

1 **RagD regulates amino acid mediated-casein synthesis and cell proliferation via mTOR**
2 **signaling in cow mammary epithelial cells**

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6 **SUPPLEMENTARY FILE**

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Supplementary materials

Western blotting analysis

The primary antibodies used in this study were shown as follow: RagD, Raptor, Deptor, PRAS40, GβL and β-actin were purchased from Santa Cruz (USA), mTOR and p-mTOR were purchased from Cell Signaling Technology (USA), Flag and Myc were purchased from Beyotime (China) and β-casein was purchased from Abbiotec (USA). The HRP-conjugated secondary antibodies were purchased from ZSGB-BIO (Beijing, China). Antibodies purchased from Santa Cruz and Abbiotec were used at 1:200 dilutions, purchased from Cell Signaling Technology and Beyotime were used at 1:1000 dilutions, and the secondary antibodies were used at 1:2000 dilutions.

Co-immunoprecipitation

About 200 mg of cell lysates were used for immunoprecipitation and incubated with anti-RPL35 antibodies. As the negative control, the equal volumes of lysates were incubated with control resin in the same way. The identity of the proteins in the immunoprecipitates was determined by WB with anti-mTOR, anti-p-mTOR, anti-Raptor, anti-Deptor, anti-PRAS40 and anti-PRAS40 GβL.

For the Co-IP used Flag antibody, CMECs were co-transfected with Raptor-Flag/RagD-Myc. Then, 200 mg of each cell lysates were used for immunoprecipitation with anti-Flag antibody. The identity of proteins in the immunoprecipitates was determined by WB with anti-Myc and anti-Flag antibodies.

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Supplementary Tables

Tab. S1. Primer sequences for qRT-PCR analysis.

Gene	Primer Sequences (5' → 3')	
Name	Forward Primer	Reverse Primer
mTOR	ATGCTGTCCTGGTCCTTATG	GGGTCAGAGAGTGGCCTT
CSN2	AACAGCCTCCCACAAAAC	AGCCATAGCCTCCTTCAC
Raptor	GACCGTCAGTGTTGCCTTAGT	GACAGAGGATCAATCCAGCAT
RagD	GGGAGTTCTGGACTTTAGCG	AACAGAGTTTCGTTGGGAGA
β-actin	AAGGACCTCTACGCCAACAC	TTTGCGGTGGACGATGGAG

Tab. S2. Primer sequences for plasmid construction.

Gene	Primer Sequences (5' → 3')	
Name	Forward Primer	Reverse Primer
Raptor	CGGAATTCATGGAGTCCGAG ATGCTGC the <i>EcoR</i> I site is underlined	ACGCGTCGACTCTGACACGCT TCTCCACCG the <i>Sal</i> I site is underlined
RagD	CGGAATTCACCACCAGGGACC CCCGTTG the <i>EcoR</i> I site is underlined	ACGCGTCGACCAGCAGCACCC TAGGCGTCCC the <i>Sal</i> I site is underlined