Lrf suppresses prostate cancer through repression of a Sox9-dependent pathway for cellular senescence bypass and tumor invasion

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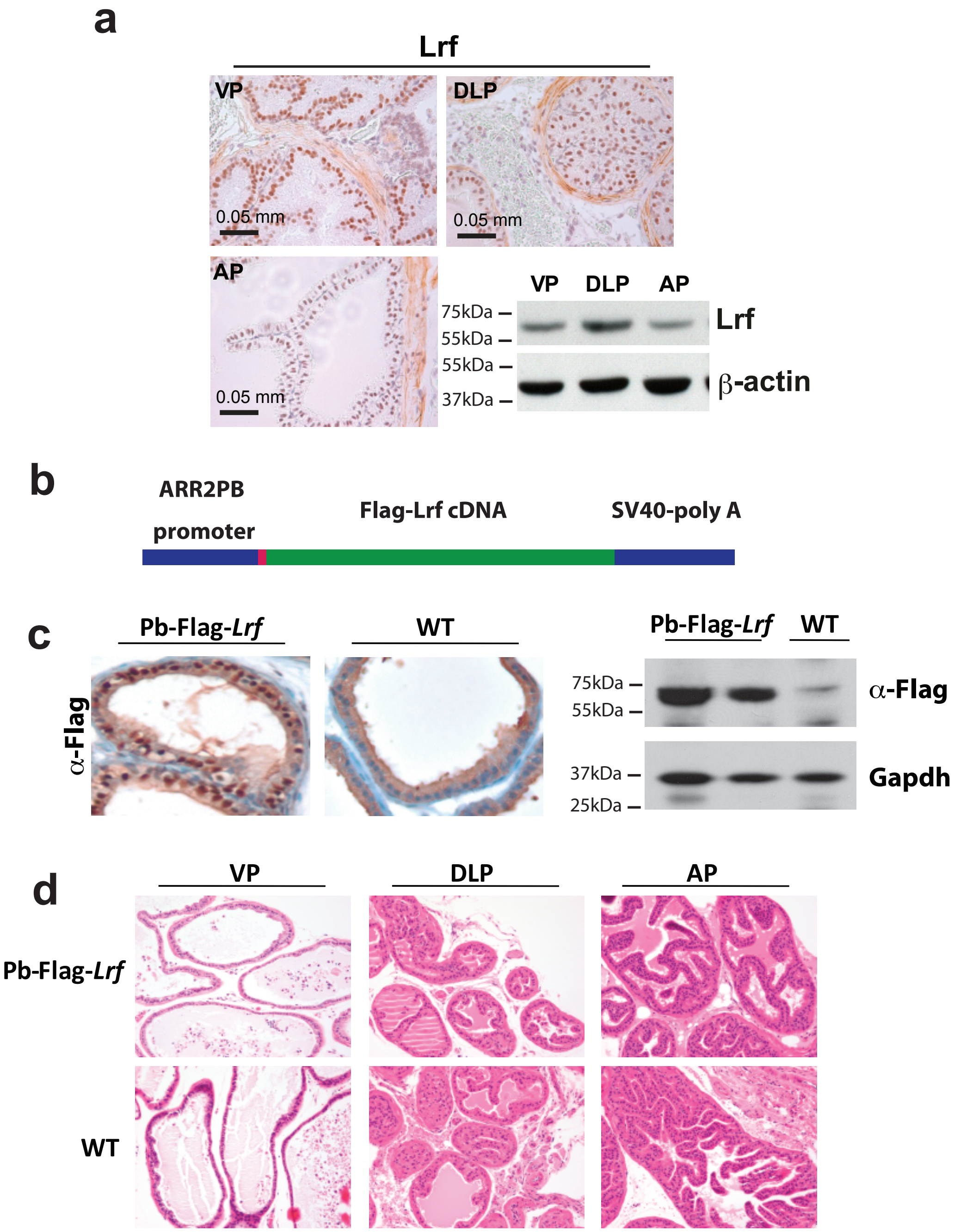
Supplementary Information:

Supplementary Figures: 7

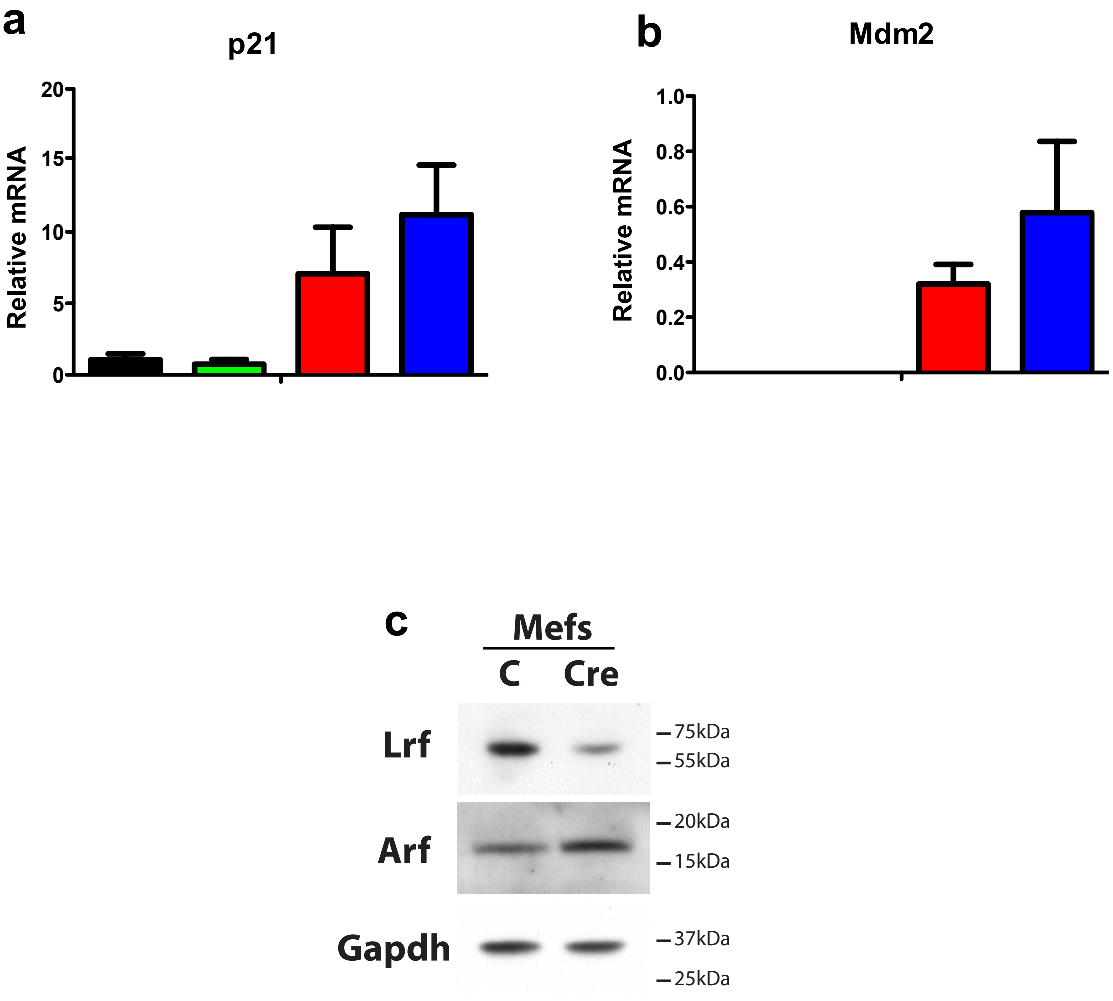
Supplementary Tables: 8



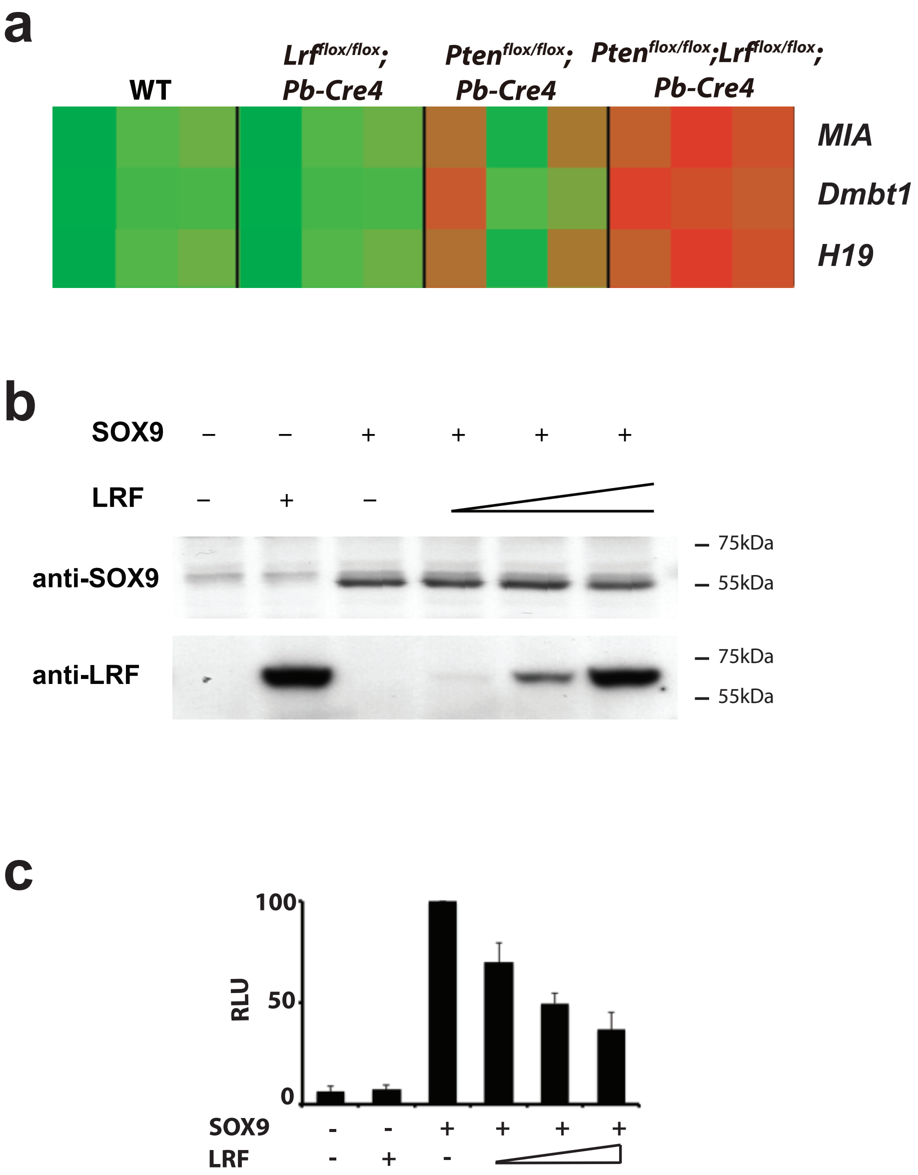
e

18-19 months	Hyperplasia	PIN
WT	1/20	0/20
Flag-Lrf	1/17	0/17

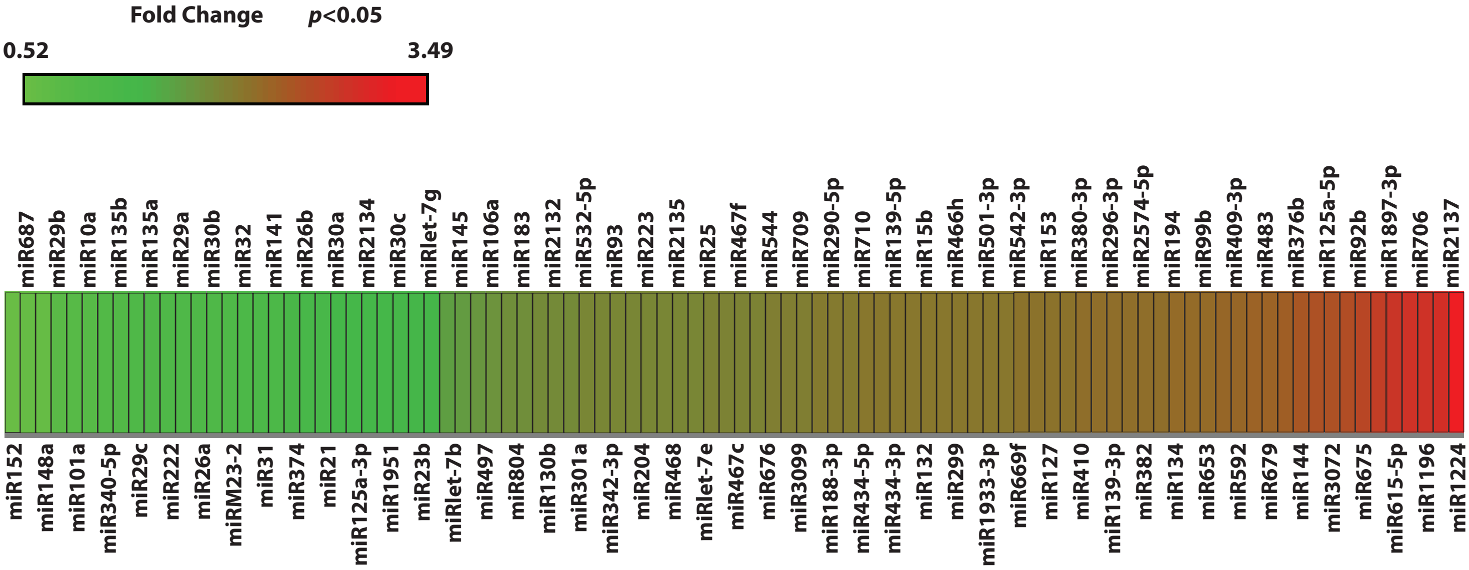
Suppl. Figure 1. Lrf transgenic mice show no signs of prostate neoplasia. (a) IHC and Western Blot analysis for Lrf expression in the three prostate lobes of 12 week-old WT mice. **(b)** Schematic representation of ARR2PB-Lrf construct. **(c)** IHC and Western Blot analysis for Flag-Lrf expression in the three prostate lobes of 16-18 month-old transgenic mice. **(d)** H&E of Anterior, Ventral, and Dorso-Lateral prostate lobes of WT and Lrf transgenic 16-18 month-old mice. **(e)** Incidence of prostate hyperplasia and PIN in WT and Lrf transgenic 16-18 month-old mice.



