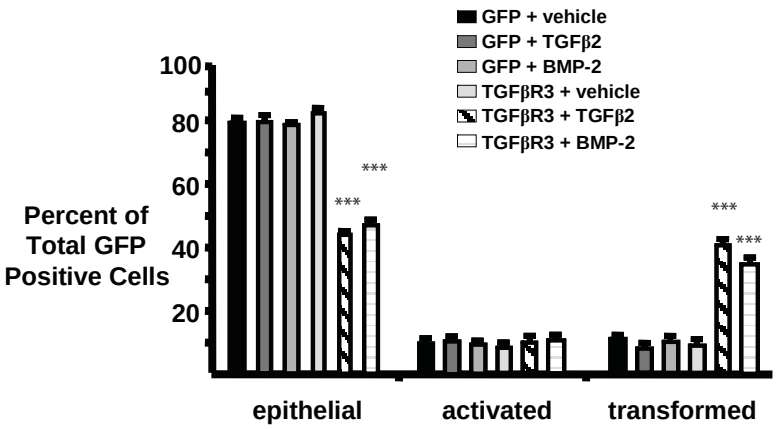
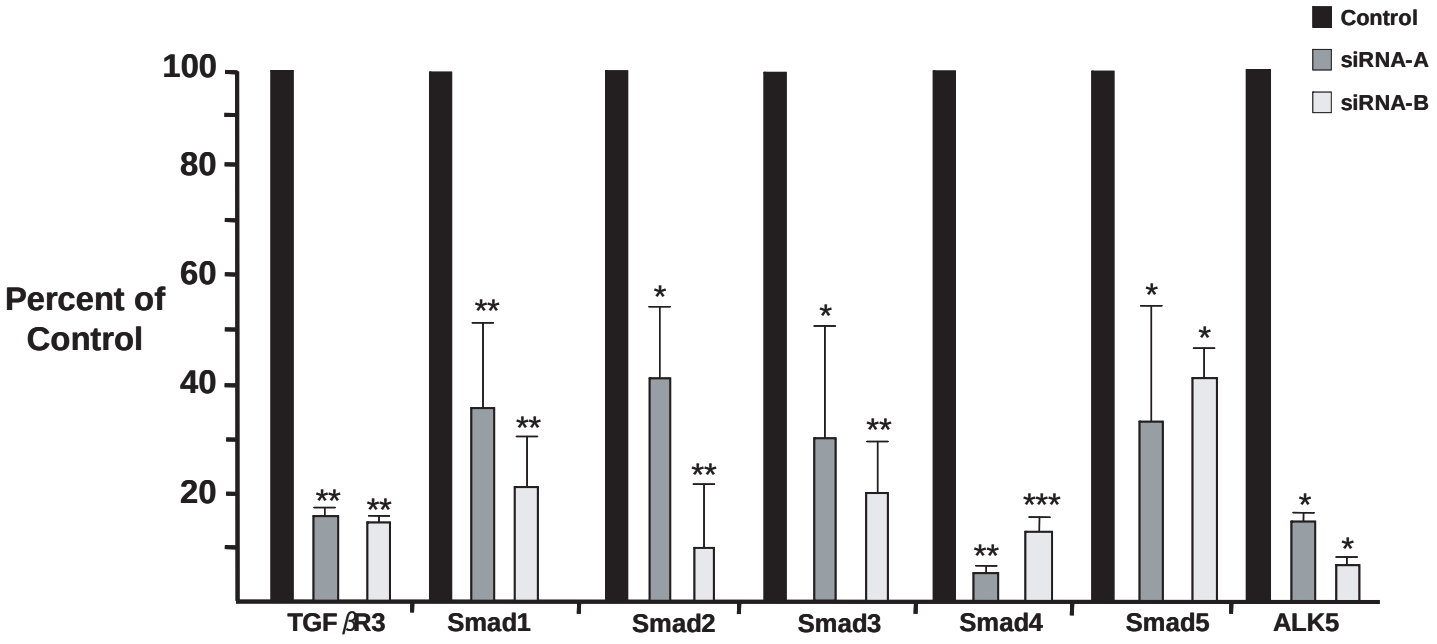

GFP + vehicle
GFP + TGFβ2
GFP + BMP-2
TGFβR3 + vehicle
TGFβR3 + TGFβ2
TGFβR3 + BMP-2
Percent of Total GFP Positive Cells
100
80
60
40
20
***
***
***
***
epithelial
activated
transformed


