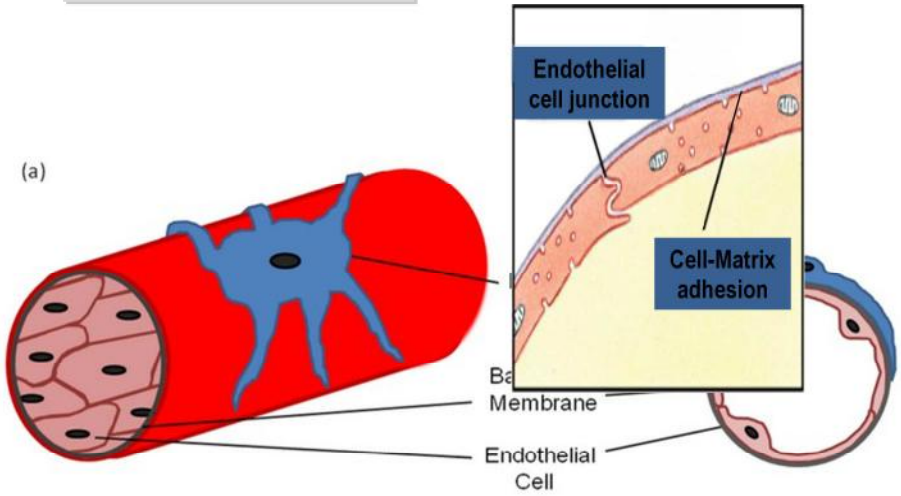
ZO-1 ROLE IN ENDOTHELIAL CELLS

Olga Tornavaca

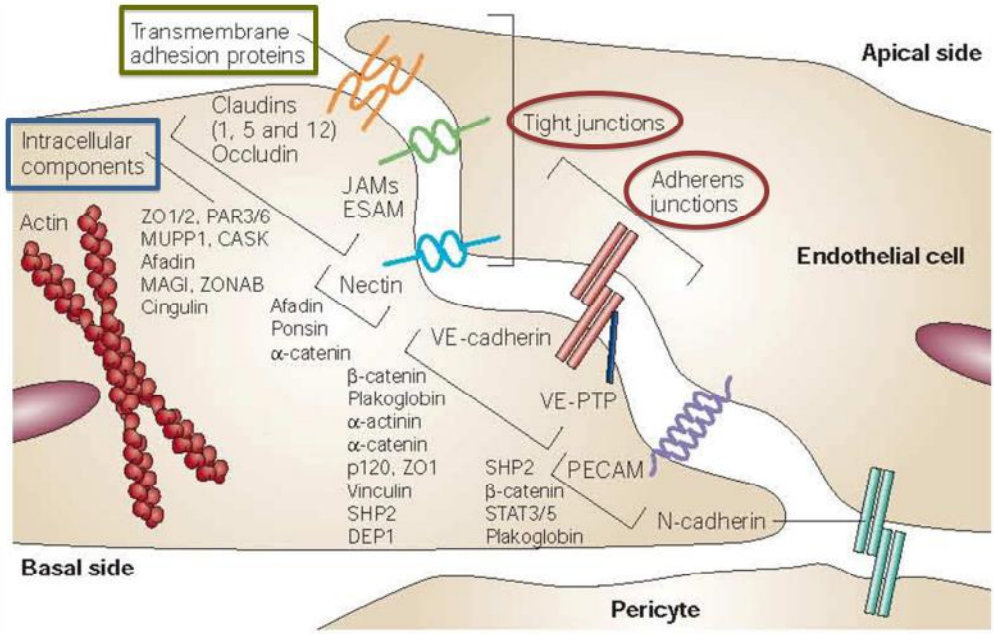
Maria S. Balda & Karl Matter Lab

Department of Cell Biology, Institute of Ophthalmology
University College London. London. United Kingdom

