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# Supplemental information

# Phase separation of the LINE-1 ORF1 protein is mediated by the N-ter-

# minus and coiled-coil domain

Jocelyn C. Newton, Mandar T. Naik, Grace Y. Li, Eileen L. Murphy, Nicolas L. Fawzi, John M. Sedivy, and Gerwald Jogl

**Table S1. Nucleotide and Protein Sequences of Human LINE-1 ORF1**<sub>1-338</sub> (UniProt Q9UN81)</sub> The nucleotide sequence was codon optimized for expression in *E. coli* and subcloned into pET26b using Ndel and XhoI (cut sites italicized in the nucleotide sequence).

# E. coli codon optimized ORF1 gene sequence used in this study

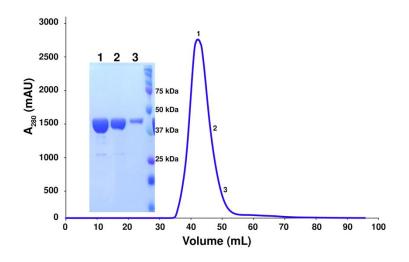
*CAT*ATGGGTAAAAAACAGAATCGTAAGACCGGTAACAGCAAAA CGACCGAGCAGAGCTGGATGGAAAACGACTTCGATGAGCTGCGTG AGGAAGGTTTTCGTCGTAGCAACTACAGCGAGCTGCGTGAAGACA TCCAAACCAAGGGCAAAGAGGTGGAAAACTTTGAAAAGAACCTGG AGGAATGCATCACCCGTATTACCAACACCGAGAAGTGCCTGAAAG AGCTGATGGAACTGAAGACCAAAGCGCGTGAACTGCGTGAGGAAT GCCGTAGCCTGCGTAGCCGTTGCGACCAGCTGGAGGAACGTGTGA GCGCGATGGAGGATGAAATGAACGAGATGAAGCGTGAGGGTAAAT TCCGTGAGAAGCGTATCAAACGTAACGAACAGAGCCTGCAAGAGA TTTGGGATTACGTTAAGCGTCCGAACCTGCGTCTGATCGGTGTGC CGGAGAGCGACGTTGAAAACGGCACCAAACTGGAAAACACCCTGC AGGATATCATTCAAGAGAACTTTCCGAACCTGGCGCGTCAAGCGA ACGTGCAGATCCAAGAAATTCAGCGTACCCCGCAACGTTATAGCA GCCGTCGTGCGACCCCGCGTCACATCATTGTGCGTTTCACCAAGG TTGAGATGAAGGAAAAAATGCTGCGTGCGGCGCGTGAGAAAGGTC GTGTTACCCTGAAGGGCAAACCGATTCGTCTGACCGCGGATCTGA GCGCGGAAACCCTGCAGGCGCGTCGTGAGTGGGGGTCCGATCTTCA ACATTCTGAAGGAGAAGAACTTTCAACCGCGTATCAGCTACCCGG CGAAACTGAGCTTCATTAGCGAGGGCGAAATCAAGTACTTCATCG ACAAGCAGATGCTGCGTGATTTCGTTACCACCCGTCCGGCGCTGA AGGAGCTGCTGAAAGAAGCGCTGAATATGGAACGCAATAACCGCT ACCAACCGCTGCAAAATCACGCGAAAATGTAACTCGAG

# Protein Sequence of ORF1<sub>1-338</sub> used in this study

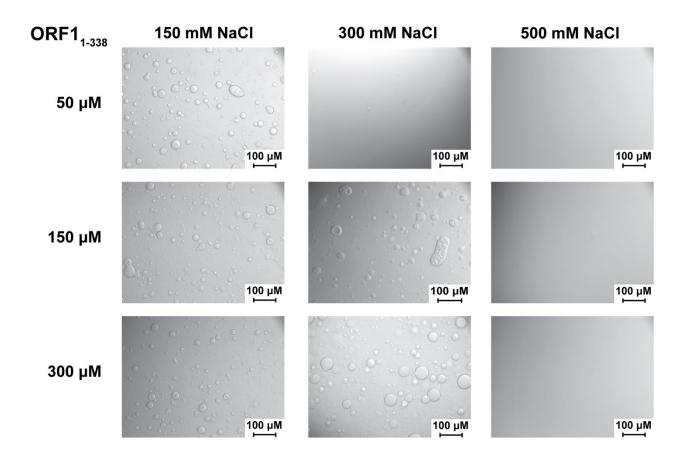
MGKKQNRKTGNSKTQSASPPPKERSSSPATEQSWMENDFDELREEGFRRSNYSELREDIQTKGKEVENFE KNLEECITRITNTEKCLKELMELKTKARELREECRSLRSRCDQLEERVSAMEDEMNEMKREGKFREKRIK RNEQSLQEIWDYVKRPNLRLIGVPESDVENGTKLENTLQDIIQENFPNLARQANVQIQEIQRTPQRYSSR RATPRHIIVRFTKVEMKEKMLRAAREKGRVTLKGKPIRLTADLSAETLQARREWGPIFNILKEKNFQPRI SYPAKLSFISEGEIKYFIDKQMLRDFVTTRPALKELLKEALNMERNNRYQPLQNHAKMLEHHHHHH

Table S2. Concentration measurements of the dilute phase of ORF1. Concentrations in  $\mu$ M of ORF1 measured in the dilute phase of the assays plotted in the main text Figures 3G and 4B.

ORF1 Construct	150 mM NaCl	200 mM NaCl	250 mM NaCl	300 mM NaCl	350 mM NaCl	400 mM NaCl	450 mM NaCl	500 mM NaCl
ORF1 <sub>1-338</sub>	9 ± 1	22 ± 1	76 ± 4	220 ± 10	311 ± 10	308 ± 11	305 ± 10	293 ± 8
ORF1 <sub>66-338</sub>	234 ± 24	324 ± 6	335 ± 7	340 ± 3	339 ± 4	332 ± 8	340 ± 3	335 ± 7
ORF1 <sub>1-131</sub>	340 ± 32	319 ± 9	314 ± 34	31 ± 5	337 ± 28	322 ± 2	318 ± 8	326 ± 1
ORF1 <sub>1-141</sub>	77 ± 7	130 ± 9	255 ± 5	323 ± 10	317 ± 7	311 ± 20	319 ± 1	323 ± 2
ORF1 <sub>1-338</sub> L93P	4 ± 2	8 ± 0.4	33 ± 2	115 ± 3	288 ± 11	294 ± 5	308 ± 29	323 ± 15
ORF1 <sub>1-338</sub> S27D	4 ± 1	10 ± 2	30 ± 3	152 ± 15	301 ± 4	304 ± 2	300 ± 4	293 ± 16
ORF <sub>1-338</sub> K3A/K4A	6 ± 1	17 ± 5	61 ± 16	190 ± 38	293 ± 31	298 ± 24	297 ± 34	296 ± 31
ORF <sub>1-338</sub> K3E/K4E	13 ± 2	35 ± 1	119 ± 9	323 ± 10	316 ± 2	320 ± 7	320 ± 0.3	316 ± 4
ORF <sub>1-338</sub> R7E/K8E	11 ± 2	42 ± 9	147 ± 17	303 ± 6	274 ± 22	276 ± 25	265 ± 21	277 ± 9
ORF <sub>1-338</sub> K3A/K4A/ R7A/K8A	11 ± 3	40 ± 4	133 ± 10	278 ± 20	293 ± 21	258 ± 21	277 ± 39	313 ± 6
ORF <sub>1-338</sub> K3E/K4E/ R7E/K8E	11 ± 2	37 ± 2	133 ± 5	316 ± 1	312 ± 3	316 ± 3	316 ± 2	315 ± 2

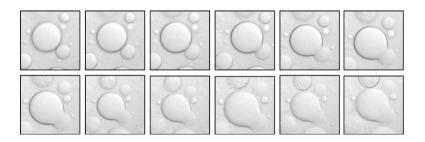


**Figure S1. Gel Filtration Profile of ORF1**<sub>1-338</sub>**.** ORF1<sub>1-338</sub> elutes as a trimer on a Hi-Prep 16/60 Sephacryl S300 in 20 mM Tris pH8.0, 1 M NaCl, 1 mM DTT with little evidence of hexamers or higher order oligomers.



