To better understand the mouse embryonic development, especially the gastrulation stage, we established an *in vitro* mouse embryonic stem cell (mESC) platform allowing the investigation of the critical processes and involved genes during embryoid body (EB) differentiation. Wnt/β-catenin is a crucial signaling pathway that modulates the differentiation during the gastrulation process. We generated the Wnt3, Wnt8a, and Wnt2b knock-out mESCs and differentiated them into EBs, in order to unveil the effects caused by the loss of these genes and find out the molecular mechanisms underlying the distinct phenotypes obtained.

To achieve that, we utilized TALEN and CRISPR DNA-editing tools. Besides, we developed a culture of three-dimensional EBs aggregating mESCs as an *in vitro* cell differentiation model.

Our results highlighted the importance of Wnt8a and Wnt2b in the mesodermal and cardiac differentiation, with Wnt8a being more essential, which was evidenced by the delayed EB morphogenesis upon the loss of Wnt8a or Wnt2b. Our results also suggested that the defects in Wnt/β-catenin signaling affected the exit from naïve to primed pluripotency during lineage-commitment differentiation, as well as the EMT progression. Moreover, our data illustrated the influence of Wnt/β-catenin signaling on other fundamental signaling pathways.

Finally, we also explored the biological functions of a series of novel genes including epithelial membrane protein 2 (Emp2). We found that Emp2 participated in the gastrulation-like and cardiac-relevant differentiation. Furthermore, we discovered a connection between Emp2 and Wnt/β-catenin pathway by activating the Wnt/β-catenin signaling to rescue the phenotype of Emp2 knock-out EBs. We also attempted to find out the potential link between Emp2 and FAK/Src, which was analyzed based on the specific inhibition of FAK/Src EB models.

In this thesis, we characterized a series of genes involved in the gastrulation-like and cardiac differentiation of mESC using the EB formation and differentiation platform.