SUPPLEMENTARY TABLES

Supplementary Table 1. Detailed clinical features of the six affected individuals.

	Subject 1	**Subject 2**	**Subject 3**	**Subject 4**	**Subject 5**	**Subject 6**	**Subject 7**
***RHOA* change**	c.139G>A p.(Glu47Lys)	c.139G>A p.(Glu47Lys)	c. 211C>T p.(Pro71Ser)	c.139G>A p.(Glu47Lys)	c.139G>A p.(Glu47Lys)	No mutation found (DNA not analyzable due to failed quality control)	c.139G>A p.(Glu47Lys)
Sex	Female	Male	Female	Female	Female	Female	Female
Age (age at last visit)	18 years (15 years)	28 years (25 years)	14 years (10 years)	6 years (3 years)	16 years (15 years)	9 years (10 years)	2 years (2 years)
Skin anomalies	Linear hypopigmentation Patchy scalp hair scarring alopecia.	Linear hypopigmentation on neck and upper and lower limbs with pilar dysplasia. Patchy scalp hair scarring alopecia.	Linear hypopigmentation on neck and upper and lower limbs with pilar dysplasia.	Linear hypopigmentation on upper and lower limbs.	Linear hypopigmentation on upper and lower limbs Patchy scalp hair scarring alopecia (right occiput and left frontoparietal regions)	Linear hypopigmentation on limbs	Linear hypopigmentation on upper and lower limbs Alopecia not reported
Facial dysmorphy	Facial asymmetry. Marked malar hypoplasia with retrognathic maxilla and malocclusion. Broad nasal bridge with thick alae nasi.	Facial asymmetry with left malar hypoplasia. Broad nasal bridge with thick alae nasi. Short philtrum.	Facial asymmetry with malar hypoplasia, microtia, and narrower palpebral fissure on the left side. Thick alae nasi. Cleft lip.	Facial asymmetry with left microsomia, left deviation of the maxilla and the mandible, and soft tissue atrophy.	Right malar hypoplasia and moderate right hemifacial microsomia.	Left hemifacial microsomia	Right malar hypoplasia Moderate right hemifacial microsomia, broad nasal bridge
Acral anomalies	Asymmetrical III, IV, V toes.	Bilateral brachydactyly. Bilateral short II, III, IV, and V toes Broad first toes.	Left foot postaxial polydactyly. Left III-IV syndactyly	Brachydactyly of the right third toe	Bilateral clinodactyly of second toes.	Bilateral brachydactyly of third toes.	Bilateral clinodactyly, brachydactyly, broad toes, sandal gap.
Teeth anomalies	Conical teeth, oligodontia and microdontia of deciduous and permanent teeth. Persistent decidual dentition. Linear enamel dysplasia.	Conical teeth, oligodontia and microdontia of deciduous and permanent teeth.	Agenesis of twelve tooth: two on the right, six on the left, and the four third molars.	Infraocclusion and conical teeth.	Multiple right dental agenesis	Multiple dental agenesis and bilateral microdontia.	Premature eruption of right teeth
Ocular anomalies	Right peripapillary chorioretinal atrophy, high-degree myopia, and strabismus.	Bilateral congenital nystagmus, myopia, astigmatism, asymmetric posterior capsular cataract. Right eye visual acuity: 20/100; Left: hand motion. Normal cornea, corectopia, bilateral pale fundus with diffuse retinal atrophy, papillary dysversion.	Strabismus. No microphthalmia on CT-scan.	Left microphthalmia Atrophy of the left optical nerve (visual evoked potentials) and oculo-motor dyssynergia.	Normal examination (visual acuity, visual evoked potentials, fundus ophthalmoscopy, and slit lamp examination).	Myopia. Normal ophthalmoscopy.	Right congenital cataracts Right congenital glaucoma Bilateral retinal dystrophy Myopia
Brain anomalies	Non-progressive cystic leukoencephalopathy.	Leukoencephalopathy. Posterior fossa arachnoid cyst.	No brain MRI available. Enlarged lateral ventricle with no calcifications on CT-scan	Leukoencephalopathy with enlarged perivascular spaces. Posterior fossa arachnoid	Bilateral white matter anomalies predominating in the right hemisphere CT-scan: no calcifications	No MRI available	Normal MRI and CT at 6 weeks

