

Cumulin: a new oocyte-secreted factor of potential use as an IVM media additive

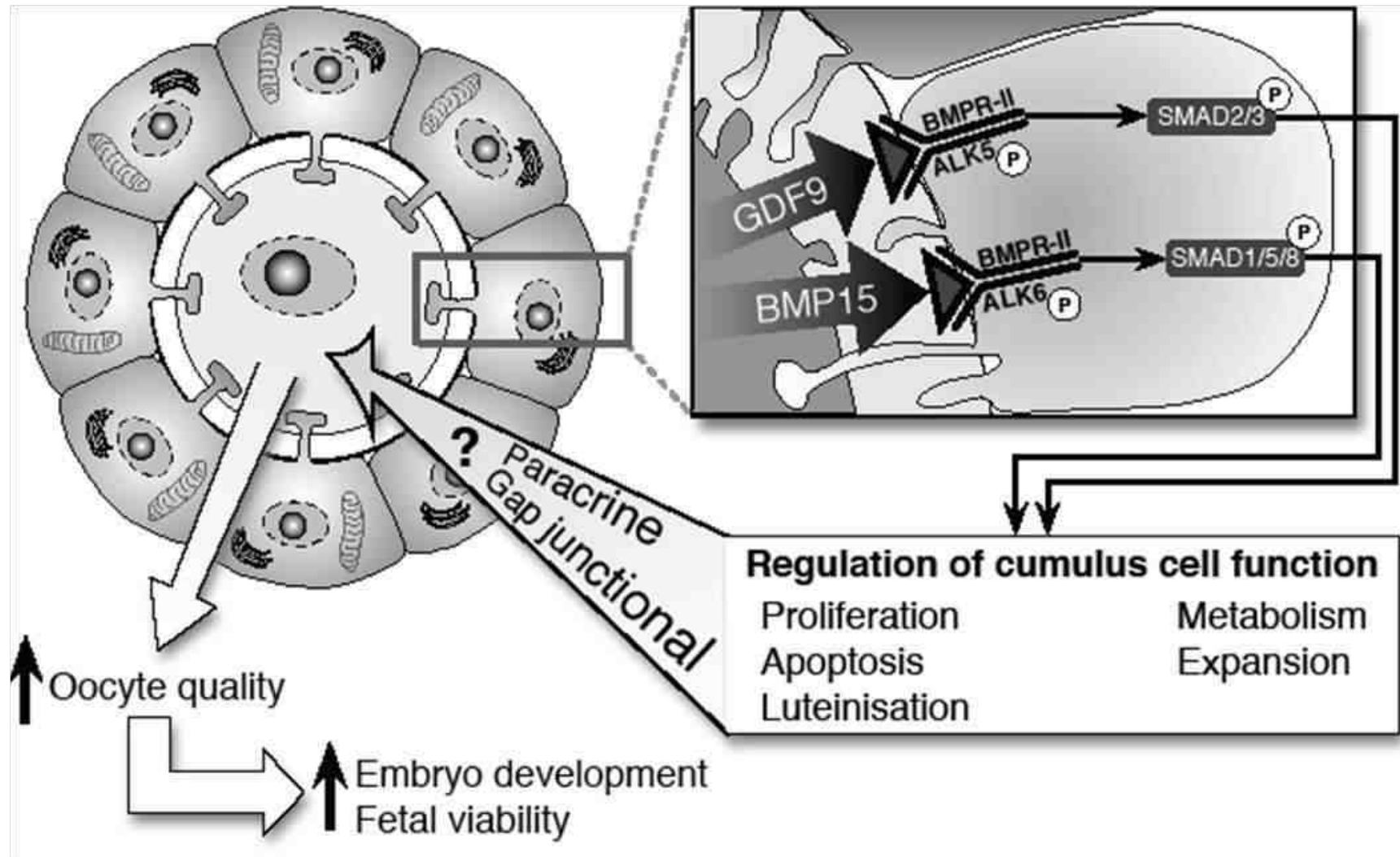
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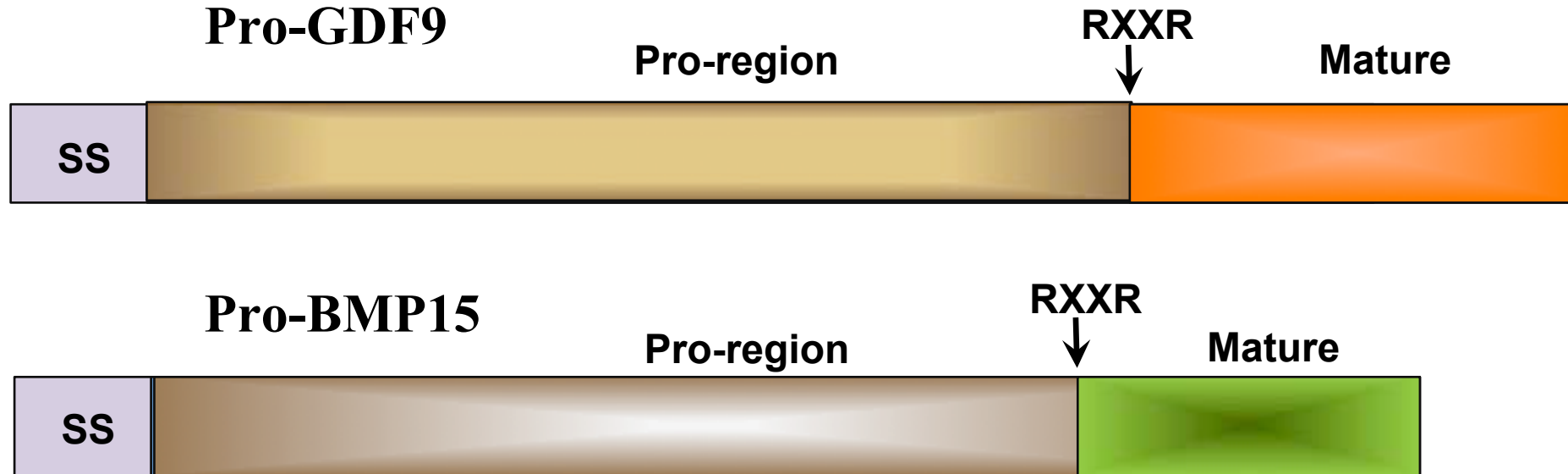
**Robinson Research Institute,
School of Medicine, University of Adelaide, Australia.**



Oocyte-Secreted Factors GDF9 & BMP15 Regulate Granulosa Cells and Oocyte Developmental Competence



GDF9 and BMP15 as TGF- β family members



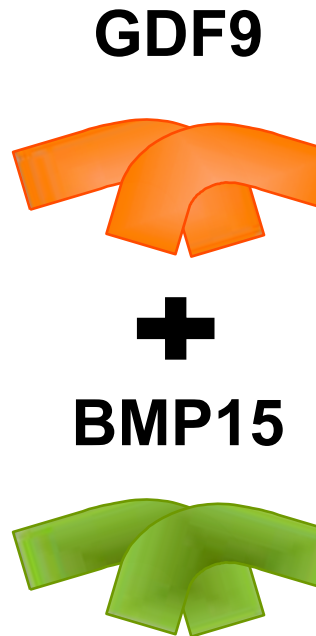
- TGF- β family mature regions normally form covalent dimers
- GDF9 & BMP15 lack 4th Cys of the 7 conserved Cys usually found in the mature region, hence they form **non-covalent dimers**



