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Supplemental Information

RNA-Modified T Cells Mediate Effective Delivery of Immunomodulatory Cytokines to Brain Tumors

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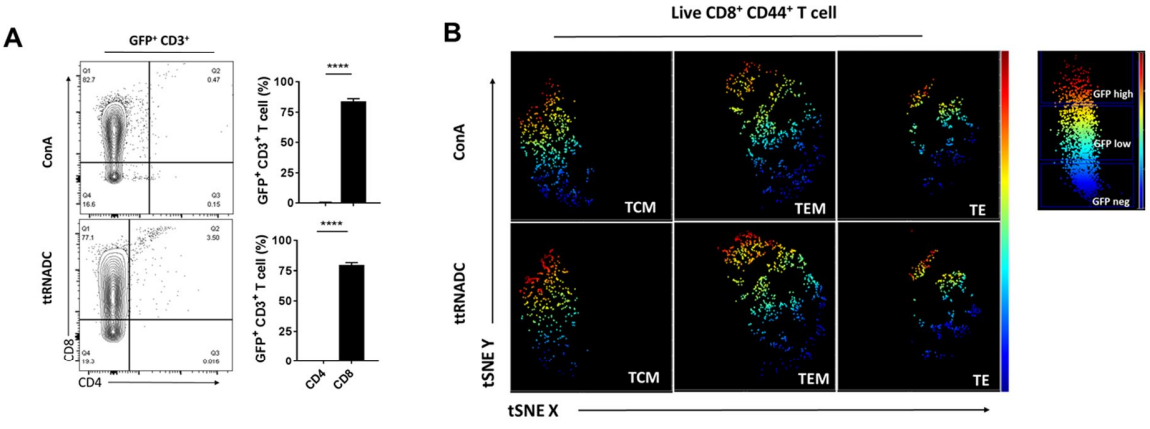
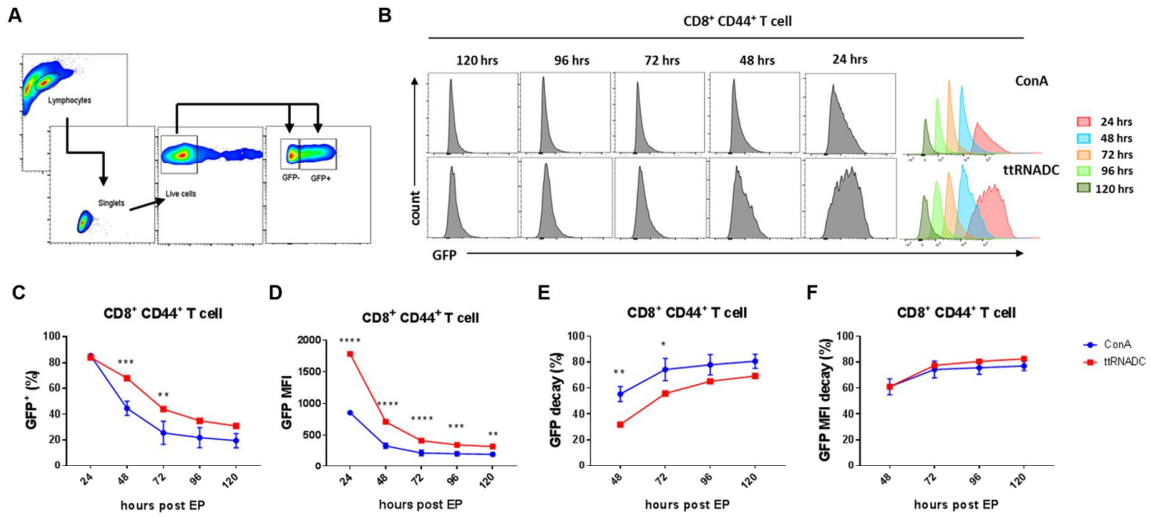
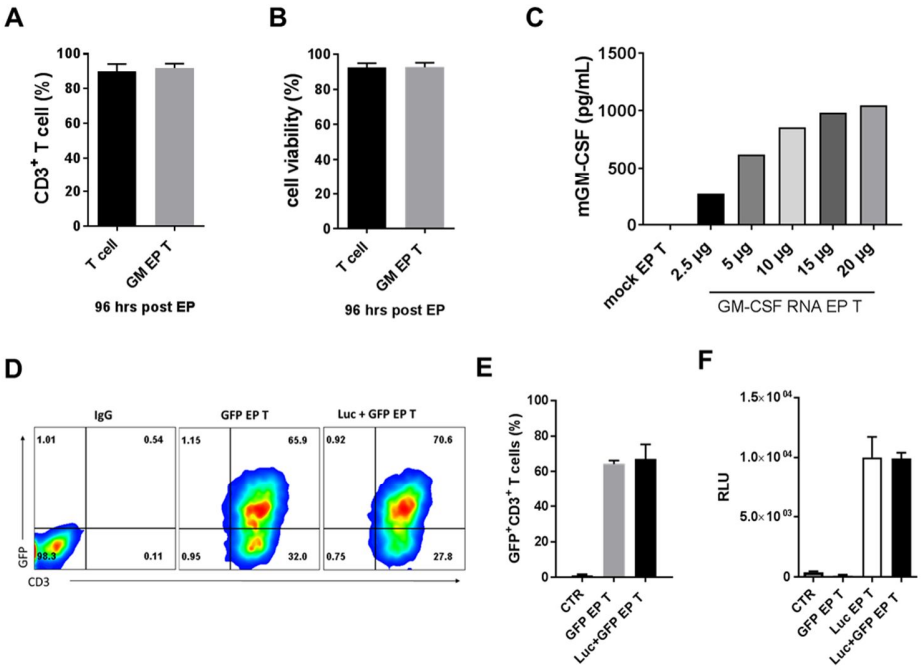
Supplementary figure 1



