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Supplemental Information

Exosomal Delivery of AntagomiRs Targeting Viral and Cellular MicroRNAs Synergistically Inhibits Cancer Angiogenesis

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Supplemental Figures and Legends:

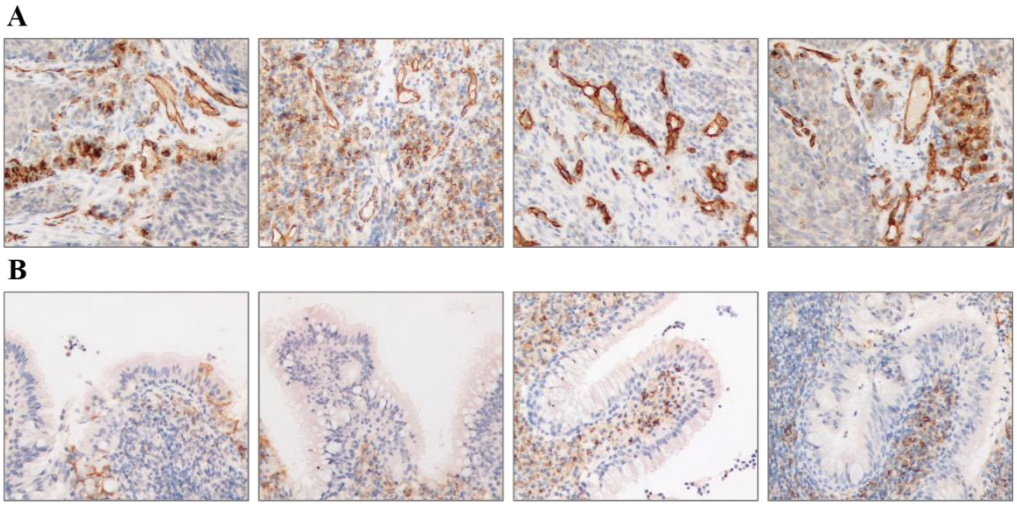


Figure S1 The expression of CD31 in NPC (A) and NP (B) (200x).

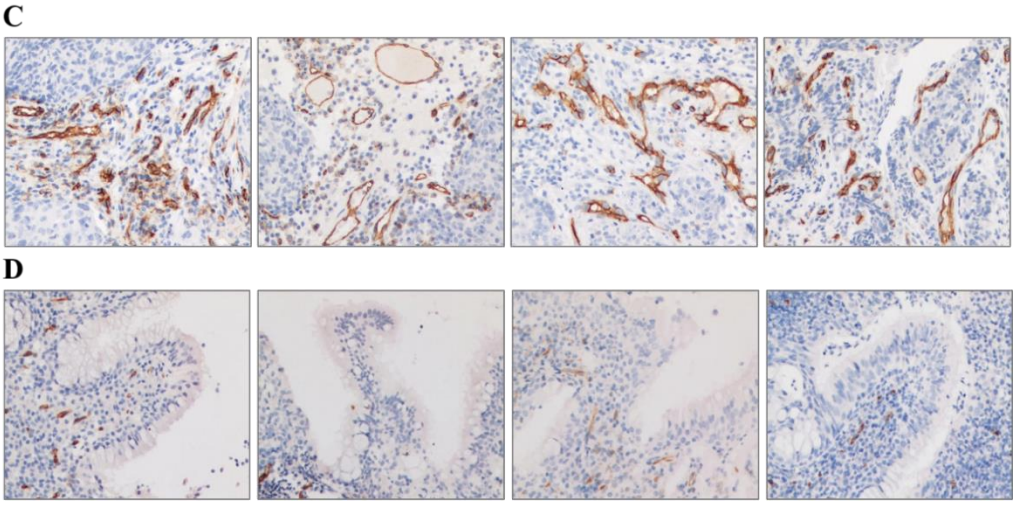


Figure S1 The expression of CD34 in NPC (C) and NP (D) (200x).

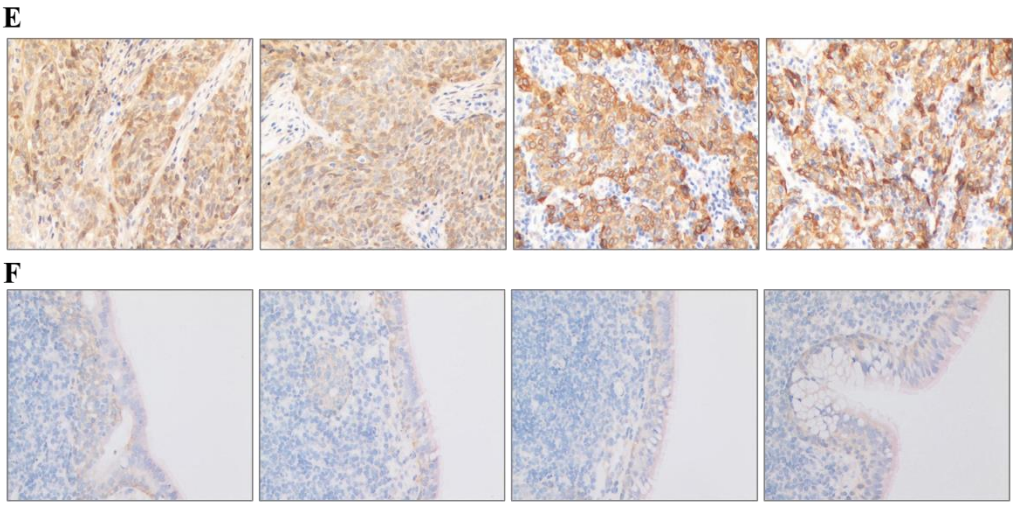
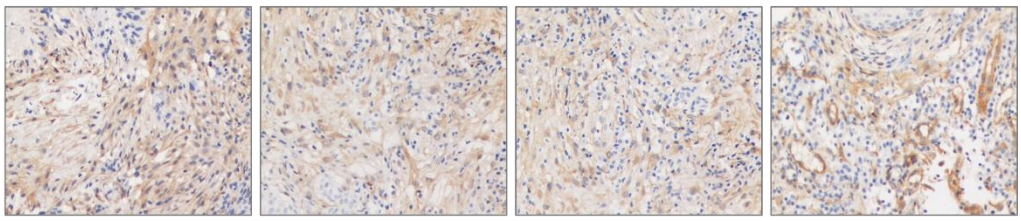


Figure S1 The expression of αv in NPC (E) and NP (F) (200x).

G



H

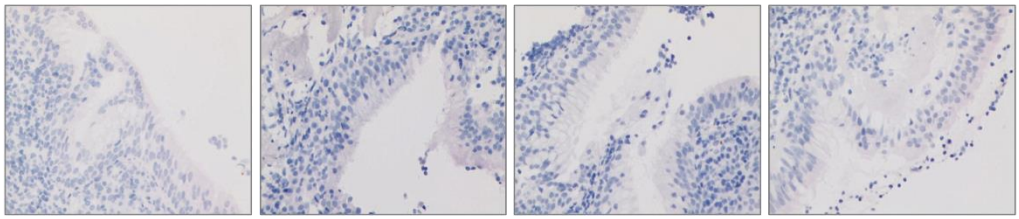
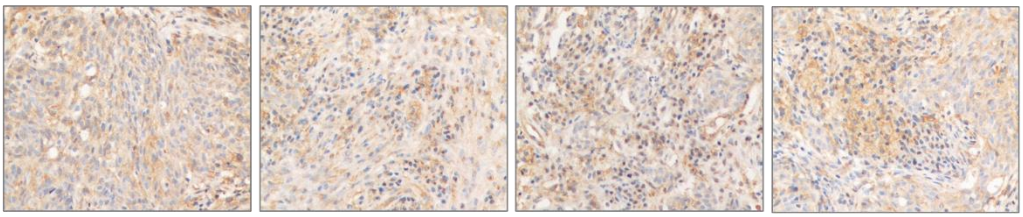


Figure S1 The expression of β3 in NPC (G) and NP (H) (200x).

