

## **Early-stage IM treatment with the host-derived immunostimulant CPDI-02 increases curative protection of healthy outbred mice against subcutaneous infection with CA-MRSA USA 300**

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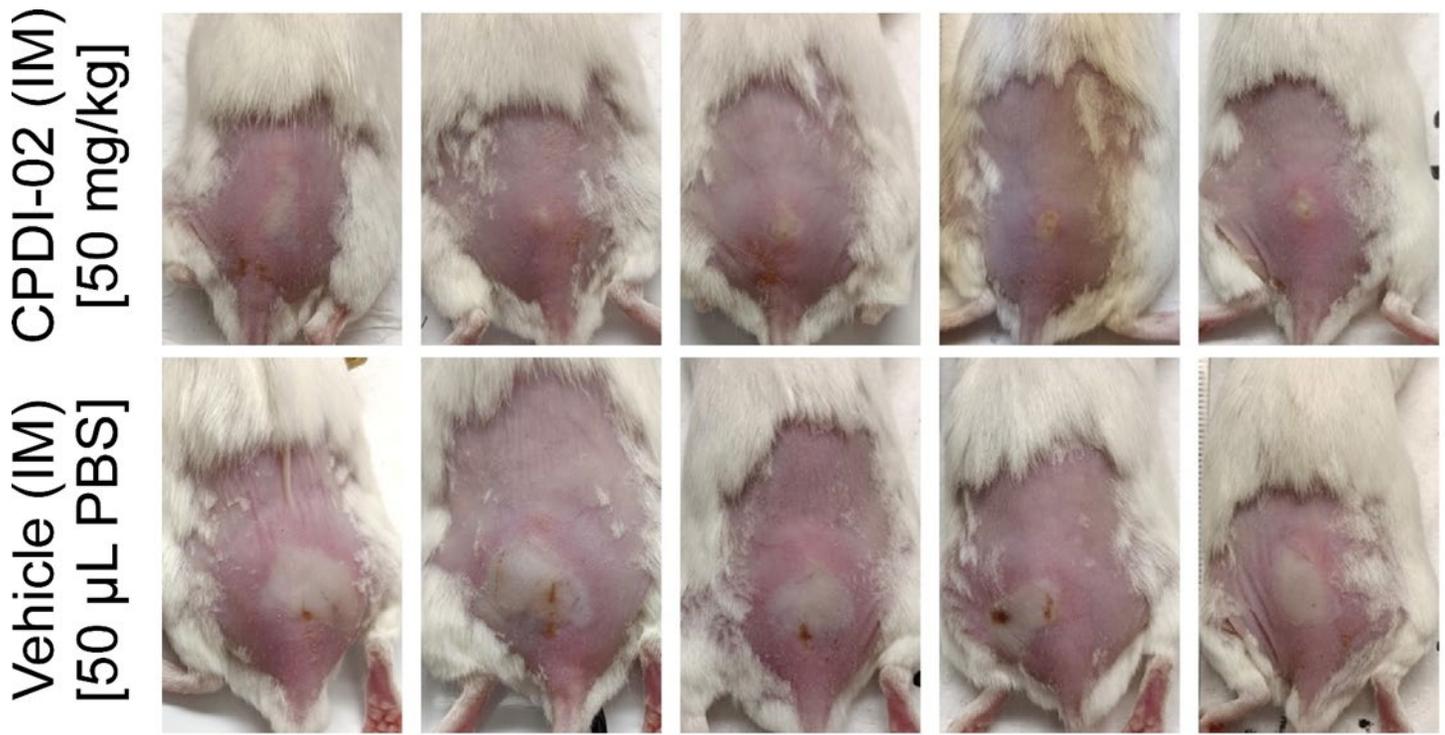
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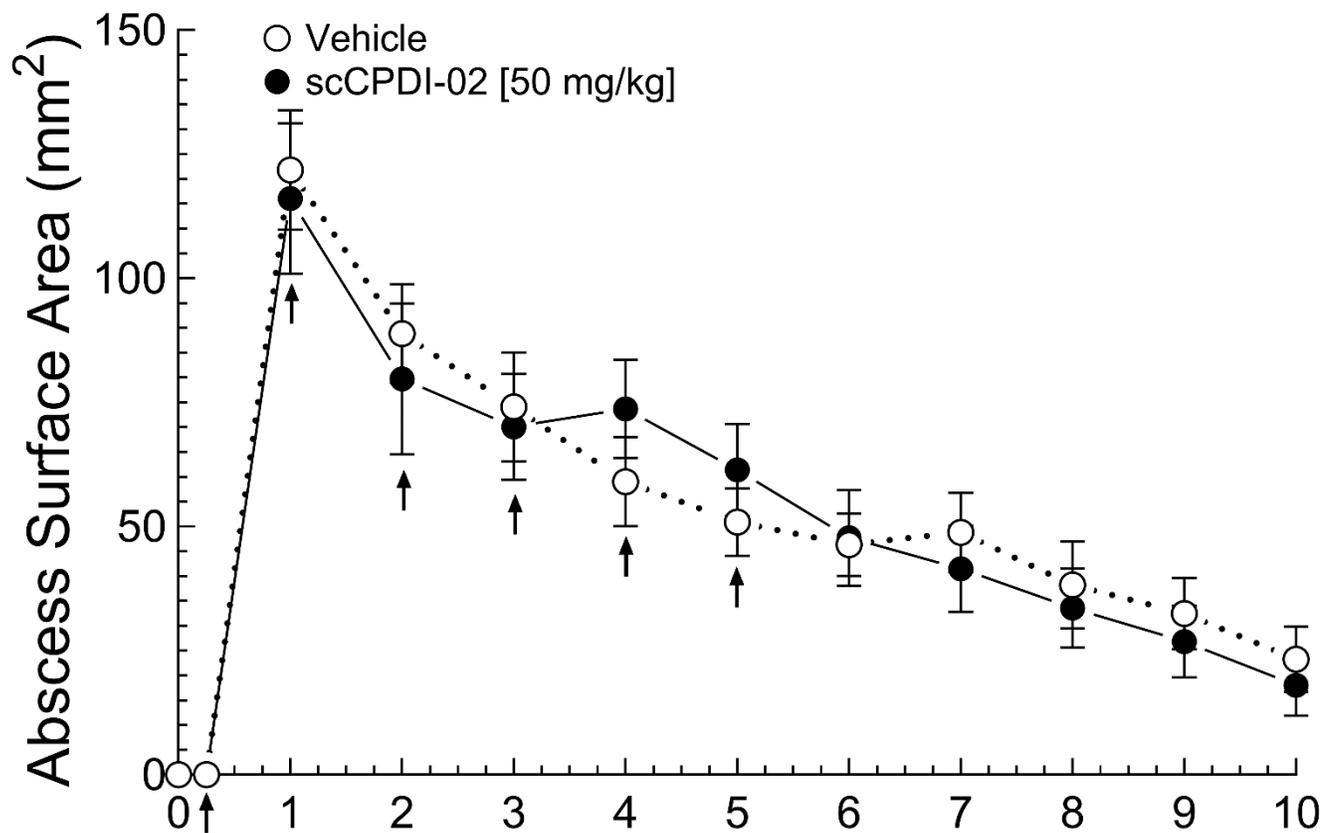
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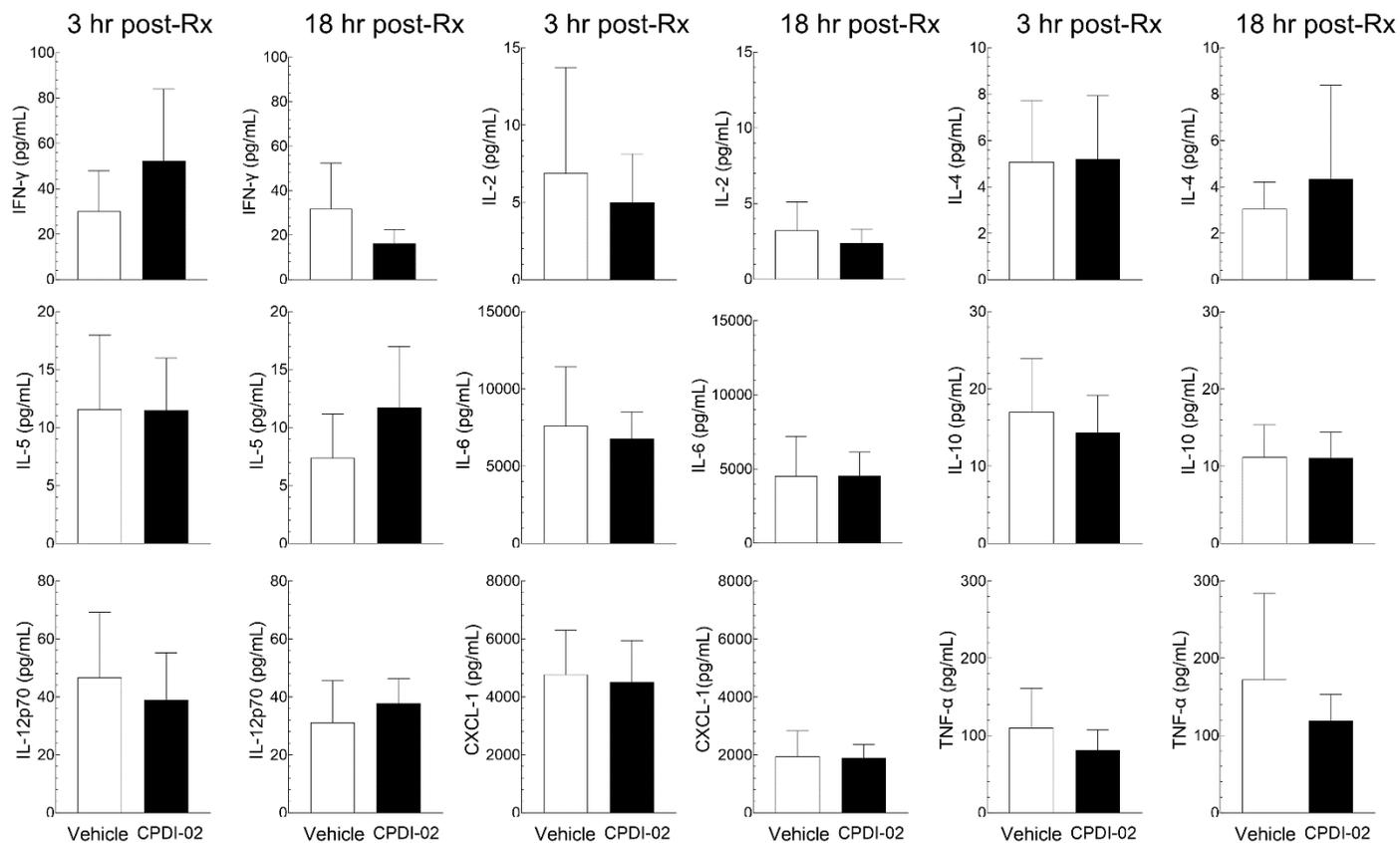
