Online supplemental materials and methods:

List of primary antibodies used for immunohistochemistry

Antibody against	Origin	Dilution	Sample
a-tubulin	Mouse monoclonal, Sigma	1/200	Cryosections
APN	Rabbit polyclonal from E.M. Danielsen	1/500	Paraffin Carnoy
cordon-bleu	Rabbit polyclonal from J. Klingensmith	1/250	Paraffin Carnoy
DPPIV	Rabbit polyclonal from A. Hubbard	1/500	Paraffin Carnoy
E-cadherin	Mouse monoclonal, Transduction	1/200	Paraffin PFA
EEA1	Goat polyclonal, Santa Cruz	1/50	Paraffin Carnoy
Eps8	Mouse monoclonal, BD Biosciences	1/200	Paraffin MetOH
ezrin	Rabbit polyclonal from M. Arpin	1/250	Paraffin Carnoy
giantin	Rabbit polyclonal, Covance	1/200	Paraffin MetOH
IAP	Rabbit polyclonal from our laboratory	1/500	Paraffin Carnoy
LPH	Mouse monoclonal from A. Quaroni (Gift from R. Jacob)	1/500	Paraffin Carnoy
myosin-1a	Rabbit polyclonal from M. Mooseker	1/250	Paraffin MetOH
Na/K ATPase	Rabbit serum from F. Jaisser	1/50	Paraffin PFA
pan-cytokeratins	Rabbit polyclonal, Dako	1/200	Paraffin PFA
pepT1	Rabbit polyclonal from G. Kellett	1/500	Paraffin Carnoy
Rab11	Mouse monoclonal, BD Biosciences	1/100	Paraffin MetOH
Rab6	Rabbit polyclonal, Santa Cruz Biotech	1/200	Cryosections
Rab8	Mouse monoclonal, BD Biosciences	1/500	Paraffin MetOH
SI	Mouse monoclonal from A. Quaroni (Gift from R. Jacob)	1/500	Paraffin Carnoy
ZO-1	Rabbit polyclonal, Zymed	1/200	Paraffin PFA

Brush border shedding assay

A flow chamber was made of a polylysine coated coverslip fixed with double-sided tape on a microscope slide. Isolated brush borders kept on ice were injected into the slit and incubated for few minutes to allow adherence before washing extensively with solution B. The adherent isolated brush borders were observed by differential interference contrast microscopy. Movies were recorded with one frame every 5 seconds for 15 minutes. At approximately 3 minutes of recording, a solution B supplemented with 200µM ATP was injected into the flow chamber to replace the former solution free of ATP. The acquisitions were made with an epifluorescence microscope (Leica DM 6000B) coupled to a CCD camera (Roper CoolSnap HQ) and driven by the software Metamorph.

Statistical analysis of growth curves

We measured the evolution of the weight of 86 mice of three different genotypes distributed as follows:

- WT (wild type) genotype: 6 litters, 18 males, 17 females
- VP-/- genotype: 2 litters, 6 males, 10 females
- VEP-/- genotype: 4 litters, 17 males, 18 females

Model:

In order to assess the significance of the genotype effect we adjusted a mixed-effects model to the growth curves. Usually mixed effects models with a random effect "individual" are used when analyzing growth curves because they enable us to model the natural increase of variance with time using auto-covariance structures. Unfortunately here we were not able to follow up individually the evolution of the weights so we could not introduce a random effect "individual" in our model. The fixed effects are the genotype (VP for VP-/-, VEP for VEP-/- or WT for wild type) and the sex, and the only random effect is the litter. In order to stabilize the variance we applied a logarithmic transformation to the data. The model incorporates a linear and a logarithmic growth rate, and the litter as a random effect on both the birth weight of the animals and the linear growth rate. The model is written as follows:

$$Y_{ijkl}(t) = \alpha_{0,i} + \beta_{0,j} + f_{0,k} + \alpha_{1,i}t + \beta_{1,j}t + f_{1,k}t + \alpha_{2,i}\log(t) + \beta_{2,j}\log(t) + \varepsilon_{ijkl}$$

