Supplementary Material

Biallelic *TRIP13* mutations predispose to Wilms tumor and chromosome missegregation

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Supplementary Figure 1: Case reports, pedigrees and chromatograms of individuals with mutations in TRIP13

Supplementary Figure 2: *TRIP13* p.Arg354X mRNA is degraded by nonsense-mediated mRNA decay

Supplementary Figure 3: *TRIP13* p.Arg354X leads to absence of TRIP13 protein

Supplementary Figure 4: *TRIP13* p.Arg354X expressed from cDNA in HeLa cells is present at a much lower level compared to wild-type TRIP13

Supplementary Figure 5: MDS analysis strongly suggests the families with the *TRIP13* c.1060C>T_p.Arg354X mutation are from Pakistan

Supplementary Figure 6: *TRIP13* p.Arg354X mutant patient cell lines exit mitosis within 1 hour

Supplementary Figure 7: *TRIP13* p.Arg354X cells have increased p31^{comet} and reduced MAD2 expression

Supplementary Figure 8: TRIP13 protein levels of HCT116 wt and TRIP13 KO cells with or without ectopic expression of TRIP13 wt or p.Arg354X

Supplementary Figure 9: Sanger validation of homozygous alternate *TRIP13* c.1064dupA in the HCT116 TRIP13 KO clone

Supplementary Table 1: Summary of samples from MVA families included in exome sequencing experiment

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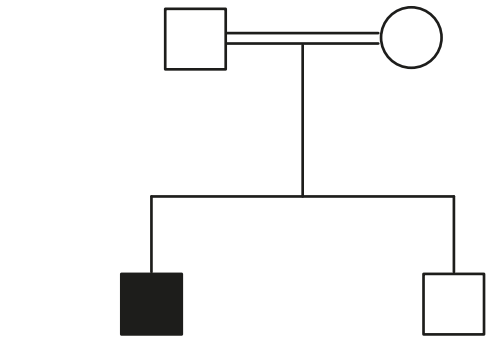
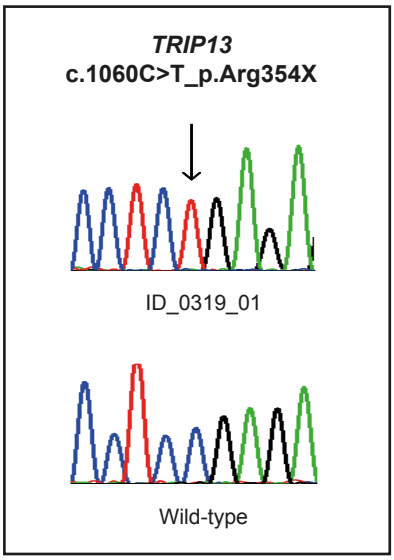
Supplementary Figure 1. Case reports, pedigrees and chromatograms of individuals with mutations in *TRIP13*

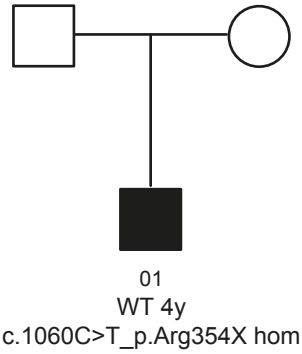
TRIP13

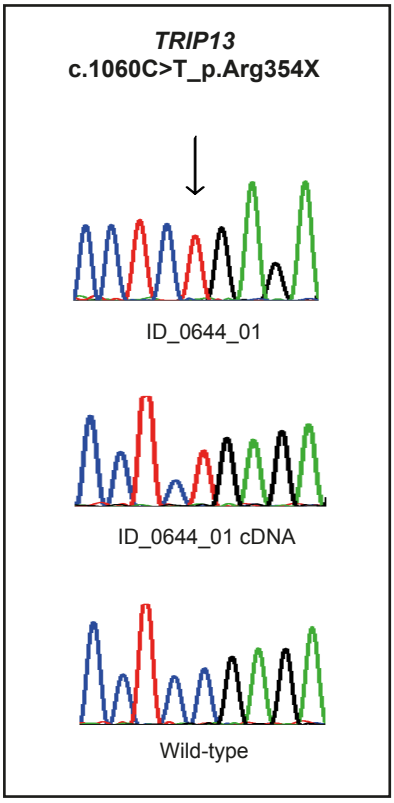
ID_0319. The proband was the first child of consanguineous parents of Asian origin. He had microcephaly and severe global developmental delay. An MRI scan showed multiple cerebral cysts and neuronal migration defects. Dysmorphic features included large low-set ears, a beaked nose and cleft palate. He also had arthrogryposis, severe cone-rod dystrophy and nystagmus. He had a horseshoe kidney and developed Wilms tumor (WT) at 2 years. Chromosome analysis confirmed a diagnosis of MVA, although karyotypic details were not provided. Parental samples were not available. The proband was alive at 6 years of age.



