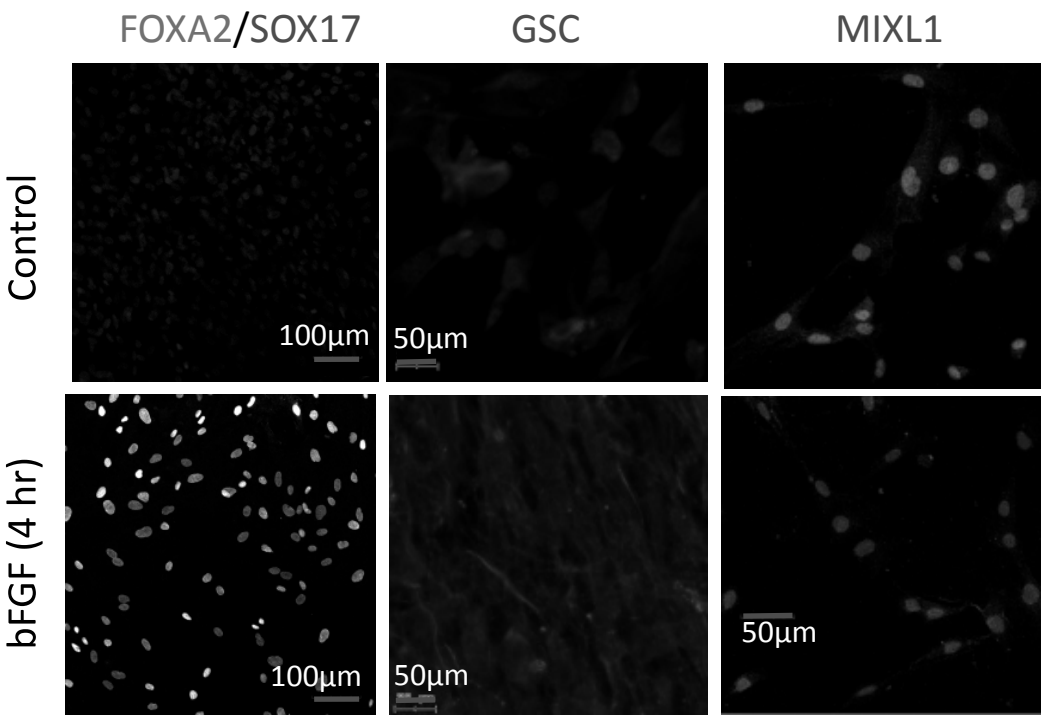
FIG. 1A (first column)

FIG. 1B (middle column)

FIG. 1C (right column)



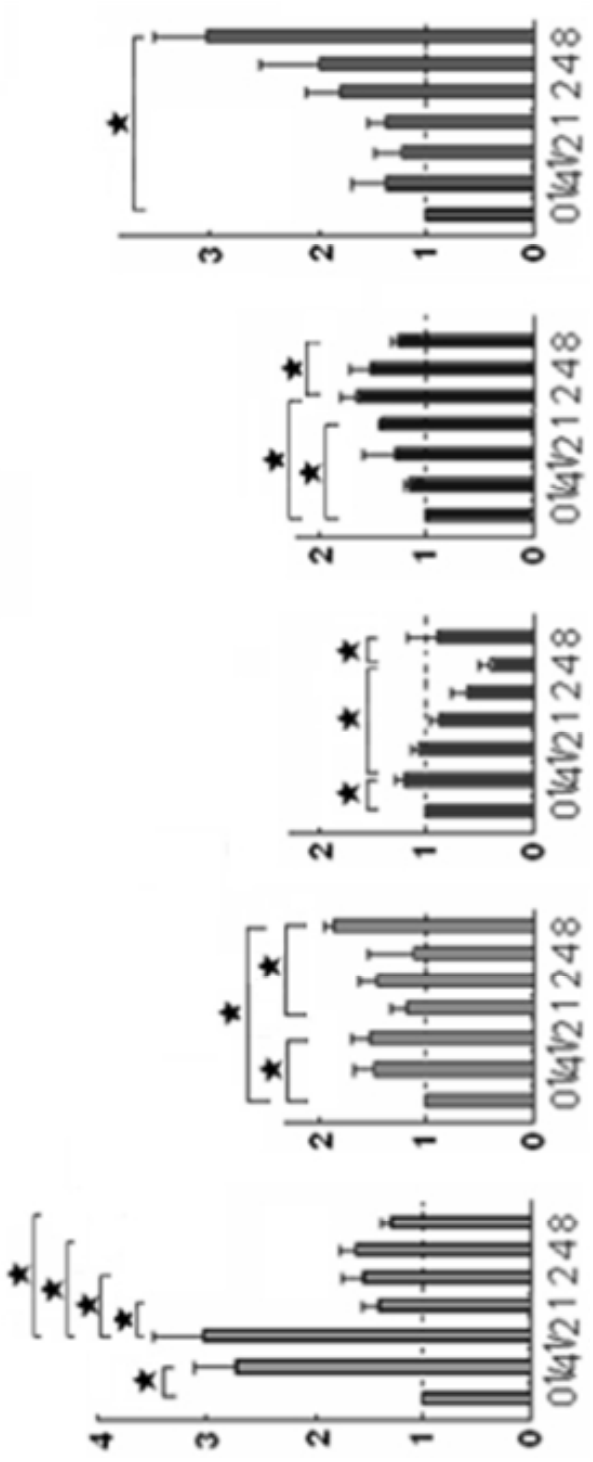

Relative protein expression
Time after induction (hr)
0 1/4 1/2 1 2 4 8

FIG. 1D

FIG. 1E

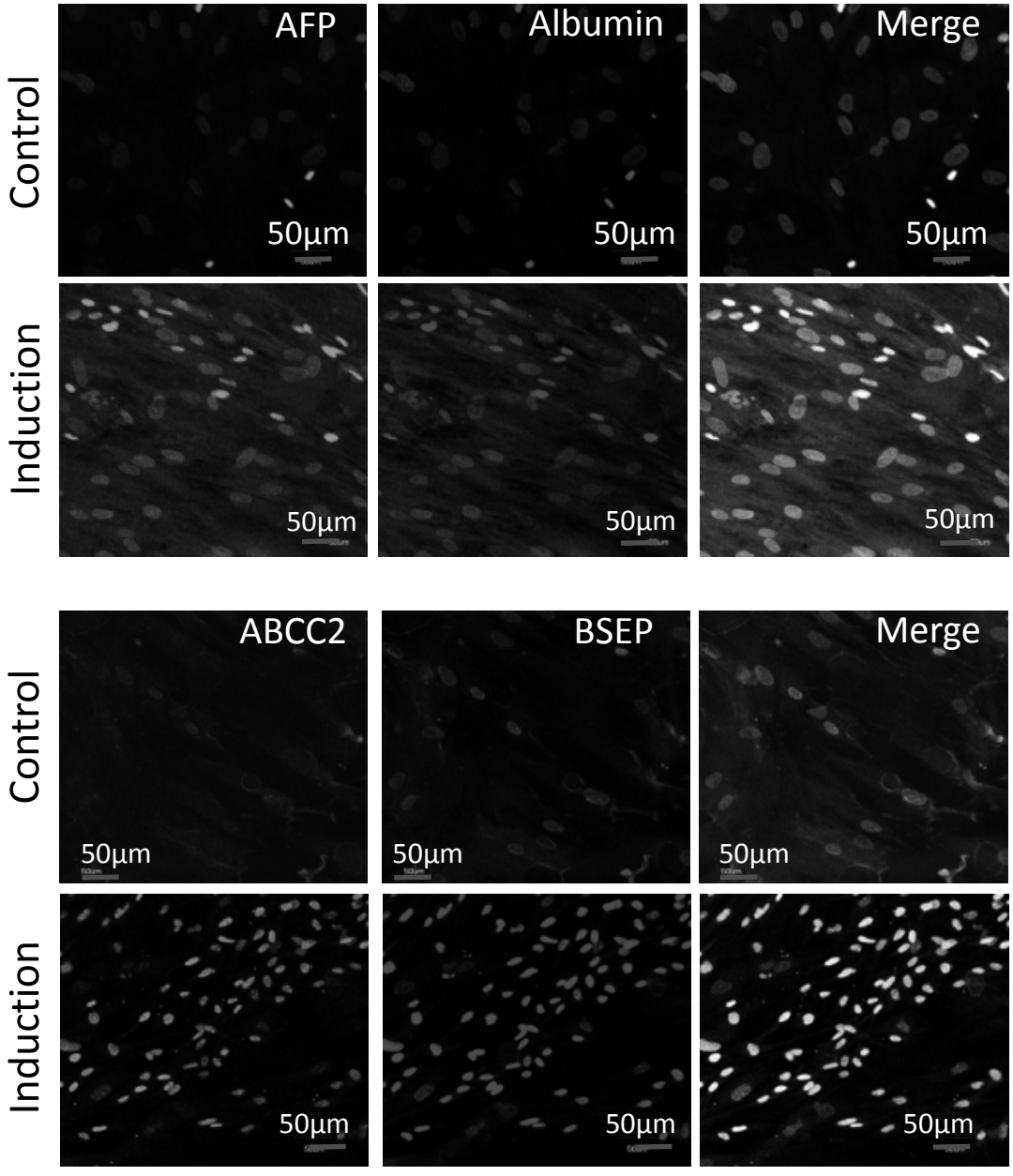

Control
AFP
Albumin
Merge
50μm
50μm
50μm
Induction
50μm
50μm
50μm
Control
ABCC2
BSEP
Merge
50μm
50μm
50μm
Induction
50μm
50μm
50μm

FIG. 1F

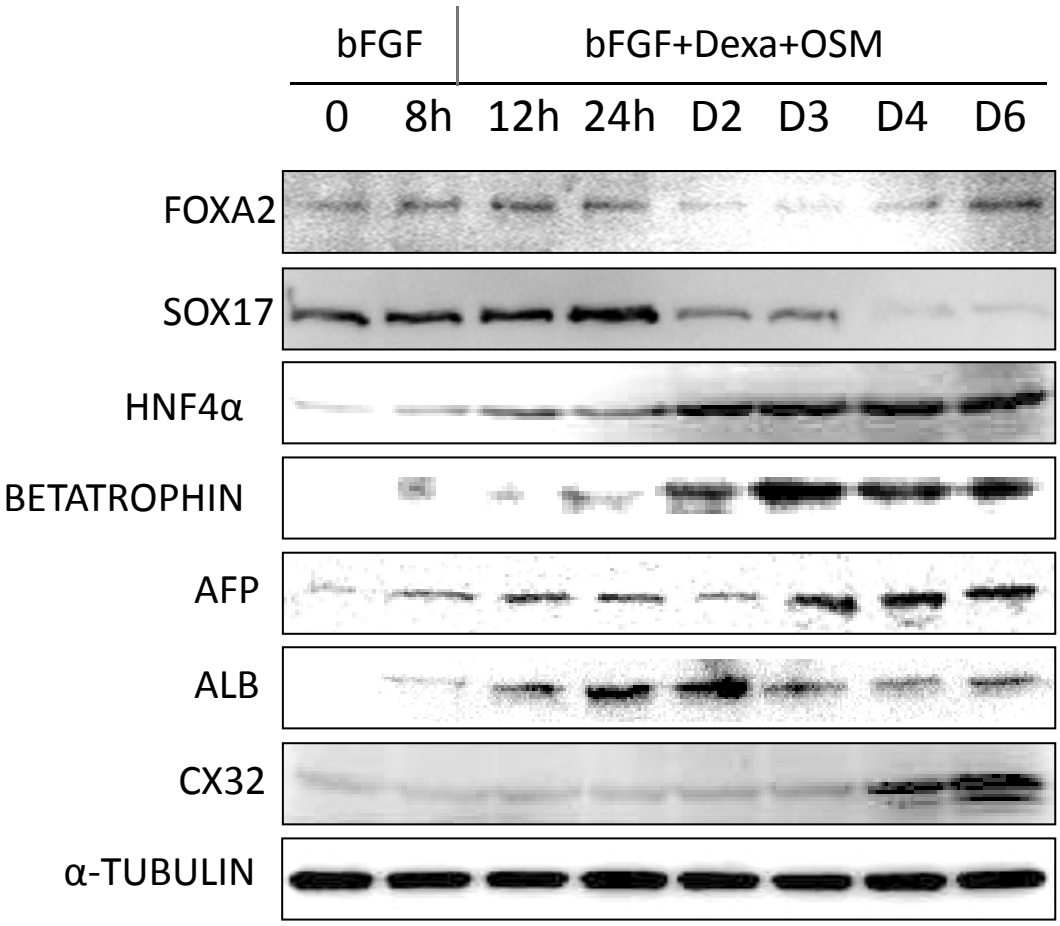

bFGF
bFGF+Dexa+OSM
0
8h
12h
24h
D2
D3
D4
D6
FOXA2
SOX17
HNF4α
BETATROPHIN
AFP
ALB
CX32
α-TUBULIN

FIG. 2A

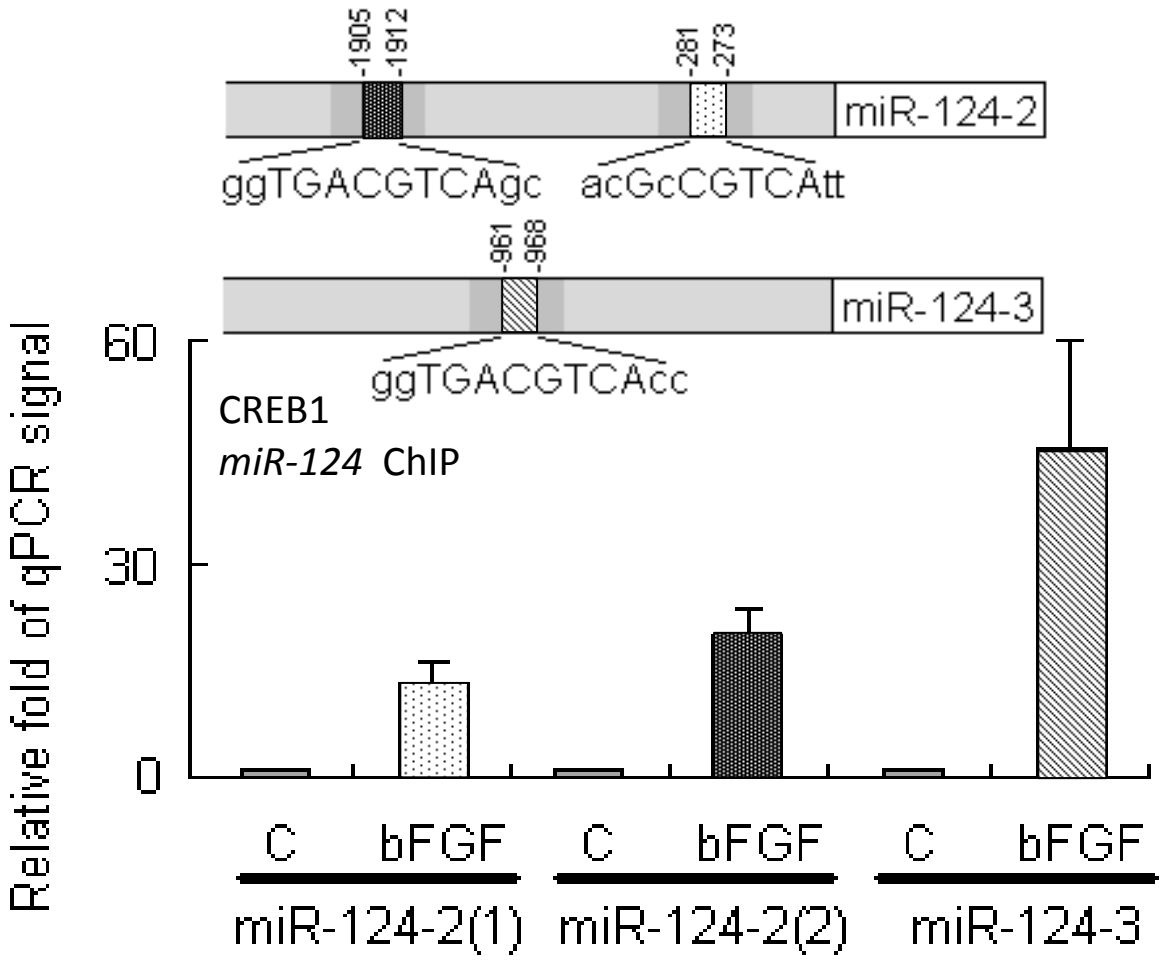

-1905
-1912
-281
-273
miR-124-2
ggTGACGTCAgc
acGcCGTCAtt
-961
-968
miR-124-3
ggTGACGTCAcc
CREB1
miR-124 ChIP
Relative fold of qPCR signal
60
30
0
C
bFGF
C
bFGF
C
bFGF
miR-124-2(1)
miR-124-2(2)
miR-124-3

FIG. 2B

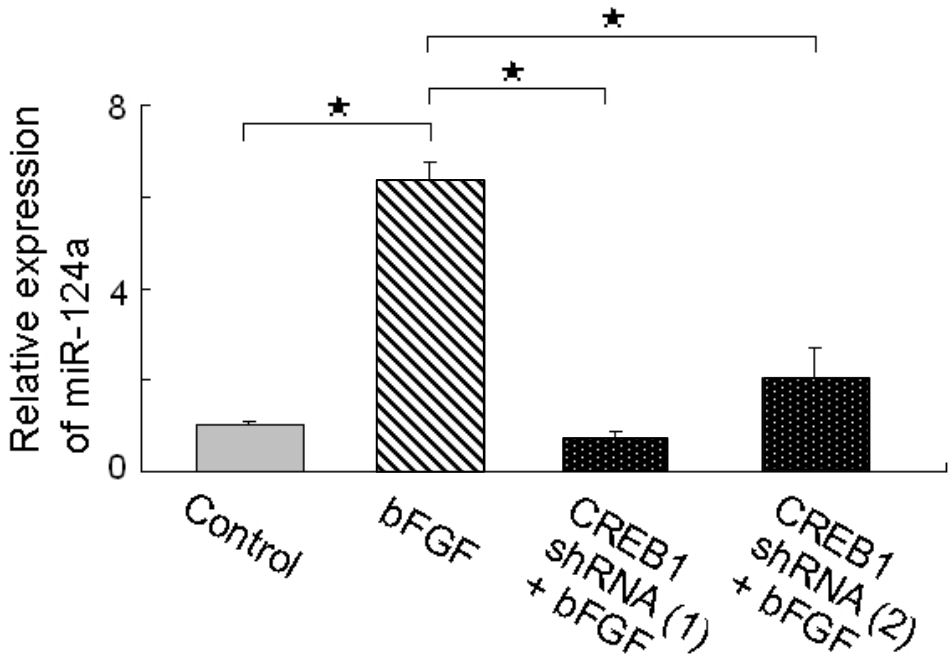

Relative expression of miR-124a
8
4
0
Control
bFGF
CREB1 shRNA (1) + bFGF
CREB1 shRNA (2) + bFGF

