Supplemental Data

Figure S1

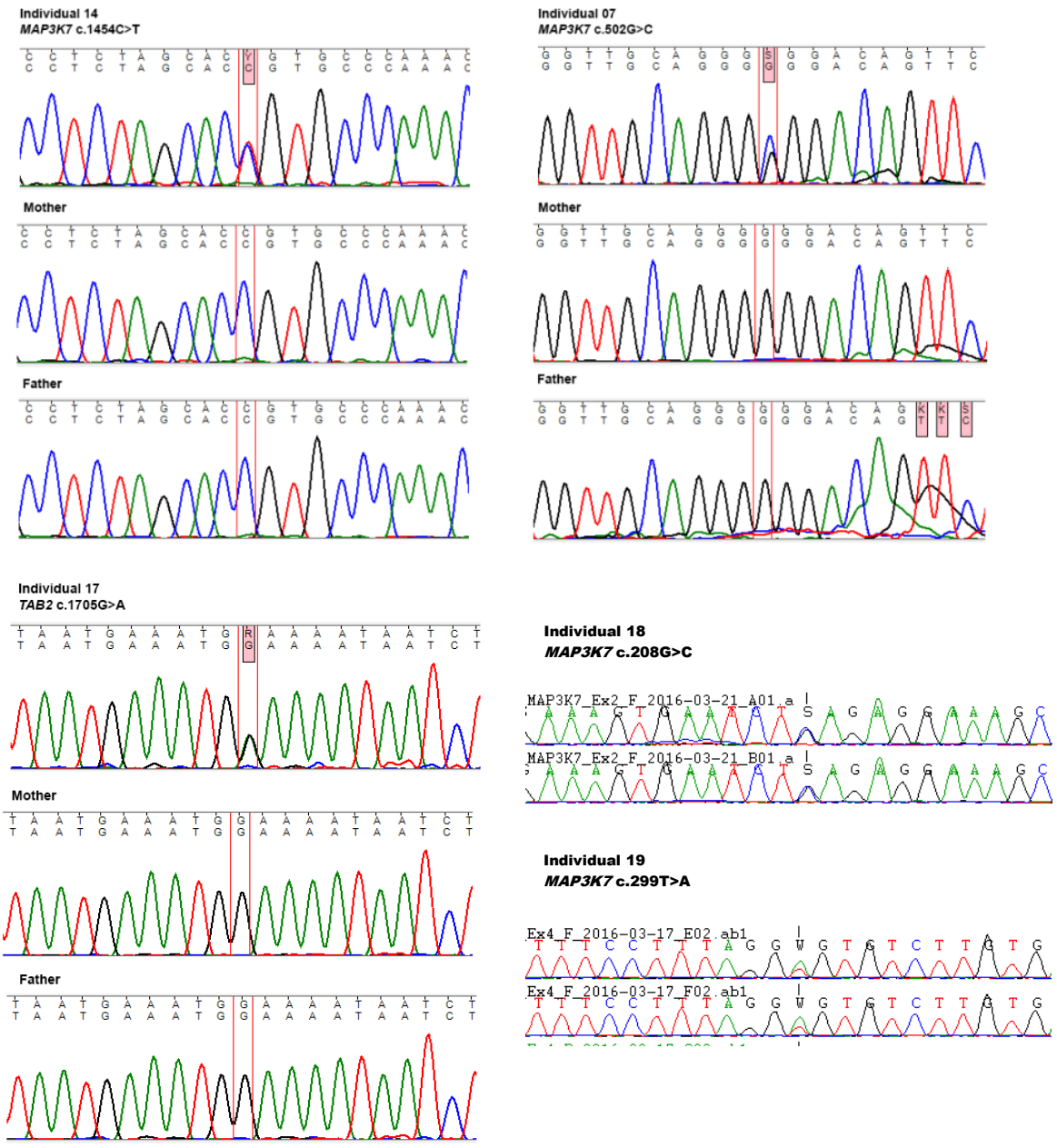

Figure S1. Chromatograms showing mutations in *MAP3K7* and *TAB2:* Chromatograms of individual 07 showing the heterozygous c.502G>C mutation in *MAP3K7*, and the parents who are homozygous for the reference allele; individual 14 showing the heterozygous c.1454C>T mutation in *MAP3K7*, and the parents who are both homozygous for the reference allele; individual 17 showing the heterozygous c.1705G>A mutation in *TAB2*, and the parents who are homozygous for the reference allele; individual 