**Supplementary Material**

**Actin from the apicomplexan *Neospora caninum* (NcACT) has different isoforms in 2-D electrophoresis**

Luciana Baroni1; Letícia Pollo-Oliveira1; Albert JR Heck2; AF Maarten Altelaar2; Ana Patrícia Yatsuda1,3

1 Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Av. do Café, s/n, 14040-930, Ribeirão Preto, SP, Brazil.

2 Biomolecular Mass Spectrometry and Proteomics, Utrecht Institute for Pharmaceutical Sciences and Bijvoet Centre for Biomolecular Research, Utrecht

University, Padualaan 8, Utrecht 3884 CH, The Netherlands

**Supplementary table 5**. Actin peptides from mass spectrometry analysis. Western blot bands detected by the C4 antibody were localized with their correspondent bands at 1-D SDS-PAGE for excision and analysis by MS/MS. Significant and top ranking tryptic fragments (peptides) matches are exposed. The bold peptides are identical between Swissprot and ToxoDB searches for the same band.

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| --- | --- | --- | --- | --- | --- |
| **Cell extract** | **Band**  | **Swissprot ID** | **Peptides Swissprot** | **ToxoDB ID** | **Peptides ToxoDB** |
| Vero | 1 | P53478Actin, cytoplasmic type 5 (*Gallus gallus*) | 20 K.AGFAGDDAPR.A 29185 R.DLTDYLmK.I 192330 K.IKIIAPPER.K 336198 R.GYSFTTTAER.E 207317 K.EITALAPSTMK.I 32752 K.DSYVGDEAQSK.R 6230 R.AVFPSIVGRPR.H 4041 R.HQGVmVGmGQK.D 5152 K.DSYVGDEAQSKR.G 63**86 K.IWHHTFYNELR.V 96**361 K.QEYDESGPSIVHR.K 373198 R.GYSFTTTAEREIVR.D 211240 K.SYELPDGQVITIGNER.F 255**97 R.VAPEEHPVLLTEAPLNPK.A 114**293 K.DLYANTVLSGGTTmYPGIADR.M 313292 R.KDLYANTVLSGGTTMYPGIADR.M 31341 R.HQGVMVGMGQKDSYVGDEAQSK.R 62 | NCLIV\_003440 | 286 K.CDVDIR.K 29163 K.RGILTLK.Y 69208 K.EIVRDIKEK.L 216**86 K.IWHHTFYNELR.V 96****97 R.VAPEEHPVLLTEAPLNPK.A 114** |
| *N. caninum* | 2 | P53476 Actin (*Toxoplasma gondii*) | **330 K.VVAPPERK.Y 337** §**20 K.AGVAGDDAPR.A 29** §**317 K.ELTSLAPSTmK.I 327** §**41 K.NPGImVGmEEK.D 51** §**52 K.DCYVGDEAQSK.R 62** §**258 R.CPEALFQPSFLGK.E 270** §**361 K.EEYDESGPSIVHR.K 373** §**314 R.LTKELTSLAPSTmK.I 327** §**198 R.GYGFTTSAEKEIVR.D 211** §**179 R.LDLAGRDLTEYmmK.I 192** §**240 K.SYELPDGNIITVGNER.F 255** §**97 R.VAPEEHPVLLTEAPLNPK.A 114** §**292 R.KDLYGNVVLSGGTTmYEGIGER.L313** §**41 K.NPGImVGmEEKDCYVGDEAQSK.R 62** §**41 K.NPGImVGmEEKDCYVGDEAQSKR.G 63** § | NCLIV\_003440 | 286 K.CDVDIR.K 291**330 K.VVAPPERK.Y 337** §**20 K.AGVAGDDAPR.A 29** §278 R.TTFDSImK.C 285 §230 K.AAEDSSDIEK.S 239 §328 K.IKVVAPPERK.Y 337 §30 R.AVFPSIVGKPK.N 40 §**317 K.ELTSLAPSTmK.I 327** §**41 K.NPGIMVGmEEK.D 51** §**52 K.DCYVGDEAQSK.R 62** §**258 R.CPEALFQPSFLGK.E 270** §86 K.IWHHTFYNELR.V 96**361 K.EEYDESGPSIVHR.K 373** §**314 R.LTKELTSLAPSTmK.I 327** §**198 R.GYGFTTSAEKEIVR.D 211** §361 K.EEYDESGPSIVHRK.C 374 §**179 R.LDLAGRDLTEYMMK.I 192** §**240 K.SYELPDGNIITVGNER.F 255** §278 R.TTFDSIMKCDVDIRK.D 292 §198 R.GYGFTTSAEKEIVRDIK.E 214 §**97 R.VAPEEHPVLLTEAPLNPK.A 114****292 R.KDLYGNVVLSGGTTmYEGIGER.L 313** §**41 K.NPGImVGmEEKDCYVGDEAQSK.R 62** §**41 K.NPGImVGmEEKDCYVGDEAQSKR.G 63** § |
| 3 | P53505  Actin, cytoplasmic type 5 (*Xenopus laevis*) | 20 K.AGFAGDDAPR.A 29185 R.DLTDYLmK.I 192328 K.IKIIAPPER.K 337198 R.GYSFTTTAER.E 207317 K.EITALAPSTmK.I 32752 K.DSYVGDEAQSK.R 6230 R.AVFPSIVGRPR.H 4041 R.HQGVmVGmGQK.D 5152 K.DSYVGDEAQSKR.G 63361 K.QEYDESGPSIVHR.K 373314 R.mQKEITALAPSTmK.I 327240 K.SYELPDGQVITIGNER.F 2552 M.ADEEIAALVIDNGSGmCK.A 19 ¥ **97 R.VAPEEHPVLLTEAPLNPK.A 114**293 K.DLYANTVLSGGTTmYPGIADR.M 31341 R.HQGVmVGmGQKDSYVGDEAQSK.R 6241 R.HQGVmVGmGQKDSYVGDEAQSKR.G 63217 K.LCYVALDFEQEmATAASSSSLEK.S 239149 R.TTGIVmDSGDGVTHTVPIYEGYALPHAILR.L 178 | NCLIV\_003440 | 288 K.CDVDIR.K 291208 K.EIVRDIKEK.L 21686 K.IWHHTFYNELR.V 96179 R.LDLAGRDLTEYMMK.I 192 §**97 R.VAPEEHPVLLTEAPLNPK.A 114** |

