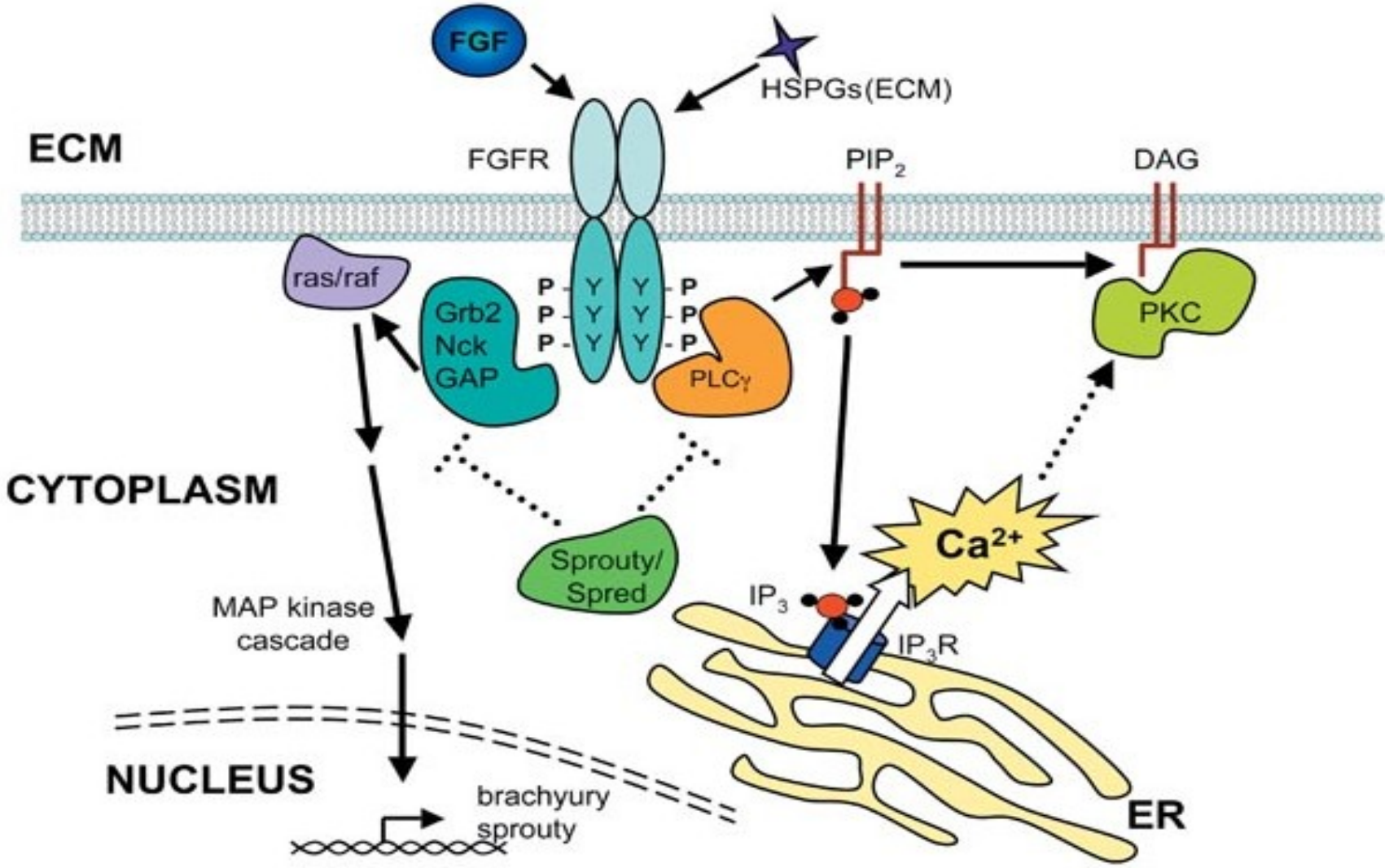
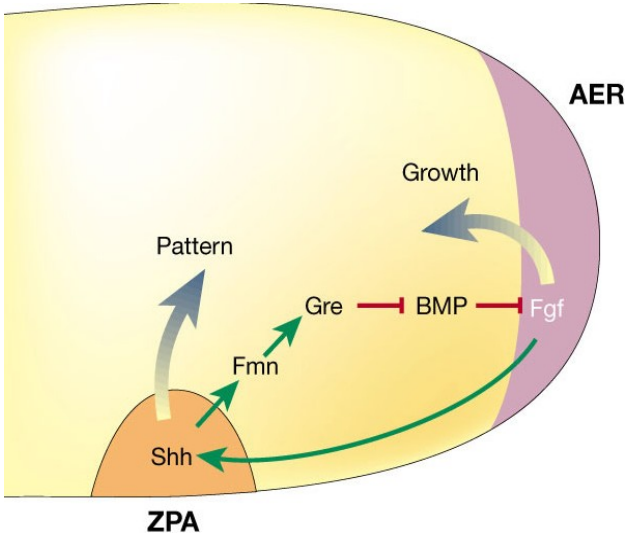
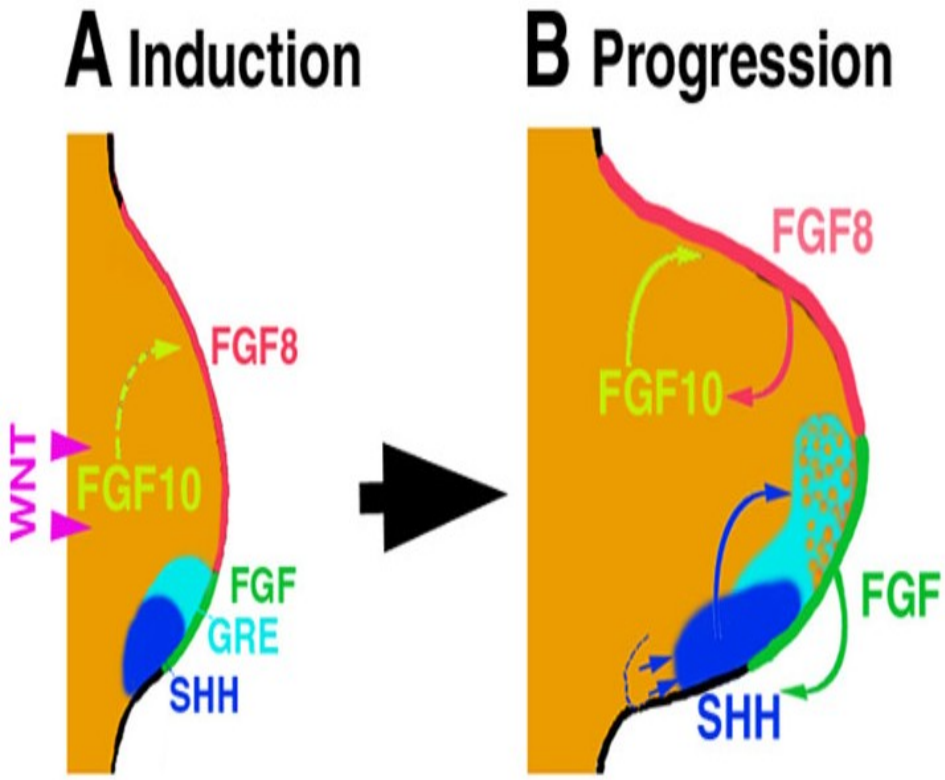
REGULATION OF LIMB DEVELOPMENT BY FIBROBLAST GROWTH FACTOR (FGF) SYSTEM

Pavel Krejci

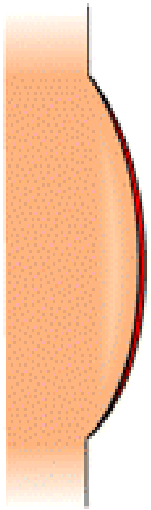
4 receptors: FGFR1-4
22 ligands: FGF1-23

