

1 Supplementary Figures

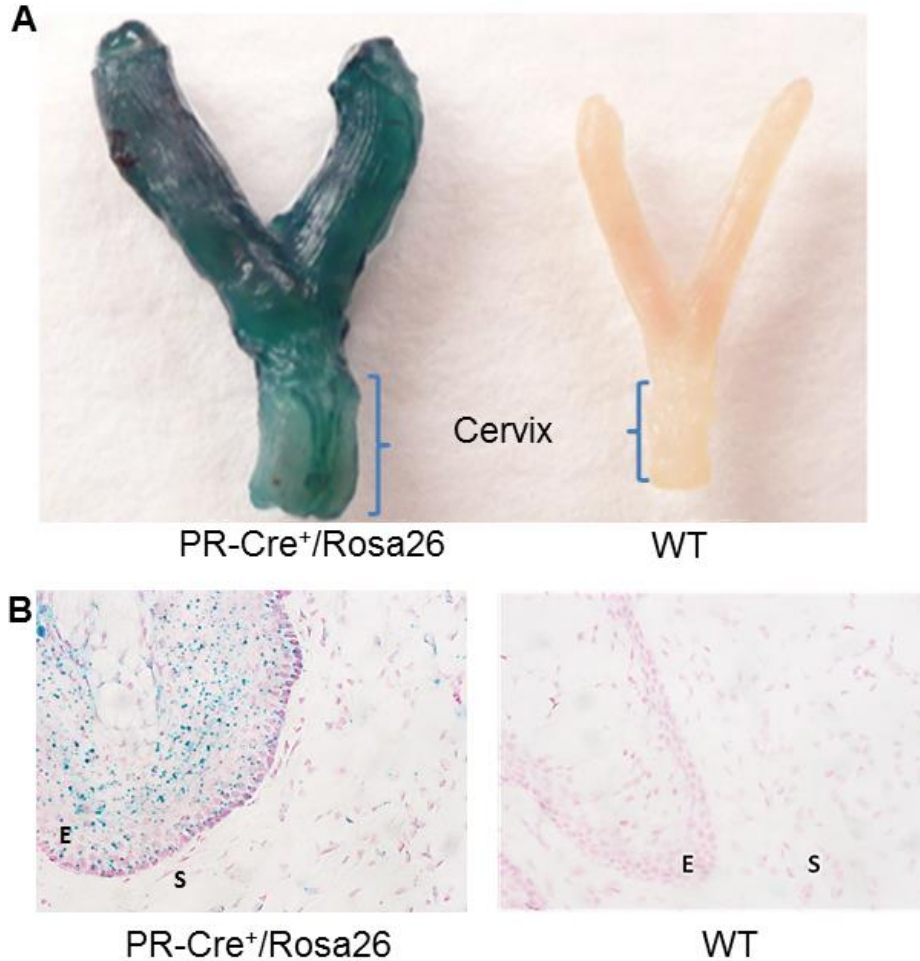


Fig. S1- (A) Progesterone receptor (PR) is expressed in uterus and cervix as evidenced in PR-Cre⁺/Rosa26 tissues stained with Cre reporter, β -Galactosidase. (B) Representative cervical sections from PR-Cre⁺/Rosa26 and WT mice were stained with β -Galactosidase (blue) and counter-stained with Nuclear Fast Red to demonstrate that PR is expressed in both cervical epithelia and stroma. E-epithelia, S-stroma. n=3

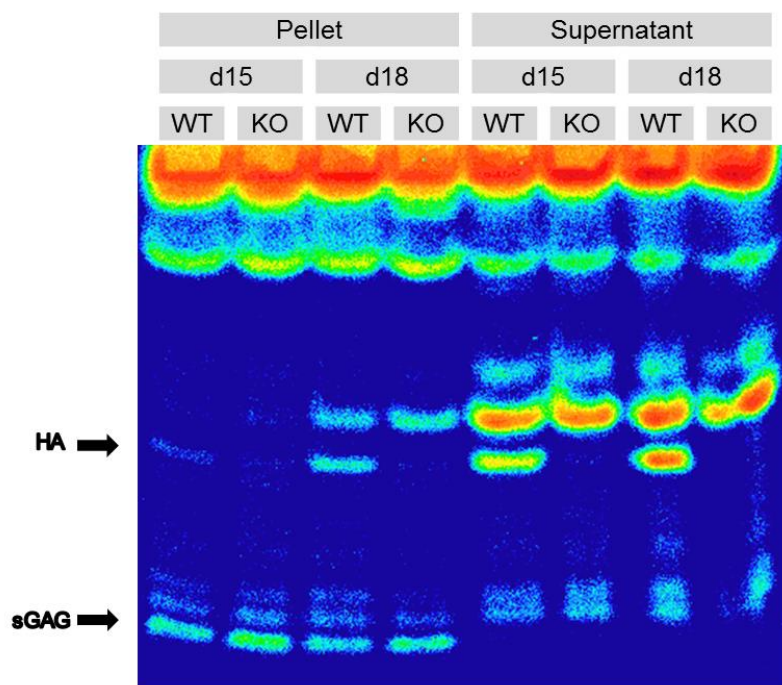


Fig. S2. Over 93% of HA is soluble and not associated with the cell fraction in WT cervixes. HA and Chondroitin/dermatan sulfate glycosaminoglycans (sGAGs) in pellet and supernatant were quantified by FACE in gestation day 15 and 18 cervixes from wild type (WT) and KO (*Has1/2/3* KO) mice. n= 3 mice per genotype. Data were analyzed using one-way ANOVA with pair wise multiple comparisons performed with Tukey's test for data normally distributed.

