Cell Reports Medicine, Volume *3*

Supplemental information

SU086, an inhibitor of HSP90, impairs glycolysis and represents a treatment strategy for advanced prostate cancer

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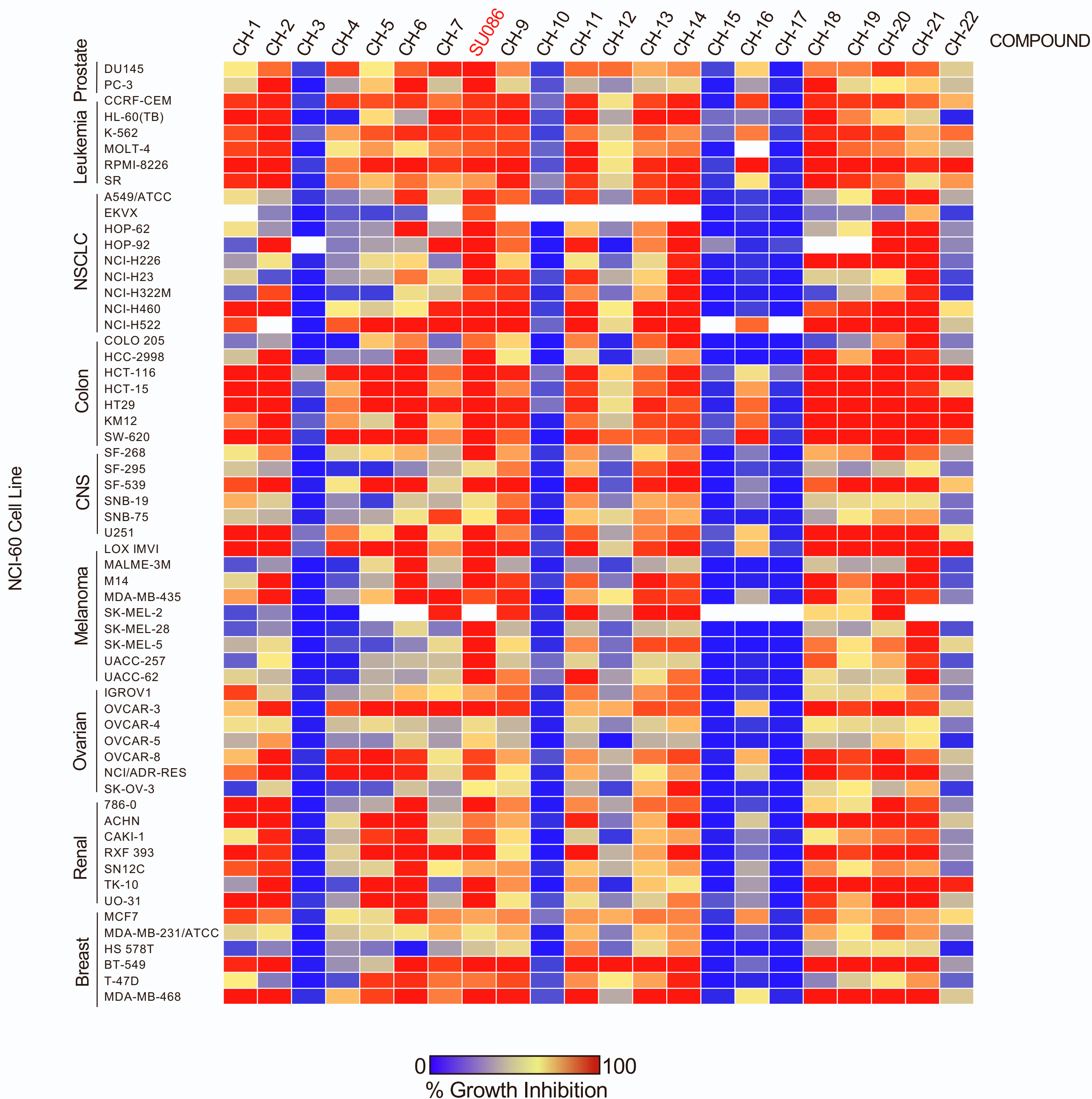


Figure S1. Assay of chalcones in NCI-60 cell line panel. Related to Figure 1. Twenty-two chalcone analogues were assayed against the NCI-60 cell line panel at 10μM for 48 hours. Growth inhibition was measured and displayed as a heatmap from no inhibition (blue), to complete growth inhibition (red). White indicates no measurements were acquired. Raw data is included in Table S1.

