Supplementary Material

Differences in intracellular fate of two spotted fever group *Rickettsia* in macrophage-like cells

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Supplementary Movie 1 | THP-1-derived macrophages at 60 minutes post infection with *R. montanensis*
This movie corresponds to Figure 5A and represents a 360 degrees rotation movie of the 3D projection of the stack of images (generated with 3D Viewer, ImageJ). Frame rate, 14 fps. (MOV, 1.35 MB)

Supplementary Movie 2 | THP-1-derived macrophages at 24 hours post infection with *R. montanensis*
This movie corresponds to Figure 5C and represents a 360 degrees rotation movie of the 3D projection of the stack of images (generated with 3D Viewer, ImageJ). Frame rate, 14 fps. (MOV, 1.79 MB)

Supplementary Movie 3 | Vero cells at 60 minutes post infection with *R. montanensis*
This movie corresponds to Supplementary Figure 4A and represents a 360 degrees rotation movie of the 3D projection of the stack of images (generated with 3D Viewer, ImageJ). Frame rate, 14 fps. (MOV, 1.04 MB)

Supplementary Movie 4 | Vero cells at 24 hours post infection with *R. montanensis*
This movie corresponds to Supplementary Figure 4C and represents a 360 degrees rotation movie of the 3D projection of the stack of images (generated with 3D Viewer, ImageJ). Frame rate, 14 fps. (MOV, 739.35 KB)

Supplementary Movie 5 | THP-1-derived macrophages at 60 minutes post infection with *R. conorii*

This movie corresponds to Figure 6A and represents a 360 degrees rotation movie of the 3D projection of the stack of images (generated with 3D Viewer, ImageJ). Frame rate, 14 fps. (MOV, 1.3 MB)

Supplementary Movie 6 | THP-1-derived macrophages at 24hours post infection with *R. conorii*

This movie corresponds to Figure 6C and represents a 360 degrees rotation movie of the 3D projection of the stack of images (generated with 3D Viewer, ImageJ). Frame rate, 14 fps. (MOV, 1.9 MB)

Supplementary Movie 7 | Vero cells at 60 minutes post infection with *R. conorii*

This movie corresponds to Supplementary Figure 5A and represents a 360 degrees rotation movie of the 3D projection of the stack of images (generated with 3D Viewer, ImageJ). Frame rate, 14 fps. (MOV, 747.97 KB)

Supplementary Movie 8 | Vero cells at 24 hours post infection with *R. conorii*

This movie corresponds to Supplementary Figure 5C and represents a 360 degrees rotation movie of the 3D projection of the stack of images (generated with 3D Viewer, ImageJ). Frame rate, 14 fps. (MOV, 2.13 MB)

Supplementary Movie 9 | THP-1-derived macrophages at 60 minutes post infection with *R. montanensis*

This movie corresponds to Figure 7B and represents a 360 degrees rotation movie of the 3D projection of the stack of images (generated with 3D Viewer, ImageJ). Frame rate, 14 fps. (MOV, 1.02 MB)

Supplementary Movie 10 | THP-1-derived macrophages at 24 hours post infection with *R. montanensis*

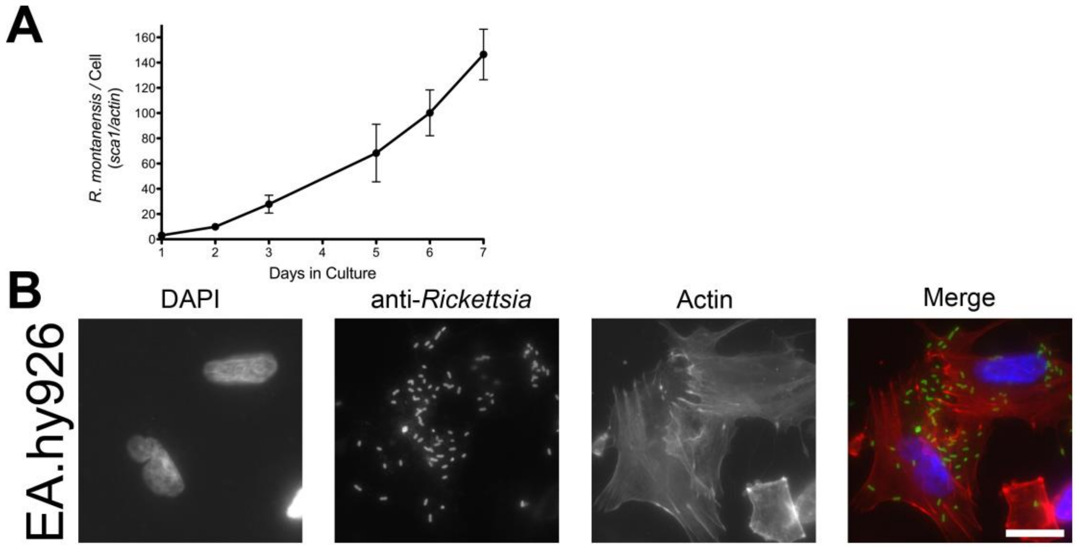
This movie corresponds to Figure 7C and represents a 360 degrees rotation movie of the 3D projection of the stack of images (generated with 3D Viewer, ImageJ). Frame rate, 14 fps. (MOV, 2.77 MB)

Supplementary Movie 11 | THP-1-derived macrophages at 60 minutes post infection with *R. conorii*

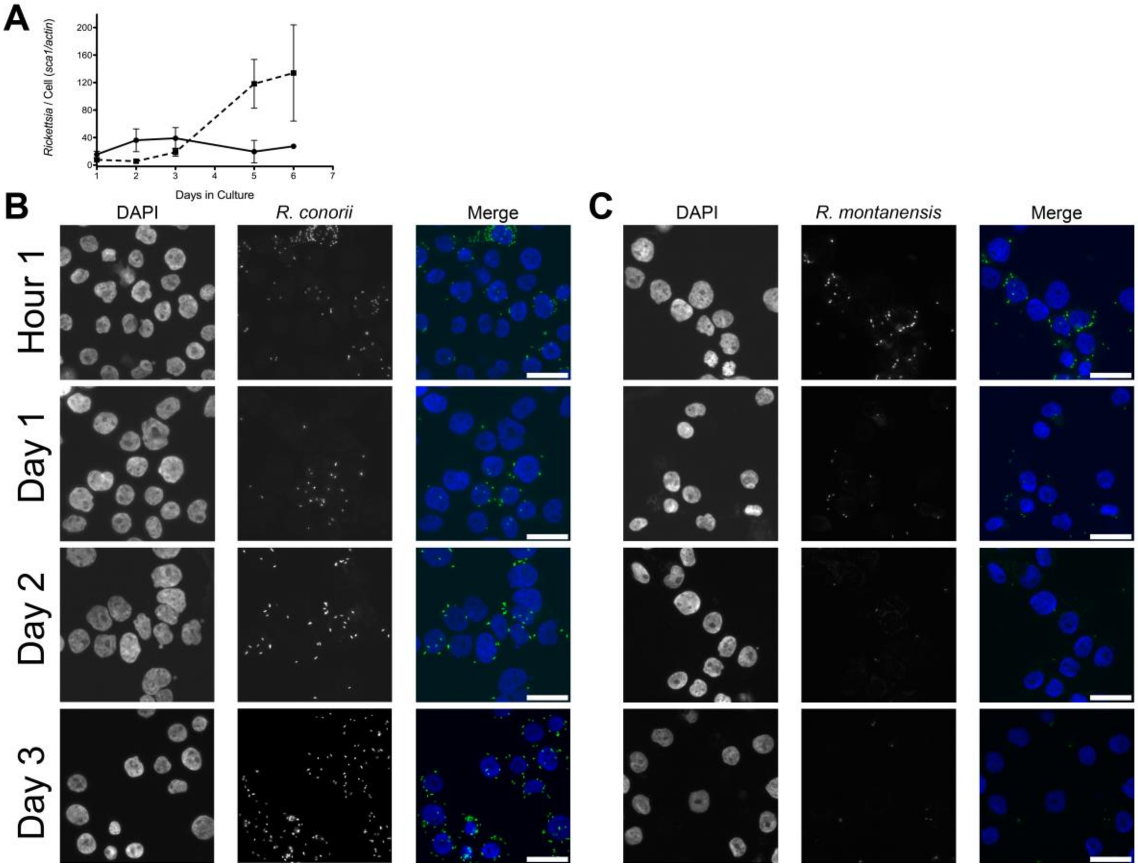
This movie corresponds to Figure 8B and represents a 360 degrees rotation movie of the 3D projection of the stack of images (generated with 3D Viewer, ImageJ). Frame rate, 14 fps. (MOV, 1.98 MB)

Supplementary Movie 12 | THP-1-derived macrophages at 24 hours post infection with *R. conorii*

