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# Functional Assessment of the Induced Pluripotent Stem Cells (iPSC)-derived $\beta$ -like Cells

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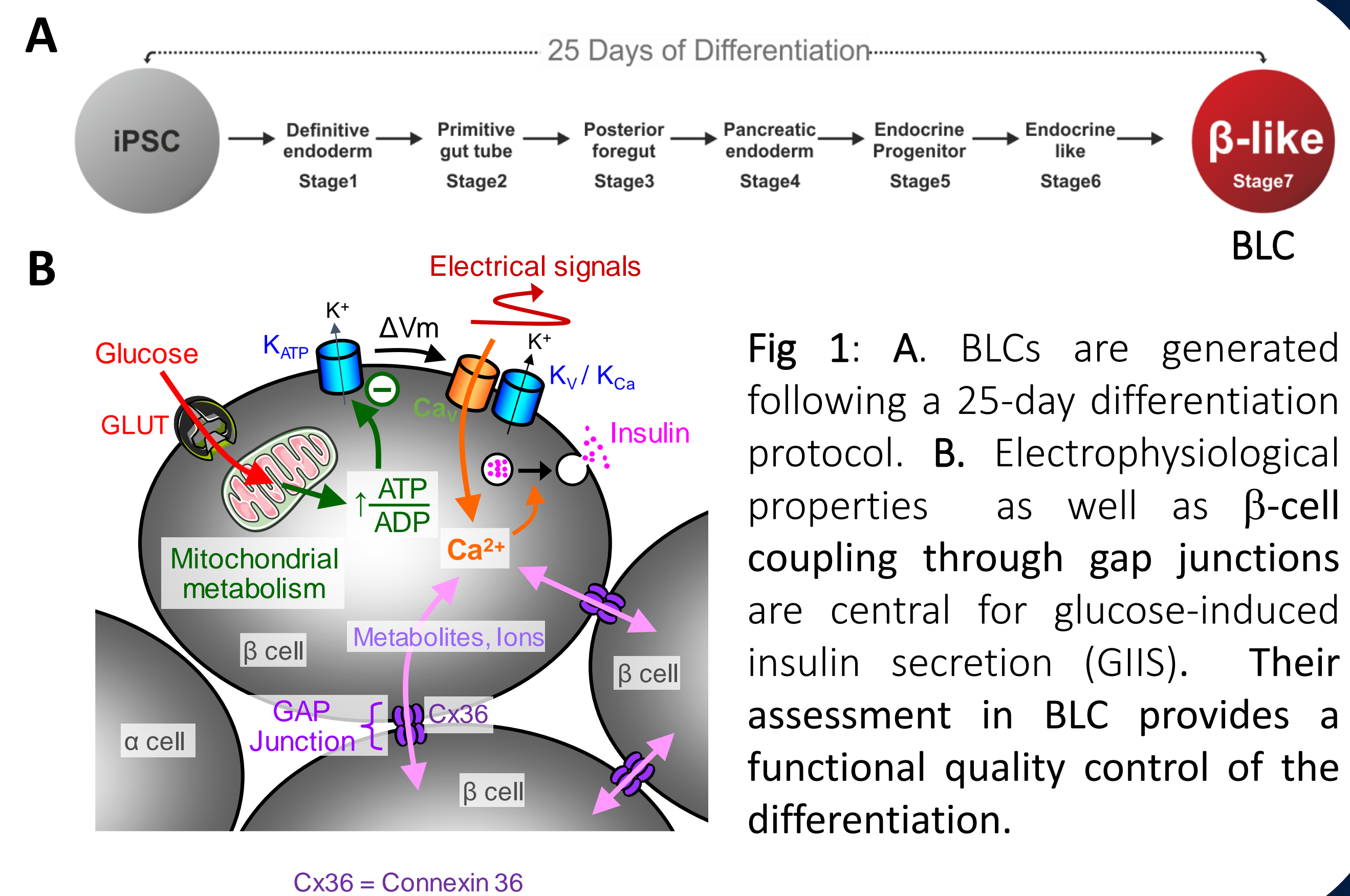
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## Background and Aim

Human  $\beta$ -like cells (BLCs) derived from induced Pluripotent Stem Cells (iPSC) are an emerging tool to study human  $\beta$ -cell development and function.

