Supplementary figures and tables

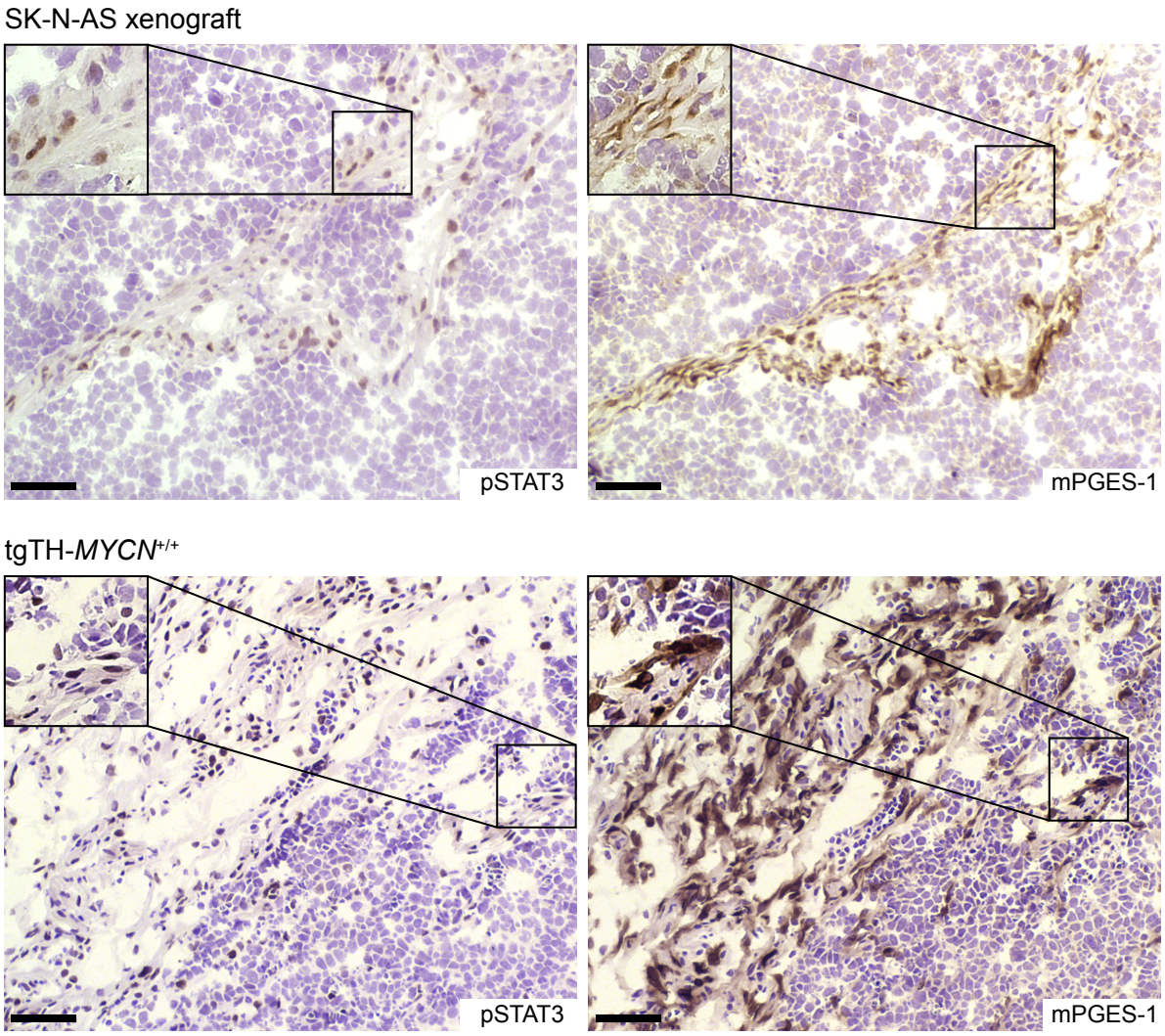

Figure S1. Expression of phosphorylated STAT3 (pSTAT3) and mPGES-1 in the tumor stroma of neuroblastoma mouse models. Representative images from immunohistochemical staining of mPGES-1 and pSTAT3 (DAB, brown staining) in consecutive sections from xenograft tumors generated from an 11q-deleted neuroblastoma cell line (SK-N-AS) and MYCN-driven transgenic neuroblastoma model (tgTH-*MYCN*). Sections were counterstained with Mayer's hematoxylin (blue staining). Scale bars indicate 100 µm.

