

Multifunctional Exosomes Construction by Precision Cell Engineering

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Abstract

Introduction: Codiak BioSciences has leveraged natural exosome biology to develop a therapeutic platform based on precisely engineered exosomes. Our engEx™ platform utilizes the unique exosome scaffolds PTGFRN and Baspl that allow surface display and luminal loading of an array of structurally and biologically different proteins. Here we sought to expand the capabilities of the engEx platform for the precise engineering of multifunctional exosomes by constructing combinatorial extracellular vesicles (EVs) derived from our lead therapeutic candidate exoIL-12.

Methods: We generated exoIL-12 cells and EVs using random and site-specific integration (RI and SSI) and tested their expression stability and activity. Then, we constructed double and triple exosomes of IL-12 with the immunomodulators CD40L and FLT3L using a combination of RI and SSI in different safe-harbors. We tested protein expression and biological activity of the combo EVs, and confirmed co-expression of protein ligands by co-IP, Western blot, ELISA and activity assays.

Results: While SSI resulted in more homogenic and stable cell pools, both methods yielded exosomes of similar protein levels and activity, and thus were both applied to construct multifunctional EVs. Combinatorial exoIL-12/CD40L/FLT3L showed similar protein levels and activity as their single EV counterparts with some variability between different safe-harbors used for SSI. Most importantly, all proteins were efficiently co-expressed in combo EVs, demonstrating the capability of PTGFRN for high-density display of multiple ligands on exosome surface.

Summary/Conclusion: Future in vivo studies will investigate the potential synergistic activity of these multifunctional exosomes when applied as combinatorial therapeutic agents in mouse cancer models. Overall, this POC study demonstrates the potential of our engEx™ platform to engineer multifunctional exosomes and has broad therapeutic applications for immuno-oncology, vaccines development and targeted functional delivery.

