

Vaccinia Virus Expressing Interferon Regulatory Factor 3 Induces Higher Protective Immune Responses against Lethal Poxvirus Challenge in Atopic Organism

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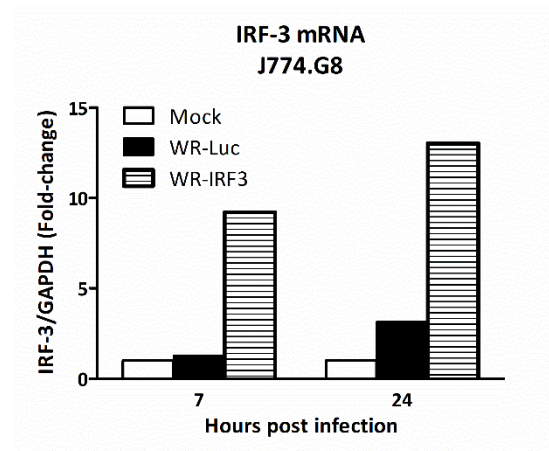
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Supplements

Figure S1

(a)



(b)

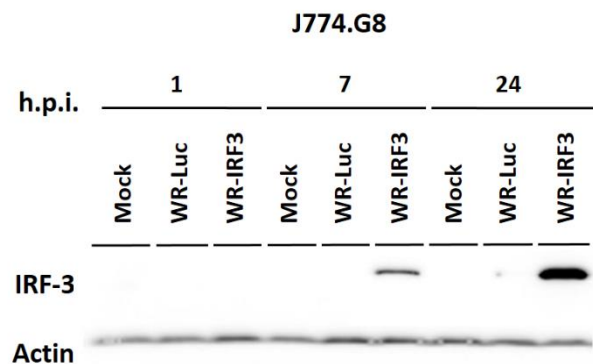


Figure S1. Expression of mouse IRF-3 by WR-IRF3 in J774.G8 monocyte/macrophage cell line. The cells were mock-infected or infected with WR-Luc or WR-IRF3 at M.O.I. 1. At individual time points, the cells were collected and used for further analysis. (a) 1-step RT-qPCR analysis at 24 h.p.i.. IRF-3 mRNA was normalized to GAPDH mRNA and expressed relatively as a fold-change compared to mock-infected cells. (b) Western blot analysis. The cell lysates were resolved by 12 % SDS-PAGE. IRF-3 and control β -actin were detected by western blot analysis and chemiluminescence. Mock – mock-infected cells; WR-Luc – cells infected with VACV expressing luciferase; WR-IRF3 – cells infected with VACV expressing Mu IRF-3.

Figure S2

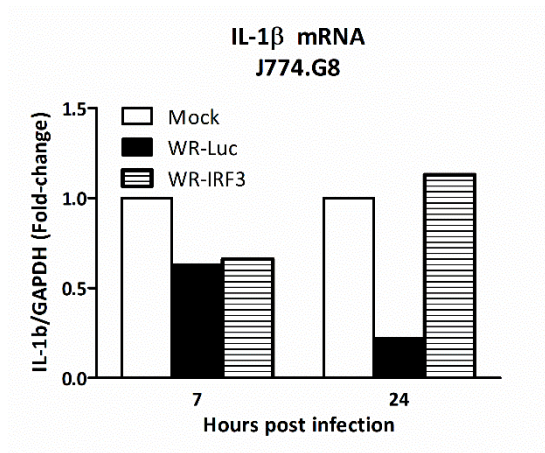


Figure S2. Infection with WR-IRF3 increases mRNA levels of IL-1 β in J774.G8 monocyte/macrophage cell line. The cells were mock-infected or infected with WR-Luc or WR-IRF3 at M.O.I. 1. At indicated time points, the cells were collected and used for 1-step RT-qPCR analysis. IL-1 β mRNA was normalized to GAPDH mRNA and expressed relatively as a fold-change compared to mock-infected cells. Mock – mock-infected cells; WR-Luc – cells infected with VACV expressing luciferase; WR-IRF3 – cells infected with VACV expressing Mu IRF-3.

Figure S3

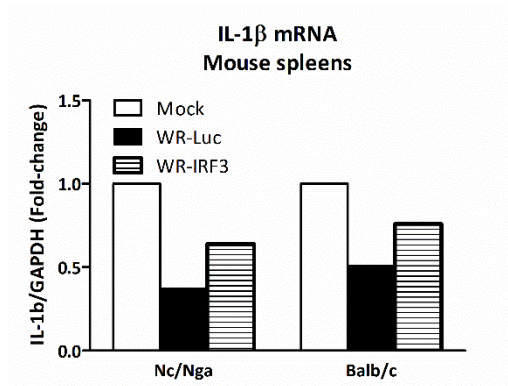


Figure S3. Intranasal infection with WR-IRF3 supports higher mRNA levels of IL-1 β in spleens of Nc/Nga and Balb/c mice. The mice were i.n. mock-infected with PBS or infected with 4.37×10^5 PFU of a purified stock of WR-Luc or WR-IRF3. At 3 days p.i., the mice were sacrificed and spleens were used for 1-step RT-qPCR analysis. IL-1 β mRNA was normalized to GAPDH mRNA and expressed relatively as a fold-change compared to mock-infected samples. n = 3 mice/group. Mock – mock-infected mice; WR-Luc – mice infected with VACV expressing luciferase; WR-IRF3 – mice infected with VACV expressing Mu IRF-3.

