Molecular Cell, Volume *48*

Supplemental Information

Control of Proinflammatory Gene Programs by Regulated Trimethylation and Demethylation of Histone H4K20

Joshua D. Stender, Gabriel Pascual, Wen Liu, Minna U. Kaikkonen, Kevin Do, Nathanael J. Spann, Michael Boutros, Norbert Perrimon, Michael G. Rosenfeld, and Christopher K. Glass

Inventory:

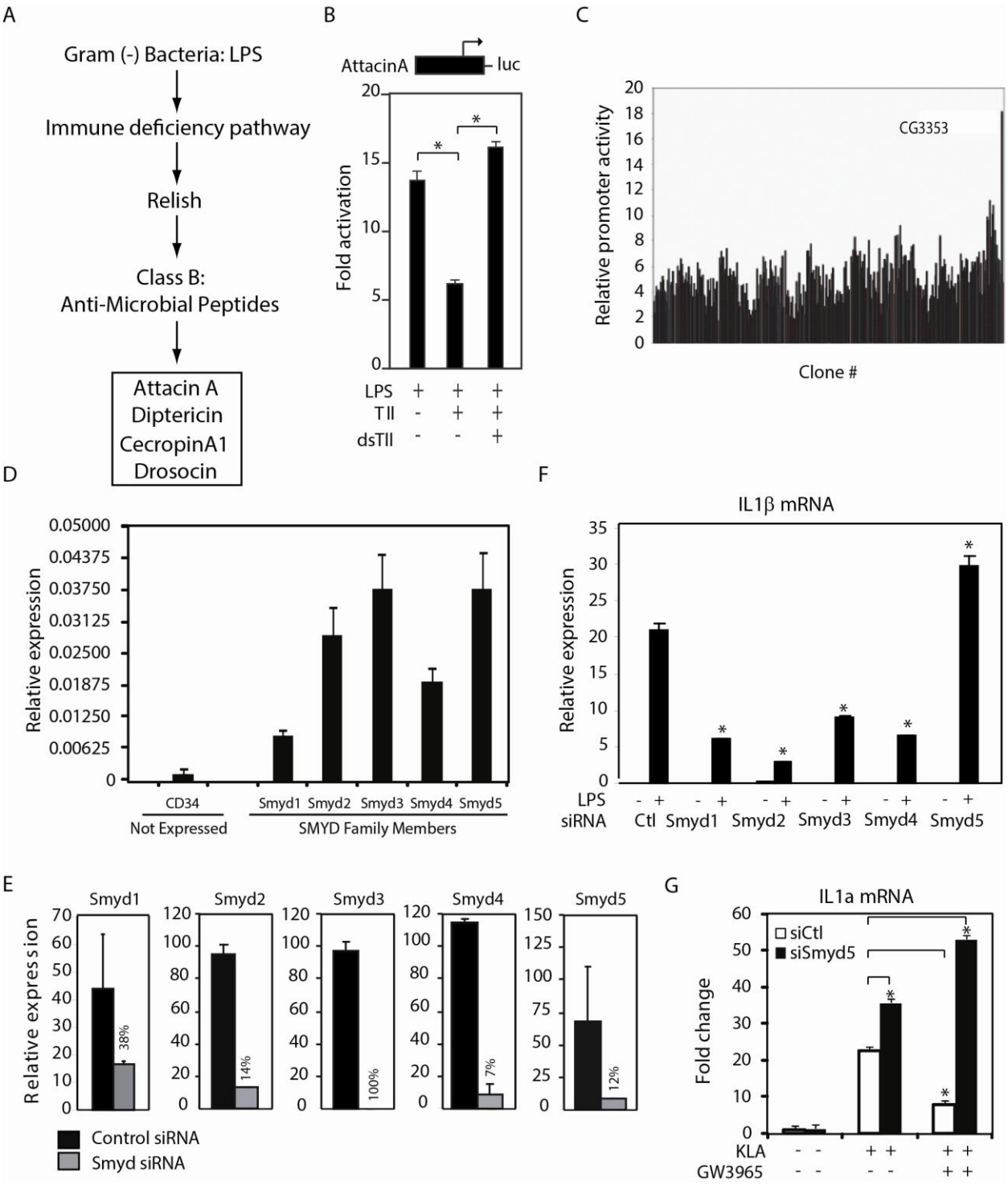

Figure S1, Related to Figure 1. Identification of CG3353 as a Negative Regulator of LPS-Induced Gene Expression in *Drosophila* S2 Cells

(A) Diagram for the response of *Drosophila melanogaster* to gram negative bacteria.

