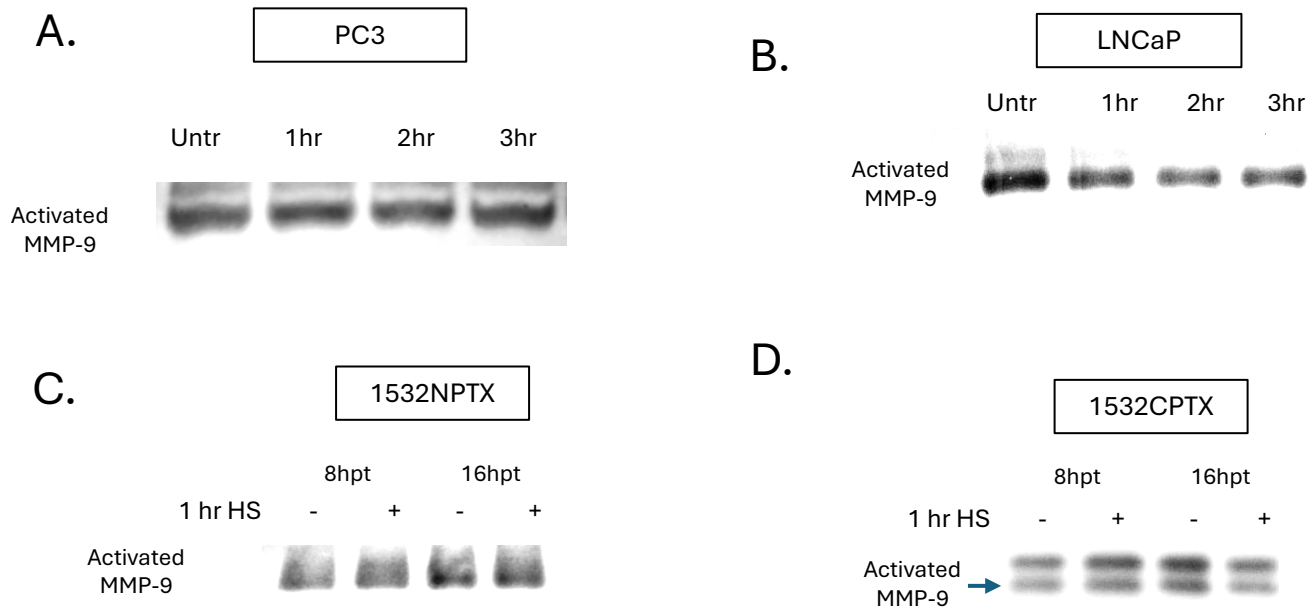
Supplementary Information to the manuscript:

