Phospholipid profiling identifies acyl chain elongation as a ubiquitous trait and potential target for the treatment of lung squamous cell carcinoma

Supplementary Material

Table S1: Clinical characteristics of lung SCC patients

Variable	Statistic	Set 1	Set 2
Age	N	30	31
	Mean	63.9	64.1
	Std	9.76	8.66
	Median	62.5	64.0
	IQR	(57.0; 73.3)	(59.0; 70.0)
	Range	(43.0; 81.0)	(46.0; 80.0)
Sex			
Male	n/N (%)	25/30 (83.33%)	28/31 (90.32%)
Female	n/N (%)	5/30 (16.67%)	3/31 (9.68%)
Smoking status			
Smoker	n/N (%)	9/30 (30.00%)	17/31 (54.84%)
Non smoker	n/N (%)	0/30 (0.00%)	0/31 (0.00%)
StoppedS<6mo	n/N (%)	8/30 (26.67%)	2/31 (6.45%)
StoppedS>6mo	n/N (%)	13/30 (43.33%)	12/31 (38.71%)
Tumor stage			
I	n/N (%)	7/30 (23.33%)	8/31 (25.81%)
II	n/N (%)	9/30 (30.00%)	14/31 (45.16%)
III	n/N (%)	14/30 (46.67%)	9/31 (29.03%)

7th ed. of TNM classification was used. StoppedS<6mo = Stopped smoking less than 6 months; StoppedS>6mo = Stopped smoking more than 6 months

Table S2: Primers used for RT-qPCR

hELOVL1	Fw	CTGTGGCACAACCCTACCTT
	Rv	CTGGGAGATGTGCAGTGAGA
hELOVL2	Fw	TCTTTGGACCAACACTGAACA
	Rv	GTGATGGTGAGCACGAACTG
hELOVL3	Fw	CCTTGCAATCTTCAGTATCCT
	Rv	GATGATGAAGGCTGTGTCTC
hELOVL4	Fw	CCGAGAACCTTTTCAGATGC
	Rv	TCCACACTCTGGCAAATATAGC
hELOVL5	Fw	GTGCACATTCCCTCTTGGTT
	Rv	TTCAGGTGGTCTTTCCTTCG
hELOVL6	Fw	CAAAGCACCCGAACTAGGAG
	Rv	TGGTGATACCAGTGCAGGAA
hELOVL7	Fw	GCGCAAGAAAAATAGCCAAG
	Rv	GAATGTTCCCAAACCACCTG
mELOVL1	Fw	TTGGCTGAGTACCTACACCTG
	Rv	CTCGAACCATCCGAAGTGCTT
mELOVL2	Fw	AAGTCAATGCTTTCTTGGACAAC
	Rv	TACTTGTTACCCAGCCATATCG
mELOVL3	Fw	TTCTCACGCGGGTTAAAAATGG
	Rv	GAGCAACAGATAGACGACCAC
mELOVL4	Fw	GGGATCATACAACGCAGGATAC
	Rv	GTCCAAATACTCAACGCCTTTC
mELOVL5	Fw	GAACATTTCGATGCGTCACTCA
	Rv	GGAGGAACCATCCTTTGACTCTT
mELOVL6	Fw	GCAGAGAACACGTAGCGAC
	Rv	CGCAGAAAACAGGAAAGACTTC
mELOVL7	Fw	CATCGAGGACTGTGCGTTTTT
	Rv	CCAGGATGATGGTTTGTGGCA
18S	Fw	CGCCGCTAGAGGTGAAATTC
	Rv	TTGGCAAATGCTTTCGCTC

