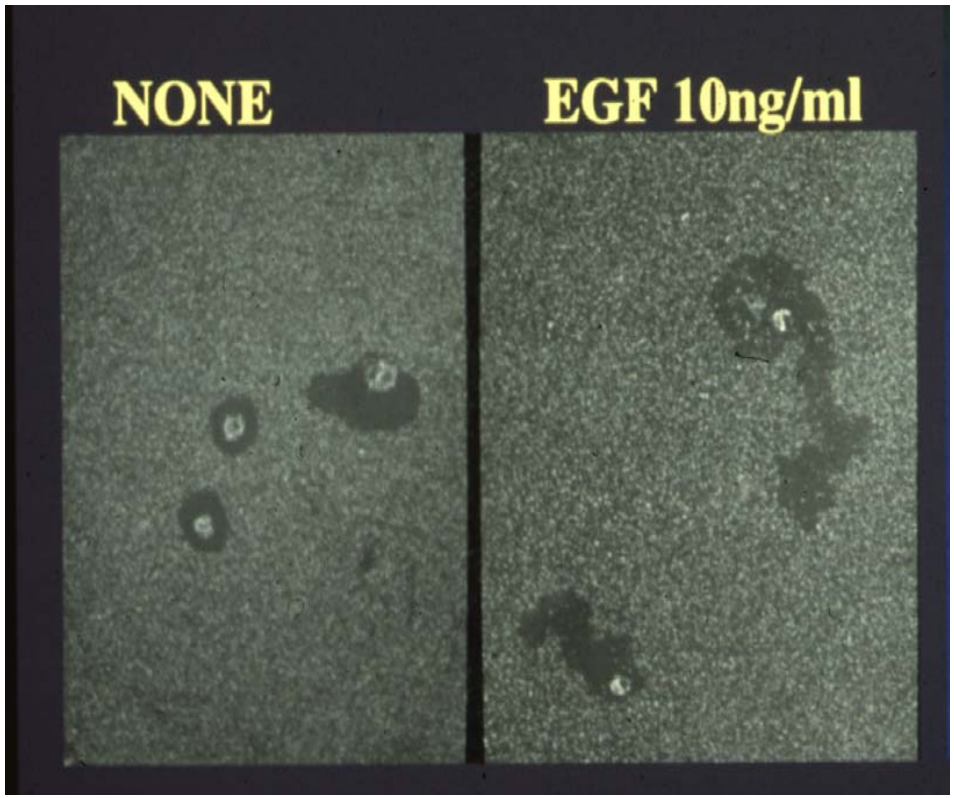
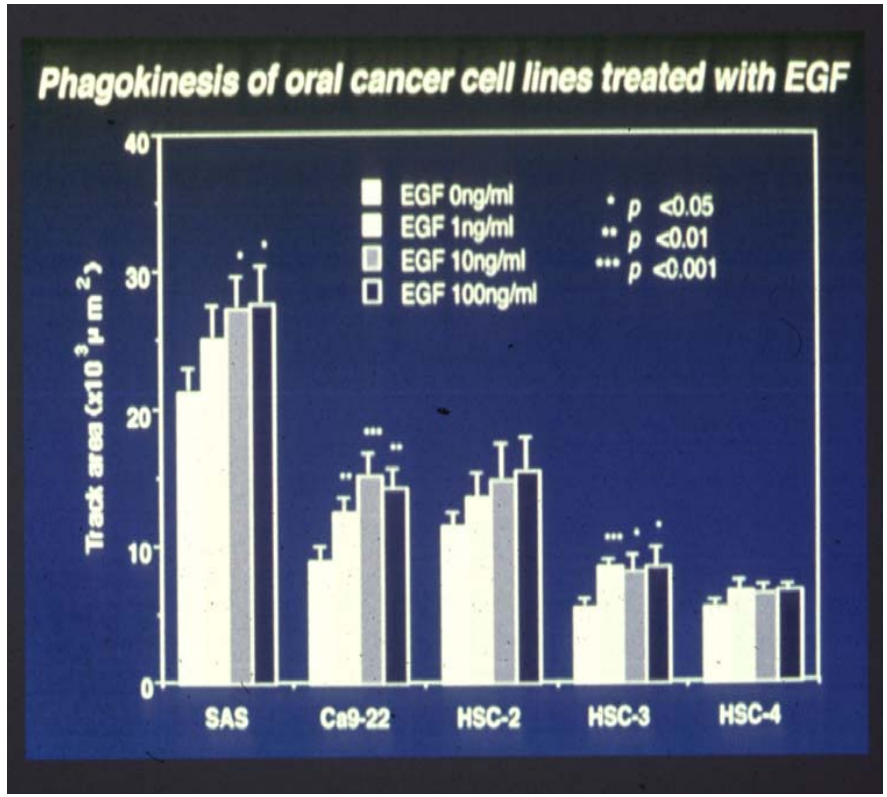
EGF受容体・シグナル伝達を分子標的とする口腔がん治療の基礎的検討

