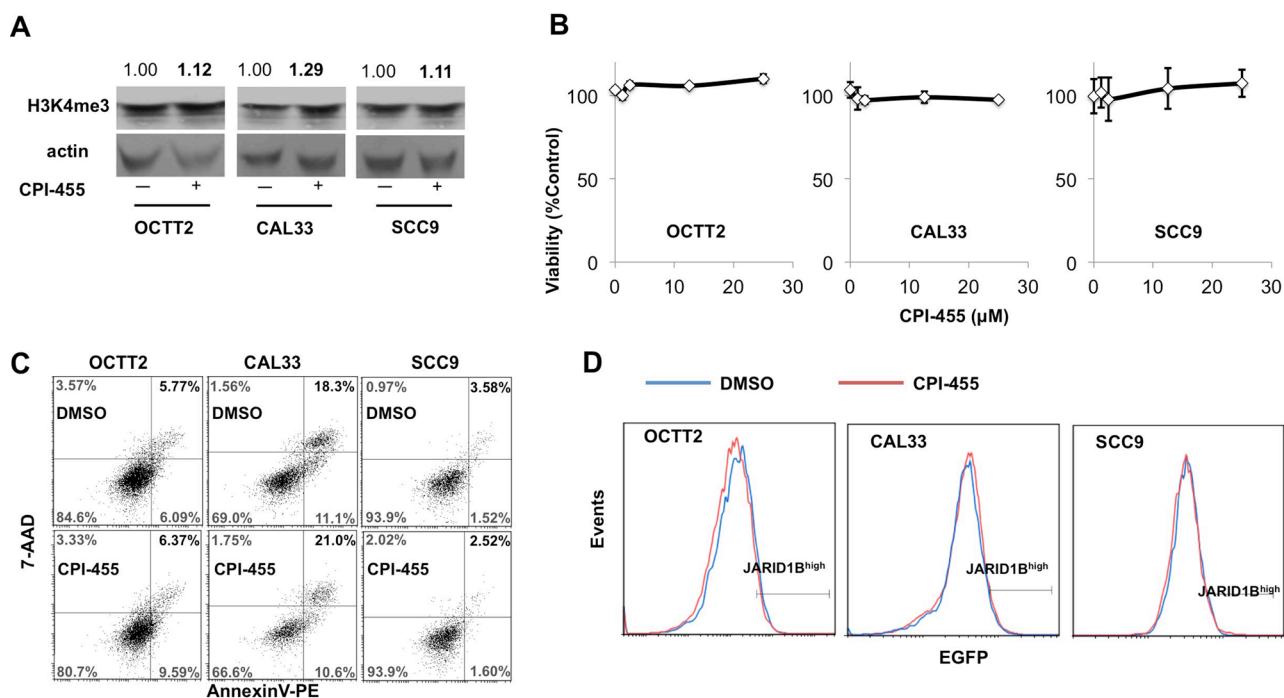
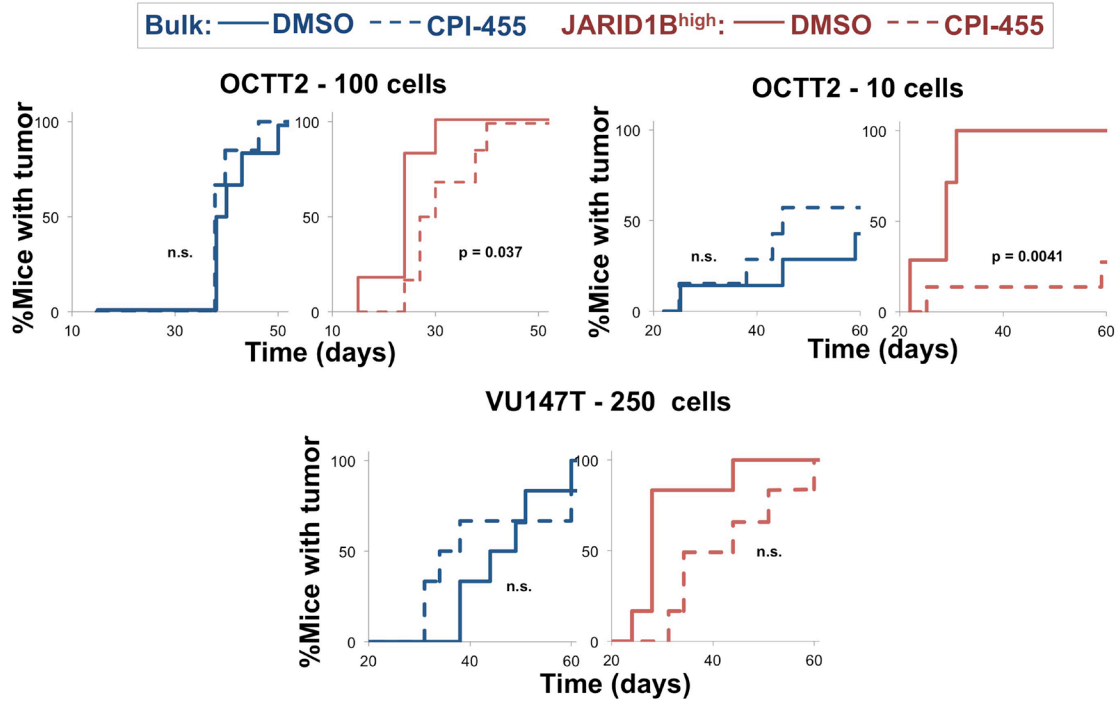