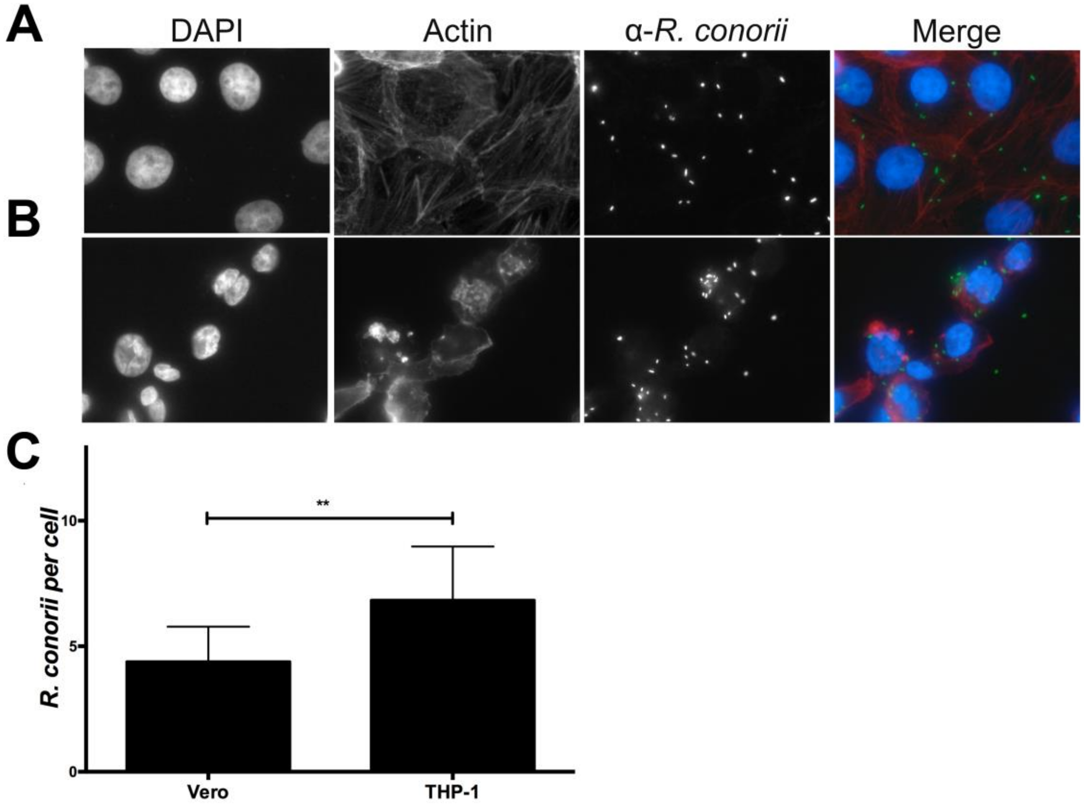
This movie corresponds to Figure 8D and represents a 360 degrees rotation movie of the 3D projection of the stack of images (generated with 3D Viewer, ImageJ). Frame rate, 14 fps. (MOV, 1.68 MB)



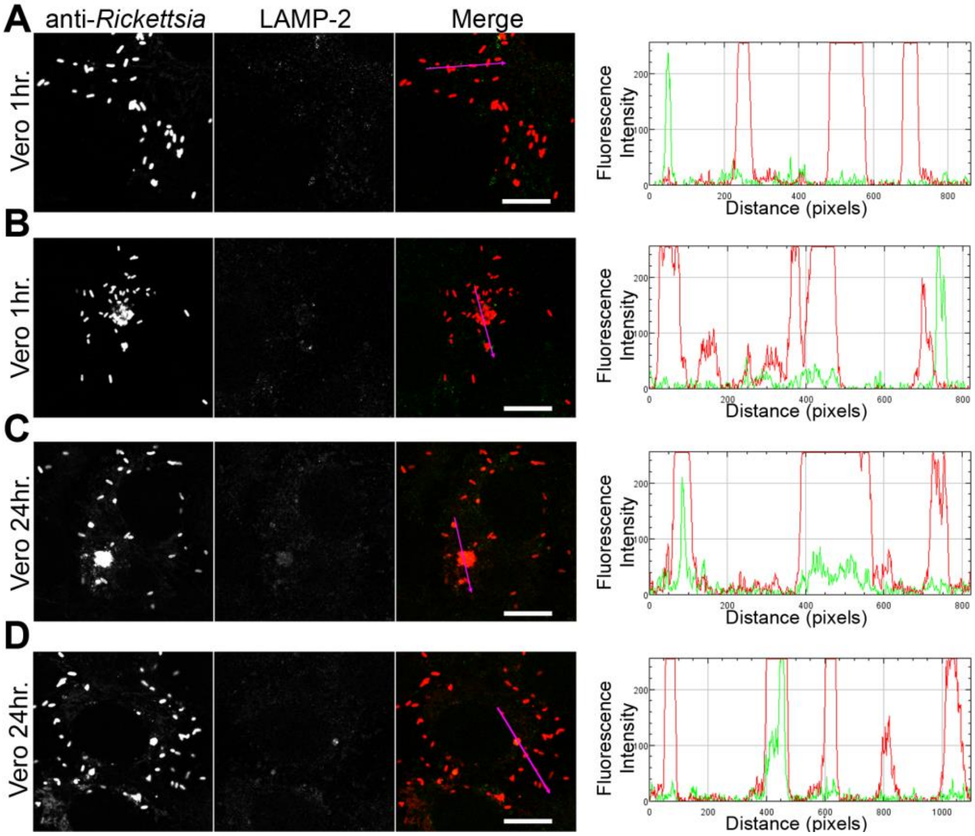
Supplementary Figure 1 | Ability of *R. montanensis* to invade and proliferate within human endothelial cells, EA.hy926. (A) EA.hy926 cells were infected with *R. montanensis* and genomic DNA was extracted at different time-points after infection. Quantitative PCR data are expressed as the ratio of *R. montanensis sca1* versus *actin* DNA content. **(B)** Immunofluorescence microscopy of EA.hy926 cells infected with *R. montanensis* at 3 days post-infection. Cells were stained with DAPI (blue) to identify host nuclei, Phalloidin (red) to stain actin and NIH/RML I7198 followed by Alexa Fluor 488 (green) to stain *R. montanensis*. Scale bar = 10 µm.



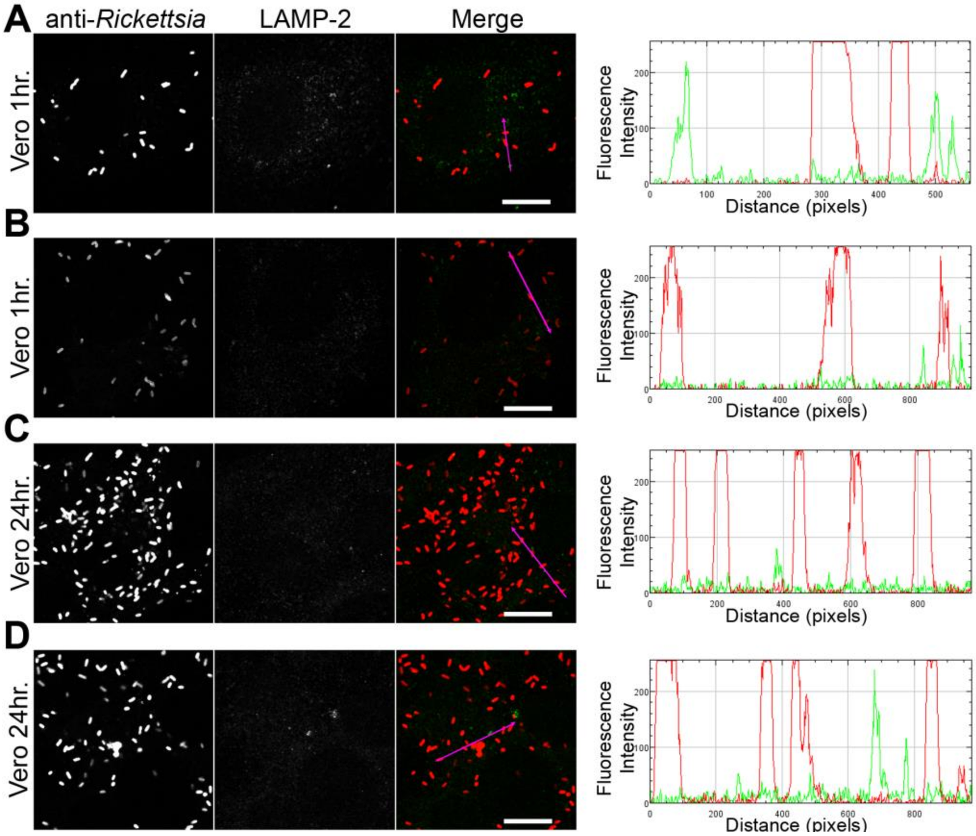
Supplementary Figure 2 | *R. conorii* and *R. montanensis* show a different ability to proliferate within undifferentiated THP-1 cells. (A) Undifferentiated THP-1 cells were infected with *R. montanensis* (solid line) and *R. conorii* (dashed line) and genomic DNA was extracted at different time-points after infection. Quantitative PCR data are expressed as the ratio of *R. montanensis* or *R. conorii sca1* versus *actin* DNA content. **(B)** Immunofluorescence microscopy of undifferentiated THP-1 cells infected with *R. conorii* at 1 h, and 1, 2, and 3 days post-infection, respectively. **(C)** Immunofluorescence microscopy of undifferentiated THP-1 cells infected with *R. montanensis* at 1 h, and 1, 2, and 3 days post-infection, respectively. Cells were stained with DAPI (blue) to identify host nuclei, and anti-Rc_{PFA} or NIH/RML I7198, followed by Alexa Fluor 488 (green) to stain *R. conorii* or *R. montanensis*, respectively. Scale bar = 10 µm.