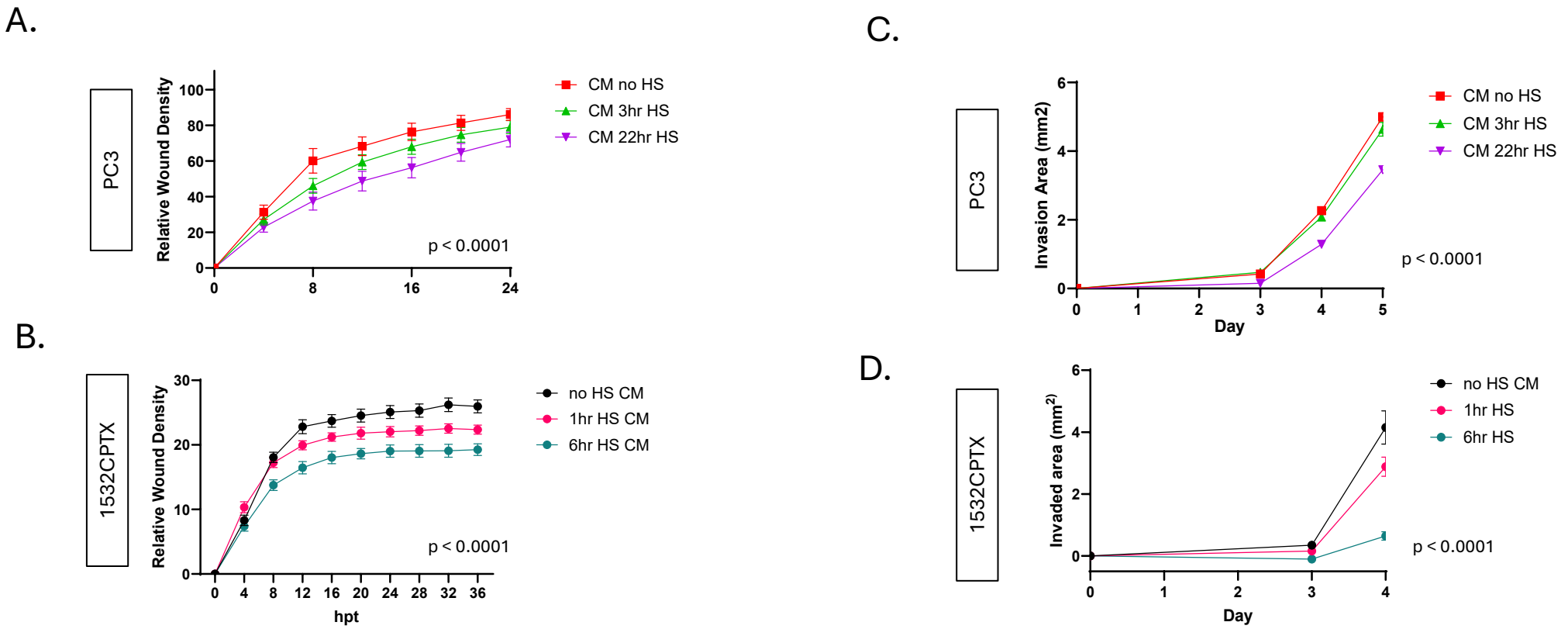
HSP90α and KLK6 Co-regulate Stress-Induced Prostate Cancer Cell Motility

Authors: Katelyn L. O'Neill, Johnny W. Zigmond, Raymond Bergan

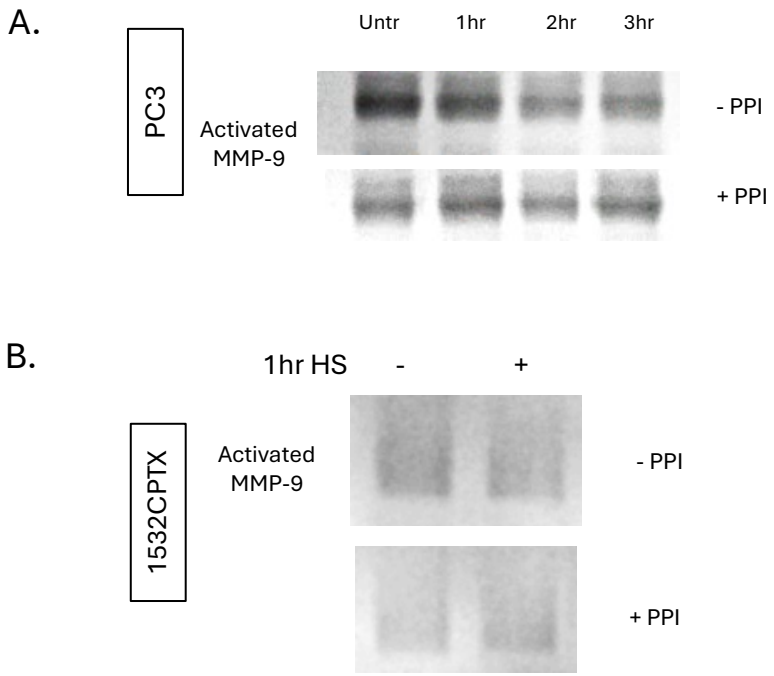
Supplemental Figures S1-S8



Supplemental Figure S1. Effects of cellular stress on MMP-9 in prostate cell lines. MMP-9 activity in conditioned media (CM) from (**A**) PC3 (**B**) LNCaP (**C**) 1532NPTX and (**D**) 1532CPTX cells subjected to stress as indicated by gelatin zymography *n*=2-3. CM was collected from 24 h post-stress PC3 and LNCaP and 8 h and 16 h post-stress from1532NPTX and 1532CPTX cells. (Samples/gels are the same as Figure 1.)



