

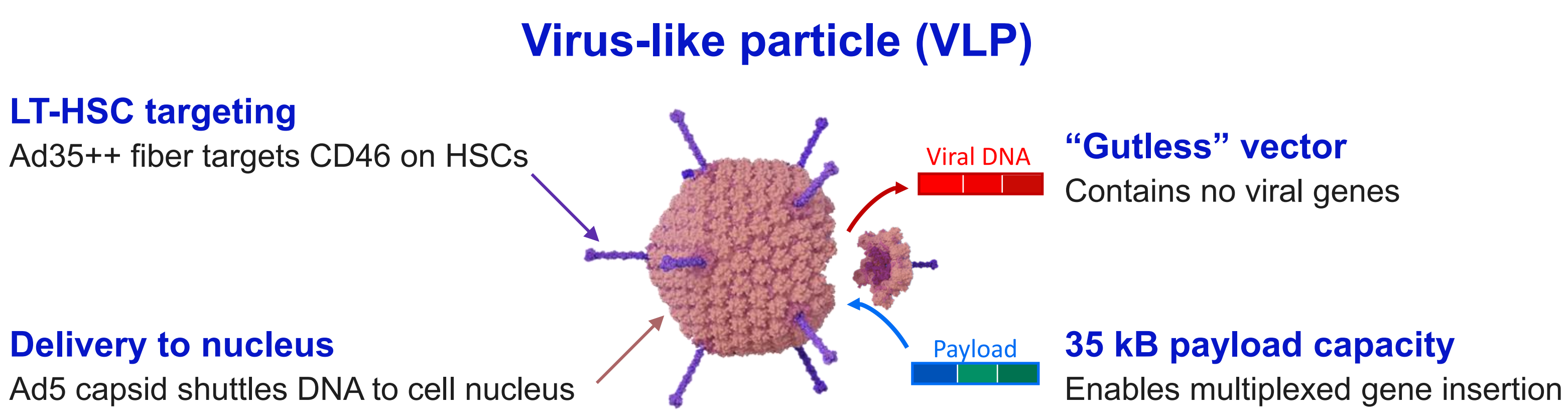
# In vivo engineered and lineage-restricted multiplexed CAR-M, -NK, and -T cell therapy mounts robust solid tumor control in pre-clinical models

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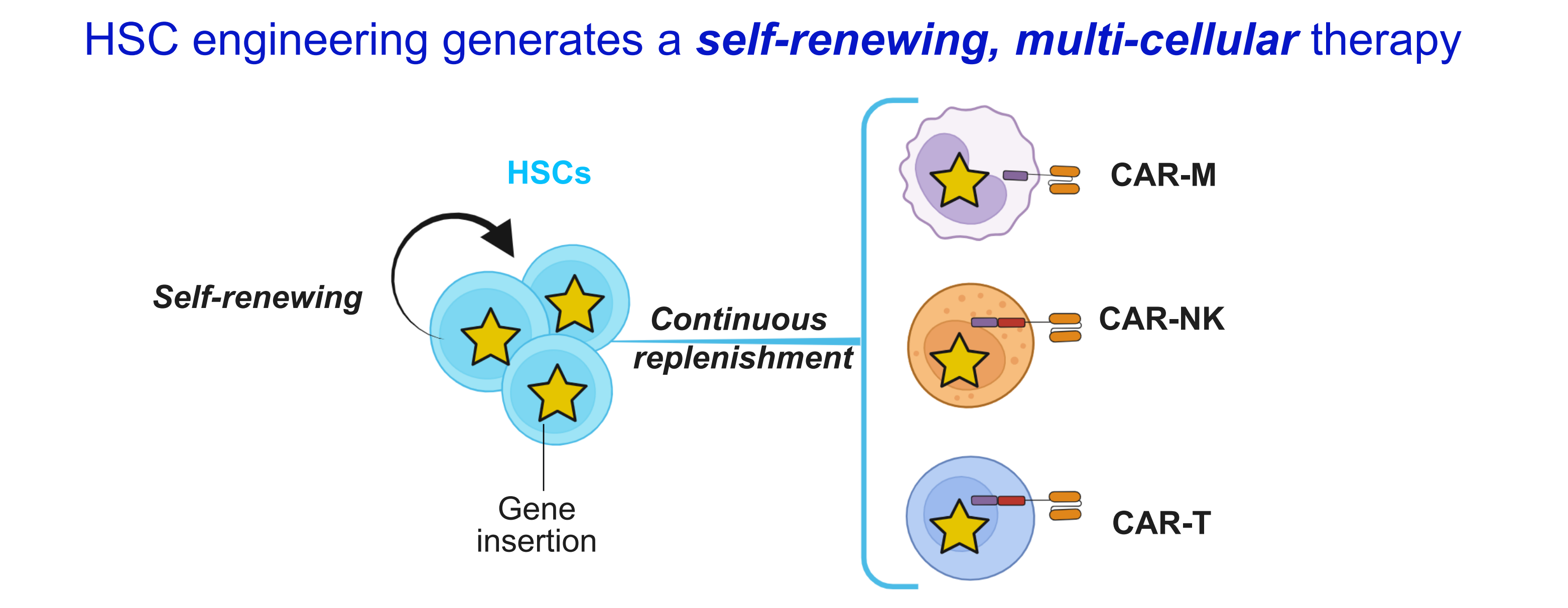
### Abstract

