Supplemental Material

Table S1. Differential expression profile.

Gene Symbol	KRAS 1	KRAS 2	KRAS 3	CTRL 1	CTRL 2	CTRL 3	FC	p value
CD34	2.78	3.71	3.99	105.27	103.21	112.91	0.032608	0.000688
SELP	0.06	0.09	0.21	2.43	1.8	1.98	0.057971	0.006521
CLDN3	0.19	0.17	0.06	2.7	2.59	2.39	0.054688	0.000262
EMCN	4.99	5.09	5.62	71.93	79.63	75.76	0.069066	0.000916
JUP	25.68	41.6	34.55	287.51	277.68	279.08	0.120613	0.000006
LMO2	5.33	6.45	6.18	38.12	35.47	35.23	0.165043	0.002893
CXADR	0.83	0.73	0.59	2.27	3.09	2.82	0.262836	0.009590
VWF	120.89	169.09	151.46	735.51	818.25	837.84	0.184579	0.000513
SELE	0.12	0.19	0.15	0.53	0.55	0.79	0.245989	0.024812
GPR116	3.69	6.77	6.23	25.91	30.51	34.77	0.183024	0.005277
VCAM1	0.08	0.15	0.07	0.54	0.39	0.57	0.200000	0.009082
SOX18	27.61	25.78	22.21	109.16	100.69	99.73	0.244202	0.000165
TP53	10.92	15.23	13.09	49.57	49.25	57.8	0.250543	0.013516
FLI1	32.37	41.76	37.75	135.21	139.09	159.53	0.257889	0.002122
KRT19	24.97	20.38	19.89	86.97	82.35	76.5	0.265397	0.000363
KDR	19.17	27.52	25.46	84.29	99.13	104.84	0.250295	0.002824
MTUS1	26.39	32.91	30.24	80.4	88.24	93.1	0.342095	0.000851
LYVE1	2.37	5.02	3.2	10.63	9.78	11.5	0.331871	0.002974
CAV1	243.98	271.89	276.2	526.92	543.02	581.02	0.479763	0.000326
CDH5	174.44	190.03	183.27	404.63	427.82	444.67	0.428887	0.000696
ICAM2	260.54	243.75	230.44	650.95	571.68	552.96	0.413795	0.004524
TIE1	247.88	265.89	264.02	427.84	430.15	453.18	0.593203	0.000119
TJP1	12.8	14.72	14.52	19.69	22.43	25	0.626341	0.020490
SOX7	19.8	19.57	19.08	30.29	33.62	35.75	0.586494	0.011984
EGFL7	619.84	550.46	539.99	948.31	854.67	791.48	0.659208	0.009872
TAGLN	20.72	19.46	18.27	8.31	7.4	6.61	2.618728	0.000305
NEXN	10.82	12.33	10.89	4.94	7.63	5.66	1.867252	0.008621
NT5E	152.88	175.22	182.04	91.03	97.93	106.35	1.727473	0.005686
CDH2	16.29	15.65	15.94	9.43	11.01	11.09	1.518554	0.000744
SPOCK1	74.74	93.35	89.57	41.91	56.97	55.51	1.668890	0.010465
SRF	32.2	37.31	36.6	19.38	18.92	21.3	1.780369	0.004006
PLAU	39.06	41.15	41.57	27.43	25.7	25.66	1.545628	0.000197
AHNAK	128.93	171.33	180.61	63.82	82.65	93.29	2.005631	0.020056
SRGN	996.7	907.46	907.13	518.38	477.33	456.26	1.936190	0.000592
MSN	526.71	585.26	599.11	254.49	276.63	293.45	2.075118	0.001349
ZEB1	42.17	57.2	63.85	20.17	27.56	28.84	2.131644	0.031349
SERPINE2	19.61	18.15	17.69	6.25	6.15	6.14	2.990831	0.002137
IGFBP3	12.84	12.88	12.82	5.31	5.23	5.81	2.357187	0.000541
SMTN	140.52	132.86	129.02	59.2	57.42	54.55	2.350879	0.000523
PRKCA	6.14	7.37	8.11	2.51	2.74	3.19	2.561611	0.010092
SNAI1	20.35	20.54	19.94	9.49	9.82	9.72	2.095419	0.000011
JAG1	16.39	19.27	18.26	6.96	8.2	7.92	2.336222	0.002242
TWIST2	0.9	0.99	0.89	0.59	0.19	0.39	2.376068	0.035535
PLAUR	183.82	161.96	167.04	50.03	43.32	45.59	3.690946	0.001403
KLF4	32.84	34.07	36.1	4.63	4.51	4.13	7.762622	0.000782

PLEK2	5.74	5.42	5.68	1.97	1.45	1.75	3.257253	0.000085
CTGF	848.3	776.53	759.02	135.39	126.45	137	5.976958	0.001491
SERPINE1	2882.5	2733.5	2714.01	578.74	540.88	568.17	4.935454	0.000350
ADAM12	0.59	1.12	0.88	0.08	0.04	0.08	12.950000	0.034210
PLAT	203.08	192.17	197.34	38.7	37.65	37.44	5.207751	0.000324
THY1	1.24	1.92	1.5	0.05	0.11	0.11	17.259259	0.017040
CD44	60.42	69.61	60.54	2.07	5.09	3.17	18.448209	0.001310
PTX3	1377.37	1159.73	1186.43	82.42	75	69.88	16.381566	0.003366

FC indicates fold change.

Table S2. Primers used for RT-qPCR.

Primers for genes	Sequence
CDH5-F	AAGCGTGAGTCGCAAGAATG
CDH5-R	TCTCCAGGTTTTCGCCAGTG
VWF-F	CCGATGCAGCCTTTTCGGA
VWF-R	TCCCCAAGATACACGGAGAGG
CDH2-F	TGCGGTACAGTGTAACTGGG
CDH2-R	GAAACCGGGCTATCTGCTCG
SNAI2-F	TGTGACAAGGAATATGTGAGCC
SNAI2-R	TGAGCCCTCAGATTTGACCTG
NOTCH1-F	GAGGCGTGGCAGACTATGC
NOTCH1-R	CTTGTACTCCGTCAGCGTGA
NOTCH2-F	CAACCGCAATGGAGGCTATG
NOTCH2-R	GCGAAGGCACAATCATCAATGTT
NOTCH3-F	TGGCGACCTCACTTACGACT
NOTCH3-R	CACTGGCAGTTATAGGTGTTGAC
NOTCH4-F	TGTGAACGTGATGTCAACGAG
NOTCH4-R	ACAGTCTGGGCCTATGAAACC
HEY1-F	GTTCGGCTCTAGGTTCCATGT
HEY1-R	CGTCGGCGCTTCTCAATTATTC
HEY2-F	AAGGCGTCGGGATCGGATAA
HEY2-R	AGAGCGTGTGCGTCAAAGTAG
GAPDH-F	AATGACCCCTTCATTGAC
GAPDH-R	TCCACGACGTACTCAGCGC

