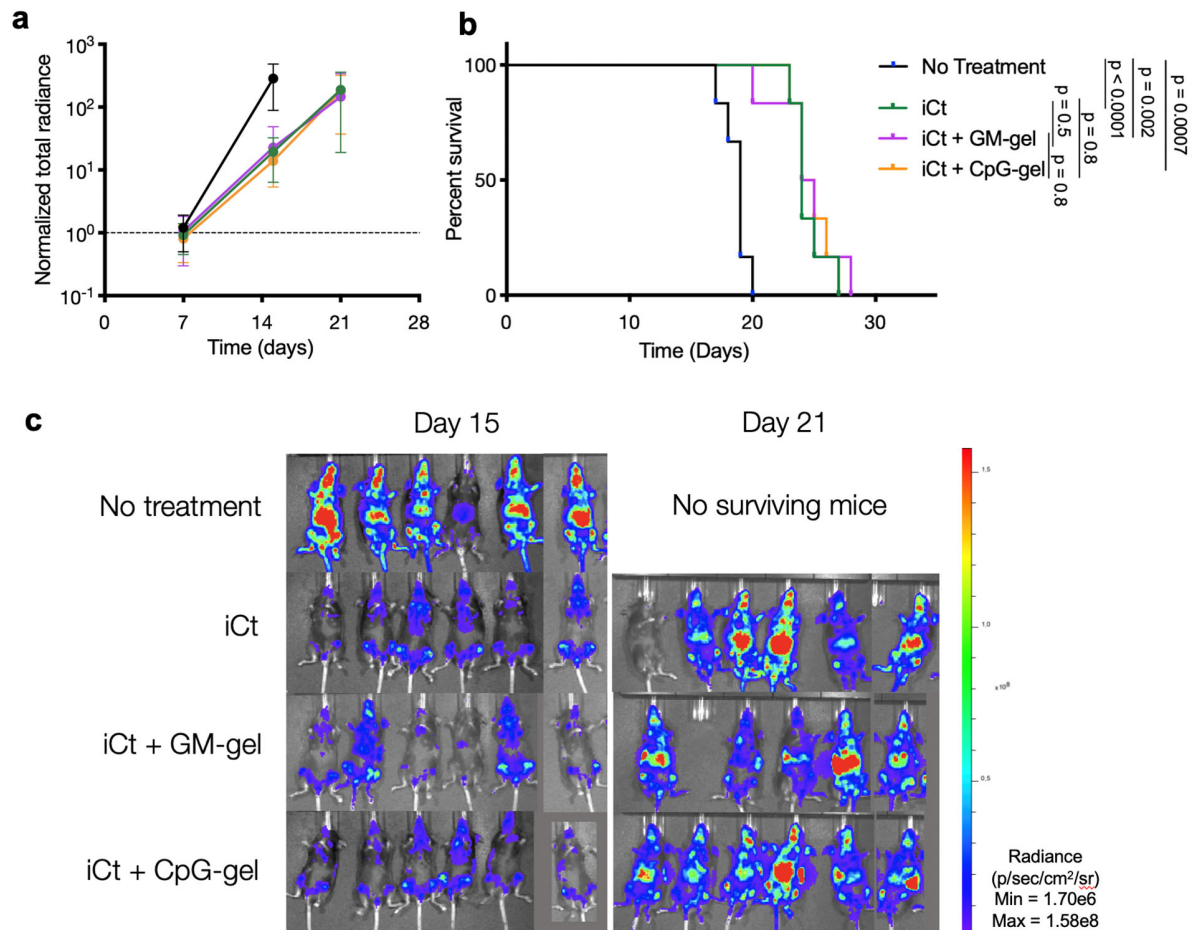
Supplementary Figure 7. Comparison of AML progression between mice that were naïve and non irradiated, sublethally irradiated, and sublethally irradiated with bone marrow transplant. (a) Whole body radiance from luciferase expressing AML cells (analysis of variance (ANOVA) with a Tukey post hoc test, two-tailed t test for two groups at last time point; p values comparing naïve to irradiated shown above, p values comparing naïve to irradiated + BMT shown below); means depicted; error bars, s.d., (b) survival rate (one-sided log-rank (Mantel-Cox) test, not adjusted for multiple comparisons) and (c) bioluminescence images from mice quantified in (a). Results shown from one experiment; $n = 5$ biologically independent animals per group.

