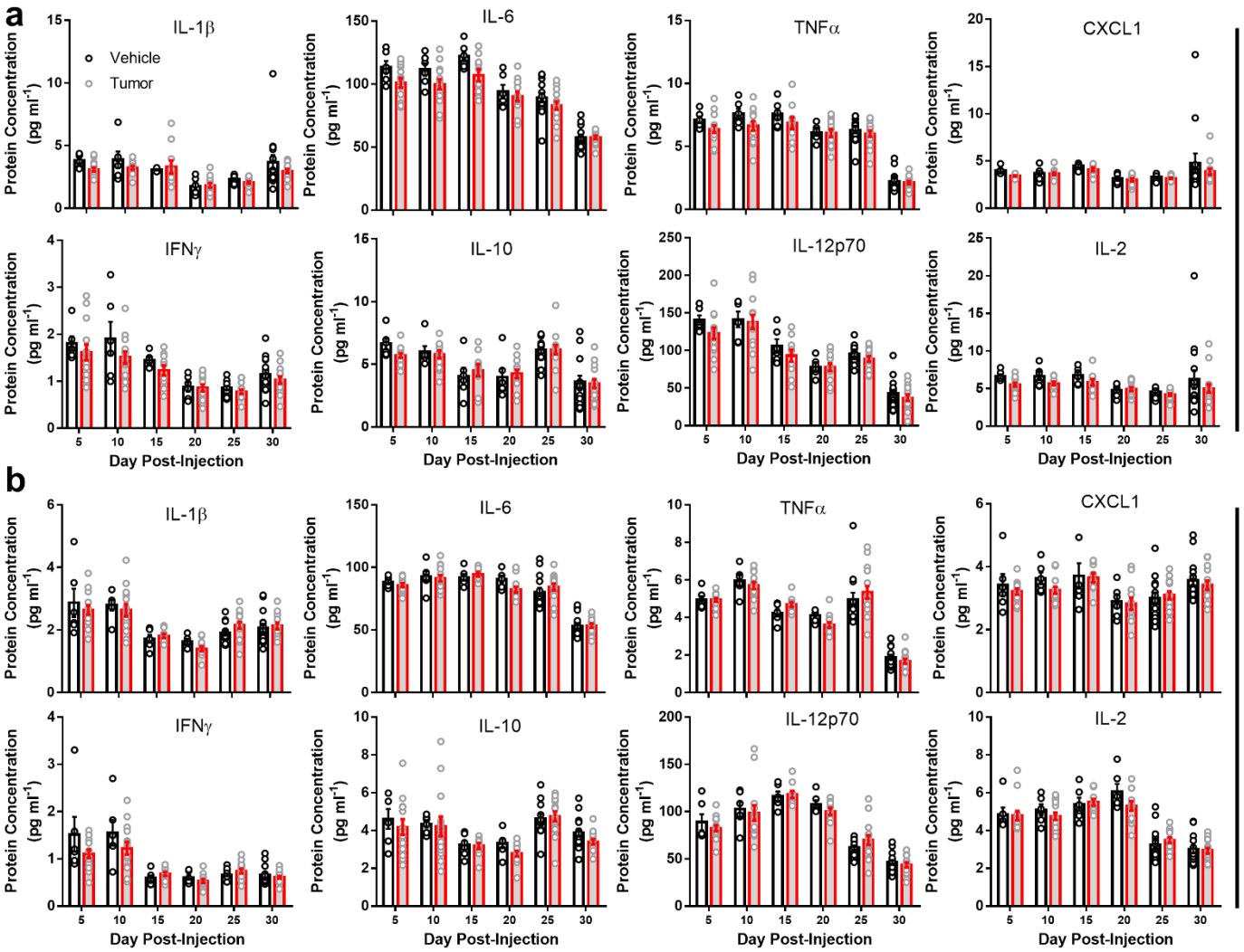
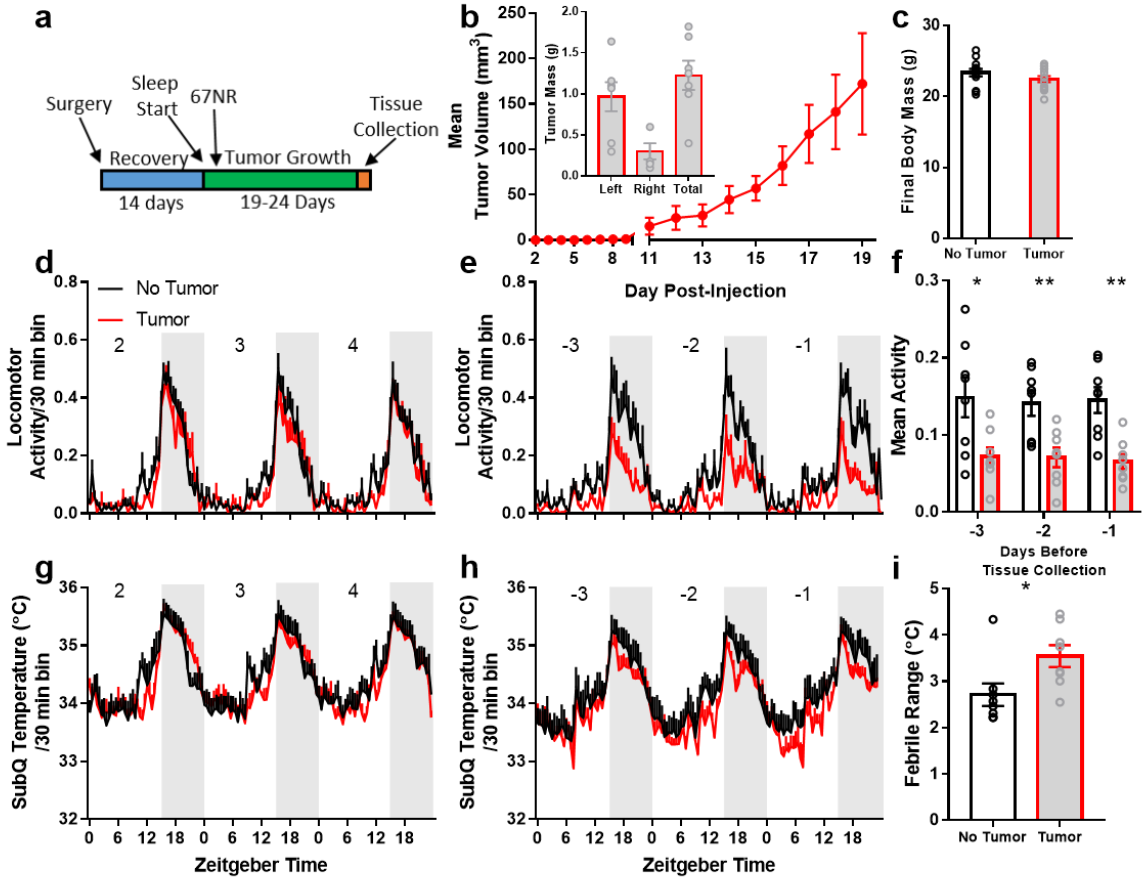
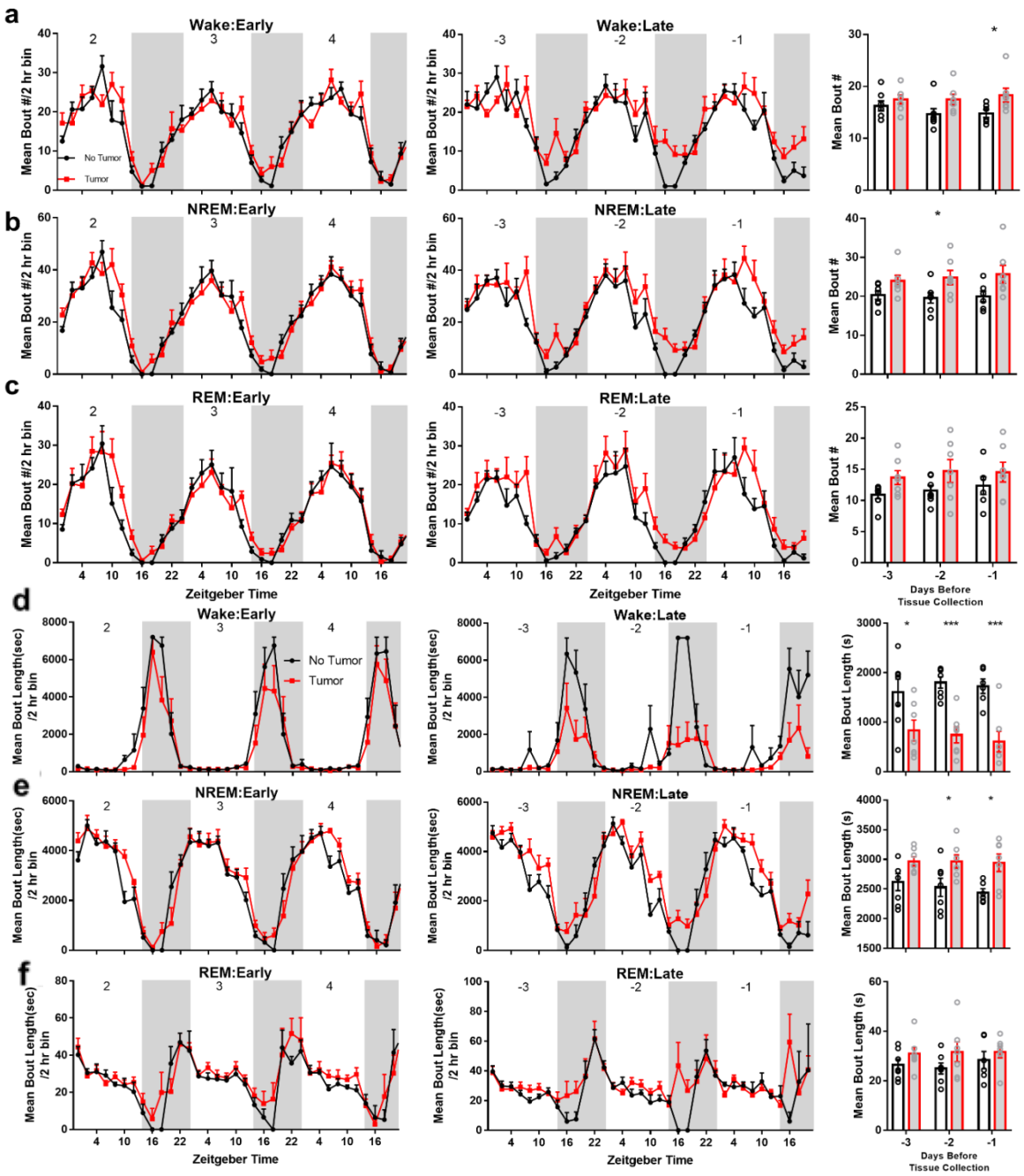


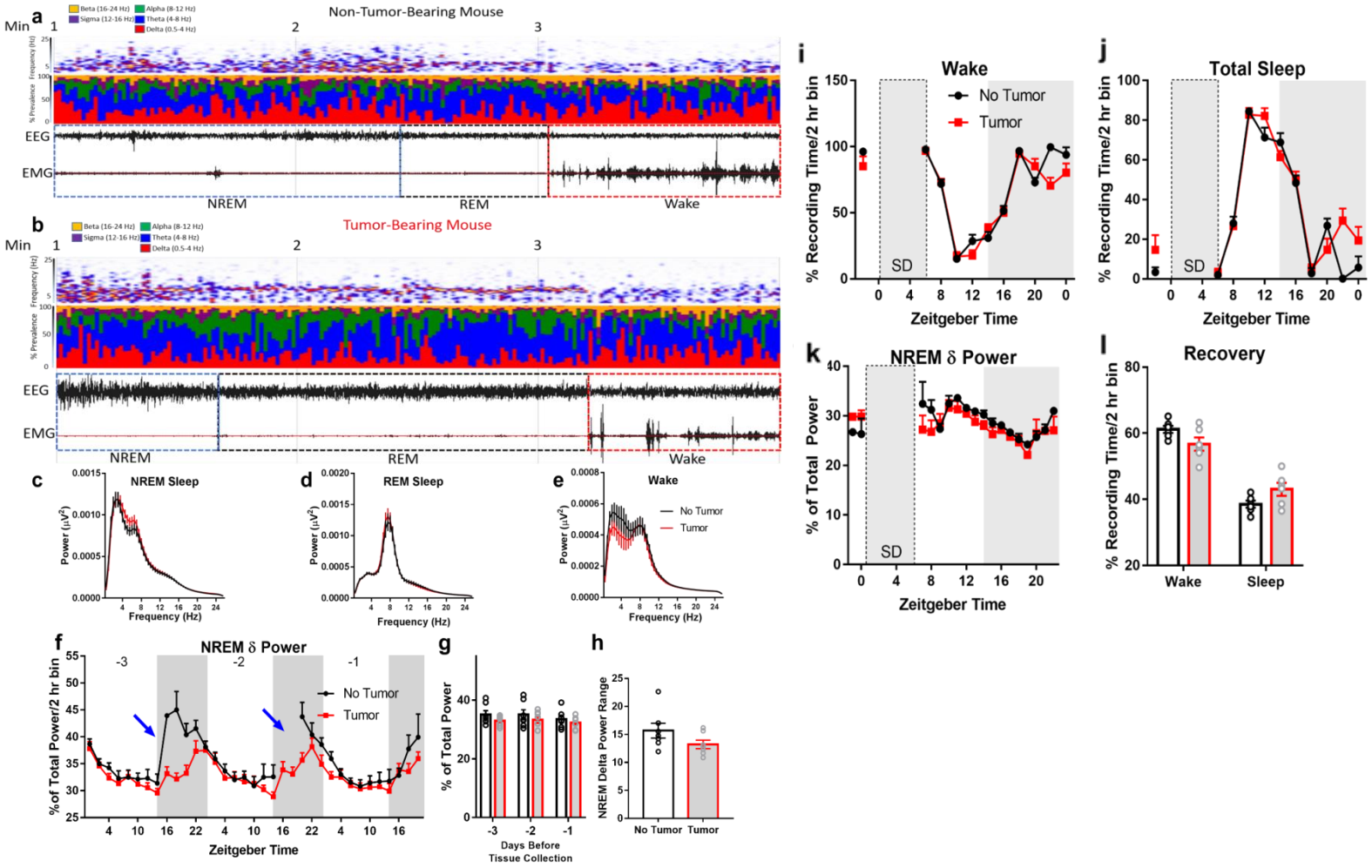
Supplementary Figure 1, related to Figure 1. Peripheral non-metastatic tumors do not induce neuroinflammation. No changes in any of the cytokines examined were detected in (a) the cortex or (b) the hippocampus throughout the course of tumor development (days 5, 10, 15, 20, 25, or 30). (error bars represent SEM, N = 5-6 for vehicle treated mice on days 5, 10, 15, 20, and N = 14-15 for days 25 and 30; N = 13-15 for tumor-bearing mice on all days).



