Supplemental data

Figure S1

COX-2 expression increased in the stroma and epithelia in some human colonic adenomas. Original magnification: x 25 (left panel) and x 250 (right panel).

Figure S2

11βHSD2 activity in CT26 cells was inhibited by GA or 11βHSD2 knockdown. (**A**) 11βHSD2 knockdown was more potent than GA (10 μM) in inhibition of 11βHSD2 activity (*P<0.01 versus vehicle, †P<0.01 versus CT26 plus GA, n = 4). (**B**) Representative immunoblots indicating that corticosterone (CS)-induced COX-2 inhibition in CT26 cells was augmented by 11ßHSD2 knockdown. 10 nM CS in 11ßHSD2 knockdown CT26$^{shRNA3\text{-}31}$ cells was as effective as 1,000 to 10,000 nM CS in parental CT26 cells to inhibit COX-2 expression. Also included is densitometric quantification of COX-2 immunoreactive protein in response to CS administration, normalized to ß actin expression and represented as fold of expression of control cells (n=2).

Figure S3

11βHSD2 inhibition enhanced corticosterone (CS)-induced inhibition of cell migration. (**A**) 11βHSD2 knockdown enhanced low dose CS (10 nM)-induced inhibition of CT26 cell migration (*P<0.0001, n = 3). (**B**) GA treatment enhanced CS (10 nM)-induced inhibition of CT26 cell migration (*P<0.0001 versus GA group, †P<0.0001 versus CS group, n = 3).

Figure S4

GA treatment inhibited CT26 tumor growth. (**A**) GA dose-dependently inhibited CT26 tumor growth. Tumor growth in the vehicle treated group was taken as 100%. *P < 0.01 versus vehicle, **P<0.0001 versus vehicle (n = 6). (**B**) GA treatment did not further inhibit CT26 tumor growth in the presence of COX-2 inhibition (SC-58236, 2 mg/kg/day, i.p.). *P<0.0001, n = 5.

Figure S5

GA was efficiently converted to its metabolite glycyrrhetinic acid (GE) in mice. Mice were sacrificed 24 h after last GA i.p. injection and tissues were dissected and stored at -80^{o}C. Tissue GA and GE levels were determined using LC-MS/MS. GA levels were 162 ± 79, 170 ± 89, and 402 ± 96 ng/g while GE levels were 1093 ± 309, 2958 ± 1206, and 1108 ± 206 ng/g, respectively, in kidney, colon and CT26 tumors (n = 4 in each group).

Figure S6

Inhibition of 11βHSD2 activity with GA suppressed human colon carcinoma HCA-7 tumor growth. (**A**) Human colon carcinoma HT-29 cells and HCA-7 cells expressed similar levels of 11βHSD2, but HT-29 cells expressed significantly lower levels of COX-2 than HCA-7 cells. Protein lysates from HCA-7 and HT-29 cells were transferred and the same immunoblot was successively probed for COX-2, 11βHSD2 and ß-actin. (**B**) Selective COX-2 inhibition with SC-58236 or NS-398 inhibited HCA-7 but not HT-29 cell proliferation (*P<0.01 vs. vehicle, n = 6 in each group). (**C**) GA treatment (10 mg/kg/day, i.p.) had no effect on HT-29-derived tumor growth in athymic nude mice. P = 0.4766, n = 6. (**D**) GA inhibited HCA-7-derived tumor growth (*P<0.01, n = 6), COX-2 and mPGES-1 expression (original magnification: x 400).

Figure S7

Inhibition of 11βHSD2 activity with GA suppressed vascularization in CT-26 tumors. (**A**) GA reduced CT26 tumor VEGF expression (*P<0.0001, n = 6). (**B**) GA reduced CT26 tumor vascular density (green=CD31 immunofluorescent staining, blue=DAPI staining) (original magnification: x160).

Figure S8

Long-term GA treatment did not inhibit PGI_2 production or promote atherogenesis. (**A**) GA treatment (3 to 30 mg/kg/day, i.p.) for 4 weeks trended to increase urinary excretion of the major murine PGI_2 metabolite 2,3-dinor-6-keto $PGF_{1\alpha}$ (PGI-M) and had no effect on excretion of the thromboxane metabolite, 2,3-dinor TxB_2 (Tx-M). *: P<0.05 versus vehicle, n = 6. (**B**) GA treatment (10 mg/kg/day, i.p.) for 10 weeks had no effect on the development of atherosclerosis (n = 4). (left) Atherosclerotic lesion size in the proximal aorta; (right) lesion percentage of en *face* aorta.

