SUPPLEMENTAL FIGURE 1. **Biotin Cyclopentenone PG Analogs Modify Cellular HDAC via Michael Addition.** (A) Immunoblots of HDAC1 protein in HL-60 cell lysates and carbonylated HDAC1 (HDAC1:PG-biotin) conjugates isolated by biotin capture. Cells were treated with 10 μ M 15d-PGJ₂-B (lane 2), PGA₁-B (lane 3), or vehicle (lane 1) for 30 minutes. (B) Similar immunoblots of total and carbonylated HDAC1 from cells treated with 10 μ M 15d-PGJ₂-B (lane 2), sodium borohydride treated 15d-PGJ₂-B (lane 3), or cells pretreated with 30 μ M Nethylmaleimide for 30 minutes before treatment with 10 μ M 15d-PGJ₂-B (lane 4). (C) Schematic representation of a Michael addition reaction between cysteinyl thiolate of HDAC and electrophilic β -carbon of an α , β -enone. (D) Immunoblots of RPD3 protein in S. cerevisiae cell lysates and carbonylated RPD3 (RPD3:PG-biotin) conjugates isolated by biotin capture. Growing cells were treated either vehicle or 15d-PGJ₂-B (3 or 10 μ M) for 60 minutes.

SUPPLEMENTAL FIGURE 2. Carbonylation of HDAC1 by J-series Dehydration Products of PGD₂. (A) Schematic of PGD₂ dehydration into electrophilic PGJ metabolites. (B) Time-dependent carbonylation of HDAC1. (C) Immunoblots of HDAC1 (*arrows*), from HCT116 cells treated with 15d-PGJ₂-B or its precursor, PGD₂-B. Lower immunoblot panels depict total HDAC1 in cell lysates while the upper immunoblot panels depict carbonylated HDAC1 (i.e.HDAC1:15d-PGJ₂-B covalent adducts isolated by biotin capture on NeutrAvidin beads). HDAC1:15d-PGJ₂-B covalent adducts were detected within 10 minutes in cells treated with 1 μ M 15d-PGJ₂-B (*C* - left) or with 15 μ M PGD₂-B, the precursor for electrophilic J-series prostaglandins (*C* - right). The graph in panel B represents the signal density of the immunoblots in panel C.









Supplemental Figure 1



Supplemental Figure 2