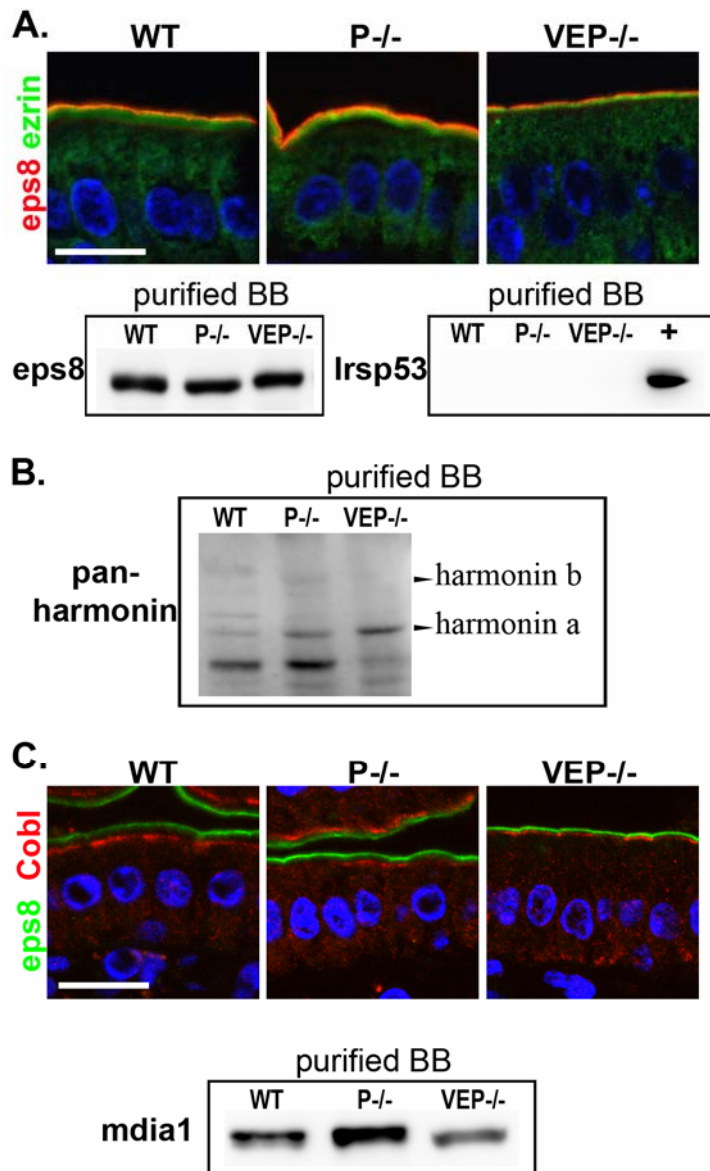


Figure S1

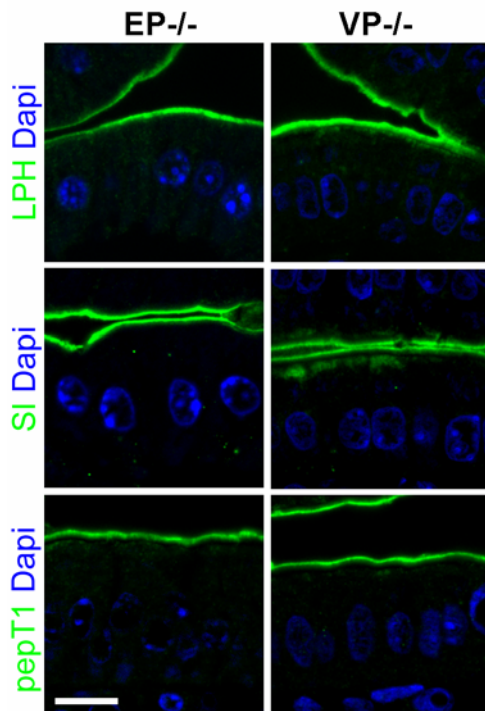


Supplementary figure 1

Distribution of potential actors of microvilli morphogenesis identified by proteomics analysis