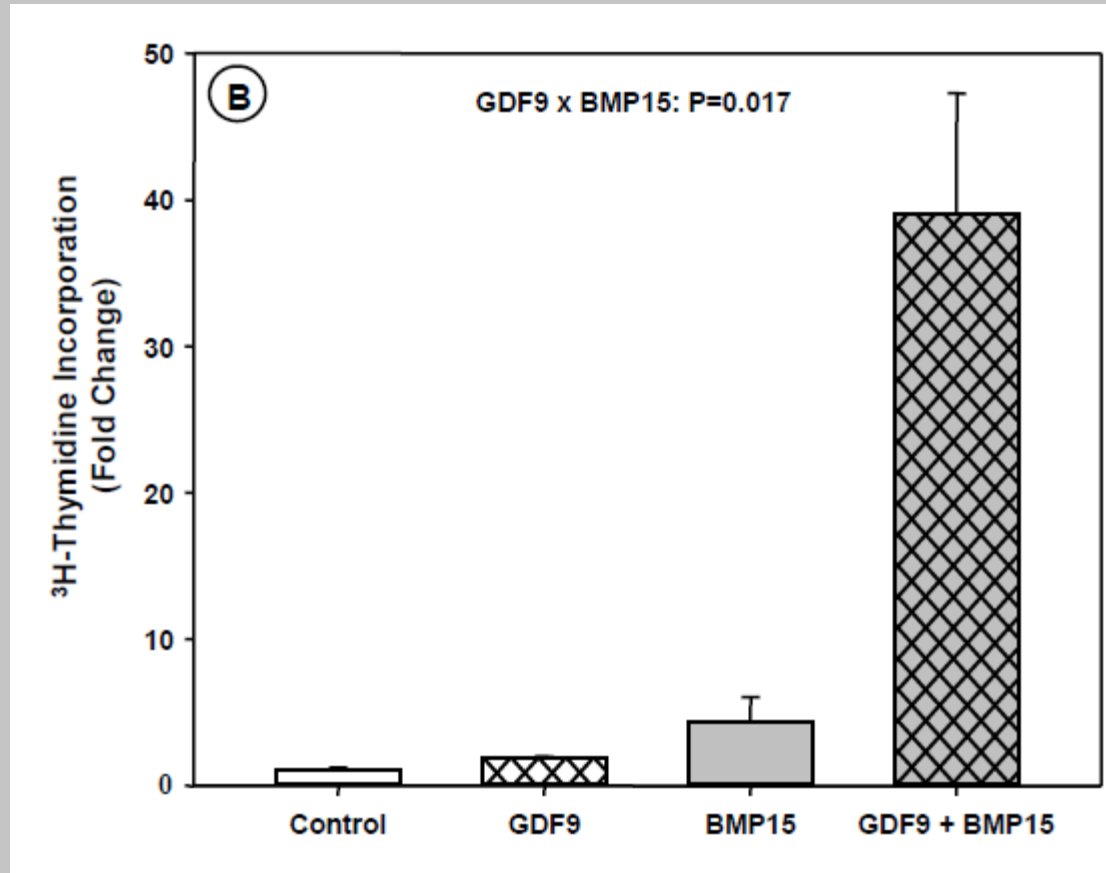
GDF9/BMP15 Synergism

- utilizing mouse primary g

purified mature regions of GDF9 and BMP15 (R&D Systems)



GDF9/BMP15 Synergism on Cumulus Cells

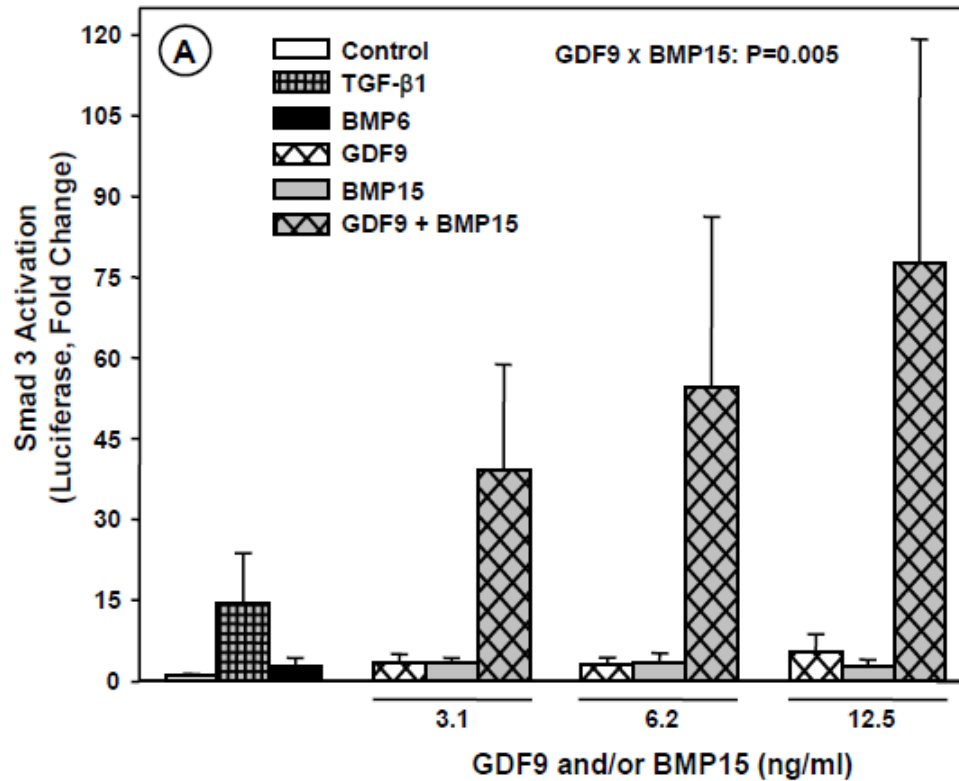


(Mottershead et al., 2012)

