# Supporting Information

## Ubelmann et al. 10.1073/pnas.1218446110

### SI Material and Methods

**Genotyping.** Mice genotypes were determined by PCR on genomic DNA extracted from mouse tail tips. Villin knockout was assessed using the following oligonucleotides: 5′-GGA AAC CCG ATA GTA TCC TG-3′ and 5′-GAC TAC ATA GCA GTC ACC ATC G-3′ amplifying a fragment of the villin knockout allele; 5′-CGA ATT CGC CAA TGA CAA GAC-3′ and 5′-GAC TAC ATA GCA GTC ACC ATC G-3′ amplifying a fragment of villin WT allele. A fragment of the monomeric Cherry-villin (mCherry-villin) plasmid was amplified with 5′-CCA GAC CGT TCA GCT GGA TAT TAC GGC CTT-3′ and 5′-CCT GAA TCG CCA GCG GCA TCA GCA- 3′. Finally, the villin:creERT2 transgene was determined using the oligonucleotides 5′-CAA GCC TGG CTC GAC GGC C-3′ and 5′-CGC GAA CAT CTT CAG GTT CT-3′.

**Tissue Cell Lysis and Western Blotting.** The intestine of adult mice was isolated and divided into three parts of identical length corresponding to the duodenum, jejunum, and ileum. For particular experiments, the colon was isolated and divided in two parts of identical length corresponding to proximal and distal colon. Frozen tissues were homogenized with a Dounce homogenizer in a solution containing 50 mM Tris·HCl (pH 7.5), 150 mM NaCl, 20 mM MgCl2, 5 mM EDTA, 1% Triton X-100, 1% Nonidet P-40, 0.5% SDS, and protease inhibitor mixture (Sigma-Aldrich). Cells grown at confluence were lysed in the same lysis solution. Proteins were extracted after centrifugation at 15,000 × *g* for 10 min at 4 °C. Thirty micrograms of total protein extract were used for electrophoresis on 7.5% SDS-polyacrylamide gels under reducing conditions and were transferred to a nitrocellulose membrane using standard procedures. Immunogens were visualized using the enhanced chemiluminescence method (Thermo Scientific). The primary antibodies used for immunoblots in these experiments are described in Table S1. Densitometry measurements were carried out using ImageJ software and were normalized to the loading controls.

**Plasmids.** The mCherry-villin and photoactivatable GFP plasmids used in this study were described previously (1,2). Actin-enhanced yellow fluorescent protein (EYFP) plasmid was obtained from Clontech. For endogenous villin depletion, the plKO vector (Addgene) was used. shRNA sequences specific for endogenous porcine villin were cloned inside the multiple cloning site and verified by sequencing. Two different hairpin sequences were used: for shRNA1, forward oligonucleotide: 5′-CCG GAA CCA GGC ACT GAA CTT TAT CCT CGA GGA TAA AGT TCA GTG CCT GGT TTT TTT G-3′; reverse oligonucleotide: 5′-AAT TCA AAA AAA CCA GGG CAC TGA ACT TTA TCC TCG AGG ATA AAG TTC AGT GCC TGG TT-3′; for shRNA2, forward oligonucleotide: 5′-CCG GAA GCC AGC CAG GAT GAA ATC ACT CGA GTG ATT TCA TCC TGG CTG GCT TTT TTT G-3′; reverse oligonucleotide: 5′-AAT TCA AAA AAA GCC AGC CAG GAT GAA ATC ACT CGA GTG ATT TCA TCC TGG CTG CTT-3′.

