

1 **Engineered chimeric T cell receptor fusion construct (TRuC)-expressing T** 2 **cells prevent translational shutdown in SARS-CoV-2-infected cells**

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18 19 20 **Abstract**

21 SARS-CoV-2, the causative agent of Covid-19, is known to evade the immune system by
22 several mechanisms. This includes the shutdown of the host cellular protein synthesis, which
23 abrogates the induction of antiviral interferon responses. The virus initiates the infection of
24 susceptible cells by binding with its spike protein (S) to the host angiotensin-converting
25 enzyme 2 (ACE2). Here we applied the T cell receptor fusion construct (TRuC) technology to
26 engineer T cells against such infected cells. In our TRuCs an S-binding domain is fused to the
27 CD3 ϵ component of the T cell receptor (TCR) complex, enabling recognition of S-containing
28 cells in an HLA independent manner. This domain either consists of the S-binding part of
29 ACE2 or a single-chain variable fragment of an anti-S antibody. We show that the TRuC T
30 cells are activated by and kill cells that express S of SARS-CoV-2 and its alpha (B.1.1.7) and
31 beta (B.1.351) variants at the cell surface. Treatment of SARS-CoV-2 infected cells with our
32 engineered T cells did not lead to massive cytotoxicity towards the infected cells, but resulted
33 in a complete rescue of the translational shutdown despite ongoing viral replication. Our data
34 show that engineered TRuC T cell products might be used against SARS-CoV-2 by exposing
35 infected cells to the host innate immune system.

36 **Introduction**

37

38 Covid-19 is caused by a new virus of the beta-coronavirus family, the severe acute respiratory
39 syndrome coronavirus 2 (SARS-CoV-2). Most infections are rather mild and are controlled by
40 adaptive immune responses. Both, strong CD4 and CD8 responses as well as neutralizing
41 antibodies are important (Grifoni et al., 2020; Schulien et al., 2021; Zheng et al., 2020) and
42 might provide a certain degree of protection for re-infections. However, a significant
43 proportion of patients require hospitalization and mortalities occur, especially in patients with
44 pre-existing medical conditions, such as obesity, cardiovascular disease or type 2 diabetes,
45 immunocompromised individuals (Docherty et al., 2020; Richardson et al., 2020) or those of
46 older age (Docherty et al., 2020; Grasselli et al., 2020). In several severe cases SARS-CoV-2-
47 specific CD4+ and CD8+ T cell responses failed to develop although neutralizing antibodies
48 were found, but were insufficient to control disease (Rydyznski Moderbacher et al., 2020).
49 Indeed, cases were reported in which T cells could control the infection well in the absence of
50 detectable antibodies (Rydyznski Moderbacher et al., 2020). With more than 3.7 million
51 deaths world-wide (June 2021) there is an urgent need for the treatment of ongoing severe
52 COVID-19 cases, and personalized treatments might be required.

53 To enter host cells, the receptor binding domain (RBD) of the SARS-CoV-2 spike protein (S)
54 binds to the host cell angiotensin-converting enzyme 2 (ACE2) (Wrapp et al., 2020; Yan et
55 al., 2020; Zhou et al., 2020). Cleavage of S by the cellular serine protease TMPRSS2 at the
56 cell surface initiates membrane fusion, so that the viral RNA is released into the cytosol for
57 viral replication (Hoffmann et al., 2020). Alternatively, in the absence of TMPRSS2, the
58 virion is endocytosed and S cleavage as well as membrane fusion takes place in endosomes
59 (Hoffmann et al., 2020; Simmons et al., 2005).

60 One of the major innate antiviral defence mechanisms is the type I interferon (IFN-I) system,
61 the induction of which stimulates the expression of a large number of interferon-stimulated
62 genes (ISGs). In case of coronaviruses the viral RNA is sensed by retinoic acid-inducible gene
63 I (RIG-I), thereby activating innate immune signaling (Hu et al., 2017; Sparrer and Gack,
64 2015). However, these viruses evade the innate immune response by abrogating host mRNA
65 translation, thus preventing expression of IFN-I and ISGs (Hsu et al., 2021; Kamitani et al.,
66 2009; Lei et al., 2020; Narayanan et al., 2008; Puray-Chavez et al., 2020; Thoms et al., 2020;
67 Xia H et al., 2020; Yuen et al., 2020). In SARS-CoV-1 and SARS-CoV-2 multiple viral
68 proteins are responsible for this translational shutdown (Hsu et al., 2021; Kamitani et al.,
69 2009; Narayanan et al., 2008; Puray-Chavez et al., 2020; Thoms et al., 2020). For example,

70 the non-structural protein 1 (Nsp1) inhibits nuclear export of host mRNA, but also blocks
71 ribosomal translation by binding to the 40S ribosomal subunit (Kamitani et al., 2009;
72 Narayanan et al., 2008; Thoms et al., 2020; Zhang et al., 2021) (Lapointe et al., 2021; Yuan et
73 al., 2020b). In addition, Nsp5, Nsp14 and Nsp15 can also halt translation of host mRNA (Hsu
74 et al., 2021; Lei et al., 2020; Xia H et al., 2020; Yuen et al., 2020). Indeed, SARS-CoV-2
75 infection as well as overexpression of these Nsps in Vero E6 or HEK293T cells inhibits the
76 production of IFN-I and induction of ISGs (Lei et al., 2020; Puray-Chavez et al., 2020;
77 Thoms et al., 2020; Xia H et al., 2020; Yuen et al., 2020). However, viral proteins can still be
78 produced, most likely due to a special feature of the 5' untranslated region of viral mRNAs
79 (Tanaka et al., 2012). Thus, impairment of host mRNA translation may allow SARS-CoV-2
80 to evade host immunity. How host cells can counteract this translational shutdown is poorly
81 understood.

82 T cells play an important role in the protection from infections and tumors through their
83 production of cytokines and other immune mediators as well as through their cytotoxic
84 activity. However, in chronic or severe viral infections as well as in the development of
85 tumors, T cells have failed to control the disease. Thus, one possible approach for a treatment
86 is to strengthen the T cell response by engineering the T cells to better recognize the target
87 cells (infected or tumor cells) and to mount a stronger, yet more balanced immune response.

88 One strategy is to redirect polyclonal T cells to recognize tumor cells by expressing a
89 chimeric antigen receptor (CAR) in the patients T cells. CARs consist of a tumor antigen-
90 binding extracellular part, typically a single-chain variable fragment (scFv) of an antibody, a
91 transmembrane domain and cytoplasmic regions derived from the TCR's CD3 ζ chain and co-
92 stimulatory receptors, such as CD28 or 4-1BB (Guedan et al., 2019; Rafiq et al., 2020). Thus,
93 the engineered T cells recognize tumors in an HLA-independent manner and couple this to T
94 cell activation signals. CAR T cells are highly effective at eradicating certain hematological
95 malignancies, such as acute B-lymphoblastic leukaemia and non-Hodgkin's lymphoma, but
96 fail against solid tumors (Glover et al., 2021). Further, over-activation and the dangerous
97 cytokine release syndrome are important safety concerns (Fitzgerald et al., 2017; Morris et al.,
98 2021).

99 A significant improvement of the CAR design was to employ the complete TCR machinery
100 (Baeuerle et al., 2019; Hardy et al., 2020; Liu et al., 2021; Rana et al., 2021; Xu et al., 2018),
101 instead of only the isolated cytoplasmic tail of CD3 ζ . One such example are chimeric
102 receptors called TCR fusion constructs (TRuCs). The TCR itself is a complicated multiprotein
103 machine composed of the antigen-binding TCR $\alpha\beta$ subunits and the signal transduction

104 CD3 $\epsilon\gamma$, CD3 $\epsilon\delta$ and $\zeta\zeta$ subunits. Ligand-binding leads to the exposure of signaling motifs in
105 the cytoplasmic tails of CD3, such as a proline-rich sequence, basic-rich sequences, the
106 receptor-kinase motif and the immuno-tyrosine based activation motifs (ITAMs) (Hartl et al.,
107 2020; Reth, 1989; Schamel et al., 2019). These conformational changes, the recruitment of
108 signaling molecules to the motifs and phosphorylation of the ITAMs are delicately controlled
109 to determine the quantity and quality of the signal, eventually leading to the activation and
110 differentiation of the T cell in a proper way. Importantly, mechanisms also control that in the
111 absence of ligand the TCR is held in a closed, inactive conformation (Swamy, Schamel JI).
112 In TRuCs, the anti-tumoral scFv is directly engineered onto the TCR (Baeuerle et al., 2019;
113 Gil et al., 2002; Minguet et al., 2007; Schamel and Reth, 2012). Thus, in stark contrast to the
114 CARs, TRuCs use the full signaling potential of the TCR. In case of the ϵ TRuC, where the
115 scFv is fused to the ectodomain of the TCR's subunit CD3 ϵ , it was shown that the ϵ TRuC T
116 cells had a more physiological and controlled signaling than CAR T cells (Baeuerle et al.,
117 2019; Rana et al., 2021) and were consequently more effective against B cell lymphoma and
118 leukemia (Baeuerle et al., 2019). Importantly, the ϵ TRuCT cell were also effective against
119 solid tumors (Baeuerle et al., 2019; Liu et al., 2021) - situations where CAR-T cells fail.
120 We sought to apply the lessons learned from engineering anti-tumor ϵ TRuC T cells to address
121 viral infections, with SARS-CoV-2 as a model case. Hence, the aim of this study was to
122 construct and test the ability of ϵ TRuC T cells to control and counteract SARS-CoV-2 viral
123 infection *in vitro*.

124

125

126 **Results**

127

128 **SARS-CoV-2-specific ϵ TRuCs redirect T cells to recognize the S protein**

129 To re-program T cells to recognize SARS-CoV-2-infected cells, we here constructed ϵ TRuCs
130 able to bind the SARS-CoV-2 S protein. To this end, the S-binding part of the extracellular
131 domain of human ACE2 (aa 18-615) (Yan et al., 2020) was genetically fused to human CD3 ϵ
132 in a lentiviral vector. A short or a long linker were used to connect the two proteins, resulting
133 in ACE2_s- ϵ TRuC and ACE2_l- ϵ TRuC (Fig. 1A and B). Besides ACE2, the human antibody
134 CR3022 also binds to SARS-CoV-2 S (ter Meulen et al., 2006; Wrapp et al., 2020; Yuan et
135 al., 2020a). Thus, a single chain fragment of the V_L and V_H regions (scFv) of CR3022 was
136 fused to CD3 ϵ , yielding the α S- ϵ TRuC (Fig. 1A and B); the linker that was used before to

137 construct the α CD19- ϵ TRuC (Baeuerle et al., 2019) was chosen. All lentiviruses also encode
138 for GFP fused with a T2A peptide to the ϵ TRuC.

139 Jurkat T cells that lack CD3 ϵ do not express a TCR on their surface (Fig. 1C, untransduced).
140 When they were lentivirally transduced with the S-specific ϵ TRuCs, a TCR containing these
141 ϵ TRuCs was expressed on the surface as seen by anti-CD3, anti-ACE2 and anti-IgG staining
142 (Fig. 1C, D, E, S1). As controls, we used lentiviruses that only encode for GFP (mock) or for
143 the α CD19- ϵ TRuC. Co-purification of the endogenous TCR subunits TCR β and CD3 ζ with
144 the ϵ TRuCs demonstrates that the ϵ TRuCs assemble into a TCR (Fig. 1F). Since CD3 ζ is the
145 last subunit to be added during TCR assembly (De Waal Malefyt et al., 1989; Minami et al.,
146 1987), the data shows that the ϵ TRuCs integrated into full TCRs. The Western blot confirmed
147 the expected sizes of the ϵ TRuCs (Fig. 1F). Hence, the SARS-CoV-2-specific ϵ TRuCs are
148 expressed as part of a TCR on the surface of Jurkat T cells.