I



J

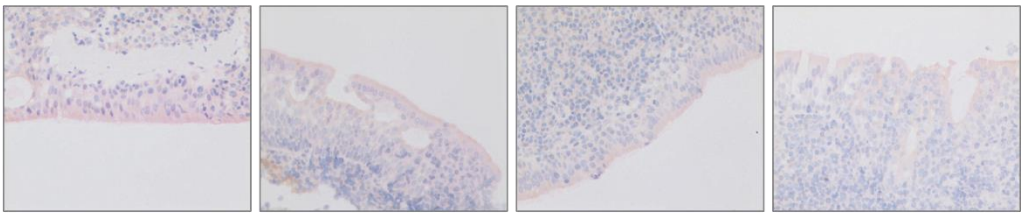


Figure S1 The expression of VEGFR1 in NPC (I) and NP (J) (200x).

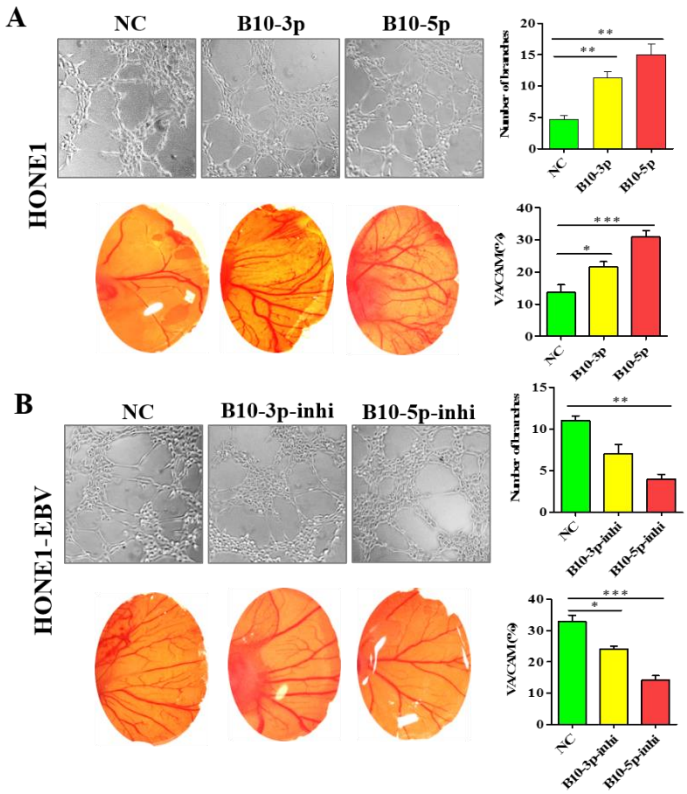


Figure S2 EBV-miR-BART10-5p possesses a superior angiogenesis-promoting effect compared with EBV-miR-BART10-3p.

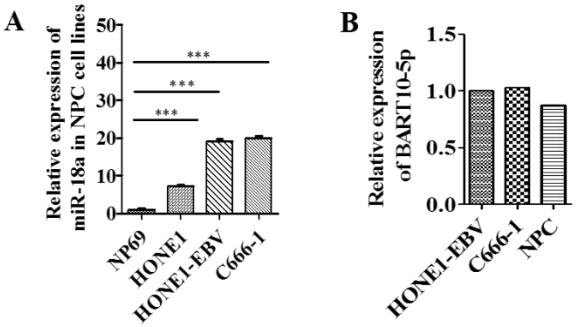


Figure S3 Expression of BART10-5p and miR-18a in NPC cell or NPC tissues.

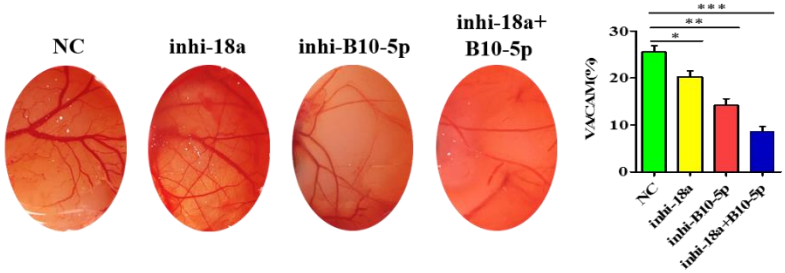


Figure S4 Influence upon angiogenesis after inhibition of BART10-5p and miR-18a in NPC C666-1 cell .

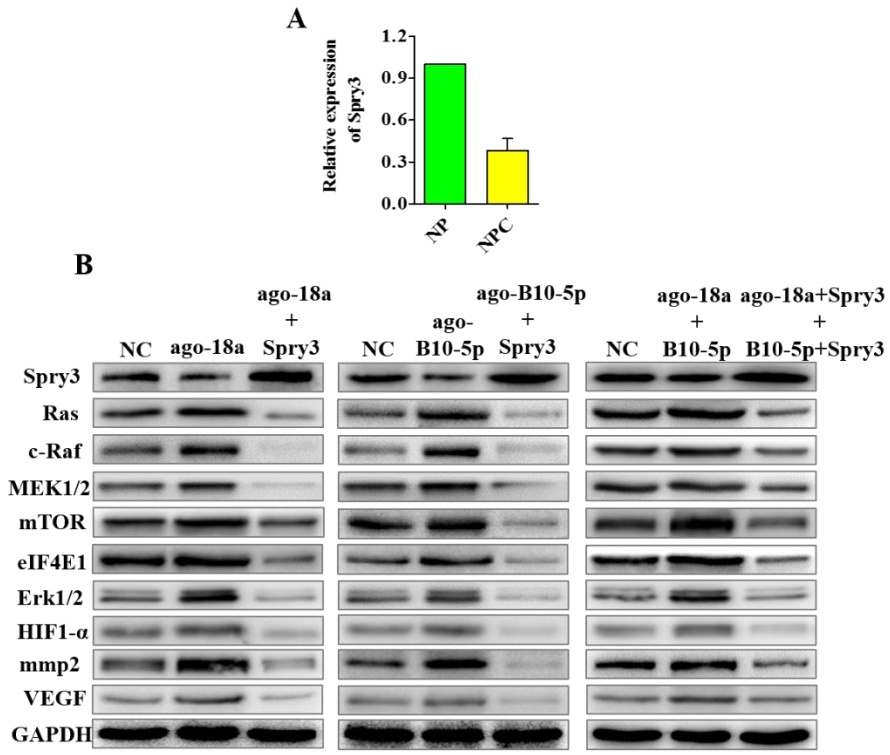


Figure S5A Expression of Spry3 in NPC tissues; Figure S5B Influence upon angiogenesis-associated pathway after recovery of Spry3.

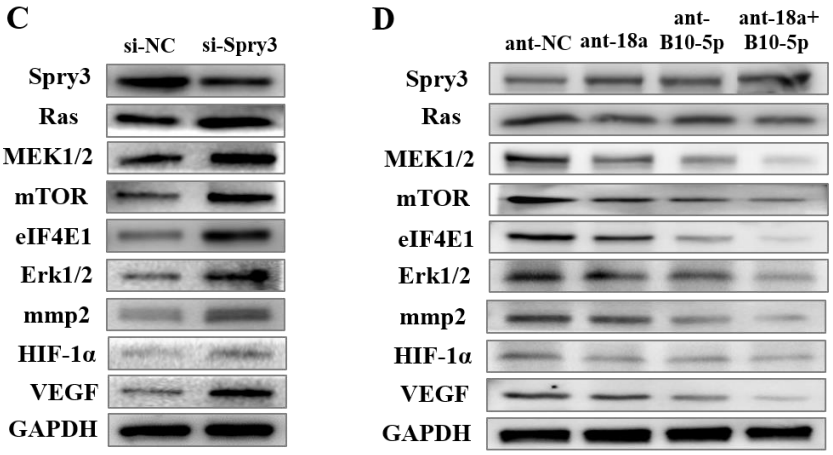


Figure S5C, S5D Influence upon angiogenesis-associated pathway after inhibition of Spry3, miR-18a, BART10-5p in C666-1 cells.

