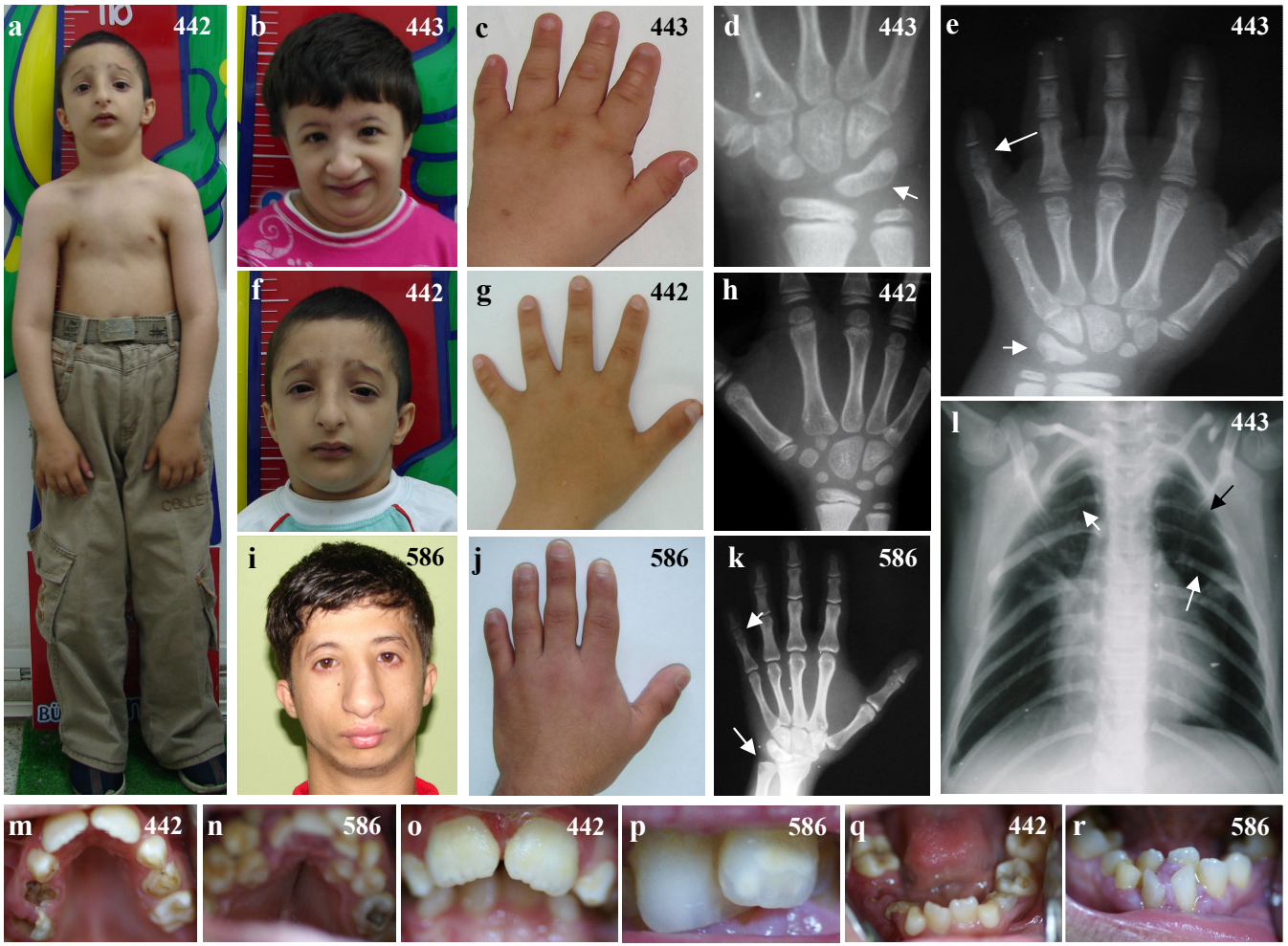
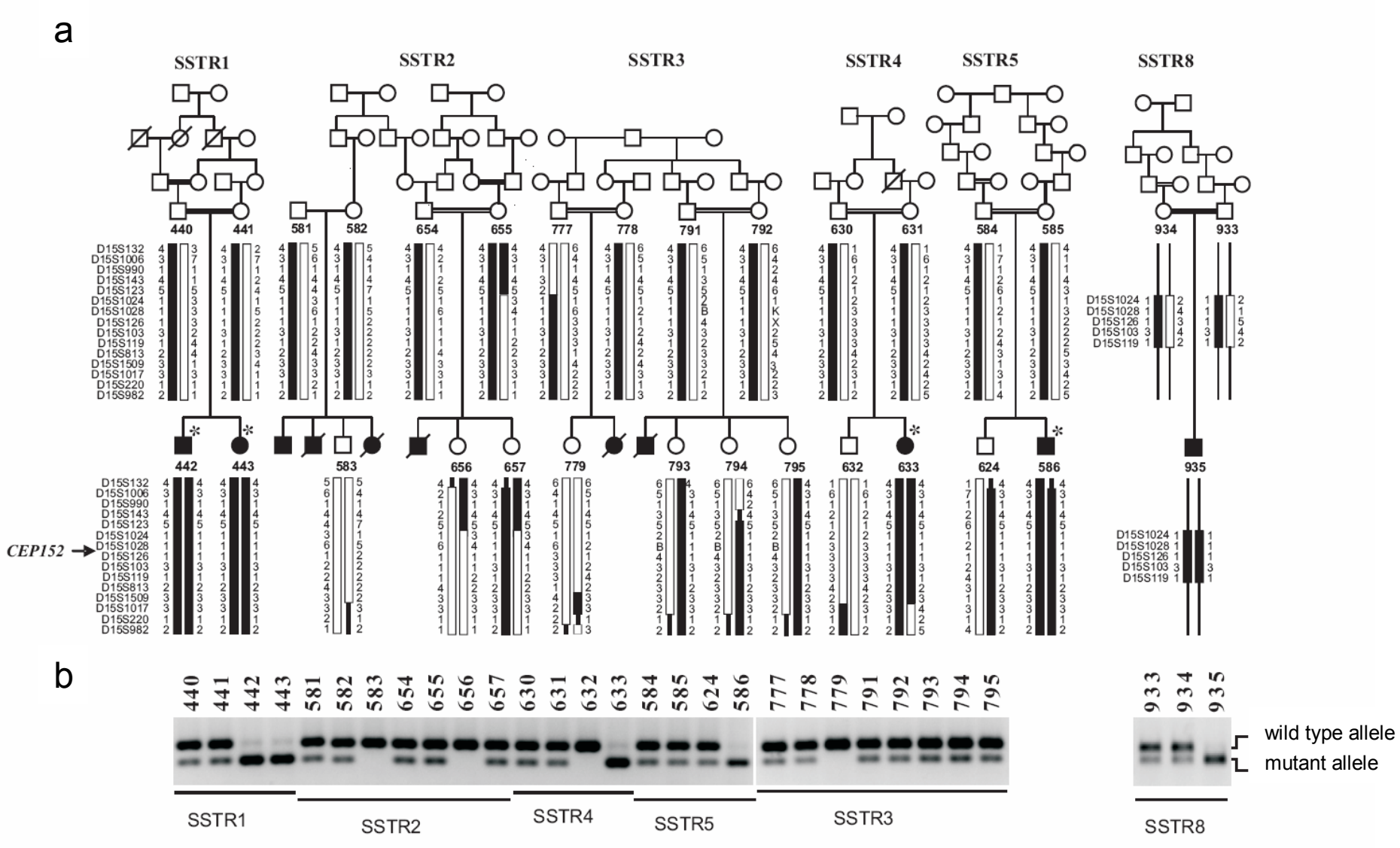
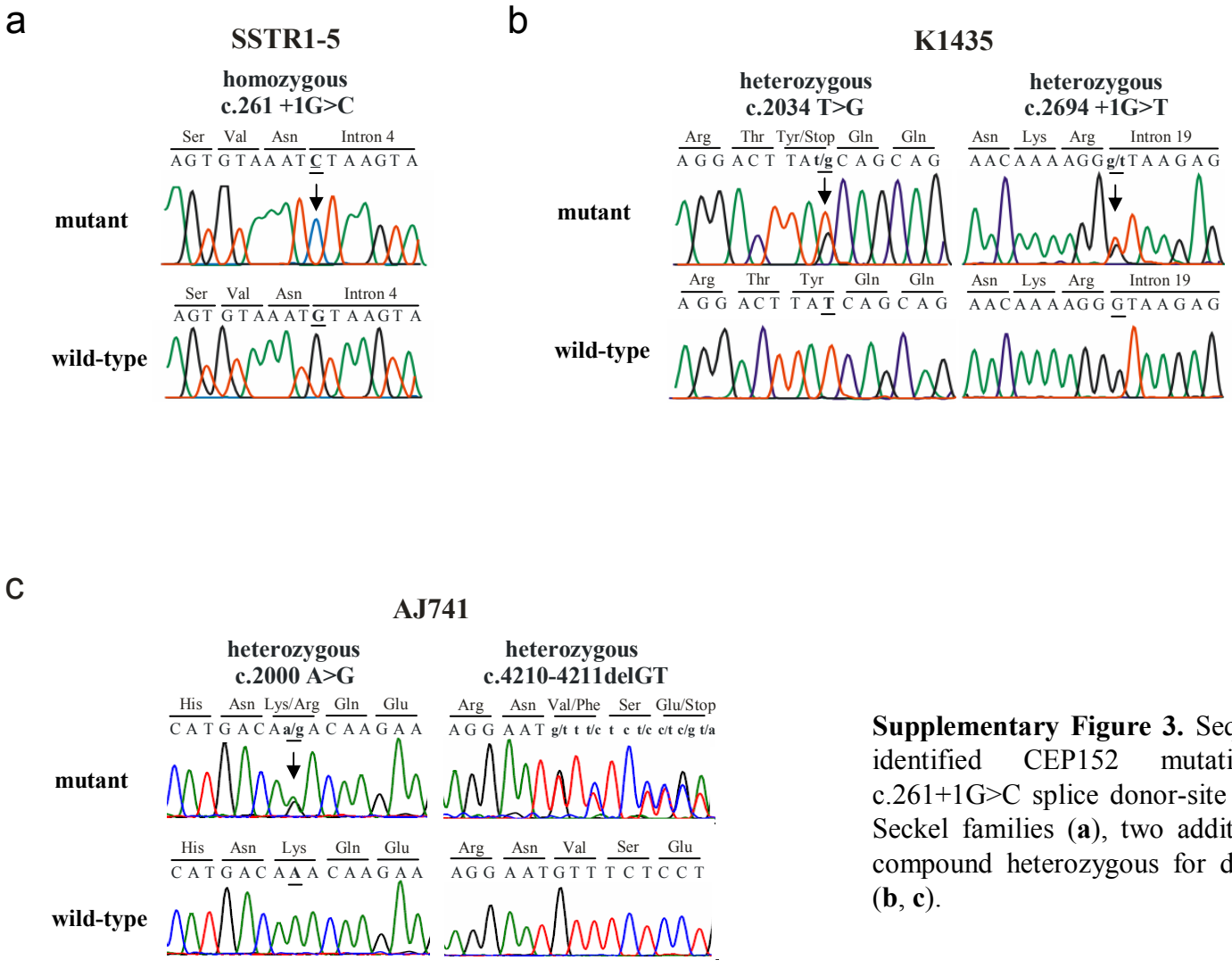
Supplementary Figure 1. Representative clinical details of *CEP152*-Seckel patients. **(a)** The 10 year-old Turkish patient 442 showed markedly short stature and narrow thorax. **(b, f, i)** Cases 443, 442 and 586 had microcephaly, micrognathia, low-set ears, prominent nose and **(c, g, j)** fifth finger clinodactyly. X-ray radiography of hands **(d, e)** showed fusion of the carpal bones (coalition of lunate and triquetrum) and hypoplasia of the middle phalanx of the fifth digit in 8 year-old patient 443, **(h)** delayed bone age (consistent with 6 years of age) in patient 442, and **(k)** fusion of the lunate and triquetral bones, osteonecrosis of the scaphoid bone, hypoplasia of the ulnar styloid process, and hypoplasia of the middle phalanx of the fifth digit in 20 year-old patient 586. **(l)** Chest X-ray radiography of patient 443 showed bilateral eleven ribs, abnormal and fragmented clavicles, bilateral hypoplasia of upper ribs, lucent ossification gaps in some of the posterior ribs (black arrow), and asymmetric short ribs (short tail arrow). **(m-n)** High-arched palate, **(m, o, p, q)** enamel hypoplasia, **(n, p, q, r)** dental crowding observed in patients 442 and 586. Informed consents to publish the photographs were obtained from the subject's parents.