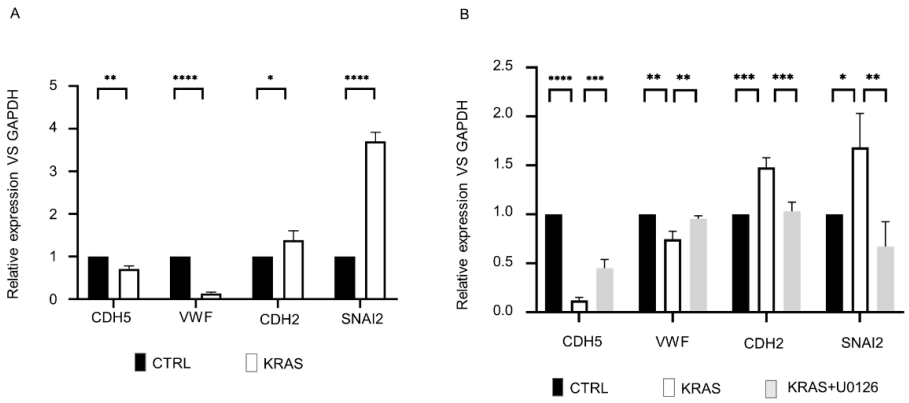

Figure S1. A, Expression of endothelial (CDH5, VWF) and mesenchymal markers (CDH2, SNAI2) in HUVECs overexpressing $KRAS^{G12D}$ and control adenovirus after 24 hours as shown by real-time PCR (n=3). **B,** Expression of endothelial and mesenchymal markers in HUVECs overexpressing $KRAS^{G12D}$, HUVECs overexpressing $KRAS^{G12D}$ treated with U0126 (KRAS+U0126), and control after 48 hours as shown by real-time PCR (n=3). *P<0.05, **P<0.01, ****P<0.0001.

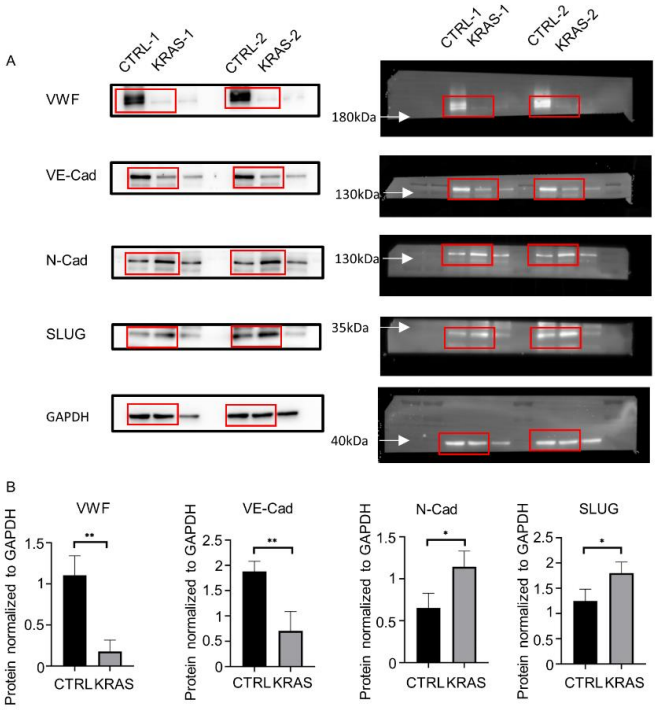

Figure S2. Effects of KRAS mutation on EndMT markers at the protein level. **A**, Two additional western blot (left) and full unedited gel (right) image. The red box is the target protein region. **B,** quantification showing decrease in endothelial markers (VWF, VE-Cad) and increase in mesenchymal markers (N-Cad, SLUG) after infected with KRAS mutant adenovirus. *: p≤0.05, **: p≤0.01.

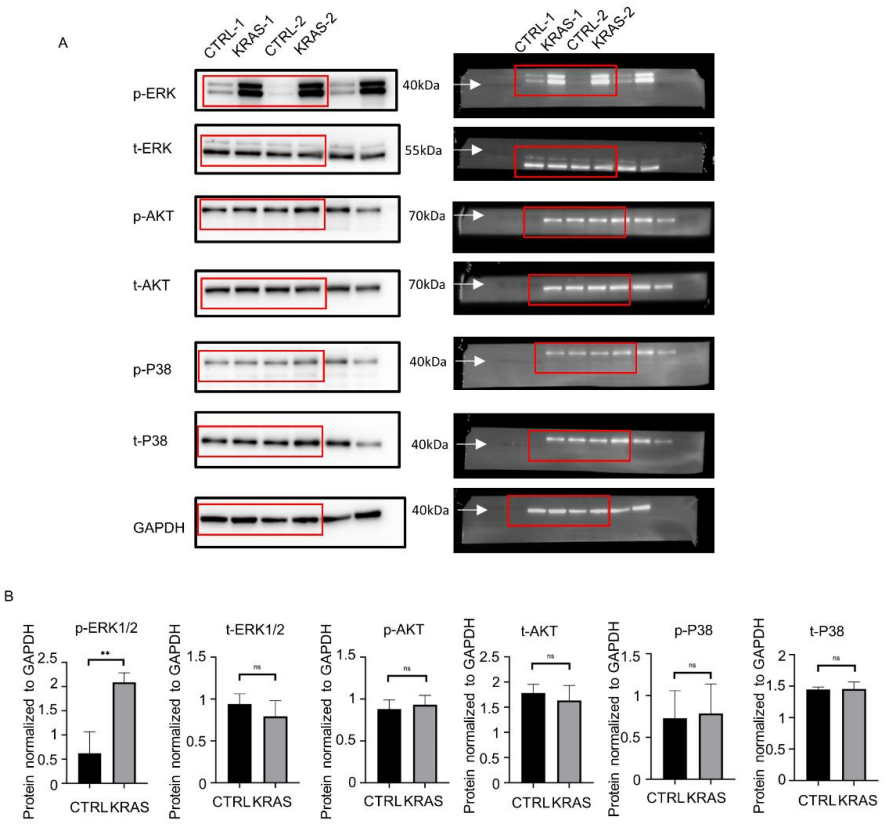

Figure S3. Detection of ERK Phosphorylation in KRAS mutant HUVECs. **A**, Two additional western blot (left) and full unedited gel (right) image. The red box is the target protein region. **B,** quantification showing increased phosphorylation of ERK in KRAS mutant HUVECs but not of P38 or AKT. ns: $p>0.05$, **: $p\leq0.01$.

