Cullin5 deficiency promotes small cell lung cancer metastasis by stabilizing integrin β1

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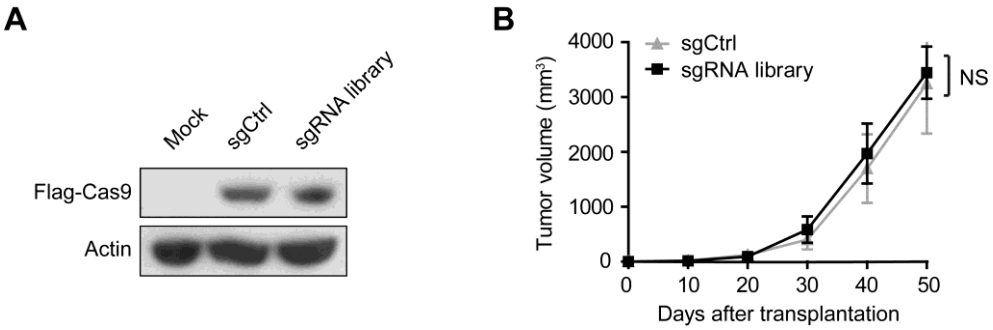
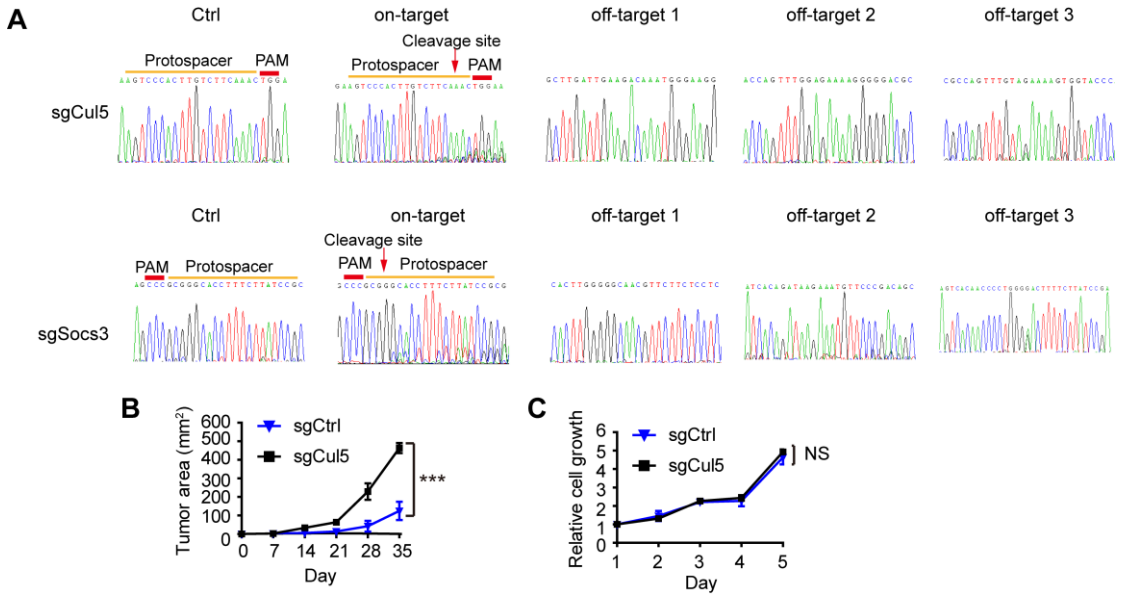
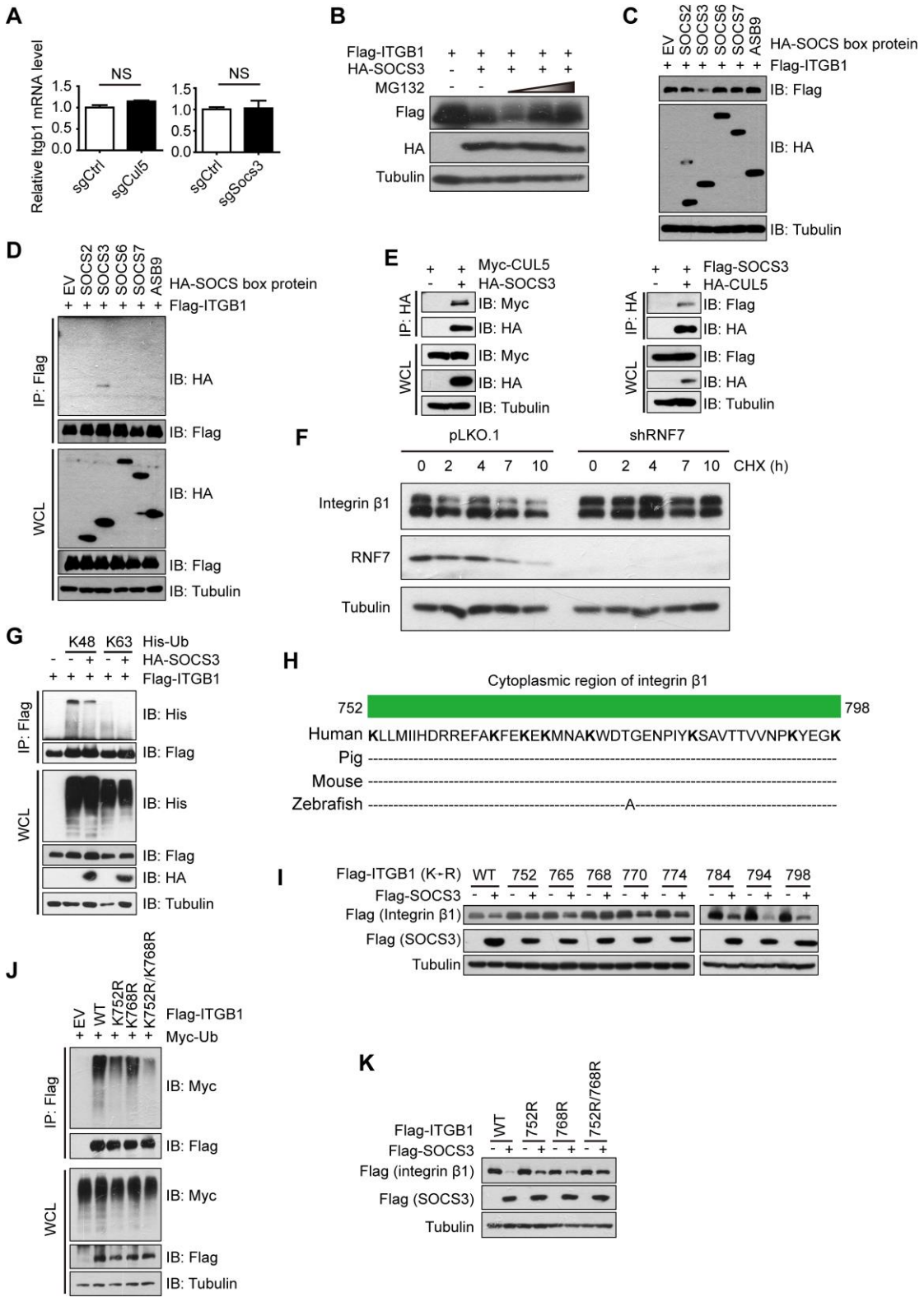


