

Supplemental Material

Table S1. qPCR primer sequences.

qPCR primer sequences

Gene	Forward primer 5'-3'	Reverse primer 5'-3'
β-actin	CATCCTCTTCCTCCCTGGAGAAGA	ACAGGATTCCATACCCAAGAAGGA
NOX1	CATAAGGGGAGTTGCAGGCA	AATCACGACCTTCTGCTGGG
NOX2	ACCTCCTCTTCCCAGATCC	ACCCTAGCCTGCTTATGGGA
NOX4	TAGGCTCCAGGCAAACACTG	TTAGGAACACTGCAGCGAGG
SOD1	CAAGCGGTGAACCAGTTGTG	CAGTCACATTGCCAGGTCT
SOD2	ACCGAGGAGAAGTACCACGA	GCCTCCAGCAACTCTCCTTT
CAT	TTATTCCTGTGCTGTGCGGT	GCAACCAAACACGGTCCTTC
IL-1β	CAACCAACAAGTGATATTCTCCATG	GATCCACACTCTCCAGCTGCA
IL-10	TGAGGCGCTGTCGTCATCGATTTCTCCC	ACCTGCTCCACTGCCTTGCT

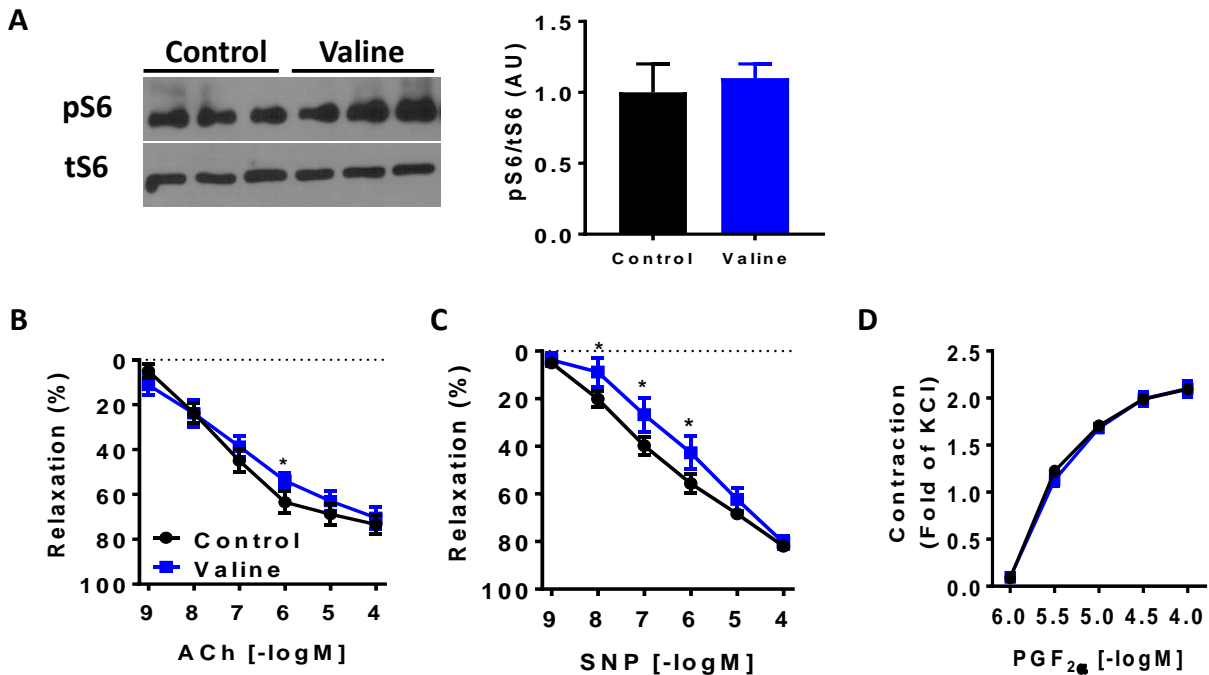
Primer sequences used for qPCR experiments in this study.

Table S2. Aortic ring EC₅₀ and E_{max} values.