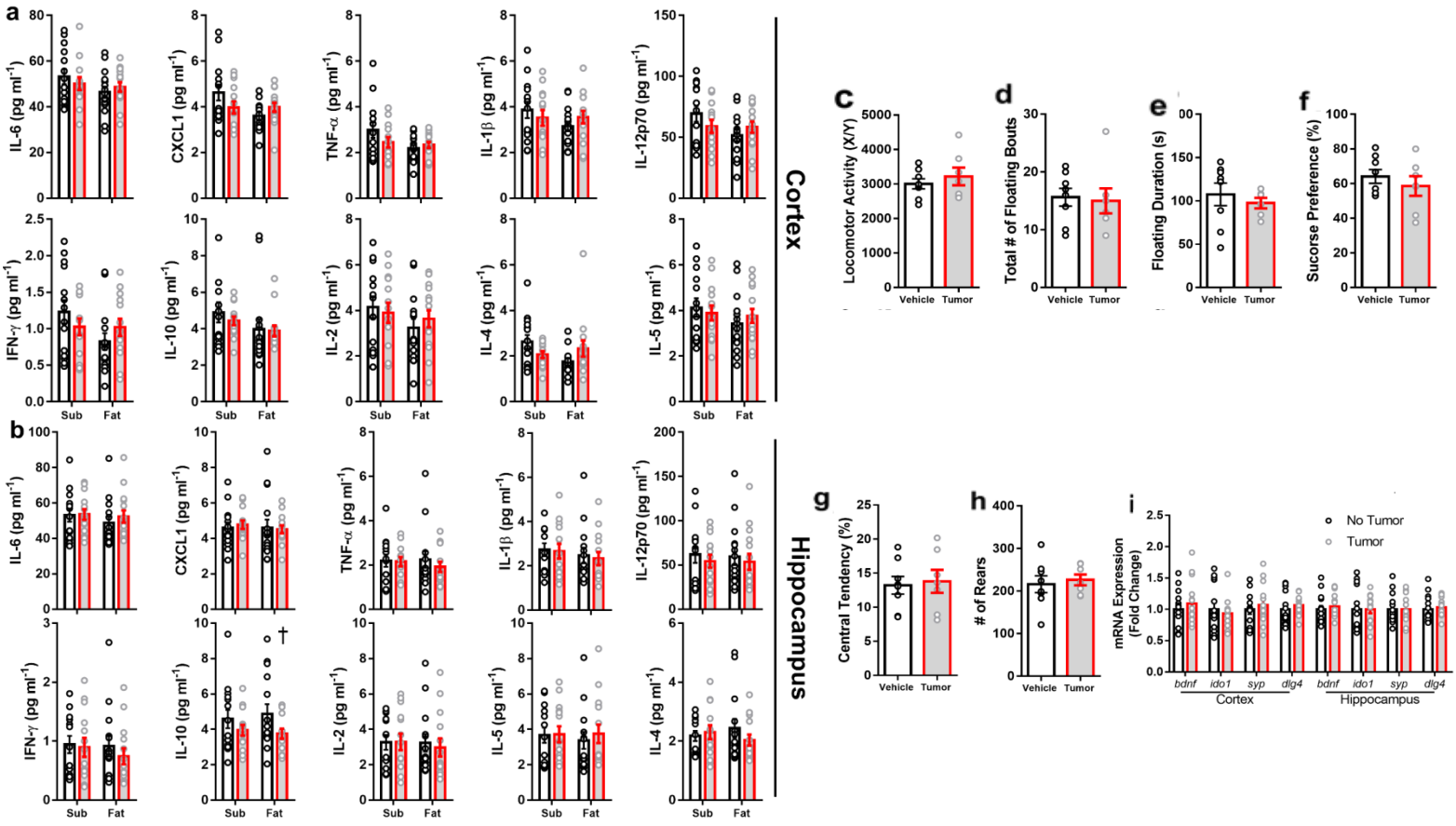
Supplementary Figure 2, related to Figure 3. Peripheral tumors disrupt locomotor activity rhythms and temperature regulation in mice. (a) schematic experimental design; (b) Mean tumor volumes throughout the course of the study (calculated using $V = (LW^2)/2$, (c) tumors did not alter body mass. (d) early after 67NR inoculation (days 2,3,4), locomotor activity was equivalent between groups, however (e,f) during the final days of tumor development (-3,-2,-1 days before tissue collection), tumor-bearing mice significantly reduced their daily activity. (N = 8/group, day -3 t = 2.676, p = 0.018, -2 t = 3.411, p = 0.004, -1 t = 4.095, p = 0.0011) (g) body temperature was unchanged during early timepoints following 67NR injection, (h,i) however, towards the end of the study tumor-bearing mice showed a large range in body temperatures indicating impaired temperature regulation (N = 8/group, t = 2.444, p = 0.028). (error bars represent SEM; *p < 0.05, **p < 0.01),student's t-test).



