

Figure S1

CSFV-7381_for
→

5' -**GCTCAGGGGGATGTGCAGAGATGTG**TGGAAGCAGTGACCAATTATGCGAGAGAGGGGCATCCAATTCATGAAGTCGCAGGGCACTGAAAGTGAGAGAAACCCCTACCTATAAAGAGACAATGAACACCGTGGCAGATTATGTGAAAAAGTTTATTGAGGGCACTGACGGATAGCAAGGAAGACATCATTAATATGGGCTGTGGGGGGGCACATACGGCATTGTATAAGAGCATTGGTGCCAGGCTTGGTCACGAAACCGCGTTTCGCAACTCTAGTTGTGAAGTGGTTGGCATTTTGGGGGGGAGTCAATATCAGACCACATAAAGCAAGCGGCCACAGACTTGGTCGTTTTATTACATTATTAACAGACCTCAATTCCCAGGAGACACAGAAACACAACAAGAAGGGAGAAAATTTGTTGCCAGCCTGCTAGTCTCAGCTCTAGCGACTTATACATAACAAGAGCTGGAACTACAATAATCTGTCCAAAATAGTTGAACCGGCTTTGGCTACCCTGCCCTATGCCGCTAAAGCCCTCAAGCTATTTGCTCCTACCCGACTGGAGAGCGTTGTCATACTGAGCACTGCAATCTACAAAACATACCTATCAATAAGGCGAGGCCAAAAGTGATGGTCTGCTAGGTACAGGGGTTAGCGCGGCCATGGAAATTATGTCACAAAACCCAGTATCTGTGGGTATAGCAGTTATGCTAGGGGTAGGGGCTGTAGCAGCCCACAATGCAATTGAAGCCAGTGAGCAAAAAAGAACACTACTTATGAAAGTCTTTGTGAAAAACTTCTTAGACCAGGCCGCCACCGACGAAGTAGTCAAAGAGAGCCCTGAGAAAATAATAATGGCTTTGTTCGAAGCGGTGCAAACGGTGGGCAACCCTCTTAGATTAGTGTACCACCTCTATGGAGTTTTCTATAAAGGGTGGGAAGCAAAAGAGTTGGCCCAAAGAACAGCCGGCAGGAACCTTTTCACCTTGATAATGTTTCGAGGCTGTGGAACTACTGGGAGTAGACAG**TGAGGGGAAAAATTCGCCAGCTA**-3'

← CSFV-8421_rev

Figure S1: Sequence of the NS4B gene from cp CSFV-Ubi* after passage 5. After virus infection, the surviving cells were trypsinized and total RNA extracted. An RT-PCR amplicon was generated using oligonucleotides CSFV-7381_for and CSFV-8421 rev. The PCR product was inserted into a T-vector and sequenced using M13 and M13 rev. A pinpoint reversion by homologous recombination to the parental ncp CSFV strain was documented.

