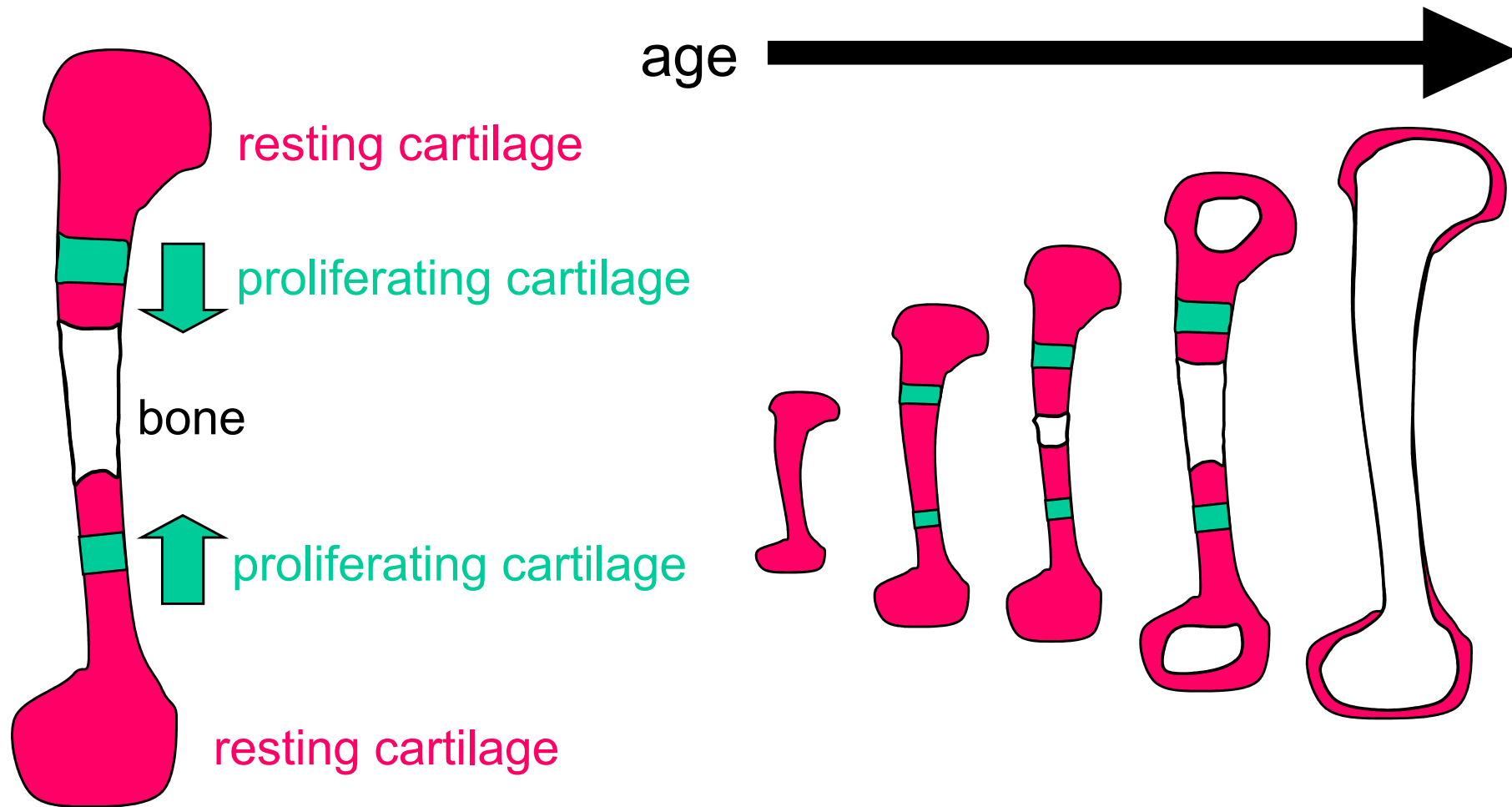


# **9. MECHANISMS OF DEVELOPMENT I – FIBROBLAST GROWTH FACTORS (FGF) IN LIMB GROWTH**

Pavel Krejci

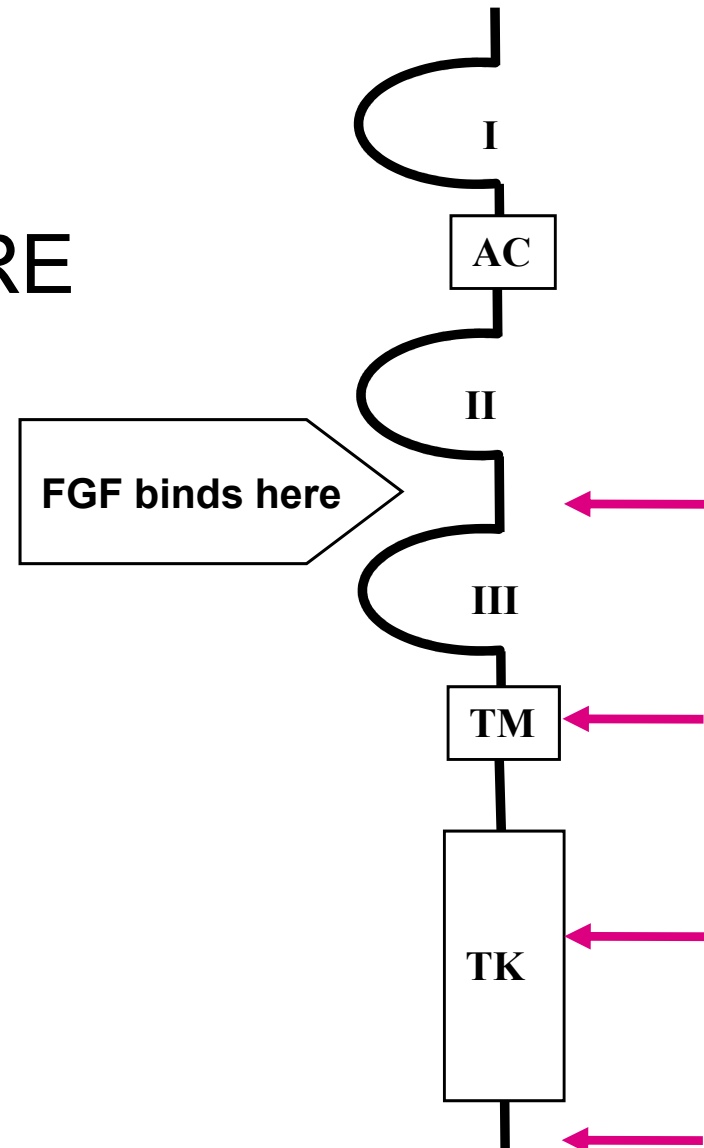
# How do the limbs grow?



# FGFR3-related skeletal dysplasia

Hypochondroplasia  
Achondroplasia  
SADDAN  
Thanatophoric Dysplasia

STATURE



# FGFR3-related skeletal dysplasia



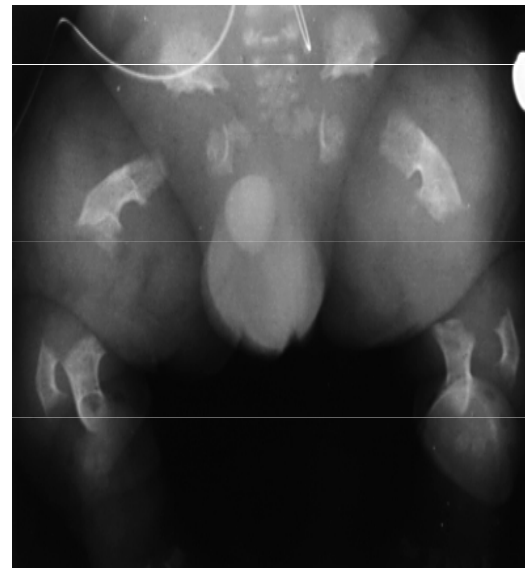
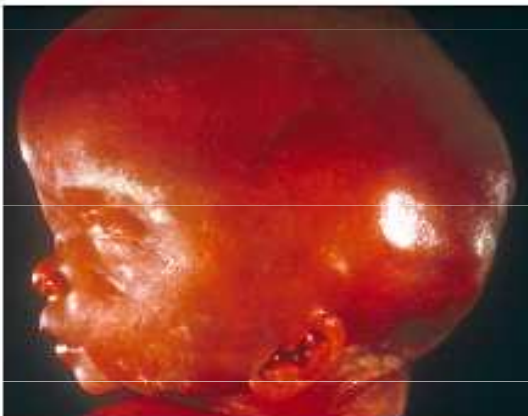
Achondroplasia

# Thanatophoric Dysplasia



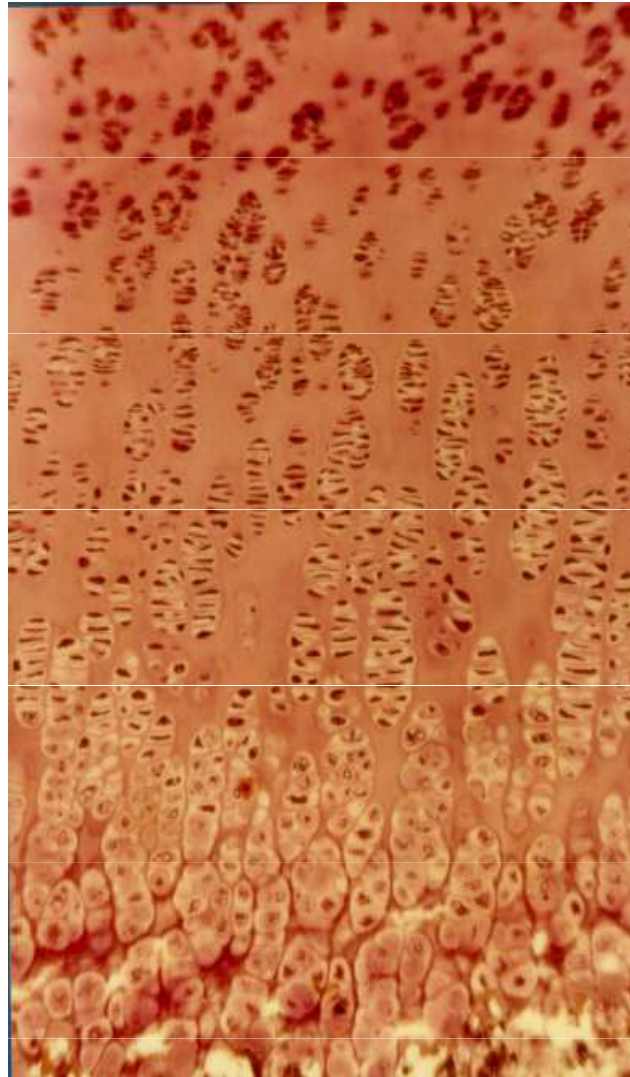
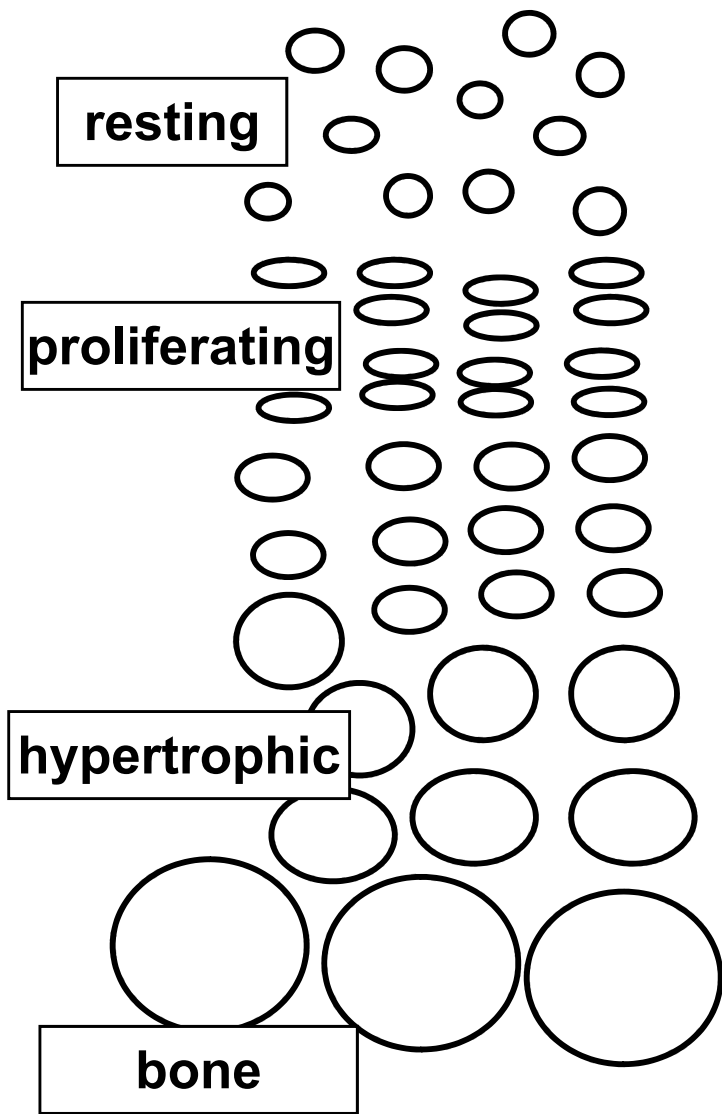
**healthy**

