

Supplemental Figures

Figure Legends

Figure S1. Cordycepin inhibits growth of uveal melanoma in an adenosine deaminase (ADA) dependent manner.
A, Cell growth assays for cordycepin treatment for 5 days. Uveal melanoma cells are seeded onto 96 cell culture plate and treated with 0, 4, 20 and 40 μ M of cordycepin. After 5 days incubation, MTT, or Cell Titer Blue solution was used for measuring fluorescence. **B-C**, Effects of cordycepin treatment on the soft agar colony formation of uveal melanoma cells. **D-E**, the effect of cordycepin on the anchorage-dependent colony formation abilities. **F**, Body weight changes of vehicle- or cordycepin treated mice. 0mg/kg, cordycepin 0mg/kg b.w. treatment; 10mg/kg, cordycepin 10mg/kg b.w. treatment; 20mg/kg, cordycepin 20mg/kg b.w. treatment. Values represent mean \pm SD of experiments conducted in sextuplicate. ** $P < 0.01$ and **** $P < 0.0001$ by one-way ANOVA analysis of variance compared with control group.

Figure S2. Combination treatment with cordycepin and an ADA inhibitor enhances inhibition of anchorage independent-, dependent colony formation and migration abilities in uveal melanoma

A, Cell growth assays for combination treatment with cordycepin and EHNA for 5 days. **B-E**, Effects of combination treatment of cordycepin and EHNA treatment on the anchorage independent- (B) and anchorage dependent colony formation of uveal melanoma cells. **F and G**, Inhibition of migration abilities of uveal melanoma cells by treatment with 10 μ M cordycepin and combination treatment of 1 μ M EHNA (or pentostatin) and 10 μ M cordycepin. The cells were seeded onto the Transwells coated with gelatin. After incubation for 12 hours, the migratory cells on the bottom of the membrane were stained with crystal violet solution and counted. Values represent mean \pm SD of experiments conducted in sextuplicate. **H**, Body weight changes of vehicle- or compound treated mice. Cordy, cordycepin; CT, control; Pento, pentostatin; Combo1, combination treatment with pentostatin 1mg/kg b.w. and cordycepin 2mg/kg b.w.; Combo2, combination treatment with pentostatin 2mg/kg b.w. and cordycepin 2mg/kg b.w.. Values represent mean \pm SEM of experiments conducted in quadruplicate. *** $P < 0.01$ and **** $P < 0.0001$ by one-way ANOVA analysis of variance compared with control group.

Figure S3. Cordycepin binds Hsp90 and disrupts its function, resulting in degradation of client proteins.

A, 92.1 cells were treated with various combinations of vehicle, 10 μ M MG-132, and 10 μ M cordycepin/1 μ M of EHNA (combination) then incubated for 6 hours. Expression of ubiquitination was analyzed by Western blot analysis. **B and C**, 92.1 cells were treated with vehicle 10 μ M MG-132, and 10 μ M cordycepin/1 μ M of EHNA (combination) (**B**), or with various combinations of vehicle, 10 μ M MG-132, and 160 μ M cordycepin (**C**), and then incubated for 6 hours. Total cell lysates were prepared and co-immunoprecipitated (Co-IP) with HiF-1 α and anti-mCherry antibodies. The interaction between HiF-1 α (or mCherry-Akt1) and Hsp90 was analyzed by Western blot analysis. **D**, Akt1 recombinant protein expressions after treatment of cordycepin in a dose dependent manner in 92.1 and MP46 expressing mCherry-Akt1 recombinant protein. **E**, Cells were treated with 160 μ M cordycepin for 0, 0.5, 1, 2, 4, 8, 12, and 24 hours. Total Akt, total ERK and EGFR protein expression levels were analyzed by Western blot analysis.

Figure S4. Antitumor effects of cordycepin in mice xenograft tumor models.

A, VEGF mRNA expression after treatment of 160 μ M cordycepin. Cells were treated with cordycepin and incubated under 1% O₂ conditions for 1.5 hours. **B**, VEGF protein expression in 92.1 and MP46 mice bearded xenograft tumors. CT, control; Combo2, combination treatment with pentostatin 2mg/kg b.w. and cordycepin 2mg/kg b.w.. Values represent mean \pm SD of experiments. **** $P < 0.0001$ by one-way ANOVA analysis of variance compared with control group.