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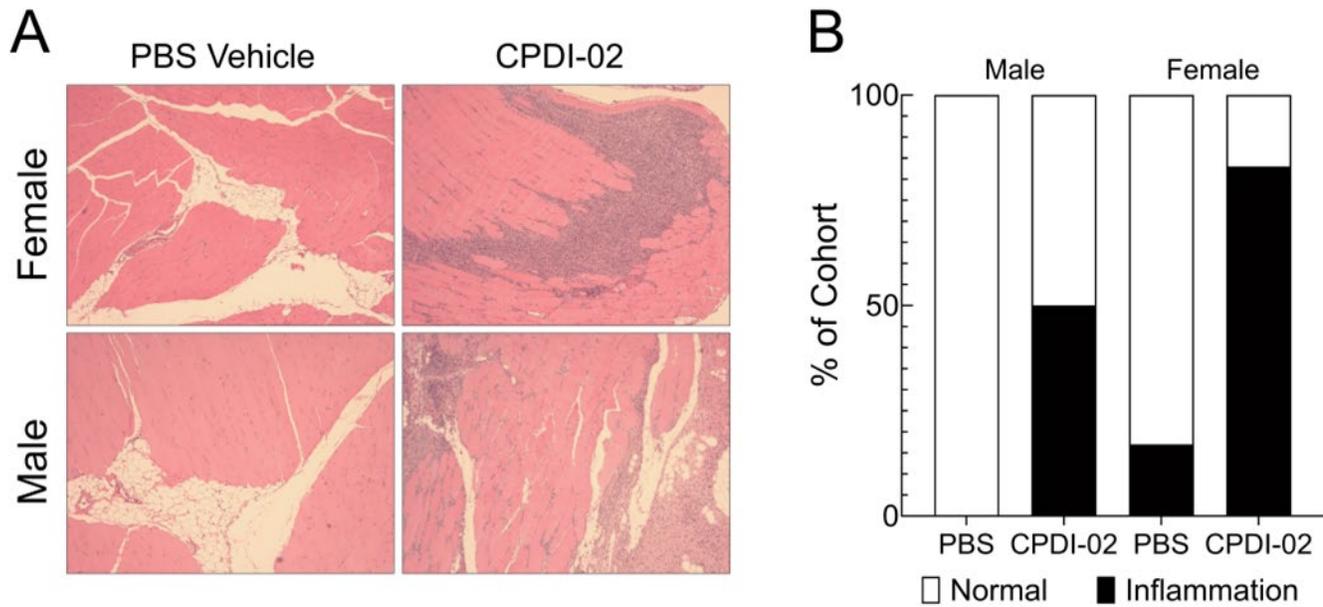
**Figure S1.** Representative images of dermal abscesses on the rear dorsal region of CPDI-02-treated and untreated healthy female outbred mice 24 hours after subcutaneous challenge with CA-MRSA. On Day 0, community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA, USA300 strain,  $5 \times 10^7$  CFU) was administered SQ (0.1 mL sterile PBS) from the left rear flank into the dorsal side of 4 to 6-week-old healthy female outbred CD-1 mice (Figs.2&3). At 6 hours post-challenge, vehicle alone (sterile PBS, 50 µL) or vehicle containing CPDI-02 [50 mg/kg] was administered in the caudal thigh muscle and abscess surface areas (n=10 mice per cohort; 5 representative mice shown) were determined 24 hours post-challenge by image analysis.



