

Supplementary tables:**Table S1.** shRNA constructs used for knockdown of Aldh1a1 and Aldh1a3.**Table S2.** siRNA oligos, primers and antibodies used in the study.

Table S 1. shRNA constructs used for knockdown of Aldh1a1 and Aldh1a3.

Gene	Name	Vector	Hairpin sequence
Non-silencing	shNS	pLKO.1	CCTAAGGTTAAGTCGCCCTCGCTC GAGCGAGGGCGACTTAACCTTAGG
Aldh1a1	shAldh1a1	pLKO.1	CCCAGTTCTTATCCAAGAATACTC GAGTATTCTTCGGATAAGAAGTGGG
Aldh1a3	shAldh1a3	pLKO.1	CGAATCCAAGAGTGGAAGAACTC GAGTTTCTTCCACTCTTGGATTCTG

Table S2. siRNA oligos, primers and antibodies used in the study.

siRNA oligos used for knockdown of gene expression		
Gene	siRNA name	Sequence
Scrambled siRNA	siSCR	ss GCAGCUAUAUGAAUGUUGUdTdT as ACAACAUUCAUAUAGCUGCdTdT
ALDH1A1	siALDH1A1 #1	ss GAUCCAGGGCCGUACAAUAdTdT as UAUUGUACGGCCCUGGAUCdTdT
ALDH1A1	siALDH1A1 #2	ss AGGUAGAAGAAGGAGAUAdTdT as ACAACAUUCAUAUAGCUGCdTdT
ALDH1A3	siALDH1A3 #1	ss AGGAAAUGGCAGAGAACUAdTdT as UAGUUCUCUGCCAUUUCCUdTdT
ALDH1A3	siALDH1A3 #2	ss UCGUGGAGGAGCAGGUCUAdTdT as UAGACCUGCUCCUCCACGAdTdT
Scrambled siRNA pool	siSCR	ss GCAGCUAUAUGAAUGUUGUdTdT as ACAACAUUCAUAUAGCUGCdTdT ss UGCGCUAGGCCUCGGUUGCdTdT as GCAACCGAGGCCUAGCGCAdTdT
AR	siAR	ss CCAAAGGGCUAGAAGGCGAdTdT as UCGCCUUCUAGCCCUUUGGdTdT ss AUUGAUAAAUUCCGAAGGAdTdT as UCCUUCGGAAUUUAUCAAUdTdT
CTNNB1	siCTNNB1	ss GGGUACGAGCUGCUAUGUUDdTdT as AACAUAGCAGCUCGUACCCdTdT ss GGUGGUGGUUAAUAAGGCUdTdT as AGCCUUAUUAACCACCACCDdTdT

Primers used for RT-qPCR

Gene	Sequence (5'→3')
ACTB	F 5'- ATGGAGTCCTGTGGCATCCA -3' R 5'- AGTACTTGCGCTCAGGAGGA -3'
RPLP0	F 5'- CTCAACATCTCCCCCTTCTCCTT -3' R 5'- TGATGCAACAGTTGGGTAGCC -3'
ALDH1A1	F 5'- GAATGGCATGATTCAGTGAGTGG-3' R 5'- CAGCCAACCTTGTATAATAGTCG-3'
ALDH1A3	F 5'- TCTCGACAAAGCCCTGAAGT-3'

AR	R 5'- TATTCGGCCAAAGCGTATTC -3' F 5'- CATCTTGTCGTCTTCGGAAATGTTA -3'
CTNNB1	R 5'- GAAGCCTCTCCTTCCTCCTGTAGTT -3' F 5'- ATTTGATGGAGTTGGACATGGC-3' R 5'- CCAGCTACTTGTTCTTGAGTGAAGG -3'
SMAD3	F 5'- GCGTGCGGGTCTACTACATC -3' R 5'- GCACATTCGGGTCAACTGGTA -3'
TGFB1	F 5'- CAGCAGGGATAACACACTGC -3' R 5'- CACGCAGCAGTTCTTCTCC -3'
Mouse/rat Gapdh	F 5'- TTCAACGGCACAGTCAAGG -3' R 5'- ACATACTCAGCACCAGCATCAC -3'
Mouse Aldh1a1	F 5'- AACTTGTTCGGATTTAGGAGGCT-3' R 5'- GGGCCTATCTTCCAAATGAACA -3'
Mouse Aldh1a3	F 5'- GGGTCACACTGGAGCTAGGA -3' R 5'- CTGGCCTCTTCTTGGCGAA -3'

Antibodies used for Immunofluorescence

Antibody	Dilution and host	Vendor and catalogue number
Endomucin	1:200, Goat	Thermo Fisher Scientific, #PA5-47648
GFP Tag	1:200, Rabbit	Thermo Fisher Scientific, #A6455
Anti-Goat IgG (Alexa Fluor 555)	1:350, Donkey	
Anti-Rabbit IgG (Alexa Fluor 488)	1:350, Donkey	

Supplementary Figures:

Figure S1. ALDH1A1 and ALDH1A3 as regulators of radiosensitivity.

Figure S2. Correlation analysis of ALDH1A1 or ALDH1A3 expression in the TCGA PRAD dataset with RT2 signatures.

Figure S3. qPCR validation of 84 extracellular matrix and adhesion genes analysed using DNA RT² Profiler PCR Array.

Figure S4. Disease-free survival of prostate cancer patients with high or low ALDH1A1 and ALDH1A3 expression.

Figure S5. Survival and extravasation potential of color-coded PC3 cells.

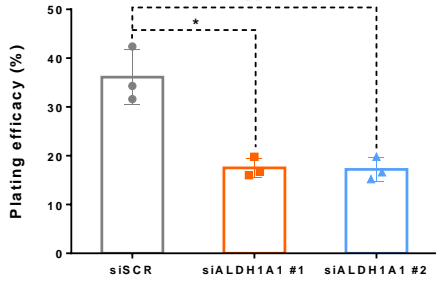
Figure S6. Comparison of the iPSA serum level and nuclear AR between tumors with or without ALDH1A1 or ALDH1A3 expression.

Figure S7. Analysis of the osteogenesis-related genes.

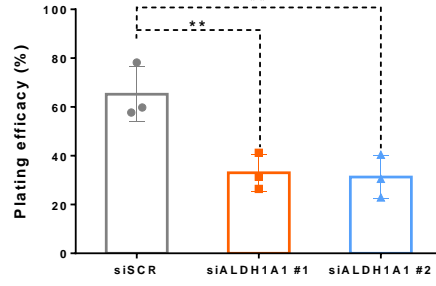
Figure S8. Interconnection of ALDH genes with MMPs.

