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Supplemental Data

SHORT Syndrome with Partial Lipodystrophy

Due to Impaired Phosphatidylinositol 3 Kinase Signaling

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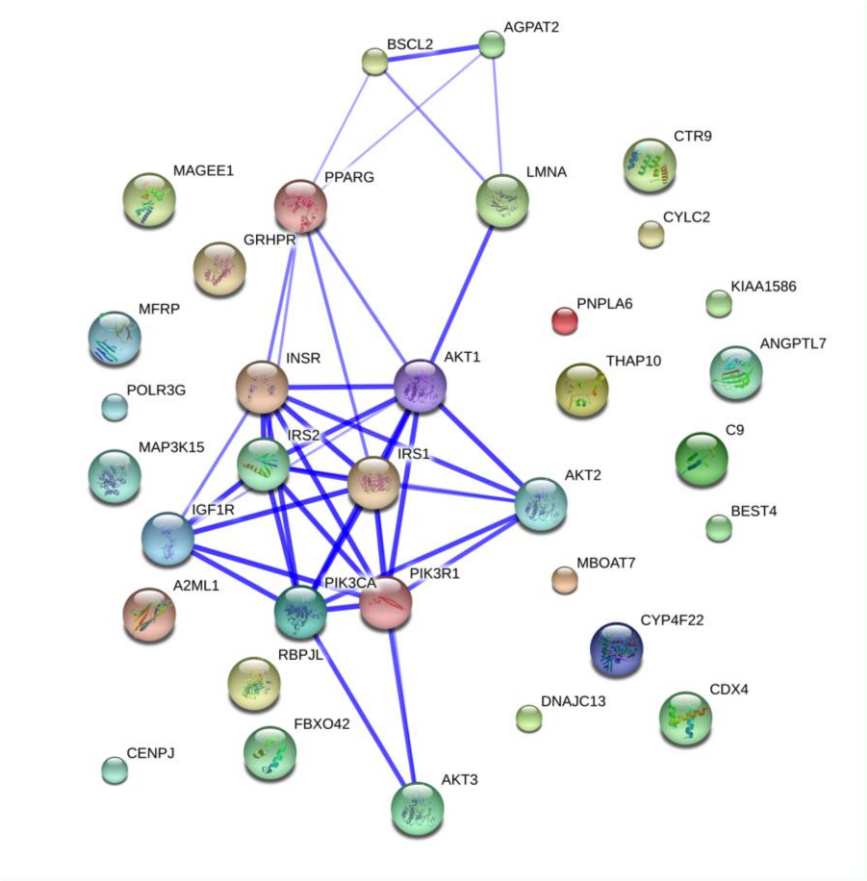

Figure S1. Protein-Network Analysis (STRING) by Searching for Physical or Functional Associations

We performed protein-network analysis with information from genomic context, reported experiments, co-expression and PubMed (http://www.string-db.org). We included the 22 candidate genes resulting from the exome sequencing and 12 genes related to lipodystrophy and insulin signaling (*AGPAT2, BSCL2, IGF1R, LMNA, PPARG, INSR, IRS1, IRS2, PIK3CA, AKT1, AKT2 and AKT3).* Only one of our candidate genes, *PIK3R1*, clustered with genes involved in insulin signaling and fat metabolism.