Figure S4

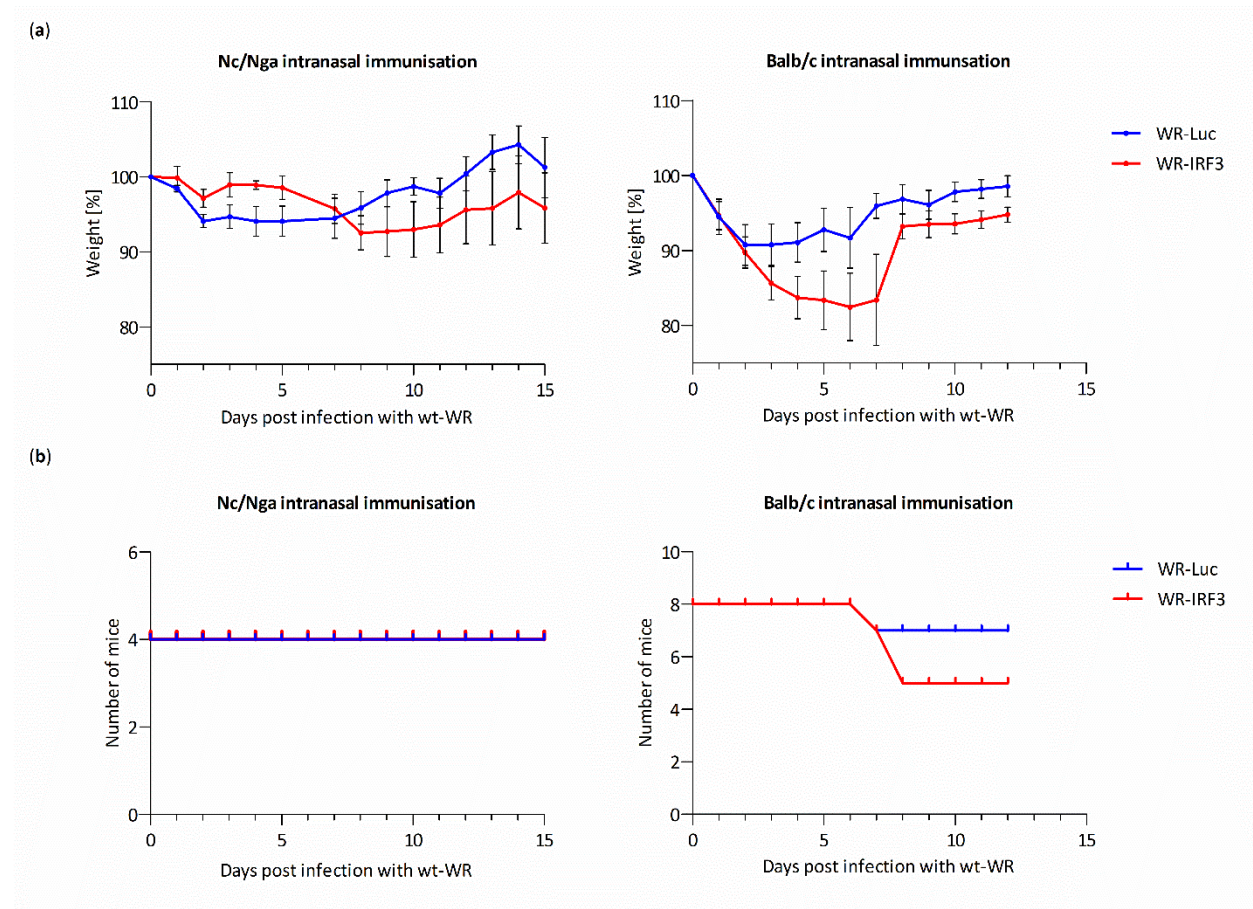
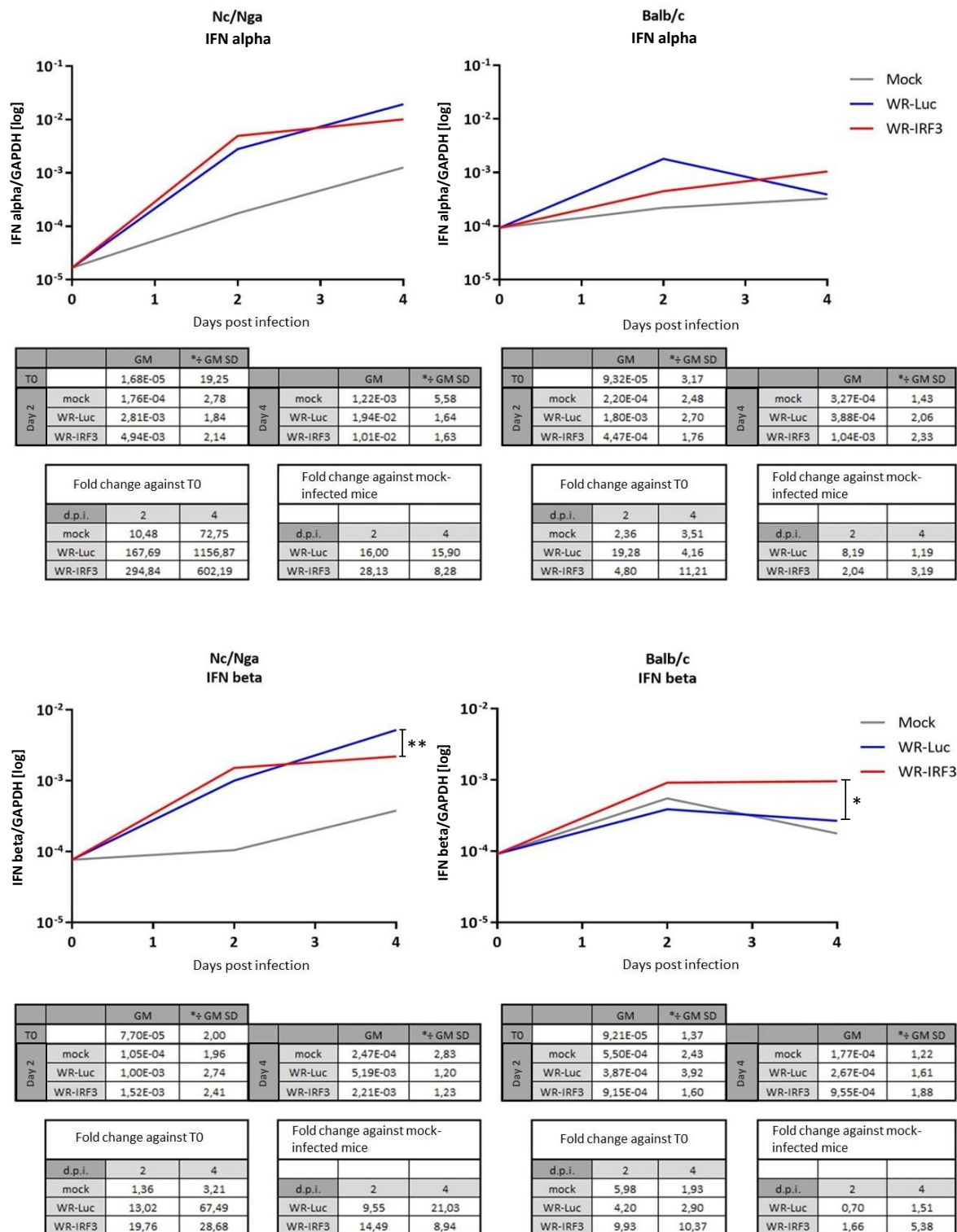