Supplementary Figure S1

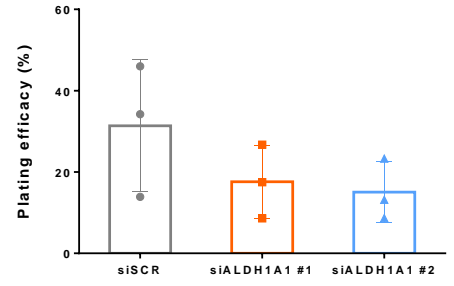
LNCaP



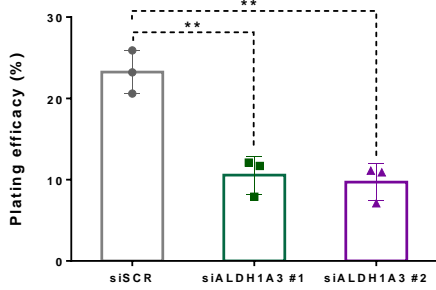
C42B



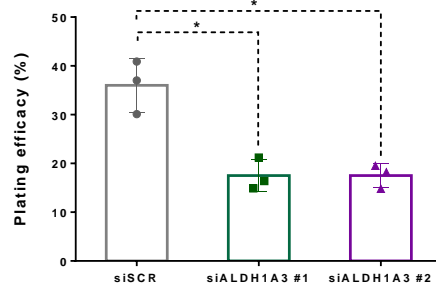
PC3



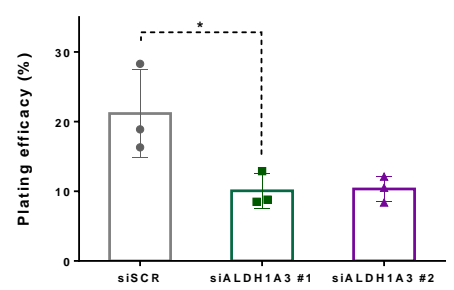
LNCaP



C42B

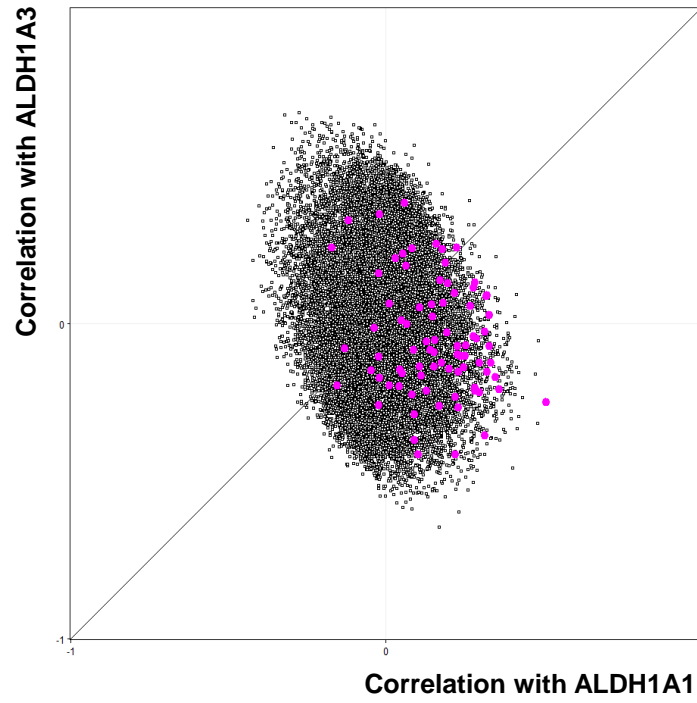


PC3

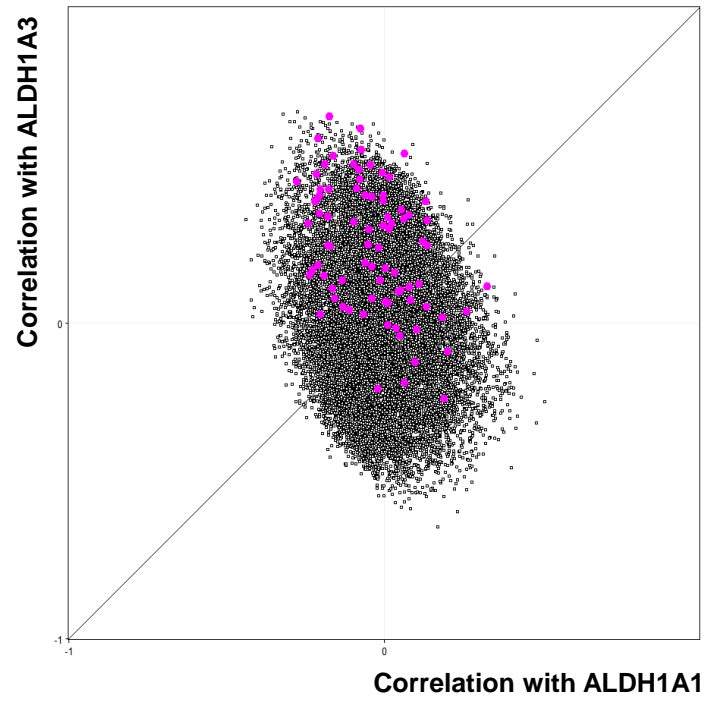


Supplementary Figure S2

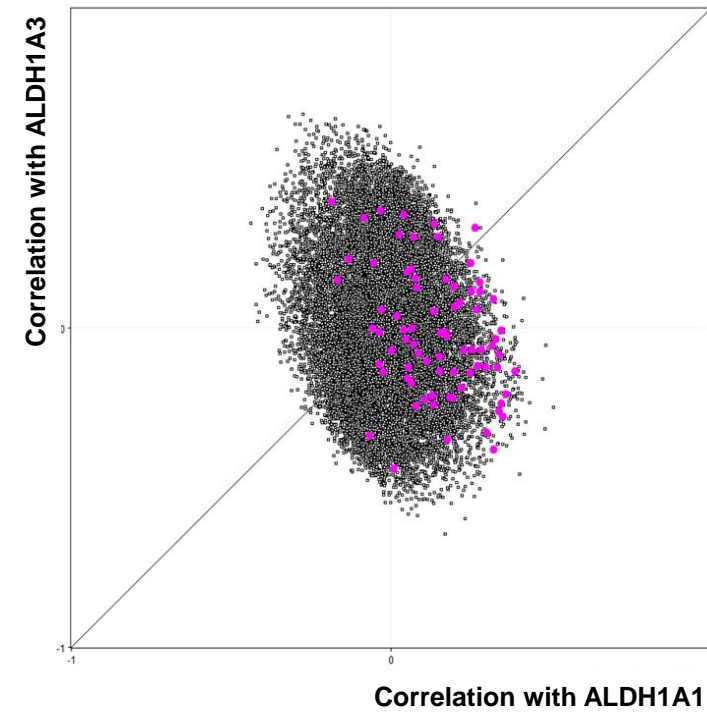
Extracellular Matrix and Adhesion Molecules



