Supplementary Material

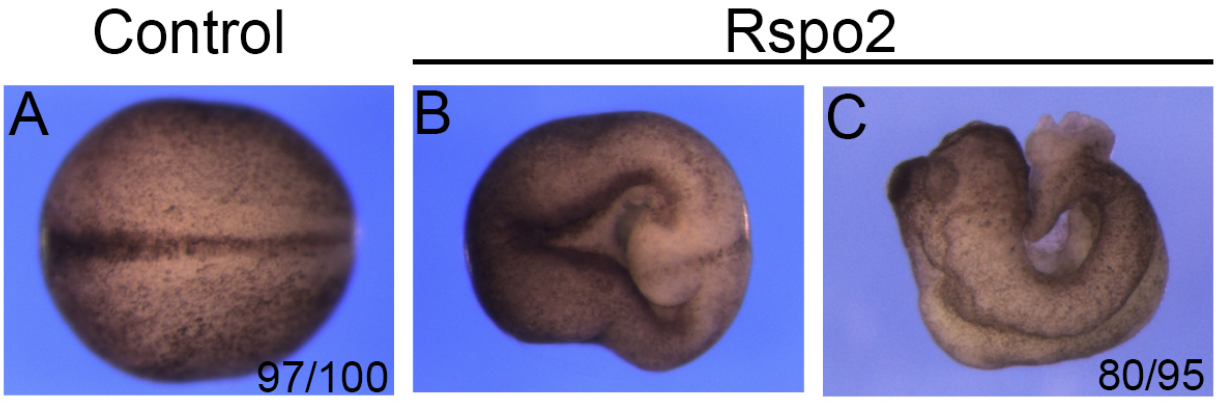

Fig. S1. Phenotypes of embryos injected with Rspo2 RNA.

Four-cell stage embryos were injected into two dorsal blastomeres with Rspo2 RNA (0.5 ng) and allowed to develop until neurula (A, B) or tailbud (C) stages. (A) Uninjected control embryo, stage 19. (B-C) Rspo2-expressing embryos. Open blastopore and posterior defects are apparent. Representative embryos are shown, with more than 20 embryos per group from five separate experiments. The number of embryos displaying the phenotype and the total number of embryos are indicated.

