**Supplementary Material**

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

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**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.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Cell extract** | **Band** | **Swissprot ID** | **Peptides Swissprot** | **ToxoDB ID** | **Peptides ToxoDB** | |
| Vero | 1 | P53478  Actin, cytoplasmic type 5 (*Gallus gallus*) | 20 K.AGFAGDDAPR.A 29  185 R.DLTDYLmK.I 192  330 K.IKIIAPPER.K 336  198 R.GYSFTTTAER.E 207  317 K.EITALAPSTMK.I 327  52 K.DSYVGDEAQSK.R 62  30 R.AVFPSIVGRPR.H 40  41 R.HQGVmVGmGQK.D 51  52 K.DSYVGDEAQSKR.G 63  **86 K.IWHHTFYNELR.V 96**  361 K.QEYDESGPSIVHR.K 373  198 R.GYSFTTTAEREIVR.D 211  240 K.SYELPDGQVITIGNER.F 255  **97 R.VAPEEHPVLLTEAPLNPK.A 114**  293 K.DLYANTVLSGGTTmYPGIADR.M 313  292 R.KDLYANTVLSGGTTMYPGIADR.M 313  41 R.HQGVMVGMGQKDSYVGDEAQSK.R 62 | NCLIV\_003440 | | 286 K.CDVDIR.K 291  63 K.RGILTLK.Y 69  208 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 29  185 R.DLTDYLmK.I 192  328 K.IKIIAPPER.K 337  198 R.GYSFTTTAER.E 207  317 K.EITALAPSTmK.I 327  52 K.DSYVGDEAQSK.R 62  30 R.AVFPSIVGRPR.H 40  41 R.HQGVmVGmGQK.D 51  52 K.DSYVGDEAQSKR.G 63  361 K.QEYDESGPSIVHR.K 373  314 R.mQKEITALAPSTmK.I 327  240 K.SYELPDGQVITIGNER.F 255  2 M.ADEEIAALVIDNGSGmCK.A 19 ¥  **97 R.VAPEEHPVLLTEAPLNPK.A 114**  293 K.DLYANTVLSGGTTmYPGIADR.M 313  41 R.HQGVmVGmGQKDSYVGDEAQSK.R 62  41 R.HQGVmVGmGQKDSYVGDEAQSKR.G 63  217 K.LCYVALDFEQEmATAASSSSLEK.S 239  149 R.TTGIVmDSGDGVTHTVPIYEGYALPHAILR.L 178 | NCLIV\_003440 | | 288 K.CDVDIR.K 291  208 K.EIVRDIKEK.L 216  86 K.IWHHTFYNELR.V 96  179 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.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Cell extract** | **Spot** | **NCBI ID** | **Peptides NCBI** | **ToxoDB ID** | **Peptides ToxoDB** |
| Vero | 1 | P53505  Actin, cytoplasmic type 5 (*Xenopus laevis*) | 20 K.AGFAGDDAPR.A 29  185 R.DLTDYLmK.I 192  328 K.IKIIAPPER.K 337  198 R.GYSFTTTAER.E 207  317 K.EITALAPSTMK.I 327  52 K.DSYVGDEAQSK.R 62  30 R.AVFPSIVGRPR.H 40  41 R.HQGVmVGmGQK.D 51  52 K.DSYVGDEAQSKR.G 63  361 K.QEYDESGPSIVHR.K 373  314 R.mQKEITALAPSTmK.I 327  240 K.SYELPDGQVITIGNER.F 255  2 M.ADEEIAALVIDNGSGMCK.A 19 ¥  **97** **R.VAPEEHPVLLTEAPLNPK.A 114**  293 K.DLYANTVLSGGTTmYPGIADR.M 313  292 R.KDLYANTVLSGGTTmYPGIADR.M 313  41 R.HQGVmVGmGQKDSYVGDEAQSK.R 62  41 R.HQGVmVGmGQKDSYVGDEAQSKR.G 63  217 K.LCYVALDFEQEmATAASSSSLEK.S 239  149 R.TTGIVMDSGDGVTHTVPIYEGYALPHAILR.L 178 | NCLIV\_003440 | 286 K.CDVDIR.K 291  208 K.EIVRDIKEK.L 216  86 K.IWHHTFYNELR.V 96  **97 R.VAPEEHPVLLTEAPLNPK.A 114** |
