

Fig. S1

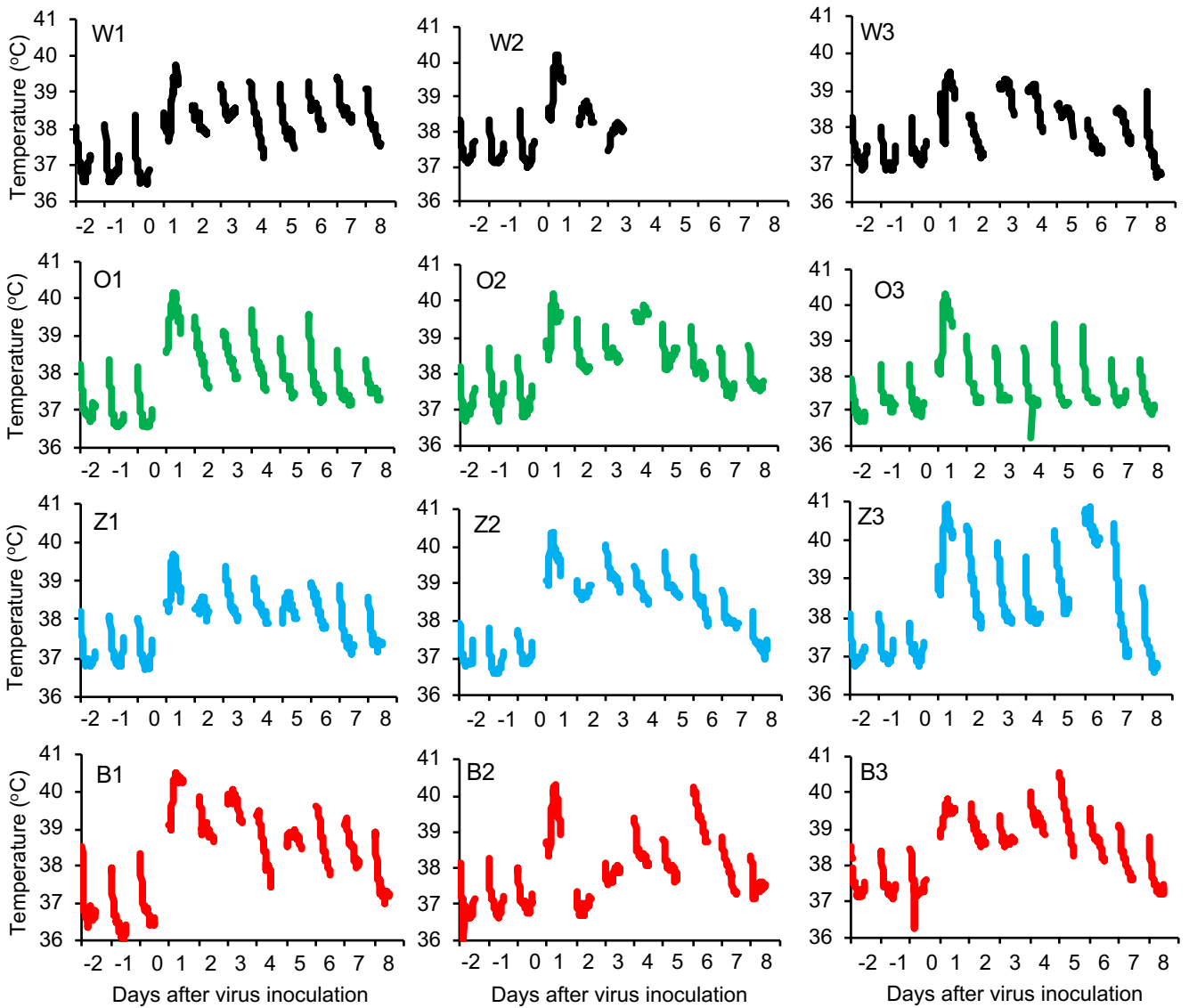


FIG S1 Body temperatures of macaques infected with Dk/HE29-22.

Cynomolgus macaques (n = 3 in each group) were inoculated with Dk/HE29-22. Body temperatures of the macaques were recorded using telemetry transmitters and a computer. Temperatures from 8 p.m. to 8 a.m. are shown in the graphs since temperatures during the daytime were affected by anesthesia.