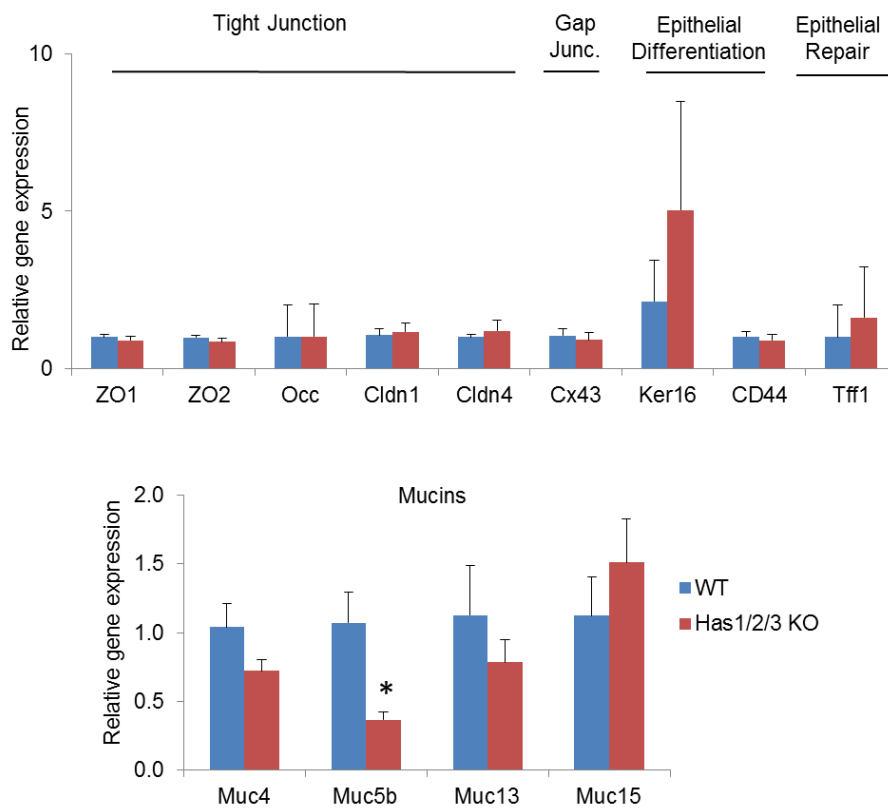


Fig. S3- Expression of epithelia related genes in gestation d18 cervix; tight junction (ZO1/2, zonula occludens 1 and 2; Occ, occludin; Cldn1/4, claudin 1 and 4), Gap junction (Cx43, connexin 43), Epithelial differentiation (Ker16, Keratin 16 and CD44), Epithelial repair (TFF1, Trefoil factor 1) and mucins (Muc4, 5b and 13 and 15). Error bars represent SEM. * $p < 0.05$, $n = 4$ per genotype. Data were analyzed using one-way ANOVA with pair wise multiple comparisons performed with Tukey's test for data normally distributed.