Endothelial cells

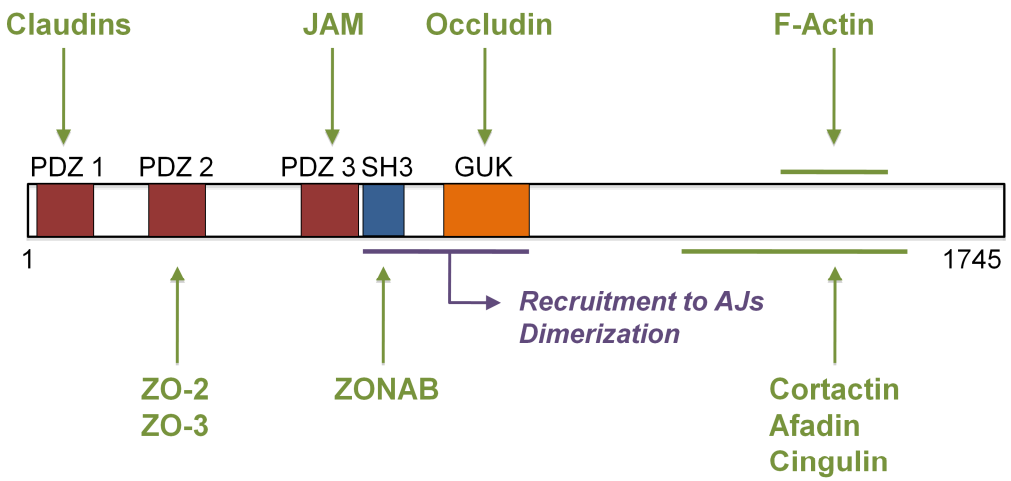


Molecular organization of endothelial junctions

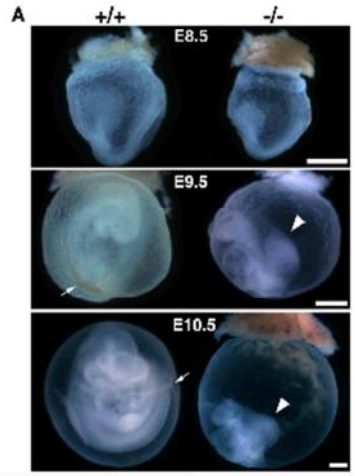


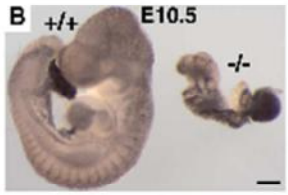
INTRODUCTION

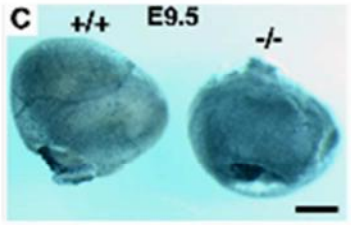
ZO-1, a TJ-associated protein



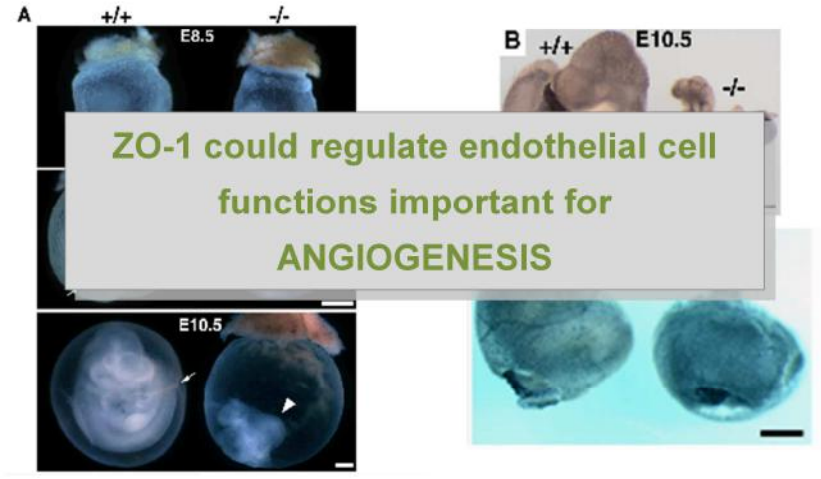
ZO-1 knockout mice are embryonic letal (E9.5-10.5) and ZO-1 is required for normal blood vessel formation in the yolk sac of mice (Katsuno, T., 2008).





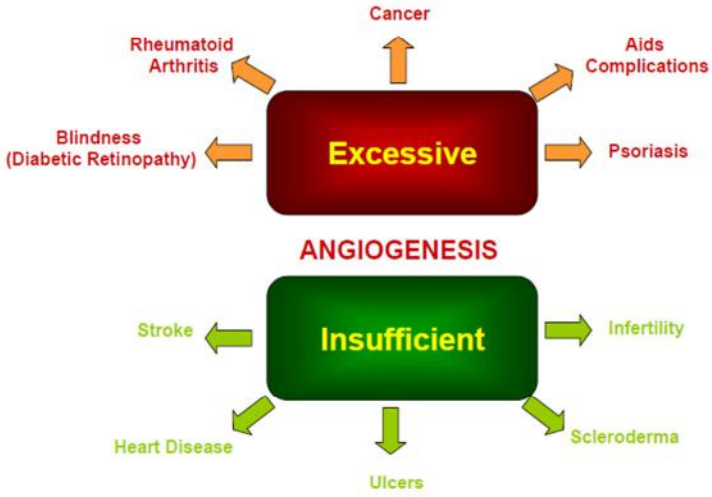


ZO-1 knockout mice are embryonic letal (E9.5-10.5) and ZO-1 is required for normal blood vessel formation in the yolk sac of mice (Katsuno, T., 2008).



Angiogenesis

Dysregulation in disease states



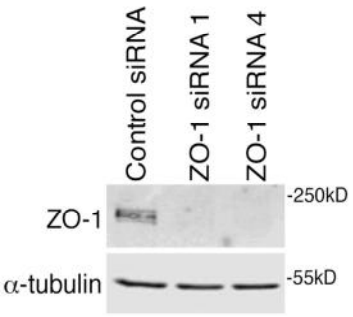
OBJECTIVE

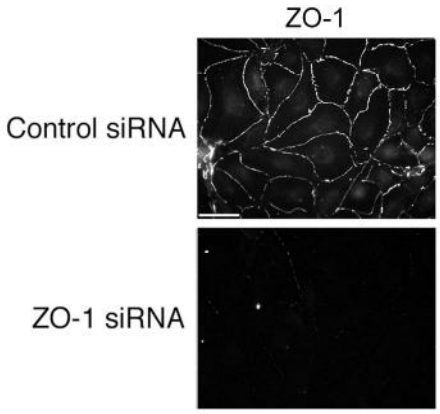
Is ZO-1 an important determinant of endothelial cell behaviour?

