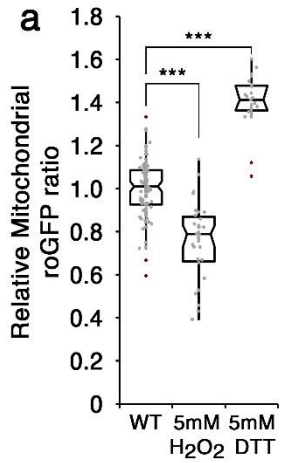
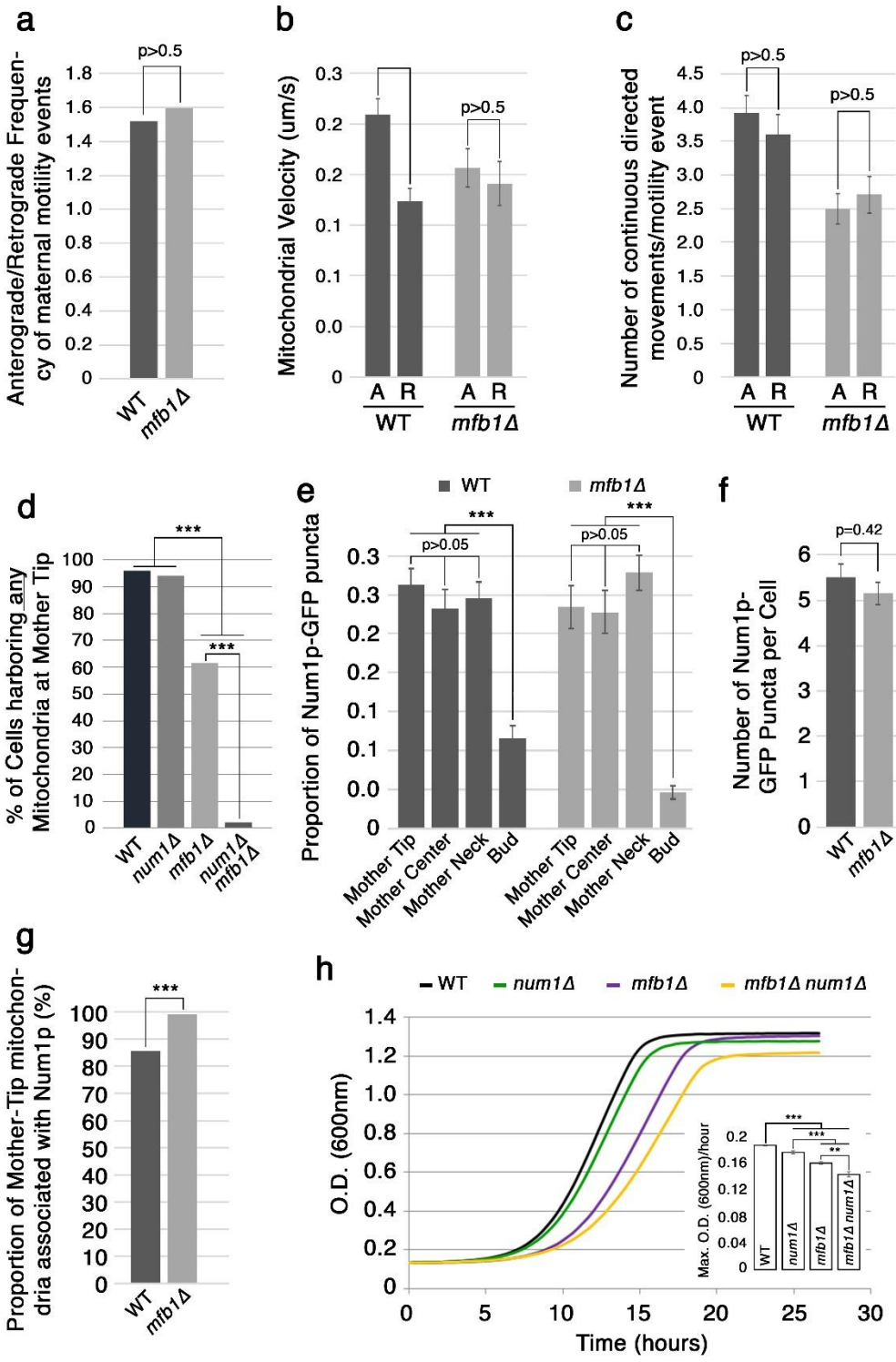
Supplementary Figures:

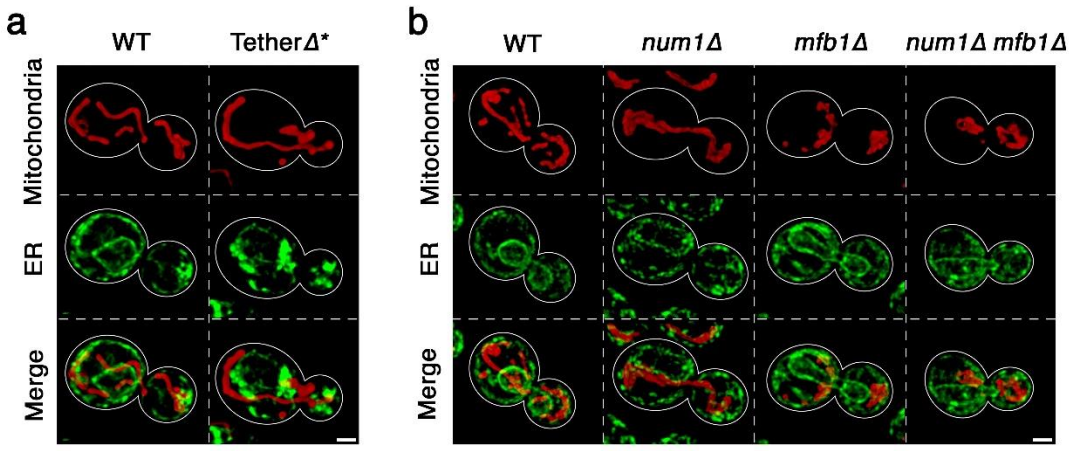


Supplementary Figure 1: roGFP allows ratiometric assessment of mitochondrial redox state a) Notched box plot of the average reduced/oxidized mito-roGFP1 ratio in WT cells treated with H_2O_2 (5 mM) or DTT (5 mM) relative to untreated cells. n > 40 cells per condition. The central band in the box represents the median, boxes indicate the middle quartiles, and whiskers extend to the 5^{th} and 95^{th} percentiles; red dots indicate data points beyond this range. Statistical significance was determined using the Student's *t*-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$.

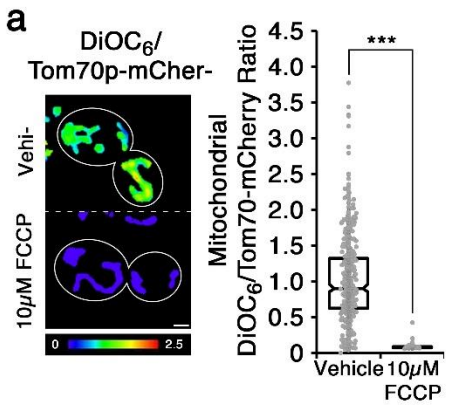


Supplementary Figure 2: Independent function of Num1p and Mfb1p a-c. To test for changes in motility upon deletion of MFB1 that could explain the lack of mitochondrial accumulation at the mother tip (either increased anterograde trafficking or decreased retrograde trafficking), **a)** ratio of frequency of anterograde events to that of retrograde events, **b)** velocity and **c)** processivity of anterograde and

