

Leukotriene B4 in PAH

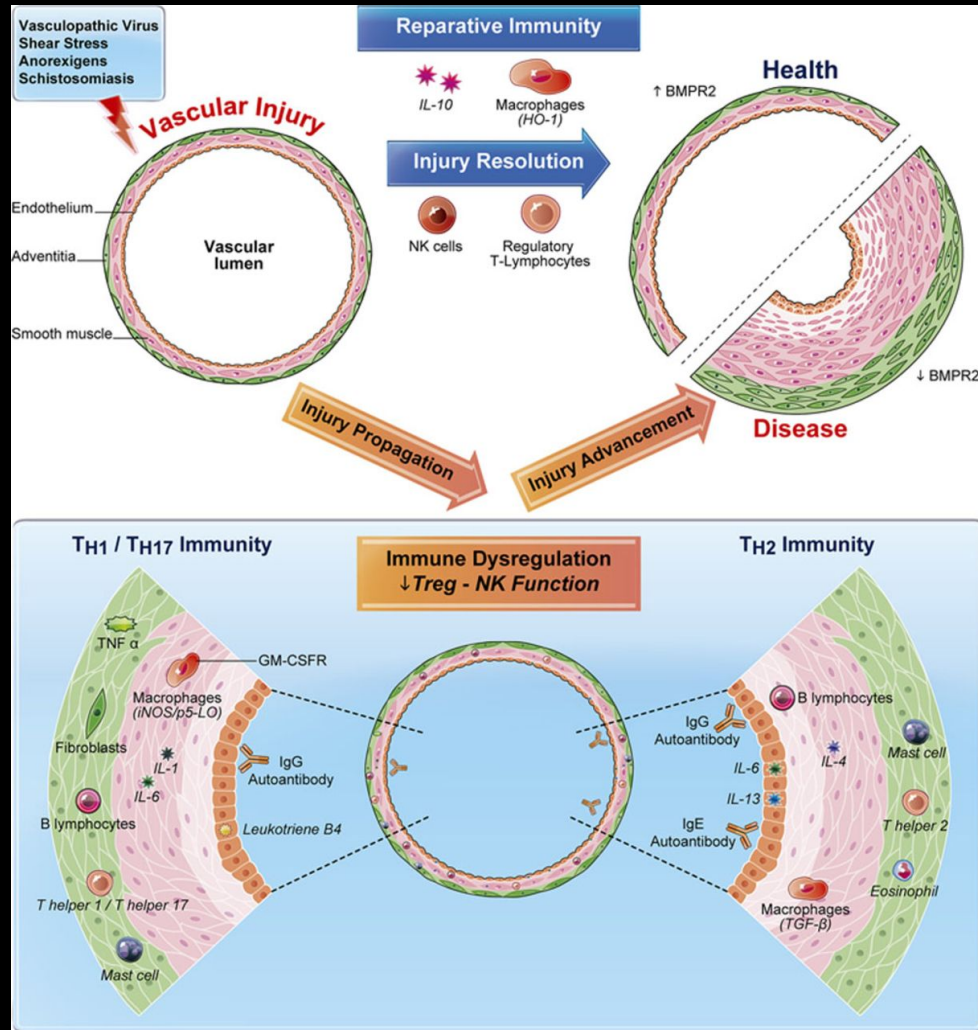
Amy Tian, PhD

02/13/2017

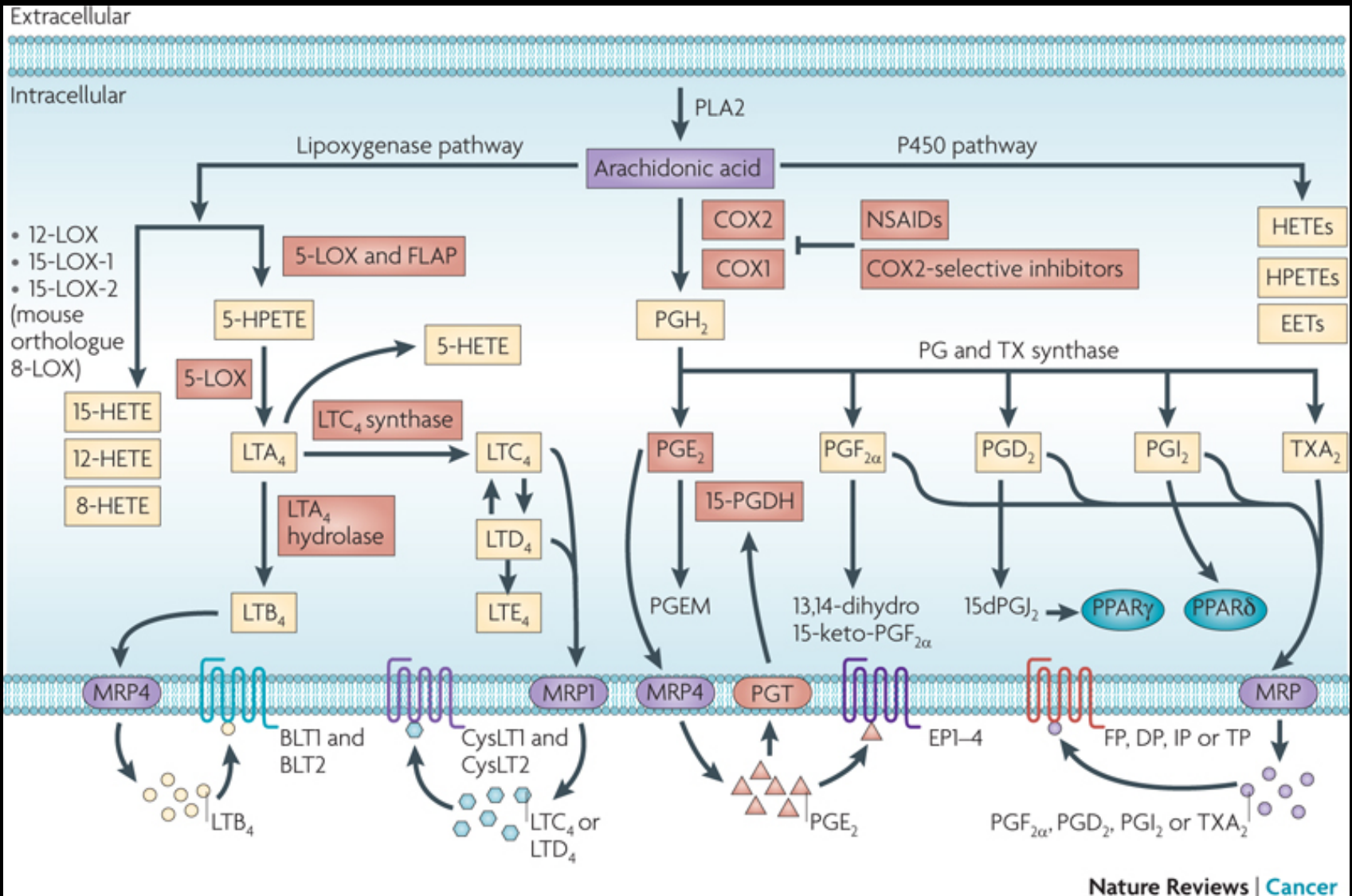
Conflict of Interests

- I am a co-founder of Eicco LLC

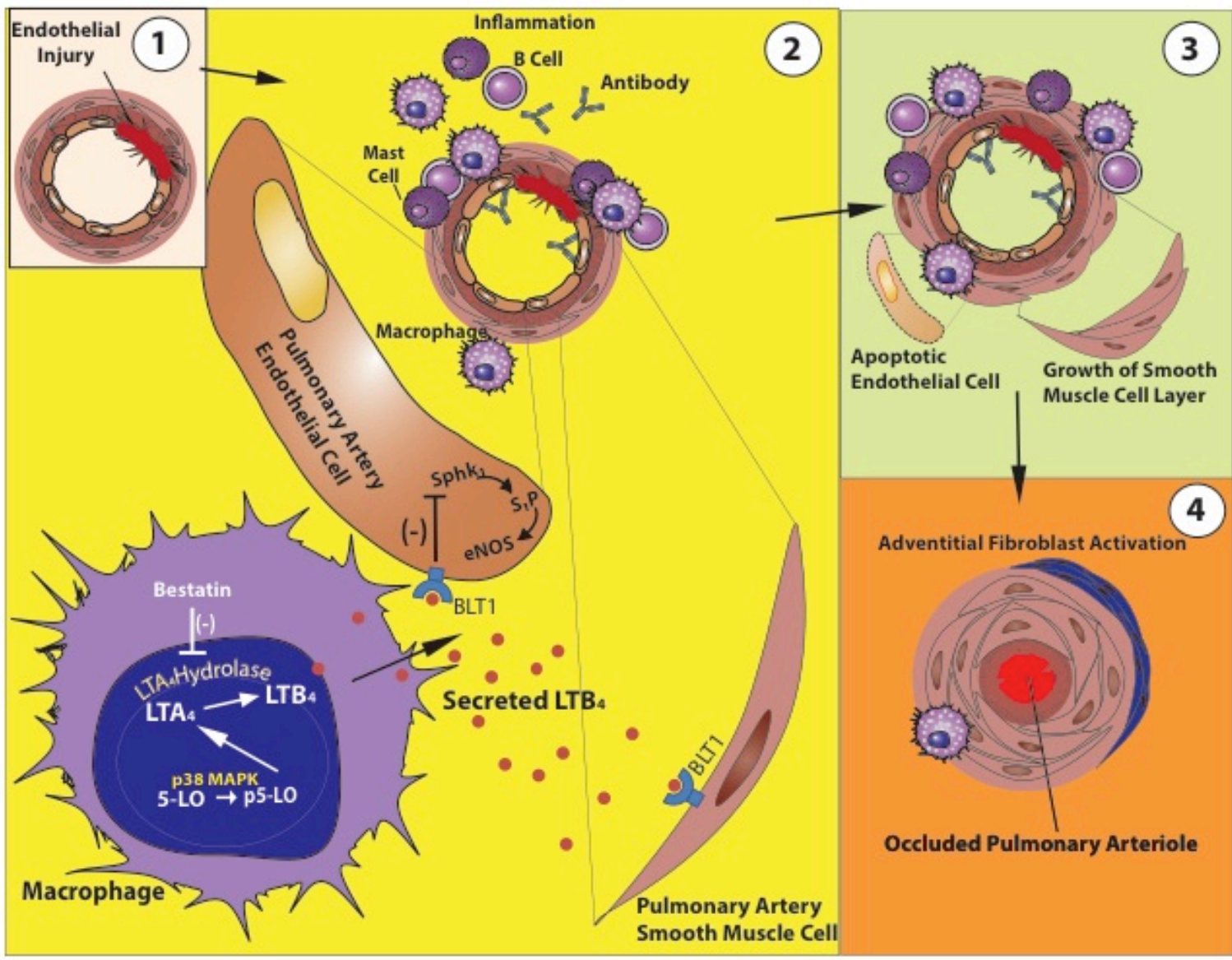
Pulmonary Hypertension and dysregulated immunity



Eicosanoid Pathway



Macrophage Derived LTB₄ Causes Experimental PH

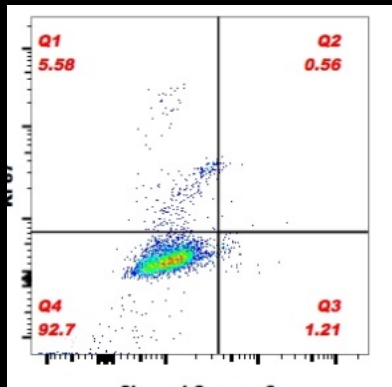


Tian. et al. Science Translational Medicine. 2013
 Qian. et al. Hypertension. 2015

