SUPPLEMENTARY MATERIAL

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Downregulation of E-cadherin but not of β-catenin or SNAIL1 proteins after disruption of PCNA and DMAP1 binding domains of DNMT1. Quantification of the Integrated Density (the product of Area and Mean Gray Value) of protein expression signals obtained in semiquantitative immunoblot analysis of the expression of E-cadherin, β-catenin and SNAIL1 relative to the expression of β-tubulin in HCT116 *DNMT1*$^{+/+}$, *DNMT1*$^{\Delta E3-6}$, and *DNMT3b*$^{-/-}$ cells. Analyses were performed in triplicate and the mean ± SD are shown. **: p<0.001.

Supplementary Figure 2. Epithelial-mesenchymal transition marker proteins are expressed after disruption of PCNA and DMAP1 binding domains of DNMT1. Semiquantitative analysis by immunoblot of the expression level of the indicated proteins in HCT116 *DNMT1*$^{+/+}$and *DNMT1*$^{\Delta E3-6}$ cells. Results are representative of three separate experiments.

Supplementary Figure 3. Deletion of PCNA and DMAP1 domains of DNMT1 or of DNMT3b does not alter the unmethylated status of *E-cadherin* and *SNAIL1* promoters in HCT116 cells. A) Analysis by bisulfite sequencing of the status of CpG methylation in the promoter sequences of *E-cadherin* or *SNAIL1* genes. The two promoters are equally demethylated in HCT116 DNMT1$^{+/+}$, DNMT1$^{\Delta E3-6}$, and DNMT3b$^{-/-}$ cell lines. Ten different clones were analyzed in each case. B) Analysis of *E-cadherin* and *SNAIL1* promoter methylation in different cell lines by DNA restriction with a methylation-sensitive endonuclease followed by PCR. The isoschizomers Hpa II (methylation-sensitive) and MspI (methylation-insensitive) were used. The breast cancer cell lines MDA-MB-231 and MDA-MB-435 were used as positive control samples for *E-cadherin* methylation. *E-cadherin* and *SNAIL1* promoters are unmethylated in HCT116 DNMT1$^{+/+}$, DNMT1$^{\Delta E3-6}$, DNMT3b$^{-/-}$, DNMT^{DKO} cells

and normal lymphocytes. “In vitro” methylated DNA (IMD) was also used as positive control.

Supplementary Figure 4. Deletion forms of *DNMT1* cDNA lacking the catalytic domain (*DNMT1*$^{\Delta Cat}$), or the catalytic and BAH domains (*DNMT1*$^{\Delta Cat/BAH}$) are located in the cell nucleus. Immunolocalization analysis of the distribution of different DNMT1 cDNA forms fused to RFP (red) and co-localization with DAPI-stained chromatin (blue).