				cyst vs. mega cisterna magna. Small calcifications in the basal ganglia, especially putamen, on CT-scan.			
Other features	NR	Asymmetrical hearing loss (left: 70% loss; right: normal), consecutive to an abnormal cochlear development.	Scoliosis, inequality of the lower limbs Bilateral vesicoureteral reflux	NR	Sigmoid and rectal atresia	NR	Bilateral hearing loss Inequality of the lower limbs: right leg > Left leg by 3 cm
Intelligence	Normal development and intellect.	Normal development and intellect.	Normal development and intellect.	Normal development and intellect.	Normal development and intellect.	Normal development and intellect.	Normal development and intellect.

Abbreviations: CT-scan: Computerized tomography scan; MRI: Magnetic resonance imaging.

Supplementary Table 2. Metrics of trio-based whole-exome sequencing (WES) experiments.

Subject	Tissue	Mapped sequences[a]	Mean sequencing depth[b]	Percent target ≥ 10X[b]	Percent target ≥ 100X[b]
S1	Fresh skin	5.5	160.3	94.6	68.1
Father S1	Blood	2.4	70.4	92.4	24.7
Mother S1	Blood	4.1	119.4	94.2	55.1
S2	Fibroblasts[c]	6.5	187.1	95.0	74.1
Father S2	Blood	4.5	130.4	94.4	59.2
Mother S2	Blood	3.9	113.3	94.0	52.4
S3	Fresh skin	5.8	169.3	95.8	71.9
Father S3	Blood	2.4	70.5	94.3	23.0
Mother S3	Blood	2.2	65.0	93.9	17.9

[a]In gigabases. [b]Based on RefSeq coding exons and splice junctions. Depth-of-coverage metrics were calculated with the Genome Analysis Toolkit (GATK)[1] DepthofCoverage tool using reads with mapping quality ≥ 20 and bases with base quality ≥ 30. [c]Cultured skin fibroblasts.

Supplementary Table 3. De novo candidates after WES in 3 subjects.

