Supplementary Figures and Figure Legends

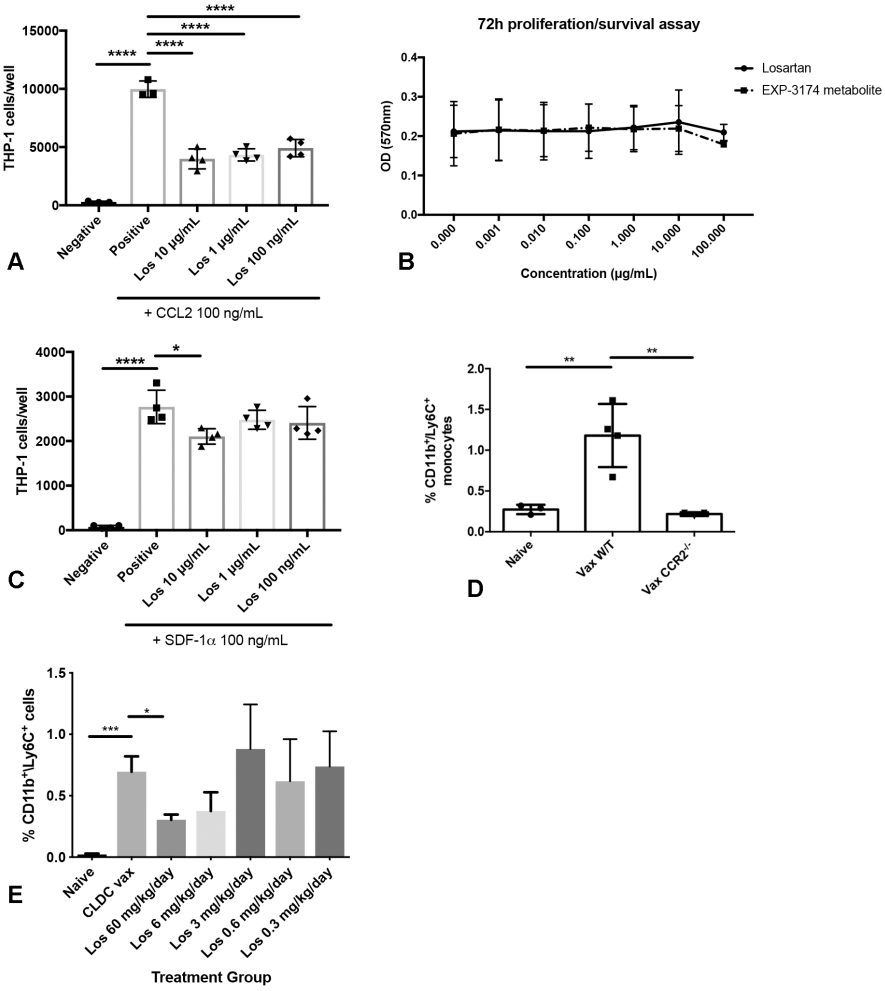


Figure S1. Losartan demonstrates dose-dependent inhibition of CCL2-CCR2 monocyte recruitment *in vitro* and *in vivo*. **(A)** Quantification of *in vitro* CCL2-directed THP-1 monocyte migration across a range of three 10-fold dilutions of losartan treatment. ****p < 0.0001, one-way ANOVA. (**B**) Dose-response curve quantifying the effects of losartan or EXP-3174 metabolite on proliferation and survival of THP-1 cells over 72 hours, assessed via MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric assay. (**C**) Quantification of the effects of losartan on *in vitro* SDF-1α-directed THP-1 migration across the same range of three 10-fold dilutions of losartan treatment shown in (A). ****p < 0.0001, *p = 0.022, one-way ANOVA. (**D**). Flow cytometric quantification of % *Ly6G*$^{-}$*/CD11b*$^{+}$*/Ly6C*^{Hi} IMs in the popliteal lymph nodes of naïve (un-injected) C57BL/6 w/t mice as compared to C57BL/B6 w/t (Vax W/T) or *CCR2*$^{-/-}$ (VAX *CCR2*$^{-/-}$) mice 24 hours post-footpad injection of a liposome-TLR3 agonist adjuvant, demonstrating that recruitment of inflammatory monocytes (IMs) to vaccine draining LNs is entirely dependent on the chemokine receptor *CCR2.* **p < 0.01, one-way ANOVA. **(E)** Flow cytometric quantification of inflammatory monocytes in vaccine-draining popliteal LNs of mice, demonstrating a dose dependent effect of losartan on inhibition of *in vivo* CCL2-CCR2 monocyte recruitment. ***p = 0.0003, *p = 0.036, *t* test. Data (means ± SEM) are from one representative of three to five independent experiments (A, B, D), three independent experiments (C), or are pooled from two independent experiments (E, *n* = 3-6 mice/group).

