Supporting Information

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SI Methods

Exome Analysis. Picard, version 1.27, SamTools, version 0.1.16 (1), Dindel, version 1.01 (2), Genome Analysis Toolkit (GATK), version 1.1-20 (3), and Variant Effect Predictor (VEP), version 2.1 (4), were used to map sequence reads to the human genome reference sequence [National Center for Biotechnology Information (NCBI) build 37], to calculate read quality, to call single-nucleotide variants (SNVs) and insertion/deletions, and to annotate against Ensembl build 64. Variants were filtered to include only those with a minimum depth of 4× to a maximum depth of 1,200× and were reported in variant calling file (VCF) format, version 4.0. For proband 1 (P1) and proband 2 (P2), 7.6 and 11.9 Gb of sequence, respectively, was produced in total, at a mean depth of 87 and 130, and there was 85% and 88% coverage at a depth of 10, using high mapping quality alignments only (i.e., mapping quality, ≥30). Equivalent figures for the father and mother of P1 were 8.3 Gb, 98% and 96%, and 8.1 Gb, 95% and 86%, respectively, and 8.8 Gb, 94% and 86%, and 8.4 Gb, 90% and 86%, for the father and mother of P2.

De Novo and Compound Heterozygous Mutation Analysis. Exome data for each patient was merged with that for her parents, and variants with a high posterior probability of being de novo (cutoff value, 0.8) were extracted using DeNovoGear, version 5.0 (https://sourceforge.net/projects/denovogear/), which takes genotype likelihood into account in detecting likely de novo variants from sequence data from family trios (5). Python scripts were also used to extract potential compound heterozygous mutations. Variants were then annotated with the NCBI dbSNP database build 137 (ftp://ftp.ncbi.nih.gov/snp/database/organism_data/human_9606), allele frequencies from the 1000 Genomes Project Final Phase 1 Release (ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/), Exome Variant Server, National Heart, Lung, and Blood Institute (NHLBI) Grand Opportunity (GO) Exome Sequencing Project (ESP) (Seattle, WA) (http://evs.gs.washington.edu/EVS/) (April 2013), the U.K.10K Cohorts group (www.UK10K.org) (REL-2012-06-02), and exome-wide sequencing data from 409 control individuals from the CoLaus Cohort (6) (December 2010). Variants that failed to pass additional quality filters were removed (minimum genotyping or mapping quality of 20; maximum contiguous homopolymer run of 5; maximum strand bias of −0.1 and maximum of three SNVs found in each 10-bp stretch). Variants were defined as functional if they were nonsynonymous, resulted in loss or gain of a stop codon, an in-frame insertion/deletion or a frameshift, or occurred within essential splice sites. Those unlikely to have a functional impact were removed as were all variants found with a nonreference allele frequency greater than 1% in individuals from any of the four control populations previously described (1000 Genomes, NHLBI exomes, U.K.10K Cohorts genomes, and CoLaus exomes). The remaining variants were then evaluated using Integrative Genome Viewer (IGV) (7) to assess depth and quality of coverage at the variant locus and reconfirmed in each index case and her parents by bidirectional Sanger sequencing of PCR products using the BigDye Terminator, version 3.1, Cycle Sequencing Kit according to the manufacturer's instructions (Applied Biosystems; Life Technologies) and run on ABI 3730 DNA Sequencers (Applied Biosystems). Sequence analysis was performed using the Mutation Surveyor package, version 2.3 (SoftGenetics).

Haplotype Analysis. The Illumina HumanOmniExpress BeadChip array was used to genotype both probands and their parents. Consensus SNP genotype calls were generated from the GenCall (8) and Illuminus (9) genotype calls (46,240 SNPs with 99.9% measure of concordance on chromosome 3). All samples passed quality control (QC) with a call rate of >0.996. Plink, version 1.0.7 (http://pngu.mgh.harvard.edu/purcell/plink/) (10), was used to perform SNP QC (minor allele frequency, ≤0.01; SNP call rate, ≤0.95; Hardy–Weinberg equilibrium, <0.0001) and to merge with the genotypes for the previously unidentified *PCYT1A* variants obtained by whole-exome sequencing. SHAPEIT2 (www.shapeit.fr/) (11) was used to phase the genotypes for chromosome 3 and calculate the most likely haplotype for the probands.

