Supplementary Figures and Tables

Metronomic cyclophosphamide schedule-dependence of innate immune cell recruitment and tumor regression in an implanted glioma model

Junjie Wu and David J. Waxman

Division of Cell and Molecular Biology, Department of Biology,
Boston University, 5 Cummington Mall, Boston, MA 02215

Supplemental Table S1. Forward and reverse primer sets used for qPCR for the indicated mouse gene transcripts. Gene names are shown in parentheses. Sequences for the following primer sets were reported previously [21]: TNF receptor superfamily member 6 (*Fas*), natural cytotoxicity triggering receptor 1 (Nkp46), perforin 1 (*Prf1*), granzyme B (*Gzmb*), killer cell lectin-like receptor subfamily K, member 1 (Nkg2d or *Klrk1*), Cd68 antigen (*Cd68*), Cd74 antigen (*Cd74*), Cd207 antigen (Cd207), EGF-like module containing, mucin-like, hormone receptor-like sequence 1 (Emr1). Fas ligand (Fasl) was reported in [22].

Supplemental Fig. S1. Time course of changes in tumor expression levels of the indicated genes assayed by qPCR. The experiments were conducted as in Fig. 2, except that Icam2 and Vcam1 were assayed using pooled cDNA from n = 4 to 6 individual tumors**.**

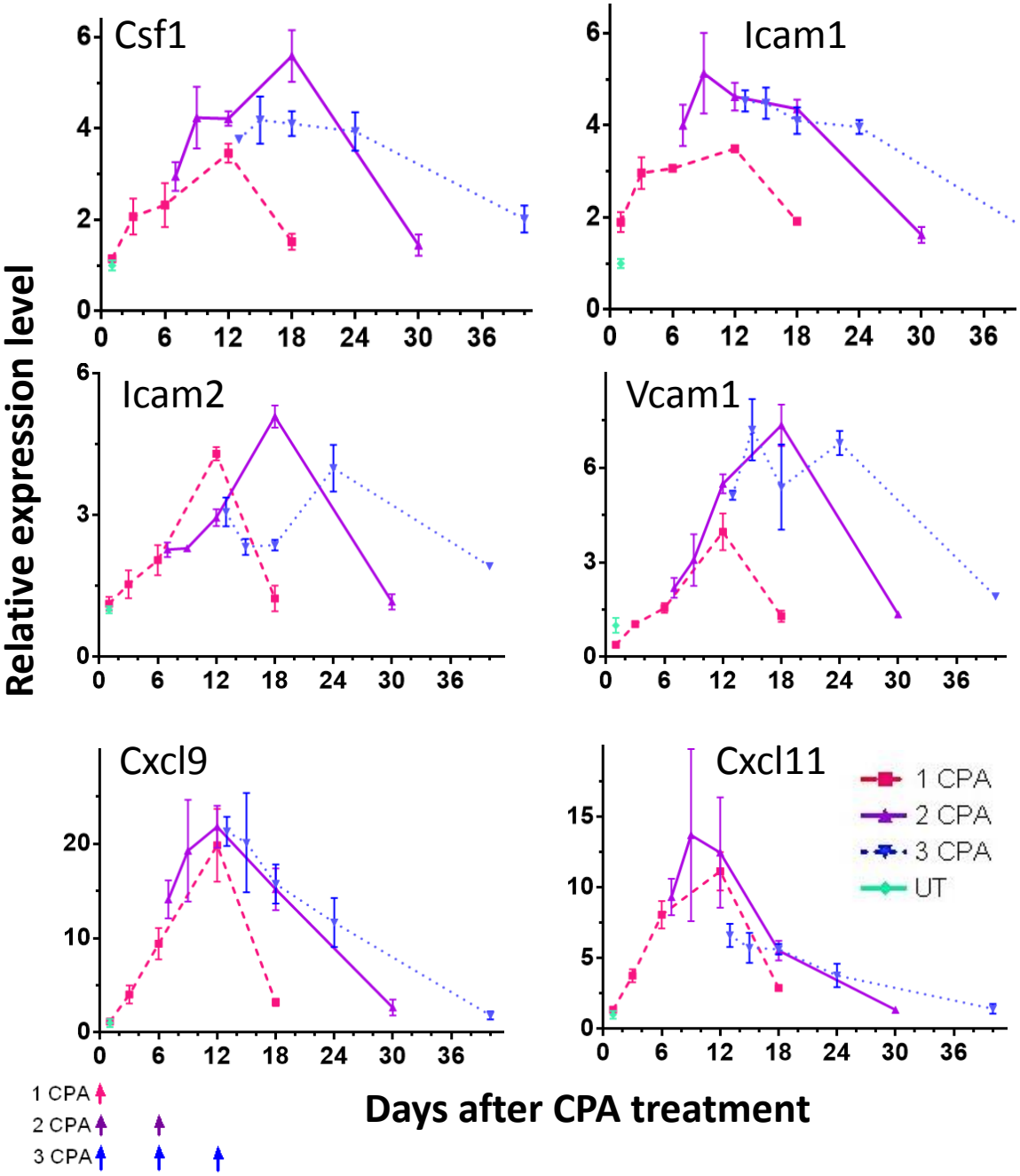
Supplemental Fig. S2. Individual GL261 tumor growth curves from four metronomic CPA treatment schedules (CPA/6d, CPA/9d, CAP/6-9d, and CPA(210)/9d). Rebounding and regressing tumor numbers are as indicated.