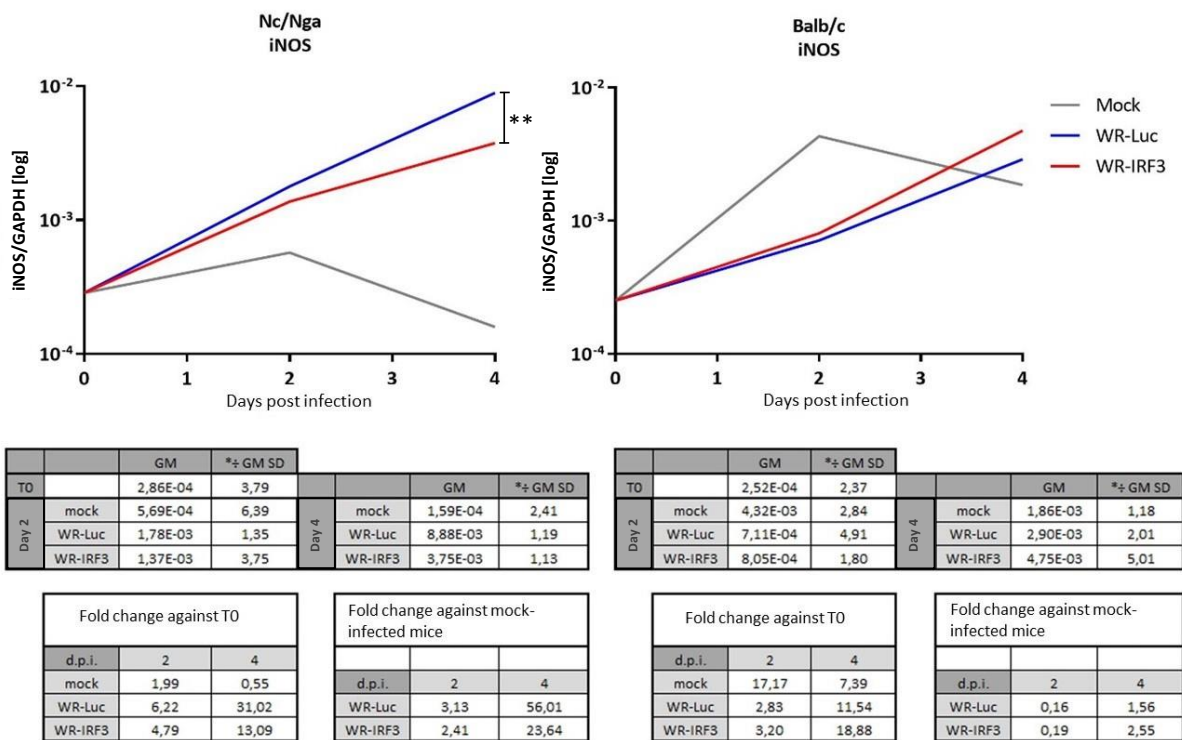
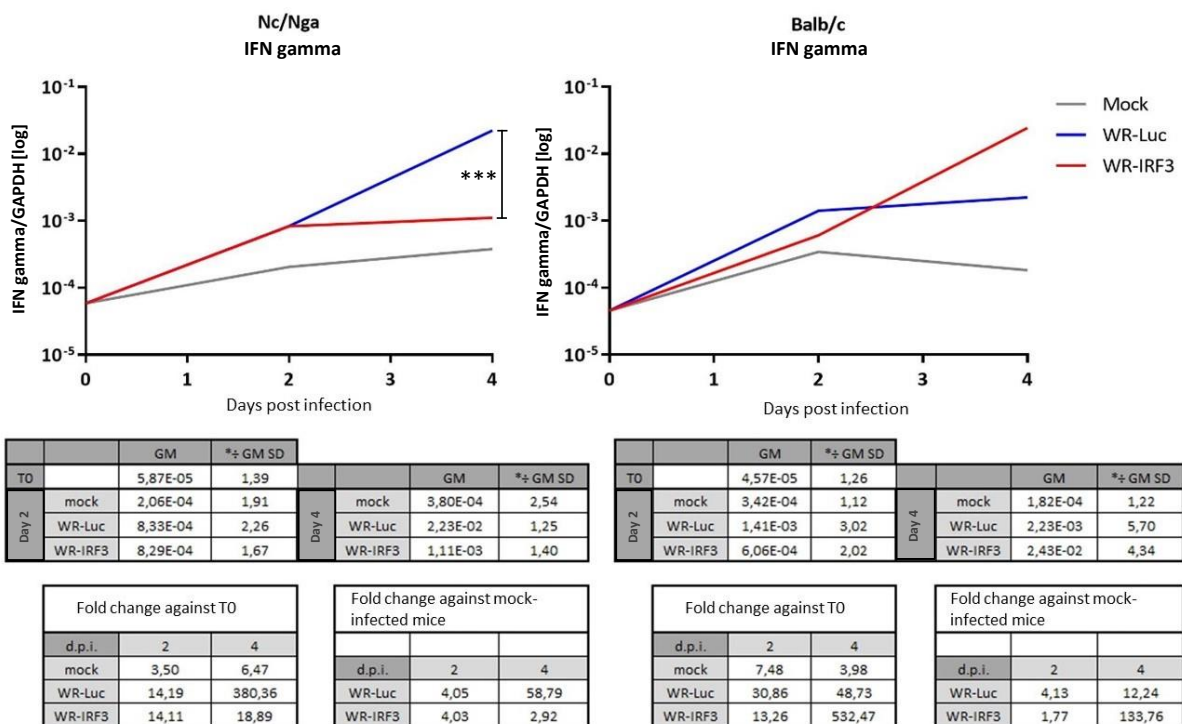


