Supplementary Materials

Early-stage IM treatment with the host-derived immunostimulant CPDI-02 increases curative protection of healthy outbred mice against subcutaneous infection with CA-MRSA USA300

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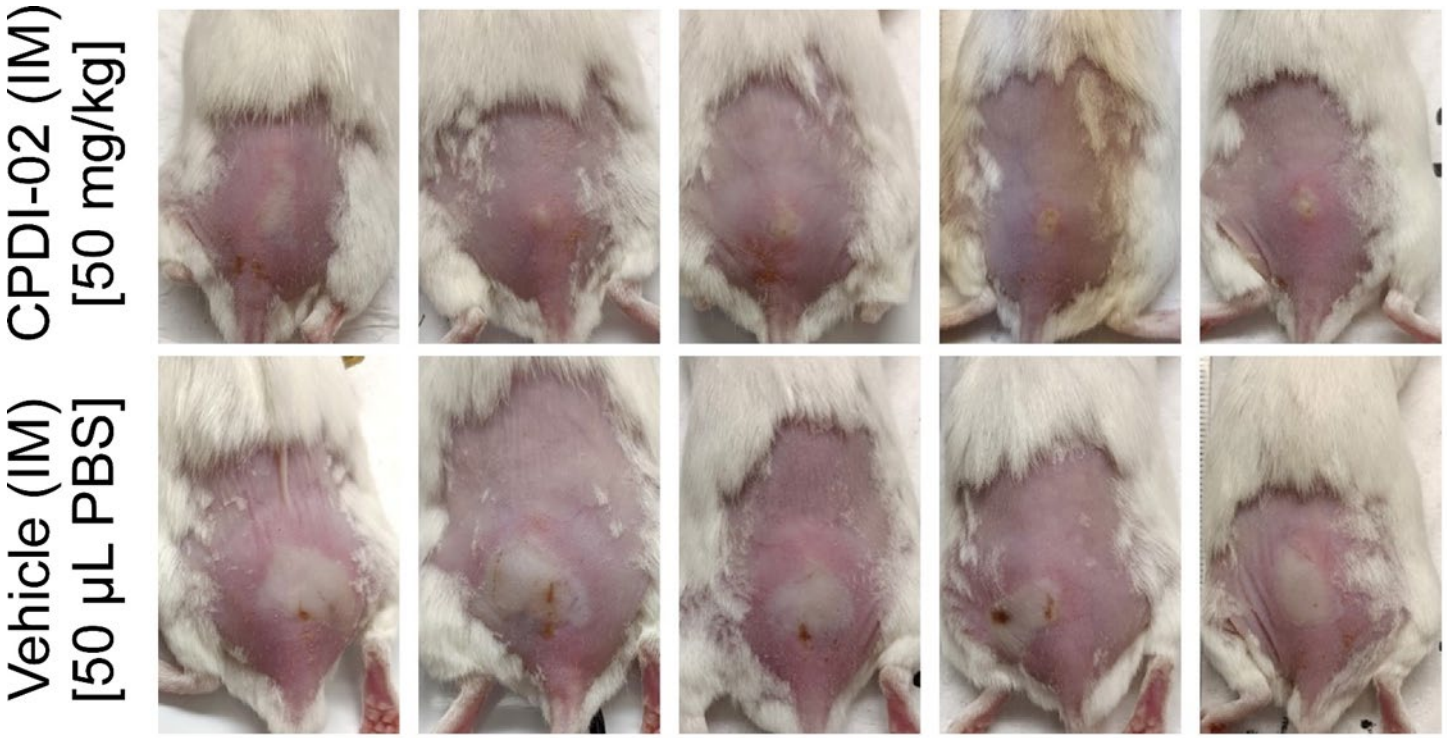

Figure S1. Representative images of dermal abscesses on the rear dorsal region of CPDI-02-treated and untreated healthy female outbred mice 24 hours after subcutaneous challenge with CA-MRSA. On Day 0, community-acquired methicillin-resistant Staphylococcus aureus (CA-MRSA, USA300 strain, 5×10^7 CFU) 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.