(B) Transrepression of the *Attacin A* promoter by Tailless (Tll) nuclear receptor in S2 cells. Overexpression of *Tll* results in decreased luciferase signal, while *dsTll* abrogates this repression. Values represent the average of three experiments -/+ SEM,*p<0.05.

(C) Raw data generated from the dsRNA screen. Knocking down clone CG3353 reversed Tll-dependent repression of the *Attacin A* luciferase reporter construct.

(D) Comparison of mRNA expression for mammalian SMYD family members in primary mouse macrophages. The mRNA expression is normalized to the housekeeping gene *β-actin* generated from polyA RNA-sequencing of primary thioglycollate elicited macrophages.

(E) Quantitative real time PCR for *Smyd1*, *Smyd2*, *Smyd3*, *Smyd4*, *Smyd5* mRNAs in siRNA treated thioglycollate elicited macrophages.

(F) Quantitative real time PCR for *Il1b* mRNA isolated from thioglycollate elicited macrophages cells treated with siRNA for *Control*, *Smyd1*, *Smyd2*, *Smyd3*, *Smyd4*, and *Smyd5* for 48 hours and subsequently treated with LPS for 4 h, p<0.05 as compared to *siCtl*, LPS treated sample.

(G) Effect of SMYD5 knockdown on LXR repression of *Il1a* mRNA in thioglycollate elicited macrophages treated with GW3965 for 1 hour followed by 4 hours of KLA treatment. Values represent the average of three experiments -/+ SEM, *p<0.05 relative to *siCtl*, KLA treated sample.

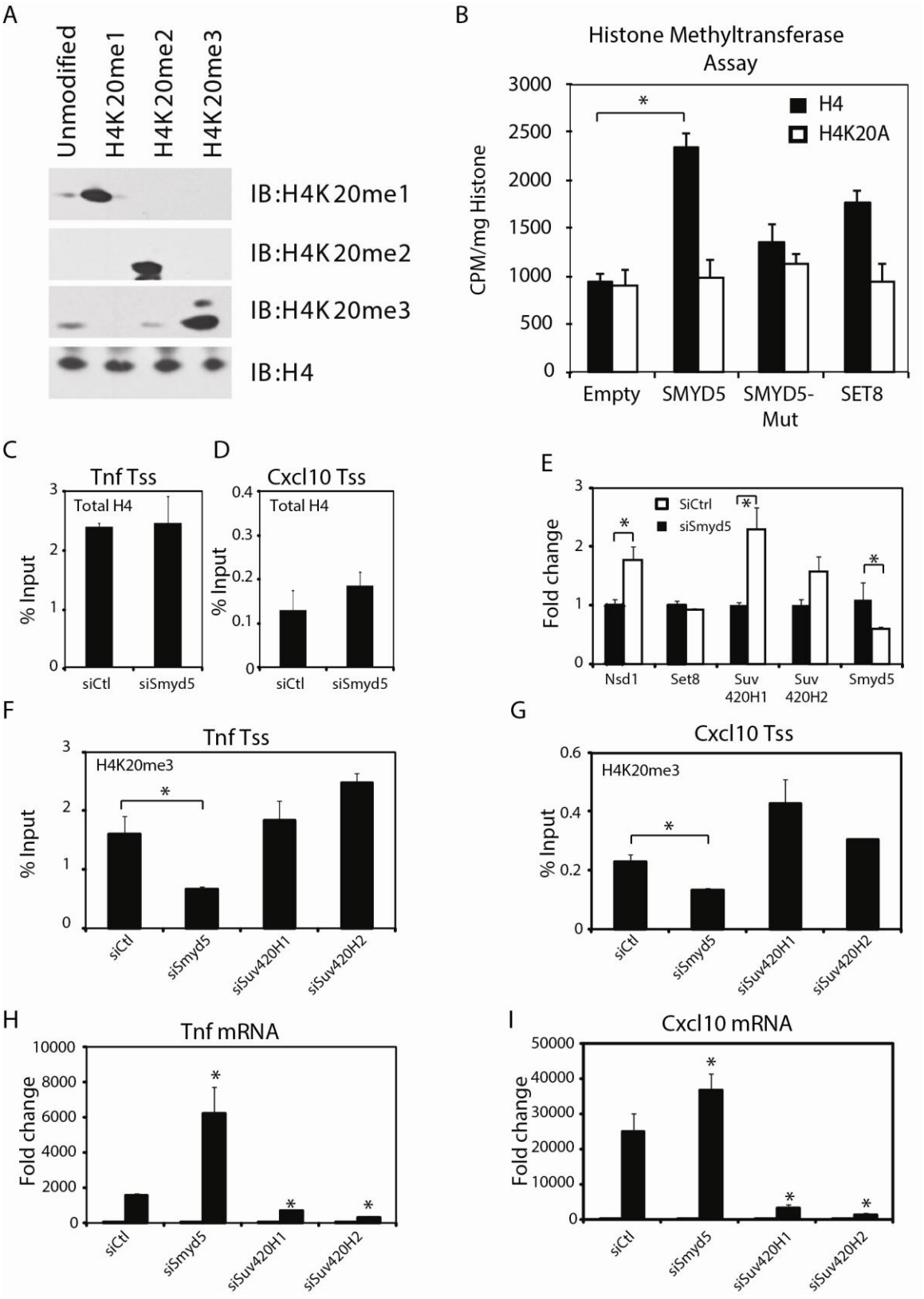
A
Unmodified
H4K20me1
H4K20me2
H4K20me3
IB:H4K20me1
IB:H4K20me2
IB:H4K20me3
IB:H4
B
Histone Methyltransferase Assay
CPM/mg Histone
H4
H4K20A
Empty
SMYD5
SMYD5-Mut
SET8
C
Tnf Tss
Total H4
% Input
siCtl
siSmyd5
D
Cxcl10 Tss
Total H4
% Input
siCtl
siSmyd5
E
SiCtrl
siSmyd5
Fold change
Nsd1
Set8
Suv 420H1
Suv 420H2
Smyd5
F
Tnf Tss
H4K20me3
% Input
siCtl
siSmyd5
siSuv420H1
siSuv420H2
G
Cxcl10 Tss
H4K20me3
% Input
siCtl
siSmyd5
siSuv420H1
siSuv420H2
H
Tnf mRNA
Fold change
siCtl
siSmyd5
siSuv420H1
siSuv420H2
I
Cxcl10 mRNA
Fold change
siCtl
siSmyd5
siSuv420H1
siSuv420H2

