YMTHE, Volume *29*

Supplemental Information

lnc-Rps4l-encoded peptide RPS4XL regulates RPS6 phosphorylation and inhibits the proliferation of PASMCs caused by hypoxia

Yiying Li, Junting Zhang, Hanliang Sun, Yujie Chen, Wendi Li, Xiufeng Yu, Xijuan Zhao, Lixin Zhang, Jianfeng Yang, Wei Xin, Yuan Jiang, Guilin Wang, Wenbin Shi, and Daling Zhu

Supplemental material
Figure S1

5'3' Frame 1

```
TPLVLWEGEGRVSACSSAMARGPKKHLKRVAAPRHWMLDKLTGVFAPRPSAGPHRLRECLPLAIFLRNRLKYA
LTGDEVKKICMQRLIKVDGKVRTDVAYPAGFMDVISIDKSGENFRLVYDTKGRFAVHRITPEEAKYKLCKVRK
VFVGTKGIPHLVTHDARTIRYPDPLIKVNDTVQISLDSGKITDAIKFDTGNLCMVTGGANLGRIGVITNRERH
PGSFDVVHVKDANGNGFATRLSNIFVIGKGNKPWISLPRGKGIRLTIAEERDKRLAAKQSG-SGL-GTLRGRG
VYVCLYLKVF-AAC-IKKESK
```

5'3' Frame 2

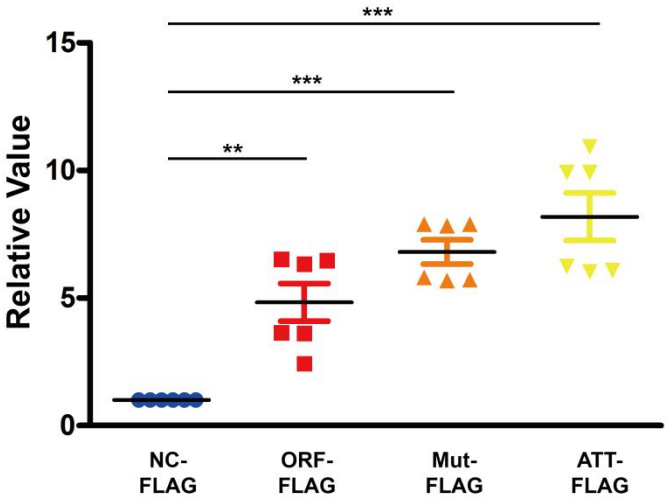
```
LRLCSGKEKGVFPLAPPPWLVVPRNT-SVWRPHATGCWTN-PVCSRPVRLPARTACGSACRSPSS-GTGSSTP
-PATR-RRSACSGSLRSTARSEPMWPTQLASWMSSASTRAARTSASSTTPRAASRYTASRPRRPSTSCAR-GK
SSWVPRASRTSSRTTRVPSATLTRSSRSTTPCRSR-TAEKSPMPSSLIPATCAW-PEAPTWVASASSPTASAT
PAPLTWST-KMPTAMASLPASPTSS-SGRATSRGSPFPGEKESASPLPKRETRGWRPSRAGEVDSEGHCGEGA
FTFVYT-KFFKQHVELKKKAS
```

5'3' Frame 3

```
SACALGRRRACFRLLLRHGSWSQETPEACGGPTPLDAGQTDRCVRAPSVCRPAPPAGVPAARHLPEEQAQVRP
DRRRGEEDLHAAAH-GRRQGQNRCGLPSWLHGCHQHRQERRELPPRLRHQGPLRGTPHHARGGQVQAVQGEES
LRGYQGHPAPRHARRAYHPLP-PAHQGQRHRADLARQRKNHRCHQV-YRQPVHGNRRRQPGSHRRHHQPRAPP
RLL-RGPRERCQRQWLRYPPLQHLRDREGQQAVDLPSPGKRNPPHHCRRERQEAGGQAERVKWTLRDIAGKGR
LRLFILKSFLSSMLN-KRKQA
```

