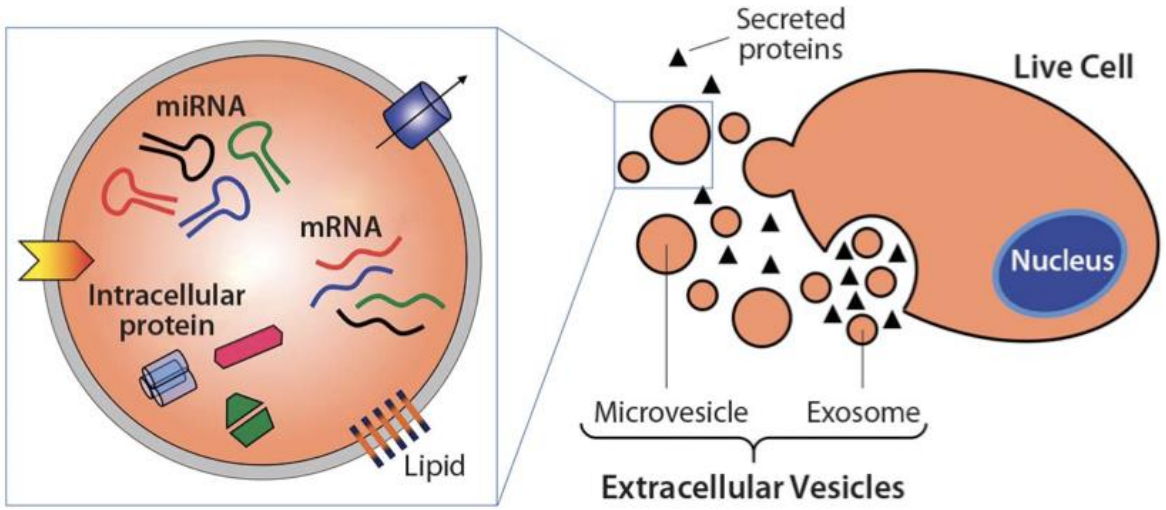
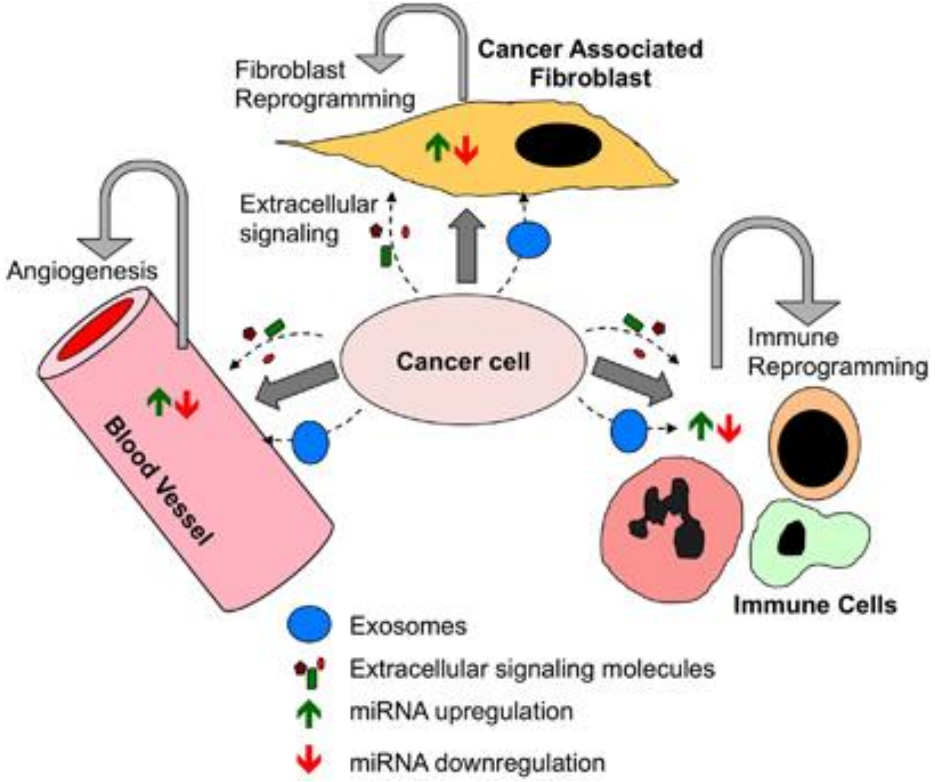
Tumor-derived exosomes promote tumor growth through the modulation of RCAN1.4

이대목동병원 호흡기내과
박소정

Exosome

Exosomes derived from lung cancer cell are known to regulate tumor growth, angiogenesis, metastasis, drug resistance, and immune escape





Down's syndrome and solid tumor

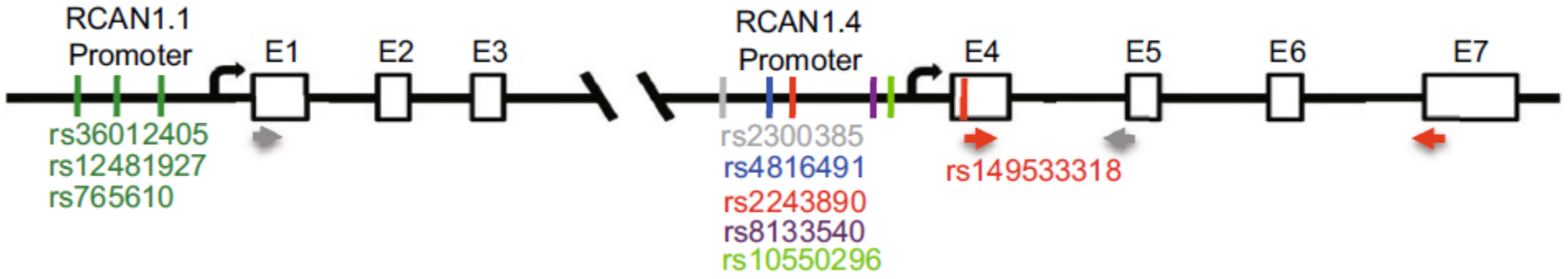
Age-group (years)	Years at risk	Observed	Expected	SIR (95% CI)
0–29 years				
0–4	6848	1	0·89	
5–9	6440	0	0·53	
10–14	6209	0	0·54	
15–19	5938	1	0·96	0·44 (0·09–1·28)
20–24	5311	0	1·56	
25–29	4506	1	2·33	
⩾30 years				
30–34	3494	2	2·87	
35–39	2716	2	3·60	
40–44	2247	2	4·99	
45–49	1812	3	6·53	0·51 (0·32–0·78)
50–54	1343	5	7·19	
55–59	901	3	7·09	
>60	688	4	8·69	
Total	48 453	24	47·77	0·50 (0·32–0·75)

Table 2: **Person-years at risk and observed and expected numbers of solid tumours in 5-year age-groups**

Lancet 2000;355:165-9

RCAN1 gene

- Human regulator of calcineurin 1 (RCAN1) gene
- Also known as Down's syndrome critical region 1 (DCR1)
- Located on chromosome 21
- Composed of seven exons that are alternatively spliced and/or transcribed by differential promoters to produce different isoforms.



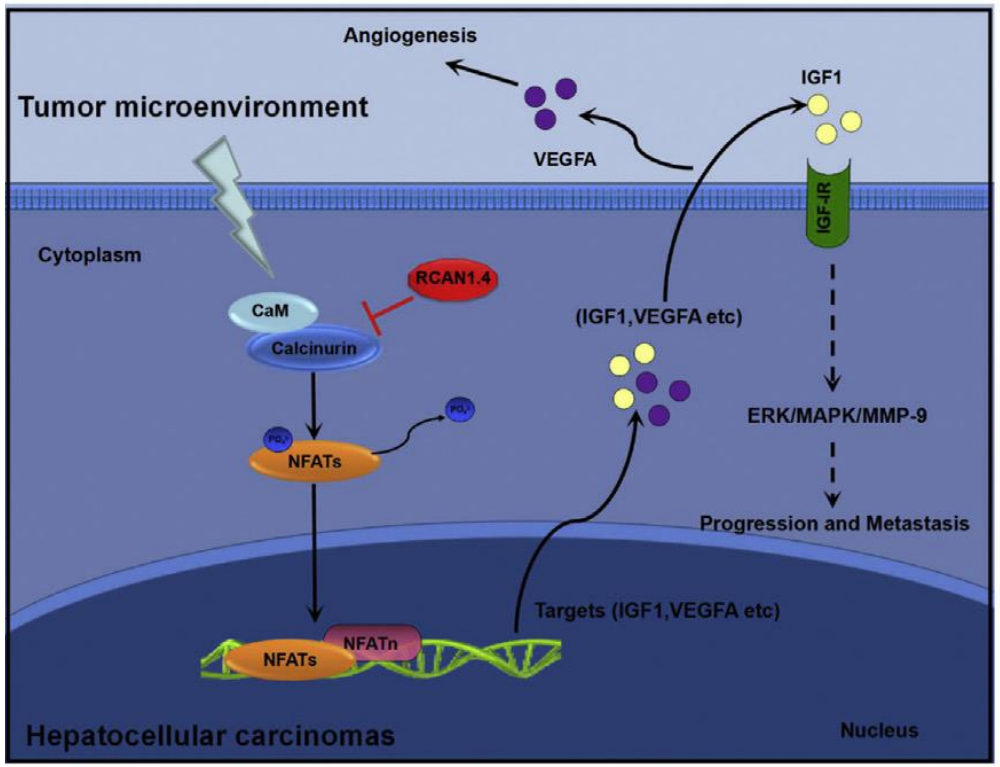
RCAN1 gene

- RCAN1.1 (NM_004414) and RCAN1.4 (NM_203418) are the two major isoforms differentially expressed in many tissues and cells

