

SUPPLEMENTAL TABLE 1. Quantitative qRT-PCR primer list.

SUPPLEMENTAL FIGURE 1. eHsp90 mediated migration requires LRP1. *A*, Representative results of a scratch wounding assay with PC3 following treatment with NPGA (1 μ M) or either of two different eHsp90 antibodies (SPA-830, Ab1; SPS-771, Ab2). *B*, Immunoblot validation of LRP1 shRNA knockdown in DU145 cells. Representative images of cell motility from a wounding assay with scrambled (scr) shRNA or shLRP1 in DU145 prostate cancer cells. Magnification is 100x. Asterisks (*) indicate significance $p \leq 0.05$.

SUPPLEMENTAL FIGURE 2. eHsp90 promotes LRP1 mediated motility and ERK phosphorylation. *A*, Immunoblot analysis of LRP1 and indicated EMT effectors in ARCaPE/ARCaPM and M12/P69 cell pairs. *B*, *C*, Wounding assay in ARCaPE/P69 following a 3 day pretreatment with eHsp90 protein, and in ARCaPM/M12 with NPGA treatment for 20 h. *D*, Immunoblot validation of LRP1 expression in ARCaPE shLRP1 cells, and wounding assay to evaluate the effects upon eHsp90 directed cell motility. Magnification is 100x. *E*, Time dependent analysis of MEK/ERK activation from ARCaPE cells treated with 3 μ g/ml eHsp90.

SUPPLEMENTAL FIGURE 3. eHsp90 regulates the localization of junctional proteins and cell morphology. *A*, Confocal microscopy was utilized to evaluate E-cadherin and ZO-1 cellular localization in P69 control (lac Z transduced) and P69-eHsp90 modified cells. *B*, Evaluation of the effects of eHsp90 inhibition (5 day NPGA treatment) upon E-cadherin and ZO1 membrane localization in ARCaPM and M12 prostate mesenchymal cell lines. Scale bar is 50 μ m.

SUPPLEMENTAL FIGURE 4. Proteolytic activity and ERK signaling is required for eHsp90 mediated cell migration. Wounding assay using ARCaPE-eHsp90 prostate cancer cells pretreated with NPGA (1 μ M), GM6001 (1 μ M), MMP2/9 inhibitor (SB-3CT, 1 μ M) MMP3 inhibitor (inhibitor IV, 5 μ M) and ERK inhibitor (UO126, 10 μ M) for three days. Magnification is 100x.

<u>Name</u>	<u>Accession#</u>		<u>Sequence</u>
CDH1	NM_004360	Sense	TGGGCCAGGAAATCACATCCTACA
		Antisense	TTGGCAGTGTCTCTCCAAATCCGA
SNAI1	NM_005985	Sense	TTTCTGGTTCTGTGTCCTCTGCCT
		Antisense	TTCCCAGTGAGTCTGTGTCAGCCTTT
SNAI2	NM_003068	Sense	TTTCTGGGCTGGCCAAACATAAGC
		Antisense	ACACAAGGTAATGTGTGGGTCCGA
ZEB1	NM_030751	Sense	ATGCACAACCAAGTGCAGAAGAGC
		Antisense	TTGCCTGGTTCAGGAGAAGATGGT
ZEB2	NM_014795	Sense	ATATGGTGACACACAAGCCAGGGA
		Antisense	GTTTCTTGCAGTTTGGGCACTCGT
MMP2	NM_004530	Sense	AGAAGGATGGCAAGTACGGCTTCT
		Antisense	AGTGGTGCAGCTGTGCATAGGATGT
MMP3	NM_002422	Sense	AGGTGTGGAGTTCCTGATGTTGGT
		Antisense	TACAGCCTGGAGAATGTGAGTGGA
MMP9	NM_004994	Sense	ATTTCTGCCAGGACCGCTTCTACT
		Antisense	CAGTTTGTATCCGGCAAACCTGGCT
GAPDH	NM_002046	Sense	TCGACAGTCAGCCGCATCTTCTTT
		Antisense	ACCAAATCCGTTGACTCCGACCTT