- Is it important for the endothelial angiogenic potential?
- What is the molecular mechanism involved in ZO-1-regulated angiogenesis?

Does ZO-1 regulate the angiogenic potential of endothelial cells?

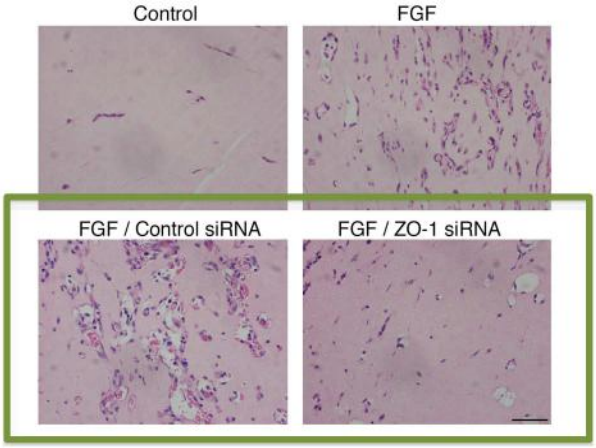
Loss-of-function approach

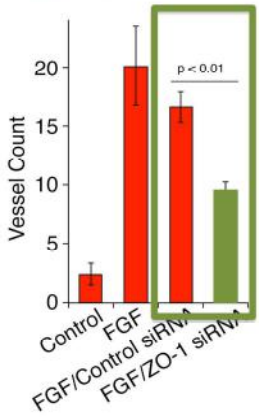




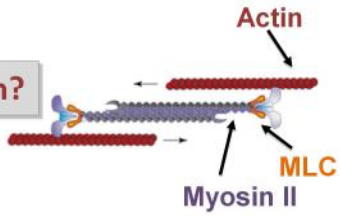
Does ZO-1 regulate the angiogenic potential of endothelial cells?

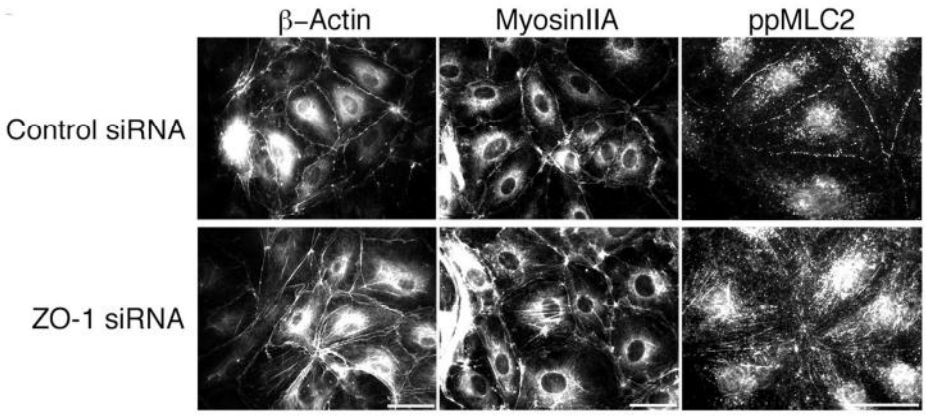
ZO-1 downregulation inhibits angiogenesis *in vivo*



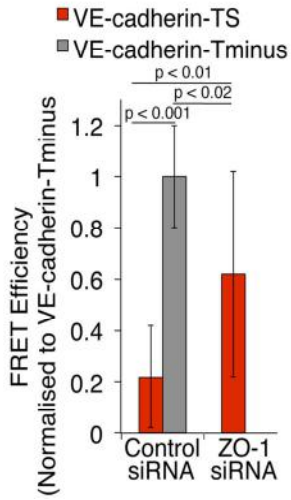


Does ZO-1 regulate actomyosin distribution?

