BMP9 is a potent activator of ALK1 signalling in endothelial cells

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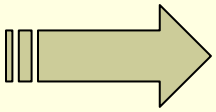
ALK1 is an orphan type I receptor

- ALK1ca phosphorylates Smad1, 5 and 8
- TGFß1 and 3 have been shown to bind to ALK1 and phosphorylate Smad1, 5 and 8 in certain cells
- TGFß1 and 3 binding to ALK1 need the presence of ALK5
- TGFß1 and 3 affinities for ALK1 are much lower than for ALK5
- TGFß1 does not activate BRE (BMP Response Element) of Id1 in endothelial cells or 3T3 fibroblasts

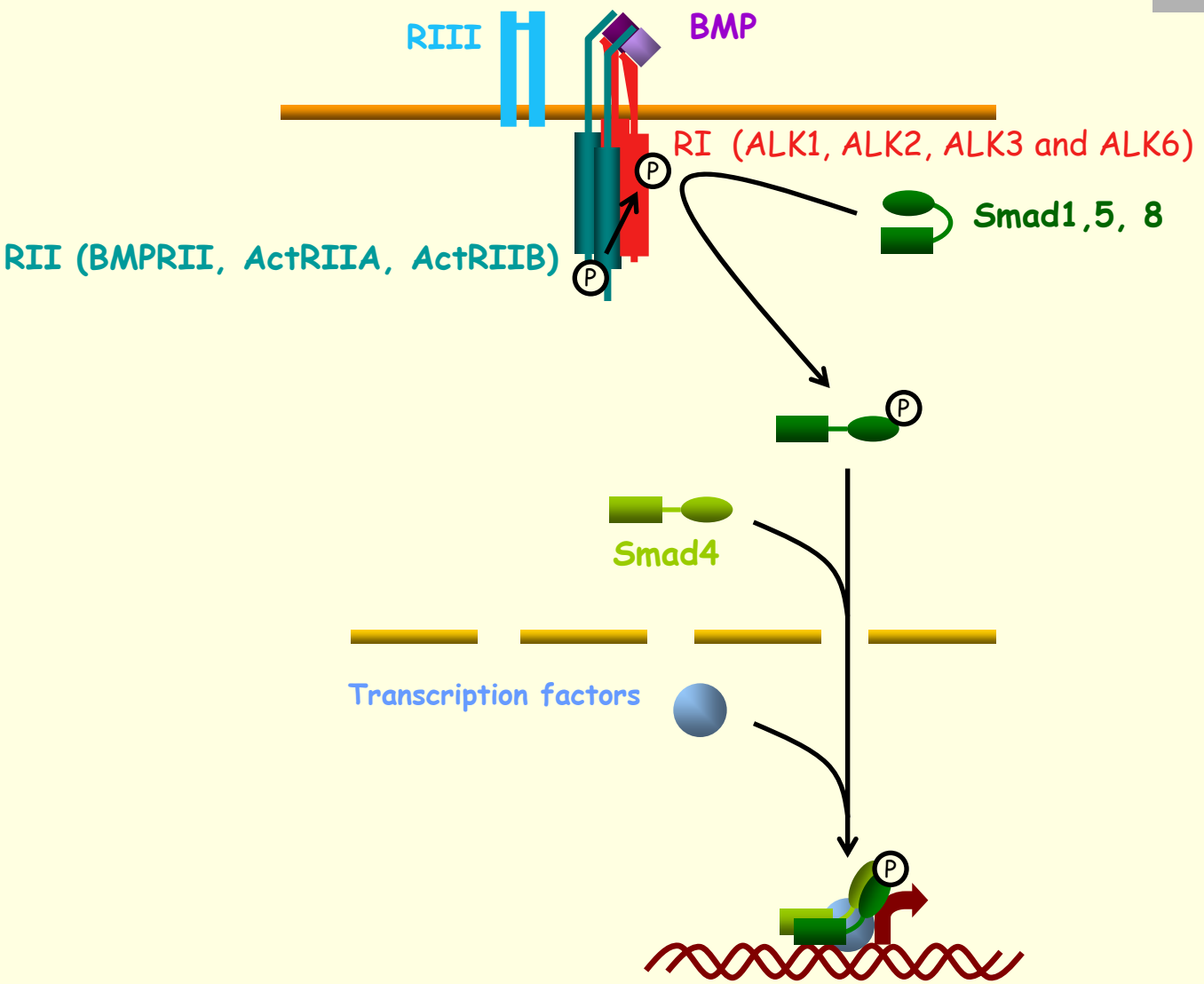
There is another ligand for ALK1



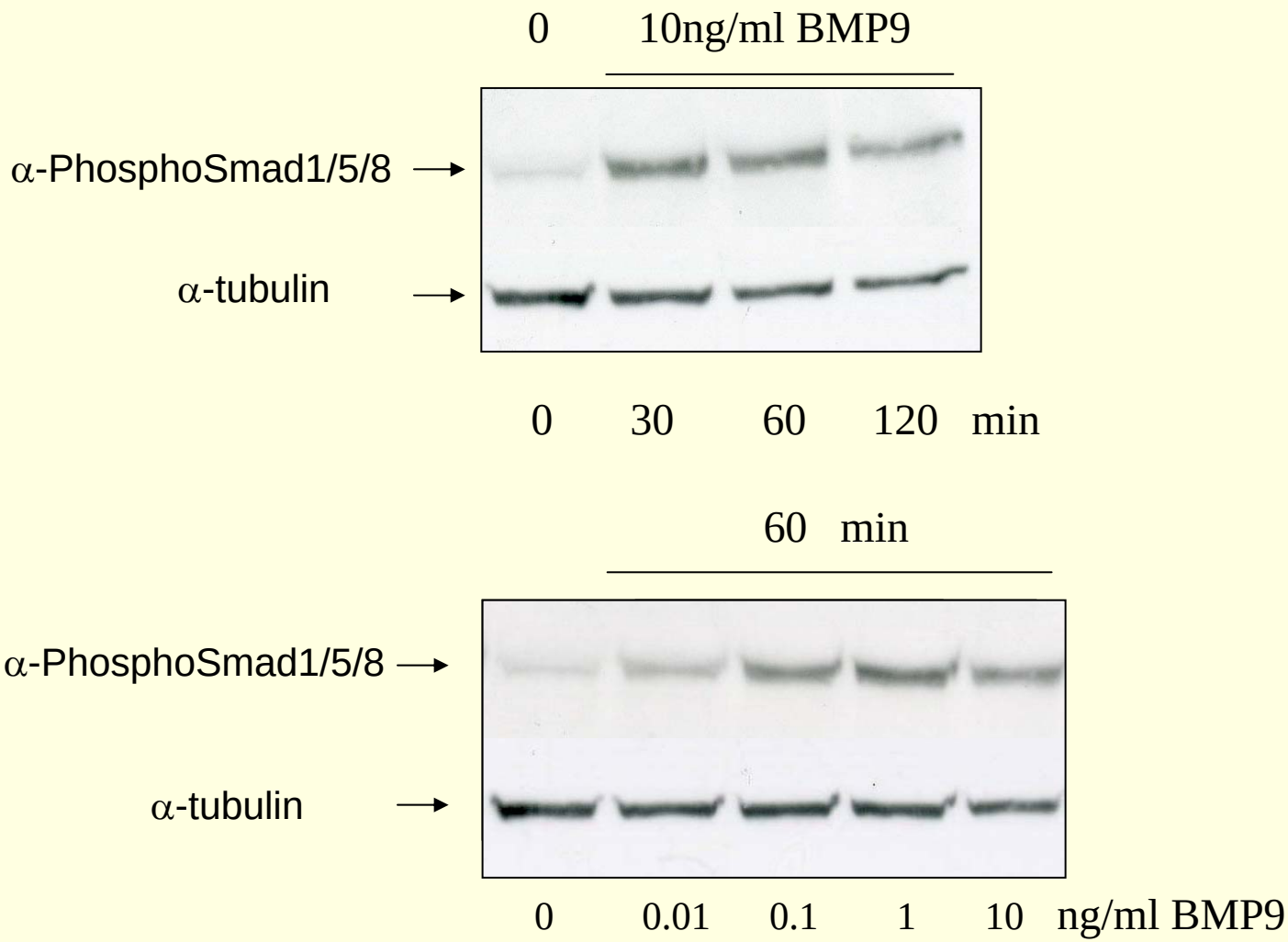
ALK1 was identified as a potential receptor for BMP9 (BIAcore) and soluble ALK1 blocked BMP9 activity in myoblasts (Brown, J. Biol. Chem., 2005)

Is BMP9 a ligand for ALK1 in endothelial cells ?

