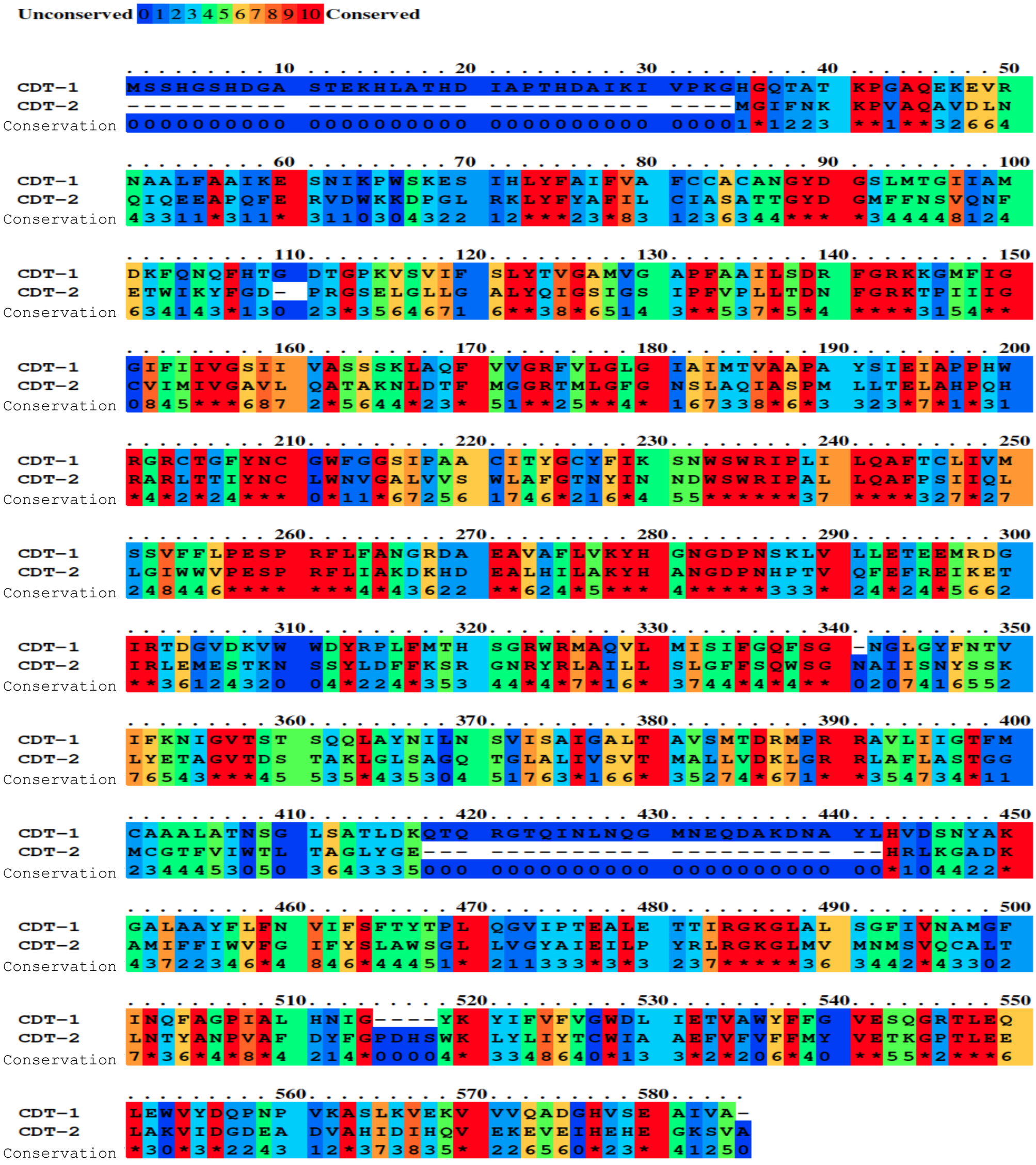


Fig. S1: Amino acid sequence alignment of *N.crassa* CDT-1 and CDT-2 transporters using the PRALINE multiple sequence alignment program because of low sequence identity between CDT-1 and CDT-2 (1). Residues are color-coded based on conservation.

