

A conserved TLR5 binding and activation hot spot on flagellin

- Supplementary Figures S1 and S2

- Supplementary Table S1

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Supplementary Table S1. Crystallographic statistics of the *bsflagellin*^{cent}-rTLR5^{N14} structure.

<i>bsflagellin</i> ^{cent} -rTLR5 ^{N14}	
<u>Data collection</u>	
Space group	<i>P2</i> ₁
Cell parameters	<i>a</i> = 78.15 Å, <i>b</i> = 54.28 Å, <i>c</i> = 165.98 Å <i>α</i> = 90.00°, <i>β</i> = 98.62°, <i>γ</i> = 90.00°
Wavelength (Å)	0.9796
Resolution (Å)	30.00 - 2.10
Highest resolution (Å)	2.18 - 2.10
No. observations	274,773
No. unique reflections	80,115
<i>R</i> _{merge} (%) ^a	9.1 (51.4) ^b
<i>I</i> /σ	16.8 (2.7) ^b
Completeness (%)	98.1 (95.2) ^b
Redundancy	3.4 (3.3) ^b
<u>Refinement</u>	
Resolution (Å)	30.00 - 2.10
No. of reflections (working)	75,311
No. of reflections (test)	3,987
<i>R</i> _{cryst} (%) ^c	19.5
<i>R</i> _{free} (%) ^d	23.6
Average B-value (Å ²)	33.3
No. protein atoms	9,429
No. water molecules	465
No. ligand atoms	140 (N-linked glycan) 26 (Polyethylene glycol)
RMSD bonds (Å)	0.016
RMSD angles (°)	1.51
Ramachandran ^e (favored)	96.1%
(outliers)	0%

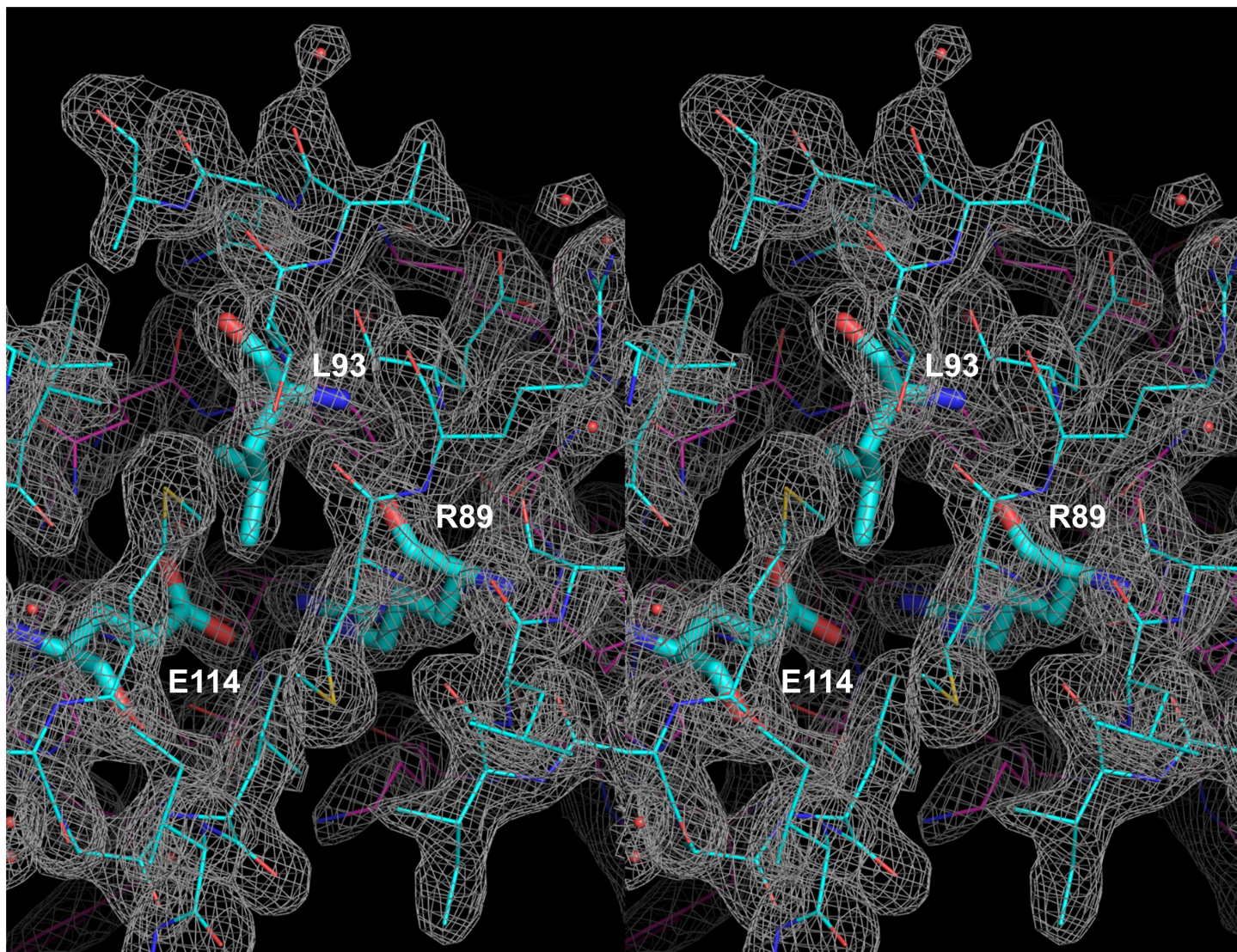
^a $R_{\text{merge}} = \frac{\sum_{\text{hkl}} \sum_i |I_i(\text{hkl}) - \langle I(\text{hkl}) \rangle|}{\sum_{\text{hkl}} \sum_i I_i(\text{hkl})}$

^bNumbers in parenthesis were calculated from data of the highest resolution shell.