Fig . S2

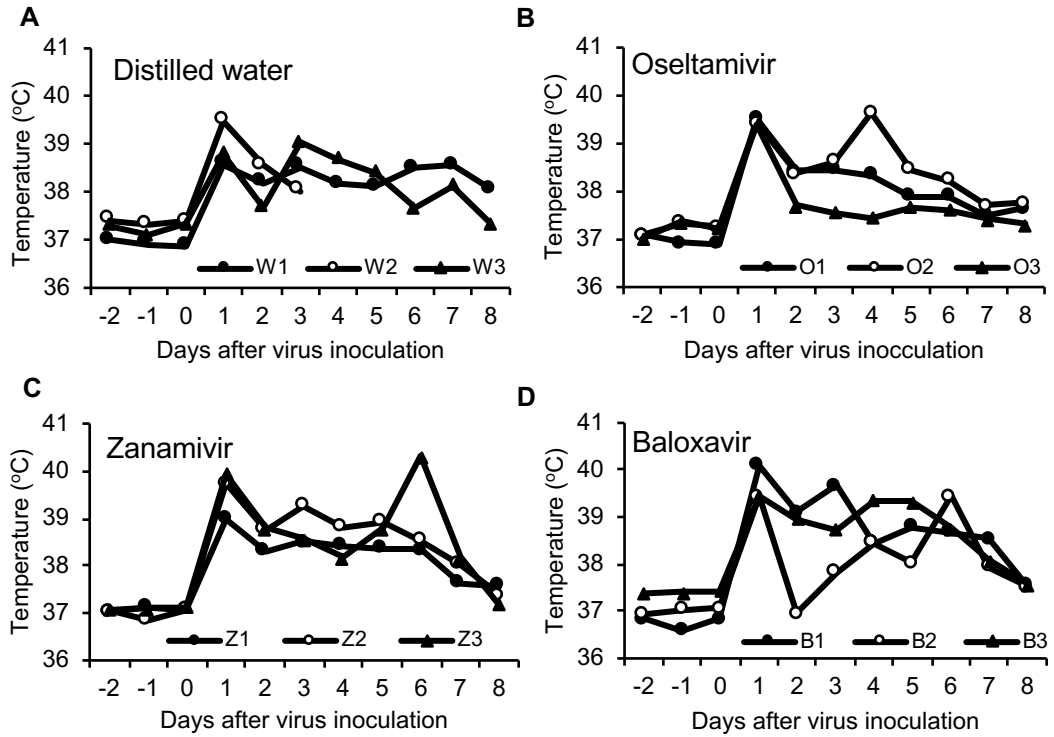


FIG S2 Average body temperatures of macaques infected with Dk/HE29-22.

Cynomolgus macaques were inoculated with Dk/HE29-22. (A - D) Average temperatures from 8 p.m. to 8 a.m. the next day were calculated on the basis of data for individual macaques (Fig. S1).

Table S1. Clinical scoring used in the present study.

Parameter	Degree of parameter	Possible score
Fever	Normal (< 39°C)	0
	Elevated temperature (39 - 40°C)	3
	High temperature (> 40°C)	5
Posture	Piloerection of body hair	1
	Decreased activity, decreasing normal behavior/Occasionally lying down, huddled, active when people in room	2
	Huddled on camera, active when people in room/Lying down, getting up when approached, using cage for support	3
	Huddled when people in room, shaking, toes and hands clenched/Lying down, not getting up when approached or prompted	5
Respiration	Increased or decreased; mild cough and clear nasal discharge	3
	Labored breathing through mouth; severe cough and severe nasal discharge	5
Appetite	Slightly decreased	1
	Decreased	2
	Severely decreased	5
Skin	Flushed appearance	2
	Visible rash	2
	Bleeding	5

Animals were monitored every day during the study to be clinically scored. Animals would be scheduled to be euthanized if their clinical scores reached 15 (humane endpoint).