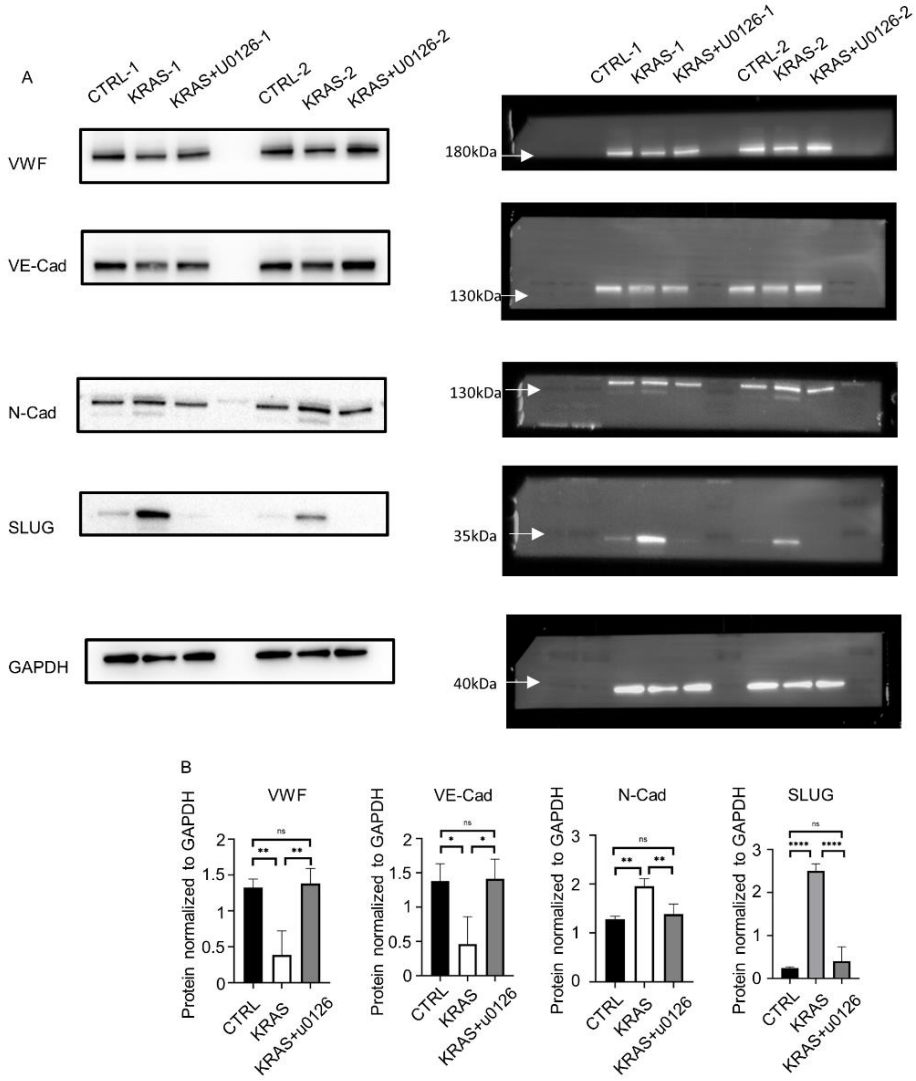

Figure S4. Effects of MAPK inhibitor U0126 on EndMT markers in KRAS mutant HUVECs. **A,** Two additional western blot (left) and full unedited gel (right) image. **B,** quantification showing the decrease of endothelial markers (VWF, VE-Cad) and increase of mesenchymal markers (N-Cad, SLUG) by KRAS mutant were reversed by U0126 (KRAS+U0126). ns: p>0.05, *: p≤0.05, **: p≤0.01, ****: p≤0.0001.

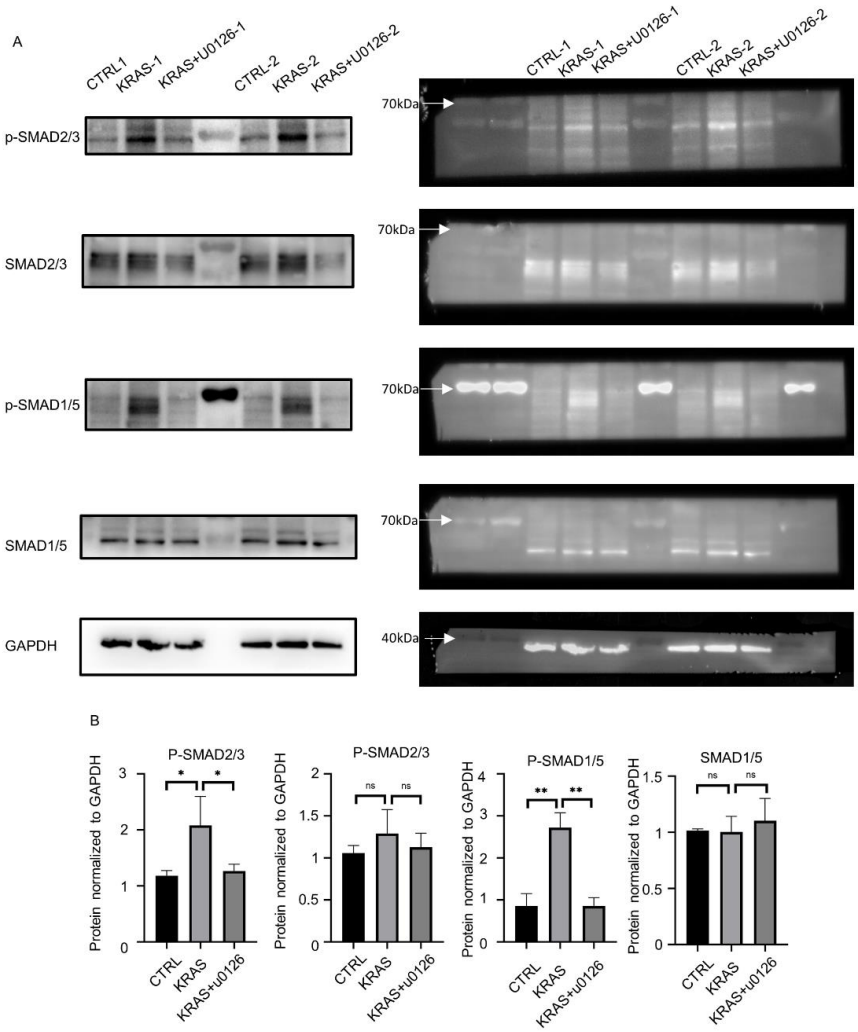

Figure S5. Effect of U0126 on SMAD2/3 and SMAD1/5 Phosphorylation in KRAS mutant HUVECs. **A,** Two additional western blot (left) and full unedited gel (right) image. **B,** quantification showing increased phosphorylation of SMAD2/3 and SMAD1/5 in KRAS mutant HUVECs, which was reversed by U0126 (KRAS+U0126). ns: $p>0.05$, *: $p\leq0.05$, **: $p\leq0.01$.

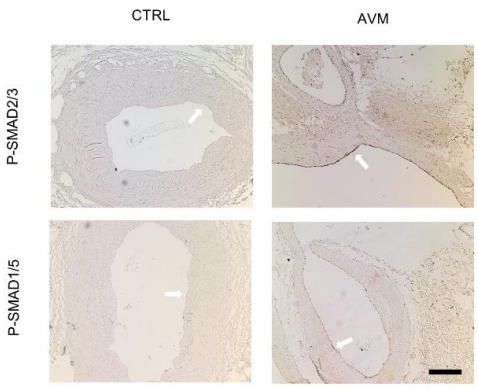

Figure S6. Immunohistochemical staining for p-SMAD2/3 or p-SMAD1/5 in bAVM tissue (n=3). The white arrows indicate vascular ECs. The scale bar corresponds to 200 μm.