Figure S4. Intranasal immunization with WR-Luc and WR-IRF3 and protective immunity against a lethal challenge with wt-WR. Nc/Nga and Balb/c mice were immunized i.n. (10^4 PFU/mouse) with purified WR-Luc or WR-IRF3. 24 days later (day 0 in the graphs), they were infected i.n. with a lethal dose of wt-WR (10^5 PFU/mouse). Changes in body weight (a) and survival (b) of mice are presented. (a) Graphs represent means \pm S.E.M.

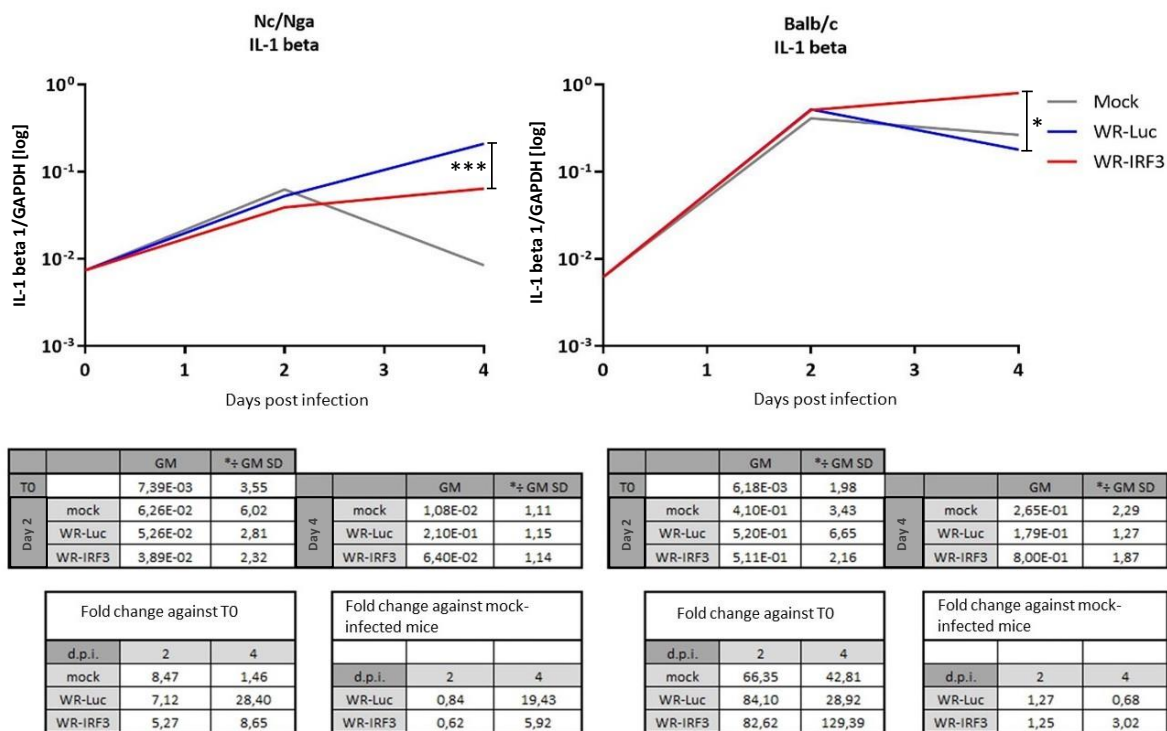
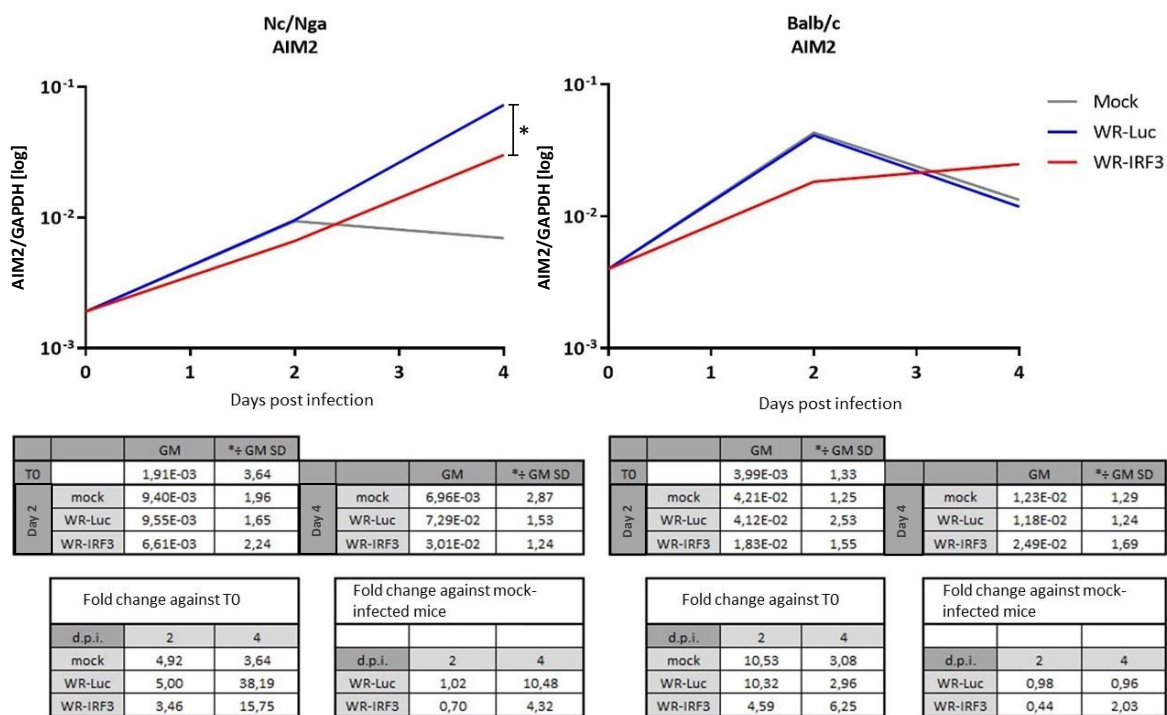
Figure S5
(a)