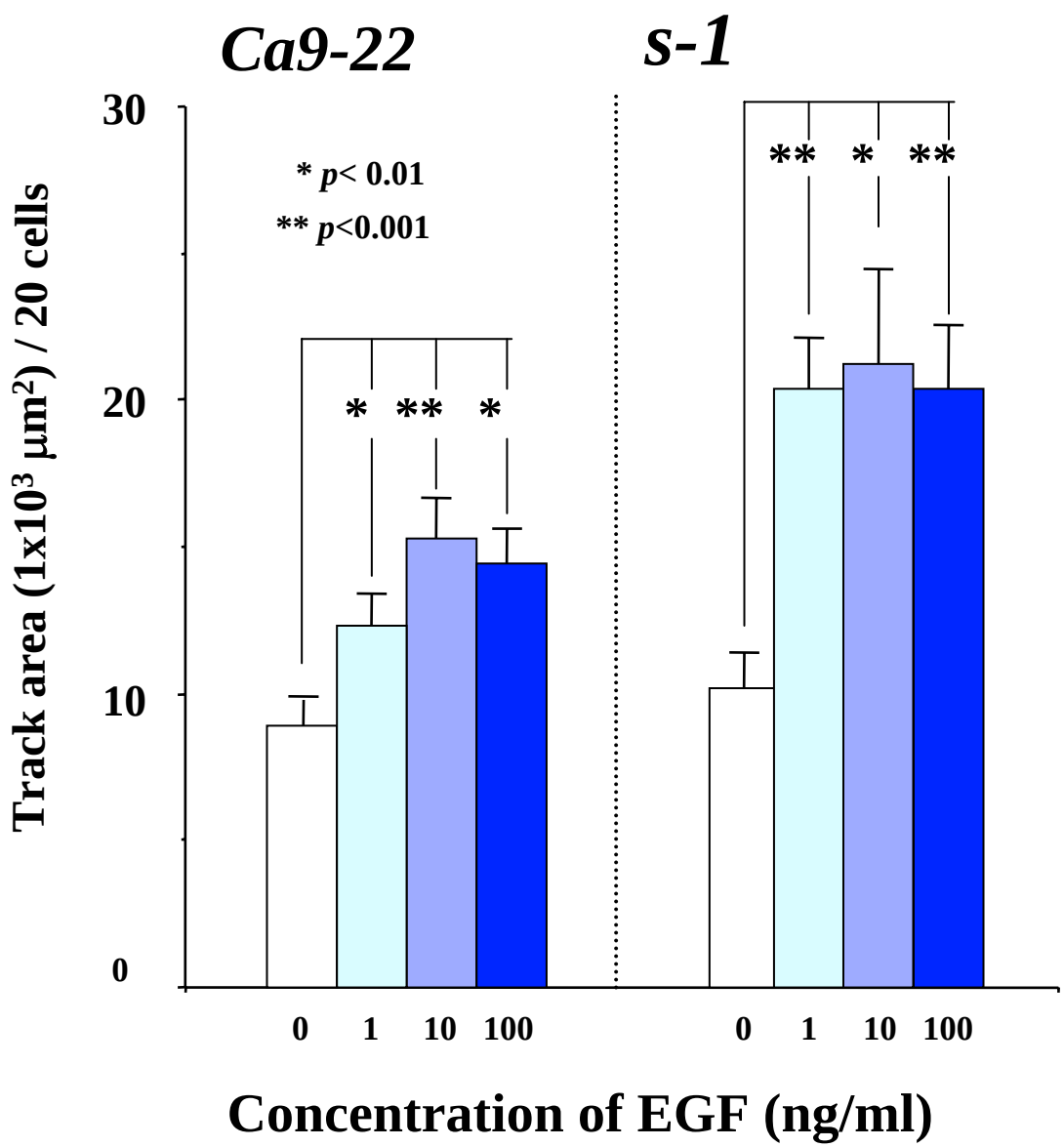
ヒト口腔がん細胞の浸潤シグナルの伝達

EGF刺激による運動能の促進（金コロイド法による評価）

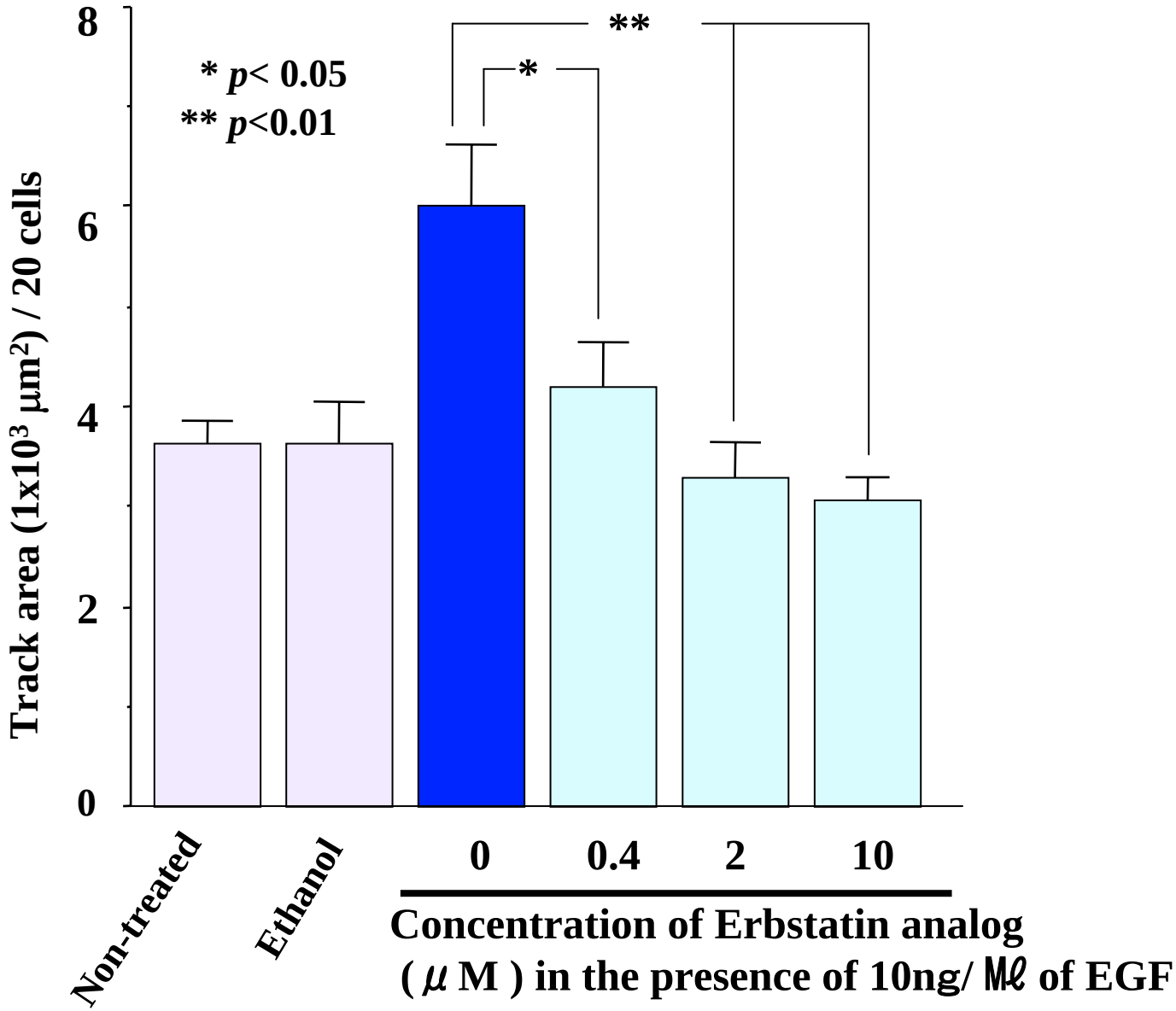




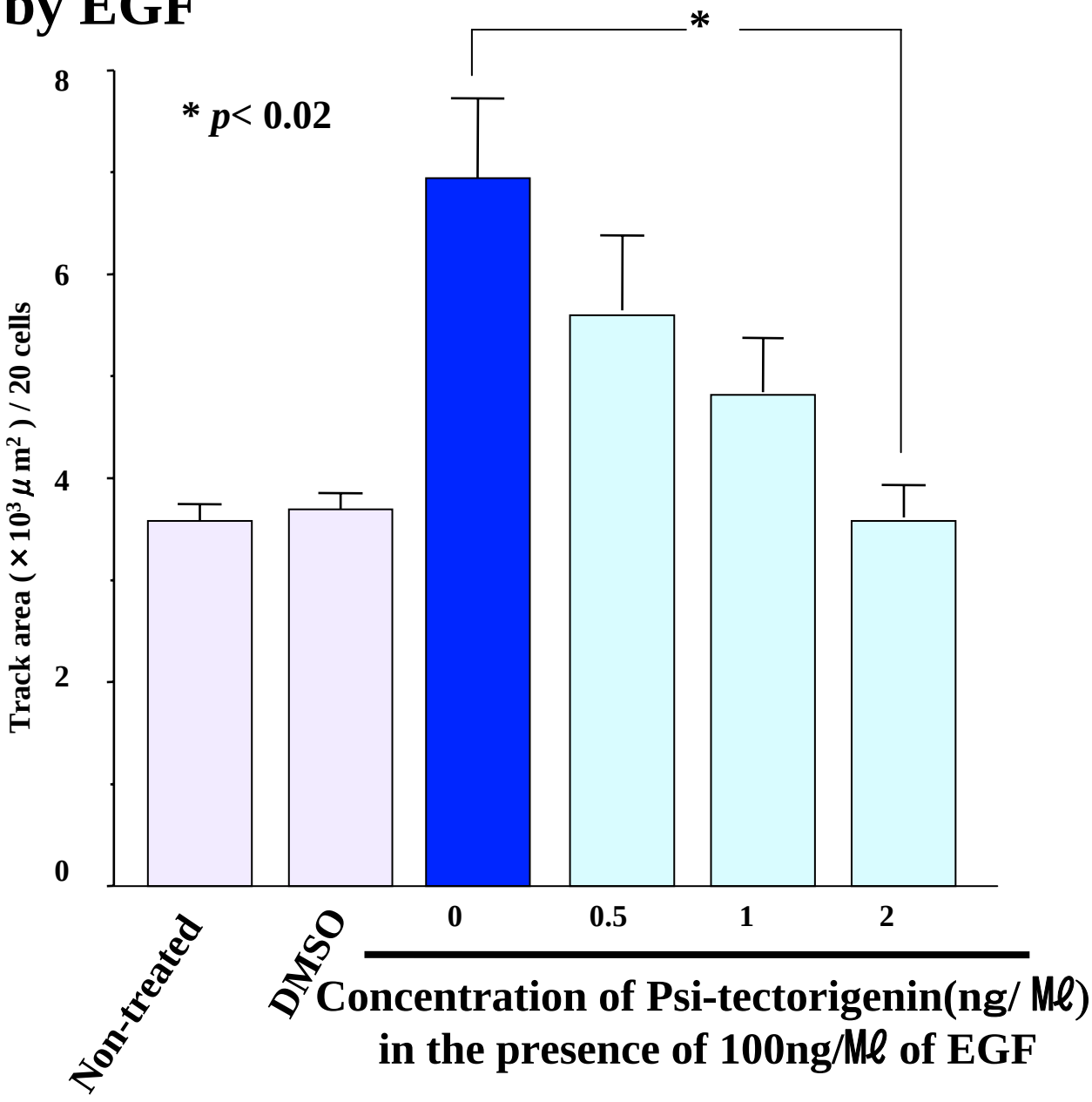
Enhanced effect of EGF stimulation on the motility of Ca9-22 and s-1 cells



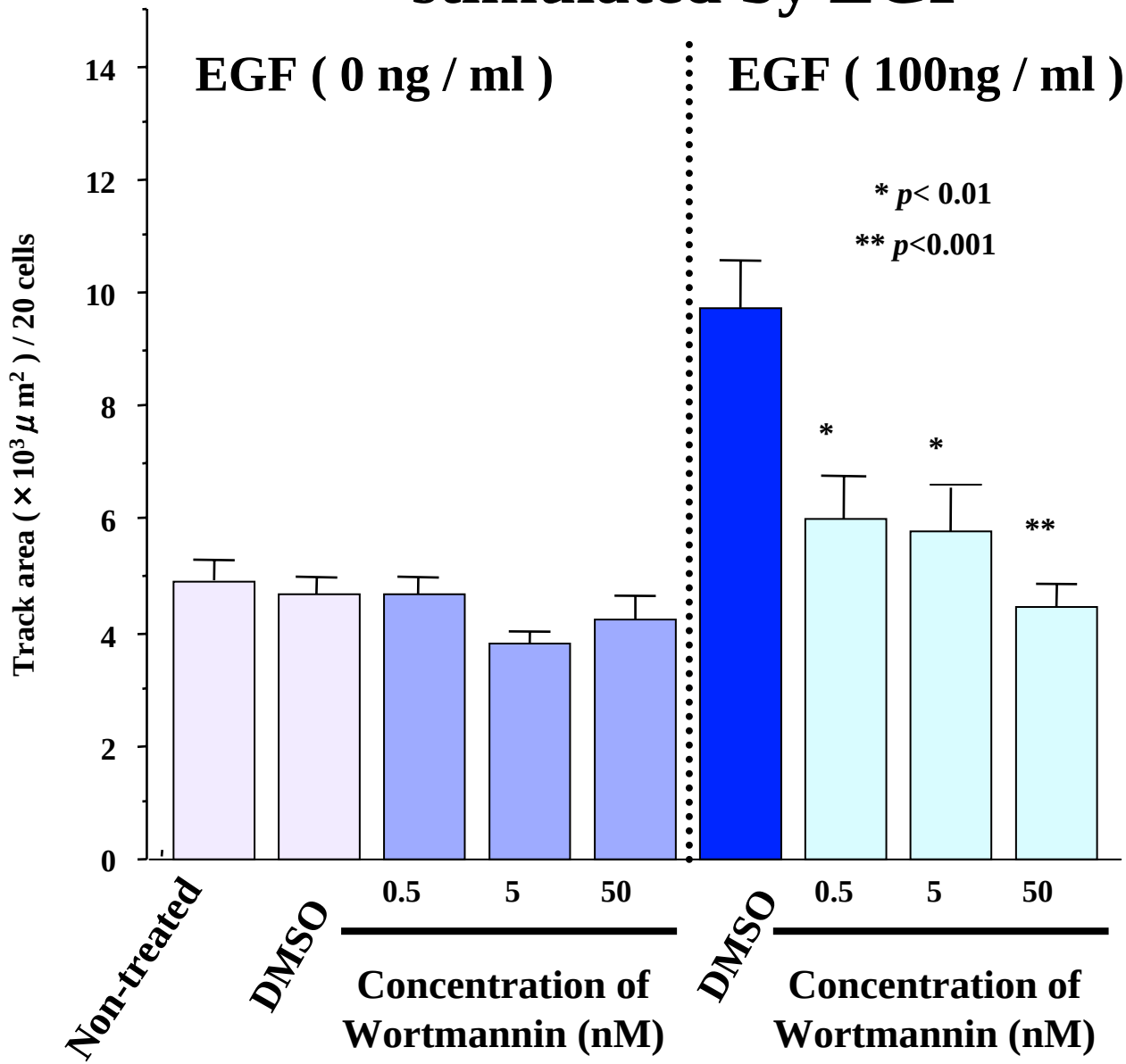
Effect of Erbstatin analog on the motility of s-1 cells stimulated by EGF



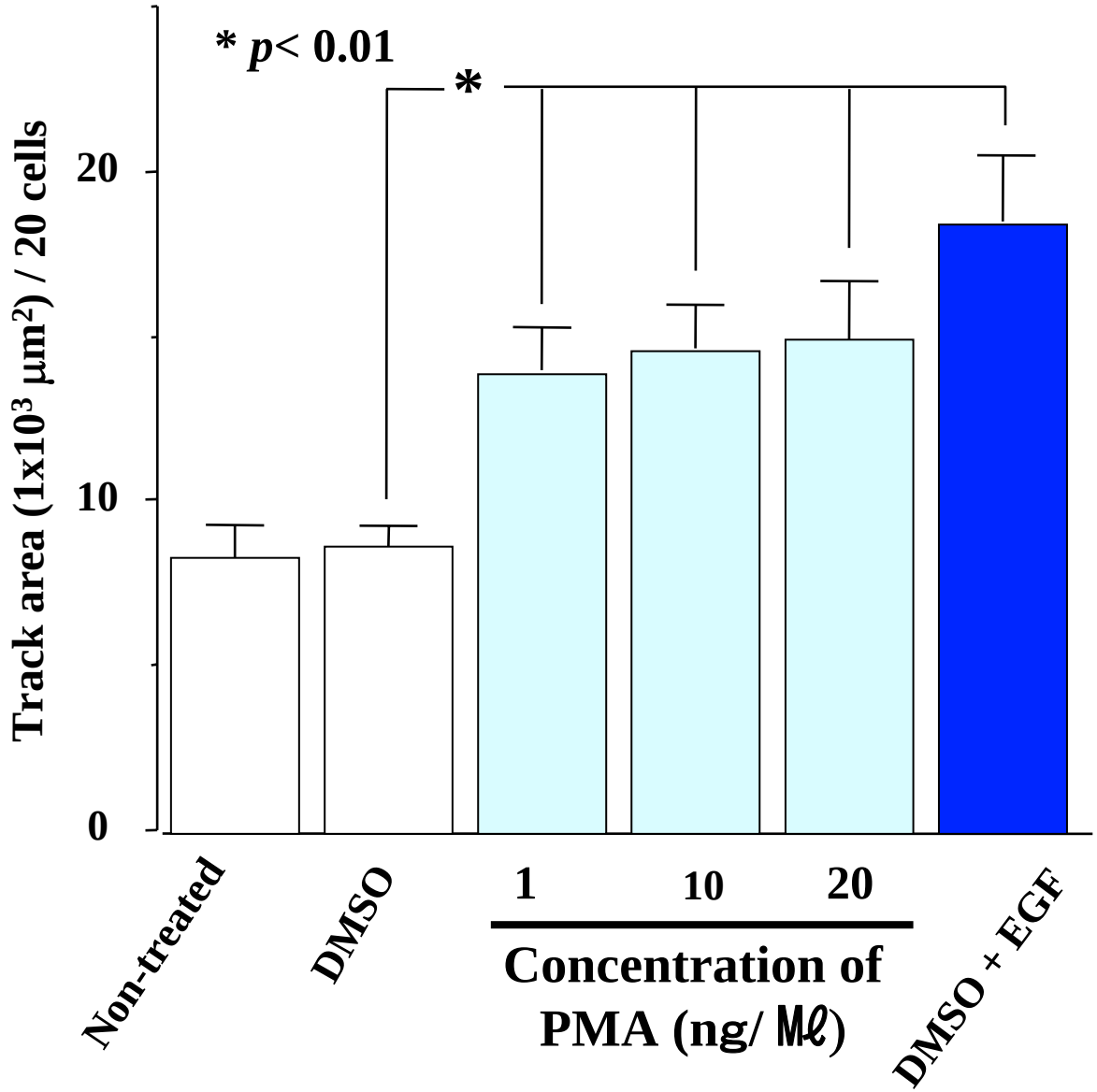
Effect of Psi-tectorigenin on the motility of s-1 cells stimulated by EGF



