

699 **SUPPORTING INFORMATION**

700 Additional supporting information for this file includes ten Supplemental Figures and one
701 supplemental table.

702 **Supplemental Figure 1.** MCF reproducibly pulls down native ARF1.

703 HEK293T cells were transfected with dt-cMCF^{CA} in triplicate. For each replicate, MCF was
704 immunoprecipitated from lysate using anti-HA beads and analyzed by Western blot. dt-cMCF^{CA}
705 was detected by anti-HA and endogenous ARF1 detected by anti-ARF1 antibodies.

706

707 **Supplemental Figure 2.** MCF does not cleave or directly modify ARF1 or ARF3.

708 A-G. Bottom-up mass spectrometry was performed on ARF1 and ARF3 samples recovered from
709 recombinant proteins incubated with (A and B), or anti-Myc IPs (C-G) from HEK293T cells
710 transfected with and without MCF or MCF^{CA}. Modifications that were detected are denoted. Any
711 modifications found on either ARF1 or ARF3 were not reproducible across replicates.
712 Furthermore, there were no modifications detected that were found in ARF + MCF samples that
713 were not found in ARF samples alone and thus not attributable to MCF.

714

715 **Supplemental Figure 3.** Edman degradation is blocked in MCF recovered from cells.

716 A. Reads from Edman degradation analysis completed on dt-fIMCF immunoprecipitated from
717 HEK293T cell lysate using anti-HA antibody. No signal and very little background was detected.

718 B. Gel of sample used for analysis.

719

720 **Supplemental Figure 4.** Mass Spectrometry analysis shows MCF is acetylated inside cells.

721 Representative mass spectrometry spectra for each modification detected per population in the
722 replicates of the two-dimensional analysis of dt-fIMCF expressed in HEK293T cells recovered
723 by anti-HA IP shown in Supplemental Fig. 5. Asterisk above peptide sequence denotes residue
724 acetylated in that particular spectra.

725

726 **Supplemental Figure 5.** MCF is N-terminally acetylated inside host cells.

727 Replicates of two-dimensional gel analysis of dt-fIMCF expressed in HEK293T cells, recovered
728 by anti-HA immunoprecipitation. Amino acids acetylated in each circled population indicated in
729 corresponding color on sequence. Representative mass spectrometry data for the acetylations
730 shown in Supplemental Fig. 4.

731

732 **Supplemental Figure 6.** Golgi staining is more diffuse in cells expressing MCF.

733 A. Area in μm^2 of Golgi staining by IF measured for individual Cos7 cells ectopically expressing
734 the specified vector. B. The Golgi of Cos7 cells intoxicated with *V. vulnificus* strains was scored
735 on a scale of 1-3 for extent of dispersal. Images representative of the amount of dispersion each
736 score signifies are shown.

737

738 **Supplemental Figure 7.** MCF does not alter normal endoplasmic reticulum structure.

739 Cos7 cells were transfected with empty vector or MCF-EGFP (green) for 18 hours, fixed, and
740 stained for DAPI (blue), and endoplasmic reticulum marker (calreticulin) (red).

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742 **Supplemental Figure 8.** MCF induces fragmentation of host mitochondria

743 Replicates for cells intoxicated, as in Fig 6, with *V. vulnificus* strains and stained with Mitotracker.

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745

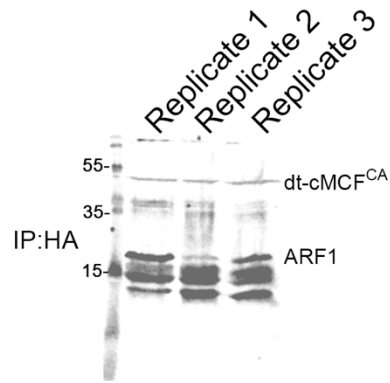
746 **Supplemental Figure 9.** Transmission electron microscopy shows Golgi vesiculation induced
747 by MCF.

748 A, B. Electron microscopy tomograms taken of HeLa cells ectopically expressing MCF for 10 (A)
749 or 15 hours (B). A-D. On right, higher magnification of boxed region on left showing Golgi (G),
750 herniated Golgi (red arrows), vesicles (V), and autolysomes (A).

751 **Supplemental Figure 10.** Western blot of subcellular fractions from HEK 293T cells ectopically
752 expressing dt-fIMCF. Each fraction probed with standards for membrane (CD-44), mitochondria
753 (Hsp-60), and nucleus (HistoneH3), and MCF (anti-HA).