Individual	Gene	gene accessions	gDNA change (hg19)	cDNA change	Amino acid change	Allelic Balance	ExAC (Allele count - Allele frequency)	gnomAD (Allele count - Allele frequency)	OMIM
S1	***RHOA***	**NM_001664.2**	**chr3:g.49412884C>T**	**c.139G>A**	**p.(Glu47Lys)**	**AD=140,44;AB=0.31**	**none**	**none**	**non OMIM**
	ANKRD36	NM_001164315.	chr2:g.97827852C>T	c.1406C>T	p.(Pro469Leu)	AD=43,6;AB=0.12	263 - 0.01526	743 - 0.005736	non OMIM
	MDC1	NM_014641.2	chr6:g.30681077_30681081delGAAGG	c.638_642delCCTTC	p.(Ala213Glufs*5)	AD=26,10;AB=0.28	207 - 0.00182	560 - 0.002110	non OMIM
			chr6:g.30681083_30681101delAAAAGGCGGCCCAAGGCCG	c.618_636delCGGCCTTGGGCCGCCTTTT	p.(Gly207Profs*4)	AD=24,9;AB=0.27	206 - 0.001848	560 - 0.002135	non OMIM
	SNAP29	NM_004782.3	chr22:g.21224808C>A	c.421C>A	p.(Gln141Lys)	AD=16,4;AB=0.20	none	none - 1 variant affecting the same AA (1 - 0,000004070)	MIM609528 - unrelated phenotype - autosomal recessive
	BIK	NM_001197.4	chr22:g.43525234_43525235insTGCTGCTGGCGCTGCTGC	c.406_407insTGCTGCTGGCGCTGCTGC	p.(Leu143_Leu148dup)	AD=7,4;AB=0.36	738 - 0.006323	74 - 0.0002769	non OMIM
S2	***RHOA***	**NM_001664.2**	**chr3:g.49412884C>T**	**c.139G>A**	**p.(Glu47Lys)**	**AD=6/222;AB=2.6**	**none**	**none**	**non OMIM**
	PML	NM_002675.3	chr15:g.74290351C>T	c.136C>T	p.(Pro46Ser)	AD=15,15;AB=0.50	none	none	MIM102578 - unrelated phenotype
	ISLR2	NM_001130136.1	chr15:g.74425772C>A	c.677C>A	p.(Pro226His)	AD=24,6;AB=0.20	none	none	non OMIM
	TSHZ3	NM_020856.2	chr19:g.31768792G>A	c.1907C>T	p.(Ser636Phe)	AD=99,17;AB=0.15	none	none	non OMIM
	RASSF2	NM_014737.2	chr20:g.4771164G>A	c.470C>T	p.(Thr157Met)	AD=58,46;AB=0.44	9 - 0.00007673	10 - 0.00004665	non OMIM
	AKAP4	NM_003886.2	chrX:g.49957329C>T	c.2035G>A	p.(Glu679Lys)	AD=203,26;AB=0.11	none - 1 variant affecting the same AA (1 - 0.00001143)	none - 1 variant affecting the same AA (7 - 0,00003931)	non OMIM
	EDA2R	NM_001199687.2	chrX:g.65824274G>A	c.341C>T	p.(Ser114Phe)	AD=117,14;AB=0.11	none - 1 variant affecting the same AA (1 - 0.00001173)	none - 1 variant affecting the same AA (1 - 0,000005698)	non OMIM
	PLXNB3	NM_001163257.1	chrX:g.153032499C>T	c.286C>T	p.(Arg96Cys)	AD=133,18;AB=0.12	1 - 0.00001187	5 - 0,00002828	non OMIM
S3	***RHOA***	**NM_001664.2**	**chr3:g.49405927G>A**	**c.211C>T**	**p.(Pro71Ser)**	**AD=87:28;AB=0.24**	**none**	**none**	**non OMIM**

NBPF10	NM_001039703.5	chr1:g.145297661C>A	c.536C>A	p.(Ala179Asp)	AD=39:6;AB=0.13	12 - 0.00009978	1421 - 0.005642	non OMIM
LOR	NM_000427.2	chr1:g.153233747_153233836delGGGGGCGGCTCCGGCTGCTTCTCCTCCGGTGGCGGCGGCTCCTCCGGGGGCGGCTCCGGCTGCTTCTCCAGCGGTGGGGGCGGCTCCTCC	c.322_411delGGGGGCGGCTCCGGCTGCTTCTCCTCCGGTGGCGGGCGGCTCCTCCGGGGGCGGCTCCGGCTGCTTCTCCAGCGGTGGGGGCGGCTCCTCC	p.(Ser121_Gly150del)	AD=26:6;AB=0.19	none	2 - 0.00007407	MIM604117 - unrelated phenotype
CAB39	NM_001130849.1	chr2:g.231678762_231678763delAG	c.628-2_628-1delAG	intronic	AD=29:4;AB=0.12	37 - 0.0003182	98 - 0.0004175	non OMIM
FRG1	NM_004477.2	chr4:g.190883039T>C	c.692T>C	p.(Leu231Pro)	AD=161:18;AB=0.10	none	4 - 0.0001317	non OMIM
POLR3B	NM_001160708.1	chr12:g.106820973delA	c.928-2delA	intronic	AD=79:19;AB=0.19	none	10 - 0.00005319	MIM614381 - unrelated phenotype - autosomal recessive
SETD8	NM_020382.3	chr12:g.123880924_123880925delTT	c.542_543delTT	p.(Leu181Hisfs*20)	AD=44:7;AB=0.14	none	none	non OMIM
TSC2	NM_000548.3	chr16:g.2137925_2137958delCCCTGCAGTGCAGGAAAGGTAGGGCCGGGTGGGG	c.5051_5068+16delCCCTGCAGTGCAGGAAAGGTAGGGCCGGGTGGGG	intronic	AD=17:20;AB=0.54	270 - 0.002265	571 - 0.002071	MIM613254 - unrelated phenotype
EPG5	NM_020964.2	chr18:g.43460190T>C	c.5519-2A>G	intronic	AD=42:36;AB=0.46	none	1 - 0.000004578	MIM242840 - unrelated phenotype - autosomal recessive
VCX	NM_013452.2	chrX:g.7811958_7812017delGAGTCAGGAGAGCGAGATGGAAGAACCACTGAGTCAGGAGAGCCAGGTGGAGGAACCACC	c.522_581delGAGTCAGGAGAGCGAGATGGAAGAACCACTGAGTCAGGAGAGCCAGGTGGAGGAACCACC	p.(Pro183_Glu202del)	AD=84:39;AB=0.32	514 - 0.006246	439 - 0.002395	non OMIM

