Searching for new functional properties in traditional foods of Japan and South America

November 8, 2010
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Most of functional factors in food are non-nutrients.

Nutrients (proteins, sugars and lipids) undergo decomposition to produce energy (catabolism) in liver. This indicates that nutrients cannot exhibit bio-functions.

Contrarily, non-nutrients do not undergo catabolism, and therefore can exist in mostly their unchanged forms in food and can exhibit diverse bio-functions. However, most of non-nutrients undergo a different metabolism, conjugations, in intestinal surface cells.

The conjugations occur on the functional groups, masking with glucuronic acid and/or sulfate, and then invalidate active non-nutrients.

A strategy for finding out biofunctional factors

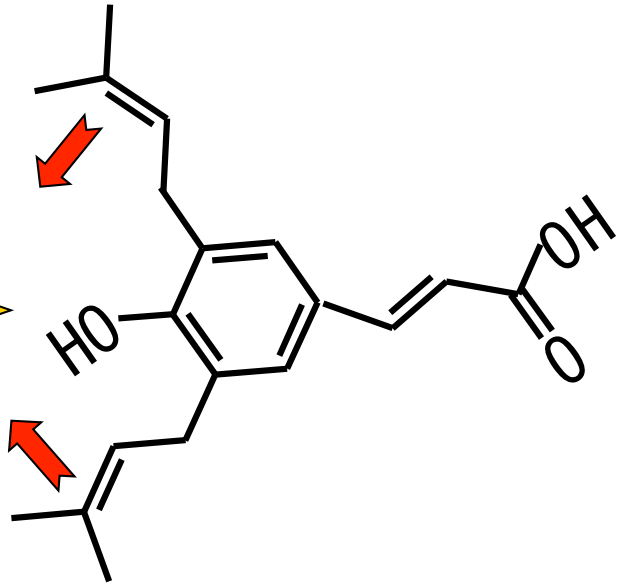
To find out endogenously active functional non-nutrients, difficult forms to undergo the conjugations should be searched according to the following strategy:

1) Compounds retaining the activity even after the conjugations
2) A hard chemical to undergo conjugations
3) Unchanged chemical during absorption process
4) Metabolically activated chemicls when absorbed
5) Available chemicls after de-conjugation with endogenous β-glucuronidase
6) Compounds that can interact with intestinal surface cells and transfer immune signals without absorption

The second idea

Propolis includes a hard chemical to be conjugated, artepillin C

Conjugate enzymes



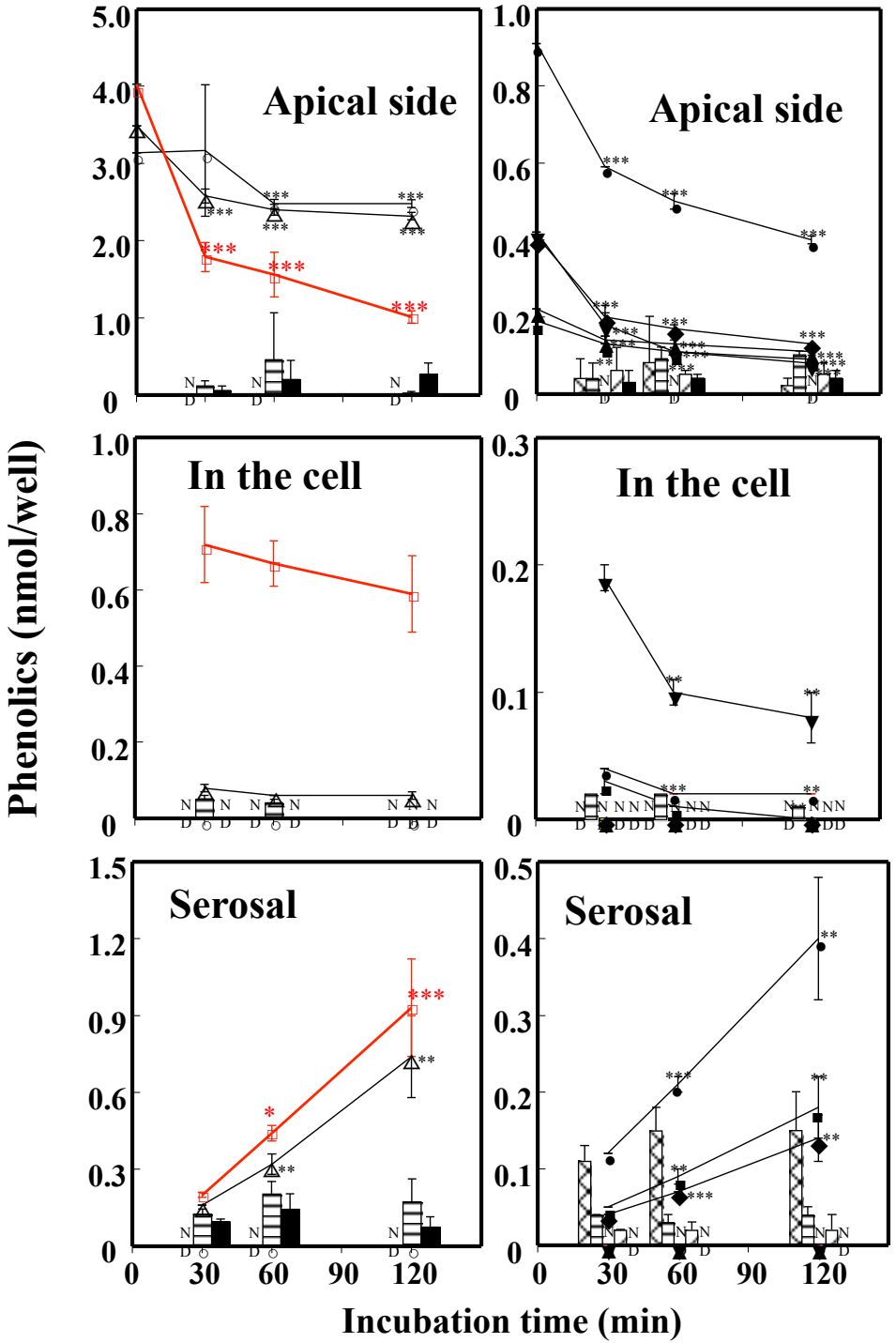
Prenyls

Bee collects from *Baccharis dracunculifolia* (chilca)

Bioavailability of artepillin C in Caco-2 monolayers

Free of ;
○; Ferulic acid
△; *p*-Coumaric acid
□; Artepillin C

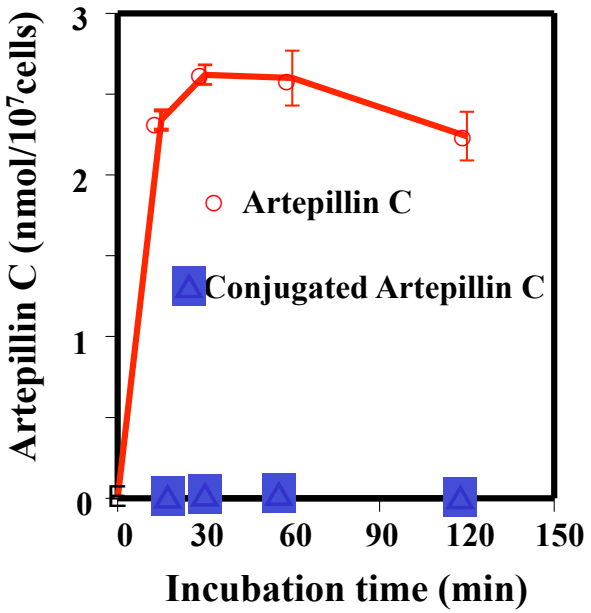
Conjugated of ;
Ferulic acid
p-Coumaric acid
Artepillin C

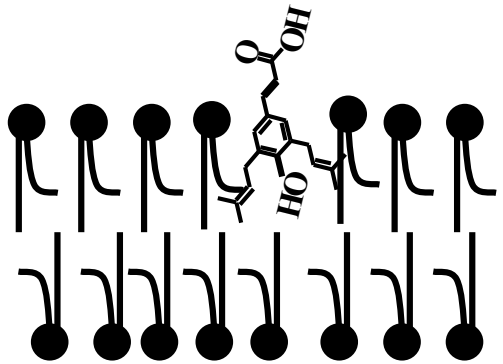


Free of ;
●; Naringenin
▲; Kaempferol
■; Isosakuranetin
◆; Chrysin
▼; Kaempferide

Conjugated of ;
Naringenin
Kaempferol
Isosakuranetin
Chrysin
Kaempferide

Artepillin C can Enter HepG2 Cells and Suppress Oxidative Stress

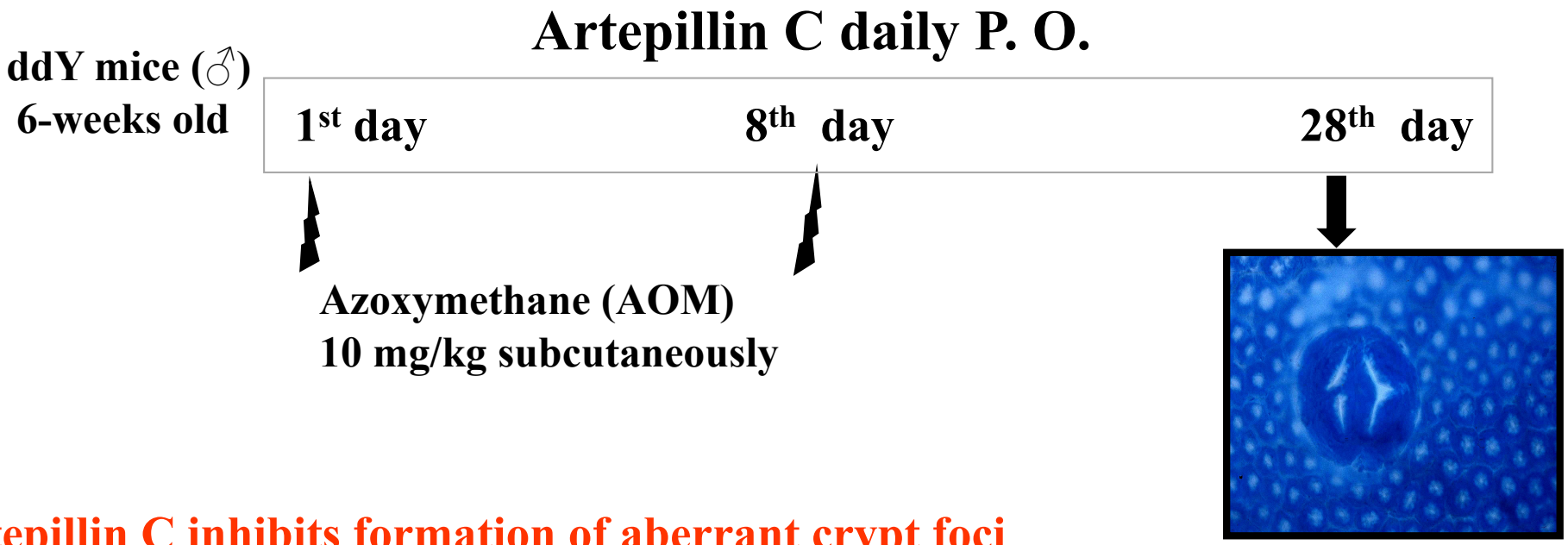




Speculation

HepG2 cells Exposed to O_2^-

Artepillin C (μM)	Lipid peroxides (nmol/mg protein)
0	0.468±0.013
5	0.426±0.010*
10	0.446±0.006*
20	0.392±0.013*
	8-OHdG/2'-dG X 10^{-5}
0	8.22±1.089
5	7.14±0.411
10	5.35±0.724*
20	5.23±0.176*
Mixed Polyphenols	7.18±0.815
Mixed polyphenol + 10 μM of Artepillin C	4.86±0.481*

