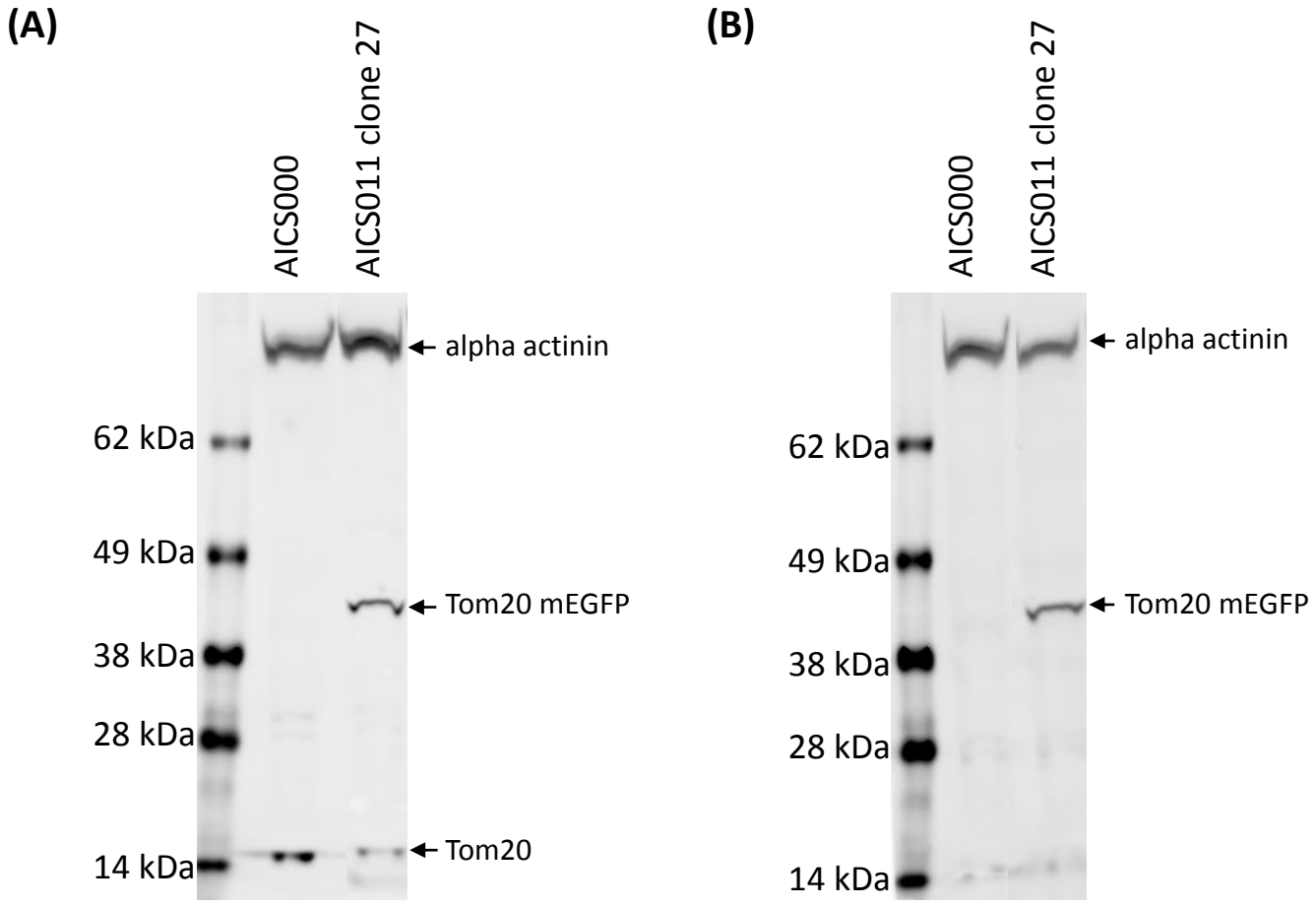

Whole cells lysate were extracted from AICS000 and AICS011 cells using M-PER buffer supplemented with protease inhibitors and nuclease. 27 µg whole lysate was separated on 4-12% Bolt Bis-Tris Plus gels and transferred onto 0.45 µm nitrocellulose membranes. Untagged and GFP-tagged Tom20 was detected using (A) mouse monoclonal anti-Tom20 antibody (Santa Cruz Biotechnologies #sc-17764 1:250 in PBS-T with 5% milk, overnight at 4°C) and (B) mouse monoclonal anti-GFP antibody (Sigma-Aldrich #11814460001; 1:250 in PBS-T with 5% milk, overnight at 4°C). Alpha actinin was used as loading control (mouse monoclonal anti-alpha actinin; ThermoFisher, #MA1-91860; 1:2,000 in PBS-T with 5% milk, overnight at 4°C). A goat polyclonal anti-mouse Alexa 647-conjugated secondary antibody was used (1:10,000 in in PBS-T with 5% milk, 1.5 hrs at room temperature). Blots were imaged at different exposure times using the ChemiDoc MP system (BioRad). Appropriate exposure times were used for semi-quantification of protein levels. Multiple clones of AICS011 were analyzed on the same blot and the blot was cropped to only show the final clone of AICS011.

	AICS000	AICS011 clone 27
relative levels of total Tom20	1.00	1.44*
relative levels of mEGFP-tagged Tom20 in AICS011 clone 27	N/A	0.54

* total protein levels of Tom20 in AICS011 clone 27 might be lower than reflected in the semi-quantitative analysis as the molecular weight difference between untagged and mEGFP-tagged Tom20 is significant. Therefore, transfer efficiency and the amount of protein per band exposed to antibody might be different between the untagged and the mEGFP-tagged protein.