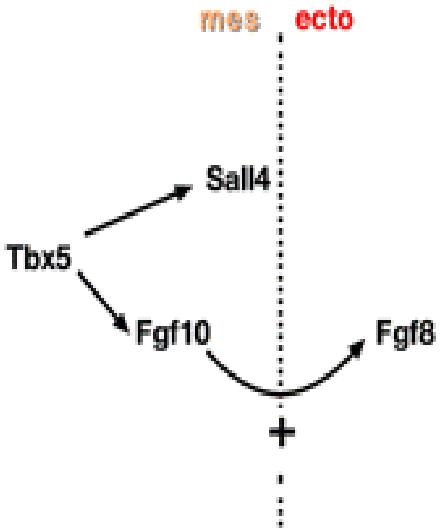




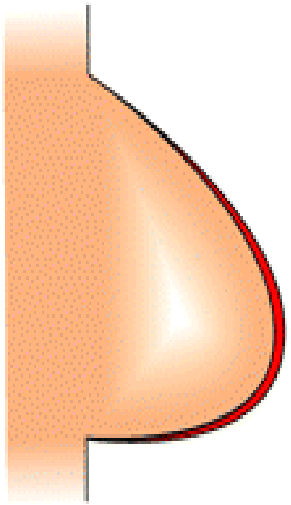


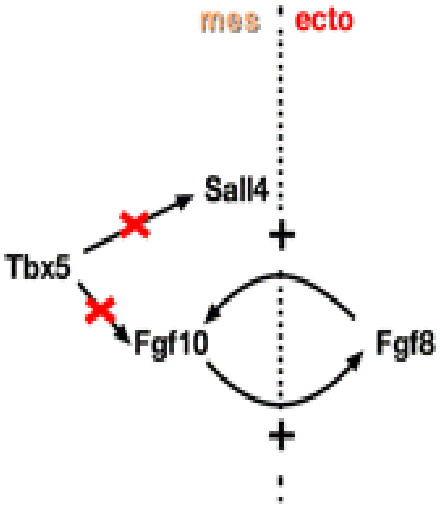
Initiation: *Tbx5* - dependent





Outgrowth: *Tbx5* - independent



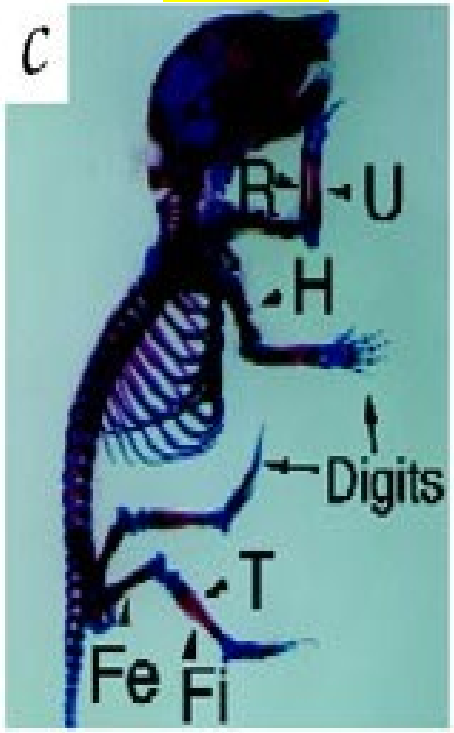


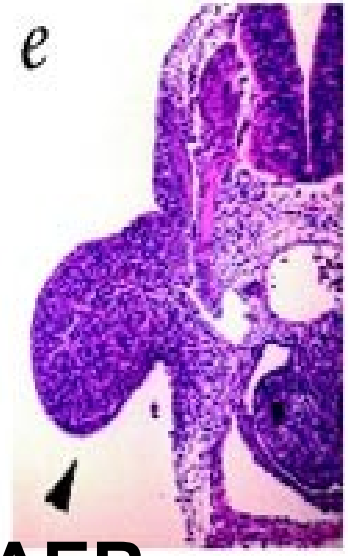
wt

Fgf10-/-

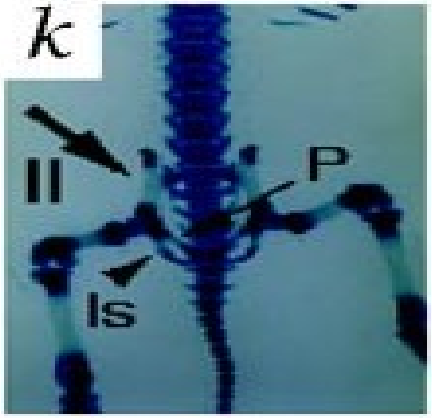
wt

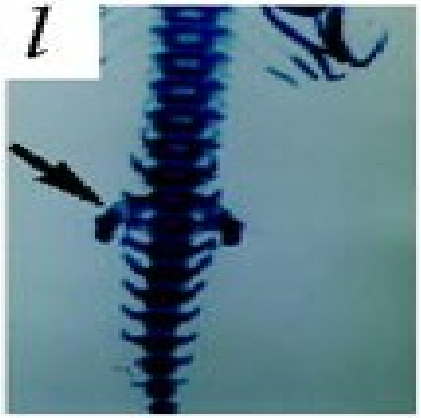
Fgf10-/-





AER

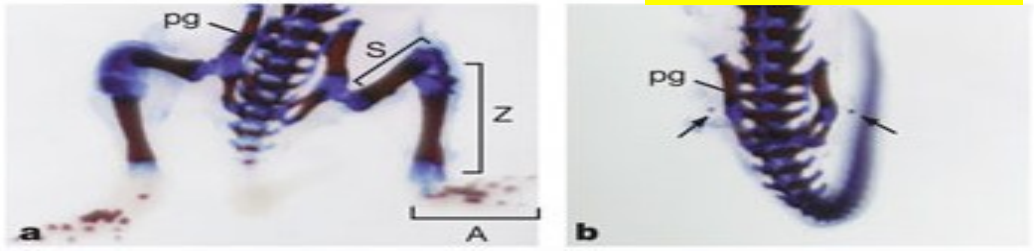




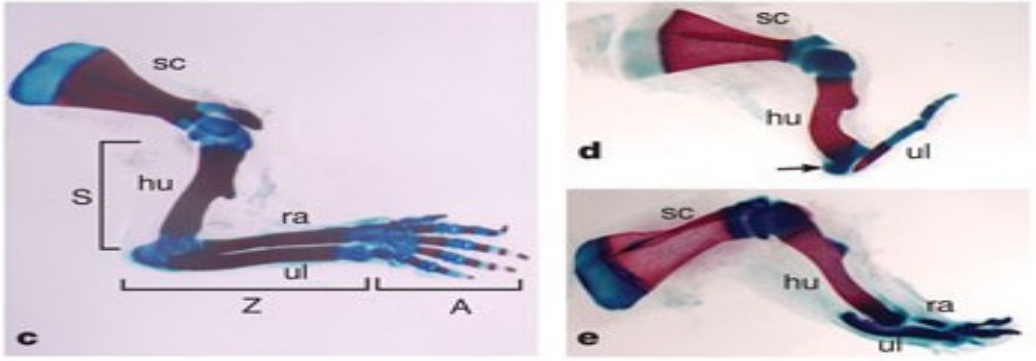
wt

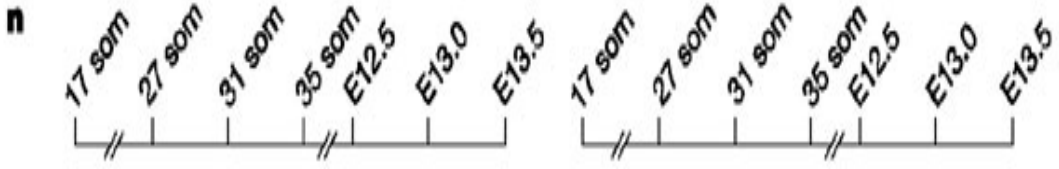
Hindlimb

Fgf8/Fgf4$^{-/-}$



Forelimb





Normal

Fgf8

Fgf4

Fgf8

Fgf4

Cre function

Cre function

Double KO

No *Fgf8* expression

No *Fgf4* expression

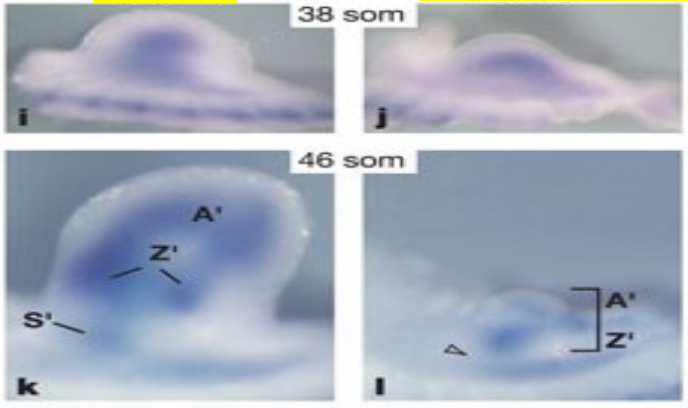
Transient *Fgf8* expression

Precocious, transient *Fgf4* expression

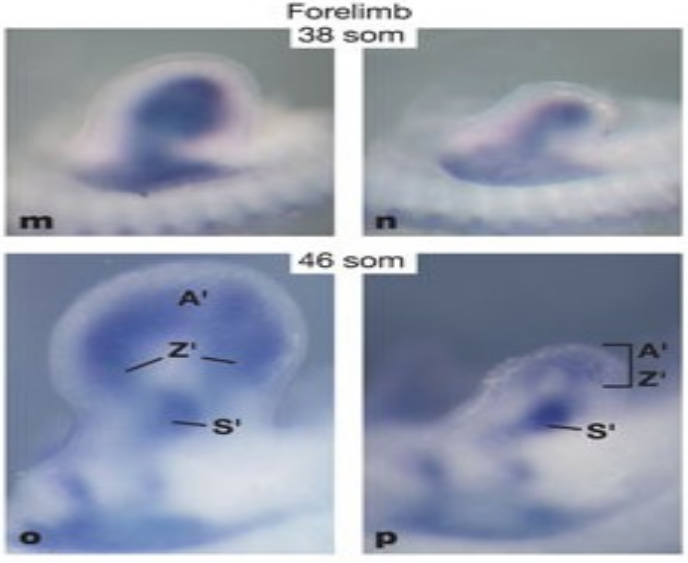
wt

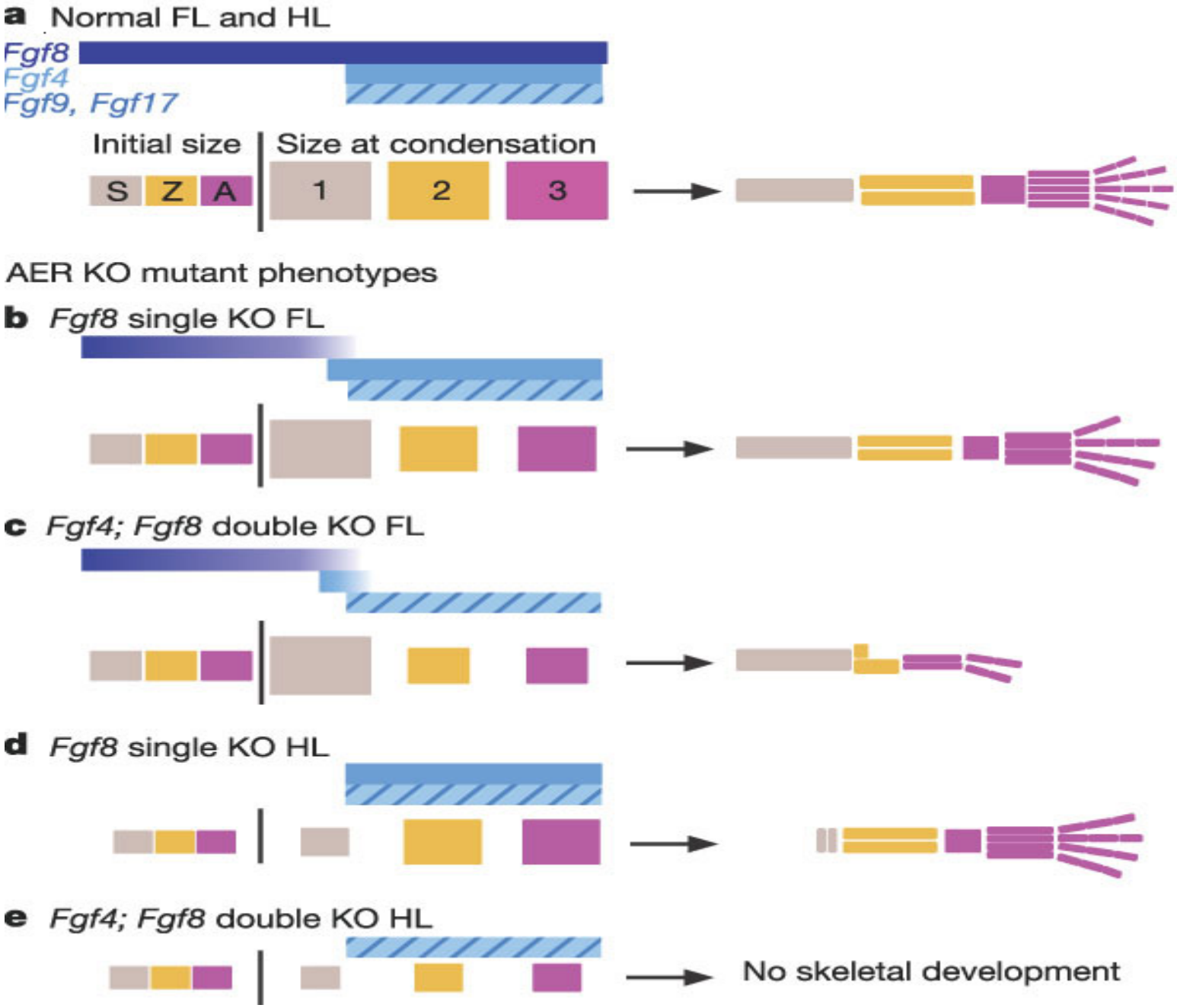
Hindlimb

Fgf8/Fgf4$^{-/-}$

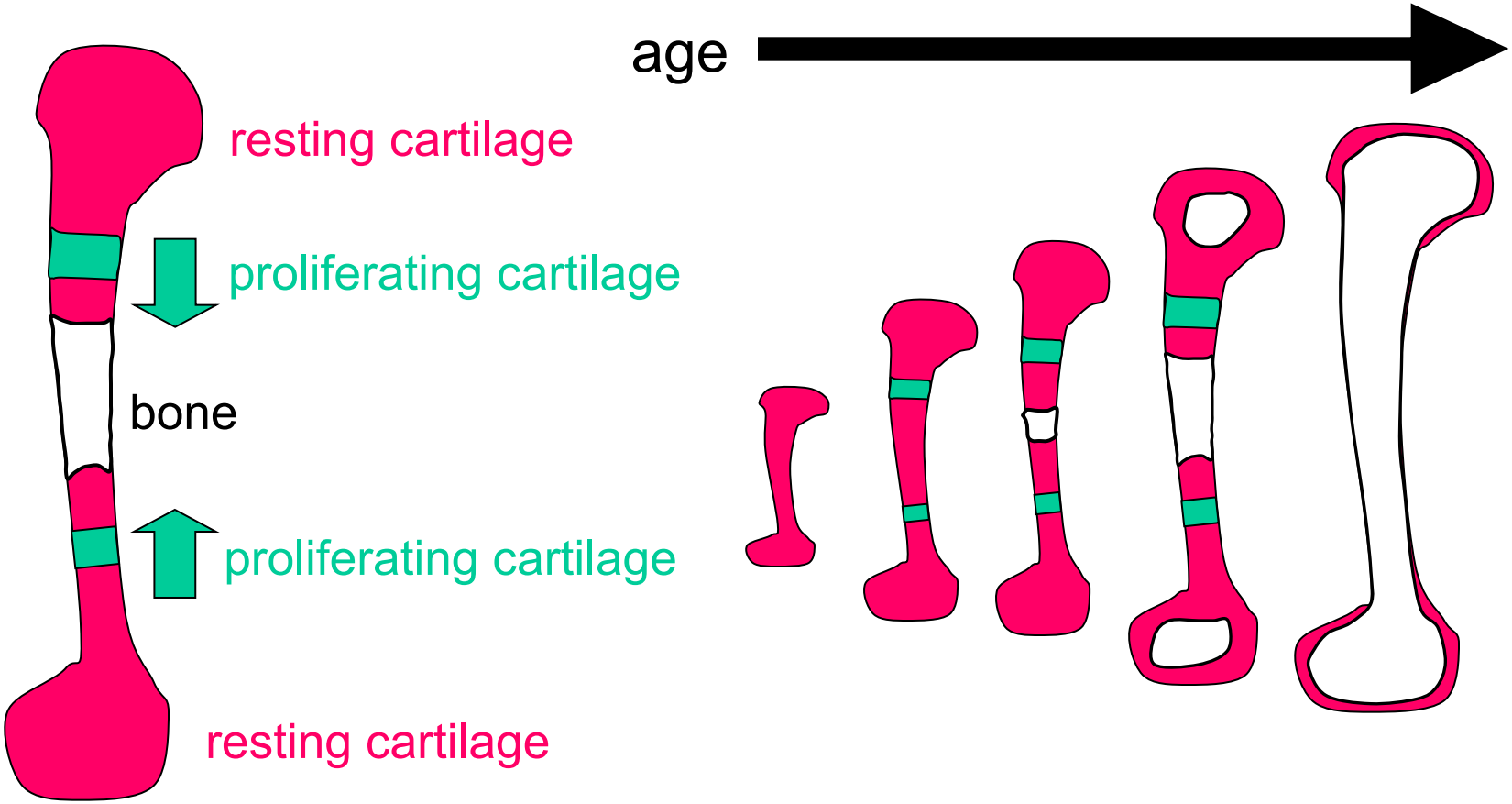


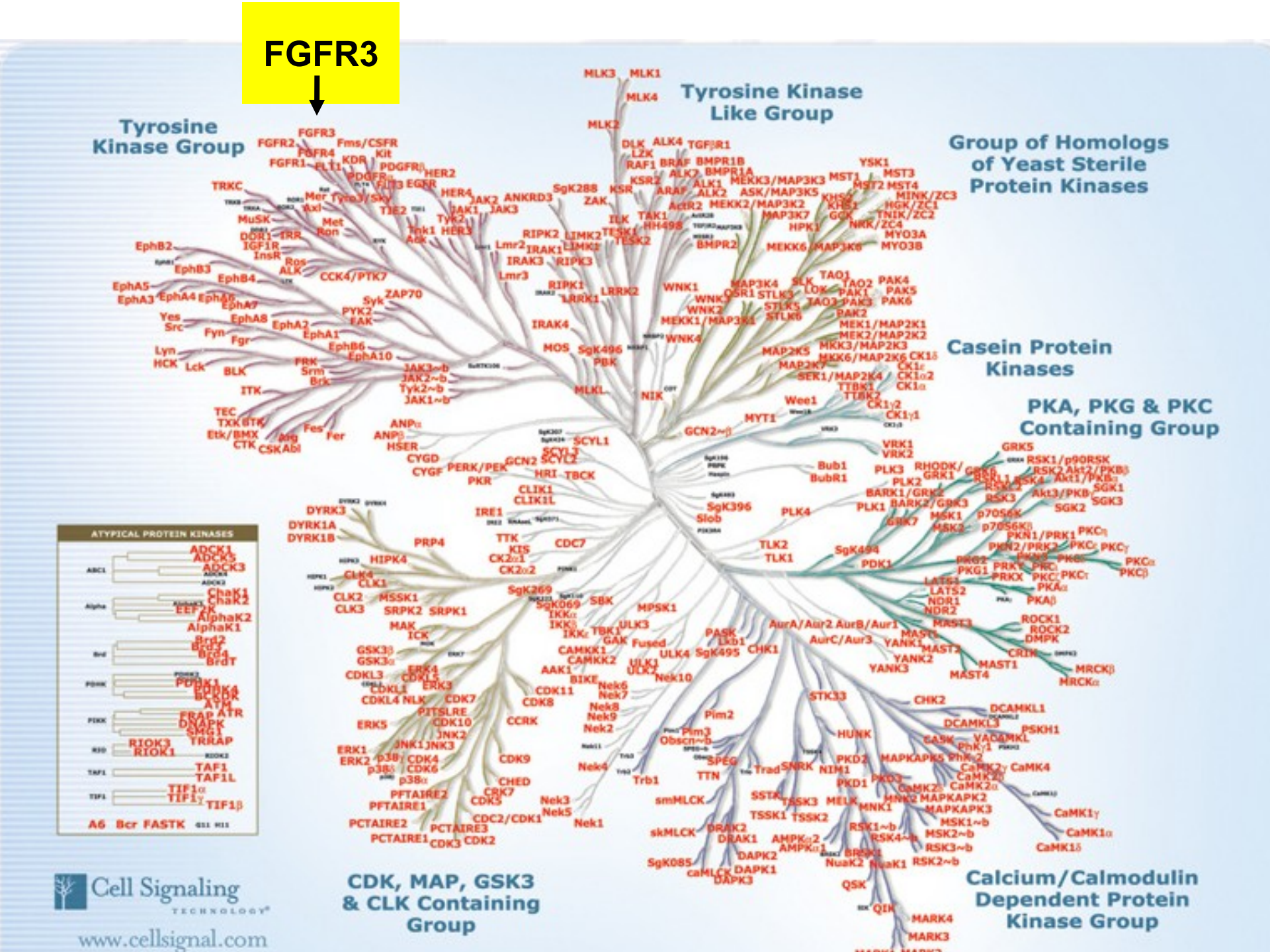
Forelimb

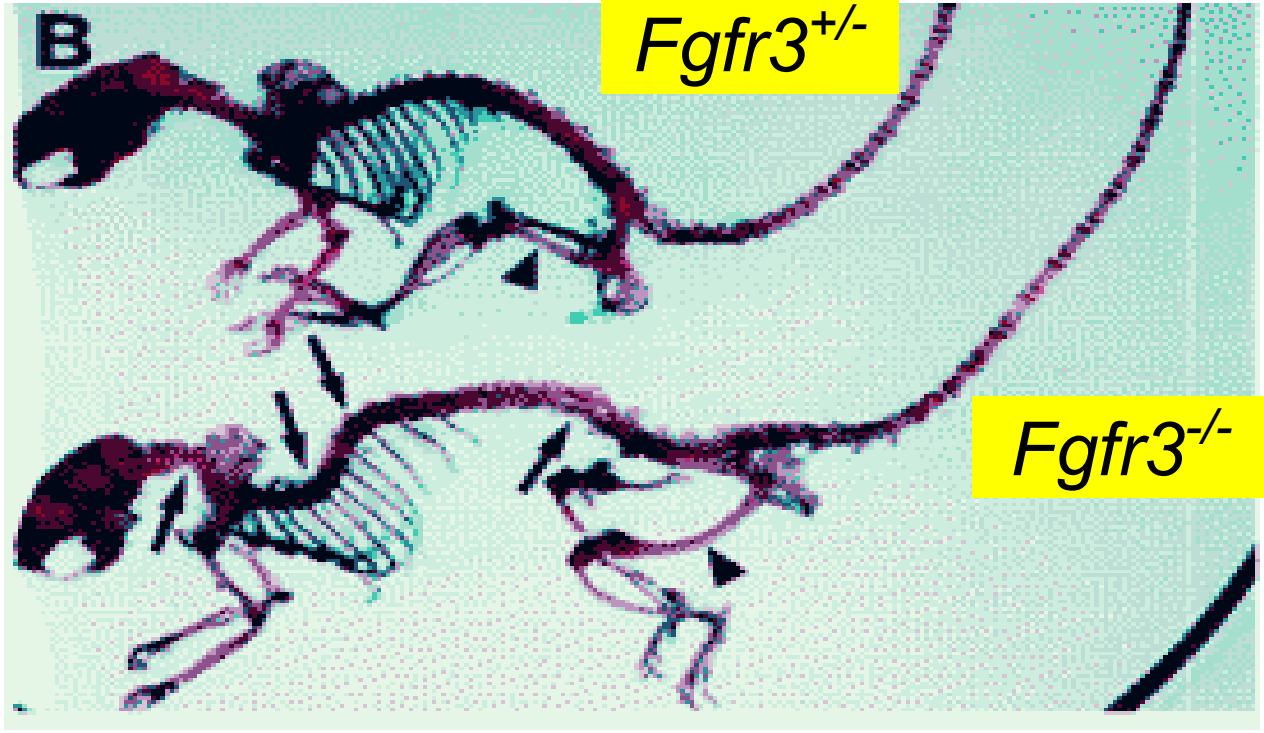


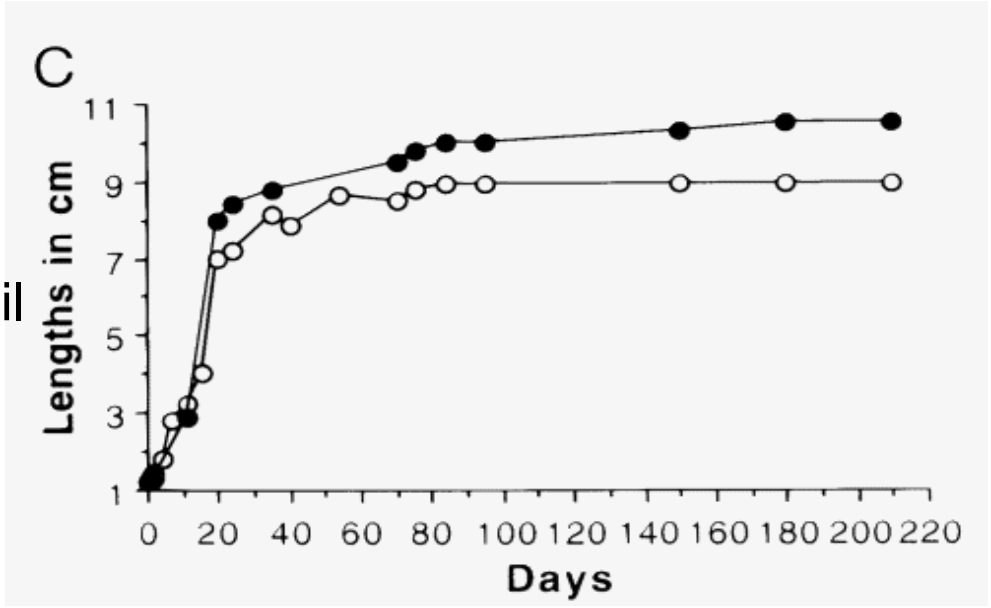

a Normal FL and HL
Fgf8
Fgf4
Fgf9, Fgf17
Initial size
Size at condensation
S
Z
A
1
2
3
AER KO mutant phenotypes
b Fgf8 single KO FL
c Fgf4; Fgf8 double KO FL
d Fgf8 single KO HL
e Fgf4; Fgf8 double KO HL
No skeletal development

How do the limbs grow?




