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Mediator, a large multi-subunit co-activator complex, that relays information from gene-specific transcription factors to RNA polymerase II, is grouped into 4 modules: head, middle, tail, and a dissociable four subunit kinase module consisting of MED13, MED12, CDK8, and Cyclin C (CCNC). MED12 acts as an activator of CDK8-CCNC in Mediator which in turn activates cell-type specific transcription factors[1]. Because of its essential role in key developmental pathways, mutations in MED12 leads to tumorigenesis and genetical disorders[2]. The concept concerning the structural and functional impact of MED12 on the module is still unclear. This research investigates the structural integrity of the Mediator complex in the presence and absence of MED12 in human adipose stem cells (hASCs). ASCs are multipotent stem cells that can self-renew and differentiate into different lineages with the help of transcription machinery, making them of great interest as a potential source in the fields of tissue engineering and regenerative medicine[3]. These considerations highlight the importance of studying kinase module subunits interactions, to better understand how MED12 contributes to specific genetic disorders and suggest new therapeutic strategies for human tumorigenesis.

We are testing the hypothesis that the loss of MED12 in the Mediator complex leads to loss of other subunits in the kinase module ultimately causing major structural changes in the complex. To determine effects on the Mediator complex following the loss of MED12, we use co-immunoprecipitation (CoIP) to pull down MED12 in hASCs grown under standard culture conditions and use western blots to determine Mediator subunit interactions. The MED12 antibody is used to pull down MED12 and its associated proteins. Analysis of the pull-down of MED12 is done by running an SDS-PAGE and probing for different subunits using specific subunit antibodies to detect each subunit interaction. The results of Co-IP of MED12 show that MED12 is not still attached CDK8 as we can see the presence of MED12 in elution sample and CDK8 in unbound lysate (Fig. 1A), whereas the results Co-IP of CDK8 show that MED12 is still bound to CDK8 (Fig. 1B) as we can detect the presence of both proteins in elution sample. We still have to redo the same process to confirm the stated results. To determine the interactions within kinase module in the absence of MED12, we performed siRNA mediated knockdown of MED12. Validation of knockdown of MED12 and its associated subunits was done by qRT-PCR and western blot. We will perform additional Co-IP and knockdown for each of the subunits that make up the kinase domain to investigate the interactions.