# Supplementary Materials

**Table S1.** Individual RABV-specific VNA titers (IU/mL) obtained at different time points post vaccination as measured by RFFIT. VNA > 0.5 IU/mL were considered as positive (threshold of positivity).

| | dpv: | 0 | 14 | 28 | 58 | 118 | 178 | 268 | 365 | 377 | 379 | 393 | 455 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Animal ID** | | | | | | | | | | | | |
| **Orally vaccinated (Group 1)** | **30** | 0.04 | 19.55 | 9.36 | 12.99 | 10.69 | 9.81 | 10.27 | 35.65 | - | 40.11 | 43.4 | 15.23 |
| | **31** | 0.06 | 8.28 | 5.83 | 44.2 | 24.59 | 43.83 | 24.8 | 36.36 | - | 63.69 | 50.66 | 18.41 |
| | **33** | 0.02 | 8.97 | 8.48 | 30.84 | 24.07 | 18.57 | 21.5 | 24.4 | - | 64.96 | 50.66 | 43.78 |
| | **34** | 0.04 | 0.02 | 0.06 | 0.12 | 0.06 | 0.08 | 0.07 | 0.03 | 49.8 | - | - | - |
| | **35** | 0.03 | 1.71 | 1.27 | 2.13 | 1.72 | 2.47 | 2.69 | 2.41 | - | 64.96 | 32.25 | 13.72 |
| | **38** | 0.13 | 1.97 | 0.81 | 3.41 | 1.22 | 3.45 | 2.03 | 2.73 | - | 53.69 | 14.39 | 3.28 |
| | **41** | 0.02 | 0.01 | 0.01 | 0.05 | 0.04 | 0.03 | 0.06 | 0.07 | 3.34 | - | - | - |
| | **42** | 0.06 | 26.56 | 8.4 | 9.47 | 15.83 | 6.56 | 8.51 | 12.85 | - | 47.72 | 24.13 | 7.12 |
| | **43** | 0.03 | 0.76 | 1.37 | 3.33 | 5.02 | 7.4 | 3.19 | 2.2 | - | 73.14 | 34.43 | 6.55 |
| | **44** | 0.25 | 0.84 | 0.22 | 0.32 | 0.42 | 0.35 | 0.35 | 0.27 | - | 8.76 | 4.05 | 0.43 |
| | **45** | 0.06 | 8.28 | 5.83 | 44.2 | 24.59 | 43.83 | 24.8 | 36.36 | - | 63.69 | 50.66 | 18.41 |
| | **GMT** | 0.05 | 1.56 | 1.14 | 3.19 | 2.52 | 2.90 | 2.55 | 2.90 | 12.90 | 47.04 | 27.44 | 8.38 |
| **Parenterally vaccinated (Group 2)** | **47** | 0.38 | 36.69 | 19.12 | 47.55 | 70.77 | 20.03 | 61.53 | 53.97 | - | 386.9 | 68.44 | 59.74 |
| | **48** | 0.13 | 20.82 | 11.6 | 38.29 | 29.62 | 29.6 | 24.79 | 21.86 | - | 23.31 | 54.76 | 13.55 |
| | **49** | 0.01 | 34.9 | 31.8 | 29.33 | 37.72 | 46.18 | 53.32 | 109.36 | - | 31.77 | 53.29 | 34.9 |
| | **50** | 0.06 | 6.07 | 62.65 | 43.92 | 20.93 | 21.62 | 43.83 | 20.67 | - | 57.04 | 10.8 | 9.35 |
| | **51** | 0.04 | 10.63 | 28.86 | 55 | 34.69 | 39.68 | 30.5 | 43.73 | - | 40.69 | 16.04 | 19.91 |
| | **52** | 0.03 | 13.24 | 8.93 | 18.55 | 21.3 | 30.89 | 41.05 | 38.02 | - | 33.11 | 16.15 | 13.51 |
| | **GMT** | 0.06 | 16.84 | 22.02 | 36.57 | 32.70 | 29.98 | 40.58 | 40.53 | - | 52.94 | 28.70 | 20.35 |
| **Control (Group 3)** | **29** | 0.08 | 0.03 | 0.07 | 0.14 | 0.02 | 0.06 | 0.09 | 0.06 | 6.32 | - | - | - |
| | **36** | 0.05 | 0.06 | 0.09 | 0.02 | 0.02 | 0.07 | 0.08 | 0.07 | 57.67 | - | - | - |
| | **37** | 0.09 | 0.24 | 0.1 | 0.08 | 0.02 | 0.17 | 0.1 | 0.14 | 62.2 | - | - | - |
| | **39** | 0.07 | 0.1 | 0.25 | 0.13 | 0.02 | 0.07 | 0.09 | 0.08 | 62.75 | - | - | - |
| | **46** | 0.13 | 0.02 | 0.11 | 0.04 | 0.04 | 0.02 | 0.09 | 0.01 | 3.22 | - | - | - |
| | **GMT** | 0.08 | 0.06 | 0.11 | 0.07 | 0.02 | 0.06 | 0.09 | 0.05 | 21.49 | - | - | - |