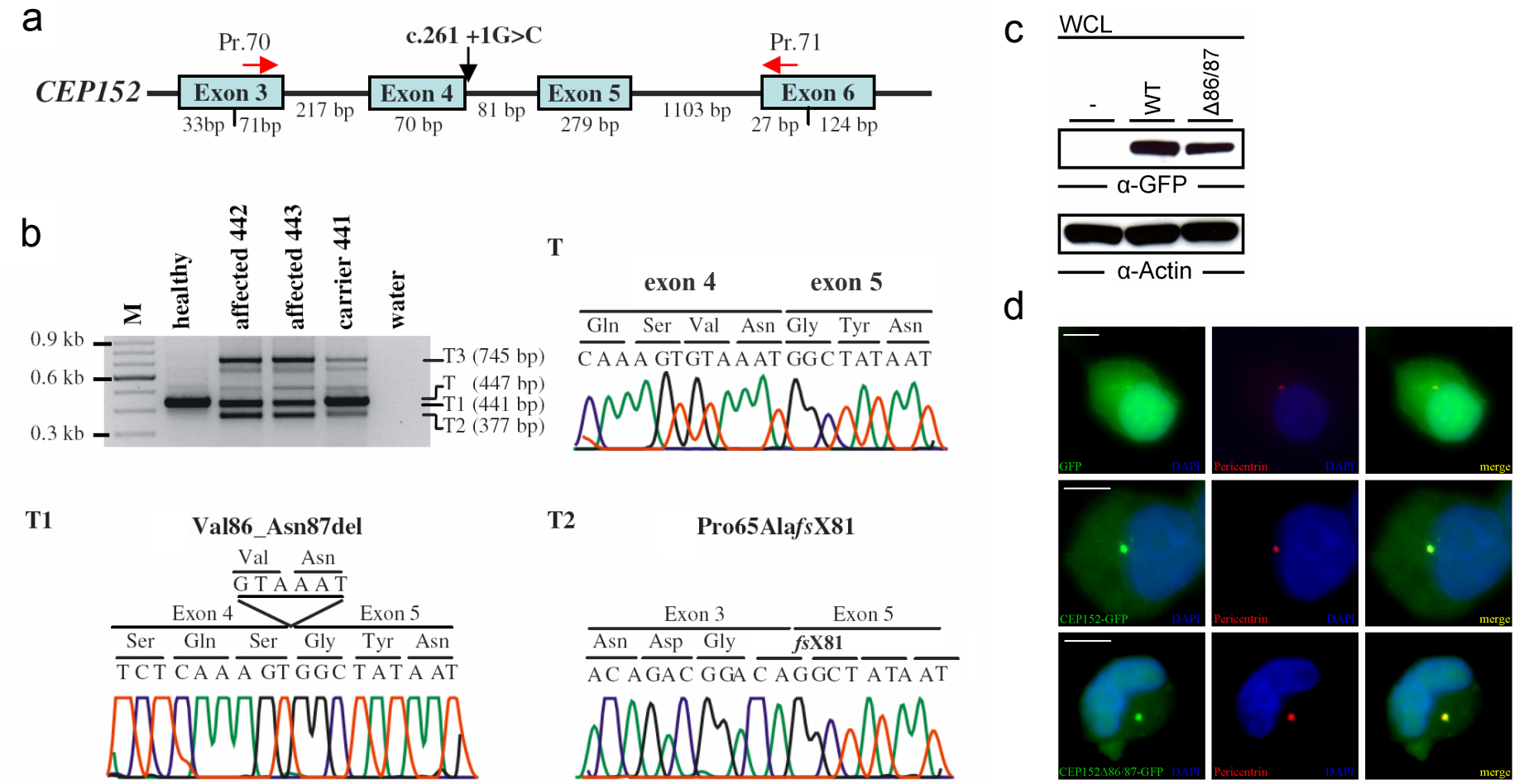
Supplementary Figure 2. (**a**) Haplotypes of Turkish Seckel families linked to chromosome 15q21.1-q21.2. Black symbols represent affected individuals. The critical interval was defined by the recombinations observed for markers *D15S123* in individuals 655 and 657 and marker *D15S1017* in the affected individual 633. The disease haplotype is indicated by black bars and family members included in the initial genome-wide homozygosity mapping are marked by an asterisk. A founder haplotype was present in families SSTR1, SSTR2, SSTR3, SSTR4, SSTR5, and SSTR8. **(b)** The c.261+1G>C mutation creates a *Dde*I restriction site and digestion of 328 bp mutant amplicon by *Dde*I yielded in fragments of 274 bp and 54 bp in size. Analysis of restriction enzyme digestion showed co-segregation of the mutant allele with disease in the tested families.



