frontiers

Supplementary Material

Isoprinosine as a foot-and-mouth disease vaccine adjuvant elicits robust host defense against viral infection through immunomodulation

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Supplementary Table legends

Supplementary Table 1. List of primer sequences for qRT-PCR.

Supplementary Figure legends

Supplementary Figure 1. Cytotoxicity of isoprinosine measured by cell viability assay in BHK-21, LF-BK, ZZ-R, murine PECs and porcine PBMCs.

(A–E) Cell viability of BHK-21 **(A)**; LF-BK **(B)**; ZZ-R cells **(C)**; murine PECs **(D)**; and porcine PBMCs **(E)**.

Data have been represented as the mean ± SEM of triplicate measurements (n = 3/group). Statistical analyses were performed using one-way ANOVA with Dunnett's *post hoc* test.

Supplementary Figure 2. Isoprinosine alone-mediated host defense in early stage of FMDV infection on mice.

C57BL/6 mice (6–7 weeks-old, n = 5/group) were administered intramuscularly an isoprinosine alone. Mice were challenged with FMDV O (100 LD_{50} O/VET/2013) or FMDV A (100 LD_{50} A/Malay/97) at 3 or 7 days post-injection (dpi) using an intraperitoneal injection. Survival rates and body weights were monitored for 7 days post-challenge (dpc) with the respective viruses. **(A–E)** experimental workflow **(A)**; survival rates in 3 dpi challenged group with O/VET/2013 **(B)** or A/Malay/97 **(C)**; changes in body weight 3 dpi challenged group with O/VET/2013 **(D)** or A/Malay/97 **(E)**; survival rates in 7 dpi challenged group with O/VET/2013 **(F)** or A/Malay/97 **(G)**; and changes in body weight 7 dpi challenged group with O/VET/2013 **(H)** or A/Malay/97 **(I)**. Data are presented as mean ± SEM of triplicate measurements (n = 5/group).

Supplementary Figure 3. FMD vaccine containing isoprinosine-mediated antibody titers by SP O ELISA using PrioCheck™ kit in pigs.

For the challenge experiments, FMDV type O and type A antibody-seronegative pigs (8–9 weeks old, n = 5–6/group) were administered FMD vaccine including FMDV type O (O PA2) and type A (A YC) antigen (15+15 μg/dose/mL, one dose for cattle and pig use) with Isoprinosine (1 mg/dose/pig), ISA 206 (oil-based emulsion, 50%, w/w), 10% $Al(OH)_3$, and 150 μg Quil-A. One milliliter vaccine was prepared as a single dose and introduced into the animals via intramuscular (I.M.) injection. The positive control (PC) group and negative control (NC) group of pigs were treated with an equal volume of commercial FMD vaccine (O Primorsky+A Zabaikalski, ARRIAH-VAC® by FGBI "ARRIAH", Vladimir, Russia) and PBS, respectively, via the same route. Blood samples were collected at 0 and 28 days post-vaccination (dpv) in pigs for serological assays. Vaccinated pigs were challenged with FMDV type O (O/SKR/JC/2014) on the heel bulb at 10^5 $TCID_{50}$/100 μL at 28 dpv. Data are represented as the mean ± SEM of triplicate measurements (n = 5–6/group). Statistical analyses were performed using two-way ANOVA followed by Tukey's *post-hoc* test. $^{****}p < 0.0001$.