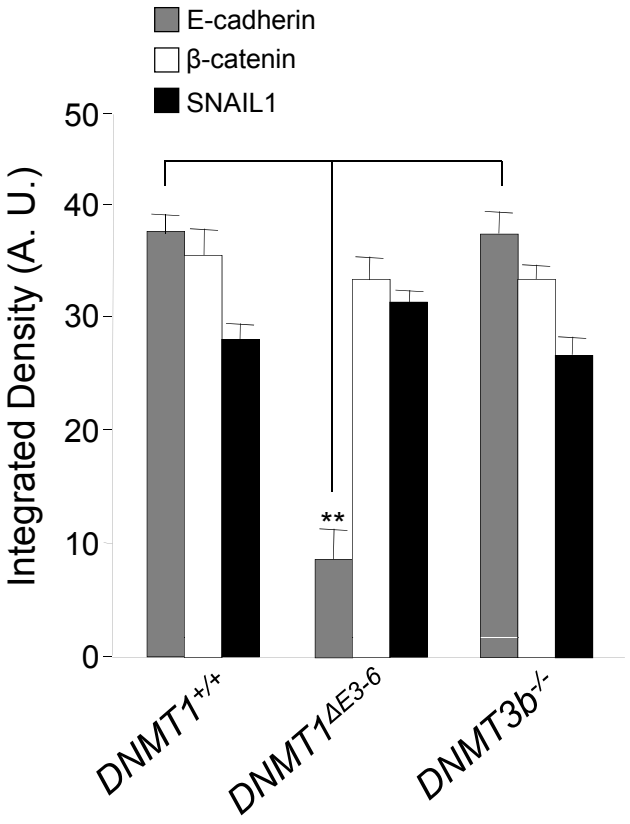
Supplementary Figure 5. *DNMT*^{DKO} cells, showing that combined deletion of PCNA and DMAP1 domains of *DNMT1* and of both copies of *DNMT3b* in HCT116 cells results in E-cadherin downregulation, nuclear translocation of β-catenin, and activation of β-catenin-dependent transcriptional signaling. This phenotype reverts to that of the parental cells after introduction of full-length *DNMT1*. A) Phase-contrast images of living cultured cells (left panels) and Ca^{2+}-dependent fast cell-cell aggregation assays in the presence or absence of a functional antibody against E-cadherin (Decma1) and the Ca^{2+}-chelating agent EDTA (right panel) in HCT116 *DNMT1*$^{+/+}$ and *DNMT*^{DKO} cells. B) Immunolocalization of β-catenin in HCT116 *DNMT1*$^{+/+}$ and *DNMT*^{DKO} cells. Bar: 10 μm. C) Quantitative analysis of *E-cadherin* mRNA expression by qRT-PCR in HCT116 *DNMT1*$^{+/+}$, *DNMT*^{DKO} cells and *DNMT*^{DKO} cells transfected with full-length *DNMT1*. Relative expression levels are normalized to that of actin expression. D) Normalized *Luciferase/Renilla* activities of reporter vectors transiently transfected in HCT116 *DNMT1*$^{+/+}$, *DNMT*^{DKO} cells and *DNMT*^{DKO} cells transfected with full-length DNMT1, containing either TOP multimerized promoter sequences recognized by β-catenin-Lef/Tcf complexes, or the human cyclinD1 promoter. Analyses were performed in triplicate and the mean ± SD are shown. **: $p<0.001$.

Supplementary Figure 6. DNMT1 and SNAIL1 do not interact in HCT116 DNMT1$^{\Delta E3-6}$ cells. A) Co-immunoprecipitation assay of endogenous DNMT1 showing interaction with SNAIL1 in DNMT1$^{+/+}$but not in DNMT1$^{\Delta E3-6}$ cells. B) Direct immunofluorescence analysis of SNAIL1 and DNMT1 distribution in the

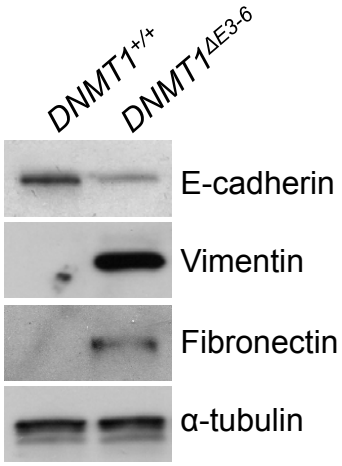
nucleus of in DNMT1$^{\Delta E3-6}$ cells, showing lack of co-localization of both proteins. Images are of 0.1-µm-thick sections in the z-plane obtained by confocal microscopy. Bar: 5 µm.

Supplementary Figure 7. DNMT1 and SNAIL1 can interact in the cell nucleus. A) An additional example of an extended biomolecular fluorescence complementation assay (ExBiFC) to demonstrate the interaction of DNMT1 and SNAIL1 in the cell nucleus showing cells expressing DNMT1-RFP+C-YFP and SNAIL1-CFP+N-YFP. Both tagged proteins showed a homogeneous distribution pattern in the cell nucleus. By contrast the yellow emission corresponding to the N+C-YFP fusion was located in discrete regions of the nuclear compartment. Bar: 5 µm. B) No complementation of YFP was detected SNAIL1-CFP+N-YFP and DNMT1 construct lacking the DMAP1 and PCNA domains (DNMT1$^{\Delta N}$-RFP+C-YFP) were co-expressed. Bar: 5 µm. C) Control experiments for ExBIFC assays, showing co-expression of DNMT1-RFP+C-YFP and Empty-CFP+N-YFP (upper panels; bar: 10 µm) or Empty vector-RFP+C-YFP and SNAIL1-CFP+N-YFP (lower panels; bar: 10 µm), but absence of YFP emission.

