

SUPPLEMENTAL MATERIAL

Table S1. Primers used for genotyping and gene expression analyses

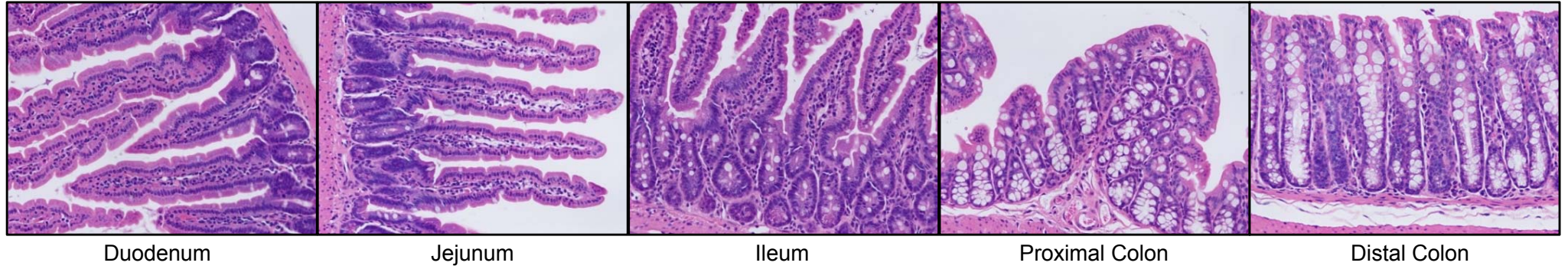
MR genotyping primers		
MR-7	5'-CTGGAGATCTGAACTCCAGGCT-3'	
MR-8	5'-CCTAGAGTTCCTGAGCTGCTGA-3'	
MR-10	5'-TAGAAACACTTCGTAAAGTAGAGCT-3'	

Cre genotyping primers		
Forward primer	5'-CCGCAGAACCTGAAGATGTTTCGC-3'	
Reverse primer	5'-CAGATTACGTATATCCTGGCAGCG-3'	

qPCR primers		
Gene product name	Forward primer	Reverse primer
18S rRNA	5'-TTCTGGCCAACGGTCTAGACAAC-3'	5'-CCAGTGGTCTTGGTGTGCTGA-3'
MR	5'-ATGGGTACCCGGTCCTAGAG-3'	5'-ACCAAGCAGATCTTGGAAAGG-3'
GR	5'-CTGGTGTGCTCCGATGAAGC-3'	5'-GAGACTCCTGCAGTGGCTTG-3'
α -ENaC	5'-GCACCCTTAATCCTTACAGATACTG-3'	5'-CAAAAAGCGTCTGTTCCGTG-3'
β -ENaC	5'-GGGTGCTGGTGGACAAGC-3'	5'-ATGTGGTCTTGGAAACAGGAATG-3'
γ -ENaC	5'-AACCTTACAGCCAGTGCACAGA-3'	5'-TTGGAAGCATGAGTAAAGGCAG-3'
SGK1	5'-CCAAACCCTCCGACTTTCAC-3'	5'-CCTTGTGCCTAGCCAGAAGAA-3'
NHE3	5'-CCACACACTGCAACAGTACC-3'	5'-ATAGGCAGTTTCCCATTAGG-3'
NKCC2	5'-CCTTTGACTTTGAGATTGGCGTG-3'	5'-GGGCTGGCTTGGTAATGTTTCCAG-3'
NCC	5'-CGTGGTGCCGGCCTACGAAC-3'	5'-AGGTGCCACCCGACTTGACCTT-3'
SGLT1	5'-GGGTGGCTTTGAATGGAA-3'	5'-CCTTGATGTAAATCGGGACAA-3'
SGLT2	5'-GCTGGATTTGAGTGGAAATGC-3'	5'-CGGTCAGATACTGGCACA-3'

MR: mineralocorticoid receptor; GR: glucocorticoid receptor; ENaC: epithelial sodium channel;
 SGK1: serum/glucocorticoid regulated kinase 1; NHE3: sodium-hydrogen exchanger 3;
 NKCC2: sodium-potassium-chloride cotransporter; NCC: sodium-chloride cotransporter;
 SGLT1: sodium-glucose cotransporter 1; SGLT2: sodium-glucose cotransporter 2

