Supporting Information

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SI Material and Methods

Histological Analysis. For routine histology, embryos were fixed in 4% paraformaldehyde and embedded in paraffin. For immunohistochemistry, embryos were fresh-frozen in tissue freezing medium, and 7-μm sections were stained with an anti-α4 antibody (BD 553314; 1:50) or an anti-Abi1 antibody (clone 1B9; 1:100; MBL). For whole-mount immunohistochemistry, embryos were fixed in zinc fixative (BD 550523) and stained with an anti-VCAM antibody (BD 550547; 1:25). For ultrastructural analysis, embryos were fixed in buffered glutaraldehyde, osmium and uranyl acetate, then embedded in PolyBed 812, and 1-μm thin sections stained with toluidine blue. Ultrathin sections (90 nm) were processed for routine transmission EM (Duke EM Service, Pathology Department, Duke University Medical Center), and imaged on a Philips CM12 electron microscope.

Co-Precipitation and In Vitro Binding Assays. GFP-tagged Abi1 WT and deletion mutants were previously described (1). GFP-Abi1, GFP-paxillin, or GFP alone were expressed in 293T cells, and following cell lysis (20 mM Pipes, pH 6.8, 50 mM NaCl, 3 mM $MgCl_2$, 150 mM sucrose, 50 mM NaF, 40 mM $Na_4P_2O_7$*$10H_2O$, 1% Tx100), the clarified extracts were mixed with purified, bacterially expressed, His-Avi-labeled α4 or αIIb cytoplasmic tail immobilized on NeutrAvidin agarose as described (2). GFP-fusion proteins were identified by Western blotting with an anti-GFP antibody (1814460; Roche), and equal loading of mimics was determined by Coomassie staining of SDS/PAGE gels. Quantification of coprecipitated proteins was carried out with ImageJ software. For endogenous coimmunoprecipitation experiments, 1 mg lysate from A7r5 cells was incubated with 5 μg of anti-α4 (clone HP2/1; Immunotech 0764), or an isotype control IgG followed by immunoblotting with an anti-Abi1 or phosphoserine 988-α4 antibody. For in vitro binding assays, GST fusions of the full-length WT Abi1 and the N-terminal fragment 1–111 in pGEX-3X were purified using glutathione sepharose 4B according to the manufacturer's instructions (GE Healthcare). Eluted GST fusion proteins (in 10 mM glutathione, 50 mM Tris-HCl, pH 8.0) were incubated with NeutrAvidin agarose-immobilized α4 cytoplasmic tails in lysis buffer containing 0.1% Tx100. Integrin-bound GST fusion proteins were resolved by SDS/PAGE and detected by Western Blotting with an anti-GST antibody (sc-138; Santa Cruz Biotechnology).

Statistical Analysis. Statistical significance of measurements described was determined by using the two-tailed Student *t* test with resulting histograms generated by GraphPad Prism software.

Cell Culture, Cell Spreading Assays, and Immunofluorescence Microscopy. Immortalization by SV40 or high passage of $Abi1^{-/-}$ MEFs often resulted in up-regulation of the *Abi2* gene. Accordingly, for experiments in which the α4 integrin was expressed, an $Abi1^{-/-}$/$Abi2^{+/-}$ immortalized MEF cell line (mutant) was used together with an $Abi1^{+/-}$/$Abi2^{+/-}$ MEF cell line (control) derived from a littermate embryo. MEF cells were grown in DMEM with 10% heat-inactivated FBS (16140; Gibco) and penicillin/streptomycin. Abi1 control and mutant MEFs were transduced with full-length human α4 integrin or pBabe control vector and used to analyze α4-mediated cell spreading. For analysis of cell spreading, cells were trypsinized briefly, then subjected to FACS before plating in media containing 0.1% heat-inactivated FBS on a 10 μg/mL fibronectin (FN; F1141; Sigma) or 5 μg/mL VCAM1 (862-VC; R & D Systems)-coated coverslip (Warner Instruments). Cells were allowed to spread for 2 h, fixed briefly in 4% paraformaldehyde, washed with PBS solution, then blocked/permeabilized no less than 5 min with PBS solution containing 3% BSA and 0.5% Triton X-100. Cells were stained with an antibody to paxillin (BD 612405, 1:500) followed by an Alexa 568-labeled secondary antibody together with Alexa 488-phalloidin (Molecular Probes) to reveal F-actin. To obtain control and mutant cell populations expressing equivalent levels of α4 integrin, cells were stained with a PE-coupled α4 antibody (BD 555503), or with an isotype control antibody (BD 555749), and subjected to FACS. Cells expressing equal levels of α4 integrin/cell were used immediately after sorting. Cells were plated and assayed as described earlier. Measurement of the area of cell spreading was determined using the automeasure module of Zeiss Axiovision 4.7. For rescue experiments, control or mutant MEFs expressing α4 integrin were transfected with Lipofectamine 2000 (Invitrogen) and pEGFP vector (Clontech) in the case of the control MEFs, or pEGFP, pEGFP-WT Abi1, or pEGFP-Δ1–145 Abi1 in the case of mutant MEFs, then subjected to FACS sorting to obtain cell populations expressing similar levels of GFP as well as α4 integrin, and analyzed for cell spreading as described earlier. Images were taken with a Zeiss Axiovert 200M using a 63×/1.4NA Plan Apo objective or a 40×/0.75 NA Plan Neofluor objective. For colocalization studies of endogenous Abi1 and α4 integrin, we used the 3AsubE choriocarcinoma cell line, which endogenously expresses both proteins. 3AsubE cells were plated on VCAM1-coated coverslips and processed for immunofluorescence as detailed earlier. In brief, cells were stained with a mouse monoclonal anti-Abi1 and a rabbit polyclonal anti-phosphoserine 988 α4 (AB1919; Chemicon) and Alexa 488 and 568 secondary antibodies, respectively. Images were taken with a Leica SP5 confocal microscope using a 100×/1.4–0.7 NA Plan Apochromat objective.