- RCAN1.1 is constitutively expressed, whereas expression of RCAN1.4 is induced by diverse stimuli

RCAN1

- RCAN1 is an endogenous protein which interacts with calcineurin
- Overexpression of RCAN1 affords a generalized cancer protection via attenuating tumor angiogenesis by inhibiting the calcineurin pathway in the vascular endothelium



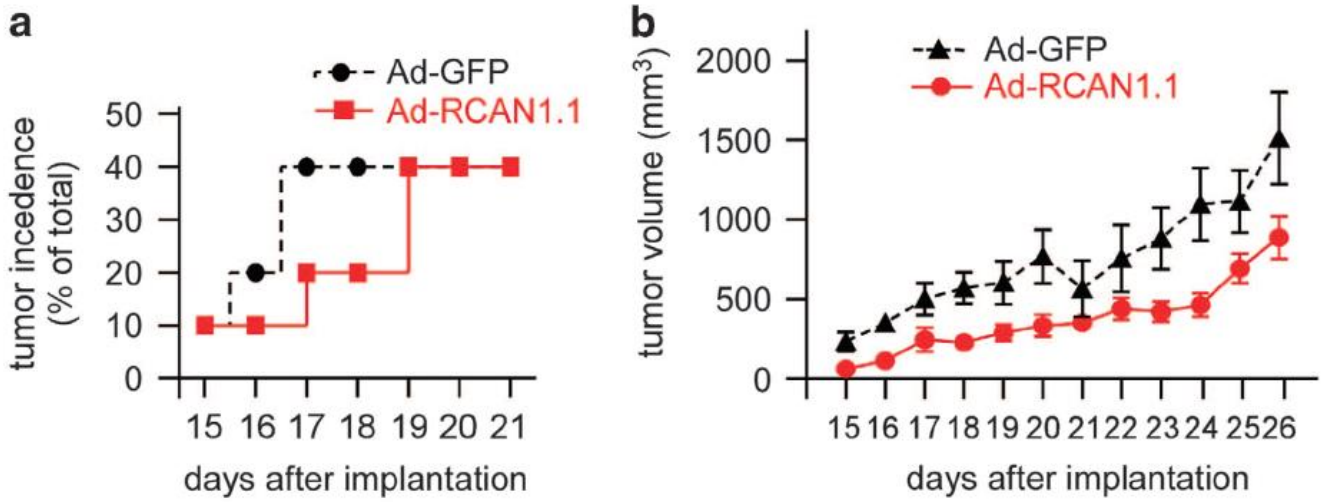
RCAN1.4 and solid tumor

Increased RCAN1 level promoted lymphoma cell apoptosis

Cell Death Dis 2015;6:e1929

Reduced RCAN1.4 mRNA levels were associated with thyroid cancer growth and metastasis suppressor *in vivo*

JCI Insight 2017;2:e90651



RCAN1.4 and solid tumor

RCAN1.4 mRNA and protein levels were significantly decreased in hepatocellular carcinoma

Reduced RCAN1.4 mRNA levels were associated with advanced tumor stages, larger tumor size, vascular invasion, poor survival and early recurrence in hepatocellular carcinoma

Gastroenterology 2017;153:799-811

Downregulation of RCAN1 resulted in enhanced invasive and migratory ability in small-cell lung cancer

Tumour Biol 2017

Goal

- The function of RCAN1.4 has scarcely been investigated in tumor development and prognosis of NSCLC
- Selection of miRNA which target the RCAN1.4 in exosome excreted from NSCLC cell
- The role of the RCAN1.4/targeted miRNAs on tumor growth *in vitro*
- The relationship between RCAN1.4/targeted miRNAs and tumor growth, metastasis, survival rate in NSCLC patients

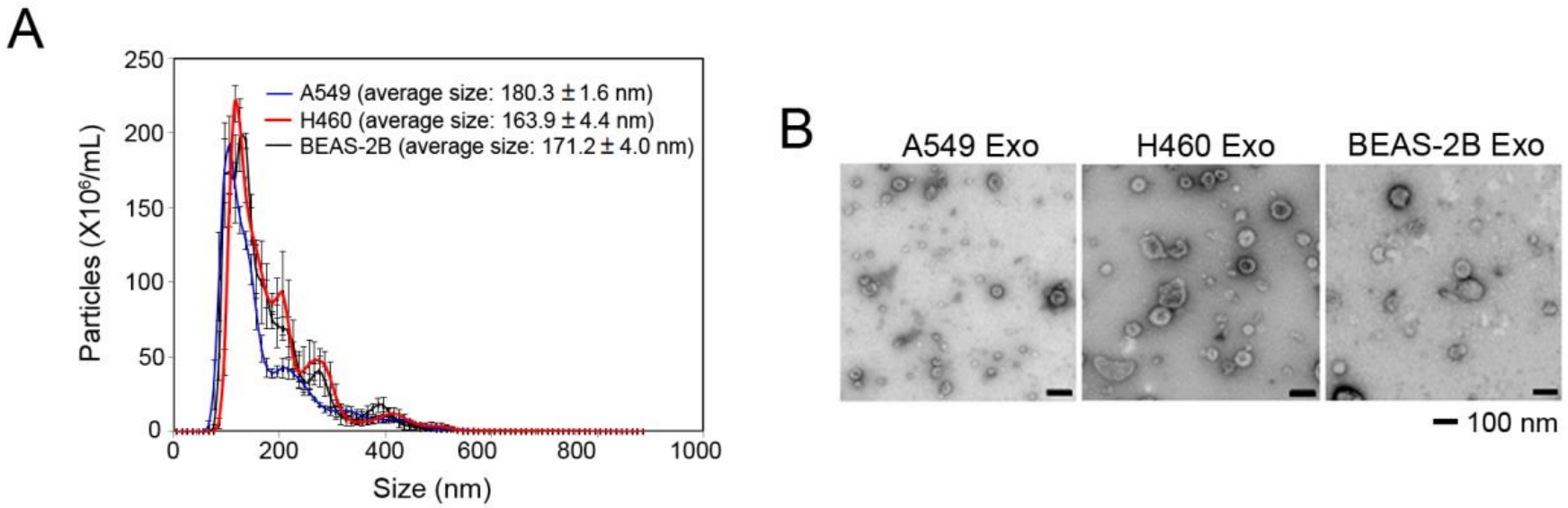
Methods

- Human NSCLC cell lines - A549, H460
- Human bronchial epithelial cell line - BEAS-2B

