ONLINE METHODS

Patient cohort, pre-sequencing of known WWS genes, CNV and homozygosity mapping studies (Supplementary Fig. 1). Informed consent was received after institutional ethical board approval for the research project was obtained from the Radboud University Nijmegen Medical Centre, CMO Regio Arnhem-Nijmegen Approval 2011/155. A cohort of 94 families with one or more children affected with WWS/MEB were available for study. Of these, mutations for one of the six screened WWS genes were identified in 35 affected individuals (37%). For 30 of the remaining 59 families, CNV analysis and homozygosity mapping was carried out using SNP arrays. Genomic DNA (250 ng) was digested by *Nsp*I before hybridization to the GeneChip Mapping 250K SNP Array Set (Affymetrix, Santa Clara, CA, USA), following standard protocols to perform CNV analyses, as described[29]. Briefly, a Hidden Markov Model was used to predict the copy numbers and start and end position of each CNV. To define losses and gains, a cut-off of four and seven consecutive SNP probes was used, respectively. For homozygosity mapping, genotype files were generated by the Affymetrix GTC software and analysed using an in-house algorithm (J.V.R., unpublished data). Regions of excess homozygosity were identified using an algorithm adapted from Woods *et al*.[30] which calculated the number of contiguous homozygous SNPs required for significance in relation to degree of consanguinity for each individual. Haplotypes consistent with homozygosity in affected individuals were overlapped.

Next generation sequencing and Sanger sequencing. The exome of one child affected with WWS from a consanguineous Turkish family (WWS-25) was sequenced using the SOLiD™ 4 System and the resulting DNA variants were integrated with familial homozygosity data in a variant prioritization scheme. Next generation sequencing and analysis was carried out as described[18] with the following alterations. Exome enrichment was performed using the SureSelect

Human All Exon 50 Mb Kit (Agilent, Santa Clara, CA, USA), covering ~21,000 genes. The enriched exome library was equimolarly pooled in a set of four samples including three other samples unrelated to WWS. The pool was based on a combined library concentration of 1 pM. Subsequently, the obtained pool was used for emulsion PCR and bead preparation using the EZbead system, following manufacturer's instructions (Life Technologies, Carlsbad, CA, USA). For each pool of four exome libraries, a full sequencing slide was used on a SOLiD™ 4 System, thereby anticipating that all four samples would be represented as a quarter of the total beads sequenced (Life Technologies, Carlsbad, CA, USA). A total of 5.56 Gb of mappable sequence was obtained. Raw sequencing statistics are available on request. Colour space reads were mapped to the hg19 reference genome with the SOLiD bioscope software v1.3. Quality control settings included the presence of at least 5 unique variant reads (i.e. different start sites), as well as the variant being present in at least 20% of all reads. Exclusion of known variants was based on dbSNPv132 and an in-house database including 177 in-house analyzed exomes. The next filtering step was based on a recessive model of disease, keeping those variants that occurred in more than 80% of all reads, suggestive for homozygous changes.

Sequencing of exons of the *ISPD* gene was performed using traditional Sanger sequencing on ABI 310 or ABI 3100 Genetic Analyzers (Applied Biosystems, Foster City, California, USA). The sequences of oligonucleotides used for exon amplification are provided in **Supplementary Table 2**.

Control cohort. A control cohort of 3712 haploid genomes was interrogated for the presence of the identified mutations. This cohort includes the results from 679 control individuals from the 1000-genomes project, approximately 2000 control chromosomes from the University of Washington (Exome Variant Server) as well as the 177 diploid exomes present in the in-house

exome database.

Zebrafish studies

Antisense morpholino oligonucleotides (MO). To simultaneously knockdown both zebrafish *ispd* isoforms, two antisense MOs (Gene Tools) were designed against the exon-intron splice sites common to both *ispd* transcripts (**Supplementary Fig. 5**). For sequences of *ispd* MO1 and *ispd* MO2, see **Supplementary Table 2**. Sequences of standard control, *p53, dag1*, *fktn,* and *fkrp* MOs have been described[31-33]. MOs were injected into 1- or 2-cell-stage with concentrations specified in the figure legends.

Microsome preparation and western blot analysis. Zebrafish embryos were de-yolked as described[32], followed by homogenization in pyrophosphate buffer containing protease inhibitors cocktail (Roche) and centrifugation (14,000*g*)[34]. Proteins were quantified using a Qubit Fluorometer (Invitrogen). Equal amounts of total protein were ultra-centrifuged (142,000*g*) to obtain microsome pellets, which were then solubilized in Tris-HCl buffered saline containing 1% Triton X-100 and protease inhibitors. The microsome supernatant and solubilised pellets were separated by 4-12% Bis-Tris gels (Invitrogen); transferred to PVDF membranes (Immobilon); probed with primary antibodies: glycosylated α-Dag1 IIH6 (Millipore 05-593; 1:2000), ß-Dag1 (Novocastra NCL-b-DG; 1:50), γ-tubulin (Sigma T5326; 1:1000) and acetylated tubulin (Sigma T6793; 1:1000). SuperSignal West chemiluminescent substrates (Pierce) were used to develop signals from HRP-conjugated secondary antibodies.

RT-PCR and mRNA synthesis. Total RNA was extracted from control and MO-injected embryos using TRIzol (Invitrogen), followed by first-strand cDNA synthesis using SuperScript III

(Invitrogen) with either oligo dT or random primers. Primers for PCR are specified in **Supplementary Table 2**. To label cell membranes in developing zebrafish embryos, a construct encoding membrane-localized red fluorescent protein (mRFP; gift from AF Schier) was used to synthesize sense-strand capped mRNA[34].

Zebrafish immunohistochemistry and Evans blue dye (EBD). Zebrafish embryos were fixed and incubated with primary antibodies, including rabbit anti-laminins (Sigma L9393; 1:400) and mouse anti-γ-tubulin (Sigma T5326; 1:100) and anti-dystrophin (Sigma D8043; 1:100) using methods described previously[31]. Stainings were visualized by Alexa Fluor 488, 546 or 594 conjugated antibodies to rabbit or mouse immunoglobulin (Molecular Probes; 1:250), followed by incubation in mounting media with DAPI (Vector Labs) before analysis under confocal and differential interference contrast (DIC) microscopy. Zebrafish embryos were fixed and incubated with Alexa Fluor 488 phalloidin (Molecular Probes; 1:100) to label F-actin[15]. 0.1% EBD (Sigma) was injected into the peri-cardiac sinus of zebrafish embryos at 48 or 72 h.p.f. as previously described[15,23,35], followed by analysis under confocal and DIC microscopy.

Statistical analysis. Eye width measurements were plotted as mean ± s.d. and statistical significance was determined by the Student's t-test assuming unequal variance. A *P* value smaller than 0.05 was considered statistically significant.

Protein modeling. ISPD protein modeling was carried out through the HOPE web server which analyzes the structural and functional effects of point mutations by data mining information relating to the amino acid sequence (UniProt), predictions (DAS), homology models (YASARA) and a calculation of the three dimensional protein structure (WHAT IF)[36] using the available *E.*

coli model of ISPD as a template[37].