ACh	CONTROL	LEUCINE	LEUCINE +S6KDN
-logEC ₅₀	7.2 ± 0.1	6.7 ± 0.1*	7.0 ± 0.2
E _{max}	74.2 ± 4.3	63.9 ± 5.3	67.9 ± 2.3
ACh	Ad-GFP	Ad-S6KCA	Ad-S6KCA +Tempol
-logEC ₅₀	7.6 ± 0.1	6.4 ± 0.3*	6.9 ± 0.1†
E _{max}	77.9 ± 2.4	70.2 ± 3.5	74.6 ± 2.0

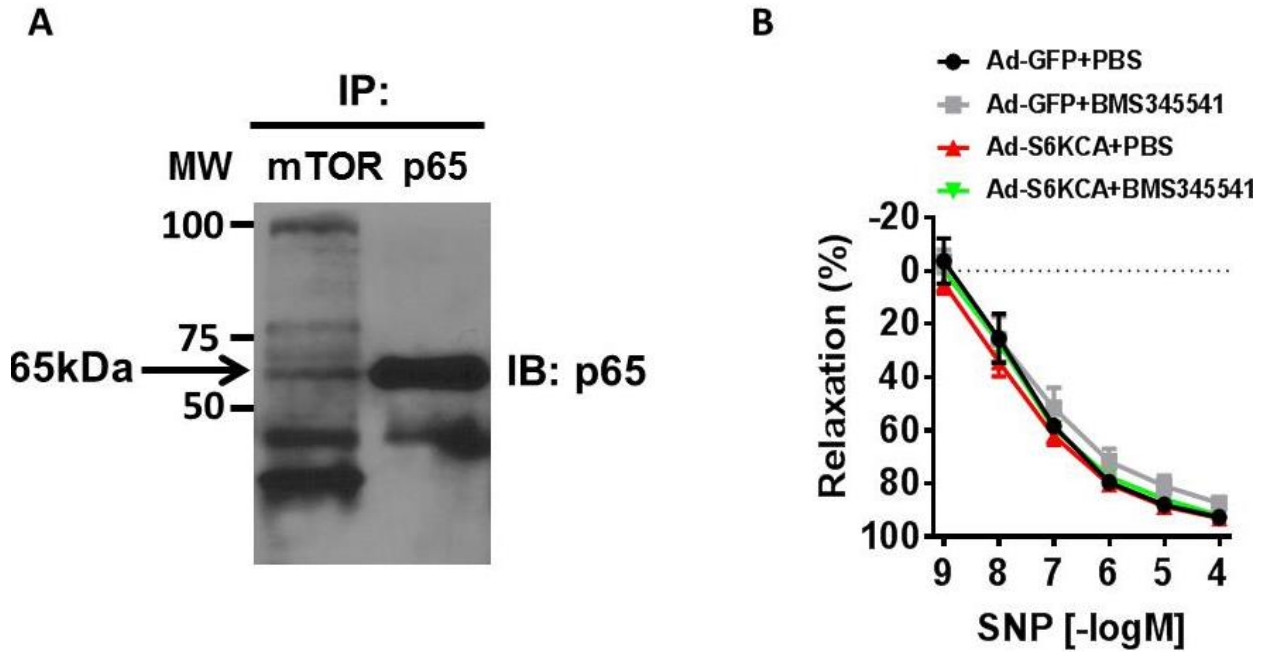
Calculated EC₅₀ and E_{max} values for leucine stimulated and Ad-S6KCA infected aortic rings. *p<0.05 vs control; †p<0.05 vs Ad-S6KCA.

Figure S1. Valine does not activate mTORC1 signaling in aortic rings ex vivo.



(A) Representative western blot images of phospho- and total S6 expression and quantification data (expressed as arbitrary units) of aortic rings cultured in control (0.45mM) or valine-supplemented (10mM) media. Vascular reactivity responses of aortic rings to **(B)** endothelial-dependent acetylcholine, **(C)** endothelial-independent sodium nitroprusside, and contractile responses to **(D)** prostaglandin F_{2α} (n=10/group). Of note, the control group is reproduced from Figures 1C-E. *p<0.05 vs control.

Figure S2. mTOR physically interacts with the p65 subunit of the NF κ B transcriptional complex.



(A) Representative western blot image of MLECs immuno-precipitated with antibodies for mTOR and p65 subunit of the NF κ B transcriptional complex. (B) Vascular reactivity responses of adenoviral infected aortic rings treated with BMS-345541 (300nM) or vehicle (PBS) for 16hrs to endothelial-independent sodium nitroprusside (n=5/group).