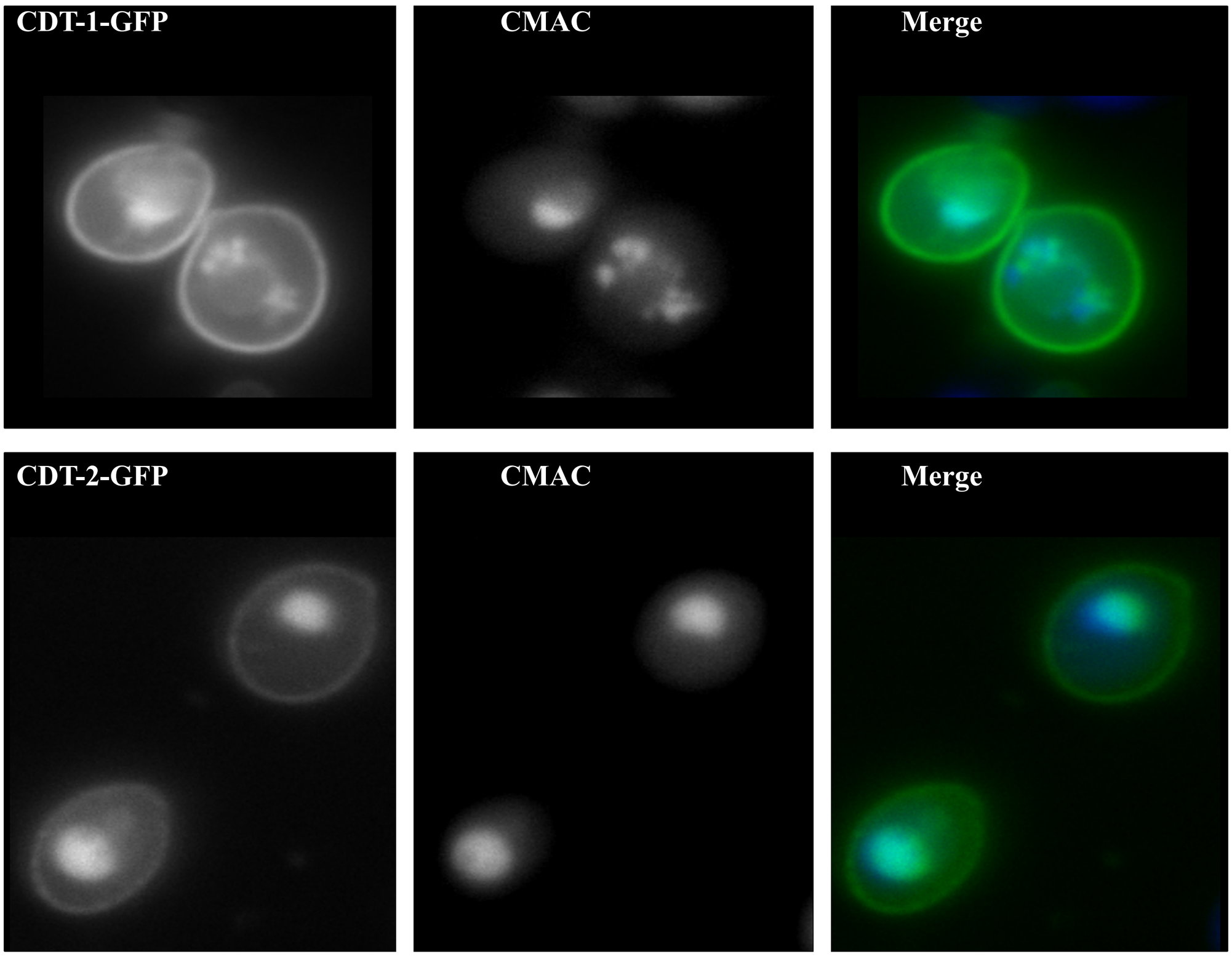


Fig. S2: To visualize vacuoles, WT cells expressing CDT-1-GFP or CDT-GFP were incubated with 250 μM of the vacuolar lumen marker CMAC (Life Technologies, Carlsbad, CA) for 20 min, washed and resuspended prior to imaging.