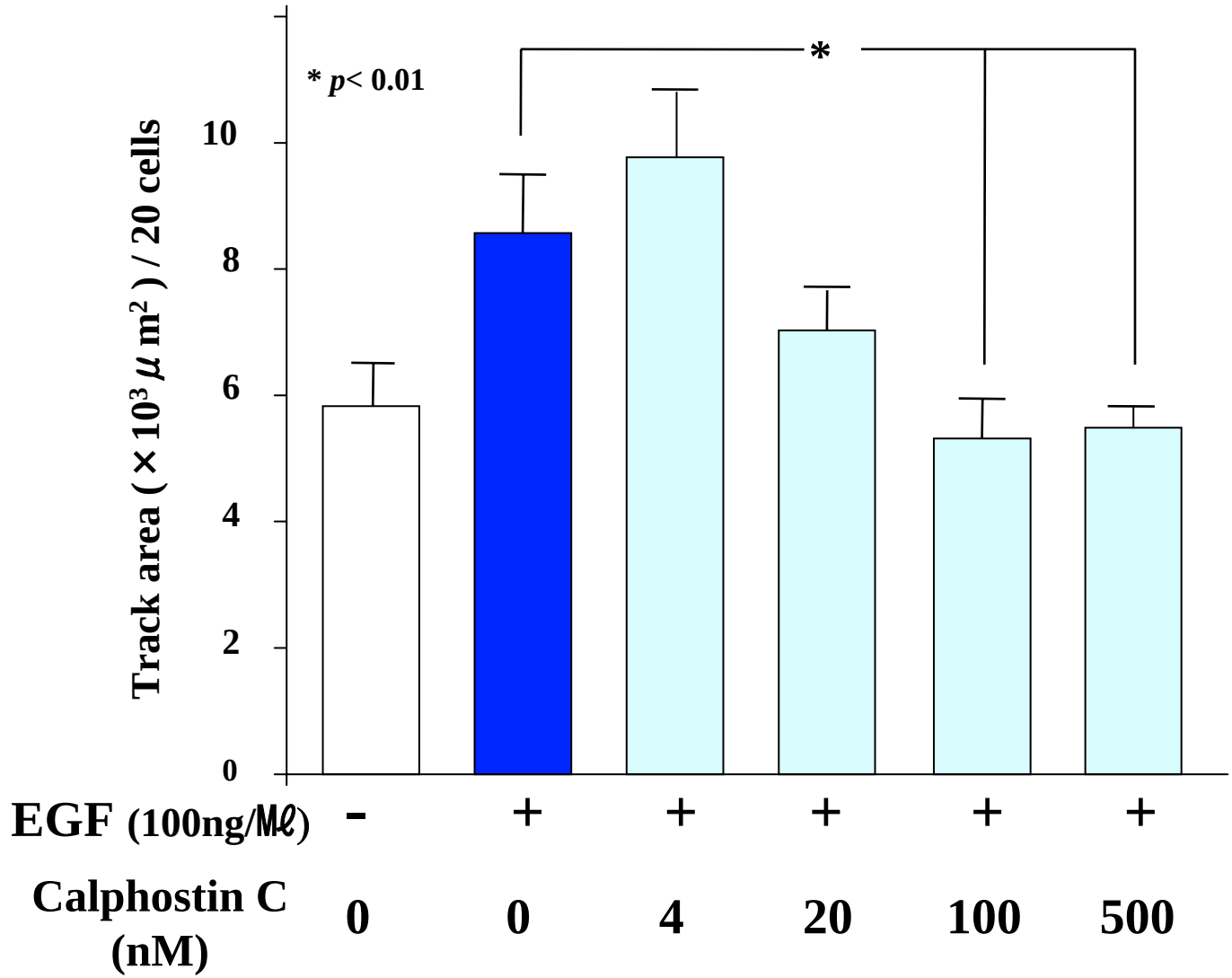
Effect of Wortmannin on the motility of s-1 cells stimulated by EGF



Effect of PKC activator on the motility of s-1 cells

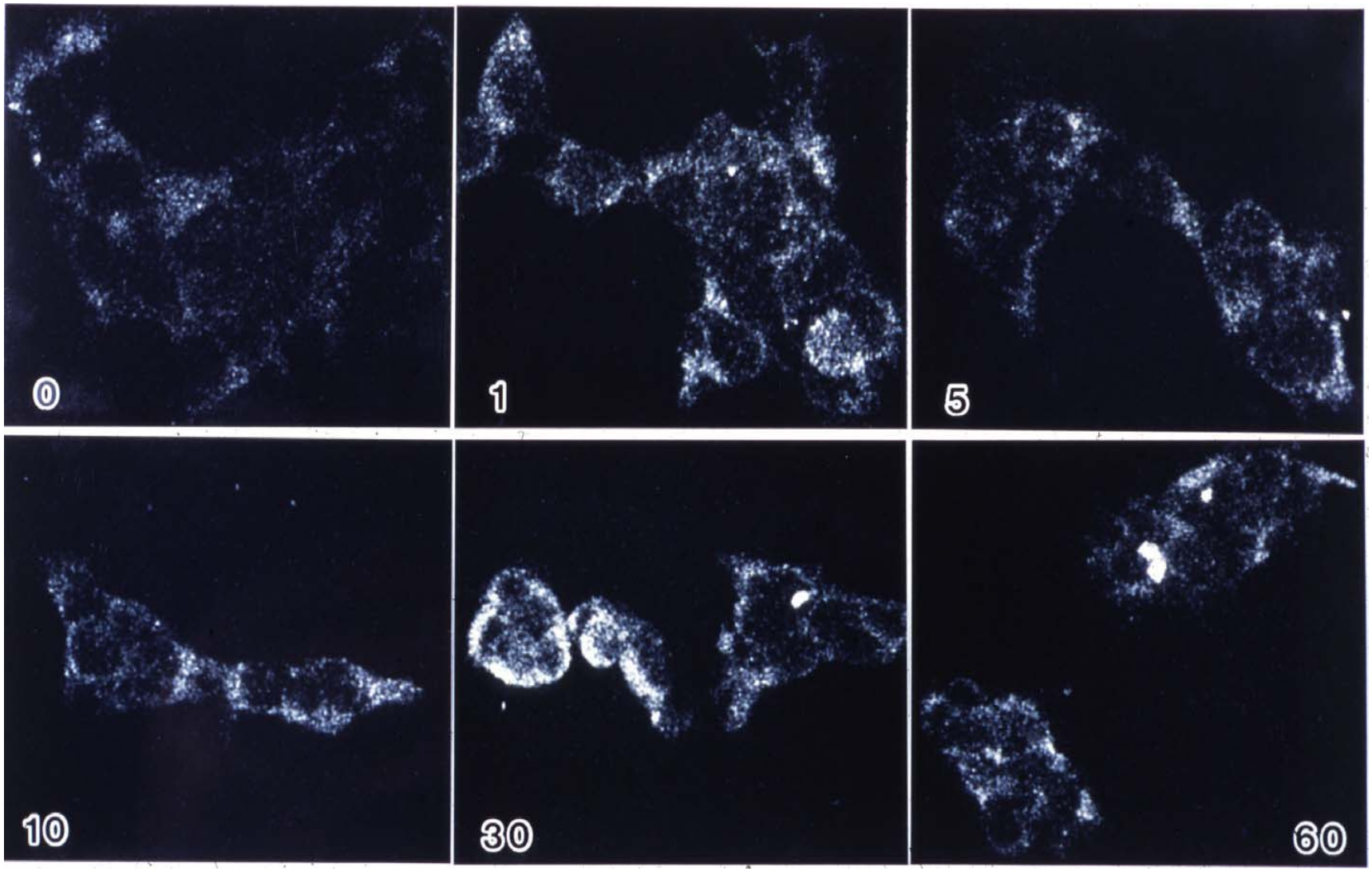


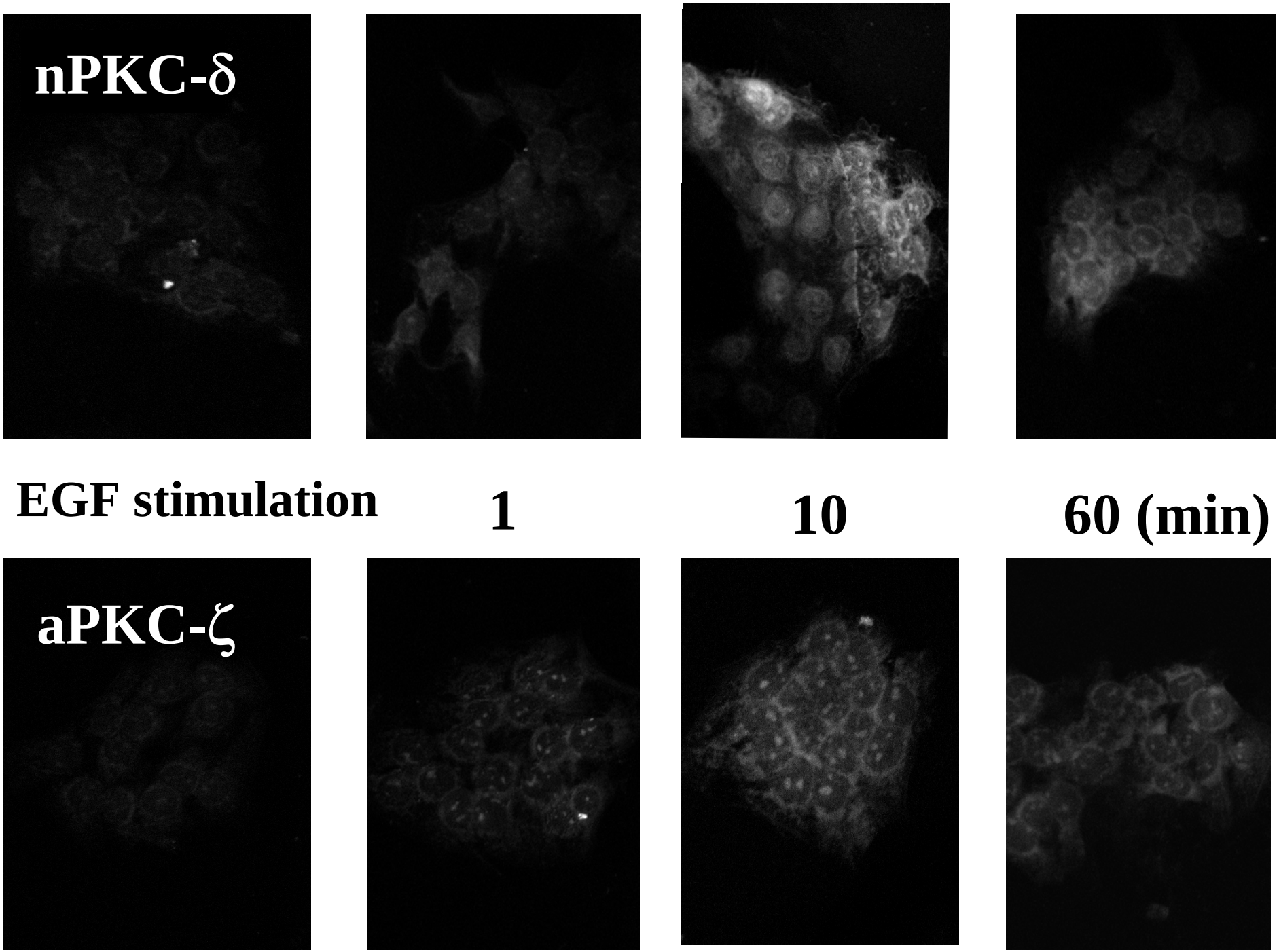
Effect of PKC inhibitor on the motility of s-1 cells stimulated by EGF