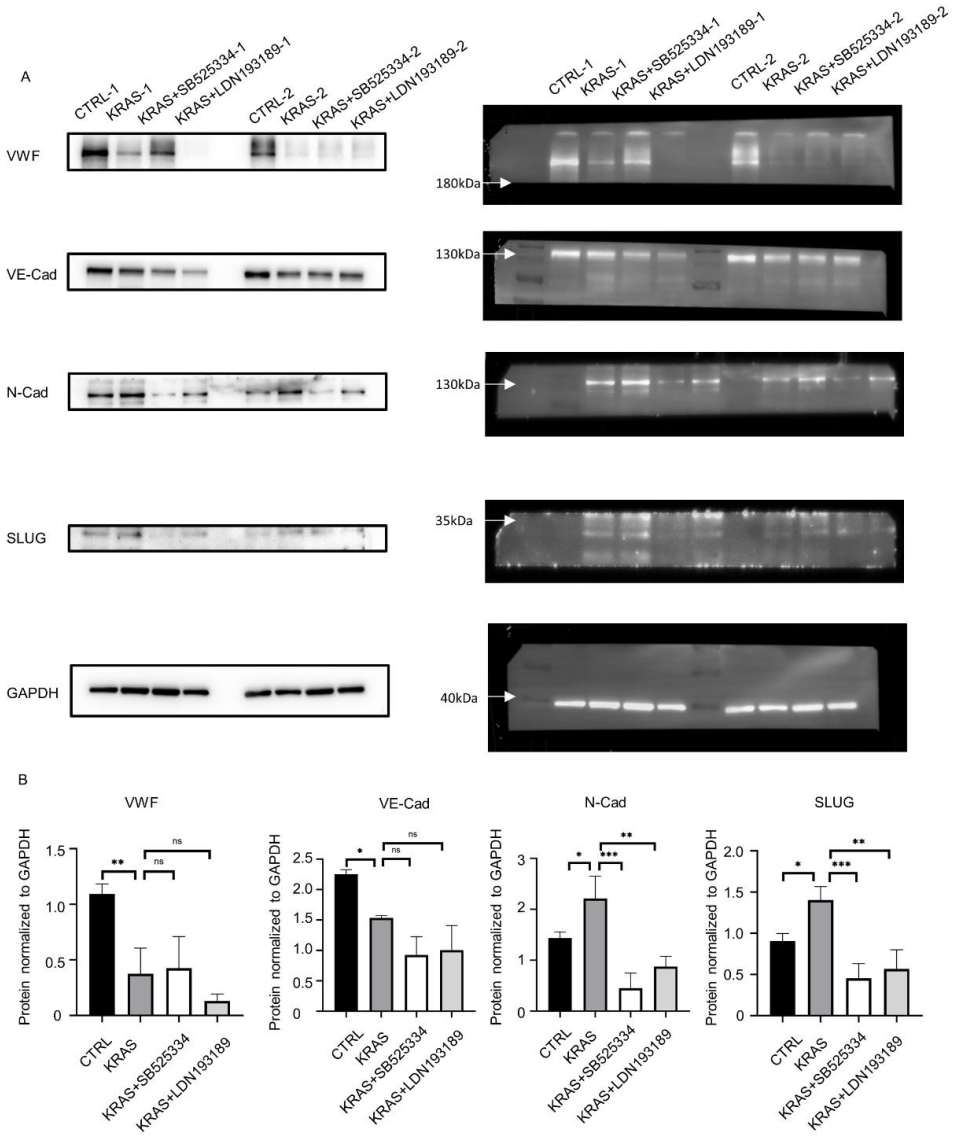

Figure S7. Effects of TGF inhibitor (SB525334) and BMP inhibitor (LDN193189) on EndMT markers in KRAS mutant HUVECs. **A,** Two additional western blot (left) and full unedited gel (right) image. **B,** quantification showing the decrease of endothelial markers (VWF, VE-Cad) and increase of mesenchymal markers (N-Cad, SLUG) by KRAS mutant, and only mesenchymal markers were reversed by SB525334 (KRAS+ SB525334) or LDN193189 (KRAS+ LDN193189). ns: p>0.05, *: p≤0.05, **: p≤0.01, ***: p≤0.001.

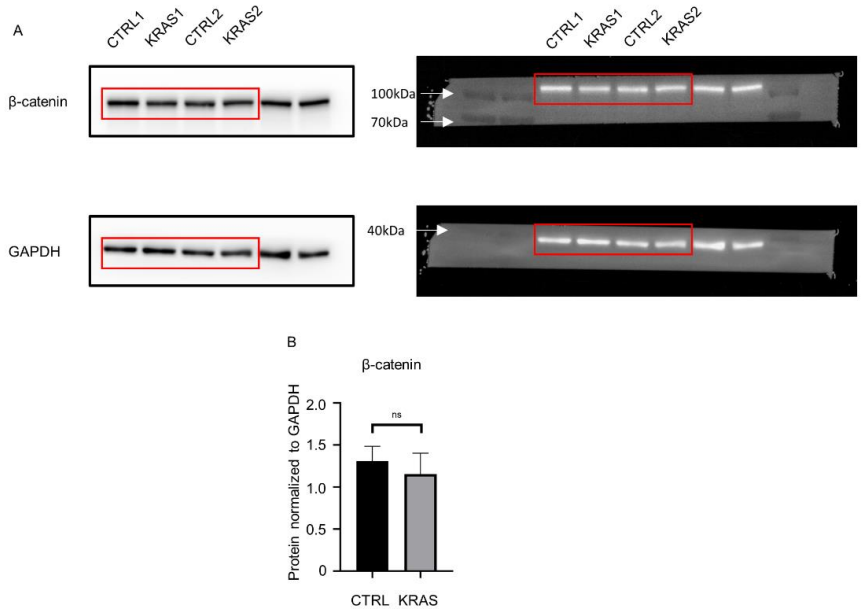

Figure S8. Effects of KRAS mutation on β-catenin at the protein level. **A**, Two additional western blot (left) and full unedited gel (right) image. The red box is the target protein region. **B,** quantification showing KRAS mutation had no effect on β-catenin expression. ns: p>0.05.

