The SALL2 transcription factor: a new regulator of cell migration through integrin β1 expression

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INTRODUCTION

Migration is a multistep process involved in development, maintenance of multicellular organisms and diseases, such as cancer. This process involves cellular polarization, generation of membranous protrusions and focal adhesión, disassembly of previously existing focal adhesions, and cell retraction. SALL2, a member of the *spalt-like* family of proteins, is a poorly characterized transcription factor that has been indirectly related to cell migration due to its crucial function in optic fissure closure and neurite outgrowth. However, the role of SALL2 in cell migration remains unclear. Understanding whether SALL2 plays a role in cell migration is relevant to understanding its role in physiology and disease.

METHODS

To determine the role of SALL2 in cell migration, we used *Sall2* knockout (KO) and *Sall2* wild-type Mouse Embryo Fibroblasts (MEFs). Additionally, we used gain of function experiments. The effects of Sall2 in different aspects of cell migration was measured by wound healing, cell detachment, focal adhesion dynamics, and immunofluorescence assays. We used Western blot, real-time PCR, flow cytometry, chromatin immunoprecipitation, and bioinformatics analysis for mechanistic insights. Data were compared in unpaired Student's t-tests by using the GraphPad Prism software.

RESULTS

1. Sall2 promotes mouse embryo fibroblasts migration

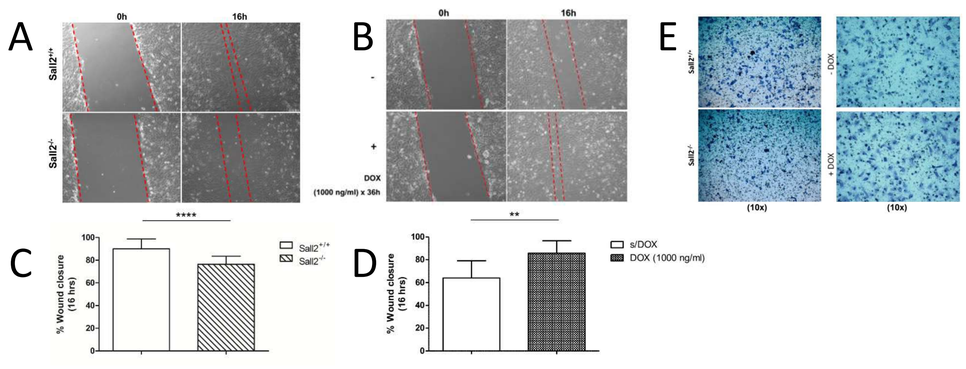


Figure 1: (A, B) Representative phase-contrast images (10X) at 0 and 16 hours of wound healing from *Sall2-/-* and *Sall2+/+* iMEFs and the Sall2 doxycycline (dox)-inducible Tet-On iMEF model. (C, D) Quantification of wound closure (as percentage) from A and B. (E) Representative images (10X) of transwell migration assay.

2. Loss of Sall2 alters celular protrusions

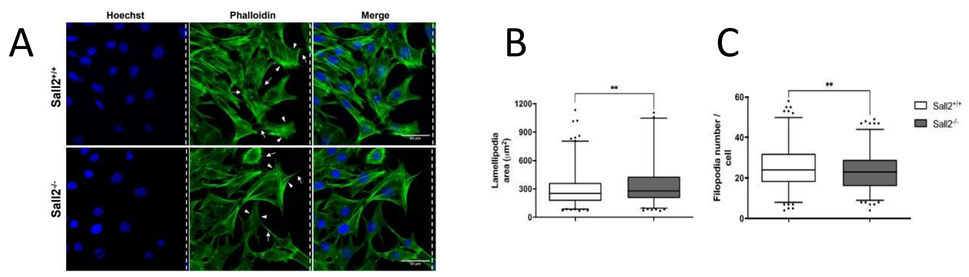


Figure 2: (A) Representative confocal images (40X) from *Sall2-/-* and *Sall2+/+* iMEFs after cell migration induction at 16 hours. F-actin, and nuclei stained with phalloidin (green) and Hoechst (blue). Dashed lines indicate the position of the wound; arrowheads and arrows show the lamellipodium and filopodium, respectively. (B, C) Quantification of lamellipodia area and filopodia number per cell.

3. Sall2 promotes cell detachment

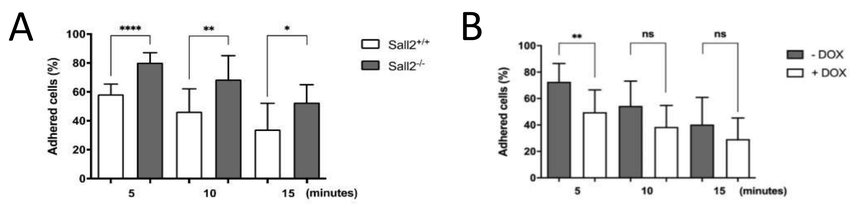


Figure 3. (A, B) Adherent cells quantification at 5, 10, and 15 minutes after EDTA incubation from *Sall2-/-* and *Sall2+/+* iMEFs and the Sall2 doxycycline (dox)-inducible Tet-On iMEF model, respectively.