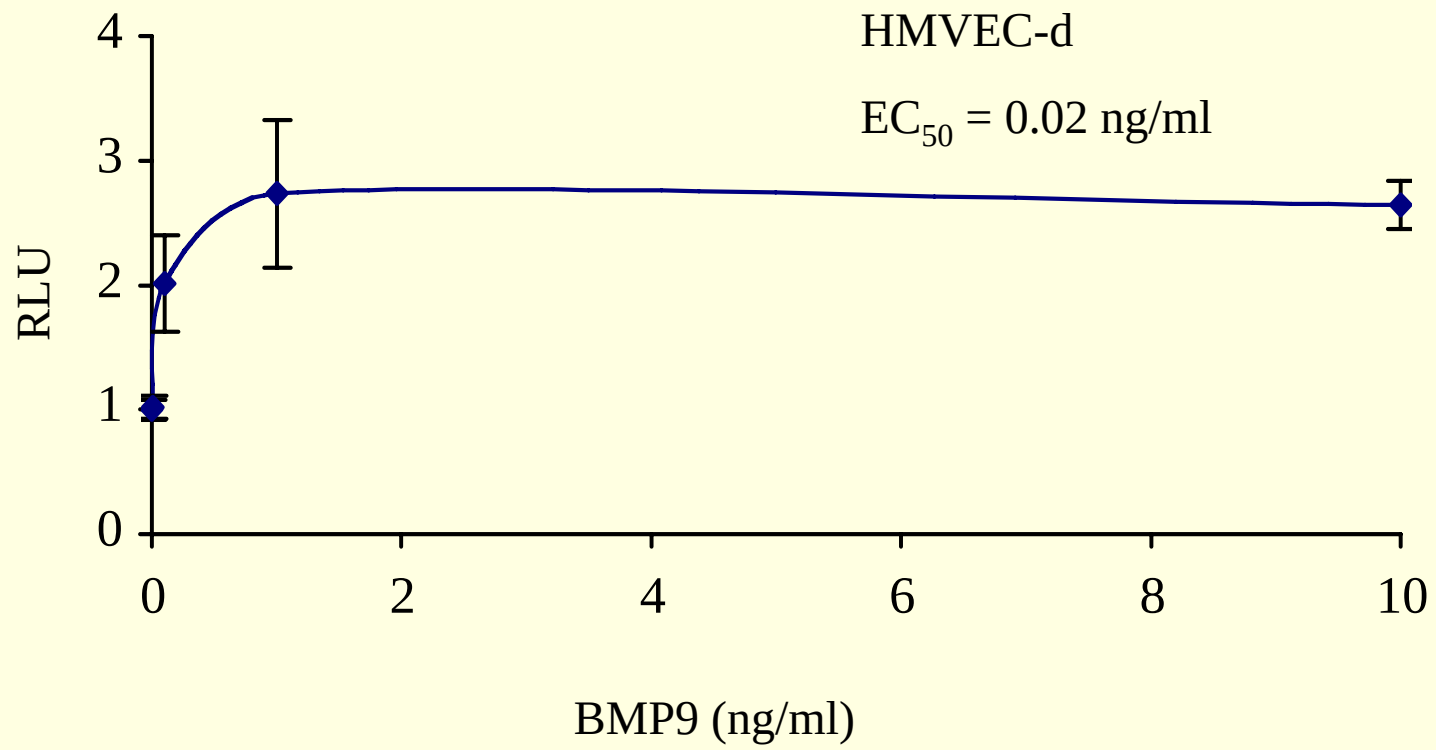
BMP signal transduction pathway



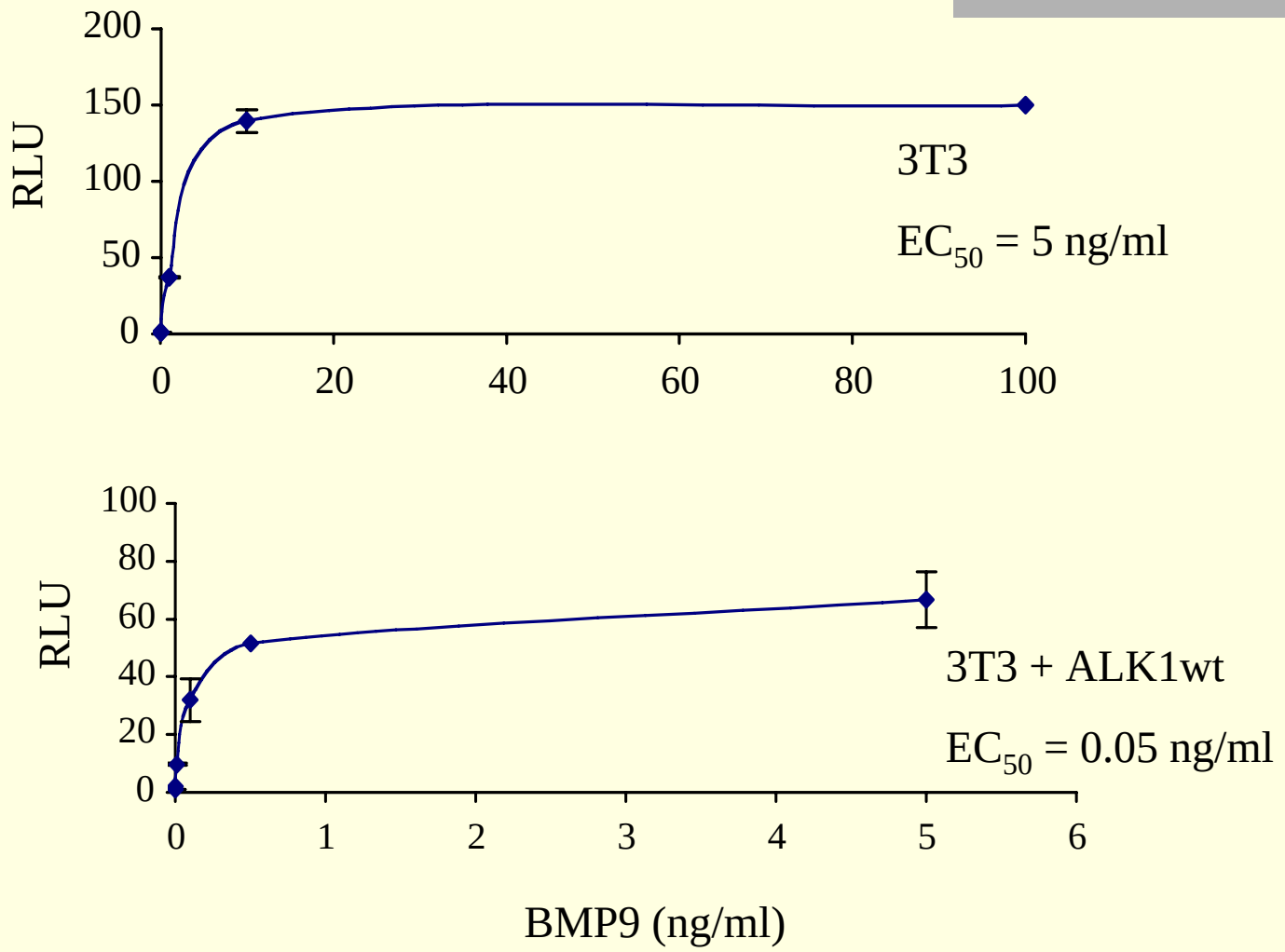
BMP9 phosphorylates Smad1/5/8 in Human microvascular endothelial cells from the dermis (HMVEC-d)