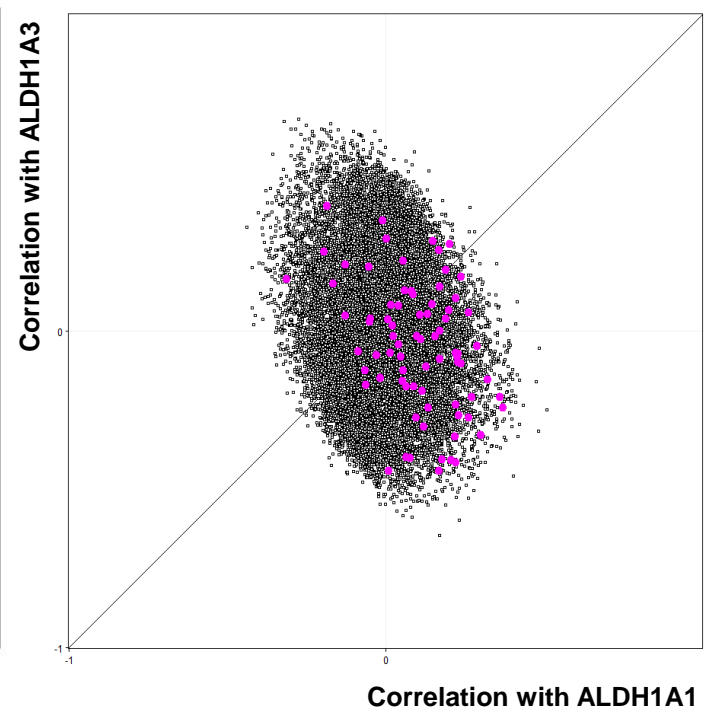
Androgen Receptor Signaling



Osteogenesis



