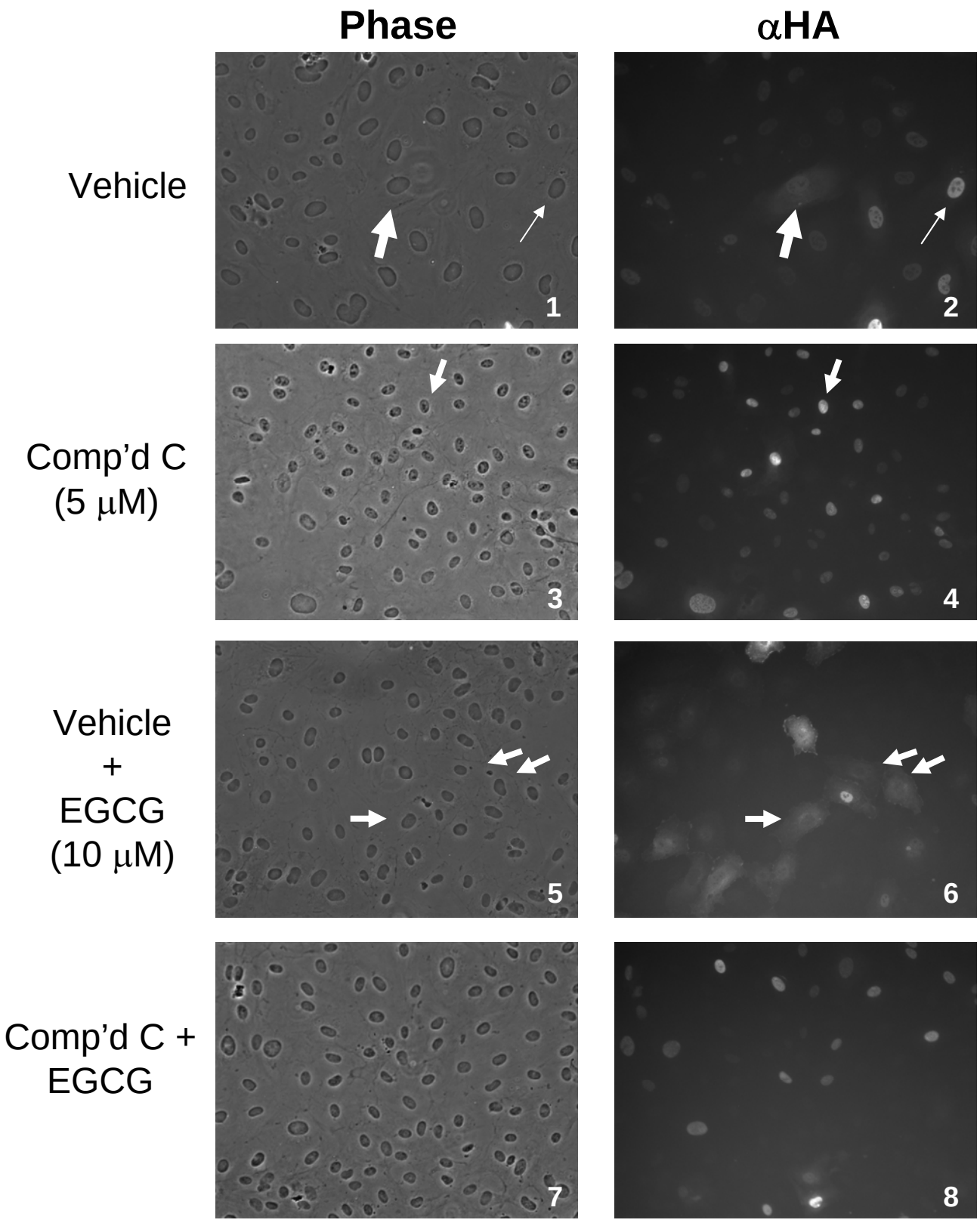
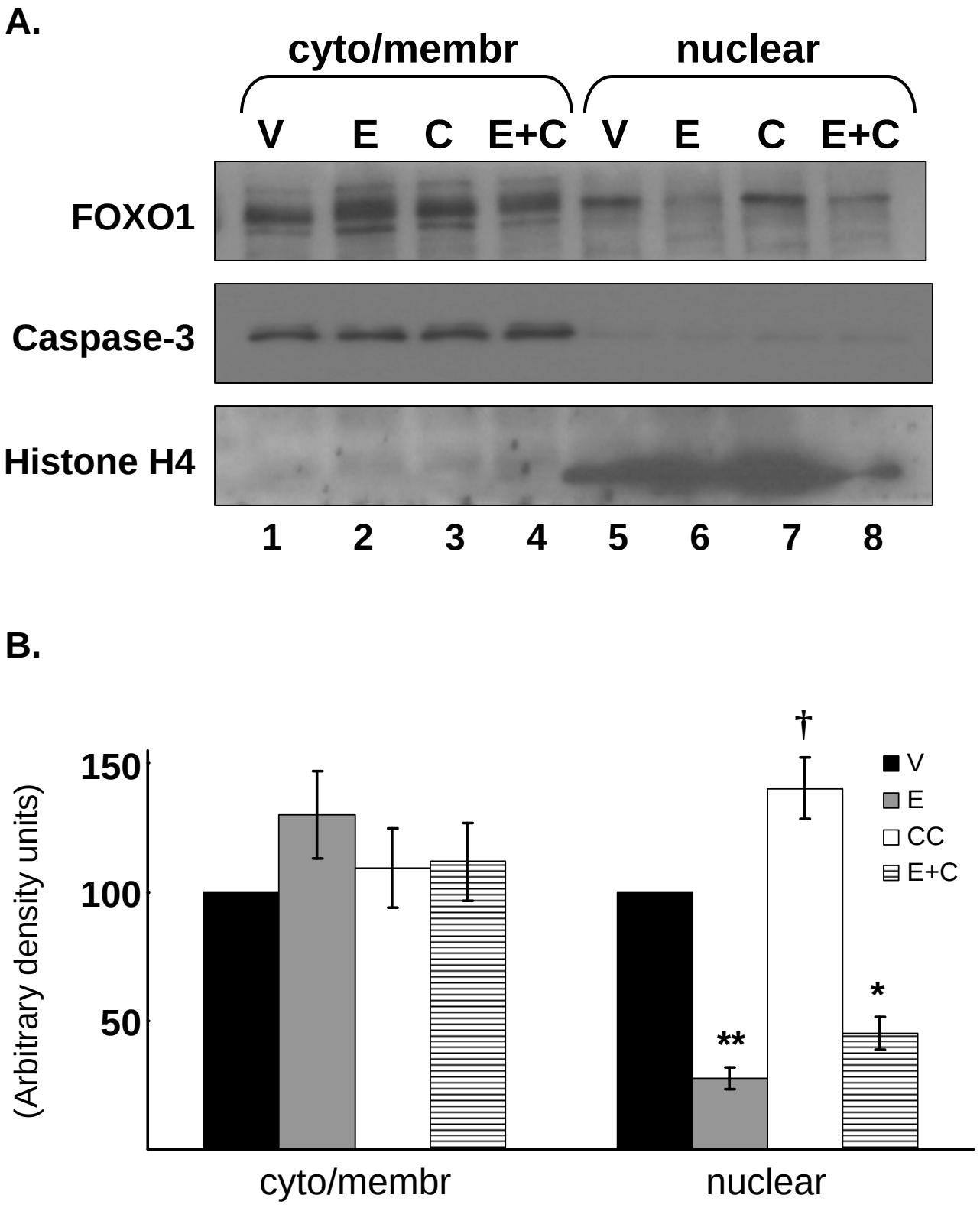
Supplemental Figure 1