Immunostainings and western blots on WT, P^{-/-} and VEP^{-/-} samples. **A-** Eps8 localisation at the tips of microvilli analysed by immunostainings and counterstained for the microvillus protein ezrin (top). Scale bar 10 μm. Detection of Eps8 and IRSp53 by immunoblots performed on isolated brush border lysates loaded for equal protein content (bottom). For IRSp53, a lysate of caco-2 cells has been used as positive control (+). **B-** Immunoblots using a pan-harmonin antibody performed on isolated brush border lysates. The expected sizes of isoforms a and b are highlighted. **C-** Analysis of the nucleators of parallel actin networks detected by mass spectrometry in WT, P^{-/-} and VEP^{-/-} enterocytes. Immunostainings for cordon-bleu (Cobl) counterstained with Eps8 to identify microvilli tips (top) show a terminal web localisation of the nucleator in all samples. Scale bar 10 μm. Immunoblots against mDia1 performed on isolated brush border lysates loaded for equal protein content (bottom) confirming its presence in the brush borders of the 3 samples.

Figure S2

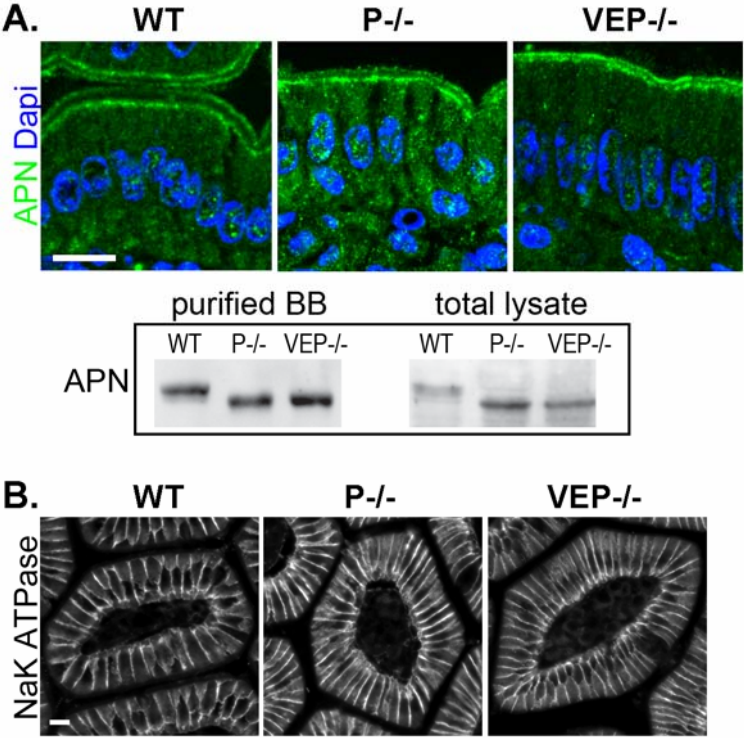


Supplementary figure 2

Apical enzyme localisation in EP-/- and VP-/- enterocytes

Immunostainings against the apical enzymes LPH, SI and PepT1 (green) on espin/plastin-1 (EP-/-) and villin/plastin-1 (VP-/-) samples. Dapi (blue) labels nuclei. Scale bar 10 μ m.

