Figure S1

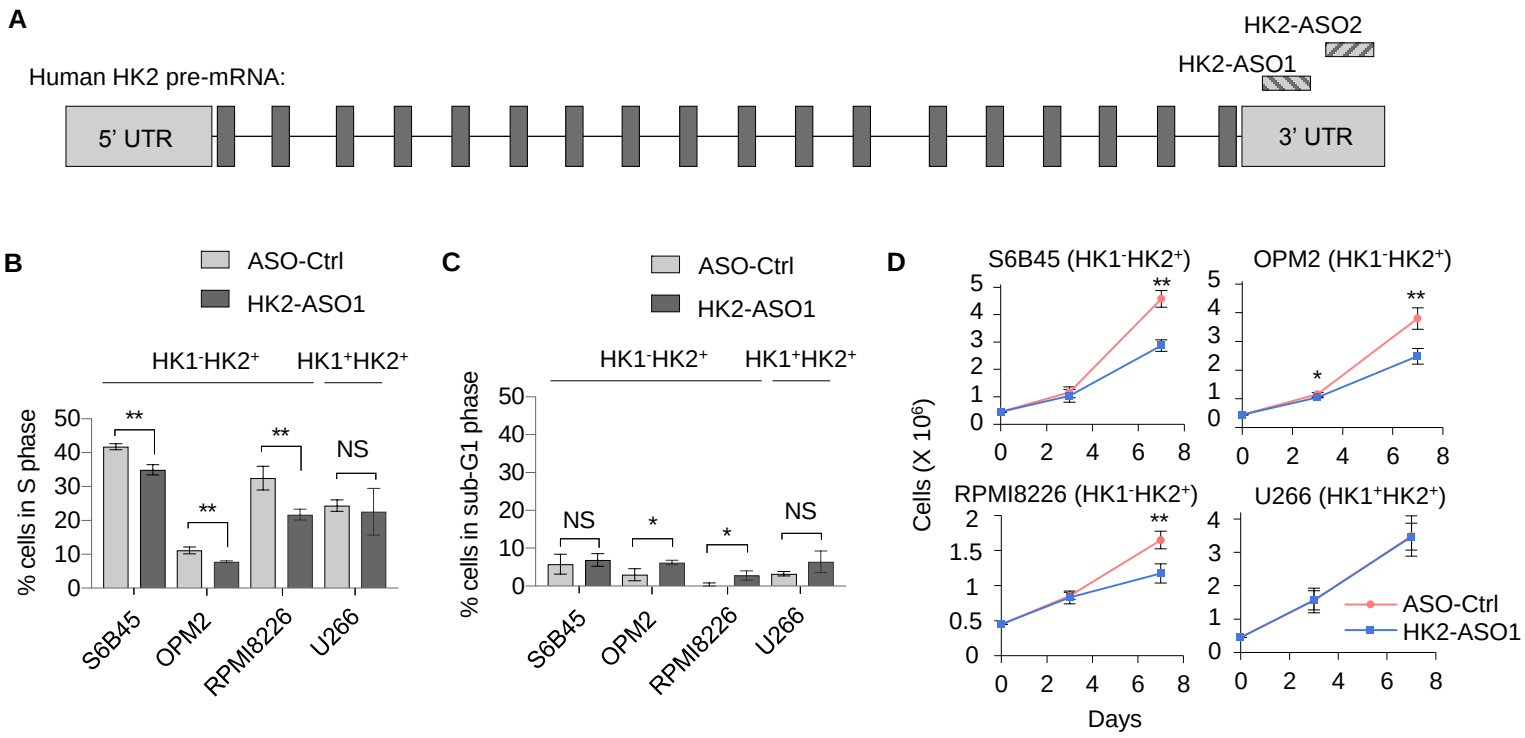

Fig. S1. HK2 ASOs selectively inhibit the proliferation of HK1$^-$HK2$^+$ MM cells. (A) Locations of targeted sequences for HK2-ASO1 and HK2-ASO2 in the human HK2 pre-mRNA. (B, C) Effects of HK2-ASO1 on cell cycle S phase (B) and sub-G1 populations (C). MM cells were treated with 10 μM ASOs for 6 days, then stained with propidium iodide (PI) for cell cycle analysis. HK2-ASO1 selectively inhibits proliferation (D) and colony formation (E) of HK1$^-$HK2$^+$ MM cells. (D) HK2-ASO1 selectively inhibits proliferation of HK1$^-$HK2$^+$ MM cells. Cells were treated with 10 μM ASO-Ctrl or HK2-ASO1, and cell numbers were counted on day 3 and day 7. (E) HK2-ASO1 selectively inhibits the colony formation of HK1$^-$HK2$^+$ MM cells. *, $P < 0.05$. **, $P < 0.01$. NS, not significant.