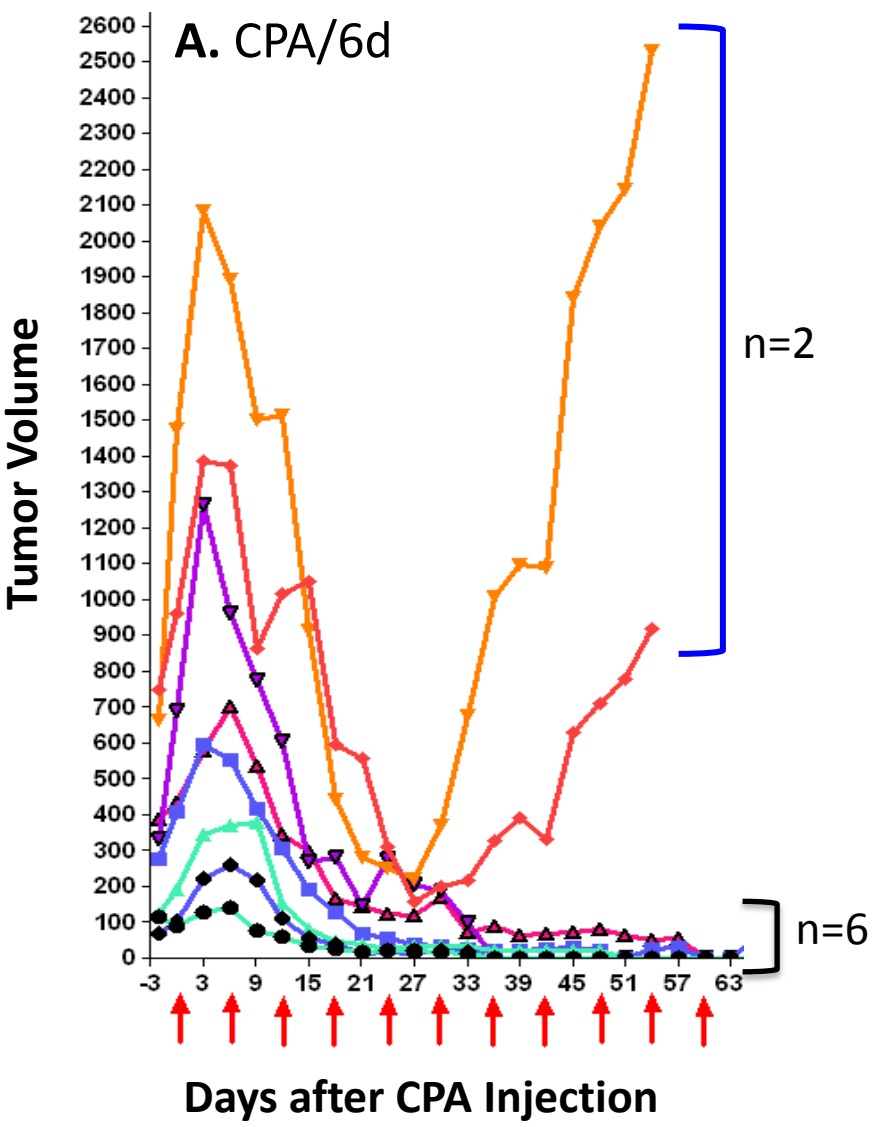
Supplemental Fig. S3. Body weights for GL261-bearing mice treated on the indicated metronomic CPA schedules. Data are normalized to the first treatment day (day 0). Body weight data are mean ± SEM, n = 2, 4, 6, 5, 7 mice for untreated, CPA/6d, CPA/9d, CPA(210)/9d, CAP/6-9d, CPA/12d treatment groups, respectively. The CPA/12d and CPA/9d schedules were associated with higher total body weight than the CPA/6d or CPA(210)/9d schedules; however, these increases primarily reflect the increased tumor sizes in the CPA/12d and CPA/9d treated mice.

