Cell Reports, Volume *25*

Supplemental Information

Esrp1-Regulated Splicing of Arhgef11 Isoforms Is Required for Epithelial Tight Junction Integrity

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Figure S1

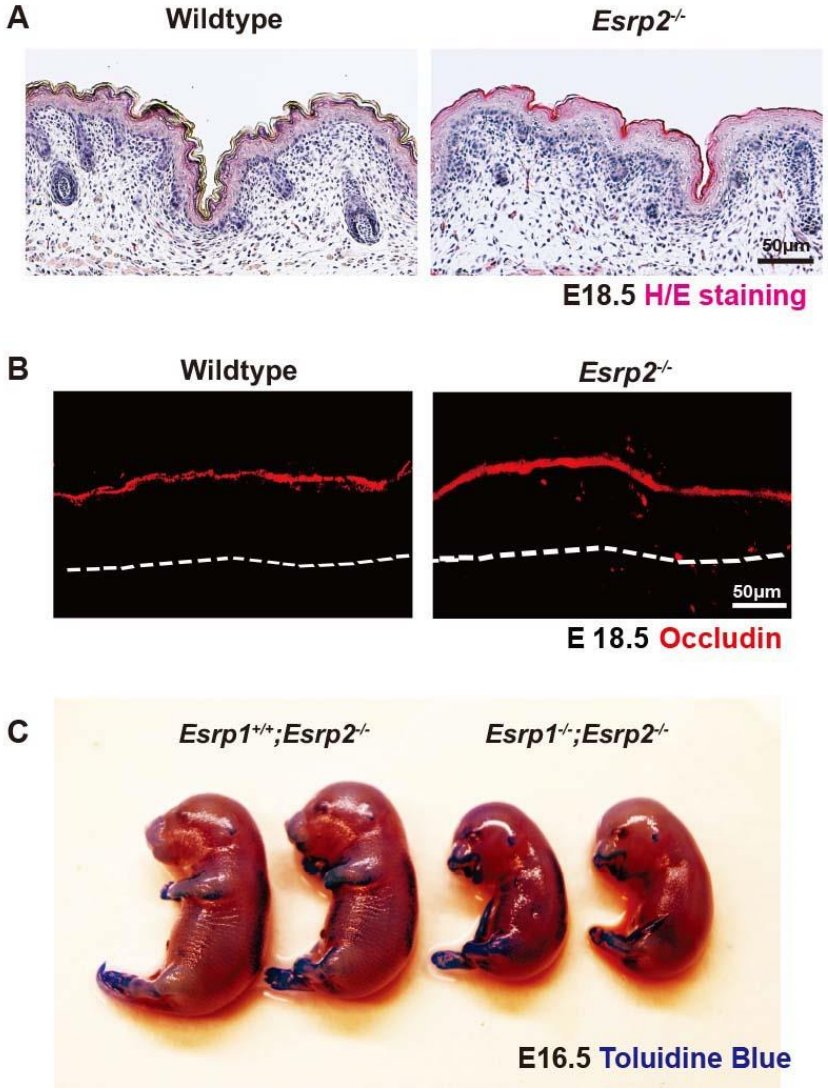

Figure S1. Absence of Epidermal defects in Esrp2$^{-/-}$ mice. Related to Figure 1. (A) H and E staining shows no difference between wildtype (*Esrp1*$^{+/+}$*;Esrp2*$^{+/-}$) and *Esrp2*$^{-/-}$ (*Esrp1*$^{+/+}$*;Esrp2*$^{-/-}$) epidermis (left panels). (B) Intact linear occludin staining in wildtype and Esrp2$^{-/-}$ epidermis (right panels). (C) *Esrp* DKO (*Esrp1*$^{-/-}$*;Esrp2*$^{-/-}$*)* epidermis show no apparent stratum corneum based barrier defect. Toluidine blue staining in E16.5 control (*Esrp1*$^{+/+}$*;Esrp2*$^{-/-}$) and Esrp DKO (*Esrp1*$^{-/-}$*;Esrp2*$^{-/-}$*)* embryos. Note the increased perioral staining is likely due to increased oral mucosal exposure due to the facial clefting defect.

