

Gerodermia osteodysplastica is caused by mutations in a novel golgin, SCYL1BP1

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Introduction

Skin wrinkling is a feature of several uncommon monogenic disorders so far mainly assigned to mutations in extracellular matrix components¹⁻⁸.

Supplementary Methods

Subjects

Several of our GO patients have been described in the literature. Family CD1 is family V described by Hunter et al.⁹. The phenotype of the sporadic patient IG is mentioned in a case report from Picco et al.¹⁰. Family OA is family B in our detailed report on the clinical features of GO and autosomal recessive cutis laxa type II (ARCL2; ARCL Debré type; wrinkly skin syndrome; OMIM 219200) in the Omani population and the differential diagnosis¹¹. In summary, an often cachexic appearance, failure to thrive, a wide open fontanelle with delayed closure, a typical facial dysmorphism with downslanting palpebral fissures, and a stronger and more generalized skin wrinkling distinguishes ARCL2 from GO during infancy. During adolescence the skin wrinkling in ARCL2 becomes less pronounced and a patchy distribution of subcutaneous fat tissue becomes apparent. Mental impairment is highly variable in ARCL2 and ranges from mild speech delay to neurodegenerative deterioration¹². None of our GO patients had mental retardation or neurodegeneration. Furthermore, GO can be distinguished from autosomal dominant and autosomal recessive cutis laxa type 1 (ARCL1) by a complete absence of pulmonary and cardiovascular involvement.

We have done ultrastructural investigations of samples from one GO case. The collagen fibrils were round and regular in contour, of normal diameter and well packed. The elastin fibers were also normal in any respect. We have excluded N-

glycosylation abnormalities by capillary electrophoresis of serum transferrin in a total of four patients from Oman and two patients from Libya. O-glycosylation analysis by ApoCIII isoelectric focussing was performed in two patients from Oman and did not reveal any abnormality.

All the families were recruited after written informed consent. The study was conducted according to the standards of the Ethics Committees of the participating clinics.

Genotyping and linkage analysis

DNA was extracted from EDTA blood samples using standard procedures. DNA samples were genotyped with 375 microsatellites evenly distributed over the genome. Data were checked using the program Graphical Representation of Relationships (GRR)¹³ and PedCheck¹⁴. Parametric linkage analysis was performed with the program package LINKAGE v5.2¹⁵, assuming autosomal recessive inheritance and full penetrance. Multipoint lod score analysis was done with Simwalk2¹⁶. Most likely haplotypes were constructed either manually or with Simwalk2.

Mutation analysis

Positional candidate genes were obtained from the GenBank (<http://www.ncbi.nlm.nih.gov/mapview/>) and Ensembl (<http://www.ensembl.org>) databases. Genes were analyzed by direct sequencing of DNA with primers flanking each exon. Primer sequences were based on the reference sequences of each gene as obtained from the afore mentioned databases. The primer sequences can be provided upon request. Sequence analysis was done with the BigDye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA), and products were run on a 3730 DNA Analyzer (Applied Biosystems, Foster City, CA). Sequence alignments were done with ClustalW¹⁷. Protein domains were analysed using the Pfam¹⁸ and InterPro¹⁹ databases. Coiled-coil domains were predicted with the programmes Coils²⁰ and Paircoil2²¹.

Quantitative PCR and Western blot analysis

RNA was isolated from organs of P5 mice using the Trizol (Invitrogen) method. 1µg of RNA was reverse transcribed using the Revert Aid kit (Fermentas) and random

hexamers. Quantitative PCR was performed with SYBR green (Applied Biosystems) and the following primers: Scyl1bp1-f TCGAGCTCCAAAGCCAGATT, Scyl1bp1-r TCTTTCCGCAATGGCTTGAG, Gapdh-f GGAAGCCCATCACCATCTT, Gapdh-r CGGCCTCACCCCATTTG. Whole cell lysates of passage 4-8 primary skin fibroblasts were resolved by electrophoresis in SDS polyacrylamide gels and transferred onto PVDF (Amersham). For Western blot analysis membranes were probed with the antibodies: anti-Actin A5060 (Sigma) 1:1000 and rabbit anti-SCYL1BP1 1:300. The anti-SCYL1BP1 antibody was generated by immunization with the peptide H2N-CKQTKDPFEPQRRLPA-CONH2.

