

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1. HDAC inhibitors induce histone H3 hyperacetylation in UM cells. A, acetyl-histone H3 (Ac-H3) immunofluorescence of 92.1 and OCM1A cells untreated (UT) or HDAC inhibitor-treated for 72 hours. Magnification 200X. B, acetyl-histone H3 (Ac-H3, upper panel) and total histone H3 (lower panel) western blots of 92.1 cells untreated (UT) or HDAC inhibitor-treated.

Supplementary Figure S2. HDAC inhibitors induce differentiation of UM cells. HDAC inhibitors induce differentiation of UM cells. 92.1, OCM1A and Mel202 cell lines were either untreated (UT) or HDAC inhibitor-treated for 72 hours. A, representative phase contrast images of morphologic changes. Magnification 100X. B, spindle morphology index determined using the maximal cell length versus width ratio. * $P < 0.05$.

Supplementary Figure S3. Cultured cells from class 2 tumors express low levels of BAP1 mRNA compared to those from class 1 tumors. RNA expression of BAP1 in primary UM cells derived from class 1 (MM131; blue dot) and class 2 (MM137, MM138, MM151, MM161, MM162; red dots) tumors measured by qPCR. The box-and-whiskers plots represent the expression of BAP1 in eight class 1 and twenty-eight class 2 primary UM tumors. Fold change was calculated using the sample with the lowest BAP1 expression as a calibrator. Middle line represents median; box, 25th to 75th percentiles; outer bars, minimum and maximum values.

Supplementary Figure S4. HDAC inhibitors shift class 2 UM cells and UM cell lines toward a class 1 expression signature in a dose-dependent fashion. A, Support vector machine (SVM) discriminant scores of primary UM cells treated with VPA (left panel) or LBH-589 (right panel) for 72 hours. B, SVM discriminant scores of UM cell lines treated with VPA for 72 hours. The more negative the number, the more class 1-like; the more positive the number, the more class 2-like. Black dots correspond to untreated cells and red dots to HDAC inhibitor-treated cells; x-axis represents HDAC

inhibitor concentrations and *y*-axis represents discriminant score values in SVM units. **P*<0.05, ***P*<0.005.

Supplementary Figure S5. Effects of HDAC inhibitors on gene expression signature. A, mRNA levels of the multi-gene prognostic assay of MM151 class 2 cells treated with increasing concentration of VPA for 72 hours (left panel) and of three class 2 cells (MM137, MM138, MM151) treated with 2 mM VPA for 72 hours (right panel), presented as relative change in class 2 treated/untreated cells. B, mRNA levels of the multi-gene clinical prognostic assay of MM151 class 2 cells (left panel) and MM131 class 1 cells (right panel) treated with 2 mM VPA for 7 days, presented as relative change in treated/untreated cells. C, mRNA levels of the multi-gene clinical prognostic assay of three class 2 cells (MM137, MM138, MM151) treated with LBH-589 for 72 hours, presented as relative change in class 2 treated/untreated cells. **P*<0.05. Blue boxes indicate genes with higher expression in class 1 tumors, and red boxes indicate genes with higher expression in class 2 tumors.

Supplementary Figure S6. SAHA has similar effects to other HDAC inhibitors in UM cells. A, MTS cell viability assays after 72 hours treatment. The absorbance of control cells at 490 nm was taken as 100%. B, BrdU incorporation assays after 72 hours treatment. The absorbance at 370 nm of control cells was taken as 100%. C, cell cycle analysis by flow cytometry using propidium iodide staining. Cells were either untreated (UT) or SAHA-treated for 48 hours; *x*-axis represents DNA content and *y*-axis represents cell number. D, mean number of dendritic arborizations in primary cultured UM cells that were untreated (UT) or SAHA-treated for 72 hours. E, Support vector machine (SVM) discriminant scores showing the effect of SAHA treatment (1 μM) on gene expression signature. The more negative the number, the more class 1-like; the more positive the number, the more class 2-like. MM137, MM161 and MM162 cells were from class 2 tumors. Red spheres represent the untreated cells and blue spheres the SAHA-treated cells. **P*<0.05.