

Fucosylation Enhances Activity of Chimeric Antigen Receptor T-cells Against Lung Cancer

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Introduction

Fucosylation is a process by which fucose sugar groups are added to cell surface receptors. This process is mediated by fucosyl transferases that attach terminal fucose groups to acceptors on the cell surface. Fucosylation of cord blood stem cells and human regulatory T cells (Treg) were shown to enhance cord blood engraftment and Treg homing to inflamed tissues. Since tumor tissues have a component of inflammation, we hypothesized that fucosylation of antigen specific chimeric antigen receptor T-cells (CAR-T) will enhance their migration into tumors and subsequent killing of tumor cells. Specifically, we tested whether *in vivo* fucosylation of CART that target EGFR, a molecule over-expressed in non-small cell lung cancer (NSCLC), enhances EGFR-CAR-T cell migration and cytotoxic functions.

Experimental Design

- **EGFR-CAR-T cell line preparation:** Human T cells were purified from the blood cell fractions of normal blood donors and activated with CD3/28 beads on day 0, transduced with lentiviral vectors encoding EGFR-CAR (provided by Dr. Marcela Maus) on day 1, and cultured for a week in RPMI-10% FBS with 20 IU/ml rIL-2. Transduction of CAR-T cell products was verified and quantified by staining for the m-cherry reporter encoded in the vector
- **Fucosylation:** EGFR-CAR-T cell fucosylation was achieved by incubating the cells with FTVII enzyme (FTVII was generously supplied by Targazyme, Inc., Carlsbad, CA) and GDP fucose for 30 min at room temperature in phosphate buffered solution and confirmed by flow cytometry after staining EGFR-CAR-T cells with HECA-452 antibody.
- **Migration assay:** Cytoselect standard leukocyte migration assays were used to investigate the effects of fucosylation on EGFR-T migration through a HUVEC monolayer barrier.
- **Cytotoxicity assay:** Standard Calcein AM cytotoxicity assays were performed to test the effects of CAR-T fucosylation on target cell killing. Fucosylated and non-fucosylated EGFR-CAR-T cells were co-cultured with H358 lung cancer cells. After 4 hours, intracellular Calcein AM was measured as an indicator of live cells. Cytotoxicity was measured at multiple Effector:Target (E:T) ratios.
- **Xenograft mouse model:** Sublethally irradiated NSG mice were engrafted orthotopically with NSCLC tumor cells. On day 1, mice received IV injections of 2×10^6 fucosylated or non-fucosylated EGFR-CAR-T cells and were followed closely for tumor size as assessed by bioluminescence imaging. Mice were sacrificed on week 4 and assessed for tumor and CAR-T homing.

Summary of Key Findings

- EGFR-CAR-T cells were efficiently generated via transduction (Figure 1)
- EGFR-CAR-T cells were efficiently fucosylated by FTVII enzyme (Fig 2).
- Fucosylation significantly increased migration of EGFR-CAR-T cells into tumor cells (Fig. 4).
- Fucosylation enhanced cytolytic activity of EGFR-CAR-T cells against A549 lung cancer cells (Fig. 5).
- *In vivo* experiments showed that the frequency of CD8+ CART- that were human CD45+/mouse CD45- was significantly higher in the primary tumors that were treated with fucosylated EGFR-CAR-T cells (Fig. 6).
- *In vivo* experiments using H358 lung cancer xenografts showed that fucosylated EGFR-CAR-T reduced tumor size more than for the non-fucosylated group (Fig. 6).

Conclusion

Fucosylation of antigen-specific EGFR-CAR-T cells enhance cell migration, as well as homing and cytotoxic functions *in vitro* and *in vivo*. These data demonstrate a novel approach to enhance the efficacy of antigen specific T cells that could be used in adoptive cellular immunotherapy approaches for lung cancer.