- short long bones
- brachydactyly
- macrocephaly

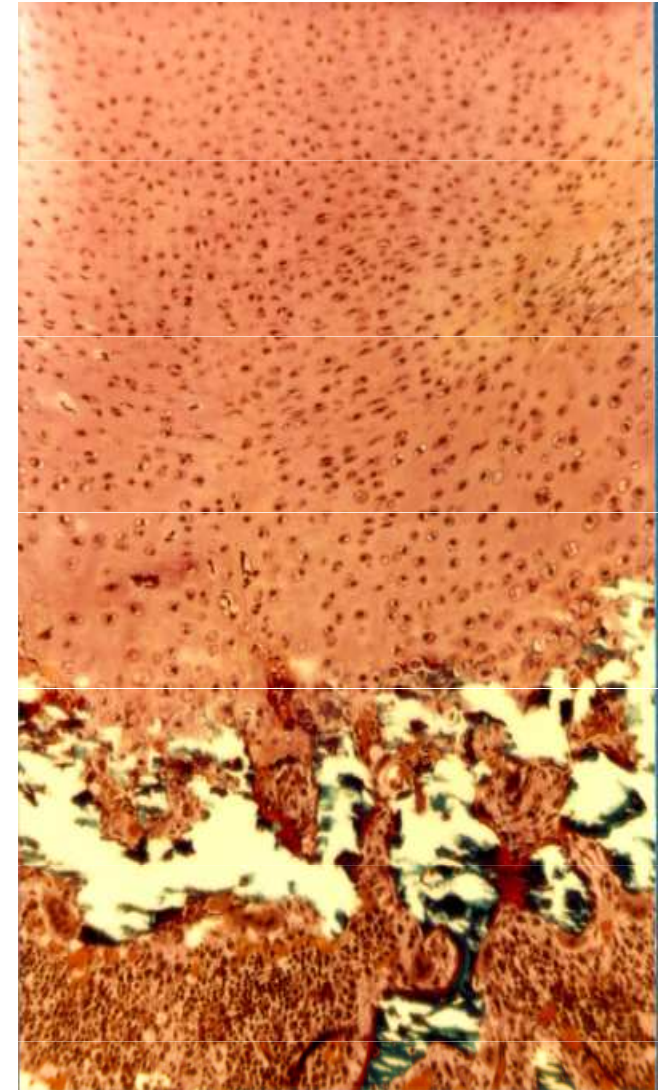


**TD**

- low nasal bridge
- spinal stenosis
- temporal lobe malformations



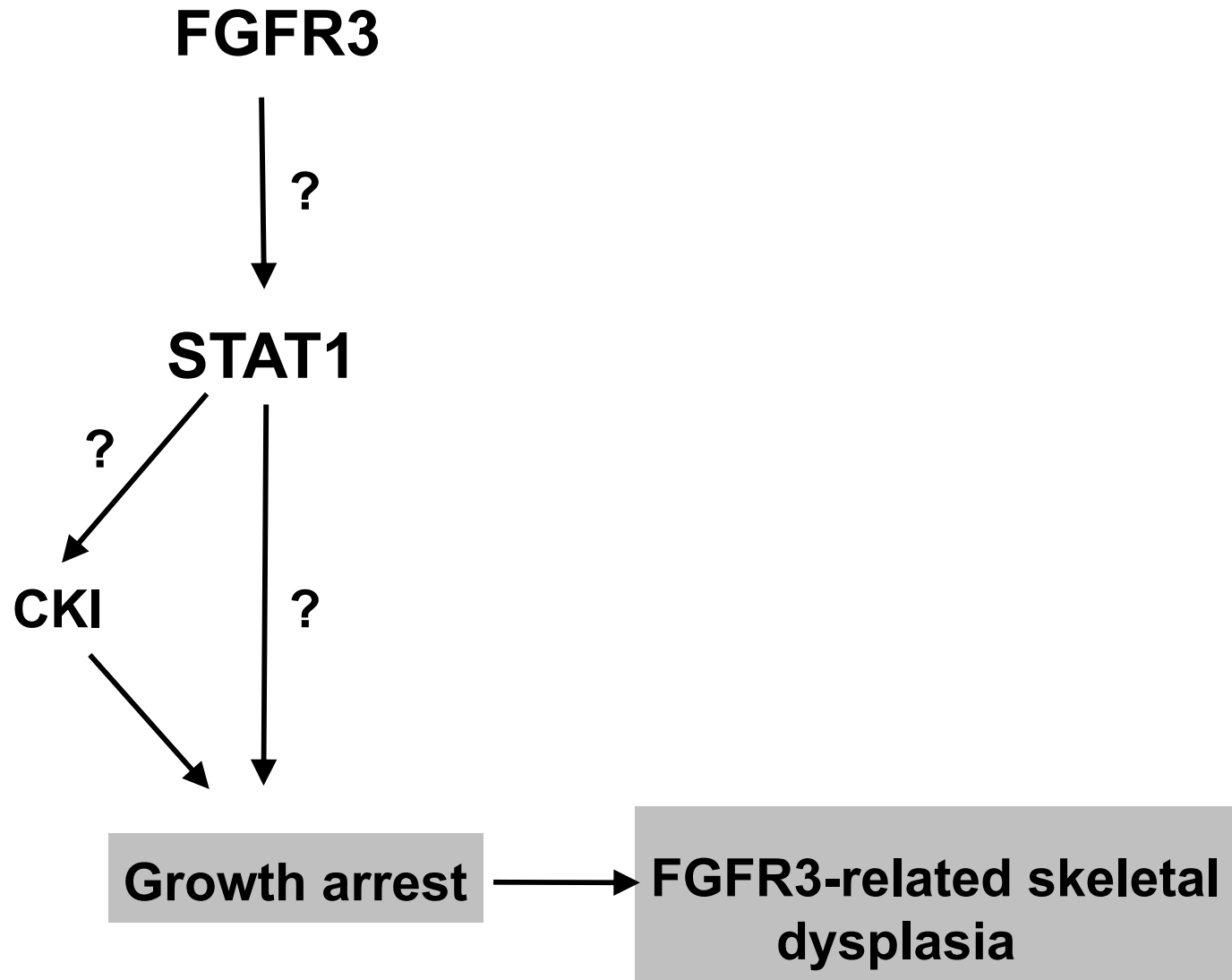
**healthy**



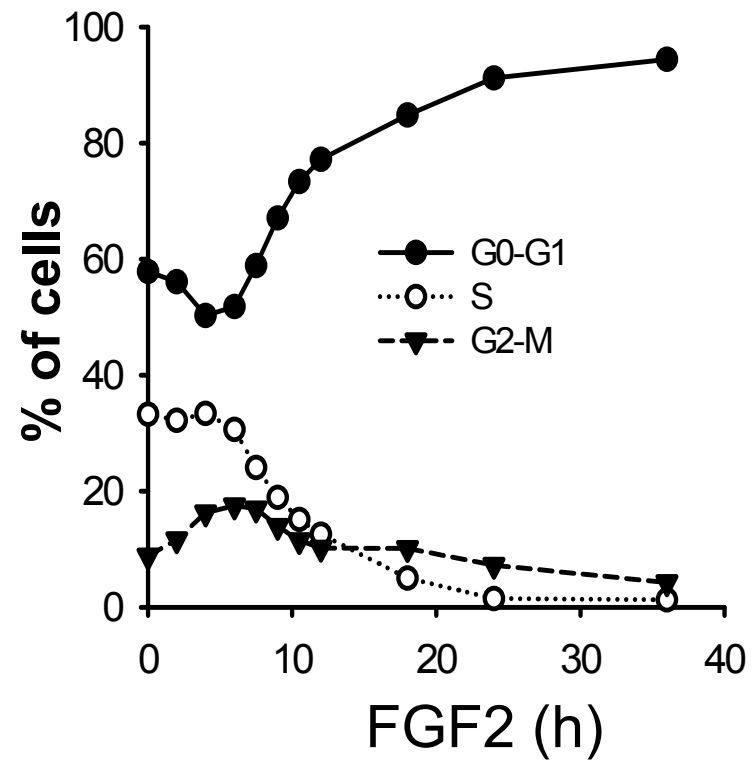
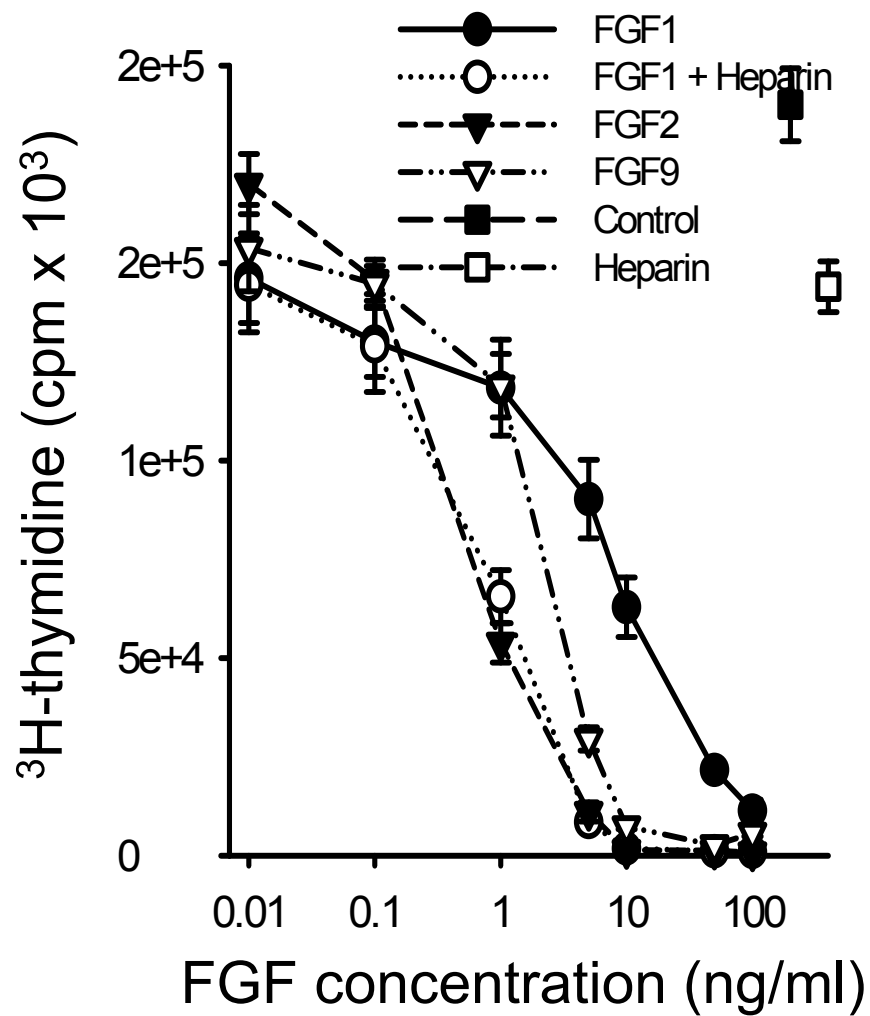
**TD**

Sahni *et al.*, *Genes Dev* 1999, 13, 1361-66.

Sahni *et al.*, *Development* 2001, 128, 2119-29.

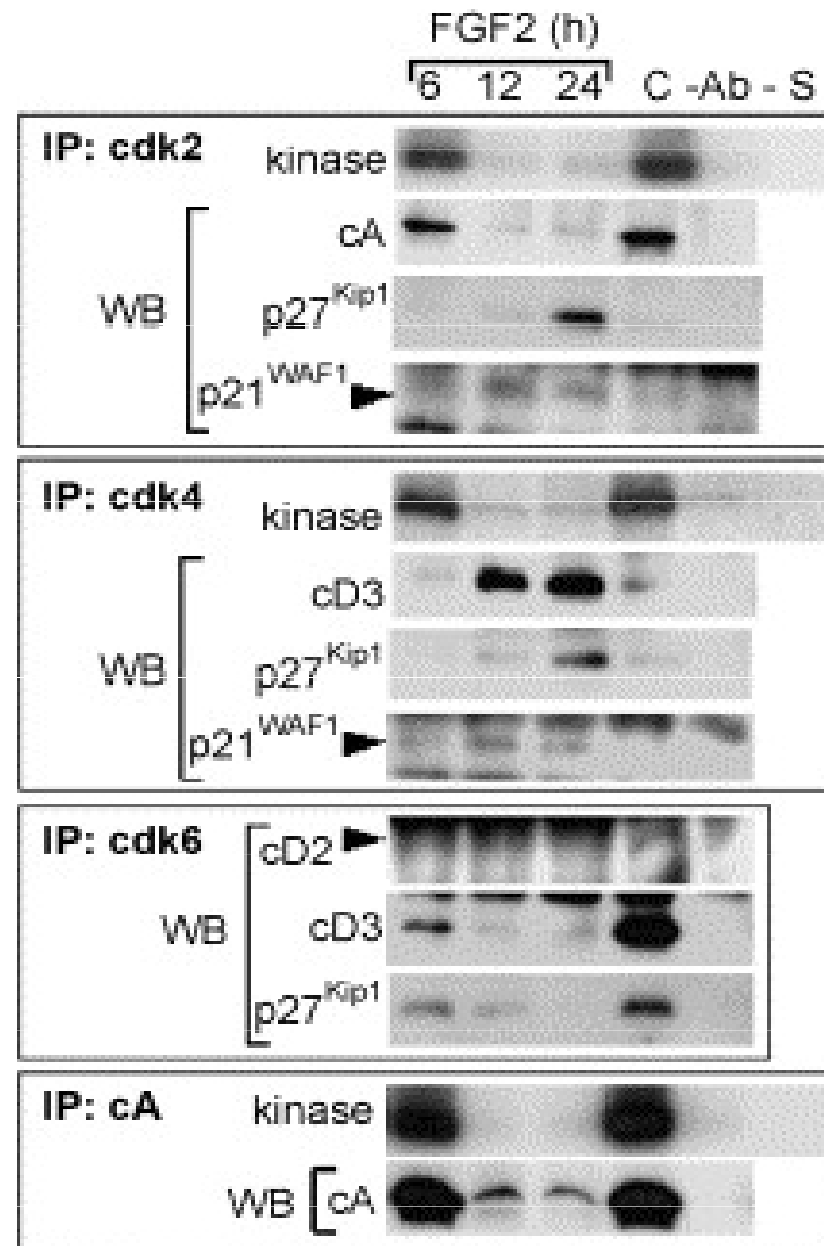
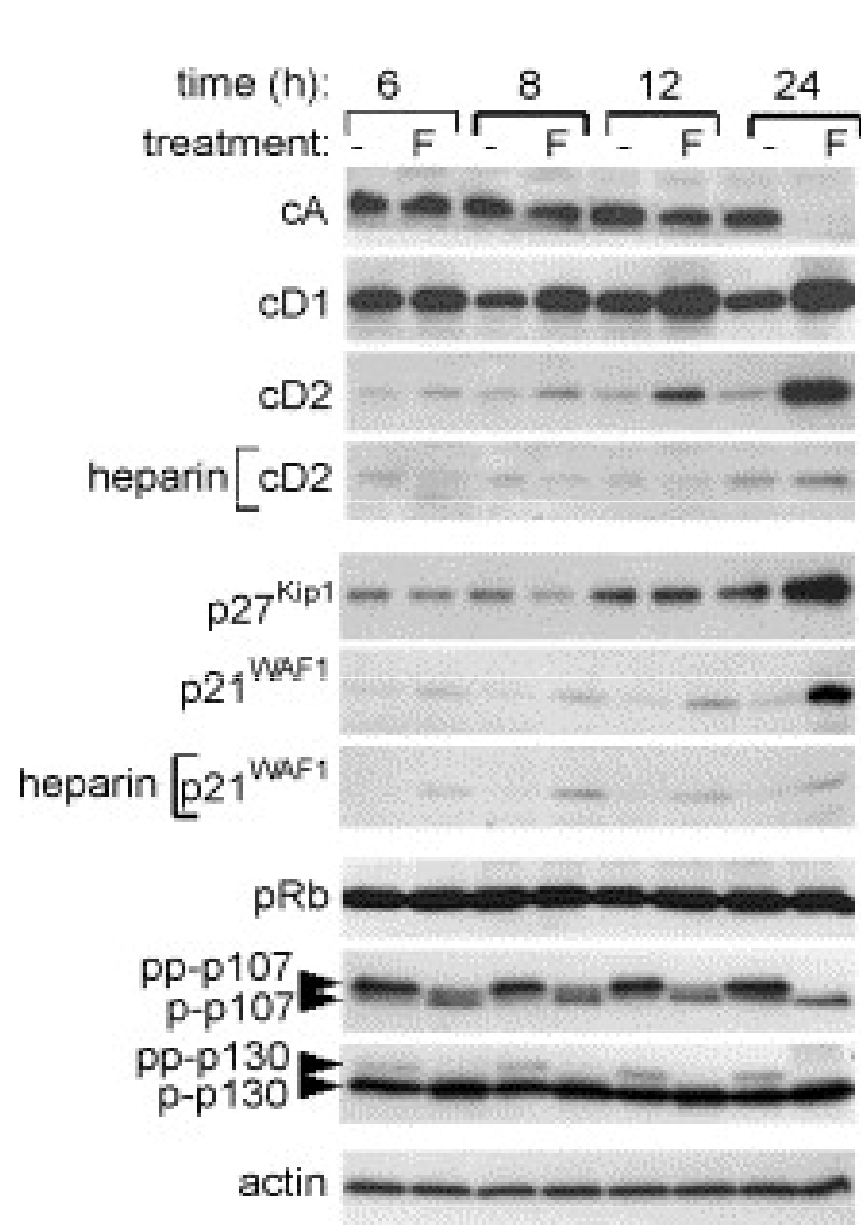