§ Peptides that did not match with *Cercopithecus aethiops* beta-actin (Vero cell beta-actin) but matched with NcACT.

¥ Peptides that did not match neither with Vero cell beta-actin nor NcACT.

**Supplementary table 6**. Actin hits from mass spectrometry analysis of Vero cell extract. Western blot-correspondent spots detected by the C4 antibody were localized in their correspondent spots at 2-D SDS-PAGE for excision and analysis by MS/MS. Significant and top ranking tryptic fragments (peptides) are exposed. The bold peptides are identical between Swissprot and ToxoDB searches for the same band.

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| **Cell extract** | **Spot**  | **NCBI ID** | **Peptides NCBI** | **ToxoDB ID** | **Peptides ToxoDB** |
| Vero | 1 | P53505 Actin, cytoplasmic type 5 (*Xenopus laevis*)  | 20 K.AGFAGDDAPR.A 29185 R.DLTDYLmK.I 192328 K.IKIIAPPER.K 337198 R.GYSFTTTAER.E 207317 K.EITALAPSTMK.I 32752 K.DSYVGDEAQSK.R 6230 R.AVFPSIVGRPR.H 4041 R.HQGVmVGmGQK.D 5152 K.DSYVGDEAQSKR.G 63361 K.QEYDESGPSIVHR.K 373314 R.mQKEITALAPSTmK.I 327240 K.SYELPDGQVITIGNER.F 2552 M.ADEEIAALVIDNGSGMCK.A 19 ¥**97** **R.VAPEEHPVLLTEAPLNPK.A 114**293 K.DLYANTVLSGGTTmYPGIADR.M 313292 R.KDLYANTVLSGGTTmYPGIADR.M 31341 R.HQGVmVGmGQKDSYVGDEAQSK.R 6241 R.HQGVmVGmGQKDSYVGDEAQSKR.G 63217 K.LCYVALDFEQEmATAASSSSLEK.S 239149 R.TTGIVMDSGDGVTHTVPIYEGYALPHAILR.L 178 | NCLIV\_003440 | 286 K.CDVDIR.K 291208 K.EIVRDIKEK.L 21686 K.IWHHTFYNELR.V 96**97 R.VAPEEHPVLLTEAPLNPK.A 114** |
| 2 | P79818 Actin, cytoplasmic(*Oryzias latipes*) | 19 K.AGFAGDDAPR.A 28197 R.GYSFTTTAER.E 206316 K.EITALAPSTmK.I 32651 K.DSYVGDEAQSK.R 6129 R.AVFPSIVGRPR.H 3940 R.HQGVmVGmGQK.D 5051 K.DSYVGDEAQSKR.G 62360 K.QEYDESGPSIVHR.K 372313 R.MQKEITALAPSTMK.I 326197 R.GYSFTTTAEREIVR.D 210239 K.SYELPDGQVITIGNER.F 25496 R.IAPEEHPVLLTEAPLNPK.A 11319 K.AGFAGDDAPRAVFPSIVGRPR.H 39292 K.DLYANTVLSGGTTmYPGIADR.M 312291 R.KDLYANTVLSGGTTmYPGIADR.M 31240 R.HQGVmVGmGQKDSYVGDEAQSK.R 6140 R.HQGVmVGmGQKDSYVGDEAQSKR.G 62 | NCLIV\_003440 | 286 K.CDVDIRK.D 29186 K.IWHHTFYNELR.V 9697 R.VAPEEHPVLLTEAPLNPK.A 11497 R.VAPEEHPVLLTEAPLNPKANR.E 117 |
| 3 | P18600 Actin (*Artemia* sp.)  | 20 K.AGFAGDDAPR.A 29185 R.DLTDYLmK.I 192198 R.GYSFTTTAER.E 20752 K.DSYVGDEAQSK.R 6241 R.HQGVmVGmGQK.D 5152 K.DSYVGDEAQSKR.G 63**86 K.IWHHTFYNELR.V 96**198 R.GYSFTTTAEREIVR.D 211240 K.SYELPDGQVLTIGNER.F 255 ¥97 R.VAPEEHPILLTEAPLNPK.A 114¥293 K.DLYANTVLSGGTTmYPGIADR.M 313292 R.KDLYANTVLSGGTTmYPGIADR.M 31341 R.HQGVmVGmGQKDSYVGDEAQSK.R 62 | NCLIV\_003440 | **86 K.IWHHTFYNELR.V 96**97 R.VAPEEHPVLLTEAPLNPK.A 114 |

¥ Peptides that did not match neither with Vero cell beta-actin nor NcACT.

**Supplementary table 7.** Actin hits from mass spectrometry analysis of *N. caninum* extract. Western blot spots detected by C4 antibody were localized with their correspondent spots at 2-D SDS-PAGE for excision and analysis by MS/MS. Significant and top ranking tryptic fragments (peptides) are exposed. The bold peptides are identical between Swissprot and ToxoDB searches for the same band.