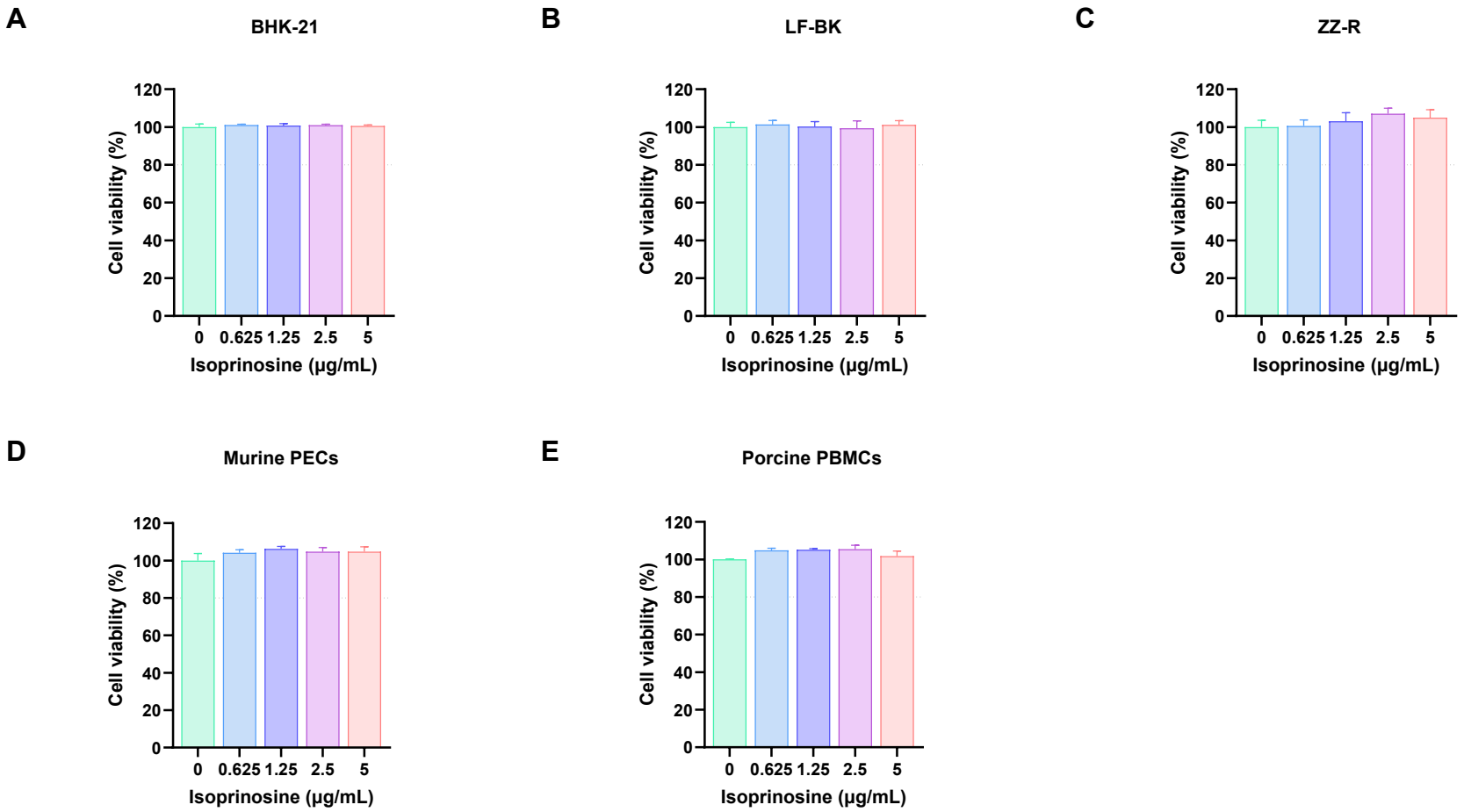
Supplementary Table 1

Target	Forward/Reverse	Sequence (5'- 3')	Length (mer)
RIG-I	RIG-I F	GCACCTCATACTTACAGCCCA	21
	RIG-I R	CCACAACCAGTAGGAGCACAT	21
TLR9	TLR9 F	TCCTCTACGACTGCATCACCA	21
	TLR9 R	GTAATTGAAGGACAGGTTGAGCTT	24
STAT1	STAT1 F	TGCACGATGGTCTCAGCTTT	20
	STAT1 R	CAGCAGTGGGACCAAGAAGT	20
STAT4	STAT4 F	ACATGTCAAAGCCATGTCCA	20
	STAT4 R	ATGTGACAGCCCTCATTTCC	20
MyD88	MyD88 F	CCATTCGAGATGACCCCCTG	20
	MyD88 R	TGCACAAACTGGGTATCGCT	20
TBX21	TBX21 F	ATCATCACCAAGCAGGGACG	20
	TBX21 R	CGTCCACGAACATCCGGTAA	20
EOMES	EOMES F	CGACTCCATGTACACCGCTT	20
	EOMES R	CGGGCTTGAGGTAAGGTGTT	20
NF-κB	NF-κB F	TCGCTGCCAAAGAAGGACAT	20
	NF-κB R	AGCGTTCAGACCTTCACCGT	20
IFNα	IFNα F	CATCTGCTCTCTGGGCTGTG	20
	IFNα R	TGAGGGGATCCAAAGTCCCT	20
IFNβ	IFNβ F	TGCAACCACCACAATTCCAGA	21
	IFNβ R	GGTTTCATTCCAGCCAGTGC	20
IFNγ	IFNγ F	GCCATTCAAAGGAGCATGGAT	21
	IFNγ R	CTGATGGCTTTGCGCTGGAT	20
IL-1β	IL-1β F	AGCCAGTCTTCATTGTTCAGGT	22
	IL-1β R	TCATCTCTTTGGGGCCATCAG	21
IL-6	IL-6 F	CTGCAGTCACAGAACGAGTG	20
	IL-6 R	CGGCATCAATCTCAGGTGCC	20