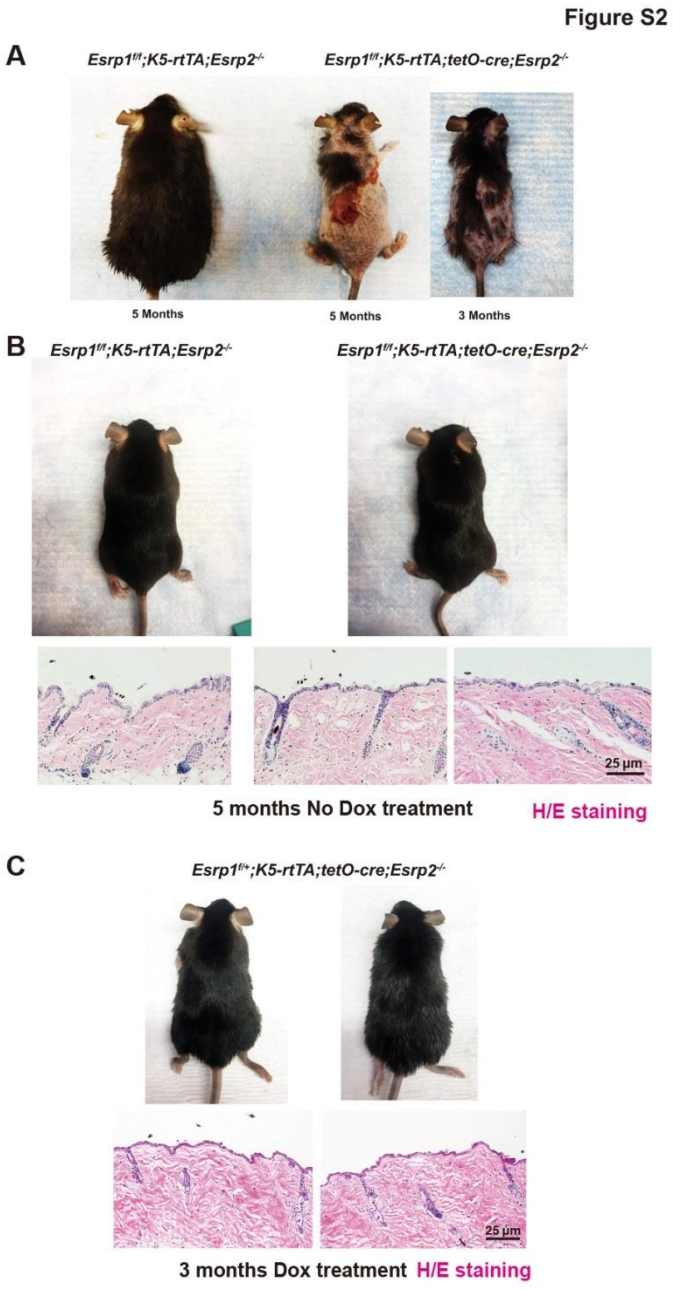

Figure S2. Hair loss and epidermal inflammation in mice with inducible ablation of Esrp1 and Esrp2. Related to Figure 2. (A) 5 month control *Esrp1$^{f/f}$; K5-rtTA; Esrp2$^{-/-}$* and 5 month or 3 month *Esrp1$^{f/f}$;Esrp2$^{-/-}$;K5-rtTA;tetO-Cre* mice**.** (B, C) Additional controls showing no epidermal defects in mice due to Cre toxicity or leaky Cre expression. Two *Esrp1$^{f/f}$; K5-rtTA;tetO-Cre;Esrp2$^{-/-}$* mice were evaluated at 5 months of age without doxycycline treatment and compared to an *Esrp1$^{f/f}$; K5-rtTA; Esrp2$^{-/-}$* control (photographic image of the second mouse *Esrp1$^{f/f}$; K5-rtTA;tetO-Cre;Esrp2$^{-/-}$* mouse not available. (C) Two *Esrp1$^{f/+}$; K5-rtTA;tetO-Cre;Esrp2$^{-/-}$* mice (containing one non-floxed wild-type Esrp1 allele) were evaluated at age 3 months after 3 months treatment with doxycycline.