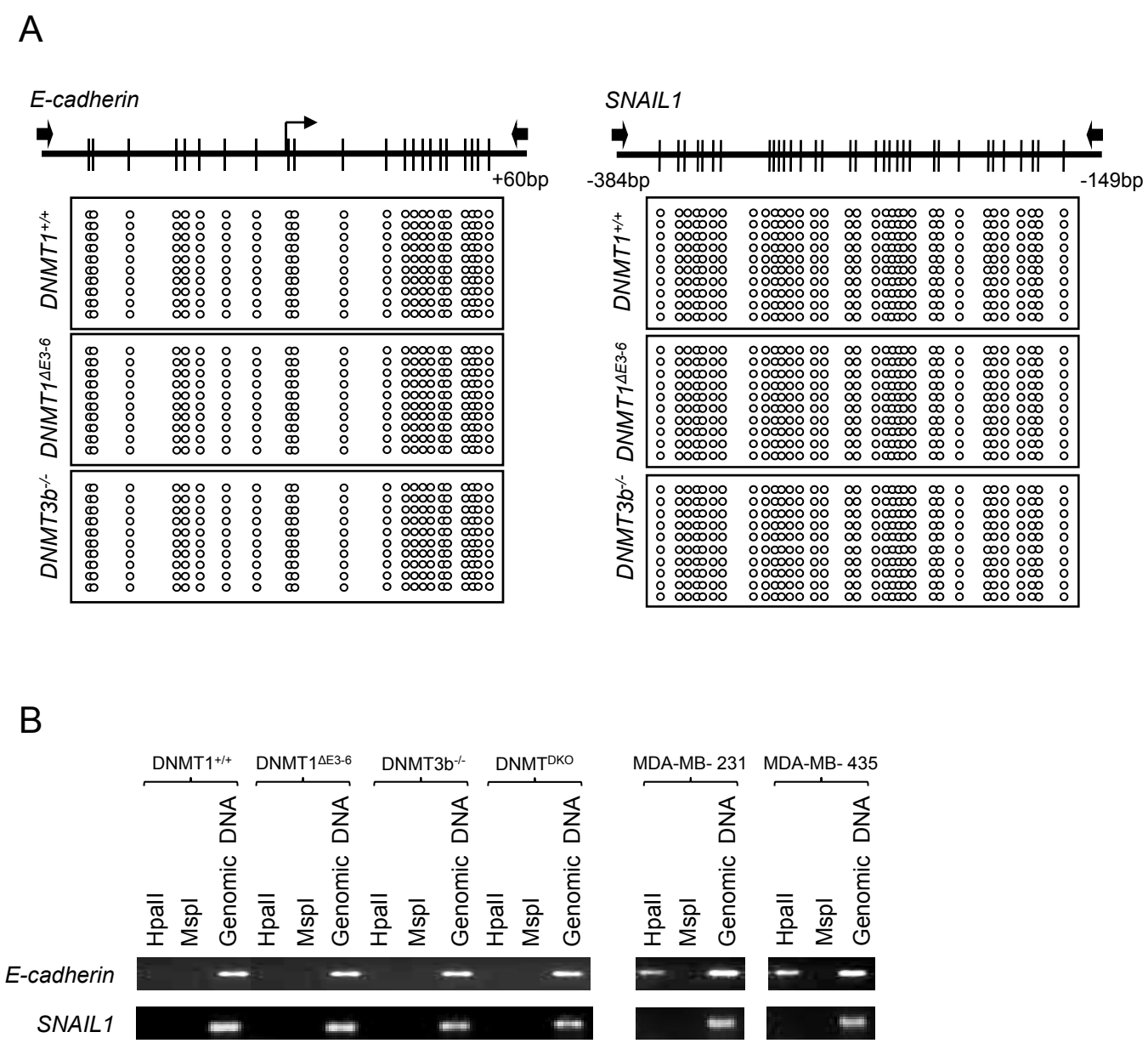
Supplementary Figure 8. Interaction between DNMT1 and SNAIL1 in human embryonic kidney cells (HEK-293T). Constructs of human SNAIL1 cDNA fused to a His tag and of human DNMT1 fused to a Myc tag were transiently transfected in 293T cells. Pull-down assay of the interaction of SNAIL1-His and DNMT1-Myc are shown. Results are representative of two different experiments.



