

Supplemental information

mRNA vaccine-induced IgG mediates nasal

SARS-CoV-2 clearance in mice

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Supplemental information:

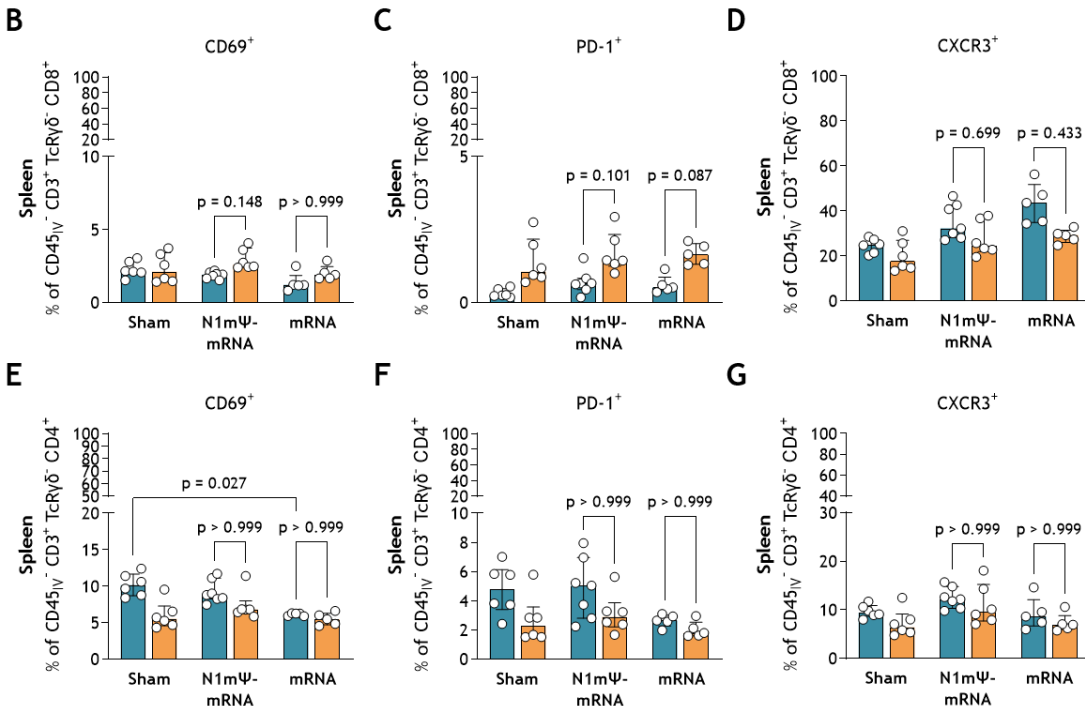
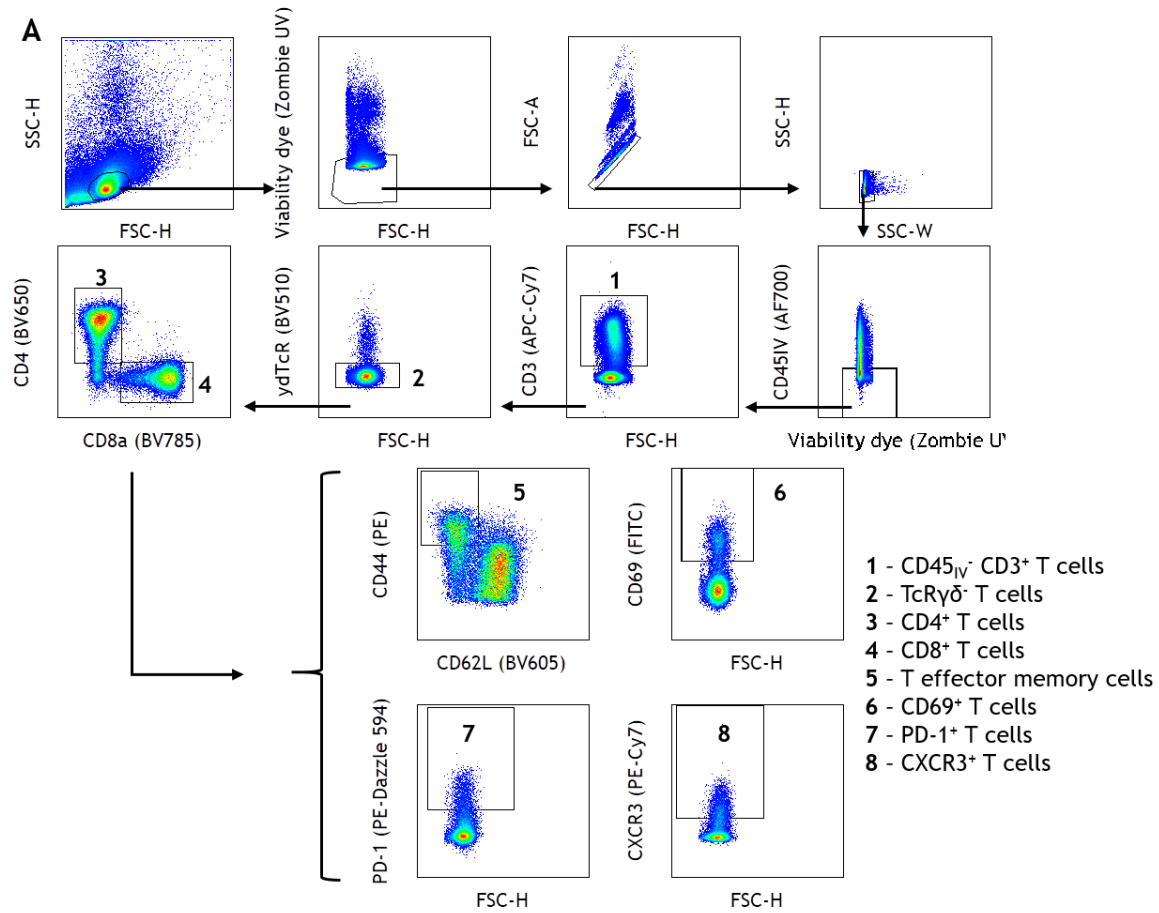


Fig. S1 | Flow cytometry gating strategy and analysis for CD45^{IV-} T cells in the lungs and spleen. **A:** Lung and spleen lymphocytes were identified using FSC-H against SSC-H gating. FSH-H/FSC-A and SSC-W/SSC-H gating eliminated doublets. The fixable live/dead viability dye (Zombie UV, BioLegend GmbH) was used to exclude dead cells. During lethal anaesthesia, an intravenous injection of anti-mouse CD45 antibodies was administered to distinguish parenchymal (CD45^{IV-}) from vascular cells (CD45^{IV+}). Before gating on CD4⁺ helper T cells and CD8⁺ cytotoxic T cells, CD3⁺ $\gamma\delta$ TCR⁺ T cells were excluded. Effector memory T cells (T_{em}) were characterized as CD44^{hi} and CD62L⁻. The flow plots were created from one representative mouse sample. **B-G:** Pre-challenge CD4⁺ and CD8⁺ T cells from the spleen of mice (d56) were analyzed for CD69 (**B and E**), PD-1 (**C and F**) and CXCR3 (**D and G**) expression. Each data point represents one animal. n = 6 per vaccine and mouse group. n = 7 for N1m Ψ -mRNA WT. n = 5 for mRNA. The median with the interquartile range is displayed. p Values were determined by nonparametric one-way ANOVA and Dunn's multiple comparison test.