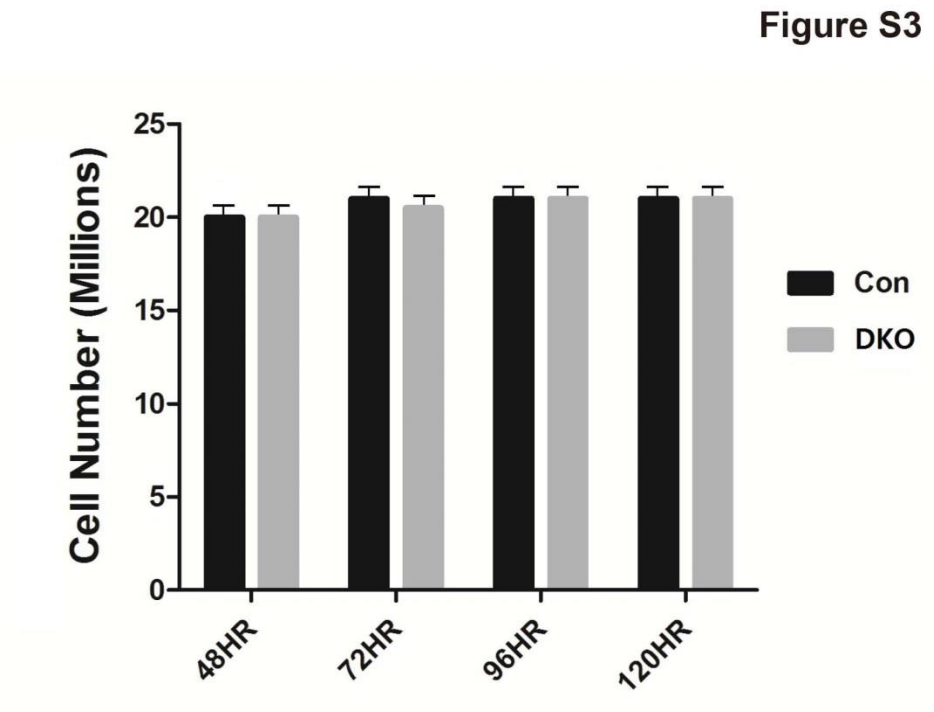

Figure S3. No apparent differences in cell numbers between control and Esrp1-/-;Esrp2-/- (DKO) Py2T cells when plated on transwells. Related to Figure 4. Py2T cells were independently plated on transwells in the presence of calcium under the same conditions used in Figure 4 D-F and harvested and counted at the indicated times.

