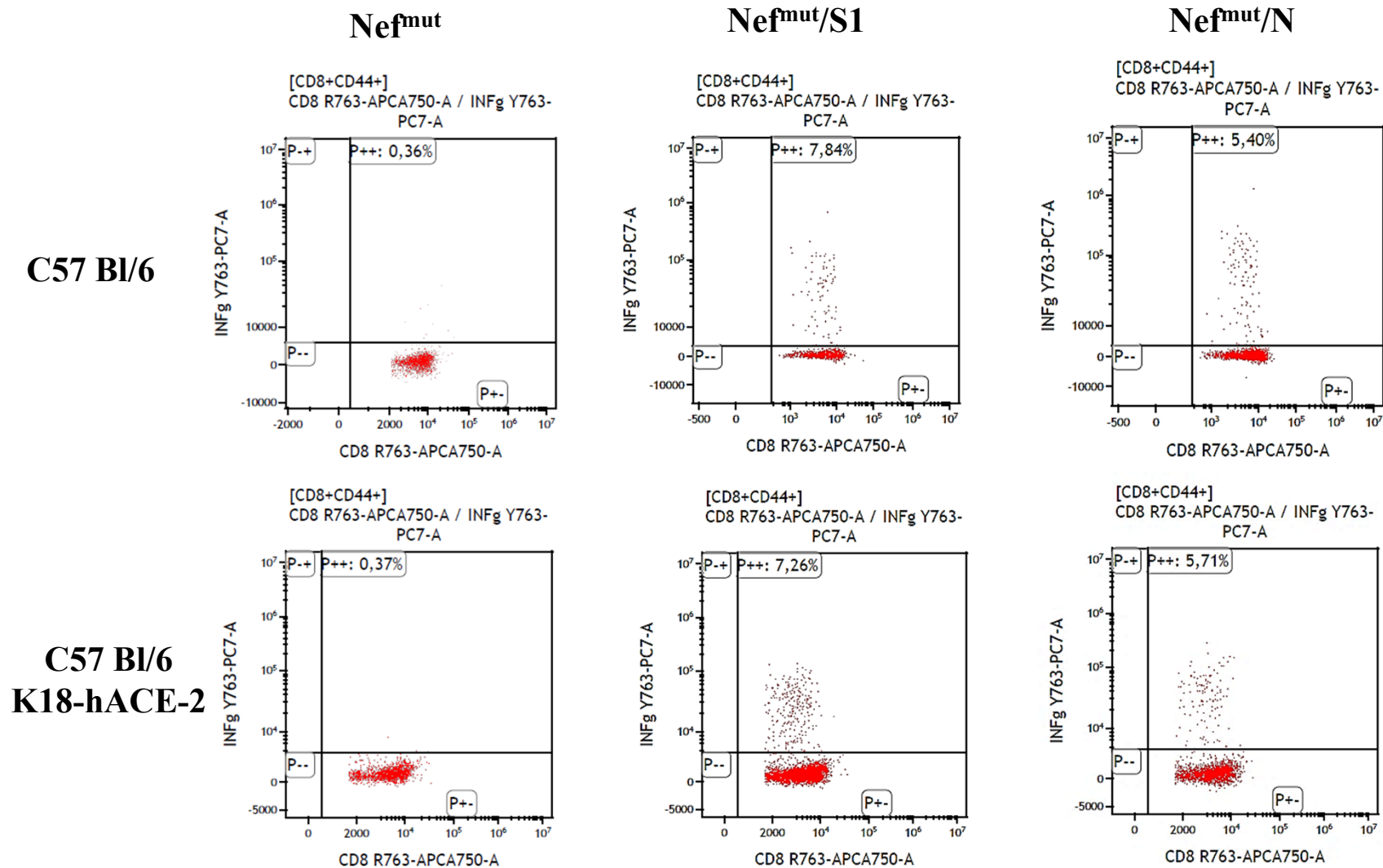
