

mLN fold difference gene expression to WT SPF																																		
gene	WT SPF			WT GF			WT ABX						<i>Ifnb1</i> ^{-/-} SPF				<i>Ifnb1</i> ^{-/-} ABX																	
	Mean	SEM	N	Mean	SEM	N	p value to WT SPF			Mean	SEM	N	p value to WT SPF			p value to <i>Ifnb1</i> ^{-/-} SPF			Mean	SEM	N	p value to WT SPF			p value to <i>Ifnb1</i> ^{-/-} ABX									
<i>Mx1</i>	1.02	0.04	25	0.73	0.14	7	0.0311 (*)			0.68	0.07	13	0.0073 (**)			0.7648 (ns)			0.83	0.06	17	0.1552 (ns)			17	0.69	0.09	8	0.0419 (*)			0.8966 (ns)		
<i>Ifit1</i>	1.04	0.06	27	0.83	0.08	11	0.0527 (ns)			0.61	0.06	12	0.0002 (***)			0.9997 (ns)			0.63	0.05	21	<0.0001 (****)			21	0.67	0.09	7	0.0138 (*)			0.9979 (ns)		
<i>Ifit2</i>	1.03	0.04	27	0.81	0.07	11	0.0311 (*)			0.75	0.11	12	0.0797 (ns)			0.9791 (ns)			0.68	0.04	21	0.0025 (**)			21	0.72	0.22	8	0.1184 (ns)			0.9978 (ns)		
<i>Ifit3</i>	1.04	0.06	27	0.68	0.07	11	0.0046 (**)			0.58	0.10	12	<0.0001 (****)			0.9986 (ns)			0.56	0.04	21	<0.0001 (****)			21	0.43	0.07	7	<0.0001 (****)			0.8118 (ns)		
<i>Oasl2</i>	1.01	0.03	26	0.73	0.06	11	0.0005 (****)			0.66	0.06	11	<0.0001 (****)			>0.9999 (ns)			0.66	0.03	21	<0.0001 (****)			21	0.66	0.08	8	<0.0001 (****)			>0.9999 (ns)		
<i>Rsad2</i>	1.03	0.05	25	0.74	0.06	11	0.0047 (**)			0.63	0.06	10	<0.0001 (****)			0.8457 (ns)			0.56	0.03	20	<0.0001 (****)			20	0.56	0.05	7	<0.0001 (****)			>0.9999 (ns)		
<i>Irf7</i>	1.02	0.03	27	0.73	0.05	11	0.0005 (****)			0.77	0.06	12	0.0009 (***)			0.8858 (ns)			0.69	0.03	21	<0.0001 (****)			21	0.75	0.05	8	0.0024 (**)			0.901 (ns)		
<i>Gbp4</i>	1.01	0.03	28	0.78	0.06	14	0.0009 (****)			0.81	0.07	12	0.0197 (*)			0.3071 (ns)			0.96	0.05	19	0.6544 (ns)			19	0.85	0.06	8	0.1917 (ns)			0.7471 (ns)		

Table S1. Statistical analysis of mLN ISG expression. Related to Figure 1. mLNs were harvested from age- and gender-matched C57BL/6 WT GF mice and from WT or *Ifnb1*^{-/-} SPF mice with and without broad spectrum antibiotics (ABX) treatment. RNA was isolated from whole tissue samples and qRT-PCR was performed to analyze ISG expression levels. Fold change gene expression was calculated compared to WT SPF mice using the $\Delta\Delta CT$ method, with *ActB* as the reference gene. Statistical analysis with unpaired t-test (WT GF) or one-way ANOVA followed by Tukey's multiple comparisons test (WT ABX, *Ifnb1*^{-/-} SPF, *Ifnb1*^{-/-} ABX). N= number of samples analyzed, SEM=standard error of the mean, ns= not significant, *p<0.05, **p<0.01, ***p<0.001, **** p<0.0001.