Fig. S3

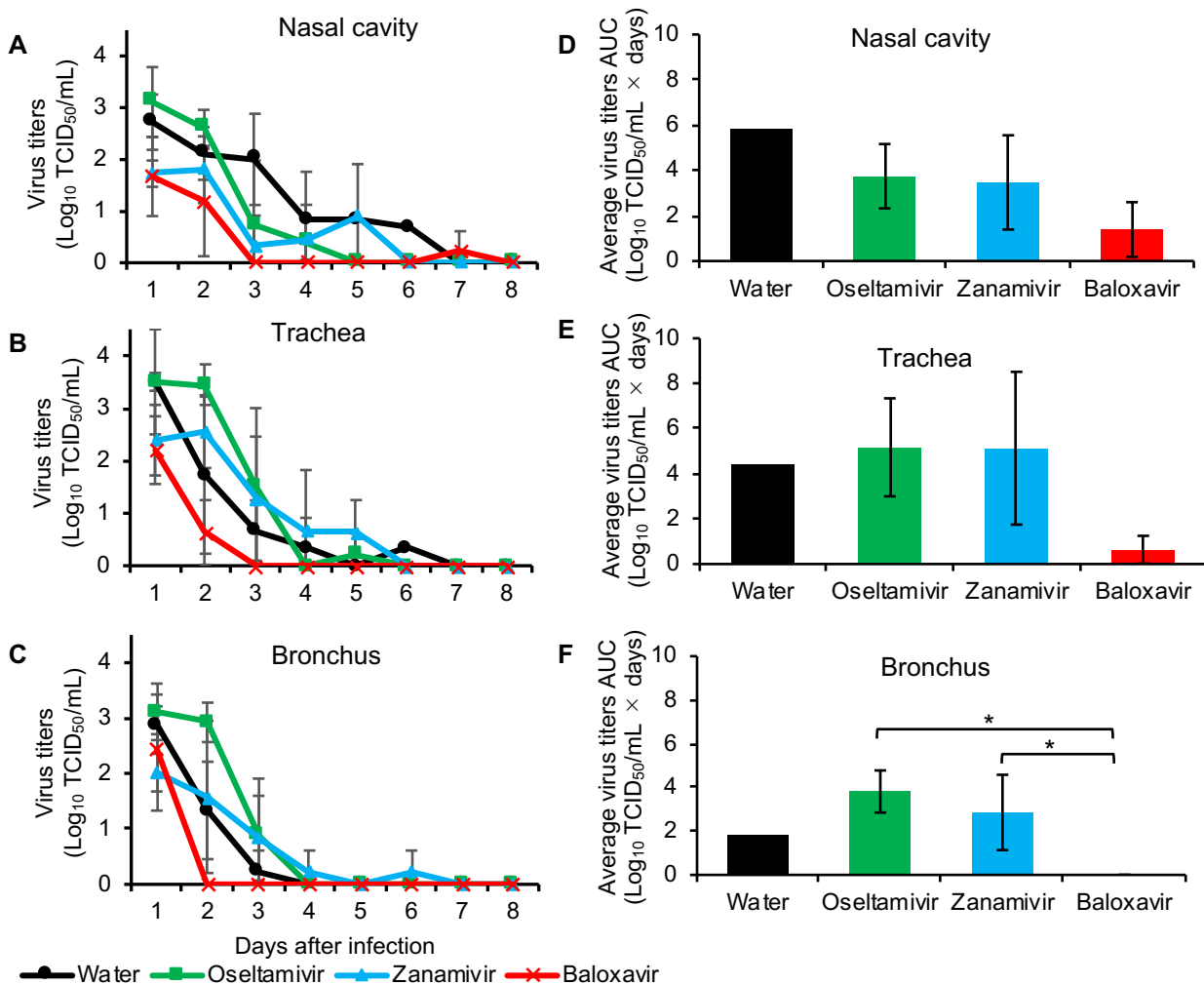


FIG S3 Virus titers in swabs from macaques infected with Dk/HE29-22.

Cynomolgus macaques were inoculated with Dk/HE29-22 on day 0. (A - C) Nasal, tracheal and bronchial samples were collected on the indicated days. Averages of virus titers in the nasal (A), tracheal (B), and bronchial (C) samples were calculated on the basis of the individual titers listed in Table 1. (D - F) Virus titer AUC values after treatment (2 to 8 days after virus inoculation) in the nasal (D), tracheal (E) and bronchial (F) samples were calculated on the basis of the individual titers listed in Table 1. Averages and standard deviations of the results of three macaques are shown except for the DW group. Virus titer AUC in the DW group was calculated with exclusion of W2. Virus titers under the detection limit were calculated as 0. Asterisks indicate significant differences between two groups ($P < 0.05$, Mann-Whitney U test). Black: DW group, green: oseltamivir group, blue: zanamivir group, and red: baloxavir group.