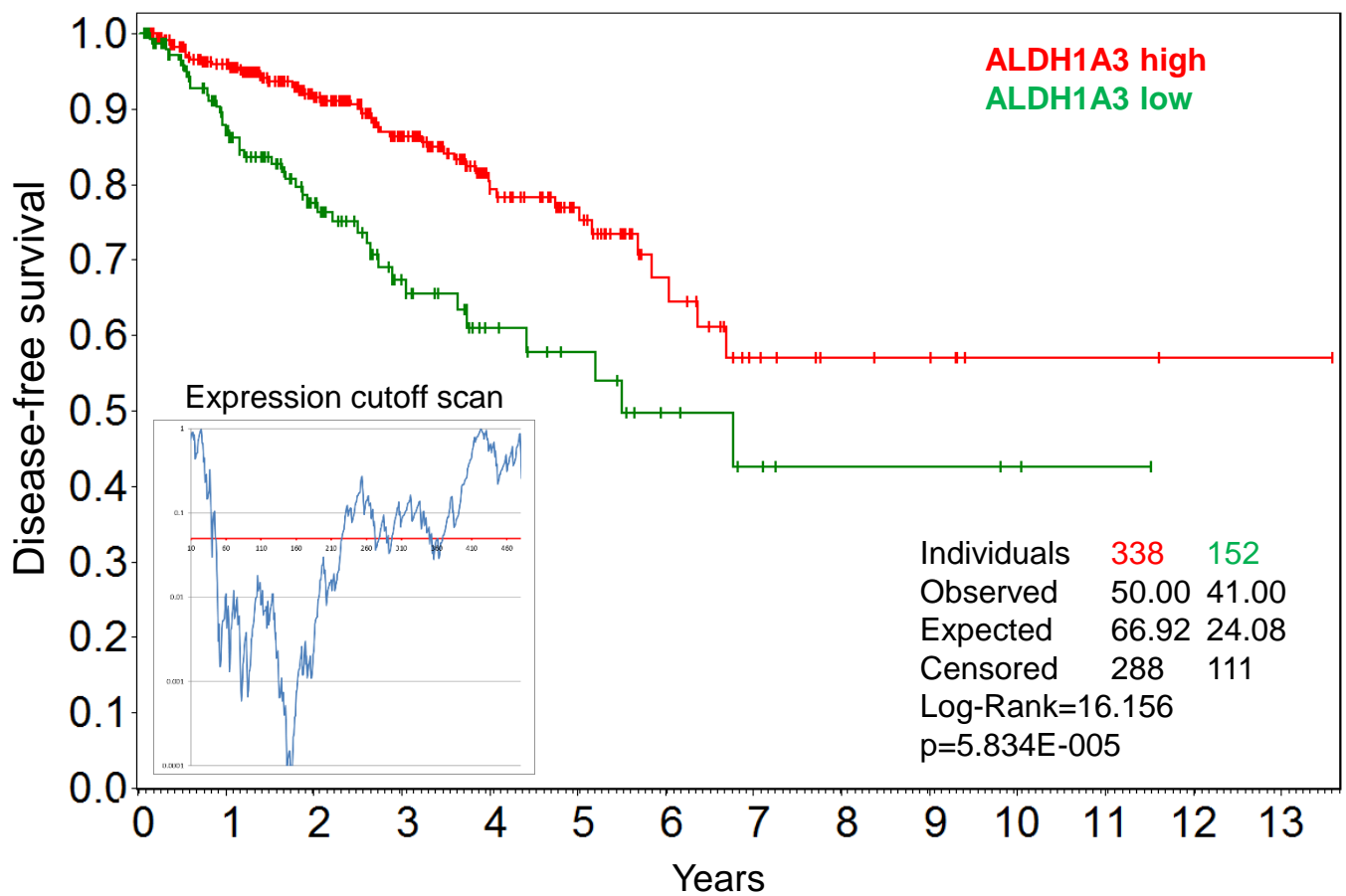
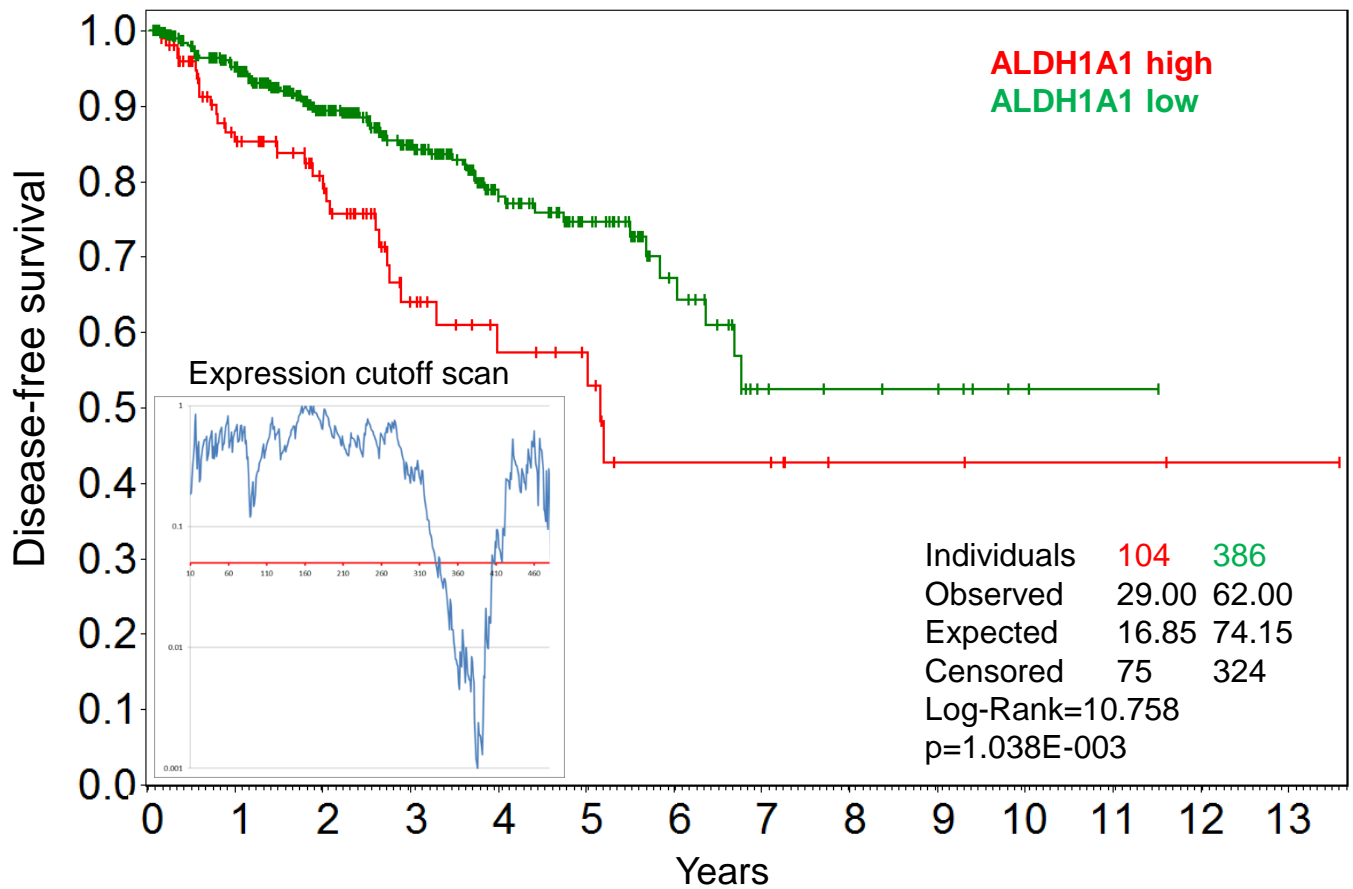
Angiogenesis



