**Supplementary Figures**

**Figure S1**

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**Figure S1. Mutual co-immunoprecipitation of HPV16 E6 and KDM5C in HCT116 cells.**

**Figure S2**

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**Figure S2. HPV16 E6 attenuates the KDM5C protein level and KDM5C mRNA quantification**

1. Protein levels of KDM5A, KDM5B and KDM5C in HCT116 cells when overexpressing HPV16 E6 or vector.

(B) Quantification of KDM5A, KDM5B and KDM5C expression in HCT116 cells transfected with HPV16 E6 or vector based on blot intensities in (A).

(C) KDM5C mRNA expression level in HCT116 cells overexpressing HPV16 E6 or vector was measured by qRT-PCR.

**Figure S3**

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**Figure S3. HPV16 E6 destabilizes KDM5C and alters the global H3K4 methylation**

1. HPV16 E6 or vector was transfected into U2OS cells. H3K4me3, H3K27me3 and H3K36me3 were detected by using antibodies against the corresponding histone methylation mark.
2. HPV16 E6 and KDM5C were co-expressed in U2OS cells and H3K4me3 levels were analysed by immunoblots.
3. Immunoblot analyses of H3K4me3 levels in U2OS co-expressing HPV16 E6 and p53.

**Figure S4**

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**Figure S4. KDM5C does not visibly alters HPV 16E6, HPV 16E7 mRNA and protein levels**

1. mRNA levels of HPV 16E6 and HPV 16E7 in both CaSki-vector and CaSki-KDM5C cells are quantified.
2. Immunoblots detecting HPV 16E6, HPV 16E7 and p53 protein levels. No significant alterations of the above proteins were detected.

**Figure S5**

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**Figure S5. KDM5C knock out increases tumorigenicity in mice xenograft assay**

1. KDM5C-deficient CaSki cells were generated using CRISPR-Cas9. Knock out efficiency was confirmed by immunoblot.
2. KDM5C knock out results in visibly larger tumors in nude mice xenograft assay.
3. Comparison of tumor volumes in mice xenograft assays with CaSki and KDM5C deficient CaSki-KDM5C KO cells.
4. Confirmation of KDM5C knock out in tumors excised from xenograft assays by immunoblot.

**Figure S6**

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**Figure S6**. H3K27Ac recruitment to chromatin in the parental cells was examined by the genomic analysis of ChIP-seq signals.  We identified a total of 313 super-enhancers in parental CaSki cell

 based on the signal of H3K27Ac by using ROSE algorithm, which stitches constituent enhancers

within 12.5 kb (excluding TSS ± 2 kb) and ranks the enhancers by input-subtracted signal of

H3K27Ac.

**Figure S7**

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**Figure S7**

1. Normalized ChIP-seq signal for H3K27Ac across the *EGFR* gene (upper panel) and c-*MET* gene (lower panel). The regions in red dashed box are selected regions corresponding to **Figure 6B**. The black bars denote the ChIP-PCR of KDM5C amplified region.
2. ENCODE H3K27Ac ChIP-seq data of indicated 7 cell lines obtained from dbSUPER

([www.bioinfo.au.tsinghua](http://www.bioinfo.au.tsinghua).edu.cn/dbsuper), clearly display tandem H3K27Ac peaks, suggesting the existence of *EGFR* and *c-MET* super-enhancers.

**Figure S8**

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**Figure S8.** Normalized H3K4me1 levels at KDM5C-bound super enhancers in CaSki-vector and CaSki-KDM5C cells. *p*-values by ANOVA test were shown.

**Figure S9**

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**Figure S9** **HPV 16E6 depletion leads to *EGFR* and *c-MET* super-enhancer inhibition.**

1. Specific siRNA targeting HPV16 E6 was introduced into CaSki cells. Alterations in *EGFR* and *c-MET* mRNA expression were measured by qRT-PCR.
2. and (C) *EGFR* and *c-MET* super-enhancers eRNA transcription levels in HPV16 E6-depleted CaSki cells relative to parental CaSki cells at three selected super-enhancer regions were examined by qPCR.
3. siRNA targeting KDM5C was introduced into C33A cells. Expression changes of KDM5C were examined by Immunoblots.
4. mRNA expression of *EGFR* and *c-MET* in C33A and KDM5C-depleted C33A cells was examined by RT-qPCR.
5. And (G)The eRNA transcription levels at *EGFR* and *c-MET* super-enhancers were examined by qPCR of three selected super-enhancers in parental C33A and KDM5C depleted C33Acells.