Figure S2, Related to Figure 2. SMYD5 Is Required for LXR-dependent Transrepression

(A) Western blot analysis for H4K20me1, H4K20me2, H4K20me3 and H4 for chemically methylated H4K20 proteins.

(B) Flag-SMYD5, Flag-SMYD5-mut (H315L) or recombinant SET8 were incubated with recombinant His-H4 or His-H4K20A. Activity was measured as CPM/μg histone. Values represent the average of three experiments -/+ SEM, *p<0.05 relative to Empty, histone H4 treatments.

(C) Chromatin immunoprecipitation assays assessing the total H4 levels on the *Tnf* promoter after treatment of thioglycollate elicited macrophages with siRNA targeting *Control* or *Smyd5* for 48 hours.

(D) Chromatin immunoprecipitation assays assessing the total H4 levels on the *Cxcl10* promoter after treatment of thioglycollate elicited macrophages with siRNA targeting *Control* or *Smyd5* for 48 hours.

(E) Quantitative real time PCR for genes known to methylate H4K20 including *Set8*, *Nsd1*, *Suv420H1*, *Suv420H2*, and *Smyd5* from mRNA isolated from thioglycollate elicited macrophages treated with siRNA for *Control* or *Smyd5*. Values represent the average of three experiments -/+ SEM,*p<0.05.

(F) H4K20me3 ChIP on the *Tnf* promoter in THEM cells treated with siRNA for *Control*, *Smyd5*, *Suv420H1*, and *Suv420H2* for 48 hours. Values represent the average of three experiments -/+ SEM,*p<0.05.

(G) H4K20me3 ChIP on the *Cxcl10* promoter in THEM cells treated with siRNA for *Control*, *Smyd5*, *Suv420H1*, and *Suv420H2* for 48 hours. Values represent the average of three experiments -/+ SEM,*p<0.05.

(H) Quantitative real time PCR for *Tnf* mRNA in thioglycollate elicited macrophages treated with siRNA for *Ctl*, *Smyd5*, *Suv420H1*, or *Suv420H2* for 48 hours and subsequently treated with KLA for 4 hours. Values represent the average of three experiments -/+ SEM,*p<0.05, compared to *siCtl* KLA treated samples.

