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Supplemental Information

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

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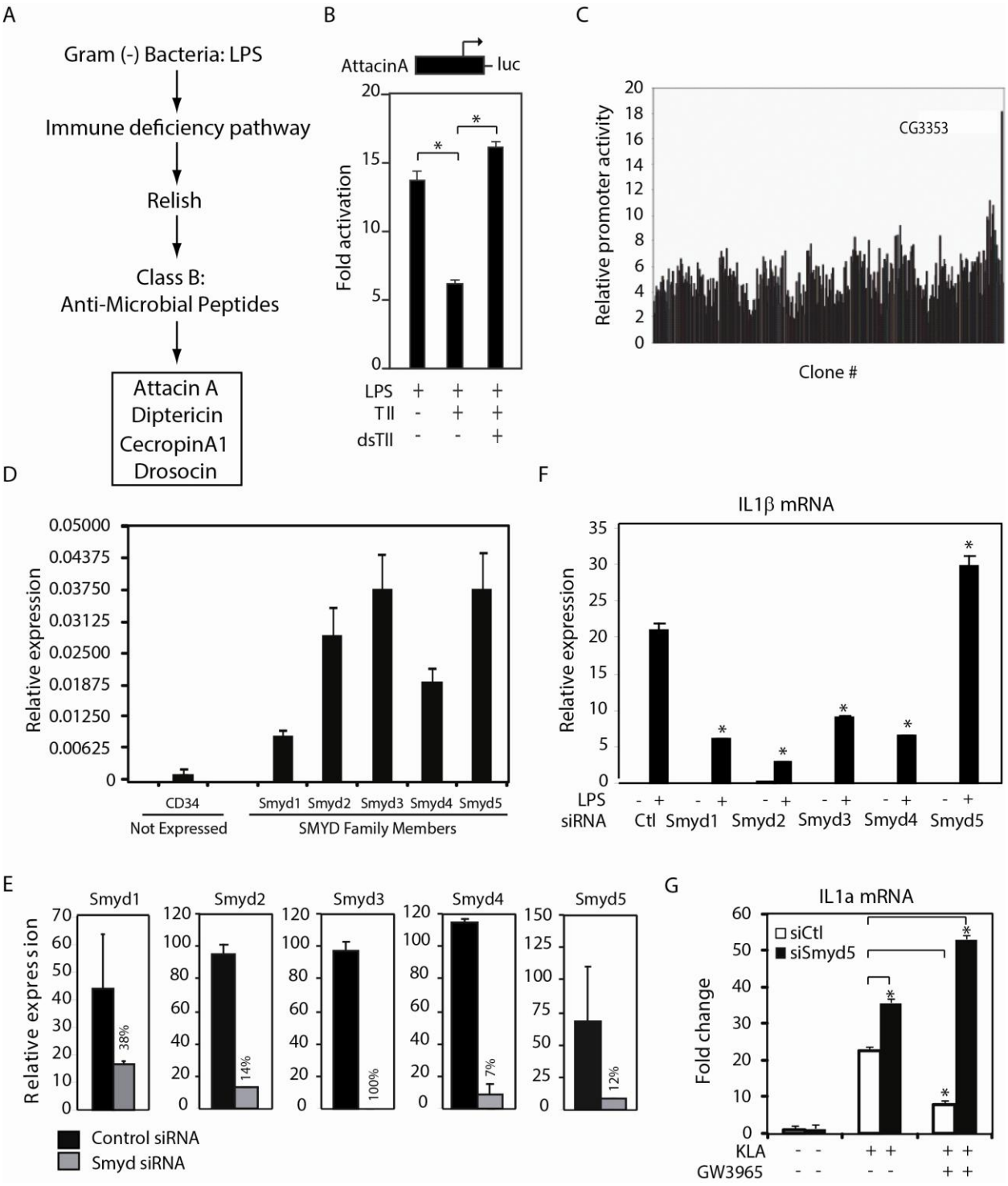

Figure S1, Related to Figure 1. Identification of CG3353 as a Negative Regulator of LPS-Induced Gene Expression in *Drosophila* S2 Cells

(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.