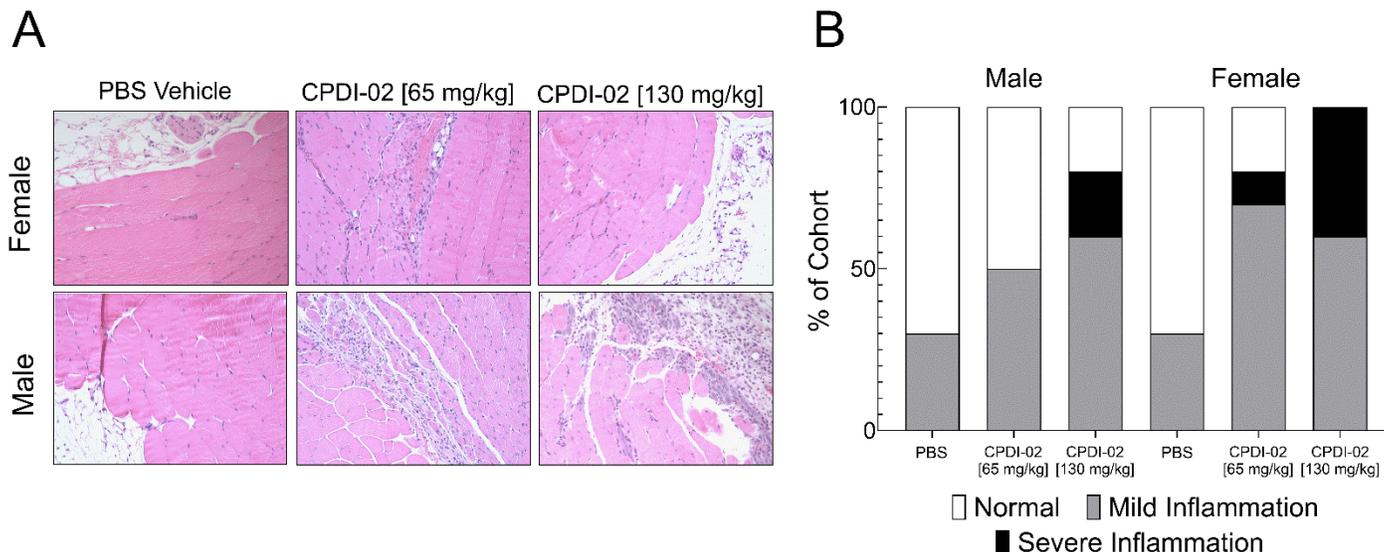
**Figure S2.** Repeat IM treatment with inactive, scrambled CPDI-02 does not increase curative protection of healthy female outbred mice against subcutaneous challenge with CA-MRSA. On Day 0, community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA, USA300 strain,  $5 \times 10^7$  CFU) was administered SQ (0.1 mL sterile PBS) from the rear left flank into the dorsal side of 6-week-old female outbred CD-1 mice (**Fig.2**). At 6 hours post-challenge ( $\uparrow$ ), vehicle alone (50  $\mu$ L sterile PBS, black circles) or vehicle containing 50 mg/kg inactive, scrambled CPDI-02 (scCPDI-02, white circles) was administered IM (left caudal thigh muscle). Average dermal abscess surface areas  $\pm$  SEM (n=10 mice) were determined daily starting 24 hours post-challenge by quantitative image analysis and compared between doses by repeated measurement 2-Way ANOVA with Geisser-Greenhouse correction and Tukey post-test