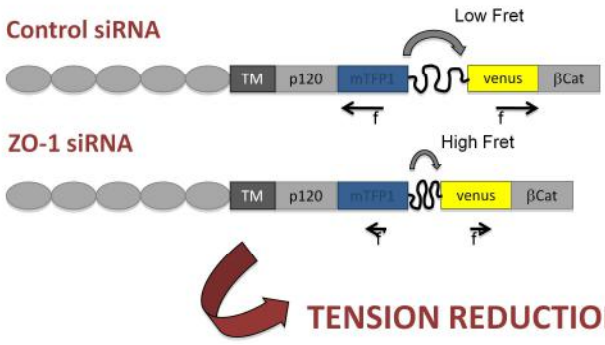


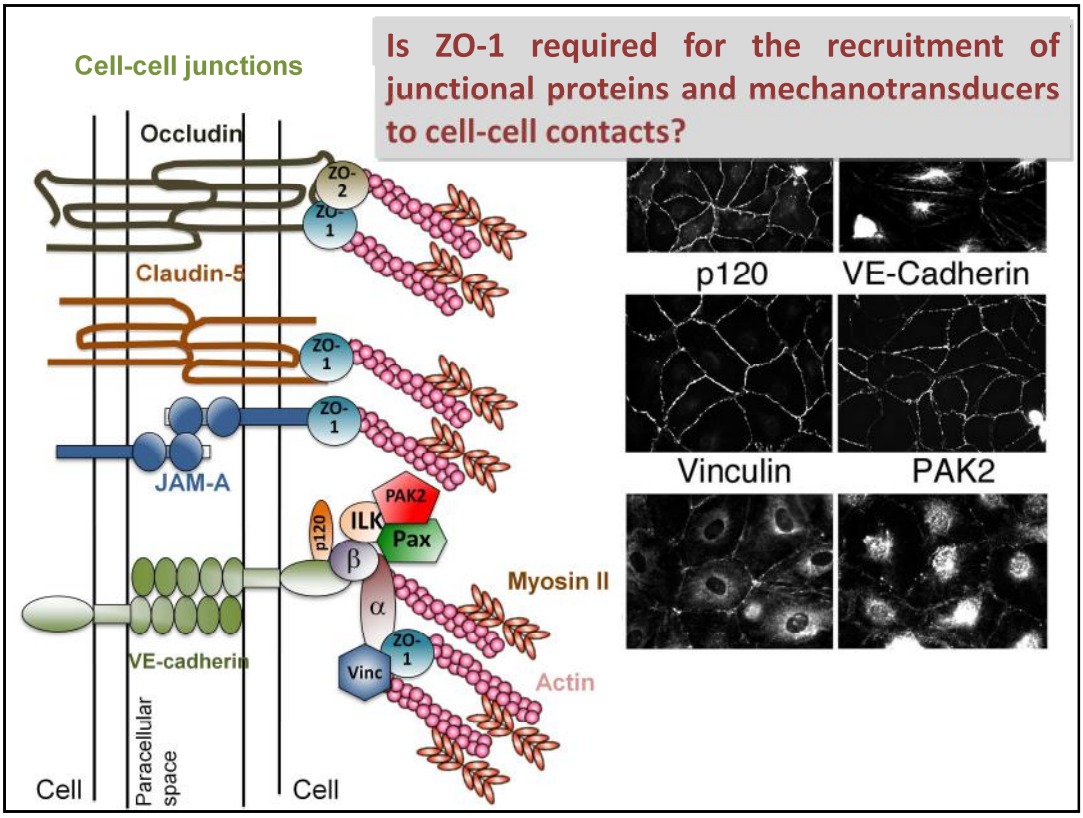


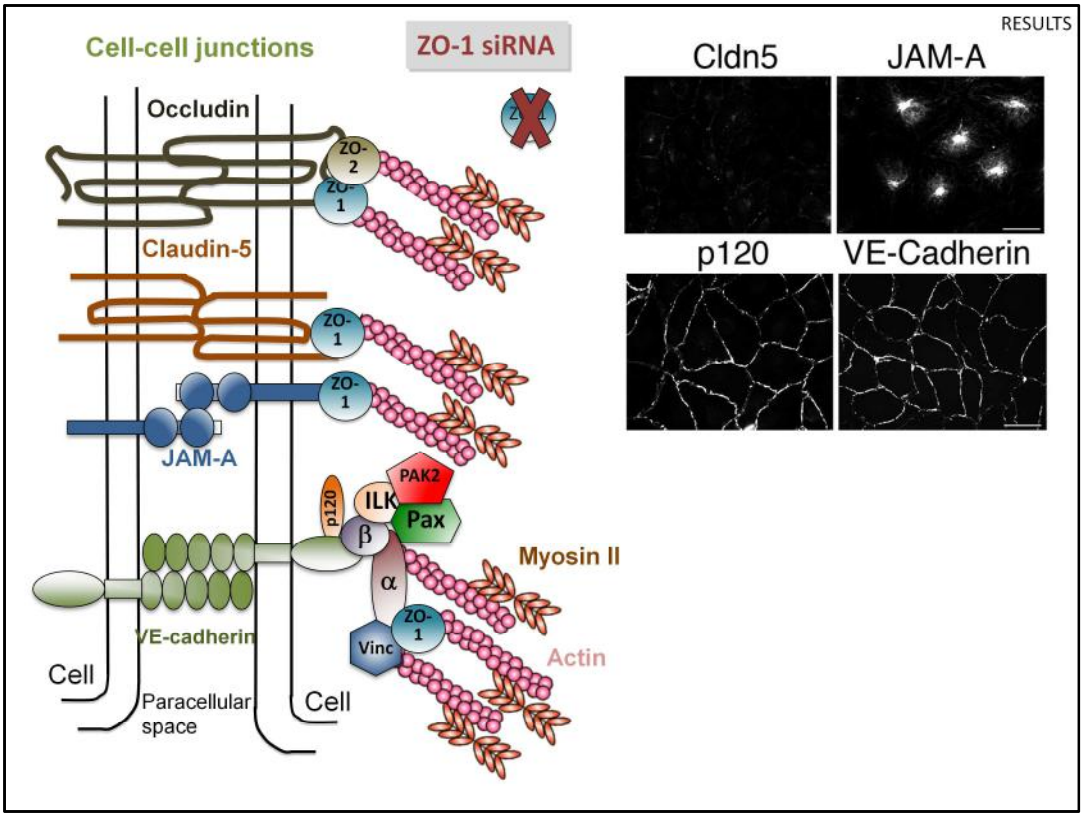
Does ZO-1 downregulation reduce tension on junctional complex?