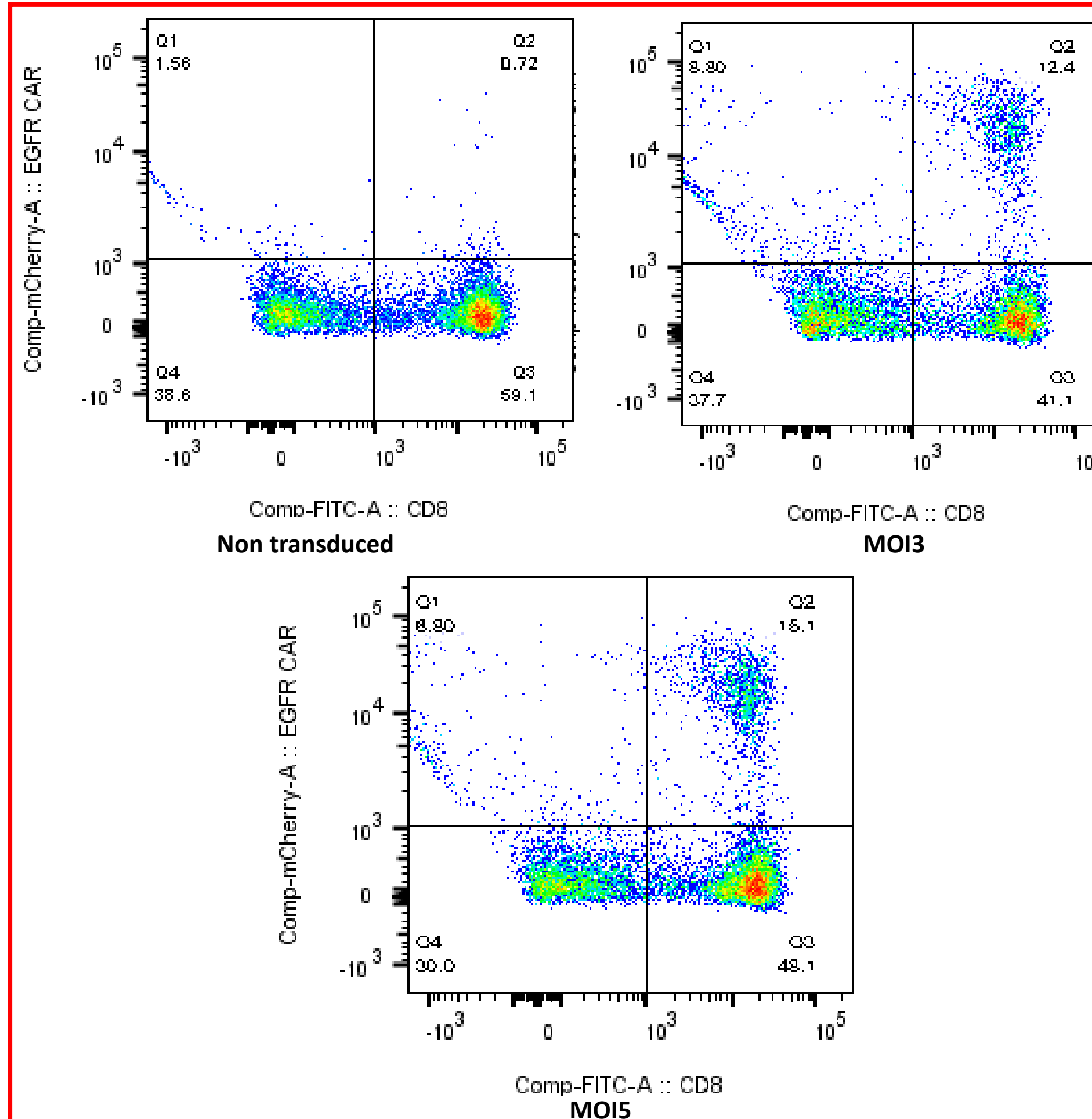


Figure 1. EGFR CAR-T cells are efficiently transduced. Transduced EGFR-CAR-T cells displayed the highest efficiency of transduction of EGFR CAR into CD8+ cells. As expected, the efficiency was higher for MOI5 than MOI3. This data indicates the successful synthesis of EGFR-CAR-T cells.

