Supplemental Material

Six Figures (Figures S1-S6)

Four Tables (Tables S1-4)

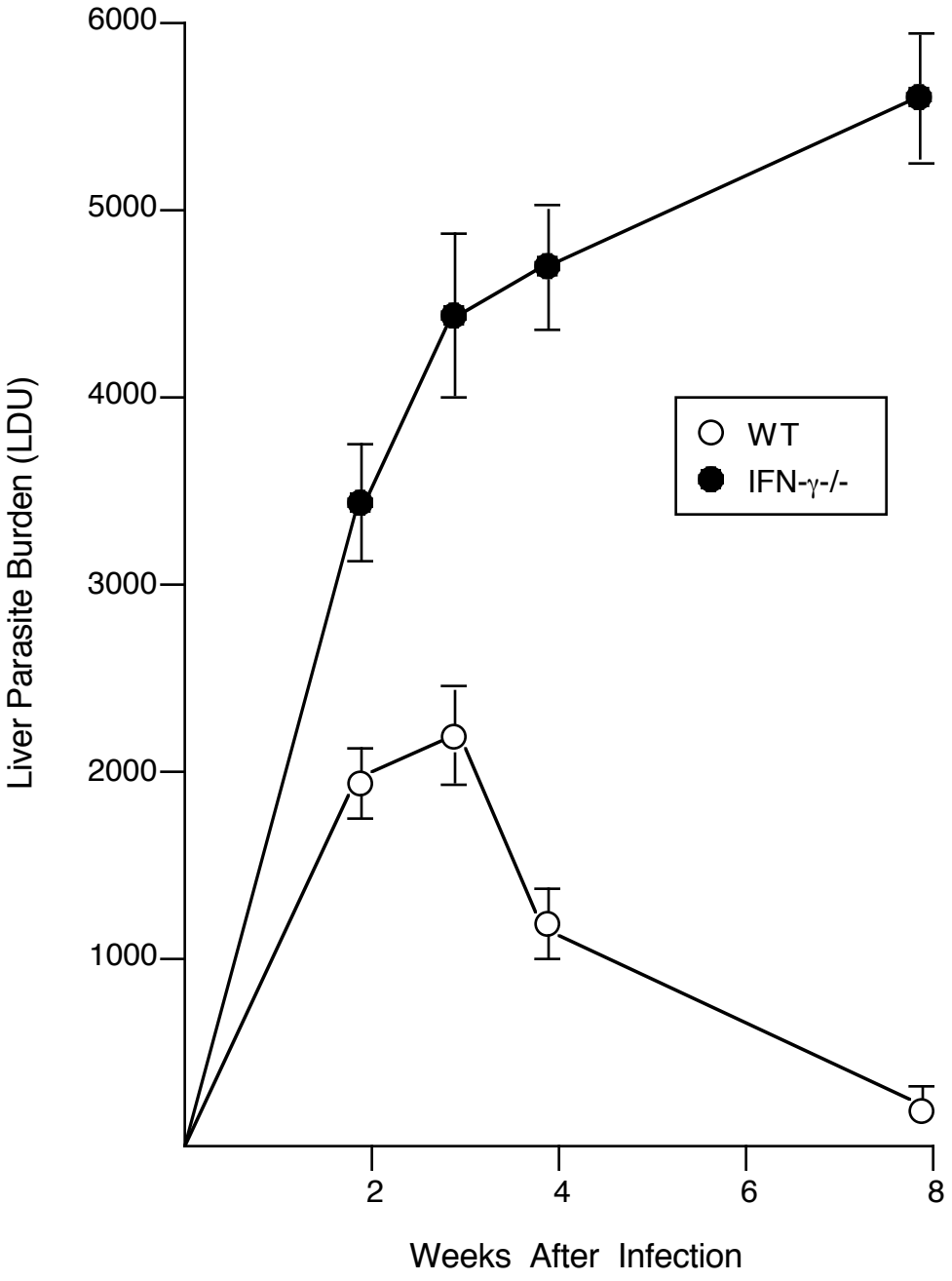

Figure S1. Course of *L. donovani* infection 2-8 weeks after challenge in livers of WT and IFN-γ-/- mice. Results, from 2 experiments, indicate mean ± SEM values for 8 mice per group at each time point. *$p < .05$ for IFN-γ-/- vs. WT liver parasite burdens (LDU) at all time points.

