Supplemental information

Biodistribution and environmental safety
of a live-attenuated YF17D-vectored
SARS-CoV-2 vaccine candidate

Li-Hsin Li, Laurens Liesenborghs, Lanjiao Wang, Marleen Lox, Michael Bright Yakass, Sander Jansen, Ana Lucia Rosales Rosas, Xin Zhang, Hendrik Jan Thibaut, Dirk Teuwen, Johan Neyts, Leen Delang, and Kai Dallmeier
Fig. S1 (related to Fig. 1 and Fig. 2). YFV-specific humoral immune responses in one-dose YF17D and YF-S0 vaccinated WT hamsters (primary pharmacodynamics). Serum samples for determination of YFV-specific neutralizing antibodies (nAb) collected at the respective endpoint of experiments assessing vaccine virus biodistribution (Fig.1A, 7 dpi) and shedding (Fig. 2A, 29 dpi). Sample number in biodistribution experiment: Sham n=4, YF17D n=6, and YF-S0 n=6, and in shedding experiment: Sham n=4, YF17D n=5, and YF-S0 n=5. 50% serum neutralizing titers (SNT\textsubscript{50}) were presented as median ± IQR for each group at logarithmic scale. Mann-Whitney test was used for the statistical analysis, with p >0.05 marked as non-significant (ns).
Fig. S2 (related to Fig. 2B). Infectious virus loads in serum (viremia) in selected YF17D or YF-S0 vaccinated hamsters. Serum samples collected at 3 days after vaccination. Sample number for YF17D vaccinated WT hamster, n=2; for YF-S0 vaccinated WT hamster, n=1; and (3) YF17D vaccinated STAT2 knockout (STAT2 KO) hamster, n=2. Selected samples included specimen with respectively highest viral RNA copies numbers detected (YF17D in WT and STAT2 KO hamsters) or, in case of YF-S0, the only PCR positive specimen.
Fig. S3 (related to Fig. 2). Cumulative detection rates of viral RNA by RT-qPCR in all specimens (serum, urine, faeces or buccal swab) collected from vaccinated WT or STAT2 knockout (STAT2 KO) hamster. Specimens were collected according to sampling scheme depicted in Fig. 2A. Heat map showing ratios of the total number of all PCR-positive samples versus the total number of all samples tested per study group over the course of 29 days after vaccination.
Supplementary Table 1. (Related to Fig. 1B, RT-qPCR)

| Sample | Genotype | vaccine | spleen | liver | brain | kidney | ileum | parotid gland | lung |
|--------|----------|---------|--------|-------|-------|--------|-------|---------------|------|
| 581    | WT       | sham    | n.d.   | n.d.  | n.d.  | n.d.   | n.d.  | n.d.          | n.d. |
| 582    | WT       | sham    | n.d.   | n.d.  | n.d.  | n.d.   | n.d.  | n.d.          | 1,85E+02 |
| 583    | WT       | sham    | n.d.   | n.d.  | n.d.  | n.d.   | n.d.  | n.d.          | 1,85E+02 |
| 584    | WT       | sham    | n.d.   | n.d.  | n.d.  | n.d.   | n.d.  | n.d.          | n.d. |
| 585    | WT       | YF 17D  | 2,75E+02 | n.d. | n.d.  | n.d.   | n.d.  | n.d.          | n.d. |
| 586    | WT       | YF 17D  | 3,26E+02 | n.d. | n.d.  | n.d.   | n.d.  | n.d.          | n.d. |
| 587    | WT       | YF 17D  | 1,93E+03 | n.d. | 6,24E+02 | n.d.   | n.d.  | 8,36E+02      | 1,25E+02 |
| 588    | WT       | YF 17D  | n.d.   | n.d.  | n.d.  | n.d.   | n.d.  | n.d.          | n.d. |
| 589    | WT       | YF 17D  | 1,66E+03 | n.d. | n.d.  | n.d.   | n.d.  | n.d.          | n.d. |
| 590    | WT       | YF 17D  | n.d.   | n.d.  | n.d.  | n.d.   | n.d.  | n.d.          | n.d. |
| 591    | WT       | YF-S0   | n.d.   | n.d.  | n.d.  | n.d.   | n.d.  | n.d.          | n.d. |
| 592    | WT       | YF-S0   | n.d.   | n.d.  | n.d.  | n.d.   | n.d.  | n.d.          | 1,14E+02 |
| 593    | WT       | YF-S0   | n.d.   | n.d.  | n.d.  | n.d.   | n.d.  | n.d.          | n.d. |
| 594    | WT       | YF-S0   | n.d.   | n.d.  | n.d.  | n.d.   | n.d.  | n.d.          | n.d. |
| 595    | WT       | YF-S0   | n.d.   | n.d.  | n.d.  | 1,86E+02 | n.d.  | n.d.          | n.d. |
| 596    | WT       | YF-S0   | n.d.   | n.d.  | n.d.  | 1,73E+02 | n.d.  | n.d.          | n.d. |
| 597    | STAT2/-  | YF 17D  | 1,85E+06 | 1,92E+05 | 9,94E+07 | 9,41E+06 | 3,12E+07 | 2,15E+05      | 6,31E+06 |
| 598    | STAT2/-  | YF 17D  | 5,23E+07 | 2,59E+06 | 1,49E+08 | 1,91E+07 | 4,80E+07 | n.d.          | 1,65E+07 |