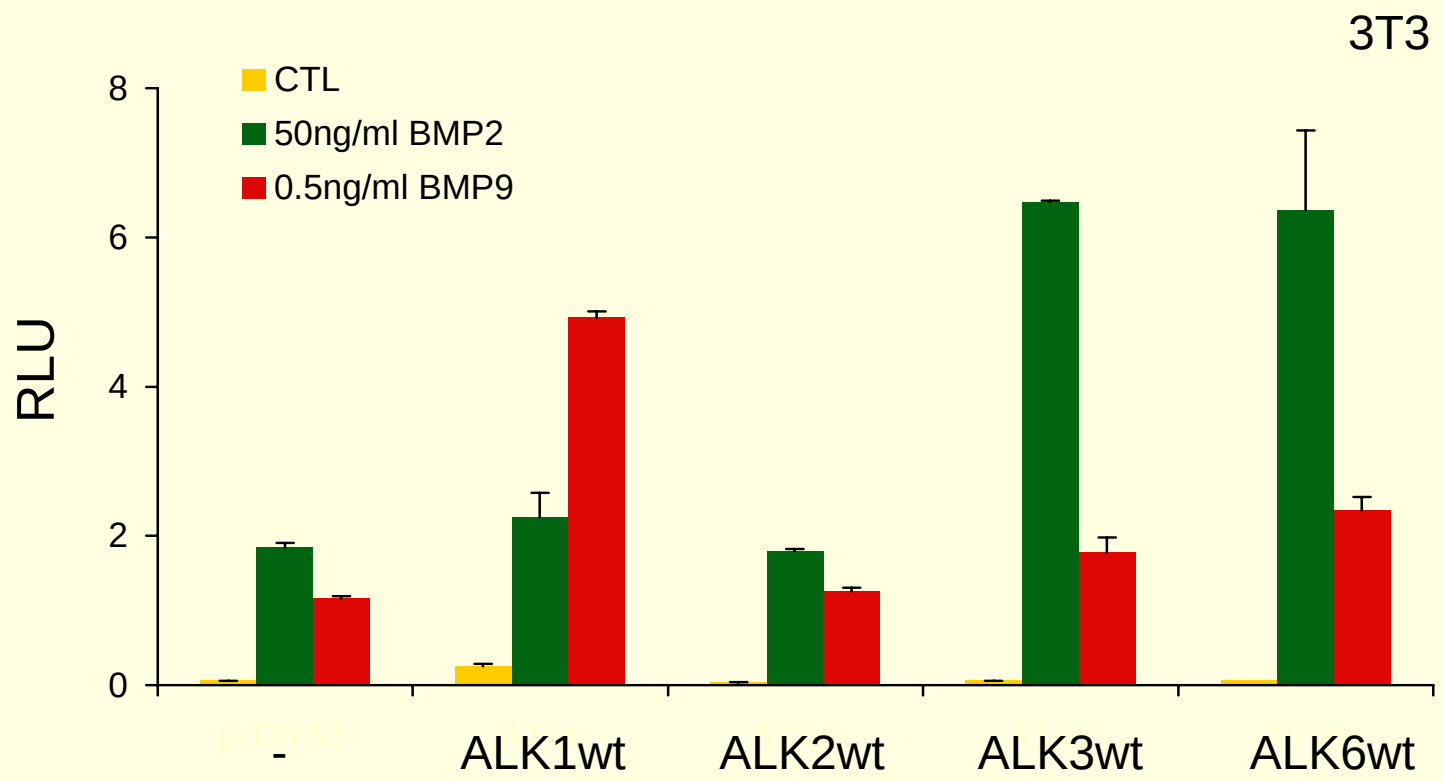
BMP9 activates BMP Response Element (BRE) of Id1 in HMVEC-d



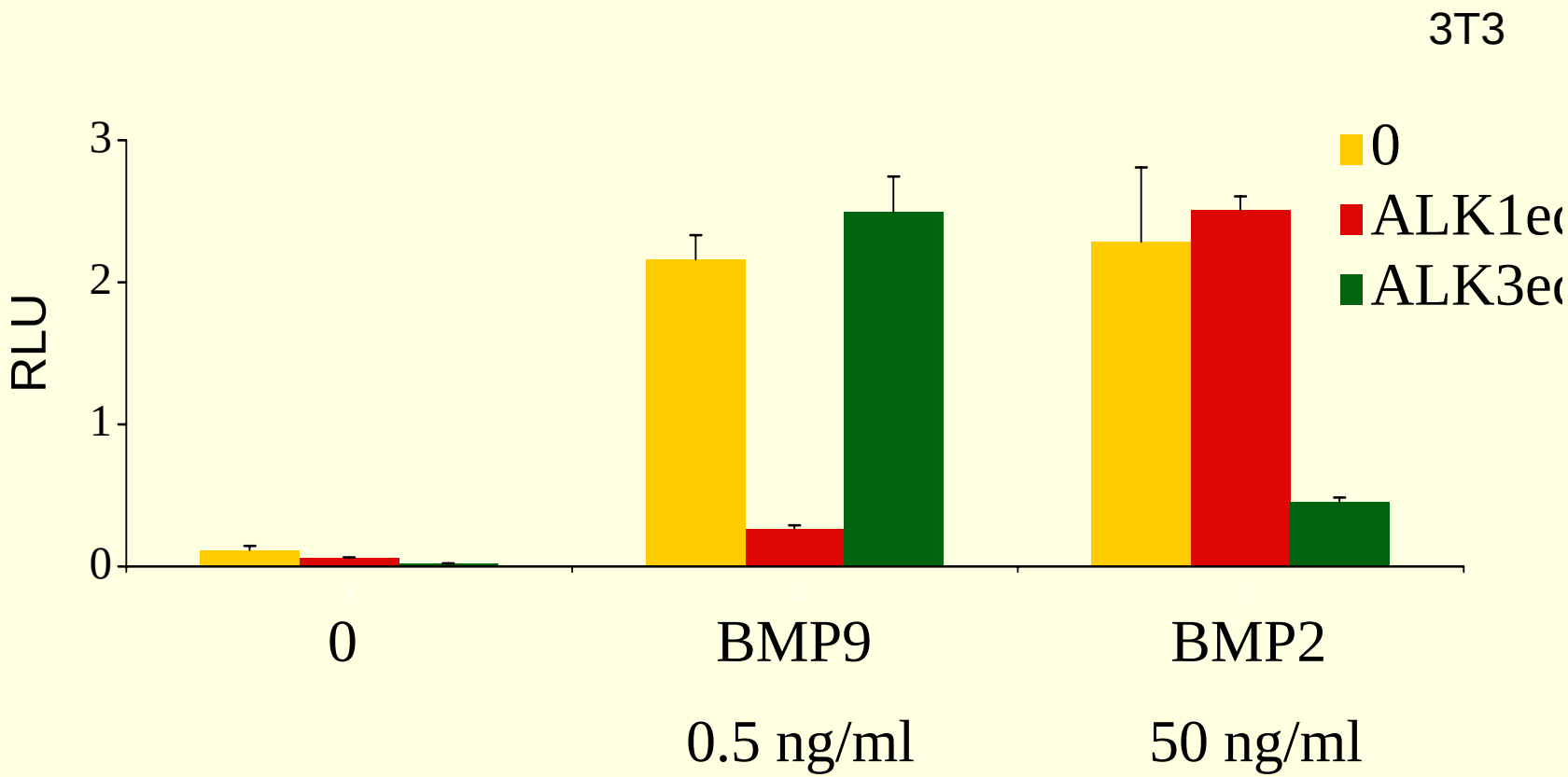
BMP9 activation of BRE in 3T3 cells is increased by ALK1wt expression