Candidate de novo mutational events were identified by focusing on protein-altering and splice-site changes as follows: (1) supported by at least three reads and 10% of total reads in the proband; (2) absent in both parents, as defined by variant reads representing less than 5% of total reads; (3) at base-pair positions covered by at least four reads in the entire trio; and (4) present at a frequency less than 1% in dbSNP (build 147) and 0.1% in the Exome Aggregation Consortium (ExAC). *RHOA* was the only candidate gene mutated in all three subjects, and no variants in other genes were found in more than one subject. Most candidate genes were rejected because on the high frequency of variants in public databases (*ANKRD36*, *BIK*, *CAB39*, *NBPF10*, *MDC1*, *RASSF2*, *TSC2*, *VCX*), their previous involvement in either unrelated autosomal recessive

(*EPG5*, *POLR3B*, *SNAP29*) or dominant (*LOR)* phenotypes, or their tolerance to variants in public databases (*AKAP4*, *FRG1*, *ISLR2*, *PLXNB3*, *TSHZ3*). *EDA2R* encodes a receptor for an isoform of ectodysplasin, mutated in hypohidrotic ectodermal dysplasia. However, the reference *EDA2R* sequence includes a high number of missense variants according to ExAC (z = -1,63). *SETD8*, a gene intolerant to loss-of-function variants in ExAC (pLI = 0.95), was found mutated in one subject only, and no evidence was found in the literature for its involvement in the observed phenotype.

Supplementary Table 4. Amplicons used for *RHOA* ultra-deep sequencing.

Amplicon	Sequence	Chromosomal coordinates[a]	Amplicon size
Exon 2	Forward: GATTGCAGAATGTCTACCCAAAC Reverse: ATATGCTAAGGACACCATGTCAA	chr3:49412185-49414215	2,031 bp
Exon 3	Forward: GAGCTTGATGCTCATAAAAGTGA Reverse: CTACAATCCCAAACTCCAGGAC	chr3:49405714-49406784	1,071 bp
Exon 4-5	Forward: TTGTGTTGTGGATCTTTTGCTC Reverse: GAGACAGGTTATGCCATCTTCC	chr3:49396766-49400342	3,577 bp

Abbreviations: bp: base pair. [a]Based on the human genome reference sequence GRCh37/hg19. *RHOA* RefSeq accession number: NM_001664.2. Exon 1 is non coding.

Supplementary Table 5. Summary of *RHOA* ultra-deep sequencing.