Supplementary Figure S3

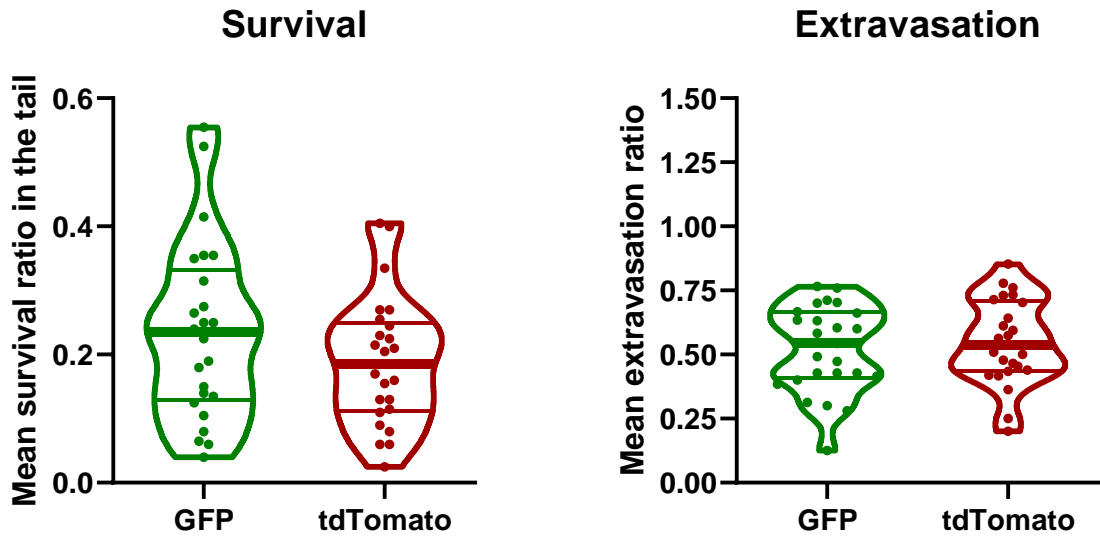
	LNCaP				PC3	
	siALDH1A1	siALDH1A3			siALDH1A1	siALDH1A3
ITGA8	3,4457424	13,176562		ADAMTS1	2,5456312	1,2379519
ITGA1	3,3190363	2,5670124		ADAMTS13	2,4899838	1,7253882
SELE	2,8402345	1,9934559		COL7A1	2,4816593	3,2576491
LAMB1	1,8026658	1,7948206		COL16A1	2,4191095	3,4794358
MMP8	1,7800653	1,8855528		VTN	1,9934757	0,9320517
COL14A1	1,6748544	3,5283427		ITGAM	1,9313438	1,3742763
VTN	1,6677947	1,6008062		COL14A1	1,91886	1,7008488
ITGB3	1,597696	2,1582947		VCAN	1,9041017	0,9428615
MMP3	1,5931042	0,7725707		COL12A1	1,7694796	1,2398371
MMP10	1,5807678	0,3869222		LAMB1	1,7525492	1,0384546
MMP9	1,4836756	0,9496065		ITGA7	1,6752365	1,5290232
ITGA2	1,3291003	0,6567818		LAMA3	1,6398663	1,1025826
PECAM1	1,2995651	1,7689802		LAMA1	1,553781	0,3770434
ICAM1	1,2680028	1,0782503		MMP7	1,5237628	0,733172
COL4A2	1,2524879	1,2067284		TIMP2	1,5073309	0,5509773
THBS3	1,1880757	0,8636981		THBS3	1,4554457	1,4294116
SPG7	1,1627652	1,9019644		ICAM1	1,4349864	1,1119441
COL7A1	1,1585684	1,2383869		MMP12	1,4180188	1,7192085
ITGB1	1,1448859	1,14006		ITGAV	1,4154902	1,4296441
ADAMTS13	1,1315893	1,1552432		VCAM1	1,4075145	3,412206
MMP13	1,122938	0,6891376		LAMC1	1,3905187	1,1085608
TGFBI	1,0978999	0,8807619		MMP15	1,3893775	0,7165384
COL1A1	1,0977011	0,9863823		THBS2	1,3845623	1,6625503
ITGA4	1,0878769	0,2124813		SPG7	1,3782269	1,009498
LAMC1	1,0401072	1,0576065		SELE	1,33	0,7924925
TIMP2	1,034769	0,7548247		TIMP1	1,3329303	0,6703062
LAMA3	1,0317555	1,8088347		ITGA2	1,3112203	0,8404221
ADAMTS8	1,0021507	1,4579233		HAS1	1,2904627	0,9911907
ITGAV	0,9775376	1,0227054		MMP16	1,2864494	1,5395976
NCAM1	0,9751716	1,1230701		CLEC38	1,2562167	1,2960533
COL6A2	0,9688324	1,1562884		ITGA5	1,2492804	1,3850067
ITGA7	0,9675012	0,851476		ECM1	1,1973863	2,011028
COL16A1	0,9626171	1,1075554		SGCE	1,1905576	0,8772027
MMP12	0,9606135	0,8372479		CTNNB1	1,161265	1,0290504
CDH1	0,9400942	1,0851935		ITGB4	1,1529279	1,3502799
MMP16	0,9373515	2,9326135		ADAMTS8	1,1280559	1,2660885
ADAMTS1	0,9283781	1,147903		ITGA4	1,0983899	1,6256578
MMP15	0,9188098	1,2483633		COL1A1	1,090569	2,1716679
CTNNB1	0,9066081	0,9445517		CCN2	1,0466456	0,6457103
COL12A1	0,8844819	1,4549272		COL4A2	1,0433044	0,8014654
ITGAL	0,8809342	0,8944822		COL5A1	1,0384563	0,7542075
THBS1	0,8725112	2,3957273		ITGB1	1,0339793	0,9750346
ECM1	0,869554	1,8347866		LAMA2	1,0326569	1,283846
ITGA5	0,8644463	1,0651375		CNTN1	1,0259969	1,6086714
MMP11	0,8582322	0,7557293		COL6A1	1,0210848	1,4432137
HAS1	0,8576526	0,7661647		SELL	1,01	1,4612911
LAMB3	0,8434573	2,437513		MMP14	1,0027862	0,6310447
CTNND1	0,8338764	1,1585378		CTNND1	0,9969843	0,9894442
ITGA6	0,8330478	0,9970363		ITGA3	0,9852726	1,5170697
SGCE	0,8213126	0,9780573		CCNNA1	0,9794878	1,7519162
CCNNA1	0,8209316	1,0206037		MMP9	0,968892	0,9657469
ITGB5	0,8047896	0,7803178		MMP11	0,9649789	1,5369416
LAMA2	0,8009228	0,9926408		TGFBI	0,9321974	0,8030554
COL6A1	0,7879602	1,0667271		COL6A2	0,9293094	0,9736498
MMP7	0,7840893	0,7566001		ITGB5	0,9088361	0,6444732
MMP1	0,7831983	0,6783706		SPP1	0,8934002	0,460759
ITGA3	0,7761631	1,7270719		CDH1	0,8517873	5,0105759
FN1	0,7647732	1,0024687		MMP3	0,8382109	0,4833455
COL5A1	0,7641728	0,7017601		ITGA6	0,8282865	1,1035918
ITGB4	0,6895869	1,0211152		ITGB3	0,8094661	0,3686243
LAMA1	0,6640285	0,4835741		MMP10	0,7854759	0,4220869
ITGB2	0,6415707	1,0311173		THBS1	0,7723966	1,8800374
SELL	0,6392187	1,3916102		CD44	0,7711538	1,2881271
TIMP3	0,6117086	1,272633		FN1	0,7458408	0,686763
CLEC38	0,5540223	0,7528494		TNC	0,720417	0,6379045
CCN2	0,4157619	0,6769479		ITGA1	0,7046165	1,0064237
TNC	0,4044734	0,7966553		ITGB2	0,6361033	1,3870517
SPP1	0,336207	0,3857541		CTNND2	0,6086106	0,8425904
SPARC	0,2264424	0,606537		ANOS1	0,4683601	0,6615725
				MMP13	0,4479699	0,518483
				COL8A1	0,3564923	1,4662142
				MMP1	0,3429869	0,3899497
				PECAM1	0,33	0,6189817
				SPARC	0,2649995	0,5830115