149 To test whether this binding would lead to T cell activation, we used human Ramos-null B
150 cells (with a deletion of the B cell receptor (He et al., 2018)) expressing SARS-CoV-2 S on
151 their surface (Fig. 2A and S2A). These cells could activate our S-specific ϵ TRuC-expressing
152 Jurkat cells as seen by upregulation of the activation marker CD69 (Fig. 2B and S2B).
153 Similarly, expression of the alpha (B.1.1.7) and beta (B.1.351) variants of the S protein of
154 SARS-CoV-2, but not of the common cold human corona viruses HCoV-OC43 or -229E, led
155 to activation of our ϵ TRuC-expressing Jurkat cells (Fig. 2B). The S protein of SARS-CoV-1
156 or HCoV-NL63 weakly stimulated our T cells. This is in line with the ability of the different S
157 proteins to bind to ACE2 and the antibody CR3022 (Delmas et al., 1992; Hofmann et al.,
158 2005; Tian X et al., 2020; Yeager et al., 1992). As a control, Ramos cells without S did not
159 activate these Jurkat cells. Since Ramos cells express CD19, the α CD19- ϵ TRuC Jurkat cells
160 were activated by all Ramos cell lines (Fig. 2B). In conclusion, Jurkat cells expressing the
161 SARS-CoV-2-specific ϵ TRuCs recognize and are activated by target cells expressing S from
162 SARS-CoV-2.

163

164 **The novel primary ϵ TRuC T cells lyse S-containing target cells**

165 Next, expanded primary human T cells were transduced to express the ϵ TRuCs, in order to
166 test for the cytotoxic capability of these T cells. All ϵ TRuCs were expressed well on the
167 surface of the T cells (Fig. 3A), which were CD8⁺ and CD4⁺ T cells (Suppl. Fig. S3A).

168 As an example to look at target cell killing, we chose the α S- ϵ TRuC T cells which were co-
169 cultured with Ramos cells that express a chimeric SARS-CoV-2 S protein with a C-terminal

170 (cytosolic) fusion to the red fluorescent protein mScarlet (S-mScarlet, Fig. 3B). Hence,
171 transduced T cells (green) can be distinguished from the target cells (red) by confocal
172 fluorescent imaging (Fig. 3C). After contact of the α S- ϵ TRuC T cells with the S-expressing
173 Ramos cells, a stable cell conjugate was formed that lasted on average 153 min (Fig. 3 D).
174 Most likely an immunological synapse was formed as seen by the polarisation of the
175 lysosomes (stained in magenta) towards the target cells (Fig. 3C, upper row, second image).
176 In most cases, the S-expressing Ramos cells were killed as detected by membrane blebbing
177 (upper row, last image) that is typical for apoptosis. When the target cell was killed, this took
178 on average 121 min (Fig. 3E). As a control, both mock transduced T cells and untransduced T
179 cells, had only short contact to the Ramos cells (average 10 min) and left without cytotoxic
180 activity (Fig. 3C and D).

181 All S-specific ϵ TRuC and control engineered primary T cells were co-cultured with the
182 Ramos cells, which served as target cells, expressing or not the SARS-CoV-2 S, to quantify
183 the cytotoxic activity. The target cells also expressed the firefly luciferase and BFP (Suppl.
184 Fig 2C). If they were lysed, luciferase activity was lost, serving as a readout for the killing of
185 the target cells by the T cells. Indeed, all ϵ TRuC T cells lysed the S-expressing Ramos cells
186 very efficiently, whereas mock T cells did not (Fig. 3F). As expected only the α CD19- ϵ TRuC
187 T cells killed the Ramos cells that did not express the S protein. Ramos cells expressing S of
188 the alpha (B.1.1.7) and beta (B.1.351) variants were also lysed very efficiently, but not those
189 expressing S from HCoV-NL63, -OC43 or -229E (Fig. 3F). There was a weak, but clear,
190 activity also against Ramos cells expressing S from SARS-CoV-1. As above, we did not
191 observe any differences between the ACE2l-, ACE2s- and α S- ϵ TRuCs. Together these data
192 demonstrate that we were able to redirect primary T cells to kill efficiently target cells that
193 display SARS-CoV-2 S.

194

195 **The S-specific primary ϵ TRuC T cells are activated by S-expressing target cells**

196 Incubation of our engineered primary T cells with the Ramos cells expressing S from different
197 coronaviruses showed that the SARS-CoV-2 S protein led to upregulation of CD69 on the T
198 cells (Fig. 4A). The same was observed for S of the alpha (B.1.1.7) and beta (B.1.351)
199 variants. The ones from SARS-CoV-1 and HCoV-NL63 caused a slight CD69 upregulation,
200 whereas the ones from HCoV-OC43 or -229E were inactive. In the T cells that are not
201 specific for the SARS-CoV-2 S, CD69 was not upregulated by any S-expressing Ramos cell
202 (Fig. 4A). We also show that the engineered T cells that were activated by SARS-CoV-2 S-
203 expressing target cells secreted the cytokines IL2, IFN γ , IFN α and TNF α (Fig. 4B). As a

204 control, the mock T cells did not produce any of those cytokines nor did a co-culture with
205 Ramos cells that do not express any S (Fig. S4A). In conclusion, our re-programmed T cells
206 with the new S-specific ϵ TRuCs could be activated by S-expressing target cells.

207

208 **The new S-specific primary ϵ TRuC T cells kill SARS-CoV-2-infected cells**

209 Having shown that the S-specific ϵ TRuC T cells kill S-expressing Ramos cells, we wanted to
210 test whether these TRuC T cells are also able to kill SARS-CoV-2-infected Vero E6 cells
211 (Fig. 5A, upper panel). To quantify killing as above, Vero E6 cells were lentivirally
212 transduced to express luciferase and BFP bicistronically separated by an IRES sequence
213 (called VeroLucBFP cells, Fig. S5A) and were infected with the authentic B.1 SARS-CoV-2
214 using a multiplicity of infection (MOI) of 0.05. After one hour, remaining virus was washed
215 away, T cells were added and the luciferase signal was recorded (Fig. 5A, lower panel). When
216 the T cells had been transduced with the S-specific ϵ TRuCs (Fig. 5B, green and blue) the
217 luciferase activity of the infected VeroLucBFP culture was reduced at 2 and 4 hours
218 compared to the one treated with the mock and α CD19- ϵ TRuC T cells (red and orange). The
219 reduction of luciferase activity at early time points indicated that S-specific ϵ TRuC T cells
220 killed infected VeroLucBFP cells specifically, since treatment with the S-specific ϵ TRuC T
221 cells did not cause a decrease in luciferase activity in uninfected VeroLucBFP cells beyond
222 background (Fig. 5C). Elimination of infected cells within 2-4 hours is in line with the fast
223 killing of Ramos cells expressing the S (Fig. 3E).

224 Similar results were obtained when the human colorectal adenocarcinoma cell line Caco-2 or
225 the human lung epithelial cell line Calu-3 cells were transduced with luciferase and taken as
226 host cells for the virus infection (Fig. S5B and C). In conclusion, engineered primary human
227 T cells expressing ACE2s-, ACE2l- and α S- ϵ TRuCs were successfully re-programmed to
228 recognize and kill cells infected with SARS-CoV-2.

229

230 **S-specific ϵ TRuC T cells prevent the loss of luciferase activity in infected cells**

231 Next, we repeated the treatment of infected VeroLucBFP cells with our T cells, but recording
232 luciferase activity for 72 hours. With the control T cells (mock and α CD19- ϵ TRuC) the
233 luciferase signal started to decrease 6 hours post infection (Fig. 5D, red and orange). A similar
234 drop in luciferase activity was observed when no T cells were added (black line), showing
235 that T cells *per se* did not recognize SARS-CoV-2-infected cells. At 72 hours post infection
236 no luciferase activity was detected anymore. This loss of luciferase signal was prevented by

237 the presence of the S-specific ϵ TRuC T cells (green and blue). In fact, at 48 and 72 hours post
238 infection the infected and treated cultures had similar luciferase activity as the uninfected ones
239 (Fig. 5D and E), demonstrating that the TRuC treatment resulted in a sustained luciferase
240 expression.

241 Similar results were obtained when a different ratio of T cell to VeroLucBFP cells was used
242 (Fig. S6) or when Caco-2 or Calu-3 cells were infected (Fig. S6). In all cases, our treatment
243 completely rescued the infected cell cultures in terms of the virus-induced loss of luciferase
244 activity.

245

246 **SARS-CoV-2 causes a translation shutdown in VeroLucBFP cells**

247 In theory, the loss of luciferase signal may result from three different mechanisms. (i) By
248 killing of VeroLucBFP cells by the S-specific TRuC T cells (as above). (ii) By reducing the
249 VeroLucBFP cell number by either SARS-CoV-2-induced cell death (Park et al., 2020) or
250 reduced cellular proliferation rates (our graphs depict the luciferase activity of the wells in
251 relation to the wells with the uninfected cells). (iii) By the SARS-CoV-2-mediated
252 suppression of host protein synthesis (Hsu et al., 2021; Lapointe et al., 2021; Puray-Chavez et
253 al., 2020; Thoms et al., 2020; Yuan et al., 2020b), called translation shutdown. This
254 mechanism acts at the single cell level.

255 To monitor the effect of translation shutdown, we blocked protein synthesis in VeroLucBFP
256 cells with puromycin, which causes premature chain termination during mRNA translation
257 (Pestka, 1971). Indeed, puromycin-treatment suppressed luciferase activity before the
258 occurrence of cell death (Fig. 6A). To directly examine whether a strong translation shutdown
259 in infected cells occurred, we determined the BFP expression in SARS-CoV-2-infected
260 VeroLucBFP cells. BFP allows us to investigate the translation shutdown at the single cell
261 level by flow cytometry. Indeed, at 72 hours post infection when no luciferase activity was
262 detectable (Fig 5D), the BFP expression was similarly lost (in the control cultures that were
263 treated with mock or α CD19- ϵ TRuCs T cells) (Fig. 6B). This suggests that a complete
264 translation shutdown is induced by SARS-CoV-2 in the VeroLucBFP cells, as seen earlier in
265 other cells (Hsu et al., 2021; Lapointe et al., 2021; Puray-Chavez et al., 2020; Thoms et al.,
266 2020; Yuan et al., 2020b). This loss of BFP-expression was not observed in uninfected
267 VeroLucBFP cells (Fig. 6B and S5A). SARS-CoV-2 infection also led to slightly reduced
268 VeroLucBFP cell numbers at 72 hours (Fig. S7A), most likely due to viral lysis of the host
269 cells (Park et al., 2020).

270

271 **S-specific ϵ TRuC T cells prevent the translation shutdown, but not viral replication**

272 The loss of luciferase activity at later time points post infection, which is indicative for the
273 translation shutdown, was drastically reduced upon S-specific ϵ TRuC T cell treatment (Fig.
274 5D and S6). Indeed, treatment of infected VeroLucBFP cells with ACE2s- or α S- ϵ TRuC T
275 cells, also prevented the loss of BFP expression (Fig. 6B). The infected and treated
276 VeroLucBFP cells had similar BFP values as the uninfected ones. Assuming that the S-
277 specific ϵ TRuC-treatment would prevent the translation shutdown by an killing of all infected
278 VeroLucBFP cells immediately post infection, we expected no viral replication and spread in
279 the tissue culture. Surprisingly, although some killing of infected cells was detected at early
280 time points (Fig. 5B and S5), SARS-CoV-2 replicated equally well independent of our
281 treatment. This was seen by determining the virus titer of the culture supernatants at different
282 time points post infection (Fig. 6C) and by SARS-CoV-2 nucleocapsid protein (N) staining in
283 infected cells (Fig. 6E). In conclusion, at later time points post infection (8 to 72 hours) the
284 virus replicates, infects new VeroLucBFP cells and leads to a suppression of the host protein
285 synthesis in the VeroLucBFP cell culture. Importantly, this shutdown was completely
286 prevented by treatment with our engineered T cells.

