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Supplemental information

mRNAs encoding IL-12 and a decoy-resistant variant of IL-18 synergize to engineer T cells for efficacious intratumoral adoptive immunotherapy

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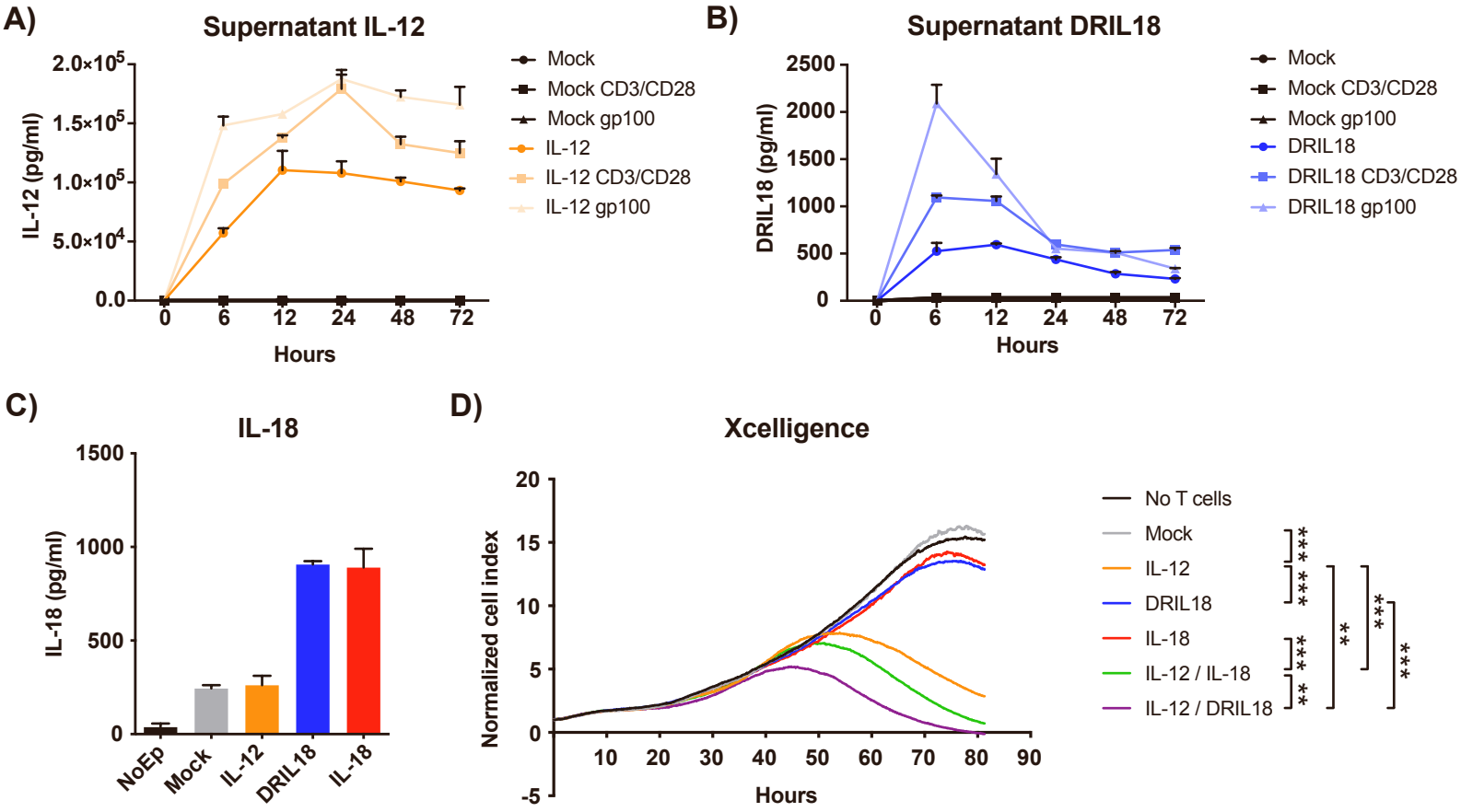

Figure S1. Electroporated mRNAs encoding IL-18, IL-12 and DRIL18 confer expression of these cytokines to Pmel-1 CD8 T lymphocytes and enhance their cytotoxic function. Related to Figure 1.

(A and B) Time course of IL-12 and DRIL18 concentrations in supernatants of pmel-1 cells electroporated with the indicated mRNAs. Pmel-1 cells were preactivated by cognate gp100 peptide and restimulated with CD3+CD28 or gp100 during the cultures.

(C) TCR-transgenic splenic Pmel-1 T cells activated by gp100 cognate peptide for 48h and maintained another 48h with IL-2 were transfected with in-vitro synthetized mRNAs encoding IL-12, DRIL18 and IL-18 concentrations in the supernatant were determined 48 h later.

(D) Cytotoxicity experiment with Pmel-1 electroporated with the indicated cytokine-encoding mRNAs that were cocultured with B16-OVA cells at 1:5 ratio. IL-12/IL-18 and IL-12/DRIL18 indicate Pmel-1 cells electroporated separately with each mRNA and mixed together one to one before the experiment.

Two-way ANOVA tests were used for comparisons. Experiments are representative of at least two similarly performed. Biological duplicates were performed in all experiments. Data is represented as mean +/- SD.