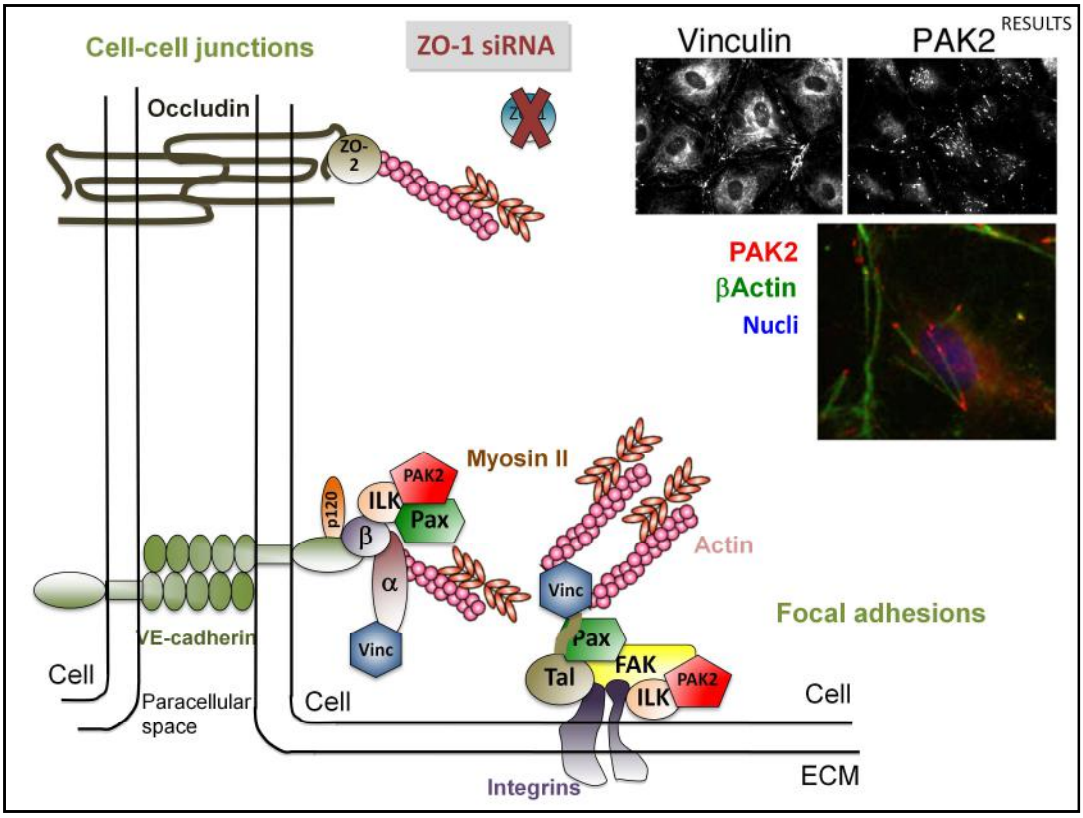


Biosenser VE-cadherin-TS

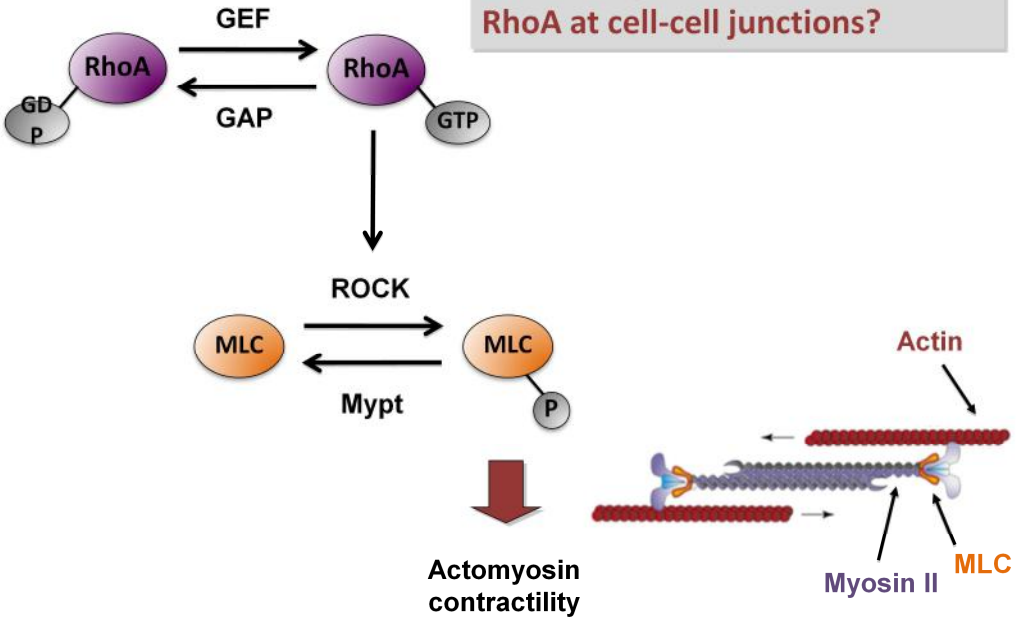



Cell-cell junctions
Is ZO-1 required for the recruitment of junctional proteins and mechanotransducers to cell-cell contacts?
Occludin
ZO-2
ZO-1
Claudin-5
ZO-1
ZO-1
JAM-A
PAK2
ILK
Pax
p120
β
α
Myosin II
VE-cadherin
ZO-1
Vinc
Actin
Cell
Paracellular space
Cell
p120
VE-Cadherin
Vinculin
PAK2