1. Limit of detection (LoD) is around 44 copies per mg tissue, values below marked as non-detectable (n.d.)
2. Values below LoD represented as the square root of the LoD on the figure
Supplementary Table 2. (Related to Fig. 2B, Serum)

| Sample | Genotype | vaccine | Day 1   | Day 2   | Day 3   | Day 4   | Day 5   | Day 7   | Day 9   | Day 11  | Day 15  | Day 22  | Day 29  |
|--------|----------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| 165    | WT       | sham    | n.d.    | n.d.    | n.d.    | n.d.    | n.d.    | n.d.    | n.d.    | n.d.    | N/A     | n.d.    |         |
| 166    | WT       | sham    | n.d.    | n.d.    | n.d.    | n.d.    | n.d.    | n.d.    | n.d.    | n.d.    | N/A     | n.d.    |         |
| 167    | WT       | sham    | n.d.    | n.d.    | n.d.    | n.d.    | n.d.    | n.d.    | n.d.    | n.d.    | N/A     | n.d.    |         |
| 168    | WT       | sham    | n.d.    | n.d.    | n.d.    | n.d.    | n.d.    | n.d.    | N/A     | n.d.    | n.d.    |         |         |
| 169    | WT       | YF 17D  | 4.87E+04| n.d.    | n.d.    | n.d.    | n.d.    | n.d.    | n.d.    | N/A     | n.d.    |         |         |
| 170    | WT       | YF 17D  | n.d.    | 1.61E+05| n.d.    | n.d.    | n.d.    | n.d.    | N/A     | n.d.    |         |         |         |
| 171    | WT       | YF 17D  | 4.48E+04| 2.54E+04| 9.55E+04| n.d.    | n.d.    | n.d.    | N/A     | n.d.    |         |         |         |
| 172    | WT       | YF 17D  | n.d.    | 4.81E+04| 1.48E+05| 1.39E+04| N/A     | n.d.    | n.d.    | N/A     | n.d.    |         |         |
| 173    | WT       | YF 17D  | 2.96E+04| 4.34E+04| 1.70E+05| 1.43E+04| n.d.    | n.d.    | N/A     | n.d.    |         |         |         |
| 174    | WT       | YF 17D  | n.d.    | 4.73E+04| 9.20E+04| N/A     | n.d.    | n.d.    | N/A     | n.d.    |         |         |         |
| 831    | WT       | YF-S0   | n.d.    | n.d.    | 4.30E+04| n.d.    | n.d.    | n.d.    | n.d.    | N/A     | n.d.    |         |         |
| 832    | WT       | YF-S0   | n.d.    | n.d.    | n.d.    | n.d.    | n.d.    | n.d.    | n.d.    | N/A     | n.d.    |         |         |
| 833    | WT       | YF-S0   | n.d.    | n.d.    | n.d.    | n.d.    | n.d.    | n.d.    | n.d.    | N/A     | n.d.    |         |         |
| 834    | WT       | YF-S0   | n.d.    | n.d.    | n.d.    | n.d.    | n.d.    | n.d.    | n.d.    | N/A     | n.d.    |         |         |
| 835    | WT       | YF-S0   | n.d.    | n.d.    | n.d.    | n.d.    | n.d.    | n.d.    | n.d.    | N/A     | n.d.    |         |         |
| 836    | WT       | YF-S0   | n.d.    | n.d.    | n.d.    | n.d.    | n.d.    | n.d.    | n.d.    | N/A     | n.d.    |         |         |
| 837    | STAT2/-  | YF 17D  | 6.16E+04| 1.53E+06| 2.43E+07| 1.40E+07| 3.06E+06| 6.69E+06|         |         |         |         |         |
| 838    | STAT2/-  | YF 17D  | N/A     | 1.61E+06| 3.41E+07| 1.24E+07| 1.72E+06| 1.24E+07|         |         |         |         |         |
| 839    | STAT2/-  | YF 17D  | n.d.    | 1.60E+06| 1.38E+07| 9.89E+06| 2.01E+06| 7.92E+06|         |         |         |         |         |