Suppl. Figure 2. Loss of Lrf does not affect p53 functions. (a-b) qPCR analysis of p21 and Mdm2 expression in *WT* (black bars), *Lrf$^{flox/flox}$;Pb-Cre4* (green bars), *Pten$^{flox/flox}$;Pb-Cre4* (red bars), and *Pten$^{flox/flox}$;Lrf$^{flox/flox}$;Pb-Cre4* (blue bars) prostates of n=3/genotype 12 week-old mice. Data are presented as mean ± standard deviation. **(c)** WB analysis for Lrf and p19Arf in *Lrf$^{flox/flox}$* mouse embryonic fibroblasts (MEFs) infected by Cre-recombinase or control vector.

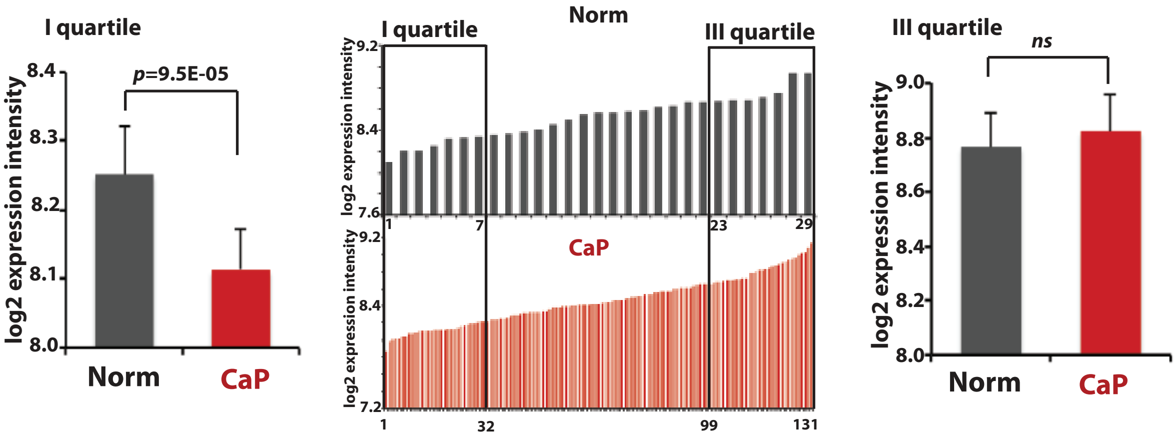


Suppl. Figure 3. Loss of Lrf dictates Sox9 transcriptional hyper-activity. (a) Heat-map of three different Sox9 transcriptional target genes (Mia, Dmbt1, and H19) in *WT*, *Lrf*$^{flox/flox}$*;Pb-Cre4*, *Pten*$^{flox/flox}$*;Pb-Cre4*, and *Pten*$^{flox/flox}$*;Lrf*$^{flox/flox}$*;Pb-Cre4* prostates of n=3/genotype 12 week-old mice. **(b)** WB analysis of SOX9 and LRF over-expression for the luciferase assay in PC3.
(c) Dual-luciferase assay with a Sox9-reporter in DU145 cells shows that LRF suppresses the transcriptional activity of SOX9 in a dose-dependent manner.

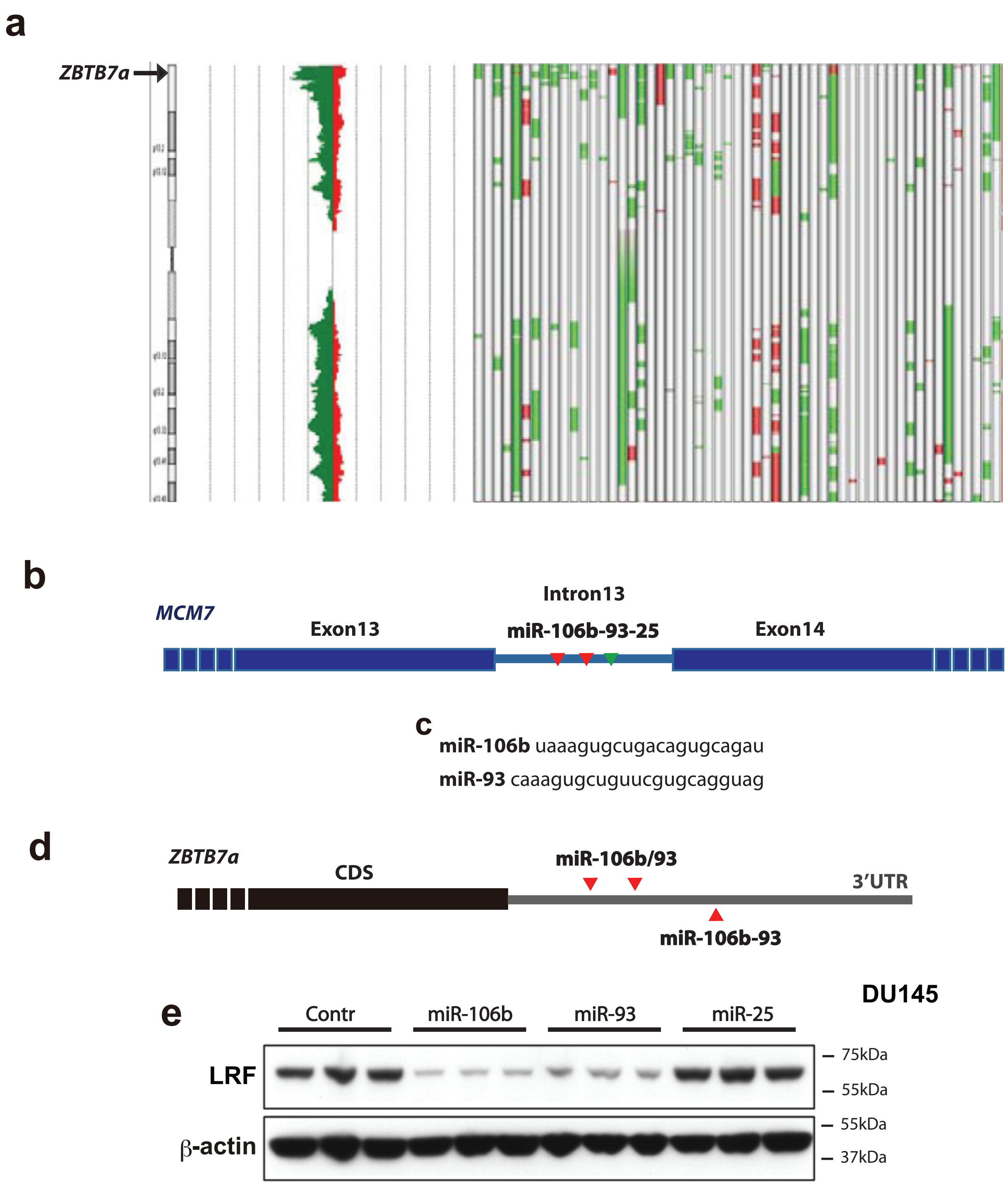


Suppl. Figure 4. microRNA profiling. Nanostring nCounter® Mouse miRNA Expression Assay Kit technology was used to analyze the miRNAs differentially expressed ($p<0.05$) in *Pten*$^{flox/flox}$*;Lrf*$^{flox/flox}$*;Pb-Cre4* versus *Pten*$^{flox/flox}$*;Pb-Cre4* prostates of n=3/genotype 12 week-old mice.

