```
          S1        H1
HsTUBE1 -MTQSVVVQVGQCGNQIGCCFWDLALREHAAVNQKGIYDEAISSFFRNVDTRVVGDGGSI
MmTUBE1 -MTQSVVVQVGQCGNQIGCCFWDLALREHAAVNQKGIYDDAISSFFRNVDTRAVGDGGSI
XlTUBE1 -MTQSIVLQVGQCGNQIGCRFWDLALREHASVNKKGIYDEALCSFFRNVDTRAEAGGADA
TtETU1  -MRELITIQVGQCGNQIGAKFWELAIKEHSKYNKSSVYDDALSSFFRNIDK-SSKGHELK
PtPT1   MPRELIFVQVGQCGNQIGMRFWDLALKEHAKAKQSLLYDESLSSFFRNVN---SKNQELK
CrTUE   MPRELVTIQVGQCGNQVGCRFWELALREHAAYNTKGVYDEALSSFFRNVDTRVEPPRNLP
                     +                                  HHHH

                    S2       H2              S3
HsTUBE1 SK---GKICSLKARAVLIDMEEGVVNEILQGPLRDVFDTKQLITDISGSGNNWAVGHKVF
MmTUBE1 SK---GRISSLKARAVLIDMEEGVVNEILQGPLRDVFDSKQLITDISGSGNNWAVGHKVF
XlTUBE1 QR---GKICSLKARAVMIDMEEGVVNEILQGPLRDLFDSKQLITDVSGSGNNWAVGHKLY
TtETU1  VG---SEIINLKARSIIVDMEEGVTNQLLKSDIGDLFDQRQFINDVSGAGNNWAHGHFFY
PtPT1   IG---DPIQNLKARALIVDMEEGVINQIMKSPLGELFEERQCINDVSGAGNNWAHGFYHY
CrTUE   VGEGRGAIRTLKARSVIVDMECGVINEMLKGPLGEVLDTRQLVSDVSGAGNNWAHGHHEY
                              +  +        HHHH H+++++++

             H3                 S4           H4             S5
HsTUBE1 GSLYQDQILEKFRKSAEHCDCLQCFFIIHSMGGGTGSGLGTFLLKVLEDEFPEVYRFVTS
MmTUBE1 GCLYREQILEKLRKSAEQCDCLQCFFIIHSMGGGTGSGLGTFLLKVLEDEFPEVYRFVTA
XlTUBE1 GGQYREHIVENLRRTAEQCDCLQCFFVIHSMGGGTGSGLGTFVLNVLEEEFPEVYRFVTS
TtETU1  GNKYRDNMSERIRKAVEQCDSLQCFFLMHSLGGGTGSGLGTFLLSLIEDEYPDVYRFTAS
PtPT1   GNQYEKHIEERLRKTVEQCDSLQCFFMTHSIGGGTGSGLGSRIVGLLEDNYPEVFRFTAS
CrTUE   GPRYHDAILDKIRLTAEDCDSLQSFMVLHSLGGGTGSGVGTYIVRMLADEFPGVFRFTGS
         HHHHHHHHHHHHHHHHHH - -                       HHHH

                 H5                S6      H6
HsTUBE1 IYPSGEDDVITSPYNSILAMKELNEHADCVLPIDNQSLFDIISKIDLMVNSGKLGTTVKP
MmTUBE1 VYPSSEDDVITSPYNSMLAMKELNEHADCVLPIDNQSLFDIISKIDLVVNSGKLGSAVKP
XlTUBE1 VYPSAEDDVITSPYNSVLAMKELTEHADCVLPIENQSLVDIVNKINQMANTGKLG-NVKP
TtETU1  VFPQKDDDVITSPYNSFFSLYELAKHADCVFPIDNQALMNICYQIDNPNKVKLAKDNINV
PtPT1   VFPSGDDDVVTSPYNSLFSLYELAKHADCVFPIDNQALINIVDQIDKPNKARLVKENAHE
CrTUE   VFPSEDDDVVTSPYNAMLALGQLVEHADCVLPIENQALIDIVNRTEAARDRAAAADAAAS
            + ++  +                      +  +   MMMM    M+

                                        H7                  H8
HsTUBE1 K----------SLVTSSSGALKKQHKKPFDAMNNIVANLLLNLTSSARFEGSLNMDLNEI
MmTUBE1 K----------SLITSNMGAVKKHHKKPFDAMNNIVANLLLSLTSSARFEGSLNMDLNEI
XlTUBE1 N----------SLVTSSKGGVK-ENEKPFDAMNNIVANLLLNLTSSARFEGSLNMDLNEI
TtETU1  SKEEEIKIMMNKGSKITQAGTEEKKEKPFDKMNNIIAHLLSNITCPMRFEGVLNVDLNEI
PtPT1   G---------SVGIKITQFGEEEKRQKPFDKMNSLIAHLLSHITCSMRFEGALNVDLNEI