18 showing the heterozygous c.208C>T mutation in *MAP3K7;* and individual 19 showing the heterozygous c.299T>A mutation in *MAP3K7*. All parental relationships were confirmed with micro-satellite genotyping

Figure S2

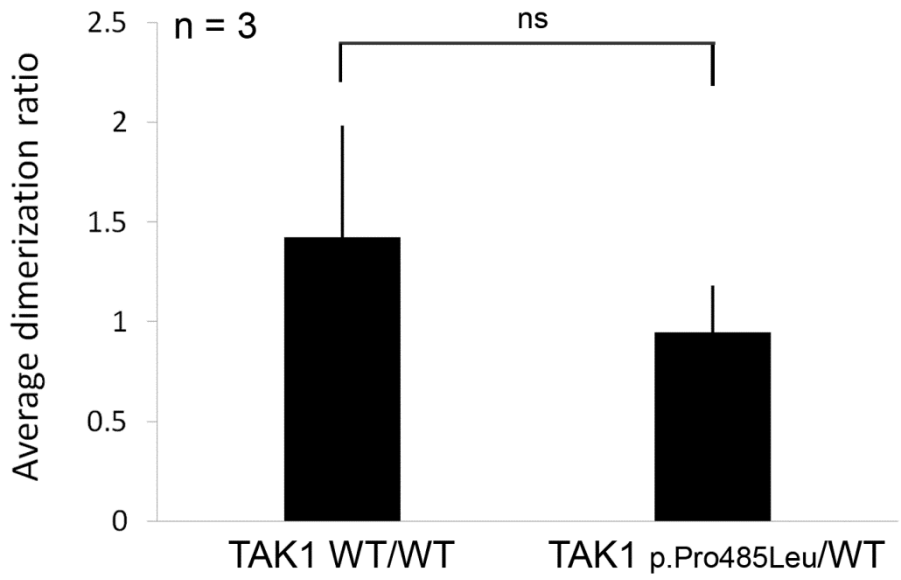

Figure S2. TAK1 dimerisation is not affected by TAK1$^{p.Pro485Leu}$: Competitive co-immunoprecipitation, in HEK293ft cells, showed that TAK1$^{p.Pro485Leu}$ did not have a significantly different capacity to homodimerise with TAK1^{WT} than TAK1^{WT} (unpaired t test, error bars show standard deviation)

