

**Symbionts exploit complex signaling to educate the immune system**

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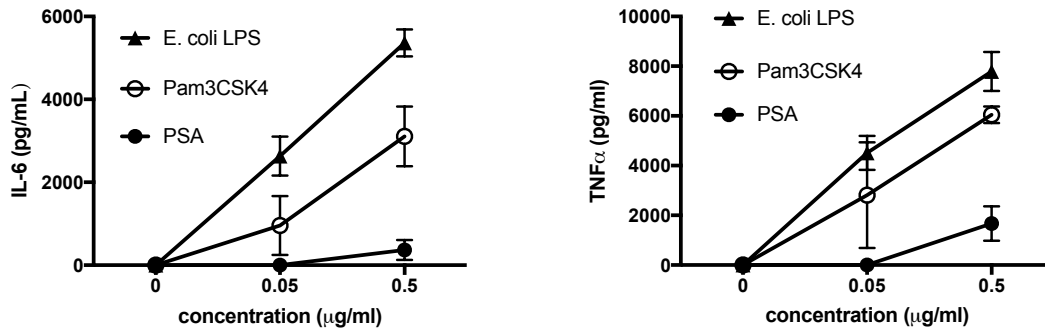
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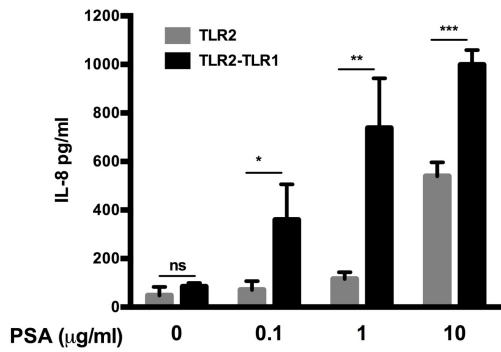
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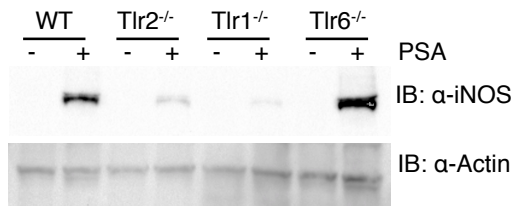
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Tables S1  
Legends for Figures S1 to S15  
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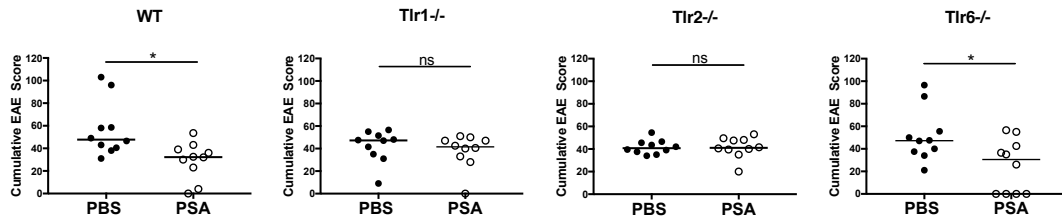
**Figure S1. PSA weakly induces inflammatory responses in APCs.** BMDCs from wild-type mice were stimulated with *E. coli* LPS, Pam<sub>3</sub>CSK<sub>4</sub>, or PSA for 24 hours. IL-6 and TNFα were quantitated by ELISA. Error bars represent SD values.



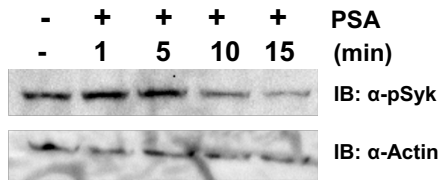
**Figure S2. PSA activates the TLR1/TLR2 heterodimer.** HEK cells stably expressing TLR2 alone or the TLR2/TLR1 heterodimer were stimulated with different concentrations of PSA for 24 hours. The concentration of IL-8 in the cell supernatant was measured by ELISA. Error bars indicate SD values. Scores were assessed for statistical significance by t test. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; ns, not significant. Data represent the average of two experiments.