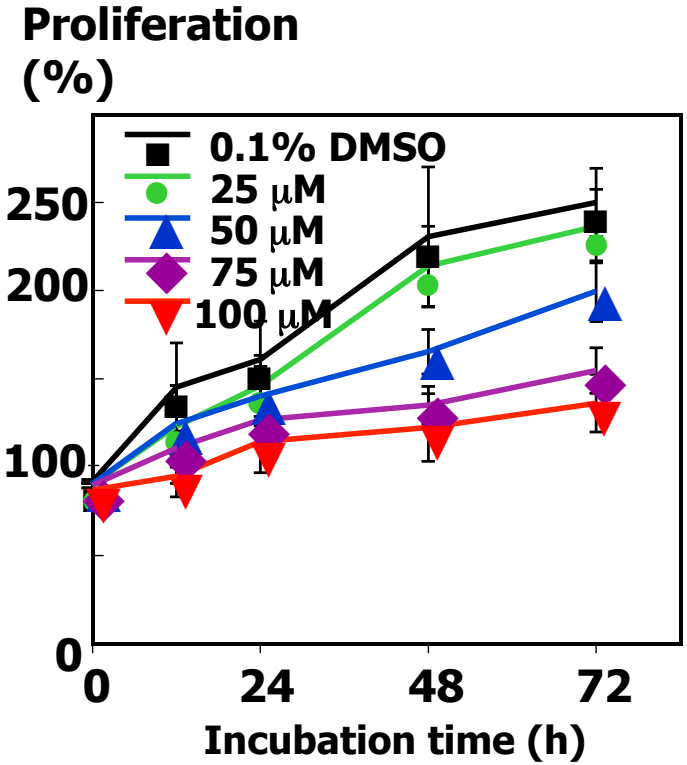


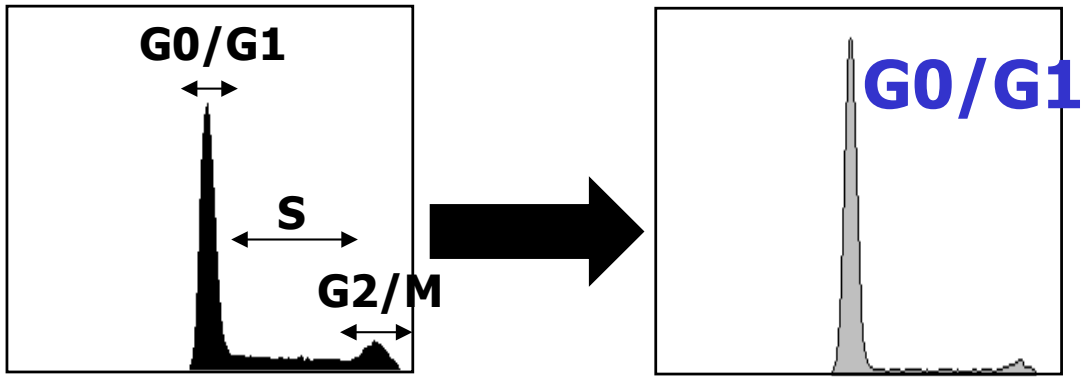
Artepillin C inhibits formation of aberrant crypt foci

Treatment	Number of ACF	% of Control	ACS/Focus
Negative control (non-treatment)	0	0	0
Positive control (vehicle alone)	63.25 ± 16.5	100	1.50 ± 0.18
80 mg/Kg propolis	38.4 ± 14.5*	60.8	1.40 ± 0.20
160 mg/Kg propolis	35.6 ± 6.8*	56.3	1.57 ± 0.14
10 mg/Kg artepillin C	35.8 ± 15.2*	56.6	1.49 ± 0.19