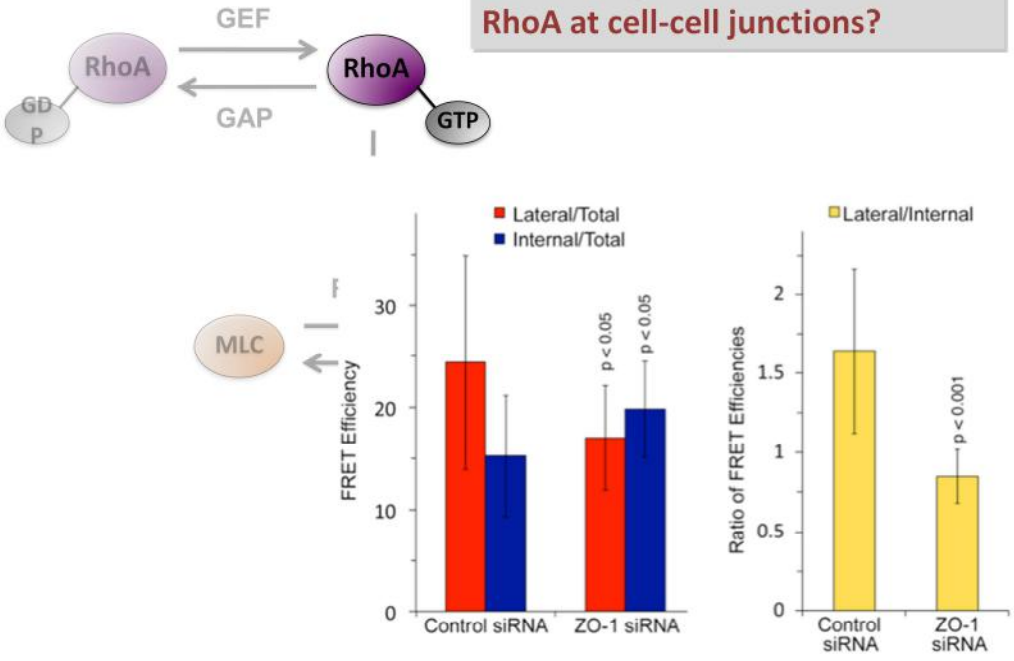

RESULTS
Cell-cell junctions
ZO-1 siRNA
Occludin
ZO-2
ZO-1
Claudin-5
ZO-1
ZO-1
JAM-A
p120
PAK2
ILK
Pax
β
α
Myosin II
VE-cadherin
ZO-1
Vinc
Actin
Cell
Paracellular space
Cell
Cldn5
JAM-A
p120
VE-Cadherin


Cell-cell junctions
ZO-1 siRNA
RESULTS
Vinculin
PAK2
Occludin
ZO-2
PAK2
βActin
Nucli
Myosin II
PAK2
ILK
Pax
p120
β
α
Vinc
Actin
Vinc
Focal adhesions
VE-cadherin
Pax
FAK
Tal
PAK2
ILK
Cell
Paracellular space
Cell
Cell
ECM
Integrins

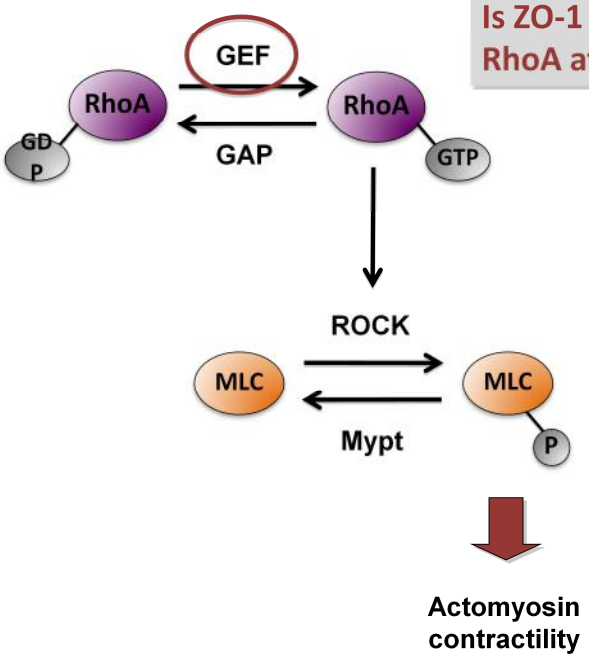
Is ZO-1 required for localize active RhoA at cell-cell junctions?