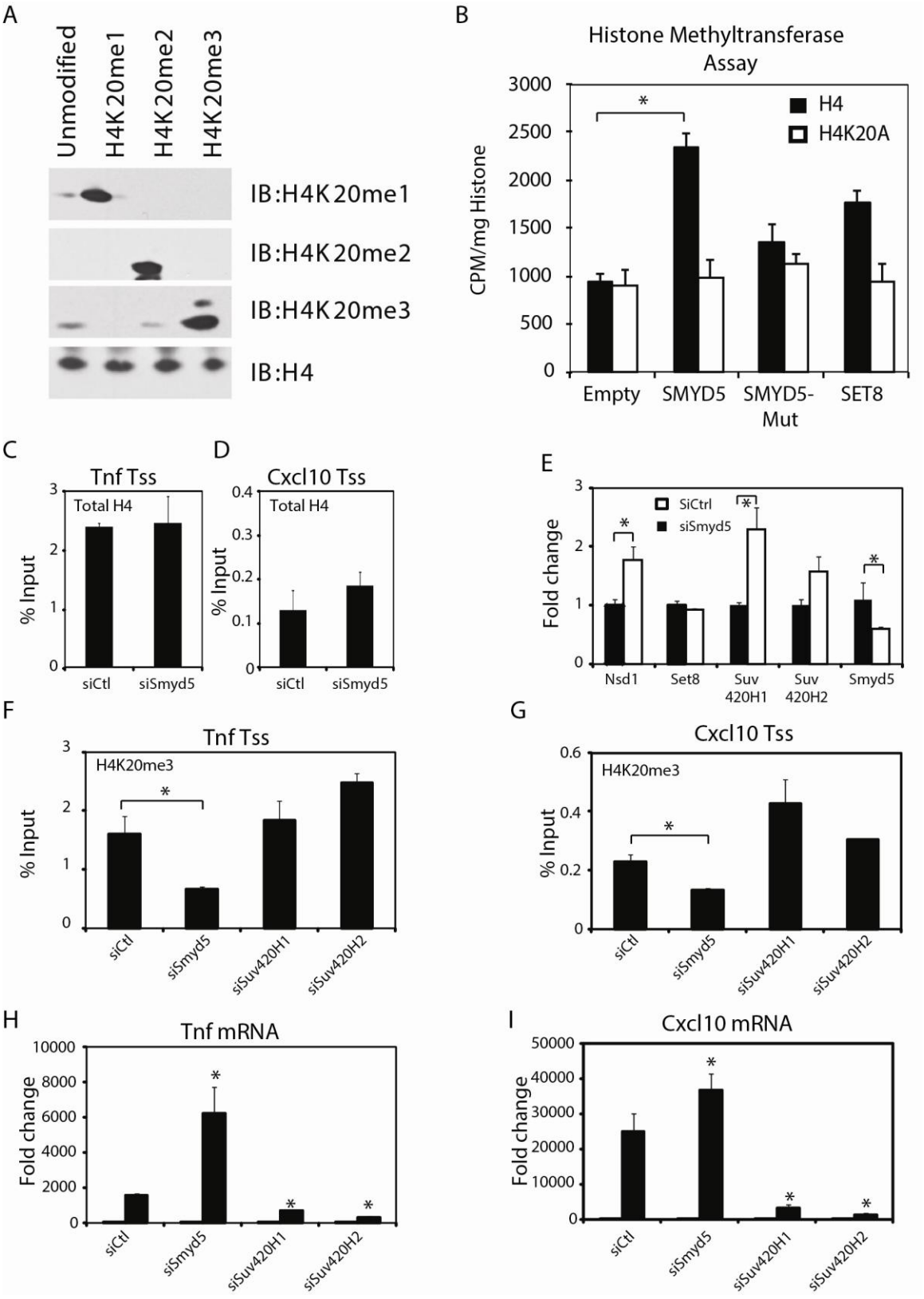
A
Unmodified
H4K20me1
H4K20me2
H4K20me3
IB:H4K20me1
IB:H4K20me2
IB:H4K20me3
IB:H4
B
Histone Methyltransferase Assay
CPM/mg Histone
H4
H4K20A
Empty
SMYD5
SMYD5-Mut
SET8
C
Tnf Tss
Total H4
% Input
siCtl
siSmyd5
D
Cxcl10 Tss
Total H4
% Input
siCtl
siSmyd5
E
SiCtrl
siSmyd5
Fold change
Nsd1
Set8
Suv 420H1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

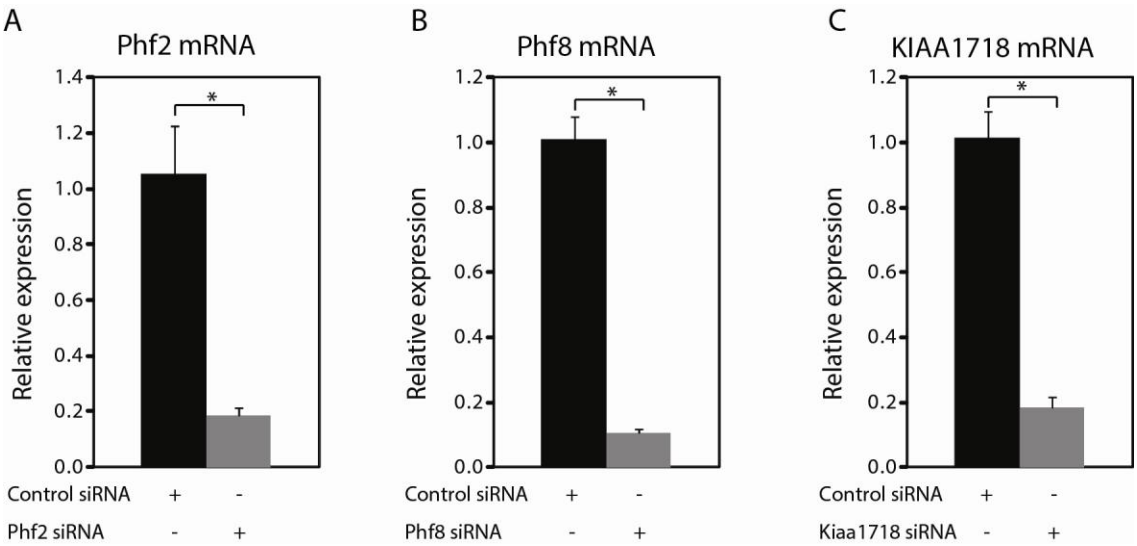

Figure S3, Related to Figure 4. Knockdown of Potential H4K20me3 Demethylases

(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.