# FGF inhibits chondrocyte proliferation by arresting their cell cycle in G1 phase

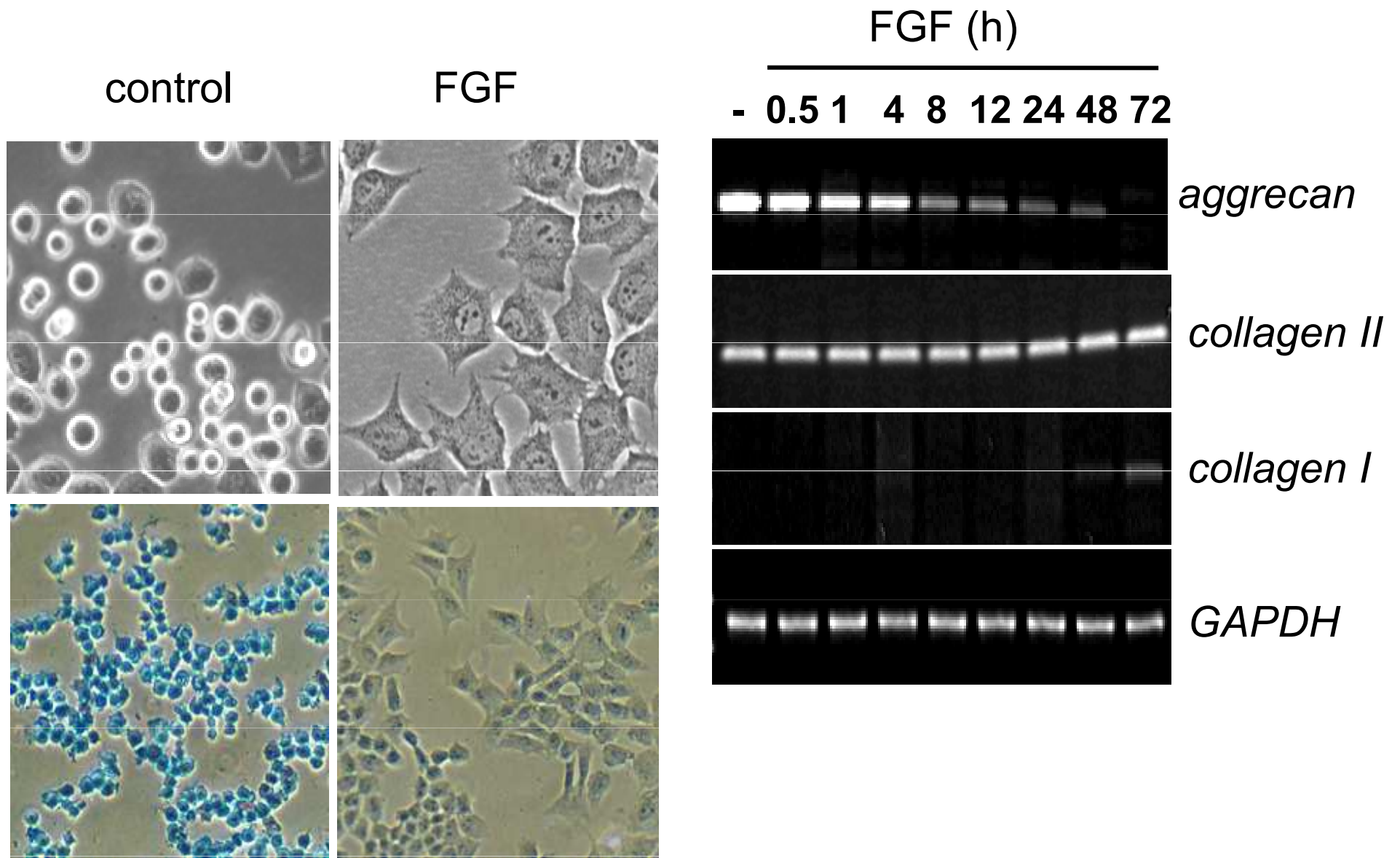




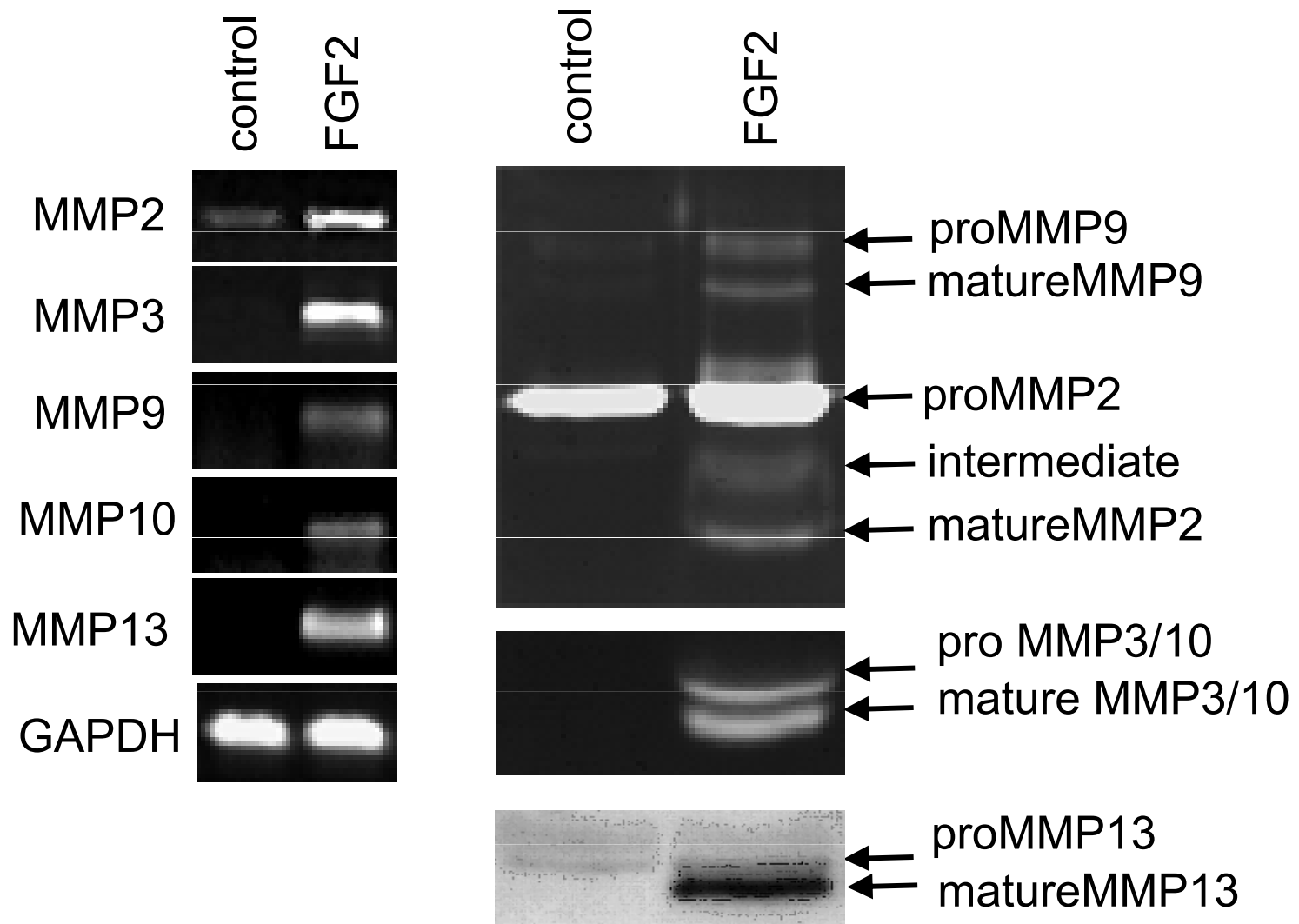
....via inhibition of cdk activity necessary for progression through the G1 phase of a cell cycle



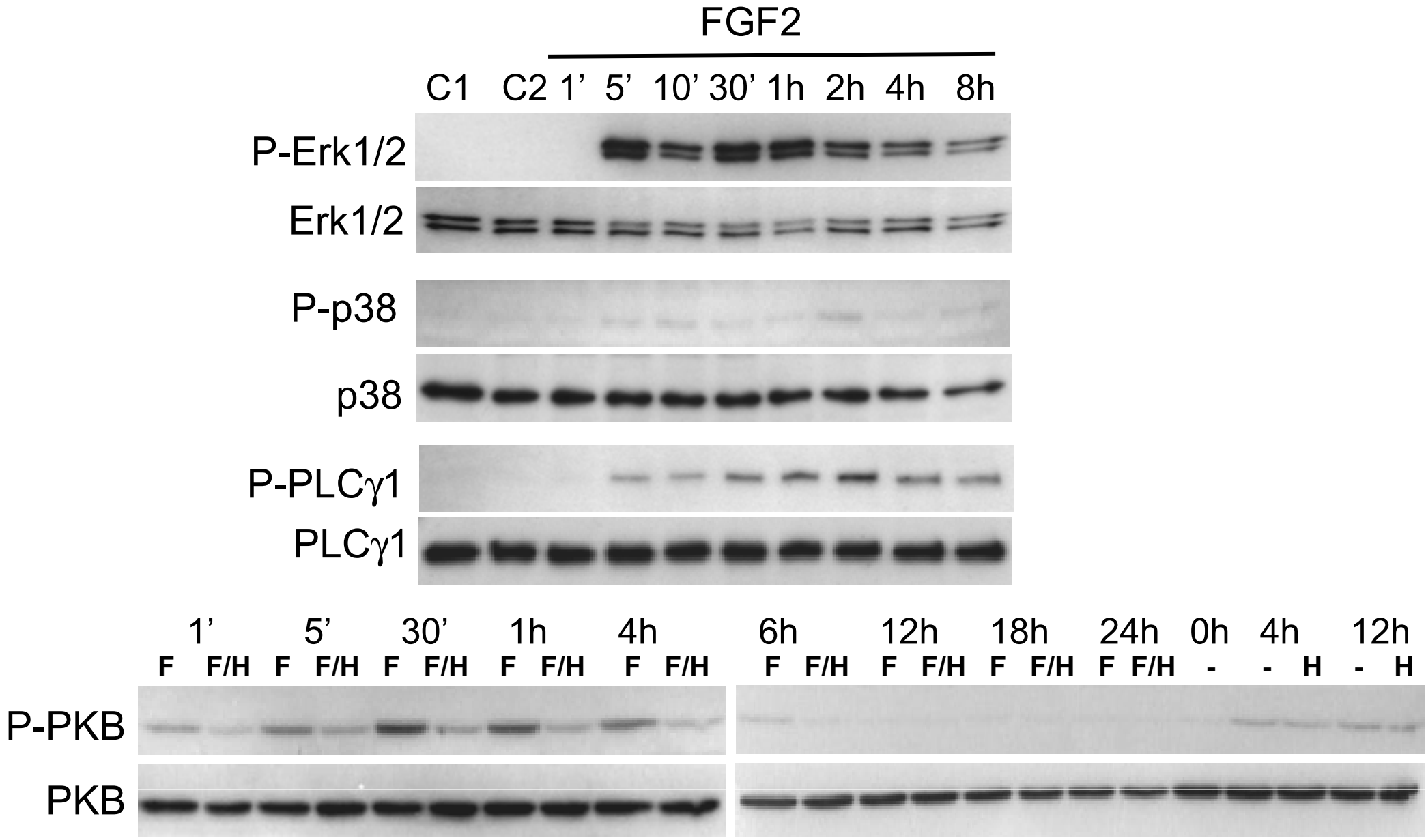
# FGF alters the cartilage-like phenotype of chondrocytes