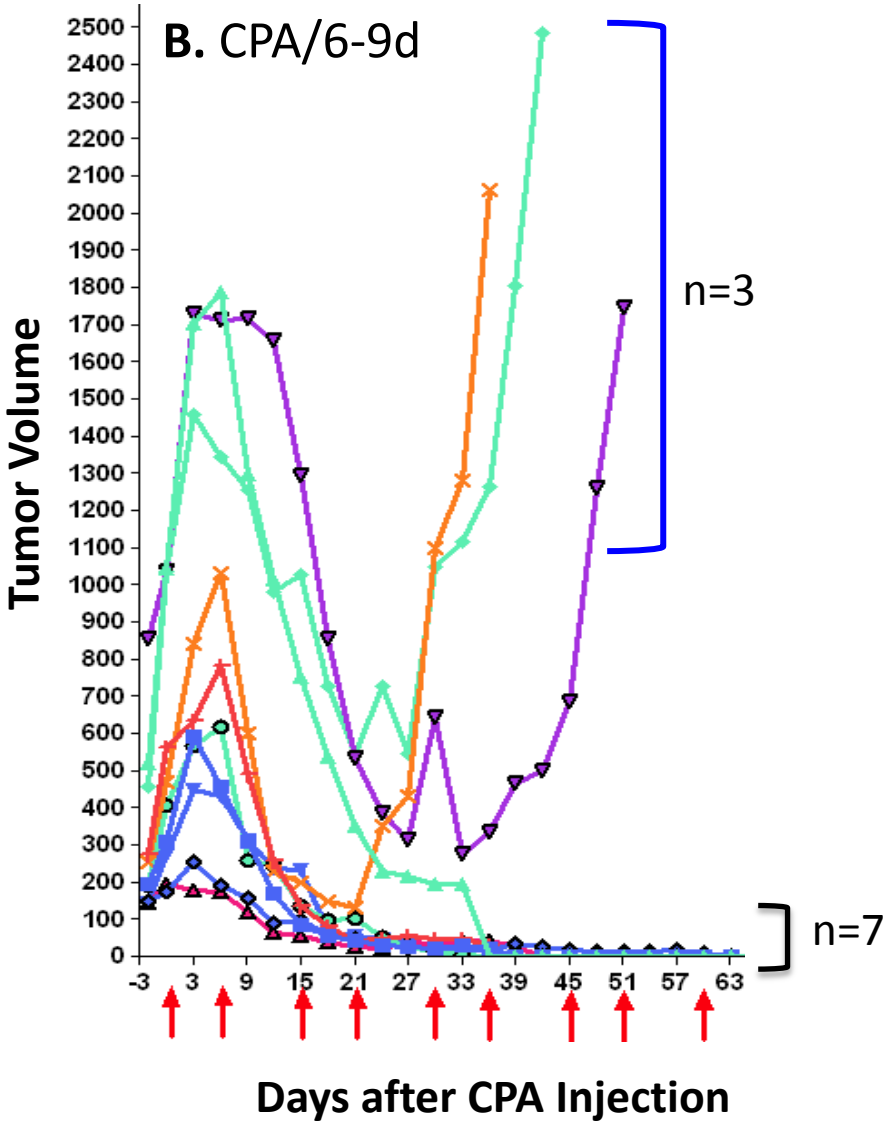
Supplemental Table S1.