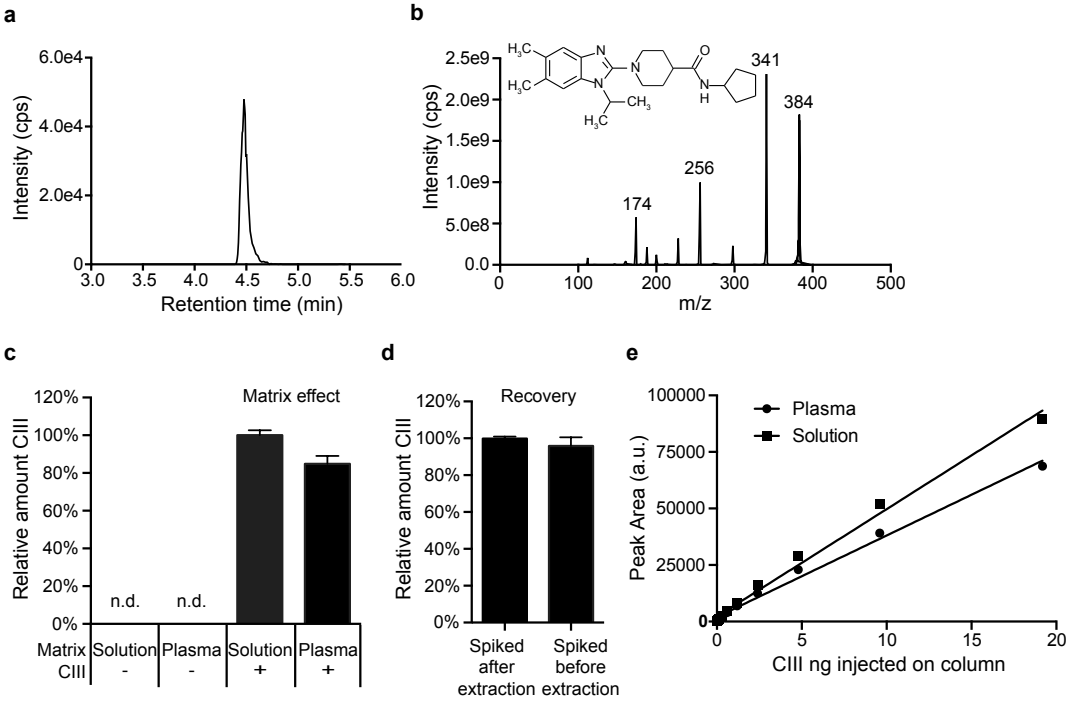

Figure S2. A targeted LC-MS/MS method for CIII detection was developed and validated in plasma. (a) Representative multiple reaction monitoring (MRM) chromatogram for CIII (m/z 384 > 341) spiked in plasma. Intensity is measured as counts per second (cps). (b) Mass spectrum showing the fragmentation pattern of CIII (insertion). (c) The matrix effect was determined by spiking CIII in plasma sample after extraction. Minor signal suppression was observed in plasma (88 ± 1%, mean ± SEM, n = 6). (d) Recovery was determined by spiking CIII in plasma samples before or after extraction. We found close to full recovery of CIII in plasma (96 ± 5%, n = 6). (e) Our LC-MS/MS method for CIII quantification gave good linearity in both plasma ($R^2 = 0.98$) and in solution ($R^2 = 0.99$) for the dynamic range of 0.1-20 ng injected on column. The limit of quantification (signal-to-noise > 10) in solution was 4 pg injected on column.

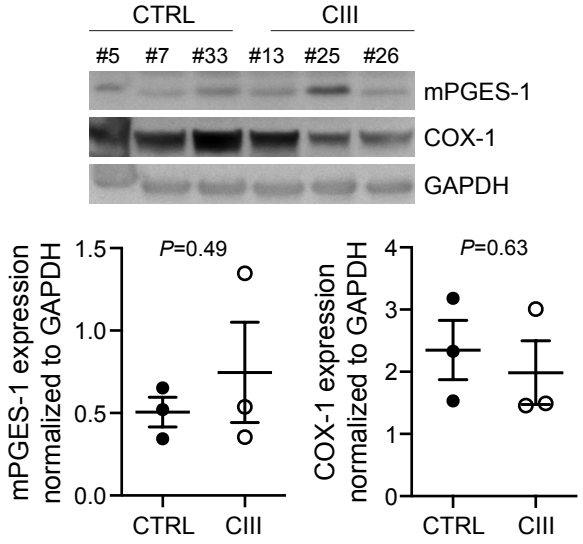

Figure S3. Expression of mPGES-1 and COX-1 in xenograft tumors. Western blot analysis of mPGES-1 and COX-1 as well as the housekeeping protein GAPDH in neuroblastoma xenograft tumors from CIII treated mice (mice number (#) 13, 25 and 26) and untreated mice (# 5, 7 and 33). Intensity of bands corresponding to mPGES-1 and COX-1 were quantified and normalized to the band corresponding to GAPDH using Image Lab software.