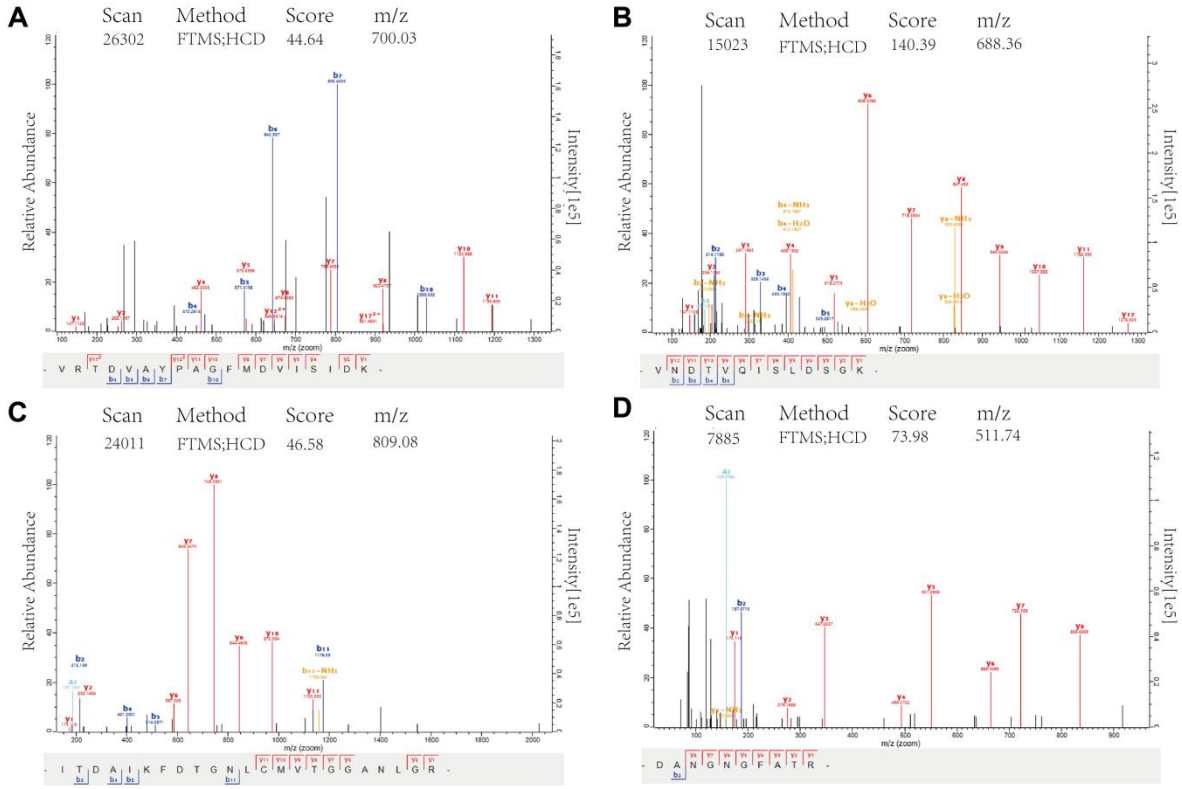
Supplemental figure 1. Rps4l's open reading frame and coding sequence were detected by ExPASy.

Figure S2



Supplemental figure 2. Overexpression efficiency of the plasmid were used. All values are represented as the mean ± SEM (**$p < 0.01$, and ***$p < 0.001$; $n \geq 3$).