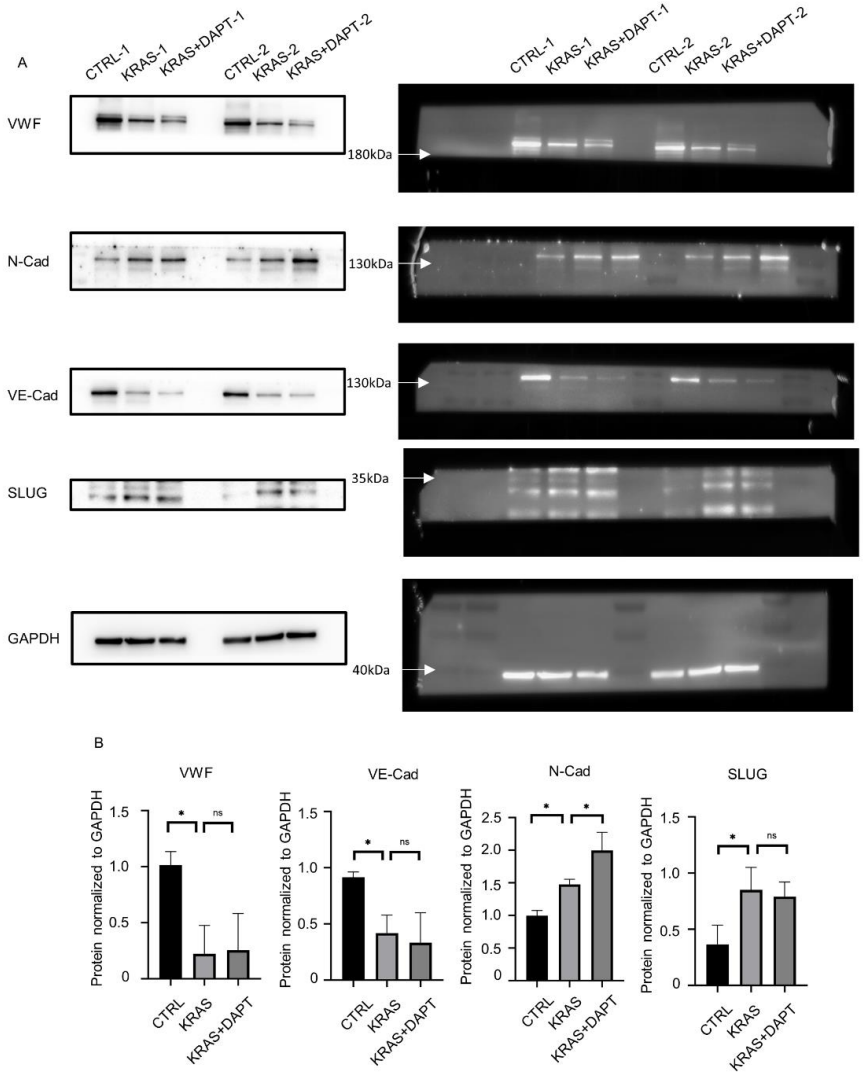

Figure S9. Effects of Notch inhibitor DAPT on EndMT markers in KRAS mutant HUVECs. **A,** Two additional western blot (left) and full unedited gel (right) image. **B,** quantification showing the decrease of endothelial markers (VWF, VE-Cad) and increase of mesenchymal markers (N-Cad, SLUG) by KRAS mutant and DAPT (KRAS+DAPT) had no effect on it. ns: $p>0.05$, *: $p\leq0.05$.

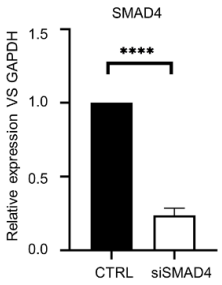

Figure S10. Knockdown efficiency of Smad4 by PCR.

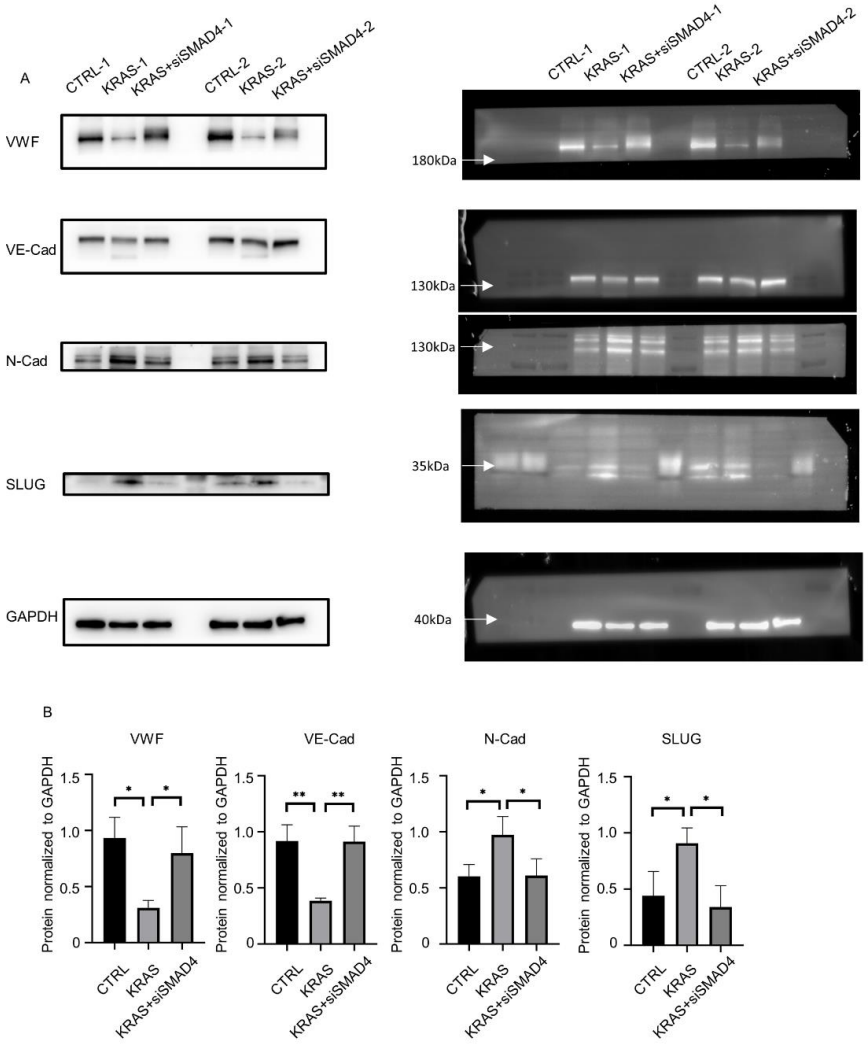

Figure S11. Effects of siSMAD4 on EndMT markers in KRAS mutant HUVECs. **A,** Two additional western blot (left) and full unedited gel (right) image. **B,** quantification showing the decrease of endothelial markers (VWF, VE-Cad) and increase of mesenchymal markers (N-Cad, SLUG) by KRAS mutant were reversed by siSMAD4 (KRAS+siSMAD4). *: p≤0.05, **: p≤0.01.