Immunofluorescence analysis

Cells were fixed in 3-4% (wt/vol) paraformaldehyde, quenched for 10min with 50nM ammonium chloride and permeabilized with 0.1% (vol/vol) Triton-X100 or Saponin for 5-10min. Antibody labelling was performed for 1h or over night. Secondary antibodies were conjugated to Alexa 488, Alexa 555 or Cy3 and DNA was stained with DAPI. Coverslips were mounted with 10% (wt/vol) Moviol 4-88 1mg/ml DAPI, 25% (wt/vol) glycerol in PBS or with Fluoromount. Antibody dilutions were: rabbit anti Rab6 (C-19, Santa Cruz) 1:250, mouse anti Rab6 (kind gift from Dr. Barnekow) 1:600, rabbit anti SCYL1BP1 1:300, goat anti GM130 (kind gift from Dr. Barr) 1:800, mouse anti γ -Adaptin (Transduction Laboratories) 1:500, sheep anti TGN46 (Serotec) 1:500.

Yeast two-hybrid analysis

The yeast reporter strain PJ69-4A was transformed with pAct2-Scyl1bp1 constructs (pAct2: Clontech, Heidelberg, Germany) as prey and the respective Rab-/Arf-/Arl-GTPases constructs in the pFBT9 bait vector (pGBT9 [Clontech] modified to carry kanamycin resistance) following the Clontech laboratories yeast protocol handbook. Yeast was plated onto synthetic medium (-LW) and grown for 3d at 30°C. Colonies were picked and restreaked onto synthetic QDO medium and grown for another 2-3d. Growth indicated interaction of bait and prey proteins.

GST pull-down

For pulldown assays, 500mg recombinant GST-Rab6 wild type, Rab6/T27N GDP-locked mutant or Rab1 wild type protein were coupled to 50ml of packed glutathione-Sepharose beads (GE Healthcare) for 1h at 4°C in a total volume of 500ml PBS.

Beads were washed three times in 500ml NE100 buffer (20mM Hepes/NaOH pH 7.5, 100mM NaCl, 10mM EDTA, 0.1% Triton-X100) and then mixed with 1.5ml HeLa-L extract (1.5mg protein) in NL100 buffer (20mM Hepes/NaOH pH 7.5, 100mM NaCl, 5mM MgCl₂, 0.1% Triton-X100, protease inhibitor cocktail (Roche Diagnostics) and supplemented with a final concentration of 100mM GDP or GTP as described by Christoforidis and Zerial²². Beads were incubated at 4°C for 2h and washed three times with 500ml NL100 buffer that included 100mM GDP or GTP. Bound protein was subsequently eluted by rotating beads for 10min in 500ml NE200 buffer (20mM Hepes/NaOH pH 7.5, 200mM NaCl, 20mM EDTA, 0.1% Triton-X100) at 4°C. After pelleting the beads by centrifugation for 3min at 400xg and 4°C, the supernatant was incubated once with 50ml glutathione-Sepharose for 10min at 4°C in order to remove resident recombinant Rab protein. The beads were again pelleted by centrifugation at 400xg 4°C for 3min and the supernatant subjected to protein precipitation using trichloric acetic acid and analysed by Western blotting analysis. For HeLa-L cell extracts, six 15cm dishes with cells were grown to 80-90% confluency. Cells were scraped off the dishes into PBS and washed with PBS. Cells were pelleted for 5min at 400xg and lysed on ice for 30min in 10ml NL100 buffer supplemented with protease inhibitor cocktail. Cell debris was removed by centrifugation for 15min at 3500xg and 4°C and the supernatant transferred to a fresh tube.

