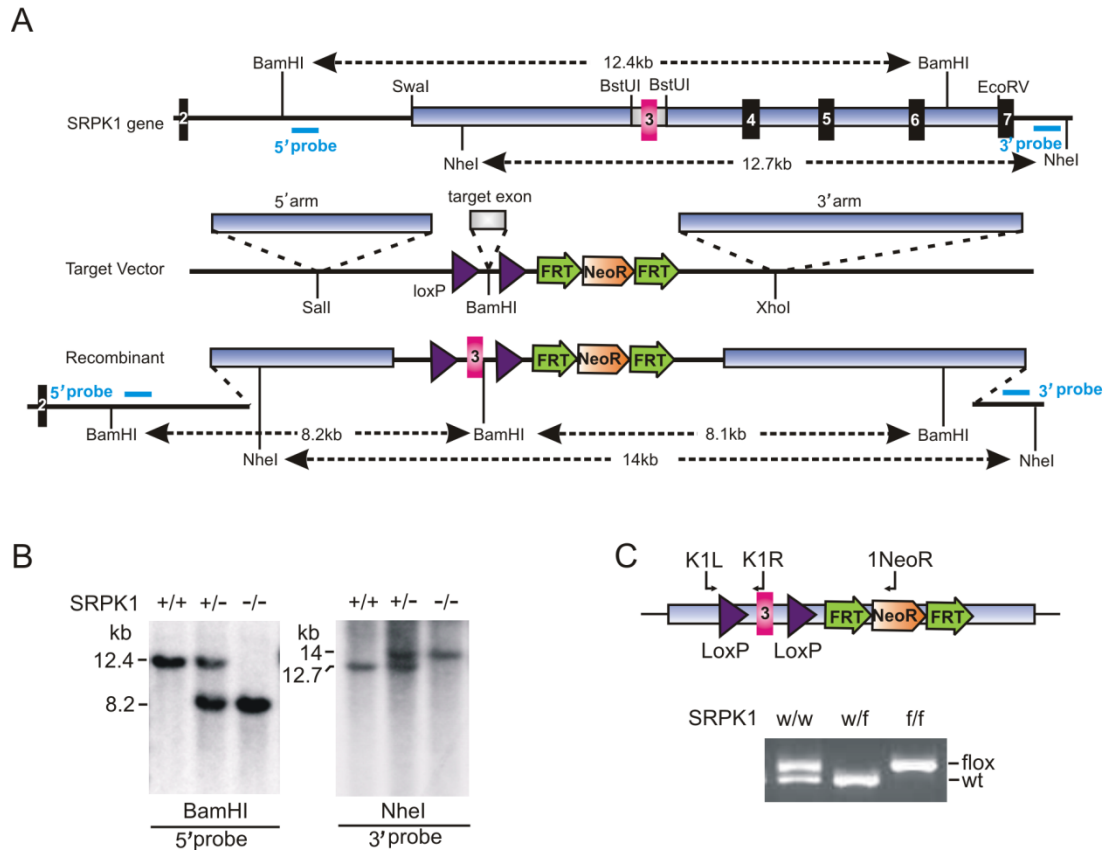


## Supplemental Information

## Supplemental Figures:

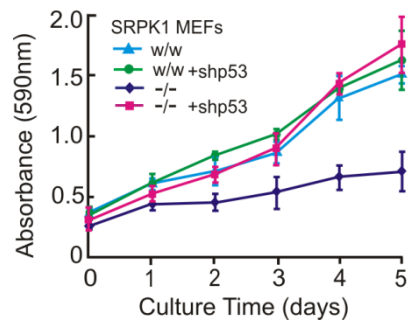


**Figure S1, Generation of SRPK1 conditional knockout mice, Related to Figure 1**

A. Schematic presentation of the mouse SRPK1 gene and the targeting strategy.

B. Southern blot analysis of targeted ES cells, confirming site-specific integration of the targeting construct by using 5' and 3' probes outside the targeting fragments.