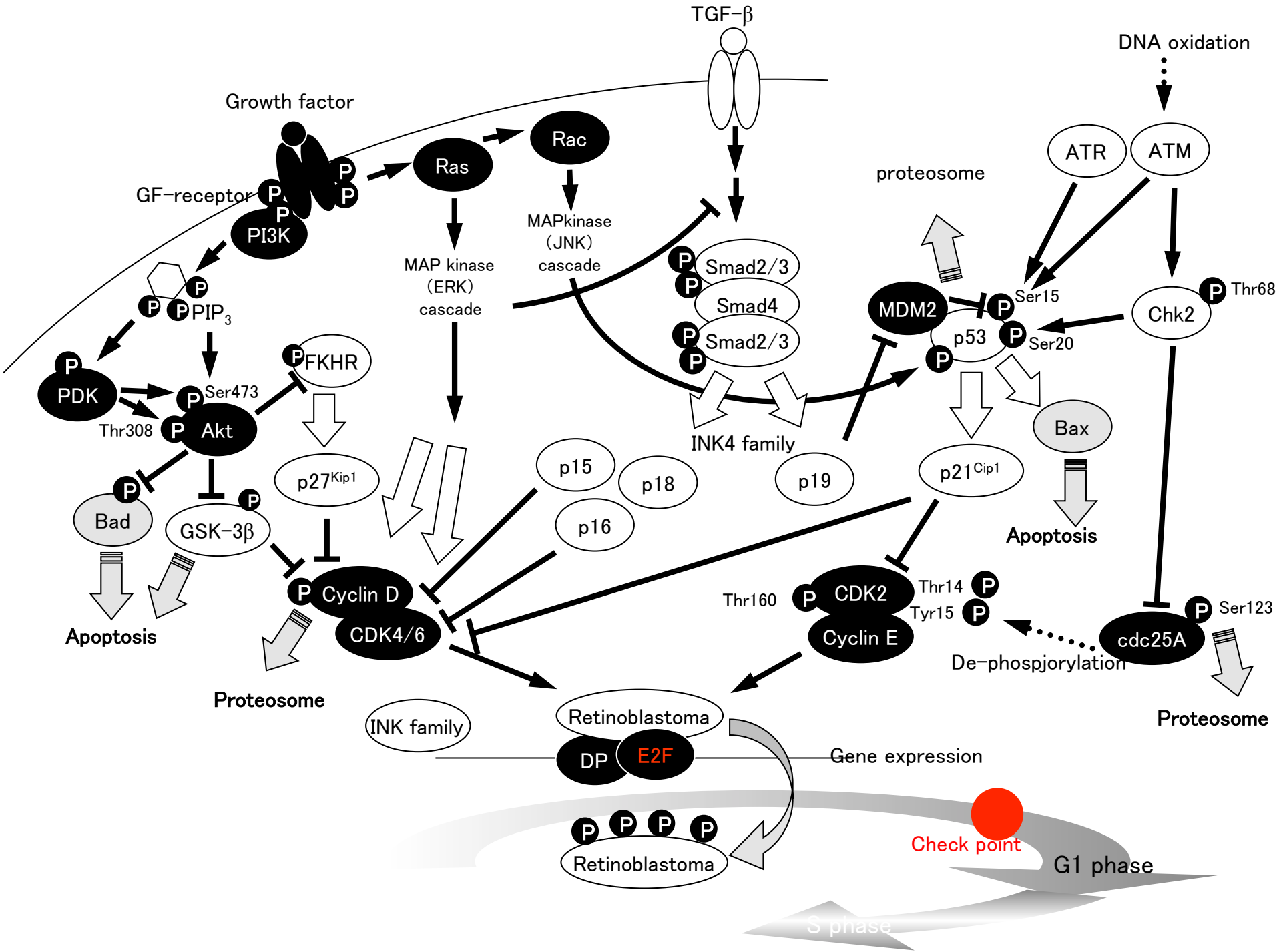
n=5, $p<0.05$

Artepillin C Induces G0/G1 Arrest in WiDr

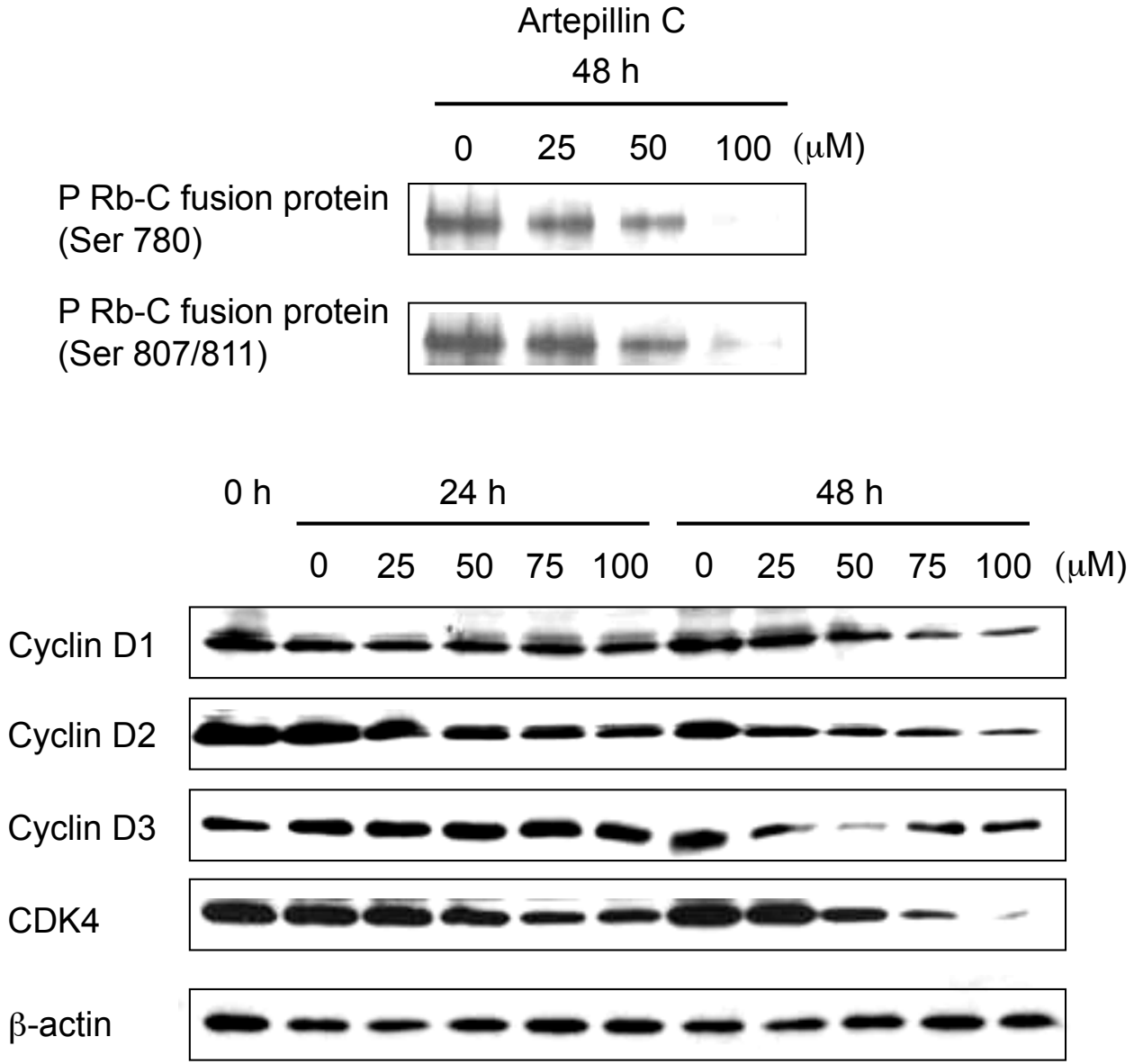




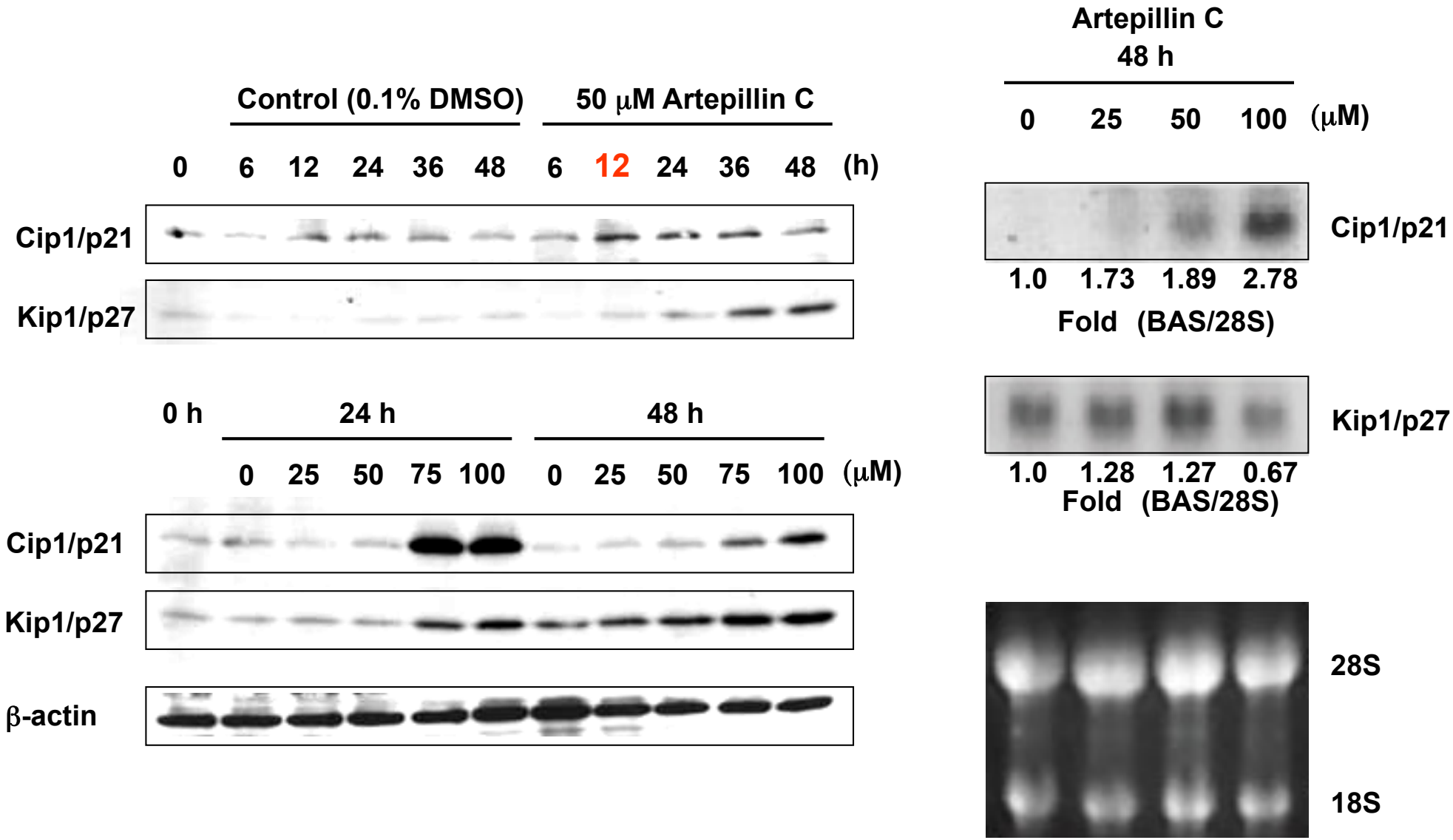
Artepillin C (μM)	G0/G1 (%)	S (%)	G2/M (%)
0 h 0	61.6 ±0.8	33.0 ±1.7	5.4 ±1.0
24 h 0	61.6 ±4.8	29.0 ±4.3	9.4 ±2.2
25	62.5 ±1.7	27.8 ±0.6	9.7 ±1.0
50	68.8 ±0.4	22.1 ±0.4	9.1 ±0.5
100	82.1 ±3.2	13.4 ±2.3	4.5 ±1.0
48 h 0	63.2 ±1.4	29.9 ±1.3	6.9 ±0.1
25	77.9 ±0.6	17.1 ±0.5	5.0 ±0.5
50	80.3 ±0.2	14.4 ±0.5	5.3 ±0.7
100	86.9 ±0.2	6.7 ±0.3	6.4 ±0.1


TGF-β
DNA oxidation
Growth factor
GF-receptor
PI3K
Ras
Rac
MAP kinase
(ERK)
cascade
MAPkinase
(JNK)
cascade
Smad2/3
Smad4
Smad2/3
INK4 family
ATR
ATM
proteosome
MDM2
p53
Ser15
Ser20
Chk2
Thr68
PIP3
PDK
Ser473
Thr308
Akt
FKHR
p27Kip1
p15
p18
p16
p19
p21Cip1
Bax
Apoptosis
Bad
GSK-3β
Apoptosis
Cyclin D
CDK4/6
Proteosome
Thr160
CDK2
Cyclin E
Thr14
Tyr15
De-phospjorylation
cdc25A
Ser123
Proteosome
INK family
Retinoblastoma
DP
E2F
Gene expression
Retinoblastoma
Check point
G1 phase
S phase

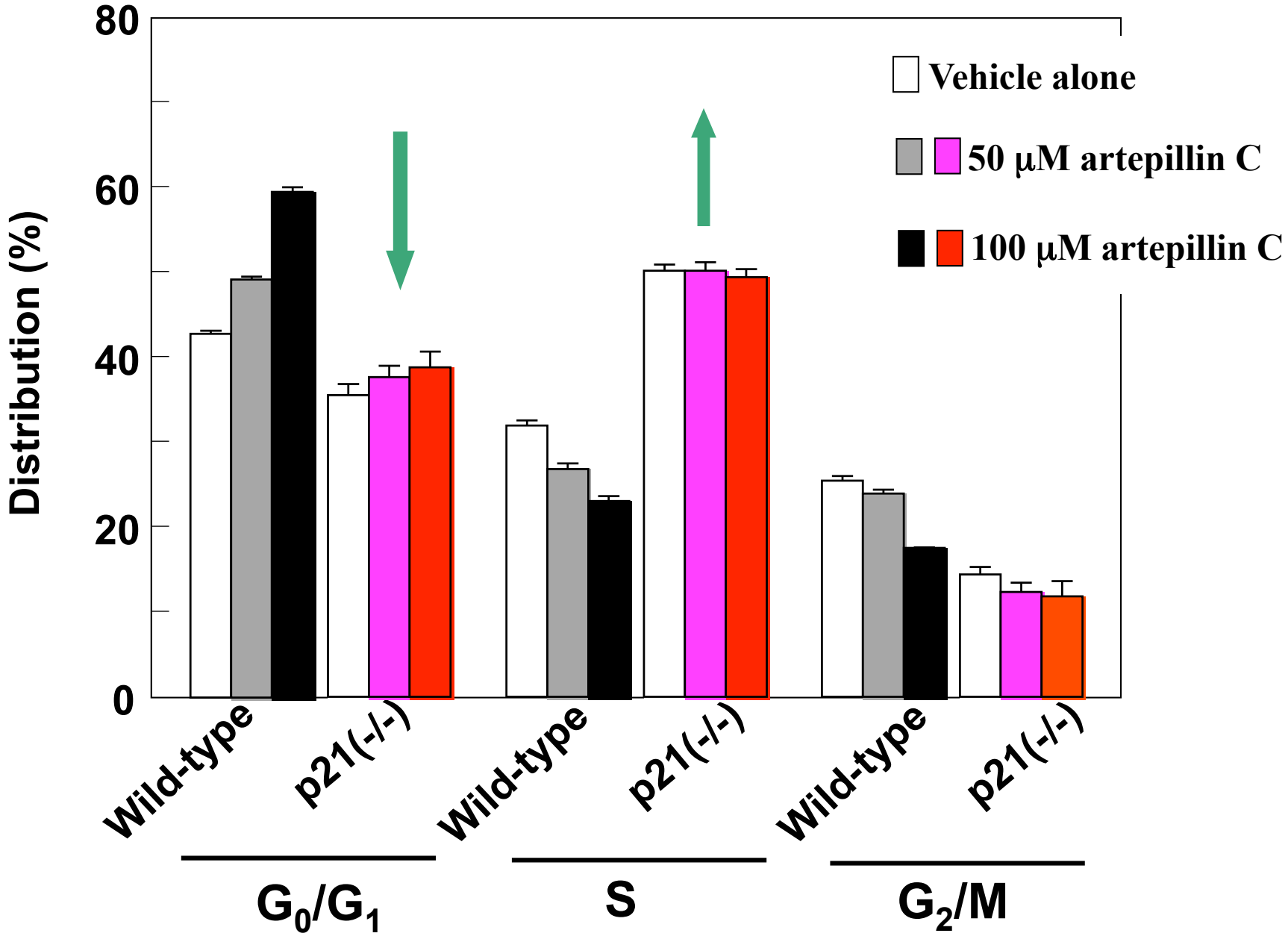
Artepillin C Regulates Cell-Cycle Related Proteins



