



Figure S1. Images of apparently healthy free-ranging pigs (kindly provided by Dr. Sergio Masala, AHS).

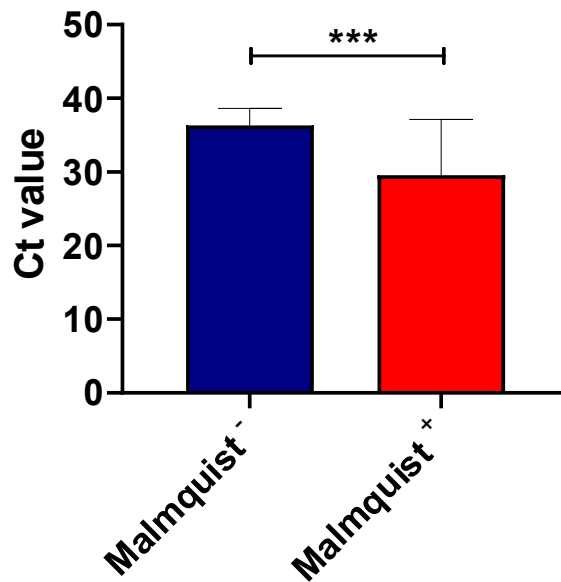


Figure S2. Threshold cycle values of real-time PCR+ free-ranging pig samples. Threshold cycle (Ct) values of Malmquist⁺ and Malmquist⁻ samples were compared using a student T test; *** p < 0.001. Mean values and SD are shown.

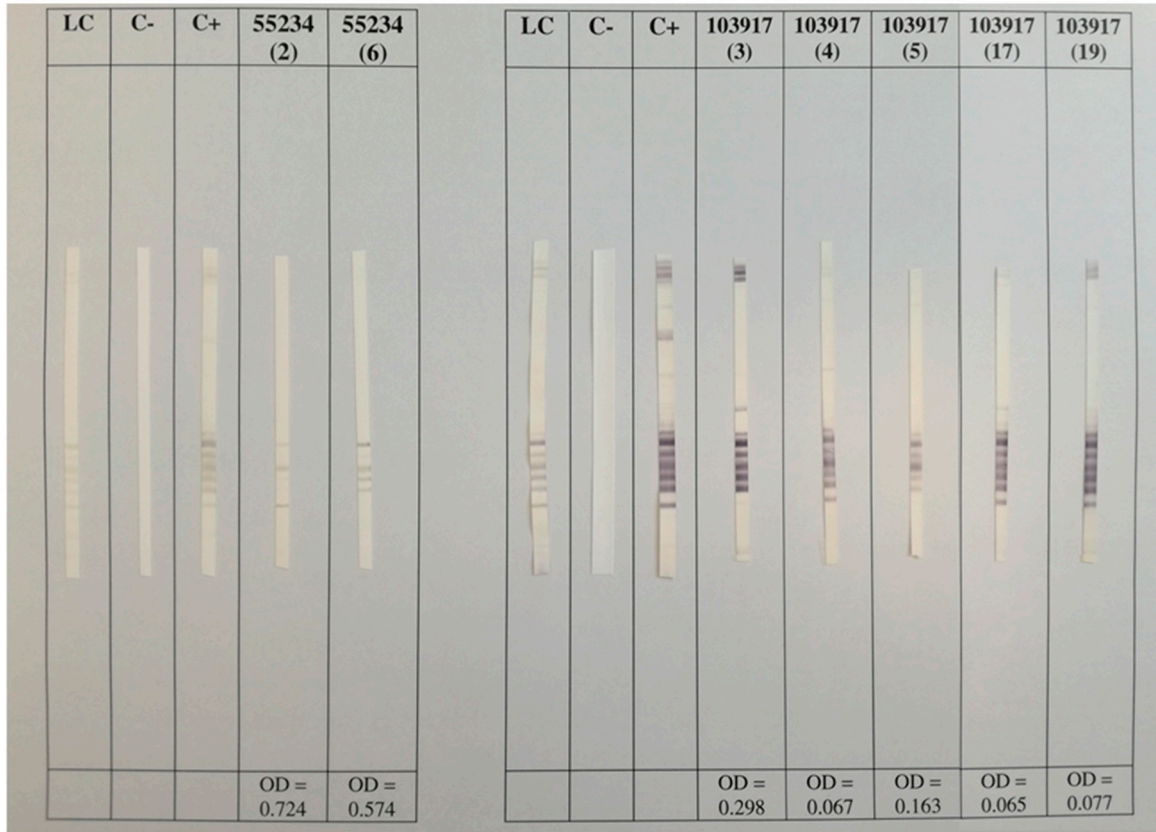


Figure S3. ASFV-antibodies of Malmquist+ free-ranging pigs. Immunoblotting strips incubated with the sera of the Malmquist+ free-ranging pigs culled in Desulo (55234) or Talana (103917), alongside positive (C+), negative (C-) and low positive (LC) controls.