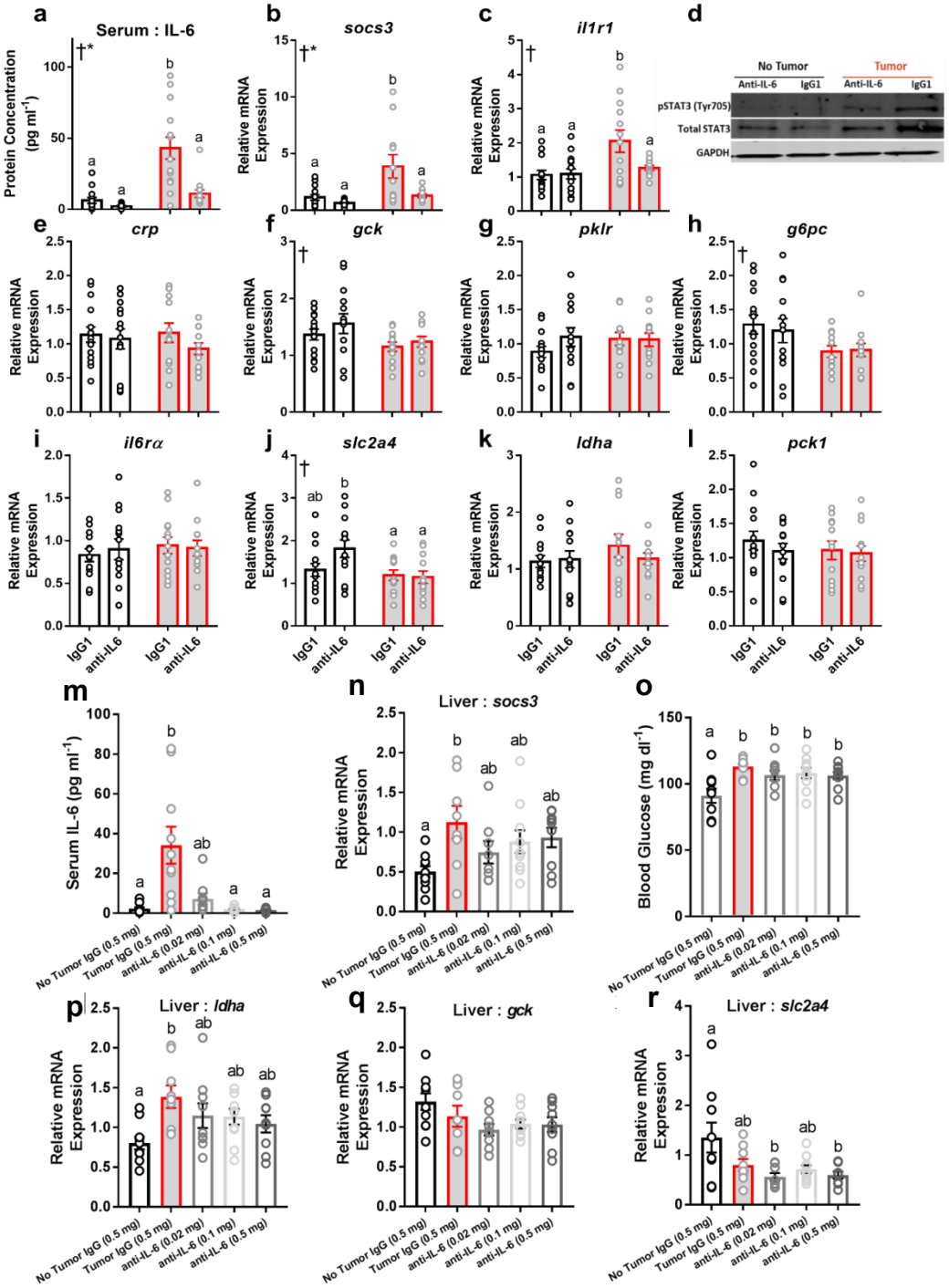
Supplementary Figure 3, related to Figure 3. Tumor-bearing mice have increased bouts of wakefulness and NREM sleep during the active phase. (a) early after 67NR inoculation (days 2, 3, 4), wakefulness bout numbers were equivalent between groups, however during the final days of tumor development (-3,-2,-1 days before tissue collection), tumor bearing mice increased their number of wake bouts indicating fragmentation of vigilance states (N = 6 no tumor, 7 tumor, day -1 t = 2.203, p = 0.049). (b) the same pattern was evident for NREM bouts (N = 7/group, day -2 t = 2.331, p = 0.038) but not (c) REM sleep bouts, and the greatest difference was observed during the dark (active phase). Tumor-bearing mice cannot maintain long bouts of wakefulness during the active phase. (d) early after 67NR injection, wake bout lengths were equivalent between groups, however during the last stages of tumor development (days -3,-2,-1 before tissue collection), mice with tumors were unable to maintain extended bouts of wakefulness during the active phase (N = 7/group, day -3 t = 2.292, p = 0.041; day -2 t = 5.342, p = 0.0002; day -1 t = 4.302, p = 0.0013). (e) this was accompanied by an increase in the length of NREM bouts during the same time period (N = 7/group, day -2 t = 2.317, p = 0.039; day -1 t = 2.925, p = 0.014), without significant changes in (f) REM sleep bout duration (Error bars represent SEM, *p < 0.05, ***p < 0.005 student's t-test). (Error bars represent SEM, *p < 0.05; student's t-test).