Gene	Primer #	Primer Sequence (5' to 3')
interleukin 15 (IL15)	5095	forward: GAAGGCTGAGTTCCACATCTAACA
	5096	reverse: CTGCTGACATGGGTTTCTGTGTT
interleukin 18 (IL18)	5099	forward: TGTGACCCTCTCTGTGAAGGATAGT
	5100	reverse: GTTTCTGAAAGAATATGAGATCACTTTG
colony stimulating factor 1 (Csf1)	5089	forward: GAGGCTCCAGGAACTCTCCAAT
	5090	reverse: CAAGGAGATTCTTTGTTTCATTAAAGAAG
intercellular adhesion molecule 1 (Icam1)	4842	forward: CAAGGGCTGGCATTGTTCTCT
	4843	reverse: GGGTGTCGAGCTTTGGGATG
vascular endothelial growth factor A (VEGFA)	3612	forward: CGACAGAAGGAGAGCAGAAGTCC
	3613	reverse: GACGGCAGTAGCTTCGCTGG
chemokine (C-X-C motif) ligand 10 (Cxcl10)	3373	forward: ACCATGAACCCAAGTGCTGCC
	3374	reverse: CTATGGCCCTCATTCTCACTGGCC
Toll-like receptor 7 (Tlr7)	5141	forward: GGATGATCCTGGCCTATCTCTGA
	5142	reverse: TCTCTTCCGTGTCCACATCGA
intercellular adhesion molecule 2 (Icam2)	4846	forward: TTGCTGGAGCCTGTCTCTTCTTA
	4847	reverse: GCAGTTGGTGCTGCAGTTGAT
vascular cell adhesion molecule 1 (Vcam1)	4840	forward: TCTGGGAAGCTGGAACGAAGT
	4841	reverse: CAGCCTGTAAACTGGGTAAATGTCT
chemokine (C-X-C motif) ligand 9 (Cxcl9)	5076	forward: GAAGAAGCTGATGAAAGAATGGGAA
	5077	reverse: ATTCAGGGTGCTTGTTGGTAAAGTAA
chemokine (C-X-C motif) ligand 11(Cxcl11)	4878	forward: GCTGAGATGAACAGGAAGGTCACA
	4879	reverse: TGCCATTTTGACGGCTTTCAT