Sequence Comparisons. Seventy-eight metazoan paralogous sequences from OMA browser database (www.omabrowser.org) were supplemented with 11 obtained using the human sequence as a query in a Blast search (http://blast.ncbi.nlm.nih.gov/Blast.cgi) in the NCBI (www.ncbi.nlm.nih.gov/) and UniProt (www.uniprot.org/) databases. The putative paralogy was checked in a reciprocal Blast search. The sequences were aligned with T-coffee [http://tcoffee.crg.cat/ (12)]. The alignments and the Protein Data Bank structure 3HL4 were displayed and analyzed with JalView [www.jalview.org/ (13)] and BrowserPro (MolSoft) software.

Cell Culture. Human primary skin fibroblasts were cultured in DMEM supplemented with 10% (vol/vol) FBS, 2 mM L-glutamine, 1 mM sodium pyruvate, 1× nonessential amino acids, penicillin, and streptomycin. Epstein–Barr virus-transformed lymphocytes (EBVLs) were cultured in RPMI 1640 medium supplemented with 10% (vol/vol) heat-inactivated FBS, 2 mM L-glutamine, 10 mM Hepes, penicillin, and streptomycin. African Green monkey kidney cells (COS7) cells were cultured in DMEM supplemented with 10% (vol/vol) FBS, 2 mM L-glutamine, 1 mM sodium pyruvate, penicillin, and streptomycin. COS7 cells were seeded 1 d before transfection with 500 ng of plasmid DNA using Lipofectamine LTX (Life Technologies). Protein lysates and mRNA were collected 24 h posttransfection.

Murine 3T3L1 preadipocytes were maintained below 70% confluence in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% (vol/vol) heat-inactivated newborn calf serum (NCS), 2 mM L-glutamine, penicillin, and streptomycin. During adipogenic differentiation, NCS was replaced with FBS. To differentiate 3T3L1 preadipocytes into adipocytes, hormonal mixture composed of 1 μM dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), and 1 μM insulin was added to 3T3L1 preadipocytes grown to 2 d postconfluence. Two days thereafter, the medium was replaced with complete DMEM with FBS supplemented with insulin for a further 2 d. Development to mature adipocytes (days 8–10) was achieved by maintaining 3T3 cells with complete DMEM with FBS.

To knockdown endogenous PCYT1A expression in 3T3-L1 adipocytes, cells were either transfected with Silencer Negative Control siRNA (Life Technologies) or *Pcyt1a* siRNA (Life Technologies; s64594) using Lipofectamine RNAiMAX (Life Technologies) to achieve a final concentration of 30 nM every 2 d after the cells reached confluency. mRNA and protein lysates were collected on day 10 of differentiation. Oil red O staining was performed on mature (day 10) adipocytes.

S2 Cell Culture and Microscopy. S2 cells were cultured in Schneider's *Drosophila* medium (Gibco) with 10% heat-inactivated FBS. Cells were transfected with 1 μg of plasmid DNA encoding N-terminal

mCherry-Pcyt1a fusion proteins with Effectene according to the manufacturer's instruction (Qiagen) and treated with 1 mM oleic acid conjugated to BSA for 15 h to induce lipid droplet (LD) formation.

Cells were stained with BODIPY and prepared for microscopy as described previously (14). Sixteen-bit images and fluorescence recovery after photobleaching (FRAP) movies were recorded on a spinning-disk confocal microscope [100×, 1.4 N.A. oil immersion objective (Olympus), iMIC (Till), CSU22 (Yokugawa), iXonEM 897 (Andor)] with Image iQ software (version 2.6; Andor). Data were fitted to exponential curves using MATLAB, version 7.14.0 (R2012a) (The MathWorks).