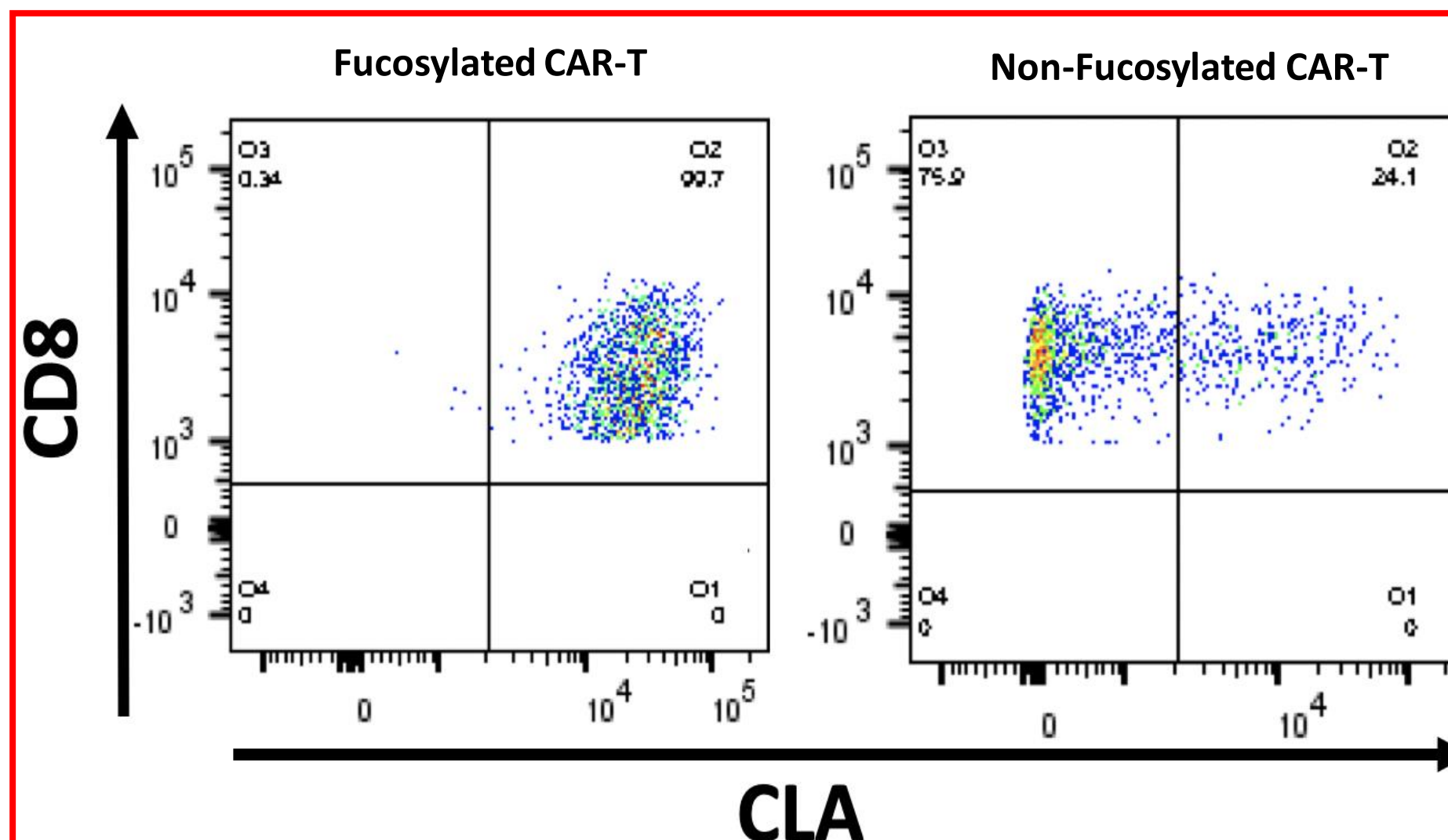


Figure 2. TZ102 fucosylates WT EGFR-CAR-T cells. The efficiency of fucosylation of WT EGFR-CAR-T cells was measured by HECA-452 antibody raised against cutaneous lymphocyte antigen (CLA), the standard marker of fucosylation. After fucosylation, the pool of EGFR-CAR-T cells (CD8+CLA+) was increased to 99.7% compared with 24.1% in the non-fucosylated group (pre-fucosylation).