Wang *et al.* Suppl. Fig. 5



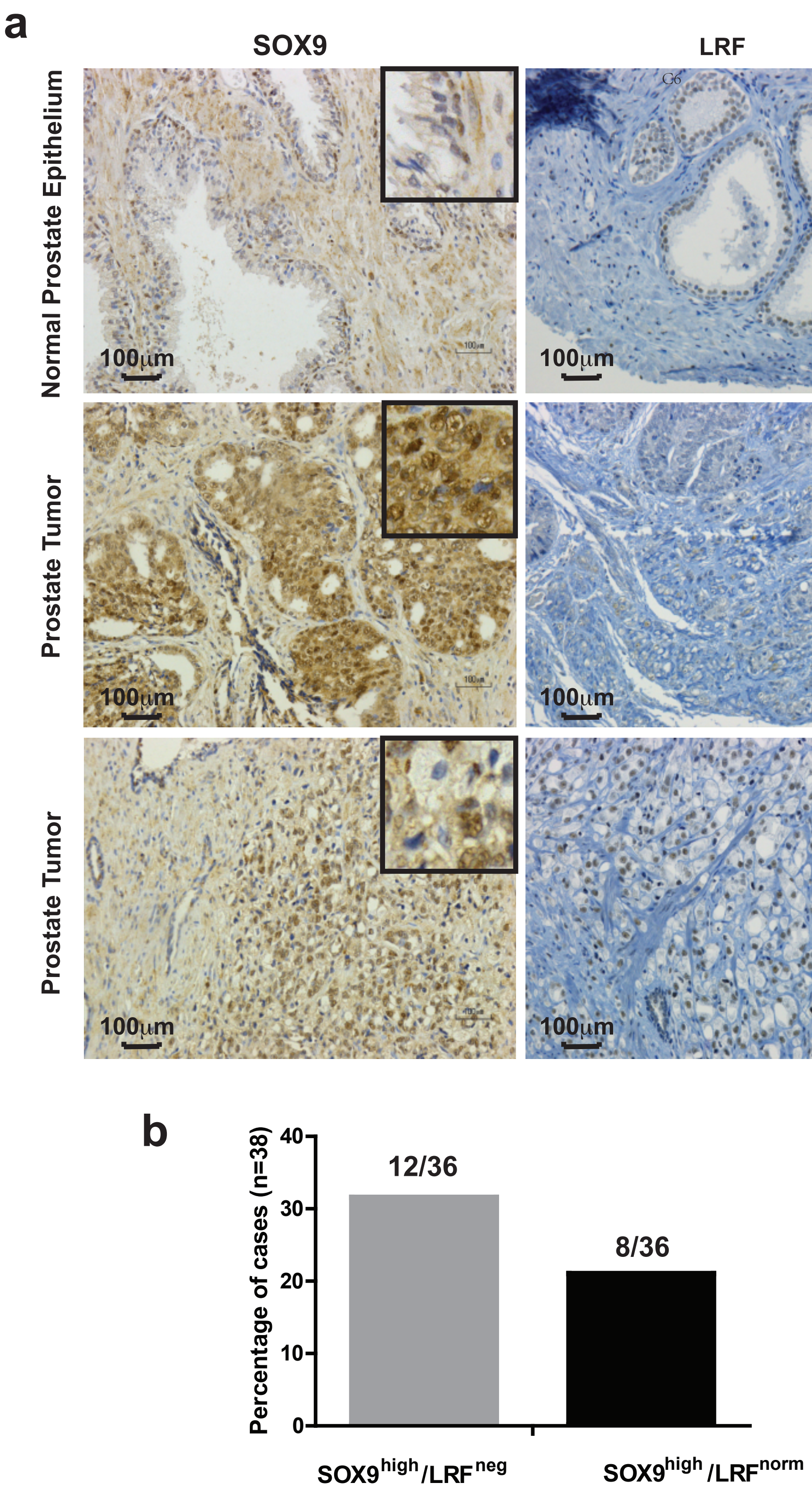
Suppl. Figure 5. LRF mRNA is significantly down-regulated in a subset of primary human prostate cancers (Oncomine).



miR-106b uaaagugcugacagugcagau

miR-93 caaagugcuguucgugcagguag

Suppl. Figure 6. LRF downregulation by the oncogenic miR106b~25 cluster. (a) aCGH analysis showing genetic loss of LRF in 18% of advanced human prostate cancer. **(b)** Schematic representation of miR-106b~25 cluster inside the Intron 13 of the MCM7 gene. **(c)** miR-106b and miR-93 sequences. **(d)** Schematic representation of ZBTB7a RNA with the miR-106b/93 target sites highlighted (red triangles) in the 3'UTR. **(e)** Western blot analysis showing reduction in the levels of LRF in DU145 prostate cell lines transfected by miR-106b, miR-93, and miR-25.



Suppl. Figure 7. Immunohistochemical staining for LRF and SOX9 in high Gleason score (8-10) prostate tumors. **(a)** Representative image of immunohistochemical staining of LRF and SOX9 in human prostate tumors. **(b)** Percentage of SOX9hi/LRFnorm and SOX9hi/LRFneg in high Gleason score (8-10) prostate cancer (n=38).

Suppl. Table 2: oPOSSUM analysis

a

TF	Background gene hits	Background gene non-hits	Target gene hits	Target gene non-hits	Background TFBS hits	Background TFBS rate	Target TFBS hits	Target TFBS rate	Z score	Fisher score	Fisher
HNF1A	2552	12598	93	287	3659	0.0022	146	0.003	13.43	1.199e-04	1.20E-04
Foxa2	8526	6624	242	138	29104	0.0152	908	0.0159	5.253	2.231e-03	2.23E-03
HLF	5181	9969	168	212	9448	0.0049	305	0.0054	5.095	4.129e-05	4.13E-05
ELF5	12185	2965	329	51	73478	0.0287	2250	0.0296	4.518	1.177e-03	1.18E-03
SRY	10985	4165	298	82	68971	0.0269	2111	0.0278	4.306	5.456e-03	5.46E-03
SOX9*	8854	6296	250	130	29087	0.0114	904	0.0119	4.181	2.225e-03	2.23E-03
Ar	585	14565	19	361	619	0.0006	22	0.0007	3.948	1.582e-01	1.58E-01
REL	7798	7352	232	148	18677	0.0081	583	0.0085	3.866	1.281e-04	1.28E-04
SRF	713	14437	23	357	793	0.0004	28	0.0005	3.16	1.372e-01	1.37E-01
Foxq1	5673	9477	166	214	12614	0.006	392	0.0063	3.027	8.009e-03	8.01E-03

b

TF	Background gene hits	Background gene non-hits	Target gene hits	Target gene non-hits	Background TFBS hits	Background TFBS rate	Target TFBS hits	Target TFBS rate	Z score	Fisher score	Fisher
SRF	713	14437	29	248	793	0.0004	46	0.001	20.17	7.083e-05	7.08E-05
TAL1-TCF3	5459	9691	132	145	10058	0.0052	293	0.0061	8.706	5.608e-05	5.61E-05
Roaz	4281	10869	110	167	7214	0.0047	205	0.0053	6.756	3.172e-05	3.17E-05
Sox5	11256	3894	226	51	73449	0.0223	1954	0.0236	6.584	2.827e-03	2.83E-03
Ar	585	14565	20	257	619	0.0006	21	0.0008	6.422	6.531e-03	6.53E-03
TLX1-NFIC	811	14339	26	251	879	0.0005	29	0.0007	5.449	4.646e-03	4.65E-03
Pax6	1024	14126	24	253	1165	0.0007	36	0.0009	4.597	1.310e-01	1.31E-01
Hand1-Tcfe2a	10659	4491	225	52	44947	0.0195	1179	0.0203	4.556	2.877e-05	2.88E-05
Foxq1	5673	9477	120	157	12614	0.006	341	0.0065	4.399	2.701e-02	2.70E-02
NHLH1	4534	10616	117	160	7563	0.0039	207	0.0043	4.191	1.125e-05	1.13E-05