**Wound-Healing Assays.** In all time-lapse experiments cells were kept at 37 °C in a 5% $CO_2$ atmosphere during acquisition. For quantification of cell migration, 36 $\times 10^5$ cells were seeded and grown at confluence overnight in Ibidi small-culture inserts (Ibidi) placed on glass-bottomed dishes. Cells were serum starved for 90 min with DMEM/F12 (without phenol red) medium, and culture inserts were removed to induce cell migration. Medium then was replaced by DMEM/F12 (without phenol red) supplemented with 20% FCS. Then the wound-healing process was monitored by time-lapse video microscopy using a 10× CFI PLAN objective of an Eclipse Ti inverted wide-field microscope allowing multipositional acquisitions (Nikon) coupled to a Coolsnap HQ2 camera (Photometrics). Wounded area colonized by the monolayer was quantified carefully at the different time points using ImageJ. All quantifications were carried out on data obtained in the same experiment including the different experimental conditions being compared. To quantify the rate of lamellipodia formation, the number lamellipodia per 10× field was evaluated at 40-min intervals. To monitor wound healing at high resolution, cells were seeded and grown at confluence ($1 \times 10^6$ cells) overnight in glass-bottomed dishes. Cells were serum starved with DMEM/F12 medium. Wounds were induced by scratching the cell monolayer with a 0.6-gauge needle. After cells were washed with medium, the medium was replaced by DMEM/F12 (without phenol red) supplemented with 20% FCS. Then time-lapse image acquisition was performed using the 60×/1,4 OIL N2 PL APO VC objective of an inverted confocal spinning-disk Eclipse Ti Roper/Nikon microscope coupled to a Coolsnap HQ2 camera. Stacks were acquired with a z interval of 0.6 μm. To evaluate the number of migrating cells that had disassembled their microvilli, scratch assays were performed on cells grown on glass coverslips as described previously. After 30 min of migration, cells were washed and fixed in 4% paraformaldehyde (PFA). The number of cells developing a lamellipodium with or without microvilli was assessed by the presence or the absence of mCherry-positive microvilli and was quantified. All experiments were performed at least three times.

1. Revenu C, et al. (2007) Villin severing activity enhances actin-based motility in vivo. *Mol Biol Cell* 18(3):827–838.

2. Patterson GH, Lippincott-Schwartz J (2002) A photoactivatable GFP for selective photolabeling of proteins and cells. *Science* 297(5588):1873–1877.

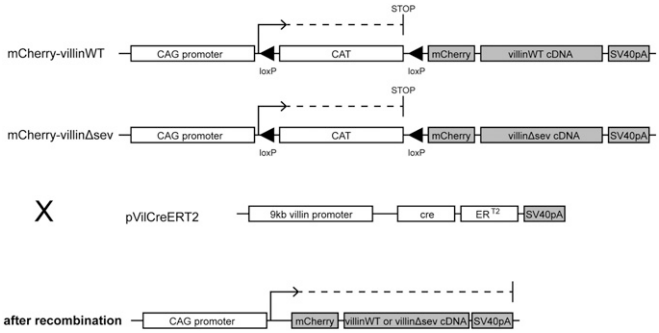


**Fig. S1.** Schematic representation of the transgenesis plasmids. The mCherry-villin constructs are inserted into the multiple cloning site of the pCAGloxCATlox transgenesis plasmid driven by the CAG promoter. Without recombination, the stop codon in the CAT sequence prevents transcription of the inserted constructs. Mice harboring the transgenesis plasmids are crossed with mice expressing the creERT2 protein under the villin promoter (1). Upon tamoxifen injection, recombination occurs in tissues where villin is normally expressed, leading to expression of the inserted constructs.

1. el Marjou F, et al. (2004) Tissue-specific and inducible Cre-mediated recombination in the gut epithelium. *Genesis* 39(3):186–193.

