

ONLINE APPENDIX

Supplement Figure S1: miR-125b alignment with potential targets. (A) miR-125b alignment with mouse (*Suv39h1*) and human SUV39H1 3'UTRs. (B) miR-125b alignment with human PPAR α 3'UTR. Alignment and score based on MIRANDA miR database at Memorial Sloan-Kettering Cancer Center, New York, NY

A

Suppressor of variegation SUV39H1

3' agUGUUCAAUCCAGAGUCCCu 5' Mouse miRNA: mmu-miR-125b-5p
| | | : | | | | | | | | Alignment score: 156.0
1000:5' auACAGAUU - - - GUCUCAGGGa 3' Mouse transcript *Suv39h1*

3' aguGUUCA - AUCCAGAGUCCCu 5' Human miRNA: hsa-miR-125b
| | | | | | | | | | Alignment score: 154.0
929:5' gucCACGUGGAUUGUCUCAGGGa 3' Human transcript SUV39H1

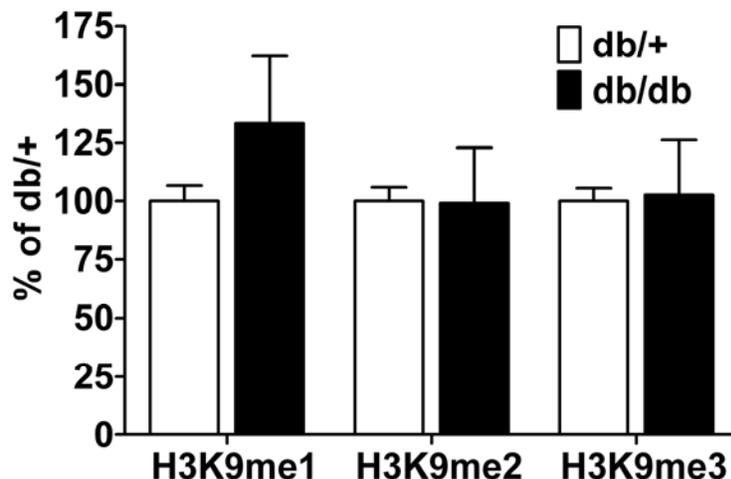
B

Peroxisome proliferator activated receptor alpha

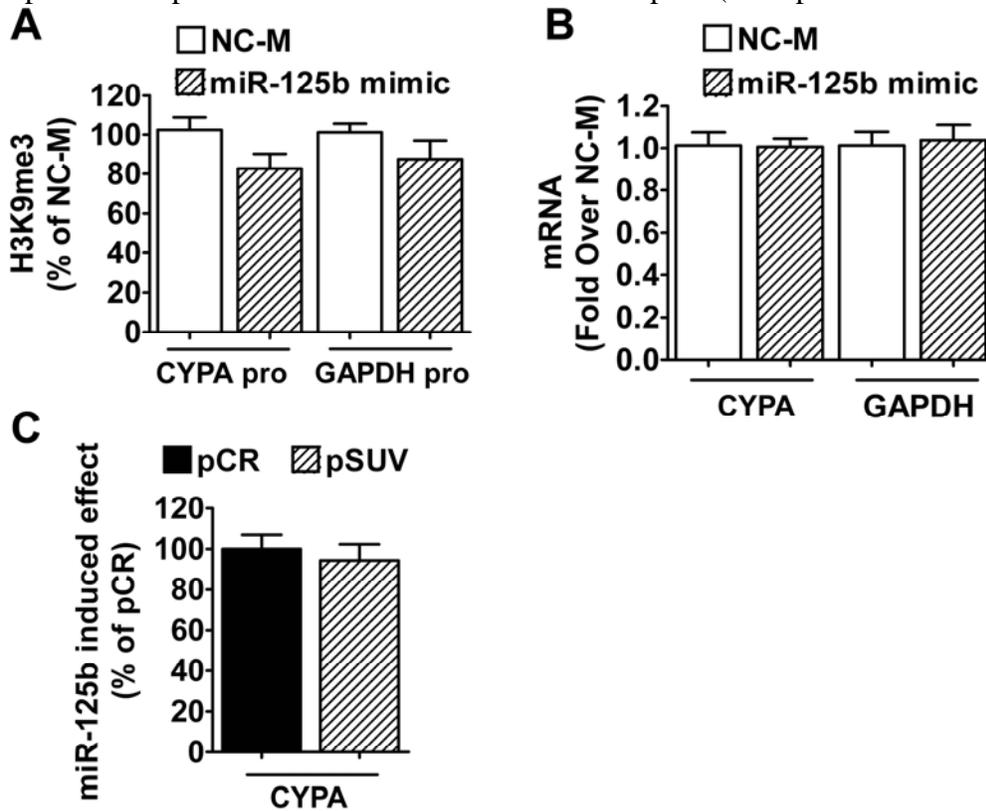
3' agUGU - UCAAUCCAGAGUCCCu 5' Human miRNA: hsa-miR-125b
| | | | | | | | | | Alignment score: 144.0
8307:5' guACAGAGUUA - - AUUUCAGGGu 3' Human transcript PPARA

