

Multiplex Prime Editing and PASSIGE™ for Non-Viral Generation of an Allogeneic CAR-T Cell Product

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Background

Multiplex Prime Editing may be able to address limitations of CAR-T cell therapy:

- > Manufacturing time, costs, and yield for autologous cell therapy cell quantity and quality issues could be addressed by using allogeneic T cells
- > Safety risks associated with semi-random integration and double strand breaks at multiple genomic loci

Current strategies for delivery and expression of CAR transgenes are limited by:

- > Semi-random integration via lentivirus or transposons risks unintended gene disruption or activation of proto-oncogenes
- > Targeted integration using nuclease + template for HDR limited by low efficiency and risks associated with DSB induction (e.g., chromothripsis, p53 activation)

Limitations of current strategies for multiplex editing

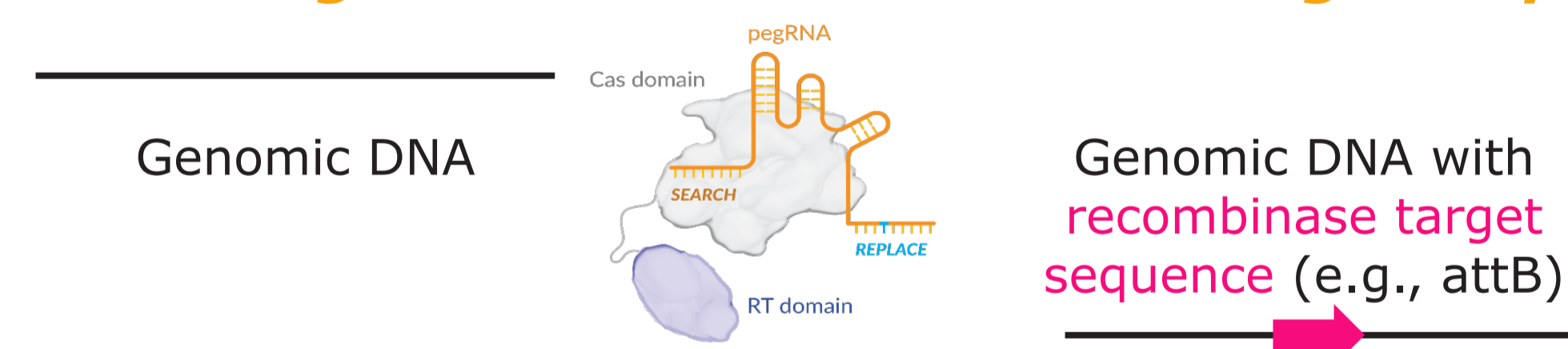
- > Targeted gene disruption at multiple loci simultaneously with nucleases carries a risk of chromosomal rearrangements
- > Base editing to disrupt splicing or introduce pmSTOP codons is limited in scope, risks pmSTOP readthrough, and cannot support targeted integration

PASSIGE™ in combination with multiplex Prime Editing (PE) maybe be able to overcome these challenges to create a potentially best-in-class allogeneic CAR-T cell product

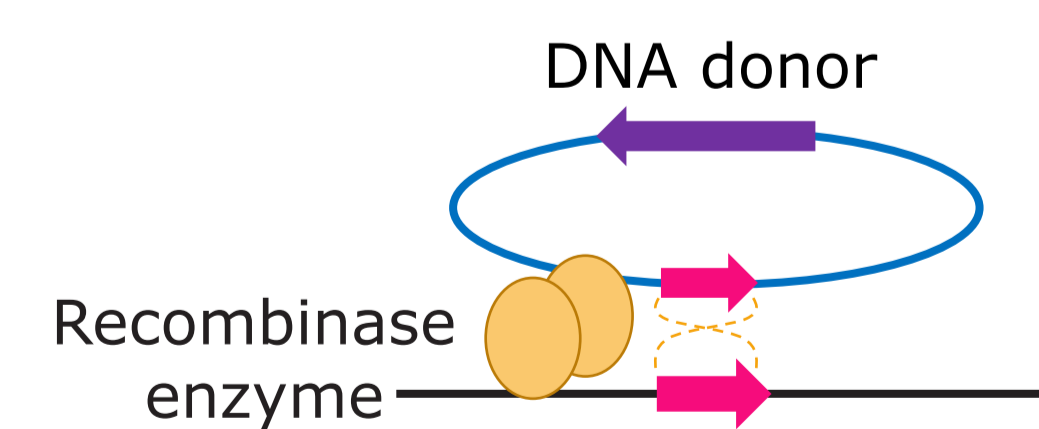
Methods

Prime Editing Assisted Site-Specific Integrase Gene Editing (PASSIGE): Prime Editing in combination with recombinases for targeted integration of gene-sized DNA