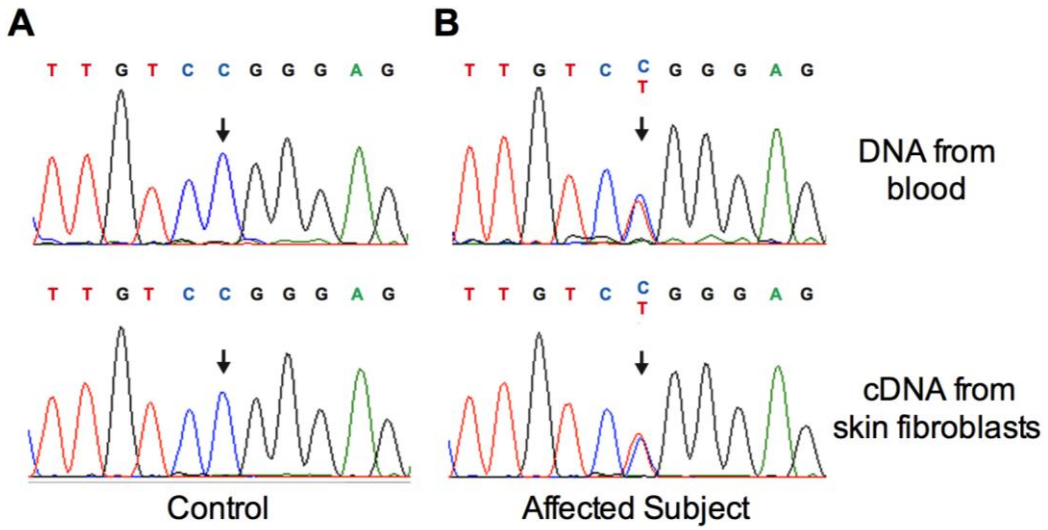

Figure S2. Sequence Analysis of DNA and cDNA of Affected and Healthy Individuals

Cultured skin fibroblasts were prepared from punch biopsies of the skin of one affected (Subject II-2) and one unaffected (Subject II-3) member of Family 1, and messenger RNA extracted, followed by cDNA synthesis. DNA sequences of the *PIK3R1* region of interest in a patient (B) (Family 1, Subject III-2) compared with a control (A). The results obtained from DNA in peripheral blood are shown in the top panel while the results of the lower panel are from cDNA made of RNA from skin fibroblasts. The right panels show the heterozygous missense mutation (c.1945C→T; marked with an arrow) in exon 15, corresponding to an amino acid change from arginine to tryptophan (p.Arg649Trp).

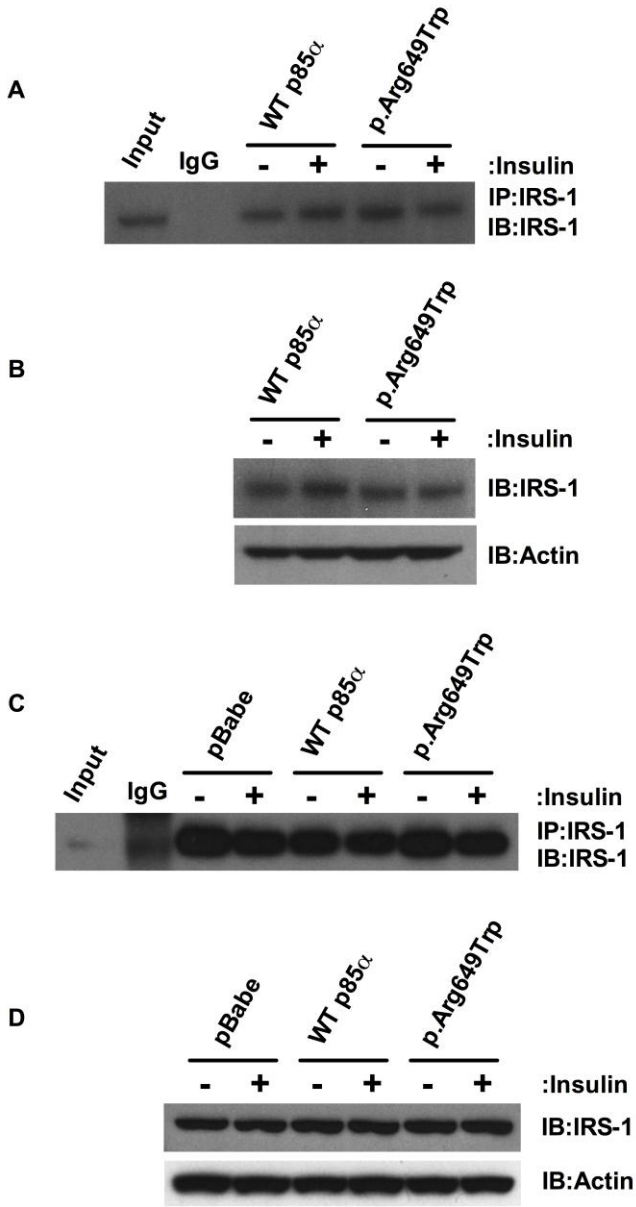

Figure S3. Expression and Immunoprecipitation Efficiency of IRS-1 Is Unaltered in Control and p.Arg649Trp Fibroblasts and p85α Reconstituted Cell Lines

(A) IRS-1 was immunoprecipitated from whole cell lysates obtained from control and p.Arg649WTrp human fibroblasts before or following insulin stimulation (10nM) for 10 minutes, resolved by SDS-PAGE and subsequently immunoblotted using IRS-1-specific antibodies.

