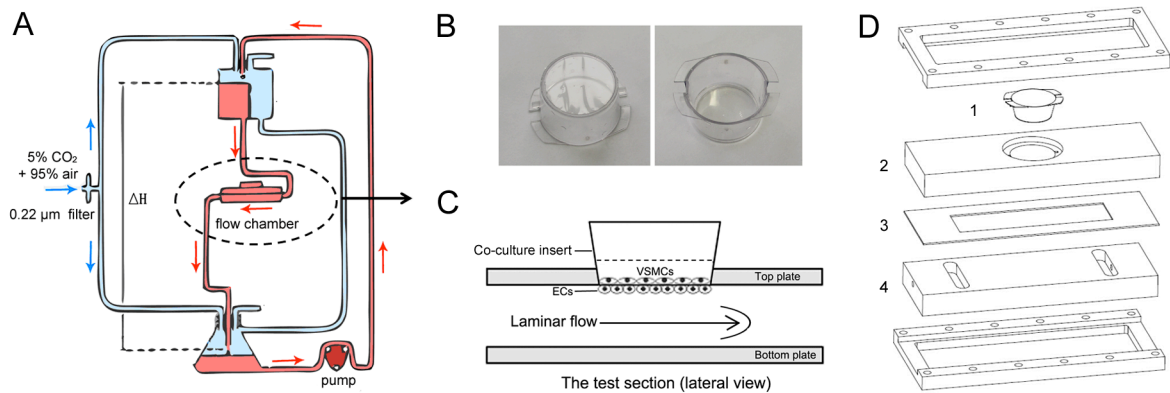


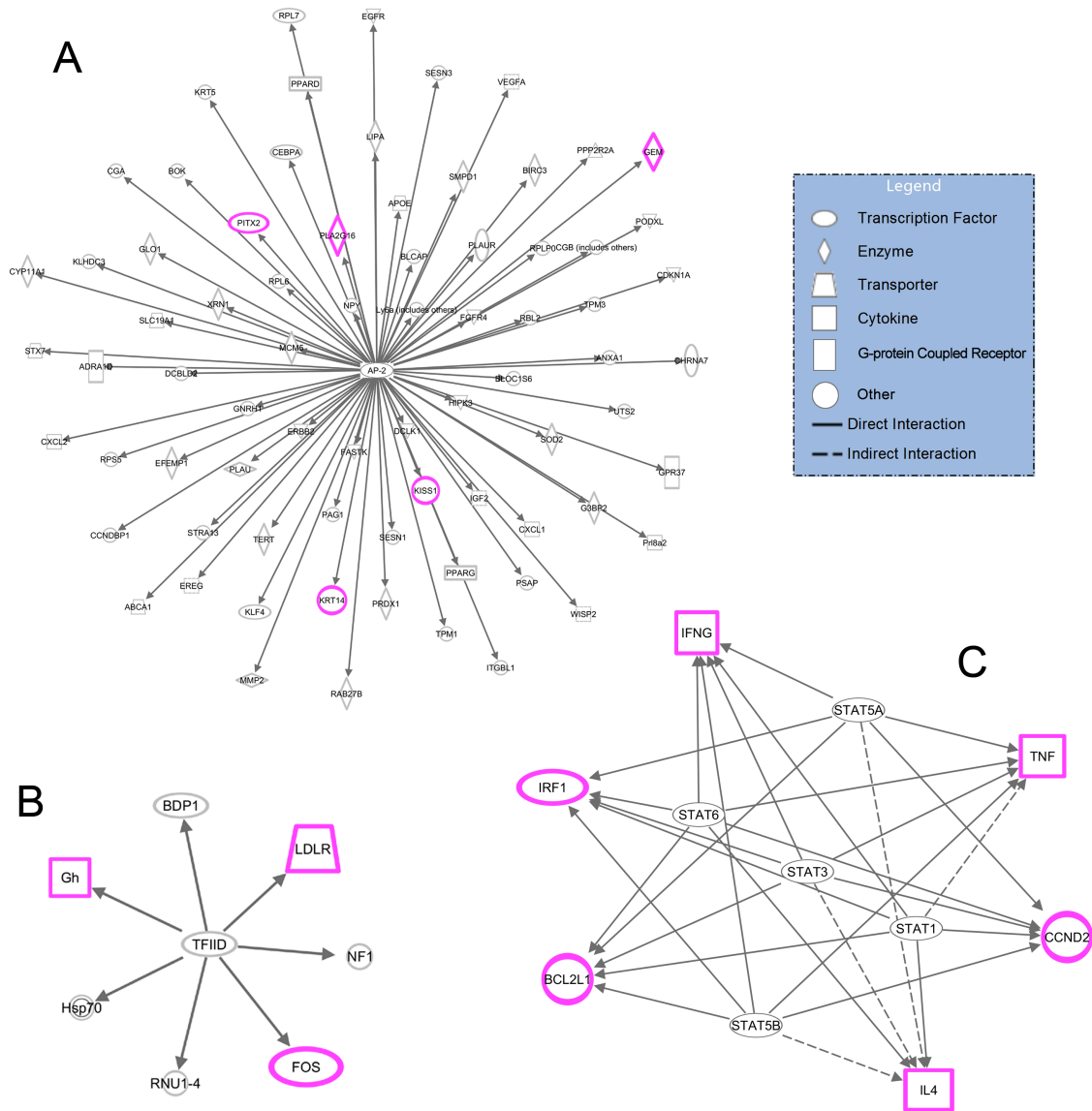
## BBAMCR-14-486\_R1

Han Y et al. Nuclear envelope proteins Nesprin2 and LaminA regulate proliferation and apoptosis of vascular endothelial cells in response to shear stress

