**Epigenome Profiling Reveals an Essential Role for Jun-N-Terminal Kinase Signaling During Human Trophoblast Stem Cell Specification and Differentiation into Terminal Lineages**

Joseph E. Zemke[1,2], Gareth Chapman[1,2], Laura A. Fischer[1,2], Rowan M. Karvas[1,2], Kyoung Park[1,2], Lauren Boggs[2], Brittany Meyer[1,2], Kristen L. Kroll[1,2], and Thorold W. Theunissen[1,2]

1. Department of Developmental Biology, Washington University in St. Louis, St. Louis, USA
2. Center of Regenerative Medicine, Washington University in St. Louis, St. Louis, USA

Due to ethical and practical constraints on access to first-trimester placental tissues, the study of human trophoblast specification and differentiation into specialized lineages has remained limited. The recent derivation of human trophoblast stem cells (hTSCs) from naive human pluripotent stem cells (hPSCs) allows for mechanistic investigation of developmental time points and cell fate decisions that we lack access to *in vivo*. By mapping genome-wide chromatin accessibility and active and repressive histone marks in the naïve hPSC and hTSC states, we have produced an epigenetic atlas of cis-regulatory regions driving hTSC specification *in vitro*. This analysis revealed an enrichment of Jun-N-Terminal Kinase (JNK) binding motifs in regions associated with hTSC-specific active histone marks. Chemical inhibition of JNK signaling in hTSCs and trophoblast organoids caused spontaneous differentiation into terminal trophoblast lineages as assessed by morphology and gene expression analysis. When attempting to differentiate hTSCs from the naïve state in the presence of this inhibitor, the cells displayed growth restriction and abrogated hTSC specification. To investigate how JNK-associated factors contribute to hTSC specification and maintenance, we have identified specific JNK targets via differential phosphorylation status upon inhibitor treatment. We are currently exploring these transcription factors in greater detail to assess their individual and combinatorial impact on hTSC specification and maintenance using a deactivated Cas9 system. By integrating these genetic studies of JNK-associated factors with genome-wide location analyses to identify DNA binding regions, we will gain a comprehensive understanding of the role of the JNK signaling pathway during hTSC specification and differentiation into terminal trophoblast lineages.