LTB4 Induces an Apoptosis-Resistant PAEC Phenotype

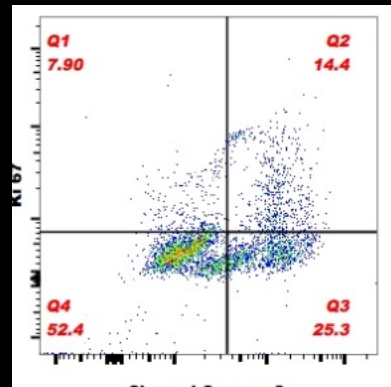
Ki67
Cleaved Cas-3

CTR

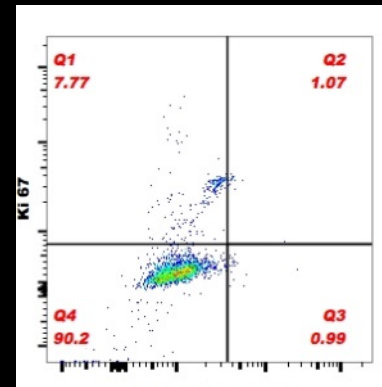


LTB4

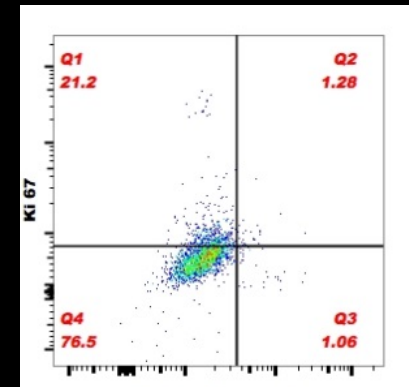
24h



48h



72h

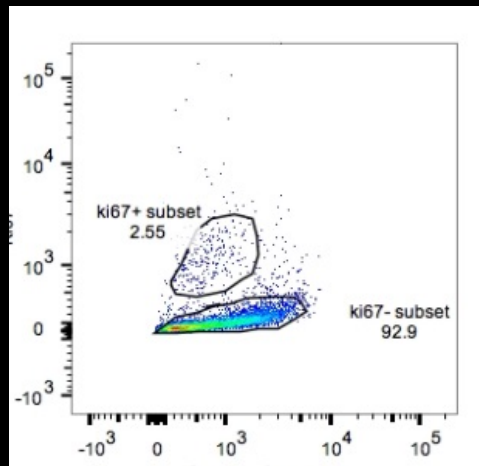


LTB4 Induces an Apoptosis-Resistant PAEC Phenotype

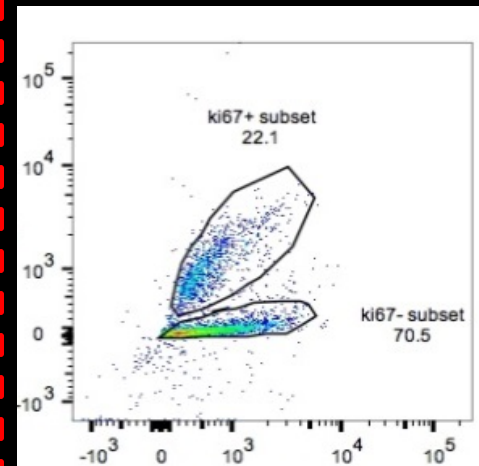
Ki67
Cleaved Cas-3

Day 9

CTR



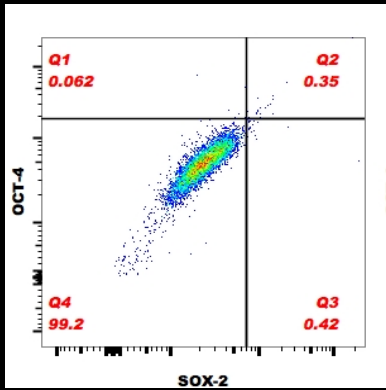
LTB4



LTB4 Induces Stemness in PAECs

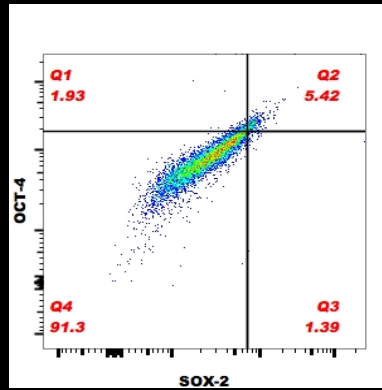
Oct4
Sox2

CTR

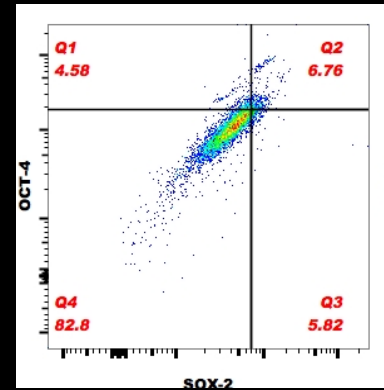


LTB4

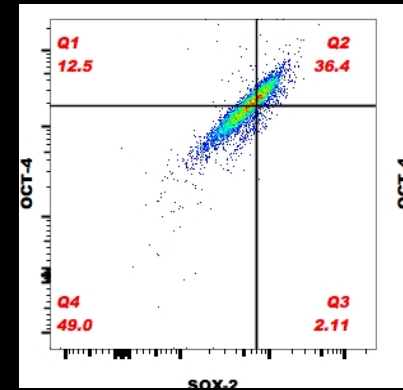
24h



48h



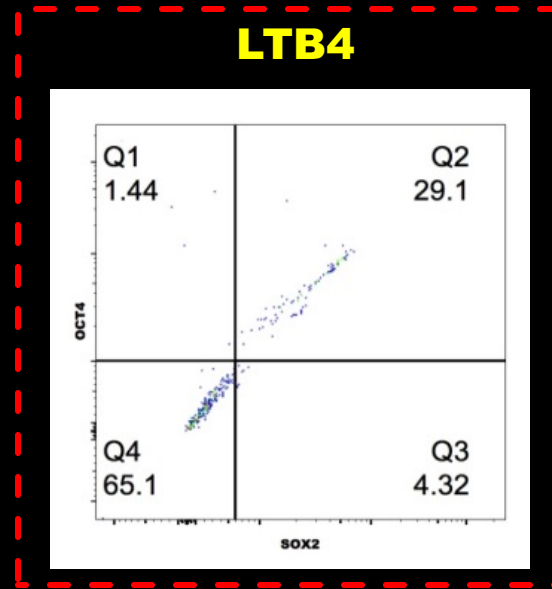
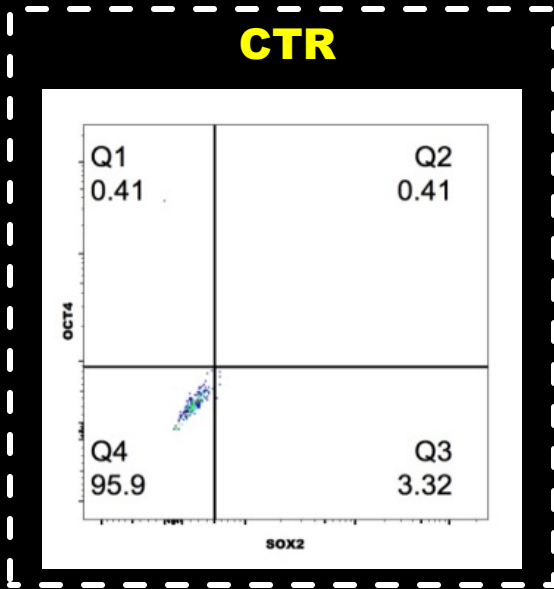
72h



LTB4 Induces Stemness in PAECs

Oct4
Sox2

Day 9



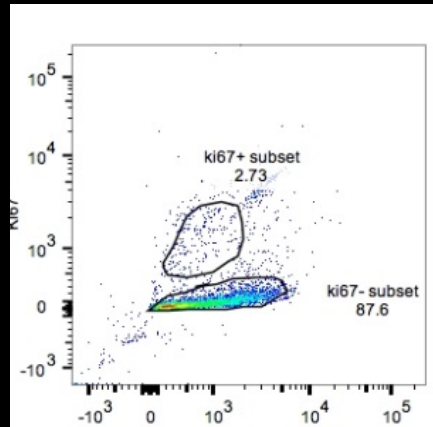
LTB4 Causes PAEC De-differentiation (in a BMPR2-Dependent Fashion)

W/O BMPR2
(*shbmpr2*)

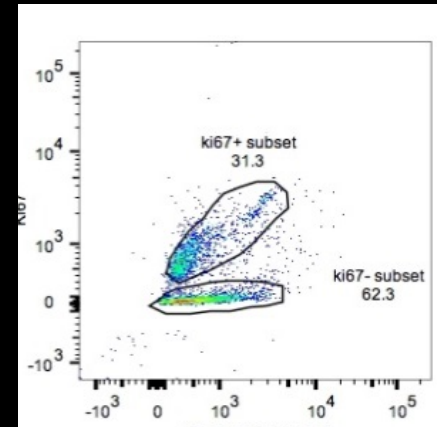
Day 9

Ki67
Cleaved Cas-3

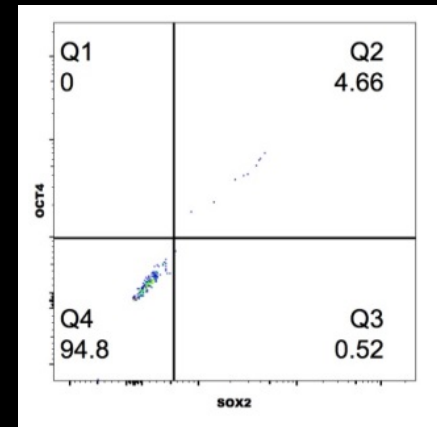
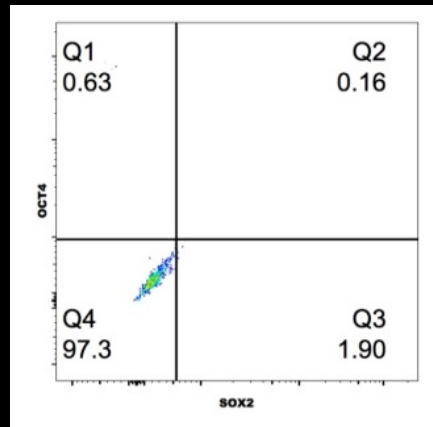
CTR



LTB4



Oct4
Sox2



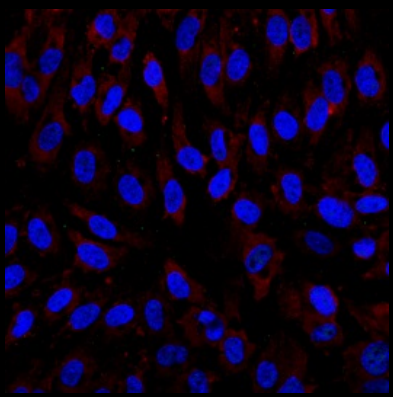
LTB4 Mediates PAEC De-differentiation /Differentiation (in a BMPR2-Dependent Fashion)