287 Lastly, we sought to get insight into how the S-specific ϵ TRuC T cell blocks the virus-
288 induced translation shutdown. To this end, we activated ACE2s- or α S- ϵ TRuC T cells by co-
289 culturing them with S-expressing Ramos cells for 24 hours. We then collected the medium
290 that should contain factors that these T cells secrete and treated uninfected and SARS-CoV-2-
291 infected VeroLucBFP cells with this “conditioned” medium. Upon treatment of SARS-CoV-
292 2-infected cells with the ACE2s- or α S- ϵ TRuC T cell “conditioned” medium, the loss of
293 luciferase activity and of BFP expression was reduced compared to cells incubated with
294 “conditioned” medium derived from mock T cells (Fig. 7A and 7B). These data suggest that
295 our engineered T cells prevent virus-induced protein shutdown in VeroLucBFP cells by
296 secreting a protective factor or a cocktail of different factors instead of killing infected cells.
297 This is in line with the observation that the T cells also prevented the translation shutdown,
298 when using a low MOI, where the killing of infected cells at the early time point was not
299 visible (Fig. S7B).

300

301

302 **Discussion**

303

304 Our results demonstrate that the TRuC technology can be used to re-program T cells to
305 recognize SARS-CoV-2-infected cells in an HLA-independent manner. TRuC T cells have
306 been developed against tumor cells, now widening their potential to viral infections, such as
307 Covid-19. Instead of recognizing a tumor antigen, the new ϵ TRuCs recognize the SARS-
308 CoV-2 S protein, and thus distinguish healthy from infected cells. In fact, the here presented
309 ϵ TRuCs were generated by either fusing the S-binding part of the ectodomain of ACE2 (Lan
310 et al., 2020; Yan et al., 2020; Yuan et al., 2020a; Zhou et al., 2020) or the scFv of the anti-S
311 antibody CR3022 (ter Meulen et al., 2006; Wrapp et al., 2020; Yuan et al., 2020a) to the
312 TCR's CD3 ϵ chain. The resulting S-specific ϵ TRuCs integrated into complete TCRs, as seen
313 by surface expression of the ϵ TRuC (in wild-type and CD3 ϵ KO T cells) and co-
314 immunoprecipitation of endogenous TCR subunits. Importantly, the engineered T cells
315 specifically lysed SARS-CoV-2-infected cells and secreted anti-viral cytokines.
316 Ramos tumor cells exogenously expressing SARS-CoV-2 S on their surface were lysed as
317 efficiently by our novel ϵ TRuC T cells as by the α CD19- ϵ TRuC T cells targeting the tumor
318 antigen CD19 that is also expressed by Ramos. Since the α CD19- ϵ TRuC T cells are very
319 potent against B cell-derived tumors (Bauerle et al., 2019), the new S-specific ϵ TRuCs
320 demonstrated full functionality. The observed killing events were very fast (between 30 min
321 and 4 hours) and included recognition of the target cells as seen by prolonged conjugate
322 formation. Ramos cells expressing S from the alpha (B.1.1.7) and beta (B.1.351) variants
323 were also killed efficiently, suggesting that these currently circulating variants of concern
324 might be equally well targeted by our ϵ TRuCs. Importantly, while escape from neutralising
325 antibody binding is frequently observed for SARS-CoV-2 variants (Hoffmann et al., 2021),
326 the reliance on ACE2-mediated entry into the host cells by SARS-CoV-2 suggests that escape
327 from binding to ACE2- ϵ TRuC will impose an unlikely strong fitness cost. Ramos cells
328 expressing S from SARS-CoV-1 and HCoV-NL63 were recognized to a certain degree, since
329 S of these viruses also binds to ACE2, albeit with lower affinity (Horndler et al., 2021; Wu et
330 al., 2009; Xie et al., 2020). Ramos expressing S from HCoV-OC43 or -229E that do not bind
331 to ACE2 (Delmas et al., 1992; Yeager et al., 1992) were not killed, showing again the
332 specificity of our approach. Together our data are in line with two recent unreviewed
333 preprints. They report that expressing S-specific CARs based on the antibodies CR3022 or
334 S309 in natural killer (NK) cells led to the killing of S-expressing target cells (Ma et al., 2021;
335 Ma et al., 2020).

336 Cytotoxic activity of our ϵ TRuC T cells was also seen against SARS-CoV-2-infected Vero
337 E6, CaCo-2 and Calu-3 cells, indicating that the amount of S being displayed on the surface
338 of the infected cells unleashed recognition and killing by the novel ϵ TRuC T cells. Indeed, T
339 cell activation via the TCR can be mediated by very low amounts of antigen, such as 3-7
340 copies per target cell (Irvine et al., 2002; Purbhoo et al., 2004). Lysis of the infected cells
341 occurred within 2 hours post infection, suggesting that the post-fusion and membrane
342 remaining S protein, rather than newly produced S by the host cell was recognized. However,
343 the latter possibility cannot be ruled out and indeed the wild-type S protein can reach the cell
344 surface when exogenously expressed (Horndler et al., 2021; Lapuente et al., 2021).

345 Although infected cells were killed quicker than the complete virus replication cycle takes
346 (which is around 8 hours), the virus could replicate and spread within the cultures. Hence,
347 either not all infected cells were killed or killing occurred too late, so that virions were already
348 formed. This is a clear difference in the usage of ϵ TRuC T cells targeting tumor cells, since in
349 the latter case, it does not matter if killing takes longer.

350 SARS-CoV-2 induces a stop in translation of endogenous proteins by the infected cells
351 (Banerjee et al., 2020; Hsu et al., 2021; Lapointe et al., 2021; Puray-Chavez et al., 2020;
352 Thoms et al., 2020; Yuan et al., 2020b). In fact, it was shown that the viral protein Nsp1
353 blocks the ribosome (Thoms et al., 2020; Yuan et al., 2020b) and in our experiments this was
354 detected by both a loss of luciferase activity and a loss of BFP expression at the individual
355 cell level. Puromycin, which also prevents protein translation, led to a loss of luciferase
356 activity too and this occurred earlier than cell death, indicating again that luciferase activity is
357 a read out for the translation shutdown in our experiments. Indeed, luciferase activity and
358 fluorescence of a reporter (GFP) were used earlier to quantify this shutdown (Hsu et al., 2021;
359 Lei et al., 2020; Puray-Chavez et al., 2020; Thoms et al., 2020; Xia H et al., 2020; Yuen et al.,
360 2020).

361 Treatment of the infected cultures with the S-specific ϵ TRuC T cells completely prevented the
362 virus-induced translation shutdown; and 72 hours post infection infected and treated cultures
363 resembled the non-infected controls in terms of luciferase activity and BFP expression. Thus,
364 the engineered T cells provided long-term protection from the translational arrest. This effect
365 of the T cells was robustly seen with various MOIs and T cell:infected cell ratios. A reduction
366 in the shutdown was also evident when only the supernatant of activated ϵ TRuC T cell was
367 used, suggesting that one or more soluble factor(s) mediated this protective effect. Thus, our
368 hypothesis is that upon recognition of infected cells, the S-specific ϵ TRuC T cells are
369 activated (as seen by the killing in the first hours) and secrete cytokines or other factors that

370 can rescue the infected cells from the protein synthesis shutdown. Indeed, we show that these
371 T cells produce certain cytokines, such as $INF\gamma$, $IFN\alpha$, $TNF\alpha$ and IL-2, upon stimulation with
372 S-expressing Ramos cells. However, the identity of the factor counteracting the shutdown
373 upon infection remains enigmatic.

374 With or without translation shutdown the same amount of virus was detected in the
375 supernatant of the infected Vero E6 cells, indicating that a stop in the translation of host
376 proteins does not impair viral growth. Thus, the main aim of the shutdown might be to
377 prevent the innate anti-viral response by the host cell, as suggested earlier (Lei et al., 2020;
378 Puray-Chavez et al., 2020; Thoms et al., 2020; Xia H et al., 2020; Yuen et al., 2020).
379 Importantly, by preventing this shutdown our treatment might enable a potent anti-viral
380 response, helping in the noncytolytic clearance of the virus, thereby preventing strong tissue
381 damage. The latter is relevant, because in some viral infections (such as in the lymphocytic
382 choriomeningitis or hepatitis B viruses (Guidotti et al., 1999; Guidotti and Chisari, 2006))
383 most of the cells in a certain organ are infected and massive killing of the infected cells might
384 destroy the organ. Surprisingly, although the S-specific ϵ TRuC T cells killed infected cells in
385 the first hours, they spared infected cells later on and allowed those to survive and prevent the
386 translation shutdown.

387 CARs have been successfully used against hematological but not solid tumors. In contrast,
388 ϵ TRuC T cells are active against solid tumors and show enhanced efficacy against
389 hematological malignancies (Baeuerle et al., 2019). This is most likely due to the employment
390 of the full TCR signal, in contrast to the CARs that only use part of the TCR signal (namely
391 the one by the isolated CD3 ζ chain). Furthermore, the strength of the TCR (and TRuC-TCR)
392 signal is autoregulated and finely balanced by conformational changes of the full TCR
393 complex (Gil et al., 2002; Hartl et al., 2020; Minguet et al., 2007; Swamy et al., 2016). For
394 example, the strong tonic signaling by the CARs that might lead to CAR T cell exhaustion
395 (Ajina and Maher, 2018; Frigault et al., 2015) is not seen with the ϵ TRuCs. Lastly, ϵ TRuC
396 expression is limited by its incorporation into the TCR, whereas CARs are often
397 overexpressed (Baeuerle et al., 2019). Thus, CAR T cells cannot benefit from 400 million
398 years of TCR evolution.

399 In conclusion, with our ϵ TRuC technology, derived from the cancer field, we re-programmed
400 human T cells to specifically recognize SARS-CoV-2-infected cells. This might be the basis
401 to develop an effective treatment for severely diseased Covid-19 patients.

402

403

404 **Methods**

405

406 **Molecular cloning of the ϵ TRuC constructs**

407 The plasmids used in this study were generated using standard molecular cloning techniques,
408 such as PCR, restriction digest and Gibson assembly (Gibson et al., 2009). pOSY120,
409 encoding for the ACE2l- ϵ TRuC, was generated by Gibson assembly of the XhoI-XbaI
410 fragment of p526 anti- α CD19 (Baeuerle et al., 2019) and the PCR fragment using the primers
411 O293 and O294 (see table 1) on the human ACE2 sequence as a template. pOSY121,
412 encoding for the ACE2s- ϵ TRuC, was generated by Gibson assembly of the XhoI-XbaI
413 fragment of above and the PCR fragment using the primers O293 and O295 on the human
414 ACE2 sequence. pOSY123, encoding the α S- ϵ TRuC, was generated by Gibson assembly of
415 the XhoI-XbaI fragment of above and, the PCR fragment using the primers O304 and O305
416 from a gBlock of CR3022 V_L (Integrated DNA Technologies) and the PCR fragment using
417 O306 and O307 from a gBlock of CR3022 V_H (Integrated DNA Technologies). All plasmid
418 sequences were verified by Sanger sequencing (Eurofins Genomics).