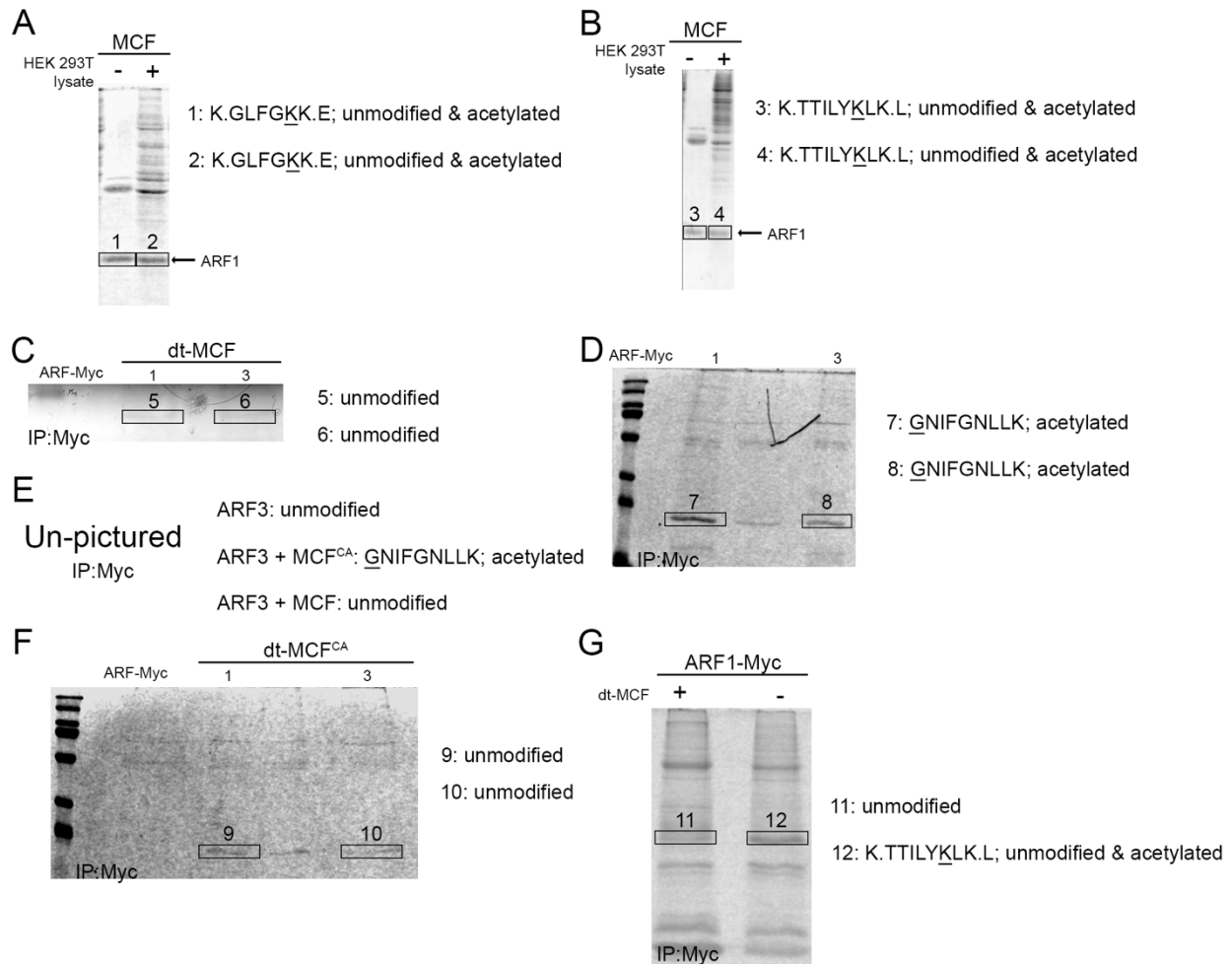
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755 **Supplemental Table 1.** Sequences of gBlocks used for plasmid construction



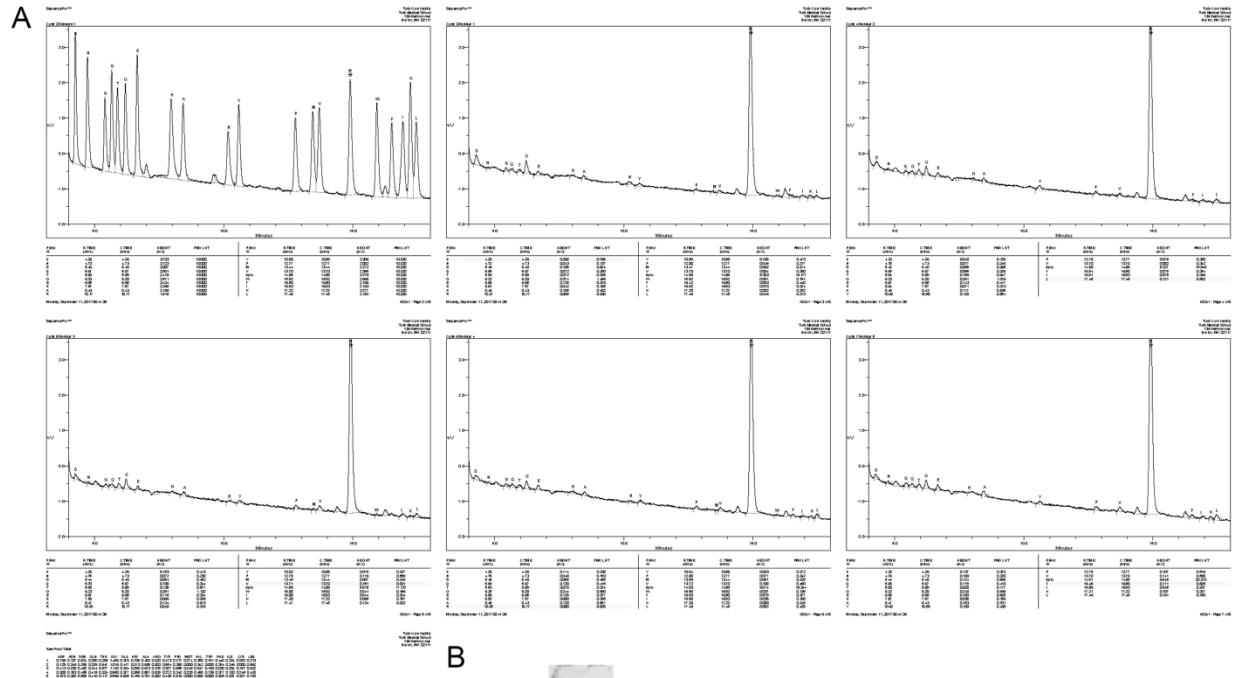
Supplemental Figure 1. MCF reproducibly pulls down native ARF1.

HEK293T cells were transfected with dt-cMCF^{CA} in triplicate. For each replicate, MCF was immunoprecipitated from lysate using anti-HA beads and analyzed by Western blot. dt-cMCF^{CA} was detected by anti-HA and endogenous ARF1 detected by anti-ARF1 antibodies.



Supplemental Figure 2. MCF does not cleave or directly modify ARF1 or ARF3.

A-G. Bottom-up mass spectrometry was performed on ARF1 and ARF3 samples recovered from recombinant proteins incubated with (A and B), or anti-Myc IPs (C-G) from HEK293T cells transfected with and without MCF or MCF^{CA}. Modifications that were detected are denoted. Any modifications found on either ARF1 or ARF3 were not reproducible across replicates. Furthermore, there were no modifications detected that were found in ARF + MCF samples that were not found in ARF samples alone and thus not attributable to MCF.