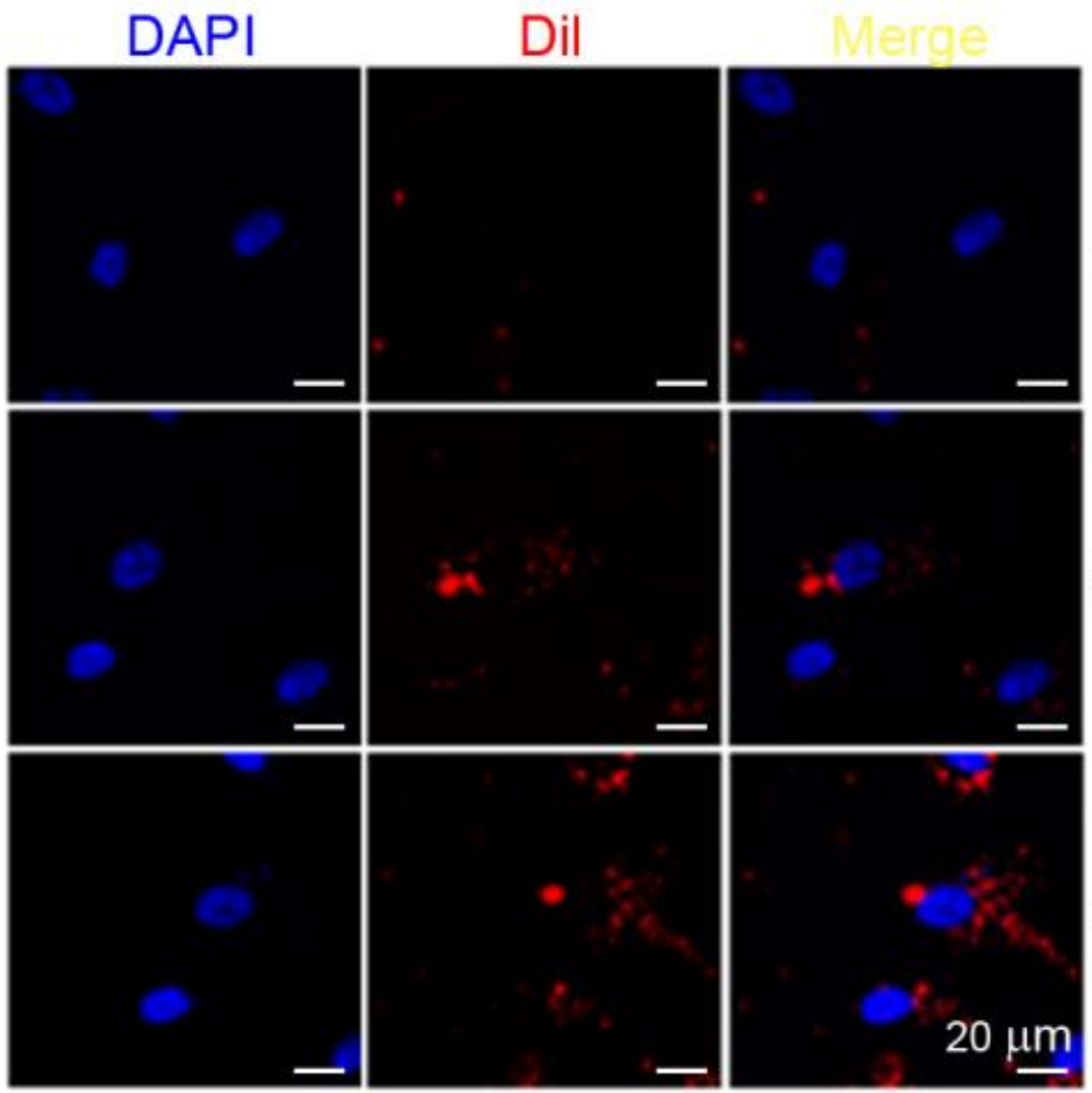
- Human umbilical vein endothelial cells (HUVECs)

- 130 paired samples of human NSCLC and noncancerous lung tissues
- Plasma samples from 48 NSCLC patients and 48 healthy volunteers

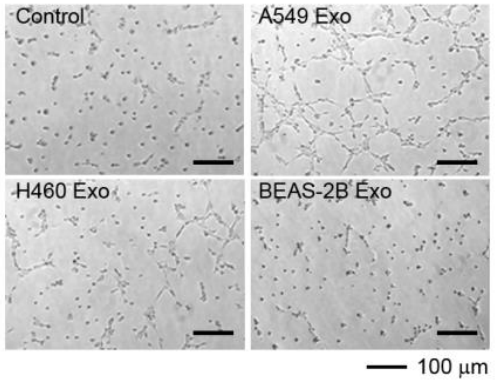
Identification of exosomes

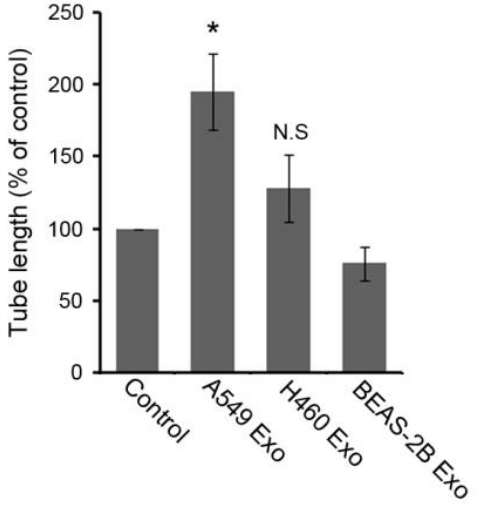


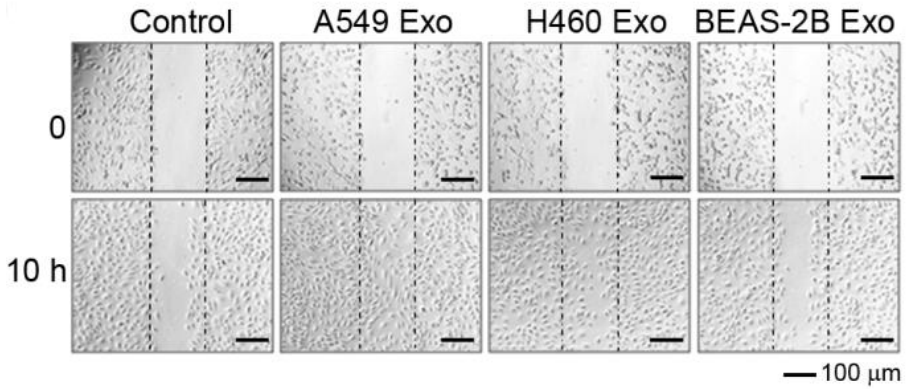
Uptake of exosomes into endothelial cells