Prime Editing to install a recombinase target sequence



Site-specific recombination



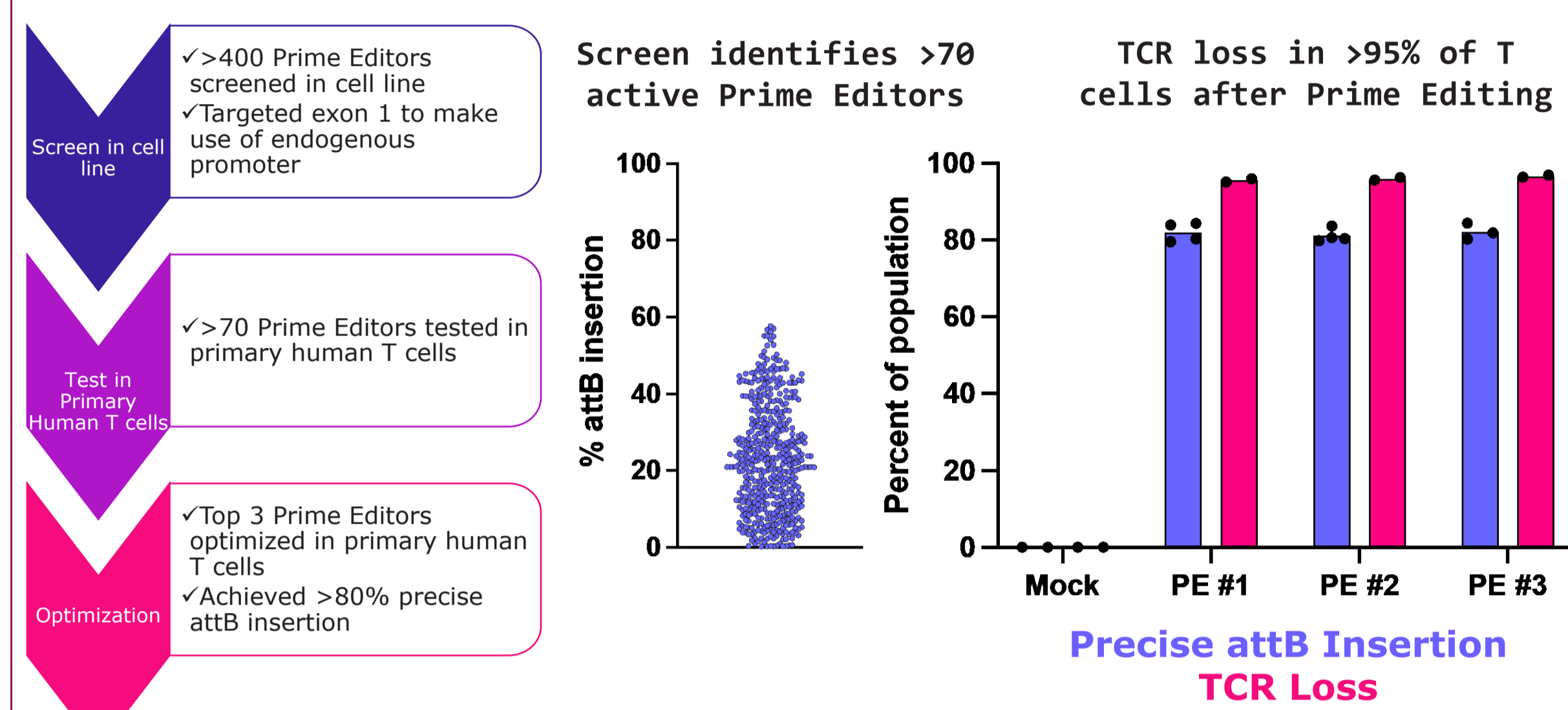
Gene-sized DNA inserted at precise genomic location