FGFR3
Tyrosine Kinase Group
Tyrosine Kinase Like Group
Group of Homologs of Yeast Sterile Protein Kinases
Casein Protein Kinases
PKA, PKG & PKC Containing Group
Calcium/Calmodulin Dependent Protein Kinase Group
CDK, MAP, GSK3 & CLK Containing Group
ATYPICAL PROTEIN KINASES
A6 Bcr FASTK
Cell Signaling TECHNOLOGY
www.cellsignal.com





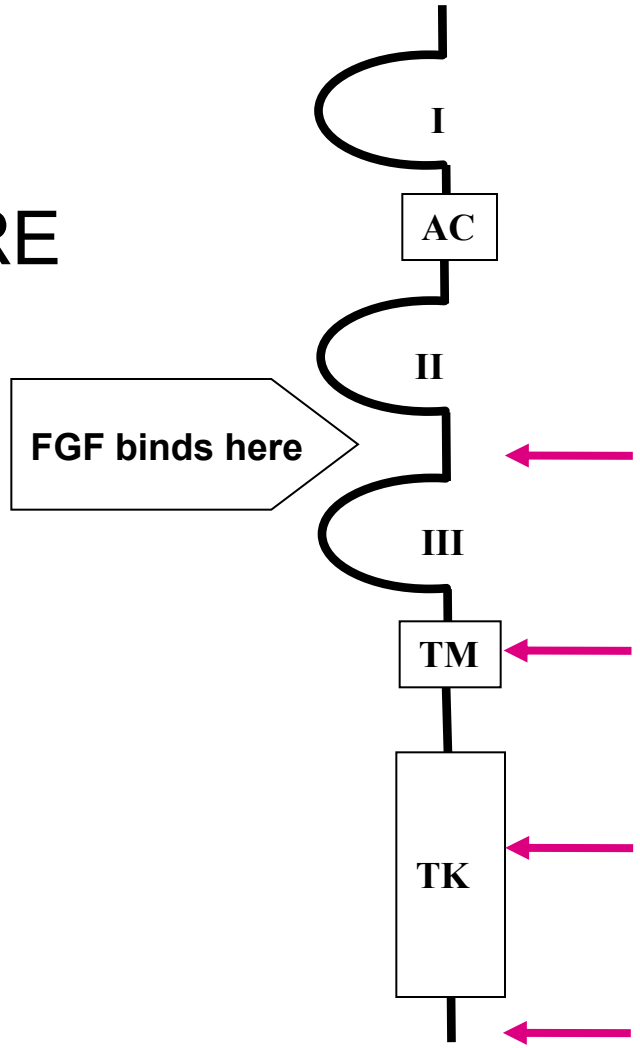
Tail

Fgfr3+/-
Fgfr3-/-

FGFR3-related skeletal dysplasia

STATURE

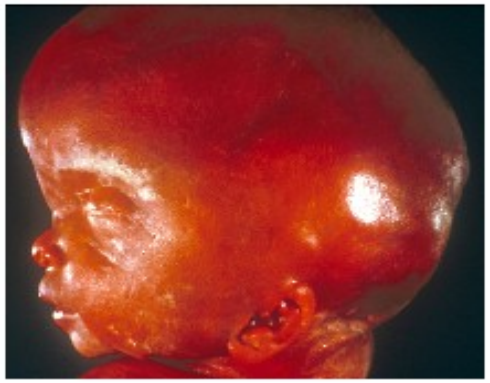
Hypochondroplasia
Achondroplasia
SADDAN
Thanatophoric Dysplasia



FGFR3-related skeletal dysplasia

Achondroplasia

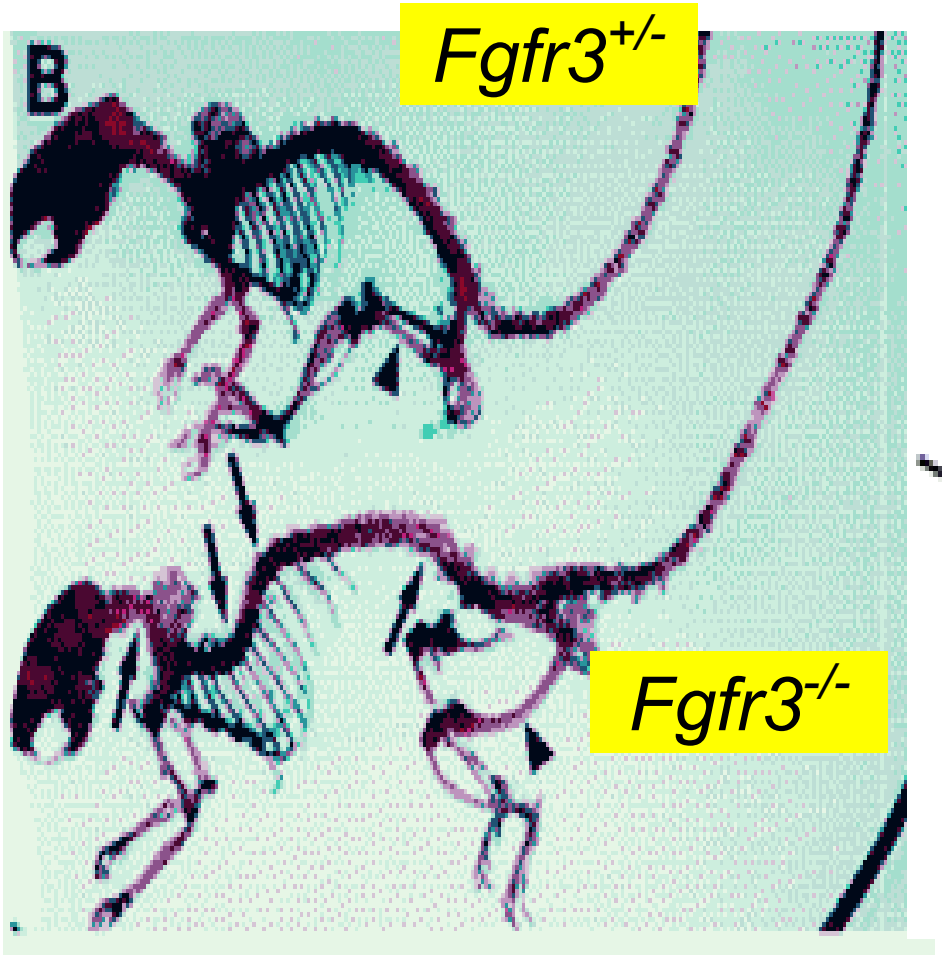
Thanatophoric Dysplasia

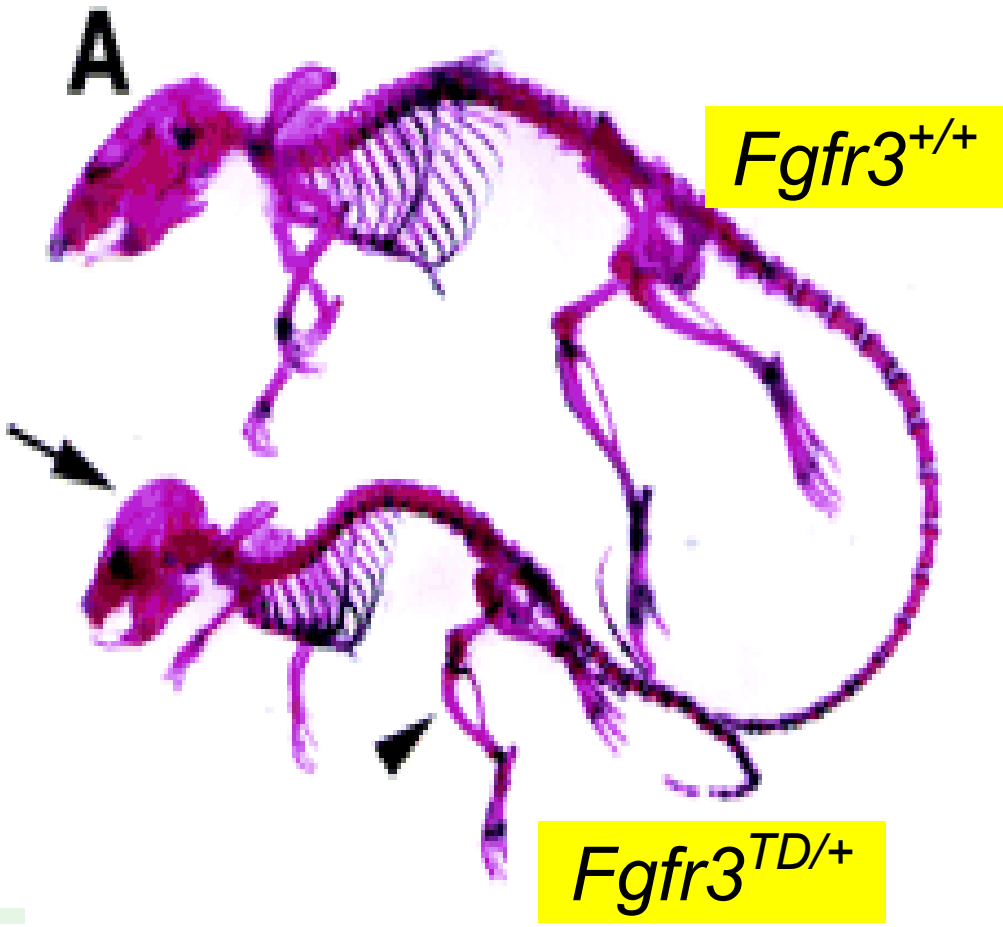


healthy

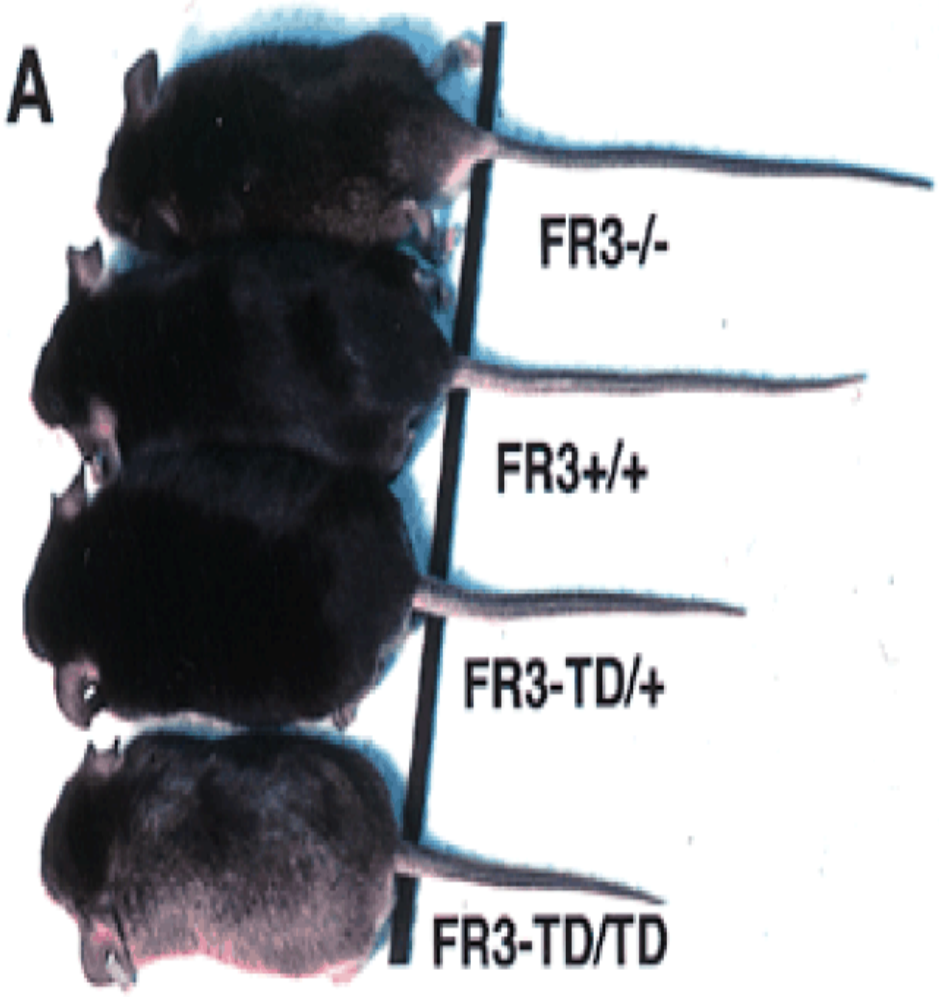
TD

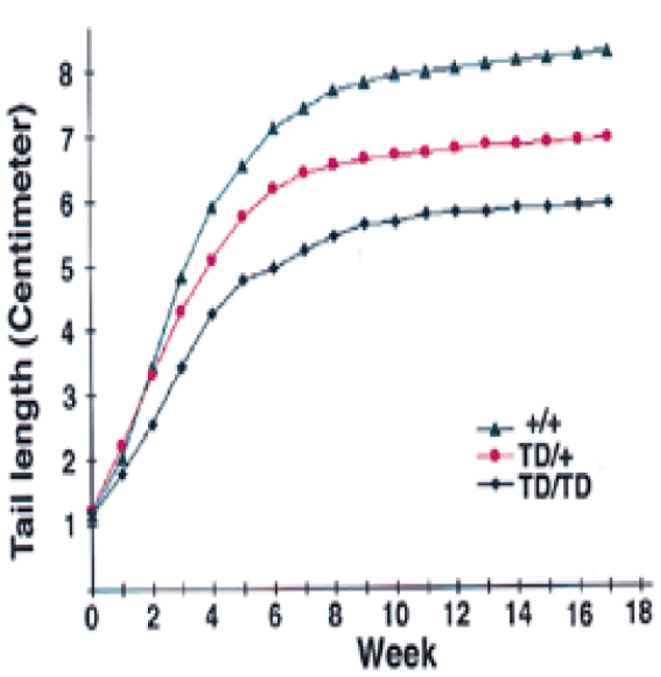
- short long bones
- brachydactyly
- macrocephaly
- low nasal bridge
- spinal stenosis
- temporal lobe malformations

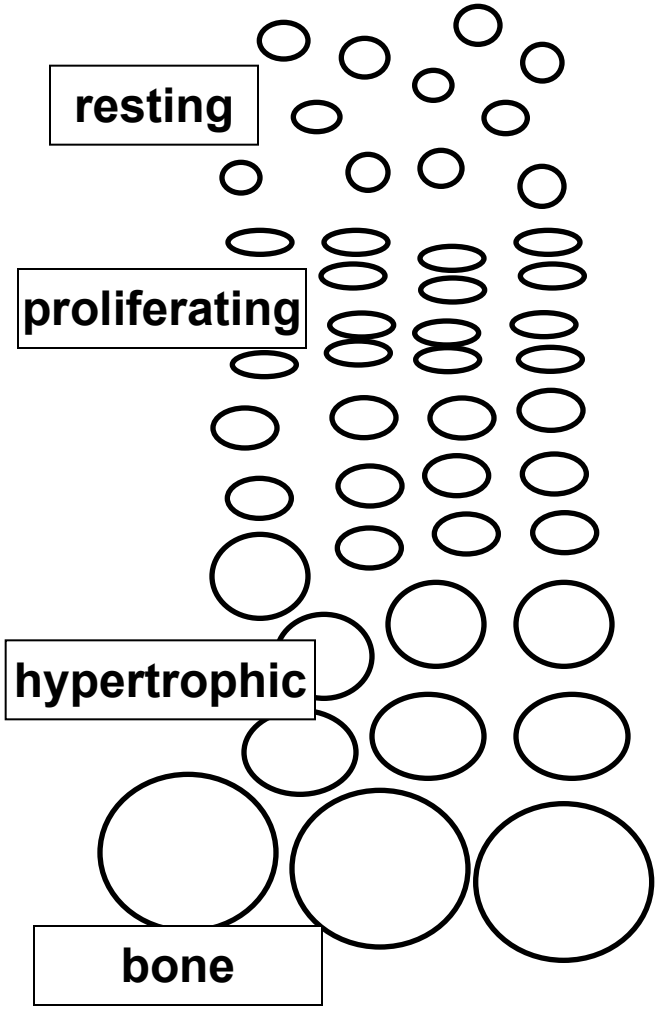


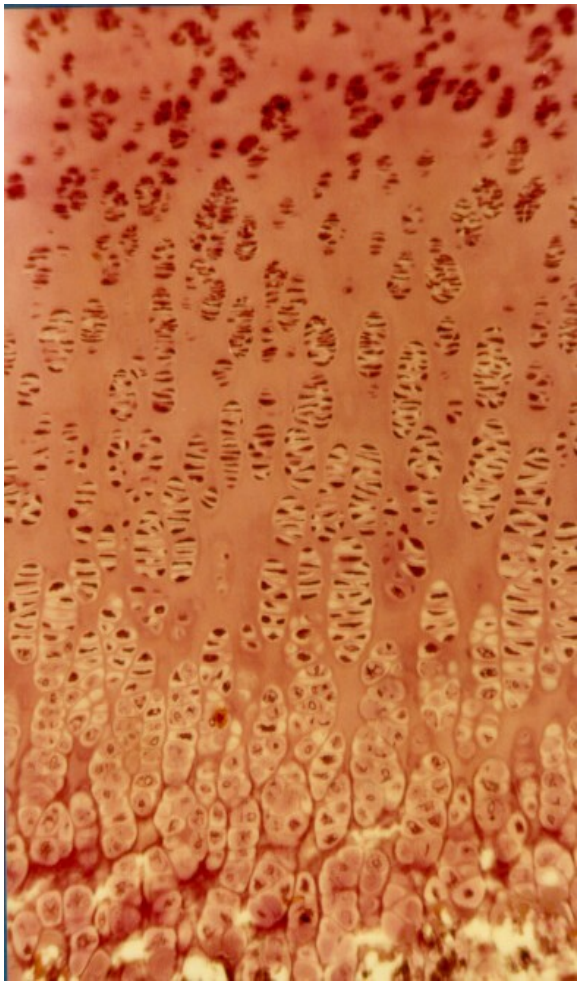


Loss-of-function vs. Gain-of-function

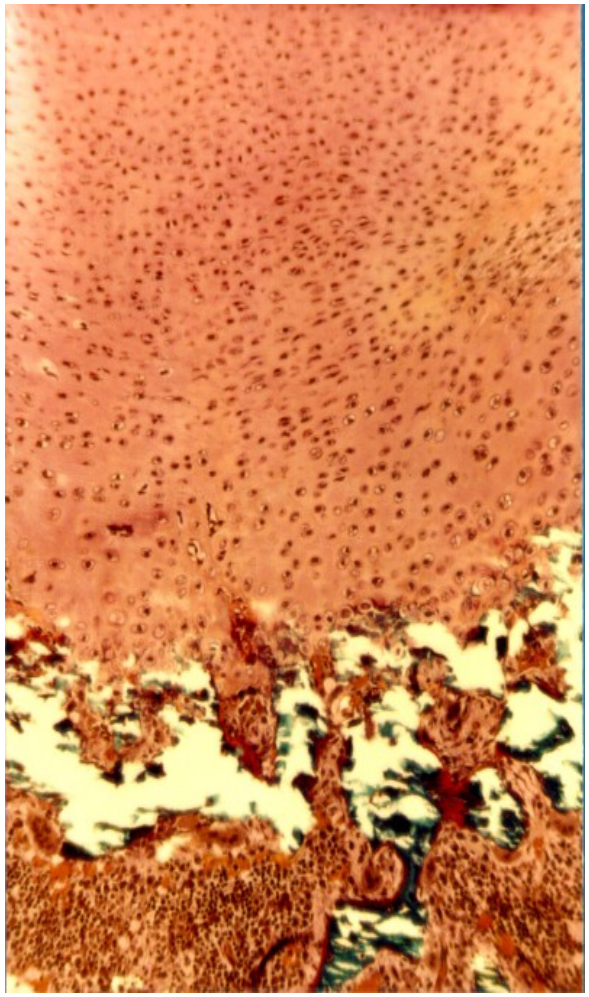

A
FR3-/-
FR3+/+
FR3-TD/+
FR3-TD/TD


B
Tail length (Centimeter)
1
2
3
4
5
6
7
8
0
2
4
6
8
10
12
14
16
18
Week
+/+
TD/+
TD/TD