• proteins tested
at 12.5 ng/ml

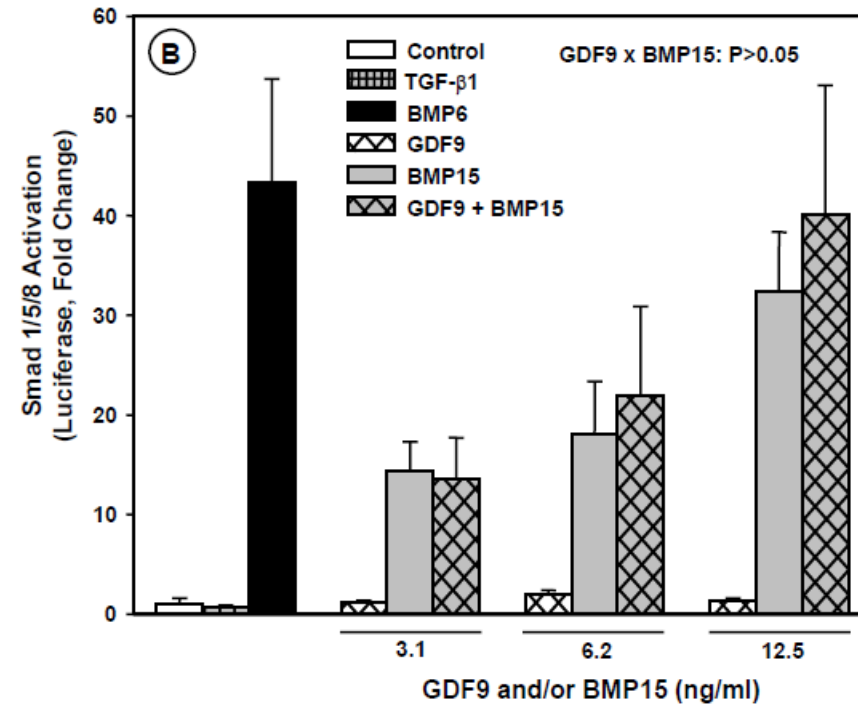


GDF9/BMP15 synergism is mediated via the SMAD3 pathway



SMAD3 (CAGA) pathway

SMAD1/5/8 (BRE) pathway



GDF9



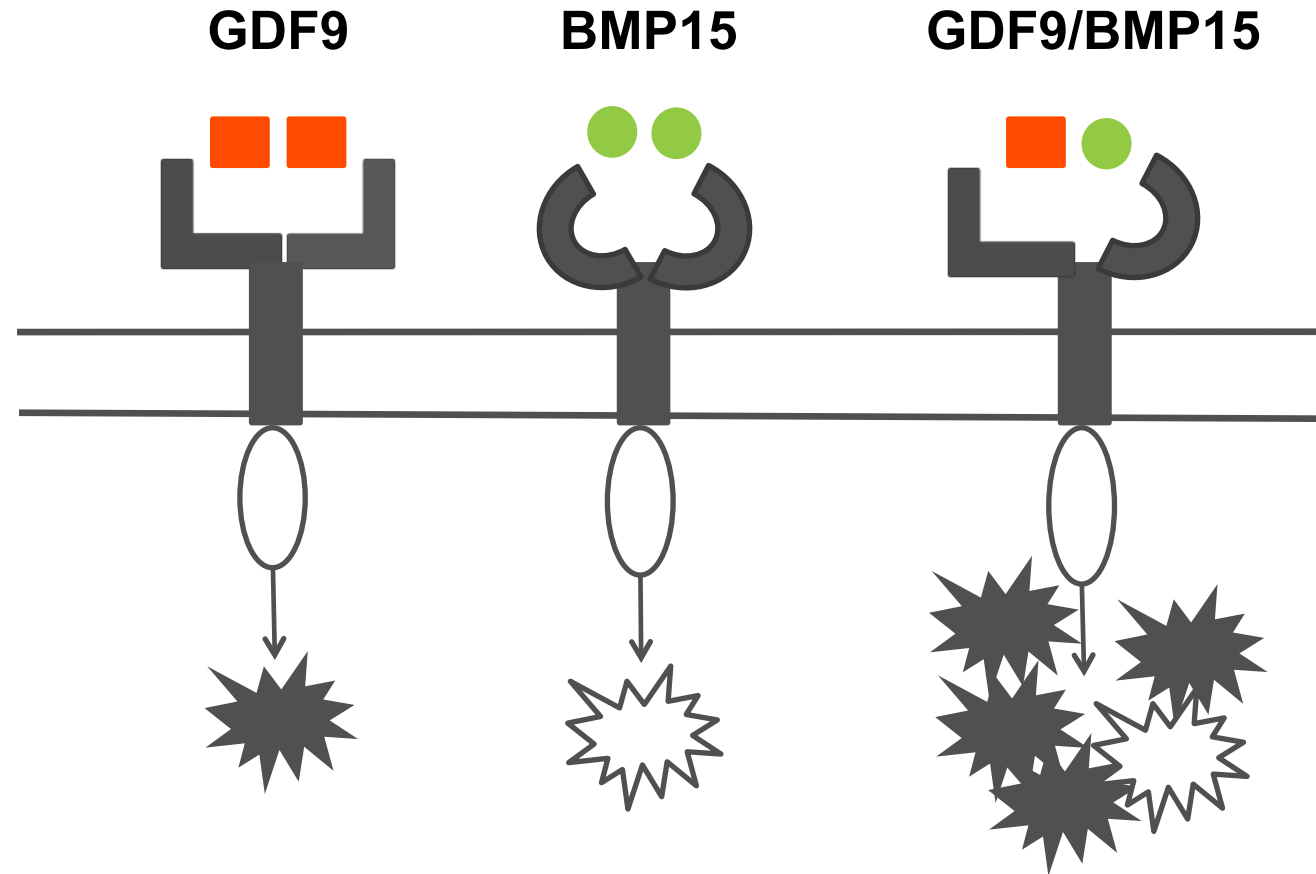
BMP15



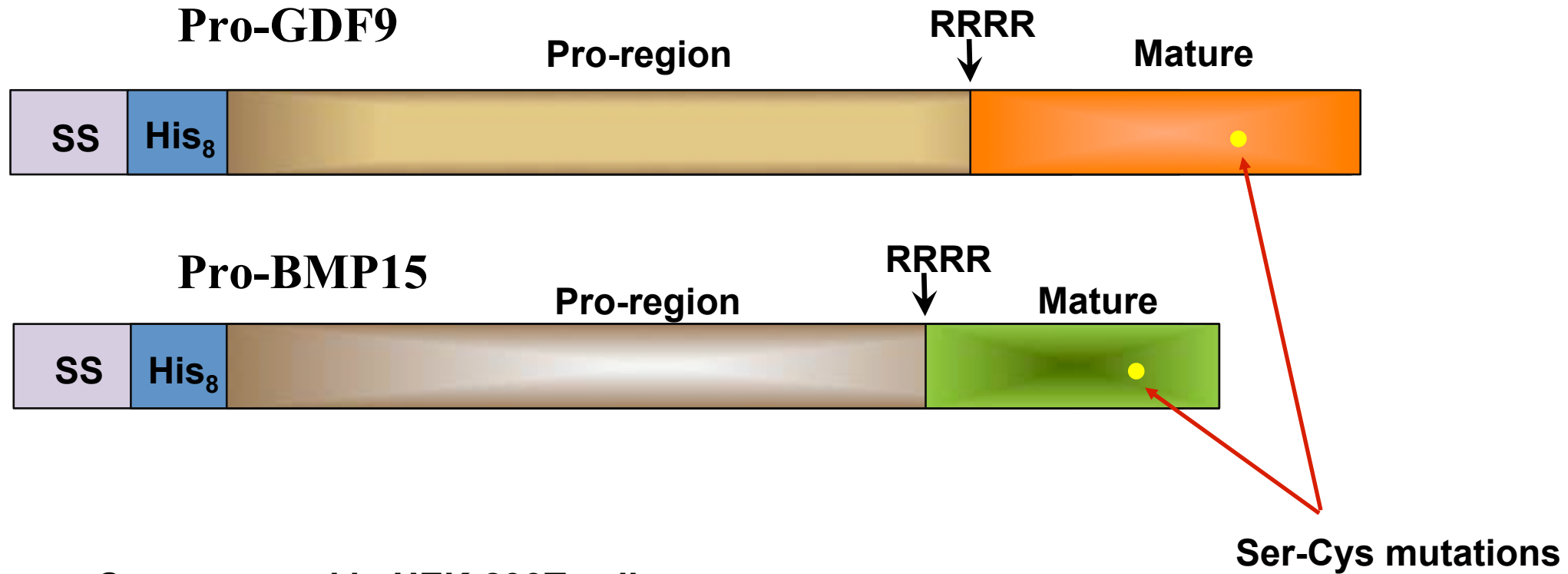
(Mottershead et al., 2012)



What is the mechanistic basis of GDF9 / BMP15 synergism?



