Winsor et al., https://doi.org/10.1083/jcb.201609071

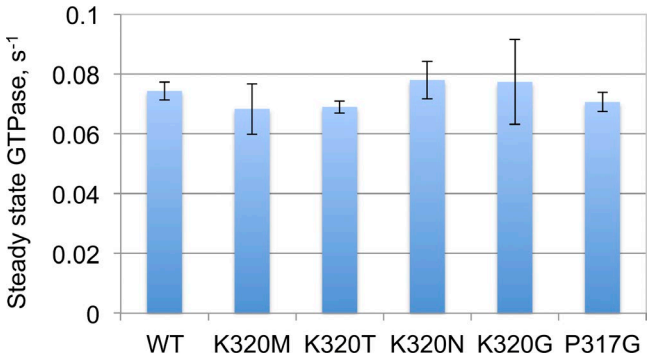

Figure S1. **All mutant variants have similar steady-state GTPase activity.** 1 µM of the indicated cytoDATL mutant variants was incubated with 1 mM GTP and subjected to a coupled enzyme assay (Materials and methods) measuring the production of GDP over time (micromoles GDP s^{-1} per micromole cytoDATL ± SD). WT, wild type.

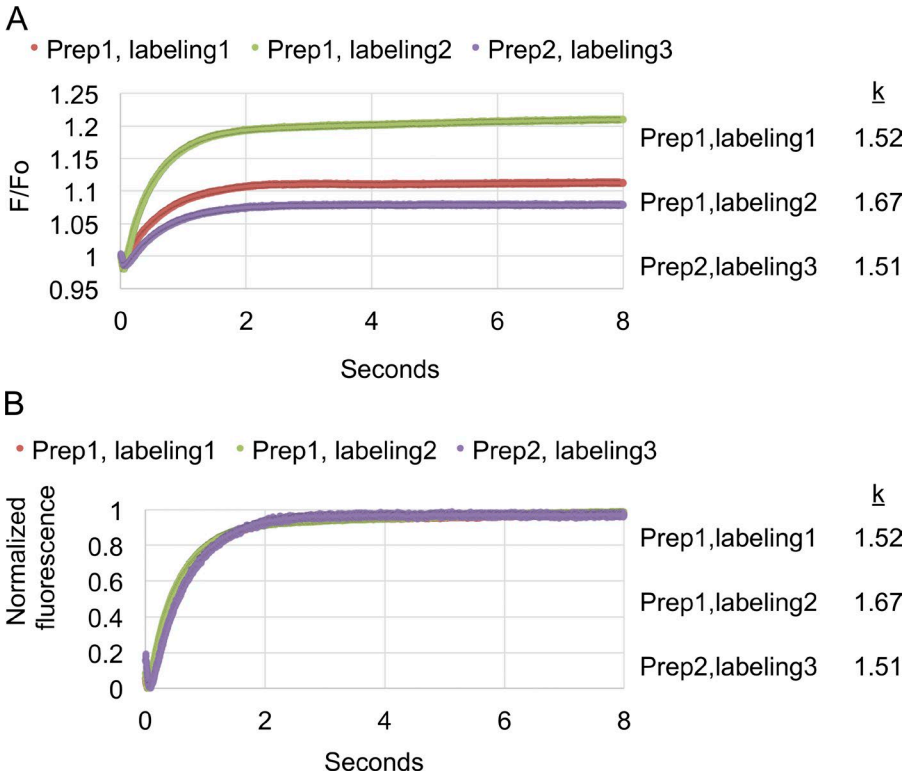

Figure S2. **Normalization has no effect on reaction kinetics of PIFE.** (A) PIFE, or fluorescence enhancement (F/Fo), over time when 1 µM K320G Cy3-cytoDATL from three independent labeling reactions and two independent protein preparations is mixed with 1 mM GTP. (B) The same data in A after normalization. A comparison of the reaction rates, k, from fitting the rising component of each trace to a single exponential decay equation, for each trace either before (A) or after (B) normalization, indicates that normalization has no effect on the reaction kinetics.