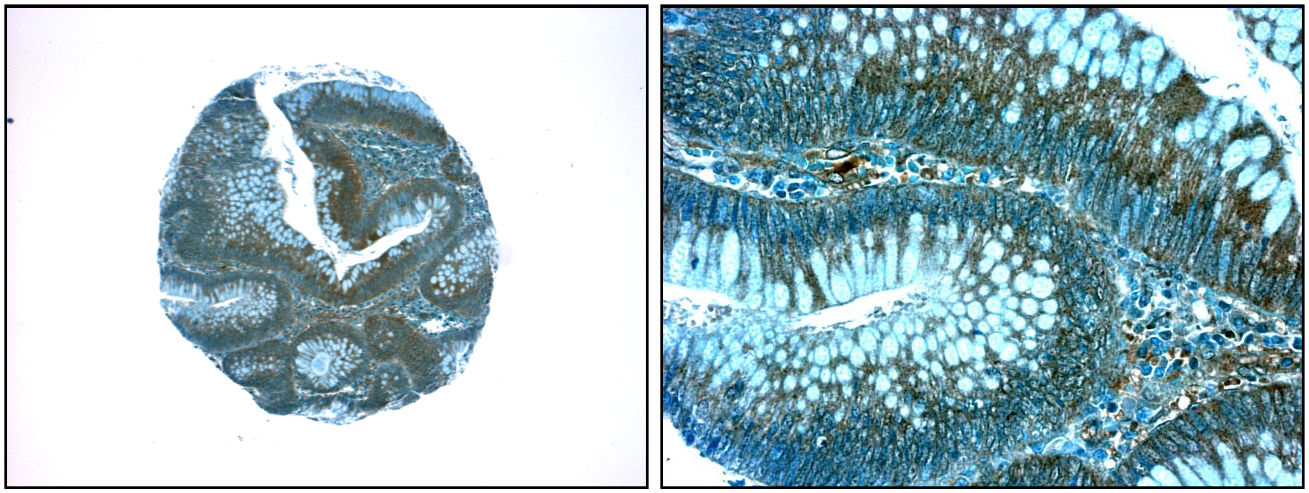


Figure S1

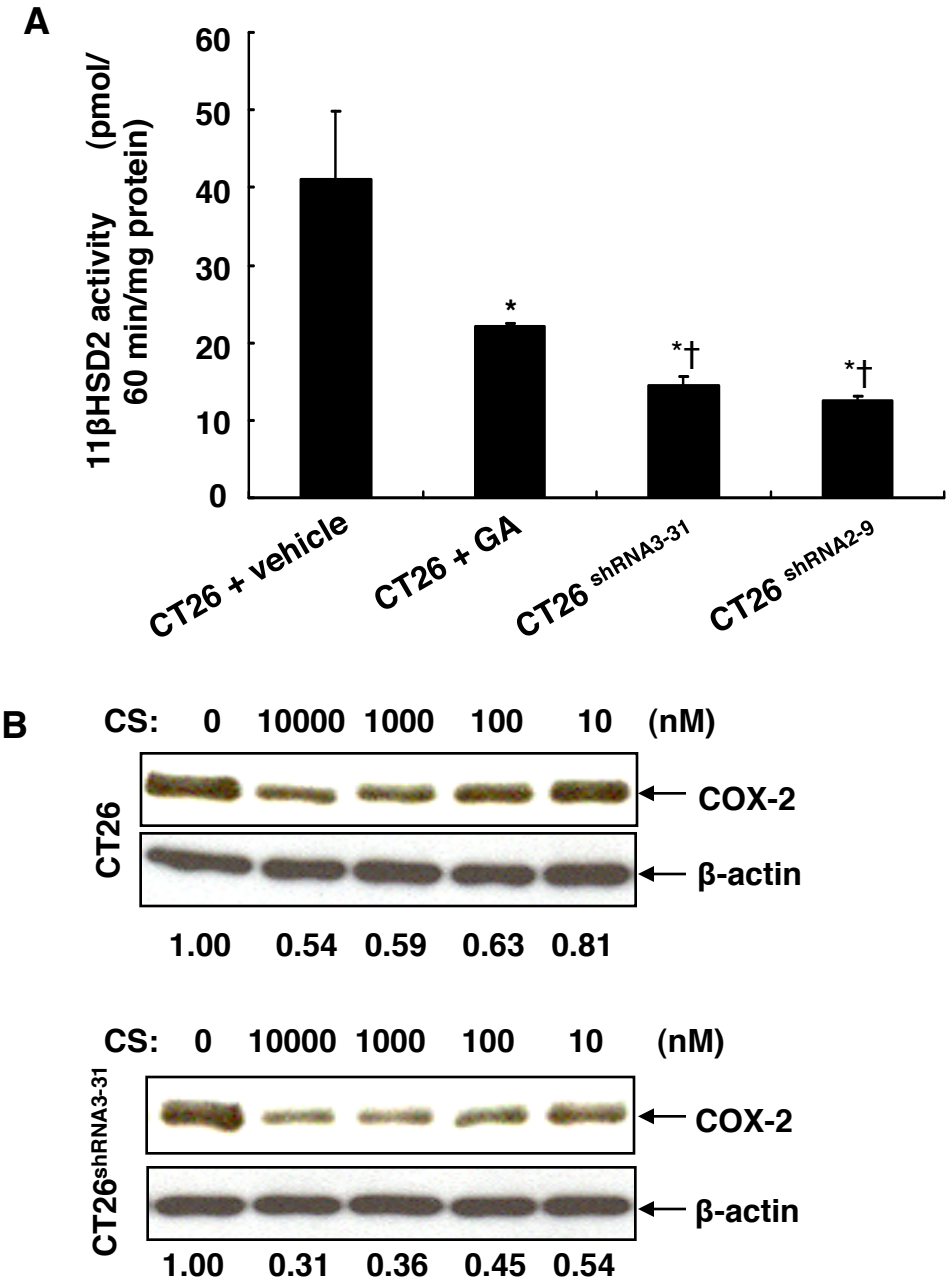

A
11βHSD2 activity (pmol/ 60 min/mg protein)
60
50
40
30
20
10
0
*
*†
*†
CT26 + vehicle
CT26 + GA
CT26 shRNA3-31
CT26 shRNA2-9
B
CS: 0 10000 1000 100 10 (nM)
CT26
COX-2
β-actin
1.00 0.54 0.59 0.63 0.81
CS: 0 10000 1000 100 10 (nM)
CT26shRNA3-31
COX-2
β-actin
1.00 0.31 0.36 0.45 0.54