Supplementary Figure 8



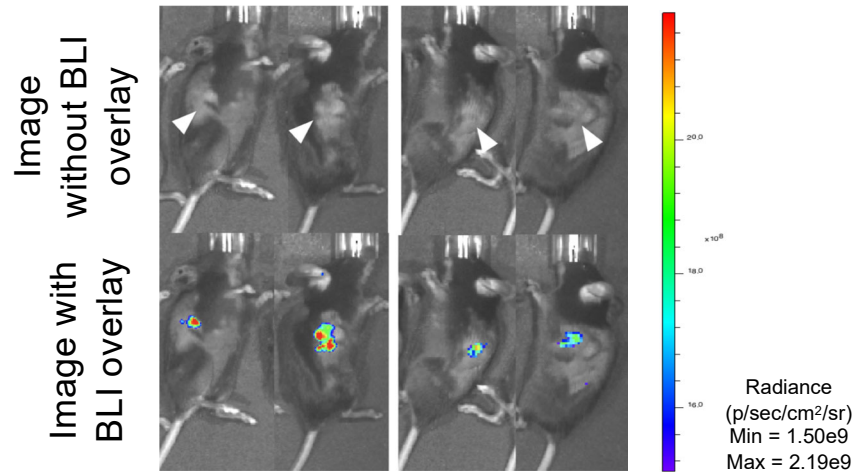
Supplementary Figure 8. Survival rate in AML models and expression of a subset of AML associated genes. (a) Survival rate in MLL-AF9 AML and (b) HoxA9-Meis1 models ($n = 10$ biologically independent animals per group; means depicted; error bars, s.d.; one-sided log-rank (Mantel-Cox) test). (c) Expression of a subset of AML associated genes on Day 28 in AML cells harvested and pooled from the bone marrow, liver and spleen in relapsed mice. Transcripts with lower expression in vaccine groups are indicated by a blue arrow. Transcripts with higher expression in vaccine groups are indicated by a red arrow. AML cells from untreated mice and mice treated with the combination cryogel vaccine and iCt were not included as none of the mice survived at the time of analysis for the former, and treatment efficacy led to no residual AML cells in the latter. Results shown from one experiment; $n = 5$ biologically independent animals per group.

Supplementary Figure 9



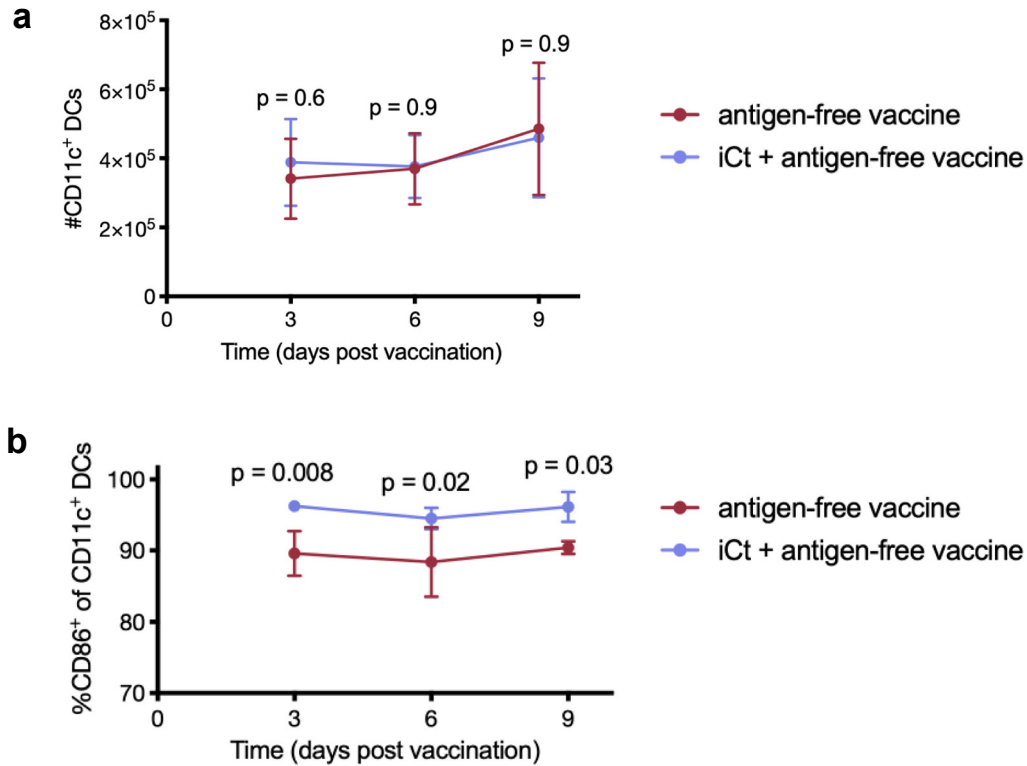
Supplementary Figure 9. Therapeutic efficacy of single-factor, antigen-free cryogel vaccines. (a) Progression of AML in treated study groups, measured as whole body radiance from luciferase expressing AML cells. Means depicted; error, s.d. (b) Survival rate in mice challenged with MLL-AF9 cells given treatment as indicated (GM-gel and CpG-gel refer to antigen-free vaccines containing only GM-CSF or CpG, respectively). $n = 6$ biologically independent animals per group (a-c); one-sided log-rank (Mantel-Cox) test (b). (c) Bioluminescence images from mice quantified in (a). Results shown from one experiment.