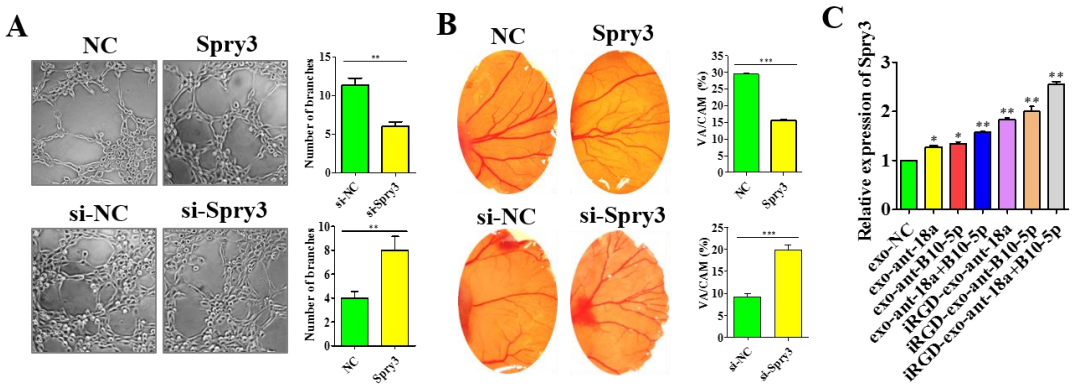


Figure S6 Spry3 is closely linked with angiogenesis in NPC.

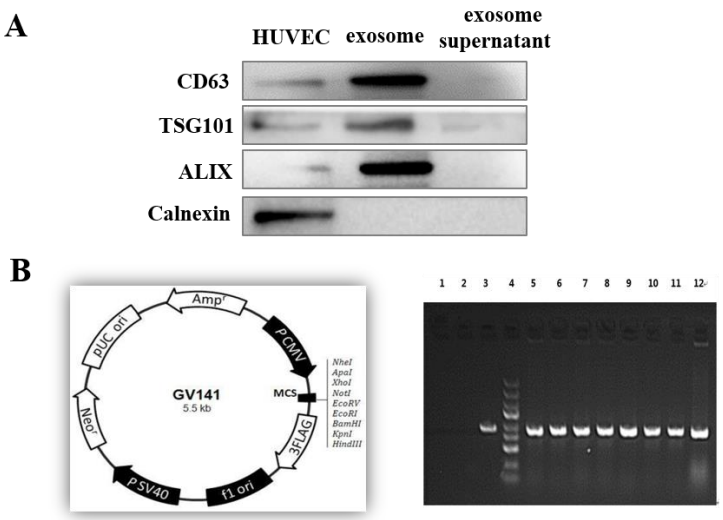


Figure S7A Identification of protein markers in exosome;
Figure S7B Construction of iRGD-Lamp2b plasmid.

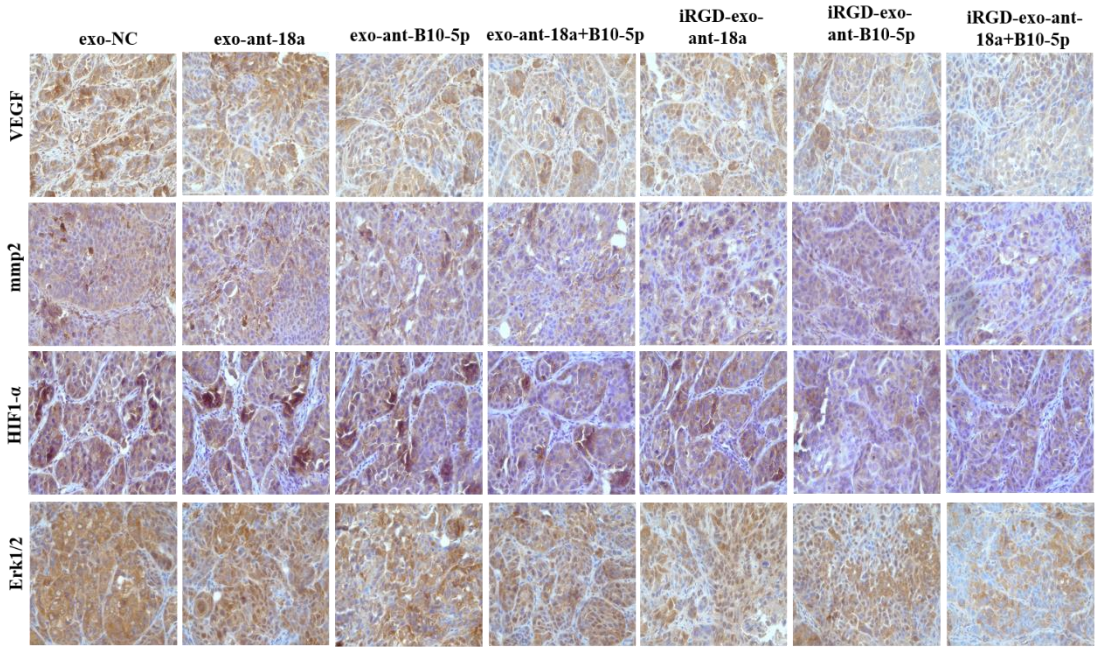


Figure S8 Down-regulation of regulating factors namely; VEGF, mmp2, HIF1-α, and Erk1/2 which are downstream of Spry3 in iRGD-exo-antagomiRs groups compared the free exosome control or exo-antagomiRs groups.

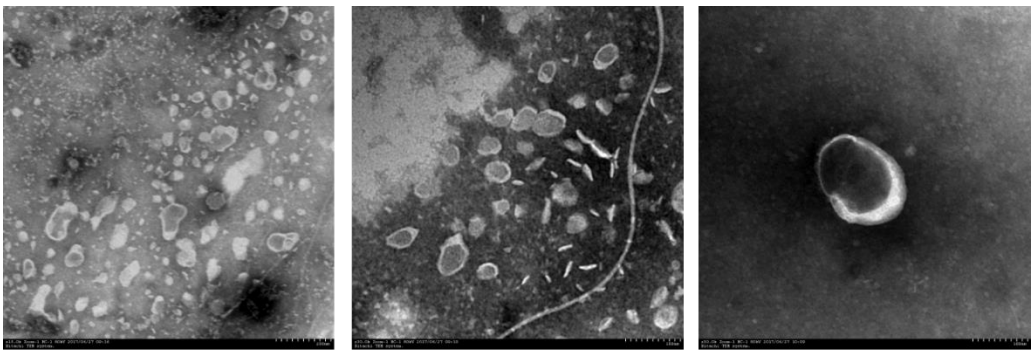
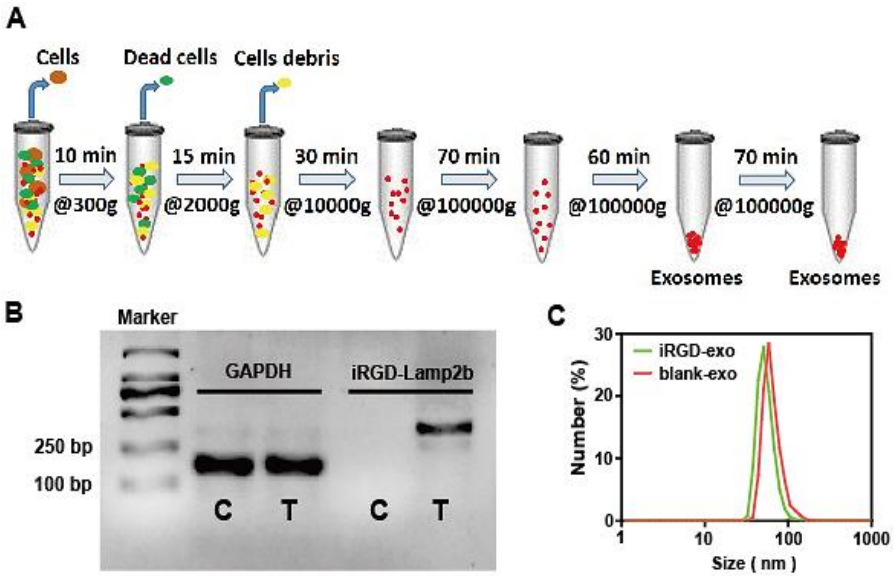


Figure S9 TAM of exosome.



