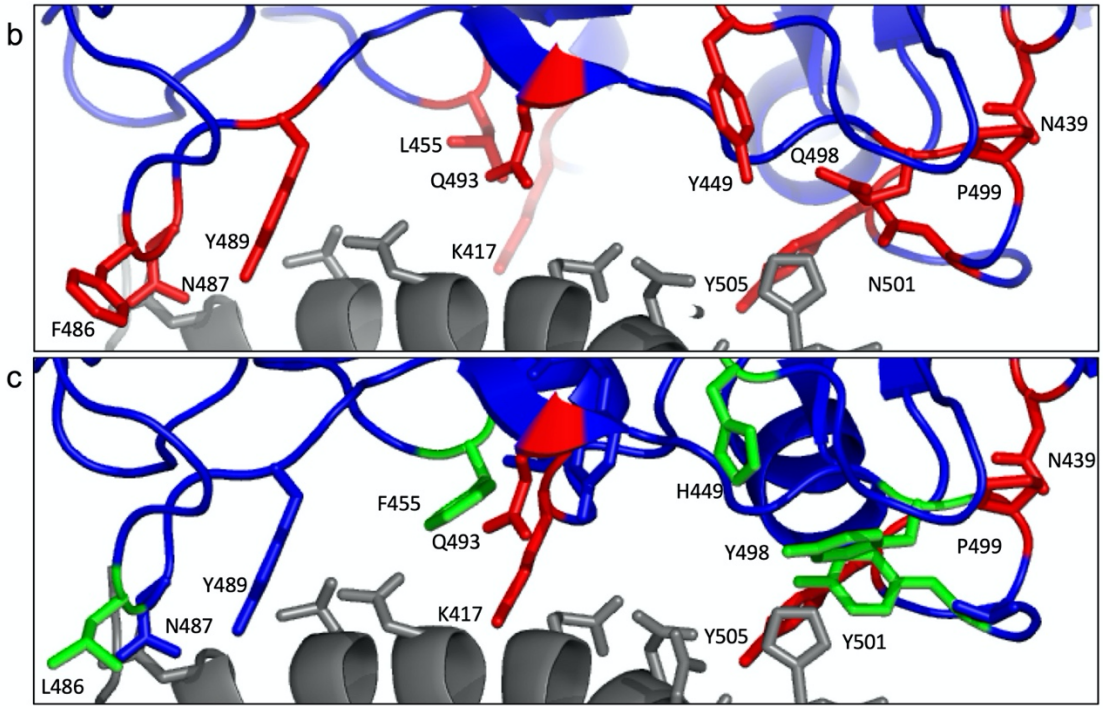
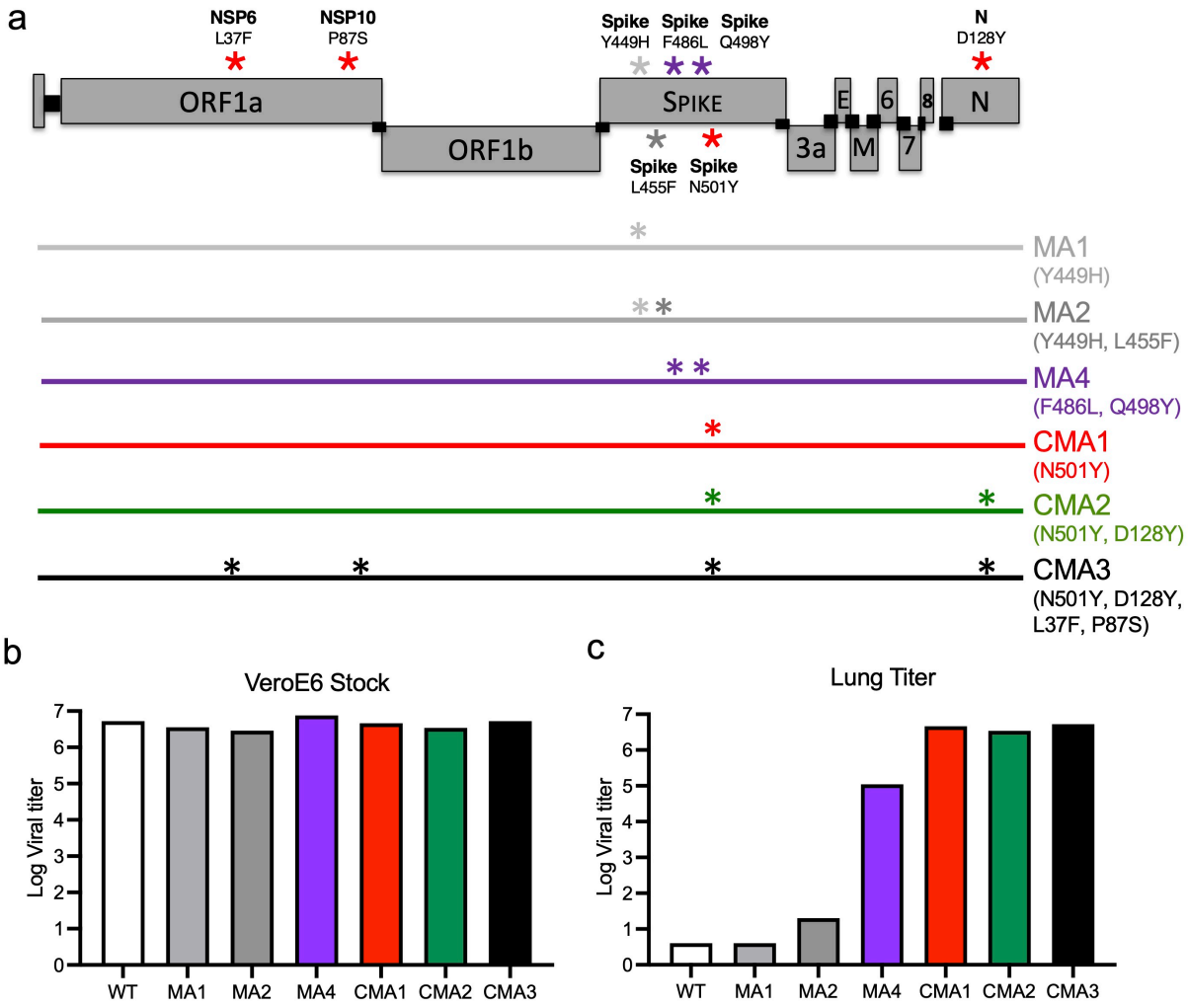
a

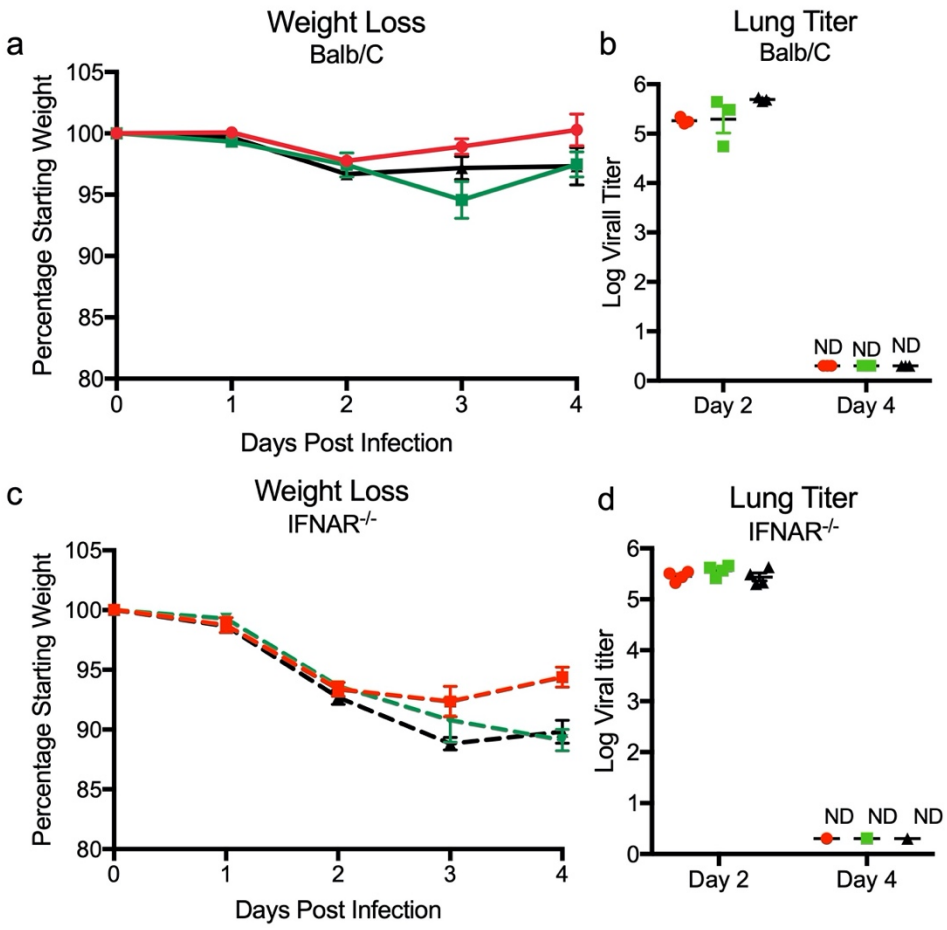
Strain	404	426	436	442	472	473	475	479	484	485	486	487	491
SARS	V	R	Y	Y	L	N	Y	N	Y	T	T	T	Y
MA15	V	R	H	Y	L	N	Y	N	Y	T	T	T	Y
MA20	V	R	Y	L	L	N	Y	K	Y	T	T	T	Y
MA25	V	R	H	F	L	N	Y	N	Y	T	T	T	Y
Strain	**417**	**439**	**449**	**455**	**486**	**487**	**489**	**493**	**498**	**499**	**500**	**501**	**505**
SARS2	K	N	Y	L	F	N	Y	Q	Q	P	T	N	Y
MA1	K	N	H	L	F	N	Y	Q	Q	P	T	N	Y
MA2	K	N	H	F	F	N	Y	Q	Q	P	T	N	Y
MA4	K	N	Y	L	L	N	Y	Q	Y	P	T	N	Y
CMA1-3	K	N	Y	L	F	N	Y	Q	Q	P	T	Y	Y



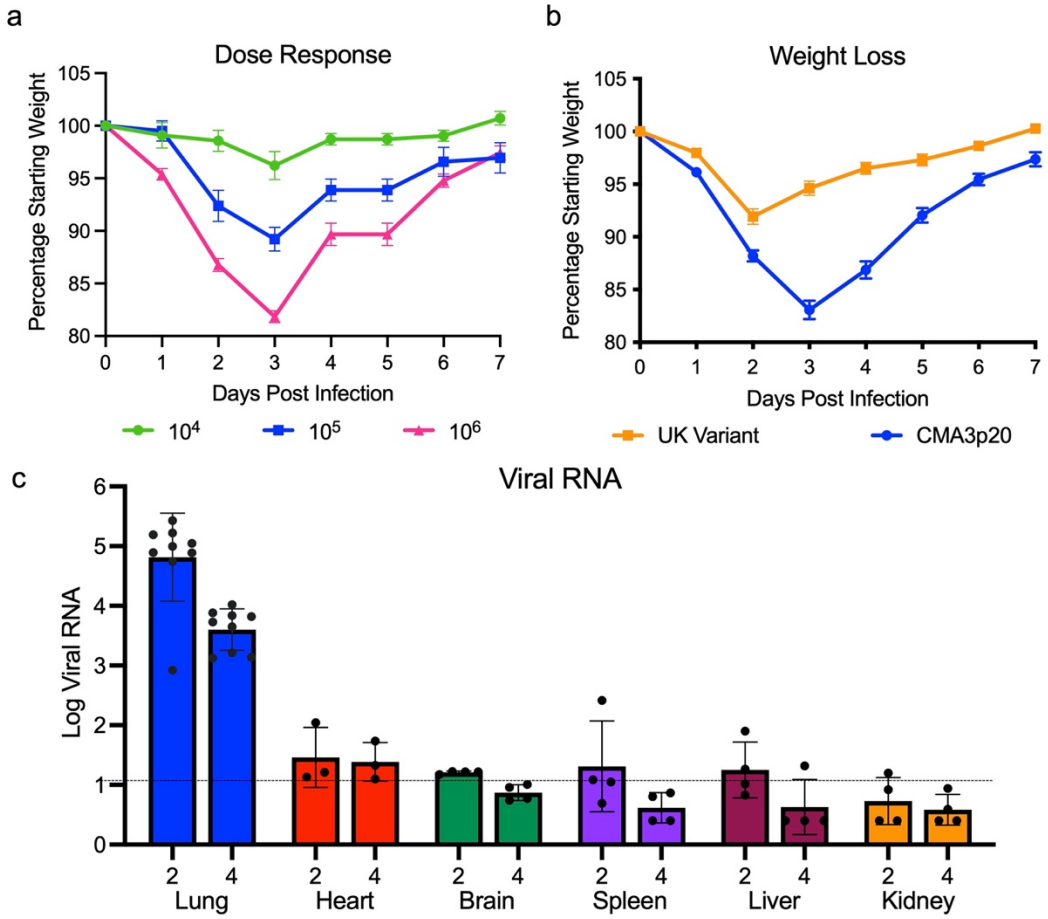