C. PCR genotyping of germ line transmitted mice. Locations of specific primers are indicated on the diagram above.



**Figure S2, Immortalization of SRPK1 conditional knockout MEFs with p53 RNAi, Related to Figure 2**

Wild type and SRPK1<sup>-/-</sup> MEFs were immortalized with p53 RNAi. Cell growth was monitored by the MTT assay based on absorbance at 590nm. SRPK1 deletion retarded cell growth and immortalization by p53 RNAi prevented such effect. Data are shown as mean ±SD.

A

	Gene Symbol (Name)	Peptide Sequence	Phospho-Sites	1st Experiment			2nd Experiment		
				Heavy (WT)	Light (KO)	L/H	Heavy (WT)	Light (KO)	L/H
PI3K/Akt Pathway	Akt1	SGSP <sup>S</sup> DNSGAEEMEVS <sup>L</sup> LAKPK	S126	2.90E+06	4.38E+06	1.51	1.22E+06	1.68E+06	1.51
	Akt1	TIT <sup>p</sup> PPDQDMSMECPVDSER	T450	1.04E+07	1.49E+07	1.43	—	—	—
	Akt2	TIT <sup>p</sup> PPDRYDSLPLELDQR	T450	1.02E+06	1.45E+06	1.42	1.20E+06	1.88E+06	1.57
	IRS1	RHS <sup>p</sup> SETFSAPTR	S1096	1.14E+07	1.66E+07	1.46	1.34E+07	3.35E+07	2.50
	IRS1	HS <sup>p</sup> ETFSAPTR	S1097	2.48E+06	3.63E+06	1.46	2.54E+06	8.45E+06	3.33
	IRS1	RS <sup>p</sup> EDLSNYASI	S1213	2.35E+06	4.06E+06	1.73	—	—	—
	IRS1	AS <sup>p</sup> DSDGEGTMSRPA	S324	3.08E+06	5.64E+06	1.83	—	—	—
	IRS1	QSYVDTS <sup>p</sup> PVAPVSYADMR	S999	1.06E+06	2.29E+06	2.16	7.41E+05	2.54E+06	3.43
	PDPK1(PDK1)	SQTEPGS <sup>p</sup> PGIPSGVSR	S37	9.52E+06	3.62E+07	3.80	—	—	—
	PDPK1(PDK1)	ANS <sup>p</sup> FGTAQYVSPPELLTEK	S244	5.77E+07	5.97E+07	1.03	4.11E+07	5.84E+07	1.42
	PI3K3c2a	SQ <sup>p</sup> LIIR	S339	—	—	—	4.47E+05	1.01E+06	2.26
	PI3K3r1	SREYDRL <sup>p</sup> EYETR	Y467	—	—	—	1.61E+05	8.97E+05	5.57
	PI3K3r4	ANAVDQSHLD <sup>p</sup> SQK	S813	2.01E+06	4.40E+06	2.19	—	—	—
	PPP2r5d	QSP <sup>p</sup> PFNLNK	S82	1.87E+07	2.75E+07	1.47	9.95E+06	2.17E+07	2.18

	Gene Symbol (Name)	Peptide Sequence	Phospho-Sites	Heavy (WT)	Light (KO)	L/H	Heavy (WT)	Light (KO)	L/H
mTOR Pathway	mTOR	TD <sup>p</sup> YSAGQSVEILDGVELGEPAAHKK	S2448	5.00E+05	9.34E+05	1.87	—	—	—
	Rictor	S <sup>p</sup> NSVSLVPPGSSHTLPR	S1281	2.11E+06	4.12E+06	1.95	—	—	—
	Rictor	SN <sup>p</sup> VSLVPPGSSHTLPR	S1283	1.85E+06	3.78E+06	2.04	—	—	—
	Rictor	ALS <sup>p</sup> YAS <sup>p</sup> LDKEDLLSPINHNTLQR	S1387	—	—	—	5.01E+06	8.46E+06	1.69
	Tsc1	GV <sup>p</sup> IFGGQLPPYDHLFEVALPK	S591	—	—	—	4.75E+05	9.47E+05	1.99

