Supplemental Methods

Patient tissue acquisition

All research participants were counseled regarding the possible outcomes of WES and signed a consent form approved by the Johns Hopkins University School of Medicine Institutional Review Board. DNA samples were obtained via a skin biopsy from the proband while in disease remission, while peripheral blood or saliva was used for unaffected individuals using the Gentra Puregene kit (Qiagen). Samples were de-identified and entered into the PhenoDB database (www.mendeliangenomics.org) for the Baylor-Hopkins Center for Mendelian Genomics (BH CMG) (1,2). When possible, samples from deceased family members were obtained from archived bone marrow aspirates or biopsies with permission from next of kin. Detailed exome sequencing methods have been described previously (3) and coverage is detailed in Table S2. Sequence data will be deposited into dbGaP (NCBI) per the BH CMG.

Identification of variants and segregation analysis

To identify the inherited variant predisposing to disease, WES was performed by the BH CMG. Germline DNA from the proband was isolated from cultured dermal fibroblasts derived from a skin biopsy. No other DNA samples from affected individuals in this family were of sufficient quality for WES, thus sequence from the proband was compared to six unaffected family members with the goal of identifying a rare variant present in the proband and absent in all unaffected individuals. To ensure absence of the variant in unaffected samples, the oldest living unaffected family members born from unaffected parents were selected, with an age range of 72-79 years (Figure 1A). We used the PhenoDB Variant Analysis tool to design the prioritization strategy (1). We applied filtering designed
to prioritize heterozygous rare functional variants (missense, nonsense, splice site variants, and indels) that fit the autosomal dominant mode of inheritance and were present in the proband but not in the other six unaffected family members. We excluded variants with a minor allele frequency (MAF) > 0.01 in dbSNP 126, 129, and 131 or in the Exome Variant Server (release ESP6500SI-V2) or 1000 Genomes Project (4). We also excluded all variants found in our in-house controls (CIDRVaR 51Mb).

This filtering generated a list of 109 candidate variants (Table S3) that were ranked based on conservation (GERP++ score) (5) and predicted pathogenicity of the amino acid change (PolyPhen-2 score) (6). PCR primers were generated to amplify 15 candidate variants (all PCR product sizes <100bps) and DNA from a bone marrow aspirate from affected individual II-03 (Figure 1A) was isolated and amplified using REPLI-g FFPE kit (Qiagen). This sample was used as template for PCR reactions to screen candidate variants, resulting in identification of a shared missense variant in the ERBB3 gene. Sanger sequencing of PCR products was performed by the Johns Hopkins Genetic Resources Core facility (www.grcf.med.jhu.edu). Sequencing of an additional affected individual (III-05) was uninformative due to poor DNA quality isolated from an archived bone marrow biopsy.

Plasmids and cell culture

TF-1 cells (ATCC CRL-2003) and BaF3 cells were maintained in RPMI-1640 media supplemented with 10% fetal bovine serum and either 2 ng/ml recombinant human granulocyte macrophage colony-stimulating factor (GM-CSF) or 2ng/ml mouse interleukin-3 (IL-3). TF-1 cells are typically maintained in GM-CSF, however when placed in erythropoietin (EPO), they can be induced to differentiate along the erythroid lineage (7). Total RNA was isolated from cells of the proband (Qiagen) and converted to cDNA using
SuperScript III (Invitrogen). Stable cell lines expressing either the ERBB3 A1337T mutant or wild type ERBB3 were generated by cloning into a piggyBac transposon vector previously described (8). Approximately 1x10^6 cells were transfected with 1ug of DNA (0.5ug piggyBac vector and 0.5ug transposase vector) using the Lonza Nucleofector 4D following the Lonza optimized protocol (www.biolonza.com). Cells were culture for 48-hours prior to addition of 1ug/ml puromycin and then assayed for ERBB3 expression by flow cytometry after 10 days of selection. To generate cells co-expressing ERBB2, cells expressing either mutant or wild type ERBB3 were transduced with lentivirus expressing the wildtype human ERBB2 gene. Vector pLX304-ERBB2 (gift from David Root, Addgene plasmid # 25890 (9)) was generated via Gateway cloning using pDONR223-ERBB2 (gift from William Hahn & David Root, Addgene plasmid # 23888 (10)) as the donor plasmid. Lentivirus generation and transduction procedure were performed as previously described (11). Blasticidin selection at 5ug/ml was added to culture media after 48-hours, and flow cytometry for ERBB2 expression was performed after 7 days using an antibody described below. Cell expressing ERBB2 on the cell surface were sorted by flow cytometry, and all experiments were performed within 7 days of sorting.