h = human primers; m = mouse primers

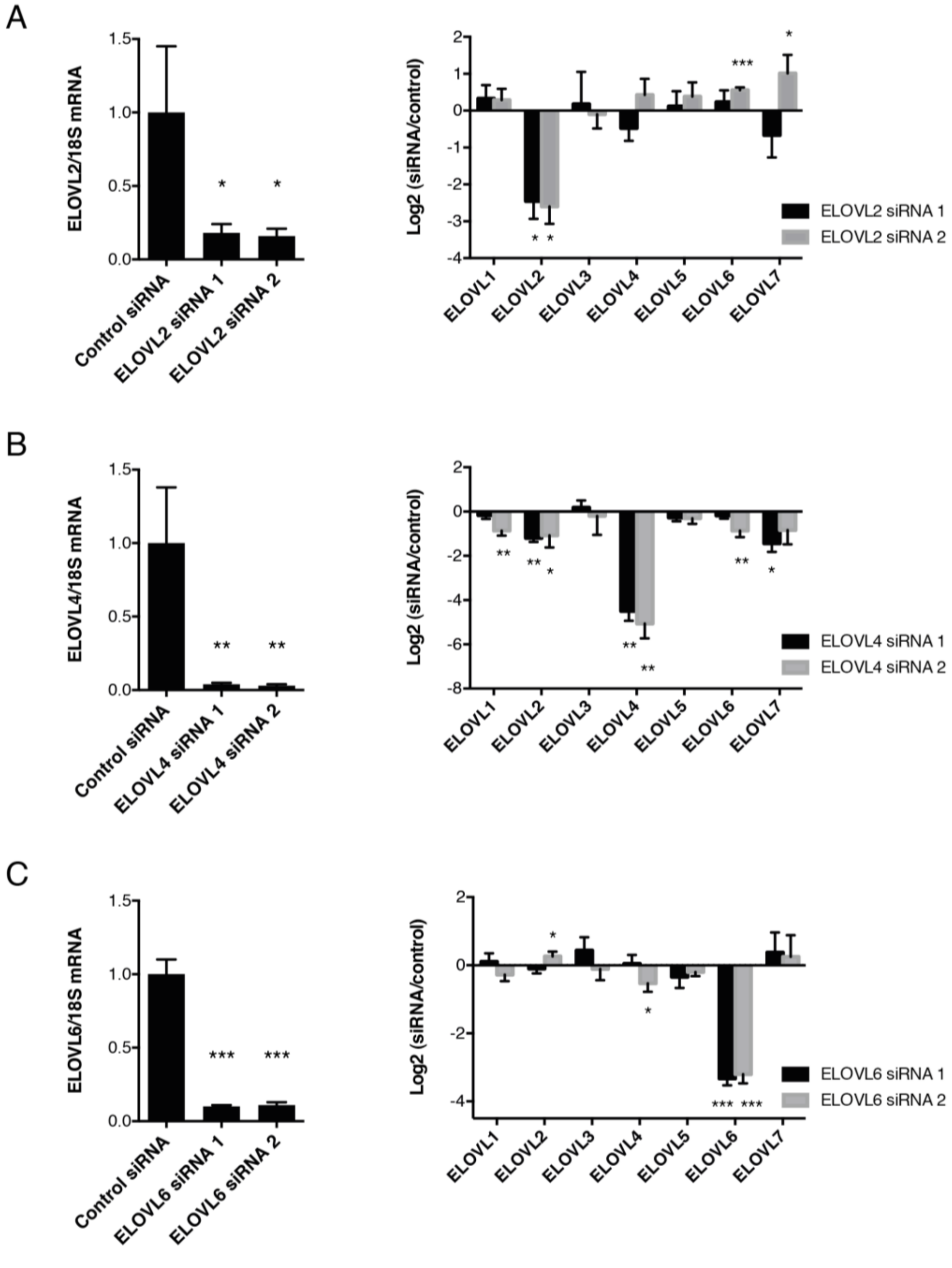


Figure S1: Effect of knockdown of ELOVL2, 4 and 6 on ELOVL expression in the SCC cell line 2427PT

2427PT cells were treated with two independent siRNAs specific for **(A)** ELOVL2, **(B)** ELOVL4 and **(C)** ELOVL6 for 48h. Cells treated with scrambled siRNA were used as a control. mRNA levels of ELOVL1-7 were analyzed by RT-qPCR. 18S was used for normalization. Data represent mean (n=3) ± standard deviation. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Student's test).