**With BMPR2
(control shRNA)**

**W/O BMPR2
(shbmpr2)**

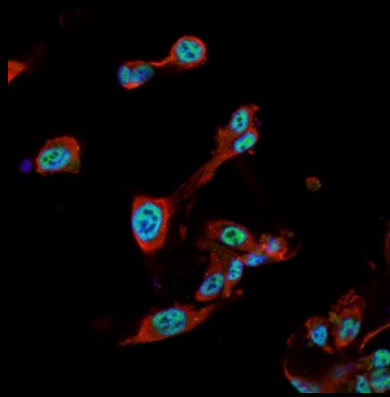
CTR

DAPI/OCT-4/a-SMA



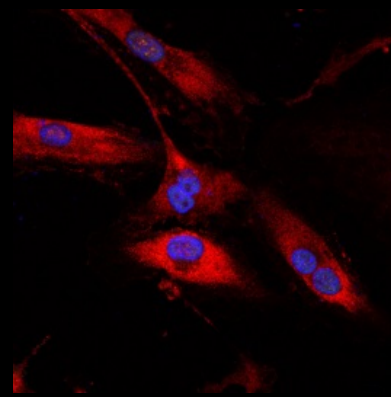
LTB4

DAPI/OCT-4/a-SMA



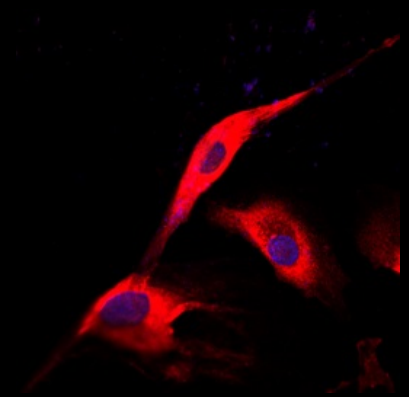
CTR

DAPI/OCT-4/a-SMA



LTB4

DAPI/OCT-4/a-SMA

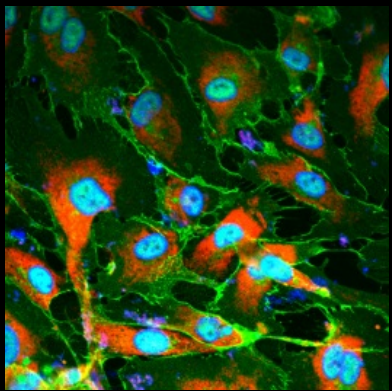


LTB4 Mediates Differentiation (in a BMPR2-Dependent Fashion)

**With BMPR2
(control shRNA)**

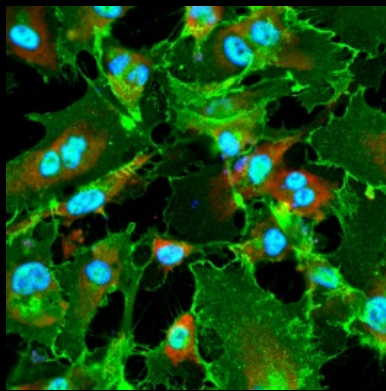
CTR

DAPI/VE-CAD/a-SMA



LTB4

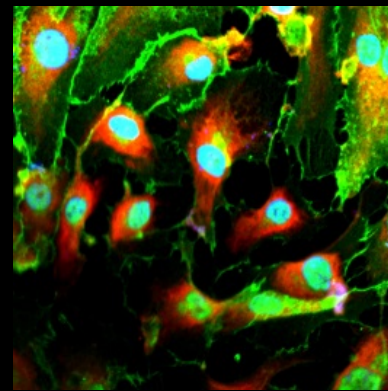
DAPI/VE-CAD/a-SMA



**W/O BMPR2
(shbmpr2)**

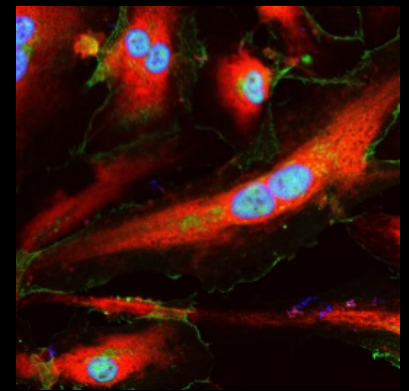
CTR

DAPI/VE-CAD/a-SMA



LTB4

DAPI/VE-CAD/a-SMA



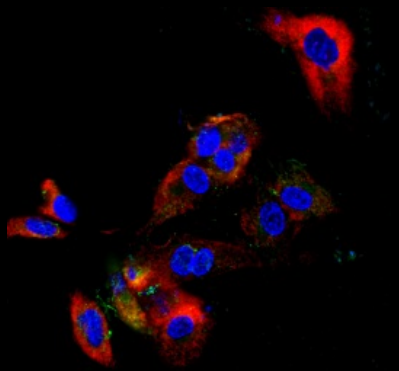
LTB4 Mediates PAEC Endothelial to Mesenchymal Transition (EndoMT) (in a BMPR2-Dependent Fashion)

**With BMPR2
(control shRNA)**

**W/O BMPR2
(shbmpr2)**

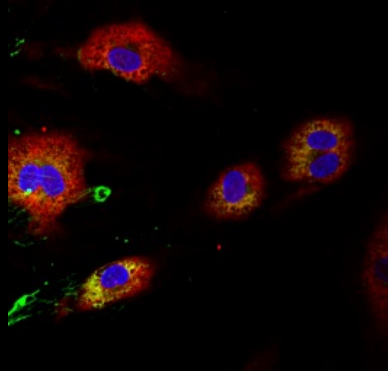
CTR

DAPI/Fibronectin/a-SMA



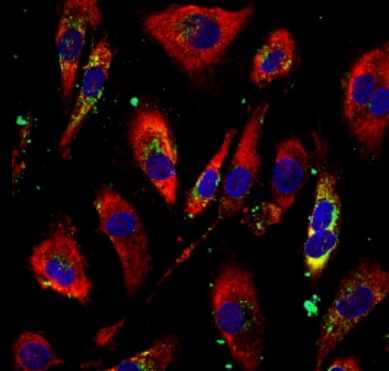
LTB4

DAPI/Fibronectin/a-SMA



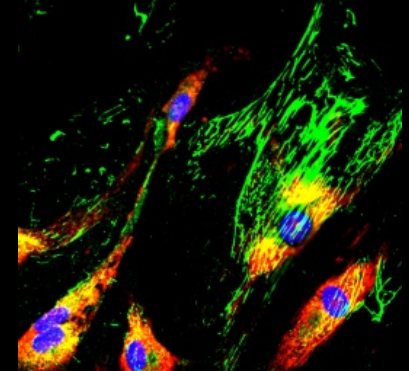
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DAPI/Fibronectin/a-SMA