**Background** *Ex vivo* chimeric antigen receptor (CAR)-T cell therapy has achieved remarkable clinical success in hematologic malignancies. Such transformative efficacy, however, has yet to be realized in solid tumors where CAR-T activity is hindered by poor trafficking and infiltration, the immunosuppressive tumor microenvironment (TME), and limited persistence. CAR-myeloid cells (CAR-M) can infiltrate and modulate the TME, enhancing T cell activity, but their short persistence limits clinical efficacy. Complex manufacturing, high cost, and need for lymphodepletion present additional obstacles that limit patient accessibility to *ex vivo* cell therapy. To address these challenges, we are pursuing an *in vivo* multicellular CAR therapy to enable durable and synergistic anti-tumor activity in solid tumors. **Methods** We developed a virus-like particle (VLP) platform using an optimized helper-dependent adenovirus that preferentially targets long-term hematopoietic stem cells (LT-HSCs) via CD46 and harbors a large 35-kilobase cargo capacity. Mobilization of HSCs into the blood enables *in situ* transduction. Co-transduction of the therapeutic payload with a VLP harboring Sleeping Beauty transposase facilitates stable transgene integration in HSCs. **Results** Lineage-specific HER2 CAR expression was validated *in vitro* in both human and murine primary cells. Mice expressing human HER2 (huHER2 mice) were transplanted with HSPCs transduced with VLPs encoding lineage-restricted HER2 CARs. Following enrichment, HSC-derived HER2 CAR-expressing monocytes, NK cells, and T cells were observed in peripheral blood. HER2 CAR significantly controlled the tumor growth of human HER2-expressing EO771 mammary fat tumors and showed significant survival benefit. No health issues occurred in the huHER2 mice during the period of tumor control, and body weight remained stable for the period of observation for over two months. **Conclusions** Here, we establish a robust platform for *in vivo* HSC-derived multicellular CAR immune cell engineering that combines the tumor remodeling potential of CAR-M with the potent cytotoxicity of CAR-T and -NK cells in a single therapeutic payload administered in one *in vivo* dose. Unlike other *in vivo* cell therapy platforms that target mature immune cells, engineering at the HSC level generates a potentially long-lived, self-renewing pool of CAR-expressing effectors. Collectively, this technology delivering a multiplexed CAR-M, -NK, and -T cell payload offers an off-the-shelf therapy with the potential to mediate long-term anti-tumor activity.