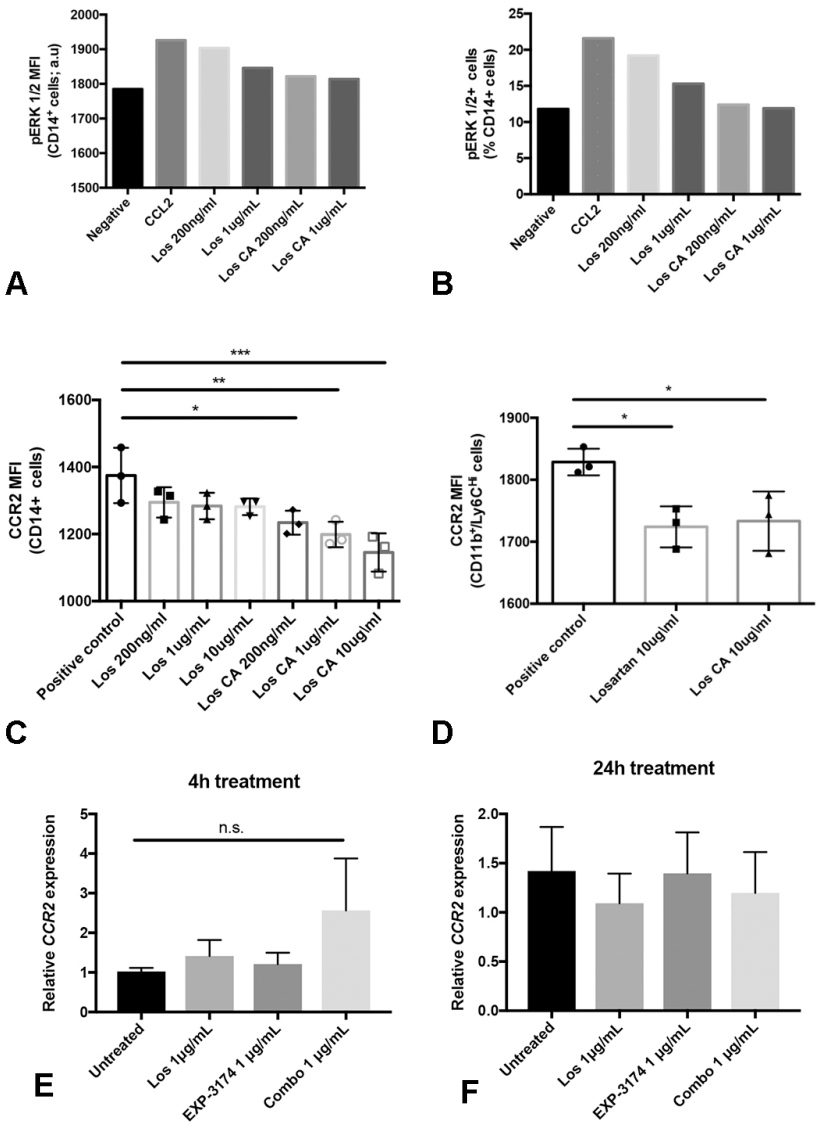


Fig. S2. Losartan and its metabolite EXP3174 decrease CCL2-induced ERK phosphorylation and CCR2 cell surface expression in primary human peripheral blood monocytes and murine bone marrow monocytes. **(A, B)** Graphs showing flow cytometric quantification of *pERK1/2* geometric MFI in $CD14^+$ monocytes (A), and *pERK1/2*$^+$ cells as % of total gated $CD14^+$ monocytes (B). (**C, D**) Bar graphs of flow cytometric quantification of cell surface *CCR2* expression (expressed as geometric mean fluorescence intensity, MFI) in human peripheral blood $CD14^+$ monocytes (**C**; three replicates from one donor, $^*p < 0.05$,$^{**}p < 0.01$, and $^{***}p < 0.001$, one-way ANOVA) and $Ly6G^-/CD11b^+/Ly6C^+$ murine bone marrow monocytes (**D**; $^*p < 0.05$, one-way ANOVA, n = 3 mice/group), following 4 hours of treatment with a range of clinically relevant concentrations of losartan and EXP3174 metabolite. (**E** and **F**) Effects of losartan (los), EXP3174 metabolite, or combination treatment on CCR2 mRNA expression in THP-1 cells, relative to vehicle control treated cells, following 4 h (**E**) and 24 h (**F**) of drug treatment. N.S., not significant, $p > 0.05$, one-way ANOVA. Data (means ± SD) are from one representative of two independent experiments (A, B, C, and D), or are pooled from two independent experiments (E and F, $n = 4$ to 5).

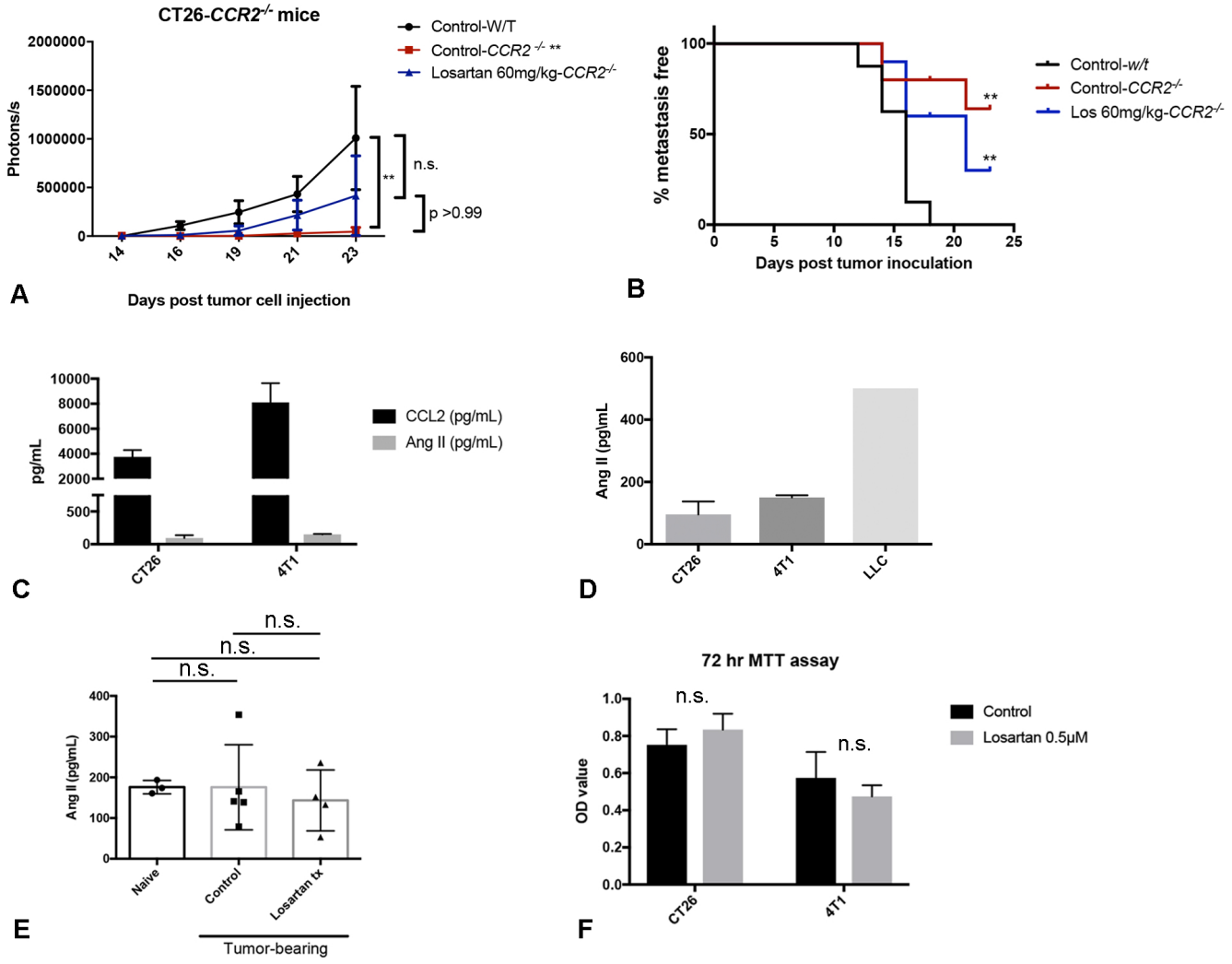
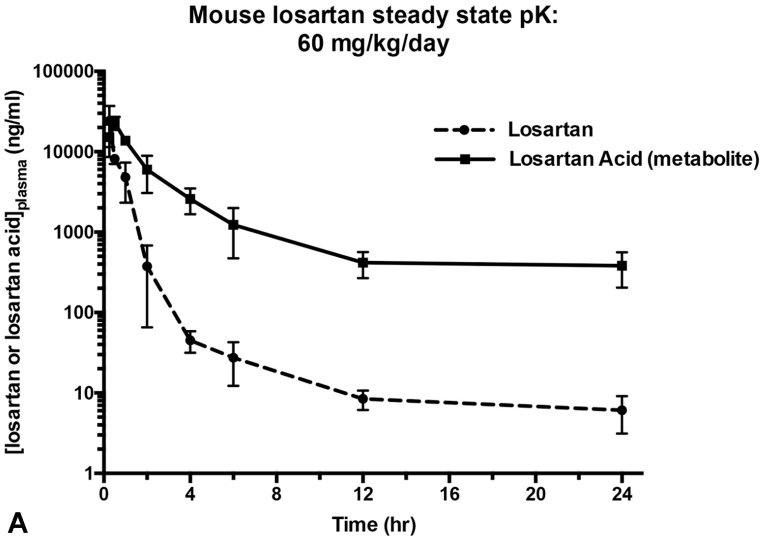