Figure S2

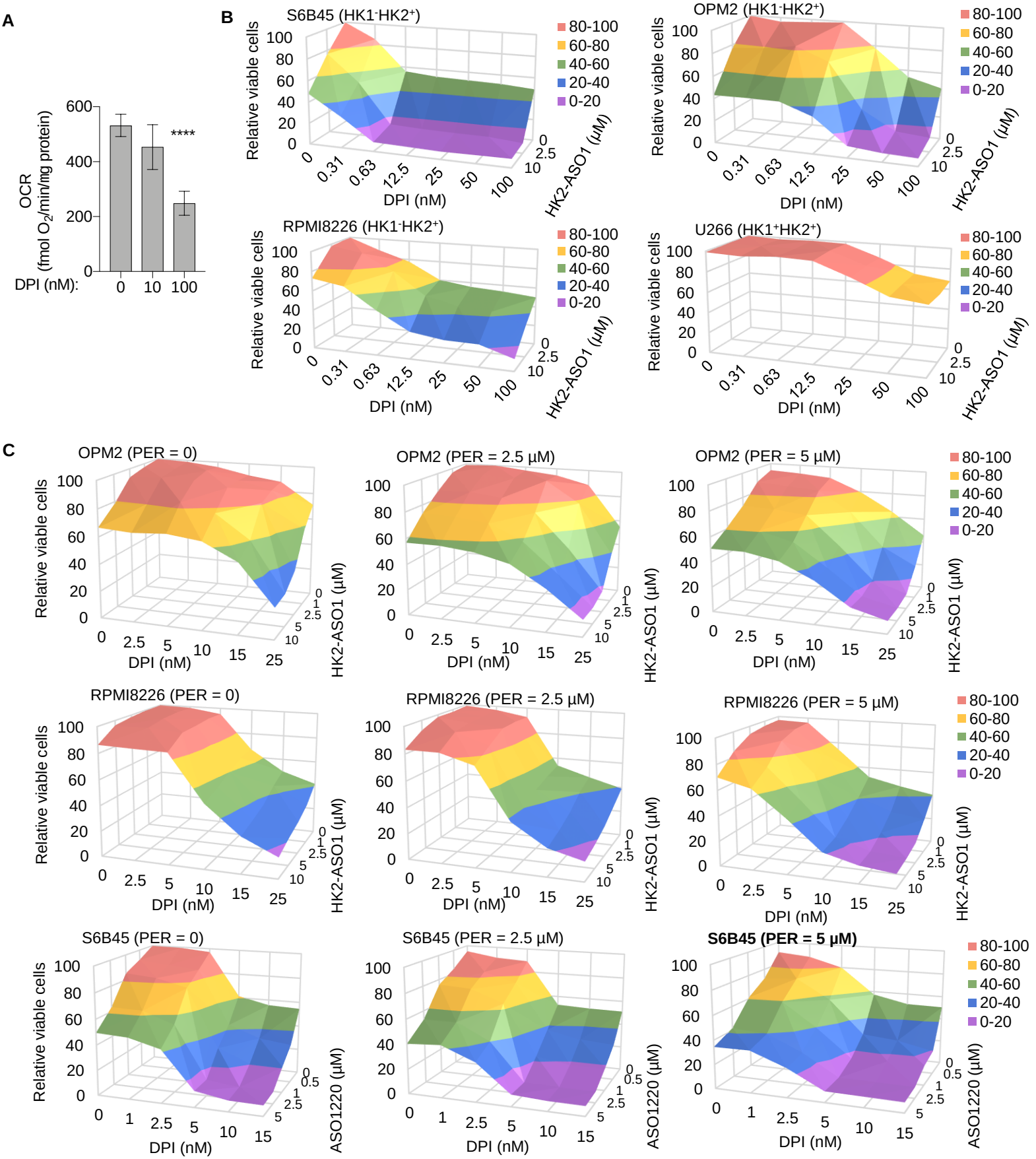

Fig. S2. The HK2-ASO1/DPI/PER combination causes synthetic lethality in HK1^{-}HK2^{+} cells. (A) DPI partially inhibits mitochondrial respiration. DPI treatment (2 h) at 10 nM and 100 nM reduced mitochondrial oxygen consumption in HK1^{-}HK2^{+} Hep3B cells by 14.8% and 53.2%, respectively, determined by the Seahorse assay. ****, P < 0.0001. (B) Dose response surfaces for the combination HK2-ASO1 and DPI. MM cells were pre-treated with ASOs for 3 days, followed by the addition of DPI for another 3 days. (C) Dose response surfaces for the combination HK2-ASO1, DPI and PER. MM cells were pre-treated with ASOs for 3 days, followed by the addition of DPI and PER for another 3 days.