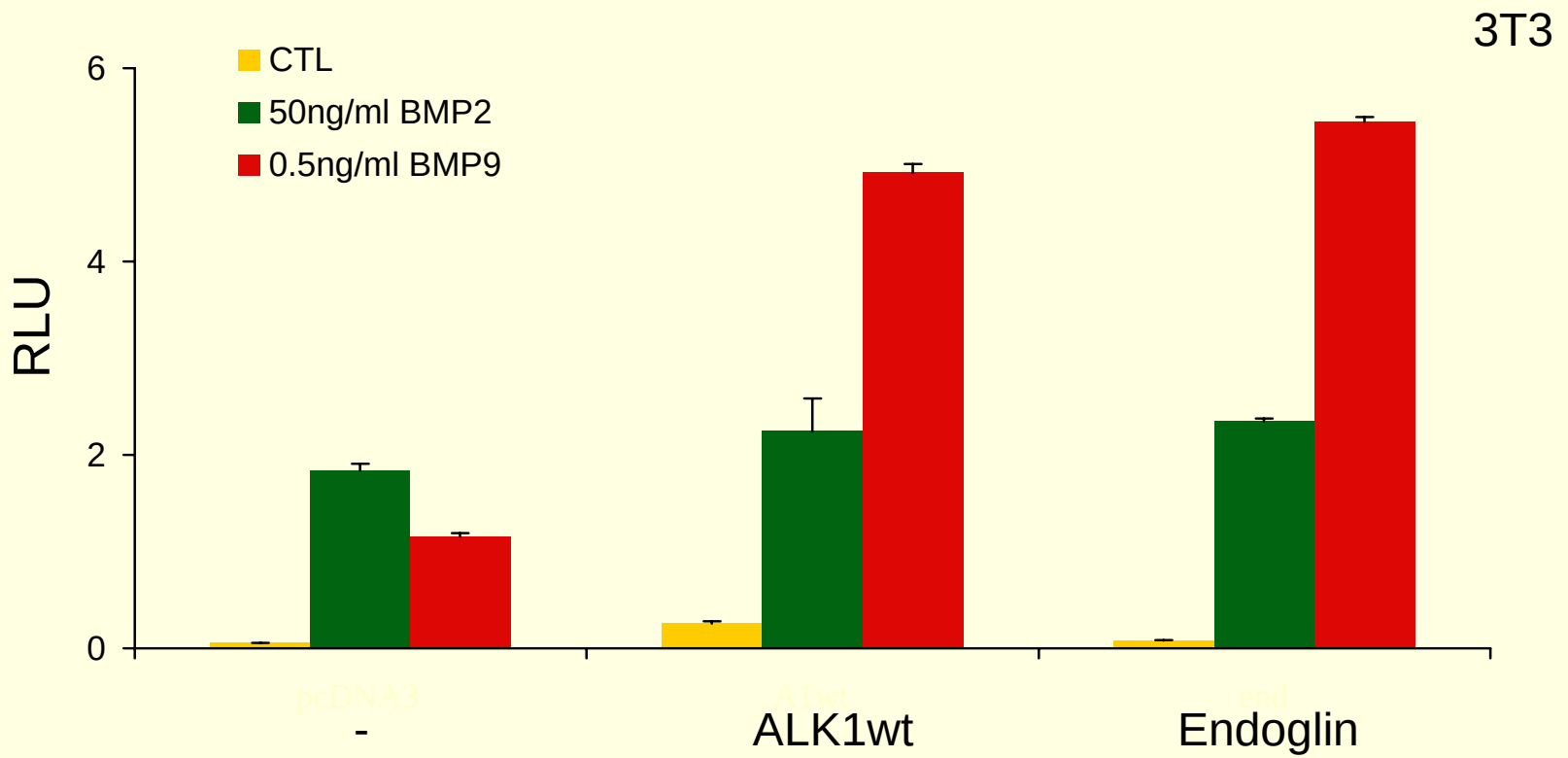
ALK1 is the only type 1 receptor for BMP9



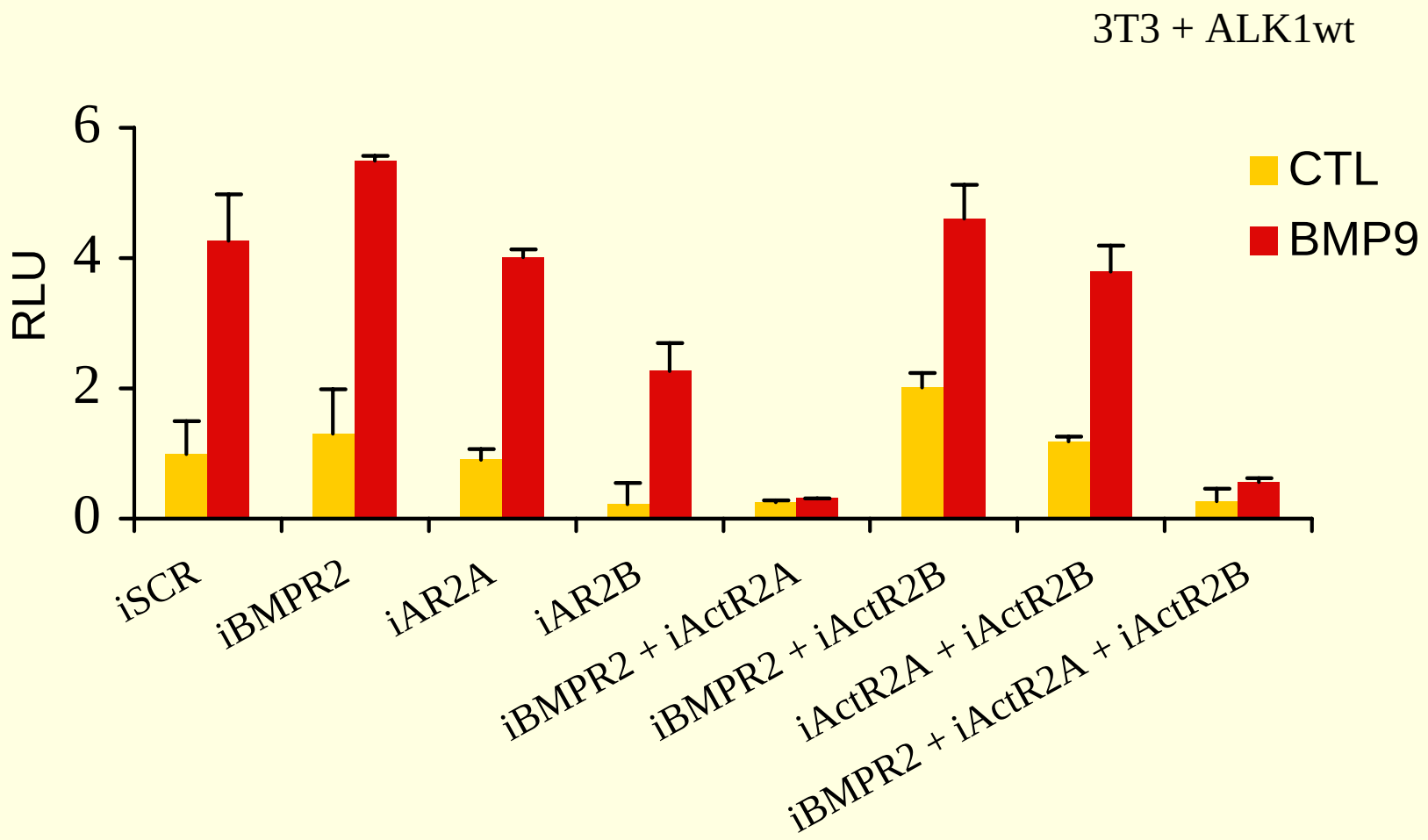
BMP9 activation is inhibited by soluble ALK1 ectodomain but not by soluble ALK3 ectodomain (20 fold m/m)