Table S1

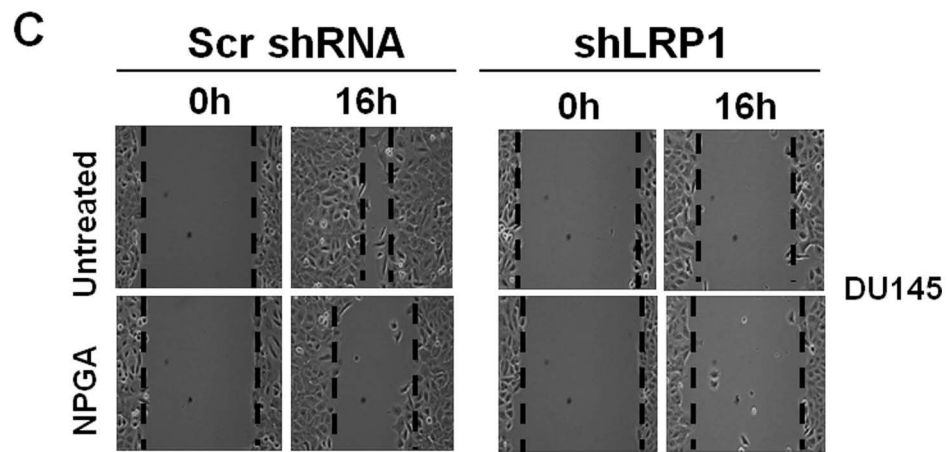
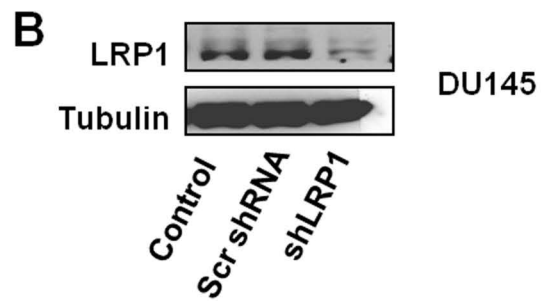
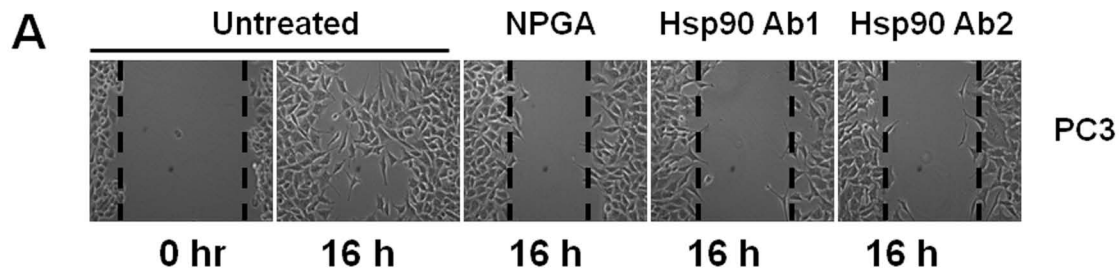