Helical Wheel Projections. The amphipathic helices of WT and E280del PCYT1A were analyzed using the HeliQuest tool (15).

Measurement of de Novo Phosphatidylcholine Synthesis. EBVLs or fibroblasts were loaded with 2 μCi of methyl[^{3}H]choline per mL (NET109001MC; Perkin-Elmer) for 3 h at 37 °C. Loading was then terminated by putting the cells on ice. After two washes in cold PBS, the cells were scraped in 1 mL of PBS and mixed with 3.75 mL of chloroform/methanol [2:1 (vol/vol)], 1.25 mL of chloroform, and 1.25 mL of water to extract total lipids. After centrifugation (3,300 × *g* for 15 min at room temperature), the lower organic phase was dried under nitrogen flow. Dried lipids containing newly synthesized phosphatidylcholine (PC) were suspended in 100 μL of chloroform and radioactivity was quantified with liquid scintillation. The measured radioactivity was normalized to total protein content.

Measurement of PC:Phosphatidylethanolamine Ratio. Lipids were extracted from cell lysates by a modification of the Bligh and Dyer method (16) and dissolved in 30 μL of chloroform/methanol [2:1 (vol/vol)]. Phospholipids were resolved by TLC on a silica glass plate (Sigma-Aldrich) using a solvent containing chloroform/methanol/ammonium hydroxide [65:25:4 (vol/vol/vol)] and detected by primuline staining (Sigma-Aldrich). Spots were identified by comparing with standards and quantified by Image Laboratory 4.1 software (Bio-Rad).

Immunoblotting. Cells were lysed in RIPA buffer (Sigma) supplemented with Complete-Mini Protease Inhibitor mixture (Roche) and PhosSTOP Phosphatase Inhibitor mixture (Roche). Protein was quantified using Bio-Rad DC protein quantification kit, and 25–40 μg of protein lysate was diluted in NuPAGE 4× LDS sample buffer (Life Technologies) containing 0.05% β-mercaptoethanol and subjected to SDS/PAGE. Following transfer onto a nitrocellulose membrane, membranes were washed in Tris-buffered saline containing 0.1% (vol/vol) Tween 20 (TBS-T; Sigma), and then blocked for 1 h at room temperature in 3% (wt/vol) BSA or 5% (wt/vol) powdered skimmed milk diluted in TBS-T. Membranes were further incubated with appropriate primary antibodies diluted in blocking buffer for 16 h at 4 °C. Primary antibodies were used as follow: anti-PCYT1A (3403-1; Epitomics), 1:1,000, in 5% (wt/vol) milk–TBS-T; anti-perilipin (4854; Vala Sciences), 1:2,000, in 5% (wt/vol) BSA–TBS-T; anti-aP2 (sc-18661; Santa Cruz), 1:5,000, in 5% milk–TBS-T; anti-myc (4A6; Millipore), 1:1,000, in 3% (wt/vol) BSA–TBS-T; anti-actin (ab8227; Abcam), 1:5,000, in 5% (wt/vol) milk–TBS-T; and anti-calnexin (ab75801; Abcam), 1:1,000, in 5% (wt/vol) milk–TBS-T.

[^{35}S]Methionine/Cysteine Labeling of Primary Human Fibroblasts. EasyTag EXPRESS ^{35}S Protein Labeling Mix was obtained from Perkin-Elmer. Primary human fibroblasts were seeded in 140-mm culture dishes 1 d before [^{35}S]methionine/cysteine labeling so that they were ∼80–90% confluent on the next day. Fibroblasts were washed three times with prewarmed PBS and starved in L-methionine– and L-cysteine–free fibroblast culturing medium for 30 min at 37 °C. Fibroblasts were then pulse labeled for 4 h in the same medium to which 35.7 μCi/mL [^{35}S]methionine/cysteine was added. Fibroblasts were either washed with cold PBS and harvested with RIPA lysis buffer immediately after 4 h pulse labeling or chased with fibroblast culturing medium for 0–4 h.

