Dcf1 deficiency attenuates the role of activated microglia during neuroinflammation

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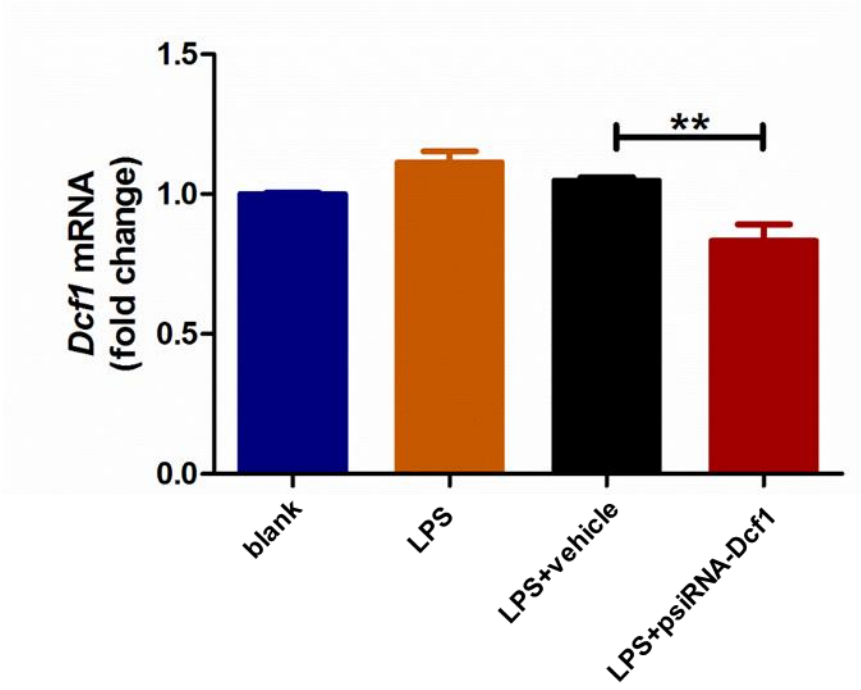
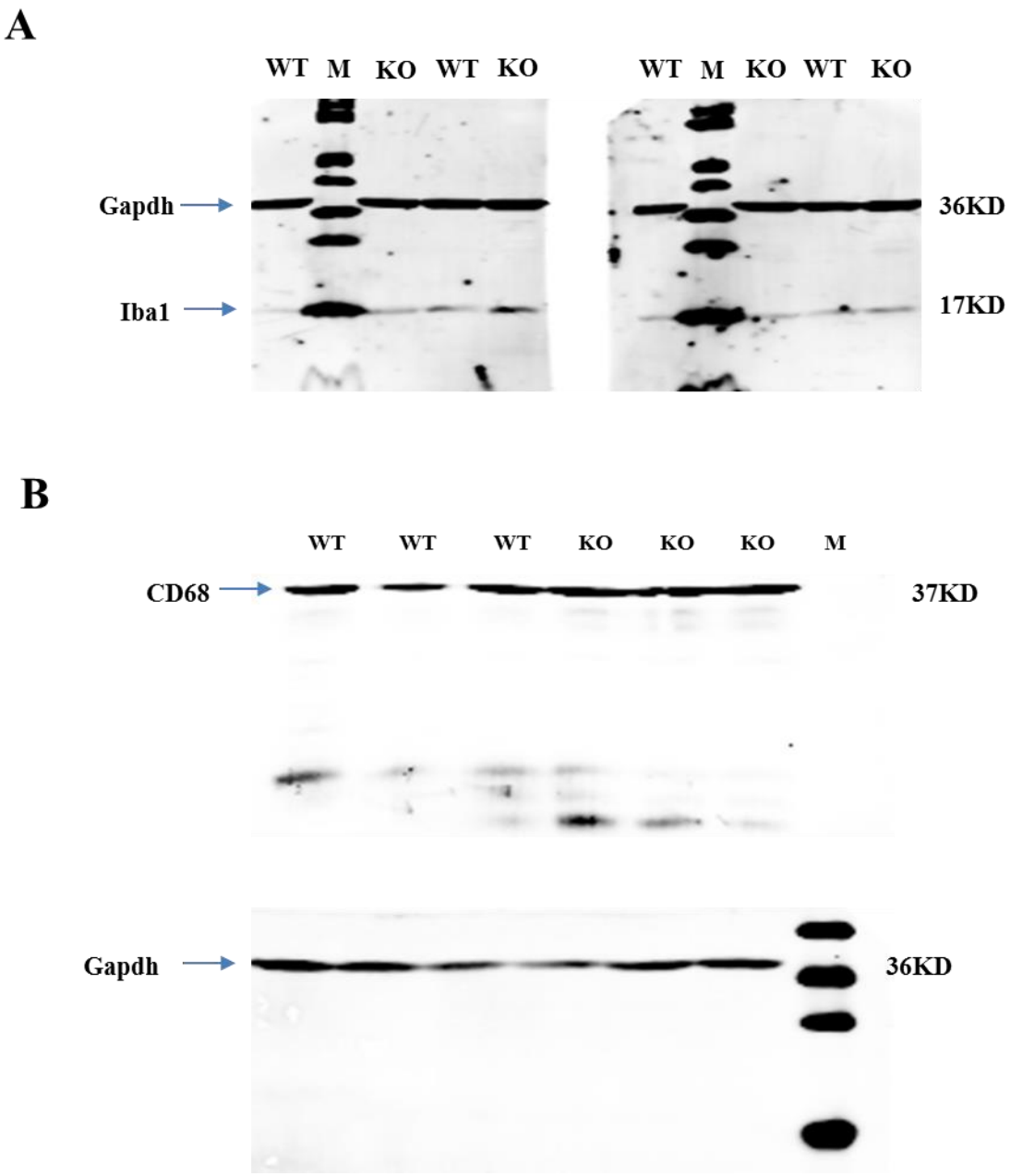
Supplementary Figures:



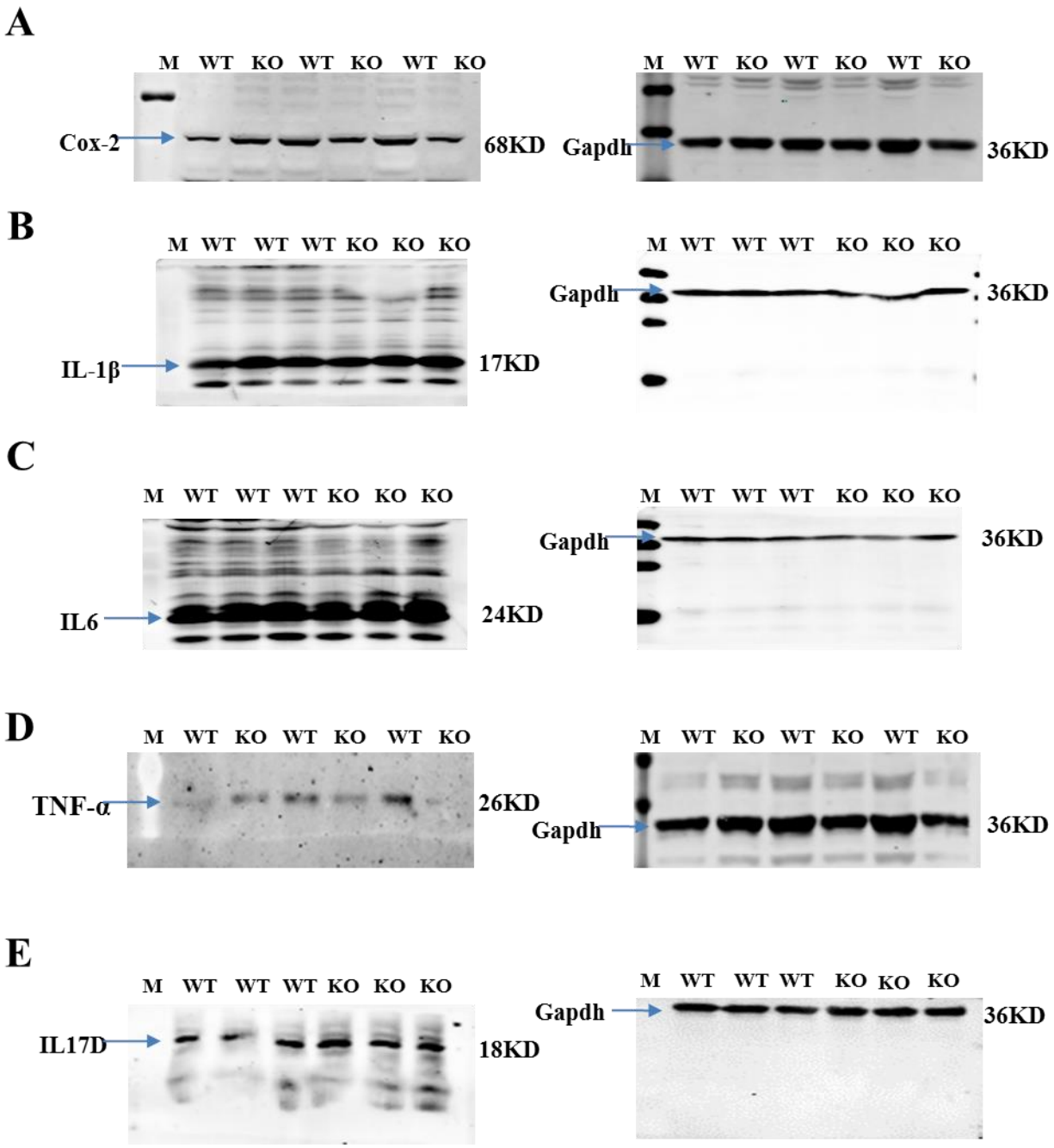
Supplementary Figure S1. *Dcf1* knockdown efficiency in BV2 cells.

