

Supplementary Materials for

Interferon- λ restricts West Nile virus neuroinvasion by tightening the blood-brain barrier

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Other Supplementary Material for this manuscript includes the following:

(available at www.sciencetranslationalmedicine.org/cgi/content/full/7/284/284ra59/DC1)

Primary data tables. (Excel)

Supplementary Figures

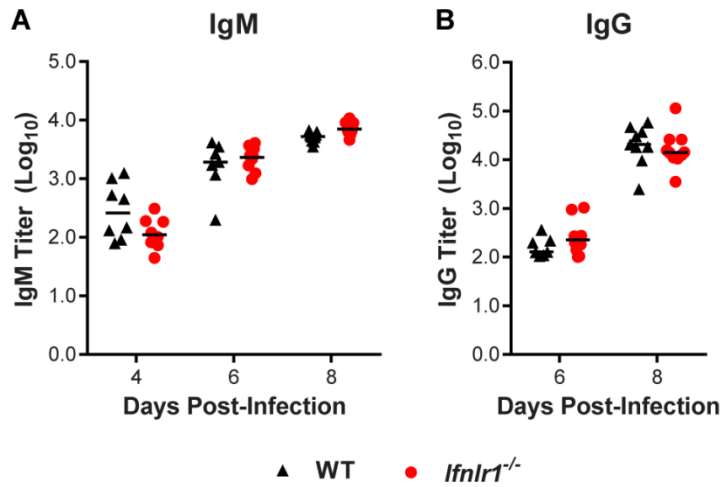


Fig. S1. Serum antibody responses in wild-type and *Ifnlr1*^{-/-} mice. Eight to ten week-old mice were infected with 10² PFU of WNV via a subcutaneous route in the footpad. Serum IgM (A) and IgG (B) against WNV E protein were measured by ELISA. Results represent the mean ± SEM of 6 to 10 mice per group. The differences between wild-type (WT) and *Ifnlr1*^{-/-} mice were not statistically significant (2-way ANOVA, $P > 0.05$).

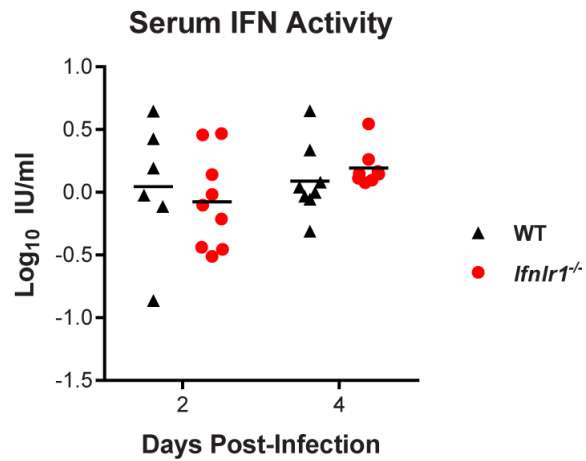


Fig. S2. Serum type I IFN responses in wild-type and *Ifnlr1*^{-/-} mice. Eight to ten week-old mice were infected with 10² PFU of WNV via a subcutaneous route in the footpad. IFN- α/β activity in serum was measured by an EMCV cytopathic inhibition bioassay. The differences between wild-type (WT) and *Ifnlr1*^{-/-} mice were not statistically significant (2-way ANOVA, $P > 0.05$).

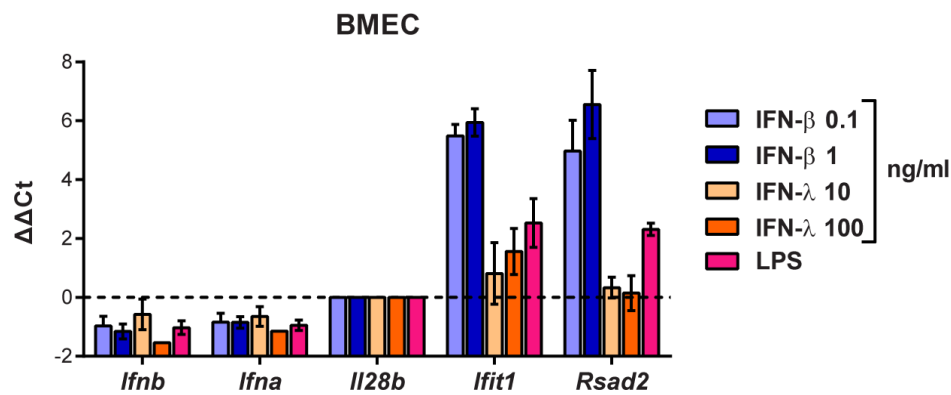


Fig. S3. ISG induction in BMECs. Wildtype BMEC were treated with IFN-β (0.1 or 1 ng/ml), IFN-λ (10 or 100 ng/ml) or LPS (100 ng/ml) for 6 h then expression of the indicated genes was measured by qRT-PCR from total cell RNA. Gene expression was normalized to 18s rRNA and to mock-treated cells. Results represent mean ± SEM of 6 samples from two independent experiments.

