Suppressor of Fused chaperones Gli proteins to generate transcriptional responses to Sonic Hedgehog signaling

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Supplementary Figure Legends

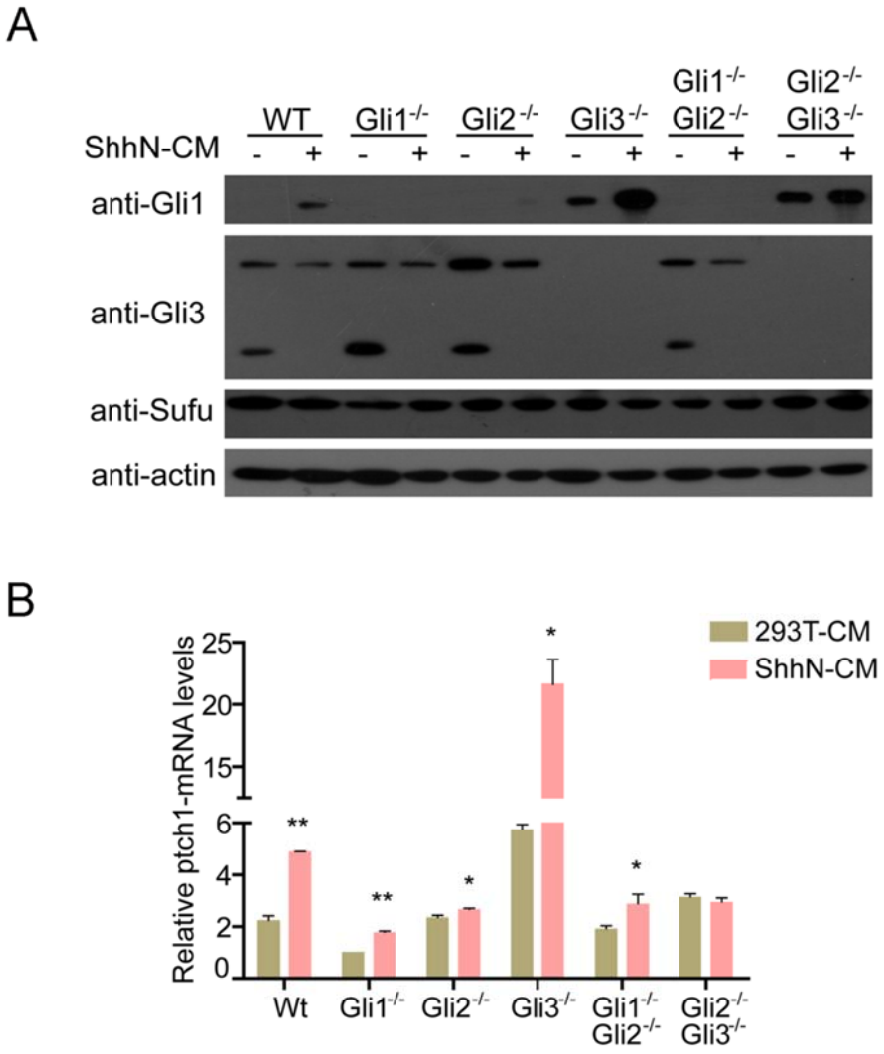
sFig.1 Shh induction of different Glis and Ptch1 in Gli mutant MEFs. (A) Western analyses of Gli expression in mutant MEFs in response to ShhN-CM treatment. Whole cell lysates from various Gli mutant MEFs were isolated following ShhN-CM treatment for 24 hours. Actin was used as a loading control. (B) qRT-PCR analyses of Ptch1 mRNA in Gli mutant MEFs following ShhN-CM treatment for 24 hours.

sFig.2 Sufu is required for stabilizing Gli1. (A) qRT-PCR analysis of Gli 1, Gli2, Gli3 and Ptch1 mRNAs and (B) Western analyses of Gli1 protein induced by ShhN-CM in normal (Wt) or $Sufu^{-/-}$ MEFs. In the absence of Sufu, Gli1 is constitutively induced. (C) Western analysis and quantification of Gli1 turnover in normal MEFs in which Sufu expression was silenced by siRNA. The stability of Gli1 decreased significantly following the knockdown of Sufu.