1. Echarri A, Lai MJ, Robinson MR, Pendergast AM (2004) Abl interactor 1 (Abi-1) wave-binding and SNARE domains regulate its nucleocytoplasmic shuttling, lamellipodium localization, and wave-1 levels. *Mol Cell Biol* 24:4979–4993.

2. Han J, Rose DM, Woodside DG, Goldfinger LE, Ginsberg MH (2003) Integrin α4β1-dependent T cell migration requires both phosphorylation and dephosphorylation of the α4 cytoplasmic domain to regulate the reversible binding of paxillin. *J Biol Chem* 278:34845–34853.

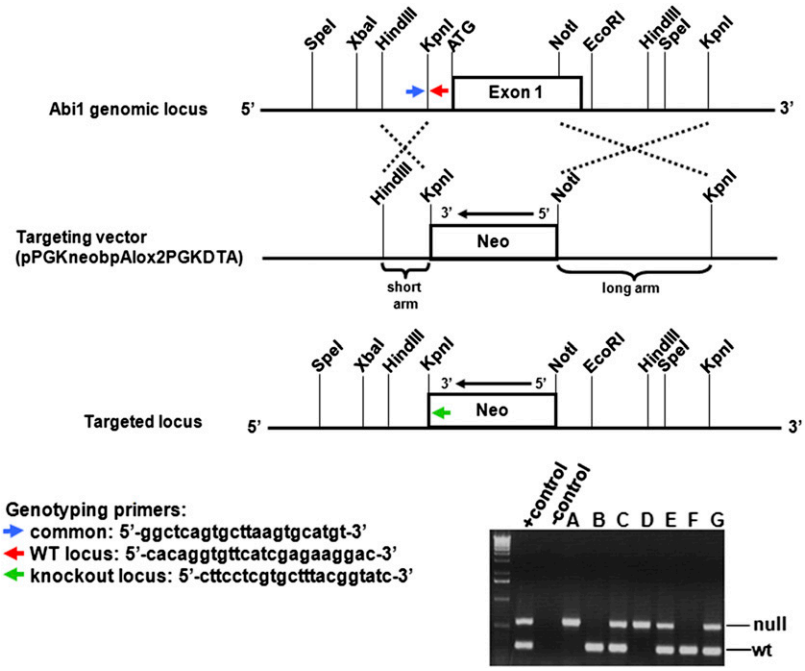

Fig. S1. *Abi1* targeting strategy and PCR identification of genotype. Exon 1 of the mouse *Abi1* locus was targeted using standard methods with the strategy depicted. Correctly targeted ES cells were verified by PCR and Southern blotting and injected into C57BL/6 blastocysts. F1 mice were backcrossed to C57BL/6 or CD1 strains. Genotyping was carried out by PCR using the three indicated primers allowing simultaneous detection of WT and homo- and heterozygous genotypes. An example of a gel from a representative litter is shown. No live pups were detected; timed matings were performed to analyze the stage of lethality.

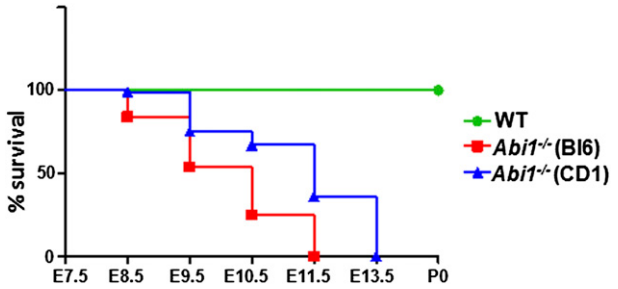

Fig. S2. Survival of $Abi1^{-/-}$ mice on the C57BL/6 and CD1 strains. $Abi1^{-/-}$ mice on the CD1 strain survive longer than those on the C57BL/6 strain, and show a similar delay of onset of phenotypic abnormalities.

