Molecular Pharmacology

Histone deacetylase inhibitors stimulate histone H3 lysine 4 methylation, in part, via transcriptional repression of histone H3 lysine 4 demethylases

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Supplemental Figure 1

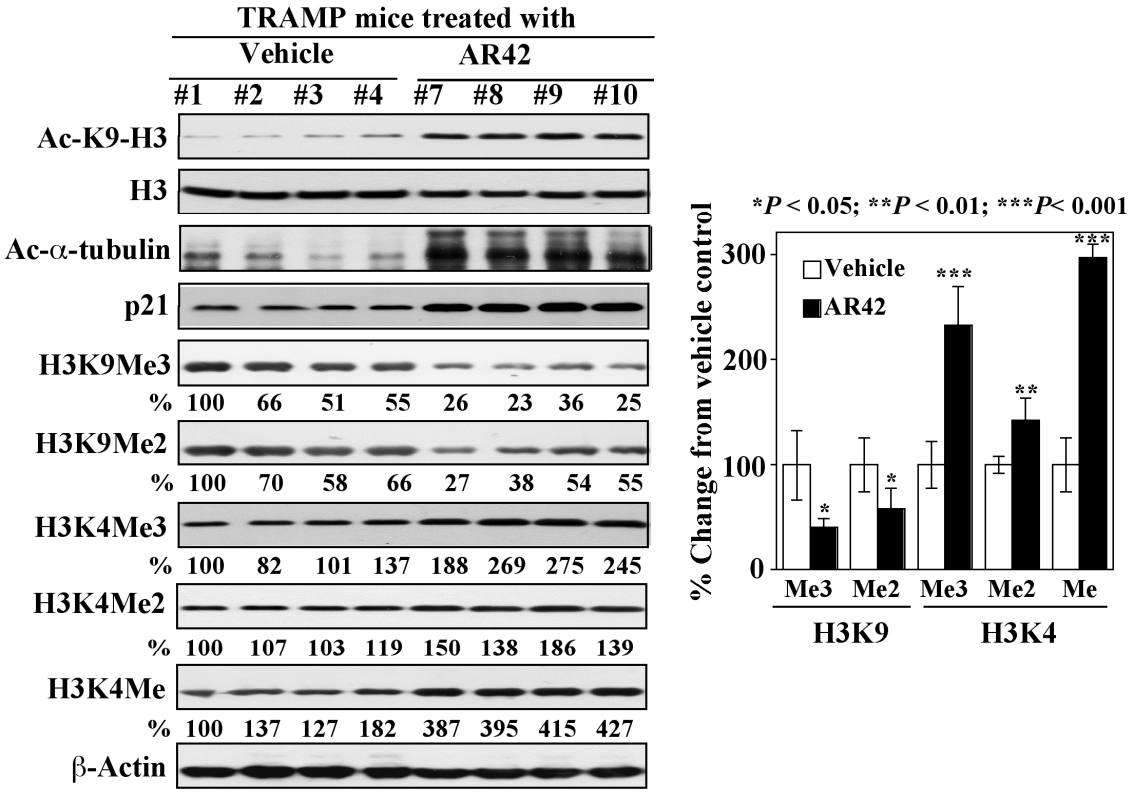

Fig. S1. **Oral administration of AR42 for 18 weeks altered the methylation status of H3K4 and H3K9 in the prostate of TRAMP mice**. AR42 was administered via an AIN-76A rodent diet containing 208 ppm of AR42 which we previously reported can suppress prostate tumorigenesis in TRAMP mice (Sargeant et al., 2008). Control (Vehicle) mice were given a placebo diet. The expression levels of acetyl-H3, acetyl-α-tubulin, H3K9Me3, H3K9Me2, H3K4Me3, H3K4Me2, and H3K4Me in the prostate of these mice (N = 4 for each group) were assessed by immunoblotting. Proteins were isolated from the homogenates of prostate tissue from each mice by ethanol precipitation after the removal of DNA and RNA using Trizol according to the manufacturer's instruction. Expression levels of the indicated proteins were assessed by Western blotting. Left panel, Western blots of acetylated and methylated proteins from the prostate of each mouse. Right panel, relative changes in the level of the methylation marks on H3K4 and H3K9 in drug-treated groups expressed as a percentage of that in the corresponding vehicle control group. Columns, mean (N = 3); Error bars, SD. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Supplemental Experimental Procedures. Data in Fig. S1 were generatd from archieved prostate tissue samples collected from TRAMP mice used in a previously published study of the chemopreventive activity of the novel HDAC inhibitor AR42 (Sargeant et al, 2008). Briefly, these TRAMP mice (C57BL/6TRAMPxFVB) were administered AR42 via an AIN-76A rodent diet containing 208 ppm of AR42 from 6 weeks of age to 24 weeks of age. Control mice received the same diet without the added AR42. At sacrifice, prostate tissues were harvested and snap-frozen. Proteins were extracted from prostate tissue lysates as previously described (Sargeant et al., 2008) and immunblotting for biomarkers associated with HDAC inhibition and histone methylation was performed as described in the main text.