Figure S3

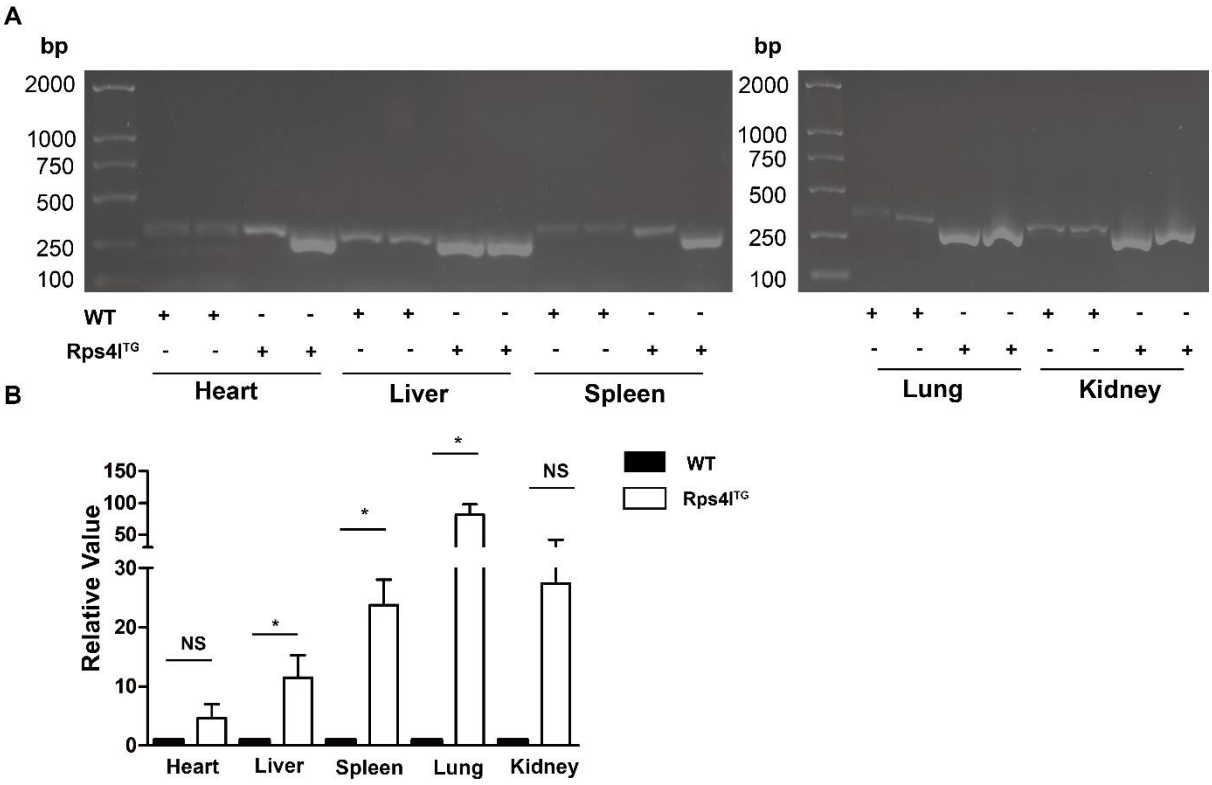


Supplemental figure 3. Mass spectrum of the RPS4XL. (A) Mass spectrum of RPS4XL amino acids 76-94. (B) Mass spectrum of RPS4XL amino acids 156-168. (C) Mass spectrum of RPS4XL amino acids 169-191. (D) Mass spectrum of RPS4XL amino acids 212-221.

Figure S4

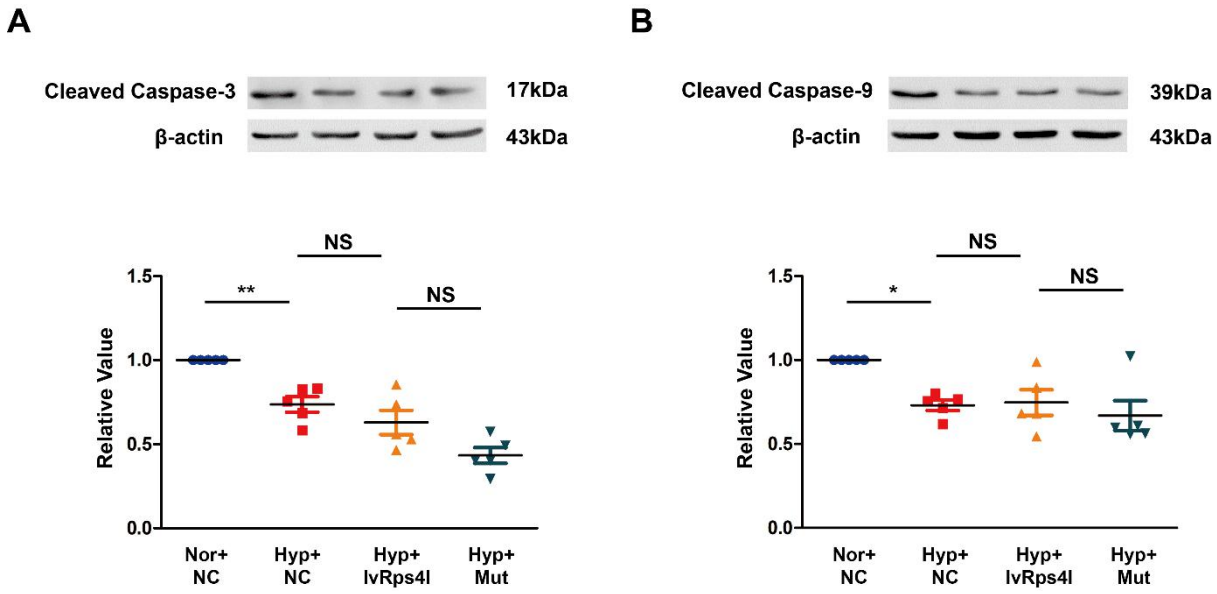
Supplemental figure 4. Detection of the specificity of anti-RPS4XL antibody. (A) Western blot analysis of expression of RPS4XL detected by the antibody in PASMCs treated with lvRps4l or NC. (B) Western blot analysis of expression of RPS4XL detected by the antibody in PASMCs treated with si-Rps4l or si-NC.

