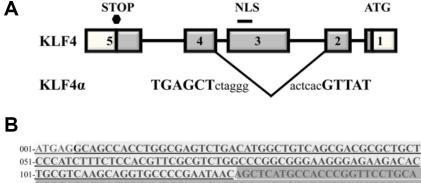
KLF4a stimulates breast cancer cell proliferation by acting as a KLF4 antagonist

Supplementary Materials



- 151-TGCCAGAGGAGCCCAAGCCAAAGAGGGGAAGACGATCGTGGCCCCGGAAA 201-AGGACCGCCACCCACACTTGTGATTACGCGGGCTGCGGCAAAAACCTACAC
- 251-AAAGAGTTCCCATCTCAAGGCACACCTGCGAACCCACACAG<mark>GTGAGAAAC</mark> 301-CTTACCACTGTGACTGGGACGGCTGTGGATGGAAATTCGCCCGCTCAGAT
- 350-GAACTGA-357

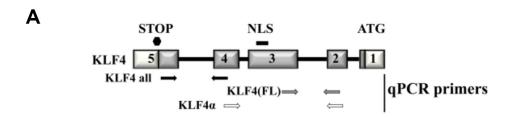
C KLF4(FL), Isoform 2

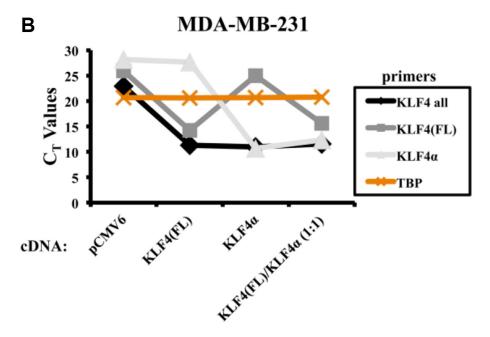
MRQPPGESDMAVSDALLPSFST <u>FASGPAGREKTLRQAGAPNN</u>R WREELSHMKRLPPVLPGRPYD LAAATVATDLESGGAGAACGG SNLAPLPRRETEEFNDLLDLDFI LSNSLTHPPESVAATVSSSASAS SSSSPSSSGPASAPSTCSFTYPIR AGNDPGVAPGGTGGGLLYGRE SAPPPTAPFNLADINDVSPSGGF VAELLRPELDPVYIPPOOPOPPG GGLMGKFVLKASLSAPGSEYG SPSVISVSKGSPDGSHPVVVAP YNGGPPRTCPKIKQEAVSSCTH LGAGPPLSNGHRPAAHDFPLGR QLPSRTTPTLGLEEVLSSRDCHP ALPLPPGFHPHPGPNYPSFLPD QMQPQVPPLHYQELMPPGSCM PEEPKPKRGRRSWPRKRTATHT CDYAGCGKTYTKSSHLKAHLR THTGEKPYHCDWDGCGWKFA RSDELTRHYRKHTGHRPFQCQ KCDRAFSRSDHLALHMKRHF*

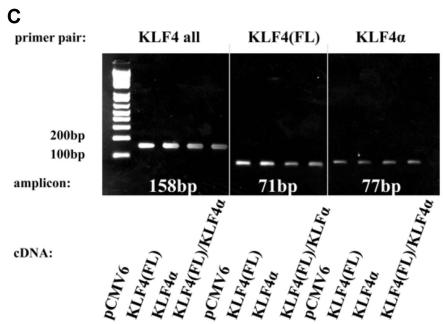
KLF4α 118aa

MRQPPGESDMAVSDALLPSFST FASGPAGREKTLRQAGAPNNSS CHPVPACQRSPSQRGEDDRGPG KGPPPLVITRAAAKPTQRVPISR HTCEPTQVRNLTTVTGTAVDGN SPAQMN*