Table S1: Transcriptional profiling of DCs treated with IFN- β or IFN- λ .

Gene Name	Log ₂ IFN-treated/mock	
	IFN- β	IFN- λ
Ifi44	5.49	1.12
Isg15	4.94	1.01
Plac8	3.81	1.00
Mx2	3.91	0.92
Ifit3	5.41	0.90
Oas3	3.39	0.82
Slfn3	3.65	0.79
Ifit1	4.70	0.78
Oas11	5.02	0.74
Ddx60	3.65	0.73
Rsad2	5.65	0.69
Herc6	3.49	0.68
Oas12	3.31	0.68
Ifit2	5.38	0.65
Ube2l6	3.18	0.64
Stat2	2.89	0.62
Irf7	5.06	0.60
Stat1	2.79	0.53
H2-T24	2.91	0.52
Zbp1	3.19	0.51
Usp18	3.62	0.50
Trim30d	2.55	0.49
Cmpk2	4.99	0.48
Gbp4,Gbp9	4.09	0.48
Nt5c3	3.58	0.48
Pyhin1	3.58	0.48
Dhx58	2.90	0.47
Ifih1	3.19	0.47
Slfn5	3.31	0.47
Gm12250	3.23	0.46
Mir1932	3.26	0.45
Uba7	2.92	0.45
Serpina3f	3.74	0.45
Gbp5	3.91	0.45
Oas1g	2.96	0.44

Oas1a	2.71	0.43
Cxcl10	4.69	0.43
Socs1	3.08	0.43
Slfn1	3.11	0.43
1600014C10Rik	2.94	0.42
Ccl5	1.89	0.41
Ch25h	2.94	0.41
2010002M12Rik,Gm14446	6.60	0.40
Rasa4	2.50	0.40
Parp12	2.88	0.40
Mxd1	2.38	0.40
Rilp	1.95	0.39
Phf11a	3.86	0.39
Irgm1	3.52	0.39
Nlrc5	2.70	0.39
Ccnd2	2.35	0.39
Psmb9	2.20	0.39
Helz2	2.18	0.39
Mx1	3.10	0.38
Xaf1	3.16	0.38
Casp4	2.62	0.38
Irgm2	2.92	0.37
Bst2	2.88	0.36
Ifi47	3.77	0.36
Zufsp	2.23	0.36
Gm5431	2.91	0.35
Trafd1	2.16	0.35
Tmem184b	1.81	0.35
Mthfr	3.03	0.34
Parp14	2.39	0.34
Ly6c1	2.04	0.33
Gbp6	3.57	0.32
Trim30a	2.35	0.32
Cant1	1.91	0.32
Etnk1	1.94	0.30
March5	2.27	0.30
Znfx1	2.78	0.30
Trim34a	1.94	0.29
Tor1aip2	1.94	0.29

Igtp	3.39	0.29
Sp100	2.23	0.29
Ifi204	3.25	0.29
Ddx58	2.68	0.28
AW112010	2.90	0.28
Parp9	2.20	0.28
Sp110	2.30	0.28
Il15	2.56	0.28
Trim12a	1.77	0.28
Phf11c	2.31	0.27
Tap1	2.39	0.27
Dtx3l	2.41	0.26
Lgals9	2.42	0.26
Ly6a	3.43	0.25
Cnp	2.16	0.25
Slfn8,Slfn9	2.84	0.25
Hmgn3	2.02	0.25
Gbp2	3.37	0.25
Gbp3	3.36	0.24
Pttg1	2.41	0.24
Adar	1.67	0.24
Tor1aip1	1.61	0.24
Ifi35	2.17	0.24
Fgl2	3.70	0.24
H2-T22,H2-T23,H2-T9	1.99	0.24
Ifi203	2.77	0.23
Fcgr4	3.01	0.23
Rgl2	1.77	0.23
Apol7c	1.86	0.22
Ifitm3	1.67	0.22
Trex1	2.06	0.21
Daxx	2.85	0.20
Aida	2.11	0.20
Trim21	2.50	0.20
Trem12	1.60	0.20
S1pr2	1.59	0.20
Rtp4	2.57	0.19
Car13	1.83	0.19
C2,Cfb	2.84	0.19