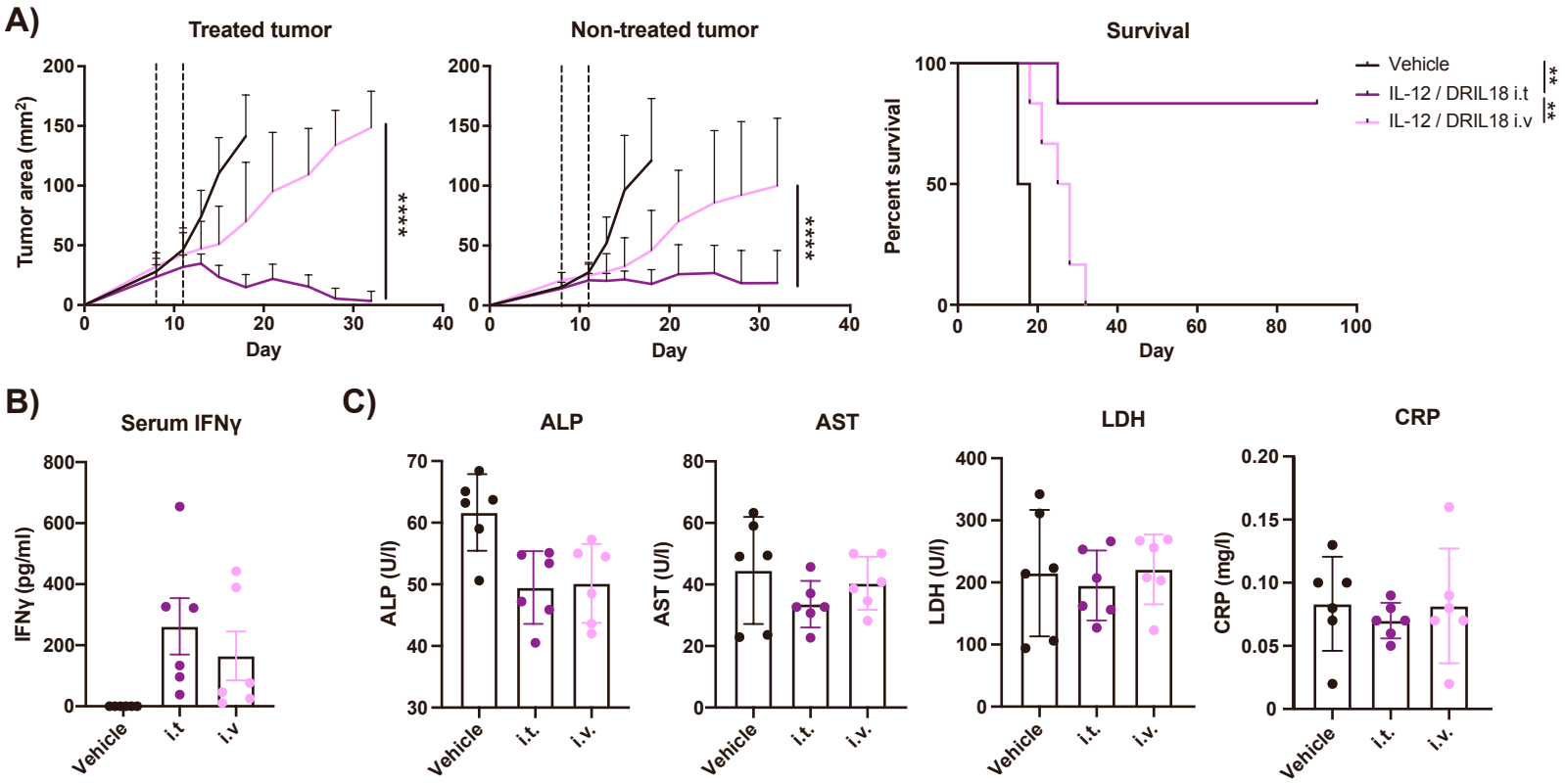

Figure S2. Unilateral intratumoral delivery is more efficacious than intravenous delivery in the B16OVA bilateral mouse model. Related to FIgure 1.

(A) Experiments as in figure 1H comparing intratumoral versus intravenous delivery of identical doses of IL-12/DRIL18 electroporated pmel-1 cells.

(B) Concentrations of IFNγ in the serum of treated mice 48h after the second T-cell dose given intravenously or intratumorally as indicated.

(C) Concentrations of ALP (alkaline phosphatase), AST (aspartate aminotransferase) LDH (lactate dehydrogenase) and CRP (C-reactive protein) in the serum of treated mice 48h following the second dose given as indicated.

Statistical comparisons were performed using two-way ANOVA and log-rank test. Experiments are representative of two similarly performed. For each experiment, we randomly assigned 6 mice per group. Data is represented as mean +/- SD.