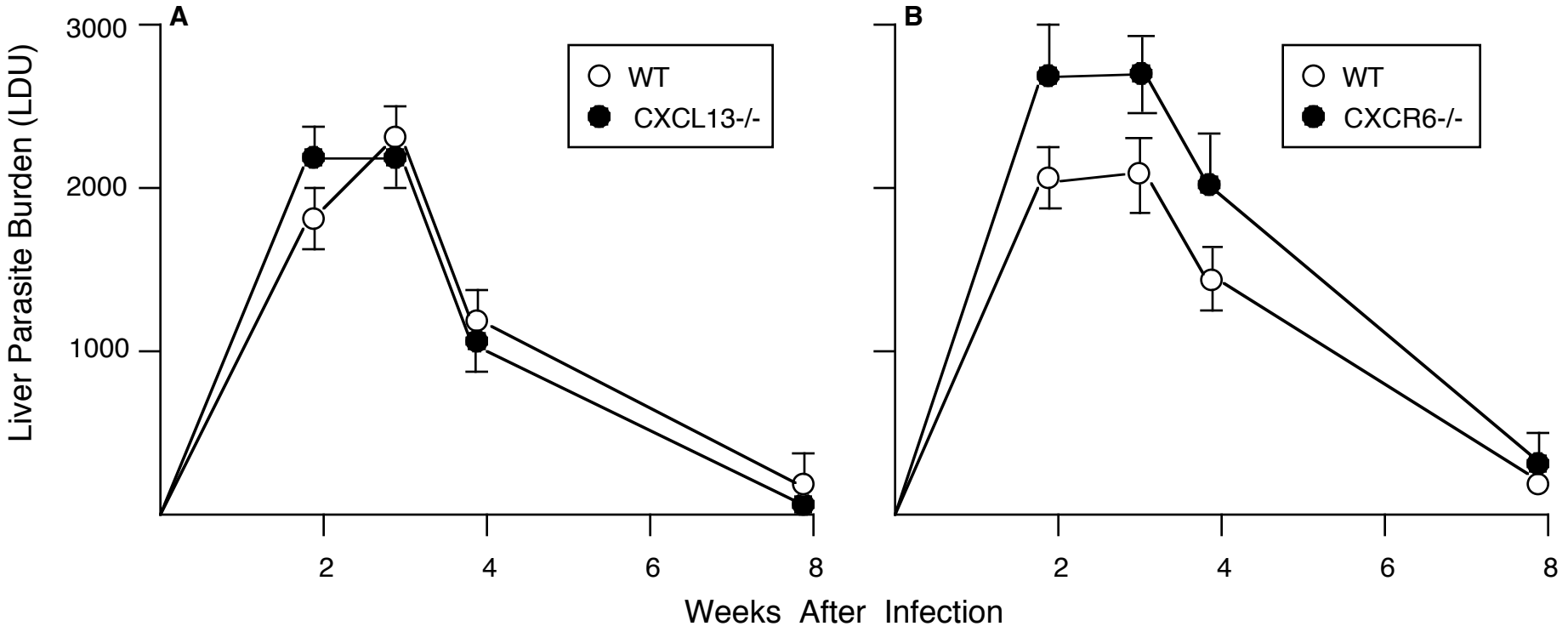

Figure S2. Course of *L. donovani* infection 2-8 weeks after challenge in livers of WT, CXCL13-/- (A) and CXCR6-/- mice (B). Results, from 2 experiments, indicate mean ± SEM values for 7-8 mice per group at each time point.

