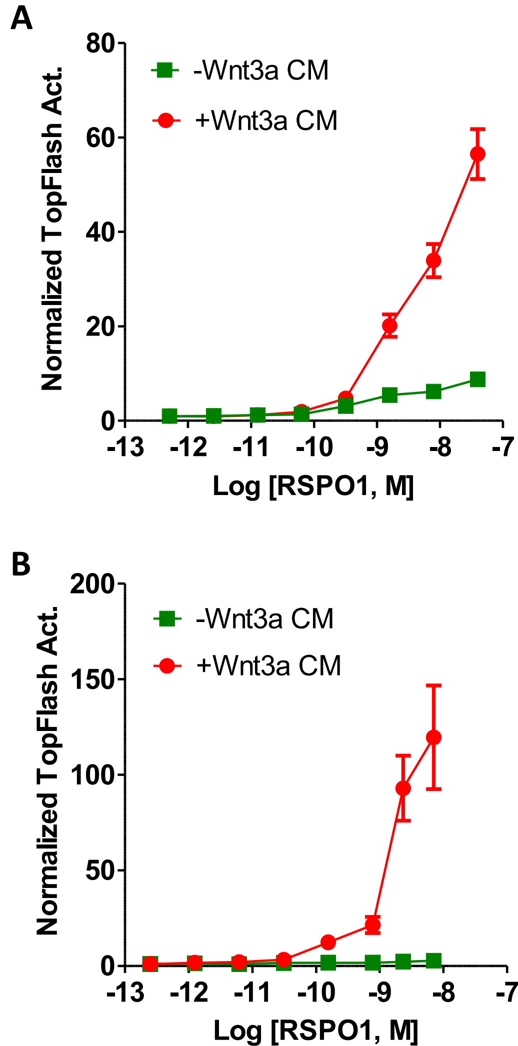
Supplementary Figures

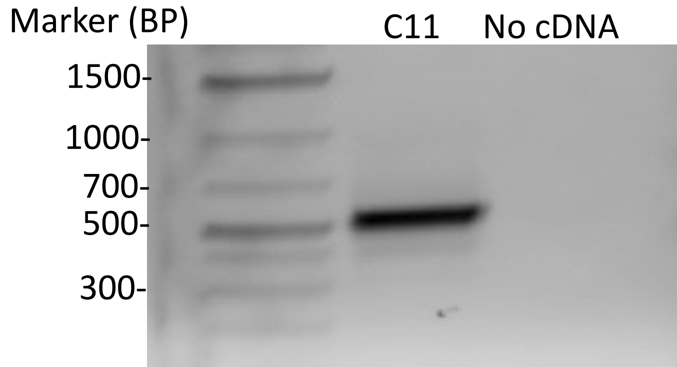


Figures S1. HEK293-STF cells show dose-dependent response to RSPO1,which also required co-stimulation with Wnt ligands. A, TOPFlash luciferase activity in STF cells in response to RSPO1 in the presence and absence of Wnt3a conditioned media (CM). B, Normalized TOPFlash activity in STF cells that were pretreated with the porcupine inhibitor LGK974 for 2 days and then treated with RSPO1 in the presence and absence of Wnt3a CM.

	Sequence of LGR4 Exon 5	No. nucleotides changed
WT	5’- GGCTGGATGACAACAGCTTGACGGAGGTGCCTGTGCACCCCCTCAGCAATCTGCCCACCCTACAGGCGCT-3’	
C6-1	5’- GGCTGGATGACAACAGCTT-------GTGCCTGTGCACCCCCTCAGCAATCTGCCCACCCTACAGGCGCT-3’	-7
C6-2	5’- GGCTGGATGACAACAG---G-----GGTGCCTGTGCACCCCCTCAGCAATCTGCCCACCCTACAGGCGCT-3’	-8
C6-3	5’- GGCTGGATGACAACA-------------------GCACCCCCTCAGCAATCTGCCCACCCTACAGGCGCT-3’	-19
C6-4	5’- GGCTGGATGACAACAGCT---------------//-----------------------TCCCTGACTTTG-3’	-80
C11-1	5’- GGCTGGATGACAACAGCTTG-CGGAGGTGCCTGTGCACCCCCTCAGCAATCTGCCCACCCTACAGGCGCT-3’	-1
C11-2	5’- GGCTGGATGACAACAGCTTG**A**ACGGAGGTGCCTGTGCACCCCCTCAGCAATCTGCCCACCCTACAGGCGCT-3’	+1