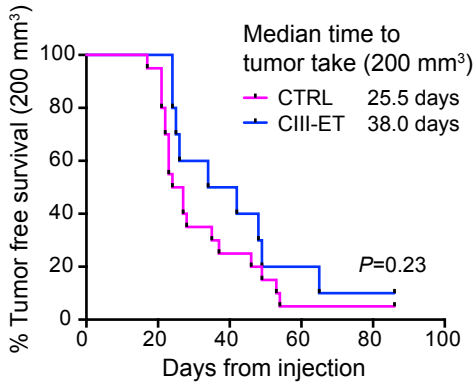

Figure S4. Effect of mPGES-1 inhibition with CIII on xenograft tumor development. Kaplan-Meier analysis of tumor development comparing tumor free (< 200 mm^3) survival probability from tumor cell injection, of CIII treated mice (CIII-ET) or untreated mice (CTRL), see Figure 4a for experimental set up. Median time to tumor take in CIII-ET was 38 days compared to 25.5 days for CTRL (P = 0.2, Log-rank test (Mantel-Cox)).

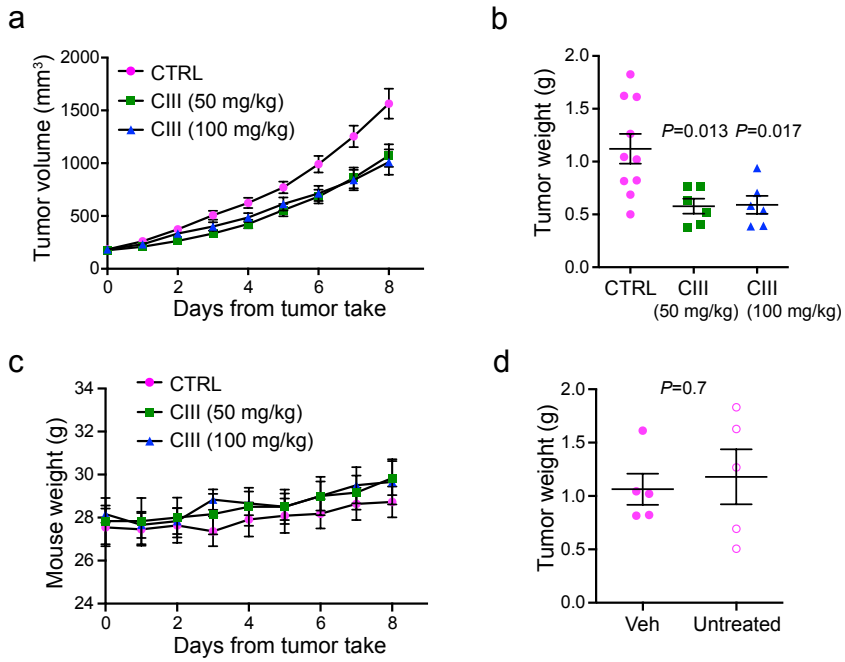

Figure S5. Investigation of CIII dose and anti-tumor effects in the xenograft model. Mice were injected with an 11q-deleted cell line (SK-N-AS, 10^7 cells, $n = 22$). At tumor take (150 mm^3) mice were randomized to CIII treatment at different doses, vehicle control or left untreated ($n = 10$, CTRL). The mPGES-1 inhibitor CIII was administered with daily intraperitoneal injections for 8 days. Two doses of CIII were tested, 50 mg/kg ($n = 6$) and 100 mg/kg ($n = 6$). (a) Data is represented as mean tumor volume (calculated as $(width)^2 \times length \times 0.44$) ± SEM. (b) Weight of tumors at the day of sacrifice (8 days post tumor take, $P = 0.013$ and $P = 0.017$ for CIII 50 mg/kg and CIII 100 mg/kg respectively compared to CTRL). (c) Wight of mice during treatment represented as mean weight ± SEM. (d) Tumor weight of CTRL mice comparing vehicle treated mice ($n = 5$) and untreated mice ($n = 5$). Data represented as mean ± SEM, $P = 0.7$, unpaired t-test.