Figure S2

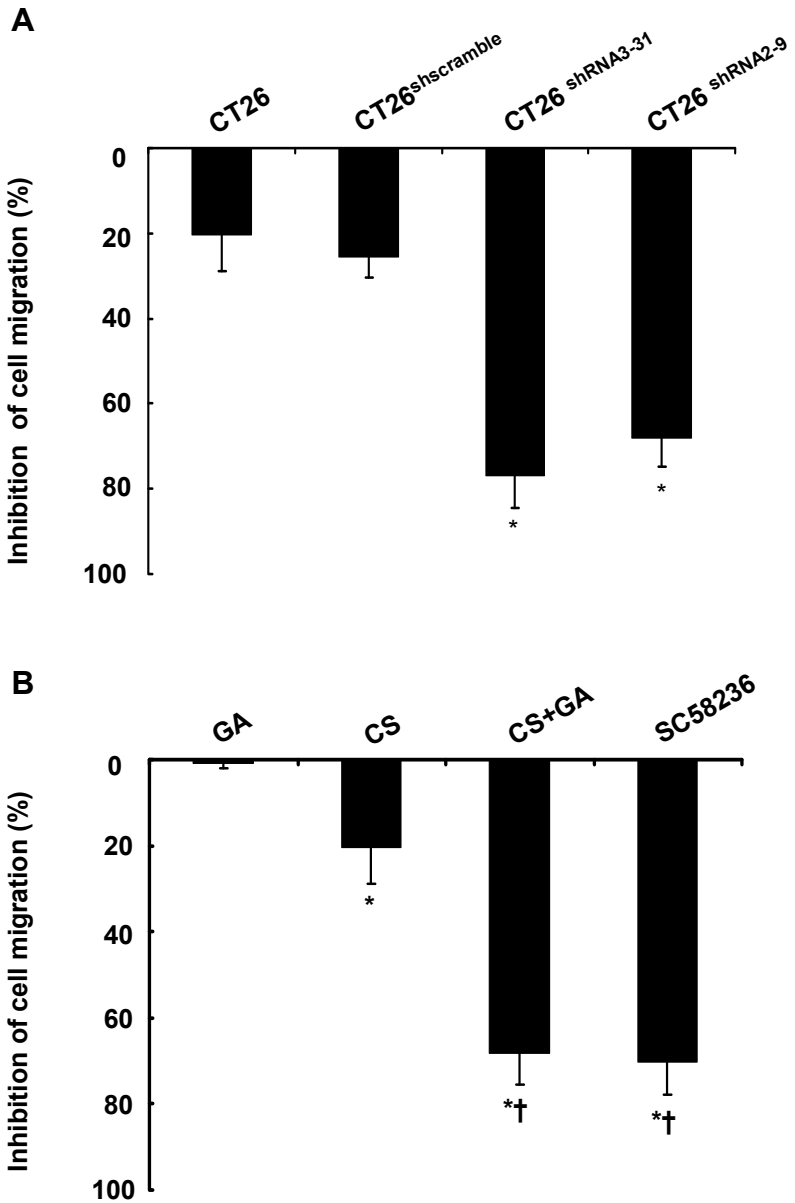

A
CT26
CT26shscramble
CT26 shRNA3-31
CT26 shRNA2-9
Inhibition of cell migration (%)
0
20
40
60
80
100
*
*
B
GA
CS
CS+GA
SC58236
Inhibition of cell migration (%)
0
20
40
60
80
100
*
*†
*†