(I) Quantitative real time PCR for *Cxcl10* mRNA in thioglycollate elicited macrophages treated with siRNA for *Ctl*, *Smyd5*, *Suv420H1*, or *Suv420H2* for 48 hours and subsequently treated with KLA for 4 hours. Values represent the average of three experiments -/+ SEM,*p<0.05, compared to *siCtl* KLA treated samples.

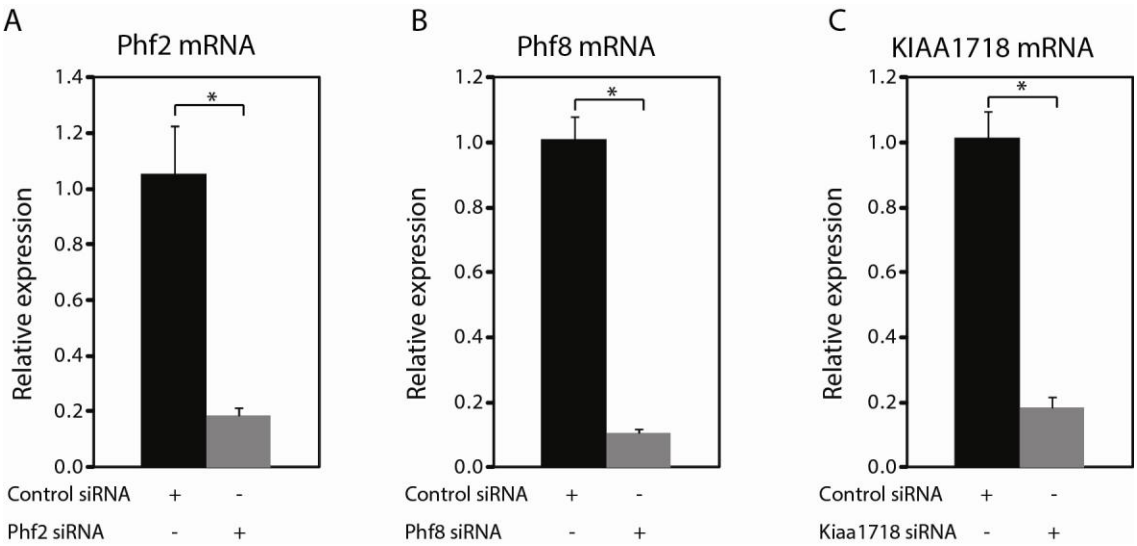

Figure S3, Related to Figure 4. Knockdown of Potential H4K20me3 Demethylases

(A) Quantitative real time PCR for *Phf2* mRNA isolated from thioglycollate elicited macrophages treated with siRNA for *Control* or *Phf2*. Values represent the average of three experiments -/+ SEM,*p<0.05.

(B) Quantitative real time PCR for *Phf8* mRNA isolated from thioglycollate elicited macrophages treated with siRNA for *Control* or *Phf8*. Values represent the average of three experiments -/+ SEM,*p<0.05.

(C) Quantitative real time PCR for *Kiaa1718* mRNA isolated from thioglycollate elicited macrophages treated with siRNA for *Control* or *Kiaa1718*. Values represent the average of three experiments -/+ SEM,*p<0.05.

A

PHF2 Targets

GO Term	Benjamini	# Genes
Cytokine Activity	1.90E-11	21
Chemokine Receptor Binding	1.90E-06	9
Chemotaxis	1.86E-04	8

B

SMYD5 Targets

GO Term	Benjamini	# Genes
Immune Response	2.49E-04	12
Defense Response	6.18E-04	11
Inflammatory Response	2.12E-03	8

Figure S4, Related to Figure 5. Gene Ontology Analysis for SMYD5 and PHF2 Target Genes

(A) Gene ontology analysis for the 63 mRNAs that demonstrate hyper-activation by KLA upon SMYD5 knockdown in thioglycollate elicited macrophages.

(B) Gene ontology analysis for the 214 mRNAs that demonstrate hypo-activation by KLA upon PHF2 knockdown in thioglycollate elicited macrophages.

Table S1, Related to Figure 5. RNA-Seq Expression for SMYD5 and PHF2 Targets

RNA-Seq analysis of thioglycollate elicited macrophages treated with *siCtl*, *siSmyd5* and *siPhf2* followed by 4 hours of Veh or KLA treatments. The spreadsheet has values for all significant KLA-stimulated genes and denotes which ones were targets of SMYD5 and PHF2.