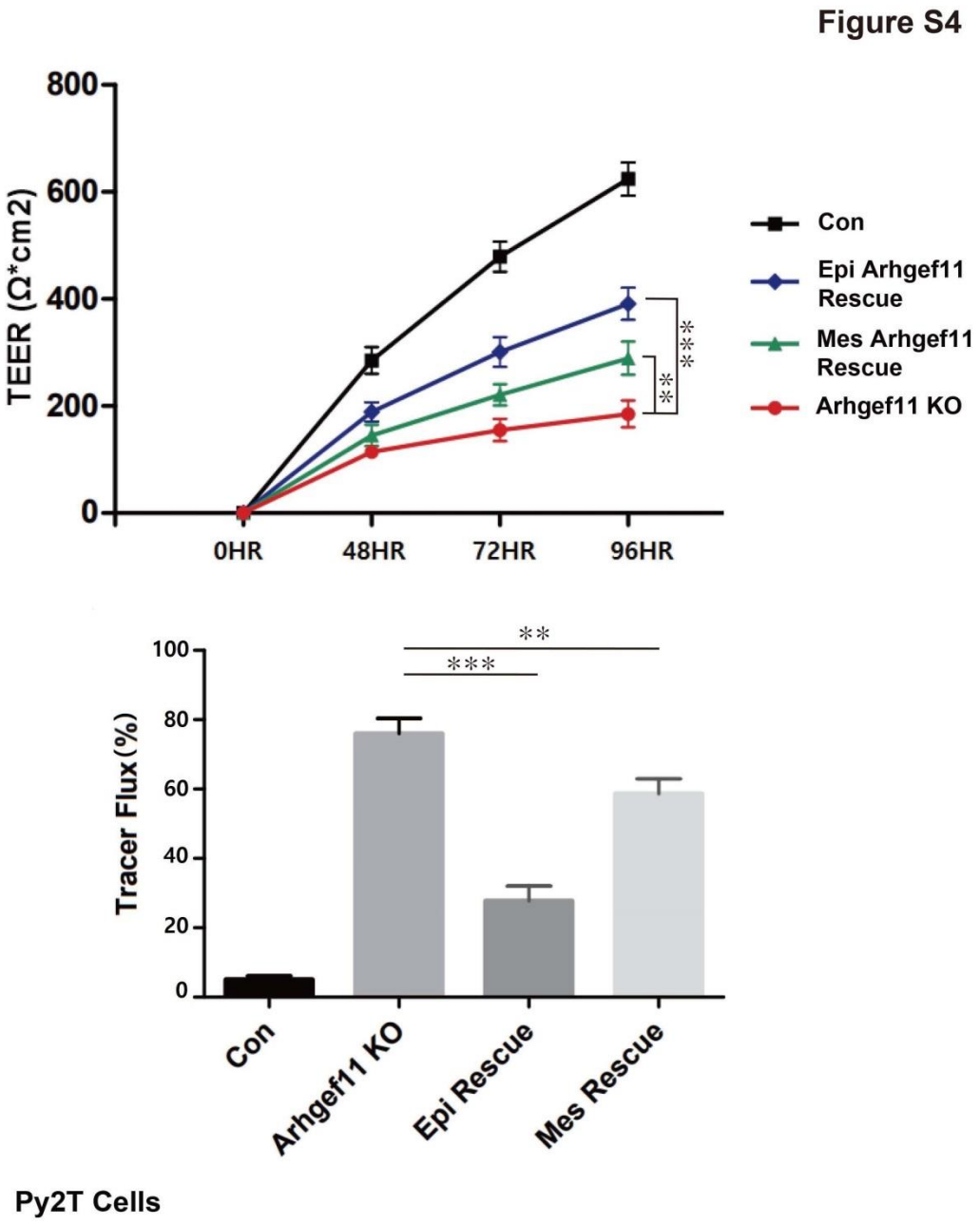

Figure S4. The alternative isoforms of mouse Arhgef11, like the human isoforms, also show preferential rescue of TEER and tracer flux with the epithelial isoform. Related to Figure 5. (A and B) TEER and FITC-dextran flux assay recovery difference between murine Arhgef11 isoforms. Error bars indicate mean ± SD. n = 3. Statistical significance comparing Epi/Mes rescue with Arhgef11 KO was determined by t-test. P <0.005; **, P<0.001; ***, P <0.0001; ****