4. Loss of *Sall2* increases focal adhesion maturation

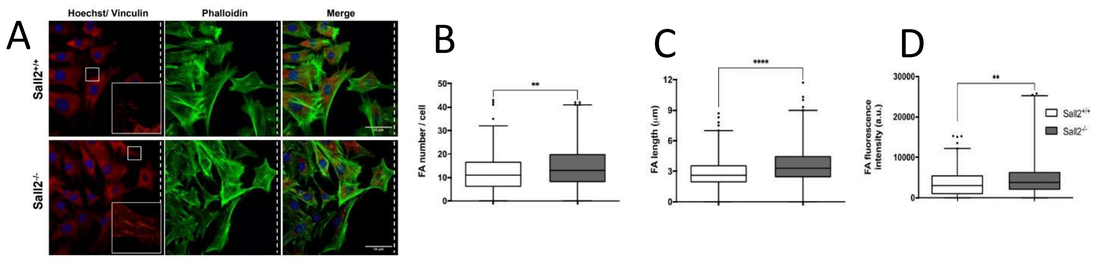


Figure 4: Representative confocal images (40X) from *Sall2-/-* and *Sall2+/+* iMEFs after 16 hours of cell migration induction. Focal adhesions (FA), F-actin, and nuclei were stained with anti-vinculin (red), phalloidin (green), and Hoechst (blue) respectively. Dashed lines indicate the position of the wound. (B, C, D) Quantification of FA number, length, and fluorescence intensity, respectively.

5. Sall2 promotes focal adhesion dynamics

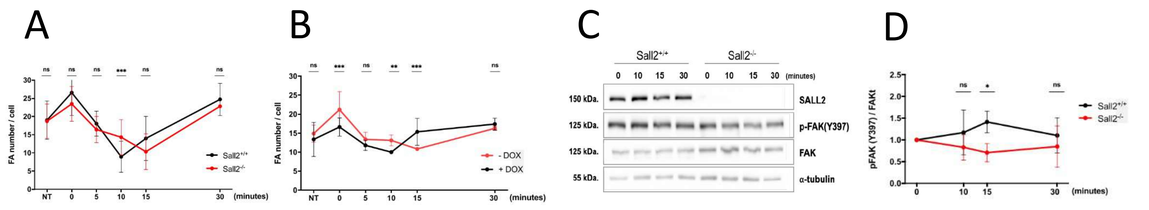


Figura 5: (A,B) Quantification of focal adhesions number per cell from *Sall2-/-* and *Sall2+/+* iMEFs and *Sall2* KO iMEFS with *Sall2* restored by Tet-On inducible system, respectively. (C) AK autophosphorylation (Y397) was evaluated by western blot at different times of nocodazole wash out. (D) FAK autophosphorylation levels were quantified by densitometric analysis respect t= 0 minute of nocodazole wash out treatment. FAK(Y397) was normalized respect to FAK and α-tubulin expression.

6. Sall2 positively regulates integrin β1 expression

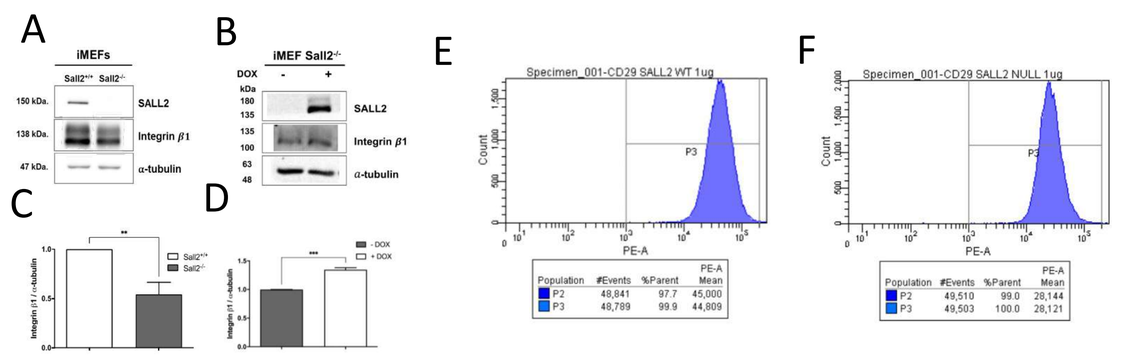


Figure 6. (A, B) Representative blots of integrin β1 at 15 minutes of nocodazole washout treatment from *Sall2-/-* and *Sall2+/+* iMEFs, and the Sall2 doxycycline (dox)-inducible Tet-On iMEF model. (C, D) Integrin β1 densitometry normalized to α-tubulin from blots as in A and B. (E, F) Percentage of cell surface integrin β1 positive cell populations evaluated by flow cytometry of *Sall2-/-* and *Sall2+/+* iMEFs, and the Sall2 doxycycline (dox)-inducible Tet-On iMEF model.