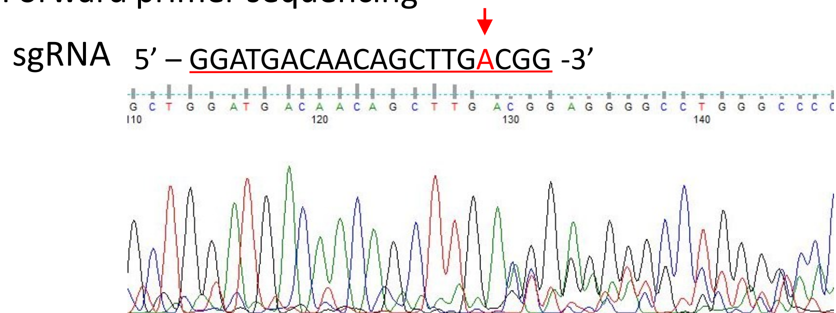
Figures S2. CRISPR/Cas9-generationed mutations in the two HEK293-STF-LGR4KO clones (#6 and #11). WT sequence is shown on top with the target sgRNA sequence underlined. Deletion of a nucleotide is marked with “-”, and addition of a nucleotide is highlighted.

A



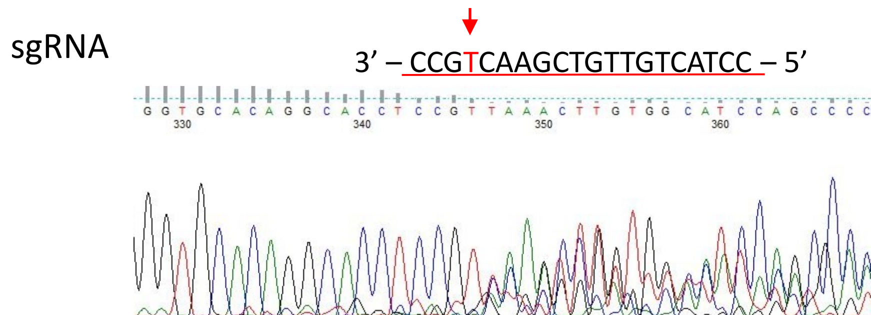
B

Forward primer sequencing



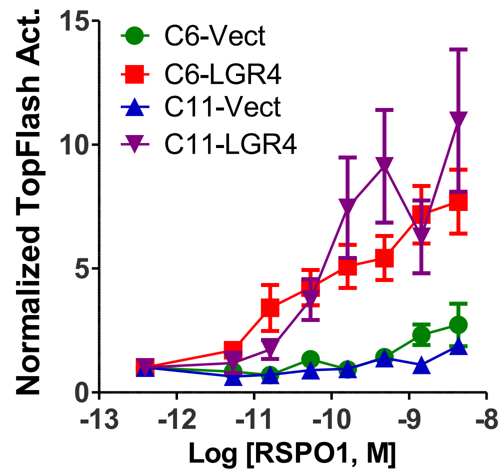
C

Reverse primer sequencing

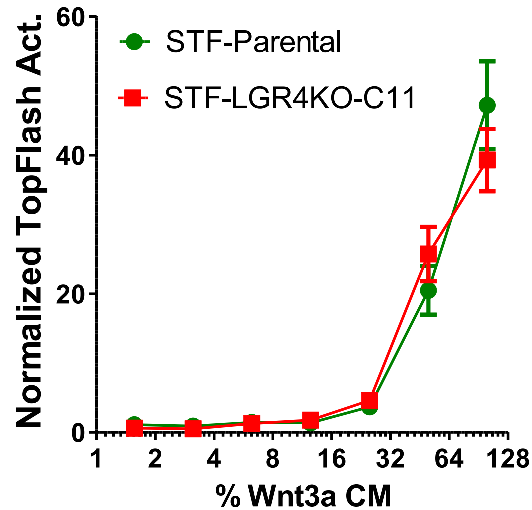


Figures S3. PCR and sequencing results of cDNA from STF-LGK4KO-C11 cells. A, DNA agarose gel results of RT-PCR products of mRNA from C11 cells by semi-nested PCR (1st round PCR primer pair: CAG AAT AAT CAG TTG AAA ACA GT/TTG CAC CAC GAA TGA CTA, 2nd round PCR primer pair: CAG AAT AAT CAG TTG AAA ACA GT/ATG CTG AGT TCC CCA CAA). The forward and primers pairs are located in the two exons flanking the exon that contains the sgRNA sequence. The major band has the expected size of 537-bp. No band was seen with the size of 321-bp, the expected size if the sgRNA-containing exon were