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| **Cell extract** | **Spot**  | **Swissprot ID** | **Peptides - Swissprot** | **ToxoDB ID** | **Peptides - ToxoDB** |
| *N. caninum* | 1 | P60712 Actin, cytoplasmic 1 (*Bos taurus*) | 19 K.AGFAGDDAPR.A 28360 K.QEYDESGPSIVHR.K 362239 K.SYELPDGQVITIGNER.F 254 | NCLIV\_003440 | 20 K.AGVAGDDAPR.A 29 §52 K.DCYVGDEAQSK.R 62 § |
| 2 | P68556Actin (*Diphyllobothrium dendriticum*) | **21 K.AGFAGDDAPR.A 30**199 R.GYSFTTTAER.E 208**317 K.EITSLAPSTmK.I 327**241 K.SYELPDGQVITIGNER.F 256292 R.KDLYANTVLSGGTTmYPGIADR.M 31341 R.HQGVMVGMGQKDSYVGDEAQSKR.G 63 | NCLIV\_003440 | 330 K.VVAPPERK.Y 337 §**20 K.AGVAGDDAPR.A 29 §**328 K.IKVVAPPERK.Y 337 §**317 K.ELTSLAPSTmK.I 327** §52 K.DCYVGDEAQSKR.G 63 §86 K.IWHHTFYNELR.V 96314 R.LTKELTSLAPSTmK.I 327 § 179 R.LDLAGRDLTEYmmK.I 192 § 97 R.VAPEEHPVLLTEAPLNPK.A 114 |
| 3 | P53476 Actin (*Toxoplasma gondii*) | **20 K.AGVAGDDAPR.A 29** § **276 R.TTFDSImK.C 285** § **30 R.AVFPSIVGKPK.N 40** § **317 K.ELTSLAPSTmK.I 327** §**41 K.NPGImVGmEEK.D 51** §**52 K.DCYVGDEAQSK.R 62** §**217 K.LCYIALDFDEEmK.A 229** §**240 K.SYELPDGNIITVGNER.F 255** §**97 R.VAPEEHPVLLTEAPLNPK.A 114**70 K.YPIEHGIVTNWDDmEK.I 85**292 R.KDLYGNVVLSGGTTmYEGIGER.L 313** §**41 K.NPGImVGmEEKDCYVGDEAQSK.R 63** § | NCLIV\_003440 | 286 K.CDVDIR.K 291**20 K.AGVAGDDAPR.A 29** §**278 R.TTFDSImK.C 285** §185 R.DLTEYmmK.I 192 §**30 R.AVFPSIVGKPK.N 40** §**317 K.ELTSLAPSTmK.I 327** §**41 K.NPGImVGmEEK.D 51** §**52 K.DCYVGDEAQSK.R 62** §86 K.IWHHTFYNELR.V 96361 K.EEYDESGPSIVHR.K 373 §**217 K.LCYIALDFDEEmK.A 229** §**240 K.SYELPDGNIITVGNER.F 255** §256 R.FRCPEALFQPSFLGK.E 270 §**97 R.VAPEEHPVLLTEAPLNPK.A 114** **292 R.KDLYGNVVLSGGTTmYEGIGER.L 313** §**41 K.NPGImVGmEEKDCYVGDEAQSK.R 62** § |