7. Integrin β1, a novel Sall2 transcriptional target

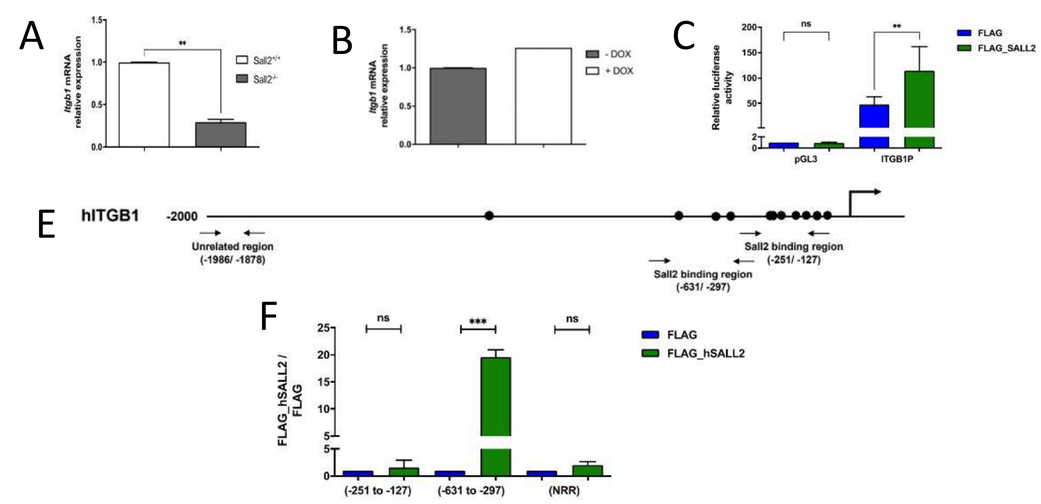
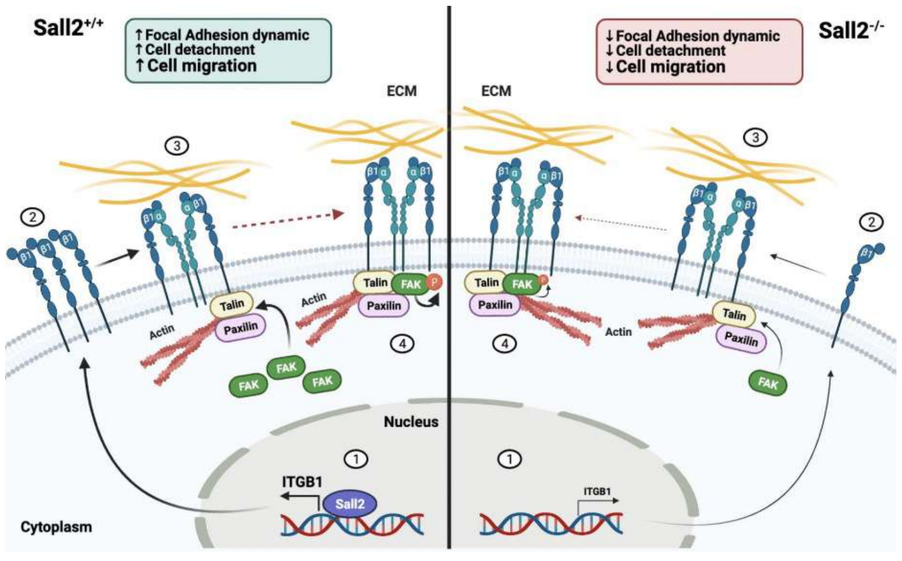


Figure 7. (A, B) *Itgb*1 mRNA levels from Sall2-/- and *Sall2+/+* iMEFs and the Sall2 doxycycline (dox)-inducible Tet-On iMEF model under nocodazole washout treatment (15 minutes). (C) h*ITGB1* promoter activity (luciferase) of HEK293 cells cotransfected with the h*ITGB1* promoter without (FLAG vector) and with FLAG_SALL2 normalized to β-galactosidase activity, promoter activity expresses as relative luciferase units (R.L.U). pGL3 vector served as control. (E) Chromatin from HEK293 cells was immunoprecipitated 24 hours after FLAG hSALL2 transfection using the FLAG antibody. Specific regions of the h*ITGB1* promoter and a nonrelated promoter region (NRR) were analyzed by real-time PCR. Graphs show quantification of the amplified DNA relative to FLAG.

8. Proposed model of the Sall2/ITGB1/migration axis



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