Figure S2

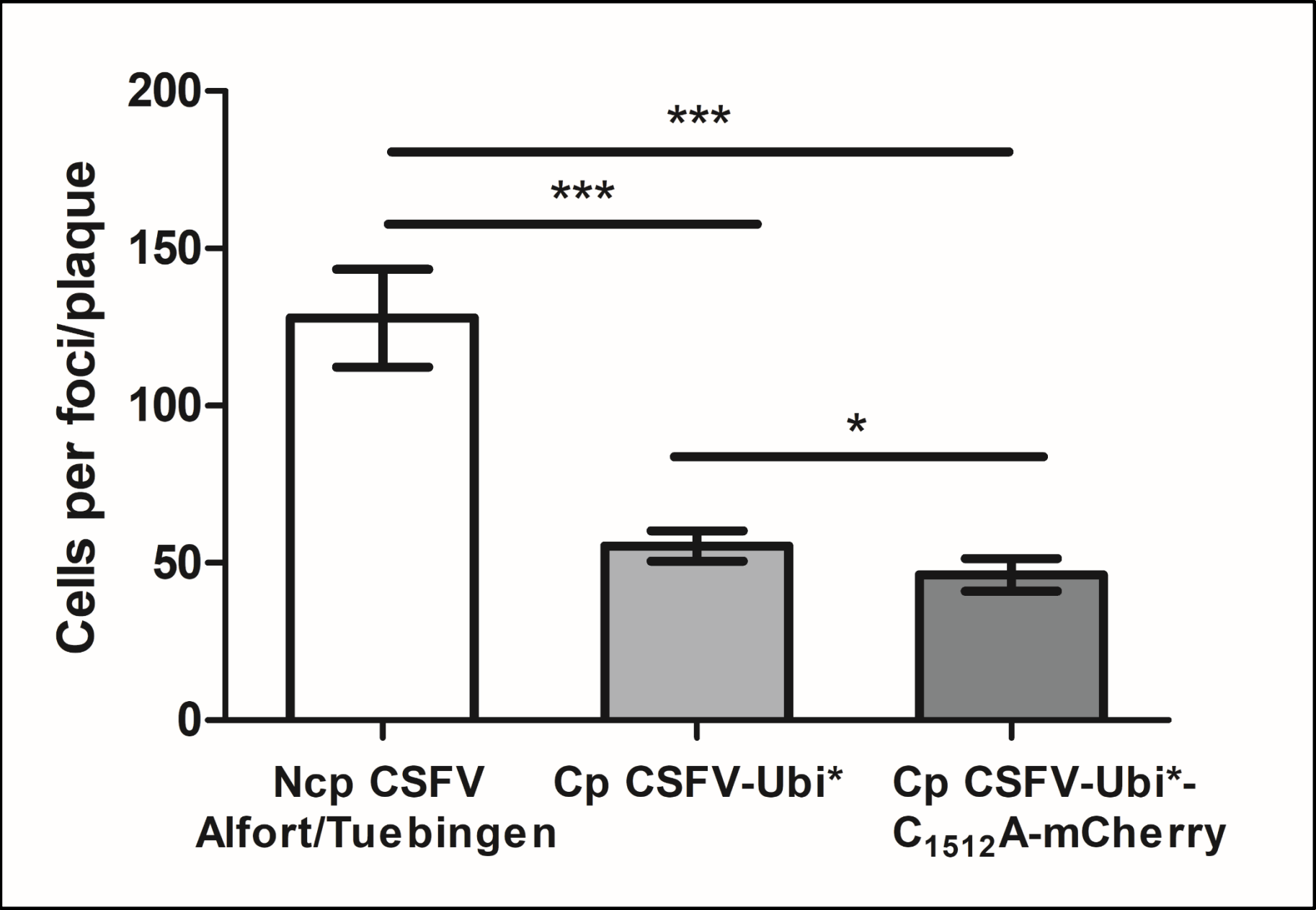


Figure S2: Focus and plaque size of the different CSFV strains. After infection of SK-6 cells, the supernatant was removed and replaced by fresh DMEM containing 1% car-boxymethyl cellulose. The infected cells of 50 individual foci were counted for each virus strain, the mean focus size was calculated, and the 95% confidence interval was determined. The values were compared in the unpaired T-test.

A SVNA for positive serum 2016/01/0441/027

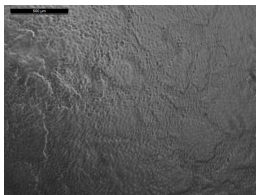
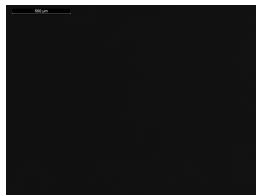
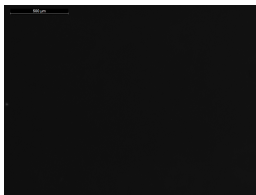

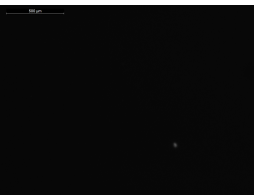
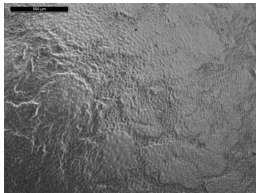
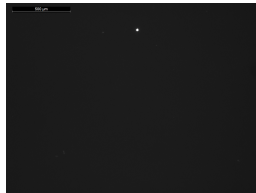
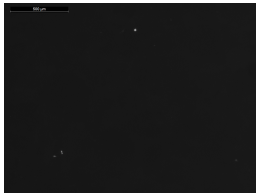
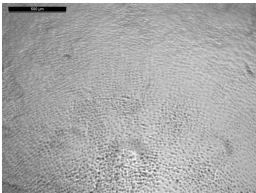
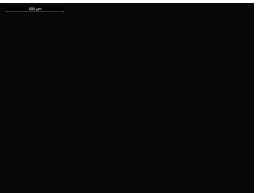