### Engenius™ Platform for *in vivo* gene delivery



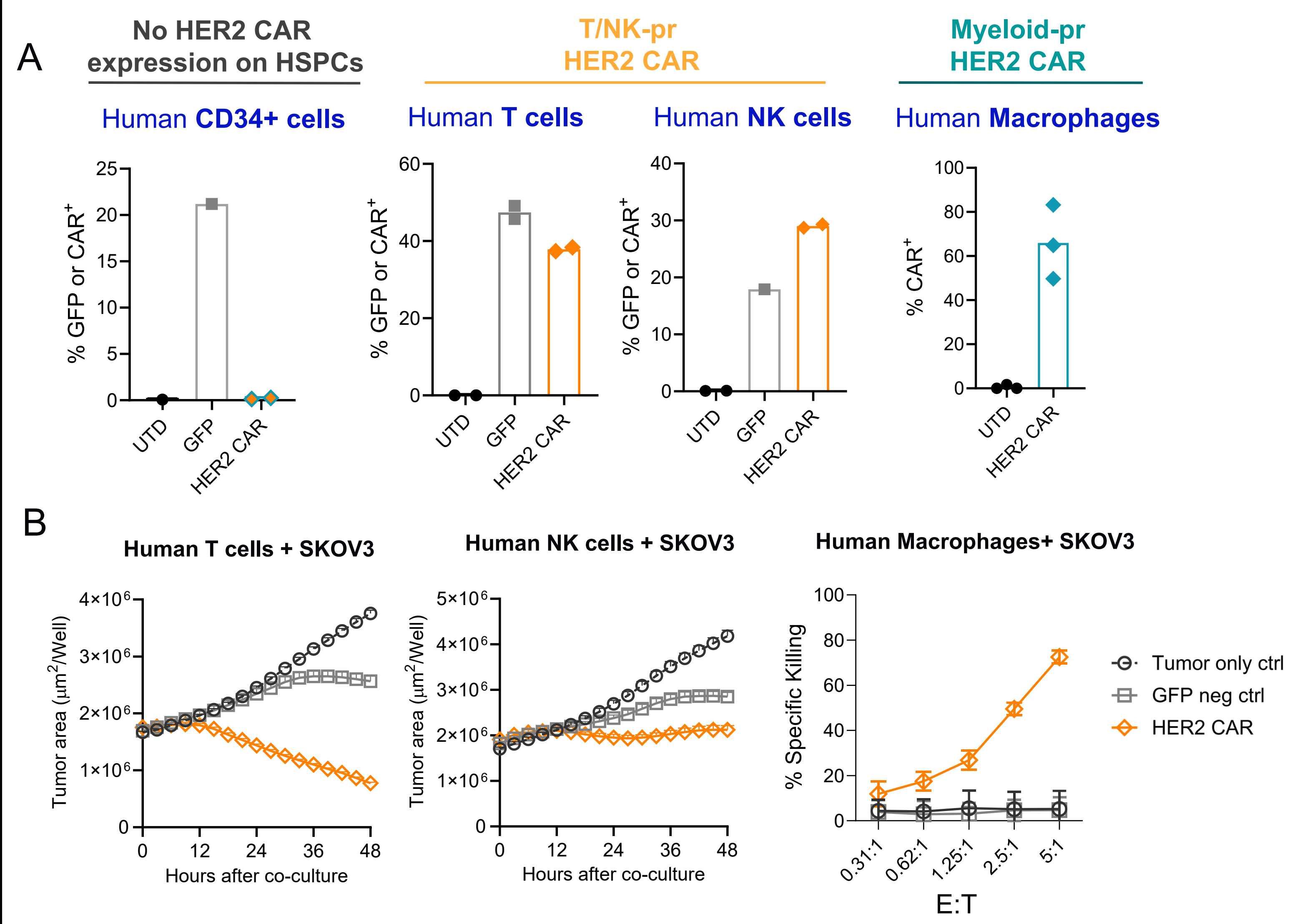
### Multi-lineage CAR strategy for solid tumors



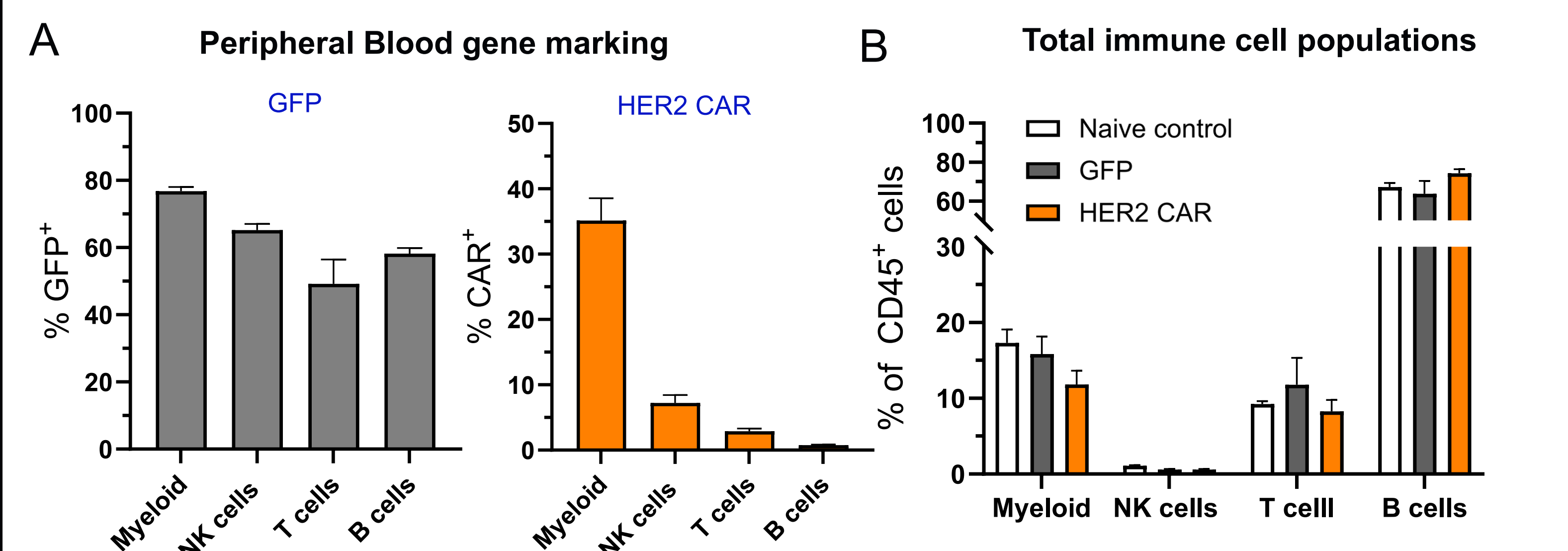
### Multiplexed, lineage-restricted vector design to generate HER2 CAR-M, NK, & T cells from a single transgene



