Supplemental Data

LKB1 regulates PRMT5 activity in breast cancer

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Supplemental method

Immunofluorescence

MCF-7 cells ($7x10^4$) were grown on coverslips in 12-well plates. After treatment, cells were fixed in methanol for 2 min and washed twice in PBS. Non-specific binding was blocked using a 1% gelatin solution for 30 min at room temperature and cells were incubated with primary antibodies for 1 hr at 37°C. After PBS washes, the cells were incubated for another 1 hr at 37°C with the secondary antibody Alexa Fluor 488 (Molecular Probes) in Dako diluent, then washed in PBS and mounted on glass slides in mounting solution (Dako). The images were acquired using a fluorescent microscope.

Supplemlentary Table 1. siRNA targeted sequences

siRNA	Sequence (5'-3')
siPRMT5	CACAGUACUACAUGGCUUU[dT][dT]
siMEP50	CUCCUUACCAUUAAACUGA[dT][dT]
siLKB1 E2	CGAAGAGAAGCAGAAAAUGU[dT][dT]
siLKB1 E4	AAGGACAUCAAGCCGGGGAAC[dT][dT]
siLKB1 E14	GAGGAGGUUACGGCACAAAAA[dT][dT]

Supplementary Table 2.

List and dilutions of antibodies used for each method

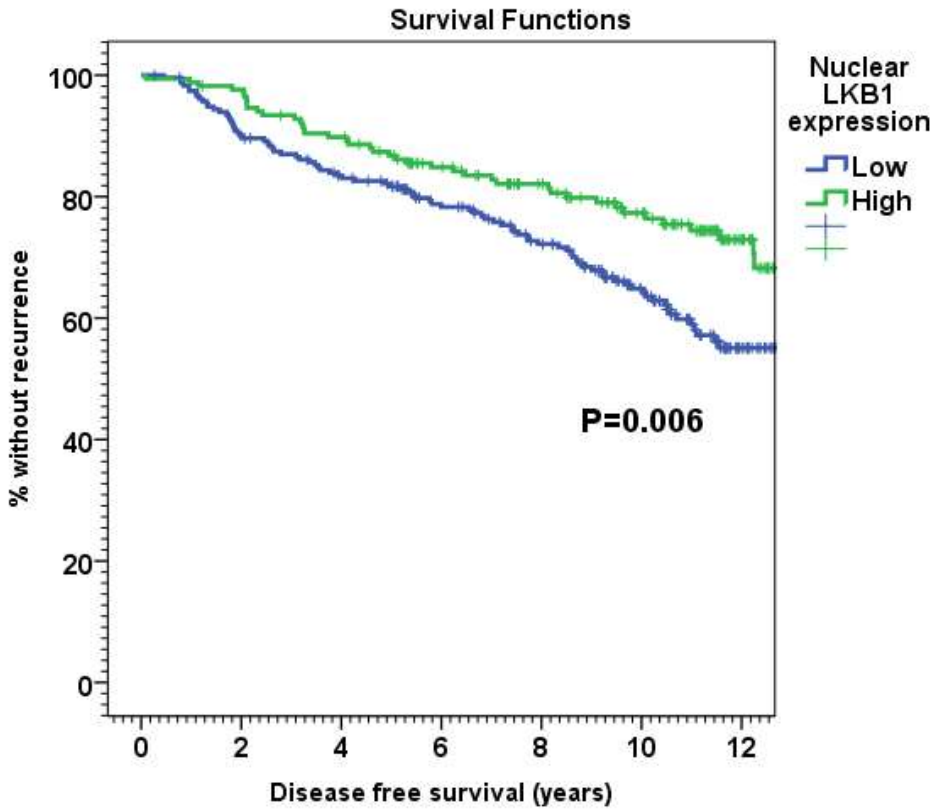
Antibody	Company	Catalog Number	Species	WB	PLA	IF	IP	IHC
Flag	Euromedex	EL1-B11	Mouse	1:1000		1:500		
GAPDH	Meridian Life Science	H86504M	Mouse	1:25000				
LKB1	Santa Cruz Biotechnology	Sc-32245	Mouse	1:1000	1:100			
LKB1	Millipore	07-694	Rabbit				2µg	
LKB1	Abcam	Ab58786	Rabbit					1:50
MEP50	Cell Signaling Technology	2823S	Rabbit	1:1000	1:100			
Pan Phospho-T	Cell Signaling Technology	9381S	Rabbit	1:1000			8µg	
pICln	Santa Cruz Biotechnology	Sc-393525	Mouse	1:1000				
PRMT5	Santa Cruz Biotechnology	Sc-376937	Mouse	1:1000	1:100			
PRMT5	Millipore	07-405	Rabbit	1:1000	1:100			1:400
PRMT5	Gift from J. Coté		Rabbit				1µg	
RiOK1	Bethyl Laboratories	A302-456A	Rabbit	1:5000				
Tubulin	Sigma Aldrich	T6074	Mouse	1:10000				
V5	Invitrogen	P/N 46-0705	Mouse	1:5000		1:200	2µg	
V5	Cell Signaling Technology	13202S	Rabbit	1:1000			2ug	