URLs

Genbank: <http://www.ncbi.nlm.nih.gov/Genbank/>

Mapviewer: <http://www.ncbi.nlm.nih.gov/mapview/>

Ensembl: <http://www.ensembl.org>

EMBnet: <http://www.ch.embnet.org>

ExpASY Proteomics: <http://www.expasy.org/>

InterPro: <http://www.ebi.ac.uk/interpro/>

Pfam: <http://pfam.sanger.ac.uk/>

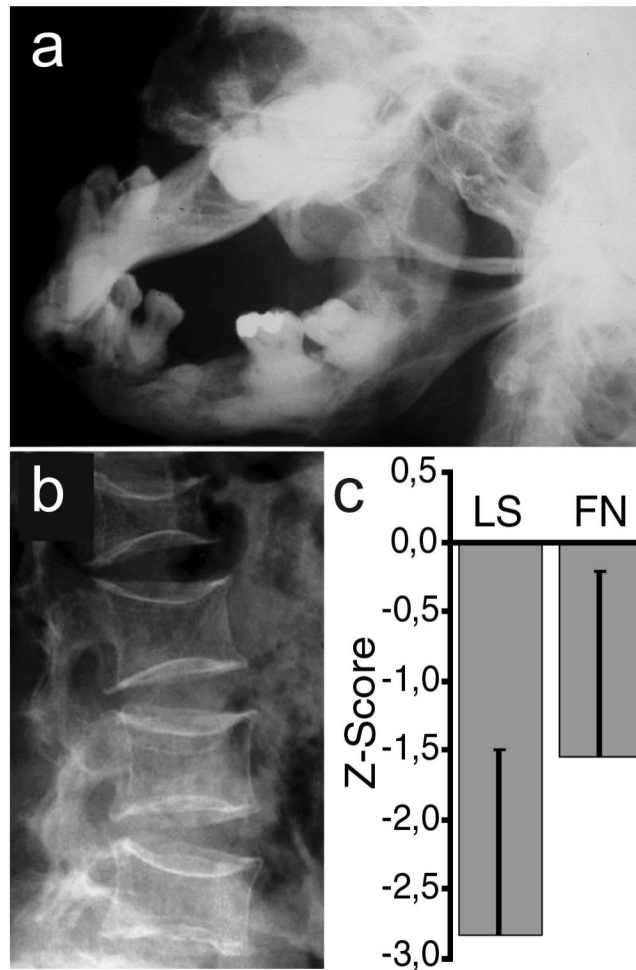
Supplementary References

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Supplementary Table 1. Sequences of primers for mutation screening.

Location		Sequence	Fragment Size
Exon 1	forward	GTTTGTTCAGGCACTGCTG	
	reverse	CTTCCGCTAACACAAACGTAG	350 bp
Exon 2	forward	GAAACTCTACAGGAAGATGGCTG	
	reverse	TCCAGCTTATATTTAGGCAGTAGC	700 bp
Exon 3	forward	AATGAACTGCAGATGGGTTTC	
	reverse	TGGGAGCACTAATGAAGGTC	527 bp
Exon 4	forward	TGTTTCAATGTAAAGGGAACACAG	
	reverse	TGCTCTAATGGCACACGAAC	678 bp
Exon 5	forward	AGTCTGGCCAACATGTAGAC	
	reverse	GTCAGGGCATGATCTACTG	683 bp



Supplementary Figure 1. Clinical features of geroderma osteodysplastica (GO).

(a) Radiograph of a 45-year old patient showing thinning of the mandibula and tooth loss.