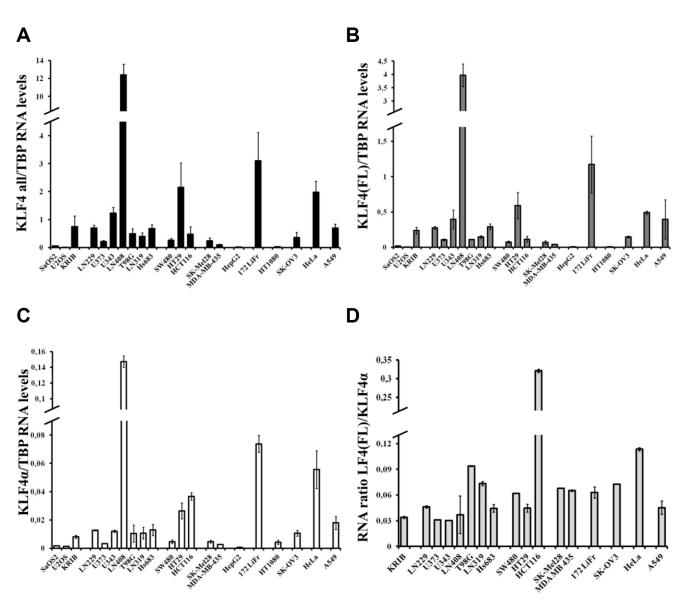
Supplementary Figure S1: cDNA and protein sequence of KLF4 α in MDA-MB-231 cells. (A) Schematic representation of the *KLF4* gene. Splicing site sequence for *KLF4* α is indicated below. Letters in uppercase: exon part, letters in lowercase: intron part. (B) cDNA sequence of KLF4 α . The different gray shades indicate the 4 different exons present. (C) Comparison of the KLF4(FL) and KLF4 α protein sequence. Underlined sequence: 42 aa, which are common between KLF4 α and KLF4(FL).







Supplementary Figure S2: KLF4 α primer specificity. (A) Schematic representation of the *KLF4* gene and the binding sites of the specific primer pairs. (B) qPCR analysis of transiently transfected MDA-MB-231 cells with the expression plasmids pCMV6, KLF4(FL), KLF4 α , and a 1:1 mix of KLF4(FL) and KLF4 α 24 h after transfection using specific primers that recognize all KLF4 isoforms (*KLF4 all*), KLF4(FL) only (*KLF4(FL)*), and KLF4 α (*KLF4* α). Note that the primers show the expected specificities. TBP: TATA-Box binding protein. (C) qPCR amplicons were analyzed on an agarose gel. For each primer pair, a single band was detectable corresponding to the expected size.



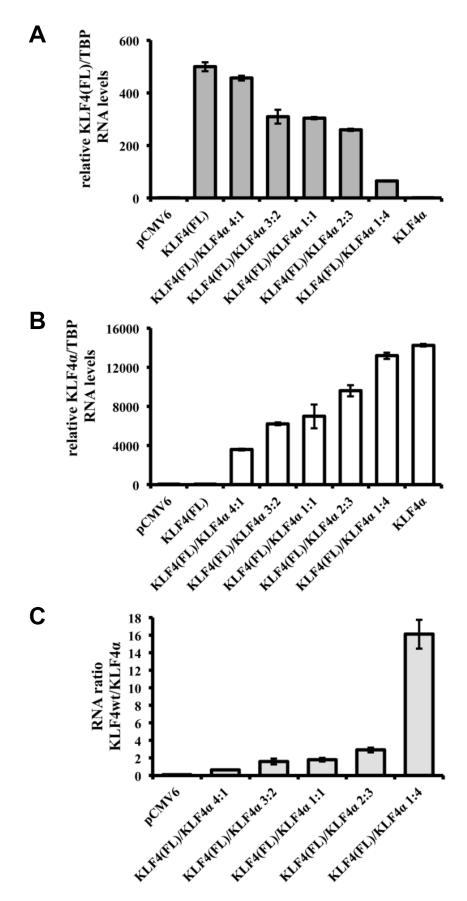
Supplementary Figure S3: $KLF4\alpha$ expression in different cancer cell lines. A panel of 21 human cancer cell lines was analyzed by qPCR for KLF4 all (top left), KLF4 (top right), $KLF4\alpha$ (bottom left) and the RNA ratio $KLF4\alpha$ /KLF4 (bottom right). In most of the cell lines (except for SaOS2, U2OS, HepG2) $KLF4\alpha$ was detectable. Levels of KLF4 and $KLF4\alpha$, as well as the resulting $KLF4\alpha$ /KLF4 (FL) ratio vary across the cell lines analyzed. TBP: TATA-Box binding protein.