Supplementary Figure 3 | Unlike *R. montanensis*, adherence of *R. conorii* to THP-1-derived macrophages is not decreased. PMA-differentiated THP-1 cells and Vero cells were infected with *R. conorii* (MOI=10). After 60 min of infection, cells were fixed and stained for immunofluorescence analysis with anti-Rc$_{PFA}$ followed by Alexa Fluor 488 (green) to stain *R. conorii*, DAPI to visualize the host nuclei (blue) and Phalloidin to illustrate the host cytoplasm (red). **(A and B)** Representative immunofluorescence images of *R. conorii* association assays in Vero (A) and macrophage-like (B) cells. Each row shows, from the left to right, nuclei staining, actin staining, rickettsia staining and merged images. Scale bar = 10 µm. **(C)** Rickettsia and mammalian cells were counted and results are expressed as the ratio of rickettsia to mammalian cells. At least 200 nuclei were counted for each experimental condition. Results are shown as the mean ± SD (P values: ** <0.01).



Supplementary Figure 4 | *R. montanensis* is maintained mostly as morphologically intact bacteria in Vero cells. Vero cells were infected with *R. montanensis* (MOI=10). At 60 min or 24 h post infection, cells were fixed, permeabilized and double stained for immunofluorescence confocal microscopy analysis with NIH/RML I7198 followed by Alexa Fluor 546 (red) to stain *R. montanensis* and the monoclonal antibody for LAMP-2, lysosomal membrane protein followed by Alexa Fluor 488 (green). (A-D) Representative images of a single slice from the z stacks. Vero cells at 60 min post infection (A and B) and 24 h post infection (C and D). Each row shows, from left to right, *Rickettsia* staining, LAMP-2 staining, the merged image, and a RGB plot profile illustrating the fluorescence intensity along the magenta arrow. Scale bar = 10 µm. Supplementary movies 3 and 4 represent 360 degrees rotation movie of the 3D projection of the stack images shown in panels 4A and 4C, respectively.



