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Supplemental Information

Salvia miltiorrhiza-Derived Sal-miR-58 Induces Autophagy and Attenuates Inflammation in Vascular Smooth Muscle Cells

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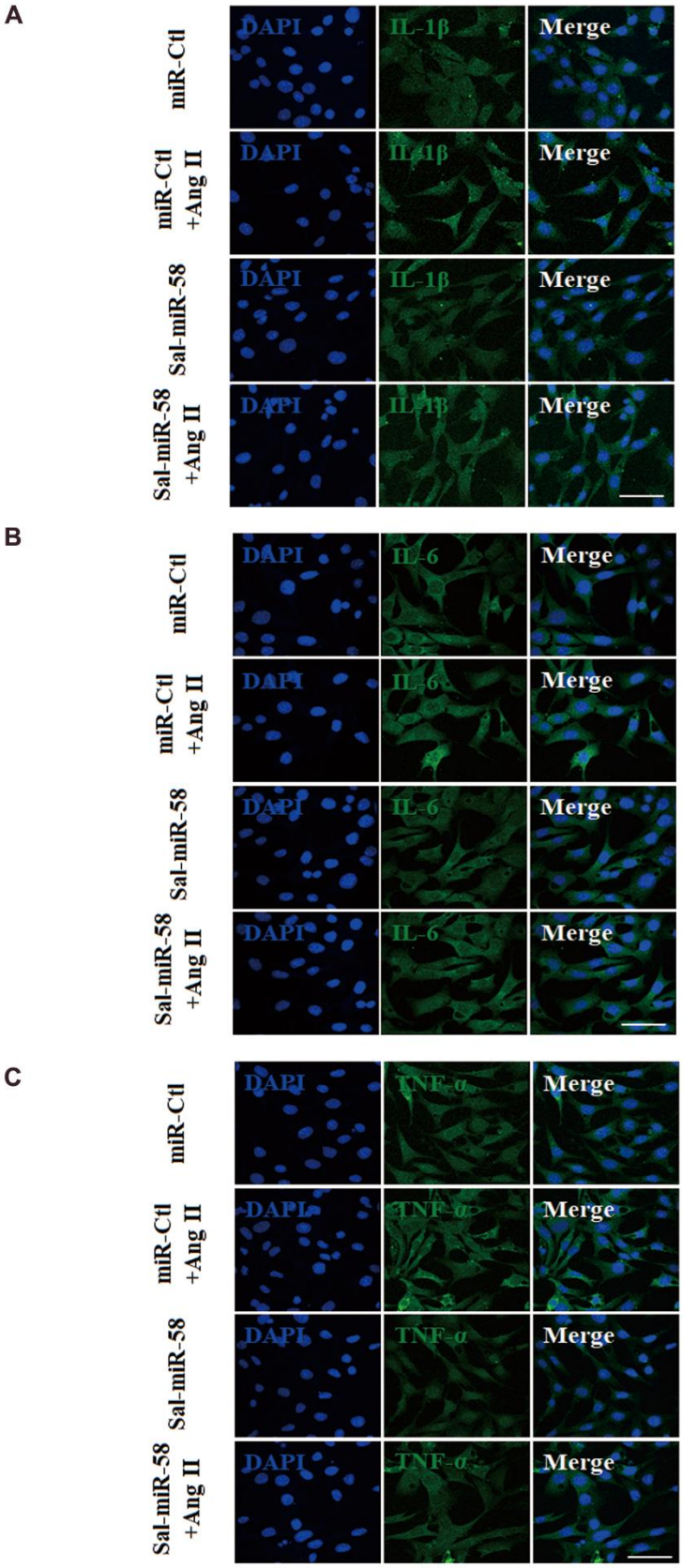
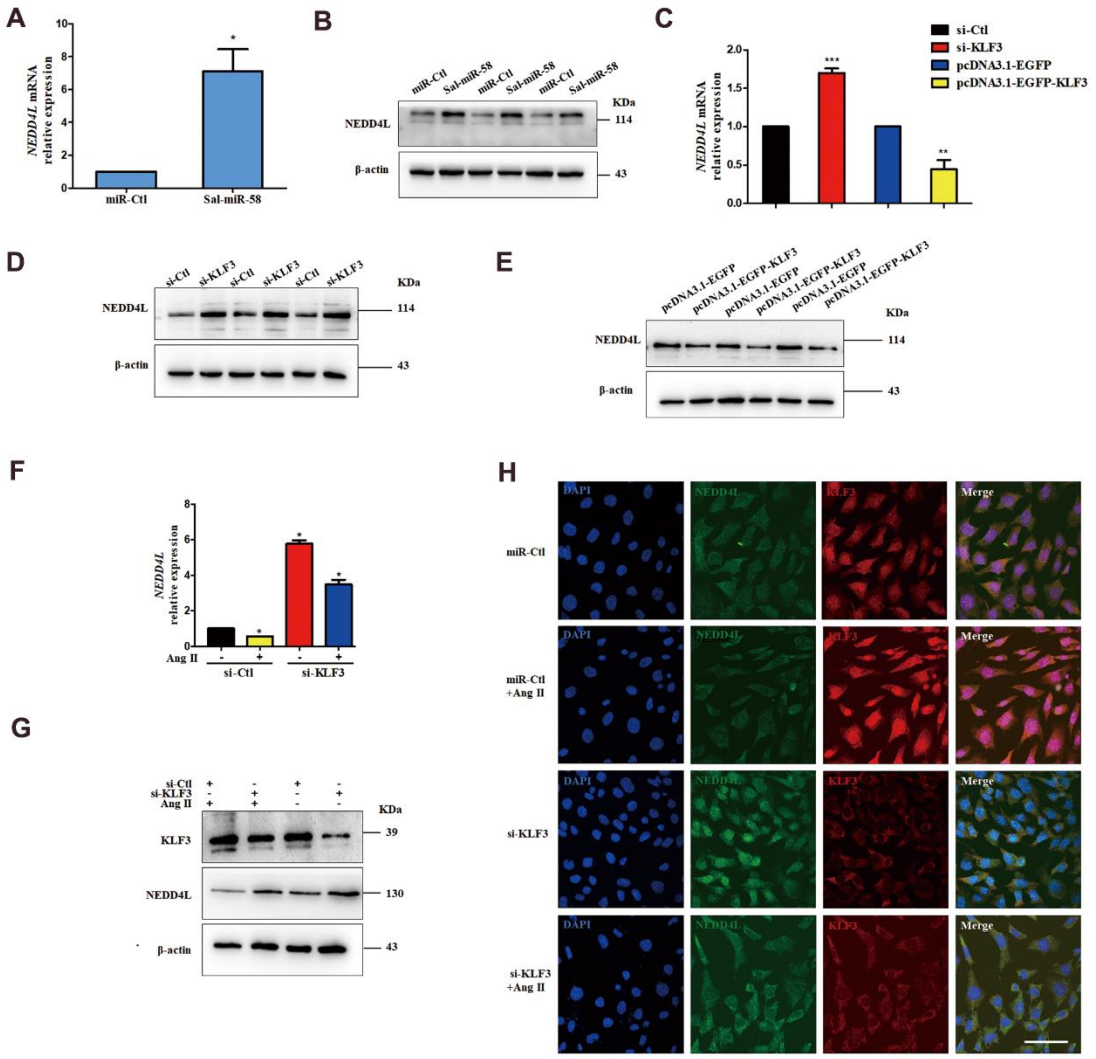


