

Cell Reports, Volume 33

Supplemental Information

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A Highly Susceptible Model for a Rapid
and Fatal Course of SARS-CoV-2 Infection**

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The Roborovski dwarf hamster – a highly susceptible model for a rapid and fatal course of SARS-CoV-2 infection

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Supplementary Materials

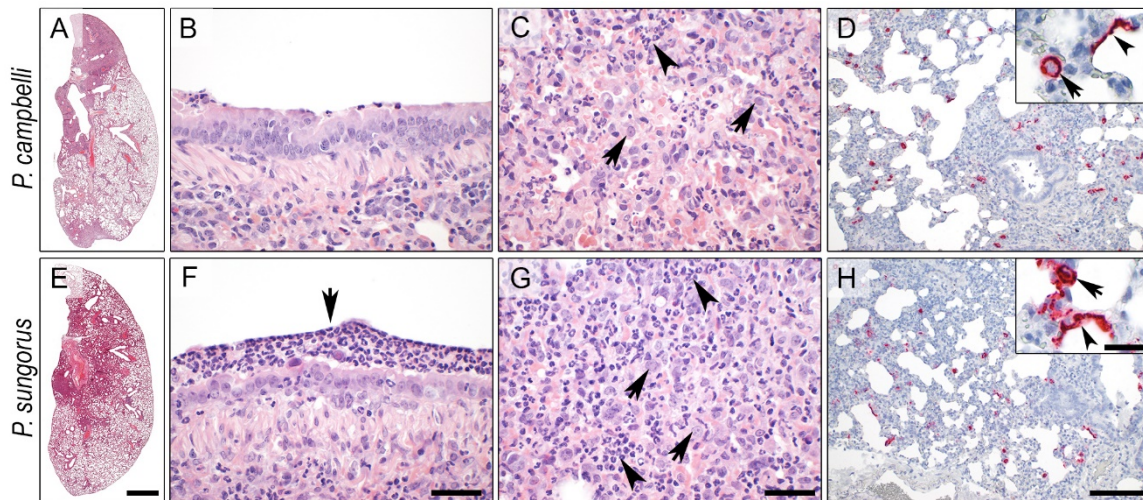


Figure S1: Histopathology of further *Phodopus* species (related to Figure 4). *Phodopus* (*P. campbelli* and *P. sungorus*) developed pneumonia after SARS-CoV2-standard dose infection similar to low dose infected *P. roborovskii* with multifocal consolidated areas (A, E). *P. campbelli* had only minimal bronchitis (B), necrosuppurative pneumonia with infiltration of mainly neutrophils (C, arrowhead) and strong initiation of regeneration by alveolar epithelial type II cells (AEC-II, C, arrows) at 3 dpi. *In situ*-hybridization localized viral RNA in bronchial epithelial cells, AEC-I (arrowhead), AEC-II (arrow) and alveolar macrophages associated with areas of inflammation. *P. sungorus* developed marked necrosuppurative bronchitis (F, arrow) and pneumonia at 3 dpi with stronger infiltration of neutrophils (G, arrowheads) and also prominent regeneration of AEC-II (G, arrows) at 3 dpi. Viral RNA was detected with virtually identical distribution and cellular tropism as observed in *P. campbelli*. Red, viral RNA signals; blue, hemalaun counterstain. Bars 1 mm (A, E), 50 μ m, (B, C, F, G), 200 μ m (D, H) and 20 μ m (insets in D, H).