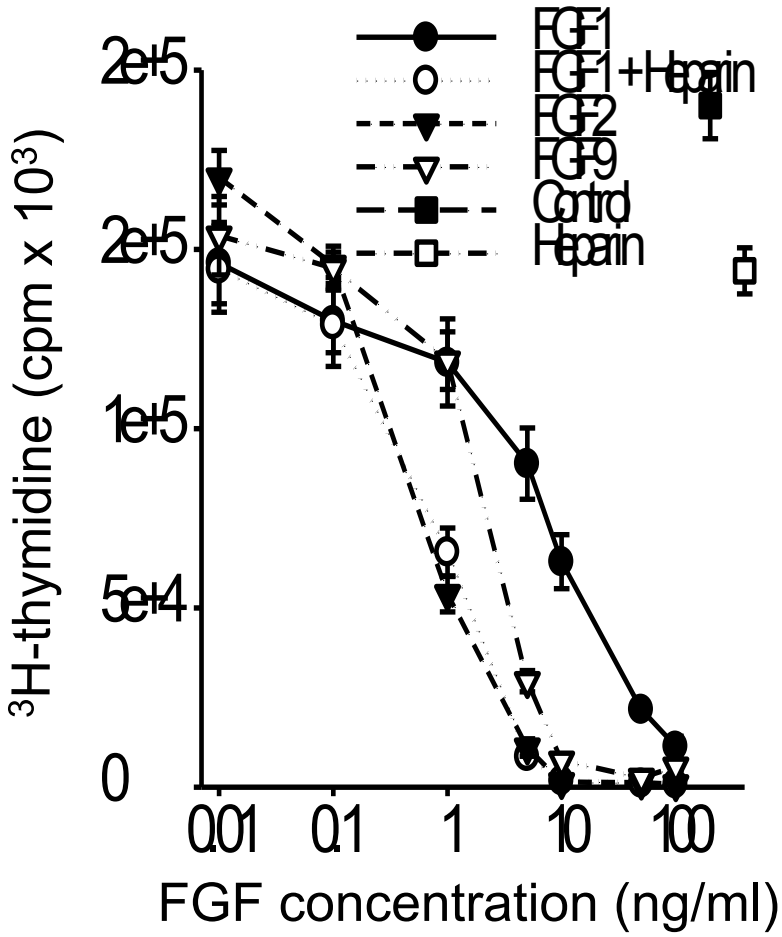


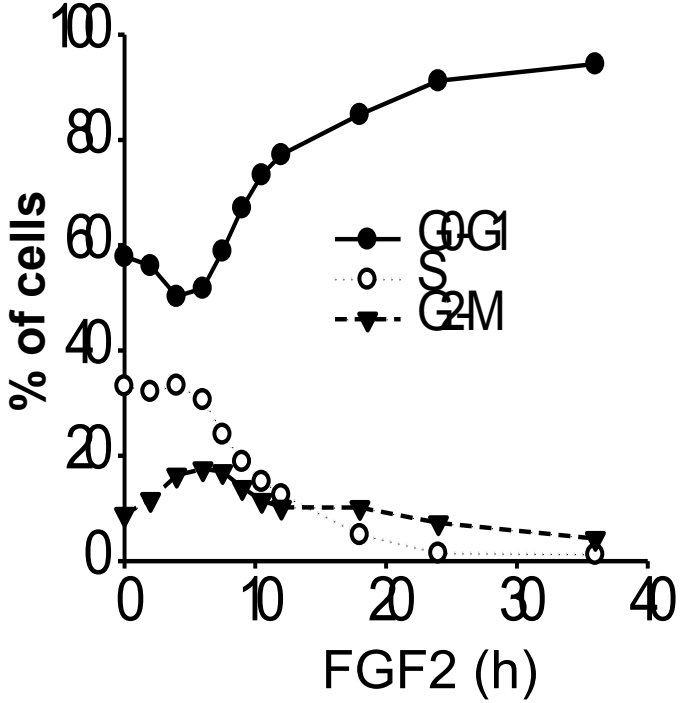
healthy



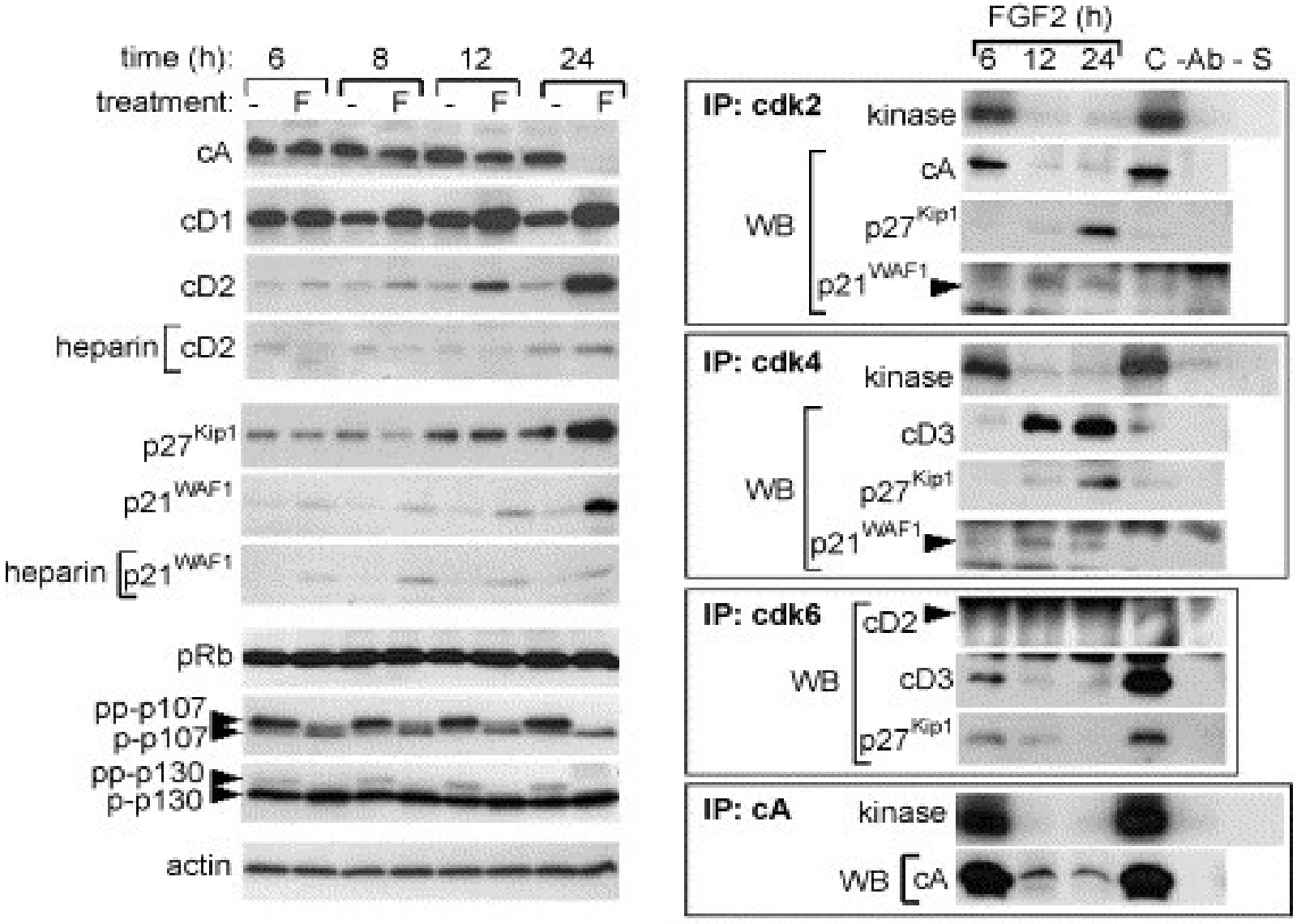
TD

FGFR3 inhibits chondrocyte proliferation by arresting their cell cycle in G1 phase

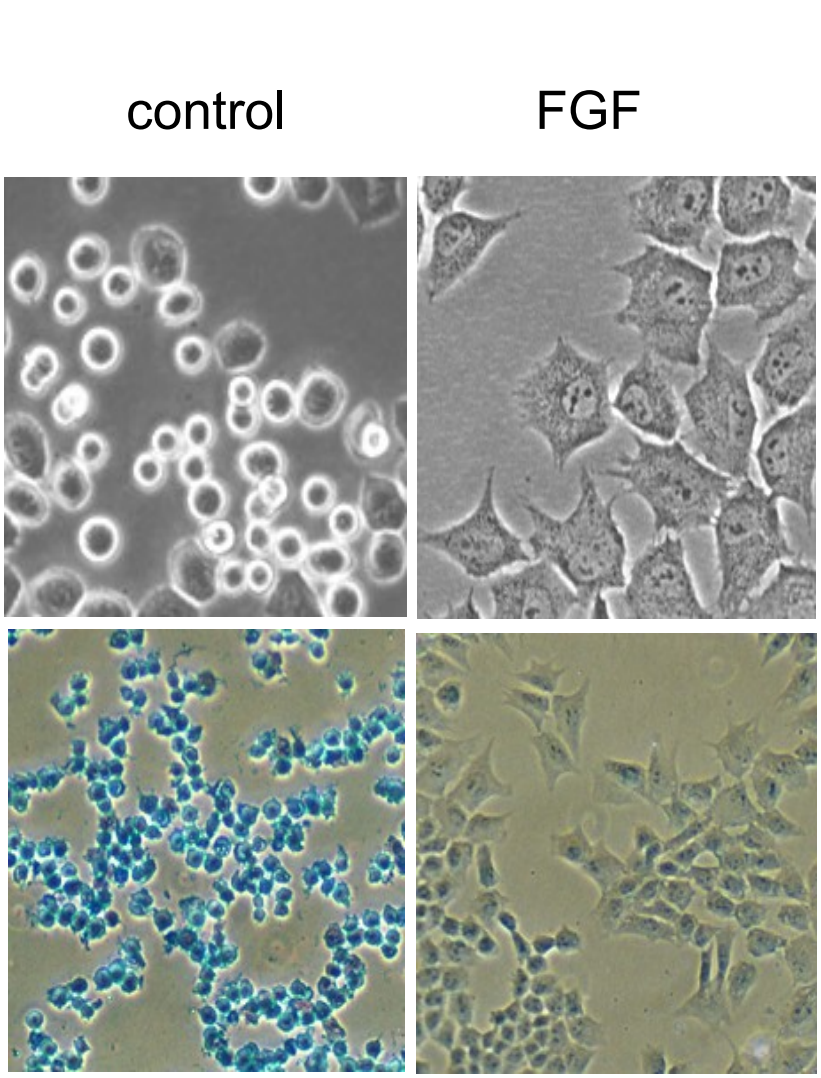


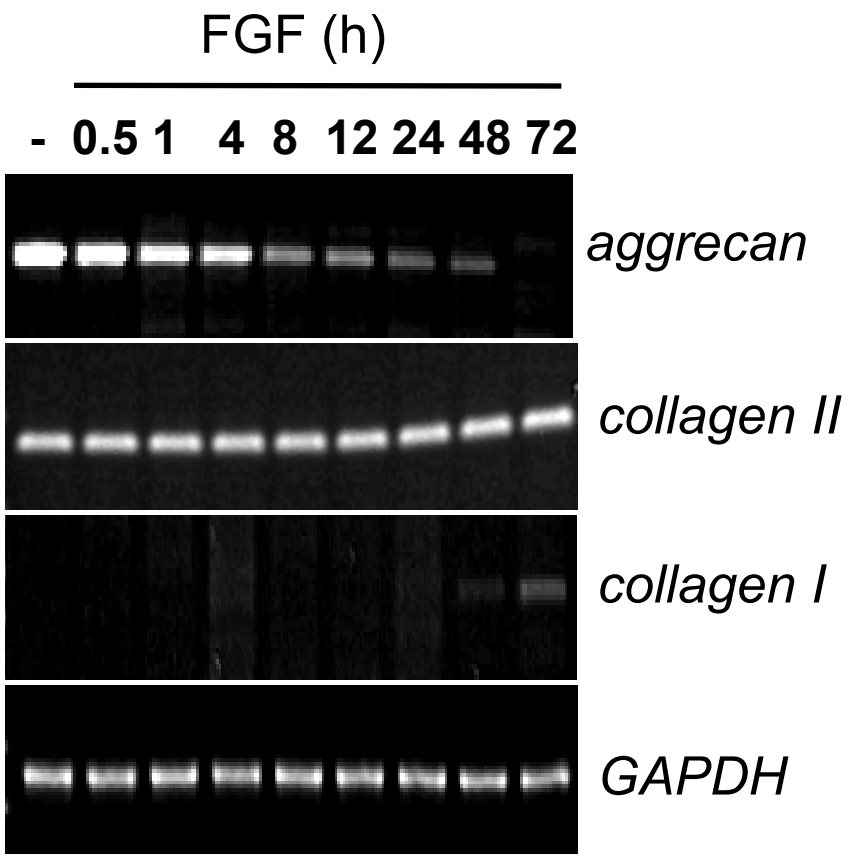


….via inhibition of cdk activity necessary for progression through the G1 phase of a cell cycle