**Figure S3.** Single curative IM treatment with CPDI-02 does not affect early levels of many cytokines and chemokines potentially involved in the inflammation of dermal abscesses after subcutaneous infection of healthy female outbred mice with CA-MRSA. On Day 0, community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA, USA300 strain,  $5 \times 10^7$  CFU) was administered SQ (0.1 mL sterile PBS) from the left rear flank into the dorsal side of 6-week-old female outbred CD-1 mice. At 6 hours post-challenge, vehicle alone (white bars, sterile PBS, 50  $\mu$ L) or vehicle containing CPDI-02 at 50 mg/kg (black symbols) was administered IM to the left caudal thigh muscle. Average concentrations of pro-inflammatory markers  $\pm$ SD (n=5 mice per time point) in dermal abscesses at (A) 3 hours post-treatment (9 hours post-challenge) or (B) 18 hours post-treatment (24 hours post-challenge) were then determined by multiplex ELISA and compared by two-tailed t test with Mann-Whitney post-test (P value shown)



**Figure S4.** Histological comparison of inflammation around the injection site in caudal thigh muscles from healthy male and female outbred mice after IM dose escalation of CPDI-02. Vehicle alone (endotoxin-free PBS) or vehicle containing increasing doses of CPDI-02 was injected biweekly into the left caudal thigh muscle of healthy male and female outbred CD-1 mice over 28 days (**Fig.6**). Four days after the final injection, inflammation around the injection site was assessed by (**A**) histochemistry (HC) with H&E staining (4X magnification) and (**B**) proportion of each cohort with signs of inflammation was determined. HC images are representative of 10 mice from each treatment group



**Figure S5.** Histological comparison of inflammation around the injection site in caudal thigh muscles from male and female outbred mice after repeat IM dosing of CPDI-02. Vehicle alone (endotoxin-free PBS) or vehicle containing CPDI-02 (65 mg/kg or 130 mg/kg) was injected biweekly into the left caudal thigh muscle of healthy male and female outbred CD-1 mice over 28 days (**Fig.7**). Four days after the final injection, signs of inflammation around the injection site were assessed by (**A**) histochemistry (HC) with H&E staining (4X magnification) to determine (**B**) proportions of each cohort with signs of mild to severe inflammation. HC images are representative of 10 mice from each treatment group.

