The naïve pluripotent state of mouse embryonic stem cells (mESCs) is maintained by a precise balance of key signaling pathways. The Jak/Stat3 as well as the PI3K/Akt/mTOR pathway, both activated by LIF (Leukemia Inhibitor Factor), are essential to sustain a network of pluripotency-associated transcription factors. Although the Jak/Stat3 pathway has been intensively studied, little attention has been focused on the PI3K/Akt/mTOR pathway. In order to exit the pluripotent state and enter differentiation, the network of transcription factors needs to be dismantled. Notably, molecular events acting in this context are still poorly understood. Several genome-wide loss-of-function screens have been performed by my lab and by others to shed light on this early differentiation process. Interestingly, among high confidence hits were found two negative regulators of the PI3K/Akt/mTOR pathway, Pten and Tsc2. A function for those factors in regulating the exit from naïve pluripotency has been already reported, although molecular mechanisms underlying their action have not been elucidated yet.

Taking advantage of knock-out (KO) ES cell lines, we observed that cells lacking Pten or Tsc2 display a differentiation defect. Intriguingly, Tsc2 KO cells show a stronger phenotype compared to Pten KO cells. Consistently, Tsc2 absence determines a stronger impact on ES cell transcriptome. By performing cellular, molecular and biochemical assays, as well as genetic screens, we aim to dissect the actual mechanistic role of Pten and Tsc2 in regulating the exit from naïve pluripotency. This will provide a better understanding of molecular events orchestrating the early differentiation process of mESCs.

**Hypotheses:**

1. Dissect how the PI3K/Akt pathway coordinates the exit from naïve pluripotency.
2. Identify key players of the PI3K/Akt-pathway-dependent regulation of the exit from naïve pluripotency.