PTGFRN is a novel EV scaffold for protein surface display

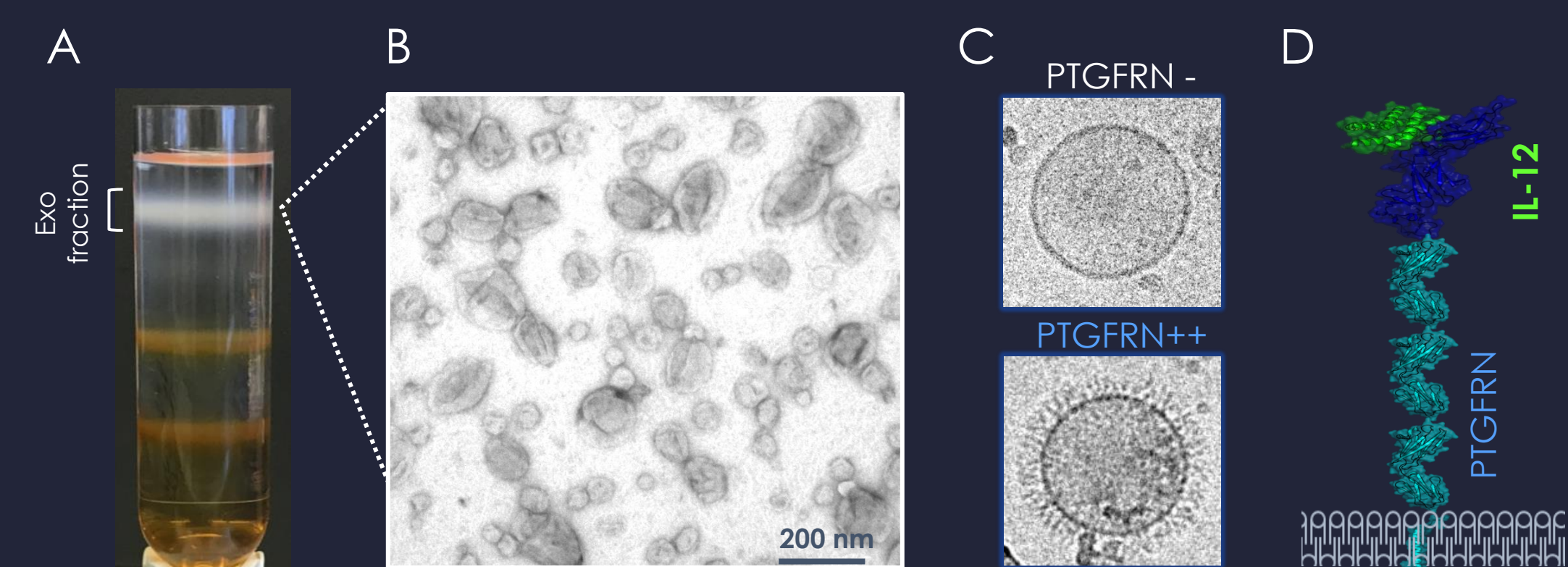


Figure 1. Identification of PTGFRN as a novel EV scaffold protein. OptiPrep density gradient centrifugation was used to purify exosomes from high density suspension cell culture (A). Transmission electron microscopy (TEM) images confirmed purity and morphology (B). Proteomic analysis led to the identification of a highly abundant exosomal protein PTGFRN, highly enriched in exosomes purified from the producer cells. Cryo-electron microscopy analysis showed ~25 nm projections densely packed on the surface of exosomes overexpressing PTGFRN (C). PTGFRN is a single-pass type-I transmembrane glycoprotein composed of six tandem extracellular IgV domains and a short cytoplasmic tail. Recombinant proteins, like 60 kDa single chain hIL-12 (scIL-12) can be efficiently anchored to the exosome surface by PTGFRN fusion (D).

engEx platform for the multifunctional EV engineering

30-200 nm vesicles released and taken up by all cells

Crucial mechanism for intercellular communication

Convey and protect complex macromolecules which can alter the function of recipient cells

Intrinsically non-immunogenic

Natural or engineered tropism to specific cells and tissues

engEx utilizes exosome scaffolds PTGFRN and Baspl for surface display and luminal loading

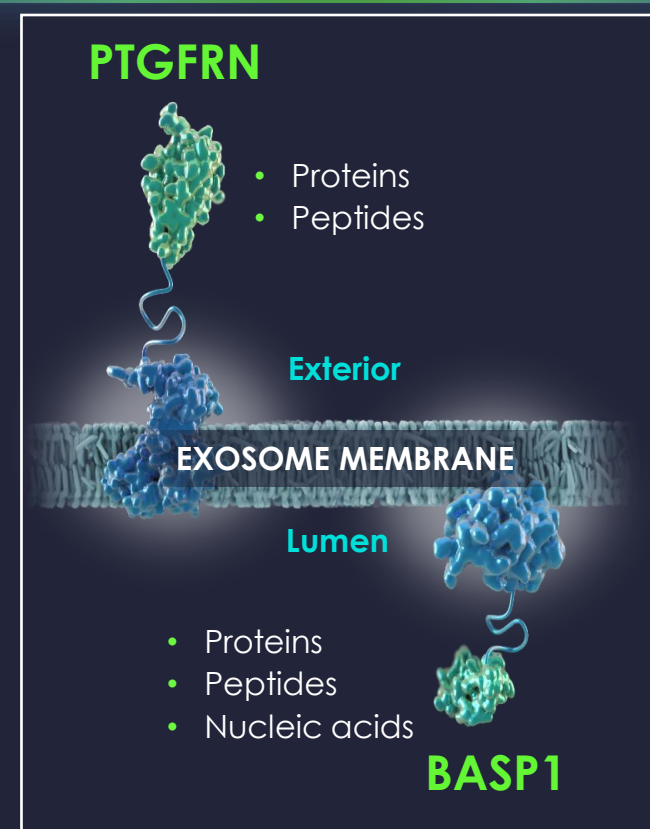
Combo EVs bear multiple functional moieties (proteins, peptides, nucleic acids) luminally or on the surface

Expand exosome engineering toolkit and demonstrate novel capabilities of EngEx platform

