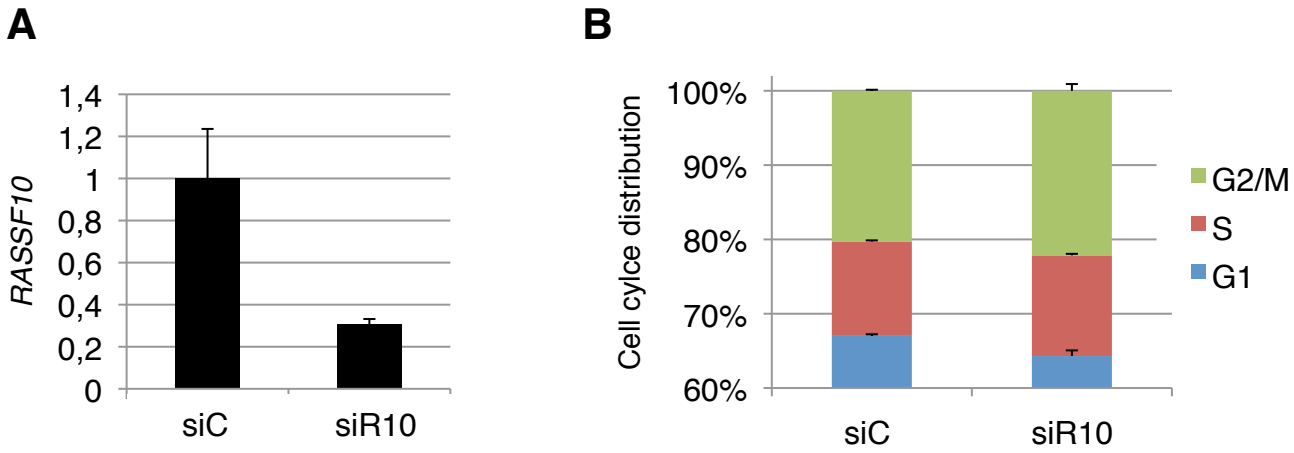

Fig. S1 RASSF10 knockdown increases rate of cells entering mitosis and decreases cells in G1. In A549 cells knockdown of RASSF10 by siRNA was performed and DNA content was determined by propidium iodide staining using FACS analysis (FACSCanto, BDBiosciences, Heidelberg, Germany). A) The knockdown of *RASSF10* was verified by RT-PCR and normalised to *ACTB*. B) Distribution of cells in the different stages of the cell cycle is shown (blue = G1, red = S and green = G2 and mitosis). Experiment was performed in triplicate and representative result is shown. A549 cells were transfected with siRNA. After 4 d cells were ethanol fixed overnight at -20°C. Then cells were 50 μg/ml RNase A treated for 30 min at 37°C and stained with 50 μg/ml propidium iodide to measure DNA content of cells with FACS CantoII (BDBiosciences, Heidelberg, Germany). Data was analyzed with FACSDiva Version 6.1.2.