Supplementary Table 3. Sequences of primers used to clone PRMT5 domains

Protein Domain	Primer's Type	Sequence (5'-3')
PRMT5 D1	Forward	GGATCCatggcggcgatggcggtcggggg
	Reverse	CTCGAGagtgtggatgtggttggtcaaaac
PRMT5 D2	Forward	GGATCCggccatcactcttccatgttctgg
	Reverse	CTCGAGgcttaagtattccaggtattggagg
PRMT5 D3	Forward	GGATCCcagaaccgtcctccacctaatgcc
	Reverse	CTCGAGatcatcttttaggaagtgctgggc
PRMT5 D4	Forward	GGATCCggtgtgagcatccccggggagtac
	Reverse	CTCGAGgaggccaatggtatatgagcggcc
PRMT5 D1a	Reverse	CTCGAGtcccacaattagcgtattccagtc
PRMT5 D1b	Forward	GGATCCaagctttctccatggattcgtcc

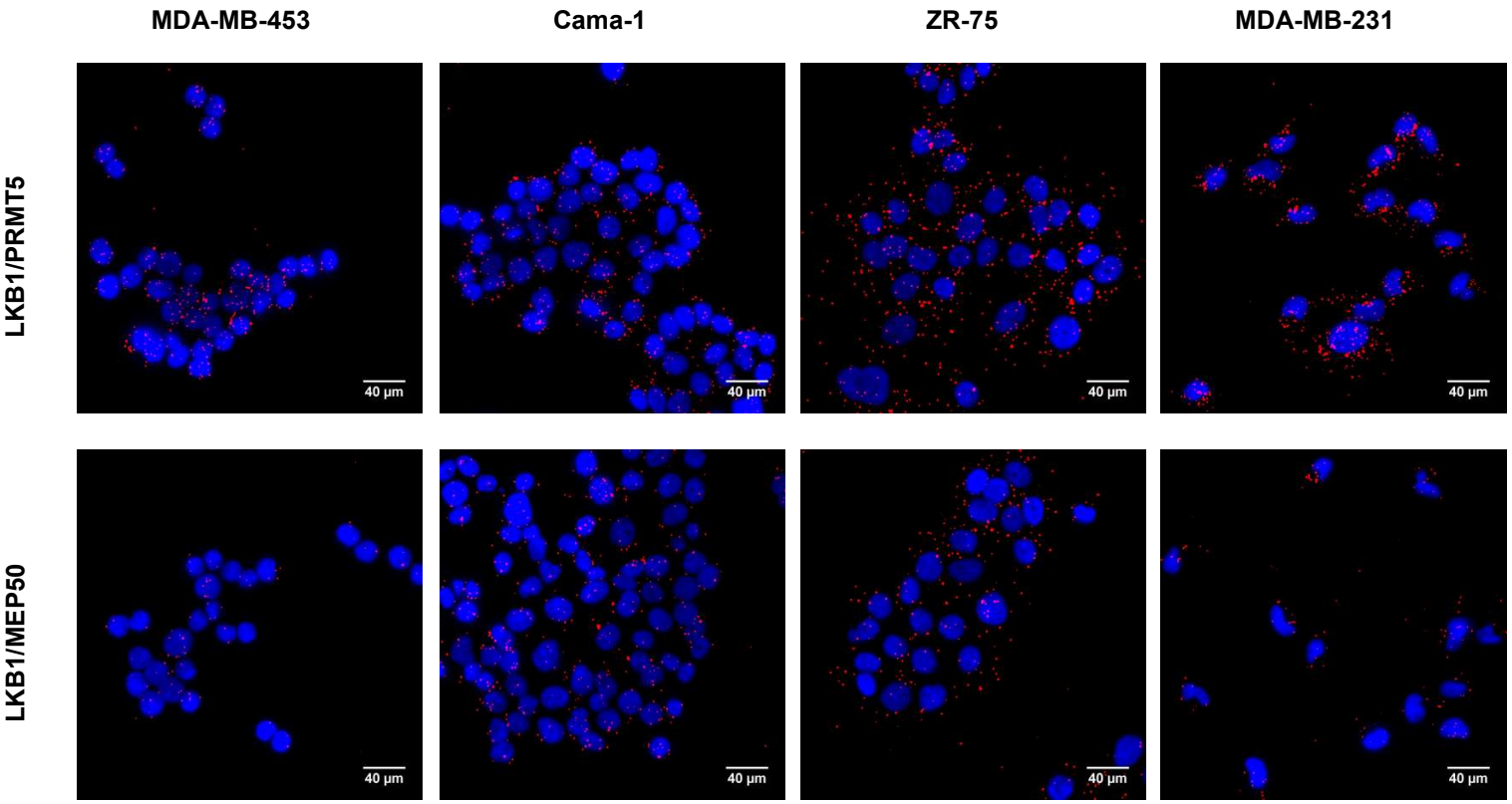
Supplementary Table 4.
Baseline characteristics for the 433 patients included in the TMA study.

Characteristics		Number	Percent
Age group	*≤ 50 years*	113	26.1%
	>50 years	320	73.9%
Menopausal status	*Premenopausal*	121	28.5%
	Post-menopausal	303	71.5%
	Unknown	9	
BMI	*≤ 25 kg/m²*	258	61.9%
	> 25 Kg/m²	159	38.1%
	Unknown	16	
Tumor size	*≤2cm*	252	58.2%
	>2cm	181	41.8%
Axillary LN metastasis	*No*	184	42.5%
	Yes	249	57.5%
SBR grade	*I*	82	18.9%
	II	207	47.8%
	III	144	33.3%
ERα status	*Negative*	56	12.9%
	Positive	377	87.1%
PR status	*Negative*	109	25.2%
	Positive	324	74.8%
HER2 status	*Negative*	397	92.8%
	Positive	31	7.2%
	Missing	5	
Breast cancer subtype	*Luminal A*	243	56.1%
	Luminal B	134	30.9%
	HER2 enriched	11	4.6%
	TNBC	45	10.4%
Adjuvant hormonal regimen	*Tamoxifen*	173	46.6%
	Sequential Tamoxifen-Aromatase Inhibitor	198	53.4%
	Unknown	62	
Nuclear PRMT5 expression	*Low (H score ≤ 70)*	141	36.2%
	High (H score >70)	249	63.8%
	Unknown	43	