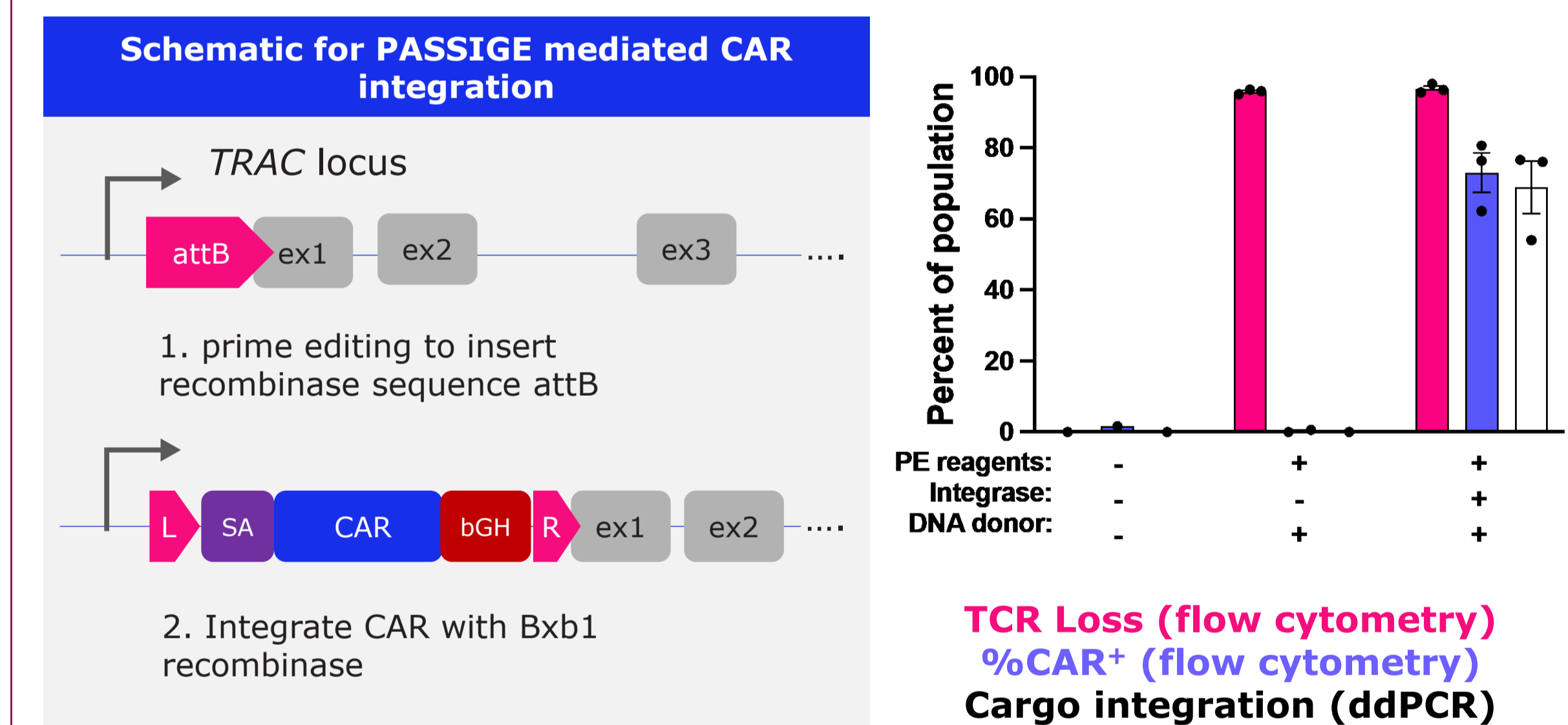
- ✓ Targeted integration of DNA in a **single delivery step**
- ✓ **No double strand break (DSB)** as integrase catalyzes recombination directly
- ✓ **Integration can be irreversible:** e.g., attL and attR products are distinct from initial attB and attP sequences

Results

High performing Prime Editors precisely insert attB sequence at TRAC locus with >80% efficiency