Supplementary Figure 5 | *R. conorii* maintains an intact morphology in Vero cells. Vero cells were infected with *R. conorii* (MOI=10). At 60 min or 24 h post infection, cells were fixed, permeabilized and double stained for immunofluorescence confocal microscopy analysis with anti-Rc$_{\text{PFA}}$ followed by Alexa Fluor 546 (red) to stain *R. conorii* and the monoclonal antibody for LAMP-2, lysosomal membrane protein followed by Alexa Fluor 488 (green). (A-D) Representative images of a single slice from the z stacks. Vero cells at 60 min post infection (A and B) and 24 h post infection (C and D). Each row shows, from left to right, *Rickettsia* staining, LAMP-2 staining, the merged image, and a RGB plot profile illustrating the fluorescence intensity along the magenta arrow. Scale bar = 10 µm. Supplementary movies 7 and 8 represent 360 degrees rotation movie of the 3D projection of the stack images shown in panels 5A and 5C, respectively.

```
rco_RC1273      MANISPKLFQKAIQQGLKAALFTTSTAAIMLSSSGALGIAVSGVIATNNNAAFSDNVGNN
rmo_MCI_03620   MANISPKLFQKVIQQGLKAALFTTSTAAIMLSSSGAFGVVAG-VISNNDAAFSNLAAANN
                ***********.************************:*:... **:.*: *  .  ..**

rco_RC1273      WNEITAAGVANGTPARGPQNNWAFTYGGDYTITADVADHIITAINVADTTPIGLNIAQNT
rmo_MCI_03620   WNEITVGGVTNGTPADGPRDNVAFTYGGDHTITADKVGRIITAINVAGTTPVGLDITQNT
                *****..**:***** **::* *******:***** ..:********.***:**:*:***

rco_RC1273      VVGSIVTGGNLLPVTITAGKSLTLNGNNADAANHGFGAPADNYTGLGNIALGGANAALII
rmo_MCI_03620   VVGSIVTGGNLLPVTIIAGKSLTLNGANAIVANHGFDAPADNYTGLGNITLGGANAELII
                **************** ********* ** .*****.************:****** ***

rco_RC1273      QSAAPAKITLAGNINGGGIITVKTDAAINGTIGNTNALATVNVGAGIATLEGAIIKATTT
rmo_MCI_03620   QSATPAKITLAGNIDGGGIITVNTDAAINGTIGNVNPAAQISVGASTLSLGGAVIKATTT
                ***:**********:*******:***********.*. * :.***.  :* **:******

rco_RC1273      KLTNAASVLTLTNVNAVLTGAIDNTTGVDNVGVLNLNGALSQVTGNIGNTNALATISVGA
rmo_MCI_03620   KLTDNASVLTLTNANSVLTGAIDNTTGGDNVGVLNLNGALSQVTGNIGDTNSLATISVGA
                ***: ********.*:*********** ********************:**:********

rco_RC1273      GKATLGGAVIKATTTKLTDNASAVTFTNP-VVVTGAIDNTGNANNGIVTFTG--DSTVTG
rmo_MCI_03620   GTATLGGAVIKATTTKLTDNVSVLTLTNANAVLTGAIDNTTGGDNVGVLNLNGALSQVTG
                *.******************.*.:*:**. .*:******* ..:*  *   .   * ***

rco_RC1273      NIGNTNALATISVGAGKATLGGAIIKATTTKLTDNASAVTFTNP----------------
rmo_MCI_03620   NIGDTNSLATISVGAGTATLGGAVIKATTTKLTDNVSVLTLTNANSVLTGAIDNTTGGDN
                ***:**:*********.******:***********.*.:*:**.

rco_RC1273      ------------------------------------------------------------
rmo_MCI_03620   VGVLNLNGALSQVTGNIGGTNSLATISVGAGTATLGGAVIKATTTKLTNNVSVLTLTNAN


rco_RC1273      ------------------------------------------------------------
rmo_MCI_03620   SVLTGAIDNTTGGDNVGVLNLNGALSQVTGNIGGTNSLATISIGAGTATLGGAVIKATKT


rco_RC1273      ---------------VVVTGAIDNTGNANNGIVTFTG--DSTVTGNIGNTNALATISVGA
rmo_MCI_03620   ELTDAASVLTLTNANAVLTGAIDNTTGGDNVGVLNLNGALSQVTGNIGDTNSLATISVGA
                               .*:******* ..:*  *   .   * ******:**:********

rco_RC1273      GKATLGGAIIKATTTKLTDNASAVTFTNP-VVVTGAIDNTGNANNGIVTFTG--DSTVTG
rmo_MCI_03620   GTATLGGAVIKATTTKLTDNVSVLTLTNANAVLTGAIDNTTGGDNVGVLNLNGALSQVTG
                *.******:***********.*.:*:**. .*:******* ..:*  *   .   * ***

rco_RC1273      NIGNTNALATISVGAGKATLGGAIIKATTTKLTDNASAVTFTNP-VVVTGAIDNTGNANN
rmo_MCI_03620   NIGDTNSLATISVGAGTATLGGAVIKATTTKLTDNVSVLTLTNANSVLTGAIDNTTGGDN
                ***:**:*********.******:***********.*.:*:**.  *:******* ..:*

rco_RC1273      GIVTFTG--DSTVTGNIGNTNALATISVGAGKATLGGAIIKATTTKLTDNASAVTFTNP-
rmo_MCI_03620   VGVLNLNGALSQVTGNIGDTNSLATISVGAGTATLGGAVIKATTTKLTDNASVLTLTNAN
                  *   .   * ******:**:*********.******:*************.:*:**.

rco_RC1273      VVVTGAIDNTGNANNGIVTFTG--------------------------------------
rmo_MCI_03620   AVLTGAIDNTTGGDNVGVLNLNGALSQVTGNIGDTNSLATISIGAGTATLGGAVIKATKT
                .*:******* ..:*  *   .

rco_RC1273      ---------------------------------------NSTVTGNIGNTNALATVNVGA
rmo_MCI_03620   ELTDAASVLTLTNANAVLTGAIDNTTGGDNVGVLNLNGALSQVTGNIGDTNSLATISVGA
                                                        * ******:**:***:.***

rco_RC1273      GIATLEGAVIKATTTKLTNAASVLTLTNVNAVLTGAIDNTTGVDNVGVLNLNGALSQVTG
rmo_MCI_03620   GTATLGGAVIKATTTKLTDNASVLTLTNANSVLTGAIDNTTGGDNVGVLNLNGALSQVTG
                * *** ************: ********.*:*********** *****************

rco_RC1273      NIGNTNALATISVGAGKATLGGAVIKATTTKLTDNASAVTFTNPVVVTGAIDNTGNANNG
rmo_MCI_03620   NIGDTNSLATISIGAGTATLGGAVIKATTTKLTDNASAVTFTNPVVVTGAIDNTGNANNG
                ***:**:*****:***.*******************************************

rco_RC1273      IATFTGDSTVTGNIGNTNALATVNVGAGLLRVQGGVVKSNTINLTDNASAVTFTNPVVVT
rmo_MCI_03620   IATFTGNSTVTGNIGNTAALATVNVGAGLLRVQGGVVKANAISLTDDASAVTFTNPVVVT
                ******:********** ********************:*:*.***:*************

rco_RC1273      GAIDNTGNANNGIVTFTGDSTVTGNIGNTNALATISVGAGKATLGGAIIKATTTKLTDNA
rmo_MCI_03620   GAIDNIGNADNGIATFTGNSTVTGNIGNTAALATVNVGAGLLQIQGGVVKANAINLTDDA
                ***** ***:***.****:********** ****:.****   : *.::**.: :***:*

rco_RC1273      SAVTFTNPVVVTGAIDNTGNANNGIVTFTGDSTVTGNIGNTNALATVNVGAGVTLQAGGS
rmo_MCI_03620   SAVTFTNPVVVTGAIDNIGNADNGIATFTGNSTVTGNIGNTATLATVNVGAGITLQAGGS
                ***************** ***:***.****:********** :*********:*******

rco_RC1273      LDANNIDFGARSTLEFNGPLDGGGNAIPYYFKGAIANGNNAILNVNTKLLTAYHLTIGTV
rmo_MCI_03620   LAANNIDFGVGSTLEFNGPLDGGGDIIHYHFKGAIANGNNATLNVNTKSLTAYHSTIGTV
                * *******. *************: * *:*********** ****** ***** *****

rco_RC1273      AEINIGAGNLFAIDASAGDVTILNAQDIHFRALDSALVLSNLTGVGVNNILLAADLVAPG
rmo_MCI_03620   AEINIGADSFFTIDASAGDVTILNAQDINFRAQNSTLMLSNLTGVGVKNILLAADLVAPG
                *******..:*:****************:*** :*:*:*********:************

rco_RC1273      VDEGTVVFDGGVNGLNIGSNVAGAARNIGDVGGNKFNTLLIYNAVTITDDVNLEGIQNVL
rmo_MCI_03620   ADEGCVVFDGGMNGLNIGSNVAGTARNIGDGGGDKFNNLFIYNVVKVTDDVNLEGIKNVF
                .*** ******:***********:****** **:***.*:***.*.:*********:**:

rco_RC1273      INNNADFTSSTAFNAGTIQINDATYTIDANNGNLNIPAGNIKFAHADAQLILQNSSGNDR
rmo_MCI_03620   IGNDAYFTSSTACNAGTIQINNATYAIDANNGNLNVPAGNIQFVHAGAQLVLQNSSENDR
                *.*:* ****** ********:***:*********:*****:*.**.***:***** ***

rco_RC1273      TITLGANIDPDNDDEGIVILNSVTAGKKLTIAGGKTFGGAHKLQDIVFKGEGDFGTAGTT
rmo_MCI_03620   TITLGANIDPDNDGDGIVILNSVTAGKKLTIAGGKTFGGAHKLQAIVFKGAGNFGVAGTT
                *************.:***************************** ***** *:**.****

rco_RC1273      FNTTNIVLDITGQLELGATTANVVLFKDAVQLTQTGNIGGFLDFNAKNGTVTLNNNVNVA
rmo_MCI_03620   FNATDIVLDITSQLELGATTANVVLLNDAVQLTQTGDIGGFLDFNARNGTVTLNNNVNVV
                **:*:******.*************::*********:*********:************.

rco_RC1273      GTVKNTGGTNNGTLIVLGASNLNRVNGIAMLKVGAGNVTIAKGGNVKIGEIQGTGTNTLT
rmo_MCI_03620   RAVQNTGGTNNGTLIVLGASNLNSVNGIAMLKVGAGNVTIAKGGDVKIGEIQGTGTNTLT
                 :*:******************* ********************:***************

rco_RC1273      LPAHFKLTGSINKTGGQALKLNFMNGGSVSGVVGTAANSVGDITTAGATSFASSVNAKGT
rmo_MCI_03620   LPANFNLIGSINKTGGQALKLNFTNGGSVSGVVGTAANSVGDITTAGATSFASSVNAKGT
                ***:*:* *************** ************************************

rco_RC1273      ATLGGTTSFAHTFTNTGAVTLAKGSITSFAKNVTATSFVANSATINFGNSLAFNSNITGS
rmo_MCI_03620   VTLGGTTSFADTFTNTGAVTLAKGSITNFAKNVTATSFVANSATINFGNSLAFNSNITGS
                .*********.****************.********************************

rco_RC1273      GTTLTLGANQVTYTGTGSFTDTLTLNTTFDGAAKSGGNILIKSGSTLDLSGVSNLALVVT
rmo_MCI_03620   GTTLTLGANQVTYTGTGSFTDTLTLNTTFDRAAKSGGNILIKSGSTLDLSGVSTLALVVT
                ****************************** **********************.******

rco_RC1273      ATNFDMNNISPDTKYTVISAETAGGLKPTPKENVKITINNDNRFVDFTFDASTLTLFAED
rmo_MCI_03620   ATNFDINNISPDTKYTVISAETAGGLKPTSKENVKITINNDNRFVDFTFDASTLTLFAED
                *****:***********************.******************************

rco_RC1273      IAAGVIDEDFAPGGPLANIPNAANIKKSLELMEDAPNGSDARQAFNNFGLMTPLQEADAT
rmo_MCI_03620   IAADVIDEDFEPGGPLANIPNAANIKKSLKLMEDAPNGSDARQAFNNFGLMTPLQEADAT
                ***.****** ******************:******************************

rco_RC1273      THLMQDVVKPSDTIAAVNNQVVASNISSNITALNARMDKVQAGNKGPVSSGDEDMDAKFG
rmo_MCI_03620   THLMQDVVKPSDTIAAVNNQVVASNISSNITALNARMDEVQAVNKGPVSSGDEDMDVKFG
                **************************************:*** *************.***

rco_RC1273      AWISPFVGNATQKMCNSISGYKSDTTGGTIGFDGFVSDDLVLGLAYTRADTDIKLKNNKT
rmo_MCI_03620   AWISPFVGNATQKMCNNISGYKSDTTGGTIGFDGLVSDDLALGLAYTRADTDIKLKNNKM
                ****************.*****************:*****.******************

rco_RC1273      GDKNKVESNIYSLYGLYSVPYENLFVEAIASYSDNKIRSKSRRVIATTLETVGYQTANGK
rmo_MCI_03620   GDKNKVESNIYSLYGLYSVPYENLFVEAIASYSDNKIRSKSRRVIATALETVGYQTANGK
                ***********************************************:************

rco_RC1273      YKSESYTGQLMAGYTYMMSENINLTPLAGLRYSTIKDKSYKETGTTYQNLTVKGKNYNTF
rmo_MCI_03620   YKSEGYTGQLMAGYTYMMPENINLTPLAGLRYSTIKDKGYKETGTTYQNLTVKGKNYNTF
                ****.*************.*******************.*********************

rco_RC1273      DGLLGAKVSSNINVNEIVLTPELYAMVDYAFKNKVSAIDARLQGMTAPLPTNSFKQSKTS
rmo_MCI_03620   DGLLGARVSSNINVNEIVLTPELYAMVDYAFKNKVSAIDARLQGMAAPLPTNSFKQSKTS
                ******:**************************************:**************

rco_RC1273      FDVGVGVTAKHKMMEYGINYDTNIGSKYFAQQGSVKVRVNF
rmo_MCI_03620   FDIGVGVTAKHKMMEYGINYDTNIGSKYFAQQGSVKVRVNF
                **:**************************************
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Supplementary Figure 6 | Amino acid sequence alignment of Sca0 protein from *R. conorii* and *R. montanensis.* Amino acid sequences of rickettsial Sca0 from *R. conorii* (RC1273) and *R. montanensis* (MCI_03620) were aligned using the ClustalW software. The alignment resulted in a percentage of sequence identity of 75.36 %.

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rco_RC0019         MNKLTEQHLLKKSRFLKYSLLASISVG--AAIPFEGMAMSKEAFRIDLSNKLLNHVSQLN
rmo_MCI_04265      MNKLTEQHLLKKSRFLKYSLLASISVGAIIAIPFEGMAMSKEAFCIDLSSKLLNSLSQLN
                   ***************************   ************** ****.**** :****

rco_RC0019         GYKDTTN-----NTPQQIGKVIVSEPKINTYTPSEIREMKISNKPKASNPLKDVPIEDHY
rmo_MCI_04265      GNKDTTNTPDTTNTPQQIGTVIVPEPEINAYIPSEIREMKISNKPKASNPLKDVPVEDHY
                   * *****     *******.***.**:**:* ***********************:****

rco_RC0019         KVVARSKSDVGTARKVRPITRCKTFAGIEKTEQSQNTYTPESTEQMPQKPEIIITASSPT
rmo_MCI_04265      KVVARSKSEVGKARKVRPITRRKTFVGTEKT------------EQMPQKPEIIITASSPT
                   ********:**.********* ***.* ***            *****************

rco_RC0019         VSPASNSFITAPNTPNTTLTSPEHYTTAPGTPSSTPATPYQSTSDSKPNDSLGANTPPNI
rmo_MCI_04265      VSPASNSFVTAPNTPNTTLTSPEHYATAPGTPSSTPVTPYQPTPDSKPNYSLGANTPPNI
                   ********:****************:**********.****.*.***** **********

rco_RC0019         NTNSKAVRRLSFSSSGPQQQAVQSSSQVKSEVPPKPTFVPLPIKKSSTEIVAGMVSNISR
rmo_MCI_04265      NTNSKAARRLSFSSSDPQQQTVQSSSQVKPEVLPESTFVPLPTKKSSTEIAAGVVSNISR
                   ******.********.****:********.** *:.****** *******.**:******

rco_RC0019         VNEMIGIKLAEVTQAIDTTD-KKDKERLQKLYTQLTSTQKTTEKLKSRAEEIETKIKIGE
rmo_MCI_04265      VNEMIGIKLAEVTQAIKDTTGKKNKKPLQKLYTQLTSAQKTTEALKSRVEEIETRIKIGE
                   ****************. *  **:*: **********:***** ****.*****:*****

rco_RC0019         NKDKIKKLEKELTSKNNKADRLFQKIEKIDIPANKVSIKSQETVPVTTASTEVSAFQAQQ
rmo_MCI_04265      NKNKIKKLEKELTSKNNEADRLFQKIEKIDIPTNKVSIKSQETVPVTTASTKVSAFQAQQ
                   **:**************:**************:******************:********

rco_RC0019         ARINEARQGVFNKNKSSGGNARKSSAG-----TKREKKKQEAQKQLSEIKKQEKAIKTAS
rmo_MCI_04265      ARINEAQQVVQNQQYIAAKKTKKQRQAEDKQRAEREKKKQEAQKQLSEIKKQEKAIKTAA
                   ******:* * *::  :. :::*.  .     ::*************************:

rco_RC0019         DKAKEVAASAKKETSRTALRAMQDKMNGDSEQLNKIEENLKLLTPVVYNSSTGPTYKQSP
rmo_MCI_04265      DKAKEVAASAKKETSRTALRAMQDKK---------------LLTPVVYNSSTGSTYKQPP
                   *************************                ************.****.*

rco_RC0019         KATPTIPLSHGVQRILGEQPEDEEGYLVPIKVQQQPYQEIEDP-----------------
rmo_MCI_04265      RAKPTMPLSHRVQRILGEQPEDEEGYLVPMKVQQQPYTVMLPSQEIEDPKSMGSGLGSLS
                   :*.**:**** ******************:*******  :  .

rco_RC0019         --------------------------------------------------------IPSH
rmo_MCI_04265      SGTLGKETDEDVGFYSGWGNMPYTQAANVNSSLEGNLVLGMPQQQIQENRSEVSGQLPSH
                                                                           :***

rco_RC0019         SKDIYEAKVSQYINYLNSIQPNQSNQAQIDSVIDGLATEMRKFSADQFSQKLGEIAHLAS
rmo_MCI_04265      SKDIYEAKASLHINYLNSIQPNQSNQAQIDGVIDSLVAAMRKLSADQFNQKLGDVAHLAS
                   ********.* :******************.***.*.: ***:*****.****::*****

rco_RC0019         IKAYEGLFEKLYEIQQARILETQKVYEQAEISQSYAEYEENSRKSSIPVLSRSSSAKSVI
rmo_MCI_04265      IKAYEGLYSKLYEIQQARILETQKAYEQAEIFQSYAEYEENSRKSGTPVLSRSPSAKSVI
                   *******:.***************.****** *************. ******.******

rco_RC0019         SSNFEEKSALLQTTTTDESLRSDNNWKNSAPYSSSPKLDKRGLEYLDLAGDAFVQNLKQP
rmo_MCI_04265      SSNFEEKSALLQTTTTDESLRSDKNWKNPAPYSSSPKLDK--------------------
                   ***********************:****.***********

rco_RC0019         DTLTIETLGLITPTQNTTVAKSDSSRKNNVSGSISEIQQLQSEKMRTETLGVQDDLGLDL
rmo_MCI_04265      ---------IVTPTQNTTVAKSDSSRKNNASGSIPEIQQLQSEKMRTETLGMQNDLGLDL
                            ::******************.****.****************:*:******

rco_RC0019         HYT-----------------------------------------PQETLTEKSTYLVSSK
rmo_MCI_04265      HSQEERFDSGFRSLESQNLSSAYDFDQLSASLDAAYNQRMNTNAPQETLTEESTYSVSSK
                   *                                           *******:*** ****

rco_RC0019         KKQGNIIKRAVSKVGSILQTNYAENRRRKRDGETSKQRTVDQEGEFGHAWGNENHKESSL
rmo_MCI_04265      KKQGNIIKRAVSKAGSILQTNGAENRKRKRDGETSKQRTVDQEGGFGHAWGNENHKESSL
                   *************.******* ****:***************** ***************

rco_RC0019         SVVSGCIKKATQLISLLDAKRTAILQTTSPSQRRSVSLVLQEIENDYREAIKISQKLQQV
rmo_MCI_04265      SVVSGFTKKATQLISLLDAKRTAILQTTSPSQKKSLNLALQEIENDYQEAIKISQELQQV
                   *****  *************************::*:.*.********:*******:****

rco_RC0019         LIRKPEDIKAYNAKAEKKLDAIKSRADKHFNNIETDVDVGFNPNGGNSHSMPTANMDILP
rmo_MCI_04265      LIRKPEDIKAYNAKAEKKLDAIKSRADKHFNNIETDADVGFNPNWGNSHSMPTANMNNLP
                   ************************************.******* ***********: **

rco_RC0019         KNLAVTPPTNVGSLYNSPQAQQFQEDHKNIIINDRSQG----------------------
rmo_MCI_04265      KNLAIN----VGSLYNSPQAQQFREDHKNIIINAISSISSSGVPKESIDSTITKSLPYLD
                   ****:.    *************:*********  *.

rco_RC0019         -------------------------RLNLVDSTIVRELTDVDASIYNTAPPEVLKEAEAL
rmo_MCI_04265      NDQYQEVVLRVEKVRDINTRGYLLNKLKQLGASEVRELTDVDASIYNIAPPEVLKEAEAL
                                            :*: :.:: ************* ************

rco_RC0019         LDRSQGRLNLVDNTIKKGTQPLSNLSTIYESVSYENLASETIYKTEQPKPSISYTNTSKR
rmo_MCI_04265      LDRSQGRLNLVDSTIKKGTQPLSNLSTIYESVSYENLASETTYKTEQPKPSISYKNTSKR
                   ************.**************************** ************.*****

rco_RC0019         KLPIPLFRSAELDKKLEYLDLEDKLLEVEEARIVKEKEAIAKLNQYQDPENLEFKRLAME
rmo_MCI_04265      KLPIPLLRSAELDKKLDYLDLEDKLLKVEEARIVKEKQAIAKLNQYQDPENLEFKRLAIE
                   ******:*********:*********:**********:********************:*

rco_RC0019         ALDLSSKESQLKQKRKAIEAEFSLNEKSSSTDVSILRSYSIDDISGVLSDAESNLSRSPS
rmo_MCI_04265      ARDLSSKESQLKEKRKAMEAAFSLNKEYSSTDVSMSRSYSIGDISSVPSDAESNLSRSPS
                   * **********:****:** ****:: ******: *****.***.* ************

rco_RC0019         VSGLEDLNNSNVMQLEELKSKHEKIANDYN------------------KELELDTLNKEK
rmo_MCI_04265      VSGLEDLNNSNVMKLGELKSKHEKIANDYKAKELELDTLNHDDPKFKKLKLEVFEILKEK
                   *************:* *************:                   :**:  : ***

rco_RC0019         IWLEGEIKHLDTEFKPKVTESKPVFSCSSSVGSINSFSNDDDLSSRDVVTPVDTLNIEIN
rmo_MCI_04265      IWLEGEIKHLDTESKPKVTESKPVFSCSSSVGSINSLSNGDDLSSR-------DVTIEID
                   ************* **********************:**.******        :.***:

rco_RC0019         KDYVDTYIRVLSNKIQKIEEIQKIEELSGSSSRSEELNHIKEAMAYISNRIQDVEELDEK
rmo_MCI_04265      KDYVDTYIRVLS------NKIQKIEELPGSSSRSEELNHIKEAMAYISNRIQDVEELDEK
                   ************      ::*******.********************************


rco_RC0019         MLLAINAQLQEHDEKISSLLREEAT-----DILAQLLLQEMLVSDGKSESTLPAGDEEQE
rmo_MCI_04265      MLLAINARLQEHDEKISSLLLEKYKGEEAADILAQLLLQDMLASDGKSESTLPAGDKEQE
                   *******:************ *: .     *********:**.*************:***

rco_RC0019         D---------TEVSRQLSSLPALASSNESALALSDDREKECLALGDSSEDEESYDSGFEE
rmo_MCI_04265      DTEDTEDTEVKEVSRQLSSLPALASSNECALALSDDREKECLALGDGSEDEESYDSGFEE
                   *         .*****************.*****************.*************

rco_RC0019         EEETIGQLSDSDGDNLKITEVDTVIPLEQEAKKEMQTQISENAPTLNQAKVVNTIVNNMI
rmo_MCI_04265      EEETIGQLSDSDGGNLKITEVDTAIPLEQEAKKEMQTQISENAPTLNQAKVVNTIVNNMI
                   *************.*********.************************************

rco_RC0019         RNRLDASMNMSNNMVAVGAGDEEESHIKRGLWMRGMYGTNNHGRVENMTGYRGTNKGATI
rmo_MCI_04265      RNRLDASMNMSNNMVAVGAGDEEESHIKRGLWMRGMYGTNNHGRVDNMTGYRGTNKGATI
                   *********************************************:**************

rco_RC0019         GFDAEIDNNIVGIAYSNVHSVFKFKNSKNNDKELINSHVVSIYGQKELPKNFALQALVSA
rmo_MCI_04265      GFDAEIDNNIVGIAYSNVHSVFKFKNSKNNDKELINSHVVSIYGQKELPKNFALQALVSA
                   ************************************************************

rco_RC0019         SKNFIKDKTTYSYGDTKIKSNVKHRNHSYNAEALLHYNYLLQSKLVITPNIGLRYGKSRD
rmo_MCI_04265      SKNFIKDKTTYSYGDTKIRSNVKHRNHSYNVEALLNYNYLLQSKLLITPNIGLRYGKSRD
                   ******************:***********.****:*********:**************

rco_RC0019         GVYNETGVNVQEIALTMKENNILSGIVGTKVTVPLKDALKFNNLGLIFQGAVEHNFKEKT
rmo_MCI_04265      GVYNETGINVQEIALTMKENNILSGIVGTKVTVPLKDALKFNNLGLTFQGAVEHNFKEKT
                   *******:************************************** *************

rco_RC0019         QRINRVVKIFDNTFKHNYLIPKQPKTSYNLGTGIIGSIKNTTISLDYNYYLNKHYRSHQG
rmo_MCI_04265      QRINRVVKIFDNTFKHNYLIPKQPKTSYNLGTGIIGSIKNTTISLDYNYYLNKHYRSHQG
                   ************************************************************

rco_RC0019         SVKLKVNL
rmo_MCI_04265      SVKLKVNL
```

