Electronic Supplementary Information

A novel screening strategy to identify histone methyltransferase inhibitors reveals a crosstalk between DOT1L and CARM1

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For the High Content Screening, U2OS cells are firstly seeded into a 96-well plate and treated with different compounds from the inhibitor library (Figure S1b). After treatment, cells are fixed and different histone marks inside the cells are labeled with the antibody coupled with corresponding fluorophores and followed by immunofluorescence. Images are taken using Olympus IX83 fully-motorized microscope (Figure S1C), and multiple images are processed for quantitative analysis to evaluate the compound activity on selective mark using the Fiji software, an automated analysis step was developed to process multiple images at the same time (Figure S1FG).

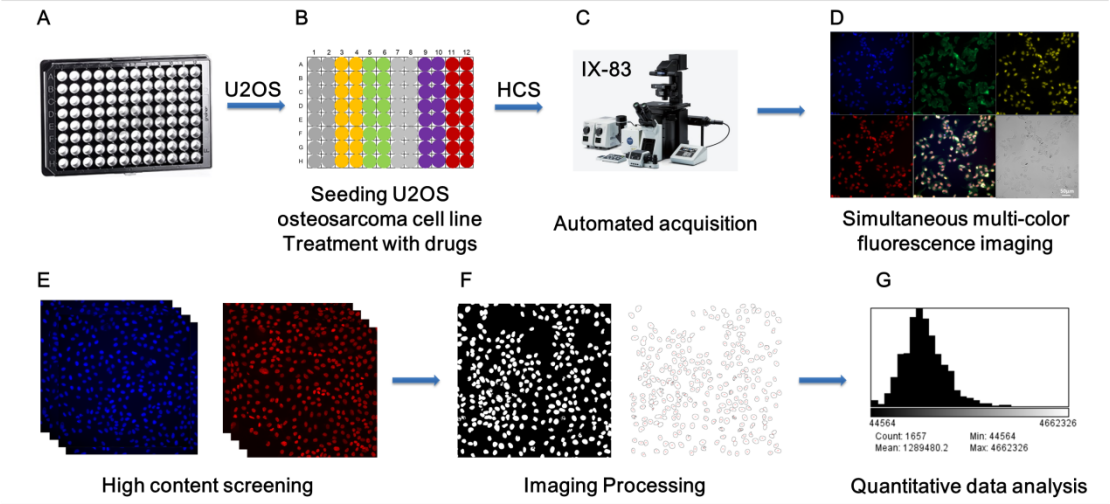

Fig S1: **Methodology of the HCS process.** Top panel: U2OS cells seeded into a 96-well plate (A) and treated with compounds (B). Use of Olympus IX83 fully-motorized microscope (C) to take simultaneous multi-color images (D). Bottom panel: Multiple images of DAPI stain-blue and antibody labelling cells-red (E) are analyzed by the Fiji software (F). Quantitative integrated fluorescence intensity analysis (G).

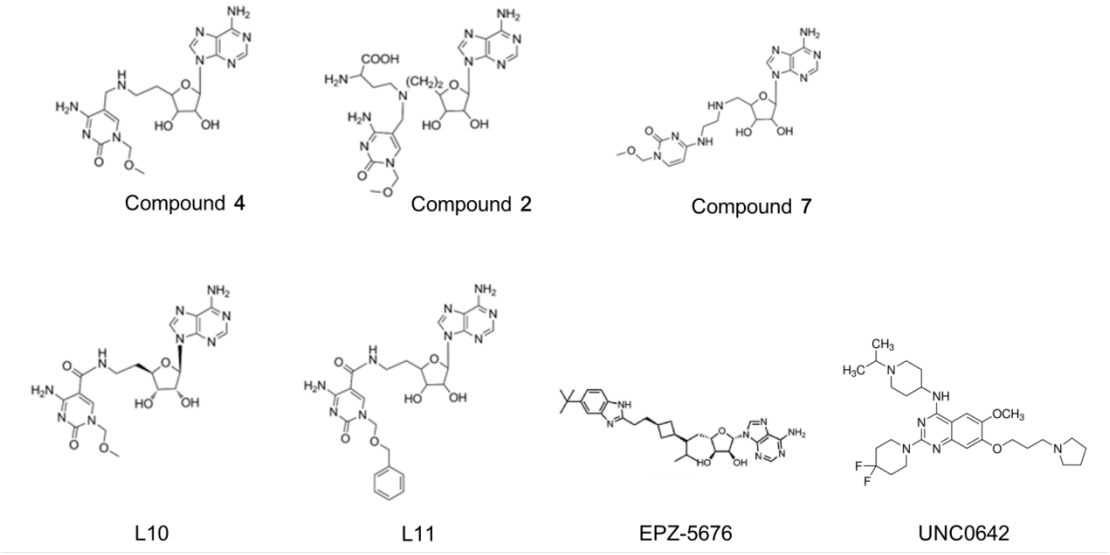

Fig S2: Selected bisubstrate and transition state analogues: compound **2**, **4**, **7** (Halby *et al,* 2018), **L10, L11** (corresponding to compound 1 and compound 2, respectively, in (Pechalrieu *et al,* 2020)), and control molecules: EPZ-5676 and UNC0642.

