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Supplementary Materials for

A commensal strain of *Staphylococcus epidermidis* protects against skin neoplasia

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Other Supplementary Material for this manuscript includes the following:
(available at advances.sciencemag.org/cgi/content/full/4/2/eaao4502/DC1)

- table S3 (Microsoft Excel format). List of unique marker genes that are present in *S. epidermidis* strains producing 6-HAP.

Supplementary Materials

Supplementary Data

table S1. Mutagenic activity of 6-HAP.

A. Mutagenic activity of 6-HAP on Mouse Lymphoma Assay

Agent	Mutant cell number	Total cell number	Mutant frequency (%)
Vehicle	8	402,432	0.0020
Methyl methanesulfonate (10 µg/mL)	141	352,512	0.040
6-HAP (0.25 µg/mL)	8	296,448	0.0027

Mouse lymphoma T5178Y$tk^{+/-}$ (2 x 10^5 cells/mL) were exposed to 6-HAP (0.25 µg/mL) for 24 hours. Methyl methanesulfonate (10µg/mL) was used as a positive control for this assay. After exposure, cells were incubated in normal media for 48 hours. Mutagenic activity of 6-HAP was determined by detecting mutagenic events at the thymidine kinase (*tk*) locus of L5178Y $tk^{+/-}$ mouse lymphoma cells. The raised mutant cells ($tk^{-/-}$) was screened in the presence of triflurothymidine, which allows only $tk^{-/-}$ cells grow, for 1 week.

B. Mutagenic activity of 6-HAP on Ames test

Agent	Number of positive well
Back ground	22
NaN_3 (5 μg/mL)*	95
6-HAP (1 μg/mL)	14
6-HAP (0.5 μg/mL)	13

*Served as a positive control.

To assess the mutagenic activity of 6-HAP, Ames test was performed with Muta-ChromoPlate^{TM} (environmental bio-detection products inc., Ontario, Canada) according to the manual provided. The histidine-dependent mutant of *Salmonella typhimurium* (TA100) was incubated with indicated agent in histidine-deficient media in a 96-well microtiter plate at 37°C for 4 days. The number of wells in which bacteria were grown was counted.

table S2. Systemic toxicity of repeated intravascular administration with 6-HAP in mice.

A. Complete blood cell counts in mice after intravenous injection with 6-HAP or vehicle.

		Treatment		
Parameter	Unit	6-HAP	Vehicle	No treatment
WBC	K/uL	6.17	8.41	4.59
NE#	K/uL	1.86	1.965	1.015
LY#	K/uL	3.93	5.76	3.19
MO#	K/uL	0.32	0.36	0.28
EO#	K/uL	0.06	0.24	0.08
BA#	K/uL	0.01	0.08	0.025
NE%	%	30.15	23.38	22.14
LY%	%	63.61	68.51	69.48
MO%	%	5.23	4.305	6.11
EO%	%	0.85	2.9	1.72
BA%	%	0.17	0.915	0.555
RBC	M/uL	9.24	8.69	8.87
HB	g/dL	14.70	13.6	14.15
HCT	%	50.95	49.4	49.2
MCV	fL	55.20	56.85	55.45
MCH	Pg	15.95	15.65	15.95
MCHC	g/dL	28.85	27.55	28.75
RDW	%	16.60	15.65	15.4
PLT	K/uL	756.50	451	600.5
MPV	fL	4.00	4.25	4

Data are mean from 2 mice.

B. Serum asparate aminotransferase (AST) activity in mice after intravenous injection with 6-HAP or vehicle.

Treatment		
6-HAP	Vehicle	No treatment
62.61±6.94	73.9±20.76	91.54±24.41

Data are mean±s.e.m from 3 individual mice.

C57/B6 mice received repeated intravascular administration with 6-HAP (20mg/kg) or with an equal volume of vehicle (2.5% DMSO in 0.9% NaCl) every 48 hours for 2 weeks. Forty eight hours after the final injection, whole blood or serum was collected. Whole blood was subjected to CBC test (A) and serum was subjected to AST test (B).