419

Primer	Sequence
O293	ATCCAAGCTGTGACCGGCGCCTACTCTAGAGCCACCATGTCAAGCTCT TC
O294	GATGCCGCCCATTTCCTCGTTGCCATCCTCGAGGGATCCTCCGCCGCCG TCTGCATATGGACTCCAGTCG
O295	GATGCCGCCCATTTCCTCGTTGCCATCCTCGAGGGATCCTCCGCCGCCA CTTCCTCCGCCACCAGAACCACCGCCTCCGTCTGCATATGGACTCCAGT CG
O304	TACAGATCCAAGCTGTGACCGGCGCCTACTCTAGAGCCGCCACCATGG TGTTGCAGACCCAGG
O305	ACTTCCTCCGCCACCAGAACCACCGCCTCCTTTGATTTCCACCTTGGTC CCC
O306	GGAGGCGGTGGTTCTGGTGGCGGAGGAAGTGGCGGCGGAGGATCCCA GATGCAGCTGGTGCAATCTG
O307	GTGATGCCGCCCATTTCCTCGTTGCCATCCTCGAGGGATCCTCCGCCGC CACTTCCTCCGCCACCAGAACCACCGCCTCCGACGGTGACCGTGGTCC

420

421 Table 1 Primer sequences

422

423 **Molecular cloning of the luciferase-BFP and S protein-mScarlet vectors**

424 For the molecular cloning of the pHRSIN-CS-Luc-IRES-mTagBFP2 vector, the previously
425 described pHRSIN-CS-Luc-IRES-emGFP vector (a kind gift from A. Rodriguez, Universidad
426 Autonoma Madrid, Spain) was digested with BstX1 and Not1 restriction enzymes to remove
427 the emGFP. mTagBFP2 was amplified from the previously described pHRSIN-CS-IRES-

428 mTagBFP2 plasmid (Dang et al., 2020) and BstX1- and Not1-specific overhangs were added
429 using PCR. The final expression vector was generated by Gibson assembly using the
430 pHRSIN-CS-Luc-IRES as recipient vector and the amplified mTagBFP2 as insert. The
431 integrity of the plasmid was verified by Sanger sequencing.

432 The retroviral expression vector encoding the S protein of SARS-CoV-2 fused at its
433 cytoplasmic tail to mScarlet was cloned as follows. The cDNA of the S protein was taken
434 from the plasmid pCG1-CoV-2019-S with a codon-optimized sequence (Lapiente et al.,
435 2021) and cloned into the pMIG vector by Gibson assembly and the cDNA of mScarlet was
436 ligated into the vector.

437 All plasmid sequences were verified by Sanger sequencing (Eurofins Genomics).

438

439 **Generation of lentiviruses**

440 Lentiviruses were prepared as described (Hartl et al., 2020). In brief, HEK293T cells were
441 transfected with the ϵ TRuC-encoding lentiviral plasmids and the packaging plasmids
442 pMD2.G (envelope) and pCMVR8.74 (gag/pol) using PEI transfection. The virus-containing
443 supernatant was collected, concentrated by centrifugation and the virus pellet was
444 resuspended in RPMI 1640 medium and stored at -80°C . If MOI is explained in Frederikes
445 Nat Immunol paper, then we can cite this here. If not we need to write a few sentences.

446

447 **Cells**

448 Jurkat (human T cell line), Ramos (human Burkitt's lymphoma line), VeroE6 (kidney
449 epithelial cell line from the African green monkey), CaCo-2 (human colorectal
450 adenocarcinoma line) and CaLu-3 (human lung epithelial cell line) cells and their derivatives
451 were grown with complete RPMI 1640 medium and 10% fetal calf serum (FCS) at 37°C and
452 5% CO_2 . Jurkat CD3 ϵ knock out (KO) cells were generated by standard CRISPR/Cas9
453 technology and the generation of the Ramos cells expressing the different S proteins will be
454 described elsewhere. Jurkat CD3 ϵ KO, Ramos, Vero E6, CaCo-2 and CaLu-3 cell lines were
455 transduced with a multiplicity of infection (MOI) of 5 with the lentiviruses indicated and sorted
456 by flow cytometry when necessary.

457 To obtain the expanded human T cells, peripheral blood mononuclear cells (PBMCs) were
458 isolated from blood of a healthy donors by density-gradient centrifugation and grown in
459 RPMI 1640 medium supplemented with 10% FCS and 1000 U/ml recombinant IL-2
460 (PeproTech) and activated with 1 $\mu\text{g}/\text{ml}$ anti-CD3 and anti-CD28 antibodies. At 48-72 h the
461 remaining PBMCs were mostly T cells ($> 99\%$) and lentivirally transduced by spin infection

462 with 5 µg/ml of protamine sulfate with a MOI of 4. Transduced T cells were tested using flow
463 cytometry by staining with anti-Fab for the αCD19-εTRuC, anti-ACE2 for the ACE2s- and
464 ACE2I-εTRuCs, and anti-human IgG for the αS-εTRuC. Cells were expanded in complete
465 RPMI 1640 medium with 10% FCS and were given 100 U/ml IL-2 every third day. Cells
466 were used until day 17 post transduction.

467 To generate S-mScarlet expressing Ramos cells, the retroviral vector pMIG encoding S-
468 mScarlet and a vector encoding the ecotropic packaging protein were co-transfected (500 ng
469 each) into Plat-E cells with the PolyJet transfection reagent (Signagen). After two days, the
470 supernatant was collected, filtered and mixed 1:1 with 300.000 Ramos-null cells that express
471 the ecotropic receptor. Cells were sorted for mScarlet expression.

472 The transduction of Ramos cells with LucBFP was done as follows. Retroviral transduction of
473 the human Ramos-null B cells (with a deletion of the B cell receptor (He et al., 2018)) with
474 vectors encoding for the different S proteins will be published elsewhere. These Ramos-null
475 cells expressing S proteins or not were lentivirally transduced with pHRSIN-CS-Luc-IRES-
476 mTagBFP2 as briefly described above.

477

478 **Flow cytometry**

479 The following antibodies were used for flow cytometry staining in a 96-well format: PE-
480 labelled anti-human CD4 (Beckman Coulter, #A07752), APC-labelled anti-human CD8
481 (Beckman Coulter, #IM2469), APC- or PacificBlue-labelled anti-human CD3 (BioLegend,
482 #300434), PE-labelled anti-human CD69 (Life Technologies, #MHCD6904), anti-human
483 ACE2 (R&D Systems, #HK0320042), PE-labelled anti-human IgG (Southern Biotech, #2040-
484 09) and biotin-labelled anti-Fab (Invitrogen, #31803). APC-coupled Streptavidin (Biolegend,
485 #105213) and APC-labelled donkey anti-goat IgG (Southern Biotech, #6420-05) served as a
486 secondary reagent. Cells were measured on the flow cytometer Attune NxT and the data were
487 analyzed by FlowJo.

488 For flow cytometry of virus-infected cells post-treatment, the cells were fixed with methanol
489 and paraformaldehyde and then washed 4 times before measuring on AttuneNxT.

490

491 **Co-immunopurification and Western blotting**

492 Following antibodies were used for biochemical analysis: anti-TCRα (clone H-1, Santa Cruz,
493 #sc-515719), anti-TCRβ (clone H-197, Santa Cruz, #sc-9101), anti-CD3γ (clone EPR4517,
494 Epitomics, #3256-1), anti-CD3δ (clone F-1, Santa Cruz, #sc-137137.), anti-CD3ε (clone
495 M20, Santa Cruz, #sc-1127), anti-CD3ζ (serum 449), horseradish peroxidase (HRPO)-

496 coupled anti-mouse IgG (Thermo Fisher, #32430), HRPO-coupled anti-goat IgG (Thermo
497 Fisher, #31402), and HRPO-coupled anti-rabbit IgG (Thermo Fisher, #31460). Protein G-
498 coupled sepharose (#17-0618-01) and Protein A-coupled sepharose (#17-5138-01) beads
499 were from GE Healthcare and the protease inhibitor cocktail was from Sigma.

500 3×10^7 cells were lysed in 0.4 ml lysis buffer containing 20 mM Tris-HCl pH8, 137 mM
501 NaCl, 2 mM EDTA, 10% glycerol, 1x protease inhibitor cocktail, 1 mM PMSF, 5 mM
502 iodoacetamide, 0.5 mM sodium orthovanadate, 1 mM NaF, and 0.5% Brij96 for 30 min at
503 4 °C followed by 15 min centrifugation to pellet the nuclei and insoluble material. For the
504 anti-CD3 ϵ immunoprecipitation 370 μ l cleared cell lysate was incubated with 5 μ l 50%
505 protein A and protein G sepharose slurry (1:1) and 2 μ g anti-CD3 ϵ UCHT1 for 4 h at 4 °C.
506 After three washes, the immunoprecipitated material was separated by 12% reducing SDS-
507 PAGE. The separated proteins were transferred to PVDF membranes by semi-dry transfer.
508 After blocking with 5% milk in PBS containing 0.1% Tween-20 the membranes were
509 incubated with antibodies against TCR α (1:1000), TCR β (1:100), CD3 γ (1:1000), CD3 δ
510 (1:100), CD3 ϵ (1:1000), CD3 ζ (1:1000) in PBS-T followed by incubation with HRPO-
511 conjugated secondary antibodies (1:10000). Western blot signals were recorded using an
512 Image Quant LAS 4000 Mini from GE Healthcare Life Sciences, Boston, MA.

513

514 **Activation assays**

515 Ramos cells expressing or not the different S proteins were co-cultured with the different
516 Jurkat transductant cells at a 1:3 target-to-effector ratio for 9 h. Cells were stained with anti-
517 CD69 antibodies and measured by flow cytometry. The BFP-positive Ramos cells were gated
518 out to ensure that only the T cells are analysed.

519 To quantify cytokines by ELISA, ϵ TRuC-expressing primary T cells and S-expressing Ramos
520 cells were co-cultured for 24 h. The following cytokines were measured according to the
521 instructions of each ELISA kit: TNF α (Invitrogen, #88-7346-88), IFN γ (Invitrogen, #88-
522 7316-88), IFN α (Invitrogen, #BMS216) and IL2 (Invitrogen, #88-7025-88).

523

524 **Cytotoxicity assay**

525 To test for the T cell-mediated lysis of the Ramos target cells expressing firefly luciferase and
526 the S proteins indicated, a bioluminescence-based cytotoxicity assay was performed. 100.000
527 Ramos cells were plated in 100 μ l of complete RPMI 1640 medium with 10% FCS in a 96-
528 well flat bottom plate (Corning). 75 μ g/ml D-firefly luciferin potassium salt (Biosynth) was
529 added and bioluminescence was measured in the luminometer (Tecan infinity M200 Pro) to

530 establish a baseline. Then, 300,000 ϵ TRuC-expressing T cells (effector cells) were added at
531 an effector-to-target ratio of 3:1 and incubated for 24 h at 37°C. Relative light units (RLUs)
532 signals from target cells treated with 1% Triton X-100 indicate maximal cell death. RLU
533 signals from target cells without added T cells determine spontaneous cell death. The percent
534 of specific lysis was calculated with the following formula:

535
$$\text{percentage specific lysis} = 100 \times (\text{average spontaneous death RLU} - \text{test RLU}) /$$

536
$$(\text{average spontaneous death RLU} - \text{average maximal death RLU}).$$

537

538 **Time-lapse microscopy**

539 Ramos B cells were retrovirally transduced with a construct encoding spike protein tagged
540 with the fluorophore mScarlet at the C-terminus and sorted by FACS (Bio-Rad S3e Cell
541 Sorter). For the experiment, mScarlet-spike-expressing Ramos B cells were washed 3 times in
542 PBS. 15,000 cells in PBS were seeded into both wells of a 2 well-culture insert placed on a 35
543 mm dish (Ibidi). PBS facilitated the attachment of Ramos B cells onto the ibi-treated dish
544 surface. T cells were incubated with 50 nM Lysotracker Deep Red (Invitrogen) for 15 min at a
545 37°C incubator with 5% CO₂, washed twice and resuspended in phenol red-free RPMI
546 containing 2 mM GlutaMAX (Gibco), 10% FCS (PAN), 50 U/ml Pen-Strep (Gibco) and 10
547 mM HEPES (Gibco). 7,500 mock- and anti-spike-transduced T cells were seeded into the 2
548 well-culture insert with Ramos B cells after removing the PBS. Mock- and anti-spike-
549 transduced T and Ramos B cell interactions were recorded in parallel using a Zeiss Observer
550 microscope equipped with a 40x oil objective and Zen Blue software. Single plane images in
551 4 channels (GFP, mScarlet, Lysotracker deep red, brightfield) were acquired every 2 or 3 min
552 for 4 h within an incubator set to 37°C and 5% CO₂. Multi-position videos were converted to
553 TIFF and transferred to ImageJ. Duration of interaction between T and B cells and the time it
554 takes for an anti-spike-transduced T cell to kill mScarlet-spike-expressing Ramos B cell were
555 quantified manually. Cell death was assessed by apoptotic morphology and the subsequent
556 halt in cellular movement from the brightfield images.