Y_{ijkl} = log(weight) of the mouse l with genotype i, sex j and belonging to the litter k

$\alpha_{0,i}$ = genotype effect on the birth log(weight), $i \in \{WT,VP,VEP\}$

$\beta_{0,j}$ = sex effect on the birth log(weight), $j \in \{M,F\}$

$\alpha_{1,i}$ = genotype effect on the linear growth rate, $i \in \{WT,VP,VEP\}$

$\beta_{1,j}$ = sex effect on the linear growth rate, $j \in \{M,F\}$

$\alpha_{2,i}$ = genotype effect on the logarithmic growth rate, $i \in \{WT,VP,VEP\}$

$\beta_{2,j}$ = sex effect on the logarithmic growth rate, $j \in \{M,F\}$

$$f_k = \begin{Bmatrix} f_{0,k}: \text{random litter effect on the birth log(weight)} \\ f_{1,k}: \text{random litter effect on the linear growth rate} \end{Bmatrix} \sim N(0,\Sigma), \quad \Sigma = \begin{pmatrix} \sigma_0 & cov_{01} \\ cov_{10} & \sigma_1 \end{pmatrix}$$

Results:

We tested the significance of the fixed effects in comparison to a reference, which is the wild type, female animals. The statistical tests are the following:

- Does the genotype have an effect on the birth log(weight) ?
  H0: $\alpha_{0,i} = \alpha_{0,WT}$ **for** $i \in \{VEP, VP\}$ **vs H1 :** $\exists\, i \in \{VEP, VP\} / \alpha_{0,i} \neq \alpha_{0,WT}$ **(test 1)**
- Does the sex have an effect on the birth log(weight) ?
  H0: $\beta_{0,M} = \beta_{0,F}$ **vs H1 :** $\beta_{0,M} \neq \beta_{0,F}$ **(test 2)**
- Does the genotype have an effect on the linear growth rate?
  H0: $\alpha_{1,i} = \alpha_{1,WT}$ **for** $i \in \{VEP,VP\}$ **vs H1 :** $\exists\, i \in \{VEP,VP\} / \alpha_{1,i} \neq \alpha_{1,WT}$ **(test 3)**
- Does the sex have an effect on the linear growth rate?
  H0: $\beta_{1,M} = \beta_{1,F}$ **vs H1 :** $\beta_{1,M} \neq \beta_{1,F}$ **(test 4)**

- Does the genotype have an effect on the logarithmic growth rate?
  H0: $\alpha_{2,i} = \alpha_{2,WT}$ **for** $i \in \{VEP, VP\}$ **vs H1 :** $\exists\, i \in \{VEP, VP\} / \alpha_{2,i} \neq \alpha_{2,WT}$ **(test 5)**
- Does the sex have an effect on the logarithmic growth rate?
  H0: $\beta_{2,M} = \beta_{2,F}$ **vs H1 :** $\beta_{2,M} \neq \beta_{2,F}$ **(test 6)**
- Does the litter have an effect on the birth log(weight) and on the linear growth rate ?

$$H0 : \Sigma = \begin{pmatrix} \sigma_0 & \mathrm{cov}_{01} \\ \mathrm{cov}_{10} & \sigma_1 \end{pmatrix} \text{ vs } H1 : \Sigma = \begin{pmatrix} 0 & 0 \\ 0 & 0 \end{pmatrix} \quad \textbf{(test 7)}$$