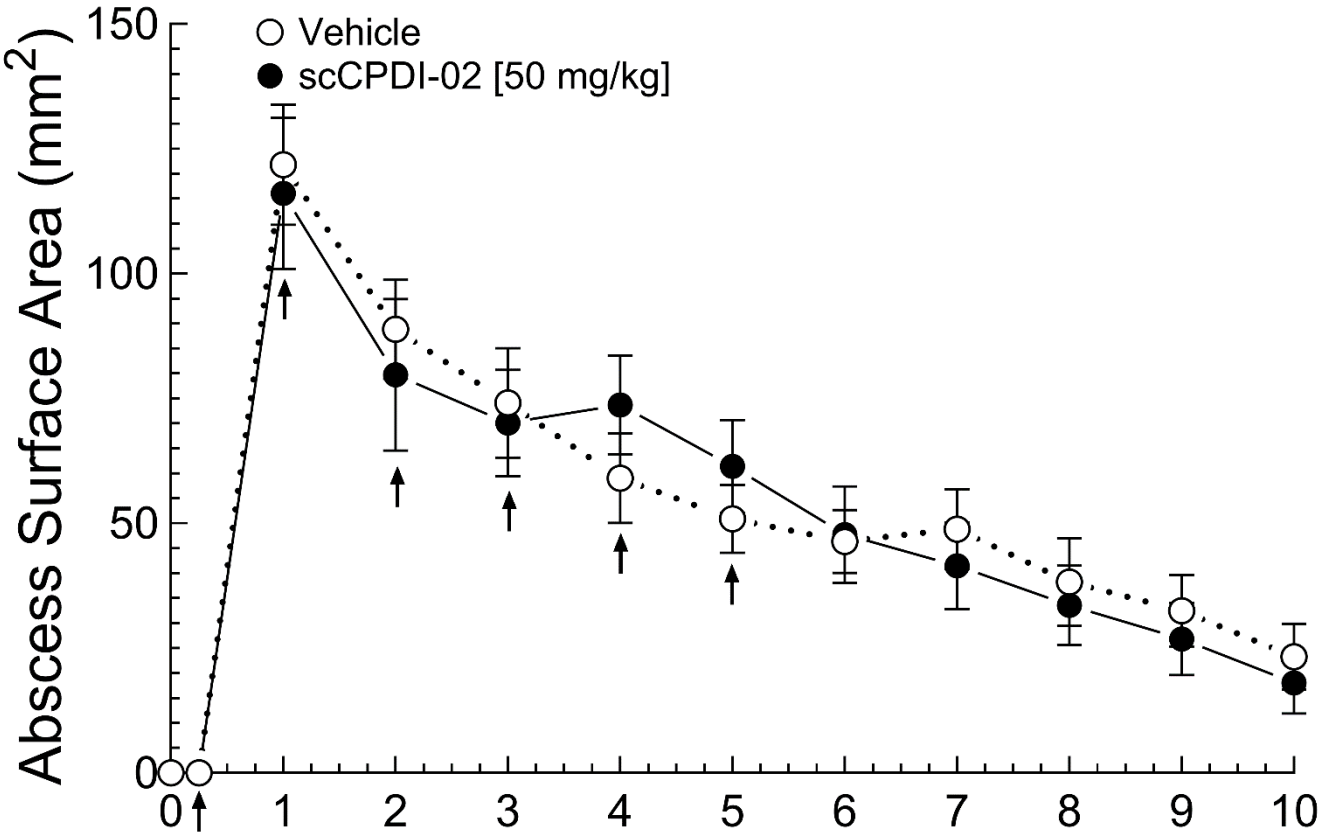

Figure S2. Repeat IM treatment with inactive, scrambled CPDI-02 does not increase curative protection of healthy female outbred mice against subcutaneous challenge with CA-MRSA. On Day 0, community-acquired methicillin-resistant Staphylococcus aureus (CA-MRSA, USA300 strain, 5×10^7 CFU) was administered 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 (↑), vehicle alone (50 μL sterile PBS, black circles) or vehicle containing 50 mg/kg inactive, scrambled CPDI-02 (scCPDI-02, white circles) was administered IM (caudal thigh muscle). Average dermal abscess surface areas ± 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

