

SUPPLEMENTAL DATA

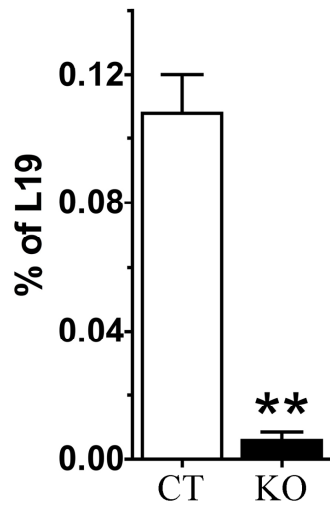


Fig. 1. Quantitative PCR analyses of RNA extracted from intestinal epithelia of *villin*^{Cre} induced homozygous CaSR KO mice (KO) and control littermates (CT) was performed with primers flanking the junction of exons 6 and 7 of *Casr* [Chang et al., (2008) *Sci Signal* **1**, ra1]. The levels of RNA expression are presented as the percentage of expression of the gene encoding the mitochondrial ribosomal protein L19 (***p* < 0.001, *n* = 6 mice per group).

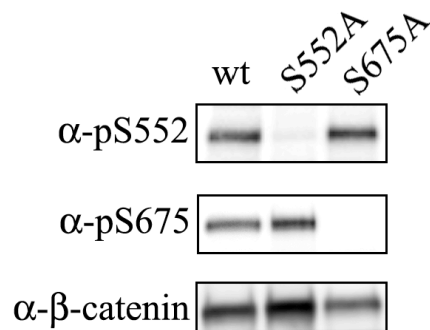


Fig. 2. Site-directed mutagenesis of a mammalian expression vector containing a β -catenin human cDNA (NM_001904) obtained from Origene (SC107921) was used as a template to replace Ser-552 for Ala or Ser-675 for Ala in the β -catenin cDNA and the obtained plasmids verified by DNA sequence analysis. HEK-293 cells in 33-mm dishes (3×10^5 cells/dish) were transfected with 1.0 μ g/dish of plasmids encoding encoding β -catenin wt, or mutants S552A or S675A. Transfections were carried out in Opti-MEM using Lipofectamine Plus according to the manufacturer suggested conditions (Invitrogen). After 18 hours, cell lysates were obtained and analyzed by Western blot using rabbit antibodies that recognizes phospho-Ser-552 or phospho-Ser-675 in β -catenin (Cell Signaling Technology) or a murine monoclonal antibody that recognizes β -catenin independently of its phosphorylation (BD Transduction). Signals were detected with a luminescent image analyzer.

Table 1: Morphometric analysis of *Casr* KO mice colonic crypts

	Control	KO	<i>P</i> value
Crypt height (μm)	121.4 \pm 1.7	181.1 \pm 2.4	< 0.0001
Crypt height (No. cells)	20.3 \pm 0.2	30.7 \pm 0.3	< 0.0001
Cell size (Crypt height [μm]/ crypt height [No. cells])	5.8 \pm 0.1	5.9 \pm 0.1	NS
Crypt circumference (No. cells)	14.5 \pm 0.1	15.53 \pm 0.2	NS
Total No. cells per crypt	296 \pm 4.9	476 \pm 8.9	< 0.0001

Full-length, longitudinally cut crypts (at least 20 per mouse) from 5 *Casr* KO and 5 control littermates were analyzed for crypt height (μm) and number of cells per crypt height. Cross-section of crypts (at least 20/mouse) was used to determine the average crypt diameter (μm) and circumference (in number of cells). These data were used to calculate cell size (crypt height in μm /crypt height in cell number) and estimate the total cells per crypt (mean cells per crypt column \times mean crypt circumference). Data from KO and control littermates were represented as mean \pm SEM and compared by unpaired Student's *t* test using SigmaPlot v 9 (Systat Software Inc.). NS: not significant different.

Table 2: Quantitative analysis of β -catenin localization in colonic epithelial cells

	induced-CaSR expression			non-induced CaSR expression		
	<i>Nuclear phospho-Ser552 β-catenin</i>					
	Control	Ca ²⁺ (5 mM)	R-568 (100 nM)	Control	Ca ²⁺ (5 mM)	R-568 (100 nM)
<i>Mean</i>	115.73	87.4	86.5	112.1	120.5	109.9
<i>SEM</i>	5.14	3.89	2.3	4.44	5.57	3.31
<i>N</i>	30					
<i>p</i>		0.004	0.0015		NS	NS
	<i>Plasma membrane β-catenin</i>					
<i>Mean</i>	83.14	110.89	105.8	76.23	79.7	81.56
<i>SEM</i>	6.8	8.1	4.43	3.8	3.5	4.3
<i>N</i>	20					
<i>P value</i>		0.035	0.023		NS	NS

NCMiCaR cells incubated without or with doxycycline (0.1 $\mu\text{g}/\text{ml}$) to induce the expression of the CaSR were challenged with 5.0 mM Ca²⁺ or 100 nM R-568 in a background of 1.4 mM Ca²⁺ for 1h and processed for immunofluorescence using rabbit anti- β -catenin phospho-Ser-552 and a mouse monoclonal anti-total β -catenin as primary antibodies and AlexaFluor 488 chicken anti-mouse IgG and Alexa Fluor 568 goat anti-rabbit IgG as secondary antibodies. Images were acquired with a 2048 x 2048 active pixels Spot Pursuit CCD Camera (Diagnostic Instruments, Inc) and regions of interest (ROI) corresponding to the nuclear and plasma membrane (cell's periphery) were defined using the imaging software SP2 V 4.0 (Carls Zeiss Microimaging GmbH). Quantification of the mean pixel intensity of the Red and Green channels corresponding to phosphoSer-552 β -catenin and β -catenin, respectively, were also determined using the imaging SP2 V 4.0 software. Values represent the mean pixel intensity \pm SEM and they were compared by unpaired Student's *t* test using SigmaPlot v 9 (Systat Software Inc.). NS: not significant different.