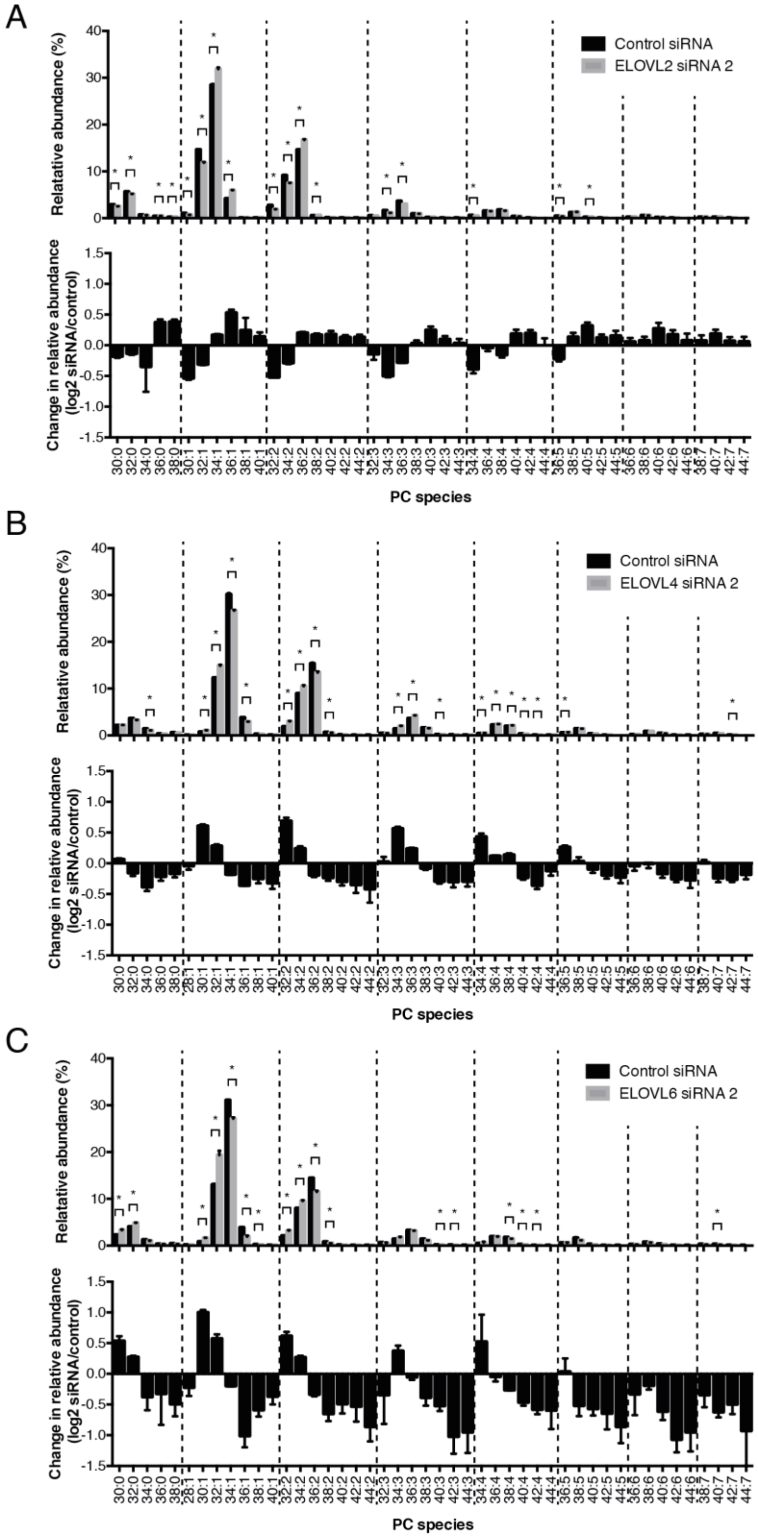


Figure S2: Effect of knockdown of ELOVL2, 4 and 6 by a second independent siRNA (siRNA 2) on PC profiles in 2427PT lung SCC cells

2427PT cells were transfected with a second siRNA specific for **(A)** ELOVL2, **(B)** ELOVL4 and **(C)** ELOVL6 for 72h. Control cells were transfected with scrambled siRNA. The relative abundance and changes in relative abundance (log2) of PC after modulation of the specific ELOVL are shown. Changes in lipids were analyzed and presented as in Figure 5. Data represent mean (n=3) ± standard deviation. * Indicates statistical significance after multiple testing using the Holm-Sidak method with alpha=0.05.