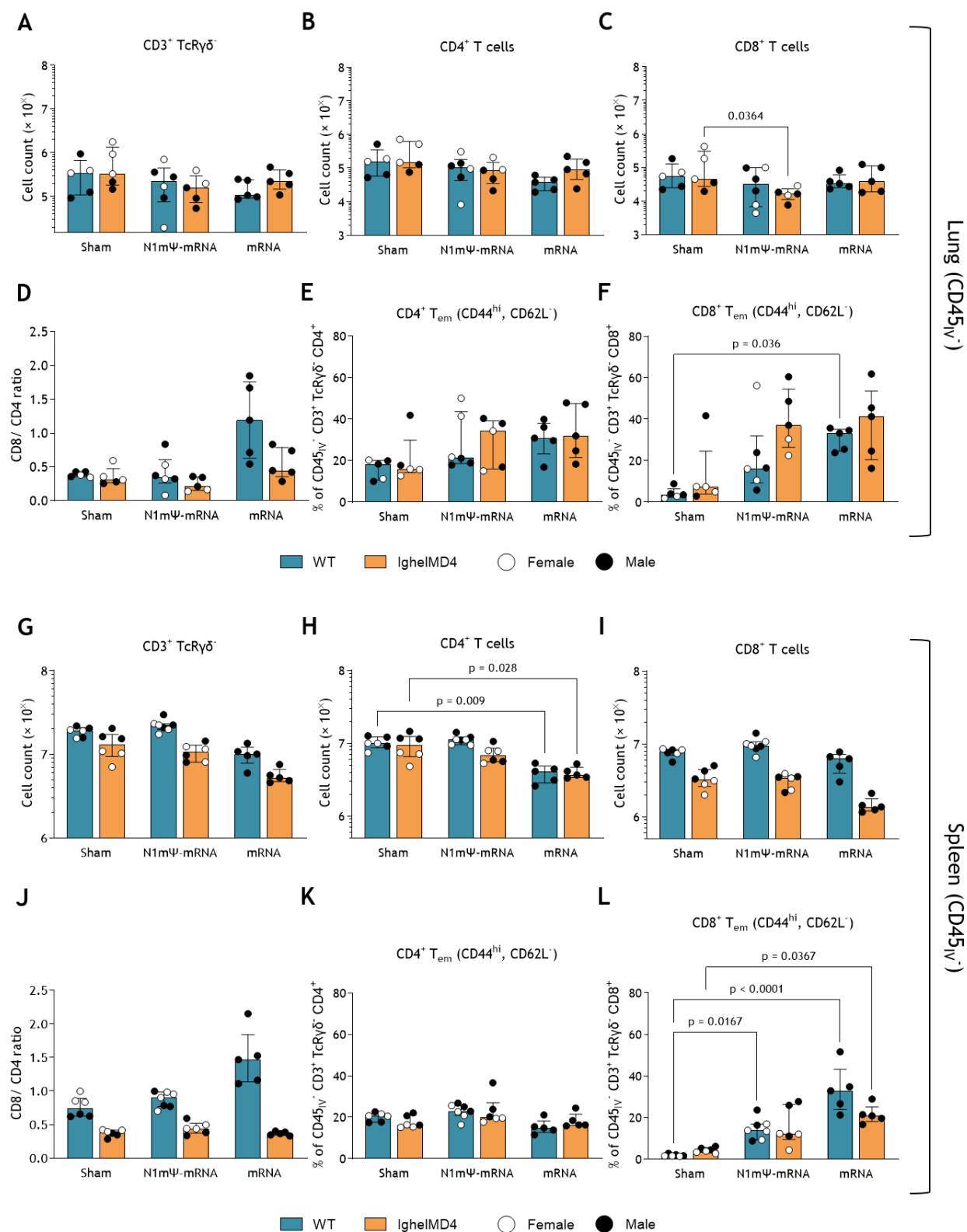


Fig. S2 | Analysis of lung and spleen T cell populations in mRNA-vaccinated WT and IgheIMD4 mice pre-challenge infection (d56). A-C: Counts of T cell populations in the lungs gated on $CD45^{IV-}$, $CD3^+$, $TcR\gamma\delta^-$ (A)

and CD4⁺ (**B**) or CD8⁺ (**C**). **D**: CD8/ CD4 T cell ratio in the lungs. **E-F**: CD4⁺ (**E**) and CD8⁺ (**F**) T_{em} cells in the lungs. **G-I**: Counts of T cell populations in the spleen gated on CD45^{IV}⁻, CD3⁺, TcRγδ⁻ and CD4⁺ (**H**) or CD8⁺ (**I**). **J**: CD8/ CD4 T cell ratio in the spleen. **K-L**: CD4⁺ (**K**) and CD8⁺ (**L**) T_{em} cells in the spleen. **A-F**: Number of animals: n = 5 per group, n = 6 for N1mΨ-mRNA WT. **G-L**: Number of animals: n = 6 per group, n = 7 for N1mΨ-mRNA WT, n = 5 for mRNA WT and ighelMD4. **A-L**: p values were determined by nonparametric one-way ANOVA and Dunn's multiple comparison test. Each data point represents one female (white dot) or male (black dot) animal.

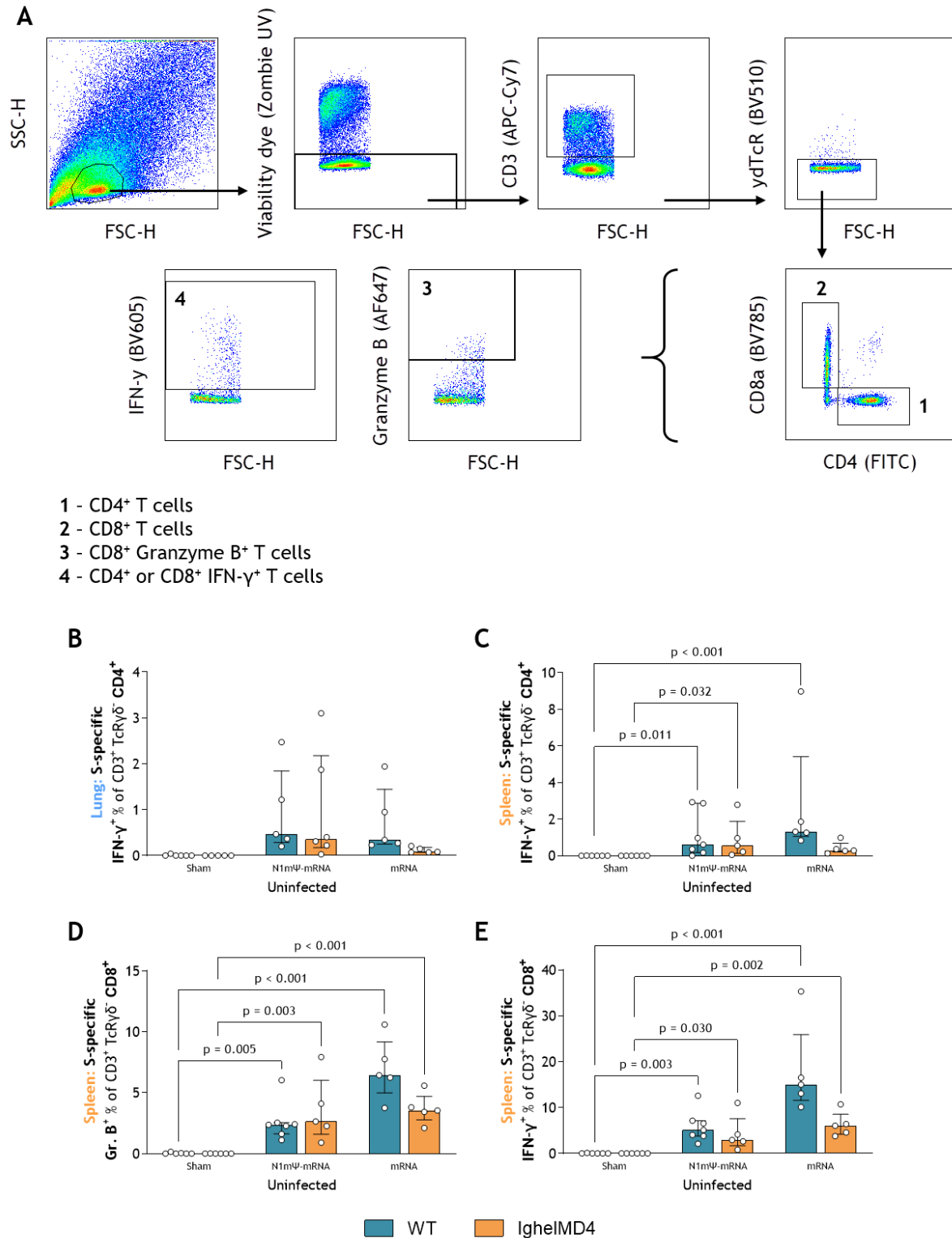


Fig. S3 | Analysis of S-specific lung and spleen T cells after vaccination pre-challenge infection. A: Flow cytometry gating strategy for S-specific T cell responses. Lung and spleen lymphocytes were identified by FSC-H against SSC-H gating. Dead cells were excluded using the fixable live/dead viability