Supplementary Figure 7 | Amino acid sequence alignment of Sca1 protein from *R. conorii* and *R. montanensis.* Amino acid sequences of rickettsial Sca1 from *R. conorii* (RC0019) and *R. montanensis* (MCI_04265) were aligned using the ClustalW software. The alignment resulted in a percentage of sequence identity of 60.15 %.

```
rco_RC0110         MNLQNSHSKKYVLTFFMSTCLLTSSFLSTSARAASFKDLVSKTPAWEKHNSTQQQNIWKD
rmo_MCI_04765      MNLQNSYSKKYVLTFFMSTCLLTSSFLSTSARAASFKDLVSKNPAWEKHNEIQQQNIWKD
                   ******:***********************************.*******. ********

rco_RC0110         LTPNEKIKKWQEAALVPSFTQAQNDLGIKYKETDLSSFLDNTRHKARQARAEILLYIERV
rmo_MCI_04765      LTPNEKIKKWQAADLVPSFTQAQDDLGIKYKETDLSSFLDNTRHKARQARAEILLYIERI
                   *********** * *********:***********************************:

rco_RC0110         KQQDFDTKKQAYINQGVVPTDIEAATNLGISYDPSKIDNNVEHDQKVRRAEKDKKAVIEL
rmo_MCI_04765      KQQDFDTKKQEYINQGVVPTDIEAATNLGISYDPSKIDNNVENDQKVRRAEKDKKAVIGL
                   ********** *******************************:*************** *

rco_RC0110         YVSSINRGIKYKHYVDNDIIPEIQEVRTALNMNKDDAQSFVASIRTEIMENAKGQYIADS
rmo_MCI_04765      YISSINRAIKYKHYVDNDIIPAMKEVRTALNMNKDDAESFVASIRTEIMENAKRQYIADS
                   *:*****.************* ::*************:*************** ******

rco_RC0110         HIPTEKELKKKFGISRDDNRDGYIKSIRLKVMDKEKPQYIADSHIPTEKELEQKFGADKG
rmo_MCI_04765      HIPTEQEFKNRFGISRDDNRDGYIKSIRLKVMDKEKPQYIAGNSIPTEKELEQKFGADKG
                   *****:*:*::******************************.. ****************

rco_RC0110         EATNYIASIATQMMLDKKSYYIDNNIIPNADELMNEFKIGPVKATSYINQIRAGIEANQF
rmo_MCI_04765      EATNYIASIATQMMLGKKSYYIDNNIIPNADELMNEFKIGKIKANSYIDQIRAGMKANQF
                   ***************.************************ :**.***:*****::****

rco_RC0110         LNNNDTTKPSTGRSQKKSGSKNDHWYMSNQSINNTGTSARIVTGREKKQRYFFDPISTFK
rmo_MCI_04765      LNNNDTTKPSAGHSQKKSGSKNDNWYMSNQGTNNTGTSSGVSTGRKKKQPYFFDPISTFK
                   **********:*:**********:******. ******: : ***:*** **********

rco_RC0110         TYFNTKASKGNLTQSQHNINRIIQQEENIEEFKNLIKTDPIAALTLQVDSSYKQEAVTTI
rmo_MCI_04765      THFNNKESKGNLTQSQRNINRIIQQEENIEEFKNLIKTDPIAALNLEVGSSYKQEAVTTI
                   *:**.* *********:***************************.*:*.***********

rco_RC0110         LSDFNDDTIQRVLFSNDKGKLDFNTNIDVKNRPILQELLENSSSEEKTKFAERIQDYATR
rmo_MCI_04765      LSDFNDDTIQRVLFSNDKEQLDFNTNIDVKNRPILKELLENSSSEEKTKFAERIQDYATR
                   ****************** :***************:************************

rco_RC0110         NISNSQFEEKARLDLIKLAASKDKSSVENFLTLQLELKNRMQPYVVNSVYILTPEIVKEI
rmo_MCI_04765      NIFNSQFEEKARLDLIKLAASKDKSSVENFLALQLELKNRMQPYIVKSVYILTPEIVKEI
                   ** ****************************:************:*:*************

rco_RC0110         NIELKNKGLIRDSLTKDYMIKLAKEVNNHTLNSVIKVILSDSKILSNETNKILGLAVSNN
rmo_MCI_04765      NIELKNKGLIIDSLTKDDMIKLAKAVNKQTLNSVIKVILSDSNMLSNETNKILGLAAGNN
                   ********** ****** ****** **::*************::************..**

rco_RC0110         ANNLEQTQSGIPNPPPLPLNGGIPNPPPLPLNGSMPPPP--LHSQGFSSNSKHFDLNQLQ
rmo_MCI_04765      ANNLEQTQSGIPNPPPLPLNGGIPNPPPLPLNGSMPPPPPPLNSQGFSSNSNNFDLNKLQ
                   ***************************************  *:********::****:**

rco_RC0110         TEYPHIHSLYVQFTHNTTVQSKAPLQPTASSATSTGRSTPETAYAKLYAEYRTETGGTKA
rmo_MCI_04765      AEYPHIHSLYIQFTR-NTVQPKVPSQPTTSSATSTDRSTPETAYAKLYTEYRTETGGKKA
                   :*********:***: .***.*.* ***:******.************:********.**

rco_RC0110         NDLQDQLIKRQADLTNVIRQILTESYANQGADEKTLLNLFSISTPEIAEKAKEAFNTLAQ
rmo_MCI_04765      DDLQDQLIKRQADLTNVIRQILTESYANQGADEKTLVNLFSISTPEIAEKAKEAFNTLVQ
                   :***********************************:*********************.*

rco_RC0110         DQYIKDITVNGKKTITSEEIIKNLFNEDTDDAIKRILLSSCKISEELKRPIKLEFNKSEL
rmo_MCI_04765      DPYIQDITVNGKKTITSEEIIKNLFNEDNDDAVKRILLSSCKISEELKKPIKLEFNKSEL
                   * **:***********************.***:***************:***********

rco_RC0110         IRELQGKQNPFKQLEFAYINTKNFDQDIFGNRIDELINNPNILTIVQQATFLTKEDTNLR
rmo_MCI_04765      IRELQSKQNPFEQLEFAYTNAKNFDQDIFGNRVKELINNPNILTIGQQATFLITEDTNLR
                   *****.*****:****** *:***********:.*********** ****** .******

rco_RC0110         KTINSDQAQAKLDDLRTAILSTIKIEELITANLPQHDFIAIVKEKDPELLKEFLKATTLT
rmo_MCI_04765      KTINSDQAQAKLDDLRTAILSTIKFEELITANLPQHEFIAIVKEKKPELLKEFLKATTIK
                   ************************:***********:********.************:.

rco_RC0110         VTGNNNLDQLRLALPSFTGMSNEQIRILSNKLKMSIILKALKECSQEKATQYIHTGNMPP
rmo_MCI_04765      LTGNNNLDQLRLALPSFTDMSNEQVRILASKLNMPIILKALKEHSQEKAKKHIHTGSMPP
                   :*****************.*****:***:.**:*.******** *****.::****.***

rco_RC0110         PPPPPPPLPDSQDLELAYLKSLGITKANT--STFKTTPKTYHFSSDIALRYKEFTLSGQK
rmo_MCI_04765      PPPP---LPDSQDLELAYLTSLGITKFNANTSTFKTTPKIYNFSSDIAVRYKEFTLSGQK
                   ****   ************.****** *:  ******** *:******:***********

rco_RC0110         SAGYKAKYSDADLLKKAIVESVAFEHSKNLSKAHQNNKYFEQIQKAVNTMYSSFIGHRTE
rmo_MCI_04765      SAGHKAKYSDANLFKKAIVESVAFEHSKNLSKAHQNNKYFAKIQEAVDTMHSSFIGPRTE
                   ***:*******:*:************************** :**:**:**:***** ***

rco_RC0110         LEQKIHNIYTSKLLELTKDKEFIKYVEDNIILNKKLTKAFTSADSDFIDSRTELEQKIHN
rmo_MCI_04765      IGQKIHNIYTSKLLELTKDKEFIKYVEDDIILSKKLTEAFTSADSDFIGPRTEIGQKIHN
                   : **************************:***.****:**********..***: *****

rco_RC0110         IYIQQLTKYPEEEVKEAFNTASLDFIGPRTEIGQEVHNIYKSQLLELTKDTELCLFTQQV
rmo_MCI_04765      IYTQQLTKYPEEAVKEAFNTANSDFIGPRTEIGQEVHNIYKSQLLELAKDKELFLFVEQL
                   ** ********* ********. ************************:**.** **.:*:

rco_RC0110         LAEATELEQKYGSDIQSENSNNEKKVERLDQEKLQLFKQENEATNDESSTKDDTQ-----
rmo_MCI_04765      LVESTELEQKYGSDIQSENSNNEKKIGRLDPKKLRLFQQKNEATNDESSTKDDTQSENSN
                   *.*:*********************: *** :**:**:*:***************

rco_RC0110         -----------------------PEDSNKKSEQSDSKTALSPRLLSSNDSKNDKSSDDKK
rmo_MCI_04765      KKSEQSEENEATNDESLTKDDTQPEDSNKKSEQSDSKTALSPRLLSSNDSKNDKSSDDKK
                                          *************************************

rco_RC0110         SLLALRSSDEDDTGYATDEEELEESN-----STTDEELKKDVVLESEDEAIDVSFKTEAI
rmo_MCI_04765      SLLALRSSDEDDKGYETNEEELEESNSTTDEKLTDEELKKDIVLESEDEAIDVSFKTEAI
                   ************.** *:********     . ********:******************

rco_RC0110         TEQDEVTQRQQVSDDTSGKVAILVQATSTLHKPVHYNIN-DRLTVAAIGAGDEETSINRG
rmo_MCI_04765      TEQNEATQRQQVSDDTSRKVAILVKATSTLHKPVHYNILSDRLKVAAIGAGDEEASINRG
                   ***:*.************ *****:*************  ***.**********:*****

rco_RC0110         VWISGLYGINKQRIWKNIPKYQNRTTGITIGTDAEFINSHDVIGIAYSRLESQIKYNKKL
rmo_MCI_04765      VWISGLYDINKQGTWKNIPKYQSRTTGITIGTDAEFINSHDVIGIAYSRLESQIKYNKKL
                   *******.****  ********.*************************************

rco_RC0110         GKTTVNGHLLSIYSLKELIKGFSLQTITSYGHNYIKNRSKNINNIIGKYQNNSLSFQTLL
rmo_MCI_04765      GKTAVNGHLLSIYGLKELIKGFSLQAITSYGHNYIKNRSKSINNIIGKYQNNNLSFQTLL
                   ***:*********.***********:**************.***********.*******

rco_RC0110         NYKYRTKYDLHFIPNIGFQYDYSRASNYKEYNVDIENLMIQKKSNQLFESSLGGKIVFKP
rmo_MCI_04765      NYKYRTKYDLHFIPNIGFKYDYSRASNYKEYNVDIENLLIQKKSNQLFESSLGSKIVFKP
                   ******************:*******************:**************.******

rco_RC0110         IVTTNNIVLTPSLYGNIEHHFNNKNTKVNAKATFKGQTLQETIITLKQPKLGYNIGSNIL
rmo_MCI_04765      IVTTNNIVLTPSLYGNIEHHFNNKNTKVNAKATFKGQTLHEKIIIPKQPKLGYNIGSNIL
                   ***************************************:*.**  **************

rco_RC0110         MSRKNINVLLEYNYYTHRKYQSHQGLIKLKVNL
rmo_MCI_04765      MSRKNINVLLEYNYYTHRKYQSHQGLIKLKVNL
                   *********************************
```

