# CHAPTER 5 FUTURE EXPERIMENTS

## 5.1. Unknown effects on penetrance of *GATA6* heterozygous mutants using various DE or pancreatic specification protocols

The discrepancy observed at the DE stage between different protocols suggests that the specification protocols used to differentiate the cells can have a significant impact on the DE phenotype observed. Hence, future work involving the identification of growth factors and/or signalling pathways that cause this discrepancy can be useful in further investigating the role of *GATA6* in the formation of the DE.

Moreover, it must be acknowledged that adherent differentiation fails to achieve the 3D complexity of human endoderm formation *in vivo*. Thus, future studies involving specification of pancreatic progenitors into functional and mature β-cells will likely benefit from 3D organoid systems which will more closely represent the *in vivo* environment of developing organs in humans, enabling the interactions between different pancreatic cell types and the interplay with possible niche signals.

With the availability of robust commercially available DE and pancreatic progenitor differentiation kits, perhaps studies of early pancreatic lineage commitment can be standardized intra- and inter-laboratory in an effort to minimize line-to-line and protocol-to-protocol differences.

### 5.2. Unexplored role of *GATA6* in other endoderm-derived organs

Although my work is focused on the pancreatic lineage, it is likely that *GATA6* haploinsufficiency plays a role in the development of other endoderm-derived organs such as the gall bladder, intestine and liver given that results from my study have established a critical role of *GATA6* in early endoderm formation. Indeed, preliminary results from my study where *GATA6* heterozygous mutants were specified toward the liver lineage have indicated that *GATA6* haploinsufficiency gives rise to defects in liver formation. The role of *GATA6* in these organs can be studied more closely using an inducible knockout system, such as a tetracycline-inducible system, where gene knockdown in hPSCs and even in differentiated cells can be rapid and tightly controlled, thus providing a unique opportunity for functional analyses in multiple cell types relevant for the study of human development.

### 5.3. Other possible roles of *GATA6*

Additional roles of *GATA6* such as whether *GATA6* haploinsufficiency can impair the proliferation and maintenance of pancreatic progenitors during their maturation into β-cells, or whether *GATA6* dosage may influence β-cell mass and function remain to be investigated. Utilising xenograft models such as grafting or transplanting β-like cells under the kidney capsules of mice can be used to further address these questions.

# FINAL CONCLUSIONS

The transcription factor *GATA6* has recently been identified as the most common cause of pancreatic agenesis in humans. My work has revealed dosage-dependent requirements for *GATA6* in lineage specification leading to the formation of pancreatic progenitors and immature β-cells using hPSCs as an *in vitro* system for disease modelling. The similarities in DE and pancreatic phenotypes observed between TALEN-derived lines and patient-derived lines indicate the success in disease modelling using genome editing tools coupled with hPSCs, and establish the suitability of using genome editing tools such as TALENs in the study of human diseases. On the molecular level, *GATA6* directly regulates the development of the DE and pancreatic progenitors. Thus, this work provides evidence that *GATA6* is involved in the development of the human definitive endoderm and pancreas as well as the molecular mechanisms by which it regulates this developmental process.