Supplementary Figure 10



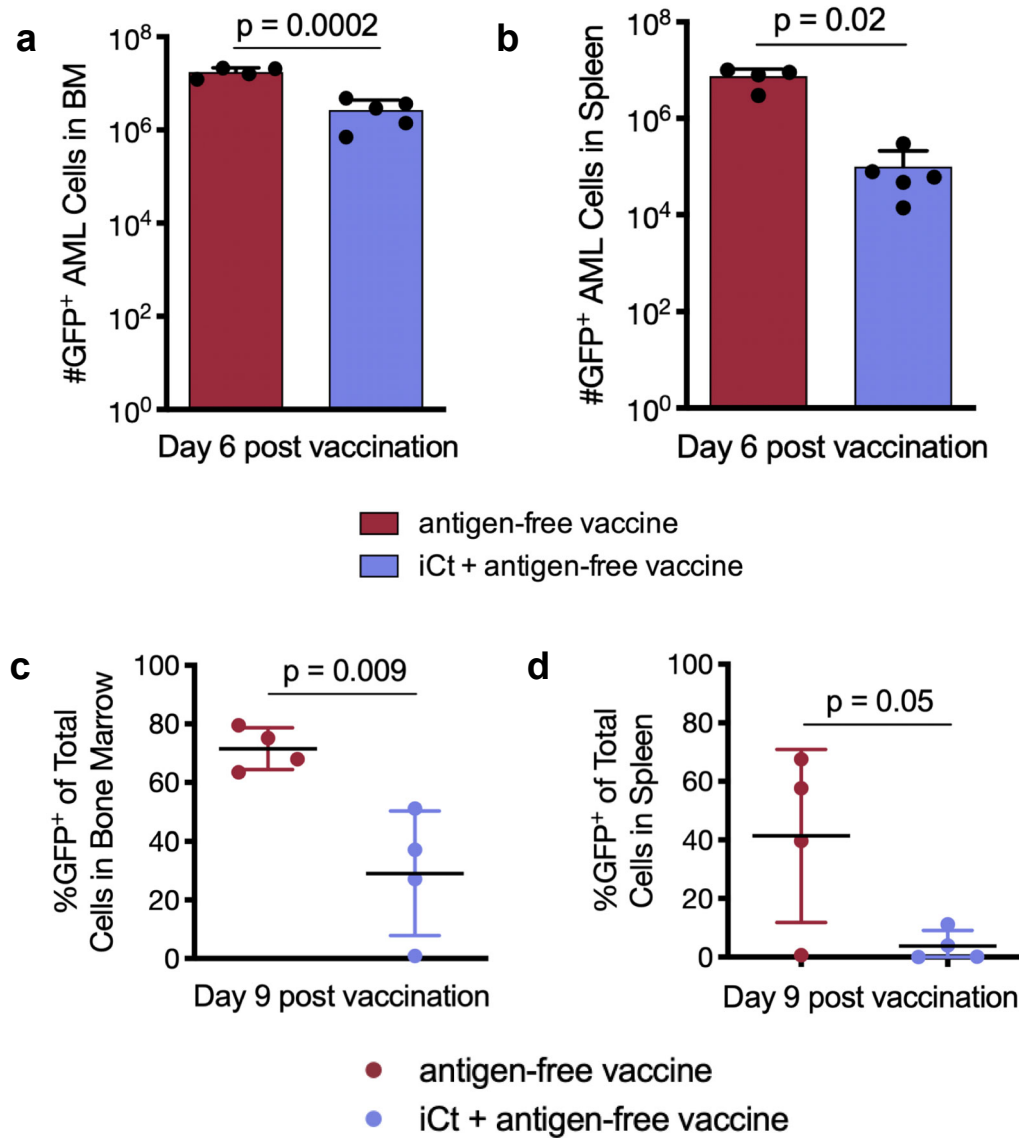
Supplementary Figure 10. Localization of AML bioluminescence signal at cryogels. Photographs (top row) and bioluminescence images (bottom row) of luciferase expressing AML cells in antigen-free cryogels in two mice 21 days following AML inoculation. White arrows in the top row indicate scaffold site. Results from one experiment.