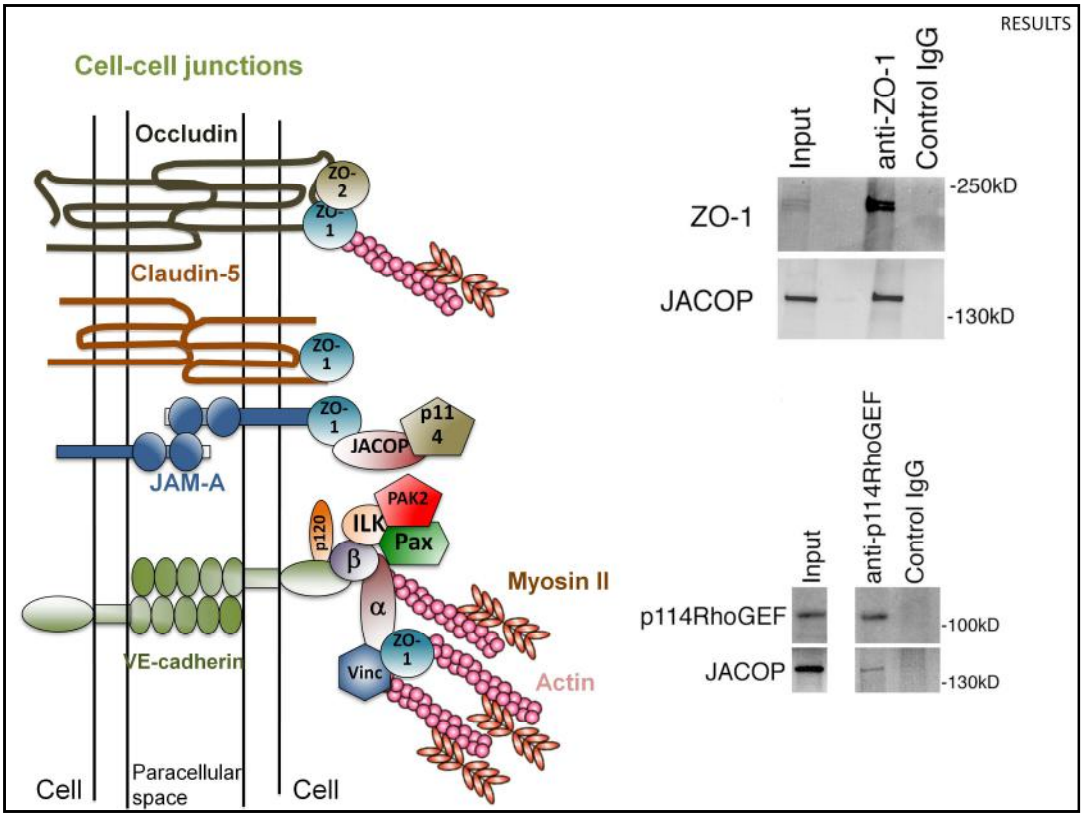


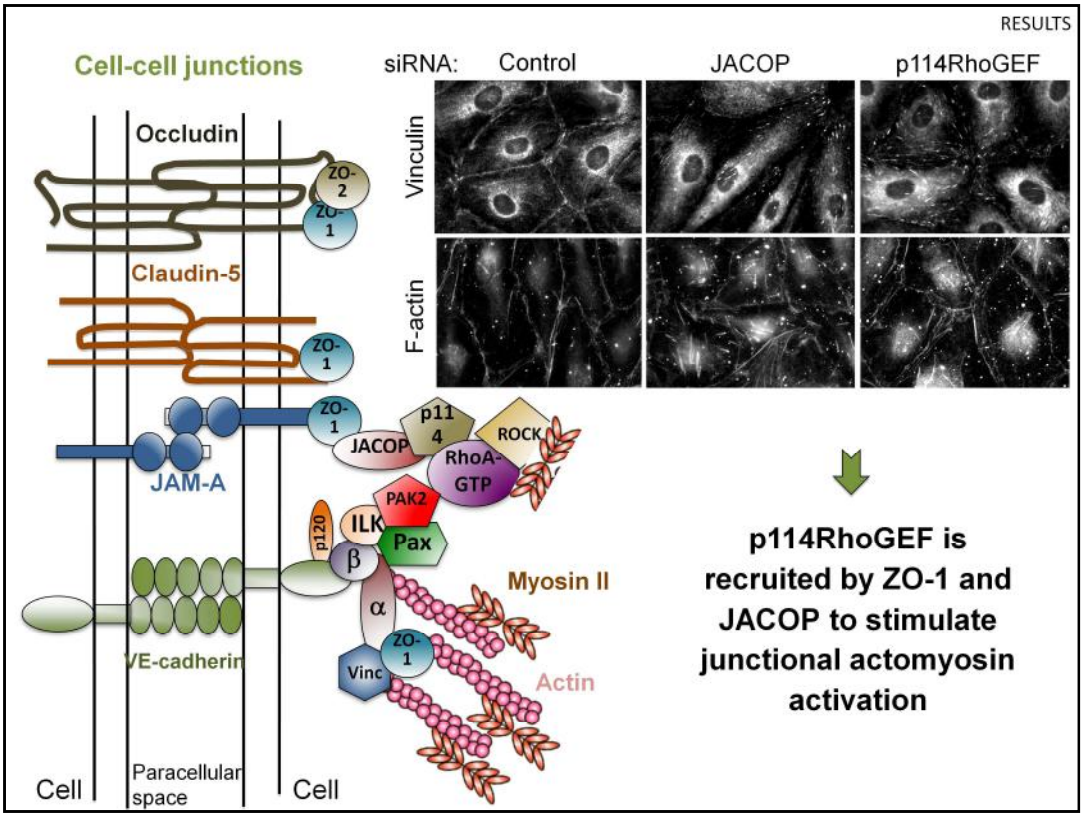
Is ZO-1 required for localize active RhoA at cell-cell junctions?