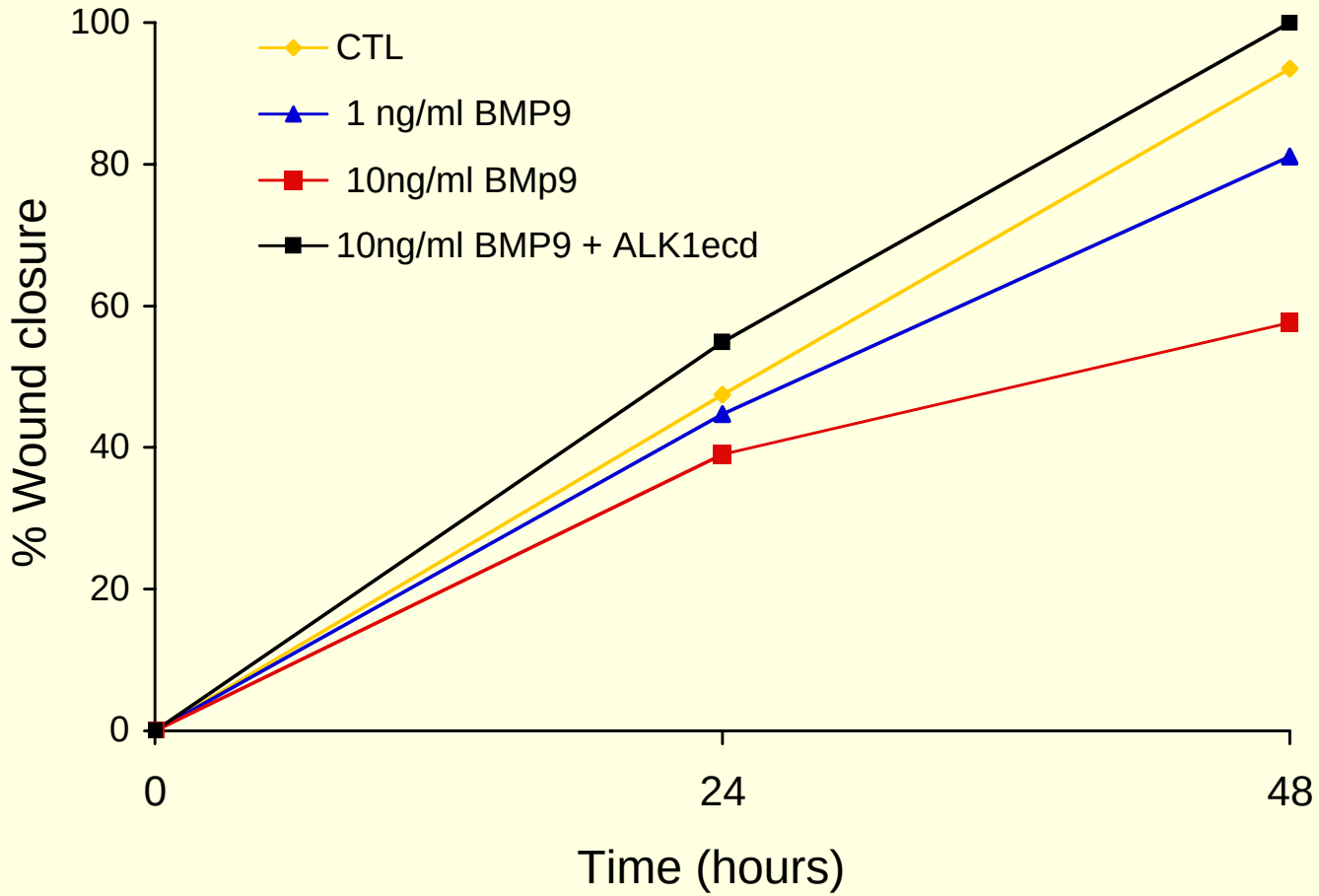
Endoglin increases BMP9 response



BMPRII and ActRIIA are implicated in BMP9 signalling

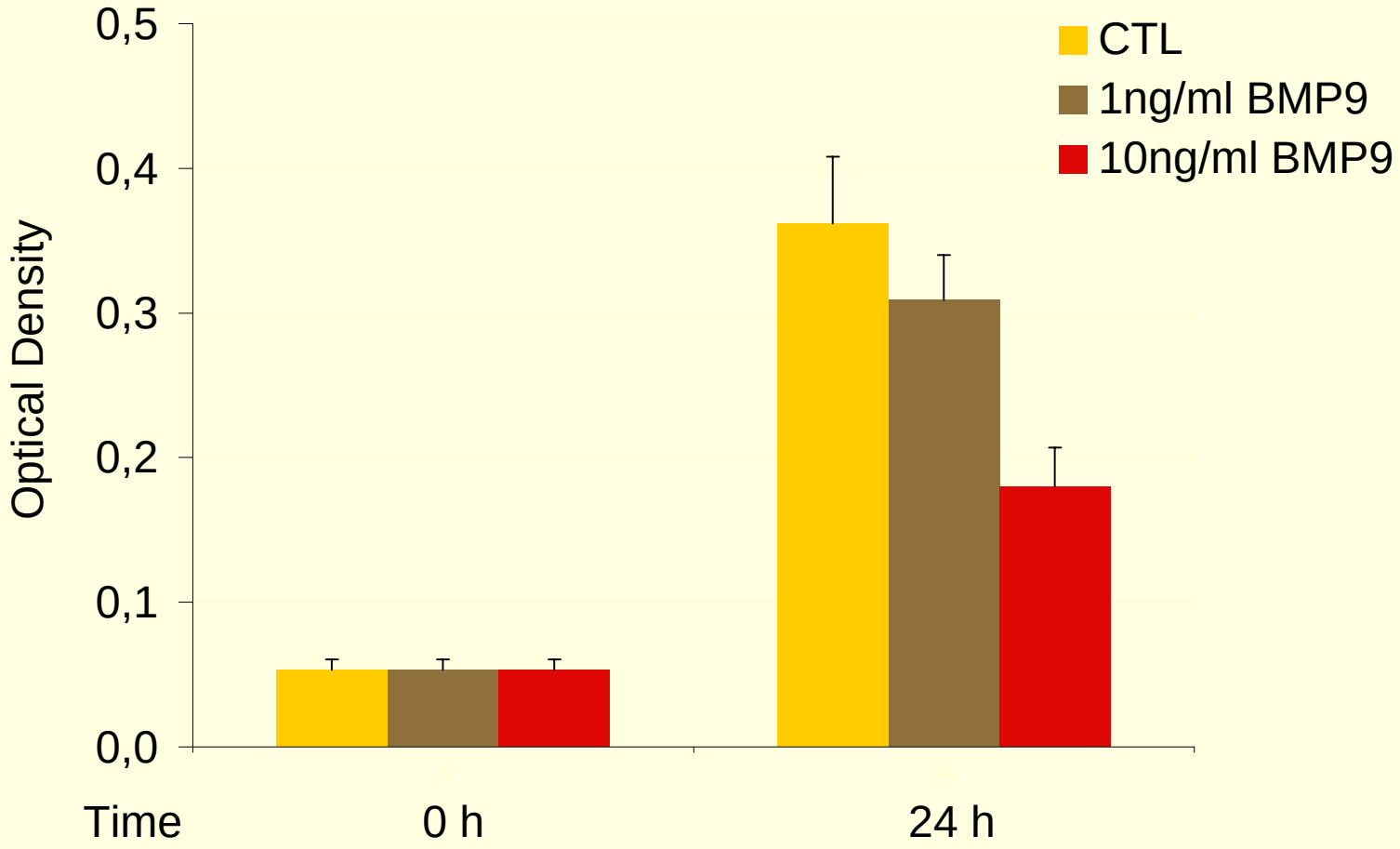


BMP9 inhibits HMVEC-d migration in the wound closure assay

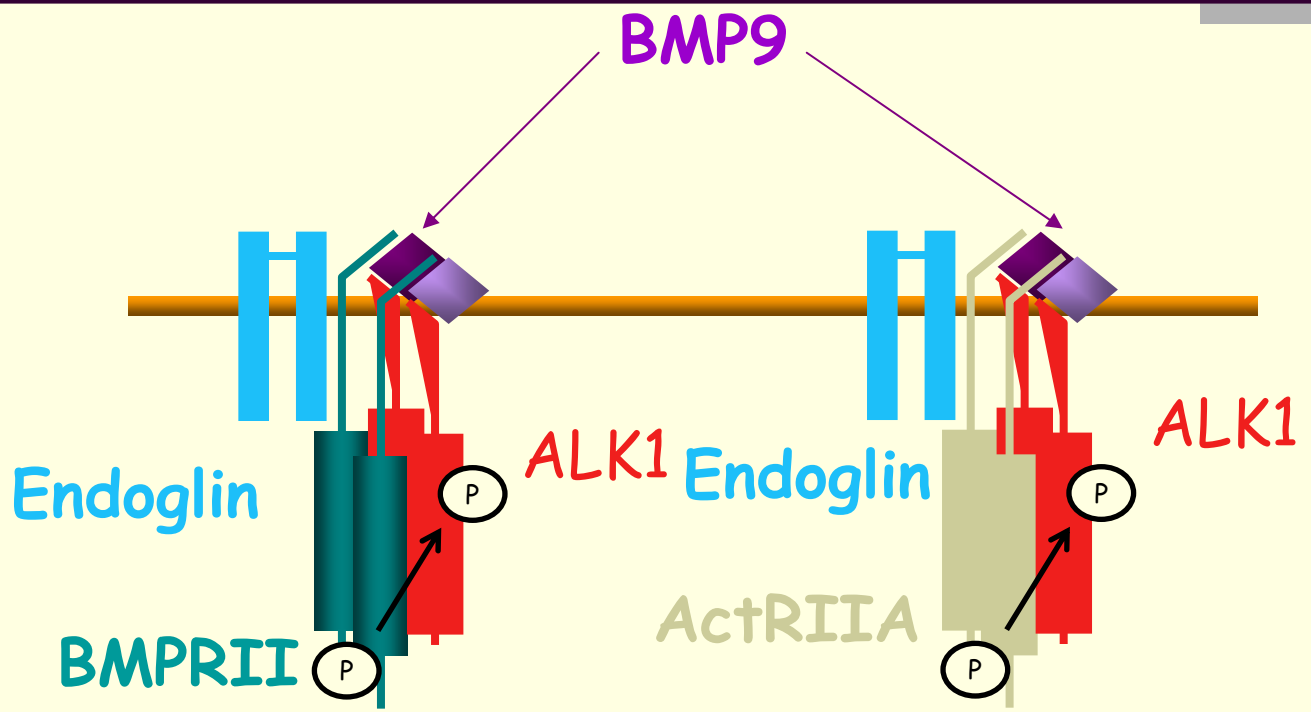


BMP9 inhibits HMVEC-d proliferation

(WST-1, colorimetric assay)



BMP9 is a ligand for ALK1 in endothelial cells



Inhibition of endothelial cell migration

Inhibition of endothelial cell proliferation

BMP9 and HHT ?

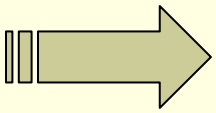
- BMP9 is predominantly expressed in the liver