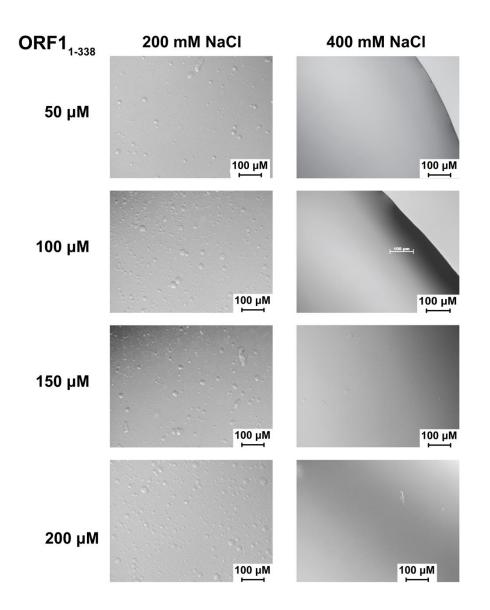
#### Figure S2. ORF1<sub>1-338</sub> phase separation is dependent on protein and salt concentration.

At lower NaCl concentrations, ORF1<sub>1-338</sub> phase separates more readily than at higher NaCl concentrations. This phenomenon is dependent on both the protein concentration and salt concentration. Images collected at room temperature with noted concentration of ORF1<sub>1-338</sub> in 20 mM Tris pH 8.0, 1 mM DTT and the NaCl concentration listed.



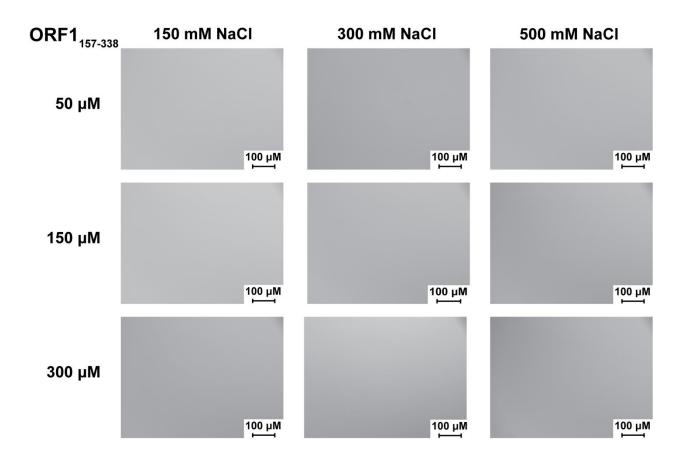
# Figure S3. ORF1 droplets behave as liquids.

Images were taken in succession (within approximately 10 seconds) to demonstrate that the ORF1 droplets are capable of flowing and fusing in solution.



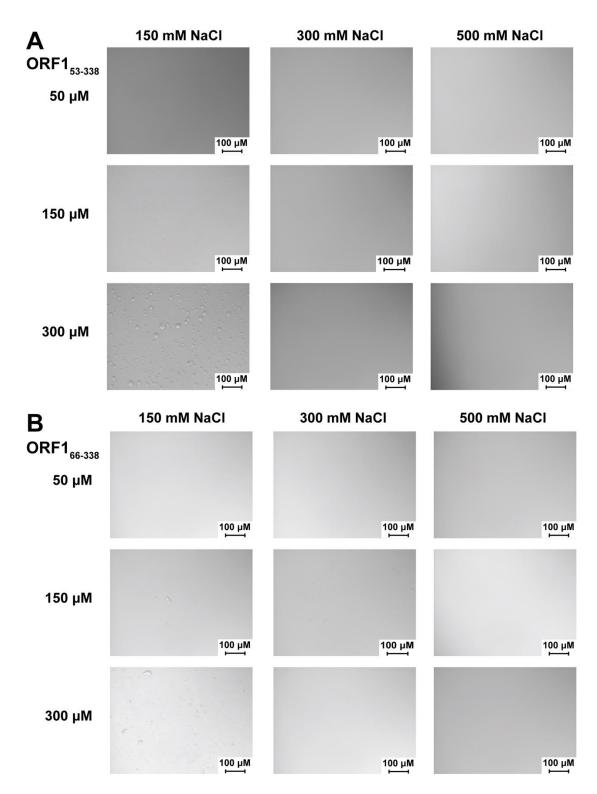
# Figure S4. ORF1<sub>1-338</sub> phase separation at 200 and 400 mM NaCl.

At higher protein concentrations, some amorphous aggregates or hydrogels can be observed in the 200 mM NaCl conditions as well as low levels of phase separation in the 400 mM NaCl conditions.



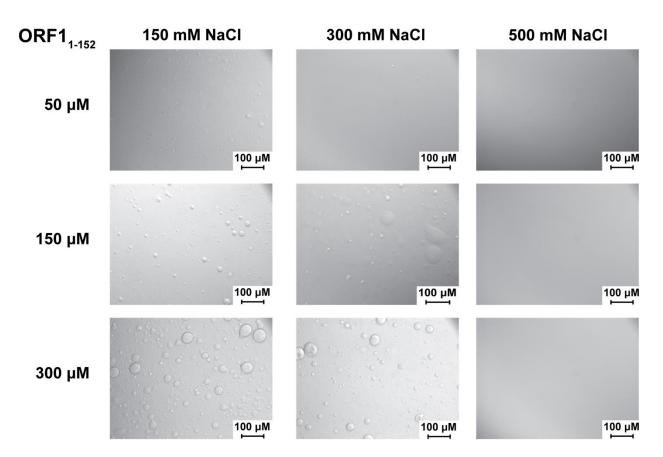
# Figure S5. The RRM and CTD domains do not contribute to ORF1 phase separation.

ORF1<sub>157-338</sub> does not phase-separate in any condition tested. Images were collected with noted concentrations of ORF1<sub>157-338</sub> at room temperature in 20 mM Tris pH 8.0 and 1 mM DTT with NaCl concentration listed.



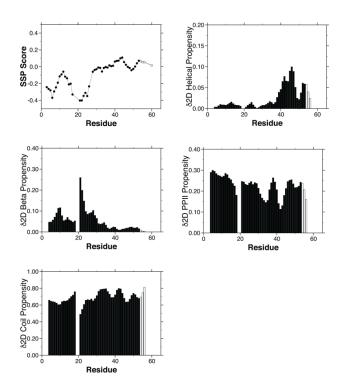
#### Figure S6. Truncation of the disordered N-terminus reduces ORF1 phase separation.

Truncation of the ORF1 N-terminus at residues 52 (**A**) or 65 (**B**) reduces ORF1 phase separation but does not abolish phase separation in the lowest NaCl concentrations tested. Images were collected with noted concentration of  $ORF1_{52-338}$  or  $ORF1_{66-338}$  at room temperature in 20 mM Tris pH 8.0, 1 mM DTT with NaCl concentration listed.



# Figure S7. A minimal construct of the N-terminus and coiled coil domain retains the ability to phase separate.

The ORF1<sub>1-152</sub> construct contains both the disordered N-terminus and coiled coil domains and is capable of phase separating in conditions similar to that of the full-length  $ORF_{1-338}$  protein. Images were collected with noted concentration of  $ORF1_{1-152}$  at room temperature in 20 mM Tris pH 8.0, 1 mM DTT with NaCl concentration listed.



# Figure S8. Additional NMR Data

The secondary structure propensity (SSP) score and  $\delta$ 2D Helical Propensity,  $\delta$ 2D Beta Propensity,  $\delta$ 2D PPII Propensity, and  $\delta$ 2D Coil Propensity scores show that residues 41-49 have slight  $\alpha$ -helical character. Open circles and open bars correspond to residues from the histidine tag.

