SUPPLEMENTAL MATERIAL

Fukui et al., http://www.jem.org/cgi/content/full/jem.20082316/DC1

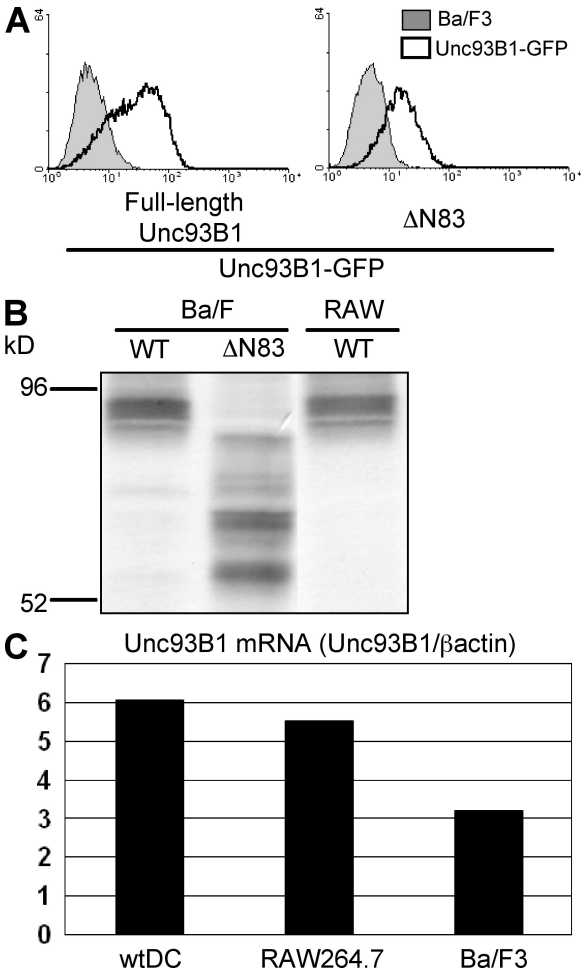

Figure S1. Expression of full-length and truncated Unc93B1. (A) Green fluorescence from Ba/F3 cells expressing full-length (left) or N-terminally truncated (right) Unc93B1 was analyzed by flow cytometry (open histograms). Shaded histograms show autofluorescence from plain Ba/F3 cells. (B) HA-tagged WT Unc93B1 and ΔN83 were transfected into Ba/F3 cells expressing cathepsin B+L or RAW264.7 cells, as indicated. The cells were subjected to detergent lysis, immunoprecipitation, and immunoblotting with anti-HA antibody. (C) Real-time RT-PCR analyses were conducted with total RNA from WTDC, RAW264.7, or Ba/F3 cells. The results represent mean values from triplicate wells. These experiments were repeated twice and the represented data are shown.