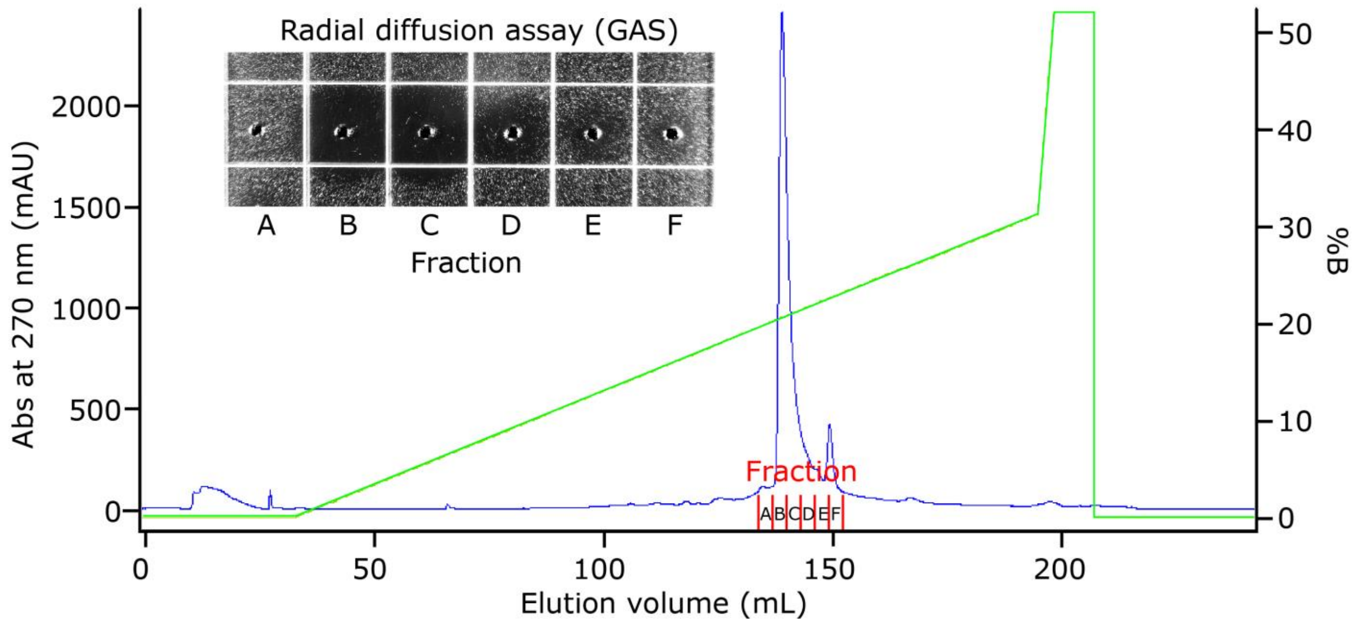

fig. S1. Purification of a unique antimicrobial compound from the culture supernatant of *S. epidermidis* strain MO34 isolated from normal human skin. Elution profile of the antimicrobial compound purified from culture supernatant of *S. epidermidis* MO34 by HPLC using a PolyHYDROXYMETHYL. The last step of 5 purification steps is shown. The insert panel represents antimicrobial activity of each fraction on radial diffusion assay against group A streptococcus. The black area represents zone of growth inhibition of bacteria. Green line represents a gradient of H_2O in acetonitrile.