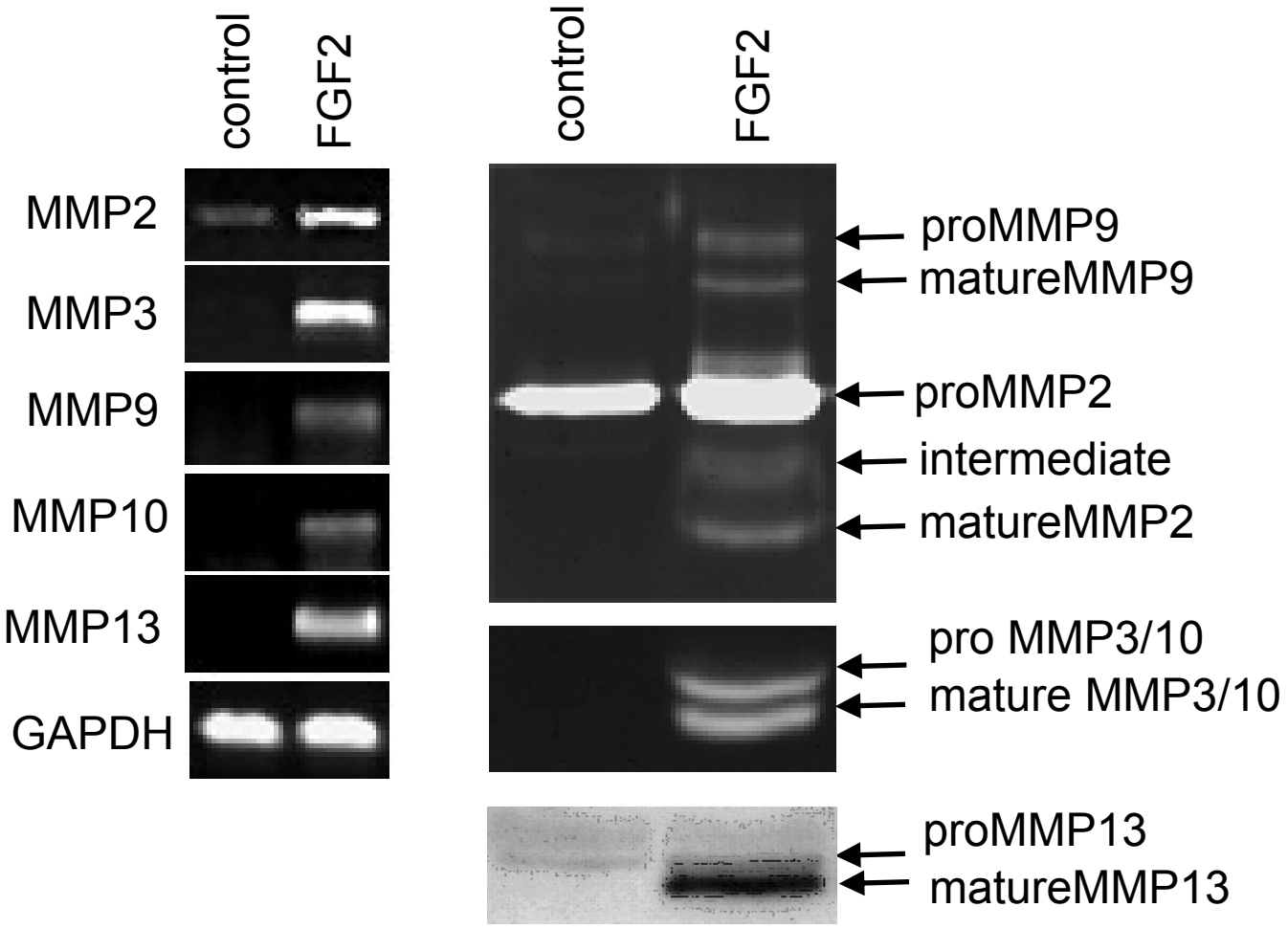


FGF alters the cartilage-like phenotype of chondrocytes

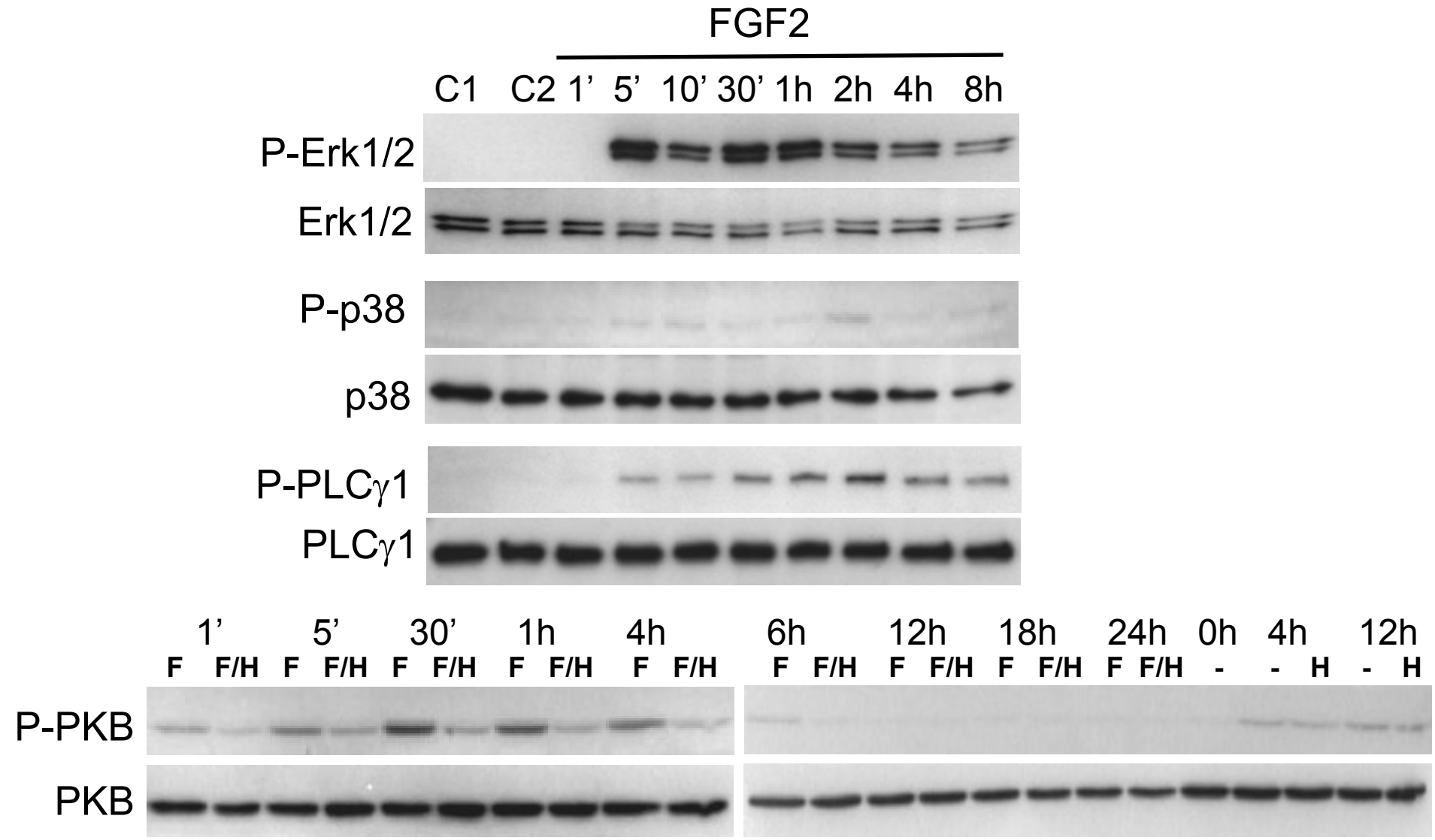




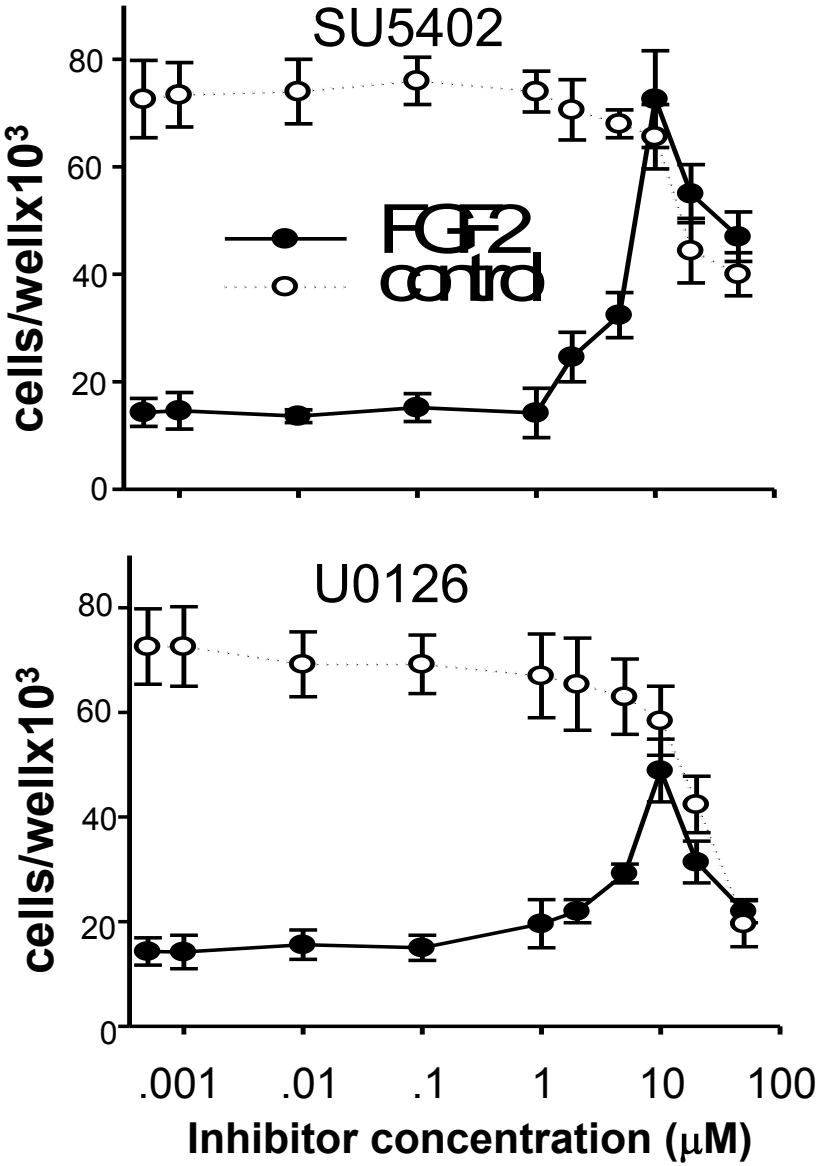
……via MMP-mediated degradation of extracellular matrix

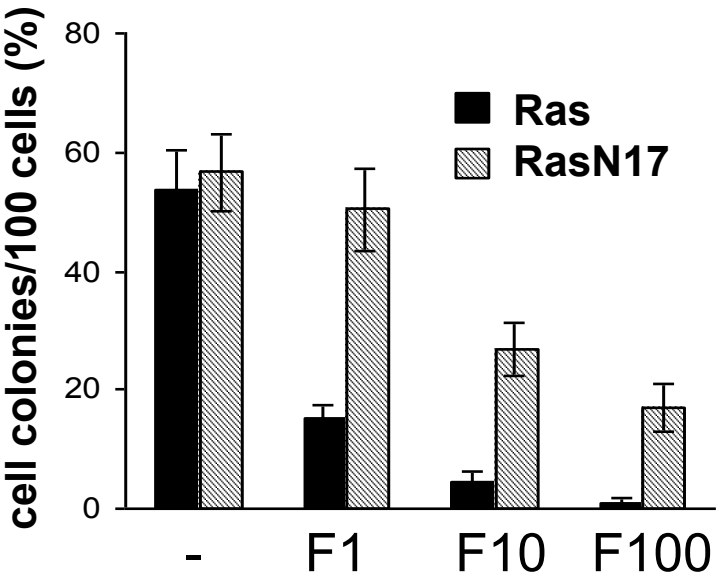


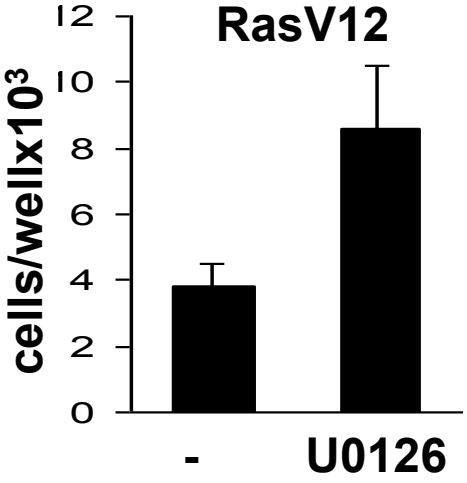
FGF2 activates Erk and p38 MAPK, PLCγ and PKB in chondrocytes



……but only Ras/Erk activity is involved in FGF-induced growth arrest



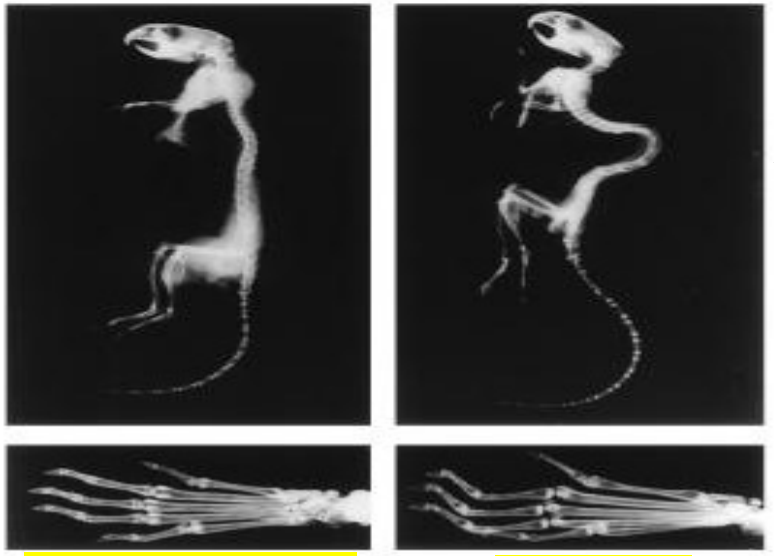


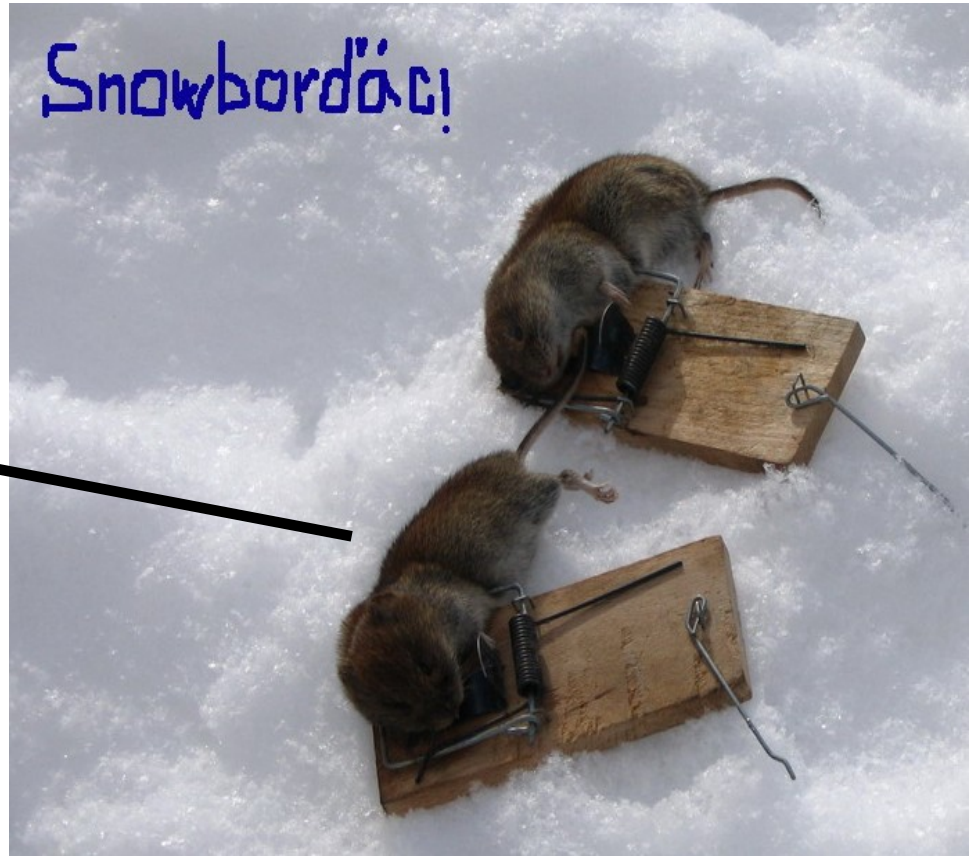


Erk MAP kinase activity is necessary for FGFR3 phenotype in cartilage

FGF2
↓
FGFR3
↓
FRS2
↓
Ras
↓
Raf-1
↓
MEK
↓
Erk →

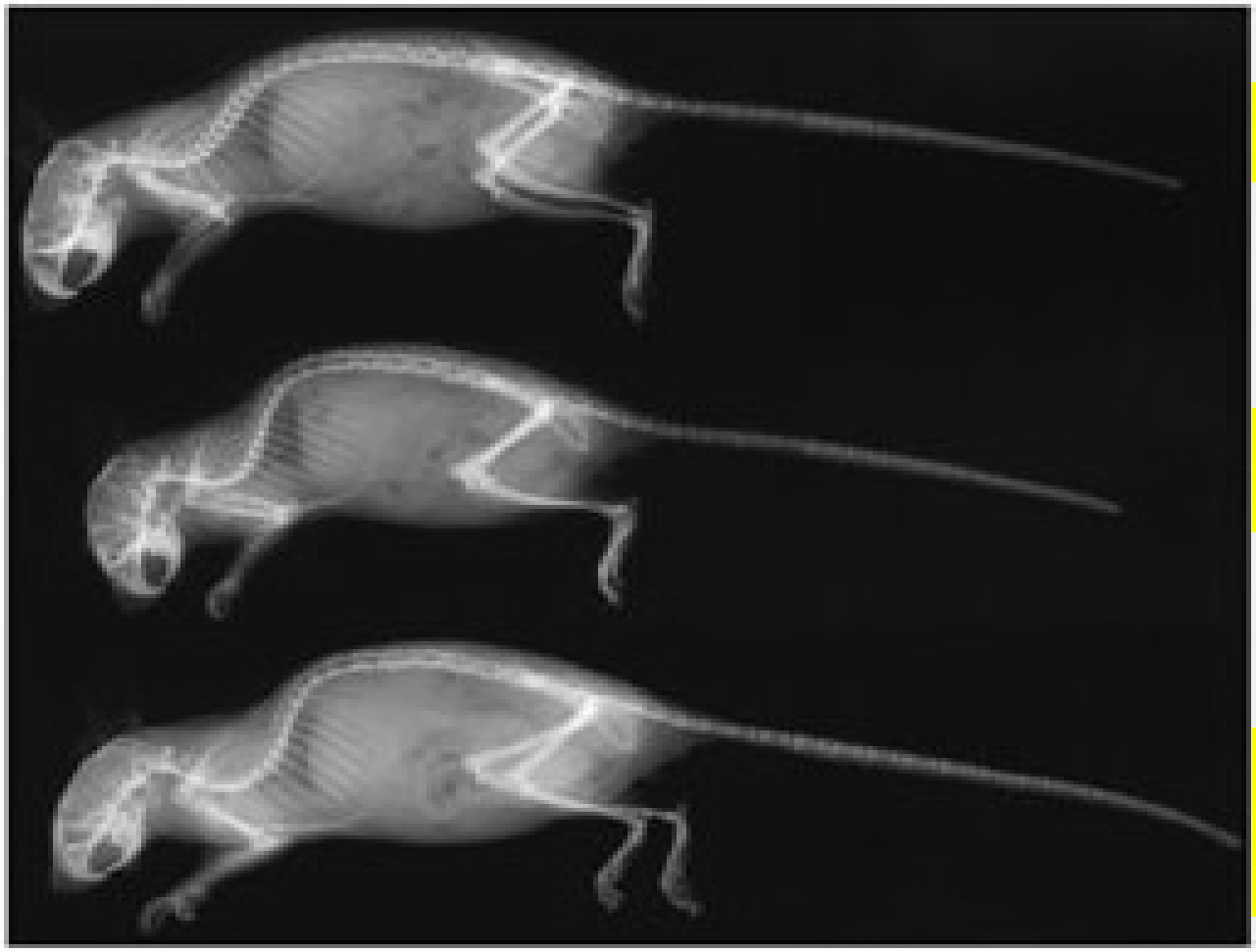
C-type Natriuretic Peptide (CNP) over-expression results in skeleton overgrowth in mice





CNP over-expression???