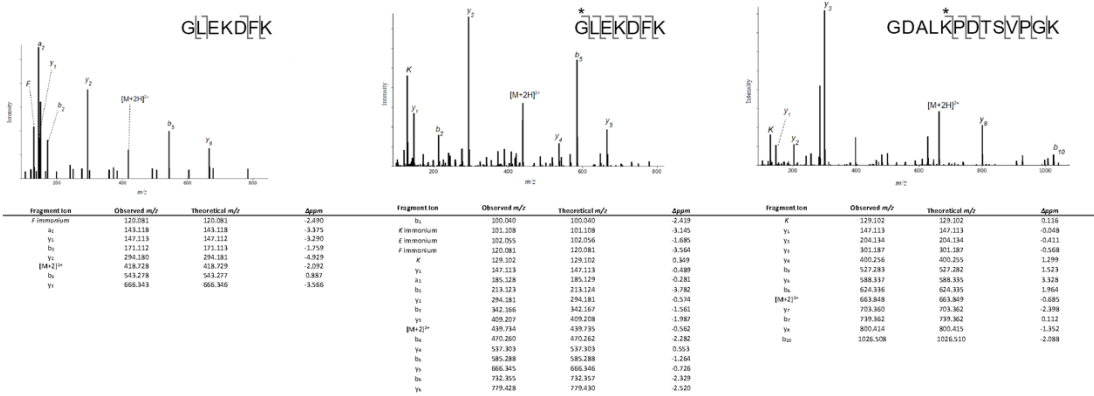


Supplemental Figure 3. Edman degradation is blocked in MCF recovered from cells.

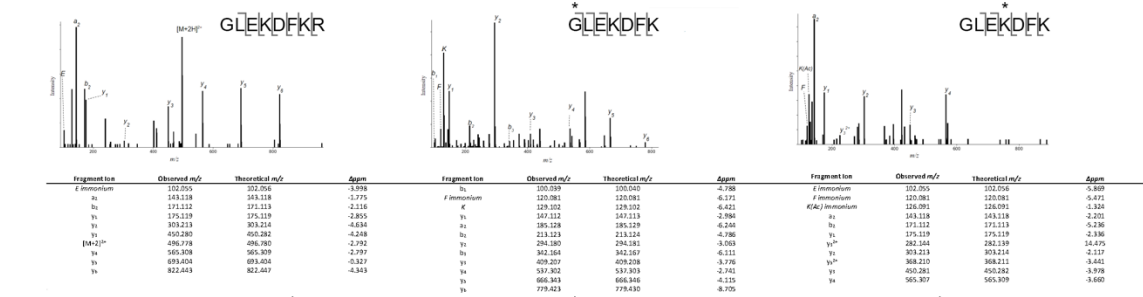
A. Reads from Edman degradation analysis completed on dt-fIMCF immunoprecipitated from HEK293T cell lysate using anti-HA antibody. No signal and very little background was detected.

B. Gel of sample used for analysis.

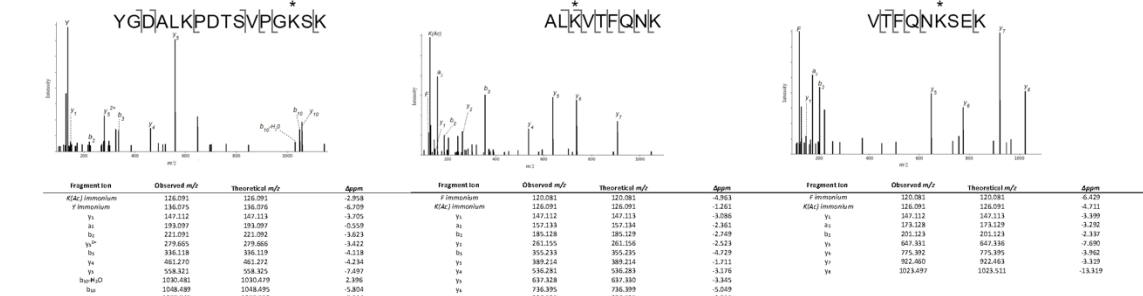
Population 2 Spectra



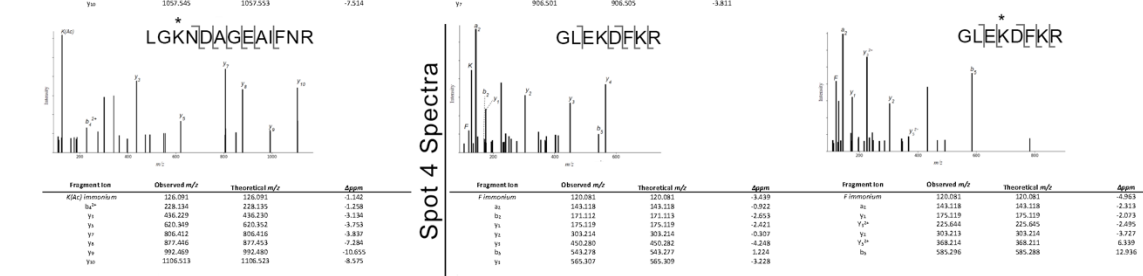
Population 3 Spectra



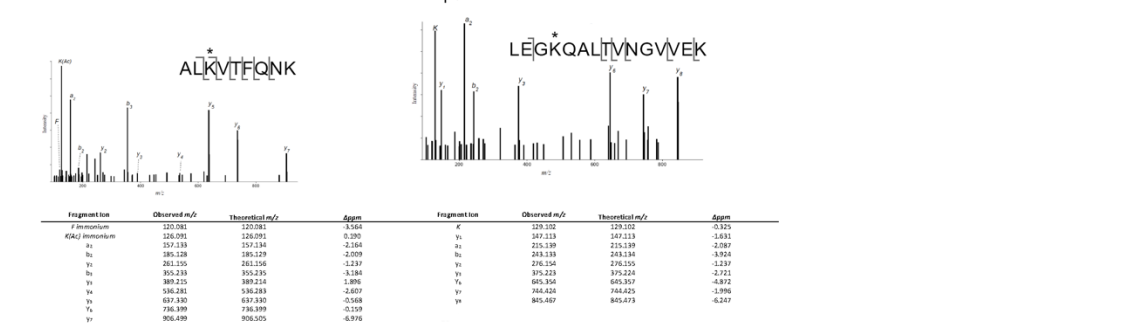
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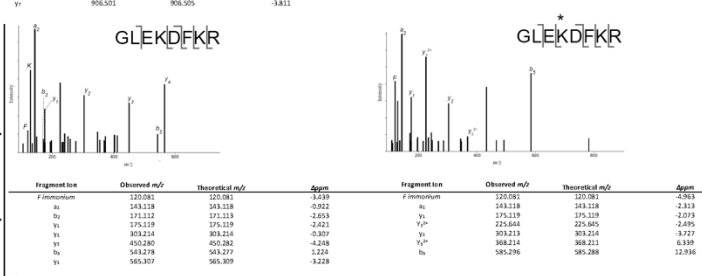
Population 3 Spectra