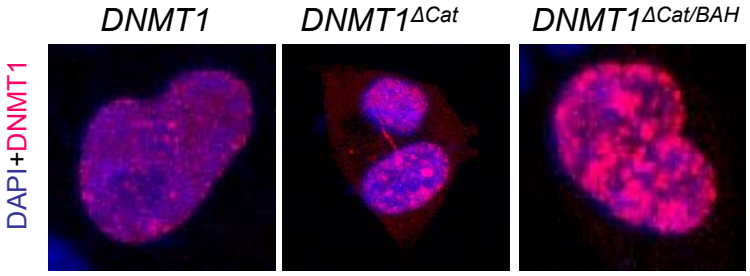
Supplementary Figure 1. Espada et al., 2011



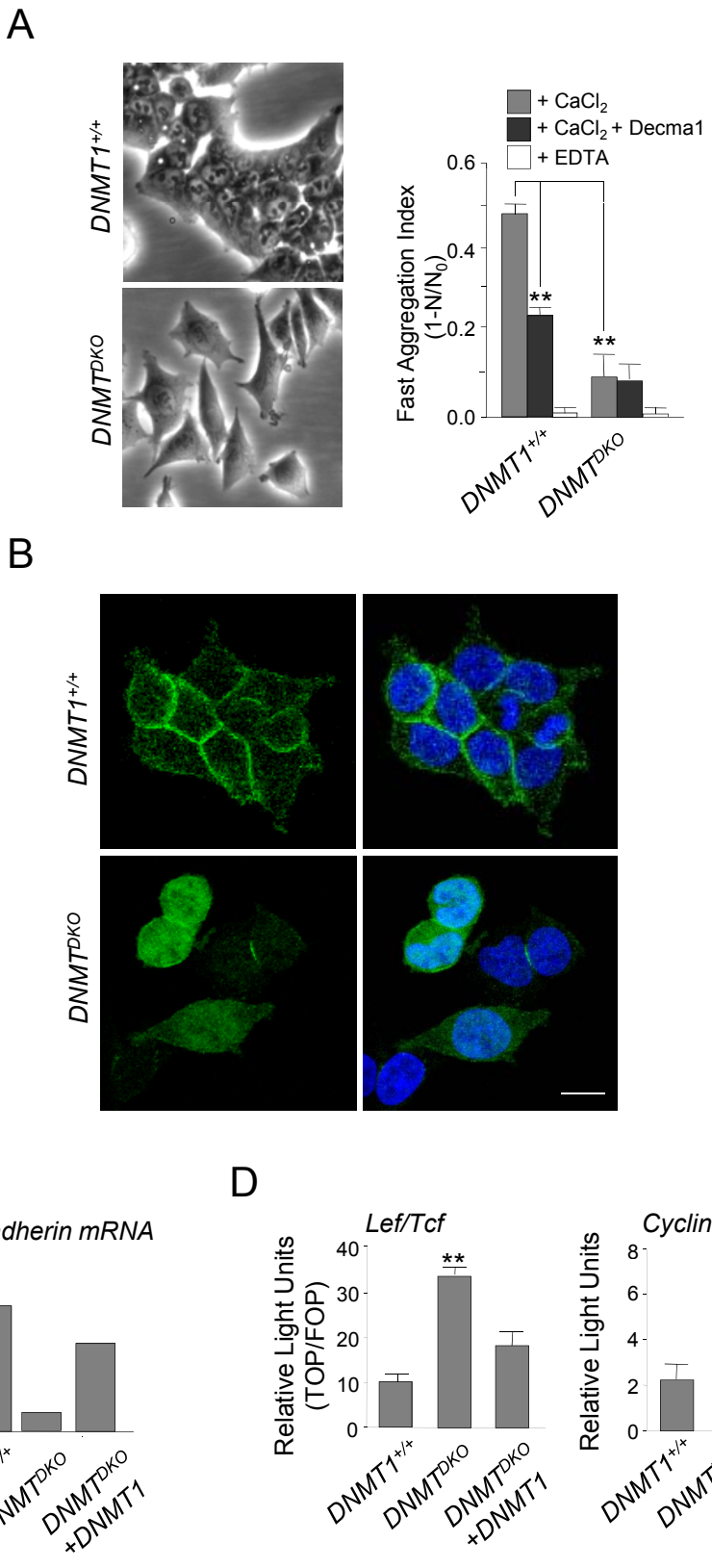
Supplementary Figure 2. Espada et al., 2011



