

Supplemental Materials

Molecular Biology of the Cell

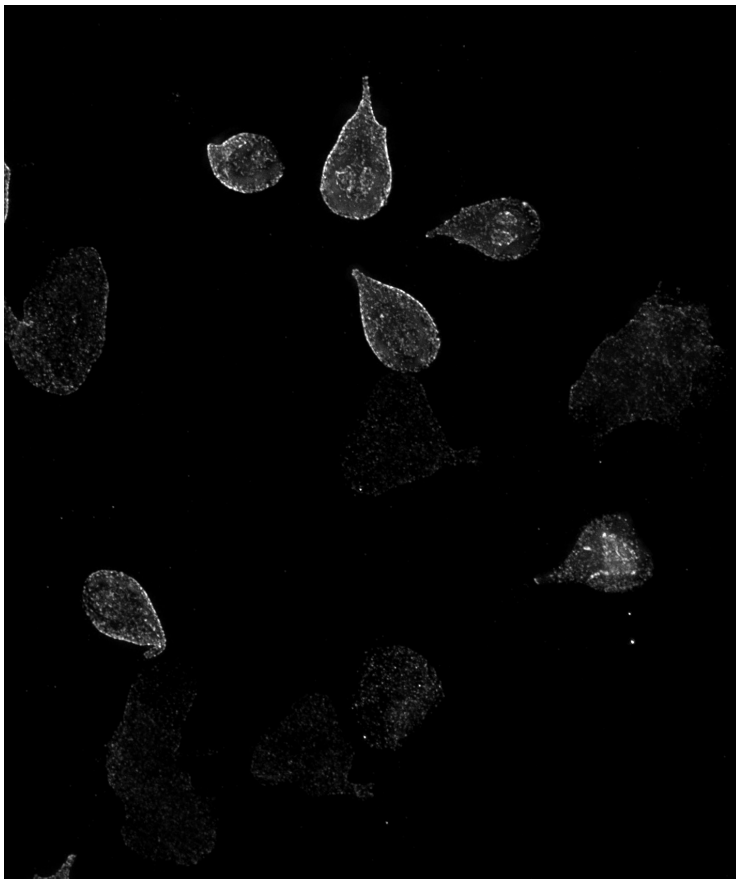
Hennessey et al.

Figure S1 Phenotype of Nek8445 knockdown cells at 48 hours post morpholino treatment. Nek8445-HA stained in red, tubulin in green (6-11B-1 antibody), and nuclei in blue. Asterisks mark six cells with abnormal nuclear number and altered cell shape. These defects correlate with reduced Nek8445 levels as indicated by staining intensity. The presence of cells with more than 4 nuclei indicates that although these cells failed cytokinesis they continue to proceed through mitosis. Scale bar= 10 μm .

Figure S2 Start point for long-term DIC movies. (A) Few cells are able to attach to the cover glass at 22 hours post Nek8445 knockdown. (B) Quantification of multinucleate (>2 nuclei) cells that are either in the process of dividing or have failed to divide (Control Morpholino=6.4%, KD=51.9%; n=2400 for each treatment). (C) Control morpholino treated cells at 12 hours post electroporation. (D) Nek8445 antisense morpholino-treated cells 12 hours post knockdown. Scale bar= 5 μm .

Figure S3. Rab11 and Nek8445 localization throughout the cell cycle. HA-Rab11 in cyan, Nek8445-mNG in red, and tubulin (6-11B-1 antibody) in green. During mitosis Nek8445 is associated with the nuclear envelope and loads onto cytoplasmic axonemes. HA-Rab11 also loads onto cytoplasmic axonemes at specific times during mitosis, but Rab11 and Nek8445 are not frequently observed to co-localize. White arrowhead shows an example of HA-Rab11 and Nek8445-mNG co-localization on the cytoplasmic axonemes of the posterolateral flagella. Scale bar= 5 μm .