(<http://www.microrna.org/microrna/home.do>).

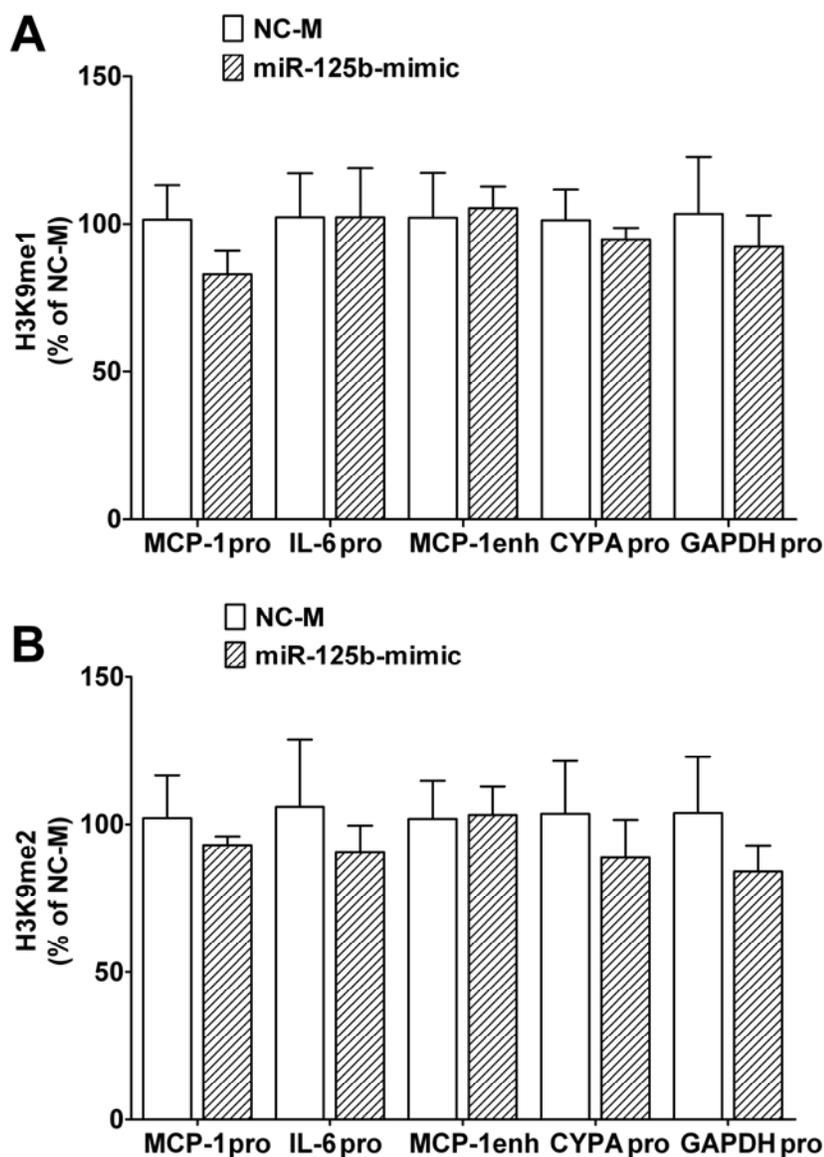
Supplement Figure S2. MVSMCs from *db/+* and *db/db* do not show significant changes in global H3K9 methylation. Cell lysates from *db/+* and *db/db* MVSMCs were immunoblotted with indicated antibodies. Intensities of protein bands were quantified using a calibrated densitometer and results normalized with internal control β -actin were expressed as % of *db/+* MVSMCs. Results showed that there were no significant differences in the global H3K9me1, H3K9me2 or H3K9me3 levels in *db/db* MVSMCs relative to *db/+* MVSMCs (Mean \pm SE, from four different batches of MVSMCs each performed in triplicate).