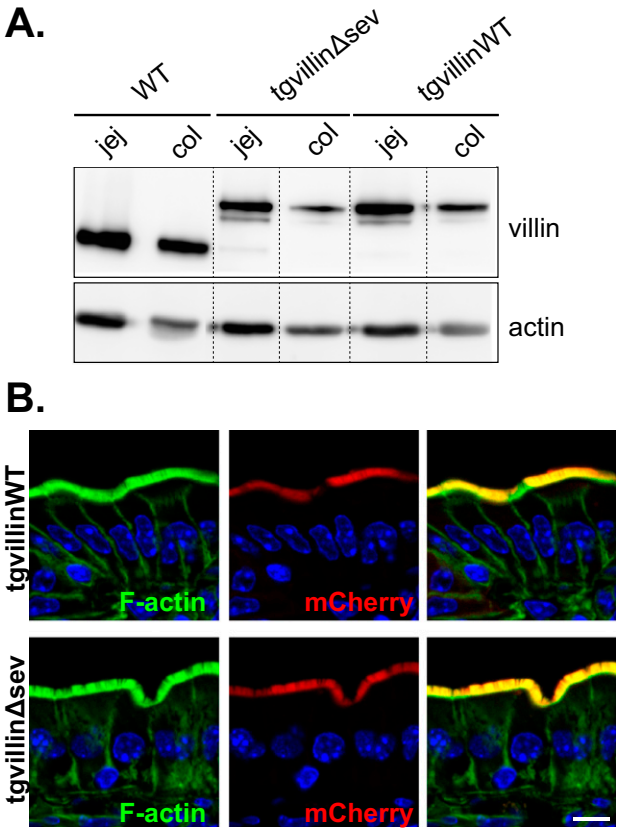


**Fig. S2.** Transgenic mouse models expressing WT villin or an actin-severing mutant recapitulate endogenous villin expression. (*A*) Western blot against villin and actin was performed on total extract from jejunum or colon of WT mice, villin knockout mice expressing transgenic WT villin (tgvillinWT), or villin knockout mice expressing tgvillinΔsev (tgvillinΔsev). The shift in villin molecular mass of tgvillinWT and tgvillinΔsev mice is caused by the mCherry tag. (*B*) Higher magnification of histological frozen sections of jejunum from tgvillinWT and tgvillinΔsev mice stained for F-actin by phalloidin (green). mCherry-villin fluorescence is shown (red). DAPI labels nuclei (blue). (Scale bar: 10 μm.)

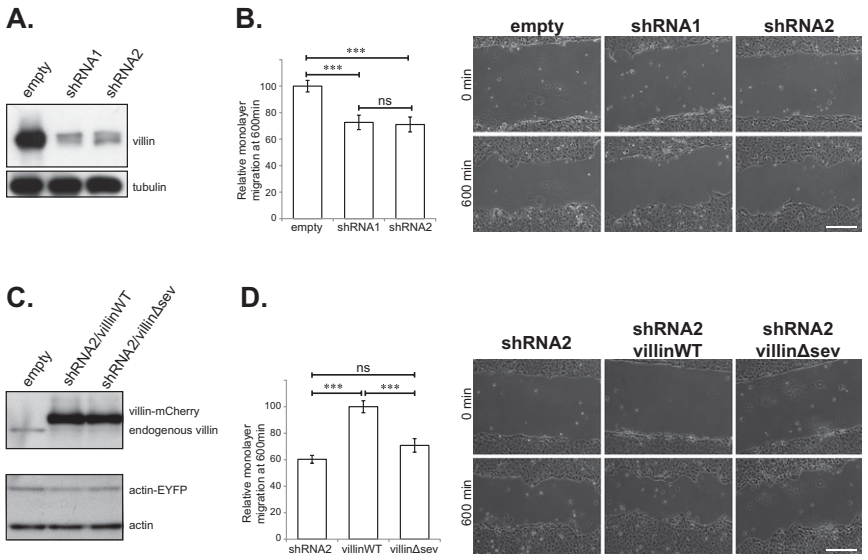


**Fig. S3.** Villin regulates cell migration positively through its actin-severing property. (*A*) Western blot against villin and tubulin performed against LLCPK-1 cells infected with lentiviral particles of shRNA empty plasmid or containing an shRNA sequence directed against porcine villin. Two different shRNA were used. Tubulin represents the loading controls. (*B*) (*Left*) Quantification of the area colonized by the LLCPK-1 monolayer at 10 h after wounding, normalized to 100 for the fastest cells. Values are shRNA1, 72.6 ± 5.5%; shRNA2, 71 ± 5.6%; ***$P < 0.001$; ns, nonsignificant ($P > 0.05$); *t* test. (*Right*) Serial micrographs from transmission light microscopy time-lapse recordings of wound-healing assays on a monolayer of LLCPK-1 cells infected with lentiviral particles of shRNA empty plasmid or containing shRNA sequences directed against porcine villin. (Scale bar: 100 μm.) (*C*) Western blot against villin and actin performed on LLCPK-1 cells infected with lentiviral particles of shRNA empty plasmid or containing shRNA sequences directed against porcine villin and reexpressing villinWT or villinΔsev human villin. (*D*) (*Left*) Quantification of the area colonized by the LLCPK-1 monolayer at 10 h after wounding, normalized to 100 for the fastest cells. Values are shRNA2, 60 ± 3%; villinΔsev, 71 ± 5%; ***$P < 0.001$; ns, nonsignificant ($P > 0.05$); *t* test. (*Right*) Serial micrographs from transmission light microscopy time-lapse recordings of wound-healing assays on a monolayer of LLCPK-1 cells infected with lentiviral particles containing shRNA sequences directed against porcine villin or reexpressing villinWT or villinΔsev human villin. (Scale bar: 100 μm.)