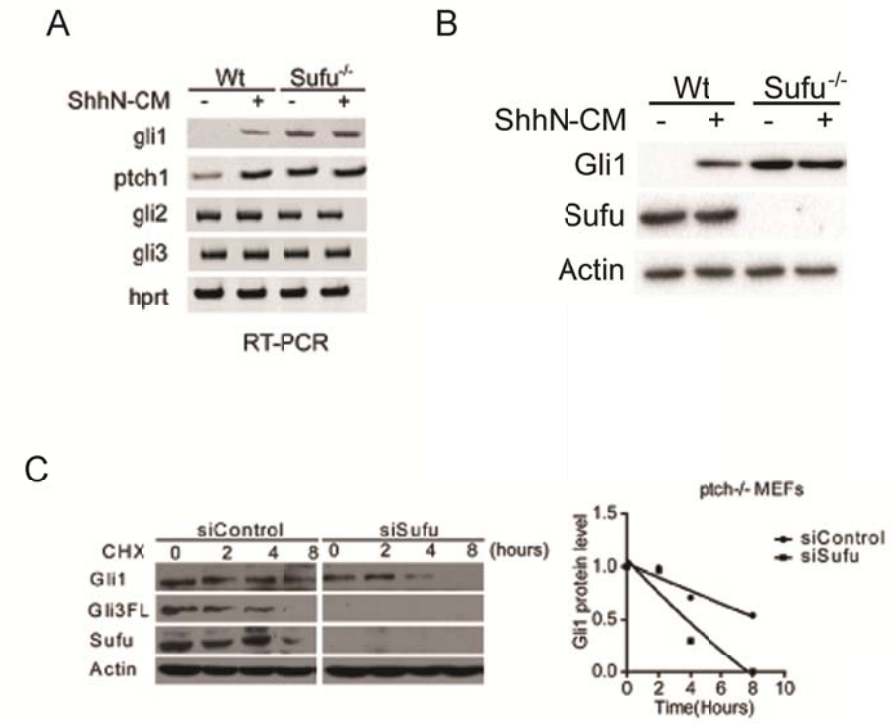
sFig.3 Shh-induced nuclear accumulation of Sufu is dependent on Gli proteins, particularly Gli1. (A) IHC staining of Sufu in $Gli2^{-/-}$;$Gli3^{-/-}$ MEFs transiently transfected with a second Gli1-specific siRNA, siGli1-2. The cells were treated with either control 293T cell conditioned medium or ShhN-CM. (B) Quantification of (A). n=30. (C) Western analysis of Gli1 expression to show the knock down efficiency of siGli1-2. Kinetics of nuclear accumulation of Sufu-GFP in (D) $Gli2^{-/-}$, (E) $Gli3^{-/-}$, and (F) $Gli1^{-/-}$;$Gli2^{-/-}$ MEFs were shown.

sFig.4 Sufu binds the chromatin with both Gli activators and repressors. (A) ChIP assays for ShhN-CM induced Sufu and SAP18 binding of the chromatin on alternate Gli-recognition sites in normal and $Sufu^{-/-}$ MEFs. The conditional medium treatment was for 24 hours. (B) ChIP assays for ShhN-CM induced dissociation of $Gli3^{R}$ from the chromatin in normal and $Gli3^{-/-}$ MEFs. (C) Quantification of ChIP assays shown in Fig.5C for the time course of ShhN-CM induced binding or dissociation.