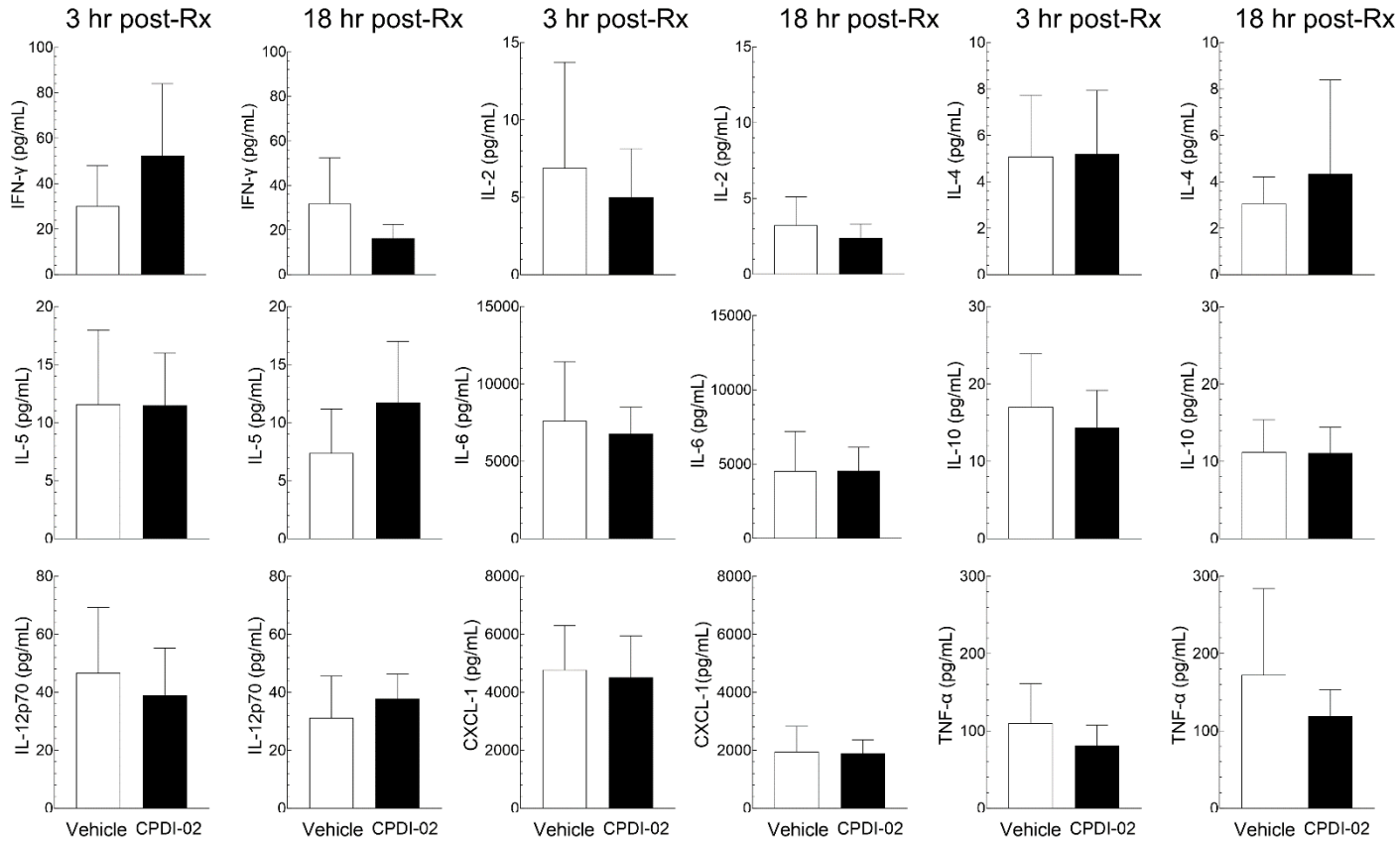

Figure S3. Single curative IM treatment with CPDI-02 does not affect early levels of many cytokines and chemokines potentially involved in the inflammation of dermal abscesses after subcutaneous infection of healthy female outbred mice with CA-MRSA. On Day 0, community-acquired methicillin-resistant Staphylococcus aureus (CA-MRSA, USA300 strain, 5×10^7 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 μL) 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 ±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)

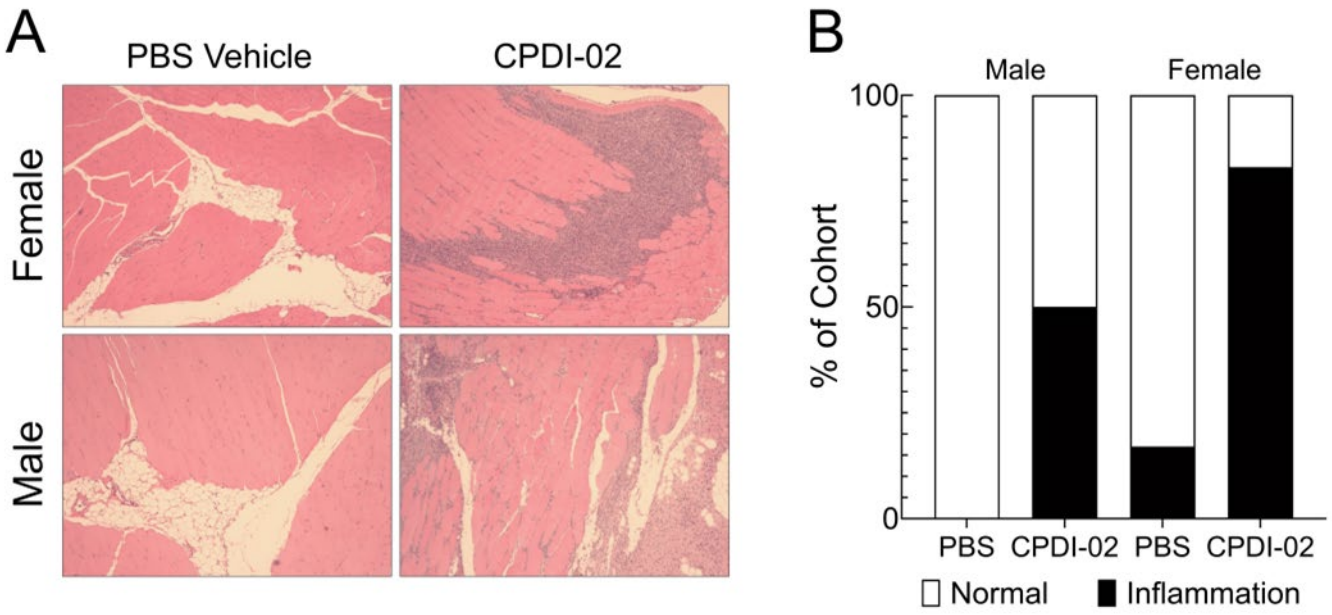

Figure S4. Histological comparison of inflammation around the injection site in caudal thigh muscles from healthy male and female outbred mice after IM dose escalation of CPDI-02. Vehicle alone (endotoxin-free PBS) or vehicle containing increasing doses of CPDI-02 was injected biweekly into the (left) 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