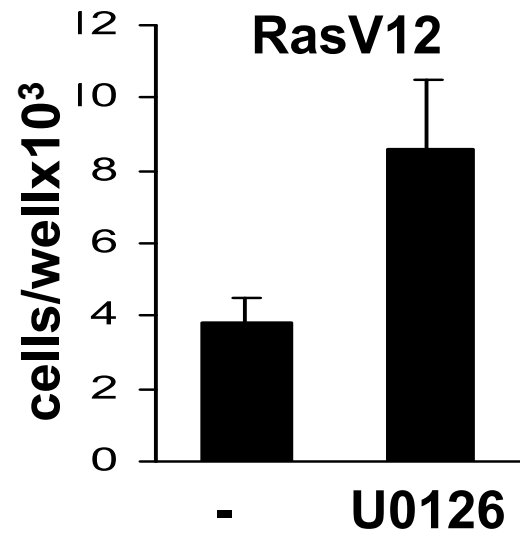
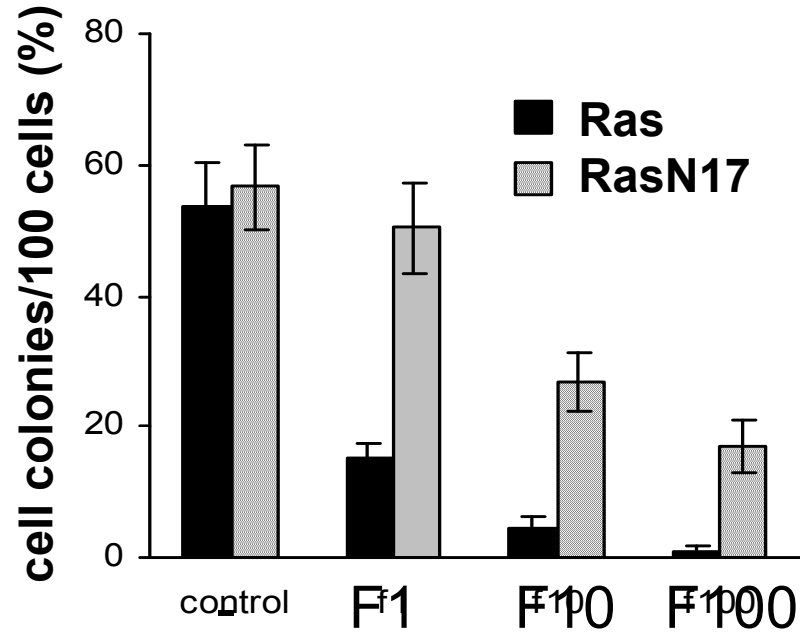
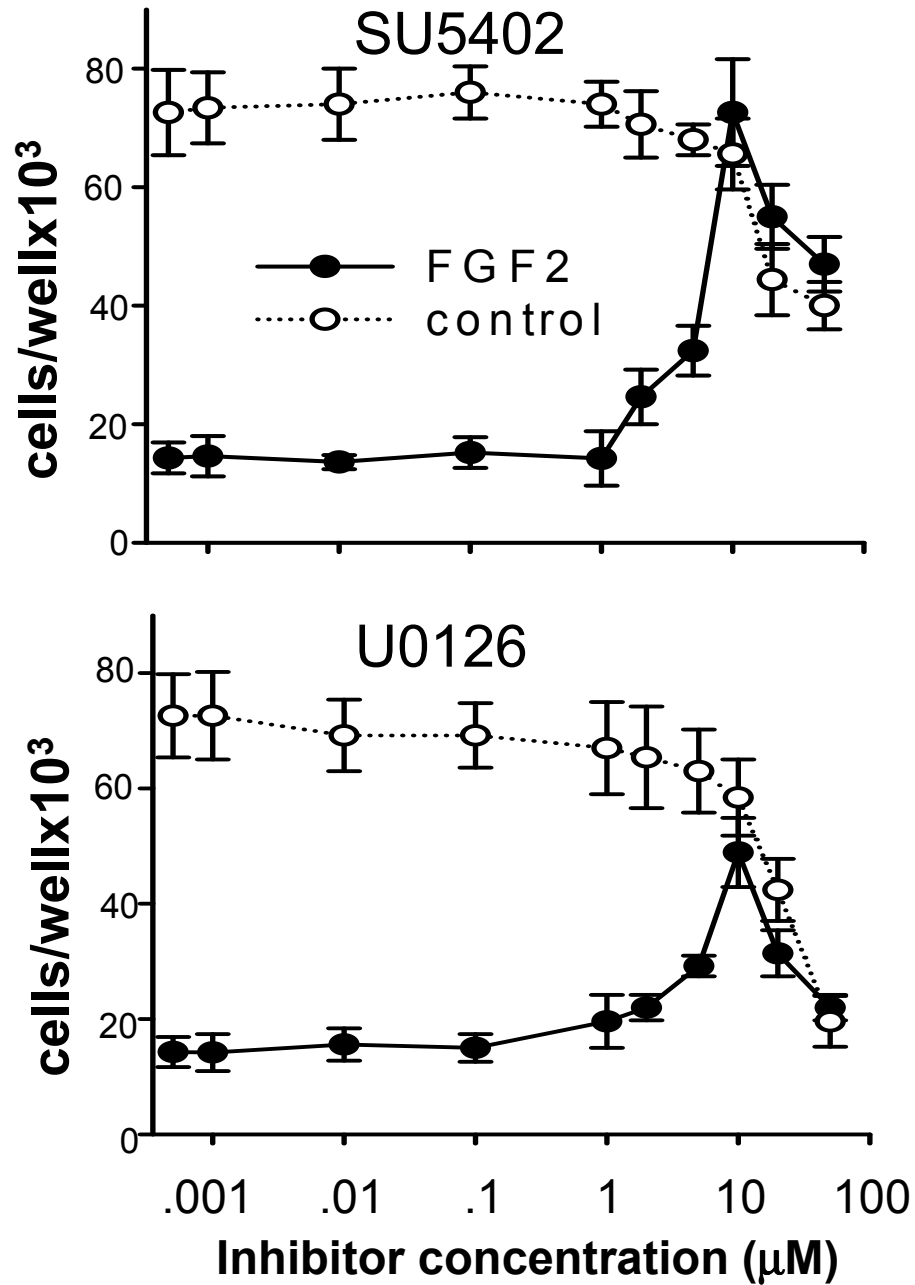
.....via MMP-mediated degradation of extracellular matrix



# FGF2 activates Erk and p38 MAPK, PLC $\gamma$ 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

Murakami *et al.*, *Genes Dev* 2004, 18, 290-305.

Rauci *et al.*, *J Biol Chem* 2004, 279, 1747-1756.

Krejci *et al.*, *Exp Cell Res* 2004, 297, 152-164.

Murakami *et al.*, *Genes Dev* 2004, 18, 290-305.

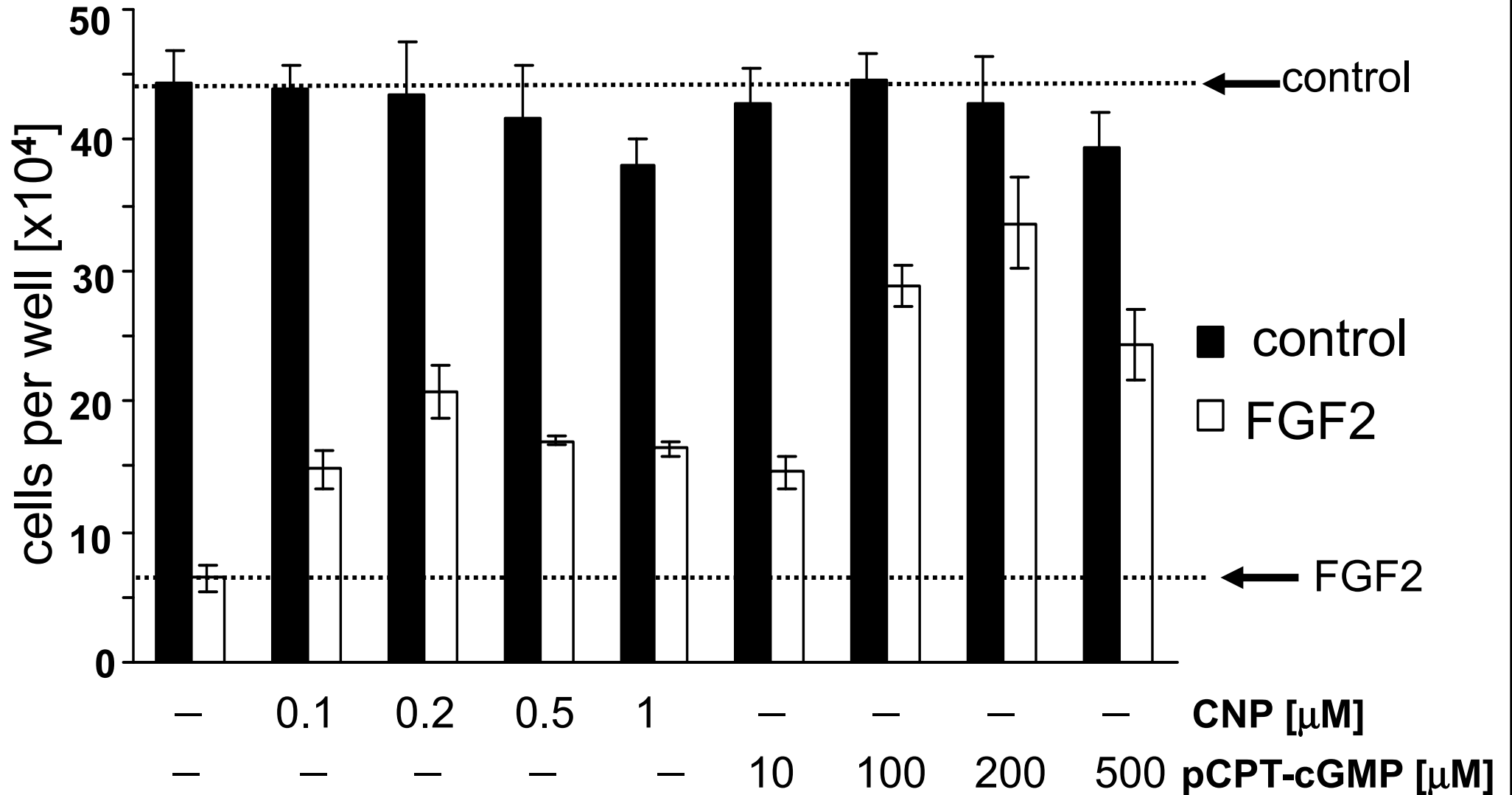
Rauci *et al.*, *J Biol Chem* 2004, 279, 1747-1756.

Krejci *et al.*, *Exp Cell Res* 2004, 297, 152-164.

C-type Natriuretic Peptide (CNP) rescues  
achondroplastic phenotype in FGFR3-ACH mice.

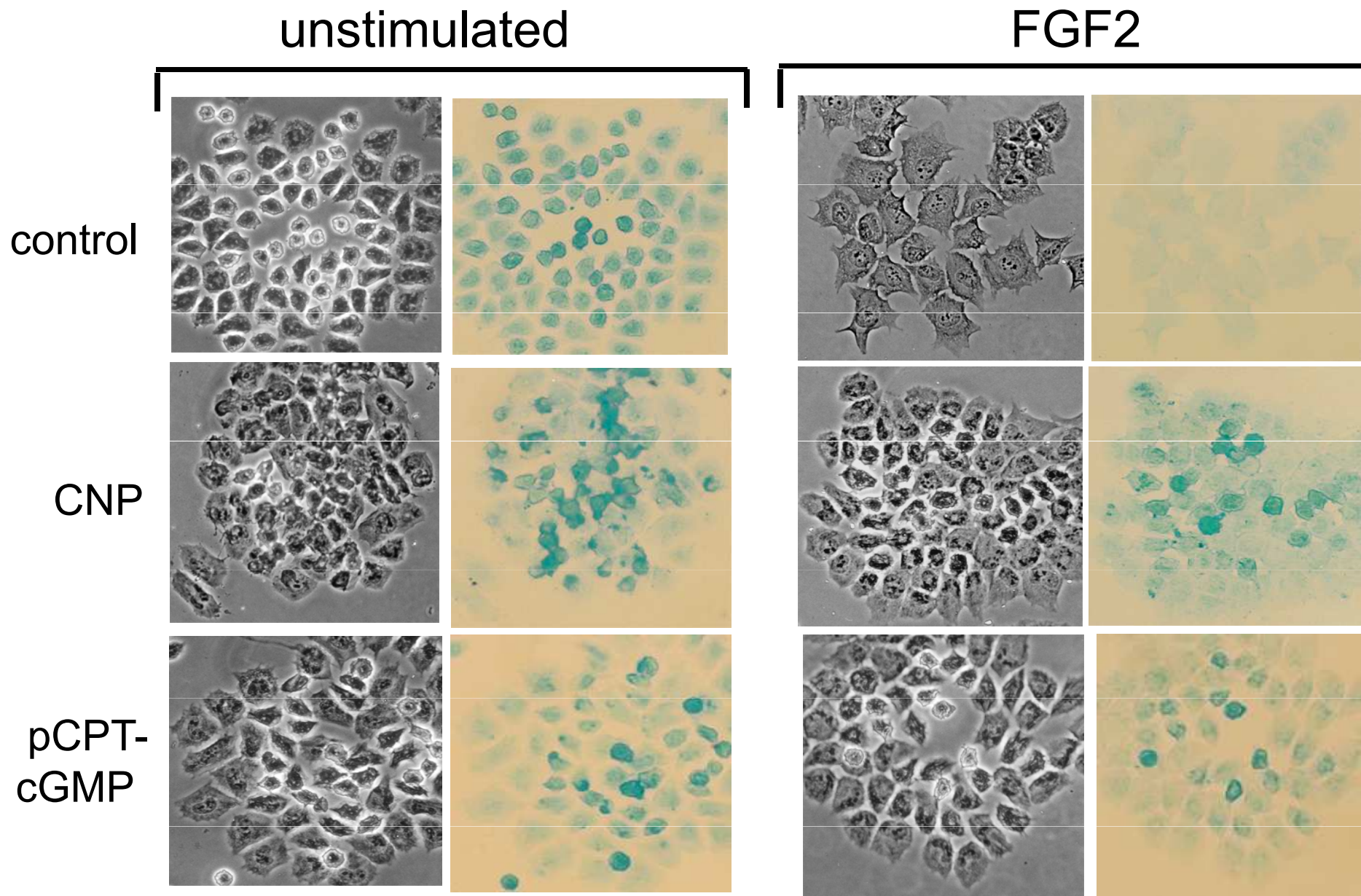
*Yasoda et al., Nature Medicine 2004, 10, 80-86*