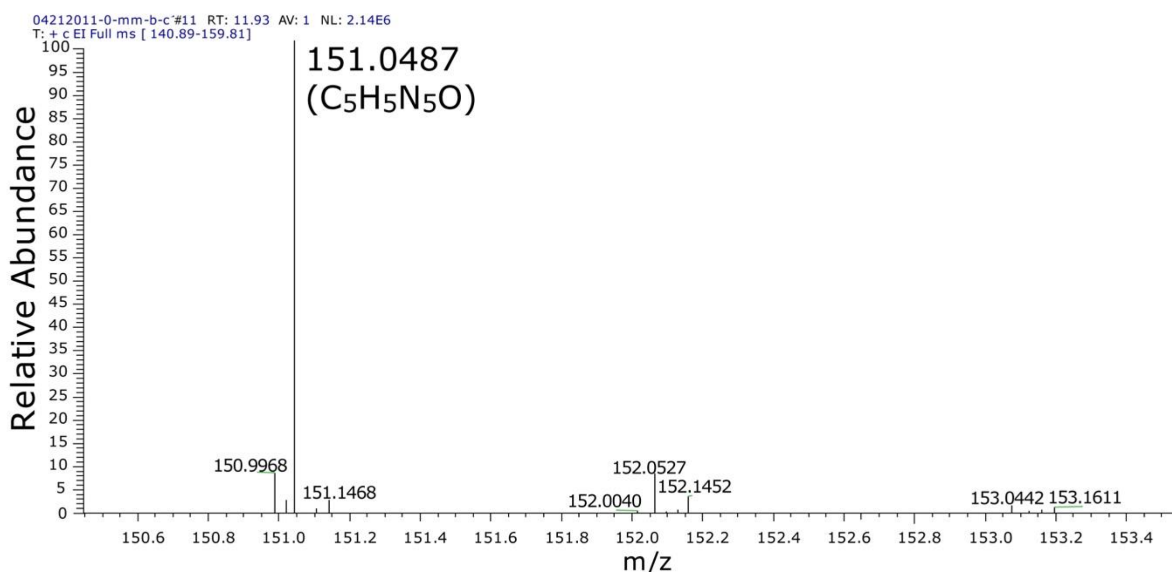

fig. S2. Molecular mass of purified antibiotic from the *S. epidermidis* MO34 strain analyzed by HR-ESI-MS. Observed molecular mass (151.0487) was identical to that of a molecular formula of $C_5H_5N_5O$ (calculated: 151.0489) .

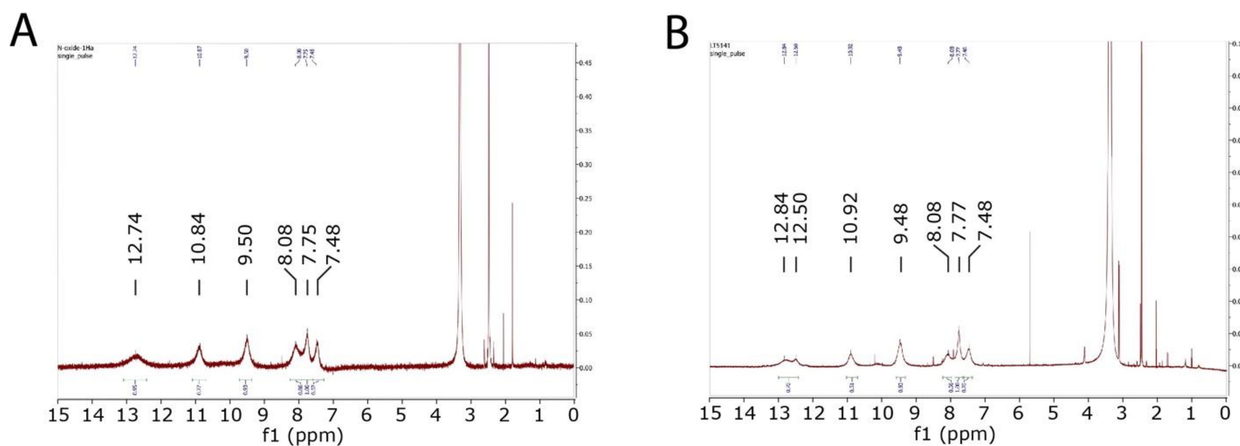

fig. S3. Comparison of chemical shifts of purified antibiotic with those of synthetic 6-HAP in 1H-NMR. (A-B) The ^{1}H NMR spectrum of both purified compound (**A**) and synthesized 6-HAP (**B**) displayed two proton signals in the aromatic region (δH = 8.19, 8.17), whereas six signals in DMSO-d5 (δH=12.74, 1H; 10.87 0.7H; 9.50, 1H; 8.08, 1H; 7.75, 1H; 7.48, 0.4H).