Supplemental Figure 1. A genome-wide pooled CRISPR/Cas9 screening for genes involved in mouse SCLC spontaneous metastasis in allograft assays. (**A**) Immunoblot (IB) analysis of Cas9 expression using anti-Flag antibodies. Actin served as loading control. (**B**) Primary tumor growth curve in nude mice transplanted with RT cells transduced with control sgRNA (sgCtrl) (n = 5 mice) or mGeCKOa sgRNA library (n = 60 mice). Data were shown as mean ± SEM. Statistical analysis was performed using Student's t test. NS, non-significant.



Supplemental Figure 2. Deletion of *Cul5* promotes mouse SCLC metastases in allograft assays. (**A**) The on-target or off-target cleaving activities for sg*Cul5* or sg*Socs3* were analyzed. Sequencing analyses revealed the efficacy of Cas9-mediated cleavage at protospacers. Red arrows indicated putative cleavage sites. (**B**) Primary tumor growth curve in nude mice transplanted with RT cells transduced with sg*Ctrl* or sg*Cul5* (n=5 for each group). Data were shown as mean ± SEM. Statistical analysis was performed using Student's t test. ***$P < 0.001$. (**C**) MTS assay of RT cells transduced with sgCul5. Data were shown as mean ± SEM. Statistical analysis was performed using Student's t test. NS, non-significant.