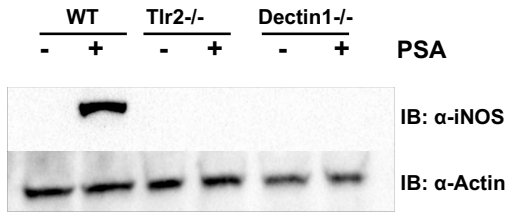
**Figure S3. PSA-induced upregulation of iNOS requires TLR2 and TLR1 but not TLR6.** Western blot analysis of 50 μg of protein extract from BMDMs 24 hours after PSA stimulation is shown. iNOS was detected with anti-iNOS. Anti-actin was used as a loading control.



**Figure S4. PSA protection from EAE requires TLR2 and TLR1.** Cumulative EAE scores were calculated as the sum of all of the daily EAE clinical scores divided by the number of mice per group. Graphs represent the combination of two independent experiments. Bars indicate the median. Scores were assessed for statistical significance by t test. \* $p < 0.05$ ; ns, not significant.

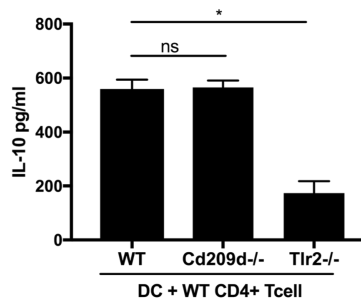


**Figure S5. PSA activates the Dectin-1 pathway.** Western blot analysis of 50  $\mu$ g of protein extract from BMDMs at different time points after PSA stimulation is shown. Activation of the Dectin-1 pathway was detected by phosphorylation of Syk with anti-phosphoSyk. Anti-actin was used as a loading control.

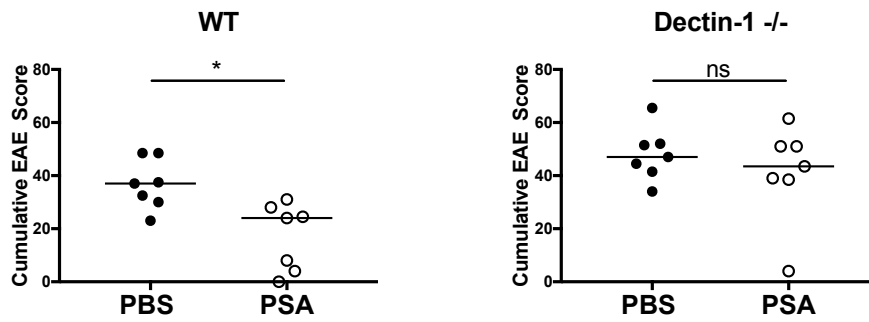


**Figure S6. PSA-induced upregulation of iNOS requires Dectin-1.** Western blot analysis of 50  $\mu$ g of protein extract from macrophages 24 hours after PSA stimulation is shown. Activation of the iNOS gene was detected with anti-iNOS. Anti-actin was used as a loading control.