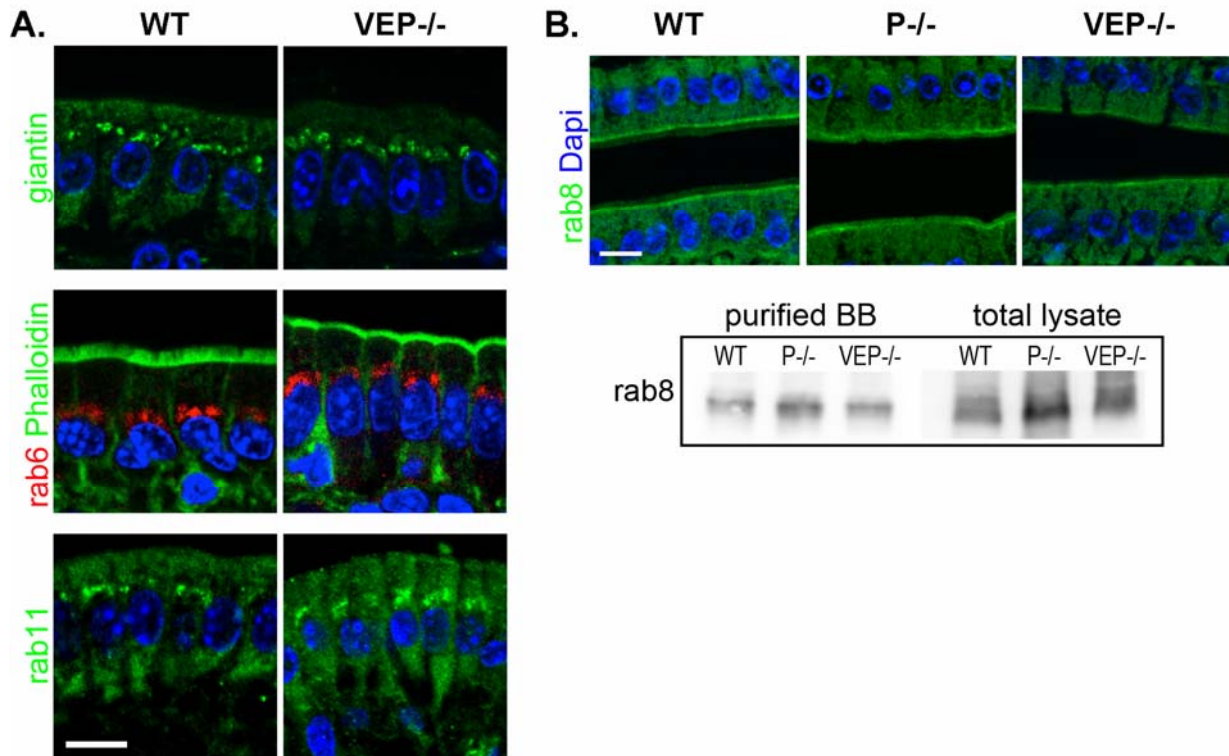
Figure S3



Supplementary figure 3
Not all polarity markers are affected in VEP-/- mice

A- Western blots and immunostainings against the apical enzyme aminopeptidase N (APN, green) on WT, P-/- and VEP-/- samples. Dapi labels nuclei (blue). Immunoblots are performed on total and isolated brush border lysates. **B-** Immunostainings for the basolateral pump Na⁺/K⁺ ATPase. Scale bars 10 μm.

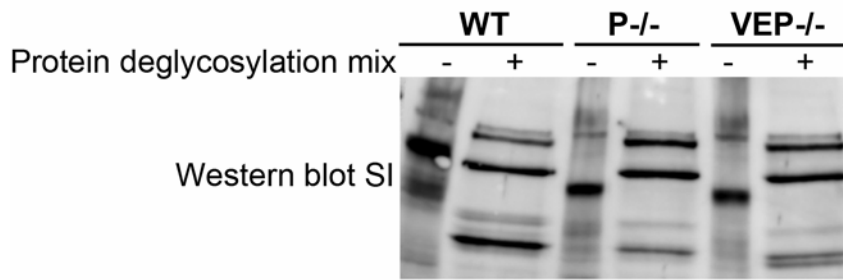
Figure S4



Supplementary figure 4
Unaffected apical trafficking markers in VEP-/- mice

A- Immunostainings for the Golgi marker giantin, for Rab6, a GTPase involved in intracellular transport at the level of the Golgi and for Rab11, a GTPase involved in apical recycling. **B-** Immunostainings and Western blots against the GTPase Rab8 on WT, P-/- and VEP-/- samples. Western blots are performed on total and isolated brush border lysates. Dapi labels nuclei (blue). Scale bars 10 μm.