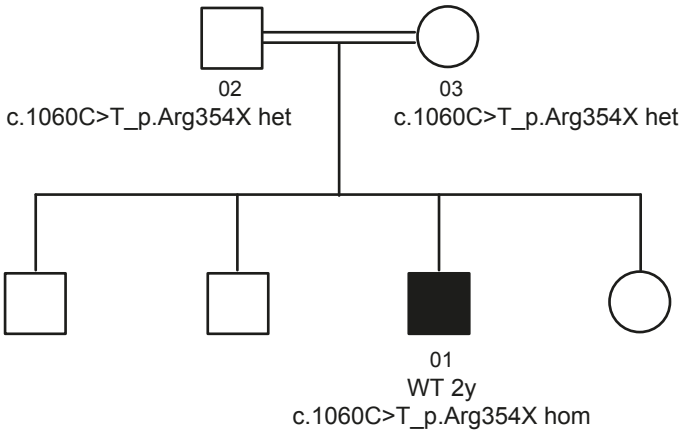


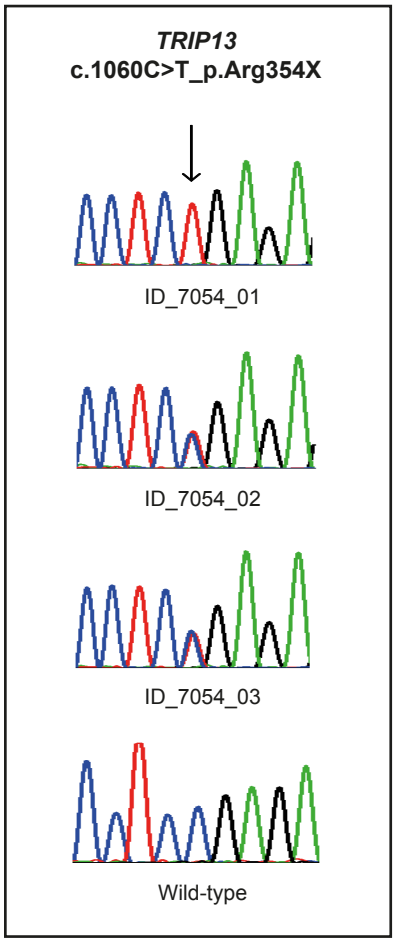
ID_0644. The proband, of Asian origin, had short stature and developed WT at 4 years and relapsed at 5 years. Tumor histology is unknown. Chromosome analysis of cells from an EBV-transformed lymphoblastoid cell line showed gains and losses of various chromosomes. Cells from two different passages were analyzed. Aneuploidy was present in 13/28 (46%) cells from the initial passage and in 19/25 (76%) cells following four subsequent passages. Premature chromatid separation (PCS) was also observed. Parental samples were not available. The proband was alive at 43 years of age.



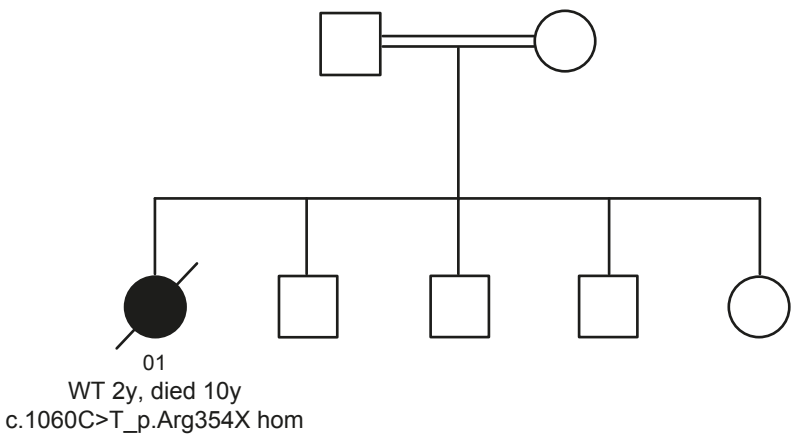


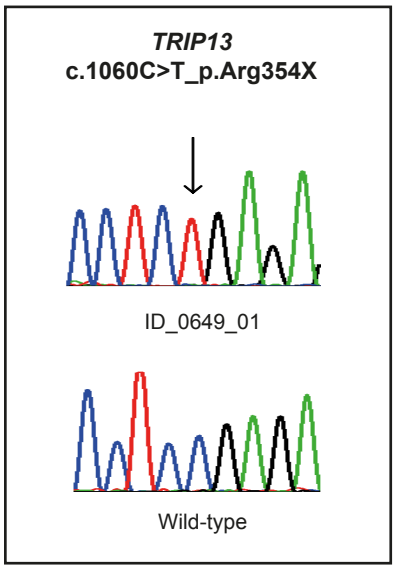
ID_7054. The proband was the third child of consanguineous parents from Pakistan. He had multiple café au lait patches. He did not have microcephaly or developmental delay. He was diagnosed with a Stage III unilateral WT of the left kidney at 2 years. Histology revealed a triphasic tumor of intermediate risk with no evidence of anaplasia or nephrogenic rests. Chromosome analysis of cultured lymphocytes revealed aneuploidy in 10% of cells involving chromosomes 12, 13, 18, 22 and Y. PCS was also noted. The proband was alive at 5 years of age.