Figure S3

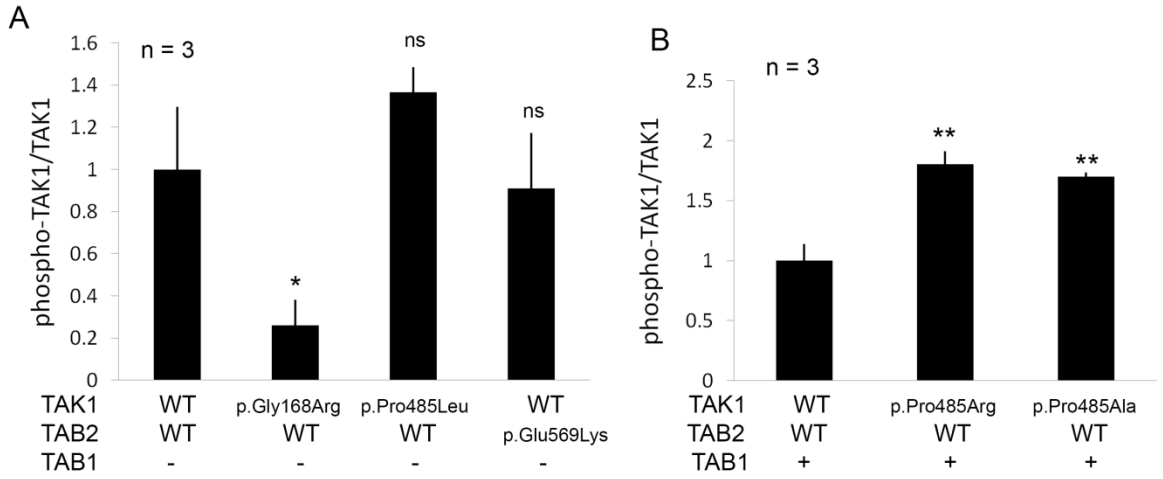

Figure S3. TAK1$^{p.Pro485Leu}$ increased phosphorylation is dependent on TAB2; in the presence of TAB2 other Pro485 positional mutants are hyperphosphorylated: (A) When co-expressed with TAB2^{WT}, and not TAB1, in HEK293ft cells TAK1$^{p.Pro485Leu}$ was not significantly more phosphorylated than TAK1^{WT}, also TAK1^{WT} was not more phosphorylated when co-expressed with TAB2$^{p.Glu569Lys}$, without TAB1. (B) The non-FMD2 causing Pro485 substitutions TAK1$^{p.Pro485Arg}$ and TAK1$^{p.Pro485Ala}$ are significantly more phosphorylated than TAK1^{WT} when expressed with TAB1 and TAB2^{WT} in HEK293ft cells (unpaired t tests, error bars show standard deviation)