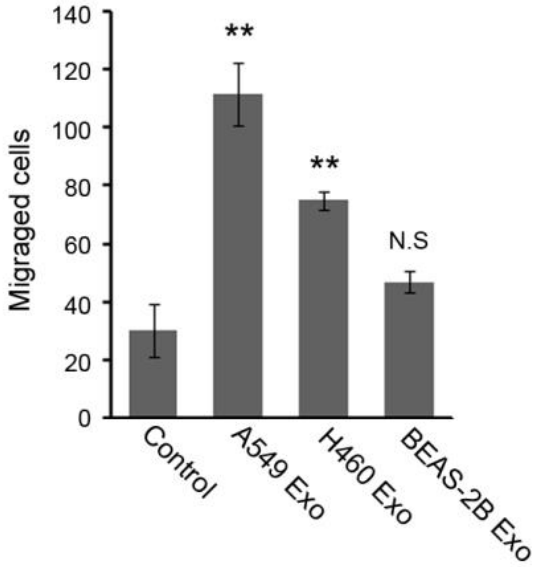


Tube formation in HUVECs with exosomes released from NSCLC cell-lines

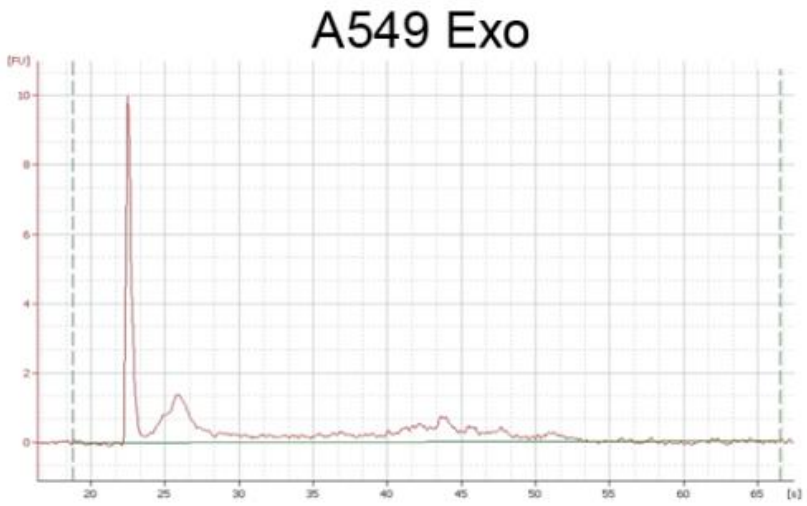


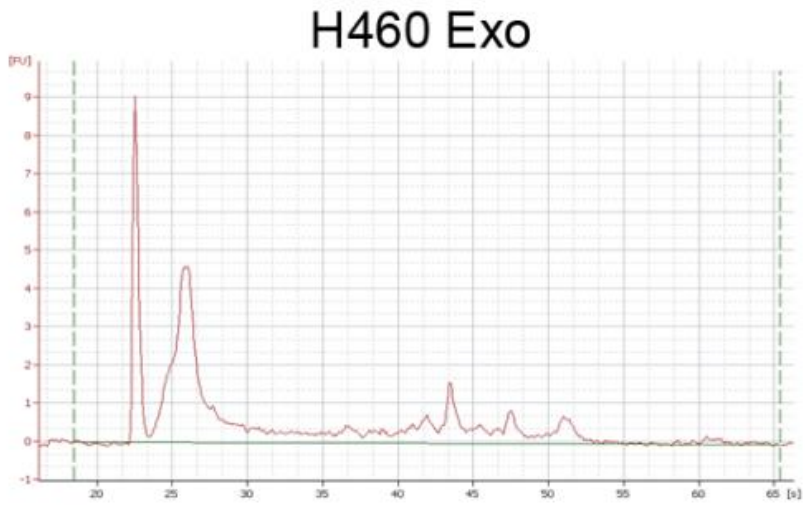


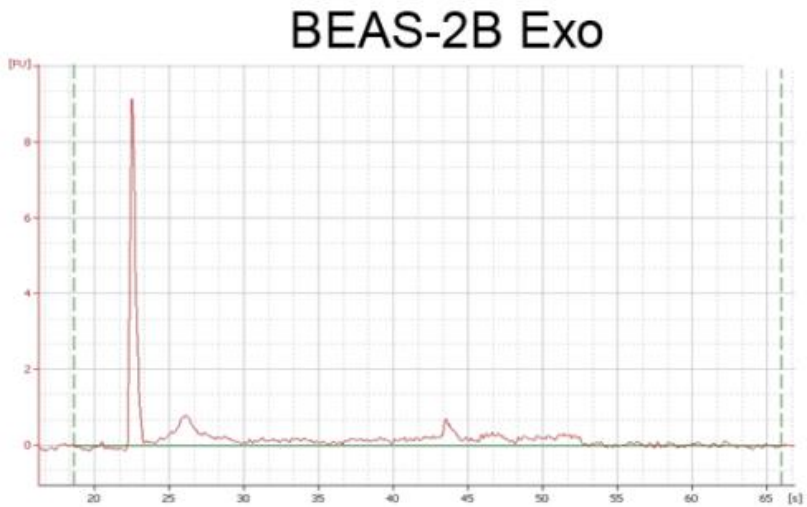




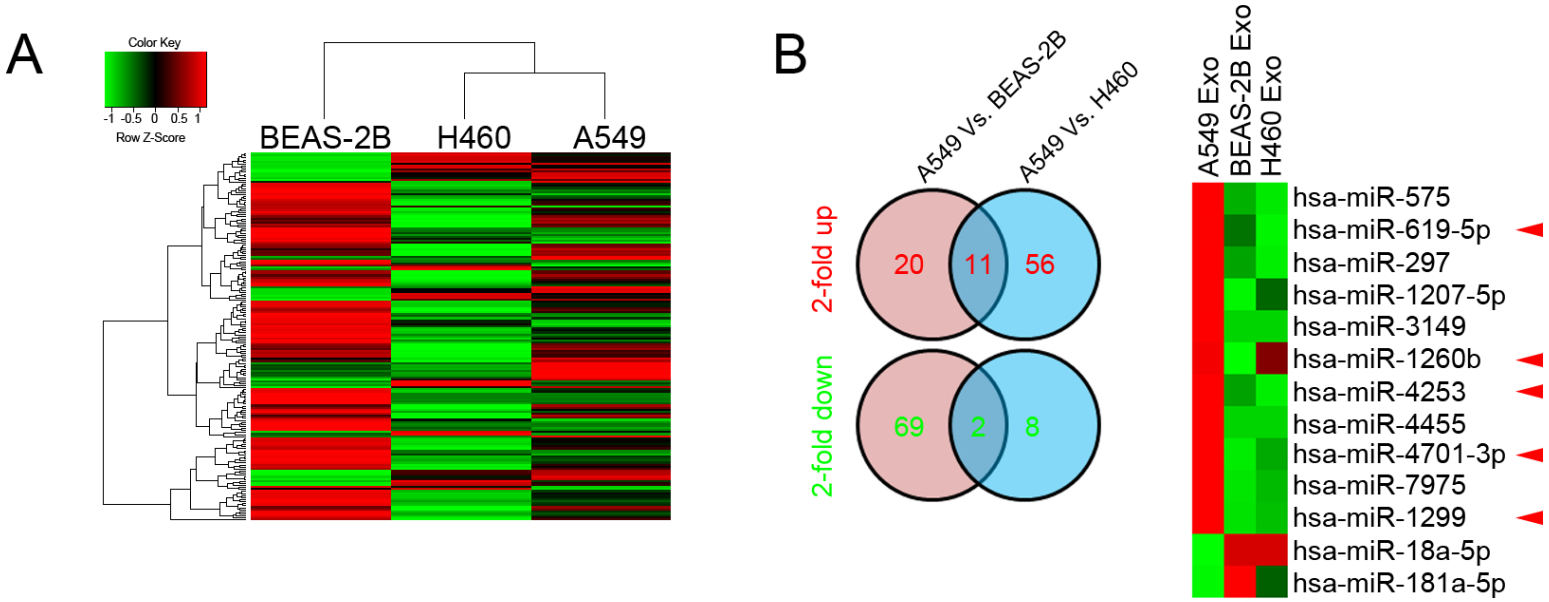
Quantitation and quality of nucleic acid within NSCLC cell-derived exosomes



