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Scalable production of small extracellular vesicles (sEV) for immune therapy: integrating management of cellular stress in upstream processing

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HAL Authorization

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Introduction

Current advances in the development of sEV-based therapeutics highlight the need for scalable production in serum-free medium. Stirred tank bioreactors allow culturing adherent cells on microcarriers or in aggregates at large scale, but potentially engender cellular stress responses due to shearing forces and serum starvation that may affect sEV immune properties. Hence, alleviating cellular stress upstream is key to streamline robust manufacturing of therapeutic sEV. Using a murine beta cell line, we first characterized the impact of required process parameter modifications in order to produce massive quantities of therapeutics sEV in comparison to the standard laboratory cell culture conditions. Then, the operating process parameters of the scalable process have been optimized in order to mitigate cellular stress.

Material & Methods

Beta cell culture conditions. Mouse Insulinoma 6 (MIN6) beta cells were either grown as monolayers (ML) or as pseudo-islet (PI) aggregates under static or stirred conditions. Prior to sEV production, cells were washed in PBS and switched to serum-free medium. **sEV isolation and characterization.** After sequential centrifugation, beta-sEV were isolated using polyethylene glycol precipitation or tangential filtration followed by size exclusion chromatography. Particle concentrations were determined by TRPS. Images of purified sEV were obtained by cryo-electron microscopy. Tetraspanin detection was performed by flow cytometry after sEV capture on CD81-coated magnetic beads.

Glucose stimulated insulin secretion. Cells were switched to basal medium w/o glucose followed by incubation w/20 mM Glucose and 10 mM Theophyllin. Stimulation indexes were calculated as the ratio of stimulated / basal insulin. **Viability.** Cell viability was determined by trypan blue cell counts and lactate dehydrogenase quantification of dead cells in culture supernatants: Viability (%) = Trypan Blue- / (Trypan Blue- + LDH) X 100. **RT-qPCR analysis.** Total RNA extracted from cells were analysed by RT-qPCR. Relative quantities inferred from standard curves were normalized to HPRT1 and GAPDH expression.

From lab to scalable process : impact of process parameters on sEV quality attributes