We established a functional assessment of each of our differentiations on the following properties:

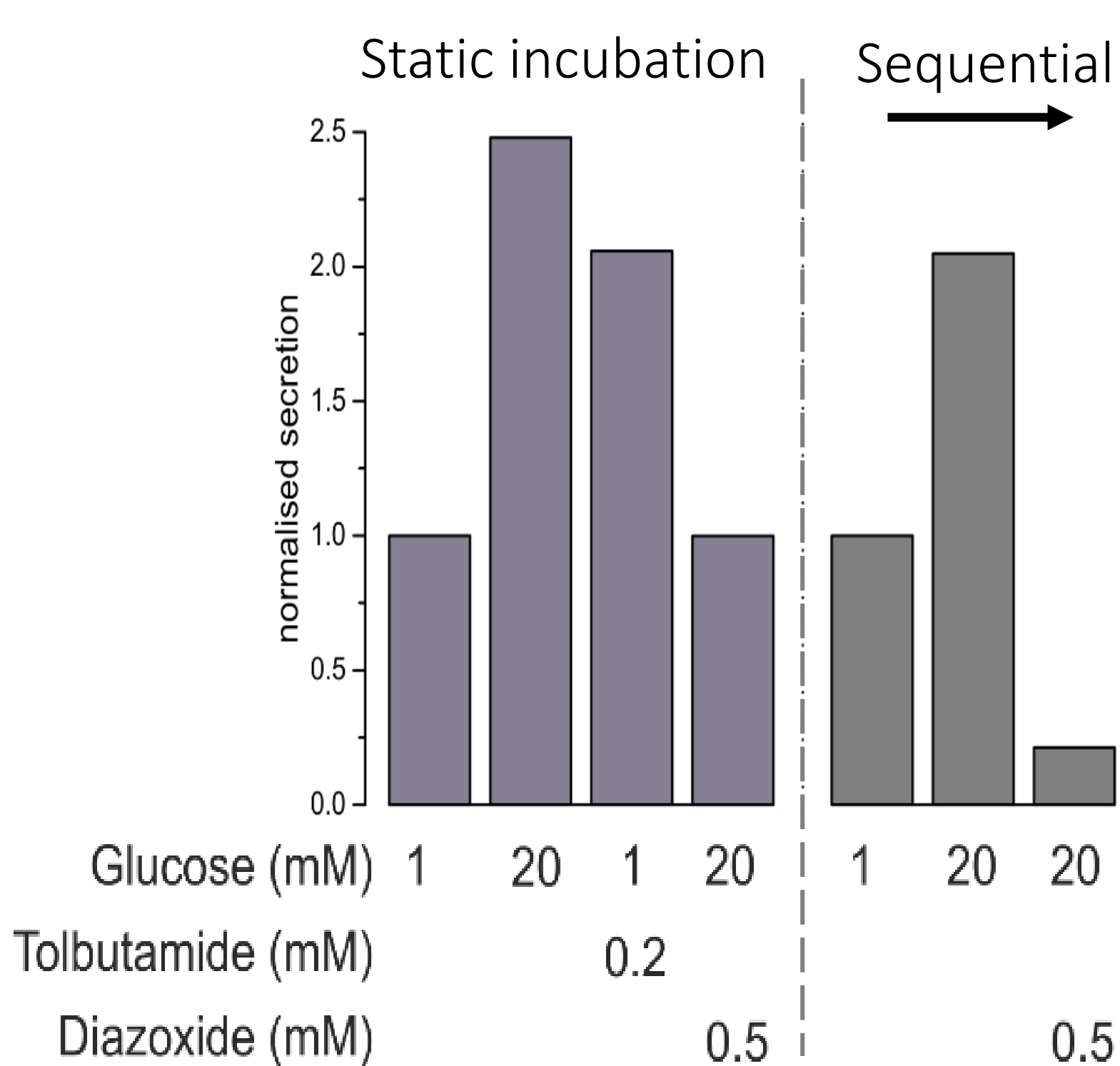
- Secretory response (insulin secretion assay)
- Exocytosis kinetics (capacitance measurement)
- Ultrastructure (Electron microscopy)
- $\beta$ -cells electrical coupling (Multi-Electrode Array, MEA)



**Fig 1:** A. BLCs are generated following a 25-day differentiation protocol. B. Electrophysiological properties as well as  $\beta$ -cell coupling through gap junctions are central for glucose-induced insulin secretion (GIIS). Their assessment in BLC provides a functional quality control of the differentiation.