557

558 **SARS-CoV-2 infection and measurement of luciferase activity**

559 A bioluminescence based cytotoxicity assay was performed with transduced primary cells co-
560 cultured with VeroE6 (kidney epithelial cells extracted from an African green monkey),
561 CaCo-2 (human colorectal adenocarcinoma cells) and CaLu-3 (human lung cancer cell line)
562 cell lines. Each of these cell lines were infected with the B.1 SARS-CoV-2 virus at the
563 indicated MOI. A control setup had the same cell lines without the infection. 10⁴/μl of target

564 (Vero E6, CaCo-2 and CaLu-3) cells were plated in a 96-well flat bottom plate (Corning,
565 #3917). 75 µg/ml D-firefly luciferin potassium salt (Biosynth) was added to it and
566 bioluminescence (BLI) was measured in the luminometer (Tecan infinity M200 Pro) to
567 establish the BLI baseline. Right after, TRuC-expressing T cells (effector cells) were added at
568 varying effector-to-target ratio (as indicated) and incubated until 72 h post infection (as
569 indicated) at 37°C. BLI was measured as relative light units (RLUs). RLU signals from cells
570 treated with 1% Triton X-100 indicate maximal cell death. RLU signals from tumor cells
571 without TRuC T cells determine spontaneous cell death. Percent specific lysis (specific
572 cytotoxicity) was calculated with the following formula:

573
574
$$\text{percentage specific lysis} = 100 - (100 \times (\text{average spontaneous death RLU} - \text{test RLU}) /$$

575
$$(\text{average spontaneous death RLU} - \text{average maximal death RLU})).$$

576 Here, spontaneous death was considered of only the target cells without the virus and without
577 T-cells.

578

579 **Immunofluorescence and viral titers**

580 Virus containing supernatant was harvested at the indicated time points and the viral titer was
581 determined on VeroLucBFP cells by indirect-immunofluorescence. Briefly, VeroLucBFP
582 cells were seeded in 96-well plates at a density of 0.04×10^6 cells/well 24 h prior to infection.
583 Infectious cell supernatants were diluted in tenfold dilution series in PBS containing 2% BSA
584 in a 100 µl volume and subsequently incubated on VeroLucBFP. At 20 h post infection, the
585 infectious supernatant was removed and cells were fixed using 4% formaldehyde for 30
586 minutes. Virus-infected cells were subsequently detected using anti-SARS-CoV nucleocapsid
587 (N) rabbit antiserum (Rockland Immunochemicals, #200-401-A50) and secondary anti-rabbit
588 IgG-coupled to Cyanine Cy3 (Jackson ImmunoResearch). Nuclei were stained with DAPI.
589 Viral endpoint titers were evaluated by fluorescence microscopy.

590 To monitor SARS-CoV-2 spread upon εTRuC T cell-treatment, infected VeroLucBFP cells
591 were fixed at the indicated time points post infection with 4% formaldehyde for 30 minutes
592 and subjected to indirect-immunofluorescence as described. Fluorescence images were
593 acquired using a Zeiss Observer.Z1 inverted epifluorescence microscope (Carl Zeiss)
594 equipped with an AxioCamMR3 camera using a ×20 objective.

595

596 **Statistics**

597 Statistical significance of one sample in comparison to another (a control) was determined
598 using a paired Student's t-test. All p values indicated with stars (* < 0.05, ** < 0.005, *** <

599 0.0005) were calculated using Prism v.6 software (GraphPad). Error bars show standard
600 deviation in all graphs.

601

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611

612 **Author contributions**

613 I.G. performed the Jurkat experiments, and the ones with the Ramos cells, O.S.Y. and I.G.
614 cloned the TRuC plasmids, K.C. and I.G. performed all experiments including SARS-CoV-2
615 whereas K.C. performed all titer analysis and N staining experiments, S.C.P. did the co-IP,
616 P.S. and N.G. helped in the flow cytometry, D.S. performed the microscopy killing
617 experiment, N.V. cloned the S-mScarlet fusion and generated all the S-expressing Ramos
618 cells, J.S. cloned the Luciferase-BFP plasmid, M.R., M.S. and W.W.S. supervised the work.
619 All authors analysed data and helped in preparing the manuscript.

620

621 **Competing interests**

622 W.W.S. serves on the scientific advisory board of TCR² Therapeutics. Patent application is
623 pending.

624

625 **Additional information**

626 Correspondence and requests for materials should be addressed to W.W.S.

627

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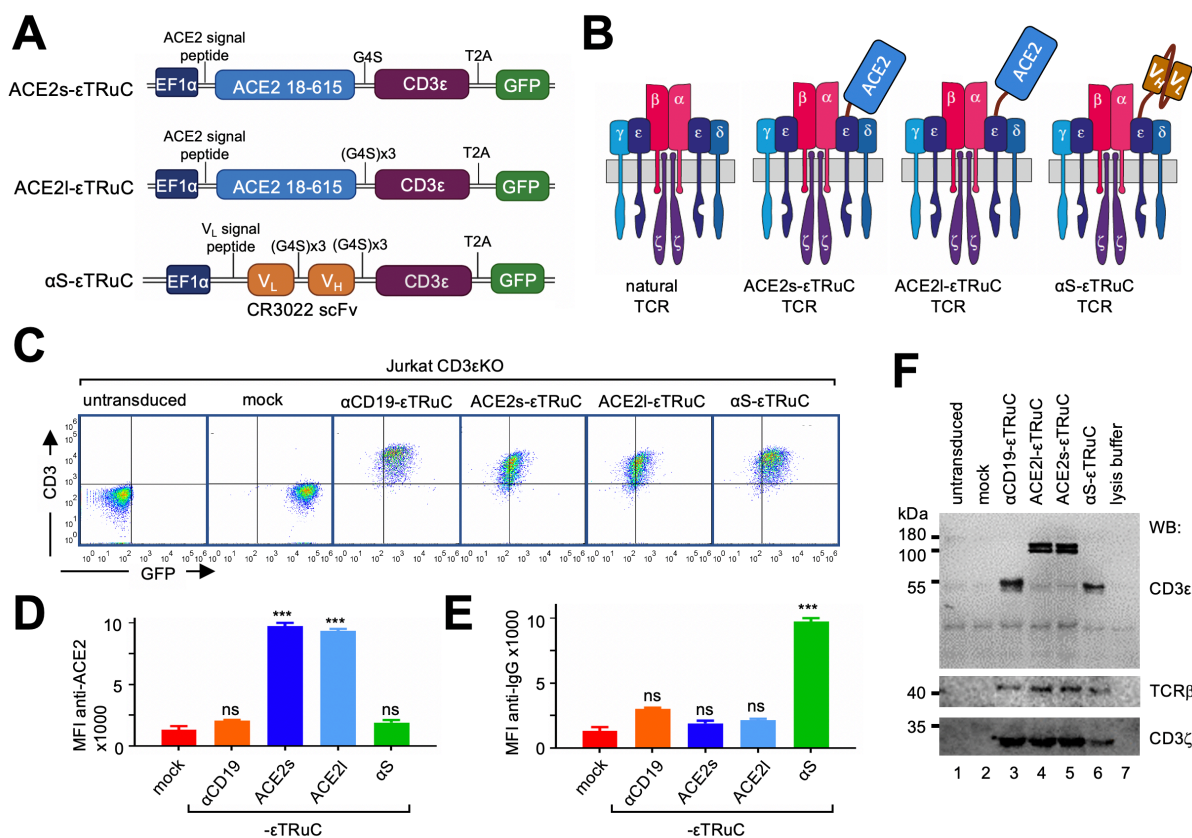


Fig. 1 The S-specific εTRuCs are expressed on the T cell surface as part of a TCR.

A, Schematics of the lentiviral vectors expressing the εTRuC transgenes. **B**, The natural TCR and the three εTRuC-containing TCRs. **C**, Jurkat CD3εKO cells were transduced with the different εTRuCs or the vector encoding for GFP only (mock) or were left untransduced. Cells were stained with an anti-CD3 antibody and measured by flow cytometry (n>3). **D** and **E**, The cells from **C** were stained with anti-ACE2 and anti-IgG antibodies. The mean fluorescence intensity (MFI) of triplicates is shown (the experiment was repeated more than 3 times, n>3). **F**, The cells from **C** were lysed and TCRs immunopurified using an anti-CD3 antibody. After reducing SDS-PAGE, the CD3ε, TCRβ and CD3ζ subunits were visualized by Western blotting (n=3).

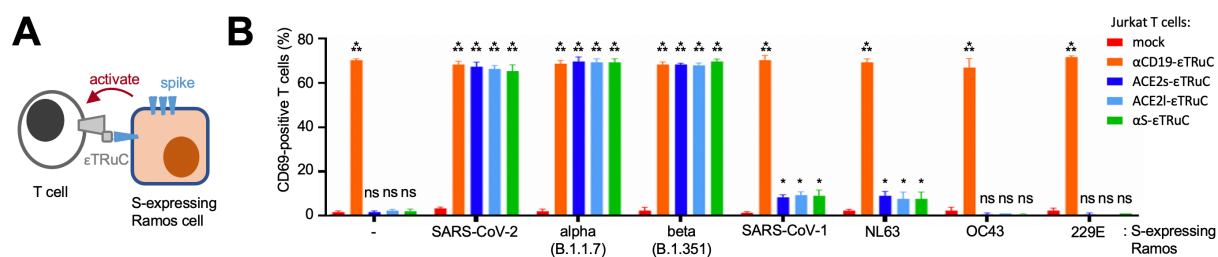


Fig. 2 The new ϵ TRuCs re-program Jurkat cells to recognize S-expressing cells.

A, By binding to the S-specific ϵ TRuCs, Ramos cells expressing S activate the ϵ TRuC Jurkat cells. **B**, Ramos cells without S and those expressing SARS-CoV-2 S were co-cultured with the Jurkat CD3 ϵ KO transductants from A and stained with anti-CD69 antibodies. After flow cytometric measurement, T cells were gated and the percentage of CD69-positive cells of triplicates is shown (the experiment was repeated more than 3 times, n>3).

