# Next-Generation Signal Peptides for Tuneable CAR-T Expression

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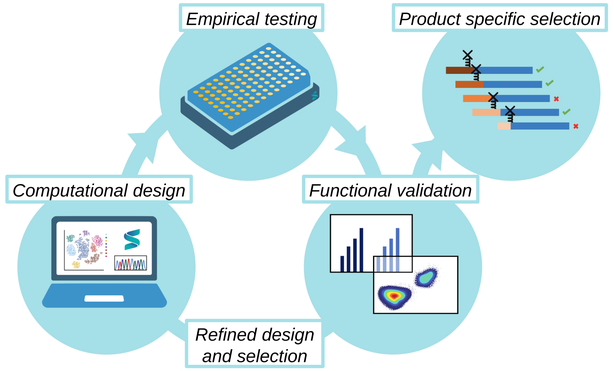
## Synthetic signal peptide design to enhance CAR translocation and activity

**A comprehensive platform for enhanced signal peptide design.**

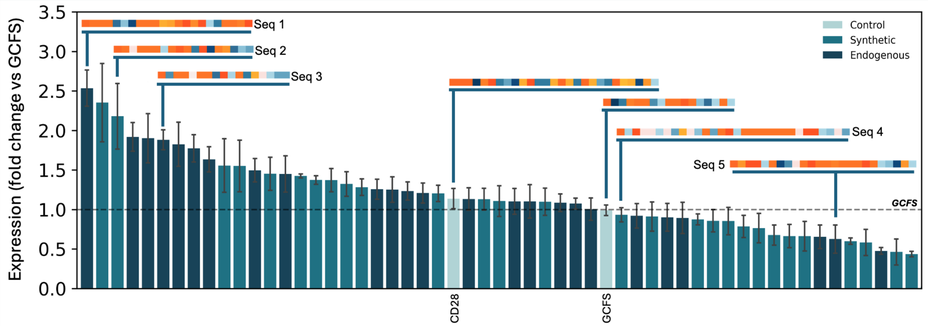
Traditional signal peptide selection relies on a "one-size-fits-all" methodology when no native signal peptide is available, often leading to suboptimal translocation and reduced yields. To provide tailored signal peptide solutions, which maximize protein secretion and functionality, SynGenSys employs a two-stage strategy encompassing product-independent and product-specific analyses:

- ► Endogenous signal peptides are mined from large protein datasets, producing an extensive library for empirical high throughput testing.
- ► Analysis of these results enables an iterative design process, leading to a refined library of endogenous and synthetic signal peptide candidates with desirable features.

This approach identifies highly efficient signal peptides which improve protein yield without compromising product functionality.




## Optimised signal peptides give 2.5-fold increase in CAR ScFv expression




SynGenSys signal peptides:

- ✓ Achieve titratable activity of CAR ScFv in a T-cell line (Jurkat E6.1), with significant relative expression fold changes compared to commonly used industry standard signal peptides (e.g. GCFS, CD28).
- ✓ Have high amino acid diversity facilitating targeted modifications, ensuring efficient product-specific optimisation.

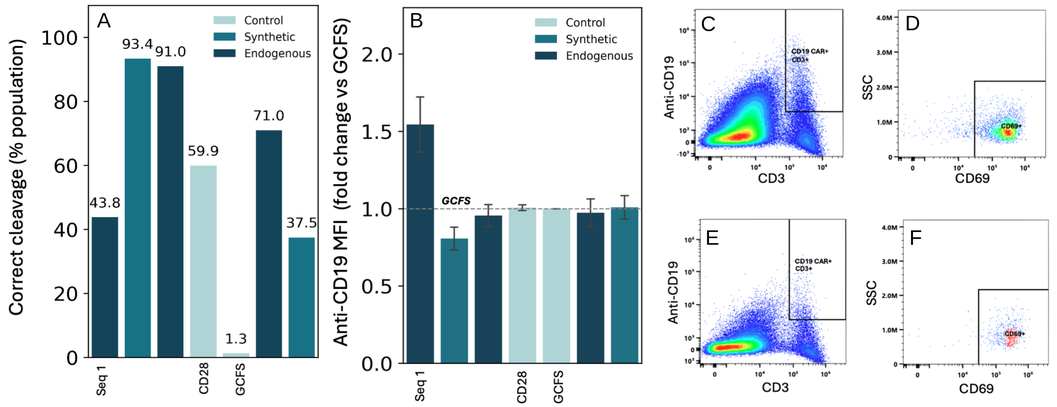
**Editing signal peptides gives expression control.**

Fine-tuning protein expression is critical for therapeutic applications to ensure their safety and efficacy. Optimisation of the signal peptide is a powerful approach for meeting application-specific requirements.

## Optimised signal peptides improve cleavage fidelity, retaining CAR-T functionality

**Altering signal peptides enables precise control of CAR surface presentation without compromising CAR functionality.**

Achieving high cleavage fidelity is a crucial consideration given the evolving regulation landscape for cellular therapeutics. LC-MS analysis of CAR ScFv cleavage reveals significant variations in cleavage efficiency ranging from 1.3%-93.4% correct cleavage (A)**.** SynGenSys signal peptides are identified with improved cleavage compared to the industry-standard signal peptides (CD28, GCFS). A 1.5-fold increase in CAR presentation was also achieved (B-C) when compared with GCFS (E), demonstrating that signal peptide choice can boost cell surface presentation though low cleavage fidelity is a focus for further iterative improvements. SynGenSys signal peptides also maintained CD69+ activation in the presence of CD19+ target B-cell line (Ramos RA1) (F, H). This data indicates that a systematic and iterative signal peptide design and selection approach can optimize CAR ScFv expression with enhanced cleavage fidelity, maximizing CAR-T efficacy and ensuring compliance with evolving regulatory standards.




SynGenSys signal peptides:

- ✓ Demonstrate more efficient cleavage compared to commonly used industry signal peptides (e.g. GCFS, CD28).
- ✓ Show comparable levels and even a marked increase (Seq1) in cell surface presentation to industry standard signal peptides

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