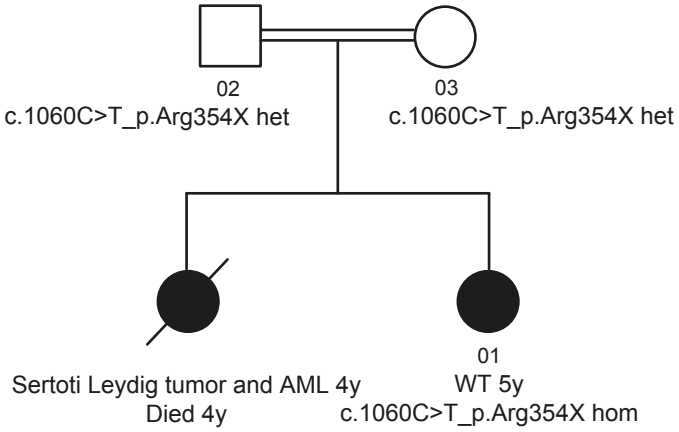


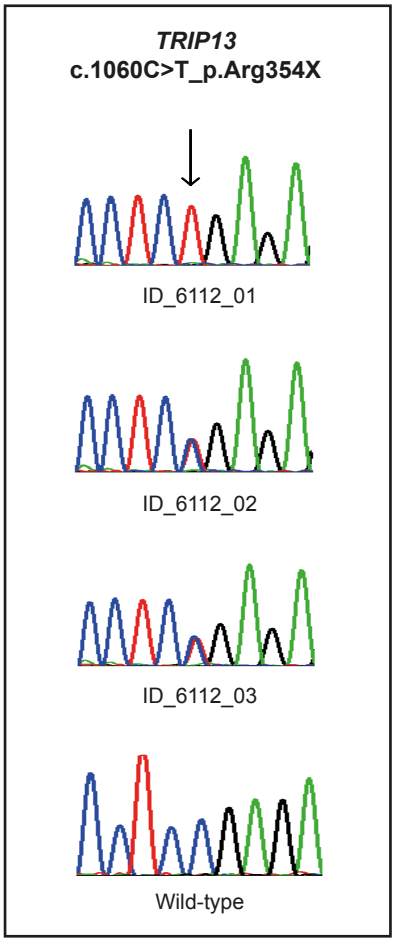
ID_0649. The proband was the first child of consanguineous parents of Asian origin. She had microcephaly, growth retardation, deep-set eyes and skin pigmentation on her trunk. At 2 years she presented with a Stage II unilateral WT of the left kidney. During her treatment she developed seizure-like episodes. She died at 10 years following a relapse of her tumor. Approximately 50% of cultured lymphocytes demonstrated PCS but no chromosomal gains or losses were observed. Cells from her tumor showed a hypertetraploid clonal karyotype. Parental samples were not available.



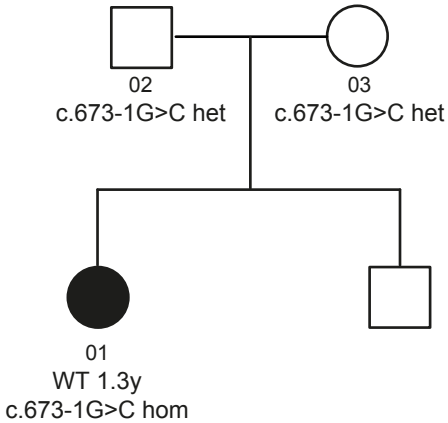


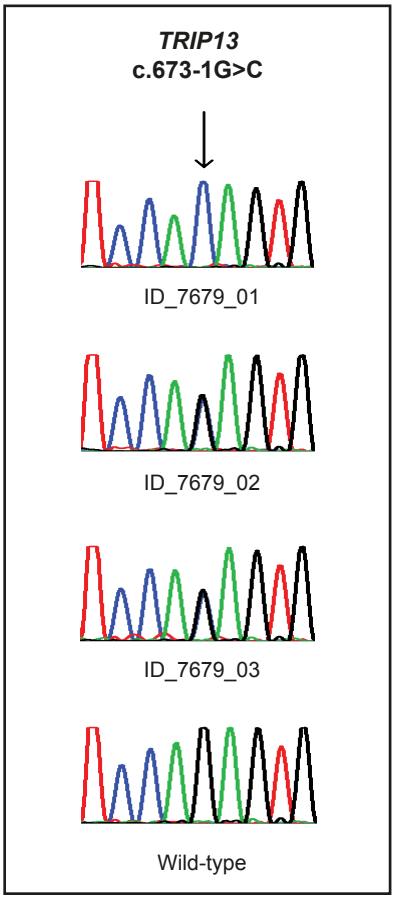
ID_6112. The proband was the second child of consanguineous parents of Asian origin. She was diagnosed with a Stage I unilateral WT of the right kidney at 5 years. Histology revealed a triphasic tumor of intermediate risk with no evidence of anaplasia. Her sister was diagnosed with Sertoli Leydig cell tumor of the left ovary and acute myeloid leukemia at 4 years and died at the same age; no DNA sample was available. Chromosome analysis was not performed for either child. The proband was alive at 6 years of age.



