

Supplementary Information

Pin1 Is Involved in HDAC6-mediated Cancer Cell Motility

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Key resources table

RAGENT or RESOURCE	SOURCE	IDENTIFIER	WORKING STATUS
Antibodies			
Anti-acetylated α Tubulin (6-11B-1)	Santa Cruz	Cat# sc-23950	2k dilution
Anti-beta-Actin	SIGMA	Cat# SI-A5441-.2 ml	5k dilution
Anti-GFP	GeneTex	Cat# GTX113617	10k dilution
Anti-human HDAC6 Antibody (D-11)	Santa Cruz	Cat# sc-28386	500 dilution
Anti-human Pin1 (G-8)	Santa Cruz	Cat# sc-46660	1k dilution
Cell lines			
H1299	ATCC	ATCC® CRL-5803	RPMI 1640 + 10%FBS
HEK293	ATCC	ATCC® CRL-1573	DMEM + 10%FBS
HeLa	ATCC	ATCC® CCL-2	DMEM + 10%FBS
PC9	ATCC		RPMI 1640 + 10%FBS
Chemicals, Enzymes and Materials			
DpnI	Agilent	Cat# 500402	
Dulbecco's Modified Eagle Medium	Gibco	Cat# 12100-061	
Fetal bovine serum	Gibco	Cat# 10437-028	
G418 (Geneticin)	Thermo Fisher	Cat# 10131035	800 μ g/mL
GFP-Trap®_A	chromotek	Cat# gta-20	
Lipofectamine 2000	Invitrogene	Cat# 11668019	
Matrigel	CORNING	Cat# 354234	
Millicell® Cell Culture Inserts	Merck Millipore	Cat# MCEP24H48	
Phusion high fidelity DNA polymerase	Thermo Fisher	Cat# F530S	
Puromycin	InvivoGen	Cat# ant-pr-1	2 μ g/mL
RPMI medium1640	Gibco	Cat# 31800-089	
T4 DNA Ligase (5 U/ μ L)	Thermo Fisher	Cat# EL0014	
Recombinant DNA			
pcDNA3.1(+)-flag-HDAC6	Addgene	Cat# 13823	
pEGFP-C1	Clontech	Cat# 6084-1	
GFP-HDAC6	this study	N/A	
GFP-HDAC6 S22E	this study	N/A	
GFP-HDAC6 S22A	this study	N/A	
GFP-HDAC6 S43E	this study	N/A	
GFP-HDAC6 S43A	this study	N/A	
GFP-HDAC6 S146E	this study	N/A	
GFP-HDAC6 S146A	this study	N/A	
GFP-HDAC6 S412E	this study	N/A	
GFP-HDAC6 S412A	this study	N/A	
GFP- Δ NLS-HDAC6	this study	N/A	
GFP- Δ SE14-HDAC6	this study	N/A	
GFP- Δ NLS/ Δ SE14-HDAC6	this study	N/A	
pCMVdeltaR8.91	RNAi Core Facility of Academia Sinica		
pMD.G	RNAi Core Facility of Academia Sinica		
Luciferase shRNA	RNAi Core Facility of Academia Sinica		
HDAC6 shRNA	RNAi Core Facility of Academia Sinica	TRCN0000004839	
HDAC6 shRNA	RNAi Core Facility of Academia Sinica	TRCN0000314910	
PIN1 shRNA	RNAi Core Facility of Academia Sinica	TRCN0000010577	
Other			
Leica DMI6000 B microscope	Leica	N/A	
Nikon ECLIPSE Ti microscope	Nikon	N/A	