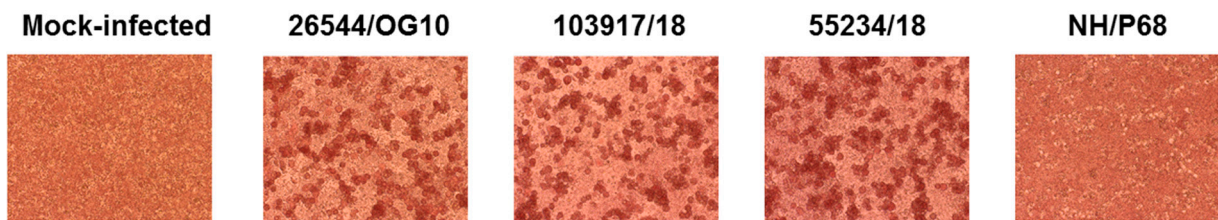


Figure S4. Haemoadsorbing effect of 103917/18 and 55234/18. Two days old monocytes were infected with the virulent HAD 26544/OG10, the two tested strains (103917/18 and 55234/18), and the non-HAD NH/P68, alongside mock-infected controls. Autologous erythrocytes were added to culture media; 24 hpi haemoadsorption effect was visible for 26544/OG10, 103917/18 and 55234/18.

| | | | | |
|------------------------------|-------|---------------------------------|-------------------------------|----------|
| 140/Or/85 | 1985 | Sardinia (Italy) | Domestic pig | MN270972 |
| 139/Nu/81 | 1981 | Sardinia (Italy) | Domestic pig | MN270971 |
| 57/Ca/79 | 1979 | Sardinia (Italy) | Domestic pig | MN270970 |
| 56/Ca/78 | 1978 | Sardinia (Italy) | Domestic pig | MN270969 |
| BA71 | 1971 | Spain | Domestic pig | NC044942 |
| BA71V | | Spain | Tissue culture adapted (Vero) | U18466 |
| Benin 97/1 | 1997 | Republic of Benin (West Africa) | Domestic pig | NC044956 |
| E75 | 1975 | Spain | Domestic pig | NC044958 |
| L60 | 1960 | Portugal | Domestic pig | NC044941 |
| NH/P68 | 1968 | Portugal | Domestic pig | NC044943 |
| OUTR 88/3 | 1988 | Portugal | Tick | NC044957 |
| Belgium/Etalle/wb/2018 | 2018 | Belgium | Wild Boar | MK543947 |
| Pig/HLJ/2018 | 2018 | China | Domestic pig | MK333180 |
| ASFV-wbBS01 | 2019? | China | Wild Boar | MK645909 |
| ASFV/pig/China/CAS19-01/2019 | 2019 | China | Domestic pig | MN172368 |
| Moldova 2017/1 | 2017 | Moldova | Wild Boar | LR722599 |
| ASFV/POL/2015/Podlaskie | 2015 | Poland | Wild Boar | MH681419 |
| ASFV CzechRepublic 2017/1 | 2017 | Czech Republic | Wild Boar | LR722600 |
| Estonia 2014 | 2014 | Estonia | Wild Boar | LS478113 |
| ASFV/LT14/1490 | 2014 | Lithuania | Wild Boar | MK628478 |
| ASFV Wuhan 2019-1 | 2019 | China | Domestic pig | MN393476 |
| Georgia 2007/1 | 2007 | Georgia | Domestic pig | NC044959 |
| Ken06.Bus | 2006 | Kenya | Domestic pig | NC044946 |
| Ken05/Tk1 | 2005 | Kenya | Tick | NC044945 |
| Zaire | 1977 | Zaire | Domestic pig | MN630494 |
| RSA_2_2008 | 2008 | South Africa | Tick | MN336500 |
| LIV_5_40 | 1983 | Zambia | Tick | MN318203 |
| CN/2019/InnerMongolia-AES01 | 2019 | Mongolia | Wild Boar | MK940252 |
| Georgia 2008/2 | 2008 | Georgia | Domestic pig | MH910496 |
| Warthog | 1980 | Namibia | Warthog | AY261366 |
| Warmbaths | 1987 | Republic of South Africa | Tick | AY261365 |
| Tengani 62 | 1962 | Malawi | Domestic pig | AY261364 |
| Pretorisuskop/96/4 | 1996 | Republic of South Africa | Tick | AY261363 |
| Mkuzi 1979 | 1979 | Zululand | Tick | AY261362 |
| Malawi Lil-20-1 (1983) | 1983 | Malawi | Tick | AY261361 |
| Kenya 1950 | 1950 | Kenya | Domestic pig | AY261360 |

Table S2. Information on ASF virus+ free-ranging pigs, including municipality and month of the culling action, virological (tested organs, Ct values of real-time PCR, Malmquist) and serological (ELISA and immunoblotting) results.