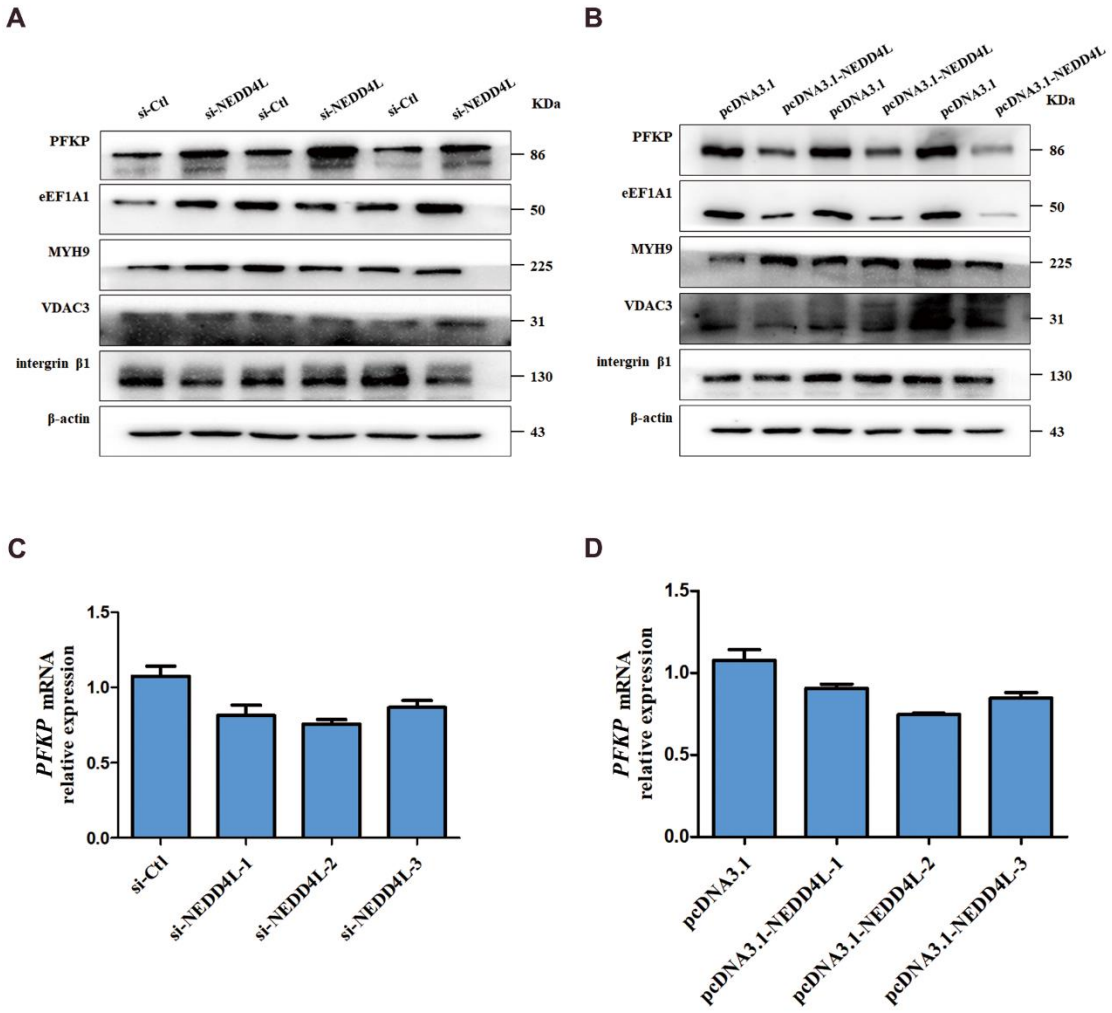
Supplementary Figure 1. Sal-miR-58 suppresses Ang II-stimulated inflammation

