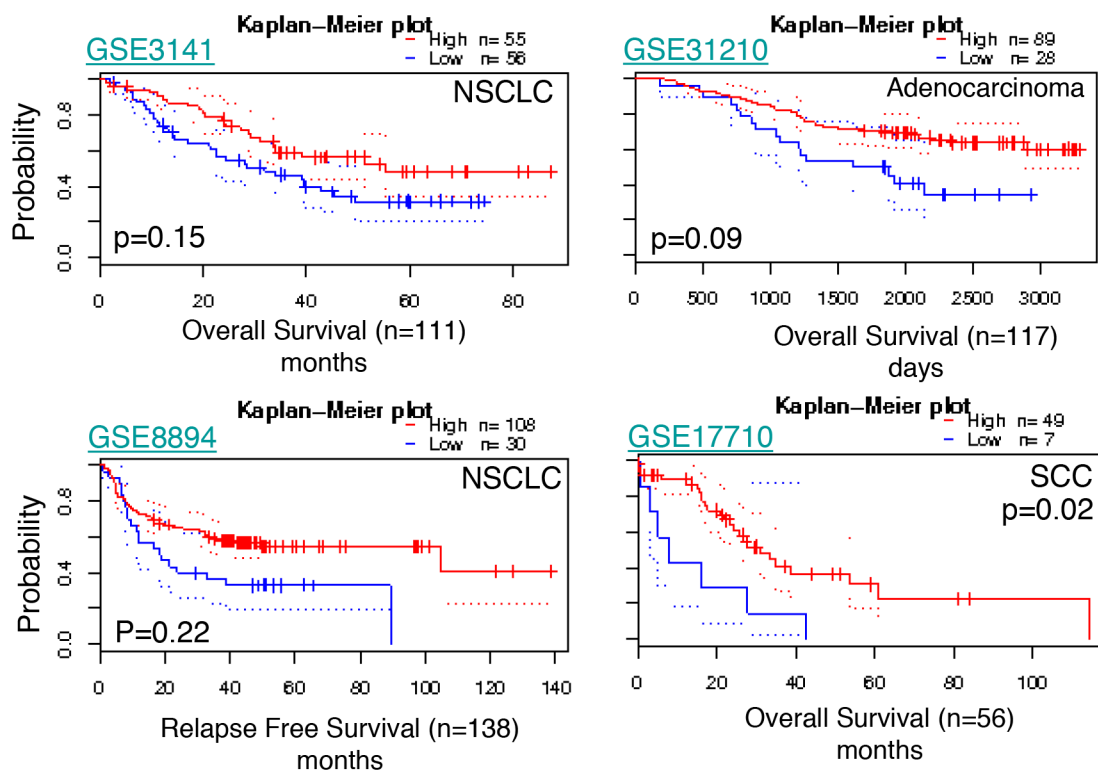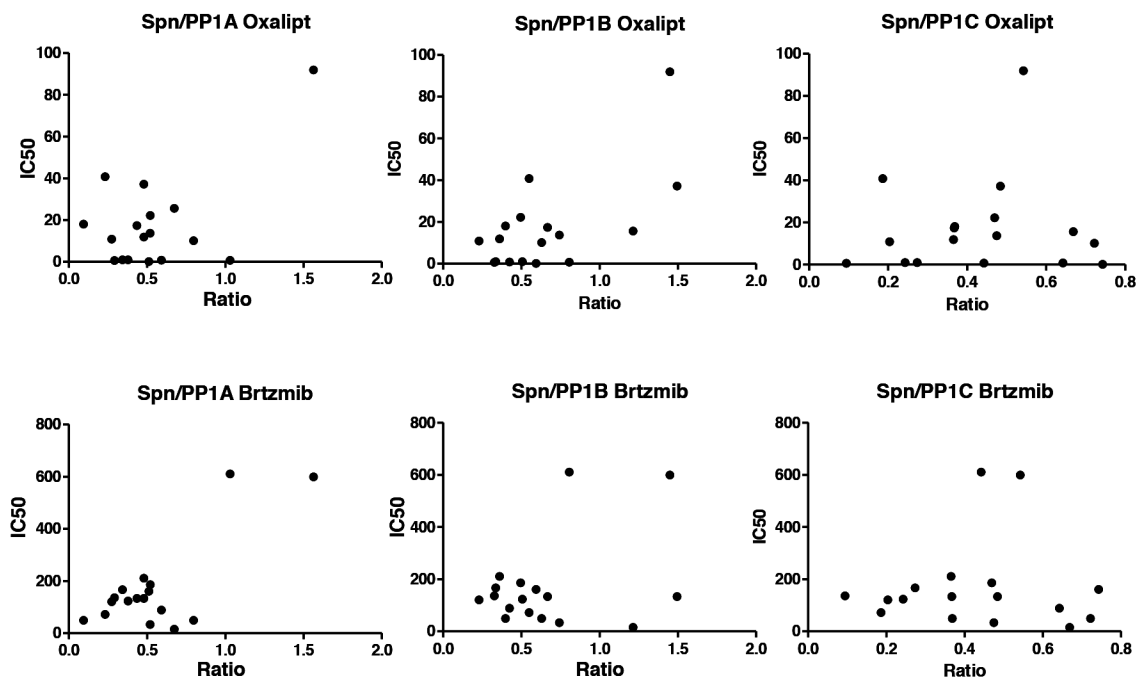# Coordinated downregulation of Spinophilin and the catalytic subunits of PP1, PPP1CA/B/C, contributes to a worse prognosis in lung cancer