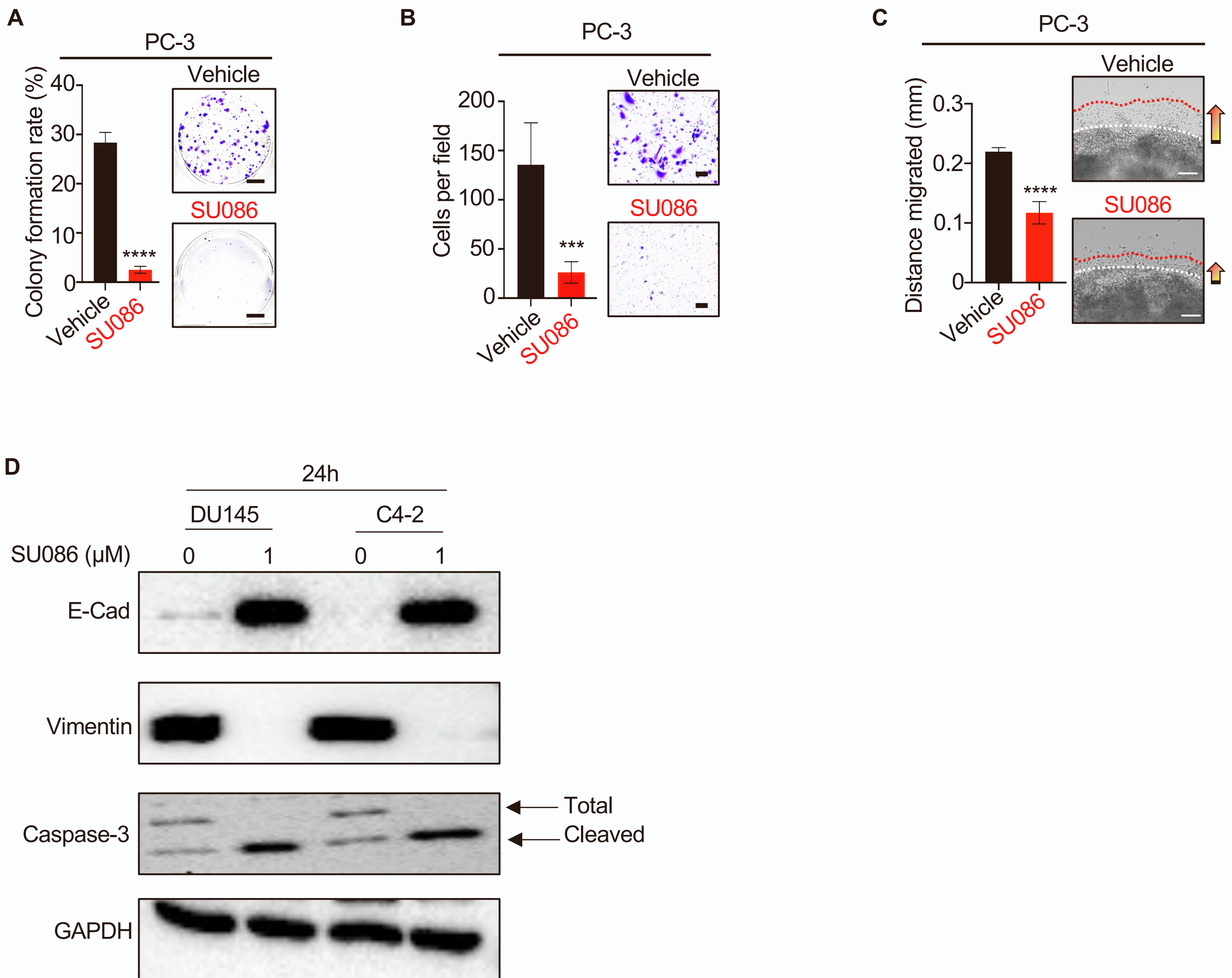
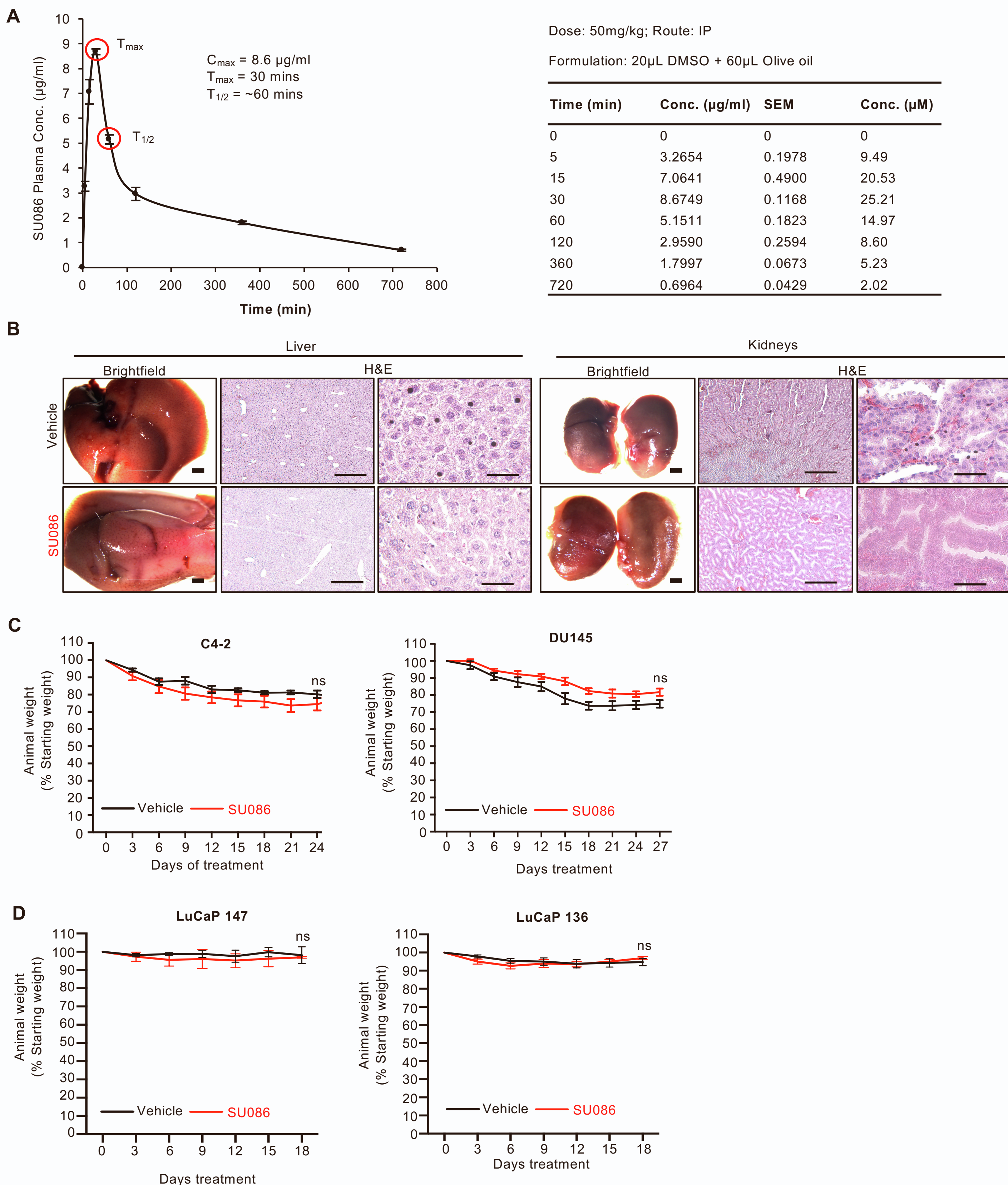