S. Figure 1. Modeling changes to mouse-adapt SARS-CoV-2. a) Key amino acid residues found in the receptor binding domain (RBD) of mouse adapted strains of SARS-CoV were aligned to SARS-CoV-2 and used to design mouse-adapted mutations 13. Key interaction sites between SARS-CoV spike and ACE2 molecules highlight in red 30. b-c) Modeling of key RBD residue interactions with mouse ACE2 (PDB:2AJF) comparing b) WT SARS-Cov-2 residues versus c) mutations (green) predicted to improve binding.



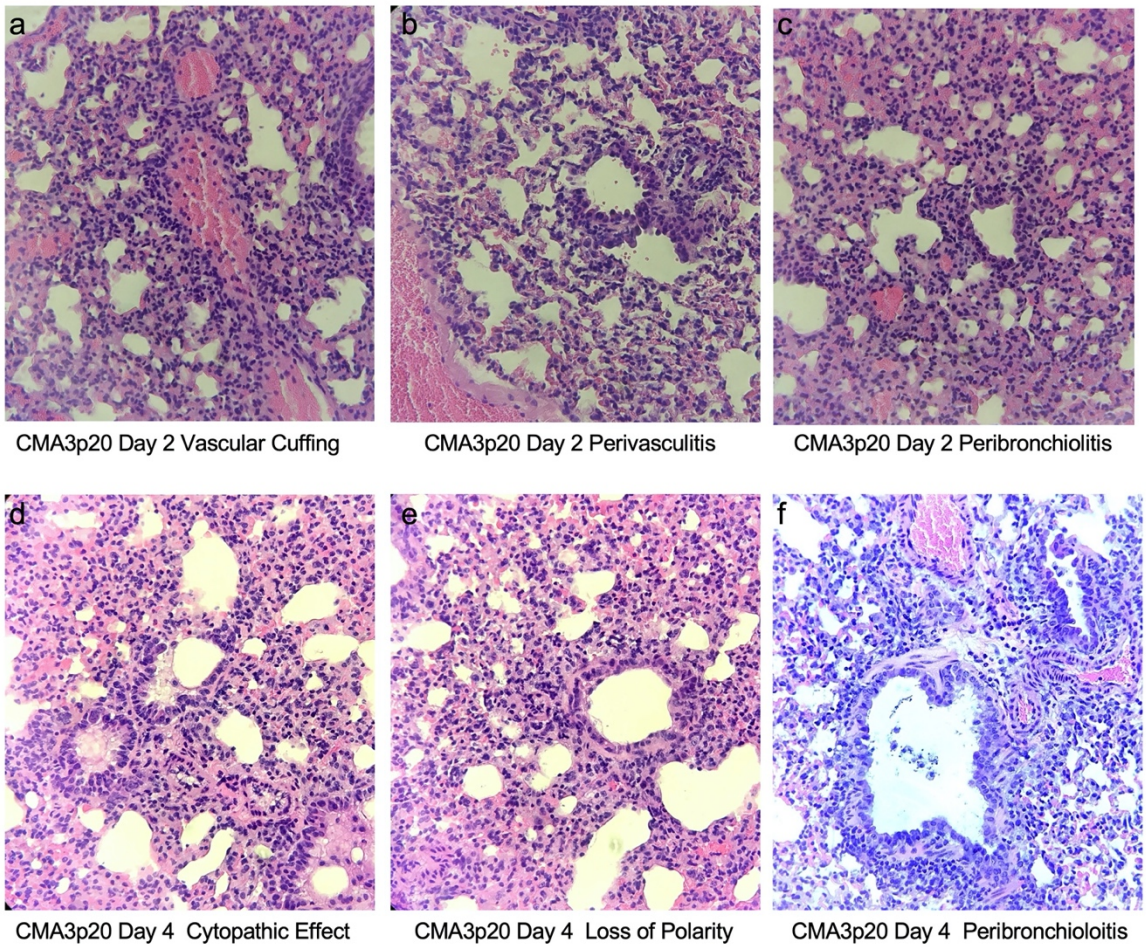