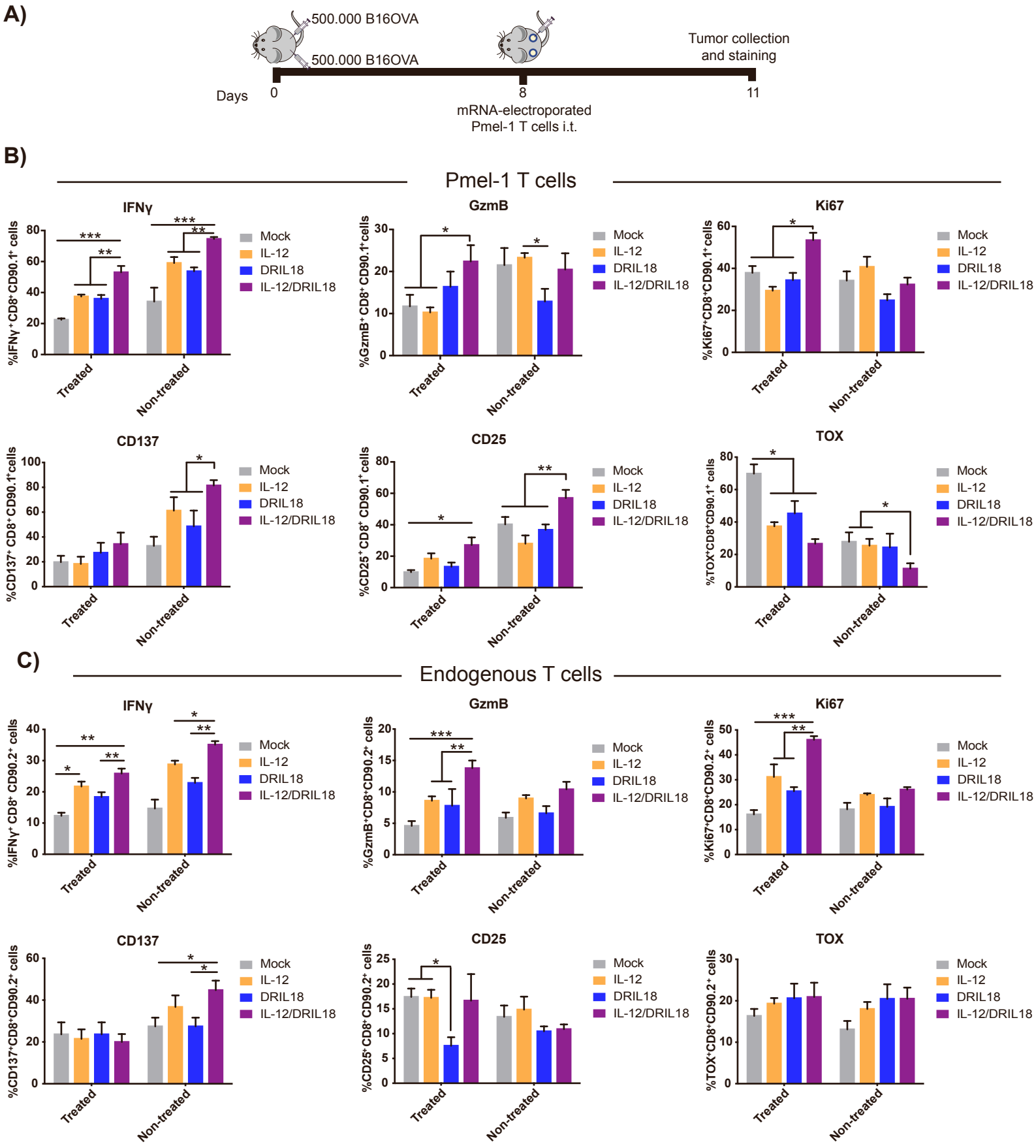

Figure S3. Adoptively transferred cells upon IL-12/DRIL18 mRNA electroporation show stronger signs of proliferation and activation. Related to Figure 1.

(A) Schematic representation of the experiments. At the end of the experiment, the directly injected and contralateral tumors were collected and a multicolor flow cytometry was performed on cell suspensions electronically gating CD90.1+ adoptively transferred ells and endogenous CD90.2+ CD8+ T lymphocytes.

(B) Percentages of expression of the indicated markers in pmel-1 cells that had been electroporated with the indicated mRNAs. (

C) Similar determination in the tumor-infiltrating CD8 T-cell compartment. Statistical comparisons were performed using one-way ANOVA tests. Experiments are representative of two similarly performed. We randomly assigned 6 mice per group before tumor injection. Data is represented as mean +/- SD.