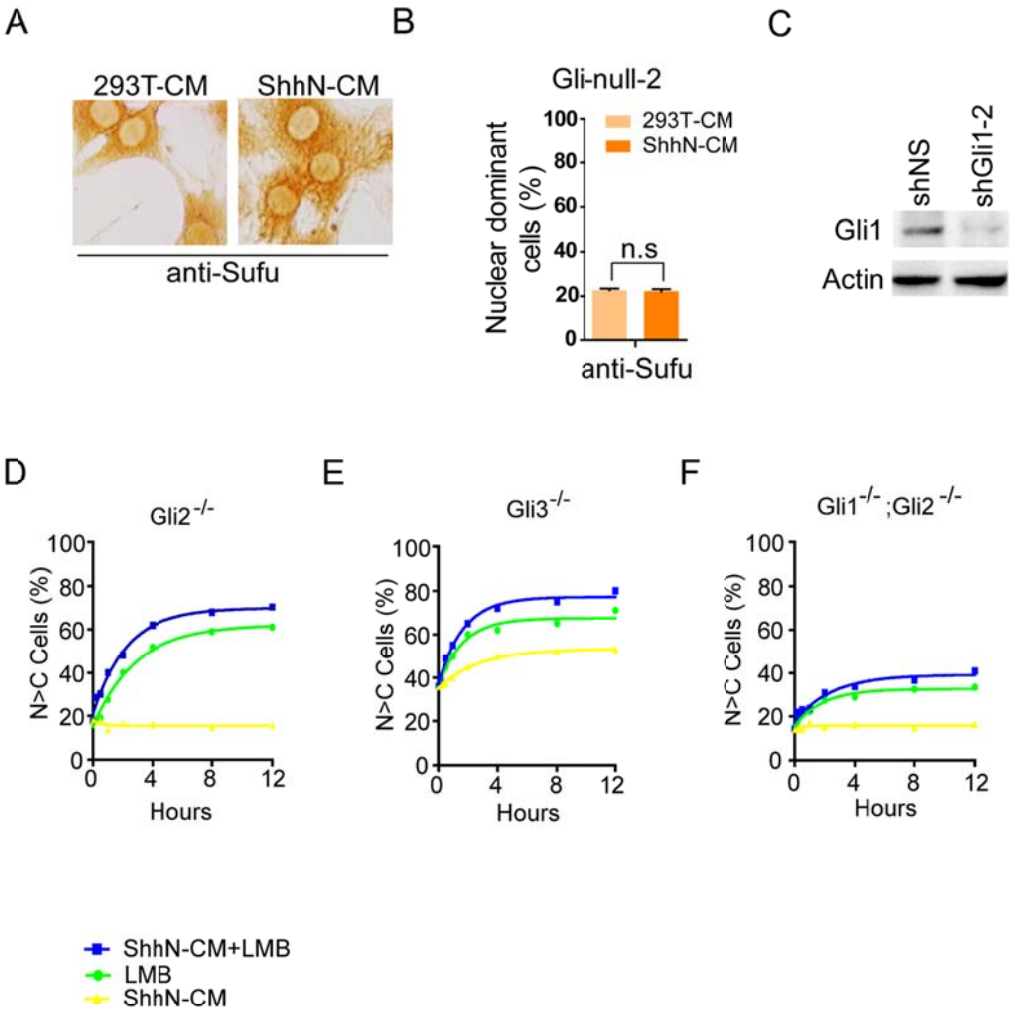
sFig.5 Association of the Gli1-Sufu and dissociation of the Gli3-Sufu-SAP18 complexes on DNA are regulated by nuclear export. ChIP assays for Gli1, Gli3, Sufu, and SAP18 were done as in Fig.5 in Gli1-only ($Gli2^{-/-}$;$Gli3^{-/-}$, left) or Gli3-only ($Gli2^{-/-}$;$Gli3^{-/-}$, right) MEFs. A Gli-binding site in Gli1 and Ptch1 promoter was assayed. ShhN-CM treatment was carried out for 24 hours and LMB treatment was given 8 hours prior to harvest.