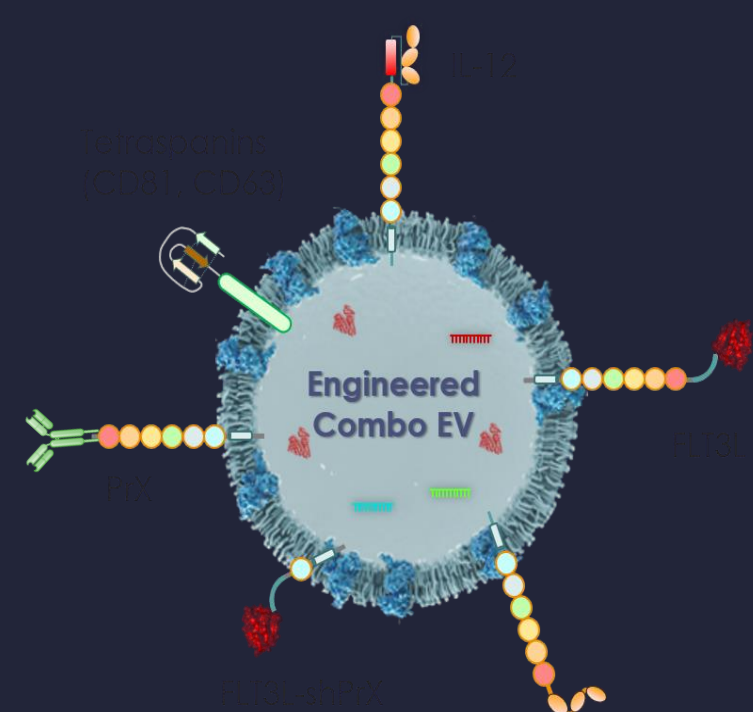
Combo EVs may have synergism of action, broader functionality and increased potency

Broad applications, including combinatorial therapy, vaccines development, targeted functional delivery

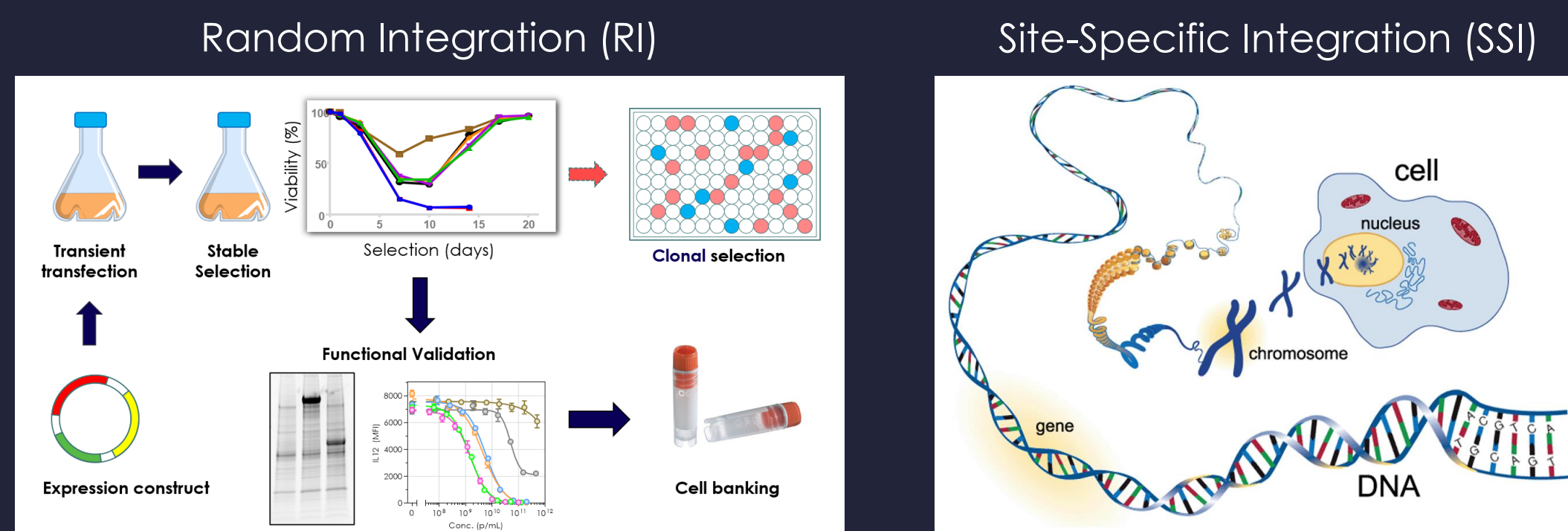
Exo Engineering Platform (engEx)