Plasmids were transfected 48 h prior to treatment with LPS for 12 h. *Dcf1* knockdown identification by qPCR. Abundance of the *Dcf1* mRNA transcript is expressed relative to *Gapdh* as an internal control. Data were expressed as the mean ± SEM. n= 4. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ (**Supplementary Table 13)**.



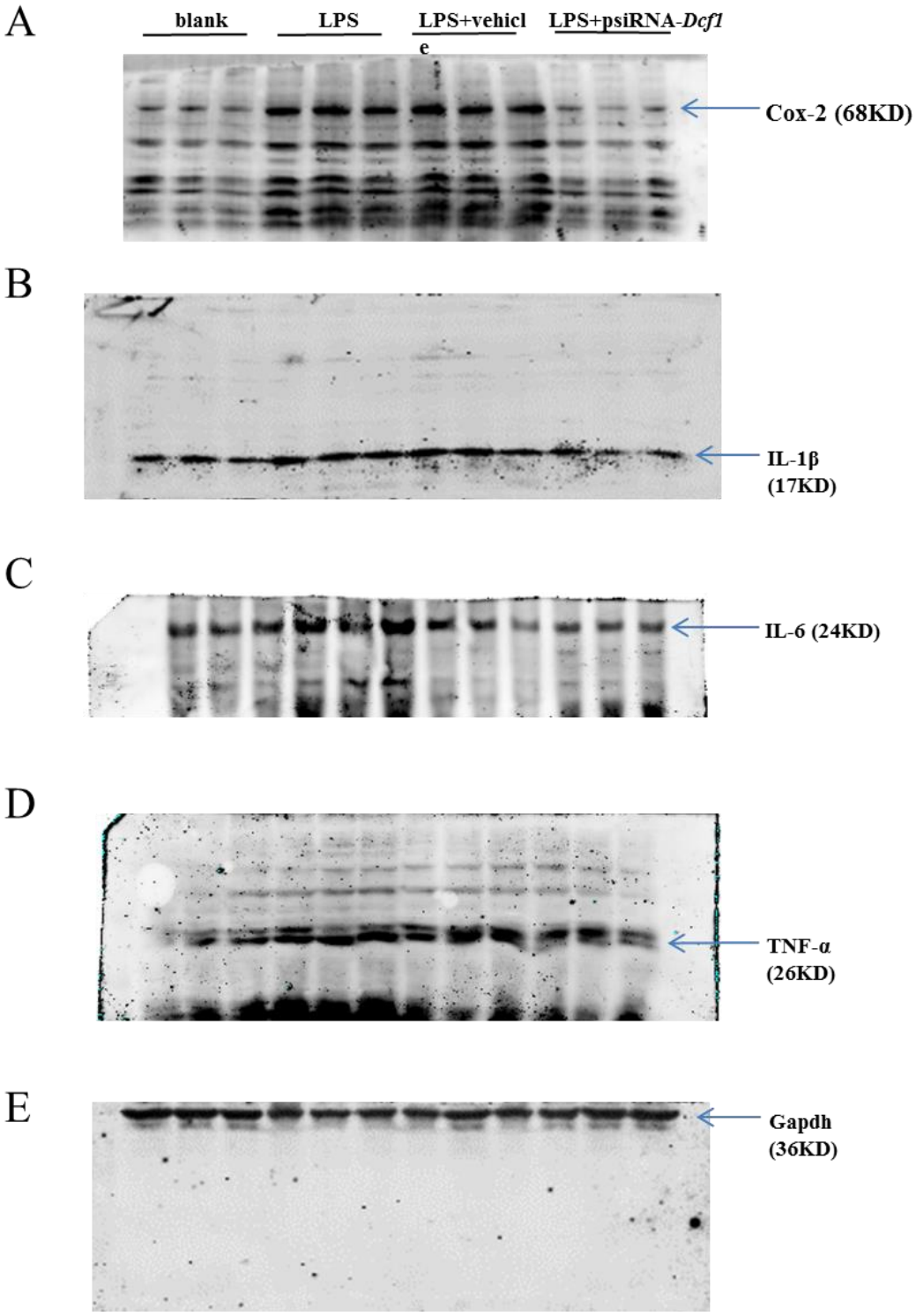
Supplementary Figure S2. *Dcf1* deletion induces upregulation of microglial activation markers *in vivo*

Protein expression of microglial activation markers Iba1 **(F)** and CD68 **(G)** in WT and *Dcf1*-KO mouse brain tissue. Quantification of protein expression levels normalized to Gapdh.



Supplementary Figure S3. Expression of proinflammatory cytokines in WT and *Dcf1*-KO mice

Protein expression of Cox-2**(A)**, IL-1β**(B)**, IL-6**(C)**, TNF-α**(D)** and IL17D**(E)**were assessed by Western blotting in WT and KO mice.



Supplementary Figure S4. Effects of *Dcf1* downregulation on proinflammatory cytokines expression in LPS-stimulated BV2 microglia

BV_2 microglial cells were transfected with the psiRNA-hH1neo plasmid or the psiRNA-*Dcf1* plasmid. 24 h post-transfection, BV2 microglia were stimulated with LPS (1000 ng/ml) and incubated for 12 h. Protein expression of COX-2**(A)**, IL-1β**(B)**, TNF-α **(C)** and IL-6**(D)** were assessed by Western blotting.

Table 1: t-test analysis of Figure. 2C

Unpaired t-test	p value	Significant? p<0.05	Summary
WT vs KO	0.0084	Yes	**

Data are expressed as the mean ± SEM. n = 3.

