

Figure S1. Schematic diagram of the TLR3-dsRNA binding assay. (A) Microplate wells were coated with goat anti-mouse IgG (blue) followed sequentially by mouse anti-GFP mAb (red), and cell lysate containing TLR3-YFP. Increasing concentrations of biotin-labeled dsRNA (bio-dsRNA) were then added, and bound bio-dsRNA was quantified by using streptavidin-HRP and HRP Substrate Reagent. Near saturating amounts of the anti-GFP mAb and TLR3-YFP were used in the standard binding assay. In the experiment of Fig 4, limiting amounts of the anti-GFP or TLR3-YFP were used in order to determine how the surface density of immobilized TLR3-YFP molecules affects dsRNA binding. In (B) limiting amounts of TLR3-YFP were bound to saturating amounts of immobilized anti-GFP, and in (C), saturating amounts of TLR3-YFP were added to limiting amounts of bound anti-GFP. In B, most of the bound TLR3-YFP molecules are unable to bind dsRNA because at low surface density they are unable to form dimers. By contrast, in C, where all anti-GFP mAbs bind two TLR3-YFP molecules, dsRNA binding is more efficient.

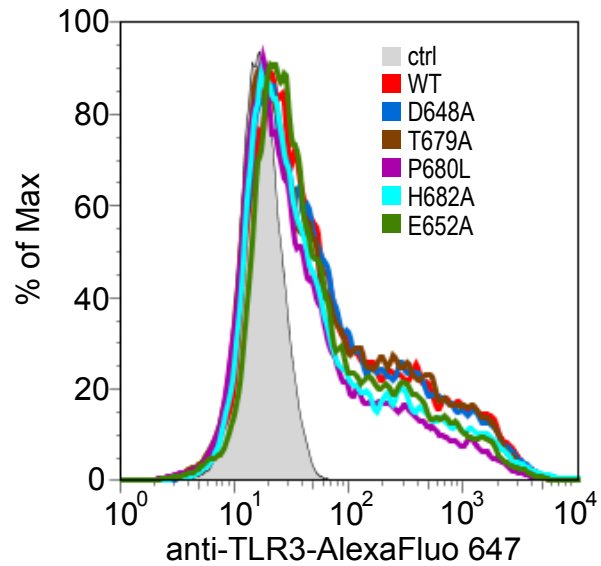


Figure S2. Expression of mutant TLR3 proteins. HEK 293 cells were transfected with plasmids encoding WT TLR3 or dimerization site mutants (D648A, T679A, P680L, H682A, and E652A), or empty vector (Ctrl). After 48 hr, cells were fixed and permeabilized, stained with mouse anti-hTLR3 mAb (eBioscience) followed by goat-anti-mouse IgG Alexa Fluor 647 (Invitrogen), and analyzed using a BD FACSCalibur (BD Biosciences).

Table S1. Effects of mutations in the dsRNA binding sites on ligand-induced TLR3 activation.

Mutation¹	Activation	References
<i>N-terminal dsRNA binding site</i>		
H39A	+/-	(1-3)
H39E	-	(1,2)
H39D	-	(3)
H39R	++	(3)
H60A	-	(1-3)
H60E	-	(1,2)
H60D	-	(3)
H60R	-	(3)
H108A	+	(1,2)
H108E	-	(1,2)
<i>C-terminal dsRNA binding site</i>		
N515A	+	(4,5)
N515D	++	(6)
N515L	++	(6)
N517A	++	(4,6)
H539A	+	(2,6)
H539E	-	(2,4,6)
N541A	-	(4,6)
R544A	++	(5,6)

¹Published data for residues making contact with dsRNA in the crystal structure.

++, activation at least 80% of WT TLR3.

+, activation 20- 80% of WT TLR3.

-, activation less than 20% of WT TLR3.

Supplemental References

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