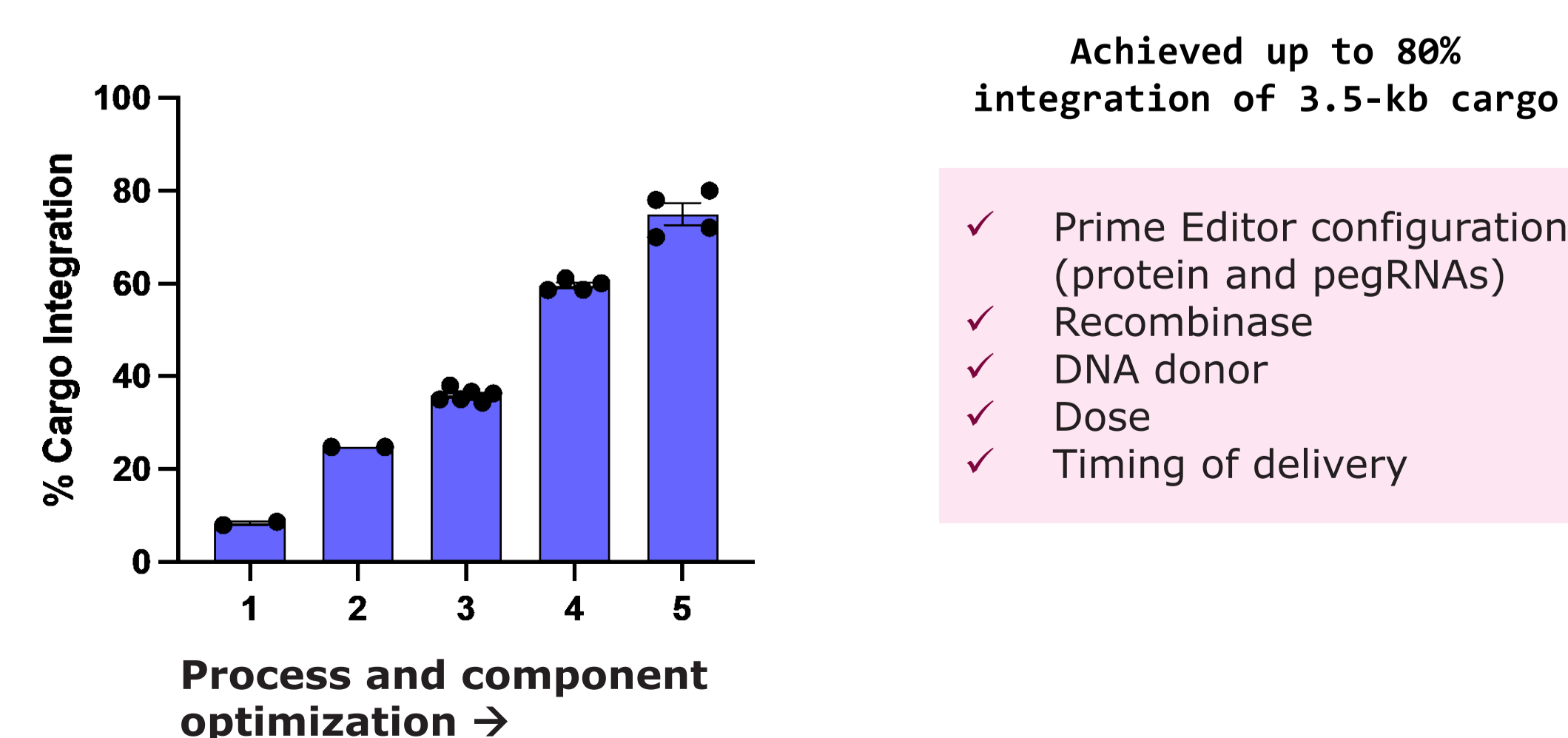


Non-viral PASSIGE delivery supports integration of >3.5 kb CD19 CAR construct at TRAC locus in >70% of T cells