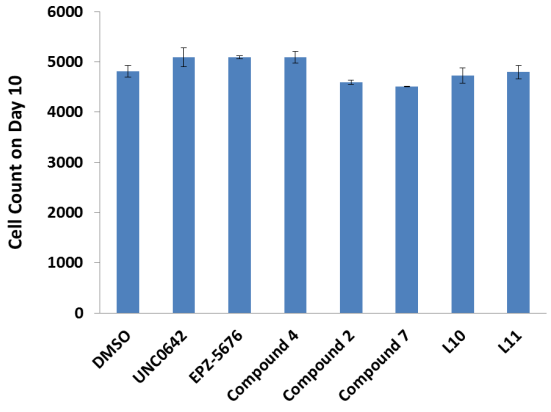

Fig S3- Toxicity. Bar graph representation of cell number counting of a DAPI staining of the nuclei in each well of U2OS cells in a 96 plate following a 10 days of treatment of each compound at concentration of 1 µM. Cells treated with DMSO were used as negative control. The error bars indicate the standard deviation between three technical replicates from the same plate.

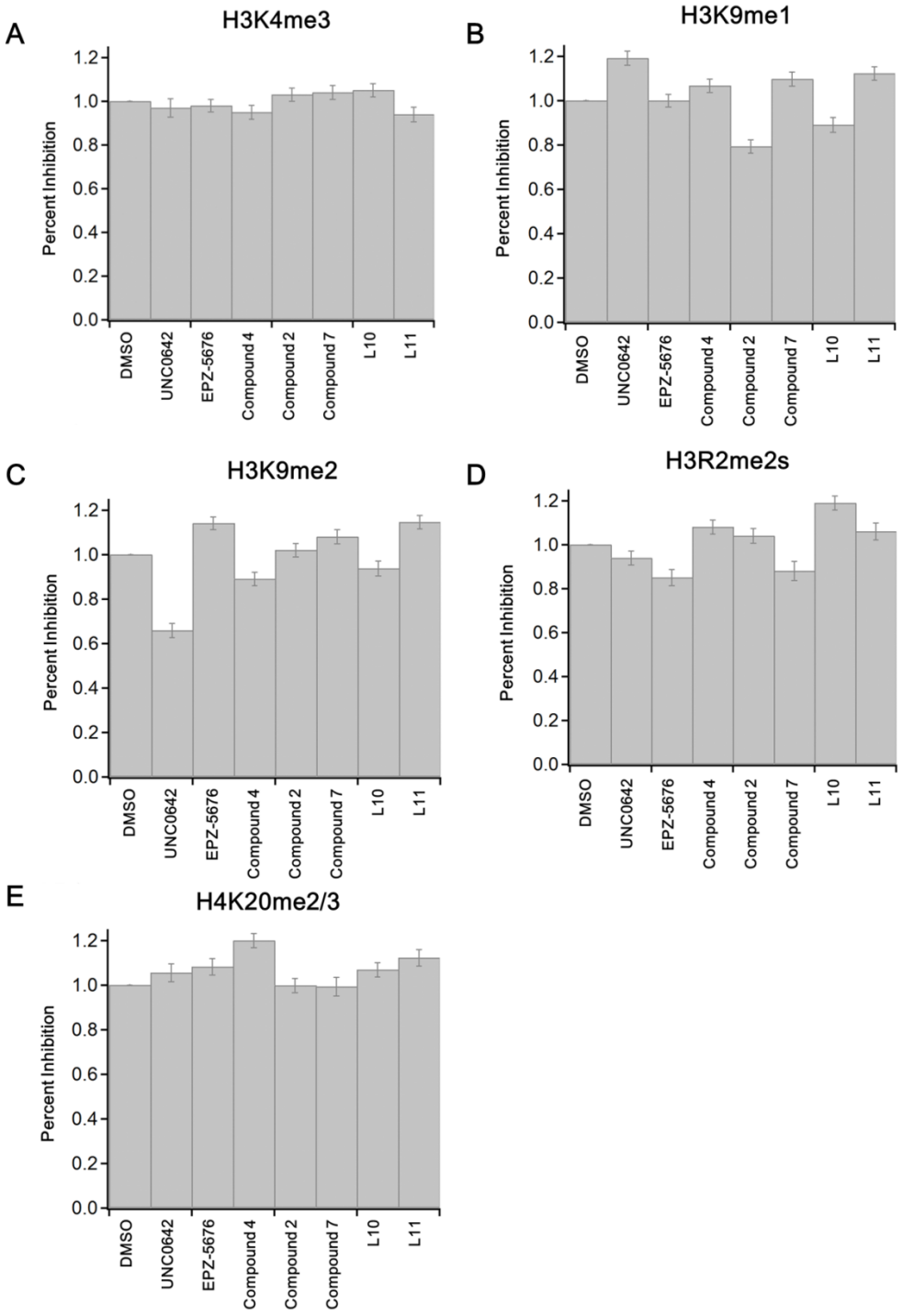