Figure S5

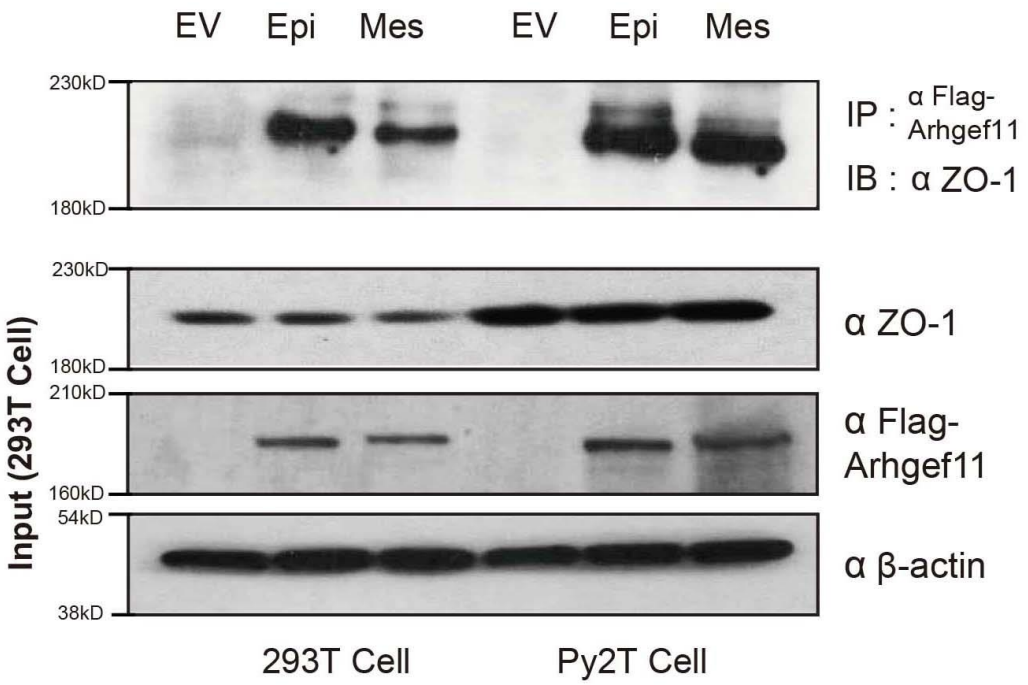

Figure S5. Stably expressed epithelial and mesenchymal ARHGEF11 isoforms both interact with ZO-1. Related to Figure 6. The indicated cells were transfected with cDNAs encoding the epithelial or mesenchymal isoforms of ARHGEF11 and pooled stably expressing cells were immunoprecipitated with anti-FLAG antibodies and blotted for ZO-1.