**Table S1 Pain and Distress Scoring in Mice**

<b>Score</b>	<b>Pain</b>	<b>Examples</b>
1	No indication of pain or distress	Normal; well groomed; alert; active; good condition; asleep or calm; normal appetite
2	Mild or anticipated pain and distress	Not well groomed; awkward gait; slightly hunched; looks at injection site or pulls away when area is touched; mildly agitated
3	Moderate pain and distress	Rough hair coat; dirty incision; squinted eyes; moves slowly; walks hunched and/or slowly; depressed or moderately agitated; slight dehydration; pruritic; restless; uncomfortable; not eating or drinking.
4	Severe pain and distress	Very rough hair coat; eyes sunken (severe dehydration); slow to move or non-responsive when coaxed; hunched; large abdominal mass; dyspnea; self-mutilating; violent reaction to stimuli or when approached

**Table S2 Complete Blood Count with Differential 4 Days after IM Dose Escalation of CPDI-02 in Healthy Male and Female Outbred CD-1 Mice**

Analyte	Unit ( $\pm$ SD)	PBS (M)	PBS (F)	CPDI-02 (M)	CPDI-02 (F)
RBC Count	$\times 10^3/\mu\text{L}$	$10 \pm 2$	$10 \pm 2$	$9 \pm 1$	$9 \pm 2$
Hematocrit	%	$52 \pm 11$	$53 \pm 10$	$45 \pm 7$	$44 \pm 9$
MCV	fL	$50 \pm 3$	$54 \pm 4$	$51 \pm 3$	$51 \pm 4$
MCH	pg	$15 \pm 1$	$15 \pm 2$	$15.4 \pm 0.5$	$14.3 \pm 0.5$
MCHC	g/dL	$29 \pm 3$	$27 \pm 2$	$30 \pm 2$	$28 \pm 2$
RDW	%	$18.4 \pm 0.9$	$19.1 \pm 0.8$	$17.8 \pm 0.5$	$18.6 \pm 0.6$
Hemoglobin	g/dL	$15 \pm 3$	$14 \pm 3$	$13 \pm 2$	$12 \pm 3$
Platelet Count	$\times 10^3/\mu\text{L}$	$320 \pm 150$	$223 \pm 93$	$135 \pm 100$	$106 \pm 66$
PCT	%	$0.2 \pm 0.1$	$0.16 \pm 0.07$	$0.11 \pm 0.06$	$0.08 \pm 0.06$
MPV	fL	$7.1 \pm 0.8$	$7.4 \pm 0.5$	$7 \pm 1$	$7 \pm 2$
PDW	fL	$11 \pm 3$	$11 \pm 3$	$11 \pm 4$	$10 \pm 2$
WBC Count	$\times 10^3/\mu\text{L}$	$2.7 \pm 0.8$	$3.4 \pm 0.7$	$4 \pm 1$	$3 \pm 2$
Neutrophil Count	$\times 10^3/\mu\text{L}$	$0.5 \pm 0.3$	$0.5 \pm 0.4$	$0.5 \pm 0.5$	$0.2 \pm 0.1$
Neutrophils	%	$16 \pm 6$	$14 \pm 10$	$15 \pm 10$	$10 \pm 6$
Monocyte Count	$\times 10^3/\mu\text{L}$	$0.11 \pm 0.05$	$0.2 \pm 0.1$	$0.2 \pm 0.1$	$0.13 \pm 0.07$
Monocytes	%	$3.9 \pm 0.7$	$6 \pm 4$	$4 \pm 2$	$5 \pm 4$
Lymphocyte Count	$\times 10^3/\mu\text{L}$	$2.1 \pm 0.5$	$2.7 \pm 0.5$	$3.1 \pm 0.7$	$2 \pm 2$
Lymphocytes	%	$80 \pm 6$	$80 \pm 13$	$80 \pm 11$	$85 \pm 9$

Complete blood cell count (CBC) with differential was determined four days after the final injection (**Fig.6**). (F) – female CD-1 mice; (M) – male CD-1 mice; MCV – mean corpuscular volume; MCH – mean corpuscular hemoglobin; MCHC – mean corpuscular hemoglobin concentration; RDW – red cell distribution width; PCT – plateletcrit; MPV – mean platelet volume; PDW – platelet distribution width; WBC – white blood cell.