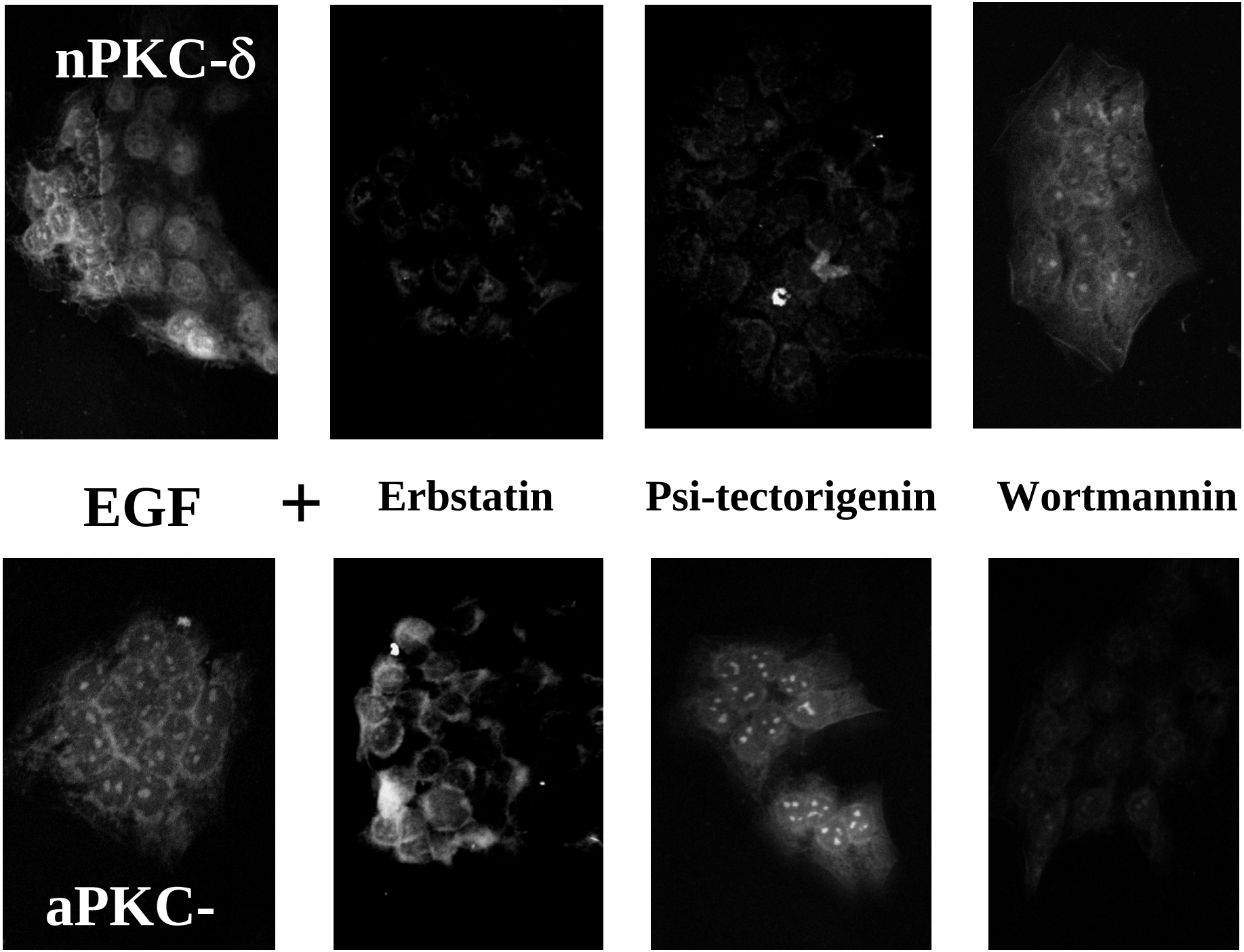


Translocation of PKC in EGF stimulated s-1 cells

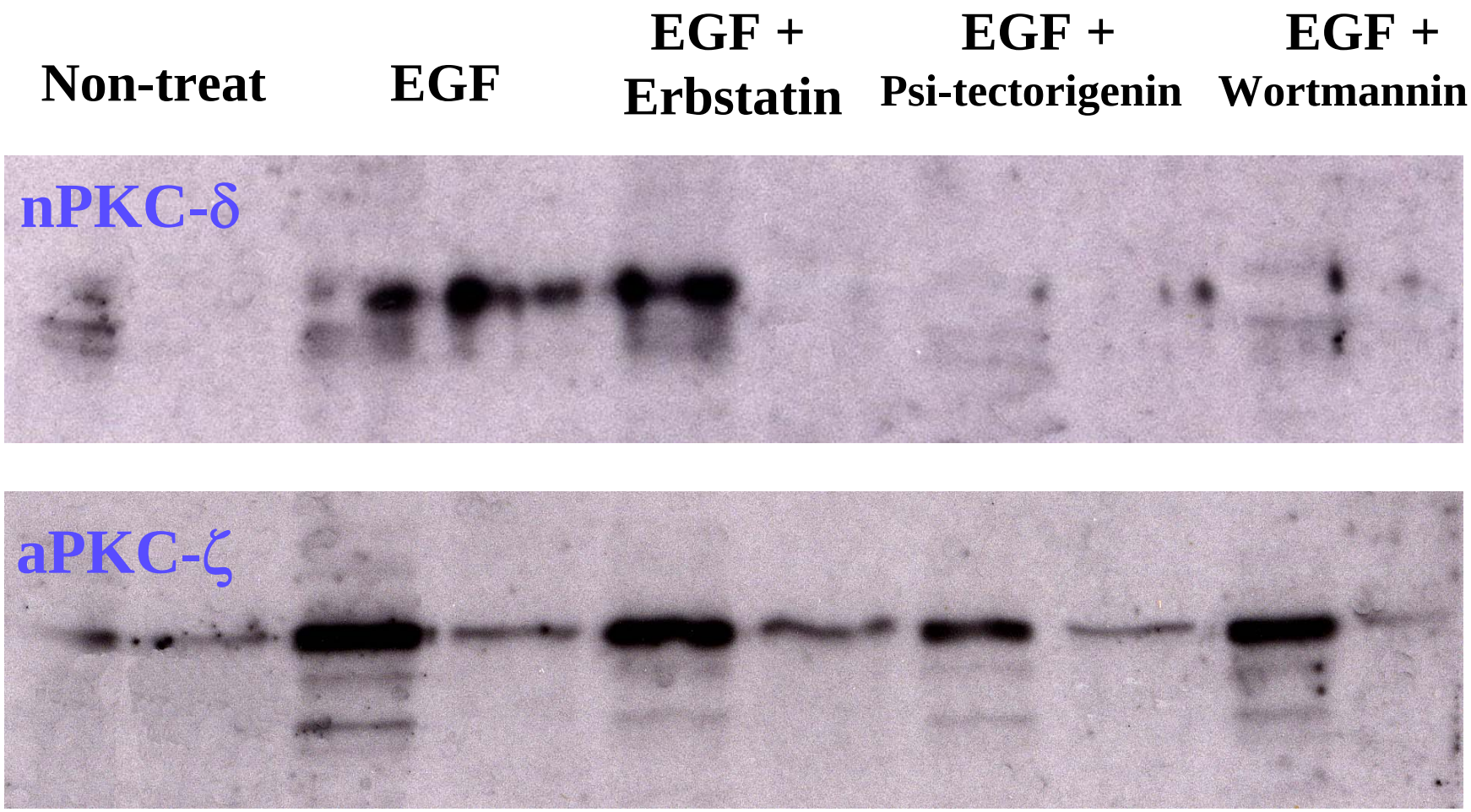
pan PKC MoAb staining



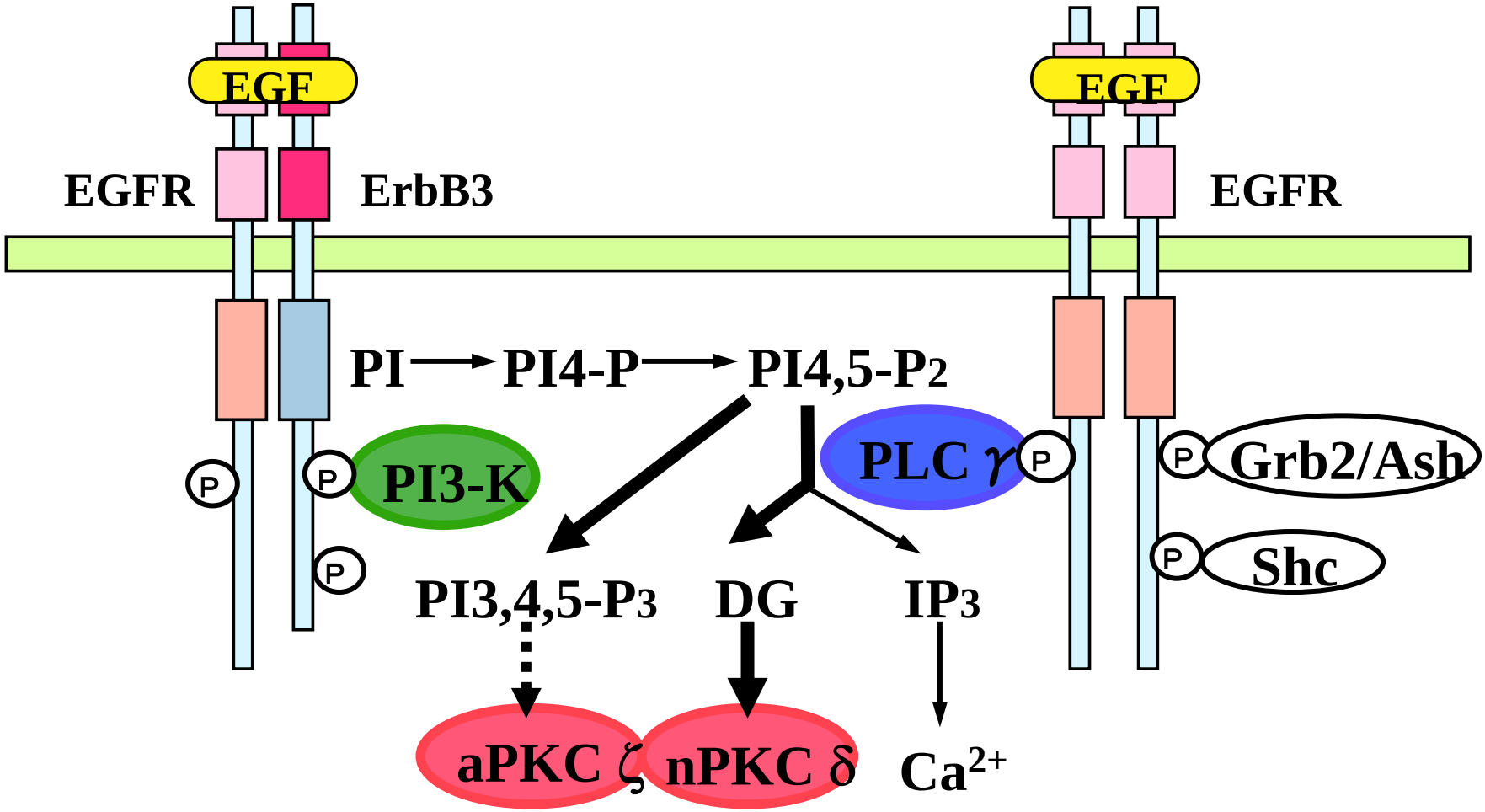

nPKC-δ
EGF stimulation
1
10
60 (min)
aPKC-ζ


nPKC-δ
EGF
+
Erbstatin
Psi-tectorigenin
Wortmannin
aPKC-

Localization of intracellular PKC-δ and PKC-ζ in EGF and signal transduction inhibitors treated s-1 cells.