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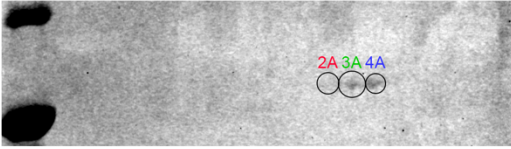
Spot 4 Spectra



Supplemental Figure 4. Mass Spectrometry analysis shows MCF is acetylated inside cells.

Representative mass spectrometry spectra for each modification detected per population in the replicates of the two-dimensional analysis of dt-fIMCF expressed in HEK293T cells recovered by anti-HA IP shown in Supplemental Fig 5. Asterisk above peptide sequence denotes residue acetylated in that particular spectra.

Replicate 1



FNAEQAAVEAGEVLK: GLEKDFKRYGDALKPD
 TSVPGKSKDIRTTKDFLNGYKNDHAKAIVDGFRRS
 DMSIKQLVDFVKGNWSAEQK GALAWAIE SRALK
 VTFQNKSEKYNRLFREIASAGVVDAKATEQLAPQ
 LMLLNLSNDGFGGRCDPLSKLVLVAKQLENDGQV
 GVARQLLEKMYSAAAVLSNPTLYSDSEKANASKL
 LSSLAAIHAKNPMHDTSMKVWQEKLEGKQALTVN
 GVVEKITDASANGKPVLLELDAPGHAMA AWAKGS
 GDDR VYGFYDPNAGIVEFSSAEKFGDYLTRFFGK
 SDLNMAQSYKLGKNDAGEAIFNRVVVMDGNTLAS
 YKPTFGDKTTMQGILDLPVFDATPIKKPTGGVAS
 DLEAL

Replicate 2



FNAEQAAVEAGEVLK: GLEKDFKRYGDALKPD
 TSVPGKSKDIRTTKDFLNGYKNDHAKAIVDGFRRS
 DMSIKQLVDFVKGNWSAEQK GALAWAIE SRALK
 VTFQNKSEKYNRLFREIASAGVVDAKATEQLAPQ
 LMLLNLSNDGFGGRCDPLSKLVLVAKQLENDGQV
 GVARQLLEKMYSAAAVLSNPTLYSDSEKANASKL
 LSSLAAIHAKNPMHDTSMKVWQEKLEGKQALTVN
 GVVEKITDASANGKPVLLELDAPGHAMA AWAKGS
 GDDR VYGFYDPNAGIVEFSSAEKFGDYLTRFFGK
 SDLNMAQSYKLGKNDAGEAIFNRVVVMDGNTLAS
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 DLEAL