	Gene Symbol (Name)	Peptide Sequence	Phospho-Sites	Heavy (WT)	Light (KO)	L/H	Heavy (WT)	Light (KO)	L/H
Translational Control	eIF4E-BP1	VALGDGVQLPPGDY <sup>p</sup> TPGGT <sup>p</sup> L FSTTPGGTRIYYDRK	S34, T40	—	—	—	2.29E+06	4.28E+06	1.87
	eIF4E-BP1	FLMECP <sup>RNS</sup> <sup>p</sup> PVA	S65	2.77E+07	4.25E+07	1.53	—	—	—
	eIF4E-BP1	NS <sup>p</sup> VPVAK <sup>Tp</sup> PPKDLPAIPGVTSPTS	S65, T69	2.01E+06	3.06E+06	1.52	—	—	—
	eIF4E-BP1	RVALGDGVQLPPGDYSTTPGG TLFST <sup>p</sup> TPGGTR	T45	3.20E+05	6.61E+05	2.07	—	—	—
	eIF4E-BP3	LNL <sup>T</sup> <sup>p</sup> PK	S1678	1.19E+08	1.61E+08	1.35	5.96E+06	1.31E+07	2.20
	eIF4E-BP3	TGGGGGASG <sup>p</sup> DEDEVSEVESFI LDQEDLDNPVLK	S99	—	—	—	4.08E+05	1.03E+06	2.52
	eIF4ENIF1	SG <sup>p</sup> PLEKDSGLR	S137	—	—	—	3.06E+06	7.78E+06	2.54
	eIF4ENIF1	SS <sup>p</sup> LGSTPHEELER	S352	8.35E+06	9.79E+06	1.17	1.53E+06	2.23E+06	1.46
	RPS6Ka1 (p90RSK)	TPRD <sup>p</sup> SPGIPPSAGAHQLFR	S352	—	—	—	1.55E+06	5.62E+06	3.63
	RPS6Ka1	KLPST <sup>p</sup> TL	T722	—	—	—	6.23E+05	2.25E+06	3.61
	RPS6KA4 (MSK2)	SELDVGNFAEEFTRLEPVY <sup>p</sup> SPAG SPPPGDPDR	S343, S347	9.18E+05	1.41E+06	1.54	1.76E+06	4.22E+06	2.40
	RPS6KA4	SSPPLRT <sup>p</sup> PDVLESSGPAVR	T687	—	—	—	6.88E+06	1.44E+07	2.09
	RPS6Kb1 (p70S6K)	S <sup>p</sup> PDDSTLSEANQVFLGFTYVAPS VLESVKEK	S394	—	—	—	9.18E+04	2.73E+05	2.97
	RPS6Kb1	TPV <sup>p</sup> SPVKFSPGDFWGR	S447	3.18E+06	3.42E+06	1.08	8.87E+06	1.59E+07	1.79