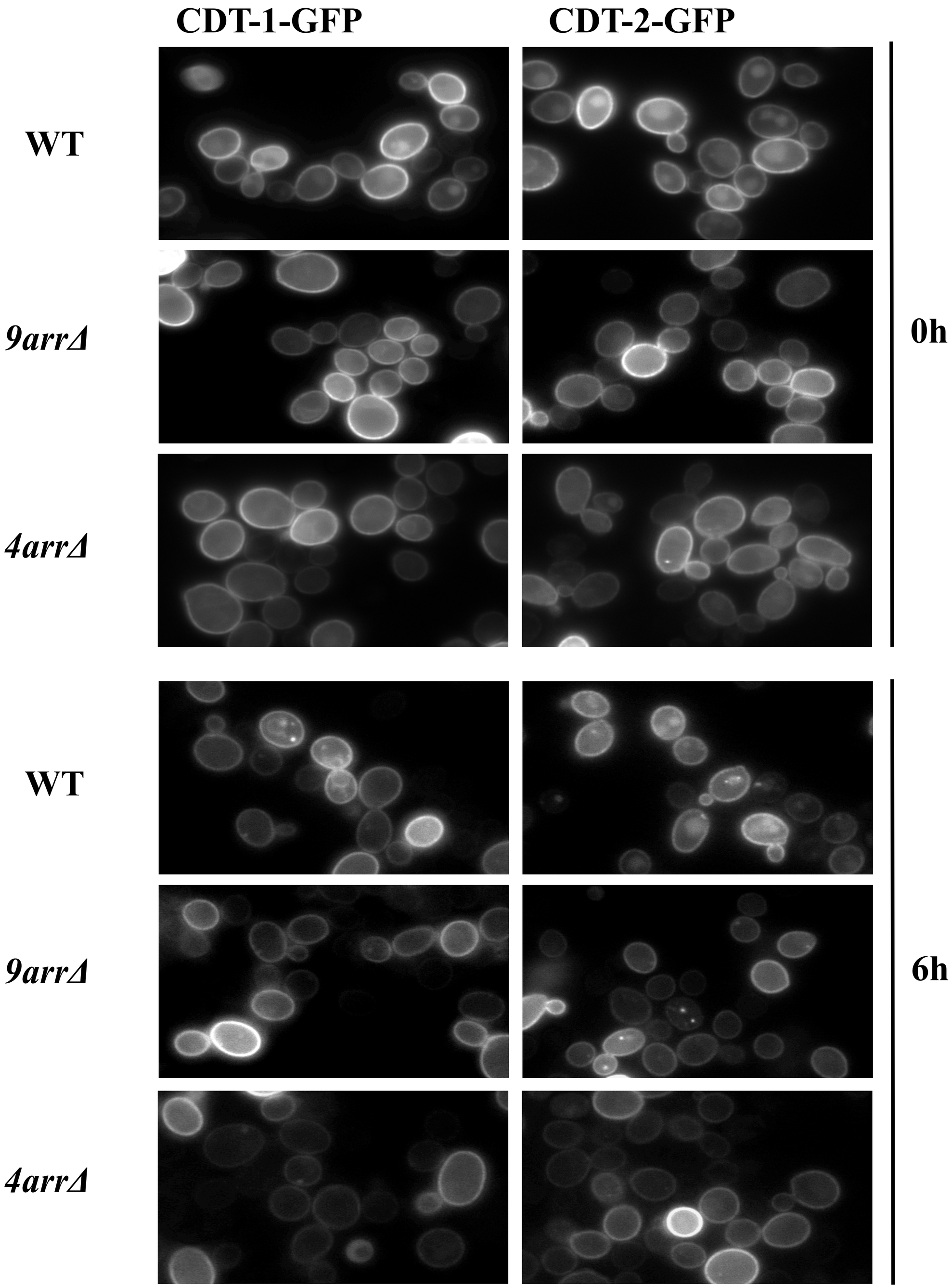


Fig. S3: Localization of WT, *9arrΔ, or 4arrΔ* cells expressing CDT-1 or CDT-2 under normal conditions, or after 6 hours of anaerobic growth.

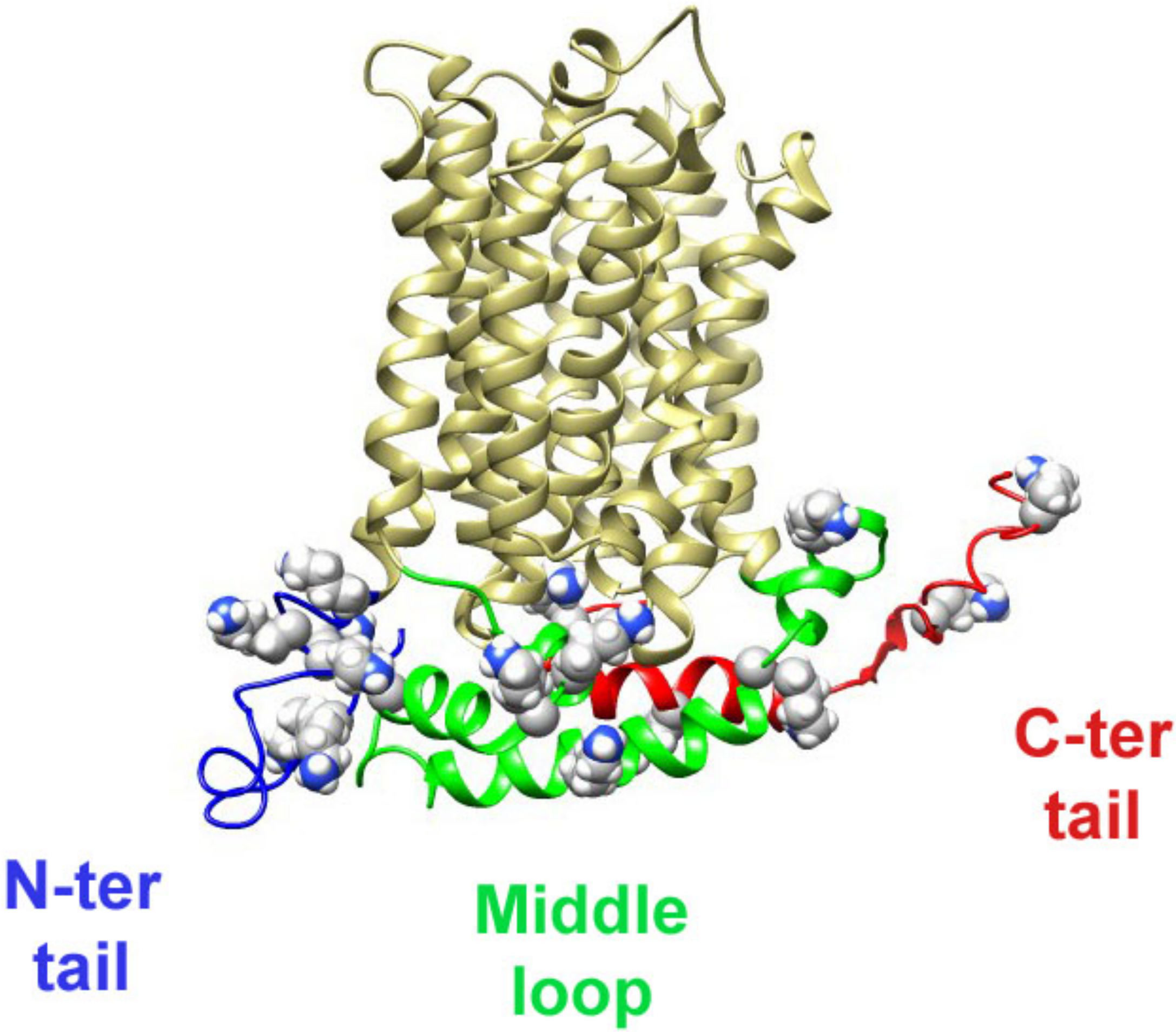


Fig. S4: I-TASSER model for CDT-2 showing Lys residues mutated to Arg in the N-terminal tail (blue), the interdomain middle loop (green), and the C-terminal tail (red).

```
FGTrunc         MGIKEIMGLGADEKTQRLRDEAPKFEKVTWYRDPALRKLTFYACVLCASSMGTGWDGMYM    60
FG              MGIKEIMGLGADEKTQRLRDEAPKFEKVTWYRDPALRKLTFYACVLCASSMGTGWDGMYM    60
CDT-2Trunc      MGIFNKKPVAQAVDLNQIQEEAPQFERVDWKKDPGLRKLYFYAFILCIASATTGYDGMFF    60
CDT-2           MGIFNKKPVAQAVDLNQIQEEAPQFERVDWKKDPGLRKLYFYAFILCIASATTGYDGMFF    60
                *** :   :.   . ::::::***:**:* * :**.**** *** :** :*  **:***::

FGTrunc         NNVQNFDSWKNFFDKPEGERLGLLIALYQIGSVASIPLVPLITDRWGRRPSIALGFIIMA    120
FG              NNVQNFDSWKNFFDKPEGERLGLLIALYQIGSVASIPLVPLITDRWGRRPSIALGFIIMA    120
CDT-2Trunc      NSVQNFETWIKYFGDPRGSELGLLGALYQIGSIGSIPFVPLLTDNFGRKTPIIIGCVIMI    120
CDT-2           NSVQNFETWIKYFGDPRGSELGLLGALYQIGSIGSIPFVPLLTDNFGRKTPIIIGCVIMI    120
                *.******:* ::* .*.*..**** *******:.***:***:**.:**:  * :* :**

FGTrunc         IGAGLQAGAPDYATFSGGRVLLGFGNSFAQIASPMLLAELCHPQHRARFTTVYNCLWNLG    180
FG              IGAGLQAGAPDYATFSGGRVLLGFGNSFAQIASPMLLAELCHPQHRARFTTVYNCLWNLG    180
CDT-2Trunc      VGAVLQATAKNLDTFMGGRTMLGFGNSLAQIASPMLLTELAHPQHRARLTTIYNCLWNVG    180