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

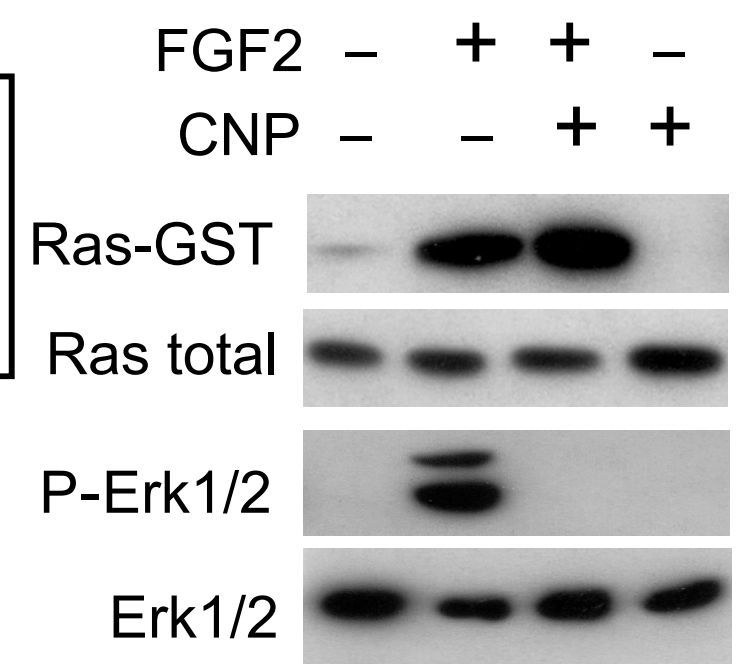
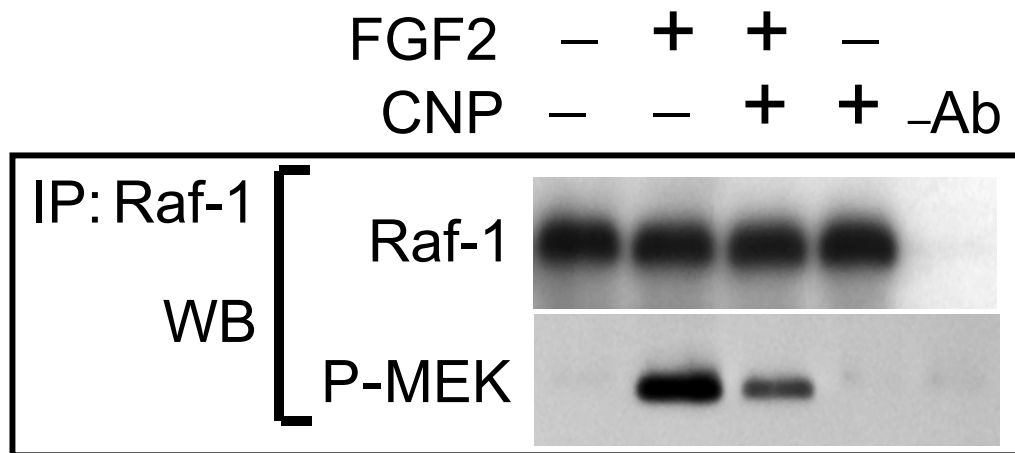
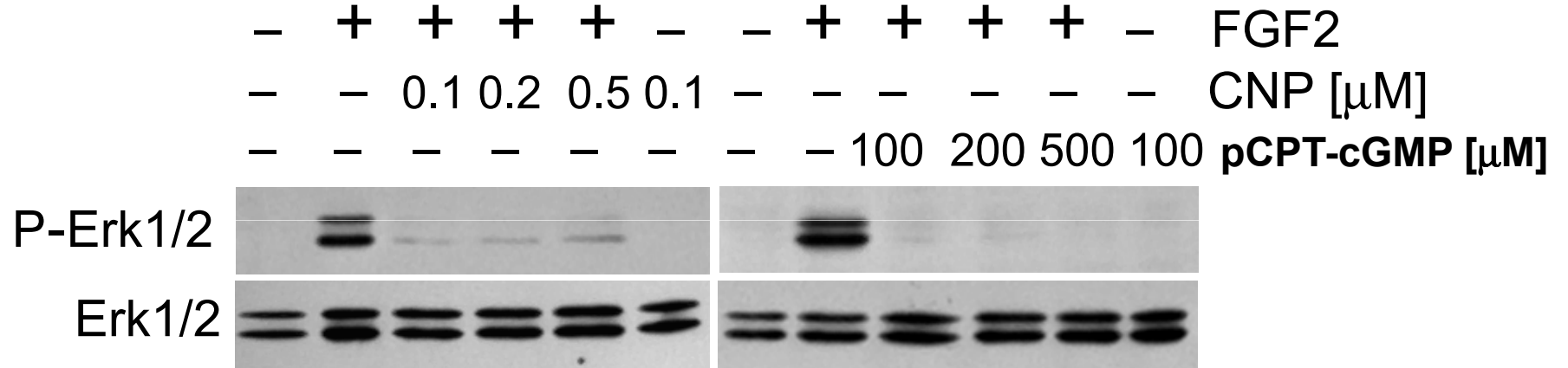
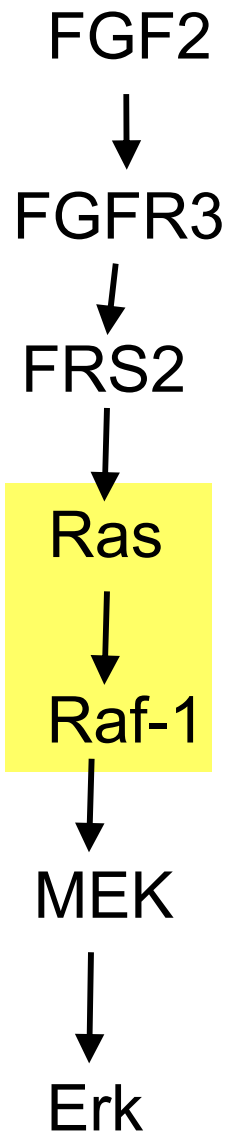




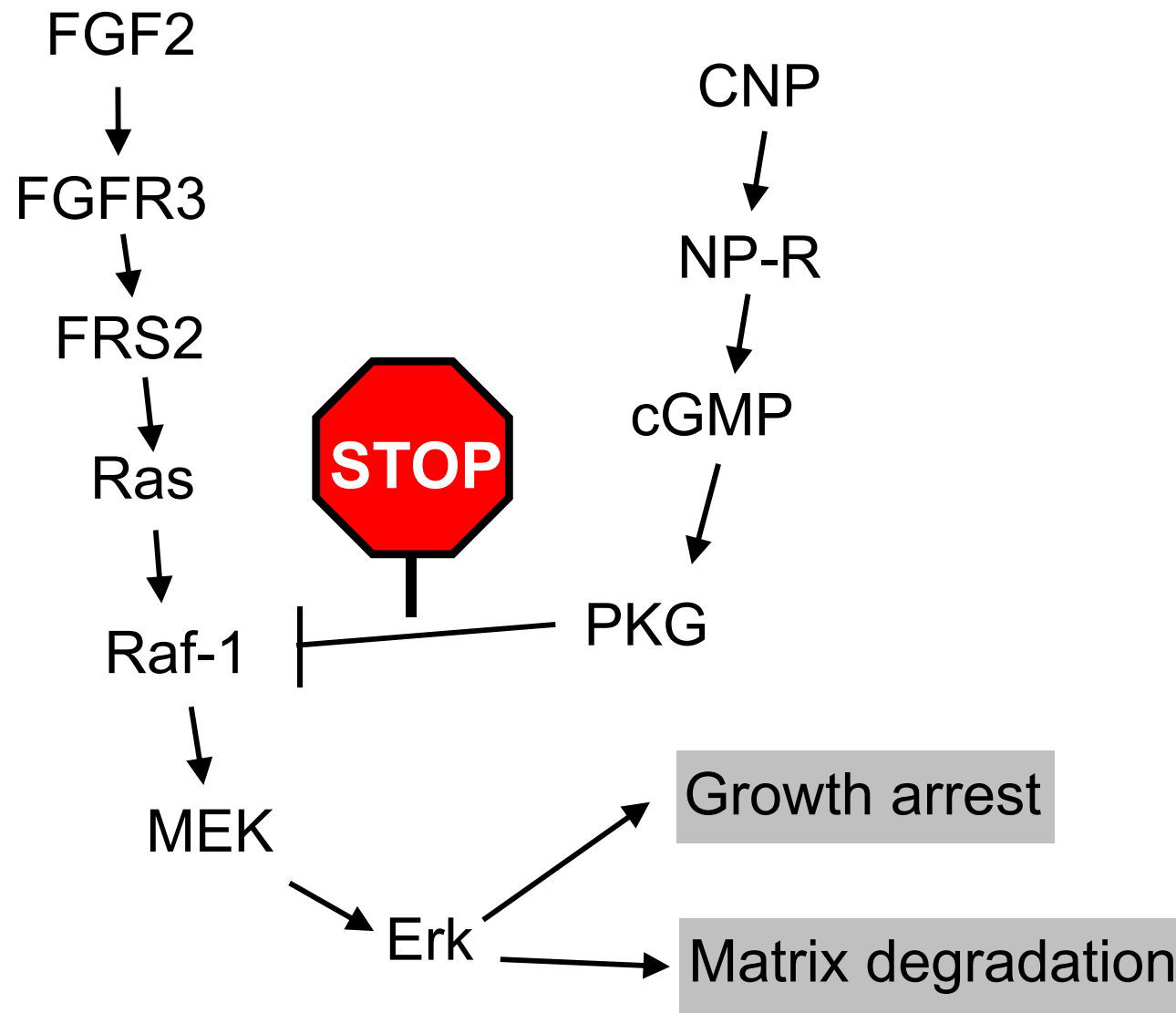
# CNP antagonizes FGF2-mediated loss of cartilage extracellular matrix in chondrocytes



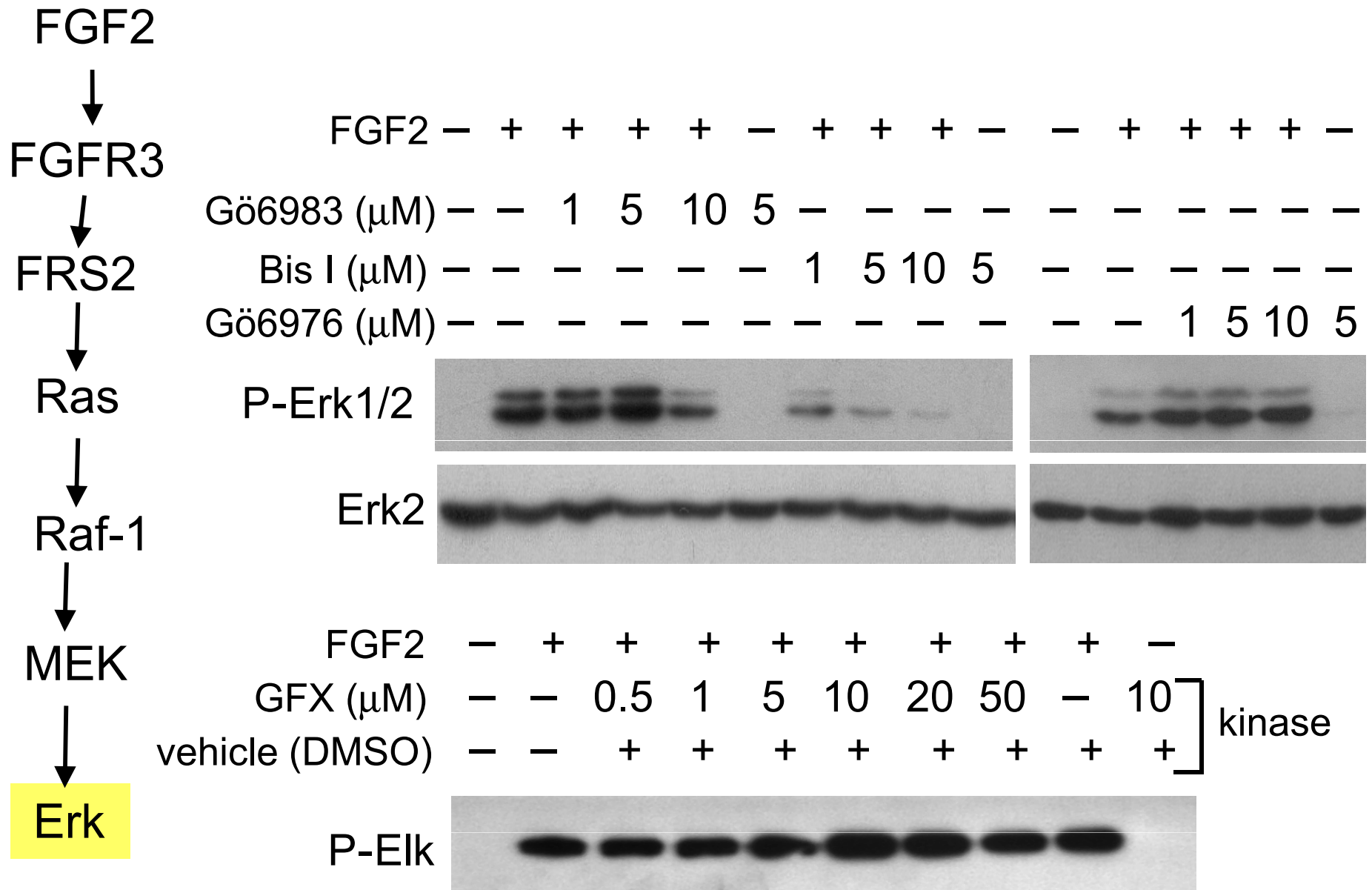
# CNP counteracts FGF2-mediated activation of Erk MAP kinase in chondrocytes



