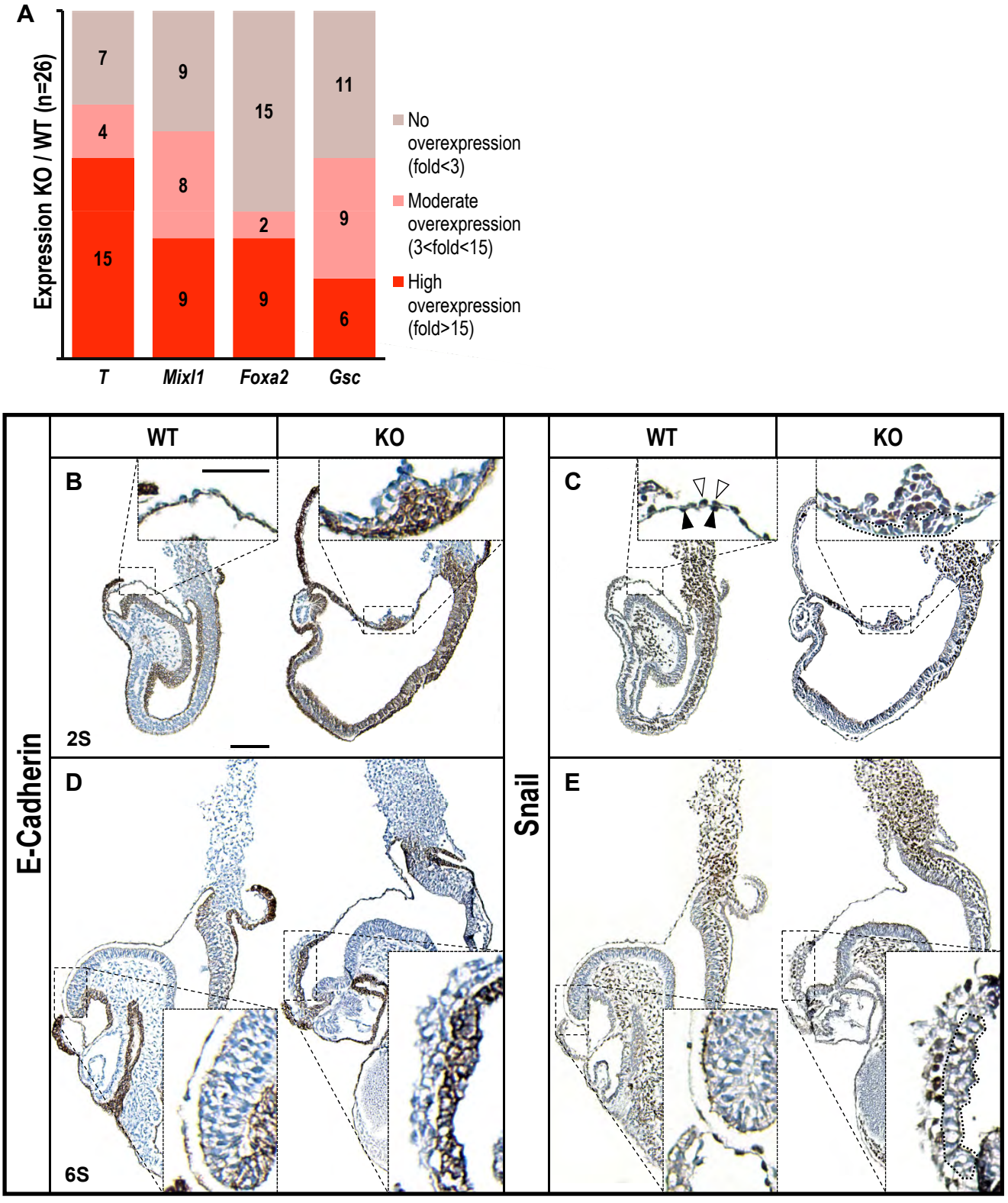


**Fig. S1. Ectopic expression of mesoderm markers in the *Smad5* mutant amnion.** (**A**) Bar graph summarizing the expression of *T, Mixl1*, *Gsc* and *Foxa2* by quantitative RT-PCR in *Smad5* knockout compared with control littermate amnions at E7.5 (*n*=26 amnion pairs), well before the morphological appearance of the cell clump. (**B-E**) E-cadherin and Snail localization in wild-type and *Smad5* knockout embryos at the 2S and 6S stage, respectively. Insets are magnifications of the boxed areas. In wild-type embryos, Snail is present both in the amniotic mesoderm (open arrowheads) and in the amniotic ectoderm (arrowheads). In the clump in *Smad5* KO amnion, Snail is absent from most cells located in the outlined region. Scale bars: 100 µm on figure panels; 25 µm in the insers.

