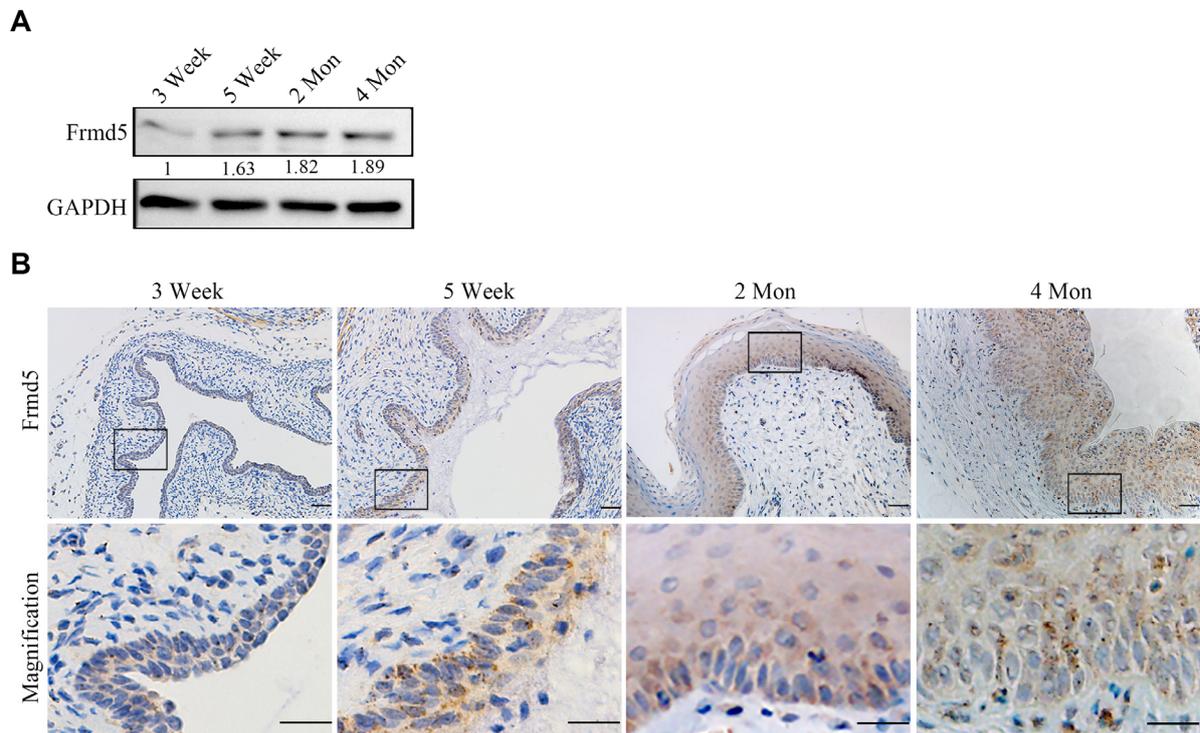


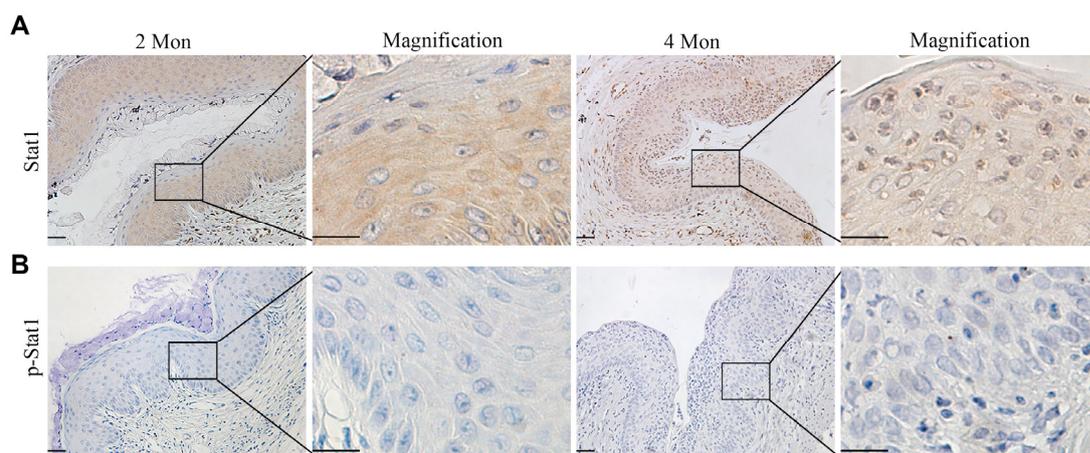
Supplementary Materials for

**Loss of Frmd5 Inhibits Jak2-Stat3 Signalling Pathway and Impairs Cell Apoptosis During  
Vagina Luminal Formation in Puberty Mice**