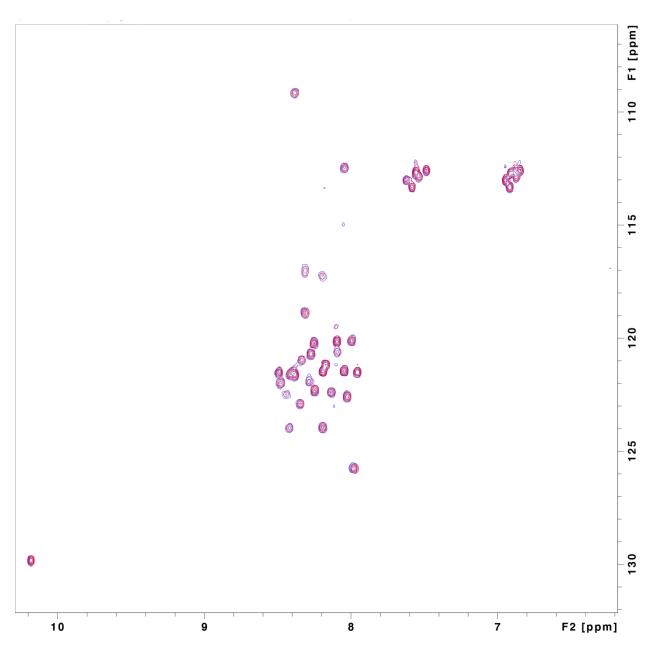
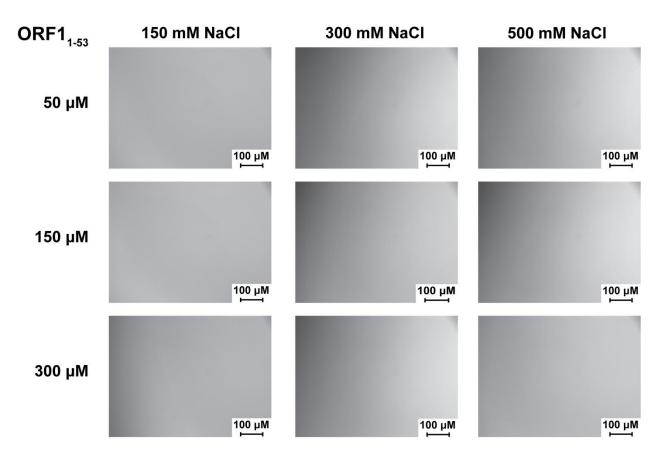


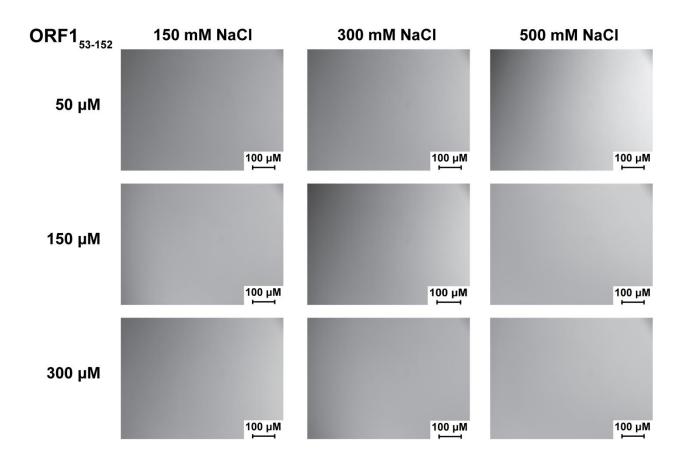
Figure S9. No chemical shift perturbations are observed with increasing concentrations of ORF1<sub>1-53</sub>.

ORF1<sub>1-53</sub> is shown at 20  $\mu M$  (blue) and 1 mM (red) concentration in 200 mM NaCl and 20 mM HEPES at pH 7.25 at 25 °C.



# Figure S10. ORF1<sub>1-53</sub> is not sufficient for phase separation.

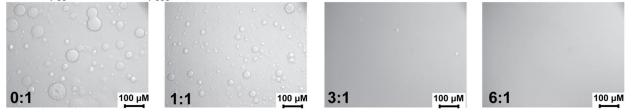
 $ORF1_{1-53}$  does not phase separate in any condition tested by DIC microscopy. Images were collected with noted concentration of  $ORF1_{1-53}$  at room temperature in 20 mM Tris pH 8.0, 1 mM DTT with NaCl concentration listed.



# Figure S11. The ORF1 coiled-coil domain is not sufficient for phase separation.

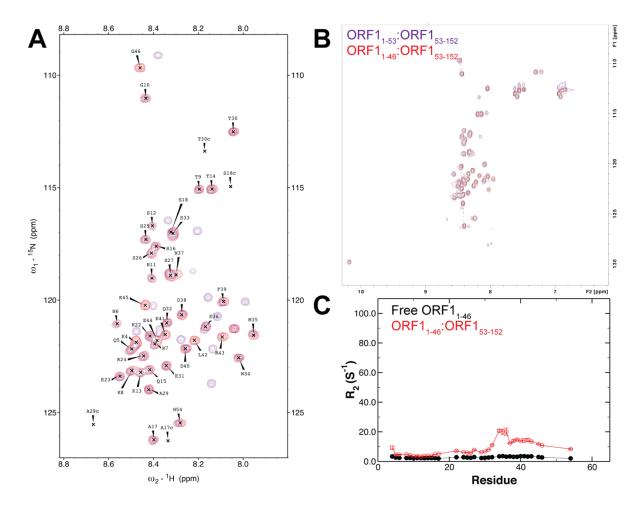
ORF1<sub>53-152</sub> isolates the coiled-coil domain and does not phase separate in any condition tested. Images were collected with noted concentration of ORF1<sub>53-152</sub> at room temperature in 20 mM Tris pH 8.0, 1 mM DTT with NaCl concentration listed.

# ORF1<sub>1-53</sub> into ORF1<sub>1-338</sub>



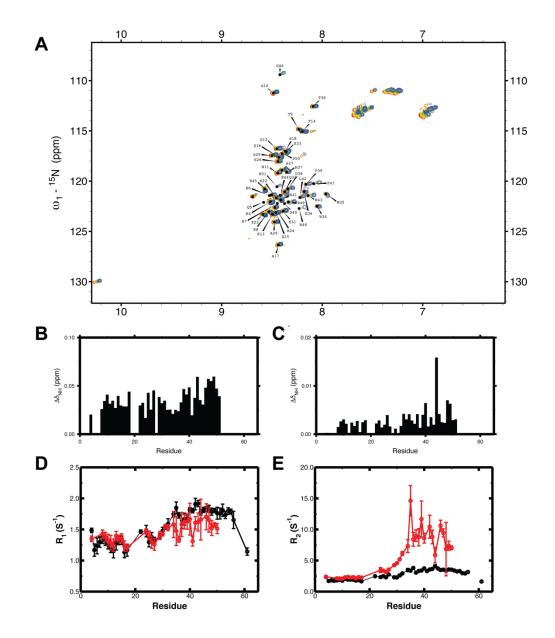
# Figure S12. Titration of ORF1<sub>1-53</sub> into ORF1<sub>1-338</sub> disrupts phase separation in a concentration dependent manner.

Increasing the concentration of ORF1<sub>1-53</sub> disrupts phase separation of full-length ORF1<sub>1-338</sub> in a concentration dependent manner. Ratios in the image panels describe the molar ratio of ORF1<sub>1-53</sub>:ORF1<sub>1-338</sub>. Images were collected with 300  $\mu$ M ORF1<sub>1-338</sub> at room temperature in 20 mM Tris pH 8.0, 300 mM NaCl, 1 mM DTT with increasing concentrations of ORF1<sub>1-53</sub> as indicated by the molar ratio in the figure panels.





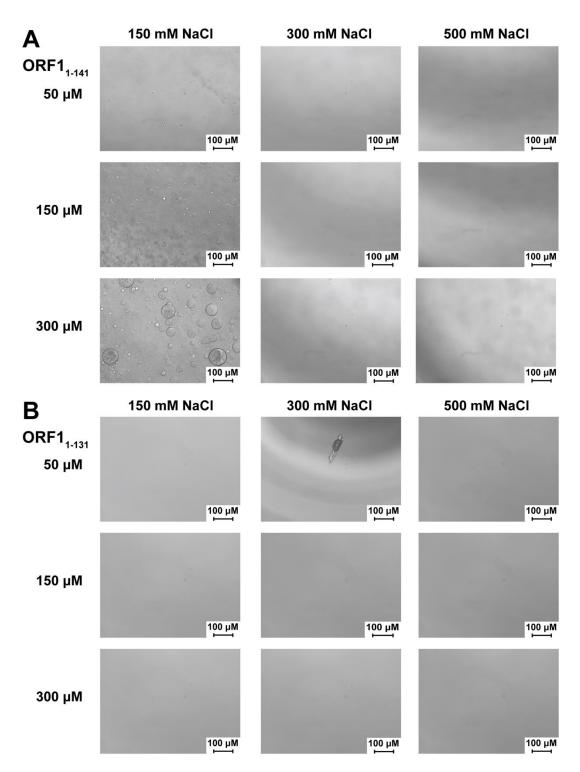
**A.** The <sup>15</sup>N-<sup>1</sup>H HSQC correlation spectra overlay nicely between ORF1<sub>1-53</sub> (purple) and ORF1<sub>1-46</sub> (red). **B.** ORF1<sub>1-46</sub> no longer displays significant line broadening in the presence of ORF1<sub>53-152</sub> (red) as observed with ORF1<sub>1-53</sub> (purple), indicating that the interaction is disrupted by removing residues 47-53. **C.** The values of  $R_2$  are slightly elevated in ORF1<sub>1-46</sub> in the presence of ORF1<sub>53-152</sub> (red) compared to free ORF1<sub>1-46</sub> (black), unlike the highly elevated values observed for ORF1<sub>1-53</sub> in the presence of ORF1<sub>53-152</sub>. Open circles correspond to residues from the histidine tag. These data were acquired at 700  $\mu$ M ORF1<sub>1-53</sub> or ORF1<sub>1-46</sub> and their respective 1:1 molar ratio complexes with 700  $\mu$ M ORF1<sub>53-152</sub> (red).