Figure S1.
A

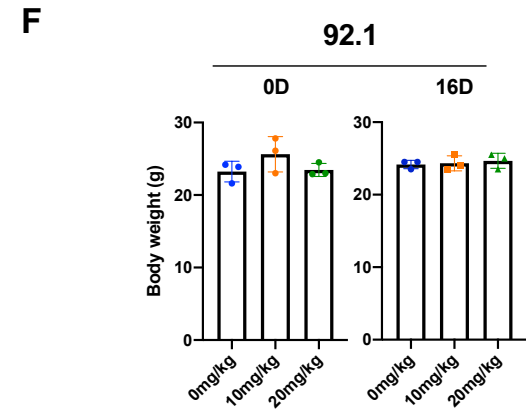
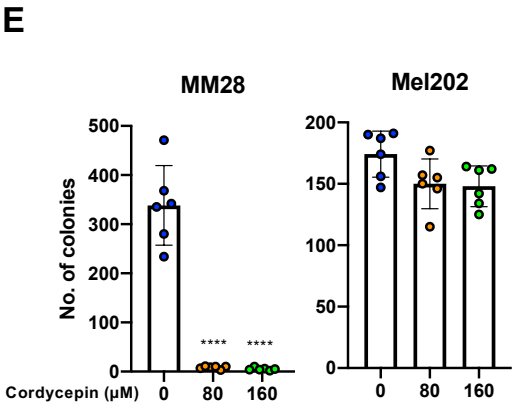
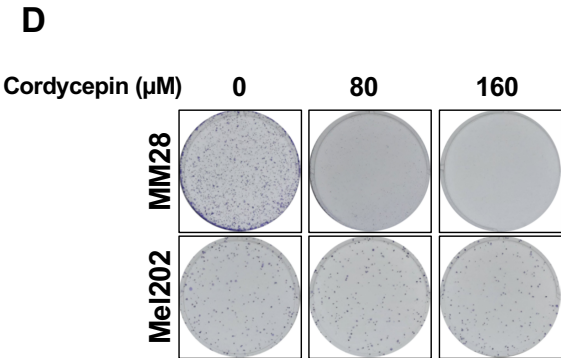
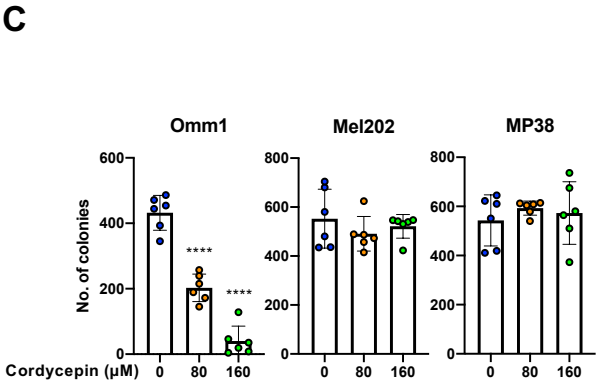
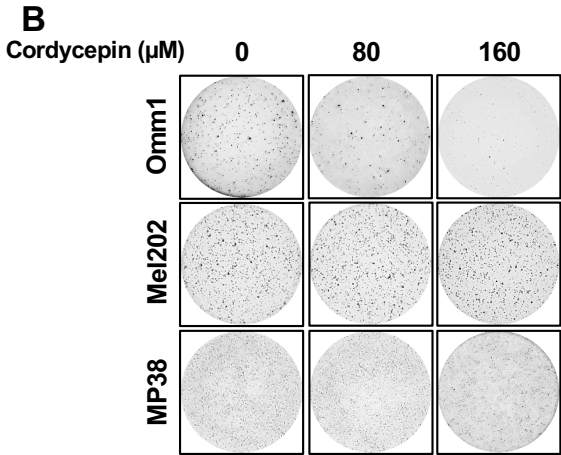
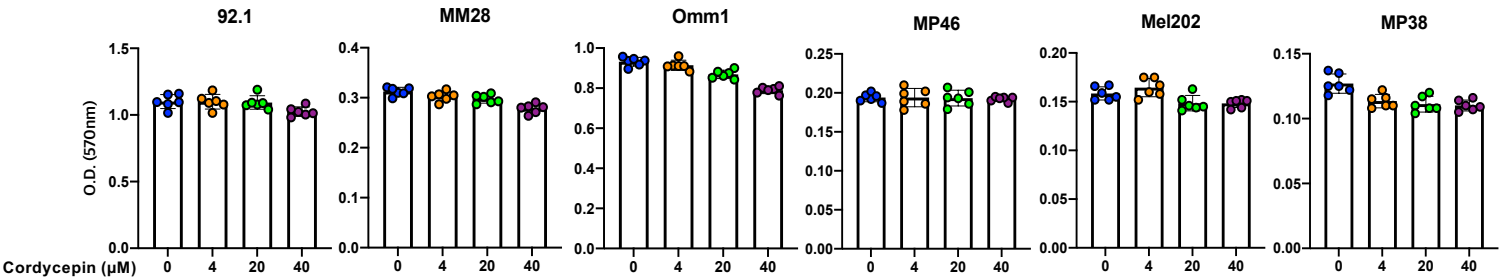