Figure S4

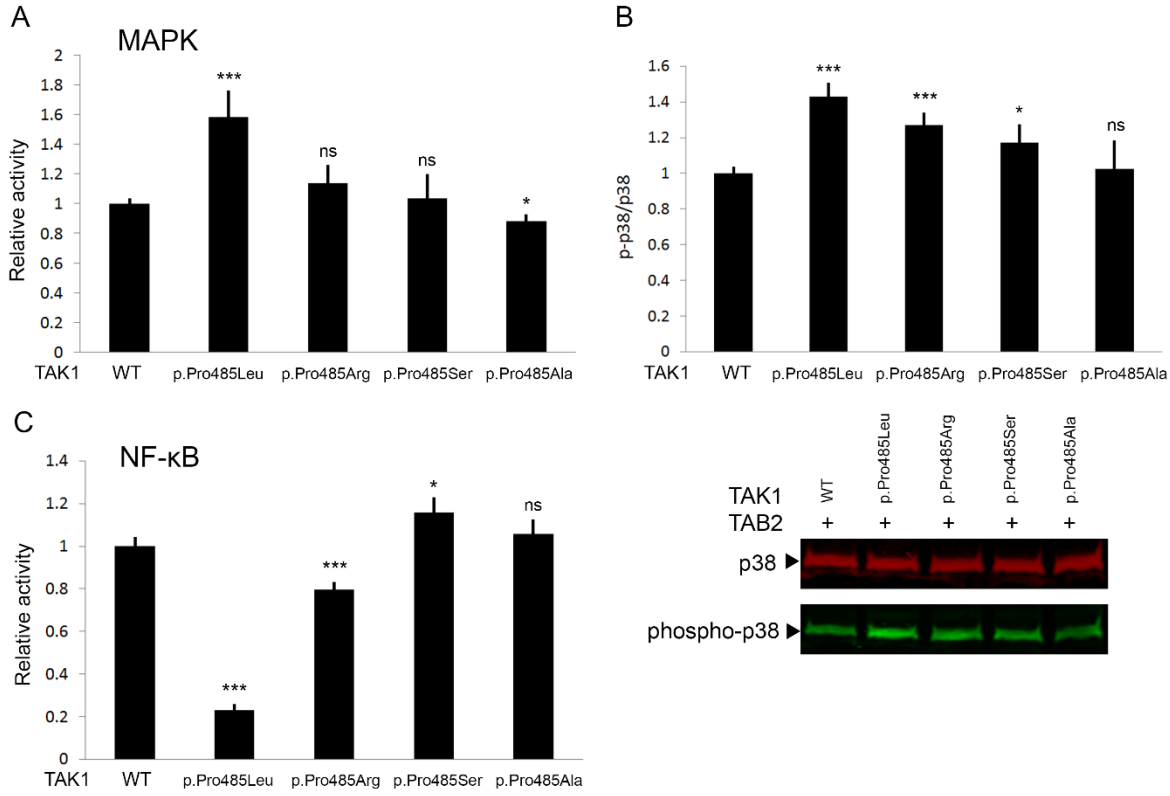

Figure S4: The effect on TAK1 signalling at Pro485 varies with the identity of the substituting residue. TAK1 constructs containing different substitutions at the 485 position were expressed alongside $TAB2^{WT}$ in HEK293 cells, (A) Only $TAK1^{p.Pro485Leu}$ significantly increases MAPK luciferase reporter activity however (B) a specific immunoblot to measure phosphorylated-p38 shows that $TAK1^{p.Pro485Arg}$ and $TAK1^{p.Pro485Ser}$ also have increased activity, although not as pronounced as that conferred by $TAK1^{p.Pro485Leu}$. $TAK1^{p.Pro485Ala}$ was not significantly different from $TAK1^{WT}$. (C) NF-κB luciferase reporter assay showing that $TAK1^{p.Pro485Arg}$ has decreased NF-κB activity compared to $TAK1^{WT}$ but this was not as reduced as $TAK1^{p.Pro485Leu}$. $TAK1^{p.Pro485Ser}$ has a small but significant increase in NF-κB activity and $TAK1^{p.Pro485Ala}$ is not significantly different from $TAK1^{WT}$. Error bars show standard deviation.

