Supporting Information for

Regulation of Wnt Signaling Target Gene Expression by the Histone Methyltransferase DOT1L

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METHODS

Cell culture and compound treatment

HEK293, SW480, or LS174T cells were cultured in DMEM (Life Technologies), 10% (v/v) fetal bovine serum (FBS; FisherBrand), and 1% Penicillin/Streptomycin (v/v) (Life Techlonogies) in the presence of EPZ004777 or DMSO control and were split and replated with fresh media and compound every 3-4 days. HEK293 cells were treated with 5 or 10 μM SB-261763 (Selleck Chemicals) for 24 hr prior to harvesting.

β-catenin-TCF/LEF luciferase reporter (TOPflash) assay

Upon pretreatment of cells with indicated concentrations of EPZ004777 or DMSO, cells were transfected with TOPflash or FOPflash reporter plasmids in conjunction with a renilla-luciferase reporter as a transfection control using Fugene6 (Roche) following manufacturer recommendations. After 24 hr HEK293 cells were treated with 5 μM SB-216763 after an additional 24 hr cell lysate was collected and luciferase measured using the dual luciferase reporter assay (Promega).

qRT-PCR analysis

RNA was isolated by Trizol (Invitrogen) and purified by RNeasy mini kit (Qiagen). cDNA was synthesized using SuperScript III first-strand synthesis (Invitrogen). qPCR analysis was carried out using Power SYBR green PCR master mix (Applied Biosystems) on an 7500 RT-PCR system instrument (Applied Biosystems).

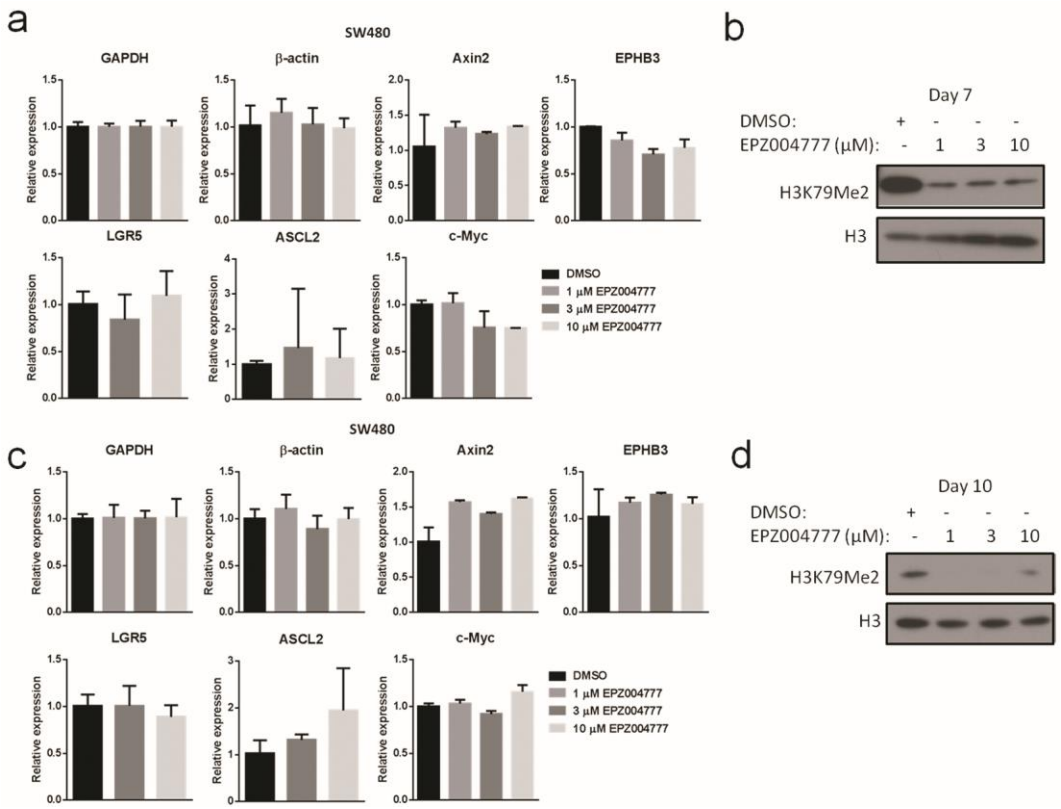
Western blot analysis of H3K79 methylation