Figure S2. Growth, migration and invasion of PC-3 prostate cancer cells upon treatment with SU086 *in vitro,* EMT markers and caspase-3 cleavage upon treatment with SU086 in prostate cancer cells. Related to Figure 2. A. Colony formation assay of PC-3 cells grown for nine days in the presence of 1 µM SU086 or vehicle control. The quantified colony area was graphed as colony formation rate (colony number/500 cells plated x 100). Scale bar = 100 mm. B. Invasion assay with PC3 cells. PC-3 cells were pretreated with 1 µM SU086 or vehicle control for 72 hours. $4x10^5$ PC-3 were plated in serum-free medium with 1 µM SU086 or vehicle control in Matrigel-coated Boyden chambers. After 20 hours incubation, invaded cells were imaged and quantified (3 images per well and two wells per condition). Representative experiments are shown. C. 3D Matrigel Drop invasion assay of PC-3 cells in presence of 1 µM SU086 or vehicle control. The distance migrated outside the Matrigel Drop was measured and graphed as distance (mm). Scale bar = 250 microns. For all, error bars represent standard deviation. *** = P<0.005, **** = P<0.001 determined by Student's t-test. D. Prostate cancer cells (DU145 or C4-2) were treated with vehicle (0) and SU086 (1 uM) for 24 h, and total cell lysates were subject to western blot analysis for Epithelial-Mesenchymal Transition (EMT), apoptosis markers (caspase-3) and GAPDH.



Time (min)	Conc. (µg/ml)	SEM	Conc. (µM)
0	0	0	0
5	3.2654	0.1978	9.49
15	7.0641	0.4900	20.53
30	8.6749	0.1168	25.21
60	5.1511	0.1823	14.97
120	2.9590	0.2594	8.60
360	1.7997	0.0673	5.23
720	0.6964	0.0429	2.02

Figure S3. Pharmacokinetic analysis of SU086 and toxicity assays of SU086. Related to Figure 3 and 4. A. Mice were treated with 50 mg/kg SU086 i.p. at time zero. Blood was collected retro-orbitally at 5, 15, 30, 60, 120, 360 and 720 minutes after injection in three mice per time point. Plasma concentration of SU086 was detected at the indicated concentrations and graphed as µg/µl ± SEM. C_{max} (maximum serum concentration), T_{max} (time to reach Cmax) and $T_{1/2}$ (elimination half-life of compound). Values were then defined on a table calculating final concentration (µM) of SU086 in plasma of treated animals. B. Liver and kidney tissues were harvested from mice treated with SU086 or vehicle in Figure 4. Fresh tissue samples were imaged on brightfield on stereomicroscope. Scale bar represents 1 mM. Tissue was subsequently fixed in 10% formalin overnight at 4 °C, processed and embedded. Samples were stained with H&E. Scale bars = 500 microns left, 50 microns enlarged. C. Animal weight was evaluated every third day from experiments shown in Figure 3B,C. D. Animal weight was measured every third day from experiments shown in Figure 4B,C.