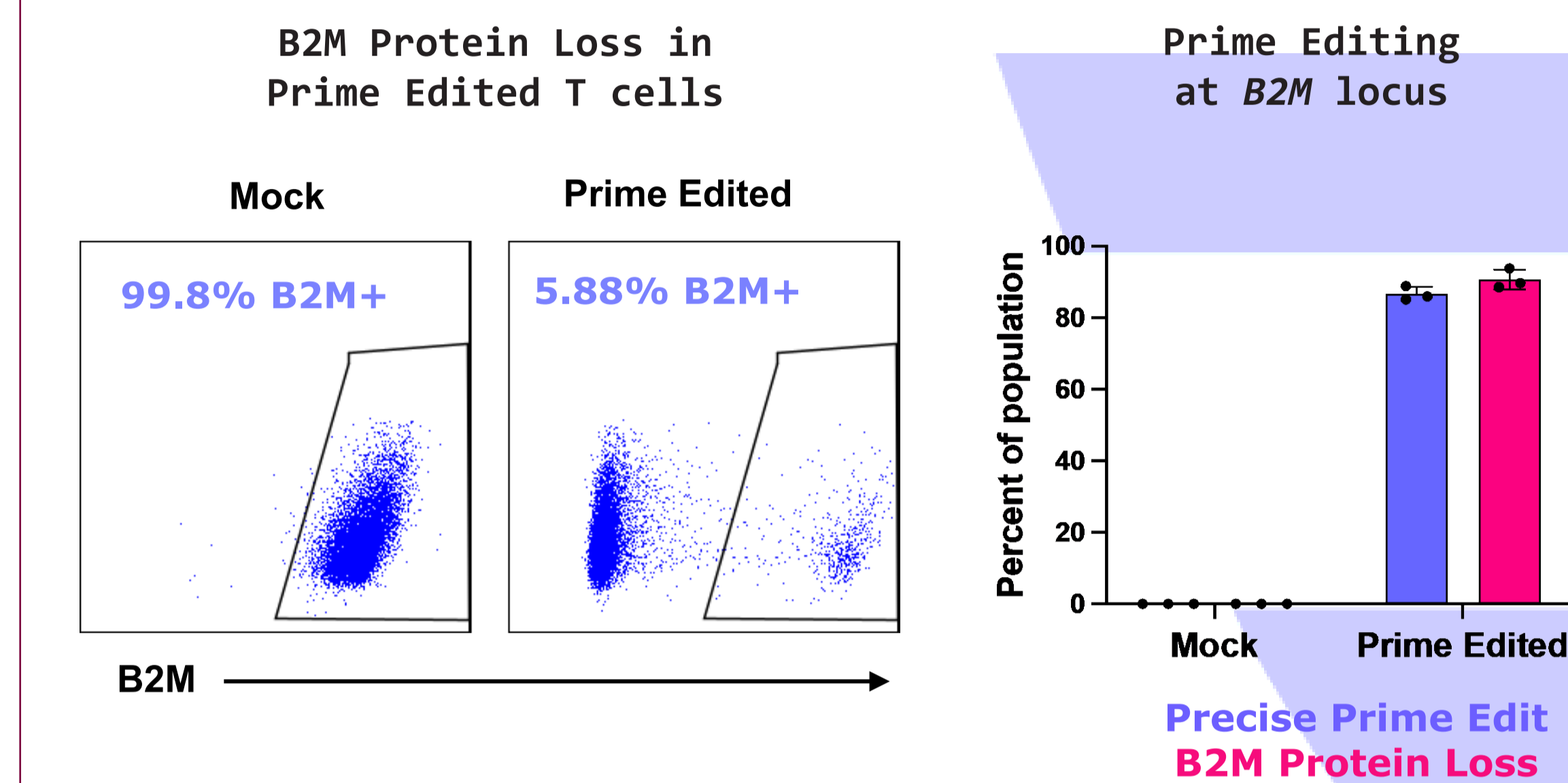
- ✓ Loss of endogenous TCR with attB insertion in TRAC exon 1
- ✓ Use of endogenous TRAC promoter allows for tuned regulation of expression
- ✓ Promoter-less cargo will not express if integrated elsewhere in genome

Ongoing PASSIGE component and process optimization leads to higher cargo integration efficiency