SUPPLEMENTARY MATERIALS

Supplementary Figure 1: Survival probability of patients with lung cancer according to Spinophilin levels in different lung cancer patient cohorts as indicated.

Supplementary Figure 2: Graphs of the correlation between Oxaliplatin (Oxlipt) or Bortezomib (Brtzmib) and the ratios Spinophilin/PPP1CA (Spn/PP1A), Spinophilin/PPP1CB (Spn/PP1B), Spinophilin/PPP1CC (Spn/PP1C). (Pearson correlation).

Supplementary Table 1: Clinicopathological characteristics of the NSCLC cohort number 1

See Supplementary File 1

Supplementary Table 2: Characteristics of the lung cancer patient cohort used in the methylation assay. Cohort number 2

	Patients (N=47)
Gender	
Male	76.6 (36)
Female	23.4 (11)
Age (years)	67 [60–73]
Smoking status	
Smokers	40.4 (19)
Ex-smokers	44.7 (21)
Non-smoker	14.9 (7)
Packs-year	41.0 [20.0-65.7]
Histology	
Lung adenocarcinoma	57.4 (27)
Squamous cell carcinoma	42.6 (20)
Staging	
Stage I	40.5 (19)
Stage II	38.3 (18)
Stage III-IV	21.2 (10)
Subjects with COPD	42.6 (20)

Continuous variables are expressed as median [interquartile range (IQR)] and categorical variables as number of cases (%).

Supplementary Table 3: Multivariate analysis for PFS and OS in lung cancer patients

Variable	PFS		OS	
	HR (IC 95%)	p-value	HR (IC 95%)	p-value
Age	1.013 [0.991-1.035]	0.246	0.998 [0.352-2.200]	0.895
Sex	0.753 [0.309-1.835]	0.533	0.960 [0.387-2.384]	0.931
ECOG	1.680 [1.089-2.593]	0.019	1.437 [922-2.241]	0.110
Stage	1.269 [1.086-1.483]	0.003	1.212 [1.036-1.418]	0.016
Smoking habits	1.366 [0.924-2.019]	0.118	1.190 [0.799-1.774]	0.392
SPINOPHILIN	1.683 [1.092-2.596]	0.020	1.699 [1.192-2.446]	0.022

Supplementary Table 4: Methylation of PPP1CA/B and C promoters in lung tumors

Smokers	No cancer	Cancer	p
PPP1CA	0.3168	0.2506	0.000016
PPP1CB	0.2906	0.1538	0.032025R2
PPP1CC	0.4762	0.5516	0.000007
Adc (smokers)	NO CANCER	CANCER	p
PPP1CA	0.3156	0.2616	0.011022
PPP1CB	0.2863	0.1563	0.000011
PPP1CC	0.4727	0.5399	0.005708
SCC (Smokers)	NO CANCER	CANCER	p
PPP1CA	0.3237	0.2395	0.0024789
PPP1CB	0.3053	0.1512	0.032025R21
PPP1CC	0.4794	0.5634	0.0026520
Non-Smokers	NO CANCER	CANCER	p
PPP1CA	0.2925	0.2500	0.338367
PPP1CB	0.2840	0.2568	0.499554
PPP1CC	0.4529	0.4702	0.628074

The data shows the average mean of methylation (see Methods) in samples analyzed. In all cases Student's T test was performed and the significance indicated (p).