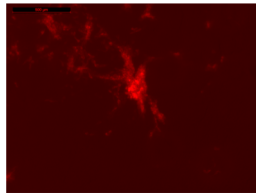
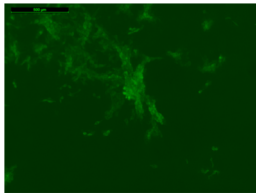
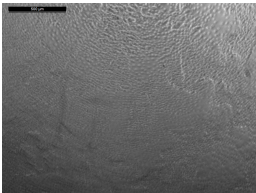
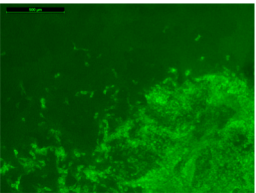

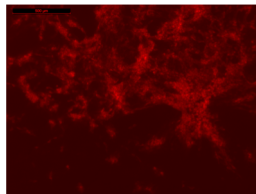
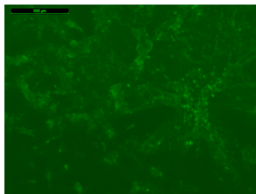
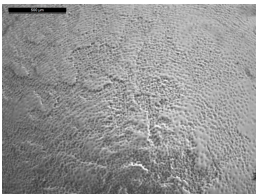
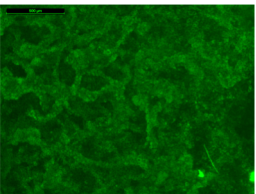

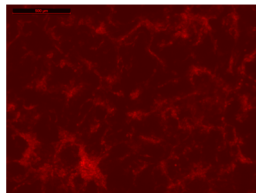
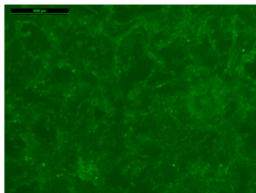
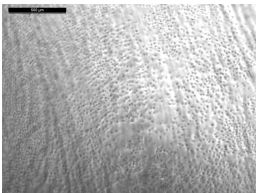
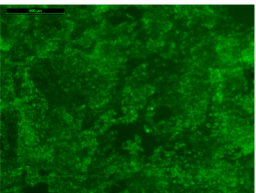

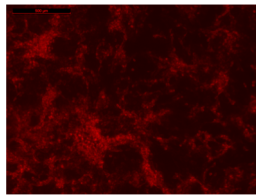
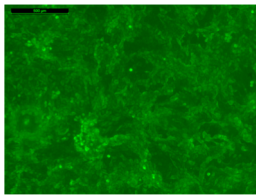
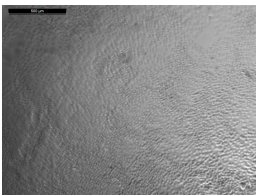
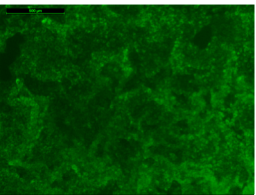