Supplementary Figure S4

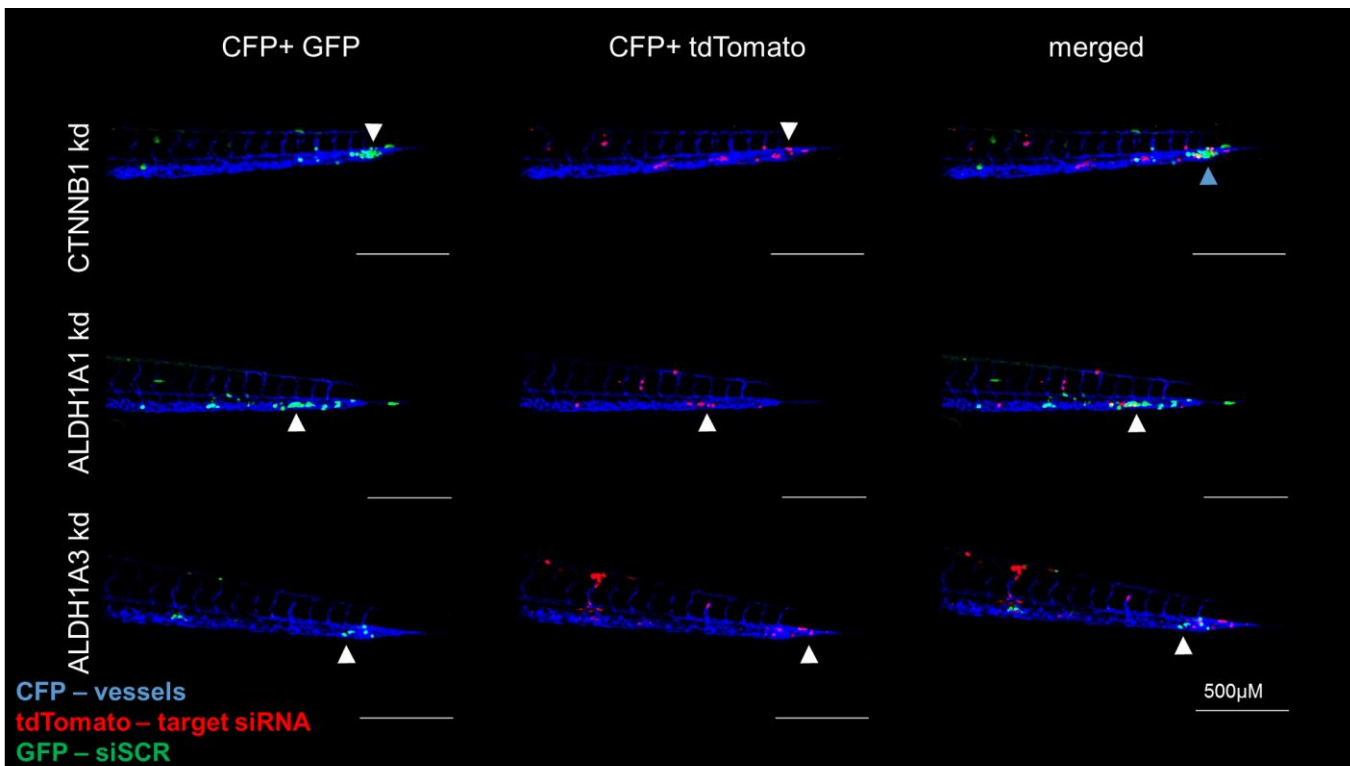


Supplementary Figure S5

A

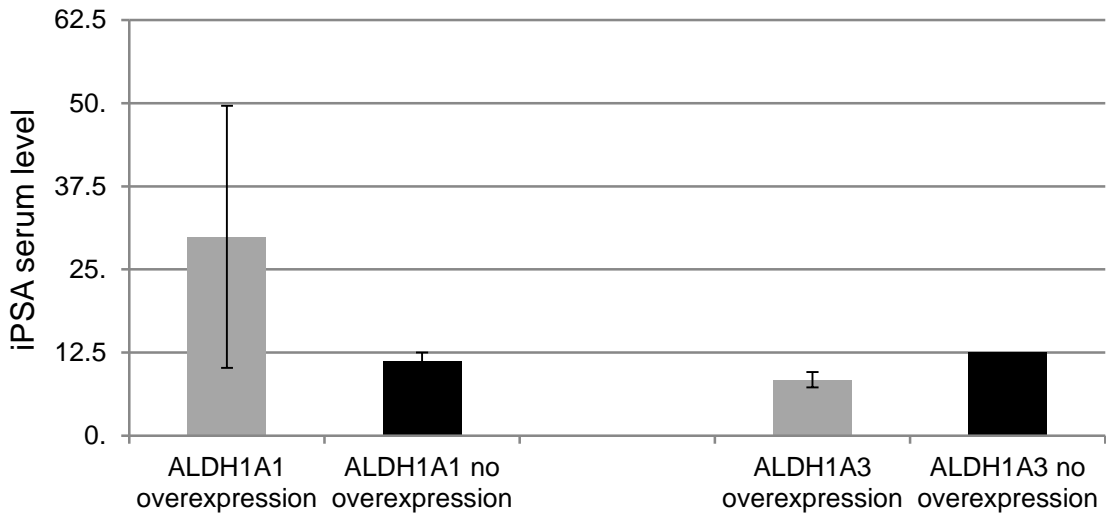


B

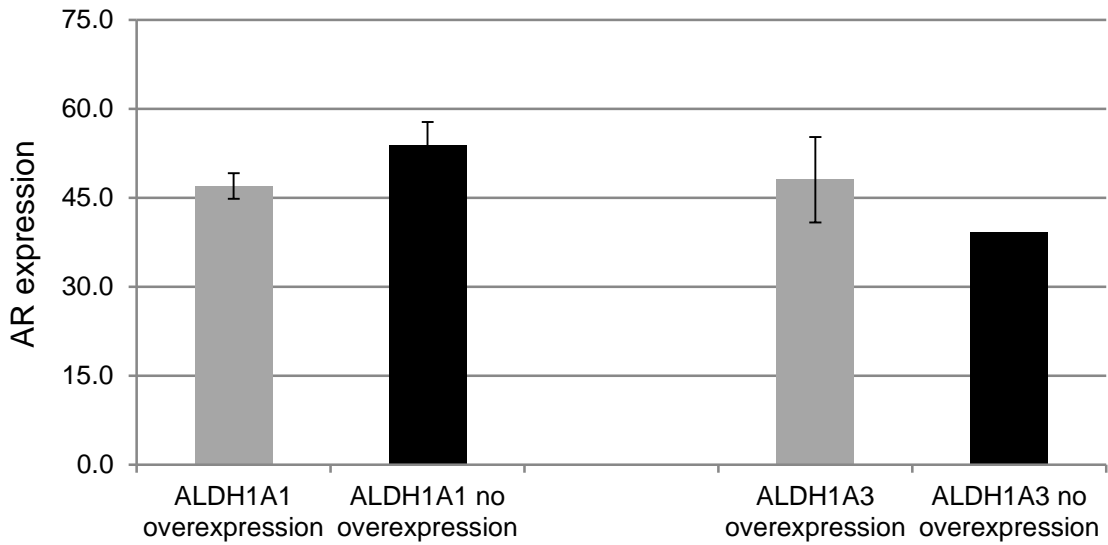