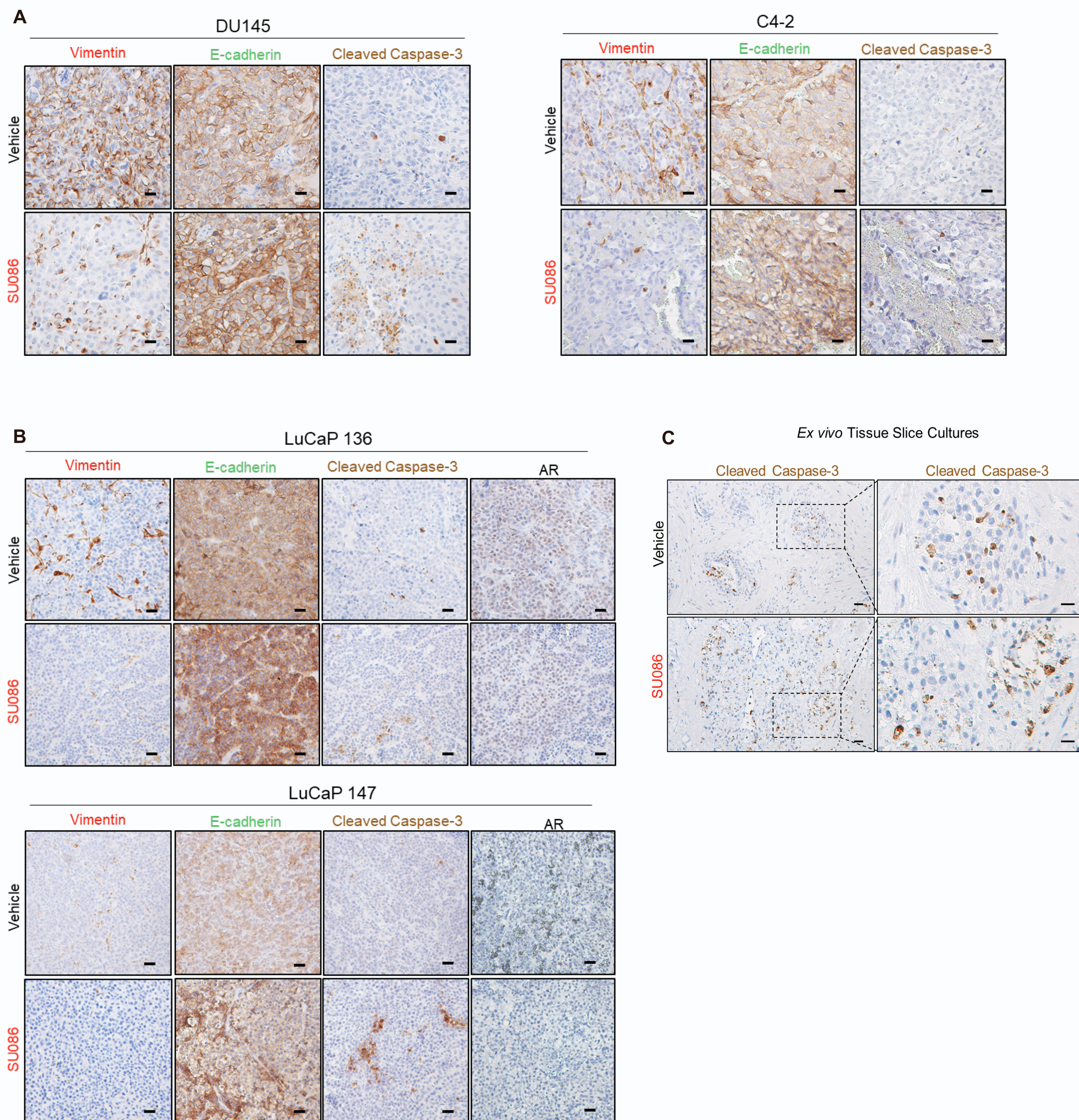


Figure S4. EMT markers, cleaved caspase-3 and AR upon treatment with SU086 in prostate cancer xenografts, Patient Derived Xenografts (PDXs) of prostate cancer and *ex vivo* tissue slice cultures of primary prostate cancer. Related to Figures 3 and 4. A. IHC for vimentin, E-cadherin and cleaved caspase-3 of DU145 and C4-2 xenografts treated with vehicle or SU086 *in vivo* from Figure 3. Scale bars indicate 20 microns. B. IHC for vimentin, E-cadherin, cleaved caspase-3 and AR of LuCaP 136 and LuCaP 147 PDXs treated with vehicle or SU086 *in vivo* from Figure 4. Scale bars indicate 20 microns. C. Cleaved caspase-3 IHC of slices from *Ex vivo* culture prostate cancer tissues with vehicle or SU086 from Figure 4. Scale bars indicate 20 microns (left) and 30 microns (right).