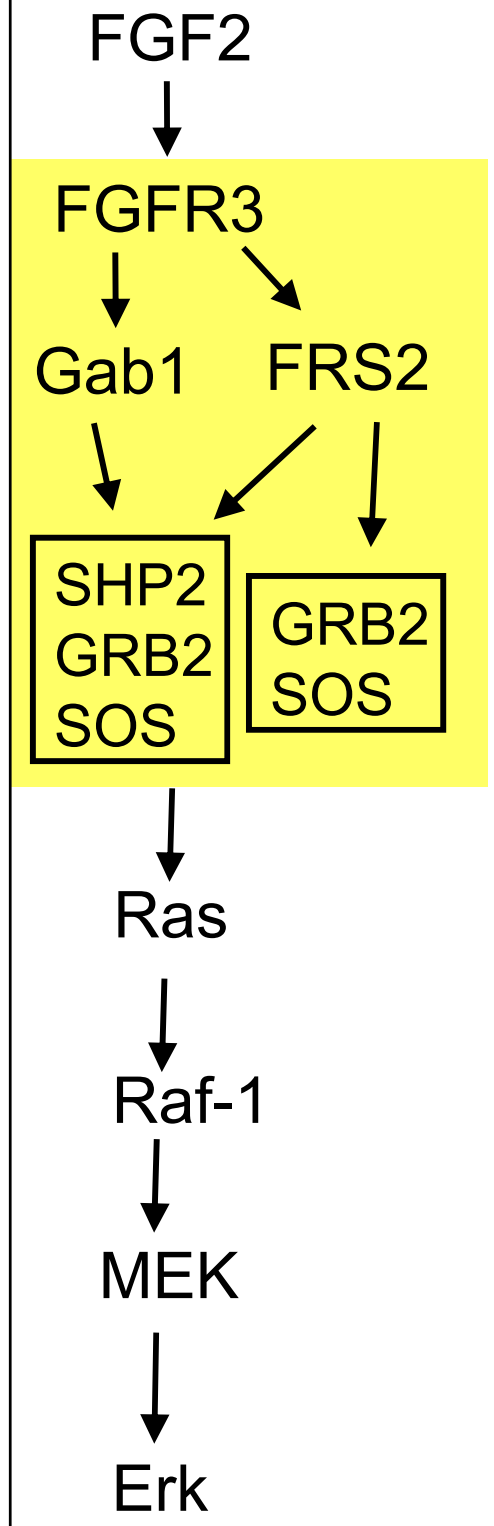
# CNP inhibits Erk MAP kinase module at the Raf level



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

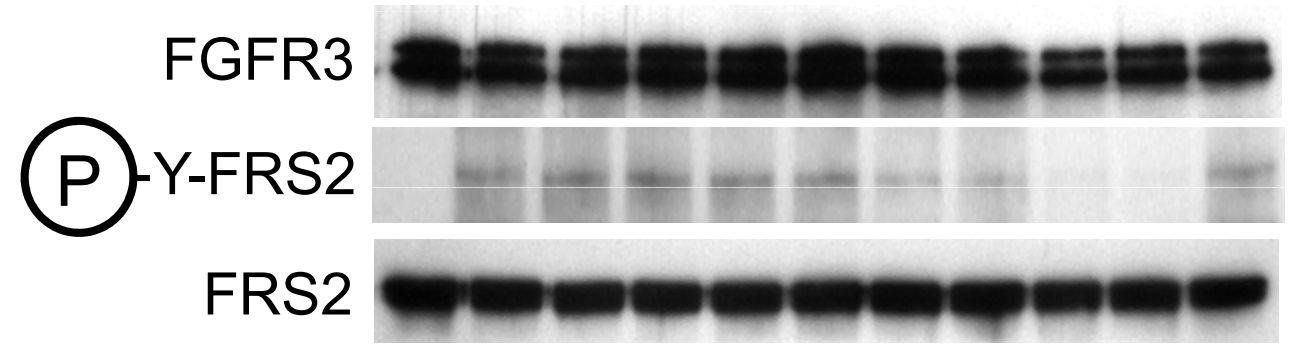




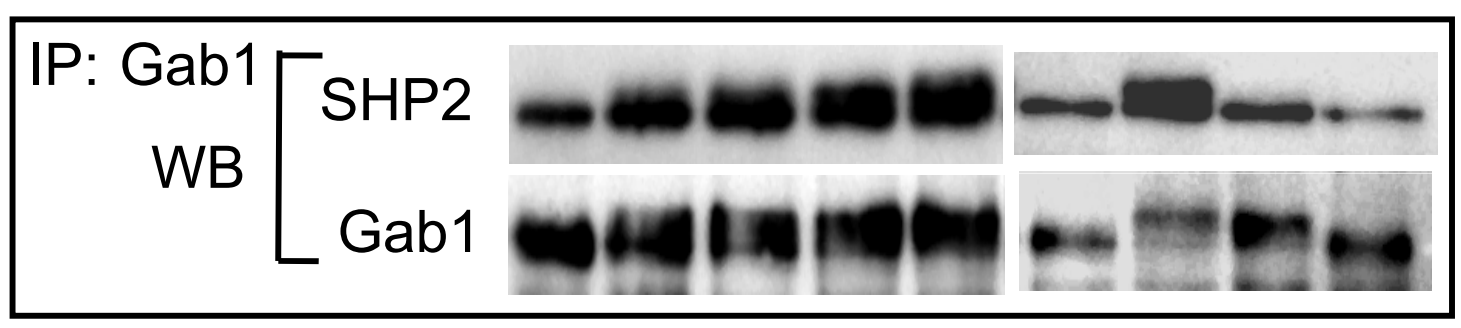
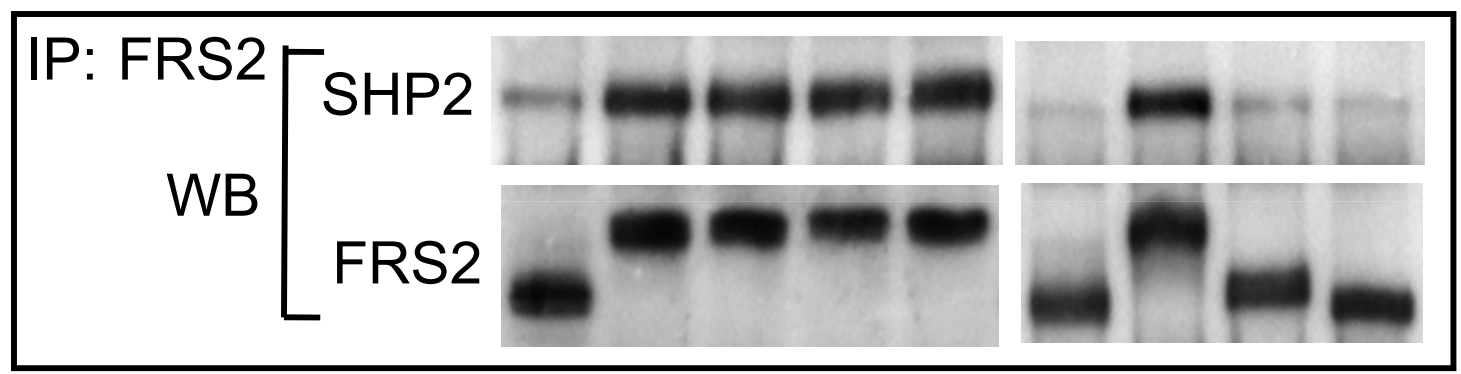


FGF2	-	+	+	+	+	+	+	+	+	+	+
Bis I (μM)	-	-	1	5	10	20	50	-	-	-	-
SU5402(μM)	-	-	-	-	-	-	-	1	10	20	-
vehicle(DMSO)	-	-	+	+	+	+	+	+	+	+	+