A
Relative Itgb1 mRNA level
NS
NS
sgCtrl
sgCul5
sgCtrl
sgSocs3
B
Flag-ITGB1
HA-SOCS3
MG132
Flag
HA
Tubulin
C
EV
SOCS2
SOCS3
SOCS6
SOCS7
ASB9
HA-SOCS box protein
Flag-ITGB1
IB: Flag
IB: HA
IB: Tubulin
D
EV
SOCS2
SOCS3
SOCS6
SOCS7
ASB9
HA-SOCS box protein
Flag-ITGB1
IP: Flag
IB: HA
IB: Flag
WCL
IB: HA
IB: Flag
IB: Tubulin
E
Myc-CUL5
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WCL
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IB: Flag
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WCL
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IB: HA
IB: Tubulin
F
pLKO.1
shRNF7
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Integrin β1
RNF7
Tubulin
G
K48
K63
His-Ub
HA-SOCS3
Flag-ITGB1
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IB: His
IB: Flag
WCL
IB: His
IB: Flag
IB: HA
IB: Tubulin
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798
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Pig
Mouse
Zebrafish A
I
Flag-ITGB1 (K-R)
WT
752
765
768
770
774
784
794
798
Flag-SOCS3
Flag (Integrin β1)
Flag (SOCS3)
Tubulin
J
EV
WT
K752R
K768R
K752R/K768R
Flag-ITGB1
Myc-Ub
IP: Flag
IB: Myc
IB: Flag
WCL
IB: Myc
IB: Flag
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752R
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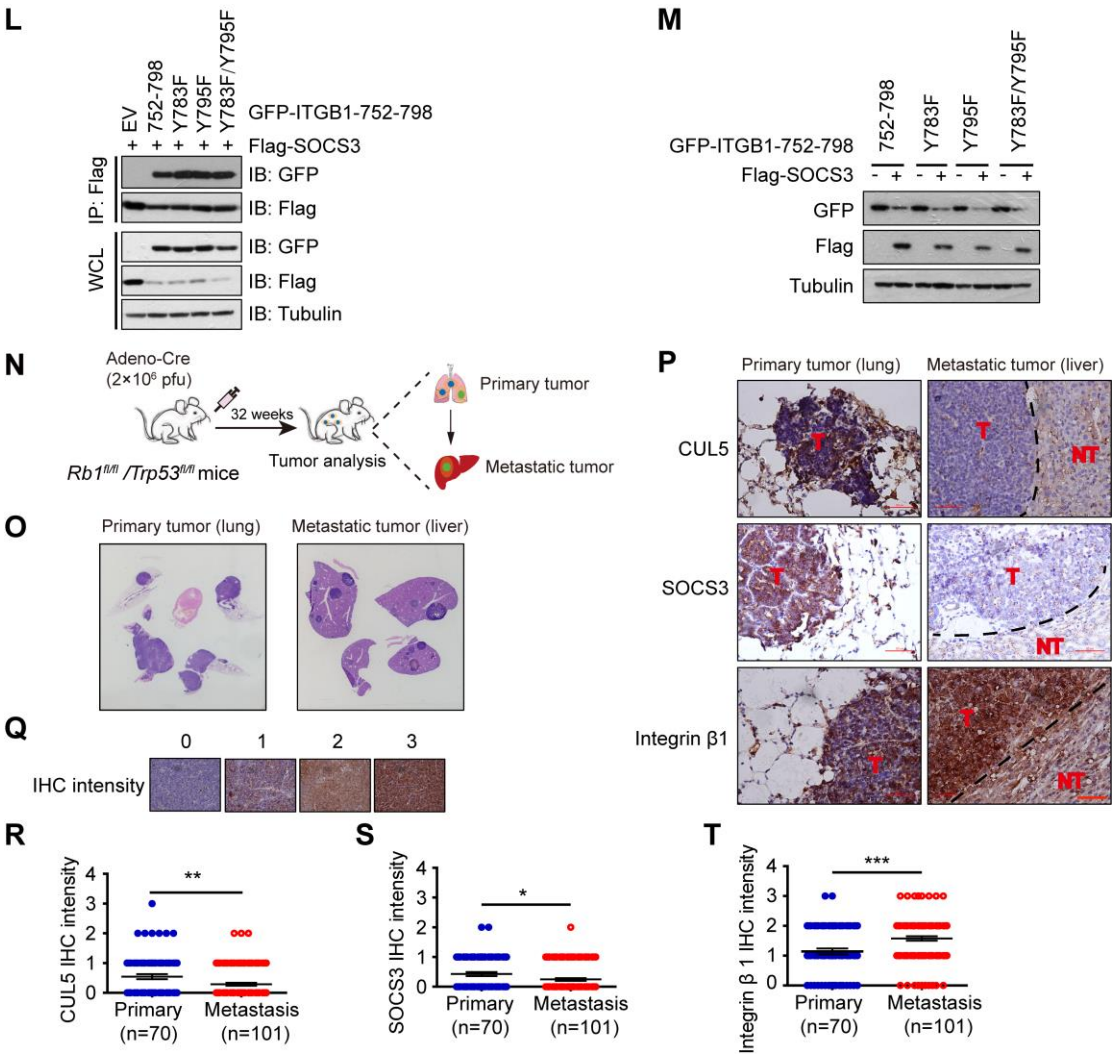
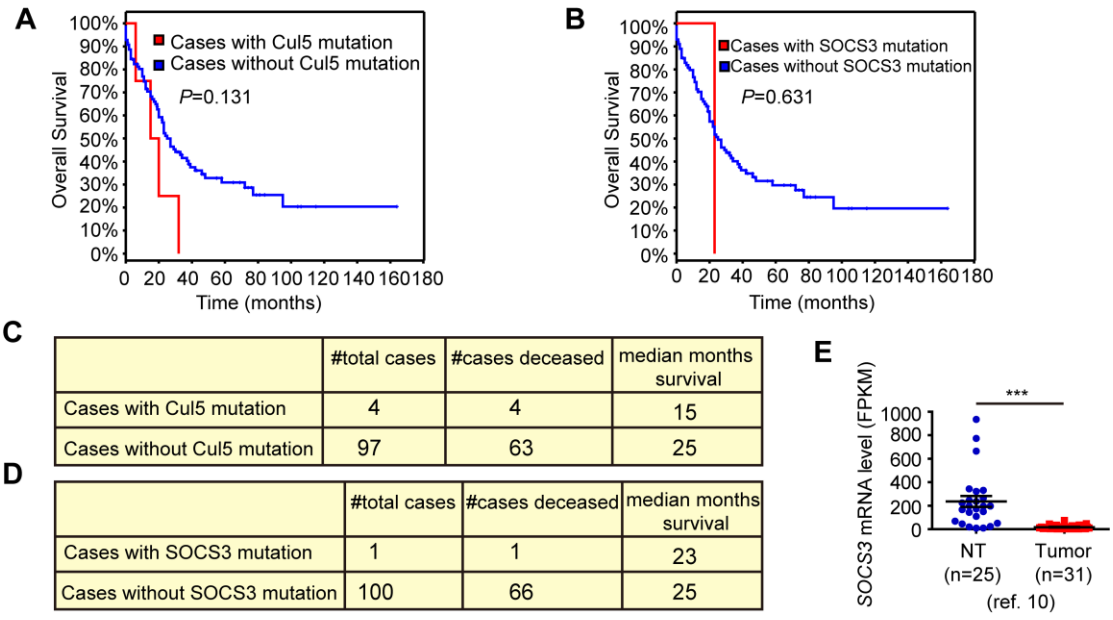
Flag-SOCS3
Flag (integrin β1)
Flag (SOCS3)
Tubulin