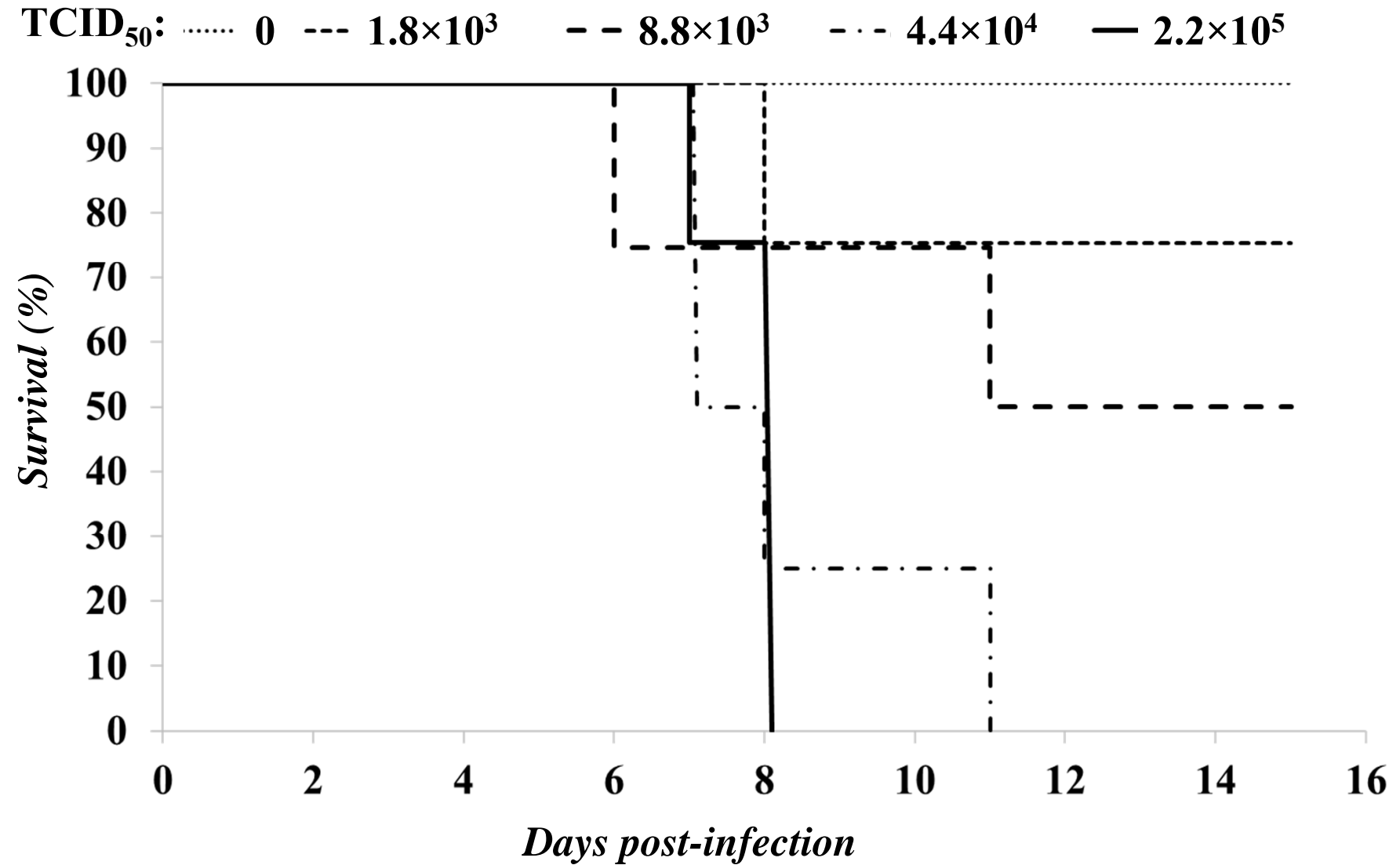