Table S2. Virus titers in tissue samples of cynomolgus macaques infected with Dk/HE29-22.

Tissue ^a	Virus titers (log ₁₀ TCID ₅₀ /g tissue)											
	Distilled water			Oseltamivir			Zanamivir			Baloxavir		
	W1	W2	W3	O1	O2	O3	Z1	Z2	Z3	B1	B2	B3
nasal mucosa	< ^b	<	<	<	<	<	1.67 ^c	<	<	<	<	<
oro-nasopharynx	<	1.67 ^c	<	<	1.83 ^c	<	1.67 ^c	<	<	<	<	<
tonsil-R	2.00 ^c	<	<	<	<	<	3.18 ^c	<	1.67 ^c	<	<	<
tonsil-L	1.67 ^c	-	3.50	4.50	<	<	1.67 ^c	<	3.50	<	<	2.33 ^c
trachea	<	<	<	<	<	<	1.67 ^c	<	<	<	<	<
bronchus-L	<	2.50 ^c	<	<	<	<	<	<	<	<	<	<
lung RU	<	4.00	<	<	<	<	<	<	<	<	<	<
lung RM	<	3.67	<	<	<	<	<	<	<	<	<	<
lung RL	<	2.50	<	<	<	<	<	<	<	<	<	<
lung LU	<	4.00	<	<	<	<	<	<	<	<	<	<
lung LM	<	3.83	<	<	<	<	<	<	<	<	<	<
lung LL	<	3.50	<	<	<	<	<	<	<	<	<	<
conjunctiva	<	<	1.67 ^c	<	<	<	1.67 ^c	<	<	<	<	<

^a The samples were collected at autopsy on day 8 and day 3 (only for W2) after virus inoculation. R: right, L: left, RU: right upper, RM: right middle, RL: right lower, LU: left upper, LM: left middle, LL: left lower. No virus was detected in bronchus-R, pulmonary hilar lymph node, heart, spleen, kidney, liver, rectum, eye, brain (frontal lobe, temporal lobe, parietal lobe, occipital lobe, cerebellum, brainstem, olfactory bulb) and muscle.

^b virus titers were under the detection limit (1.67 log₁₀TCID₅₀/g tissue).

^c Some of the four wells were positive for a CPE in quadruplicate culture of undiluted samples.

