Supporting information for

6-Thioguanine Reactivates Epigenetically Silenced Genes in Acute Lymphoblastic Leukemia Cells by Facilitating Proteasome-mediated Degradation of DNMT1

by

Bifeng Yuan, Jing Zhang, Hongxia Wang, Lei Xiong, Qian Cai, Tina Wang,

Steve Jacobsen, Sriharsa Pradhan and Yinsheng Wang

(*Cancer Res.*, 2011)

Table S1. Primers used for bisulfite sequencing

1. Sequence Name: PCDHGA12-S	5'-TTGGAGGAAGATAAGAATGATTTTTG-3'
2. Sequence Name: PCDHGA12-AS	5'-CTTAATAACCCCCATACAAAACTCC-3'
3. Sequence Name: RPIB9-S	5'-ATTGGAATTGATATAAAGTTTAGGGTT-3'
4. Sequence Name: RPIB9-AS	5'-ACCCCCTTAAACAAATATAAAAAAC-3'
5. Sequence Name: asparaginase -S	5'- GTTTTTAGAGTAGTTGGGATTATAGGTATG-3'
6. Sequence Name: asparaginase -AS	5'- AACCTCCTACTCCTAACCAAACTAAA-3'
7. Sequence Name: DCC-S	5'-TAATTTTTTGAAGGTTGAGTGTTAAATA-3'
8. Sequence Name: DCC-AS	5'-AAATAAAAAACTAAAAAAAAAAAATATAAAAAC-3'

Table S2. Primers used for real-time PCR.

1. Sequence Name: RT-ABCB1-S	5’-TGTATGCTCAGAGTTTGCAGGT-3’
2. Sequence Name: RT-ABCB1-AS	5’-TTCCAAAGATGTGTGCTTTCC-3’
3. Sequence Name: RT-DDX51-S	5’-CACACTGCTCCTGAAAGTGC-3’
4. Sequence Name: RT-DDX51-AS	5’-TTCAGTTAGCATTCGGAGGAA-3’
5. Sequence Name: RT-KCNK2-S	5’-TAACAACTATTGGATTTGGTGACTAC-3’
6. Sequence Name: RT-KCNK2-AS	5’-GCCCTACAAGGATCCAGAAC-3’
7. Sequence Name: RT-NKX6-1-S	5’-CTTCTGGCCCGGAGTGAT-3’
8. Sequence Name: RT-NKX6-1-AS	5’-TCTTCCCGTCTTTGTCCAAC-3’
9. Sequence Name: RT-NOPE-S	5’-ACAGGGCTGAAGTGCACAG-3’
10. Sequence Name: RT-NOPE-AS	5’-CTTGGTTGAGCCCAGGAGA-3’
11. Sequence Name: RT-SLC2A14-S	5’-TCCACGCTCATGACTGTTTC-3’
12. Sequence Name: RT-SLC2A14-AS	5’-CAGGCCACAAAGACCAAGAT-3’
13. Sequence Name: RT-DCC-S	5’-CCGAAAGTCCCTTACACACC-3’
14. Sequence Name: RT-DCC-AS	5’-CATGGGTCTTAGGAAGAGTGG-3’
15. Sequence Name: RT-PCDHGA12-S	5’-TGCTGTCAGGTGATTCGGTA-3’
16. Sequence Name: RT-PCDHGA12-AS	5’-AGAAACGCCAGTCCGTGTT-3’
17. Sequence Name: RT-RPIB9-S	5’-GGCCAGTCACAAGAAGGAGA-3’
18. Sequence Name: RT-RPIB9-AS	5’-GAGATCCACAGAGGCCAAGT-3’
19. Sequence Name: RT-LRP1B-S	5’-CATGATCACAACGATGGAGGT-3’
20. Sequence Name: RT-LRP1B-AS	5’-CTTGAAAGCACTGGGTCCTC-3’
21. Sequence Name: RT-DLC-1-S	5’-GCTCCTCTCCATCAGGCACAC-3’
22. Sequence Name: RT-DLC-1-AS	5’-GAATAGCCGTAGTCTTGGGTTTGG-3’
23. Sequence Name: RT-asparaginase-S	5’-GCAGAACCGTTGTGACCAGAG -3’
24. Sequence Name: RT-asparaginase -AS	5’-CCAAAGCCAGCCCAAGGAAG -3’
25. Sequence Name: RT-DNMT1-S	5’-AGGGAAAAGGGAAGGGCAAG-3’
26. Sequence Name: RT-DNMT1-AS	5’-AGAAAACACATCCAGGGTCCG-3’
27. Sequence Name: RT-LSD1-S	5’-TCGCTACACGGCTTCAGGATG-3’
28. Sequence Name: RT-LSD1-AS	5’-AACGGCTGGTGGCTGCTG-3’
29. Sequence Name: RT-GAPDH-S	5’-TTTGTCAAGCTCATTTCCTGGTATG-3’
30. Sequence Name: RT-GAPDH-AS	5’-TCTCTTCCTCTTGTGCTCTTGCTG-3’

Figure S1. HPLC traces for the separation of nucleoside mixtures produced from the digestion of genomic DNA isolated from Jurkat T cells that were treated with 3 μM ^{S}G. Shown in the insets (a) is the expanded chromatograms to visualize better the 5-mdC peak and (b) is the product-ion spectrum of the ESI-produced $[M+H]^+$ ion (*m/z* 126.1) of 5-mdC.