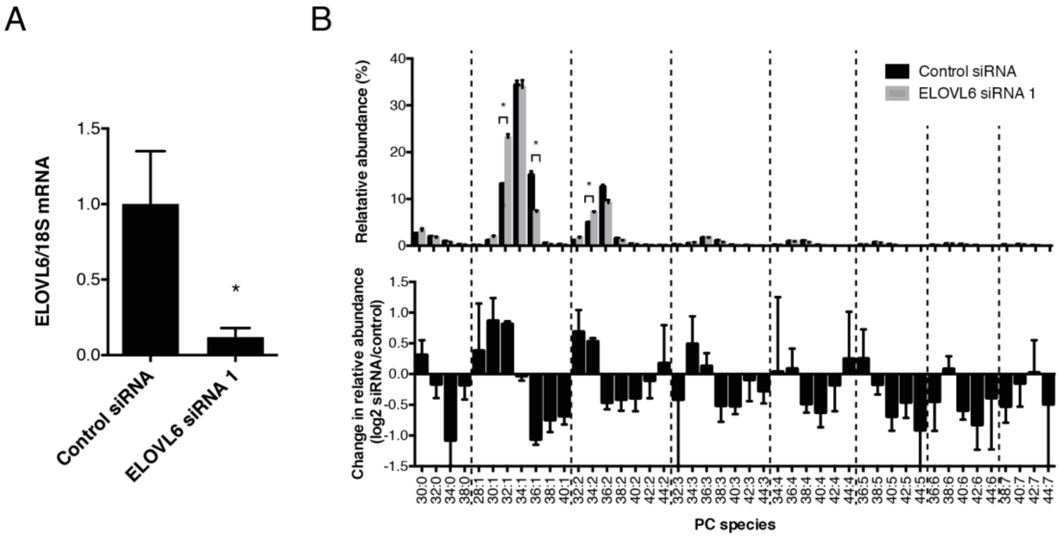
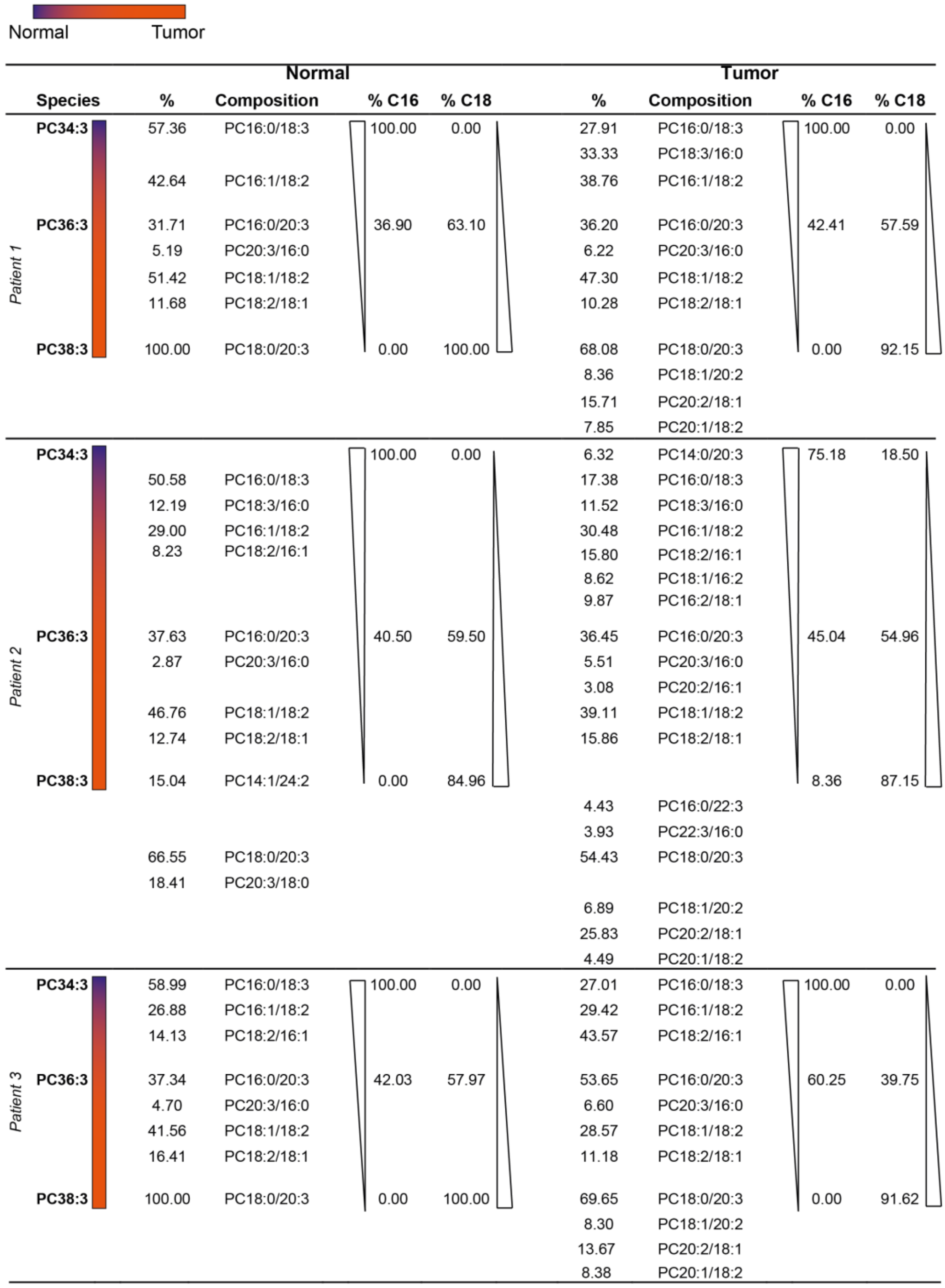