Supplementary Figure 8 | Amino acid sequence alignment of Sca2 protein from *R. conorii* and *R. montanensis.* Amino acid sequences of rickettsial Sca2 from *R. conorii* (RC0110) and *R. montanensis* (MCI_04765) were aligned using the ClustalW software. The alignment resulted in a percentage of sequence identity of 88.47 %.

```
rco_RC1085          MAQKPNFLKKLISAGLVTASTATIVASFAGSAMGAAIQQNRTTNAVATTVDGVGFDQTAV
rmo_MCI_02705       MAQKPNFLKKLISAGLVTASTATIVASFAGSAMGAATQQNRTTNGAATTVDGAGFDQTAA
                    ************************************ *******..******.******.

rco_RC1085          PANVAVPLNAVITAGVNKGITLNTPAGSFNGLFLNTANNLDVTVREDTTLGFITNVVNNA
rmo_MCI_02705       QVNAAVAPNAVITANANNGINFNTPAGSFNGLFLGTANNLAVTVSADTTLGFVTNVANNG
                     .*.**. ******..*:**.:************.***** ***  ******:***.**.

rco_RC1085          NHFNLMLNAGKTLTITGQGITNVQAAATKNANNVVAQVNNGAAIDNNDLQGVGRIDCGAA
rmo_MCI_02705       NSFNLTLGAGKTLTITGQGITNAQAAVTNNAQNVVAQFNGGVAVANNDLSGVGAIDFGAA
                    * *** *.**************.***.*:**:*****.*.*.*: ****.*** ** ***

rco_RC1085          ASTLVFNLANPTTQKAPLILGDNAVIVNGANGTLNVTNGFIKVSSKSFATVNVINIGDGQ
rmo_MCI_02705       ASTLVFNLAEPTTQKAPLILGGNAVIANGVNGTLNVTNGFIQVSDKSFATVKAINIGDGQ
                    *********:***********.****.**.***********:**.******:.*******

rco_RC1085          GIMFNTDADN-VNTLNLQANGATITFNGTDGTGRLVLLSKNAAATDFNVTGSLGGNLKGI
rmo_MCI_02705       GFIFNTNVAAGGNALNLQVGGATINFNGTDGTGRLVLLSKNGAATDFNVTGSLGGNLKGI
                    *::***:.    *:****..****.****************.******************

rco_RC1085          IEFNTVAVNGQLKANAGANAAVIGTNNGAGRAAGFVVSVDNGKVATIDGQVYAKDMVIQS
rmo_MCI_02705       IEFNTVAVAGQLIANAGPANAVIGTNNDAGRAAGFVVSVGNGNAATITGQVYAKDMVIQS
                    ******** *** ****.  *******.***********.**:.*** ************

rco_RC1085          ANAVGQVNFRHIVDVGTDGTTAFKTAASKVAITQNSNFGTTDFGNLAAQIIVPNTMTLNG
rmo_MCI_02705       ANAGGQVNFGHIVDVGTDGTTAFKTAASKVAITQNSNFGTTDFGNLAAQITVPDTMTLTG
                    *** ***** **************************************** **:****.*

rco_RC1085          NFTGDASNPGNTAGVITFDANGTLASASADANVAVTNNITAIEASGAGVVQLSGTHAAEL
rmo_MCI_02705       NFTGNASNPENTAGVITFAANGTLASASADANVAVTNNITAIEASGVGVVQLSGTHTAEL
                    ****:**** ******** ***************************.*********:***

rco_RC1085          RLGNAGSVFKLADGTVINGKVNQTALVGGALAAGTITLDGSATITGDIGNAGGAAALQGI
rmo_MCI_02705       RLGNAGSVFKLADGTVINGKVNQTAVVGGALAAGAITLDGSATITGDIGNGGGAAALQGI
                    *************************:********:***************.*********

rco_RC1085          TLANDATKTLTLGGANIIGANGGTINFQANGGTIKLTSTQNNIVVDFDLAIATDQTGVVD
rmo_MCI_02705       TLANDATKTLTLGGANIIGANGGTINFQANGGTIKLTSTQNNILVDFDLAINTDQTGVVD
                    *******************************************:******* ********


rco_RC1085          ASSLTNAQTLTINGKIGTVGANNKTLGQFNIGSSKTVLSDGDVAINELVIGNNGAVQFAH
rmo_MCI_02705       ASSLTNAQTLTISGTIGTVGANNKTLGQFNVGSSKTALNGGNVAINELVIGNNGSVQFAH
                    ************.*.***************:*****.*..*:************:*****

rco_RC1085          NTYLITRTTNAAGQGKIIFNPVVNNNTTLATGTNLGSATNPLAEINFGSKGAANVDTVLN
rmo_MCI_02705       DTYLITKTTNAAGQGKIIFNPVVNNNTTLAAGTNLGSAANPLAEINFGSK-GAHADTILN
                    :*****:***********************:*******:*********** .*:.**:**

rco_RC1085          VGKGVNLYATNITTTDANVGSFIFNAGGTNIVSGTVGGQQGNKFNTVALDNGTTVKFLGN
rmo_MCI_02705       VGKGVNLYATNITTTDANVGSFVFNAGGTNIVSGTVGGQQGSKFNTVELDNGTTVKFLGN
                    **********************:******************.***** ************

rco_RC1085          ATFNGNTTIAANSTLQIGGNYTADFVASADGTGIVEFVNTGPITVTLNKQAAPVNALKQI
rmo_MCI_02705       AMFNGGTKIEAKSTLQIGGNYTADFVASADGTGIVEFVNTGPITVTLNKQAAPVGVLKQM
                    * ***.*.* *:******************************************..***:

rco_RC1085          TVSGPGNVVINEIGNAGNYHGAVTDTIAFENSSLGAVVFLPRGIPFNDAGNRIPLTIKST
rmo_MCI_02705       TVSGPGNVVVNEIGNAGNAHGAVTGTIAFENSSLGATLFLPSGIPFNDVT------IKST
                    *********:******** *****.***********.:*** ******.       ****

rco_RC1085          VGNKTATGFDVPSVIVLGVDSVIADGQVIGDQNNIVGLGLGSDNDIIVNATTLYAGIGTI
rmo_MCI_02705       VGNETAKGFDAPKVIVSGVDSVIADGQVIGDQNNIVGLGLGSDNGIIVNATKLYAGIGTT
                    ***:**.***.*.*** ***************************.******.*******

rco_RC1085          NNNQGTVTLSGGIPNTPGTVYGLGTGIGASKFKQVTFTTDYNNLGNIIATNATINDGVTV
rmo_MCI_02705       NNNQGTVILSGGVPNTPGTVYGLGKDASASQLKQVTFTTDYNNLGNIIATNTTINDGVTV
                    ******* ****:***********.. .**::*******************:********

rco_RC1085          TTGGIAGIGFDGKITLGSVNGNGNVRFVDGILSHSTSMIGTTKANNGTVTYLGNAFVGNI
rmo_MCI_02705       TTGGIAGTVFNGKITLGSVNGNANVRFADGILSNSTSMIVTTKANNGTVTYLGGAYVGSI
                    *******  *:***********.****.*****:***** *************.*:**.*

rco_RC1085          GDSDTPVASVRFTGS----DGGAGLQGNIYSQVIDFGTYNLGISNSNVILGGGTTAINGK
rmo_MCI_02705       GASNTPVASVRFTGSGTGSDNGAVLQGNIYSQVIDFGTYDLDIVNSNVILGGGTTAINGA
                    * *:***********    *.** ***************:*.* ***************

rco_RC1085          INLRTNTLTFASGTSTWGNNTSIETTLTLANGNIGNIVILEGAQVNATTTGTTTIKVQDN
rmo_MCI_02705       IDLCTNTLTFASGTSTLGNNTSIETTLTLADGNIGHIVIAEGARVNATTTGTTTINVQDK
                    *:* ************ *************:****:*** ***:***********:***:

rco_RC1085          ANANFSGTQTYTLIQGGARFNGTLGGPNFVVTGSNRFVNYGLIRAANQDYVITRTNNAEN
rmo_MCI_02705       ANANFSGTQTYTLIQGGARFNGTLGDPDFAVTGSNRFVNYGLIRAANQDYVITRTNNVEN
                    *************************.*:*.***************************.**

rco_RC1085          VVTNDIANSSFGGAPGVGQNVTTFVNATNTAAYNNLLLAKNSANSANFVGAIVTDTSAAI
rmo_MCI_02705       VVTNDIASSTFGGALGVGQNVTTFVNATNTAAYNNLLLAKNSADSANFVGSIVTDTSAAV
                    *******.*:**** ****************************:******:********:

rco_RC1085          TNAQLDVAKDIQAQLGNRLGALRYLGTPETAEMAGPEAGAIPAAVAAGDEAVDNVAYGIW
rmo_MCI_02705       TNVQLDVAKNIQAQLGNRLGALRYLGTPETAEMAGPEAGAIPAAVAAGDEAVDNVAYGIW
                    **.******:**************************************************

rco_RC1085          AKPFYTDAHQSKKGGLAGYKAKTTGVVIGLDTLANDNLMIGAAIGITKTDIKHQDYKKGD
rmo_MCI_02705       AKPFYTDAHQSKKGGLAGYKAKTTGVVIGLDTLANDNLMIGAAIGITKTDIKHQDYKKGD
                    ************************************************************

rco_RC1085          KTDVNGFSFSLYGAQQLVKNFFAQGSAIFSLNQVKNKSQRYFFDANGNMSKQIAAGHYDN
rmo_MCI_02705       KTDVNGFSFSLYGAQQLVENFFAQGSAIFSLNQVKNKSQRYFFDANGNMSKQIAAGNYDN
                    ******************:*************************************:***

rco_RC1085          MTFGGNLTVGYDYNAMQGVLVTPMAGLSYLKSSDENYKETGTTVANKQVNSKFSDRTDLI
rmo_MCI_02705       MTFGGNLTVGYDYNAMQGVLVTPMAGLSYLKSSDENYKETGTTVANKQVNSKFSDRTDLI
                    ************************************************************

rco_RC1085          VGAKVAGSTMNITDLAVYPEVHAFVVHKVTGRLSKTQSVLDGQVTPCISQPDRTAKTSYN
rmo_MCI_02705       VGAKVAGGTMNITDLVVYPEAHAFVVHKVNGRLSKTQSVLDGQVTPCISQSDRTAKTSYN
                    *******.*******.****.********.********************.*********

rco_RC1085          LGLSASIRSDAKMEYGIGYDAQISSKYTAHQGTLKVRVNF
rmo_MCI_02705       LGLSASIRPDAKMEYGIGYDAQIASKYTAHQGTLKVRVNF
                    ********.**************:****************
```