TissueScan: Clinical data

Breast

ID	gender	age	appearance	diagnosis	stage	normal	tumor	stroma	necrosis				
N	F	47	Normal		Normal	100	0	0	0				
T1	F	52	Tumor	ductal Adenocarcinoma	IIA	0	70	30	0				
T2	n.a.	78	Tumor	ductal Adenocarcinoma	IIA	0	85	10	5				
T3	F	50	Tumor	ductal Adenocarcinoma	IIB	0	85	13	2				
T4	F	55	Tumor	ductal Adenocarcinoma	IIIC	10	80	10	0				
T5	F	68	Tumor	ductal Adenocarcinoma	IIIC	0	90	10	0				
Ki	Kidney												
ID	gender	age	appearance	diagnosis	stage	normal	tumor	stroma	necrosis				
N	M	77	Normal		Normal	100	0	0	0				
T1	M	43	Tumor	Carcinoma, renal cell, papillary	I	0	75	10	15				
T2	M	64	Tumor	Carcinoma, renal cell, papillary	II	0	95	5	0				
T3	M	4	Tumor	Nephroblastoma	II	0	95	0	5				
T4	M	59	Tumor	Carcinoma, renal cell, papillary	II	0	70	0	30				
T5	M	59	Tumor	Carcinoma, renal cell, clear cell	III	0	75	10	15				
Lu	ng												
ID	gender	age	appearance	diagnosis	stage	normal	tumor	stroma	necrosis				
					NT 1	100	0	0	0				
N	F	72	Normal		Normal	100	U	U					
N T1	F F	72 82	Normal Tumor	Adenocarcinoma	IA	0	40	55	5				
				Adenocarcinoma Squamous cell carcinoma									
T1	F	82	Tumor		IA	0	40	55	5				
T1 T2	F M	82 65	Tumor Tumor	Squamous cell carcinoma	IA IIB	0 10	40 65	55 24	5				
T1 T2 T3	F M M	82 65 76	Tumor Tumor Tumor	Squamous cell carcinoma Squamous cell carcinoma	IA IIB IIIA	0 10 0	40 65 60	55 24 10	5 1 30				
T1 T2 T3 T4 T5	F M M F	82 65 76 46	Tumor Tumor Tumor Tumor	Squamous cell carcinoma Squamous cell carcinoma Adenocarcinoma	IA IIB IIIA IV	0 10 0 25	40 65 60 40	55 24 10 35	5 1 30 0				
T1 T2 T3 T4 T5 Ov	F M M F F	82 65 76 46 64	Tumor Tumor Tumor Tumor	Squamous cell carcinoma Squamous cell carcinoma Adenocarcinoma	IA IIB IIIA IV	0 10 0 25 0	40 65 60 40 90	55 24 10 35 6	5 1 30 0				
T1 T2 T3 T4 T5 Ov	F M M F F	82 65 76 46 64	Tumor Tumor Tumor Tumor	Squamous cell carcinoma Squamous cell carcinoma Adenocarcinoma carcinoid, atypical	IA IIB IIIA IV n.a.	0 10 0 25 0	40 65 60 40 90	55 24 10 35 6	5 1 30 0 4				
T1 T2 T3 T4 T5 Ov	F M F F vary gender	82 65 76 46 64 age	Tumor Tumor Tumor Tumor Tumor	Squamous cell carcinoma Squamous cell carcinoma Adenocarcinoma carcinoid, atypical	IA IIB IIIA IV n.a.	0 10 0 25 0	40 65 60 40 90 tumor	55 24 10 35 6 stroma	5 1 30 0 4 necrosis				
T1 T2 T3 T4 T5 Ov ID	F M F F vary gender	82 65 76 46 64 age 54	Tumor Tumor Tumor Tumor Tumor Appearance Normal	Squamous cell carcinoma Squamous cell carcinoma Adenocarcinoma carcinoid, atypical diagnosis	IA IIB IIIA IV n.a. stage Normal	0 10 0 25 0 normal	40 65 60 40 90 tumor 0	55 24 10 35 6 stroma 0	5 1 30 0 4 necrosis				
T1 T2 T3 T4 T5 Ov ID N T1	F M F F Vary gender F	82 65 76 46 64 age 54 58	Tumor Tumor Tumor Tumor Tumor Appearance Normal Tumor	Squamous cell carcinoma Squamous cell carcinoma Adenocarcinoma carcinoid, atypical diagnosis Adenocarcinoma, endometrioid	IA IIB IIIA IV n.a. stage Normal IIB	0 10 0 25 0 normal 100	40 65 60 40 90 tumor 0 60	55 24 10 35 6 stroma 0 15	5 1 30 0 4 necrosis 0 25				
T1 T2 T3 T4 T5 Ov ID N T1 T2	F M F F Vary gender F F	82 65 76 46 64 age 54 58 91	Tumor Tumor Tumor Tumor Tumor Tumor Tumor appearance Normal Tumor Tumor	Squamous cell carcinoma Squamous cell carcinoma Adenocarcinoma carcinoid, atypical diagnosis Adenocarcinoma, endometrioid Adenocarcinoma, papillary serous	IA IIB IIIA IV n.a. stage Normal IIB III	0 10 0 25 0 normal 100 0	40 65 60 40 90 tumor 0 60 90	55 24 10 35 6 stroma 0 15 10	5 1 30 0 4 necrosis 0 25 0				

Supplementary Figure S4: Clinical date of the TissueScans. A TissueScan of breast, kidney, lung, and ovary cancer patients was used to study RNA levels of KLF4(FL) and $KLF4\alpha$. Clinical data of the clinical specimens are summarized. Numbers (last four columns) represent percentage of the indicated tissues.