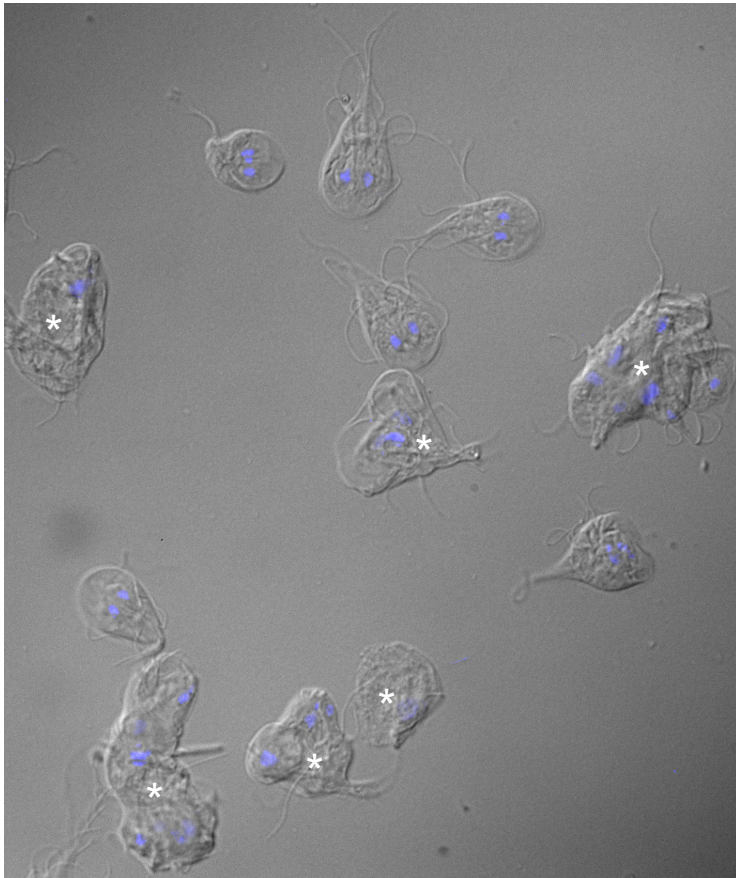
Nek8445-HA



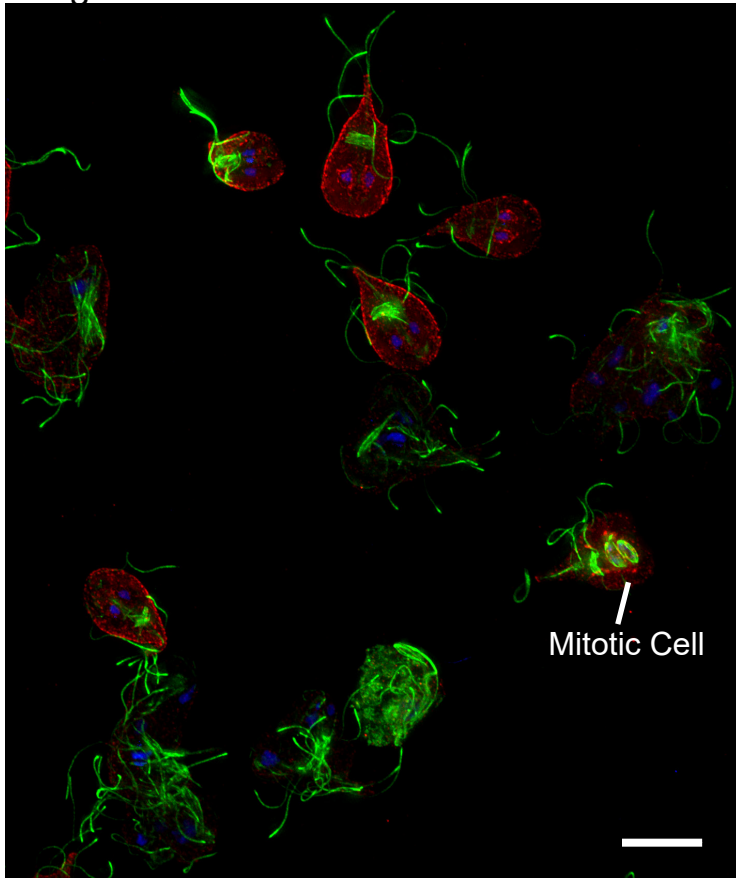
