CD36 regulates lipopolysaccharide-induced signaling pathways and mediates the internalization of *Escherichia coli* in cooperation with TLR4 in goat mammary gland epithelial cells

Authors:

Duoyao Cao , Jun Luo^{*} , Dekun Chen , Huifen Xu , Huaiping Shi , Xiaoqi Jing, Wenjuan Zang

*Correspondence to Jun Luo [luojun1@yahoo.com]

Tel: +86-29-87082892

Fax: +86-29-87082892

E-mail: luojun@nwsuaf.edu.cn

Affiliation: Shaanxi Key Laboratory of Molecular Biology for Agriculture, College of Anim

al Science and Technology, Northwest A & F University, Yangling, 712100, PR China

Supplementary Figure S1 (A) Milk samples were collected and tested for mastitis using LMT (LanZhou Mastitis Test, China). (B) Gram stain for identification of *Escherichia coli* from clinical mastitis.



Supplementary Table S1 Biochemical identification of Escherichia coli

	MacConkey medium	Eosin and methylene blue medium	Triple sugar iron medium		Glucose		Lactose		Maltose		Sucrose	
			acid production	aerogenesis	acid production	aerogenesis	acid production	aerogenesis	acid production	aerogenesis	acid production	aerogenesis
1	+	+	+	+	+	+	+	+	+	+	-	-
2	+	+	+	+	+	+	+	+	+	+	-	-
3	+	+	+	+	+	+	+	+	+	+	-	-

Supplementary Figure S2 (A–D) Incubation of cells in different concentrations of LPS (1, 10, 50, and 100 μ g/ml) for 12 hours. Cells were stained with Hochest33342 and PI to determine whether they induced apoptosis or necrosis. (E, F) Cells were incubated with LPS for 12

hours, and the relative mRNA levels of CD36 and TLR4 increased after stimulation. Quantitative PCR data were normalized to GAPDH, UXT, and MRPL39. Data are means \pm SEM from three experiments. ***P* < 0.01, and not significant (NS).



Supplementary Figure S3. Small interference RNA of CD36 in pGMECs efficiency test (A) The si-CD36-977 and si-CD36-1231 interference efficiency in pGMECs for 24 hours. (B) Changes of CD36 mRNA expression were influenced by knockdown CD36 expression in pGMECs for 24 and 48 hours. Quantitative PCR data were normalized to GAPDH, UXT, and MRPL39. Data are means \pm SEM from three experiments. **P < 0.01, and not significant (NS).



Supplementary Figure S4 Identification of the luciferase activity of pGL4-NF- κ B-RE (A) and pGL4-AP-1-RE (B) vector constructs in pGMECs. Luciferase activity is compared with pGL4 luciferase vectors. Data are means ± SEM from three experiments. ***P < 0.001.



Supplementary Figure S5 The efficiency of pef-NEO-FLAG-CD36 and pef-NEO-FLAG-CD36 were cotransfected in pGMECs. CD36 (A) and TLR4 (B) mRNA levels increased significantly compared with vector groups. (C) The anti-Myc and anti-Flag were used for detection Myc-TLR4 and Flag-CD36 protein levels in pGMECs. Quantitative PCR data were normalized to GAPDH, UXT, and MRPL39. Data are means \pm SEM from three experiments (***P* < 0.01). Immunoblotting was used to test the fusion protein (Flag-CD36 and Myc-TLR4) expressions.





Supplement Table S2. Quantitative PCR Primers

q-PCR Primer	Sequence				
GAPDH-F	GCAAGTTCCACGGCACAG				
GAPDH-R	GGTTCACGCCCATCACAA				
MRPL39-F	AGGTTCTCTTTTGTTGGCATCC				
MRPL39-R	TTGGTCAGAGCCCCAGAAGT				
UXT-F	TGTGGCCCTTGGATATGGTT				
UXT-R	GGTTGTCGCTGAGCTCTGTG				
IL6-F	AGATATACCTGGACTTCCT				
IL6-R	TGTTCTGATACTGCTCTG				
TLR4-F	AGATGGCAACACTTAGAA				
TLR4-R	ATACTGAAGGCTTGGTAG				
TNFa-F	TGGTTCAGACACTCAGGT				
TNFa-R	CGCTGATGTTGGCTACAA				
MYD88-F	CGGATGGTGGTGGTTGTCT				
MYD88-R	GGAACTCTTTCTTCATTGGCTTGT				
IL-1 β -F	GCGACTTCACTTTCACTT				
IL-1 β -R	TGTGTGACCCCATAGACGGTAG				
IL-8-F	AAGCTGGCTGTTGCTCTCTTG				
IL-8-R	GGGTGGAAAGGTGTGGAATG				
NF-Kb-F	TGGCAGCTCTTCTCAAAGCA				
NF-Kb-R	GACCCCTTCATCCTCTCCATC				
TGF-β-F	TTTCCGTGGGATACCGAGA				
TGF-β-R	CTGTTTGCGGGGAGAGTTG				
CD36-F	CGCCATAATTGACACATACA				
CD36-R	CTCCGAACACAGCATAGA				