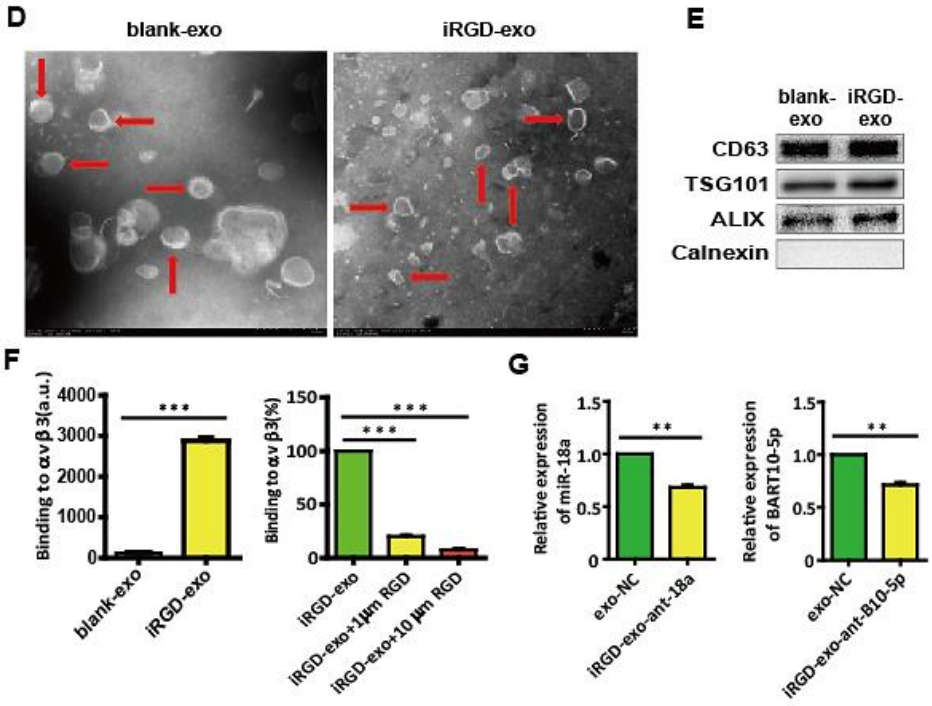


Figure S10. Isolation, identification, and characterization of exosomes.

(A) Schematic representation of isolating exosomes from the culture supernatant of HUVECs. (B) mRNA expression level of iRGD-Lamp2b in transfected HUVECs. (C) The size distributions of control (blank-exo) and iRGD-positive (iRGD-exo) exosomes. (D) Representative TEM images of control (blank-exo) and iRGD-positive (iRGD-exo) exosomes. Scale bar = 200 nm (in the lower right corner). (E) Expressions of CD63, ALIX, TSG101 and Calnexin in control (blank-exo) and iRGD-positive (iRGD-exo) exosomes derived from HUVECs. (F) Binding activity of blank-exo or iRGD-exo to immobilized αvβ3 integrin(left). (G) The relative expressions of BART10-5p and miR-18a following isolation of exosomes from co-transfection of BART10-5p/miR-18a and Lamp2b-iRGD in HUVEC.

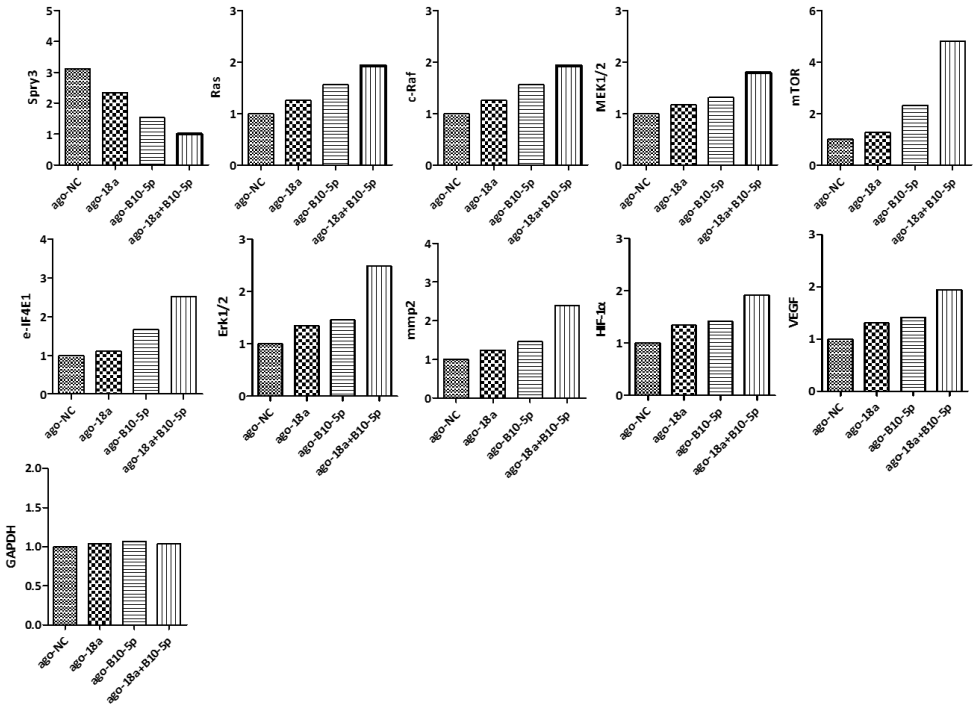


Figure S11 Quantitative analysis of western blot results by using Image J for Figure 3D .

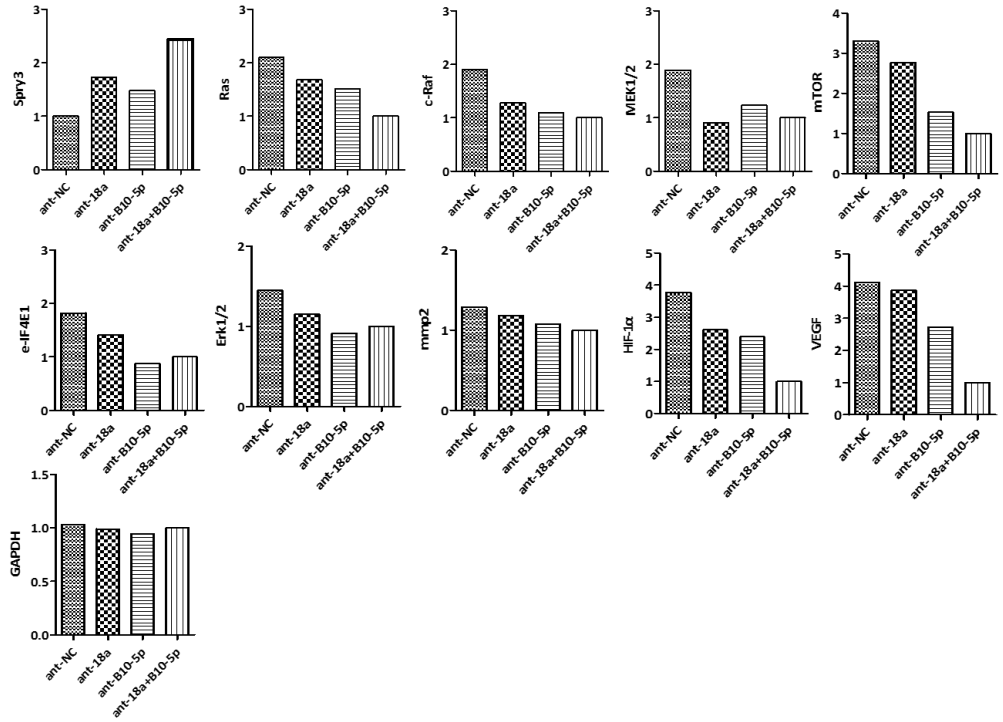


Figure S12 Quantitative analysis of western blot result by using Image J for Figure 3E .