Control mice



IEC-MR-KO mice

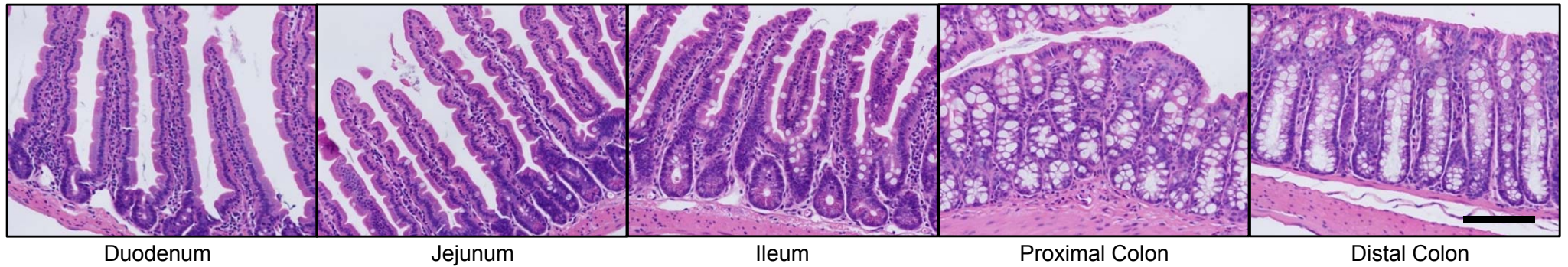


Figure S1. Histological evaluation of intestinal mucosal morphology by hematoxylin-eosin staining. Intestines, including the duodenum to the distal colon, of control (upper panels) and IEC-MR-KO mice (lower panels) are shown. Magnification: 200 \times , Scale bar: 100 μ m. IEC-MR-KO: intestinal epithelial cell-mineralocorticoid receptor-knockout.