Figure S2. Western blot analysis of Set7 with whole-cell extracts from Jurkat-T cells treated with ^{S}G for various time periods. β-actin was used as the loading control.

Figure S3. Real-time RT-PCR (A) and Western blot (B) analyses for monitoring the siRNA-induced knockdown of LSD1 gene in HEK-293T cells. GAPDH was used as an internal control for real-time RT-PCR analysis, and β-actin was used as the loading control for Western analysis.

Figure S4. (A) Western blot analysis of DNMT1 with whole-cell extracts from HEK-293T cells treated with ^{S}G (3 μM) for 24 h or 48 h. (B) Western blot analysis of DNMT1 with whole-cell extracts from HEK-293T cells after LSD1 siRNA knockdown. (C) Western blot analysis of DNMT1-K142me with whole-cell extracts from HEK-293T cells after LSD1 siRNA knockdown. β-actin was used as the loading control.

Figure S5. Western blot analysis of DNMT1-K142me with whole-cell extracts from Jurkat-T cells treated with ^{S}G (3 μM) for various time periods. β-actin was used as the loading control.

Figure S1.

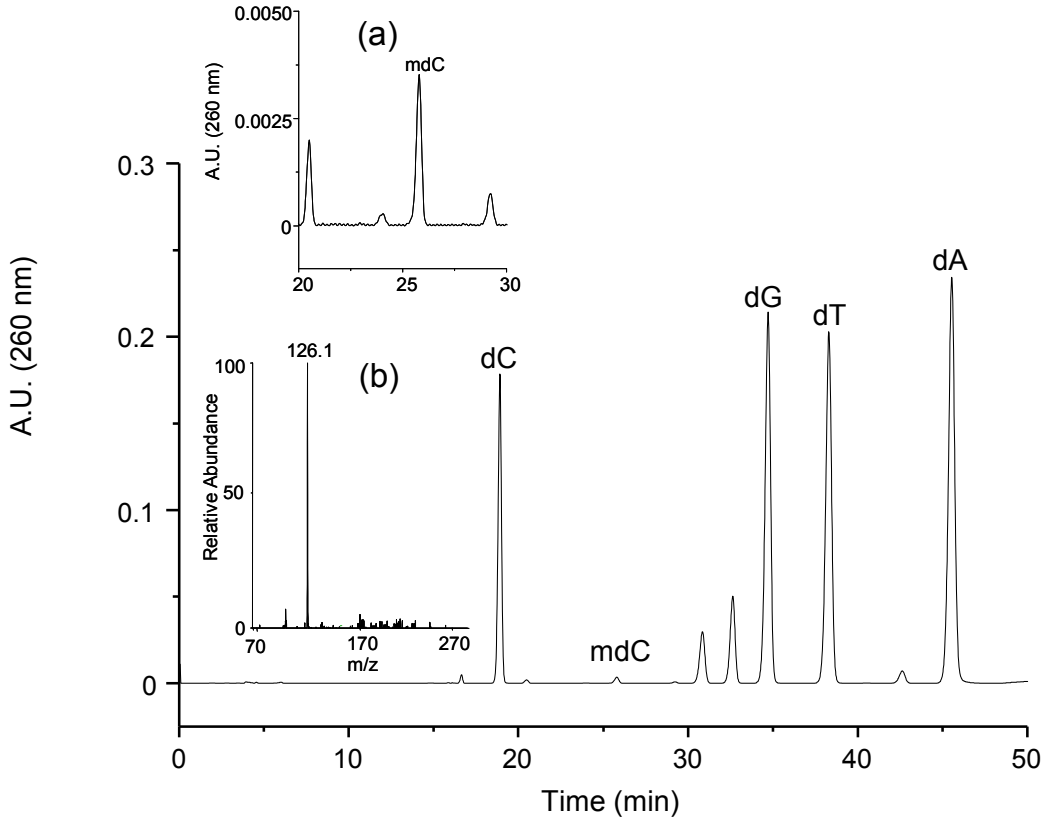
(a)
mdC
A.U. (260 nm)
0.0050
0.0025
0
20
25
30
(b)
126.1
Relative Abundance
100
50
0
70
170
270
m/z
A.U. (260 nm)
0.3
0.2
0.1
0
dC
mdC
dG
dT
dA
0
10
20
30
40
50
Time (min)

Figure S2.