skipped. B-C, ABI-chromatogram from sequencing the 537-bp using the forward primer (B) or reverse primer (C). The chromatograms showed the beginning of insertions/deletions that were consistent with the genomic sequencing results (Fig. S2). The red arrows mark the position of mutation.

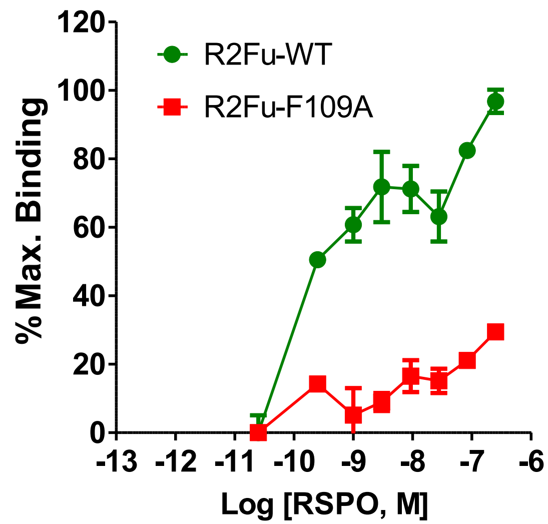
A



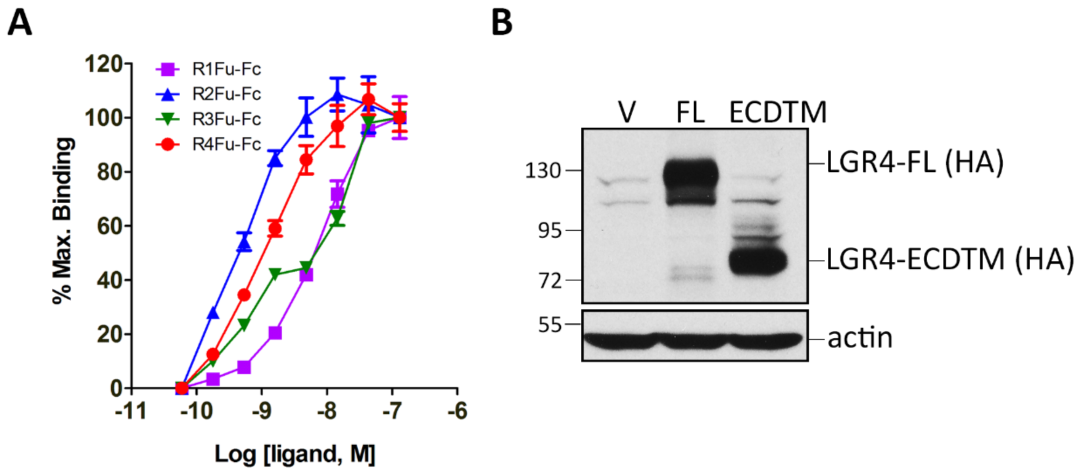
B



Figures S4. Response to Wnt3a alone and LGR4 rescue in STF-LGR4KO cells. A, TOPFlash data of LGR4KO-C6 and C11 cells transfected with either vector control (vect) or full-length human LGR4 in response to RPSO1. B, TOPFlash data of the response of STF-parental and LGR4KO –C11 cells to Wnt3a CM.



Figures S5. Binding of R2Fu-WT and R2Fu-F109A to HEK293 cells stably expressing full-length human LGR5.



Figures S6. Binding and expression of LGR4FL and LGR4ECDTM. A, Saturation binding of furin domains of RSPO1-4 to HEK293T cells overexpressing LGR4ECDTM. B, Western blot of HA-tagged LGR4 and actin in control vector, LGR4-full length (FL) or ECDTM transfected STF-LGR4KO cells from the experiment of Fig. 4.