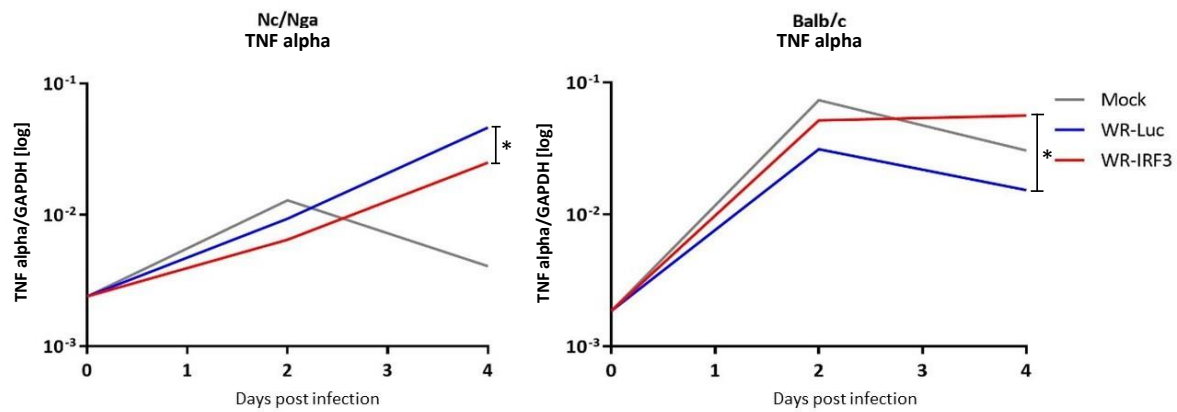
(b)



(c)



(d)



		GM	*± GM SD			GM	*± GM SD
Day 2	TO	2,39E-03	2,14	Day 4	mock	4,06E-03	1,16
	mock	1,29E-02	3,31		WR-Luc	4,62E-02	1,18
	WR-Luc	9,36E-03	1,26		WR-IRF3	2,50E-02	1,24
	WR-IRF3	6,48E-03	2,04				

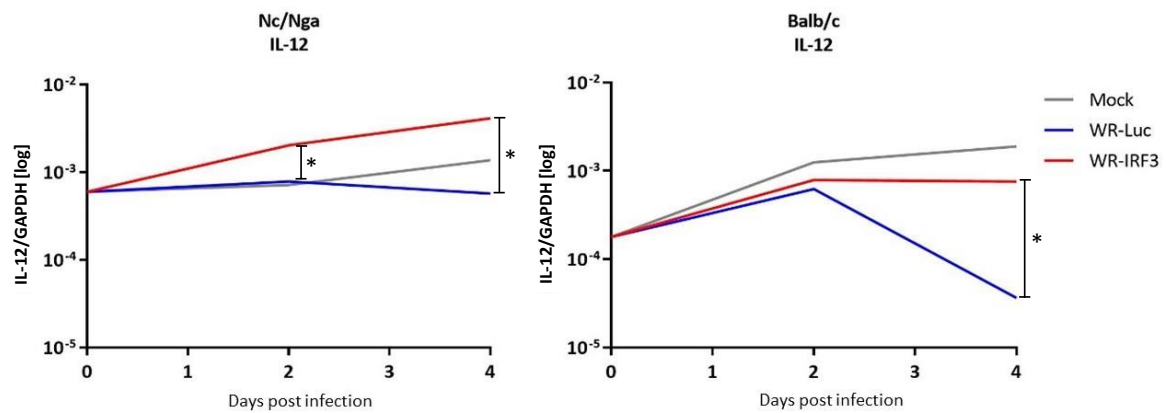
Fold change against T0		
d.p.i.	2	4
mock	5,41	1,70
WR-Luc	3,92	19,32
WR-IRF3	2,71	10,48

Fold change against mock-infected mice		
d.p.i.	2	4
WR-Luc	0,72	11,37
WR-IRF3	0,50	6,17

		GM	*± GM SD			GM	*± GM SD
Day 2	TO	1,86E-03	1,42	Day 4	mock	3,05E-02	2,57
	mock	7,35E-02	4,61		WR-Luc	1,53E-02	1,61
	WR-Luc	3,12E-02	5,42		WR-IRF3	5,62E-02	1,79
	WR-IRF3	5,15E-02	1,70				