- High frequency of hepatic AVM in HHT2

⇨ Is BMP9 the candidate gene for HHT3 ?

⇨ Is BMP9 present in the serum, could it be a marker for HHT ?

Conclusion

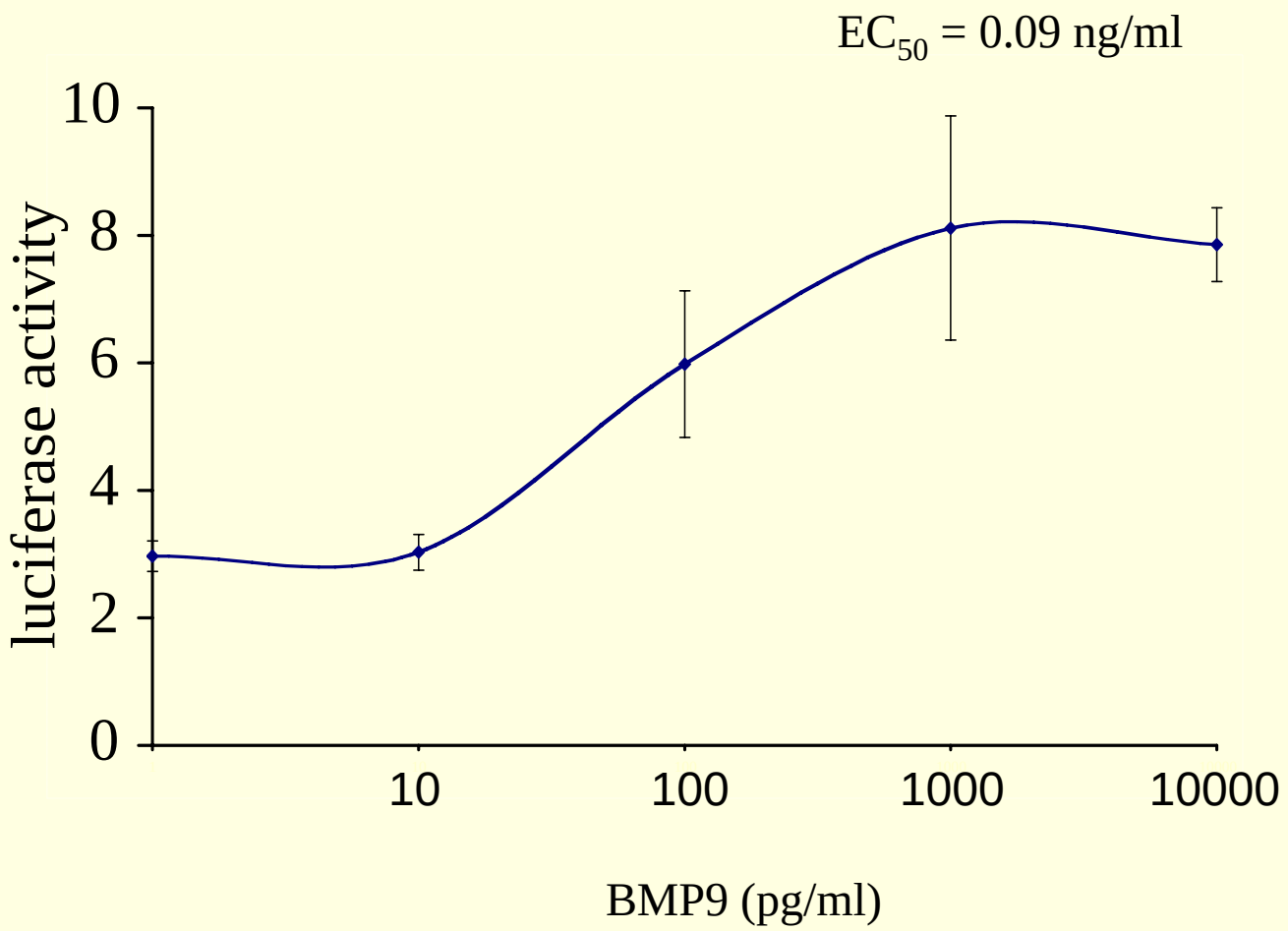
- BMP9 phosphorylates Smad1,5,8 in endothelial cells
- BMP9 binds specifically to the type I receptor : ALK1
- BMPRII and ActRII can both transduce BMP9 signalling
- Endoglin increases BMP9 signalling
- BMP9 inhibits endothelial cell migration
- BMP9 inhibits endothelial cell proliferation
- BMP9 mimics ALK1ca inhibitory effects on endothelial cells