1. Limit of detection (LoD) is around 8900 copies per ml serum, values below marked as non-detectable (n.d.)
2. Values below LoD represented as the square root of the LoD on the figure
| Sample | Genotype | vaccine | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 7 | Day 9 | Day 11 | Day 15 | Day 22 | Day 29 |
|---------|----------|---------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 165     | WT       | sham    | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | N/A   | n.d.  | N/A   | N/A   | N/A   |
| 166     | WT       | sham    | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | N/A   | n.d.  | N/A   | N/A   | N/A   |
| 167     | WT       | sham    | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | N/A   | n.d.  | N/A   | N/A   | N/A   |
| 168     | WT       | sham    | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | N/A   | n.d.  | n.d.  | N/A   | N/A   | N/A   |
| 169     | WT       | YF 17D  | n.d.  | n.d.  | N/A   | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | N/A   | n.d.  | n.d.  |
| 170     | WT       | YF 17D  | n.d.  | n.d.  | N/A   | n.d.  | 1,13E+04 | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  |
| 171     | WT       | YF 17D  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | N/A   | N/A   |
| 172     | WT       | YF 17D  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | N/A   | N/A   |
| 173     | WT       | YF 17D  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | N/A   | N/A   | n.d.  |
| 174     | WT       | YF 17D  | n.d.  | n.d.  | n.d.  | n.d.  | N/A   | n.d.  | n.d.  | n.d.  | N/A   | N/A   | n.d.  |
| 831     | WT       | YF-S0   | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  |
| 832     | WT       | YF-S0   | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | 2,56E+04 | n.d.  | n.d.  | N/A   |
| 833     | WT       | YF-S0   | n.d.  | N/A   | N/A   | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | N/A   | N/A   |
| 834     | WT       | YF-S0   | n.d.  | N/A   | N/A   | n.d.  | n.d.  | n.d.  | 1,35E+04 | n.d.  | n.d.  | n.d.  | n.d.  |
| 835     | WT       | YF-S0   | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | 1,08E+06 | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  |
| 836     | WT       | YF-S0   | n.d.  | n.d.  | n.d.  | N/A   | n.d.  | n.d.  | n.d.  | N/A   | N/A   | n.d.  | n.d.  |
| 837     | STAT2/-  | YF 17D  | n.d.  | n.d.  | n.d.  | 1,30E+08 | N/A   | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  |
| 838     | STAT2/-  | YF 17D  | n.d.  | 3,11E+05 | 1,60E+05 | 1,21E+08 | N/A   | 1,81E+06 | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  |
| 839     | STAT2/-  | YF 17D  | N/A   | n.d.  | n.d.  | 7,62E+05 | N/A   | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  |

1. Limit of detection (LoD) is around 8900 copies per ml urine, values below marked as non-detectable (n.d.)
2. Values below LoD represented as the square root of the LoD on the figure
### Supplementary Table 4. (Related to Fig. 2D, Faeces)