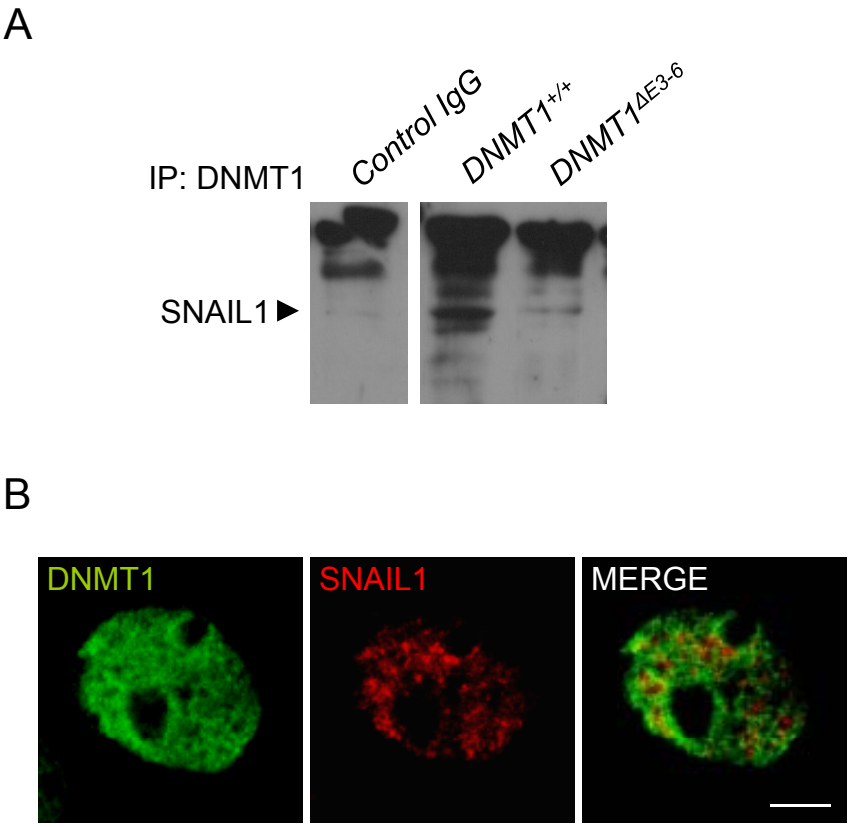
Supplementary Figure 3. Espada et al., 2011