Supplemental Figure S 2. Stress-induced cellular invasion over time. (**A, B**) Scratch-wound invasion assay for PC3 and 1532CPTX cells treated with CM from cells -/+ stress. Cell monolayers were scratched and then overlaid with Matrigel and treated with CM from cell -/+ stress. Relative wound density was measured by Incucyte S3 live cell analysis over 24-36 h post treatment. (**C, D**) Matrigel drop invasion assay with PC3 and 1532CPTX cells treated with CM from cells -/+ stress. PC3 CM was harvested 24 h post-stress and 1532CPTX CM was harvested 16 h post-stress. Error bars are SEM. Two to four individual biological replicates were performed for the above experiments, and the results were combined. Two-way ANOVA was used to determine statistical significance.

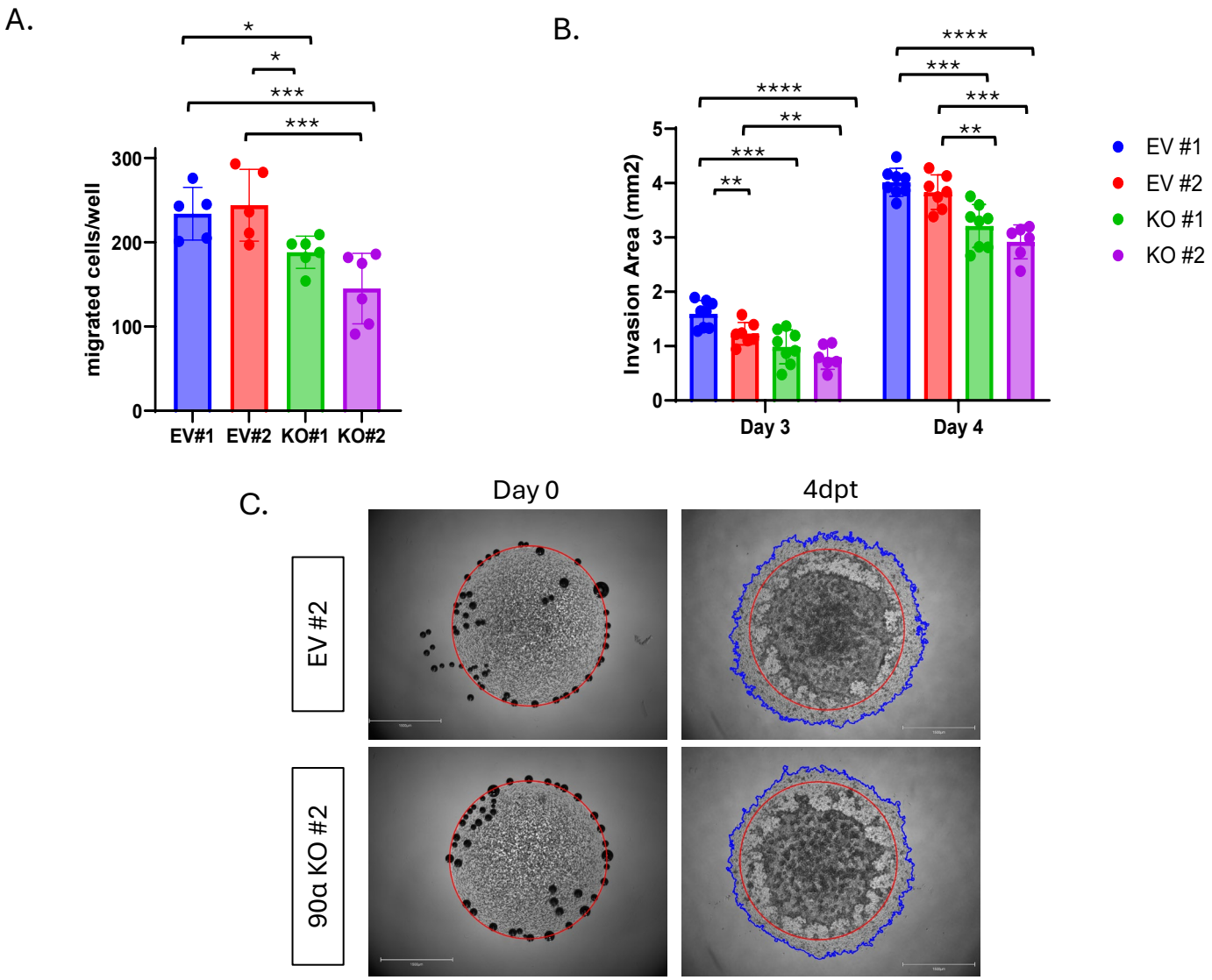