Supplementary Figure 9 | Amino acid sequence alignment of Sca5 protein from *R. conorii* and *R. montanensis*. Amino acid sequences of rickettsial Sca5 from *R. conorii* (RC0019) and *R. montanensis* (MCI_02705) were aligned using the ClustalW software. The alignment resulted in a percentage of sequence identity of 88.14 %.

```
rco_RC1270          MKRKNNKFIEISIAFILGIALGLYGQNPDYFTNLISQKSLALSALQIKHYNISELSRSKV
rmo_MCI_03585       MKRRNNKFIEISTAFILGIALGIYGQNPDYFTNLISQKSLALPALQIKHYNISELSRSKV
                    ***:******** *********:*******************.*****************

rco_RC1270          STCFTPPAGCTKFIANQIDKAEESIYMQAYGMSDALITTALINAQARGVKVRILLDRSNL
rmo_MCI_03585       STCFTPPAGCTKFIANQIDKAEESIYMQAYGMSDELITTALINAQARGVKVRILLDRSNL
                    ********************************** *************************

rco_RC1270          KQKFSKLHELQRAKIDVDIDKVPGIAHNKVIIIDKKKVITGSFNFTAAADKRNAENVIII
rmo_MCI_03585       KQKFSKLHELQQAKIDVGIDKVPGIAHNKVIIIDKKKVITGSFNFTAAADKRNAENVIII
                    ***********:*****.******************************************

rco_RC1270          EDQELAESYLQNWLNRKASN
rmo_MCI_03585       EDQELAESYLQNWLNRKASN
                    ********************
```