Supplementary Table 5: List of genes that correlated positively or negatively with Spn (PPP1R9B), Pearson correlation r>0,350 or r<-350 (p<0.05) in the Lung Squamous cell carcinoma TCGA database (n=81). Correlation was performed in R2: Genomics Analysis and Visualization Platform. Supplementary Table 3 is provided in the excel document.

See Supplementary File 2

Supplementary Table 6A: GO biological processes affected by the genes with positive correlation to Spinophilin in Squamous cell lung tumors (TCGS database). Based on Gene list from Supplementary Table 3. Performed through Enrichr web page (http://amp.pharm.mssm.edu/Enrichr/enrich)

See Supplementary File 3

Supplementary Table 6B: GO biological processes affected by the genes with negative correlation to Spinophilin in Squamous cell lung tumors (TCGS database). Based on Gene list from Supplementary Table 3. Performed through Enrichr web page (http://amp.pharm.mssm.edu/Enrichr/enrich).

See Supplementary File 3

Supplementary Table 7: Summary of the different cell lines used in our study

Cell line	Histology	Driver mutation	Reference for driver mutation
A549	ADC	KRAS p.G12S	(Helfrich, Raben et al. 2006)
H460	ADC	KRAS p.Q61H	(Helfrich, Raben et al. 2006)
H2009	ADC	KRAS p.G12A	COSMIC
H358	ADC	KRAS p.G12C	(Helfrich, Raben et al. 2006)
H1650	ADC	EGFR E746-E750 del	(Blanco, Iwakawa et al. 2009)
H1975	ADC	EGFR L858R/T790M	COSMIC
HCC827	ADC	EGFR E746-E750 del	(Helfrich, Raben et al. 2006)
H3122	ADC	EML4-ALK v1 translocation	COSMIC
H2228	ADC	EML4-ALK v3 translocation	COSMIC
H1781	ADC	TN (*L858R mutation detected in our lab)	(Helfrich, Raben et al. 2006)
H1437	ADC	TN	(Helfrich, Raben et al. 2006)
Calu-3	ADC	TN	(Helfrich, Raben et al. 2006)
Calu-1	SSC	KRAS p.G12C	COSMIC
HTB59	SSC	KRAS p-G12V	COSMIC
H520	SSC	TN	COSMIC, (Helfrich, Raben et al. 2006)
H226	SSC	TN	COSMIC, (Helfrich, Raben et al. 2006)
NL20	NT	TN	COSMIC
NuLi-1	NT	TN	COSMIC

ADC=Adenocarcinoma, SCC=Squamous Cell Carcinoma, TN=Triple Negative, NT=Non-Tumorogenic.