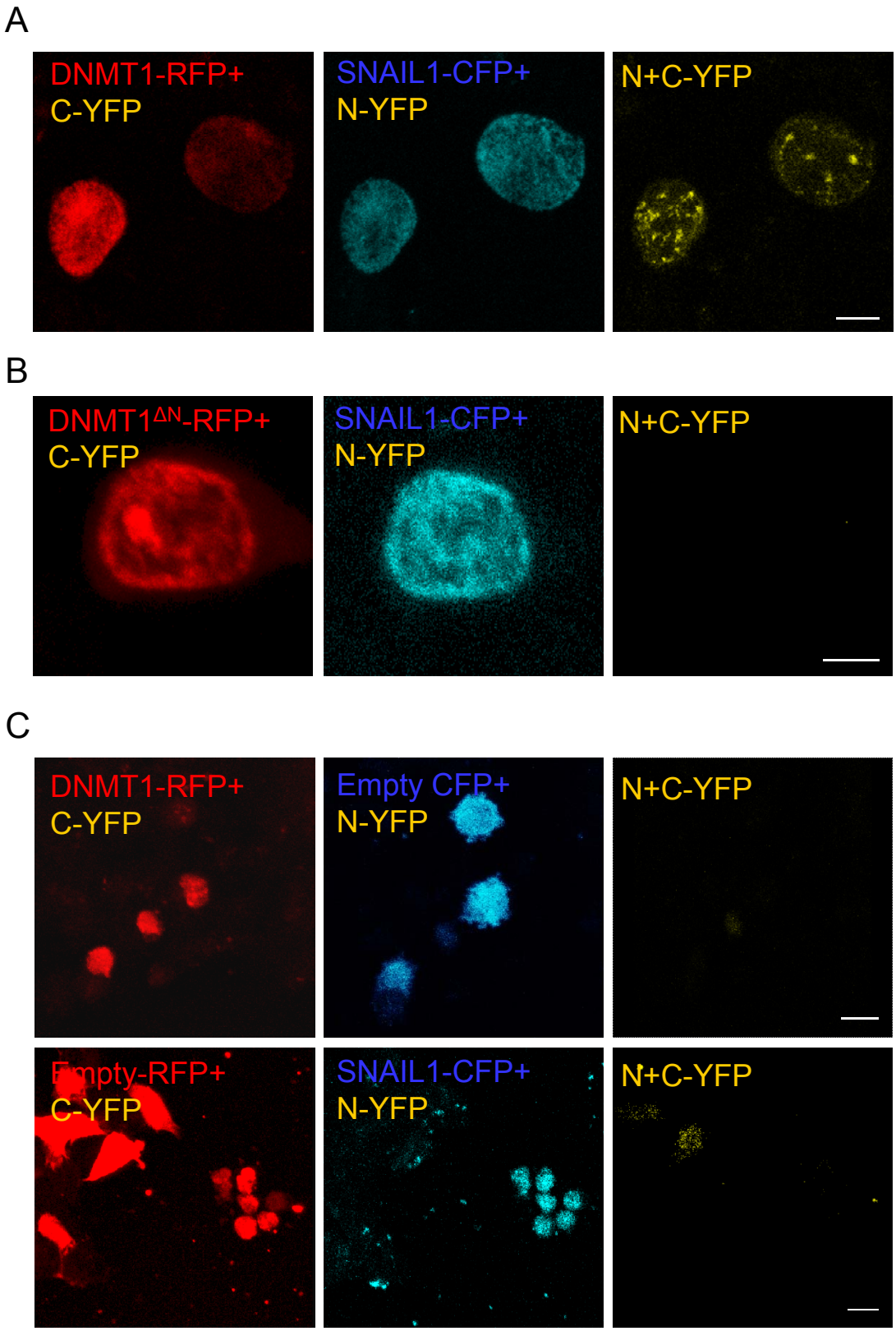
Supplementary Figure 4. Espada et al., 2011