FIG. 2C

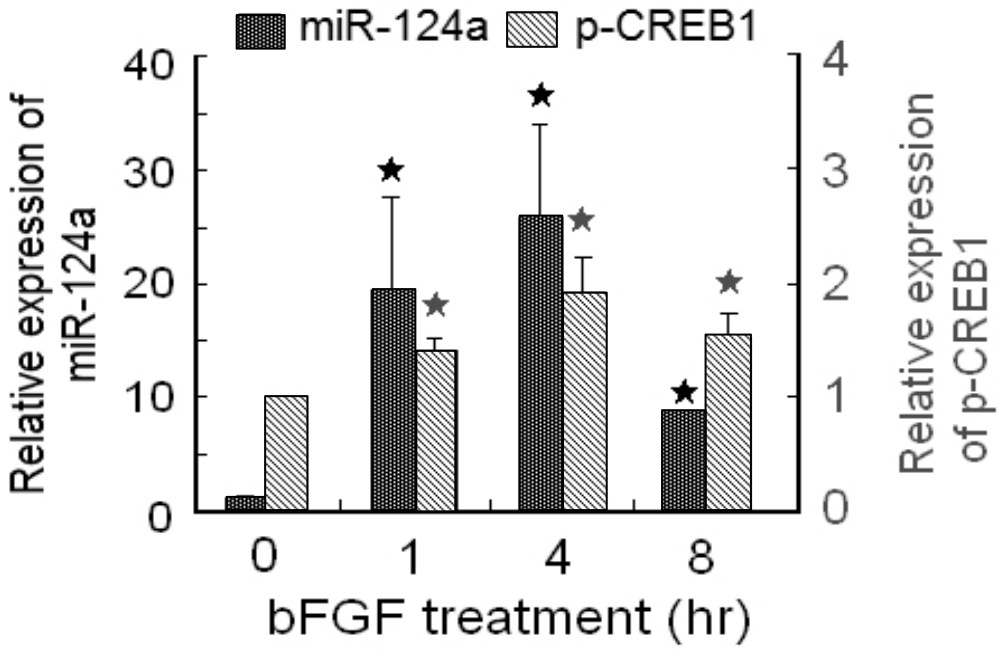

miR-124a
p-CREB1
Relative expression of miR-124a
Relative expression of p-CREB1
40
30
20
10
0
4
3
2
1
0
0
1
4
8
bFGF treatment (hr)

FIG. 2D

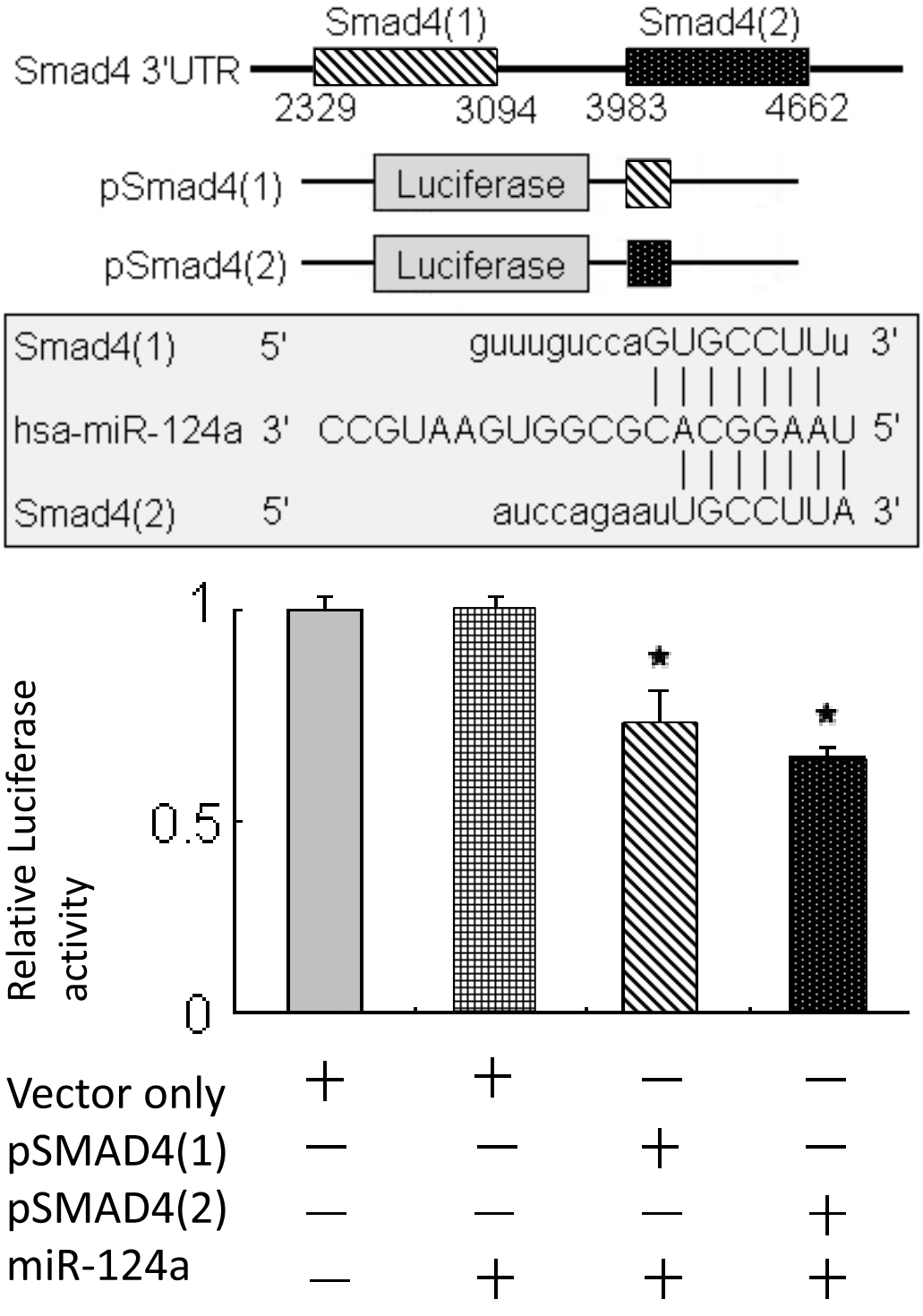

Smad4(1)
Smad4(2)
Smad4 3'UTR
2329
3094
3983
4662
pSmad4(1)
Luciferase
pSmad4(2)
Luciferase
Smad4(1) 5' guuuguccaGUGCCUUu 3'
hsa-miR-124a 3' CCGUAAGUGGCGCACGGAAU 5'
Smad4(2) 5' auccagaauUGCCUUA 3'
Relative Luciferase activity
1
0.5
0
Vector only + + − −
pSMAD4(1) − − + −
pSMAD4(2) − − − +
miR-124a − + + +

FIG. 2E

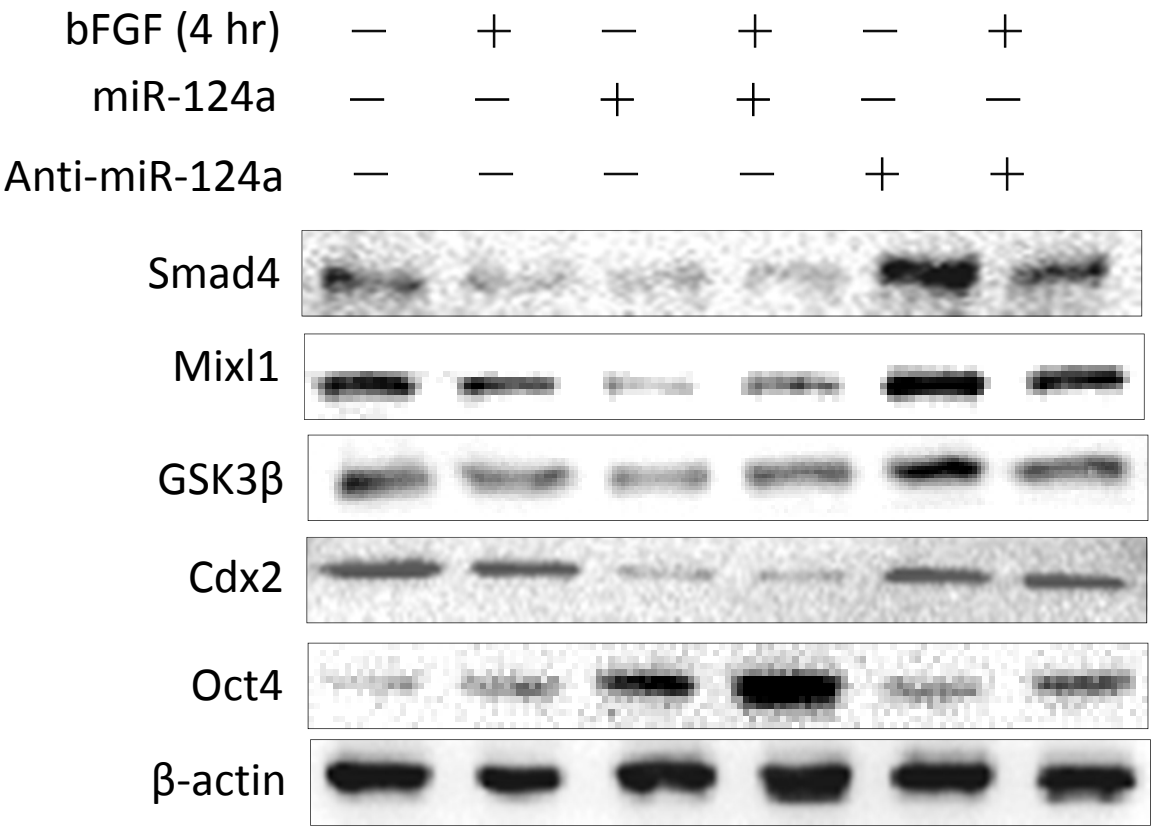

bFGF (4 hr) − + − + − +
miR-124a − − + + − −
Anti-miR-124a − − − − + +
Smad4
Mixl1
GSK3β
Cdx2
Oct4
β-actin

FIG. 2F

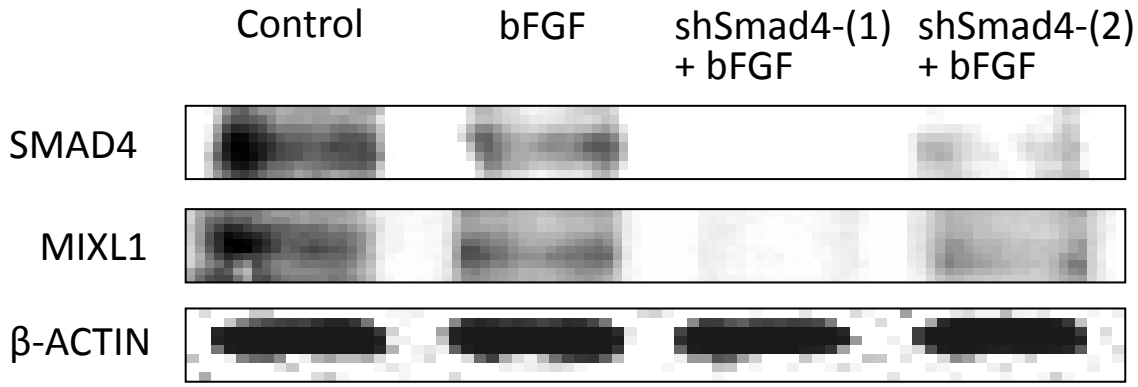

Control
bFGF
shSmad4-(1) + bFGF
shSmad4-(2) + bFGF
SMAD4
MIXL1
β-ACTIN

FIG. 2G

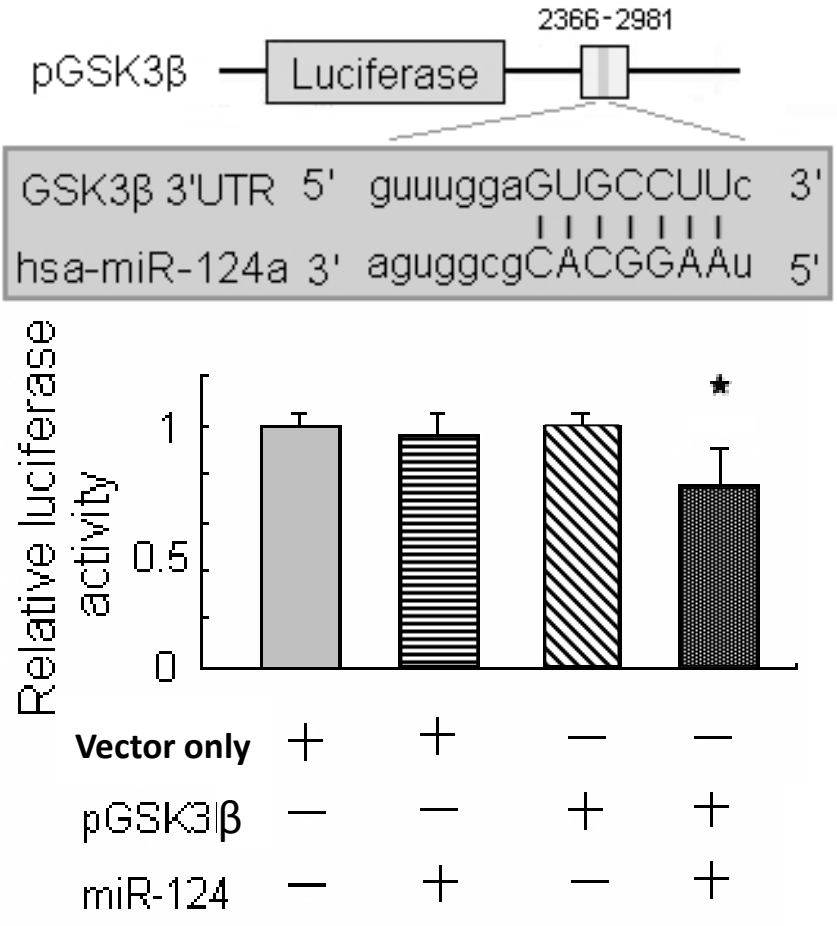

2366-2981
pGSK3β
Luciferase
GSK3β 3'UTR 5' guuuggaGUGCCUUc 3'
hsa-miR-124a 3' aguggcgCACGGAAu 5'
Relative luciferase activity
1
0.5
0
*
Vector only + + − −
pGSK3β − − + +
miR-124 − + − +

FIG. 2H

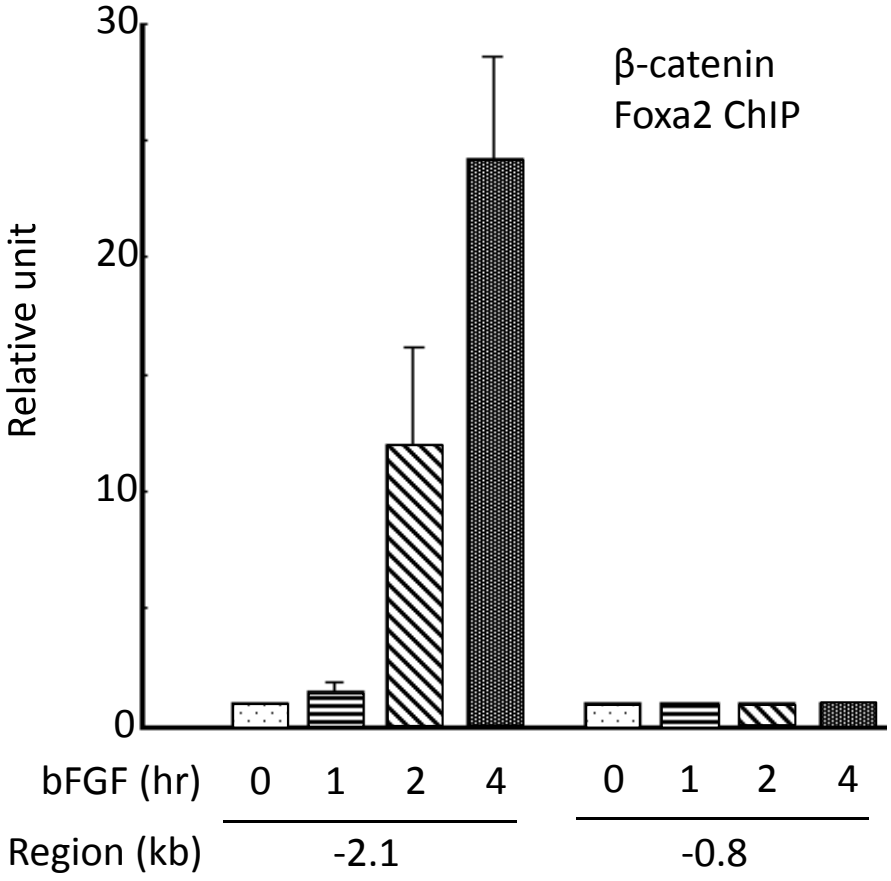

30
20
10
0
Relative unit
β-catenin
Foxa2 ChIP
bFGF (hr)
0
1
2
4
0
1
2
4
Region (kb)
-2.1
-0.8

FIG. 2I

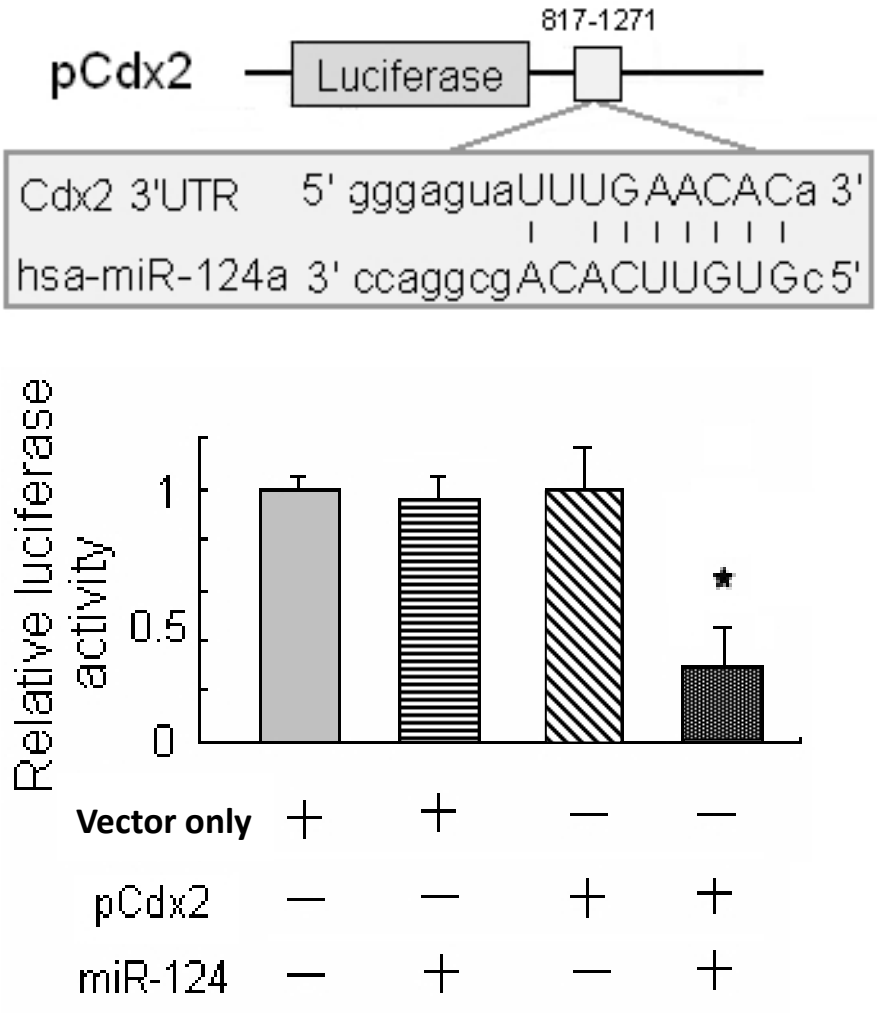

817-1271
pCdx2
Luciferase
Cdx2 3'UTR 5' gggaguaUUUGAACACAa 3'
hsa-miR-124a 3' ccaggcgACACUUGUGc 5'
Relative luciferase activity
1
0.5
0
*
Vector only + + − −
pCdx2 − − + +
miR-124 − + − +