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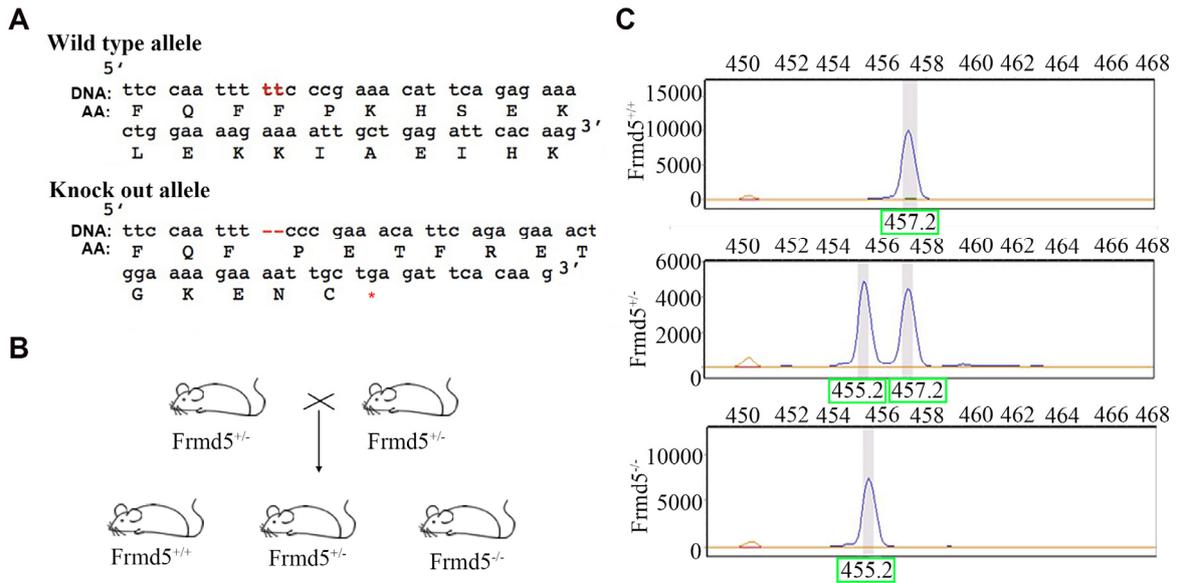


**Figure S1: Distribution of Frmd5 protein in the vagina of female mice.** (A) Western blot analysis of Frmd5 protein levels in the vagina at different developmental stages. (B) IHC detection of Frmd5 protein distribution in the vagina at different developmental stages. Brown staining indicates Frmd5-positive cells. Original image scale bar: 50  $\mu\text{m}$ ; magnified image scale bar: 20  $\mu\text{m}$ .