In all panels, qPCR data are represented as mean ± SD from three biological replicates.

\*p < 0.05; \*\*p < 0.01; *t* test.

**Supplementary Methods**

**Transfection, siRNA and MG132 treatment**

Transfection of HCT116 and U2OS cells was performed using FuGENE 6 (Promega, E2691) according to manufacturer’s instructions and harvested 48 h post transfection. For siRNA knockdown assays, CaSki cells were transfected with final concentration of 50 nM specific siRNA using Lipofectamine 2000 (Invitrogen) and incubated for 48 h before harvest. The targeting sequences are as follows: si-16E6 (5’-ACCGUUGUGUGAUUUGUUATT-3’) and (5’-CCAUAUGCUGUAUGUGAUATT-3’), si-E6AP (5’-CAACUCCUGCUCUGAGAUATT-3’) and (5’-GAUGUGACUUACUUAACAGTT-3’), si-KDM5C (5’-GCCAACCUUGUGCAGUGUATT-3’) and (5’-GC

CAGUGUAUCAAGUGCAATT-3’). For MG132 treatment, cells were exposed to 10 µM MG132 (Sigma, M7449) dissolved in dimethyl sulfoxide (DMSO) in culture medium for 4 h prior to harvest.

**Antibodies**

The following primary antibodies were used in our experiments: α-Tubulin (Santa Cruz Biotechnology, sc-8035), MBP-Tag (Beijing Ray Antibody Biotech, RM1007), KDM5A (Cell Signaling Technology, 3876), KDM5B (Cell Signaling Technology, 3273), KDM5C (Cell Signaling Technology, 5361), GFP-Tag (Abmart, M20004), HA-Tag (Abmart, M20003), FLAG-Tag (PMK001M), E6AP(Cell Signaling Technology, 7526), p53 (Servicebio , GB13029-1), H3K4me1 (ABclonal, A2355), H3K4me2 (Cell Signaling Technology, 9725), H3K4me3 (ABclonal, A2357 ; Cell Signaling Technology, 9751), H3K27me3 (Cell Signaling Technology, 9733), H3K36me3 (Cell Signaling Technology, 4909), H3K27Ac (Cell Signaling Technology, 8173),HPV16E6(Santa Cruz Biotechnology,sc-460), HPV16E7(Santa Cruz Biotechnology,sc-6981). Secondary antibodies used include Horseradish Peroxidase-HRP conjugated goat anti-mouse IgG (Servicebio, GB23301) and goat anti-rabbit IgG (Servicebio, GB23303).

**Co-immunoprecipitation and ubiquitination assays**

For HCT116 or U2OS cell line, cells were transfected with pEGFP-16E6 and pHAGE-KDM5C; For C33A cell line, cells were transfected with pEGFP-16E6 by using FuGENE 6 (Promega). After 48 hours, cells were harvested and then lysed on ice in immunoprecipitation Lysis Buffer (Beyotime, P0013) supplemented with fresh protease inhibitor cocktail (Biotool, B14002) for 30 minutes, and insoluble proteins were removed by centrifugation at 4°C at 13,000 rpm for 10 minutes. An aliquot of the cleared cell lysates was collected as input control, and the rest of the lysates were incubated with GFP-Tag primary antibody (Abmart, M20004) overnight at 4°C, then mixed with 30 μL of prewashed Protein G Sepharose beads (GE Healthcare, 17061805) for 3 h at 4°C. Protein resin complexes were washed three times in phosphate buffered saline (PBS). For immunoblot analysis, proteins were eluted into 2×SDS loading buffer by boiling beads. For ubiquitination assays, plasmids pEGFP-C1, EGFP-16E6, pHAGE-hKDM5C-Flag and pRK5-HA-Ubiquitin were transfected into HCT116 cells. 10 µM of MG132 (Sigma, M7449) were added to the culture medium for 4 h prior to harvest. Cells were lysed in IP Lysis Buffer (Beyotime, P0013) supplemented with 20 mM N-ethylmaleimide desumolyse inhibitor (Sigma, E1271) and protease inhibitor cocktail (Biotool, B14002), cleared supernatant was then incubated with KDM5C primary antibody (Cell Signaling Technology, 5361) overnight at 4°c followed by incubation with 20 μl Protein G Sepharose beads (GE Healthcare) at 4°c for 3 h on a rotator. For mutation assay of K1479R, the plasmid was transfected into HCT116 cells and performed as described above.