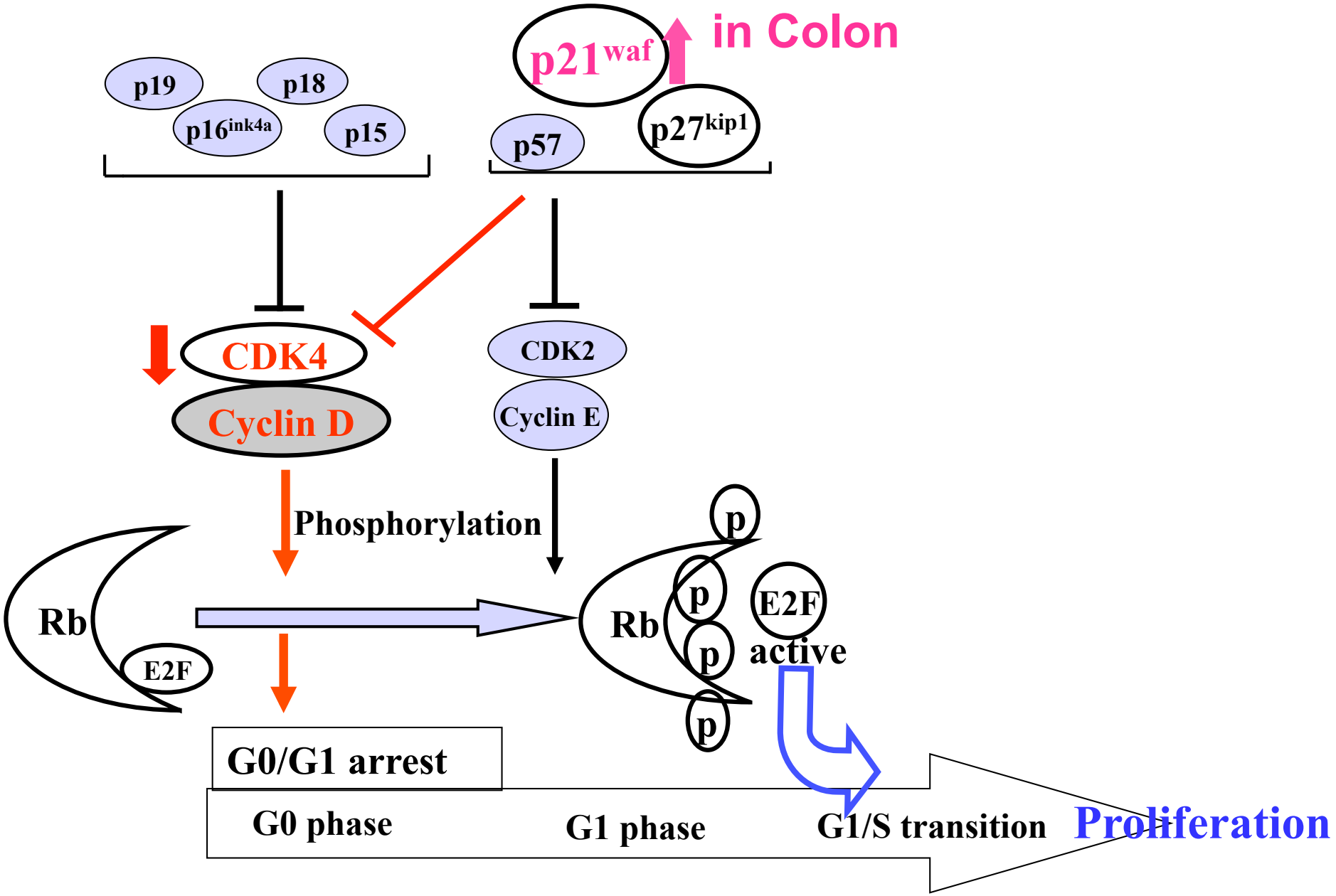
In up-stream signals



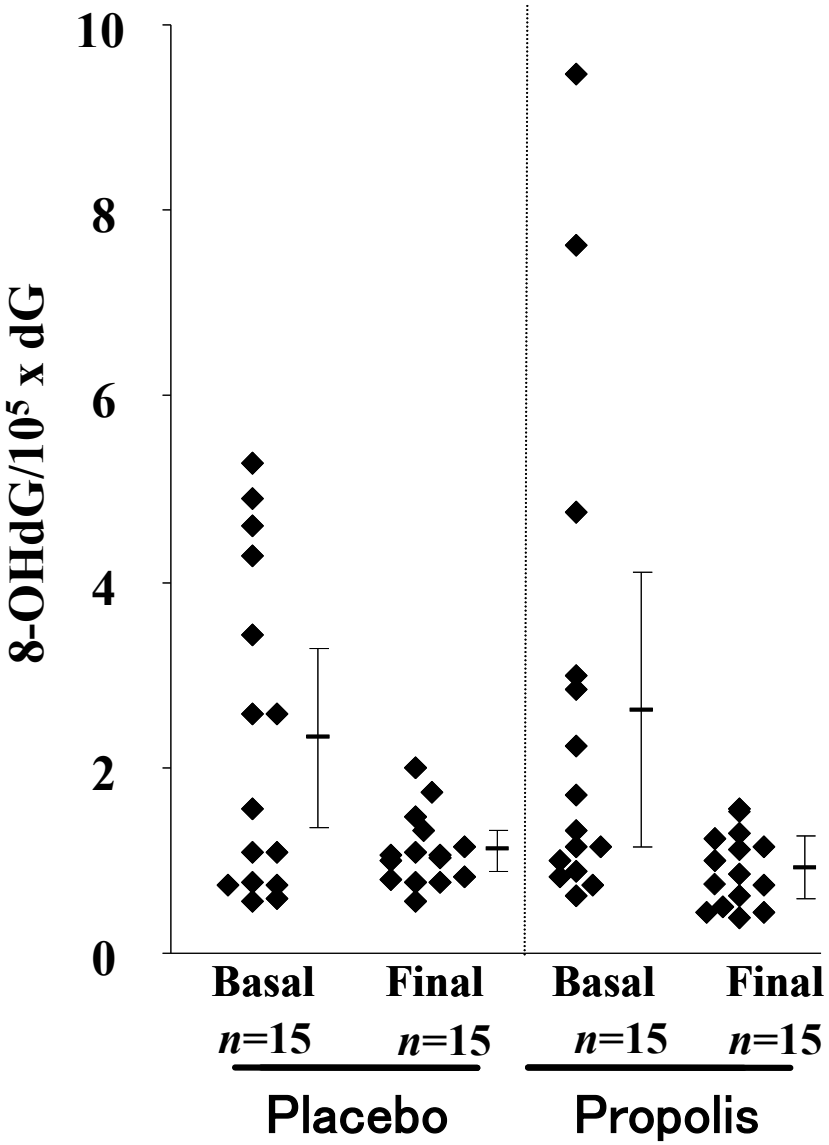
In Cip1/p21-deleted cells



Regulatory events by Artepillin



In Human trial

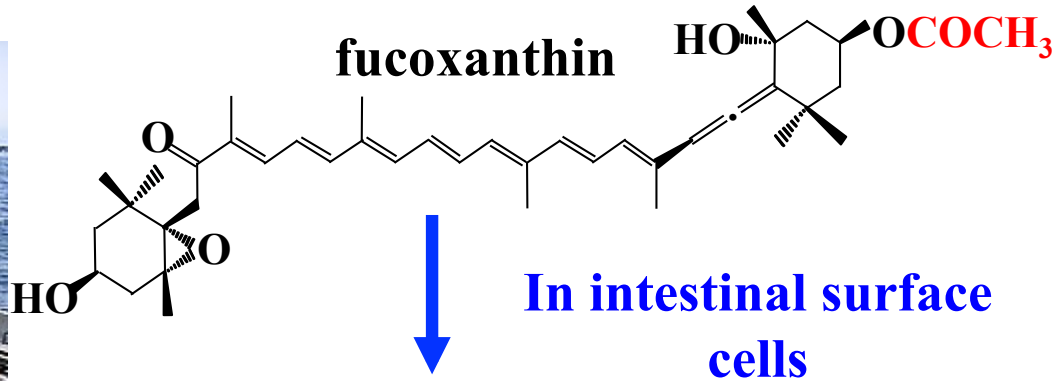


Artepillin C 20.4 μmol (6.12 mg)
Ferulic acid 0.3 μmol
***p*-Coumaric acid 3.4 μmol**
Kaempferol 8.6 μmol
Naringenin 1.8 μmol

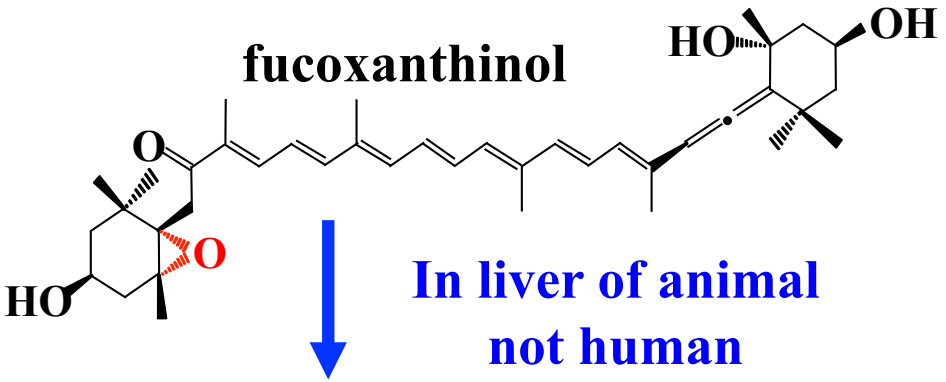
3 Capsules per day
For 3 month

Dosed amounts were too small

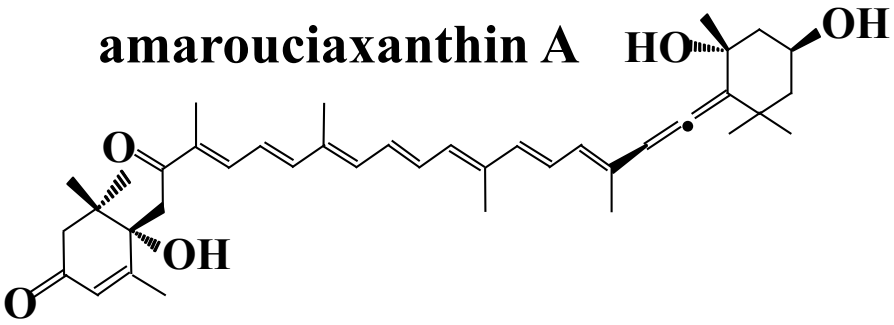
3) Unchanged chemical during absorption process