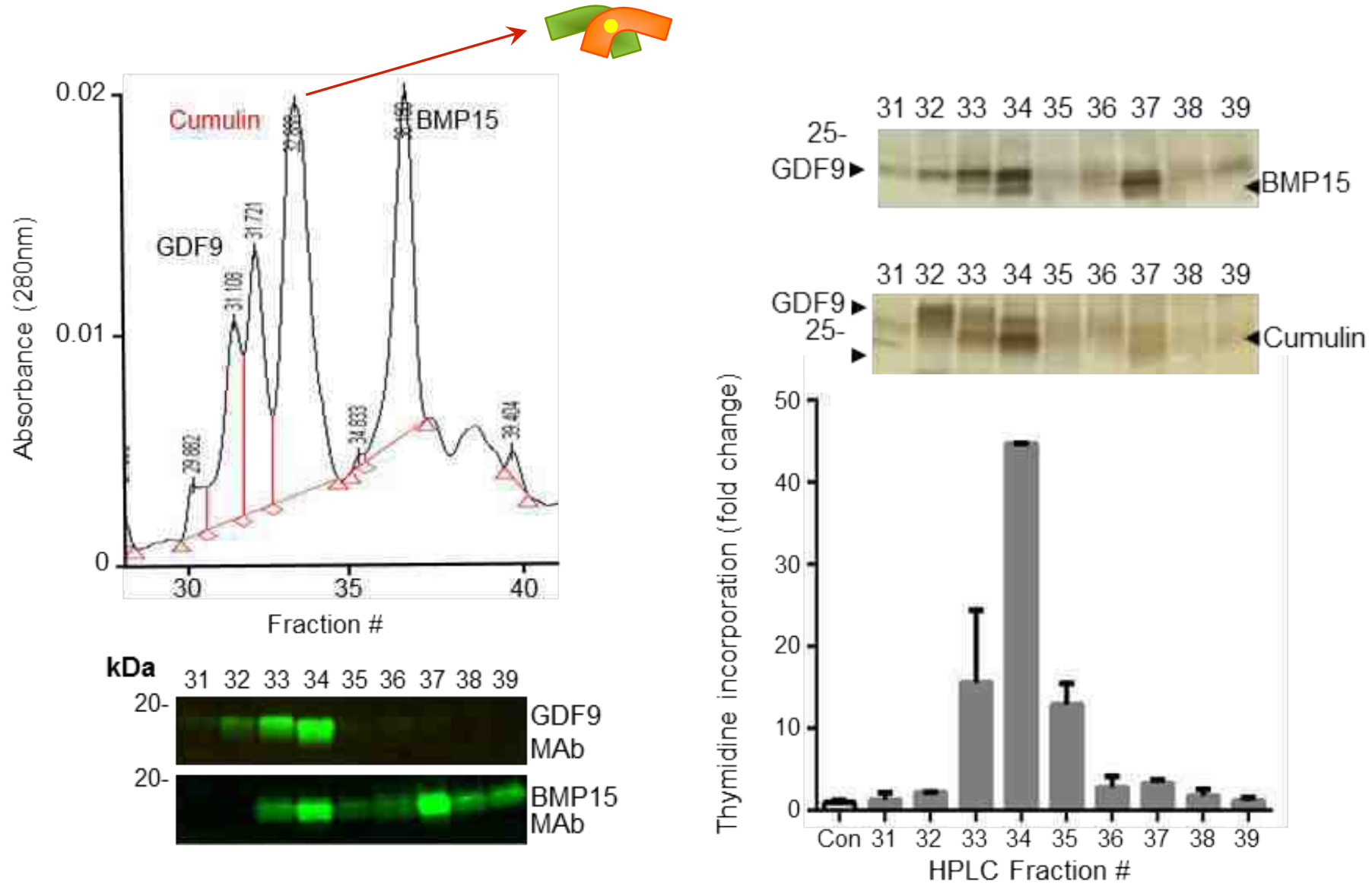
Can GDF9 and BMP15 form a covalent heterodimer?



- Co-expressed in HEK-293T cells
- Serum-free production media mixed with IMAC resin
- Final step rpHPLC