in VSMCs. (**A** to **C**) VSMCs were treated with Ang II or Sal-miR-58 alone or together, expression of IL-1β, IL-6 and TNF-α was examined by immunofluorescence staining. Green staining indicates IL-1β, IL-6 or TNF-α and blue staining indicates the nuclei, respectively. Scale bars =100 μm.



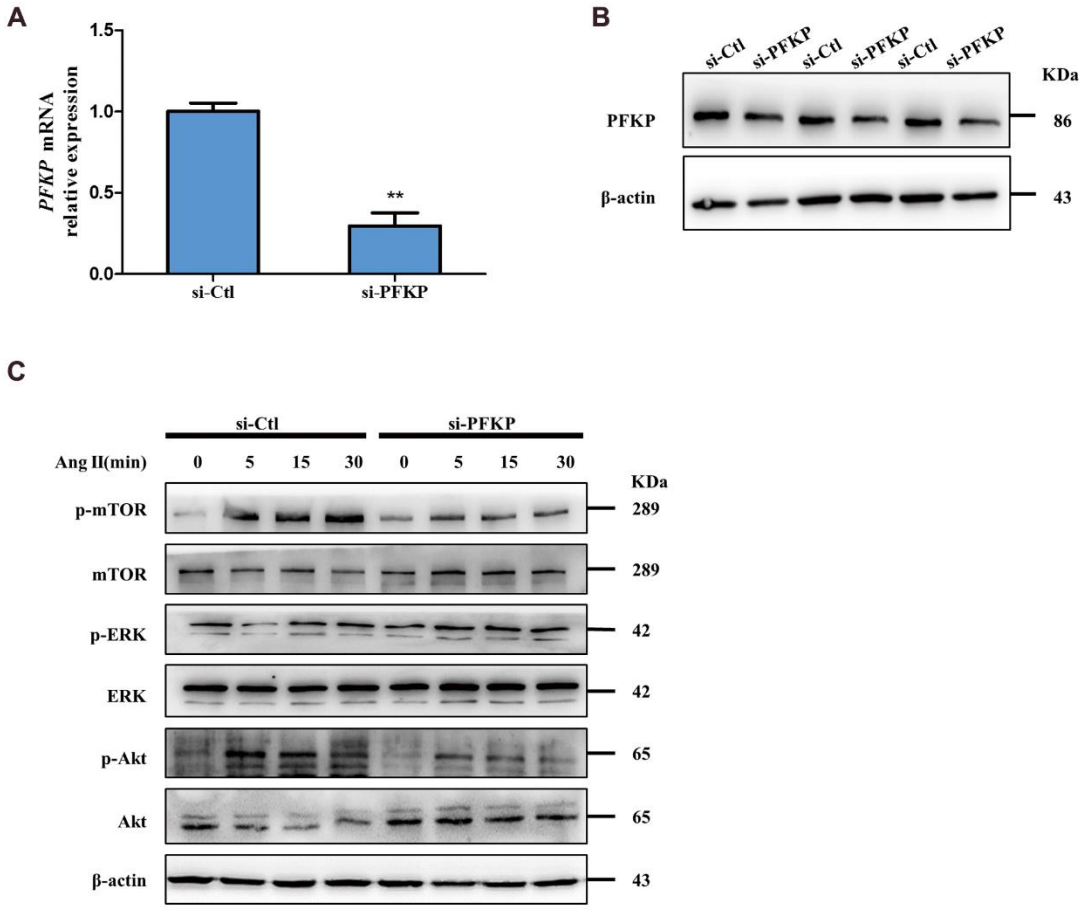
Supplementary Figure 2. Sal-miR-58 induces VSMC autophagy by KLF3-mediated downregulation of NEDD4L. (**A**) NEDD4L mRNA was determined by qRT-PCR in VSMCs transfected with Sal-miR-58. n=3; $^{*}P<0.05$ vs. miR-Ctl. (**B**) Western blot analysis of NEDD4L in VSMCs transfected with Sal-miR-58 or miR-Ctl. (**C**) NEDD4L mRNA were determined by qRT-PCR in VSMCs transfected with si-

KLF3 or pcDNA3.1-KLF3. $^{**}P<0.01$ and $^{***}P<0.001$ vs. their corresponding control. (**D**) Western blot analysis of NEDD4L in VSMCs transfected with si-KLF3 or si-Ctl. (**E**) Western blot analysis of NEDD4L in VSMCs transfected with pcDNA3.1-KLF3 or pcDNA3.1. (**F**) VSMCs were transfected with si-Ctl (100 nM) or si-KLF3 (100 nM) and treated with/without Ang II, and the total RNA was harvested and analyzed by qRT-PCR. $^{*}P<0.05$, vs. miR-Ctl. (**G**) VSMCs were transfected with si-KLF3 (100 nM) and treated with/without Ang II and the protein was harvested and analyzed by Western blotting using anti-KLF3 or anti-NEDD4L antibodies. (**H**) VSMCs were treated as in (**G**), and expression of KLF3 and NEDD4L was examined by immunofluorescence staining. Green, red and blue staining indicates NEDD4L, KLF3 and the nuclei, respectively. Scale bar=100 μm.



Supplementary Figure 3. NEDD4L knockdown or overexpression increases and

decreases, respectively, the level of PFKP protein. (**A, B**) Western blot analysis detected the expression of PFKP, eEF1A1, MYH9, VDAC3, intergrin β1 in VSMCs tranfected with si-NEDD4L or pcDNA3.1-NEDD4L. (**C, D**) VSMCs were transfected with si-NEDD4L, pcDNA3.1-NEDD4L or their corresponding control, and relative expression of PFKP mRNA was examined by qRT-PCR and presented after normalizing to 18S rRNA (mean±SEM; n=3).