Suppl. Table 3: Ingenuity pathway analysis

a

Top Canonical Pathways

Name	p-value	Ratio
Virus Entry via Endocytic Pathways	5.53E-07	16/99 (0.162)
ILK Signaling	2E-06	23/192 (0.12)
LPS/IL-1 Mediated Inhibition of RXR Function	1.1E-05	24/239 (0.1)
Tight Junction Signaling	1.56E-05	19/161 (0.118)
Integrin Signaling	1.75E-05	22/207 (0.106)

b

Top Networks

ID	Associated Network Functions	Score
1	Cancer, Cellular Development, Tumor Morphology	43
2	Post-Translational Modification, Cellular Function and Maintenance, Skeletal and Muscular System Development and Function	36
3	Drug Metabolism, Protein Synthesis, Glutathione Depletion In Liver	36
4	Neurological Disease, Psychological Disorders, Carbohydrate Metabolism	36
5	Cellular Movement, Tissue Development, Nervous System Development and Function	36

c

Top Bio Functions

Diseases and Disorders	p-value	# Molecule
Cancer	1.40E-21 - 6.71E-03	311
Reproductive System Disease	5.16E-20 - 4.87E-03	173
Gastrointestinal Disease	4.44E-12 - 6.57E-03	155
Organismal Injury and Abnormalities	4.54E-05 - 6.05E-03	63
Hereditary Disorder	6.80E-05 - 5.36E-03	40

Molecular and Cellular Functions	p-value	# Molecule
Cellular Movement	5.16E-10 - 6.70E-03	151
Cell Death and Survival	6.82E-09 - 6.19E-03	222
Cellular Growth and Proliferation	6.13E-08 - 6.25E-03	235
Cellular Assembly and Organization	3.30E-07 - 6.57E-03	146
Cellular Function and Maintenance	3.30E-07 - 6.32E-03	144

Physiological System Development and Function	p-value	# Molecule
Organismal Development	5.43E-07 - 6.82E-03	151
Cardiovascular System Development and Function	8.69E-07 - 6.05E-03	133
Organ Morphology	2.11E-06 - 6.82E-03	124
Skeletal and Muscular System Development and Function	2.11E-06 - 6.82E-03	113
Tissue Morphology	2.39E-06 - 6.58E-03	165

d

Top Molecules

Log Ratio up-regulated		**Log Ratio down-regulated**	
Molecules	Exp. Value	Molecules	Exp. Value
SPINK1	↑6.009	XAF1*	↓-6.066
REG3A*	↑5.483	TNNC2	↓-3.197
REG1A	↑4.769	PRR9	↓-3.185
MIA	↑4.340	CPA3	↓-2.781
Muc2 (includes EG:17831)	↑4.334	MYH11	↓-2.479
CLCA1	↑4.333	INMT	↓-2.459
H19	↑4.246	PCP4	↓-2.431
ONECUT2*	↑4.087	SYNPO2*	↓-2.431
CXCL6	↑4.071	Defb1*	↓-2.408
KRT20	↑3.954	ACTA2*	↓-2.303