GDF9/BMP15 covalent heterodimer is bioactive

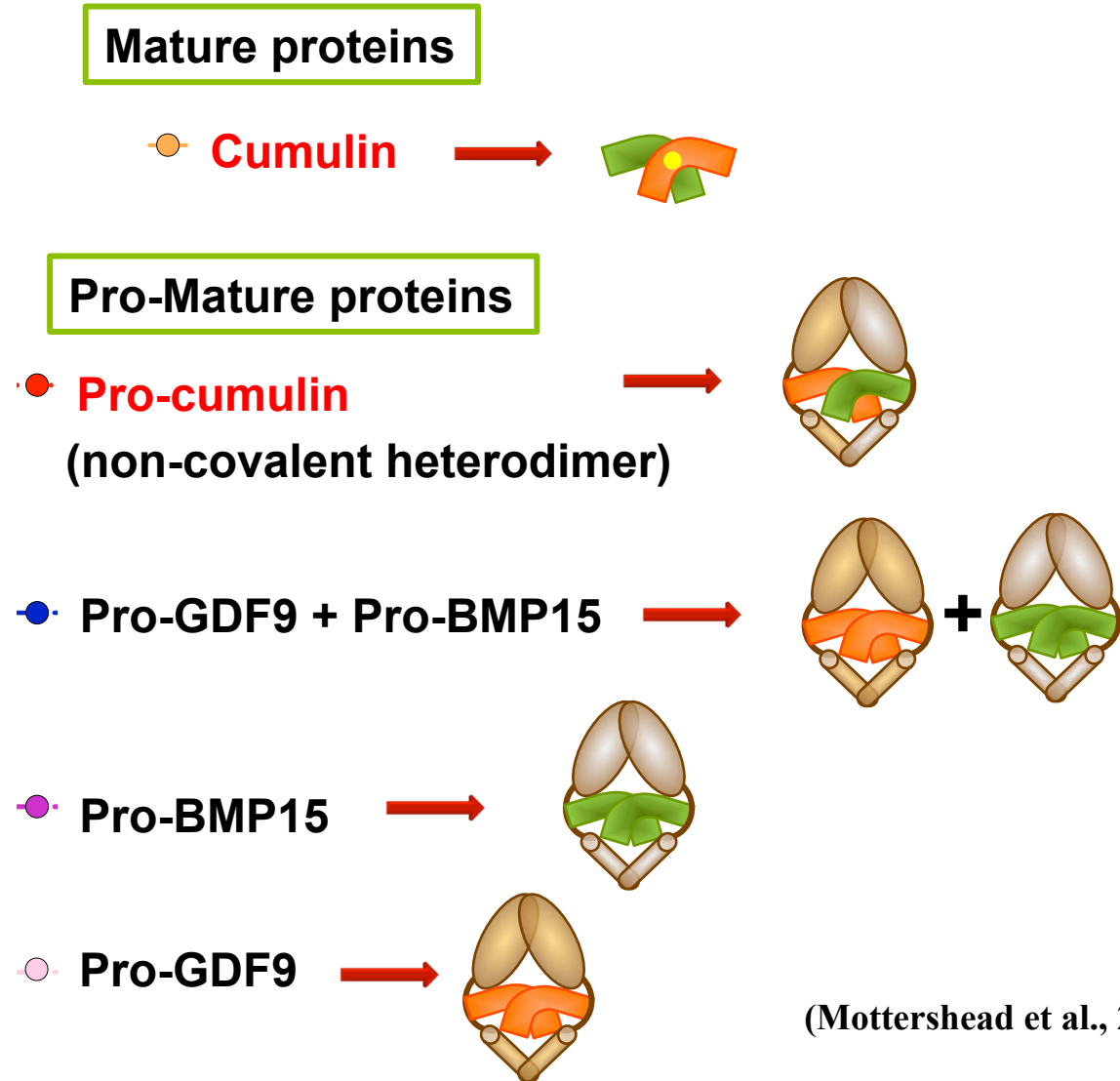
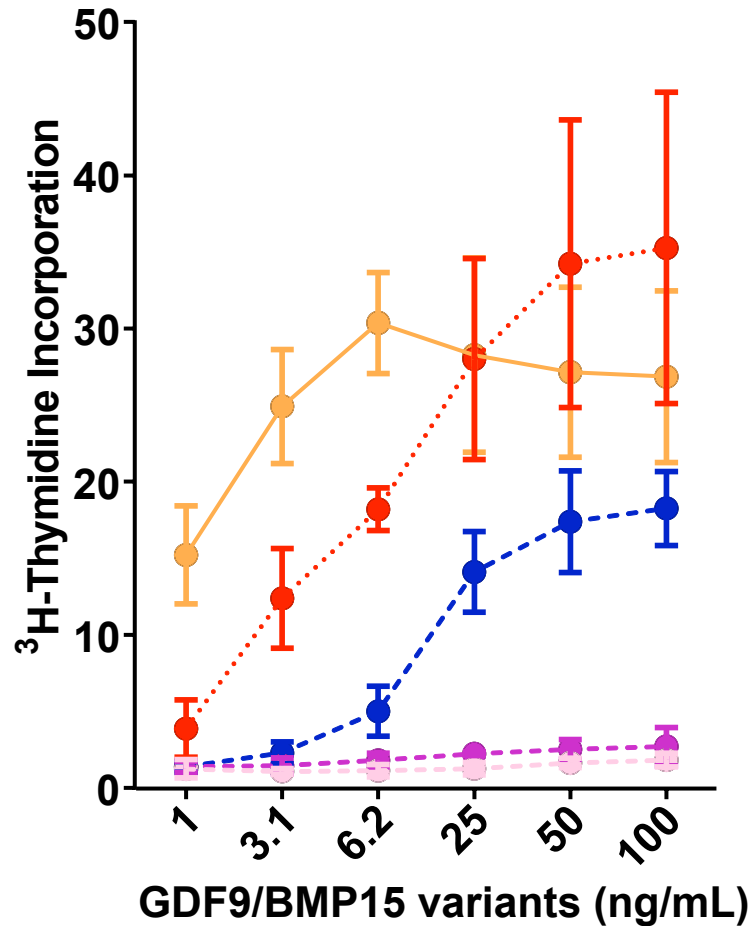


(Mottershead et al., 2015)



GDF9:BMP15 heterodimer bioactivity comparison

- on mouse primary granulosa cells

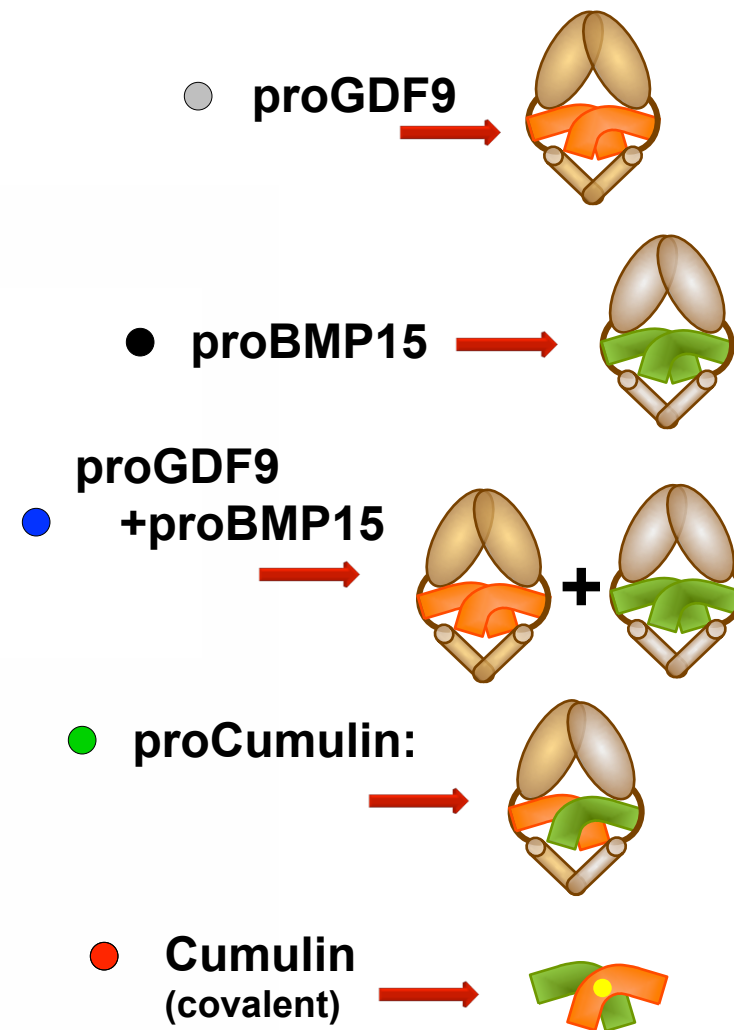
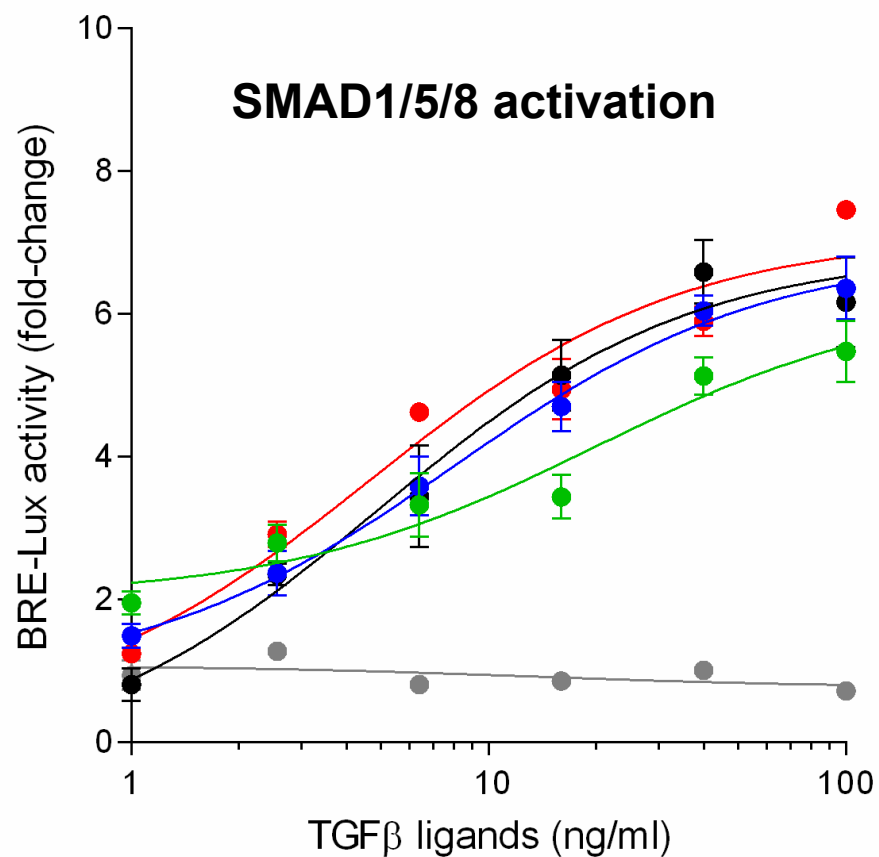


