DeSUMOylation of Gli1 by SENP1 attenuates Sonic hedgehog signaling

Huaize liu*, Sen Yan*, Jie Ding, Ting-ting Yu¶ and Steven Y. Cheng¶

Department of Developmental Genetics, School of Basic Medical Sciences, Nanjing Medical University, 101 Longmian Avenue, Nanjing, Jiangsu 211166, China

* These two authors contributed equally.

¶ Correspondence should be addressed to sycheng@njmu.edu.cn and tingting@njmu.edu.cn

SUPPLEMENTARY INFORMATION

SUPPLEMENTARY FIGURE LEGENDS

Figure S1 Knockdown efficiency of each SENP. Data are presented as means ±SD of three independent experiments. Student T test was used for statistical analysis, * P<0.05, **P<0.01.

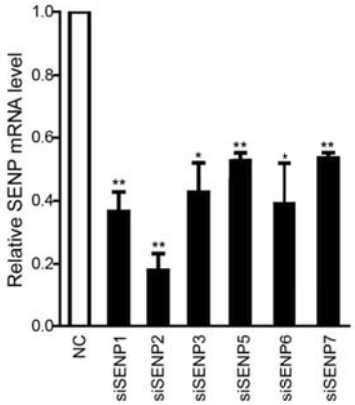
Figure S2 Proximity Ligation Assay of different mutant towards the 3 potential SUMO modifying sites. (A) Proximity Ligation Assay (PLA), a method through which we can detect the conjunction between the substrate protein and the modifier, SUMO1. The red signal requires a quite closely distance of two proteins identified by two kinds of antibodies respectively. We transfected 8 kinds of different lysine-mutant Gli1 which tagged by HA into SENP1$^{-/-}$ MEFs, and detected the red signal with anti-HA and anti-SUMO1 antibodies to ensure the presence and localization of SUMO1-modified Gli1. Except for the HA-Gli1 with full of modification and HA-Gli1-3KR without any modification at all, the other 6 mutants showed an indistinctive limited modification, indicating that all the three lysine residues could be modified and there is no dominant one. The PLA signals are quantified in (B).

Figure S3 Gli1 Ubiquitination also happens on the 3 SUMOylation lysine residues. (A) Western analysis and (B) quantification of ubiquitinated HA-Gli1 or HA-Gli1-3KR. HEK293T cells were transfected with HIS-ubiquitin, and HA-Gli1 or HA-Gli1-3KR. The cells were treated with MG132 (20μM) for 6 hours prior to harvesting for immunoprecipitation using anti-HA antibodies. Student T test was used

for statistical analysis, ** $P<0.01$.

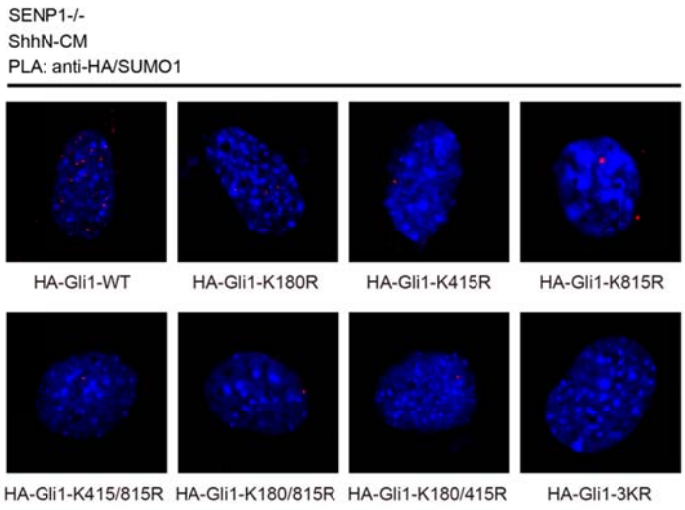
Figure S4 HA-Gli1 and HA-Gli1-3KR show different cellular localization. (A) Immunofluorescence assay shows the localization of different Gli1. HA-Gli1 or HA-Gli1-3KR is transfected into $SENP1^{-/-}$ or $SENP1^{WT}$ MEFs. MEFs are treated with conditional medium for 24 hours before fixation. (B) western shows the expression of exogenous Gli1 in $SENP1^{-/-}$ and $SENP1^{WT}$ MEFs are same. Tubulin as a loading control.