Supplementary Figure S6

A

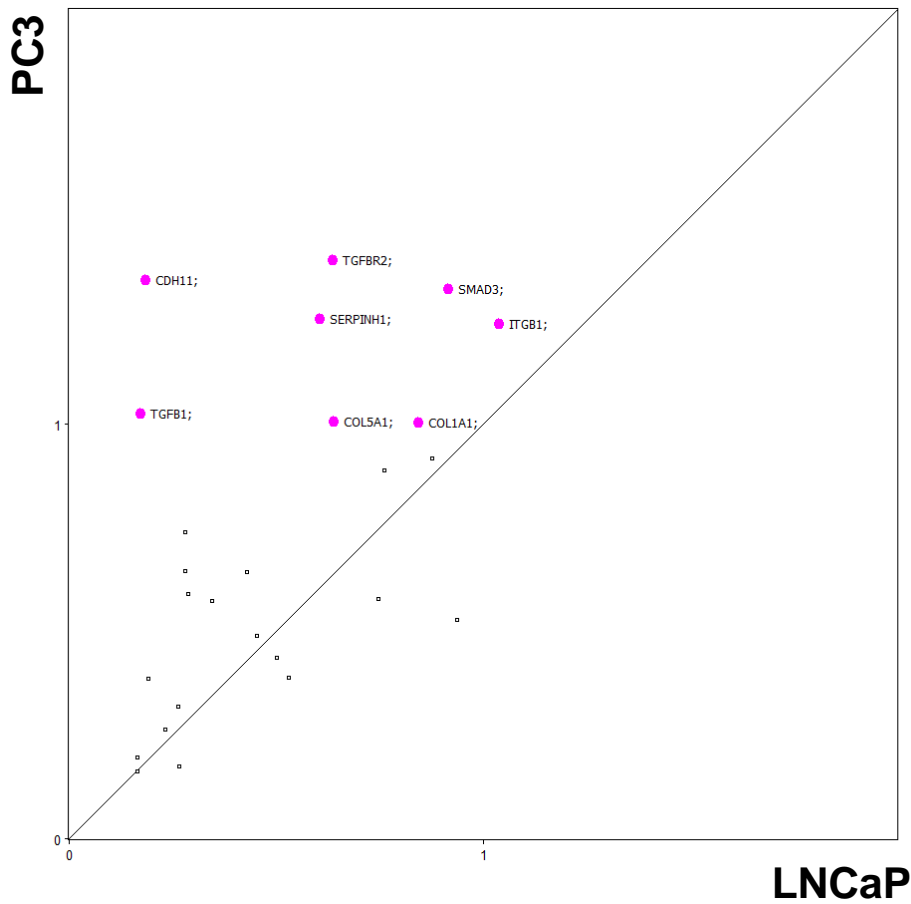


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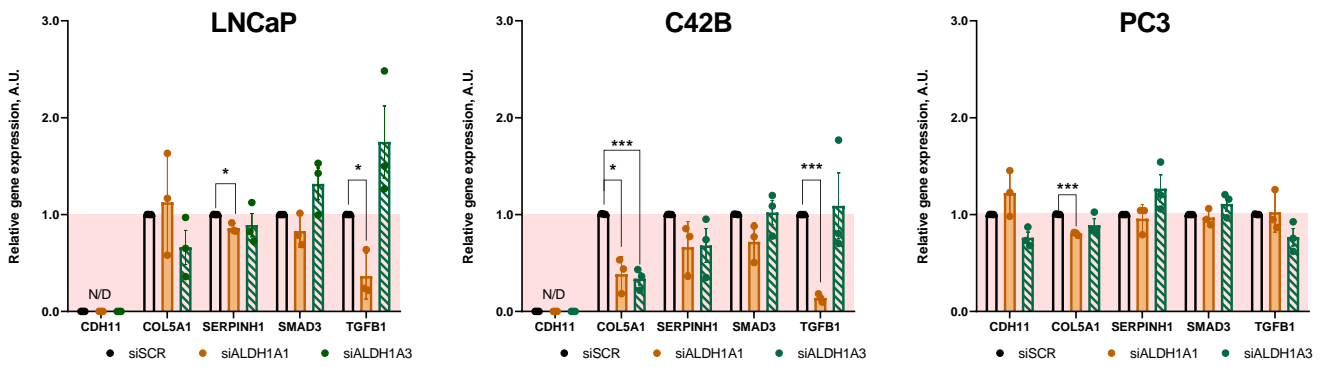