Multifunctional exosomes



Random and site-specific integration for EV engineering



- Multiple integration events
- High expression levels
- Heterogeneous growth and expression
- Possible genomic instability and silencing
- Single integration events
- Stable genomic sites (safe harbors)
- Lower expression levels
- Homogenic and stable expression

Stable and potent exoIL-12 engineered by SSI and RI

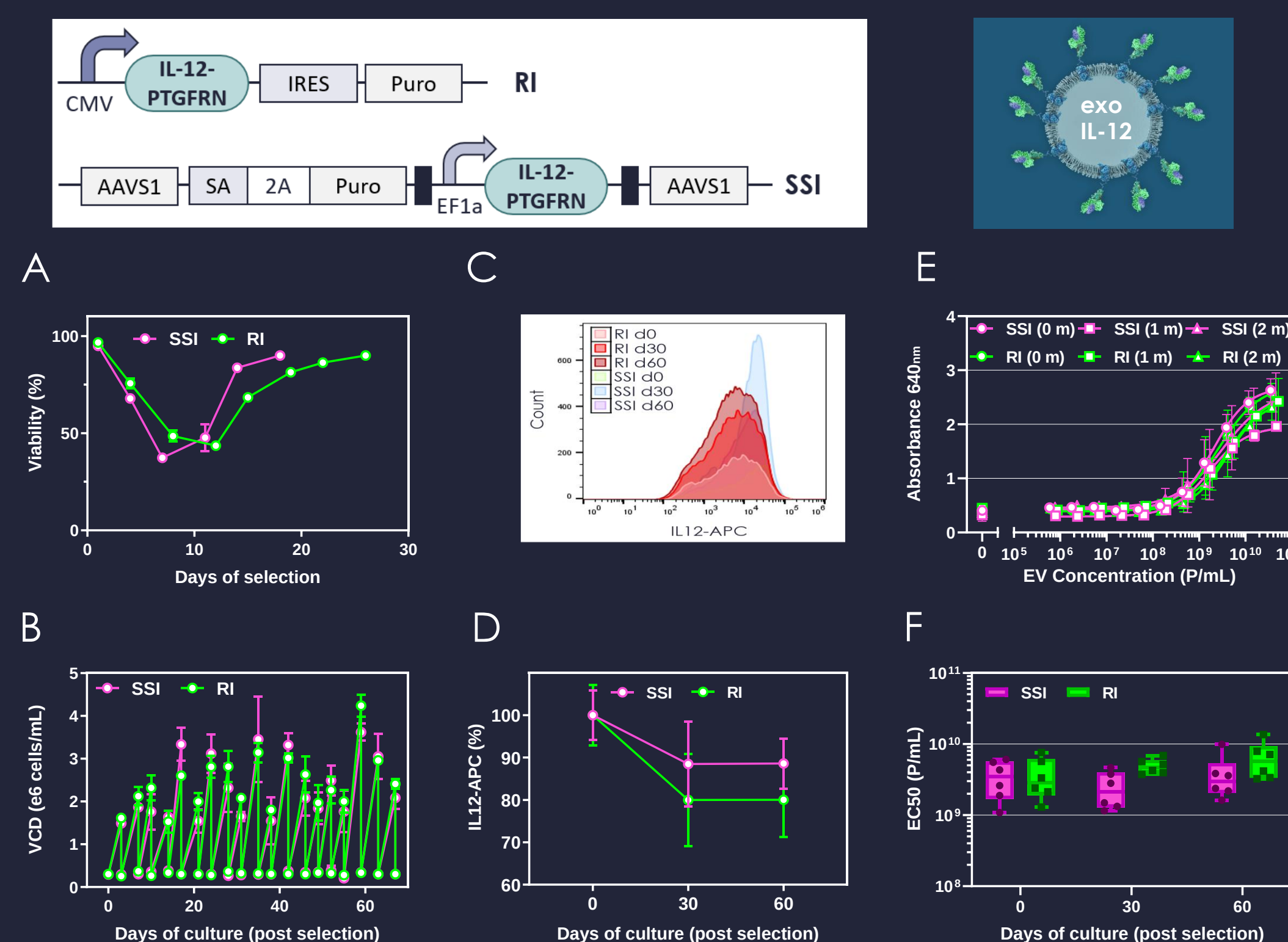
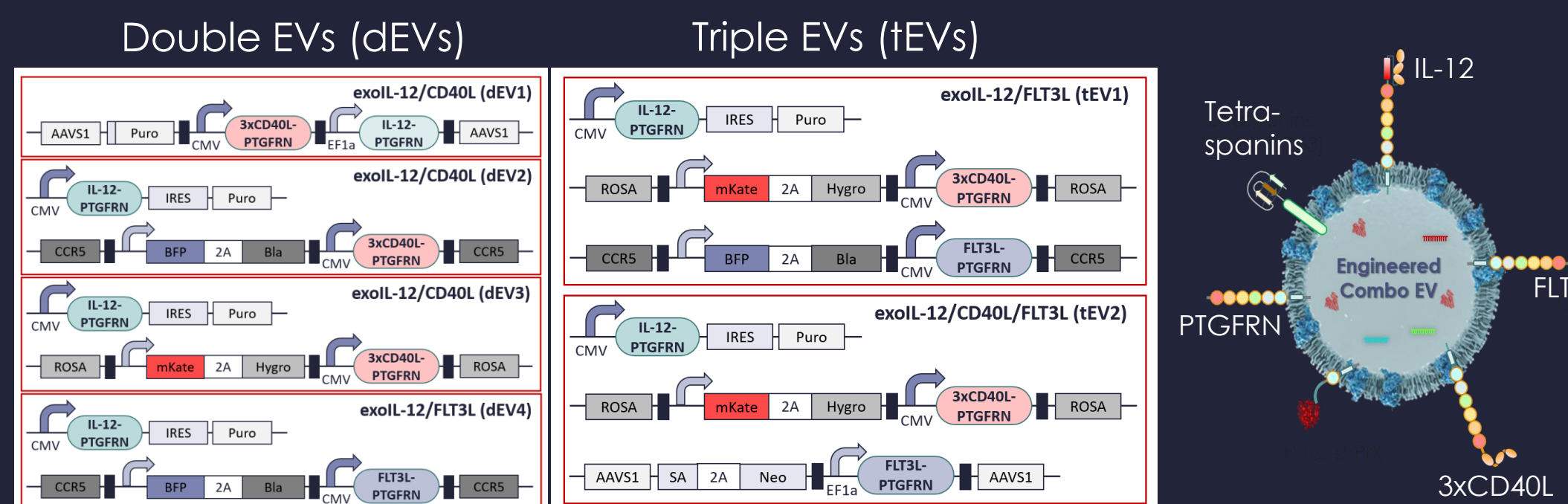


