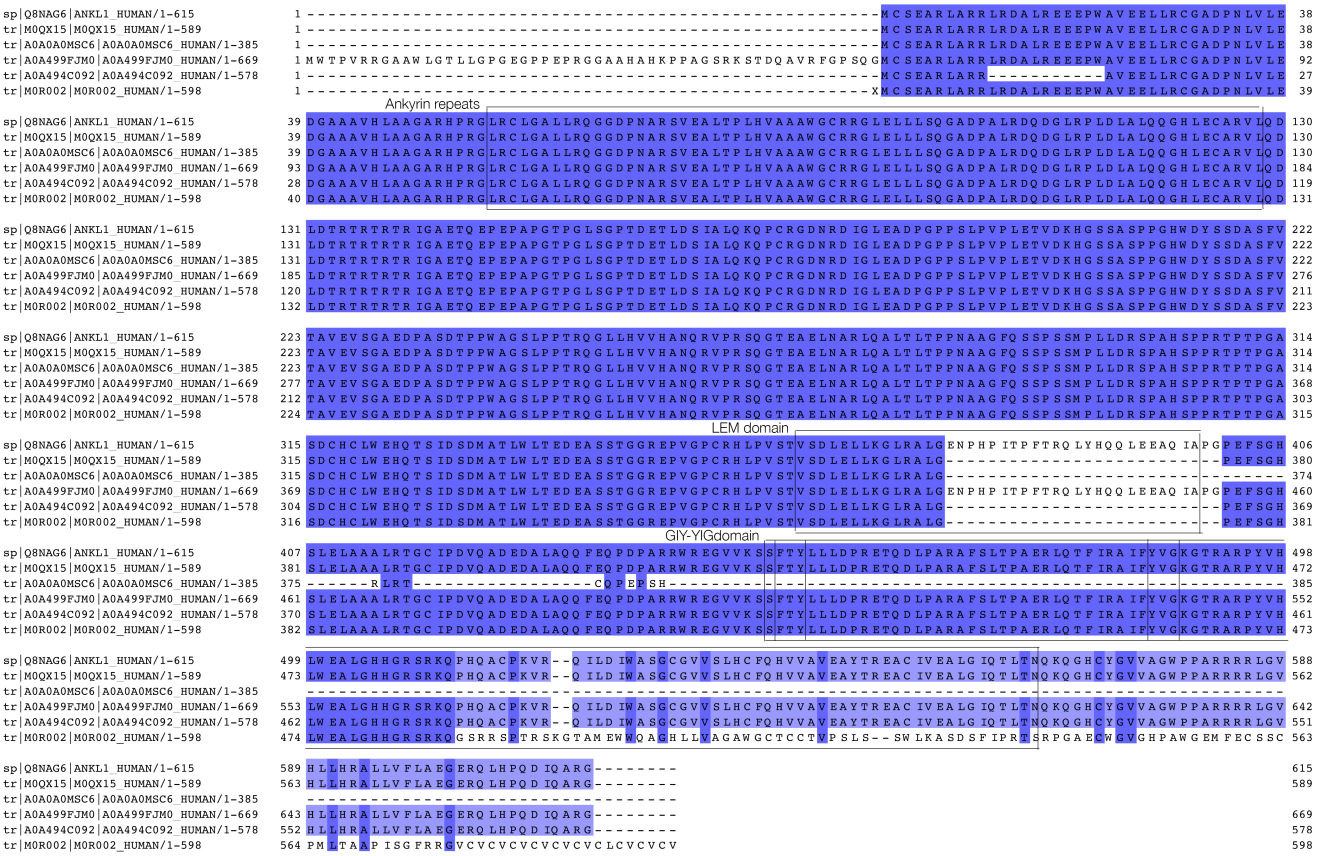
Human ANKLE1 is a nuclease specific for branched DNA

