

***Supplementary Fig 1****. Comparison of GFP intensity following eGFP-MVA (4 x 104 IU) delivery with MAF and buccal administration of GFP-MVA without the film (buccal) (A). Images were captured after 2 days of application, showing GFP expression in the whole mouse (B) and specifically in the oral mucosa (C).*



***Supplementary Fig 2****. Immunization of mice with mRNA/LNP encoding SARS-CoV-2 Spike protein via IN route. (A) Endpoint titers of total IgG antibodies against SARS-CoV-2 Spike protein with SD determined by ELISA three weeks after the prime dose and three weeks after each booster dose (blank n=6, IN-IN-IN n=5, IM-IN-IN n=6); (B) Detection of IgAs against SARS-CoV-2 Spike protein was measured with ELISA three weeks after the second booster dose. Graphs represent the mean and SEM (C) Endpoint titers of total IgG antibodies against SARS-CoV-2 Spike protein with SD determined by ELISA three weeks after the prime dose and three weeks after the boost (MAF – stomach =5, mRNA/LNP buccal =5, mRNA/LNP stomach =5, IM-MAF =5; MAF-MAF n=6) .*



**Supplementary Fig. 3**. Gating strategy and flow cytometry statistics of mouse spleen for groups IM-IN-IN and MAF-MAF-MAF. Cell subset defined as 1 A: CD19- CD3-, 1 B: CD8- CD4+ T-cells, 1 C: CD8- CD4- T-cells, 1 D: CD8+ CD4- T-cells. Figures illustrating statistically significant differences among groups.



***Supplementary Fig. 4.*** *Gating strategy and flow cytometry statistics of mouse spleen for groups IM-IM-IM, IM-MAF-MAF and IN-IN-IN. Cell subset defined as 2 A: CD19- CD3-, 2 B: CD8- CD4+ T-cells, 2 C: CD8- CD4- T-cells, 2 D: CD8+ CD4- T-cells. Figures illustrating statistically significant differences among groups.*

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Samples: IM-IM-IM; IM-MAF-MAF; IN-IN-IN | | | | | Samples: IM-IN-IN; MAF-MAF-MAF | | | | |
|  | Marker | Clone | Dye | Dilution | Cat. No. | Marker | Clone | Dye | Dilutions | Cat. No. |
| V1 | CD103 | 2E7 | BV421 | 100 | BL 121421 | CD103 | 2E7 | BV421 | 250 | BL 121421 |
| V3 | CD44 | IM7 | Pacific Blue | 100 | BL 103019 | CD44 | IM7 | Pacific Blue | 200 | BL 103019 |
| V5 | CD4 | RM4-5 | BV510 | 250 | BL 100553 | CD4 | RM4-5 | BV510 | 500 | BL 100553 |
| V10 | CD11c | N418 | BV605 | 100 | BL 117334 | CD11c | N418 | BV605 | 200 | BL 117334 |
| V11 | CD62L | MEL-14 | BV650 | 250 | BL 104453 | CD62L | MEL-14 | BV650 | 250 | BL 104453 |
| V13 | MHCII | M5/114.15.2 | BV711 | 250 | BL 107643 | CD3e\* | 145-2C11 | BV711 | 170 | BL 100349 |
| V14 | CD11b | M1/70 | BV750 | 250 | BL 101267 | CD11b | M1/70 | BV750 | 500 | BL 101267 |
| V15 | CD8a | 53-6.7 | BV785 | 250 | BL 100749 | CD8a | 53-6.7 | BV785 | 1000 | BL 100749 |
| B3 | CD161 | S17016D | KIRAVIA Blue 520 | 100 | BL 156521 | CD161 | S17016D | KIRAVIA Blue 520 | 170 | BL 156521 |
| B4 | CD86 | GL-1 | PE | 100 | BL 105007 | CD86 | GL-1 | PE | 250 | BL 105007 |
| B8 | CD3 | 145-2C11 | PCP | 50 | BL 100325 | MHCII\* | M5/114.15.2 | PerCP | 1000 | BL 107623 |
| B9 | CD64 | X54-5/7.1 | PerCP-Cy5.5 | 50 | BL 139307 | CD64 | X54-5/7.1 | PE-Cy5 | 200 | BL 139331 |
| B13 | CD69 | H1.2F3 | PE-Cy7 | 100 | BL 104511 | CD69 | H1.2F3 | PE-Cy7 | 200 | BL 104511 |
| R1 | Ly-6C | HK1.4 | APC | 250 | Milteny 130-102-341 | Ly-6C | HK1.4 | APC | 100 | Milteny 130-102-341 |
| R4 | CD19 | 6D5 | Spark NIR 685 | 250 | BL 115567 | CD19 | 6D5 | Spark NIR 685 | 1000 | BL 115567 |
| R6 | Live/Dead |  | Zombie NIR | 5000 | BL 423106 | Live/Dead |  | Zombie NIR | 5000 | BL 423106 |
| R8 | CD45 | 30-F11 | APC-Cy7 | 1000 | BL 103115 | CD45 | 30-F11 | APC-Cy7 | 1000 | BL 103115 |

***Supplementary Table 1****: List of antibodies*

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| --- | --- | --- | --- | --- | --- |
| Marker | Clone | Dye | Manufacturer | Catalogue no. | Dilution |
|  |  | ZoombieNIR | BioLegend | 77184 | 5000 |
| CD103 | 2E7 | BV421 | BioLegend | 121422 | 200, 250 |
| CD11b | M1/70 | BV750 | BioLegend | 101267 | 500 |
| CD11c | N418 | BV605 | BioLegend | 117334 | 200 |
| CD161 | S17016D | KIRAVIA Blue 520 | BioLegend | 156522 | 170, 200 |
| CD19 | 6D5 | Spark NIR 685 | BioLegend | 115567 | 500, 1000 |
| CD3e | 145-2C11 | PerCP | BioLegend | 100325 | 200 |
|  |  | BV711 | BioLegend | 100349 | 170 |
| CD4 | RM4-5 | BV510 | BioLegend | 100559 | 500 |
| CD44 | IM7 | Pacific Blue | BioLegend | 103020 | 200 |
| CD45 | 30-F11 | APC-Cy7 | BioLegend | 103115 | 200, 500, 1000 |
| CD62L | MEL-14 | BV650 | BioLegend | 104453 | 200, 250, 500 |
| CD64 | X54-5/7.1 | PerCP-Cy5.5 | BioLegend | 139307 | 100 |
|  |  | Pe-Cy5 | BioLegend | 139331 | 200 |
| CD86 | GL-1 | PE | BioLegend | 105007 | 200, 250 |
| CD8a | 53-6.7 | BV785 | BioLegend | 100750 | 500, 1000 |
| Ly-6C | HK1.4 | APC | MiltenyiBiotec | 130-102-341 | 100, 200, 500 |
| MHCII | M5/114.15.2 | BV711 | BioLegend | 107643 | 500, 1000 |
|  |  | PerCP | BioLegend | 107623 | 1000 |

***Supplementary* Table 2:** List of antibodies used in antibody cocktail.