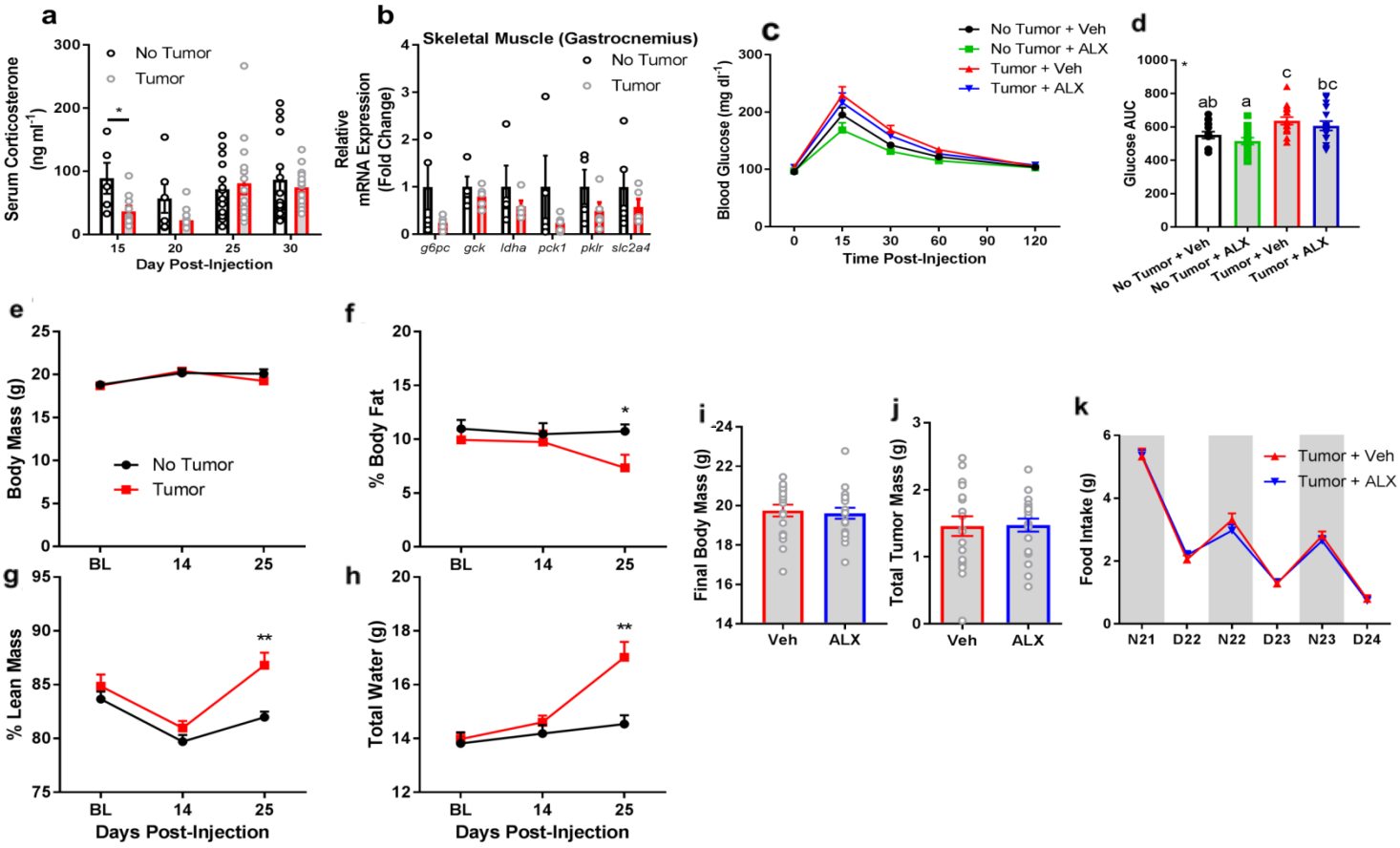
Supplementary Figure 4, related to Figure 3. The spectral components of sleep are intact in tumor-bearing mice, but they show altered temporal patterns of NREM delta power. (a and b) representative 3 minute EEG/EMG recordings from a non-tumor-bearing and a tumor-bearing mouse demonstrate normal frequency distributions during vigilance state transitions (outlined hatched boxes), and summed three final days of data recording showing the spectral power for (c) NREM sleep, (d) REM sleep, and (e) wakefulness. (f) within NREM sleep, the delta frequency shows reduced amplitude during the active phase (delineated by blue arrows), indicating lower sleep pressure due to increased sleep during this time period. (g, h) despite this temporal difference, the mean delta power was unchanged and range of values was similar between groups. (Error bars represent SEM, N =7-8/group for a-h). Tumor-bearing mice show intact responses to sleep deprivation. Mice were sleep deprived via gentle handling during the first 6 hours of the light phase (ZT 0-6) when sleep pressure is high, and allowed 18 h of recovery. Both non-tumor bearing and tumor-bearing mice showed equivalent (i,j,k) patterns of increased sleep and decreased wakefulness following sleep deprivation, and (k) normal (enhanced) NREM delta power during recovery sleep. (Error bars represent SEM, N = 6/group for i-l).