Supplementary Table 1 (continued)

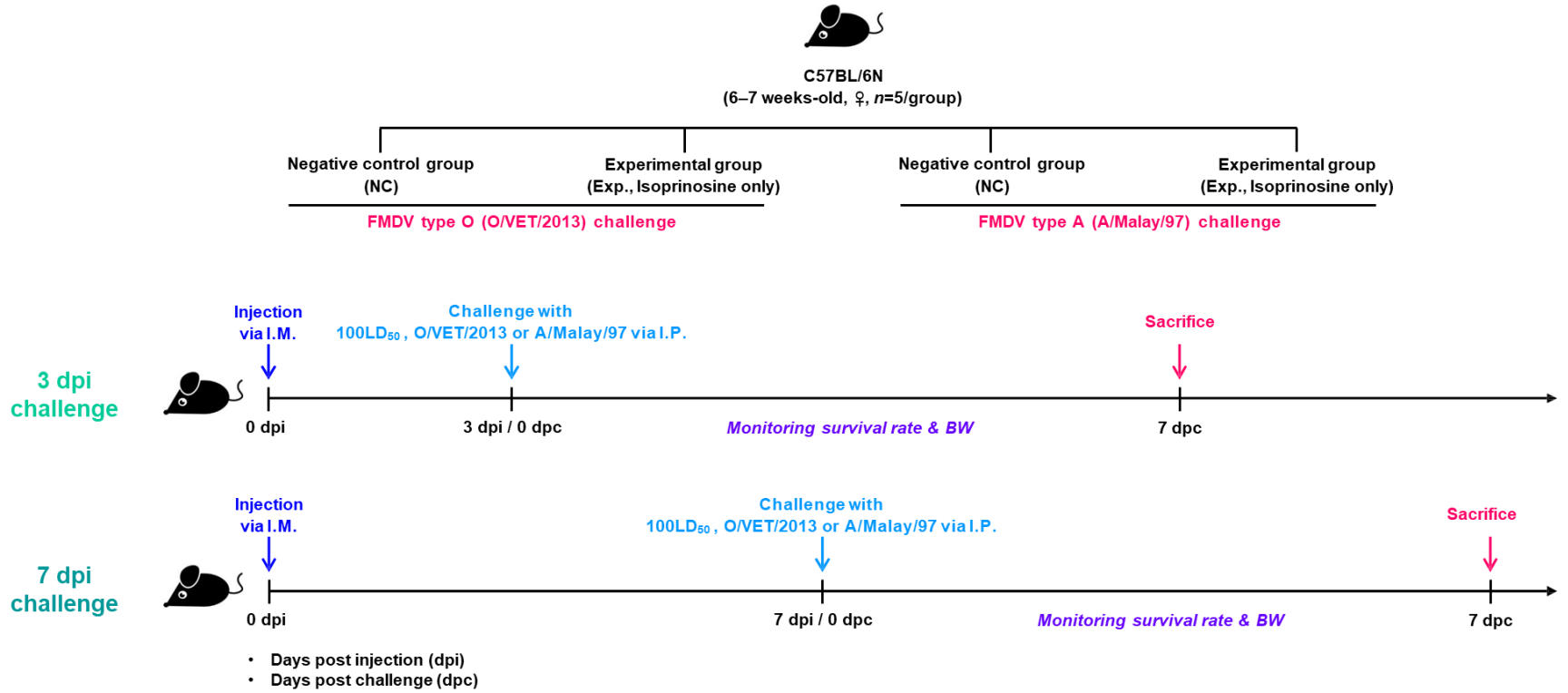
Target	Forward/Reverse	Sequence (5'- 3')	Length (mer)
IL-12p40	IL-12p40 F	GGAGTATAAGAAGTACAGAGTGG	23
	IL-12p40 R	GATGTCCCTGATGAAGAAGC	20
IL-23p19	IL-23p19 F	CCATATCCAGTGCGGGGATG	20
	IL-23p19 R	AGGCCTTGGTGGATCCTTTG	20
IL-23R	IL-23R F	TCCCTCATTGCAAAGCACAA	20
	IL-23R R	GCATCTCCTCTTGCAAGCAAAT	22
IL-17A	IL-17A F	CTCGTGAAGGCGGGAATCAT	20
	IL-17A R	GGTGTGCTCCGGTTCAAGAT	20
CD80	CD80 F	TCAGACACCCAGGTACACCA	20
	CD80 R	GACACATGGCTTCTGCTTGA	20
CD86	CD86 F	TTTGGCAGGACCAGGATAAC	20
	CD86 R	GCCCTTGTCCTTGATTTGAA	20
CD28	CD28 F	TCAAAGGAGTTCCGGGCATC	20
	CD28 R	CTGAAGCAGGCGGGAGTAAT	20
CD19	CD19 F	GGACGACAGACTTCCTGAGC	20
	CD19 R	GTTCTGGCCCATCAGGATTA	20
CD21	CD21 F	TGCCATGCCTACAAAGCTGA	20
	CD21 R	GTAGTAACCAGGGCGGCATT	20
CD81	CD81 F	TCAACAAGGACCAGATCGCC	20
	CD81 R	GAGCGTCTCGTGGAAAGTCT	20
HPRT	HPRT F	CCCAGCGTCGTGATTAGTGA	20
	HPRT R	GCCGTTCAGTCCTGTCCATA	20