Supplemental Figure S3. Protease and Phosphatase inhibition effects on stress-induced MMP-9 activity. (**A**) MMP-9 activity in CM from PC3 and (**B**) 1532CPTX cells subjected to stress -/+ protease/ phosphatase inhibitors (PPI) as indicated by gelatin zymography *n*=2-3. CM was collected 24 h post-stress from PC3 cells and 8 h post-stress from1532CPTX cells. Zymography images are from the same gels as Figure 3.

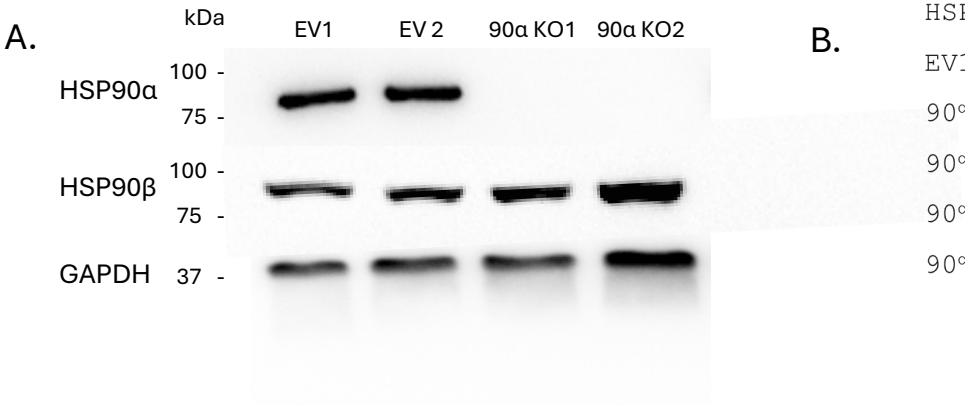


	PC3 90α KO 1	PC3 90α KO 2
Mutation	299 bp ins	1 bp del

Supplemental Figure S4. Selected pool and genomic sequencing for PC3 HSP90α KO cells. (**A**) Western with PC3 blasticidin-selected pools from CRISPR/Cas9 transient transfection. (**B**) Genomic sequencing data with PC3 EV and HSP90α KO clones 1 and 2. gRNA sequence is in green font; inserted sequence is highlighted yellow and deleted base pairs are highlighted green.



