Molecular Cell, *Volume 39*

Supplemental Information

Loss of miR-200 Inhibition of Suz12 Leads to Polycomb-Mediated Repression Required for the Formation and Maintenance of Cancer Stem Cells

Dimitrios Iliopoulos, Marianne Lindahl-Allen, Christos Polytarchou, Heather A. Hirsch, Philip N. Tsichlis, and Kevin Struhl

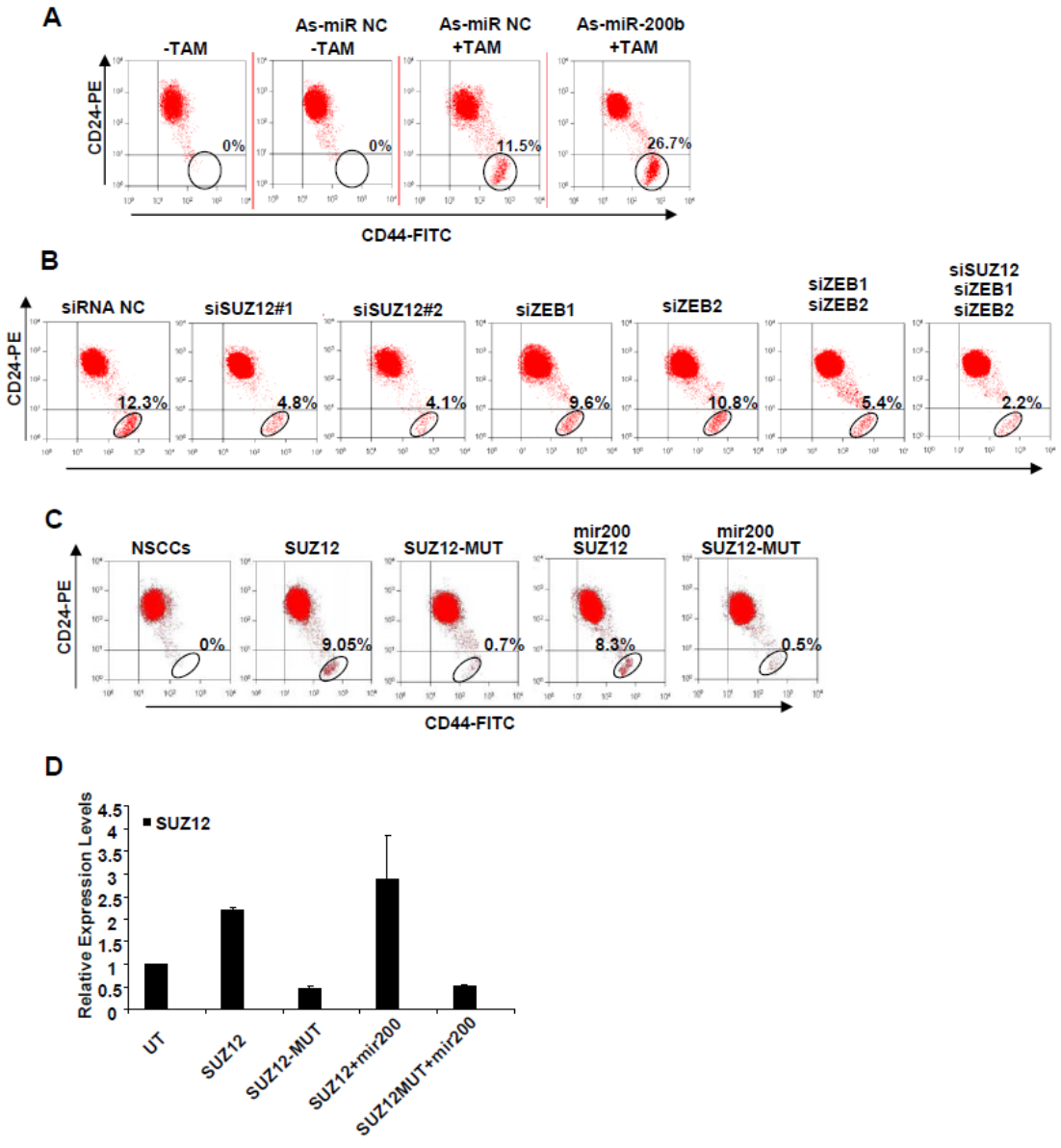

Figure S1, related to Figure 3. Flow cytometry analysis of ER-Src transformed (+TAM for 36h) cells for CD44 and CD24 antigens. A) Percentage of CSCs after transfection