| 4 | P53476 Actin (*Toxoplasma gondii*) | **20 K.AGVAGDDAPR.A 29** §**198 R.GYGFTTSAEK.E 207** §**230 K.AAEDSSDIEK.S 239** §**30 R.AVFPSIVGKPK.N 40** §**41 K.NPGImVGmEEK.D 51** §**52 K.DCYVGDEAQSK.R 62** §**52 K.DCYVGDEAQSKR.G 63** §**258 R.CPEALFQPSFLGK.E 270** §**86 K.IWHHTFYNELR.V 96**207 K.LCYIALDFDEEmK.A 229 §**240 K.SYELPDGNIITVGNER.F 255** §97 R.VAPEEHPVLLTEAPLNPK.A 11470 K.YPIEHGIVTNWDDmEK.I 85292 R.KDLYGNVVLSGGTTMYEGIGER.L 313 §41 K.NPGIMVGMEEKDCYVGDEAQSK.R 62 § | NCLIV\_003440 | 330 K.VVAPPER.K 336 §286 K.CDVDIR.K 291**20 K.AGVAGDDAPR.A 29** §278 R.TTFDSImK.C 285 §**198 R.GYGFTTSAEK.E 207** §185 R.DLTEYmmK.I 192 §**230 K.AAEDSSDIEK.S 239** §**30 R.AVFPSIVGKPK.N 40** §317 K.ELTSLAPSTmK.I 327 § **41 K.NPGImVGmEEK.D 51** §**52 K.DCYVGDEAQSK.R 62** §**52 K.DCYVGDEAQSKR.G 63** §**258 R.CPEALFQPSFLGK.E 270** §**86 K.IWHHTFYNELR.V 96**198 R.GYGFTTSAEKEIVR.D 211 §217 K.LCYIALDFDEEmK.A 229 § **240 K.SYELPDGNIITVGNER.F 255** § |
| 5 | P53476 Actin (*Toxoplasma gondii*) | **20 K.AGVAGDDAPR.A 29** §198 R.GYGFTTSAEK.E 207 §**30 R.AVFPSIVGKPK.N 40** §**317 K.ELTSLAPSTmK.I 327** §**41 K.NPGImVGmEEK.D 51** §**52 K.DCYVGDEAQSK.R 62** §**258 R.CPEALFQPSFLGK.E 270** §**314 R.LTKELTSLAPSTmK.I 327** §**198 R.GYGFTTSAEKEIVR.D 211** §**217 K.LCYIALDFDEEmK.A 229** §**240 K.SYELPDGNIITVGNER.F 255** §**97 R.VAPEEHPVLLTEAPLNPK.A 114**70 K.YPIEHGIVTNWDDmEK.I 85**292 R.KDLYGNVVLSGGTTmYEGIGER.L 313** §**41 K.NPGImVGmEEKDCYVGDEAQSK.R 62** §**41 K.NPGImVGmEEKDCYVGDEAQSKR.G 63** § | NCLIV\_003440 | 330 K.VVAPPER.K 336 §330 K.VVAPPERK.Y 337 §**20 K.AGVAGDDAPR.A 29** §278 R.TTFDSImK.C 285 §185 R.DLTEYmmK.I 192 § 328 K.IKVVAPPERK.Y 337 §**30 R.AVFPSIVGKPK.N 40** §**317 K.ELTSLAPSTmK.I 327** §**41 K.NPGImVGmEEK.D 51** §**52 K.DCYVGDEAQSK.R 62** §**258 R.CPEALFQPSFLGK.E 270** §86 K.IWHHTFYNELR.V 96**314 R.LTKELTSLAPSTmK.I 327** §**198 R.GYGFTTSAEKEIVR.D 211** §**217 K.LCYIALDFDEEmK.A 229** §**240 K.SYELPDGNIITVGNER.F 255** §