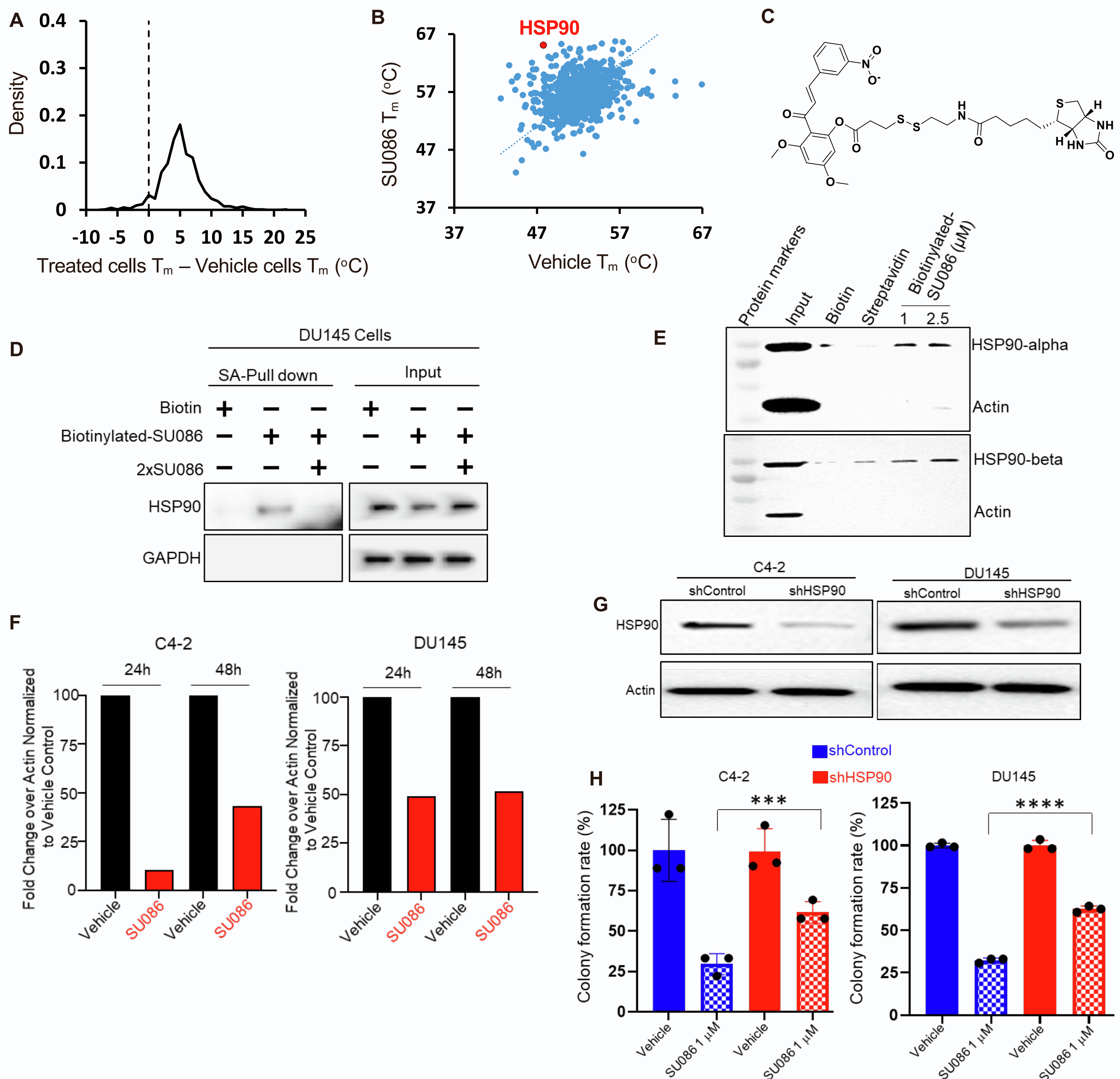


Figure S5. Testing SU086 binding to cellular HSP90 and HSP90 as a target of SU086 in prostate cancer cells. Related to Figure 5. A. Average melting temperature (T_m) of proteins in SU086 treated cells minus the T_m of vehicle control. B. Scatterplot of T_m calculated in SU086 and vehicle treated cells. HSP90 (red) identified with statistical cutoffs of $p<0.01$ and $R^2 >0.7$. C. Structure of biotinylated-SU086. D. The SU086-HSP90 interaction was further evaluated via streptavidin (SA) affinity purification with biotinylated-SU086. DU145 cells were treated with vehicle (-) or 5µM SU086 for 1 hour. Cells were then treated with biotinylated-SU086 (2.5µM for 1.5 hour). Cells were lysed and lysates were subjected to pull down with SA beads. The protein levels of HSP90 and GAPDH control in the pull down products and input controls were examined by Western blot. E. Immunoprecipitation with biotinylated SU086 followed by western blot experiments from Figure 5 for HSP90 alpha, HSP90-beta and beta-actin as a loading control following treatment (1.5 hr) of C4-2 or DU145 live cells with SU086 at the indicated doses. F. Densitometry quantification of the Western blots for HSP90 and actin loading control shown in Figure 5J. G. Western blots for HSP90 and actin on cell lysates from C4-2 (left) or DU145 (right) cells expressing control shRNA (shControl) or shRNAs targeting HSP90 (shHSP90). H. Colony formation assay with shControl and shHSP90 expressing C4-2 and DU145 cells. Cells were incubated with indicated doses of SU086 or vehicle for 10 days (C4-2) or 7 days (DU145). Colony formation (% of area normalized to the control and shown as 100) was quantified and plotted. Error bars represent standard deviation. *** = $P<0.005$, **** = $P<0.001$ determined by Student's t-test.