PCYT1A Immunoprecipitation. [^{35}S]Methionine/cysteine-labeled fibroblast lysates were precleared with GFP antibody (ab290; Abcam) conjugated to protein A-Sepharose at 4 °C for 1 h, then immunoprecipitated (IP) with PCYT1A antibody (3403-1; Epiomics) conjugated to protein A-Sepharose at 4 °C for 2.5 h. Samples were washed four times with RIPA lysis buffer (20-min washes), once with high salt wash buffer [10 mM Tris (pH 7.5), 500 mM NaCl, 1 mM EDTA, 1% (vol/vol) Triton X-100 for 20 min], and once with cold PBS briefly. IP samples were diluted in NuPAGE 4× LDS sample buffer and boiled at 95 °C for 10 min. IP samples were then subjected to SDS/PAGE, fixed [40% (vol/vol) methanol, 10% (vol/vol) acetic acid, 2% (vol/vol) glycerol] for 30 min, and dried at 80 °C for 1 h. Dried gels were exposed to autoradiography films (Kodak BioMAX MR-1; Z353949).

Statistical Analysis. Data are expressed as mean ± SE or SD. Two-tailed Student *t* tests or one-way ANOVA with post hoc Bonferroni analyses were performed on data at a minimum $P < 0.05$ threshold.

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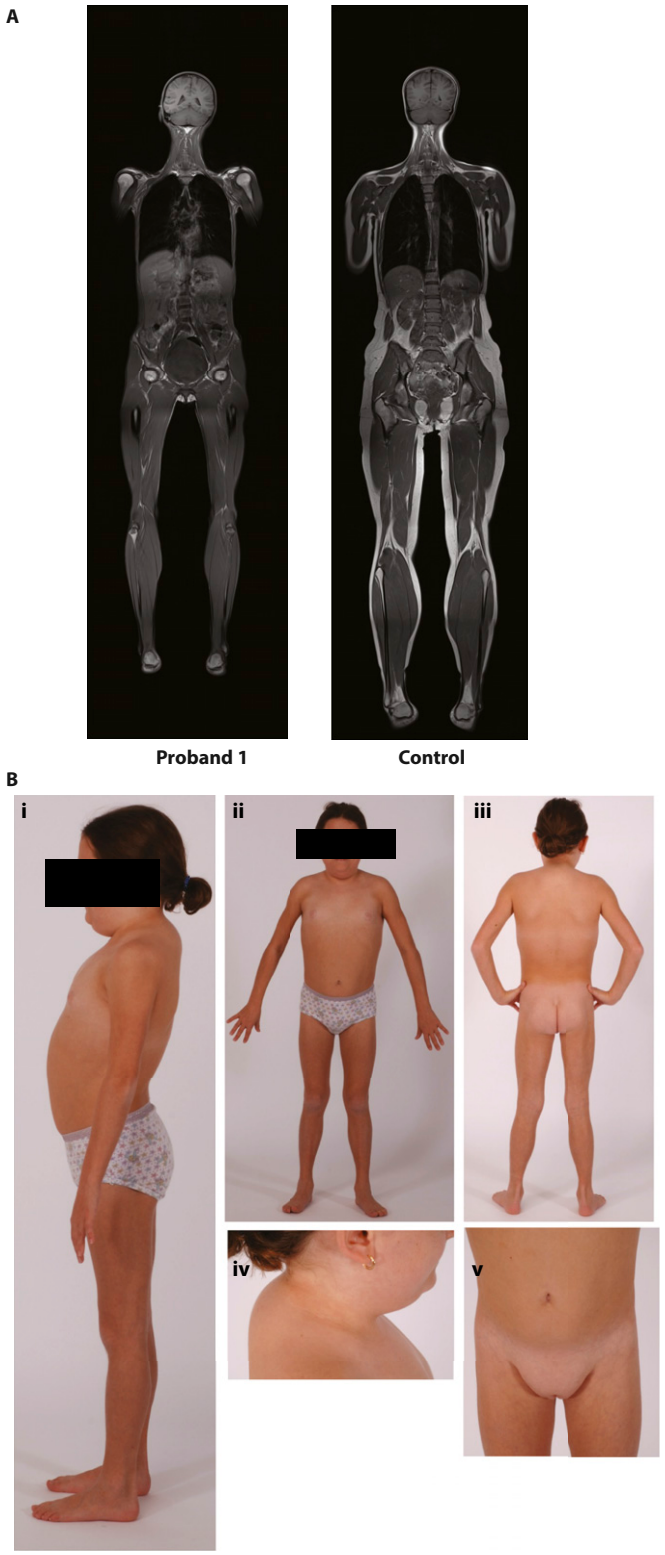