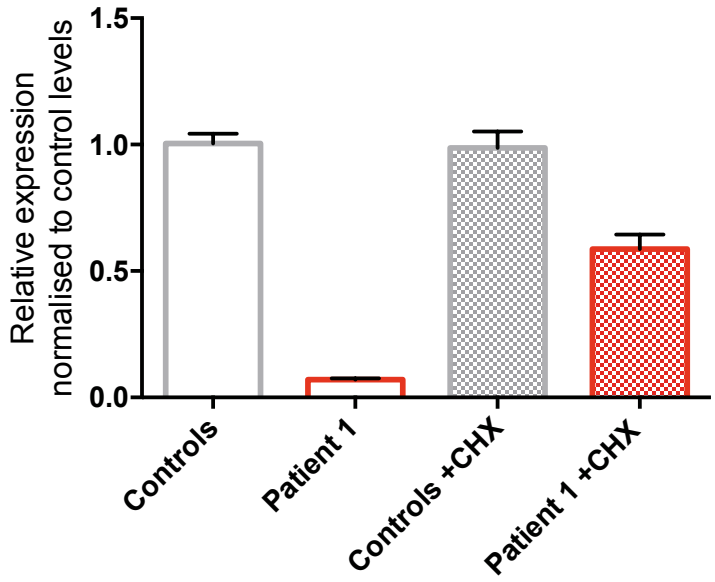


ID_7679. The proband was the first child of parents of Norwegian origin. She had microcephaly and developmental delay. She also had a congenital ventricular septal defect that closed spontaneously. She developed treatment-resistant and progressive multifocal epilepsy at 13 months. She was diagnosed with a Stage I ulilateral WT in her left kidney at 15 months which was successfully treated by surgery. Brain MRI at 18 months was normal. Chromosome analysis of cultured lymphocytes did not reveal any aneuploidies. The proband was alive at 2.5 years of age.



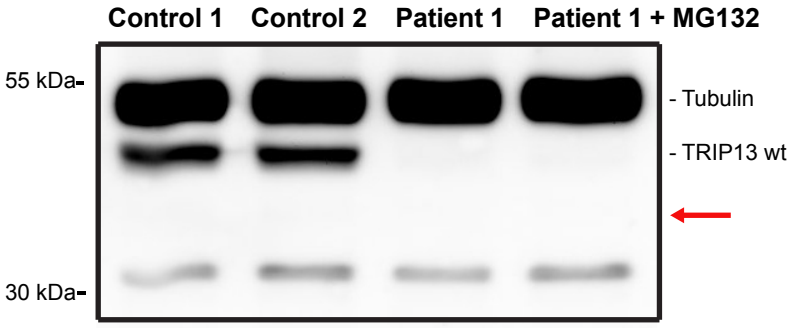


Supplementary Figure 2: *TRIP13* p.Arg354X mRNA is degraded by nonsense-mediated mRNA decay



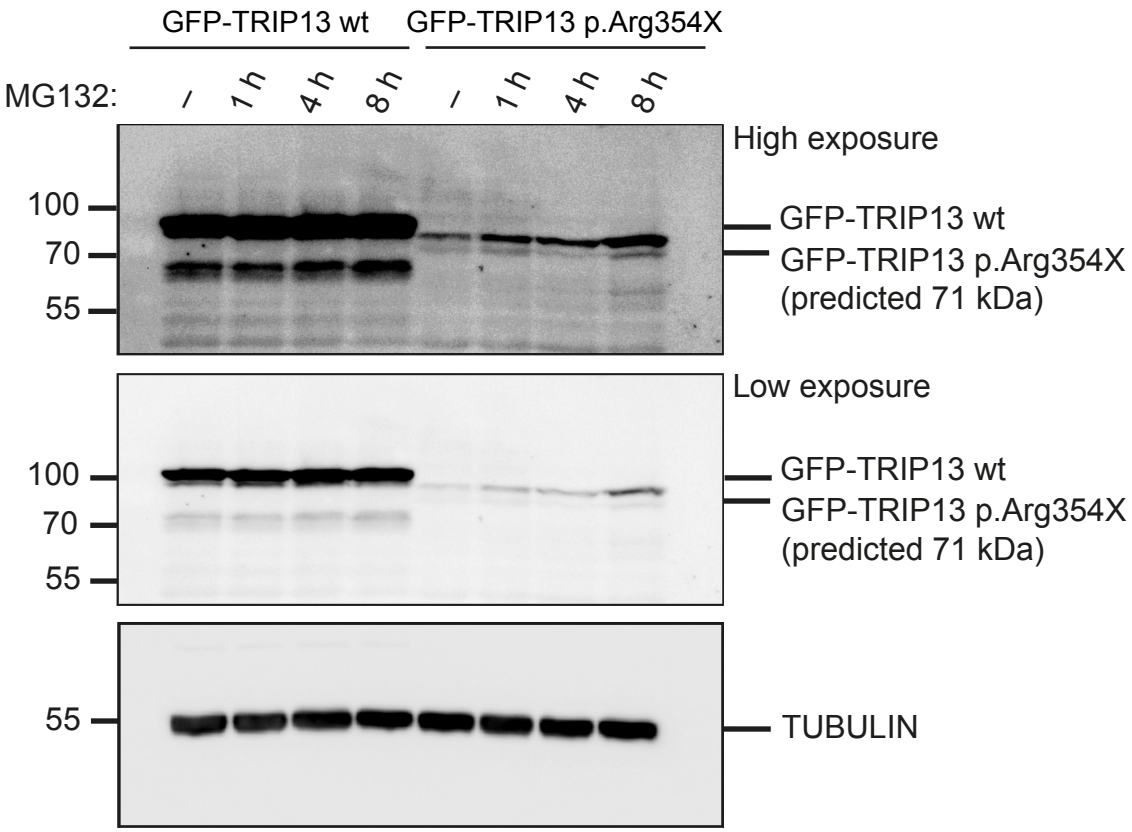
q-PCR analysis of *TRIP13* mRNA in controls and patient 1 lymphoblasts untreated or treated with the inhibitor of nonsense-mediated mRNA decay cycloheximide for 4.5 hrs. *TRIP13* p.Arg354X mRNA is degraded by nonsense-mediated mRNA decay.
Key: controls, data from control 1 and control 2; CHX, cycloheximide

