Supplementary Figures (1-6)

RNF121 inhibits angiogenic growth factor signaling by restricting cell surface expression of VEGFR-2

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Running title: RNF121 regulates cell surface expression of VEGFR-2

S. Figure 1: The predicted 3D structure of RING domain and the RING domain consensus sequence motif: (A) The predicted 3D structure of RING domain of RNF121, which was modeled based on the known RING domain of RNF7/RBX2 (RING BOX Protein2). (B) The RING domain consensus sequence motif and the corresponding putative RING domain of RNF121 is shown.

S. Figure 2: Effect of cycloheximide on RNF-121 mediated maturation of VEFR-2: (A) HEK-293 cells expressing VEGFR-2 were subjected to cycloheximide (CHX) chase by incubating cells for 30 and 90 minutes. Cells were lysed and whole cell lysates were blotted for VEGFR-2 or loading control. (B) HEK-293 cells expressing VEGFR-2 alone or with RNF121 were treated with cycloheximide for 90 minutes and cell lysates were blotted for VEGFR-2, RNF121 and loading control, PLCγ1.

S. Figure 3: **RNF121 co-localizes with KDEL-GFP**: Immunofluorescence staining of HEK-293 cells expressing VEGFR-2 with c-Myc-RNF121 and KDEL-GFP. The overlay of the same images is also shown. Scale bars, 10 μm.

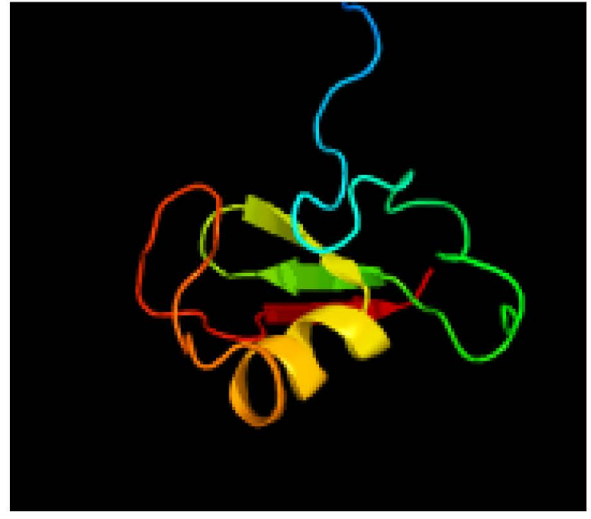
S. Figure 4: RNF121 inhibits VEGF-induced tyrosine phosphorylation of VEGFR-2: PAE cells co-expressing VEGFR-2 with empty vector (pMSCV) or RNF121 were stimulated with VEGF (100ng/ml) for 10 minutes and cells were lysed. Whole cell lysates were blotted with phosphoY1054-VEGFR-2, total VEGFR-2, phospho-p38MAPK, total p38MAPK, phospho-PLCγ1 and total PLCγ1. Graph is representative of two independent experiments.

S. Figure 5. Validation of RNF121 antibody: Whole cell lysates from HEK-293 cells expressing empty vector or Myc-RNF121 was blotted with anti-c-Myc, anti-RNF121 or lading control protein. The same cell lysates also was blotted with RNF-121 antibody pre-incubated with blocking RNF121 peptide.

S. Figure 6: RNF121 expression is regulated by ER stress and oxidative stress inducing agents: PAE cells treated with ER-stress inducing agent (tunicamycin) or hydrogen peroxide and cells were lysed and whole cell lysates blotted for RNF121 or loading control.