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256 R.FRCPEALFQPSFLGK.E 270 §**97 R.VAPEEHPVLLTEAPLNPK.A 114****292 R.KDLYGNVVLSGGTTmYEGIGER.L 313** § **41 K.NPGImVGmEEKDCYVGDEAQSK.R 62** §**41 K.NPGImVGmEEKDCYVGDEAQSKR.G 63** § |
| 6 | P53476 Actin (*Toxoplasma gondii*) | **20 K.AGVAGDDAPR.A 29** §**198 R.GYGFTTSAEK.E 207** §**240 K.SYELPDGNIITVGNER.F 255** § **97 R.VAPEEHPVLLTEAPLNPK.A 114****41 K.NPGImVGmEEKDCYVGDEAQSK.R 62** § | NCLIV\_003440 | 330 K.VVAPPER.K 336 § **20 K.AGVAGDDAPR.A 29** §**198 R.GYGFTTSAEK.E 207** §317 K.ELTSLAPSTmK.I 327 §**240 K.SYELPDGNIITVGNER.F 255** §**97 R.VAPEEHPVLLTEAPLNPK.A 114****41 K.NPGImVGmEEKDCYVGDEAQSK.R 62** § |
| 7 | Q5JAK2 Actin (*Rana lessonae*) | 19 K.AGFAGDDAPR.A 28197 R.GYSFTTTAER.E 206316 K.EITALAPSTmK.I 32651 K.DSYVGDEAQSK.R 6129 R.AVFPSIVGRPR.H 3940 R.HQGVmVGmGQK.D 5051 K.DSYVGDEAQSKR.G 62**85 K.IWHHTFYNELR.V 95**360 K.QEYDESGPSIVHR.K 372313 R.mQKEITALAPSTmK.I 326239 K.SYELPDGQVITIGNER.F 254**96 R.VAPEEHPVLLTEAPLNPK.A 113**392 K.DLYANTVLSGGTTmYPGIADR.M 312291 R.KDLYANTVLSGGTTMYPGIADR.M 31240 R.HQGVMVGMGQKDSYVGDEAQSK.R 61 | NCLIV\_003440 | 286 K.CDVDIR.K 291 52 K.DCYVGDEAQSK.R 62 §**86 K.IWHHTFYNELR.V 96****97 R.VAPEEHPVLLTEAPLNPK.A 114** |
| 8 | P53505 Actin, cytoplasmic type 5 (*Xenopus laevis*) | 20 K.AGFAGDDAPR.A 29185 R.DLTDYLmK.I 192198 R.GYSFTTTAER.E 207317 K.EITALAPSTmK.I 32752 K.DSYVGDEAQSK.R 6230 R.AVFPSIVGRPR.H 4041 R.HQGVmVGmGQK.D 5152 K.DSYVGDEAQSKR.G 63**86 K.IWHHTFYNELR.V 96**361 K.QEYDESGPSIVHR.K 373314 R.mQKEITALAPSTmK.I 327240 K.SYELPDGQVITIGNER.F 255**97 R.VAPEEHPVLLTEAPLNPK.A 114** | NCLIV\_003440 | 286 K.CDVDIR.K 29120 K.AGVAGDDAPR.A 29 §41 K.NPGImVGmEEK.D 51 §52 K.DCYVGDEAQSK.R 62 § **86 K.IWHHTFYNELR.V 96****97 R.VAPEEHPVLLTEAPLNPK.A 114** |
| 9 | P79818 Actin, cytoplasmic 1 (*Oryzias latipes*) | 19 K.AGFAGDDAPR.A 28197 R.GYSFTTTAER.E 206327 K.IKIIAPPERK.Y 336316 K.EITALAPSTmK.I 32651 K.DSYVGDEAQSK.R 6119 R.AVFPSIVGRPR.H 2840 R.HQGVmVGmGQK.D 5051 K.DSYVGDEAQSKR.G 62360 K.QEYDESGPSIVHR.K 362313 R.mQKEITALAPSTmK.I 326197 R.GYSFTTTAEREIVR.D 210239 K.SYELPDGQVITIGNER.F 254292 K.DLYANTVLSGGTTmYPGIADR.M 312 | NCLIV\_003440 | 20 K.AGVAGDDAPR.A 29 §30 R.AVFPSIVGKPK.N 40 §41 K.NPGImVGmEEK.D 51 §52 K.DCYVGDEAQSK.R 62 §86 K.IWHHTFYNELR.V 9697 R.VAPEEHPVLLTEAPLNPK.A 114 |