**Table S3 Complete Blood Count with Differential 4 Days after Repeat IM Dosing of CPDI-02 in Healthy Male and Female Outbred CD-1 Mice**

Analyte	Unit ( $\pm$ SD)	PBS (M)	PBS (F)	CPDI-02	CPDI-02	CPDI-02	CPDI-02
				65 mg/kg (M)	65 mg/kg (F)	130 mg/kg (M)	130 mg/kg (F)
RBC Count	$\times 10^3/\mu\text{L}$	$9.9 \pm 0.9$	$9 \pm 3$	$10 \pm 1$	$10 \pm 3$	$11 \pm 2$	$10 \pm 2$
Hematocrit	%	$54 \pm 6$	$46 \pm 15$	$54 \pm 7$	$54 \pm 17$	$61 \pm 12$	$55 \pm 9$
MCV	fL	$55 \pm 2$	$48 \pm 1$	$55 \pm 2$	$52 \pm 2$	$54 \pm 1$	$54 \pm 2$
MCH	pg	$15.7 \pm 0.6$	$14 \pm 4$	$15.7 \pm 0.5$	$15.6 \pm 0.7$	$15.3 \pm 0.4$	$16 \pm 1$
MCHC	g/dL	$28.5 \pm 0.9$	$30 \pm 8$	$28 \pm 1$	$30 \pm 2$	$28.1 \pm 0.9$	$29 \pm 1$
RDW	%	$23 \pm 1$	$21 \pm 2$	$23 \pm 1$	$24 \pm 3$	$25 \pm 2$	$24 \pm 2$
Hemoglobin	g/dL	$15 \pm 1$	$16 \pm 5$	$15 \pm 2$	$16 \pm 4$	$17 \pm 3$	$16 \pm 2$
Platelet Count	$\times 10^3/\mu\text{L}$	$636 \pm 188$	$759 \pm 196$	$636 \pm 146$	$659 \pm 237$	$689 \pm 170$	$857 \pm 198$
PCT	%	$0.5 \pm 0.1$	$0.6 \pm 0.2$	$0.5 \pm 0.1$	$0.5 \pm 0.2$	$0.5 \pm 0.1$	$0.7 \pm 0.2$
MPV	fL	$7.8 \pm 0.3$	$8.3 \pm 0.3$	$7.7 \pm 0.6$	$7.6 \pm 0.6$	$7.7 \pm 0.3$	$7.8 \pm 0.3$
PDW	fL	$7.4 \pm 0.3$	$7.3 \pm 0.3$	$7.6 \pm 0.6$	$7.8 \pm 0.9$	$8.2 \pm 0.7$	$7.4 \pm 0.5$
WBC Count	$\times 10^3/\mu\text{L}$	$7 \pm 2$	$7 \pm 3$	$6 \pm 2$	$7 \pm 2$	$6 \pm 2$	$8 \pm 3$
Neutrophil Count	$\times 10^3/\mu\text{L}$	$2.6 \pm 0.8$	$1.3 \pm 0.7$	$2.6 \pm 0.8$	$3 \pm 1$	$2 \pm 1$	$3 \pm 1$
Neutrophils	%	$37 \pm 5$	$20 \pm 5$	$41 \pm 10$	$30 \pm 18$	$45 \pm 17$	$33 \pm 8$
Monocyte Count	$\times 10^3/\mu\text{L}$	$0.03 \pm 0.01$	$0.02 \pm 0.01$	$0.03 \pm 0.01$	$0.02 \pm 0.01$	$0.02 \pm 0.02$	$0.03 \pm 0.01$
Monocytes	%	$0.4 \pm 0.2$	$0.2 \pm 0.1$	$0.4 \pm 0.2$	$0.3 \pm 0.2$	$0.4 \pm 0.2$	$0.3 \pm 0.1$
Lymphocyte Count	$\times 10^3/\mu\text{L}$	$4 \pm 1$	$5 \pm 2$	$4 \pm 1$	$5 \pm 1$	$3 \pm 2$	$5 \pm 2$
Lymphocytes	%	$60 \pm 6$	$77 \pm 5$	$55 \pm 11$	$62 \pm 12$	$51 \pm 17$	$63 \pm 7$

Complete blood cell count (CBC) with differential was determined four days after the final injection (**Fig.7**). (F) – female CD-1 mice; (M) – male CD-1 mice; MCV – mean corpuscular volume; MCH – mean corpuscular hemoglobin; MCHC – mean corpuscular hemoglobin concentration; RDW – red cell distribution width; PCT – plateletcrit; MPV – mean platelet volume; PDW – platelet distribution width; WBC – white blood cell.