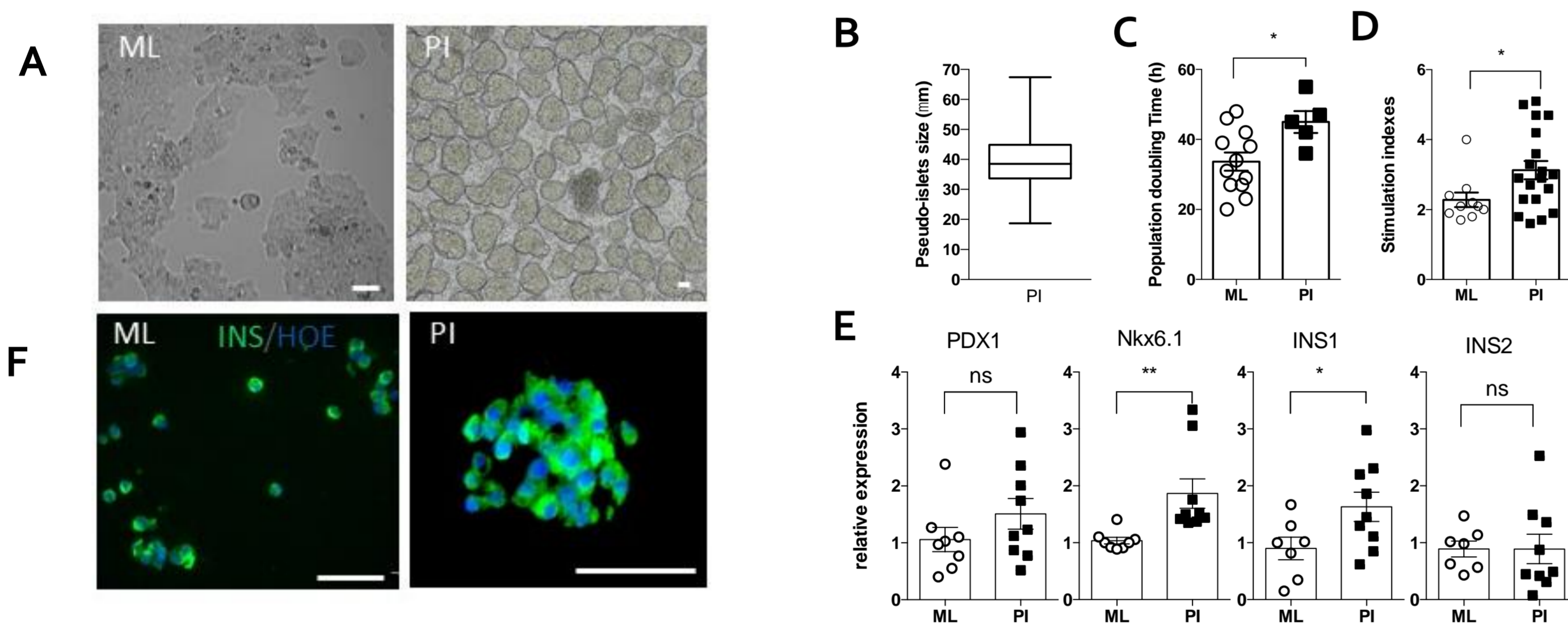


Figure 1. (A) Morphology of MIN6 cells grown as monolayer (ML) or pseudo-islets (PI) in a stirred tank bioreactor. Scale bar: 100 µm (B) PI size distribution at 72h of culture measured using ImageJ (C) Population doubling Time. Beta function appreciated by (D) Glucose-stimulated insulin secretion indexes (E) RT-qPCR analysis and (F) Immuno-fluorescent detection of insulin (green) and nuclei counterstained with Hoechst 33342 (blue).

► PI culture improves beta cell maturation as shown by decreased proliferation and enhanced expression of markers of beta cell function and insulin indexes

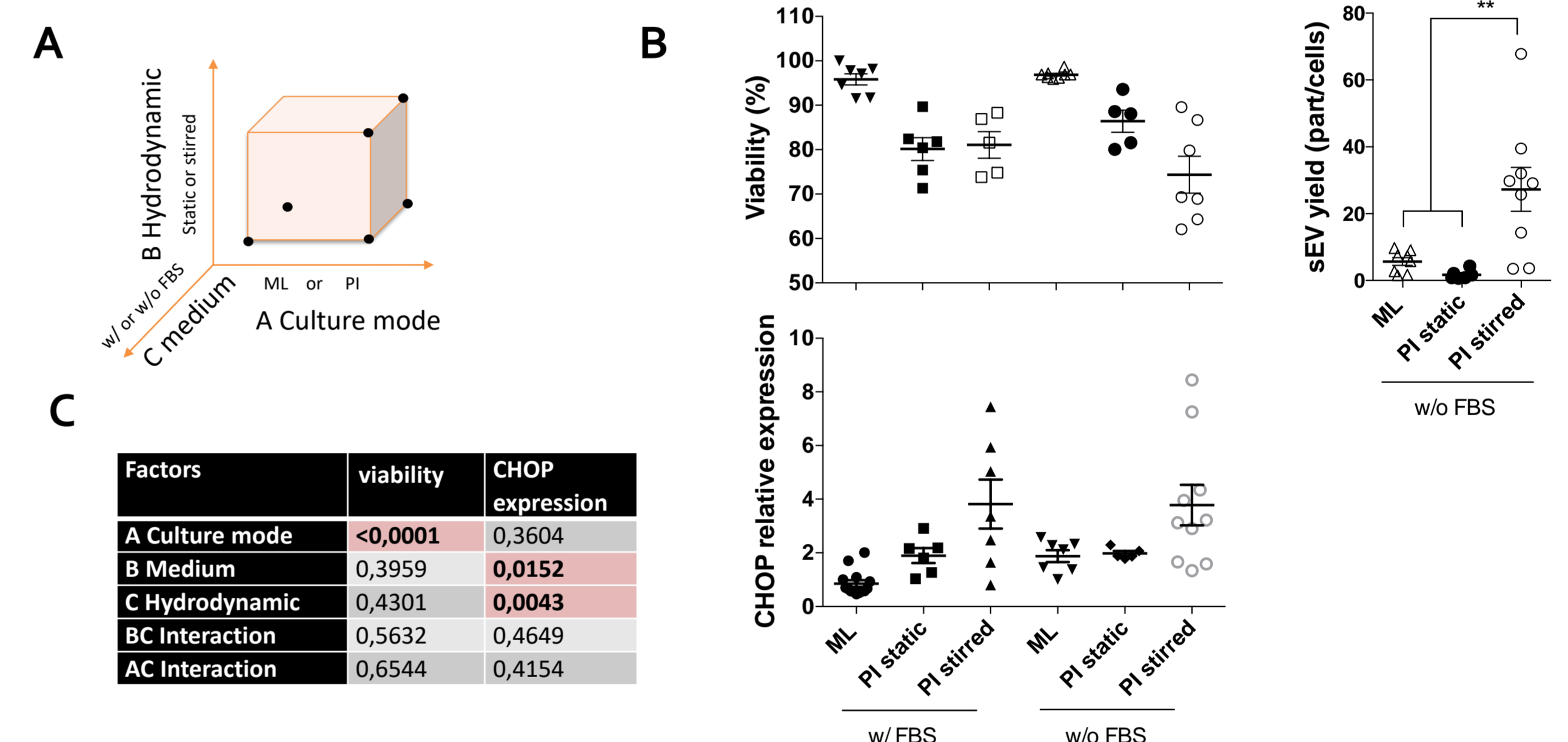


Figure 2. (A) Design of experiment including the culture mode (ML vs PI), hydrodynamic conditions (static vs stirred) and medium formulation (± FBS). Readouts were (B) Cell viability, CHOP relative expression and sEV production after 24h of culture. (C) Analysis of variance (ANOVA) using Statgraphics Centurion.