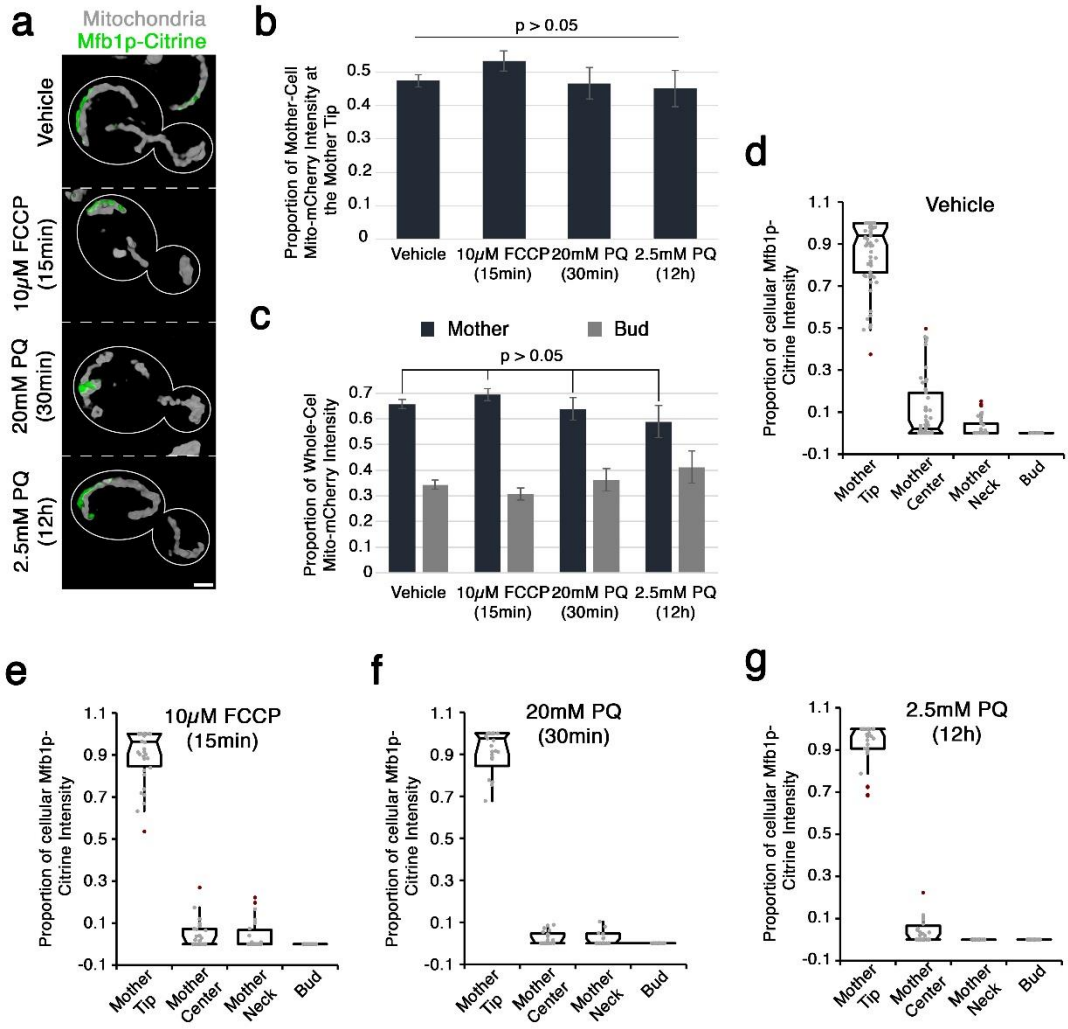
retrograde mitochondrial motility events were measured in cells expressing Cit1p-mCherry. Z-series were collected every 1 s for 30 s and analyzed as described in Experimental Procedures. **d)** The prevalence of mitochondrial localization to the mother tip was quantified in WT, *num1Δ*, *mfb1Δ* and *num1Δ mfb1Δ* cells expressing Cit1p-mCherry (n > 80). **e)** Relative Num1p-puncta distribution in WT and *mfb1Δ* cells expressing Num1p-GFP in 3 areas of the dividing mother cell (see Fig.1b) and the bud. **f)** Total number of Num1p-GFP puncta per cell. **g)** Proportion of WT and *mfb1Δ* cells displaying Cit1p-mCherry at the mother tip in which in which colocalized Num1p-GFP puncta were also observed (n > 80). **d-g.** Data is representative of three independent experiments. **h)** Growth of WT, *num1Δ*, *mfb1Δ* and *num1Δ mfb1Δ* cells in YPD medium at 30°C. Maximum growth rates were calculated for intervals of 2h. Error bars represent standard errors of the mean. Statistical significance was determined using Fisher's exact test (d,g) or Student's *t*-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$.



Supplementary Figure 3: Mitochondrial tethering occurs independent of cER a) Mitochondria accumulate at the mother tip despite loss of of cortical ER. Representative 3D renderings of WT and TetherΔ (*Ist2Δ, Tcb1/2/3Δ, Scs2Δ and Scs22Δ*) cells expressing Cit1p-mCherry (mitochondria) and Pho88-CFP and Pho88-YFP (ER) respectively. **b)** Cortical ER is present at mother cell retention sites in m*fb1Δ* and *num1Δ* cells even when mitochondria fail to accumulate there. Representative 3D renderings of WT, *num1Δ*, *mfb1Δ* and *num1Δ mfb1Δ* cells expressing Cit1p-mCherry (mitochondria) and Pho88p-GFP (ER). **a-b.** Cell outlines are shown in white; scale bars = 1 µm.