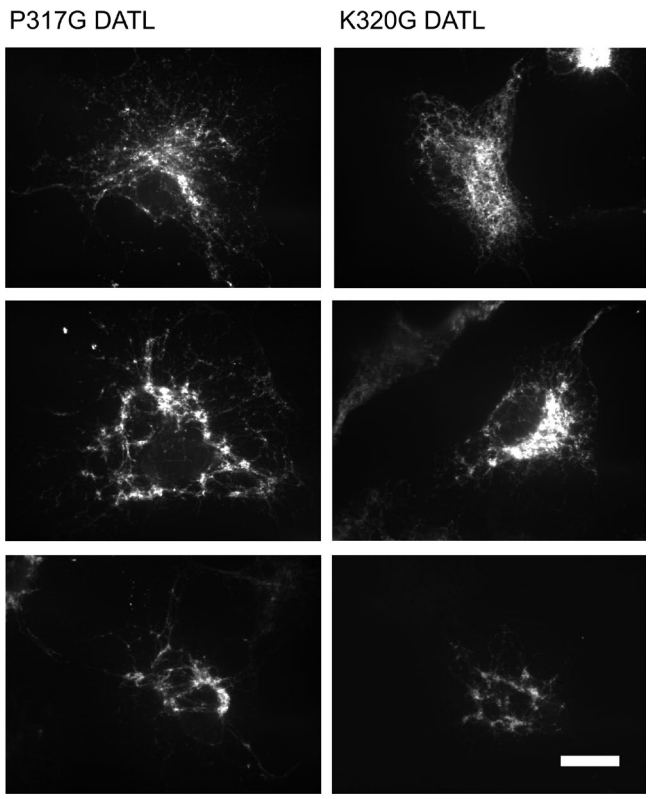

Figure S3. **Expression of P317G and K320G DATL causes a range of defective ER morphologies.** COS-7 cells transfected with either P317G or K320G full-length Venus-tagged DATL were fixed and imaged 48 h later by confocal microscopy. Bar, 10 µm.

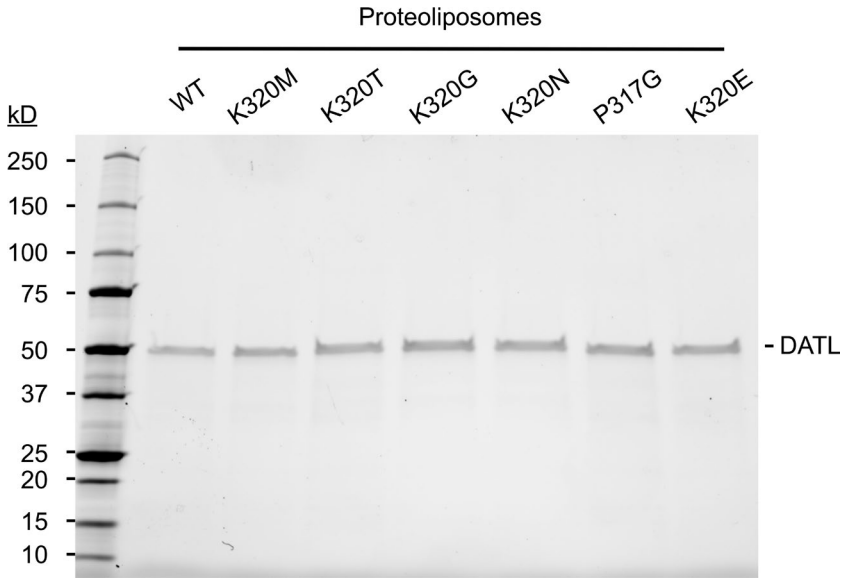

Figure S4. **All mutant variants of full-length DATL are efficiently incorporated into vesicles.** The indicated DATL proteins, purified and incorporated into labeled (donor) and unlabeled (acceptor) vesicles at a 1:1,000 protein/lipid ratio, were subjected to a (50%/45%/0%) Nycodenz flotation step gradient to separate incorporated from unincorporated protein. After desalting, equivalent amounts of each sample were resolved by SDS-PAGE and visualized with UV-induced tryptophan-bound trihalo fluorescence. Only the unlabeled vesicles are shown. WT, wild type.