S. Figure 1

A

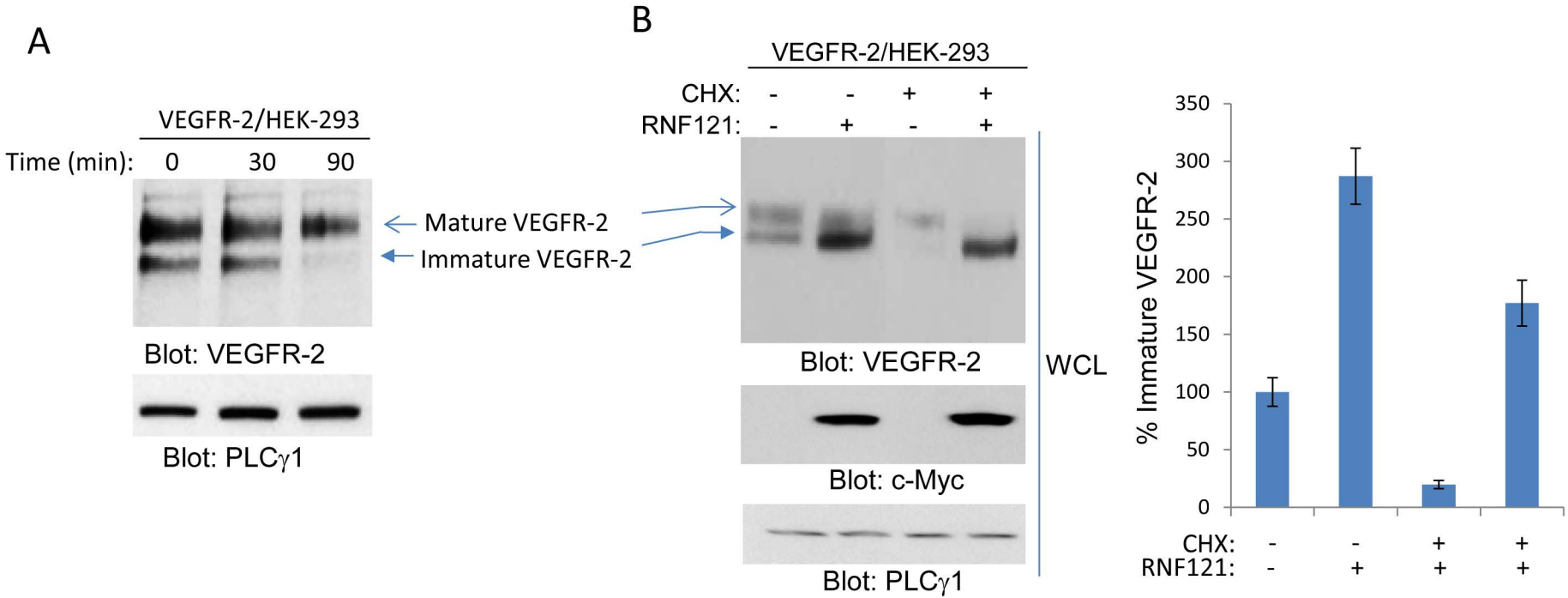


B

RING finger domain consensus sequence: **C**-X_2-**C**-$X_{[9-39]}$-**C**-$X_{[1-3]}$-**H**-$X_{[2-3]}$-**C**-X_2-**C**-$X_{[4-48]}$-**C**-X_2-**C**