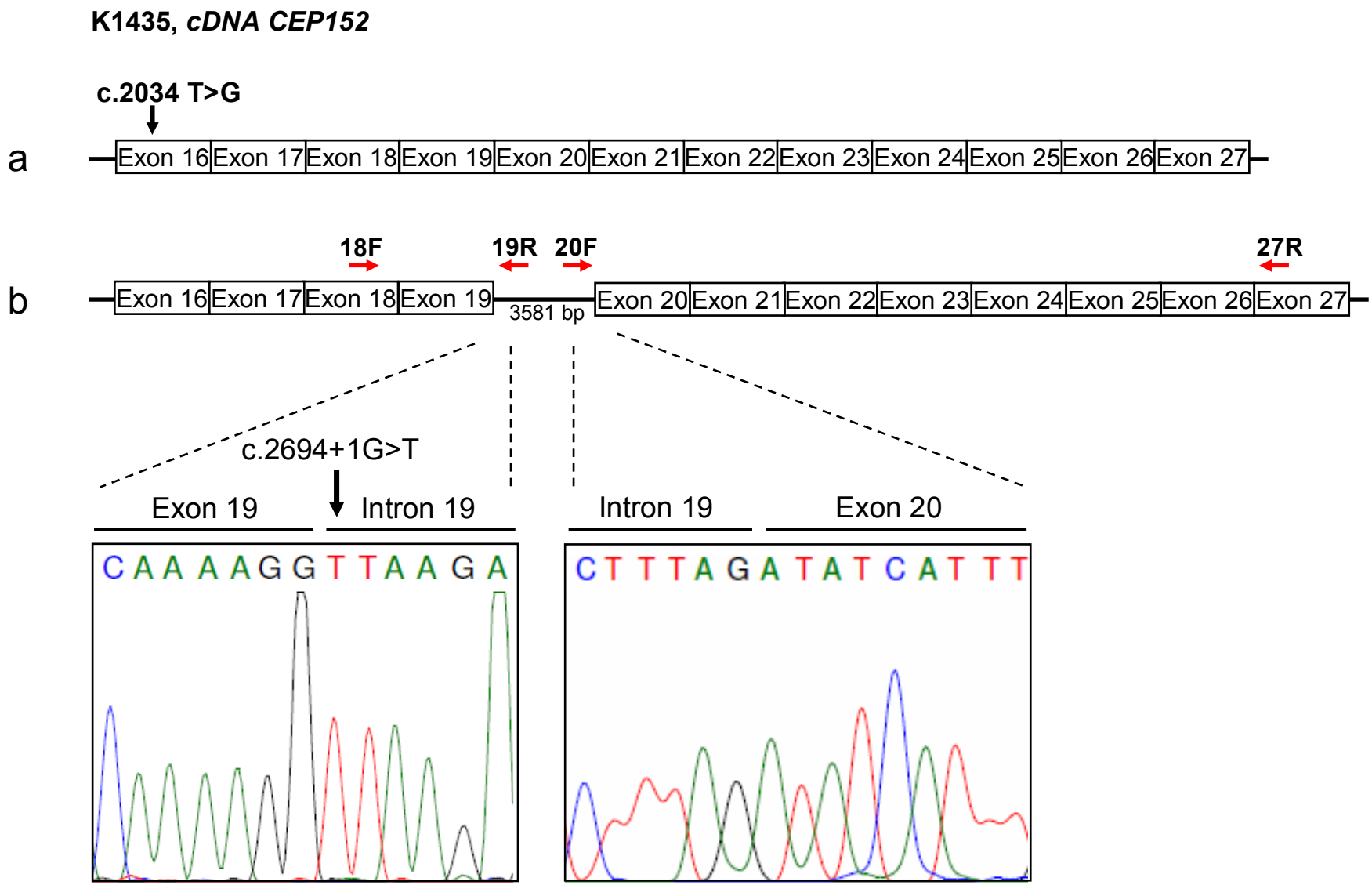


Supplementary Figure 3. Sequencing chromatograms of identified CEP152 mutations. The homozygous c.261+1G>C splice donor-site mutation found in Turkish Seckel families (**a**), two additional Seckel patients were compound heterozygous for different CEP152 mutations (**b**, **c**).