Supplementary Figure 4: The $DiOC_6$/Tom70p-mCherry ratio allows assessment of mitochondrial membrane potential a) Mid-log-phase cells expressing Tom70p-mCherry were labeled with $DiOC_6$ as described in Experimental Procedures. To determine the sensitivity of the $DiOC_6$/Tom70p-mCherry ratio to mitochondrial membrane potential, cells were treated with 10 µM FCCP or vehicle (DMSO). Panels show representative images and quantitation of the whole-cell mitochondrial $DiOC_6$/Tom70p-mCherry ratio ($n > 40$); warmer colors and higher numbers indicate higher mitochondrial membrane potential. The central band in the box represents the median, boxes indicate the middle quartiles, and whiskers extend to the 5^{th} and 95^{th} percentiles; red dots indicate data points beyond this range. Statistical significance was determined using the Wilcoxon Rank-Sum test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$. Cell Outlines are shown in white.



Supplementary Figure 5: Mfb1p localization is not sensitive to oxidative stress or loss of mitochondrial membrane potential a-g . Cells expressing Tom70p-mCherry and Mfb1-Citrine were grown to mid-log phase, treated with vehicle or drug, and visualized by fluorescence microscopy. **a)** Representative 3D renderings of mitochondria and Mfb1p-Citrine after treatment with FCCP or acute or chronic paraquat (PQ). Cell outlines are shown in white. Scale bars = 1 µm. **b)** Cit1p-mCherry intensity at the mother tip, expressed as a proportion of the total intensity in the mother cell. **c)** Cit1p-mCherry intensity in mother and daughter, expressed as a proportion of the total intensity in the entire cell (mother + daughter), after FCCP and PQ treatment. Error bars indicate standard errors of the mean (n > 40 for each condition). **d-g.** Relative distribution of Mfb1p-Citrine after FCCP and PQ treatment (n > 40 for each condition). The central band in the box represents the median, boxes indicate the middle quartiles, and whiskers extend to the 5th and 95th percentiles; red dots indicate data points beyond this range.Statistical significance was determined using Student's *t*-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$.

Supplementary Tables:

Supplementary Table 1: Strains used in this study

Strains	Genotype	Source
BY4741	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Open Biosystems
WPY0005	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ1 CIT1-mCherry-hphMX4*	This Study
WPY0006	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ2 CIT1-mCherry-hphMX4 mfb1Δ::kanMX6*	This Study
WPY0007	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ3 CIT1-mCherry-hphMX4 mmr1Δ::LEU2*	This Study
WPY0008	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ5 CIT1-mCherry-hphMX4 mfb1Δ::kanMX6 mmr1Δ::LEU2*	This Study
WPY0013	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ5 CIT1-mCherry-hphMX4 PHO88-GFP(S65T)-KanMX6*	This Study
WPY0014	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ5 CIT1-mCherry-hphMX4 PHO88-GFP(S65T)-KanMX6 mfb1Δ::LEU2*	This Study
WPY0023	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ5 CIT1-mCherry-hphMX4 PHO88-GFP(S65T)-KanMX6 num1Δ::LEU2*	This Study
WPY0024	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ5 CIT1-mCherry-hphMX4 PHO88-GFP(S65T)-KanMX6 num1Δ::LEU2 mfb1Δ::URA3*	This Study
WPY0015	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ9 [pMito-roGFP1:URA3]*	This Study
WPY0016	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ10 mfb1Δ::LEU3 [pMito-roGFP:URA3]*	This Study
WPY0017	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ10 mmr1Δ::LEU3 [pMito-roGFP:URA3]*	This Study
WPY0018	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ10 mmr1Δ::LEU3 mfb1Δ::KanMX6 [pMito-roGFP:URA3]*	This Study
JVY:66	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ10 CIT1-mCherry-hphMX4 MFB1-GFP-KanMX6*	This Study
WPY0048	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ10 CIT1-mCherry-hphMX4 MFB1-GFP-KanMX6 num1Δ::LEU3*	This Study
WPY0105	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ10 CIT1-mCherry-hphMX4 NUM1-GFP-KanMX6*	This Study
WPY0106	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ10 CIT1-mCherry-hphMX4 NUM1-GFP-KanMX6 mfb1Δ::URA3*	This Study
WPY0067-1	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ17 TOM70-mCherry-hphMX4*	This Study
WPY067	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ17 TOM70-mCherry-hphMX4 mfb1Δ::KanMX6*	This Study
WPY0068	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ17 TOM70-mCherry-hphMX4 mmr1Δ::LEU2*	This Study
WPY0069	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ17 TOM70-mCherry-hphMX4 mfb1Δ::KanMX6 mmr1Δ::LEU2*	This Study
SEY6210.1	*MAT**a** leu2-3,112 ura3-52 his3-Δ 200 trp1-Δ901 lys2-801 suc2-Δ9*	Robinson et al., 1988