*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ vs. WT.

Table 2: t-test analysis of Figure. 2E

Unpaired t-test	p value	Significant? p<0.05	Summary
WT vs KO	0.0151	Yes	*

Data are expressed as the mean ± SEM. n = 4.

*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ vs. WT.

Table 3: t-test analysis of Figure. 2F

Unpaired t-test	p value	Significant? p<0.05	Summary
WT vs KO	0.0353	Yes	*

Data are expressed as the mean ± SEM. n = 3.

*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ vs. WT.

Table 4: t-test analysis of Figure. 2G

Unpaired t-test	p value	Significant? p<0.05	Summary
WT vs KO	0.0264	Yes	*

Data are expressed as the mean ± SEM. n = 3.

*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ vs. WT.

Table 5: t-test analysis of Figure. 3A

Unpaired t-test	p value	Significant? p<0.05	Summary
● *Cox2*:			
WT vs KO	0.0433	Yes	*
● *IL-1beta*:			
WT vs KO	0.0441	Yes	*
● *Tnfsf111*:			
WT vs KO	0.7862	no	ns
● *Cxcl1*:			
WT vs KO	0.2350	no	ns
● *Ccl7*:			
WT vs KO	0.0333	Yes	*
● *IL-6*:			
WT vs KO	0.0132	Yes	*
● *IL17D*:			
WT vs KO	0.0238	Yes	*
● *TNF-alpha*:			
WT vs KO	0.0364	Yes	*
● *Csf1*:			
WT vs KO	0.0200	Yes	*

Data are expressed as the mean ± SEM. n = 3.

*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ vs. WT.

Table 6: t-test analysis of Figure. 3B

Unpaired t-test	p value	Significant? p<0.05	Summary
● Cox2:			
WT vs KO	0.047751	Yes	*
● IL-1beta:			
WT vs KO	0.048958	Yes	*
● IL-6:			

WT vs KO	0.047189	Yes	*
● IL17D:			
WT vs KO	0.035709	Yes	*
● TNF-alpha:			
WT vs KO	0.012969	Yes	*

Data are expressed as the mean ± SEM. n = 3.

*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ vs. WT.

Table 7: t-test analysis of Figure. 4C

Unpaired t-test	**p value**	**Significant? p<0.05**	**Summary**
blank vs LPS	3.45x10^-5	Yes	***

Data are expressed as the mean ± SEM. n = 6.

*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ vs. blank.

Table 8: One-way ANOVA analysis of Figure. 4D

Bonferroni's Multiple Comparison Test	**Significant? p<0.05**	**Summary**
LPS vs LPS+vehicle	No	ns
LPS vs LPS+psiRNA-*Dcf1*	Yes	*
LPS+vehicle vs LPS+psiRNA-Dcf1	Yes	*

Data are expressed as the mean ± SEM. n = 6.

*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$

Table 9: One-way ANOVA analysis of Figure. 5A

Bonferroni's Multiple Comparison Test	**Significant? p<0.05**	**Summary**
● *Cox2*:		

blank vs LPS	Yes	**
blank vs LPS+vehicle	Yes	***
blank vs LPS+psiRNA-*Dcf1*	No	ns
LPS vs LPS+vehicle	No	ns
LPS vs LPS+psiRNA-*Dcf1*	Yes	*
LPS+vehicle vs LPS+psiRNA-*Dcf1*	Yes	##

- *IL-1β*:

blank vs LPS	Yes	*
blank vs LPS+vehicle	Yes	*
blank vs LPS+psiRNA-*Dcf1*	No	ns
LPS vs LPS+vehicle	No	ns
LPS vs LPS+psiRNA-*Dcf1*	No	ns
LPS+vehicle vs LPS+psiRNA-*Dcf1*	Yes	#