Fig. S1. Severe lipodystrophy in patients with biallelic mutations in *PCYT1A*. (*A*) T1-weighted magnetic resonance image of proband 1 and a lean (body mass index, 21.5 kg/m^2) healthy female control. The proband's muscle bulk also appears to be relatively reduced. (*B*) Clinical photographs of proband 2 at age 10.9 y. The patient exhibits very little s.c. fat in the arms, legs, and buttocks, with preservation of fat in the trunk (*i*, *ii*, and *iii*). s.c. fat is preserved in the dorsocervical and submandibular regions (*iv*), as well as over the mons pubis and labia majora (*v*).

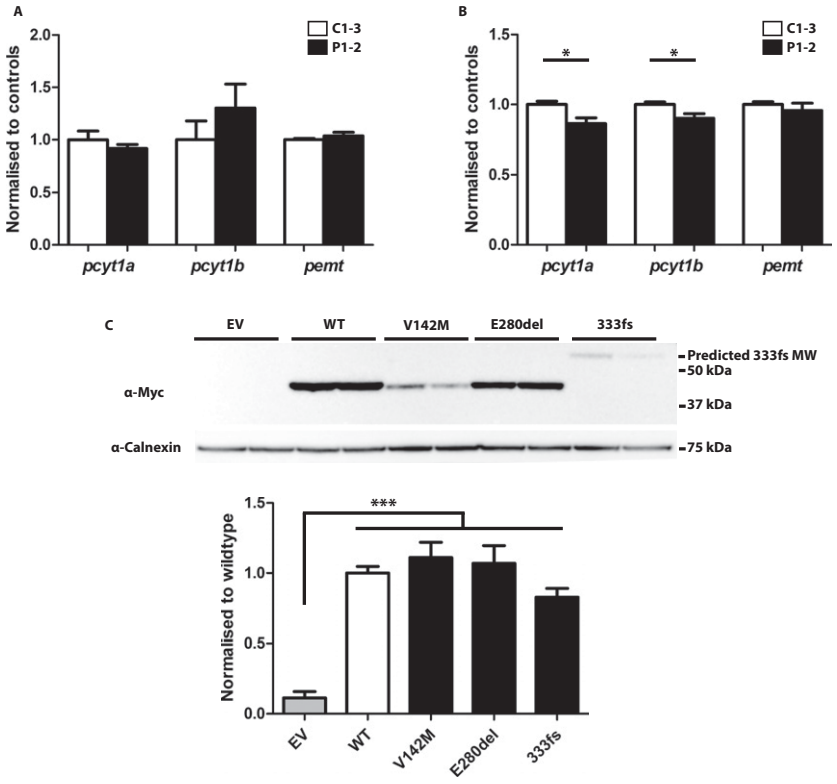


Fig. S2. Functional consequences of the mutations on *PCYT1A* expression in primary cells. (*A*) *PCYT1A*, *PCYT1B*, and *PEMT* mRNA expression levels in EBVLs from the probands (P1–2) and three healthy controls (C1–3). Data represent mean ± SE of three separate experiments and are normalized to controls for consistency. (*B*) *PCYT1A*, *PCYT1B*, and *PEMT* mRNA expression levels in skin fibroblasts from the probands (P1–2) and three healthy controls (C1–3). Data represent mean ± SE of three separate experiments and are normalized to controls for consistency. $*P < 0.05$. (*C*) COS7 cells were transfected with expression vectors for wild-type (WT) or mutant (V142M, E280del, or 333fs) myc-tagged PCYT1A. Lysates from these cells were then immunoblotted with a myc- and a calnexin antibody (loading control). *PCYT1A* mRNA