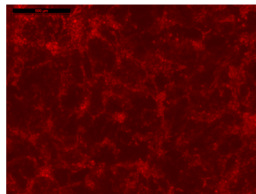
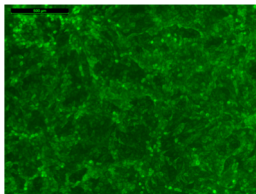
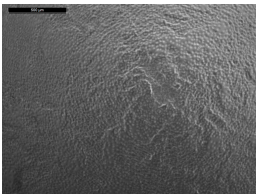
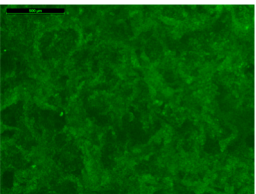
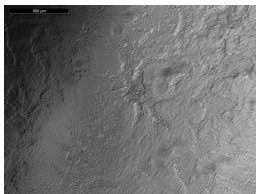
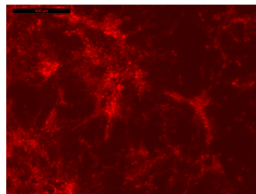
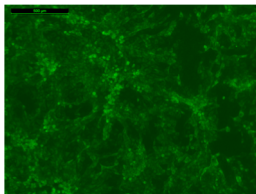
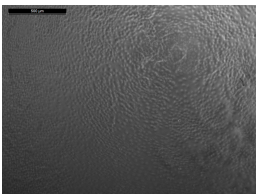
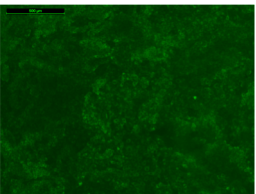
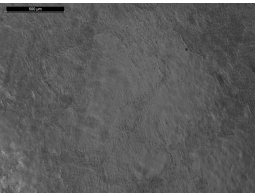
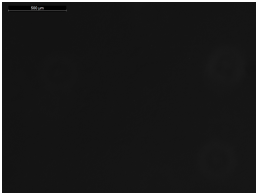
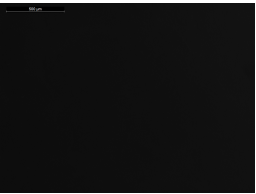
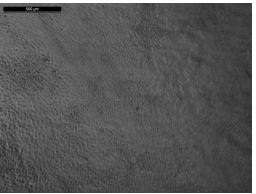
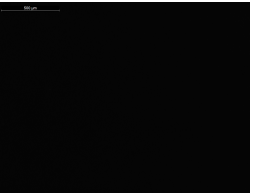
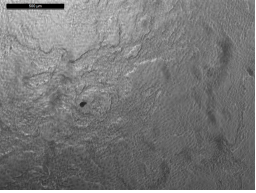
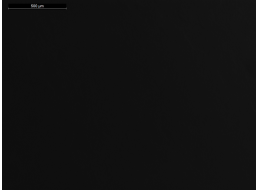
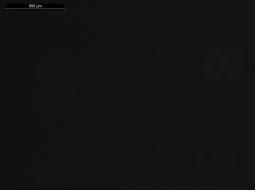
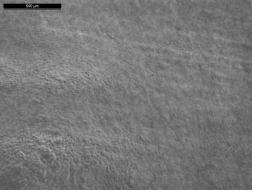
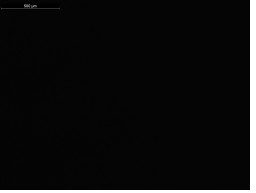
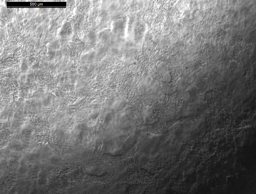
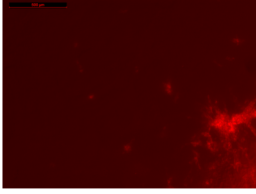
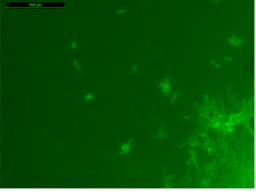
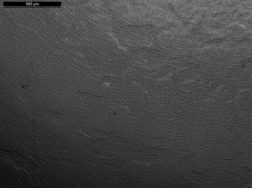
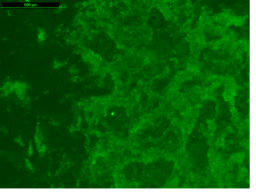

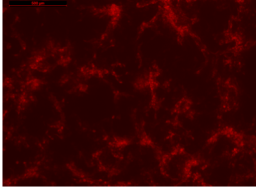
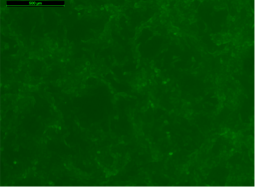

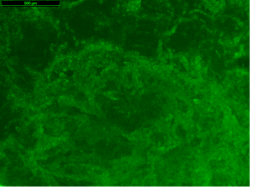
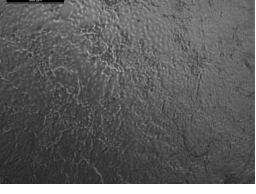
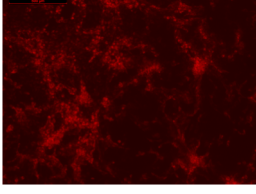
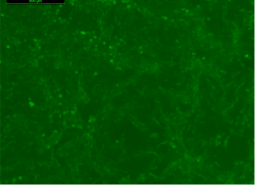
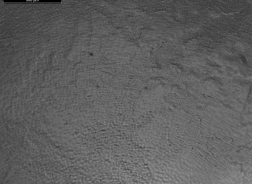
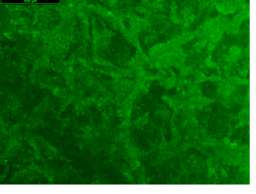
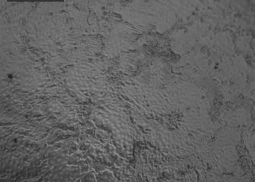
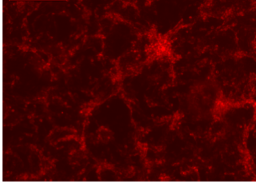
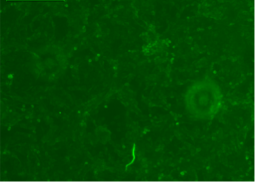