Proliferation assays

TF-1 cells were counted, washed twice in PBS and 4x10^3 cells were plated in 100ul of RPMI media containing GM-CSF and/or recombinant human Neuregulin-β1 (NRG-β1, Peprotech) as indicated in a round-bottom 96-well plate. Four replicates were done for each experimental condition. Cell proliferation reagent WST-1 (Roche) was added at either at 0h or after culture for 24h (BaF3) or 48h (TF-1), followed by incubation at 37°C for 2h. Absorbance was measured using a microplate reader at 450nm, using the absorbance at
650nm as a reference, per manufacturer protocol. Relative growth was calculated by taking the ratio at 24h or 48h compared to 0h. The average of four replicates was used for each experimental condition, and each experiment was repeated a minimum of three times. P-values were calculated using a two-tailed student's t-test using the free software environment R.

**Cell Cycle Assays**

Assays were performed using Click-iT® EdU Flow Cytometry Assay Kits (Invitrogen) following the manufacture’s protocol. Cells were cultured for 24hrs either with or without NRG-1β, followed by Edu labeling by incubating with a 10uM solution for 1hr prior to fixation, permeabilization and staining with anti-Edu Alexa Fluor® 647azide. Propidium iodide (PI) was used to measure DNA content following treatment with Ribonuclease A. Cells were analyzed by flow cytometry and p-values were calculated from four independent experiments using a two-tailed student’s t-test.

**Western blot**

TF-1 cells were cultured in EPO for 48hrs and incubated with or without NRG-β1 for 1h at 37°C. Cells were then washed twice in PBS and protein lysates were isolate using RIPA buffer (Sigma) containing protein and phosphatase inhibitors. Lysates were quantitated using BCA Protein Assay (Pierce) and 10ug total lysate was run on NuPage TrisAcetate gels (Invitrogen). Transfers were performed using the iBlot System (Invitrogen), and membranes were probed overnight at 4°C with primary antibody at the recommended concentration. Visualization was done using chemiluminescent substrate.

**Antibodies**
The following antibodies were used for flow cytometry: ERBB3 APC-conjugated and ERBB2 (R&D Systems), AlexaFluor 488 (Life Technologies), CD235a PE-conjugated (Invitrogen). Flow cytometry was performed using FACSCalibur (BD Biosciences) and analysis with Flowjo software (www.flowjo.com). The following antibodies were used for Western blots: ERBB3 (C-17) and pERBB3 (Tyr1328) (Santa Cruz Biotechnology), pERBB2 (Tyr 1248) (Millipore), ERBB2, AKT (pan), pAKT (Ser473), GAPDH, and Anti-Rabbit IgG HRP-linked secondary antibody (Cell Signaling Technology).

**Cloning and PCR primers**

Reference sequences used throughout for DDX41 are NM_016222.2 and NP_057306.2, and genomic coordinates refer to hg19. PCR primers for the genomic ERBB3 c.4009G>A variant: 5’ AGCAGGCTTTTCCCAAG 3’ and 5’ CGGTACCCTCTAAAGGCACT 3’. ERBB3 cloning primers 5’ GCCGCTAGCATGAGGGCAAGAC 3’ and 5’ CCGGTCGACTTACGTTCTCTGGGCA 3’.

**Supplemental References**

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