Supplementary Information

JAM-A interacts with α3β1 integrin and tetraspanins CD151 and CD9 to regulate collective cell migration of polarized epithelial cells

Cellular and Molecular Life Sciences

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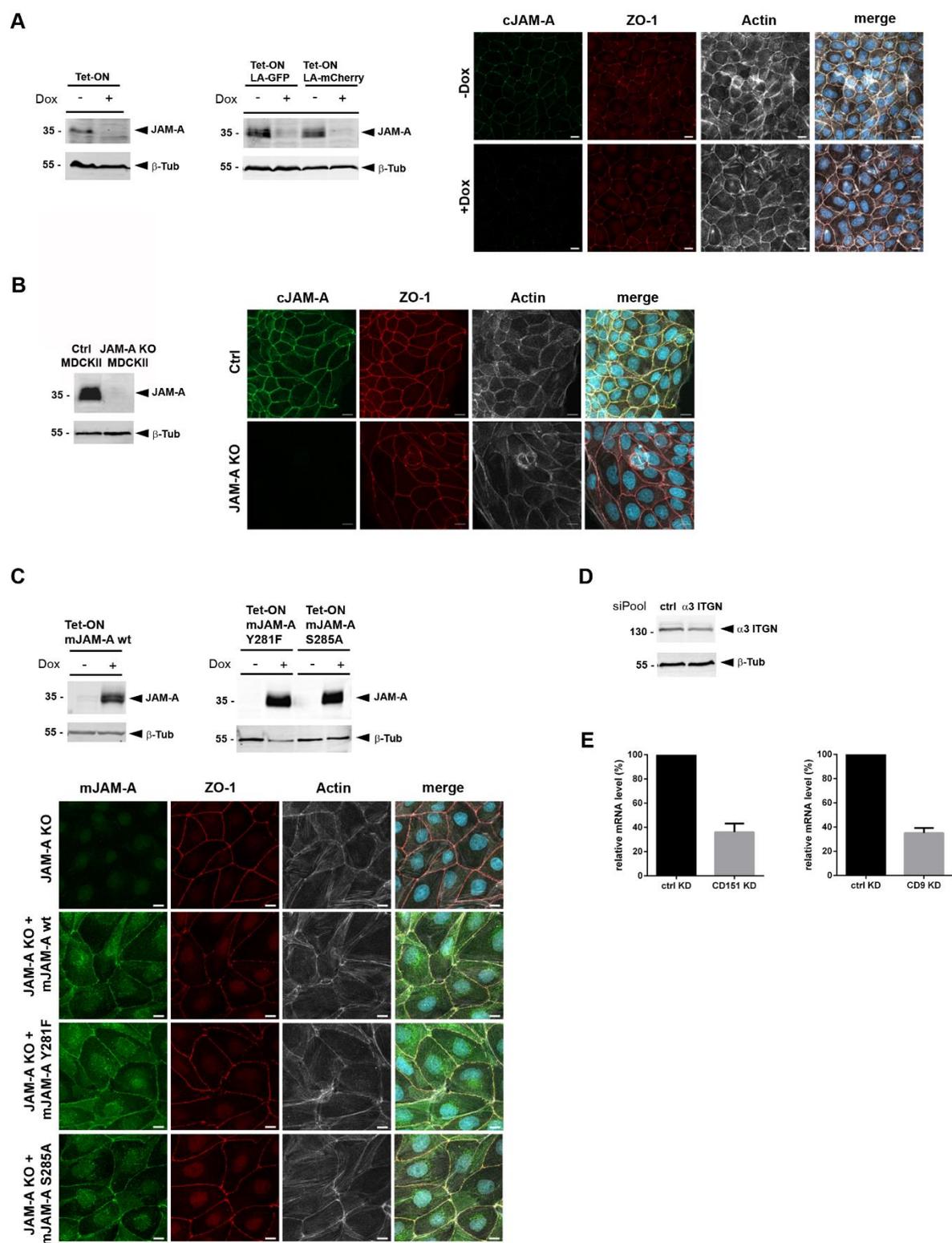

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Supplementary Figures S1 – S3