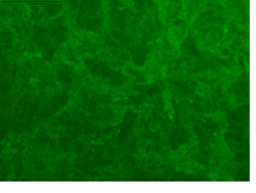

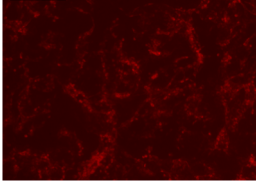
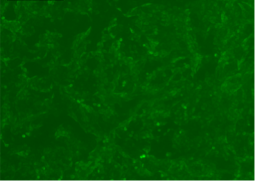

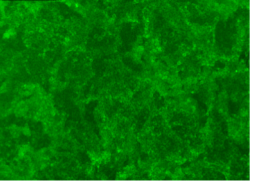

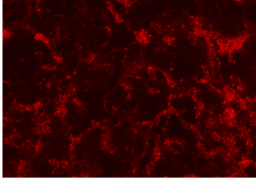
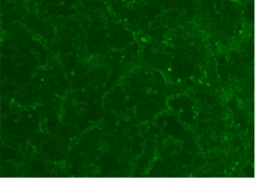

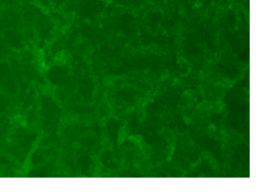
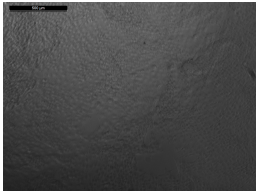
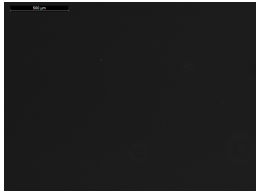
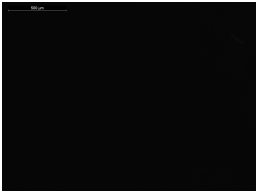
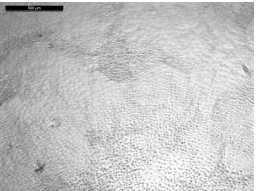
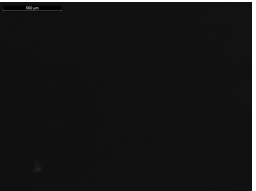
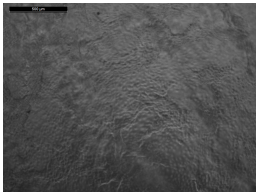
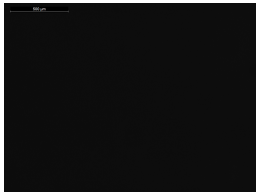
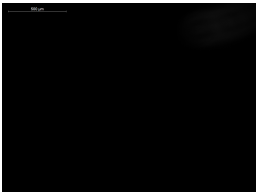
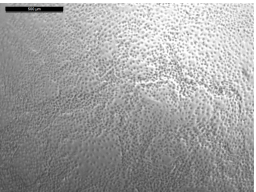
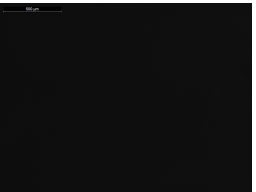
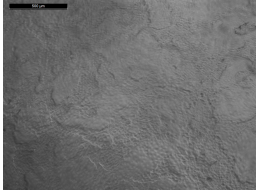
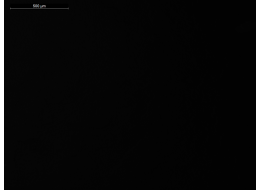
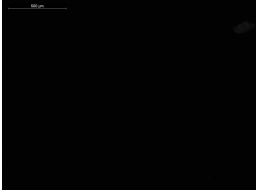
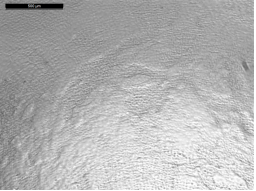
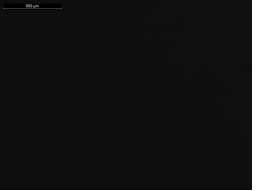
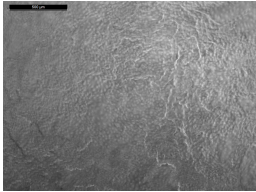
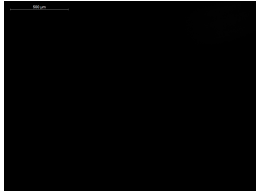
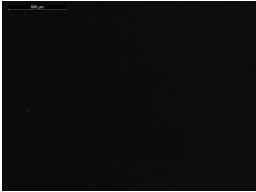
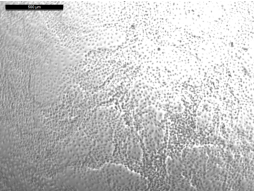