Tubulin

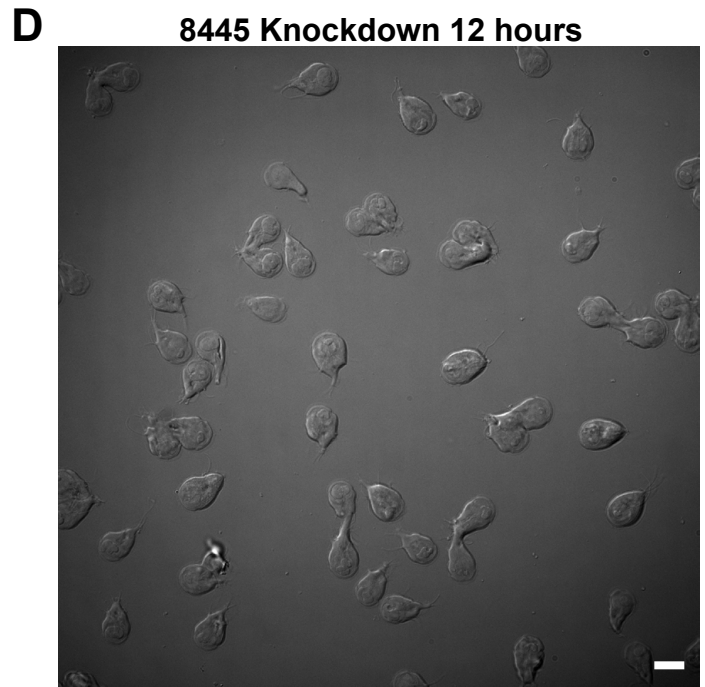
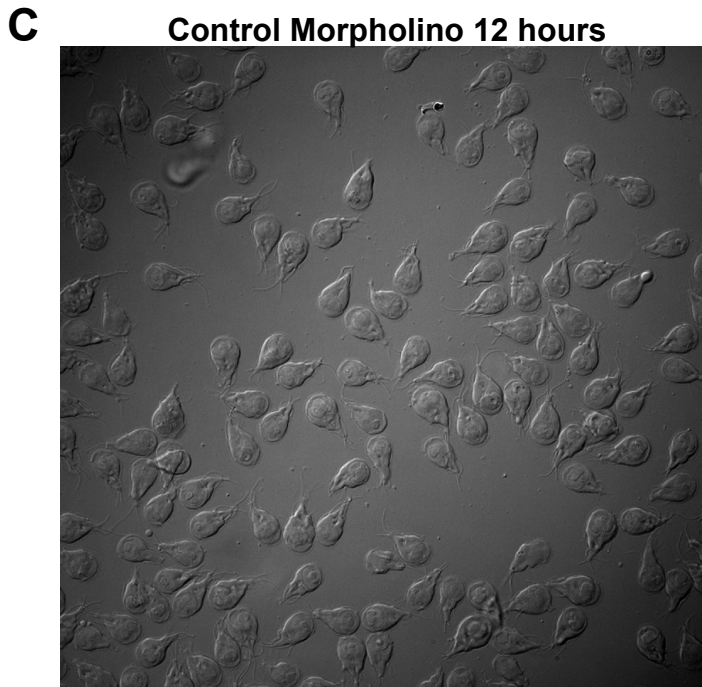