Cricetulus griseus	MSSSSWLLLSLVAVTTAQSII EEQAKTFLDKFNQEAEDLSYQSALASWNYNTNI TEENAQ	60
Mesocricetus auratus	MSSSSWLLLSLVAVTTAQSII EEQAKTFLDKFNQEAEDLSYQSALASWNYNTNI TEENAQ	60
Phodopus campbelli	MSSSSWLLLSLVAVTTAQSII EEQAKTFLDKFNQEAEDLSYQSALASWNYNTNI TEENAQ	60
Phodopus sungorus	MSSSSWLLLSLVAVTTAQSII EEQAKTFLDKFNQEAEDLSYQSALASWNYNTNI TEENAQ	60
Phodopus roborovskii	MSSSSWLLLSLVAVTTAQSII EEQAKTFLDKFNQEAEDLSYQSALASWNYNTNI TEENAQ *****	60
Cricetulus griseus	KMNEAAKWSAFYEEQSKLAKNYSLQEVQNLII KRQLQALQQSGSSALSADKKNQLNTIL	120
Mesocricetus auratus	KMNEAAKWSAFYEEQSKLAKNYSLQEVQNLII KRQLQALQQSGSSALSADKKNQLNTIL	120
Phodopus campbelli	KMNEAAKWSAFYEEQSKLAKNYSLQEVQNLII KRQLQALQQSGSSALSADKKNQLNTIL	120
Phodopus sungorus	KMNEAAKWSAFYEEQSKLAKNYSLQEVQNLII KRQLQALQQSGSSALSADKKNQLNTIL	120
Phodopus roborovskii	KMNEAAKWSAFYEEQSKLAKNYSLQEVQNLII KRQLQALQQSGSSALSADKKNQLNTIL *****	120
Cricetulus griseus	NTMSTIYSTGKVCNPKNFQECLELLEPGLDDIMATSTDYNERLWAWEGWRAEVGKQLRPLY	180
Mesocricetus auratus	NTMSTIYSTGKVCNPKNFQECLELLEPGLDDIMATSTDYNERLWAWEGWRAEVGKQLRPLY	180
Phodopus campbelli	NTMSTIYSTGKVCNPKNFQECLELLEPGLDDIMATSTDYNERLWAWEGWRAEVGKQLRPLY	180
Phodopus sungorus	NTMSTIYSTGKVCNPKNFQECLELLEPGLDDIMATSTDYNERLWAWEGWRAEVGKQLRPLY	180
Phodopus roborovskii	NTMSTIYSTGKVCNPKNFQECLELLEPGLDDIMATSTDYNERLWAWEGWRAEVGKQLRPLY *****	180
Cricetulus griseus	EYVVLKNEMARANNYKDYGYWRGDYEAEGADGYNYNGNQLIEDVERTFKEIKPLYEQL	240
Mesocricetus auratus	EYVVLKNEMARANNYKDYGYWRGDYEAEGADGYNYNGNQLIEDVERTFKEIKPLYEQL	240
Phodopus campbelli	EYVVLKNEMARANNYKDYGYWRGDYEAEGADGYNYNGNQLIEDVERTFKEIKPLYEQL	240
Phodopus sungorus	EYVVLKNEMARANNYKDYGYWRGDYEAEGADGYNYNGNQLIEDVERTFKEIKPLYEQL	240
Phodopus roborovskii	EYVVLKNEMARANNYKDYGYWRGDYEAEGADGYNYNGNQLIEDVERTFKEIKPLYEQL *****	240
Cricetulus griseus	HAYVRTKLMDTYPSYISPTGCLPAHLLGDMWGRFNTNLYPLTVFPFGQKFNIDVTDAMVQ	300
Mesocricetus auratus	HAYVRTKLMNTYPSYISPTGCLPAHLLGDMWGRFNTNLYPLTVFPFGQKFNIDVTDAMVQ	300
Phodopus campbelli	HAYVRTKLVNTYPSYISPTGCLPAHLLGDMWGRFNTNLYPLTVFPFGQKFNIDVTDAMVQ	300
Phodopus sungorus	HAYVRTKLVNTYPSYISPTGCLPAHLLGDMWGRFNTNLYPLTVFPFGQKFNIDVTDAMVQ	300
Phodopus roborovskii	HAYVRTKLMDTYPSYISPTGCLPAHLLGDMWGRFNTNLYPLTVFPFGQKFNIDVTDAMVQ *****	300
Cricetulus griseus	GWDAERIFKEAEKFFVSVGLPHMTQGFQWNSMLTDPGDRKVVCHPTAWDLGKGFRIKM	360
Mesocricetus auratus	GWNAERIFKEAEKFFVSVGLPYMTQGFQWNSMLTDPGDRKVVCHPTAWDLGKGFRIKM	360
Phodopus campbelli	GWGAERIFKEAEKFFVSVGLPHMTQGFQWNSMLTDPGDRKVVCHPTAWDLGKGFRIKM	360
Phodopus sungorus	GWDAERIFKEAEKFFVSVGLPHMTQGFQWNSMLTDPGDRKVVCHPTAWDLGKGFRIKM	360
Phodopus roborovskii	GWDAERIFKEAEKFFVSVGLPHMTQGFQWNSMLTDPGDRKVVCHPTAWDLGKGFRIKM *****	360
Cricetulus griseus	CTKVTMDNFLTAAHEMGHIQYDMAYATQPFLLRNGANEGFHEAVGEIMLSAATPKHLKS	420
Mesocricetus auratus	CTKVTMDNFLTAAHEMGHIQYDMAYATQPFLLRNGANEGFHEAVGEIMLSAATPEHLKS	420
Phodopus campbelli	CTKVTMDNFLTAAHEMGHIQYDMAYATQPFLLRNGANEGFHEAVGEIMLSAATPEHLKS	420
Phodopus sungorus	CTKVTMDNFLTAAHEMGHIQYDMAYATQPFLLRNGANEGFHEAVGEIMLSAATPEHLKS	420