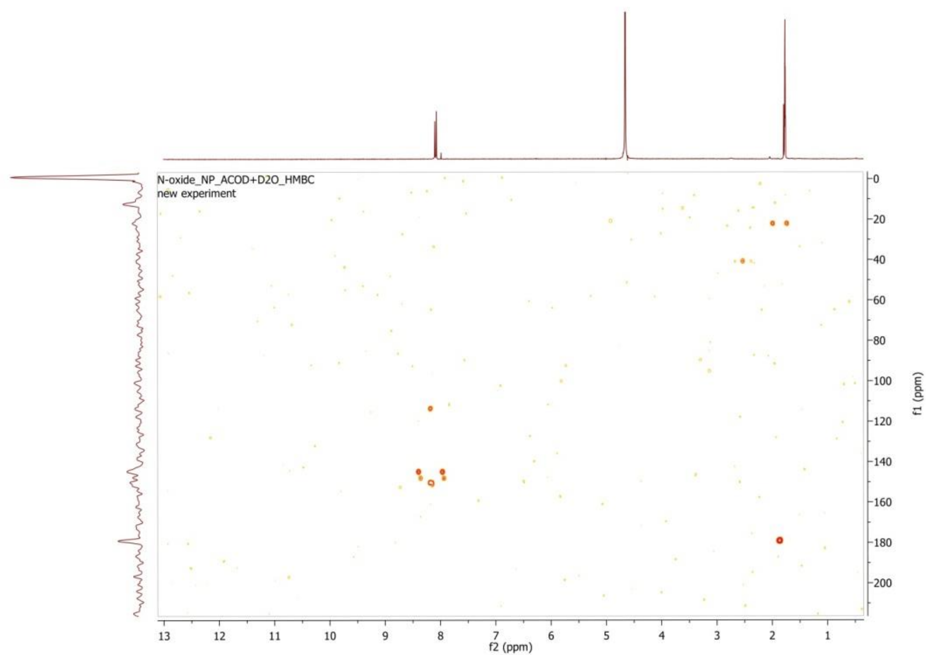

fig. S4. The gHMBC spectrum (500 MHz) of 6-HAP in AcOD-D2O. The carbon spectrum of 6-HAP was measured indirectly by the gHMBC experiment. The gHMBC spectral data was recorded on a Mercury Pluss 500 (Varian) spectrometer. FID file was processed using MestRenova 8.1 (MestreLab Research). The gHMBC spectrum of 6-HAP in AcOD-D_2O (1:5 v/v) revealed five carbon signals in the aromatic region (δ_C = 113.60, 144.94, 148.17, 150.28, 150.45).