dye (Zombie UV, BioLegend GmbH) before gating on CD3⁺ T cells. $\gamma\delta$ TCR⁺ T cells were excluded. Expression analysis of Granzyme B and IFN- γ was performed for CD8⁺ and CD4⁺ T cells. Flow plots were generated from one representative sample. **B-E:** Single-cell suspensions obtained from the lungs and spleen of mice were restimulated in vitro with S peptides. The percentage of CD8⁺ T cells that expressed Granzyme B (**D**) or CD4⁺ and CD8⁺ T cells that expressed IFN- γ (**B, C and E**) in the lungs (**B**) and spleen (**C-E**) at day 56 is presented. The values were obtained by subtracting percentage [unstimulated] from percentage [S peptide stimulated]. Each data point represents one animal. The median with the interquartile range is displayed. p Values were determined by nonparametric one-way ANOVA and Dunn's multiple comparison test. **B:** n = 5 per vaccine and mouse group. n = 6 for Sham WT and N1m Ψ -mRNA IghelMD4. **C-E:** n = 5 per vaccine and mouse group. n = 6 for Sham, n = 7 for N1m Ψ -mRNA WT, n = 5 for N1m Ψ -mRNA IghelMD4 and mRNA.

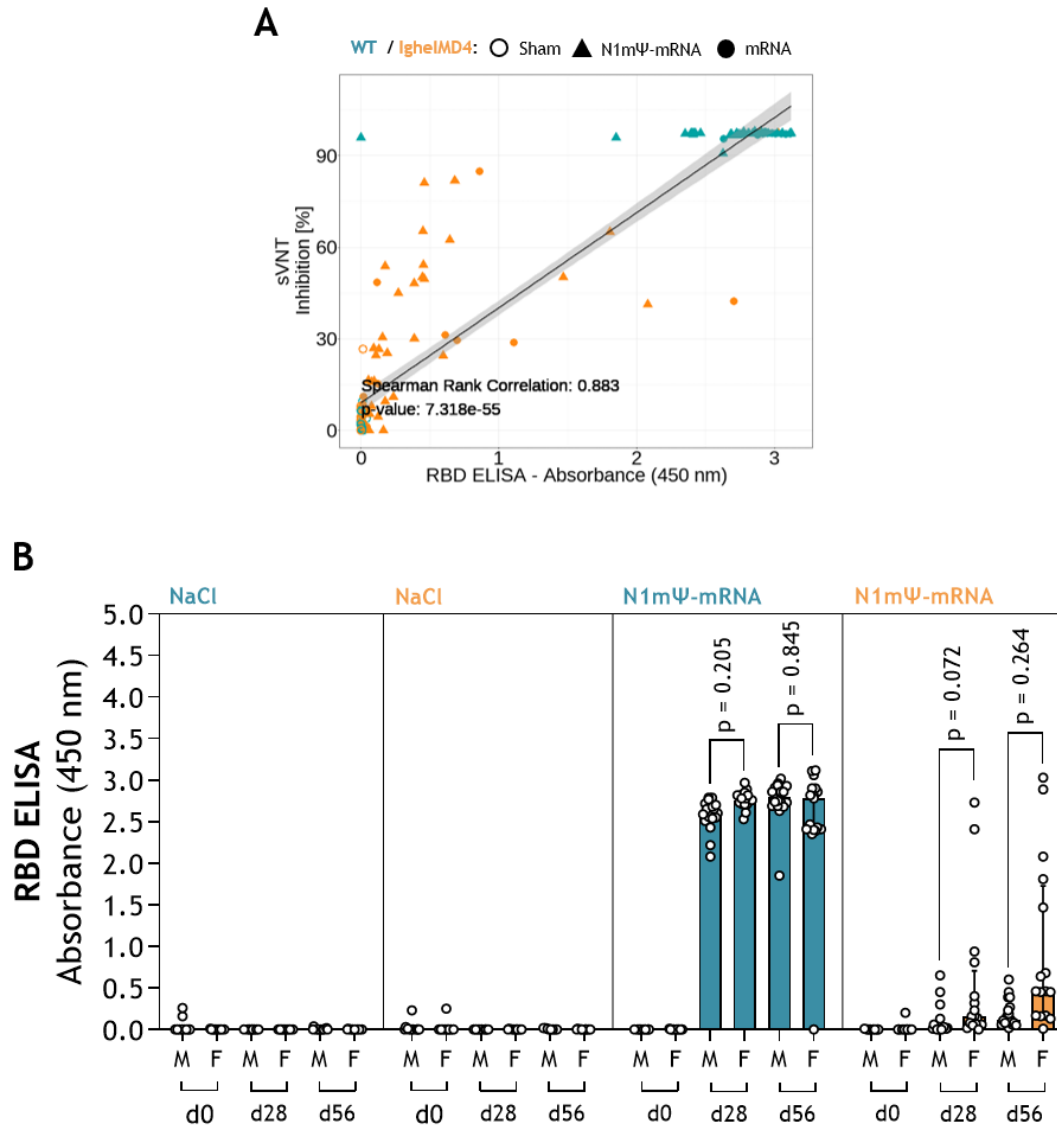


Fig. S4 | Analysis of humoral immune responses in serum and nasal conchae pre-challenge infection. A: RBD ELISA absorbance linear regression and correlation analysis with inhibition measured in sVNT (d56). Number of animals: $n = 34$ for Sham WT, $n = 30$ for Sham IghelMD4, $n = 35$ for N1mΨ-mRNA WT, $n = 34$ for N1mΨ-mRNA IghelMD4, $n = 15$ for mRNA WT, $n = 15$ for mRNA IghelMD4. Two-tailed Spearman rank correlation was performed with R (version 4.3.2) in R studio (2023.12.1 build 402) using `cor.test()`. The regression line was added using `geom_smooth()` and the linear model (`lm`) method within the `ggplot2` visualization package. The grey area represents the 95 % confidence interval. **B:** Comparison of male and female mice RBD ELISA data. The median with the interquartile range is displayed. p

Values were determined by nonparametric one-way ANOVA and Dunn's multiple comparison test. **A and B:** Each data point represents one animal.