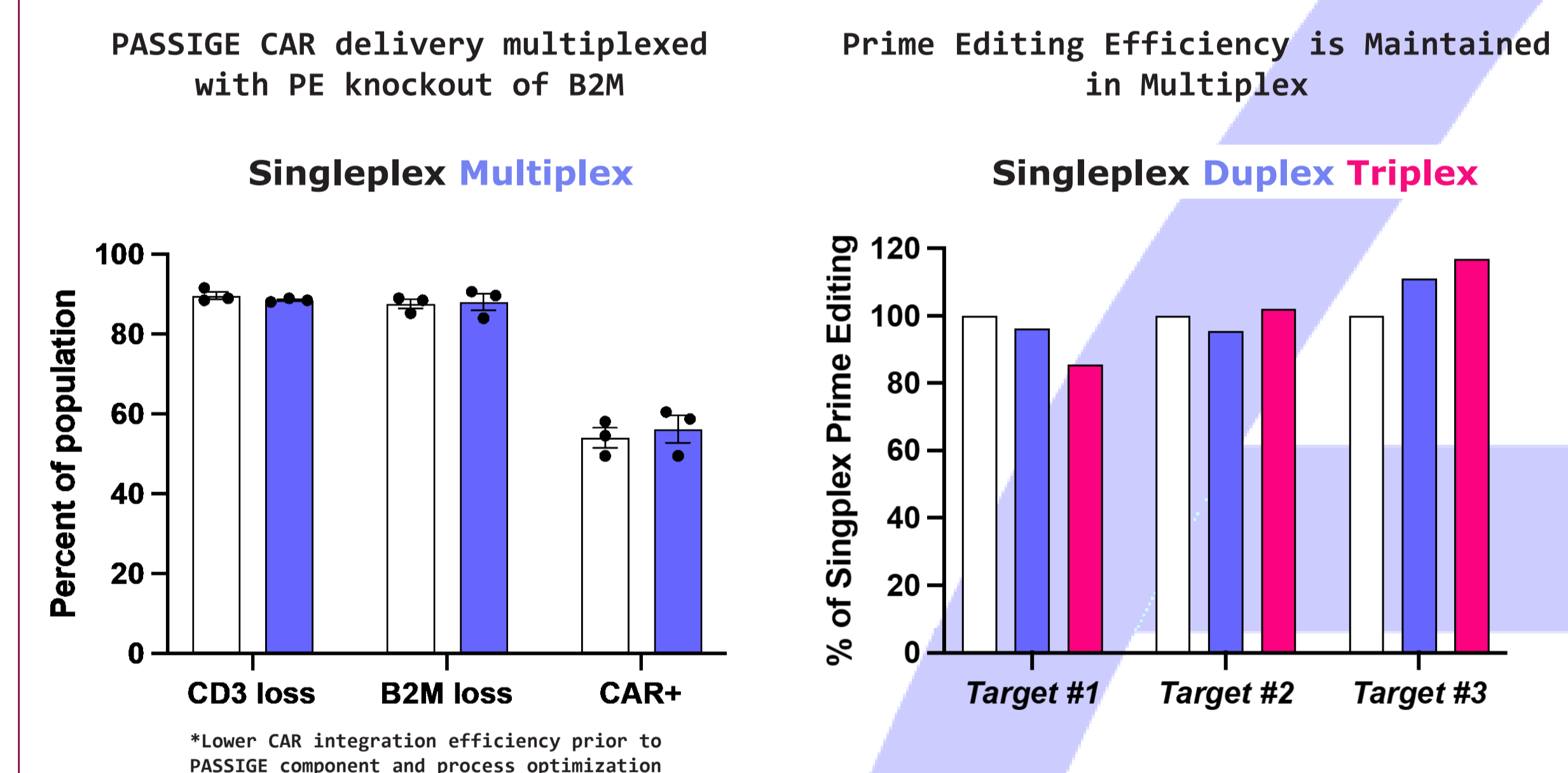


Results

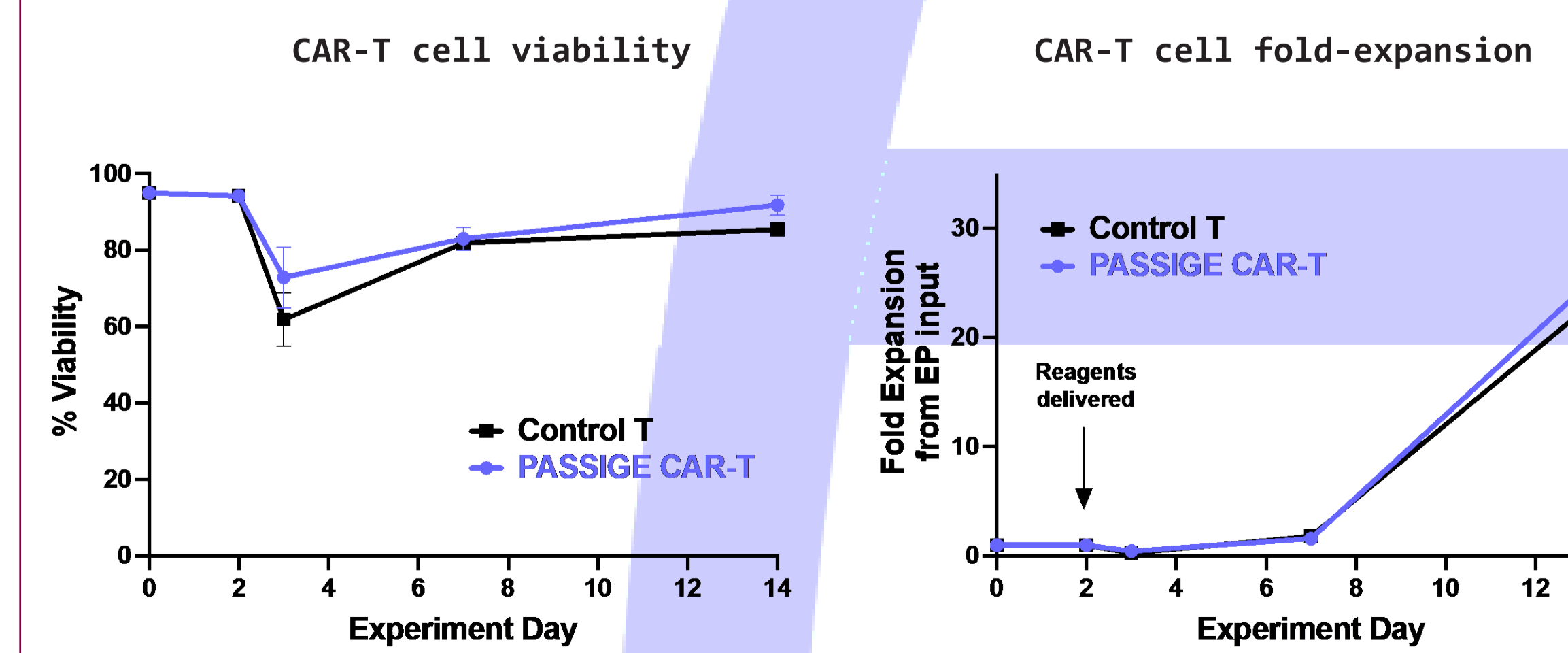
High performing Prime Editors disrupt B2M expression with >90% efficiency