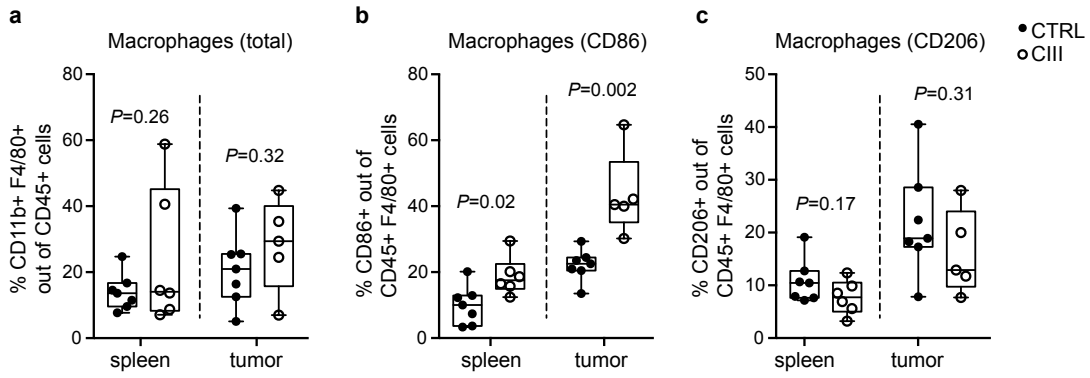

Figure S6. Macrophage polarization in transgenic mice treated with CIII. Flow cytometry analysis of macrophage polarization markers in F4/80+CD45+ population of cells in tumors and spleen from untreated (CTRL) and CIII treated (CIII) tgTH-*MYCN* mice. (a) Percentage of CD45+CD11b+F4/80+ cells representing total population of macrophages in spleen and tumor. (b) CD45+F4/80+CD86+ cells corresponding to M1 polarized macrophages ($P = 0.02$ and $P = 0.002$ for CIII compared to CTRL in spleen and tumors respectively, unpaired t-test). (c) CD45+F4/80+CD206+ M2 macrophages in spleen and tumor. Data is presented as a Box and whiskers plot showing minimum to maximum range and each mouse as an individual data point.

Table S1 Antibodies used for immunohistochemical analysis

Antigen	Antibody ID	Dilution
COX-1	Cayman Chemical Cat# 160109, RRID:AB_10077936	1:3000
COX-2	Cayman Chemical Cat# 160126, RRID:AB_10079419	1:400
mPGES-1	In house (Westman et al., 2004)	1:5000
EP1	Cayman Chemical Cat# 101740, RRID:AB_10079426	1:200
EP2	Cayman Chemical Cat# 101750, RRID:AB_10078697	1:200
EP3	Cayman Chemical Cat# 101760, RRID:AB_10077931	1:200
EP4	Cayman Chemical Cat# 101775, RRID:AB_10077932	1:200
GD2	Millipore Cat# MAB2052, RRID:AB_94520	1:15000
PDGFRβ	Cell Signaling Technology Cat# 3175, RRID:AB_2162494	1:100
PDGFRβ	Thermo Fisher Scientific Cat# 14-1402-82, RRID:AB_467493	1:500
IL-1RI	R&D Systems Cat# AF771, RRID:AB_355587	1:20
pSTAT3	Cell Signaling Technology Cat# 9145, RRID:AB_2491009	1:200
Ki-67	Lab Vision Cat# RM-9106-S1, RRID:AB_149792	1:500
CD31	BD Biosciences Cat# 550274, RRID:AB_393571	1:400
CD206	BioLegend Cat# 141701, RRID:AB_10900263	1:200

Table S2 Antibodies used for FACS analysis

Antigen	Antibody ID	Fluorochrome
CD11b	BioLegend Cat# 101212, RRID:AB_312795	APC
CD45	BioLegend Cat# 103111, RRID:AB_312976	APC
CD45	BioLegend Cat# 103129, RRID:AB_893343	PerCP
CD45	BioLegend Cat# 103138, RRID:AB_2563061	Pacific Orange
CD86	BioLegend Cat# 105109, RRID:AB_313162	FITC
CD206	BioLegend Cat# 141703, RRID:AB_10900988	FITC
F4/80	BioLegend Cat# 123110, RRID:AB_893486	PE

Westman, M., Korotkova, M., af Klint, E., Stark, A., Audoly, L.P., Klareskog, L.*, et al.* (2004). Expression of microsomal prostaglandin E synthase 1 in rheumatoid arthritis synovium. *Arthritis Rheum 50*(6), 1774-1780.