Figure S2.

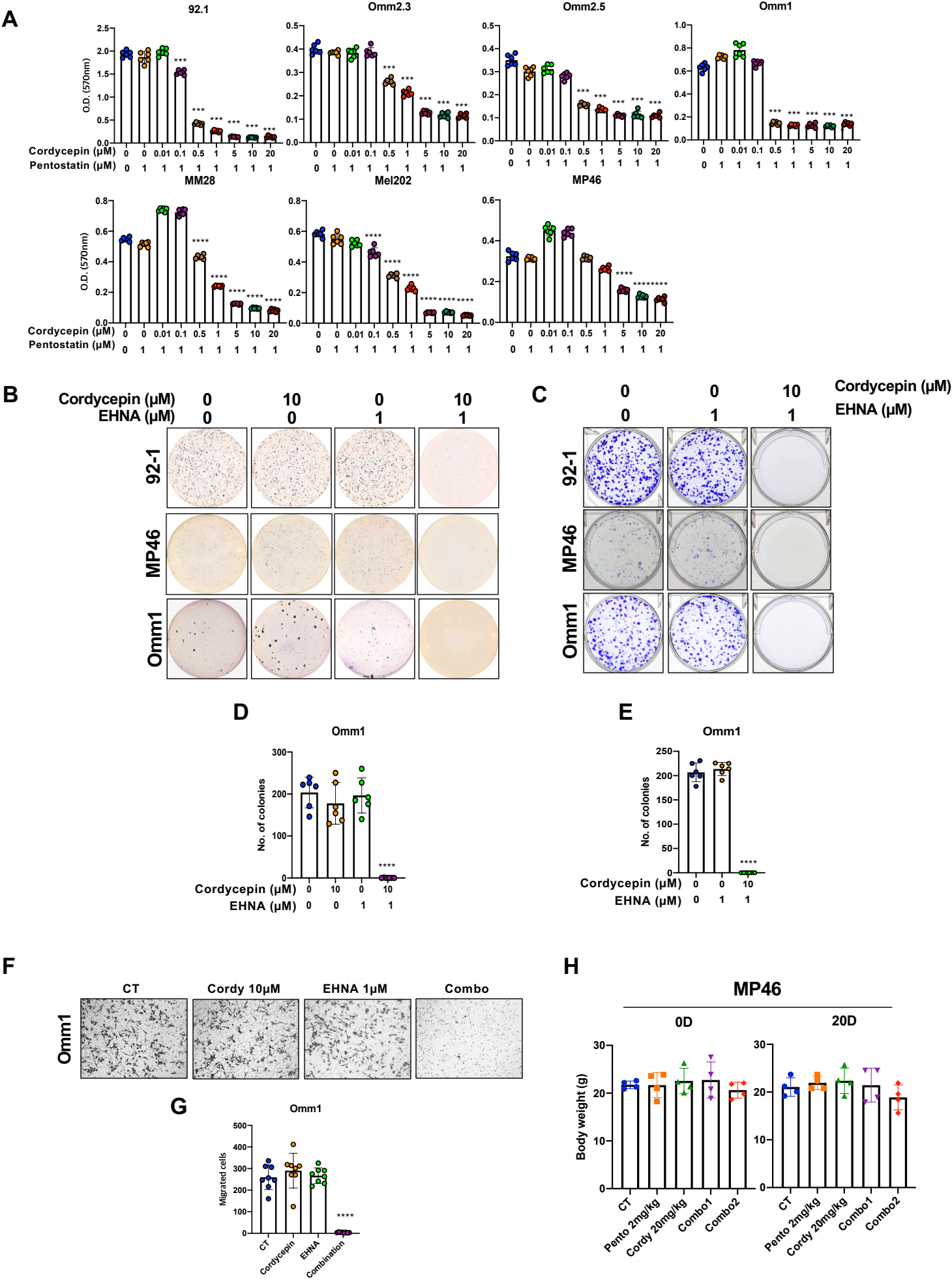


Figure S3.