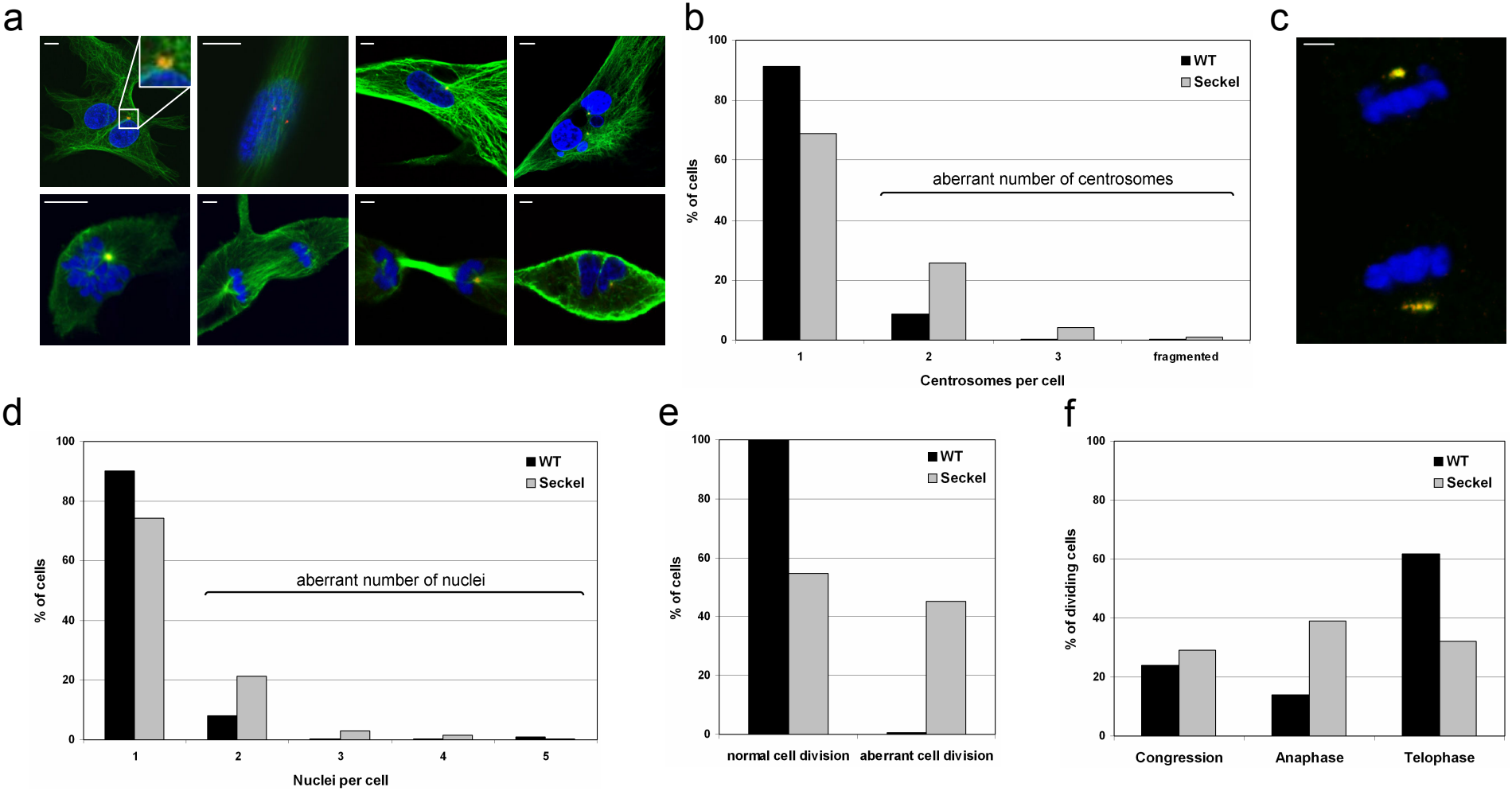
Supplementary Figure 4. RT-PCR analysis and sequencing of *CEP152* cDNA. RNA samples isolated from lymphocyte cells of a healthy individual, two affected (442 and 443) and one carrier (441) were used to synthesize cDNA. **(a)** Amplification of *CEP152* from cDNA carried out by using primers located in exon 3 and exon 6 designated with red arrows. Black arrow shows the position of splicing donor-site mutation c.261+1G>C in *CEP152*. **(b)** Three amplicons T1, T2, T3 of different length were amplified from cDNA of affected and carrier individuals. Only a single amplicon (T) was obtained from cDNA of a healthy control. Sequencing chromatograms of the different amplicons T1, T2, and T3 identified the exon composition of the three different derivative transcripts generated as a result of the c.261+1G>C mutation. **(c)** HEK293T cells were transfected with expression constructs coding for GFP-tagged wt CEP152, CEP152Δ86/87 or left untransfected as control. Equal amounts of proteins from whole cellular lysates (WCL) were analyzed by anti-GFP and anti-Actin immunoblotting. **(d)** Subcellular localization of wt and Δ86/87 variant of CEP152. Centrosomal localization of CEP152 was monitored by immunofluorescence microscopy of HEK293T transfectants expressing either GFP-tagged wt CEP152 (middle panel), CEP152Δ86/87 (lower panel) or GFP only as control (upper panel). Cells were stained with antibodies against pericentrin (red) and nuclei were counterstained with DAPI (blue). Scale bar, 5µm.



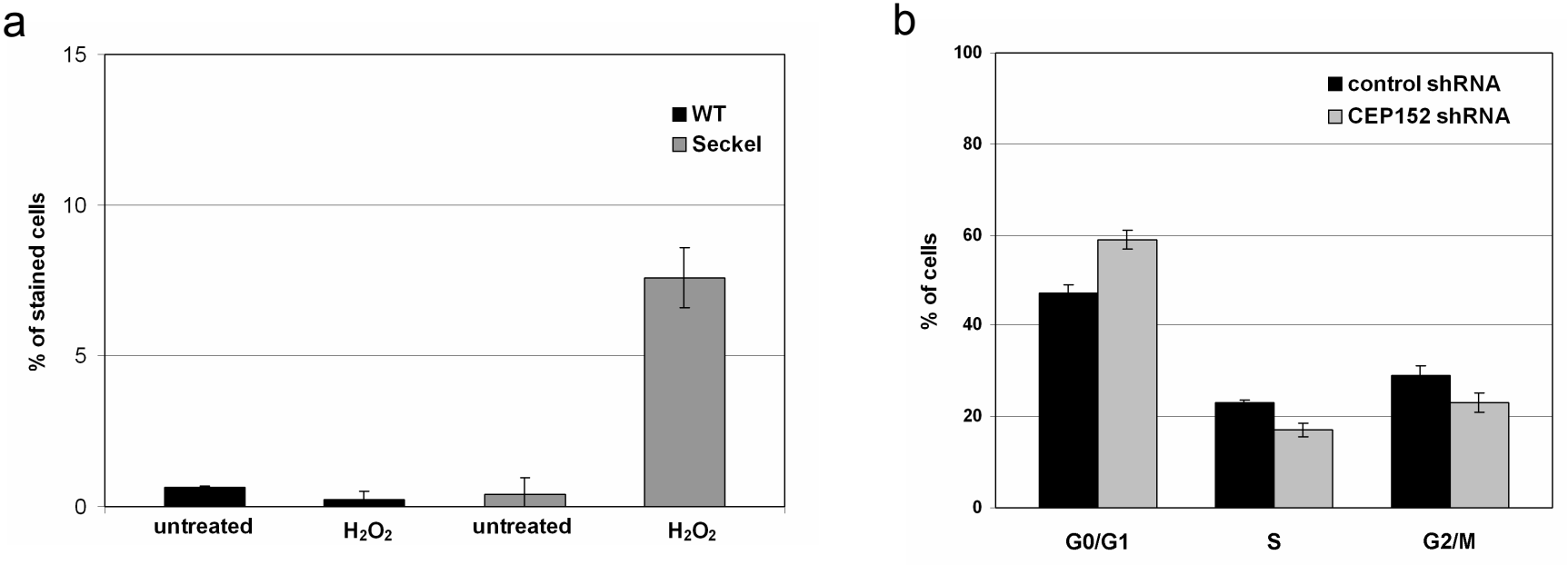
Supplementary Figure 5. *CEP152* transcripts identified in the German patient with Seckel syndrome (K1435). The C-terminal part of transcripts encoded by exon 16 to exon 27 are shown. (**a**) The location of the c.2034T>G nonsense mutation is indicated. (**b**) The c.2694+1G>T donor-site mutation caused a retention of intron 19 in the transcript. Chromatograms showing intron/exon borders on cDNA are shown. Location of primers used for amplification are indicated by arrows.