Individual	Tissue	Target	Mapped sequences[c]	Mean sequencing depth[d]	Percent target ≥ 1000X[d]
S1	Fresh skin	Exon 2	12.9	80,873	100
	Fibroblasts (A)[a]	Exon 2	9.1	56,693	100
	Fibroblasts (N)[b]	Exon 2	1.6	10,336	100
	Fibroblasts (N)[b]	Exon 2	9.3	58,155	100
	Blood	Exon 2	9,0	56,261	100
Father S1	Blood	Exon 2	9.0	55,971	100
Mother S1	Blood	Exon 2	7.5	46,805	100
S2	Fresh skin	Exon 2	4.7	29,564	100
	Fibroblasts (A)[a]	Exon 2	4.1	25,954	100
	Blood	Exon 2	8.2	51,099	100
Mother S2	Blood	Exon 2	9.5	59,360	100
Father S2	Blood	Exon 2	7.8	48,453	100
S3	Fresh skin	Exon 3	1.4	11,482	100
	Blood	Exon 3	1.2	9,292	100
Mother S3	Blood	Exon 3	1.0	8,291	100
Father S3	Blood	Exon 2	1.5	11,871	100
S4	Fresh Skin	Exon 2	1.7	10,590	100
	Blood	Exon 2	1.4	8,506	100
S5	Fresh Skin	Exon 2	2.4	14,739	100
	Blood	Exon 2	2.0	12,225	100
S6	Fresh Skin	All coding exons	1.5	2,439	100

[a]Cultured skin fibroblasts derived from a biopsy of an affected (hypopigmented) skin lesion. [b]Cultured skin fibroblasts derived from a biopsy in normal skin. [c]In megabases. [d]Based on targeted coding bases and splice junctions. Depth-of-coverage metrics were calculated with the GATK DepthofCoverage tool using reads with mapping quality ≥ 20 and bases with base quality ≥ 30. *RHOA* RefSeq accession number: NM_001664.2.

Supplementary Table 6. Mutant allele fraction of the two *RHOA* mutations in all tested samples.

Individual	Tissue	Sequencing method	Alternative allele count[c]	Total reads count[c]	Mutant allele fraction[c]
chr3:g.49412884C>T (c.139G>A; p.(Glu47Lys))					
S1	Fresh skin	WES	44	144	30.6
		Targeted	30,761	91,492	33.5
	Fibroblasts (A)[a]	Targeted	11,534	63,041	18.3
	Fibroblasts (N)[b]	Targeted	1,632	11,924	13.7
	Fibroblasts (N)[b]	Targeted	1,186	63,208	1.9
	Blood	Targeted	56	58,126	0.1
Father S1	Blood	WES	0	89	0
		Targeted	44	60,811	<0.1
Mother S1	Blood	ES	0	173	0
	Blood	Targeted	57	49,274	0.1
S2	Fibroblasts	WES	6	228	2.6
		Targeted	776	28,774	2.7
	Fresh skin	Targeted	7,729	33,501	23.1
	Blood	Targeted	115	56,144	0.2
Father S2	Blood	WES	0	170	0
		Targeted	31	51,869	0
Mother S2	Blood	WES	0	123	0
		Targeted	32	64,442	0.1
S4	Fresh skin	Targeted	2,939	9,733	30.2
	Blood	Targeted	4	7,674	0.1
S5	Fresh skin	Targeted	3,103	15,639	19.8
	Blood	Targeted	5	11,261	0.1
chr3:g.49405927G>A (c.211C>T; p.(Pro71Ser))					
S3	Fresh skin	WES	28	115	24.3
		Targeted	3,381	11,651	29
	Blood	Targeted	7	9,305	0.1
Father S3	Blood	WES	0	79	0
		Targeted	10	11,774	0.1
Mother S3	Blood	WES	0	60	0
		Targeted	1	8,353	<0.1

Abbreviations: WES: whole exome sequencing. Targeted: targeted ultra-deep sequencing of *RHOA*. [a]Cultured skin fibroblasts derived from a biopsy of an affected (hypopigmented) skin lesion. [b]Cultured skin fibroblasts derived from a biopsy in normal skin. [c]Only bases with base quality ≥ 30 were considered.

Supplementary Table 7. *RHOA* mutations, frequency in public variant databases, and *in silico* predictions.