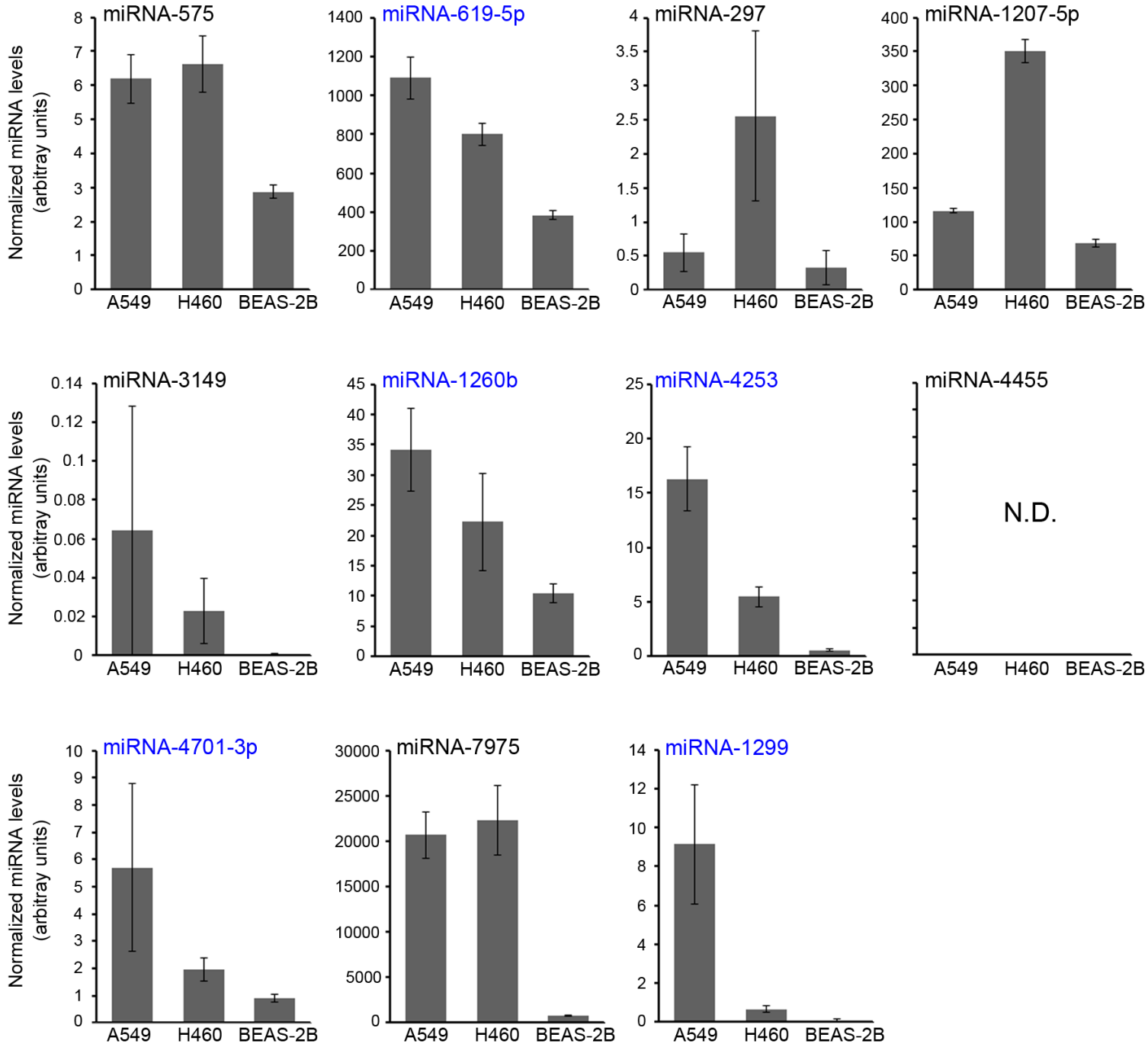




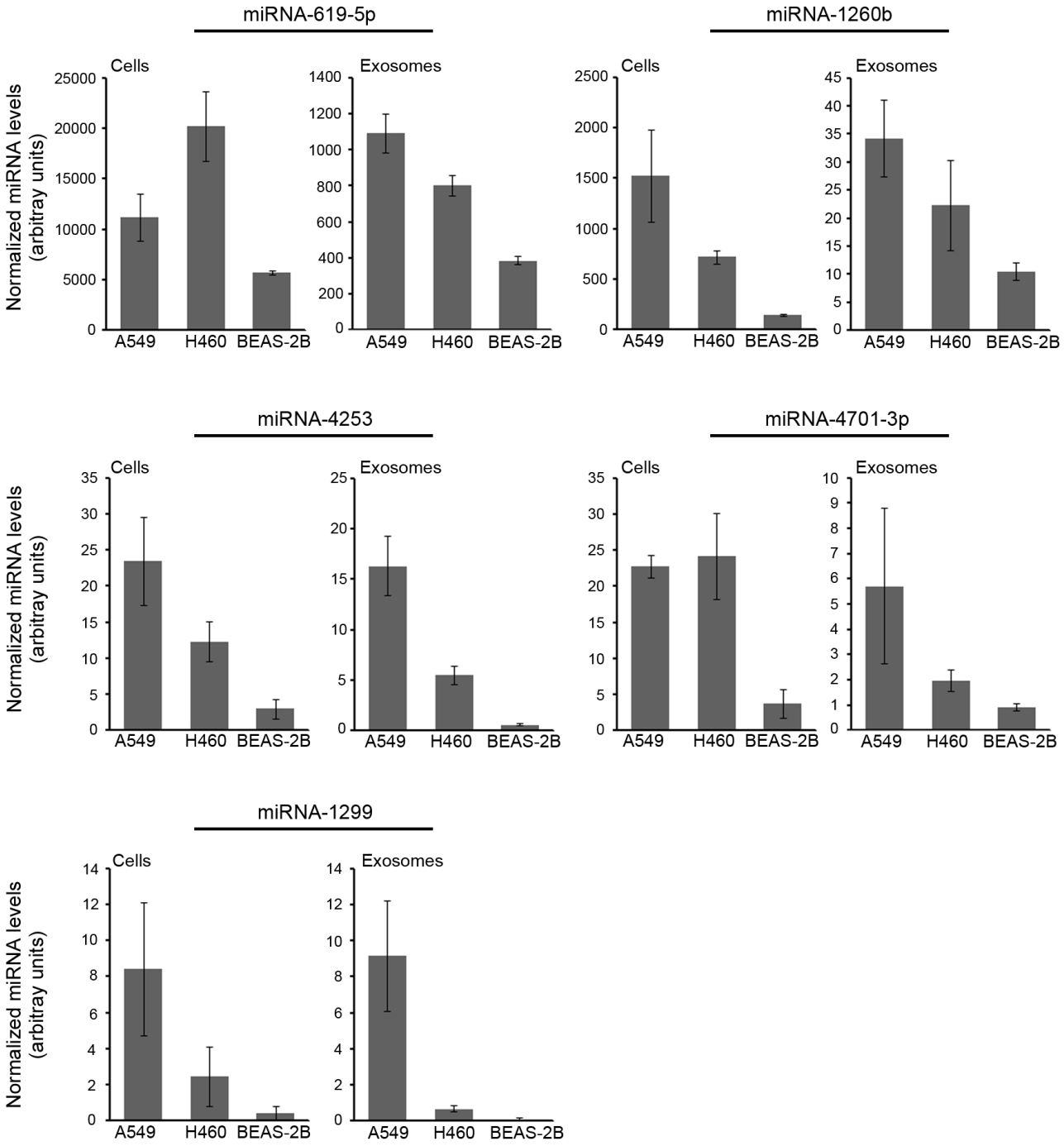
miRNA array in NSCLC cell-derived exosome



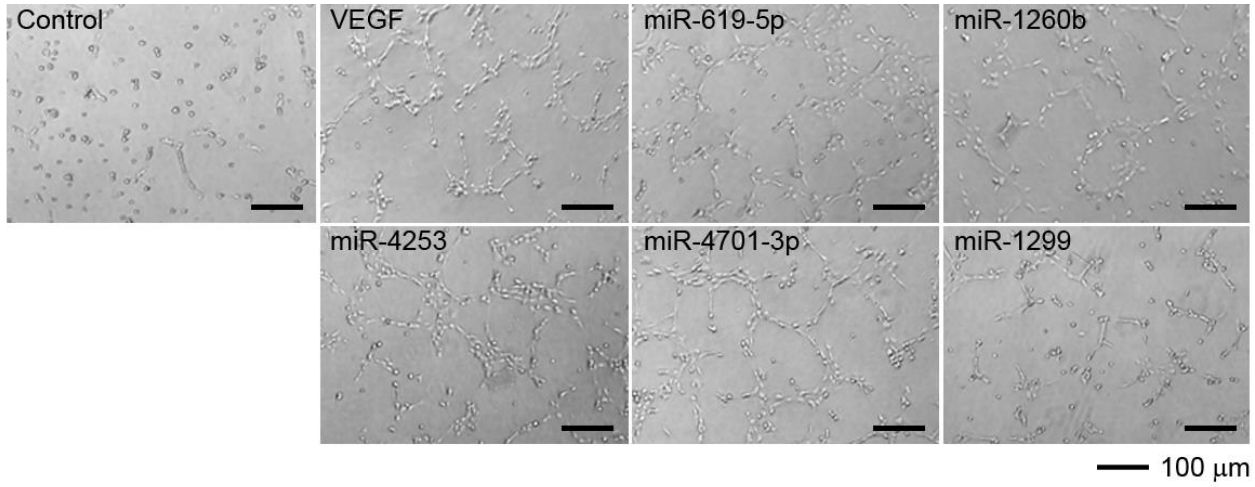
Validation of false positive results in miRNA array

