

Supplemental figure 1. Purification of fission yeast Act1 and human non-muscle actins

(A) Endogenous *P. pastoris* actin does not co purify with the expressed recombinant actin. Proteins bound on nickel beads were eluted with SDS-PAGE sample buffer just before chymotrypsin cleavage and analysed by SDS-PAGE and CBB staining (Lane 1). Eluates collected after chymotrypsin treatments were also loaded on SDS-PAGE gel (Lane 2). Note that a 43 kDa protein is visualized only after cleavage with chymotrypsin and not in the uncleaved sample, establishing that *P. pastoris* actin does not co-purify detectably.

(B) Fluorescent labelling of arginylated beta-actin with Alexa Fluor 488 C5 maleimide and TMR-5-maleimide.

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ACT_KOMPG/1-376 1 MDGEDVAALVINDNGSGMCKAGYAGDDAPHTVFPSSVGRPRHQGVVMGMGQKDSFVGDEAQSQRGILTLKYP IEHGIV 77
ACTG_HUMAN/1-375 1 - MEEEIAALVINDNGSGMCKAGFAGDDAPRAVFPSSVGRPRHQGVVMGMGQKDSYVGDEAQSQRGILTLKYP IEHGIV 76
ACTB_HUMAN/1-375 1 - MDDDI AALVVDNGSGMCKAGFAGDDAPRAVFPSSVGRPRHQGVVMGMGQKDSYVGDEAQSQRGILTLKYP IEHGIV 76
R-ACTB_HUMAN/1-374 1 - - RDDIAALVVDNGSGMCKAGFAGDDAPRAVFPSSVGRPRHQGVVMGMGQKDSYVGDEAQSQRGILTLKYP IEHGIV 75

ACT_KOMPG/1-376 78 TNWDDMEKIWHHTFYNELRLAPEEHPVLLTEAPMNPKNREKMTQIMFETFNVPAYVSIQAVLSLYASGRITGIVL 154
ACTG_HUMAN/1-375 77 TNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPLNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRITGIVM 153
ACTB_HUMAN/1-375 77 TNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPLNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRITGIVM 153
R-ACTB_HUMAN/1-374 76 TNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPLNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRITGIVM 152

ACT_KOMPG/1-376 155 DSGDGVTHVVP IYAGFSLPHAILRLDLACRDLDYLMKILSERGYTFTSAEREIVRDIKEKLCYVALDFDQELQTS 231
ACTG_HUMAN/1-375 154 DSGDGVTHVTP IYEGYALPHAILRLDLACRDLDYLMKILTERGYSFTTTAEREIVRDIKEKLCYVALDFEQEMATA 230
ACTB_HUMAN/1-375 154 DSGDGVTHVTP IYEGYALPHAILRLDLACRDLDYLMKILTERGYSFTTTAEREIVRDIKEKLCYVALDFEQEMATA 230
R-ACTB_HUMAN/1-374 153 DSGDGVTHVTP IYEGYALPHAILRLDLACRDLDYLMKILTERGYSFTTTAEREIVRDIKEKLCYVALDFEQEMATA 229