Supplemental Figure 3. CUL5/SOCS3 complex interacts with and promotes integrin β1 protein ubiquitination and degradation. (**A**) The mRNA levels of *Itgb1* were analyzed by real-time quantitative PCR (qPCR). Data were shown as mean ± SEM. Statistical analyses were performed using Student's t test. NS, non-significant. (**B**) HEK293T cells expressing Flag-ITGB1 and HA-SOCS3 were treated with different concentration of MG132 (10, 20, 30 μM) for 10 hours before subjected to IB assay. Tubulin served as loading control. (**C**) IB analyses of HEK293T cells transfected with empty vector (EV) or various SOCS box proteins with Flag-ITGB1. Tubulin served as loading control. (**D**) HEK293T cells transfected with various SOCS box proteins and Flag-ITGB1 were treated with 10 μM MG132 for 10 hours before subjected to Co-immunoprecipitation (Co-IP) and IB assays. (**E**) HEK293T cells transfected with Myc-CUL5 and HA-SOCS3 or Flag-SOCS3 and HA-CUL5 were treated with 10 μM MG132 for 10 hours before subjected to Co-IP and IB assays. (**F**) Hela cells transfected with pLKO.1 or shRNF7 were

treated with 100 μg/ml cycloheximide (CHX) for indicated time before IB analysis. Tubulin served as loading control. (**G**) HEK293T cells expressing Flag-ITGB1 together with His-tagged Ub K48 or K63 with or without HA-SOCS3 were treated with 10 μM MG132 for 10 hours before Co-IP and IB assays. (**H**) The schematic structure showed the amino acid sequence of cytoplasmic region of integrin β1. The lysine (K) residues were shown in bold. The amino acid sequence of cytoplasmic region of integrin β1 was conserved across different species. (**I**) IB analyses of HEK293T cells transfected with wild type (WT) or various indicated lysine-to-arginine (K-R) mutants of ITGB1 with or without SOCS3. Tubulin served as loading control. (**J**) HEK293T cells transfected with WT ITGB1 or various lysine-to-arginine (K-R) mutants of ITGB1 were treated with 10 μM MG132 for 10 hours before Co-IP and IB. (**K**) IB analyses of HEK293T cells transfected with wild type (WT) or various indicated lysine-to-arginine (K-R) mutants of ITGB1 with or without SOCS3. Tubulin served as loading control. (**L**) HEK293T cells transfected with various tyrosine-to- phenylalanine (Y-F) mutants of ITGB1 and Flag-SOCS3 were treated with 10 μM MG132 for 10 hours before subjected to Co-IP and IB assays. (**M**) IB analyses of HEK293T cells transfected with ITGB1-752-798 or various tyrosine-to- phenylalanine (Y-F) mutants of ITGB1 with or without SOCS3. Tubulin served as loading control. (**N**) Schematic illustration of genetically engineered mouse SCLC from *Rb1*$^{fl/fl}$;*Trp53*$^{fl/fl}$ (*RT*) model. (**O**) Representative photographs of hematoxylin and eosin (H&E) staining in *RT* mouse lung and liver. (**P**) Representative images of CUL5, SOCS3 and integrin β1 *immunohistochemistry* (IHC) staining in RT mouse primary tumor and metastatic tumor. T, tumor; NT, non-tumor. Scale bar, 50 μm. (**Q**) IHC scores of CUL5, SOCS3 and integrin β1. Representative photos were shown. (**R-T**) IHC scores of CUL5, SOCS3 and integrin β1 in primary lung tumors and liver metastases from *Rb1*$^{fl/fl}$;*Trp53*$^{fl/fl}$ SCLC mouse model. Data were shown as mean ± SEM. Statistical analyses were performed using Student's t test. **P*<0.05, ***P* < 0.01, ****P* < 0.001.