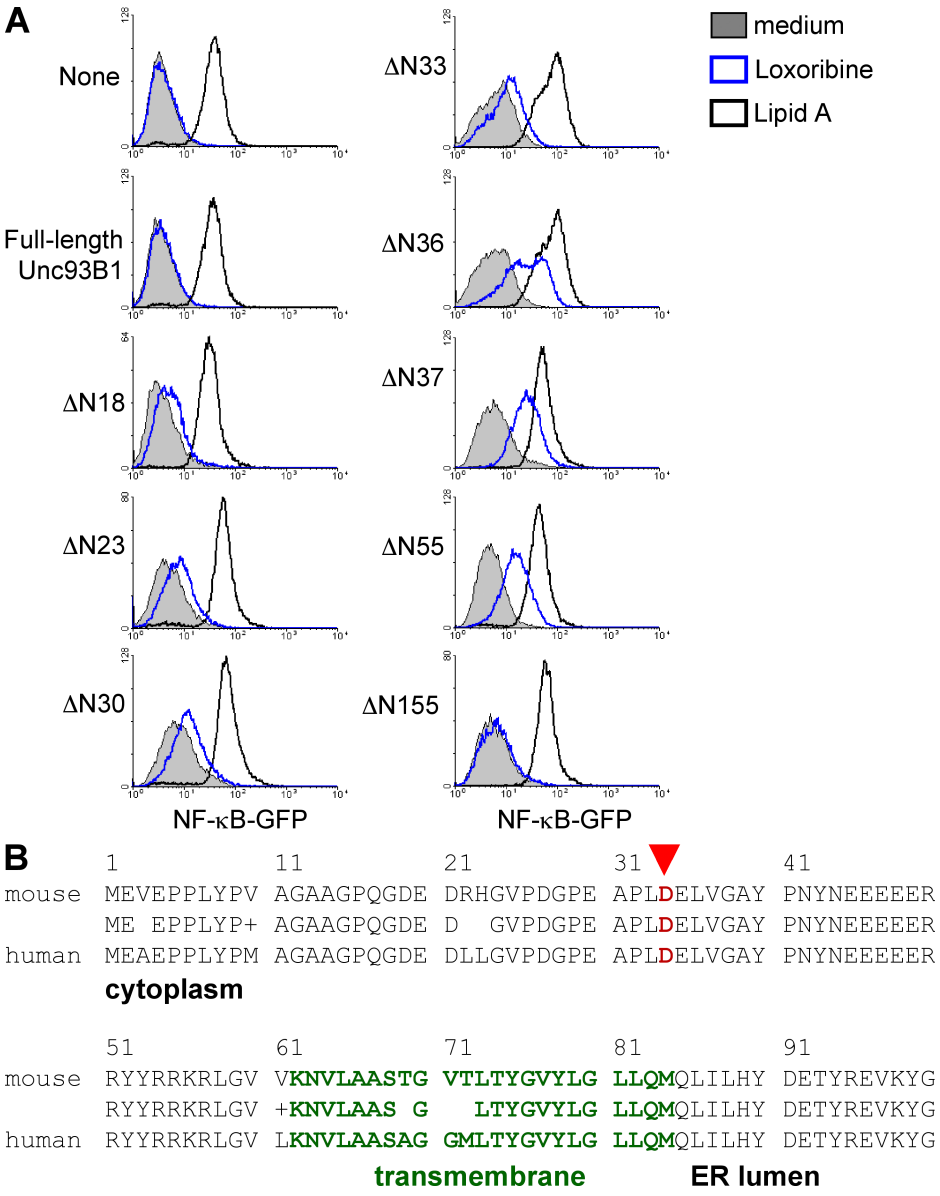

Figure S2. The effect of N-terminal deletion of Unc93B1 on TLR7 responses. (A) Ba/F3 cells expressing CD14/TLR4/MD-2, NF-κB–GFP, cathepsin B+L, and TLR7 were further transfected with cDNA encoding full-length Unc93B1 or its mutants lacking N-terminal 18 aa (ΔN18), 23 aa (ΔN23), 30 aa (ΔN30), 33 aa (ΔN33), 36 aa (ΔN36), 37 aa (ΔN37), 51 aa (ΔN51), or 155 aa (ΔN155). These cells were stimulated with 1 µg/ml lipid A (black lines) or 100 µM TLR7 ligand loxoribine (blue lines). Gray histograms show GFP expression in unstimulated cells. (B) Amino acid sequences of the N-terminal region of mouse and human Unc93B1. These experiments were repeated three times and the represented data are shown.