Control
siRNA-A
siRNA-B
Percent of Control
100
80
60
40
20
**
**
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*
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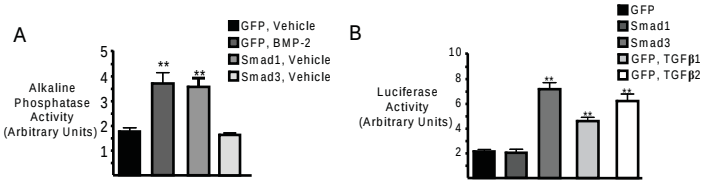
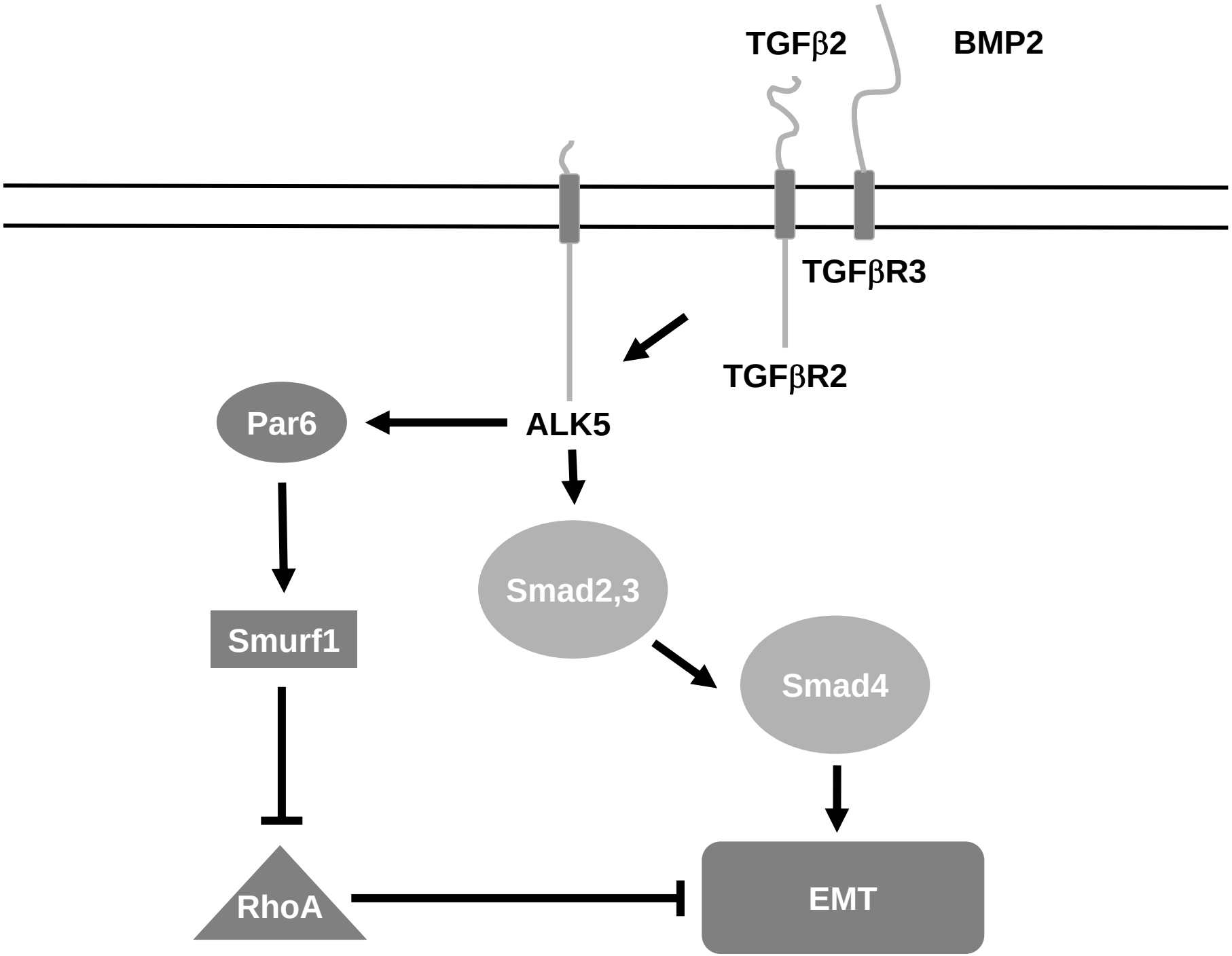
*
**
**
***
*
*
*
*
TGF βR3
Smad1
Smad2
Smad3
Smad4
Smad5
ALK5