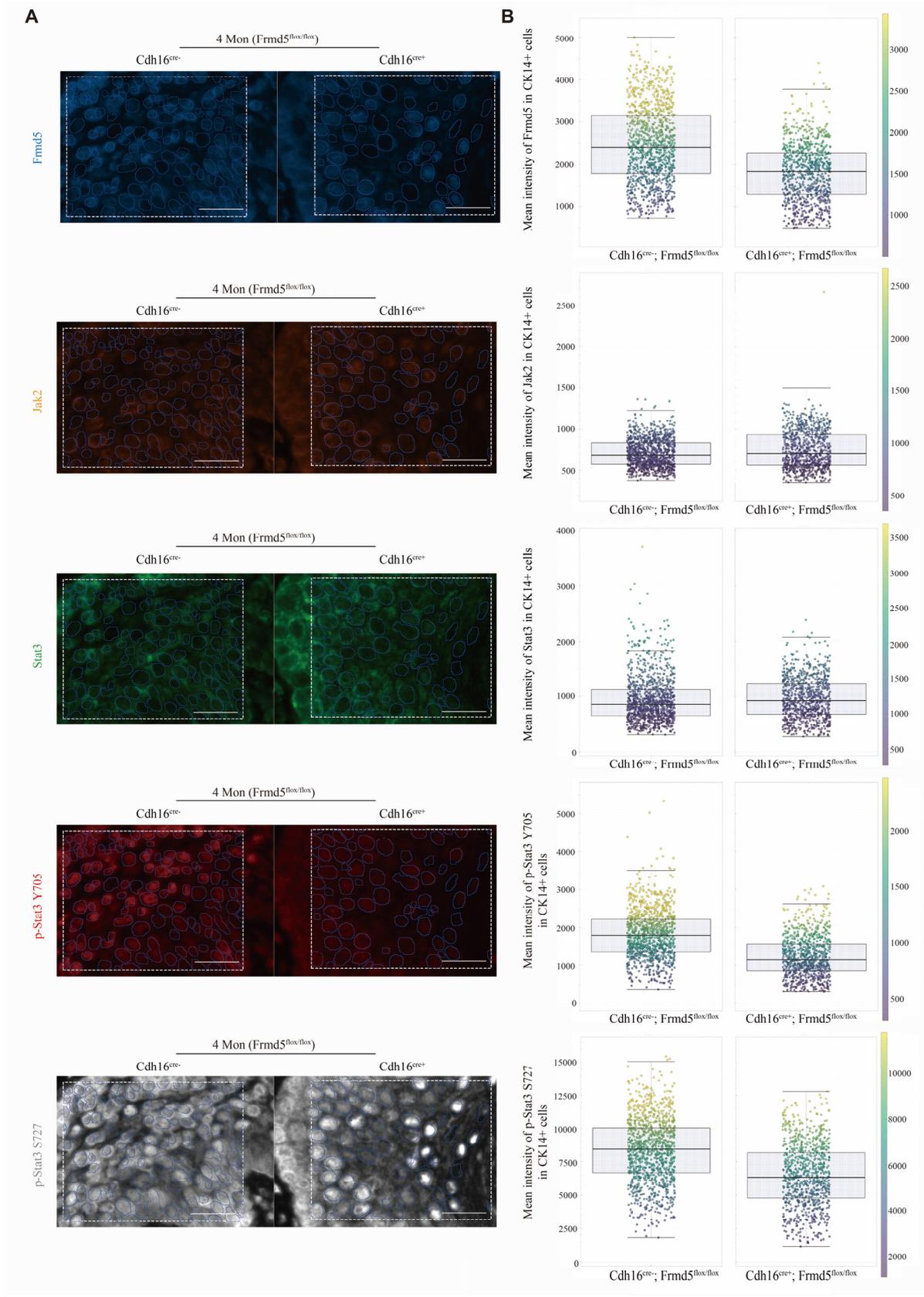


**Figure S2. Distribution of Stat1 and p-Stat1 in vaginal epithelial cells of female mice.** (A)

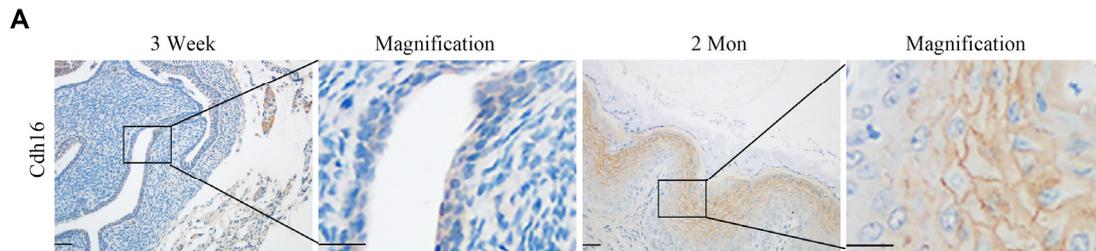
Immunohistochemical staining showing the distribution of Stat1 protein at different developmental stages, with Stat1-positive cells shown in brown. (B) Immunohistochemical staining of p-Stat1 protein at various developmental stages. Original image scale bar: 50  $\mu$ m; magnified image scale bar: 20  $\mu$ m.



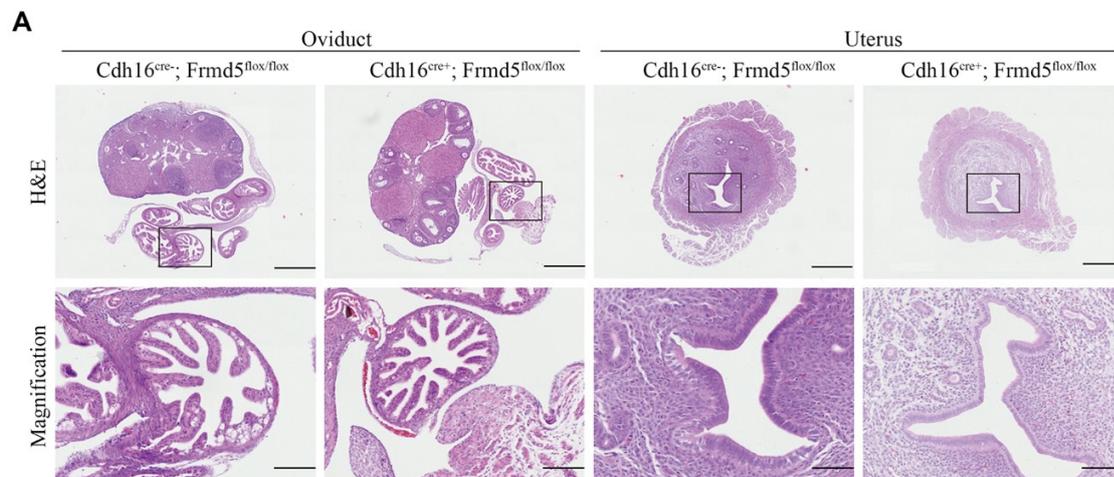
**Figure S3. Generation of the global *Frmd5* knockout mice model.** (A) Schematic representation of the gene-targeting strategy used to generate the global *Frmd5* knockout model. The knockout mice harbor a deletion of two nucleotides in the coding region, resulting in a frameshift mutation and the introduction of a premature stop codon shortly downstream, leading to premature translation termination. (B) Breeding strategy for generating the global *Frmd5* knockout mice. (C) Genotype confirmation of the global *Frmd5* knockout model using PCR and sequencing.