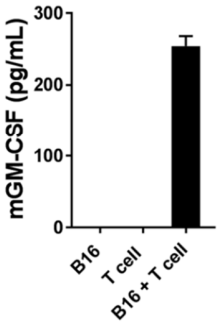
Supplementary figure 2



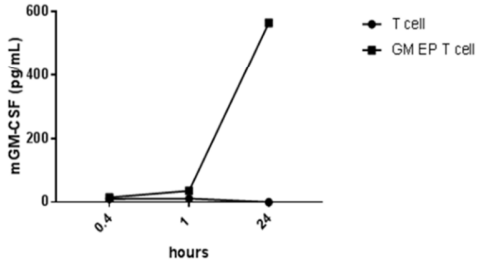
Supplementary figure 3



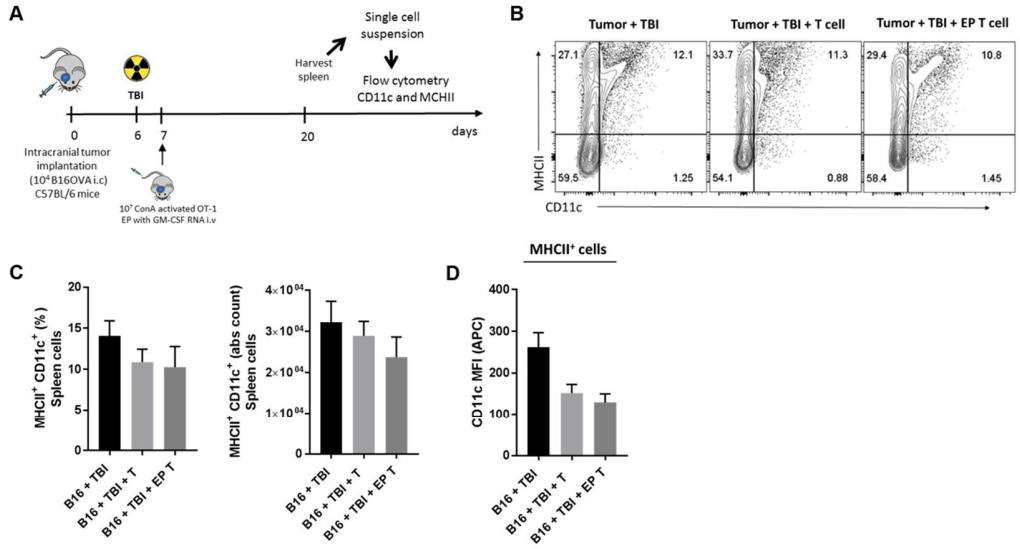
Supplementary figure 4



Supplementary figure 5



Supplementary figure 6



SUPLEMENTARY FIGURE LEGENDS

Supplementary figure 1. Phenotypic analysis of GFP RNA-modified T cell. Spleen was harvested from naïve or ttRNADC vaccinated C57BL/6 mice. Single cell suspension was expanded with ttRNADC or ConA for 5 and 8 days, respectively. Activated T cells were electroporated with 10 µg of GFP RNA. Cells were harvested at indicated time points for phenotypic analysis of GFP+ cells by flow cytometry. (**A**) Percentage of CD8+ and CD4+ T cell subtypes under GFP+CD3+ gate. (**B)** Representative t-SNE analysis of the percentage of TCM, TEM and TE cells (gated on CD8+ CD44+ T cell) negative for GFP, expressing low or high GFP 24 hours after electroporation (gating strategy showed in Figure 2). TCM, central memory T cell; TEM, effector memory T cell; TE, effector T cell; t-SNE, t-distributed stochastic neighbor embedding; ttRNADC, total tumor RNA-pulsed dendritic cells. (****$p < 0.0001$, unpaired *t* test). Values indicated are the mean ± SEM.