► PI culture in stirred tank in serum-free medium increases cell stress and sEV production

Scalable process optimisation for sEV production from healthy beta cells and validation

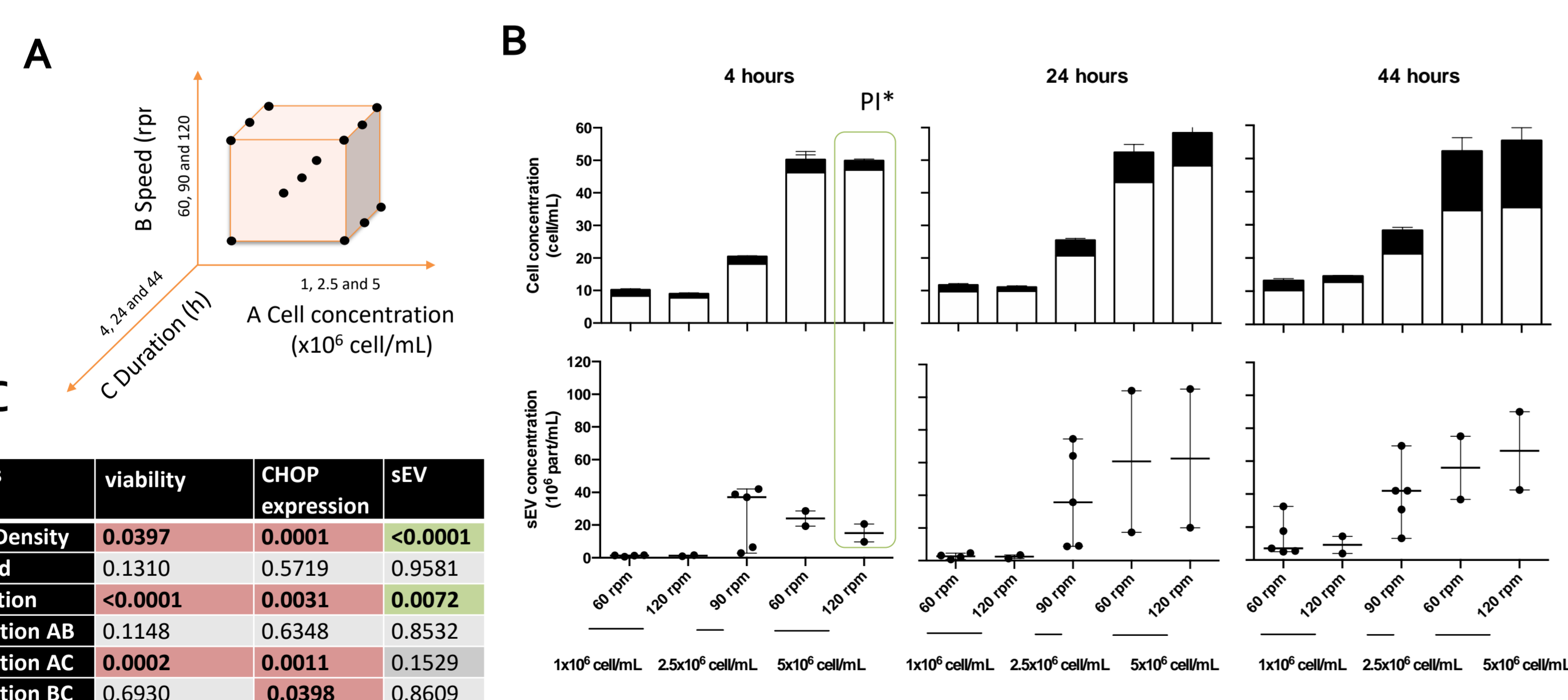


Figure 3. (A) Design of experiment including the culture of PI at a concentration of 1.0, 2.5 and 5.0 x10⁶ cell/mL with stirring at 60, 90 and 120 rpm in medium w/o FBS for 4, 24 and 44h. Read outs were (B) Concentration of viable (white) and dead (black) cells, sEV production and CHOP relative expression (data not shown). (C) Analysis of variance (ANOVA) using Statgraphics Centurion.