expression levels were similar in the WT and V142M or E280del mutant transfected cells, but slightly lower in cells expressing the PCYT1A 333fs mutant (*Lower*). Data represent mean ± SE of three separate experiments and are normalized to WT for consistency. $***P < 0.001$.

Table S1. De novo mutations found in proband 1

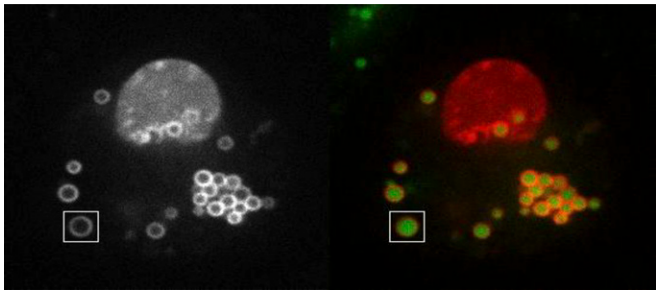
Gene	Mutation	Chr	Position GRCh37	Ref	Alt	PP DNM	1000 Genomes				NHLBI Exomes		U.K.10K Cohorts	ODEX Exomes	dbSNP v137
							EUR	AMR	AFR	ASN	EA	AA			
GEMIN5	S301C	5	154308099	G	C	1	a	a	a	a	a	a	a	a	a
BRF1	R57Q	14	105695163	C	T	1	a	a	a	a	0.0002	0.0002	0.0001	a	a

Chromosome positions are relative to NCBI build 37. Ref refers to the NCBI reference sequence allele, and Alt refers to the alternate allele. Nonreference allele frequencies are given for (*i*) 1000 Genomes (release 2012-07-19)—EUR (European ancestry), AMR (from the Americas), AFR (West African ancestry), ASI (East Asian ancestry); (*ii*) NHLBI Exome Variant Server (release ESP6500SI-V2, Apr-2013)—EA (European American ancestry), AA (African American ancestry); (*iii*) 3,781 Genomes from the U.K.10K Cohorts group (U.K.10K Cohorts, release 2012-06-02)—all of European ancestry; and (*iv*) 409 CoLaus Exomes (ODEX, release Dec-2010)—all of European ancestry. Variants absent from the control populations are labeled "a." PP DNM refers to the posterior probability that the mutation is de novo, as calculated by DeNovoGear, version 0.5.