Fold change against T0		
d.p.i.	2	4
mock	39,47	16,37
WR-Luc	16,76	8,21
WR-IRF3	27,64	30,19

Fold change against mock-infected mice		
d.p.i.	2	4
WR-Luc	0,42	0,50
WR-IRF3	0,70	1,84



		GM	*± GM SD			GM	*± GM SD
Day 2	TO	5,96E-04	3,25	Day 4	mock	1,37E-03	1,50
	mock	7,15E-04	1,75		WR-Luc	5,70E-04	1,95
	WR-Luc	7,82E-04	1,57		WR-IRF3	4,12E-03	1,94
	WR-IRF3	2,02E-03	1,39				

Fold change against T0		
d.p.i.	2	4
mock	1,20	2,29
WR-Luc	1,31	0,96
WR-IRF3	3,40	6,91

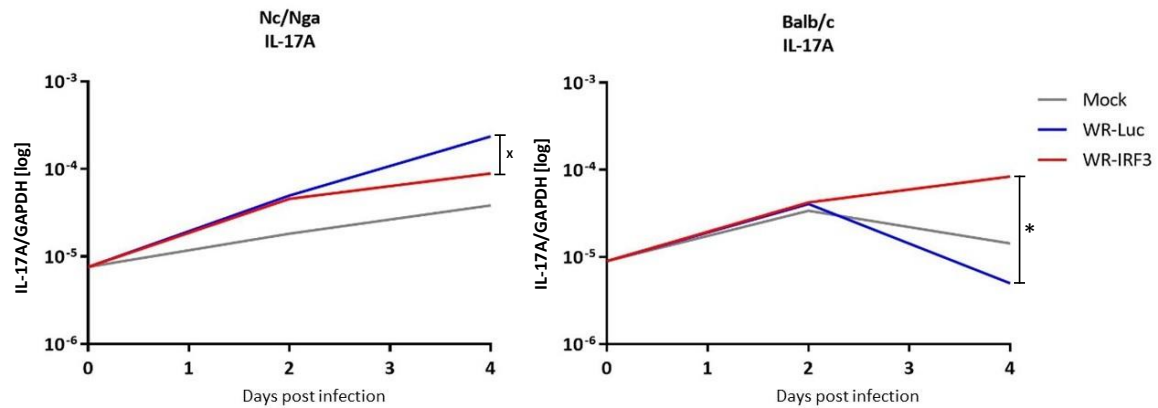
Fold change against mock-infected mice		
d.p.i.	2	4
WR-Luc	1,09	0,42
WR-IRF3	2,83	3,02

		GM	*± GM SD			GM	*± GM SD
Day 2	TO	1,78E-04	2,67	Day 4	mock	1,89E-03	1,52
	mock	1,25E-03	1,70		WR-Luc	3,65E-05	1,95
	WR-Luc	6,22E-04	10,14		WR-IRF3	7,58E-04	3,78
	WR-IRF3	7,88E-04	2,62				

Fold change against T0		
d.p.i.	2	4
mock	7,01	10,64
WR-Luc	3,50	0,21
WR-IRF3	4,44	4,26

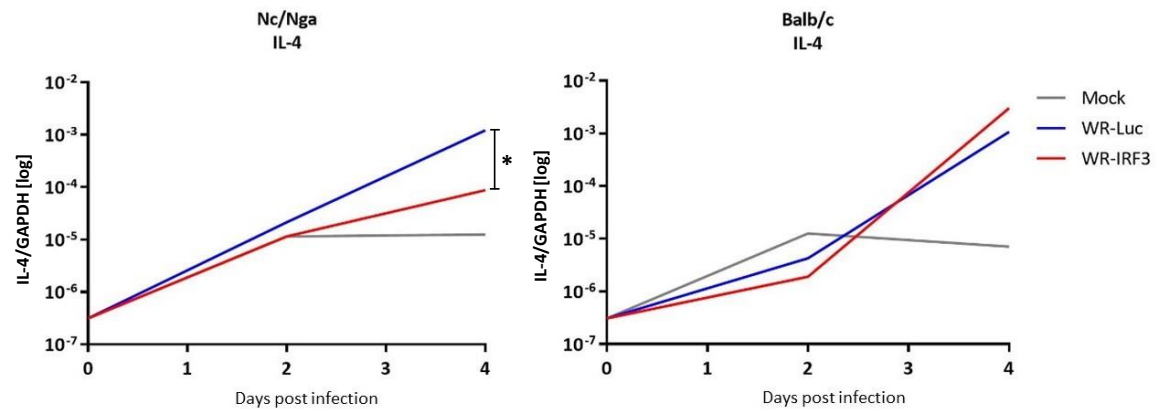
Fold change against mock-infected mice		
d.p.i.	2	4
WR-Luc	0,50	0,02
WR-IRF3	0,63	0,40

(e)



Nc/Nga IL-17A				Balb/c IL-17A			
		GM	*± GM SD			GM	*± GM SD
T0		7,61E-06	1,88	T0		5,08E-06	2,83
Day 2	mock	1,83E-05	1,41	Day 2	mock	3,39E-05	1,97
	WR-Luc	4,96E-05	2,55		WR-Luc	4,06E-05	3,93
	WR-IRF3	4,91E-05	2,62		WR-IRF3	4,24E-05	3,17
		GM				GM	
Day 4	mock	1,64E-05	2,07	Day 4	mock	1,43E-05	4,30
	WR-Luc	2,35E-04	1,80		WR-Luc	5,00E-06	3,48
	WR-IRF3	8,91E-05	1,61		WR-IRF3	8,38E-05	2,87