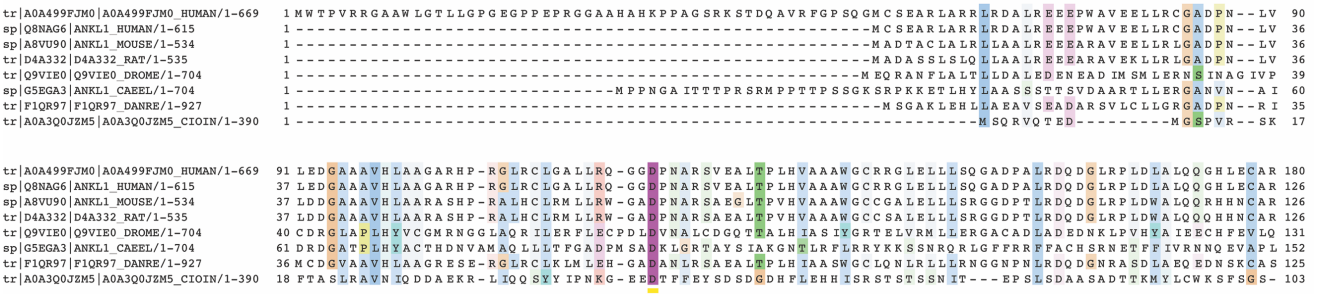
SUPPLEMENTARY INFORMATION

SUPPLEMENTARY FIGURES

A.



B

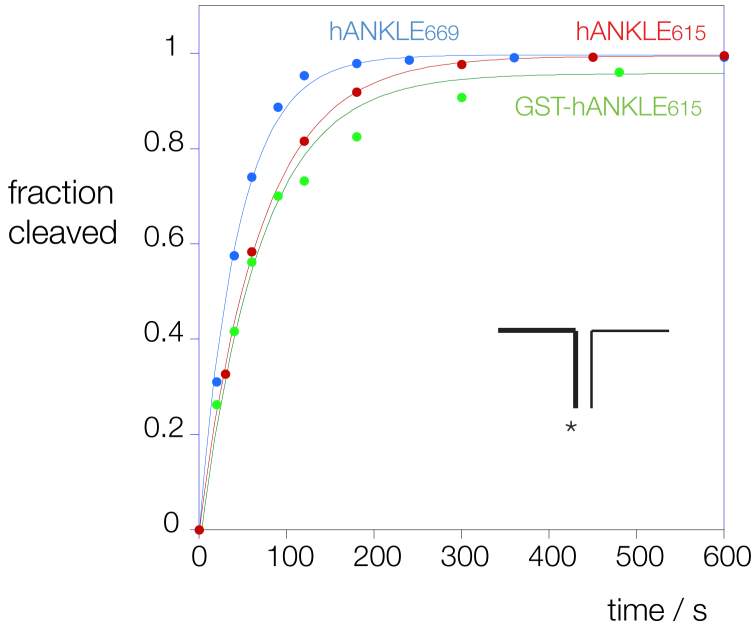


Appendix Figure S1. Alignment of human isoforms of ANKLE1.