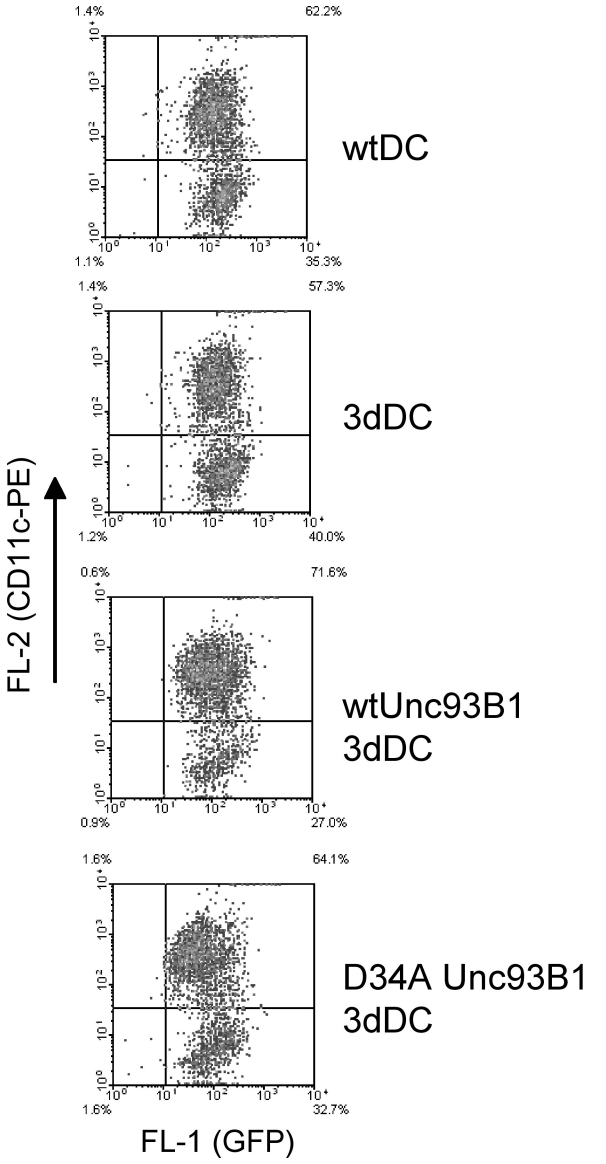

Figure S3. Expression of Unc93B1–GFP and CD11c in BM–DCs. Dot plot analyses show expression of GFP (WTDC or 3dDC) or Unc93B1-GFP (WT Unc93B1-3dDC or D34A Unc93B1-3dDC) in BM-DCs from WT or 3d mice. CD11c expression is also shown. These experiments were repeated three times and the represented data are shown.

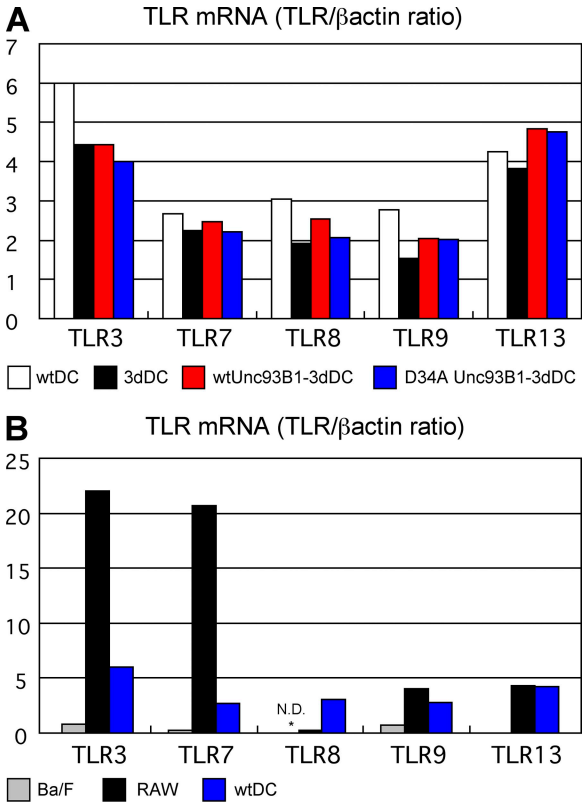

Figure S4. TLR mRNA expression in BM–DCs, Ba/F3 cells, and RAW264.7 cells. (A) BM-DCs from WT mice were transduced with retrovirus vector encoding GFP (WTDC). BM-DCs from 3d mice (3dDC) were also transduced by retroviral vector encoding GFP (3dDC), WT Unc93B1-GFP (WT Unc93B1-3dDC), or D34A Unc93B1-GFP (D34A Unc93B1-3dDC). Total RNA from these DCs were prepared and used for real-time PCR for the indicated TLR and β-actin. (B) Total RNA from Ba/F3, RAW264.7, or WTDCs was prepared and used for real-time PCR for TLR3/7/8/9/13 and β-actin. The results were represented by mean values from triplicate wells. These experiments were repeated twice and the represented data are shown. N.D., not detectable.