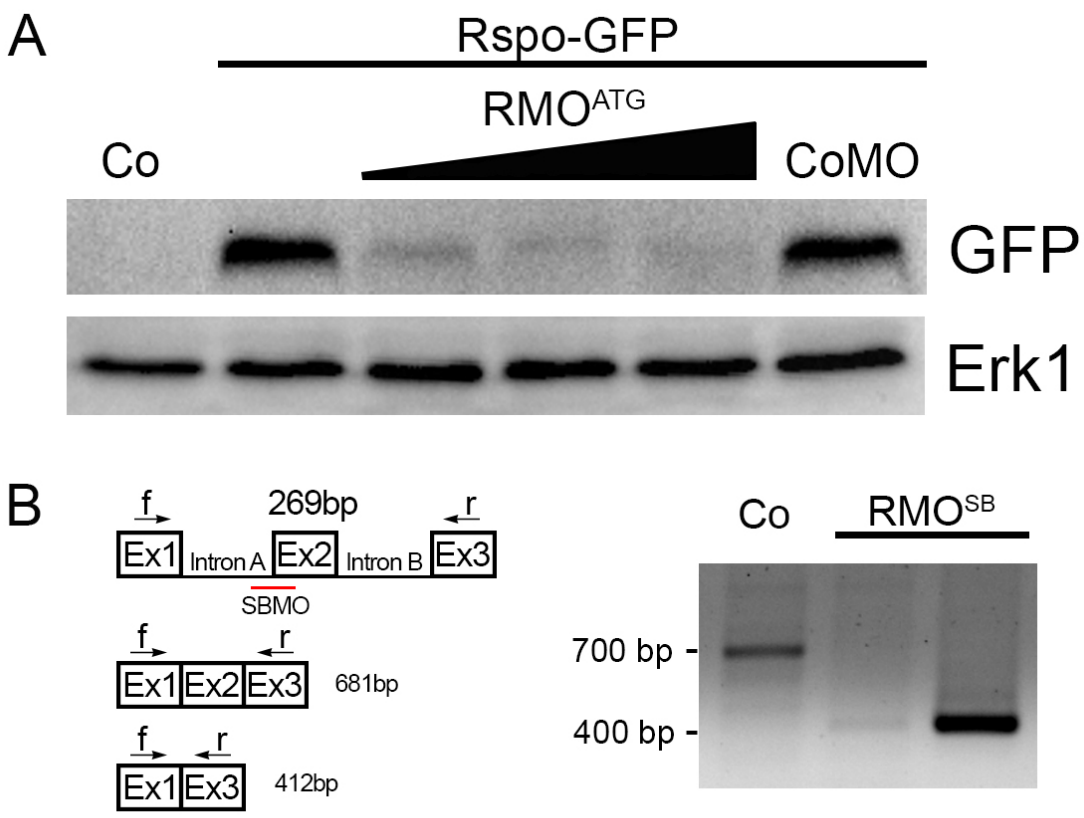

Fig. S2. Validation of Rspo2 knockdown *in vivo*. A, Embryos were injected with Rspo2-GFP RNA (500 pg) alone or coinjected with increasing amounts of RspoMO^{ATG} (10, 20, and 30 ng). Lysates were prepared from injected embryos at stage 11 for immunoblotting with anti-GFP antibody. CoMO (control MO). Co, control uninjected embryo. Erk1 is a control for loading. B, Schematic of RT-PCR to detect changes in Rspo2 RNA splicing. The PCR fragment of 681 bp corresponds to three exons expected in a control embryo. The 412 bp DNA fragment is expected for Rspo2.L RNA with un-spliced exon 2. RT-PCR was carried out with RNA prepared from stage 11 embryos previously injected with RspoMO^{SB} (20 ng). PCR fragments corresponding to a control embryo (Co) and two different embryos injected with RspoMO^{SB} are shown.