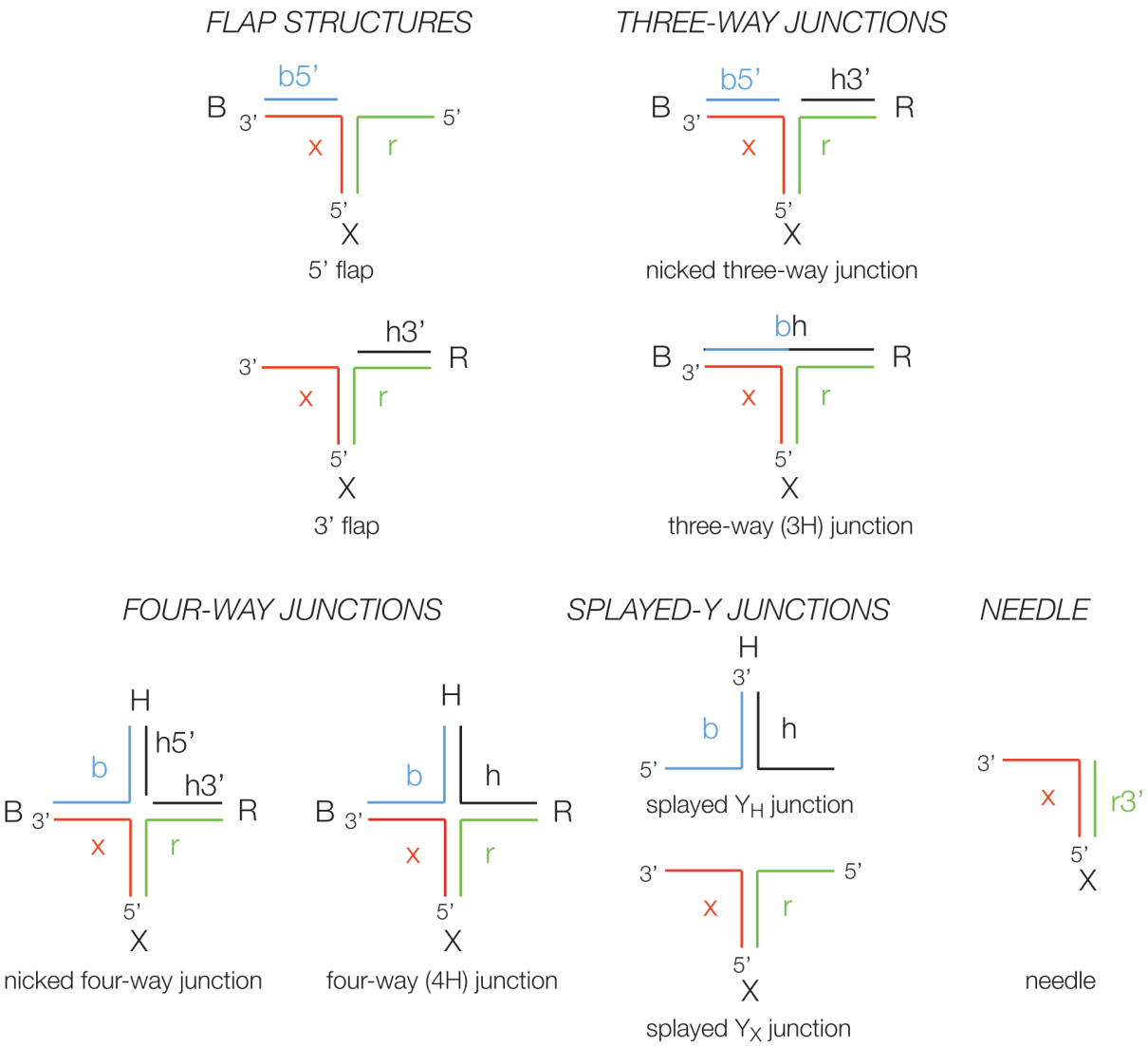
A. The sequences of six human ANKLE1 isoforms were obtained from the UniProt database. Multiple sequence alignment was performed using Clustal via Jalview. The alignment shows that two isoforms Q8NAG6 and A0A499FJM0 contain three functional domains highly conserved in ANKLE1 homologs in vertebrate, N-terminal Ankyrin repeats, LEM domain and a GIY-YIG domain (all boxed). A0A499FJM0 comprises 669 amino acids, and shares 100% sequence identity with Q8NAG6 (615 aa), except for an extension of 54 amino acids in its N-terminus.

B. Alignment of the N-terminal of ANKLE1 sequences from other species suggests the extra 54 amino acid residues in A0A499FJM0 are not present in all other species, from *C. elegans* to mouse. It therefore indicates that extra N-terminus sequence in Q8NAG6 may be not required activity. The majority of experiments here were performed using Q8NAG6, the 615 amino acid form.

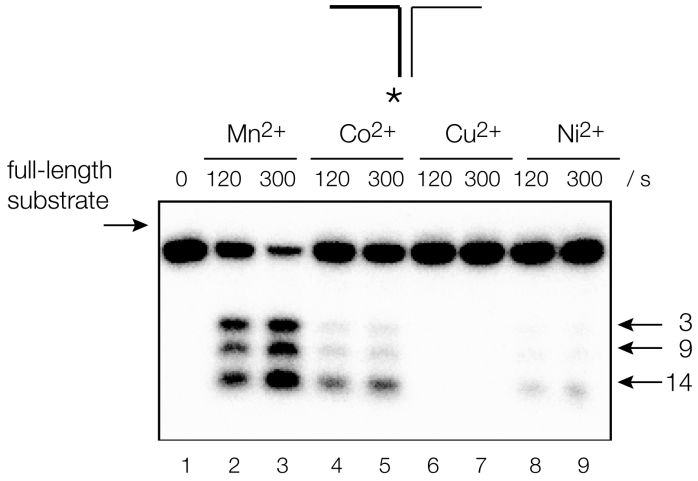
Appendix Figure S2. Mass spectrometric characterisation of human ANKLE1 expressed in insect cells. The band from the polyacrylamide gel shown in Figure 1B was excised and analysed by peptide fragmentation and mass spectrometry. Peptides matching the sequence of hANKLE1 are colored red; these correspond to 72% of the total hANKLE1 protein sequence.