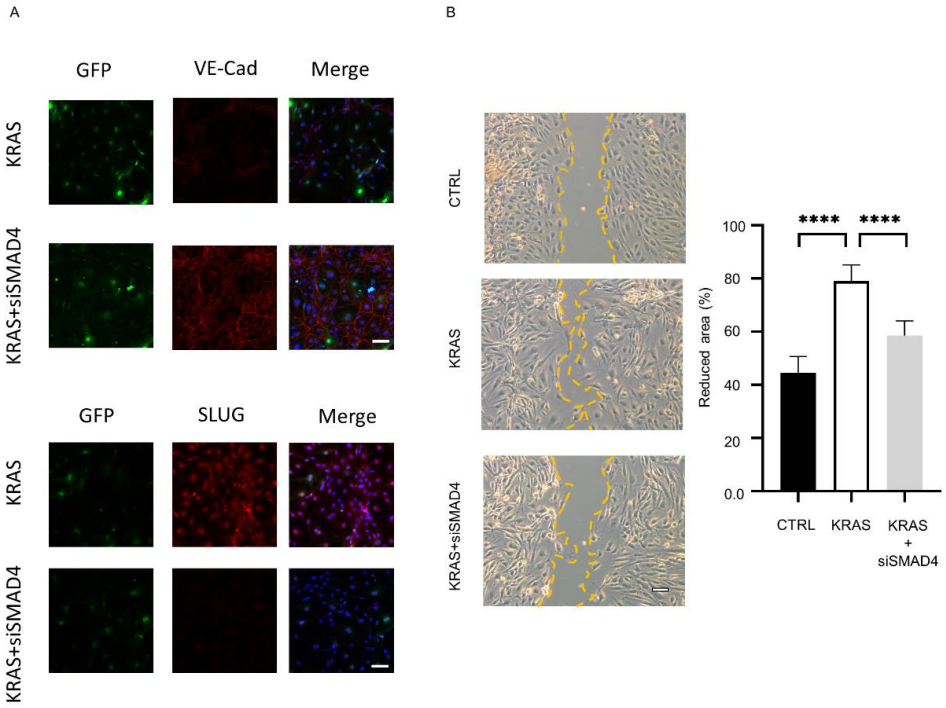

Figure S12. A, Immunofluorescence staining for VE-Cadherin and SLUG in KRAS-mutant HUVECs transfected with siSmad4 or siCTRL (n=3). Scale bar: 100 μm. **B,** Effects of siSmad4 on KRAS-mutant HUVEC migration (left). The scale bar corresponds to 100 μm. The right panel shows the statistical analysis of the reduced area (n=9). The T bars represent standard deviation.

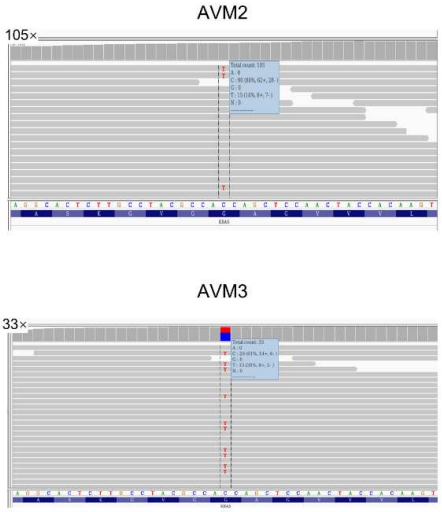

Figure S13. Whole exon sequencing for KRAS^{G12D} mutation in bAVM endothelial cells.

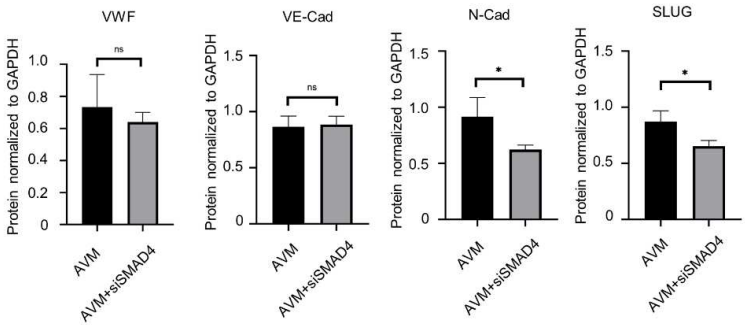

Figure S14. Effects of siSMAD4 on EndMT markers in KRAS mutant primary AVM ECs. ns: $p>0.05$, *: $p\leq0.05$.

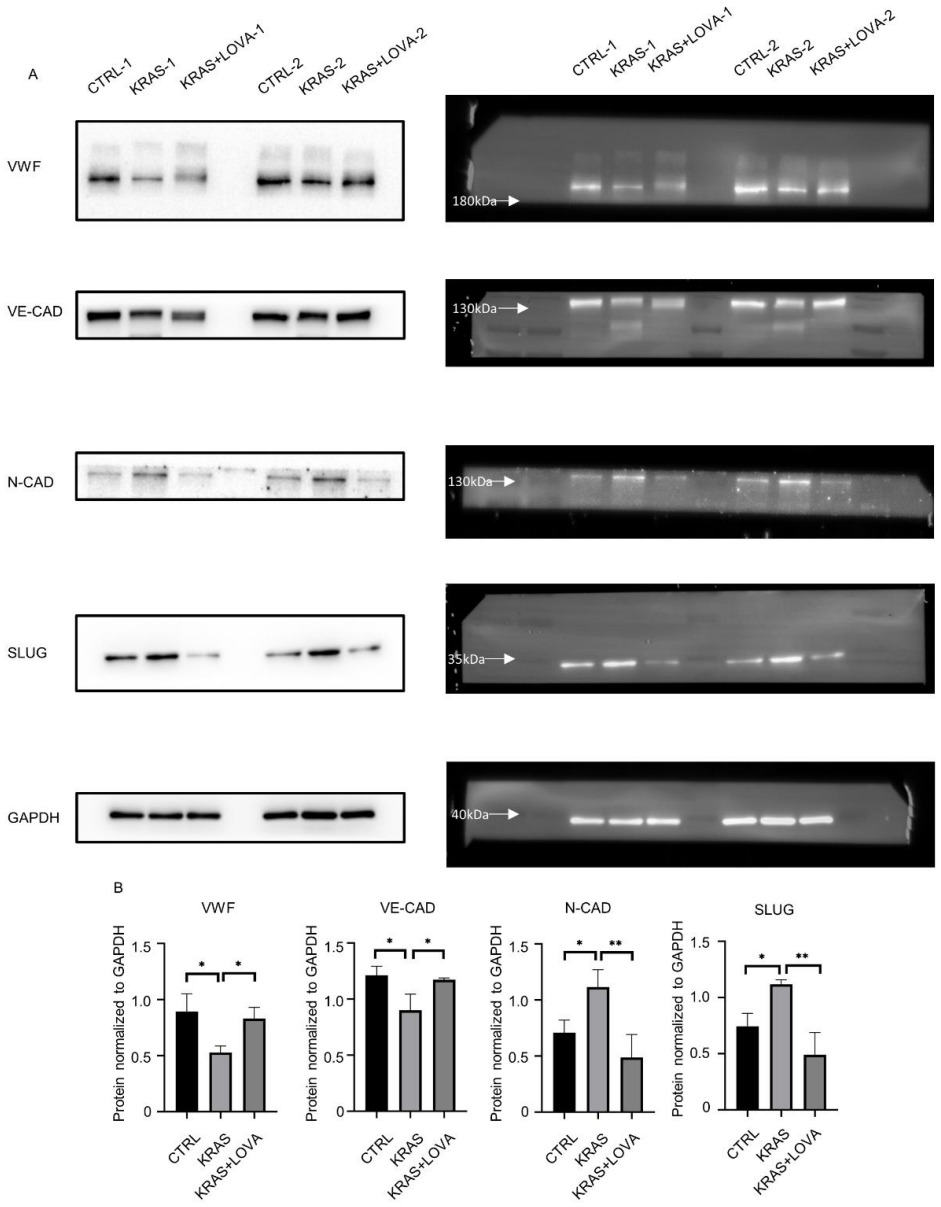

Figure S15. Effects of lovastatin (LOVA) on EndMT markers in KRAS mutant HUVECs. **A,** Two additional western blot (left) and full unedited gel (right) image. **B,** quantification showing the decrease of endothelial markers (VWF, VE-Cad) and increase of mesenchymal markers (N-Cad, SLUG) by KRAS mutant were reversed by LOVA (KRAS+LOVA). *: p≤0.05, **: p≤0.01.