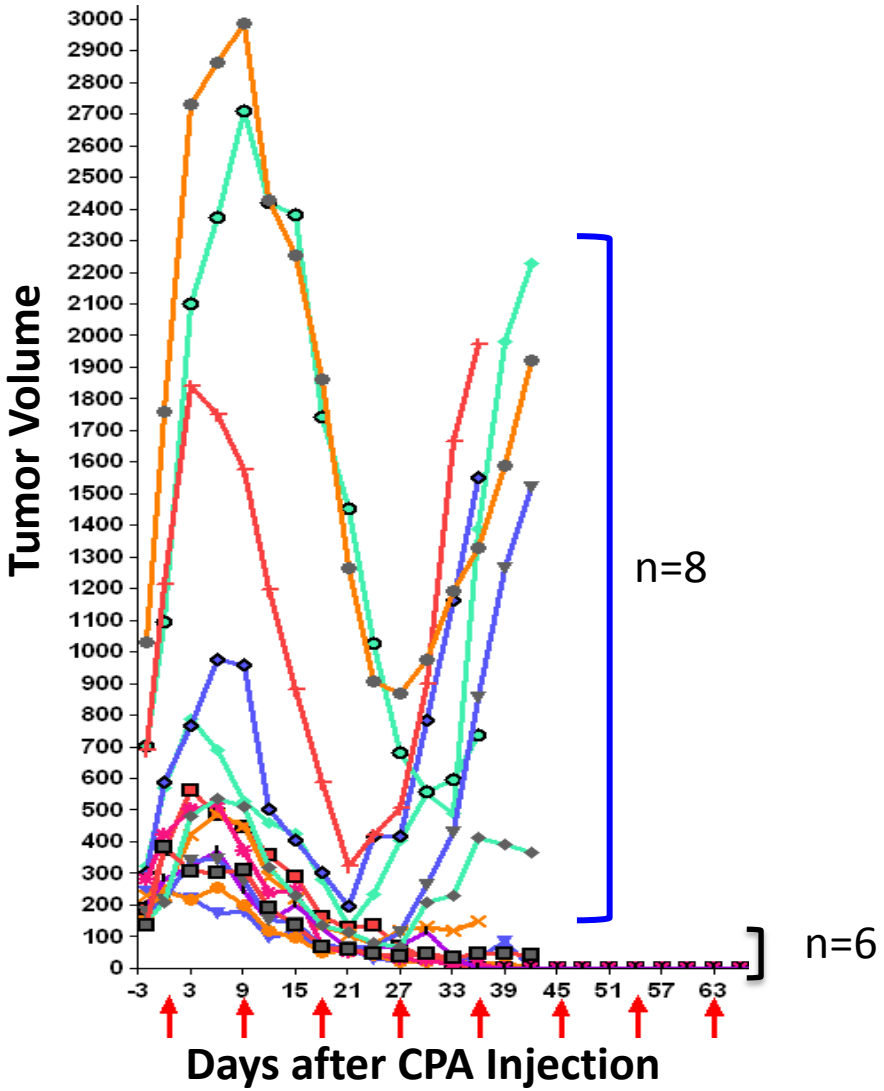
Fig. S1


Csf1
Icam1
Icam2
Vcam1
Cxcl9
Cxcl11
1 CPA
2 CPA
3 CPA
UT
Relative expression level
Days after CPA treatment
1 CPA
2 CPA
3 CPA


A. CPA/6d
Tumor Volume
2600
2500
2400
2300
2200
2100
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-3 3 9 15 21 27 33 39 45 51 57 63
n=2
n=6
Days after CPA Injection


B. CPA/6-9d
Tumor Volume
2500
2400
2300
2200
2100
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-3 3 9 15 21 27 33 39 45 51 57 63
n=3
n=7
Days after CPA Injection