Tap2	1.76	0.18
9930111J21Rik1	2.12	0.18
Il18bp	3.09	0.18
Cd69	3.26	0.18
Usp25	1.71	0.17
Ms4a6d	2.83	0.17
Eif2ak2	1.64	0.17
Fbxw17	1.88	0.17
Themis2	2.28	0.17
Chpt1	1.91	0.17
Tor3a	2.41	0.17
Xdh	2.32	0.17
Sell	2.10	0.17
Ogfr	1.93	0.16
Usb1	1.83	0.16
Amical	1.71	0.16
Cd40	3.17	0.16
Samhd1	2.43	0.16
Epsti1	2.46	0.16
Tdrd7	1.71	0.15
Ly6i	1.66	0.15
Ehd4	1.77	0.15
Mlkl	2.62	0.15
Tapbpl	2.06	0.15
Gbgt1	1.73	0.15
Ly6c2	1.81	0.15
Samd9l	2.27	0.14
Slamf7	1.84	0.14
Slc7a8	1.80	0.14
Peli1	1.67	0.14
Gm4759	1.69	0.14
Irf1	1.65	0.13
5730508B09Rik	1.79	0.13
Fcgr1	3.51	0.13
Ms4a6c	3.53	0.12
Clic4	1.60	0.12
Tagap	1.61	0.11
Tnfsf8	2.03	0.11
Cd86	1.86	0.11

Gnb4	1.65	0.10
Ncoa7	1.97	0.10
Hpse	1.82	0.10
1110018G07Rik	1.86	0.10
Parp3	1.73	0.09
Sepw1	1.68	0.09
Psmb10	2.11	0.09
Nmi	2.38	0.09
Dhcr24	-1.67	0.09
Stard3	1.62	0.08
Irg1	2.43	0.07
Gbp7	2.58	0.07
Mitd1	2.01	0.07
H2-Q4	1.65	0.07
Abcg1	1.65	0.07
Pdk3	1.59	0.06
Cd274	1.96	0.06
Acs11	2.27	0.05
Slfn2	1.64	0.05
Fndc3a	1.85	0.05
Lap3	2.34	0.05
A530064D06Rik	2.22	0.05
Dck	2.34	0.05
Pnp	2.76	0.03
Ppbp	-1.85	0.03
Tpst1	2.39	0.02
Rgs2	1.87	0.02
Rnf34	1.74	0.02
Ly86	1.78	0.02
Sqle	-1.82	0.01
Ccl12	3.68	0.01
C3ar1	1.74	0.01
Ccnd1	2.48	0.01
Adap2	1.81	0.01
Scimp	2.32	0.00
Dpy19l1	2.00	-0.01
Hk3	1.76	-0.01
Tfrc	-1.67	-0.01
AA467197	1.95	-0.02

Aldh1b1	2.84	-0.02
Slamf8	1.75	-0.02
Rabggtb	5.12	-0.02
Ppa1	1.77	-0.02
Ctsc	1.68	-0.04
Casp1	1.72	-0.04
Cd180	1.74	-0.04
Ccr12	1.76	-0.05
Srm	-2.03	-0.06
Ccdc23	1.73	-0.08
Mmp13	2.04	-0.08
Il18	1.70	-0.11
Prpf38a	1.85	-0.13
Ccl7	2.25	-0.17
Xrcc6	-2.56	-0.54
Bcl	-2.01	-0.79

RNA-Seq analysis of wildtype DCs treated with IFN- β (20 ng/ml) or IFN- λ 3 (10 ng/ml) for 6 h. Gene expression differences are expressed as Log₂ fold change relative to mock-treated cells and are shown in order of induction level following IFN- λ treatment.

Table S2: Serum cytokines after WNV infection.