# Figure S14. NMR Characterization of the disordered N-terminal region of ORF1<sub>1-152</sub> at varying salt concentrations and protein concentrations.

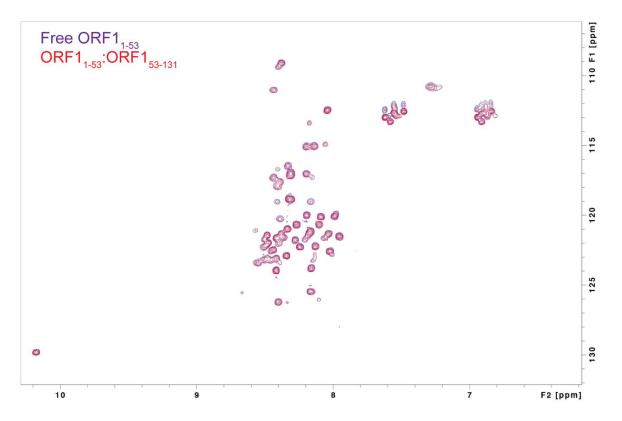
**A.** The <sup>15</sup>N-<sup>1</sup>H HSQC correlation spectra of <sup>15</sup>N labeled ORF1<sub>1-152</sub> in 20 mM MES pH 6.0, 1 mM DTT, 7% D<sub>2</sub>O at 400 mM (blue), 600 mM (green), 800 mM (yellow), and 933 mM (red) NaCl (collected in 3 mm NMR tubes to avoid issues with long pulse lengths in high salt conditions) are similar, showing no qualitative changes in the spectra arising from additional interactions. Only the disordered N-terminal region until about residue 50 is visible. We note that samples were all initially created at 200  $\mu$ M ORF1<sub>1-152</sub>. At 100 mM and 200 mM NaCl measurements were attempted but there remained no observable protein by spectrophotometry after clearing the supernatant. At higher salt, 17  $\mu$ M remained at 400 mM NaCl, 67  $\mu$ M at 600 mM, while no apparent phase separation occurred and approximately 200  $\mu$ M remained after centrifugation at both 800 mM and 933 mM NaCl. **B.** Chemical shift differences between 400 mM and 933 mM NaCl for ORF1<sub>1-152</sub> indicate to significant regional difference, suggesting we cannot directly observe the

contacts using this approach in the dispersed phase samples. **D.** Chemical shift differences for ORF1<sub>1-152</sub> at 600 mM NaCl between 67  $\mu$ M (near the saturation concentration) and 33  $\mu$ M (below the saturation concentration) are small and uniform, suggesting again that this approach cannot observe interactions leading to phase separation. **D.**  $R_1$  and **E.**  $R_2$  for 179  $\mu$ M (below saturation concentration) ORF1<sub>1-152</sub> at 933 mM NaCl shown in red compared to ORF1<sub>1-53</sub> acquired at 700  $\mu$ M protein and 200 mM NaCl shown in black. (Heteronuclear NOE was attempted but the signal to noise ratio in 3 mm tubes for weak peaks was prohibitive.)  $R_2$  is enhanced in the region of residues 35-50 in ORF1<sub>1-152</sub> with only small change in  $R_1$ . This elevated  $R_2$  suggests some interaction at this site within the trimeric ORF1<sub>1-152</sub> assembly, mirroring what we observed mixing ORF<sub>1-53</sub> with ORF1<sub>53-152</sub>. The lack of large chemical shifts compared to the disordered 1-53 and the small change in  $R_1$  do not support a large structural change. The uniformity of the values across this region suggests the elevation is more than can be explained by simple tethering of the region to the coiled-coil trimer.



# Figure S15. Truncation of the C-terminus of the coiled-coil domain disrupts ORF1 phase separation.

Truncation of the C-terminus of the coiled-coil domain at residues 141 (**A**.) or 131 (**B**.) reduces ORF1 phase separation but does not abolish phase separation in the ORF1<sub>1-141</sub> construct. Images were collected with noted concentration of ORF1<sub>1-141</sub> or ORF1<sub>1-131</sub> at room temperature in 20 mM Tris pH 8.0, 1 mM DTT with NaCl concentration listed.



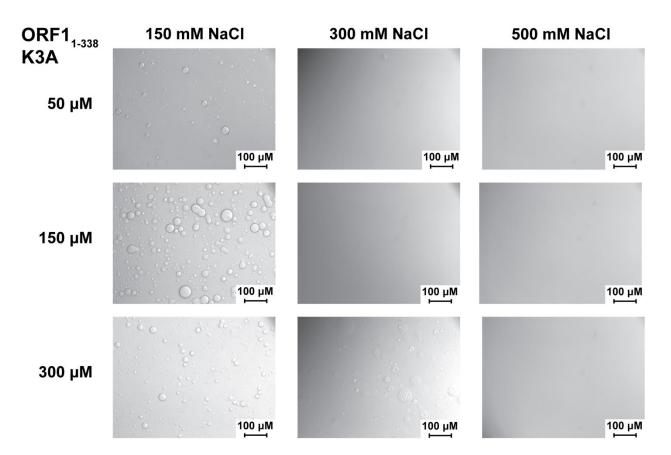
# Figure S16. ORF1<sub>1-53</sub> cannot bind to the C-terminal portion of the coiled-coil domain after truncating residues 132-152.

No differences are observed between the <sup>15</sup>N-<sup>1</sup>H HSQC correlation spectra of free ORF1<sub>1-53</sub> (790  $\mu$ M) and ORF1<sub>1-53</sub> (300  $\mu$ M) in the presence of the ORF1<sub>53-131</sub> (300  $\mu$ M) truncated coiled-coil domain, demonstrating that the C-terminal 22 residues are necessary for that interaction.