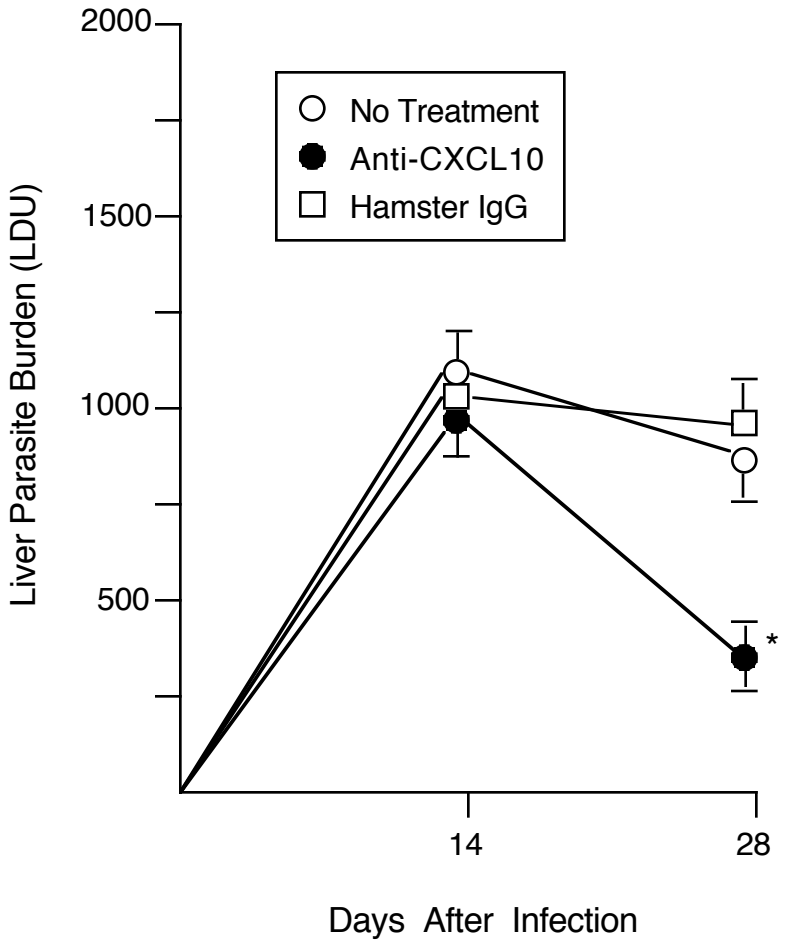

Figure S3. Effect of anti-CXCL10 mAb treatment in *L. donovani*-infected WT mice. Starting 4 hours after challenge and on alternate days thereafter to day +26, mice were injected IP with 200 ug of hamster anti-mouse CXCL10 mAb or hamster IgG. Results, from 2 experiments, indicate mean $\pm$ SEM values for 6-7 mice per group at each time point. *$p < .05$ vs. untreated and hamster IgG-treated liver parasite burden (LDU).