Fig. S4

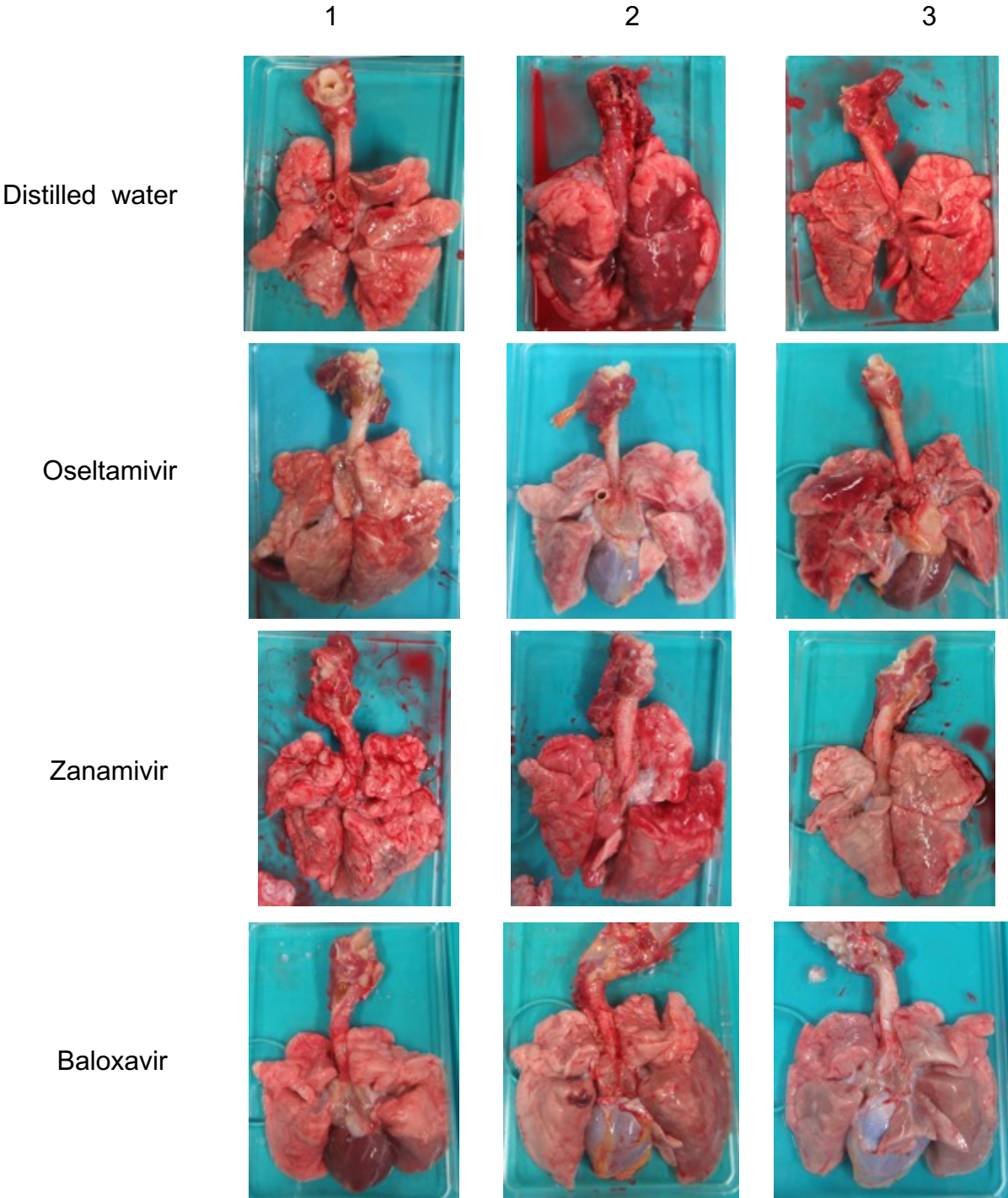


FIG S4 Gross appearance of lungs of cynomolgus macaques infected with Dk/HE29-22. The organs were collected at autopsy 8 days or 3 days (only for W2) after virus inoculation.

Table S3. Lung tissue inflammation scoring used in the present study

Histological change	Score			
	0	1	2	3
BALT	-	+		
Infiltrating eosinophils	-	+	++	+++
Infiltrating neutrophils	-	+	++	+++
Infiltrating lymphocytes	-	+	++	+++
Thickened alveolar wall	-	+ (focal)	++	+++ (diffuse)
Bleeding	-	+ (focal)	++	+++ (diffuse)
Exudate	-	+ (focal)	++	+++ (diffuse)

Table S4. Levels of cytokines and chemokines in plasma and CSF of W2.

Cytokine (pg/mL)	Days after virus infection			CSF ^b
	0 ^a	1	3	
IFN- α	ND ^c	6785.25 ^d	1959.76	ND
IFN- β	ND	195.07	15.61	4.43
IFN- γ	2.28	39.13	9.53	0.21
MIP-1 α	ND	ND	ND	11.59
IL-6	0.72	954.50	47.63	32.33
TNF- α	ND	ND	ND	3.83
MCP-1	117.25	981.87	365.34	1125.55
IL-13	4.80	ND	ND	10.60
IL-2	ND	ND	ND	1.22
IL-12	3.46	5.62	1.30	ND
IL-10	13.11	45.28	12.18	ND
IL-8	251.36	390.10	283.38	71.02
IP-10	28.72	28.88	64.90	ND
Eotaxin	ND	8.49	ND	ND
MIP-1 β	ND	ND	ND	ND

^a Plasma was collected on day 0 before virus infection and on days 1 and 3 after virus inoculation.

^b Cerebrospinal fluid (CSF) was collected on day 3 after virus inoculation at autopsy.

^c not detected.

^d The concentrations (pg/mL) of cytokines and chemokines in plasma collected from W2 were indicated.