In intestinal surface cells



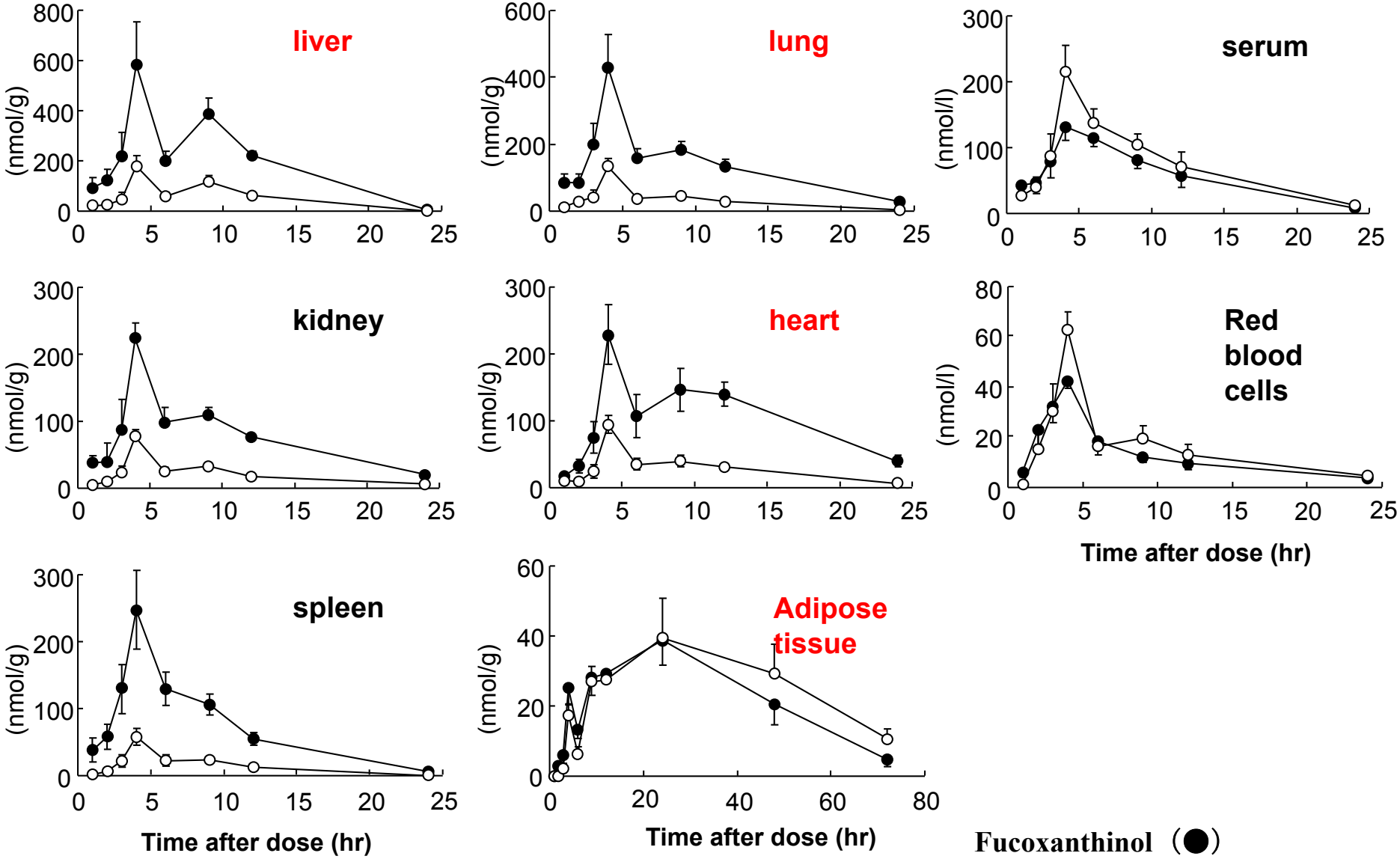
In liver of animal not human



Fucoxanthin Contents

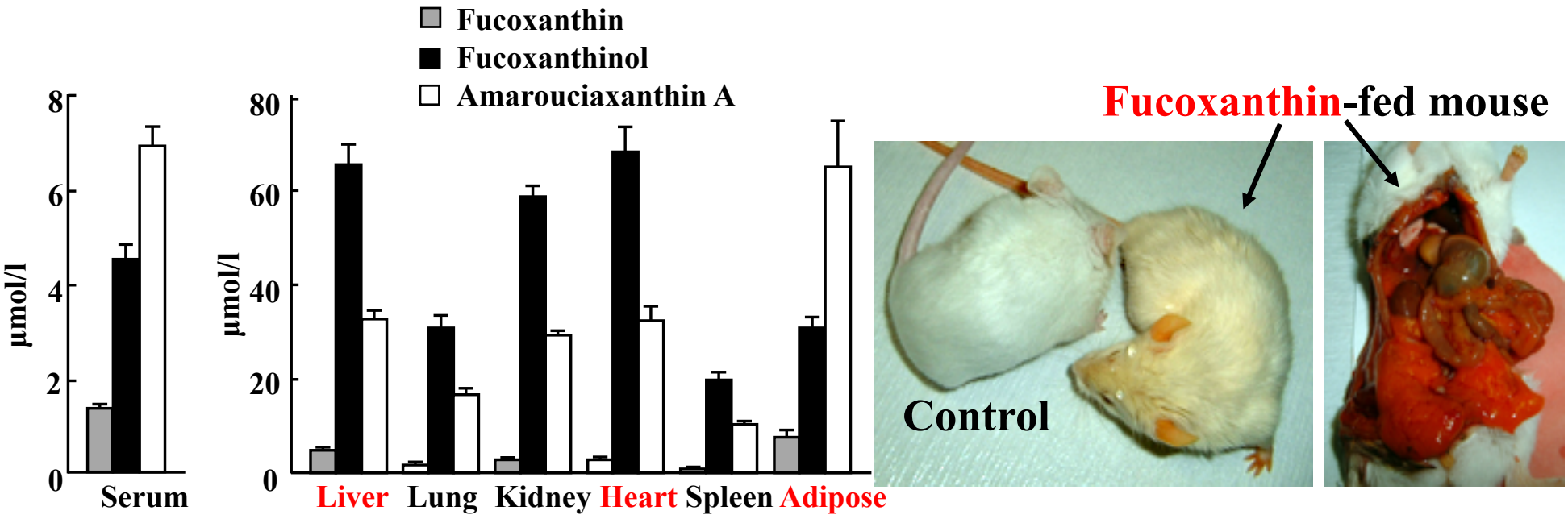
Brown algae	Fucoxanthin per 100 g fresh
Wakame (*Undaria pinnatifida*)	**11.1 mg**
Arame (*Eisenia bicyclis*)	**7.7 mg**
Hondawara (*Sargassum fulvellum*)	**6.5 mg**
Kombu (*Laminalia japonica*)	**17.7 mg**
Hijiki (*Hizikia fusiformis*)	**2.2 mg**

Single dose of fucoxanthin 0.1 mg (160 nmol)/mouse



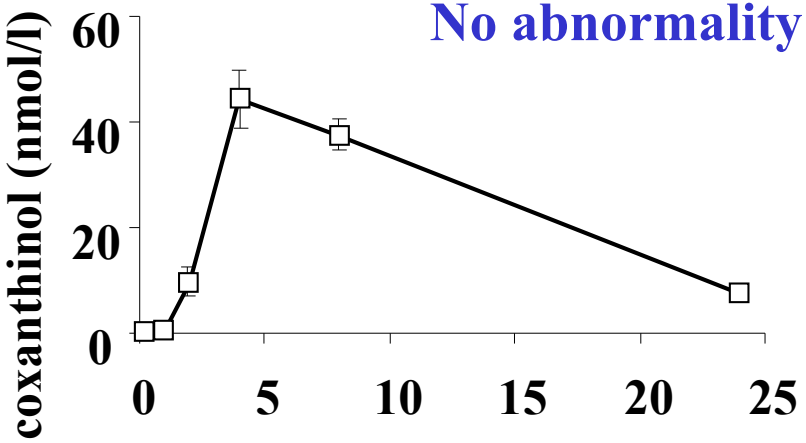
Hashimoto et al., Br. J. Nutr.

Fucoxanthinol (●)
Amarouciaxanthin A (○)
Fucoxanthin was not detectable.



Dose of fucoxanthin 3.5 mg (5.6 µmol)/day/mouse for 1 month
（equal to 760 g per kg bw of raw kombu)

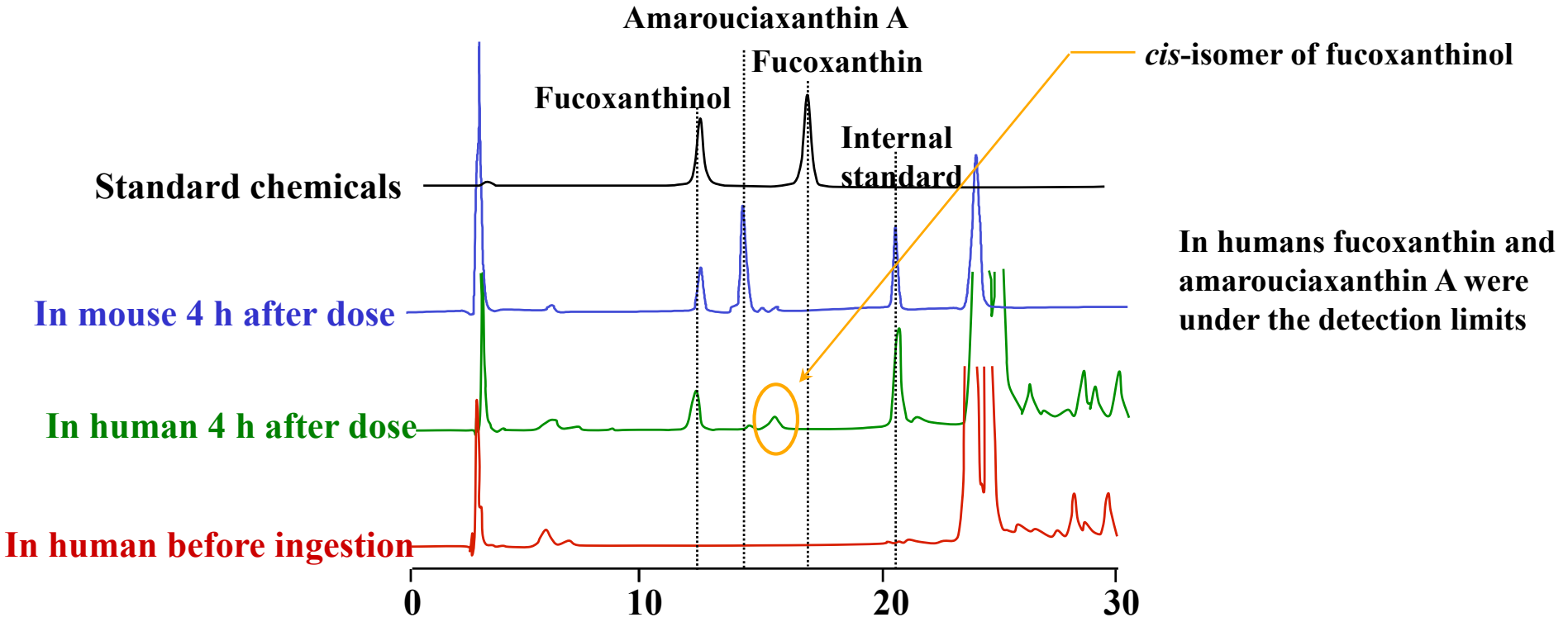
In another experiments (20 times more),
F344 fed rats on 0, 17.5, 35, 70 mg/day/mouse of fucoxanthin for 90 days and found NOAEL was more than 70 mg/day/mouse.



No abnormality; more than 8 mg/day for 5 weeks in humans

Pharmaceutical parameter in human	
Cmax	44.2 nml/L
Tmax	4 h
t1/2	7.0 h

No accumulation indicates that produces no side effects and no toxicity.