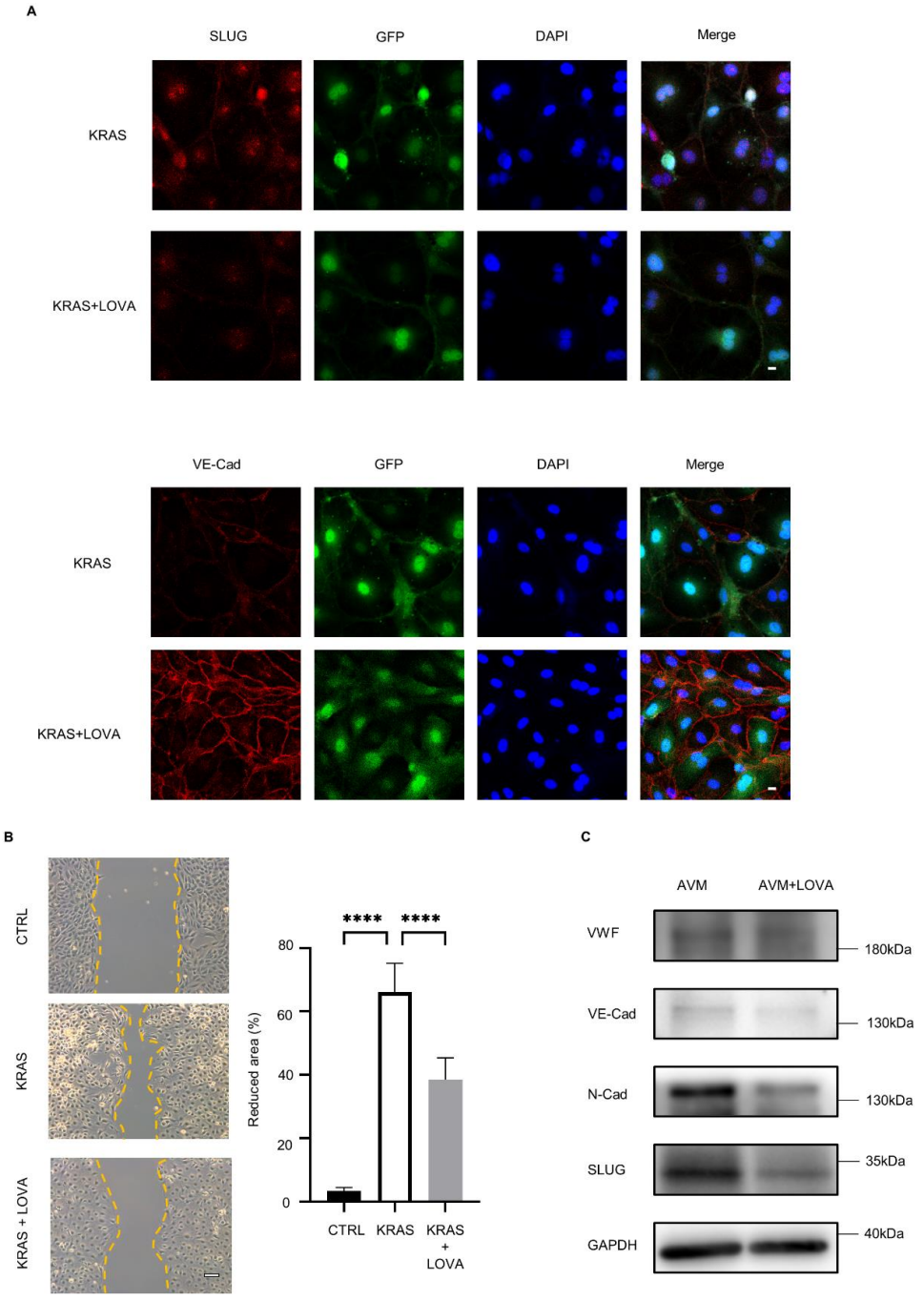

Figure S16. A, Immunofluorescence staining for VE-Cadherin and SLUG in KRAS-mutant HUVECs treated with lovastatin or 0.1% DMSO (n=3). Scale bar: 100 μm. **B,** Effects of lovastatin on KRAS-mutant HUVEC migration (left). The scale bar corresponds to 100 μm. The right panel shows the statistical analysis of the reduced area (n=9). The T bars represent standard deviation. **C,** Western blot showing expression of mesenchymal markers and endothelial markers in KRAS-mutant bAVM ECs and ECs treated with lovastatin (AVM1).

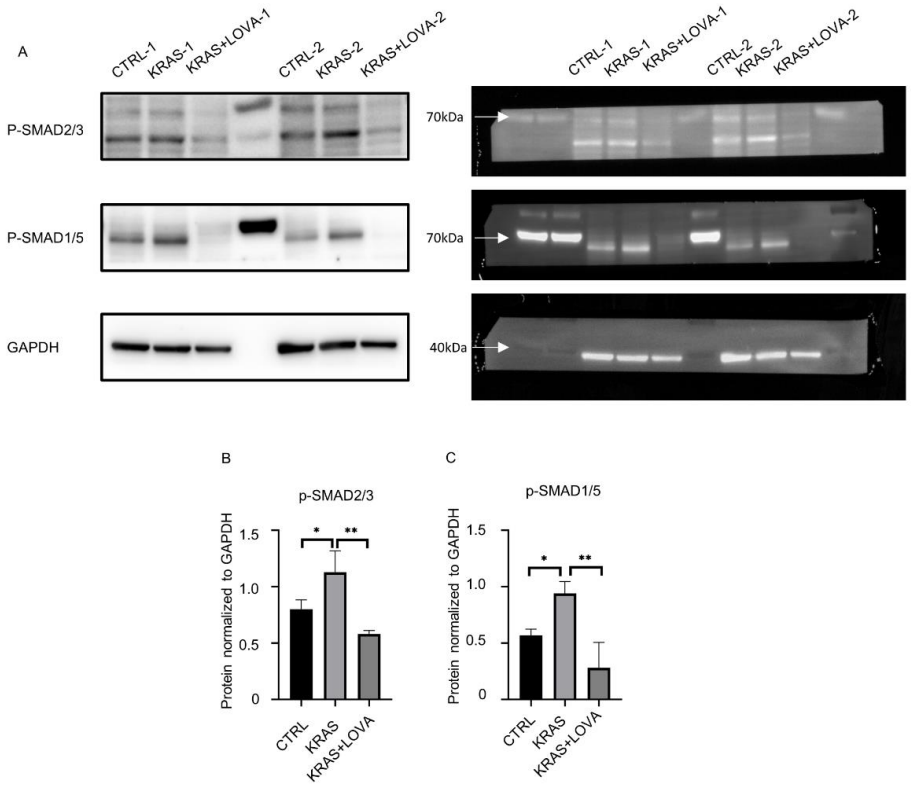

Figure S17. Effect of lovastatin (LOVA) on SMAD2/3 and SMAD1/5 Phosphorylation in KRAS mutant HUVECs. **A,** Western blot (left) and full unedited gel (right) image. **B,** quantification showing increased phosphorylation of SMAD2/3 and SMAD1/5 in KRAS mutant HUVECs, which was reversed by lovastatin (KRAS+LOVA). *: $p \leq 0.05$, **: $p \leq 0.01$.