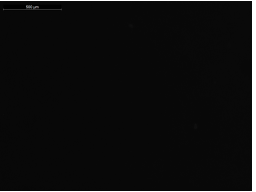
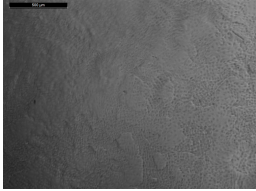
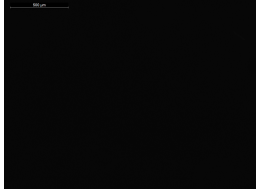
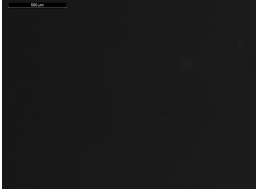
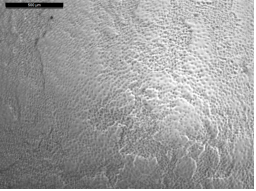
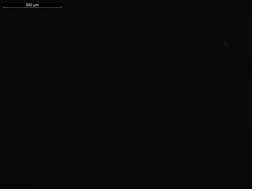
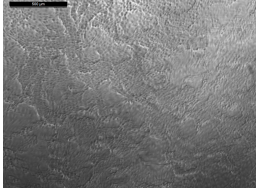
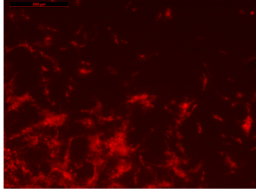
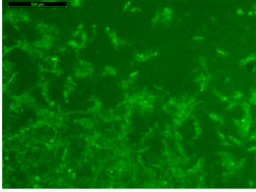
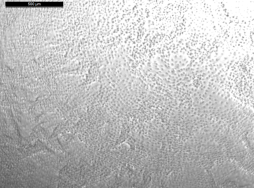
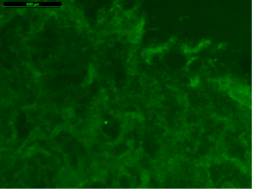
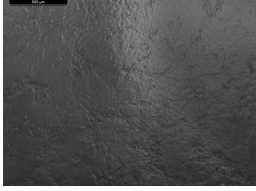
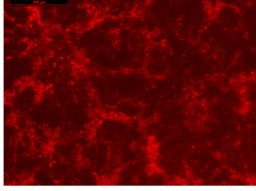
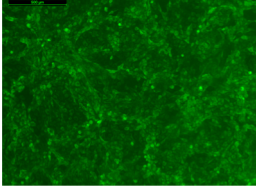
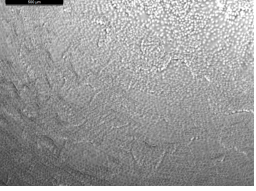
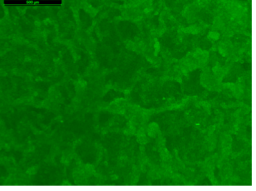
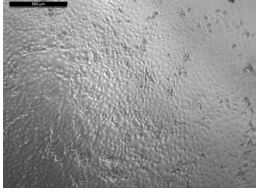
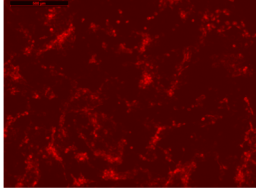
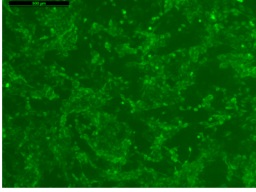
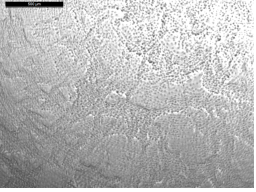
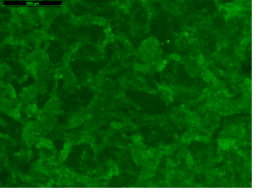
Cp CSFV-Ubi*-C ₁₂₁₅ A-mCherryE2				Ncp CSFV Alfort/Tuebingen	
	Bright field	mCherry	FITC	Bright field	FITC
1:4					
1:16					
1:64					
1:256					
1:1,024					
1:4,096					
1:16,384					
1:65,536					