**Table S2.** Detection of individual RABV-specific binding antibodies obtained at different time points post vaccination as measured by ELISA. Values are expressed as percent inhibition. RABV PB > 40% inhibition were considered as positive (seroconversion). MPB = Mean percentage blocking values.

| | dpv: | 0 | 14 | 28 | 58 | 118 | 178 | 268 | 365 | 377 | 379 | 393 | 455 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Animal ID** | | | | | | | | | | | | |
| **Orally vaccinated (Group 1)** | **30** | 18.72 | 93.23 | 96.86 | 97.63 | 100.68 | 100.79 | 98.66 | 100.14 | - | 102.09 | 101.86 | 99.68 |
| | **31** | 22.05 | 91.21 | 97.2 | 98.86 | 101.61 | 101.35 | 99.42 | 100.78 | - | 103.01 | 102.49 | 100.15 |
| | **33** | 18.46 | 85.65 | 94.73 | 98.54 | 100.17 | 101.25 | 99.23 | 99.37 | - | 98.36 | 100.46 | 100.6 |
| | **34** | 15.22 | 14.7 | 10.83 | 9.92 | 22.15 | 22.8 | -4.31 | -4.3 | 73.63 | - | - | - |
| | **35** | 17.92 | 82.03 | 86.33 | 93.97 | 97.43 | 96.41 | 96.79 | 97.14 | - | 100.15 | 100.83 | 100.68 |
| | **38** | 18.19 | 81.61 | 85.54 | 94.83 | 98.93 | 97.97 | 95.41 | 96.42 | - | 100.66 | 100.7 | 99.51 |
| | **41** | 18.46 | 0.11 | 8.31 | 12.92 | 9.97 | 20.81 | -5.4 | -2.83 | 70.19 | - | - | - |
| | **42** | 16.75 | 78.26 | 92.19 | 96.82 | 98.96 | 101.76 | 101.36 | 98.74 | - | 99.69 | 102.29 | 99.44 |
| | **43** | 12.35 | 84.81 | 93.68 | 97.76 | 100.04 | 95.07 | 99.82 | 73.72 | - | 99.86 | 103.31 | 99.87 |
| | **44** | 13.43 | 68.97 | 83.25 | 90.35 | 94.18 | 101.35 | 96.31 | 93.33 | - | 98.61 | 99.92 | 93.61 |
| | **45** | 21.51 | 88.31 | 95.56 | 98.45 | 100.5 | 100.37 | 98.67 | 99.92 | - | 99.89 | 102.55 | 100.6 |
| | **MPB** | 17.55 | 69.90 | 76.77 | 80.91 | 84.06 | 85.45 | 79.63 | 77.49 | 71.91 | 100.26 | 101.60 | 99.35 |
| **Parenterally vaccinated (Group 2)** | **47** | 23.57 | 91.13 | 99.97 | 96.47 | 101.65 | 101.55 | 101.76 | 101.92 | - | 103.6 | 102.9 | 101.13 |
| | **48** | 19.26 | 85.96 | 99.08 | 101.14 | 101.63 | 100.1 | 101.36 | 101.33 | - | 102.18 | 103.35 | 100.6 |
| | **49** | 17.11 | 91.14 | 99.49 | 101.28 | 99.69 | 102.25 | 102.29 | 102.1 | - | 102.53 | 102.79 | 100.5 |
| | **50** | 21.6 | 93.57 | 100.69 | 101.23 | 100.67 | 101.13 | 101.48 | 100.25 | - | 102.28 | 103.07 | 100.52 |
| | **51** | 13.43 | 92.26 | 100.05 | 101.61 | 101.6 | 101.2 | 101.52 | 100.14 | - | 101.13 | 103.17 | 100.58 |
| | **52** | 14.32 | 91.49 | 98.07 | 100.52 | 101.75 | 101.46 | 101.47 | 102.19 | - | 99.25 | 103.83 | 99.96 |
| | **MPB** | 18.22 | 90.93 | 99.56 | 100.38 | 101.17 | 101.28 | 101.65 | 101.32 | - | 101.83 | 103.19 | 100.55 |
| **Control (Group 3)** | **29** | 13.61 | 9.68 | 11.14 | 6.66 | 19.51 | 13.74 | -15.07 | -9.24 | 71.8 | - | - | - |
| | **36** | 14.86 | 20.03 | 10.54 | 3.63 | 16.45 | 14.34 | -15.96 | -5.58 | 64.87 | - | - | - |
| | **37** | 11.54 | 11.78 | 3.84 | -0.84 | 18.95 | 10.82 | -7.42 | -9.87 | 80.81 | - | - | - |
| | **39** | 20.16 | 15.01 | 21.11 | 22.46 | 23.37 | 18.64 | 7.2 | 2.55 | 74.57 | - | - | - |
| | **46** | 23.04 | 9.35 | 15.25 | 32.12 | 21.71 | 13.21 | 2.06 | -4.36 | 56.79 | - | - | - |
| | **MPB** | 16.64 | 13.17 | 12.38 | 12.81 | 20.00 | 14.15 | -5.84 | -5.30 | 69.77 | - | - | - |

