Appendix S1

SUPPLEMENTARY MATERIALS AND METHODS

Plasmid construction

cDNAs containing the *PLEC* coding region (NM_201384.3, plectin isoform 1a) encoding Met1 to Glu315 were subcloned into the pcDNA3.1V5-His vector (PLEC1a_1-315, wild-type). Constructs with *PLEC* cDNA harbouring p.Leu319Pro (equivalent to p.Leu292Pro in plectin 1a) or p.Leu319dup (equivalent to p.Leu292dup in plectin 1a) were generated using the GeneTailor Site-Directed Mutagenesis System (Invitrogen, Waltham, MA, USA) on PLEC1a_1-315 (L319P and L319dup, respectively). Note that the nomenclature of p.Leu319Pro and p.Leu319dup is based on NM_000445.5, corresponding to plectin isoform 1c. In addition, *ITGB4* cDNA (NM_001005731.3) encoding Gln1115 to Arg1355 was subcloned into the pCMV-3Tag vector (3xFLAG-tagged, ITGB4_1115-1355).

Immunoblotting

HEK293 cells were maintained in Dulbecco's Modified Eagle medium (DMEM) supplemented with 10% bovine serum (Sigma-Aldrich, St Louis, MO, USA) and 1% penicillin-streptomycin-amphotericin (Wako, Osaka, Japan). Plasmids were transfected into HEK293 cells using Lipofectamine 2000 (Invitrogen). Cell lysates were obtained using NP-40-containing buffer (1% NP-40, 25 mM Tris-HCl, 4 mM EDTA, 100 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, and proteinase inhibitor cocktail (Sigma-Aldrich, St Louis, MO, USA)) 48 h after transfection. Cell debris was removed by centrifugation, and the supernatant was collected and mixed with Laemmli's sample buffer. After boiling, samples were separated by gel electrophoresis on a NuPAGE 4–12% Bis-Tris gel (Invitrogen) and transferred to a PVDF membrane. The membrane was incubated with the primary antibody (anti-tubulin (Abcam, Cambridge, UK)), followed by incubation with secondary antibodies conjugated with horseradish peroxidase (HRP), or was incubated with HRP-conjugated anti-FLAG (M2, Invitrogen) or anti-V5 (Invitrogen) antibodies. The blots were detected using Clarity Western ECL Substrate (Bio-Rad, Hercules, CA, USA).