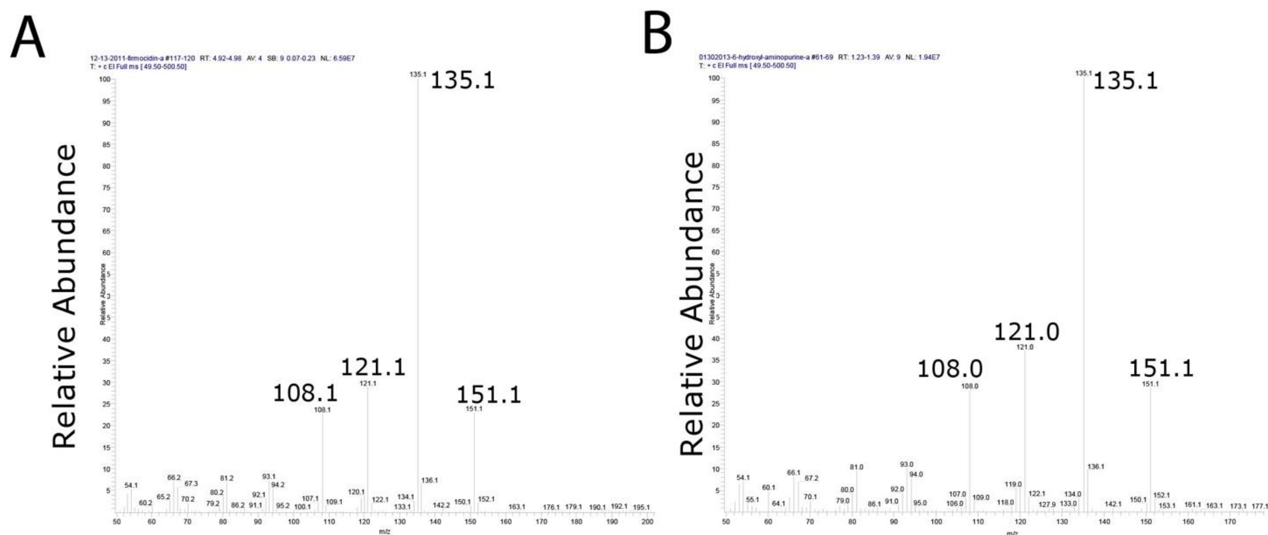

fig. S5. Comparison of the fragmentation profile of purified antibiotic with that of synthetic 6-HAP on electron impact mass spectrometry. Comparison of the fragmentation profile of purified antibiotic (**A**) with that of synthetic 6-HAP (**B**) on electron impact mass spectrometry.

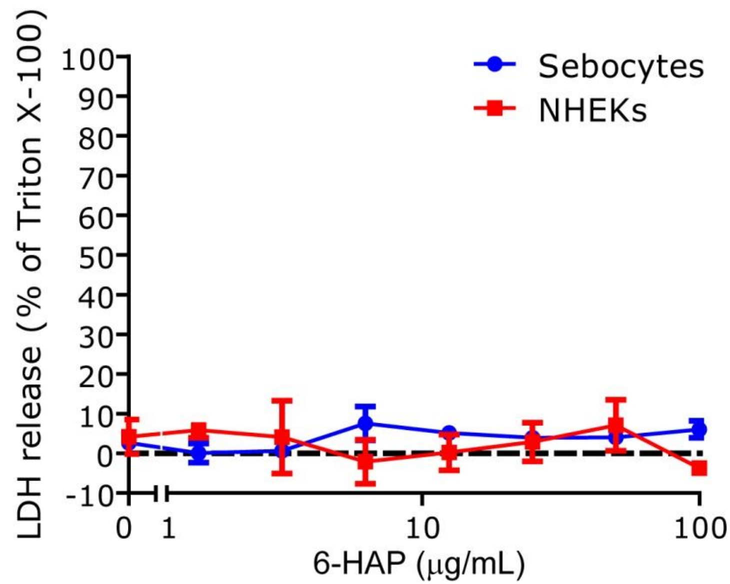

fig. S6. Capacity of 6-HAP to directly disrupt plasma membrane of human keratinocytes and sebocytes. Normal human epidermal keratinocytes (NHEKs) or immortalized human sebocytes (SZ95) (1×10^5 cells) were incubated with the indicated concentrations of 6-HAP in Epilife or Sebmed medium, respectively, at 37°C for 6 hrs. Vehicle (0.5% DMSO) or Triton X-100 (0.1%) was added to achieve 0% or 100% of LDH release, respectively. LDH release was determined with Cytotoxicity Detection Kit (LDH) (Roche, Mannheim, Germany) according to the protocol provided. Data represent mean ± s.e.m. of three individual experiments.