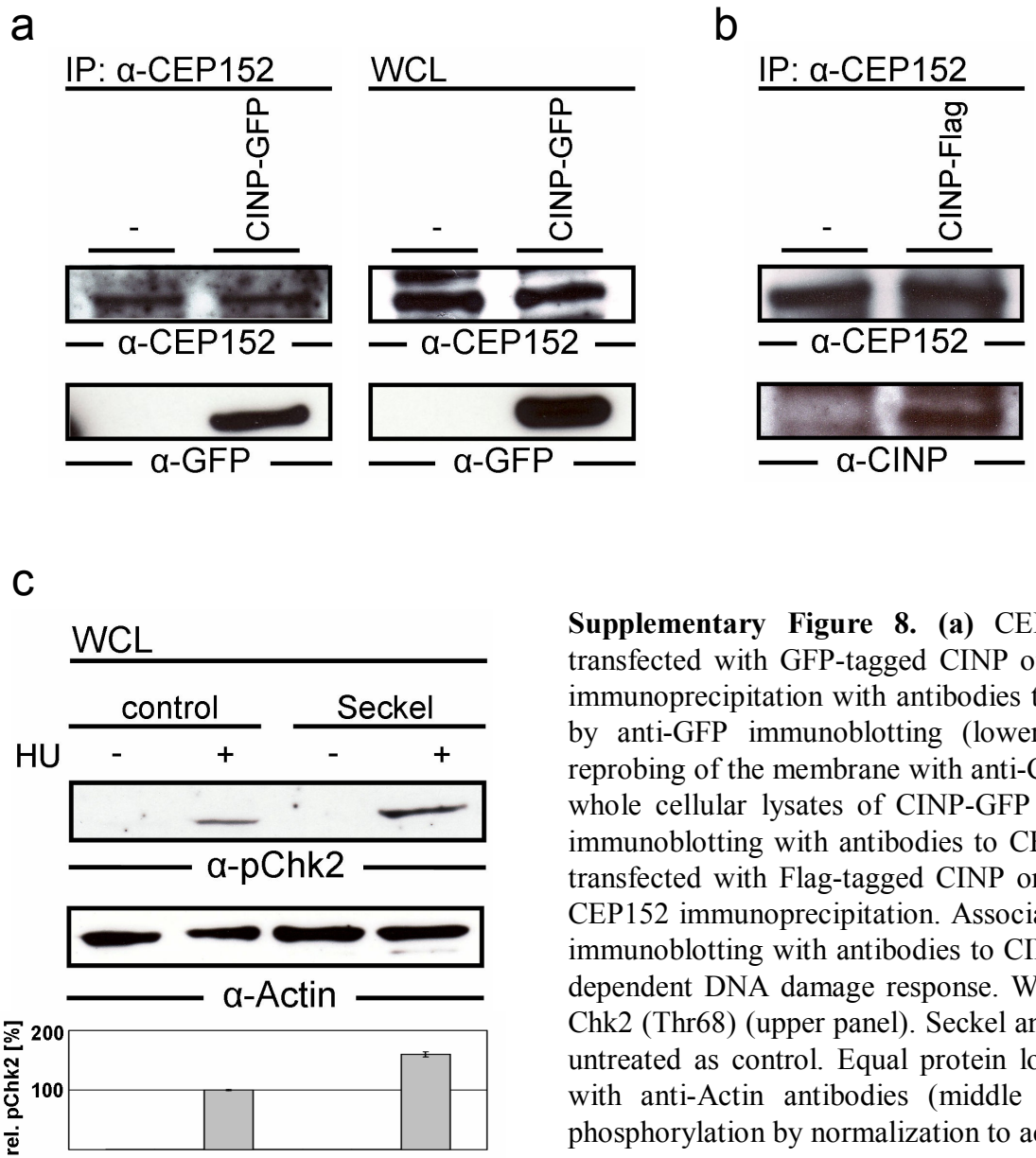


Supplementary Figure 6. (a) Additional images of aberrant interphase and mitotic morphology observed in *CEP152*-Seckel fibroblasts. (Upper panel, from left to right) Seckel interphase cells with two equally sized nuclei and double centrosomes, two centrosomes without astral microtubules and one nucleus, very faint DAPI stained second nucleus adjacent to a normal interphase nucleus suggesting that the second nucleus was present but the DNA has broken down or been degraded, and multiple, different sized nuclei and micronuclei. (Lower panel) Metaphase with misaligned spindle poles, Seckel telophase lacking the cytokinetic constriction, telophase cell with extended telophase cleavage furrow without contractile ring, and telophase without an organized spindle. Inset shows 3 fold magnification. Scale bar, 5 µm. **(b)** Comparison of the observed centrosomal abnormalities in interphase wildtype and Seckel primary fibroblasts. **(c)** Centrin (red)/pericentrin (green) co-staining of *CEP152*-Seckel fibroblasts with fragmented centrosome in telophase.DNA was counterstained with DAPI. Overlay showed a co-localization in all analyzed centrosomes including centrosomal fragments. Scale bar, 5 µm. **(d)** Comparison of the observed abnormalities in Seckel and control cells revealed a significantly higher proportion of multinucleated Seckel cells. **(e)** During mitotic division 45% of the Seckel cells showed detectable abnormalities. **(f)** Comparison of mitotic phases in fixed populations of Seckel and control cells.



Supplementary Figure 7. (**a)** Seckel cells show increased sensitivity to oxidative stress. Wild-type and Seckel fibroblasts were treated with H_20_2, and apoptotic cell death was quantified by TUNEL assay 15 h after oxidative stress. Whereas wild-type (black) and Seckel (gray) fibroblasts showed no differences in apoptosis under normal culture conditions (untreated), a significant increase in apoptosis was observed in Seckel cells 15 h after incubation with 500 µM H_2O_2. **(b)** Effects of *CEP152*-knockdown on cell cycle. HEK293T cells were transfected with shRNA expression vectors specific for *CEP152*. 96 h after transfection, cells were harvested, stained with propidium iodide, and subjected to FACS analysis (lower image). After exclusion of doublets, *CEP152* shRNA expressing cells were gated upon their co-expression of GFP, and cell cycle of GFP-positive cells was analyzed.





Supplementary Figure 8. (a) CEP152 interaction with CINP. HEK293T cells were transfected with GFP-tagged CINP or left untransfected as control, lysed, and subjected to immunoprecipitation with antibodies to CEP152 (left panel). Purified proteins were analyzed by anti-GFP immunoblotting (lower panel). Equal protein recovery was confirmed by reprobing of the membrane with anti-CEP152 antibodies (upper panel). As expression control, whole cellular lysates of CINP-GFP transfected and untransfected cells were subjected to immunoblotting with antibodies to CEP152 and GFP (right panel). **(b)** HEK293T cells were transfected with Flag-tagged CINP or left untransfected and lysates were subjected to anti-CEP152 immunoprecipitation. Association of CEP152 with CINP was detected by sequential immunoblotting with antibodies to CINP (lower panel) and CEP152 (upper panel). **(c)** ATM-dependent DNA damage response. Western blot analysis of HU-induced phosphorylation of Chk2 (Thr68) (upper panel). Seckel and control fibroblasts were treated with 1mM HU or left untreated as control. Equal protein loading was confirmed by reprobing of the membranes with anti-Actin antibodies (middle panel). Lower panel shows quantification of Chk2 phosphorylation by normalization to actin. Phosphorylation levels in control was set to 100%.