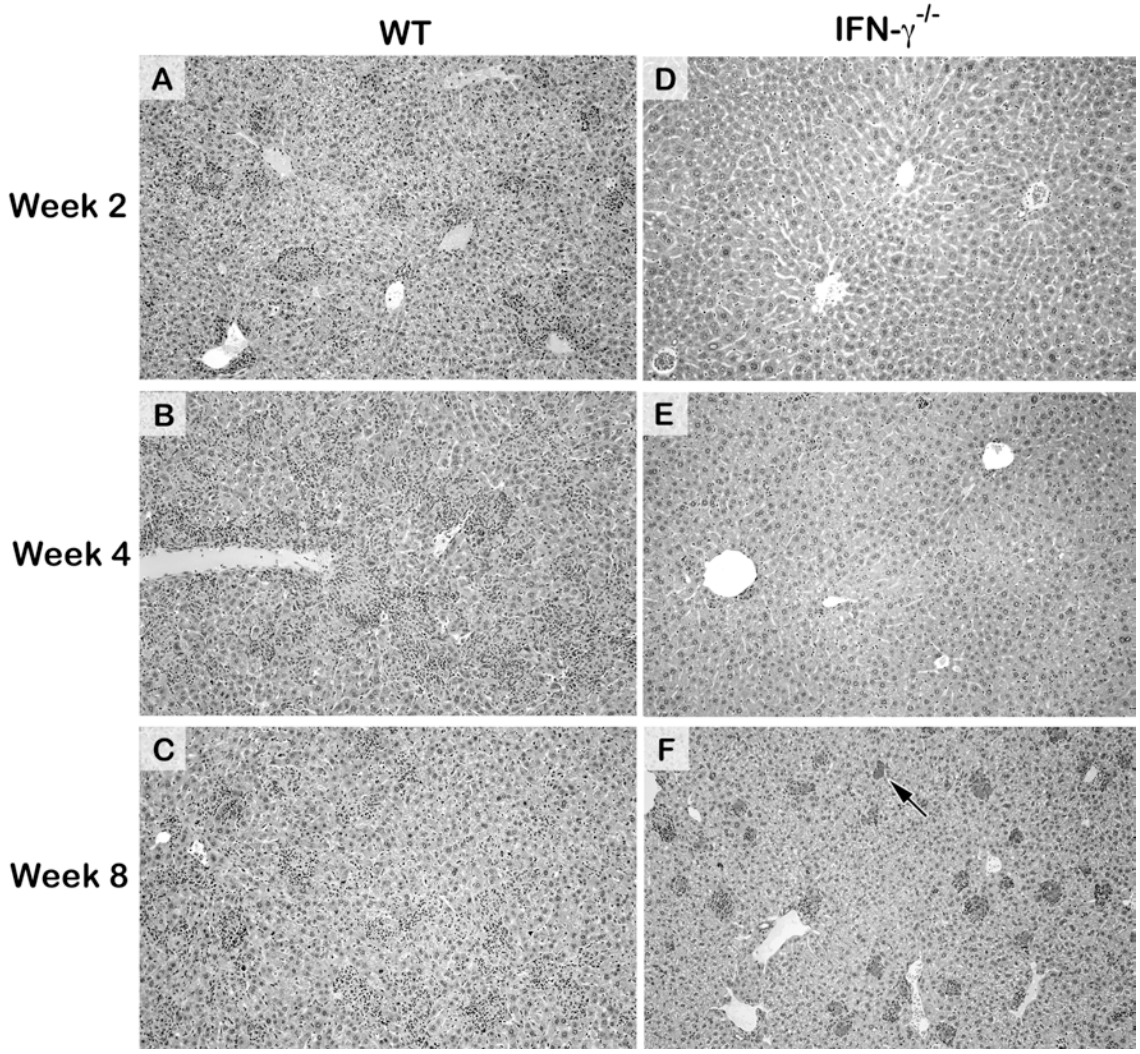

Figure S4. Low-power photomicrographs of livers of WT (A-C) and IFN-$\gamma^{-/-}$ mice (D-F) 2-8 weeks after infection demonstrating: (i) the intact inflammatory granulomatous reaction in WT mice, expressed at week 2 (A), fully-established at week 4 (B) and maintained but involuting by week 8 (C) vs. (ii) the essentially absent inflammatory response in IFN-$\gamma^{-/-}$ mice at both weeks 2 and 4 (D, E) with some mononuclear cell recruitment to heavily-infected foci at week 8 (F). In (F), certain foci (arrows) are comprised of remarkably parasitized Kupffer cells alone (see Figure 4F). Original magnification, x100.