Possible Signal Transduction Pathways Involved in Cell Motility Induced by EGF



EGF受容体を分子標的とした薬剤

	薬剤	標的分子・特徴	
低分子化合物	ZD1839	EGF-R Tyrosine K	Iressa
	OSI-774	EGF-R Tyrosine K	PI3K MAPK
	EKB-569	EGF-R Tyrosine K	
	Cl-1033	EGF-R Tyrosine K	
	PKl-116	EGF-R Tyrosine K	
	PD183805	EGF-R Tyrosine K	
	CGP-59362A	EGF-R Tyrosine K	
抗体	IMC-C225	キメラ抗EGF-R 抗体	Cetuximab
	h-R3	ヒト化抗EGF-R 抗体	
	MDX-447	抗EGF-R, CD64抗体	
	ABX-EGF	ヒト化抗 EGF-R抗体	

Tumorigenicity of ER-1 cells treated with EGF

- 24 hours treatment

EGF	Tumor take
None	0/5
100ng/ml	5/5
100ng/ml (4 days EGF free)	0/5

- 1 month treatment

EGF	Tumor take
None	0/5
100ng/ml (1 month EGF free)	4/5
100ng/ml (2 month EGF free)	5/5

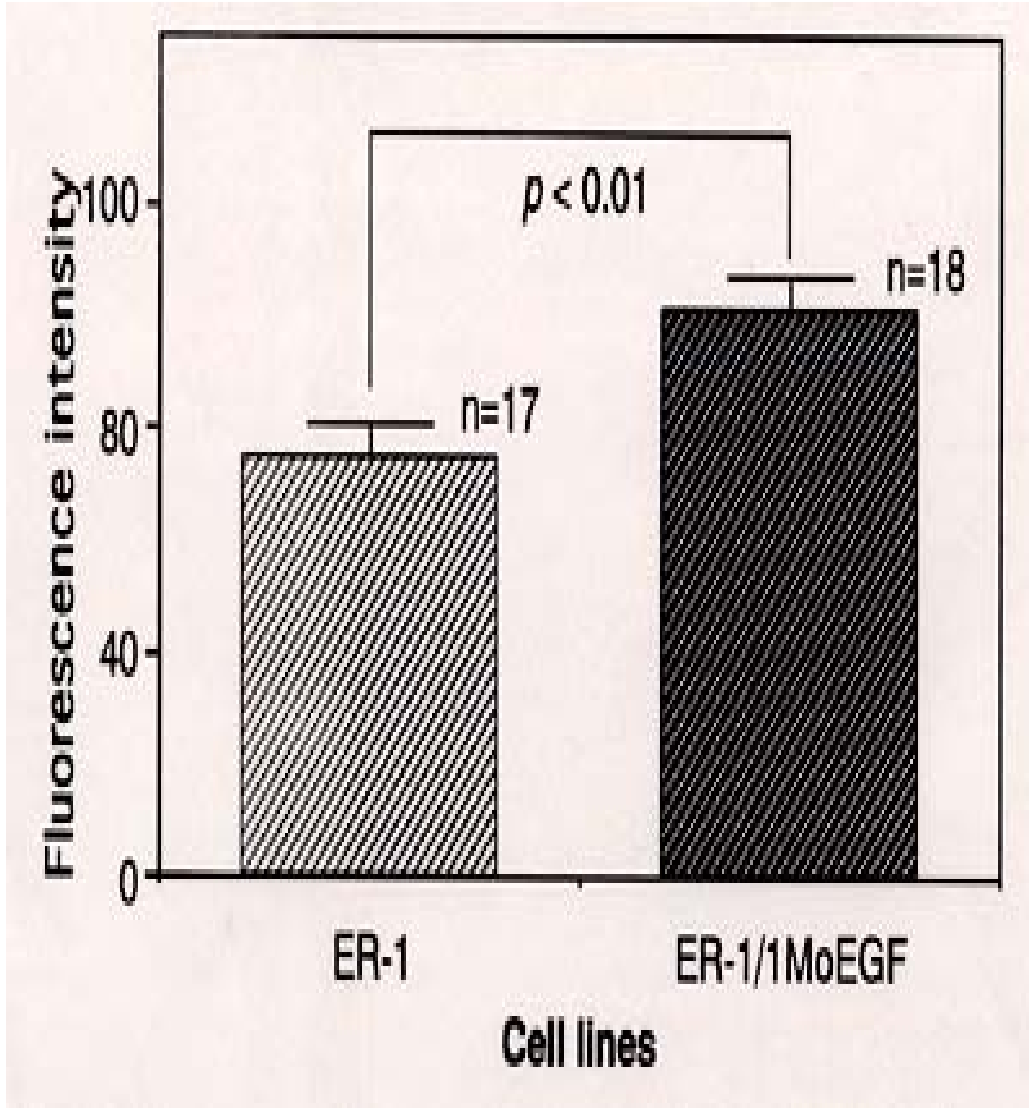
ER-1 cells $1x10^5$ ip

DCFDA

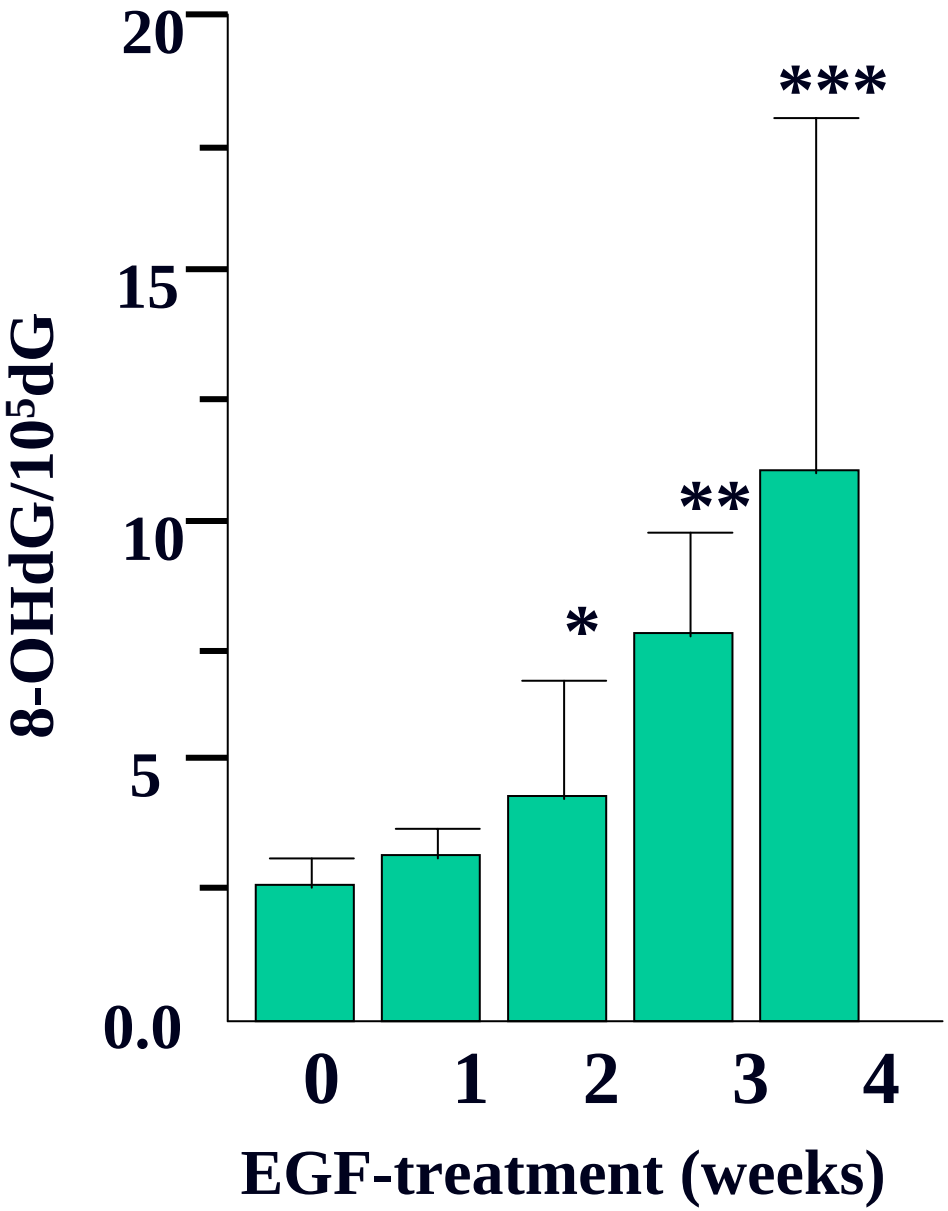
Intracellular oxidized state in ER-1 cells

ER-1

EGF 1 mo treated ER-1



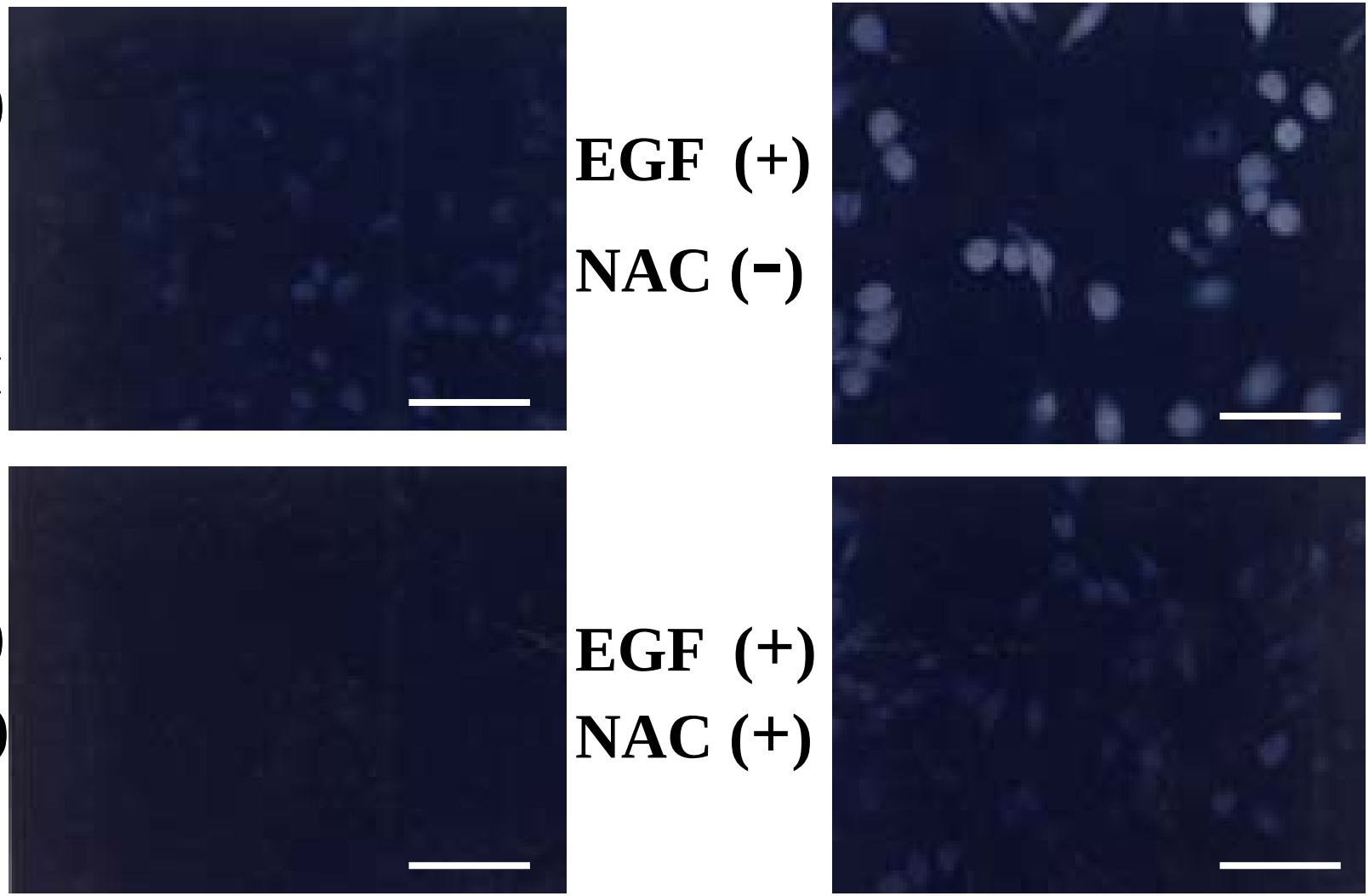
Levels of 8-OHdG in ER-1 cells depending on the length of EGF tre