CrTUE   A---------VSGLKGSGGGS-----KPFDSMNGVAASLLLHLTASVRFEGPLNVDLNDI
                                  ++++                  --- - --

                  S7                     H9
HsTUBE1 SMNLVPFPQLHYLVSSLTPLYTLTDVNIPPRRLDQM--FSDAFSKDHQLLRADPKHSLYL
MmTUBE1 SMNLVPFPKLHYLVSSLTPLYTLADVNIPPRRLDQM--FSDAFSKDHQLIQADPRHSLYL
XlTUBE1 SMNLVPFPQLHYLTSSLTPLYTLADVNVPSRRLDQM--FSDAFSKDHQLIRADPKHSLYL
TtETU1  TMNLVPYPDLHFLISSIAPLYSLSDSNLQPRRLDQM--FKDCYHPDYQLVSVNPFQNKYL
PtPT1   TMNLVPYPDLHFLISSMAPLYSLLDSKLQPRRLDQM--FNDIYHPDFQMITGQPSLHKYL
CrTUE   TMNLVPYPRMHFLLSSMSPLQPPP-KDKDPRTLDQVRVFGDVFSREHQLIRADPRAATYL
        --- ---                 MMMMMMMMMM MMM  MMMMMMMMMM

          S8           H10                 S9              S10
HsTUBE1 ACALMVRG-NVQISDLRRNIERLKPSLQFVSWNQEGWKTSLCSVPPVGHSHSLLALANNT
MmTUBE1 ACALIVRG-NVQISDLRRNIERLKPALQFVSWNQEGWKTSLCSVPPVGHSHSLLALANNT
XlTUBE1 ACALMVRG-NVQISDLRRNIERLKPSLQFVSWNQEGWKTGLCSVPPVGHSHSLLALANNT
TtETU1  AVGLMVRG-SVPFSDVNRNIKKLRSELNMIYWNQEGFKYGICNTPPIGQPYSLLCLANNT
PtPT1   AVGLLVRG-DVAFSDVNRNIKKLKDQLKMIYWNQEGFKYGICNQPPIGQQYSMLCLANNT
CrTUE   ACGLIARGPTATMADINRNVARLRPQLKMVHWNSEGFKLGICSTPPVGCPFGLLCLANNT
                M---   -    -         -----

             H11                             H12
HsTUBE1 CVKPTFMELKERFMRLYKKKAHLHHYLQVEGMEESCFTEAVSSLSALIQEYDQLDATKNM
MmTUBE1 CVKPTFMELRERFTRLYKKKAHLHHYLQVDGMEESTFTEAVSSLSALIQEYSDLDATKSL
XlTUBE1 CVKPTFIELRDRFSKLYRKKAHLHHYLQVDGMEQHCFTEAISSLSSLIEEYSQLDASKGI
TtETU1  AIRDQFSTMVERFNKLYKRKVFVHHYTEY--MDAKHFDEALNSITSLISKYEELHKAEEP
PtPT1   CIKDTFQEMTDRFQKLYKRKVYVHHYKQY--MEQSHFDETLNGIQNLMVKYQDLEMAQ-P
CrTUE   AIAHTFTTMRERFDKLYKRRFYTHHYEQY--MDPGGFTSAMEVVGDLTAQYRALEGATQA
                            ++ ++

HsTUBE1 PVQDLPRLS---IAM
MmTUBE1 PVPDVPRLS---VAL
XlTUBE1 HVLDPPRLN---IV-
TtETU1  SSQPRLKP----LI-
PtPT1   KKYQRIQP----IF-
CrTUE   PPLTRLRPRGLSFLP
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Fig. S1. Sequence alignment of ε-tubulin homologues. ε-tubulin amino acid sequences from *H. sapiens* (HsTUBE1), *M. musculus* (MmTUBE1), *X. laevis* (XlTUBE1), *T. thermophila* (TtETU1), *P. teraurelia* (PtPt1), and *C. reinhardtii* (CrTUE) were aligned using ClustalW 2.0 (Larkin et al., 2007). The predicted tubulin structural motifs, sheets and helices, are designated above the sequences (Inclan and Nogales, 2001). The residues predicted to be part of the ML surface (M), H3 surface (H), (+)-end (+), and (-)-end (-) are labeled below the sequences.