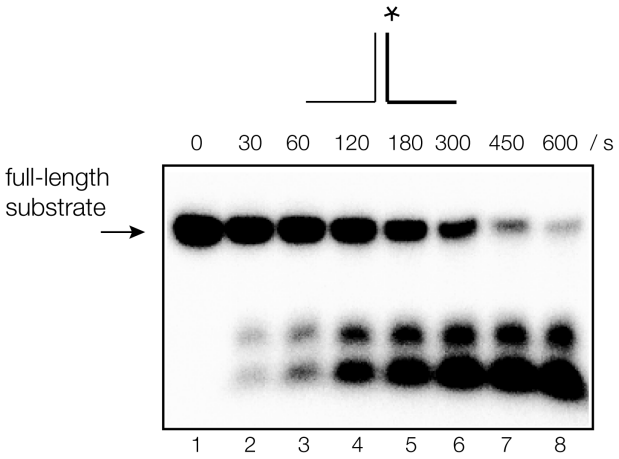
Appendix Figure S3. Reaction progress plotted for cleavage of splayed Y_X junction by three forms of human ANKLE1. The three forms are the 669 amino acid polypeptide (blue), and the 615 amino acid polypeptide with (green) and without (red) an N-terminal GST peptide. Each reaction proceeds with a similar rate and each achieved near-full conversion to cleaved product. Fitting to single exponential functions (lines) gives rates of unfused 615 aa $k_{obs} = 0.015\ s^{-1}$, GST-615 aa $k_{obs} = 0.015\ s^{-1}$, and 669 aa $k_{obs} = 0.022\ s^{-1}$. Reactions were performed under single-turnover conditions in 20 mM cacodylate (pH 6.5), 50 mM KCl, 2 mM $MnCl_2$, 0.1mg/ml BSA. The insert represents the structure of the Y_X junction, in the same manner as the depictions in Figure S4. In this and other figures the asterisk shows the labelled terminus.



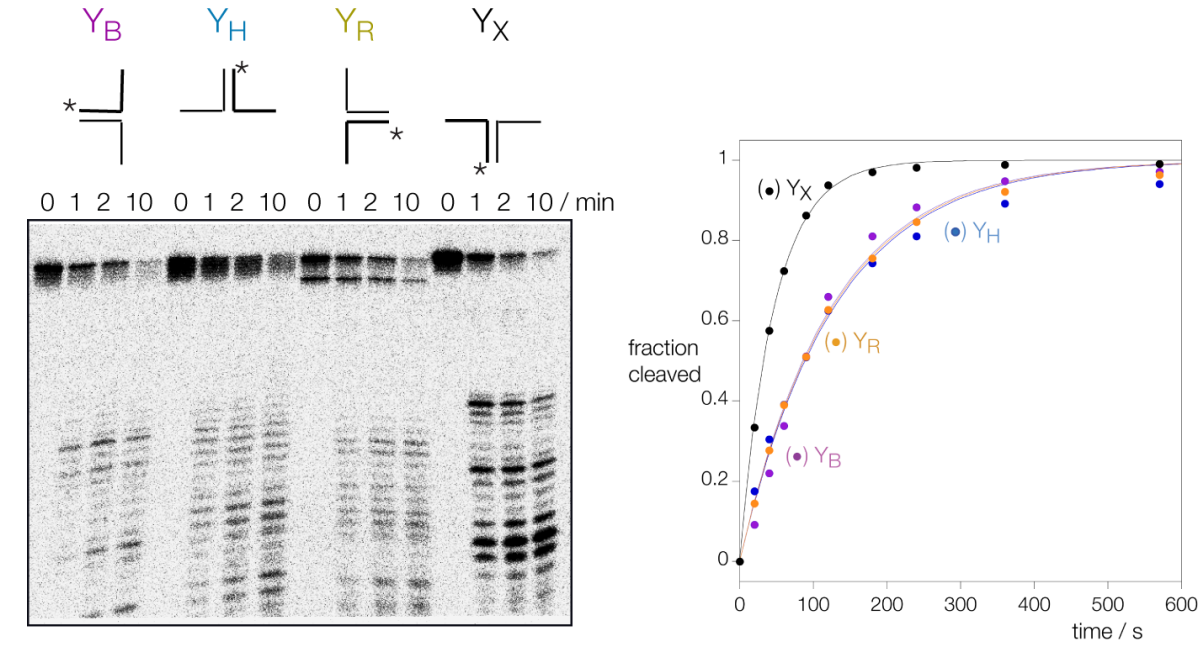
Appendix Figure S4. Relationship between the DNA junctions used as substrates for ANKLE1 in this work. All the junctions may be derived from the parental four-way junction 3, comprising four strands called b, h, r and x. These generate the four helical arms B, H, R and X named from the component 5' strand. The other junctions are generated by hybridising sub-sets of these strands, half-strands (e.g. *b5'* in the 5' flap structure) or composite strands (e.g. *bh* in the three-way junction). In the majority of the experiments we have used a radioactively-[5'-^{32}P] labelled x strand, shown red here. The sequences of the strands are tabulated in Table S1.