Fig. S5

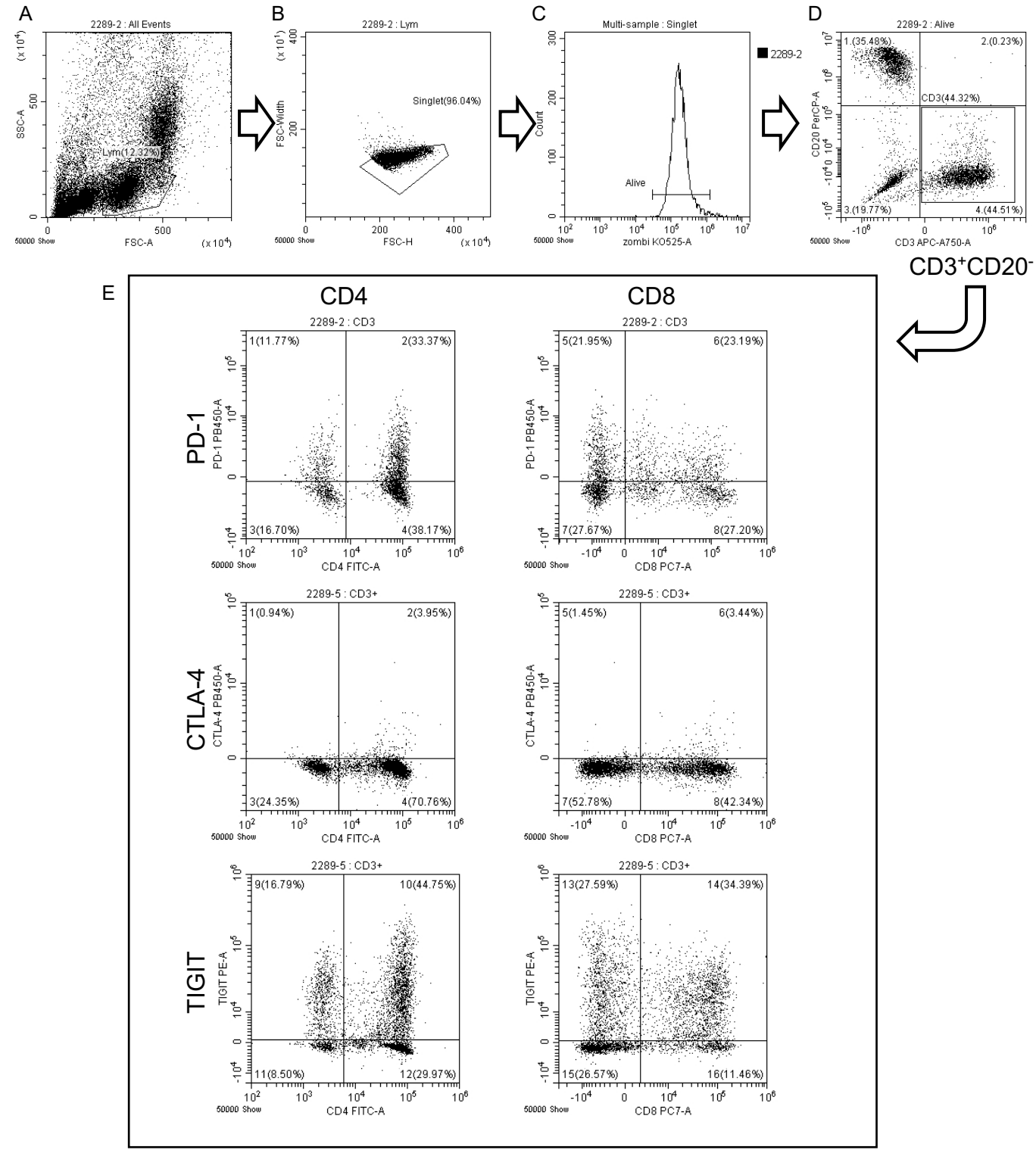


FIG S5 Gating for flow cytometry analysis of surface molecules

(A) Lymphocytes in peripheral blood cells (low FSC/low SCC) were selected to exclude granulocytes. (B) Single cells not doublet cells (low FSC width) were selected. (C) Live cells (negative for Zombie) were selected. (D) CD3 positive and CD20 negative cells (T lymphocytes) were analyzed in E. A representative analysis for W2 on day 3 is shown.