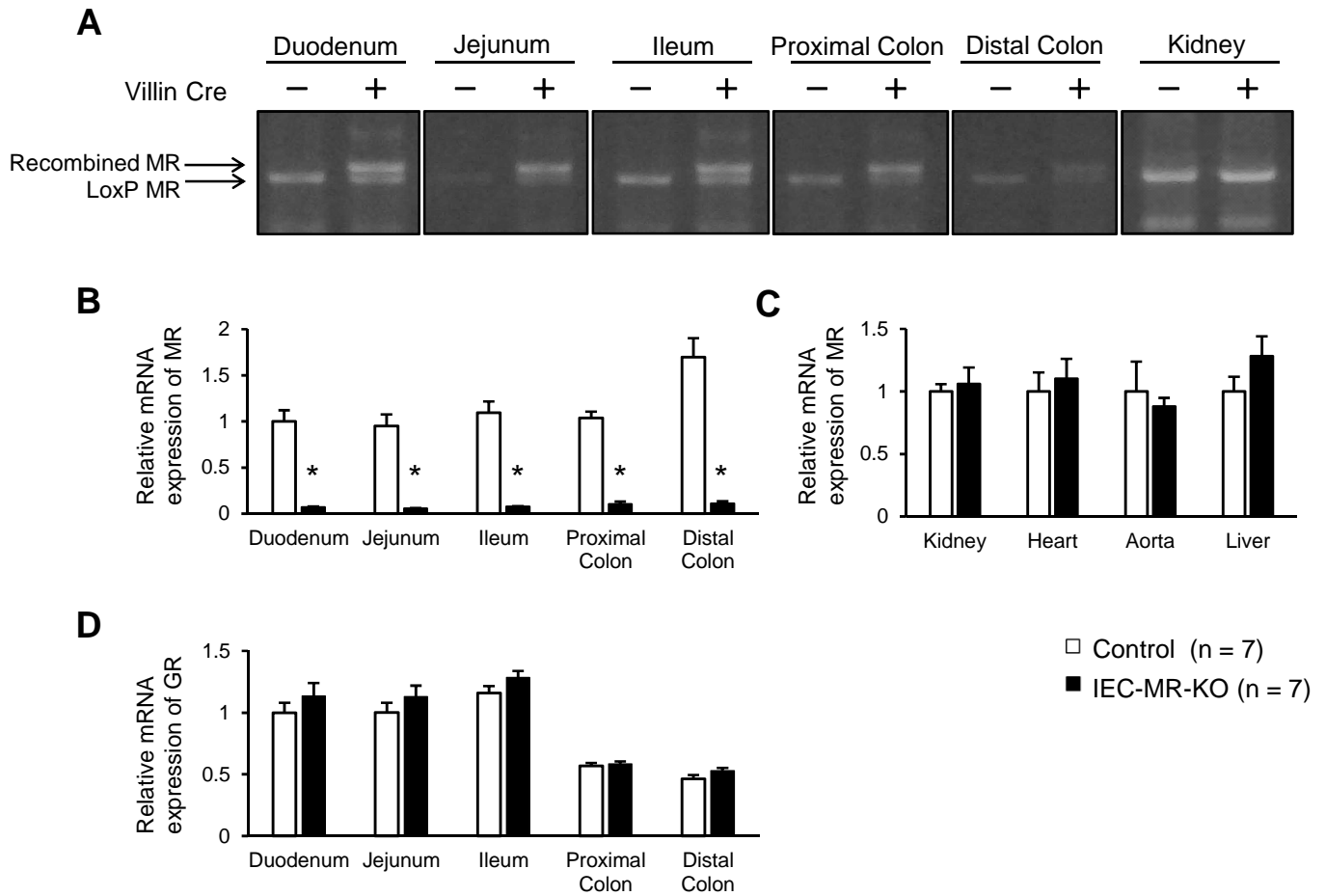


Figure S2. Specific deletion of MR from intestinal epithelial cells in IEC-MR-KO mice.

A. Genomic DNA was amplified with primers specific for LoxP MR or recombined MR. Recombination in MR occurs throughout the intestine of IEC-MR-KO mice (+), including the duodenum, jejunum, ileum, proximal colon, and distal colon. Recombination does not occur in the intestine of control mice (-) or the kidneys of either mouse line. **B,C.** mRNA expression level of MR throughout the intestine (**B**) and in the kidney, heart, aorta, or liver (**C**) of IEC-MR-KO and control mice. **D.** mRNA expression of GR in each intestinal portion between genotypes. The expression level in each intestinal portion was normalized to that in the duodenum of control mice. Data are presented as means \pm SEMs. * $P < 0.05$ vs. Control. IEC-MR-KO, intestinal epithelial cell-mineralocorticoid receptor-knockout.

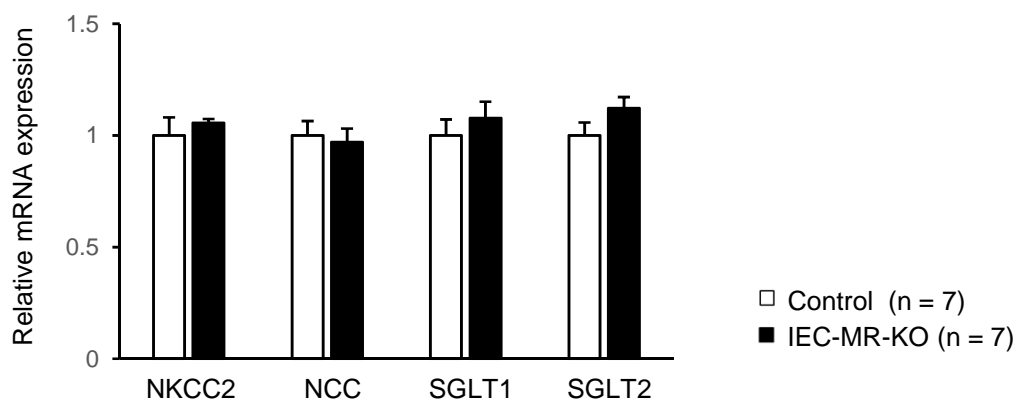


Figure S3. mRNA expression levels of NKCC2, NCC, SGLT1, and SGLT2 in the kidneys.

mRNA expression in the kidneys of NKCC2, NCC, SGLT1, and SGLT2 under standard diet as measured by qPCR. Gene expression was normalized to that of 18S rRNA and is expressed relative to that in control mice. Data are expressed as means \pm SEMs. n = 7 per genotype. IEC-MR-KO, intestinal epithelial cell-mineralocorticoid receptor-knockout.