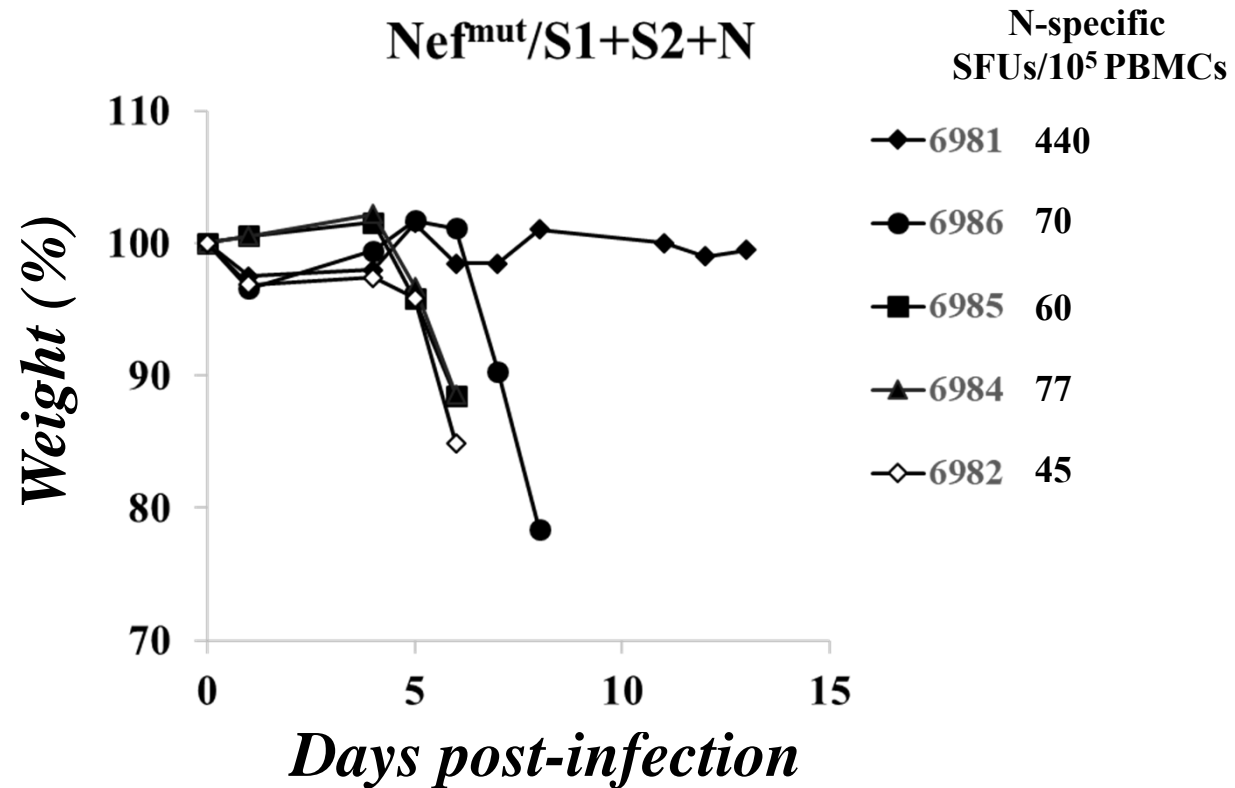
Supplementary Figure S1. Gating strategy carried out in ICS/flow cytometry analysis of splenocytes from injected mice. Shown is the analysis on PMA-treated cells.



