METHODS

Primer pairs used to amplify NEMO coding region cDNA for sequencing, including the 3′ UTR:

Forward	Reverse
TCACCAAACTTGACTGCGCTCT	CCAGAGCCTGGCATTCCTTAG
AGGACAAGGCCTCTGTGAAA	GACAGCTGGCCTTCAGTTTGC
GCCGAGCAGCACAAGATT	GGAGAGGAAAGCGCAGACT

Primer pairs used for 5′ upstream genomic PCR for sequencing:

Forward	Reverse
AACGGATACTACTCAGCAACACTG	CTGGAAGGGGGCAGTAAGTAC
CCAGAAATGTTCTGAGGAAAGG	CGTGTAATTTGAGATGAAGCCCTT
CGCACGATGTGGAAGAACTAACTA	AGACAACATCTGCCTATCGTCA
TTTCTACTCCTCCCTCCTCCTC	GAAGAGCCAACTGTGTGAGATGG

Sequencing primers for 5′ upstream genomic sequencing:

GGAGTCTCACTCTGTCGGCC	CATGGTGAGACCCCGTTTC
GCCAGGCAGTTAGGAAGC	GACTGGTCTGCTGAGTCAC
CACAAGGTGACTTAGTAGA	CCATCATTGGGATGCGTCC
CTAGGTCATGCTGAGCTTGT	CGAGGCTCTTCAGAGAGAGG
TCAGAGTCCTGGCTGTTAAG	AGTGCTGGGATTACAGACGT
CTCTTCTGAGGGGACCAG	AGTCTCACTGCCCCATGG
GGTGGCTCATGCCTGTCA	CCCATGATGATGAATATGTG
CCTGGAGCATGGGAGATG	TGCTCTGCATCCCCAATT
CCCACAGCTATGACACCG	ATCGTTCTAGCAGTGGTGG
CATTCACAGCTACCAACTTC	CTCACCGCAACCTCCATC
GTGGATTTGCCTGTTGTAGA	ATGGATTCGCCATCAGCT
CGTGTCACCACACTCTGC	GGAGACTAGAAGTCCAAAACC
TTCCAGCCTGGAGCTAGG	

Primers for cDNA transcript PCR (adds BamHI site in forward primers and HindIII site on reverse):
Exon 1A forward: 5′ ATGGATCCCATGGCCCTTGTGATCCAG 3′
Exon 1B forward: 5′ ATGGATCCGACACCGGAAGCCGGAAG 3′
Exon 1C forward: 5′ ATGGATCCAGCCCGTTCCTGCTCCG 3′
Exon 10 reverse: 5′ ATAAGCTTCTCAATGCACTCCATGACATGTATC 3′

FIG E1. Normal ectodermal development in the index patient at 11 years of age. Notice normal dentition and hairline.

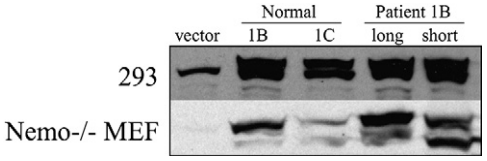

FIG E2. Western blot showing expression of NEMO cDNA isoforms amplified from normal and patient cells. NEMO cDNA using exons 1B and 1C were amplified from cDNA isolated from normal fibroblasts, and the misspliced 1B isoforms were amplified from cDNA isolated from patient fibroblasts. The PCR products were cloned into the pCMV-Tag4a vector and expressed in T293 HEK cells *(top)* or in $NEMO^{-/-}$ mouse embryonic fibroblasts *(bottom)*. Note the presence of the endogenous NEMO band in T293 cells but not in NEMO-deficient murine embryonic fibroblasts (MEFs).

TABLE E1. Lymphocyte counts of the index patient

Cell counts per mm^3	11/2001	Normal range
Absolute lymphocyte count	5140	1700-6900
CD3	3084	900-4500
CD4	596	500-2400
CD8	709	300-1600
CD16/CD56	1182	100-1000
CD19	668	200-2100
$CD27^+IgD^+IgM^+$ (nonswitched memory)	29 (4.4% of $CD19^+$)	30-98 (7% to 14%)
$CD27^+IgD^-IgM^-$ (switched memory)	10 (1.5% of $CD19^+$)	22-76 (5% to 12.3%)
$CD27^-IgD^+IgM^+$ (naive)	625 (93.6% of $CD19^+$)	260-716 (70.7% to 85%)

These measurements were taken when the patient was first seen at 4 years of age. Similar numbers have been obtained on subsequent evaluations. Reference range values (10th-90th percentile) for B-cell subsets: Huck K, Feyen O, Ghosh S, Beltz K, Bellert S, Niehues T. Memory B-cells in healthy and antibody-deficient children. Clin Immunol 2009;131:50-9.

TABLE E2. Serum immunoglobulin levels from the index patient

Serum immunoglobulins (mg/dL)	11/2001	Normal range
IgG	653	441-1135
IgG_1	369	360-810
IgG_2	248	60-310
IgG_3	38	9-160
IgG_4	8	9-160
IgA	901	22-159
IgM	31	47-200

Immunoglobulin levels measured when the patient was first seen at age 4 years, before intravenous immunoglobulin therapy was initiated.

TABLE E3. Antibody response to immunization

Serum titers	6/2003	5/2004	1/2005	9/2006	6/2008
Tetanus (IU/mL)	0.2	—	—	0.09	0.1
Measles (IU/mL)	—	Absent	—	Absent	Absent
Mumps (AU/mL)	—	Absent	—	Absent	Absent
Pneumococcus (mg/L)	40	—	150	90	30
Hepatitis B virus surface antibody (mIU/mL)	—	Absent	—	Absent	Absent

The patient received Measles/Mumps/Rubella vaccinations 8/1999 and 9/2005, tetanus 7/1998, 9/1998, 3/1999, 8/1999, 6/2003, and 12/2005, 23-valent pneumococcus vaccine 6/2003 and 12/2005, hepatitis B virus 7/1998, 9/1998, 3/1999 and 3/2007. Protective titers after immunization with tetanus toxoid and pneumococcus are >0.1 IU/mL and >60 mg/L, respectively. Dashes indicate the test was not done on that day.

TABLE E4. Proliferation of PBMCs, ^{3}H counts per minute

Stimulus	Patient	Healthy control
Medium, day 3	455	316
PHA	33,376	34,506
PWM	25,564	36,794
Anti-CD3 (OKT3)	25,267	34,111
Medium, day 6	282	187
Tetanus	2,165	9,601

Measured 5/2006, blood sample was taken before intravenous immunoglobulin infusion. Cells were examined for proliferation to phytohemagglutinin *(PHA)*, pokeweed mitogen *(PWM)*, and anti-CD3 after 3 days of culture, and to tetanus after 6 days of culture.