Figure S3

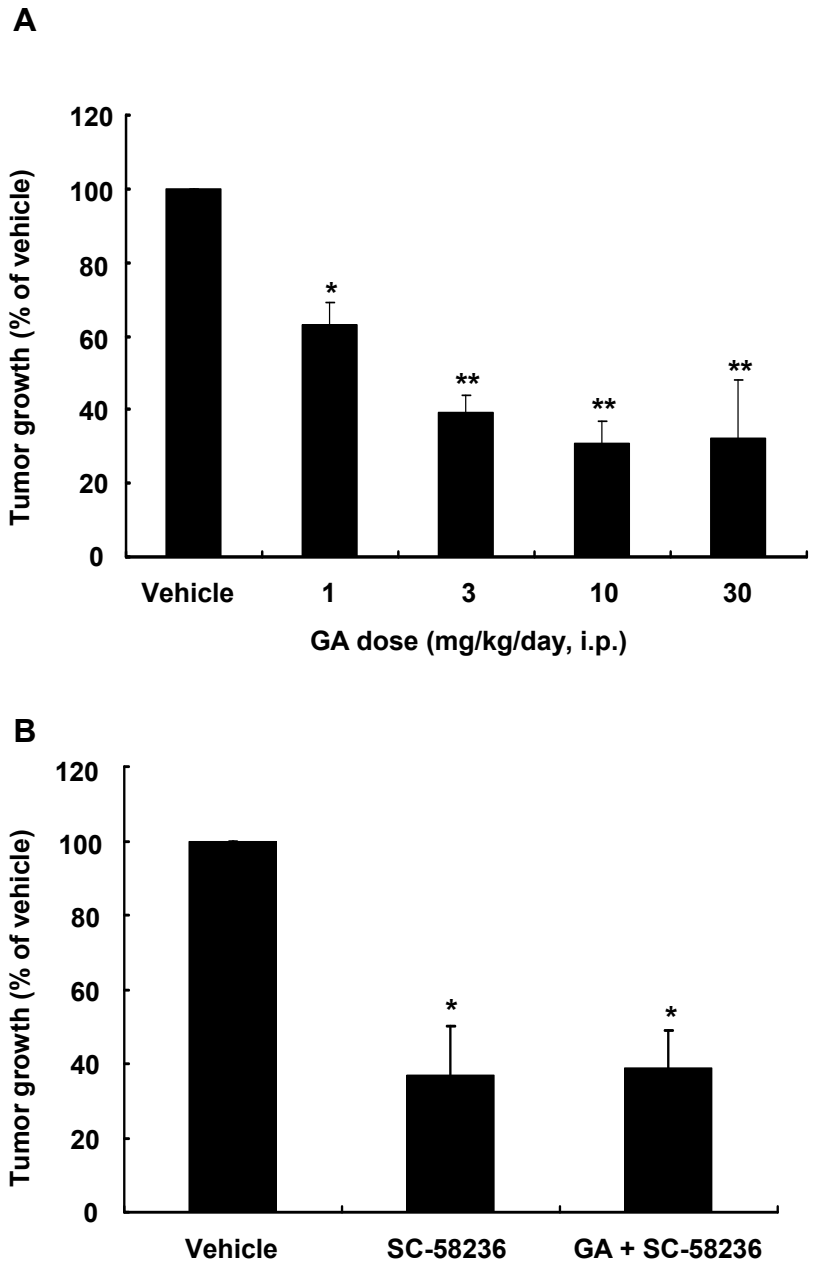

A
120
100
80
60
40
20
0
Tumor growth (% of vehicle)
*
**
**
**
Vehicle
1
3
10
30
GA dose (mg/kg/day, i.p.)
B
120
100
80
60
40
20
0
Tumor growth (% of vehicle)
*
*
Vehicle
SC-58236
GA + SC-58236

Figure S4

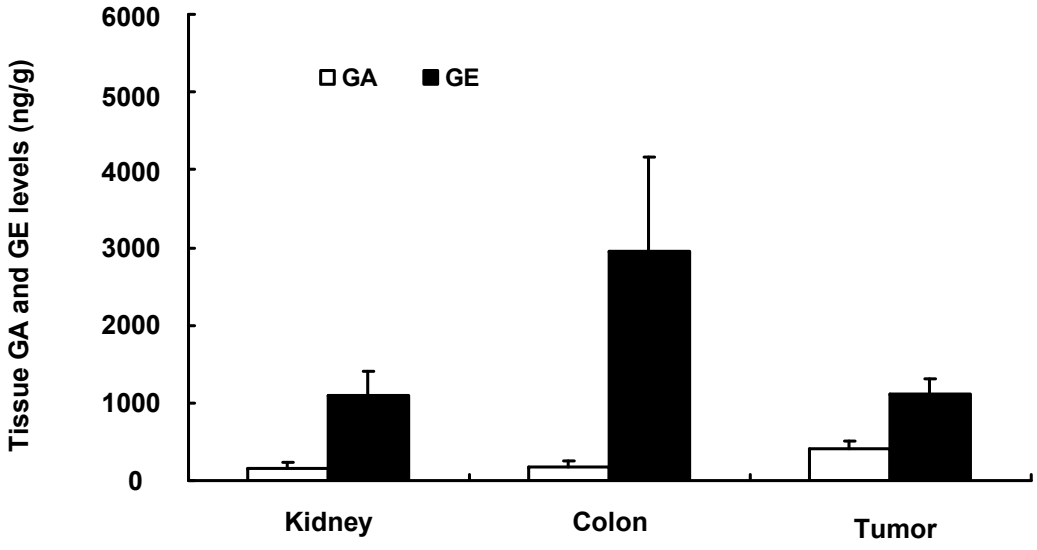

Tissue GA and GE levels (ng/g)
6000
5000
4000
3000
2000
1000
0
GA
GE
Kidney
Colon
Tumor