CNP rescues dwarfism caused by ACH mutation in FGFR3

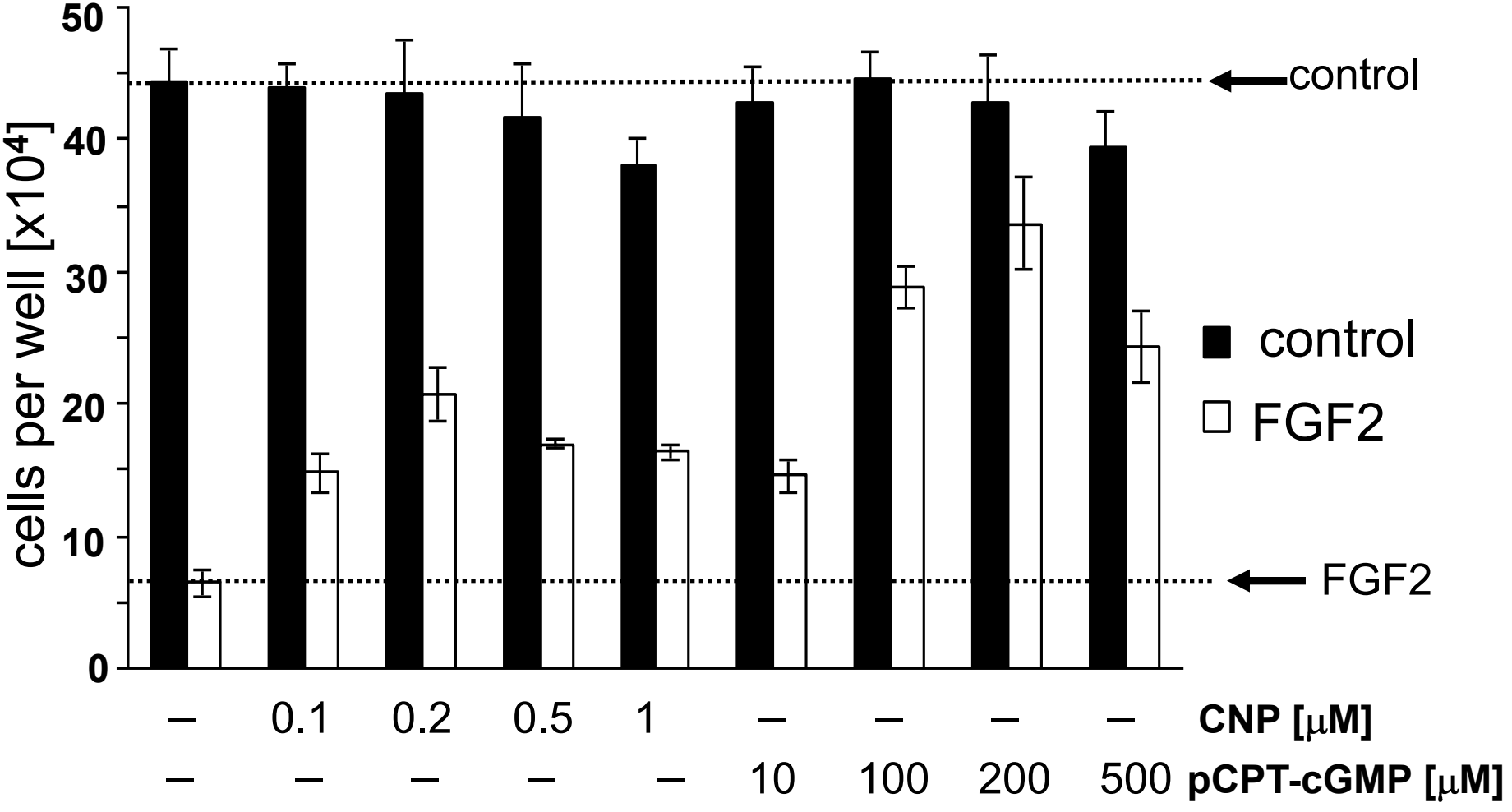


wild-type

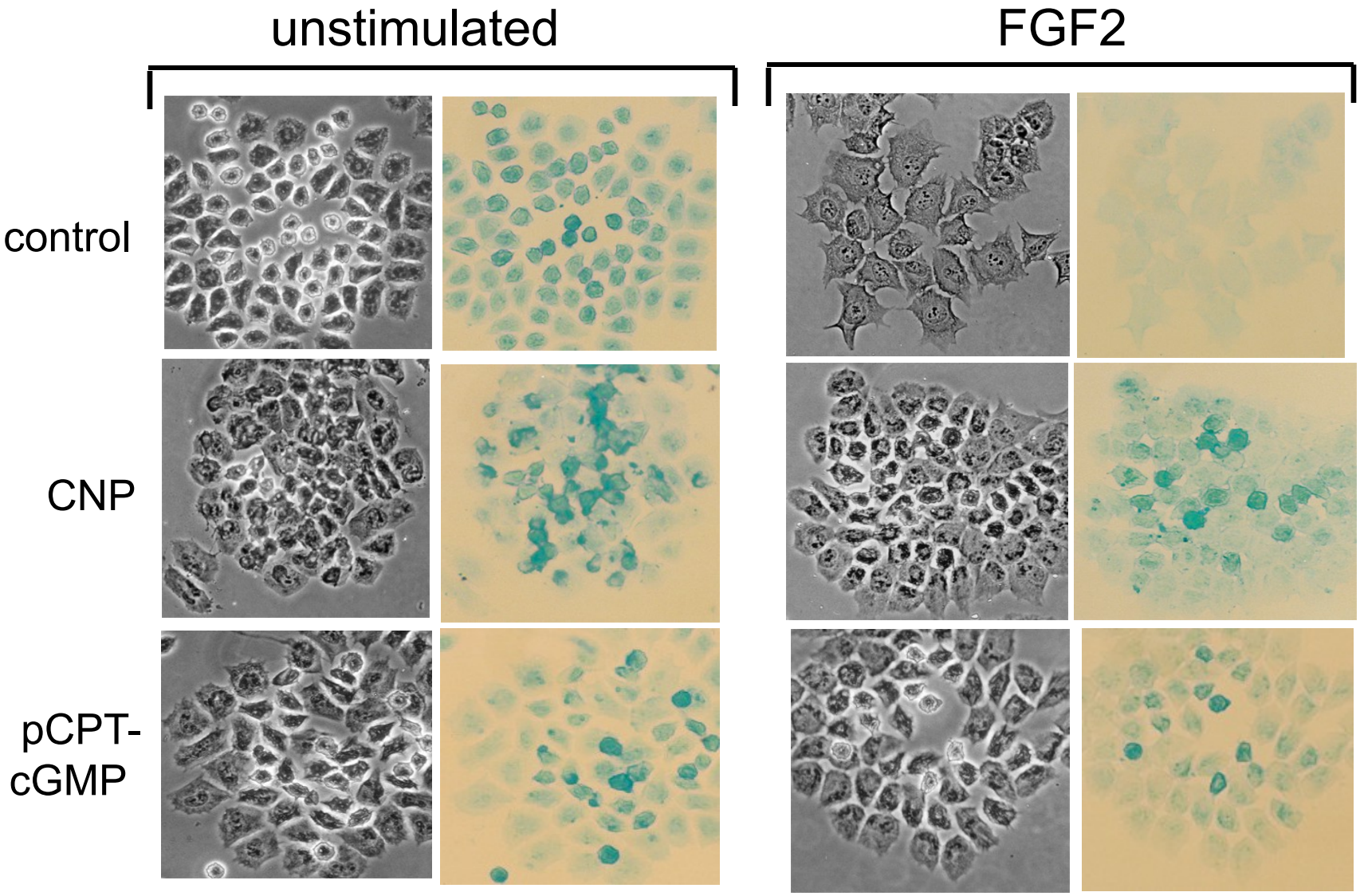
Fgfr3^{Ach}

Fgfr3$^{Ach/CNP}$ ↑

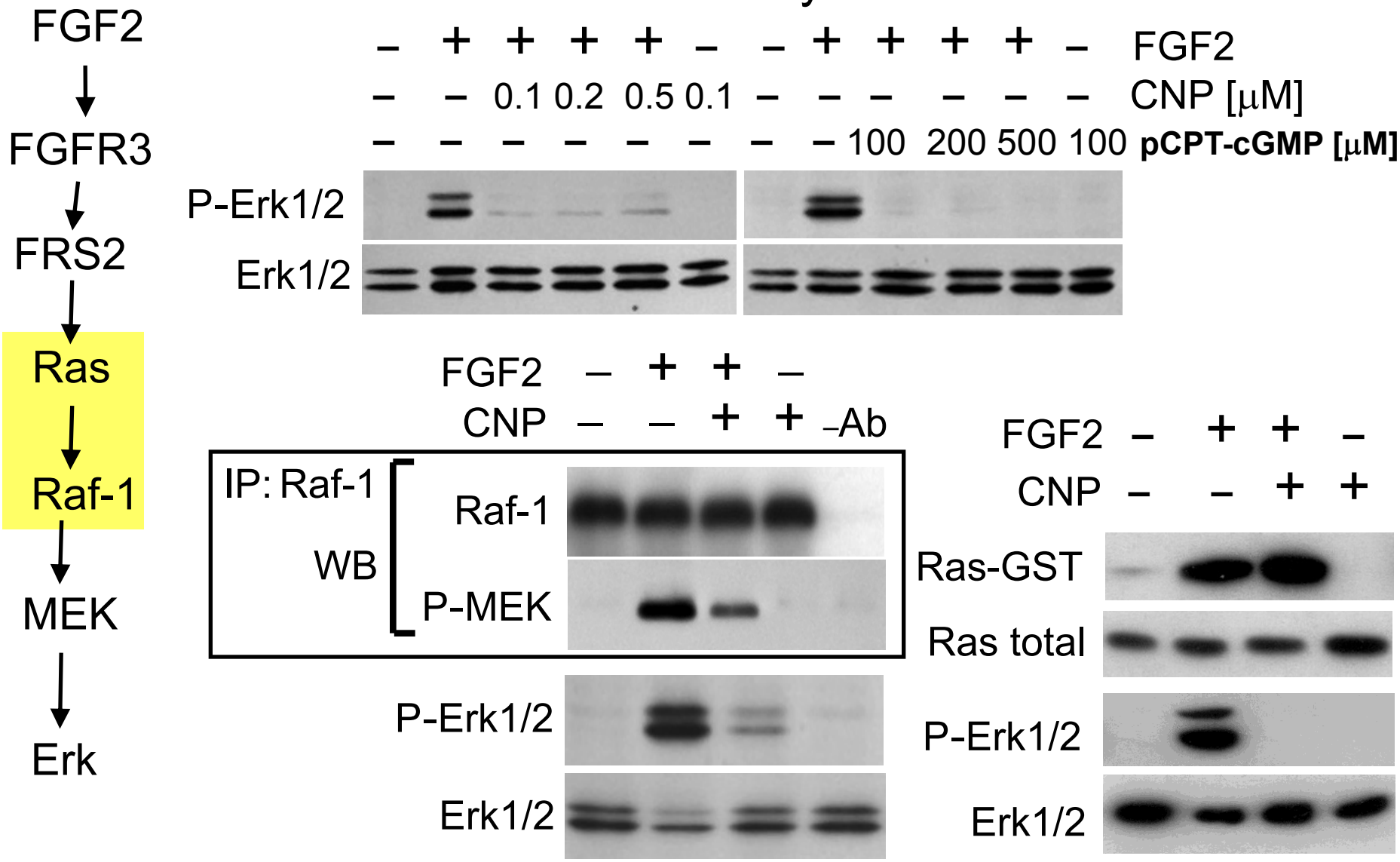
CNP counteracts FGF2-mediated chondrocyte growth arrest through cGMP-dependent pathway



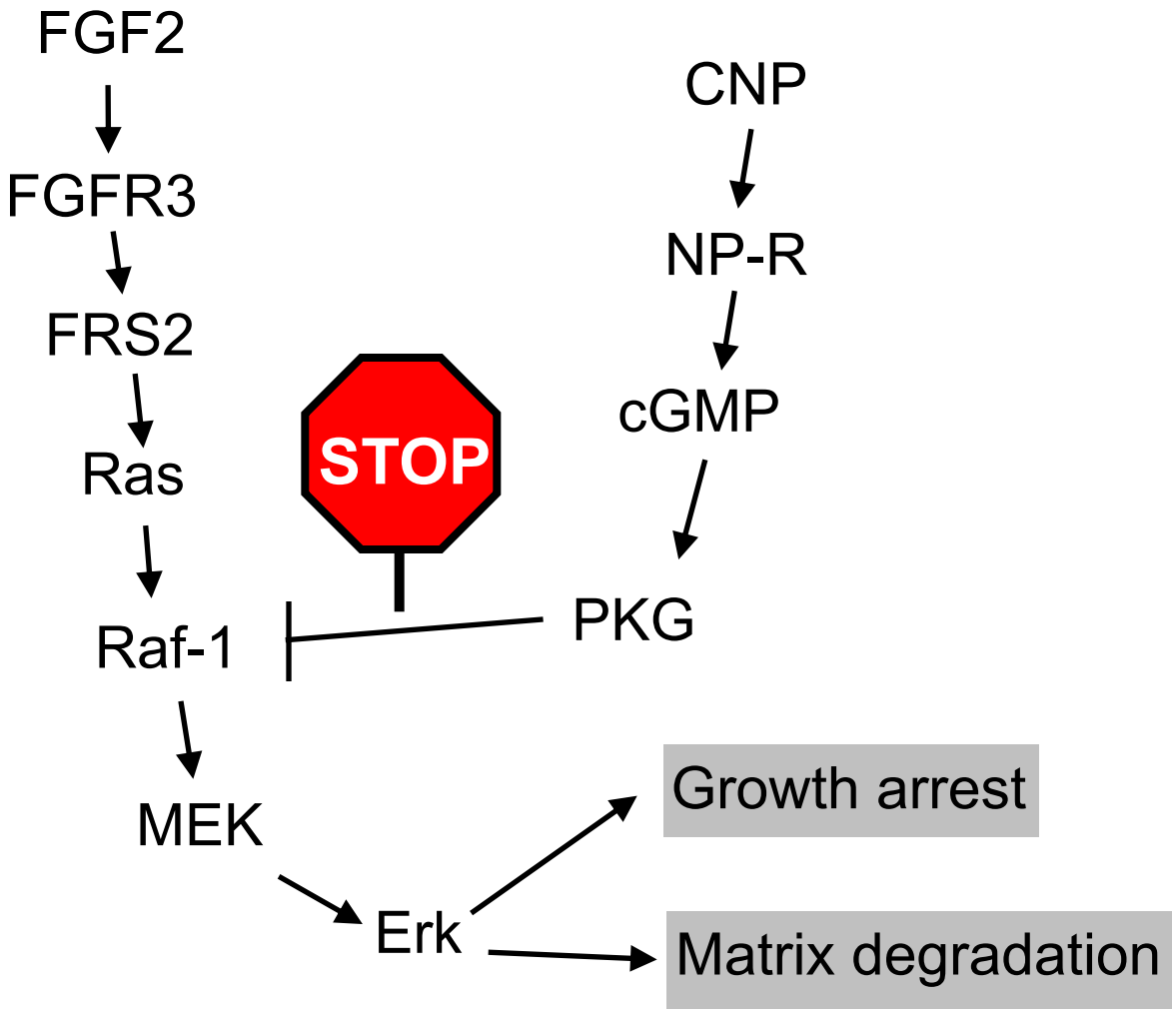
CNP antagonizes FGF2-mediated loss of cartilage extracellular matrix in chondrocytes



CNP counteracts FGF2-mediated activation of Erk MAP kinase in chondrocytes

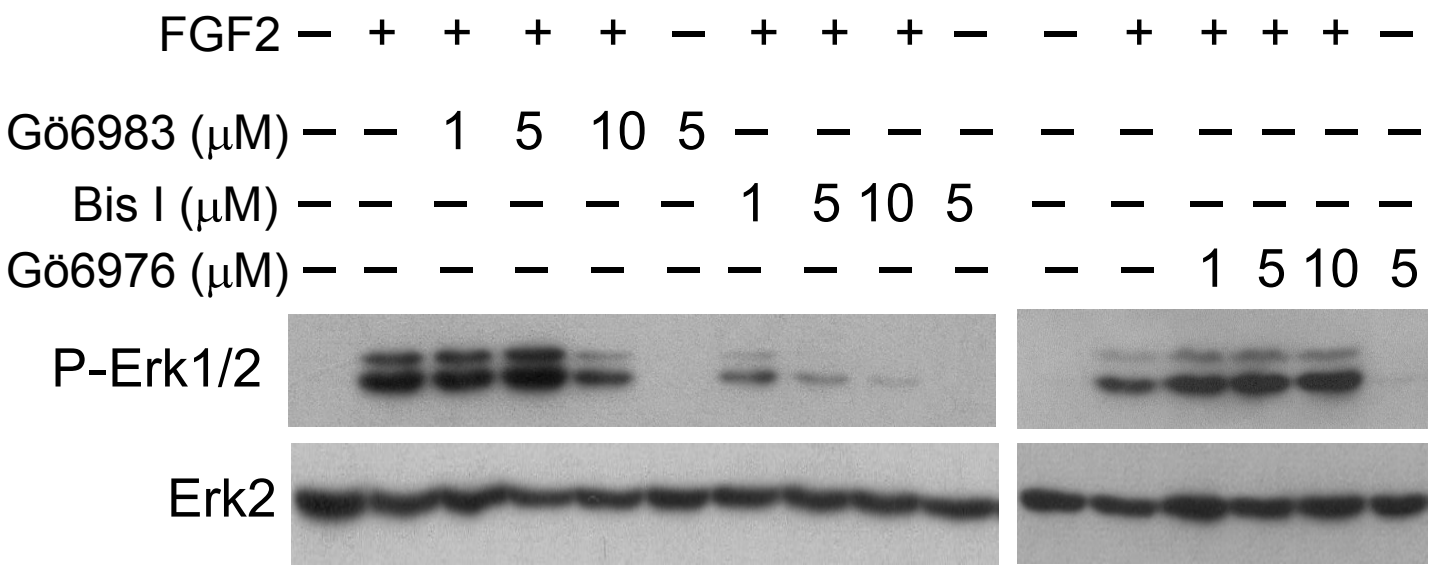


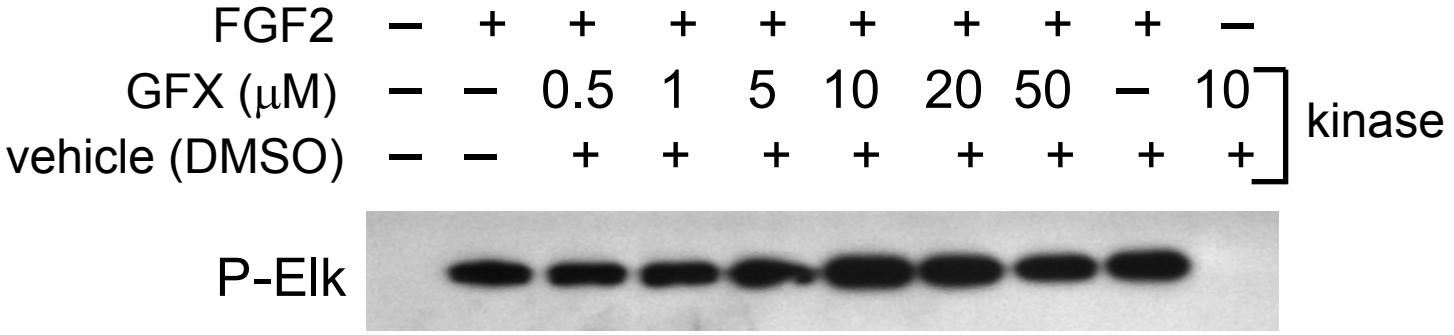
CNP inhibits Erk MAP kinase module at the Raf level