Cytokine	Genotype	3 dpi		5 dpi	
		pg/ml	<i>P</i>	pg/ml	<i>P</i>
IL-1 α	WT	8.8 (\pm 1.6)	0.37	10.6 (\pm 2.0)	0.90
	<i>Ifnlr1</i> ^{-/-}	11.3 (\pm 2.1)		10.9 (\pm 1.0)	
IL-1 β	WT	424.8 (\pm 25.7)	0.52	406.7 (\pm 25.1)	0.09
	<i>Ifnlr1</i> ^{-/-}	393.0 (\pm 39.4)		344.6 (\pm 20.1)	
IL-3	WT	2.3 (\pm 0.2)	0.67	5.3 (\pm 1.6)	0.13
	<i>Ifnlr1</i> ^{-/-}	2.6 (\pm 0.6)		2.4 (\pm 0.4)	
IL-4	WT	5.2 (\pm 0.0)	0.95	5.9 (\pm 0.2)	0.50
	<i>Ifnlr1</i> ^{-/-}	5.2 (\pm 0.4)		5.6 (\pm 0.2)	
IL-6	WT	4.7 (\pm 0.8)	0.98	8.3 (\pm 1.5)	0.30
	<i>Ifnlr1</i> ^{-/-}	4.6 (\pm 2.0)		17.5 (\pm 8.0)	
IL-9	WT	195.7 (\pm 11.4)	0.45	222.5 (\pm 8.3)	0.09
	<i>Ifnlr1</i> ^{-/-}	173.7 (\pm 25.4)		190 (\pm 14.7)	
IL-10	WT	60.1 (\pm 4.5)	0.06	63.2 (\pm 5.5)	0.67
	<i>Ifnlr1</i> ^{-/-}	40.1 (\pm 8.0)		59.8 (\pm 5.2)	
IL-12 (p40)	WT	177.2 (\pm 13.1)	0.26	136.3 (\pm 9.0)	0.12
	<i>Ifnlr1</i> ^{-/-}	150.3 (\pm 17.6)		115.4 (\pm 8.1)	
IL-12 (p70)	WT	50.0 (\pm 6.0)	0.96	54.0 (\pm 5.8)	0.32
	<i>Ifnlr1</i> ^{-/-}	49.4 (\pm 10.5)		62.2 (\pm 5.2)	
IL-13	WT	69.6 (\pm 7.3)	0.92	102.1 (\pm 8.1)	0.69
	<i>Ifnlr1</i> ^{-/-}	72.4 (\pm 24.9)		112.2 (\pm 22.6)	
IL-17	WT	24.2 (\pm 2.7)	0.73	28.3 (\pm 2.0)	0.56
	<i>Ifnlr1</i> ^{-/-}	25.8 (\pm 3.3)		30.9 (\pm 3.6)	
Eotaxin	WT	585.4 (\pm 117.5)	0.83	345.3 (\pm 49.6)	0.73
	<i>Ifnlr1</i> ^{-/-}	641.0 (\pm 244.9)		370 (\pm 46.1)	
G-CSF	WT	73.2 (\pm 4.1)	0.04	66.8 (\pm 5.3)	0.51
	<i>Ifnlr1</i> ^{-/-}	56.4 (\pm 5.1)		72.9 (\pm 6.9)	
GM-CSF	WT	130.4 (\pm 20.5)	0.25	143.4 (\pm 21.9)	0.93
	<i>Ifnlr1</i> ^{-/-}	184.9 (\pm 44.6)		140.6 (\pm 23.0)	
IFN- γ	WT	8.6 (\pm 0.9)	0.64	7.7 (\pm 0.8)	0.61
	<i>Ifnlr1</i> ^{-/-}	7.8 (\pm 1.3)		8.6 (\pm 1.2)	
KC	WT	100.7 (\pm 9.1)	0.17	68.3 (\pm 1.8)	0.03
	<i>Ifnlr1</i> ^{-/-}	79.4 (\pm 10.7)		84.4 (\pm 5.8)	
MCP-1	WT	143.0 (\pm 16.6)	0.10	137.5 (\pm 8.2)	0.94
	<i>Ifnlr1</i> ^{-/-}	91.4 (\pm 21.9)		134.8 (\pm 32.1)	
MIP-1a	WT	19.8 (\pm 0.9)	0.07	18.1 (\pm 1.4)	0.47
	<i>Ifnlr1</i> ^{-/-}	16.1 (\pm 1.5)		20.2 (\pm 2.4)	
MIP-1b	WT	28.3 (\pm 6.7)	0.59	33 (\pm 5.2)	0.04
	<i>Ifnlr1</i> ^{-/-}	23.3 (\pm 5.9)		56.4 (\pm 7.9)	
RANTES	WT	33.4 (\pm 3.5)	0.06	19.7 (\pm 0.9)	0.02
	<i>Ifnlr1</i> ^{-/-}	24.3 (\pm 2.3)		24.3 (\pm 1.2)	