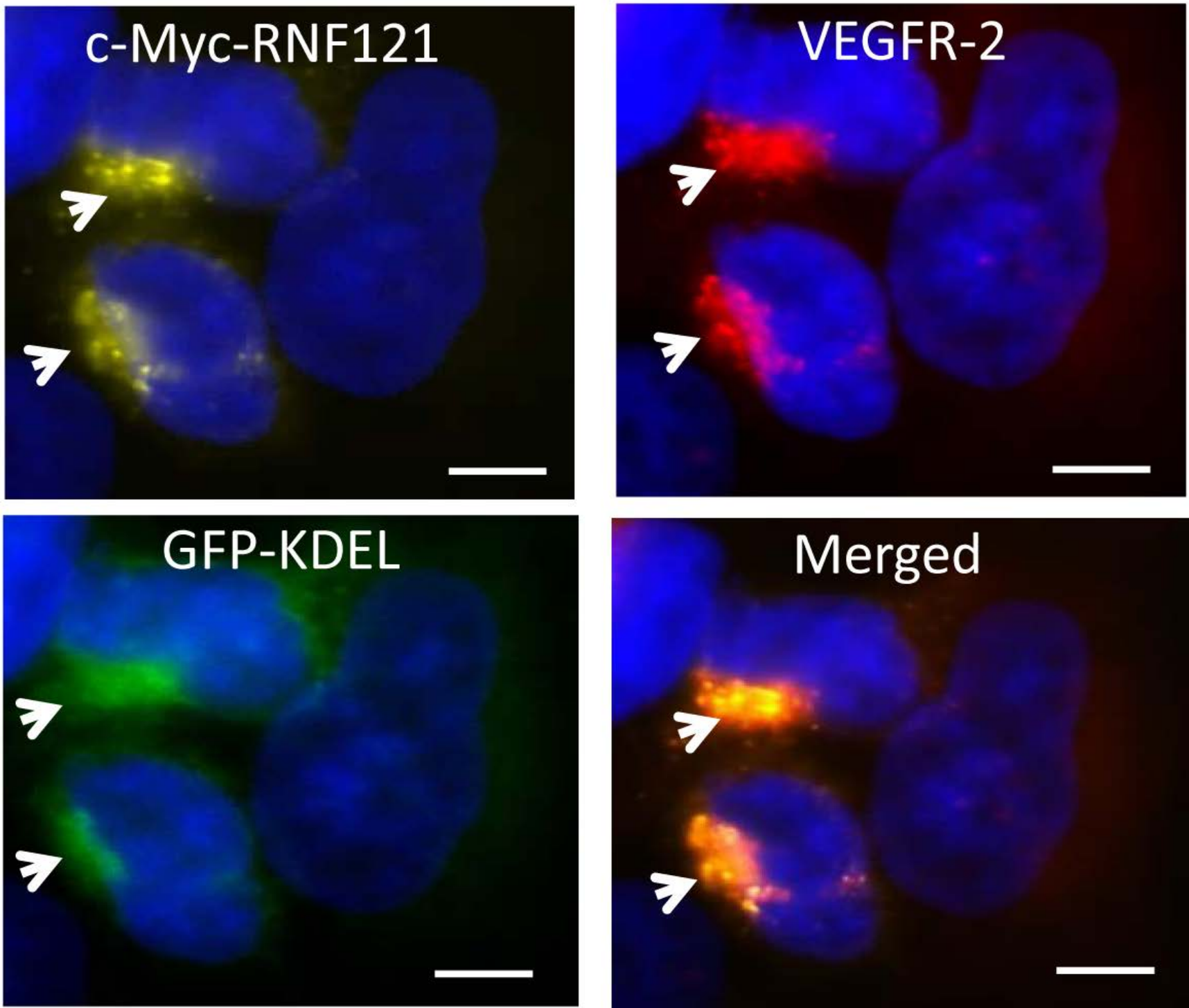
Human RNF121 …IGFYSESGMPTKHLSDSVCAVCGQQIFVDVSEEGIIENTYRLSCNHVFHEFCIRGWCIVGKKQTCPYCKEKVDLKRMFSNPW…