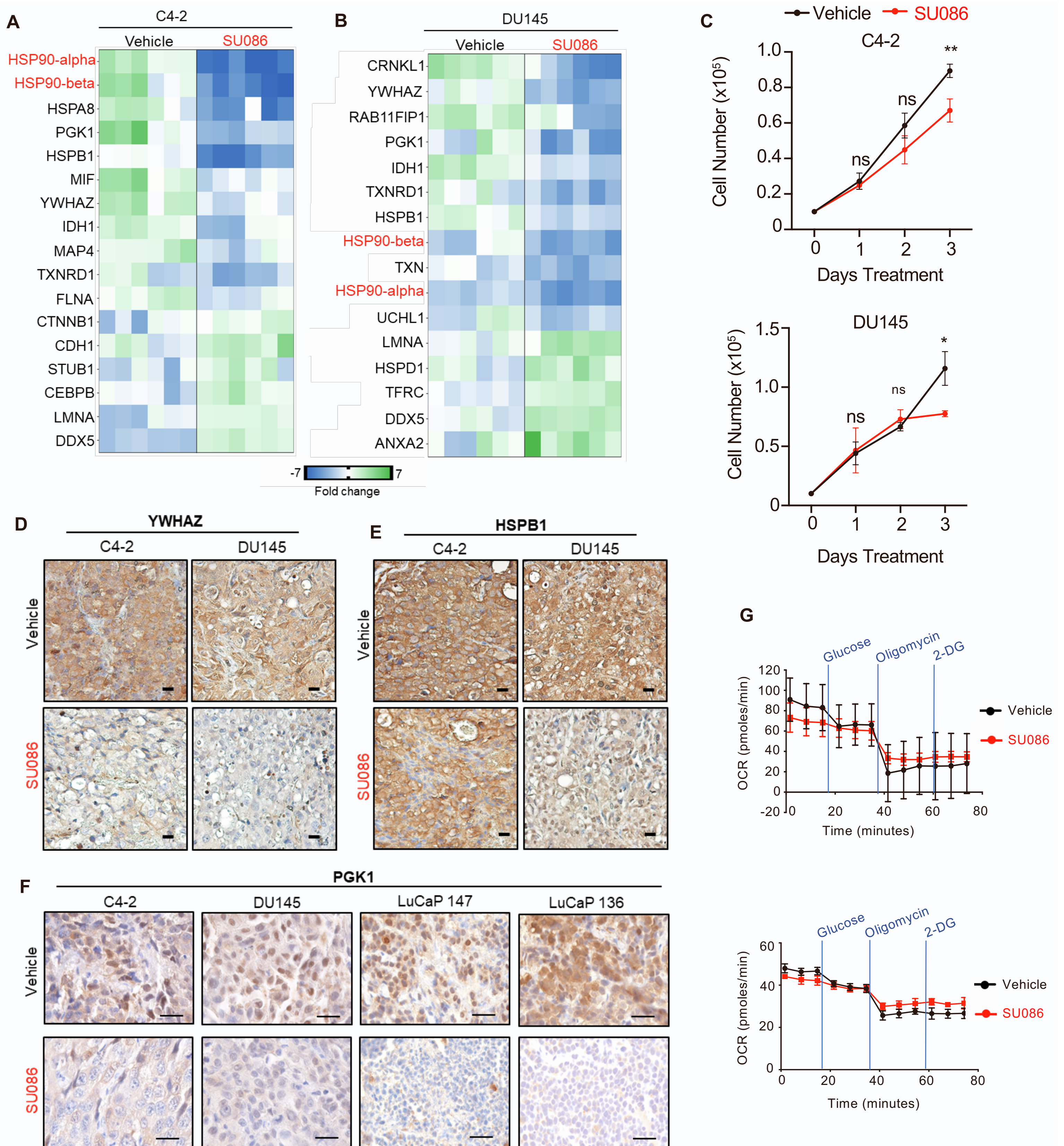


Figure S6. Changes in HSP90 targets upon treatments with SU086, SU086 anti-proliferative effects and levels of HSP90 clients, YWHAZ, HSPB1 and PGK1, in prostate cancer xenografts and measuring effects of SU086 on OCR reflecting oxidative phosphorylation. Related to Figure 6. Heatmap of (A) C4-2 and (B) DU145 HSP90 interactors extracted from proteomic data shown in Figure 6A. C. Proliferation of C4-2 and DU145 cells at 1, 2, and 3 days of treatment with 1 µM SU086 or vehicle control and counted by trypan blue exclusion assay. D. Immunohistochemical (IHC) evaluation of YWHAZ in vehicle or SU086 treated C4-2 and DU145 xenograft shown in Figure 3. Scale bars = 20 microns. E. IHC analysis for HSPB1 levels in vehicle or SU086 treated C4-2 and DU145 xenograft shown in Figure 3. Scale bars = 20 microns. F. IHC evaluation of PGK1 in vehicle or SU086 treated xenograft and PDX specimens from Figures 3 and 4. Scale bars = 25 microns. G. OCR (oxygen consumption rates) of C4-2 and DU145 cells post treatment with SU086 or Vehicle control by Seahorse glycolytic stress test.