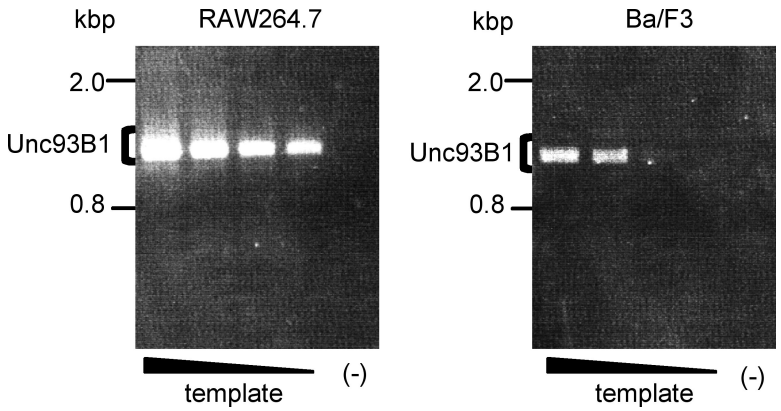

Figure S5. No difference in Unc93B1 mRNA transcription start between Raw264.7 and Ba/F3 cells. Total RNA from Ba/F3 cells (right) or RAW264.7 cells (left) was subjected to oligo capping as described in Materials and methods. Oligo-capped mRNA were reverse transcribed with the Super Script II RT kit with an oligo-dT primer. Synthesized cDNA were used as the PCR template. The PCR reaction was performed by forward primer on oligo capping and reverse primer on Unc93B1 (1,038–1,063). The amount of template was sequentially diluted, as indicated in the figure. PCR products were subjected to agarose electrophoresis and visualized by ethidium bromide staining. The expected size is ~1,100 bp. These experiments were repeated twice and the represented data are shown.