Human		
asmanian Devil	MGKKQNRKTGNSKTQSTSPPPKERSSSPATDQSWMENDFDE MSKQKKNMSGKGYYSGRD-DPDSSEASGKSKKGANWSQGQQEILDELKKEI-R	<b>41</b> 51
louse	MAKGKRKNPTNRNOPHS-PSSERSTPTPPSPGHPNT-TENLDPDLKTFLM	48
Rat	MARGKRRNPSNRNQDYM-PSSEPNSPTKTNMEYSNT-PEKQDLVSKSYLI	48
Rabbit	SQEYEDDEIEE	38
log	PTELQNLDYNS	29
lorse	MRRHKSTSSSNMKKYIKSPEQKESNKYTENNPKENEIYN	39
Cow	PNQTKEEEIGN	28
Sheep	PNQTKEEEVGN	28
	ł	
Human	Coiled-Coil LREEGFRRSNYSELREDIQTKGKEVENFEKNLEECITRI-TNTEKC	86
Tasmanian Devil	VVEEKLGREMRVMQENYEKKITTLAKQAQKNTEENNTLKNKIGQMAKEAC	10
louse	MMIEDIKKDFHKSLKDLQESTAKELQALKEKQENTAKQVM-EMNKTILELKGEVDTI	10
lat	MMPEDFKKDMNTL-RETQENINKQVEAYREESQKSLKEFQENTIKQLKELKMEIEAI	10
Rabbit	MQDTDFKKFMIRTFRSFQKQILELQKSLLDKIENLSRENEILRKSQNETQKLVEQESVIV	98
og	MSEIQFRSTMIQLLVALEKSIKDSRDFMTAAEF	61
lorse	LNDDDFKTAIIKILTELRENSDRQLNEFRSYVTKEFDTIKKN	81 74
ow heep	LPDKEFRIMIVKLIQNLEIKMESQINSLETRIEKMQERFNKDLEEI LPEKEFRILIVKMIQNLEIKMETQINSLETRIEKMQERFNKDLEEI	74
	: : Coiled-Coil	
Human	LKELMELKTKARELREECRSLRSQCDQLEERVSAMEDEMNEMK	12
Casmanian Devil	KSTEEKNSLKTRVGQMEKDVQKFSEEKNFLRSRIGQMEKEVQNLTEEKNSLKMRIGQVEA	16
louse	KKTQSEATLEIETLGKRSGTIDASISNRIQEMEERISGAEDSIENID	15
lat	KKEHMETTLDIENQKKRQGAVDTSFTNRIQEMEERISGAEDSIEMID	15
abbit log	KRNQNEMKSSIDQMTNTLESLKNRMGEAEERISDLEDRAQENI RANQAEIKNQLNEMQSKLEVLTTRVNEVEERVSDLEDKLIAKR	14
lorse	QT-EILEMKNTIEEIKKNLDALNSRADNMEERISNLEDGNIELL	12
Cow	KKSQYIMNNAINEIKNTLEATNSRITEAEDRISELEDRMVEIN	11
Sheep	KKSQNIMNNTINEIRNTLEATNSRITEAEDRISEIEDRMVEIN	1:
	· · · · · · · · ·	
	Coiled-Coil RRM	
Human	QEGKFREKRIKRNEQSLQEIWDYVKRPNLRLIGVPESDAENGTKLEN	17
asmanian Devil	NDNMRHQETVRQSRKNERIEENVKYLIGKTNDLENRSRRDHLRIIGLPECHD-QKKSLET	22
louse lat	TTVKENTKCKRILTQNIQVIQDTMRRPNLRIIGIDENEDFQLKGPAN	19
Rabbit	STVKDNVKQKKLLVQNIQEIQDSMRRSNLRIIGIEESEDSQLKGPVN QSNORKEEEIRNLKNIVGNLQDTIKKTNIRVLGVPEGME-KEKGLEG	18
labere	ETEEKRDKQLKDHEDRLRE INDSLRKKNLRLIGVPEGAE-RDRGPEY	15
lorse	QAEEEREARLKRNEETLRELSDTIRRCNVRIIGIPEGEE-KEKGAEN	17
Cow	ESERIKEKRIKRNEDNLRDLQDNIKRYNIRIIGVPEEED-KKKDHER	10
Sheep	ESERKQEKRIKRNEDNLRDLQDNMKRSNIRIIGVPEEED-RKKDHEK . : : : : : : : *: *	1
	RRM	
Human	TLQDIIQETFPNLAR-QANVQIQEIQRTPQRYSSRRATPRHIIVRFTKVEMKEKMLRAAR	23
Tasmanian Devil	IFQEIIKENCPNILYPEGKIEIERIHRSPPEKDPKMKGARDVIAKFQNPLMKERILQAVR	28
louse	IFNKIIEENFPNIKK-EMPMIIQEAYRTPNRLDQKRNSSRHIIIRTTNALNKORILKAVR	25
Rat Rabbit	IFNKI IEENFPNLKK-EIPIDIQEAYRTPNRLDQKRNTSRHIIVKTPNAQNKERILKAVR	24
log	LFSEILAENFPGLEK-DRDILVQEAHRTPNKHDQKRSSPRHVVIKLTTVKHKEKILKCAR VFEQILAENFPNLGR-ETGIQIQEIERSPPKINKNRSTPRHLIVKLANSKDKEKILKAAR	20
lorse	LFKE IMAENFPNLVR-EMDLQVTEANRSPNFINARRPTPRHIVVKLAKVNDKEKILRTAR	22
Cow	ILEEIIVENFPKMGK-EIITQVQETQRVPNRINPRQNTPRHILIKLTNIKHKEQILKAAR	2
Sheep	ILEEIIVENFPKMGK-EIITQVQETQRVPNRINPRRNTPRHILIKLTKIKHKEQILKAAR	2
	······································	
	RRM CTD	
	EKGRVTLKGKPIRLTADLSAETLQARREWGPILNILKEKNFQPRISYPAKLSFISEGEIK	
Tasmanian Devil	EKGRVTLKGKPIRLTADLSAETLQARREWGPILNILKEKNFQPRISYPAKLSFISEGEIK KR-PFKYRGATVRITQDLAPSTLKERRAWNVIFRRAKELGLQPRITYPAKLSIIIQGRRW	3
Tasmanian Devil Mouse	EKGRVTLKGKPIRLTADLSAETLQARREWGPILNILKEKNFQPRISYPAKLSFISEGEIK KR-PFKYRGATVRITQDLAPSTLKERRAWNVIFRRAKELGLQPRITYPAKLSIIIQGRRW EKGQVTYKGRPIRITPDFSPETMKARRAWTDVIQTLREHKCQPRLLYPAKLSITIDGETK	3
Tasmanian Devil Mouse Rat	EKGRVTLKGKPIRLTADLSAETLQARREWGPILNILKEKNFQPRISYPAKLSFISEGEIK KR-PFKYRGATVRITQDLAPSTLKERRAMVVIFRAAKELGLOPRITYPAKLSIIIQGRW EKGQVTYKGRPIRITPDFSPETMKARRAWTDVIQTLREHKCQPRLLYPAKLSITIOGETK EKGQVTYKGRPIRITPDLSPETMKARRAKSVTDVIQTLREHKCQPRLLYPAKLSINIDGETK	3 3 3
Tasmanian Devil Mouse Rat Rabbit	EKGRVTLKGKPIRLTADLSAETLQARREWGPILNILKEKNFOPRISYPAKLSFISEGEIK KR-PFKYRGATVRITODLAPSTLKERRANNVIFRRAKELGLOPRITYPAKLSIIIOGRRW EKGQVTYKGRPIRITDFSPETMKARRANTDVIOILKEHKCOPRLLYPAKLSITIOGEIK EKGQVTYKGRPIRITPDLSPETMKARRANTDVIOILKEHKCOPRLLYPAKLSINIOGEIK EKRQITLRGSPIRLTADFSSETLQARREWRDIAQVLREKNCOPRILYPAKLSFVNEGEIK	3 3 3 3
Tasmanian Devil Mouse Rat Rabbit Dog	EKGRVTLKGKPIRLTADLSAETLQARREWGPILNILKEKNFQPRISYPAKLSFISEGEIK KR-PFKYRGATVRITQDLAPSTLKERRAMVVIFRAAKELGLOPRITYPAKLSIILGGRW EKGQVTYKGRPIRITPDLSPETMKARRAWTDVIQTLREHKCQPRLLYPAKLSITLGGETK EKGQVTYKGRPIRITPDLSPETMKARRAWTDIQTLREHKCQPRLLYPAKLSINIGEIK EKKQITLGSPIRLTADFSSETLQARREWDIAQVLREKKCQPRILYPAKLSFKNEGEIK DKKSLTFMGRSIRVTADLSTETWQARKGWQIFRVLNEKNMQPRILYPAKLSFKNEGEIK QK-KLTYKGTPIRLSADFSAETLQARREWNDIFNVLNEKNMQPRILYPAKLSFKNEGEIK	3 3 3 2
Tasmanian Devil Mouse Rat Rabbit Dog Horse Cow	EKGRVTLKGKPIRLTADLSAETLQARREWGPILNILKEKNFOPRISYPAKLSFISEGEIK KR-PFKYRGATVRITCDLAPSTLKERRANNVIFRAAKELGUPRISYPAKLSFIIGERK EKGQVTYKGRPIRITPDSPSTLKARRANTDVIGTLREHKCOPRLLYPAKLSITIGETK EKGQVTYKGRPIRITPDLSPETMKARRSNTDVIGTLREHKCOPRLLYPAKLSINIGGEIK EKKQUTHKGPIRITADSFSTLGARREWGDIGVLREKKCOPRLLYPAKLSKNEGEIK DKKSLTFMGRSIRVTADLSTETWQARKGWQDIFRVLNEKNMQPRILYPAKLSFKNEGEIK QK-KLTYKGTPIRITADLSTETLQARREWDDIGVLRKKNUOPRILYPAKLSFKNEGEIK EKQQITHKGTPIRITADLSTETLQARREWDDIFKNLKUKKUOPRILYPAKLSFKNEGEIK	3 3 3 2 2 2
Human Tasmanian Devil Mouse Rat Rabbit Dog Horse Cow Sheep	EKGRVTLKGKPIRLTADLSAETLQARREWGPILNILKEKNFQPRISYPAKLSFISEGEIK KR-PFKYRGATVRITQDLAPSTLKERRAMVVIFRAAKELGLOPRITYPAKLSIILGGRW EKGQVTYKGRPIRITPDLSPETMKARRAWTDVIQTLREHKCQPRLLYPAKLSITLGGETK EKGQVTYKGRPIRITPDLSPETMKARRAWTDIQTLREHKCQPRLLYPAKLSINIGEIK EKKQITLGSPIRLTADFSSETLQARREWDIAQVLREKKCQPRILYPAKLSFKNEGEIK DKKSLTFMGRSIRVTADLSTETWQARKGWQIFRVLNEKNMQPRILYPAKLSFKNEGEIK QK-KLTYKGTPIRLSADFSAETLQARREWNDIFNVLNEKNMQPRILYPAKLSFKNEGEIK	3: 3: 3: 2: 2: 2:
Tasmanian Devil Mouse Rat Rabbit Dog Horse Cow	EKGRVTLKGKPIRLTADLSAETLQARREWGPILNILKEKNFQPRISYPAKLSFISEGEIK KR-PFKYRGATVRITQDLAPSTLKERRAMNVIFRAAKELGJQPRITYPAKLSIIGGETK EKGQVTYKGRPIRIEDFSPETMKARRAMTDVIGTLREHKCQPRLLYPAKLSIIGGETK EKGQVTYKGRPIRITPDLSPETMKARRAMTDVIGTLREHKCQPRLLYPAKLSINIGGETK EKKQITLKGSPIRLTADFSSETLQARREWDIAQVLREKKCQPRLLYPAKLSFVNGGEIK DKKSLFFMGSIRVTADLSTETWQARKGWDIFKVLKEKNVQPRLLYPAKISFKYGGEIK QK-KLTYKGTPIRLSADFSAETLQARREWDIFKNLKDKNLQPRLLYPAKISFKYGGEIK EKQQITHKGIPIRITADLSIETLQARREWDILKVMKENNLQPRLLYPAKISFKYGGEIK EKQQITHKGIPIRITADLSIETLQARREWDILKVMKENNLQPRLLYPAKISFKYGGEIK	3: 3: 3: 2: 2: 2:
Tasmanian Devil Mouse Rat Rabbit Dog Horse Cow Sheep	EKGRVTLKGKPIRLTADLSAETLQARREWGPILNILKEKNFOPRISYPAKLSFISEGEIK KR-PEKYRGATVRITCDLAPSTLKERRANNVIFRAAKELGUPRISYPAKLSFIIGERK EKGOVTYKGRPIRITPDISPETMKARRANTDVIOTLREHKCOPRLLYPAKISINIDGETK EKGOVTYKGRPIRITPDISPETMKARRSNTDVIOTLREHKCOPRLLYPAKISINIDGETK EKKOVTYKGRPIRITPDISPETMKARRSNTDVIOTLREHKCOPRLLYPAKISINEGEIK EKKOVTYKGRPIRITPDISPETMKARRSNTDVIOTLREHKCOPRLLYPAKISK EKKOVTYKGRPIRITADESSTLGARRENNDIFKULREKNCOPRLLYPAKISK EKKOVTYKGRPIRITADESSTLGARRENNDIFKULREKNCOPRLLYPAKISK EKKOVTYKGRPIRITADISTETVARRENNDIFKULREKNCOPRLLYPAKISK EKKOVTYKGRPIRITADISTETJARRENNDIFKULREKNCOPRLLYPAKISK EKKOVTYKGRPIRITADISTETJARRENNDIFKULREKNCOPRLLYPAKISK EKKOVTYKGRPIRITADISTETJARRENNDIFKULREKNCOPRLLYPAKISK EKKOVTYKGRPIRITADISTETJARRENNDIFKULREKNCOPRLLYPAKISK EKKOVTYKGRPIRITADISTETJARRENNDIFKULREKNCOPRLLYPAKISK EKKOVTYKGRPIRITADISTETJARRENNDIFKULREKNCOPRLLYPAKISK EKKOVTYKGRPIRITADISTETJAR	3: 3: 3: 2: 2: 2:
Tasmanian Devil Mouse Rat Rabbit Dog Horse Cow Sheep <b>Human</b>	EKGRVTLKGKPIRLTADLSAETLQARREWGPILNILKEKNFOPRISYPAKLSFISEGEIK KR-PEKYRGATURITQDLAPSTLKERRANNUTFRAAKELGUPRISYPAKLSFIIGERK EKGOVTYKGRPIRITPDISPETMKARRANTDVIOTLREHKOPRLLYPAKLSINIDGETK EKGOVTYKGRPIRITPDISPETMKARRSNTDVIOTLREHKOPRLLYPAKLSINIDGETK EKKGUTYKGRPIRITPDISPETMKARRSNTDVIOTLREHKOPRLLYPAKLSINIDGETK EKKGUTYKGRPIRITADSSETLGARRENNDIGVULREKHOOPRLLYPAKISKNEGEIK OK-KLTYKGTPIRITADISTETUGARKGNOIFRULREKNMOPRILYPAKISKNEGEIK EKGOTTHKGIPIRITADISTETUGARRENNDIFKULREKNMOPRILYPAKISKNEGEIK EKGOTTHKGIPIRITADISTETUGARRENNDIFKULREKNMOPRILYPAKISKNEGEIK EKGOTTHKGIPIRITADISTETUGARRENNDIFKULREKNMOPRILYPAKISKNEGEIK EKGOTTHKGIPIRITADISTETUGARRENNDIFKULREKNNOLPRLLYPAKISKNEGEIK CTD	3: 3: 3: 2: 2: 2:
Tasmanian Devil Mouse Rat Rabbit Dog Horse Cow Sheep Ruman Tasmanian Devil	EKGRVTLKGKPIRLTADLSAETLQARREWGPILNILKEKNFOPRISYPAKLSFISEGEIK KR-PFKYRGATVRITQDLAPSTLKERRAMNVIFRAAKELGUPRITYPAKLSIIGGEK EKGOVTYKGRPIRITPDLSPETMKARRAMTDVIOTLREHKCOPRLLYPAKLSINIDGETK EKGOVTYKGRPIRITPDLSPETMKARRSWTDVIOTLREHKCOPRILYPAKLSINIDGETK EKROITLRGSPIRITADSSETLQARREWDIAULREKKOPRILYPAKLSINIDGETK EKROITLRGSPIRITADLSTETWGARGWODIFRVLKEKNOPRILYPAKISKYNGEIK EKQOITHKGIPIRITADLSIETLQARREWDIFKVLKEKNOPRILYPAKISKYNGEIK EKQOITHRGIPIRITADLSIETLQARREWODIKMKENNLQPRILYPARISKYNGEIK EKQOITHRGIPIRITADLSIETLQARREWODIKMKENNLQPRILYPARISKYNGEIK EKQOITHRGIPIRITADLSIETLQARREWODIKMKENNLQPRILYPARISKYNGEIK	3: 3: 3: 2: 2: 2:
Tasmanian Devil Mouse Rat Rabit Dog Horse Cow Sheep <b>Human</b> Tasmanian Devil Mouse Rat	EKGRVTLKGKPIRLTADLSAETLQARREWGPILNILKEKNFOPRISYPAKLSFISEGEIK KR-PFKYRGATVRITQDLAPSTLKERRAMNVIFRAAKELGJQPRITYPAKLSIIGGEK EKGQVTYKGRPIRITPDLSPETMKARRAWTDVIQTLREHKCQPLLYPAKLSINIDGETK EKRQITLRGSPIRITADFSBETLGARREWDIAQVLREKKOQPRILYPAKLSKNRGEIK OKKLTFKGRSIRVTADLSTETWGARKGWDIFKVLREKKNQPRILYPAKISFKYRGEIK EKQITHKGIPIRITADLSIETLQARREWDIFKVLREKKNQPRILYPAKISFKYRGEIK EKQQITHKGIPIRITADLSIETLQARREWODILKWKKENNLQPRILYPAKISFKYRGEIK :*:::::::::::::::::::::::::::::::::	33 33 30 21 21
Tasmanian Devil Mouse Rat Rabbit Dog Horse Cow Sheep Human Tasmanian Devil Mouse Rat Rabbit	EKGRVTLKGKPIRLTADLSAETLQARREWGPILNILKEKNFQPRISYPAKLSFISEGEIK KR-PFKYRGATURITQDLAPSTLKERRAMNVIFRAAKELGUPRISYPAKLSFISEGEIK EKGQVTYKGRPIRITPDISPETMKARRAWTDVIQTLREHKCOPRLLYPAKLSINIDGETK EKKQVTYKGRPIRITPDISPETMKARRSWTDVIQTLREHKCOPRLLYPAKLSINIDGETK EKKQUTIKGSPIRITADLSIETUQARREWNDIAQVLREKNCOPRLLYPAKLSFNNEGEIK DKKSITFMGSIRVTADLSIETUQARREWNDIFRVLREKNCOPRLLYPAKISFNNEGEIK DKKSITFMGSIRVTADLSIETUQARREWODIKNURKKINDLPRLLYPAKISFNEGEIK EKQQITHKGIPIRITADLSIETUQARREWODIKNURKKINDLPRLLYPAKISFNEGEIK EKQQITHKGIPIRITADLSIETLQARREWODIKNURKKINDLPRLLYPAKISFNEGEIK *.:::::::::::::::::::::::::::::::	33 33 30 21 21
Tasmanian Devil Mouse Rat Rabbit Dog Horse Cow Sheep Human Tasmanian Devil Mouse Rat Rabbit Dog	EKGRVTLKGKPIRLTADLSAETLQARREWGPILNILKEKNFQPRISYPAKLSFISEGEIK KR-PFKYRGATKVITQDLAPSTLKERRAMNVIFRAAKELGUPRITYPAKLSIIGGERW EKGQVTYKGRPIRITPDISPETMARRAMTDVIGTLREHKQPRLLYPAKLSINIGGETK EKGQTTYKGRPIRITPDISPETMARRAMTDVIGTLREHKQPRLLYPAKLSINIGGEIK OK-KLTYKGTPIRITADLSIETWQARRGWQDIFWILKEKNQPRILYPAKLSFVNEGEIK EKQQITHKGIPIRITADLSIETUQARREWNDIFWILKEKNQPRILYPAKISKYSGEIK EKQQITHKGIPIRITADLSIETUQARREWODIKWMKENNLQPRILYPAKISKYSGEIK EKQQITHKGIPIRITADLSIETUQARREWODIKWMEXNLQPRILYPARISKYSGEIK EKQQITHKGIPIRITADLSIETLQARREWODIKWMEXNLQPRILYPARISKYSGEIK EKQQITHKGIPIRITADLSIETLQARREWODIKWMEXNLQPRILYPARISKYSGEIK IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	33 31 30 26 28
Tasmanian Devil Mouse Rat Rabbit Dog Horse Cow Sheep Human Tasmanian Devil Mouse Rat Rabbit	EKGRVTLKGKPIRLTADLSAETLQARREWGPILNILKEKNFQPRISYPAKLSFISEGEIK KR-PFKYRGATURITQDLAPSTLKERRAMNVIFRAAKELGUPRISYPAKLSFISEGEIK EKGQVTYKGRPIRITPDISPETMKARRAWTDVIQTLREHKCOPRLLYPAKLSINIDGETK EKKQVTYKGRPIRITPDISPETMKARRSWTDVIQTLREHKCOPRLLYPAKLSINIDGETK EKKQUTIKGSPIRITADLSIETUQARREWNDIAQVLREKNCOPRLLYPAKLSFNNEGEIK DKKSITFMGSIRVTADLSIETUQARREWNDIFRVLREKNCOPRLLYPAKISFNNEGEIK DKKSITFMGSIRVTADLSIETUQARREWODIKNURKKINDLPRLLYPAKISFNEGEIK EKQQITHKGIPIRITADLSIETUQARREWODIKNURKKINDLPRLLYPAKISFNEGEIK EKQQITHKGIPIRITADLSIETLQARREWODIKNURKKINDLPRLLYPAKISFNEGEIK *.:::::::::::::::::::::::::::::::	25 33 31 30 26 28 28