(Mottershead et al., 2015)



Cumulin activates both SMAD signaling pathways

- in human COV434 granulosa tumour cells

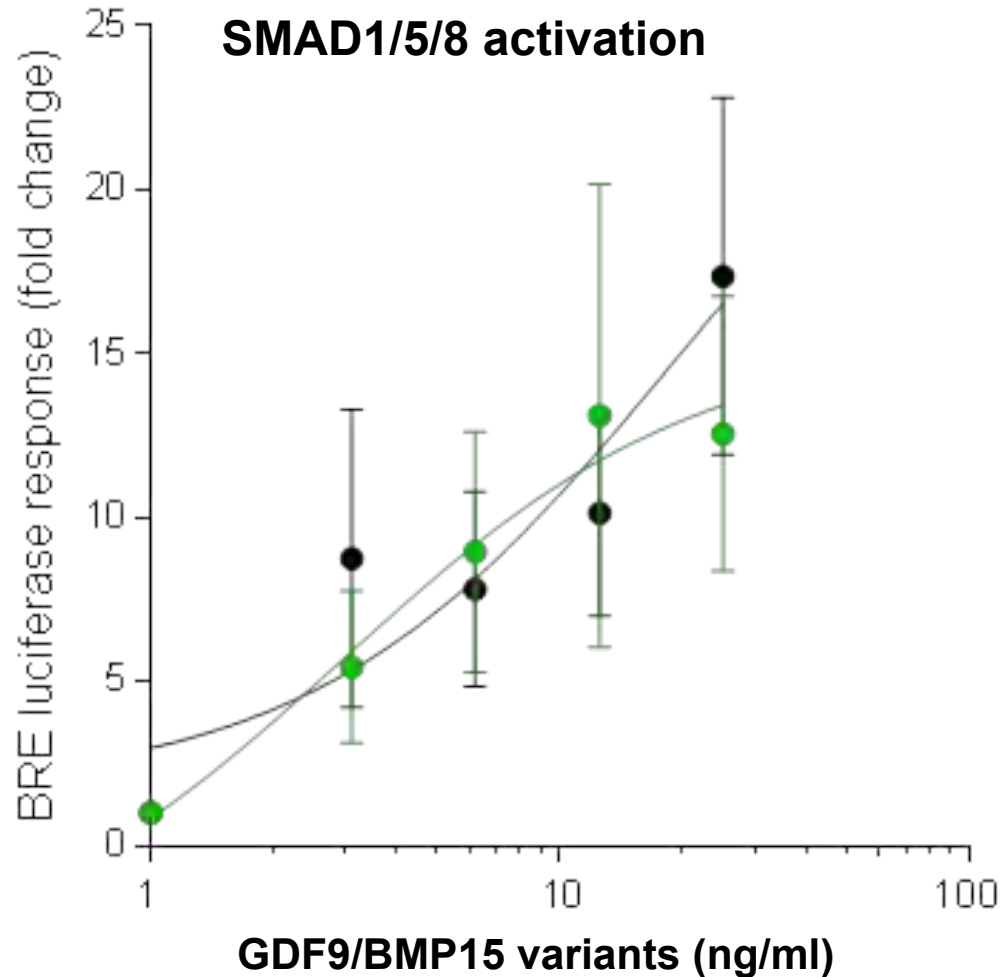


(Mottershead et al., 2015)