Spleen fold difference gene expression to WT SPF																																		
gene	WT SPF			WT GF			WT ABX						<i>Ifnb1</i> ^{-/-} SPF				<i>Ifnb1</i> ^{-/-} ABX																	
	Mean	SEM	N	Mean	SEM	N	p value to WT SPF			Mean	SEM	N	p value to WT SPF			p value to <i>Ifnb1</i> ^{-/-} SPF			Mean	SEM	N	p value to WT SPF			p value to <i>Ifnb1</i> ^{-/-} ABX									
<i>Mx1</i>	1.10	0.09	38	0.67	0.08	21	0.0208 (*)			0.83	0.07	15	0.2171 (ns)			0.0658 (ns)			0.47	0.04	25	<0.0001 (****)			17	0.47	0.07	11	0.0002 (***)			>0.9999 (ns)		
<i>Ifit1</i>	1.07	0.07	29	1.00	0.17	8	0.9639 (ns)			0.61	0.05	20	<0.0001 (****)			0.871 (ns)			0.52	0.05	23	<0.0001 (****)			21	0.37	0.06	11	<0.0001 (****)			0.7177 (ns)		
<i>Ifit2</i>	1.04	0.06	31	1.10	0.14	16	0.9639 (ns)			0.87	0.07	18	0.5135 (ns)			0.2127 (ns)			0.64	0.06	20	0.0012 (**)			21	0.56	0.13	8	0.0085 (**)			0.9879 (ns)		
<i>Ifit3</i>	1.02	0.04	41	0.81	0.05	24	0.0063 (**)			0.75	0.05	21	0.0001 (***)			0.5977 (ns)			0.58	0.04	27	<0.0001 (****)			21	0.43	0.07	11	<0.0001 (****)			0.7042 (ns)		
<i>Oasl2</i>	1.03	0.04	38	0.93	0.07	21	0.6714 (ns)			0.59	0.07	21	<0.0001 (****)			>0.9999 (ns)			0.54	0.05	27	<0.0001 (****)			21	0.43	0.05	11	<0.0001 (****)			0.5122 (ns)		
<i>Rsad2</i>	1.08	0.09	27	0.93	0.13	13	0.8667 (ns)			0.25	0.04	13	<0.0001 (****)			0.1149 (ns)			0.63	0.11	20	0.0064 (**)			20	0.31	0.07	7	0.0007 (***)			0.4625 (ns)		
<i>Irf7</i>	1.03	0.04	38	1.06	0.08	21	0.9639 (ns)			0.69	0.03	21	<0.0001 (****)			0.83 (ns)			0.62	0.05	27	<0.0001 (****)			21	0.46	0.05	11	<0.0001 (****)			0.3749 (ns)		
<i>Gbp4</i>	1.02	0.04	27	0.65	0.05	15	<0.0001 (****)			0.89	0.04	21	0.2753 (ns)			0.9962 (ns)			0.92	0.07	16	0.5894 (ns)			19	0.85	0.07	11	0.2112 (ns)			0.9356 (ns)		

Table S2. Statistical analysis of spleen ISG expression. Related to Figure 1. Spleens were harvested from age- and gender-matched C57BL/6 WT GF mice and from WT or *Ifnb1*^{-/-} SPF mice with and without broad spectrum antibiotics (ABX) treatment. RNA was isolated from whole tissue samples and qRT-PCR was performed to analyze ISG expression levels. Fold change gene expression was calculated compared to WT SPF mice using the $\Delta\Delta CT$ method, with *ActB* as the reference gene. Statistical analysis with unpaired t-test (WT GF) or one-way ANOVA followed by Tukey's multiple comparisons test (WT ABX, *Ifnb1*^{-/-} SPF, *Ifnb1*^{-/-} ABX). N= number of samples analyzed, SEM=standard error of the mean, ns= not significant, *p<0.05, **p<0.01, ***p<0.001, **** p<0.0001.

mLN fold difference gene expression to WT SPF												
gene	WT SPF			WT SPF+Met				<i>Tlr4</i> ^{-/-} SPF				
	Mean	SEM	N	Mean	SEM	N	p value to WT SPF	Mean	SEM	N	p value to WT SPF	
<i>Mx1</i>	1.03	0.08	12	0.74	0.08	10	0.0168 (*)	0.57	0.14	6	0.0079 (**)	
<i>Ifit1</i>	1.04	0.09	12	0.72	0.10	8	0.0354 (*)	0.65	0.13	6	0.0218 (*)	
<i>Ifit2</i>	1.03	0.08	12	0.68	0.09	8	0.0113 (*)	0.62	0.12	6	0.0113 (*)	
<i>Ifit3</i>	1.04	0.09	12	0.67	0.11	8	0.0201 (*)	0.76	0.24	6	0.2109 (ns)	
<i>Oasl2</i>	1.01	0.03	12	0.83	0.05	8	0.0082 (**)	0.76	0.10	6	0.0107 (*)	
<i>Rsad2</i>	1.02	0.06	12	0.75	0.05	7	0.0286 (*)	0.72	0.14	6	0.0363 (*)	
<i>Irf7</i>	1.02	0.05	12	0.85	0.07	10	0.0805 (ns)	0.86	0.08	6	0.1074 (ns)	

Table S3. Statistical analysis of mLN ISG expression. Related to Figure 4. mLN s were harvested from age- and gender-matched WT SPF mice with and without metronidazole (Met) treatment and *Tlr4*^{-/-} SPF mice. RNA was isolated from whole tissue samples and qRT-PCR was performed to analyze ISG expression levels. Fold change gene expression was calculated compared to WT SPF mice using the $\Delta\Delta CT$ method, with *ActB* as the reference gene. N= number of samples analyzed, SEM=standard error of the mean, ns= not significant, *p<0.05, **p<0.01.