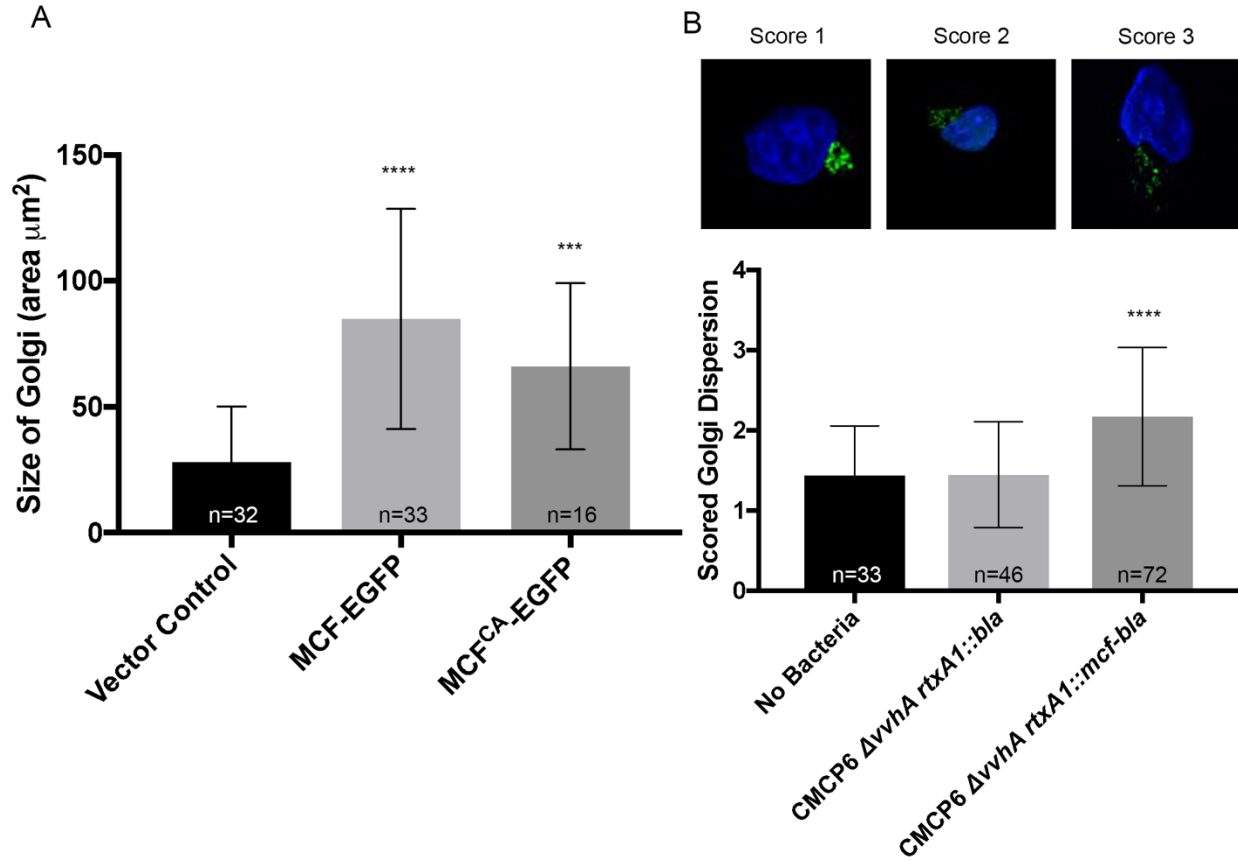
Replicate 3



FNAEQAAVEAGEVLK: GLEKDFKRYGDALKPD
 TSVPGKSKDIRTTKDFLNGYKNDHAKAIVDGFRRS
 DMSIKQLVDFVKGNWSAEQK GALAWAIE SRALK
 VTFQNKSEKYNRLFREIASAGVVDAKATEQLAPQ
 LMLLNLSNDGFGGRCDPLSKLVLVAKQLENDGQV
 GVARQLLEKMYSAAAVLSNPTLYSDSEKANASKL
 LSSLAAIHAKNPMHDTSMKVWQEKLEGKQALTVN
 GVVEKITDASANGKPVLLELDAPGHAMA AWAKGS
 GDDR VYGFYDPNAGIVEFSSAEKFGDYLTRFFGK
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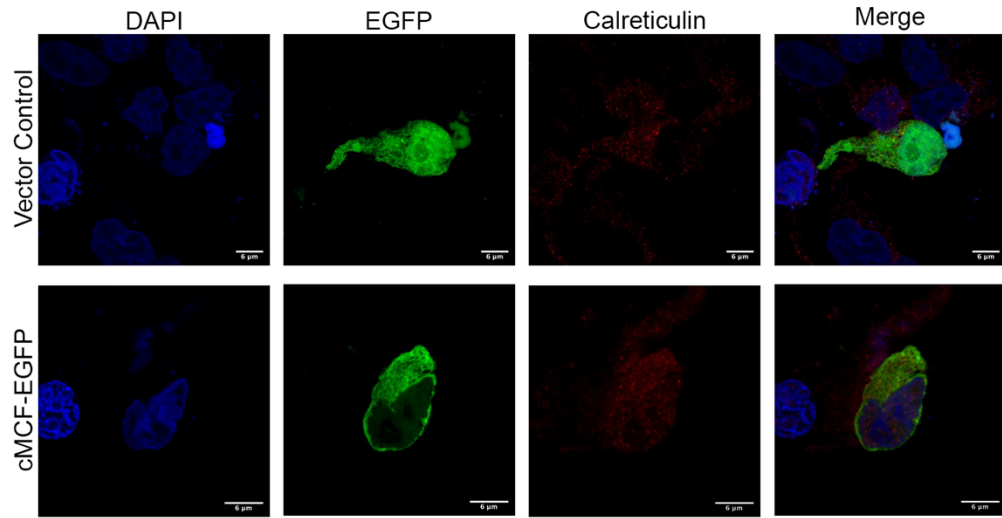
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Replicates of two-dimensional gel analysis of dt-flMCF expressed in HEK293T cells, recovered by anti-HA immunoprecipitation. Amino acids acetylated in each circled population indicated in corresponding color on sequence. Representative mass spectrometry data for the acetylations shown in Supplemental Fig 4.