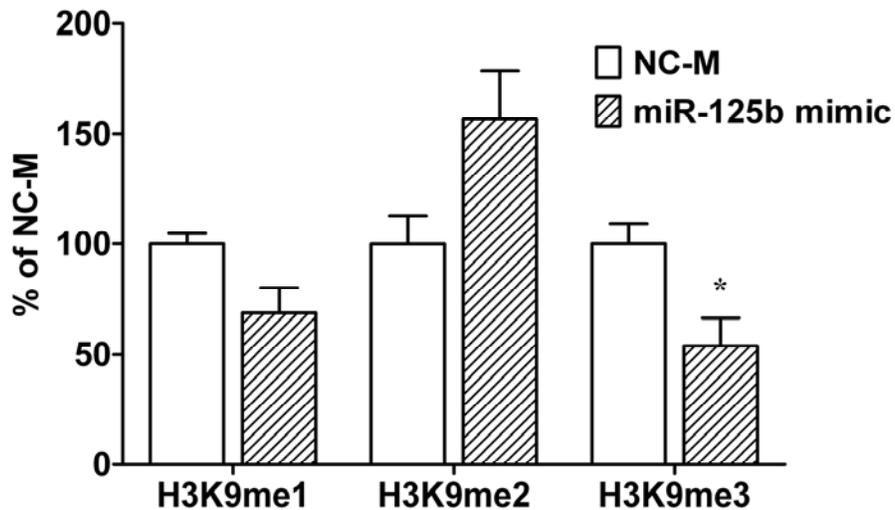
Supplement Figure S3: The effect of miR-125b mediated knockdown of SUV39H1 is specific for key inflammatory genes. HeLa cells were transfected with miR-125b mimic oligo or control NC-M oligonucleotides. Cells were either formaldehyde fixed for ChIP assays or total RNA harvested for RT-qPCR. There were no significant changes in promoter H3K9me3 levels for CYPA (n=4) or GAPDH (n=5) (A) nor in their mRNA expression levels (n=3) (B). (C) Additionally, miR-125b mimic or NC-M were co-transfected along with pCR3.1 empty vector (pCR) or FLAG-SUV39H1 (pSUV). After overnight incubation, RNA was extracted and gene expression examined by RT-qPCR. Results demonstrate no effect of miR-125b mimic on CYPA expression in pSUV co-transfected cells relative to pCR (% of pCR control vector, n=3).



Supplement Figure S4. MiR-125b mimics do not affect H3K9me1 and H3K9me2 levels at inflammatory gene promoters in HeLa cells. ChIP assays were performed with NC-M and miR-125b mimic transfected HeLa cells using H3K9me1 and H3K9me2 antibodies. ChIP DNAs were analyzed by QPCR using indicated promoter primers. Data normalized to input samples was expressed as % of NC transfected cells. Results showed no significant differences in promoter H3K9me1 and H3K9me2 levels in HeLa cells transfected with miR-125b mimics compared to NC transfected cells (Mean \pm SE, n=3).