Figure S5

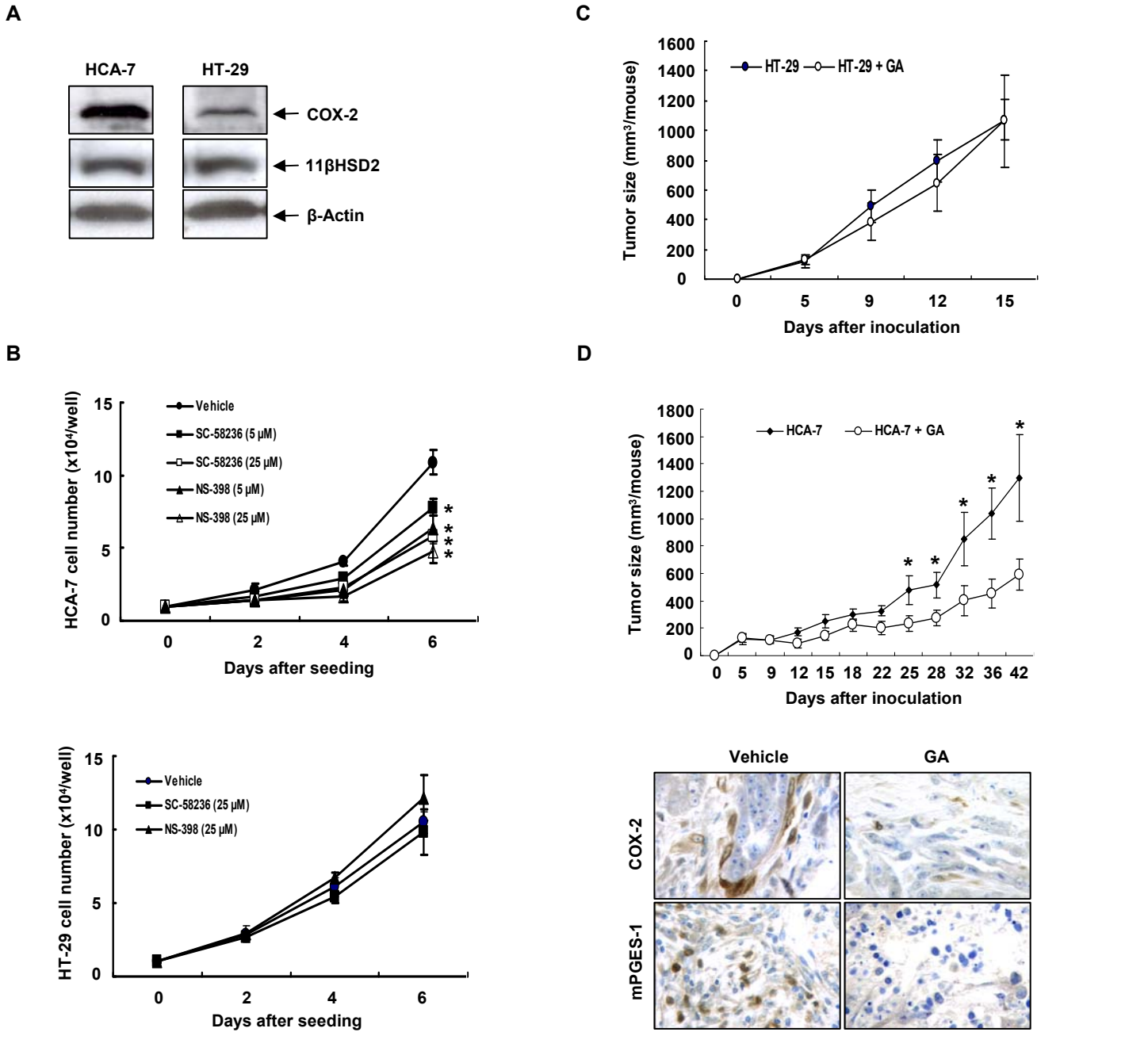

A
HCA-7
HT-29
COX-2
11βHSD2
β-Actin
B
HCA-7 cell number (x10⁴/well)
Vehicle
SC-58236 (5 μM)
SC-58236 (25 μM)
NS-398 (5 μM)
NS-398 (25 μM)
Days after seeding
HT-29 cell number (x10⁴/well)
Vehicle
SC-58236 (25 μM)
NS-398 (25 μM)
Days after seeding
C
Tumor size (mm³/mouse)
HT-29
HT-29 + GA
Days after inoculation
D
Tumor size (mm³/mouse)
HCA-7
HCA-7 + GA
Days after inoculation
Vehicle
GA
COX-2
mPGES-1