Supplementary figure 2. Transgene expression decay is correlated with cell proliferation.

ConA or ttRNADC activated T cells were electroporated with 10 µg of GFP RNA at 5-8 days after T cell expansion. At 4 hours post transfection, cells were enriched by GFP expression using FACS. Sorted cells were collected at indicated time points and stained with anti-CD8 and anti-CD44 antibodies for flow cytometry analysis. Decay was considered the transgene expression from each time point, after T cell electroporation, relative to the transgene expression at 24 hours. (**A**) Scheme of cell sorting strategy. (**B**) Representative histogram, (**C**) percentage and (**D**) MFI of GFP+ CD8+ CD44+ T cell expression over time. (**E**) Percentage of GFP (**F**) and MFI decay of GFP+ CD8+ CD44+ T cells. EP, electroporation; FACS, fluorescent-activated cell sorting; MFI, median fluorescence intensity. (*$p<0.005$; (**$p<0.01$; ***$p<0.001$; ****$p<0.0001$, two-way ANOVA). Values indicated are the mean ± SEM.

Supplementary figure 3. RNA-modified T cell transfection with increasing amount of RNA or multiple RNAs.

Spleen was harvested from naïve C57BL/6 mice and expanded with 1 µg/mL of concanavalin A (ConA). Single cell suspension was electroporated with 10 µg of GFP, Gaussia luciferase (Luc, secreted version of the firefly luciferase) or GM-CSF RNA at day 8. Cells were harvested 24 hours after transfection unless otherwise indicated. (**A**) Percentage and (**B**) cell viability of CD3+ T cells detected by flow cytometry 96 hours post electroporation. (**C**) Murine GM-CSF levels detected in the supernatant by ELISA 24 hours after T cell transfection with increasing concentrations of RNA. (**D**) Representative contour plot and (**E**) percentage of GFP+ CD3+ activated T cells co-transfected with GFP RNA alone or in combination with Luc RNA. (**F**) Relative light units detected in the supernatant by luciferase assay 24 hours after electroporation

of ConA activated T cell with GFP RNA alone or in combination with Luc RNA. EP, electroporated; CTR, non-transfected cells. Values indicated are the mean ± SEM.

Supplementary figure 4. Unmodified T cells mediate GM-CSF secretion *in vitro* upon target tumor cell recognition.

Spleen cells were harvested from OT-1 transgenic mice. Cells were activated with ConA for 8 days and co-cultured with B16F10-OVA (ratio of 10:1). GM-CSF levels were detected by ELISA in the supernatant at 24 hours after co-culture. B16, B16F10-OVA. Values indicated are the mean ± SEM.

Supplementary figure 5. GM-CSF RNA-modified T cells secrete transgene *in vitro.*

Spleen was harvested from OT-1 transgenic mice. Single cell suspension was expanded with ConA for 8 days. Activated T cells were electroporated with 10 µg of murine GM-CSF RNA. Supernatant was harvested and cytokine levels were detected by ELISA at indicated time points post electroporation.

Supplementary figure 6. DC phenotype remained unchanged following systemic GM-CSF RNA-modified T cell administration.

Spleen was harvested from OT-1 transgenic mice. Single cell suspension was expanded with ConA for 8 days and EP with 10 µg of murine GM-CSF RNA. $10x10^6$ of GM-CSF-expression OT-1 T cells were injected i.v (shortly after RNA electroporation) into tumor-bearing C57BL/6 mice at 7 days post i.c B16F10-OVA implantation ($1x10^4$ cells/mouse). Mice received TBI (5 Gy) 24 hours prior to ACT. Mice were sacrificed and spleen was harvested 13 days post T cell injection. DCs were detected by flow cytometry using anti-CD11c and anti-MHCII antibodies. (**A**) Schematic figure illustrating the experimental design. (**B**) Representative contour plot, (**C**) percentage and absolute count. (**D**) MFI of CD11c+ cells under the MHCII+ gate detected in the

spleen. ACT, adoptive cell transfer; EP, electroporated; Gy, gray; TBI, total body irradiation; T, T cell; B16, B16F10-OVA; MFI, median fluorescence intensity. Values indicated are the mean ± SEM.