FIG. 2J

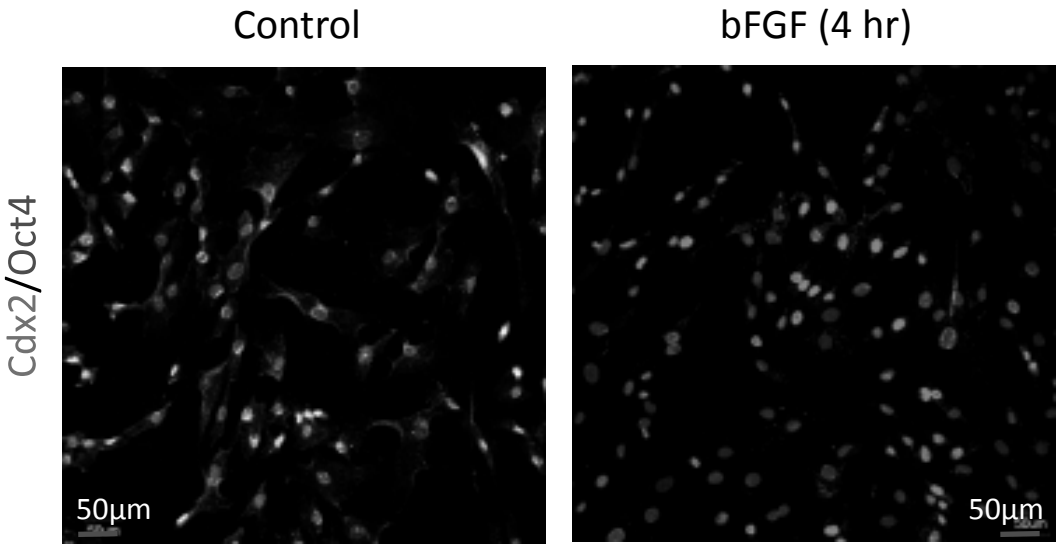


FIG. 2K

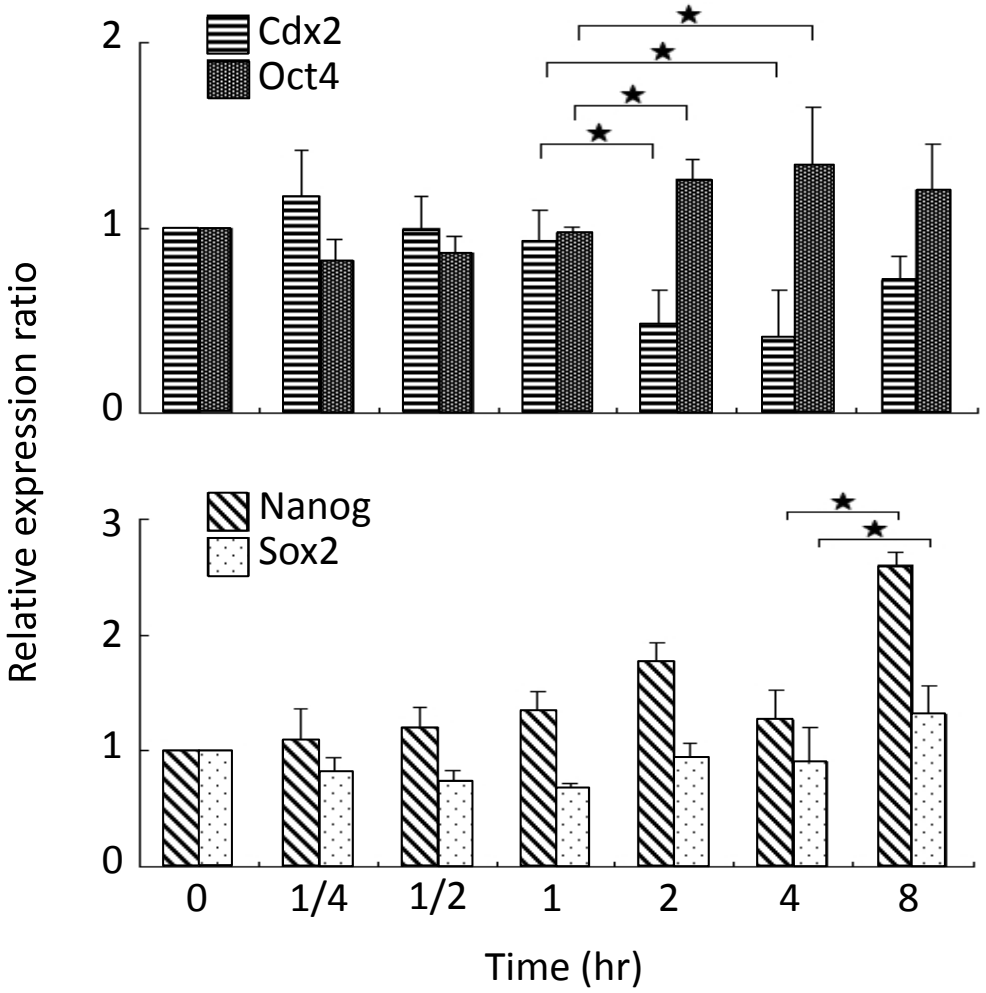

Cdx2
Oct4
Nanog
Sox2
Relative expression ratio
Time (hr)
0
1/4
1/2
1
2
4
8

FIG. 2L

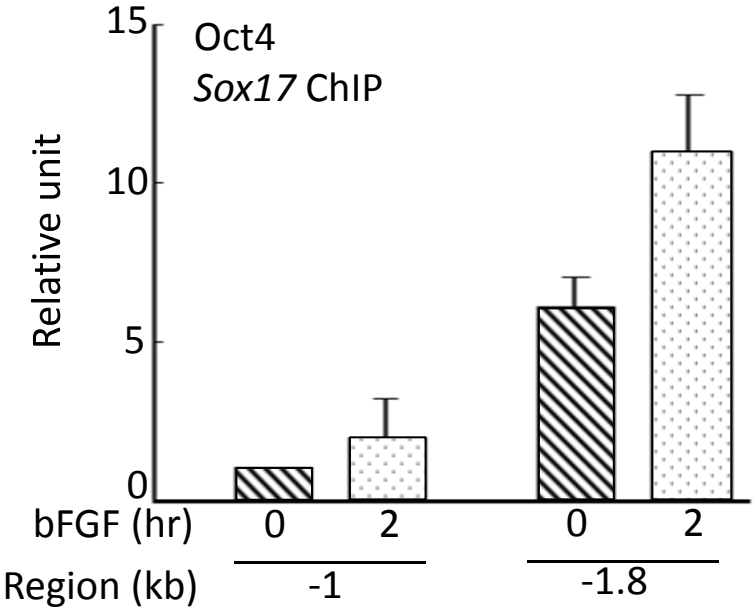

15
10
5
0
Relative unit
Oct4
Sox17 ChIP
bFGF (hr)
0
2
0
2
Region (kb)
-1
-1.8

FIG. 2M

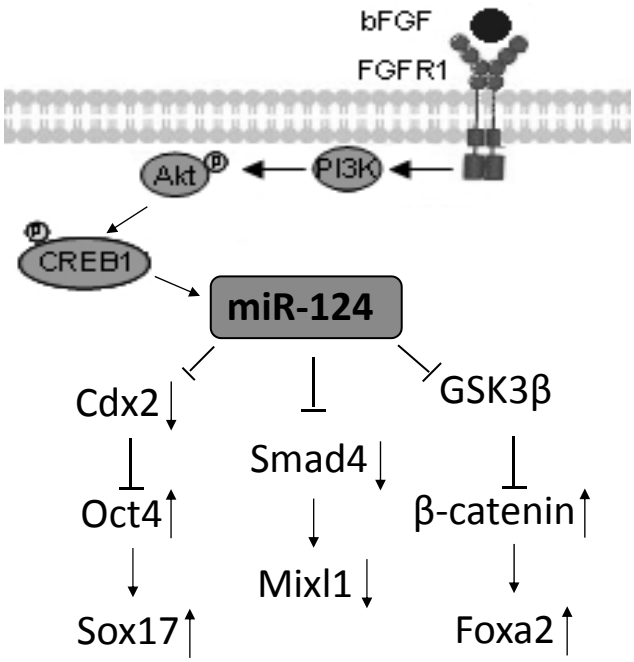

bFGF
FGFR1
Akt
P
PI3K
CREB1
P
miR-124
Cdx2
Smad4
GSK3β
Oct4
β-catenin
Mixl1
Sox17
Foxa2

FIG. 3A

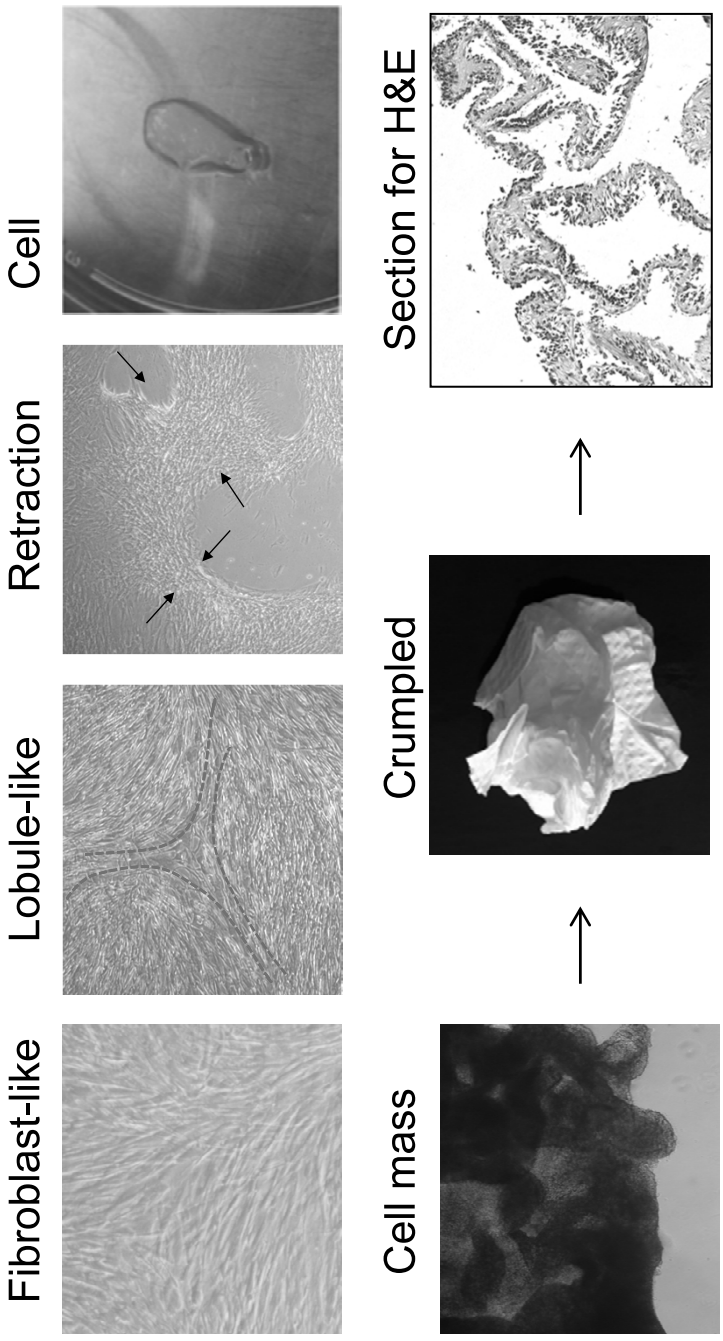

Cell
Retraction
Lobule-like
Fibroblast-like
Section for H&E
Crumpled
Cell mass

FIG. 3B

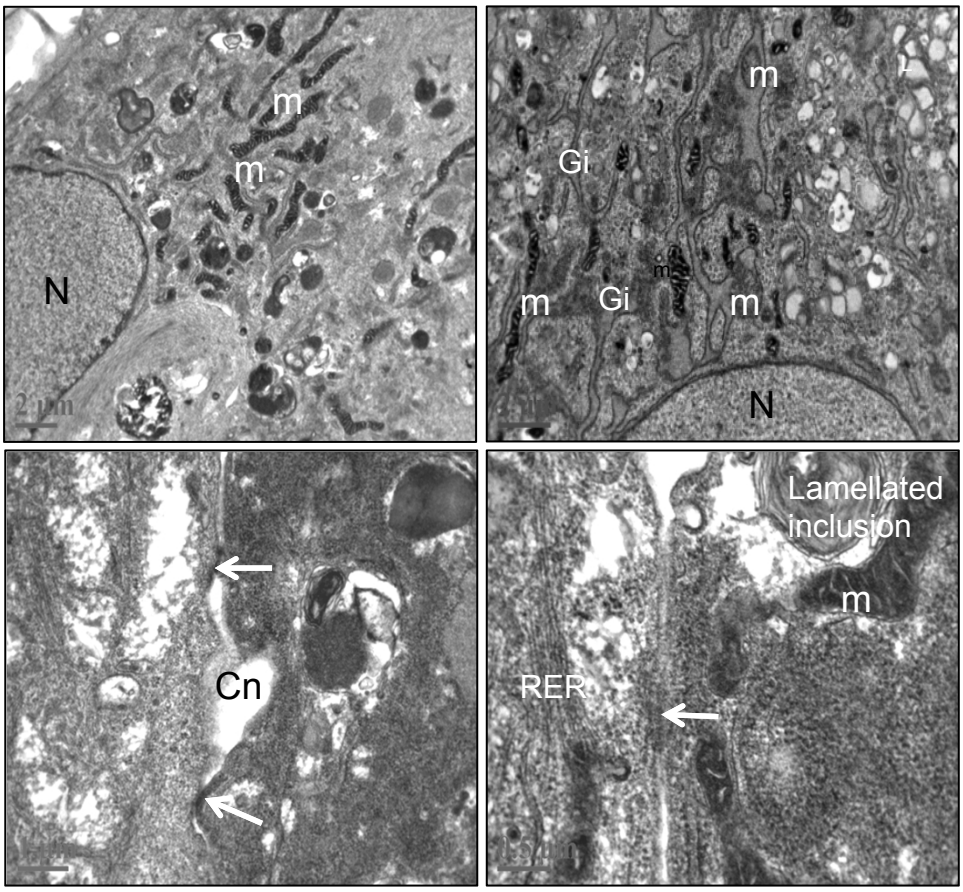


FIG. 3C

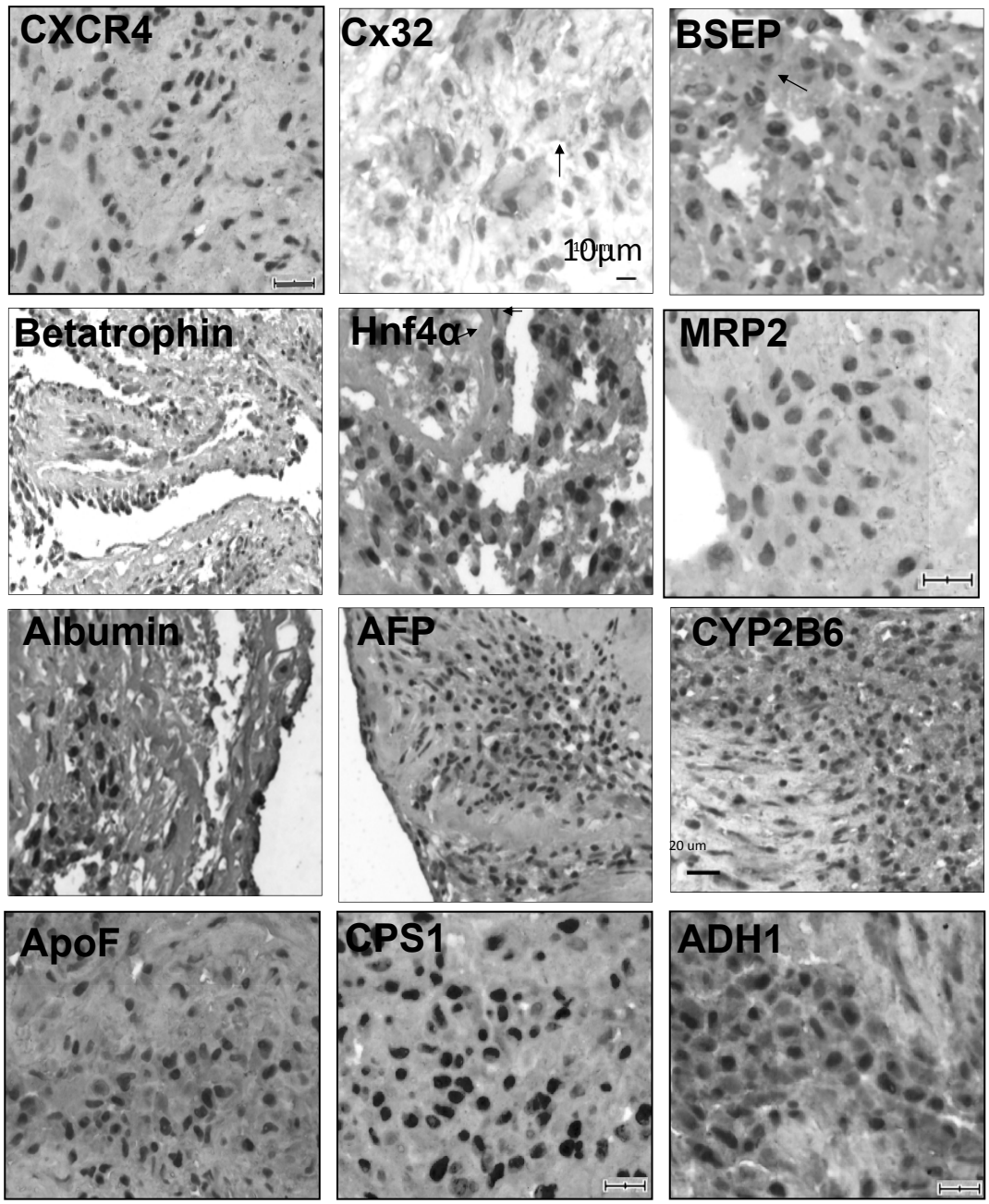

CXCR4
Cx32
10μm
BSEP
Betatrophin
Hnf4α
MRP2
Albumin
AFP
CYP2B6
20 um
ApoF
CPS1
ADH1