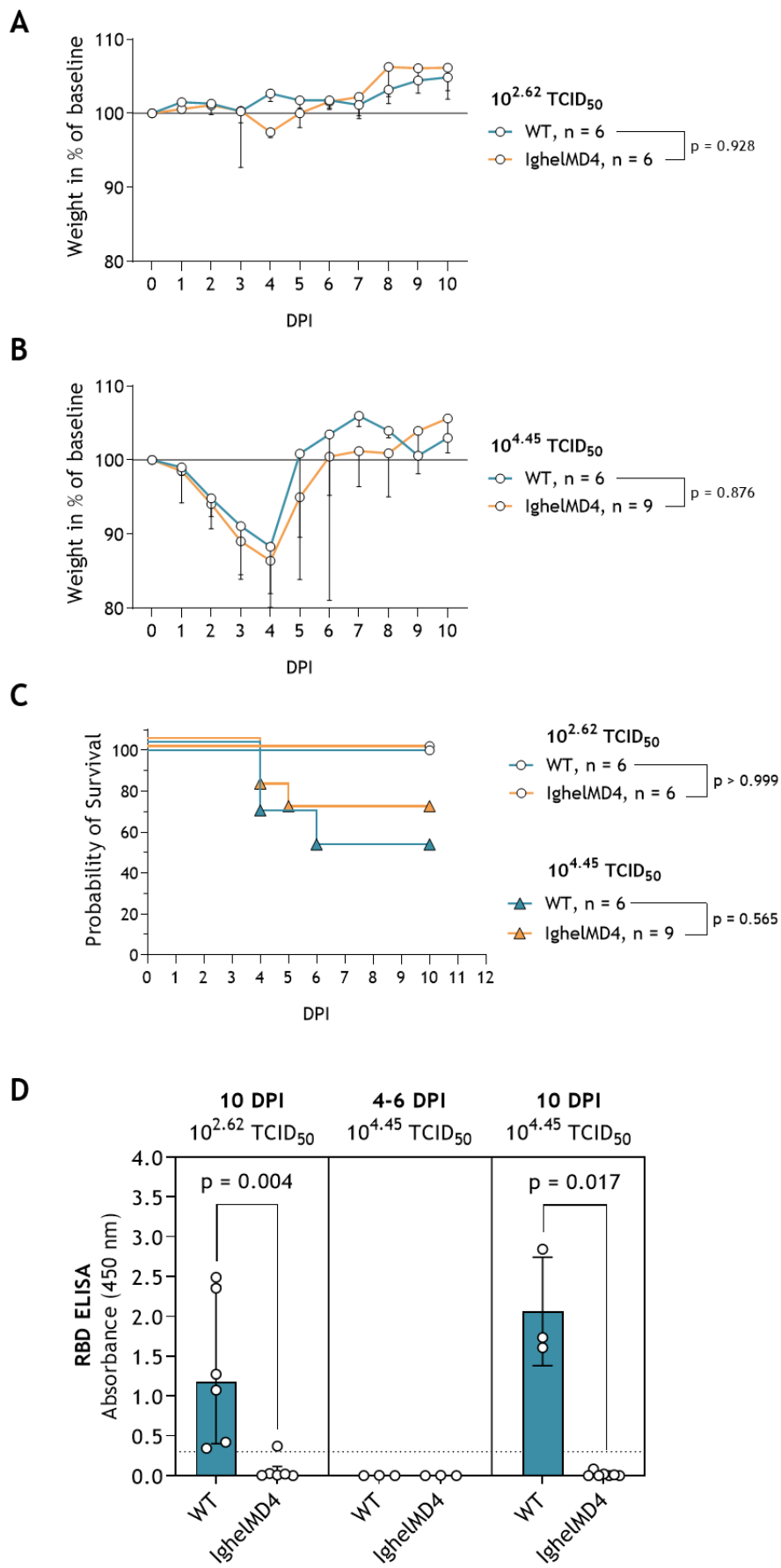
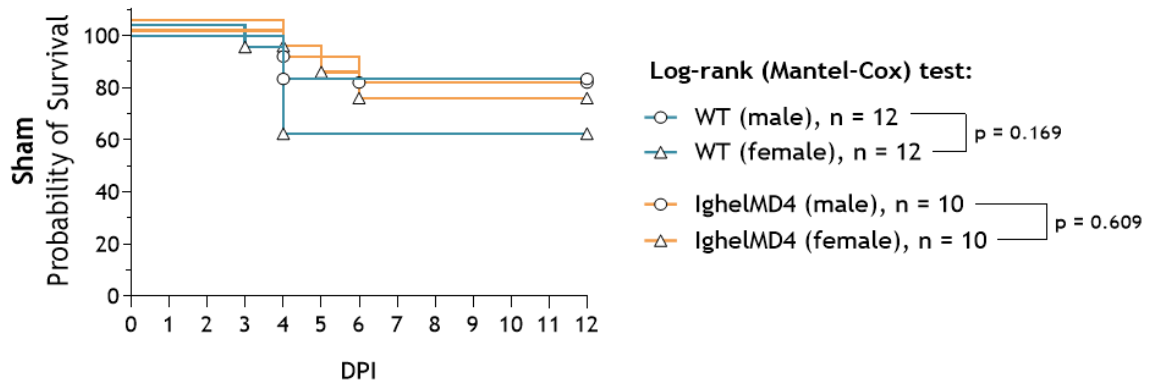
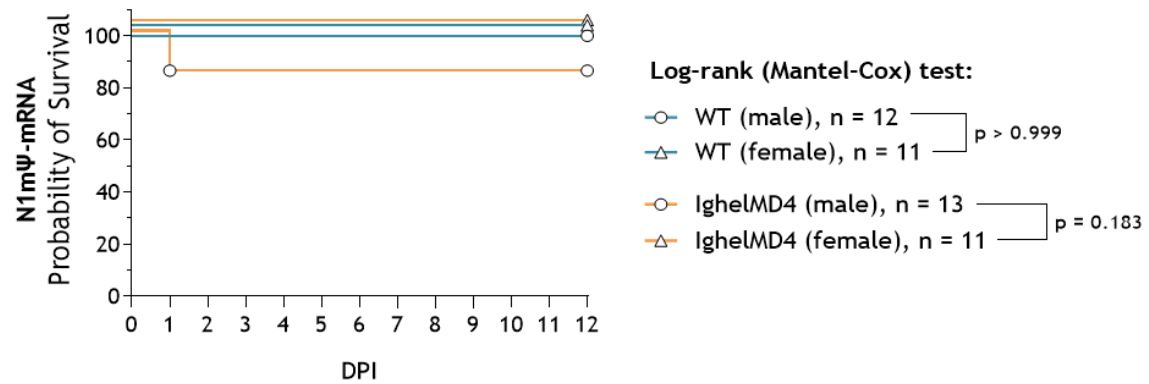


Fig. S5 | Prior dose finding experiment. A and B: Weight changes of WT and IghelMD4 mice intranasally infected with $10^{2.62}$ (**A**) or $10^{4.45}$ TCID₅₀ (**B**) of SARS-CoV-2 MA20 until 10 DPI. Each data point represents the median with the interquartile range. p Values were determined by two-way ANOVA (mixed effects analysis). **A:** n = 6 for WT, n = 6 for IghelMD4. **B:** Number of animals: n = 6 for WT, n = 9 for IghelMD4. **C:** Survival analysis for WT and IghelMD4 mice infected with $10^{2.62}$ or $10^{4.45}$ TCID₅₀ of SARS-CoV-2 MA20 up to 10 DPI. p Values were determined by direct curve comparison (Kaplan Meier) using the log-rank (Mantel-Cox) test. **D:** RBD ELISA of WT and IghelMD4 mice infected with $10^{2.62}$ or $10^{4.45}$ TCID₅₀ of SARS-CoV-2 MA20. The dotted line (...) represents the cutoff for seroconversion (OD = 0.3). Number of animals: n = 6 for WT and IghelMD4 ($10^{2.62}$ TCID₅₀), n = 3 for WT and IghelMD4 ($10^{4.45}$ TCID₅₀, 4-6 DPI), n = 3 for WT ($10^{4.45}$ TCID₅₀, 10 DPI), n = 7 for IghelMD4 ($10^{4.45}$ TCID₅₀, 10 DPI). Each data point represents one animal. The median with the interquartile range is displayed. p values were determined by the nonparametric Mann-Whitney test.