Supplementary Table 1. Clinical findings in Seckel patients (n.a.=not available/unknown).

	Patient 442	**Patient 443**	**Patient 586**	**Patient 633**	**Patient 935**	**Patient K1435**	**Patient AJ170**	**Patient AJ741**
Sex	male	female	male	female	male	female	male	male
Growth								
Length and Height	at birth -5/-6 SD at 10 y. -8/-9 SD	at birth -6/-7 SD at 8 y. -4/-5 SD	at 20 y. -6/-7 SD	at birth -7 SD at 5,5 y. -7/-8 SD	at birth -6/-7 SD at 5 y. -5/-6 SD	at birth -2.3 SD	at birth -5 SD at 7y. -5/-6 SD	at birth -3/-4 SD at 13.5 y. -3/-4 SD
Head circumference	at birth -3/-4 SD at 10 y. -7/-8 SD	at birth -4/-5 SD at 8 y. -7/-8 SD	at 20 y. -10/-11 SD	at birth -5/-6 SD	at birth -4 SD at 5 y. -8 SD	at birth -3.6 SD	at birth -9 SD at 3 y. -11 SD	at birth -7/-8 SD at 13.5 y. -6/-7 SD
Facial dysmorphism								
Sloping forehead	+	+	+	+	+	+	+	+
Micrognathia	+	+	+	+	+	+	+	+
Facial asymmetry	-	-	-	-	-	-	+	+
Low/malformed ears	-	+/-	+/-	+	+	+	+	-
Downsl. palp. fissures	+	+	+	+	-	+	+	-
Strabismus	-	-	-	right	-	-	+	-
Blepharophimosis	+	+	-	+	-	-	-	-
Beaked nose	+	+	+	+	+	+	+	+
Cleft palate	-	-	-	+	-	-	-	-
High-arched palate	+	+	+	+	+	+	-	+
Selective tooth agenesis	+	+	+	+	+	n.a.	n.a.	-
Enamel hypoplasia	+	hypodontia	oligodontia	+	-	n.a.	n.a.	-
Skeletal findings								n.a.
11 pairs of ribs	11/11	11/12	11/11	12/11	11/12	n.a.	n.a.	12/12
Delayed bone age	+	-	-	+	+	+	n.a.	n.a.
Scoliosis	-	-	-	-	-	-	n.a.	+
Fifth finger clinodactyly	+	+	+	+	+	+	n.a.	+
Brachymesophalangia	-	-	-	-	-	-	n.a.	-
Pes planus	+/+	+/+	+/+	-	-	-	n.a.	+/+
Other findings								
Genital anomalies	cryptorchidim	clitoromegaly	hypospadia cryptorchidism	clitoromegaly	-	-	-	-
Mental retardation	+	+	+	+	+ (mild)	+	+	+
Simplified gyri	+	+	+	+	+	n.a.	n.a.	n.a.
Molecular basis:								
***CEP152* mutation**	c.261+1G>C	c.261+1G>C	c.261+1G>C	c.261+1G>C	c.261+1G>C	c.2034T>G/ c.2694+1G>T	c.261+1G>C	c.2000 A>G/ c.4210-4211delGT

Supplementary Table 2. Annotation of variants identified in AJ170 exome sequencing (Novel: not in dbSNP129 or 1000 genomes pilot 1 release). Genes in which the seven novel and homozygous nonsense and essential splice site variants were identified are listed in the table below. Genotype Quality was defined as the Phred-scaled probability of the genotype call being correct.

Annotation	All variants	Novel	Novel and
Synonymous	9202	422	52
Nonsynonymous	9186	1137	136
Nonsense	125	48	5
Stop lost	14	1	0
Essential splice site	49	7	2
Splice site	1513	109	18
Total	***20089***	***1724***	***213***

Gene	Chromosome	Chr. Position	Reference	Change	Mutation Type	Genotype Quality
CEP152	15	46877149	C	G	ESSENTIAL SPLICE SITE, INTRONIC	99
CHD6	20	39487230	A	T	ESSENTIAL SPLICE SITE, INTRONIC	43
HEPHL1	11	93458804	C	A	STOP GAINED	33
LIPH	3	186717640	A	T	STOP GAINED	20
PGM2	4	37528209	C	A	STOP GAINED	40
TSG101	11	18480694	G	T	STOP GAINED	20
UPP1	11	48113040	C	A	STOP GAINED	27

Supplementary Table 3. Frequency of spontaneous and mitomycin-C induced sister chromatid exchanges (SCE) in a lymphocyte cell culture of a patient with Seckel syndrome and controls.

	average SCE per metaphase without MMC (50 metaphases)	**average SCE per metaphase with MMC (50 metaphases)**
control 1	6,06 ± 2,66	13,04 ± 3,42
control 2	6,17 ± 2,50	13,23 ± 3,55
patient	5,92 ± 2,72	17,08 ± 3,32
	p>0.05	**p<0.0001**

Supplementary Table 4. Primer sequences used for amplification and sequencing of the *CEP152* gene from genomic DNA.

Primer	Sequence	Length	Annealing temp.
CEP152_Ex1F	CCGTGAGTTCGGTTGTGAC	275	57
CEP152_Ex1R	GTAAAAGCTGAGCCCGCAAC		
CEP152_Ex2F	TTTCTCCCAGTCTGTTTCTGTC	356	54
CEP152_Ex2R	AGCAAGCAGATTTTAGGGTTG		
CEP152_Ex3F	CTTTGGCTTGGTGTCTTGTG	300	54
CEP152_Ex3R	CTTTTTCCTTCCAATCTTCACC		
CEP152_Ex4F	TTTGGGTGAAGATTGGAAGG	238	54
CEP152_Ex4R	AACAAAAGCACGAAGATCCAG		
CEP152_Ex5F	TGAGCTGGAATGAGCAAATG	476	55
CEP152_Ex5R	TGAACACACACAAACATCACG		
CEP152_Ex6F	TTGGAAGAAAAGGCCAAATC	443	54
CEP152_Ex6R	ATTCCCACCCAACATTACCC		
CEP152_Ex7F	GTCACAGTTTGAAAAACACTGC	398	54
CEP152_Ex7R	AATCCAAGGCCAACACTCAG		
CEP152_Ex8F	GATTGGGATTGTGACATTGG	356	54
CEP152_Ex8R	GAAGCAAACTATTGCAGTCAGG		
CEP152_Ex9F	AGCGGAGACCCCTTCTCTAC	474	55
CEP152_Ex9R	TTCACTCACCTCACACACAATC		
CEP152_Ex10F	GCTCCCATGTGATGATCTTG	372	54
CEP152_Ex10R	TGTGTTTTCTGTCCCTTCTGG		
CEP152_Ex11F	GCCTCAGTGGATTCAAGTCTTC	300	54
CEP152_Ex11R	GGCTGGTTTGAAGGTGGTC		
CEP152_Ex12F	CACCACTTTCCATGGAGGAC	494	54
CEP152_Ex12R	ACCATGCATCATCAGCATTAC		
CEP152_Ex13F	CTGTGGTCAGTGCTTTTAGGG	381	54
CEP152_Ex13R	ACAGAGTGACGCCCTGTCTC		
CEP152_Ex14F	TGGGGGAAGATGAGTAAAAGC	356	54
CEP152_Ex14R	CTCACATTGCCTCTTTATTTGC		
CEP152_Ex15F	CAAAGCGTGAAGAGACTTTTTC	337	54
CEP152_Ex15R	CAGGGACTTAGAGGGGAAGG		
CEP152_Ex16F	GAGGTGAGGAGGAAGGAAGG	355	54
CEP152_Ex16R	TTGGATACACAAAACCAGCAAC		
CEP152_Ex17F	AGTTTAGCTGCCTTTTAAGCTG	584	54
CEP152_Ex17R	CACCGAAAATGAGGATCTGTG		
CEP152_Ex18F	CAGCTGTGGTTGCTTTTCTG	559	56
CEP152_Ex18R	GGGTGGCAGCTTTATGACAG		
CEP152_Ex19F	TCCACTGTTTGGGATAGGTAGG	353	54
CEP152_Ex19R	TGAGATAAGAGAAGGGGTCAGG		
CEP152_Ex20F	TTTTGCGAAGGAGGAGAATC	872	54
CEP152_Ex20R	ATGCAAACCTTTGGATTTCG		
CEP152_Ex21F	GGCGATAGATAGTTCAGGCAAG	322	54
CEP152_Ex21R	TCCTCAAAGCAACCCTTCAG		
CEP152_Ex22F	CTTTCAGTCGTGCTGTAGCC	400	54
CEP152_Ex22R	TCCCAAAAGAGGATCAGTGC		
CEP152_Ex23F	GGGAACTTAAGGGAAAAGAAGG	296	54
CEP152_Ex23R	GGTTTTGGGGGATTTTCTTC		
CEP152_Ex24F	TTTCTACACATACTCCACATGCAC	570	54

