

Supplementary Tables

Table S1. Inflammatory gene expression relative to untreated control skins. RT-qPCR of thermally burned skin samples infected with MRSA biofilm with or without DJK-5 treatment. Statistical analysis comparing each condition (3–4 replicates) to untreated skin was performed using one-way ANOVA, Dunnett's multiple comparison test (* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$).

Gene	Fold change in gene expression (mean \pm SD)				
	DJK-5 control	Burned skin	Burned skin + DJK-5	Burned skin + MRSA biofilm	Burned skin + MRSA biofilm + DJK-5
IL-8	1.1 \pm 0.30	0.9 \pm 0.28	1.1 \pm 0.23	24.9 \pm 2.33 ****	4.2 \pm 3.50
IL-1 β	1.0 \pm 0.30	1.2 \pm 0.12	2.1 \pm 0.32	53.7 \pm 4.67 ****	10.8 \pm 1.02 ****
IL-1 α	1.2 \pm 0.17	1.0 \pm 0.48	1.5 \pm 0.26	20.3 \pm 1.69 ****	3.8 \pm 2.75 *
IL-6	0.7 \pm 0.12	0.7 \pm 0.13	1.0 \pm 0.18	1.6 \pm 0.26 **	1.3 \pm 0.31

Table S2. Human RT-qPCR Primer Sequences used in Supplementary TABLE 1.

Gene	Forward	Reverse
IL-8	GCCAGGAAGAAACCACCGGAAGG	GGCTGCCAAGAGAGCCACGG
IL-1 β	ACGAATCTCCGACCACCACT	CCATGGCCACAACAACCTGAC
IL-1 α	CGCCAATGACTCAGAGGAAGA	AGGGCGTCATTCAGGATGAA
IL-6	GGTACATCCTCGACGGCATCT	GTGCCTCTTTGCTGCTTTCAC
B2M	GATGAGTATGCCTGCCGTGTG	CAAACCTCGGGTAGCATCAT
RPLP0	CACCATTGAAATCCTGAGTGATGT	TGACCAGCCCAAGGAGAAG

Table S3. DJK-5 Suppressed Inflammatory Cytokine and Chemokine Production from MRSA Biofilm Infected Burned Skin. ELISA of MRSA biofilm infected burned skin samples with or without DJK-5 treatment. Statistical analysis comparing DJK-5 treated biofilm to MRSA biofilm infected skin (15–18 replicates) was performed using unpaired t-test (* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$).

Cytokine	Cytokine levels quantified from skin culture medium (mean \pm SD)		
	Burned skin + MRSA biofilm (pg/mL)	Burned skin + MRSA biofilm + DJK-5 (pg/mL)	Burned skin control (pg/mL)
IL-8	3796.2 \pm 1368.9	1437.6 \pm 897.7 ****	472.9 \pm 158.1
IL-1 β	665.5 \pm 216.3	77.2 \pm 33.8 ****	2.0 \pm 1.7

Table S4. Differential Gene Expression of Representative Genes in Different Biological Functions in RNA-Seq Analysis.

Function	Comparison	Burned skin + MRSA biofilm vs. Burn skin			Burned skin + MRSA biofilm + DJK-5 vs. Burned skin + MRSA biofilm	
		Gene	Fold change	Adjusted p-value	Fold change	Adjusted p-value
Inflammatory cytokines		IL-17C	803.80	2.62×10^{-12}	-24.0	2.16×10^{-4}
		IL-23A	68.30	6.42×10^{-14}	-6.2	1.47×10^{-3}
Keratins		KRT1	-4.95	5.13×10^{-6}	3.67	2.58×10^{-4}
		KRT2	-18.43	2.73×10^{-9}	9.23	7.77×10^{-6}
		KRT9	-5.36	5.22×10^{-11}	2.50	5.83×10^{-4}
		KRT10	-8.63	3.09×10^{-8}	5.48	1.65×10^{-5}
ECM proteins		LAMA1	5.97	4.45×10^{-31}	-3.75	2.48×10^{-17}
		LAMA3	5.70	6.67×10^{-18}	-3.15	2.11×10^{-8}
		ITGA2	7.22	3.16×10^{-18}	-3.48	6.35×10^{-8}
		ITGA5	14.31	2.22×10^{-32}	-7.41	1.08×10^{-18}
		ITGB4	6.60	1.01×10^{-36}	-5.00	7.89×10^{-27}
		MMP1	3.90	3.92×10^{-8}	-1.74	3.05×10^{-2}
		MMP3	12.63	1.05×10^{-14}	-11.05	4.85×10^{-13}
		MMP9	5.01	1.04×10^{-10}	-2.70	9.61×10^{-5}