Figure S3: Effect of knockdown of ELOVL6 on acyl chain elongation in H2170 lung SCC cells

H2170 cells were treated with siRNA specific for ELOVL6 (siRNA 1) for **(A)** 48h and **(B)** 72h. Cells treated with scrambled siRNA were used as a control. **(A)** mRNA levels of ELOVL6 were analyzed by RT-qPCR. 18S was used for normalization. Data represent mean (n=2) ± standard deviation. * $p < 0.05$ (Student's test). **(B)** The relative abundance and changes in relative abundance (log2) of PC after modulation of ELOVL6 are shown. Changes in lipids were analyzed and presented as in Figure 5. Data represent mean (n=3) ± standard deviation. * Indicates statistical significance after multiple testing using the Holm-Sidak method with alpha=0.05.



		Normal				Tumor			
	Species	**%**	**Composition**	**% C16**	**% C18**	**%**	**Composition**	**% C16**	**% C18**
Patient 1	**PC34:3**	57.36	PC16:0/18:3	100.00	0.00	27.91	PC16:0/18:3	100.00	0.00
						33.33	PC18:3/16:0		
		42.64	PC16:1/18:2			38.76	PC16:1/18:2		
	PC36:3	31.71	PC16:0/20:3	36.90	63.10	36.20	PC16:0/20:3	42.41	57.59
		5.19	PC20:3/16:0			6.22	PC20:3/16:0		
		51.42	PC18:1/18:2			47.30	PC18:1/18:2		
		11.68	PC18:2/18:1			10.28	PC18:2/18:1		
	PC38:3	100.00	PC18:0/20:3	0.00	100.00	68.08	PC18:0/20:3	0.00	92.15
						8.36	PC18:1/20:2		
						15.71	PC20:2/18:1		
						7.85	PC20:1/18:2		
Patient 2	**PC34:3**			100.00	0.00	6.32	PC14:0/20:3	75.18	18.50
		50.58	PC16:0/18:3			17.38	PC16:0/18:3		
		12.19	PC18:3/16:0			11.52	PC18:3/16:0		
		29.00	PC16:1/18:2			30.48	PC16:1/18:2		
		8.23	PC18:2/16:1			15.80	PC18:2/16:1		
						8.62	PC18:1/16:2		
						9.87	PC16:2/18:1		
	PC36:3	37.63	PC16:0/20:3	40.50	59.50	36.45	PC16:0/20:3	45.04	54.96
		2.87	PC20:3/16:0			5.51	PC20:3/16:0		
						3.08	PC20:2/16:1		
		46.76	PC18:1/18:2			39.11	PC18:1/18:2		
		12.74	PC18:2/18:1			15.86	PC18:2/18:1		
	PC38:3	15.04	PC14:1/24:2	0.00	84.96			8.36	87.15
						4.43	PC16:0/22:3		
						3.93	PC22:3/16:0		
		66.55	PC18:0/20:3			54.43	PC18:0/20:3		
		18.41	PC20:3/18:0						
						6.89	PC18:1/20:2		
						25.83	PC20:2/18:1		
						4.49	PC20:1/18:2		
Patient 3	**PC34:3**	58.99	PC16:0/18:3	100.00	0.00	27.01	PC16:0/18:3	100.00	0.00
		26.88	PC16:1/18:2			29.42	PC16:1/18:2		
		14.13	PC18:2/16:1			43.57	PC18:2/16:1		
	PC36:3	37.34	PC16:0/20:3	42.03	57.97	53.65	PC16:0/20:3	60.25	39.75
		4.70	PC20:3/16:0			6.60	PC20:3/16:0		
		41.56	PC18:1/18:2			28.57	PC18:1/18:2		
		16.41	PC18:2/18:1			11.18	PC18:2/18:1		
	PC38:3	100.00	PC18:0/20:3	0.00	100.00	69.65	PC18:0/20:3	0.00	91.62
						8.30	PC18:1/20:2		
						13.67	PC20:2/18:1		
						8.38	PC20:1/18:2		