CEP152_Ex24R	CCCTAGAAGAACTACTGCTGGTAAAC		
CEP152_Ex25F	AAGTCTGCCCCTCCTGTTTC	370	54
CEP152_Ex25R	GGGTAGCACAGCCATAAACC		
CEP152_Ex26F	ACCCTAAATGTTGGCAGAGC	300	54
CEP152_Ex26R	TCCTTACCTTCCCTGCCTTC		
CEP152_Ex27F1	GGGCAGGATTGAAAACATTG	664	54
CEP152_Ex27R1	GCCTCCTTCACCTTCACAAG		
CEP152_Ex27F2	AAAGCATGTGGGATCCAAAG	555	54
CEP152_Ex27R2	TAACCGGATGGTGAAAACTG		
CEP152_Ex27F3	TCCTTTGACAGTCGTGAAGC	458	54
CEP152_Ex27R3	TCAGTATGAGGTCTTCCCTTCC		

Primer sequences used for amplification and sequencing of *CEP152* from cDNA.

Primer	Sequence	Length	Annealing temp.
18F	GAGAAGGACATAGCCATCAAGG	381	58
19R	TGATGATGACAAGAACACTTGC		
20F	TGAGTCCGCTTTTGCTTTAG	1829	58
27R	TCCAAATGTGTCTCTTTGG		
Pr.70	ATGCTGGATGACGACCTCTC	447	58
Pr.71	CGGTTCCAAACCTTGACAAC		

Material and methods

Subjects. All subjects or their legal representatives gave written informed consent to the study. The study was performed in accordance to the Declaration of Helsinki protocols and approved by the ethic committee of the Faculty of Medicine of Karadeniz Technical University in Trabzon, Turkey. DNA from participating family members was extracted from peripheral blood lymphocytes by standard extraction procedures.

Linkage analysis. Genome-wide linkage analysis in four affected individuals (442, 443, 586 and 633) from three consanguineous Seckel families (SSTR1, SSTR4, SSTR5) was performed using the Affymetrix GeneChip® Human Mapping 250K SNP Array. Genotypes were called by the GeneChip® DNA Analysis Software (GDAS v3.0, Affymetrix). We verified sample genders by counting heterozygous SNPs on the X chromosome. Relationship errors were evaluated with the help of the program Graphical Relationship Representation (16). The program PedCheck was applied to detect Mendelian errors (17) and data for SNPs with such errors were removed from the data set. Non-Mendelian errors were identified by using the program MERLIN (18) and unlikely genotypes for related samples were deleted. Linkage analysis was performed assuming autosomal recessive inheritance, full penetrance, consanguinity and a disease gene frequency of 0.0001. Multipoint LOD scores were calculated using the program ALLEGRO (19). Haplotypes were reconstructed with ALLEGRO and presented graphically with HaploPainter (20). All data handling was performed using the graphical user interface ALOHOMORA (21). For subsequent fine

mapping, microsatellite markers for the critical region were genotyped in additional family members and additional families.

Mutation screening. We identified candidate genes in the critical region using the ENSEMBL (http://www.ensembl.org) and UCSC (http://www.genome.ucsc.edu) human genome databases. All exons and intron-exon boundaries of the *CEP152* gene (primers are listed in Supplementary Table 4) were amplified from DNA of index patients from all families and sequenced the PCR products by BigDye Terminator method on an ABI 3100 sequencer. Identified mutations were re-sequenced in independent experiments, tested for co-segregation within the families, and 250 healthy control individuals from Turkey and 300 controls from Germany were screened for each mutation by PCR/restriction digestion or direct sequencing. Clinical findings in the *CEP152*-Seckel patient K1435 have been described (22).

Exome sequencing. Coding sequences were captured using the Agilent SureSelect protocol, targeting 50 megabases of functional sequences annotated by the GENCODE consortium (as of April 2009). Paired-reads of 54 bp were generated on the Illumina GA2 platform. Sequence data were mapped using MAQ (23). Duplicate reads were removed, after which mean depth across exons was 43X. Variant sites (single nucleotide variants and indels) were called using SAMtools (24). Only variants with PHRED-scaled confidence ($-10\log_{10}$ (probability of being wrong)) greater than 25 were retained for downstream analyses.

cDNA analysis. RNA was extracted from fresh whole-cell blood by using Paxgene Blood RNA system (Becton Dickinson, Heidelberg, Germany). After cDNA transcription, PCR was used to amplify *CEP152* cDNA (primers are listed in Supplementary Table 4). Primers were designed according to the reference sequence.

Karyotyping and sister chromatid exchange analysis. A heparinized blood sample was obtained from a 20 years old male Seckel patient 586 (family SSTR5) and from two sex and age matched controls. Different lymphocyte cell lines were established for karyotyping, analysis of spontaneous sister chromatide exchange (SCE), and mitomycin-C induced SCE. Lymphocytes were cultured in PBMax Karyotyping medium (GIBCO Invitrogen Corporation, NY). For spontaneous SCE, 4.3 µg/ml BrdUrd (Sigma Chemical, St. Louis, MO) was added to cell cultures 24 hours after setup. To determine mitomycin-C induced SCE, 3.6 ng/ml mitomycin-C was added to cell cultures 24 hours after setup. BrdUrd and mitomycin-C treated cells were protected from day light and two hours before metaphase chromosome preparation 0.1 mg/ml of colcemid was applied. Air dried chromosome preparations were stained with GTG banding for conventional karyotyping. BrdUrd-incorporated metaphase chromosomes were stained using the Hoechst-Giemsa method after photodegradation of BrdUrd-labeled DNA under 356 nm UV-light to obtain harlequin chromosomes for SCE. One hundred metaphases were analyzed to determine the karyotype of the patient with Seckel syndrome obtained from native cultures. To determine the SCE frequencies 50 metaphases were analyzed.

Cell lines and cell cultures. HEK293T cells and two independent primary fibroblast cell lines established from patients P443 and AJ170 were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum (FCS, Gibco), and antibiotics. Hydroxyurea (HU, Sigma-Aldrich, Germany) was used with concentrations of 1 mmol/L for the indicated times.