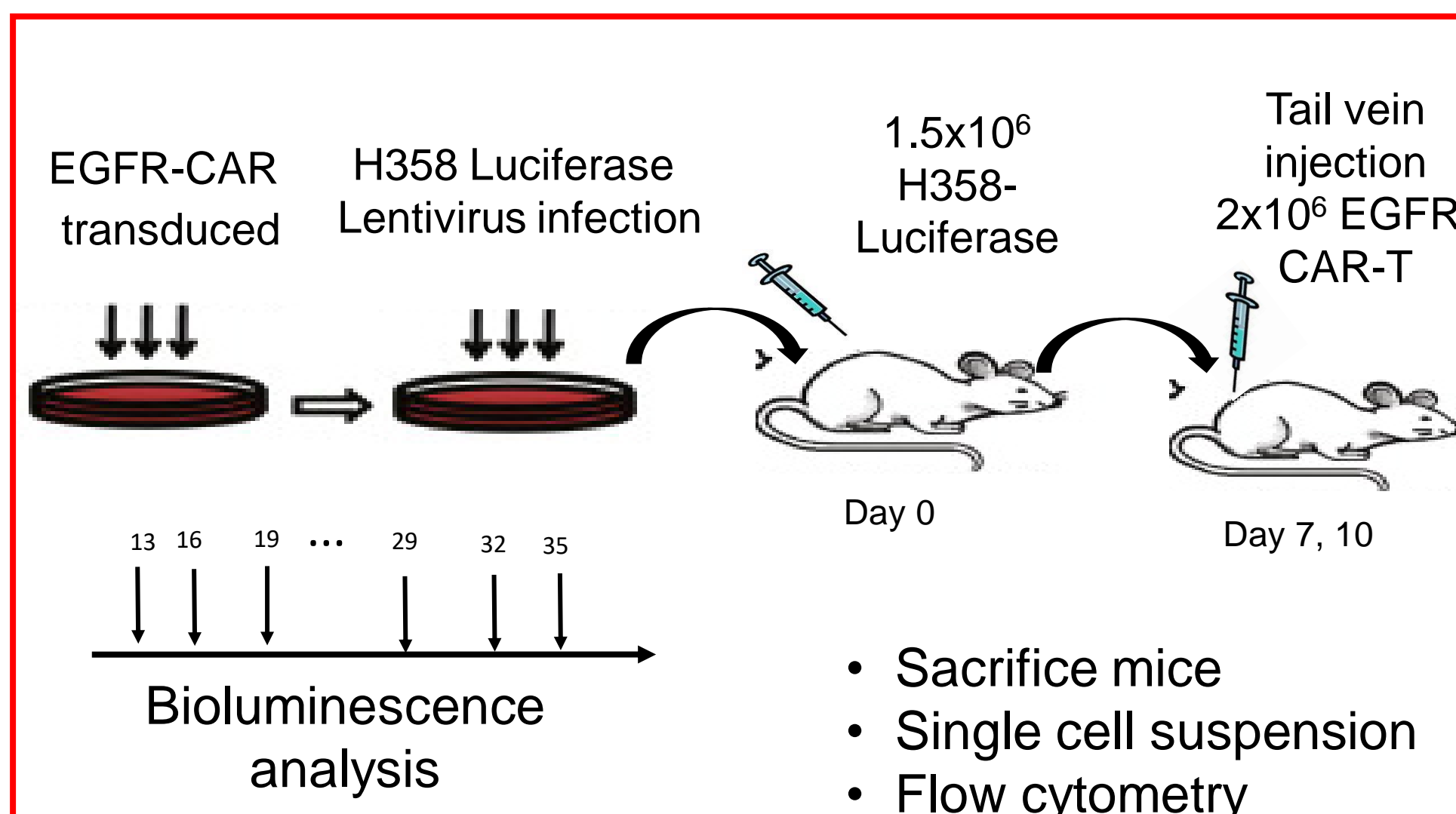


Figure 3. In vivo experimental design schema.