Table S1: Primer pairs used to sequence the exons of TAK1 and TAB2

Primer	Sequence	Primer	Sequence
TAK1 1F	GGAACCGGAAGTGTAGTGGG	TAB2 1F	AGGAGGAGGAGAGACAGAGG
TAK1 1R	CACTCCCACGTTACTGAGGG	TAB2 1R	AGGAGGCTGAATGAATGTAGGT
TAK1 2F	TGATTCCGATTCTTGTGGTGT	TAB2 2F	TCTTGTTCTGCCTACCTCTAGT
TAK1 2R	ACCGAATTGCTGATGTTCATGT	TAB2 2R	GGCAGTTACACAGGCTAAGC
TAK1 3F	CATTCTGGGCAGTCACTTGG	TAB2 3F	AAAGAATCTGGGAAGACAACCT
TAK1 3R	TGCCATGTCATTTCTCACAGG	TAB2 3R	CAGAAAGCTCCATCTTGCCA
TAK1 4F	TCACATAGTAGGGGCCAAAAGA	TAB2 4aF	AGCCAGTCACTTGGTAATCATG
TAK1 4R	TTTTGGGACTCTGACGGCA	TAB2 4aR	ACATGAGAGGTCTGGTGGC
TAK1 5F	AAGCAGGACTGATGGAGCAT	TAB2 4bF	ACCTCATCTCAACAGCCAAA
TAK1 5R	GAATTAGAGAGTGGGTTCGGG	TAB2 4bR	AACCATCCCACCTTTAGTATTCA
TAK1 6F	TGTTGGCCTTGTAACTTTTGATG	TAB2 5-6F	TTAAAGTGGGTGGGTCCTCA
TAK1 6R	ACAAGGAGTGAGGGAGATTATCA	TAB2 5-6R	GCCACAGAAAAACTGCTGGT
TAK1 7F	TCCCTGATACCCATTCTCAAAAT	TAB2 7F	AGGGGCAAAATAAATGAAAAGCA
TAK1 7R	TTAGGGAAGGGGACAGGAGT	TAB2 7R	AGAGAGGAAGGTTGCTGAAC
TAK1 8F	AGAGTTTGCATCATTCTTTGGGT	TAB2 8F	AAATGGTGTTTAAAGTTGCCTGT
TAK1 8R	CCCCTAAATCCAAAGCCCCT	TAB2 8R	AGAGAAGATACAGGGCCATTTG
TAK1 9F	TTCTTAGGTAGGTGCATGGC		
TAK1 9R	ATGAAGAATGACGCACCTGT		
TAK1 10F	CCTGCCTGGCTAGTTTATAAAAG	TAK1 cDNA F	GTGAGCAGTAGGTCATCCAGTCCCAGT
TAK1 10R	GCTCTTTTGCATGTTTCAGGA	TAK1 cDNA R	ATCATGAAGTGCCTTGTCGTTTC
TAK1 11F	CCCATTTGCCATCTTCCTCC		
TAK1 11R	TCACTCTTCAGACTTGTAGTTCC		
TAK1 12F	TCCGTGTTACTGTTTGTGGT		
TAK1 12R	TCATTATTCCGGTTCCACAGC		
TAK1 13F	GATCCAAGGAGCAACAAATTCC		
TAK1 13R	ACAGCGCTAATGAACAATTTACT		
TAK1 14F	GGATCTCTAAGTCTCTTCTTGCC		
TAK1 14R	GTATGAAGAAACAGACTGGTCAC		
TAK1 15F	TGGGGAGTTTGATTAGATAGGGT		
TAK1 15R	TTTAAGGAGGCCAACTAAAAGGA		
TAK1 16F	TCTGTCTTGACTCTCATCTTAGC		
TAK1 16R	AACTACGTTTTCATTGCACCTT		
TAK1 17F	GCTTCCATCATCAGCTACTAAGG		
TAK1 17R	GCATCAAAGCCCTTACACGG		

Number in primer name refers to target exon

Table S2: Primer pairs used to generate TAK1 and TAB2 expression constructs

Initial amplification primers – TAK1		
Pair 1 pcDNA3.1	F: TTCGAATTCGCGAGGGATCATGTCTACAG	R: CCGGGATCCCAGAGAATCATGAAGTGC
Pair 2 pCMV-Myc	F: TATGGCCATGGAGGCCCGATCTACAGCCTCTGCCGCCTCC	R: TCATGTCTGGATCCCCGCTCATGAAGTGCCTTGTCGTTTCTG
Pair 3 pCMV-HA	F: TATGGCCATGGAGGCCCGATCTACAGCCTCTGCCGCCTCCTC	R: AGAGATCTCGGTCGACCGTCATGAAGTGCCTTGTCGTTTCTG
Pair 4 p3xFLAG	F: GACAAGCTTGCGGCCGCGTCTACAGCCTCTGCCGCCTCC	R: GGATGCCACCCGGGATCCTTCATGAAGTGCCTTGTCGTTTCTG
Initial amplification primers – TAB2		
Pair 5 pCMV-HA	F: TATGGCCATGGAGGCCCGAGCCCAAGGAAGCCACCAAATTGAT	R: AGAGATCTCGGTCGACCGTCAGAAATGCCTTGGCATCTCACACTG
Mutagenesis primers		
TAK1 G168R	F: GTTGCAGGGCGGACAGTTCTAAAAATTTGTG	R: GAACTGTCCGCCCTGCAACCAGCAGTAAG
TAK1 P485L	F: CTCTAGCACTGTGCCCAAACTCCAAAGAATC	R: GTTTGGGCACAGTGCTAGAGGCTGTAGTTGG
TAK1 P485A	F: CTCTAGCAGCGTTGCCCAAACTCCAAAGAATC	R: GTTTGGGCACGCTGCTAGAGGCTGTAGTTGG
TAK1 P485R	F: CTCTAGCACGGTTGCCCAAACTCCAAAGAATC	R: GTTTGGGCACCGTGCTAGAGGCTGTAGTTGG
TAK1 P485S	F: CTCTAGCATCGTTGCCCAAACTCCAAAGAATC	R: GTTTGGGCACGATGCTAGAGGCTGTAGTTGG
TAB2 E569K	F: GTTAATGAAATGAAAAATAATCTAACTC	R: AGTTAGATTATTTTTCATTTCATTAACC