Supplementary Figure 3: *TRIP13* p.Arg354X leads to absence of TRIP13 protein



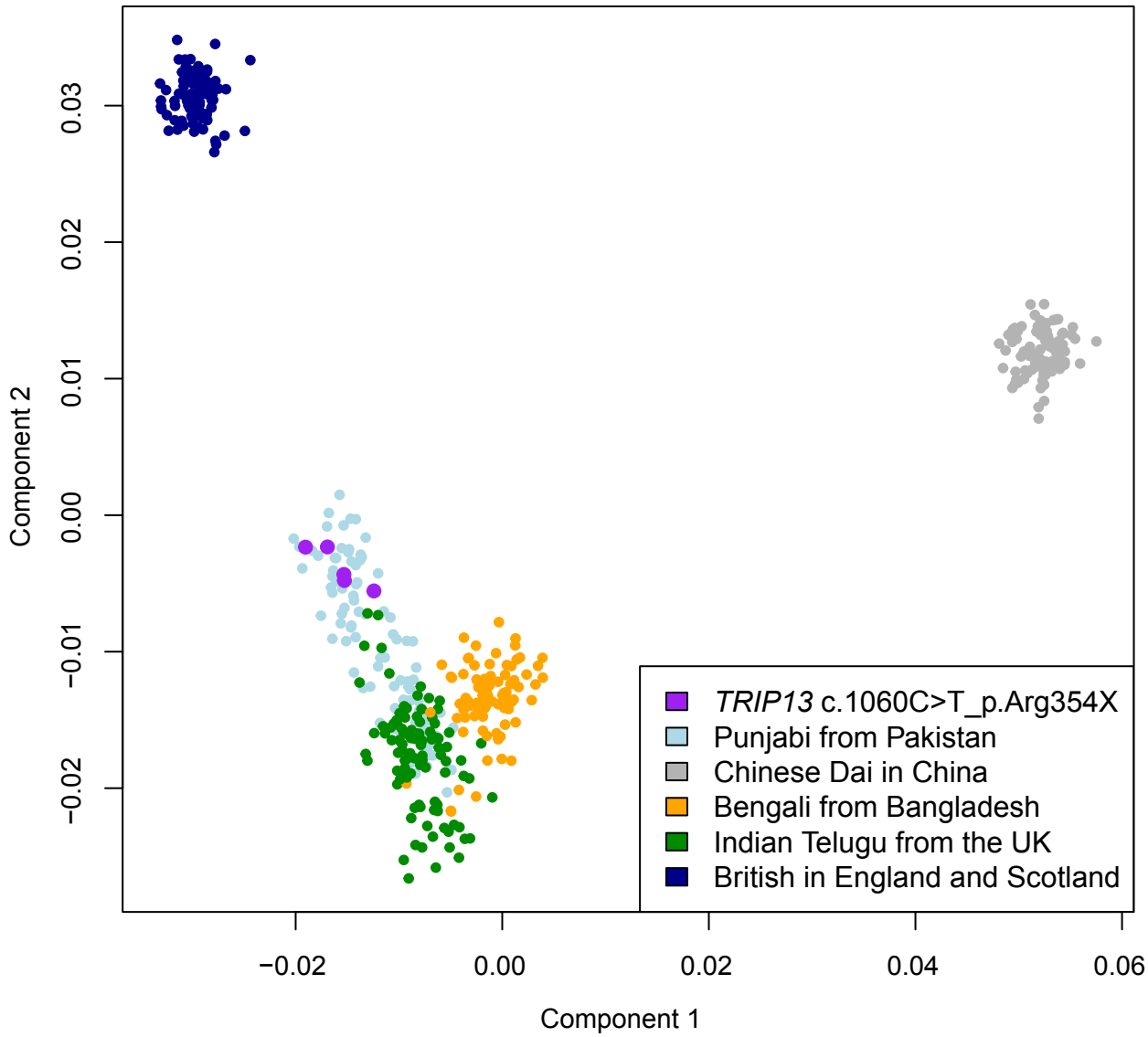
Control 1, control 2 and patient 1 lymphoblast whole cell lysates were immunoblotted for TRIP13 and tubulin. Right-most lane of patient 1 lymphoblasts were treated with the proteasome inhibitor MG132 for 8 hours. The red arrow indicates the expected position of the truncated TRIP13 protein resulting from the p.Arg354X patient mutation. No TRIP13 protein can be detected in patient lymphoblasts.
Key: wt, wild-type

Supplementary Figure 4: *TRIP13* p.Arg354X expressed from cDNA in HeLa cells is present at a much lower level compared to wild-type TRIP13.