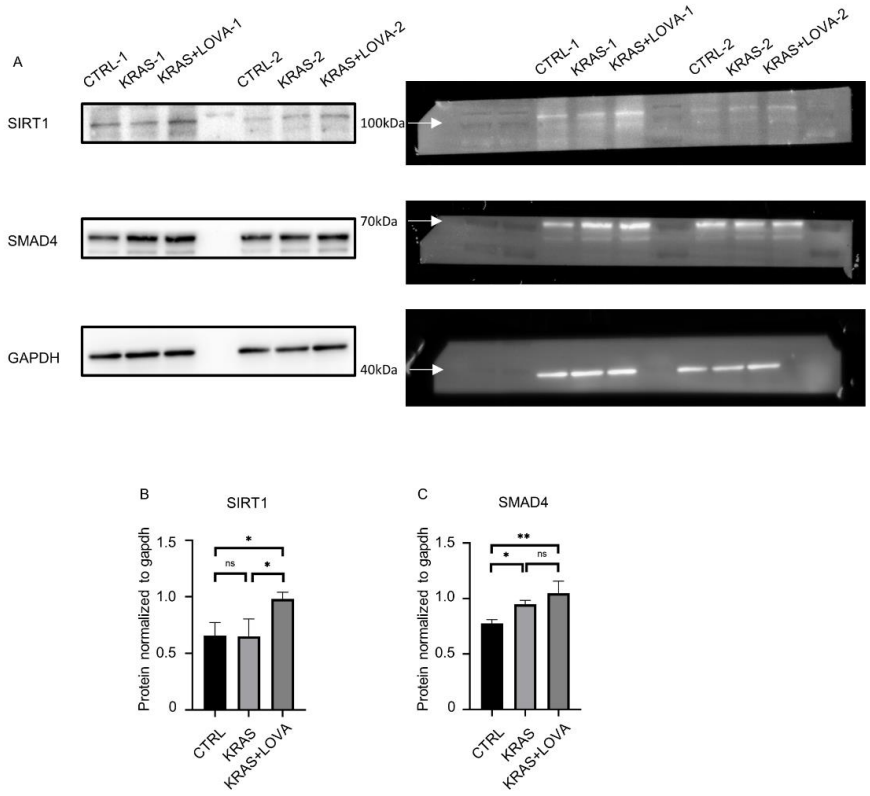

Figure S18. Effect of lovastatin (LOVA) on the expression of SIRT1 and SMAD4 in KRAS mutant HUVECs. **A,** Western blot (left) and full unedited gel (right) image. **B,** quantification showing increased level of SIRT1 in KRAS mutant HUVECs treated with lovastatin (KRAS+LOVA). **C,** quantification showing increased level of SMAD4 in KRAS mutation HUVECs and lovastatin had no effect on it. ns: $p>0.05$, *: $p\leq0.05$, **: $p\leq0.01$.

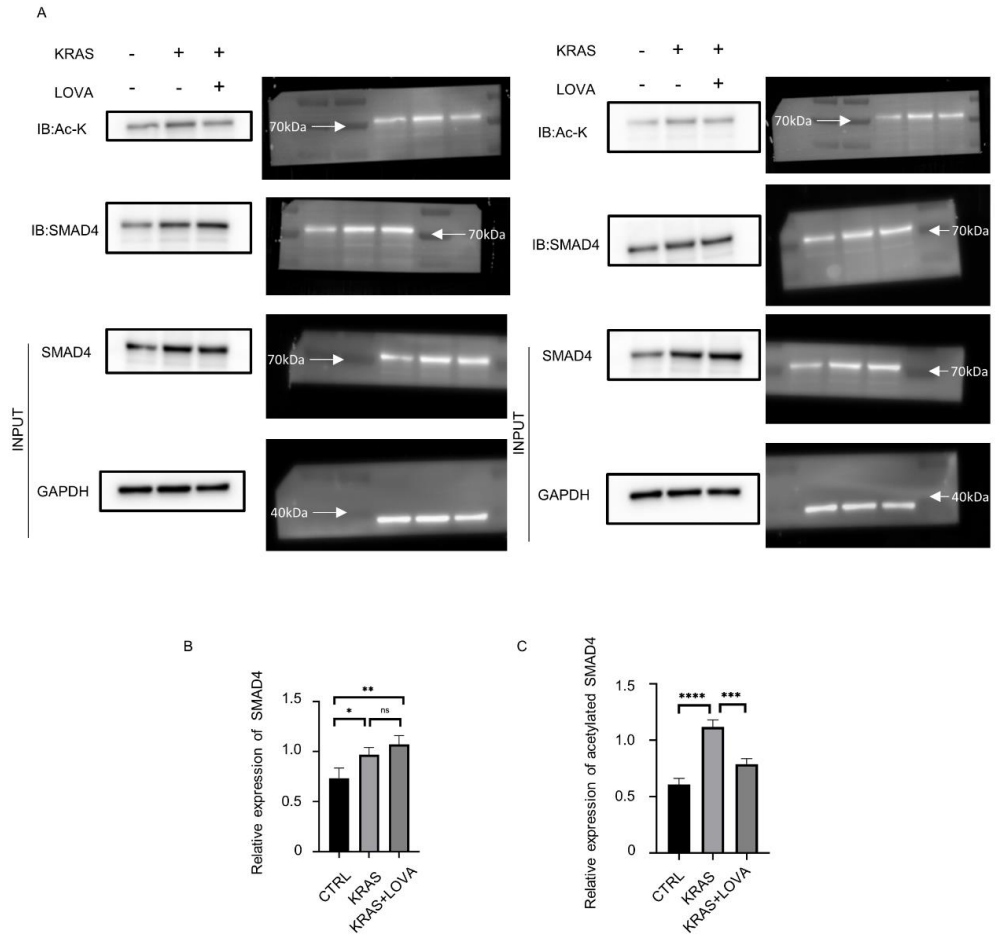

Figure S19. Effect of lovastatin (LOVA) on the expression of acetylated SMAD4 in KRAS mutant HUVECs. **A,** Immunoprecipitation of SMAD4 acetylation after lovastatin treatment. **B,** quantification showing lovastatin had no effect on SMAD4 expression in KRAS mutant HUVECs. **C,** quantification showing increased level of acetylated SMAD4 in KRAS mutation HUVECs, which was reversed by lovastatin. ns: $p>0.05$, *: $p\leq0.05$, **: $p\leq0.01$, ***: $p\leq0.001$, ****: $p\leq0.0001$.