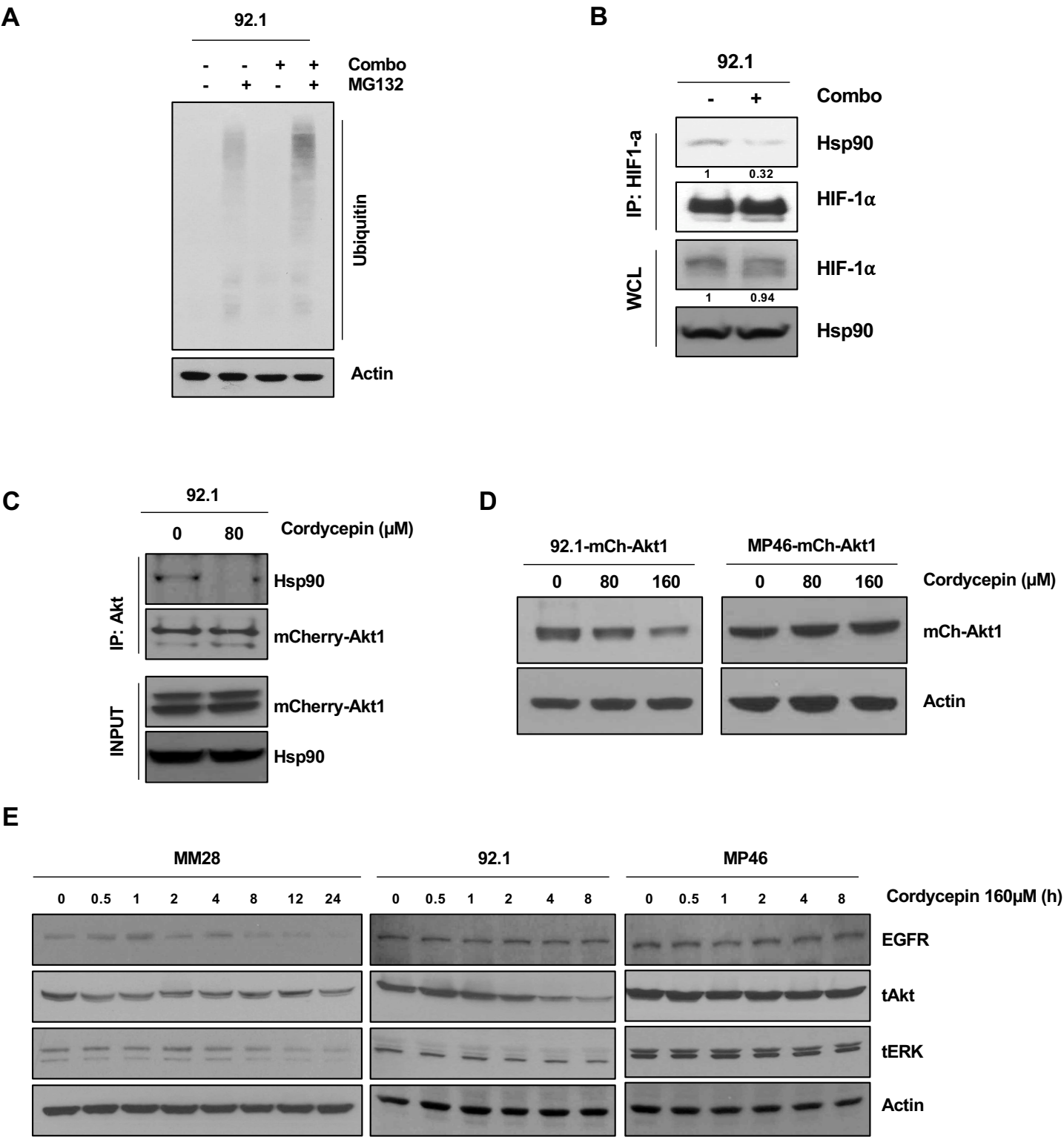
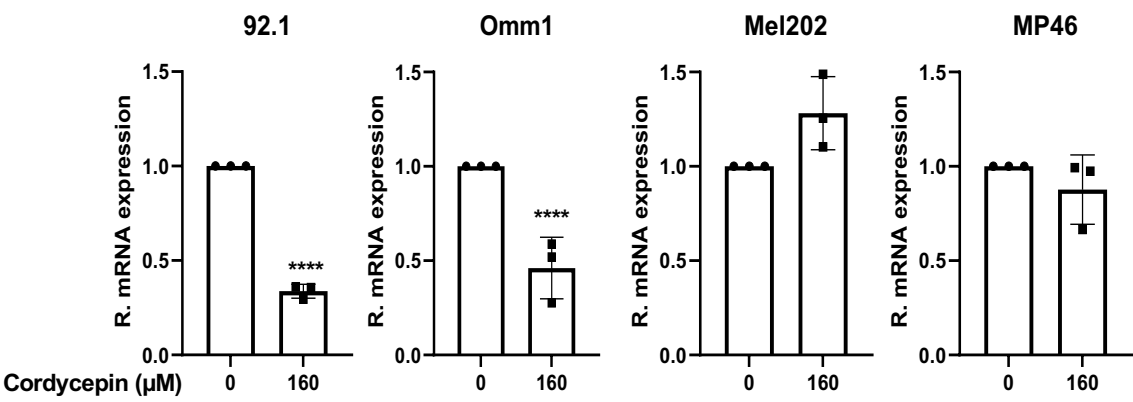