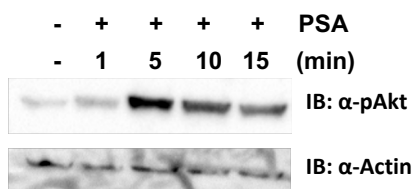




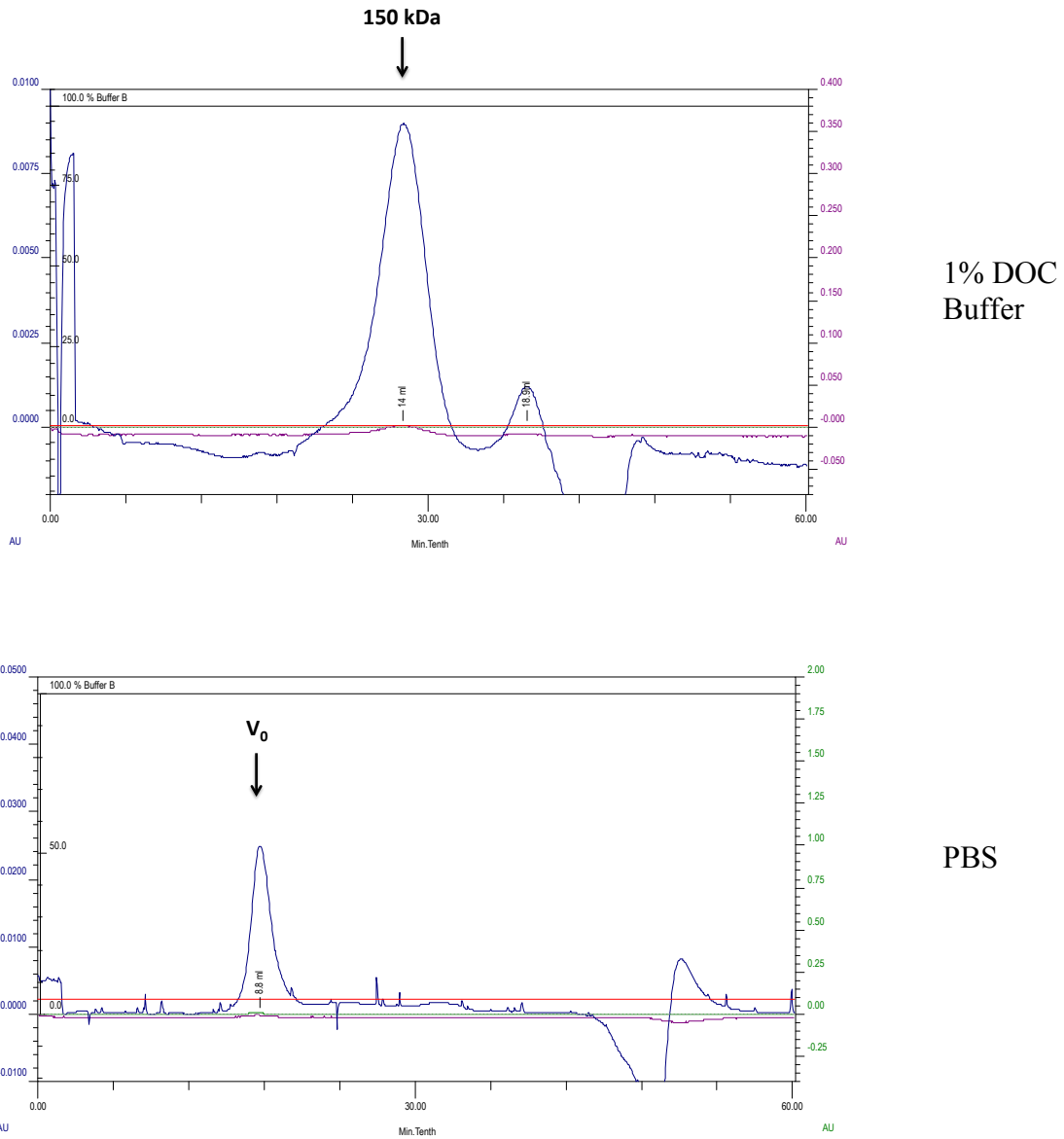
**Figure S7. SIGNR3 (CD209d) is not required for PSA signaling.** IL-10 levels were measured by ELISA of culture supernatants of splenic DCs co-cultured with CD4<sup>+</sup> T cells for 5 days in the presence of anti-CD3. Co-cultures were either treated or not treated with PSA (50  $\mu$ g/ml), and IL-10 levels in the supernatant were normalized by subtracting the medium control. Error bars indicate SD values. Scores were assessed for statistical significance by t test. \* $p < 0.05$ ; ns, not significant. Data represent the average of two experiments.