ACT_KOMPG/1-376 232 SQSSSIEKSYELPDGQVITIGNERFRCEALFHPSSLGLGEASGIDQTTYSIMKCDVDVRKELYSNIVMSGGTTMFP 308
ACTG_HUMAN/1-375 231 ASSSSLEKSYELPDGQVITIGNERFRCEALFQPSFLGMESCGIHETTFNSIMKCDVDIRKDLANTVLSGGTTMFP 307
ACTB_HUMAN/1-375 231 ASSSSLEKSYELPDGQVITIGNERFRCEALFQPSFLGMESCGIHETTFNSIMKCDVDIRKDLANTVLSGGTTMFP 307
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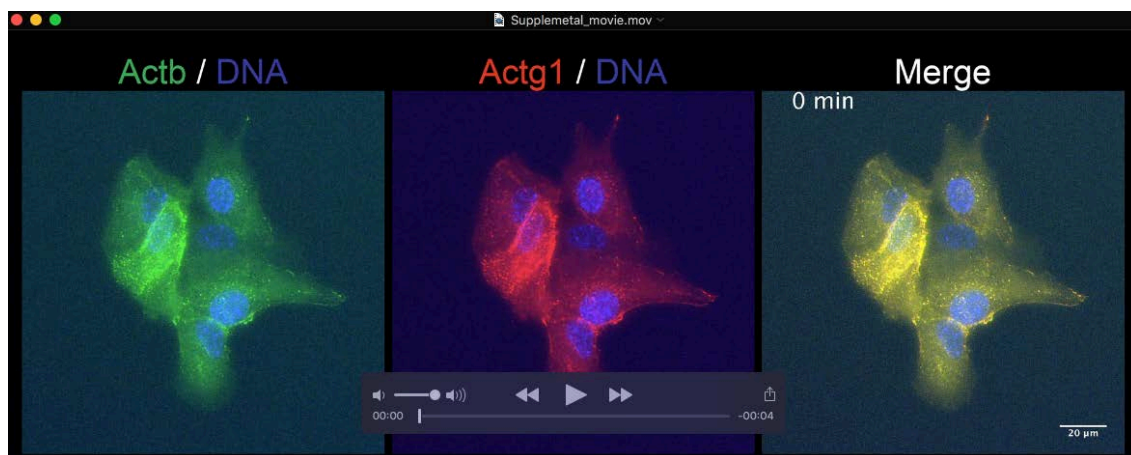
ACT_KOMPG/1-376 309 GIAERIMQKELTALAPSSMKYKHSAPPERKYSVWIGGSILASLSTFQQMWISKQEYDESGPSIVHVKCF 376
ACTG_HUMAN/1-375 308 GIADRMQKEITLALAPSTMKIKIAPPERKYSVWIGGSILASLSTFQQMWISKQEYDESGPSIVHVKCF 375
ACTB_HUMAN/1-375 308 GIADRMQKEITLALAPSTMKIKIAPPERKYSVWIGGSILASLSTFQQMWISKQEYDESGPSIVHVKCF 375
R-ACTB_HUMAN/1-374 307 GIADRMQKEITLALAPSTMKIKIAPPERKYSVWIGGSILASLSTFQQMWISKQEYDESGPSIVHVKCF 374

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Supplemental figure 2. NanoLC-ESI-MS/MS analysis of actin proteins

Alignment of amino acid sequence of *P. pastoris* Act1 (ACT_KOMPG), β -actin (ACTB_HUMAN), γ -actin (ACTG_HUMAN), and arginylated β -actin (R_ACTB). Mass spectrometry identified tryptic peptides that covered the entire human β , γ , and R- β -actins. The only exception was the C-terminal CF residues, which were identified as part of a larger C-terminal peptide recovered upon cleavage the SDS-PAGE purified sample containing unfolded actin with chymotrypsin (black box).

The *Pichia pastoris* actin is predicted to contain ~ 6 unique peptides upon cleavage with trypsin that differentiate it from human β and γ -actins. We only found one area in all mass spectrometry experiments that may be consistent with arising from *P. pastoris* actin, marked with red and green boxes. In this area it was possible to assigned spectra to two miscleaved peptides with suggested methylation modifications (+14 Da). However, the same spectra could also be assigned to the human actins without additional methylation because there are two amino acid differences between human and *P. pastoris* sequences in this area, both with a molecular mass difference of 14 Da (serine to threonine and valine to isoleucine). It is possible that there may be a trace of *P. pastoris* methylated actin in the recombinant actin preparations. However, the simplest explanation, consistent with all observed spectra, is that un-methylated human actin (not *P. pastoris* actin) has been identified.



Supplemental movie. Time-lapse images of RPE cells injected with fluorescent labelled β - and γ -actins.

Time lapse images of microinjected β - and γ -actins in RPE cells. β - and γ -actin were labelled with Alexa Fluor 488 and TMR, respectively and injected into human RPE cells. Scale bar =20 μ m. Images were acquired every 10 minutes.

Supplemental table 1. Peptides from trypsin-digested samples detected by mass spectrometric analysis.

[Click here to Download Table S1](#)

Supplemental table 2. Peptides from chymotrypsin-digested samples detected by mass spectrometric analysis.

[Click here to Download Table S2](#)