| 2 | P79818  Actin, cytoplasmic(*Oryzias latipes*) | 19 K.AGFAGDDAPR.A 28  197 R.GYSFTTTAER.E 206  316 K.EITALAPSTmK.I 326  51 K.DSYVGDEAQSK.R 61  29 R.AVFPSIVGRPR.H 39  40 R.HQGVmVGmGQK.D 50  51 K.DSYVGDEAQSKR.G 62  360 K.QEYDESGPSIVHR.K 372  313 R.MQKEITALAPSTMK.I 326  197 R.GYSFTTTAEREIVR.D 210  239 K.SYELPDGQVITIGNER.F 254  96 R.IAPEEHPVLLTEAPLNPK.A 113  19 K.AGFAGDDAPRAVFPSIVGRPR.H 39  292 K.DLYANTVLSGGTTmYPGIADR.M 312  291 R.KDLYANTVLSGGTTmYPGIADR.M 312  40 R.HQGVmVGmGQKDSYVGDEAQSK.R 61  40 R.HQGVmVGmGQKDSYVGDEAQSKR.G 62 | NCLIV\_003440 | 286 K.CDVDIRK.D 291  86 K.IWHHTFYNELR.V 96  97 R.VAPEEHPVLLTEAPLNPK.A 114  97 R.VAPEEHPVLLTEAPLNPKANR.E 117 |
| 3 | P18600  Actin (*Artemia* sp.) | 20 K.AGFAGDDAPR.A 29  185 R.DLTDYLmK.I 192  198 R.GYSFTTTAER.E 207  52 K.DSYVGDEAQSK.R 62  41 R.HQGVmVGmGQK.D 51  52 K.DSYVGDEAQSKR.G 63  **86 K.IWHHTFYNELR.V 96**  198 R.GYSFTTTAEREIVR.D 211  240 K.SYELPDGQVLTIGNER.F 255 ¥  97 R.VAPEEHPILLTEAPLNPK.A 114¥  293 K.DLYANTVLSGGTTmYPGIADR.M 313  292 R.KDLYANTVLSGGTTmYPGIADR.M 313  41 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 28  360 K.QEYDESGPSIVHR.K 362  239 K.SYELPDGQVITIGNER.F 254 | NCLIV\_003440 | 20 K.AGVAGDDAPR.A 29 §  52 K.DCYVGDEAQSK.R 62 § |
| 2 | P68556  Actin (*Diphyllobothrium dendriticum*) | **21 K.AGFAGDDAPR.A 30**  199 R.GYSFTTTAER.E 208  **317 K.EITSLAPSTmK.I 327**  241 K.SYELPDGQVITIGNER.F 256  292 R.KDLYANTVLSGGTTmYPGIADR.M 313  41 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 96  314 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 96  361 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 114  70 K.YPIEHGIVTNWDDmEK.I 85  292 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** §   |  |  | | --- | --- | |  |  |   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 28  197 R.GYSFTTTAER.E 206  316 K.EITALAPSTmK.I 326  51 K.DSYVGDEAQSK.R 61  29 R.AVFPSIVGRPR.H 39  40 R.HQGVmVGmGQK.D 50  51 K.DSYVGDEAQSKR.G 62  **85 K.IWHHTFYNELR.V 95**  360 K.QEYDESGPSIVHR.K 372  313 R.mQKEITALAPSTmK.I 326  239 K.SYELPDGQVITIGNER.F 254  **96 R.VAPEEHPVLLTEAPLNPK.A 113**  392 K.DLYANTVLSGGTTmYPGIADR.M 312  291 R.KDLYANTVLSGGTTMYPGIADR.M 312  40 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 29  185 R.DLTDYLmK.I 192  198 R.GYSFTTTAER.E 207  317 K.EITALAPSTmK.I 327  52 K.DSYVGDEAQSK.R 62  30 R.AVFPSIVGRPR.H 40  41 R.HQGVmVGmGQK.D 51  52 K.DSYVGDEAQSKR.G 63  **86 K.IWHHTFYNELR.V 96**  361 K.QEYDESGPSIVHR.K 373  314 R.mQKEITALAPSTmK.I 327  240 K.SYELPDGQVITIGNER.F 255  **97 R.VAPEEHPVLLTEAPLNPK.A 114** | NCLIV\_003440 | 286 K.CDVDIR.K 291  20 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 28  197 R.GYSFTTTAER.E 206  327 K.IKIIAPPERK.Y 336  316 K.EITALAPSTmK.I 326  51 K.DSYVGDEAQSK.R 61  19 R.AVFPSIVGRPR.H 28  40 R.HQGVmVGmGQK.D 50  51 K.DSYVGDEAQSKR.G 62  360 K.QEYDESGPSIVHR.K 362  313 R.mQKEITALAPSTmK.I 326  197 R.GYSFTTTAEREIVR.D 210  239 K.SYELPDGQVITIGNER.F 254  292 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 96  97 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.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **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 | | |