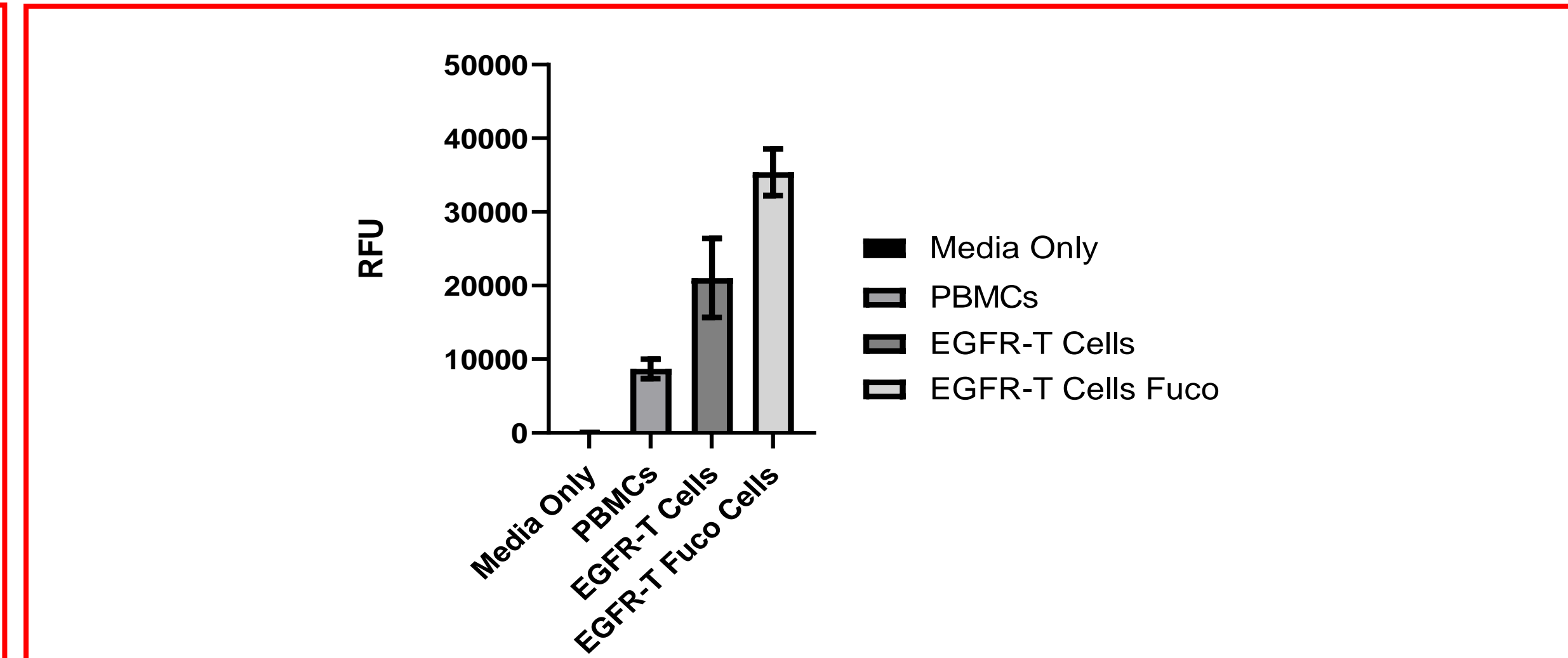


Figure 4. Fucosylation enhances the migration of EGFR-CAR-T Cells. Fucosylated and non-fucosylated EGFR-CAR-T Cells were used in migration assays. RFU values were the highest for trans fucosylated EGFR-CAR-T cells indicating successful migration into tumor cells.