Figure S5



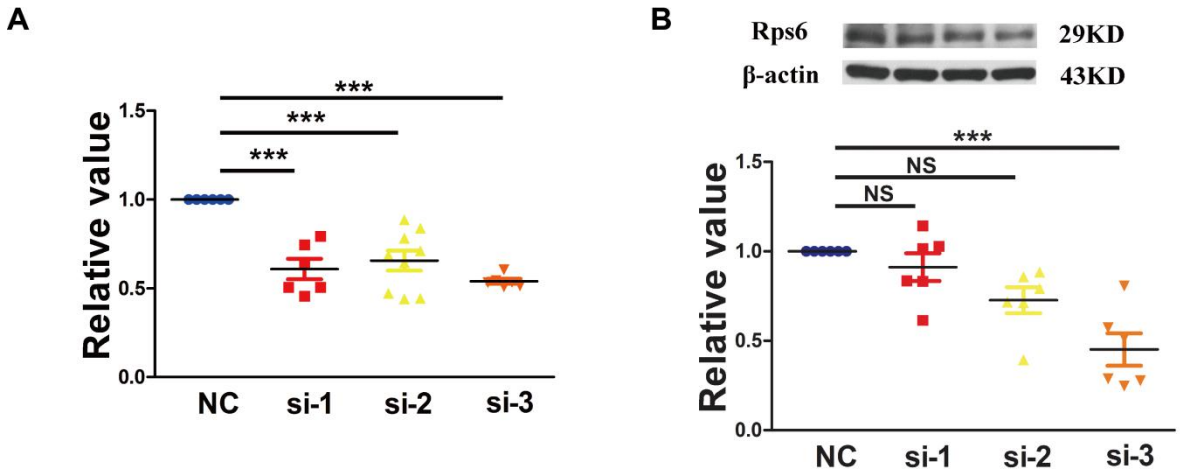
Supplemental figure 5. Expression of Rps4l in different organs of overexpression transgenic mice. (A) Agarose gel electrophoresis of the expression of Rps4l in the heart, liver, spleen, lung and kidney of WT and Rps4lTg mice. (B) qPCR analysis of the overexpression efficiency of Rps4l in the heart, liver, spleen, lung and kidney of Rps4l mice. All values are represented as the mean ± SEM (*$p<0.05$ and NS, no significance; $n \geq 3$).