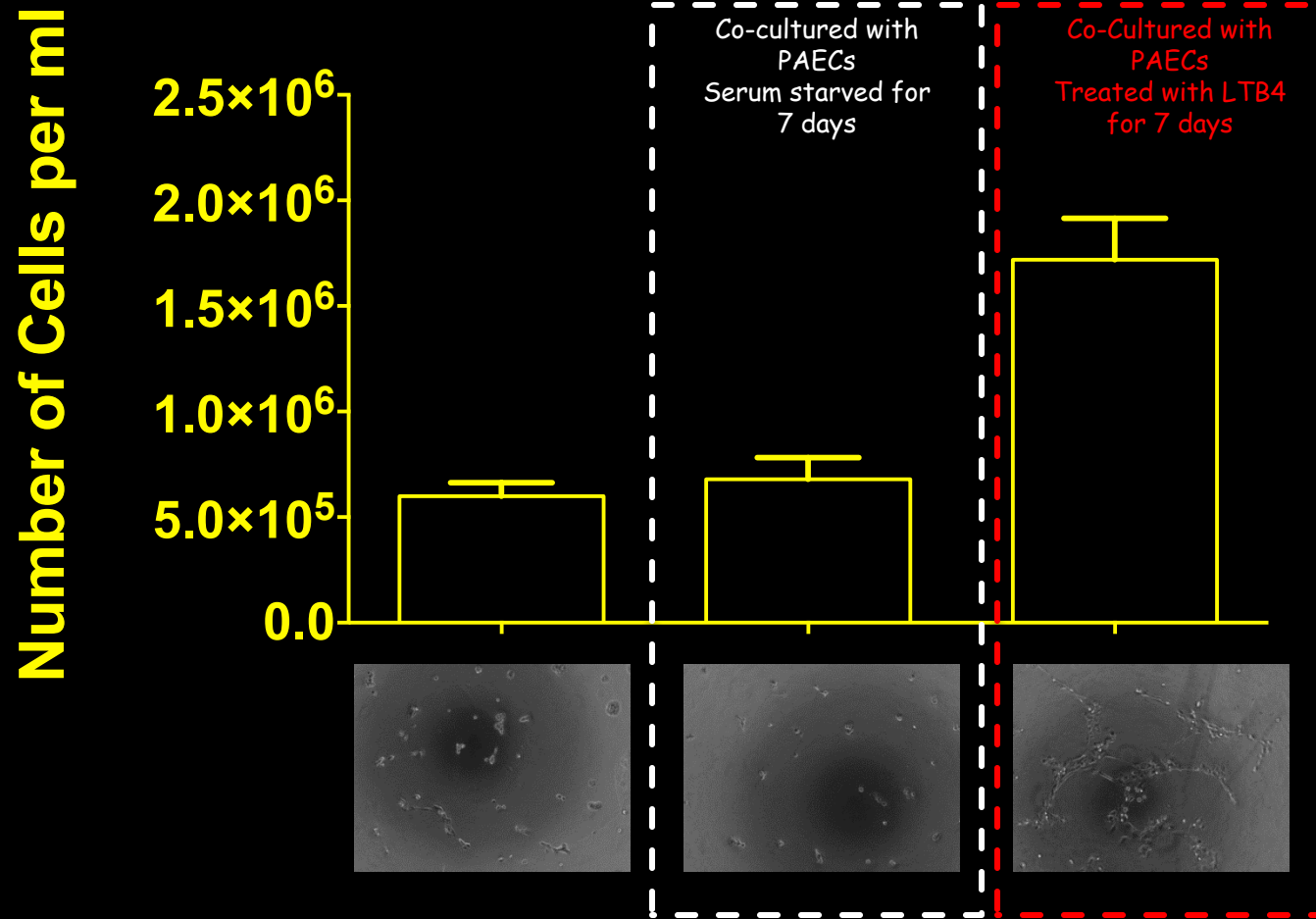
LTB4

DAPI/Fibronectin/a-SMA

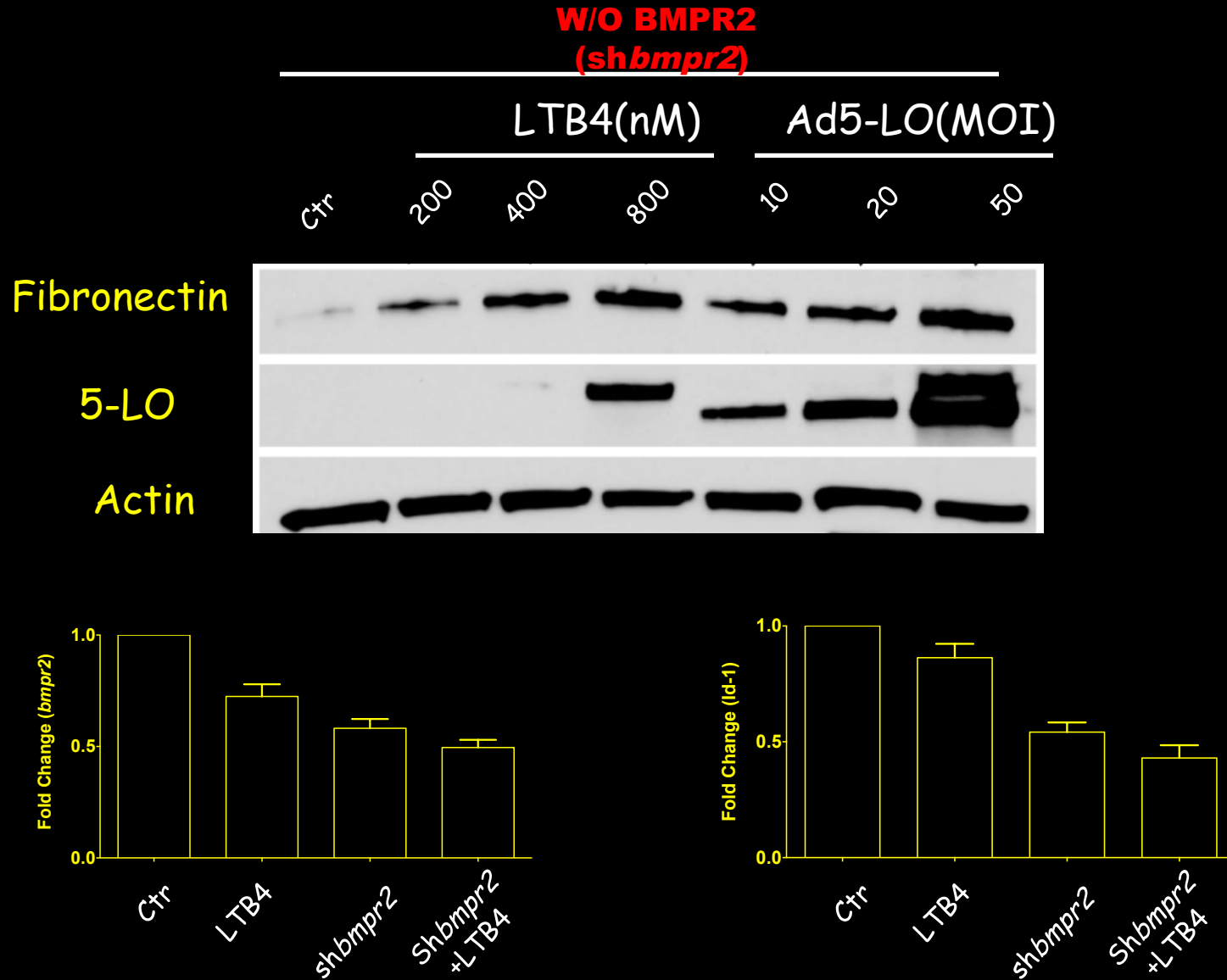


LTB4 Induced "Stem Cells" are Protective