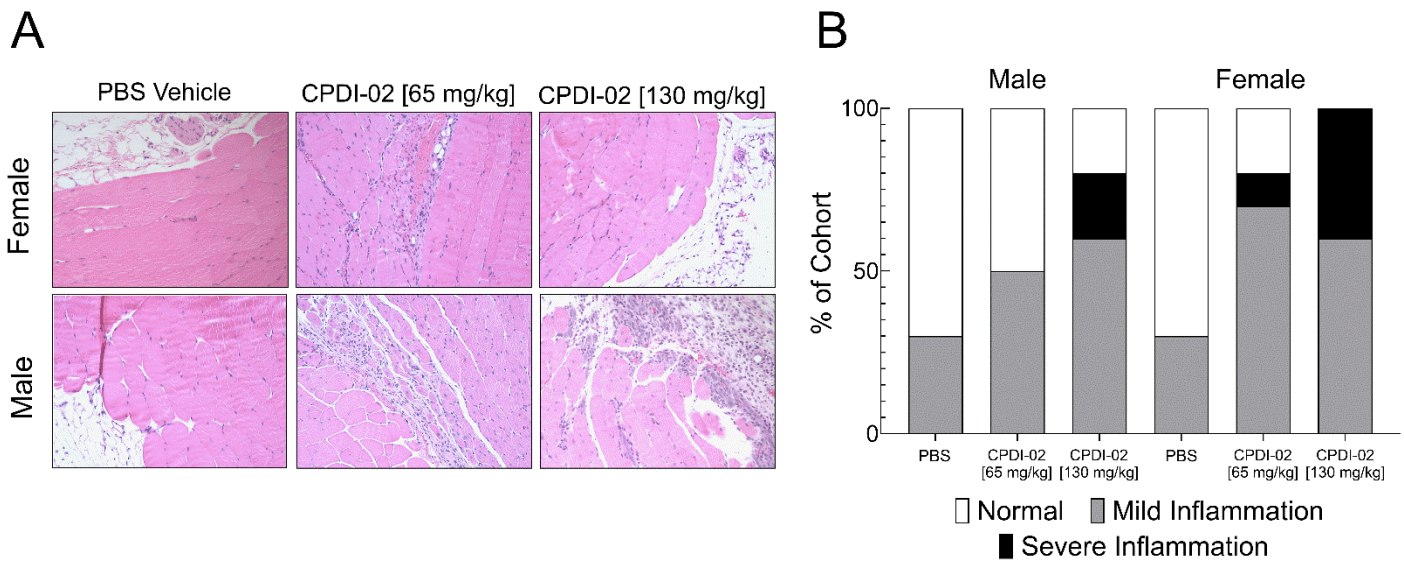

Figure S5. Histological comparison of inflammation around the injection site in caudal thigh muscles from male and female outbred mice after repeat IM dosing of CPDI-02. (Are there histological slides for 130 mg CPDI-02/kg in female CD-1 mice that show more evidence of inflamation as observed for male mice?) 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

Score	Pain	Examples
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 (±SD)	PBS (M)	PBS (F)	CPDI-02 (M)	CPDI-02 (F)
RBC Count	$x10^3/\mu L$	10 ± 2	10 ± 2	9 ± 1	9 ± 2
Hematocrit	%	52 ± 11	53 ± 10	45 ± 7	44 ± 9
MCV	fL	50 ± 3	54 ± 4	51 ± 3	51 ± 4
MCH	pg	15 ± 1	15 ± 2	15.4 ± 0.5	14.3 ± 0. 5
MCHC	g/dL	29 ± 3	27 ± 2	30 ± 2	28 ± 2
RDW	%	18.4 ± 0.9	19.1 ± 0.8	17.8 ± 0.5	18.6 ± 0.6
Hemoglobin	g/dL	15 ± 3	14 ± 3	13 ± 2	12 ± 3
Platelet Count	$x10^3/\mu L$	320 ± 150	223 ± 93	135 ± 100	106 ± 66
PCT	%	0.2 ± 0.1	0.16 ± 0.07	0.11 ± 0.06	0.08 ± 0.06
MPV	fL	7.1 ± 0.8	7.4 ± 0.5	7 ± 1	7 ± 2
PDW	fL	11 ± 3	11 ± 3	11 ± 4	10 ± 2
WBC Count	$x10^3/\mu L$	2.7 ± 0.8	3.4 ± 0.7	4 ± 1	3 ± 2
Neutrophil Count	$x10^3/\mu L$	0.5 ± 0.3	0.5 ± 0.4	0.5 ± 0.5	0.2 ± 0.1
Neutrophils	%	16 ± 6	14 ± 10	15 ± 10	10 ± 6
Monocyte Count	$x10^3/\mu L$	0.11 ± 0.05	0.2 ± 0.1	0.2 ± 0.1	0.13 ± 0.07
Monocytes	%	3.9 ± 0.7	6 ± 4	4 ± 2	5 ± 4
Lymphocyte Count	$x10^3/\mu L$	2.1 ± 0.5	2.7 ± 0.5	3.1 ± 0.7	2 ± 2
Lymphocytes	%	80 ± 6	80 ± 13	80 ± 11	85 ± 9