Figure S3: Documentation of the fluorescence-verified plaque reduction assay. (A) Low positive serum 2016/01/0441/027. (B) Low positive serum 2008/03/0225/032. (C) High positive serum 2006/07/0056/086. (D) Negative control serum. The sera were tested against the viruses cp CSFV-Ubi*-C1512A-mCherryE2 (left) and ncp CSFV Alfort/Tuebingen (right). Indicator cells were fixed 48 h post infection and stained with A18 anti E2 and FITC conjugate. A representative area of each 96-well was documented in brightfield, red and green fluorescence channels at 4x magnification. A scale bar (500 μm) is used for comparison between images.

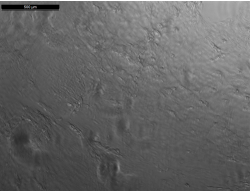
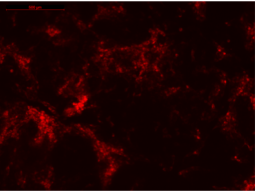
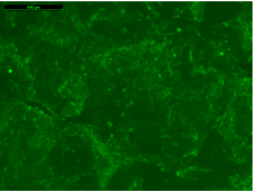
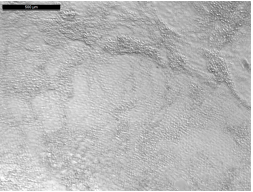
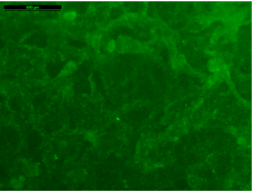
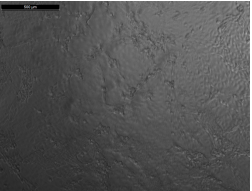
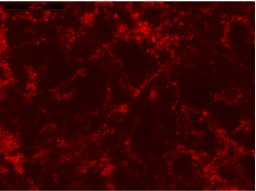
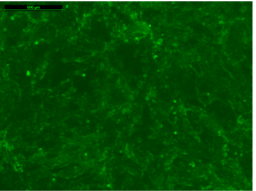
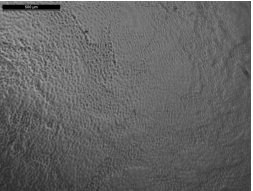
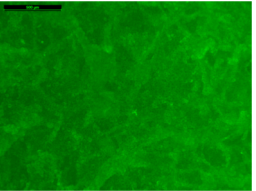
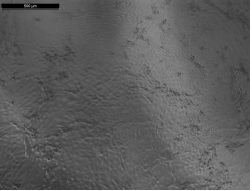
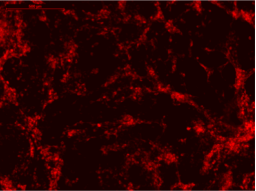
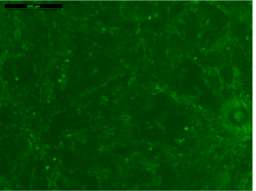
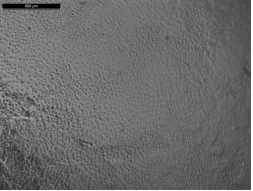
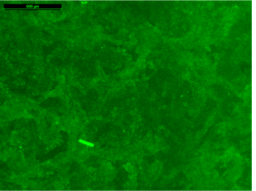
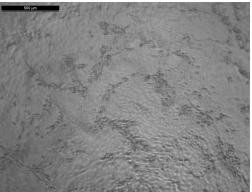
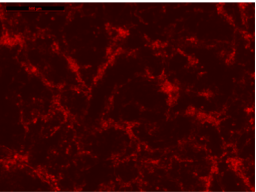
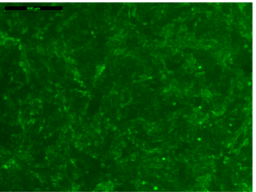
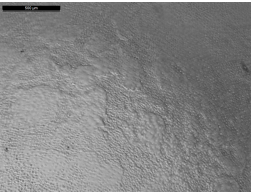