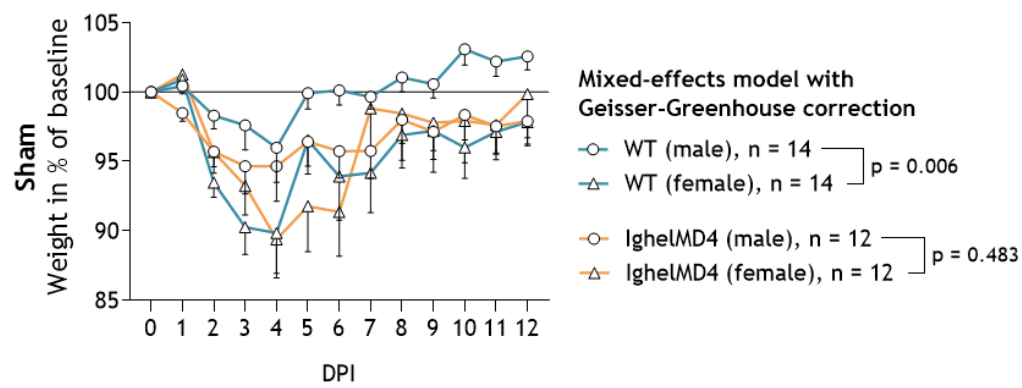
A



B



C



D

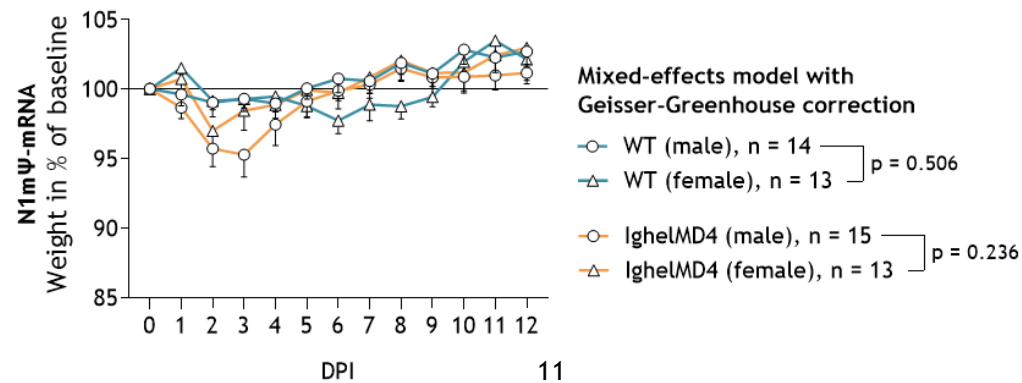


Fig. S6 | Sex-based analysis of survival and weight changes upon infection with SARS-CoV-2 MA20. A and B: Sex-based survival analysis for Sham (**A**) and N1m Ψ -mRNA (**B**) vaccinated WT and IghelMD4 mice after infection with SARS-CoV-2 MA20 up to 12 DPI. p Values were determined by direct curve comparison (Kaplan Meier) using the log-rank (Mantel-Cox) test. **A:** Number of animals: n = 12 for male and female WT, n = 10 for male and female IghelMD4. **B:** Number of animals: n = 12 for male and n = 11 for female WT, n = 13 for male IghelMD4, n = 11 for female IghelMD4. **C and D:** Sex-based analysis of weight changes until 12 DPI for Sham (**C**) and N1m Ψ -mRNA (**D**) vaccinated WT and IghelMD4 mice. The data set passed the D'Agostino-Pearson normality test ($\alpha = 0.05$). Each data point represents the mean with SEM. p Values were determined by two-way ANOVA (mixed effects analysis). **C:** Number of animals: n = 14 for male and female WT, n = 12 for male and female IghelMD4. **D:** Number of animals: n = 14 for male WT, n = 13 for female WT, n = 15 for male IghelMD4, n = 13 for female IghelMD4. **A-D:** Exclusion of animals which reached the predetermined study endpoint at 3 DPI (n = 4 per group for Sham and N1m Ψ -mRNA) from survival analysis caused differences in the number of animals in survival (**A and B**) and weight change plots (**C and D**) for each group.

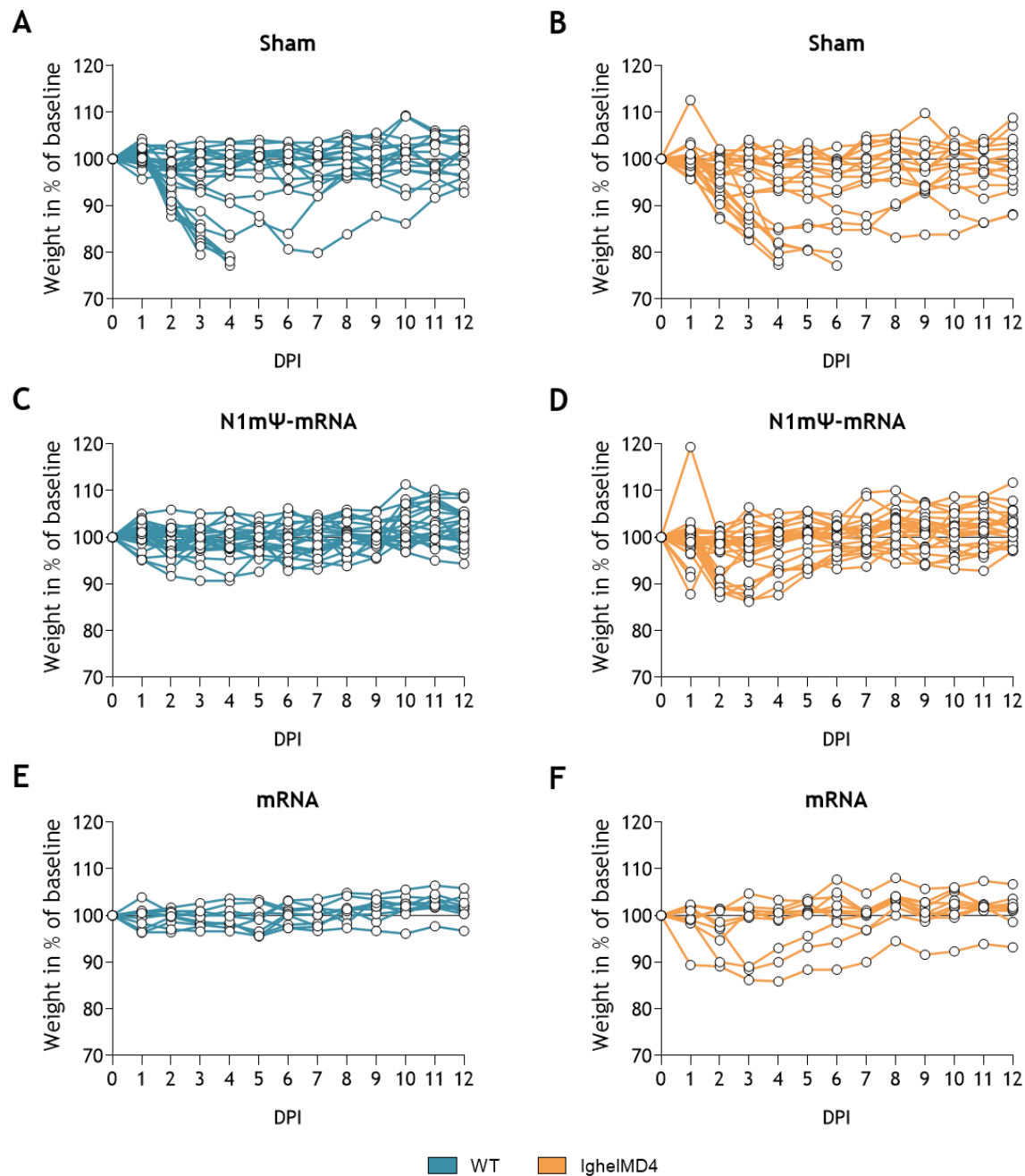


Fig. S7 | Weight changes of individual mice upon SARS-CoV-2 MA20 infection. A-F: Individual mice vaccinated with NaCl (Sham) (A and B), N1mΨ-mRNA (C and D) or mRNA (E and F) were challenged with SARS-CoV-2 MA20 and monitored for weight changes up to 12 DPI. Each line represents one animal. A-F: Number of animals: n = 28 (A), n = 24 (B), n = 27 (C), n = 28 (D), n = 10 (E) and n = 10 (F).