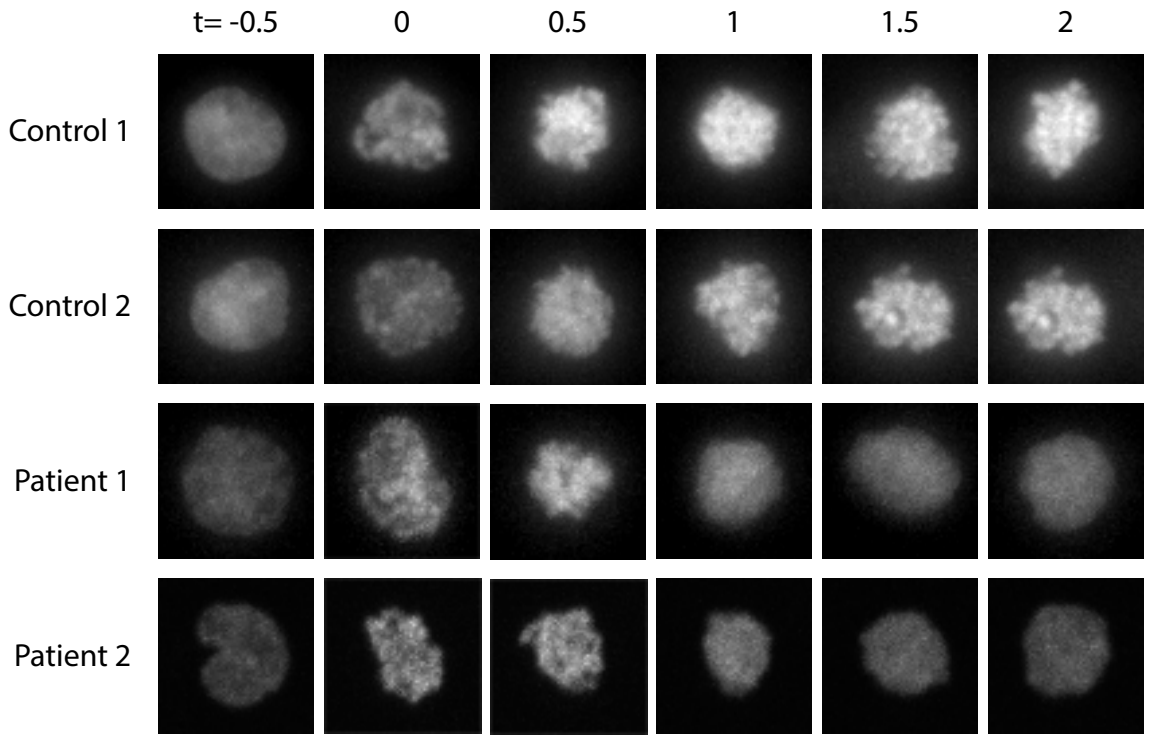
Whole cell lysates of HeLa T-REx Flp-In cells ectopically expressing GFP-TRIP13 wt or p.Arg354X from the same genomic locus were immunoblotted for TRIP13 and tubulin after treatment without (-) or with the proteasome inhibitor MG132 for indicated amount of hours. GFP-TRIP13 p.Arg354X was expressed at significantly lower levels compared to wild type, even after being treated with MG132 for 8 hours.
Key: wt, wild-type; GFP, green fluorescent protein

Supplementary Figure 5: MDS analysis strongly suggests the families with the *TRIP13* c.1060C>T_p.Arg354X mutation are from Pakistan