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§ Peptides that did not match with *Cercopithecus aethiops* beta-actin (Vero cell beta-actin) but matched with NcACT.

**Supplementary table 8.** Predicted post-translational modifications (PTMs) sites in NcACT.

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| **PTM** | **Position (amino acid)** | **Score** | **Cutoff value** |
| Ubiquitination | 85 (K) | 0.99 | 0.75 |
| S-nytrosylation | 286 (C) | 2.614 | 2.443 |
| K-acetylation | 214 (K) | 1.359 | 1.348 |
| Phosphorylation | 54 (Y) | 0.944 | 0.75 |
| 61 (S) | 0.957 |
| 67 (T) | 0.878 |
| 121 (T) | 0.857 |
| 142 (S) | 0.769 |
| 150 (T) | 0.851 |
| 202 (T) | 0.811 |
| 203 (T) | 0.945 |
| 235 (S) | 0.965 |
| 240 (S) | 0.978 |
| 295 (Y) | 0.976 |
| 305 (T) | 0.834 |
| 315 (T) | 0.926 |
| 324 (S) | 0.961 |
| 325 (T) | 0.764 |
| 339 (S) | 0.811 |
| 363 (Y) | 0.982 |
| S-glutathionylation | 258 (C) | 0.863 | 0.75 |
| 375 (C) | 1.0 |
| Carbonylation | 29 (R) | - | - |
| 69 (K) |
| 114 (K) |
| 203 (T) |
| 319 (T) |
| Sumoylation | 19 (K) | 0.76 | 0.75 |
| 69 (K) | 0.8 |
| 285 (K) | 0.8 |