**Table S3**. *In silico* analysis of consensus between Ig-related mRNA and protein sequences of fox and dog. Alignments were performed using MUSCLE alignment on sequences of fox (VulVul2,0) and dog (CanFam3,1) genome annotations.

| Species | Component | Gene ID | mRNA no. | Protein no. | Consensus [%] |
|---|---|---|---|---|---|
| Fox | FCAMR | 112910995 | XM_025987320.1 | XP_025843105.1 | |
| Dog | FCAMR | 490267 | XM_022420846.1 | XP_022276554.1 | 87.65 |
| Fox | FCAR | 112909385 | XM_025985292.1 | XP_025841077.1 | |
| Dog | FCAR | 106559252 | XM_014116440.1 | XP_013971915.1 | 81.85 |
| Fox | FCGR1A X1 | 112907678 | XM_025982978.1 | XP_025838763.1 | |
| Dog | FCGR1A X1 | 403462 | XM_022404314.1 | XP_022260022.1 | 98.12 |
| Fox | FCGR1A X2 | 112907678 | XM_025982979.1 | XP_025838764.1 | |
| Dog | FCGR1A X2 | 403462 | XM_014120558.2 | XP_013976033.1 | 75.54 |
| Fox | FCGR2B X1 | 112914296 | XM_025991239.1 | XP_025847024.1 | |
| Dog | FCGR2B X1 | 100856270 | XM_022415353.1 | XP_022271061.1 | 92.86 |
| Fox | FCGR2B X2 | 112914296 | XM_025991241.1 | XP_025847026.1 | |
| Dog | FCGR2B X2 | 100856270 | XM_022415354.1 | XP_022271062.1 | 92.65 |
| Fox | FCGR2B X3 | 112914296 | XM_022415355.1 | XP_025847027.1 | |
| Dog | FCGR2B X3 | 100856270 | XM_025991242.1 | XP_022271063.1 | 85.76 |
| Fox | FCGRT | 112931350 | XM_026014003.1 | XP_025869788.1 | |
| Dog | FCGRT | 476414 | XM_005616309.3 | XP_005616366.1 | 99.44 |
| Fox | FCGRT | 112931350 | XM_026014004.1 | XP_025869789.1 | |
| Dog | FCGRT | 476414 | XM_533618.7 | XP_533618.2 | 99.15 |
| Fox | FCMR | 112910996 | XM_025987321.1 | XP_025843106.1 | |
| Dog | FCMR X1 | 490266 | XM_547385.6 | XP_547385.3 | 91.35 |
| Dog | FCMR X2 | 490266 | XM_005622301.3 | XP_005622358.1 | 89.54 |
| Fox | JCHAIN | 112923688 | XM_026003517.1 | XP_025859302.1 | |
| Dog | JCHAIN | 475166 | XM_532398.6 | XP_532398.2 | 98.39 |

**Table S4.** Individual numbers of IFN-γ specific SFU/$10^6$ cells counted at different time points pre- and post challenge after stimulation with 2.5 and 5.0 µg/mL antigen (RABV SAD L16), Numbers of counted SFU/$10^6$ cells were normalized to unstimulated controls.