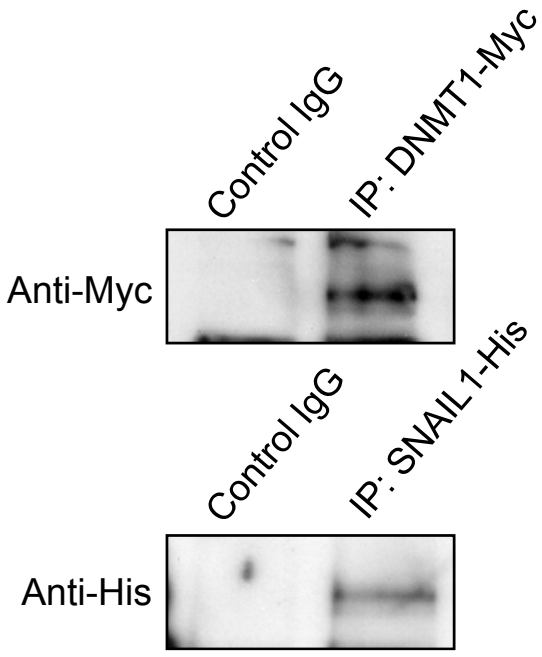
Supplementary Figure 5. Espada et al., 2011



Supplementary Figure 6. Espada et al., 2011



Supplementary Figure 7. Espada et al., 2011



Supplementary Figure 8. Espada et al., 2011

Supplementary Table 1.

Cloning Primers

Human SNAIL1

SNAIL1-Forward EcoRI:
AAAAAAAAGAATTCGCCGCCACCATGCCGCGCTCTTTCCTCGTCAGGAAGCCCTCCGACC
CCAATCGG

SNAIL1-Reverse NotI:
AAAAAAAAGCGGCCGCTCAGCGGGGACATCCTGAGCAGCCGGACTCTTGGTGCTTGTGGA
GCAGGGA

Annealing: 72ºC

Human SNAIL1-CFP

SNAIL1-Forward SacII:
AAAAAAAACCGCGGGCCGCCACCATGCCGCGCTCTTTCCTCGTCAGGAAGCCCTCCGACC
CCAATCGG

SNAIL1-Reverse Nhe I':
AAAAAAAAGCTAGCGCGGGGACATCCTGAGCAGCCGGACTCTTGGTGCTTGTGGAGCAGG
GA

Annealing: 70ºC

Human DNMT1

DNMT1-Forward EcoRI:
AAAAAAAAGAATTCGCCGCCACCATGCCGGCGCGTACCGCCCCAGCCCGGGTGCCCA

DNMT1-Reverse NotI:
AAAAAAAGCGGCCGCCTAGTCCTTAGCAGCTTCCTCCTCCTTTATTTTAGCT

Annealing: 69ºC

Human DNMT1$^{\Delta Cat/BAH}$

DNMT1$^{\Delta Cat/BAH}$–Forward EcoRI:
AAAAAAAAGAATTCGCCGCCACCATGCCGGCGCGTACCGCCCCAGCCCGGGTGCCCA

DNMT1$^{\Delta Cat/BAH}$ Reverse-NotI:
AAAAAAAGCGGCCGCCTAGGCCCCGAGGACTGTGTCTGTCCCAGCGCAGAACCAGTGGG
CGTGAA

Annealing: 73ºC

Human DNMT1$^{\Delta cat}$

DNMT1$^{\Delta cat}$-Forward EcoRI:
AAAAAAAAGAATTCGCCGCCACCATGCCGGCGCGTACCGCCCCAGCCCGGGTGCCCA

DNMT1$^{\Delta cat}$-Reverse NotI:
AAAAAAAGCGGCCGCCTACCACAGCGTGTCAGAGATGCCTGCTTGGTGGAATCCCTCCG
ACA

Annealing: 70ºC

Human DNMT1$^{\Delta N}$

Human DNMT1$^{\Delta N}$ Forward EcoRI-Kozak-NLS:
AATTCGCCGCCACCATGGGCTCTGGCAAACGGAAACCTCAGGAAGAGTCTGAAAGAGCC
AAATCGGATGAGTCCATCAAGGAAGAAGACAAAGACCAGGATGAGAAGAGACGTATG