with antisense-miR negative control (as-miR NC) or antisense-miR-200b (as-miR-200b). B) Flow cytometry analysis of ER-Src transformed cells after transfection with siSuz12 siZEB1 and 2 or control siRNAs. Data represented as mean SD± of three biological replicates. C) Flow cytometry analysis on transformed non-stem cell cancer cells (NSCC) CD24+/CD44- after transfection with a vector expressing Suz12 and control vectors. Data represented as mean SD± of at least two biological replicates. D) SUZ12 relative mRNA expression levels in NSCCs transfected with the relevant constructs assessed by real-time PCR analysis.

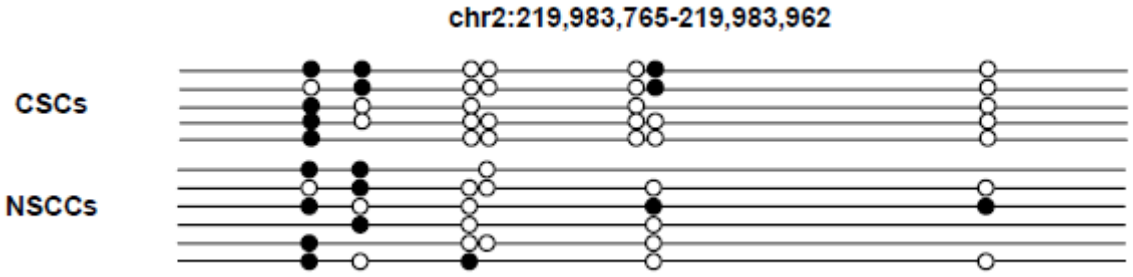

Figure S2, related to Figure 4. DNA methylation analysis by bisulfite conversion and sequencing of control region. For each horizontal line, open circles indicate non-methylated residues and black circles indicated methylated residues in a given clone that was sequenced after bisulfite conversion.

Table S1, related to Figure 4: Primers used in this study.

Gene	Forward	Reverse
ChIP		
HNRPA2	ACGGCCTGACGTAGCGGAA	CAACTCTGCGAGGAGCACCT
CDH1	AGAGGGGCATCCGTAGAAAT	ACCTCCTCCGACCTCACTTT
SOX1	GGGAAAACGGGCAAAATAAT	TTTTGCGTTCACATCGGTTA
WNT1	AGGGTGGGACTCCTAAGCAT	CTAGGTCCGGAGACTGGACA
HOXA13	ATGGCTGGCTTAGTTCTGGA	CAAAGAAGCGTGGCTTTAGG
GATA4	GATCTTCGCGACAGTTCCTC	CATGGCCAAGCTCTGATACA
PDX1	CCGGGTCGGACTAAACTACA	GGTGGGAAAGATGCTTCAAA
SOX2	CAAGATGCACAACTCGGAGA	GCTTAGCCTCGTCGATGAAC
Bisulfite		
CDH1 -181	GAGGGTCACCGCGTCTATGC	CCCCCGTACCGCTGATTGGCTGAG
CDH1 +490	GAAGGGGTGTTTTGGTGTAAGT	TTCAATCTCCTTTCTCATTTTATTAAAA
CDH1 +600	AAGGTATTTGTTATGTTAAGAAAGGT	AAAATACACCACTCCTCAAAAC
RT-PCR		
SUZ12	GATAAAAACAGGCGCTTACAGCTT	AGGTCCCTGAGAAAATGTTTCGA
EZH2	TGCAGTTGCTTCAGTACCCATAAT	ATCCCCGTGTACTTTCCCATCATAAT
b-ACTIN	CCTGTACGCCAACACAGTGC	ATACTCCTGCTTGCTGATCC
BMI1	AATCTAAGGAGGAGGTGA	AAACAAGAAGAGGTGGA
CDH1	GGAGGAGAGCGGTGGTCAAA	TGTGCAGCTGGCTCAAGTCAA