Supplemental Figure 2



Supplementary Figure 1. Inhibition of AMPK increases FOXO1 nuclear localization and blocks EGCG-mediated translocation to the cytoplasm. HAECs infected with adenoviral constructs for HA-tagged WT Foxo1 were pre-treated with vehicle or compound C (AMPK inhibitor) for 30 min in the absence or presence of EGCG treatment for 30 min as described in Methods. FOXO1 localization was assessed by immunocytochemistry using anti-HA antibodies as described in Methods. Representative phase-contrast views and epifluorescent images of cells revealing anti-HA immunostaining are shown for experiments that were repeated independently four times. In vehicle-treated cells (panels 1 and 2), the thick arrow highlights a cell where HA staining is present in both the nucleus and cytoplasm. The thin arrow shows a cell with only nuclear HA immunoreactivity. In Compound C-treated cells (panels 3 and 4), the arrows indicate a cell with only nuclear HA immunoreactivity. In the panels with EGCG-treated cells (panels 5 and 6), three arrows highlight cells in which HA immunoreactivity is both cytoplasmic and perinuclear (no nuclear HA immunoreactivity).

Supplementary Figure 2. EGCG-induced reduction of FOXO1 in nuclear-enriched cell fractions is inhibited by Compound C. A. HAECs were treated and analyzed by subcellular fractionation and immunoblot analysis as described in Methods following vehicle (V), EGCG (E), Compound C (C), or Compound C pretreatment followed by EGCG treatment (E+C). The figure is representative of three independent experiments. The membrane/cytoplasmic-enriched fractions are presented in lanes 1-4, and the nuclear-enriched fractions are presented in lanes 5-8. Immunoblot analysis of pro-caspase 3 and histone H4 demonstrate consistency of the subcellular preparations. **B.** Quantification of the FOXO1 immunoblots in A. Vehicle-treated fractions are arbitrarily set to 100. No statistical difference was detected in the cytoplasmic/membrane fractions following treatment with EGCG or Compound C. However, EGCG treatment of HAECs significantly reduced the appearance of FOXO1 in the nuclear-enriched fraction (Triton X-100-insoluble; lane 6 and 8 in A; ** $p < 0.0001$ and * $p = 0.019$ by two-way ANOVA). Compound C increased FOXO1 appearance in the nuclear-enriched fraction (lane 7; † $p = 0.0001$ by two-way ANOVA).