Supplementary Figure S2. ICS/flow cytometry analysis for the expression of IFN- γ in splenocytes from either C57 Bl/ or K18-hACE-2 mice. The mice were injected with vectors expressing either $Nef^{mut}/S1$, Nef^{mut}/N or, as control, Nef^{mut} alone, and cultivated overnight with SARS-CoV-2 specific peptides. Shown are rough data representative of the results obtained with splenocytes pooled from two mice per condition. Quadrants were set on the basis of cell fluorescence of samples treated with an unrelated peptide.



Supplementary Figure S3. Kaplan–Meier survival curve. It was calculated for groups (n=4) of C57 Bl/6 K18-hACE-2 mice infected with 5-fold dilutions of the SARS-CoV-2 preparation.



Supplementary Figure S4. Relative weight loss in mice injected with combination vectors. Mice were injected with 10 µg each of Nef^{mut}/S1, Nef^{mut}/S2 and Nef^{mut}/N expressing vectors, and then infected with 5 LD₅₀ of SARS-CoV-2. Identification numbers for each mouse are reported on the right together with the counts of SFUs/10⁵ PBMCs after incubation with the N₂₁₉₋₂₂₈ peptide. S2-specific SFU counts as measured using a pool of S2 peptides were similar in each mouse.

Linear Regression (SFU vs. Survival)

Number of points = 7

Best-fit Standard 95% confidence interval

Parameter Value Error from to

Slope 28.411 6.115 12.690 44.131

Y intercept -162.40 69.372 -340.75 15.956

X intercept 5.716

Correlation coefficient (r) = 0.9011. r squared = 0.8119

Standard deviation of residuals from line (Sy.x) = 60.355

Test: Is the slope significantly different from zero?

The P value is 0.0056, considered very significant.

This result was obtained from the following ANOVA table.

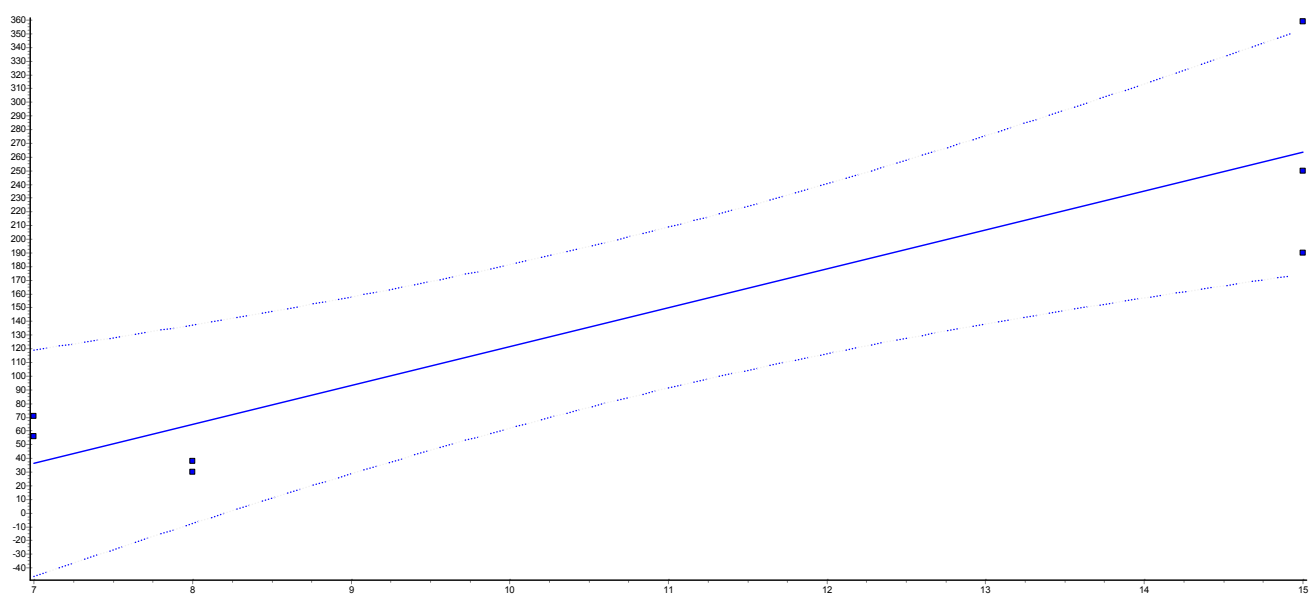
Source of variation	Degrees of freedom	Sum of squares	Mean square
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Linear regression (Model)	1	78640	78640
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Deviations from linearity (Residual)	5	18214	3642.7
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Total	6	96854	
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F = 21.588



Linear Regression (SFU vs. Weight)

Number of points = 7

Best-fit Standard 95% confidence interval

Parameter Value Error from to

Slope 0.06887 0.01014 0.04280 0.09495

Y intercept 83.948 1.870 79.140 88.757

X intercept -1218.9

Correlation coefficient (r) = 0.9498. r squared = 0.9022

Standard deviation of residuals from line (Sy.x) = 3.157

Test: Is the slope significantly different from zero?