**Table S4 Blood Chemistry 4 Days after Repeat IM Dosing of CPDI-02 in Healthy Male and Female Outbred CD-1 Mice**

Analyte	Unit ( $\pm$ SD)			CPDI-02	CPDI-02	CPDI-02	CPDI-02
		PBS (M)	PBS (F)	65 mg/kg (M)	65 mg/kg (F)	130 mg/kg (M)	130 mg/kg (F)
ALB	g/L	41 $\pm$ 2	42 $\pm$ 3	39 $\pm$ 2	41.1 $\pm$ 0.7	40 $\pm$ 2	41 $\pm$ 2
ALP	U/L	71 $\pm$ 22	86 $\pm$ 19	66 $\pm$ 18	74 $\pm$ 28	61 $\pm$ 13	77 $\pm$ 49
ALT	U/L	132 $\pm$ 285	136 $\pm$ 153	41 $\pm$ 24	39 $\pm$ 19	100 $\pm$ 134	200 $\pm$ 257
AMY	U/L	1005 $\pm$ 440	1199 $\pm$ 988	1032 $\pm$ 582	734 $\pm$ 169	867 $\pm$ 138	659 $\pm$ 62
TBIL	$\mu$ M	4.1 $\pm$ 0.8	4.1 $\pm$ 0.8	4 $\pm$ 1	6 $\pm$ 1	4 $\pm$ 1	4.4 $\pm$ 0.8
BUN	mM	12 $\pm$ 1	10 $\pm$ 2	11 $\pm$ 1	10 $\pm$ 1	11 $\pm$ 1	9 $\pm$ 2
CA	mM	2.5 $\pm$ 0.1	2.31 $\pm$ 0.05	2.5 $\pm$ 0.2	2.4 $\pm$ 0.1	2.46 $\pm$ 0.07	2.3 $\pm$ 0.1
PHOS	mM	1.9 $\pm$ 0.4	3 $\pm$ 1	2.4 $\pm$ 0.5	2.6 $\pm$ 0.9	2.2 $\pm$ 0.4	2.7 $\pm$ 0.7
GLU	mM	15 $\pm$ 3	13 $\pm$ 4	17 $\pm$ 2	15 $\pm$ 3	13 $\pm$ 5	14 $\pm$ 4
Na	mM	151 $\pm$ 6	148 $\pm$ 3	152 $\pm$ 7	148 $\pm$ 4	147 $\pm$ 2	146 $\pm$ 2
K	mM	5 $\pm$ 1	5 $\pm$ 1	5 $\pm$ 1	4.1 $\pm$ 0.3	5.6 $\pm$ 0.8	5 $\pm$ 1
TP	g/L	51 $\pm$ 3	49 $\pm$ 4	50 $\pm$ 2	51 $\pm$ 4	50 $\pm$ 1	49 $\pm$ 3
GLOB	g/L	10 $\pm$ 2	7 $\pm$ 2	11 $\pm$ 2	9 $\pm$ 3	11 $\pm$ 1	7 $\pm$ 2

Average analyte concentration  $\pm$ SD (n=7 to 10 mice) was determined four days after the final injection (**Fig.7**). (F) – female CD-1 mice; (M) – male CD-1 mice; ALB – albumin (kidney/liver); ALP – alkaline phosphatase (liver/bone); ALT – alanine transaminase (liver); AMY – amylase (pancreas); TBIL – bilirubin (liver); BUN – blood urea nitrogen (kidneys); CA – calcium (bone/thyroid/parathyroid/kidneys); PHOS – phosphate (kidney/bone/parathyroid); GLU – glucose (pancreas); Na – sodium; K – potassium; TP – total protein (liver/kidney); GLOB – globulin (liver/kidney).