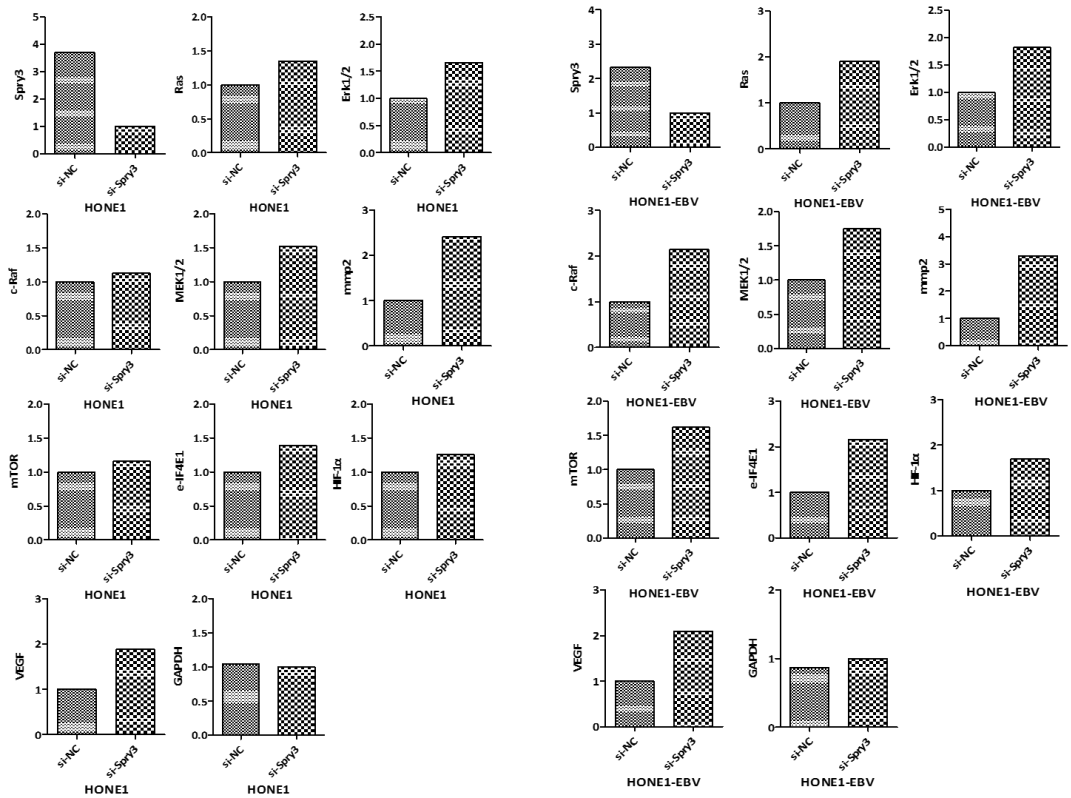


Figure S13 Quantitative analysis of western blot result by using Image J for Figure 3F .

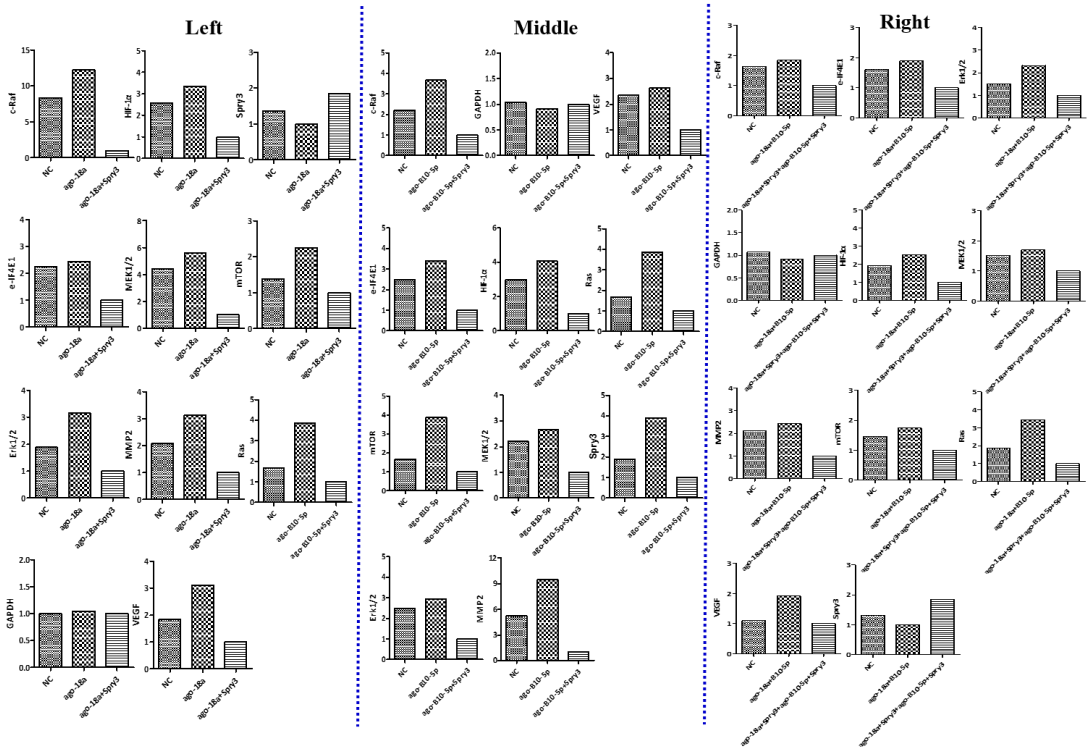


Figure S14 Quantitative analysis of western blot result by using Image J for Figure S5B .

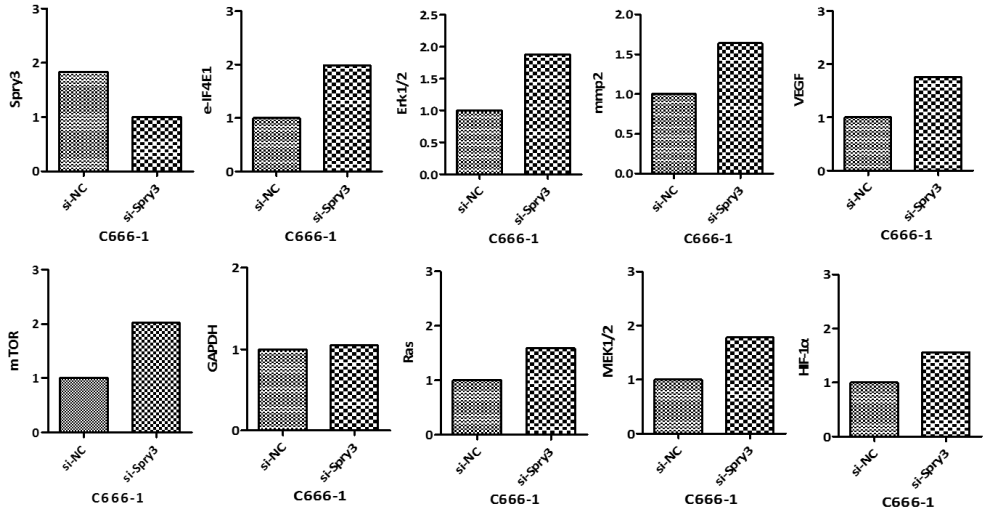


Figure S15 Quantitative analysis of western blot result by using Image J for Figure S5C .