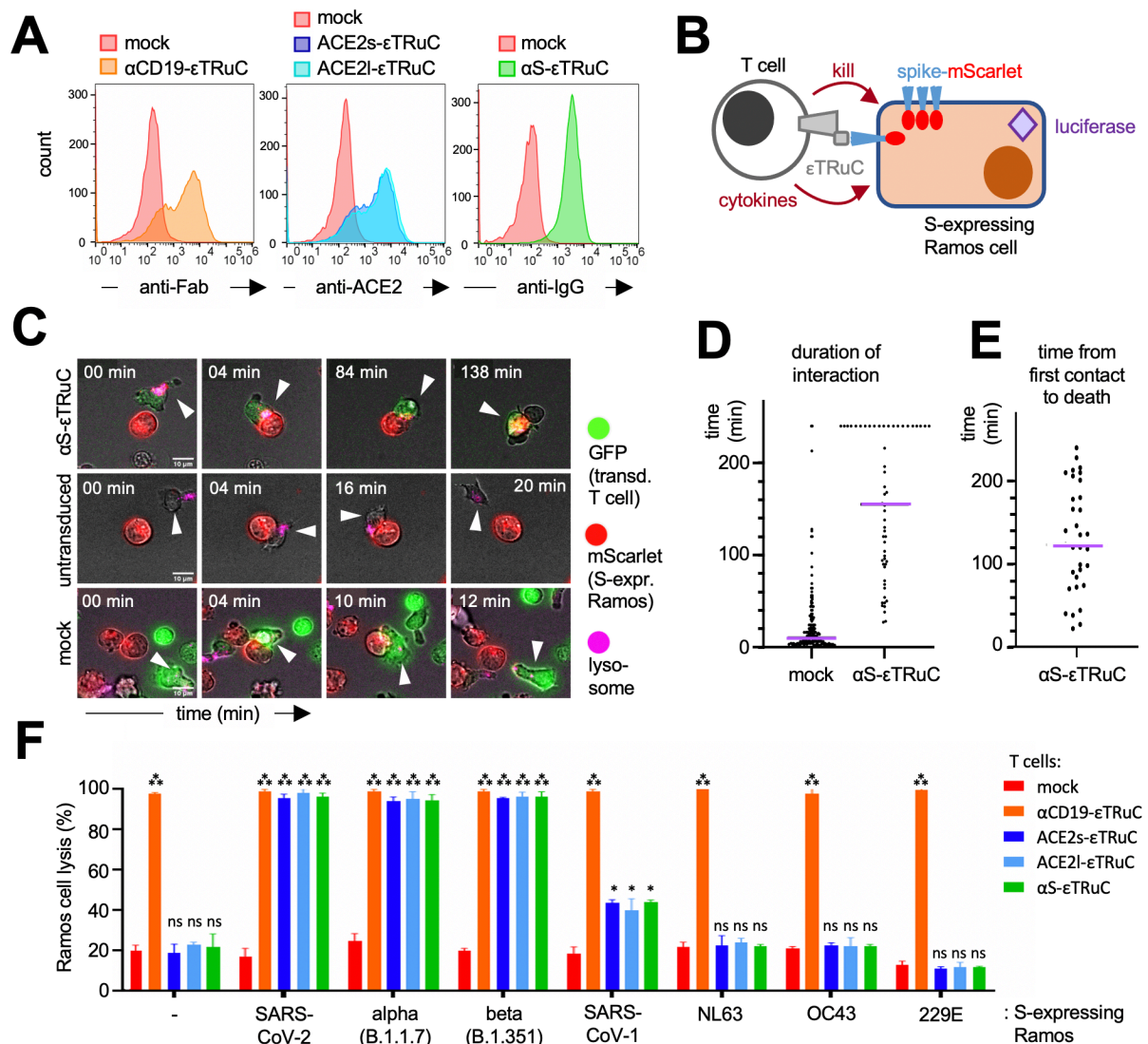


Fig. 3 S-specific ϵ TRuC T cells selectively eliminate S-expressing cells.

A, Primary human T cells were transduced with the lentiviral vectors encoding for the ϵ TRuCs or the mock vector and expanded with IL-2. Surface expression of the ϵ TRuCs was determined by anti-Fab, anti-ACE2 and anti-IgG staining as indicated. **B**, S- and luciferase-expressing Ramos cells are killed by the new ϵ TRuC T cells. **C**, An α S- ϵ TRuC T cell (green) with the lysosomes stained in pink and an S-mScarlet-expressing Ramos cell (red) were imaged and selected frames of the given times are shown (upper panel). Non-transduced (middle panel) and mock-transduced (green, lower panel) T cells were imaged together with the S-mScarlet-expressing Ramos cells (red). **D**, Quantification of the duration of interaction between S-mScarlet-expressing Ramos cells and α S- ϵ TRuC or mock T cells from the 4 h videos. **E**, Quantification of the time it takes for an α S- ϵ TRuC T cell to kill an S-mScarlet-expressing Ramos cell (the purple lines in D and E depict the median). **F**, Ramos cells expressing luciferase, BFP and the different S-proteins were co-cultured with the ϵ TRuC T cells for 24 h at a 1:1 ratio. Target cell lysis was measured by a loss of luciferase activity in triplicates (the experiment was repeated more than 3 times, $n > 3$).

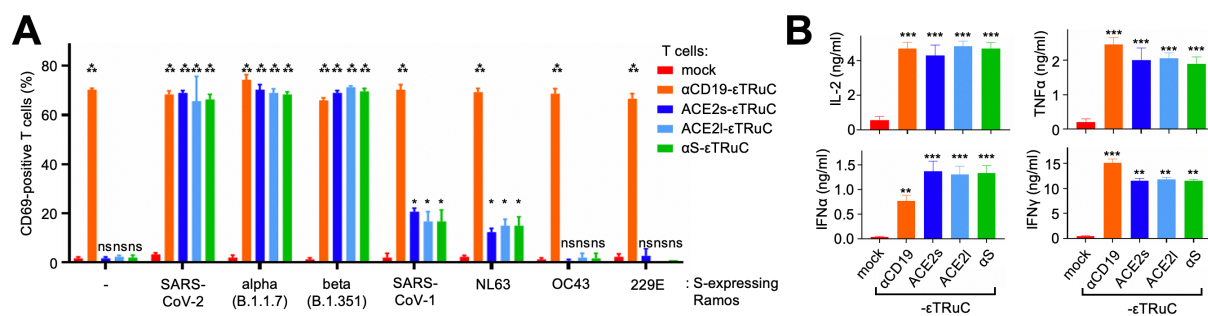


Fig. 4 S-specific εTRuC T cells are activated and secrete cytokines upon stimulation with S-expressing cells.

A, Percent of CD69-positive εTRuC T cells after co-culture with the different S-expressing Ramos cells was determined by flow cytometry in triplicates. **B**, Secretion of cytokines by the εTRuC T cells following co-culture with the Ramos cells expressing SARS-CoV-2 S was quantified by ELISA in triplicates. A and B were repeated more than 3 times (n>3).

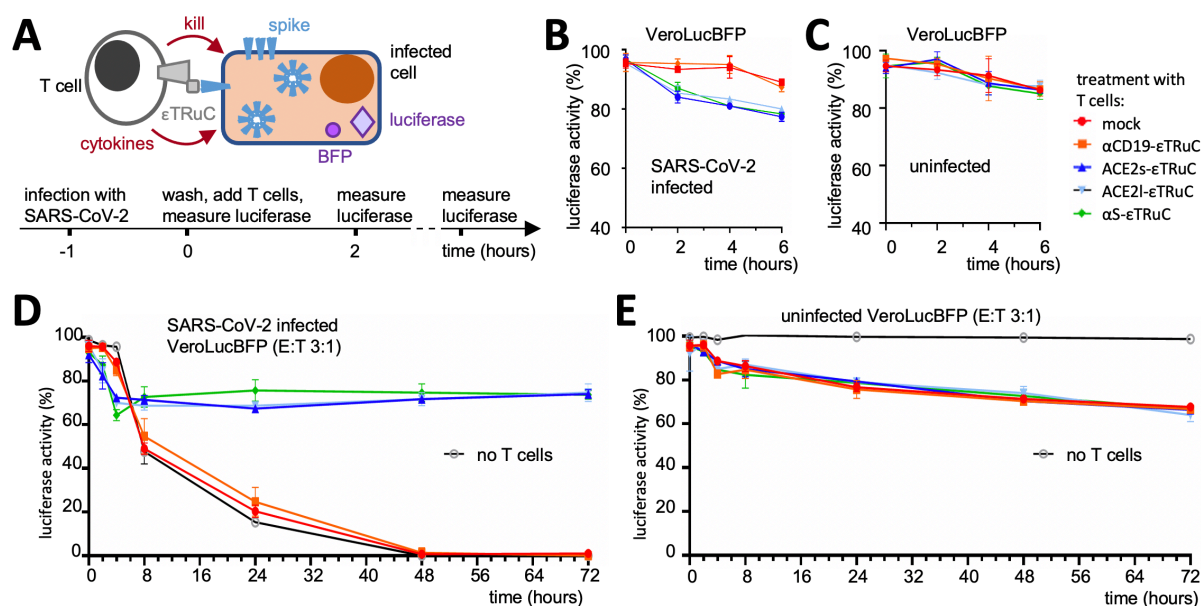


Fig. 5 SARS-CoV-2-infected cells are killed by S-specific εTRuC T cells.

A, The new εTRuC T cells recognise and kill VeroLucBFP cells infected with SARS-CoV-2 (upper panel); scheme of the infection and T cell treatment (lower panel). **B** and **D**, VeroLucBFP cells were infected with SARS-CoV-2 at an MOI of 0.05 for 1 h. After washing the εTRuC-transduced or mock T cells were added at a T : VeroLucBFP cell ratio of 3:1 (E:T). The luciferase activity was determined for 6 h (**B**) or 72 h (**D**). Samples without the addition of T cells were included in **D**. Triplicates are shown. **C** and **E**, The experiments were performed as in **B** and **D**, but the VeroLucBFP cells were not infected. **B** to **E** was done with three different T cell donors. **B** to **E** were repeated more than 3 times (n>3).

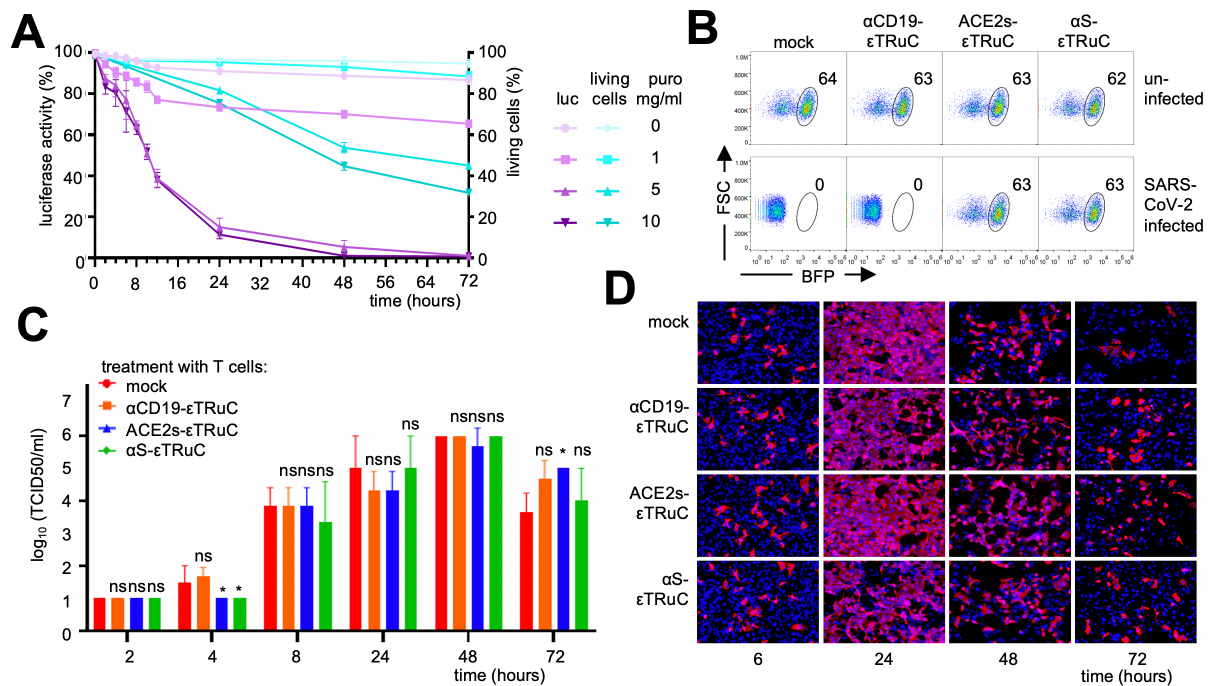


Fig. 6. εTRuC T cell treatment prevents the protein shutdown in VeroLucBFP cells, but does not limit viral replication