Expression constructs. Wild-type human *CEP152* cDNA was purchased from Thermo Fisher Scientific (Clone Id 40125733) and ligated into peGFP-N1 vector (Clontech, Germany)

via *BglII* and *KpnI* restriction sites. The *CEP152 Δ86/87* variant was obtained using site-directed mutagenesis and verified by sequencing. Coding sequences for HA tag was introduced at the 3´-site of *CEP152* cDNA by PCR. cDNA for Flag tagged *CINP* was kindly provided by David Cortez (Nashville, USA) and CINP-GFP fusion protein was generated by PCR amplification of the coding sequence of *CINP* and ligation into the peGFP-N1 vector. Expression of all introduced cDNAs was confirmed by western blot analysis. shRNA contructs against CEP152 (TG313982) and control contructs (TR30007, TR30013) were purchased from OriGene Technologies (USA) and knockdown of *CEP152* expression was confirmed by western blot analysis.

Protein isolation and analysis. All cells were solubilized using ice-cold RIPA buffer (10 mM Tris, pH: 8.0; 150 mM NaCl; 1 mM EDTA; 10 mM NaF; 1 mM Na_3VO_4; 10 µM Na_2MoO_4; 1% NP-40; protease inhibitors P 2714 (Sigma-Aldrich, St Louis, MO, USA)) and total protein concentration of extracts was determined by BCA Protein Assay Kit (Pierce Protein Research Products, Thermo Fischer Scientific, Rockford, IL, USA). 15 µg of total protein from each sample was separated by 4-12% SDS-PAGE (Invitrogen, Germany) and blotted onto nitrocellulose membrane (GE Healthcare, Germany).

Protein detection was performed using phospho-specific antibodies to Chk1 (Ser345), Chk2 (Thr68) (Cell Signaling Technology, USA) and to γH2AX (Ser139) (Upstate Biotechnology, Dundee, UK). Antibodies to Chk1 and H2AX were purchased from Cell Signaling Technology (USA) and Calbiochem (USA), respectively. Anti-Actin (NH3) and anti-Centrin-1 (N-15) antibodies were from Sigma-Aldrich, anti-HA (3F10) and anti-GFP antibodies from Roche (Switzerland). Anti-CEP152 antibody was purchased from Bethyl Laboratories (USA) and antibody specific for flag peptide (M2) was from Sigma-Aldrich. Polyclonal antibody to human CINP (VU235) was kindly provided by David Cortez (Nashville, USA).

Peroxidase conjugated secondary antibodies were used (Santa Cruz Biotechnology Inc.) and blots were developed using an enhanced chemiluminescence system, ECL Plus (Amersham, UK), followed by exposure on autoradiographic film (GE Healthcare, Germany).

Immunocytochemistry. Fibroblasts from skin biopsies (patient and control) were grown on ethanol-sterilized borosilicate glass cover slips (VWR, thickness 1) to 30% confluence. Cells were fixed with fresh 2% paraformaldehyde in PBS (Invitrogen, UK) for 20 minutes, given three 3-minute washes in PBS and permeabilized for 5 minutes with 0.4% Triton X-100 (Sigma) in PBS. Cells were given two 3-minute washes in PBS and one 5-minute wash in Wash Buffer (PBS containing 0.2% BSA Faction V, 0.05% Tween 20 (Sigma, UK). Cells were blocked for 30 minutes on a 100 ml drop of Blocking Buffer (PBS containing 2.5% BSA Fraction V, 0.05% Tween 20 and 10% Natural Goat Serum (Vector laboratories) placed on Nescofilm in a humid chamber), rinsed briefly with Wash Buffer and incubated for 1.5 hours on 100 ml drops of Blocking Buffer containing primary antibodies to α-tubulin (Abcam, ab11303), pericentrin (Abcam, ab4448) and centrin-1 (Santa Cruz), diluted according to manufacturers instructions. After three 5-minute washes in Wash Buffer, cells were incubated for 30 minutes in blocking buffer containing 1:400 dilutions of Alexa Fluor 488 anti-rabbit (Invitrogen, A11034) and Alexa Fluor 568 anti-mouse (Invitrogen, A11004) secondary antibodies. Cells were given two 5-minute washes in Wash Buffer and one 5-minute wash in PBS and then mounted in Vectorshield (Vector Laboratories) containing 0.5 mg/ml DAPI (Sigma, D9542). Cells were analysed and confocal images acquired using a Leica SP1 confocal microscope using a 100x objective.

For immunofluorescence detection of CEP152 and pericentrin, HEK293T cells grown on coverslips were transiently transfected with expression constructs for GFP-tagged wild-type CEP152 or Δ86/87 variant. 24 h after transfection cells were washed with PBS, fixed with 4% paraformaldehyde for 15 min and permeabilized with 0.5% Triton X-100 for 10 min. Upon

blocking for 30 min in PBS 3% BSA, slides were incubated with pericentrin antibodies (Abcam, Cambridge, MA), followed by incubation with Cy3 conjugated sheep-anti-rabbit-F(ab′)2-fragment (Sigma). For detection of γH2AX primary fibroblast grown on coverslips were treated with 1 mmol/L HU for 24 h and then stained for γH2AX using antibodies to γ-H2AX and FITC-conjugated anti-mouse IgG (Santa Cruz Biotechnology Inc). All cells were mounted in Vectashield with DAPI (H 1200; Vector Laboratories) and viewed with a Zeiss Axioplan2 fluorescence microscope using a 100x objective.

TUNEL assay. Analysis of apoptotic wild-type and patient cells was performed by Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) according to the manufacturer's instructions (Roche). TUNEL-positive cells with dark green fluorescent staining indicating apoptosis were analyzed and counted using a Zeiss Axioplan2 fluorescence microscope. Proportion of apoptotic cells was calculated by dividing the number of TUNEL-positive cells by the total number of cells. At least 250 cells per slide were analyzed and the average number of TUNEL-positive cells was calculated.

Cell cycle analysis. Cells were harvested by trypsinization, washed with cold PBS, fixed in ice-cold 70% ethanol, and stored at 4°C. Cell pellets were washed with PBS, resuspended in 50 µL PBS containing 2 mg/mL RNase (Sigma-Aldrich) and incubated at 30°C for 15 minutes. After addition of 450 µL PBS containing 50 µg/ml propidium iodide (Sigma-Aldrich), cells were incubated at 4°C for 30 minutes and analyzed immediately using a FACSCalibur flow cytometer (Becton Dickinson, USA).

Yeast-two-hybrid (Y2H) library screening. Automated yeast-two-hybrid screens were essentially done as described (25). In brief, human cDNA libraries from human kidney, human brain (Clontech, Germany) as well as a library of individually cloned full-length open

reading frames from cDNAs of 10.070 different genes (26) in pGAD424 were screened to a coverage of 12.2, 12.2 and 6.6 million clones per library, respectively. Yeast strains harbouring the bait protein and the prey library were mated in YPDA medium containing 20% PEG 6000 before selection of positive colonies in selective medium containing 2.0 mM 3-amino triazole. Positives were identified by activation of the HIS3 and MEL1 reporter as described, and library inserts were isolated by PCR and analyzed by DNA sequence analysis. As a bait, a fragment of CEP152 encompassing amino acids 1 to 152 was expressed from the vector pGBT9 (Clontech, Germany). For the generation of a high-confidence dataset, interaction pairs were selected which were isolated at least twice and which did not involve promiscuous preys. CINP was identified as an interaction partner of CEP152 in three independent hits from screens done with the library of full-length open reading frames.

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