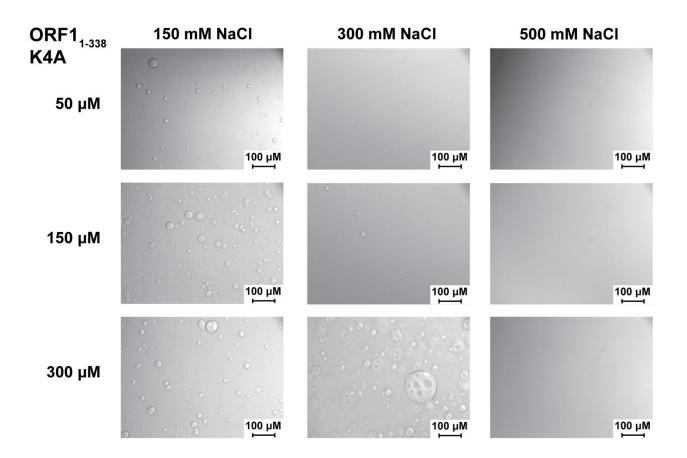
Figure S17. The basic residues at the N-terminus of ORF1 are highly conserved.

Accession numbers for the sequences are as follows: Human, AAA36590.1; Mouse, P11260.2; Tasmanian Devil, NW 003849619.1; Rat, AAY88219.1. The remaining sequences were obtained from L1Base2 (1). Domain architecture placed above the alignments corresponds to the human ORF1 domain boundaries.



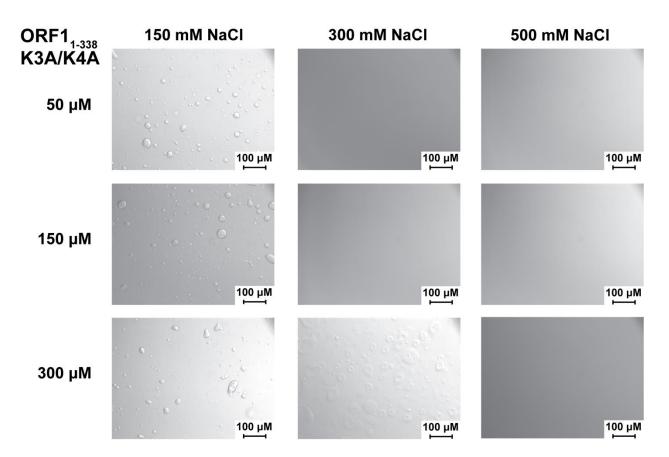
### Figure S18. Phase separation of ORF1<sub>1-338</sub> K3A.

Mutations in the disordered N-terminal region alter the behavior of ORF1 phase separation. Images were collected with noted concentration of ORF1<sub>1-338</sub> K3A at room temperature in 20 mM Tris pH 8.0, 1 mM DTT with NaCl concentration listed.



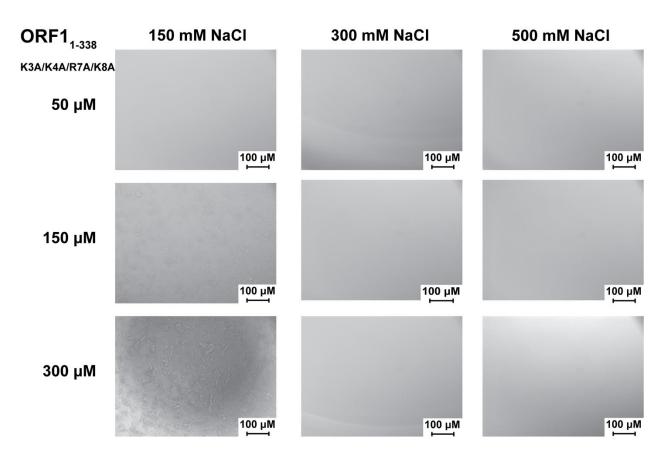
# Figure S19. Phase separation of ORF1<sub>1-338</sub> K4A.

Mutations in the disordered N-terminal region alter the behavior of ORF1 phase separation. Images were collected with noted concentration of ORF1<sub>1-338</sub> K4A at room temperature in 20 mM Tris pH 8.0, 1 mM DTT with NaCl concentration listed.



