

Table S1A: Oligonucleotides used for subcellular localization and antisense construct creation.

	Sequence (5' – 3')	Construct
TMTS 5.1	GGATCCATCATGATTTTCAATGTCG	Subcellular localization
TMTS 3.1	CCACTAGTTGATGATGATGTTGTTGG	Subcellular localization
TAS 5.1	GAATTCATCGATATCATGATTTTCAATGTCG	Antisense
TAS 3.1	CTGCAGATCGATTGTTGTAGATTGTGGTGATG	Antisense

Table S1B: Oligonucleotides used for qPCR.

	Sequence (5' – 3')
<i>twm1</i> q5.1	GTTGGTGGTGAACCTGAAAATAATC
<i>twm1</i> q3.1	CTGCAGATCGATTGTTGTAGATTG
<i>twm1</i> q5.2*	GGTATGGAAGGTGCTGCAAAATTCTC
<i>twm1</i> q3.2*	GCATCGTTTGCATCTTTTGGACC
<i>tubB</i> q5.1	GCCAAAGGTGCCTCGTCATACAATC
<i>tubB</i> q3.1	ATTGTGTGGATCGGAAGCAGCCATC
<i>rns</i> q5.1	AGTTTGACTGACAGTTGGCGAAGGC
<i>rns</i> q3.1	TCAGACTACTCGGGTCTCTAATCCG
<i>talB</i> q5.1	TGGGCGAAACAGCAGAGCAACG
<i>talB</i> q3.1	TTGAGATACGGCCCGGAACCAG
<i>kanR</i> q5.1	CCACCATGATATTCGGCAAGCAG
<i>kanR</i> q3.1	CTCGCGCCAGCCGAACCTG

* Primers used for *twm1* mRNA quantification in antisense transformants

Table S1C: Oligonucleotides used for fluorescent dsDNA substrates.

	Sequence (5' – 3')
FHA5.1	[6-FAM]GTAAAACGACGGCCA
FHA5.2	[6-FAM]GTAAAACGACGGCCATTTTTTTTTTTTTCG
FHA3.1	TGGCCGTCGTTTTAC
FHA3.2	ACCTGTTTGGTGTGTGGCCGTCGTTTTAC
FHA3.3	TGGCCGTCGTTTTACGTTGTGGTTTGTCCA
FHA3.4	CAACACCAAACAGGT
FHA3.5	CGCAAAAAAAAAAAAA
FHA3.6	TGGACAAACCACAAC

Table S1D: dsDNA substrates created for fluorescent helicase assays.

	Primer 1*	Primer 2	Primer 3	dsDNA feature	Figure
FHA0	FHA5.1	FHA3.1	N/A	None	Fig. 6A
FHAOF	FHA5.2	FHA3.2	N/A	Open fork	Fig. 6A
FHAOF5	FHA5.2	FHA3.2	FHA3.4	Open fork with duplex 5' overhang	Fig. 6B
FHAOF3	FHA5.2	FHA3.2	FHA3.5	Open fork with duplex 3' overhang	Fig. 6B
FHA5	FHA5.1	FHA3.2	N/A	5' overhang	Fig. 6C
FHA3	FHA5.1	FHA3.3	N/A	3' overhang	Fig. 6C
FHA3D	FHA5.1	FHA3.3	FHA3.6	Duplex 3' overhang	Fig. 6C

* Includes 5' 6-FAM label