| | | Pre-Challenge | | | | | | | | | Post-Infection | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **dpv:** | | **268** | | | **365** | | | **377** | | | **379** | | | **393** | | | **455** | | | |
| **$c_{antigen}$ [µg/mL]:** | | **2.5** | **5.0** | **3 ConA** | **2.5** | **5.0** | **3 ConA** | **2.5** | **5.0** | **3 ConA** | **2.5** | **5.0** | **3 ConA** | **2.5** | **5.0** | **3 ConA** | **2.5** | **5.0** | **3 ConA** | **non-stimulated controls** |
| | **Animal ID** | | | | | | | | | | | | | | | | | | | |
| **Orally vaccinated (Group 1)** | **30** | 18 | 18 | 373 | 0 | 5 | 350 | - | - | - | 15 | 50 | 630 | 60 | 38 | 1075 | 63 | 68 | 435 | 0 |
| | **31** | 38 | 18 | 648 | 25 | 68 | 778 | - | - | - | 60 | 88 | 540 | 53 | 38 | 1483 | 90 | 23 | 455 | 0 |
| | **33** | 8 | 28 | 388 | 120 | 220 | 1220 | - | - | - | 68 | 88 | 465 | 53 | 75 | 1788 | 153 | 125 | 305 | 0 |
| | **34** | 0 | 3 | 335 | 5 | 13 | 553 | 2508 | 2643 | 343 | - | - | - | - | - | - | - | - | - | 0 |
| | **35** | 15 | 10 | 490 | 45 | 40 | 830 | - | - | - | 110 | 90 | 688 | 50 | 33 | 1388 | 150 | 135 | 398 | 0 |
| | **38** | 18 | 10 | 378 | 20 | 15 | 460 | - | - | - | 100 | 83 | 710 | 128 | 408 | 2723 | 163 | 188 | 73 | 0 |
| | **41** | 0 | 0 | 628 | 33 | 8 | 435 | 1173 | 1068 | 320 | - | - | - | - | - | - | - | - | - | 0 |
| | **42** | 43 | 43 | 278 | 88 | 108 | 640 | - | - | - | 93 | 90 | 640 | 35 | 183 | 2155 | 130 | 108 | 735 | 0 |
| | **43** | 40 | 40 | 650 | 65 | 65 | 530 | - | - | - | 178 | 183 | 685 | 100 | 208 | 1985 | 158 | 143 | 673 | 0 |
| | **44** | 10 | 40 | 513 | 55 | 50 | 388 | - | - | - | 205 | 223 | 665 | 148 | 465 | 2215 | 100 | 98 | 573 | 0 |
| | **45** | 28 | 30 | 373 | 60 | 25 | 775 | - | - | - | 173 | 140 | 360 | 693 | 840 | 3265 | 125 | 133 | 715 | 0 |
| | **mean** | 20 | 22 | 459 | 47 | 56 | 633 | - | - | - | 111 | 115 | 598 | 147 | 254 | 2008 | 126 | 113 | 484 | 0 |
| | **median** | 18 | 18 | 388 | 45 | 40 | 553 | - | - | - | 100 | 90 | 640 | 60 | 183 | 1985 | 130 | 125 | 455 | 0 |
| **parenterally vaccinated (Group 2)** | **47** | 60 | 75 | 755 | 35 | 45 | 425 | - | - | - | 83 | 68 | 645 | 78 | 150 | 2750 | 20 | 63 | 278 | 0 |
| | **48** | 60 | 105 | 818 | 23 | 10 | 383 | - | - | - | 275 | 293 | 618 | 60 | 60 | 2180 | 93 | 145 | 825 | 0 |
| | **49** | 80 | 103 | 518 | 173 | 140 | 613 | - | - | - | 68 | 125 | 695 | 55 | 25 | 1803 | 83 | 108 | 448 | 0 |
| | **50** | 100 | 58 | 978 | 60 | 103 | 600 | - | - | - | 155 | 158 | 550 | 113 | 143 | 2018 | 183 | 165 | 630 | 0 |
| | **51** | 183 | 150 | 1045 | 88 | 53 | 418 | - | - | - | 50 | 73 | 715 | 83 | 63 | 2080 | 73 | 45 | 503 | 0 |
| | **52** | 28 | 18 | 230 | 73 | 80 | 275 | - | - | - | 170 | 188 | 568 | 55 | 63 | 1920 | 133 | 188 | 540 | 0 |
| | **mean** | 85 | 85 | 724 | 75 | 72 | 452 | - | - | - | 133 | 150 | 632 | 74 | 84 | 2125 | 97 | 119 | 537 | 0 |
| | **median** | 70 | 89 | 786 | 67 | 67 | 421 | - | - | - | 119 | 142 | 631 | 69 | 63 | 2049 | 88 | 127 | 521 | 0 |
| **Control (Group 3)** | **29** | 15 | 15 | 715 | 43 | 38 | 718 | 205 | 123 | 190 | - | - | - | - | - | - | - | - | - | 0 |
| | **36** | 15 | 8 | 735 | 20 | 38 | 483 | 1393 | 1213 | 358 | - | - | - | - | - | - | - | - | - | 0 |
| | **37** | 20 | 20 | 410 | 15 | 8 | 793 | 2548 | 2560 | 243 | - | - | - | - | - | - | - | - | - | 0 |
| | **39** | 10 | 8 | 353 | 25 | 15 | 555 | 665 | 528 | 405 | - | - | - | - | - | - | - | - | - | 0 |
| | **46** | 220 | 345 | 965 | 45 | 45 | 160 | 625 | 900 | 1575 | - | - | - | - | - | - | - | - | - | 0 |
| | **mean** | 56 | 79 | 636 | 30 | 29 | 542 | 1087 | 1065 | 554 | - | - | - | - | - | - | - | - | - | 0 |
| | **median** | 15 | 15 | 715 | 25 | 38 | 555 | 665 | 900 | 358 | - | - | - | - | - | - | - | - | - | 0 |

