Supplementary information

Electrogenic *Staphylococcus epidermidis* colonizes nasal cavities and alleviates IL-6 progression induced by SARS-CoV-2 nucleocapsid phosphoprotein

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Running Head: Nasal *S. epidermidis* reduces SARS-CoV-2-induced IL-6.

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Materials and methods:

Passive neutralization of NPP by antisera to NPP

To evaluate whether serum of mice immunized with lysates of recombinant NPP can hinder the production of IL-6 level, the macrophage cells were pre-treated with 0.5% anti-serum to NPP for 1 h prior to being treated to cells. Furthermore, pre-treatment with serum to NPP significantly lowered the levels of IL-6 in macrophage cells (**Fig. S1**). This data suggests that mice immunized with recombinant NPP produce neutralizing antibodies that can fight off the NPP induced IL-6 production.

Minimum bactericidal concentration (MBC)

To determine toxicity level of Roseoflavin (0, 1, 2, 4, 6, 8, 10 μM) pretreatment with 10^7 CFU/ml of *S. epidermidis* ATCC 12228 in a 1.5 ml Eppendorf tube for 12 h at 37°C. Bacteria samples after incubation were serially diluted $1:10^0$-$1:10^5$ in a 96 well plate after incubation. 10 μl of serially diluted bacteria were dropped on the surface of the TSB agar plate. The counting CFUs of bacteria was used for determining the number of bacteria.

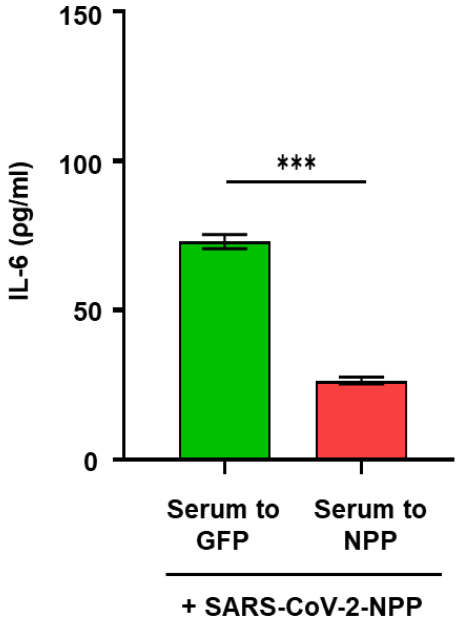

Fig. S1: Suppression of IL-6 in macrophages by anti-serum to NPP: The macrophage cells (10^5 cells/well) pre-treated with 0.5% inactivated anti-serum to GFP, or NPP. The level of pro-inflammatory IL-6 were determined. Data shown represent the mean ± SD of experiment performed in triplicate. *** $p < 0.0001$ (two-tailed t-test).

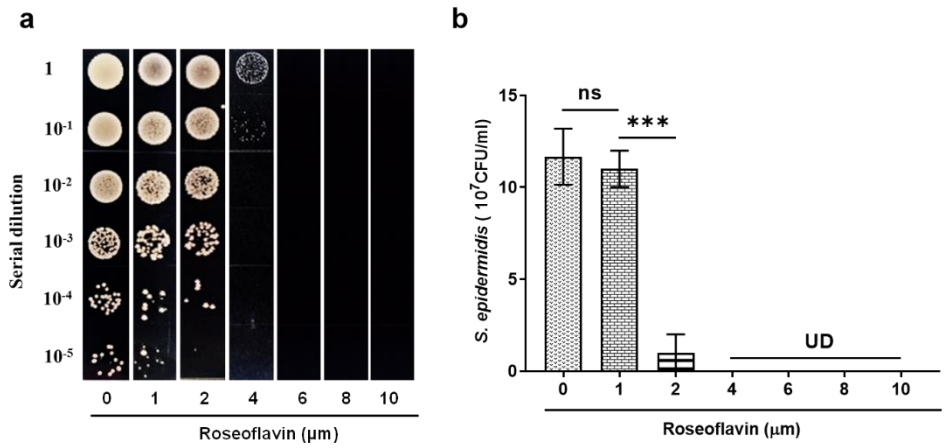

Fig. S2: Determination of antimicrobial concentration of Roseoflavin in *S. epidermidis*: *S. epidermidis* ATCC 12228 (10^7 CFU/ml) were pre-treated with 0, 1, 2, 4, 6, 8, 10 µM for 12 h. Serially diluted ($1:10^0$-$1:10^5$) bacterial culture was placed on TSB agar plates for CFU counts. Data are the mean ± SD of experiments performed in triplicate. ****p < 0.001* (two-tailed t-test). ns = non-significant.