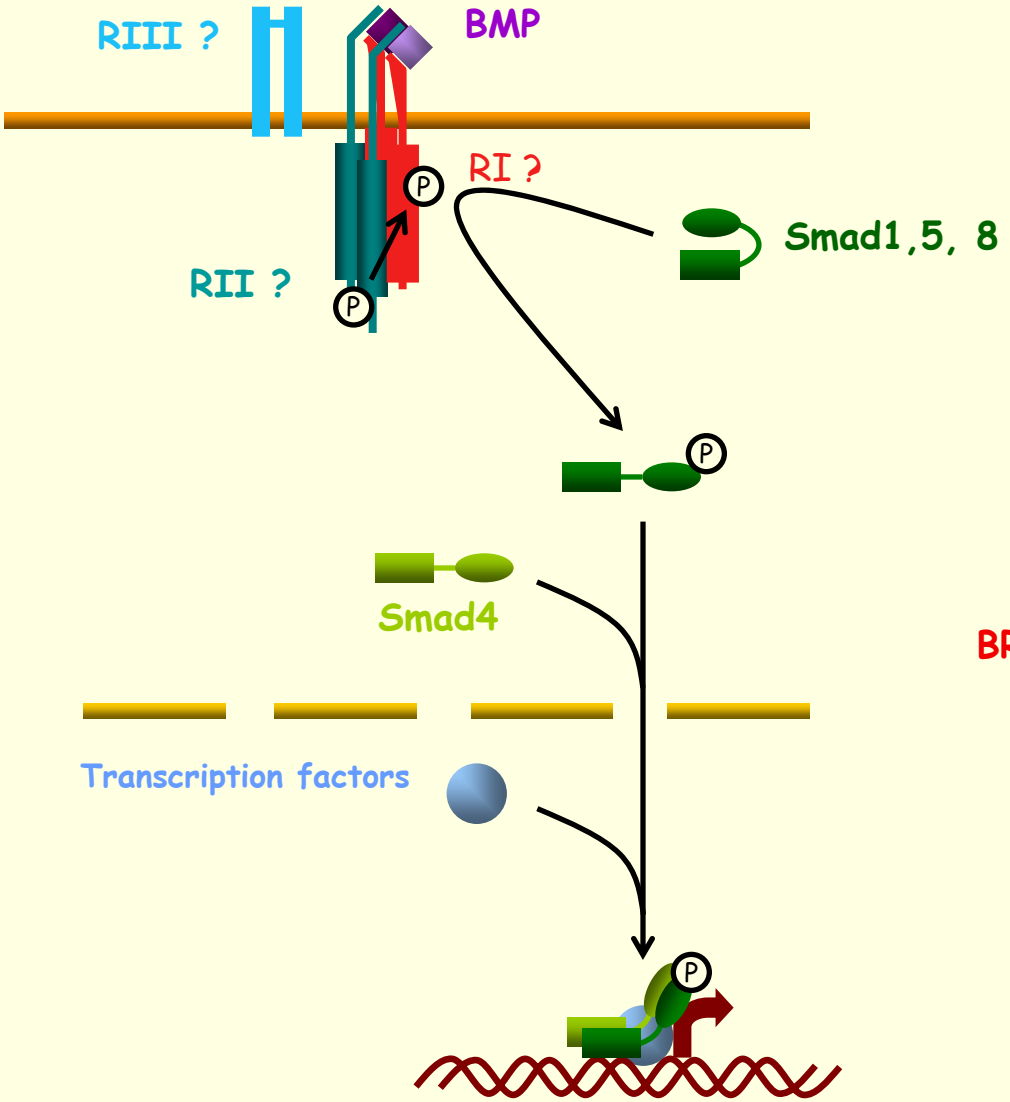
BMP9 is a ligand for ALK1 in endothelial cells

Role of BMP9 in HHT ?

BMP9 activates BMP Response Element (BRE) of Id1 in HMVEC-d



BMP signal transduction pathway



Test

HMVEC-d

3T3 Fibroblastes

Transfected

Firefly luciferase

BRE (Bone morphogenic Response Element)

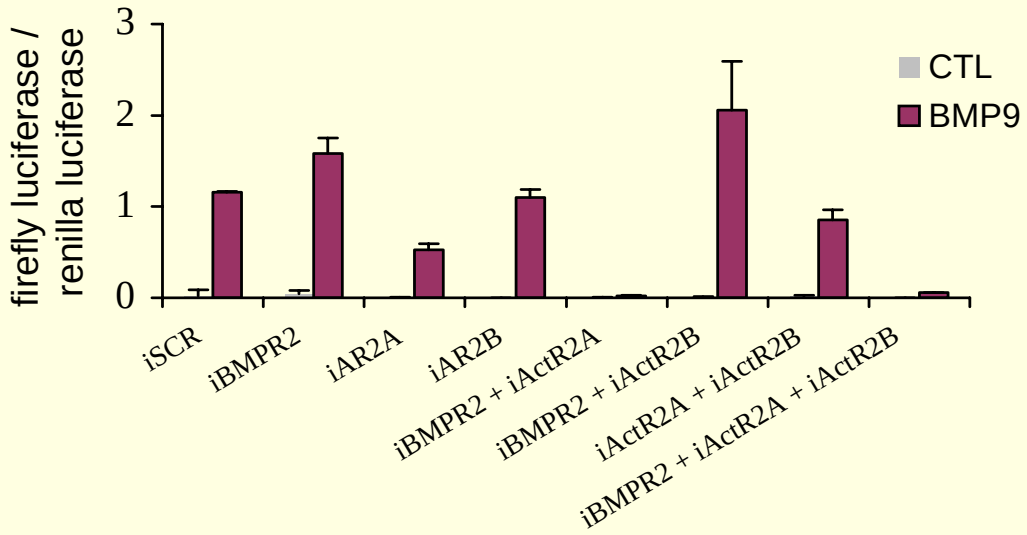
Renilla luciferase

normalization

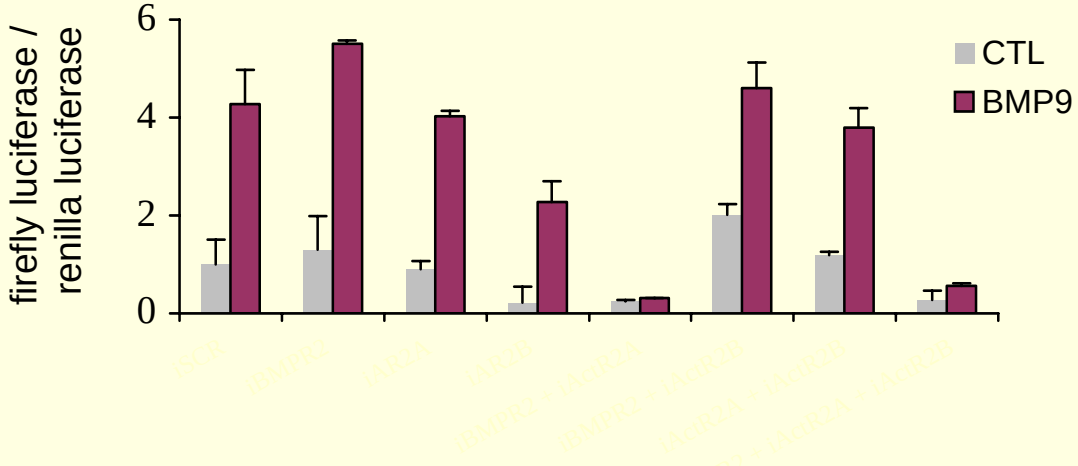
± ALK1wt or another receptor

Which type 2 receptor for BMP9 ?