Complete blood cell count (CBC) with differential was determined four days after the final injection. (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 (±SD)	PBS (M)	PBS (F)	CPDI-02 65 mg/kg (M)	CPDI-02 65 mg/kg (F)	CPDI-02 130 mg/kg (M)	CPDI-02 130 mg/kg (F)
RBC Count	$x10^3/\mu L$	9.9 ± 0.9	9 ± 3	10 ± 1	10 ± 3	11 ± 2	10 ± 2
Hematocrit	%	54 ± 6	46 ± 15	54 ± 7	54 ± 17	61 ± 12	55 ± 9
MCV	fL	55 ± 2	48 ± 1	55 ± 2	52 ± 2	54 ± 1	54 ± 2
MCH	pg	15.7 ± 0.6	14 ± 4	15.7 ± 0.5	15.6 ± 0.7	15.3 ± 0.4	16 ± 1
MCHC	g/dL	28.5 ± 0.9	30 ± 8	28 ± 1	30 ± 2	28.1 ± 0.9	29 ± 1
RDW	%	23 ± 1	21 ± 2	23 ± 1	24 ± 3	25 ± 2	24 ± 2
Hemoglobin	g/dL	15 ± 1	16 ± 5	15 ± 2	16 ± 4	17 ± 3	16 ± 2
Platelet Count	$x10^3/\mu L$	636 ± 188	759 ± 196	636 ± 146	659 ± 237	689 ± 170	857 ± 198
PCT	%	0.5 ± 0.1	0.6 ± 0.2	0.5 ± 0.1	0.5 ± 0.2	0.5 ± 0.1	0.7 ± 0.2
MPV	fL	7.8 ± 0.3	8.3 ± 0.3	7.7 ± 0.6	7.6 ± 0.6	7.7 ± 0.3	7.8 ± 0.3
PDW	fL	7.4 ± 0.3	7.3 ± 0.3	7.6 ± 0.6	7.8 ± 0.9	8.2 ± 0.7	7.4 ± 0.5
WBC Count	$x10^3/\mu L$	7 ± 2	7 ± 3	6 ± 2	7 ± 2	6 ± 2	8 ± 3
Neutrophil Count	$x10^3/\mu L$	2.6 ± 0.8	1.3 ± 0.7	2.6 ± 0.8	3 ± 1	2 ± 1	3 ± 1
Neutrophils	%	37 ± 5	20 ± 5	41 ± 10	30 ± 18	45 ± 17	33 ± 8
Monocyte Count	$x10^3/\mu L$	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.02	0.03 ± 0.01
Monocytes	%	0.4 ± 0.2	0.2 ± 0.1	0.4 ± 0.2	0.3 ± 0.2	0.4 ± 0.2	0.3 ± 0.1
Lymphocyte Count	$x10^3/\mu L$	4 ± 1	5 ± 2	4 ± 1	5 ± 1	3 ± 2	5 ± 2
Lymphocytes	%	60 ± 6	77 ± 5	55 ± 11	62 ± 12	51 ± 17	63 ± 7

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

Average analyte concentration ±SD (n=7 to 10 mice) was determined four days after the final injection and treatment groups were compared within each sex by Kruskal-Wallis test. (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).