(B) IRS-1 expression was assessed in control and p.Arg649Trp human fibroblasts in the absence or presence of insulin (10nM) for 10 minutes by performing immunoblot analysis on whole cell lysates using IRS-1-specific antibodies.

(C) IRS-1 was immunoprecipitated from whole cell lysates obtained from pBabe, WT or p.Arg649Trp reconstituted p85α/p85β-deficient cell lines in the absence or presence of insulin (10nM) for 10 minutes, resolved by SDS-PAGE and subsequently immunoblotted using anti-IRS-1 antibodies.

(D) IRS-1 expression was assessed in whole cell lysates obtained from pBabe, WT or p.Arg649Trp cells in the absence or presence of insulin (10nM) for 10 minutes by performing immunoblot analysis using anti-IRS-1 antibodies.

			Fam1		Fam2				
Chr	dbSNP	pos	III-2	II-2	III-2	II-1	Haplo Fam1	Haplo Fam2	Not shared
5	rs10515070	67525575	2 2	2 2	1 1	1 2	1	2	x
5	rs16897511	67526313	1 2	2 2	2 2	2 2	2	2	
5	rs6881033	67529191	1 2	2 2	1 1	1 2	1	2	x
5	rs4122269	67545624	1 2	2 2	2 2	2 2	2	2	
5	rs40318	67545753	2 2	2 2	1 1	1 2	1	2	x
5	rs10940159	67547276	2 2	2 2	2 2	2 2	2	2	
5	rs173703	67547326	1 2	2 2	1 1	1 2	1	2	x
5	rs831229	67553016	2 2	2 2	1 1	1 2	1	2	x
5	rs6893676	67553771	2 2	2 2	2 2	2 2	2	2	
5	rs863818	67554023	2 2	2 2	1 1	1 2	1	2	x
5	rs831121	67554926	2 2	2 2	1 1	1 2	1	2	x
5	rs13167294	67555580	2 2	2 2	2 2	2 2	2	2	
5	rs16897561	67560716	2 2	2 2	2 2	2 2	2	2	
5	rs6863431	67561015	2 2	2 2	2 2	2 2	2	2	
5	rs10072475	67568728	2 2	2 2	2 2	2 2	2	2	
5	rs2302975	67569479	2 2	2 2	2 2	2 2	2	2	
5	rs2302976	67569602	2 2	2 2	2 2	2 2	2	2	
5	rs171649	67569746	2 2	2 2	1 1	1 2	1	2	x
5	**rs251409**	**67573463**	**2 2**	**2 2**	**1 1**	**1 2**	**1**	**2**	**x**
5	rs17318918	67573919	2 2	2 2	2 2	2 2	2	2	
5	rs34308	67579223	2 2	2 2	1 2	1 2	1/2	2	
5	rs12657050	67579576	1 2	1 2	1 2	1 2	1/2	1/2	
5	**Mutation**	**67592129**	**x**	**x**	**x**	**x**			
5	rs895304	67592281	2 2	2 2	2 2	2 2	2	2	
5	rs7714169	67592640	2 2	2 2	2 2	2 2	2	2	
5	rs3729981	67593363	2 2	2 2	2 2	2 2	2	2	
5	rs1043526	67594324	1 2	1 2	2 2	2 2	2	1/2	
5	rs7722222	67595785	2 2	2 2	2 2	2 2	2	2	
5	rs3756668	67596088	1 2	1 2	1 2	1 2	1/2	1/2	
5	rs7701952	67602847	2 2	2 2	2 2	2 2	2	2	
5	rs695166	67604628	1 2	1 2	1 2	1 2	1/2	1/2	
5	rs411751	67605884	2 2	2 2	1 2	1 2	1/2	2	
5	rs440292	67609408	1 2	1 2	1 2	1 2	1/2	1/2	
5	rs453940	67610482	1 2	1 2	1 2	1 2	1/2	1/2	
5	rs10515077	67613801	2 2	2 2	2 2	2 2	2	2	
5	rs391974	67614893	2 2	2 2	1 2	1 2	1/2	2	
5	**rs17253786**	**67615528**	**1 1**	**1 2**	**2 2**	**2 2**	**2**	**1**	**x**
5	rs6859287	67628886	2 2	2 2	2 2	2 2	2	2	
5	rs256505	67629059	2 2	2 2	2 2	2 2	2	2	
5	rs16897697	67633340	2 2	2 2	2 2	2 2	2	2	
5	rs16897699	67636057	2 2	2 2	2 2	2 2	2	2	
5	rs10515080	67643213	2 2	2 2	2 2	2 2	2	2	
5	rs40055	67647834	1 1	0 0	2 2	2 2	2	1	x