Supplementary Movie Legends

Supplementary Figures

Suppl. Fig. S1: Analysis of knockdown efficiencies and characterization of JAM-A knockout MDCKII cells. (**A**) Knockdown efficiencies of JAM-A. Left: Western blot analysis of JAM-A in MDCKII

cells stably transfected with a conditional shRNA expression vector that expresses JAM-A shRNAs under a doxycycline-regulated promoter (pEmU6-proT, Tet-ON) without or with addition of doxycycline (-Dox or +Dox, respectively). Middle: Western blot analysis of JAM-A expression in pEmU6-proT/JAM-A shRNA-transfected MDCKII cells stably transfected with LA-EGFP or LA-mCherry without or with addition of doxycycline (-Dox or +Dox, respectively). Right: IF analysis JAM-A expression in pEmU6-proT/JAM-A shRNA-transfected MDCKII cells. Cells were stained for JAM-A, ZO-1 and F-Actin as indicated. Scale bars: 10 µm. (**B**) Characterization of JAM-A KO MDCKII cells. Left: Western blot analysis of JAM-A in MDCKII cells with a CrisprCas9-mediated inactivation of the JAM-A gene. Right: IF analysis of JAM-A KO MDCKII cells. Cells were stained for JAM-A, ZO-1 and F-Actin as indicated. Scale bars: 10 µm. (**C**) Characterization of JAM-A KO MDCKII cells reconstituted with expression vectors encoding murine JAM-A constructs (mJAM-A/WT, mJAM-A/Y281F, mJAM-A/S285A) under a doxycycline-regulated promoter (pInducer21, Tet-ON). Top: Western blot analysis of JAM-A constructs in the absence and presence of doxycycline (-Dox, +Dox, respectively). Bottom: IF analysis of JAM-A KO MDCKII cells after doxycycline-induced expression of murine JAM-A constructs. Cells were stained for mouse JAM-A, ZO-1 and F-Actin as indicated. Note that the ectopic JAM-A constructs localize to cell-cell contacts at comparable levels. Scale bars: 10 µm. (**D**) Knockdown efficiency of α3 integrin. Western blot analysis of the α3 integrin chain in LA-EGFP-expressing MDCKII cells after transient transfection with a control siRNA pool (ctrl) or a canine α3 integrin-specific siRNA pool (α3 ITGN). (**E**) Knockdown efficiencies of CD151 and CD9. Quantitative RT-PCR analysis of CD151 (left) and CD9 (right) in LA-EGFP-expressing MDCKII cells after transient transfection with a control siRNA pool (ctrl KD) or canine CD151-specific siRNA pool (CD151 KD) or canine CD9-specific siRNA pool (CD9 KD). Data are presented as mean values ± SD (n = 3 independent experiments).
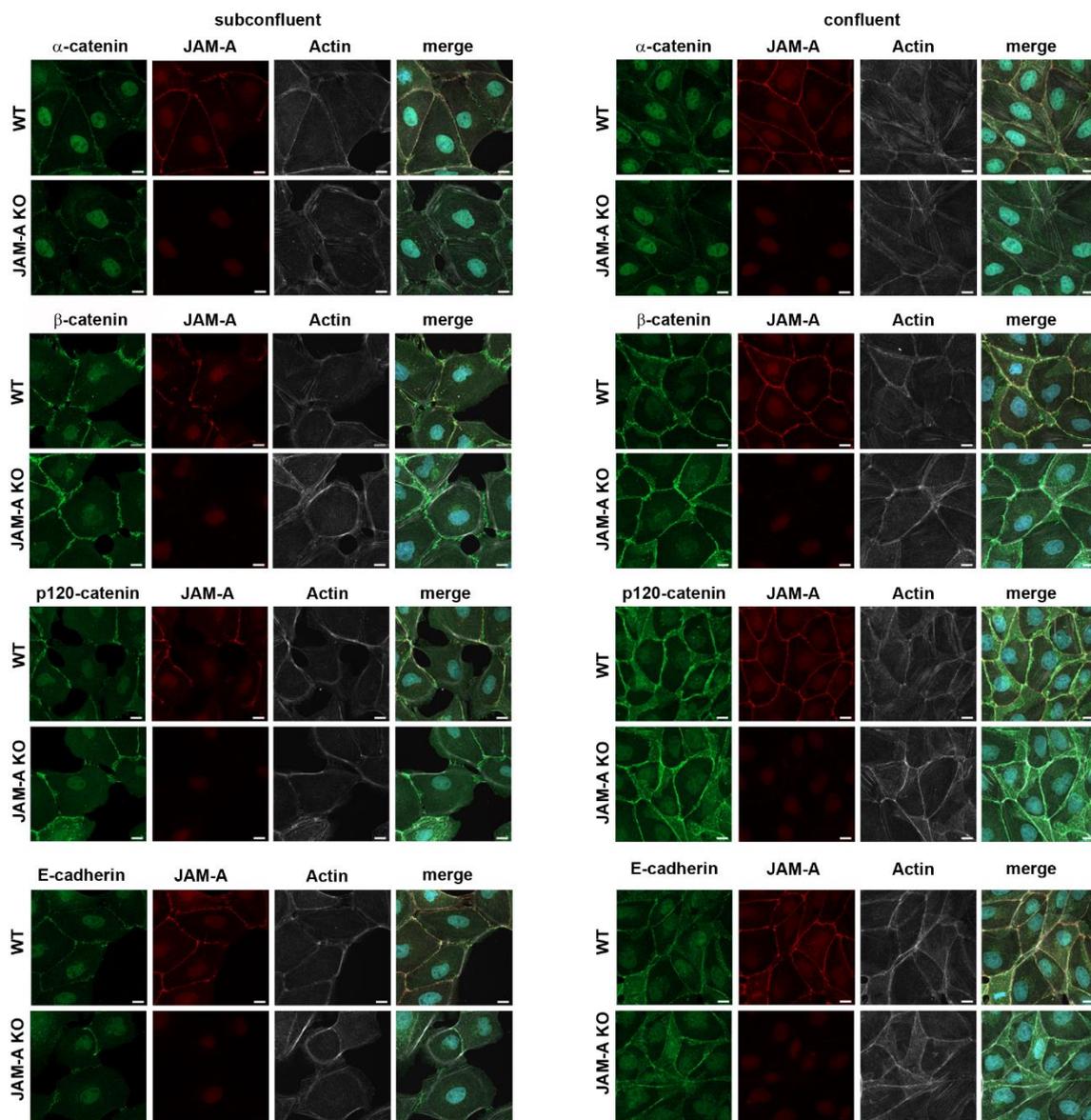
Suppl. Fig. S2: Characterization of adherens junctions proteins in JAM-A KO MDCKII cells reconstituted with mJAM-A constructs. MDCKII wildtype cells (WT) and JAM-A knockout MDCKII cells (JAM-A KO) were cultured at subconfluent conditions and confluent conditions, then fixed and stained with antibodies against α-catenin, β-catenin, p120-catenin, or E-cadherin as indicated. Note that all AJ proteins are normally localized at cell-cell contacts of JAM-A KO MDCKII cells. Scale bars: 10 µm.