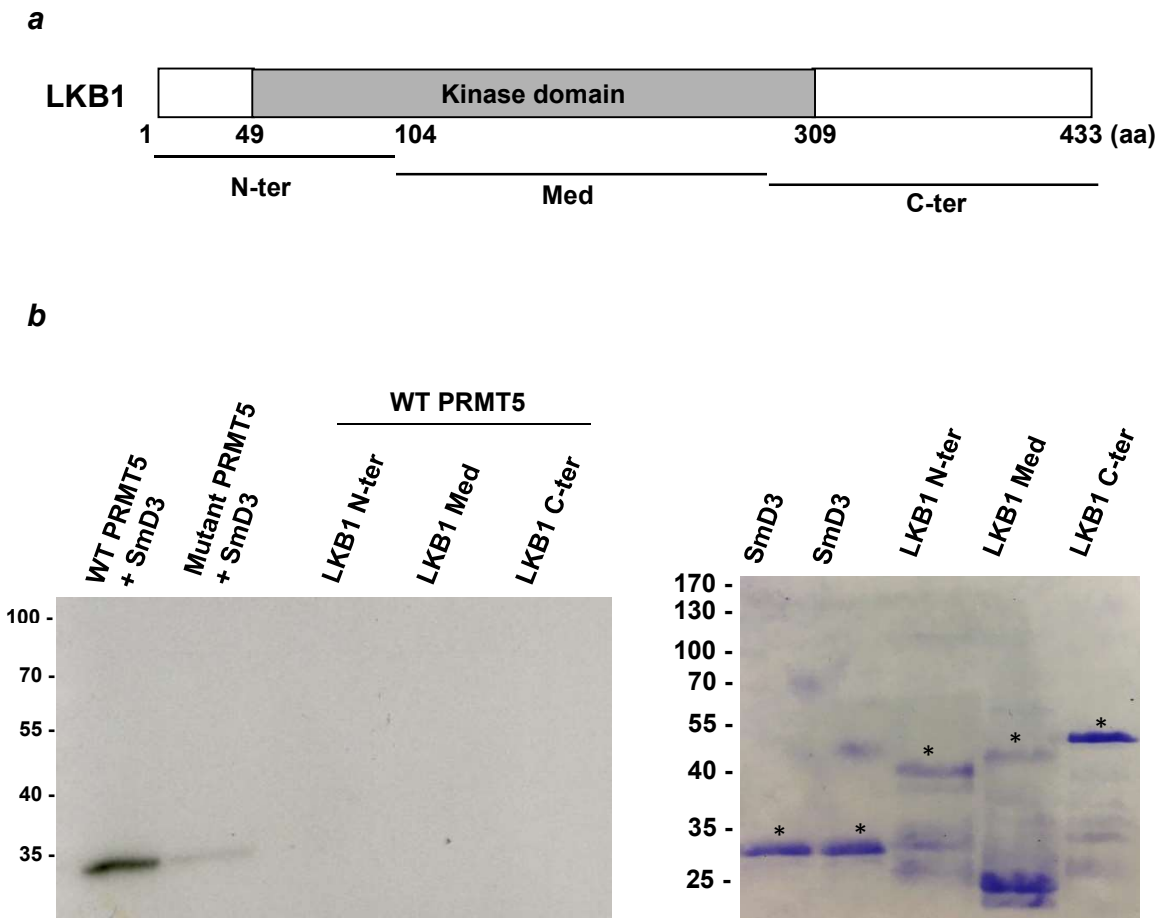
Supplementary Figure 1: LKB1 espression in breast cancer.

Kaplan-Meier plot of disease free survival (DFS) in the 433 patient samples across high LKB1 (i.e. H score > 0) in green versus low LKB1 expression (i.e. H score 0) in blue.