FIG. 4A

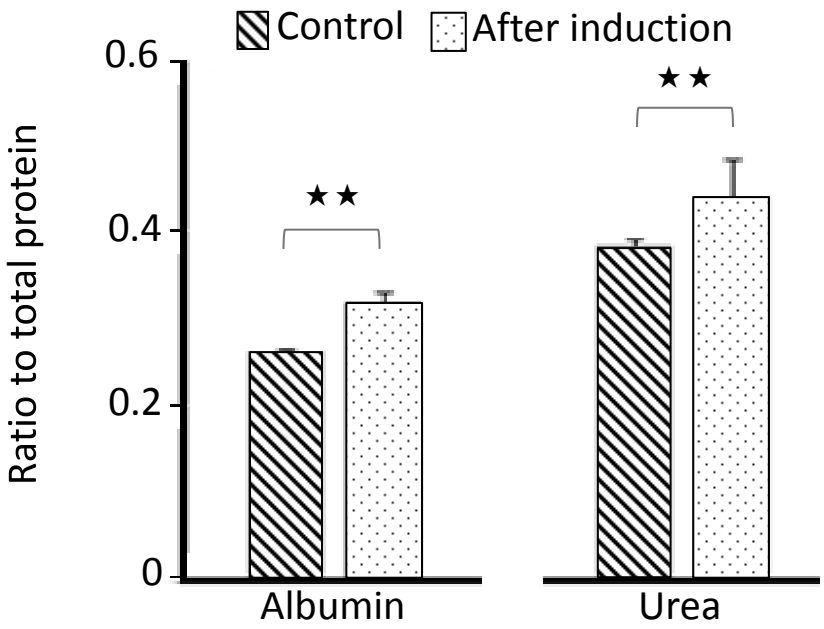

Control
After induction
Ratio to total protein
0.6
0.4
0.2
0
★★
★★
Albumin
Urea

FIG. 4B

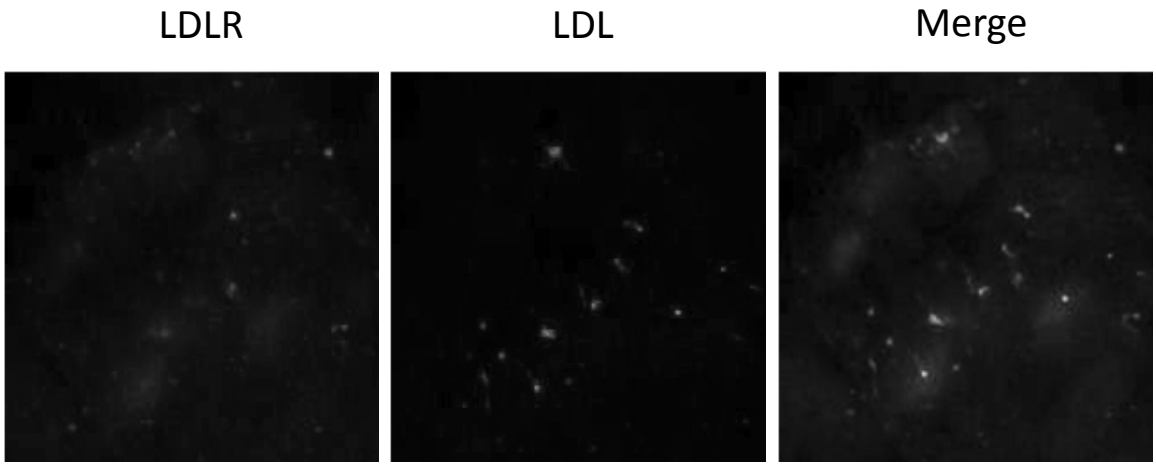

LDLR
LDL
Merge

FIG. 4C

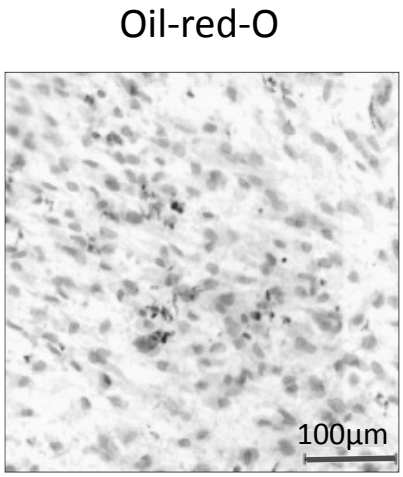

Oil-red-O
100μm

FIG. 4D

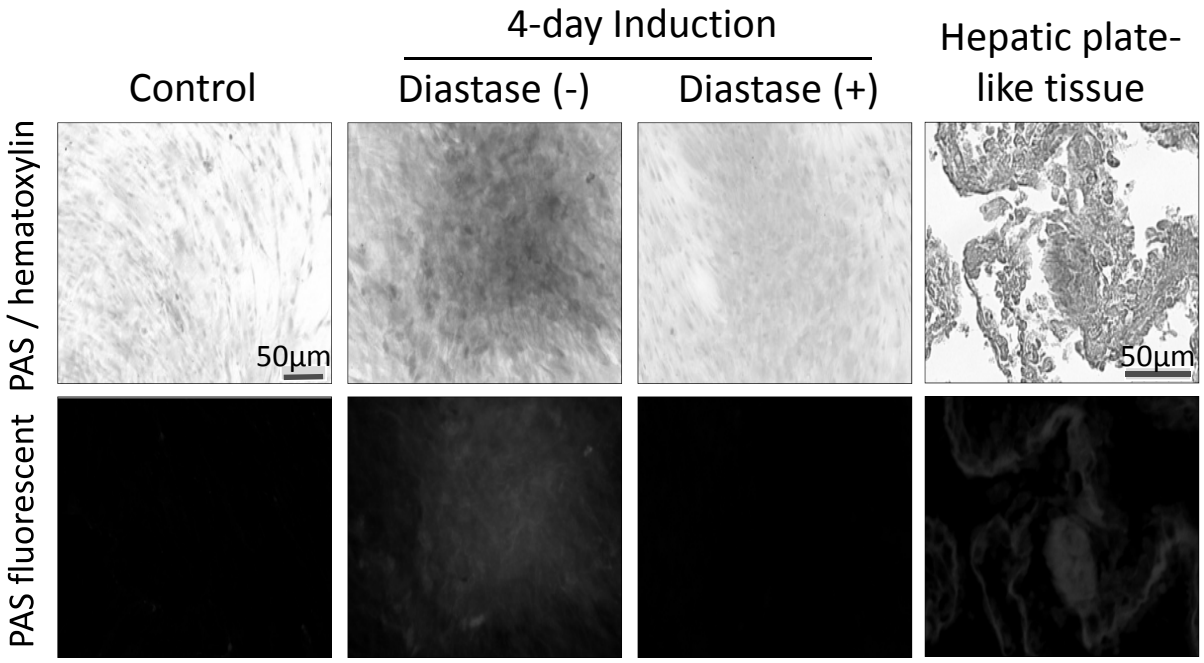

4-day Induction
Control
Diastase (-)
Diastase (+)
Hepatic plate-like tissue
PAS / hematoxylin
PAS fluorescent
50μm
50μm

FIG. 5A

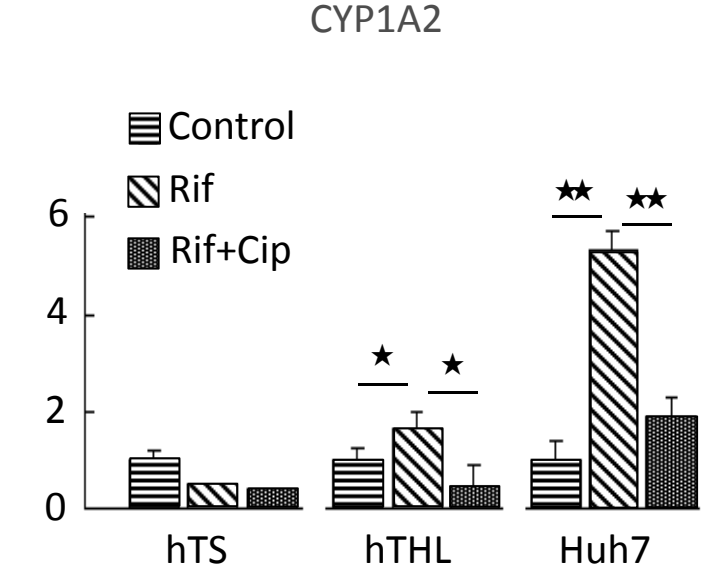

CYP1A2
Control
Rif
Rif+Cip
6
4
2
0
★
★
★★
★★
hTS
hTHL
Huh7

FIG. 5B

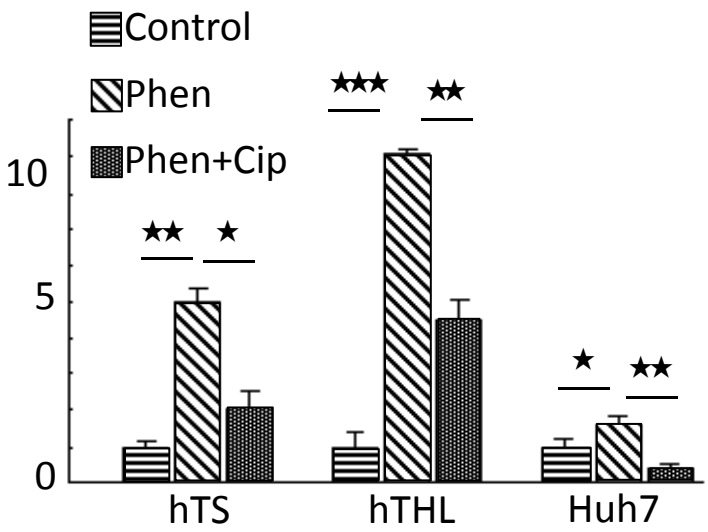

CYP2B6
Control
Phen
Phen+Cip
10
5
0
★★
★
★★★
★★
★
★★
hTS
hTHL
Huh7

FIG. 5C

CYP2C8

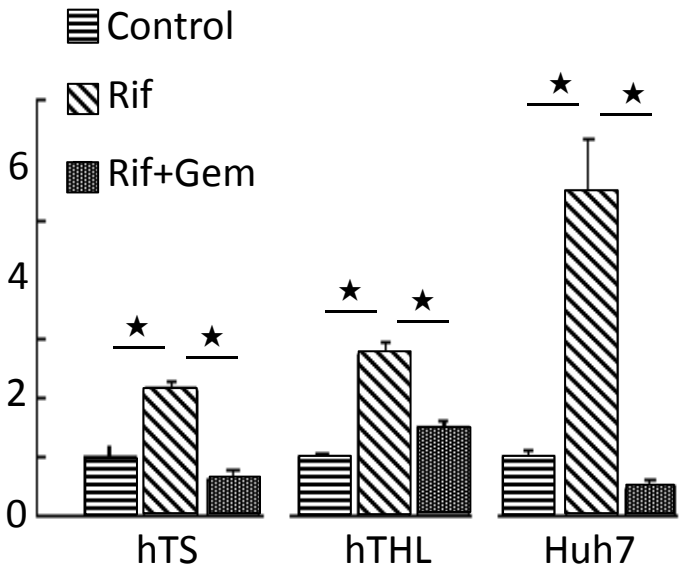


FIG. 5D

CYP2C9

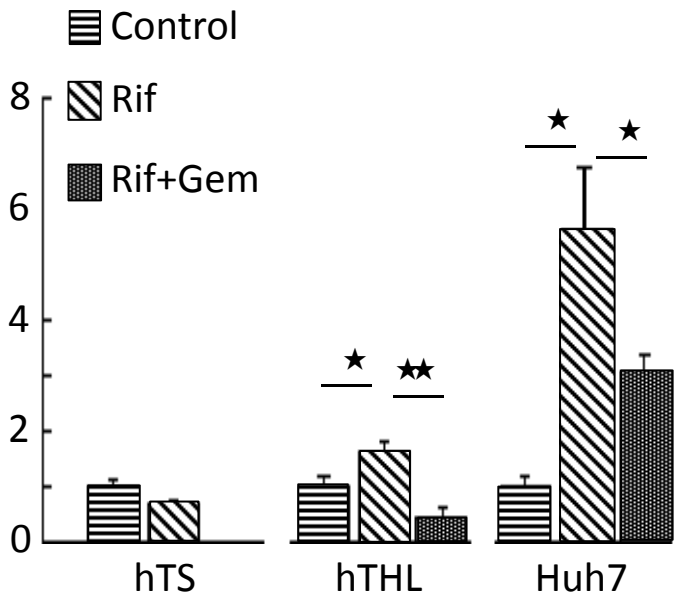


FIG. 5E

CYP2C19

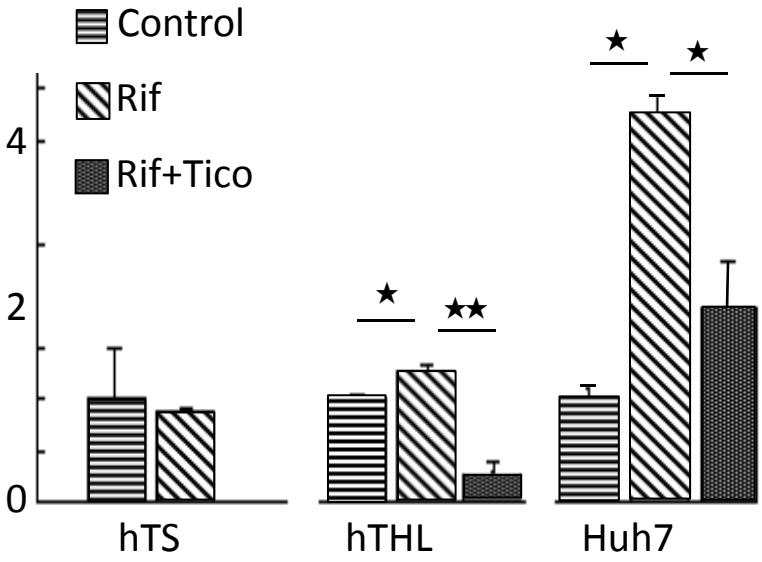


FIG. 5F

CYP2D6

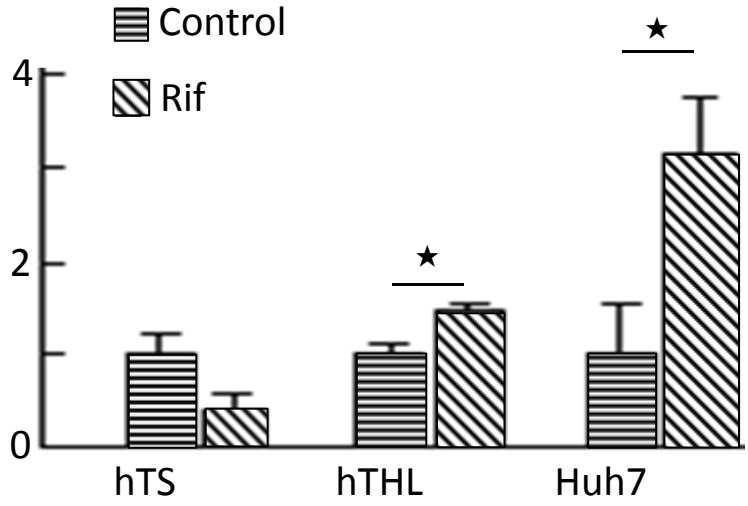


FIG. 5G

CYP2E1

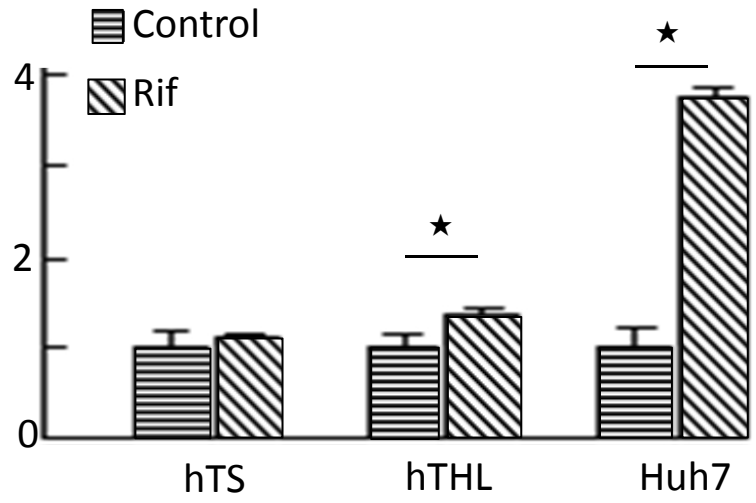


FIG. 5H

CYP3A4

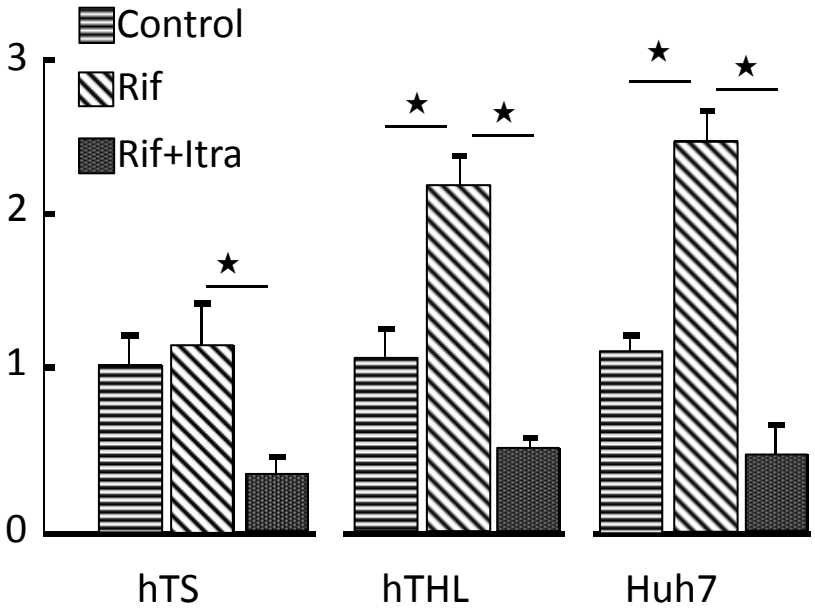


FIG. 5I

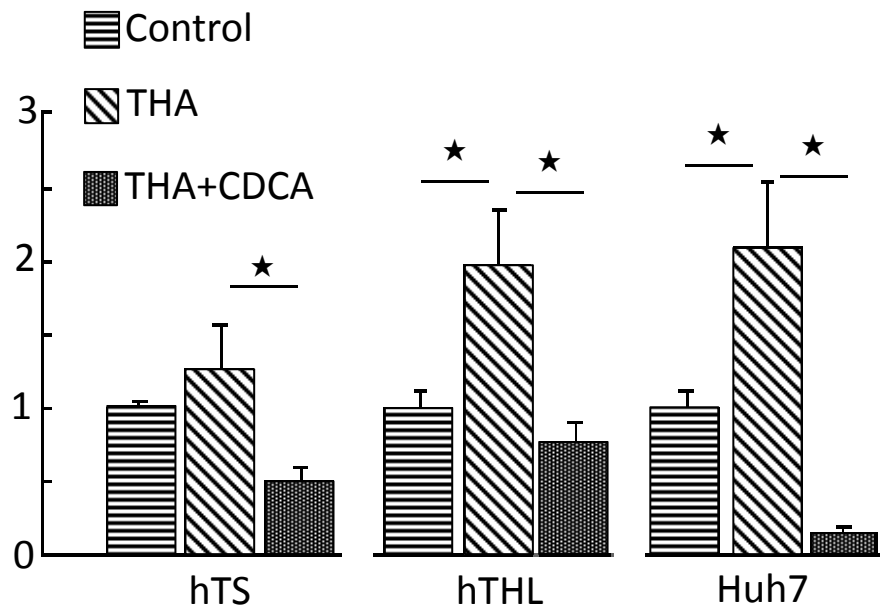


FIG. 6

Function \ Stage	Gene	Primitive cells (< 8 hr)	Hepatic endoderm lineages (8 hr to day-1)	Hepatoblasts (day 2-4)	Fetal and adult hepatocyte-like cells (≧ day-4)
DE	CXCR4	+++	+		+
	FOXA2	++++	+		+
	SOX17	++++	++		+
	HHEX	++	+	+	+++
Secretion of bile salts	BSEP		+	++	++++
Thyroxin- and retinol-binding protein	TTR		++	++	+++
Proteins carrier synthesized in the liver	ALB		++	++	+++
Tyrosine catabolism	TAT		++	++	+++
Serine protease inhibitor	SERPINA1		++	++	+++
Bile acid biosynthesis	CYP7A1		++	++	++
Enzyme of glucose homeostasis	G6PC		+	+	+++
Hepatobiliary excretion	ABCC2		+	+	+++
Adipocyte differentiation	C/EBPβ		+	+	+++
Regulator of several hepatic genes	HNF1α		+	+	+++
Regulator of several hepatic genes	HNF4α		+	+	+++
Fetal α-fetoprotein precursor	AFP		+	+	++ ★
IL-6-mediated barrier protection	CK8		+	+	++
Gluconeogenesis	PCK2		+	+	++
Drug and steroid metabolism (phase I)	CYP2B6		+	+	++
Liver glucagon synthase	GYS2		+	+	++
Hepatic transcriptional activator	HNF6		+	+	+
Enzyme of urea cycle	CPS1		+	+	+
Ethanol catabolism (phase I)	ADH1C		+		+
Hepatic gap junction	CX32		+		+
Drug and steroid metabolism (phase I)	CYP3A4		+		+
Hepatocyte migration	PROX1		+		?
Tryptophan metabolism	TDO2		+	+	
Cholesterol transport regulator	APOF		+	+	
IL-6-mediated barrier protection	CK18			+	
Organization of bile duct	CK19			+	+
Pancreatic β-cell promoter, Lipid regulator	Betatrophin			+	+