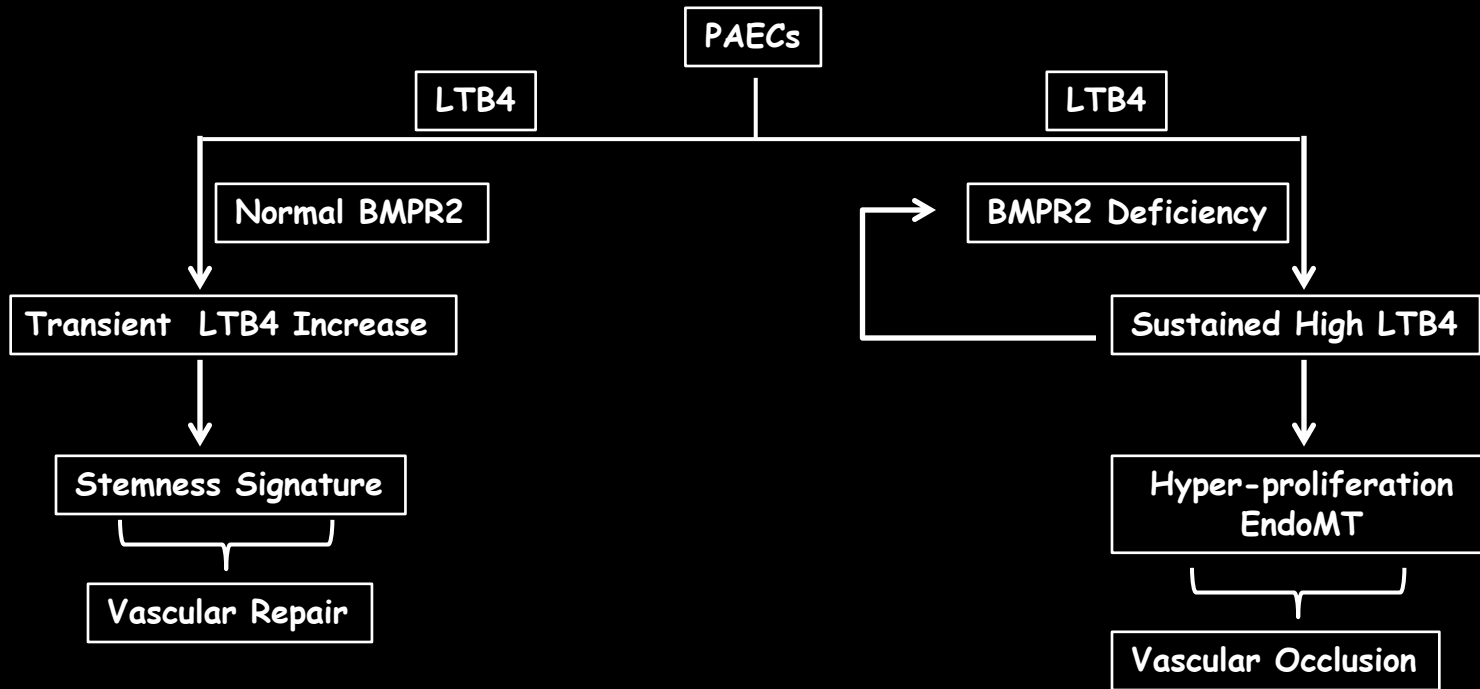
PAECs Treated with SU5416 for 24hr



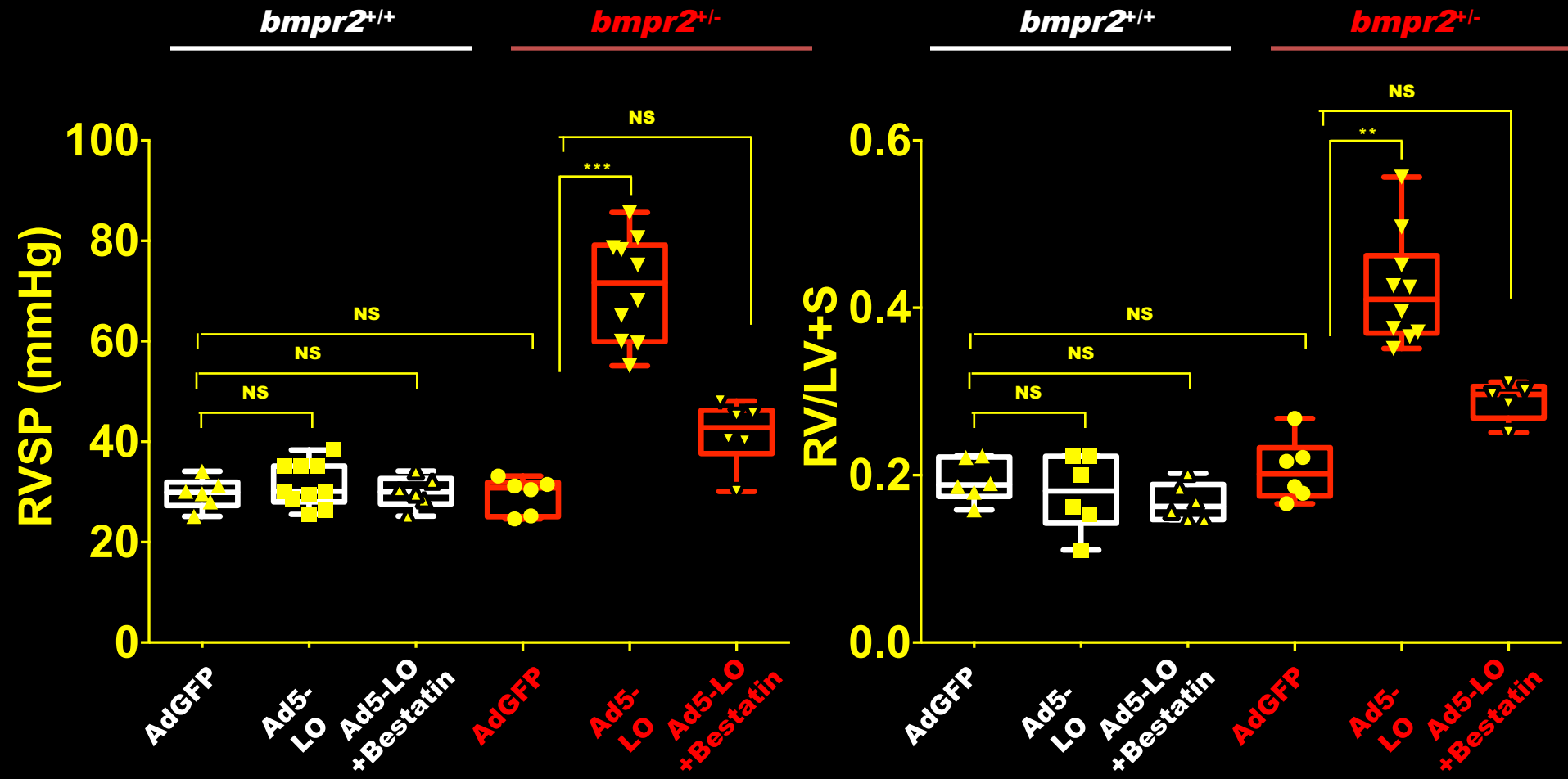
LTB4 Induced EndoMT cells have reduced BMPR2 signaling