* p=0.035
** p=0.002
*** p<0.001
Vs
EGF 1 week treatment

Inhibition of EGF-induced intracellular peroxides by N-acetylcysteine (antioxidant)

Using of DCFDA

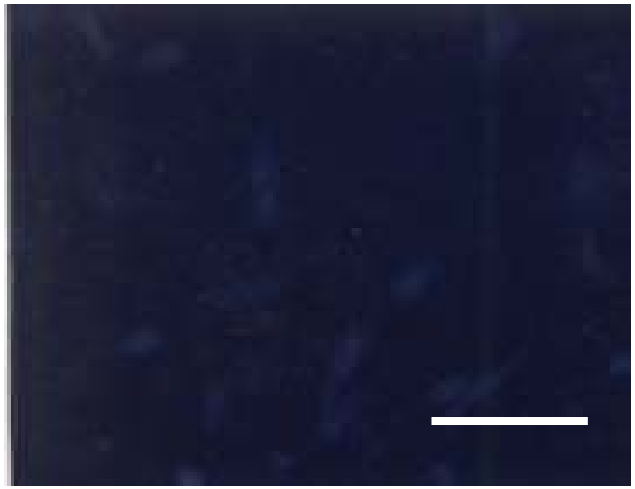


Inhibition of EGF-induced intracellular peroxides by sodium selenite (antioxidant)

Using of DCFDA

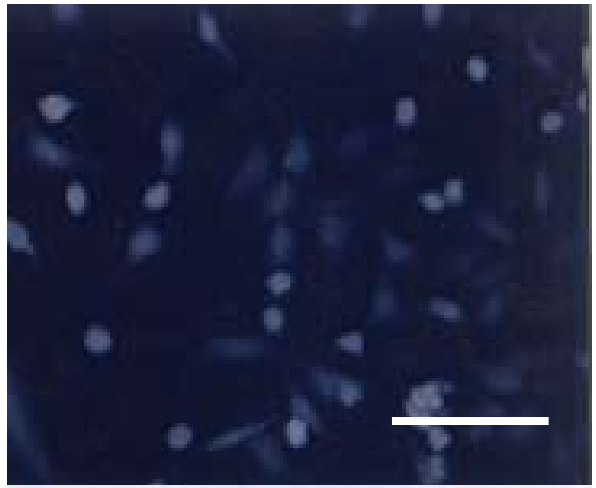
EGF(-)
100ng/ml

Se (-)
100ng/ml



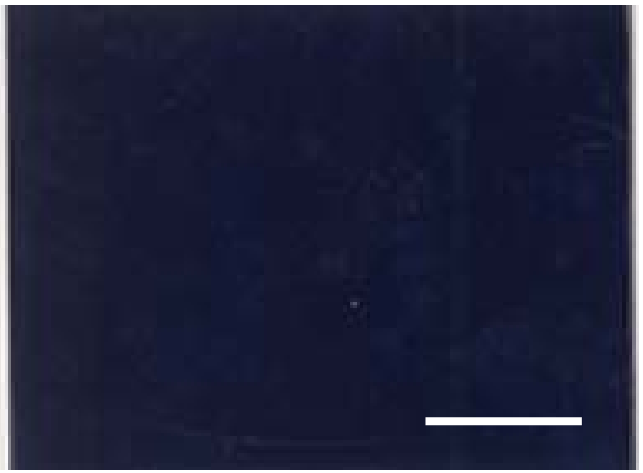
EGF(+)

Se (-)



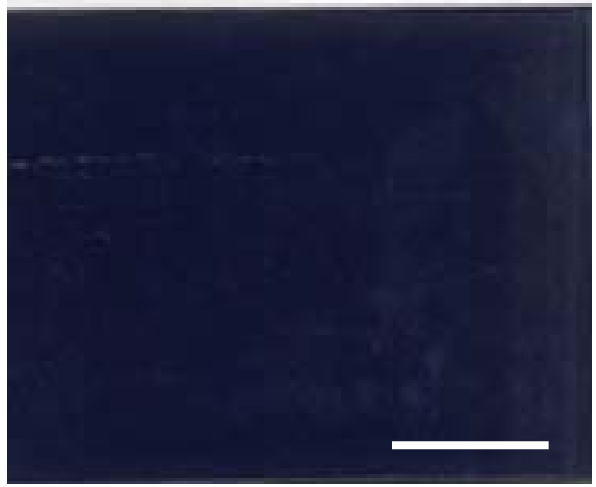
EGF(-)

Se (+)



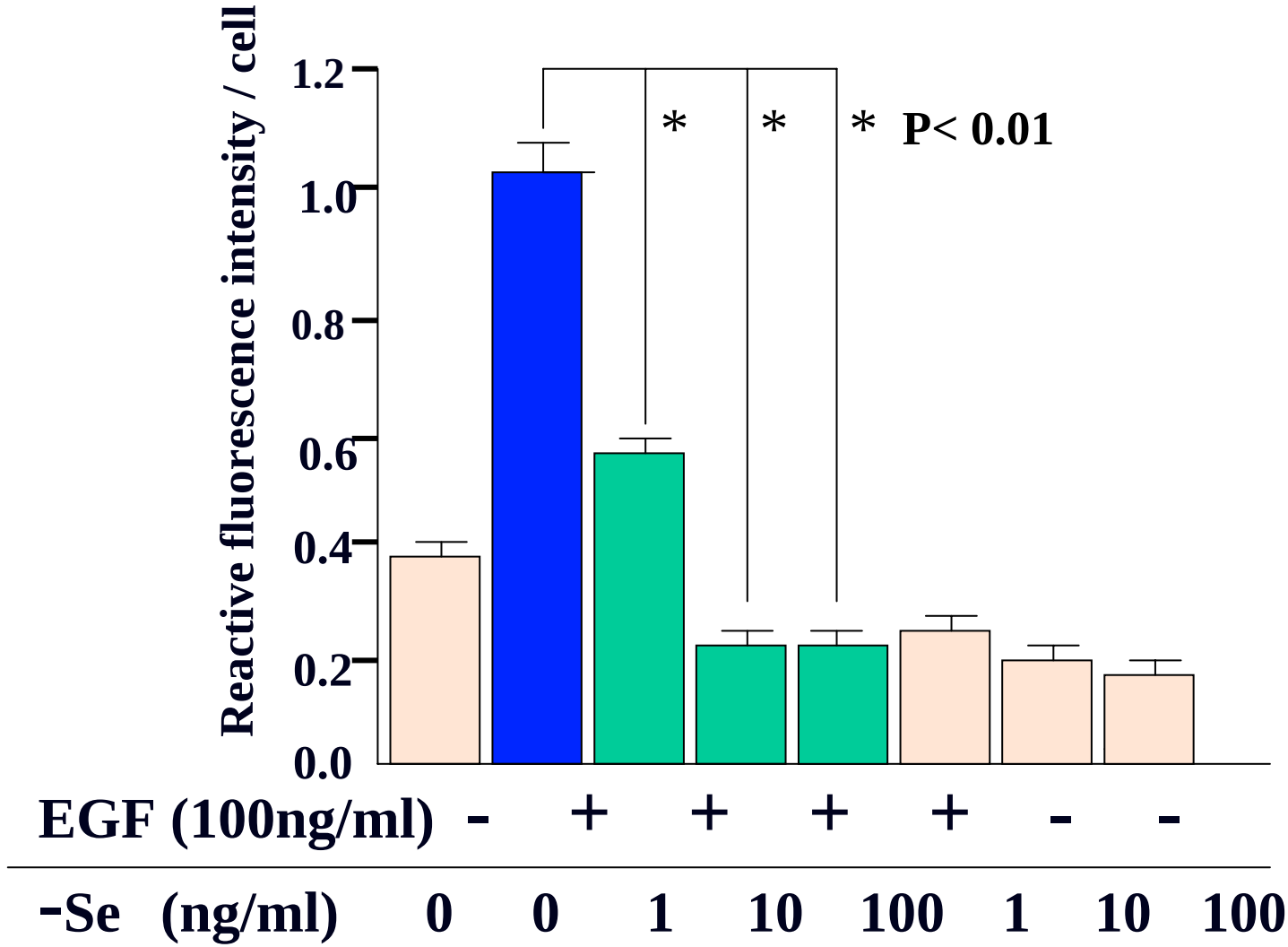
EGF(+)

Se (+)



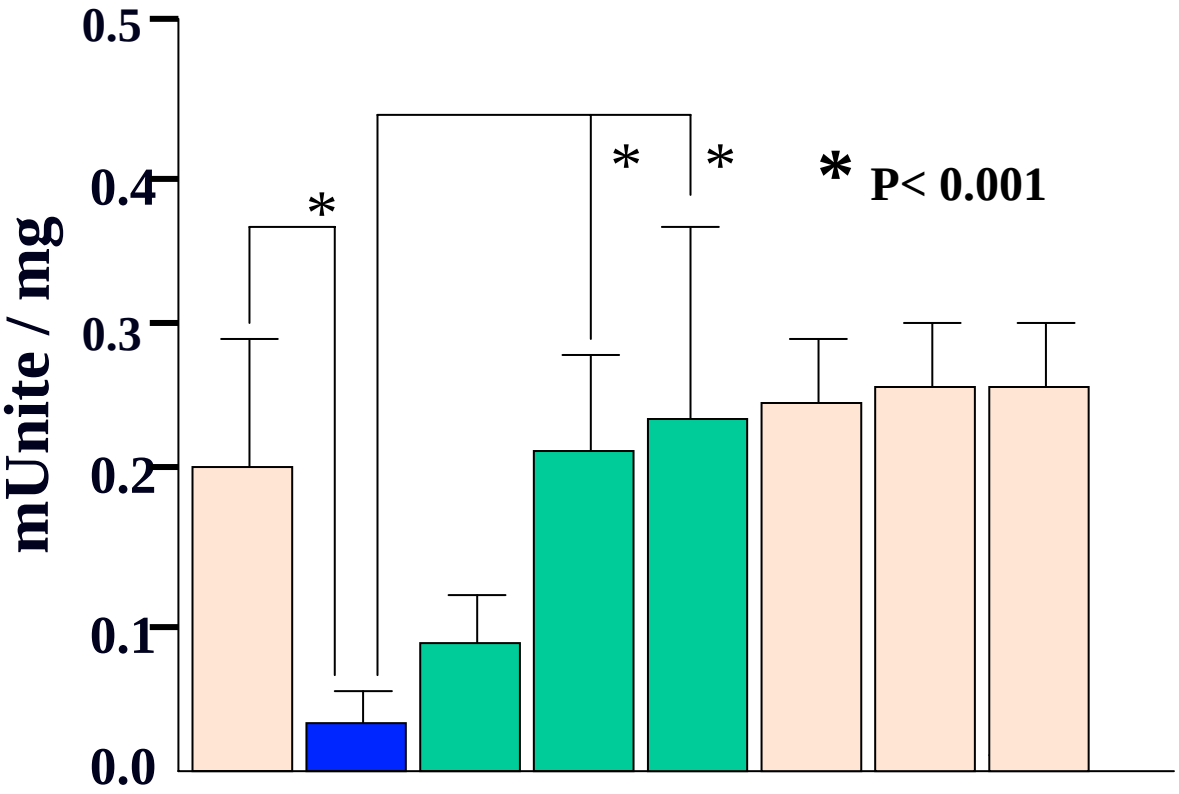
Inhibition of EGF-induced intracellular peroxides by sodium selenite

(Levels of peroxidase)