DNA repair	BRCA1	-2.55	4.12×10^{-5}	3.11	8.43×10^{-7}
	BRCA2	-4.93	2.71×10^{-5}	7.12	2.75×10^{-7}
	RFC2	-2.71	1.11×10^{-14}	2.56	6.34×10^{-13}
	RFC3	-2.49	6.30×10^{-5}	3.56	2.74×10^{-8}

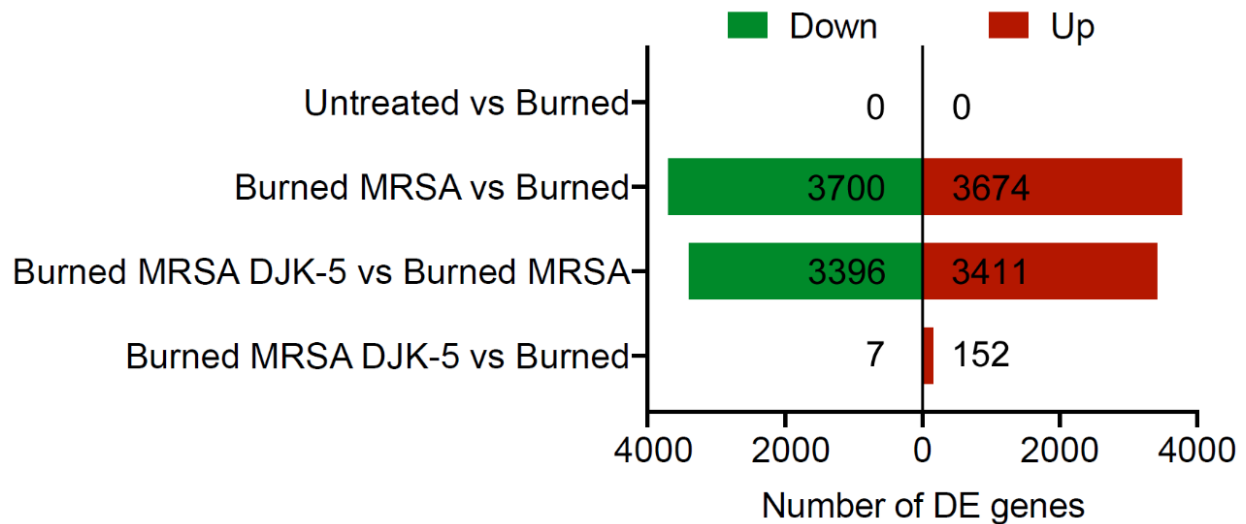


Figure S1. Differential expression analysis of MRSA biofilm infected thermally-injured skin treated with topical DJK-5. One-day MRSA USA300-LAC skin biofilms associated with thermally-damaged N/TERT epidermal skin were treated with vehicle control (water) or DJK-5 peptide at a dosage of 0.4% (120 μ g) for 24 h. RNA-Seq libraries were sequenced on a HiSeqX sequencer (Illumina). Differential expression analysis was performed using DESeq2 v1.28.1, and significance was determined based on adjusted p-value ≤ 0.05 and fold change $\geq \pm 1.5$.