



Figure S5. A. Western blot analysis shows differential protein expression levels of NAC1 in mOSE isolated from the respective genotypes. **B.** Epithelial nature of the cultured mOSE was verified by cytokeratin-14 staining. **C.** Number of multi-nucleated cells was quantified as a percentage in mOSE cultures established from different Nac1 genotypes. (Nac1 wildtype n=319, Nac1 +/- n= 429, Nac1 -/- n=304). There is a significant difference in the proportion of multi-nucleated cells between different Nac1 genotypes (***p< 0.0001, Chi-square contingency table). **D.** Immunofluorescence staining of alpha-tubulin facilitates the quantification of cells exhibiting multi-nucleation. Cells were counterstained with DAPI to reveal nuclei. Arrowheads indicate multi-nucleated cells. Scale bar, 50 μm. **E.** Double immunofluorescence staining of alpha-tubulin and V5 (to detect NAC1-V5) facilitates the quantification of cells exhibiting multi-nucleation after expression of NAC1. Cells were counterstained with DAPI to reveal nuclei. Scale bar, 100 μm. **F.** Expression of NAC1-V5 in Nac1 -/- mOSE was verified by western blot. **G.** NAC1 was ectopically expressed in Nac1 -/- mOSE cells, and its expression was associated with a significant decrease in the percentage of multi-nucleated cells as compared to control virus-treated group (**p< 0.01, Chi-square contingency table).