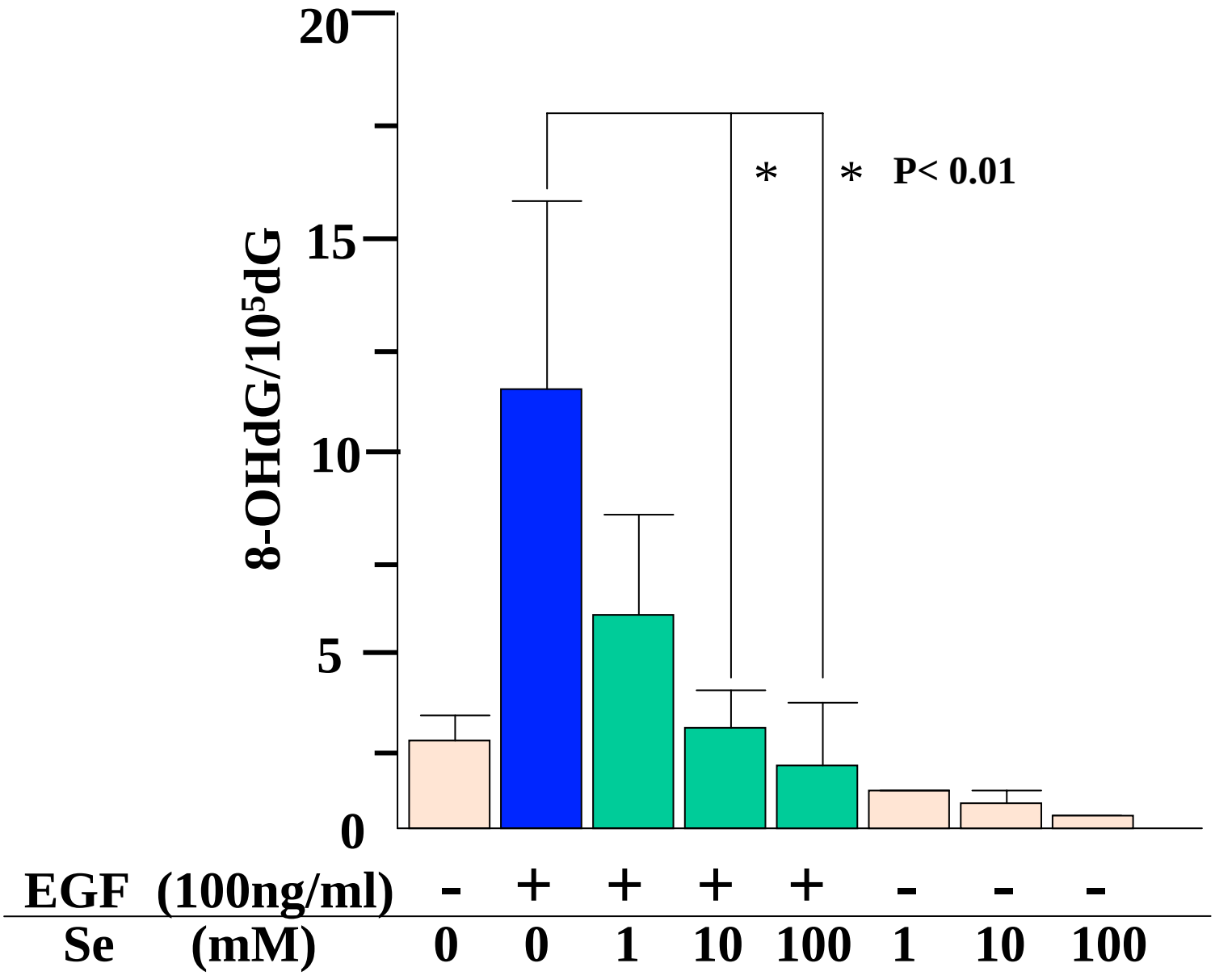


Inhibition of EGF-induced intracellular peroxides by sodium selenite

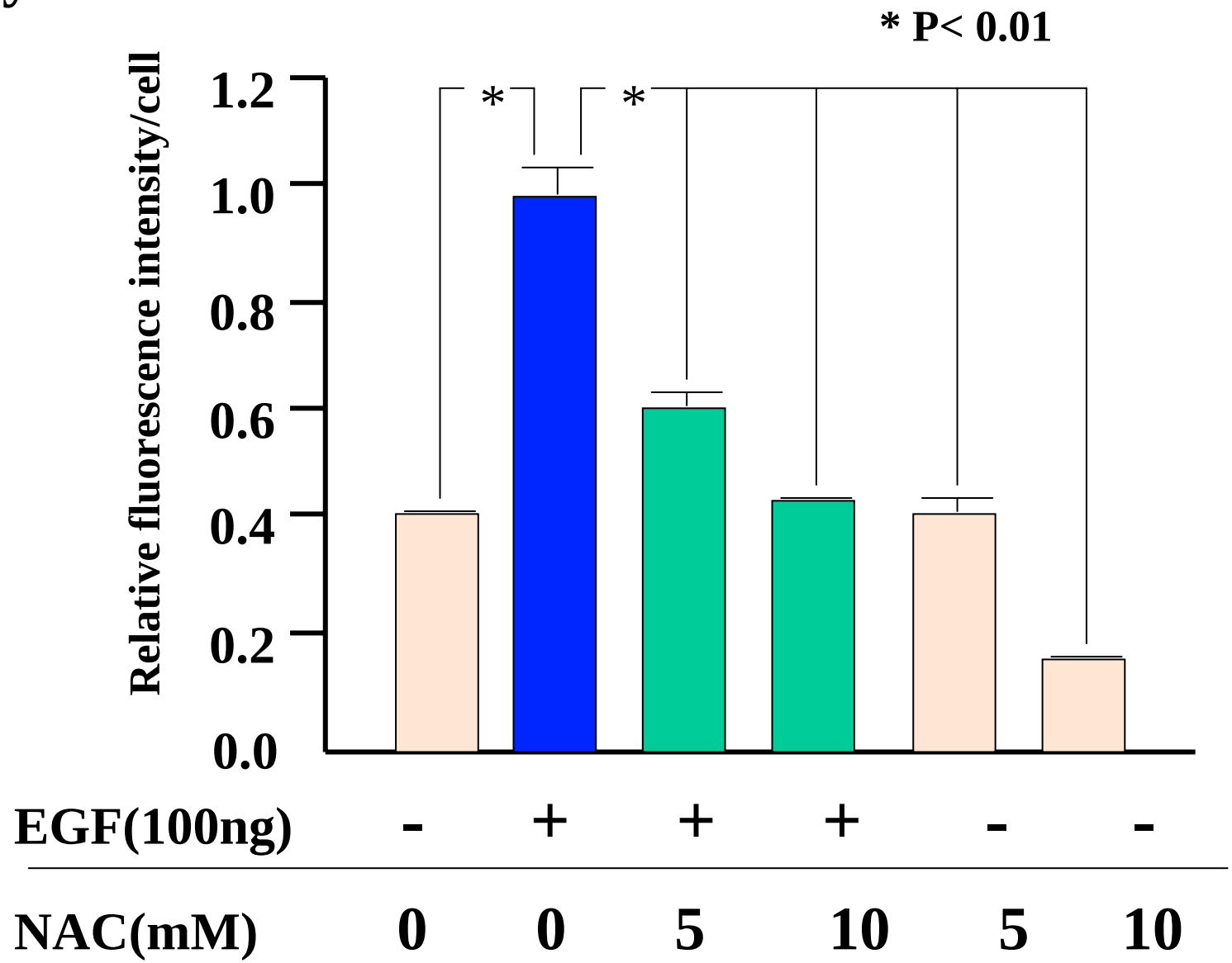
(Levels of glutathione peroxidase activity)



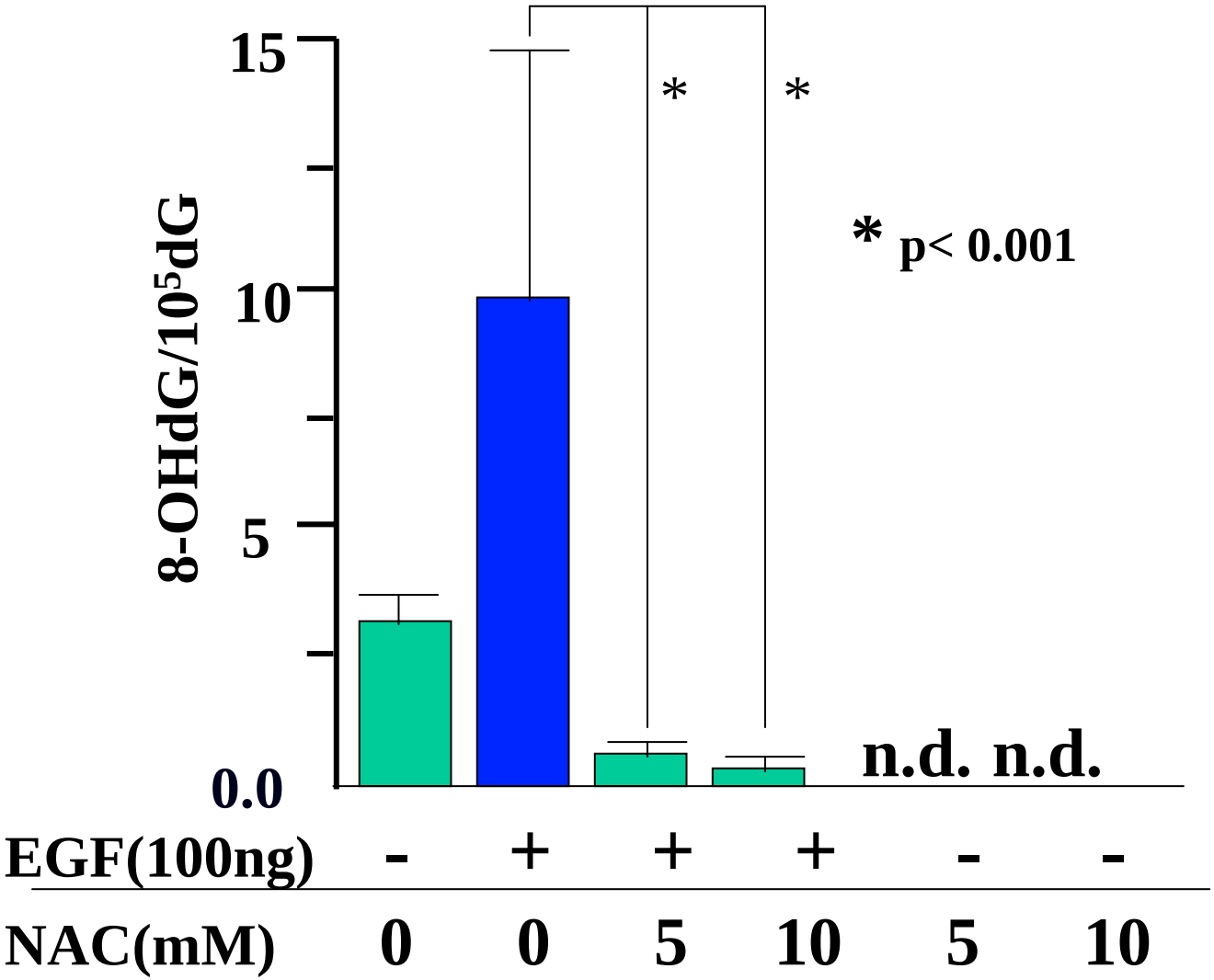
Levels of 8-OHdG in ER-1 cells treated with EGF and /or sodium selenite (Se)



Inhibition of EGF-induced intracellular peroxides by NAC



Levels of 8-OHdG in ER-1 cells treated with EGF and /or N-acetylcysteine (NAC)