Denotation: blank indicating expression <2-fold, +: >2-fold, ++: >10-fold, +++: >100-fold, and ++++: >1,000-fold ★: only at fetal and post-natal stages

FIG. 7

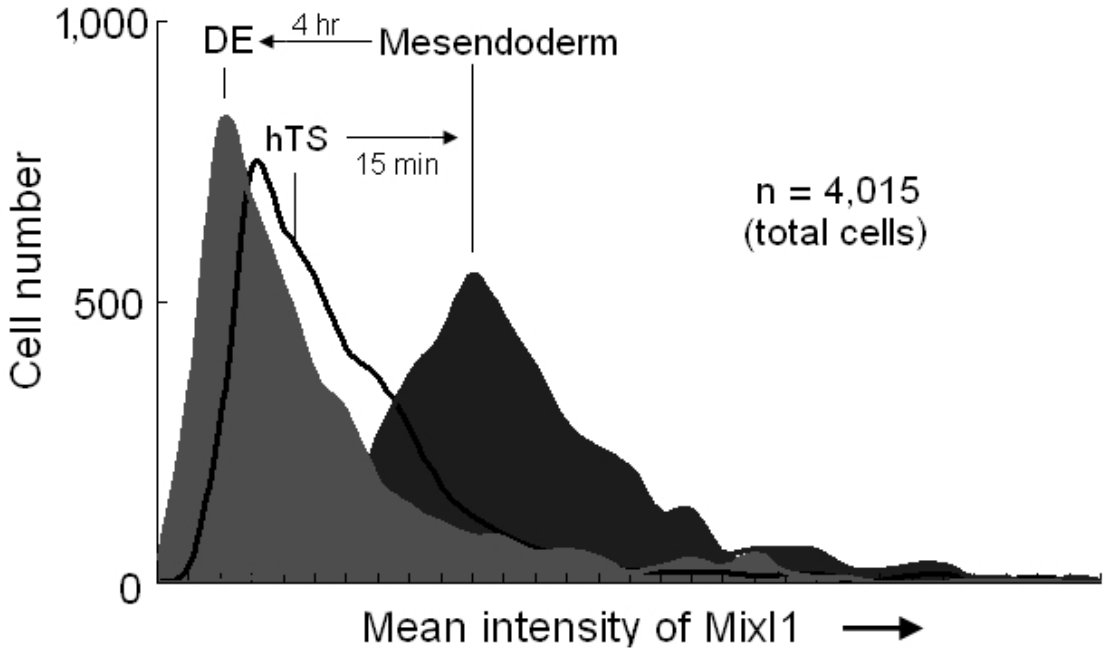

1,000
500
0
Cell number
DE
4 hr
Mesendoderm
hTS
15 min
n = 4,015
(total cells)
Mean intensity of Mixl1

FIG. 8A

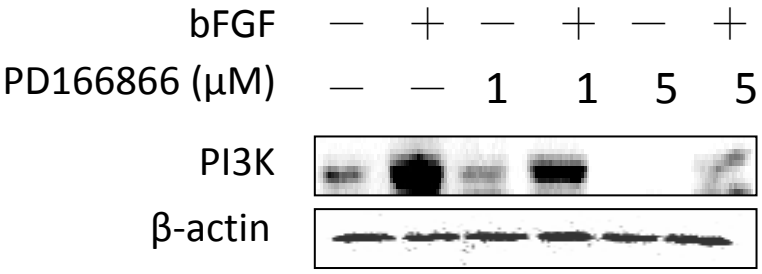

bFGF — + — + — +
PD166866 (μM) — — 1 1 5 5
PI3K
β-actin

FIG. 8B

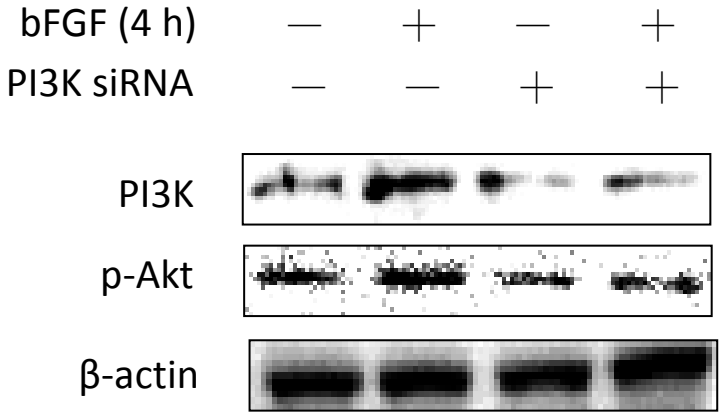

bFGF (4 h) — + — +
PI3K siRNA — — + +
PI3K
p-Akt
β-actin

FIG. 8C

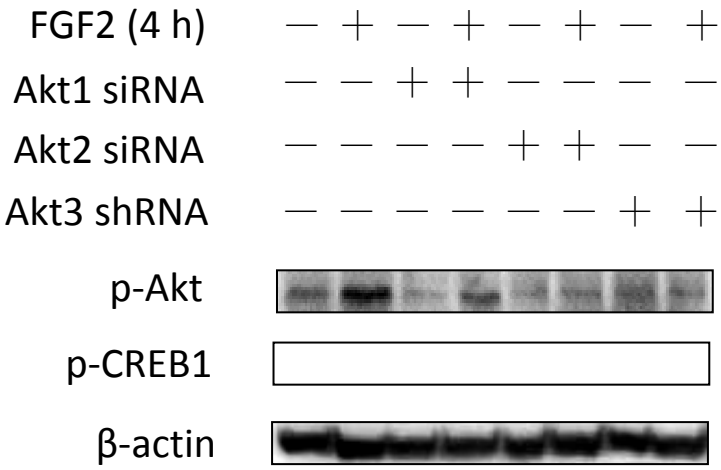

FGF2 (4 h) − + − + − + − +
Akt1 siRNA − − + + − − − −
Akt2 siRNA − − − − + + − −
Akt3 shRNA − − − − − − + +
p-Akt
p-CREB1
β-actin

FIG. 8D

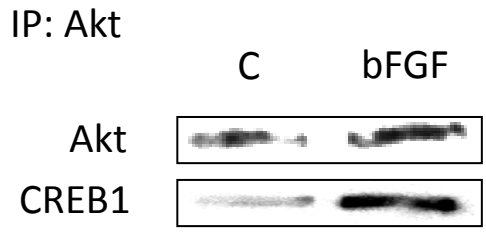

IP: Akt
C
bFGF
Akt
CREB1

FIG. 9A

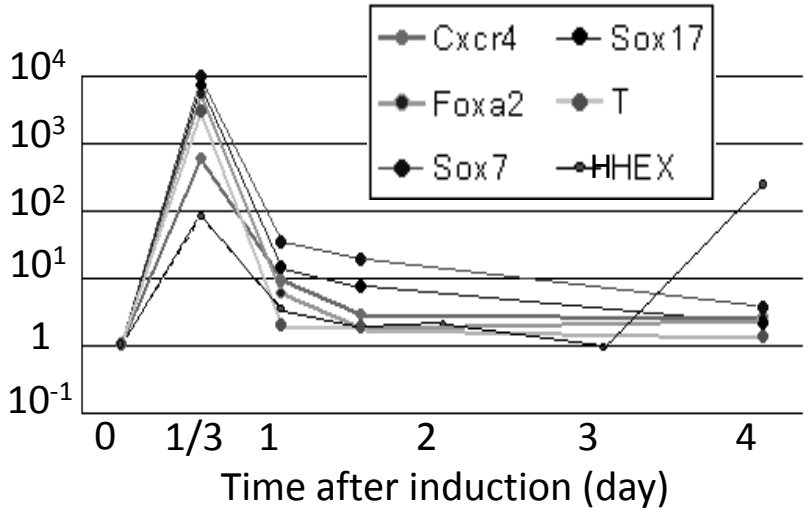

Cxcr4
Sox17
Foxa2
T
Sox7
HHEX
10^4
10^3
10^2
10^1
1
10^-1
0
1/3
1
2
3
4
Time after induction (day)

FIG. 9B

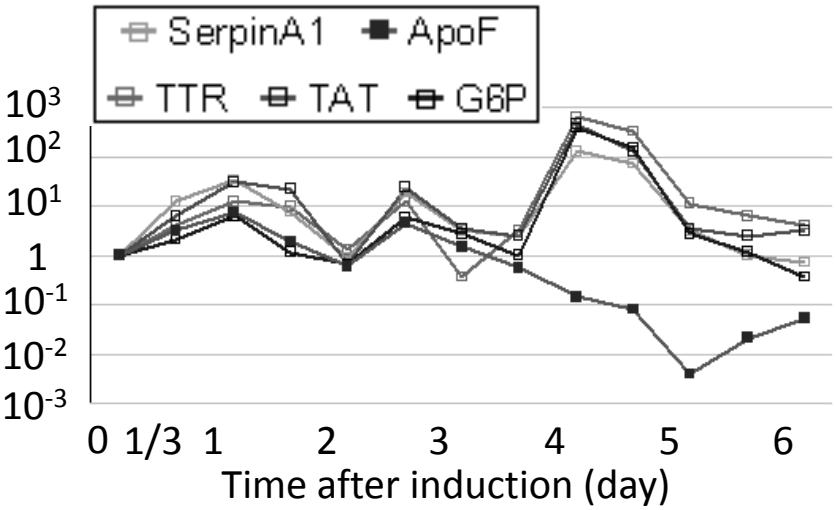

SerpinA1
ApoF
TTR
TAT
G6P
10^3
10^2
10^1
1
10^-1
10^-2
10^-3
0
1/3
1
2
3
4
5
6
Time after induction (day)

FIG. 9C

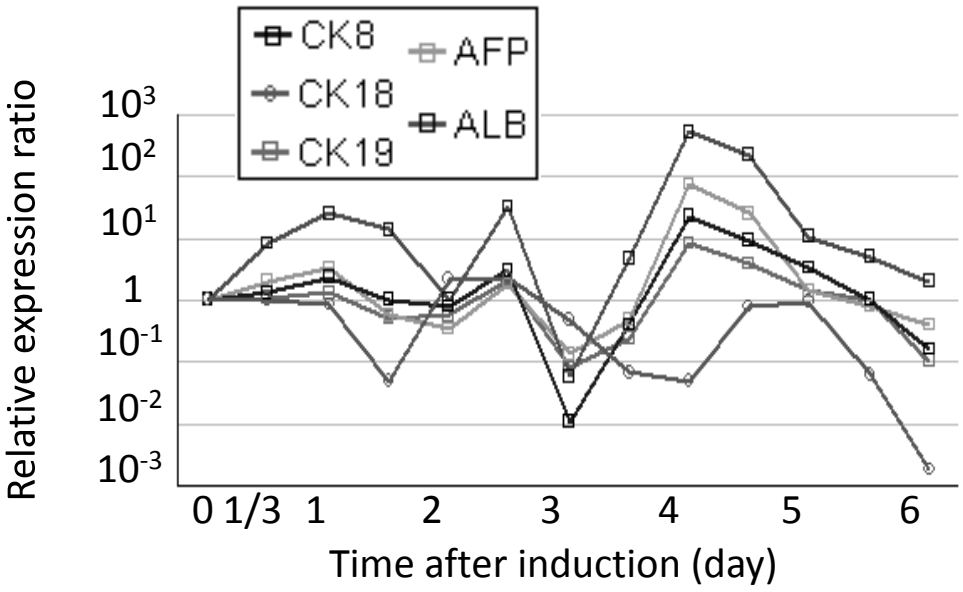

CK8
AFP
CK18
ALB
CK19
Relative expression ratio
10^3
10^2
10^1
1
10^-1
10^-2
10^-3
0 1/3 1 2 3 4 5 6
Time after induction (day)

FIG. 9D

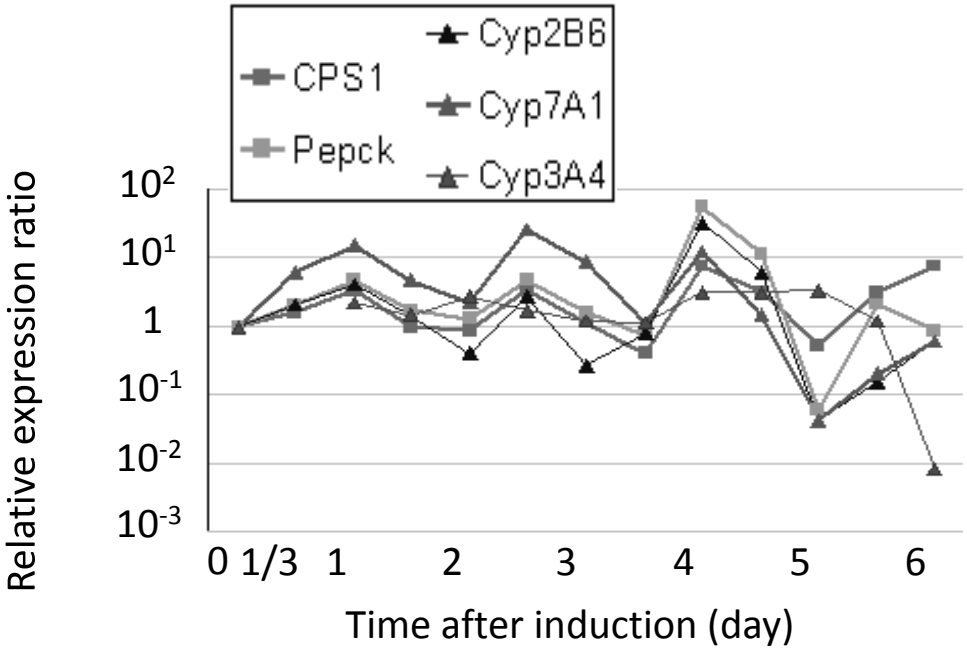

Cyp2B6
CPS1
Cyp7A1
Pepck
Cyp3A4
Relative expression ratio
10^2
10^1
1
10^-1
10^-2
10^-3
0 1/3 1 2 3 4 5 6
Time after induction (day)

FIG. 9E

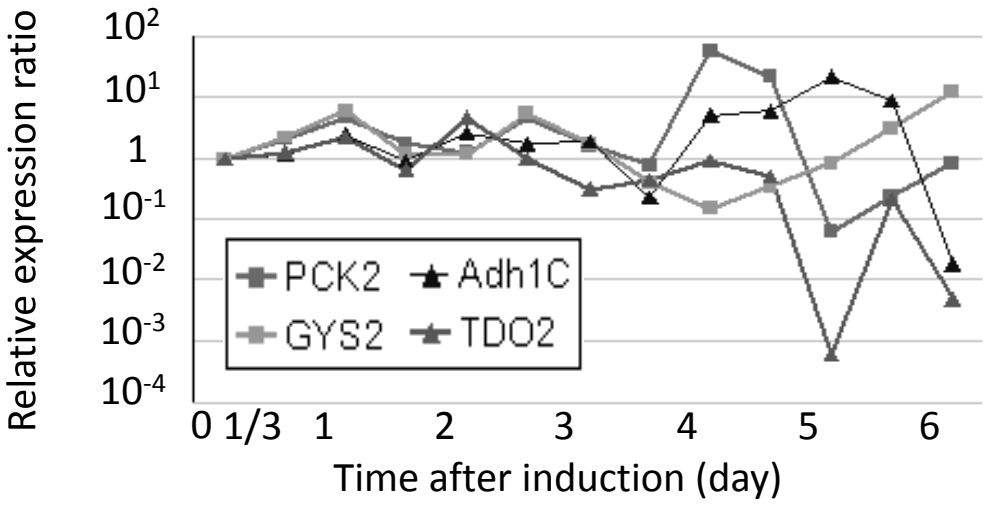

Relative expression ratio
10^2
10^1
1
10^-1
10^-2
10^-3
10^-4
PCK2
Adh1C
GYS2
TDO2
0 1/3 1 2 3 4 5 6
Time after induction (day)

FIG. 9F

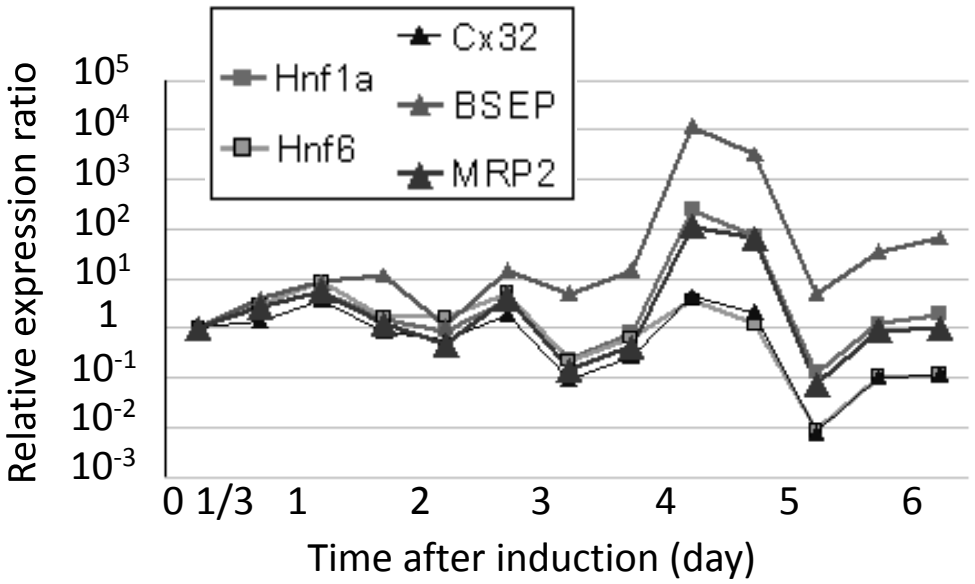

Relative expression ratio
10^5
10^4
10^3
10^2
10^1
1
10^-1
10^-2
10^-3
Hnf1a
Hnf6
Cx32
BSEP
MRP2
0 1/3 1 2 3 4 5 6
Time after induction (day)