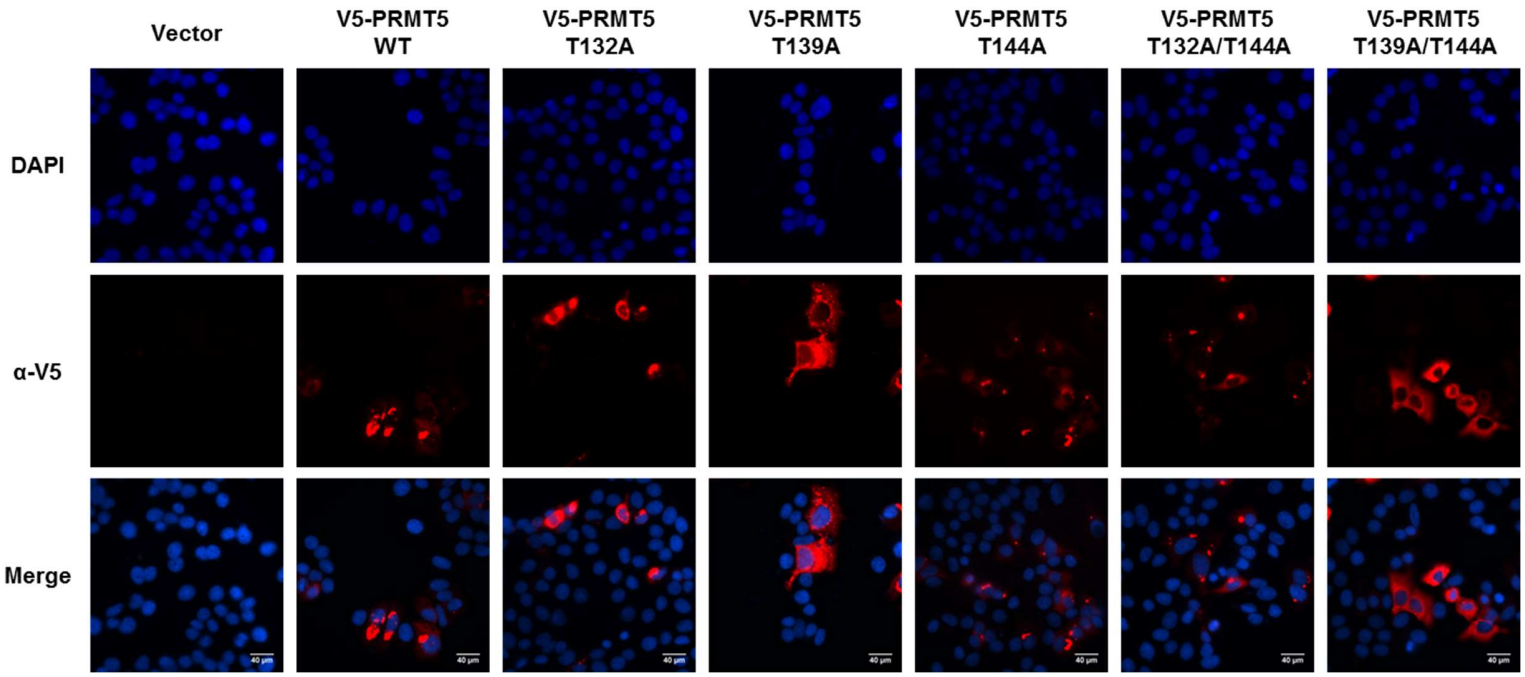


Supplementary Figure 2: LKB1 interacts with PRMT5 and MEP50 in several mammary cell lines.

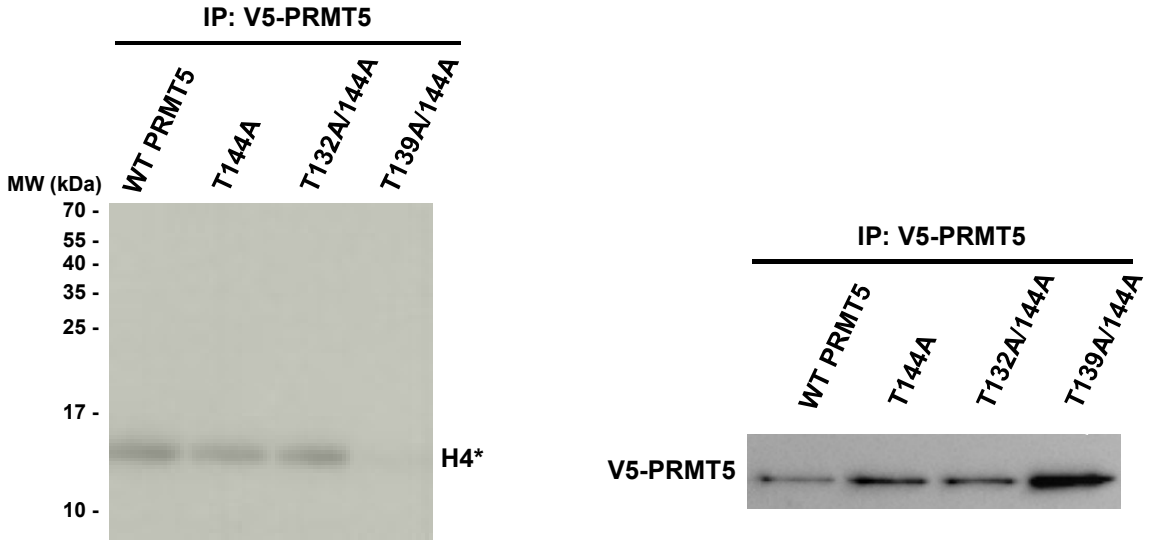
In situ Proximity Ligation Assay (PLA) for LKB1/PRMT5 and LKB1/MEP50 dimers were performed in MDA-MB-453, Cama-1, ZR-75 and MDA-MB-231 cells using LKB1, PRMT5 and MEP50 antibodies. The detected dimers are represented by red dots. The nuclei were counterstained with mounting medium containing DAPI (blue) (Obj: X60).