| Culling action (location and month) | Identification number | Organ | Ct value* | Malmquist | ELISA | IB [†] |
|-------------------------------------|-----------------------|-------|-----------|-----------|-------|-----------------|
|-------------------------------------|-----------------------|-------|-----------|-----------|-------|-----------------|

| | | | | | | |
|--|-------------|-----------------|-------|-----|-----|-----|
| Desulo December 2017 | 96355 (12) | spleen | 39.1 | neg | pos | pos |
| | 96355 (13) | spleen | 37.68 | neg | pos | pos |
| | 96355 (40) | spleen | 37.75 | neg | pos | pos |
| | 96355 (49) | spleen | 35.05 | neg | pos | pos |
| Arzana December 2017 | 96777 (1) | spleen | 38.54 | neg | pos | pos |
| | 96777 (8) | spleen | 35.85 | neg | neg | / |
| | 96777 (16) | spleen | 33.39 | neg | neg | / |
| | 96777 (18) | spleen | 33.37 | neg | pos | pos |
| Orgosolo (Montes) December 2017 | 96804 (13) | spleen | 38.82 | neg | pos | pos |
| Villagrande Strisaili December 2017 | 101337 (1) | spleen | 37.53 | neg | pos | pos |
| | 101337 (16) | spleen | 38.59 | neg | pos | pos |
| | 101337 (17) | spleen | 37.63 | neg | pos | pos |
| | 101337 (27) | spleen | 38.04 | neg | neg | / |
| Orgosolo (Pradu) December 2017 | 103306 (39) | spleen | 37.7 | neg | neg | / |
| Orgosolo (Montes) January 2018 | 2428 (48) | spleen | 37.8 | neg | neg | / |
| | 2428 (81) | spleen | 37.63 | neg | pos | pos |
| | 2428 (84) | spleen | 37.4 | neg | pos | pos |
| | 2428 (102) | spleen | 35.49 | neg | pos | pos |
| Orgosolo (Montes) January 2018 | 5703 (103) | spleen | 38.9 | neg | pos | pos |
| | 5703 (105) | spleen | 38.3 | neg | neg | / |
| Orgosolo (Biriddi - Iserrai) February 2018 | 18285 (19) | spleen lymph | 38.11 | neg | pos | pos |
| | 19185 (1) | node | 33.01 | neg | pos | pos |
| | 19185 (2) | lymph node | 37.4 | neg | pos | pos |
| Desulo June 2018 | 55232 (2) | spleen | 37.33 | neg | pos | pos |
| | 55232 (6) | spleen | 32.68 | neg | pos | pos |
| | 55232 (9) | spleen | 34.86 | neg | pos | pos |
| | 55232 (10) | spleen | 37.85 | neg | pos | pos |
| Desulo June 2018 | 55234 (1) | spleen | 20.44 | pos | neg | / |
| | 55234 (2) | spleen | 22.99 | pos | pos | pos |
| | 55234 (3) | spleen | 32.01 | neg | pos | pos |
| | 55234 (4) | spleen | 34.86 | neg | pos | pos |
| | 55234 (5) | spleen | 19.1 | pos | neg | / |
| | 55234 (6) | spleen | 23.85 | pos | pos | pos |
| | 55234 (7) | spleen | 31.64 | neg | neg | / |
| | 55234 (8) | spleen | 31.78 | neg | pos | pos |
| | 55234 (9) | spleen | 31.7 | neg | neg | / |
| | 55234 (10) | spleen | 37.37 | neg | pos | pos |
| | 55234 (11) | spleen | 35.58 | neg | pos | neg |
| Talana November 2018 | 90280 (10) | spleen | 38.1 | neg | pos | pos |
| | 90280 (18) | spleen | 34.79 | neg | pos | pos |
| | 90350 (40) | spleen | 36.72 | neg | pos | pos |
| Villagrande (Luli) November 2018 | 90307 (4) | spleen | 35.75 | neg | pos | pos |
| Villagrande (Carcaredda) November 2018 | 90335 (10) | spleen | 37.93 | neg | pos | pos |
| Talana December 2018 | 103917 (3) | spleen | 27.14 | pos | pos | pos |
| | 103917 (4) | spleen | 34.84 | pos | pos | pos |
| | 103917 (5) | spleen | 36.67 | pos | pos | pos |

| | | | | | | |
|------------------------|-------------|--------|-------|-----|-----|-----|
| | 103917 (8) | lung | 33.14 | neg | pos | pos |
| | 103917 (14) | lung | 37.98 | neg | pos | pos |
| | 103917 (16) | lung | 35.8 | pos | neg | / |
| | 103917 (17) | spleen | 36.95 | pos | pos | pos |
| | 103917 (19) | spleen | 37.98 | pos | pos | pos |
| | 103917 (20) | spleen | 39.3 | neg | pos | pos |
| Desulo January 2019 | 13750 (1) | spleen | 37.79 | neg | pos | pos |

*Ct: threshold cycle; *IB: immunoblotting; pos: positive; neg: negative; '/': not performed (IB was performed only on ELISA positive or inconclusive samples).