ANDY201	*SEY6210.1 SEC61-GFP::TRP1 ist2Δ::HISMX6 scs2Δ::TRP1 scs22Δ::HISMX6 tcb1Δ::KANMX6 tcb2Δ::KANMX6 tcb3Δ::HISMX6*	Manford et al., 2012
WPY0031	*MAT**a** leu2-3,112 ura3-52 his3-Δ 200 trp1-Δ901 lys2-801 suc2-Δ9 CIT1-mCherry-hphMX4 PHO88-CFP-LEU2*	This Study
WPY0032	*SEY6210.1 SEC61-GFP::TRP1 ist2Δ::HISMX6 scs2Δ::TRP1 scs22Δ::HISMX6 tcb1Δ::KANMX6 tcb2Δ::KANMX6 tcb3Δ::HISMX6 CIT1-mCherry-hphMX4 PHO88-YFP-LEU2*	This Study

Supplementary Table 2: Primers used in this study

Name	**Sequence**
FW *MFB1* deletion	CCAACACAGTCTTCATACACTATTATTATTCATTTTATGGcggatccccgggttaattaa
RV *MFB1* deletion	CGTATAGTAGCTCTTTTTTTGTATCGATTTATAAAAATGCgaattcgagctcgtttaaac
FW *MFB1* deletion POM	CCAACACAGTCTTCATACACTATTATTATTCATTTTATGGacgctgcaggtcgacaaccc
RV *MFB1* deletion POM	CGTATAGTAGCTCTTTTTTTGTATCGATTTATAAAAATGCttaagggttctcgagagctc
FW *MMR1* deletion pOM	AAAAAAAAAAACACAACTAATAAACTAAACAACTAAAAAAacgctgcaggtcgacaaccc
RV *MMR1* deletion pOM	GTTTGTGTAAAATAAGTTAATTTAATTTGAAGTTGACGCTttaagggttctcgagagctc
FW *NUM1* deletion pOM	CTAATAGGACCACAGGGTTGAATAGAGACGAGTAAAGACGacgctgcaggtcgacaaccc
RV *NUM1* deletion pOM	TACTCATCGGTGGCAAACGTGTTTACAGGACTAAAAATCGttaagggttctcgagagctc
FW *PHO88* tag	AGAAGCTGAAAGAGCCGGTAACGCTGGTGTTAAGGCTGAAggtgacggtgctggttta
RV *PHO88* tag	TTCTATGGCGATGTAGGAAAATAGACACAATTCGTCTAGCatcgatgaattcgagctcg
FW linker-YFP pWJ1863	GGTGACGGTGCTGGTTTAATTAACAGTatgagtaaaggagaagaacttttcactgg
FW nested *PHO88*-tag pWJ1863	AGAAGCTGAAAGAGCCGGTAACGCTGGTGTTAAGGCTGAAggtgacggtgctggtttaattaac agt
RV *PHO88* tag	AAAACTAGGAAAAAAAAATACTTCGCTTTTGATCGAATCAatcgatgaattcgagctcg
FW *NUM1*-tag pFA6	ACATAGAGTACCACAAAGCCGATCATTTGGCAATTTACGAcggatccccgggttaattaa
RV *NUM1*-tag pFA6	CATATTTATTTCAGTCACAAAACAAAATTAAAGAATTCGTgggcagatgatgtcgagg
FW *MFB1*-tag pFA6	TGTAATCAAACGGCTTGACGCTAATACCGATTTTAATATAcggatccccgggttaattaa

RV *MFB1*-tag pFA6	CGTATAGTAGCTCTTTTTTTGTATCGATTTATAAAAATGCgaattcgagctcgtttaaac
FW *MFB1*-Citrine tag	TGTAATCAAACGGCTTGACGCTAATACCGATTTTAATATAggtgacggtgctggttta
RV *MFB1*-Citrine tag	CGTATAGTAGCTCTTTTTTTGTATCGATTTATAAAAATGCatcgatgaattcgagctcg
FW *CIT1* tag	AAAATACAAGGAGTTGGTAAAGAAAATCGAAAGTAAGAACcggtgacggtgctggttta
RV *CIT1* tag	TTTGAATAGTCGCATACCCTGAATCAAAAATCAAATTTTCCcatcgatgaattcgagctcg
FW *TOM70* tag	TCAAGAAACTTTAGCTAAATTACGCGAACAGGGTTTAATGcggtgacggtgctggttta
RV *TOM70* tag	TTGTCTTCTCCTAAAAGTTTTTAAGTTTATGTTTACTGTcatcgatgaattcgagctcg