Figure S4: Fatty acyl composition of specific phospholipid species in 3 representative human SCCs and matched normal tissues

The molecular composition of PC species from tumor and matched normal tissues was characterized by MS3 fragmentation. % C16 represents the relative abundance of PC species containing C16:0 or C16:1 in one of the fatty acyl chains. Accordingly, % C18 represents the relative abundance of PC species containing C18:0 or C18:1 in one of the fatty acyl chains. Blue indicates a decrease in relative phospholipid abundance of that species in normal tissue while red represents an increase in relative phospholipid abundance in tumor tissue as indicated by the scale bar.

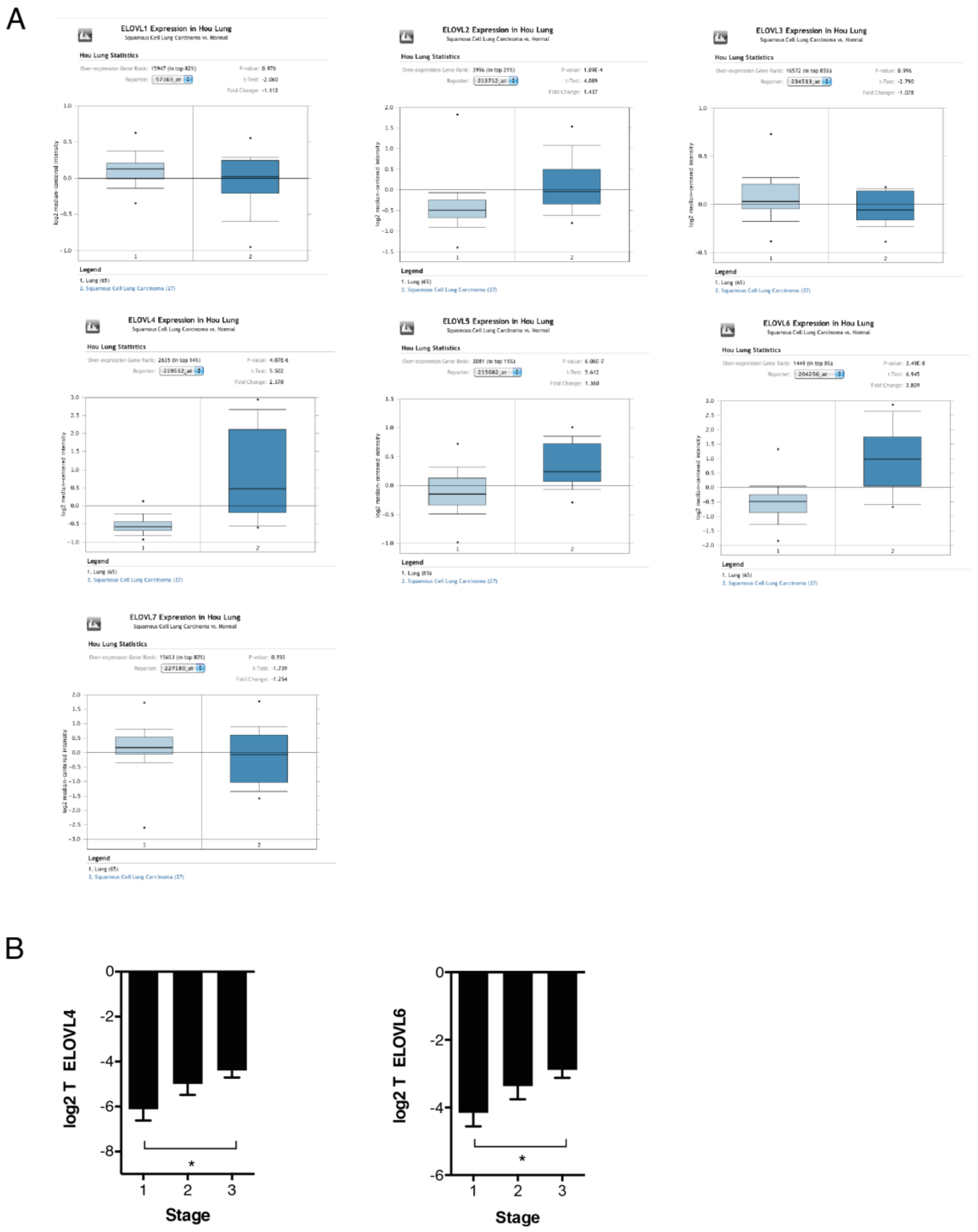


Figure S5: ELOVL6 mRNA expression in lung SCC in the Oncomine database (https://www.oncomine.org)