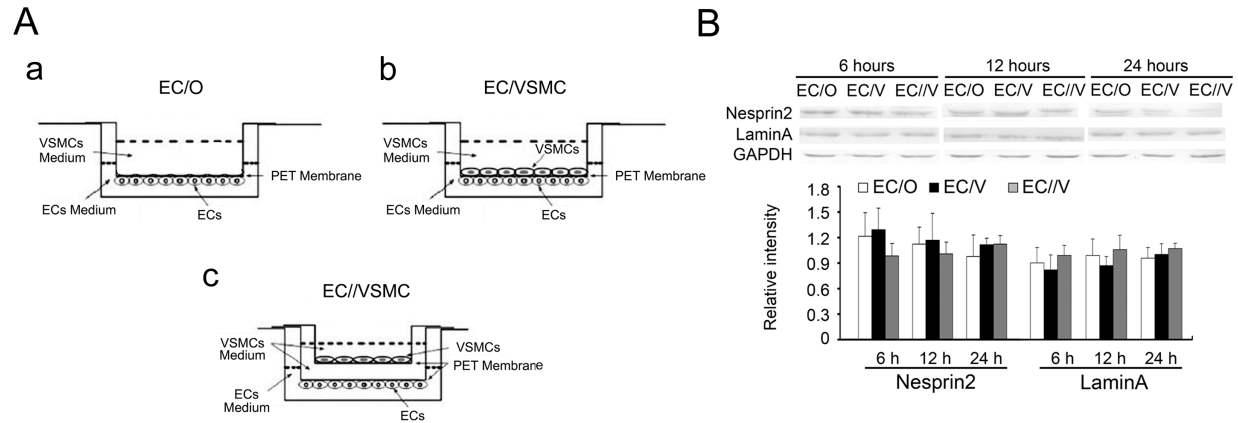
### Supplementary Material



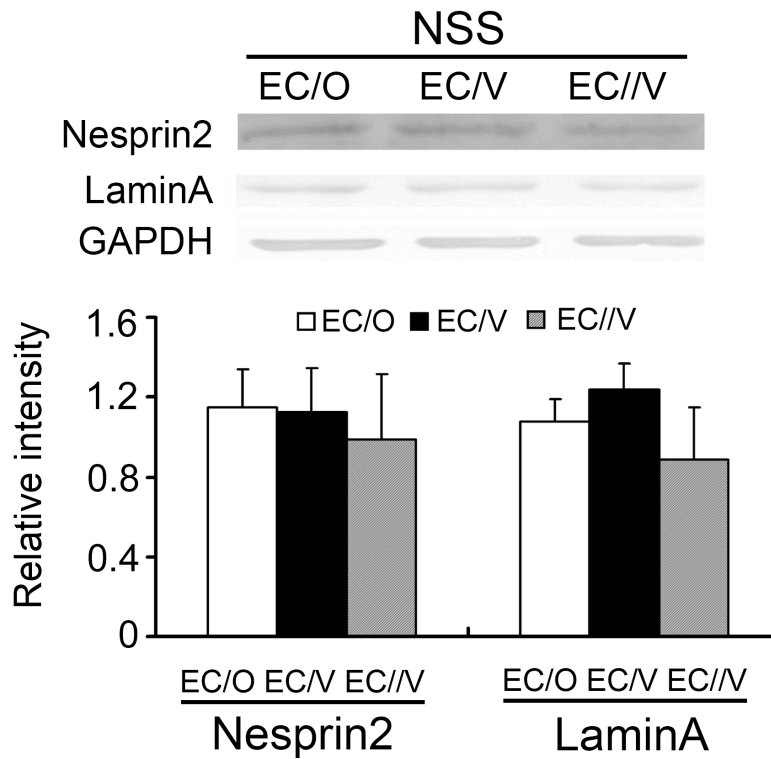
**Suppl. Fig. 1.** Schematic diagram of the parallel-plate flow chamber system. **(A)** Schematic diagram of the whole parallel-plate flow chamber system. **(B)** The pictures of the cell culture insert. **(C)** The lateral view of the parallel-plate flow chamber. **(D)** The components of the chamber. 1, the cell culture insert; 2, the top plate; 3, the flow field (28 mm in width and 0.2 mm in height); 4, the bottom plate.



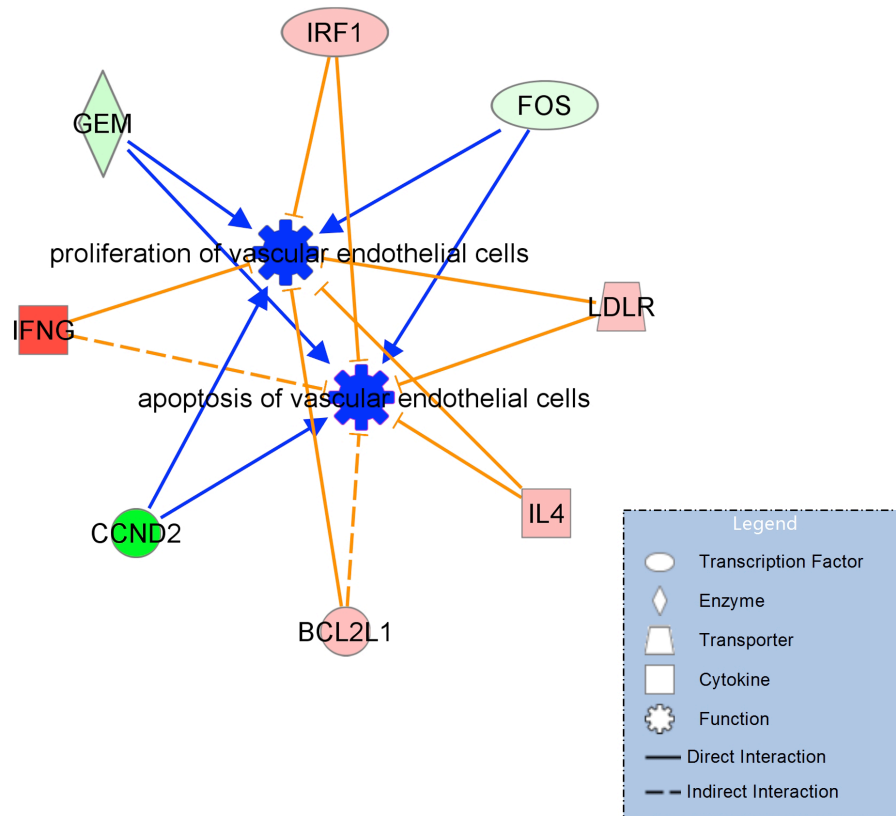
**Suppl. Fig. 2.** Networks of the downstream target genes of AP-2, TFIID, Stat-1, Stat-3, Stat-5, and Stat-6 revealed by IPA. The molecules, which were important to cellular proliferation and apoptosis (Supplementary Table 2), were selected for further research and marked in pink. **(A)** The downstream target genes of AP-2. **(B)** The downstream target genes of TFIID. **(C)** The common target genes of Stat-1, Stat-3, Stat-5, and Stat-6.