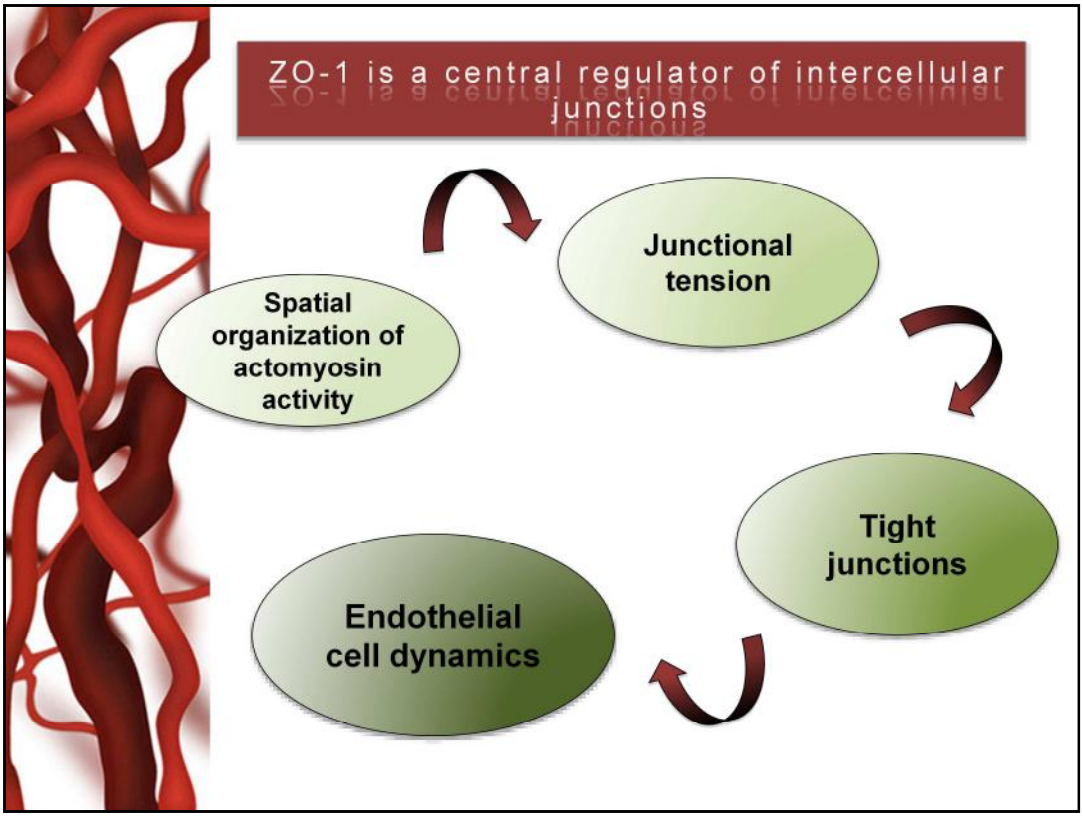


Is ZO-1 required for localize active RhoA at cell-cell junctions?




RESULTS
Cell-cell junctions
Occludin
ZO-2
ZO-1
Claudin-5
ZO-1
ZO-1
p114
JACOP
JAM-A
PAK2
ILK
Pax
p120
β
α
Myosin II
VE-cadherin
ZO-1
Vinc
Actin
Cell
Paracellular space
Cell
Input
anti-ZO-1
Control IgG
ZO-1
JACOP
-250kD
-130kD
Input
anti-p114RhoGEF
Control IgG
p114RhoGEF
JACOP
-100kD
-130kD


RESULTS
Cell-cell junctions
siRNA:
Control
JACOP
p114RhoGEF
Vinculin
F-actin
Occludin
ZO-2
ZO-1
Claudin-5
ZO-1
ZO-1
JAM-A
p114
ROCK
JACOP
RhoA-GTP
PAK2
ILK
Pax
p120
β
α
Myosin II
Actin
VE-cadherin
ZO-1
Vinc
Cell
Paracellular space
Cell
p114RhoGEF is recruited by ZO-1 and JACOP to stimulate junctional actomyosin activation


ZO-1 is a central regulator of intercellular junctions
Junctional tension
Spatial organization of actomyosin activity
Tight junctions
Endothelial cell dynamics

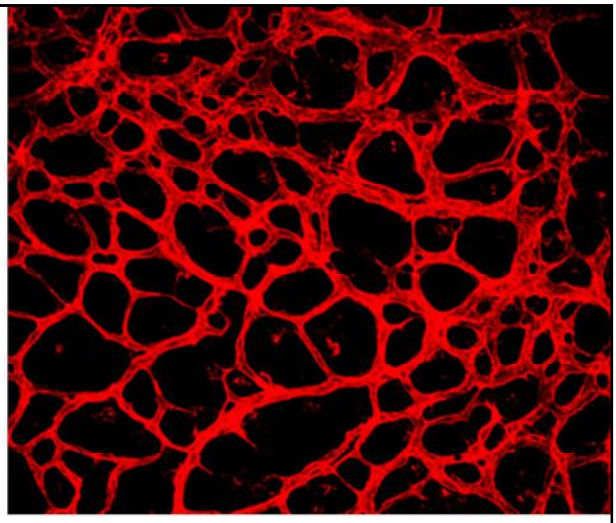
Acknowledgments

Institute of Ophthalmology, UCL
Cell Biology department

Maria S. Balda
Karl Matter

Ahmed Elbediwy
Elena Sanchez-Heras
Emily Steed
Mei Nei
Elisa Vitiello
Kasia Wisniewska
Ceniz Zihni

Mansoor Ahsan
Vivian D'Arcy
Stephen J. Terry



Collaborators

Imperial College London
Anna M. Randi
Graeme M. Birdsey

Funding

Medical Research Council
Wellcome Trust