Supplementary material to the manuscript

Title: Dsg2 via Src-mediated transactivation shapes EGFR signaling towards cell adhesion

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Supplementary Methods

Generation of Dsg2 knockout in Caco2 cells by using the CRISPR/Cas9 system

Disruption of the Dsg2 gene was carried out with the CRISPR/Cas9 system. Two sgRNA targeting exon 3 and exon 13 of human Dsg2 were designed using the Chopchop-web based sgRNA design tool [1] and subcloned into the lentiviral vectors pLentiCRISPR v2 (kind gift from Feng Zhang, Addgene plasmid # 52961) and pLKO5.sgRNA.EFS.GFP (kind gift from Benjamin Ebert, Addgene plasmid # 57822) respectively. HEK293T cells were used for production of lentiviral particles employing the second generation lentiviral packaging system comprising pPAX and pMD2 packaging plasmids. Caco2 cells were infected with viral supernatant containing both viral particles, CrisprV2 and LKO-sgRNA-EFS GFP accompanied with the addition of polybrene. Infected cells were selected using Puromycin

(15µg/ml) for 1 week and positive clones were sorted for GFP-expression using FACS. Deletion of the target gene in single clones was assessed by immunoblotting and qPCR.

Table 1: Primers used for synthesis of the Dsg2 construct and used for qPCR

Primer name	*Sequence*
hDSG2-1 f	caccgCTTTGGCGCCCTTTCCGCAA
hDSG2-1 r	aaacTTGCGGAAAGGGCGCCAAAGc
hDSG2-2 f	caccgCTAAACATCCTCATTTAGTG
hDSG2-2 r	aaacCACTAAATGAGGATGTTTAGc
qPCR primer	*Sequence*
DSG2 f	Aattgcgctcatgattttgg
DSG2 r	Gcaatggcacatcagcagta

Supplementary figure legends

Fig. S1 (A) Immunostaining for Dsg2 and EGFR in confluent Caco2 cell monolayer displayed linear localization of Dsg2 and EGFR along the cell borders in Caco2 WT cells, which was not present in Dsg2 deficient Caco2 cells. Bar 10 µm (B) Triton X-100 protein extraction revealed reduced amount of EGFR in the insoluble fraction upon loss of Dsg2 in Caco2 cells. GAPDH served as loading control. (C) Band intensity of detected EGFR was analyzed from 4 independent experiments, resulting in a significant reduction of EGFR in both fractions in Dsg2-deficient Caco2 cells. Results are shown as means ± SE * $p < 0.05$ (D) Total protein level of EGFR in Caco2 cells was assessed by Western blotting resulting in reduced levels of EGFR in Dsg2-deficient Caco2 cells. GAPDH served as loading control. (E) Band intensity of detected EGFR bands was quantified from 5 independent experiments, showing a significant reduction of total EGFR protein levels upon loss of Dsg2 in Caco2 cells. Results are shown as means ± SE. * $p < 0.05$