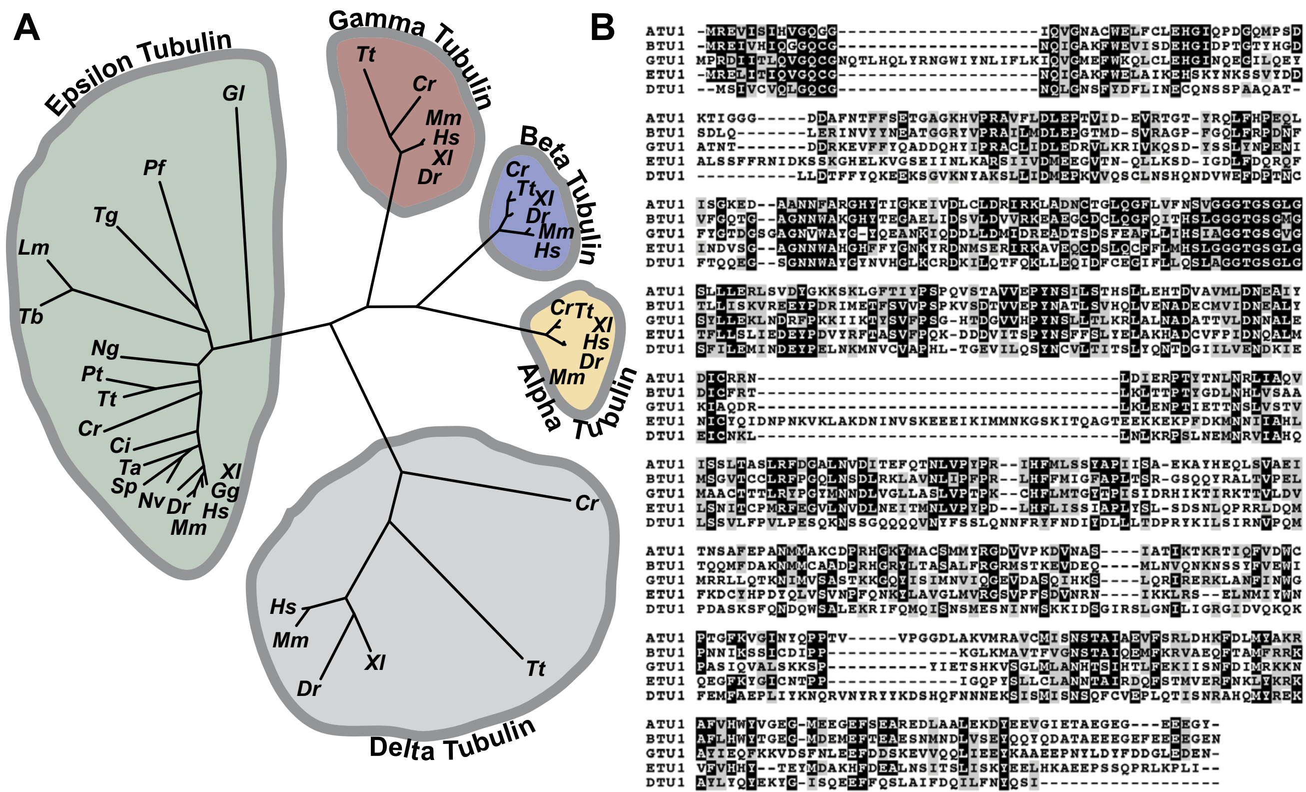

Fig. S2. Phylogenetic analysis of *ETU1*. (A) Sequences were identified and aligned for the 5 conserved tubulin family members. For α, β, γ, and δ-tubulin, 5 representative sequences were used. In the case of ε-tubulin we used as many known or predicted sequences as could be identified. The sequences were aligned using MUSCLE (Edgar, 2004) and the phylogeny was determined with PhyML (Guindon et al., 2010) using Phylogeny.fr (Dereeper et al., 2008). *Tetrahymena* ε-tubulin clustered with other ε-tubulin proteins from other organisms indicating that it is indeed a unique branch of the tubulin superfamily. *Tt=T. thermophila, Cr=C. reinhardtii, Xl=X. laevis, Hs=H. sapiens, Dr=D. rerio, Mm=M. musculus, Gl=G. lamblia, Pf=P. falciparum, Tg=t. gondii, Lm=Leishmania major, Tb=T. brucei, Ng=N. gruberi, Pt=P. tetra, Ci=C. intestinalis, Ta=T. adhaerens, Sp=S. purpuratus, Nv=N. vectensis, Gg=G. gallus*. (B) The *Tetrahymena* α, β, γ, δ, and ε-tubulin sequences were aligned. Although the family members share a high degree of identity and similarity, unique features are visible. ε-tubulin contains two unique and conserved insertions at amino acids 60 and 221 of 5 and 36 amino acids, respectively.