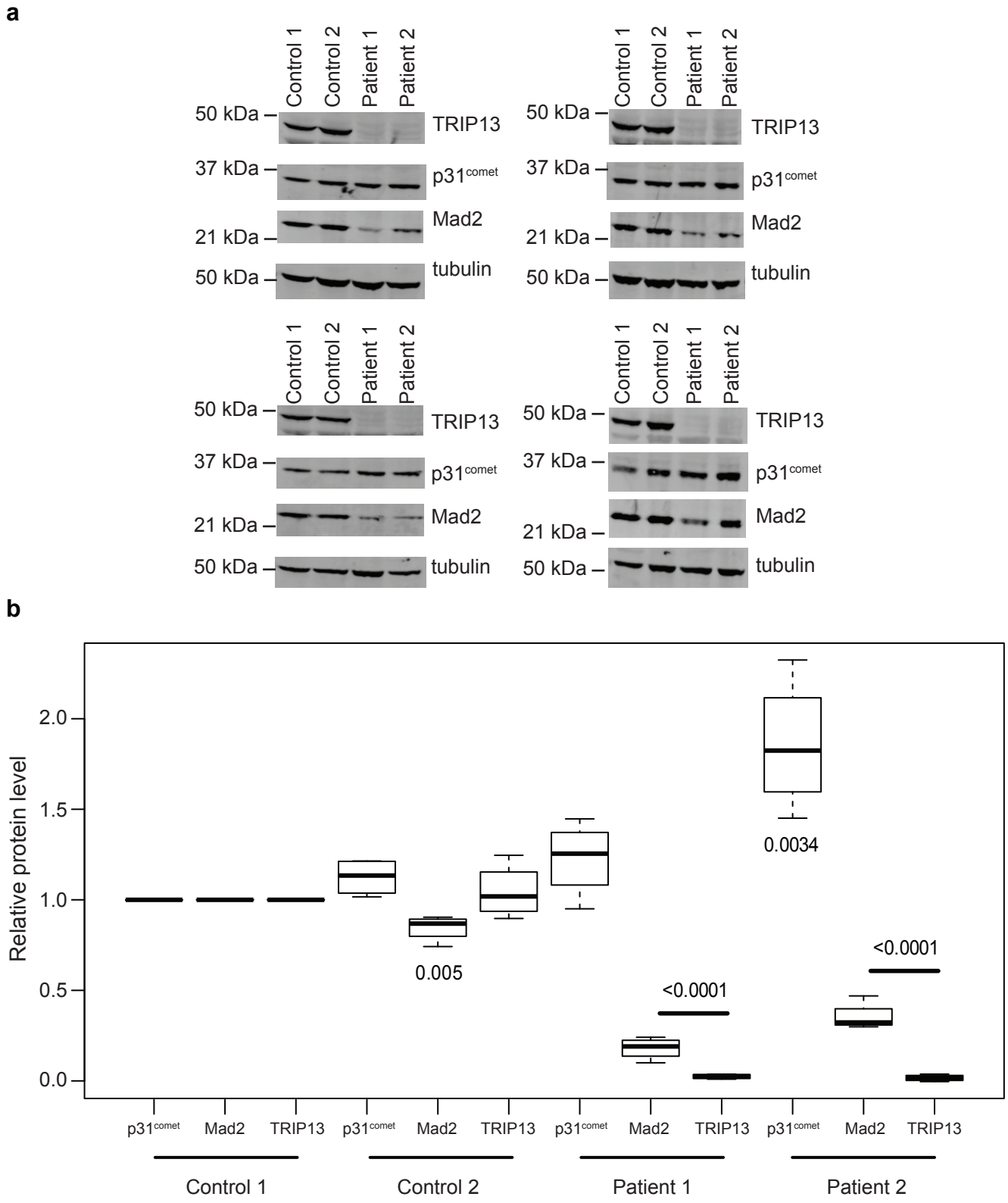
Multidimensional scaling analysis (MDS) of individuals with *TRIP13* c.1060C>T_p.Arg354X mutation and select individuals from 1000 Genomes project. Individuals with the *TRIP13* c.1060C>T_p.Arg354X mutation are closely related to the Pakistan group from the 1000 Genomes project.

Supplementary Figure 6: *TRIP13* p.Arg354X mutant patient cell lines exit mitosis within 1 hour



Representative images of H2B-mNeon expressing control (row one and two) and patient (row three and four) lymphoblasts going through mitosis (time in hours with mitotic entry at t=0.0) in the presence of nocodazole. Both patient 1 and patient 2 cells exit from mitosis after 1 hour.

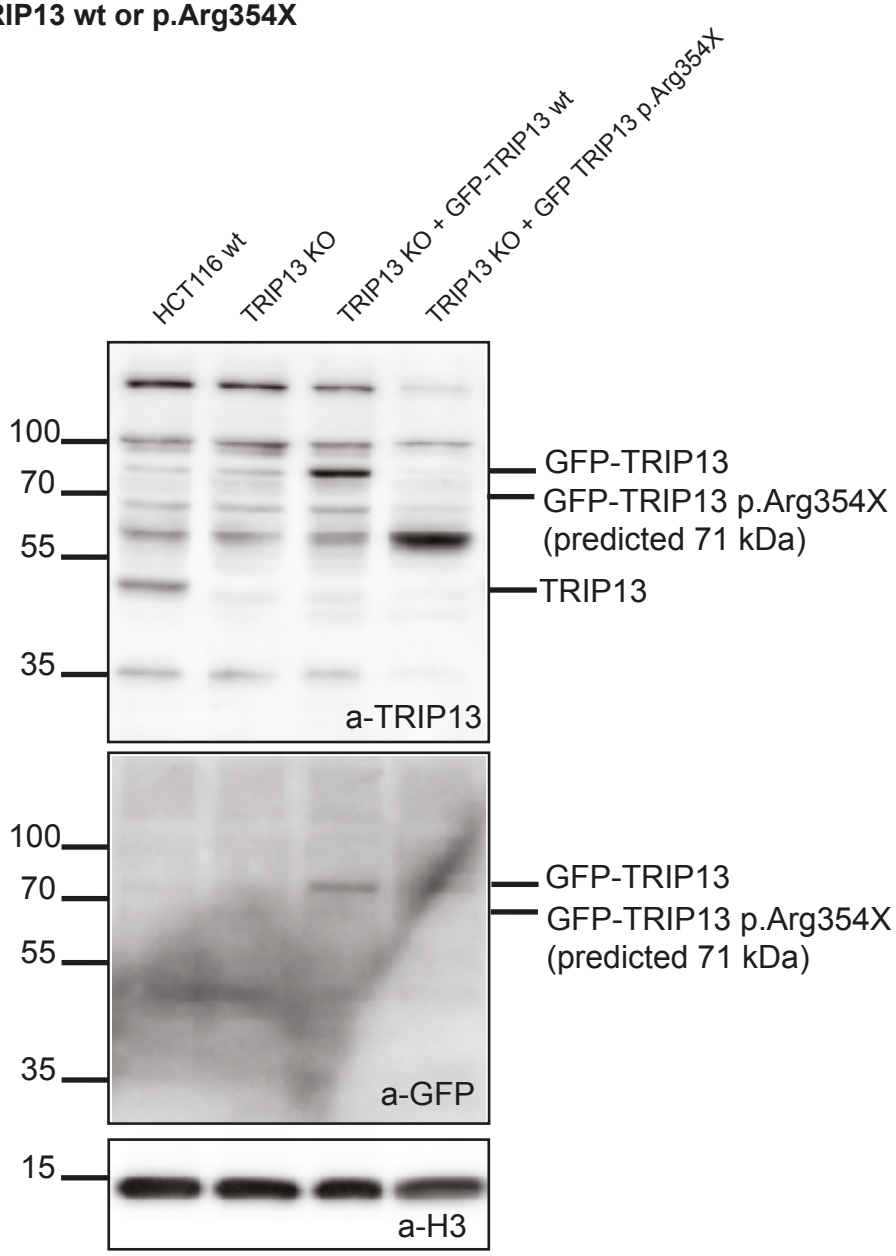
Supplementary Figure 7: *TRIP13* p.Arg354X cells have increased p31^{comet} and reduced MAD2 expression