Phodopus roborovskii	CTKVTMDNFLTAAHEMGHIQYDMAYATQPFLLRNGANEGFHEAVGEIMLSAATPEHLKS *****	420
Cricetulus griseus	IGLLPSNFHEDNETEINFLKQALITVGLPFTYMLEKWRWVMPKGDIPKEQWMEKWEM	480
Mesocricetus auratus	IGLLPSDFQEDNETEINFLKQALITVGLPFTYMLEKWRWVMPKGDIPKEQWMEKWEM	480
Phodopus campbelli	IGLLPSNFQEDSETEINFLKQALITVGLPFTYMLEKWRWVMPKGDIPKEQWMEKWEM	480
Phodopus sungorus	IGLLPSNFQEDSETEINFLKQALITVGLPFTYMLEKWRWVMPKGDIPKEQWMEKWEM	480
Phodopus roborovskii	IGLLPSNFQEDSETEINFLKQALITVGLPFTYMLEKWRWVMPKGDIPKEQWMEKWEM *****	480
Cricetulus griseus	KREIVGVVEPLPHDETYCDPAALPHVSNDFSFIRYRTTIIYQFQFQALCQAAKHDGFLH	540
Mesocricetus auratus	KREIVGVVEPLPHDETYCDPAALPHVSNDFSFIRYRTTIIYQFQFQALCQAAKHDGFLH	540
Phodopus campbelli	KREIVGVVEPLPHDETYCDPAALPHVSNDFSFIRYRTTIIYQFQFQALCQAAKHDGFLH	540
Phodopus sungorus	KREIVGVVEPLPHDETYCDPAALPHVSNDFSFIRYRTTIIYQFQFQALCQAAKHDGFLH	540
Phodopus roborovskii	KREIVGVVEPLPHDETYCDPAALPHVSNDFSFIRYRTTIIYQFQFQALCQAAKHDGFLH *****	540
Cricetulus griseus	KCDISNSTEAGQKLLNMLRLGKSEPWTLALENVVGARNMDVRPLLNYFEPLSVWLKEQNK	600
Mesocricetus auratus	KCDISNSTEAGQKLLNMLRLGKSEPWTLALENVVGARNMDVRPLLNYFEPLSVWLKEQNK	600
Phodopus campbelli	KCDISNSTEAGQKLVNMLRLGKSGPWTLALENVVGARNMDVRPLLNYFEPLSVWLKEQNK	600
Phodopus sungorus	KCDISNSTEAGQKLVNMLRLGKSEPWTLALENVVGARNMDVRPLLNYFEPLSVWLKEQNK	600
Phodopus roborovskii	KCDISNSTEAGQKLLNMLRLGKSEPWTLALENVVGARNMDVRPLLNYFEPLSVWLKEQNK *****	600
Cricetulus griseus	NSFVGWNTDWSPYADQS IKVRI SLKSALGENAYEWNDDNEMYLFRASVAYAMRVYPAKNKT	660
Mesocricetus auratus	NSFVGWNTDWSPYADQS IKVRI SLKSALGENAYEWNDDNEMYLFRASVAYAMRVYPAKNKT	660
Phodopus campbelli	NSFVGWNTDWSPYADQS IKVRI SLKSALGENAYVWDDNEMYLFRASVAYAMRVYPAKNKT	660
Phodopus sungorus	NSFVGWNTDWSPYADQS IKVRI SLKSALGENAYVWDDNEMYLFRASVAYAMRVYPAKNKT	660
Phodopus roborovskii	NSFVGWNTDWSPYADQS IKVRI SLKSALGENAYEWNDDNEMYLFRASVAYAMRVYPAKNKT *****	660
Cricetulus griseus	QTVLFGVEDIRVSDLKPRVSNFVFTSPQNMSDII PRNEVEEAVRFSRGRINDVFGLLDN	720
Mesocricetus auratus	QTVPFVGEDIRVSDLKPRVSNFVFTSPQNMSDII PRNEVEEAVRFSRGRINDVFGLLDN	720
Phodopus campbelli	QIVPFVGEDIRVSDLTFRVSNFVFTSPQNMSDII PRNEVEEAVRFSRGRINDVFGLLDN	720
Phodopus sungorus	QIVPFVGEDIRVSDLTFRVSNFVFTSPQNMSDII PRNEVEEAVRFSRGRINDVFGLLDN	720
Phodopus roborovskii	QTVPFVGEDIRVSDLTFRVSNFVFTSPQNMSDII PRNEVEEAVRFSRGRINDVFGLLDN *****	720
Cricetulus griseus	SLEFLGINPTLAPPYQPPVTIWLII FGVVMGIVVVGIVLIVTGIRAKKKNNEAKREENP	780
Mesocricetus auratus	SLEFLGINPTLSPPYQPPVTIWLII FGVVMGIVVVGII LILIFTGIGRKKKNETKREENP	780
Phodopus campbelli	SLEFLGINPTLAPPYQPPVTIWLII FGVVMGIVVVGIVLIVTGIRAKKKNNEAKREENP	780
Phodopus sungorus	SLEFLGINPTLAPPYQPPVTIWLII FGVVMGIVVVGIVLIVTGIRAKKKNNEAKREENP	780
Phodopus roborovskii	SLEFLGINPTLAPPYQPPVTIWLII FGVVMGIVVVGII LILIVTGIRAKKKNNEAKREENP *****	780
Cricetulus griseus	YDSVDIGKGESNAGFQSNDDVQTSF	805
Mesocricetus auratus	YDSVDIGKGESNAGFLSNDDAQTFS	805
Phodopus campbelli	YASTDIGKGESNAGFQSNDDAQTFS	805
Phodopus sungorus	YDSTDIGKGESNAGFQSNDDAQTFS	805
Phodopus roborovskii	YDSMDIGKGESNAGFQSNDDAQTFS *****	805