Table S2. Compound heterozygous mutations found in probands 1 and 2

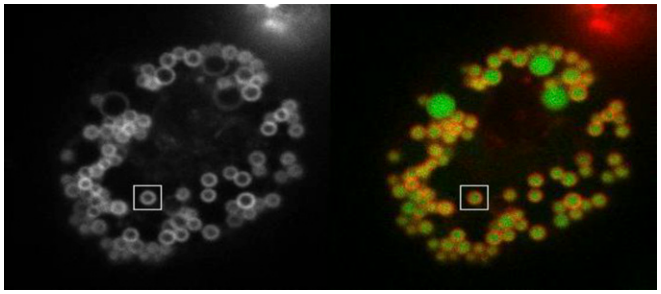
Patient	Gene	Mutation	Chr	Position GRCh37	Ref	Alt	1000 Genomes				NHLBI Exomes		U.K.10K Cohorts	ODEX Exomes	dbSNP v137
							EUR	AMR	AFR	ASN	EA	AA			
P1	PCYT1A	E280del	3	195966474	CTC	—	a	a	a	a	a	a	a	a	a
P1	PCYT1A	V142M	3	195974300	C	T	a	a	a	a	a	a	a	a	a
P2	PCYT1A	S333Lfs*164	3	195965666	G	—	a	a	a	a	a	a	a	a	a
P2	PCYT1A	E280del	3	195966474	CTC	—	a	a	a	a	a	a	a	a	a
P1	KLHDC7A	G337A	1	18808485	G	C	0.0000	0.0000	0.0081	0.0000	0.0000	0.0045	a	a	rs140251743
P1	KLHDC7A	D606A	1	18809292	A	C	a	a	a	a	a	a	a	a	a
P1	POTEI	E959Q	1	131220742	C	G	a	a	a	a	a	a	a	a	rs200829486
P1	POTEI	R816H	1	131221170	C	T	a	a	a	a	a	a	a	a	rs144934754
P2	MCTP1	L130P	5	94619891	A	G	a	a	a	a	a	a	a	a	a
P2	MCTP1	P60S	5	94620102	G	A	a	a	a	a	0.0002	0.0005	a	0.0016	rs199518217
P1	URGCP	R389Q	7	43917767	C	T	0.0013	0.0000	0.0000	0.0000	0.0045	0.0007	0.0042	0.0025	rs200826148
P1	URGCP	P242L	7	43918208	G	A	a	a	a	a	0.0012	0.0002	0.0012	0.0013	rs201996150
P1	CSPP1	S165C	8	68028252	C	G	0.0040	0.0028	0.0000	0.0000	0.0061	0.0011	0.0050	0.0051	rs146431326
P1	CSPP1	N701K	8	68062160	T	A	a	a	a	a	a	a	a	a	a
P1	MKI67	D747A	10	129909929	T	G	a	a	a	a	a	a	a	a	a
P1	MKI67	T435M	10	129913368	G	A	0.0000	0.0000	0.0061	0.0000	0.0001	0.0041	a	a	rs147894598
P1	LRRC56	R19Q	11	540740	G	A	0.0040	0.0055	0.0061	0.0000	0.0020	0.0073	0.0015	0.0025	rs144525570
P1	LRRC56	T285M	11	551708	C	T	0.0013	0.0000	0.0000	0.0070	0.0056	0.0011	0.0060	0.0069	rs113929032

Chromosome positions are relative to NCBI build 37. Ref refers to the NCBI reference sequence allele, and Alt refers to the alternate allele. Nonreference allele frequencies are given for (*i*) 1000 Genomes (release 2012-07-19)—EUR (European ancestry), AMR (from the Americas), AFR (West African ancestry), ASI (East Asian ancestry); (*ii*) NHLBI Exome Variant Server (release ESP6500SI-V2, Apr-2013)—EA (European American ancestry), AA (African American ancestry); (*iii*) 3,781 genomes from the U.K.10K Cohorts group (U.K.10K Cohorts, release 2012-06-02)—all of European ancestry; and (*iv*) 409 CoLaus Exomes (ODEX, release Dec-2010)—all of European ancestry. Variants absent from the control populations are labeled "a." P1 and P2 refer to patients 1 and 2, respectively.



Movie S1. Representative mCherry-PCYT1A wild-type FRAP experiment. The mCherry signal is shown on the *Left* [the bleached lipid droplet (LD) is boxed]; the image on the *Right* includes the LD stain (Bodipy, green).

Movie S1



Movie S2. Representative mCherry-PCYT1A E280del FRAP experiment. The mCherry signal is shown on the *Left* [the bleached lipid droplet (LD) is boxed]; the image on the *Right* includes the LD stain (Bodipy, green).

Movie S2