In humans fucoxanthin and amarouciaxanthin A were under the detection limits

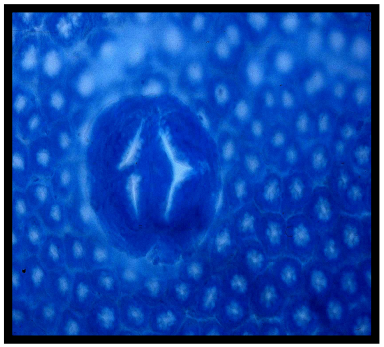
ddY mice (♂)
6-weeks old

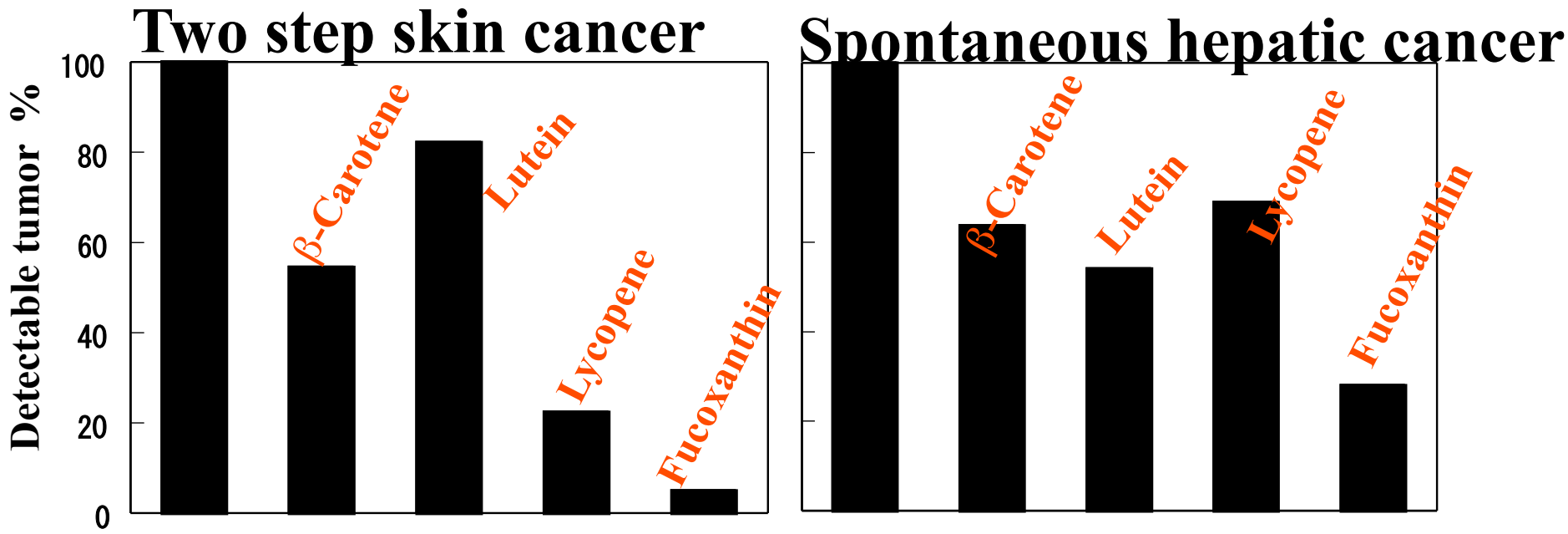
Fucoxanthin in drinking water

1st day — 8th day — 28th day

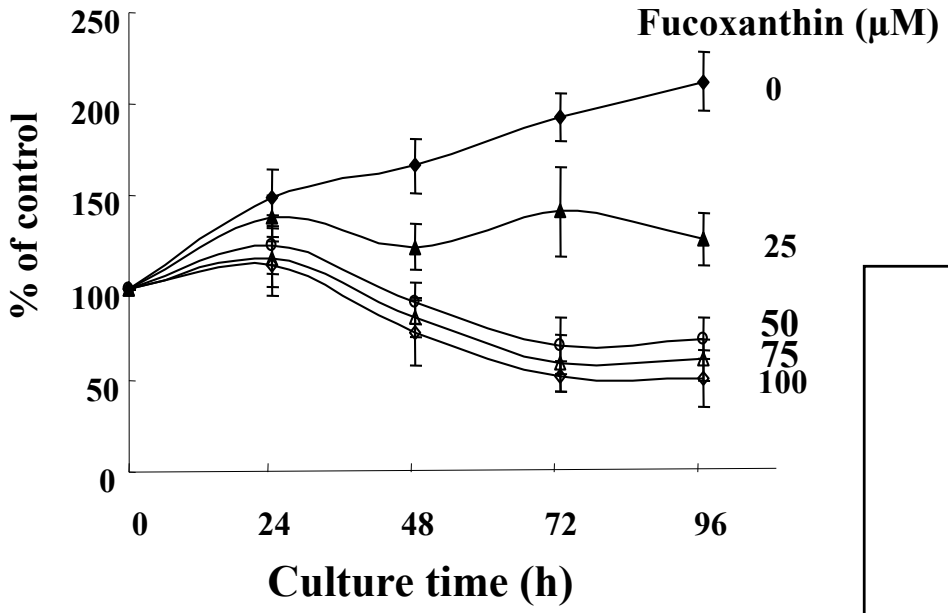
Subcutaneous injection of AOM

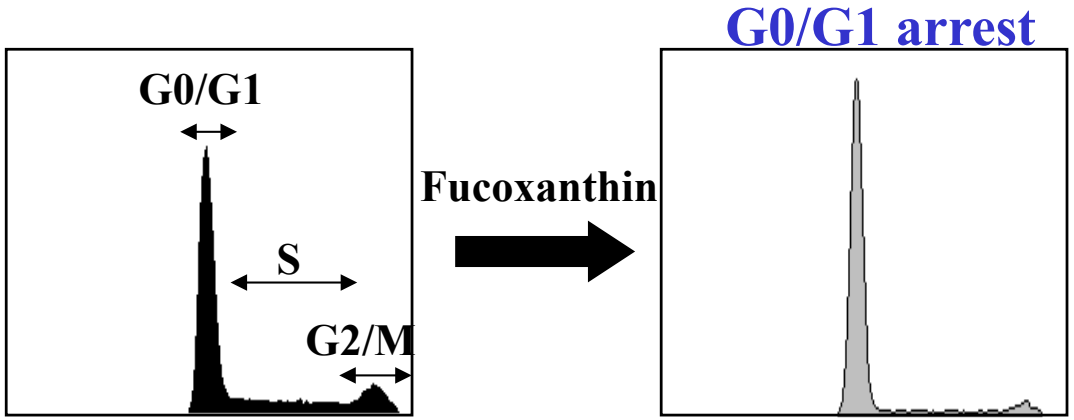
treatment	ACF	%
Negative control	0	0
Positive control	85 ± 26	100
Fucoxanthin 50 ppm	60 ± 10	70
Fucoxanthin 100 ppm	55 ± 21*	65





Fucoxanthin showed the strongest activity to prevent form colon, skin and hepatic cancers among carotenoids.