Targeting JARID1B's demethylase activity blocks a subset of its functions in oral cancer

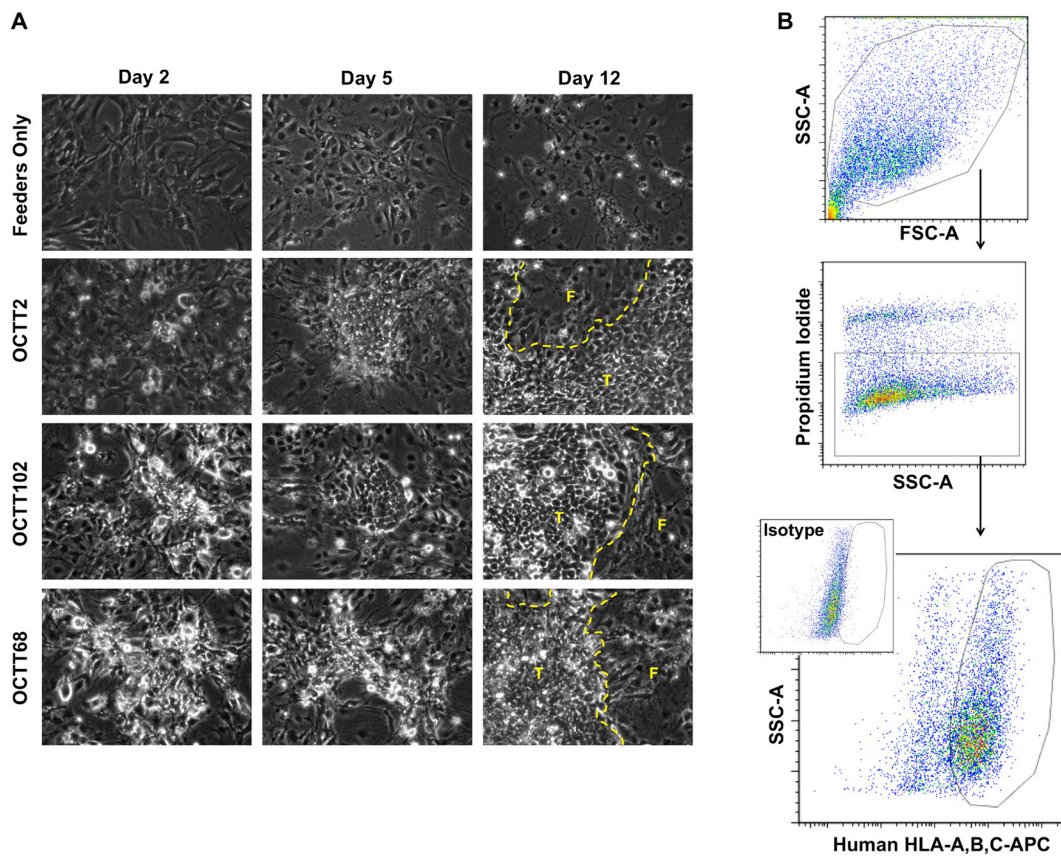
SUPPLEMENTARY MATERIALS



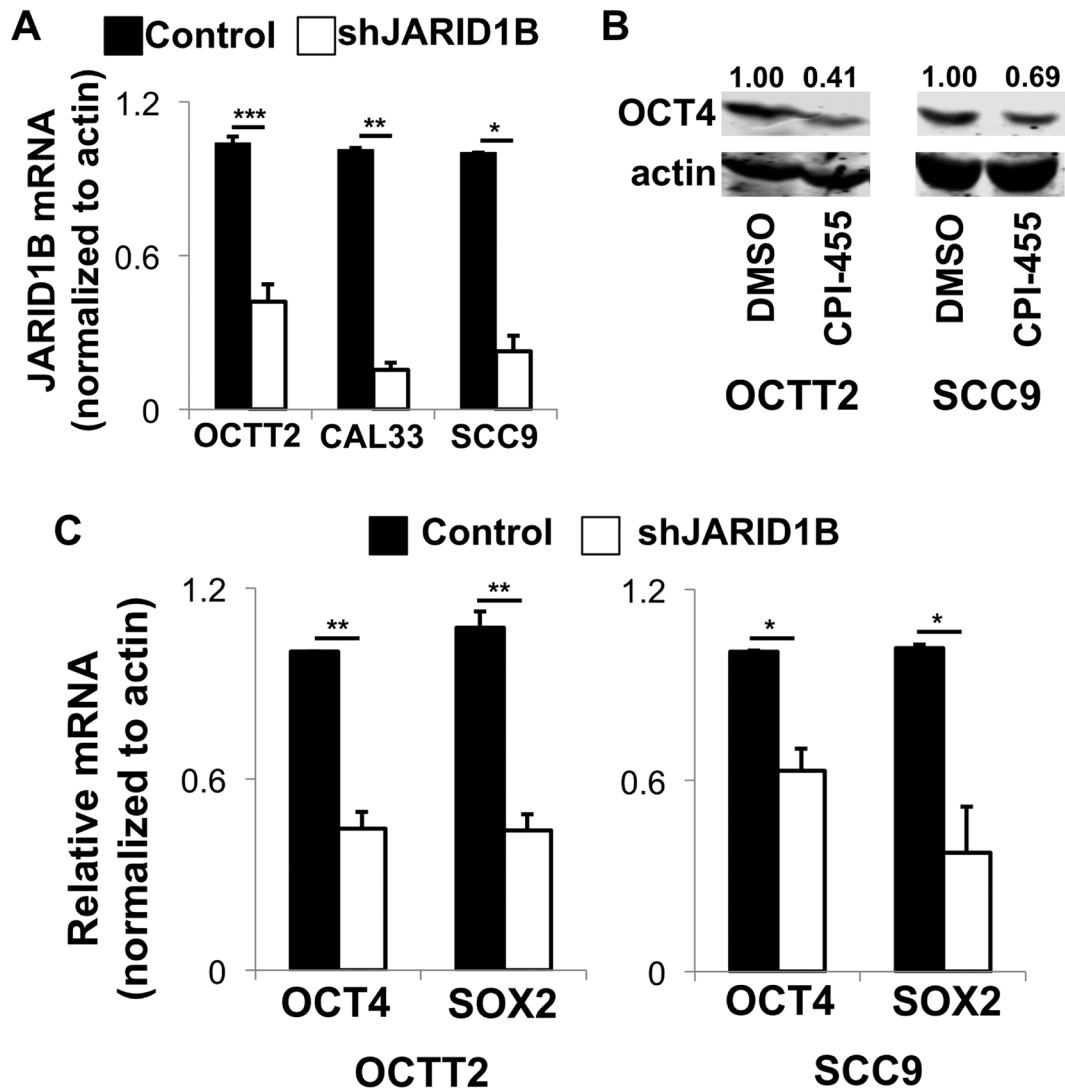
Supplementary Figure 1: CPI-455 has no effect on OSCC cell proliferation or apoptosis. (A) WB showing H3K4me3 levels in cell lines treated for 48 hours with CPI-455 (20 µM). Values are H3K4me3 band densities normalized to actin. (B) MTS assay of cell lines treated with CPI-455 for 72 hours. (C) Apoptotic cells (Annexin-V+/7-AAD+) after CPI-455 (20 µM) treatment. (D) JARID1B expression based on EGFP intensity in J1BpromEGFP reporter-containing OSCC cell lines treated for 5 days with CPI-455 (20 µM) or DMSO.