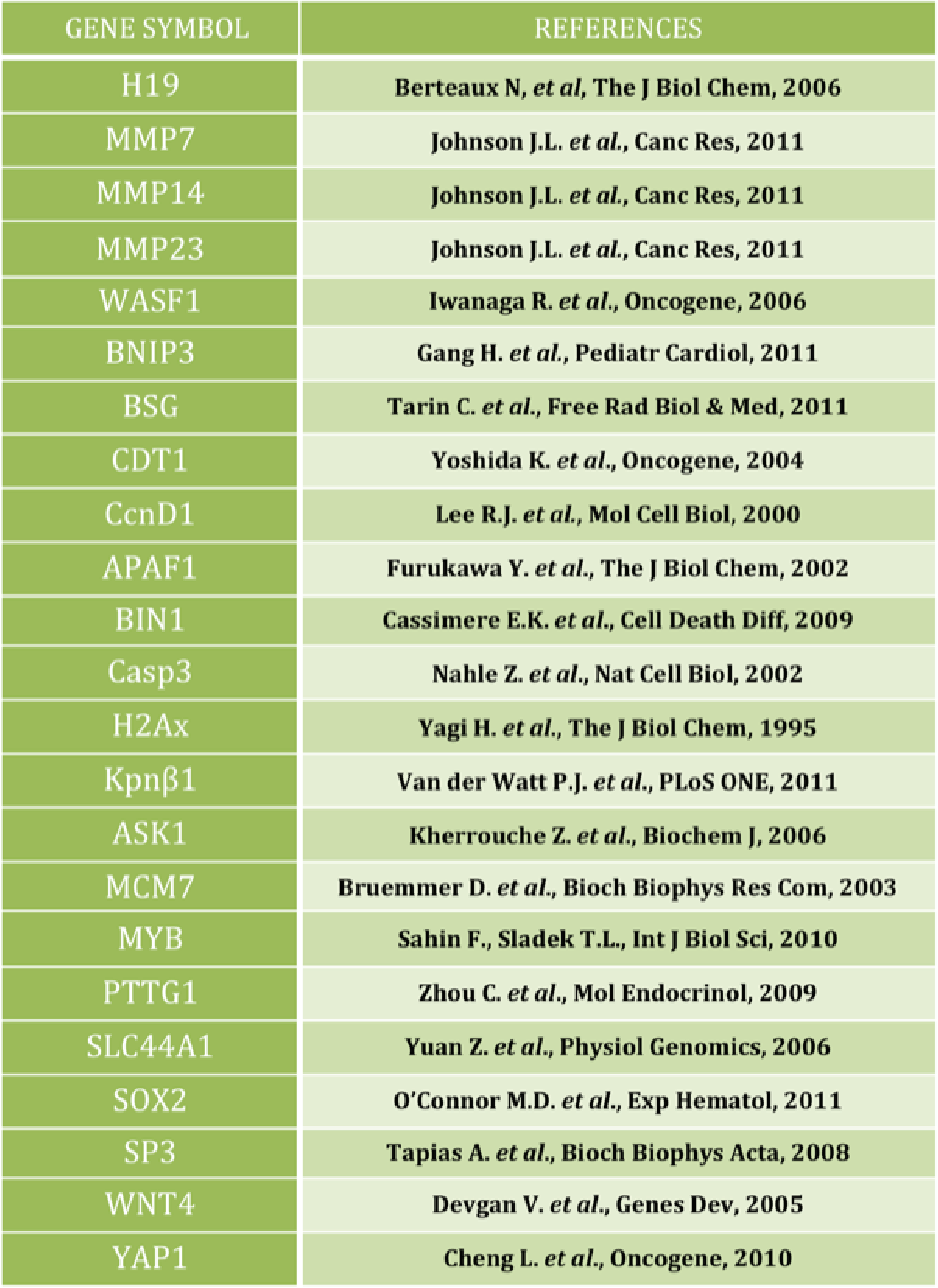
Suppl. Table 4: GSEA analysis

	C2 Gene Signature	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX	LEADING EDGE
1	HSA03022_BASAL_TRANSCRIPTION_FACTORS	15	0.71	2.08	0.000	0.074	0.064	860	tags=40%, list=13%, signal=46%
2	UVB_NHEK3_C2	30	0.57	1.98	0.000	0.152	0.234	1913	tags=63%, list=28%, signal=88%
3	LAL_KO_3MO_UP	22	0.58	1.83	0.004	0.501	0.746	960	tags=41%, list=14%, signal=47%
4	HYPOXIA_REVIEW	53	0.46	1.81	0.002	0.465	0.812	1051	tags=34%, list=15%, signal=40%
5	HYPOXIA_REG_UP	22	0.56	1.79	0.005	0.462	0.870	978	tags=41%, list=14%, signal=48%
6	AS3_FIBRO_DN	19	0.59	1.79	0.006	0.388	0.872	1547	tags=53%, list=23%, signal=68%
7	LEE_DENA_UP	41	0.47	1.78	0.004	0.359	0.896	915	tags=29%, list=13%, signal=34%
8	LAL_KO_6MO_UP	28	0.52	1.77	0.002	0.354	0.928	960	tags=39%, list=14%, signal=46%
9	CHEN_HOXA5_TARGETS_UP	112	0.39	1.77	0.000	0.318	0.929	2096	tags=46%, list=31%, signal=66%
10	DEATHPATHWAY	16	0.59	1.77	0.009	0.286	0.929	1710	tags=56%, list=25%, signal=75%
11	UVB_NHEK3_ALL	284	0.34	1.76	0.000	0.279	0.939	1631	tags=36%, list=24%, signal=45%
12	ZHAN_MULTIPLE_MYELOMA_VS_NORMAL_DN	20	0.56	1.73	0.008	0.337	0.972	84	tags=15%, list=1%, signal=15%
13	HSA03050_PROTEASOME	16	0.57	1.72	0.006	0.331	0.979	2683	tags=88%, list=39%, signal=144%
14	UVB_SCC_DN	69	0.41	1.72	0.004	0.325	0.985	1636	tags=41%, list=24%, signal=53%
15	ET743_SARCOMA_DN	171	0.36	1.71	0.000	0.311	0.986	1887	tags=41%, list=28%, signal=55%
16	UVB_SCC_UP	67	0.41	1.71	0.000	0.301	0.987	1576	tags=40%, list=23%, signal=52%
17	HINATA_NFKB_UP	59	0.42	1.71	0.000	0.287	0.988	1561	tags=39%, list=23%, signal=50%
18	FLECHNER_KIDNEY_TRANSPLANT_WELL_PBL_DN	25	0.51	1.69	0.015	0.315	0.990	1226	tags=48%, list=18%, signal=58%
19	LINDSTEDT_DEND_8H_VS_48H_UP	30	0.50	1.69	0.009	0.308	0.990	408	tags=27%, list=6%, signal=28%
20	CELL_MOTILITY	57	0.42	1.67	0.002	0.334	0.997	1759	tags=47%, list=26%, signal=63%

	C4 Gene Signature	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX	LEADING EDGE
1	GCM_CBFB	53	0.52	2.04	0.000	0.049	0.048	2063	tags=58%, list=30%, signal=83%
2	MODULE_239	85	0.46	2.02	0.000	0.032	0.062	2085	tags=52%, list=31%, signal=74%
3	GNF2_TDG	23	0.61	1.99	0.002	0.029	0.084	2027	tags=74%, list=30%, signal=105%
4	GNF2_KPNB1	53	0.50	1.97	0.000	0.029	0.107	2060	tags=62%, list=30%, signal=89%
5	GNF2_UBE2I	18	0.62	1.91	0.002	0.055	0.236	1834	tags=72%, list=27%, signal=99%
6	GNF2_XRCC5	58	0.48	1.90	0.000	0.051	0.258	2159	tags=57%, list=32%, signal=83%
7	MODULE_233	17	0.64	1.89	0.004	0.048	0.282	2062	tags=71%, list=30%, signal=101%
8	MODULE_342	115	0.40	1.83	0.000	0.077	0.457	1474	tags=43%, list=22%, signal=55%
9	MODULE_295	15	0.62	1.78	0.008	0.109	0.626	647	tags=40%, list=9%, signal=44%
10	MODULE_91	30	0.51	1.77	0.006	0.107	0.661	2713	tags=87%, list=40%, signal=143%
11	GNF2_BNIP2	23	0.55	1.76	0.006	0.108	0.698	1675	tags=65%, list=25%, signal=86%
12	MODULE_357	39	0.48	1.76	0.010	0.100	0.701	1520	tags=51%, list=22%, signal=66%
13	GNF2_APEX1	65	0.43	1.76	0.000	0.094	0.704	2027	tags=48%, list=30%, signal=67%
14	MODULE_139	48	0.45	1.76	0.002	0.088	0.708	1121	tags=40%, list=16%, signal=47%
15	MODULE_297	38	0.48	1.75	0.008	0.090	0.736	1520	tags=50%, list=22%, signal=64%
16	GCM_RAD21	27	0.52	1.74	0.008	0.089	0.752	1913	tags=56%, list=28%, signal=77%
17	GCM_ZNF198	64	0.42	1.73	0.000	0.090	0.776	2592	tags=70%, list=38%, signal=112%
18	MORF_PPP2R5E	53	0.43	1.71	0.008	0.102	0.825	1603	tags=47%, list=24%, signal=61%
19	MODULE_181	69	0.41	1.71	0.002	0.099	0.830	1010	tags=30%, list=15%, signal=35%
20	MODULE_154	35	0.48	1.71	0.008	0.095	0.833	1520	tags=51%, list=22%, signal=66%