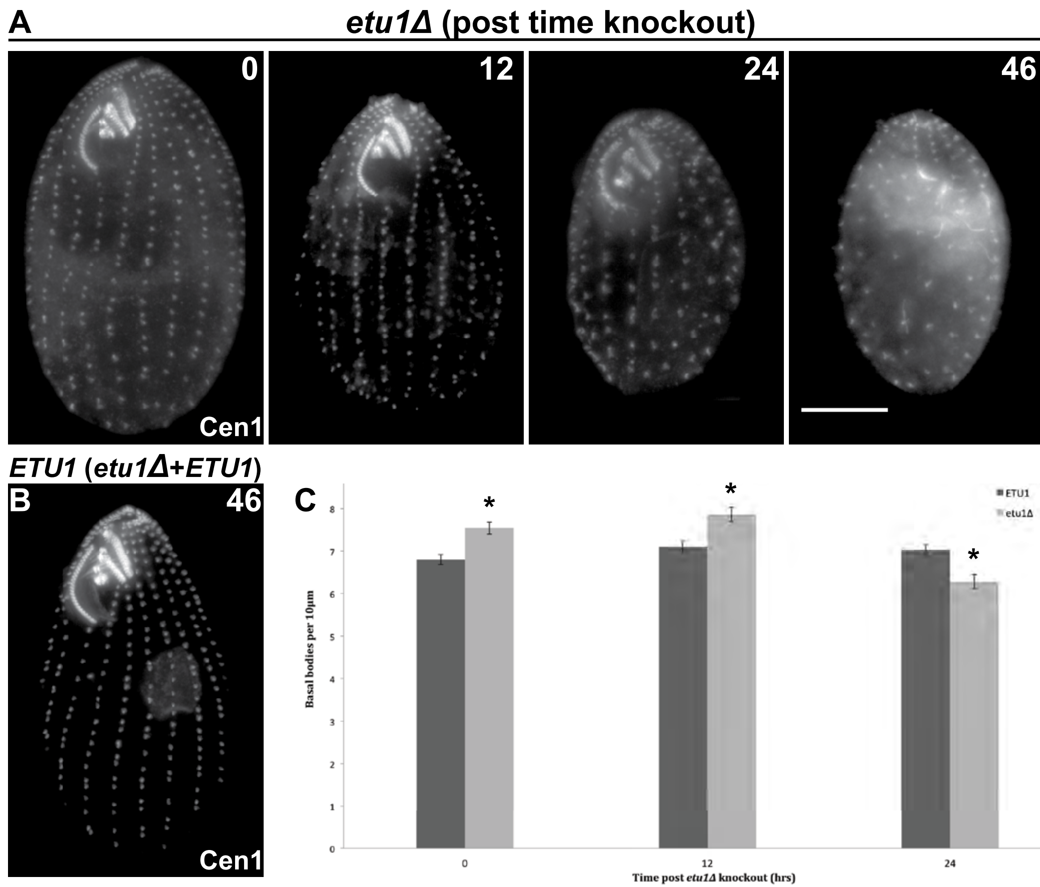

Fig. S3. *ETU1* is essential for viability and maintenance of basal body number. (A) *etu1*Δ cells were fixed and stained for centrin (Cen1) at the times indicated post knockout. Scale=10 µm. (B) An *etu1*Δ strain was mated to a wild-type strain to create an *ETU1* strain. Scale=10 µm. (C) Basal body density was measured within 10 µm of a cortical row. At 0 and 12 hrs, the *etu1*Δ strain had a significantly higher basal body density than *ETU1*. 24 hours after knockout the *etu1*Δ strain showed a significant decrease in basal body numbers. *n*=100 cortical rows from 20-22 cells. Error bars=s.e.m. *P<0.01.