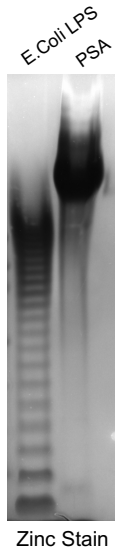
**Figure S8. PSA protection in EAE requires Dectin-1.** Cumulative EAE scores were calculated as the sum of all daily EAE clinical scores divided by the number of mice per group. Graphs represent the combination of two independent experiments. Bars indicate the median. Scores were assessed for statistical significance by t test. \* $p < 0.05$ ; ns, not significant.



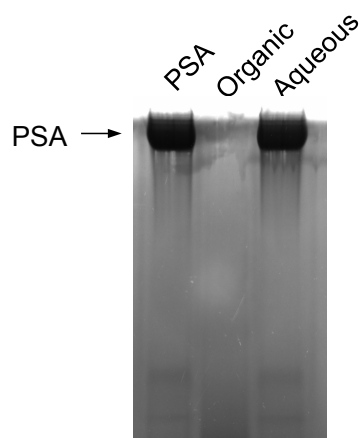
**Figure S9. PSA activates the PI3K pathway.** Western blot analysis of 50  $\mu$ g of protein extract from BMDMs at different time points after PSA stimulation is shown. Activation of the PI3K pathway was detected by phosphorylation of Akt with anti-phosphoAkt. Anti-actin was used as a loading control.



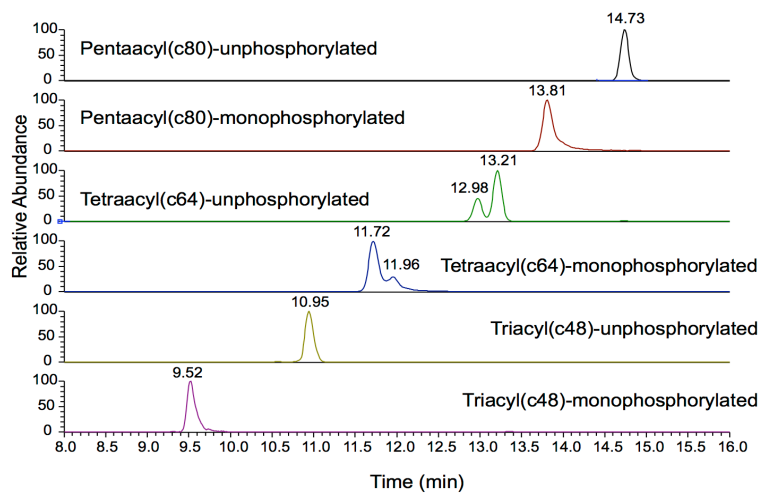
**Figure S10. Average molecular size of PSA.** Gel filtration of a 0.2-mg aliquot of PSA on a Superose 6 10/300 GL column running at a 0.5-ml/min flow rate, with 1% deoxycholate (DOC) buffer (upper panel) or PBS buffer (lower panel), is shown. The size was calculated with dextran standards.



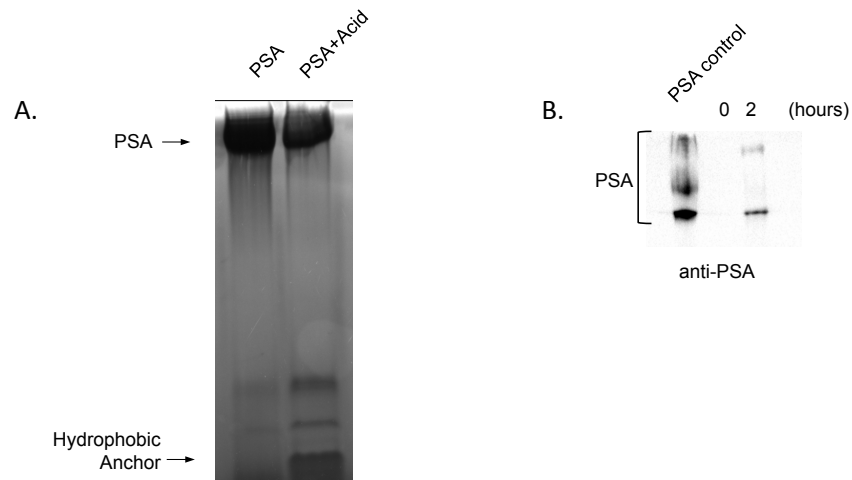
**Figure S11. Ladder-profile comparison of PSA and *E. coli* LPS.** Zinc gel analysis of PSA and ultrapure *E. coli* LPS (InvivoGen) shows that PSA does not have a ladder structure typical of bacterial LPS.