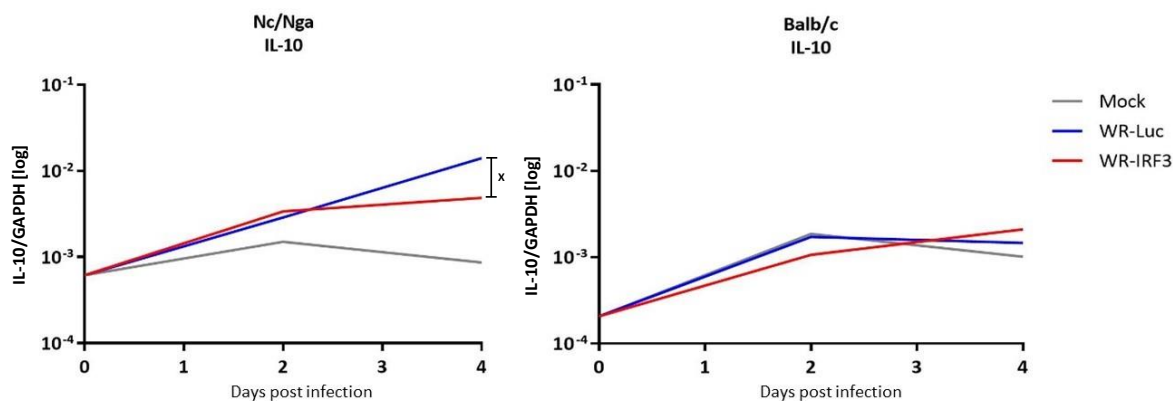
Nc/Nga IL-17A				Balb/c IL-17A			
Fold change against T0				Fold change against mock-infected mice			
d.p.i.	2	4		d.p.i.	2	4	
mock	2,40	2,16		WR-Luc	1,20	0,35	
WR-Luc	6,52	30,82		WR-IRF3	1,25	5,86	
WR-IRF3	6,45	11,71		WR-Luc	1,20	0,35	



Nc/Nga IL-4				Balb/c IL-4			
		GM	*± GM SD			GM	*± GM SD
T0		3,77E-07	2,23	T0		3,09E-07	28,50
Day 2	mock	1,15E-05	1,44	Day 2	mock	1,27E-05	3,05
	WR-Luc	2,13E-05	1,56		WR-Luc	1,38E-06	16,59
	WR-IRF3	1,15E-05	2,03		WR-IRF3	1,92E-06	36,01
		GM				GM	
Day 4	mock	1,25E-05	1,77	Day 4	mock	7,11E-06	2,49
	WR-Luc	1,22E-03	1,61		WR-Luc	1,07E-03	1,99
	WR-IRF3	8,81E-05	3,51		WR-IRF3	3,03E-03	2,53

Nc/Nga IL-4				Balb/c IL-4			
Fold change against T0				Fold change against mock-infected mice			
d.p.i.	2	4		d.p.i.	2	4	
mock	30,58	33,24		WR-Luc	1,85	97,41	
WR-Luc	56,63	3237,69		WR-IRF3	1,00	7,04	
WR-IRF3	30,62	233,96					

(f)



		GM	*± GM SD			GM	*± GM SD
T0		6,16E-04	1,99			2,06E-04	1,19
Day 2	mock	1,50E-03	1,68	Day 4	mock	8,65E-04	1,38
	WR-Luc	2,87E-03	1,68		WR-Luc	1,40E-02	2,03
	WR-IRF3	3,38E-03	3,86		WR-IRF3	4,86E-03	1,41

		GM	*± GM SD
T0		1,86E-03	1,55
Day 2	mock	1,01E-03	1,21
	WR-Luc	1,46E-03	1,36
	WR-IRF3	2,10E-03	1,81

Fold change against T0		
d.p.i.	2	4
mock	2,43	1,41
WR-Luc	4,65	22,66
WR-IRF3	5,49	7,89

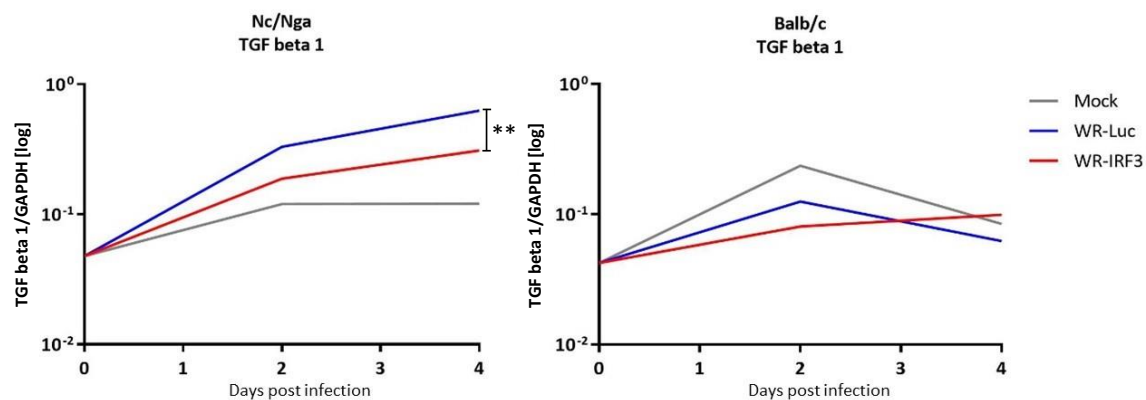
Fold change against mock-infected mice		
d.p.i.	2	4
WR-Luc	1,91	16,12
WR-IRF3	2,25	5,61

		GM	*± GM SD
T0		4,22E-02	1,49
Day 2	mock	2,36E-01	1,37
	WR-Luc	1,25E-01	5,30
	WR-IRF3	8,06E-02	1,52