► A short production phase at high cell density in the assessed range of speed allows to mitigate cellular stress while ensuring large amount of sEV produced

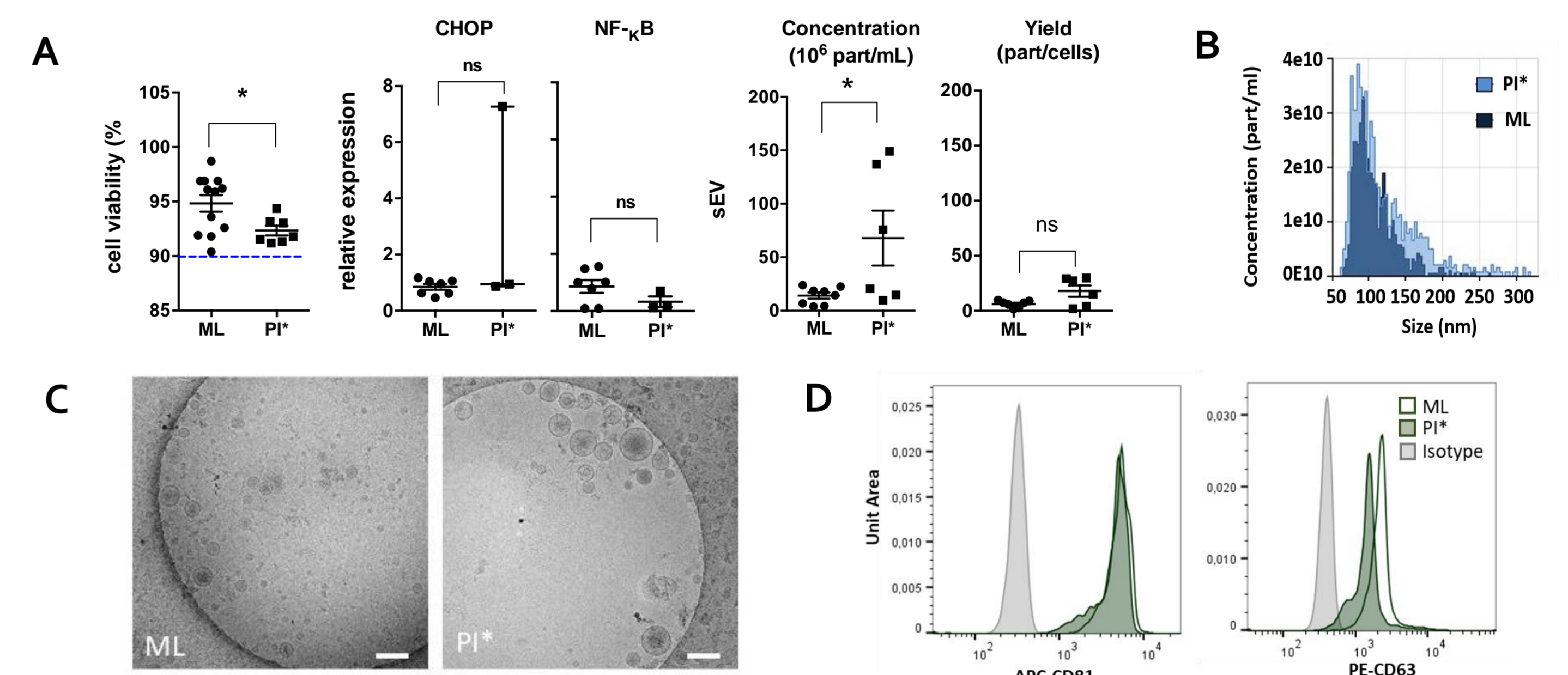


Figure 4. Performance of PI* under optimized culture conditions in comparison to cells grown in ML (A) Cell viability, RT-qPCR relative analysis of CHOP and NF-κB1, sEV concentration and yield after polyethylene glycol precipitation. (B-D) EV characterization after purification by tangential filtration followed by size exclusion chromatography (B) TRPS size distribution (C) Cryo-EM images. Scale bar: 200 nm (D) Tetraspanin CD81 & CD63 detection by flow cytometry.

► Optimized culture conditions allow to warrant cell viability >91% and to alleviate stress, all while improving recovery of sEV per unit of culture volume

Conclusion

PI culture in stirred tank bioreactors in serum free medium allows to improve beta cell function, but increases cell stress. As these conditions are required to design a GMP scalable process, a design of experiment was conducted to minimize cellular stress by tuning the impeller speed, the seeding cell density and the duration of culture. Interestingly, this optimization step allowed to reduce cell stress to levels measured in standard static two-dimensional culture. The proposed workflow should instruct rational management of cellular stress upstream in sEV-manufacturing bioprocesses for therapy. The benefit of sEV produced from healthy pancreatic beta cells will be studied as a new opportunity for type 1 diabetes multi-target immune therapy.

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Post-doc position available ↓