Figure S5 Nuclear localization of different Gli1 mutants. Immunofluorescence assay shows the localization of different Gli1 mutants. HA-Gli1 or HA-Gli1-3KR is transfected into $SENP1^{-/-}$ MEFs and then treated with ShhN-conditional medium for 24 hours before fixation. Two groups are treated with LMB during the last 6 hours.

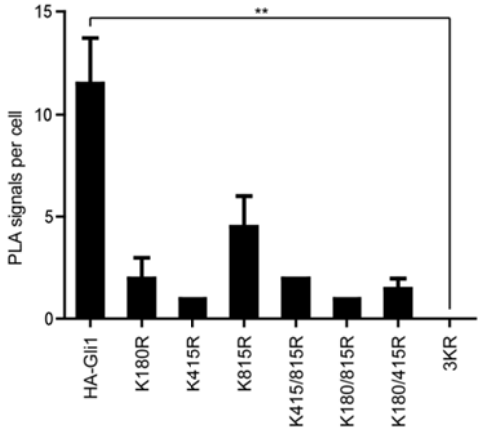

Relative SENP mRNA level
1.0
0.8
0.6
0.4
0.2
0.0
NC
siSENP1
siSENP2
siSENP3
siSENP5
siSENP6
siSENP7

sFig.1

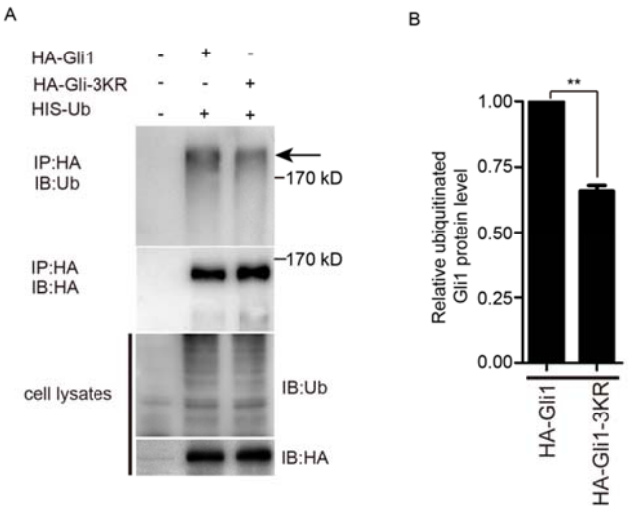
A


SENP1-/-
ShhN-CM
PLA: anti-HA/SUMO1
HA-Gli1-WT
HA-Gli1-K180R
HA-Gli1-K415R
HA-Gli1-K815R
HA-Gli1-K415/815R
HA-Gli1-K180/815R
HA-Gli1-K180/415R
HA-Gli1-3KR