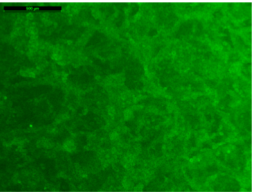
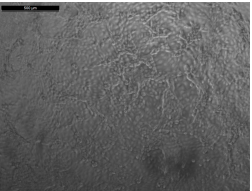
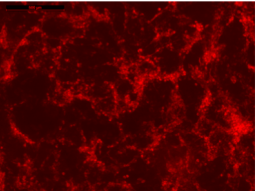
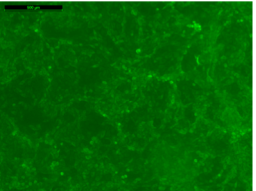
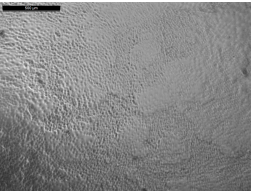
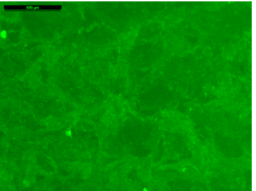
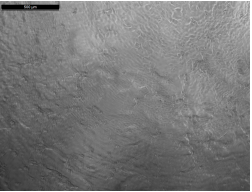
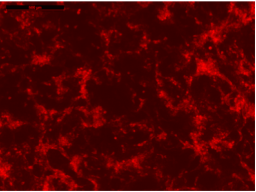
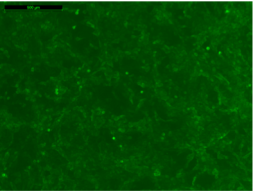
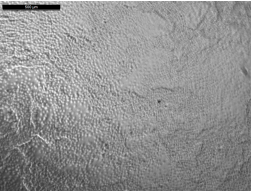
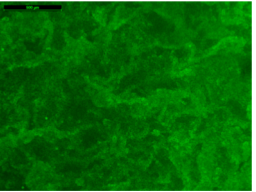
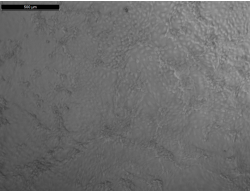
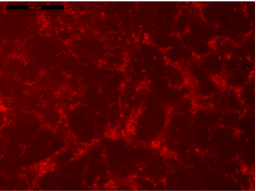
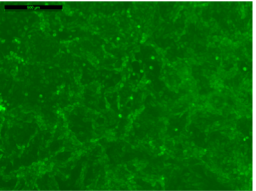
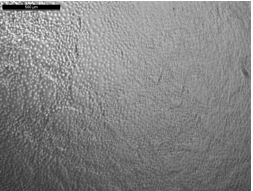
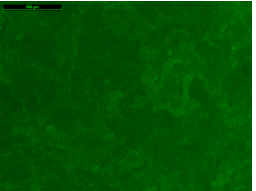
B SVNA for positive serum 2008/03/0225/032

Cp CSFV-Ubi*-C ₁₂₁₅ A-mCherryE2				Ncp CSFV Alfort/Tuebingen	
	Bright field	mCherry	FITC	Bright field	FITC
1:4					
1:16					
1:64					
1:256					
1:1,024					
1:4,096					
1:16,384					
1:65,536					

C SVNA for positive serum 2006/07/0056/086

Cp CSFV-Ubi*-C ₁₂₁₅ A-mCherryE2				Ncp CSFV Alfort/Tuebingen	
	Bright field	mCherry	FITC	Bright field	FITC
1:4					
1:16					
1:64					
1:256					
1:1,024					
1:4,096					
1:16,384					
1:65,536					

D SVNA for the negative control serum

Cp CSFV-Ubi*-C ₁₂₁₅ A-mCherryE2				Ncp CSFV Alfort/Tuebingen	
	Bright field	mCherry	FITC	Bright field	FITC
1:4					
1:16					
1:64					
1:256					
1:1,024					
1:4,096					
1:16,384					
1:65,536	