A

B
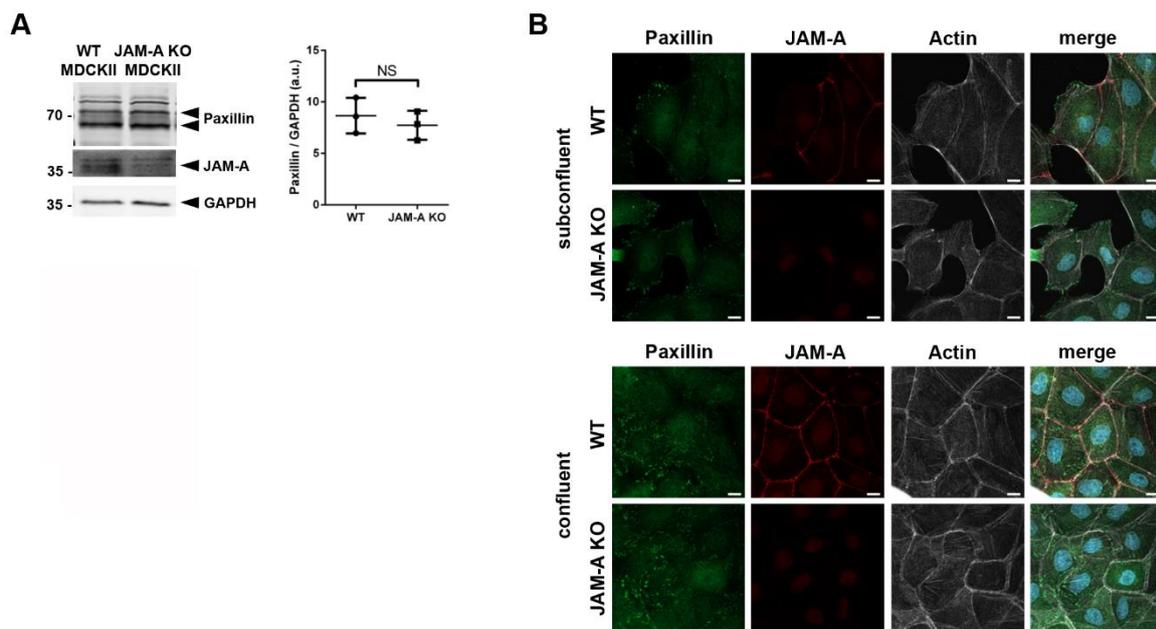
Suppl. Fig. S3: Characterization of paxillin expression and localization in JAM-A KO MDCKII cells. (**A**) Left: Western blot analysis of paxillin expression in JAM-A KO MDCKII cells. Right: Quantitative analysis of paxillin protein levels. Data are presented as mean values ± SD (n = 3 independent experiments). (**B**) IF analysis of paxillin-positive focal adhesions in JAM-A KO MDCKII cells cultured at subconfluent conditions and confluent conditions as indicated. Scale bars: 10 µm. Note that paxillin protein levels as well as paxillin-positive focal adhesions are unchanged in JAM-A KO cells.

