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EXTENDED EXPERIMENTAL PROCEDURES

Vectors

The ICR sequence was first PCR amplified using 5′-GATTTTTATGGTGAGGTTTTA-3′ and 5′-CAAAACCACCCCTACTTCTAT-3′ and blunt cloned into pRRL.TetO_PGK_GFP vector between TetO repeats and PGK promoter in a EcoRV site. The sequence 5′-GAGTA TTTAGGAGGTATAAGAATT-3′ was then added between the TetO and ICR to facilitate the vector specific bisulfite PCR. The shRNA vectors were generated from a pLKO backbone. The *puro* resistance gene was replaced for a hygromycin resistance gene. The hairpin targeting human KAP1 gene (targeting: GACCACCAGUACCAGUUCUUA) was cloned in EcoRI/AgeI sites. All the lentiviral vectors were produced and tittered as previously described (Barde et al., 2010).

Extendend Human ESC Cultures and Experimentations

Undifferentiated H1 human ES cells (Thomson et al., 1998) were used following the Swiss Federal guidelines and maintained on BD Matrigel (BD Biosciences, as recommended by the manufacturer) in mTeSR1 medium (Stemcell technologies). For transduction, hES were detached with TrypLE Express (Invitrogen) and maintained for 24hrs with the Rho-associated kinase (ROCK) inhibitor Y-27632 (Ascent Scientific) (Watanabe et al., 2007). Cells were transduced with pLV.ttrKRAB and pLV.TetO.GFP or pLV.ICR .TetO.GFP vectors (Quenneville et al., 2011) at a multiplicity of infection of 36 and 10 respectively. When indicated cells were previously transduced with a control pLKO.1.empty or a pLKO.1.shKAP1 lentivector (SIGMA, TRCN0000018002). The KAP1 knock-down was verified, after hygromycin selection, by quantitative RT.PCR during the experiment. Maintenance of the pluripotent state of the transduced cells was verified by FACS analysis using the BD human pluripotent Stem Cell Transcription Factor Analysis kit.

Pyrosequencing

DNA was extracted using from approximately 100 000 cells using DNeasy Blood & Tissue Kit (QIAGEN). 1ug of DNA was then used for bisulfite conversion using EpiTect Bisulfite kit (QIAGEN). Pyrosequencing was performed using Pyro Gold reagent (QIAGEN). The ICR PCR product was obtained by nested PCR. First, a vector specific PCR was performed using 5′-GAGTATTTAGGAGGTATAAGA ATT-3′and 5′-CTACCTCAACAATTAAATCACTTCC-3′.primers. The 600 bp product was gel purified and second PCR was produced using 5′-GGGAAAAGAAGAGTGTTGAAGAAAAATTG-3′ and 5′-biot-CTACCTCAACAATTAAATCACTTCC-3′ followed by gel purification. Sequencing was then performed with 5′-TTAAAAAAAAATGTTGAGAAGTTAA-3′ sequencing primer. PGK methylation was evaluated by amplification with 5′-AGATTAGGATGGGTATTATTT-3′ and 5′-biotAATATAAACCCTATTCCTACCC-3′. DNA was gel extracted and sequencing was performed with 5′-GTGGATTTTTTTGGGGAGAGAGG-3′ sequencing primer.

SUPPLEMENTAL REFERENCES

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Thomson, J.A., Itskovitz-Eldor, J., Shapiro, S.S., Waknitz, M.A., Swiergiel, J.J., Marshall, V.S., and Jones, J.M. (1998). Embryonic stem cell lines derived from human blastocysts. Science *282*, 1145–1147.

Watanabe, K., Ueno, M., Kamiya, D., Nishiyama, A., Matsumura, M., Wataya, T., Takahashi, J.B., Nishikawa, S., Nishikawa, S., Muguruma, K., and Sasai, Y. (2007). A ROCK inhibitor permits survival of dissociated human embryonic stem cells. Nat. Biotechnol. *25*, 681–686.

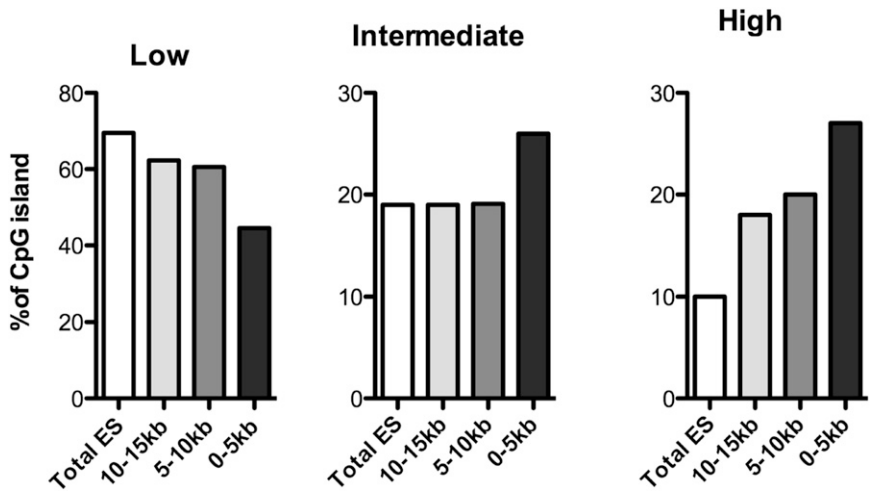

Figure S1. Confirmation of the Spreading of KRAB/KAP1-Associated DNA Methylation on Biochemically Isolated CpG Islands

Distribution of CpG islands among groups exhibiting low (<20%), intermediate (>20%, < 80%) and high (>80%) levels of methylation, comparing their entire pool (Total ES) with the subsets located at different distances from KAP1-binding sites in murine ESC. Islands identified with (Illingworth et al., 2010) data set.