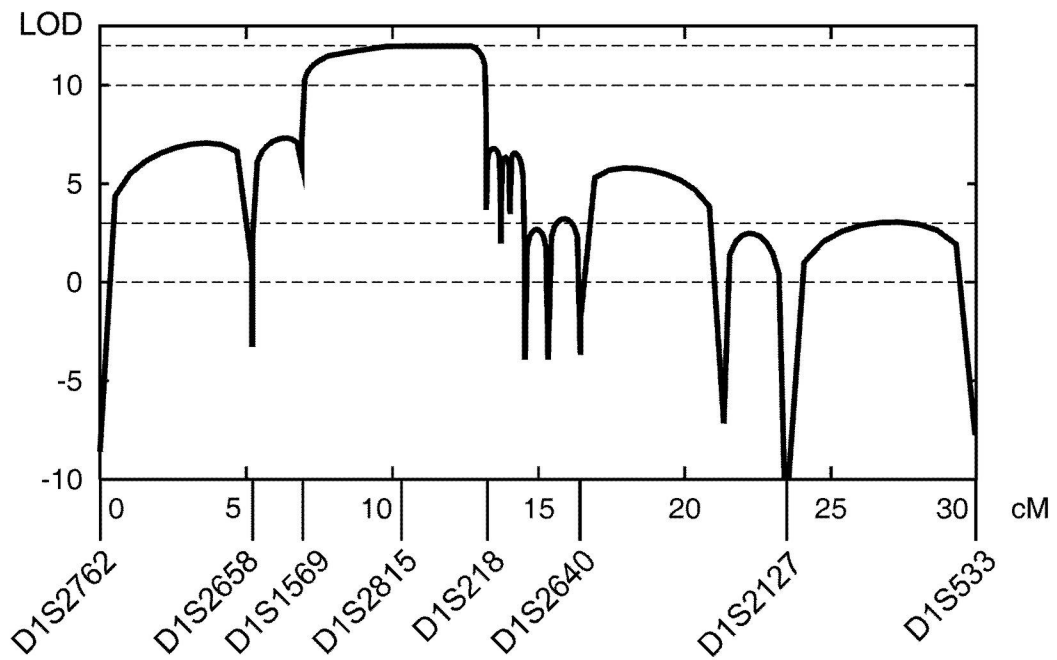
(b) X-ray of the lumbar spine from a 34-year old female patient from Oman showing

osteoporosis and cod-fish vertebrae. (c) Bone mineral density in GO shown as number of

standard deviations after age correction (Z score). While average bone mineral density

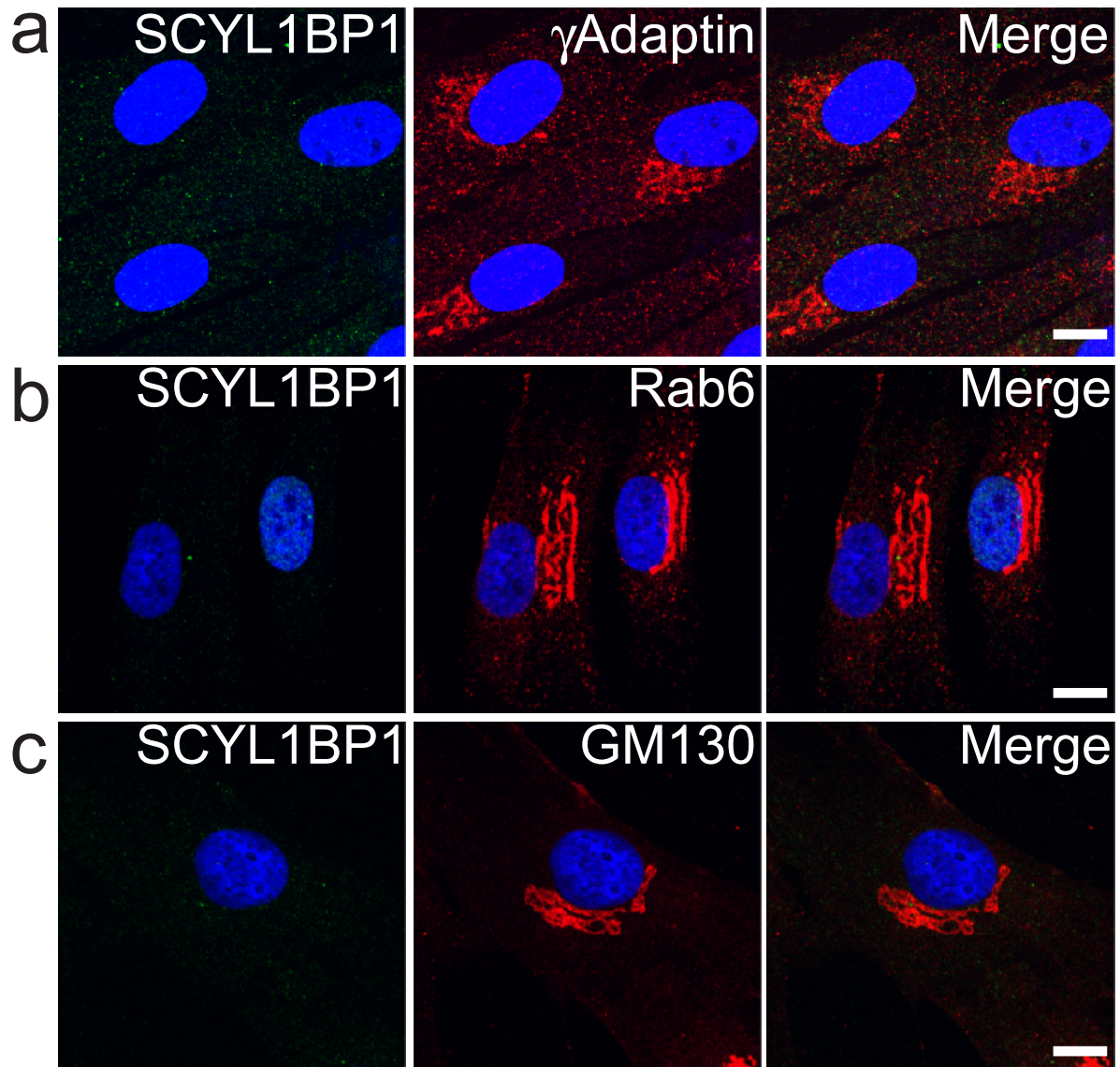
values matched the criteria for osteoporosis at the lumbar spine, the femoral neck