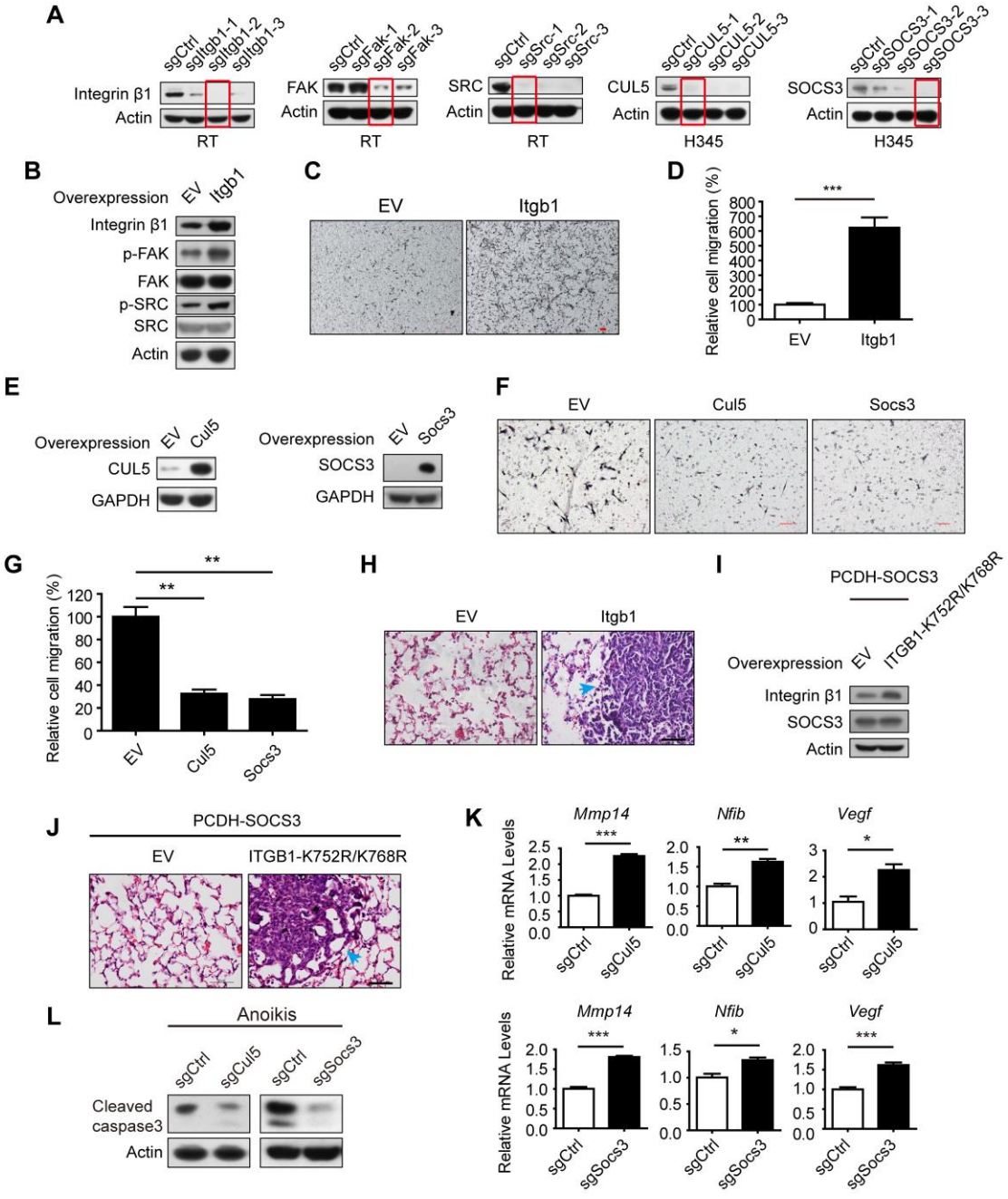
C

	#total cases	#cases deceased	median months survival
Cases with Cul5 mutation	4	4	15
Cases without Cul5 mutation	97	63	25