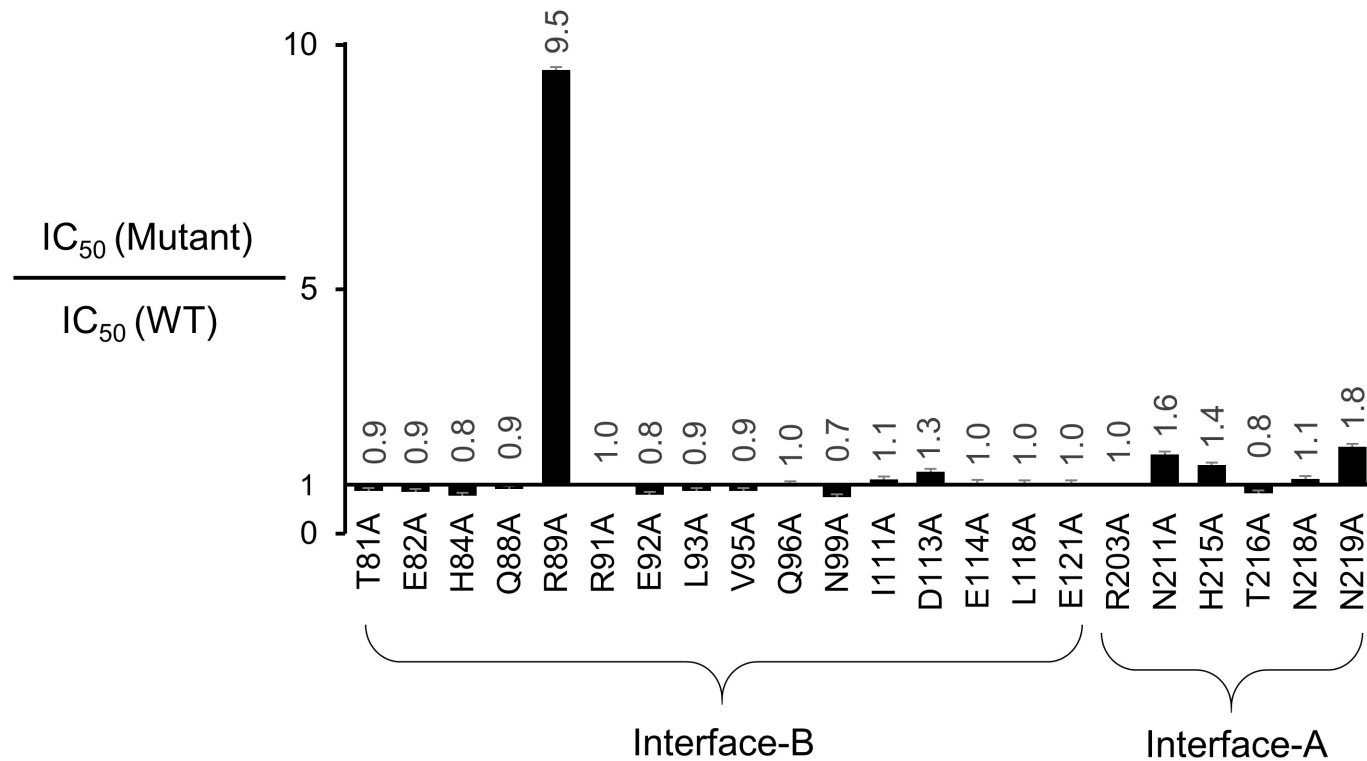
^c $R_{\text{cryst}} = \frac{\sum |F_{\text{obs}}| - |F_{\text{calc}}|}{\sum |F_{\text{obs}}|}$ where *F*_{calc} and *F*_{obs} are the calculated and observed structure factor amplitudes, respectively

^d*R*_{free} = as for *R*_{cryst}, but for 5 % of the total reflections chosen at random and omitted from refinement

^eCalculated using MolProbity (<http://molprobity.biochem.duke.edu>).



Supplementary Figure S1. Stereo view showing the intermolecular interactions of *bsflagellin* R89, E114, and L93 with TLR5 residues in the *bsflagellin*^{cent}-*rTLR5*^{N14} structure. *bsflagellin*^{cent} and *rTLR5*^{N14} are depicted as cyan and magenta lines, respectively. *bsflagellin* R89, E114, and L93 are represented by cyan sticks. Water molecules are shown as red spheres. The gray mesh represents the 2Fo-Fc map contoured at 1.3 σ .



Supplementary Figure S2. Relative TLR5-binding activity of the *bsflagellin* alanine mutants, compared to *bsflagellin*^{WT}. Activity was determined using the competitive rTLR5^{N14}-binding assay (means ± S.D.; n ≥ 2). Although the rTLR5^{N14}-binding assay was less sensitive to the mutations than the HEK293^{TLR5} reporter cell assay, the binding assay obviously showed that *bsflagellin* R89 functions as a key determinant for TLR5 binding.