CDT-2           VGAVLQATAKNLDTFMGGRTMLGFGNSLAQIASPMLLTELAHPQHRARLTTIYNCLWNVG    180
                :** *** * :  ** ***.:******:*********:**.*******:**:******:*

FGTrunc         SFLVSWTCFGTSFWGNNWSWRFPAILQGAPGLLQLVVLFWIPESPRFLIAKDRHDEALGI    240
FG              SFLVSWTCFGTSFWGNNWSWRFPAILQGAPGLLQLVVLFWIPESPRFLIAKDRHDEALGI    240
CDT-2Trunc      ALVVSWLAFGTNYINNDWSWRIPALLQAFPSIIQLLGIWWVPESPRFLIAKDKHDEALHI    240
CDT-2           ALVVSWLAFGTNYINNDWSWRIPALLQAFPSIIQLLGIWWVPESPRFLIAKDKHDEALHI    240
                :::*** .***.:  *:****:**:**. *.::**: ::*:***********:***** *

FGTrunc         LAKYHANGDVNHPTVQFEYREIRETIKAEQMADNTSSYIDFFKTKGNRYRLVVLFSLGLF    300
FG              LAKYHANGDVNHPTVQFEYREIRETIKAEQMADNTSSYIDFFKTKGNRYRLVVLFSLGLF    300
CDT-2Trunc      LAKYHANGDPNHPTVQFEFREIKETIRLEMESTKNSSYLDFFKSRGNRYRLAILLSLGFF    300
CDT-2           LAKYHANGDPNHPTVQFEFREIKETIRLEMESTKNSSYLDFFKSRGNRYRLAILLSLGFF    300
                ********* ********:***:***: *  : :.***:****::******.:*:***:*

FGTrunc         SQWSGNGVVSNYSSILYEQAGLESEQDRLIITAGKTILDMIVSIGCALFVDRLNRRFSFL    360
FG              SQWSGNGVVSNYSSILYEQAGLESEQDRLIITAGKTILDMIVSIGCALFVDRLNRRFSFL    360
CDT-2Trunc      SQWSGNAIISNYSSKLYETAGVTDSTAKLGLSAGQTGLALIVSVTMALLVDKLGRRLAFL    360
CDT-2           SQWSGNAIISNYSSKLYETAGVTDSTAKLGLSAGQTGLALIVSVTMALLVDKLGRRLAFL    360
                ******.::***** *** **: ..  :* ::**:* * :***:  **:**:* **::**

FGTrunc         FATGGMFLVLIFWTLTCGLYEQHQAAGANYGMIFLVWMHGVFYSTCWSGLLIGYAVEILP    420
