

**Supplementary information S2 (box): RNA transcripts proposed to induce DNA or chromatin modifications** (in order of discovery):

**LUC7L gene:** An antisense RNA for the  $\alpha$ -globulin gene (*HBA2*) can induce promoter DNA methylation, leading to transcriptional silencing of the  $\alpha$ -globulin gene<sup>1</sup>. Expression of *HBA2* is silenced and its CpG island becomes completely methylated during early development. Antisense RNA mediates silencing and methylation of the associated CpG island in the affected individual as well as in a mouse transgenic model.

**EF1 $\alpha$ -promoter-associated RNA:** RNA-mediated modification of the promoter region is shown for elongation factor 1 $\alpha$  (EF1 $\alpha$ )<sup>2</sup>. Low-copy promoter-associated RNAs are recognized by siRNA and function as a recognition motif to direct epigenetic silencing complexes to the corresponding sense gene promoter and, consequently, to mediate transcriptional silencing in human cells.

**P15AS transcript:** Transcriptional silencing of the tumour suppressor gene *p15* via DNA methylation and heterochromatin formation induced by antisense transcript, *p15AS*<sup>3</sup>.

**PR-AT transcript:** Promoter-directed antigen RNA (agRNA) is a class of synthetic RNA that can bind to the TSS of genes and can activate or block transcription of the target gene dependent on the cellular context<sup>4</sup>. Natural antisense transcript for progesterone receptor, *PR-AT1* and *PR-AT2*, is required for agRNA-induced gene activation or suppression. *PR-AT1* and *PR-AT2* bind to the Argonaute (Ago) protein and provide a scaffold for suppressor or activator proteins to assemble in the progesterone receptor promoter region<sup>5</sup>.

**P21-AS transcript:** Low-copy promoter-directed antisense transcript of the tumour suppressor gene *p21* mediates epigenetic modification of the sense promoter region<sup>6</sup>. Suppression of sense mRNA is directed by antisense-mediated induction of trimethylation at histone H3 lysine 27 (H3K27me3) at the *p21* sense promoter region<sup>6</sup>.

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2. Han, J., Kim, D. & Morris, K.V. Promoter-associated RNA is required for RNA-directed transcriptional gene silencing in human cells. *Proc Natl Acad Sci U S A* **104**, 12422-7 (2007).
3. Yu, W. et al. Epigenetic silencing of tumour suppressor gene p15 by its antisense RNA. *Nature* **451**, 202-6 (2008).
4. Janowski, B.A. et al. Inhibiting gene expression at transcription start sites in chromosomal DNA with antigene RNAs. *Nat Chem Biol* **1**, 216-22 (2005).
5. Schwartz, J.C. et al. Antisense transcripts are targets for activating small RNAs. *Nat Struct Mol Biol* **15**, 842-8 (2008).
6. Morris, K.V., Santoso, S., Turner, A.M., Pastori, C. & Hawkins, P.G. Bidirectional transcription directs both transcriptional gene activation and suppression in human cells. *PLoS Genet* **4**, e1000258 (2008).