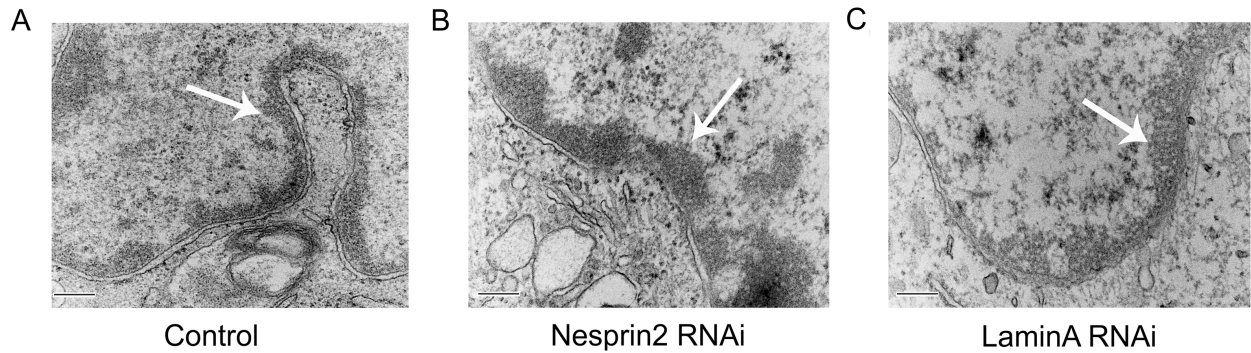
**Suppl. Fig. 3.** The effect of VSMCs on NE proteins expressions in the co-cultured ECs under the static. **(A)** Schematic diagram of the mono-cultured ECs (EC/O), the directly-contacted (EC/V) and indirectly-contacted (EC//V) co-culture model. The ECs/VSMCs co-culture system was made up of plating cells on the different sides of a 10  $\mu\text{m}$  thick porous polyethylene terephthalate (PET) membrane (Becton Dickinson Labware, USA) which contained 1.6 million pores/ $\text{cm}^2$  and each pore was 0.4  $\mu\text{m}$  in diameter. a) For the mono-cultured ECs group, ECs were cultured alone on the outer side of the PET membrane. b) For the EC/V co-culture group, ECs could communicate with VSMCs via both direct physical contact and paracrine effect. c) For the EC//V co-culture group, the physical contact between ECs and VSMCs were blocked but the paracrine effect via the continuous culture medium was still exist. **(B)** Compared with ECs cultured alone group (EC/O), co-cultured VSMCs, both directly-contacted and indirectly-contacted, had no specific effect on the expressions of Nesprin2 and LaminA in ECs. The values represent the mean  $\pm$  SD (n= 5).



**Suppl. Fig. 4.** The synergistic effect of shear stress and VSMCs on NE proteins expressions. After the NSS application for 12 hours, the expressions of Nesprin2 and LaminA in ECs cultured with, directly-contacted model (EC/V) and indirectly-contacted model (EC//V), or without VSMCs had no significant difference. The values represent the mean  $\pm$  SD (n= 5).



**Suppl. Fig. 5.** The relationship between the target genes and ECs functions. The Network displayed the molecules that were associated with the proliferation and apoptosis of ECs based on our experiment results and the Ingenuity® Knowledge Base. Orange shading indicated predicted activation and orange lines indicated a prediction of leading to activation; while blue shading indicated predicted inhibition and blue lines indicated a prediction of leading to inhibition.