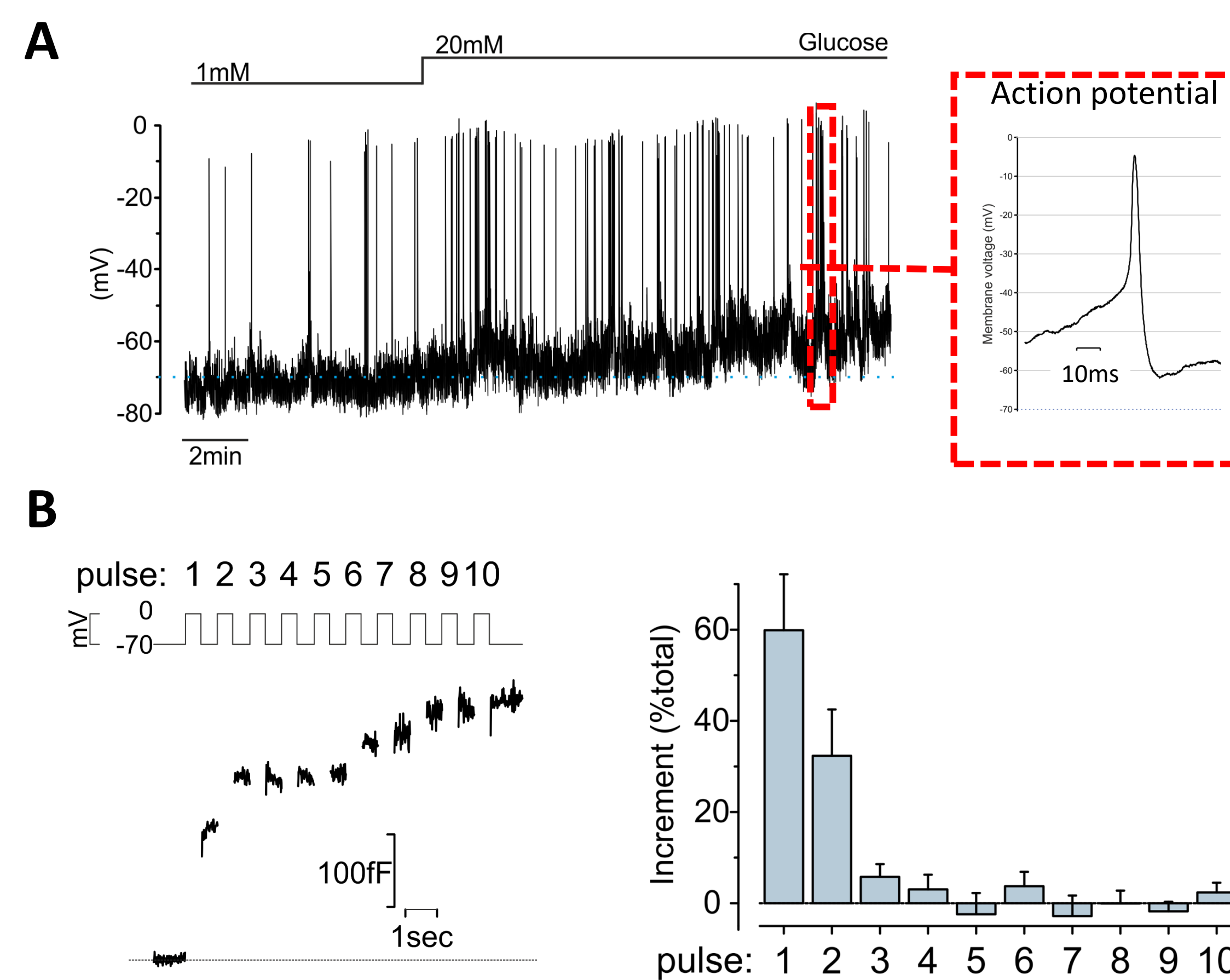
## Results

### Secretory response



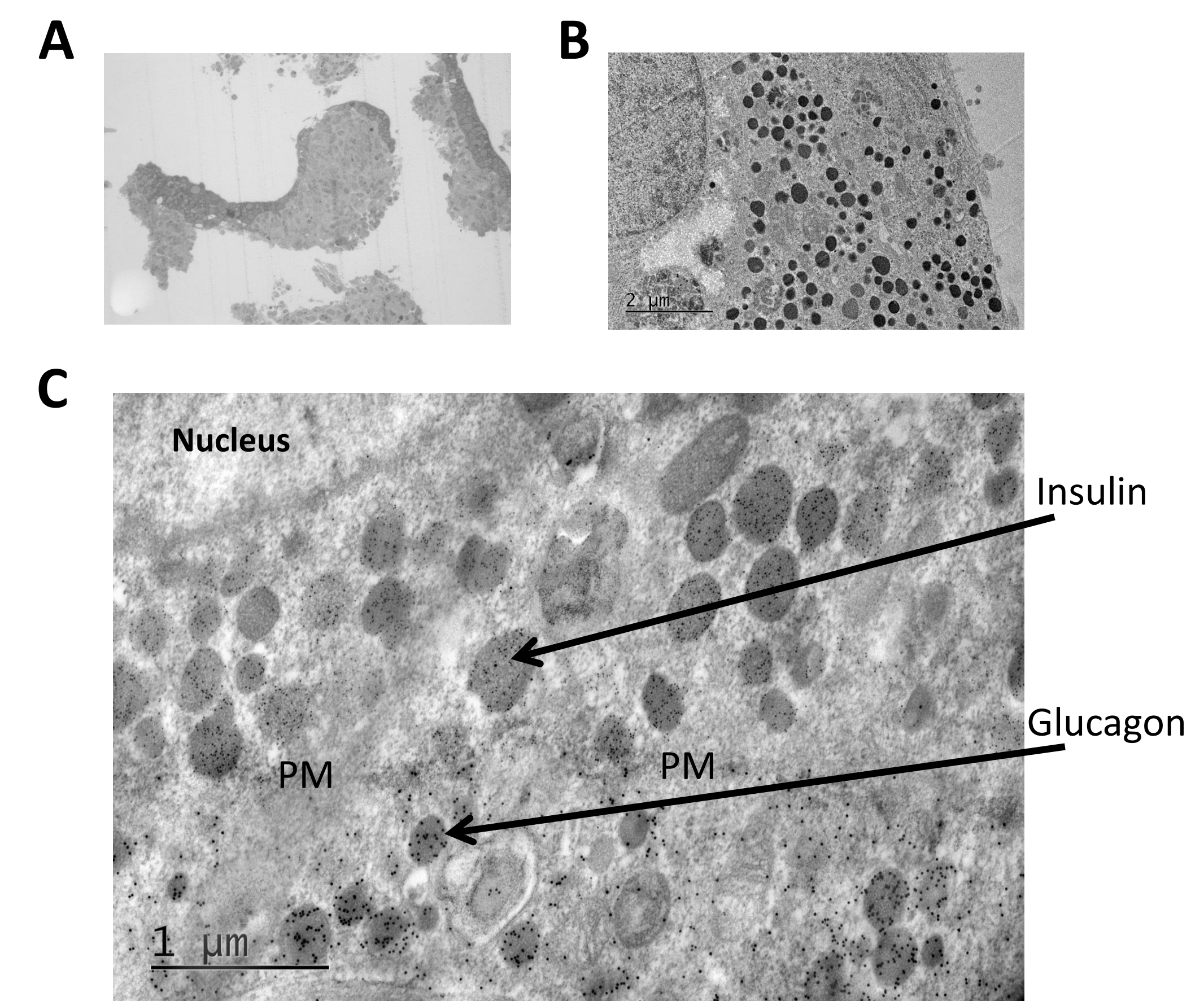
**Fig 2:** Representative GIIS induced by 1 and 20mM glucose and modulators of  $K_{ATP}$  channels activity (Tolbutamide/Diazoxide). The stimulations are either parallel static incubations (left), or sequential on the same BLC preparation (right).