	C5 Gene Signature	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX	LEADING EDGE
1	CHROMATIN	20	0.61	1.93	0.000	0.295	0.214	721	tags=30%, list=11%, signal=33%
2	TRANSFERASE_ACTIVITY__TRANSFERRING_ACYL_GROUPS	28	0.52	1.81	0.008	0.473	0.547	2522	tags=79%, list=37%, signal=124%
3	CARBON_OXYGEN_LYASE_ACTIVITY	16	0.60	1.81	0.008	0.330	0.561	647	tags=44%, list=9%, signal=48%
4	SMALL_GTPASE_REGULATOR_ACTIVITY	30	0.51	1.78	0.008	0.335	0.659	1763	tags=50%, list=26%, signal=67%
5	TRANSFERASE_ACTIVITY__TRANSFERRING_GROUPS_OTHER_THAN_AMINO_ACYL_GROUPS	22	0.55	1.77	0.011	0.281	0.679	2522	tags=82%, list=37%, signal=129%
6	CYTOPLASMIC_MEMBRANE_BOUND_VESICLE	66	0.42	1.72	0.005	0.382	0.832	1028	tags=29%, list=15%, signal=34%
7	CYTOPLASMIC_VESICLE_MEMBRANE	18	0.55	1.71	0.013	0.376	0.877	0	tags=6%, list=0%, signal=6%=
8	MEMBRANE_BOUND_VESICLE	67	0.41	1.70	0.002	0.340	0.890	1028	tags=28%, list=15%, signal=33%
9	CYTOPLASMIC_VESICLE	67	0.41	1.69	0.006	0.325	0.907	1028	tags=28%, list=15%, signal=33%
10	LIPID_METABOLIC_PROCESS	148	0.36	1.69	0.000	0.293	0.907	1112	tags=28%, list=16%, signal=32%
11	VESICLE	69	0.40	1.68	0.007	0.293	0.925	1028	tags=28%, list=15%, signal=32%
12	CYTOPLASMIC_VESICLE_PART	18	0.55	1.66	0.010	0.319	0.963	0	tags=6%, list=0%, signal=6%
13	DEFENSE_RESPONSE	81	0.38	1.65	0.002	0.325	0.972	1096	tags=25%, list=16%, signal=29%
14	ATPASE_ACTIVITY__COUPLED_TO_MOVEMENT_OF_SUBSTANCES	23	0.50	1.65	0.013	0.305	0.972	1820	tags=48%, list=27%, signal=65%
15	HYDROLASE_ACTIVITY__ACTING_ON_ACID_ANHYDRIDES__CATALYZING_TRANSMEMBRANE_MOVEMENT_OF_SUBSTANCES	22	0.51	1.64	0.010	0.298	0.977	1820	tags=50%, list=27%, signal=68%
16	VESICLE_MEMBRANE	19	0.52	1.60	0.019	0.376	0.992	0	tags=5%, list=0%, signal=5%
17	ANATOMICAL_STRUCTURE_MORPHOGENESIS	152	0.34	1.60	0.000	0.371	0.994	1120	tags=26%, list=16%, signal=30%
18	GUANYL_NUCLEOTIDE_EXCHANGE_FACTOR_ACTIVITY	19	0.51	1.54	0.031	0.528	1.000	848	tags=32%, list=12%, signal=36%
19	GTPASE_REGULATOR_ACTIVITY	65	0.37	1.54	0.011	0.509	1.000	925	tags=26%, list=14%, signal=30%
20	TRANSMEMBRANE_RECEPTOR_PROTEIN_TYROSINE_KINASE_SIGNALING_PATHWAY	47	0.40	1.53	0.024	0.529	1.000	1381	tags=38%, list=20%, signal=48%

Suppl. Table 5: E2F transcriptional target genes



GENE SYMBOL	REFERENCES
H19	**Berteaux N, *et al*, The J Biol Chem, 2006**
MMP7	**Johnson J.L. *et al.*, Canc Res, 2011**
MMP14	**Johnson J.L. *et al.*, Canc Res, 2011**
MMP23	**Johnson J.L. *et al.*, Canc Res, 2011**
WASF1	**Iwanaga R. *et al.*, Oncogene, 2006**
BNIP3	**Gang H. *et al.*, Pediatr Cardiol, 2011**
BSG	**Tarin C. *et al.*, Free Rad Biol & Med, 2011**
CDT1	**Yoshida K. *et al.*, Oncogene, 2004**
CcnD1	**Lee R.J. *et al.*, Mol Cell Biol, 2000**
APAF1	**Furukawa Y. *et al.*, The J Biol Chem, 2002**
BIN1	**Cassimere E.K. *et al.*, Cell Death Diff, 2009**
Casp3	**Nahle Z. *et al.*, Nat Cell Biol, 2002**
H2Ax	**Yagi H. *et al.*, The J Biol Chem, 1995**
Kpnβ1	**Van der Watt P.J. *et al.*, PLoS ONE, 2011**
ASK1	**Kherrouche Z. *et al.*, Biochem J, 2006**
MCM7	**Bruemmer D. *et al.*, Bioch Biophys Res Com, 2003**
MYB	**Sahin F., Sladek T.L., Int J Biol Sci, 2010**
PTTG1	**Zhou C. *et al.*, Mol Endocrinol, 2009**
SLC44A1	**Yuan Z. *et al.*, Physiol Genomics, 2006**
SOX2	**O'Connor M.D. *et al.*, Exp Hematol, 2011**
SP3	**Tapias A. *et al.*, Bioch Biophys Acta, 2008**
WNT4	**Devgan V. *et al.*, Genes Dev, 2005**
YAP1	**Cheng L. *et al.*, Oncogene, 2010**

Suppl. Table 6: Expression profile datasets analysis

Study	Platform	Data source	Probesets ID	Probeset sequence analysis[1] *(when applicable)*	Signal quality control[2] *(when applicable)*	Use	Behavior[3]	Reference
Tomlins	spotted cDNA	GEO data	Hs6-32-3-7	------	not present	no	------	Tomlins et al. Nat. Genet (2007)
	spotted cDNA	GEO data	Hs6-8-13-3	------	present	yes	DOWN *	
Vanaja	U133A	Oncomine	213299_at	ZTBT7A [a]	------	yes	NO CHANGE	Vanaja et al. Cancer Res (2003)
	U133A	Oncomine	213303_x_at	ZBTB7A	------	yes	UP *	
	U133A	Oncomine	222082_at	intronic	------	no	------	
	U133B	Oncomine	230709_x_at	ZBTB7A	------	yes	DOWN *	
	U133B	Oncomine	226554_at	ZBTB7A	------	yes	NO CHANGE	
Lapointe	spotted cDNA	Oncomine	Hs6-32-3-7	------	------	yes	NO CHANGE	Lapointe et al. PNAS (2004)
	spotted cDNA	Oncomine	Hs6-8-13-3	------	------	yes	NO CHANGE	
Stephenson	U133A	GEO data	213299_at	ZTBT7A [a]	not present	no	------	Stephenson et al. Cancer (2005)
	U133A	GEO data	213303_x_at	ZBTB7A	present	yes	DOWN *	
	U133A	GEO data	222082_at	intronic	not present	no	------	
	U133A	GEO data	219186_at	intergenic	present	no	------	
Liu	U133A	Array Express	213299_at	ZTBT7A [a]	not present	no	------	Liu P. et al. Cancer Res (2006)
	U133A	Array Express	213303_x_at	ZBTB7A	not present	no	------	
	U133A	Array Express	222082_at	intronic	not present	no	------	
	U133A	Array Express	219186_at	intergenic	present	no	------	
	U133B	Array Express	230709_x_at	ZBTB7A	not present	no	------	
	U133B	Array Express	226554_at	ZBTB7A	not present	no	------	
Luo	U95A_V2	Oncomine	39540_at	ZTBT7A [a]	------	yes	NO CHANGE	Luo JH et al. Mol Carcinog (2002)
	Hu35kSub_A	Oncomine	AF000561_at	ZTBT7A [a]	------	yes	DOWN *	
	Hu35kSub_A	Oncomine	H14210_s_at	ZBTB7A	------	yes	NO CHANGE	
	Hu35kSub_D	Oncomine	RC_F04371_at	intergenic	------	no	------	
Taylor	Human Exon 1.0 ST Array	GEO data	13564	ZBTB7A	------	yes	DOWN *	Taylor et al. Cancer Cell (2010)
LaTulippe	U95A_V2	Oncomine	39540_at	ZTBT7A [a]	------	yes	DOWN *	LaTulippe et al. Cancer Res (2002)
Arredouani	U133 Plus2	Oncomine	213299_at	ZTBT7A [a]	------	yes	NO CHANGE	Arredouani et al. Clin Cancer Res (2009)
	U133 Plus2	Oncomine	213303_x_at	ZBTB7A	------	yes	NO CHANGE	
	U133 Plus2	Oncomine	222082_at	intronic	------	no	------	
	U133 Plus2	Oncomine	230709_x_at	ZBTB7A	------	yes	UP *	
	U133 Plus2	Oncomine	226554_at	ZBTB7A	------	yes	DOWN *	
Grasso	Agilent Human Genome 44K	Oncomine	A_23_P16423	------	------	yes	DOWN *	Grasso et al. Nature (2012)
	Agilent Human Genome 44K	Oncomine	A_23_P27827	------	------	yes	DOWN *	
Wallace	U133A 2.0	Oncomine	213299_at	ZTBT7A [a]	------	yes	NO CHANGE	Wallace et al. Cancer Res (2008)
	U133A 2.0	Oncomine	213303_x_at	ZBTB7A	------	yes	NO CHANGE	
	U133A 2.0	Oncomine	222082_at	intronic	------	no	------	