Figure S3

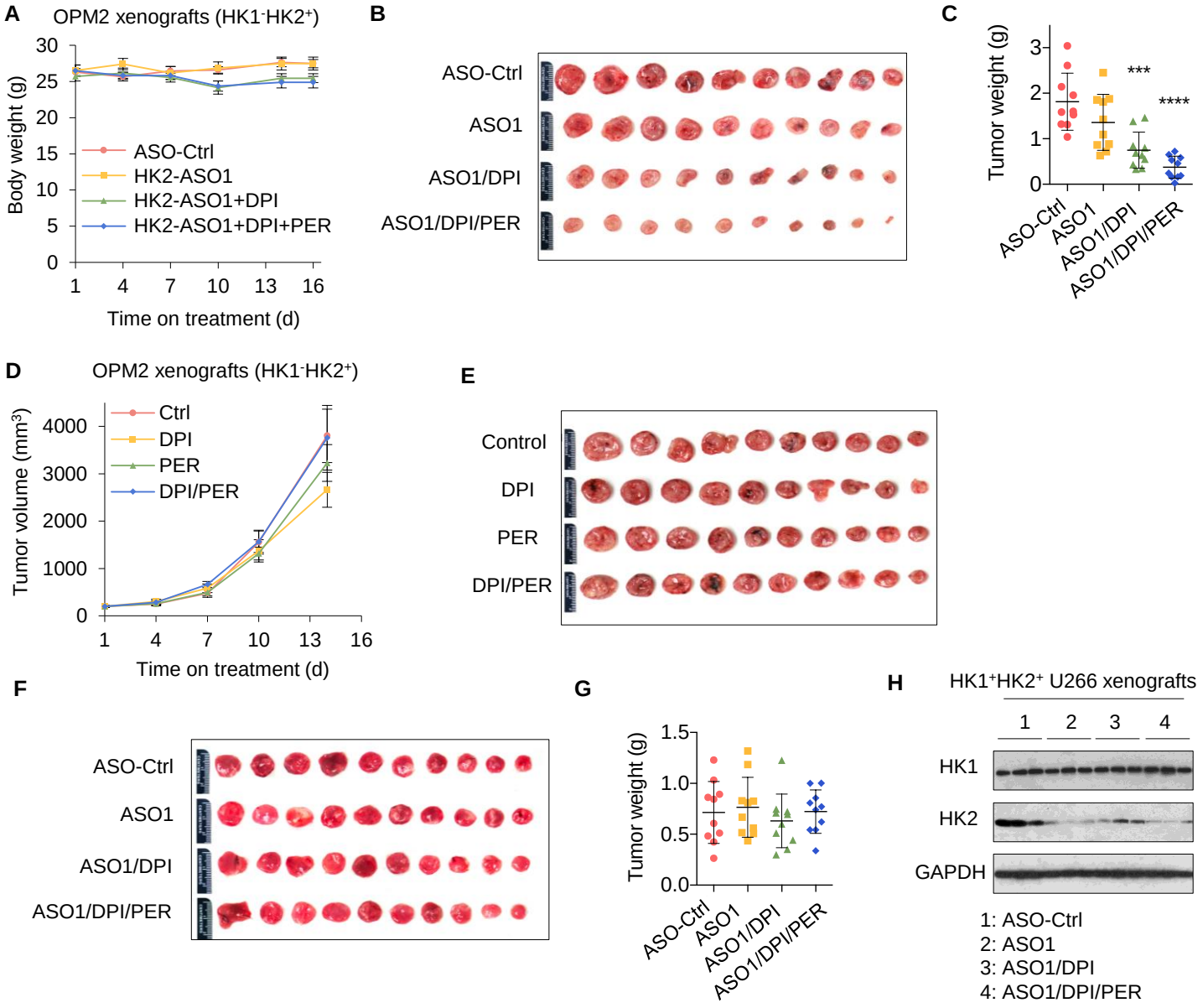

Fig. S3. *In vivo* effects of HK2-ASO1, DPI, PER on MM OPM2 xenograft models. (A) HK2-ASO1, HK2-ASO1/DPI, or HK2-ASO1/DPI/PER did not significantly reduce the body weight of mice bearing OPM2 HK1^{-}HK2^{+} MM xenografts. (B) OPM2 tumors collected at the experiment endpoint (day 16) from OPM2 xenografts of indicated groups are shown. (C) OPM2 tumor weights are shown. (D) OPM2 tumor progression and (E) tumors collected at the experiment endpoint (day 14). When tumors reached 200 mm^3 (day 1), xenografts were randomized into the indicated four groups (n = 10/group) for DPI (2 mg/kg, daily i.p.), PER (30 mg/kg, daily i.p.), and DPI+PER. Treatments were for 5 days per week, with a 2-day break between weeks. (F) U266 tumors collected at the experiment endpoint (day 23) are shown. (G) U266 tumor weights are shown. (H) Treatment effects on U266 tumor HK1 and HK2 expression.