Figure S4. Fine Mapping and Haplotype Analysis of Region Surrounding the *PIK3R1* Mutation

Two affected individuals were genotyped in each family to search for a putative shared haplotype (last three columns). Homozygous calls shown in red and yellow. Highlighted box shows the 41 kb maximal region where a shared haplotype could not be refuted.

Table S1. Key Clinical Features of Affected Subjects in Family 1 and Family 2

Features	Frequency	Description or Median Value
Partial lipodystrophy	5/5	Face, flanks, buttocks
Low body mass index	4/5	17.1 kg/m^2
Short stature	5/5	Females 152 cm Males 163 cm
Progeroid face	5/5	Midface hypoplasia, wide and deep set eyes, triangular face, hypotrichosis
Rieger anomaly	5/5	Anterior chamber and iris malformations

Table S2. Variant Filtration of Exome Sequencing Data

Filter	Remaining Number of Variants
Exomic variants identified in Subject III-2 of Family 1[a]	14,253
Excluding synonymous variants	6,467
Excluding variants found in 52 Norwegian exomes or in the 1000Genomes[b] database (minor allele frequency >0.5%)	182
Excluding variants not present in both affected subjects	49
Excluding variants present in healthy sibling or dbSNP[c] database	22

[a]Three patients from the Norwegian family (Family 1) was exome-sequenced, the affected proband, his affected aunt and his non-affected brother. Twenty-two rare genetic variants fitted a dominant disease model in the family.
[b]http://www.1000genomes.org/.
[c]http://www.ncbi.nlm.nih.gov/projects/SNP/.

Table S3. Candidate Rare Variants Identified by Exome Sequencing after Filtering[a]

Position	Type of Mutation	Gene	cDNA Change	Protein Change
Chr12: 9020954	nonsynonymous	*A2ML1*	c.4061+1G>A	Splice
Chr1: 11249707	nonsynonymous	*ANGPTL7*	c.71C>T	p.A24V
Chr1: 45253091	nonsynonymous	*BEST4*	c.200G>A	p.R67Q
Chr5: 39306775	nonsynonymous	*C9*	c.1360G>A	p.D454N
ChrX: 72673407	nonsynonymous	*CDX4*	c.557T>G	p.L186W
Chr13: 25480072	nonsynonymous	*CENPJ*	c.2104A>G	p.S702G
Chr11: 10786213	nonsynonymous	*CTR9*	c.1532G>A	p.C511Y
Chr9: 105767074	nonsynonymous	*CYLC2*	c.278C>T	p.A93V
Chr19: 15648692	nonsynonymous	*CYP4F22*	c.559C>T	p.R187W
Chr3: 132221143	nonsynonymous	*DNAJC13*	c.4547G>A	p.R1516H
Chr1: 16641889	nonsynonymous	*FBXO42*	c.25G>A	p.D9N
Chr9: 37426621	nonsynonymous	*GRHPR*	c.374G>A	p.R125Q
Chr6: 56919345	nonsynonymous	*KIAA1586*	c.2048A>G	p.N683S
ChrX: 75648697	nonsynonymous	*MAGEE1*	c. 374G>A	p.C125Y
ChrX: 19379488	nonsynonymous	*MAP3K15*	c.3826A>C	p.T1276P
Chr19: 54678024	nonsynonymous	*MBOAT7*	c.914G>A	p.R305Q
Chr11: 119213348	nonsynonymous	*MFRP*	c.1345G>A	p.G449S
Chr5: 67592129	nonsynonymous	*PIK3R1*	c.1945C>T	p.R649W
Chr19: 7625573	nonsynonymous	*PNPLA6*	c.3544T>C	p.W1182R
Chr5: 89783851	nonsynonymous	*POLR3G*	c.152G>C	p.G51A
Chr20: 43938333	nonsynonymous	*RBPJL*	c.257+1G>T	Splice
Chr15: 71184356	nonsynonymous	*THAP10*	c.256G>A	p.V86M

[a]Based on an autosomal dominant model.