Figure S5

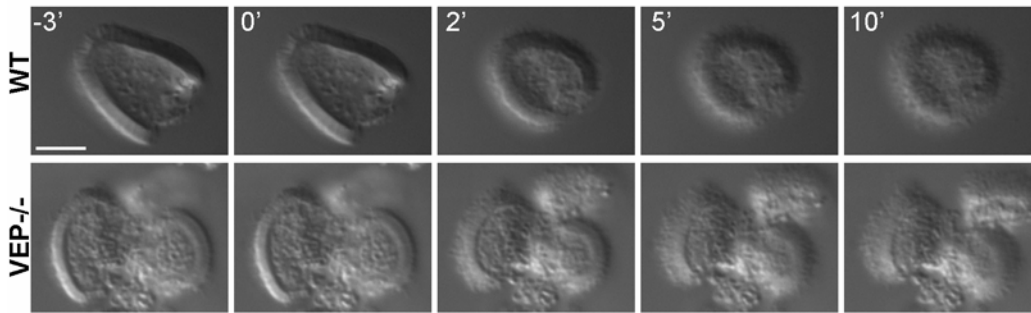


Supplementary figure 5

Altered glycosylation pattern of digestive hydrolases in P^{-/-} and VEP^{-/-} enterocytes

Immunoblot against sucrase-isomaltase (SI) performed on enterocyte total lysate from WT, P^{-/-} and VEP^{-/-} mice treated or not with a Protein Deglycosylation Mix (NEB).

Figure S6



Supplementary figure 6

Vesicular membrane extrusion is preserved in brush borders of VEP-/- mice

DIC time-lapse series of WT and VEP-/- isolated brush borders before (-3') and after (0 to 10') addition of 200 μ M ATP showing the apical shedding of vesicles in both conditions. Bar 5 μ m.

Table S1: Actin binding proteins identified in WT isolated brush borders

Identifier	Description	Matching peptides	MW	Coverage
Bundling				
gi 85986577	Plastin 1	43	70.4	46.5
gi 148667910	Villin 1	38	92.9	42.6
gi 46877084	Espin isoform 6	5	28.1	28.9
Cross linking				
gi 11230802	Actinin alpha 4	43	105	43.9
gi 61097906	Actinin alpha 1	16	103.1	20.7
gi 7304855	Actinin alpha 3	6	103	8.3
gi 38257404	Filamin B	14	277.8	8.3
gi 38257560	Filamin A	5	281.2	2.8
Capping				
gi 2833214	Eps8, EGF receptor kinase substrate 8	14	91.7	26.1
gi 83649737	Capping protein, muscle Z-line, beta isoform a	7	31.3	26
gi 6671672	Capping protein, muscle Z-line, alpha 2	5	33	25.5
gi 161086971	F-actin capping protein alpha-1 subunit	5	33	25.5
Nucleation				
gi 23956222	ARP3 actin-related protein 3 homolog	7	47.4	18.4
gi 148708705	Cordon-bleu, isoform CRA_b	4	146	3.5
gi 6681183	Diaphanous homolog 1	2	139.3	2.2
Myosins				
gi 71151983	Myosin heavy chain 14, NMHC II-C	151	228.6	54
gi 124487037	Myosin IA	54	118.7	37.8
gi 114326446	Myosin, heavy polypeptide 9, non-muscle isoform 1	51	226.4	27.3
gi 148694488	Myosin-VI	26	146.3	22.9
gi 148664634	Myosin VIIb, isoform CRA_b	22	241	11.8
gi 118026911	Myosin ID	19	116.1	18.4
gi 50510675	Myosin-11 isoform	18	228.3	9.1
gi 123262062	Myosin XVB	13	258.7	7.5
gi 17986258	Myosin light chain 6, smooth muscle and non-muscle	12	16.9	57
gi 13432181	Myosin-Ib	8	128.5	7
gi 148677575	Myosin Vb, isoform CRA_a	7	213.4	4
gi 56205559	Myosin, heavy polypeptide 10, non muscle	6	232.5	2.5
gi 15809016	Myosin regulatory light chain MRCL2	5	19.8	35.5
gi 124494242	Myosin IC isoform a	5	119.9	6.2
gi 125987842	Myosin-XVIIIa	6	232.8	5.4
gi 30410852	Myosin IE	5	126.8	5.3
Others				
gi 40849928	Plectin 1 isoform 11	112	517.3	28
gi 50881	Ezrin	17	69.3	30.9
gi 149251314	Cingulin	16	149.5	20.8
gi 41281802	Harmonin	10	102.3	15.1
gi 122890249	Coronin, actin binding protein 2A	8	61.7	10.5
gi 2851563	Adseverin	8	80.3	21.1
gi 11127935	Epithelial protein lost in neoplasm-b, eplin	8	84.1	15.5
gi 149751320	Similar to tropomyosin 3 isoform 1	5	29	18.1
gi 11528490	Flightless I homolog	6	144.8	5.8
gi 148669742	Adducin 3, isoform CRA_c	3	83.5	5.5
gi 148665000	Dynamin 1-like, isoform CRA_c	3	83.3	3.9
gi 149255823	Similar to Cofilin 1	2	18.5	15.7