Prime Editing and PASSIGE efficiency are maintained in multiplex context

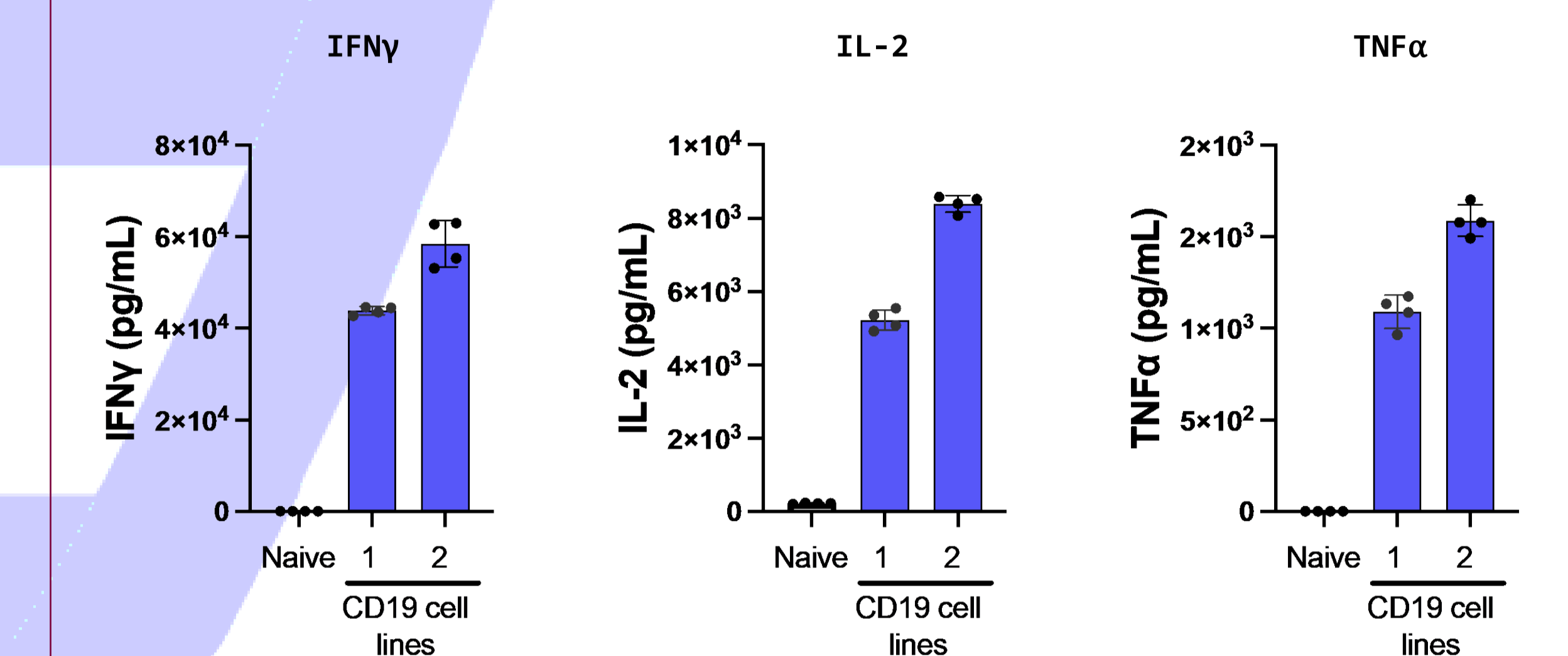


T cell health is maintained: PASSIGE-mediated integration of CD19 CAR does not reduce T cell viability or fold-expansion