BMP15 covalent homodimer does not synergize with GDF9

- utilizing mouse primary granulosa cells

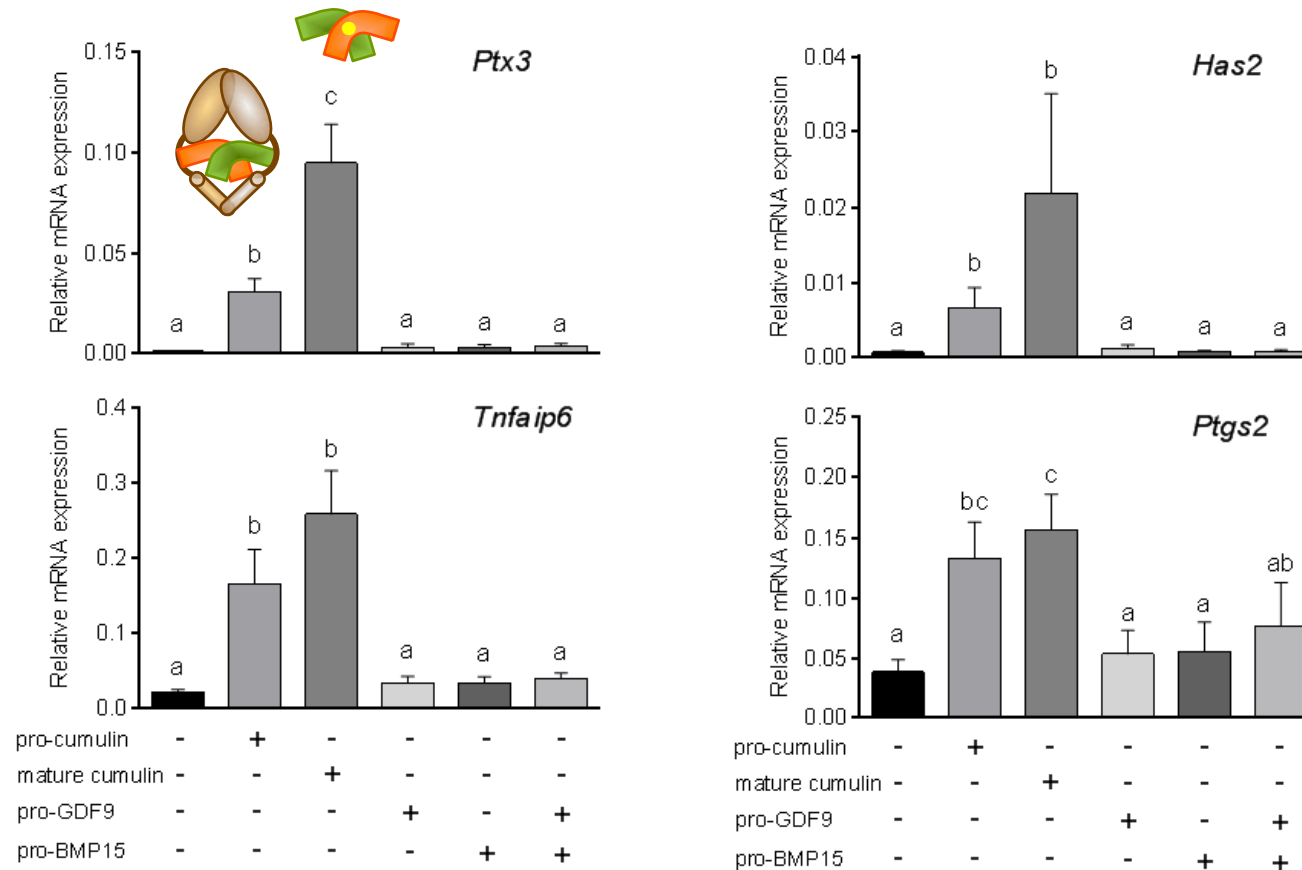


(Mottershead et al., 2015)



Cumulin activates gene expression associated with cumulus differentiation

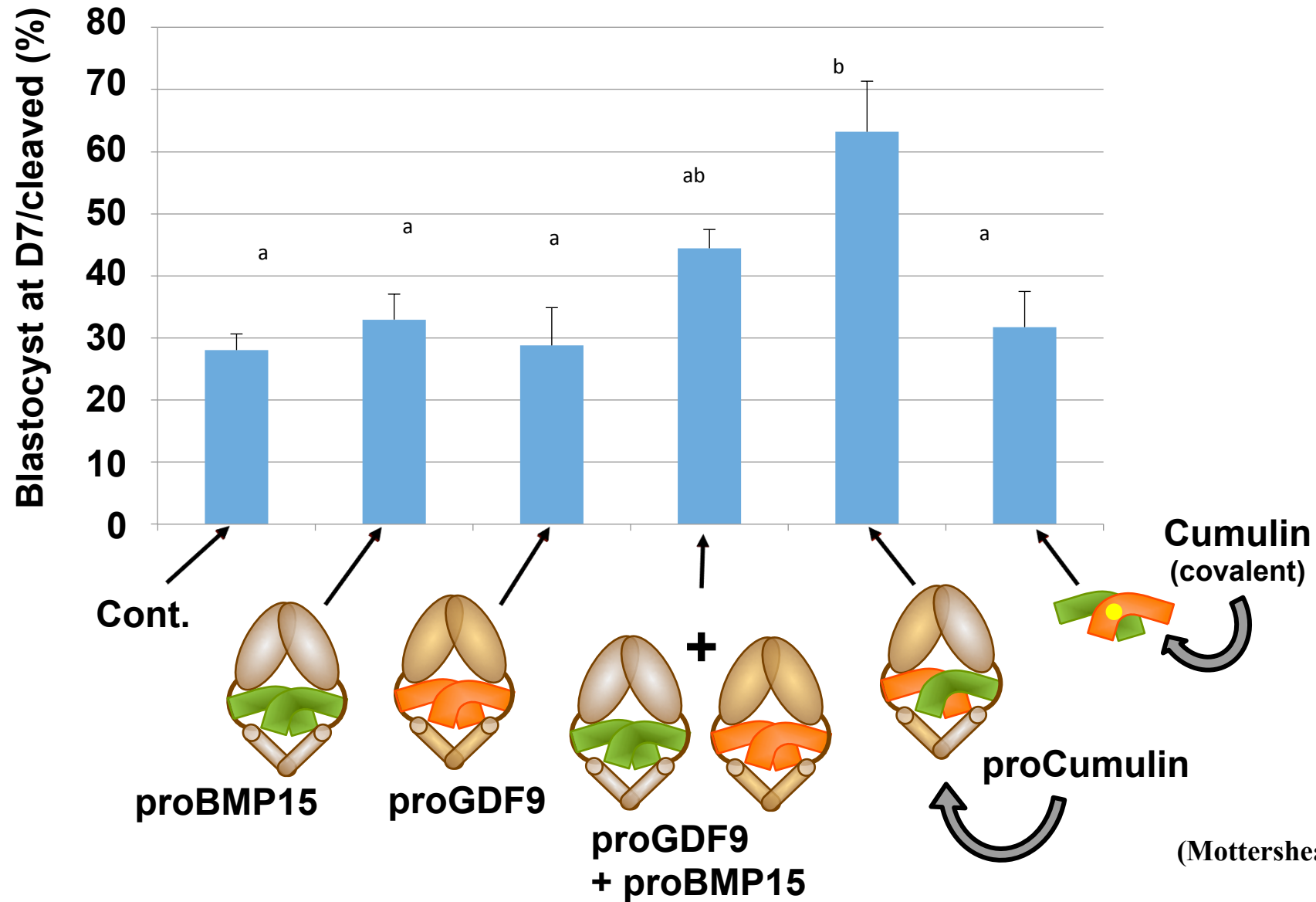
- on mouse primary mural granulosa cells



(Mottershead et al., 2015)

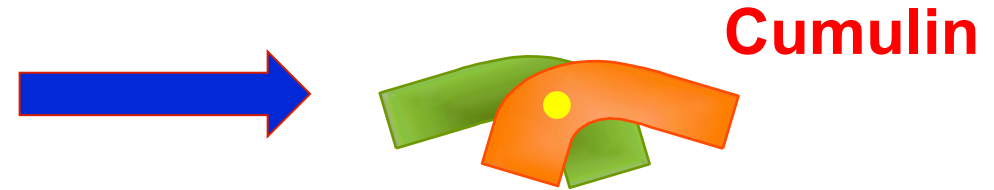


proCumulin stimulates developmental competence in the porcine IVM model



Key Findings 1

1). It is possible to produce a GDF9/BMP15 mature region **covalent heterodimer**, which is very bioactive.