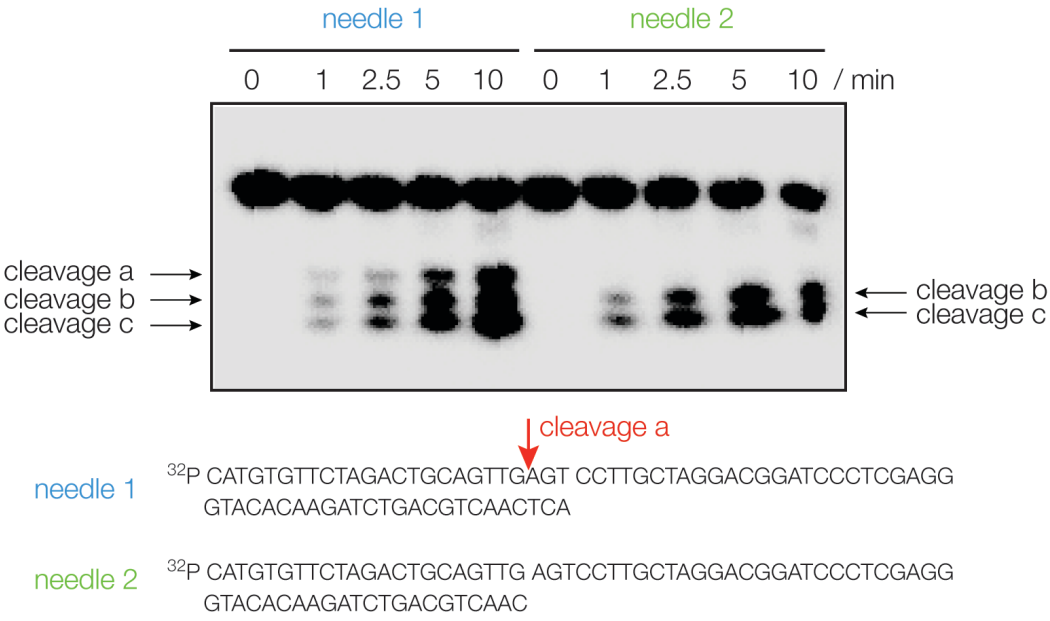
Appendix Figure S5. Cleavage of splayed Y_X DNA junction by human ANKLE1 as a function of the divalent metal ion present. Radioactively [5'-^{32}P]-labelled DNA was incubated with hANKLE1 in the presence of 20 mM cacodylate (pH 6.5), 50 mM KCl, 0.1mg/ml BSA and 2 mM indicated divalent metal ion chloride for 120 and 300 seconds. Very little activity was observed in the presence of Mg^{2+} or Ca^{2+} ions.