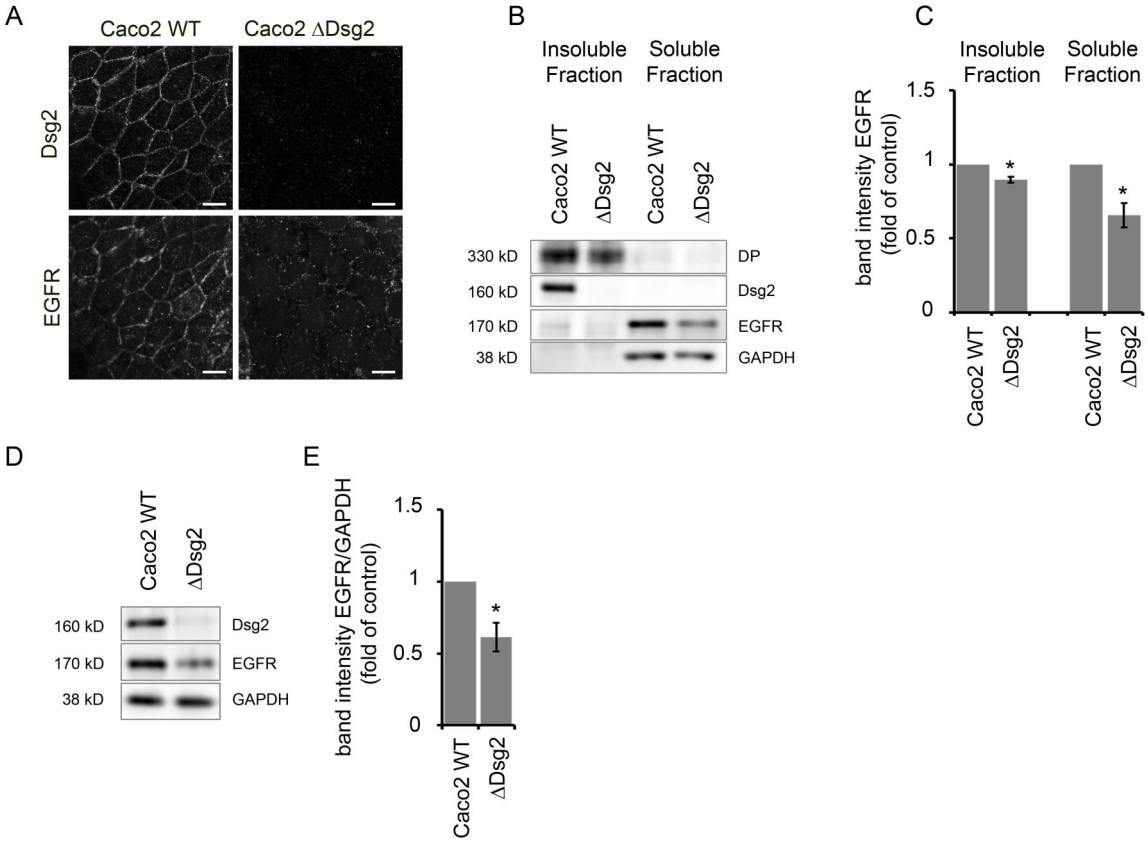
Fig. S2 (A) Phosphorylation of EGFR at Y845 in Caco2 cells was analyzed via Western blot. Reduced level of phosphorylation were observed in cells deficient for Dsg2. (B) Band intensity for detected pEGFR was quantified and normalized to total EGFR. Shown are fold change values ± SE of 6 independent experiments. * $p < 0.05$ (C) Immunostaining for Src in DLD1 cells deficient for Dsg2 and/or Dsc2 revealed similar localization of Src at cell borders in all cell lines independent of Dsg2 expression. Alexa-phalloidin was used to visualize cell borders. Bar 10 µm (D) Western blot analysis of whole cell lysates of DLD1 cells revealed unaltered protein levels of total Src as well as phosphorylated Src in all knockout cell lines. GAPDH was used as loading control. Shown is representative blot of 3 independent experiments. (E) Src is present in the TritonX-100 insoluble fraction despite loss of Dsg2 and similar in all DLD1 knockout cell lines. GAPDH was used as loading control. Shown is representative blot of 3 independent experiments.

Fig. S3 (A) Barrier recovery of Caco2 cells after Ca^{2+}-switch was monitored by measuring the TER showing impaired barrier recovery after inhibition of EGFR, Src and p38MAPK activity with respective inhibitors. Inhibitors were applied together with $CaCl_2$ after 1 h depletion with EGTA. (B) TER values were quantified 10 h after repletion with respective inhibitors revealing significantly reduced TER values after repletion with Erlotinib, PP2 and SB202190 compared to control repletion with respective vehicle. Shown are fold change values ± SE of 6 independent experiments. * $p < 0.05$; n.s. = not significant (C) Barrier recovery of DLD1 WT cells grown on filter inserts was analyzed via TER measurements. Test reagents were applied together with $CaCl_2$ from the apical or basolateral site of cell monolayer, which resulted in impaired barrier recovery after inhibition of apical as well as of basolateral localized EGFR. (D) TER values were quantified 8 h after repletion showing significantly decreased values in the presence of Erlotinib compared to control repletion. Shown are fold change values ± SE of 4 independent experiments. * $p < 0.05$; n.s. = not significant (E) Barrier recovery of DLD1 cells deficient for Dsg2 and Dsc2 after Ca^{2+}-switch was analyzed using TER measurements revealing impaired recovery after repletion with added inhibitors for EGFR, Src and p38MAPK activity. (F) TER values were quantified 10 h after repletion showing a significant reduction in the presence of Erlotinib, PP2 and SB202190. Shown are fold change values ± SE of 6-8 independent experiments. * $p < 0.05$; n.s. = not significant (G) Ca^{2+}-switch assay was performed with confluent cell monolayer of DLD1 WT cells