Figure S4

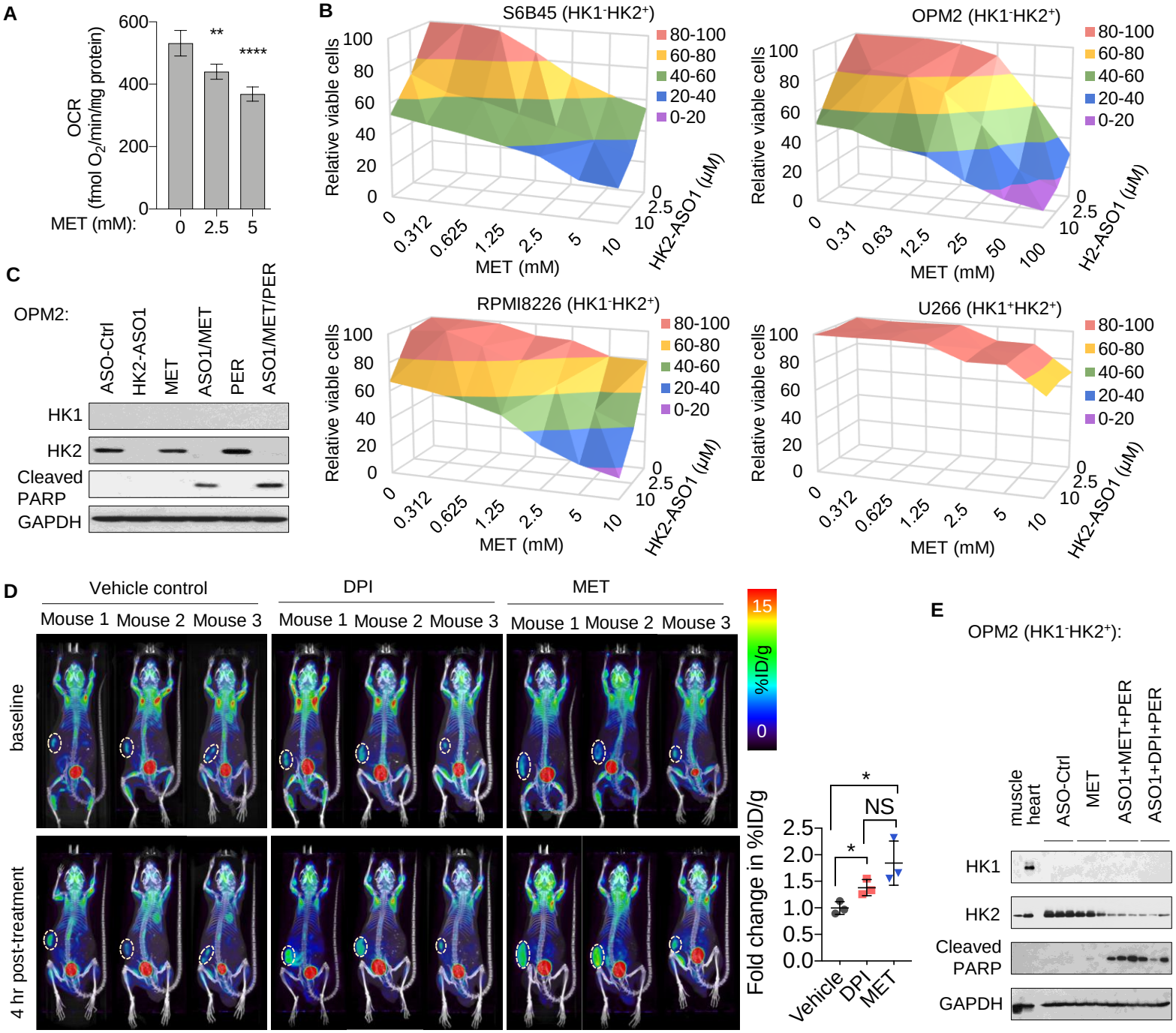

Fig. S4. MET can replace DPI for mitochondrial oxidative phosphorylation inhibition. (A) MET partially inhibits mitochondrial respiration. MET treatment (2 h) at 2.5 mM and 5 mM reduced mitochondrial oxygen consumption in HK1^{-}HK2^{+} Hep3B cells by 17.2% and 30.6%, respectively, determined by the Seahorse assay. **, P < 0.01. ****, P < 0.0001. (B) Dose response surfaces for the combination HK2-ASO1 and MET. Cells were pre-treated with ASOs for 3 days, followed by the addition of MET for another 3 days. (C) Effects of HK2-ASO1, MET and PER, alone and in combination, on PARP cleavage in HK1^{-}HK2^{+} OPM2 cells. (D) DPI and MET increase ^{18}F-FDG PET signal in HK1^{-}HK2^{+} OPM2 xenograft tumors. Three mice bearing OPM2 xenografts were imaged on day 1 for basal tumor ^{18}F-FDG PET signal. Four hours after DPI (2 mg/kg, i.p.) or MET (250 mg/kg, i.p.) on day 2, the same tumor bearing mice were imaged again for post-treatment tumor ^{18}F-FDG PET signal. Base line and post-treatment ^{18}F-FDG PET/CT images are shown in the left panel. Fold change in the mean values of tumor ROIs are quantified in the right panel. NS, not significant. *, P < 0.05. (E) Effects of HK2-ASO1/MET/PER and HK2-ASO1/DPI/PER combinations on HK1 and HK2 protein levels and PARP cleavage in OPM2 xenograft tumors.