- *Tnfsf111*:

blank vs LPS	No	ns
blank vs LPS+vehicle	No	ns
blank vs LPS+psiRNA-*Dcf1*	No	ns
LPS vs LPS+vehicle	No	ns
LPS vs LPS+psiRNA-*Dcf1*	No	ns
LPS+vehicle vs LPS+psiRNA-*Dcf1*	No	ns

- *Cxcl1*:

blank vs LPS	Yes	*
blank vs LPS+vehicle	Yes	*
blank vs LPS+psiRNA-*Dcf1*	Yes	***
LPS vs LPS+vehicle	No	ns
LPS vs LPS+psiRNA-*Dcf1*	Yes	***
LPS+vehicle vs LPS+psiRNA-*Dcf1*	Yes	###

- *Ccl7*:

blank vs LPS	Yes	***
blank vs LPS+vehicle	Yes	**
blank vs LPS+psiRNA-*Dcf1*	Yes	***
LPS vs LPS+vehicle	No	ns
LPS vs LPS+psiRNA-*Dcf1*	No	ns
LPS+vehicle vs LPS+psiRNA-*Dcf1*	Yes	#

- *IL-6*:

blank vs LPS	Yes	*
blank vs LPS+vehicle	Yes	*
blank vs LPS+psiRNA-*Dcf1*	No	ns
LPS vs LPS+vehicle	No	ns

LPS vs LPS+psiRNA-*Dcf1*	No	ns
LPS+vehicle vs LPS+psiRNA-*Dcf1*	Yes	#
● *IL17D* :		
blank vs LPS	No	ns
blank vs LPS+vehicle	No	ns
blank vs LPS+psiRNA-*Dcf1*	No	ns
LPS vs LPS+vehicle	No	ns
LPS vs LPS+psiRNA-*Dcf1*	No	ns
LPS+vehicle vs LPS+psiRNA-*Dcf1*	No	ns
● *TNF-α* :		
blank vs LPS	Yes	*
blank vs LPS+vehicle	Yes	**
blank vs LPS+psiRNA-*Dcf1*	No	ns
LPS vs LPS+vehicle	No	ns
LPS vs LPS+psiRNA-*Dcf1*	No	ns
LPS+vehicle vs LPS+psiRNA-*Dcf1*	Yes	#
● *Csf1* :		
blank vs LPS	Yes	**
blank vs LPS+vehicle	Yes	**
blank vs LPS+psiRNA-*Dcf1*	No	ns
LPS vs LPS+vehicle	No	ns
LPS vs LPS+psiRNA-*Dcf1*	Yes	**
LPS+vehicle vs LPS+psiRNA-*Dcf1*	Yes	##

Data are expressed as the mean ± SEM. n = 4.
*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ vs.blank.
#, $p<0.05$; ##, $p<0.01$; ###, $p<0.001$ vs. LPS+vehicle.

Table 10: One-way ANOVA analysis of Figure. 5B

Bonferroni's Multiple Comparison Test	**Significant? p<0.05**	**Summary**
● Cox2:		
blank vs LPS	Yes	***
blank vs LPS+vehicle	Yes	**
blank vs LPS+psiRNA-*Dcf1*	No	ns
LPS vs LPS+vehicle	No	ns
LPS vs LPS+psiRNA-*Dcf1*	Yes	**
LPS+vehicle vs LPS+psiRNA-*Dcf1*	Yes	##