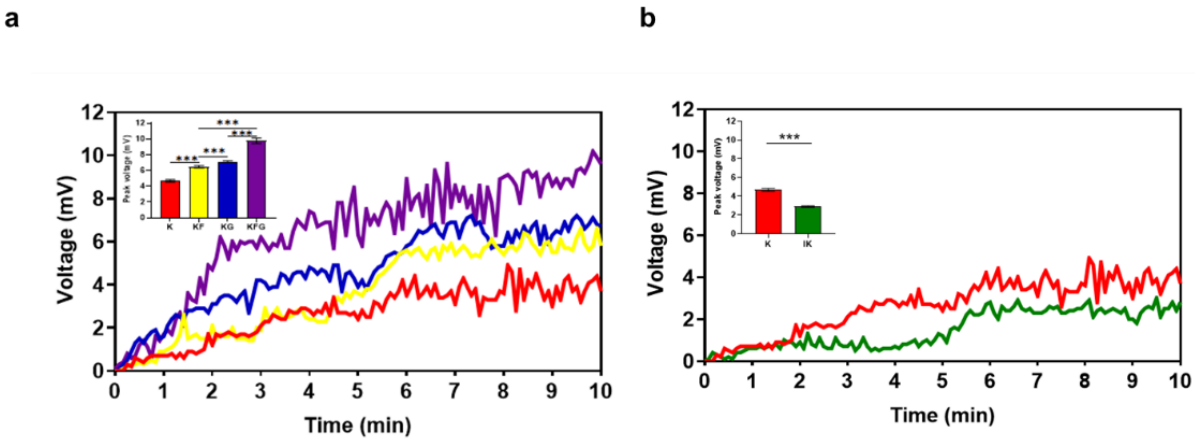

Fig. S3: Electricity production by glycerol fermentation of *S. epidermidis* K1 isolate: A) After pipetting *S. epidermidis* K1 alone (K), FMN (KF), glycerol (KG), and FMN plus glycerol (KFG) on the surface of an anode, the electricity was measured by voltage changes (mV) for 10 min. B) The *S. epidermidis* K1 isolate pre-treated with (IK) and without (K) riboflavin kinase inhibitor. Data are the mean ± SD of experiments performed in triplicate. ****p < 0.001* (two-tailed t-test). ns = non-significant.\

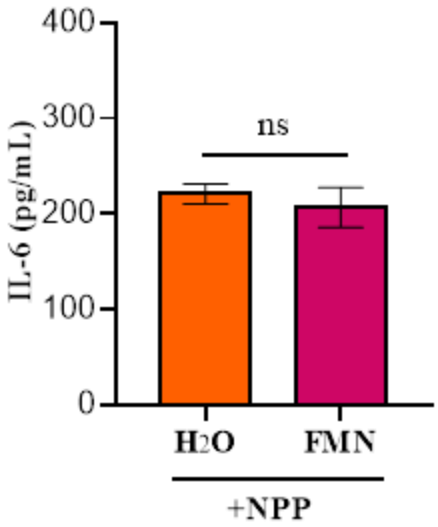

Fig. S4: Effect of FMN on IL-6 production induced by NPP in BALF of ICR mice without *S. epidermidis* colonization. 10 μM of FMN or distilled water were nasally inoculated into mice 20 minutes prior to inoculation of NPP. 6 h after inoculation of NPP, IL-6 were quantified in BALF of mice by ELISA. FMN did not induce the IL-6 production in BALF of mice 6 h after inoculation of NPP compared with distilled water inoculation. Data are represented as mean ± SD, in triplicate, two-tailed t-tests. ns = non-significant.

Supplementary tables

Table S1:

Colony name	16S rRNA sequence	Species	% Identity
K1	GGCATGGCGGCGTGCTATACATGCAGTCGAGCGAA CAGACGAGGAGCTTGCTCCTCTGACGTTAGCGGCG GACGGGTGAGTAACACGTGGATAACCTACCTATAA GACTGGGATAACTTCGGGAAACCGGAGCTAATACC GGATAATATATTGAACCGCATGGTTCAATAGTGAA AGACGGTTTTGCTGTCACTTATAGATGGATCCGCG CCGCATTAGCTAGTTGGTAAGGTAACGGCTTACCA AGGCAACGATGCGTAGCCGACCTGAGAGGGTGAT CGGCCACACTGGAACTGAGACACGGTCCAGACTCC TACGGGAGGCAGCAGTAGGGAATCTTCCGCAATG GGCGAAAGCCTGACGGAGCAACGCCGCGTGAGTG ATGAAGGTCTTCGGATCGTAAAACTCTGTTATTAG GGAAGAACAAATGTGTAAGTAACTATGCACGTCTT GACGGTACCTAATCAGAAAGCCACGGCTAACTACG TGCCAGCAGCGGGCCGGTA	*S. epidermidis*	100

Table S2:

Gene	Primer Sequence
pdh	Forward 5' CTTCTACTGATGTCGTTAATGCTTCTG 3' Reverse 5' GCAATTGCTTTGCGCATTG 3'
ndh2	Forward 5' TCTGCTAGTTGAGCACTGGG 3' Reverse 5' TGGACAGCAGGCATACAACC 3'
menA	Forward 5' CGGTAGCGAAGACCATCTGA 3' Reverse 5' ACGTACAATAGCGCCACCAA 3'
rk	Forward 5' CCAACAGCTAATGTGCAACCC 3' Forward 5' CAGCCCTAGTTGGATCGTGG 3'
16s	Forward 5' ATGCACGTCTTGACGGTACCT 3' Reverse 5' TCCATGGCAGTTCTGCACGTA 3'

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