Figure S5

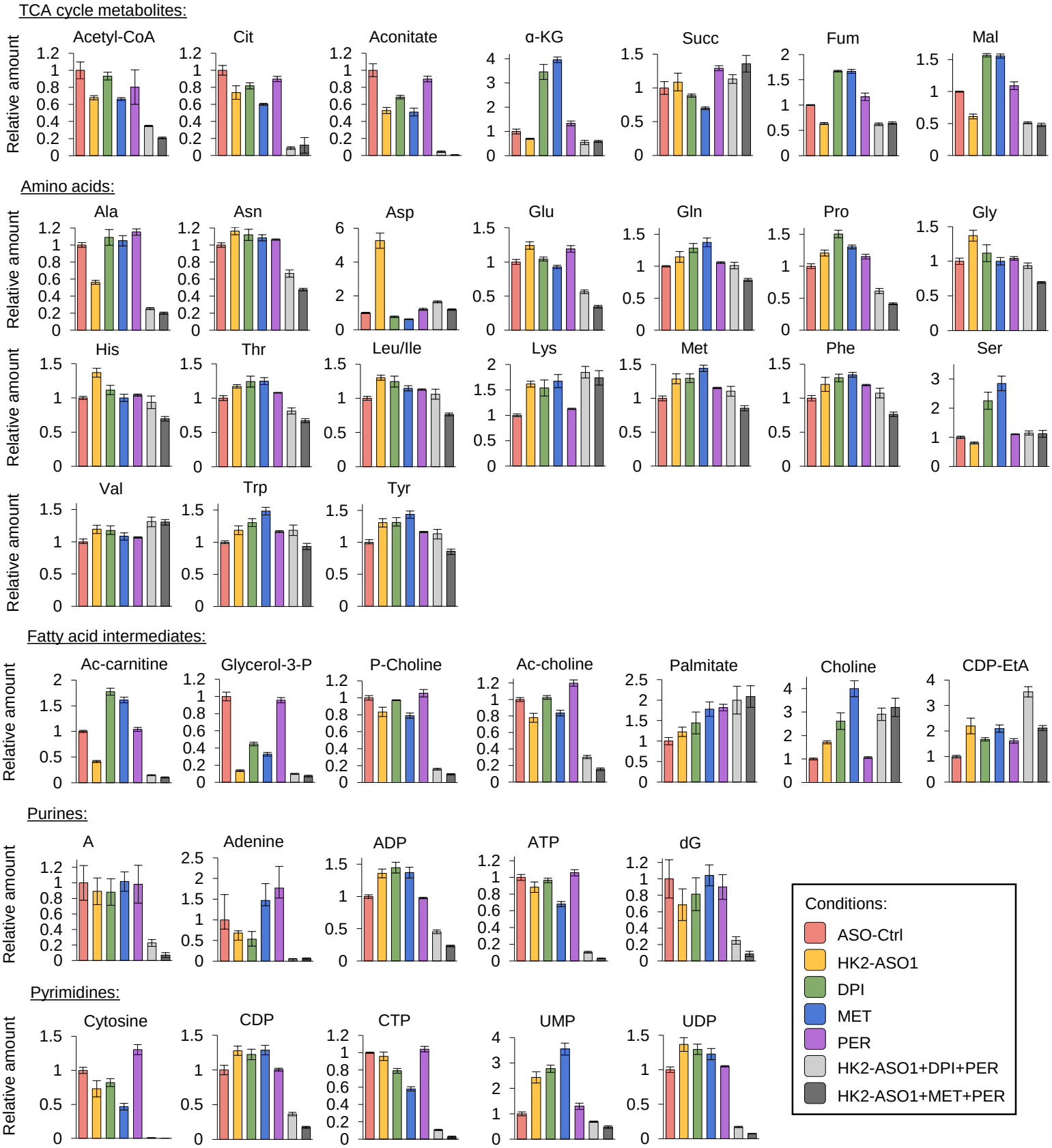

Fig. S5. Effects of 24 h treatments of HK1^{-}HK2^{+} OPM2 cells with HK2-ASO1, DPI, MET and PER, alone and in combination, on relative amounts of amino acids, TCA cycle metabolites, fatty acid intermediates, purines and pyrimidines. Triplicate samples for each group were analyzed as described in the Methods section.