The genotype has no significant effect on the birth weight (test 1: $\alpha_{0,VEPi} = \alpha_{0,WT}$ and $\alpha_{0,VP} = \alpha_{0,WT}$) whereas the sex has a significant effect on the birth weight (test 2: $\beta_{0,M} \neq \beta_{0,F}$, p-value = $5.6e^{-5}$). The effect of the VEP genotype significantly differs from the effect of the WT genotype for the linear growth rate, while the VP genotype has no effect (test 3: $\alpha_{1,VEP} \neq \alpha_{1,WT}$, p-value = 0.021, and $\alpha_{1,VP} = \alpha_{1,WT}$, p-value=0.79). The sex has a significant effect on the linear growth rate (test 4: $\beta_{1,M} \neq \beta_{1,F}$, p-value = $1e^{-14}$). Both the sex and the VEP genotype have an effect on the logarithmic growth rate (test 5 : $\alpha_{2,VEP} \neq \alpha_{2,WT}$, p-value = $2.2e^{-15}$; test 6: $\beta_{2,M} \neq \beta_{2,F}$, p-value = $1.2e^{-4}$), while the VP genotype has no effect (test 5: $\alpha_{2,VP} = \alpha_{2,WT}$, p-value=0.21). The test 7 is a log-likelihood ratio test between this model and the corresponding fixed effects model (same model without random effects). The p-value of this test is significant (p-value < $1e^{-4}$), meaning that the litter has a significant random effect on both the birth weight and the linear growth rate.

We then wanted to test the significance of the overall difference between the predicted growth curves of animals having different genotypes and the same sex. This is equivalent to test the nullity of a linear combination of the parameters. The statistical test and the corresponding combinations are defined as follow:

- Is there a difference between predicted growth curves of wild-type and VP animals having the same sex? **(test 8)**

$$\begin{aligned} \mathbf{H0}&: \Delta_{WT-VP}(t) = \alpha_{0,WT} - \alpha_{0,VP} + (\alpha_{1,WT} - \alpha_{1,VP}) * t + (\alpha_{2,WT} - \alpha_{2,VP}) * log(t) = 0 \\ \text{VS}\quad \mathbf{H1}&: \Delta_{WT-VP}(t) = \alpha_{0,WT} - \alpha_{0,VP} + (\alpha_{1,WT} - \alpha_{1,VP}) * t + (\alpha_{2,WT} - \alpha_{2,VP}) * log(t) \neq 0 \end{aligned} \quad , \; t \in [1.60]$$

- Is there a difference between predicted growth curves of wild-type and VEP animals having the same sex? **(test 9)**

$$\begin{aligned} \mathbf{H0}&: \Delta_{WT-VEP}(t) = \alpha_{0,WT} - \alpha_{0,VEP} + (\alpha_{1,WT} - \alpha_{1,VEP}) * t + (\alpha_{2,WT} - \alpha_{2,VEP}) * log(t) = 0 \\ \text{VS}\quad \mathbf{H1}&: \Delta_{WT-VEP}(t) = \alpha_{0,WT} - \alpha_{0,VEP} + (\alpha_{1,WT} - \alpha_{1,VEP}) * t + (\alpha_{2,WT} - \alpha_{2,VEP}) * log(t) \neq 0 \end{aligned} \quad , \; t \in [1.60]$$

- Is there a difference between predicted growth curves of VP and VEP animals having the same sex? **(test 10)**

$$\begin{aligned} \mathbf{H0}&: \Delta_{VP-VEP}(t) = \alpha_{0,VP} - \alpha_{0,VEP} + (\alpha_{1,VP} - \alpha_{1,VEP}) * t + (\alpha_{2,VP} - \alpha_{2,VEP}) * log(t) = 0 \\ \text{VS}\quad \mathbf{H1}&: \Delta_{VP-VEP}(t) = \alpha_{0,VP} - \alpha_{0,VEP} + (\alpha_{1,VP} - \alpha_{1,VEP}) * t + (\alpha_{2,VP} - \alpha_{2,VEP}) * log(t) \neq 0 \end{aligned} \quad , \; t \in [1.60]$$

In **Figure 1** we visualize the results of these tests in a convenient way: we have plotted $\Delta_{VP\text{-}WT}(t)$, $\Delta_{VEP\text{-}WT}(t)$, $\Delta_{VEP\text{-}VP}(t)$ for each t with error bars representing the 95% confidence interval for each value. For each of the three test functions above we obtain a "difference

curve" with a confidence interval around the curve, so that if the confidence interval enclose/wrap/contain the horizontal axis y=0 then the H0 hypothesis cannot be rejected: there is no difference between the two predicted growth curves.