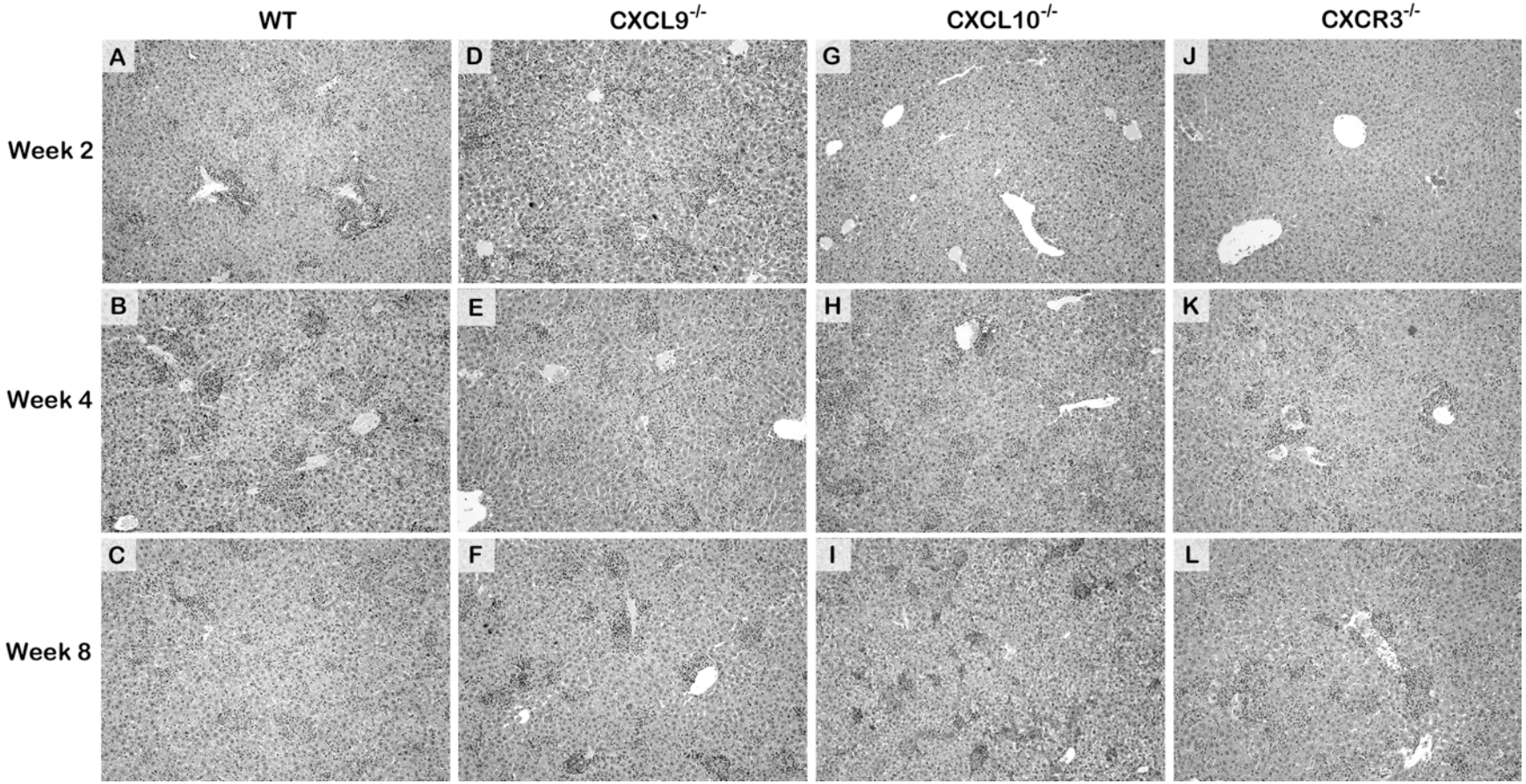

Figure S5. Low-power photomicrographs of livers of WT (A-C), CXCL9$^{-/-}$ (D-F), CXCL10$^{-/-}$ (G-I) and CXCR3$^{-/-}$ mice (J-L) 2-8 weeks after *L. donovani* infection. The WT mice shown were infected in parallel with CXCL9$^{-/-}$ mice (histologic reactions were similar in WT mice infected in parallel with CXCL10$^{-/-}$ and CXCR3$^{-/-}$ mice). Compared to inflammatory granulomatous responses in WT mice, photomicrographs demonstrate: (i) an accentuated early reaction at week 2 in CXCL9$^{-/-}$ mice (D), and in contrast, (ii) near-absent inflammatory reactions in CXCL10$^{-/-}$ and CXCR3$^{-/-}$ mice at week 2 (G, J). By weeks 4-8, mononuclear cell recruitment is expressed in both CXCL10$^{-/-}$ (H, I) and CXCR3$^{-/-}$ mice (K, L). (Original magnification, x100.)