Figure S6



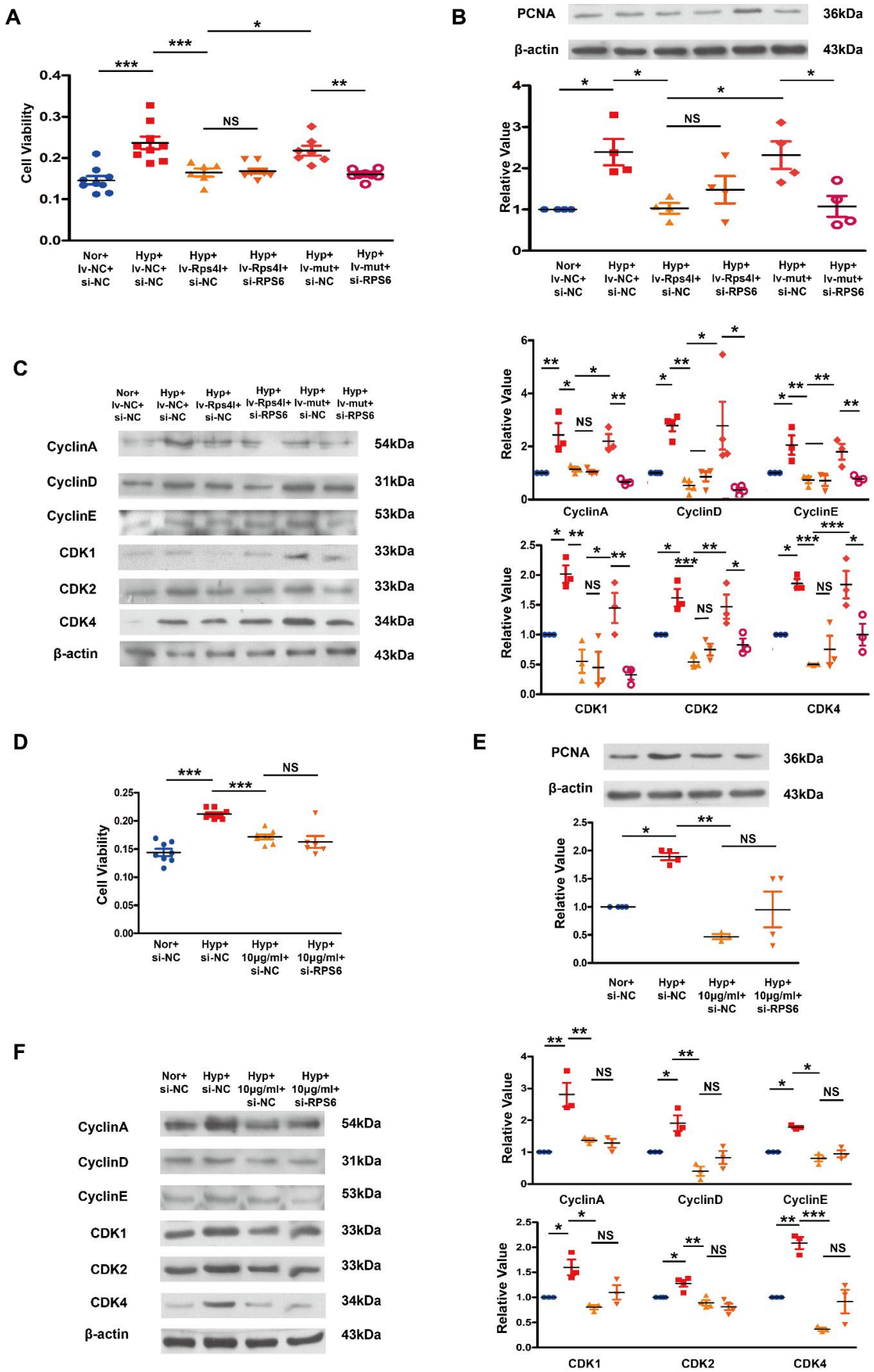
Supplemental figure 6. RPS4XL does not regulate hypoxia-induced inhibition of apoptosis in PASMCs. (A) WB analysis of cleaved caspase-3 in hypoxic and control PASMCs transfected with lvRps4l, ORFmut, or NC. (B) WB analysis of cleaved caspase-9 in hypoxic and control PASMCs transfected with lvRps4l, ORFmut, or NC. All values are represented as the mean ± SEM (*$p<0.05$, **$p < 0.01$ and NS, no significance; $n \geq 3$).

Figure S7



Supplemental figure 7. Interference efficiency of three RNAi of Rps6 in PASMCs. The si-3 was used in this study. (A) Interference efficiency of RPS6 in mRNA level. (B) Interference efficiency of RPS6 in protein level. Si-3 was used for the study. All values are represented as the mean ± SEM (***$p < 0.001$ and NS, no significance; $n \geq 3$).