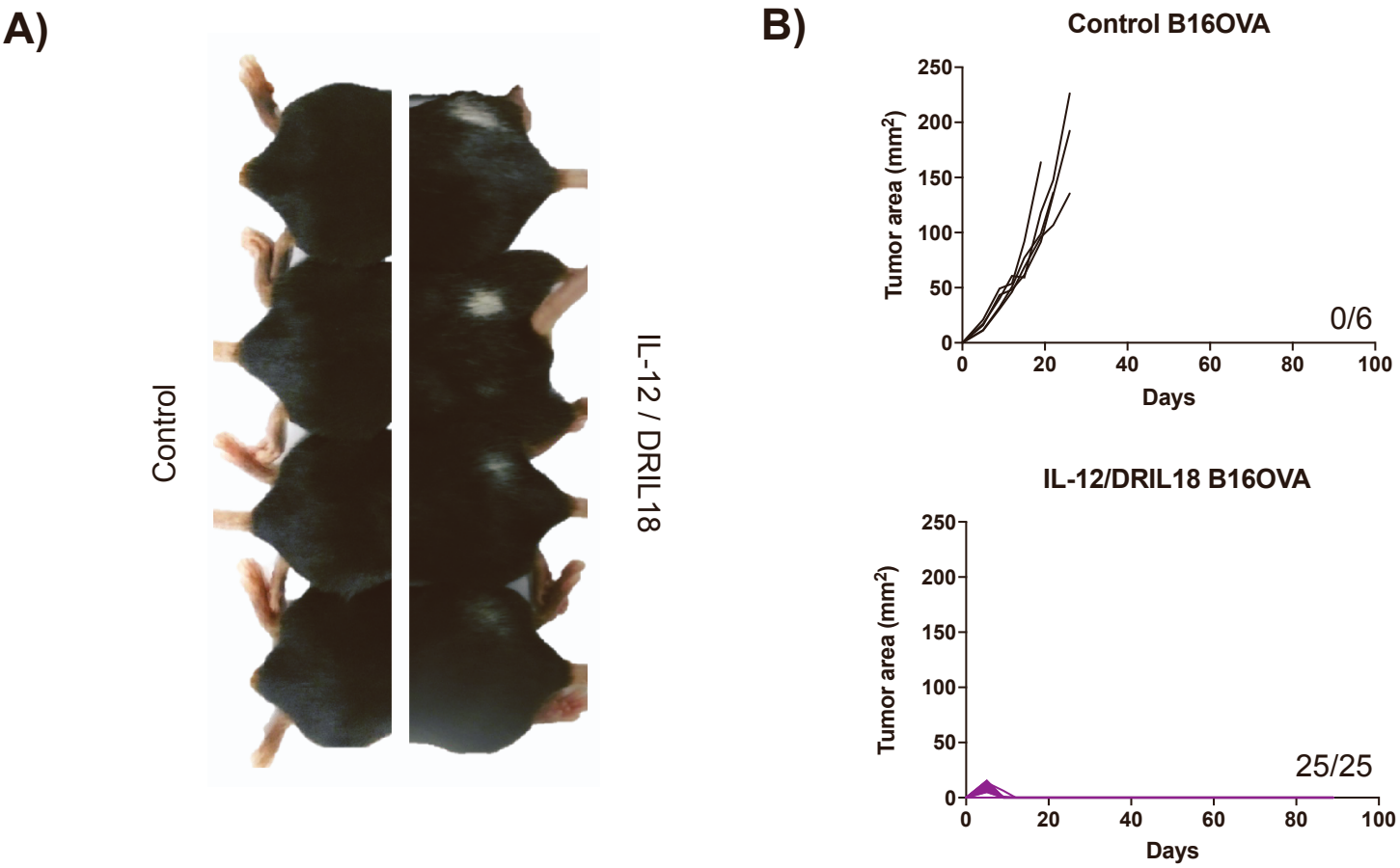

Figure S4. Vitiligo and long-term immunity in successfully treated mice. Related to Figure 1.
(A) Mice from experiments similar to those shown in Figures 1H and 1I showed de pigmentation (vitiligo) in the area of the fur where tumors had been injected and regressed. (B) Cured mice were kept at least 90 days following complete tumor regression after treatment with IL-12/DRIL18 mRNA electroporated Pmel-1 T cells and rejected a rechallenge with B16-OVA whilst a control group of naïve mice developed fast progressing lethal tumors. Experiments are representative of five similarly performed.