S. Figure 2

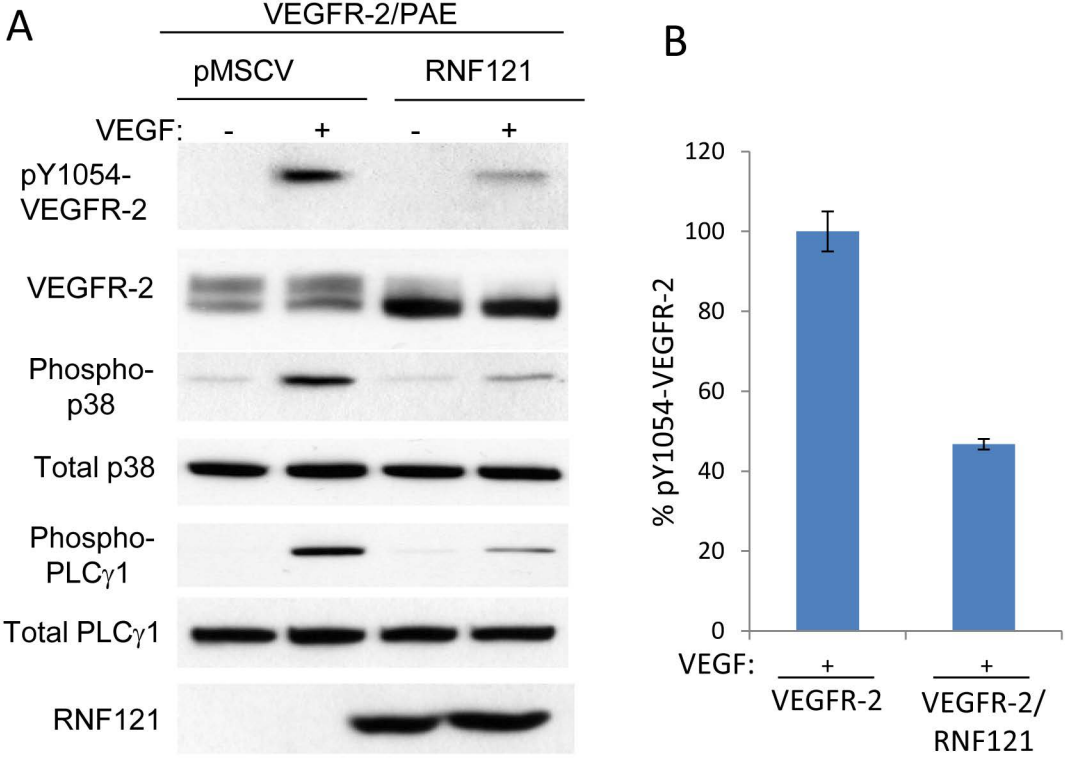

A
VEGFR-2/HEK-293
Time (min): 0 30 90
Mature VEGFR-2
Immature VEGFR-2
Blot: VEGFR-2
Blot: PLCγ1
B
VEGFR-2/HEK-293
CHX: - - + +
RNF121: - + - +
Blot: VEGFR-2
Blot: c-Myc
Blot: PLCγ1
WCL
% Immature VEGFR-2
350
300
250
200
150
100
50
0
CHX: - - + +
RNF121: - + + +