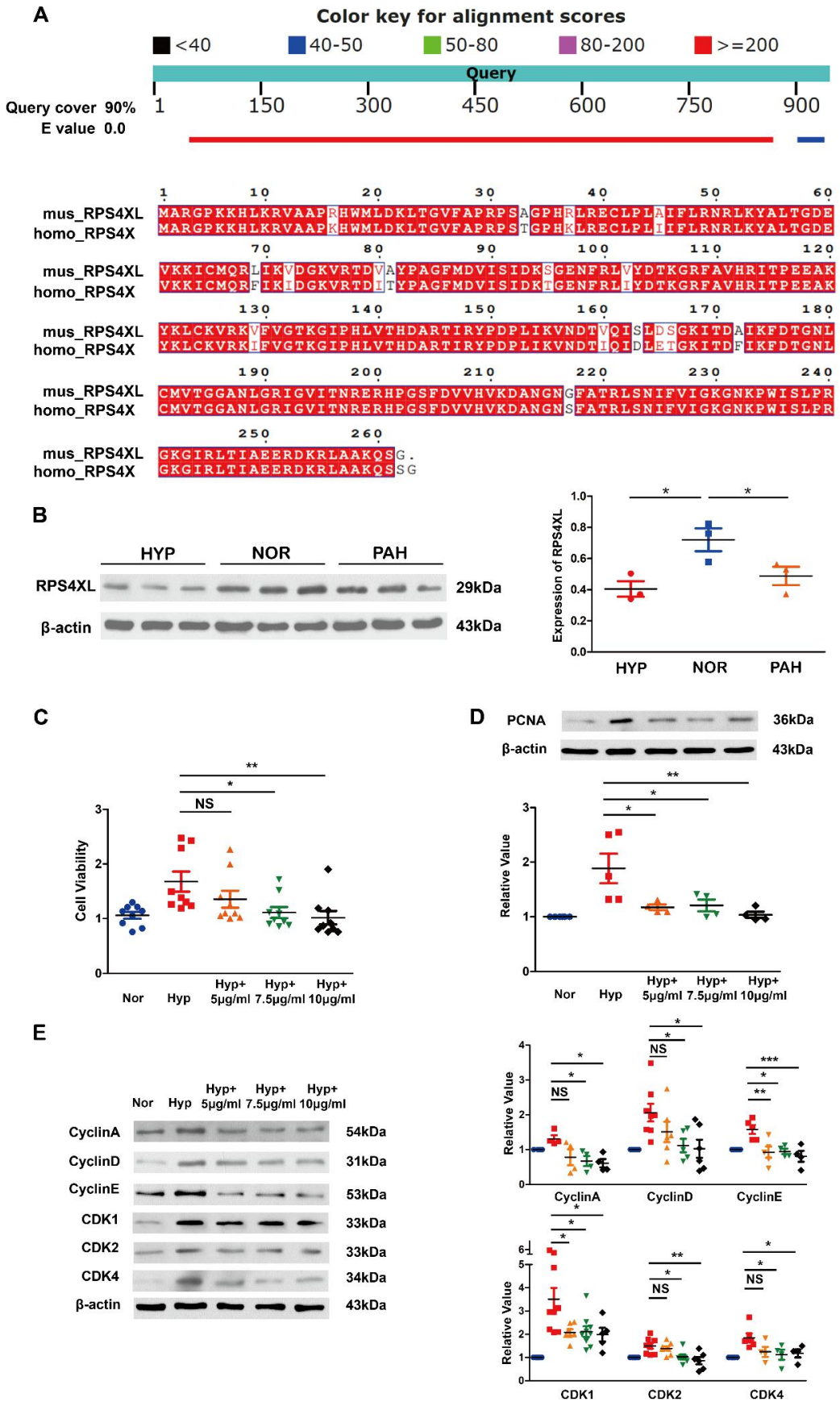
Figure S8



Supplemental figure 8. Rps4l-encoded peptide RPS4XL attenuates PASMC

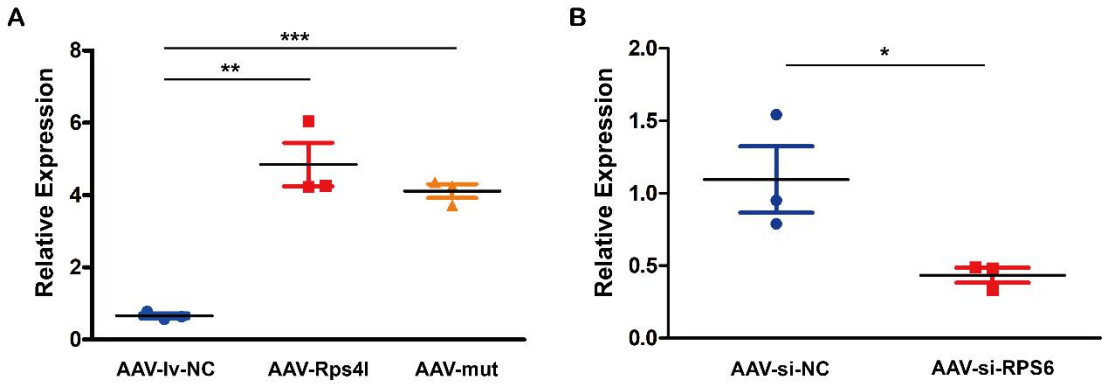
proliferation by inhibiting RPS6 phosphorylation. (A) MTT assay in hypoxic and control PASMCs co-transfected with lvRps4l, ORFmut, or lv-NC and si-RPS6 or si-NC. (B) WB analysis of PCNA in hypoxic and control PASMCs co-transfected with lvRps4l, ORFmut, or lv-NC and si-RPS6 or si-NC. (C) WB analysis of cyclin A, cyclin D, cyclin E, CDK1, CDK2, and CDK4 in hypoxic and control PASMCs co-transfected with si-RPS6 or si-NC. (D) MTT assay in hypoxic and control PASMCs simultaneously add 10ug/ml exogenous peptide RPS4XL and si-NC or si-RPS6. (E) WB analysis of PCNA in hypoxic and control PASMCs simultaneously add 10ug/ml exogenous peptide RPS4XL and si-NC or si-RPS6. (F) WB analysis of cyclin A, cyclin D, cyclin E, CDK1, CDK2, and CDK4 in hypoxic and control PASMCs simultaneously add 10ug/ml exogenous peptide RPS4XL and si-NC or si-RPS6. All values are represented as the mean ± SEM (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ and NS, no significance; $n \geqslant 3$). Nor, normoxia; Hyp, hypoxia.

Figure S9


A
Color key for alignment scores
<40
40-50
50-80
80-200
>=200
Query
Query cover 90%
E value 0.0
mus_RPS4XL
homo_RPS4X
B
HYP
NOR
PAH
RPS4XL
β-actin
29kDa
43kDa
Expression of RPS4XL
C
Cell Viability
NS
Nor
Hyp
Hyp+ 5μg/ml
Hyp+ 7.5μg/ml
Hyp+ 10μg/ml
D
PCNA
36kDa
Relative Value
E
CyclinA
CyclinD
CyclinE
CDK1
CDK2
CDK4
54kDa
31kDa
53kDa
33kDa
34kDa