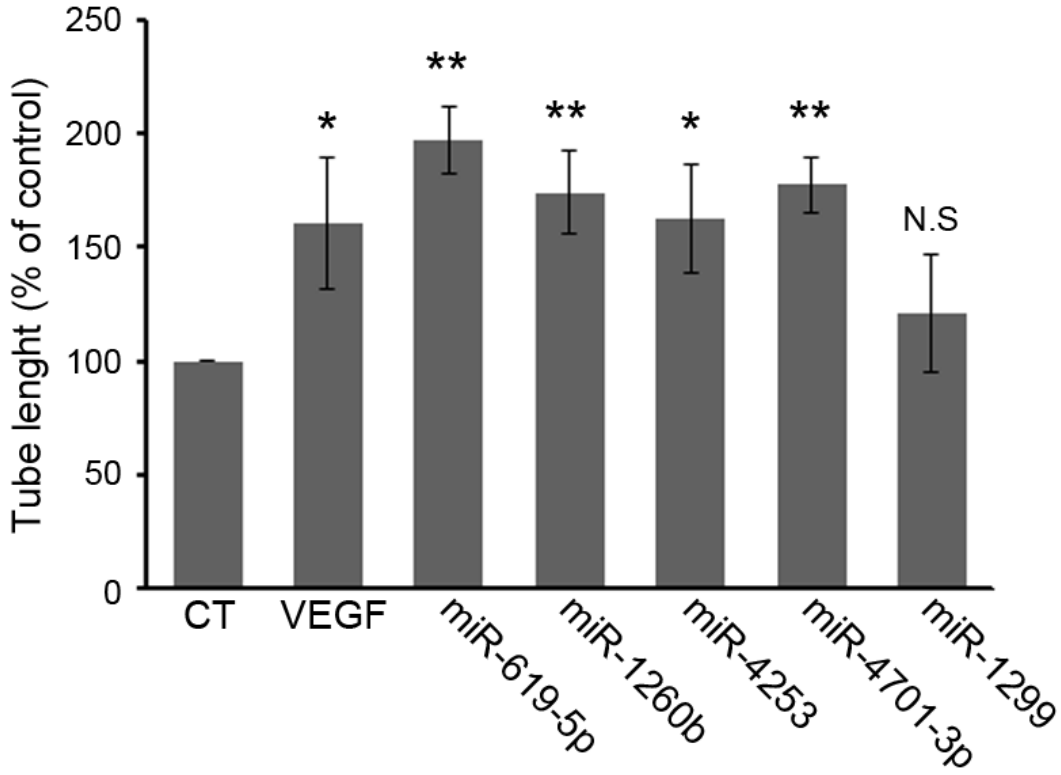


A comparison between cellular miRNAs and exosomal miRNAs

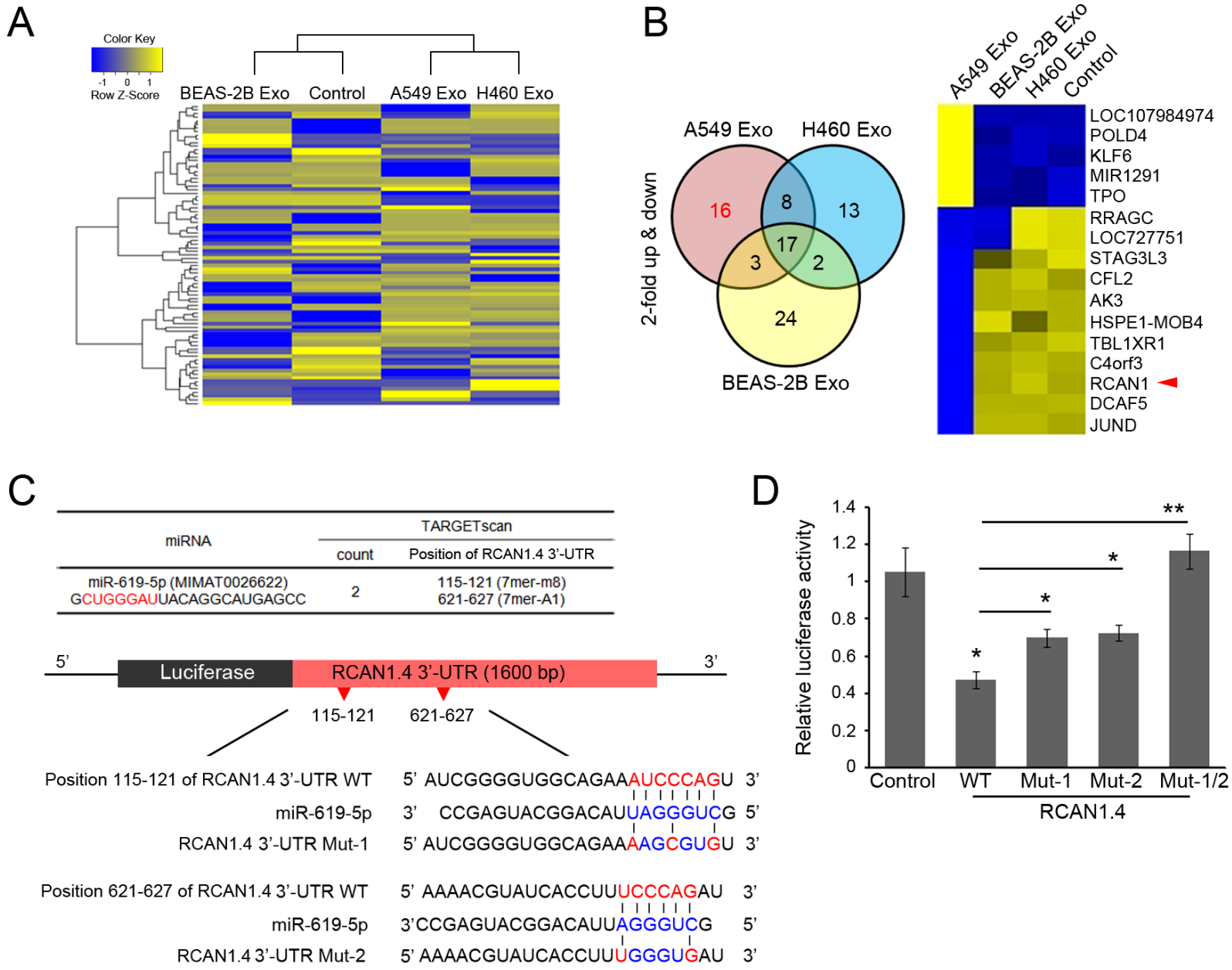


Tube formation in HUVECs with specific miRNA from A549-deprived exosomes





Exosomal miRNA-619-5p targeted RCAN1.4 in endothelial cells



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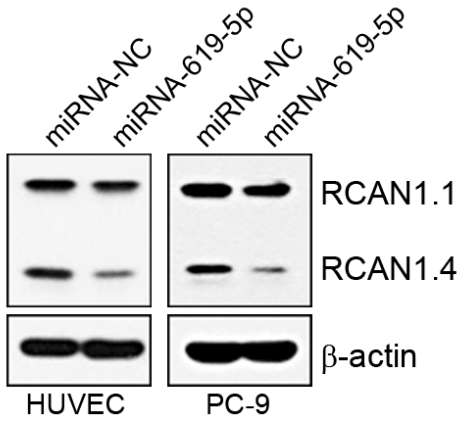
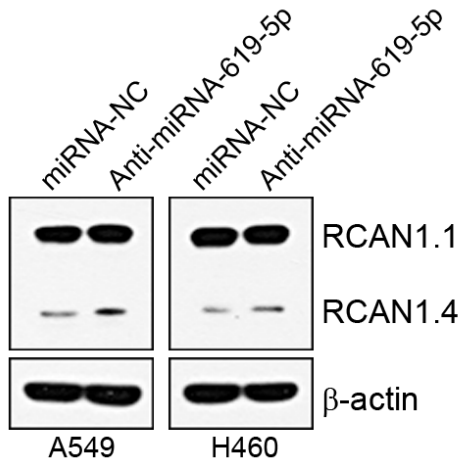
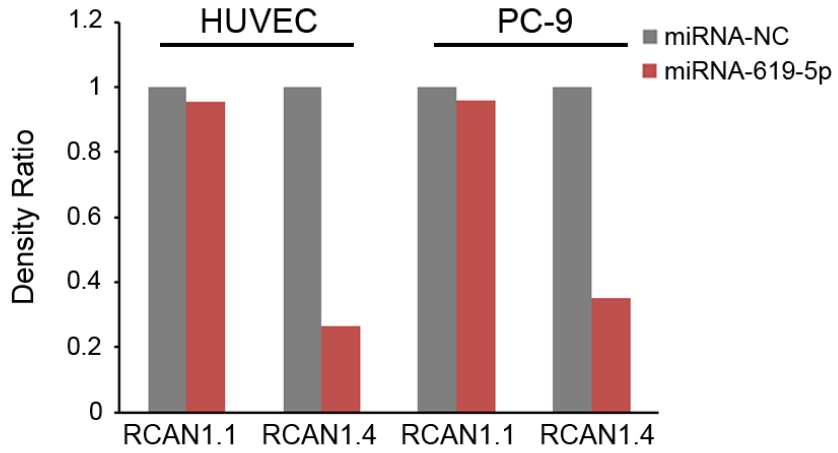
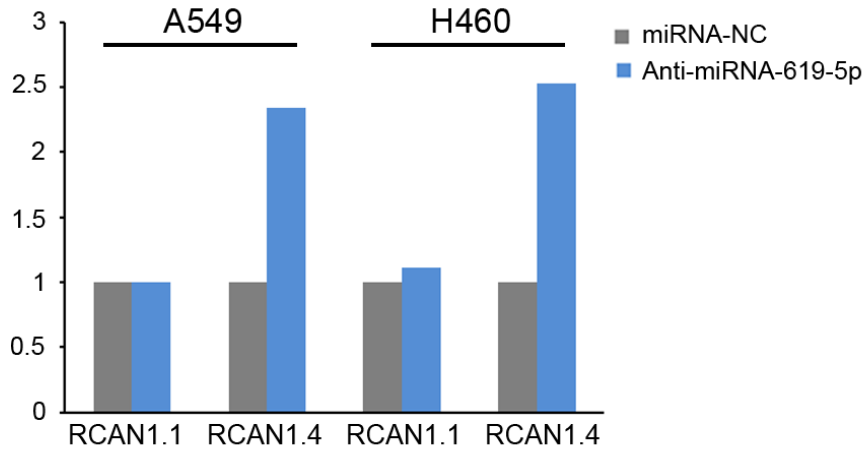
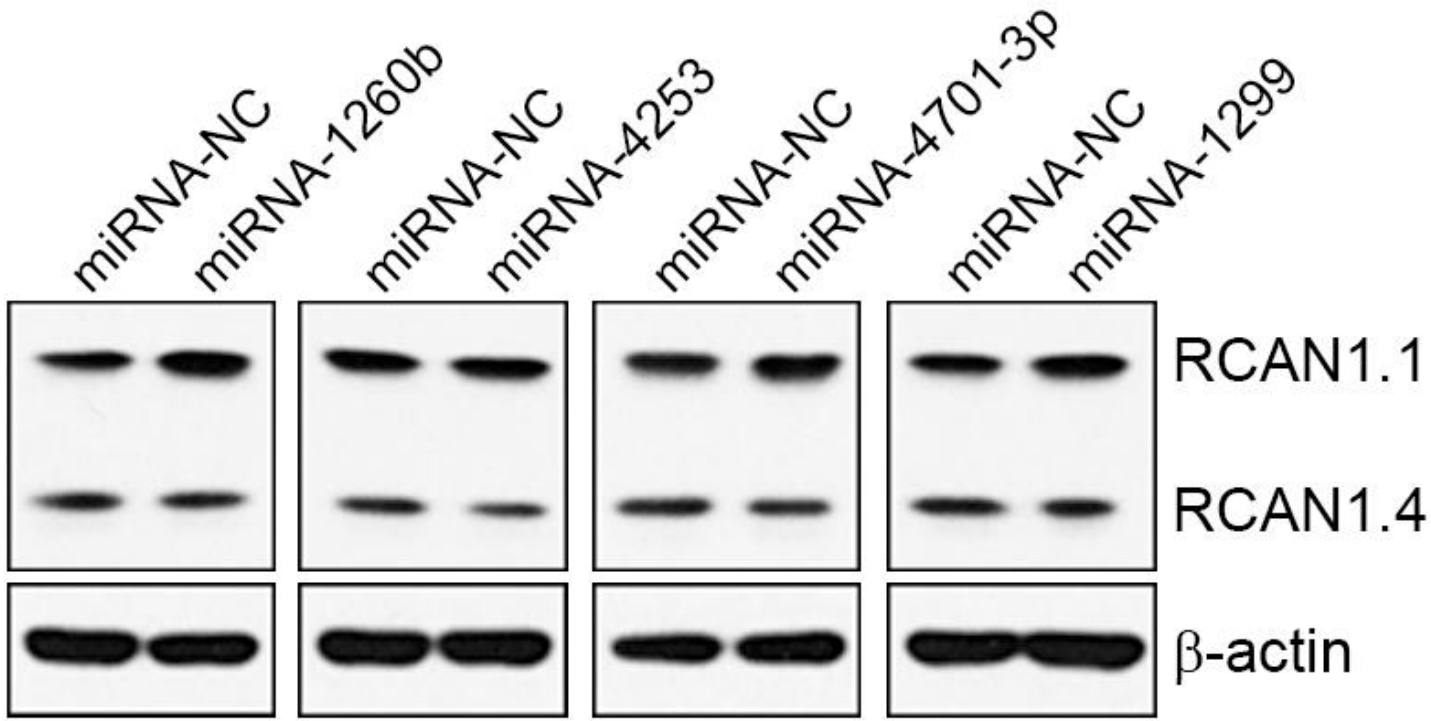