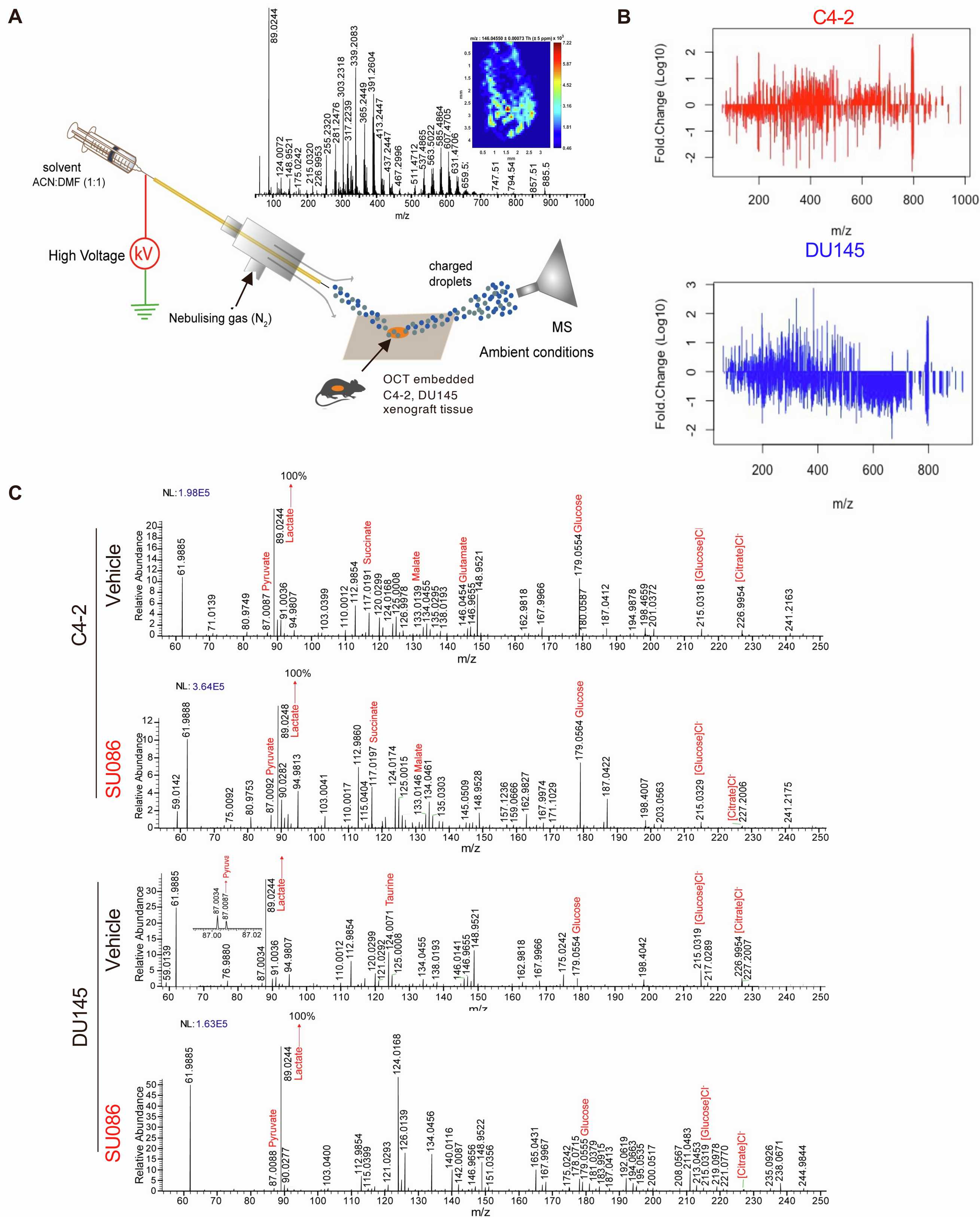
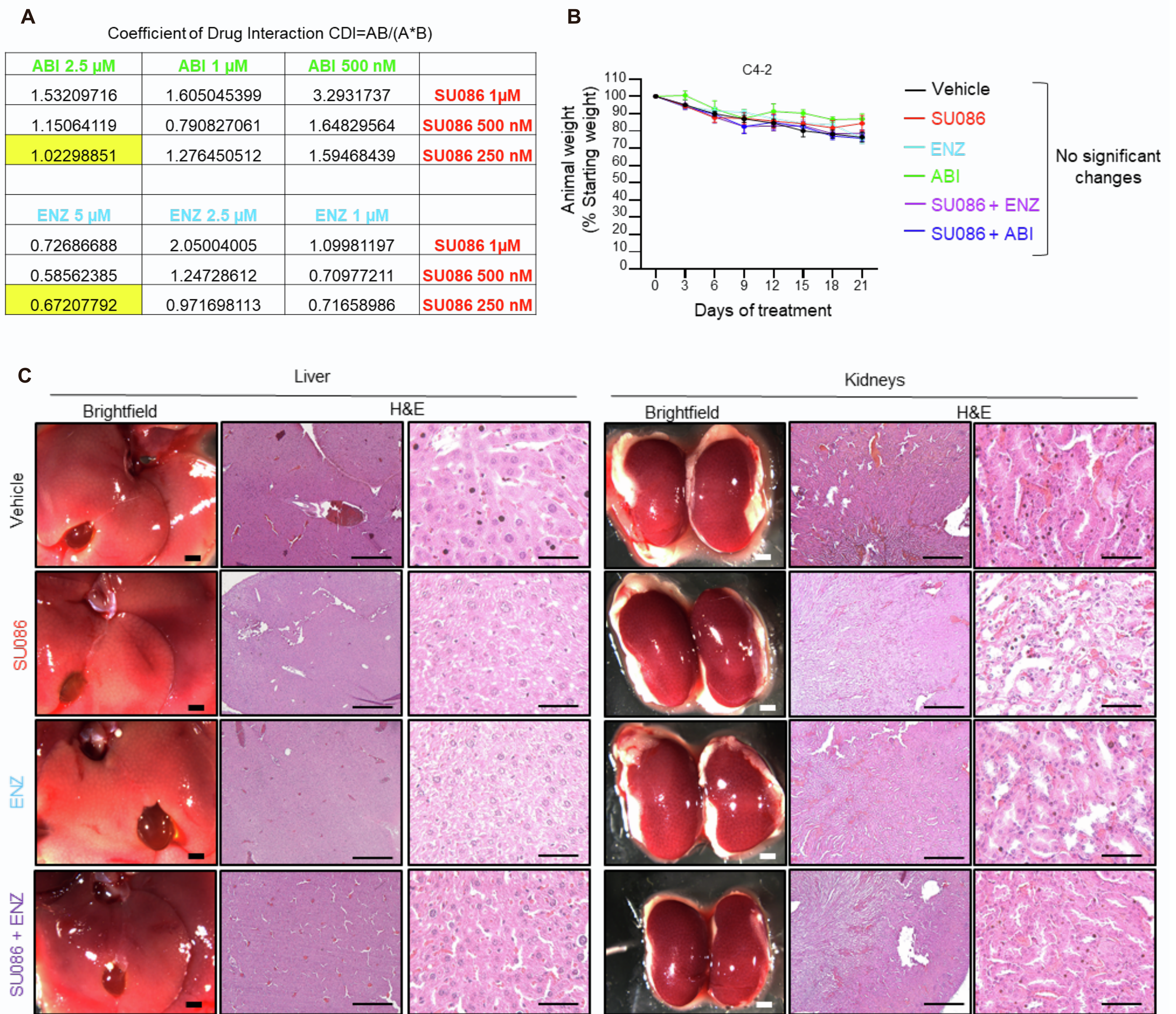