FIG. 10A

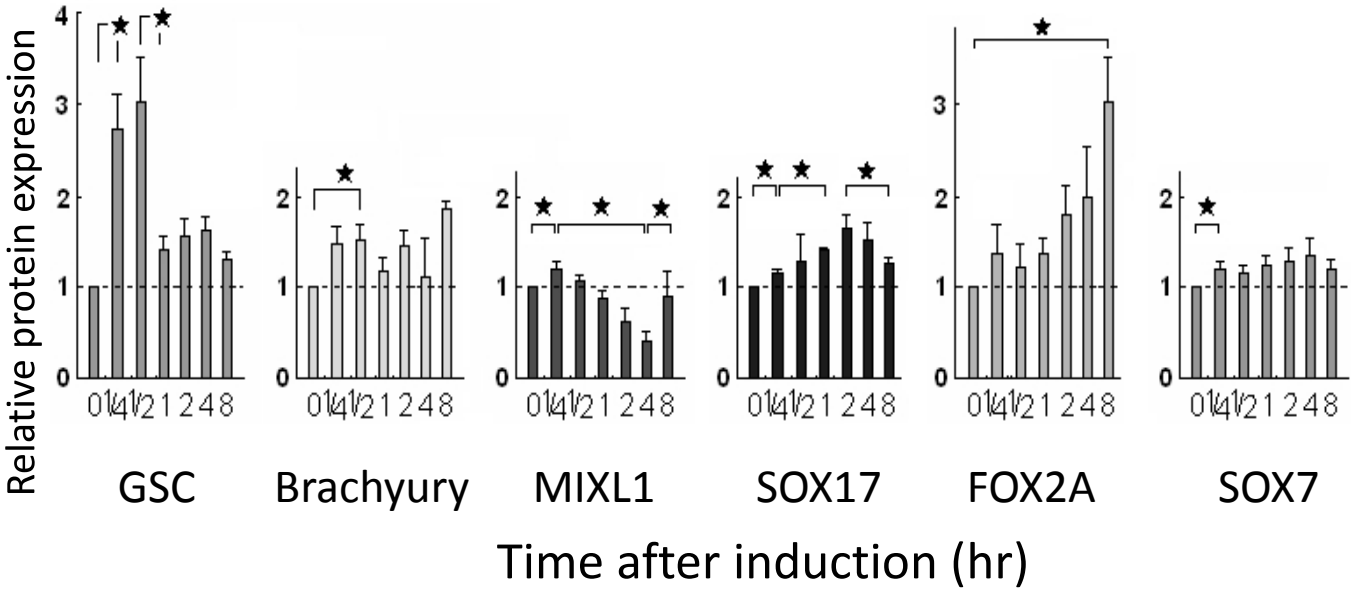


FIG. 10B

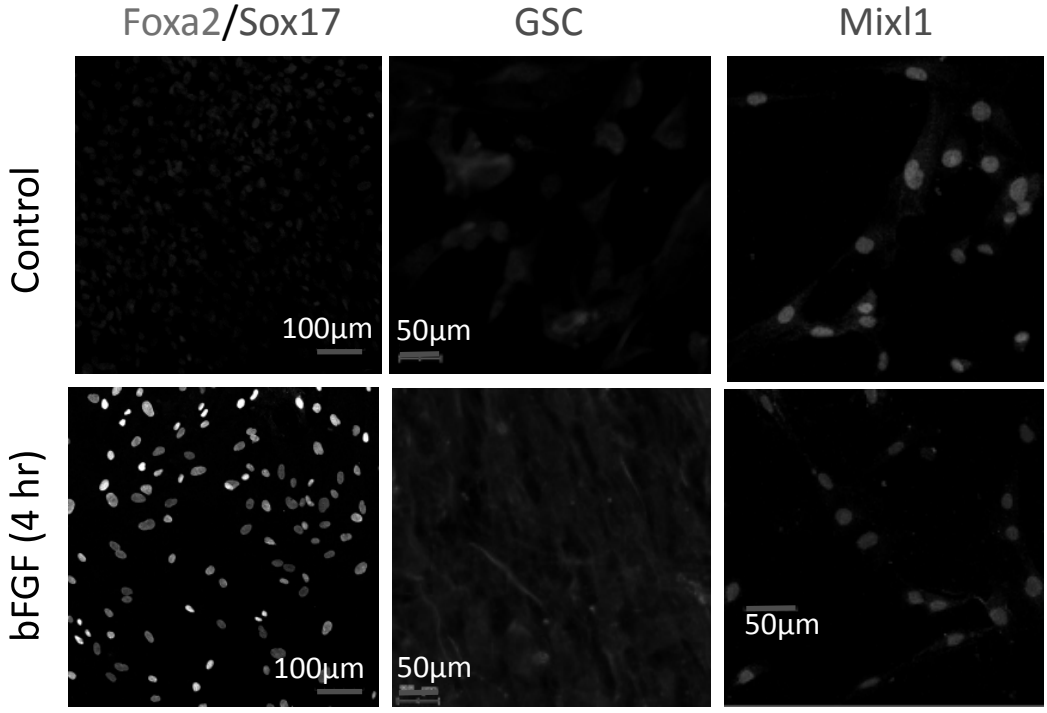


FIG. 10C

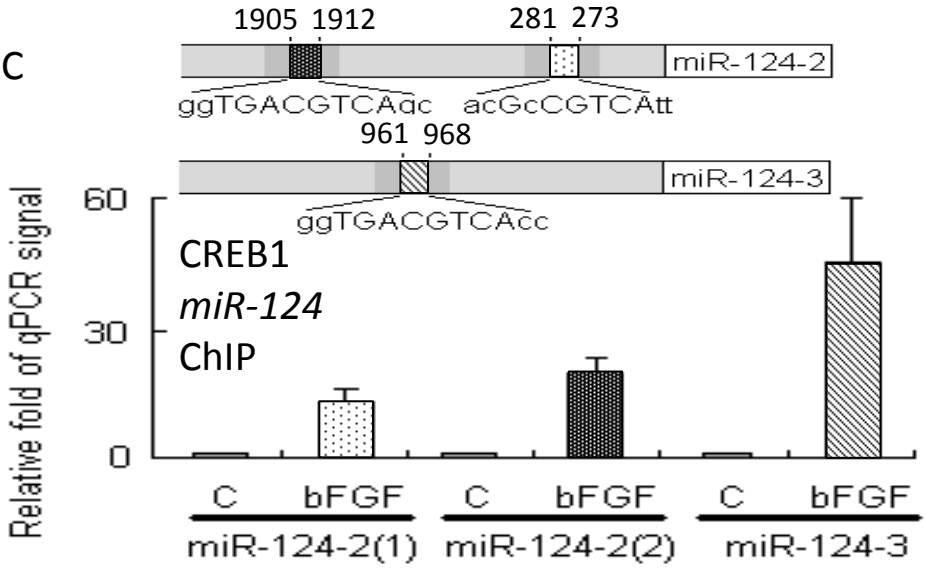


FIG. 10D

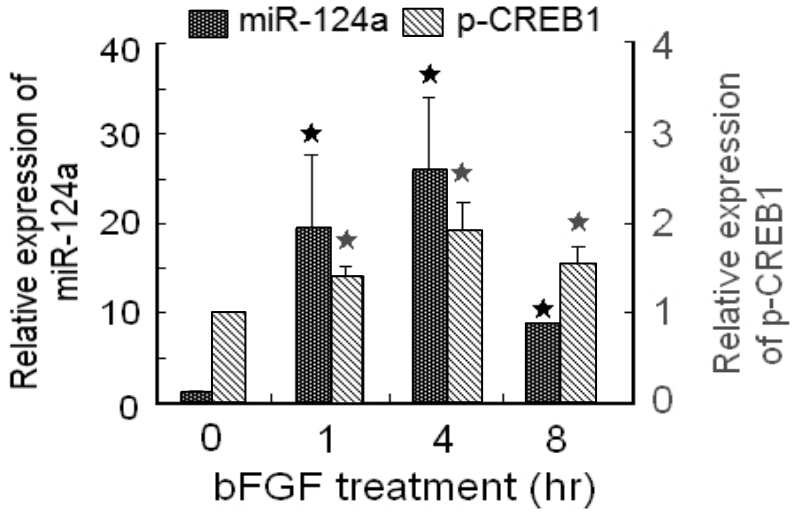


FIG. 10AS

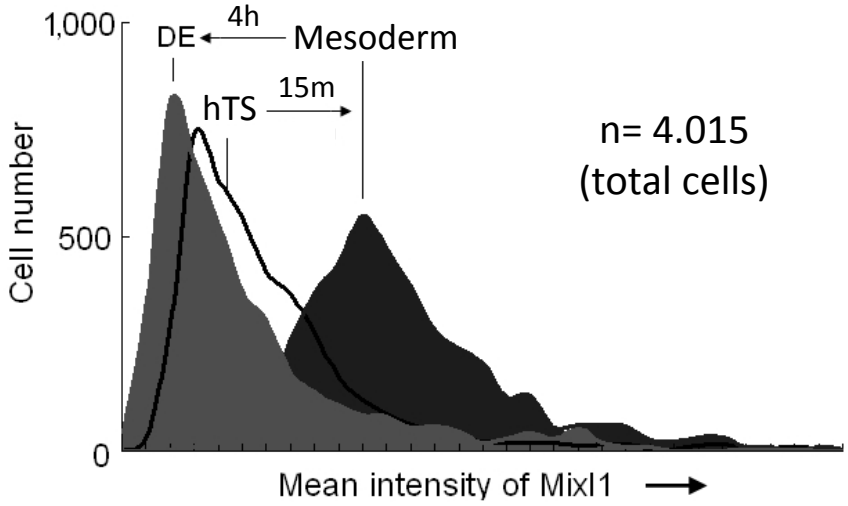


FIG. 10BS

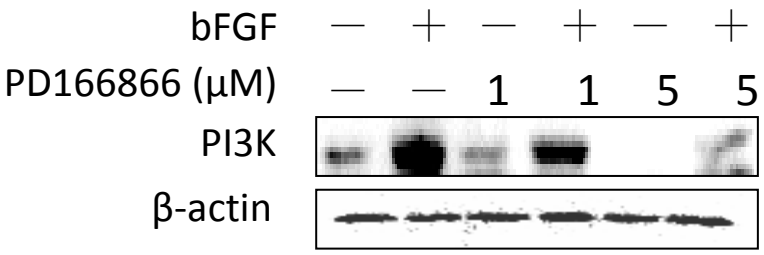


FIG. 10CS

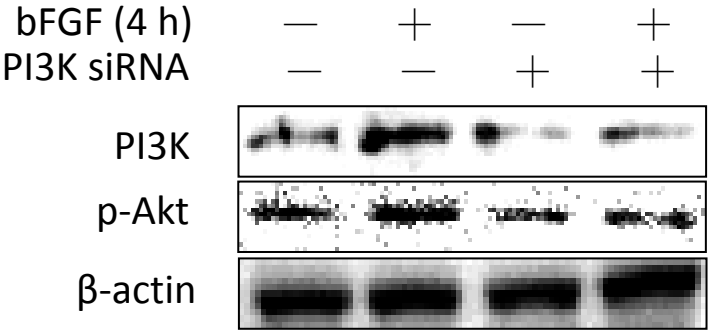


FIG. 10DS

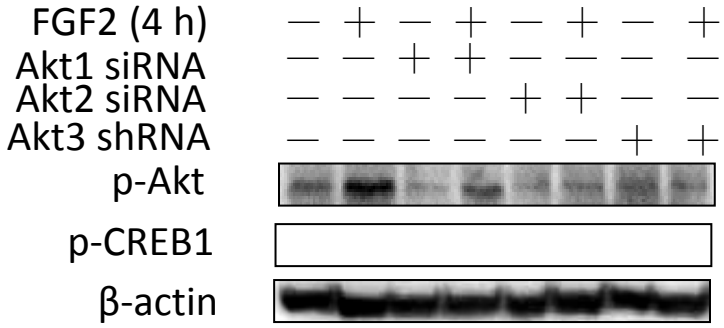


FIG. 10ES

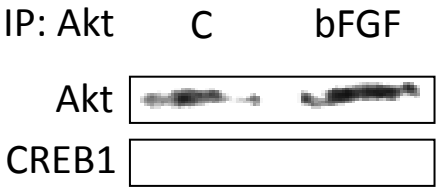


FIG. 10FS

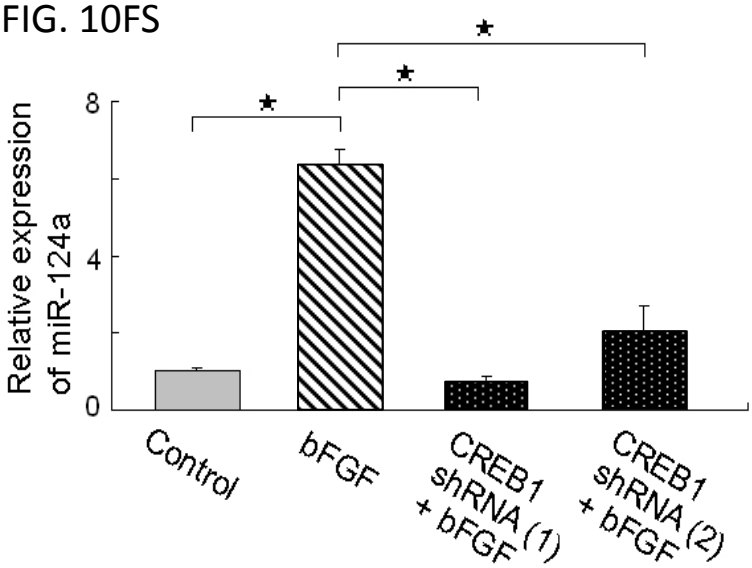


FIG. 11A

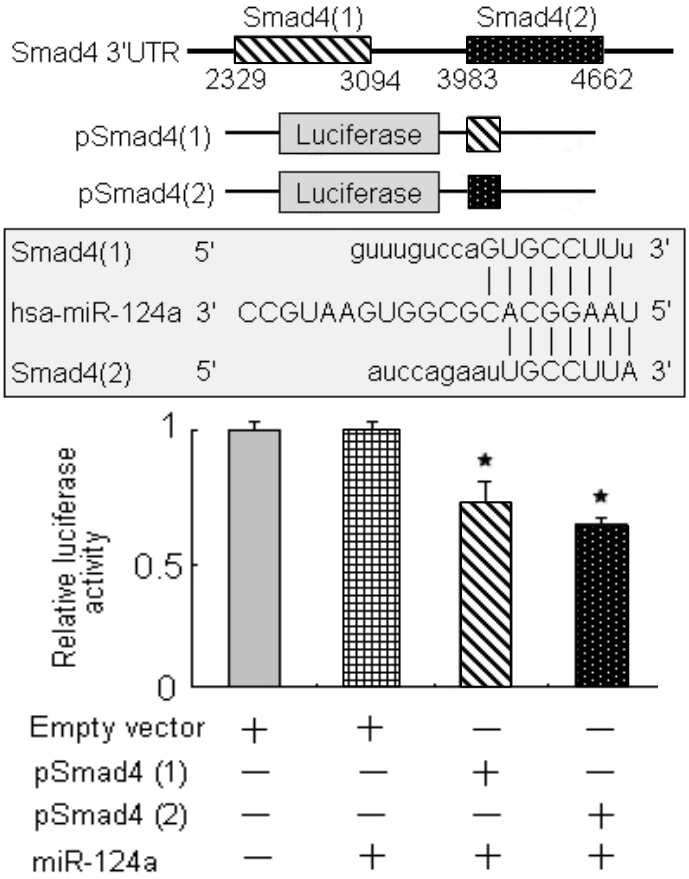


FIG. 11B

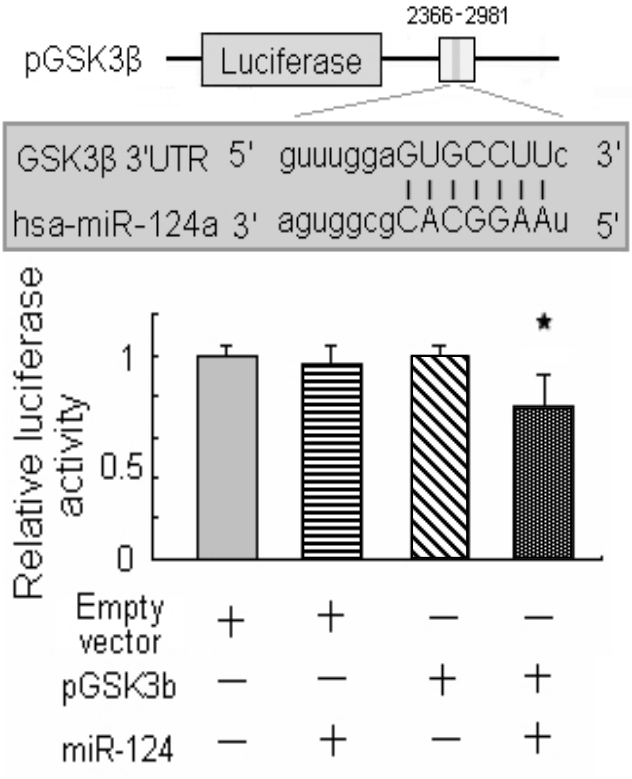


FIG. 11C

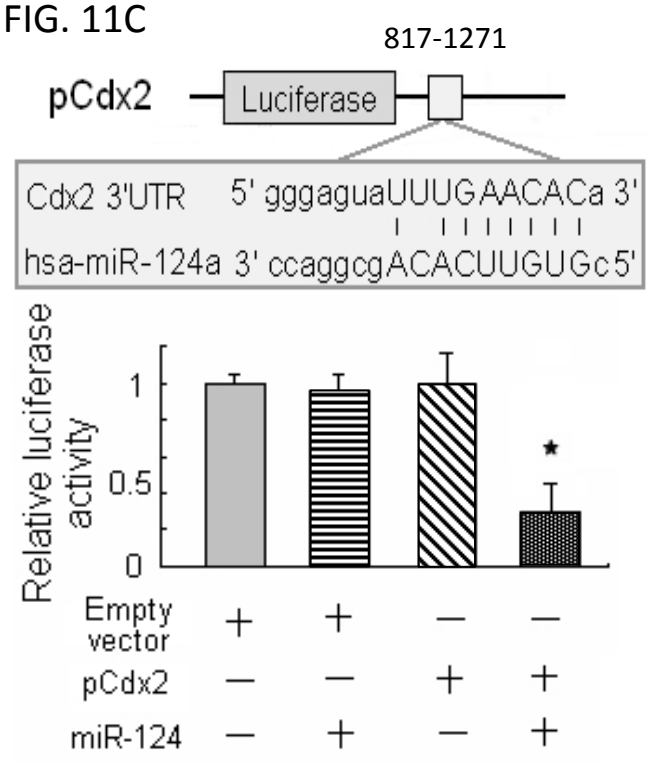


FIG. 11D

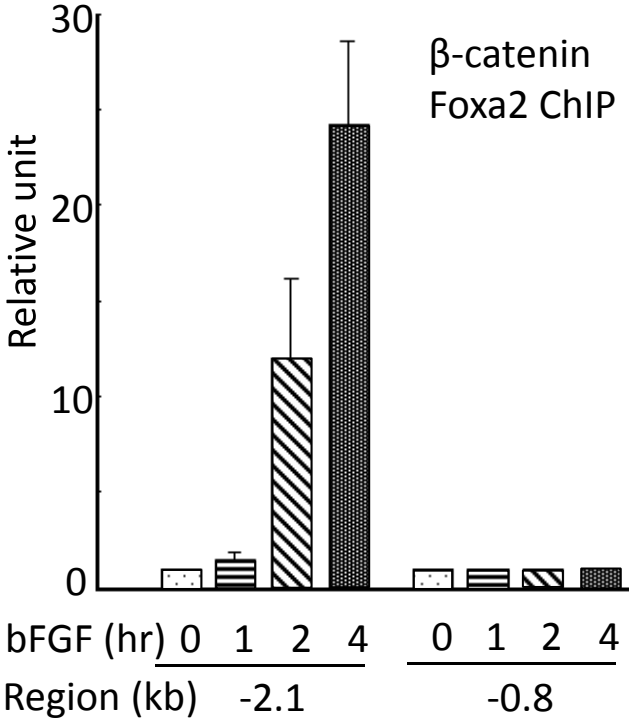


FIG. 11E

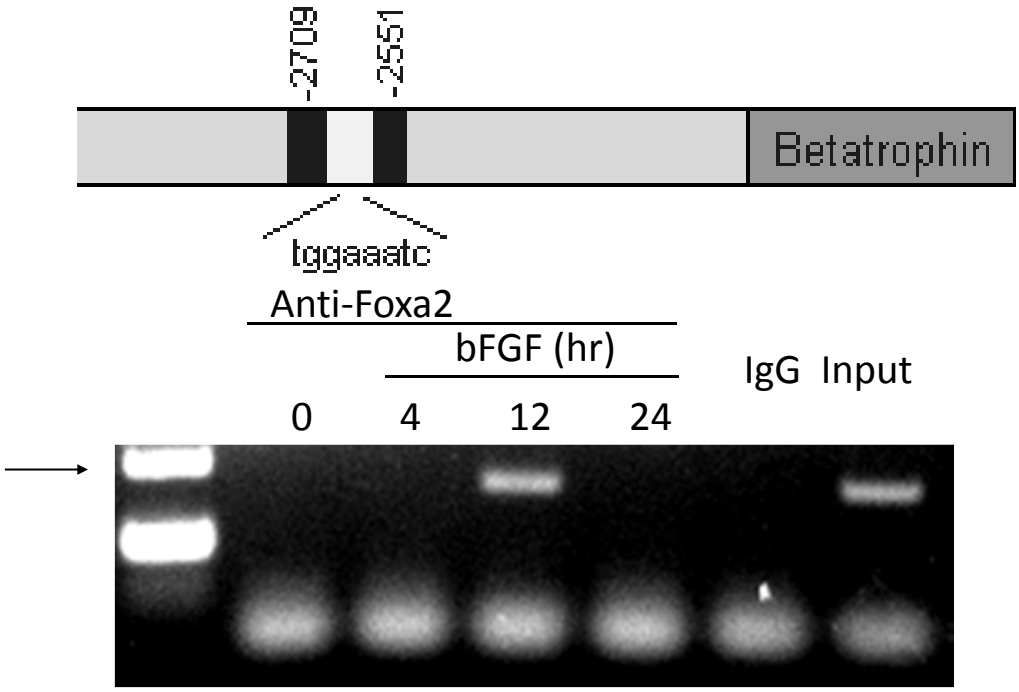


FIG. 11F

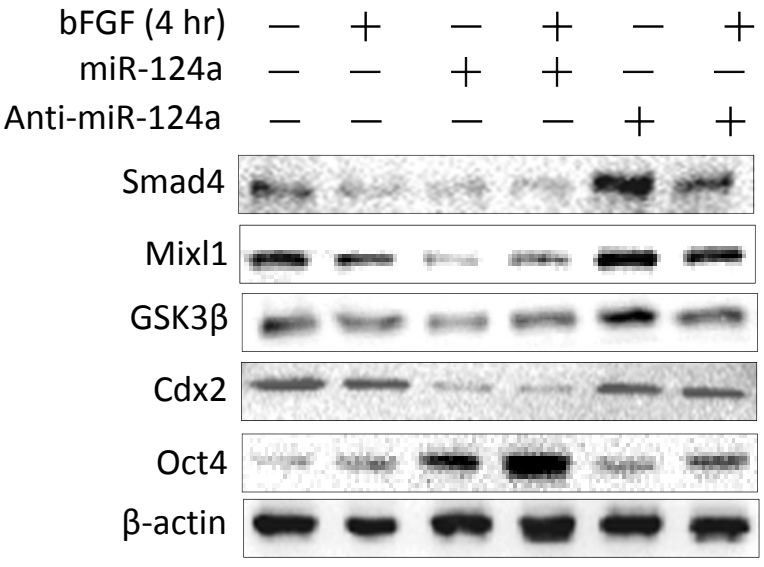