Figure S6

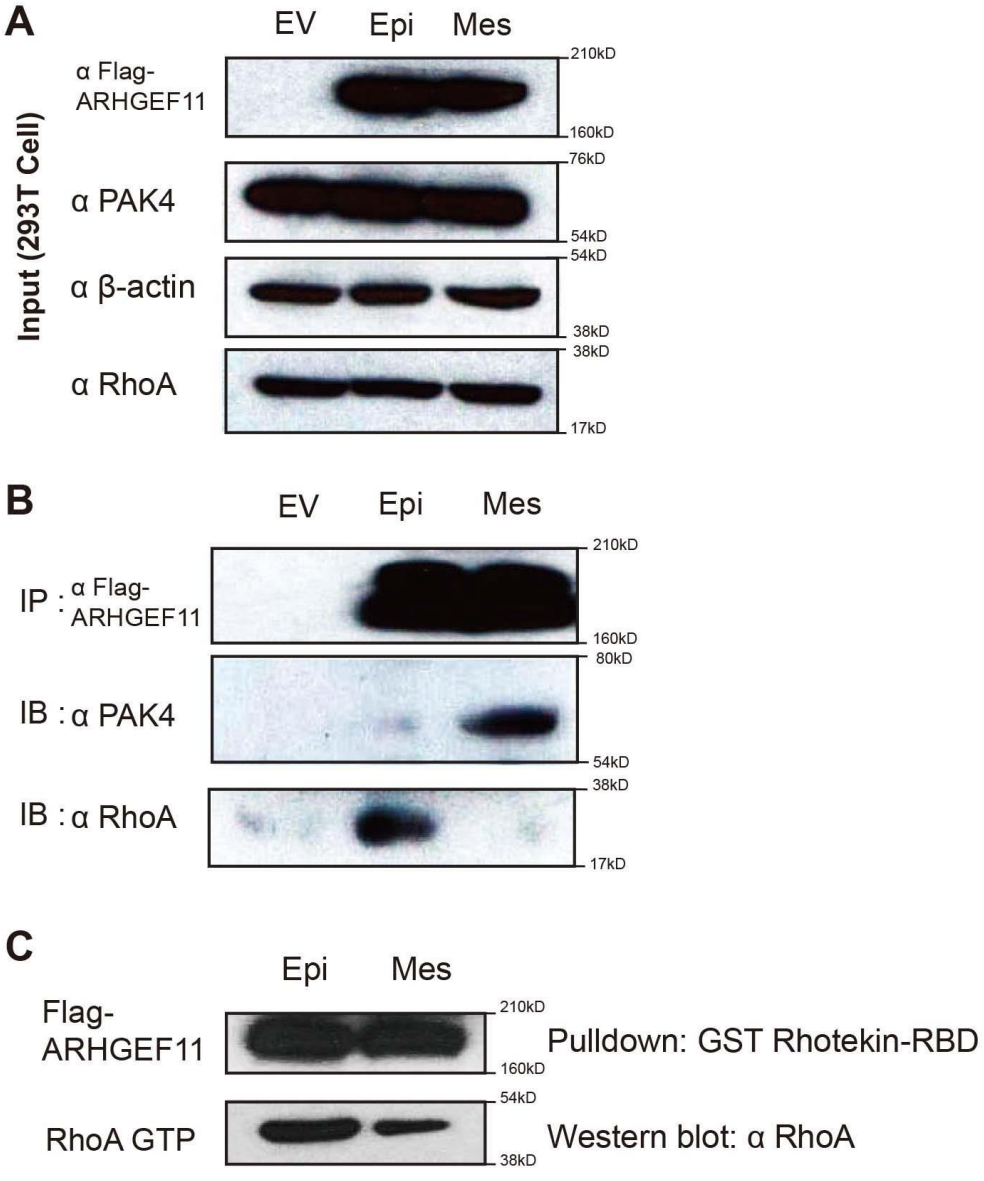

Figure S6. Differential binding of the mesenchymal isoform of ARHGEF11 to PAK4. Related to Figure 6 B, C, D). Additional replicate demonstrating the isoform-specific interaction of Arhgef11 mesenchymal isoforms with Pak4 and greater activation of RhoA by the epithelial Arhgef11 isoform than the mesenchymal isoform.

Table S1. Single-guide RNA Sequences. Related to Figures 4 and 5.

Gene	Exon Target	sgRNA Sequence	
Esrp1	Exon1	8A	GTCATGACGATAGGTGGGGA
		16A	GGCGTCTCCGGATTACTTGG
Esrp2	Exon2 and 3	guide 40	GCTCTCATTTGGCCTAGAGG
		guide 43	ACCCATCAAAATTAAGGTGG
Arhgef11	Exon2	Up crRNA1	GTATGTCTGCAGAATATCAG
		Down crRNA1	TGGGACTGGAGACTGCACCC

Table S2. PCR primer sequences used to confirm CRISPR/Cas9 mediated KO. Related to Figures 4 and 5.

Gene	Primer sequence	
Esrp1	Forward	AGGGTTTTAGCACAGGTTTTCTCG
	Reverse	GACAGGTTTTCGGCGTCTAT
Esrp2	Forward	ATACAGCCCATACGGCATCA
	Reverse	CTGCGAGTGGCCAAGTATGA
Arhgef11	Forward	GTCCTGCCGTTGTCTCTTTGTT
	Reverse	GTATGCCACTGTCTCTGCTGTA

Table S3. shRNA sequences used for Esrp1 or Esrp2 knock down experiments. Related to Figure 1.

Gene	**Sequence**	
Control	Forward	CCGGCAACAAGATGAAGAGCACCAACTCGAGTTGGTGCCTTCATCTTGTTGTTTTTG
	Reverse	AATTCAAAAACAACAAGATGAAGGCACCAACTCGAGTTGGTGCTCTTCATCTTGTTG
Esrp1 (RC1)	Forward	CCGGCCTACCGAAGCTGCCATTTATCTCGAGATAAATGGCAGCTTCGGTAGGTTTTTG
	Reverse	AATTCAAAAACCTACCGAAGCTGCCATTTATCTCGAGATAAATGGCAGCTTCGGTAGG
Esrp1 (RC5)	Forward	CCGGGAGTGCACATGGTATTGAATCCTCGAGGATTCAATACCATGTGCACTCTTTTTG
	Reverse	AATTCAAAAAGAGTGCACATGGTATTGAATCCTCGAGGATTCAATACCATGTGCACTC
Esrp2	Forward	CCGGCTTCTTTATGGCTCGTCAAAGCTCGAGCTTTGACGAGCCATAAAGAAGTTTTTG
	Reverse	AATTCAAAAACTTCTTTATGGCTCGTCAAAGCTCGAGCTTTGACGAGCCATAAAGAAG