Figure 2. Comparison of exoIL-12 vesicles generated by random and site-specific integration. scIL-12-PTGFRN is expressed from RI and SSI vector. SSI targets integration to AAVS1 genomic locus and is selected with puromycin expressed from an endogenous promoter through splice acceptor (SA) and 2A self cleaving peptide. Stable cell pools were generated by puromycin selection of HEK producing cells. Note a significantly quicker selection of SSI pools (A). Stable cell pools were cultured for 60 days post selection and showed similar stability, growth rate and viable cell density (B). Expression stability of stable pools was assayed by cell surface labeling with aIL12-APC antibody at 0, 30 and 60 days of cell culturing. Note higher homogeneity and stability of SSI pools (C, D). Purified exosomes were assayed for IL-12 activity by HEK IL12 reporter assay (E) from which EC50 values were derived (F). Note stable and similar activity of exoIL-12 from RI and SSI pools.

Construction of exoIL-12/CD40L/FLT3L combo EVs



Efficient multi-ligand co-expression in combo EVs

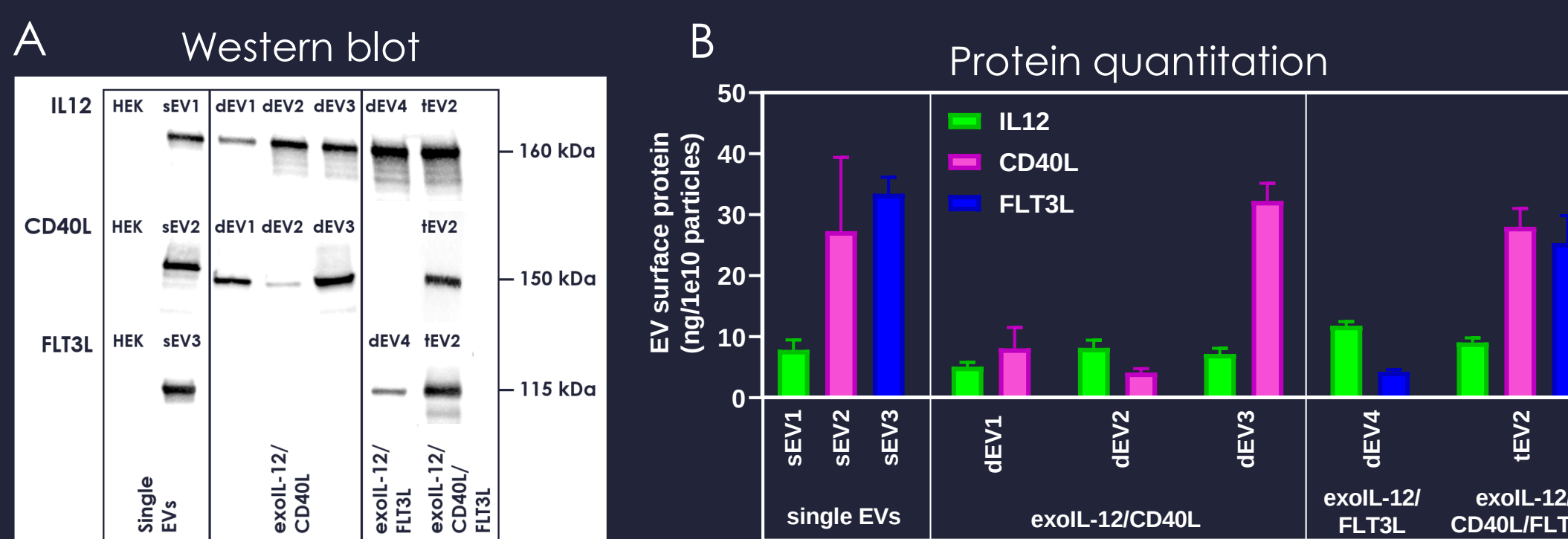


Figure 3. Construction of exoIL-12/CD40L/FLT3L combo EVs with preserved protein expression levels. Single, double and triple EVs of IL12, CD40L and FLT3L were engineered by a combination of RI and SSI in different safe-harbors. Protein expression on EV surface was measured by Western blot (A), ELISA and FACS (B). Triple exosomes tEV2 showed nonreduced expression levels of all three proteins compared to their single EV counterparts. Overall, the data shows that multiple proteins can be simultaneously anchored to the EV surface by PTGFRN fusion with no reduction in their expression levels.