Fig S4: **Screening.** HCS screening of inhibitor library to inhibit H3K4me3(A), H3K9me1(B), H3K9me2(C), H3R2me2s(D), H4K20me2/3(E) in U2OS cells following ten days of treatment at 1 μM. The error bars indicate the standard deviation between two biological replicates.

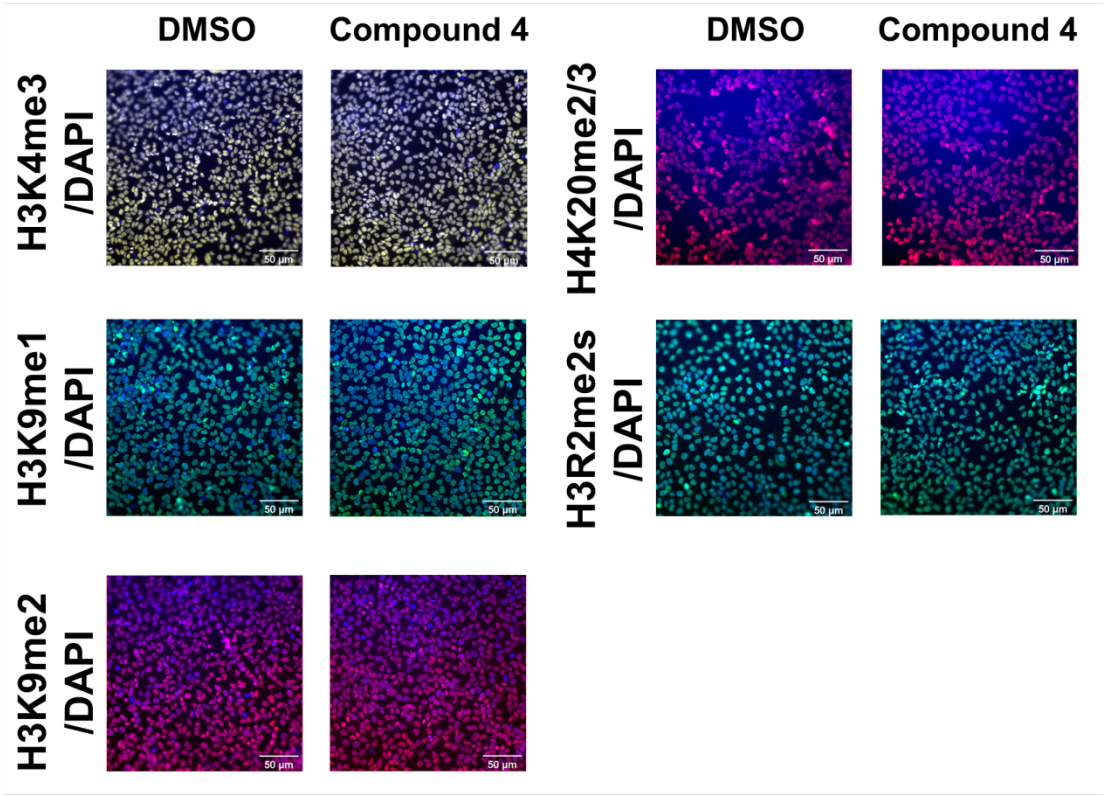

Fig S5: Stain of H3K4me3 (yellow), H3K9me1 (green), H3K9me2 (red), H4K20me2/3 (red) and H3R2me2s (green) merged with the staining of the nuclei (by DAPI, blue) in U2OS cells on day 10 treated with compound **4** (1µM) and DMSO control (0.1%).