Supplementary Figure S5: KLF4(FL) and $KLF4\alpha$ levels in transfected MDA-MB-231 cells. (A) qPCR analysis of KLF4(FL) RNA in MDA-MB-231 cells transfected with different KLF4(FL)/KLF4 α ratios. (B) qPCR analysis of $KLF4\alpha$ levels in MDA-MB-231 cells transfected with different KLF4(FL)/KLF4 α ratios. (C) Ratio of forced KLF4(FL)/KLF4 α imbalance in MDA-MB-231 cells. TBP: TATA-Box binding protein.

Supplementary Table S1: Details of the cell lines used

NAME	ATCC♯	TISSUE	DISEASE	MEDIUM
MCF10A	CRL-10317	Breast		DMEM/F12, Horse serum, cholera toxin, EGF, Hydrocortisone, Insulin
MCF7	HTB-22	Breast	adenocarcinoma; ER+	DMEM
T47D	HTB-133	Breast	ductal carcinoma; ER+, PR+	DMEM
MDA-MB-175	HTB-25	Breast	ductal carcinoma	DMEM/F12
MDA-MB-231	HTB-26	Breast	adenocarcinoma; ER+	DMEM
Saos-2	HTB-85	Bone	osteosarcoma	DMEM
U-2 OS	HTB-96	Bone	osteosarcoma	DMEM
143B (KRIB)	CRL-8303	Bone	osteosarcoma	DMEM
LN229	CRL-2611	Brain	glioblastoma	DMEM
U373	HTB-17	Brain	glioblastoma	DMEM
U343		Brain	glioblastoma	DMEM
LN408		Brain	glioblastoma	DMEM
T98G	CRL-1690	Brain	glioblastoma	DMEM
LN319		Brain	glioblastoma	DMEM/1% glutamine
Hs683	HTB-138	Brain	oligodendroglioma	DMEM/1% glutamine
SW480	CCL-228	Colon	colorectal adenocarcinoma	Leibovitz's L15
HT29	HTB-38	Colon	colorectal adenocarcinoma	McCoy's
HCT116	CCL-247	Colon	colorectal adenocarcinoma	McCoy's
Sk-Mel-28	HTB-72	Skin	malignant melanoma	DMEM
MDA-MB-435	HTB-129	Skin	melanoma	Leibovitz's L15
172			LiFraumeni (Trp53R172H/R172H)	DMEM
Hep G2	HB-8065	Liver	hepatocellular carcinoma	EMEM
HT1080	CCL-121	Connective	fibrosarcoma	DMEM
SK-OV3	HTB-77	Ovary	adenocarcinoma	McCoy's
HeLa	CCL-2	Cervix	adenocarcinoma	DMEM
A549	CCL-185	Lung	carcinoma	RPMI 1640/glutamine

Supplementary Table S2: qPCR primers

gene	Sequence (5'-3')
KLF4 all F	CAAGCCAAAGAGGGGAAGAC CGTCCCAGTCACAGTGGTAA
KLF4 all R	
KLF4(FL) F	GAGAAGACACTGCGTCAAGC
KLF4(FL) R	AGTCGCTTCATGTGGGAGA
KLF4α F	GTGCCCCGAATAACAGCTCA
KLF4α R	ACGATCGTCTTCCCCTCTTT
$p21^{Cip1} F$	TGGAGACTCTCAGGGTCGAAA
$p21^{Cip1} R$	CGGCGTTTGGAGTGGTAGAA
$p27^{Kipl} F$	CCGGTGGACCACGAAGAGT
$p27^{Kip1} R$	GCTCGCCTCTTCCATGTCTC
CDH1 F	AGAACGCATTGCCACATACACT
CDH1 R	TCTGATCGGTTACCGTGATCAA
TBP F	TGCACAGGAGCCAAGAGTGAA
TBP R	CACATCACAGCTCCCCACCA

F: forward; R: reverse.