Figure S7. DESI analysis. Related to Figure 6. A. Desorption electrospray ionization (DESI) experimental workflow involves spraying microdroplets onto C4-2 or DU145 xenograft tissues from Figure 3 that had undergone *in vivo* treatment with SU086 at 50 mg/kg or DMSO vehicle control. Charged droplets containing metabolites from tissues were then picked up by MS reflecting coordinately mapped metabolites across tissues. Samples were then run through LC/MS-MS analysis as described in the Methods section. B. Profiles of all C4-2 and DU145 spectral analyses of unique pixels. C. Metabolite spectra for a representative row of one tissue sample. Commonly occurring metabolites are marked in red reflecting m/z size. C4-2 n=2 xenografts per condition; DU145 n=6 xenografts per condition.



A

Coefficient of Drug Interaction CDI=AB/(A*B)

ABI 2.5 µM	ABI 1 µM	ABI 500 nM	
1.53209716	1.605045399	3.2931737	SU086 1µM
1.15064119	0.790827061	1.64829564	SU086 500 nM
1.02298851	1.276450512	1.59468439	SU086 250 nM
ENZ 5 µM	ENZ 2.5 µM	ENZ 1 µM	
0.72686688	2.05004005	1.09981197	SU086 1µM
0.58562385	1.24728612	0.70977211	SU086 500 nM
0.67207792	0.971698113	0.71658986	SU086 250 nM

Figure S8. Testing SU086 in combination with enzalutamide and abiraterone *in vitro* and *in vivo* animal weights and tissue histology from Figure 7. Related to Figure 7. A. Coefficient of Drug Interaction (CDI) was tested with colony formation assay of each of the indicated compounds at the indicated dose concentrations and at the indicated combination concentrations. CDI = AB (SU086 + ENZ treatment, or SU086 + ABI treatment)/ [(A, SU086 treatment alone) X (B, ENZ or ABI treatment alone)]. Values less than one specify synergism, values equal to one indicate additive effects, and values greater than one indicate antagonism. The concentrations selected for further functional assays *in vitro* are highlighted in yellow and shown in Figure 7A. B. Mice from Figure 7D were weighed every third day to assay toxicity as a result of combination therapy of SU086 with second-generation anti-androgens enzalutamide (ENZ) or abiraterone (ABI). C. At study termination, liver and kidneys were isolated, fixed in 10% formalin, stained with H&E and imaged on brightfield. Scale bar represents 1 mM. Scale bars = 500 microns left, 50 microns enlarged.