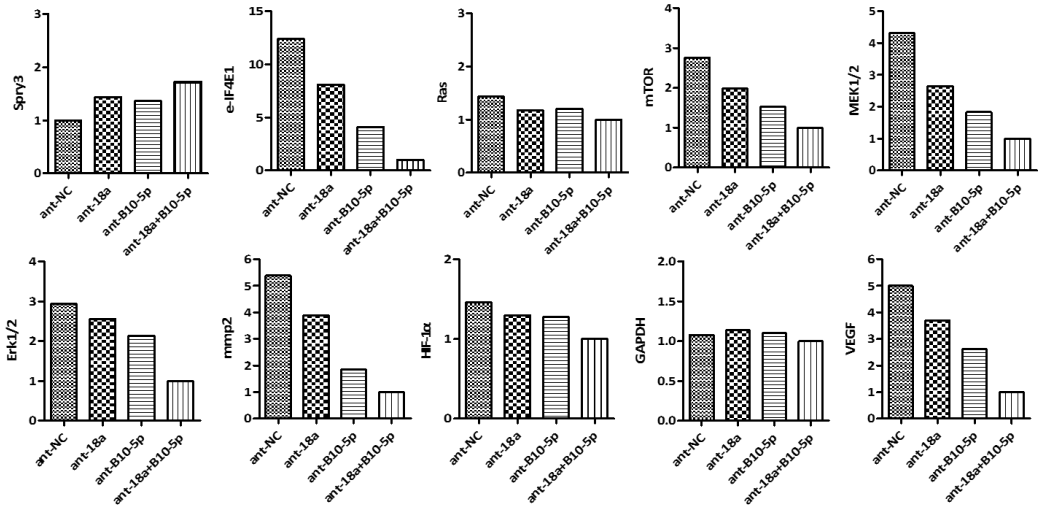


Figure S16 Quantitative analysis of western blot result by using Image J for Figure S5D .

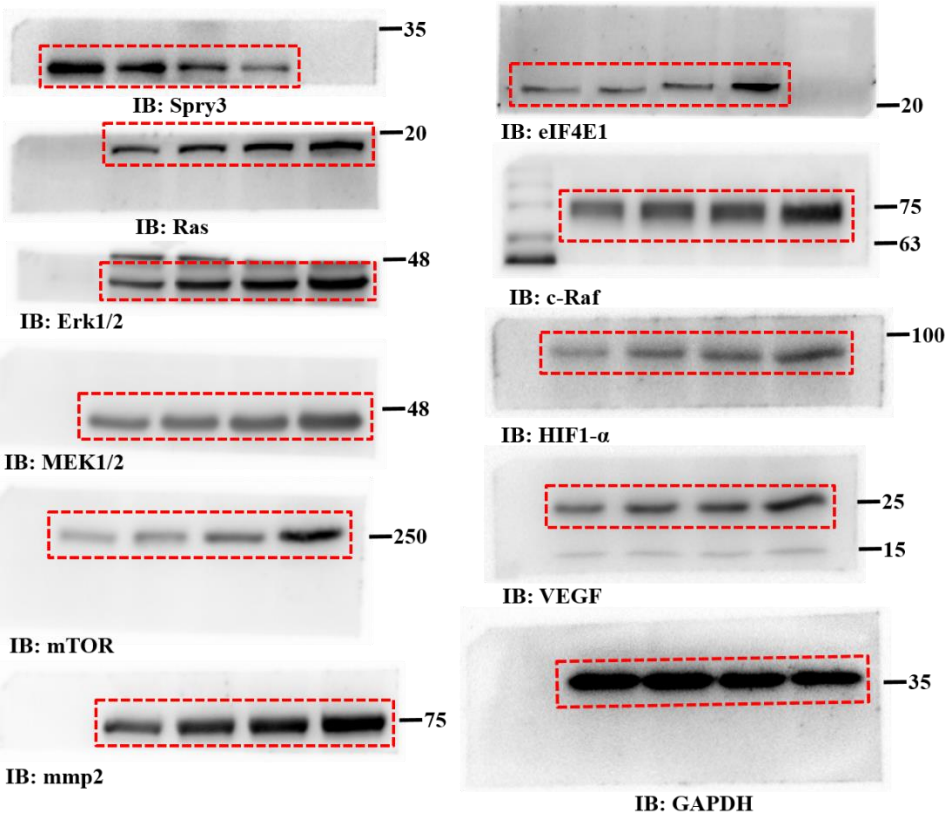


Figure S18 Original bands of western blot for Figure 3D.

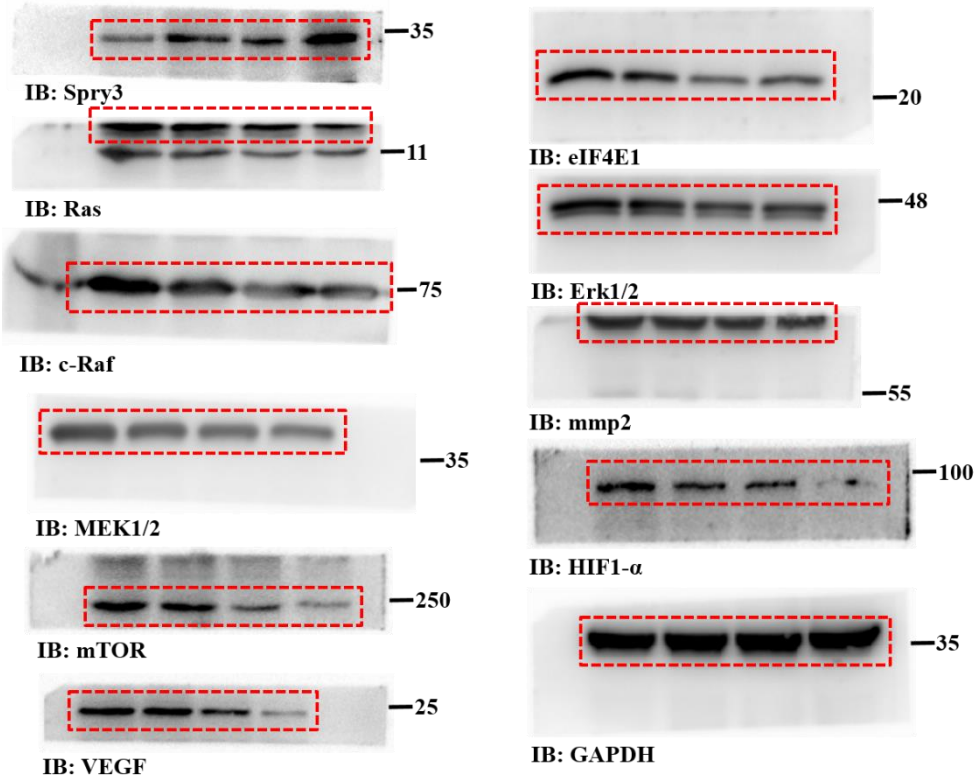


Figure S19 Original bands of western blot for Figure 3E.