**Figure S4. Multiplex immunofluorescence analysis of *Frmd5*, CK14, and Jak2-Stat3 pathway-related protein distribution.** (A) Immunofluorescence images were captured using the Zeiss Axio Scan.7 system, and cell identification was performed using the Visiopharm Image Analysis System (Version: 5.0.3.1309). Dashed lines indicate the boundaries of individual cells identified by the software. (B) Fluorescence intensity analysis of all cells identified in panel A. Each point represents a single cell, with the x-axis indicating relative position of the cell and the y-axis representing fluorescence intensity.



**Figure S5. Distribution of *Cdh16* in vaginal of female mice.** (A) Immunohistochemical staining showing the distribution of *Cdh16* protein at different developmental stages, with *Cdh16*-positive cells shown in brown. Original image scale bar: 50  $\mu\text{m}$ ; magnified image scale bar: 20  $\mu\text{m}$ .



**Figure S6. Histological analysis of the oviduct and uterus in *Frmd5*-deficient female mice.** (A) H&E-stained sections of the oviduct and uterus from *Cdh16-Cre*<sup>-</sup>; *Frmd5*<sup>flox/flox</sup> and *Cdh16-Cre*<sup>+</sup>; *Frmd5*<sup>flox/flox</sup> mice. Original image scale bar: 500  $\mu\text{m}$ ; magnified image scale bar: 100  $\mu\text{m}$ .

Gene	Forward	Reverse
<i>Frmd5</i>	CCTCTGGAAATGTGGAATTGAGA	CTGGACACTGTGCGGACTT
<i>Gapdh</i>	TGGCCTTCCGTGTTCTAC	GAGTTGCTGTTGAAGTCGCA
<i>Jak2</i>	GGAATGGCCTGCCTTACAATG	TGGCTCTATCTGCTTCACAGAAT
<i>Stat3</i>	CACCTTGGATTGAGAGTCAAGAC	AGGAATCGGCTATATTGCTGGT
<i>Bcl2</i>	GCTACCGTCGTGACTTCGC	CCCCACCGAACTCAAAGAAGG
<i>Bcl-XL</i>	ACATCCCAGCTTCACATAACCC	CCATCCCGAAAGAGTTCATTCAC
<i>Casp3</i>	CTCGCTCTGGTACGGATGTG	TCCATAAATGACCCCTTCATCA
<i>Casp8</i>	TGCTTGGACTACATCCCACAC	GTTGCAGTCTAGGAAGTTGACC
<i>Xaf1</i>	TGACACGTATTCGGGATGAAAG	GTGGGAAGATCAGCCCTCTG
<i>Il1b</i>	GAAATGCCACCTTTTGACAGTG	TGGATGCTCTCATCAGGACAG
<i>Cxcl2</i>	AGGGCGGTCAAAAAGTTTGC	CGAGGCACATCAGGTACGAT
<i>Smox</i>	TCCCACGGGAATCCTATCTATC	GCCACGGTTGGTAAGGTAGC
<i>Il1rn</i>	TAGACATGGTGCCTATTGACCT	TCGTGACTATAAGGGGCTCTTC
<i>Ucp3</i>	CTGCACCGCCAGATGAGTTT	ATCATGGCTTGAAATCGGACC
<i>Ptgs1</i>	ATGAGTCGAAGGAGTCTCTCG	GCACGGATAGTAACAACAGGGA
<i>Msx2</i>	CTAAAGGCGGTGACTTGTTTTCG	CGGCTTCTTGTCGGACATGAG

Table S1. Primers for RT-qPCR in mice vagina.