FIG. 11G

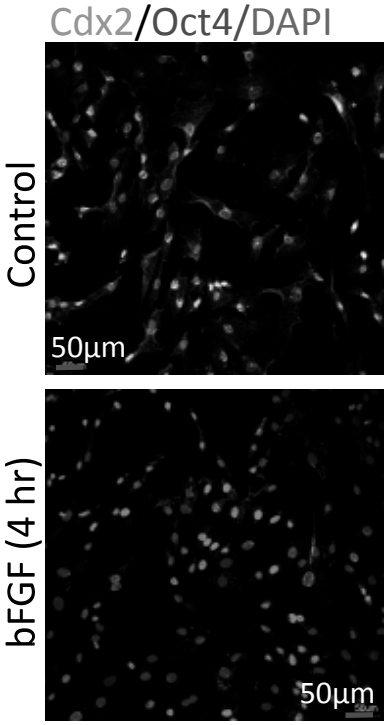


FIG. 11H

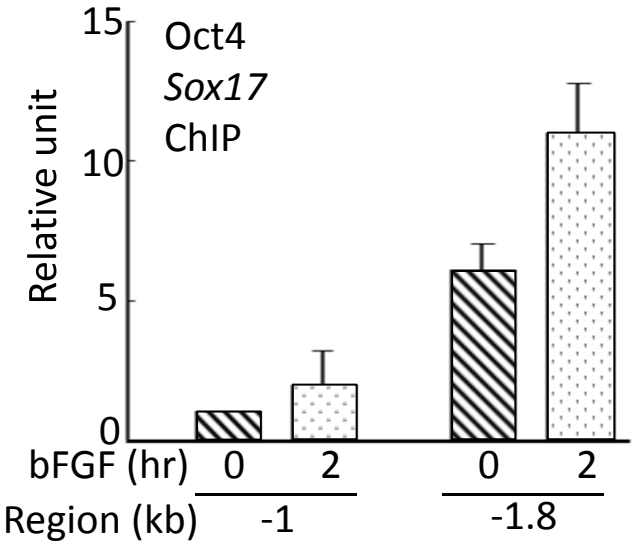


FIG. 11I

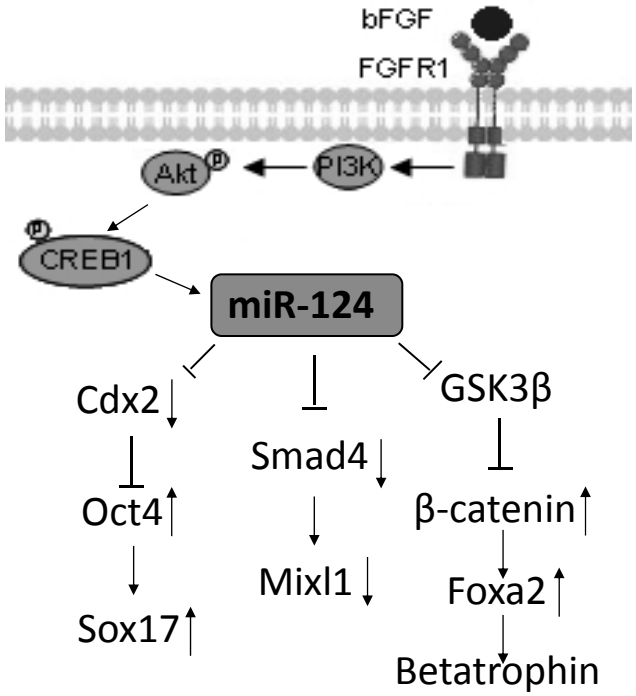


FIG. 11AS

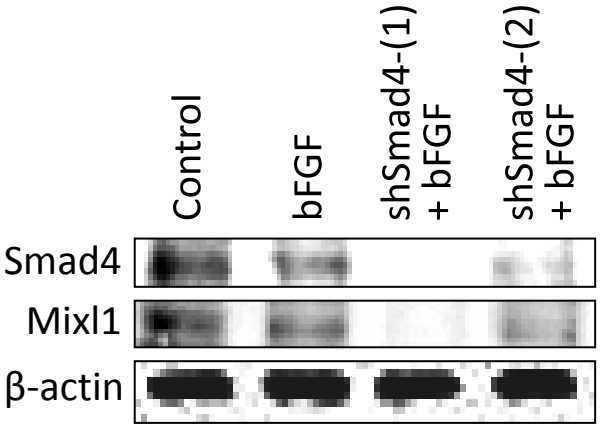


FIG. 11BS

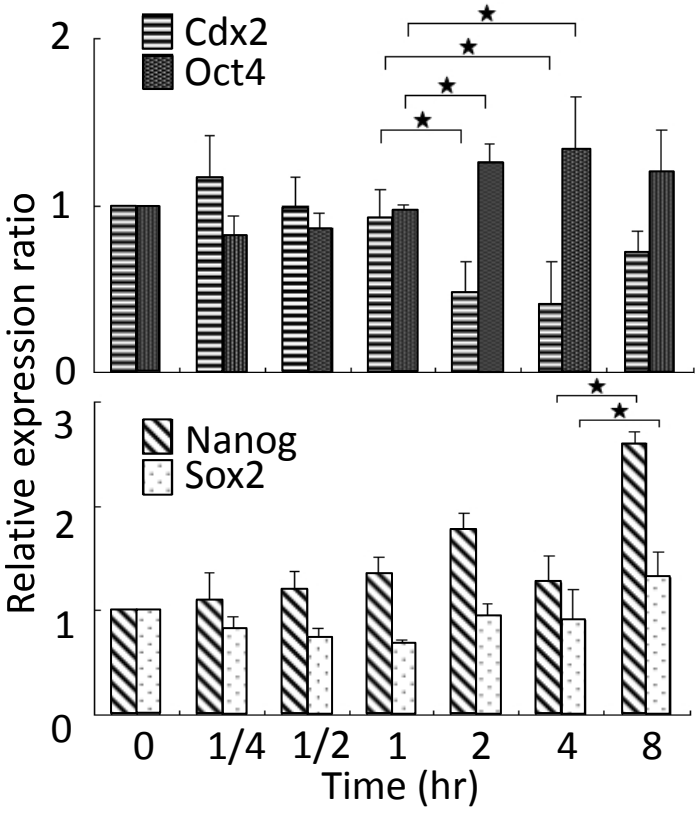


FIG. 12A

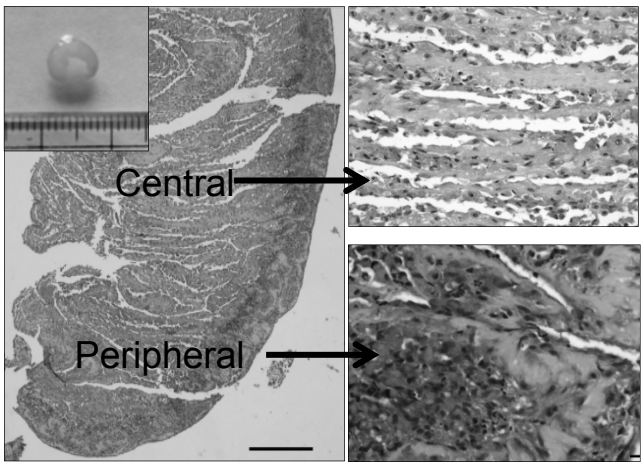


FIG. 12B

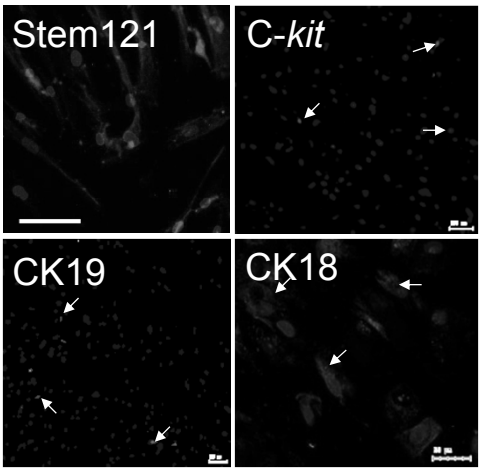


FIG. 12C

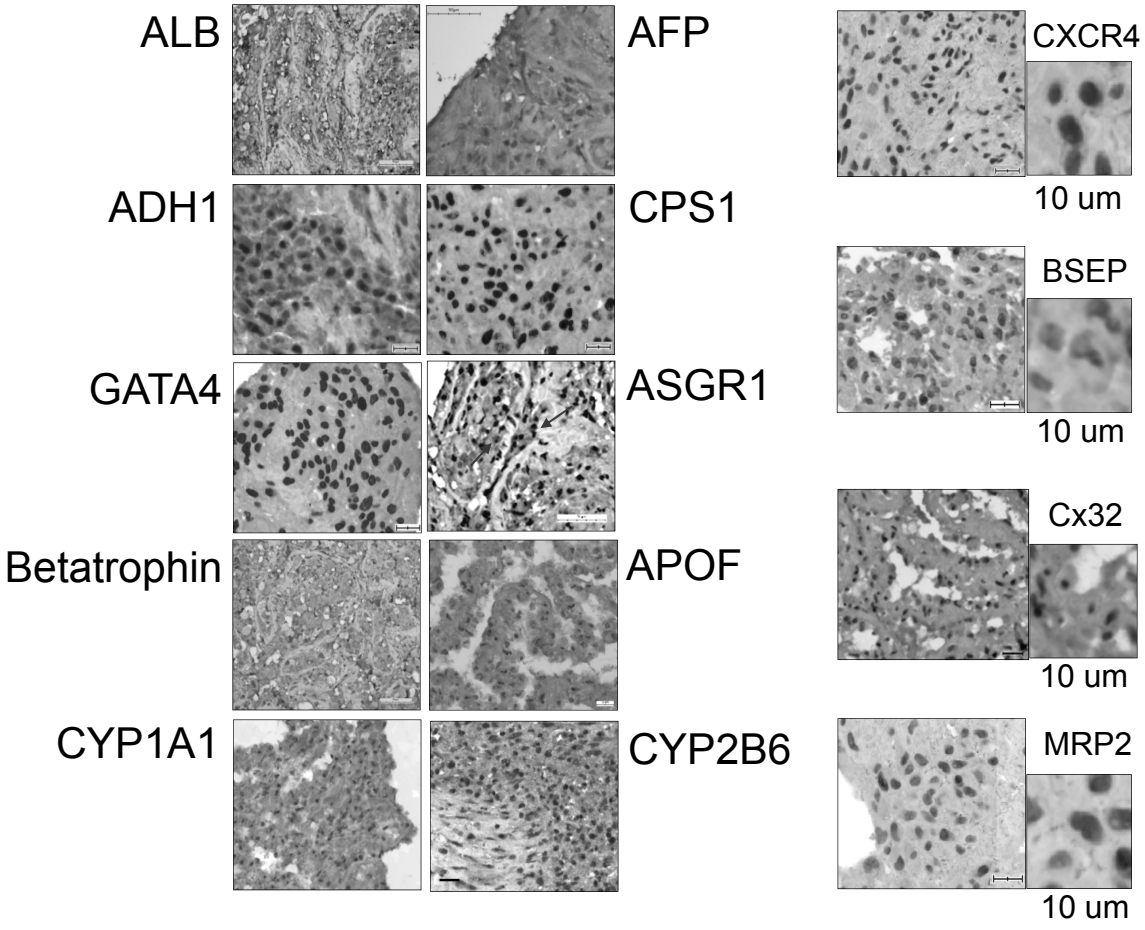


FIG. 12D

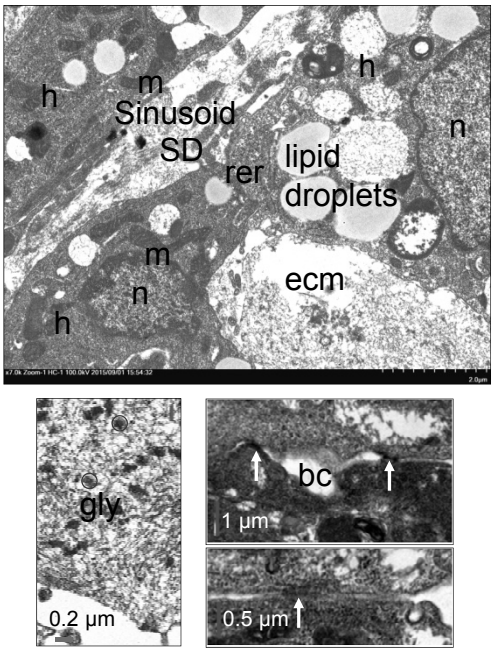


FIG. 12AS

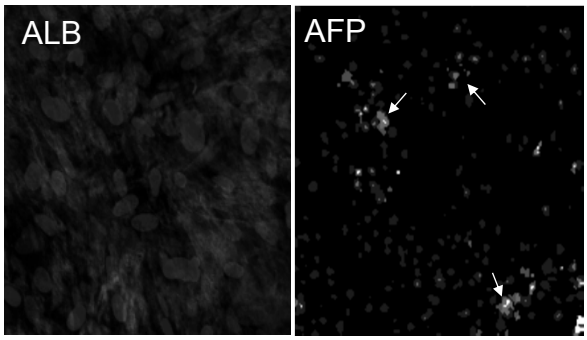


FIG. 12BS

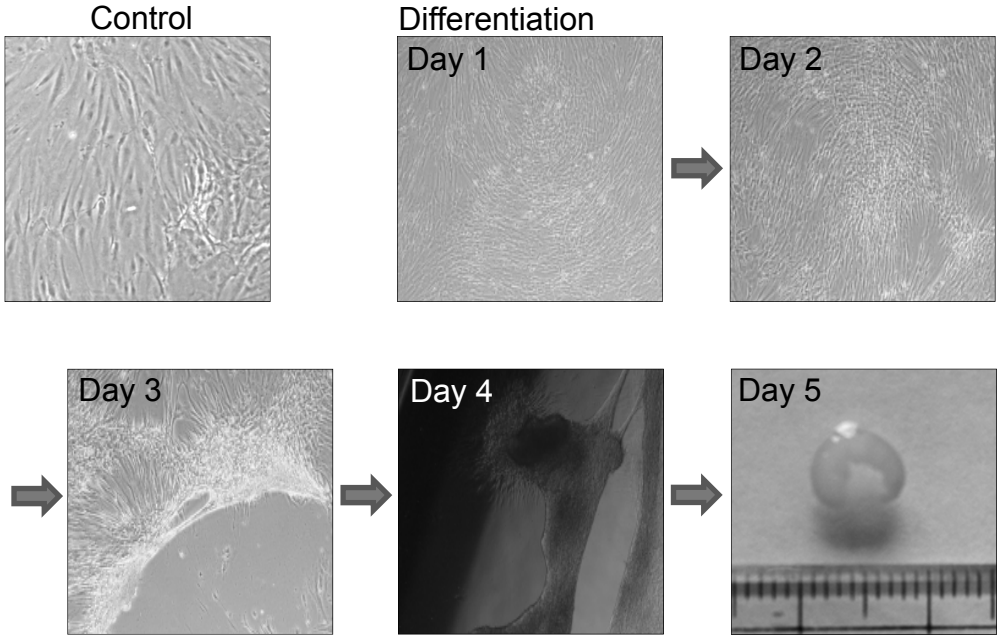


FIG. 13A

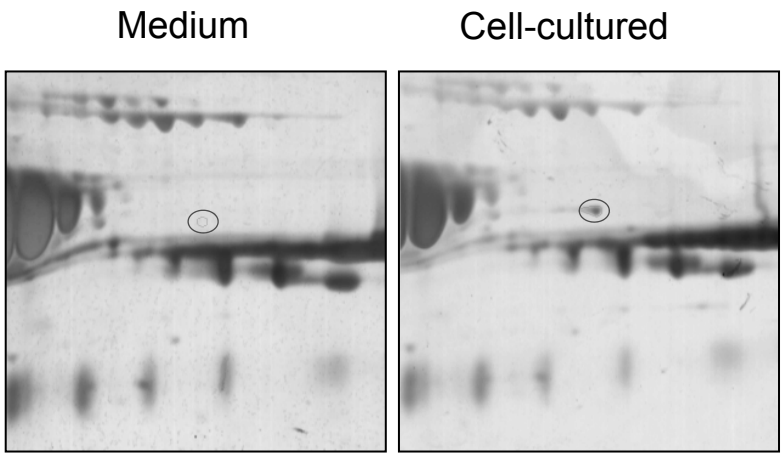


FIG. 13B

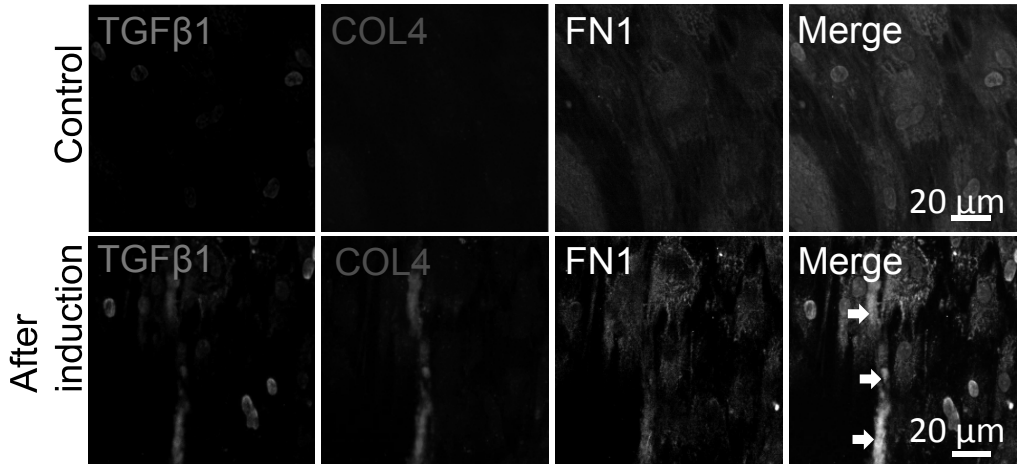


FIG. 13AS

Fixed modifications: Carboxymethyl (C)
Variable modifications: Oxidation (M)
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Sequence Coverage: 46%