**Suppl. Fig. 6.** Down-regulation of Nesprin2 or LaminA caused the degradation of nuclear envelope by transmission electron microscope (TEM) analysis. **(A)** The nuclear envelope consisting of phospholipid bilayer was continuous and clear in control group. **(B)** Nesprin2 RNAi resulted in the degradation of nuclear envelope. **(C)** LaminA RNAi resulted in the degradation of nuclear envelope. Bar = 200 nm.

**Supplementary Table 1.** Transcription factors (TFs) whose activations were changed more than 2-fold or less than 0.5-fold comparison with the respective control in ECs transfected with target siRNA or overexpression plasmid.

TFs	Fold change compared with the control	
	Nesprin2 RNAi	Nesprin2 overexpression
AP-2(2)	2.706496078	0.494234743
TFIID	2.959703778	0.252417355
HSE	2.198956842	0.46807815
	LaminA RNAi	LaminA overexpression
PRE	0.44927383	2.225749133
RAR/DR-5	0.414879962	3.422094006
SIE	0.358242155	4.551779723
Stat-1	0.38668264	3.16688076
Stat-3	0.490473553	2.586263264
Stat-5	0.287069811	2.460661287
Stat-6	0.094304661	2.182947833
TR/DR-4	0.415631481	4.830839255
VDR/DR-3	0.400824541	6.919662302
MRE	0.357176219	6.788149833

**Supplementary Table 2.** Bio-functional analysis and p-value of target genes.

Genes	Biofunction	p-value
PLA2G16	Cellular Growth and Proliferation; Cell Death and Survival; Cell Cycle; Lipid Metabolism	1.81E-16
PITX2	Tissue Morphology; Cellular Growth and Proliferation; Cell Death and Survival; Cellular Movement; Organismal Development; Organismal Survival; Cardiovascular System Development and Function; Cancer	1.20E-17
GEM	Cellular Growth and Proliferation; Cell Death and Survival; Embryonic Development; development of cardiovascular system; Cellular Assembly and Organization; development of blood vessel	4.11E-12
KISS1	Tissue Morphology; Cellular Growth and Proliferation; Cell Death and Survival; Cellular Movement; Molecular Transport; Cell-To-Cell Signaling and Interaction;	1.47E-14
KRT14	Cellular Growth and Proliferation; Cell Death and Survival; Cellular Movement; Inflammatory Response; Embryonic Development	6.09E-07
FOS	Cellular Growth and Proliferation; Cell Death and Survival; Cellular Movement; Cellular Assembly and Organization; Cancer;	1.20E-17
LDLR	Inflammatory Response; Organismal Survival; Lipid Metabolism; Cellular Growth and Proliferation; Cell Death and Survival; Digestive System Development and Function;	1.81E-16
Gh	Cellular Development; Tissue Morphology; Cell-To-Cell Signaling and Interaction; Hematological System Development and Function; Cellular Growth and Proliferation; Cell Death and Survival;	9.45E-10
BCL2L1	Lymphoid Tissue Structure and Development; Tissue Morphology; Cellular Development; Cellular Growth and Proliferation; Cell Death and Survival;	8.19E-10
CCND2	Lymphoid Tissue Structure and Development; Cellular Development; Cell Cycle; Hematopoiesis; Cell Morphology; Gene Expression; Respiratory System Development and Function; Organismal Development; Cellular Growth and Proliferation; Cell Death and Survival;	7.13E-07
IRF1	Cellular Development; Cellular Growth and Proliferation; Cell Death and Survival; Hematological System Development and Function; Cell-To-Cell Signaling and Interaction	1.05E-10
IFNG	Tumor Morphology; Cancer; Inflammatory Response; Cellular Growth and Proliferation; Cell Death and Survival; Cell-To-Cell Signaling and Interaction	2.46E-09
IL4	Cell-To-Cell Signaling and Interaction; Cancer; Immune Cell Trafficking; Liver Damage; Inflammatory Disease; Cellular Growth and Proliferation; Cell Death and Survival;	1.93E-09
TNF	Cellular Development; Cell-To-Cell Signaling and Interaction; Cell Death and Survival; Cellular Growth and Proliferation; Cell Death and Survival; Tumor Morphology	7.73E-09