RHOA variant[a]	dbSNP (build147)	COSMIC[b]	ExAC[c]	gnomAD[c]	GERP score	CADD score	PolyPhen-2 HumVar score[d]	SIFT score
chr3:g.49412884C>T c.139G>A p.Glu47Lys	Absent	Absent	None (~120,900 alleles sequenced)	None (~252,400 alleles sequenced)	5.9	35.0	0.855 (possibly damaging)	0.010 (damaging)
chr3:g.49405927G>A c.211C>T p.Pro71Ser	Absent	Absent	None (~121,400 alleles sequenced)	None (~248,700 alleles sequenced)	5.8	33.0	0.516 (possibly damaging)	0.002 (damaging)

Abbreviations: COSMIC: Catalogue of Somatic Mutations in Cancer[2]; ExAC: Exome Aggregation Consortium[3]; gnomAD: Genome Aggregation Database[4]; GERP: Genomic Evolutionary Rate Profiling[5]; CADD: Combined Annotation-Dependent Depletion[6] ; PolyPhen-2: Polymorphism Phenotyping v2[7] ; SIFT: Sorting Tolerant From Intolerant[8]. [a]Coordinates of DNA changes are based on the human genome reference sequence GRCh37/hg19. The nomenclature of nucleotide and amino acid changes is based on *RHOA* RefSeq accession number NM_001664.2. [b]Presence in COSMIC (http://cancer.sanger.ac.uk/, accessed in November 2016). [c]Number of alleles found in ExAC (http://exac.broadinstitute.org/, accessed in November 2016) and gnomAD (http://gnomad.broadinstitute.org/, accessed in November 2016). [d]Among the two scores provided by PolyPhen-2 the HumVar-trained model is the most appropriate for Mendelian diseases.

Supplementary Table 8. Phenotype of patients with hypomelanosis of Ito in the M.U.S.T.A.R.D. cohort.

Subject	*RHOA*'s related clinical manifestation							Other symptom	Test	Result
	Linear hypopigmentation	Craniofacial asymmetry	Acral anomalies	Teeth Anomalies	Ocular anomalies	Brain anomalies	Intellectual disability			
S1	+	+	+	+	+	+	-	-	WES	*RHOA*
S2	+	+	+	+	+	+	-	Asymmetrical hearing loss	WES	*RHOA* and m.11778 G>A
S3	+	+	+	+	-	+	-	Skeletal and vesicoureteral anomalies	WES	*RHOA*
S4	+	+	+	+	+	+	-	-	TUDS	*RHOA*
S5	+	+	+	+	-	-	-	Sigmoid and rectal atresia	TUDS	*RHOA*
S6	+	+	+	+	-	NA	-	-	TUDS	-
S7	+	+	+	+	+	NA	-	Bilateral hearing loss, asymmetrical lower limbs	Sanger Sequen-cing	*RHOA*
S8	+	+	+	-	-	-	-	Skeletal anomalies	WES	-
S9	+	-	-	-	+	+	+	Autism, Skeletal anomalies, seizure, spina bifida occulta	WES	Other postzygotic cause
S10	+	-	-	-	+	-	+	Atrial septal aneurysm, cryptorchidism	WES	Other constitutional cause