● IL-1β :		
blank vs LPS	Yes	**
blank vs LPS+vehicle	Yes	*
blank vs LPS+psiRNA-*Dcf1*	No	ns
LPS vs LPS+vehicle	No	ns
LPS vs LPS+psiRNA-*Dcf1*	Yes	*
LPS+vehicle vs LPS+psiRNA-*Dcf1*	Yes	#
● IL6:		
blank vs LPS	Yes	**
blank vs LPS+vehicle	Yes	*
blank vs LPS+psiRNA-*Dcf1*	No	ns
LPS vs LPS+vehicle	No	ns
LPS vs LPS+psiRNA-*Dcf1*	Yes	**
LPS+vehicle vs LPS+psiRNA-*Dcf1*	Yes	#
● TNF-α :		
blank vs LPS	Yes	**
blank vs LPS+vehicle	Yes	**
blank vs LPS+psiRNA-*Dcf1*	No	ns
LPS vs LPS+vehicle	No	ns
LPS vs LPS+psiRNA-*Dcf1*	Yes	*
LPS+vehicle vs LPS+psiRNA-*Dcf1*	Yes	#

Data are expressed as the mean ± SEM. n = 3.
*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ vs.blank.
#, $p<0.05$; ##, $p<0.01$; ###, $p<0.001$ vs. LPS+vehicle.

Table 11: One-way ANOVA analysis of Figure. 6B

Bonferroni's Multiple Comparison Test	**Significant? p<0.05**	**Summary**
● 12h:		
blank vs LPS	No	ns
blank vs LPS+vehicle	No	ns
blank vs LPS+psiRNA-*Dcf1*	No	ns
LPS vs LPS+vehicle	No	ns
LPS vs LPS+psiRNA-*Dcf1*	No	ns
LPS+vehicle vs LPS+psiRNA-*Dcf1*	Yes	**
● 24h:		
blank vs LPS	No	ns

blank vs LPS+vehicle	No	ns
blank vs LPS+psiRNA-*Dcf1*	No	ns
LPS vs LPS+vehicle	No	ns
LPS vs LPS+psiRNA-*Dcf1*	No	ns
LPS+vehicle vs LPS+psiRNA-*Dcf1*	No	ns
● 36h:		
blank vs LPS	No	ns
blank vs LPS+vehicle	No	ns
blank vs LPS+psiRNA-*Dcf1*	No	ns
LPS vs LPS+vehicle	No	ns
LPS vs LPS+psiRNA-*Dcf1*	No	ns
LPS+vehicle vs LPS+psiRNA-*Dcf1*	No	ns
● 48h:		
blank vs LPS	No	ns
blank vs LPS+vehicle	No	ns
blank vs LPS+psiRNA-*Dcf1*	Yes	***
LPS vs LPS+vehicle	No	ns
LPS vs LPS+psiRNA-*Dcf1*	Yes	***
LPS+vehicle vs LPS+psiRNA-*Dcf1*	Yes	###

Data are expressed as the mean ± SEM. n = 8.
*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ vs.blank.
#, $p<0.05$; ##, $p<0.01$; ###, $p<0.001$ vs. LPS+vehicle.

Table 12: One-way ANOVA analysis of Figure. 7B

Bonferroni's Multiple Comparison Test	**Significant? p<0.05**	**Summary**
blank vs LPS	No	ns
blank vs LPS+vehicle	No	ns
blank vs LPS+psiRNA-*Dcf1*	No	ns
LPS vs LPS+vehicle	No	ns
LPS vs LPS+psiRNA-*Dcf1*	No	ns
LPS+vehicle vs LPS+psiRNA-*Dcf1*	Yes	*

Data are expressed as the mean ± SEM. n = 4.
*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ vs.blank.
#, $p<0.05$; ##, $p<0.01$; ###, $p<0.001$ vs. LPS+vehicle.

Table 13: One-way ANOVA analysis of Supplement Figure. 1

Bonferroni's Multiple Comparison Test	Significant? p<0.05	Summary
blank vs LPS	No	ns
blank vs LPS+vehicle	No	ns
blank vs LPS+psiRNA-*Dcf1*	Yes	*
LPS vs LPS+vehicle	No	ns
LPS vs LPS+psiRNA-*Dcf1*	Yes	***
LPS+vehicle vs LPS+psiRNA-*Dcf1*	Yes	**

Data are expressed as the mean ± SEM. n = 4.
*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ vs. LPS+vehicle.