The pancreas is an organ composed of two main parts: exocrine, which produces digestive enzymes, and endocrine, where specialized cells work together to regulate blood glucose levels. When endocrine cells, particularly insulin-producing β cells, either do not work properly or are lost, the resulting disease is called diabetes. *In vitro* directed differentiation is a process in which we guide human pluripotent stem cells (hPSC) to become different kinds of mature cells. Our focus are pancreatic precursor cells and insulin-producing β-cells. *In vitro* differentiation is a promising tool in regenerative medicine to study the origin and new treatment possibilities for diabetes. This approach also allows studying developmental processes in a human context, overcoming the ethical limitations of using fetal tissues for research purposes. Over the last decade the protocols for pancreatic differentiation of hPSC were refined and now they result in robust and reliable results. Ultimately, the goal is to obtain functional β cells from hPSC that could be potentially transplanted to people with diabetes. Despite significant progress, the hPSC-derived β cells are not widely used in clinics and research because of incomplete understanding of the signals and factors controlling β cell formation. That is why it is so important to fully understand the developmental processes of the pancreas, as this knowledge can be translated into the generation of functional human β cells *in vitro*.
Based on the existing literature and preliminary data, I hypothesize that FKBP2:

- plays a significant role in the maturation of human insulin in β cells
- regulates β cell formation *in vitro*, as we identified the expression of FKBP2 not only in β cells but also in their precursor cells
- affects the endoplasmic reticulum (ER) network, the Golgi apparatus, and the extracellular matrix (ECM)

To test these hypotheses, I generated a hPSC line with turned off expression of FKBP2 gene (knockout (KO)). Upon differentiation of these cells into β cells, I observed changes in cell physiology and in the insulin maturation. Insulin maturation is a process in which protein chain is folded and cut to adopt a proper shape, which is necessary for insulin functionality. Using the hPSC differentiation platform, I want to elucidate the molecular changes that occur in FKBP2-deficient cells and their importance for the development and function of human β cells. The project will be implemented through the following objectives:

1. I will investigate in detail how FKBP2 influences the formation of human pancreatic β cells *in vitro*.
2. I will investigate how FKBP2 affects insulin maturation and secretion both *in vitro* (in hPSC-derived β cells) and *in vivo* (in mouse model). I will analyze the levels of insulin and insulin maturation byproducts, proinsulin and c-peptide, and their location in cells. Since we found FKBP2 expression in β cell precursors, we need to distinguish between the effects of FKBP2 deficiency on β-cell formation and insulin maturation. For this, I will perform inducible KO, to allow natural FKBP2 expression in β-cell precursors but to turn FKBP2 expression off in already formed β-cells. In addition, I will check β cell functionality by inducing insulin release in response to glucose in hPSC-derived β cells *in vitro.* Finally, I will transplantat hPSC-derived β cells into diabetic mice to check if they can produce enough mature insulin to reverse diabetes.
3. I will check what molecular changes underlie the improper insulin maturation in FKBP2 KO. Using single-cell RNA sequencing, I will analyze differences in gene expression between the FKBP2 KO and the control, focusing on the ER, Golgi apparatus, extracellular matrix, β-cell development, and insulin maturation.

Through this comprehensive study, I aim to advance our understanding of the role played by FKBP2 in pancreatic development and β cell function, shedding light on its potential therapeutic application in the treatment of diabetes.