### Electrical activity and exocytosis (single-cell)



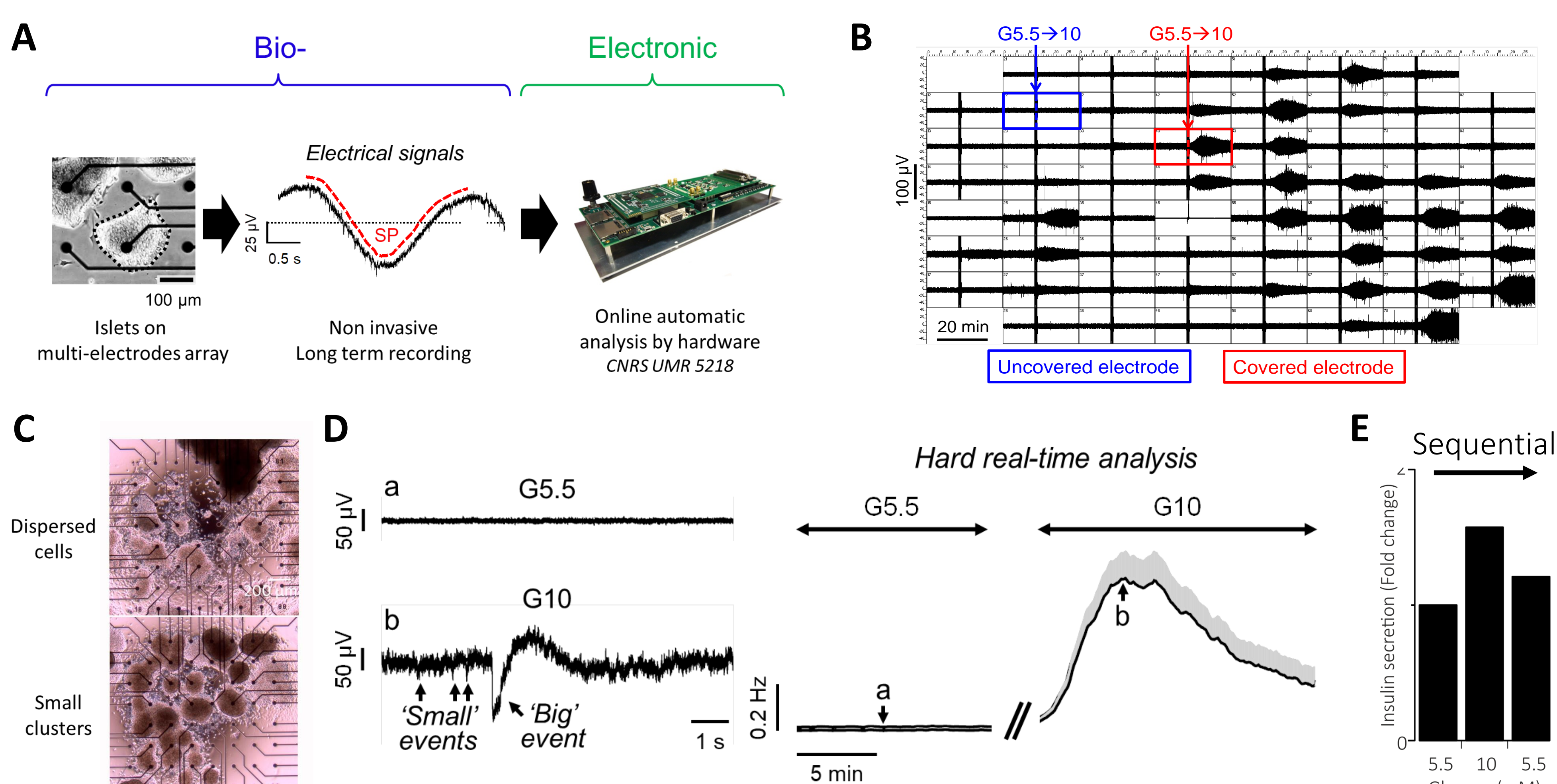
**Fig 3:** A. Glucose stimulation triggers action potential firing ( $n=3$  cells). Action potential kinetics is similar to human primary  $\beta$ -cells (10ms duration). B. Exocytosis triggered by a train of 10 depolarisations to 0mV is biphasic as in human primary  $\beta$ -cells (Raw trace left, quantification right,  $n=7$  cells).

### Ultra structure



**Fig 4:** A. Semi thin section showing cell heterogeneity. B. Ultra structure of BLC. Scale bar: 2 $\mu$ m. C. Immunogold labelling of insulin (BLC, 10nm gold particles, top) and glucagon (15nm gold particle,  $\alpha$ -like cell, bottom). PM: Plasma Membrane. Scale bar: 1 $\mu$ m.

### Multicellular activity assessed by MEA and its automatic analysis in hard real-time



**Fig 5:** A. Principle of the approach: islets on MEA generate slow potentials (SP) analysed by a microelectronic circuit. SPs are  $\beta$  cell-specific coupling signals. B. 60 recording channels showing  $\beta$ -like cells activity. C. Images of preparations. D. Representative traces at 5.5 and 10mM glucose and real-time analysis of SP frequencies (means+SEM,  $n=29$  electrodes). E. Corresponding insulin secretions.

## Conclusions

$\beta$ -like cells derived from iPSC exhibit critical human  $\beta$ -cell features:

- Glucose-induced insulin secretion
- Electrical excitability and exocytosis
- Electrical coupling

BLCs allow functional studies on human  $\beta$ -cell differentiation. The emergence of new differentiation protocols should improve their homogeneity and ultrastructure.

This pipeline will support the characterization of T2D-risk alleles on human  $\beta$ -cell function.