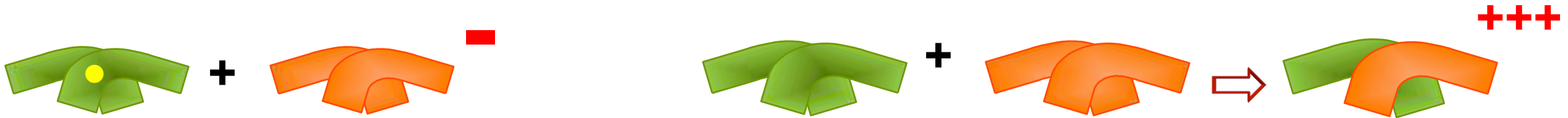


2). For DNA synthesis in granulosa cells **cumulin [1]** is the most active form, followed by **pro-cumulin [2]**, and finally the **GDF9 + BMP15 [3]** combination.

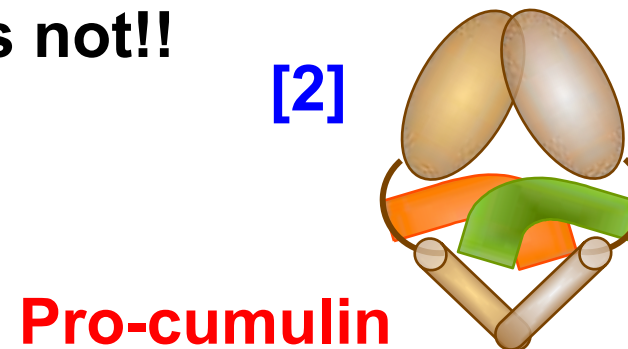


Key Findings 2

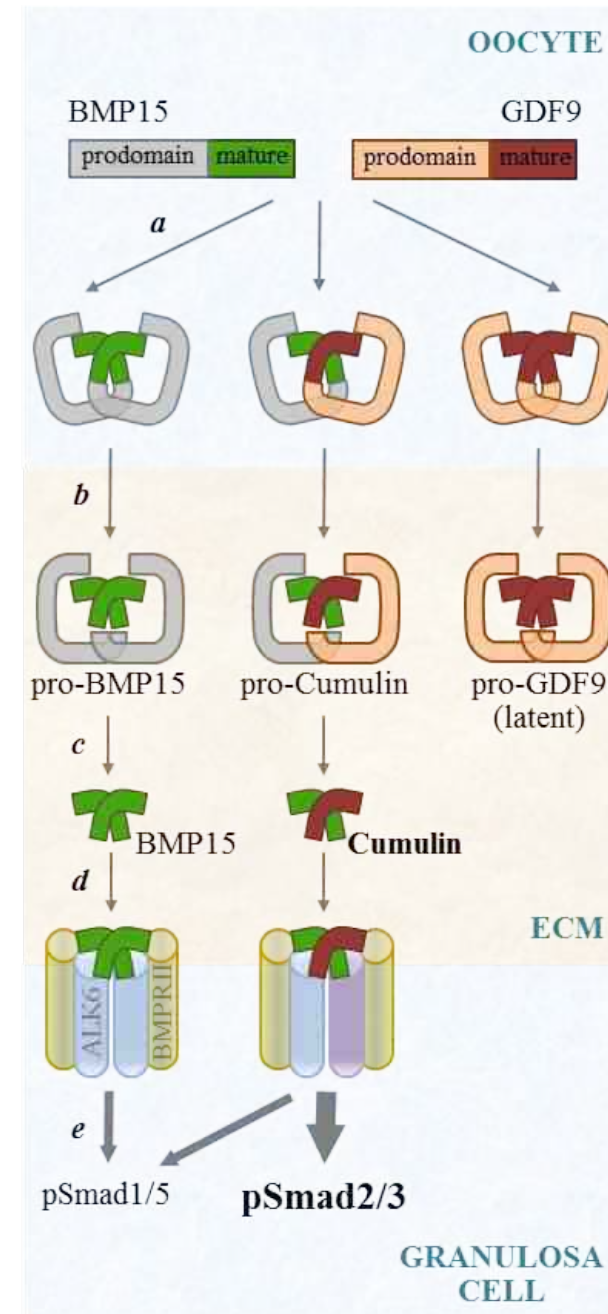
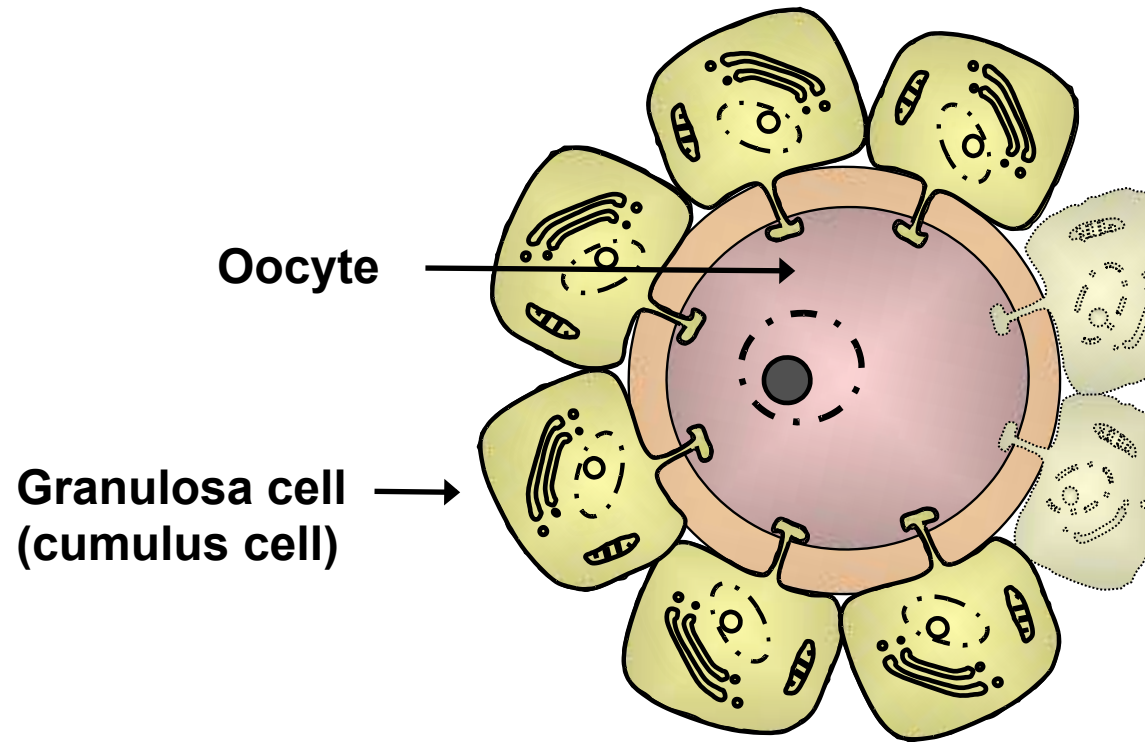
3). The BMP15 **covalent homodimer** does not synergize with **wild type GDF9**, a further indication that the basis of GDF9/BMP15 synergism is heterodimerization.



4). **Pro-cumulin [2] stimulates oocyte developmental competence**, whereas **cumulin [1] (mature region only) does not!!**



Model of human GDF9/BMP15 action *in vivo*



Potential Uses of Cumulin in Reproductive Medicine

1). IVM media development?

2). Ovarian cryopreservation?

3). Measurement of Cumulin levels for oocyte selection?



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