Figure S3. Losartan-mediated blockade of IM recruitment and associated anti-tumor effects are not associated with changes in Angiotensin II-AT1R signaling. **(A)** Quantification of CT26luc pulmonary metastatic burden over time in WT control, *CCR2*$^{-/-}$, and losartan-treated *CCR2*$^{-/-}$ mice by repeated bioluminescent imaging. **p < 0.01, n.s. p > 0.05, two-way ANOVA. **(B)** Kaplan-Meier curve demonstrating time to detection of luciferase-positive CT26 pulmonary metastases in WT control, *CCR2*$^{-/-}$, and losartan-treated *CCR2*$^{-/-}$. **p < 0.01, Log-rank test. **(C and D)** In vitro *CCL2* and *Ang II* production by 4T1, CT26, and LLC cell lines, measured by ELISA assay performed on cell culture supernatants following 24h of culture. **(E)** Ang II levels in the serum of untreated-control and losartan-treated CT26luc pulmonary metastasis-bearing mice as compared to naïve BALB/c mice, as measured by ELISA. n.s., p > 0.05, one-way ANOVA. **(F)** Results of MTT survival/proliferation assay following 72hr treatment of 4T1 or CT26 cells with losartan at the indicated concentrations. n.s., p > 0.05, *t* test. Data (means ± SD) are from one representative of two independent experiments (A-D, and F; *n* = 5 mice/group), or one independent experiment (E; *n* = 3-5 mice/group).



A

Table 1:	Parameters			
	$C_{max\ (\mu g/mL)}$	$AUC_{0-\infty\ (\mu g*hr/mL)}$	$T_{1/2,l\ (hr)}$	$C_{24h\ (ng/mL)}$
Losartan	24.3 ± 12.8	13.62	25.8	6.11 ± 2.99
Losartan carboxylic acid metabolite (EXP 3174)	22.9 ± 4.2	47.83	–	382 ± 178

B

Figure S4. Steady-state pharmacokinetics of high dose losartan in mice**. (A)** Mean plasma concentration of losartan and losartan carboxylic acid metabolite (EXP3174) in mice following 14 days of intra-peritoneal dosing at 60mg/kg once daily. Drug levels were measured by LC/MS/MS at the indicated post-dose time points following the last dose on day 14. **(B)** Table 1 summarizing PK parameters from data shown in (A). Data representative of the mean ± SD, *n* = 3 mice per time point.