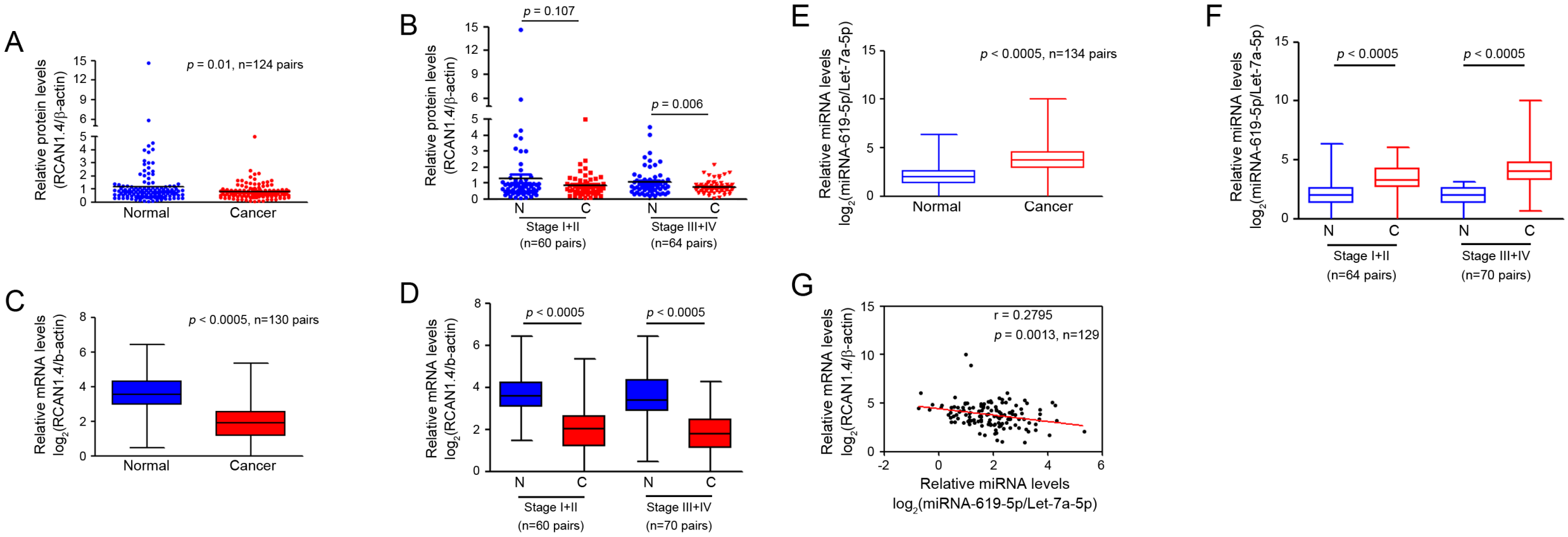




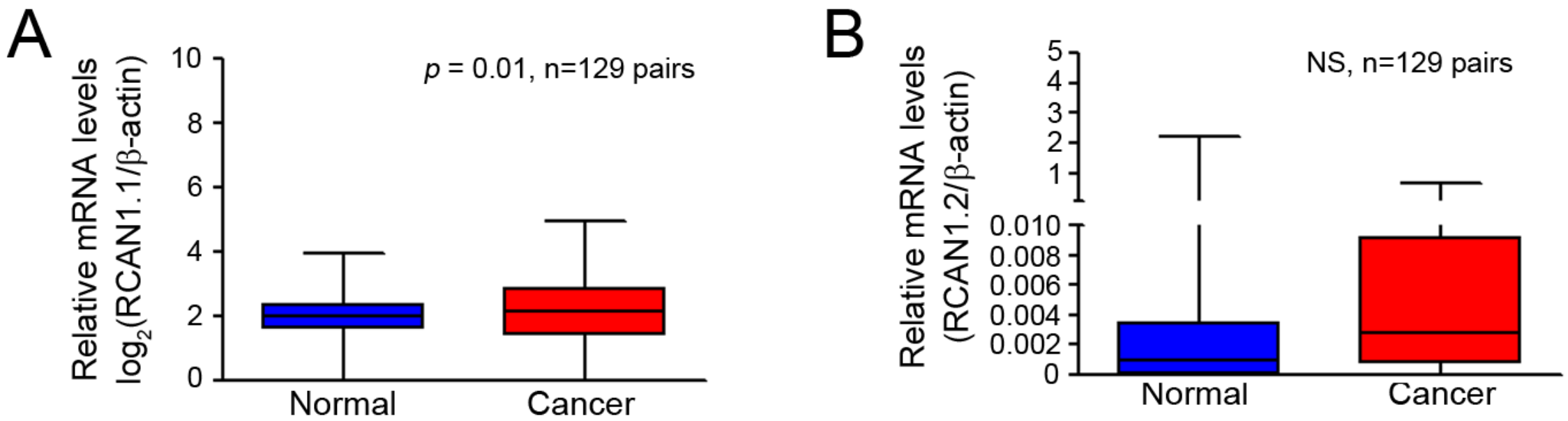
The effect of other miRNAs on RCAN1.4



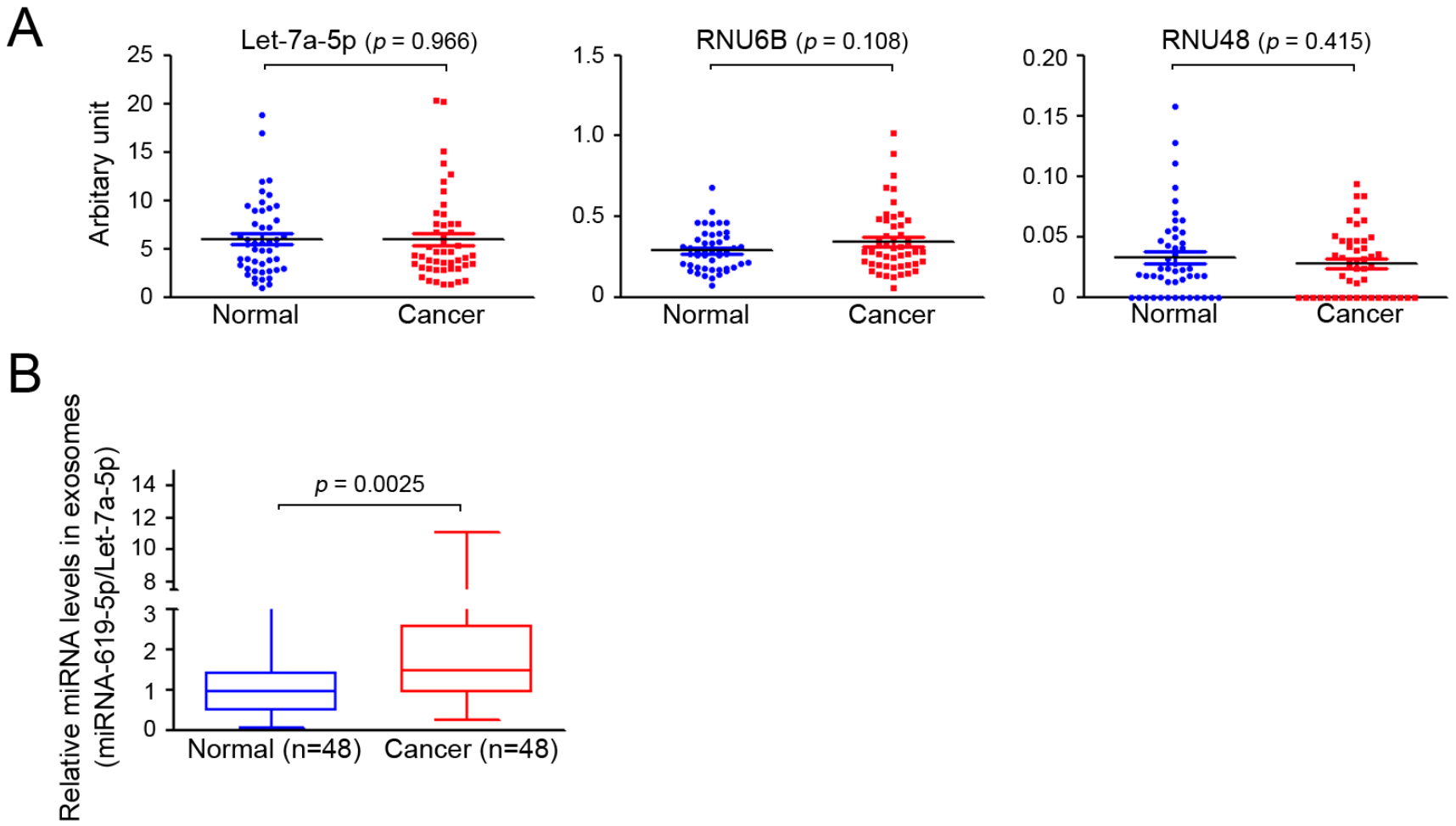
Expression of RCAN1.4 and miRNA-619-5p in patients tissue with NSCLC



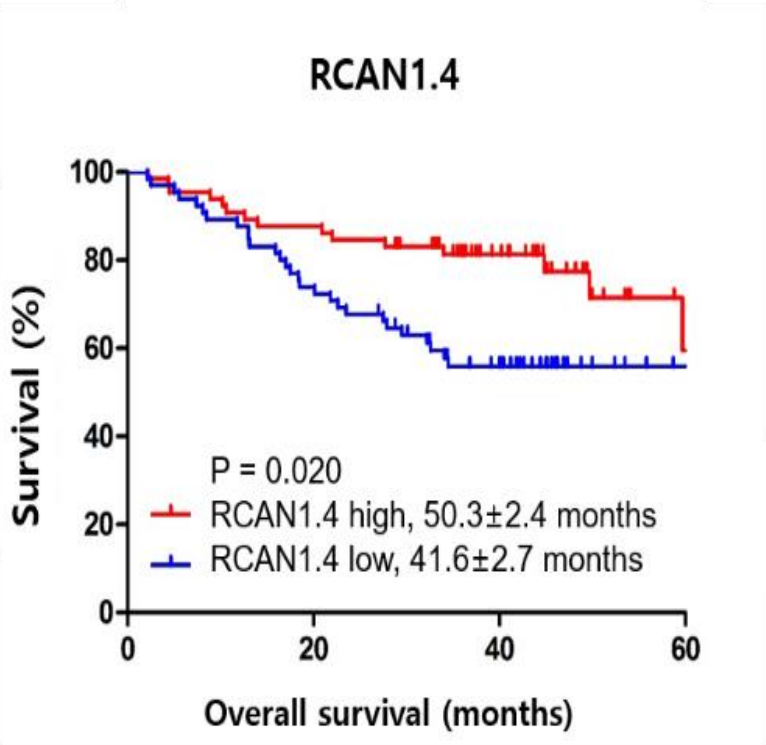
Expression of RCAN1.1 and RCAN1.2 in patients tissue with NSCLC

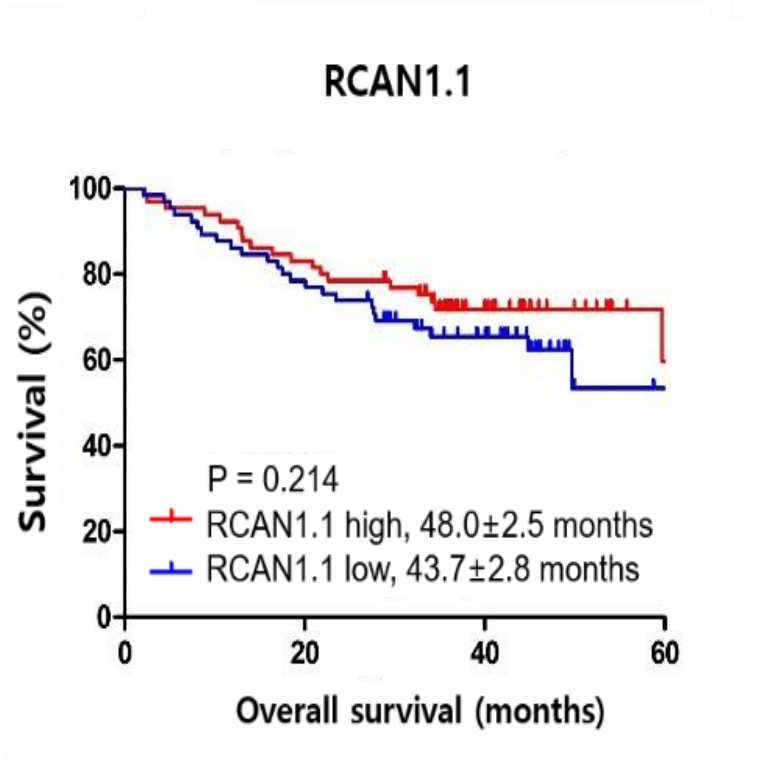


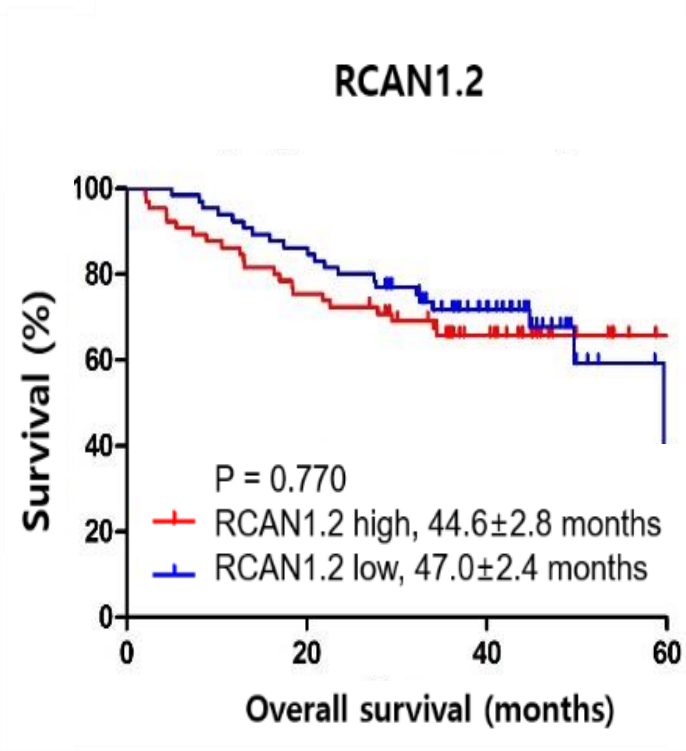
miRNA-619-5p levels within exosome from NSCLC plasma



Survival and RCAN1 expression level







Conclusion

- Tumor-derived exosomes may promote angiogenesis through the modulation of RCAN1.4
- RCAN1.4 may be an endogenous tumor suppressor of NSCLC
- RCAN1.4 may be a potential prognostic indicator of NSCLC
- RCAN1.4 and miR-169-5p may serve as a therapeutic target in patients with NSCLC

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