The P value is 0.0011, considered very significant.

This result was obtained from the following ANOVA table.

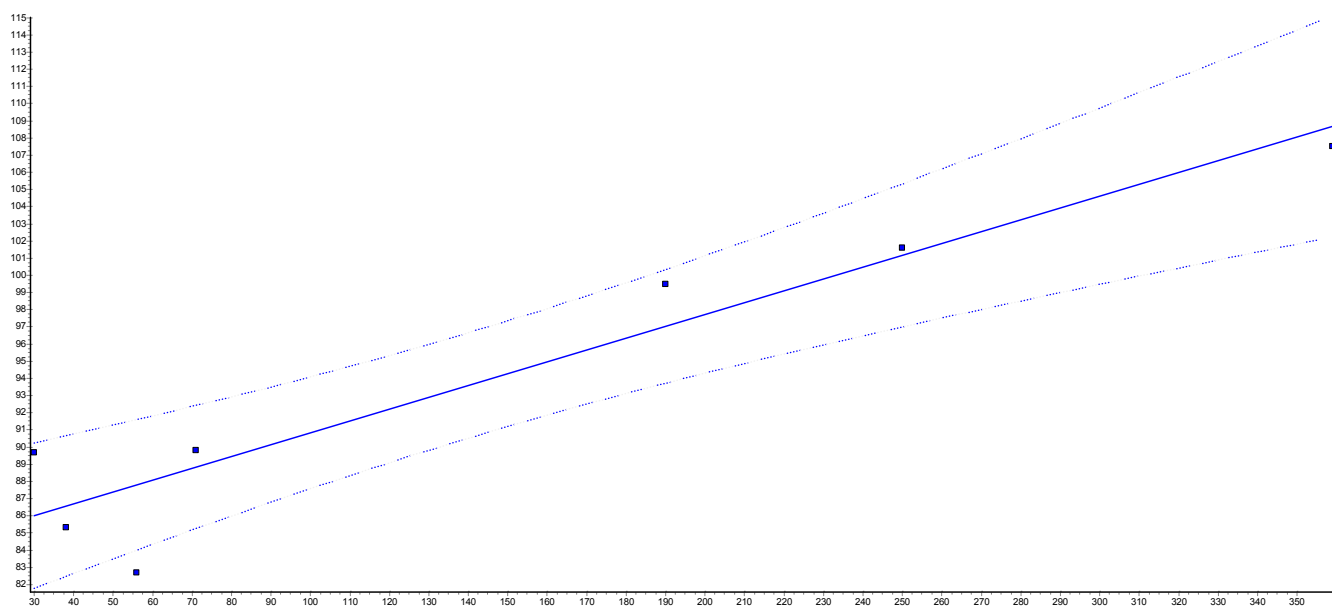
Source of variation	Degrees of freedom	Sum of squares	Mean square
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Linear regression (Model)	1	459.44	459.44
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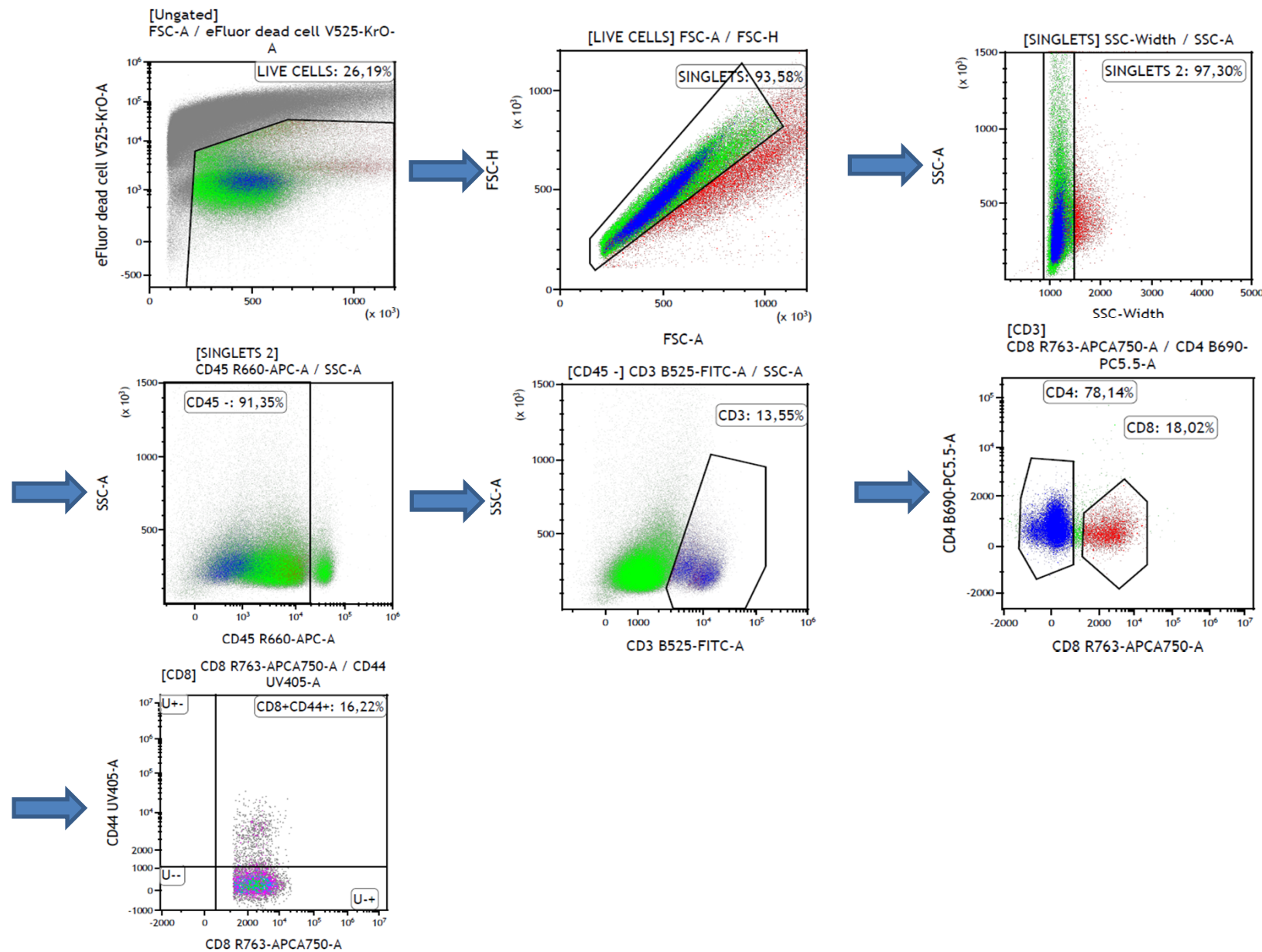
Deviations from linearity (Residual)	5	49.818	9.964
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Total	6	509.25	