Supplementary Figure 5, related to Figure 1. Peripheral 67NR tumors do not promote neuroinflammation regardless of whether they are placed subcutaneously (Sub) or orthotopically within the mammary fat pad (Fat). (a) cortical and (b) hippocampus protein concentrations of inflammatory and anti-inflammatory cytokines at day 25 post-injection, for hippocampal IL-10 N = 14 no tumor subQ, 15 no tumor fat pad, 14/group for tumor subQ and fat pad injections, main effect of tumor $F_{1,53} = 4.117$, $p = 0.0475$). (error bars represent SEM; † = main effect of tumor, N = 13-16/group, two-way ANOVA for a and b). Tumor-bearing mice do not show behavioral deficits or alterations to genes frequently deregulated in disorders of mental health. (c) tumor and non-tumor bearing mice show equivalent phenotypes in the open field arena, including (g) central tendency and (h) the number of rears, indexes of anxiety-like behavior. (d,e) tumor and non-tumor bearing mice show equivalent responses in the forced swim test, as well as (f) sucrose preference, measures of depressive-like behavior. (i) the transcription of many genes previously implicated in animal models of depression were unchanged regardless of tumor status or brain region examined. (Behavioral testing completed on days 24-25 post-injection; error bars represent SEM, N = 7-8 for behavioral tests, and 14-15 for gene expression (i)).


a
Serum : IL-6
Protein Concentration (pg ml-1)
b
socs3
Relative mRNA Expression
c
il1r1
d
No Tumor
Tumor
Anti-IL-6
IgG1
pSTAT3 (Tyr705)
Total STAT3
GAPDH
e
crp
f
gck
g
pklr
h
g6pc
i
il6rα
j
slc2a4
k
ldha
l
pck1
IgG1
anti-IL6
m
Serum IL-6 (pg ml-1)
n
Liver : socs3
o
Blood Glucose (mg dl-1)
No Tumor IgG (0.5 mg)
Tumor IgG (0.5 mg)
anti-IL-6 (0.02 mg)
anti-IL-6 (0.1 mg)
anti-IL-6 (0.5 mg)
p
Liver : ldha
q
Liver : gck
r
Liver : slc2a4

Supplementary Figure 6, related to Figure 5. A single injection of a neutralizing antibody against IL-6 at day 22 does successfully knockdown IL-6 signaling at day 25 in tumor-bearing mice. (a) tumor bearing mice that received the IgG1 isotype control had elevated IL-6 serum concentrations, but treatment with anti-IL6 normalized these values (N = 13 no tumor IgG, 14 no tumor anti-IL6, 14 tumor IgG, 13 tumor anti-IL6, main effect of tumor $F_{1,50} = 27.4$, $p < 0.0001$, main effect of antibody $F_{1,50} = 17.25$, $p = 0.0001$, interaction $F_{1,50} = 10.23$, $p = 0.0024$). Consistent with reduced IL-6 signaling, mRNA expression of IL-6 target genes (b) *socs3* (N = 14 no tumor IgG, 12 no tumor anti-IL6, 11/group for tumor IgG and anti-IL6, main effect of tumor $F_{1,44} = 11.26$, $p = 0.0016$, main effect of antibody $F_{1,44} = 9.409$, $p = 0.0037$, interaction $F_{1,44} = 4.306$, $p = 0.0439$) and (c) *il1r1* (N = 13 no tumor IgG and 14 no tumor anti-IL6, 13 tumor IgG and 12 tumor anti-IL6, main effect of tumor $F_{1,48} = 8.888$, $p = 0.0045$, interaction $F_{1,44} = 4.392$, $p = 0.0414$) and (d) protein expression of phosphorylated STAT3 (pSTAT3 (Tyr705)) were attenuated by antibody treatment. Despite reducing IL-6 signaling, liver metabolic abnormalities remained, with tumor-bearing mice showing reduced (f) *gck* (N = 15 no tumor IgG, 14 no tumor anti-IL6, 12/group tumor IgG and anti-IL6, main effect of tumor $F_{1,49} = 4.814$, $p = 0.033$), (h) *g6pc* (N = 15 no tumor IgG, 13 no tumor anti-IL6, 11 tumor IgG, 12 tumor anti-IL6, main effect of tumor $F_{1,47} = 6.295$, $p = 0.0156$), and (j) *slc2a4* (N = 14 no tumor IgG, 13 no tumor anti-IL6, 12/group for tumor IgG and anti-IL6, main effect of tumor $F_{1,47} = 6.016$, $p = 0.018$) expression regardless of which treatment (IgG1 or anti-IL6) they received. (error bars represent SEM, † = main effect of tumor, * = main effect of antibody treatment; multiple comparisons (different letters) denote bars that differ from one another at $p < 0.05$, two-way ANOVA, Tukey's multiple comparisons post-hoc test for a-l). Escalating doses of IL-6 mAb do not attenuate tumor-induced elevations in blood glucose concentrations. (m) Serum IL-6 concentrations were altered by antibody treatment ($H = 22.45$, $p = 0.0002$; Kruskal-Wallis test with Dunn's multiple comparisons). (n) *socs3* expression ($F_{4,40} = 2.847$, $p = 0.0362$). (o) blood glucose was not affected by antibody treatment regardless of dose ($F_{4,45} = 5.083$, $p = 0.0018$). (p) *ldha* expression (F4,42 = 3.217, $p = 0.0216$). (q) *gck* was unaltered, (r) *slc2a4* expression ($F_{4,40} = 3.774$, $p = 0.0107$). (n = 8-10/group; Error bars represent S.E.M., 1-way ANOVA with Tukey's post-hoc test for m-r).



Supplementary Figure 7, related to Figures 2,6: (a) Transient changes in corticosterone concentrations observed in tumor-bearing mice during early tumor-development are absent towards later stages of tumor growth. (Day 15 n = 5 no tumor, 10 tumor, t = 2.581, p = 0.023). (n = 6 no tumor, 12 tumor day 20; 14-15/group for days 25 and 30). (b) No change in skeletal muscle gene expression of enzymes involved in gluconeogenesis/glycolysis at day 25 post-injection (n = 4-5/group. Error bars represent SEM, *p < 0.05 student's t-test). Almorexant pre-treatment attenuates measures of glucose intolerance (assayed in fasting mice at ZT 1; drug administered 12 hours earlier) in tumor-bearing mice. (c) glucose tolerance test following 2 mg/kg injection of glucose, and (d) Area Under the Curve measures (*main effect of tumor: $F_{1,56} = 14.66$, p = 0.0003). (N = 15/group, error bars represent S.E.M., 2-way ANOVA with Fisher's LSD post-hoc test for c and d). Changes in body composition assessed using EchoMRI during the course of tumor growth. (e) no change in body mass (corrected for tumor mass at tissue collection), (f) change in % body fat (day 25 t = 2.463, p = 0.039). (g) change in % lean mass (day 25 t = 3.763, p = 0.0055), and (h) change in total water mass (day 25 t = 3.759, p = 0.00556). (n = 5/group/timepoint, error bars represent S.E.M., multiple student's t-tests for e, f, g, and h). Chronic almorexant treatment (days 15, 18, 21, 24; ZT 12) does not alter (i) final body masses at day 25 (corrected for tumor burden), (j) tumor burden at tissue collection, or (k) food intake in tumor-bearing mice. (Error bars represent SEM; n = 19-20/group for (i) and (j) and 14-15/group for (k)).

Supplementary Table 1, related to STAR Methods: Gene expression primer/probe information.

Gene Name	Assay ID	Amplicon Length (bp)
IL-1β	Mm00434228_m1	90
IL-6	Mm00446190_m1	78
TNFα	Mm00443258_m1	81
Socs3	Mm00545913_s1	76
Stat3	Mm01219775_m1	75
Il6rα	Mm00439653_m1	98
Il1r1	Mm00434237_m1	63
Crp	Mm00432680_g1	134
Ccl2	Mm00441242_m1	74
Ifnγ	Mm01168134_m1	100
Ido1	Mm01218006_m1	83
bdnf	Mm01334044_m1	92
Dlg4	Mm00492193_m1	114
Syp	Mm00436850_m1	60
Ldha	Mm01612132_g1	95
Pck1	Mm01247058_m1	61
G6pc	Mm00839363_m1	116
Pklr	Mm00443090_m1	62
Gck	Mm00439129_m1	69
Slc2a4	Mm00436615_m1	68
18s	Hs99999901_s1	187