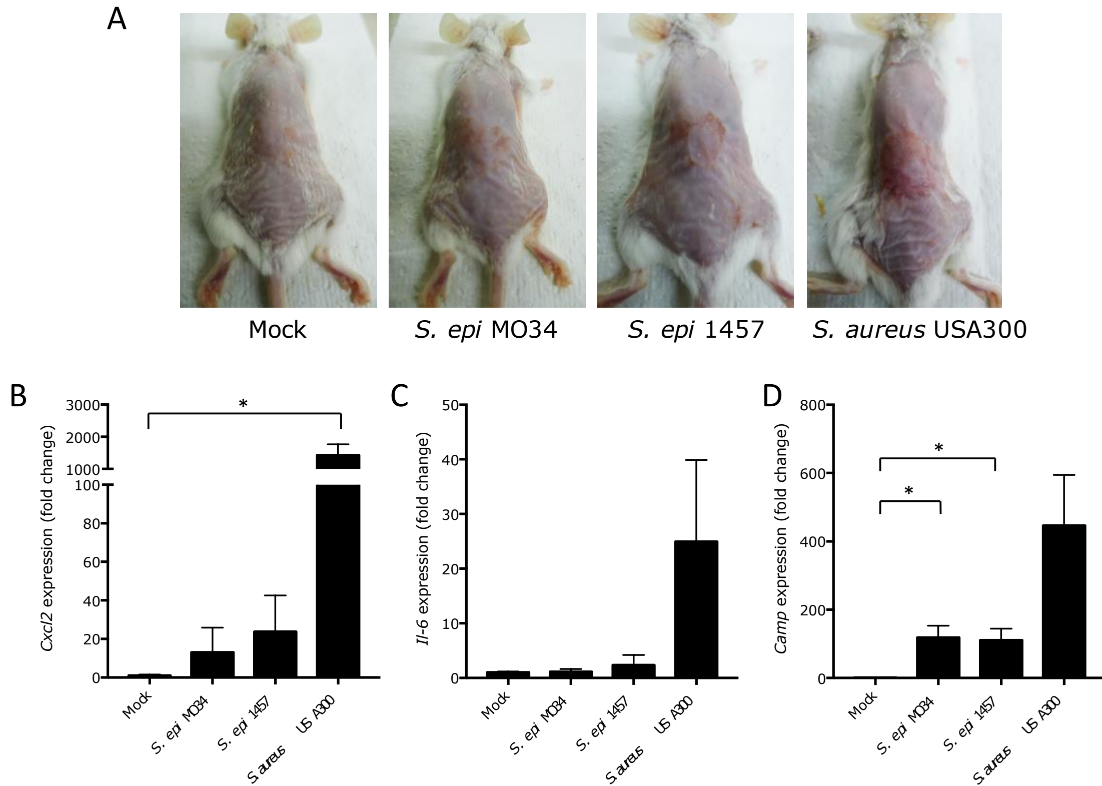

fig. S7. Effect of epicutaneous application of *S. epidermidis* strain producing 6-HAP on the cutaneous immune system in mice. Dorsal skin of Balb/c mice was shaved, treated with depilatory cream and tape stripped to disrupt the epidermal barrier. **(A)** To examine if *S. epidermidis* strain producing 6-HAP alters cutaneous immune systems, *S. epidermidis* MO34 (6-HAP strain) or *S. epidermidis* 1457 (non-6-HAP producing strain) (1×10^6 CFU) was applied with an 8 mm agar disc on the shaved back skin and covered with Tegaderm wound dressing film for 24 hours. Equal CFUs of *S. aureus* USA300 strain were served as a positive control of immune modulator and an agar disc without bacteria was served as a negative control. **(B)** Skin was harvested with a 6 mm skin biopsy and expression of indicated gene was measured with qPCR. Expression of indicated gene was normalized to that of GAPDH. Data represent mean ± s.e.m. of three (Mock) or four individual experiments (other groups) (*P<0.05 by two-tailed independent t-test).