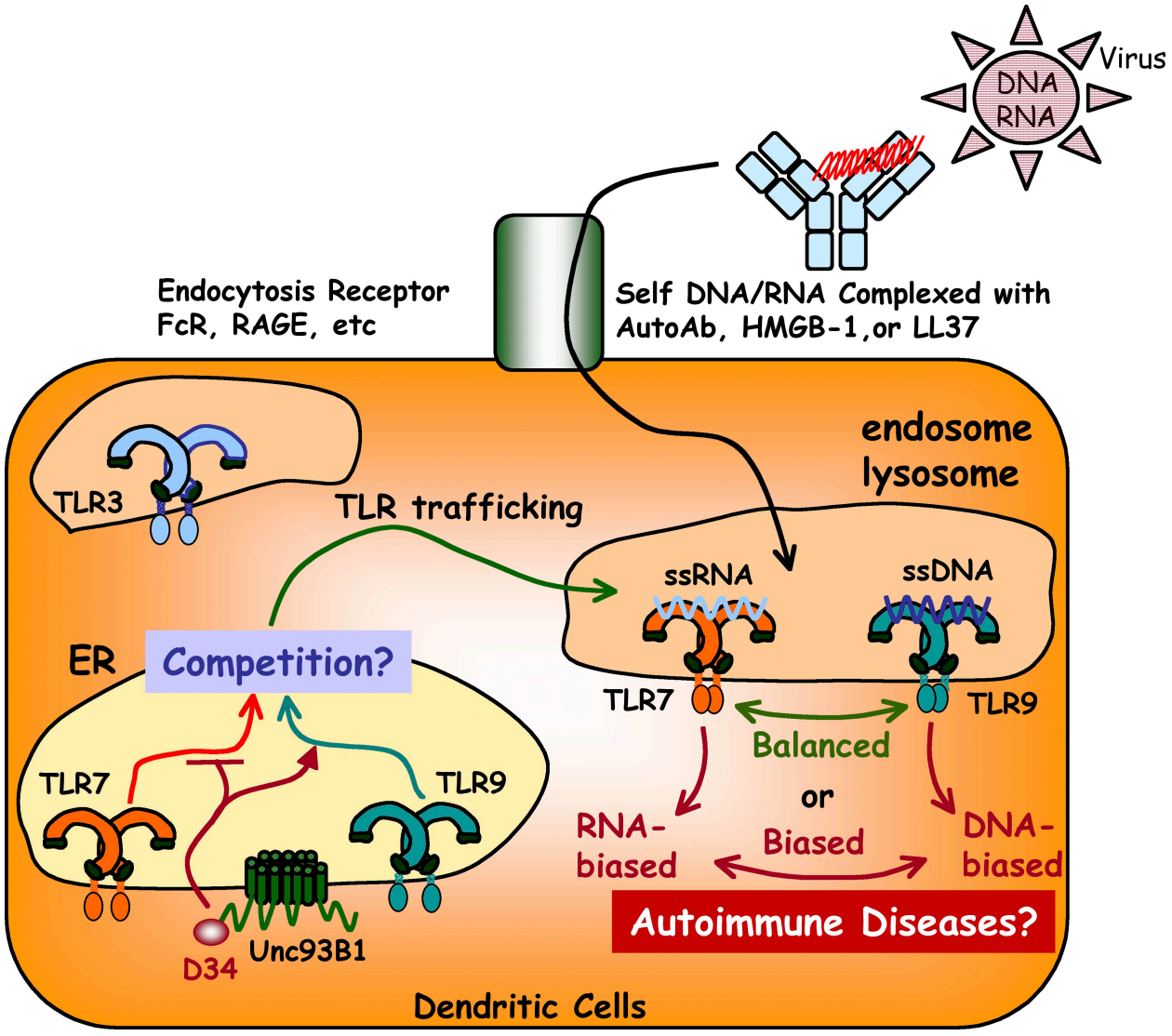

Figure S6. RNA- versus DNA-sensing in DCs controlled by Unc93B1-dependent inverse link between TLR7 and 9. TLR7 and 9 reside in the ER, whereas TLR3 resides outside the ER. Unc93B1 associates with TLR7 or 9 and transports them from the ER to endolysosomes upon activation. Because trafficking of TLR7/9 occurs simultaneously upon stimulation like LPS, TLR7 and 9 are likely to compete for association with Unc93B1. The competition may be one of the mechanism for Unc93B1-dependent balancing of TLR7 and 9 responses. Our results demonstrate that D34 in the N-terminal cytoplasmic domain of Unc93B1 inhibits its association with TLR7 by an as yet unknown mechanism, and suggest that D34 up-regulates Unc93B1 association with TLR9. Consequently, ligand-induced trafficking is down- or up-regulated in TLR7 or 9, respectively, by D34 in Unc93B1. By controlling the ratio of TLR7 to 9 in endolysosomes, Unc93B1 has a critical role in determining the relative responsiveness of TLR7 and 9. Biased response toward TLR7 or 9 may predispose to autoimmune diseases.

Table S1. The XIC-based peptide quantification for Unc93B1 and coprecipitated TLRs

unc93 homologue B1		WT		D34A	
Sequence	Score	XIC [Da*secs]	Score	XIC [Da*secs]	D34/WT
MEVEPPLYPVAGAAG PQGDEDR	36	929.941	58	810.311	0.87
SVGWGNIFQLPFK	63	19358.896	60	26622.503	1.38
TGLSTLLGILYEDK	36	322.811	52	240.814	0.75
YGNMGLPDIDSK	55	39391.940	61	42587.961	1.08
				Average 1.02	SD 0.28

toll-like receptor 13		WT		D34A	
Sequence	Score	XIC [Da*secs]	Score	XIC [Da*secs]	D34/WT
AMDLSNWELR	57	2283.680	74	7066.912	3.09
DAFTPLIK	45	4765.919	32	10941.384	2.30
ELVPALEQGSQTTFK	36	275.381	50	913.444	3.32
ENTHLIVVE	33	2012.790	33	3578.259	1.78
IDEGAFR	27	1140.681	28	838.610	0.74
ILEPNSFSGLTNLR	51	999.234	65	2232.381	2.23
LETLVFQK	52	5532.521	46	6144.449	1.11
LEWNSIWK	28	857.554	32	898.144	1.05
QLNLEGQR	32	6462.155	47	11293.174	1.75
QTFITWPDSVHQQPL FWAR	36	1292.509	32	2990.271	2.31
SFPDFAFSPLK	29	5025.059	44	10152.610	2.02
				Average 1.97	SD 0.81