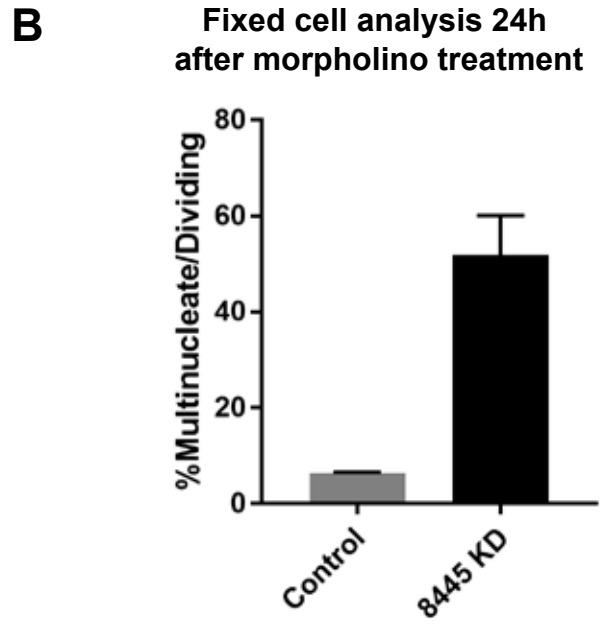
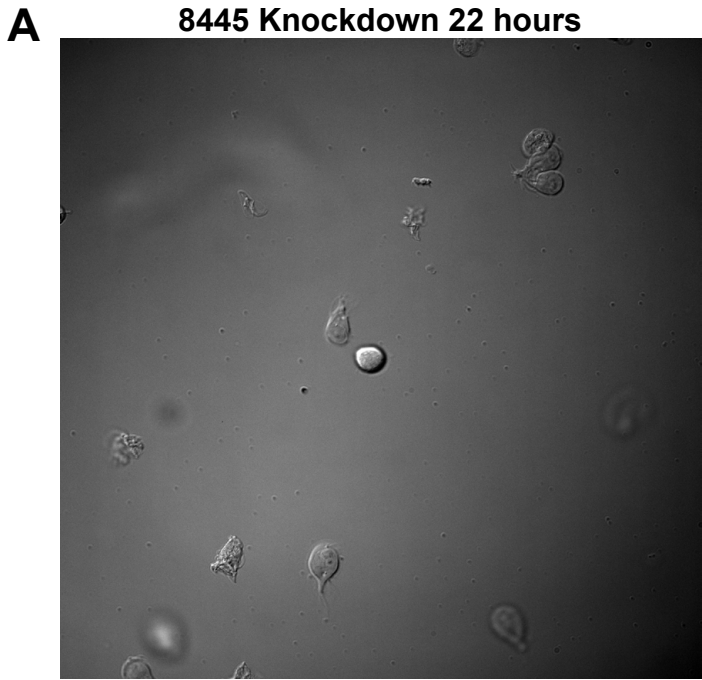