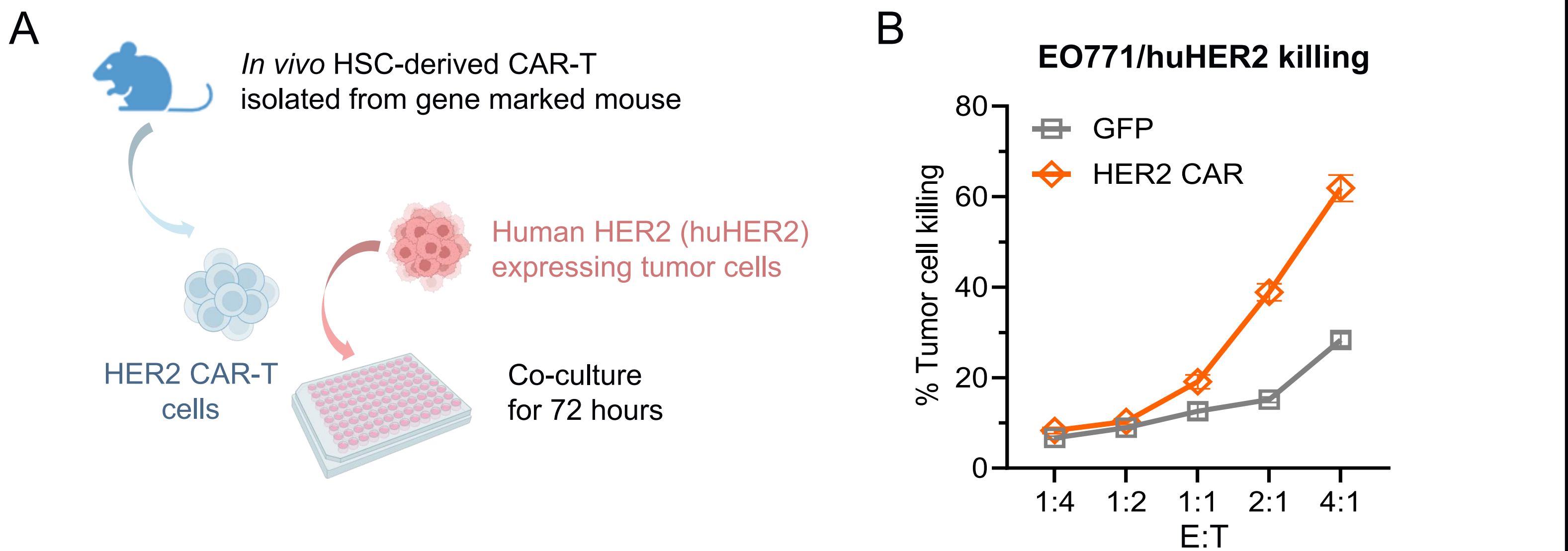
### Lineage-specific promoters drive functional HER2 CAR expression in human immune cells *in vitro*