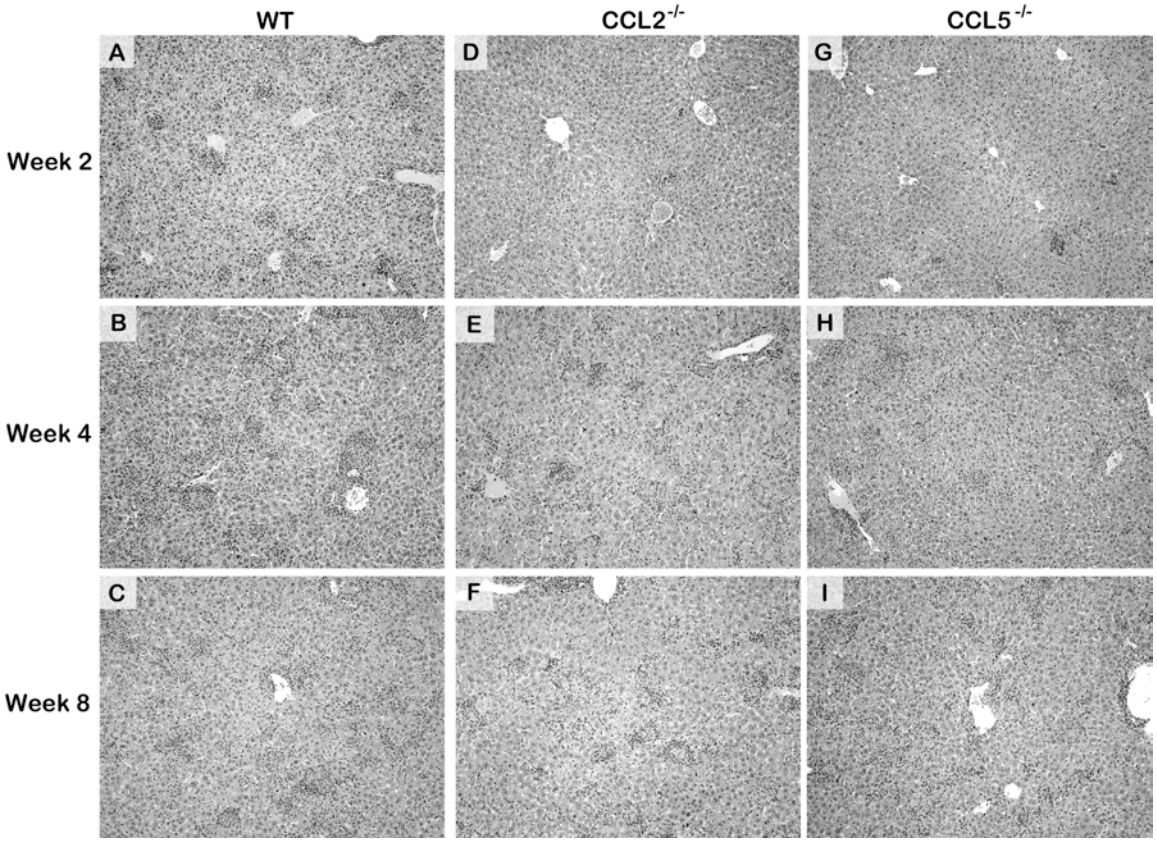

Figure S6. Low-power photomicrographs of livers of WT (A-C), CCL2$^{-/-}$ (D-F) and CCL5$^{-/-}$ (G-I) 2-8 weeks after *L. donovani* infection. WT mice shown were infected in parallel with the CCL2$^{-/-}$ mice (histologic reactions were similar in WT mice infected in parallel with CCL5$^{-/-}$ mice). Compared to inflammatory granulomatous responses in WT mice, photomicrographs demonstrate in both CCL2$^{-/-}$ and CCL5$^{-/-}$ mice: (i) overtly-deficient mononuclear cell recruitment at week 2 (D, H) with impaired responses at week 4 (E, H), but (ii) established inflammation and granuloma assembly by week 8 (F, I). (Original magnification, x100.)

Table S1. Primer Sequences Used for RT-PCR.

Gene	Forward Primer	Reverse Primer
CXCL9	5'-TCATCTTCCTGGAGCAGTGT-3'	5'-TCTCCGTTCTTCAGTGTAGC-3'
CXCL10	5'-GCTGCAACTGCATCCATATC-3'	5'-CCGGATTCAGACATCTCTGC-3'
CXCL13	5'-CTCTCCAGGCCACGGTATTC-3'	5'-GCAGCTCTTCTCTTACTCAC-3'
CXCL16	5'-GCAGTGTCGCTGGAAGTTGT-3'	5'-GTGCTCGTGTCCGAAGGTGT-3'
CCL2	5'-ACAGTTGCCGGCTGGAGAAT-3'	5'-CTGCTGCTGGTGATCCTCTT-3'
CCL5	5'-ATATGGCTCGGACACCACTC-3'	5'-CACACTTGGCGGTTCCTTCG-3'
GADPH	5'-AACTTTGGCATTGTGGAAGG -3'	5'-ACACATTGGGGGTAGGAACA -3'

Table S2. Chemokine and Chemokine Receptor Gene Expression in *L. donovani*-Infected Livers.*