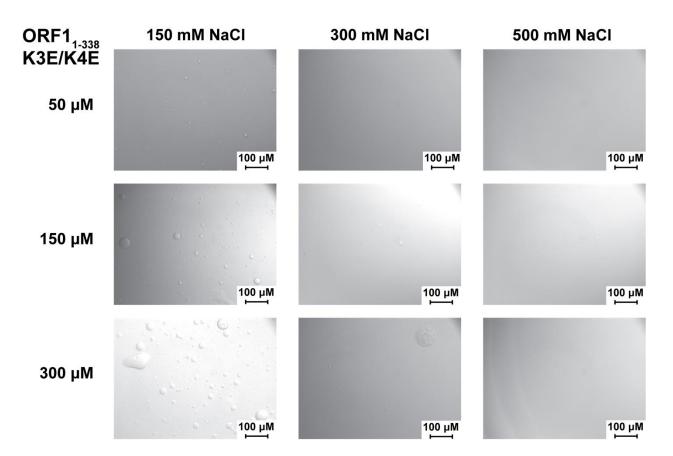
#### Figure S20. Phase separation of ORF1<sub>1-338</sub> K3A/K4A.

Mutations in the disordered N-terminal region alter the behavior of ORF1 phase separation. Images were collected with noted concentration of ORF1<sub>1-338</sub> K3A/K4A at room temperature in 20 mM Tris pH 8.0, 1 mM DTT with NaCl concentration listed.



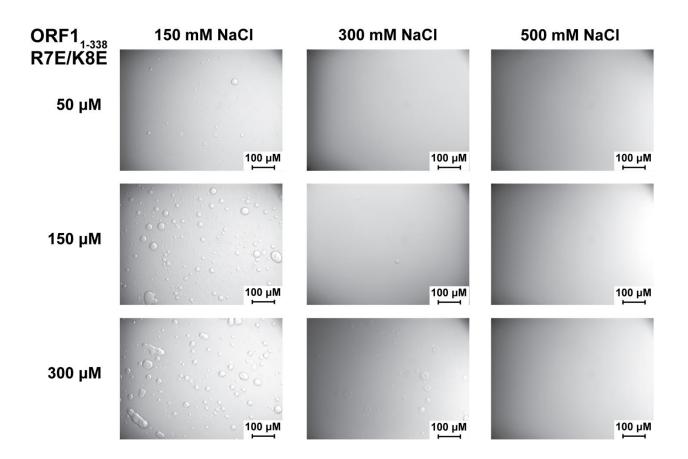
### Figure S21. Phase separation of ORF1<sub>1-338</sub> K3A/K4A/R7A/K8A.

Mutations in the disordered N-terminal region alter the behavior of ORF1 phase separation. Removing the basic charged patch drastically reduces the ability of ORF1 to form droplets. Images were collected with noted concentration of ORF1<sub>1-338</sub> K3A/K4A/R7A/K8A at room temperature in 20 mM Tris pH 8.0, 1 mM DTT with NaCl concentration listed.



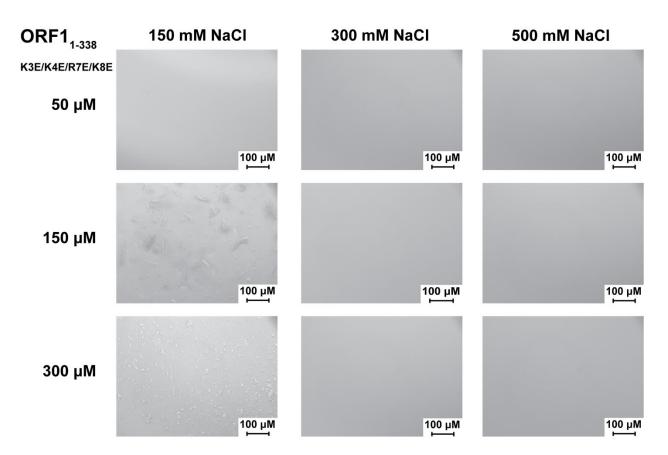
### Figure S22. Phase separation of ORF1<sub>1-338</sub> K3E/K4E.

Mutations in the disordered N-terminal region alter the behavior of ORF1 phase separation. Exchanging the basic residues for acidic residues in the N-terminus reduces phase separation. Images were collected with noted concentration of ORF1<sub>1-338</sub> K3E/K4E at room temperature in 20 mM Tris pH 8.0, 1 mM DTT with NaCl concentration listed.



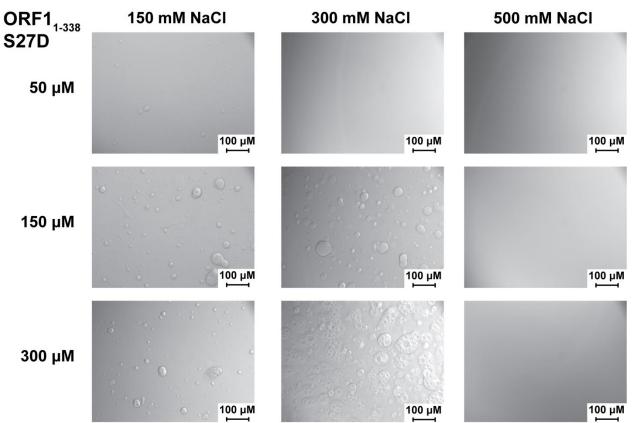
# Figure S23. Phase separation of ORF1<sub>1-338</sub> R7E/K8E.

Mutations in the disordered N-terminal region alter the behavior of ORF1 phase separation. Exchanging the basic residues for acidic residues in the N-terminus reduces phase separation. Images were collected with noted concentration of ORF1<sub>1-338</sub> R7E/K8E at room temperature in 20 mM Tris pH 8.0, 1 mM DTT with NaCl concentration listed.



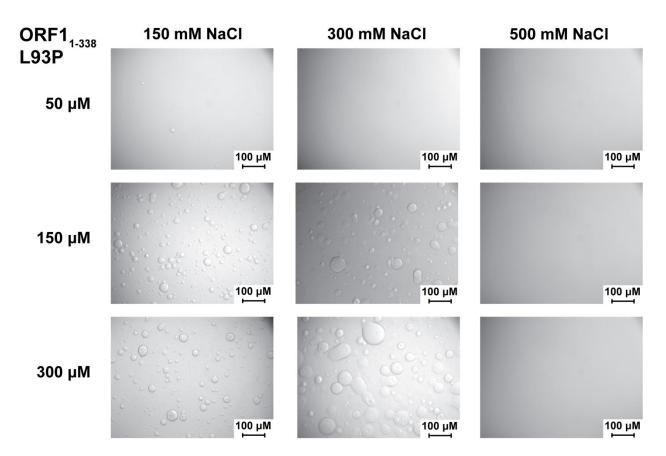
# Figure S24. Phase separation of ORF1<sub>1-338</sub> K3E/K4E/R7E/K8E.

Mutations in the disordered N-terminal region alter the behavior of ORF1 phase separation. Exchanging all of the basic residues for acidic residues in the extreme N-terminus reduces phase separation and alters the morphology of the phase separated state at 150 mM NaCI. Images were collected with noted concentration ORF1<sub>1-338</sub> K3E/K4E/R7E/K8E at room temperature in 20 mM Tris pH 8.0, 1 mM DTT with NaCI concentration listed.





Mutations in the disordered N-terminal region alter the behavior of ORF1 phase separation. Introducing a phosphomimetic mutation at residue 27 increases phase separation and changes the droplet morphology at higher protein concentrations. Images were collected with noted concentration of ORF1<sub>1-338</sub> S27D at room temperature in 20 mM Tris pH 8.0, 1 mM DTT with NaCl concentration listed.



# Figure S26. Phase separation of ORF1<sub>1-338</sub> L93P.

Mutations in the coiled-coil domain also affect the phase separation behavior of ORF1<sub>1-338</sub>. Introducing a proline mutation at residue 93 in the stammer causes an increase in phase separation. Images were collected with noted concentration of ORF1<sub>1-338</sub> L93P at room temperature in 20 mM Tris pH 8.0, 1 mM DTT with NaCl concentration listed.

# **Supporting Reference**

1. Penzkofer, T., M. Jager, M. Figlerowicz, R. Badge, S. Mundlos, P. N. Robinson, and T. Zemojtel. 2017. L1Base 2: more retrotransposition-active LINE-1s, more mammalian genomes. Nucleic Acids Res 45(D1):D68-D73.