Figure S6

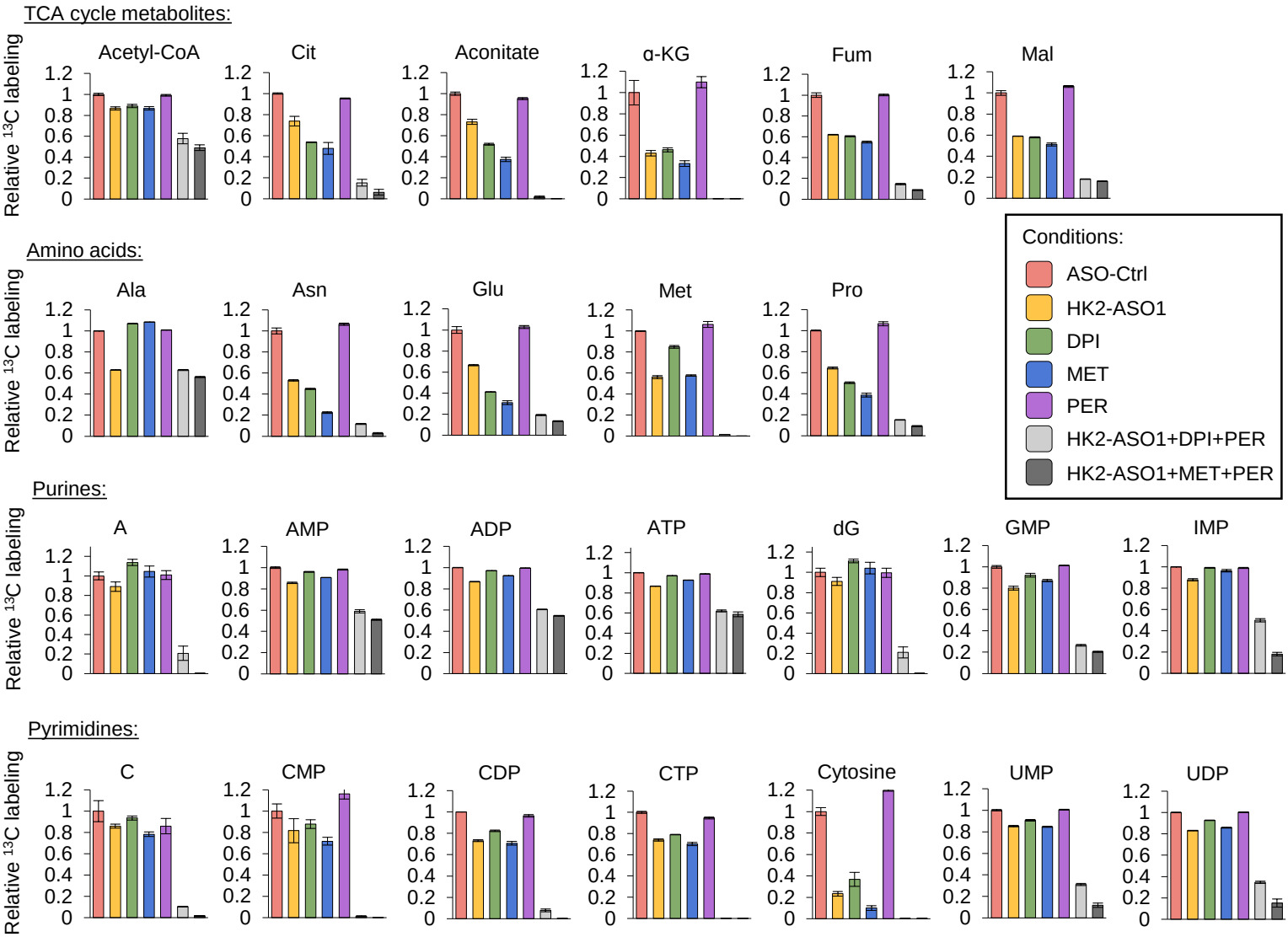

Fig. S6. Effects of 24 h treatments of HK1^{-}HK2^{+} OPM2 cells with HK2-ASO1, DPI, MET and PER, alone and in combination, on relative ^{13}C-labeled portions of TCA cycle metabolites, amino acids, purines, and pyrimidines. Triplicate samples for each group were analyzed as described in the Methods section.