Table S2: Proteins identified in VEP-/- but not in WT isolated brush borders

Identifier	Description	Matching peptides	MW	Cover-age
Actin binding				
gi 5031569	ARP1 actin related protein 1 homolog A, centractin alpha	3	42.6	12.8
Tra c				
gi 148704358	Adaptor protein complex AP-1, gamma 2 subunit, isoform CRA_a	6	91.9	9.2
gi 48734610	Sec31a protein	6	98.2	9.5
gi 67906177	Sec23A	6	86.2	11.1
gi 148669551	Sec24 related gene family, member C, isoform CRA_a	4	118.6	4.7
gi 149254026	Sec31 like 1	4	144.1	5.7
gi 23956096	USO1 homolog, vesicle docking protein	3	107	3.4
gi 55670639	Chain M, Ap1 Clathrin Adaptor Core	3	48.5	5.4
Junction				
gi 148669535	Vinculin, isoform CRA_b	5	123.9	5
gi 156255171	Adducin 1 (alpha) isoform 1	4	80.6	7.6
Others				
gi 144922656	Lethal giant larvae homolog 2	3	114.3	3.7
gi 7106301	Microtubule associated protein, RP/EB family, member1	3	30	20.5

Table S3: Proteins lost in VEP-/- compared to WT isolated brush borders

Identifier	Description	Matching peptides	MW	Coverage
Actin bundling proteins				
gi 148667910	villin 1 Mus musculus	33	92.9	41.5
gi 85986577	plastin 1, LOC102502 Mus musculus	35	70.4	46.5
gi 46877084	espin isoform 6 Mus musculus	3	28.1	20.2
Others				
gi 115704	RecName: Full=Catalase unspecified organism	8	59.8	21.4
gi 148699462	mCG12140, isoform CRA_a Mus musculus	3	8.3	31.9
gi 148679891	mCG132388 Mus musculus	3	22.2	18.6
gi 148686945	RIKEN cDNA 9030617O03, isoform CRA_b Mus	3	74	5.7
gi 148694085	calmodulin-like 4, isoform CRA_c Mus musculus	3	20.4	6.2
gi 6681727	sodium-glucose cotransporter 1 Mus musculus	3	73.1	5.6
gi 6755114	peroxiredoxin 5 precursor Mus musculus	3	21.9	10.5
gi 73622267	methylcrotonoyl-Coenzyme A carboxylase 2 (β)	3	61.4	6.9
gi 13385310	propionyl Coenzyme A carboxylase, β polypeptide	3	58.4	7.4
gi 148540106	methylmalonyl-Coenzyme A mutase Mus	3	82.8	6
gi 15126777	carnitine O-octanoyltransferase Mus musculus	3	70.2	8.2
gi 145275168	glutathione peroxidase 2 Mus musculus	3	21.8	12.6

Tables S1, S2 and S3

Tables show identifier (NCBI protein accession number), description (NCBI protein definition), matching peptides (number of peptides identified per experiment), MW (molecular weight in Daltons), coverage (% of sequence coverage identified from MS/MS data) of the proteins identified by mass spectrometry. Shown is the best experiment.