S11	+	-	+	-	+	+	+	Feeding difficulties, hypospadias, short stature, scoliosis, bilateral hearing loss, brachycephaly, hypertelorism	WES	Other postzygotic cause
S12	+	-	-	-	-	+	+	Autism, seizure	WES	Other postzygotic cause
S13	+	-	-	-	-	-	+	Fronto-nasal dysplasia, epilepsy, lower limbs hypoplasia	WES	Other constitutional cause
S14	+	-	+	-	-	-	+	Eczema, tongue protrusion	WES	-
S15	+	-	-	-	-	-	-	Skeletal anomalies, ichtiosis	WES	Other postzygotic cause
S16	+	-	-	-	-	-	+	Autism, hypotonia, feeding difficulties, hand stereotypies	WES	Other constitutional cause
S17	+	-	-	-	-	+	+	Low set hairline	WES	Other postzygotic cause
S18	+	-	-	-	-	-	+	Ptosis, Kawasaki disease, encopresia	WES	-
S19	+	-	-	-	-	+	-	Movement disorder	WES	Other postzygotic cause
S20	+	-	-	-	-	+	-	Scoliosis, kidney anomalies, leg length discrepancy	WES	-
S21	+	-	-	-	-	+	-	Progressive peripheral neuropathy	WES	Other postzygotic cause
S22	+	-	-	-	+	-	+	Seizures, autism	WES	-
S23	+	-	-	-	-	+	-	Macrocephaly	WES	-
S24	+	-	+	-	-	-	+	Leg length discrepancy, scoliosis, delayed puberty, cardiac valvular dysplasia	WES	Other postzygotic cause
S25	+	-	-	-	-	+	+	Obesity, synophris, blepharophimosis, ideomotor apraxia	WES	Other postzygotic cause

S26	+	-	-	-	-	-	-	Focal epilepsy	WES	Other postzygotic cause
S27	+	-	-	-	-	NA	+	Cryptorchidism, strabismus	TUDS	-
S28	+	-	-	+	-	NA	+	Microcephaly	TUDS	-
S29	+	-	-	-	NA	+	+	Feeding difficulties, atrial septal defect	TUDS	-
S30								-	TUDS	-
S31	+	-	-	+	+	-	+	-	TUDS	-

Supplementary Table 9. Summary of the most frequent somatic mutations of *RHOA* reported in cancer.

Amino-acid change	Samples in COSMIC	Cancer type (sample count)	Functional impact
p.Gly17Val	152	Lymphoid neoplasm (151), ovarian carcinoma (1)	Dominant-negative[9,10]
p.Gly17Glu	35	Lymphoid neoplasm (27), stomach carcinoma (6), breast carcinoma (2)	Gain of function (growth-promoting effects)[11]
p.Tyr42Cys	21	Stomach carcinoma (19), breast carcinoma (1), Large intestine carcinoma (1),	Both gain[11] (growth-promoting effects) and loss of function[12] (defective signaling) reported
p.Cys16Arg	18	Lymphoid neoplasm (18)	Gain of function (increased RhoA activity, faster GTP/GDP exchange)[9]
p.Arg5Gln	13	Lymphoid neoplasm (9), large intestine carcinoma (2), stomach carcinoma (2)	Dominant negative and reduced RhoA activity[13]
p.Arg5Trp	10	Stomach carcinoma (8), breast carcinoma (1), oesophagus carcinoma (1)	Gain of function (growth-promoting effects)[11]
p.Leu57Val	9	Stomach carcinoma (9)	Loss of function (defective signaling)[12]
p.Ala161Val	6	Lymphoid neoplasm (3), urinary tract carcinoma (2), pleural mesothelioma (1),	Gain of function (increased RhoA activity, faster GTP/GDP exchange)[9]
p.Tyr34Cys	5	Large intestine carcinoma (2), stomach carcinoma (2), oesophagus carcinoma (1)	No data available
p.Leu69Arg	5	Stomach carcinoma (3), lymphoid neoplasm (2)	Loss of function (predicted to diminish most RhoA-GEF interactions)[14]

Abbreviations: COSMIC: Catalogue of Somatic Mutations in Cancer[2]. Only mutations reported at least five times in COSMIC are shown (accessed in November 2016). The nomenclature of amino acid changes is based on *RHOA* RefSeq accession number NM_001664.2.

Supplementary Table 10. Primers used for mutagenesis.

Variant	Sequence
E47K	Forward: TATGTGGCAGATATCAAGGTGGATGGAAAG Reverse: CTTTCCATCCACCTTGATATCTGCCACATA
P71S	Forward: TGGGTAGGAGAGGGACCTCAGGCGATC Reverse: GATCGCCTGAGGTCCCTCTCCTACCCA

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