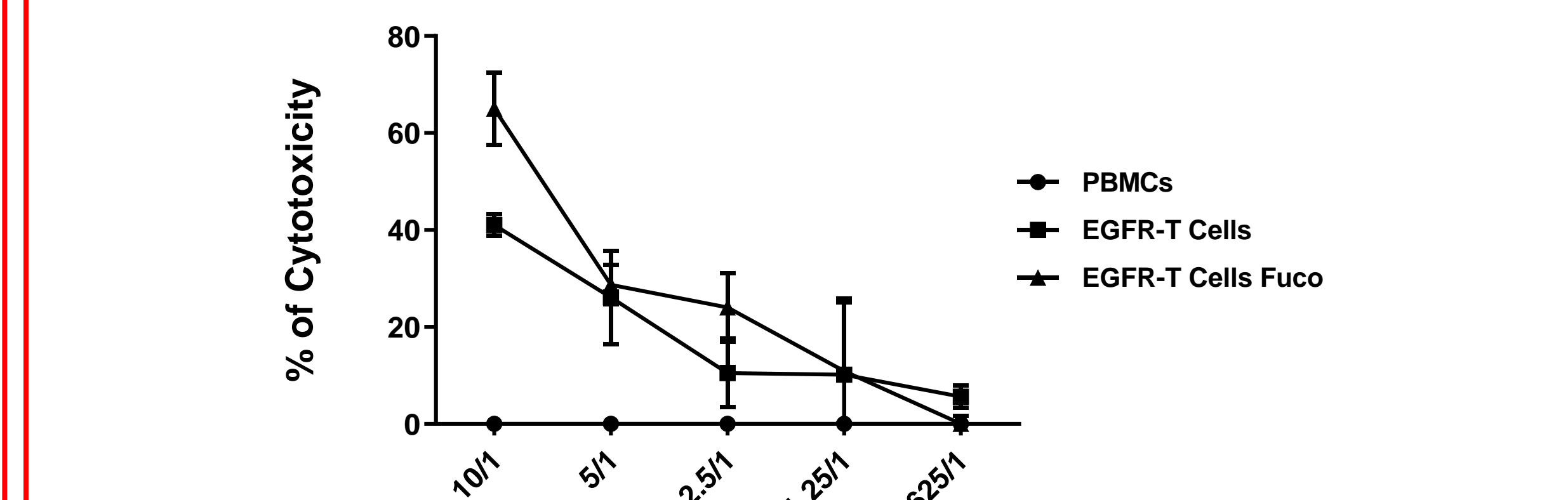


Figure 5. Fucosylation enhances cytotoxicity of EGFR-CAR-T Cells. Fucosylated and non-fucosylated EGFR-CAR-T Cells were used in cytotoxicity assays at various E:T ratios versus A549 lung cancer cells. Fucosylated EGFR-CAR-T Cells more effectively lysed tumor cells at all E:T ratios. (B) Fucosylated and non-fucosylated and non-trans non-fucosylated EGFR-CAR-T Cells were used in cytotoxicity assays at various E:T ratios versus A549 lung cancer cells. Trans fucosylated EGFR-CAR-T Cells more effectively lysed tumor cells at all E:T ratios, followed by trans non-fucosylated EGFR-CAR-T Cells. Non-trans EGFR-CAR-T Cells had no ability to lyse tumor cells.