Supplementary Figure 11



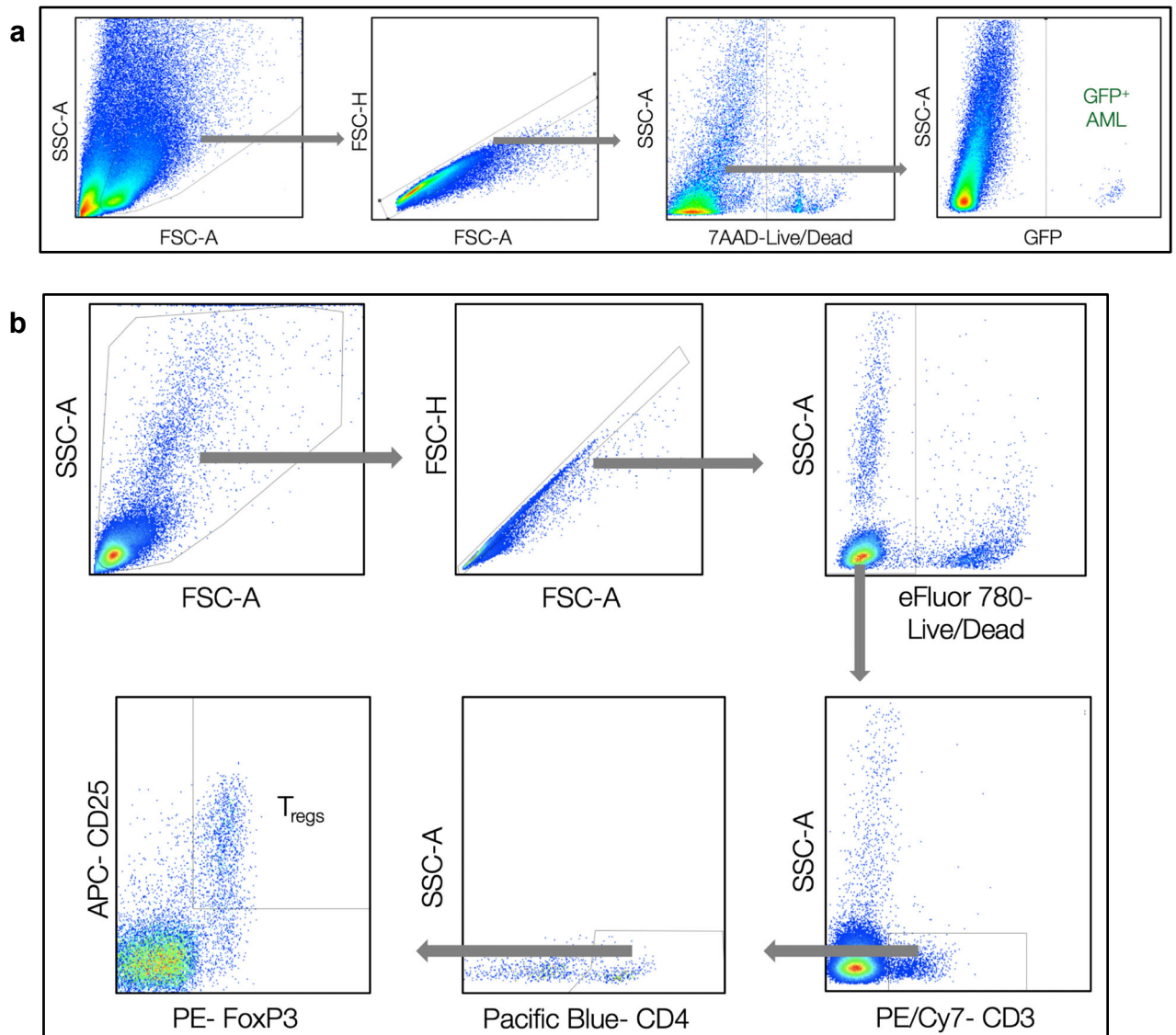
Supplementary Figure 11. Dendritic cell recruitment and activation in cryogels delivered to mice with established AML untreated or given chemotherapy. (a) CD11c⁺ DC recruitment to a cryogel vaccine containing only GM-CSF and CpG delivered in an established model of AML with or without chemotherapy (two-tailed Mann-Whitney test). (b) Percent of CD11c⁺ DCs expressing CD86 (two-tailed Mann-Whitney test). Results from one experiment; n = 5 (both groups, day 3; iCt + antigen-free vaccine group, day 6) or 4 (both groups, day 9; antigen-free vaccine group, day 6) biologically independent animals per group per time point in (a-b); means depicted; error bars, s.d.