Table S3: Coverage and mapping data from individuals sequenced on the Agilent SureSelect Human All Exon V4+UTRs chip

Individual	Subject 01	Sibling 01	Mother 01	Father 01	Subject 02	Mother 02	Father 02	Subject 05	Mother 05	Father 05
Total reads	106,493,680	63,853,341	46,674,820	81,582,899	165,020,599	35,997,633	59,559,617	140,776,595	66,035,919	72,373,797
Mapped reads [a]	92,324,081 (86.69%)	55,982,531 (87.67%)	40,387,525 (86.53%)	71,863,933 (88.09%)	142,626,619 (86.43%)	30,337,637 (84.28%)	51,966,431 (87.25%)	121,754,108 (86.49%)	57,653,931 (87.31%)	63,579,562 (87.85%)
Mean coverage	115.54X	62.72X	50.52X	89.55X	178.37X	37.87X	64.66X	152.09X	71.76X	79.21X
Coverage at 10X	97.14%	95.23%	93.46%	97.08%	98.53%	89.33%	94.50%	98.42%	95.27%	96.29%

Individual	Subject 17	Mother 17	Father 17	Subject 18	Subject 19
Total reads	64,287,941	87,174,023	65,666,191	67,993,180	63,898,139
Mapped reads [a]	60,746,791 (94.49%)	74,376,740 (85.32%)	54,114,983 (82.41%)	61,170,223 (89.96%)	57,148,928 (89.4%)
Mean coverage	46.42X	51.34X	36.50X	50.71X	47.14X
Coverage at 10X	87.98%	87.29%	83.86%	88.37%	87.77%

[a] Inside of regions – capture region size = 2.27% of human genome

Table S4: Filtering data from exome sequenced families

Family	Subject 01	Subject 02	Subject 05	Subject 17	Subject 18	Subject 19
Total variants	204,358	201,364	201,362	42,070	?	?
Rare variants[a]	5,133	3,980	4,092	3,799	6,288	6,180
De novo variants	177	106	114	15	n/a[b]	n/a[b]
Protein altering variants	6	1	4	2	42	58
Sanger confirmed candidates	2[c]	1	3[d]	2[e]	1	1
Disease causing variant	*MAP3K7*: c.1454C>T, p.Pro485Leu	*MAP3K7*: c.1454C>T, p.Pro485Leu	*MAP3K7*: c.1454C>T, p.Pro485Leu	*TAB2*: c.1705G>A, p.Glu569Lys	*MAP3K7:* c.208G>C p.Gly70Gln	*MAP3K7:* c.299T>A p.Val100Glu
Depth of coverage at disease causing variant (reads/total reads)	16/29	10/37	18/31	15/39	24/74	3/7

[a] See methods for rare variant filtering protocol
[b] No parental sequence available
[c] MAP3K7 NM_003188, c.1454C>T; PIKFYVE NM_001178000, c.977C>T
[d] MAP3K7 NM_003188, c.1454C>T; MAPK8IP2 NM_012324, c.2068G>A; NOA1 NM_032313, c.88C>G
[e] EFHC1 NM_018100.3, c.674C>G; TAB2 NM_015093.5, c.1705G>A
N.B. Data in this table is presented from sequential filtering steps