Augmenting Epithelial Resistance to Invading Bacteria using mRNA Transfections

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SUPPLEMENTAL INFORMATION

Quantitative Real-time Polymerase Chain Reaction Analysis. RNA was isolated using Trizol reagent (Life Technologies) and RNeasy plus Mini-kits (Qiagen, Valencia, CA). Total RNA was reverse transcribed with Superscript III (Life Technologies). Quantitative Real-time Polymerase Chain Reaction (qRT-PCR) was performed using PrimeTime pre-designed qRT-PCR assays (Hs.PT.42.1073747 for human *CAMP*, Hs.PT.42.3682141 for human *S100A8*, Hs.PT.42.3080635 for human *S100A9*, Hs.PT.45.227970.g for human *ACTB*; Integrated DNA Technologies, Coralville, Iowa). The expression levels of *CAMP*, *S100A8*, *S100A9 A8-IRES-A9* and *A8-nIRES-A9* mRNA were normalized to *ACTB* mRNA.

Immunofluorescence. At 16 h after *CAMP* or *S100A8*/*S100A9* mRNA transfection, cells were fixed with 4% paraformaldehyde for 10 min, washed with PBS (pH 7.4), and then permeabilized with 0.25% Triton X-100 for 10 min. After blocking for 1 h with 1% BSA in PBS/0.1% Tween 20 and rinsing 3X with PBS, cells were incubated with mouse anti-calprotectin (mAb 27E10, sc-33714, Santa Cruz Biotechnolology) or mouse anti-LL37 (sc-166770, Santa Cruz Biotechnolology) for 1 h, followed by Alexa Fluor 568-conjugated goat anti-mouse IgG or Alexa Fluor 488-conjugated goat anti-rabbit IgG (Life Technologies) for 1 h. For *EGFP* mRNA transfection, no antibody incubation was needed. Nuclei were stained with DAPI. Fluorescence images were captured using an epifluorescence microscope system. *A8-IRES-A9* and *A8-nIRES-A9* mRNA transfection for 40 h was also performed.

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37 **Supplementary Table 1: Oligonucleotides Probes**

Name		Oligonucleotide sequence (5'-3')[a]
Primers for fragments cloned into pGEM4Z. 2bgUTR.150A vector		
EGFP	*sense*	CCTAAGCTTGCCACCATGGTGAGCAAGGG
	antisense	ATTTGCGGCCGCTTACTTGTACAGCTCGTCCATGCCGAG
CAMP	*sense*	CCTAAGCTTGCCACCATGAAGACCCAAAGGGATGGCC
	antisense	ATTTGCGGCCGCCTAGGACTCTGTCCTGGGTACAAGATTCC
S100A8	*sense*	CCTAAGCTTGCCACCATGTTGACCGAGCTGGAGAAAGCC
	antisense	ATTTGCGGCCGCCTACTCTTTGTGGCTTTCTTCATGGC
S100A9	*sense*	CCTAAGCTTGCCACCATGACTTGCAAAATGTCGCAGCTG
	antisense	ATTTGCGGCCGCTTAGGGGGTGCCCTCCCC
A8-IRES-A9	*sense*	CCTAAGCTTGCCACCATGTTGACCGAGCTGGAGAAAGCC
	antisense	ATTTGCGGCCGCTTAGGGGGTGCCCTCCCC
Primers for fragments cloned into pIRES vector		
S100A8	*sense*	CTAGCTAGCGCCACCATGTTGACCGAGCTGGAGAAAGC
	antisense	CCGCTCGAGCTACTCTTTGTGGCTTTCTTCATGGC
S100A9	*sense*	GCTCTAGAGCCACCATGACTTGCAAAATGTCGCAGCTG
	antisense	ATTTGCGGCCGCTTAGGGGGTGCCCTCCCC

38 [a]Regions of oligonucleotide not derived from the genes/fragments are underlined.

Figure S1. CAMP protein expression after KB cells were transfected with ARCA, CE cap0 or cap1-capped *CAMP* mRNA. Upper, representative Western blot. Lower, quantitative interpretation, with ARCA cap expression at 8h arbitrarily set to 100. Intensity levels of each band are normalized to β-actin. Bars show the means ± SD of three to six independent experiments.