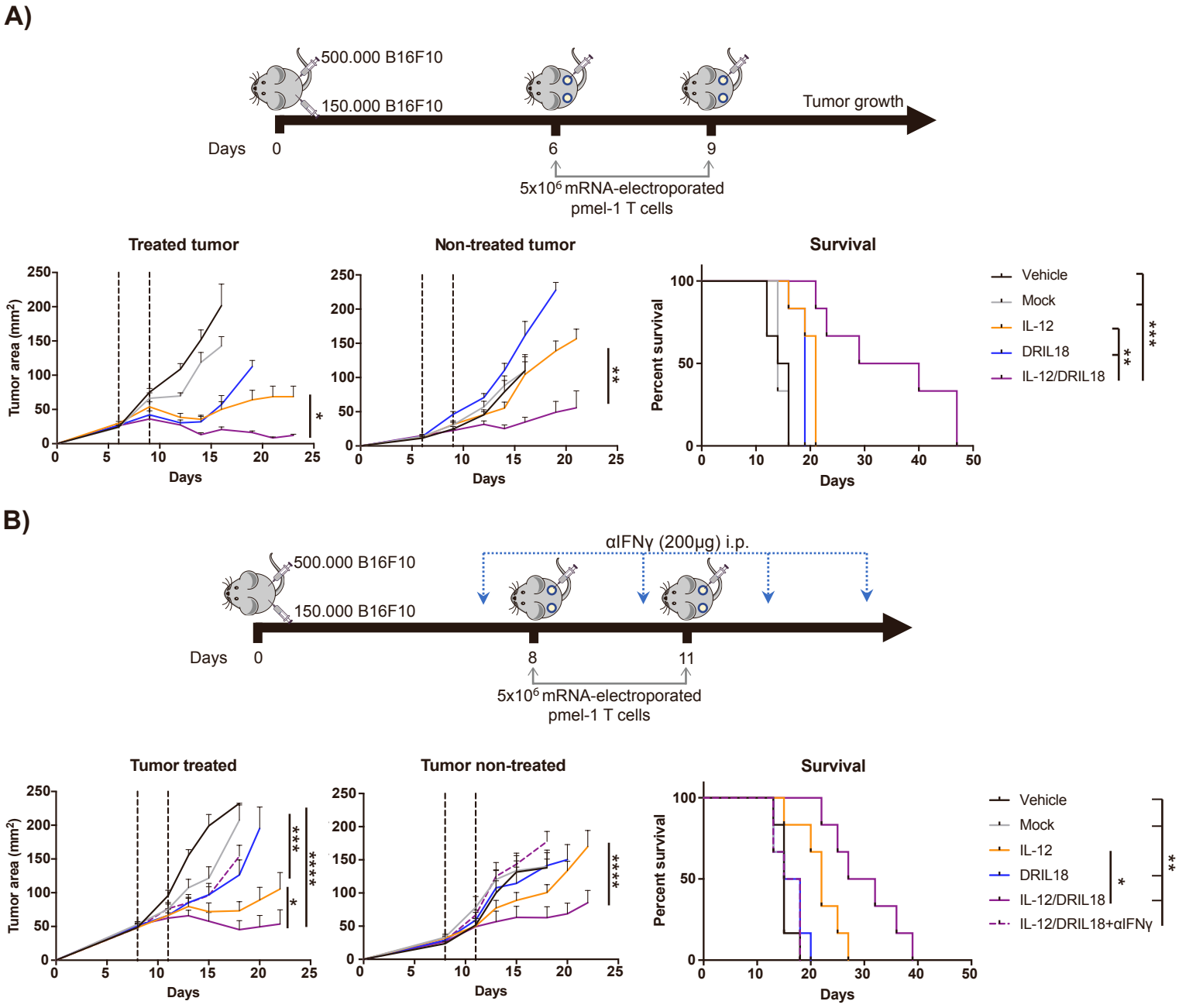

Figure S5. Efficacy of the scIL-12 and DRIL18 adoptive T cell strategy against B16F10 tumors. Related to Figure 1.
(A) Experiments as in Figure 1H were performed in mice bearing bilateral B16F10-derived tumors treated intratumorally on day +6 and +9 with mRNA-electroporated Pmel-1 cells or vehicle. Follow-up of tumor sizes of the indicated groups of treatment in the injected and non-injected tumor sites are shown. Overall survival of the mice in each group is also provided.
(B) Experiments as in A, but postponing the treatment onset to day +8 and giving to a group of mice treated with Pmel-1 (IL-12/DRIL18) a course of neutralizing anti-IFN-γ mAb.
Statistical comparisons were made with log-rank tests. Experiments are representative of at least two similarly performed. We randomly assigned 6 mice per group before tumor treatment. Data is represented as mean +/- SD.