Supplementary Figure 4. PFKP mediates Sal-miR-58-induced autophagy. (**A, B**) VSMCs were transfected with si-PFKP for 24 h, and expression of PFKP was examined by qRT-PCR and Western blotting. $^{**}P<0.01$ vs. si-Ctl, n=3. (**C**) VSMCs were transfected with si-PFKP or si-Ctl and treated with Ang II for different times. Western blot analysis detected total and phosphorylated mTOR, ERK1/2 and Akt.

Table 1. Primers for Real-time PCR and RT-PCR

name	Sequences 5' to 3'
ppt-miR-414 F	CAGTGCTGTCATCCTCATCATC
ppt-miR-414 R	TATGGTTGTTCACGACTCCTTCAC
hbr-miR-156 F	GCAGTGCGTTGACAGAAGATAGA
hbr-miR-156 R	TATGGTTGTTCACGACTCCTTCAC
gra-miR-172-5p F	TCAGTCCTGGTAGCATCATCAAG
gra-miR-172-5p R	TATGGTTGTTCACGACTCCTTCAC
Sal-miR-58 F	AAGGGGAUGUAGCUCAUC
Sal-miR-58 R	UGAGCUACAUCCCCUUUU
U6 F	CGCTTCGGCAGCACATATAC
U6 R	TTCACGAATTTGCGTGTCATC
IL-1β F	CAACCAACAAGTGATATTCTCCATG
IL-1β R	GATCCACACTCTCCAGCTGCA
IL-6 F	ACTTCCATCCAGTTGCCTTCTTGG
IL-6 R	TTAAGCCTCCGACTTGTGAAGTGG
TNF-α F	CATCTTCTCAAAATTCGAGTGACAA
TNF-α R	TGGGAGTAGACAAGGTACAACCC
KLF3 F	AGGCCTCACTCACGGGATAC
KLF3 R	AGAGAGGAAGGAGAACCGCC
KLF4 F	CAGTGGTAAGGTTTCTCGCC
KLF4 R	AAGCCAAAGAGGGGAAGAAG
KLF5 F	CACCGGATCTAGACATGCCC
KLF5 R	ACGTCTGTGGAACAGCAGAG
Beclin1 F	GGACCAGGAGGAAGCTCAGTACC
Beclin1 R	GCTGTGCCAGATGTGGAAGGTG
Atg5 F	CAGAAGCTGTTCCGGCCTGTG
Atg5 R	CAGATGCTCGCTCAGCCACTG
LC3B F	TCGCCGACCGCTGTAAGGAG
LC3B R	CGCCGGATGATCTTGACCAACTC

NEDD4L F	CAGTGGAGATTTGTGAACAGGG
NEDD4L R	CTAGAATCCACCCCTTCAAATCCTTG
PFKP F	CTCAGAGCCACCAGAGGACCTTC
PFKP R	CAGTCGGCACCGCAAGTCAAG

Table 2. Primer sequences for ChIP

name	Sequences 5' to 3'
Klf3-Nedd4l-bindingsite1-F	GGCTTTGTTGTTGTTTCACGG
Klf3-Nedd4l-bindingsite1-R	TGTTTCGCCCCACGCCCACC
Klf3-Nedd4l-bindingsite2-F	GCCCCAGCTCAGTGCTCTGTG
Klf3-Nedd4l-bindingsite2-R	TGGTGTTTCGCCCCACGC
Klf3-Nedd4l-negative primers 1-F	AGGTGCCCATTCAACATA
Klf3-Nedd4l-negative primers 1-R	ATAAGAAGACCAGGTAACGA
Klf3-Nedd4l-negative primers 2-F	GCTGGTGCAACATCCTTC
Klf3-Nedd4l-negative primers 2-R	TTGAGTGCGGTGGTAAATTA

Table 3. The siRNA sequences

si-KLF3 #1 F	CCCGUCGAAUUACAGAATT
si-KLF3 #1 R	UCA UUG ACG UCU GUG GAA CTT
si-NEDD4L F	GCAGAAAUACGACUACUUUTT
si-NEDD4L R	AAAGUAGUCGUAUUUCUGCTT
si-Control F	UUC UCC GAA CGU GUC ACG UTT
si-Control R	ACG UGA CAC GUU CGG AGA ATT
si-PFKP F	CCUGUAACUUGGCGCGCUUTT
si-PFKP R	AAGCGCGCCAAGUUACAGGTT