		GM	*± GM SD
T0		8,44E-02	3,93
Day 2	mock	6,23E-02	1,30
	WR-Luc	9,92E-02	2,10

Fold change against T0		
d.p.i.	2	4
mock	5,61	2,00
WR-Luc	2,97	1,48
WR-IRF3	1,91	2,35

Fold change against mock-infected mice		
d.p.i.	2	4
WR-Luc	0,53	0,74
WR-IRF3	0,34	1,18



		GM	*± GM SD			GM	*± GM SD
T0		4,77E-02	1,35			4,22E-02	1,49
Day 2	mock	1,20E-01	1,93	Day 4	mock	1,21E-01	3,73
	WR-Luc	3,31E-01	1,58		WR-Luc	6,28E-01	1,06
	WR-IRF3	1,88E-01	3,42		WR-IRF3	3,10E-01	1,27

		GM	*± GM SD
T0		4,22E-02	1,49
Day 2	mock	2,36E-01	1,37
	WR-Luc	1,25E-01	5,30
	WR-IRF3	8,06E-02	1,52

Fold change against T0		
d.p.i.	2	4
mock	2,52	2,53
WR-Luc	6,93	13,15
WR-IRF3	3,95	6,49

Fold change against mock-infected mice		
d.p.i.	2	4
WR-Luc	2,75	5,21
WR-IRF3	1,56	2,57

		GM	*± GM SD
T0		4,22E-02	1,49
Day 2	mock	2,36E-01	1,37
	WR-Luc	1,25E-01	5,30
	WR-IRF3	8,06E-02	1,52

		GM	*± GM SD
T0		8,44E-02	3,93
Day 2	mock	6,23E-02	1,30
	WR-Luc	9,92E-02	2,10

Fold change against T0		
d.p.i.	2	4
mock	5,61	2,00
WR-Luc	2,97	1,48
WR-IRF3	1,91	2,35

Fold change against mock-infected mice		
d.p.i.	2	4
WR-Luc	0,53	0,74
WR-IRF3	0,34	1,18

(g)

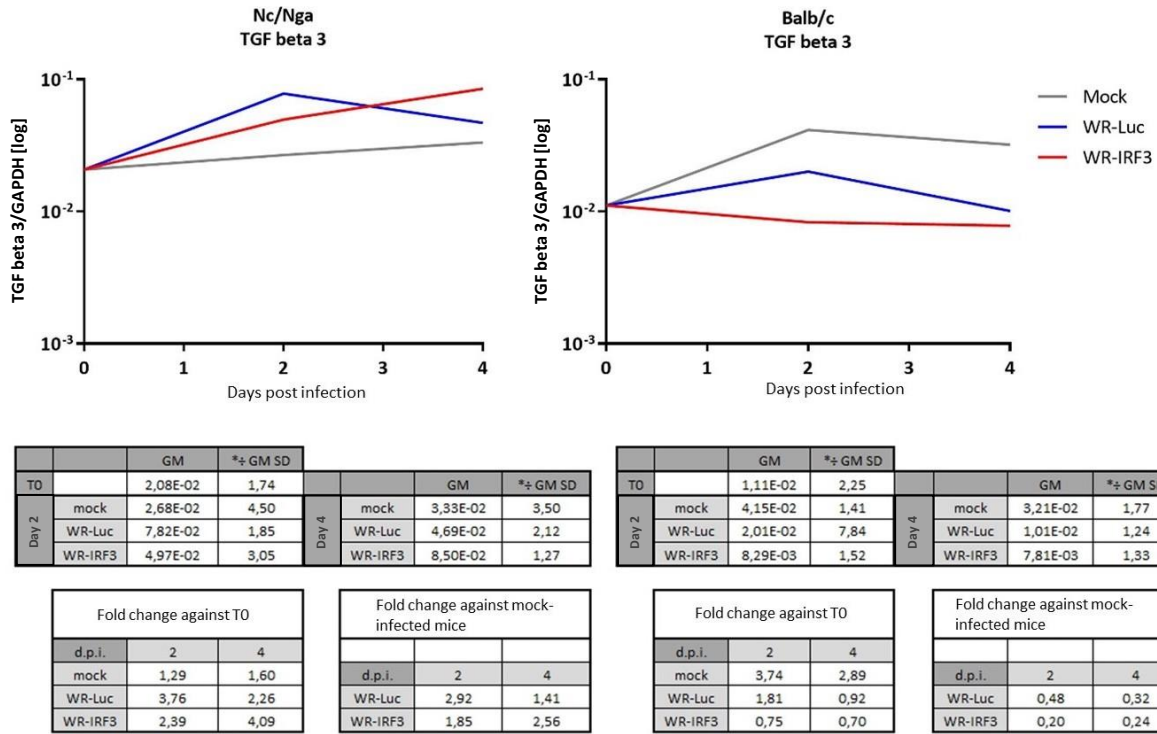


Figure S5. Changes in cytokine mRNA levels in mouse skin lesions. Nc/Nga and Balb/c mice were transdermally inoculated with PBS or 7.5×10^5 PFU/mouse of purified WR-Luc or WR-IRF3, sacrificed at indicated days p.i., and RNA from skin lesions used for 1-step RT-qPCR analysis. Cytokine mRNA was normalized to GAPDH mRNA and expressed as geometrical means; $n = 3$ mice/group. Statistically significant differences at * $P < 0.1$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (two-tailed unpaired Student t-test). GM \pm GM SD and relative changes compared to time 0 and to mock-infected samples are listed in accompanying tables. Mock – mock-inoculated mice (administration of PBS); WR-Luc – mice inoculated with VACV expressing luciferase; WR-IRF3 – mice inoculated with VACV expressing Mu IRF-3; T0 – time of inoculation; d.p.i. – days post infection; GM – geometrical mean, GM SD – geometric standard deviation.