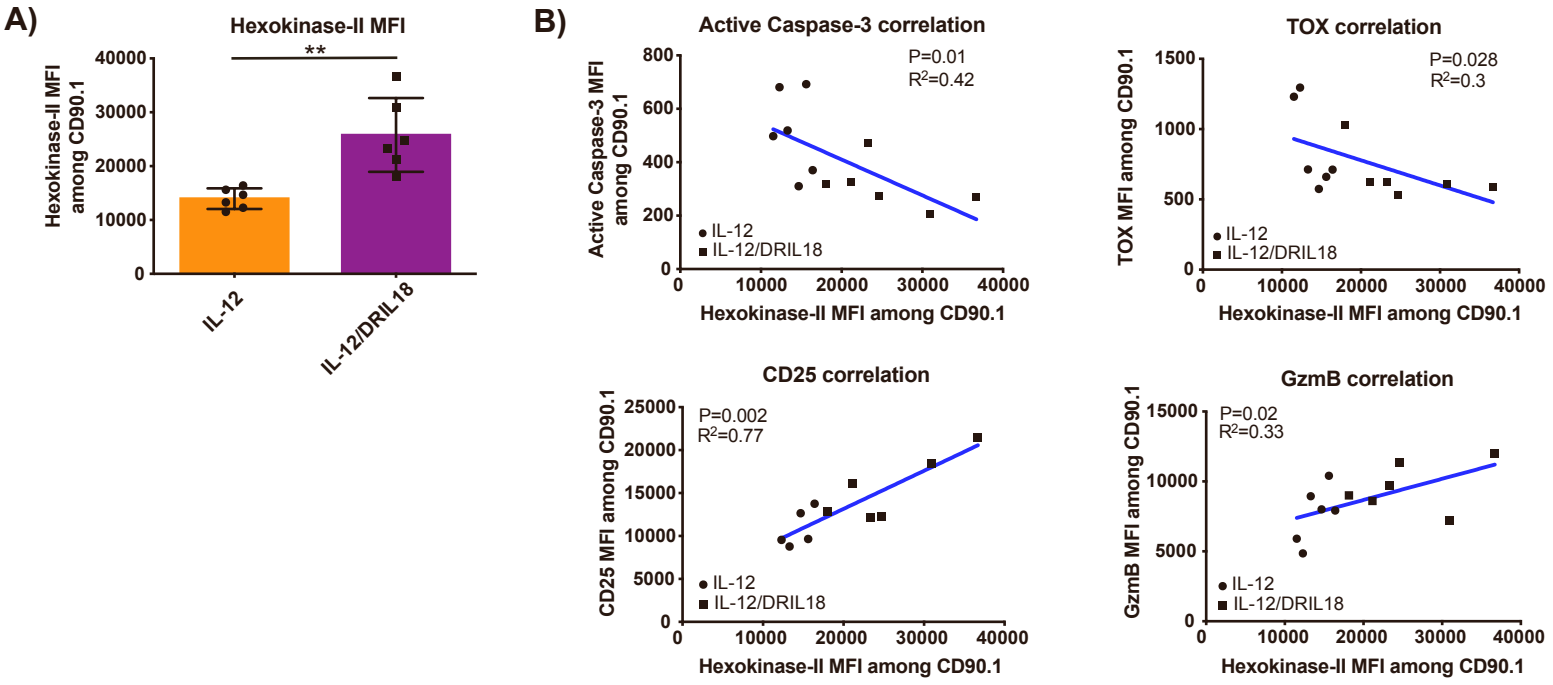

Figure S6. Hexokinase-II expression correlates with markers of functional T-cell activation. (A) Flow cytometry analysis of hexokinase -II expression in pmel-1 cells electroporated with the indicated mRNAs and recovered from B16OVA tumors 24h after being intratumorally injected. (B) Average intensity of expression of hexokinase-II in individual tumors compared with the average intensity of expression of the indicated markers (active caspase-3, CD25 surface expression, granzyme B intracellular expression and TOX expression). Regression statistics are shown in each graph. Statistical comparison in A was performed using one-way ANOVA test. Experiments are representative of two similarly performed. Related to Figure 4.

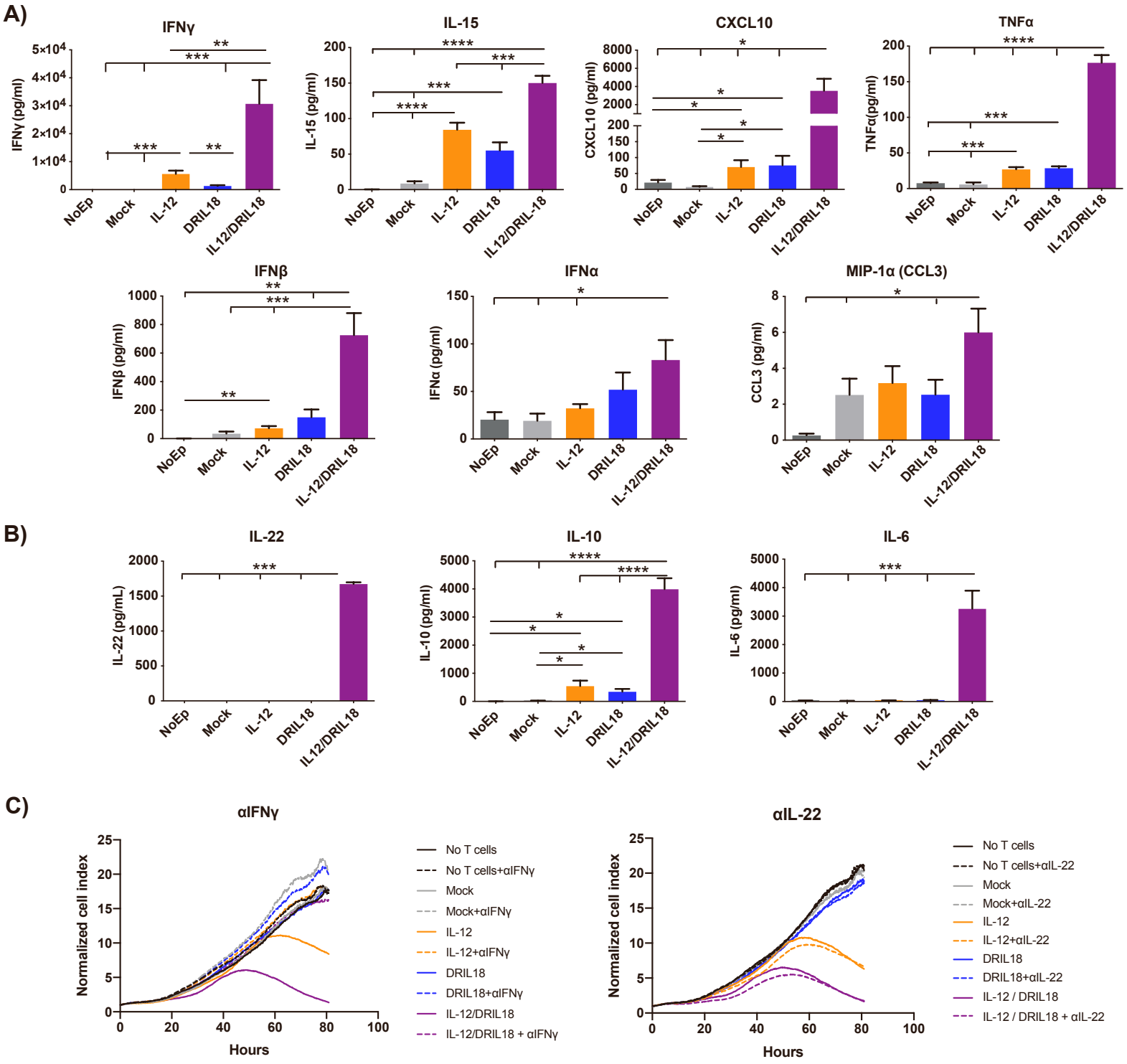

Figure S7. Electroporated DRIL18 and scIL-12 mRNA induce the production of endogenous cytokines. Related to Figure 5.
(A) Concentrations of the indicated immunostimulatory cytokines in 48h supernatants of Pmel-1 cells electroporated as indicated and assessed by a multiplex assay.
(B) Concentrations of potentially immunosuppressive cytokines in the same culture supernatants.
(C) Cytotoxicity experiments of the indicated mRNA-electroporated Pmel-1 cells against B16-OVA. Combinations of scIL-12/DRIL18 lymphocytes showed synergy. In the left graph neutralizing an anti-IFNγ mAb was added to some of the assays as well as a neutralizing anti-IL-22 mAb in some of the conditions shown in the right graph.
Experiments were repeated twice and statistical comparisons were performed with one-way ANOVA (A-B) or two-way ANOVA (C) tests. Biological duplicates were performed in all experiments. Data is represented as mean +/- SD.