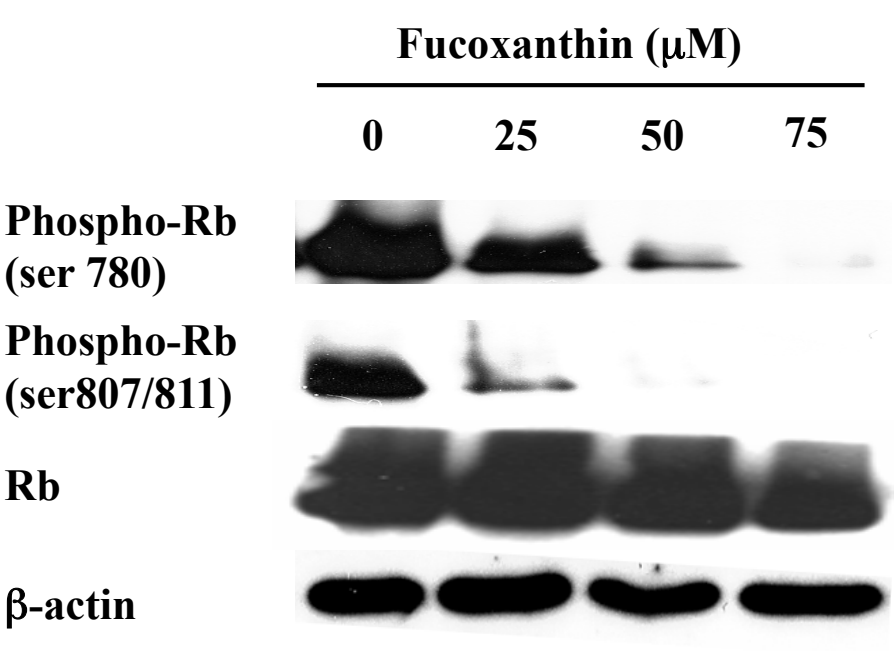


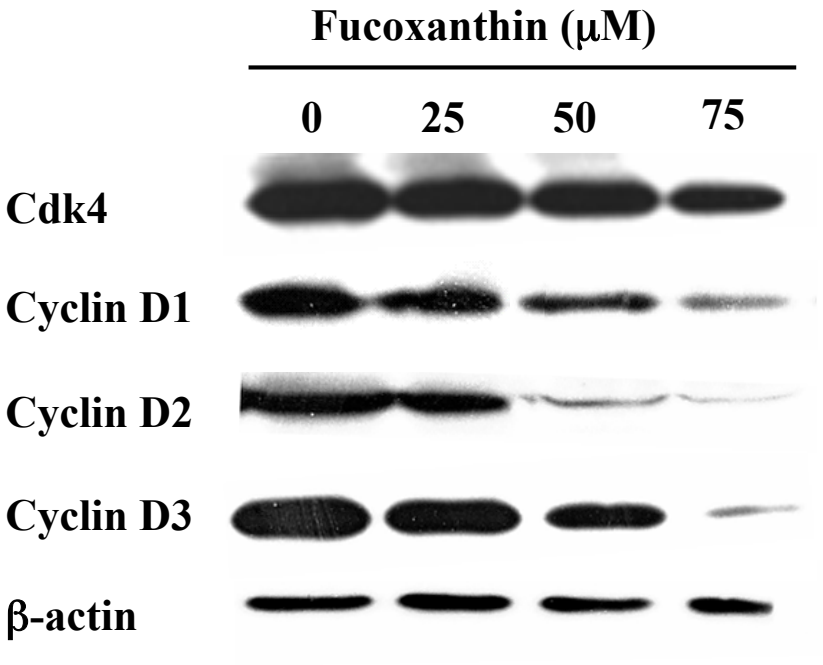


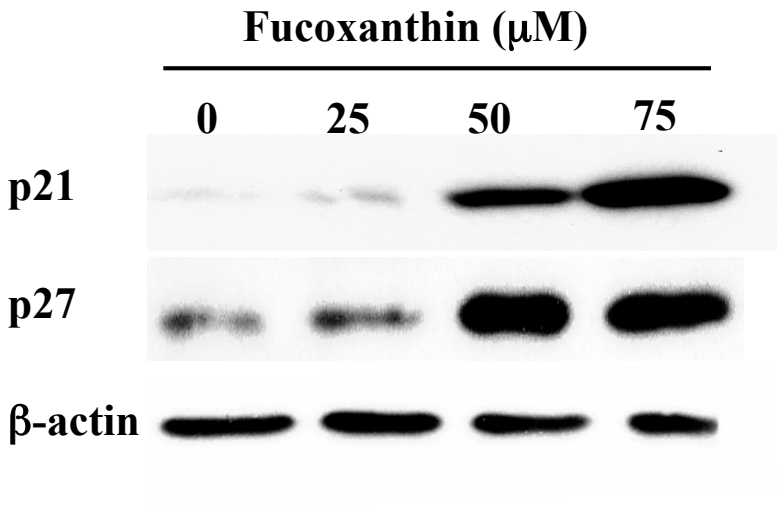
Time	Sub-G_1(%)		G_0-G_1(%)		S (%)		G_2/M (%)	
	Control	Fucoxanthin	Control	Fucoxanthin	Control	Fucoxanthin	Control	Fucoxanthin
24h			62.5±3.4	83.7±2.4*	30.1±4.1	11.9±1.9	7.5±0.9	4.25±0.7
48h			69.5±2.7	95.3±0.7*	24.5±1.9	3±0.5	5.8±0.5	1.7±0.4
72h	2.8±0.1	29.3±1*	81.8±0.6	61.2±1	5.3±0.6	3.1	8.7±0.4	4±0.1
96h	4.9±0.1	45.5±1*	78.2±2	48.2±1.9	5.3±0.2	1.8±0.3	9.9	2.2±0.3

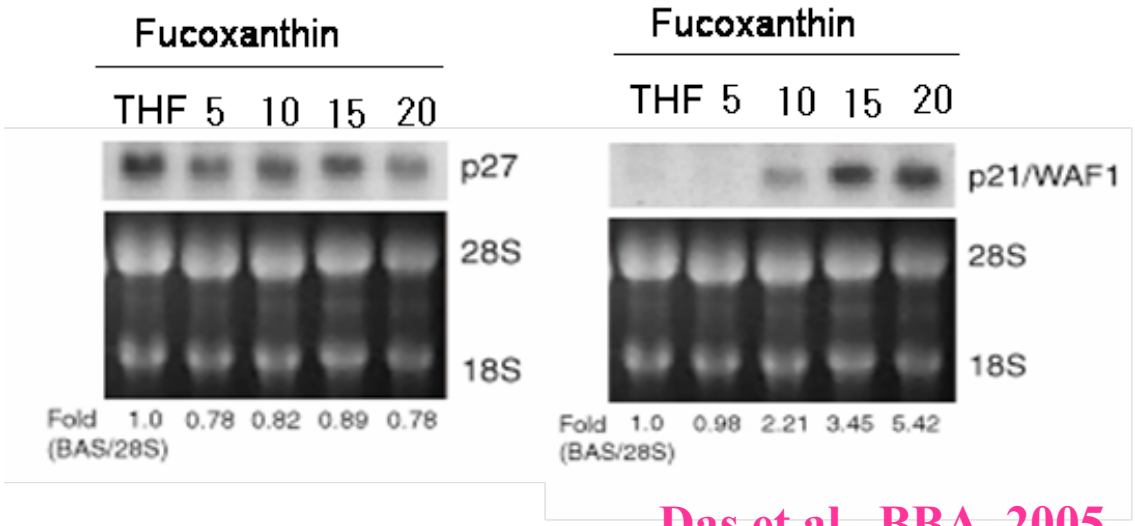
Fucoxanthin induced cell cycle arrest at G_0/G_1 phase in human WiDr cells