(A) Differences in ELOVL1-7 mRNA expression between SCC versus normal lung tissue were assessed in the Oncomine database [1] for dataset Hou *et al.* **(B)** Correlation of mRNA level of ELOVL4 and ELOVL6 with clinical stage in 61 human SCCs. * p < 0.05 (Kruskal-Wallis test/One-way ANOVA).

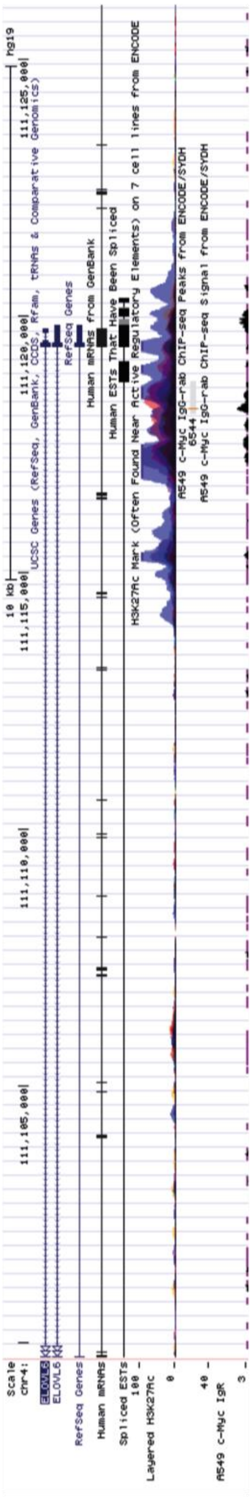


Figure S6: ChIP seq data for MYC in the lung cancer cell line A549 (http://genome.ucsc.edu)

Data were visualized using the UCSC genome browser on Human Feb. 2009 (GRCh37/hg19) Assembly [2]. (track name: A549 c-Myc IgG-rab ChIP-seq Peaks/Signal from ENCODE/SYDH)

Supplemental references

1. Rhodes DR, Yu J, Shanker K, Deshpande N, Varambally R, Ghosh D, Barrette T, Pandey A and Chinnaiyan AM. ONCOMINE: a cancer microarray database and integrated data-mining platform. Neoplasia. 2004; 6(1):1-6.

2. Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zahler AM and Haussler D. The human genome browser at UCSC. Genome Res. 2002; 12(6):996-1006.