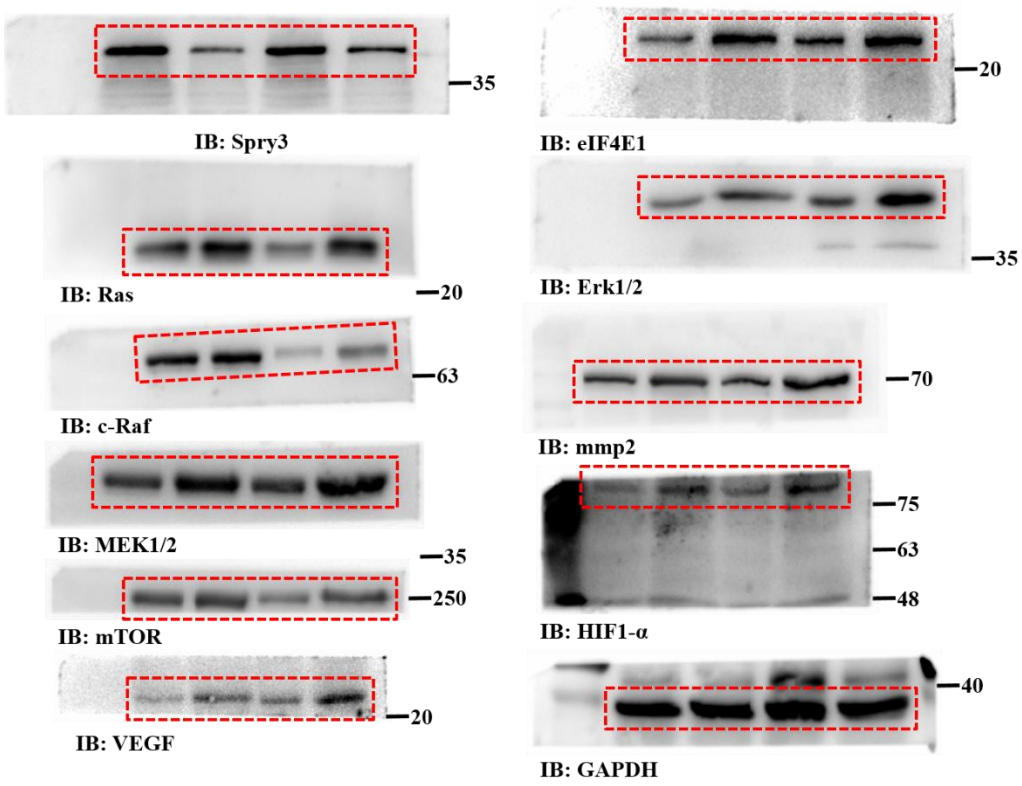


Figure S20 Original bands of western blot for Figure 3F.

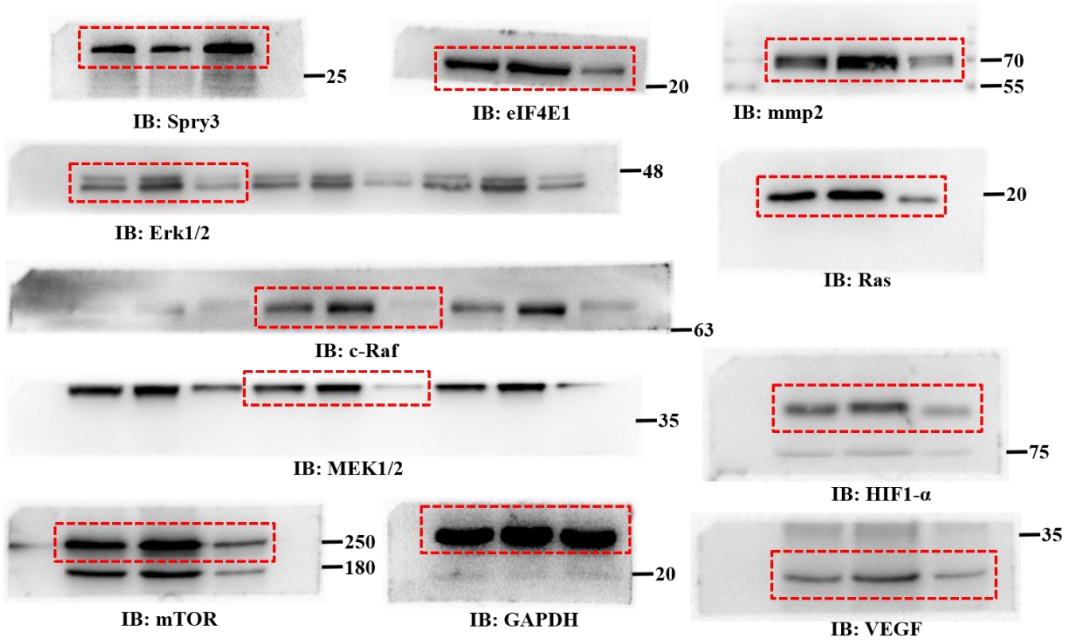


Figure S21 Original bands of western blot for Figure S5B left.

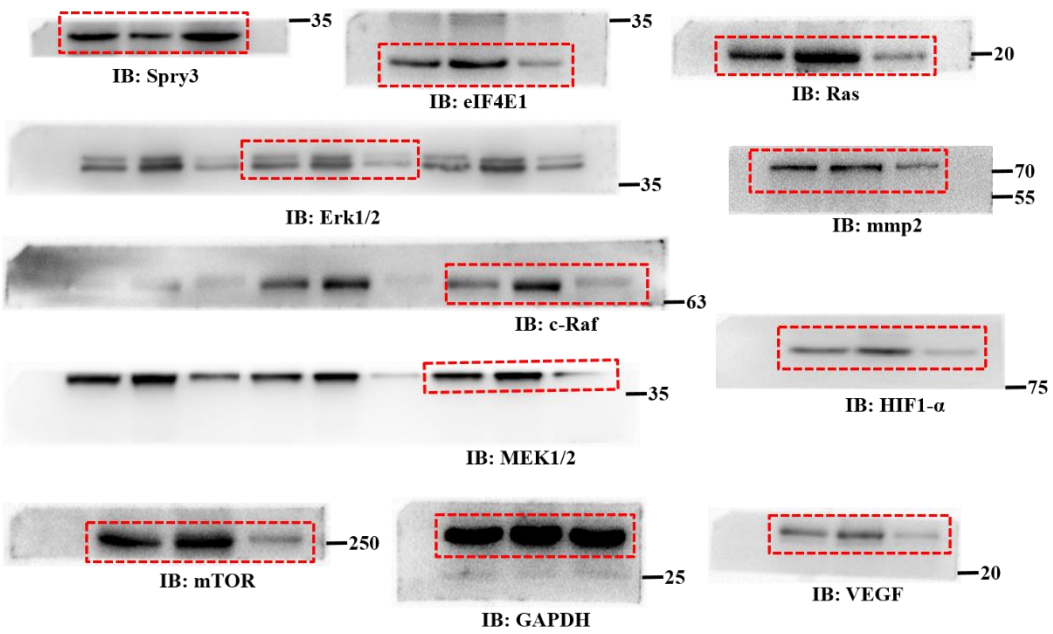


Figure S22 Original bands of western blot for Figure S5B middle.

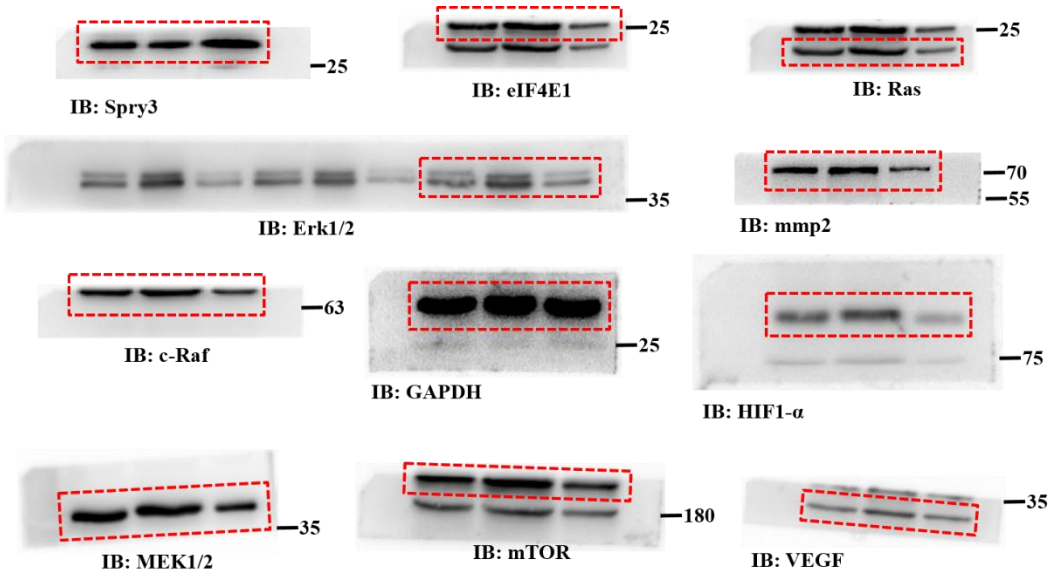


Figure S23 Original bands of western blot for Figure S5B right.