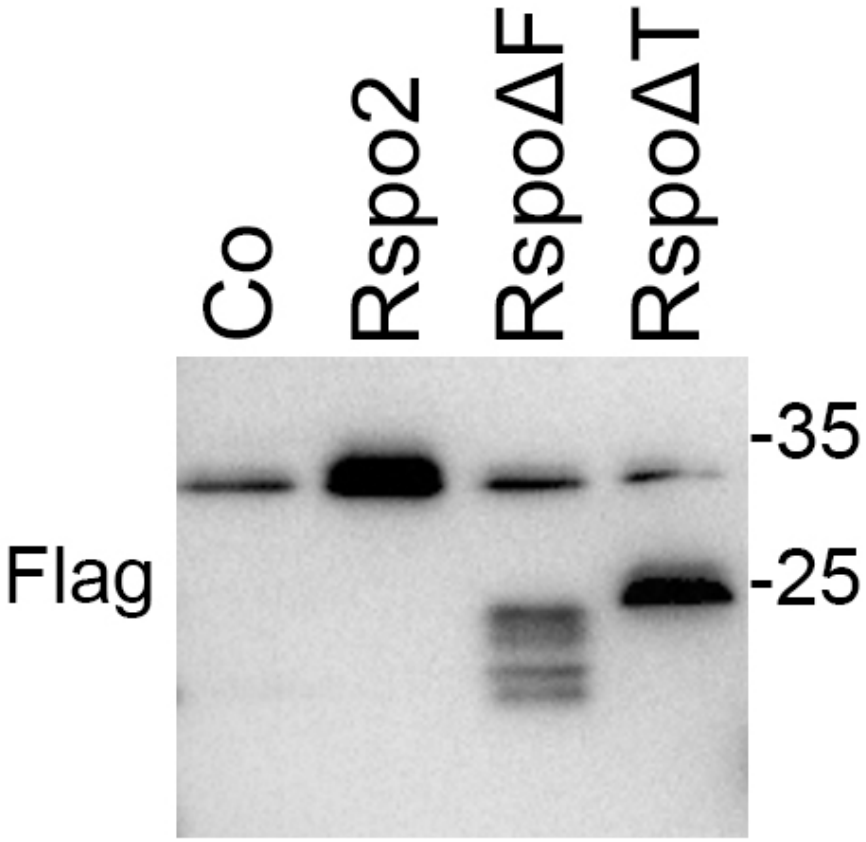

Fig. S3. Expression levels of Rspo2 constructs. RNAs encoding different Flag-tagged Rspo2 constructs (see Fig. 3A) were injected into four cell embryos, ectoderm explants were isolated at midblastula stages and cultured until stage 11 for immunoblotting with anti-Flag antibody.

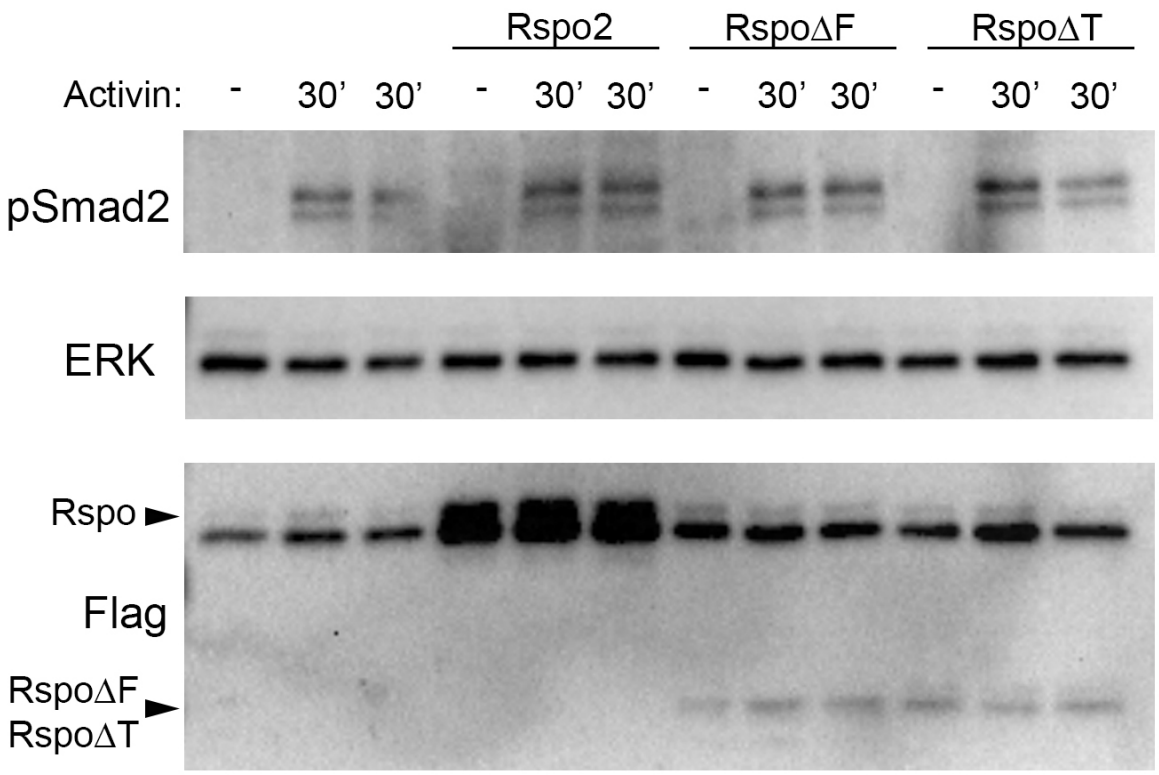

Fig. S4. Lack of Rspo2 effects on Activin/Nodal signaling. RNAs encoding different Flag-tagged Rspo2 constructs were injected into four cell embryos, ectoderm explants were isolated at midblastula stages and stimulated with 0.5 ng/ml of Activin A for 30'. Cell lysates were separated by PAGE and immunoblotted with anti-phospho-Smad2 and anti-Flag antibody.