Supplementary Movie Legends

Suppl. Movie 1 (SupplMovie1_single_cell_migration_ctrl.avi): **Single cell migration of a ctrl MDCKII cell**. Confocal laser scanning microscopy time-lapse movie of a LA-mCherry expressing MDCKII cell without induction of JAM-A shRNA expression (-Dox, ctrl). Z-stacks were taken every 10 minutes over a time-period of 10 h. Left: original video, right: analysis using the Trackmate plugin of ImageJ.

Suppl. Movie 2 (SupplMovie2_single_cell_migration_JAM-A_KD.avi): **Single cell migration of a JAM-A KD MDCKII cell**. Confocal laser scanning microscopy time-lapse movie of a LA-

EGFP expressing MDCKII cell with induction of JAM-A shRNA expression (+Dox, JAM-A KD). Z-stacks were taken every 10 minutes over a time-period of 10 h. Left: original video, right: analysis using the Trackmate plugin of ImageJ.

Suppl. Movie 3 (SupplMovie3_cryptic_lamellipodia_ctrl.avi): **Cryptic lamellipodia in ctrl MDCKII cells.** Confocal laser scanning microscopy time-lapse movie of mixed LA-EGFP and LA-mCherry expressing MDCKII cells without induction of JAM-A shRNA expression (-Dox, ctrl). Z-stacks were taken every 3 minutes over a time-period of 10 h. Left: original video with overlapping areas highlighted in yellow, right: analysis of overlapping areas by ImageJ.

Suppl. Movie 4 (SupplMovie4_cryptic_lamellipodia_JAM-A_KD.avi): **Cryptic lamellipodia in JAM-A KD MDCKII cells.** Confocal laser scanning microscopy time-lapse movie of mixed LA-EGFP and LA-mCherry expressing MDCKII cells with induction of JAM-A shRNA expression (+Dox, JAM-A KD). Z-stacks were taken every 3 minutes over a time-period of 10 h. Left: original video with overlapping areas highlighted in yellow, right: analysis of overlapping areas by ImageJ.

Suppl. Movie 5 (SupplMovie5_cryptic_lamellipodia_ctrl.avi): **Dynamic of cryptic lamellipodia in collectively migrating ctrl MDCKII cells.** Confocal laser scanning microscopy time-lapse movie of mixed LA-EGFP and LA-mCherry expressing MDCKII cells without induction of JAM-A shRNA expression (-Dox, ctrl). Z-stacks were taken every 3 minutes over a time-period of 10 h. Left: original video with overlapping areas highlighted in yellow, right: analysis of the dynamic of overlapping areas by ImageJ (increase in area shown in white, decrease in black).

Suppl. Movie 6 (SupplMovie6_cryptic_lamellipodia_JAM-A_KD.avi): **Dynamic of cryptic lamellipodia in collectively migrating JAM-A KD MDCKII cells.** Confocal laser scanning microscopy time-lapse movie of mixed LA-EGFP and LA-mCherry expressing MDCKII cells

with induction of JAM-A shRNA expression (+Dox, JAM-A KD). Z-stacks were taken every 3 minutes over a time-period of 10 h. Left: original video with overlapping areas highlighted in yellow, right: analysis of the dynamic of overlapping areas by ImageJ (increase in area shown in white, decrease in black).

Suppl. Movie 7 (SupplMovie7_single_cell_in_collective_ctrl.avi): **Migration behavior of single cells embedded in a cell collective (-Dox).** Confocal laser scanning microscopy time-lapse movie of mixed LA-EGFP and LA-mCherry expressing MDCKII cells without induction of JAM-A shRNA expression (-Dox, ctrl). Z-stacks were taken every 3 minutes over a time-period of 10 h. Left: original video, right: binary video of the single LA-mCherry expressing cell which were used to determine Jaccard indices, migration velocities and directionalities.

Suppl. Movie 8 (SupplMovie8_single_cell_in_collective_JAM-A_KD.avi): **Migration behavior of single cells embedded in a cell collective (+Dox).** Confocal laser scanning microscopy time-lapse movie of mixed LA-EGFP and LA-mCherry expressing MDCKII cells with induction of JAM-A shRNA expression (+Dox, JAM-A KD). Z-stacks were taken every 3 minutes over a time-period of 10 h. Left: original video, right: binary video of the single LA-mCherry expressing cell which were used to determine Jaccard indices, migration velocities and directionalities.