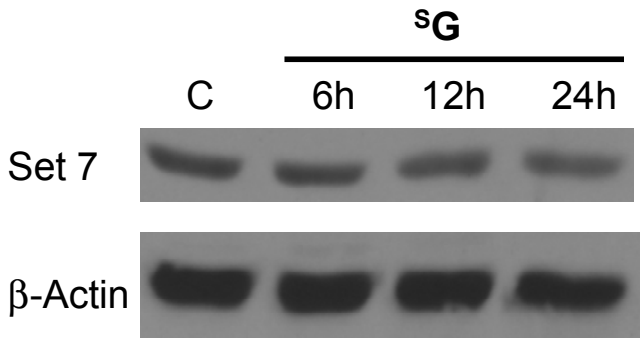

Figure S3.

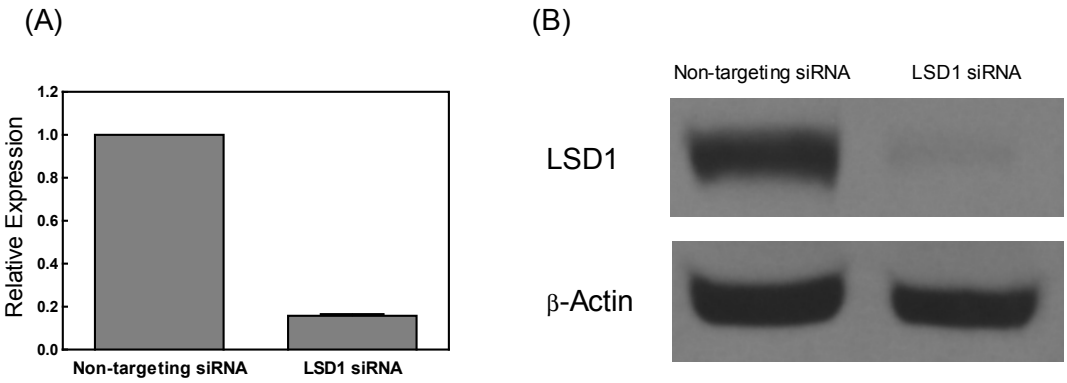
(A)
Relative Expression
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Non-targeting siRNA
LSD1 siRNA
(B)
Non-targeting siRNA
LSD1 siRNA
LSD1
β-Actin

Figure S4.

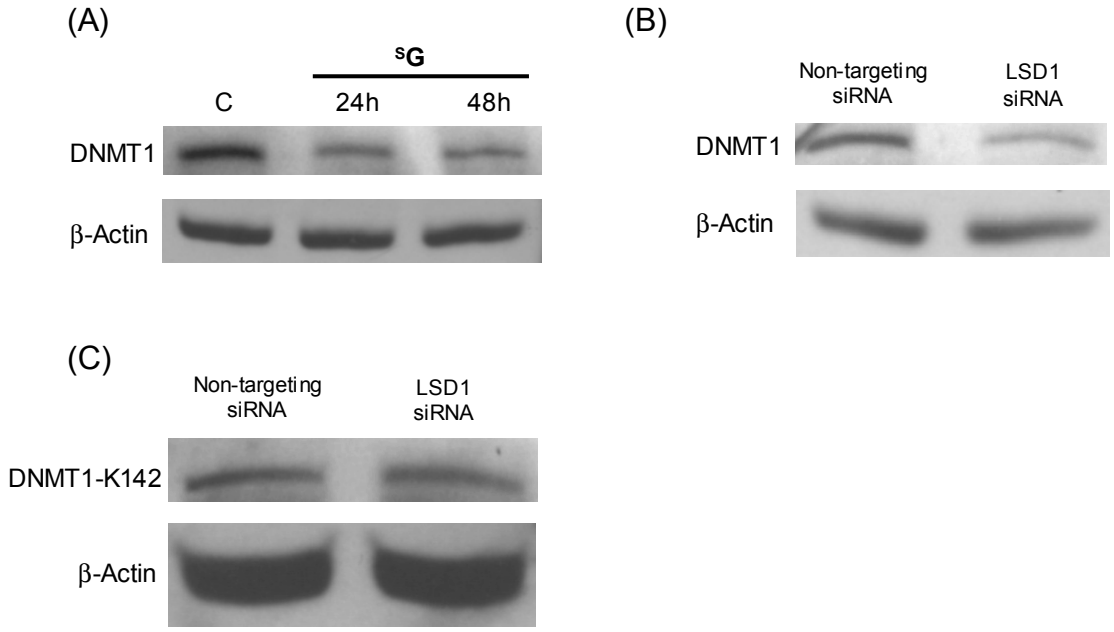
(A)
SG
C
24h
48h
DNMT1
β-Actin
(B)
Non-targeting siRNA
LSD1 siRNA
DNMT1
β-Actin
(C)
Non-targeting siRNA
LSD1 siRNA
DNMT1-K142
β-Actin

Figure S5.