The difference between VP-/- and wild type animals is not significant from the 19th day (test 8: **Figure 1**, top), confirming that the animals from these two genotypes have similar growth curves. The difference between the growth curves of VEP-/- and wild type animals is significant from the second day (test 9: **Figure 1**, middle) and the difference between the growth curves of VP-/- and VEP-/- animals is significant at every time point (test 10: **Figure 1**, bottom). In both cases, the growth curve of the VEP-/- animals is below the ones of the other genotypes. Globally, for a given sex the predicted growth curve of VEP-/- animals is significantly below the predicted growth curve of both wild type animals and VP-/- animals, while the latter two are not significantly different.

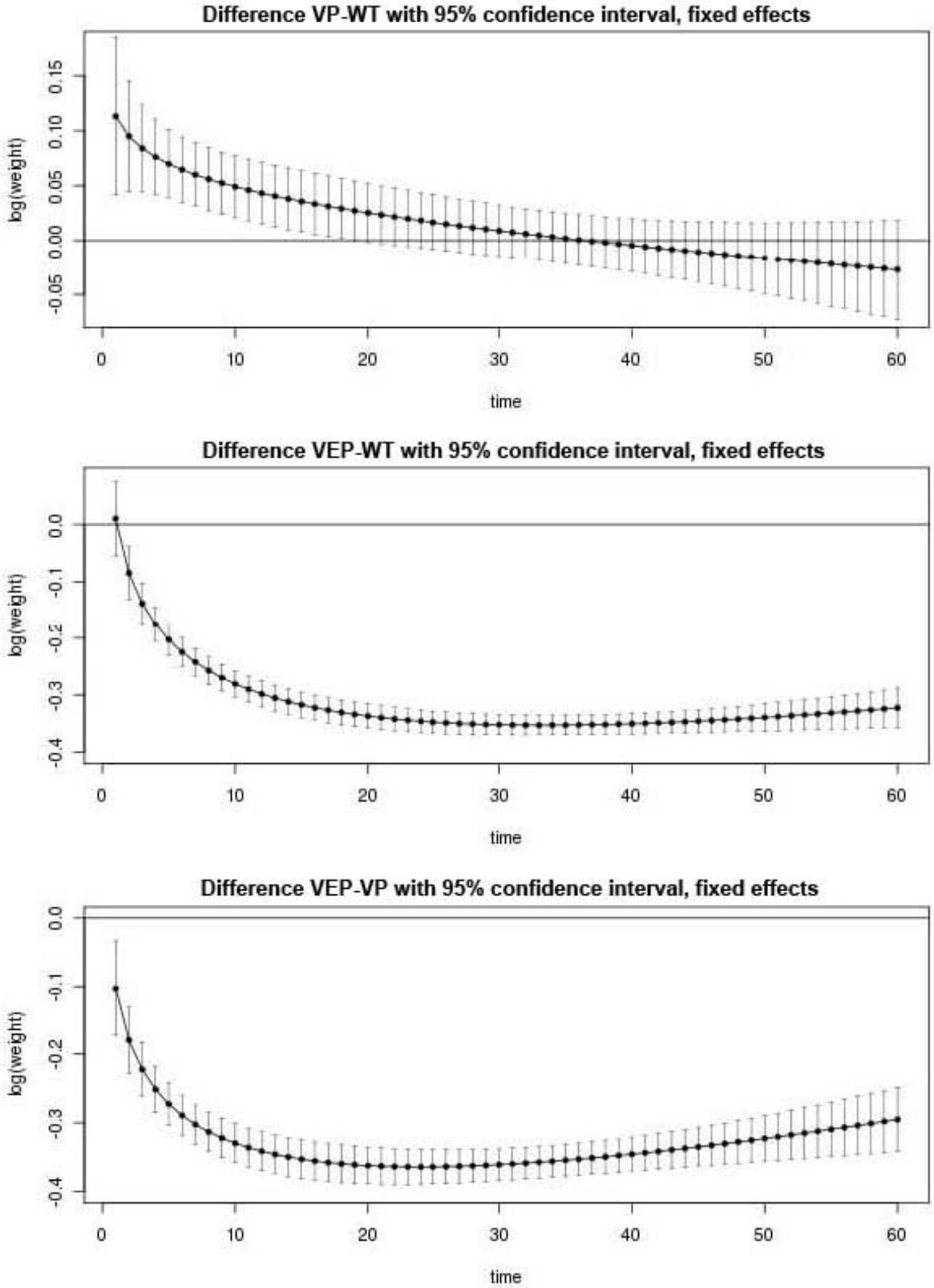

Figure 1: *Predicted difference between growth curves, genotypes pair-wise comparisons. Top figure: predicted difference between WT and VP animals having the same sex. Middle figure: predicted difference between WT and VEP animals having the same sex. Bottom figure: predicted difference between VP and VEP animals having the same sex.*

Mass spectrometry sample preparation and analysis

Trypsin Digestion and Nano-LC-MS/MS Analysis

50 µg of purified brush border (BB) extracts from WT or vep animals were run on a 7-15% SDS-PAGE gel, stained with *LabSafe* GEL Blue™ (G Biosciences) and bands were cut out into regular slices of 1 mm. In-gel digests were performed as described in standard protocols. Briefly, the dry gel slices were reduced by adding 10 mM DTT (Sigma Aldrich) prior to alkylation with 55 mM iodoacetamide (Sigma Aldrich). After washing and shrinking of the gel pieces with 100% acetonitrile, trypsin (125ng, Sequencing Grade Modified, Roche Diagnostics) was added and proteins were digested overnight in 25 mM ammonium bicarbonate at 30°C. The resulting peptides were extracted from the gel by adding 20 µL of acetonitrile/water/formic acid (60/35/5) for 10 min. The extracted peptides were dried and resolubilized in solvent A (95/5 water/acetonitrile in 0.1% [wt/vol] formic acid). In a second step, 50 µg of WT BB extract were separated before or after trypsin digestion by using the 3100 OFFGEL Fractionator (Agilent Technologies, Böblingen, Germany).