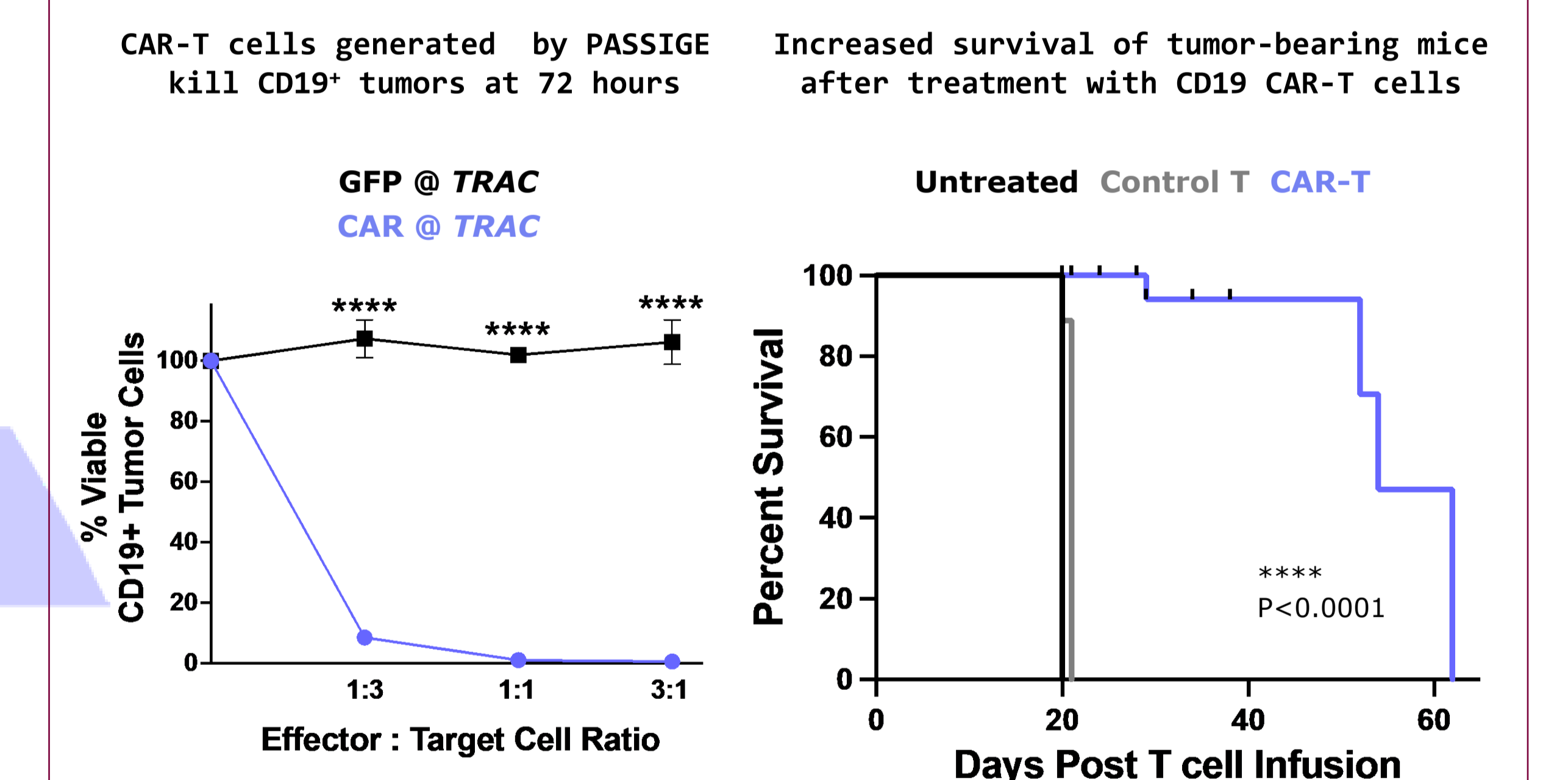


Results

PASSIGE-generated CD19 CAR-T cells produce pro-inflammatory cytokines after exposure to CD19+ cell lines



CAR-T cells generated via PASSIGE have potent cytotoxicity against CD19+ cells in vitro and in vivo



Summary & Next Steps

PASSIGE is efficient for non-viral, site-specific delivery of large cargo to primary human T cells

Achieved >80% site-specific integration of CD19 CAR through systematic PASSIGE component and process optimization

PASSIGE can be multiplexed with Prime Editing at other target sites by non-viral one-step delivery with no loss of efficiency

PASSIGE-generated CAR-T cells are healthy and show potent antigen-specific function and cytotoxicity

Next: apply Prime Medicine's comprehensive suite of assays to CAR-T product for off-target discovery

Next: potential for additional multiplex Prime Edits to improve CAR-T properties