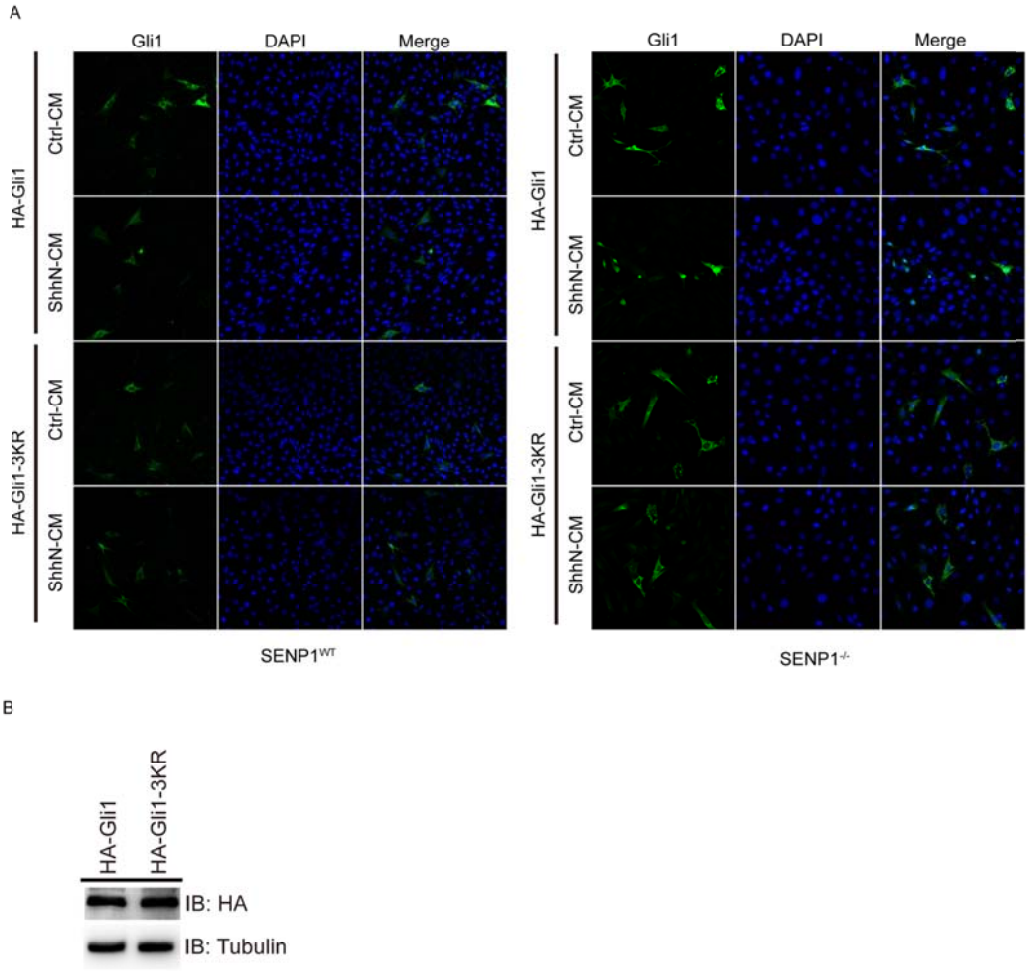
B


**
PLA signals per cell
15
10
5
0
HA-Gli1
K180R
K415R
K815R
K415/815R
K180/815R
K180/415R
3KR

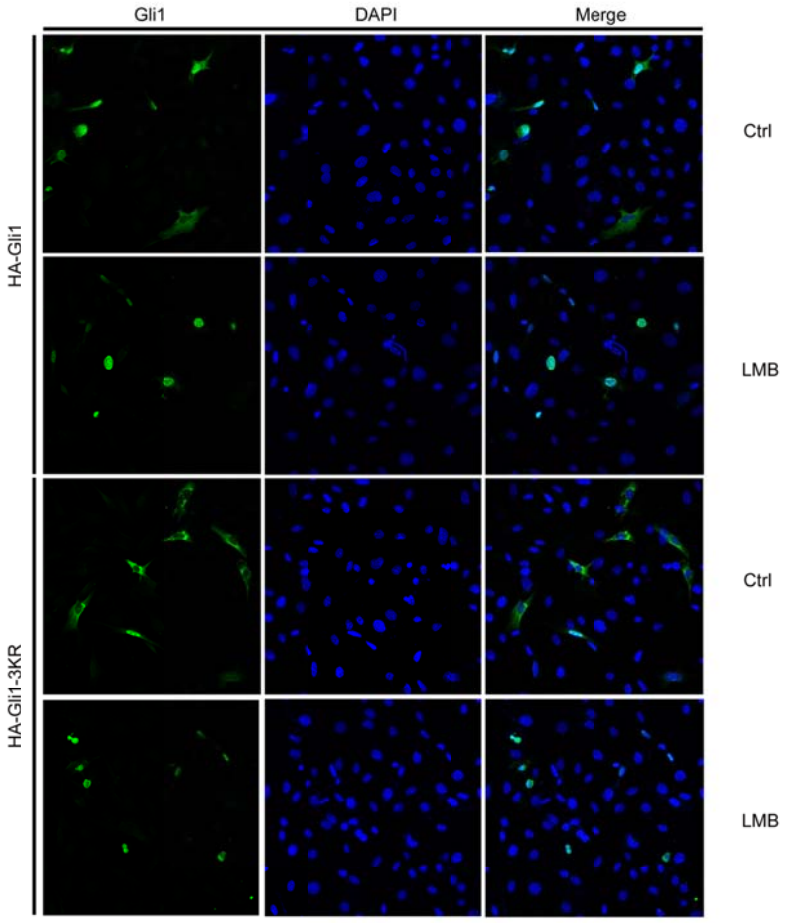
sFig.2


A
HA-Gli1 - + -
HA-Gli-3KR - - +
HIS-Ub - + +
IP:HA
IB:Ub
170 kD
IP:HA
IB:HA
170 kD
cell lysates
IB:Ub
IB:HA
B
**
1.00
0.75
0.50
0.25
0.00
Relative ubiquitinated Gli1 protein level
HA-Gli1
HA-Gli1-3KR