A
Alkaline Phosphatase Activity (Arbitrary Units)
5
4
3
2
1
**
**
GFP, Vehicle
GFP, BMP-2
Smad1, Vehicle
Smad3, Vehicle
B
Luciferase Activity (Arbitrary Units)
10
8
6
4
2
**
**
**
GFP
Smad1
Smad3
GFP, TGFβ1
GFP, TGFβ2


TGFβ2
BMP2
TGFβR3
TGFβR2
ALK5
Par6
Smad2,3
Smurf1
Smad4
RhoA
EMT

Supplemental Figure Legends

Fig S1. TGFβ2 and BMP-2 stimulate TGFβR3 mediated ventricular endocardial cell EMT. Average percent of total GFP-expressing cells scored as epithelial, activated or transformed. Means are derived from 3 separate experiments. Explants were given either vehicle (BSA/HCl), 200 pM TGFβ2, or 5 nM BMP-2 as indicated. GFP overexpression alone served as a negative control to define basal levels of transformation, which is unchanged upon ligand addition. TGFβR3 induced statistically significant increases in transformed cells with a concomitant decrease in epithelial cells when stimulated by either ligand. For actual counts and statistical analysis refer to Supplemental Table 1.

Fig S2. Quantification of siRNA Knockdown. The levels of expression of ALK5, Smad4, Smad1, Smad2, Smad3, Smad5 and TGFβR3 were assayed by quantitative real time (RT)-PCR after inhibition of mRNA by siRNA treatment of CEFs. ALK5 mRNA expression level was significantly reduced by 84% and 93% after ALK5-A and ALK5-B siRNA treatment versus control siRNA treatment respectively. Smad4 mRNA expression levels were significantly reduced by 95% and 86% by siRNA treatment with Smad4-A and Smad4-B siRNA treatment respectively. Smad1 mRNA expression levels were significantly reduced by 64% and 78% by siRNA treatment with Smad1-A and Smad1-B siRNA treatment respectively. Smad2 mRNA expression levels were significantly reduced by 59% and 87% by siRNA treatment with Smad2-A and Smad2-B siRNA treatment respectively. Smad3 mRNA expression levels were significantly reduced by 69% and 78% by siRNA treatment with Smad3-A and Smad3-B siRNA treatment respectively. Smad5 mRNA expression levels were significantly reduced by 66% and 60% by siRNA treatment with Smad5-A and Smad5-B siRNA treatment respectively. TGFβR3 mRNA expression levels were significantly reduced by 87% and 85% by siRNA treatment with TGFβR3-A siRNA treatment respectively.

Fig S3. Overexpression of Smad1 or Smad 3 activates Smad signaling. Smad1 and Smad3 adenovirus were tested in C3H10T1/2 cells that produce alkaline phosphatase in response to BMP-2 and Smad1 activation. As expected, BMP-2 or Smad1 stimulated alkaline phosphatase production while Smad3 did not. To test for Smad3 activity we transfected C3H10T1/2 cells with the p3TP-lux luciferase reporter plasmid which is known to be activated by Smad3. Smad3 overexpression activated the p3TP-lux reporter while Smad1 did not. Data are the average of at least 3 independent assays ± SEM, performed three times. (**P<0.01) *A.* C3H10T1/2 cells were infected with adenovirus encoding GFP alone, Smad1, or Smad 3. In the presence of adenovirus encoding GFP alone, 5 nM BMP-2 stimulated alkaline phosphatase expression when compared to vehicle. Smad1 also stimulated expression of alkaline phosphatase, whereas Smad3 did not. *B.* C3H10T1/2 cells were transfected with p3TP-lux and infected with adenovirus encoding GFP alone, Smad1, or Smad 3. GFP overexpression alone or with Smad1 did not induce of luciferase activity. The introduction of Smad3 or the addition of TGFβ1 or TGFβ2 ligand stimulated luciferase production by approximately 3-fold.

Fig S4. Model of TGFβR3 signaling in endocardial cells. Both TGFβ2 and BMP-2 signal EMT in endocardial cells. TGFβR3-mediated EMT requires ALK5 kinase activity as determined by both small molecule inhibitors and siRNA knockdown. Knockdown of Smad4 revealed a requirement for Smad4 downstream of TGFβR3. The Par6/Smurf1/RhoA pathway is implicated since knockdown of Par6 or Smurf1 inhibits TGFβR3-mediated EMT. The relationship of these pathways to TGFβR3 is depicted.