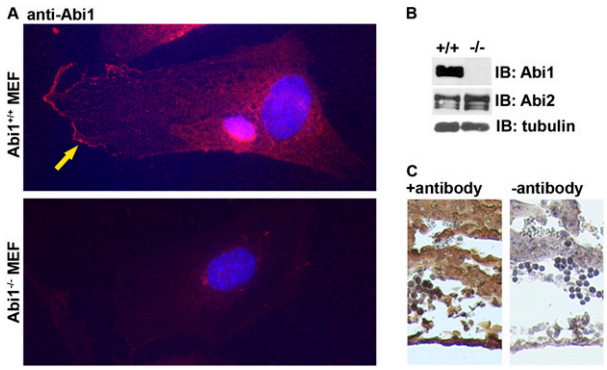

Fig. S3. Characterization and specificity of anti-Abi1 antibody 1B9. (*A*) $Abi1^{+/+}$ or $Abi1^{-/-}$ MEFs were plated on fibronectin-coated glass coverslips. MEFs were stained with an anti-Abi1 monoclonal antibody (1B9; 1:500), followed by an appropriate fluorescence-conjugated secondary antibody. Antibody specificity was verified by the lack of immunofluorescence when applied to MEFs derived from $Abi1^{-/-}$ mice. Abi1 immunofluorescence appears at the leading edge of lamellipodia of $Abi1^{+/+}$ MEFs (arrow). (*B*) Cellular lysates from $Abi1^{+/+}$ or $Abi1^{-/-}$ MEFs were resolved by SDS/PAGE followed by Western blotting with the 1B9 antibody (1:500) to detect Abi1. Note lack of immunoreactivity with lysates derived from $Abi1^{-/-}$ MEFs. Abi2 was detected by Western blotting with a goat polyclonal anti-Abi2 (P-20, sc-20327; Santa Cruz Biotechnology). Equal loading was verified with antibodies to tubulin. (*C*) Abi1 protein has widespread expression in the placenta at E9.5, as revealed by immunohistochemical staining of paraffin sections.

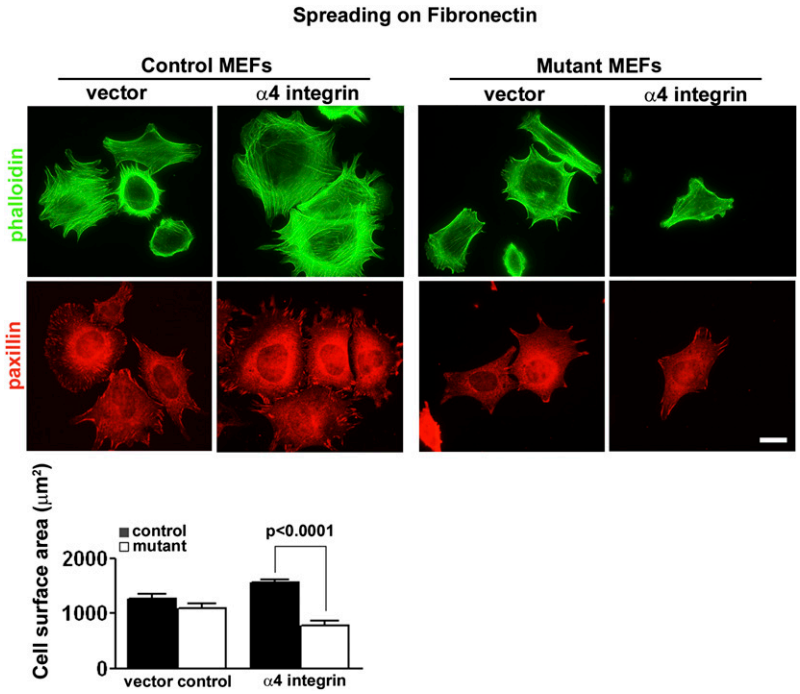

Fig. S4. *Abi1* mutant MEFs expressing α4 integrin show diminished cell spreading on fibronectin. Control (*Abi1*$^{+/-}$/*Abi2*$^{+/-}$) or mutant (*Abi1*$^{-/-}$/*Abi2*$^{+/-}$) MEFs were transduced with virus containing empty vector or vector containing WT full-length α4 integrin. Cells were sorted by FACS to obtain cell populations expressing equivalent amounts of α4, and these cells were plated immediately on coverslips coated with fibronectin. After 2 h, cells were fixed and processed for indirect immunofluorescence for detection of paxillin (red). Actin staining with phalloidin is shown (green). Quantification of cell spreading was analyzed by measuring cell surface area of phalloidin stained cells using Zeiss software (*Bottom*). Abi1 mutant cells expressing α4 integrin exhibit significantly reduced spreading compared with their control counterparts on fibronectin substratum. (Scale bar: 10 μm.)