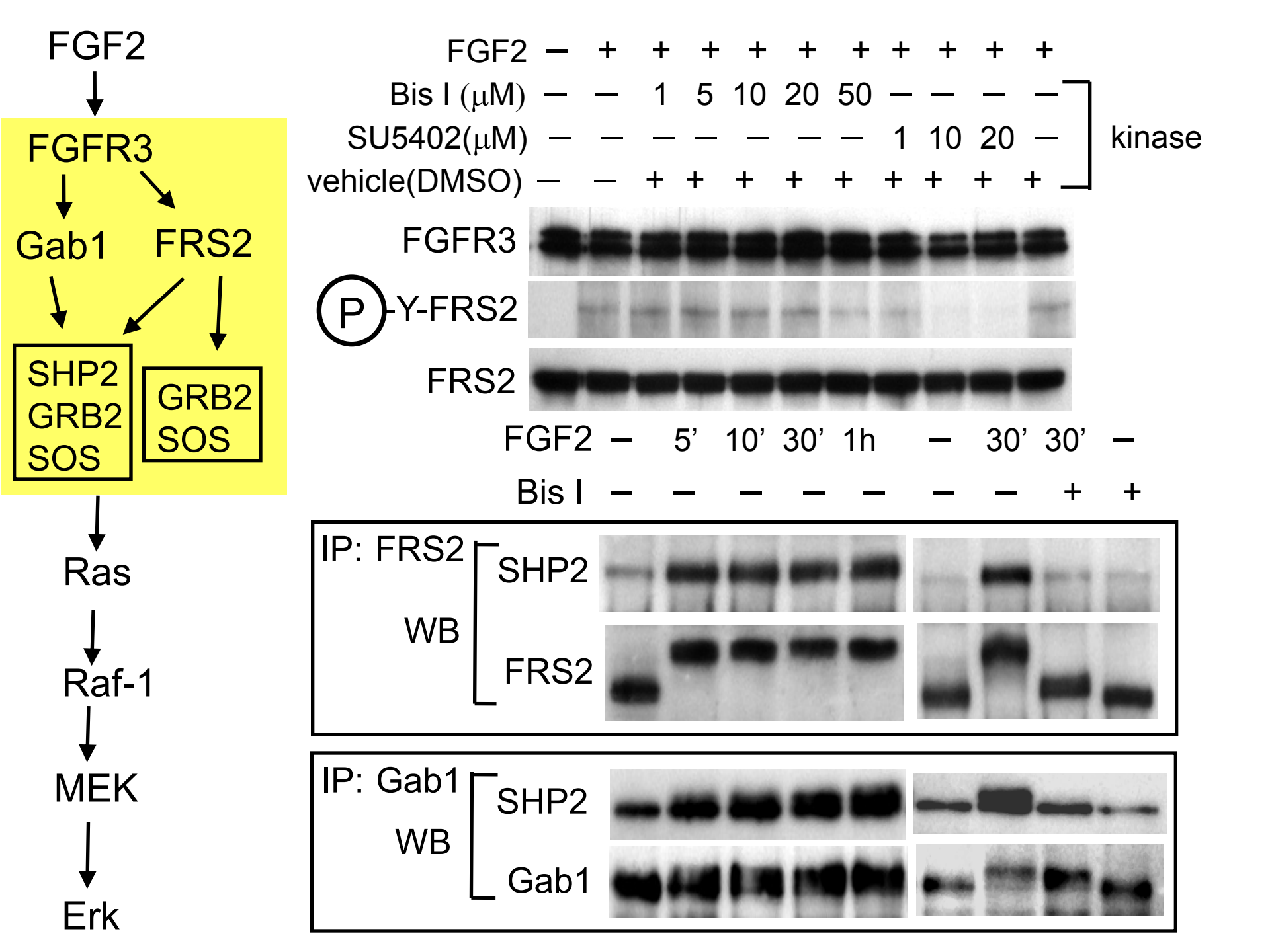


Is protein kinase C (PKC) involved in FGFR3-mediated activation of Erk in chondrocytes?

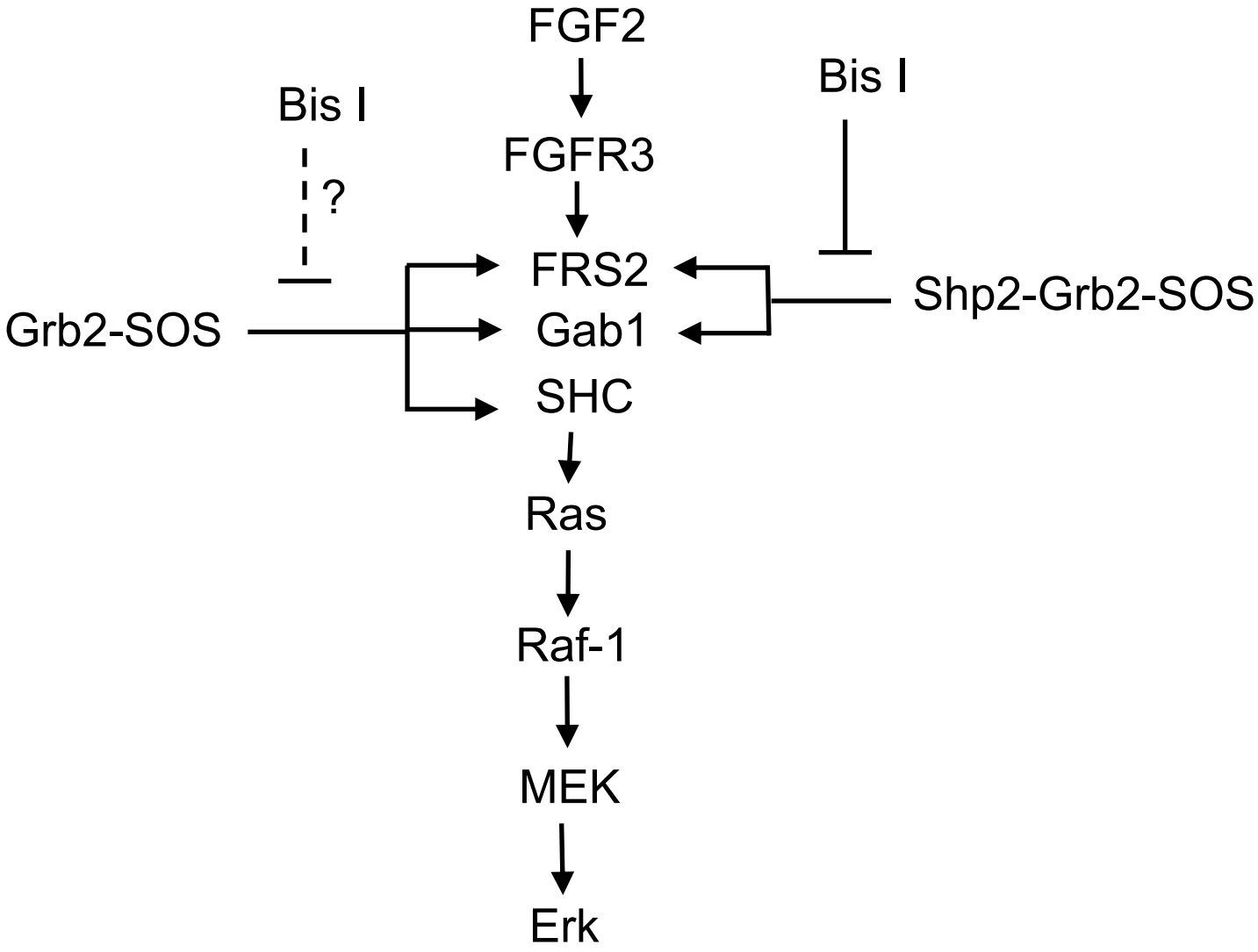
FGF2
↓
FGFR3
↓
FRS2
↓
Ras
↓
Raf-1
↓
MEK
↓
Erk



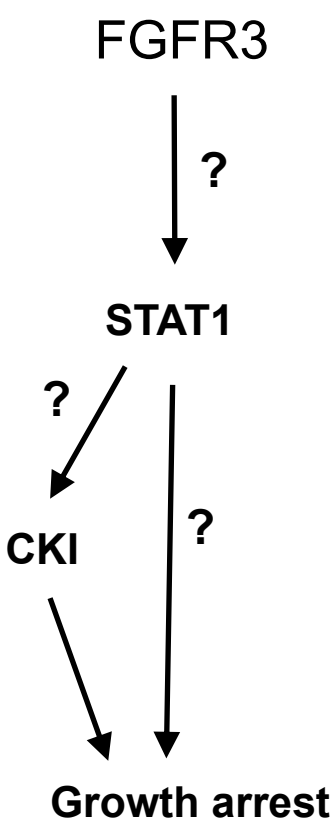


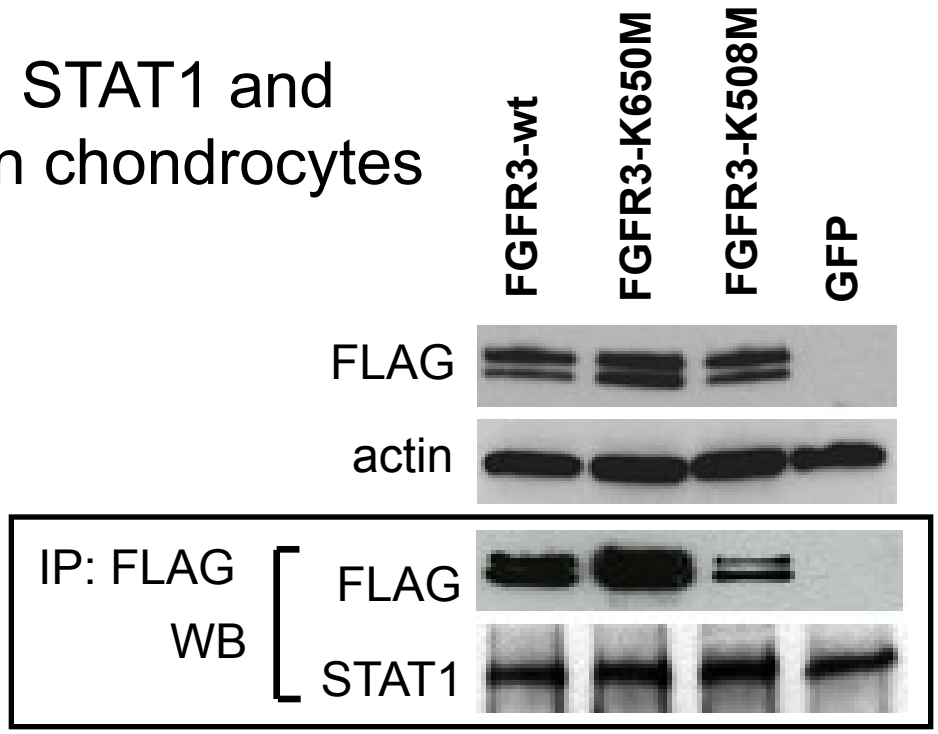

FGF2
FGFR3
Gab1
FRS2
SHP2
GRB2
SOS
GRB2
SOS
Ras
Raf-1
MEK
Erk
FGF2 – + + + + + + + + + +
Bis I (μM) – – 1 5 10 20 50 – – – –
SU5402(μM) – – – – – – – 1 10 20 –
vehicle(DMSO) – – + + + + + + + + +
kinase
FGFR3
P-Y-FRS2
FRS2
FGF2 – 5’ 10’ 30’ 1h – 30’ 30’ –
Bis I – – – – – – – + +
IP: FRS2
WB
SHP2
FRS2
IP: Gab1
WB
SHP2
Gab1

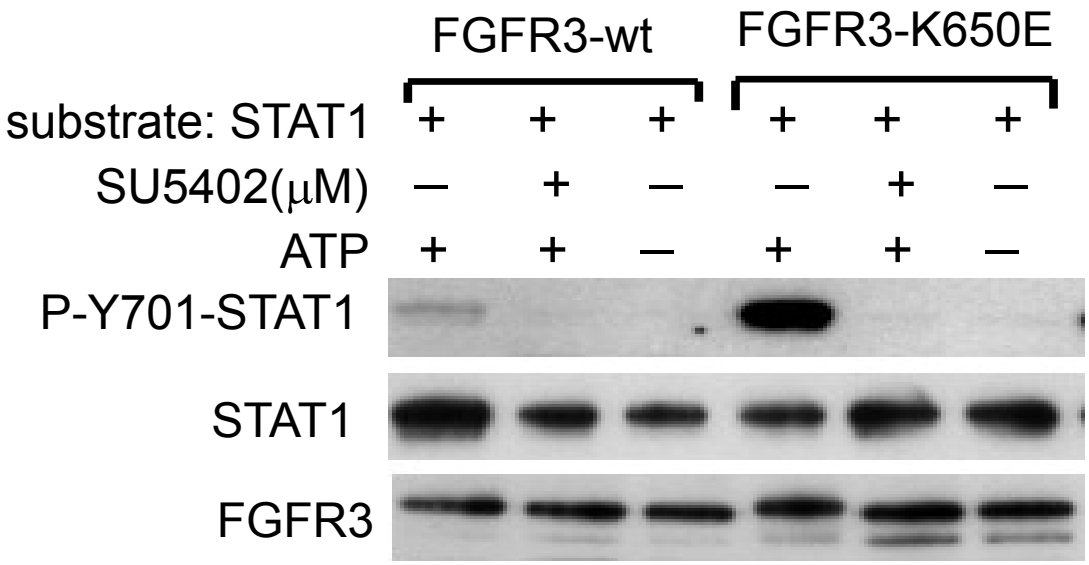
Protein kinase C inhibitor Bisindolylmaleimide I (Bis I)suppresses the FGF2-mediated activation of Erk MAP kinase pathway in chondrocytes by preventing the SHP2 association with FRS2 and Gab1 adaptor proteins



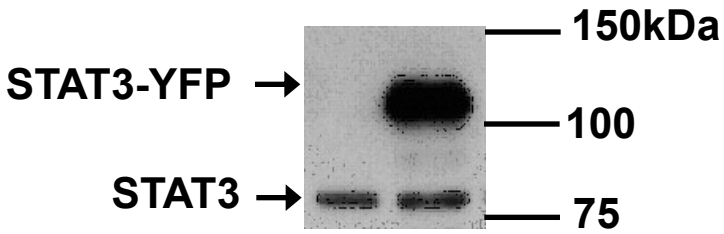
FGFR3 associates with STAT1 and acts as STAT1-kinase in chondrocytes