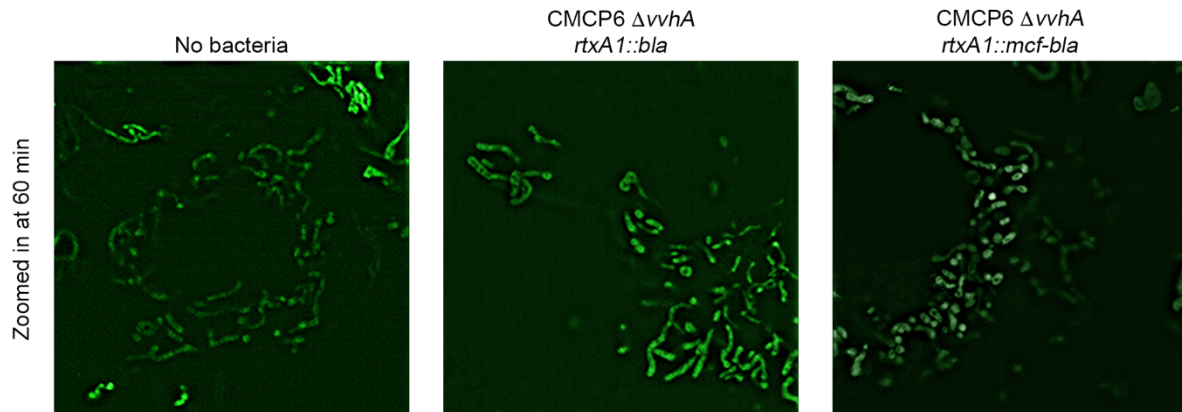
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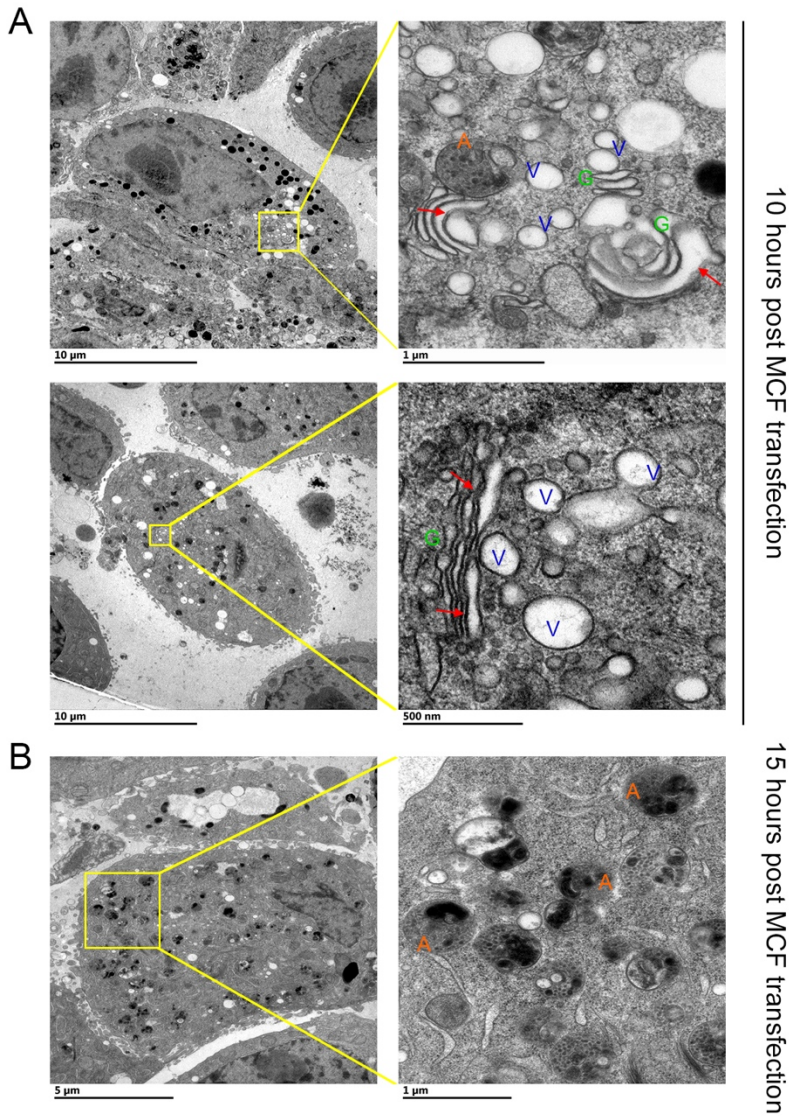
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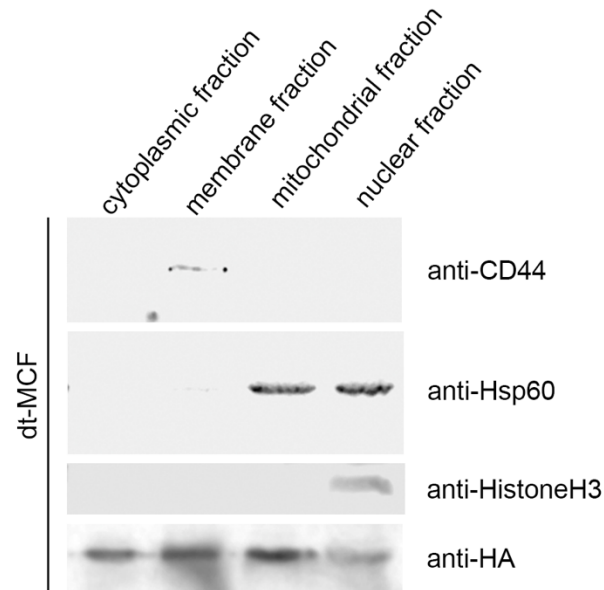
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A, B. Electron microscopy tomograms taken of HeLa cells ectopically expressing MCF for 10 (A) or 15 hours (B). A-D. On right, higher magnification of boxed region on left showing Golgi (G), herniated Golgi (red arrows), vesicles (V), and autolysosomes (A).



Supplemental Figure 10. Western blot of subcellular fractions from HEK 293T cells ectopically expressing dt-filMCF. Each fraction probed with standards for membrane (CD-44), mitochondria (Hsp-60), and nucleus (HistoneH3), and MCF (anti-HA).

