SUPPLEMENTARY INFORMATION S1 (box) | Non-histone substrates of Lys methyltransferases

An increasing number of non-histone substrates is being identified for Lys methyltransferases (KMTs; see also Supplementary information S2 (Table)), highlighting an additional layer of complexity in their regulation of biological processes.

**H3K9 methyltransferases.** Thus far, the highest number of non-histone substrates has been identified for G9a1, which was shown to also methylate itself at two Lys residues embedded in an amino-acidic stretch with marked sequence similarity to that around H3K92. Autocatalytic G9a methylation is necessary to mediate in vivo interactions with CDYL and with HP1, which recognizes methylated G9a through its chromo-domain, with an affinity similar to that for H3K9me32. In hypoxic conditions, G9a methylates the chromatin remodeler RUVBL2 (REPTIN), which in turn binds to the promoters and induces silencing of hypoxia-responsive genes3. G9a-mediated methylation negatively regulates the chromatin binding capacity of different DNA-binding proteins (Supplementary information S2 (Table)) and also methylates and inactivates p534. G9a- and GLP-mediated methylation of DNMT3A and the self-methylation of GLP lead to the binding of MPP8 (M-phase phosphoprotein 8) and result in the formation of the silencing complex DNMT3A–MPP8–GLP (or G9a) on the chromatin5. A number of other substrates of G9a were also reported (Supplementary information S2 (Table))1,6, however the biological outcome of these modifications is unknown.

SUV39H1 and SETDB1 methylate the chromatin remodelers HP1α and ING2, respectively7, but the functional consequences of this are currently unknown. SUV39H1 also methylates the Polycomb 2 protein (PC2/CBX4), thereby inducing a noncoding RNA-dependent recruitment of growth-control genes into Polycomb foci and consequently their transcriptional repression8. The upstream binding factor (UBF), which positively regulates the transcription of ribosomal DNA (rDNA), is methylated and thus inactivated by SETDB1, ultimately leading to the condensation of nucleolar chromatin and decreased rDNA transcription9.

**H3K27 methyltransferases.** EZH2 was shown to interact and methylate the cardiac transcription factor GATA4, thereby attenuating its transcriptional activity by reducing its interaction with and acetylation by p30010. EZH2-dependent methylation of orphan nuclear receptor RORα provides a binding platform for the DCAF1–DDB1–CUL4 ubiquitin ligase complex11. EZH2 binds to and methylates STAT3, which promotes STAT3 phosphorylation and activity in glioblastoma stem-like cells12.

**H4K20 methyltransferases.** The tumor suppressor Numb promotes apoptosis in a p53-dependent manner. It is methylated by PR-Set713, which disrupts Numb–p53 interactions, increases p53 ubiquitination and degradation, and abolishes Numb-mediated apoptosis. Conversely, p53 itself is methylated by PR-Set714, leading to its recognition by L3MBTL1 and to p53 target gene expression15. PR-Set7 also methylates PCNA (proliferating cell nuclear antigen) to stabilize it and support proper DNA replication16.

Strategies for the identification of methylation of non-histone proteins have so far been based on the availability of modification-specific antibodies. However, taking
advantage of the native methyl-lysine domain of L3MBTL1 — malignant brain tumor domain repeats (3xMBT) — a new tool was recently developed for identifying mono- and dimethylated Lys residues. The ability of 3xMBT to bind methylated Lys in vitro and in cells, together with its methyl selectivity and lack of sequence specificity, make it a powerful tool for the proteome-wide enrichment of protein complexes containing methyl-lysines. This approach, coupled with chemical inhibition of KMTs, promises to dramatically increase our knowledge of the spectrum of non-histone targets of KMTs in the near future.

References