Figure S6

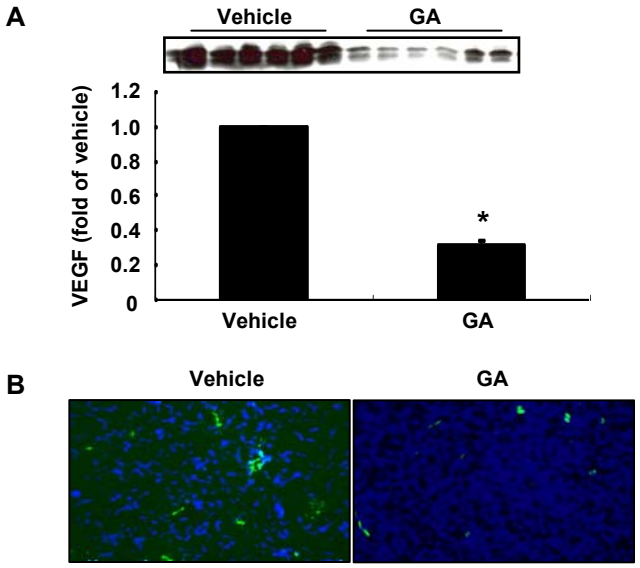
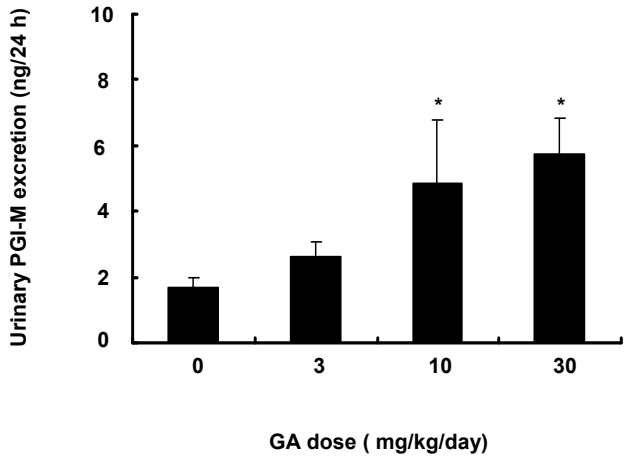
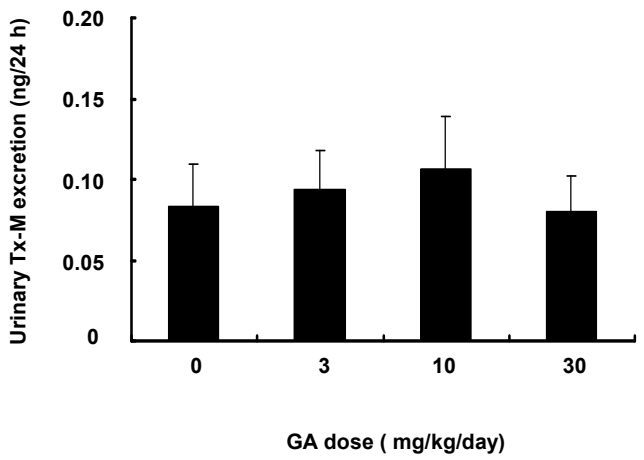

A
Vehicle
GA
VEGF (fold of vehicle)
1.2
1.0
0.8
0.6
0.4
0.2
0
*
Vehicle
GA
B
Vehicle
GA

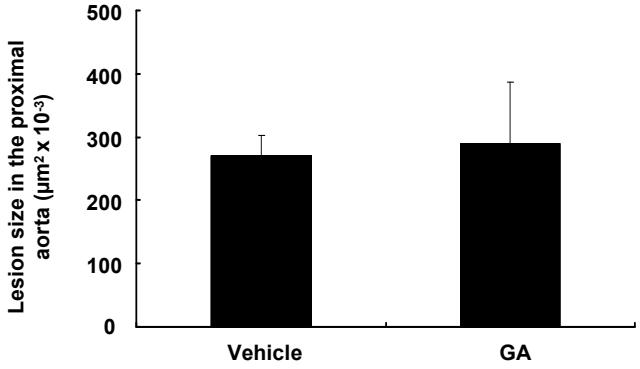
Figure S7

A


Urinary PGI-M excretion (ng/24 h)
10
8
6
4
2
0
*
*
0
3
10
30
GA dose (mg/kg/day)


Urinary Tx-M excretion (ng/24 h)
0.20
0.15
0.10
0.05
0
0
3
10
30
GA dose (mg/kg/day)

B


Lesion size in the proximal aorta (μm² x 10⁻³)
500
400
300
200
100
0
Vehicle
GA

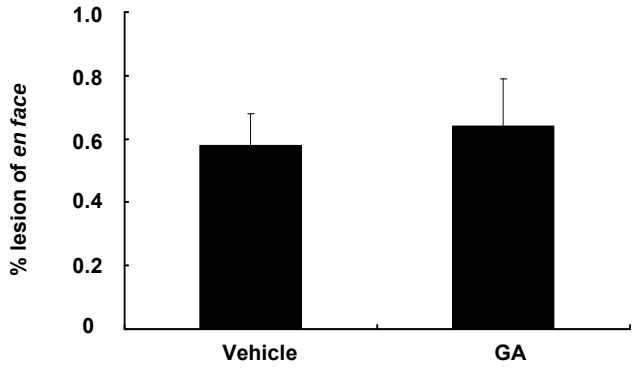

% lesion of en face
1.0
0.8
0.6
0.4
0.2
0
Vehicle
GA

Figure S8