



Whole cells lysate were extracted from AICS000 and AICS012 cells using M-PER buffer supplemented with protease inhibitors and nuclease. 5 μ g whole lysate was separated on 4-12% Bolt Bis-Tris Plus gels and transferred onto 0.45 μ m nitrocellulose membranes. Untagged (indicated with dashed boxes) and GFP-tagged (indicated with solid boxes) alpha tubulin was detected using (A) mouse monoclonal anti-alpha tubulin antibody (ThermoFisher #62204; 1:10,000 in PBS-T with 5% milk, 2 hrs at room temperature) and (B) mouse monoclonal anti-GFP antibody (Sigma-Aldrich #11814460001; 1:250 in PBS-T with 5% milk, 2hrs at room temperature). Beta actin (indicated in with dotted boxes) was used as loading control (mouse monoclonal anti-beta actin; ThermoFisher, #MA5-15739; 1:10,000 in PBS-T with 5% milk, 2hrs at room temperature). A goat polyclonal anti-mouse Alexa 654-conjugated secondary antibody was used (1:10,000 in in PBS-T with 5% milk, 1.5 hrs at room temperature) and the blots were imaged using ChemiDoc MP system (BioRad). AICS012 final clones are indicated in bold.

Quantification of levels of all alpha tubulin isoforms in AICS000 and AICS012 cells

Antibody recognizes all alpha tubulin isoforms and not only TUBA1B

	relative levels of total alpha tubulin protein
AICS000	1.00
AICS012 clone 100	0.87
AICS012 clone 101	0.83
AICS012 clone 103	0.80
AICS012 clone 105	0.83

	relative levels within clones	
	GFP-tagged alpha tubulin	untagged alpha tubulin
AICS000	0.00	1.00
AICS012 clone 100	0.29	0.71
AICS012 clone 101	0.28	0.72
AICS012 clone 103	0.26	0.74
AICS012 clone 105	0.25	0.75

	arbriatry units of		
	total alpha tubulin	GFP-tagged alpha tubulin	untagged alpha tubulin
AICS000	100	0	100
AICS012 clone 100	87	25	62
AICS012 clone 101	83	23	60
AICS012 clone 103	80	21	59
AICS012 clone 105	83	21	62

Quantification of TUBA1B-specific alpha tubulin protein levels in AICS000 and AICS012 cells

45% of all alpha tubulin isoforms in WTC cells are expressed by TUBA1B. This means that if 50% of TUBA1B-specific alpha tubulin are GFP-tagged in the clones, then the relative adjusted levels of GFP-tagged TUBA1B-specific alpha tubulin would be 0.225 (half of 0.45)

	adjusted relative levels within clones GFP-tagged TUBA1B-specific alpha tubulin
AICS000	0.00
AICS012 clone 100	0.65
AICS012 clone 101	0.62
AICS012 clone 103	0.58
AICS012 clone 105	0.55

	arbriatry units of		
	total TUBA1B-specifc alpha tubulin protein	GFP-tagged TUBA1B- specifc alpha tubulin	untagged TUBA1B-specifc alpha tubulin
AICS000	100		
AICS012 clone 100	87	57	30
AICS012 clone 101	83	51	32
AICS012 clone 103	80	46	34
AICS012 clone 105	82	45	37