toll-like receptor 7		WT		D34A	
Sequence	Score	XIC [Da*secs]	Score	XIC [Da*secs]	D34/WT
DAFLVMR	41	2021.203	36	2409.781	1.19
GNHLDVLWR	32	899.965	33	2229.619	2.48
HGPQVLEALHYFR	50	486.216	54	1107.949	2.28
HLEILDLSHNQLTK	61	7461.432	60	16412.119	2.20
IADLNIFK	50	3845.017	50	6215.617	1.62
LEVLDLGTNFIK	40	7908.494	58	13269.832	1.68
LIDLSVNK	54	8569.683	49	16652.236	1.94
LMMNDNDISTSASR	59	3185.532	97	7501.669	2.35
LNHLEEIDLR	26	2391.396	32	3031.120	1.27
LQIRPGSFSGLSDLK	42	2209.616	42	2731.083	1.24
TMESDSLR	44	2749.548	28	7853.836	2.86
TVFVMTQK	38	1194.969	35	426.406	0.36
YFLEDALQLR	63	6439.158	53	10739.245	1.67
YLDFFK	30	2784.588	34	5996.630	2.15
YLDFSNNR	47	2884.943	44	3298.954	1.14
YLDISSNK	36	6681.285	45	8859.416	1.33
				Average 1.73	SD 0.64

toll-like receptor 8		WT		D34A	
Sequence	Score	XIC [Da*secs]	Score	XIC [Da*secs]	D34/WT
DASVTDWVINELR	51	136.812	78	248.001	1.81
NVVLTENDSR	25	442.552	29	574.613	1.30
SLQNLIR	33	412.189	36	796.313	1.93
TIFVLTK	41	333.198	27	562.781	1.69
				Average 1.68	SD 0.27

toll-like receptor 3		WT		D34A	
Sequence	Score	XIC [Da*secs]	Score	XIC [Da*secs]	D34/WT
LQVALGSR	38	385.774	43	610.086	1.58
NVDISPSPFRPLR	31	518.344	40	583.801	1.13
				Average 1.35	

toll-like receptor 9		WT		D34A	
Sequence	Score	XIC [Da*secs]	Score	XIC [Da*secs]	D34/WT
AQSAVADWVYNELR	43	255.934	30	95.094	0.37
VSSHLNSNSVR	29	459.667	25	184.036	0.40
				Average 0.39	

The sequence of each peptide is shown with its corresponding score and XIC value. Scores shown are the Mascot ion scores representing the quality of the match of the identified peptide. The XIC is a measure that is proportional to the peptide's abundance. The XIC values of each peptides from Unc93B1, and TLR3, 7, 8, 9, and 13 were calculated directly without isotope labeling using the MSQuant program. The XIC values were retrieved directly for the corresponding peptides' m/z signals between the two states. The values of peptides derived from BM-DCs expressing D34A Unc93B1-GFP were divided with the values from WT Unc93B1 to compare the binding ratio. The mean and SD were calculated in each protein.

Table S2. The calculated XIC values, ions scores, and SILAC ratio (D34/WT) of each peptide from Unc93B1 and TLR7 and 9

Experiment 1

toll-like receptor 7		WT	D34A	
Sequence	Score	XIC [Da*secs]	XIC [Da*secs]	D34/WT
GNHLDVLWR	36	3770.313	2163.793	0.574
HLEILDLSHNQLTK	32	3716.439	2510.923	0.676
IADLNIFK	40	6537.026	4595.103	0.703
LEVLDLGTNFIK	32	6017.307	3893.252	0.647
LIDLSVNK	34	8599.321	5625.563	0.654
LMMNDNDISTSASR	84	4807.597	2881.716	0.599
LNHLEEIDLR	49	2365.431	1551.471	0.656
LQIRPGSFSGLSDLK	29	3330.140	1888.595	0.567
NALTTDNHVAYSQMFK	27	1486.583	1009.132	0.679
NLQELDLSQNYLAR	73	2658.184	1538.807	0.579
NSAVTEWVLQELVAK	43	526.661	392.487	0.745
RLNHLEEIDLR	38	2601.872	1375.490	0.529
TMESDSLR	30	7912.975	5569.973	0.704
TVFVMTQK	31	1726.539	1190.817	0.690
YFLEDALQLR	51	3773.247	2320.015	0.615
YLDFFK	28	4899.068	3507.844	0.716
YLDFSNNR	48	3755.365	1781.110	0.474
YLDISSNK	40	4221.156	2750.148	0.652
			Average 0.637	SD 0.07