A, VeroLucBFP cells were treated with puromycin (0-10 μg/ml). The luciferase activity (purple lines) and the percent of living cells (turquoise lines) were determined. **B**, VeroLucBFP cells were infected with SARS-CoV-2 (MOI 0.05) for 1 h or left uninfected. After washing the transduced T cells were added at a ratio of T : VeroLucBFP cell of 3:1 and at 72 h post infection the BFP-expression of the VeroLucBFP cells was quantified by flow cytometry. The percent of BFP⁺ cells is given. **C** and **D**, In an experiment as **B**, the viral titers in the supernatant of the infected and treated VeroLucBFP cells (**C**) and the amount of infected cells as seen by staining for the viral N protein in pink (**D**) was determined at several time points post infection. In **D**, the nuclei of the cells were stained with DAPI (blue).

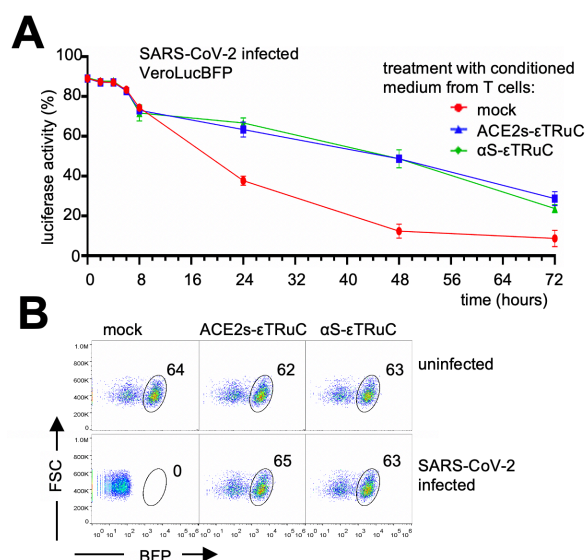


Fig. 7. Soluble factors of the ϵ TRuC T cells prevent the protein shutdown in VeroLucBFP cells

A, Conditioned medium of mock, ACE2s- and α S- ϵ TRuC T cells that were co-cultured with S-expressing Ramos cells was added to SARS-CoV-2-infected VeroLucBFP cells at 0 and again at 24 h post infection. Luciferase activity was measured in triplicates (the experiment was done twice, n=2). **B**, At 72 h post infection the BFP-expression of the VeroLucBFP cells treated with the conditioned medium was quantified by flow cytometry. The percent of BFP⁺ cells is given.

SUPPLEMENT

Engineered chimeric T cell receptor fusion construct (TRuC)-expressing T cells prevent translational shutdown in SARS-CoV-2-infected cells

Ira Godbole, Kevin Ciminski, O. Sascha Yousefi, Salma Pathan-Chhatbar, Deniz Saltukoglu, Niklas Vesper, Pavel Salavei, Juliane Strietz, Nicole Gensch, Michael Reth, Martin Schwemmler, Wolfgang W. Schamel

Content

Figures S1, S2, S3, S4, S5, S6 and S7

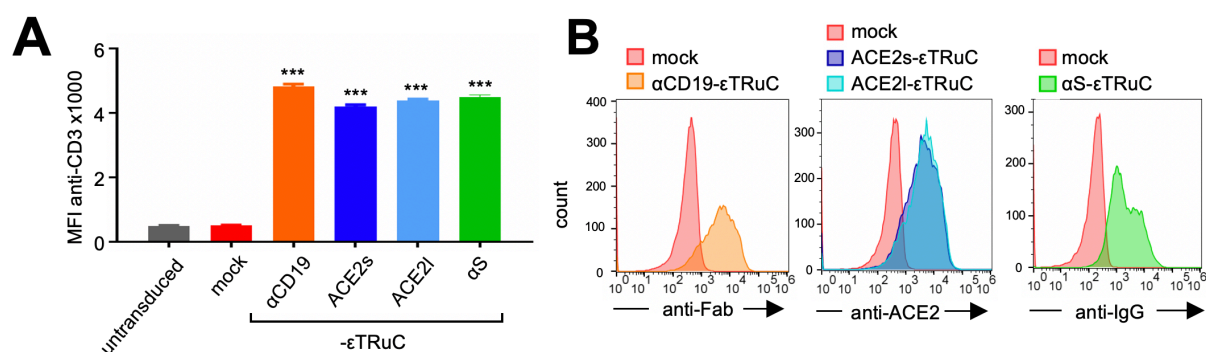


Fig. S1 The S-specific ϵ TRuCs are expressed on Jurkat T cells.

A, The Jurkat CD3 ϵ KO transductants as indicated were stained with an anti-CD3 antibody and measured by flow cytometry. The MFI of the CD3 stain is shown. This is a quantification of triplicates, as shown in figure 1C. **B**, The cells from A were stained with an anti-ACE2, anti-IgG, or anti-Fab antibody and measured by flow cytometry (the experiment was performed more than three times, $n > 3$). These are some of the original data that were used to generate figures 1D and 1E. These data show that the α CD19-, ACE2s-, ACE2l- and the α S- ϵ TRuC are expressed on the surface of Jurkat CD3 ϵ KO cells.

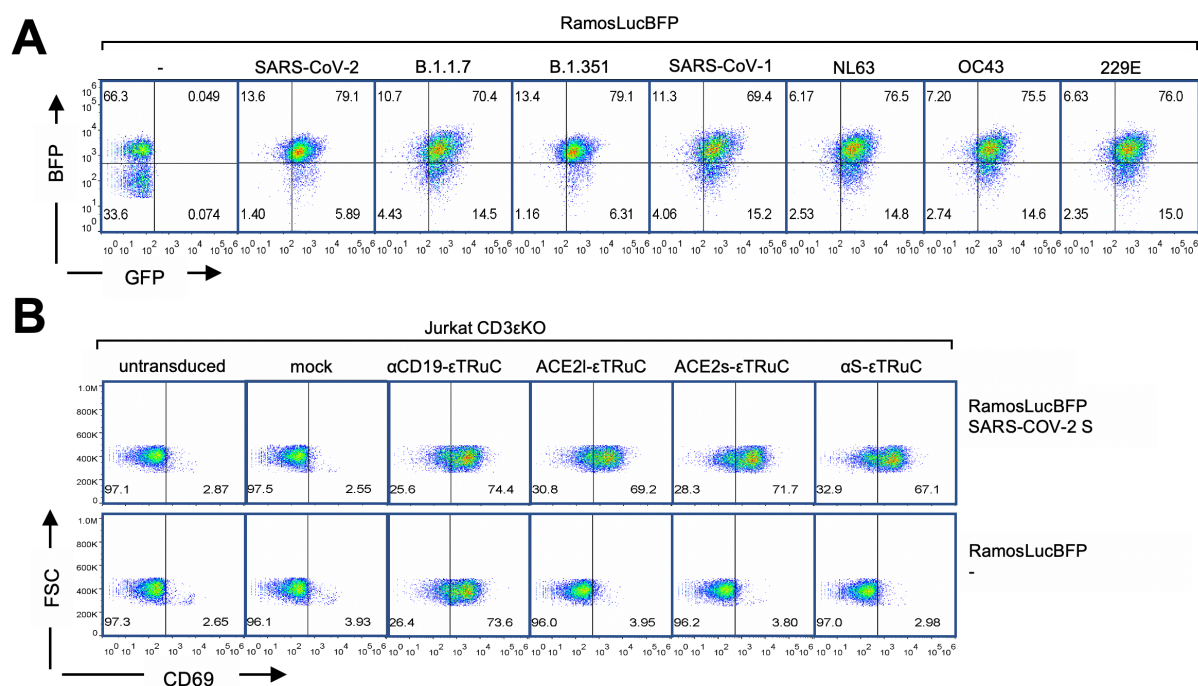


Fig. S2 The S-specific ϵ TRuC T cells bind to the S and are activated by S-expressing Ramos cells.

A, Ramos cells expressing the S of the corona viruses as indicated and GFP were lentivirally transduced to bicistronically express firefly luciferase and BFP. The flow cytometric plots show the GFP and BFP fluorescence intensity (the experiment was performed more than three times, $n > 3$). This data demonstrates that the Ramos cells express both the S and luciferase. **B**, Ramos cells without S and those expressing SARS-CoV-2 S were co-cultured for 9 h with the Jurkat CD3 ϵ KO cells expressing the ϵ TRuCs as indicated, being transduced with the GFP-expressing mock vector or left untransduced. Subsequently, cells were stained with anti-CD69 and anti-CD3 antibodies and analysed by flow cytometry. These original data were used to generate figure 2C.

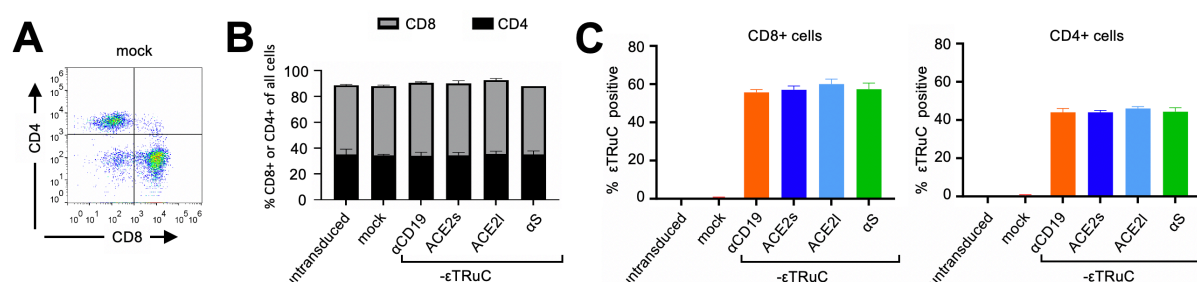


Fig. S3 The S-specific ϵ TRuCs are expressed on CD4⁺ and CD8⁺ T cells.

A, At day 5 after mock or ϵ TRuC transduction the primary, IL2-expanded T cells were stained with anti-CD4, anti-CD8 and the ϵ TRuC-specific antibodies as in figure 3A. As an example, the anti-CD4 and anti-CD8 stain is shown for the mock transduction. **B**, The ratio of CD8 to CD4 cells, of the untransduced or transduced primary T cells is shown from the data as in A. **C**, The percentage of CD8⁺TRuC⁺ (left panel) and CD4⁺TRuC⁺ (right panel) cells are shown from the data as in A ($n > 3$). These data show that the ϵ TRuC expression did not alter the ratio of CD8⁺ to CD4⁺ T cells.