displayed osteopenia. LS, lumbar spine; FN, femoral neck.

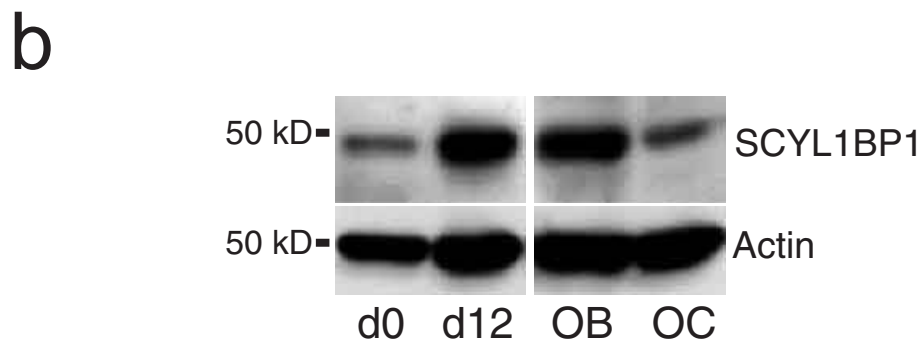
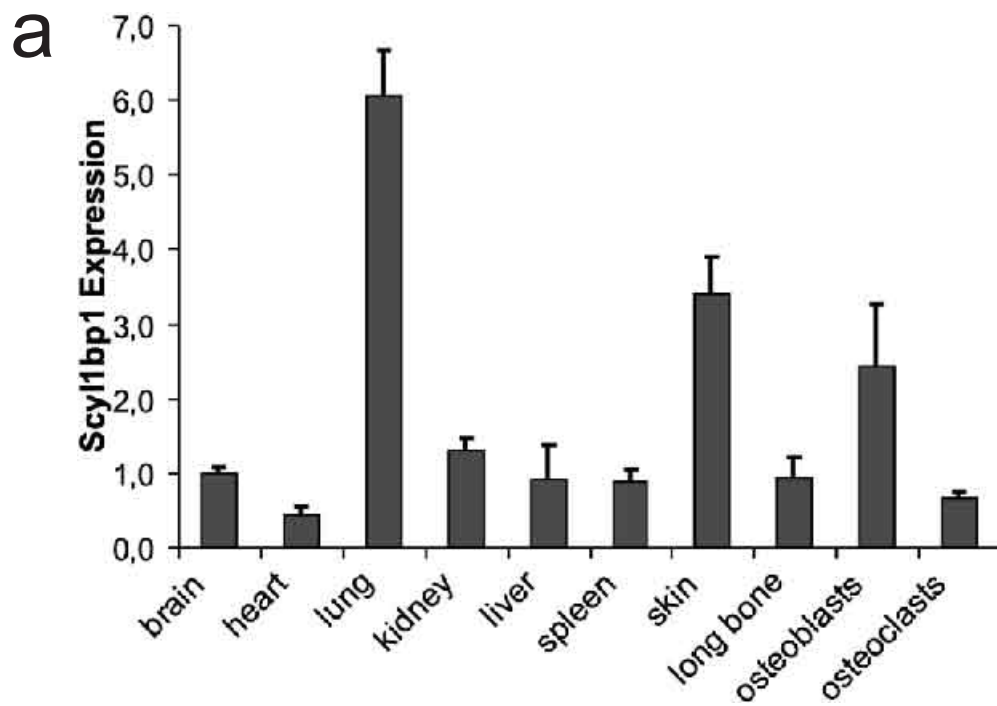