A
WT
Gli1-/-
Gli2-/-
Gli3-/-
Gli1-/- Gli2-/-
Gli2-/- Gli3-/-
ShhN-CM
- + - + - + - + - + - +
anti-Gli1
anti-Gli3
anti-Sufu
anti-actin
B
293T-CM
ShhN-CM
Relative ptch1-mRNA levels
25
20
15
6
4
2
0
**
**
*
*
*
Wt
Gli1-/-
Gli2-/-
Gli3-/-
Gli1-/- Gli2-/-
Gli2-/- Gli3-/-

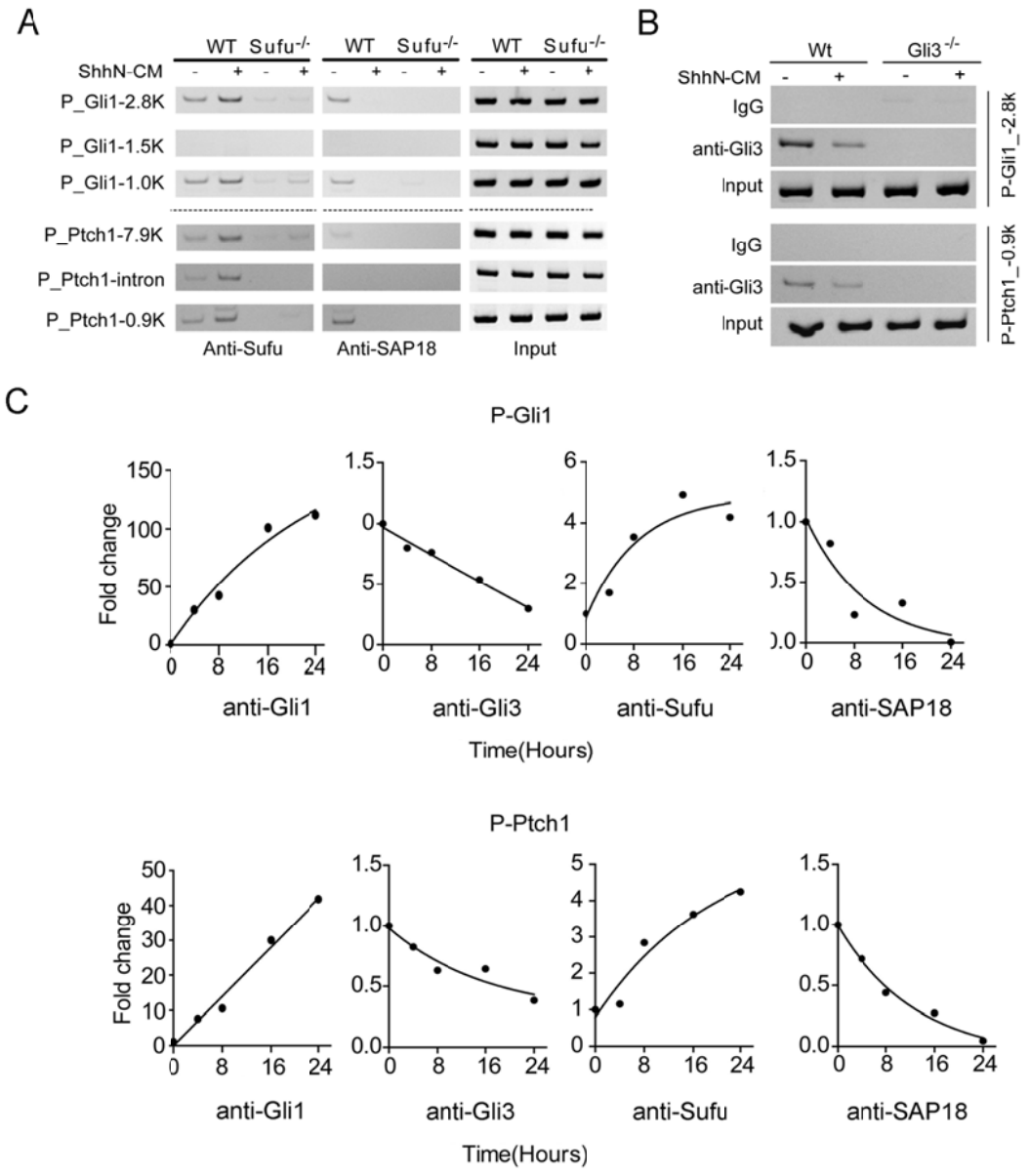
sFig.1


A
Wt
Sufu-/-
ShhN-CM
- + - +
gli1
ptch1
gli2
gli3
hprt
RT-PCR
B
Wt
Sufu-/-
ShhN-CM
- + - +
Gli1
Sufu
Actin
C
siControl
siSufu
CHX
0 2 4 8 0 2 4 8 (hours)
Gli1
Gli3FL
Sufu
Actin
ptch-/- MEFs
siControl
siSufu
Gli1 protein level
1.5
1.0
0.5
0.0
0 2 4 6 8 10
Time(Hours)

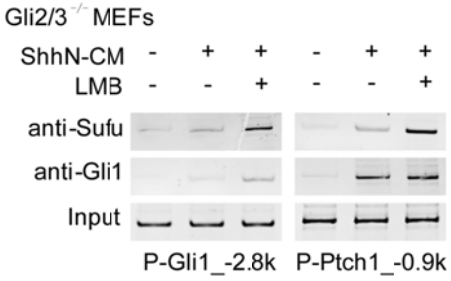
sFig.2

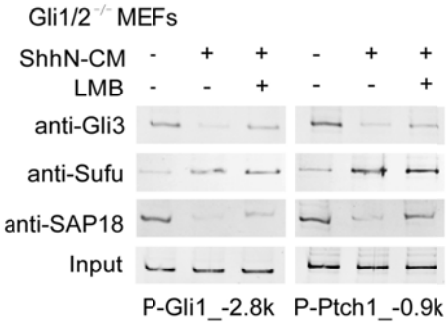

A
293T-CM
ShhN-CM
anti-Sufu
B
Gli-null-2
293T-CM
ShhN-CM
Nuclear dominant cells (%)
n.s
anti-Sufu
C
shNS
shGli1-2
Gli1