Histones were extracted as described (*1*) and protein concentration was determined using Bradford assay (Bio-Rad) and normalized. Samples were denatured by in SDS-loading buffer and heating and separated on 4-20% Tris-Glycine gel (Invitrogen). Protein was transferred to PVDF membrane (Millipore), blocked with 5% (w/v) non-fat milk in tris-buffered saline with 0.05% Tween20 (TBST) and probed with antibodies from Abcam, histone H3 (ab1791), H3K79 Me1 (ab2886), or H3K79Me2 (ab3594). Followed by goat anti rabbit HRP conjugated secondary antibody (GenScript) and signal developed with Lumi-light western blot substrate (Roche) before exposure to autoradiography film (Denville).

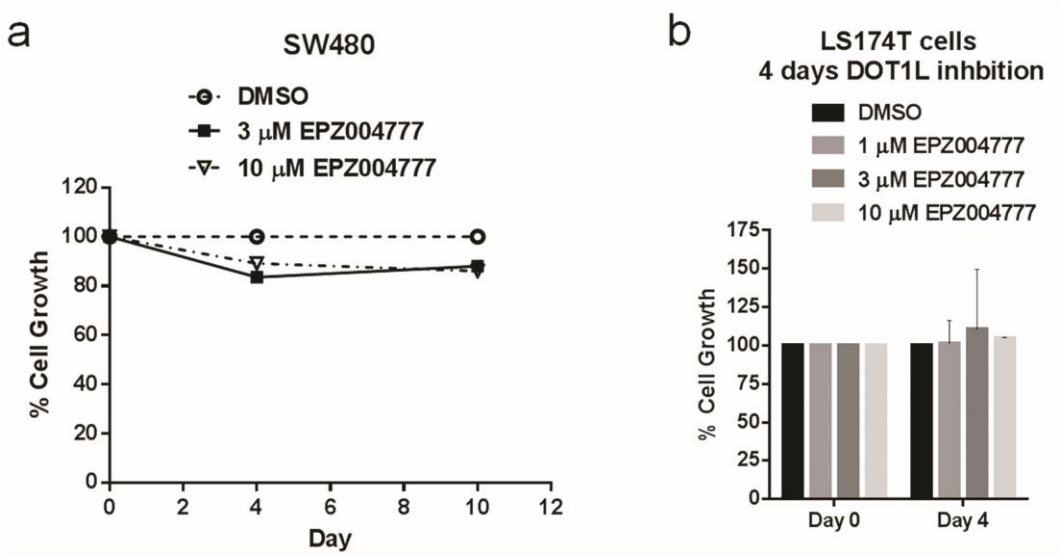
Immunohistochemical staining

Immunohistochemical staining of H3K79Me2 was carried out as previously described (*2*) on the

Dako Autostainer (Dako North America) using biotinylated goat antirabbit IgG (Invitrogen, 1:250), streptavidin-horseradish peroxidase, and diaminobenzidine as the chromogen. Deparaffinized sections of mouse formalin fixed tissue at 5-μm thickness were labeled with anti-H3K79me2 antibody (rabbit polyclonal antibody, 1:1500, ab3594, 30 minutes) after microwave citric acid epitope retrieval. Services were performed by the histology and immunohistochemistry services in the department of pathology at the University of Michigan.



Supporting Figure 1. Wnt target gene expression in SW480 cells over a time course in the absence of DOT1L enzymatic activity. qRT-PCR analysis of well characterized Wnt target genes and western blots of H3K79 dimethylation in SW480 cells upon (a) 7 days and (b) 10 days treatment with EPZ004777 at indicated concentrations.



Supporting Figure 2. Proliferation of human colon cancer cell lines is not inhibited by EPZ004777. Cell counts of (a) SW480 cells over at 10 day time course and (b) LS174T cells after 4 days of EPZ04777 treatment at the indicated concentrations.

REFERENCES

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2. Jo, S. Y., Granowicz, E. M., Maillard, I., Thomas, D., and Hess, J. L. (2011) Requirement for Dot1l in murine postnatal hematopoiesis and leukemogenesis by MLL translocation, *Blood 117*, 4759-4768.