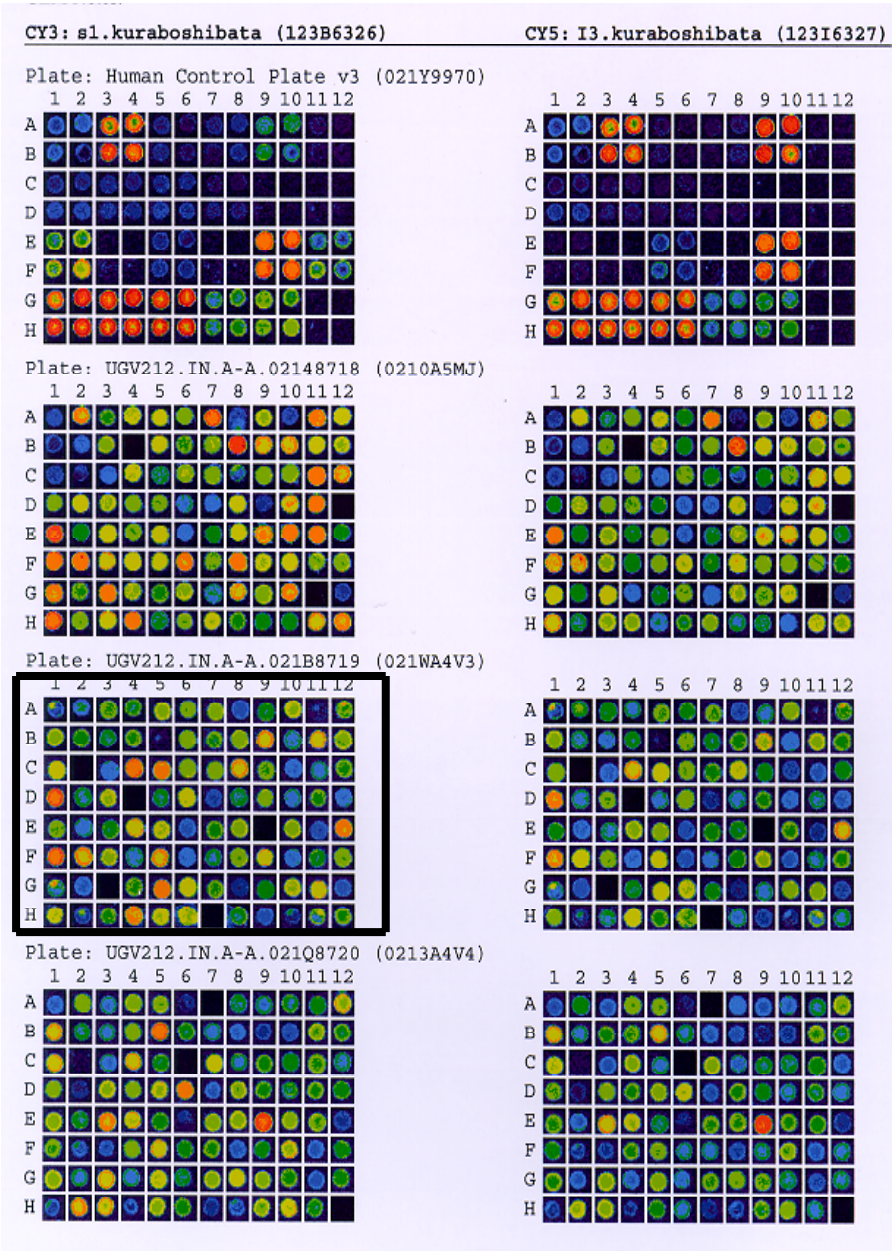
EGF (-) Se (-)	EGF (+) Se (-)	EGF (-) Se (+)	EGF (+) Se (+)

EGF(100ng/ml) +/- Sodium Se(100ng/ml) 1 mo

Tumorigenicity of ER-1 cells treated with EGF in the presence or absence of NAC or Selenite

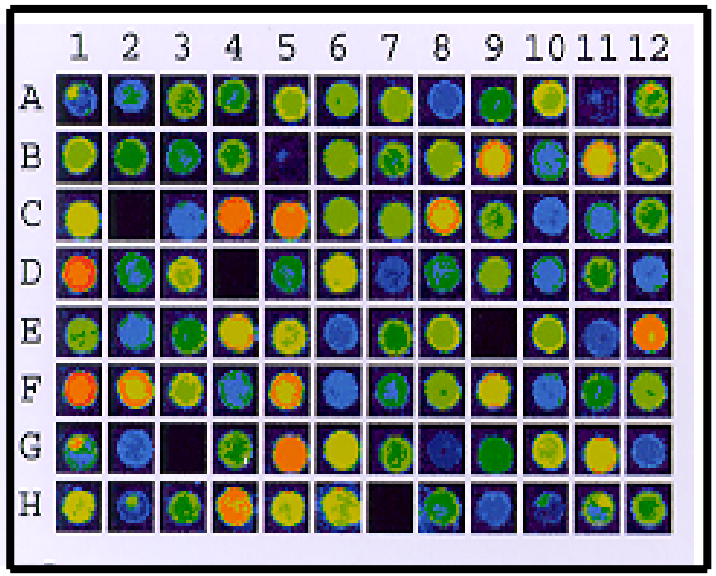
Treatment			Tumor take
EGF ng/ml	NAC mM	Selenite ng/ml	
0	0		0/6
100	0		6/6
100	5		1/6
100	10		1/6
0	5		0/5
0	10		0/5
0		0	0/6
100		0	6/6
100		1	6/6
100		10	2/6
100		100	1/6
0		1	0/6
0		10	1/6
0		100	1/6

Micro-Array Analysis ---- Comparison Between S-1 and I-3 cells



S-1 **I-3**

CY3(Red) **CY5(Green)**



Comparison of mRNA Expression between S-1 and I-3 cells

matrix metalloproteinase 1 (interstitial collagenase)

proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional protease 2)

heparin-binding growth factor binding protein

keratin 13

caveolin 1, caveolae protein, 22kD

protein kinase, cAMP-dependent, catalytic, alpha

matrilin 2

ESTs

serine/threonine kinase 15

annexin A1

KIAA0069 protein

sperm specific antigen 2

ataxia-telangiectasia group D-associated protein

centromere protein A (17kD)

cadherin 13, H-cadherin (heart)

karyopherin alpha 2 (RAG cohort 1, importin alpha 1)

integrin, alpha 6

EGF-containing fibulin-like extracellular matrix protein 1

spermidine/spermine N1-acetyltransferase

M-phase phosphoprotein 4

MHC class I region ORF

ESTs, Moderately similar to ORF derived from protease and integrase coding regions [H.sapiens]

requiem, apoptosis response zinc finger gene

Ste-20 related kinase

transcription factor AP-2 alpha (activating enhancer-binding protein 2 alpha)

high-mobility group (nonhistone chromosomal) protein 2

keratin 6B

matrix metalloproteinase 13 (collagenase 3)

Incyte EST

coagulation factor III (thromboplastin, tissue factor)

contactin 1

serine/threonine kinase 17a (apoptosis-inducing)

low density lipoprotein receptor (familial hypercholesterolemia)

laminin, gamma 1 (formerly LAMB2)

synaptophysin-like protein

butyrophilin, subfamily 3, member A2

Genome_Systems

IncyteGenomics

Fab: 7/26/2000 Hyb: 10/13/2000 Scan: 10/13/2000 Analyze: 10/13/2000 Ready: 10/17/2000

GEM Notes:

CY3: si10.kurabo.shibata (123I8109) **CY5: siegf10.kurabo.shibata (123X8110)**

Plate: Human Control Plate v3 (021Y9970)

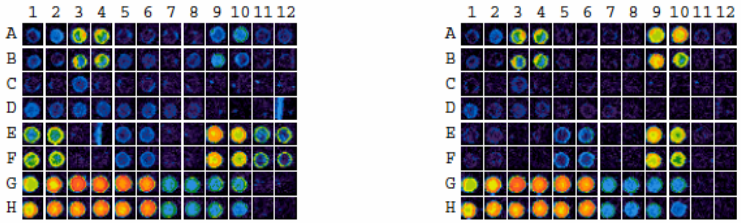
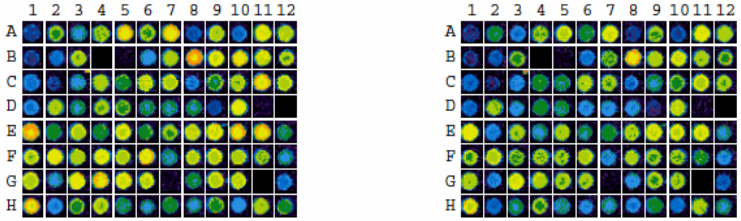


Plate: UGV2.27.IN.A-A.02148718 (0210A5MJ)



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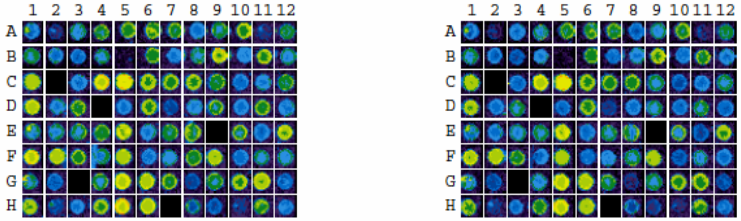
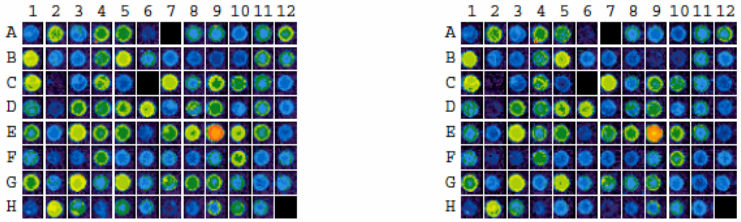


Plate: UGV2.14.IN.A-A.021Q8720 (0213A4V4)



Genome_Systems

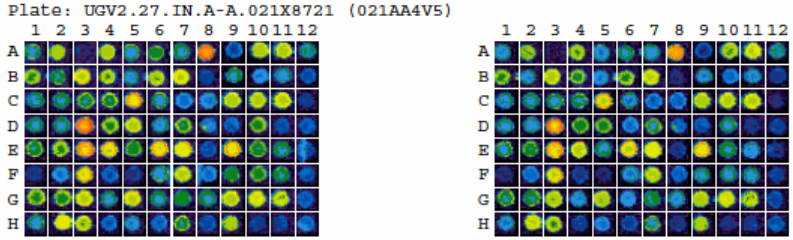
IncyteGenc

Fab: 7/26/2000 Hyb: 10/13/2000 Scan: 10/13/2000 Analyze: 10/13/2000 Ready: 10/17/2000

GEM Notes:

CY3: si10.kurabo.shibata (123I8109) **CY5: siegf10.kurabo.shibata (123X8110**

Plate: UGV2.27.IN.A-A.021X8721 (021AA4V5)



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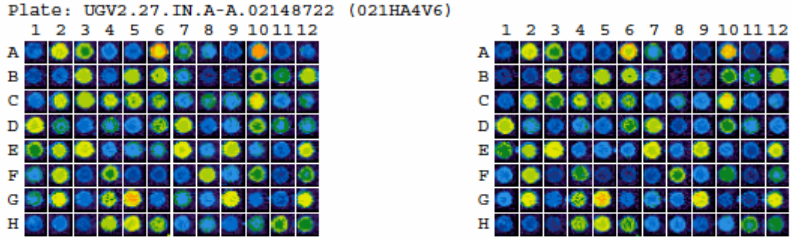

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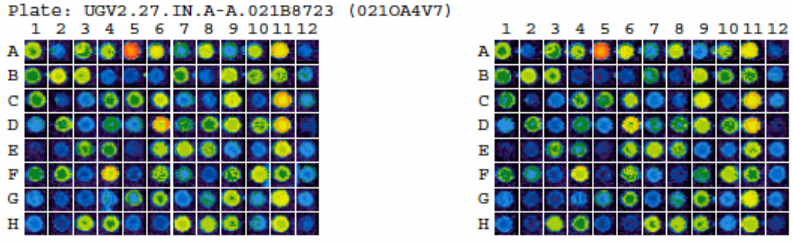

Plate: UGV2.27.IN.A-A.021I8724 (021VA4V8)