C. CPA/9d



D. CPA(210)/9d

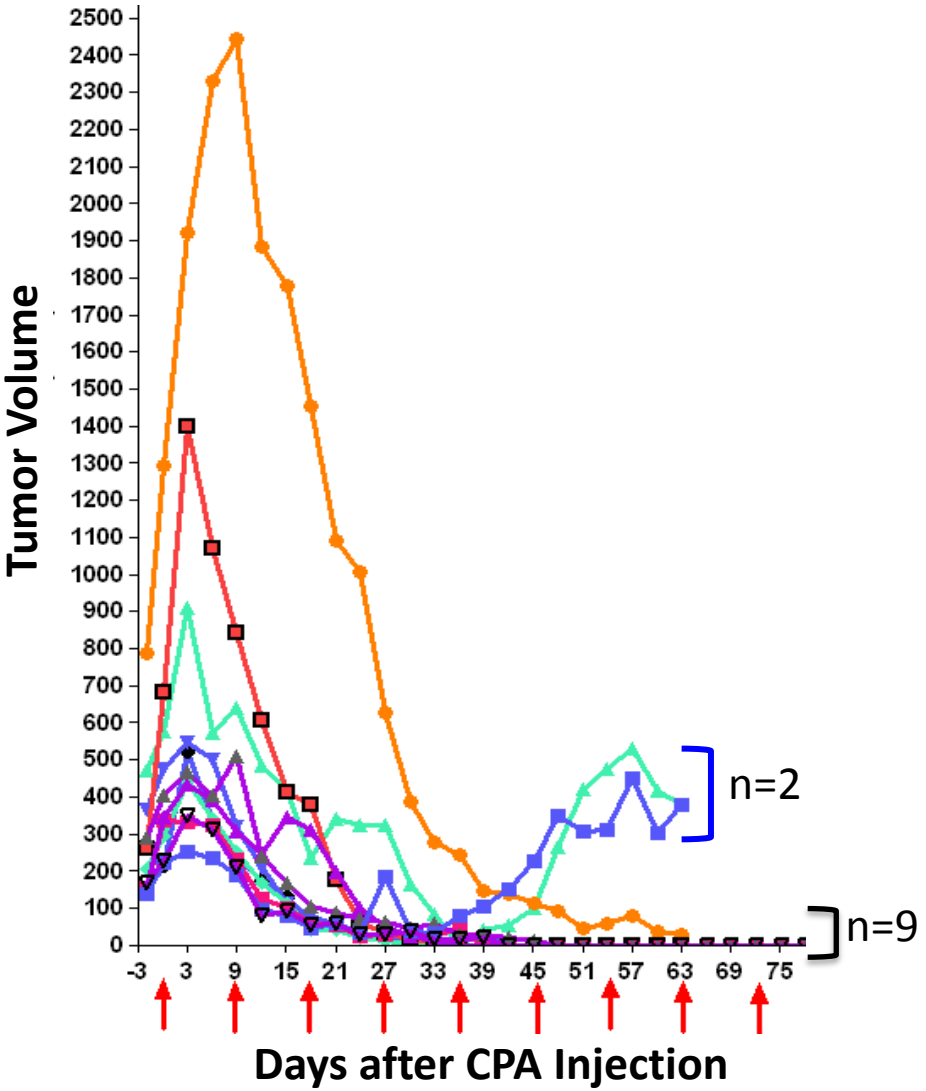
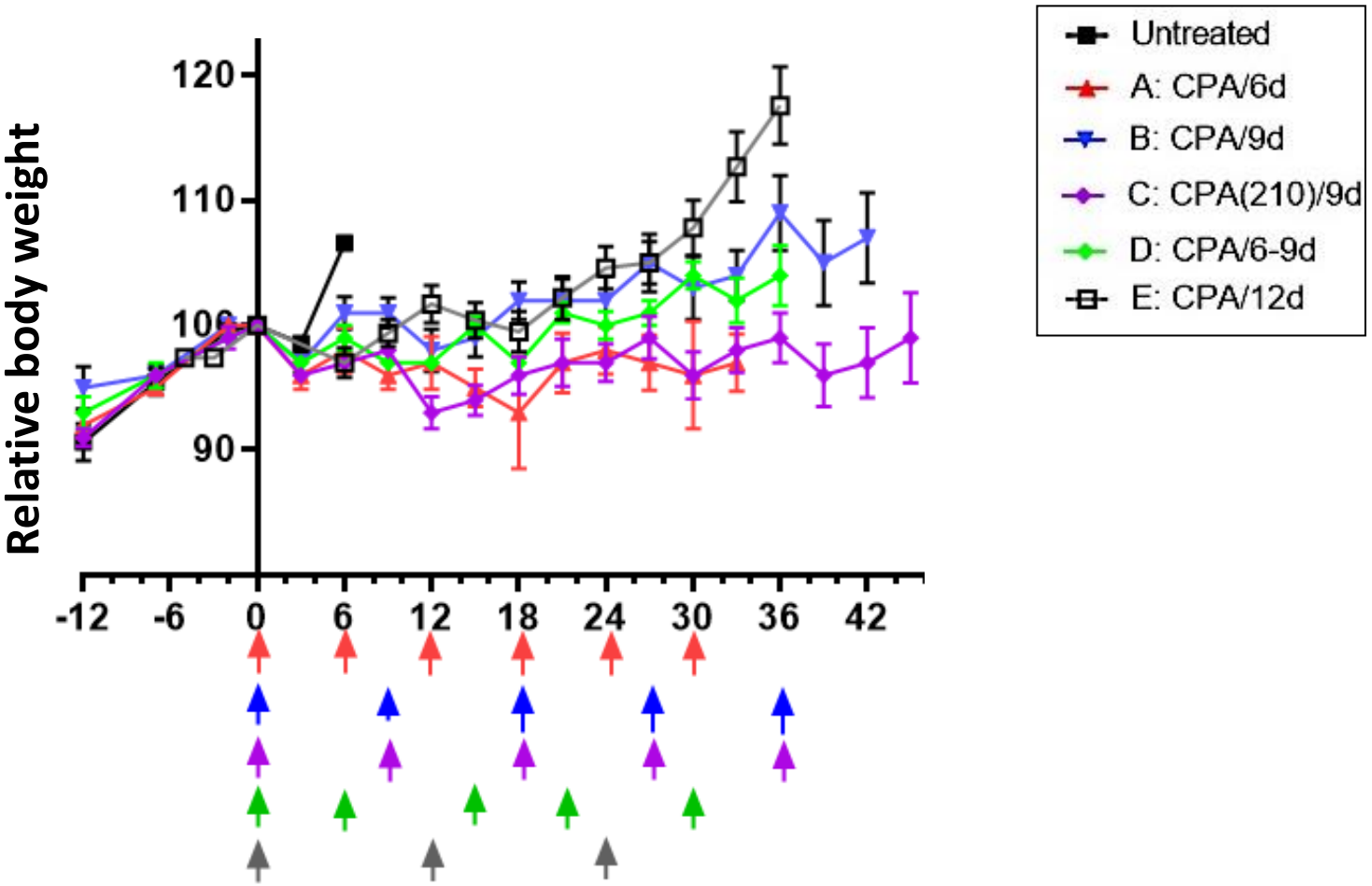


Fig. S3


Relative body weight
120
110
100
90
-12
-6
0
6
12
18
24
30
36
42
Untreated
A: CPA/6d
B: CPA/9d
C: CPA(210)/9d
D: CPA/6-9d
E: CPA/12d