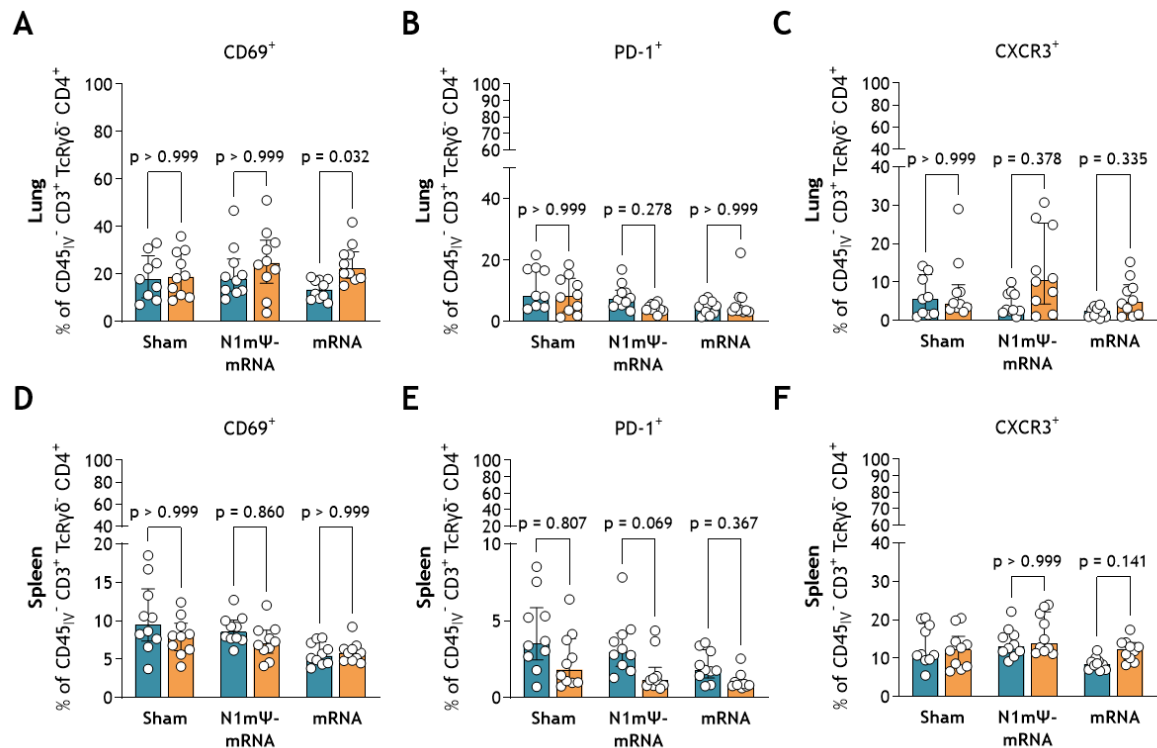


Fig. S8 | Expression analysis of activation and homing markers of lung and spleen T cells 12 DPI. A-F: Post-challenge CD4⁺ and CD8⁺ T cells from the lungs (A-C) and spleen (D-F) of mice 12 DPI were analyzed for CD69 (A and D), PD-1 (B and E) and CXCR3 (C and F) expression using flow cytometry. Each data point represents one animal. n = 10 per vaccine and mouse group, n = 9 for Sham WT (lungs). The median with the interquartile range is displayed. p Values were determined by nonparametric one-way ANOVA and Dunn's multiple comparison test.

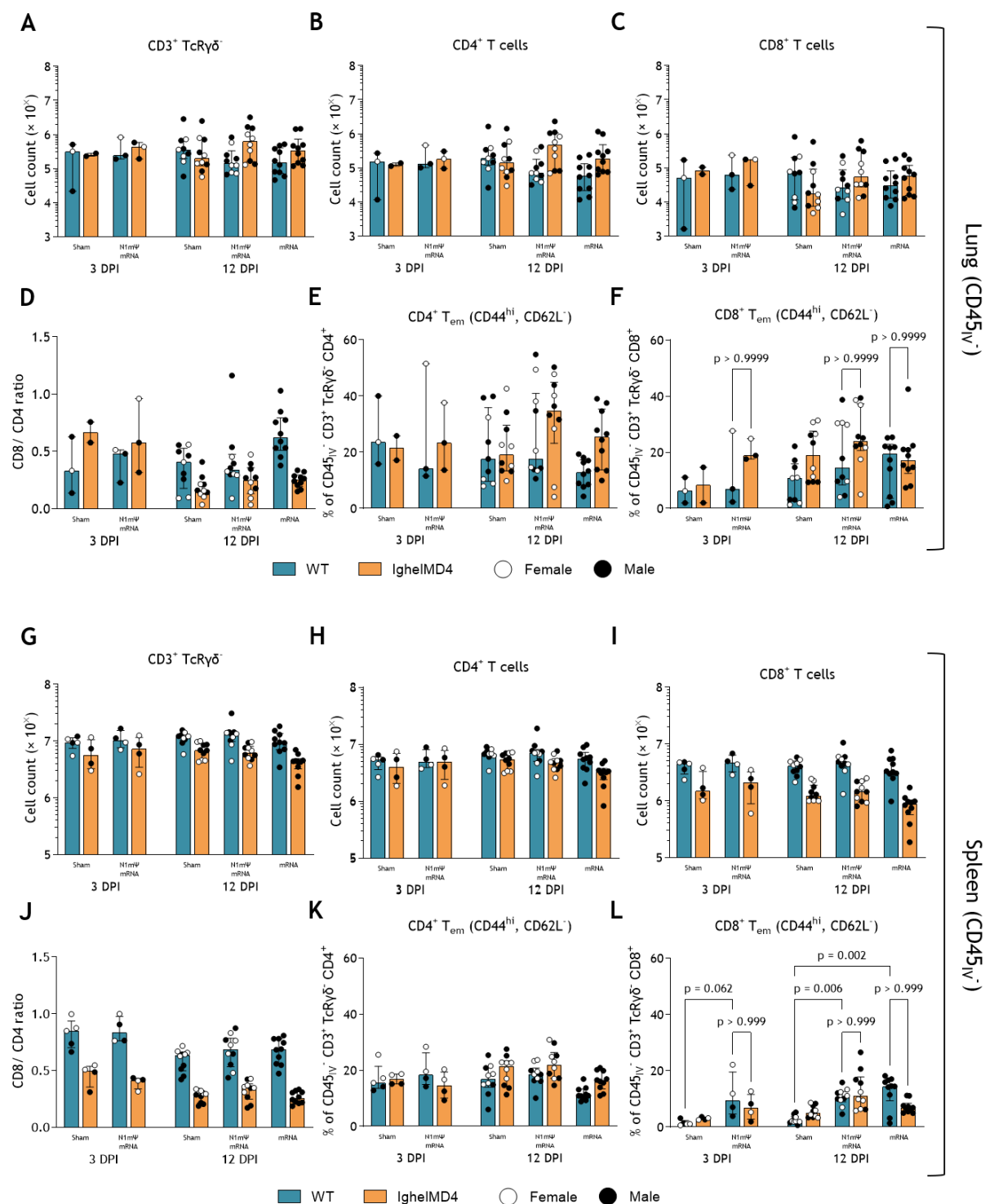


Fig. S9 | Analysis of lung and spleen T cell populations in mRNA-vaccinated WT and IghelMD4 mice post-challenge infection at 3 and 12 DPI. A-C: Counts of T cell populations in the lungs gated on CD45^{IV}-, CD3⁺, TcRγδ⁻ (A) and CD4⁺ (B) or CD8⁺ (C). **D:** CD8/CD4 T cell ratio in the lungs. **E-F:** CD4⁺ (E) and CD8⁺ (F) T_{em} cells in the lungs. **G-I:** Counts of T cell

populations in the spleen gated on CD45^{IV-}, CD3⁺, TcR $\gamma\delta$ ⁻ (**G**) and CD4⁺ (**H**) or CD8⁺ (**I**). **J**: CD8/ CD4 T cell ratio in the spleen. **K-L**: CD4⁺ (**K**) and CD8⁺ (**L**) T_{em} cells in the spleen. **A-F**: Number of animals: n = 3 per group for 3 DPI, n = 2 for Sham IghelMD4 (3 DPI), n = 10 per group for 12 DPI, n = 9 for Sham WT (12 DPI). **G-L**: Number of animals: n = 4 per group for 3 DPI, n = 5 for Sham WT (3 DPI), n = 10 per group for 12 DPI. **A-L**: p values were determined by nonparametric one-way ANOVA and Dunn's multiple comparison test. Each data point represents one female (white dot) or male (black dot) animal.