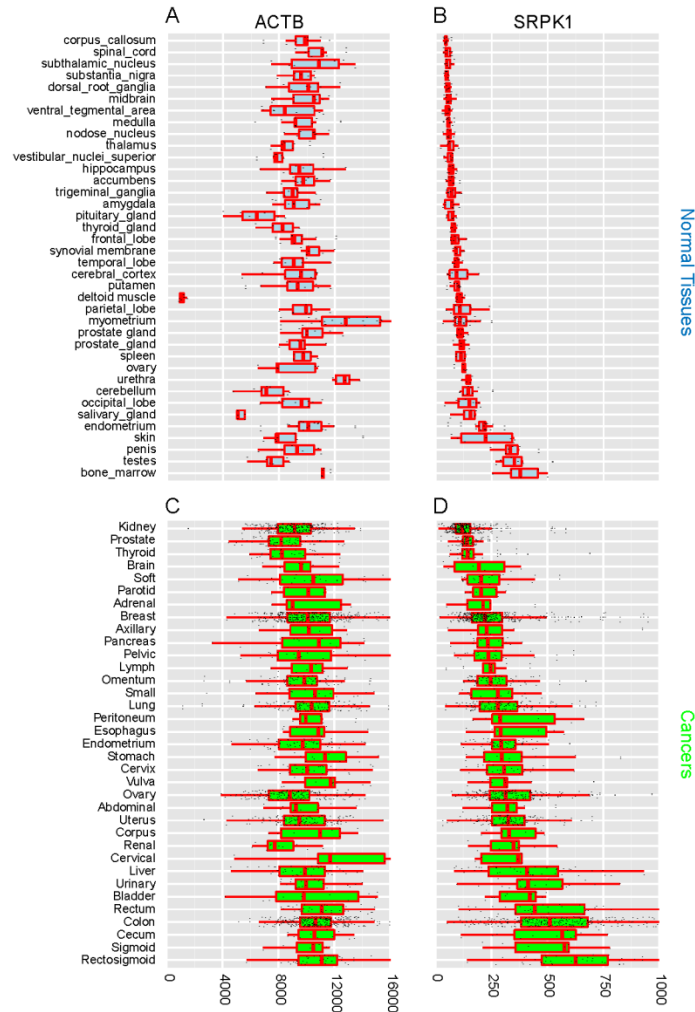
B

	Gene Symbol (Name)	Peptide Sequence	Phospho-Sites	Heavy (WT)	Light (KO)	L/H	Heavy (WT)	Light (KO)	L/H
MAPK Pathway	Raf1 (c-Raf)	RA <sup>p</sup> DDGKLTDDSSK	S43	1.32E+07	1.38E+07	1.05	3.30E+06	5.04E+06	1.53
	MAPK1(ERK2)	VADPHDHTGFLTEY <sup>p</sup> VATR	Y185	3.02E+06	2.84E+06	0.94	1.89E+07	2.12E+07	1.12
	MAPK3(ERK1)	IADPEHDHTGFLTEY <sup>p</sup> VATR	Y205	2.86E+07	3.13E+07	1.09	4.67E+06	5.51E+06	1.18
	MAP2k4	TH <sup>p</sup> SLESSGK	S78	1.51E+07	1.49E+07	0.99	1.71E+07	2.14E+07	1.25
	MAP3k7	S <sup>p</sup> IQDLTVGTGTEPGQVSSR	S412	1.60E+07	1.78E+07	1.11	9.81E+06	1.02E+07	1.04
	MAP4k4	RD <sup>p</sup> PLQGGGQQNSQAGQR	S629	9.68E+06	1.52E+07	1.57	1.63E+06	2.66E+06	1.63
	MAP4k4	NS <sup>p</sup> TSSIEPR	S646	3.50E+06	4.96E+06	1.42	—	—	—

**Figure S3, Phosphoproteomic analysis of SRPK1-deleted MEFs by SILAC, Related to Figure 3**

A. Activation of multiple components in the Akt pathway scored from two independent SILAC experiments.

B. The phosphorylation status of representative components in the MAPK pathway.



**Figure S4, Expression of SRPK1 in normal and cancer tissues in humans, Related to Figure 7**

A and B. Boxplots of  $\beta$ -actin and SRPK1 expression in different normal human tissues (data extracted from GSE7307). The expression of SRPK1 is more tightly controlled than  $\beta$ -actin in normal human tissues.

C and D. Boxplots of  $\beta$ -actin and SRPK1 expression in different human cancers (data extracted from GSE2109). SRPK1 expression is highly variable in different human cancers.

Y-axis shows normalized probeset signals from the Affymetrix U133 plus 2.0 arrays. Each tissue/cancer group is required to have at least 5 samples.