Supplemental Table 1. Sequences of gBlocks used for plasmid construction.	
Primer/Gblock	Sequence
Δ 17ARF3	CCTGTA CTTCCAATCCAATGCTATGCGCATCCTGATGGTGGGCCTGGATGCC GCAGGAAAGACCACCATCCTATACAAGCTGAAACTGGGGGAGATCGTCACCA CCATCCCTACCATTGGGTTCAATGTGGAGACAGTGGAGTATAAGAACATCAG CTTTACAGTGTGGGATGTGGGTGGCCAGGACAAGATTCGACCCCTCTGGAGA CACTACTTCCAGAACACCCAAGGGTTGATATTTGTGGTCGACAGCAATGATCG GGAGCGAGTAAATGAGGCCCGGGAAGAGCTGATGAGAATGCTGGCGGAGGA CGAGCTCCGGGATGCTGTACTCCTTGTCTTTGCAAACAACAGGATCTGCCT AATGCTATGAACGCTGCTGAGATCACAGACAAGCTGGGCCTGCATTCCCTTC GTCACCGTAACTGGTACATT CAGGCCACCTGTGCCACCAGCGGGGACGGGC TGTACGAAGGCCTGGACTGGCTGGCCAATCAGCTCAAAAACAAGAAGTGATA AATTGGAAGTGGATAACGG
Δ 17ARF4	CCTGTA CTTCCAATCCAATGCTATGCGCATT TTTGATGGTTGGATTGGATGCTG CTGGCAAGACAACCATTCTGTATAAACTGAAGTTAGGGGAGATAGTCACCAC CATTCCCTACCATTGGTTTTAATGTGGAAACAGTAGAATATAAGAACATTTGTTT CACAGTATGGGATGTTGGTGGTCAAGATAGAATTAGGCCTCTCTGGAAGCAT TACTTCCAGAATACCCAGGGTCTTATTTTTGTGGTAGATAGCAACGATCGTGA AAGAATTCAGGAAGTAGCAGATGAGCTGCAGAAAATGCTTCTGGTAGATGAAT TGAGAGATGCAGTGTCTACTTTTTGCAAACAACAGGATTTGCCAAATGCT ATGGCCATCAGTGAATGACAGATAAACTAGGGCTTCAGTCTCTTCGTAACAG AACATGGTATGTTCAAGCCACTTGTGCAACACAAGGAACTGGTCTGTATGAAG GACTTGACTGGCTGTCAAATGAGCTTTCAAACGTTAATAAATTGGAAGTGGTA TAACGG
Δ 17ARF6	CCTGTA CTTCCAATCCAATGCTATGTTGGGCCTGGACGCGGCCGGCAAGACA ACAATCCTGTACAAGTTGAAGCTGGGCCAGTCGGTGACCACCATTCCCCTG TGGGTTTTCAACGTGGAGACGGTGACTTACAAAATGTCAAGTTCAACGTATGG GATGTGGGCGGCCAGGACAAGATCCGGCCGCTCTGGCGGCATTACTACACT GGGACCCAAGGTCTCATCTTCGTAGTGGACTGCGCCGACCGCGACCGCATC GATGAGGCTCGCCAGGAGCTGCACCGCATTATCAATGACCGGGAGATGAGG GACGCCATAATCCTCATCTTCGCCAACAAGCAGGACCTGCCCGATGCCATGA AACCCACGAGATCCAGGAGAACTGGGCCTGACCCGGATTCGGGACAGGA ACTGGTATGTGCAGCCCTCCTGTGCCACCTCAGGGGACGGACTCTATGAGG GGCTCACATGGTTAACCTCTAACTACAAATCTTAATAAATTGGAAGTGGATAA CGG
ARF1	CCTGTA CTTCCAATCCAATGCTATGGGGAACATCTTCGCCAACCTCTTCAAGG GCCTTTTTGGCAAAAAGAAATGCGCATCCTCATGGTGGGCCTGGATGCTGC AGGGAAGACCACGATCCTCTACAAGCTTAAGCTGGGTGAGATCGTGACCACC ATTCCCACCATAGGCTTCAACGTGGAAACCGTGGAGTACAAGAACATCAGCT TCACTGTGTGGGACGTGGGTGGCCAGGACAAGATCCGGCCCCTGTGGCGCC ACTACTTCCAGAACACACAAGGCCTGATCTTCGTGGTGGACAGCAATGACAG AGAGCGTGTGAACGAGGCCCGTGAGGAGCTCATGAGGATGCTGGCCGAGGA CGAGCTCCGGGATGCTGTCTCCTGGTGTTCGCCAACAAGCAGGACCTCCC CAACGCCATGAATGCGGCCGAGATCACAGACAAGCTGGGGCTGCACTCACT ACGCCACAGGAACTGGTACATTCAGGCCACCTGCGCCACCAGCGGCGACGG GCTCTATGAAGGACTGGACTGGCTGTCCAATCAGCTCCGGAACCAGAAGTAA ATTGGAAGTGGATAACGG
fIARF3	CCTGTA CTTCCAATCCAATGGGCAATATCTTTGGAAACCTTCTCAAGAGCCTG ATTGGGAAGAAGGAGATGCGCATCCTGATGGTGGGCCTGGATGCCGACGGA