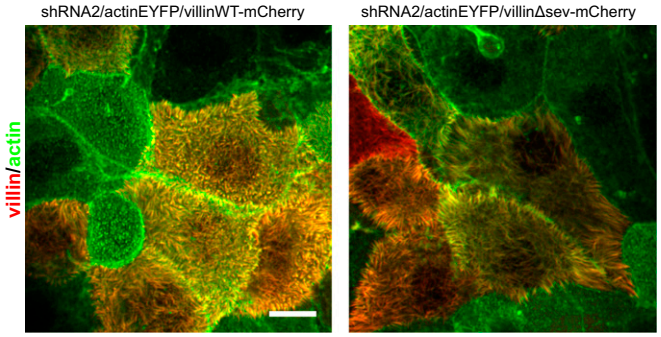


**Fig. S4.** Villin-mCherry proteins retain their microvillar distribution and morphogenetic effect. Maximal projections of *z* stacks acquired by spinning-disk microscopy. Micrographs represent overlays of EYFP-actin (green) and villinWT- or villinΔsev-mCherry (red). Note the presence of cells untransfected for villin. (Scale bar: 10 μm.)

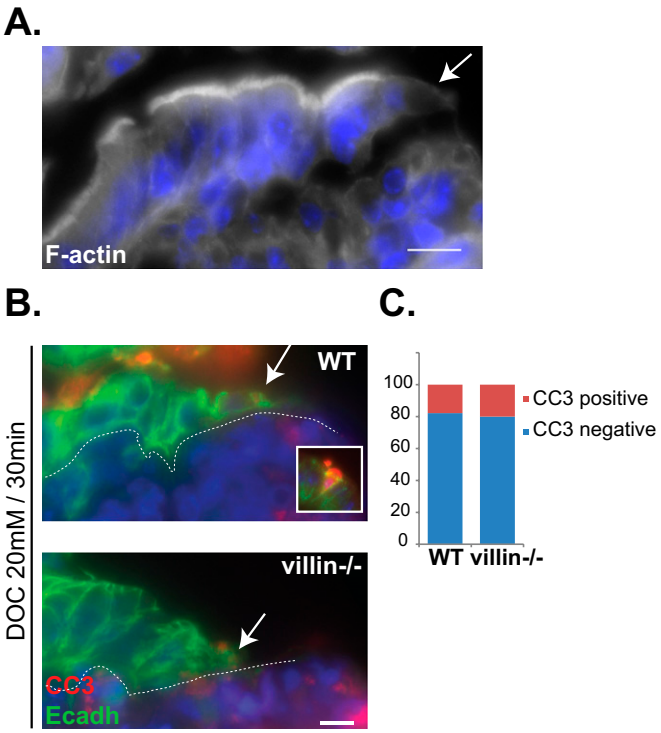


**Fig. S5.** Upon cell migration in vivo, WT cells are not apoptotic and show microvillus disassembly. (*A*) Histological frozen sections of the small intestine of WT mice luminally treated with deoxycholate sodium salt (DOC) 30 min after washout and stained for phalloidin (green). DAPI labels nuclei (blue). The arrow indicates the injury-adjacent enterocyte. (Scale bar: 10 μm.) (*B*) Histological paraffin sections of the small intestine of WT or villin$^{-/-}$ mice luminally treated with DOC 30 min after washout and stained for E-cadherin (green) and cleaved-caspase 3 (red). DAPI labels nuclei (blue). *Inset* shows a positive control which represents an apoptotic enterocyte at a villus tip. Basal dashed lines delineate the lamina propria; arrows indicate the injury-adjacent enterocytes. (Scale bar: 10 μm.) (*C*) Histogram showing the ratio of enterocytes at the wound edge positive and negative for cleaved caspase 3, quantified from histological paraffin sections of small intestine stained for cleaved caspase 3 from WT or villin$^{-/-}$ mice treated with DOC, 30 min after washout. Values are WT, 82%; villin$^{-/-}$, 80% of nonapoptotic cells.

**Table S1. List of antibodies used for immunohistochemistry and Western blotting**

| Antibody | Species | Source | Immunofluorescence: fixation used | Dilution |
|---|---|---|---|---|
| α-Tubulin | Mouse | Cell Signaling | Western blot | 1/1,000 |
| β-Actin | Mouse | Sigma | Western blot | 1/2,000 |
| Villin | Mouse | D.L. laboratory* | Western blot | 1/2,000 |
| Cleaved caspase 3 | Rabbit | Cell Signaling | Carnoy or PFA | 1/200 |
| E-cadherin | Mouse | BD | Carnoy or PFA | 1/400 |
| Ezrin | Rabbit | M. Arpin* | Carnoy | 1/800 |
| Ki-67 | Mouse | Abcys | PFA | 1/200 |
| Sucrase-isomaltase | Rabbit | B.L.N. | Carnoy | 1/1,000 |
| Villin | Rabbit | M. Arpin | PFA | 1/800 |

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