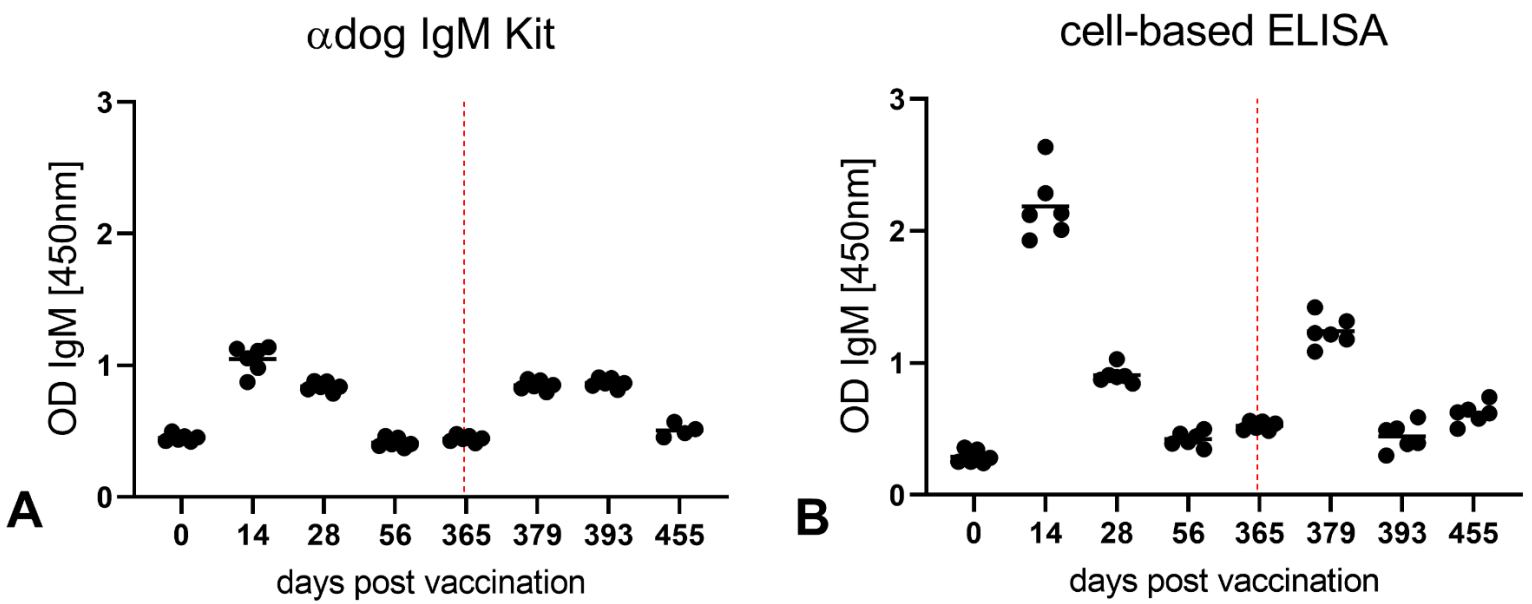


**Figure S1.** Comparison of RABV-specific IgM detection by a commercial dog-specific rabies virus IgM Antibody ELISA Kit and the cell-based ELISA. Cross-reactivity and specificity of IgM antibodies as measured by the cell -based ELISA established for foxes (**A**) was confirmed by a commercial dog-specific rabies virus IgM Antibody ELISA Kit (**B**) using 6 parenterally vaccinated animals selected at different time points dpv.