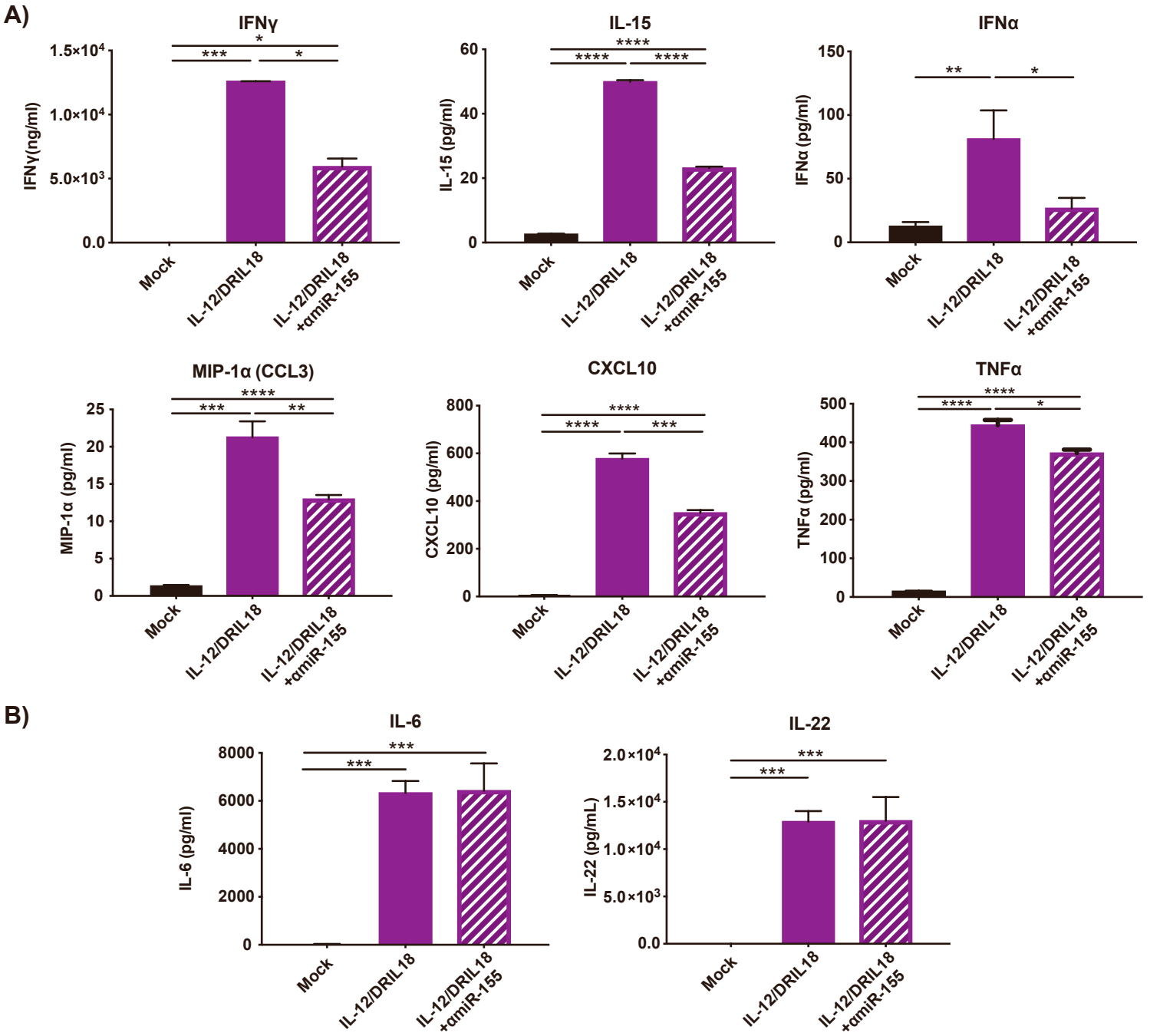

Figure S8. miR-155 degradation induced by coelectroporation of an antagomir reduces the production of T-cell stimulating cytokines. Related to Figure 5.

(A) Multiplex assessment of the concentrations of the indicated cytokines in the culture supernatants of pmel-1 cells electroporated with the indicated mRNAs with or without the antagomir.

(B) Experiments as in A testing the concentration of IL-6 and IL-12 that do not vary upon antagomir electroporation.

Statistical comparisons were performed using one-way ANOVA tests. Experiments are representative of two similarly performed. Biological duplicates were performed in all experiments. Data is represented as mean +/- SD.