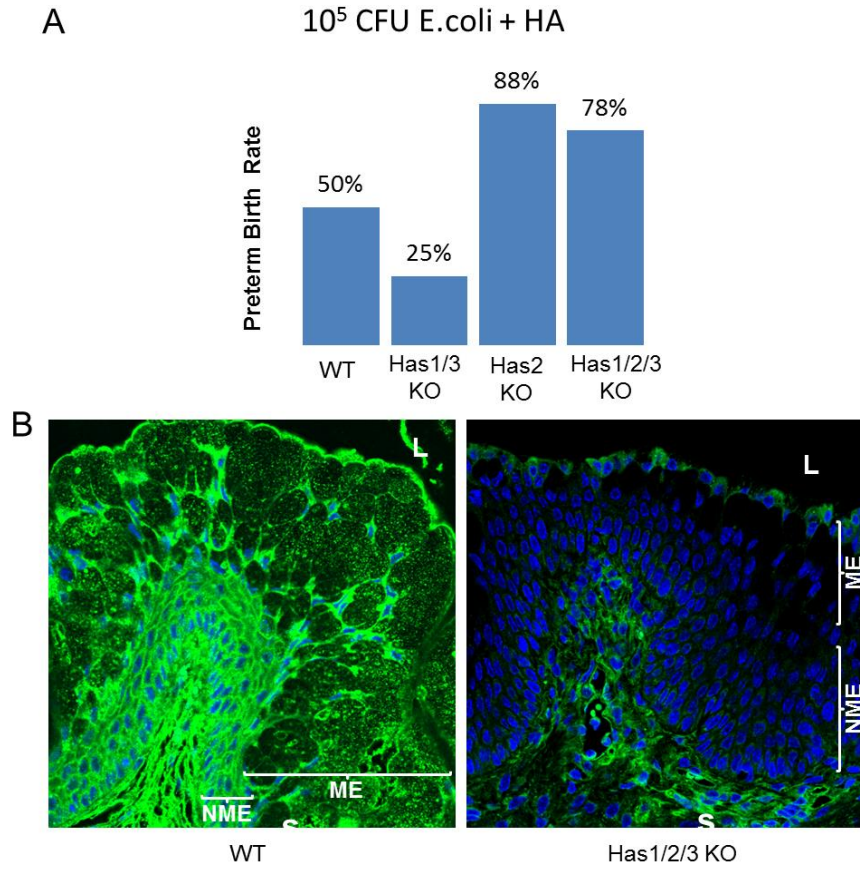


Fig. S4- (A) Effect of daily vaginal HA treatment on E.coli induced PTB rates. Mice were treated daily HA (100 μ g/mouse) from d12 to d17. Live E.coli (10^5 CFU) was inoculated into vagina on the morning of d16 and observed 48 h for preterm delivery. Differences in preterm birth rates between genotypes were analyzed using Fisher Exact test. (B) Effectiveness of HA uptake by the cervical tissue assessed by immunofluorescence staining of HA (green) 48 h following E.coli exposure and 14 h after d17 HA treatment (d18). Sections were counterstained against DAPI (Blue). N=4-9. S, Stroma; ME, mucosal epithelia; NME, epithelia with no mucus-laden vacuoles; M, mucus and L, Cervico-vaginal Lumen.

2 Supplementary Tables

Table S1- Wet cervical weight, pubic symphysis length and litter size are unaffected in *Has1/2/3* KO mice

	Cervix weight (mg)	% Cervical water content	PS length (mm)	Litter size
WT	37.01 ± 2.41	80.93% ± 0.42%	4.20 ± 0.20	7.17 ± 0.37
<i>Has1/2/3</i> KO	40.07 ± 1.48	80.26% ± 0.33%	5.88 ± 0.31	8.38 ± 1.16

Measurements of cervical weight and pubic symphysis length were recorded from gestation day 18 (term) mice. N=5-8 mice per genotype. Parturition and litter size was not affected by parity in all genotypes as mice were followed over 4 consecutive pregnancies (n=5 mice per genotype). Values presented with ± SEM

Table S2- Averages of cervical biomechanical data

	Wild type Non pregnant	Wild Type D18	Has1/2/3 KO D18
Max Force (N)	0.41 \pm 0.03	0.44 \pm 0.03	0.37 \pm 0.04
Max Stress (N/m ²)	54894 \pm 6315	11578 \pm 1570	9636 \pm 930
Distention at max force (mm)	3.71 \pm 0.19	8.71 \pm 0.73	11.44 \pm 1.62
Strain at max force	3.71 \pm 0.19	8.50 \pm 0.87	9.96 \pm 1.62
Stiffness (N/m ²)	22343 \pm 3043	2231 \pm 269	1491 \pm 274
length change	3.35 \pm 0.24	8.71 \pm 0.66	10.71 \pm 1.31

Values presented with \pm SEM

Table S3- List of genes evaluated with Tight Junction Pathway PCR Array (QIAGEN)

Focal Adhesions: Cav1, Cav2, Cav3, Itga1, Itga2, Itga3, Itga4, Itga5, Itga6, Itga7, Itga8, Itga9, Itgal, Itgam, Itgav, Itgb1, Itgb2, Itgb3, Itgb4, Itgb5, Itgb6.

Tight Junctions: Cldn1, Cldn10, Cldn11, Cldn12, Cldn14, Cldn15, Cldn16, Cldn17, Cldn18, Cldn19, Cldn2, Cldn3, Cldn4, Cldn5, Cldn6, Cldn7, Cldn8, Cldn9, Esam, F11r, Icam1, Icam2, Jam2, Jam3, Ocln, Tjp1, Tjp2, Tjp3.

Gap Junctions: Gja1, Gja3, Gja4, Gja5, Gja8, Gjb1, Gjb2, Gjb3, Gjb4, Gjb5, Gjb6, Gjc2, Gjd2, Gje1.

Adherens Junctions: Cdh1, Cdh2, Dll1, Notch1, Notch2, Notch3, Notch4, Pvrl1, Pvrl2, Pvrl4.

Desmosomes: Dsc1, Dsc2, Dsc3, Dsg1a, Dsg2, Dsg3, Dsg4, Dsp, Jup.

Hemidesmosomes: Dst, Plec.

Underlined genes were expressed robustly (Ct values below 25) in the cervix from gestation d18 mice while other genes had low to no expression.