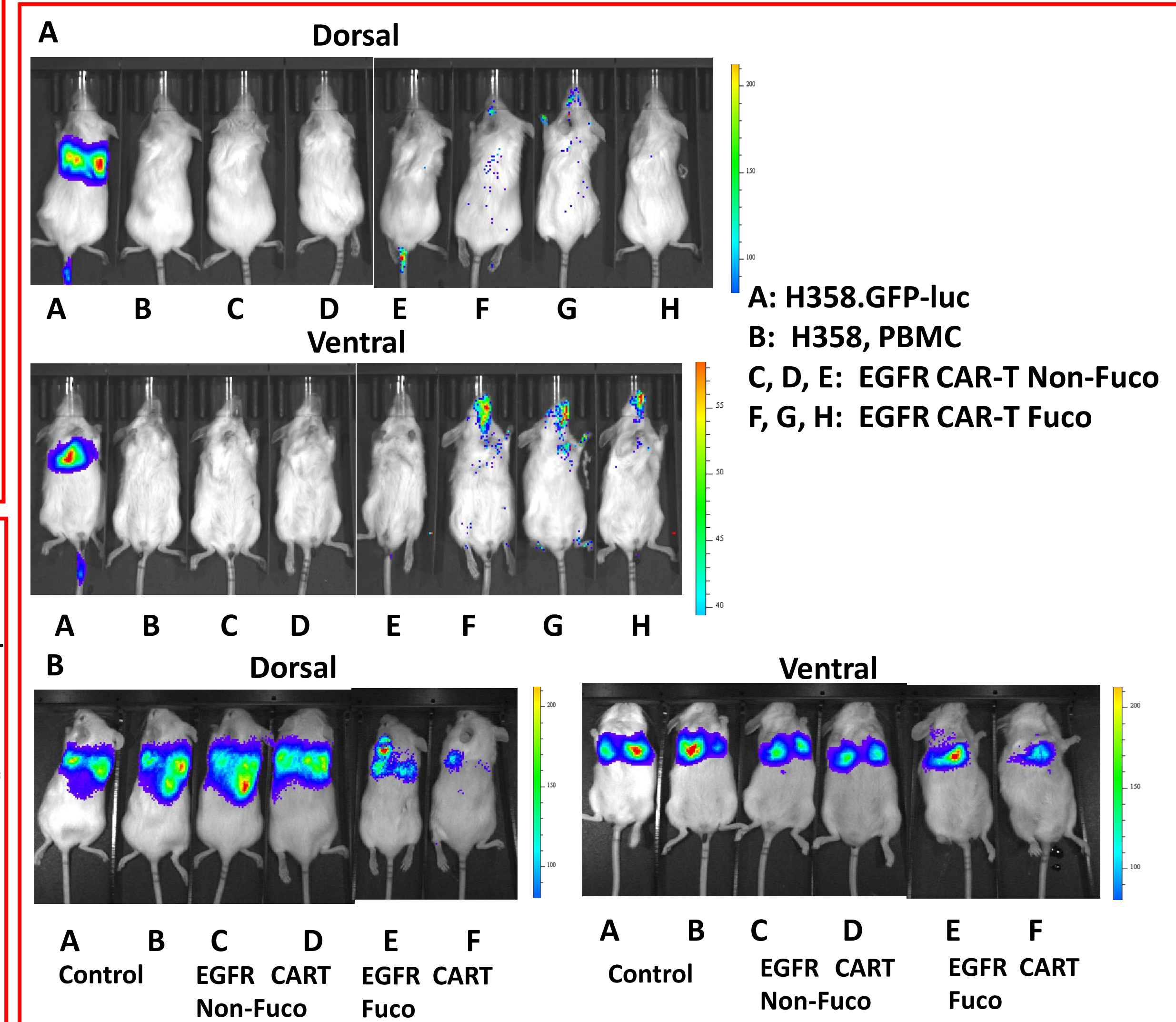


Figure 6. Fucosylation improves CAR-T cell migration and efficacy in reducing lung tumor size in xenograft mouse models. (A) Irradiated NSG tumor-bearing NSG mice treated with fucosylated or non-fucosylated CAR-T were analyzed for migration and homing into lung tumor. (B) Fucosylation improves homing of CAR-T cells to tumors in mice. Irradiated NSG tumor-bearing NSG mice treated with fucosylated or non-fucosylated CAR-T were analyzed for efficacy of reducing tumor size. Fucosylation of CAR-T cells leads to reduction of lung tumor size.