D

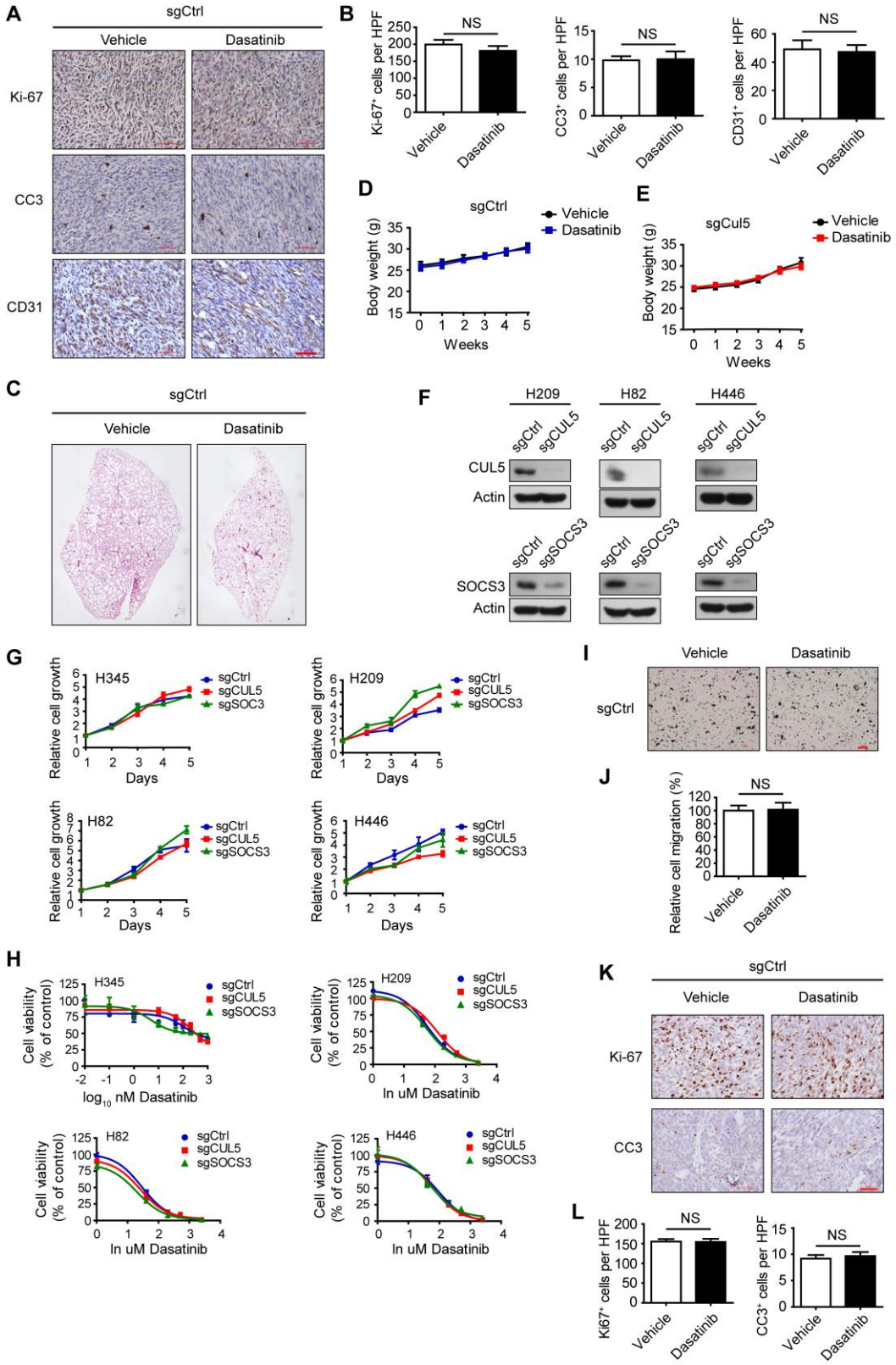
	#total cases	#cases deceased	median months survival
Cases with SOCS3 mutation	1	1	23
Cases without SOCS3 mutation	100	66	25

Supplemental Figure 4. CUL5/SOCS3 deficiency is associated with poor SCLC patient prognosis. (**A**) Kaplan-Meyer plots showed that SCLC patients with CUL5 mutations tended to have poor survival. (**B**) Kaplan-Meyer plots showed that SCLC patient with SOCS3 mutation tended to have poor survival. (**C**) Median survival of SCLC patients with or without CUL5 mutations. (**D**) Median survival of SCLC patients with or without SOCS3 mutation. (**E**) RNA sequencing of SOCS3 in human SCLC tumor and non-tumor (NT) tissue. Data were shown as mean ± SEM. Statistical analysis was performed using Student's t test. ***$P < 0.001$.