Supplement Figure S5. Global H3K9 methylation levels in HeLa cells transfected with miR-125b. Cell lysates from HeLa cells transfected with NC-M and miR-125b mimic were immunoblotted with indicated antibodies and intensities of protein bands quantified using a calibrated densitometer. . Results were normalized with internal control histone H3 and expressed as % of NC transfected cells. HeLa cells transfected with miR-125b showed significantly reduced H3K9me3 compared to NC transfected cells. On the other hand, both H3K9me1 and H3K9me2 did not show any significant differences (Mean \pm SE; *, $p < 0.04$ vs NC-M, $n=3$).



Supplement Table S1. Primer sequences.

ChIP Primers *	Forward Sequence	Reverse Sequence**	Annealing Temp., °C
mIL-6pro	CGTTTATGATTCTTTTCGATGCTAAACG	GTGGGCTCCAGAGCAGAATGAG	60
mMCP-1pro	ACCAAATCCAACCCACAGTTTC	TGCTCTGAGGCAGCCTTTTATT	58
mMCP-1enh	ATTTCCACGCTCTTATCCTACTCTG	TCACCATTGCAAAGTGAATTGG	58
HIL-6pro	CTTCGTGCATGACTTCAGCTTT	CGTCCTTTAGCATCGCAAGAC	58
HMCP-1pro	TTGGAATGTGGCCTGAAGGT	AGGGTTATTTTAAAGGATTCTGCTTTC	58
HMCP-1enh	GGCCCAGTATCTGGAATGCA	GTCAGTGCTGGCGTGAGAGA	60
HCYPApro	GCAGGGAAAGACCAGGAGCAG	cggaaCTTTCAAATGCTCTACTTTCCG	60
HGAPDHpro	cgaagCTCACGTCCCGCTCTTcG	AGGCACTCCTGGAAACCTGTG	58

miR Detection		Annealing Temp., °C
miR-125b specific	5'-CATGATCAGCTGGGCCAAGATCACAAGTTAGG-3'	60
miR-125b specific reverse	5'-TCCCTGAGACCCTAAC-3'	60
universal	5'-CATGATCAGCTGGGCCAAGA-3'	60

RT-PCR Primers *	Forward Sequence**	Reverse Sequence**	Annealing Temp., °C
mSuv39h1	cgccACAAGAACCATCTGGGcG	CAGGCCAAAGTTGGAGTCCATT	60
mGapdh	CCTGCACCACCAACTGCTTAG	catgcGGTGGCAGTGATGGCAtG	60
HSUV39H1	cggaaATATGACCTCTGCATCTTcG	CCTCCACGTAGTCCAGGTCAAAAG	58
HIL6	TCCTGCAGAAAAAGGCAAAG	GCCCAGTGGACAGGTTTCT	60
HMCP-1	cacagaCTCTGCCGCCCTTCTGtG	GCGAGCCTCTGCACTGAGAT	58-59
HCYPA	GACTGAGTGGTTGGATGGCAAAG	cgcttATTCCTGGACCCAAAGcG	58
HGAPDH	CCTGCACCACCAACTGCTTAG	cgggTCACGCCACAGTTTCCcG	58
2610203C20Rik201 (p1-p2)	p1: AACACCTCGCCCAGCCTAGAA	p2: cggaGTTGGCATGAGCGTCCG	60
pri-miR-125b-2 (p3-p4)	p3: cggacCTCTAATTCCCAAGCTGTCcG	p4: TTCTTTGCGCCTATGCAGAAATC	60
RIKEN Clones (p5-p6)	p5: cgattcCCCAAAATACAATCTTCAGAATcG	p6: CGATAATCACAGTAAATACAAACGCTAT	60

*m = mouse; H = human; pro = promoter; enh = enhancer

**upper case indicates gene sequence; lower case sequence is designed to prevent primer dimers.