Supplementary Figure 1

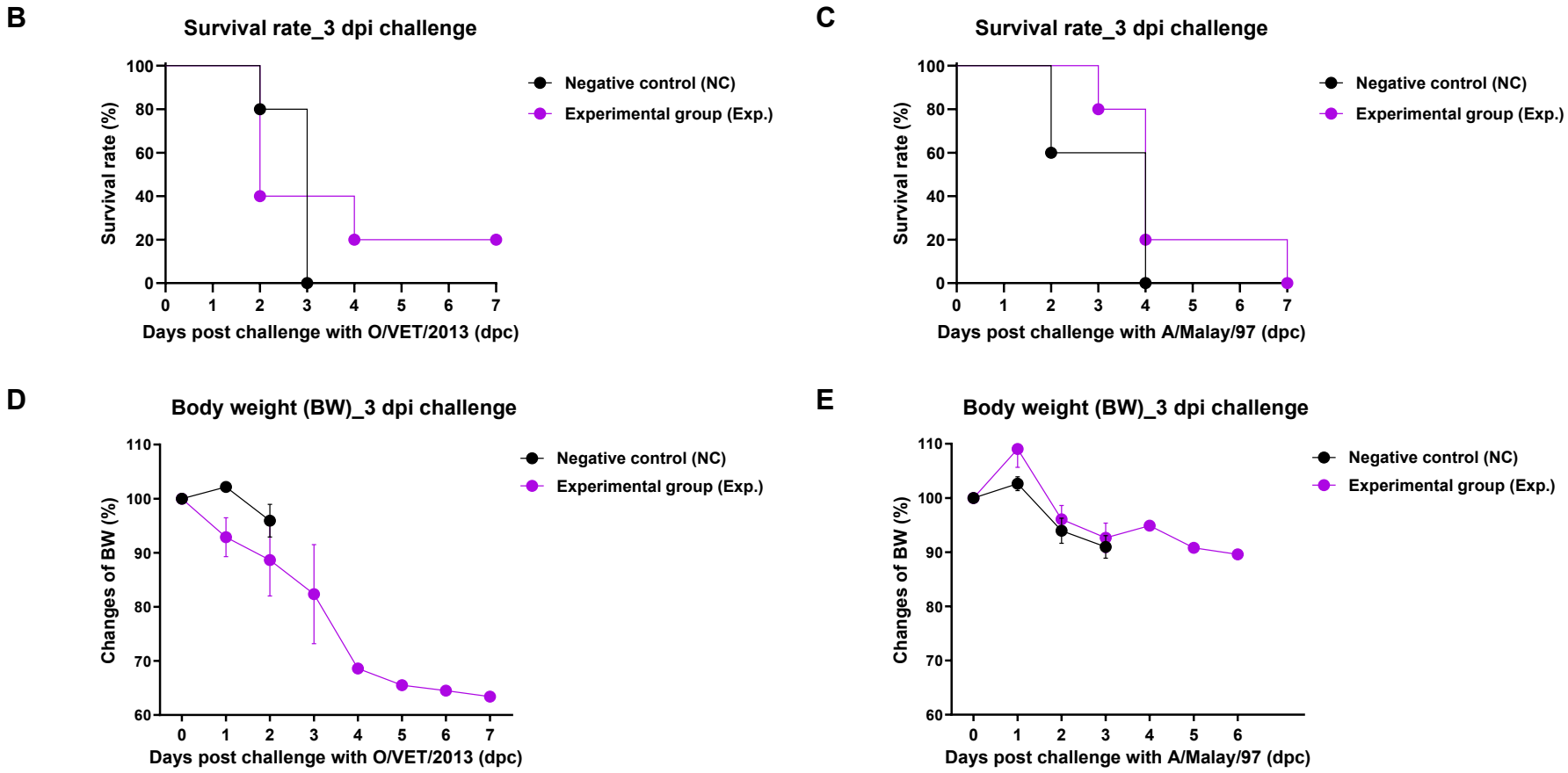


Supplementary Figure 2

A



Supplementary Figure 2 (continued)