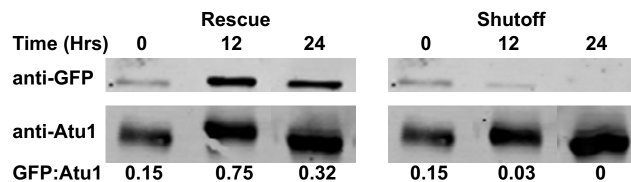

Fig. S4. Western blot analysis of *GFP-ETU1* expression. Cells were grown in the presence (rescue) or absence (shutoff) of $CdCl_2$ and then samples were taken at the indicated times after depletion of GFP-Etu1p and blotted for GFP and Atu1. The ratio of GFP to Atu1 signal was calculated and is indicated below the corresponding time.

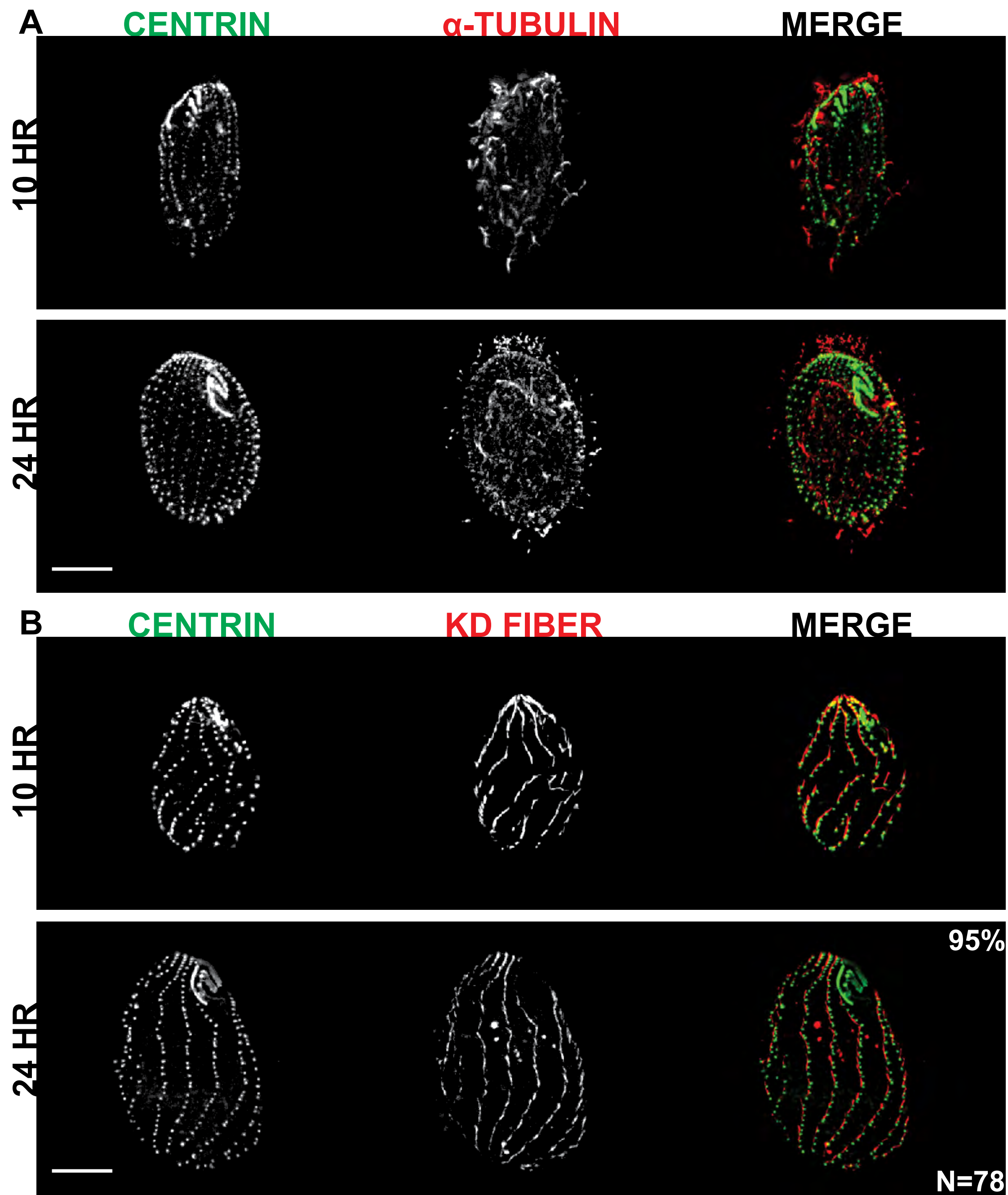

Fig. S5. Depletion of GFP-Etu1p is reversible. After 24 hrs of depletion of GFP-Etu1p, $CdCl_2$ was re-introduced to induce expression of *GFP-ETU1*. Cells were then fixed after 10 hrs and 24 hrs of induction. (A) Cells were stained for Cen1 (centrin) and Atu1 (α-tubulin). Basal body organization is nearly fully recovered after 24 hrs and the cells have cilia. (B) Staining of the KD fiber reveals that basal bodies have also regained proper orientation after 24 hrs (95%). *n*=78 cells. Scale=10 µm.

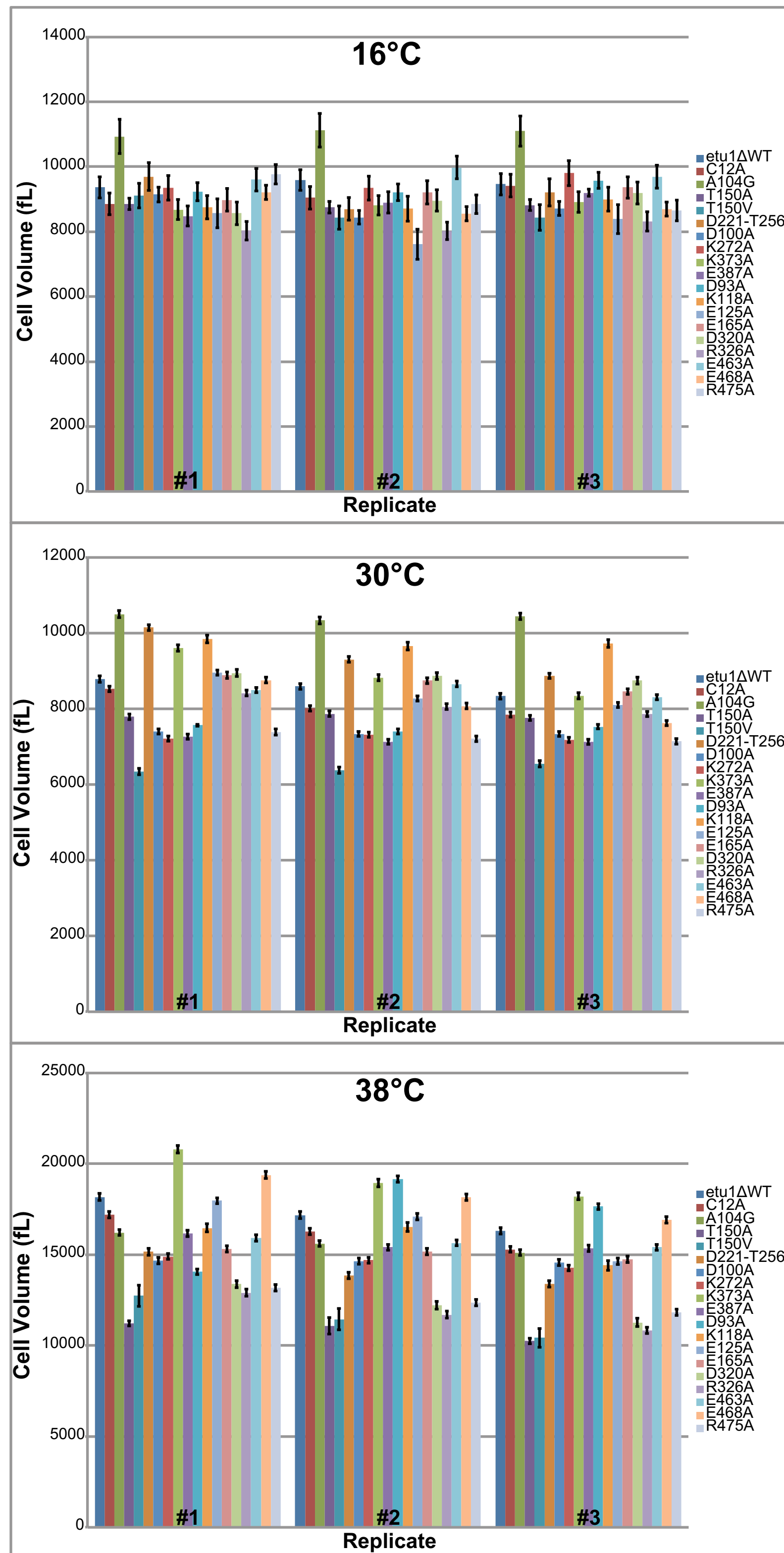

Fig. S6. Size analysis of *ETU1* mutants. *ETU1* mutant cells were grown at 16°C (A), 30°C (B), and 38°C (C) for 24 hrs then assayed for cell size using a Z2 Coulter