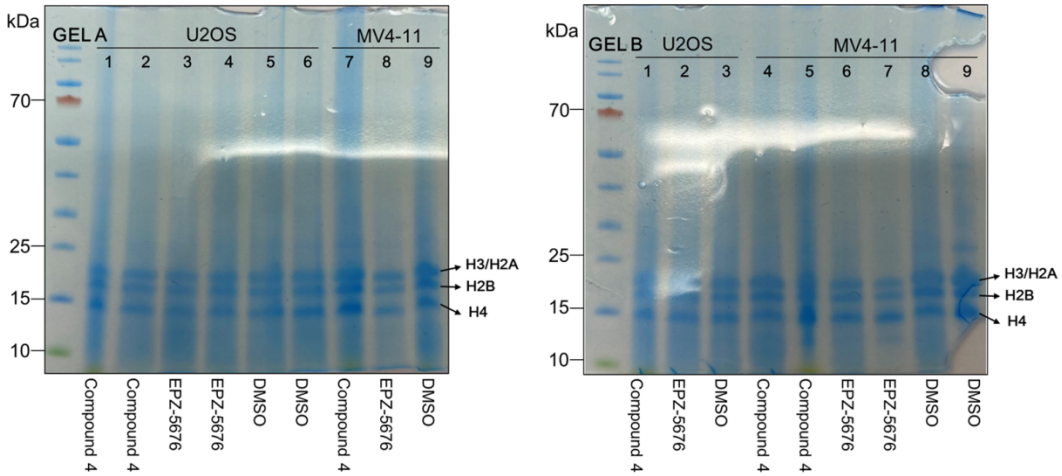

Fig S6: Purification of crude histones. Crude histones were separated by running on a NuPAGE 4-12% bis-tris gel in MES buffer and stained with instant blue (Sigma). Gel bands corresponding to H3/H2A were cut and collected for mass spectrometry analysis. U2OS cells treated with compound **4** (GELA-lane1/2, GELB-lane1), EPZ-5676 (GELA-lane3/4, GELB-lane2), control 0.1% DMSO (GELA-lane5/6, GELB-lane3); MV4-11 cells treated with Compound **4** (GELA-lane7, GELB-lane4/5), EPZ-5676 (GELA-lane8, GELB-lane6/7), control 0.1% DMSO (GELA-lane9, GELB-lane8/9).

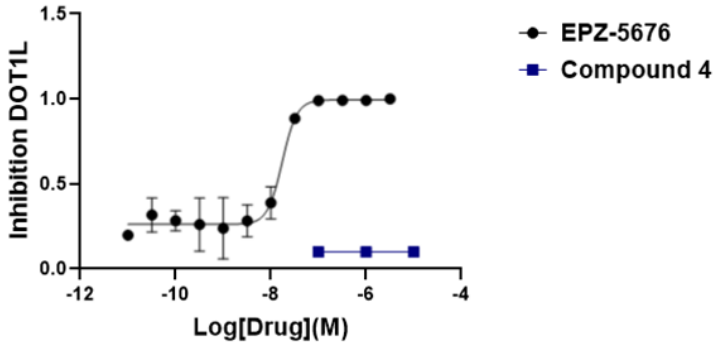

Fig S7: Evaluation of the compound **4** against DOT1L enzymatic activity compared to EPZ-5676 measured by AlphaLISA DOT1L Histone H3 Lysine-N-methyltransferase Assay(Song *et al*, 2018). The error bars indicate the standard deviation between two biological replicates.

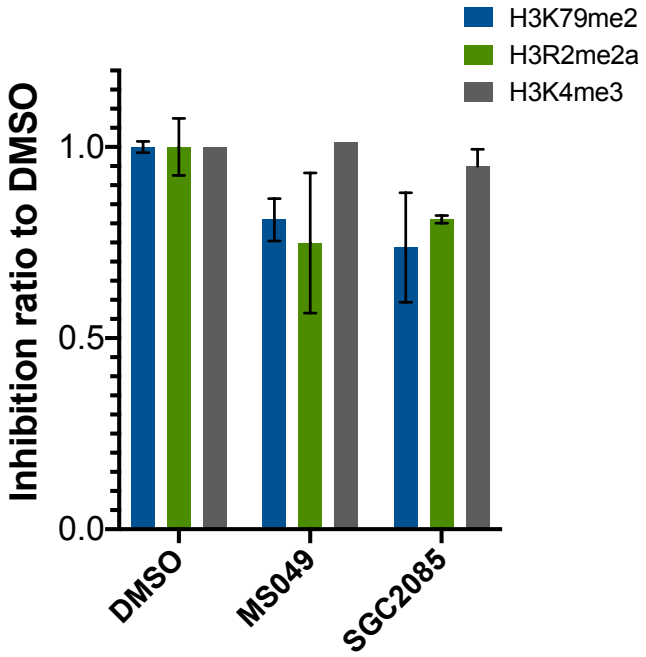

Fig S8: HCS evaluation of the commercial compounds MS049, specific inhibitor of CARM1 and PRMT6 (Shen *et al.*, 2016), and SGC2085, specific inhibitor of CARM1 (Ferreira de Freitas *et al.*, 2016). The mean value of biological replicates are shown for the dimethylation of H3K79me and the asymmetric dimethylation of H3R2 of U2OS cells treated with 1µM of compound MS049 (n=4 independent biological replicates) and compounds SGC2085 (n=3 independent biological replicates) normalized to DMSO. The trimethylation of H3K4 was used as control.

References:

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