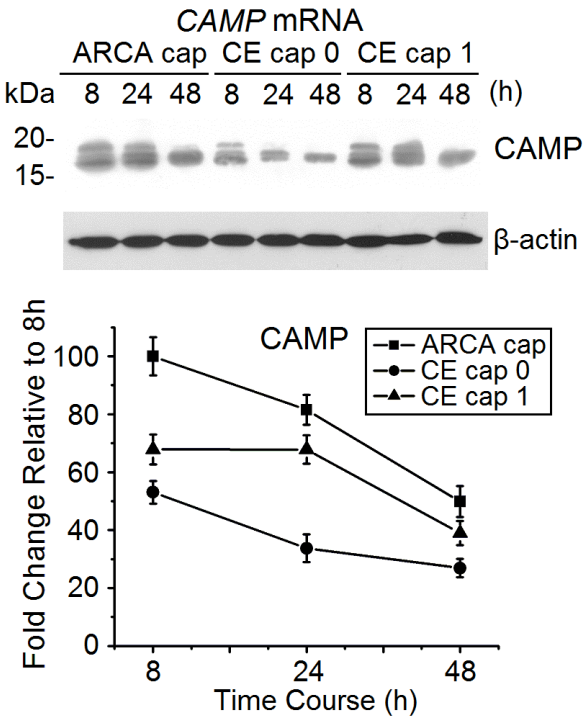

Figure S2. Real-time PCR analysis of mRNAs delivery into KB cells showing message decay. Relative quantitation of AMP mRNA was carried out as described in the Materials and Methods. **A.** *CAMP* mRNA transfection. **B.** *S100A8*/*S100A9* mRNA (mol/mol=1:1) cotransfection. **C.** *A8-IRES-A9* mRNA transfection. **D.** *A8-nIRES-A9* mRNA transfection. *A8-IRES-A9* and *A8-nIRES-A9* mRNA were detected by *S100A9* primers. mRNA abundance at 4h after mRNA transfection was assigned the arbitrary value of 100%. Bars show the means ± SD of three to six independent experiments.

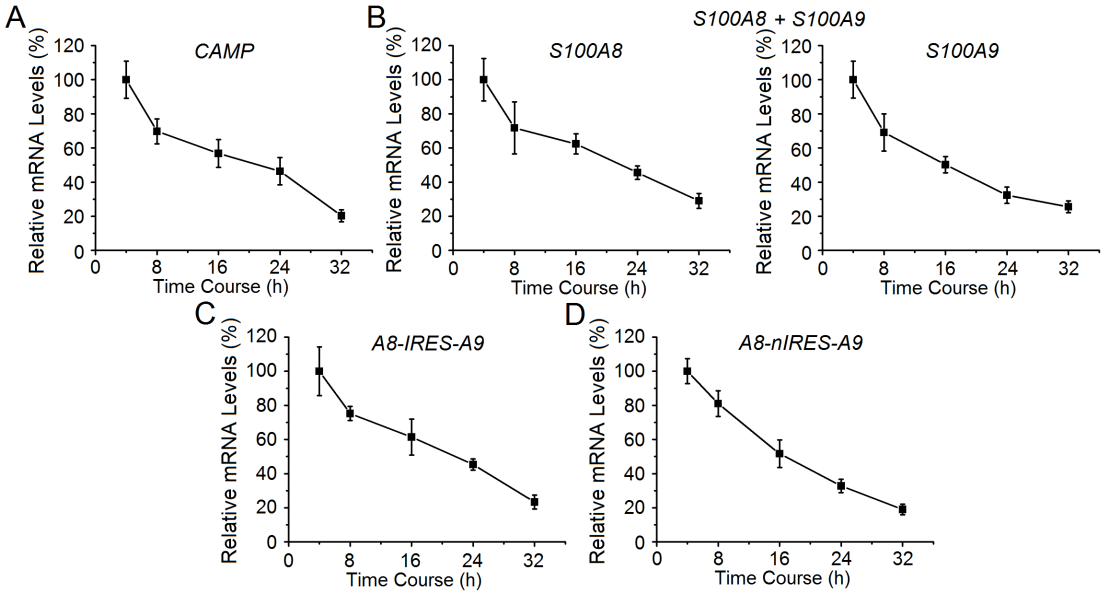

Figure S3. Immunofluorescence evidence of new protein expression after mRNA transfection. Immunofluorescent microscopy images of **(A)** *EGFP* mRNA transfection for 16h. **(B)** *CAMP* mRNA transfection for 16h. **(C)** S*100A8*/*S100A9* mRNA (mol/mol = 1:1) cotransfection for 16h, **(D)** *A8-IRES-A9* and *A8-nIRES-A9* mRNA transfection for 16h, **(E)** *A8-IRES-A9* and *A8-nIRES-A9* mRNA transfection for 40h. Vehicle, PBS. Bar = 10 μM**.** The experiments were performed three times and representative images are shown.