toll-like receptor 13		WT	D34A	
Sequence	Score	XIC [Da*secs]	XIC [Da*secs]	D34/WT
AMDLSNWELR	70	3565.387	2412.198	0.677
DAFTPLIK	32	3365.059	2844.331	0.845
HLEFLSLSR	31	1568.964	1125.584	0.717
IDEGAFR	38	4262.170	3288.453	0.772
ILEPNSFSGLTNLR	65	1423.800	883.573	0.621
ITYETTR	28	3495.475	2506.544	0.717
LDISGTKDGDR	35	768.552	622.070	0.809
LETLVFQK	33	1851.626	1401.363	0.757
QLNLEGQR	43	6942.479	4623.323	0.666
QTFITWPDSVHQQPLFWAR	50	6882.571	3690.338	0.536
SLVSLTHLSFEGNK	50	6452.203	4970.296	0.770
			Average 0.717	SD 0.09

unc93 homologue B1		WT	D34A	
Sequence	Score	XIC [Da*secs]	XIC [Da*secs]	D34/WT
EQDEQGPQQRPPR	26	2125.310	935.590	0.440
LQQGLVPR	40	8041.079	3556.698	0.442
SVGWGNIFQLPFK	79	22207.616	11593.001	0.522
YGNMGLPDIDSK	62	11732.953	5575.985	0.475
YYEYSHYKEQDEQGPQQRPPR	38	3382.064	1494.082	0.442
			Average 0.464	SD 0.04

Experiment 2

toll-like receptor 7		WT	D34A	
Sequence	Score	XIC [Da*secs]	XIC [Da*secs]	D34/WT
LMMNDNDISTSASR	96	586.290	336.876	0.575
LNHLEEIDLR	49	765.799	465.086	0.607
NLFNLEVLDISR	72	1503.953	827.530	0.550
YFLEDALQLR	37	689.200	390.851	0.567
			Average 0.575	SD 0.02

toll-like receptor 8		WT	D34A	
Sequence	Score	XIC [Da*secs]	XIC [Da*secs]	D34/WT
LGFIQNLINLR	76	534.994	463.034	0.865
SLQNLIR	28	1347.144	2022.869	1.502
			Average 1.184	

toll-like receptor 13		WT	D34A	
Sequence	Score	XIC [Da*secs]	XIC [Da*secs]	D34/WT
AMDLSNWELR	57	681.846	744.721	1.092
HLEFLSLSR	36	598.695	436.486	0.729
IDEGAFR	34	1366.523	1250.082	0.915
ITYETTR	26	1429.883	1632.820	1.142
QTFITWPDSVHQQPLFWAR	64	908.732	586.919	0.646
			Average 0.905	SD 0.22

unc93 homologue B1		WT	D34A	
Sequence	Score	XIC [Da*secs]	XIC [Da*secs]	D34/WT
EQDEQGPQQRPPR	36	4282.860	1810.431	0.423
LQQGLVPR	44	8676.055	3963.979	0.457
TGLSTLLGILYEDKER	73	1430.074	781.292	0.546
YYEYSHYKEQDEQGPQQRPPR	49	2466.434	868.565	0.352
			Average 0.445	SD 0.08

Quantification of the interaction of TLR7 and 9 with Unc93B1 was analyzed by SILAC. The XIC values of each peptide from Unc93B1 and TLR7 and 9 were calculated using the MSQuant program. SILAC ratios represent the relative abundance of the heavy to the light peptide, indicating the binding ratio (D34/WT). The ion score, SILAC ratio, and mean ratio and SD are shown. The experiments were repeated twice and all of the results are shown.