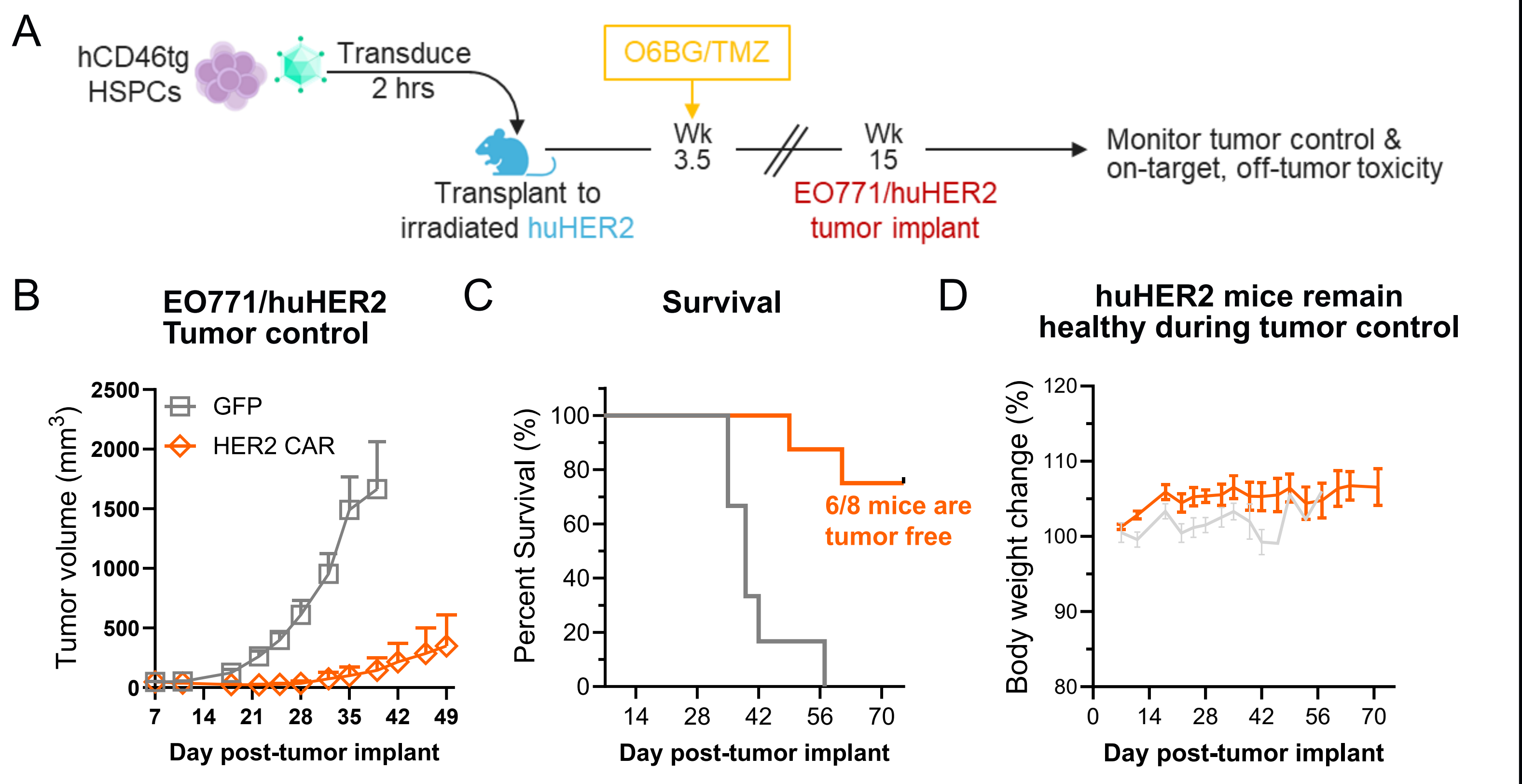
### Multiplexed, lineage-restricted vector generates HER2 CAR-M, NK, and T cells *in vivo*



### In vivo generated, HSC-derived CAR-T cells mediate potent tumor cell killing *ex vivo*



### Multiplexed HER2 CAR-M, NK, & T cell therapy mediates strong tumor control *in vivo*



### Conclusions

- Lineage-specific promoters direct CAR expression to discreet mature immune cells, enabling **regulated expression of multiplexed therapeutic payloads**
- In vivo* multiplexed, lineage-restricted vector generates **HSC-derived CAR-M, CAR-NK, & CAR-T** cells and mediates **robust anti-tumor efficacy**
- Preparing to advance development candidate **EN-002 to clinical studies in 2027**

### Related talks

**Late-Breaking Abstract:** First in vivo hematopoietic stem cell (HSC) gene addition clinical trial: Initial results from EN-374-101 in X-linked chronic granulomatous disease (X-CGD). Friday, May 15, 11:07AM – 11:33AM ET  
**Oral Abstract:** Discovery and development of engineered neutralizing antibody-evading helper-dependent adenovirus capsids as candidates for in vivo gene therapy. Wednesday, May 13, 11:15AM – 11:30AM ET