Figure S2 – Amino acid alignment of Hamster ACE-2 sequences (related to Figure 5).

Table S1: SARS-CoV-2 blood titers and virus loads in brains of infected Roborovski dwarf hamsters (related to Figures 2 and 3). Presented as means \pm standard deviations of standard dose-infected (SD, n = 10) and low dose-infected Roborovski dwarf hamsters (LD, n = 3).

d.p.i.	Blood titers (SD)	Blood titers (LD)	Virus RNA copies (brains, SD)	Virus RNA copies (brains, LD)
2	all < 100	all < 100	0	n.a.
3	$3.9 \times 10^4 \pm 7.9 \times 10^4$		$4.8 \times 10^1 \pm 1.1 \times 10^2$	

Table S2: Comparison of pathologies and distribution of viral RNA in Phodopus species following SARS-CoV-2 infection (related to Figure 4).

Evaluation criteria	Roborovski standard dose	Roborovski low dose	Campbell standard dose	Djungarian standard dose
Histopathology				
Distribution	diffuse	multifocal	multifocal	multifocal
Alveolar damage	++	+	+	+
Bronchitis	+/-	+	+	++
Pneumonia	+/-	++	++	++
Endothelialitis	-	+/-	-	-
Hyaline thrombi	++	-	-	-
Edema	+	+	+/-	-
Regeneration AEC	-	++	++	++
Regeneration BEC	-	+	-	-
SARS-CoV-2 detection by <i>in situ</i> -hybridization				
AEC I	-	-	++	++
AEC II	++	++	++	++
BEC	+	+	+	+
Macrophages	+	+	+	+

++ main lesion, + observed, +/- variable, - not observed, AEC = alveolar epithelial cells, BEC = bronchial epithelial cells

Table S3: Oligonucleotides used in this study (related to the STAR Methods section).

Primer/probe	Sequence 5'-3'
SARS-CoV-2 qPCR forward	ACAGGTACGTTAATAGTTAATAGCGT
SARS-CoV-2 qPCR reverse	ATATTGCAGCAGTACGCACACA
SARS-CoV-2 qPCR probe	FAM-ACACTAGCCATCCTTACTGCGCTTCG-BHQ
ACE2 seq forward 1	TCCTGGCTCCTTCTCAG
ACE2 seq forward 2	GACAAGTTTAACCAGGAAGCT
ACE2 seq forward 3	CCAAAAGATGAATGAGGCTG
ACE2 seq reverse 1	TTCCTTCAACTTCTTTGTCACT
ACE2 seq reverse 2	WGTACCATATGGCTGATT
ACE2 seq reverse 3	TGATGAYGYTCAGACTTCATTTTAG