FG              FATGGMFLVLIFWTLTCGLYEQHQAAGANYGMIFLVWMHGVFYSTCWSGLLIGYAVEILP    420
CDT-2Trunc      ASTGGMCGTFVIWTLTAGLYGEHRLKGADKAMIFFIWVFGIFYSLAWSGLLVGYAIEILP    420
CDT-2           ASTGGMCGTFVIWTLTAGLYGEHRLKGADKAMIFFIWVFGIFYSLAWSGLLVGYAIEILP    420
                 :****  .:::****.*** :*:  **: .***::*:.*:*** .*****:***:****

FGTrunc         YSLRAKGLMILNISVQIALLLNNYLNPLAFKAWQAGDDQIFGGNTWRLYLIYTIWVFGEV    480
FG              YSLRAKGLMILNISVQIALLLNNYLNPLAFKAWQAGDDQIFGGNTWRLYLIYTIWVFGEV    480
CDT-2Trunc      YRLRGKGLMVMNMSVQCALTLNTYANPVAFDYFGP-------DHSWKLYLIYTCWIAAEF    473
CDT-2           YRLRGKGLMVMNMSVQCALTLNTYANPVAFDYFGP-------DHSWKLYLIYTCWIAAEF    473
                * **.****::*:*** ** **.* **:**. :           .:*:****** *: .*.

FGTrunc         TFIYFMYVETRGPTLEEVAKVIDGDNAAVGHVTMERLKKRP*                     521
FG              TFIYFMYVETRGPTLEEVAKVIDGDNAAVGHVTMEKVEKEALNEEVDKISSEGQQIHEVA  540
CDT-2Trunc      VFVFFMYVETKGPTLEELAKVIDGDEADVAHIDIHRLKKRP*                     514
CDT-2           VFVFFMYVETKGPTLEELAKVIDGDEADVAHIDIHQVEKEVEIHEHEGKSVA*          525
                .*::******:******:*******:* *.*: :.::::*.

FGTrunc                 521
FG              PTKV    544
CDT-2Trunc              514
CDT-2                   525
```

Fig. S5: Clustal Omega (Ver 1.2.1) alignment (2) of amino acid residues of FG and CDT-2, along with the truncated versions for both. FG and CDT-2 share 58.7% amino acid sequence identity.