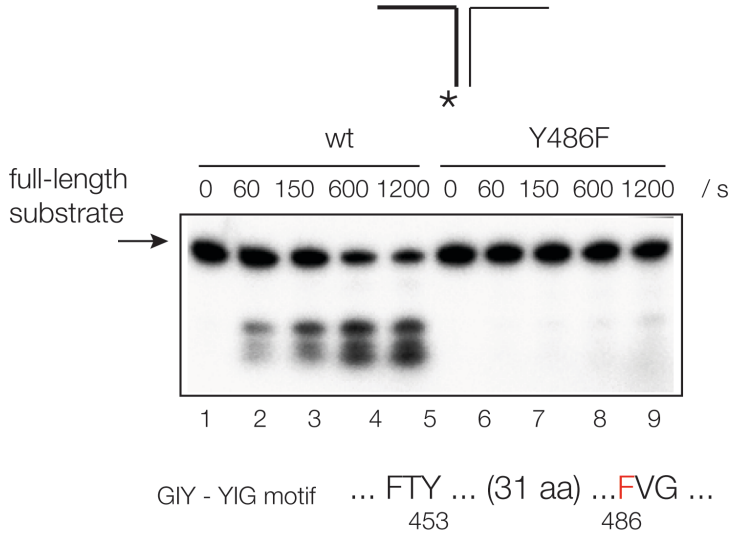
Appendix Figure S6. Cleavage of a different splayed-Y junction by human ANKLE1. The splayed Y_H junction is derived from the H arm of the four-way junction 3 (see Figure S4). Radioactively [5'-^{32}P]-r-strand labelled DNA was incubated with hANKLE1 under single-turnover conditions. Despite having a completely different sequence from the splayed Y_X DNA, it is nevertheless a good substrate for hANKLE1.



Appendix Figure S7. Comparison of the cleavage positions and rates for the four possible splayed Y-junctions derived from the four-way junction 3. Radioactively [5'-^{32}P]- labelled splayed Y-junctions were incubated with hANKLE1 for the indicated times and the positions of cleavage determined by polyacrylamide gel electrophoresis at high resolution (left). In a separate experiment the extent of cleavage of the four junctions was measured as a function of time. The data were plotted and fitted to single exponential functions (right).



Appendix Figure S8. Comparison of cleavage position of two needles that differ in the position of the junction between double- and single-stranded DNA. The lower strand of needle 2 is 3 nt shorter than that of needle 1, so that the position of the junction moves 3 nt left-wards in needle 2. Radioactively [5'-^{32}P]- labelled needles were incubated with hANKLE1 for the indicated times and the positions of cleavage determined by polyacrylamide gel electrophoresis. Note that cleavage occurs at site **a** in needle 1, but the same sequence in needle 2 is uncleaved.



Appendix Figure S9. Mutation of tyrosine 486 in the GIY-YIG motif prevents cleavage of a splayed-Y_X DNA junction by human ANKLE1. This experiment was performed using a GST-hANKLE1 (615 amino acid) fusion. Radioactively [5'-^{32}P]-labelled splayed Y_X junction DNA was incubated with hANKLE1 under single-turnover conditions.

SUPPLEMENTARY TABLES

b-strand :
CCTCGAGGGATCCGTCCTAGCAAGG GGCTGCTACCGGAAGCTTACAGATG

h-strand :
CATCTGTAAGCTTCCGGTAGCAGCC TGAGCGGTGGTTGAATTCACAGATG

r-strand :
CATCTGTGAATTCAACCACCGCTCA ACTCAACTGCAGTCTAGAACACATG

x-strand :
CATGTGTTCTAGACTGCAGTTGAGT CCTTGCTAGGACGGATCCCTCGAGG

bh-strand :
CCTCGAGGGATCCGTCCTAGCAAGG TGAGCGGTGGTTGAATTCACAGATG

b5'-strand :
CCTCGAGGGATCCGTCCTAGCAAGG

h5'-strand :
CATCTGTAAGCTTCCGGTAGCAGCC

h3'-strand :
TGAGCGGTGGTTGAATTCACAGATG

r3'-strand :
ACTCAACTGCAGTCTAGAACACATG

Complementary r-strand (used to generate double-stranded DNA) :
CATCTGTGAATTCAACCACCGCTCAGGCTGCTACCGGAAGCTTACAGATG

Appendix Table S1. The sequences of the oligonucleotides used in the construction of the hANKLE1 substrates studied in this work. All sequences are written 5' to 3'. A gap has been left at the point of strand exchange a junction is constructed from a combination of these oligonucleotides. The combinations of strands used to generate the different species are shown in Figure S4.

plasmid	vector	description	UniProt accession number
JF1	pKL	GST tagged hANKLE1669aa	A0A499FJM0
JF2	pKL	GST tagged hANKLE1615aa	Q8NAG6
JF3	pKL	GST tagged hANKLE1615aa with a point mutant, Y453F	Q8NAG6
JF4	pKL	GST tagged hANKLE1615aa with a point mutant, Y486F	Q8NAG6

Appendix Table S2. The plasmids used to express ANKLE1 in these studies.