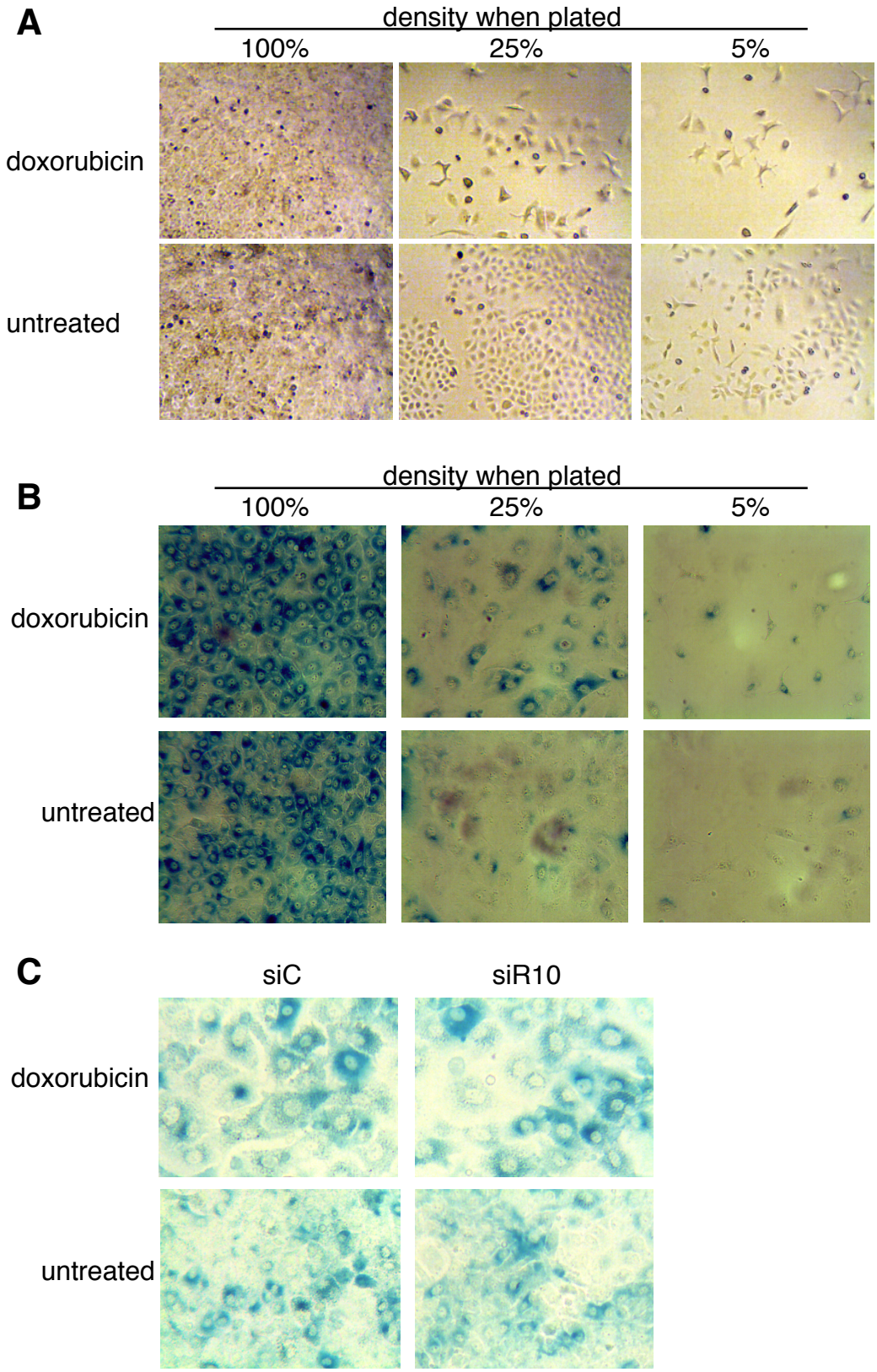

Fig. S2 Senescence-associated β-galactosidase staining is associated with increasing cellular density and doxorubicin treatment. A) Confocal microscopy of B) senescence associated β-galactosidase staining of doxorubicin treated A549 was performed. A549 cells were plated at indicated densities and left untreated or 200 nM doxorubicin treated for 72 h. Cells were fixed with 3.7% formaldehyde and stained for β-galactosidase activity (blue) overnight and embedded in MOWIOL before microscopy. Matching experiment is found in figure 5C. C) Senescence associated β-galactosidase staining of doxorubicin treated A549 after knockdown of RASSF10 is depicted. A549 were transfected with siRNA against RASSF10 (siR10) or Control (siC). After 24 h cells were treated with doxorubicin for another 72 h. Cells were fixed with 3.7% formaldehyde and stained for β-galactosidase activity (blue) overnight and embedded in MOWIOL before microscopy. Matching experiment is found in figure 5D.

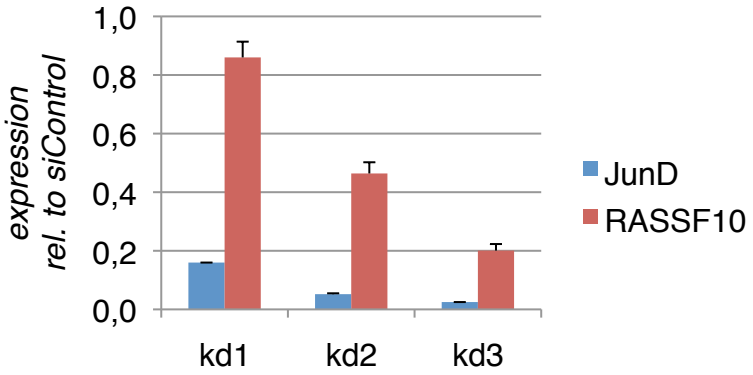

Fig. S3 JunD knockdown correlates dose dependently with downregulation of *RASSF10*. Three independent experiments (kd1-3) for knockdown of JunD and siControl were performed and analysed by qRT-PCR for *JunD* (blue), *RASSF10* (red) and *ACTB* expression. Results were normalised to *ACTB*. QRT-PCR was performed in triplicate and according SD is indicated. SiControl was set 1 for each experiment and relative *JunD* and *RASSF10* expression under siJunD are depicted. Matching experiment found in 5F.