Supplement Figure 1

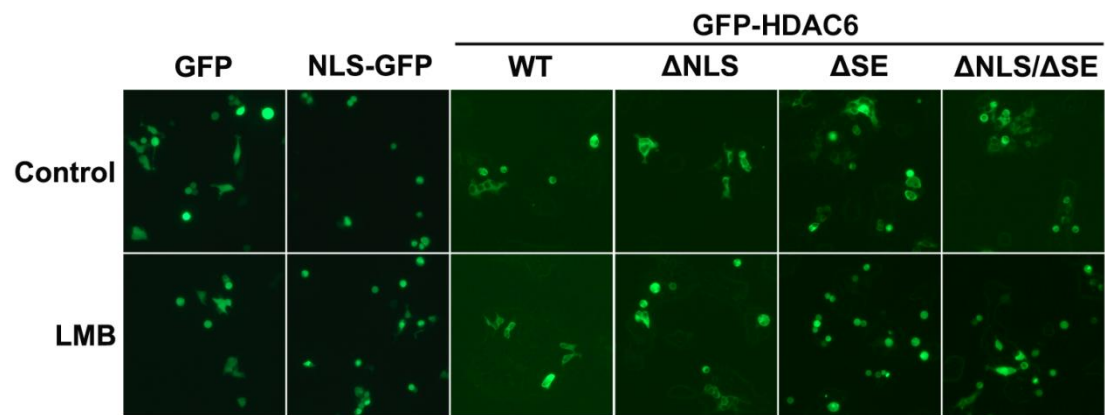
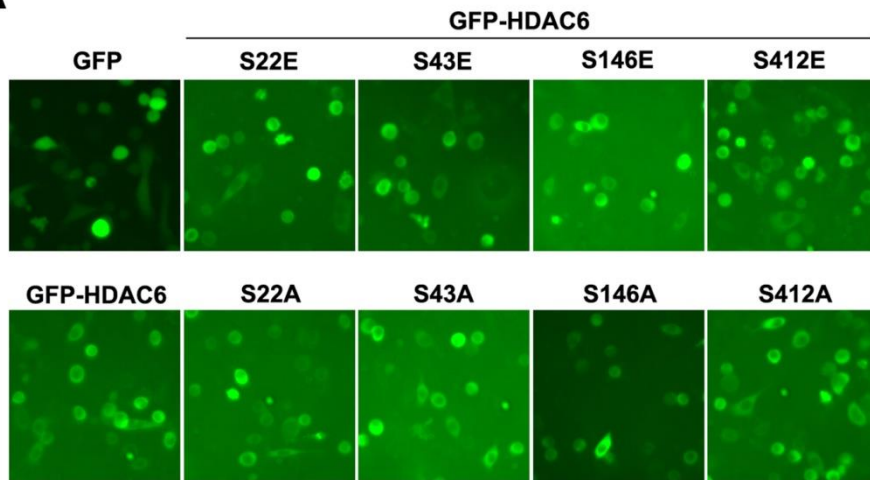


Figure supplement 1. N-ter GFP tag does not interfere the HDAC6 shuttling between cytosol and nucleus. HEK 293 cells expressing GFP fusion constructs as indicated were treated with 20 nM of leptomycin B (LMB) for 6 hours, followed by observation by fluorescence microscopy to determine the cellular localization of GFP-fusion proteins.

Supplement Figure 2

A



B

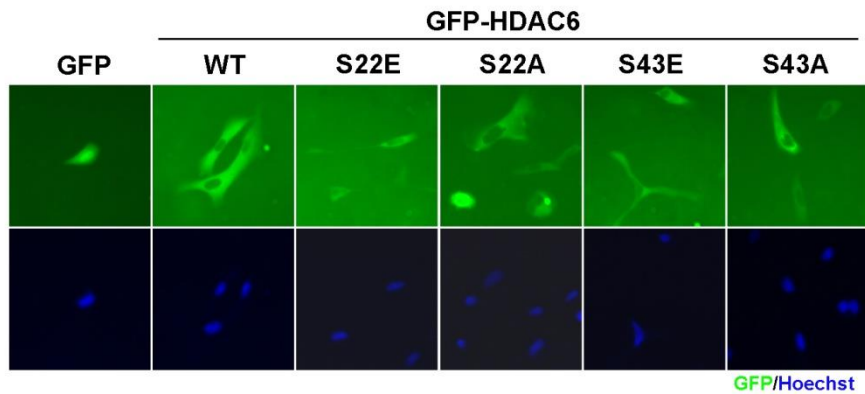


Figure supplement 2. Post-translational modifications little affect cellular localization of HDAC6. (A) PC9 and (B) HeLa cells expressing GFP, GFP-HDAC6 wild-type and various indicated mutants, respectively, were stained with Hoechst and monitored the cellular localization of GFP-fusion proteins by fluorescence microscopy.