The peptides mixtures were analyzed by nano-LC-MS/MS using an Ultimate3000 system (Dionex) coupled to an LTQ-Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Samples are loaded on a C18 precolumn (300 µm inner diameter x 5 mm; Dionex) at 40 µl/min in 5% acetonitrile, 0.1% formic acid. After 3 min of desalting, the precolumn was switched on line with the analytical C18 column (75 µm inner diameter x 15 cm; C18 PepMap™, Dionex) equilibrated in 95% solvent A and 5% solvent B (80% acetonitrile, 0.085% formic acid). Peptides were eluted using a 5–50% gradient of solvent B during 60 min at a 200 nl/min flow rate. Data-dependent acquisition was performed on the LTQ-Orbitrap mass spectrometer in the positive ion mode. Survey MS scans were acquired in the orbitrap on the 465-1600 m/z range with the resolution set to a value of 30 000. Each scan was recalibrated in real time by co-injecting an internal standard from ambient air into the C-trap ('lock mass option'). The 5 most intense ions per survey scan were selected for CID fragmentation and the resulting fragments were analyzed in the linear trap (LTQ). Target ions already selected for MS/MS were dynamically excluded for 120 s. Data were acquired using the Xcalibur software (version 2.0.5).

Database Search and Data Analysis

The resulting spectra where then analyzed via the Mascot™ Software created with Proteome Discoverer (version: 1.2.0.92, Thermo Scientific) using the "mus musculus" (house mouse) database of the National Center for Biotechnology Information nr (National Library of Medicin, Bethesda, 3rd January 2011, 143202 sequences). Carbamidomethylation of cysteines was fixed and oxidation of methionine and protein N-terminal acetylation were set as variable modifications for all Mascot searches. Specificity of trypsin digestion was set for cleavage after Lys or Arg except before Pro, and two missed trypsin cleavage site were allowed. The mass tolerances in MS and MS/MS were set to 2 ppm and 0.8 Da, respectively, and the instrument setting was specified as "ESI-Trap." All data were validated by using myProMS (Poullet *et al.*, 2007).

Reference

Poullet, P., S. Carpentier, and E. Barillot. 2007. myProMS, a web server for management and validation of mass spectrometry-based proteomic data. *Proteomics*. 7:2553-2556.