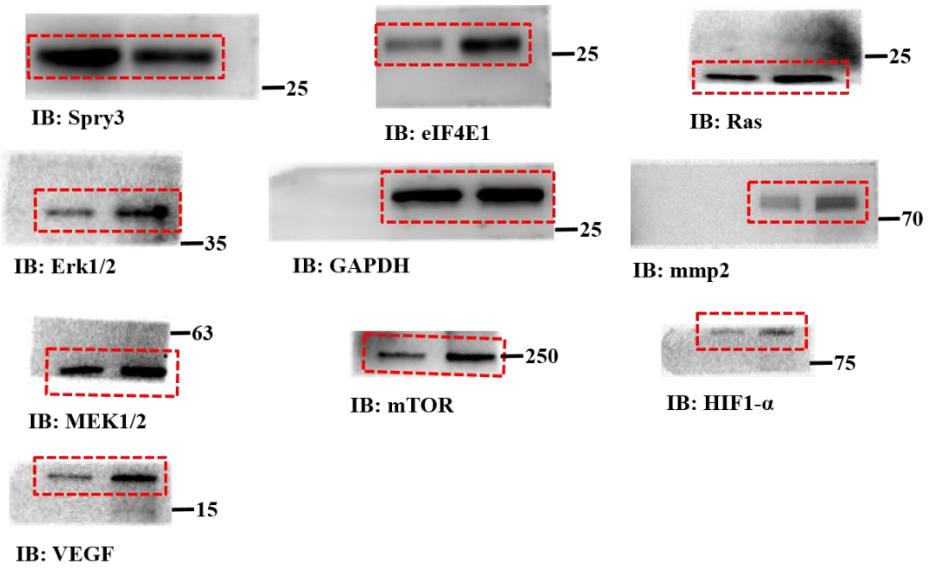


Figure S24 Original bands of western blot for Figure S5C.

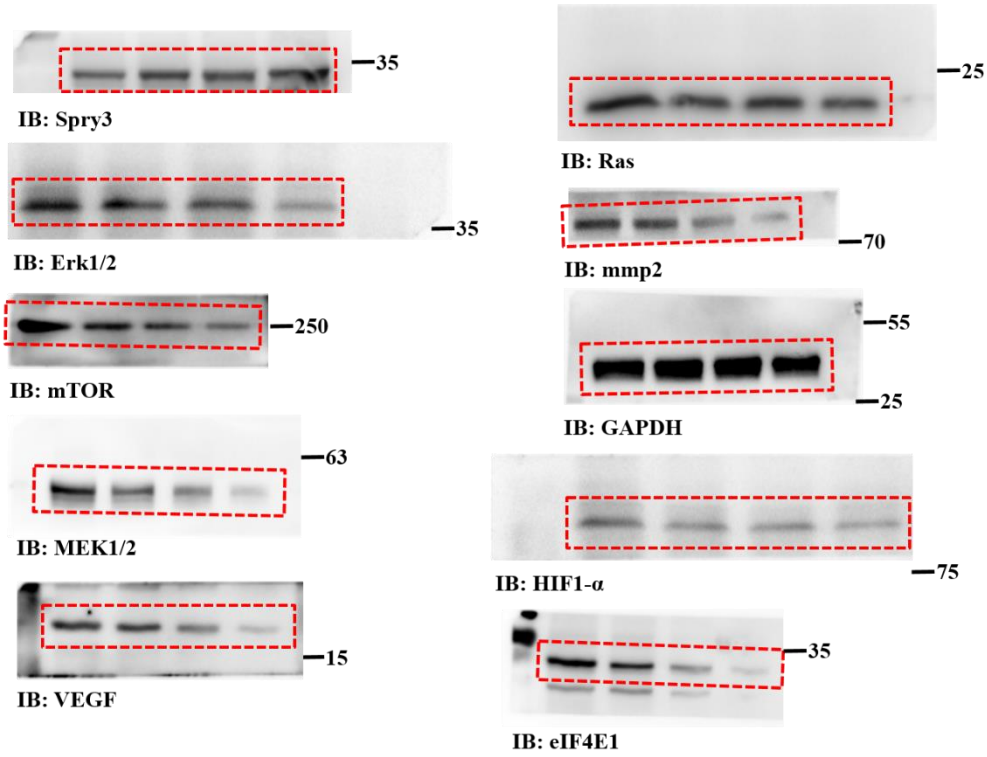


Figure S25 Original bands of western blot for Figure S5D.

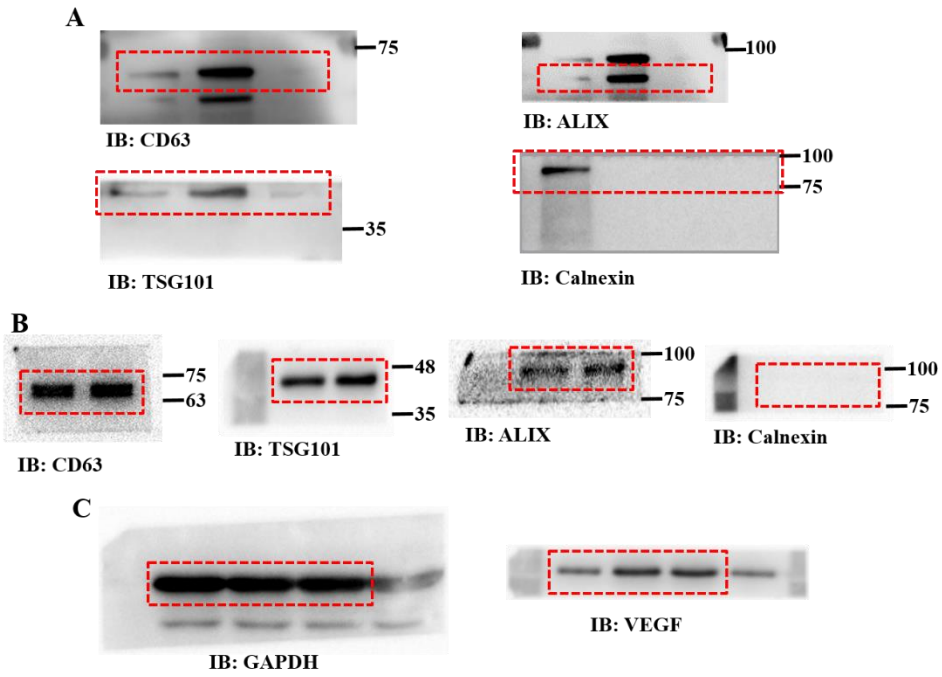


Figure S17 A) Original bands of western blot for Figure S7A, B) for Figure S10E, C) for Figure 1E.

Table S1 Antibodies and its diluted concentration involved in article.

Antibody	Brand	Cat.No	Mol weight	Dilution rate
CD63	abcam	ab134045	30-65 kDa	WB (1:1000)
TSG101	abcam	ab125011	45 kDa	WB (1:1000)
ALIX	abcam	ab186429	97, 80 kDa	WB (1:1000)
Calnexin	abcam	ab133615	90 kDa	WB (1:1000)
Spry3	abcam	ab180037	31 kDa	WB (1:1000)
Ras	abcam	ab52939	18 kDa	WB (1:10000)
c-Raf	abcam	ab181115	73 kDa	WB (1:1000)
MEK1/2	abcam	ab178876	43,44 kDa	WB (1:20000)
mTOR	abcam	ab32028	250 kDa	WB (1:1000)
eIF4E1	abcam	ab33766	30 kDa	WB (1:500)
Erk1/2	abcam	ab184699	44, 42 kDa	WB (1:10000)
HIF1-α	abcam	ab51608	93 kDa	WB (1:500)
mmp2	abcam	ab92536	74 kDa	WB (1:1000)
VEGF	abcam	ab52917	23 kDa	WB (1:1000)
GAPDH	proteintech	10494-1-AP	36 kDa	WB (1:2000)
αv	abcam	ab179475	125, 135 kDa	IHC-P (1:1000)
β3	abcam	ab210515	100 kDa	IHC-P (1:20000)