Fig. S1

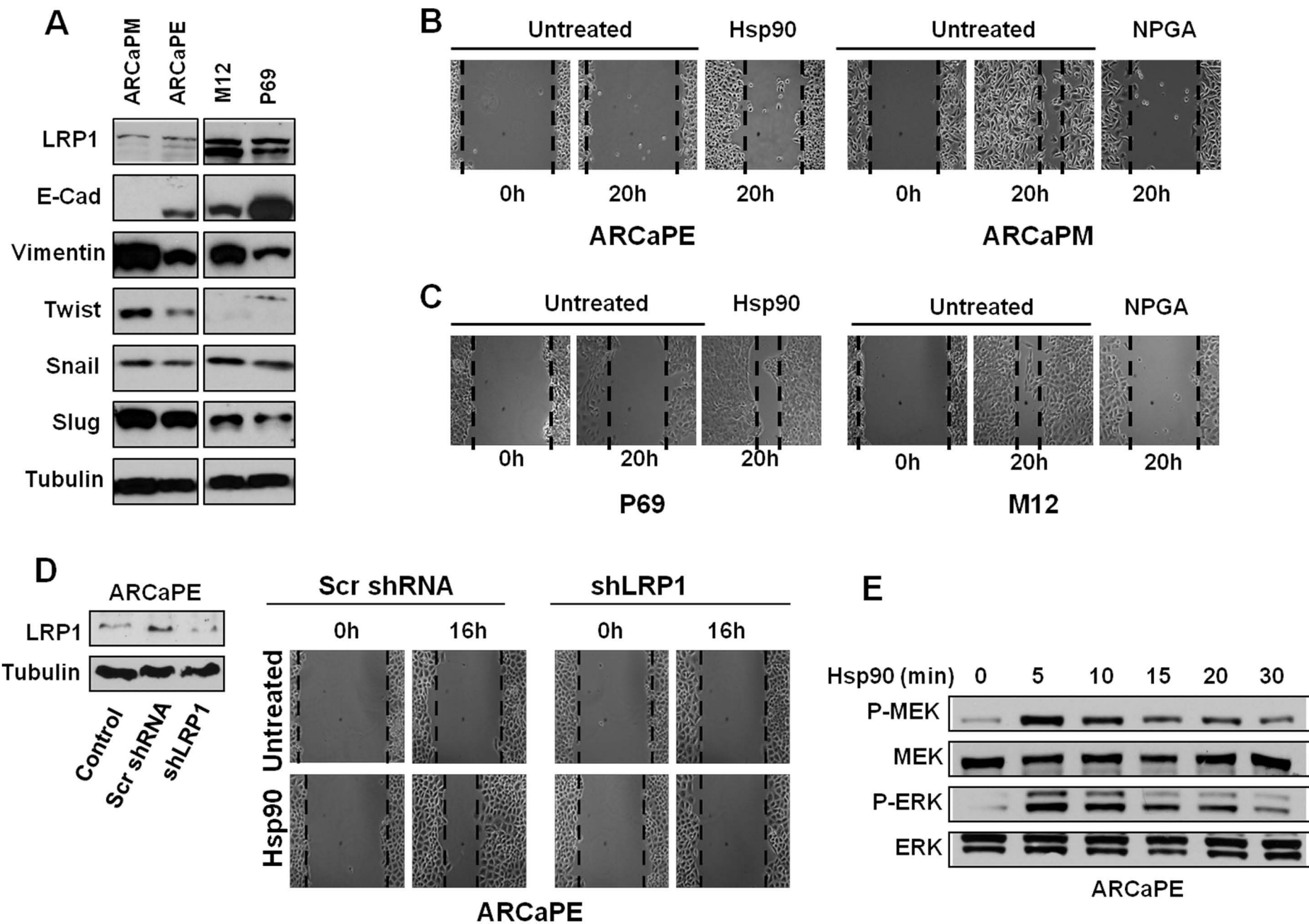
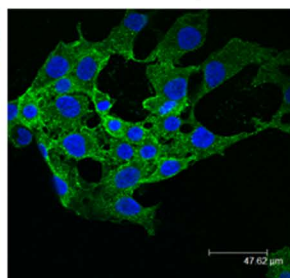
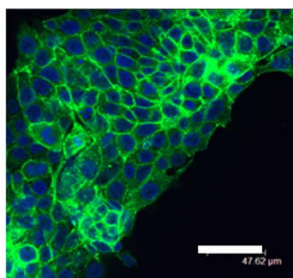
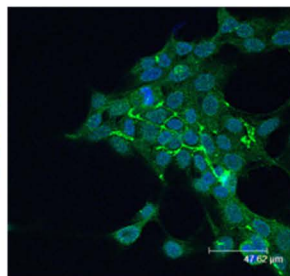
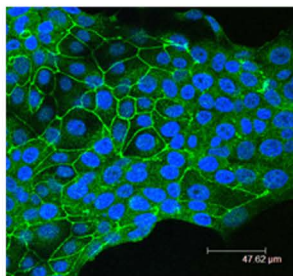
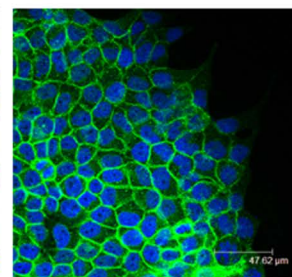
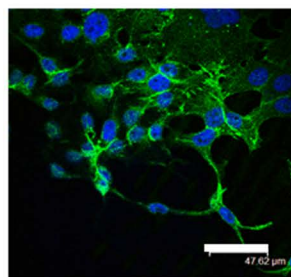
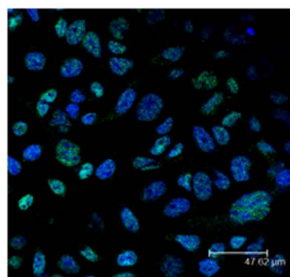
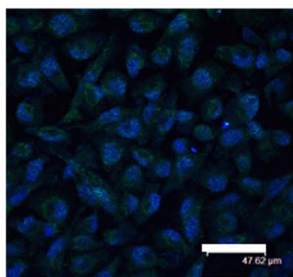
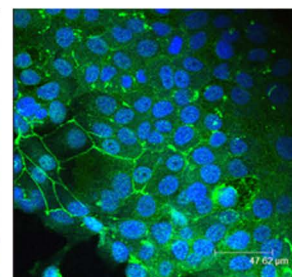
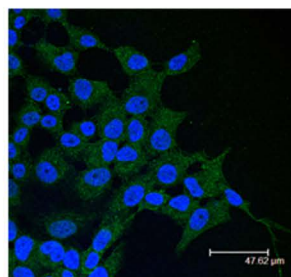
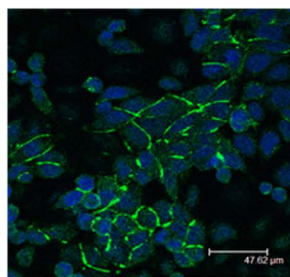
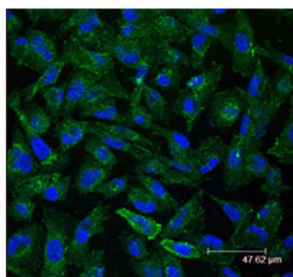
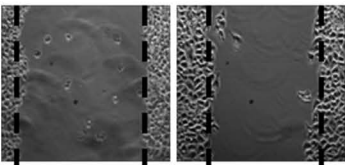


Fig. S2

A**P69 LacZ****P69-eHsp90****E-cad/Topro****ZO1/Topro****B****Untreated****5d NPGA****Untreated****5d NPGA****E-cad/Topro****ZO1/Topro****ARCaPM****M12****Fig. S3**

ARCaPE-LacZ

Untreated

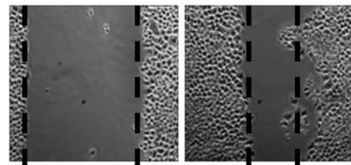


0h

20h

ARCaPE-eHsp90

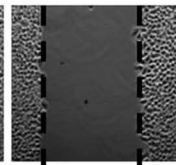
Untreated



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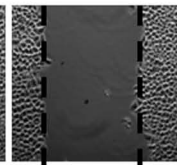
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NPGA



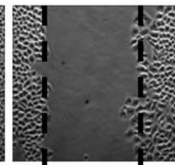
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GM6001



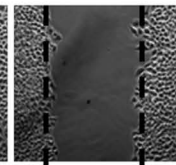
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MMP2/9 In



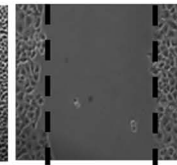
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MMP3 In



20h

UO126



20h

Fig. S4