Supplementary Figure 12



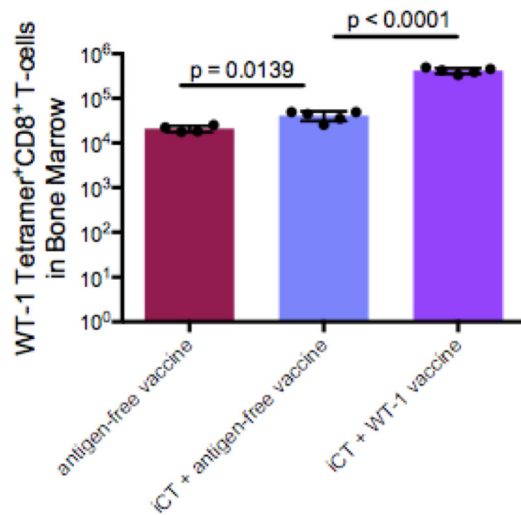
Supplementary Figure 12. AML burden in bone marrow and spleen. Number of GFP⁺ AML cells in bone marrow (a) and spleen (b) of mice treated with the antigen-free cryogel vaccine with and without chemotherapy on Day 6 post vaccination (two-tailed t test (a), two-tailed Mann-Whitney test (b); $n = 4$ (antigen-free vaccine group) or 5 (iCt + antigen-free vaccine group) biologically independent animals per group). Percent of total bone marrow (c) and spleen (d) cells expressing GFP (i.e. AML cells) on Day 9 post vaccination (two-tailed t test; $n = 4$ biologically independent animals per group). Results shown from one experiment; means depicted; error bars, s.d (a-d).

Supplementary Figure 13



Supplementary Figure 13. Representative gating strategies. Flow cytometry plots used to identify GFP⁺ AML cells (a) and CD25⁺FoxP3⁺ Tregs (b).

Supplementary Figure 14



Supplementary Figure 14. WT-1-specific T cell responses comparing full versus antigen-free cryogel vaccines. WT-1 tetramer⁺ CD8⁺ T cells in mice receiving the antigen-free vaccine only, the antigen-free vaccine + iCT, or the antigen-including vaccine + iCT 6 days post-injection of the vaccine (analysis of variance (ANOVA) with a Tukey post hoc test, $n = 4$ (antigen-free vaccine) or 5 (other groups) biologically independent animals per group). Results from one experiment; means depicted; error bars, s.d.