**Figure S12. Chloroform/methanol extraction of PSA.** Zinc gel analysis of PSA extracted with chloroform/methanol shows that the polysaccharide enters the aqueous phase and that the hydrophobic lipid moiety is not detected in the organic phase.

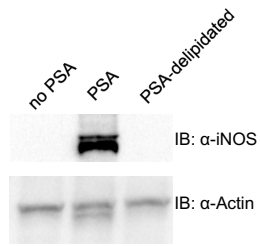


**Figure S13. Molecular diversity of *B. fragilis* lipid A from PSA.** The LC-MS/MS profile shows monophosphorylated and unphosphorylated glycolipid species with different numbers of acyl chains.



**Figure S14. Acid hydrolysis of intact *B. fragilis* bacteria releases PSA.** (A) Treatment of purified PSA with 1% acetic acid (high-molecular-weight band in the first lane) at 65°C is sufficient to cleave the lipid terminus (visible as lower-molecular-weight bands in the second lane) of purified PSA. PSA was analyzed with zinc staining before and after acid treatment. (B) Hydrolysis of intact bacteria releases PSA into the supernatant. The *B. fragilis* bacterial cell pellet was washed with water, resuspended in 1% acetic acid solution, and incubated at 65°C for 2 hours. After acid treatment, the supernatant was analyzed by western blot with monoclonal PSA antibody. The first lane shows control PSA with high-molecular-weight bands detected by anti-PSA. The second and third lanes show the supernatant before (0 hours) and after 2 hours of acid treatment of the bacterial cell pellet, respectively. The high-molecular-weight band (2 hours) is the full-length PSA repeating unit liberated by acid hydrolysis.





**Figure S15. Delipidated PSA cannot induce the iNOS gene.** Western blot analysis of 50  $\mu$ g of protein extract from BMDMs 24 hours after PSA stimulation is shown. iNOS was detected with anti-iNOS. Anti-actin was used as a loading control.

Relative abundance (%)	PSA	Acap LOS
Triacylated, unphosphorylated	3.7	4.7
Triacylated, monophosphorylated	1.9	0.7
Tetraacylated, unphosphorylated	9.4	21.4
Tetraacylated, monophosphorylated	7.0	12.6
Pentaacylated, unphosphorylated	44.5	32.9
Pentaacylated, monophosphorylated	33.6	27.7

**Table S1. Distribution of lipid A structural variants from *B. fragilis* PSA and LOS.** PSA (10  $\mu$ g) or LOS from an acapsular mutant (Acap LOS; 2  $\mu$ g) was hydrolyzed, and the relative abundances of representative tri-(c48), tetra-(c64), and pentaacylated (c80) species were determined with LC-MS-extracted ion chromatograms.

## References

1. M. J. Coyne, K. G. Weinacht, C. M. Krinos, L. E. Comstock, Mpi recombinase globally modulates the surface architecture of a human commensal bacterium. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 10446–10451 (2003).
2. A. Pantosti, A. O. Tzianabos, A. B. Onderdonk, D. L. Kasper, Immunochemical characterization of two surface polysaccharides of *Bacteroides fragilis*. *Infect. Immun.* **59**, 2075–2082 (1991).