Figure S4.

A

VEGF mRNA



B

92.1 xenograft tumors

MP46 xenograft tumors

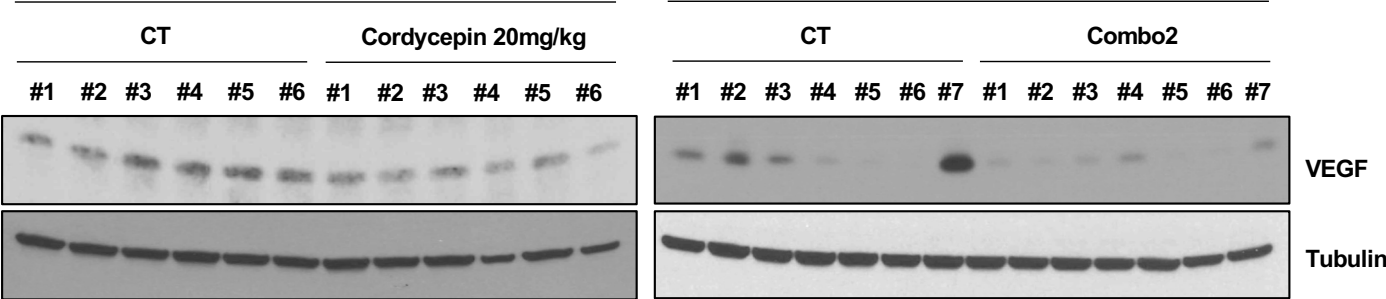


Table S1.

92.1	Omm1	MP46	Mel202	Omm2.5	Omm2.3	PANC1	MCF7	BT37
58.46 ± 6.21	134.30 ± 15.14	258.29 ± 14.71	391.12 ± 29.62	183.23 ± 7.55	190.10 ± 5.63	90.91 ± 10.71	23.75 ± 1.74	36.23 ± 2.34

Table S1. IC₅₀ of Cordycepin in various cancer cells

Determination of IC₅₀ of cordycepin in uveal melanoma, pancreatic, breast and AT/RT cancer cell lines. Mean and SD of at least six biological replicates is shown.