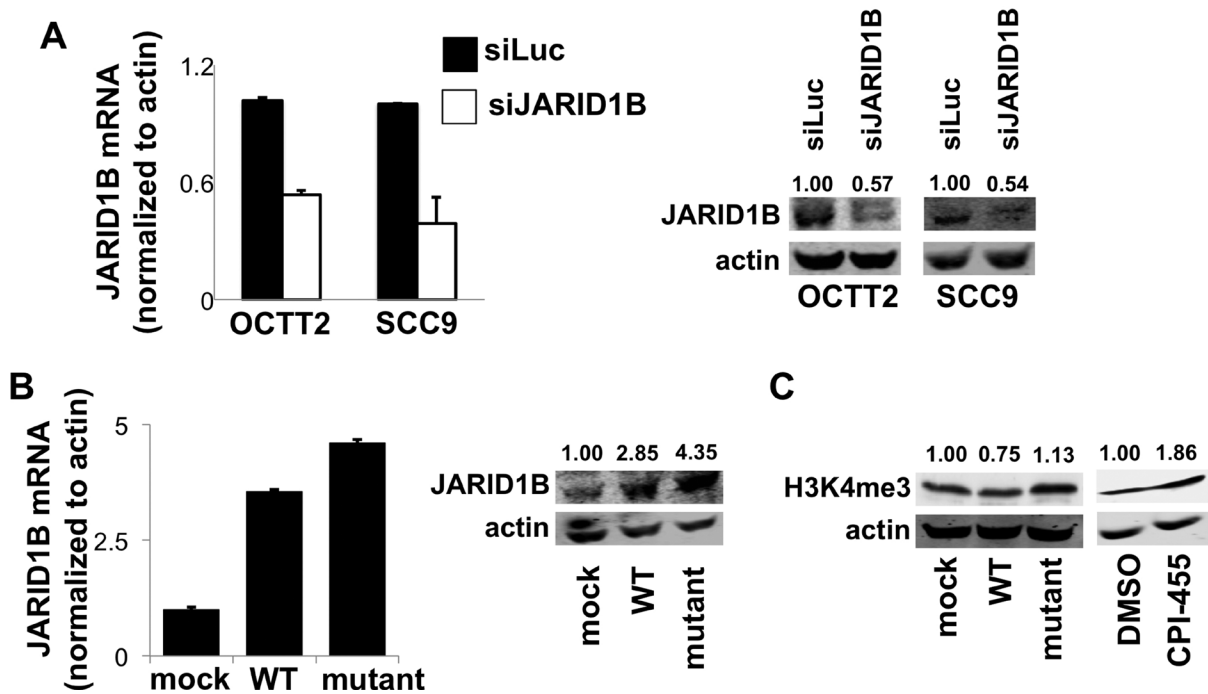
Supplementary Figure 2: CPI-455 pretreatment lengthens tumor latency by JARID1B^{high} but not bulk OSCC cells. Xenograft formation by JARID1B^{high} vs. bulk OCTT2 (left: 100 cells/mouse, $n = 6$, middle: 10 cells/mouse, $n = 7$) or VU147T (right: 250 cells/mouse, $n = 6$) cells pre-treated for 7 days with CPI-455 (20 μ M, blue) or DMSO (red). p -values are unadjusted.



Supplementary Figure 3: Growth and identification of OSCC PDX tumor cells in primary, feeder-assisted cultures.
 (A) Phase contrast images (10×) of feeder-assisted, primary PDX cultures 2, 5, and 12 days post-plating. F = fibroblasts, T = tumor cells.
 (B) Representative FC plots depicting identification of live, PDX tumor cells (PI^{negative}/humanHLA^{positive}).



Supplementary Figure 4: Inhibition of JARID1B suppresses OCT4 and SOX expression in OSCC cells. (A) JARID1B mRNA by QRT-PCR after lentiviral shRNA knockdown $*p < 0.005$, $**p < 0.001$, $***p < 0.0001$. (B) OCT4 protein by WB in OSCC cells treated with CPI-455 (20 μ M) or DMSO for 5 days. Values are band densities normalized to actin. (C) OCT4 and SOX2 mRNA by QRT-PCR in OSCC cells treated with CPI-455 (20 μ M) or DMSO for 5 days $*p < 0.05$, $**p < 0.01$.



Supplementary Figure 5: Characterization of siRNA-mediated JARID1B silencing and mutant (H499Y) JARID1B overexpression. (A) JARID1B mRNA and protein by QRT-PCR and WB, respectively, after transient siRNA knockdown in OSCC cell lines. (B) JARID1B mRNA and protein expression in OCTT2 cells overexpressing WT or mutant (H499Y) JARID1B. (C) WBs showing side-by-side comparison of H3K4me3 levels in OCTT2 cells overexpressing WT or mutant (H499Y) JARID1B or treated for 5 days with 20 μ M CPI-455 (Figure 1A). Values are H3K4me3 band densities normalized to actin.

Supplementary Table 1: List of genes differentially expressed upon stable JARID1B silencing in OCTT2 cells. See Supplementary_Table_1

Supplementary Table 2: List of genes both upregulated in shJARID1B OCTT2 cells and downregulated in JARID1B^{high} OCTT2 cells. See Supplementary_Table_2