	<p>AAGACCACCATCCTATACAAGCTGAAACTGGGGGAGATCGTCACCACCATCC CTACCATTGGGTTCAATGTGGAGACAGTGGAGTATAAGAACATCAGCTTTACA GTGTGGGATGTGGGTGGCCAGGACAAGATTCGACCCCTCTGGAGACACTAC TTCCAGAACACCCAAGGTTGATATTTGTGGTCGACAGCAATGATCGGGAGC GAGTAAATGAGGCCCGGGAAGAGCTGATGAGAATGCTGGCGGAGGACGAGC TCCGGGATGCTGTACTCCTTGTCTTTGCAAACAACAGGATCTGCCTAATGCT ATGAACGCTGCTGAGATCACAGACAAGCTGGGCCTGCATTCCCTTCGTACC GTAACCTGGTACATTCAGGCCACCTGTGCCACCAGCGGGGACGGGCTGTACG AAGGCCTGGACTGGCTGGCCAATCAGCTCAAAAACAAGAAGTAAATTGGAAG TGGATAACGG</p>
cMCF	<p>CCTGTACTIONCCAATCCAATGCTATGGGACTAGAGAAAAGACTTTAAACGCTATG GCGACGCGCTGAAACCAGATACGAGCGTGCCGGGTAATCGAAAGACATTC GCACCACTAAAGATTTCTAAATGGTTACAAAAATGACCATGCGAAAGAGATC GTTGACGGCTTCCGCTCAGATATGAGTATCAAGCAACTGGTGGATCTGTTTGT TAAAGGTAAGTGGAGTGCAGAGCAAAAAGGTGCGCTTGCTTGGGAAATCGAA AGTCGTGCACTGAAAGTGACGTTCCAGAACAAGTCTGAGAAGTACAACCGAT TGTTCCGTGAGATTGCTTCTGCTGGCGTGGTGGATGCGAAAGCGACTGAACA GCTTGCGCCACAGTTAATGCTGCTGAACCTATCGAATGACGGTTTTGGTGGG CGTTGTGATCCACTTTCTAAACTCGTTTTGGTTGCGAAACAGCTTGAAAACGA TGGTCAAGTTGGCGTGGCAAGACAAGTCTAGAAAAGATGTACTIONTCTGCGGCA GCGGTGCTGAGCAATCCAACCCTTTACTCAGACAGTAAAAAGCCAATGCAA GCAAGTTGCTCAGCAGCTTGGCGGCCATTGATGCGAAGAACCCAATGCATGA TACGTCGATGAAAGTGTGGCAGGAAAAGCTGGAAGGGAAGCAAGCGCTGAC CGTAAACGGTGTGGTTGAGAAAATCACTGATGCATCGGCTAACGGTAAACCT GTGCTGTTGGAACCTTGATGCTCCGGGGCATGCGATGGCAGCTTGGGCAAAA GGCTCAGGCGACGATCGTGTACGGCTTCTACGATCCAAATGCTGGCATCG TTGAGTTTTCGTCAGCAGAGAAGTTTGGCGACTACCTAACGCGTTTTCTTCGGC AAGTCCGATCTGAACATGGCTCAAAGCTATAAGCTGGGTAAAAACGACGCGAG GTGAAGCAATCTTCAACCGCGTGGTGGTAATGGATGGCAATACATTAGCAAG CTACAAGCCGACCTTCGGTGACAAGACCACCATGCAGGGGATCCTAGATCTA CCTGTGTTTGACGCTACACCGATTA AAAAGCCTACGGGTGGAGTCGCGAGCG ATCTCGAAGCATTGTAATTGGAAGTGGATAACGG</p>
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fIMCF ^{CA}	<p>CCTGTA CTTCCAATCCAATGCTATGGCAGCGGTAGAAGCGGGCGAAGTGCTG AAAGGACTAGAGAAAGACTTTAAACGCTATGGCGACGCGCTGAAACCAGATA CGAGCGTGCCGGGTAATCGAAAGACATTCGCACCACTAAAGATTTCTAAAT GGTTACAAAAATGACCATGCGAAAGAGATCGTTGACGGCTTCCGCTCAGATA TGAGTATCAAGCAACTGGTGGATCTGTTTGTAAAGGTAAGTGGAGTGCAGA GCAAAAAGGTGCGCTTGCTTGGGAAATCGAAAGTCGTGCACTGAAAGTGACG TTCCAGAACAAGTCTGAGAAGTACAACCGATTGTTCCGTGAGATTGCTTCTGC TGGCGTGGTGGATGCGAAAGCGACTGAACAGCTTGCGCCACAGTTAATGCTG CTGAACCTATCGAATGACGGTTTTGGTGGGCGTGCTGATCCACTTTCTAACT CGTTTTGGTTGCGAAACAGCTTGAAAACGATGGTCAAGTTGGCGTGGCAAGA CAACTGCTAGAAAAGATGTA CTTCTGCGGCAGCGGTGCTGAGCAATCCAACCC TTTACTCAGACAGTAAAAAGCCAATGCAAGCAAGTTGCTCAGCAGCTTGGC GGCCATTCATGCGAAGAACCCAATGCATGATACGTCGATGAAAGTGTGGCAG GAAAAGCTGGAAGGGAAGCAAGCGCTGACCGTAAACGGTGTGGTTGAGAAA ATCACTGATGCATCGGCTAACGGTAAACCTGTGCTGTTGGAACCTGATGCTCC GGGCATGCGATGGCAGCTTGGGCAAAAGGCTCAGGCGACGATCGTGTTTA CGGCTTCTACGATCCAAATGCTGGCATCGTTGAGTTTTCGTCAGCAGAGAAG TTTTGGCGACTACCTAACGCGTTTTCTTCGGCAAGTCCGATCTGAACATGGCTCA AAGCTATAAGCTGGGTAAAAACGACGCAGGTGAAGCAATCTTCAACCGCGTG GTGGTAATGGATGGCAATACATTAGCAAGCTACAAGCCGACCTTCGGTGACA AGACCACCATGCAGGGGATCCTAGATCTACCTGTGTTTGACGCTACACCGAT TAAAAAGCCTACGGGTGGAGTCGCGAGCGATCTCGAAGCATTGTAATTGGA AGTGGATAACGG</p>
CMCF ^{CA}	<p>CCTGTA CTTCCAATCCAATGCTATGGGACTAGAGAAAGACTTTAAACGCTATG GCGACGCGCTGAAACCAGATACGAGCGTGCCGGGTAATCGAAAGACATTC GCACCACTAAAGATTTCTAAATGGTTACAAAAATGACCATGCGAAAGAGATC GTTGACGGCTTCCGCTCAGATATGAGTATCAAGCAACTGGTGGATCTGTTTGT TAAAGGTAAGTGGAGTGCAGAGCAAAAAGGTGCGCTTGCTTGGGAAATCGAA AGTCGTGCACTGAAAGTGACGTTCCAGAACAAGTCTGAGAAGTACAACCGAT TGTTCCGTGAGATTGCTTCTGCTGGCGTGGTGGATGCGAAAGCGACTGAACA GCTTGCGCCACAGTTAATGCTGCTGAACCTATCGAATGACGGTTTTGGTGGG CGTGCTGATCCACTTTCTAAACTCGTTTTGGTTGCGAAACAGCTTGAAAACGA TGGTCAAGTTGGCGTGGCAAGACAAGTCTAGAAAAGATGTA CTTCTGCGGCA GCGGTGCTGAGCAATCCAACCTTTACTCAGACAGTAAAAAGCCAATGCAA GCAAGTTGCTCAGCAGCTTGGCGGCCATTCATGCGAAGAACCCAATGCATGA TACGTCGATGAAAGTGTGGCAGGAAAAGCTGGAAGGGAAGCAAGCGCTGAC CGTAAACGGTGTGGTTGAGAAAATCACTGATGCATCGGCTAACGGTAAACCT GTGCTGTTGGAACCTGATGCTCCGGGGCATGCGATGGCAGCTTGGGCAAAA GGCTCAGGCGACGATCGTGTTTACGGCTTCTACGATCCAAATGCTGGCATCG TTGAGTTTTCGTCAGCAGAGAAGTTTGGCGACTACCTAACGCGTTTTCTTCGGC AAGTCCGATCTGAACATGGCTCAAAGCTATAAGCTGGGTA AAAACGACGCAG GTGAAGCAATCTTCAACCGCGTGGTGGTAATGGATGGCAATACATTAGCAAG CTACAAGCCGACCTTCGGTGACAAGACCACCATGCAGGGGATCCTAGATCTA CCTGTGTTTGACGCTACACCGATTAAAAAGCCTACGGGTGGAGTCGCGAGCG ATCTCGAAGCATTGTAATTGGAAGTGGATAACGG</p>
CMV7.1.kpnI.A PEX2.f	ACGATGTTCCAGATTACGCTGGTACCGGAGGAGGATCATCATCA
CMV7.1.kpnI.A PEX2.r	ACCCGGGATCCTCTAGAGTCGACTGGTACCTTAGGCATCAGCAAACCCAAG