TNF- α	WT	356.6 (\pm 62.1)	0.56	488.1 (\pm 57.7)	0.12
	<i>Ifnlr1</i> ^{-/-}	412.7 (\pm 67.1)		626.0 (\pm 53.0)	

Wildtype (WT) and *Ifnlr1*^{-/-} mice were infected with 10² PFU of WNV in the footpad. Serum was collected at 3 and 5 days after infection and the indicated cytokines were measured by Bio-Plex array. Data represent the mean (\pm SEM) in pg/ml of 5 mice per group. Statistical significance was determined using t-tests and the Holm-Sidak method to correct for multiple comparisons.

Table S3: Primer and probe sequences used for qRT-PCR.

Target		Sequence (5'-3')	Ref
<i>Ribosome 18s</i>	Fwd	CGGCTACCACATCCAAGGAA	
	Rev	GCTGGAATTACCGCGGCT	
	Probe	/56-FAM/TGCTGGCAC/ZEN/CAGACTTGCCC/3IABkFQ/	
<i>Gapdh</i>	Fwd	AATGGTGAAGGTCGGTGTG	(54)
	Rev	GTGGAGTCATACTGGAACATGTAG	
	Probe	/56-FAM/TGCAAATGG/ZEN/CAGCCCTGGTG/3IABkFQ/	
<i>Ifnar1</i>	Fwd	GTGGAATGAGGTTGATCCGTT	
	Rev	AGAGATGTGGACTAGTATGGCT	
	Probe	/56-FAM/CCCCCAGAA/ZEN/GTACGTTTAGAAGCTGAAG/3IABkFQ/	
<i>Ifnlr1</i>	Fwd	CCTGTACTCGTCCTTTGAACT	
	Rev	GTGACCTATTTTCGTGACCTACC	
	Probe	/56-FAM/TGTGTCCCC/ZEN/TGATGTGCCTGAAG/3IABkFQ/	
<i>Il10rb</i>	Fwd	GAAAGATGAGAGAAGTCGCACT	
	Rev	GAACATTCTACAGTGGGAGGT	
	Probe	/56-FAM/CCAAAACGA/ZEN/ACCTGACTTTCACAGCT/3IABkFQ/	
<i>Ifit1</i>	Fwd	TGAAGCAGATTCTCCATGACC	
	Rev	GCAAGAGAGCAGAGAGTCAAG	
	Probe	/56-FAM/ACAGCTACC/ZEN/ACCTTTACAGCAACCAT/3IABkFQ/	
<i>Rsad2</i>	Fwd	ACACAGCCAAGACATCCTTC	(54)
	Rev	CAAGTATTCACCCCTGTCCTG	
	Probe	/56-FAM/TGTTTGAGCAGAAGCAGTCCTCGC/3IABkFQ/	
<i>Irf7</i>	Fwd	CTGGAGCCATGGGTATCGA	
	Rev	AAGCACAAGCCGAGACTGCT	
	Probe	/56-FAM/CTGGAGGGCGTGCAGCGTGA/36-TAMSp/	
<i>Ifnb</i>	Fwd	GGCTTCCATCATGAACAACAG	(54)
	Rev	GTTGATGGAGAGGGCTGTG	
	Probe	/56FAM/CTGCGTTCCTGCTGTGCTTCTC/36-TAMSp/	
<i>Ifna (non-a4)</i>	Fwd	ARSYTGSTGATGCARCAGGT	
	Rev	GGWACACAGTGATCCTGTGG	
	Probe	/56-FAM/CAGGAACCTCCTCTGACCCAGGA/36-TAMSp/	
<i>Il28b</i>	Fwd	GCTGAGTCATTTATGTTCTCCA	
	Rev	AGTTCCACCTCATCTCCA	
	Probe	/56FAM/CTGCAGGTC/ZEN/CAAGAGCGCC/3IABkFQ/	
<i>WNV-NY (E gene)</i>	Fwd	TCAGCGATCTCTCCACCAAAG	(49)
	Rev	GGGTCAGCACGTTTGTGATTG	
	Probe	/56-FAM/TGCCCCGACCATGGGAGAAGCTC/36-TAMSp/	