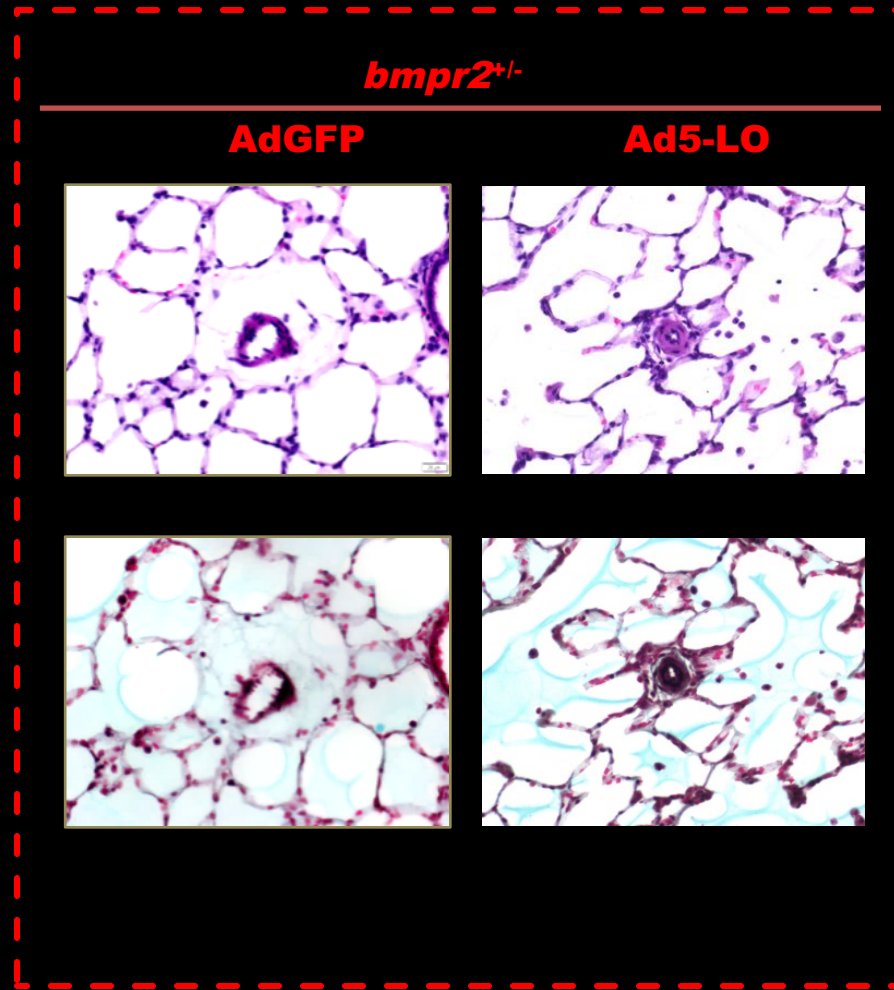
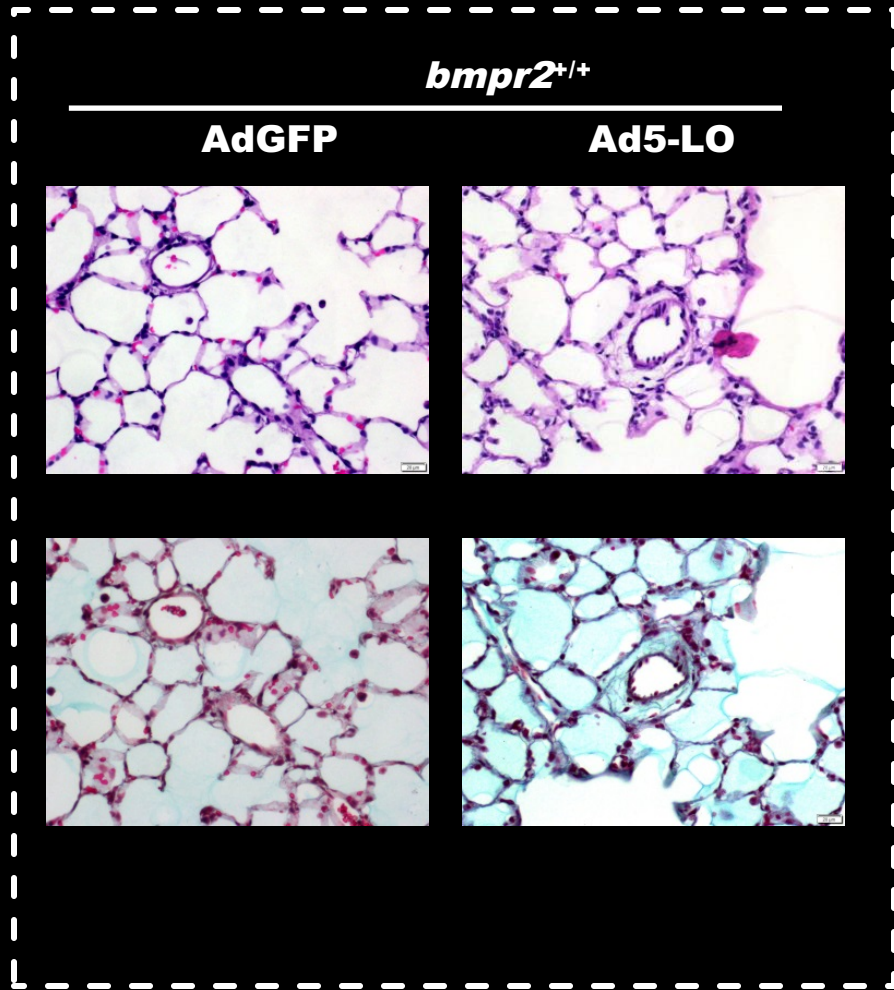
Hypothesis



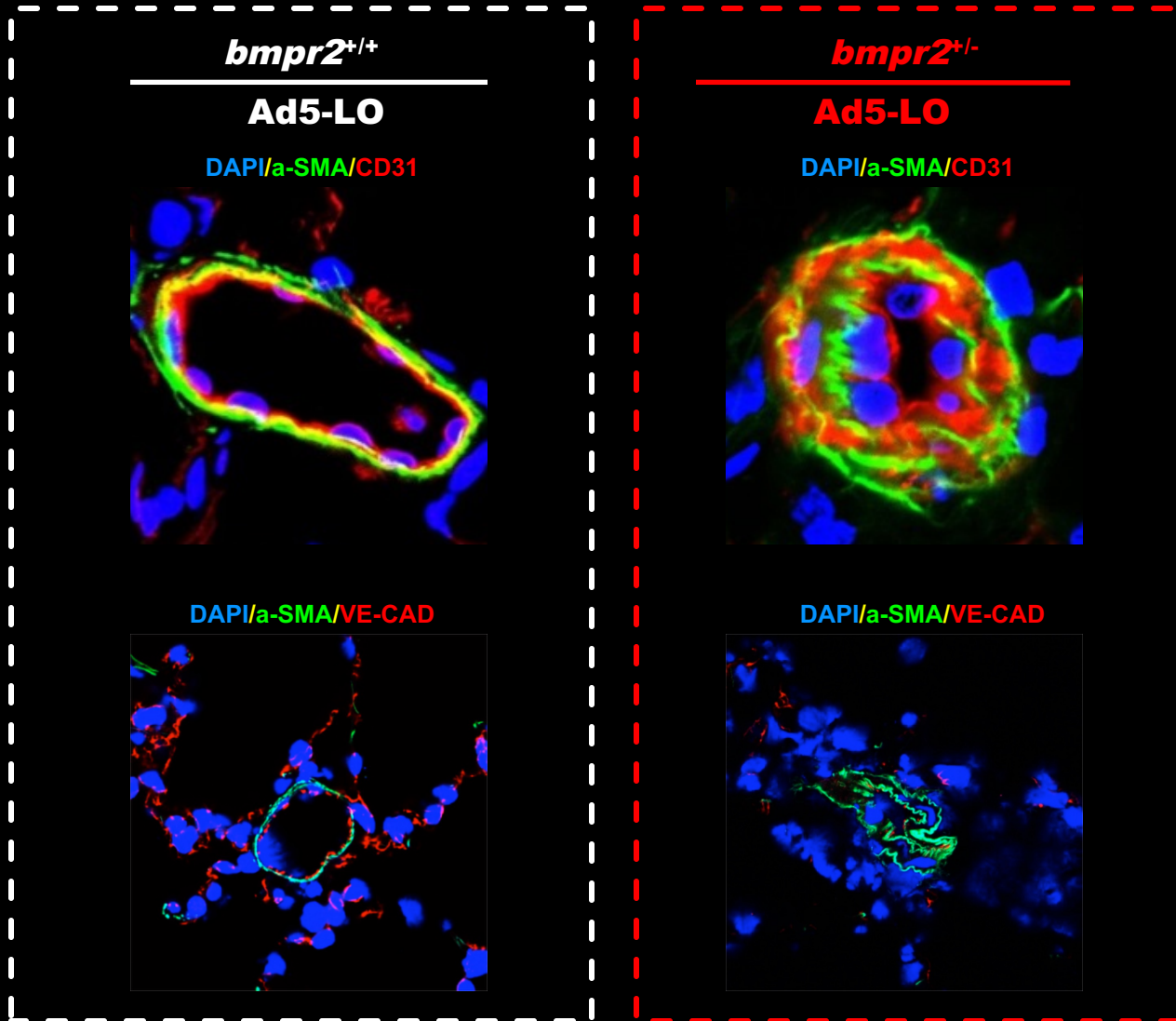
Overexpressing 5-LO Induces PH in *bmpr2*^{+/-} Rats