Supplementary Figure 3: Fragments of LKB1 are not methylated by PRMT5.
(*a*) Schematic representation of the different domains of LKB1.
(*b*) *In vitro* methylation of LKB1 was performed by incubating purified LKB1 fragments (2µg) with immunoprecipitated V5-PRMT5 (from 500µg of cell lysate overexpressing V5-PRMT5) in the presence of 0,75 µM of [methyl-^{3}H] SAM. 2µg of SmD3 was used as a positive control. Mutant PRMT5 was used as a negative control. Reaction products were analyzed by SDS-PAGE followed by fluorography for one week. The right panel shows the corresponding coomassie staining. * indicates the full length fusion proteins.

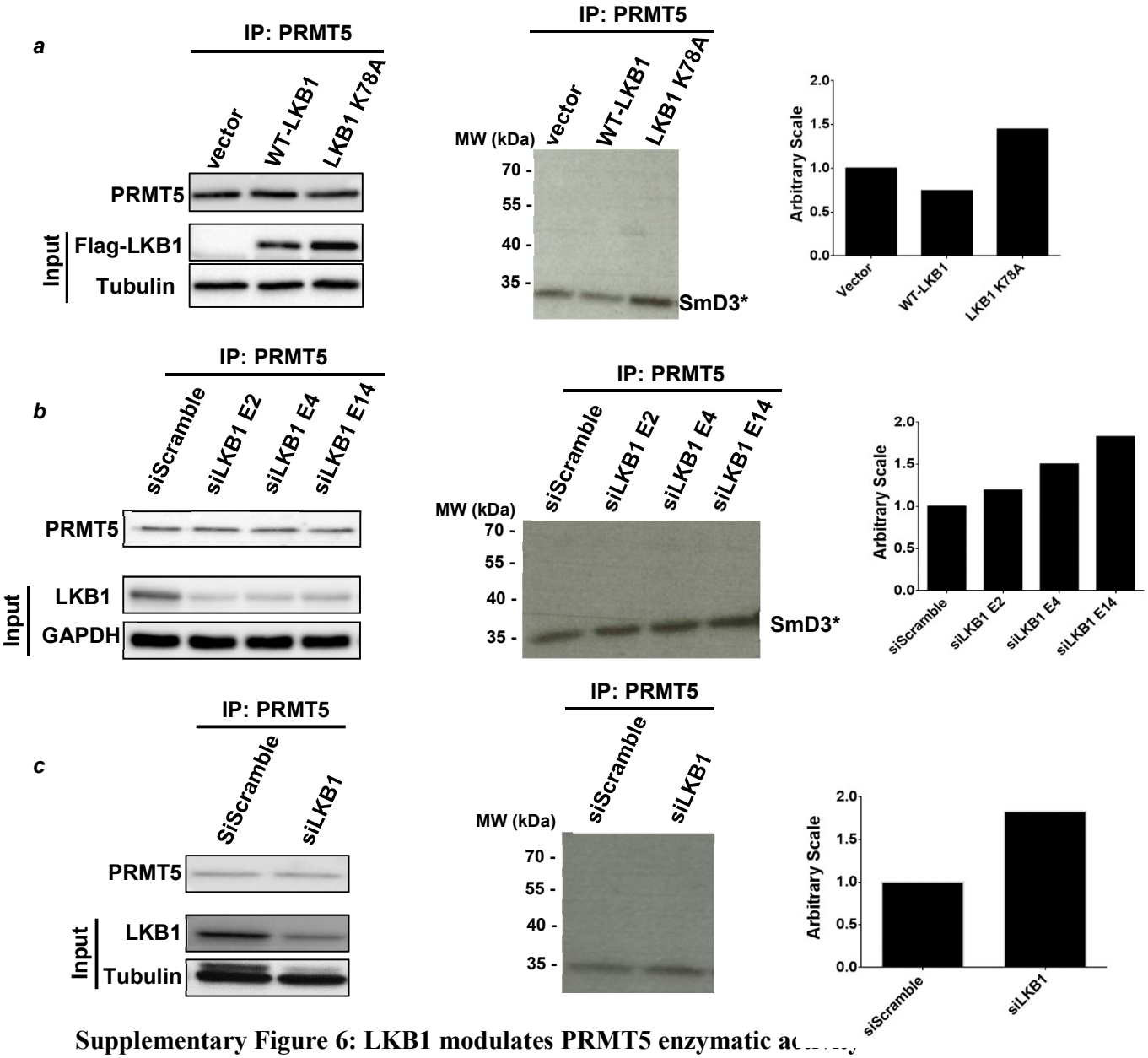


Supplementary Figure 4: Study of the localization of PRMT5 mutants.
MCF-7 cells were transfected with pcDNA3.1 vector or with V5-tagged PRMT5 WT and mutants for 48 hr, then fixed and stained with an anti-V5 antibody (red; middle panel). The nuclei were counterstained with mounting medium containing DAPI (blue; upper panel) (Obj: X60).



Supplementary Figure 5: the T139/144 mutant of PRMT5 looses its capacity to methylate histone H4

HeLa cells were transfected with the different V5-PRMT5 mutants for 48 hr. The catalytic activity of PRMT5 was assessed by immunoprecipitation using an anti-V5 antibody, followed by a radioactive *in vitro* methylation assay. 