Human DNMT1$^{\Delta N}$ Reverse BamHI:
GATCCATACGTCTCTTCTCATCCTGGTCTTTGTCTTCTTCCTTGATGGACTCATCCGATTTG
GCTCTTTCAGACTCTTCCTGAGGTTTCCGTTTGCCAGAGCCCATGGTGGCGGCG

Annealing: 72ºC

Human DNMT1-RFP

Human DNMT1-RFP Forward EcoRI:
AAAAAAAAGAATTCGCCGCCACCATGCCGGCGCGTACCGCCCCAGCCCGGGTGCCCA

Human DNMT1-RFP Reverse NheI:
AAAAAAAGCTAGCGTCCTTAGCAGCTTCCTCCTCCTTTATTTTAGCT

Annealing: 60ºC

Human DNMT1$^{\Delta N}$ -RFP

Human DNMT1$^{\Delta N}$ -RFP Forward EcoRI-NLS:
AAAAAAAAGAATTCGCCGCCACCATGGGCTCTGGCAAACGGAAACCTCAGGAAGAGTCT
GAAAG

Human DNMT1$^{\Delta N}$ -RFP Reverse NheI:
AAAAAAAGCTAGCGTCCTTAGCAGCTTCCTCCTCCTTTATTTTAGCT

Annealing: 60ºC

Bisulfite Sequencing Primers

E-cadherin

E-cadherin Forward:
TTGTTGATTGGTTGTGGT

E-cadherin Reverse:
TCCRAAATACCTACAACAACA

Annealing: 50ºC

SNAIL1

SNAIL1 Forward:
TTTTTTTTTTAGTGATGTGYGT

SNAIL1 Reverse:
AACCTTATCTACCACRCCC

Annealing: 50ºC

Methylation Specific PCR (MSP) Primers

E-cadherin

Methylated E-cadherin Forward:
TAGTTCGGTTCGATTCGATC

Methylated E-cadherin Reverse:
GAATACGTCCCTCGCAAAT

Annealing: 50ºC

Unmethylated E-cadherin Forward:
TTTTAGTTTGGTTTGATTTGATT

Unmethylated E-cadherin Reverse:
CCCAAATACATCCCTCACAAAT

Annealing: 50ºC

SNAIL1

Methylated SNAIL1 Forward:
CGTTTCGGAGGAGTTTTC

Methylated SNAIL1 Reverse:
CAATCGAAAACTCGTCTCC

Annealing: 50ºC

Unmethylated SNAIL1 Forward:
TTGTTTTGGAGGAGTTTTT

Unmethylated SNAIL1 Reverse:
CCAATCAAAAACTCATCTCC

Annealing: 50ºC

Quantitative RT-PCR Primers

E-cadherin

E-cadherin Forward:
ATTGCTCACATTTCCCAACTC

E-cadherin Reverse:
GTCACCTTCAGCCATCCT

SNAIL1

SNAIL1 Forward:

TCTAATCCAGAGTTTACCTTCC

SNAIL1 Reverse:
GAAGAGACTGAAGTAGAGGAG

RT-PCR Primers

E-cadherin

E-cadherin Forward:
CAGCACGTACACAGCCCTAA

E-cadherin Reverse:
ACCTGAGGCTTTGGATTCCT

Annealing: 52ºC

SNAIL1

SNAIL1 Forward:
GAAAGGCCTTCAACTGCAAA

SNAIL1 Reverse:
TGACATCTGAGTGGGTCTGG

Annealing: 52ºC

Chromatin Immunoprecipitation Primers

E-cadherin

E-cadherin Forward:
AGCGCTAGCAGGCTAGAGGGTCACCGCGT

E-cadherin Reverse:
CCGAAGCTTCACAGGTGCTTTGCAGTTCCG

Annealing: 61ºC

Satellite2

Satellite2 Forward:
TCGCATAGAATCGAATGGAA

Satellite2 Reverse:
GCATTCGAGTCCGTGGA

Annealing: 50ºC