[1] Based on BLAT (UCSC Genome Browser) and BLAST (NCBI)

[a] possibility of cross-hybridization with other ZBTB family genes

[2] *Present*: detectable signal in more than 60% of samples; *Non present*: detectable signal in less than 60% of samples

[3] significance (*) when p <= 0.05; NO_CHANGE => FoldChange ~ 1

Suppl. Table 7: Clinical information from human specimens collected on TMAs

Number	Age	Organ	Dignosis	Grade	Gleason	Type
1	60	Prostate	Adenocarcinoma	1	2+1	Malignant
2	70	Prostate	Adenocarcinoma	3	4+4	Malignant
3	66	Prostate	Adenocarcinoma	2	1+2	Malignant
4	76	Prostate	Adenocarcinoma	3	2+2	Malignant
5	64	Prostate	Adenocarcinoma	2	2+2	Malignant
6	75	Prostate	Adenocarcinoma	1	1+2	Malignant
7	72	Prostate	Adenocarcinoma	2	2+4	Malignant
8	61	Prostate	Adenocarcinoma	2	3+4	Malignant
9	72	Prostate	Adenocarcinoma	3	4+5	Malignant
10	54	Prostate	Adenocarcinoma	2	3+4	Malignant
11	68	Prostate	Adenocarcinoma	3	4+5	Malignant
12	70	Prostate	Adenocarcinoma	2	3+5	Malignant
13	63	Prostate	Adenocarcinoma	2	3+5	Malignant
14	80	Prostate	Adenocarcinoma	2	5+3	Malignant
15	75	Prostate	Adenocarcinoma	2	4+5	Malignant
16	65	Prostate	Adenocarcinoma	3	4+5	Malignant
17	69	Prostate	Adenocarcinoma	2	3+4	Malignant
18	67	Prostate	Adenocarcinoma	3	4+5	Malignant
19	74	Prostate	Adenocarcinoma	2	3+4	Malignant
20	76	Prostate	Adenocarcinoma	2	2+5	Malignant
21	55	Prostate	Adenocarcinoma	2	2+4	Malignant
22	57	Prostate	Adenocarcinoma	3	4+5	Malignant
23	77	Prostate	Adenocarcinoma	3	4+5	Malignant
24	73	Prostate	Adenocarcinoma	3	4+5	Malignant
25	76	Prostate	Adenocarcinoma	3	5+4	Malignant
26	82	Prostate	Adenocarcinoma	3	5+4	Malignant
27	78	Prostate	Adenocarcinoma	3	5+4	Malignant
28	63	Prostate	Adenocarcinoma	3	5+5	Malignant
29	69	Prostate	Adenocarcinoma	3	3+5	Malignant
30	73	Prostate	Adenocarcinoma	3	5+5	Malignant
31	65	Prostate	Adenocarcinoma	3	5+5	Malignant
32	83	Prostate	Adenocarcinoma	3	4+5	Malignant
33	64	Prostate	Adenocarcinoma	2	3+4	Malignant
34	64	Prostate	Adenocarcinoma	3	4+5	Malignant
35	69	Prostate	Adenocarcinoma	3	5+5	Malignant
36	73	Prostate	Adenocarcinoma	2	3+5	Malignant
37	51	Prostate	Adenocarcinoma	2	3+4	Malignant
38	71	Prostate	Adenocarcinoma	3	4+5	Malignant
39	66	Prostate	Adenocarcinoma	3	5+4	Malignant
40	70	Prostate	Adenocarcinoma	2	4+5	Malignant
41	60	Prostate	Adenocarcinoma	3	5+5	Malignant
42	70	Prostate	Adenocarcinoma	3	5+5	Malignant
43	57	Prostate	Adenocarcinoma	3	5+4	Malignant
44	73	Prostate	Adenocarcinoma	3	5+4	Malignant
45	73	Prostate	Adenocarcinoma	3	5+4	Malignant
46	60	Prostate	Adenocarcinoma	3	5+4	Malignant
47	72	Prostate	Adenocarcinoma	3	5+4	Malignant
48	62	Prostate	Adenocarcinoma	3	5+4	Malignant
49	67	Prostate	Adenocarcinoma	3	4+5	Malignant
50	55	Prostate	Adenocarcinoma	3	4+5	Malignant
51	70	Prostate	Normal prostate tissue	-	-	
52	65	Prostate	Normal prostate tissue	-	-	
53	72	Prostate	Normal prostate tissue	-	-	
54	71	Prostate	Normal prostate tissue	-	-	
55	63	Prostate	Normal prostate tissue	-	-	
56	27	Prostate	Normal prostate tissue	-	-	
57	25	Prostate	Normal prostate tissue	-	-	
58	21	Prostate	Normal prostate tissue	-	-	
59	48	Prostate	Normal prostate tissue	-	-	

Suppl. Table 8: Primers sequence

PtenLoxP_Fwd 5′-AAAAGTTCCCCTGCTGATGATTTGT-3′
PtenLoxP_Rev 5′-TGTTTTTGACCAATTAAAGTAGGCTGTG-3′

PCFW1 5′-TCTGAGGCCCGGTGCAT-3′
PCFW2 5′-AGGGTGGTGCTCCCTCTAGAC-3′
PCFW3 5′-ACCGCGGTCTAGGGATCC-3′
PCRV 5′-GCTTGGGCTCCCCATGTAG-3′
PCNeo 5′-GGATGTGGAATGTGTGCGAG-3′

MIA1_Fwd 5'-CTGGGCAACAGAGCAAGACT-3'
MIA1_Rev 5'- ACTGCAGCCTCAACCTCCTA-3'

H19_Fwd 5'-GTGTCCCCATTCTTTGGATG-3'
H19_Rev 5'- TGGTGAGACAGAAGGGGAAG-3'

DMBT1_Fwd 5'- GAACAGTGCATGGCAAAGAA-3'
DMBT1_Rev 5'-GACAGCTTTCCAGGACTTGC-3'