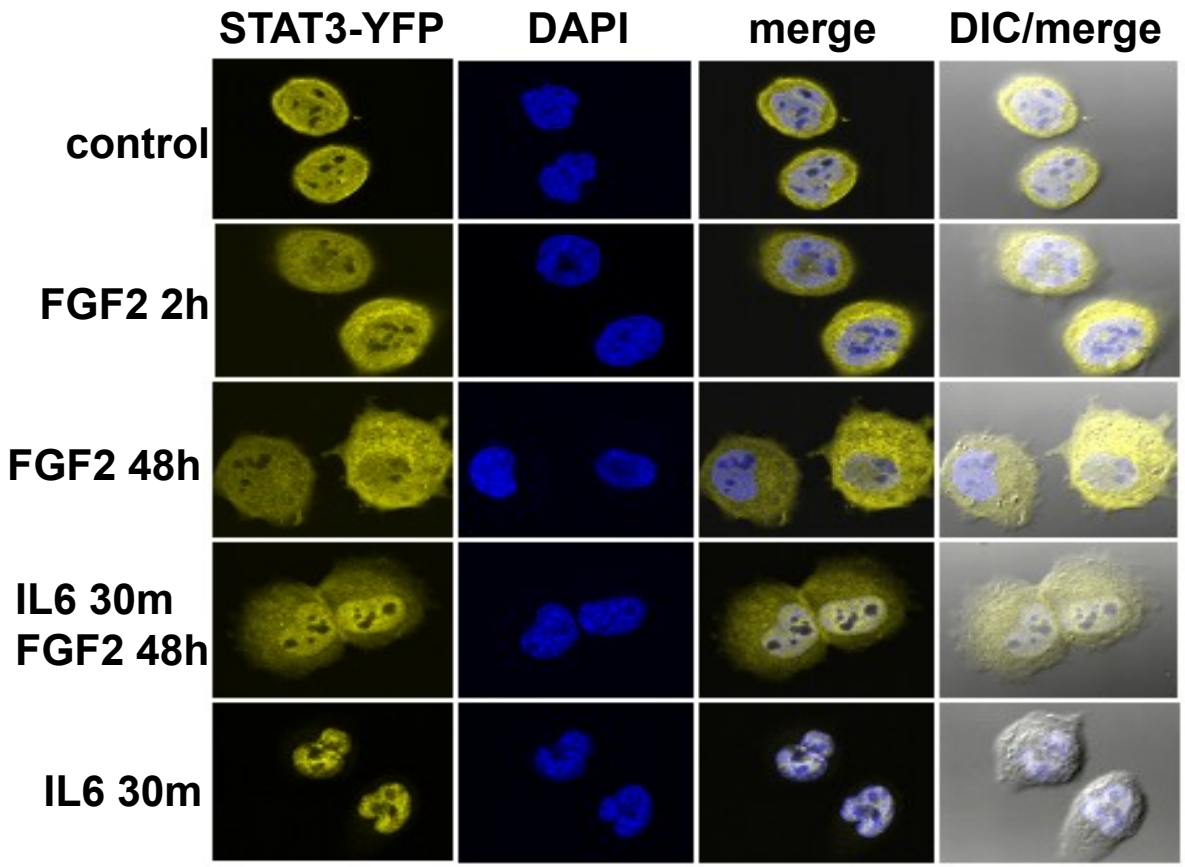




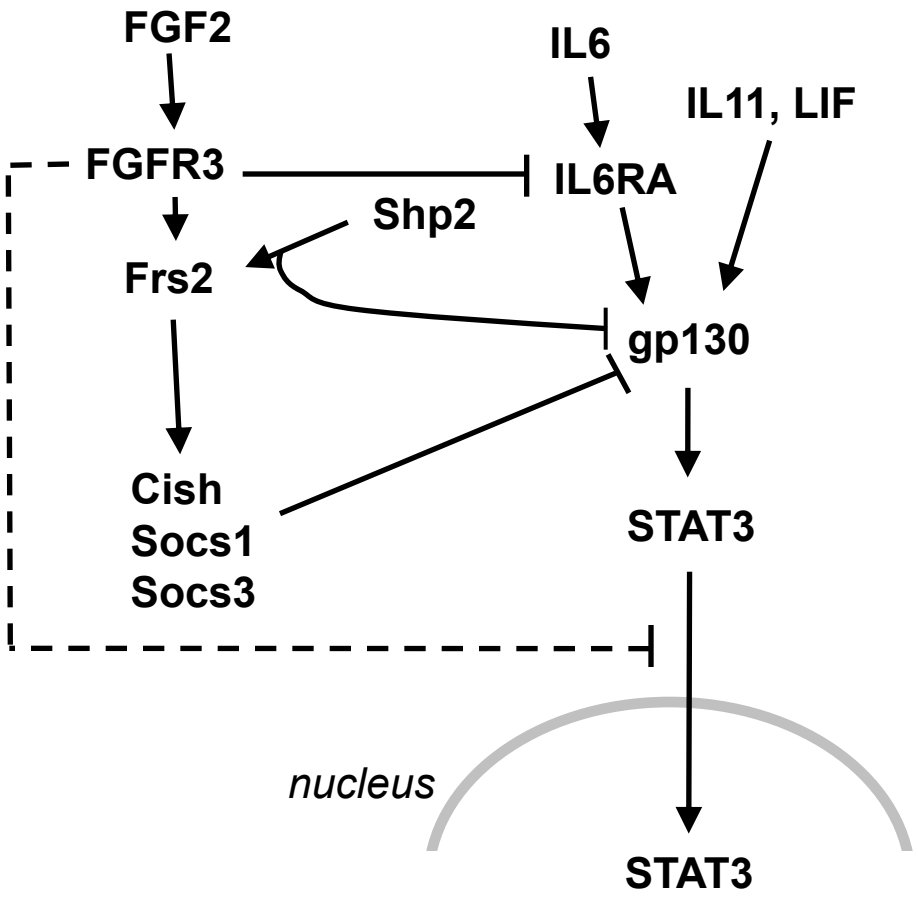


Chronic FGF stimulus inhibits cytokine/STAT signaling in chondrocytes

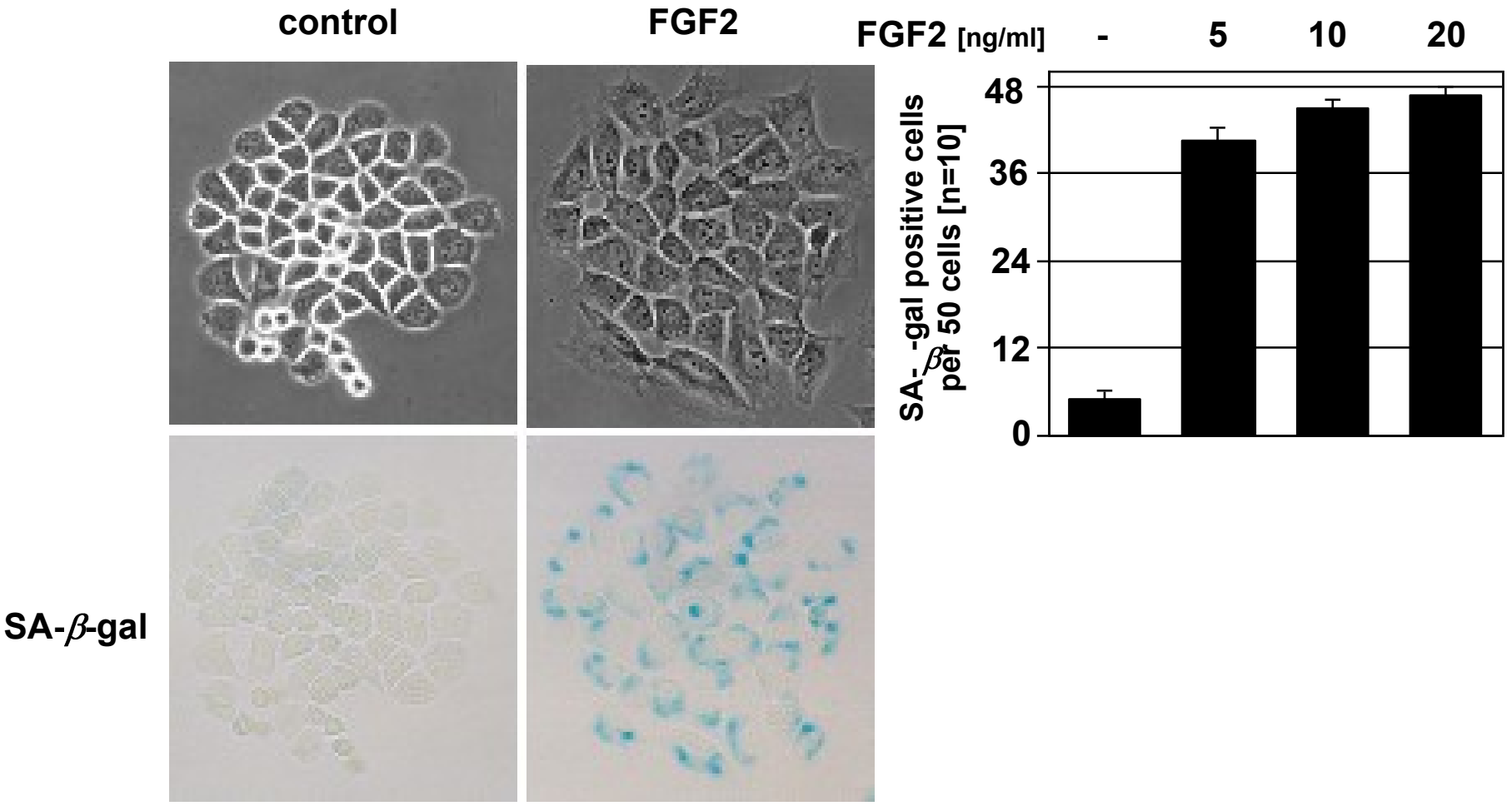




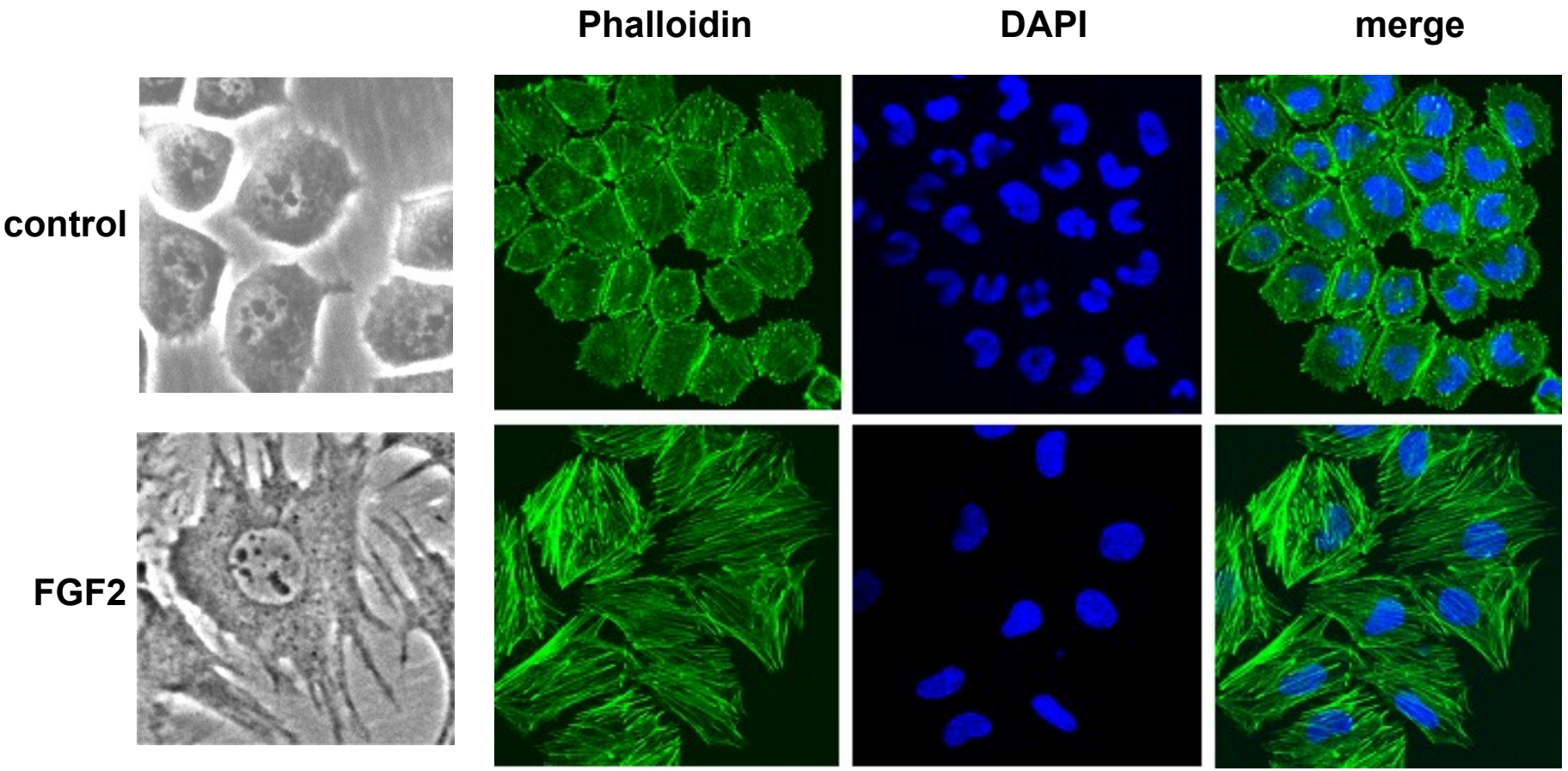
Chronic FGF stimulus inhibits cytokine/STAT signaling in chondrocytes



FGF2 causes premature senescence in chondrocytes

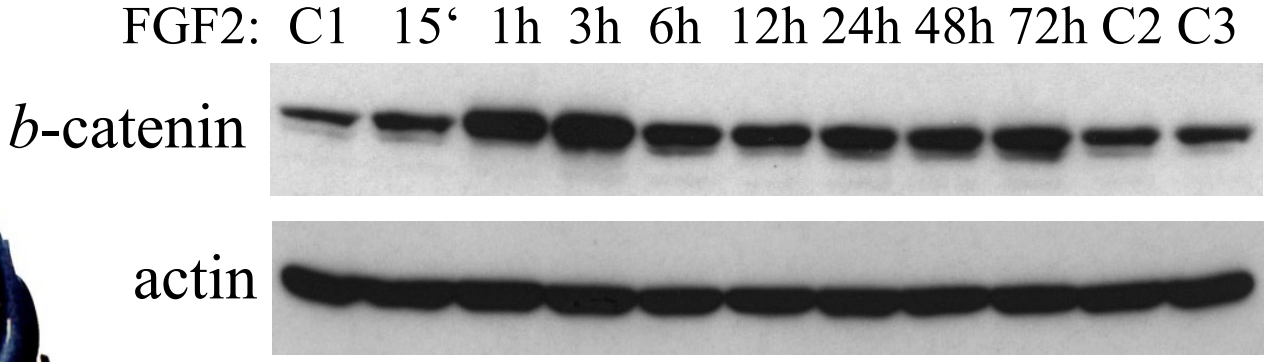


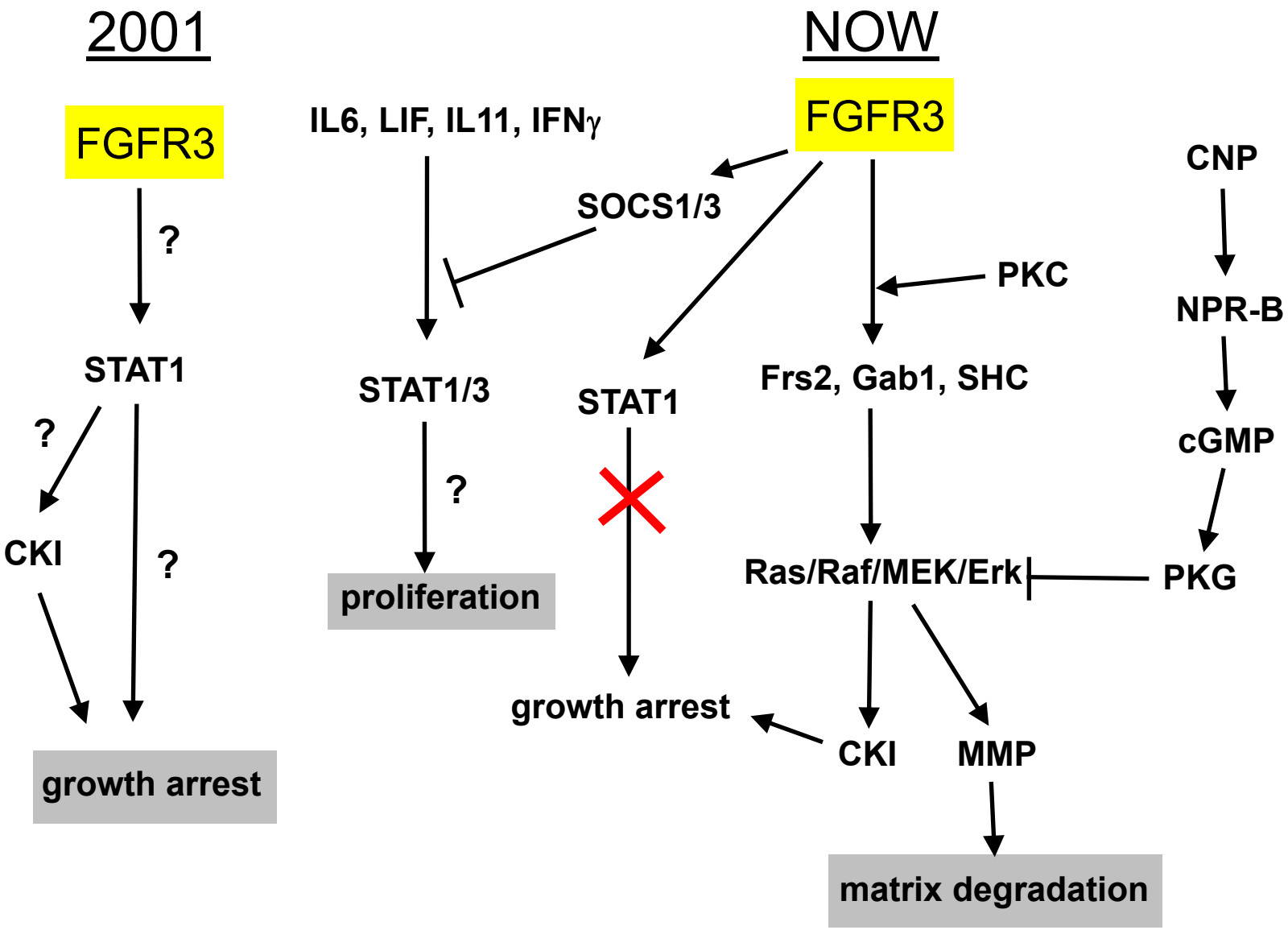
FGF2 signals towards the cytoskeleton in chondrocytes



Where is Wnt?


I Wnt YOU


FGF2: C1 15‘ 1h 3h 6h 12h 24h 48h 72h C2 C3
b-catenin
actin


2001
FGFR3
?
STAT1
?
CKI
?
growth arrest
NOW
IL6, LIF, IL11, IFNγ
FGFR3
SOCS1/3
PKC
CNP
NPR-B
cGMP
PKG
STAT1/3
?
proliferation
STAT1
Frs2, Gab1, SHC
Ras/Raf/MEK/Erk
growth arrest
CKI
MMP
matrix degradation