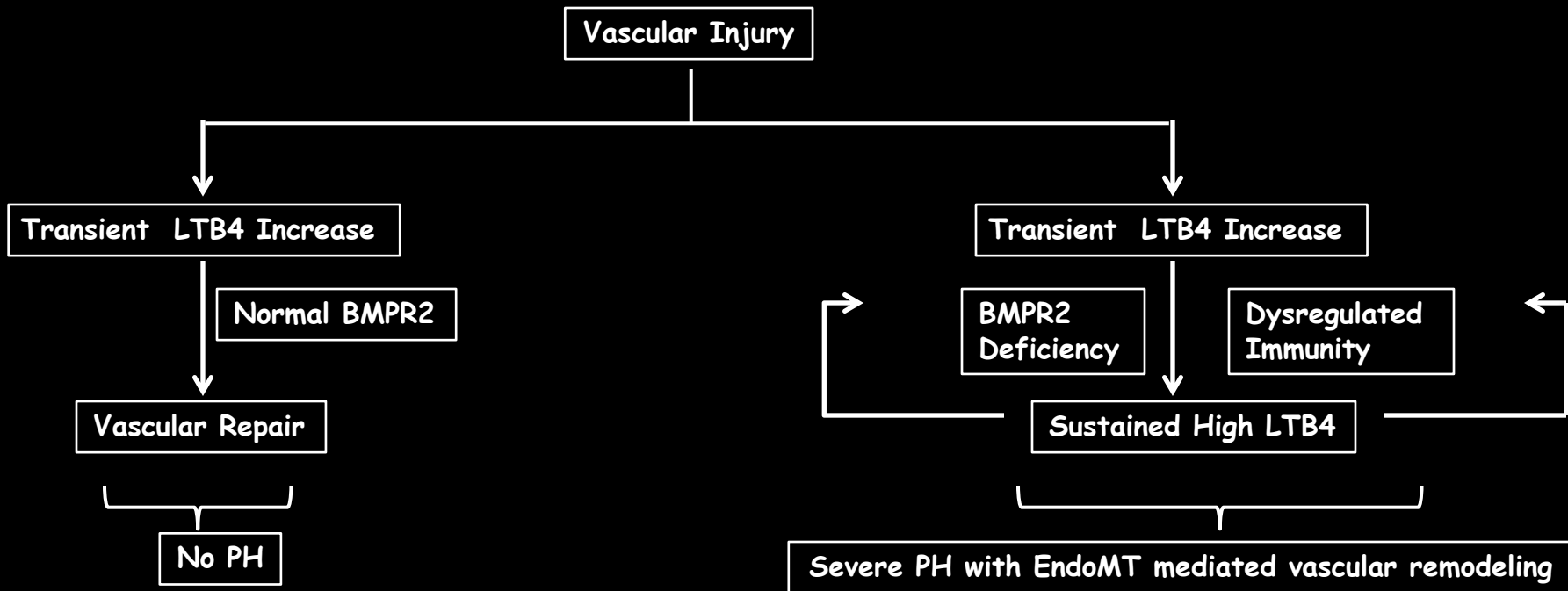
Overexpressing 5-LO Induces Vascular Remodeling in *bmpr2*^{+/-} Rats



Overexpressing 5-LO causes EndoMT in *bmpr2*^{+/-} rat



Conclusion



Acknowledgements

- Nicolls group
 - Xinguo Jiang
 - Eric Shuffle
 - Allen Tu
 - Jin Qian
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- Dr. Marlene Rabinovitch
- Dr. Roham Zamanian
- Dr. Norbert Voelkel
- Dr. Rubin Tuder
- Dr. Kurt Stenmark
- Dr. Marc Peters-Golden

Candidate Mechanism from RNA-seq

	Gene	Protein	FOLD CHANGE
inflammation	<i>SELP</i>	Selectin P CD62	38.4
	<i>IL33</i>	Interleukin 33	8.6
	<i>CXCL11</i>	C-X-C Motif Chemokine Ligand 11	6.6
	<i>SELL</i>	Selectin L	6.5
	<i>LGALS9</i>	Lectin	4.1
	<i>STAT1</i>	Signal Transducer And Activator Of Transcription 1	2.1
	<i>IL17D</i>	Interleukin 17D	2.2
	<i>JAK3</i>	Janus Kinase 3	7.4
complement	<i>C1R</i>	complement component 1 R	14.4
	<i>C1S</i>	complement component 1 S	11.7
	<i>C3</i>	Complement Component 3	8.0
	<i>CFB</i>	complement Factor B	7.3
cell differentiation	<i>HIF3A</i>	Hypoxia-inducible factor 3-alpha	6.2
	<i>IGFBP2</i>	Insuline like growth factor binding protein	6.1
	<i>FLRT2</i>	Fibronectin	5.2
	<i>LRP5</i>	Wnt receptor	2.6
	<i>ADAM9</i>	ADAM Metallopeptidase	2.1
	<i>IGF1</i>	Insulin-like growth factor I	4.6
lymphatic	<i>CCBE1</i>	Collagen And Calcium Binding EGF Domains 1	2.2
	<i>LYVE1</i>	lymphatic marker	4.3
TGFB	<i>TGFB1</i>	TGFB ligand	4.2
	<i>TGFB2</i>	TGFB ligand	2.5
	<i>TGFB2</i>	TGFB receptor 1	1.6
	<i>LTBP1</i>	Latent Transforming Growth Factor Beta	4.1
	<i>ITGB3</i>	(Integrin Subunit Beta 3)	2.3
	<i>ITGBL1</i>	Integrin Subunit Beta Like 1	2.2

Candidate Mechanism from RNA-seq

	Gene	Protein	FOLD CHANGE
Notch signaling	NOTCH1	Notch receptor	0.55
	NOTCH3	Notch receptor	0.38
	NOTCH4	Notch receptor	0.26
	HES1	Notch down stream gene	0.29
	HEY1	Notch down stream gene	0.26
	EFNB2	Notch down stream gene	0.09
	DLL4	Delta like 4	0.08
	JAG2	Jaggard 2	0.21
	FLT4	VEGFR3	0.19
Gap Junction	GJA1	connexin 43	0.54
	GJA5	connexin 40	0.06
	GJA4	connexin 37	0.03
Cell differentiation	KIF4A	Kinesin Family Member 4A	0.43
	KIF18B	Kinesin Family Member 18B	0.26
	KIF11	Kinesin Family Member 11	0.44
Metabolism	FABP4	Fatty metabolism	0.07
BMP	TGIF1	TGFB Induced Factor Homeobox 1	0.53
	ID3	Inhibitor Of DNA Binding 3	0.54
	TRAF6	TNF Receptor Associated Factor 6	0.50
	YAP1	Yes Associated Protein 1	0.41
	ID2	Inhibitor Of DNA Binding 2	0.47