Supplementary Figure 10 | Amino acid sequence alignment of membranolytic phospholipase D protein from *R. conorii* and *R. montanensis.* Amino acid sequences of rickettsial phospholipase D from *R. conorii* (RC1270) and *R. montanensis* (MCI_03585) were aligned using the ClustalW software. The alignment resulted in a percentage of sequence identity of 96.50 %.

```
rmo_MCI_02955      MLKSSKKEDSSKKNQNNKLIFTVQKLFSPIKNFFRKTKTPDNFFGVIKRLKINSQKMTLD
rco_RC1141         MLKSSKKEDSSKKNQNNKLIFTVRKLFSPIKNFFRKTKTPDNFFGVIKRLKINSQKMTLD
                   ***********************:************************************

rmo_MCI_02955      ERNILANLLELEDKTIEDIMVPRSDIAAIKLTTNLEELSESIKLEVPHTRTLIYDGTLDN
rco_RC1141         ERNILANLLELEDKTIEDIMVPRSDIAAIKLTTNLEELSESIKLEVPHTRTLIYDGTLDN
                   ************************************************************

rmo_MCI_02955      VVGFIHIKDLFKALATKQNGRLKKLIRKHIIAAPSMKLLDLLAKMRRERTHIAIVVDEYG
rco_RC1141         VVGFIHIKDLFKALATKQNGRLKKLIRKHIIAAPSMKLLDLLAKMRRERTHIAIVVDEYG
                   ************************************************************

rmo_MCI_02955      GTDGLVTIEDLIEEIVGRIDDEHDQQLDSDNFKVINNSTIISNARVEVEVLEEIIGEKLQ
rco_RC1141         GTDGLVTIEDLIEEIVGRIDDEHDQQLDSDNFKVINNSTIIANARVEVEVLEEIIGEKLH
                   *****************************************:*****************:

rmo_MCI_02955      NDDDEFDTIGGLVLTRVSSVPAIGTRIDISENIEIEVTDATPRSLKQVKIRLKNGLNGK
rco_RC1141         NDYDEFDTIGGLVLTRVSSVPAIGTRIDISENIEIEVTDATPRSLKQVKIRLKNGLNGQ
                   ** *******************************************************:
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Supplementary Figure 11 | Amino acid sequence alignment of haemolysin C protein from *R. conorii* and *R. montanensis.* Amino acid sequences of rickettsial haemolysin C from *R. conorii* (RC1141) and *R. montanensis* (MCI_02955) were aligned using the ClustalW software. The alignment resulted in a percentage of sequence identity of 98.34 %.

Supplementary Table 1 | Primers and probes used in q-PCR assays.

Primer name	**Primer sequence**	**Concentration of primer or probe in PCR reaction**
Actin-F420	5’-CCTGTATGCCTCTGGTCGTA-3’	300 nM
Actin-R681	5’-CCATCTCCTGCTCGAAGTCT-3’	300 nM
Actin-Hex	5’-/5MAXN/ACTGTGCCC/ZEN/ATCTAC-3’	200 nM
Sca1-F5271	5’-CAAGCTCGTTATTACCCCGAAT-3’	300 nM
Sca1-R5371	5’-CTACCGCTCCTTGGAATGTTAGACC -3’	300 nM
Sca1-Fam	5’-/56-FAM/TCGGCTTAA/ZEN/GATACGGGAAGT-3’	200 nM