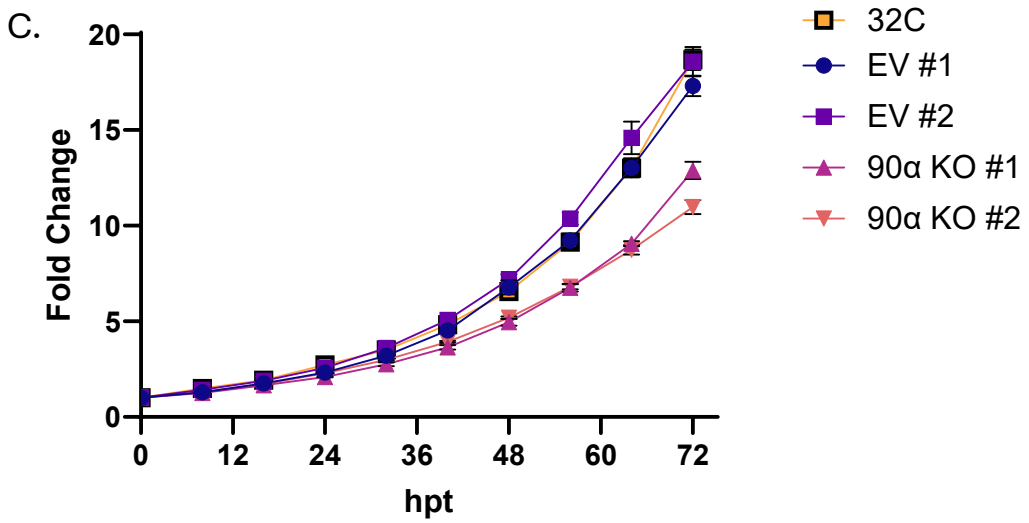
Supplemental Figure S5. HSP90α KO cells have decreased cell motility. (**A**) Boyden chamber migration assay with empty vector and HSP90α KO clones 24 h post-plating. (**B, C**) Matrigel drop invasion assay with empty vector and HSP90α KO clones. Invaded area = (area of blue outline) – (area of original drop Day 0 outlined in red). Graphs represent combined results from at least 2 biologically independent experiments. Error bars indicate SD. Statistical significance was determined by Welch's student t-test, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

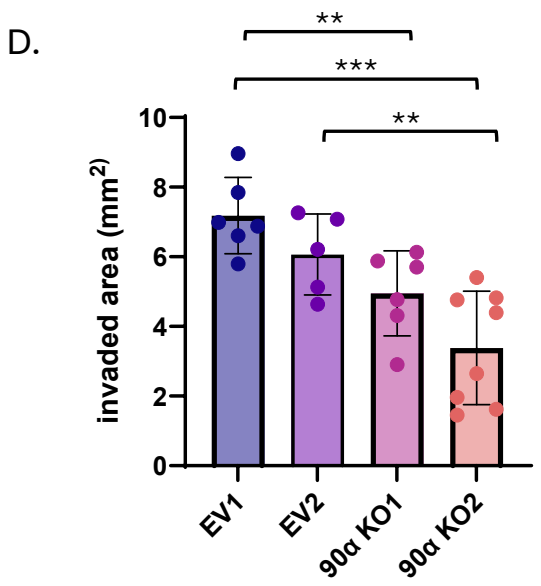


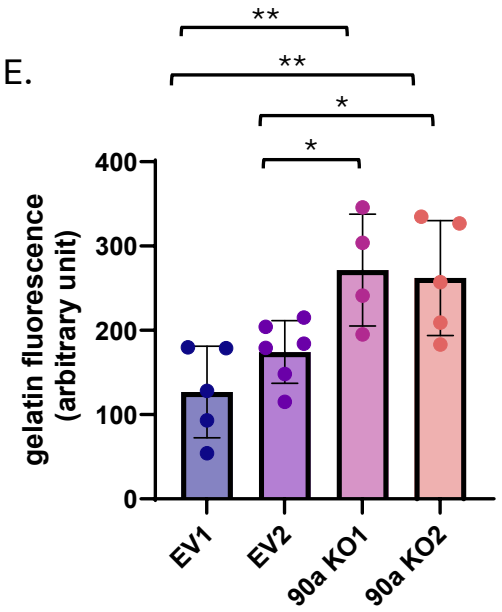
B.

```
HSP90AA1    ctcttgcaGGTGAACCTATGGGTCGTGGAACAAAAGTTATCCTACACCTGAAAGAAGACCAAACTGAGTACT
EV1         CTCTTGCAGGTGAACCTATGGGTCGTGGAACAAAAGTTATCCTACACCTGAAAGAAGACCAAACTGAGTACT
90ᵅKO 1a    CTCTTGCAGGTGAACCTATGG--CGTGGAACAAAAGTTATCCTACACCTGAAAGAAGACCAAACTGAGTACT
90ᵅKO 1b    CTCTTGCAGGTGAACCTATGG-TCGTGGAACAAAAGTTATCCTACACCTGAAAGAAGACCAAACTGAGTACT
90ᵅKO 2a    CTCTTGCAGGTGAACCTATGG-TCGTGGAACAAAAGTTATCCTACACCTGAAAGAAGACCAAACTGAGTACT
90ᵅKO 2b    CTCTTGCAGGTGAACCTA--GGTCGTGGAACAAAAGTTATCCTACACCTGAAAGAAGACCAAACTGAGTACT
```