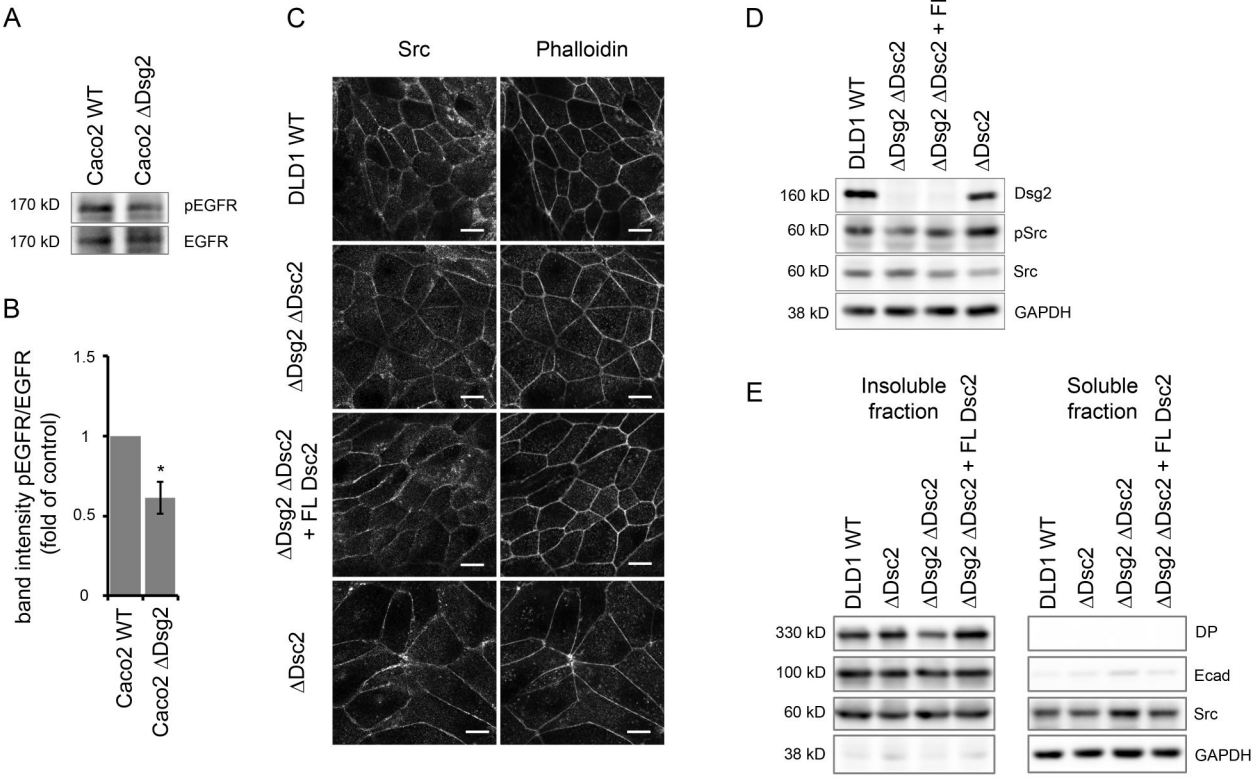
grown on coverslips and formation of TJ was assessed 2 h after repletion with several test reagents by immunostaining for Cld4. Alexa488-phalloidin was used to visualize cell borders. Bar 10 µm. (H) DLD1 double knockout cells and Caco2 Dsg2-deficient cells were transfected with Dsg2-GFP or the GFP empty vector resulting in linear localization of Dsg2-GFP along the cell borders. Bar 10 µm (I-J) Cell proliferation of Caco2 cells deficient for Dsg2 was determined by cell counting. 50000 cells were seeded and treated with Erlotinib (I) or transfected with Dsg2-GFP (J) and counted after 48 h. Shown is mean ± SE of 4 (I) and 5 (J) independent experiments. GFP-ev = empty vector, * $p < 0.05$

1. Labun, K., Montague, T.G., Gagnon, J.A., Thyme, S.B., and Valen, E. (2016). CHOPCHOP v2: a web tool for the next generation of CRISPR genome engineering. Nucleic Acids Res *44*, W272-276.

Supplemental figure 1



Supplemental figure 2



Supplemental figure 3