Supplementary Figure 2. Mapping of the gene for GO by linkage analysis.

Multipoint analysis of microsatellite markers used for refined mapping in the candidate region on chromosome 1q24 in four consanguineous Mennonite pedigrees from Germany, Canada, and Mexico revealed a maximum lod score of 12.0 between the markers D1S1569 and D1S218.

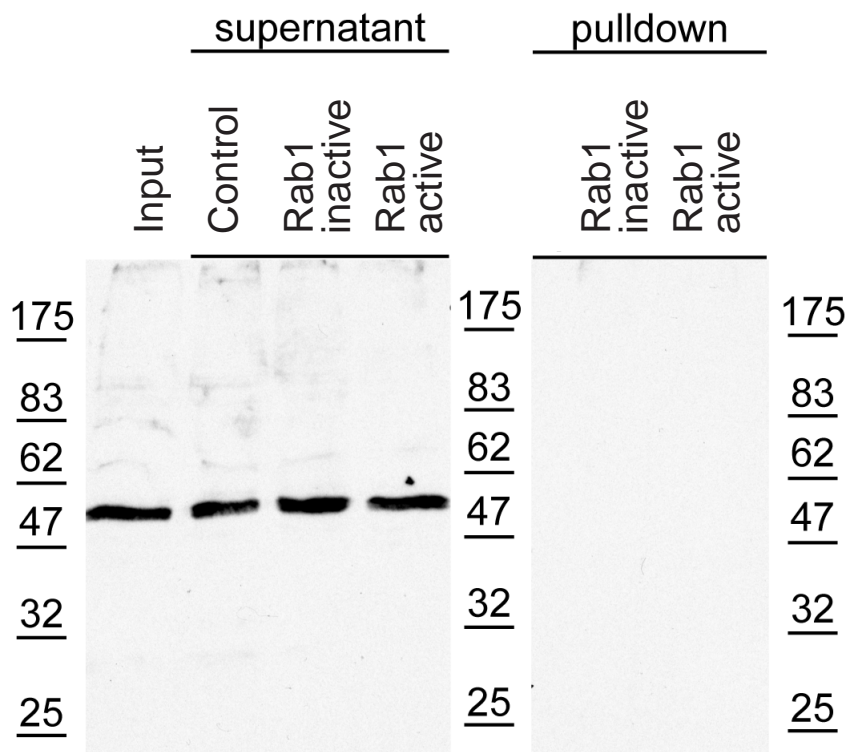


Supplementary Figure 3. Loss of porostin (SCYL1BP1, green) and unchanged distribution of Golgi markers in GO patient cells. (a) Co-staining for TGN marker γ Adaptin (red). (b) Co-staining for trans Golgi marker Rab6 (red). (c) Co-staining for cis Golgi marker GM130 (red). Scale bar 10 μ m.



Supplementary Figure 4. Expression analysis.

(a) Expression analysis of Scyl1bp1 in tissues from 5-day old mice by quantitative PCR. The highest expression levels were detected in lung, skin, and osteoblasts. (b) Western blot analysis of SCYL1BP1 synthesis in osteoblasts and osteoclasts. d0, undifferentiated murine osteoblasts, d12, differentiated murine osteoblasts, OB, human osteoblasts, OC, human osteoclasts.



Supplementary Figure 5. SCYL1BP1 does not interact with the Golgi-resident small GTPase Rab1. GST-Rab1 fusion-protein failed to pull-down SCYL1BP1 from HeLa cell lysates in the presence (active) or absence (inactive) of GTP. Blots were detected with anti SCYL1BP1 antibody.