Supplementary Figure S7

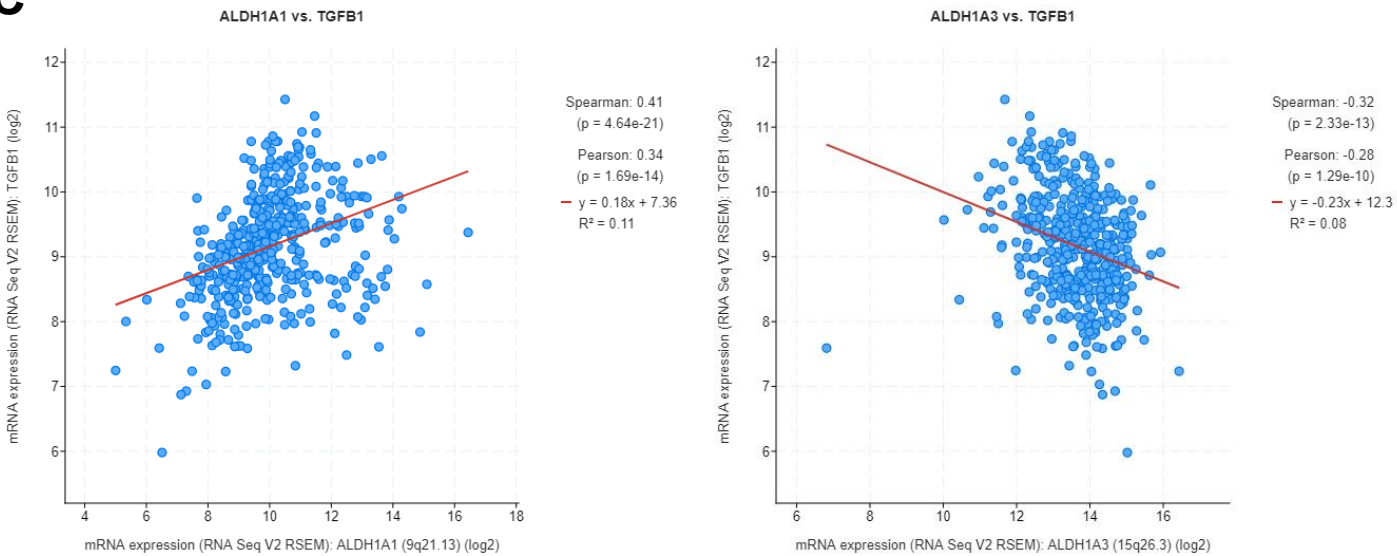
A



B



C

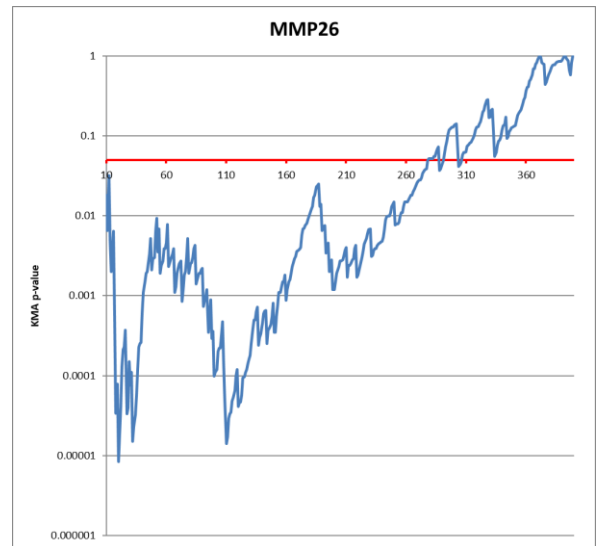
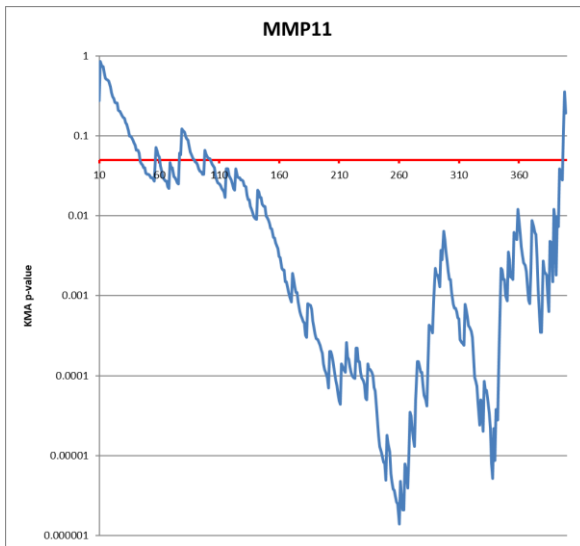


Supplementary Figure S8

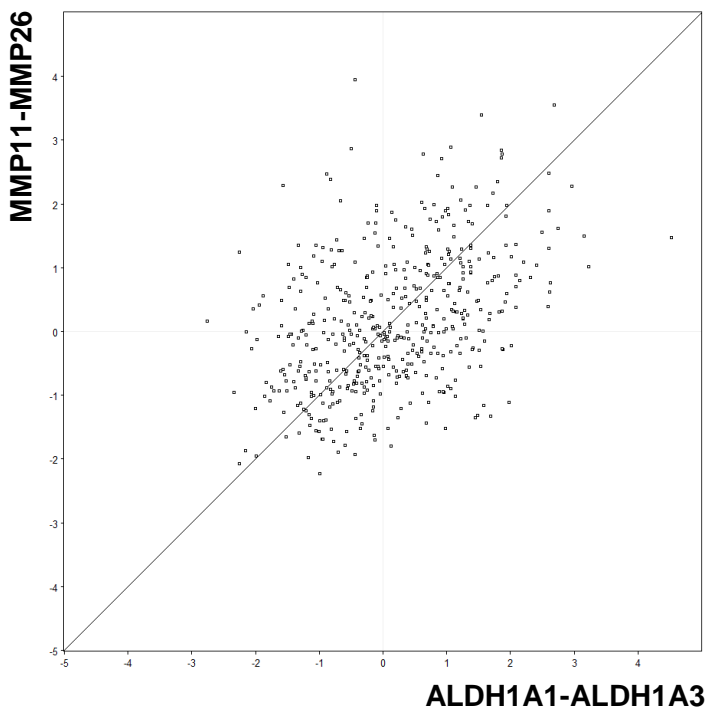
A

LNCaP	ALDH1A1kd	ALDH1A3kd	PC3	ALDH1A1kd	ALDH1A3kd
MMP1	0,783198275	0,678370572	MMP1	0,3429869	0,38994966
MMP7	0,784089265	0,756600085	MMP13	0,44796986	0,51848302
MMP11	0,858232194	0,755729342	MMP10	0,78547594	0,42208688
MMP15	0,918809791	1,248363293	MMP3	0,83821089	0,48334545
MMP16	0,937351456	2,932613542	MMP11	0,9649789	1,5369416
MMP12	0,960613538	0,837247884	MMP9	0,96889195	0,9657469
MMP13	1,122938011	0,689137573	MMP14	1,00278618	0,63104465
MMP9	1,483675634	0,949606482	MMP16	1,28644942	1,53959762
MMP10	1,580767792	0,386922226	MMP15	1,38937753	0,7165384
MMP3	1,593104239	0,772570745	MMP12	1,41801883	1,71920845
MMP8	1,780065293	1,885552818	MMP7	1,52376282	0,73317204

B



C



Pearson correlation coefficient:
 ALDH1A1-ALDH1A3 / MMP11-MMP26;
 n=490;r=0.42670; p=4.23E-023