	Fold-Increase in Expression			
	WT Mice		IFN-$\gamma^{-/-}$ Mice	
Gene	Day 14	21	Day 14	21
C-X-C Chemokines				
CXCL7	6.0	8.4	0.9	1.2
CXCL9^{+}	19.1	33.6	1.5	2.3
CXCL10^{+}	98.0	257	3.3	3.6
CXCL11	2.7	5.6	0.9	0.7
CXCL13^{+}	13.3	81.4	1.5	3.7
CXCL16^{+}	3.8	11.6	1.4	1.1
C-C Chemokines				
CCL2^{+}	3.9	28.9	2.5	1.9
CCL5^{+}	4.3	34.2	1.0	2.2
CCL6	2.0	7.5	1.1	1.5
CCL8	7.8	57.0	6.6	9.7
CCL19	2.7	4.3	0.8	1.0
C-X-C Chemokine Receptors				
CXCR3^{+}	1.3	11.2	0.8	0.9
CXCR4^{+}	19.6	47.1	0.5	1.0
CXCR6^{+}	10.8	46.8	4.6	4.1
C-C Chemokine Receptors				
CCR2^{+}	4.7	15.9	2.1	1.3

CCR3^{+}	34.9	46.7	0.6	0.6
CCR5^{+}	4.3	13.1	0.5	1.2
CCR9	5.0	11.6	2.1	2.0
CX3C Chemokine Receptors				
CX3CR1	5.0	5.7	1.1	1.4

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*DNA microarray analysis of liver tissue from WT and IFN-$\gamma^{-/-}$ mice 14 and 21 days after infection. Results, from a single experiment in which samples from 4-5 mice per group were pooled at each time point, indicate fold-increase in gene expression vs. pooled liver samples from 4-5 uninfected WT or IFN-$\gamma^{-/-}$ mice.

$^{+}$Gene expression on day 14 or day 21 designated as both prominent and IFN-γ-regulated (see text for definitions).

Table S3. Response to Pentavalent Antimony (Sb) Chemotherapy.*

Mice	Sb	Liver Parasite Burden (LDU) Day 14	Day 21[+]	% Killing
A. WT	0	1999 ± 201	2256 ± 190	0
	+		200 ± 32	90
IFN-$\gamma^{-/-}$	0	3561 ± 288	4360 ± 399	0
	+		3701 ± 357	0
B. WT	0	2418 ± 171	2555 ± 198	0
	+		197 ± 45	92
CXCL9$^{-/-}$	0	1887 ± 254	1934 ± 154	0
	+		54 ± 11	97
C. WT	0	1788 ± 159	1820 ± 127	0
	+		120 ± 22	93
CXCL10$^{-/-}$	0	1152 ± 77	1336 ± 88	0
	+		110 ± 18	90
D. WT	0	2266 ± 219	2757 ± 254	0
	+		212 ± 30	93
CXCR3$^{-/-}$	0	3048 ± 124	3090 ± 174	0
	+		158 ± 47	95
E. WT	0	1842 ± 31	2398 ± 254	0
	+		195 ± 18	89
CXCL13$^{-/-}$	0	2088 ± 262	2149 ± 126	0
	+		96 ± 32	95

F. WT	0	2037 ± 160	2215 ± 98	0
	+		106 ± 50	95
$CXCR6^{-/-}$	0	2697 ± 296	2802 ± 222	0
	+		102 ± 51	96
G. WT	0	2533 ± 205	2587 ± 201	0
	+		231 ± 87	91
$CCL2^{-/-}$	0	3254 ± 303	3928 ± 331	0
	+		214 ± 39	93
H. WT	0	2438 ± 221	2555 ± 122	0
	+		270 + 58	92
$CCL5^{-/-}$	0	2489 ± 181	3772 ± 382	0
	+		87 ± 21	97

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*2 weeks after infection (day +14), mice received no treatment or a single IP injection of Sb (500 mg/kg). Day +21 LDU in treated mice were compared to day +14 LDU to determine parasite killing (% reduction in LDU on day +21). Results (mean ± SEM values) are from 2-3 experiments for each gene-deficient group (6-12 mice per group per time point), infected in parallel with a similar number of WT controls.

Table S4. Parasite Killing at Week 4.*

Mice	% Parasite Killing at Week 4	
	Chemokine-Deficient	WT Control
$CXCL9^{-/-}$	44	26
$CXCL10^{-/-}$	49	33
$CXCL13^{-/-}$	51	46
$CXCR6^{-/-}$ (for $CXCL16^{-/-}$ mice)	29	32
$CCL2^{-/-}$	34	54
$CCL5^{-/-}$	13	44

*Calculated using the week 3 and week 4 liver parasite burdens (mean LDU) shown in Figures 2, 3 and S2 for chemokine-deficient mice and WT controls infected in parallel. % parasite killing = week 3 LDU – week 4 LDU/week 3 LDU.