| Sample | Genotype | vaccine | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 7 | Day 9 | Day 11 | Day 15 | Day 22 | Day 29 |
|--------|----------|---------|-------|-------|-------|-------|-------|-------|-------|--------|--------|--------|--------|
| 165    | WT       | sham    | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.   | n.d.   | n.d.   | n.d.   |
| 166    | WT       | sham    | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.   | n.d.   | n.d.   | n.d.   |
| 167    | WT       | sham    | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.   | n.d.   | n.d.   | n.d.   |
| 168    | WT       | sham    | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.   | n.d.   | n.d.   | n.d.   |
| 169    | WT       | YF 17D  | n.d.  | n.d.  | n.d.  | 1,04E+05 | n.d.  | n.d.  | n.d.  | n.d.   | n.d.   | n.d.   | n.d.   |
| 170    | WT       | YF 17D  | n.d.  | 5,00E+04 | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.   | n.d.   | n.d.   | n.d.   |
| 171    | WT       | YF 17D  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.   | n.d.   | n.d.   | n.d.   |
| 172    | WT       | YF 17D  | n.d.  | N/A   | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.   | n.d.   | n.d.   | n.d.   |
| 173    | WT       | YF 17D  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.   | n.d.   | n.d.   | n.d.   |
| 174    | WT       | YF 17D  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.   | n.d.   | n.d.   | n.d.   |
| 831    | WT       | YF-S0   | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.   | n.d.   | n.d.   | n.d.   |
| 832    | WT       | YF-S0   | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.   | n.d.   | n.d.   | n.d.   |
| 833    | WT       | YF-S0   | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.   | n.d.   | n.d.   | n.d.   |
| 834    | WT       | YF-S0   | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.   | n.d.   | n.d.   | n.d.   |
| 835    | WT       | YF-S0   | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.   | n.d.   | n.d.   | n.d.   |
| 836    | WT       | YF-S0   | n.d.  | n.d.  | n.d.  | 6,35E+04 | n.d.  | n.d.  | n.d.  | n.d.   | n.d.   | n.d.   | n.d.   |
| 837    | STAT2-/  | YF 17D  | n.d.  | n.d.  | 2,17E+04 | n.d.  | 5,93E+07 | 1,25E+07 | n.d. | n.d.   | n.d.   | n.d.   | n.d.   |
| 838    | STAT2-/  | YF 17D  | n.d.  | n.d.  | n.d.  | 5,96E+06 | n.d.  | 1,52E+05 | n.d. | n.d.   | n.d.   | n.d.   | n.d.   |
| 839    | STAT2-/  | YF 17D  | n.d.  | n.d.  | n.d.  | 7,28E+06 | n.d.  | 2,40E+05 | n.d. | n.d.   | n.d.   | n.d.   | n.d.   |

1. Limit of detection (LoD) is around 1500 copies per ml faeces, values below marked as non-detectable (n.d.)
2. Values below LoD represented as the square root of the LoD on the figure
Supplementary Table 5. (Related to Fig. 2E, Buccal swab)

| Sample | Genotype | vaccine | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 7 | Day 9 | Day 11 | Day 15 | Day 22 | Day 29 |
|--------|----------|---------|-------|-------|-------|-------|-------|-------|-------|--------|--------|--------|-------|
| 165    | WT       | sham    | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.   | n.d.   | n.d.   | n.d.   |
| 166    | WT       | sham    | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.   | n.d.   | n.d.   | n.d.   |
| 167    | WT       | sham    | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.   | n.d.   | n.d.   | n.d.   |
| 168    | WT       | sham    | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.   | n.d.   | n.d.   | n.d.   |
| 169    | WT       | YF 17D  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.   | n.d.   | n.d.   | n.d.   |
| 170    | WT       | YF 17D  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.   | n.d.   | n.d.   | n.d.   |
| 171    | WT       | YF 17D  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.   | n.d.   | n.d.   | n.d.   |
| 172    | WT       | YF 17D  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.   | n.d.   | n.d.   | n.d.   |
| 173    | WT       | YF 17D  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.   | n.d.   | n.d.   | n.d.   |
| 174    | WT       | YF 17D  | 3,98E+03 | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.   | n.d.   | n.d.   | n.d.   |
| 831    | WT       | YF-S0   | n.d.  | n.d.  | n.d.  | 5,08E+03 | n.d.  | n.d.  | n.d.  | n.d.   | n.d.   | n.d.   | n.d.   |
| 832    | WT       | YF-S0   | n.d.  | n.d.  | n.d.  | n.d.  | 1,66E+03 | n.d.  | n.d.  | n.d.   | n.d.   | n.d.   | n.d.   |
| 833    | WT       | YF-S0   | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.   | n.d.   | n.d.   | n.d.   |
| 834    | WT       | YF-S0   | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.   | n.d.   | n.d.   | n.d.   |
| 835    | WT       | YF-S0   | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.   | n.d.   | n.d.   | n.d.   |
| 836    | WT       | YF-S0   | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.  | n.d.   | n.d.   | n.d.   | n.d.   |
| 837    | STAT2-/- | YF 17D  | n.d.  | n.d.  | 1,28E+05 | 8,60E+05 | 2,88E+06 | 3,33E+07 |      |      |      |      |      |
| 838    | STAT2-/- | YF 17D  | n.d.  | n.d.  | 2,07E+05 | 7,92E+05 | n.d.  | 2,21E+06 |      |      |      |      |      |
| 839    | STAT2-/- | YF 17D  | n.d.  | n.d.  | 4,29E+04 | 8,95E+05 | 3,16E+07 | 1,34E+07 |      |      |      |      |      |

1. Limit of detection (LoD) is around 1500 copies per ml buccal swab, values below marked as non-detectable (n.d.)
2. Values below LoD represented as the square root of the LoD on the figure.