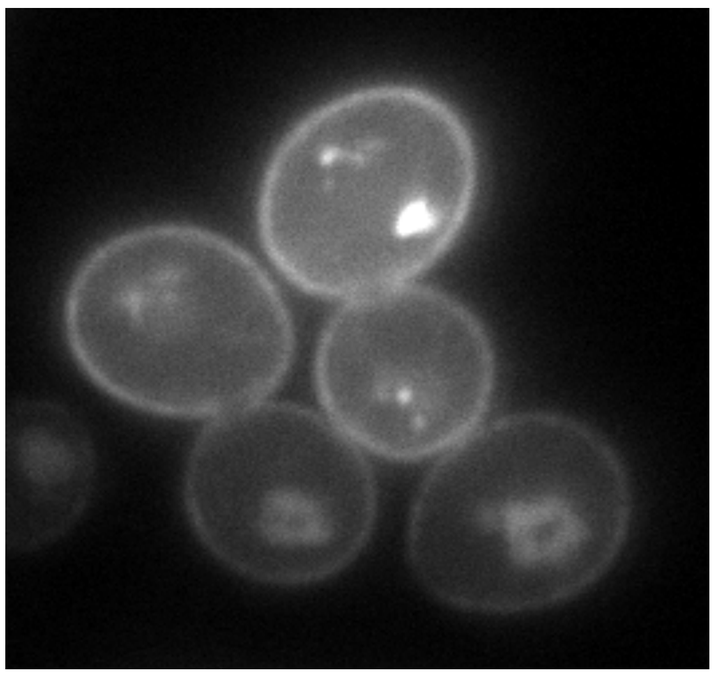
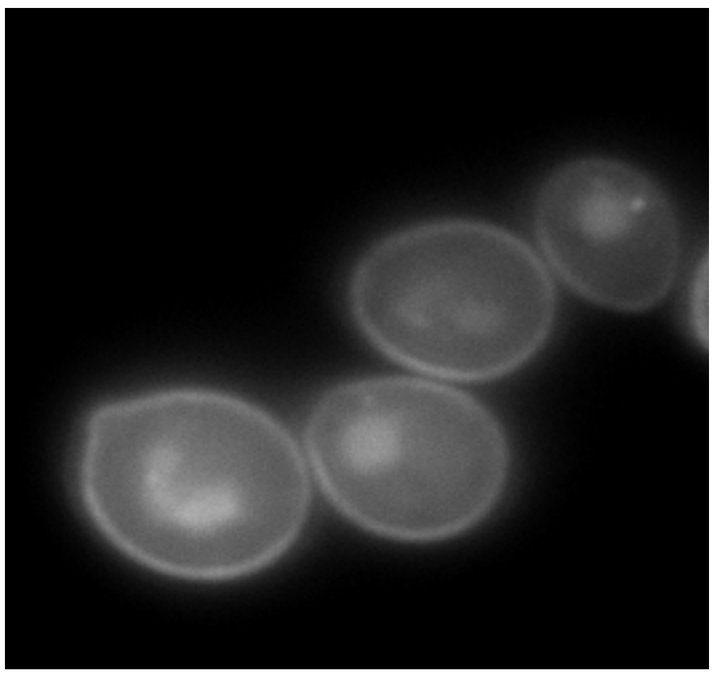
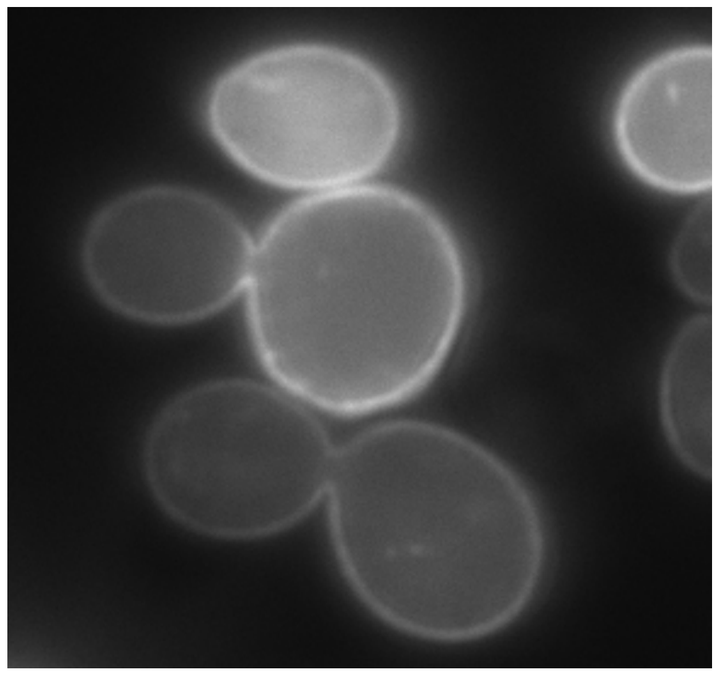
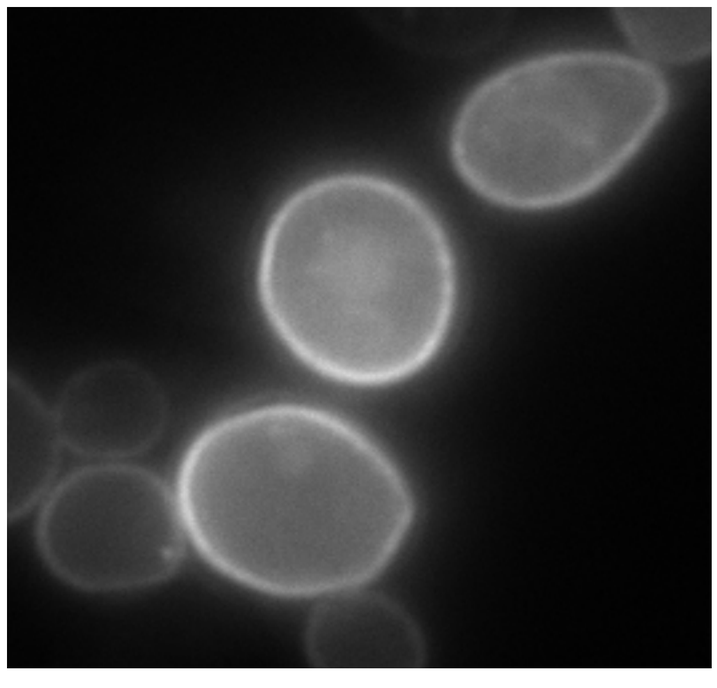
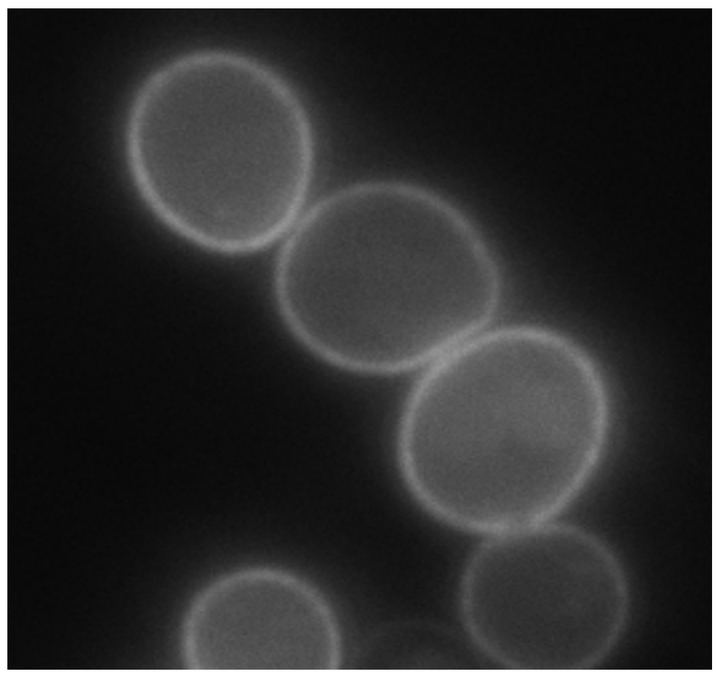
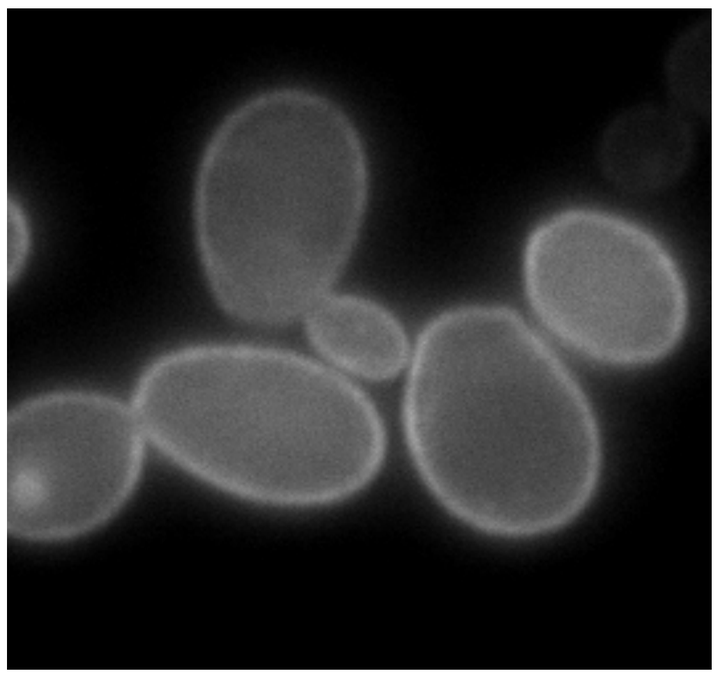
	***PGK1*$_{prom}$ CDT2-GFP**	***CCW12*$_{prom}$ CDT2-GFP**
WT		
9arrΔ		
4arrΔ		

Fig. S6: Representative images showing equivalent levels of CDT-2-GFP expression under the control of either the *PGK1prom* or the *CCW12prom*, as indicated.