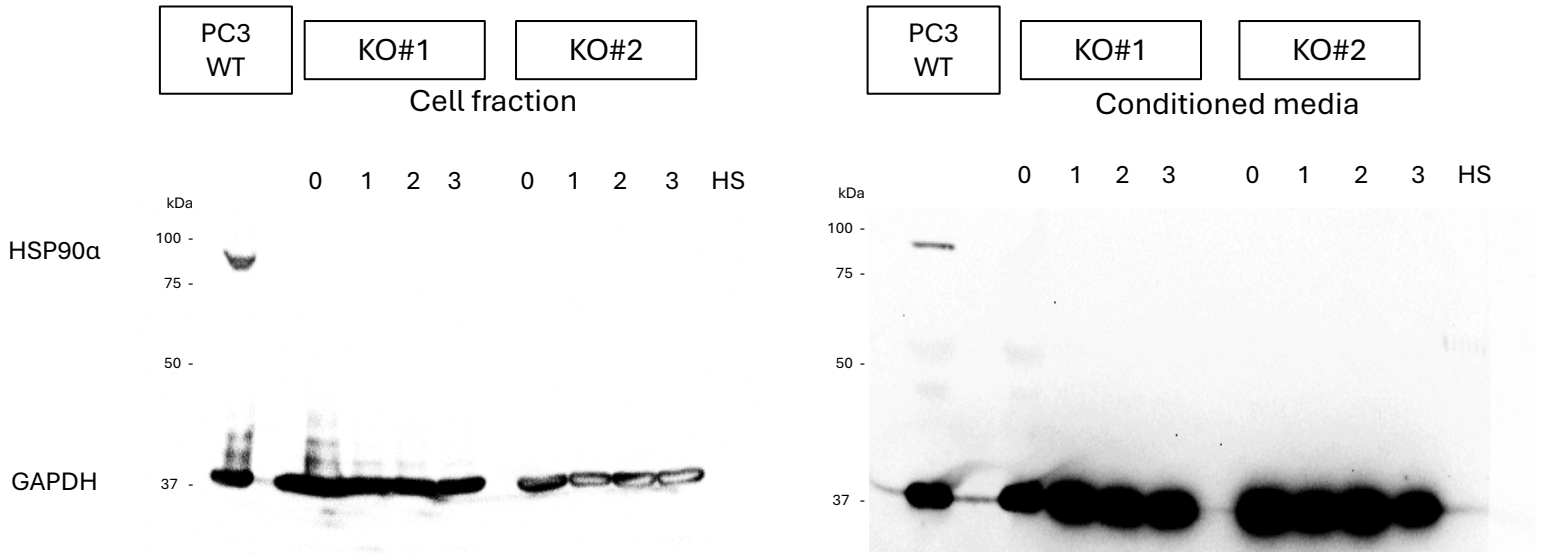
	Allele 1	Allele 2	
Hsp90α KO1	1bp del	2bp del	Early stop
Hsp90α KO2	1bp del	2bp del	Early stop