Co-IP confirms multi-ligand co-expression in combo EVs

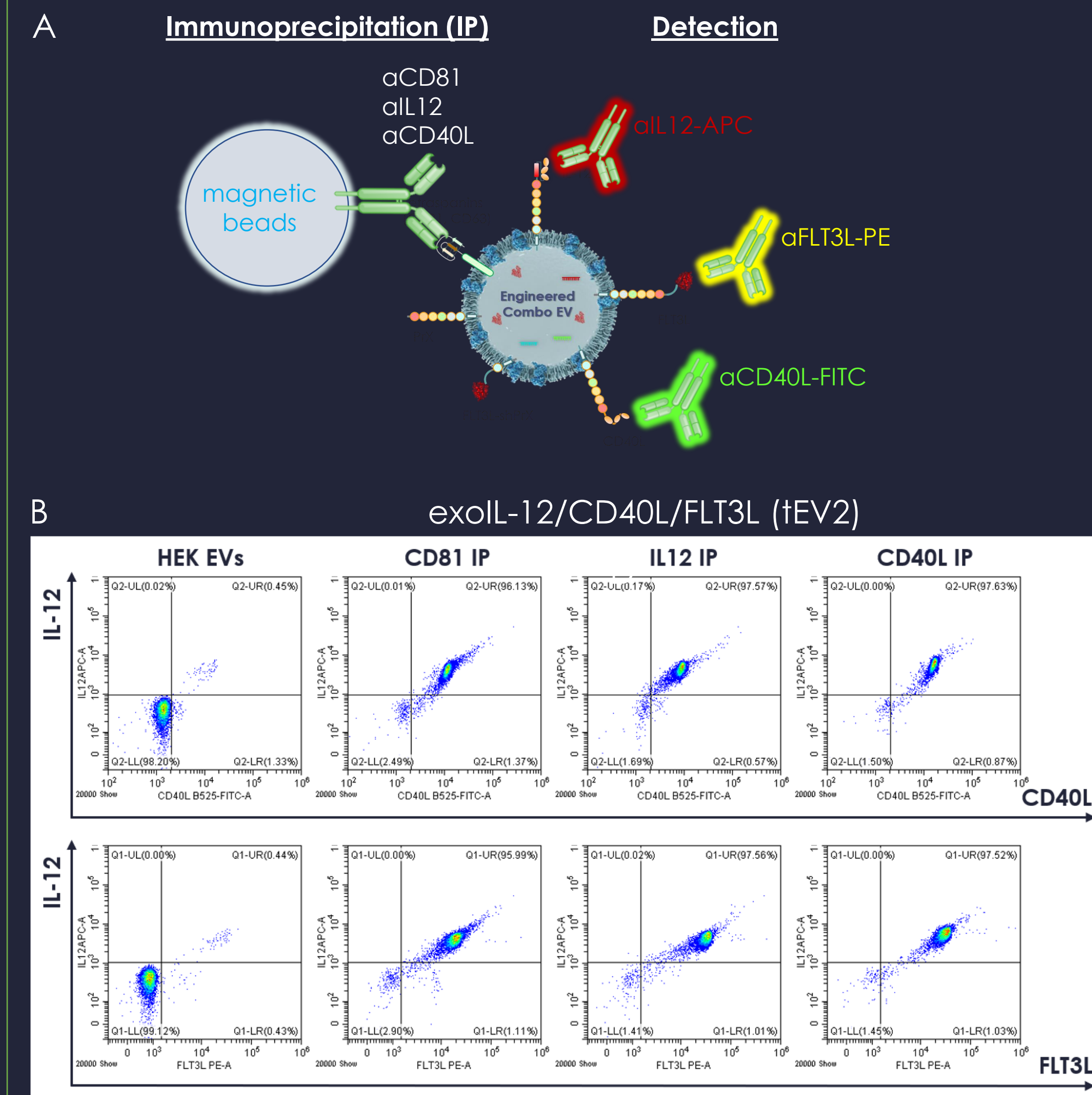


Figure 4. Co-IP confirms efficient ligand co-expression in exoIL-12/CD40L/FLT3L. Co-immunoprecipitation (co-IP) is achieved by coupling magnetic streptavidin-coated dynabeads with the biotinylated antibodies against one of the exosome surface proteins (αCD81, αIL12, αCD40L). Specific exosome populations are then immunoprecipitated with the coupled beads, co-labeled with aIL12-APC, αCD40L-FITC and αFLT3L-PE antibodies, and detected by FACS (A). FACS diagrams of exoIL-12/CD40L/FLT3L tEV2 co-IP experiment. Note >95% co-expression efficiency of all three proteins using any one of three IP methods.