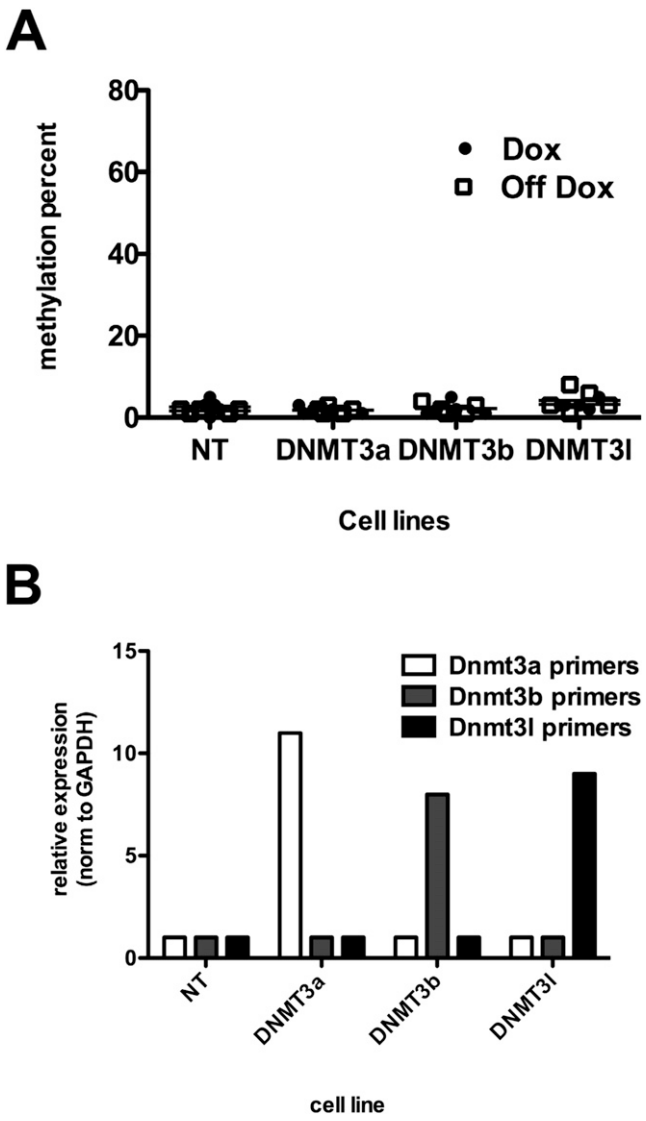

Figure S2. DNA Methylatrasferase Overexpression Does Not Recapitulate KAP1 De Novo Methylation Activity in Somatic Cells
(A) Methylation of ICR DNA, measured by pyrosequencing three weeks after introduction into NIH 3T3 cells expressing tTR.KRAB only (NT) or also overexpressing DNMT3a, DNMT3b or DNMT3l. (B) qRTPCR analysis of expression levels in cells transfected with plasmid encoding DNA methyltransferases (*x* axis).

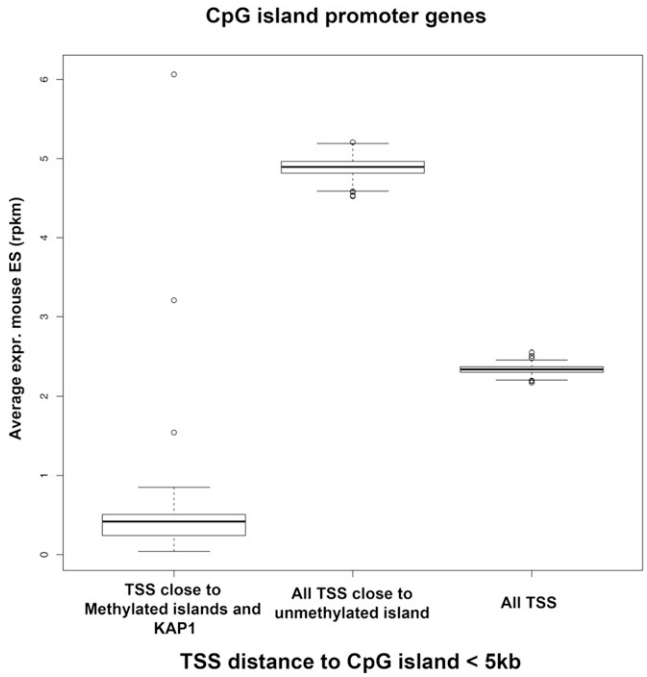

Figure S3. TSS Activity of Genes Close to KAP1 Binding Sites

Expression analysis of TSS close (<5kb) from methylated or non methylated CpG islands. CpG islands that are methylated and close (<5kb) from KAP1 binding site are compared to all TSS close to unmethylated islands or all TSS regarding the transcriptional activity (RPMK) of the resulting mRNA.