Supplementary Table 8: Levels of mRNA expresión of different subunits of PP1

	Spinophilin		PPP1CA		PPP1CB		PPP1CC	
	Average	**StDev**	**Average**	**StDev**	**Average**	**StDev**	**Average**	**StDev**
1431	0.0223	0.0018	0.0331	0.0031	0.0184	0.0013	0.0334	0.0029
1650	0.0084	0.0006	0.0105	0.0006	0.0134	0.0007	0.0116	0.0011
1781	0.0197	0.0009	0.0334	0.001	0.0467	0.0023	0.0307	0.001
1975	0.0077	0.0002	0.0822	0.0036	0.0194	0.0014	0.021	0.0013
2009	0.0146	0.0007	0.0304	0.0013	0.0405	0.0021	0.0399	0.0028
2228	0.0069	0.0002	0.0144	0.0007	0.0046	0.0001	0.0143	0.0008
226	0.0067	0.0002	0.0179	0.0007	0.0134	0.0007	0.0279	0.0011
3122	0.0081	0.0003	0.0158	0.0004	0.0136	0.0015	0.0109	0.0002
358	0.0151	0.0004	0.044	0.0012	0.0449	0.0025	0.0552	0.0043
460	0.0094	0.0009	0.0091	0.0009	0.0116	0.0012	0.0212	0.0026
520	0.0064	0.0004	0.022	0.0013	0.0197	0.0007	0.0683	0.0039
827	0.0042	0.0003	0.0155	0.0013	0.0185	0.0014	0.0208	0.0017
Calu1	0.0068	0.0004	0.0132	0.0011	0.0139	0.0008	0.0146	0.0009
Calu3	0.0283	0.0014	0.0181	0.001	0.0195	0.0008	0.0522	0.0028
A549	0.01	0.0001	0.0232	0.0009	0.0151	0.0008	0.0274	0.0008
NL20	0.005	0.0003	0.0217	0.001	0.0091	0.0006	0.027	0.0011
Nuli1	0.0125	0.0008	0.0242	0.0012	0.0169	0.0009	0.0265	0.0021

High Spn: 1431, 1781, 2009, 358, Calu3, Nuli1.
Low Spn: 1650, 1975, 2228, 226, 3122, 460, 520, 827, Calu1, A549, NL20.

Supplementary Table 9: IC50 of different drugs tested in the panel of lung cancer cell lines

| | Oxaliplatin | | Cisplatin | | etoposide | | Metformin | | Bortezomib | |
	IC50 (uM)		IC50 (uM)		IC50 (uM)		IC50 (mM)		IC50 (uM)	
	Average	StDev	Average	StDev	Average	StDev	Average	StDev	Average	StDev
A549	1.7413	0.0280	10.339	4.1085	4.0363	5.5257	3.2386	1.1515	13.3701	1.0221
Calu-1	2.2225	1.0236	0.2142	0.0665	1.1534	1.0104	11.5545	2.5617	18.6490	5.2137
Calu-3	9.1953	1.1379	0.4032	0.2184	3.5788	4.8779	15.1990	6.7110	59.9610	6.7221
H1437	2.5605	0.1175	4.3899	0.1922	2.3559	2.1435	7.0823	1.5668	15.654	0.5861
H1650	1.0148	0.2565	6.1025	2.5515	2.4579	3.1789	10.7731	1.6111	4.9663	0.8926
H1781	0.7617	0.1123	0.0638	0.0235	0.3126	0.3896	8.30844	2.3046	8.9041	0.3519
H1975	1.8063	0.3677	5.3548	0.5610	2.5096	2.5668	15.6023	1.2434	4.9767	0.6480
H2009	1.1878	0.3026	15.2050	2.5136	5.5651	8.3600	14.5725	0.2609	21.0881	4.7322
H2228	3.7237	0.1539	3.6015	0.2659	2.4931	2.0266	12.7885	4.1366	13.3823	4.9259
H226	0.9970	0.1514	4.9107	0.1462	2.0197	2.5391	12.4089	3.5138	12.3963	0.6455
H3122	0.0852	0.0109	6.3104	0.8980	2.1355	3.6157	8.8271	0.3994	16.0860	7.2781
H358	0.9965	0.3338	0.1946	0.0856	0.5083	0.4285	14.4783	1.1773	16.7320	0.3541
H460	0.7449	0.1413	1.9565	0.5650	0.9476	0.9244	10.3559	1.8699	61.1045	1.5916
H520	0.5943	0.1953	2.6998	1.0764	1.1631	1.3456	14.2986	1.6980	13.6138	2.7517
HCC827	1.0856	0.4212	6.0309	0.9970	2.5126	3.0650	48.1803	15.7150	12.0709	0.5697
HTB59	12.3497	3.5491	34.1221	6.1334	16.6736	15.7384	0.4623	0.1653	7.1309	1.0561
NL20	4.0820	1.0444	0.0453	0.0059	1.7239	2.1023	9.4784	0.6953	7.2196	0.3583
NuLi-1	1.3710	0.4178	0.0370	0.0083	0.6086	0.687	4.0813	1.8089	3.3829	0.7317

Data from 3 independent experiments performed in triplicate.