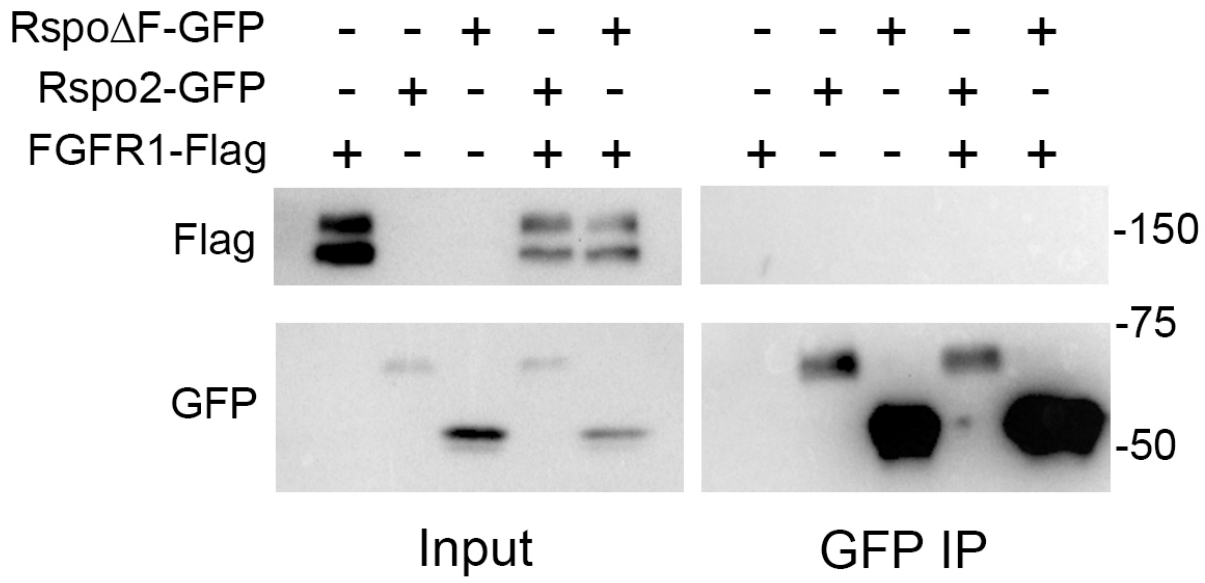

Fig. S5. Lack of Rspo2 association with FGFR1. RNAs encoding different GFP-tagged Rspo2 constructs and/or FGFR1-Flag were injected into four-cell embryos, ectoderm explants were isolated at midblastula stages and cultured until stage 12 for immunoprecipitation (IP) with GFP. FGFR1 is not pulled down with Rspo2-GFP or RspoΔF-GFP.

Table S1.

Primers for cloning Rspo2 cDNA
F: 5'- AGCGAATTCATGCAGTTTCAACTCTTTTC – 3'
R: 5'-TCAGGATCCAGTTGGCTGGACCGGTCTGTAG – 3'

Primers for Rspo2 cDNA mutagenesis
RspoΔF:
5'- AGACGGAGCAAGAGAGCCAGATCTCCATTGGATGACACCATG-3'
RspoΔT:
5'-TGCGTGGATGGCTGTGAAGCTAGCGGAGGAACAAGAACCACA-3'

Primers for RT-qPCR

cdx4.L:	Forward: 5'-TGATTTATCACCTAACCAG-3'
	Reverse: 5'-GTCCCAGATGGATGAGGAGA-3'
eef1a1.S:	Forward: 5'-ACCCTCCTCTTGGTCGTTTT-3'
	Reverse: 5'-TTTGGTTTTCGCTGCTTTCT-3'
tbxt.S:	Forward: 5'-TCACTAGCCATTCATTCCCT-3'
	Reverse: 5'-GACTATCGATTCCCTCATCC -3'
msgn1.L	Forward: 5'-GTATCCAACACTTTGCCATG-3'
	Reverse: 5'-AGCACTGGAGAAGGTTTGTG-3'