S. Figure 2. Construction of mouse-adapted SARS-CoV-2 Mutants. a) SARS-CoV-2 genome schematic indicating location of amino acid mutations for MA1, MA2, MA4, CMA1, CMA2, and CMA3. b) Viral replication of stock viruses of MA1, MA2, MA4, and CMA1-3 grown on VeroE6 cells. c) Viral replication of MA1, MA2, MA4, and CMA1-3 from lung homogenates isolated from infected mice 2 days post infection.



S. Figure 3. SARS-CoV-2 mutants CMA1, CMA2, and CMA3 replicate in laboratory mice. a-b) Ten-week-old female Balb/c mice infected with 105 PFU of CMA1 (red), CMA2 (green), or CMA3 (black) were examined for a) weight loss and b) viral lung titer following infection at days 2 and 4. c-d) Ten- to twelve-week-old female IFNAR-/- SVJ129 mice infected 105 PFU of CMA1 (red), CMA2 (green), or CMA3 (black) were examined for c) weight loss and d) viral lung titer following infection at days 2 and 4. ND- non-detected.



S. Figure 4. In vivo characterization of SARS-CoV-2 CMA3p20. a) Examination of ten-week-old female Balb/c mice infected with SARS-CoV-2 CMA3p20 at 104, 105, and106 PFU (n=5). b) Comparison of weight loss in ten0-week old female Balb/c mice infected with 106 PFU of SARS-CoV-2 CMA3p20 (blue) or SARS-CoV-2 variant B.1.1.7 (orange). c) RT-PCR of viral RNA load found in lung, heart, brain, spleen, liver, and kidney following 105 PFU infection of SARS-CoV-2 CMA3p20 2- and 4-days post infection. Dotted line signifies viral RNA value derived from mock infected samples.



S. Figure 5. SARS-CoV-2 CMA3p20 induces significant lung damage following infection. a-c) CMA3p20 infected animals 2 days post infection showing a) perivascular cuffing, b) perivasculitis and c) peribronchiolitis. d-f) CMA3p20 induced lung inflammation and damage 4 days post infection including d) cytopathic effect of the virus, e) loss of cellular polarity, and f) inflammatory cells in the lumen. Magnification at 10x for a-f.