```
creD          -MALSFFSGGGSASHAKYFDIRLDEDYIVFRGGEQEAASAHLSGKLVLCVSEPISIKHIR 59
NCU03887      MSFTNFFTSVTGKHAYTYFDIRLESDFIVFRGNEHESAGQLLQGTVVLCLPAPLKIEDVH 60
                  .**:.  .    .******:.*:***** *:*:*.  *.*.:***:  *:.*:.::
creD          LHLTGISRVCWHLPSSSAGGGRKN--WRERVFYEKTWKFRD-A-----------GKSKT 104
NCU03887      LRLTGTLHMSWNDPRVTAAGISNHKIDRTSTIYSHRWKPFVGVGAENQSIGSPNGLMSRG 120
              *:***  ::.*. *  :*.*  :.   *  .:*.: **    .             *:
creD          EILPAGNYEYPFDVILEGSMPESVEGLSDTYVTYRFKAEIGR-KYAKDIVVRRPLRIIRT 163
NCU03887      VTLPAGNYEWPFELMLPGDMTESVEGLREASLTYKLKATVARGKLAYDLHAYKRLRIIRT 180
                *******:**:::* *.* ****** :: :**::** :.* * * *: . : ******
creD          LESSALELSHAMSVENIWPNKIEYSISTPTKAVIFGTSIRVDFKLIPLLKGLGIGQIISQ 223
NCU03887      LDPSTLEFLHTMSVENIWPNKIEYSIMIPKKAVVFGSTIPLQTRFTPLLKGLELGEITIR 240
              *: *:**: *:***************  *.***:**::* :: :: ****** :*:*  :
creD          LIETHDLTLNPEDPDAIRNTYKTTRTIINDEHTIDEE--NSLEIIDEAAEGFQFSRTLDL 281
NCU03887      LLEVHEFILQSHSGYGVREHKKEREISVW-KIPIEREKHWQDVIEDTGQEGWVMNTGLDL 299
              *:*.*:: *: ..  .:*:  *  .  :  :  *:.*   .  * * . **: :.  ***
creD          PKTLTRCLQDTDTRGIKVRHKLKFRVQLLNPDGHISELRATLPVSIFISPNLAIDDNNNL 341
NCU03887      PRTLGKCVQDVNAHGIKVRHKLKVVLALHNPDGHISELRATLPVSIFISPNMPLDEEGNL 359
              *:** :*:**.:::********. : * **********************: :*:: **
creD          VDSSPQTTQRALDDLAQQAPPLYGEHQFDQLYSEVDPSGYRTPGPGSGPGTPFGTLSRNL 401
NCU03887      VRQMPNGTT--PGDVVAAPPPSYDEHTLDQLYEDMEPTGLQTPAGMA---SPLYGHSRAG 414
              * . *: *     *:.   ** * ** :****.:::*:* :**.  :   :*:   **
creD          SAENLASMNAITHTDISASALHHRLVNLDLRGHGRVSASEHDH-LGVPSDNGPPSGSNTH 460
NCU03887      SVENLAALLHMHSTAVPPAALTSRLQNVSLEPSSRSTSWNDGDSETATPNNGPADSV--- 471
              *.****::  :  * :  :**  ** *:.*.  .* :: :. .   .  :*** ..
creD          GSNTHAPGSPELSRRASDEDVHDNIPSGMATPFIPHSAELETLSRVPSYSTAVRSSVRPH 520
NCU03887      --HSSAFPSAPLTRQNSGDNITAEAMSGYHTPEHLDFSGIMELSKVPSYHTAVKTPVRPI 529
                .: *  *  *:*: * :::  :  **  **   . : :  **:**** ***:: ***
creD          ----DSDLPDYQAVVAETVHMSAPQSPQQAHIRGSGTGRGSDSY----FS---------- 562
NCU03887      AYPEGTVLPDYVAATSASG-STTPGSPELGHS---EAGDGASKVEERNQSRPEPPRRKSA 585
                   : **** *..: :   ::* **: .*     :* *:..     *
creD          ---APMDFFHRPAFLHS-----RSHSHSDDERRIR-LTQARGRA  597
NCU03887      RSRLAFTLSHIPHIHHSHSHHGHSHSESEDRRRHSSMMQAN---  626
                   : : * * : **     :***.*:*.**   : **.
```

Fig. S7: Clustal Omega (Ver 1.2.1) protein sequence alignment (2) between *A. nidulans creD* (AN4170) and *N. crassa* NCU03887. These proteins share 42% amino acid sequence identity. The arrestin N and C domains (as predicted by http://pfam.xfam.org/) are highlighted in grey; the PPxY motifs are in red.

Supplemental References

1. **Pirovano, W., Feenstra, K. A., & Heringa, J**. 2008. PRALINETM: a strategy for improved multiple alignment of transmembrane proteins. *Bioinformatics* **24**: 492-497.

2. **Sievers F, Higgins DG**. 2014. Clustal Omega, accurate alignment of very large numbers of sequences. *Methods Mol. Biol.* **1079**: 105-116.