Immunoprecipitated proteins were separated in 8% SDS-PAGE gels and transferred onto BioTrace NT Nitrocellulose Transfer Membrane (Pall, 66485). After blocking in 5% nonfat dried milk– TBS-T (Tris-buffered saline [pH7.4] with 0.1% Tween 20), blots were incubated with corresponding antibodies. Membranes were washed in TBS-T, incubated in horseradish peroxidase (HRP) conjugated anti-mouse and anti-rabbit secondary antibodies. Blots were developed with enhanced chemiluminescence (Servicebio, G2014-1, G2014-2) and visualized on film. Direct immunoblots were conducted similarly.

**Immunofluorescence Microscopy**

HCT116 cells were plated on coverslips in 6-well plates and transfected with plasmids expressing HPV16E6 (EGFP-16 E6) using FuGENE 6 Transfection Reagent (Promega) according to manufacturer’s instructions. 48 h post-transfection, cells were fixed in 4% paraformaldehyde solution, permeabilized with 0.2% Triton X-100 and blocked in 5% Bovine Serum Albumin (BSA) solution. The coverslips were incubated with relevant primary antibodies overnight at 4°c then with corresponding secondary antibodies in the dark at room temperature for 1 h, followed by incubation in DAPI (4',6-diamidino-2-phenylindole) to achieve nuclear staining, washed and mounted onto microscopic slides and visualized by confocal microscopy (Leica TCS SP8 STED) under a 63x oil immersion objective lens. Images were acquired and processed with Leica Application Suite Advanced Fluorescence (LAS AF) software. Primary antibody used in this experiment was KDM5C (Cell Signaling Technology, 5361). CY-3 conjugated goat anti-rabbit IgG (Boster, BA1032) secondary antibody was used.

**Maltose Binding Protein (MBP) Pull-down Assay**

Pull-down assay was performed as previously described with slight modifications. Overnight culture of *E. coli* strain BL21 expressing MBP and MBP-E6 fusion protein were inoculated into LB medium supplemented with 0.01M glucose and 100 μg/ml ampicillin, grown to an OD600 of approximately 0.5-0.6 followed by addition of 0.5 mM IPTG, shifted to 20°c and cultured for a further 5 h. *E. coli* were harvested, resuspended in TMN buffer (50 mM Tris pH 8.0, 12.5 mM MgCl2, 0.1% Triton X-100, 0.5 mM DTT) and sonicated in ice-water bath. Cleared supernatant were incubated with pre-washed Amylose Resin beads (NEB, E8021V) at a volume of 50 μl per 1ml supernatant for 3 h at 4°c and then washed thoroughly with TMN buffer. CaSki-KDM5C cells were pelleted and lysed in NP-40 lysis buffer (130 mM NaCl, 50mM Tris pH 8.0, 1 mM EDTA, 1% Triton X-100, 1 mM DTT). Lysate supernatant was incubated with immobilized MBP or MBP-E6 fusion protein overnight at 4°c. Beads were then washed with Wash Buffer (130 mM NaCl, 50 mM Tris pH 8.0, 1 mM EDTA, 0.1% Triton X-100, 1 mM DTT) and complexed proteins were eluted with Elution buffer (20mM maltose in TMN buffer) and detected by Western blot.