Spleen fold difference gene expression to WT SPF												
gene	WT SPF			WT SPF+Met				<i>Tlr4</i> ^{-/-} SPF				
	Mean	SEM	N	Mean	SEM	N	p value to WT SPF	Mean	SEM	N	p value to WT SPF	
<i>Mx1</i>	1.17	0.19	18	0.78	0.08	12	0.1179 (ns)	0.74	0.14	8	0.1676 (ns)	
<i>Ifit1</i>	1.09	0.13	15	0.57	0.13	8	0.0175 (*)	0.63	0.16	11	0.03 (*)	
<i>Ifit2</i>	1.04	0.08	18	0.69	0.07	12	0.0033 (**)	0.54	0.11	8	0.0011 (**)	
<i>Ifit3</i>	1.06	0.09	18	0.76	0.06	12	0.0273 (*)	1.01	0.14	8	0.7998 (ns)	
<i>Oasl2</i>	1.02	0.05	18	0.74	0.07	12	0.002 (**)	0.93	0.06	8	0.2953 (ns)	
<i>Rsad2</i>	1.11	0.12	16	0.46	0.09	12	0.0005 (***)	0.35	0.05	7	0.0007 (***)	
<i>Irf7</i>	1.02	0.06	13	0.68	0.07	12	0.0011 (**)	0.72	0.13	4	0.0322 (*)	

Table S4. Statistical analysis of spleen ISG expression. Related to Figure 4. Spleens were harvested from age- and gender-matched WT SPF mice with and without metronidazole (Met) treatment and *Tlr4*^{-/-} SPF mice. RNA was isolated from whole tissue samples and qRT-PCR was performed to analyze ISG expression levels. Fold change gene expression was calculated compared to WT SPF mice using the $\Delta\Delta CT$ method, with *ActB* as the reference gene. N= number of samples analyzed, SEM=standard error of the mean, ns= not significant, *p<0.05, **p<0.01, ***p<0.001.

Gene	Forward Primer	Reverse Primer	Source
<i>Ifit1</i>	5'-CAGAAGCACACATTGAAGAA-3'	5'-TGTAAGTAGCCAGAGGAAGG-3'	Fensterl et al., 2008
<i>Ifit2</i>	5'-GGGAAAGCAGAGGAAATCAA-3'	5'-TGAAAGTTGCCATACAGAAG-3'	Fensterl et al., 2008
<i>Ifit3</i>	5'-GCCGTTACAGGGAAATACTGG-3'	5'-CCTCAACATCGGGGCTCT-3'	Fensterl et al., 2008
<i>Oasl2</i>	5'-GGATGCCTGGGAGAGAATCG-3'	5'-TCGCCTGCTCTTCGAAACTG-3'	Sommereynset et al., 2008
<i>Rsad2</i>	5'-AACAGGCTGGTTTGGAGAAG-3'	5'-TGCCATTGCTCACTATGCTC-3'	this paper
<i>Irf7</i>	5'-GCCAGGAGCAAGACCGTGTT-3'	5'-TGCCCCACCACTGCCTGTA-3'	Bordignon et al., 2008
<i>Gbp4</i>	5'-TGGGGACACAGGCTCTACA-3'	5'-GCCTGCAGGATGGA ACTCTCAA-3'	Bordignon et al., 2008
<i>ifnb1</i>	5'-CCTACAGGGCGGACTTCAAG-3'	5'-GGATGGCAAAGGCAGTGTA ACT-3'	Chirido et al., 2005
<i>Bst2</i>	5'-GAAGTCACGAAGCTGAACCA-3'	5'-CCTGCACTGTGCTAGAAGTCTC-3'	Holmgren et al., 2015
<i>Actb</i>	5'-GATGCTCCCCGGGCTGTATT-3'	5'-GGGGTACTTCAGGGTCAGGA-3'	Diegelmann et al., 2012

Table S5. qRT-PCR primer sequences. Related to STAR methods.