sFig.3


A
Gli1
DAPI
Merge
HA-Gli1
Ctrl-CM
ShhN-CM
HA-Gli1-3KR
Ctrl-CM
ShhN-CM
SENP1WT
SENP1-/-
B
HA-Gli1
HA-Gli1-3KR
IB: HA
IB: Tubulin

sFig.4



sFig.5

sTable 1

gene	organism	Forward strain	Reverse strain
SENP1	*Mus musculus*	GCAUUUCGCCUGACCAUUATT	UAAUGGUCAGGCGAAAUGCTT
	Mus musculus	GGAAGUGACUGUGGGAUGUTT	ACAUCCCACAGUCACUUCCTT
SENP2	*Mus musculus*	GGACAAACCUAUCACAUUUTT	AAAUGUGAUAGGUUUGUCCTT
	Mus musculus	GGUAAUAAAUCUCCUAAUGTT	CAUUAGGAGAUUUAUUACCTT
SENP3	*Mus musculus*	ACGAAUUCCUUCAAACGUATT	UACGUUUGAAGGAAUUCGUTT
	Mus musculus	GCACUGAUGAGGUAGUAGATT	UCUACUACCUCAUCAGUGCTT
SENP5	*Mus musculus*	GUACAGAGCUGAUUCAUGATT	UCAUGAAUCAGCUCUGUACTT
	Mus musculus	GAGGAAAGGAAUCCACUUATT	UAAGUGGAUUCCUUUCCUCTT
SENP6	*Mus musculus*	GAAAGUGACCCUCGUUAUATT	UAUAACGAGGGUCACUUUCTT
	Mus musculus	GGACAAAUCUGCUCAGUGUTT	ACACUGAGCAGAUUUGUCCTT
SENP7	*Mus musculus*	CAAAGUACCGAGUCGAAUATT	UAUUCGACUCGGUACUUUGTT
	Mus musculus	GAUAAUGAUCUACGUACUATT	UAGUACGUAGAUCAUUAUCTT

sTable 2

gene	organisms	5’-primer	3’-primer
HPRT	*Mus musculus*	TACAGGCCAGACTTTGTTGG	AACTTGCGCTCATCTTAGGC
Gli1	*Mus musculus*	GGTCTCGGGGTCTCAAACTG	TGTAGTGCTGAGCAGGTGTG
SENP1	*Mus musculus*	ACCTTTGTGGGCAAGTCCAA	GGACTTGGGGCAGGCTTAAT
SENP2	*Mus musculus*	CAGAAGAAGTGTCTGCCCGA	AAGAGGTCTTCCGTTCTTCTG
SENP3	*Mus musculus*	ATGTGTGCAGCATTGGAGACCA	AGGGATGAGGCTGCCATAAGTT
SENP5	*Mus musculus*	TGACCAGCAGAATGGCTGTGTT	TCATCGCTGACAGCACTTGT
SENP6	*Mus musculus*	TCAGGCAGAGGTGGTCTTTGAA	TTGCGCGCAGTTTCCTTTGA
SENP7	*Mus musculus*	AAGTCAGTGTCTCAGCCCTCAA	TTACTCCCAAGCCTCCCTTAGT
β-actin	*Homo sapiens*	CATCGAGCACGGCATCGTCA	TAGCACAGCCTGGATAGCAAC
Gli1	*Homo sapiens*	AGCTAGAGTCCAGAGGTTCAA	TAGACAGAGGTTGGGAGGTAAG
SENP1	*Homo sapiens*	TACGACTCCATGGGTGGGAT	GCTGAGGAATCTCCTGGCTT
SENP2	*Homo sapiens*	TTCCCATTCCAGCTGACCAC	CCAGAACCTGTCAGTTCACAA