BMPRII and ActRIIA are necessary for BMP9 activity

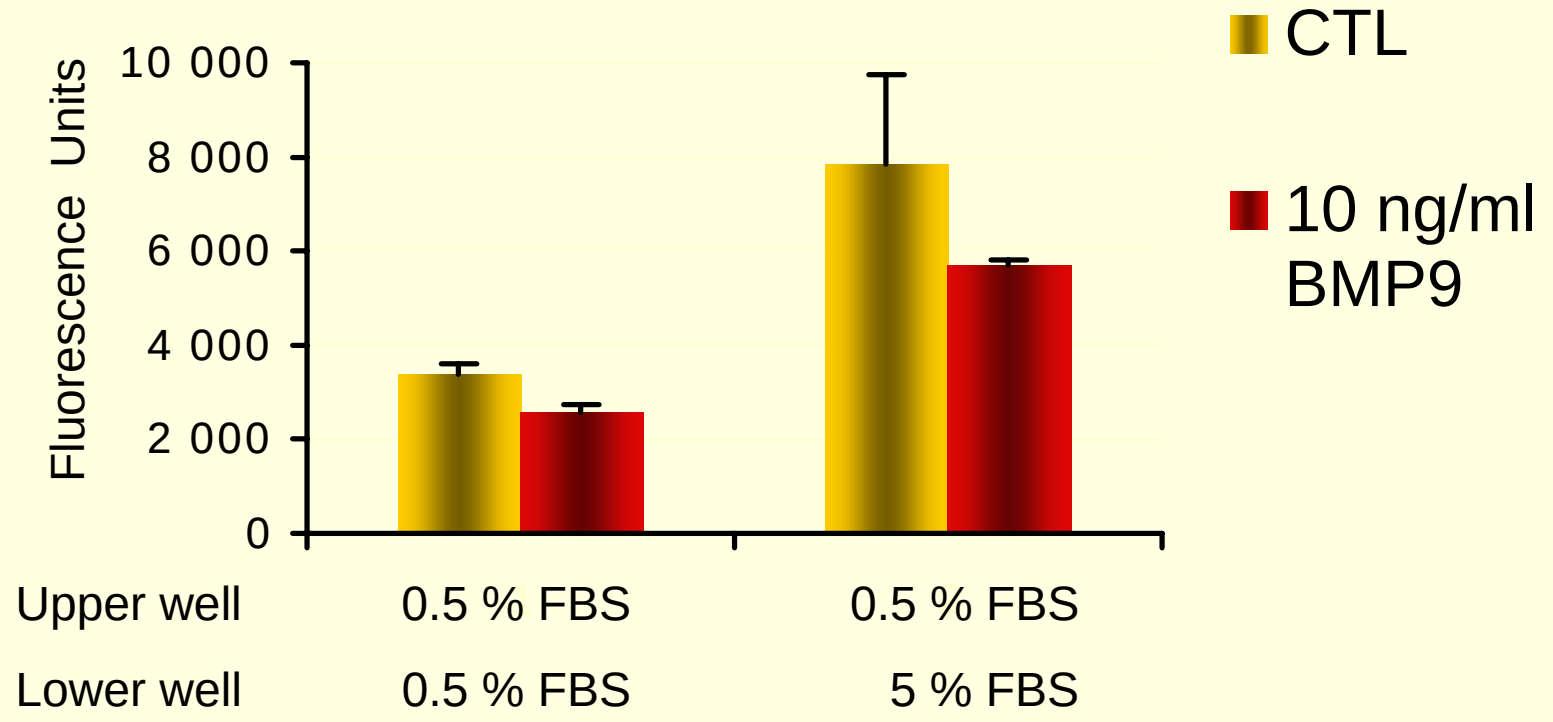


3T3

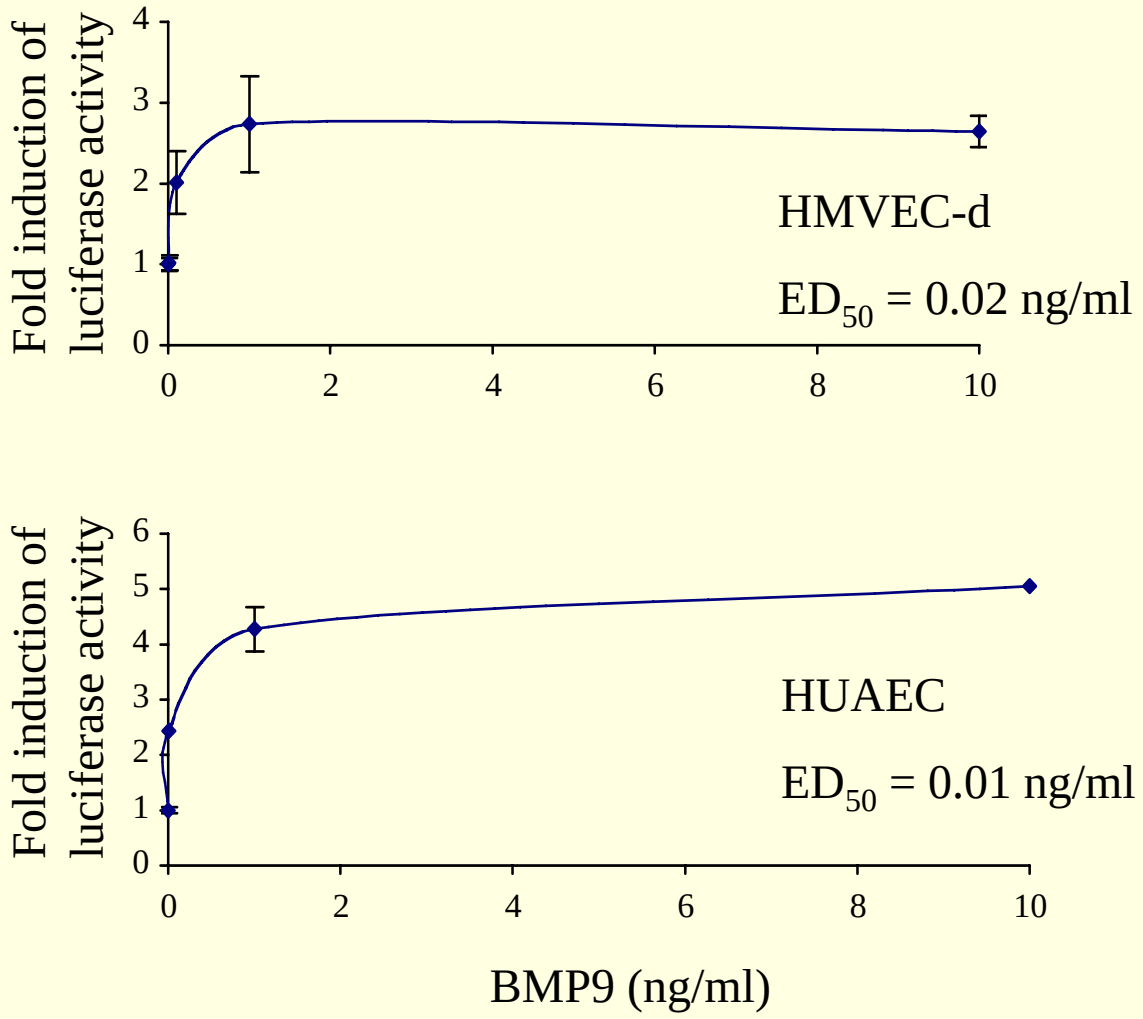


3T3 + ALK1wt

BMP9 inhibits transwell migration of HMVEC-d



BMP9 activates BMP Response Element (BRE) of Id1 in human endothelial cells



BMP9 activation of BRE in 3T3 cells is increased by ALK1wt expression