**Chromatin immunoprecipitation (ChIP)-PCR and ChIP-Seq**

Stable expressing CaSki-pHAGE and CaSki-KDM5C cells were plated in 6-well plates until confluency and fixed with 37% fresh formaldehyde solution to the final concentration of 1% for 10 minutes. The reaction was quenched with 0.125mM glycine for 5minutes before washing the cells with ice-cold PBS. Cells were trypsinized for 1 minute, after which relevant complete culture medium was added to inhibit digestion, lysed in ChIP SDS lysis buffer (1% SDS, 10mM EDTA, 50mM Tris pH 8.1, and protease inhibitor cocktail) for 10 minutes before sonication in ice-water bath to obtain DNA fragments in the range of 100-500bp. Clarified lysates were diluted with ChIP dilution buffer (0.01% SDS, 1% Triton X-100，2mM EDTA, 20mM Tris pH 8.1, 150mM NaCl and protease inhibitor cocktail) , precleared with salmon sperm DNA blocked Protein G Sepharose 4 Fast Flow (GE Healthcare, 17061805) for 1 h at 4°c and incubated overnight at 4°c with primary antibodies respectively. Protein-DNA complex is then precipitated with salmon sperm DNA blocked Protein G Sepharose beads at 4°c for 4 h. Bound complexes were washed once with each of the following buffers : low salt wash buffer (0.1% SDS, 1% Triton X-100, 2mM EDTA, 20mM Tris pH 8.1, 150mM NaCl), high salt wash buffer (0.1% SDS, 1% Triton X-100, 2mM EDTA, 20mM Tris pH 8.1, 500mM NaCl), LiCl wash buffer (0.25M LiCl, 1% deoxycholic acid, 1mM EDTA, 10mM Tris pH 8.1) and TE buffer (1M Tris pH 8.1, 0.5M EDTA). Complexes were eluted from the beads using Elution Buffer (10% SDS, 1M NaHCO3) at room temperature and cross-links were reversed by the addition of 5M NaCl into the eluent at 65°c overnight. ChIP DNA were prepared from the overnight samples with Universal DNA Purification Kit (Tiangen Biotech, DP214) according to manufacturer’s instructions and validated by qRT-PCR on the CFX Connect Real-Time PCR Detection System (Bio-Rad) using SYBR Green qPCR Master Mix (Biotool, B21802). Validated DNA samples were prepared for next-generation sequencing on HiSeq 2500 (Illumina) according to Illumina’s protocol. Sequencing reads generated were mapped to human genome (hg38) database using Burrows-Wheeler Aligner. Peak calling was performed by MACS 2.0 and significant enrichments were identified with threshold level set at *q-*value <0.05.

Primer sequences used in this assay were listed in **Table S2** in the supplemental information.

**Cell Invasion Assay**

Polycarbonate membrane of 12 Transwell inserts (24 wells, 8μm membrane pore size, Corning Costar, 3422) were coated with Matrigel Basement Membrane Matrix (Corning, 356234) diluted in chilled unsupplemented RPMI-1640 (HyClone), incubated in humidified incubator at 37°c overnight and rehydrated in unsupplemented RPMI-1640 medium. Stable CaSki-pHAGE and CaSki-KDM5C cells were harvested, resuspended in RPMI-1640 medium containing 2% FBS and 100ul of cells were seeded at a density of 2x105 cells onto the membrane-coated inserts. Inserts were carefully lowered into wells containing RPMI-1640 medium supplemented with 10% FBS to serve as chemoattractant and incubated in 37°C. 24 h later, non-invading cells were removed from the upper surface of membrane using PBS-soaked cotton swabs. Inserts were washed with PBS, fixated in 4% paraformaldehyde and stained in 0.1% crystal-violet. Migrated cells were quantified under a light microscope in 5 random fields at a magnification of 100 x. Assays were performed in triplicates.

**Real Time Cell Proliferation Assay**

Assay was carried out on an xCELLigence RTCA DP instrument (ACEA Biosciences). 100ul of stable CaSki-pHAGE and CaSki-KDM5C cells were plated at a density of 5×103 cells in a pre-calibrated E-Plate VIEW 16 electronic microtiter plates (ACEA Biosciences, USA) with 50ul of relevant culture medium. Plates were then loaded onto the instrument and set up according to manufacturer’s instructions. Data were initially collected overnight at intervals of 15 minutes per sweep, followed by 120 sweeps for 2 h at a rate of 1 sweep/min before returning to preceding rate for up to 80 h. Data were acquired using RTCA Software 2.0 paired to the RTCA DP instrument.

**Wound healing Assay**

Stable CaSki-pHAGE and CaSki-KDM5C cells were plated in 6-well plates and incubated at 37°c to obtain a confluent monolayer. A single straight line was scraped on the monolayer with a p200 pipette tip resting against ruler and washed with PBS (HyClone) before further incubation in unsupplemented RPMI-1640 (HyClone). Images were visualized at intervals of 0, 12, 24, 36 h with the IX53 Microscope Systems (Olympus) and acquired using cellSens Software (Olympus Corporation).