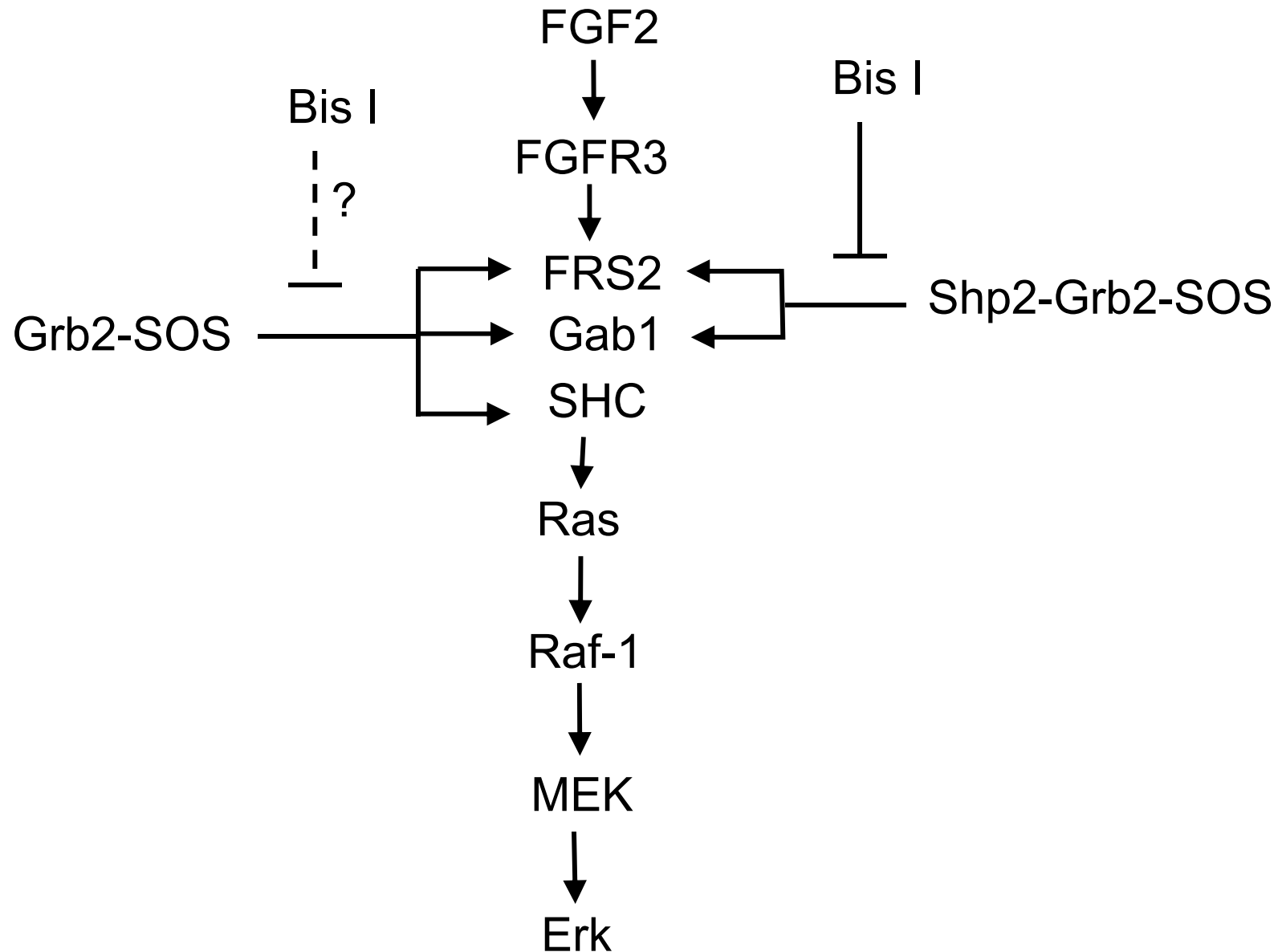
} kinase



FGF2	-	5'	10'	30'	1h	-	30'	30'	-
Bis I	-	-	-	-	-	-	-	+	+



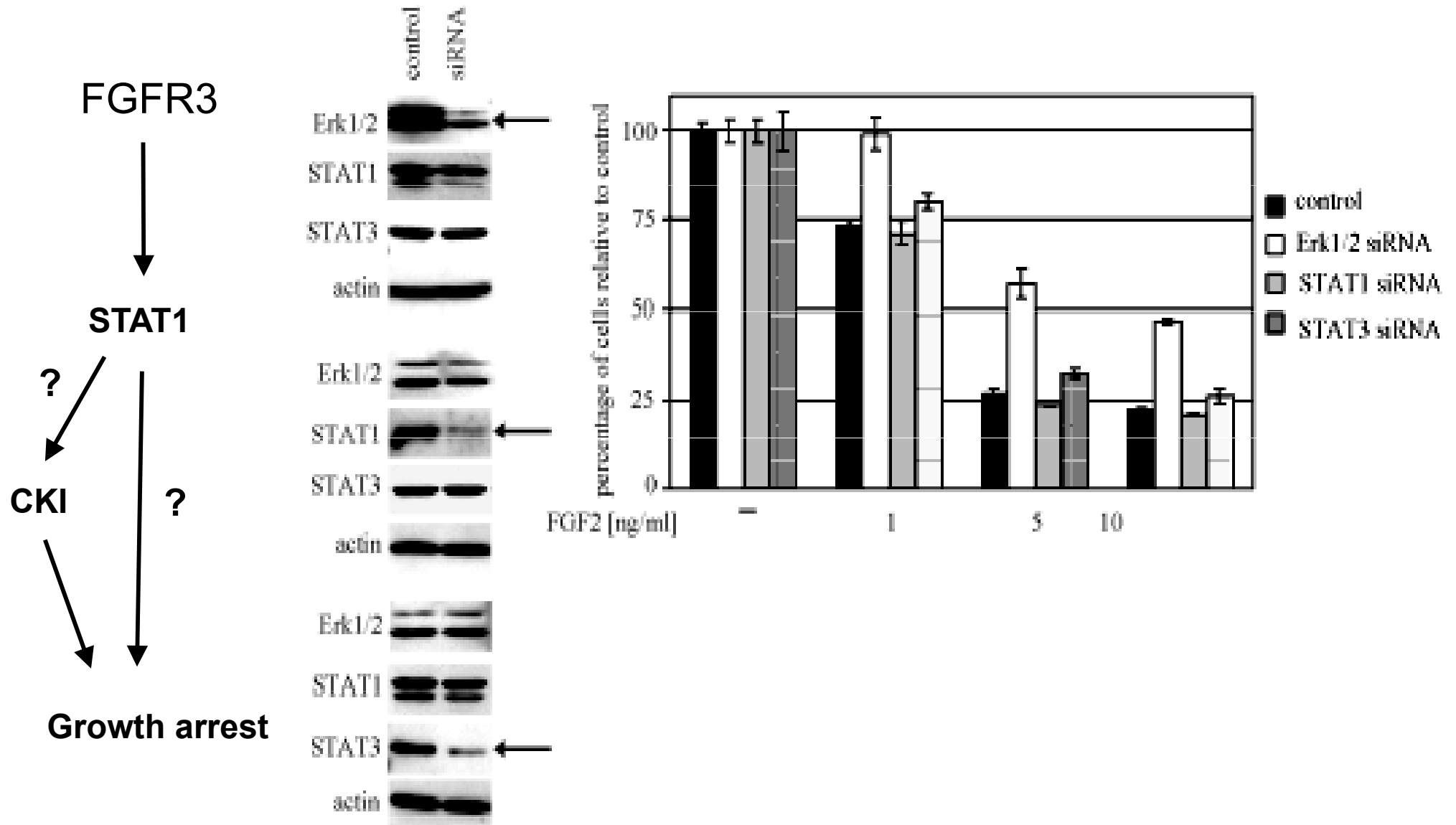
Protein kinase C inhibitor Bisindolylmaleimide I (Bis I) suppresses the FGF2-mediated activation of Erk MAP kinase pathway in chondrocytes by preventing the SHP2 association with FRS2 and Gab1 adaptor proteins



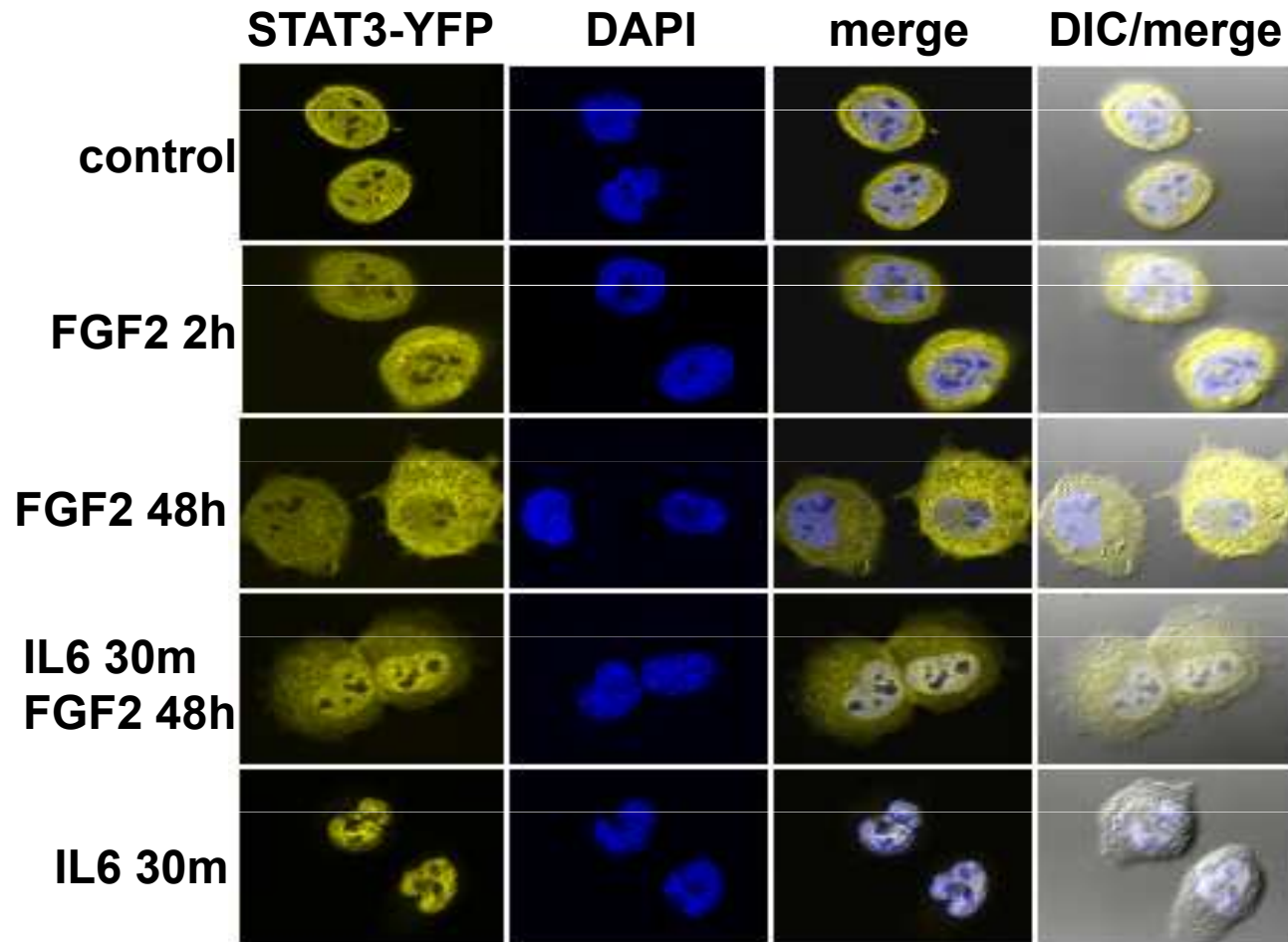
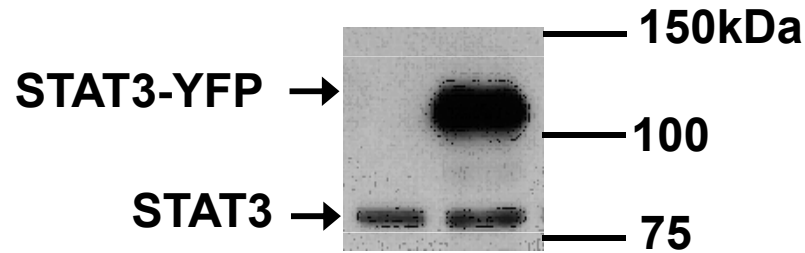




# STAT1 and STAT3 are not involved in FGFR3-mediated growth arrest in chondrocytes



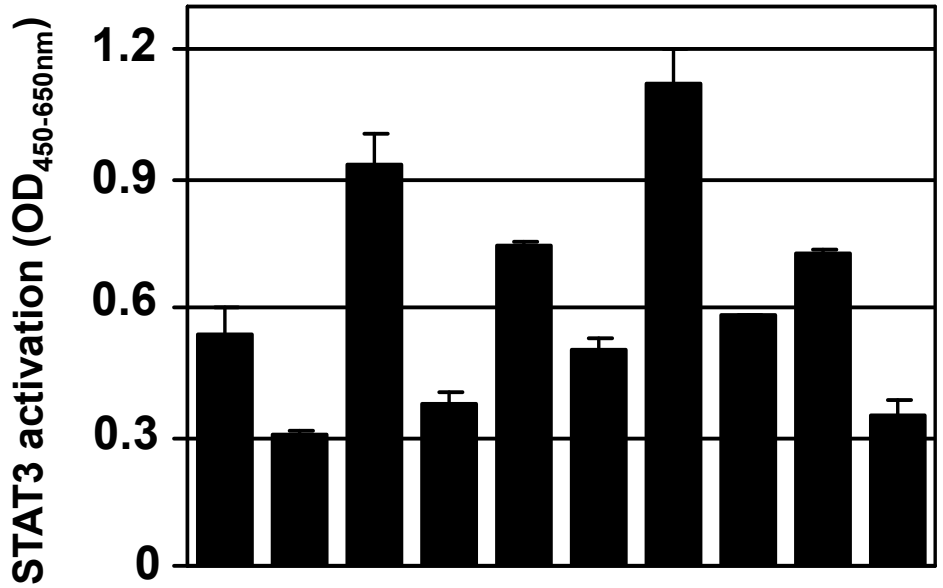
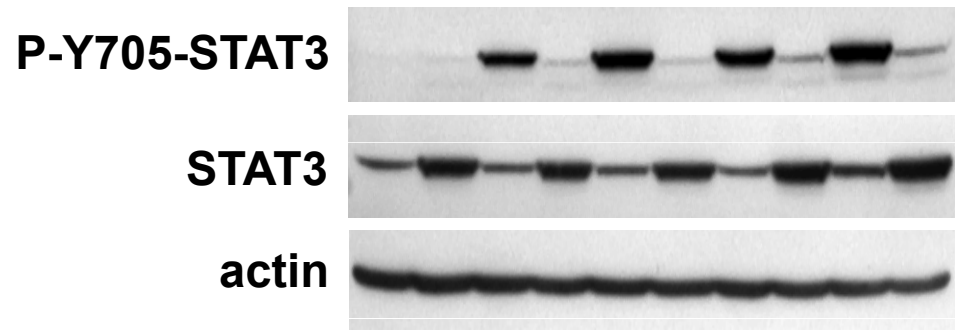
# Chronic FGF stimulus inhibits cytokine/STAT signaling in chondrocytes



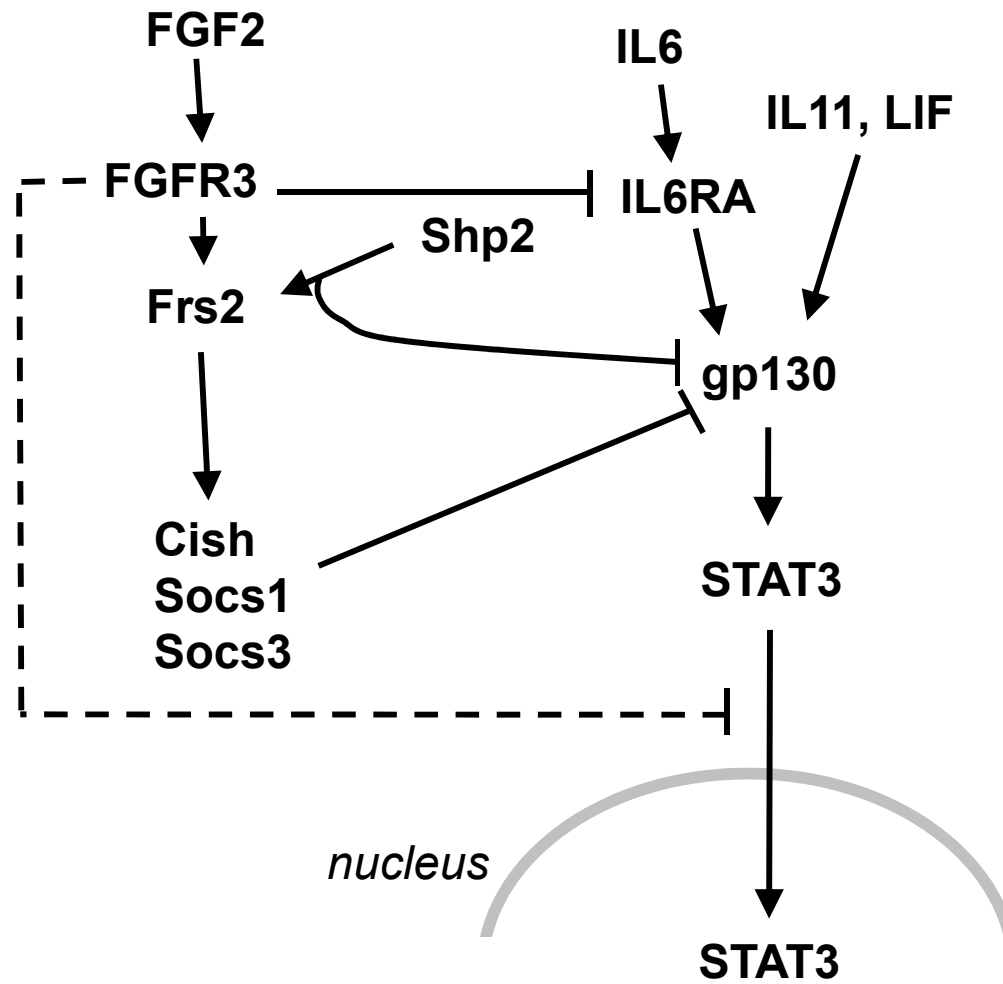
# Chronic FGF stimulus inhibits cytokine/STAT signaling in chondrocytes

FGF2 (72h)	-	+	-	+	-	+	-	+	-	+
IL6	-	-	+	+	-	-	-	-	-	-
IL11	-	-	-	-	+	+	-	-	-	-
LIF	-	-	-	-	-	-	+	+	-	-
IFN $\gamma$	-	-	-	-	-	-	-	-	+	+