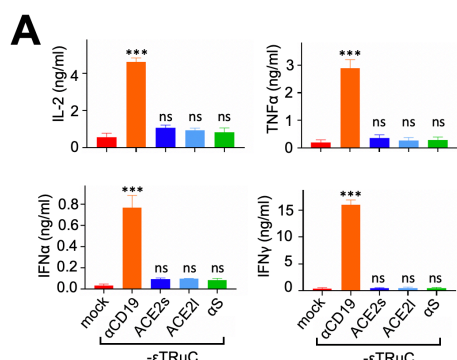


Fig. S4 The S-specific ϵ TRuC T cells do not secrete cytokines upon stimulation with Ramos cells that do not express any S.

A, Secretion of cytokines by the ϵ TRuC T cells following co-culture with the Ramos cells not expressing SARS-CoV-2 S was quantified by ELISA in triplicates (n=3). This was done in parallel to data shown in figure 4B and demonstrates that the engineered T cells are specifically activated by cells expressing S.

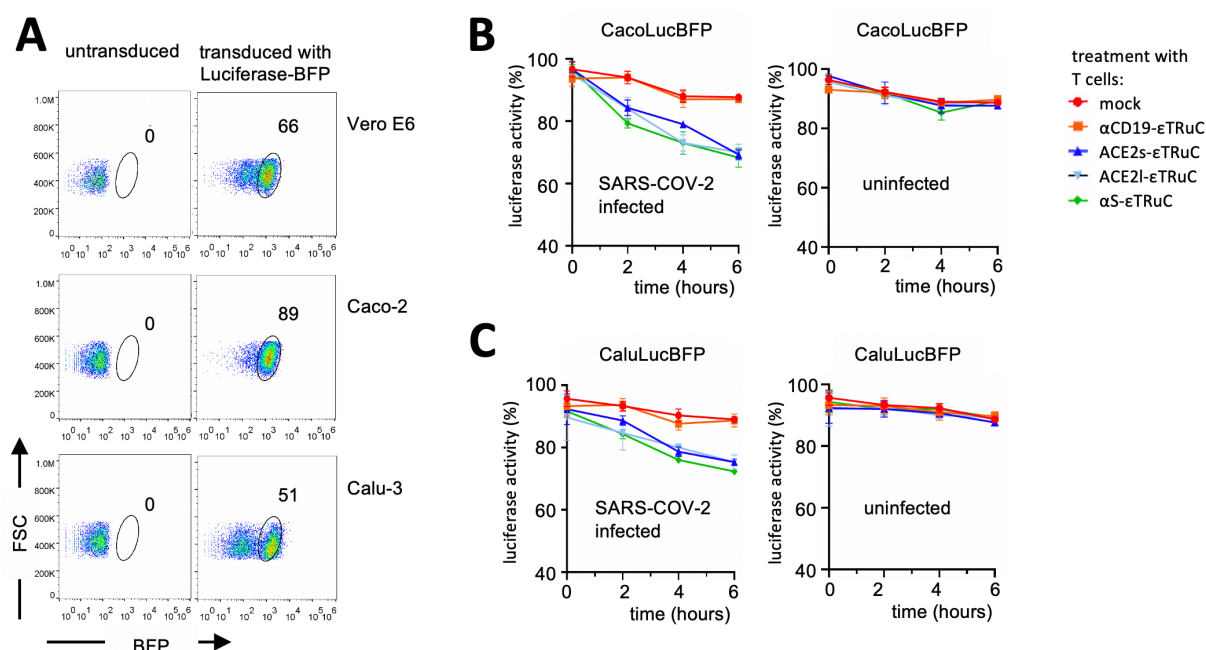


Fig. S5 Infected CacoLucBFP and CaluLucBFP cells are killed by S-specific ϵ TRuC T cells.

A, Vero E6, Caco-2 and Calu-3 cell lines were transduced to express luciferase-BFP or left untransduced and analysed by flow cytometry (n=7). In fact, 50-90% of the resulting VeroLucBFP, CacoLucBFP and CaluLucBFP cells express luciferase-BFP. These cells were used for all the SARS-CoV-2 infection experiments. **B**, The left panel shows the luciferase activity of CacoLucBFP cells when infected with SARS-CoV-2 (MOI 0.05) and treated with the primary ϵ TRuC T cells as indicated. The right panel shows the same treatment but using uninfected CacoLucBFP cells (n=2). **C**, Data shown are from an identical experiment as in (B), but with CaluLucBFP instead of the CacoLucBFP cells (n=2). This shows that SARS-CoV-2-infected Caco-2 and Calu-3 cells are killed by the primary S-specific ϵ TRuC T cells.

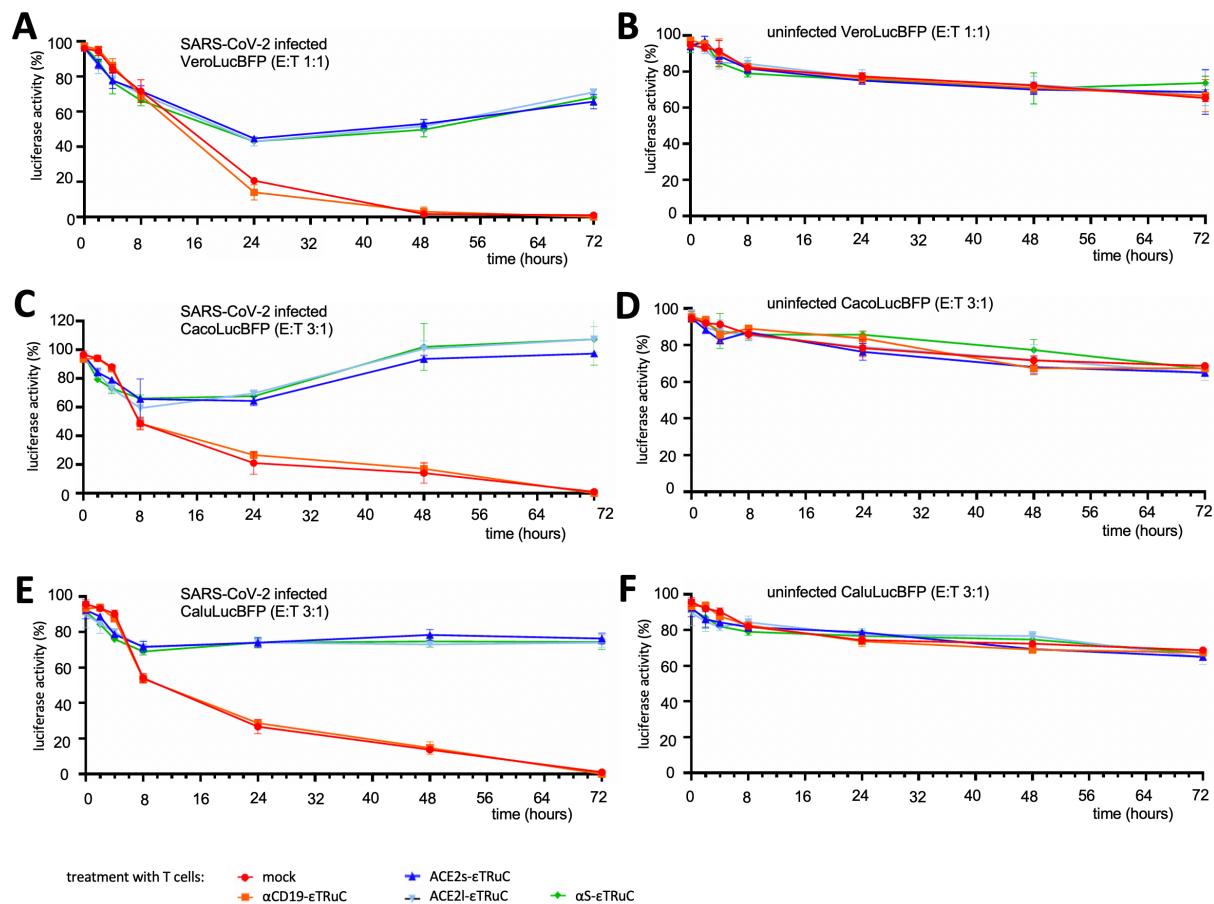


Fig. S6 Prevention of luciferase loss in infected Caco-2 or Calu-3 cells by the εTRuC T cell treatment

A, VeroLucBFP cells expressing luciferase were infected with MOI 0.05 of SARS-CoV-2 virus and treated with mock transduced, αCD19-εTRuC, ACE2s-εTRuC, ACE2l-εTRuC, and αS-εTRuC expressing primary T cells in the ratio of 1:1, i.e., one transduced T cell was added per VeroE6 cell. **B**, The negative control is the treatment of Vero E6 cells without infection but treatment with mock, αCD19-εTRuC, ACE2s-εTRuC, ACE2l-εTRuC, and αS-εTRuC primary T cells in 1:1 ratio. **C**, CacoLucBFP cells infected with SARS-CoV-2 virus (MOI 0.05) were treated with mock, αCD19-εTRuC, ACE2s-εTRuC, ACE2l-εTRuC, and αS-εTRuC primary cells in the ratio of 3:1, i.e., three transduced T cells were added per target cell. **D**, The negative control for is the treatment of CacoLucBFP cells without infection but treatment with mock, αCD19-εTRuC, ACE2s-εTRuC, ACE2l-εTRuC, and αS-εTRuC primary T cells in 3:1 ratio. **E**, CaluLucBFP cells infected with SARS-CoV-2 virus (MOI 0.05) were treated with mock, αCD19-εTRuC, ACE2s-εTRuC, ACE2l-εTRuC, and αS-εTRuC primary cells in the ratio of 3:1, i.e., three transduced T cells were added per target cell. **F**, The negative control for Fig S6E is the treatment of Calu-3 cells without infection but treatment with mock, αCD19-εTRuC, ACE2s-εTRuC, ACE2l-εTRuC, and αS-εTRuC primary cells in 3:1 ratio.

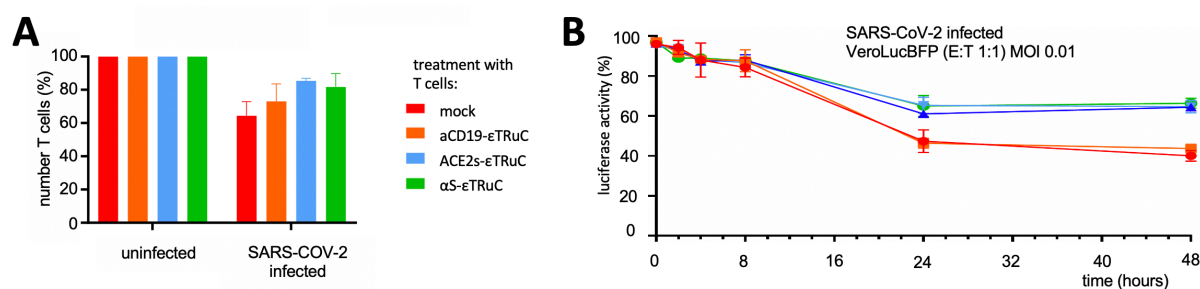


Fig. S7 SARS-CoV-2 infection reduces the number of VeroLucBFP cells at 72 h post infection independent of the T cell treatment

A, The number of infected VeroLucBFP cells measured by flow cytometry along with the data of figure 7B are shown as a relative value to the uninfected VeroLucBFP cells measured after 72 h of infection. The data show that the number of cells was unaffected by our ϵ TRuC T cell treatment. **B**, The luciferase activity of VeroLucBFP cells infected with SARS-CoV-2 (MOI 0.01) and treated with the ϵ TRuC primary T cells at ratio of T cell : VeroLucBFP cell of 1:1 (E:T) is shown. The data demonstrate that at low MOI the initial killing of infected cells cannot be detected, but the prevention of a partial translational shutdown took place.