Figure S7

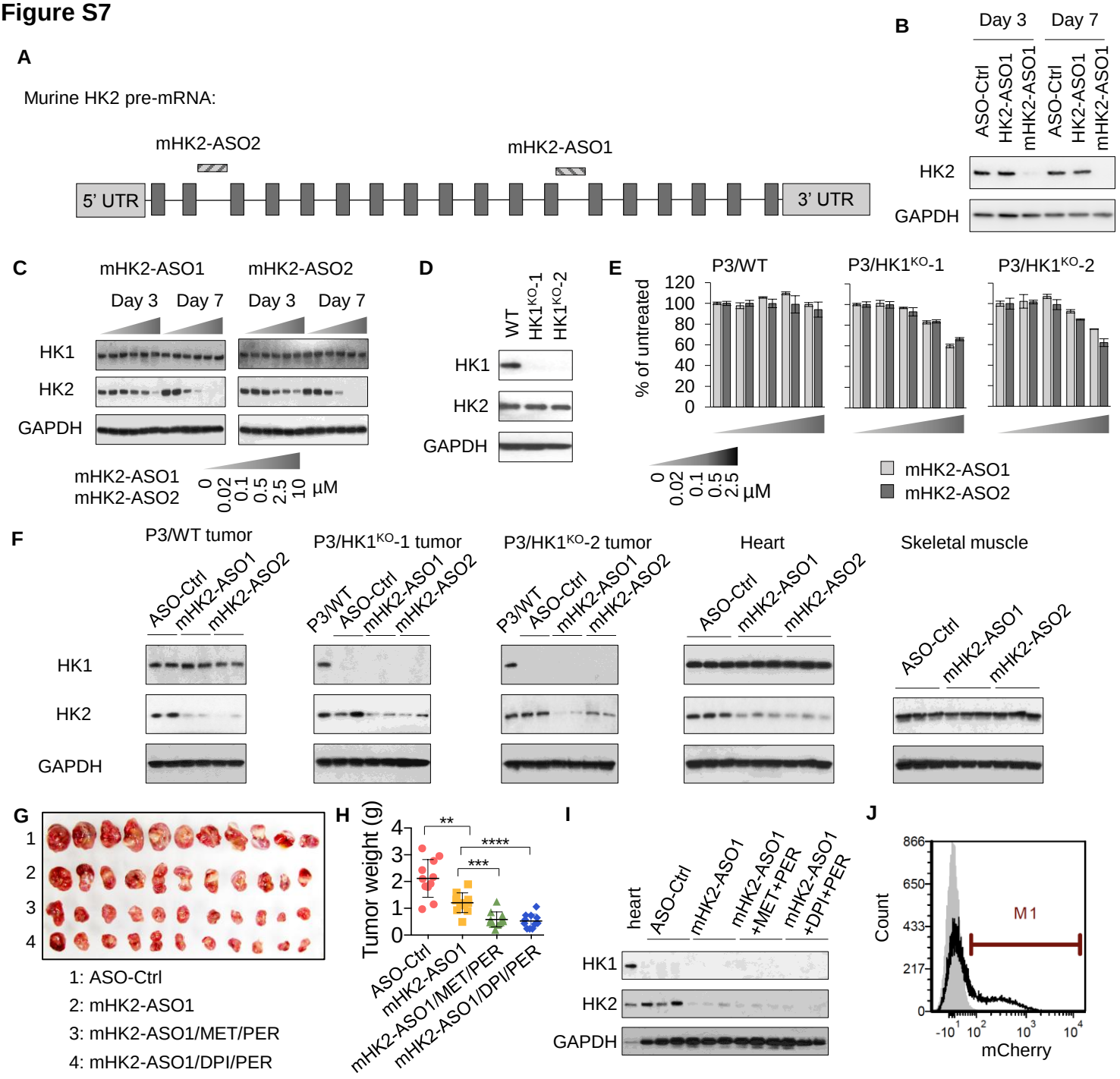

Fig. S7. *In vitro* and *in vivo* effects of mouse HK2 (mHK2) ASOs, as single agents and in combination with DPI, MET, and PER in mouse P3 ($HK1^-HK2^+$) and P3 ($HK1^+HK2^+$) isogenic MM cells. (A) Locations of targeted sequences for HK2-ASO1 and HK2-ASO2 in the murine HK2 pre-mRNA. (B) HK2-ASO1 does not affect mouse HK2 expression in mouse P3 MM cells. Cells were treated with ASO-Ctrl, human HK2-ASO1, or mHK2-ASO1, at 10 μM, for 3 days and 7 days. HK2 expression was measured by Western blotting. (C) mHK2-ASO1 and mHK2-ASO2 suppress HK2 expression in mouse P3 cells. (D) Generation of isogeneic $HK1^-HK2^+$ P3 cells using CRISPR Cas9 to knockout HK1. (E) mHK2-ASO1 and mHK2-ASO2 selectively inhibit cell proliferation in $HK1^-HK2^+$ P3 cells. Cells were treated with mHK2-ASO1 or mHK2-ASO2 for 7 days at the indicated concentrations. Cell proliferation was measured by the MTS assay on Day 7. (F) Effects of 8-day treatments with mHK2-ASO1 or mHK2-ASO2 on HK1 and HK2 levels in $HK1^+HK2^+$ P3 WT tumors, isogenic $HK1^-HK2^+$ P3 tumors, heart ($HK1^+HK2^+$), and skeletal muscle ($HK1^-HK2^+$). (G) Images of $HK1^-HK2^+$ P3 tumors collected after indicated treatments for 15 days. (H) Tumor weight was compared among the four indicated groups at the experiment endpoint. **, $P < 0.01$. ***, $P < 0.001$. ****, $P < 0.0001$. (I) Effects of ASO-Ctrl, mHK2-ASO1, mHK2-ASO1+MET+PER, or mHK2-ASO1+DPI+PER for 15 days on HK1 and HK2 protein levels in $HK1^-HK2^+$ P3 tumors. The heart lysate is from an untreated NSG mouse and used as a positive control for HK1 expression. (J) A representative histogram of percent MM cells in bone marrow extracts (study day 18) from control mice (N = 3, grey solid filled histogram) and mice with i.v. injection of P3/$HK1^-$/mCherry-LUC cells (N = 3, open histogram). M1: gating for mCherry positive cells.