



**Figure 6 - supplement 1.** Overview of BioID Strategy and Validation of Fusion Constructs. **(A)** Overview of BioID purification strategy. **(B)** MYC-BioID2-GFP and MYC-BioID2-RHOU are induced upon doxycycline treatment for 1 day. Representative MYC immunofluorescence on primary keratinocytes transduced with the BioID2 fusion constructs. Scale bars, 10  $\mu$ m. **(C)** MYC-BioID2-RHOU localize to focal adhesions as marked by VINCULIN in growth conditions (Low  $\text{Ca}^{2+}$ ) and is relocated at adherens junction as marked by E-CADHERIN upon keratinocytes differentiation (High  $\text{Ca}^{2+}$ ). Representation of MYC, VINCULIN and E-CADHERIN immunofluorescence on primary keratinocytes transduced with the BioID2 fusion constructs. Scale bars, 10  $\mu$ m. **(D)** MYC-BioID2-GFP and MYC-BioID2-RHOU are able to induce biotinylation of protein in cells in the presence of biotin. Representative immunofluorescence of Streptavidin and Phalloidin on primary keratinocytes transduced with the BioID2 fusion constructs. Scale bars, 10  $\mu$ m. **(E)** Immunoblot of lysates from *Krt14rtTA*+ keratinocytes showing expression of PAR6 in these cells. Immunoblots were probed with PAR6 and TUBULIN antibodies.