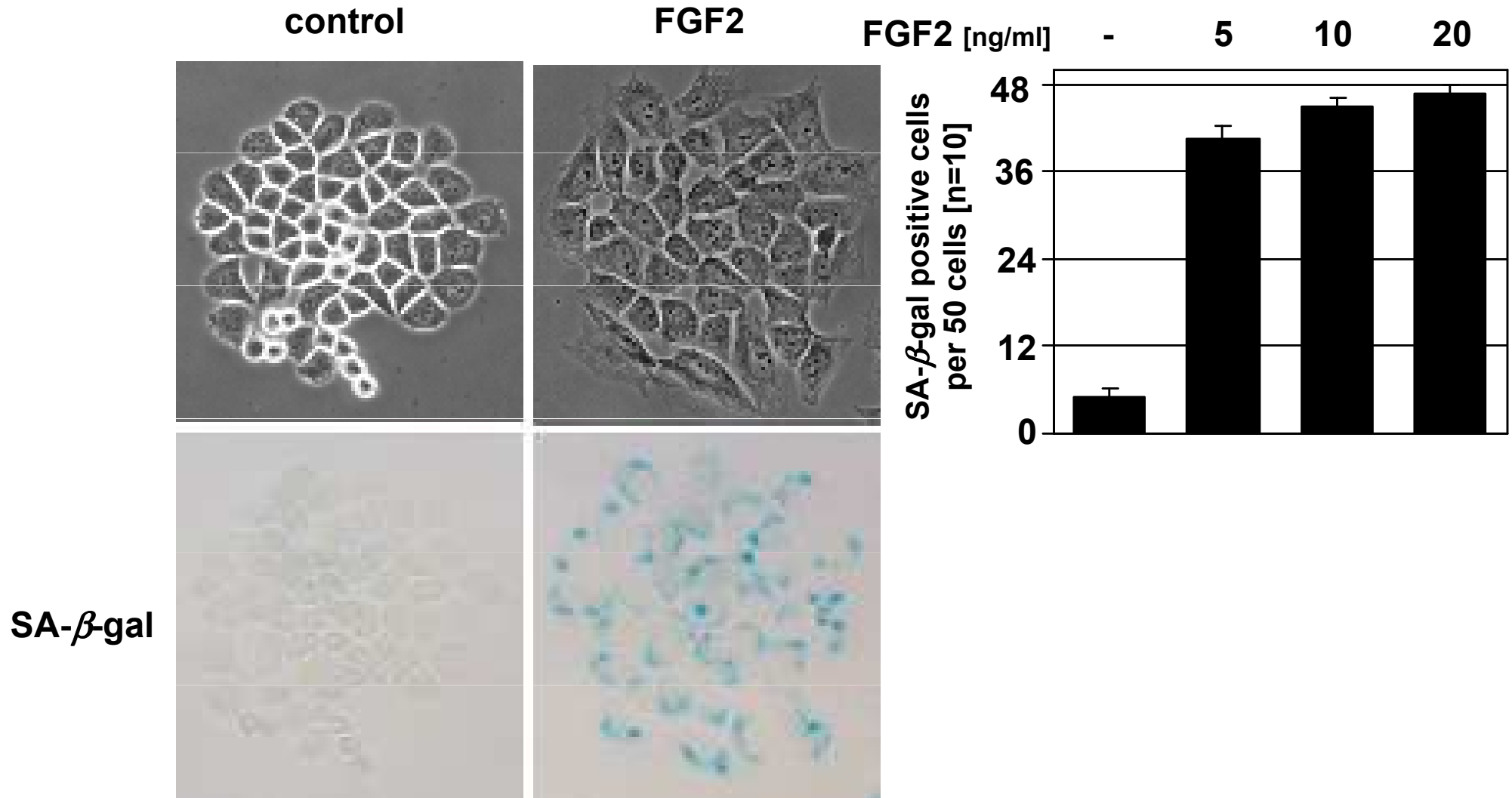
FGF2 (48h)	-	+	-	+	-	+	-	+	-	+
IL6 (minutes)	-	-	5	5	10	10	30	30	60	60



# Chronic FGF stimulus inhibits cytokine/STAT signaling in chondrocytes

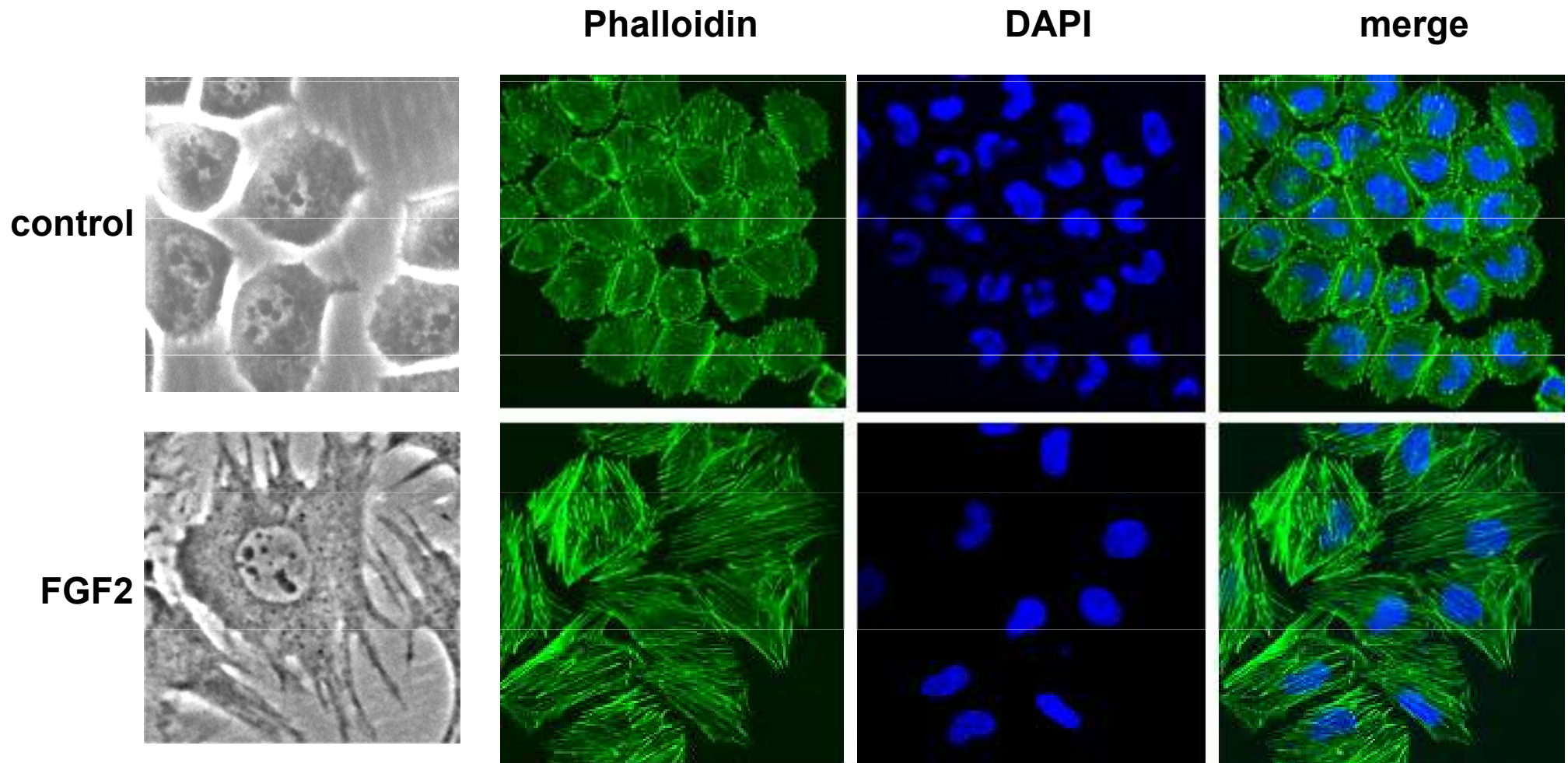


# FGF2 causes premature senescence in chondrocytes

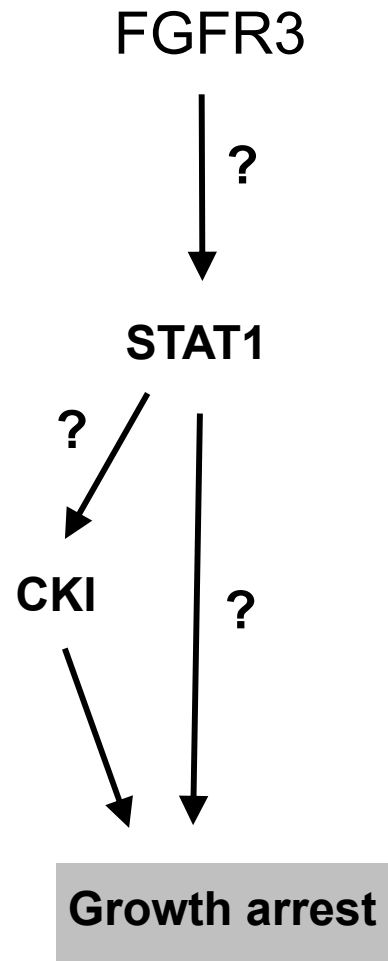




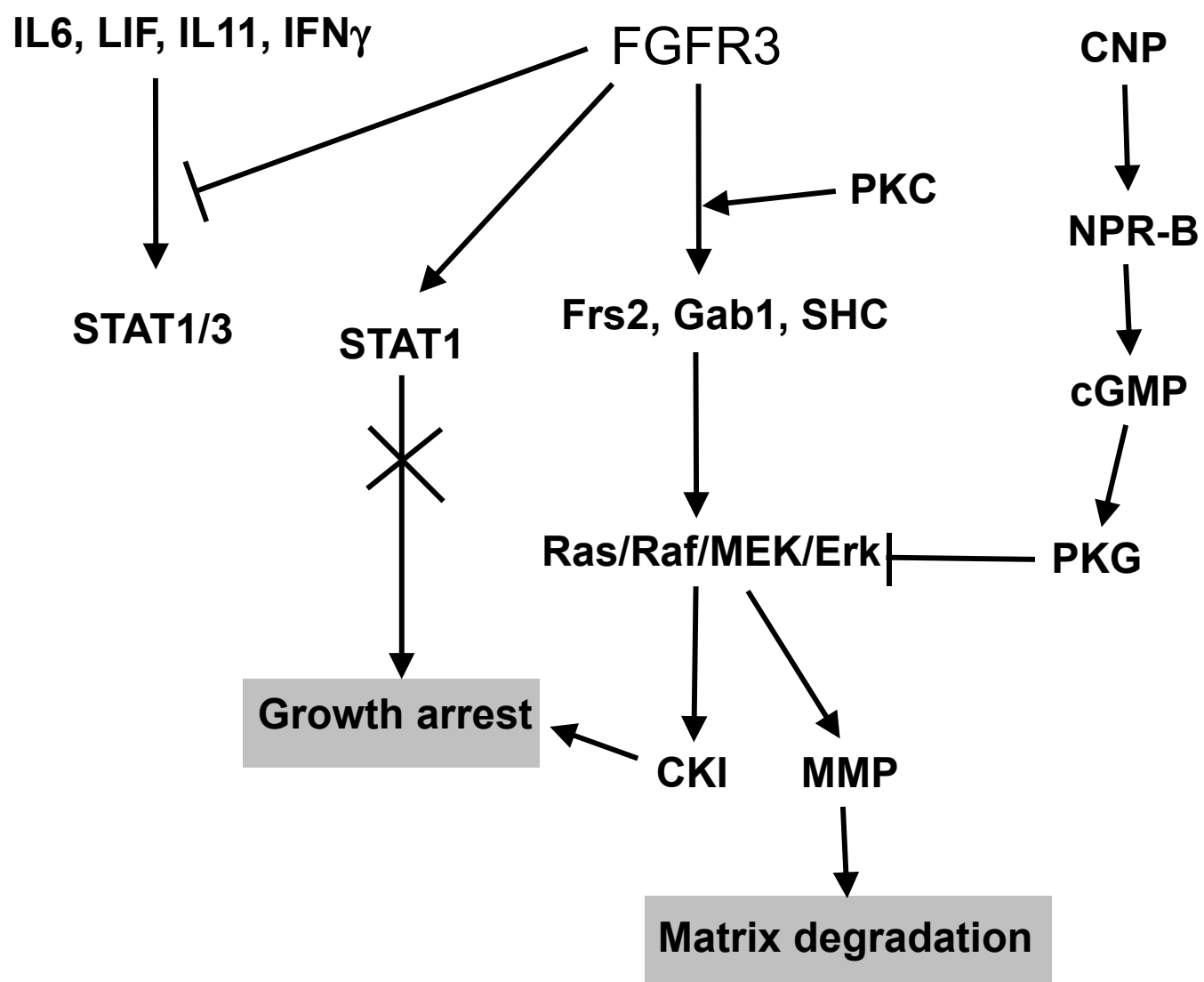
# FGF2 signals towards the cytoskeleton in chondrocytes



2001



2007





From bench to bedside:  
Strategies to treat achondroplasia

1. Stable CNP analog – Biomarin Pharmaceutical Inc.
  2. Neutralizing antibody to FGFR3
  3. Small chemical inhibitor of FGFR3
- Prochon Biotech Ltd.

*Cedars-Sinai Medical Center*

*Los Angeles, California*

William Wilcox

Katerina Pejchalova

Betty Mekikian

Patricia Lin

Matthew Rock

Claire Rock

*UCI, Irvine*

*California*

Leslie Thompson

Tamara Kashiwada

Lisa Salazar

*UCLA, Los Angeles*

*California*

Robert Pogue

Matthew Schibler

*Laboratory of Molecular Embryology*

*MZLU Brno, Czech republic*

Vita Bryja

Jiri Pachernik

*INSERM U589, Toulouse, France*

Herve Prats

Bernard Masri

Vincent Fontaine