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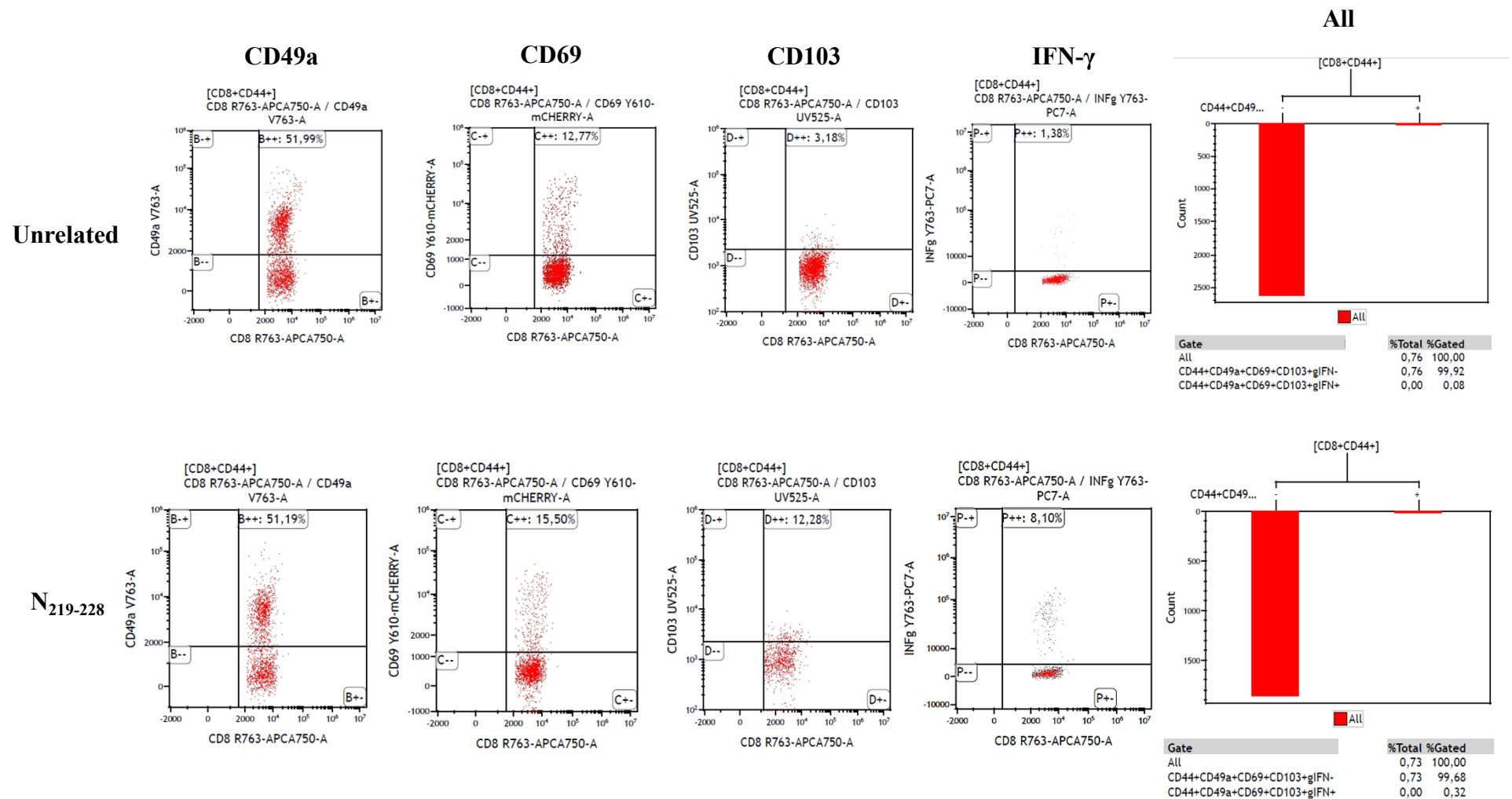
F = 46.111



Supplementary Figure S5. Correlation between anti-N SFUs and protection in terms of both mouse relative body weight at day 6/7 post-infection and survival. Regression curves and details of the analysis.



Supplementary Figure S6A. Gating strategy carried out in ICS/flow cytometry analysis of cells pooled from lungs of two mice injected with Nefmut/N expressing vector. Two micrograms of anti-CD45 mAbs (Tonbo-Bioscience CD45.2-APC) diluted in a total of 200 μ L of 1 \times PBS were i.v.injected three minutes before sacrifices. The labeling efficiency of leukocytes was 87%.



Supplementary Figure S6B. ICS/flow cytometry analysis on cells isolated from lungs of mice immunized with vectors expressing Nef^{mut}/N and injected with anti-CD45 mAbs 3 minutes before sacrifice. Shown are the rough data obtained by analyzing cells pooled from lungs of two representative mice, and treated with either unrelated or N-specific peptides. On the right, Boolean-gating based analysis for the co-expression of all considered markers.