Preserved biological activities of multi-functional EVs

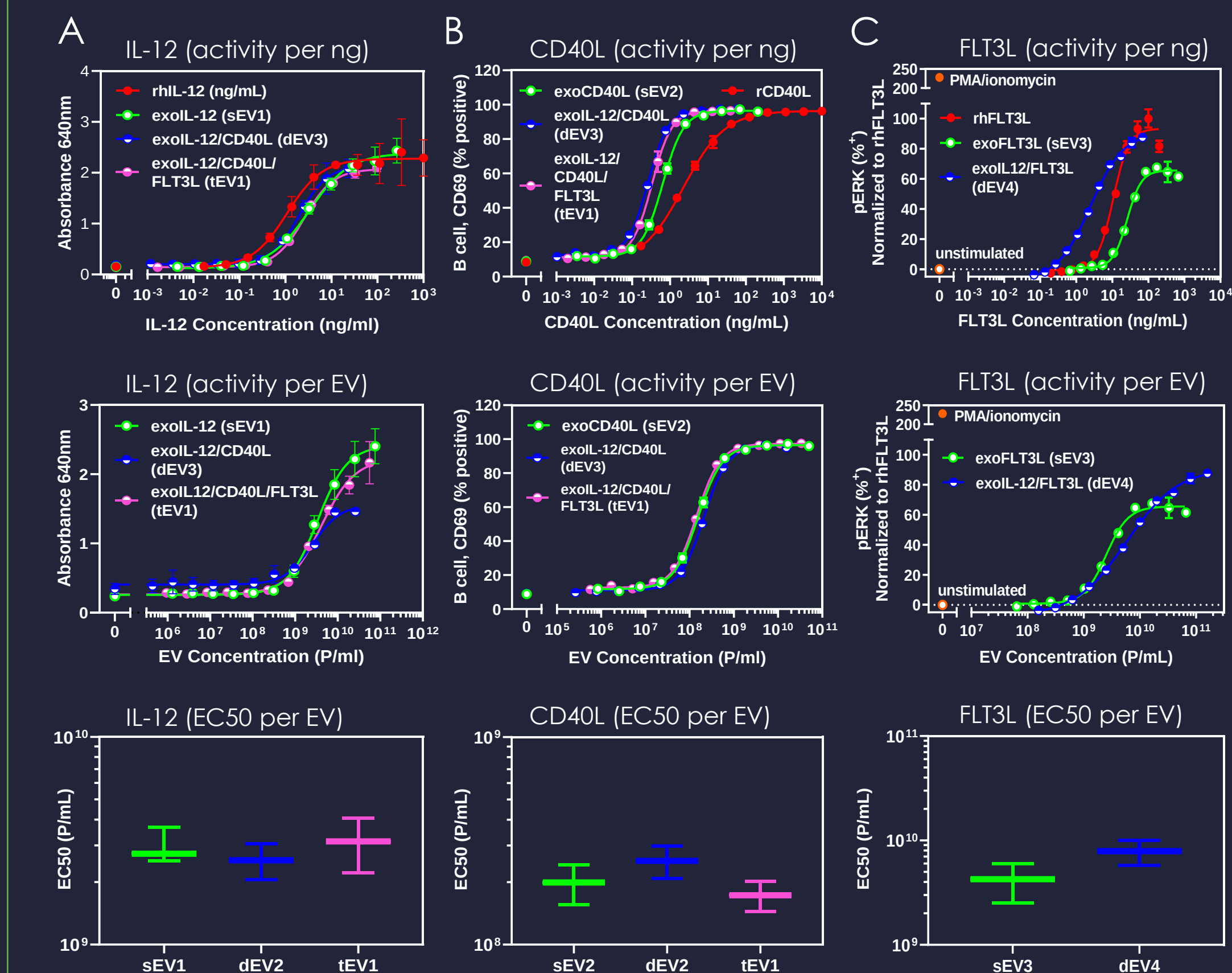


Figure 5. Multifunctional EVs show preserved biological activity. Selected multifunctional EVs with preserved expression levels were applied for in vitro activity assays. IL-12 activity was assayed with HEK-IL-12 reporter cells by measuring SEAP reporter activity in response to IL-12 binding to IL-12 receptor and activation of the STAT-4 pathway (A). CD40L activity was measured by measuring B cell activation by CD69 expression in human PBMC culture (B). FLT3L activity was assayed by measuring ERK phosphorylation in THP-1 monocytes following their stimulation by FLT3L binding or PMA/Ionomycin (full stimulation) (C). Shown are activities normalized to protein concentration (top row), EV concentration (middle row), and the derived EC50 values (bottom row). Note the preserved activity of multifunctional EVs.

Summary

- engEx platform is effective for the precision engineering of multifunctional exosomes
- PTGFRN can efficiently co-express multiple proteins on the exosome surface, with no drop in expression level and activity of individual proteins
- RI and SSI are both valid methods for the engineering of stable and potent exosomes
- SSI in different safe-harbors allows combo EV construction via precision cell engineering