**Trypsin Digestion and Mass spectrometry analysis**

Immunoprecipitation of proteins were performed as described above, substituting Protein G Sepharose beads for Dynabeads Protein G (Invitrogen,10003D), bound proteins were redissolved in 50 mM ammonium bicarbonate, then in-solution digested by trypsin as previously described. The digested the peptides were then analyzed by online nanoflow LC−MS/MS using the Ultimate 3000 Nano-LC system (Dionex, Sunnyvale, CA) connected to a LTQ-Orbitrap Elite (Thermo Scientific) mass spectrometer. Samples were injected onto the analytical C18-nanocapillary LC column (C18 resin with 3 μm particle size, 15 cm length 150 mm length × 75 μm inner diameter, Acclaim PepMap RSLC, Thermo Scientific ) and eluted at a flow rate of 300 nl/min with a 70 min gradient from 5% solvent B (90% ACN/0.1% formic acid, v/v) to 50% solvent B. The peptides were then directly ionized and sprayed into an Orbitrap Elite mass spectrometer by a nanospray ion source. Mass spectrometer was operated in a data-dependent mode with an automatic switch between MS and MS/MS acquisition. Full MS spectra from m/z 350 to 1800 were acquired with a resolution of 60,000 at m/z 400 in profile mode. Following every survey scan, up to 20 most intense precursor ions were picked for MS/MS fragmentation by collision-induced dissociation (CID) with normalized collision energy of 35%. The dynamic exclusion duration was set to be 60 s with a repeat count of one and ±10 ppm exclusion window.

All acquired raw data were processed with MaxQuant software (Version 1.5.3.30). The peak lists were searched against the NCBI Human Protein Database (https://www.ncbi.nlm.nih.gov/protein). Two missed cleavages were allowed for trypsin. The precursor and fragment ion mass tolerances were 10ppm and 0.02 Da, respectively. Carbamidomethylation (Cys) were set as a fixed modification, whereas oxidation (Met), deamidation (Asn /Gln) and Gly-Gly (Lys) were set as variable modifications. Minimum peptide length was set at 6 while the estimated false discovery rate (FDR) threshold for modification site, peptide and protein were specified at maximum 1%.

**Supplementary Tables**

**Table S1**. **List of qPCR primers used in this study**

|  |  |
| --- | --- |
| Primer Names | Sequences(5’-to-3’) |
| 16E6-mRNA-F | GAGAACTGCAATGTTTCAGGACC |
| 16E6-mRNA-R | TGTATAGTT GTTTGCAGCTCTGTGC |
| KDM5C- mRNA-F | GACTCTGCGGAAGAAAGATA |
| KDM5C- mRNA-R | GTCTTAGGCGATGTTGACTC |
| GAPDH-F | TGCACCACCAACTGCTTAGC |
| GAPDH-R | GGCATGGACTGTGGTCATGAG |
| EGFR- mRNA -F | CCTGGTCTGGAAGTACGCAG |
| EGFR- mRNA -R | CTTCGCATGAAGAGGCCGAT |
| c-MET- mRNA -F | GAGAAGGCTAAAGGAAACGAA |
| c-MET- mRNA -R | TGTGTGAAAAGTCTGAGCATCT |
| EGFR- eRNA#1 -F | CCCCTGACTCCGTCCAGTATTGA |
| EGFR- eRNA#1 -R | TTCTTTTCCTCCAGAGCCCGACT |
| EGFR- eRNA#2 -F | TGCCCAGTGTCTCAGCCTATCAT |
| EGFR- eRNA#2 -R | CCCAGACCCACCCCACTCCT |
| EGFR- eRNA#3 -F | TGTCCAAAACCAGGAAGTTCATAGG |
| EGFR- eRNA#3 -R | GAAAGGAGAGCGGGGCTAAAATC |
| c-MET - eRNA#1 -F | CTCAGGGGTTCTGCTTTCTTTG |
| c-MET - eRNA#1 -R | CCCTCCTAAGTCATACAGGTGGC |
| c-MET - eRNA#2 -F | CCTCTCCCCTTTGTCTCTCTGTCC |
| c-MET - eRNA#2 -R | CGAGGAACTATGCTCAGGATTGGA |
| c-MET - eRNA#3 -F | TCTATTGCCTCTGTTGGGTTTTTTC |
| c-MET - eRNA#3 -R | GCTTACATTGGTCTTTTCATTTCCC |

**Table S2**. **List of DNA oligonucleotides used in this study**

|  |  |
| --- | --- |
| Primer Names | Sequences(5’-to-3’) |
| EGFR-ChIP-F | GTCTGCCACTCACCATACCACA |
| EGFR-ChIP-R | CCAGTATTAAGCGAGCAATCATC |
| c-MET -ChIP-F | GGGGTTGCTTCTGATGGC |
| c-MET -ChIP-R | ACGGTGGGACGAGGATGA |