Table S1 Cell lines analysed for *RASSF10* methylation

lung cancer cell lines		cell line	R10 methylation
	NSCLC	H322	m
		H358	u
		HCC15	pm
		HCC366	u
		H1299	pm
		A549	u
		A427	pm
	SCLC	HTB171	pm
		HTB175	pm
		HTB173	pm
		CRL2062	u
		HTB184	u
		CRL5869	pm
		CRL5808	pm
		CRL5886	pm
		CRL5840	pm
		CRL5976	pm
		CRL5831	u
		CCL257	u
		HTB180	m
		CRL2066	pm
		CRL5898	pm
head and neck cancer cell lines			
		UM-SCC	u
		RPMI	m
		Hep2	pm
sarcoma cell lines			
		LMS6-93	pm
		SKLMS	pm
		A204	pm
		RD	pm
		U2OS	pm
pancreas cancer cell lines			
		PATU-S	pm
		PATU-T	pm
		PATU-02	pm
		Capan1	u
		Capan2	m
		HUP-T3	u
		HUP-T4	u
		PaCa2	m

NSCLC: non small cell lung cancer; SCLC: small cell lung cancer;
m = methylated, pm= partially, methylated, u = unmethylated;

Table S2 Primers utilised for COBRA, pyrosequencing, RT-PCR and promoter studies

COBRA PCR	
RASSF10	5'-ATAAGTAGAGGAGTTAGTAGGTTAAAGGAGA-3' 5'-AAATACAAAAAACTCAAAACCCAAACCC-3'
RASSF10USEQ	5'-GTGGAGGGATTTTTGAATTTTTTTT -3'
RT-PCR	
ACTB	5'-CCTTCCTTCCTGGGCATGGAGTC-3' 5'-CGGAGTACTTGCGCTCAGGAGGA-3'
28SrRNA	5'-GCAGGGCGAAGCCAGAGGAAACT-3' 5'-CGAGAGCGCCAGCTATCCTGAGG-3'
RASSF10	5'-GCGCCATGGATCCTTCGGAAAA-3' 5'-GGCAGCGCCTCGTCGTCGTCCT-3'
p27	5'-GTGCGAGAGAGGCGGTCGTG-3' 5'-TCCACCGGGCCGAAGAGGTT-3'
p21	5'-CCTTGTGCCTCGCTCAGGGGAG-3' 5'-GGCCCTCGCGCTTCCAGGAC-3'
JunD	5'-CGCATCTCGCGCCTGGAAGA-3' 5'-GGACTCAGTACGCGGGCACC-3'
Fra2	5'-GCGGCGGCCAGCAGAAATTC-3' 5'-GGACAGAGGCCAGGCCCGG-3'
RASSF10 promoter construct	
R10wt	5'-AGATCTTGTGACTTGGGTCTGGGCCCTGAA-3' 5'-GGTTGCGCCATGGATCCTTCG-3'
del -271 to +157	5'-AGATCTTGTGACTTGGGTCTGGGCCCTGAA-3' 5'-GCCGAGACTCAGGATGCCTGCAATGGATCCG-3'
del -900 to -776	5'-CTCACATGGCTCGACAGCCGCCTCTGAAGG-3' 5'-CCTTCAGAGGCGGCTGTCGAGCCATGTGAG-3'
del -775 to -502	5'-CTACAGGGAACGGGCAAATGCGTCTGGGGG-3' 5'-CCCCCAGACGCATTTGCCCGTTCCCTGTAG-3'
del -501 to -273	5'-CGAGGGACCAGGGCGAGCGAGGGG-3' 5'-CCCCTCGCTCGCCCTGGTCCCTCG-3'
del -272 to -218	5'-AGACTCAGGATGCCTGCAATCTGGGCCCTGA-3' 5'-TCAGGGCCCAGATTGCAGGCATCCTGAGTCT-3'
del -217 to -106	5'-GAGGGGCGGGCAGCCAGAGCGG-3' 5'-CCGCTCTGGCTGCCCGCCCCTC-3'
del -105 to -6	5'-GCACCTTGGGCAGCCAGCCTGCCTTC-3' 5'-GAAGGCAGGCTGGCTGCCCAAGGTGC-3'

Table S3 The histopathologic, clinical and *RASSF10* methylation data of 49 soft tissue sarcoma patients

Patients' characteristics	No. of cases	RASSF10	
		unmethylated	methylated
Total	46	38	8
Sex			
Male	17	15	2
Female	29	23	6
Histological subtype			
LS	12	11	1
MFH	13	11	2
FS	1	1	0
RMS	4	1	3
LMS	9	7	2
NS	4	4	0
Syn	1	1	0
Other	2	2	0
Tumour stage			
I	8	8	0
II	17	15	2
III	15	10	5
IV	6	5	1
Complete resection			
Radical (R0)	34	28	6
Not radical (R1)	12	10	2
Location			
Extremities	31	25	6
Trunk wall	3	2	1
Abdomen/ retroperitoneum	10	9	1
Multiple locations	2	2	0
Patient status			
Alive	26	20	6
Dead	20	18	2

LS-liposarcoma, MFH-malignant fibrous histiocytoma, FS-fibrosarcoma, RMS-rhabdomyosarcoma, LMS-leiomyosarcoma, NS-neuronal sarcoma, Syn-synovial sarcoma