Fucoxanthin Regulates G1 Cell Cycle Related Proteins



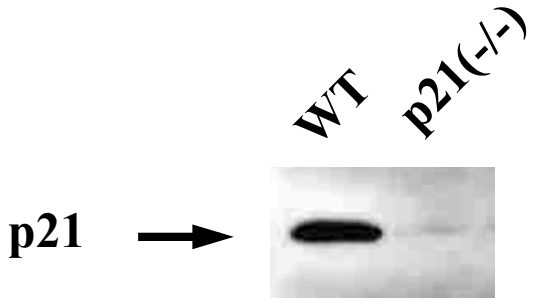




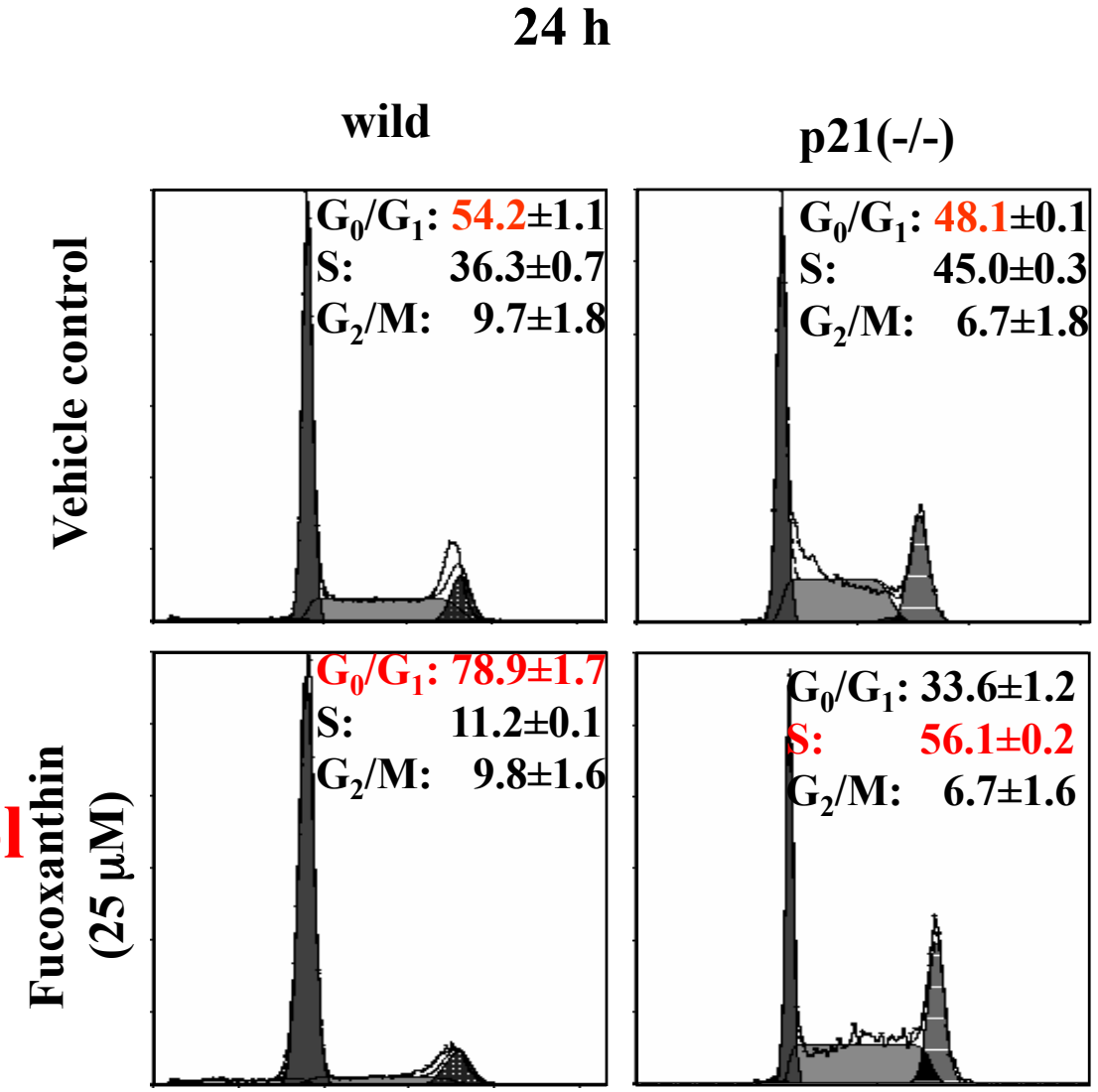


Das et al., BBA, 2005

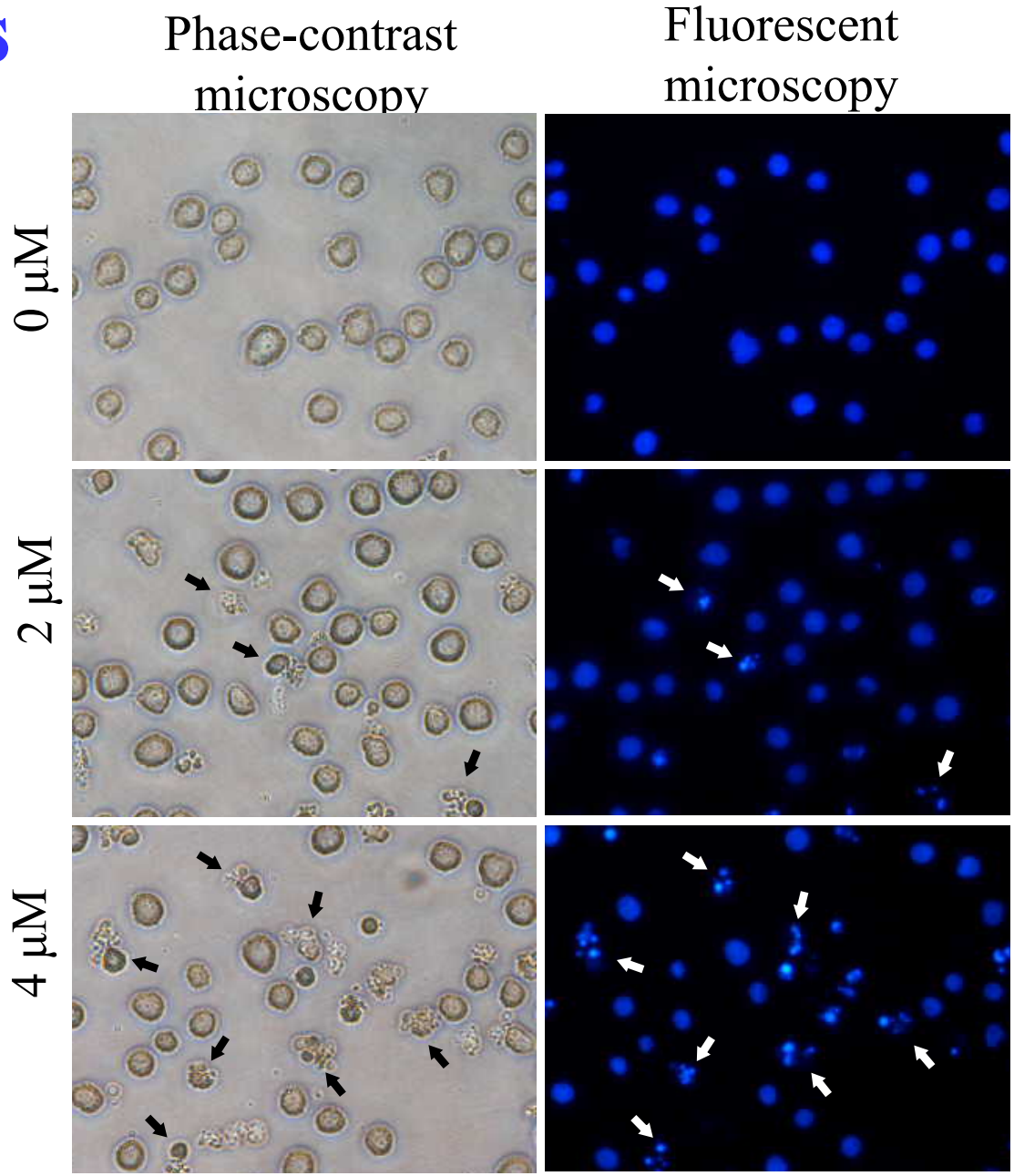
In Cip1/p21 (-/-) cells



A target protein is p21 for fucoxanthinol

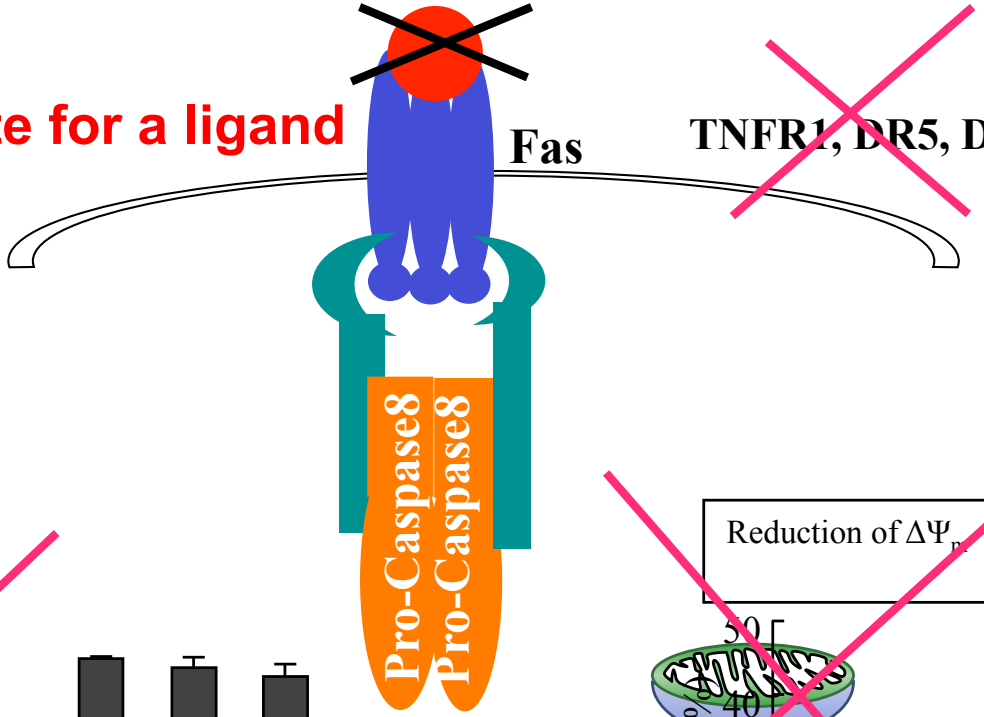


In HL-60 cells

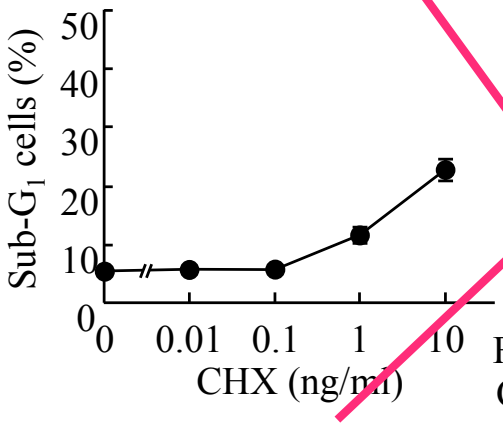


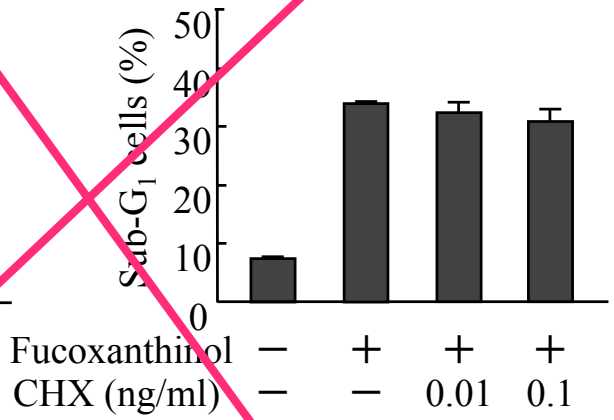
No effects of 4 μM on peripheral blood mononuclear cells of healthy volunteers

Was not candidate for a ligand

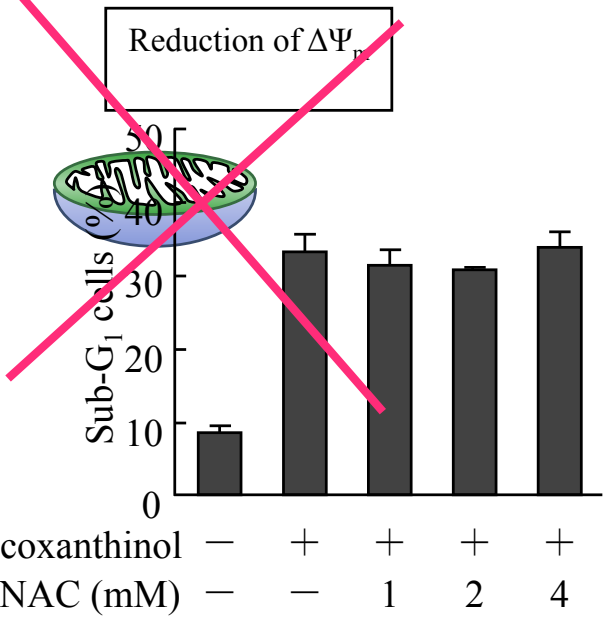


C-FLIP

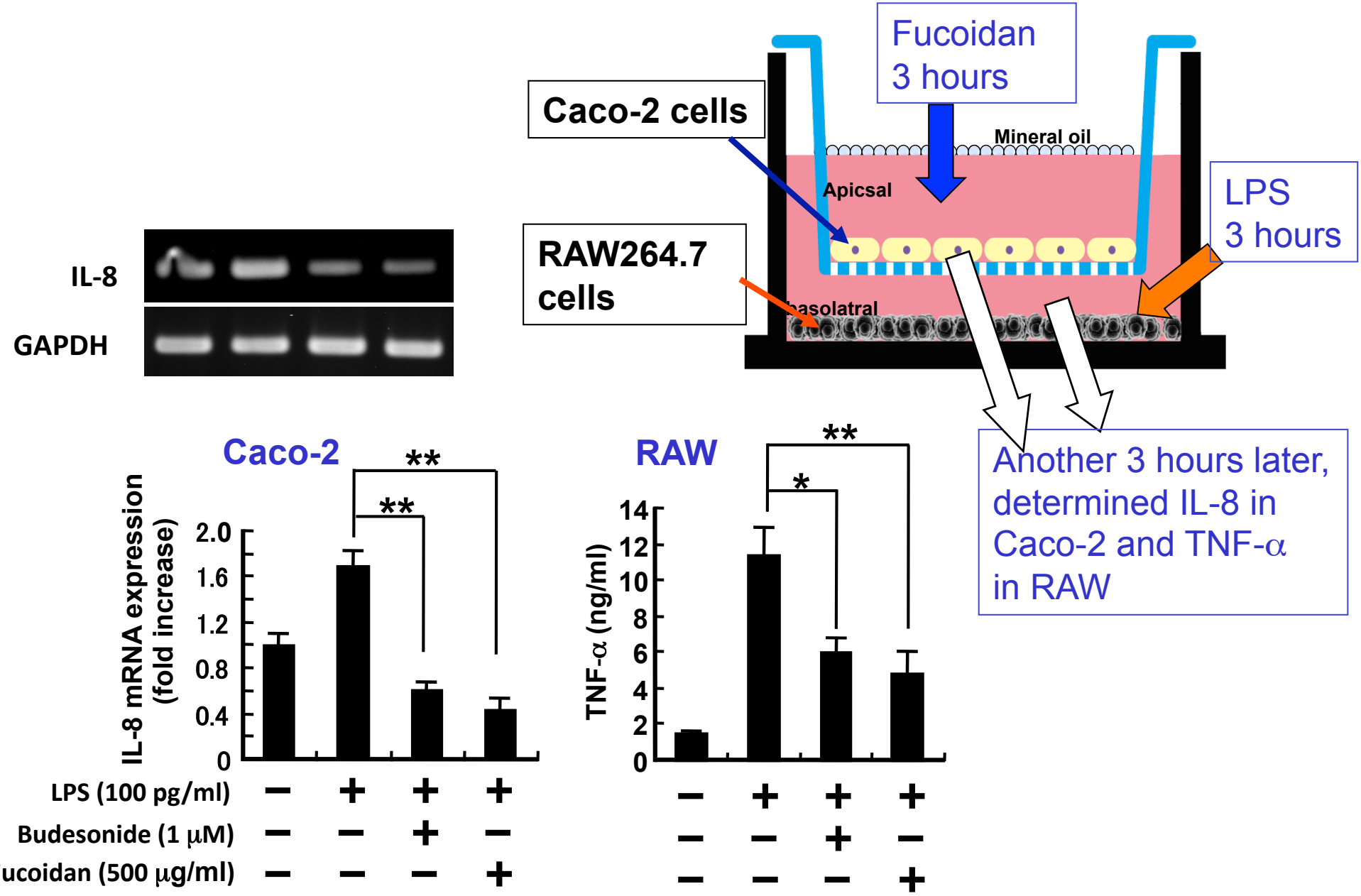




Protein synthesis did not participate in



Mitochondrial pathway did not participate in



Fucoidan modulates over response of macrophages (R) against LPS and Caco-2 (L)

Tanoue et al., Biochemical Biophysical Research Communications, 2008.

Raw kombu (Japanese kelp)

extract residual fibers

Remove
Arsenic
Salts
A part of Iodine

Remove
Arsenic
Salts
A part of Iodine

Thank you for kind attention

JAFC.2010

Anti-obesity

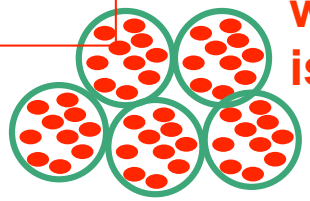
BBRC.332,392,2005

Reconstruction

Wrapping fucoxanthin with fucoidan is super kombu

BBRC. 2008

No waste parts



Functional foods being easy to eat

We are making a novel functional food