(a) Immunoblots of cell lysates that were probed with anti-Mad2, anti-p31^{comet} and anti-TRIP13 antibodies. Tubulin was used as loading control. (b) Mad2, p31^{comet} and TRIP13 levels were first normalized against tubulin and subsequently against the control cell line (control 1). Bars are the average of 4 independent experiments with SEM shown. Unpaired Student t-test against control 1 was applied for statistical analyses, shown are P-values ≤ 0.005. Both patient 1 and patient 2 cells show statistically significantly decreased levels of Mad2 and TRIP13; one patient has significantly increased levels of p31^{comet}.

Key: SEM, standard error of the mean

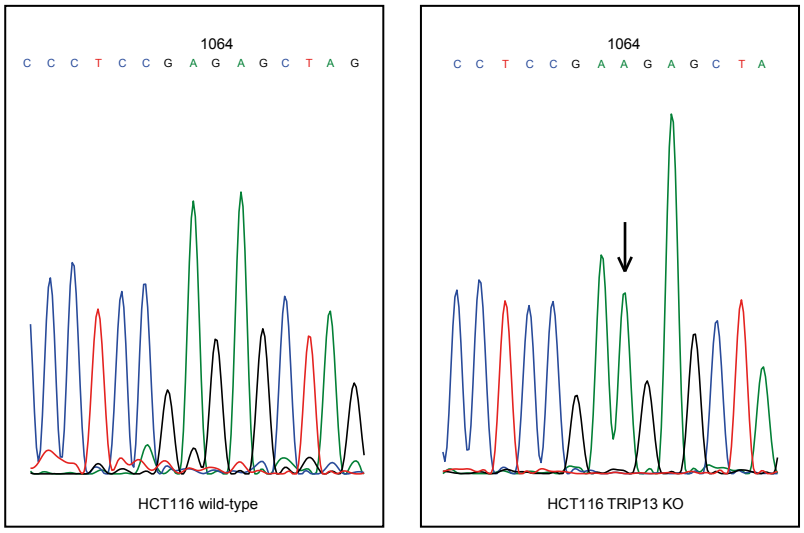
Supplementary Figure 8: TRIP13 protein levels of HCT116 wt and TRIP13 KO cells with or without ectopic expression of TRIP13 wt or p.Arg354X



Whole cell lysates of HCT116 wt and TRIP13 KO cells transduced with GFP-TRIP13 wt or GFP-TRIP13 p.Arg354X lentivirus, when indicated, were immunoblotted for TRIP13, GFP or tubulin. GFP-TRIP13 wt is indeed expressed, but GFP-TRIP13 p.Arg354X is not detected.

Key: wt, wild-type; KO, knockout; GFP, green fluorescent protein; H3, Histone H3

Supplementary Figure 9: Sanger validation of homozygous alternate *TRIP13* c.1064dupA in the HCT116 TRIP13 KO clone



Sanger sequencing reads of a PCR product amplified from the genomic DNA of HCT116 wt and TRIP13 KO cells. The TRIP13 KO cells have a single bp duplication in exon 11 causing a frameshift.
Key: KO, knockout

Supplementary Table 1: Summary of samples from MVA families included in exome sequencing experiment

Family ID	Individuals exome sequenced
ID_0641	Proband, Mother, Father
ID_0642	Proband
ID_0648	Proband
ID_0658	Proband
ID_0660	Proband, Mother, Father, Unaffected_sib
ID_0664	Proband, Mother, Father
ID_0668	Proband, Mother, Father
ID_0671	Proband, Mother, Father
ID_1992	Proband, Mother, Father
ID_2129	Proband
ID_5823	Proband
ID_5827	Proband
ID_0319	Proband
ID_0639	Proband
ID_0640	Proband
ID_0644	Proband
ID_0661	Proband, Mother, Father
ID_5728	Proband, Mother, Father, Affected_sib, Unaffected_sib
ID_6246	Proband, Mother, Father
ID_7054	Proband, Mother, Father