2 µg of histone H4 was incubated with the immunoprecipitated V5-PRMT5 proteins (WT and mutants) in the presence of [methyl-^{3}H] SAM. Reaction products were analyzed by SDS-PAGE followed by fluorography after 5 days of exposure (left panel). 1/20th of the IP was analyzed for V5-PRMT5 expression and protein expression was verified by Western blot (right panel).



Supplementary Figure 6: LKB1 modulates PRMT5 enzymatic ac...

(*a*) HeLa cells were transfected with pSG5Flag-LKB1 and the kinase dead mutant, pSG5Flag-LKB1 K78A for 48 hr. Their expression was tested by Western blot analysis (left panel). Cell extracts were immunoprecipitated with an anti-PRMT5 antibody followed by *in vitro* methylation experiment using SmD3 as an exogenous substrate (middle panel). 1/20th of the IP was analyzed for PRMT5 expression and LKB1 expression control was checked by Western blot with an anti-Flag antibody on the left panel. Quantification of the immunoprecipitated PRMT5 activity was performed by computer-assisted analysis (right panel). This result is representative of two independent experiments.

(*b*) MCF-7 cells were transfected with siScramble or different siRNA targeting LKB1 for 48 hr. The knock down efficacy was checked by Western blot analysis. PRMT5 activity was assessed as in a (medium panel). 1/20th of the IP was analyzed for PRMT5 expression (left panel). Quantification of the immunoprecipitated PRMT5 activity was performed as in a (right panel).

(c) The experiment was performed as in b), except that LKB1 was knocked down with a pool of siRNAs.