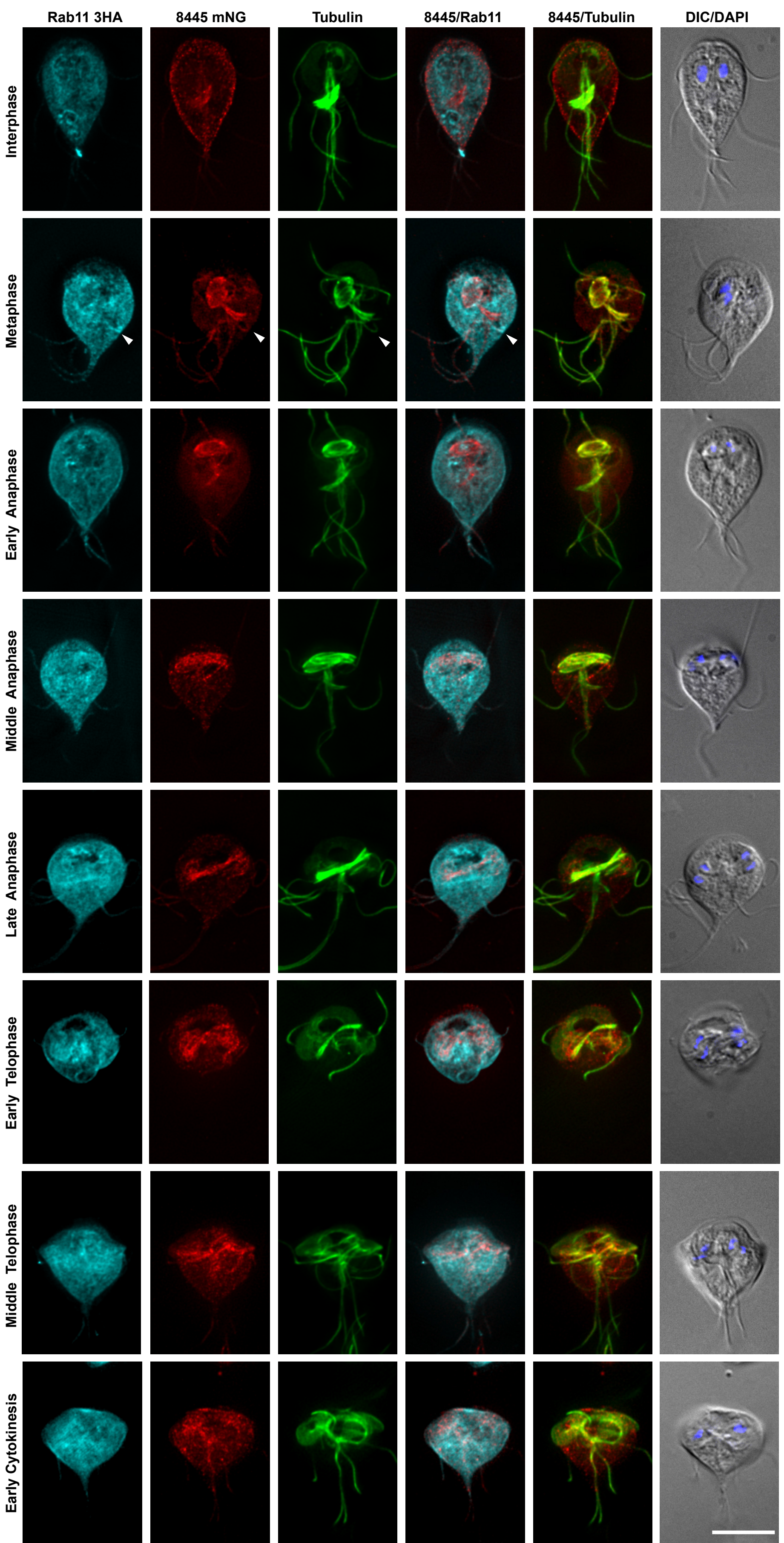
DIC/DAPI



Merge







Interphase

Metaphase

Early Anaphase

Middle Anaphase

Late Anaphase

Early Telophase

Middle Telophase

Early Cytokinesis

Rab11 3HA

8445 mNG

Tubulin

8445/Rab11

8445/Tubulin

DIC/DAPI

▲

▲

▲

▲

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PRIMER NAME	RESTRICTION ENZYME	5' TO 3' SEQUENCE
8445_VSVG_Neo R	AgeI	TTCAATATCAGTATAaccggtcttgtttagtctgtagccat
8445_VSVG_Neo F	NotI	tggagctccaccgcggtggcggccgcCATAATCTTGGCGTCCTTCG
8445_mNG_Neo F	NotI	ctccaccgcggtggcggccgcCATAATCTTGGCGTCCTTCG
8445_mNG_Neo R	BamHI	AACCGCCTCCGGATCCCTTGTTGTTAGTCTGTAGCC
8855_3HA_Neo F	SpeI	cggtggcggccgctctagaactagtTGTTGAGGTATCTGGGGATATT
8855_3HA_Neo R	AflII	gtcataaggatattcctaagAATATCAAAATCGTCGAAATCACC
pks_mNG_NEO_F	BamHI	cggccgctctagaactagtggatccGGAGGCGGTTTCAGGCGGAGG TGGCTCTatggtgagcaagggcgagga
pks_mNG_NEO_R	EcoRI	Gcaacgatgatgcaaagaattcttactgtacagctcgtccatg
Morpholino		
8445		CTGCCAGCTTCTCCGCAATACCCAT

Table S1 Oligo sequences used in this study