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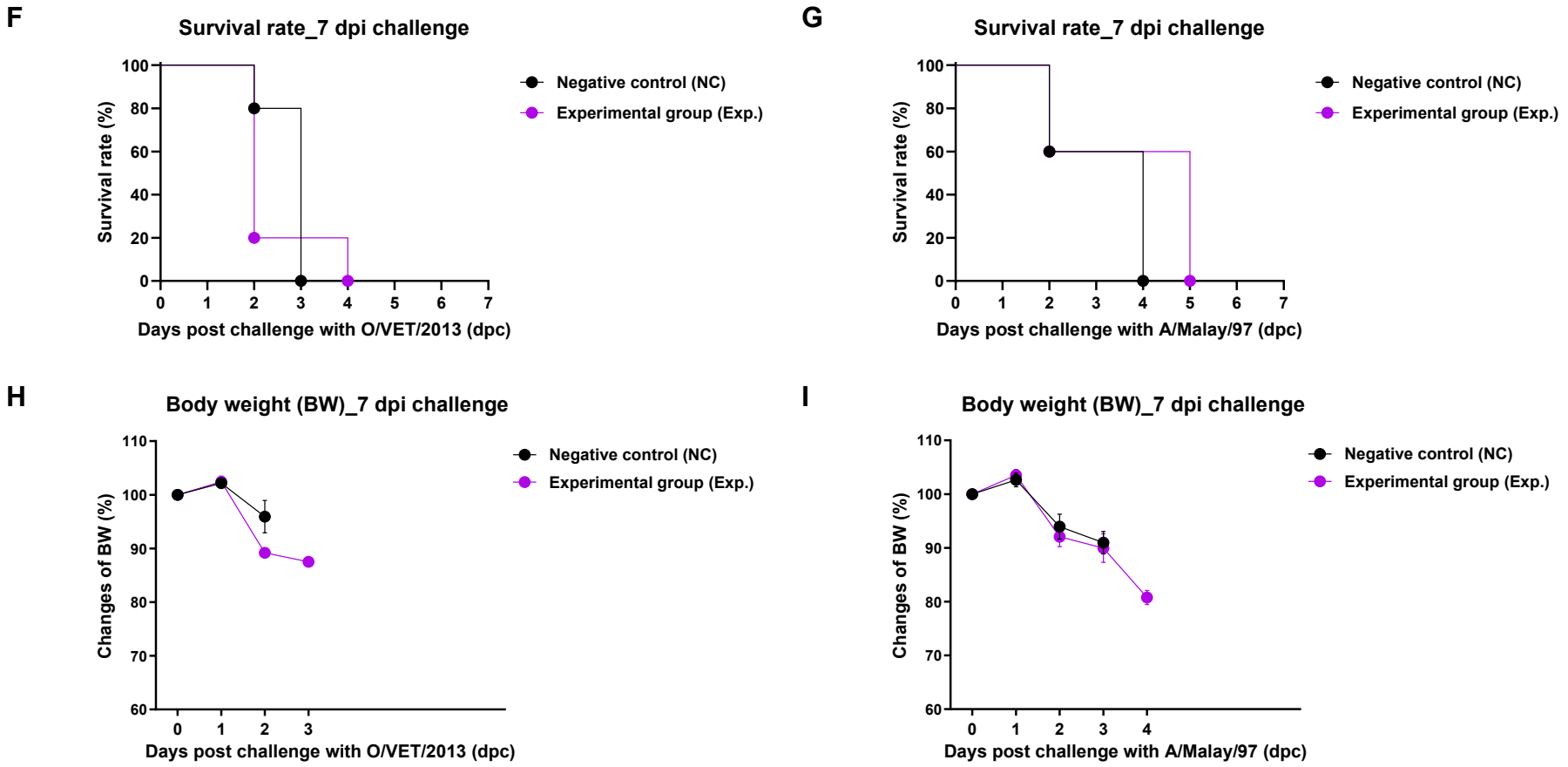

Supplementary Figure 3