Actin
D
Gli2-/-
N>C Cells (%)
Hours
E
Gli3-/-
N>C Cells (%)
Hours
F
Gli1-/-;Gli2-/-
N>C Cells (%)
Hours
ShhN-CM+LMB
LMB
ShhN-CM

sFig.3


A
WT Sufu-/- WT Sufu-/- WT Sufu-/-
ShhN-CM - + - + - + - + - + - +
P_Gli1-2.8K
P_Gli1-1.5K
P_Gli1-1.0K
P_Ptch1-7.9K
P_Ptch1-intron
P_Ptch1-0.9K
Anti-Sufu
Anti-SAP18
Input
B
Wt
Gli3-/-
ShhN-CM - + - +
IgG
anti-Gli3
Input
P-Gli1_-2.8k
IgG
anti-Gli3
Input
P-Ptch1_-0.9k
C
P-Gli1
Fold change
anti-Gli1
anti-Gli3
anti-Sufu
anti-SAP18
Time(Hours)
P-Ptch1
Fold change
anti-Gli1
anti-Gli3
anti-Sufu
anti-SAP18
Time(Hours)

sFig.4


Gli2/3 -/- MEFs
ShhN-CM - + + - + +
LMB - - + - - +
anti-Sufu
anti-Gli1
Input
P-Gli1_-2.8k
P-Ptch1_-0.9k


Gli1/2 -/- MEFs
ShhN-CM - + + - + +
LMB - - + - - +
anti-Gli3
anti-Sufu
anti-SAP18
Input
P-Gli1_-2.8k
P-Ptch1_-0.9k

sFig.5