Matched peptides shown in **BOLD**

1 **MALFVR**LLAL ALALALGPAA TLAGPAK**SPY QLVLQHSR**LR GR**QHGPNVCA**
51 **VQK**VIGTNRK YFTNCK**QWYQ RK**ICGK**STVI SYECCPGYEK** VPGEK**GCPAA**
101 **LPLSNLYETL GVVGSTTTQL YTDR**TEKLRP EMEGPGSFTI FAPSNEAWAS
151 LPAEVLDSLV SNVNIELLNA LRYHMVGRR**V LTDELK**HGMT LTSMYQNSNI
201 QIHHYPNGIV TVNCARLLKA DHHATNGVVH LIDKVISTIT NNIQQIIEIE
251 DTFETLR**AAV AASGLNTMLE GNGQYTLLAP TNEAFEKIPS ETLNRILGDP**
301 **EALR**DLLNNH ILKSAMCAEA IVAGLSVETL EGTTLEVGCS GDMLTINGKA
351 IISNK**DILAT NGVIHYIDEL LIPDSAK**TLF ELAAESDVST AIDLFR**QAGL**
401 **GNHLSGSERL TLLAPLNSVF KDGTPPIDAH TR**NLLRNHII KDQLASK**YLY**
451 **HGQTLETLGG K**KLR**VFVYRN** SLCIENSCIA AHDKRGR**YGT LFTMDRVLTP**
501 **PMGTVMDVLK** GDNR**FSMLVA AIQSAGLTET LNREGVYTVF APTNEAFRAL**
551 **PPR**ERSRLLG DAK**ELANILK** YHIGDEILVS GGIGALVRLK **SLQGDKLEVS**
601 **LKNNVVSVNK** EPVAEPDIMA TNGVVHVITN VLQPPANRPQ ER**GDELADSA**
651 **LEIFK**QASAF SRASQRSVR**L APVYQK**LLER MKH

FIG. 14A

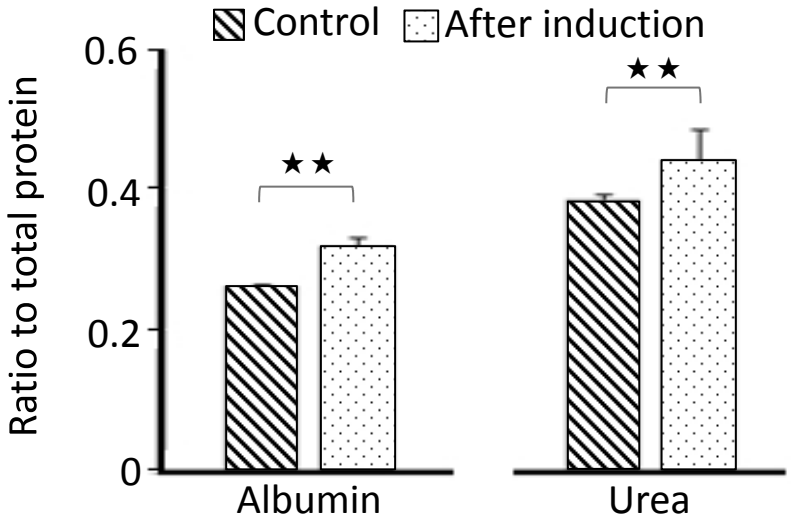


FIG. 14B

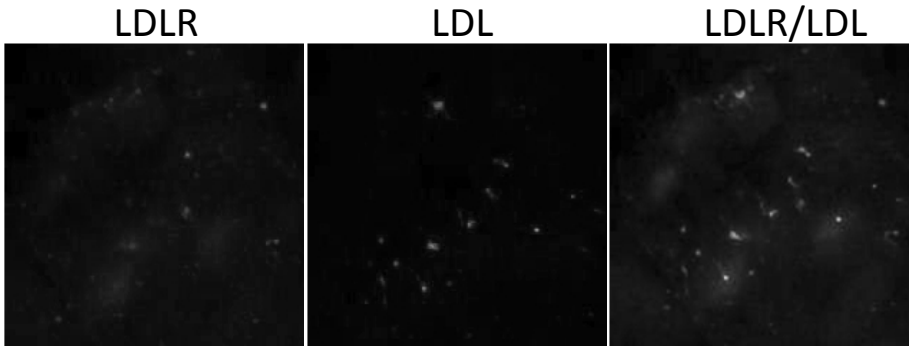


FIG. 14C

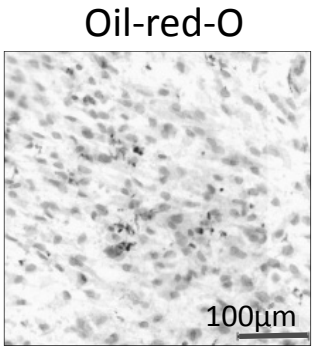


FIG. 14D

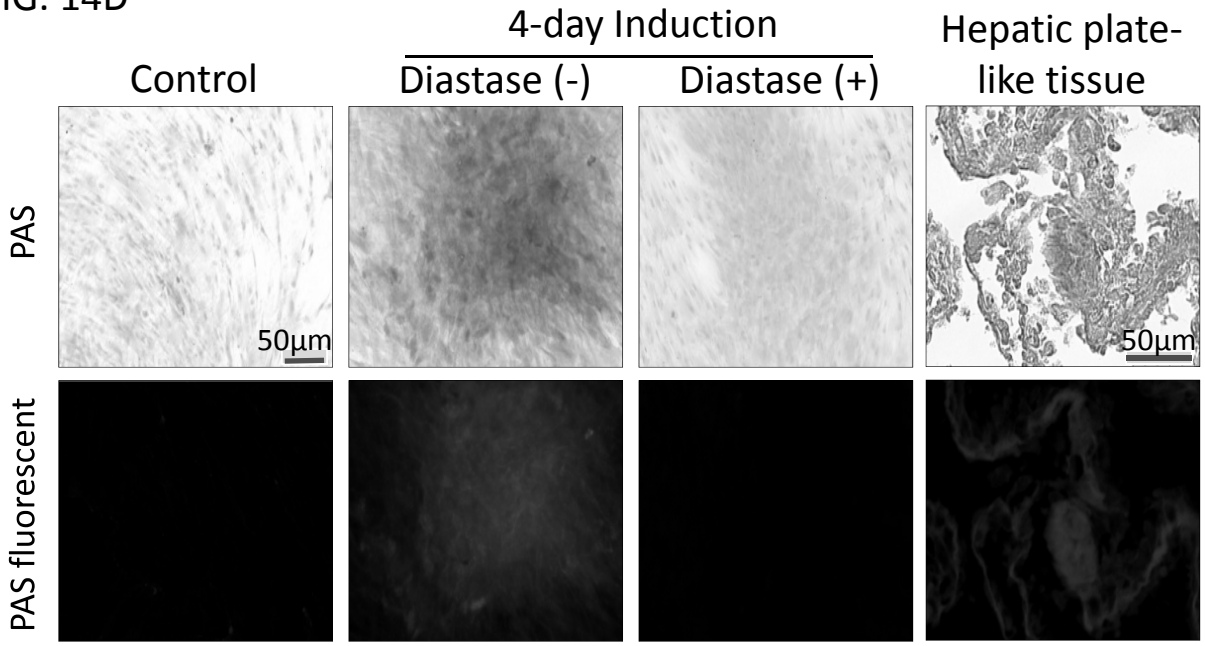


FIG. 14E

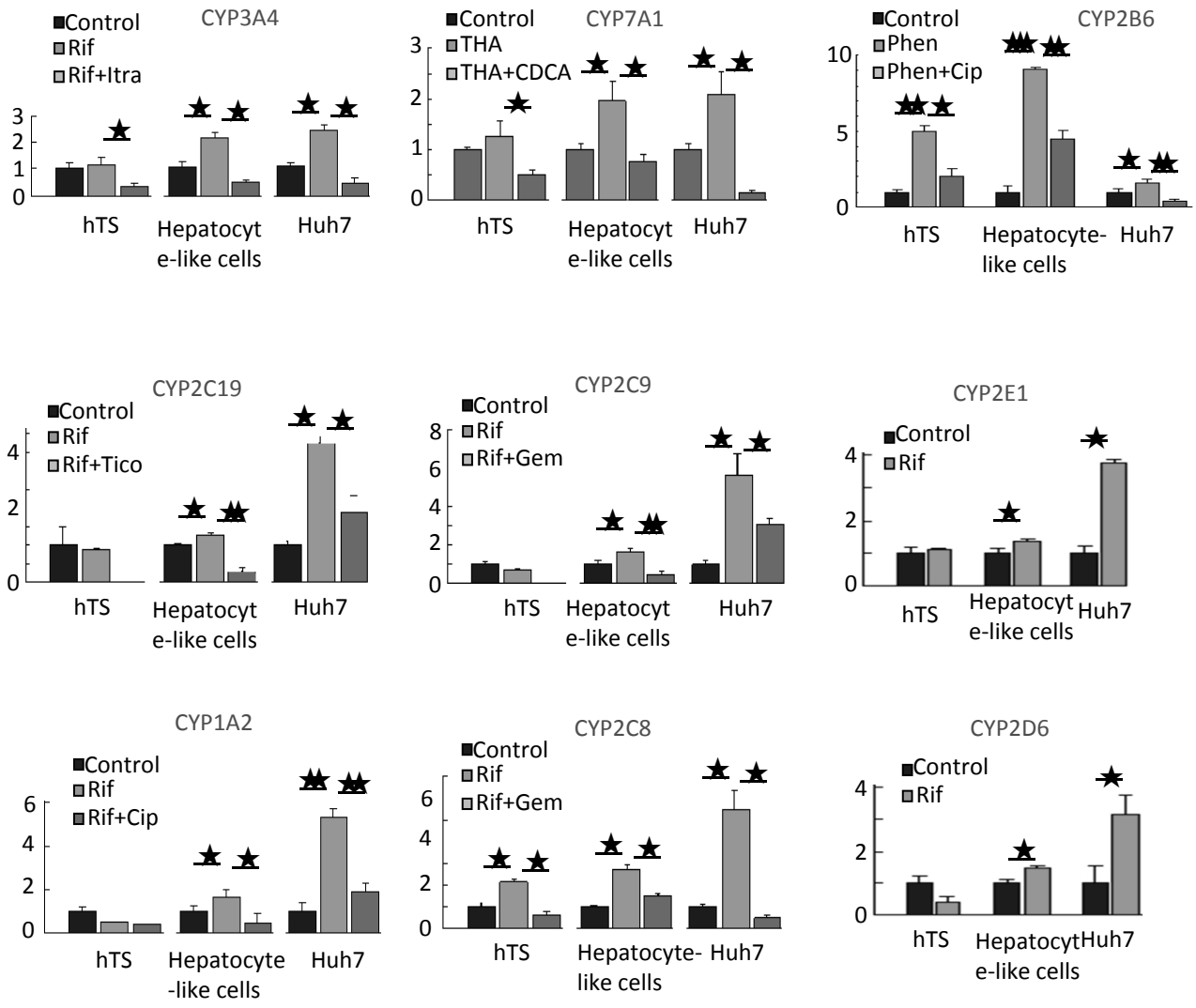

CYP3A4
Control
Rif
Rif+Itra
hTS
Hepatocyte-like cells
Huh7
CYP7A1
Control
THA
THA+CDCA
CYP2B6
Control
Phen
Phen+Cip
CYP2C19
Control
Rif
Rif+Tico
CYP2C9
Control
Rif
Rif+Gem
CYP2E1
Control
Rif
CYP1A2
Control
Rif
Rif+Cip
CYP2C8
Control
Rif
Rif+Gem
CYP2D6
Control
Rif

FIG. 15A

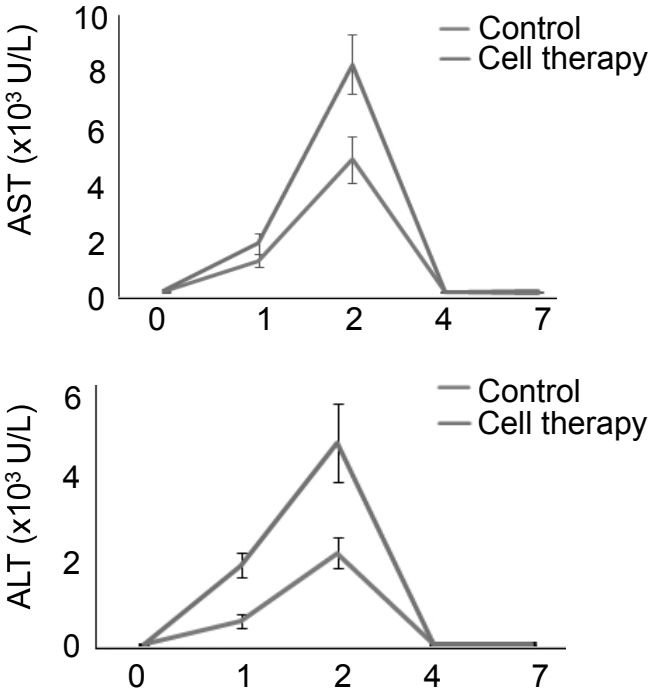


FIG. 15B

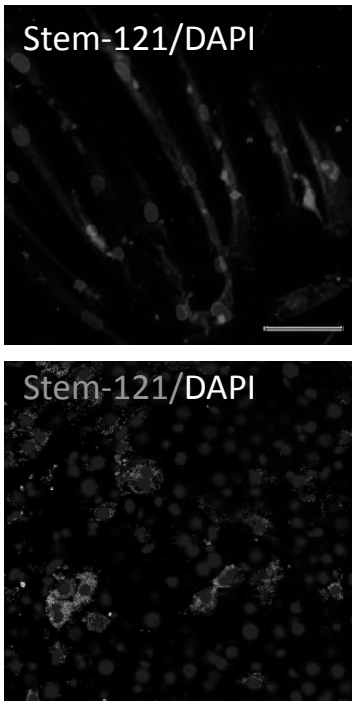


FIG. 15C

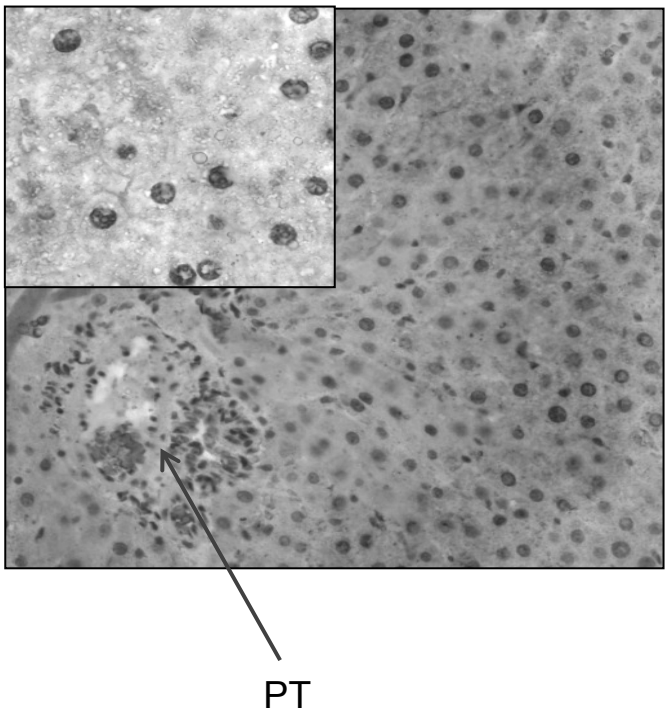


FIG. 15D

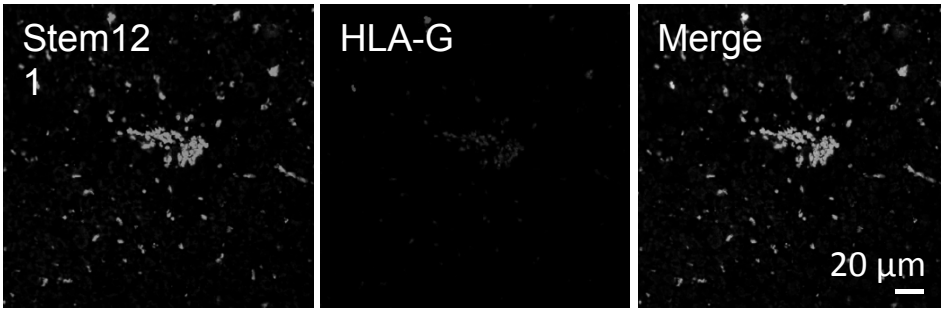


FIG. 15E

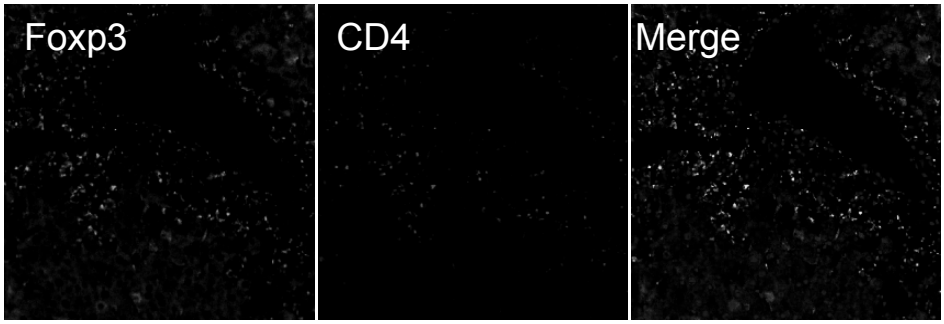


FIG. 15F