S. Figure 3

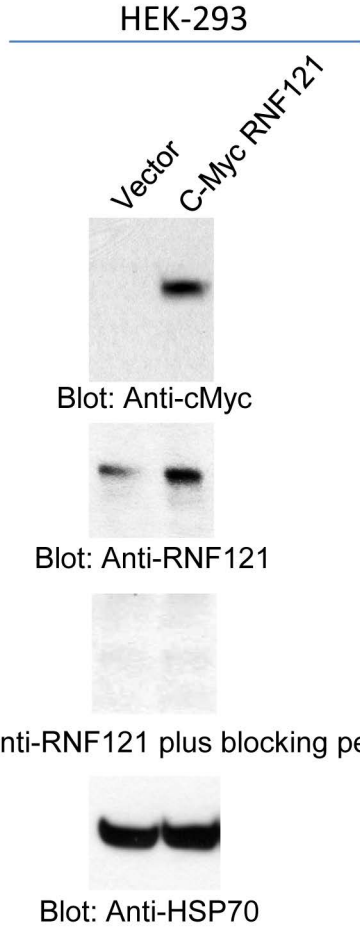
A



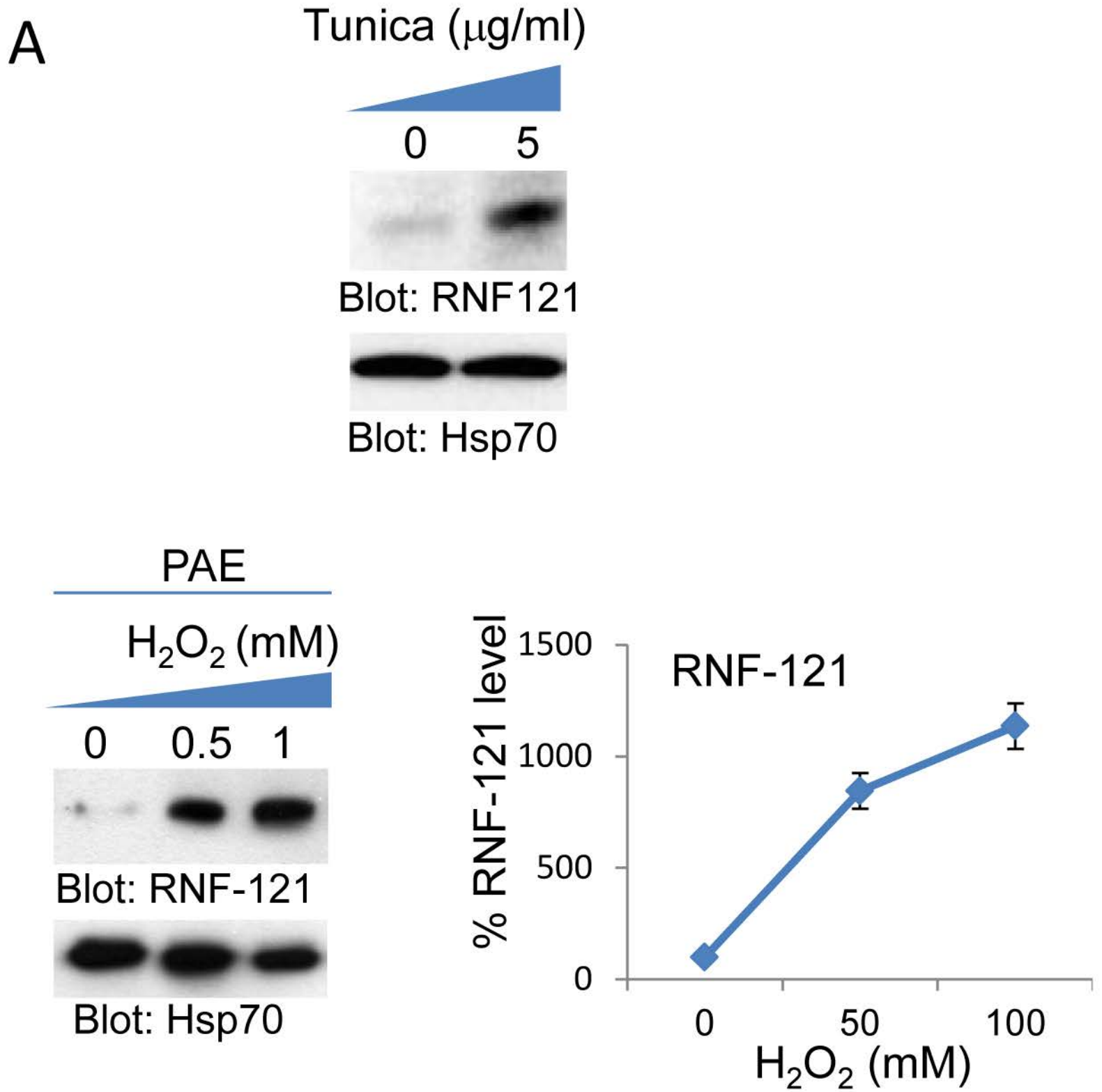
S. Figure 4


A
VEGFR-2/PAE
pMSCV
RNF121
VEGF: - + - +
pY1054-VEGFR-2
VEGFR-2
Phospho-p38
Total p38
Phospho-PLCγ1
Total PLCγ1
RNF121
B
% pY1054-VEGFR-2
120
100
80
60
40
20
0
VEGF: + +
VEGFR-2
VEGFR-2/RNF121

S. Figure 5


HEK-293
Vector
C-Myc RNF121
Blot: Anti-cMyc
Blot: Anti-RNF121
Blot: Anti-RNF121 plus blocking peptide
Blot: Anti-HSP70

S. Figure 6


A
Tunica (μg/ml)
0
5
Blot: RNF121
Blot: Hsp70
B
PAE
H2O2 (mM)
0
0.5
1
Blot: RNF-121
Blot: Hsp70
RNF-121
% RNF-121 level
1500
1000
500
0
0
50
100
H2O2 (mM)