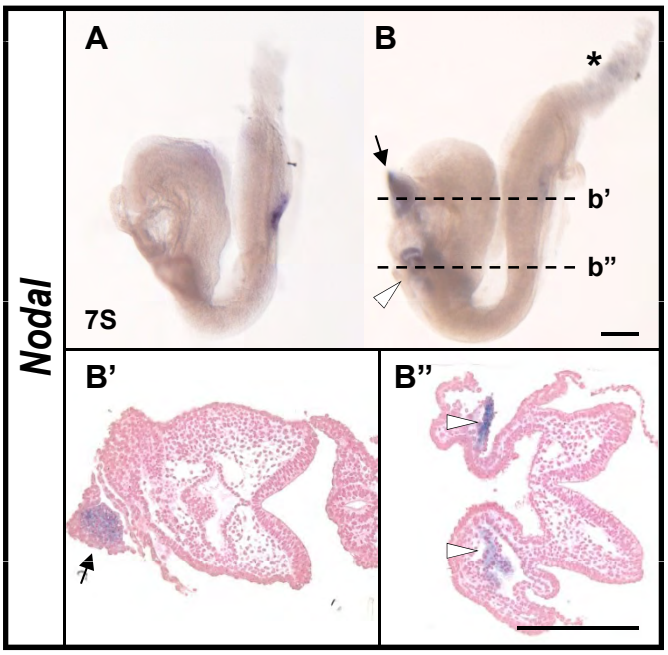


**Fig. S2. Ectopic *nodal* expression in the *Smad5* mutant at E8.5.** (**A**,**B**) *Nodal* expression in (A) wild-type and (B) *Smad5* KO embryos at 7S stage. Ectopic *Nodal* is expressed in amnion (arrow), cardiac crescent (open arrow head) and distal tip of the allantois (asterisk). (**B′**,**B″**) Transverse sections of the *Smad5* KO embryo shown in B at the indicated levels. Scale bars: 200 µm.

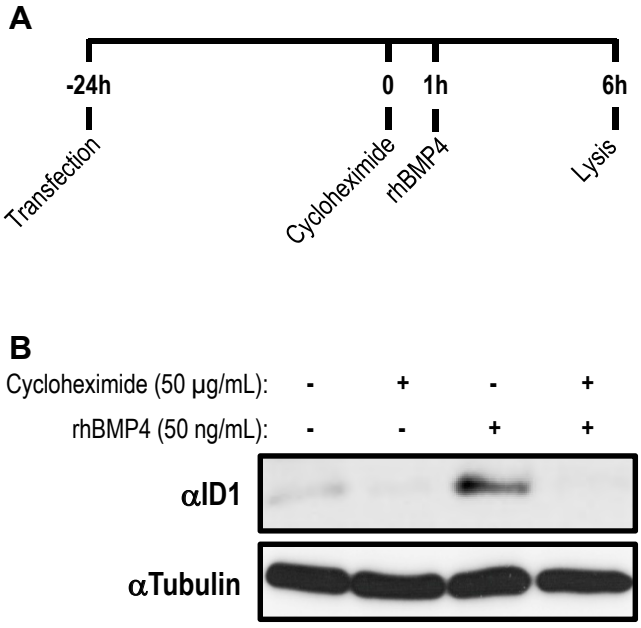


**Fig. 3. Validation of protein synthesis blockade by cycloheximide.** (**A**) Experimental setup using cycloheximide. Treatment of HEK293T cells with cycloheximide was for 1 hour before and during the 5-hour stimulation with rhBMP4. (**B**) Treatment with cycloheximide as depicted in A blocks effectively protein synthesis, as shown by western blot analysis of the BMP target gene ID1. Tubulin was used as a loading control.

| Target Gene | Primer sequence |
|---|---|
| *β-galactosidase* | **TTGTTCCCACGGAGAATCCG** |
| | **CACCACAGATGAAACGCCGA** |
| *Bmp4* | **GATCACCTCAACTCAACCAA** |
| | **TTTCAACACCACCTTGTCAT** |
| *Foxa2* | **AGTCACGAACAAAGCGGG** |
| | **TTCCTCAAAGCTCTCCCAAAG** |
| *Gapdh* | **AAGAAGGTGGTGAAGCAGGC** |
| | **GCCTCTCTTGCTCAGTGTCC** |
| *Gsc* | **AGTCAGAAAACGCCGAGAAG** |
| | **TGCAAGTAGCATCGACTGTC** |
| *Lefty2* | **TCCTTGCCCATGATTGTCAG** |
| | **CTGACGAGAGCACTAAGTTAGG** |
| *luciferase* | **ACATTTCGCAGCCTACCGTAGTGT** |
| | **GGTAATCCGTTTTAGAATCC** |
| *Mixl1* | **TGGCTCAAAGTTGGACTCC** |
| | **CAGTAAAGGCTCAGTGTCAGAG** |
| *Nodal* | **AAAAGTGTTGGCATCAGCCC** |
| | **TGGTGCTGGCGACAGGTAC** |
| *T* | **AACAGCTCTCCAACCTATGC** |
| | **TACCATTGCTCACAGACCAG** |
| *Ubc* | **TAAAAAGAGCCCTCCTTGTGCT** |
| | **AGACACCTCCCCATCACAC** |
| *Wnt3* | **AGCTGCCAAGAGTGTATTCG** |
| | **CTAGATCCTGCTTCTCATGGG** |
| Universal primer 1 | **ATATGGATCCGGCGCGCCGTCGACTTTTTTTTTTTTTTTTT** |
| Universal primer 2 | **ATATCTCGAGGGCGCGCCGGATCCTTTTTTTTTTTTTTTTT** |

**Table S1**. **Validated primers used for quantitative RT-PCR.** The primer sequences of the respective genes and poly-dT universal primers appear with the forward primer followed by the reverse primer.