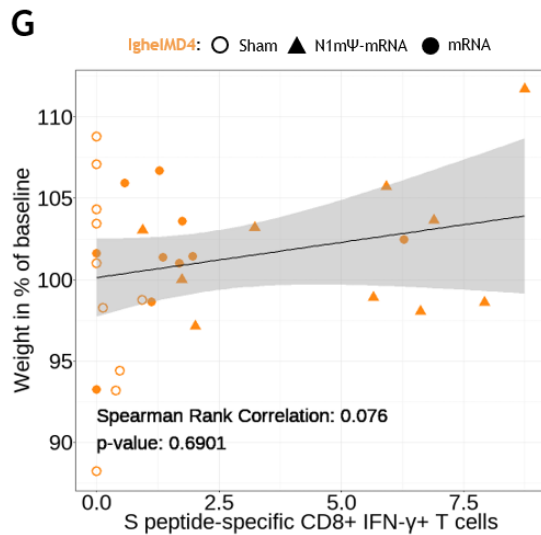
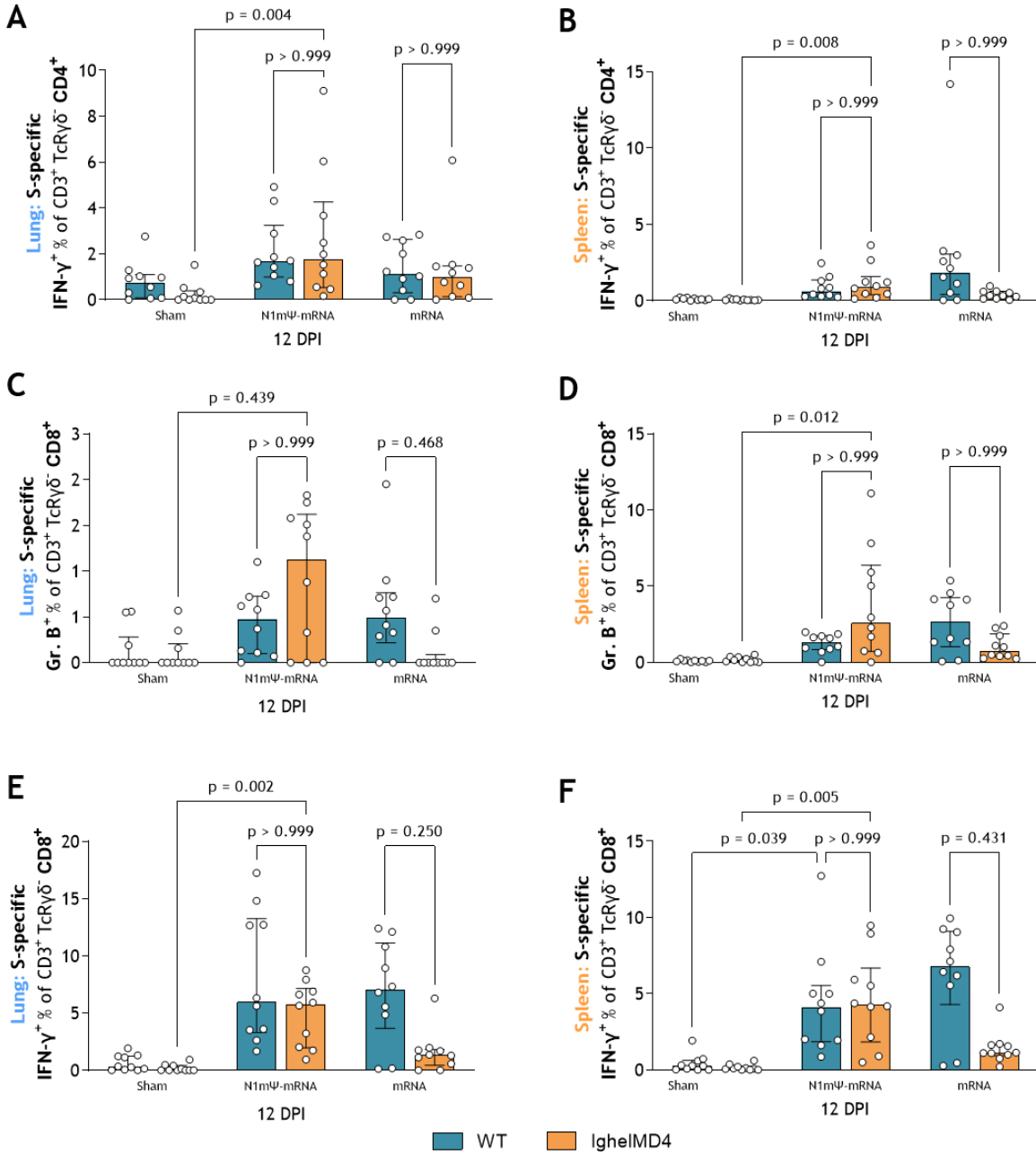


Fig. S10 | Analysis of S-specific lung and spleen T cells after vaccination post-challenge infection at 12 DPI. A-F: Single-cell suspensions obtained from the lungs and spleen of mice were restimulated in vitro with S peptides. The percentage of CD4⁺ (**A and B**) or CD8⁺ (**C-F**) T cells that expressed Granzyme B (**C and D**) or IFN- γ (**A, B, E and F**) in the lungs (**A, C and E**) and spleen (**B, D and F**) at 12 DPI is presented. Number of animals: n = 10 per group. The values were obtained by subtracting percentage [unstimulated] from percentage [S peptide stimulated]. The median with the interquartile range is displayed. p Values were determined by nonparametric one-way ANOVA and Dunn's multiple comparison test. **G:** Linear regression and correlation of S peptide-specific CD8⁺ IFN- γ ⁺ T cells with weight on the day of death for IghelMD4 mice at 12 DPI. Number of animals: n = 10 per group. Two-tailed Spearman rank correlation was performed with R (version 4.3.2) in R studio (2023.12.1 build 402) using cor.test(). The regression line was added using geom_smooth() and the linear model (lm) method within the ggplot2 visualization package. The grey area represents the 95 % confidence interval. **A-G:** Each data point represents one animal.