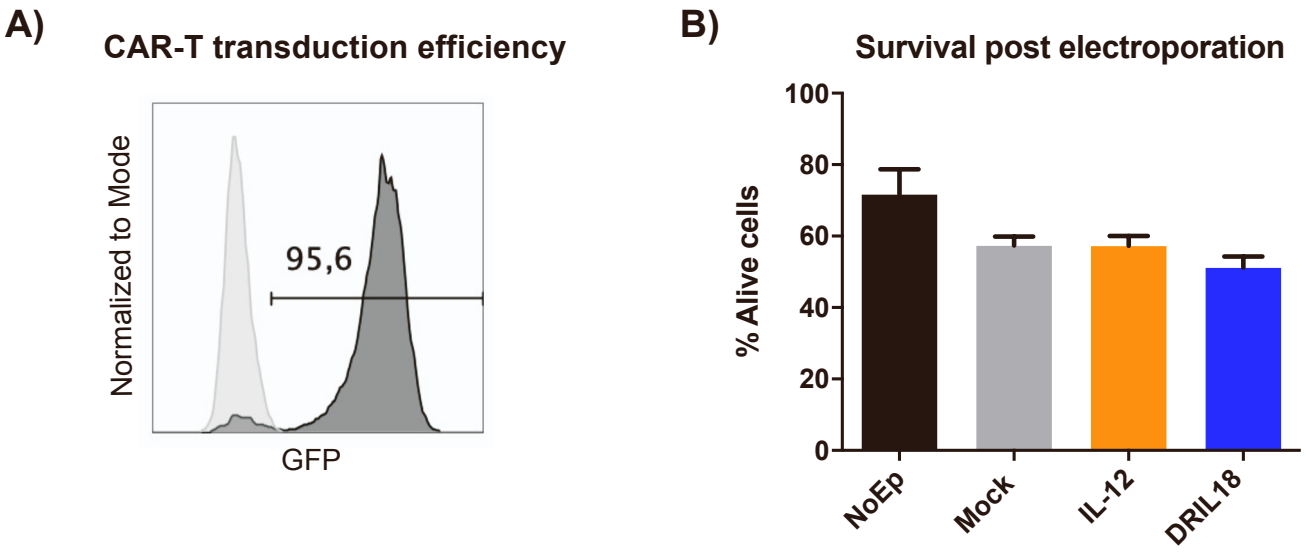

Figure S9. gp75 CAR transduction efficiency and viability following mRNA electroporation. Related to Figure 7.
(A) EGFP expression by CAR T cells transduced (black histogram) or untransduced T cell blasts (grey histogram) derived from splenocytes of C57Bl/6 mice by culture with plate-bound anti-CD3 mAb and soluble anti-CD28 mAb.
(B) Cell viability 6 h following electroporation procedures on CAR T cells.
Experiments are representative of at least two similarly performed. Biological duplicates were performed in all experiments. Data is represented as mean +/- SD.

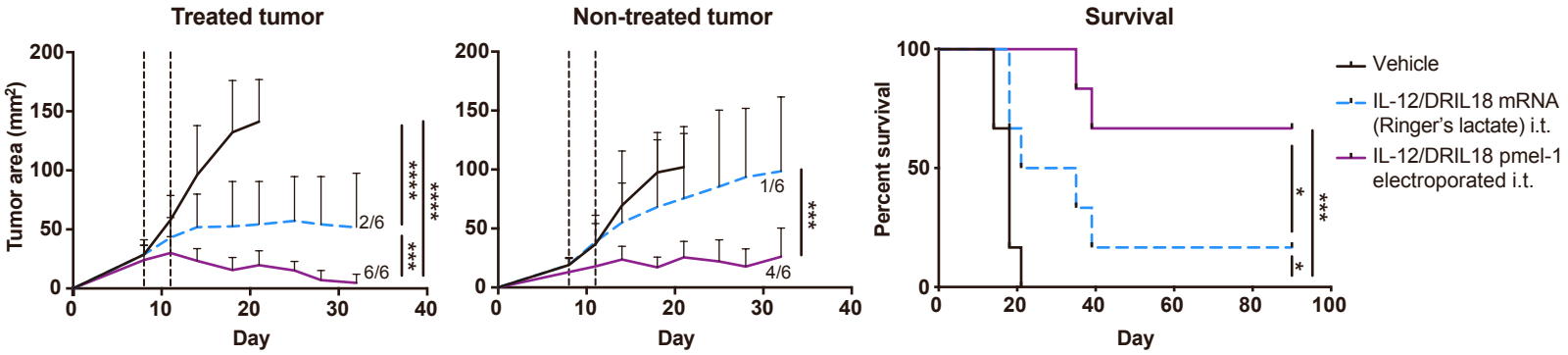

Figure S10. Comparison of naked mRNA enconding scIL-12 and DRIL18 given via intratumoral injection and intratumoral adoptive transfer of pmel-1 cells electroporated with the same mRNAs. Related to Figure 1.

Mice bearing bilateral B16OVA tumors were injected with scIL12 and DRIL18 encoding naked mRNA diluted in Ringer's lactate compared in its bilateral tumor effects with IL-12/DRIL18 electroporated pmel-1 cells given intratumorally. Both treatments were given on days +8 and +11. Black dotted lines represent the days of treatment. Tumor size follow-up of injected and contralateral tumors are provided with the overall survival achieved by the two different treatments. Tumor-free mice were also provided in each graph. Statistical comparisons were performed using two-way ANOVA test and log-rank tests for Kaplan-Meyer survival curves. Experiments are representative of two similarly performed.