Supplemental Figure 5. CUL5/SOCS3 deficiency drives SCLC metastasis by elevating integrin β1/FAK/Src signaling. (**A**) RT or H345 cells transduced with indicated sgRNA were subjected to IB analyses. Actin served as loading control. Red boxes indicated the selected sgRNAs for further studies. (**B**) IB analyses of integrin β1 level, phosphorylation levels of FAK and SRC in ITGB1-overexpressed RT cells. Actin served as loading control. (**C**) Representative images of migrated RT cells overexpressing ITGB1 in the trans-well assays. Scale bar, 50 μm. (**D**)

The quantification of migrated cells was shown as mean ± SEM. Statistical analysis was performed using Student's t test. ***P < 0.001. (**E**) RT cells transduced with PCDH-Cul5 or PCDH-Socs3 were subjected to IB analyses. GAPDH served as loading control. (**F**) Representative images of migrated RT cells overexpressing Cul5 or Socs3 in the trans-well assays. Scale bar, 50 μm. (**G**) The quantification of migrated cells was shown as mean ± SEM. P values were obtained by one-way ANOVA followed by the Newman-Keuls multiple comparison test. **P < 0.01. (**H**) Representative H&E staining of lung tumors from nude mice transplanted with RT cells transduced with indicated sgRNA. The blue arrow indicated metastatic tumor. Scale bar, 50 μm. (**I**) IB analyses of integrin β1 and SOCS3 in SOCS3 and ITGB1-K752R/K768R-overexpressed RT cells. Actin served as loading control. (**J**) Representative H&E staining of lung tumors from nude mice transplanted with RT cells transduced with PCDH-SOCS3 and ITGB1-K752R/K768R. The blue arrow indicated metastatic tumor. Scale bar, 50 μm. (**K**) qPCR analysis of mRNA levels of *Mmp14*, *Nfib* and *Vegf* in RT cells transduced with sg*Cul5* or sg*Socs3*. Data were shown as mean ± SEM (n=3). Statistical analyses were performed using Student's t test. *P < 0.05, **P < 0.01, ***P< 0.001. (**L**) IB analyses of cleaved caspase-3 in RT cells transduced with sg*Cul5* or sg*Socs3* and cultured in suspended condition for 24 hours. Actin served as loading control.



Supplemental Figure 6. SRC inhibitor dasatinib has no effect upon growth and metastasis of CUL5-competent SCLC. (**A**) Mice transplanted with RT cells transduced with sgCtrl were administered intragastrically with vehicle (1% Tween-80 and 5% PEG400 in PBS) or dasatinib (30

mg/kg) daily for 28 days. Representative photos of Ki-67, Cleaved caspase 3 (CC3) and CD31 immunostaining in allograft tumors were shown. Scale bar, 50 μm. (**B**) Statistical data of Ki-67, CC3 and CD31-positive (+) index per high-power field (HPF) were shown as mean ± SEM. Statistical analyses were performed using Student's t test. NS, non-significant. (**C**) Representative H&E staining of lung lobes from mice transplanted with RT cells transduced with sgCtrl in vehicle and dasatinib treatment groups. Scale bar, 50 μm. (**D** and **E**) Mice transplanted with RT cells transduced with sgCtrl (**D**) or sgCul5 (**E**) were administered intragastrically with vehicle (1% Tween-80 and 5% PEG400 in PBS) or dasatinib (30 mg/kg) daily for 28 days. Body weights were monitored weekly. (**F**) IB analyses of CUL5 and SOCS3 in H209, H82 and H446 cells. Actin was used as loading control. (**G**) Proliferation assays of H345, H209, H82 and H446 cells transduced with sgCUL5 or sgSOCS3. (**H**) H345, H209, H82 and H446 cells were treated with different concentrations of dasatinib for 72 h and analyzed for cytotoxicity. (**I**) Representative images of migrated H345 cells transduced with sg*Ctrl* and treated with dasatinib (5 nM) in the trans-well assay. Scale bar, 50 μm. (**J**) Quantification of the migrated cells in (**I**). Data were shown as mean ± SEM (n=3). Statistical analysis was performed using Student's t test. NS, non-significant. (**K**) Representative photos of Ki-67 and Cleaved caspase 3 (CC3) immunostaining in sgCtrl-induced xenograft tumors were shown. Scale bar, 50 μm. (**L**) Statistical data of Ki-67 and CC3-positive (+) index per high-power field (HPF) were shown as mean ± SEM. Statistical analyses were performed using Student's t test. NS, non-significant.