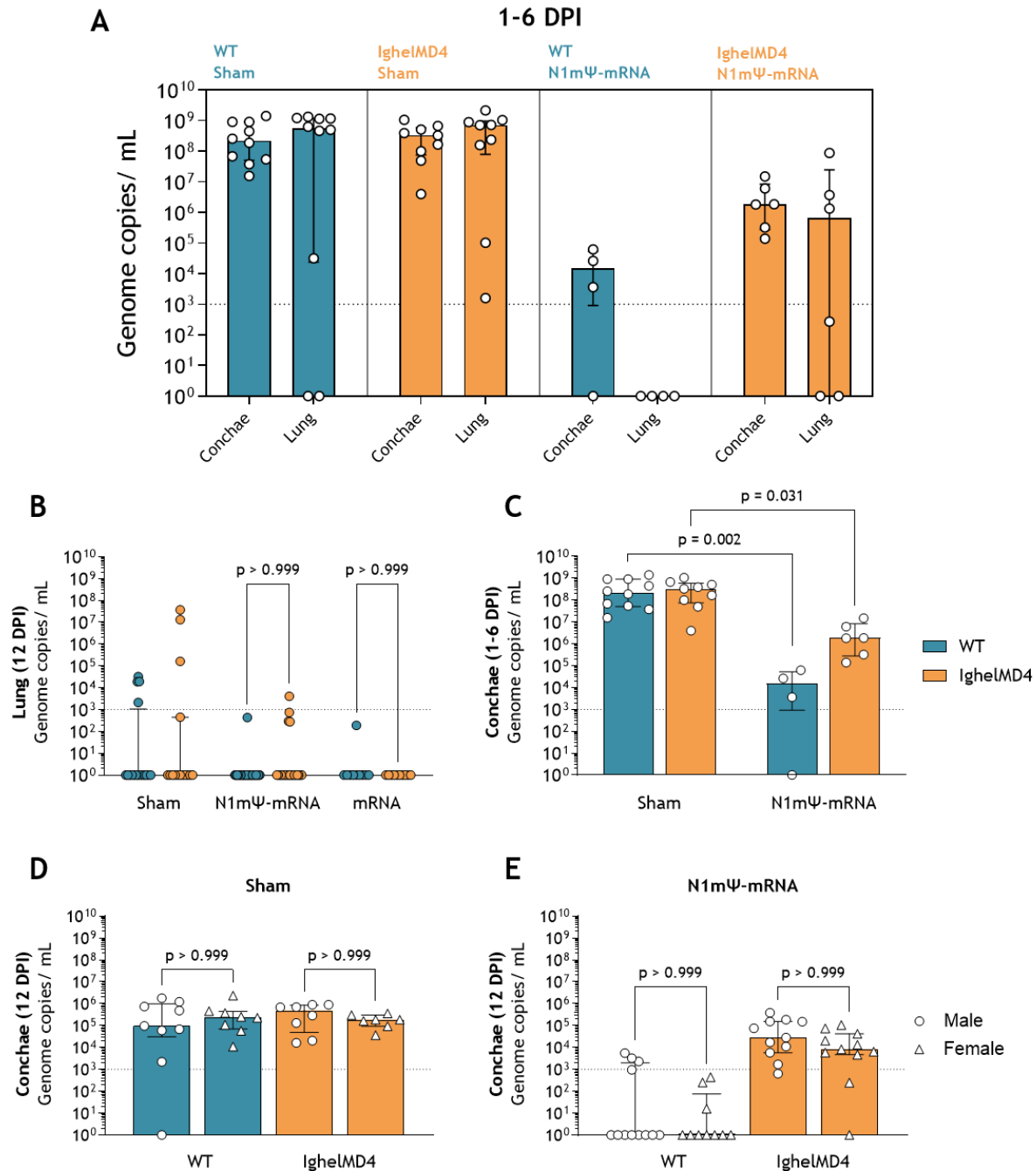


Fig. S11 | Viral loads of WT and IgheIMD4 mice infected with SARS-CoV-2 MA20. A: Viral loads in the conchae and lungs of Sham or N1mΨ-mRNA-vaccinated mice at 1-6 DPI. Number of animals: $n = 10$ for Sham WT, $n = 9$ for Sham IgheIMD4, $n = 4$ for N1mΨ-mRNA WT, $n = 6$ for N1mΨ-mRNA IgheIMD4. **B:** Lung viral loads at 12 DPI in WT and IgheIMD4 mice vaccinated with NaCl (Sham), N1mΨ-mRNA or mRNA. Number of animals: $n = 17$ for Sham WT, $n = 15$ for Sham IgheIMD4, $n = 23$ for N1mΨ-mRNA WT, $n = 22$

for N1mΨ-mRNA IghelMD4, n = 10 for mRNA WT and IghelMD4. **C:** Conchae viral loads at 1-6 DPI in WT and IghelMD4 mice vaccinated with NaCl (Sham) or N1mΨ-mRNA. Number of animals: n = 10 for Sham WT, n = 9 for Sham IghelMD4, n = 4 for N1mΨ-mRNA WT, n = 6 for N1mΨ-mRNA IghelMD4. **D and E:** Sex-based analysis of conchae (**D**) and lung (**E**) viral loads 12 DPI. **D:** Number of animals: n = 9 for male WT, n = 8 for male IghelMD4, n = 8 for female WT, n = 7 for female IghelMD4. **E:** Number of animals: n = 12 for male WT, n = 11 for male IghelMD4, n = 10 for female WT, n = 11 for female IghelMD4. **A-E:** The detection limit was set at 10^3 vRNA copies per mL, represented by a dotted line (...). Each data point represents one animal. **B-E:** p Values were determined by nonparametric one-way ANOVA and Dunn's multiple comparison test.

Table S1 | Number of mice per sex, genotype and vaccine group used pre- and post-challenge infection.

Time point	n =	Sex	Genotype	Vaccine
Pre-challenge	3	M	WT	Sham
	3		WT	N1mΨ-mRNA
	3		IghelMD4	Sham
	3		IghelMD4	N1mΨ-mRNA
	3	F	WT	Sham
	3		WT	N1mΨ-mRNA
	3		IghelMD4	Sham
	3		IghelMD4	N1mΨ-mRNA
	5	M	WT	mRNA
	5		IghelMD4	mRNA
	<u>Total: 34</u>			
Post-challenge	14	M	WT	Sham
	14		WT	N1mΨ-mRNA
	12		IghelMD4	Sham
	15		IghelMD4	N1mΨ-mRNA
	14	F	WT	Sham
	14		WT	N1mΨ-mRNA
	12		IghelMD4	Sham
	13		IghelMD4	N1mΨ-mRNA
	10	M	WT	mRNA
	10		IghelMD4	mRNA
	<u>Total: 128</u>			

Table S2 | Antibodies used for T cell flow cytometry staining.

Panel	Molecule	Fluorochrome	Isotype	Clone	Company	Cat #	Amount/ Dilution factor
T cell surface receptor analysis	CD45 (i.v.)	Alexa Fluor® 700	Rat IgG2b, κ	30-F11	BioLegend GmbH	103127	3 µg in 100 µl PBS
	CD3	APC/Cyanine7	Rat IgG2b, κ	17A2	BioLegend GmbH	100221	100
	CD4	Brilliant Violet 650™	Rat IgG2a, κ	RM4-5	BioLegend GmbH	100545	150
	CD8a	Brilliant Violet 785™	Rat IgG2a, κ	53-6.7	BioLegend GmbH	100749	100
	TCR γ/δ	Brilliant Violet 510™	Armenian Hamster IgG	GL3	BioLegend GmbH	118131	50
	CD62L	Brilliant Violet 605™	Rat IgG2a, κ	MEL-14	BioLegend GmbH	104437	100
	CD103	Brilliant Violet 711™	Armenian Hamster IgG	2E7	BioLegend GmbH	121435	100
	CD95	APC	Rat IgG1, κ	SA367H8	BioLegend GmbH	152603	150
	CD44	PE	Rat IgG2b, κ	IM7	BioLegend GmbH	103023	150
	KLRG1	Brilliant Violet 421™	Syrian Hamster IgG	2F1/KLRG1	BioLegend GmbH	138413	100
	CD183/CXCR3	PE/ Cy7	Armenian Hamster IgG	CXCR3-173	BioLegend GmbH	126515	100
	CD69	FITC	Armenian Hamster IgG	H1.2F3	BioLegend GmbH	104505	100
	PD-1	PE-Dazzle 594	Rat IgG2b, κ	RMP1-30	BioLegend GmbH	109115	100
Intracellular staining of T cells after stimulation	CD3	APC/Cyanine7	Rat IgG2b, κ	17A2	BioLegend GmbH	100221	100
	CD4	FITC	Rat IgG2a, κ	RM4-5	BioLegend GmbH	100509	100
	CD8a	Brilliant Violet 785™	Rat IgG2a, κ	53-6.7	BioLegend GmbH	100749	100
	TCR γ/δ	Brilliant Violet 510™	Armenian Hamster IgG	GL3	BioLegend GmbH	118131	50
	T-bet	Brilliant Violet 711™	Mouse IgG1, κ	4B10	BioLegend GmbH	644819	100
	GATA-3	PE	Rat IgG2b, κ	IM7	BioLegend GmbH	653803	100
	RORγT	PE-CF594	Mouse IgG2a	Q31-378	BD Biosciences	562684	100
	FoxP3	PE-Cy5.5	Rat / IgG2a, κ	FJK-16s	ThermoFisher Scientific	35-5773-80	100
	IFN-γ	Brilliant Violet 605™	Rat IgG1, κ	XMG1.2	BioLegend GmbH	505839	150
	IL-4	Brilliant Violet 650™	Rat IgG1	11B11	BD Biosciences	564004	100
	IL-17A	Brilliant Violet 421™	Rat IgG1, κ	TC11-18H10.1	BioLegend GmbH	506925	100
	IL-10	PE-Cy7	Rat IgG2b, κ	JES516E3	BioLegend GmbH	505025	50
	Granzyme B	Alexa Fluor® 647	Mouse IgG1, κ	GB11	BioLegend GmbH	515405	100