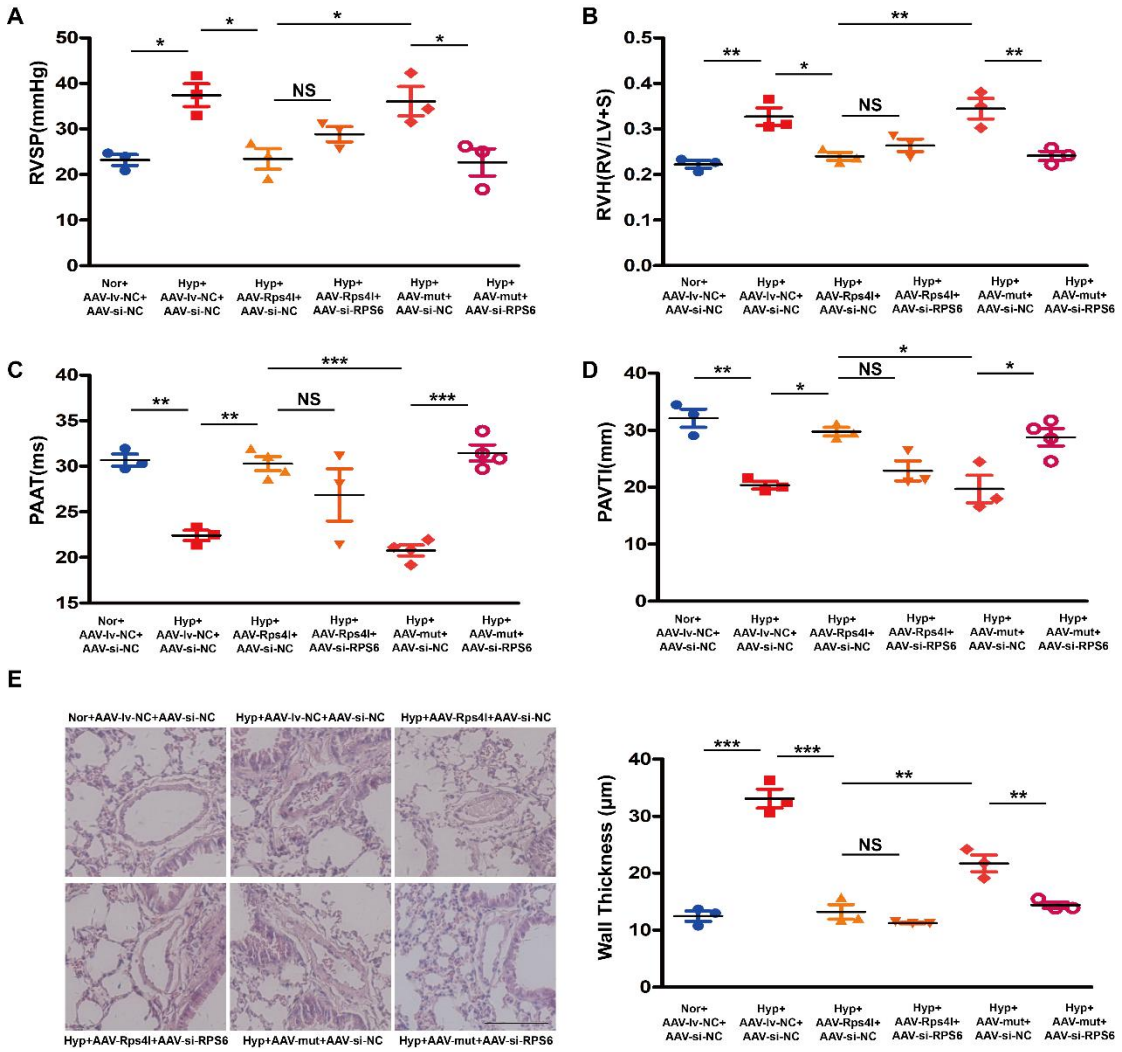
Supplemental figures 9. The peptide RPS4XL is conserved in humans and inhibits the proliferation of human pulmonary artery smooth muscle cells induced by hypoxia. (A) Bioinformatics alignment of the homologous sequences of Rps4l-encoded peptide RPS4XL in human and mouse. (B) WB analysis of RPS4XL in hypoxic, control human PASMCs and pulmonary hypertension patient PASMCs. (C) MTT assay in hypoxic and control human PASMCs treated with RPS4XL with concentrations of 5 ug/ml, 7.5ug/ml or 10 ug/ml. (D) WB analysis of PCNA. (E) WB analysis of cyclin A, cyclin D, cyclin E, CDK1, CDK2, and CDK4 in hypoxic and control human PASMCs treated with RPS4XL with concentrations of 5 ug/ml, 7.5ug/ml or 10 ug/ml. All values are represented as the mean ± SEM (*$p<0.05$, **$p<0.01$ and NS, no significance; $n \geqslant 3$). Nor, normoxia; Hyp, hypoxia.

Figure S10



Supplemental figure 10. The efficiency of AAV9 overexpresses Rps4l, mutates Rps4l and interferes with of RPS6. (A) qPCR for the efficiency of AAV9 overexpression of Rps4l and mut-Rps4l. (B) qPCR for the efficiency of AAV9 interference with RPS6. All values are represented as the mean ± SEM (*$p<0.05$, **$p<0.01$ and ***$p < 0.001$; $n \geqslant 3$).

Figure S11



Supplemental figure 11. RPS4XL blocks hypoxia-induced pulmonary hypertension in vivo through RPS6. (A-E) (A) RV systolic pressure (RVSP) (B) Right ventricular (RV)/left ventricular (LV)+S weight ratio (C) PAAT (D) PAVTI and (E) HE staining (Scale bar = 200 μm) of hypoxic mouse model infected with AAV9-lv-NC, AAV9-lv-Rps4l, AAV9-lv-mut and AAV9-si-NC, AAV9-si-RPS6. All values are represented as the mean ± SEM (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ NS, and no significance; $n \geq 3$). Nor, normoxia; Hyp, hypoxia.