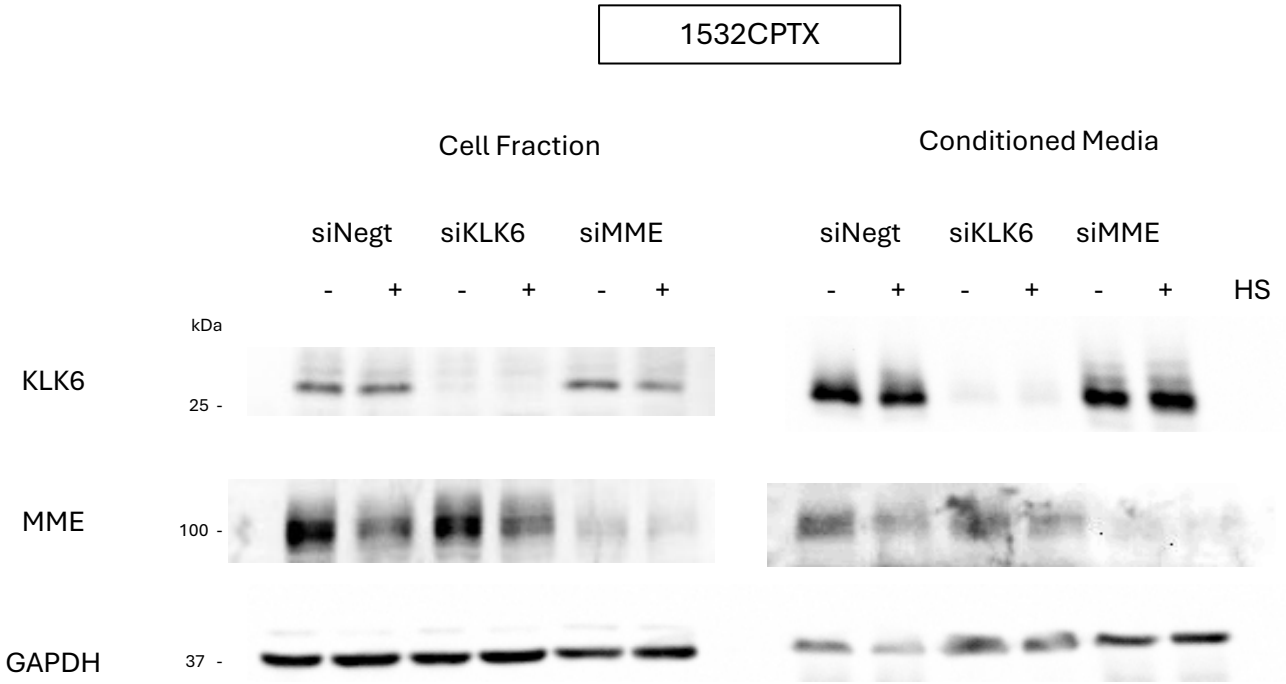




Supplemental Figure S6. Characterization of 1532CPTX HSP90α KO clones. (**A**) Western of 1532CPTX parental cell line, empty vector (EV) control single clones #1 and #2 and HSP90α KO single clones #1 and #2. (**B**) Genomic sequence analysis of 1532CPTX. gRNA is highlighted in yellow, deleted base pairs in KOs are in red font. (**C**) Proliferation assay of 1532CPTX, empty vector and HSP90α KO clones. (**D**) Matrigel Drop invasion assay with empty vector and HSP90α KO clones (*n*=2). (**E**) Gelatin dequenching assay with CM from EV and KO clones 16 h post SFM. Graphs represent combined results from at least 2 biologically independent experiments. Bar graph error bars indicate SD; XY graph error bars are SEM. Statistical significance was determined by unpaired student t-tests. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.



Supplemental Figure S7. HSP90α in KOs with cellular stress. HSP90α levels in PC3 compared to HSP90α KO CF and CM -/+ stress (same samples as Figure 5).



Supplemental Figure S8. Stress induced by siRNA transfection negates effects of stress in 1532CPTX cells. Immunoblot showing siRNA knockdown of KLK6 and MME in 1532CPTX cells -/+ stress, *n*=2.