counter. Cell counts ranged from 315-14,454 cells, depending on the strain and condition. Each mutant was assayed three times for each condition. Error bars=CI of 95%.

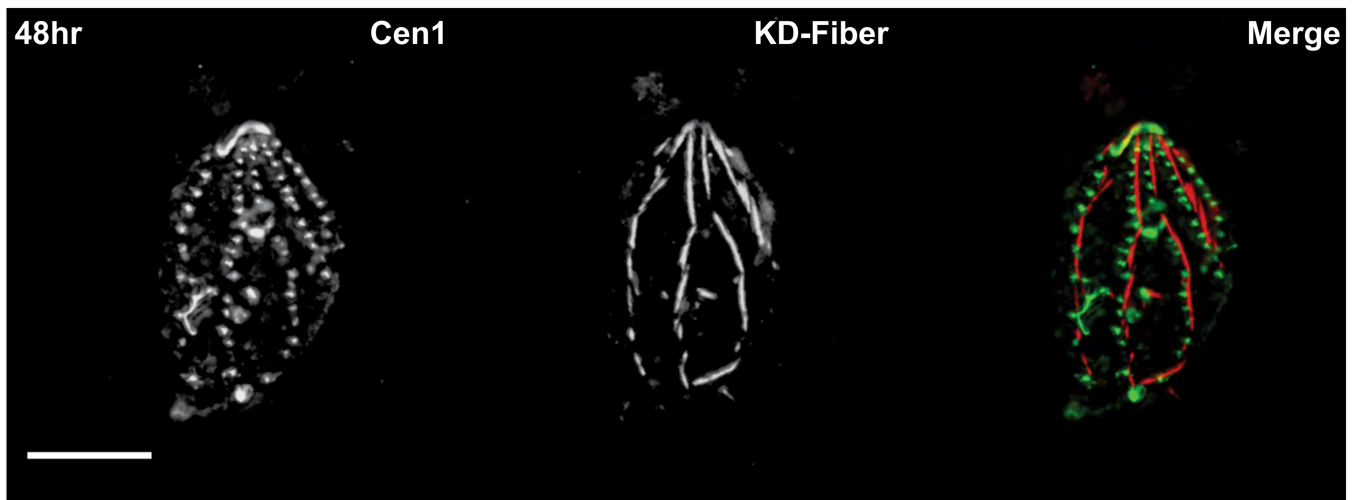

Fig. S7. Starved Etu1-T150V cells maintain basal body organization. Etu1-T150V cells lose basal body density but maintain organization within cortical rows as indicated by the KD fiber staining. This indicates that the misoriented basal bodies observed in cycling cells are likely newly assembled basal bodies that are misoriented during assembly. Scale=10 μm.