

Molecular roots of FGFR3-related skeletal dysplasia

Pavel Krejci, PhD

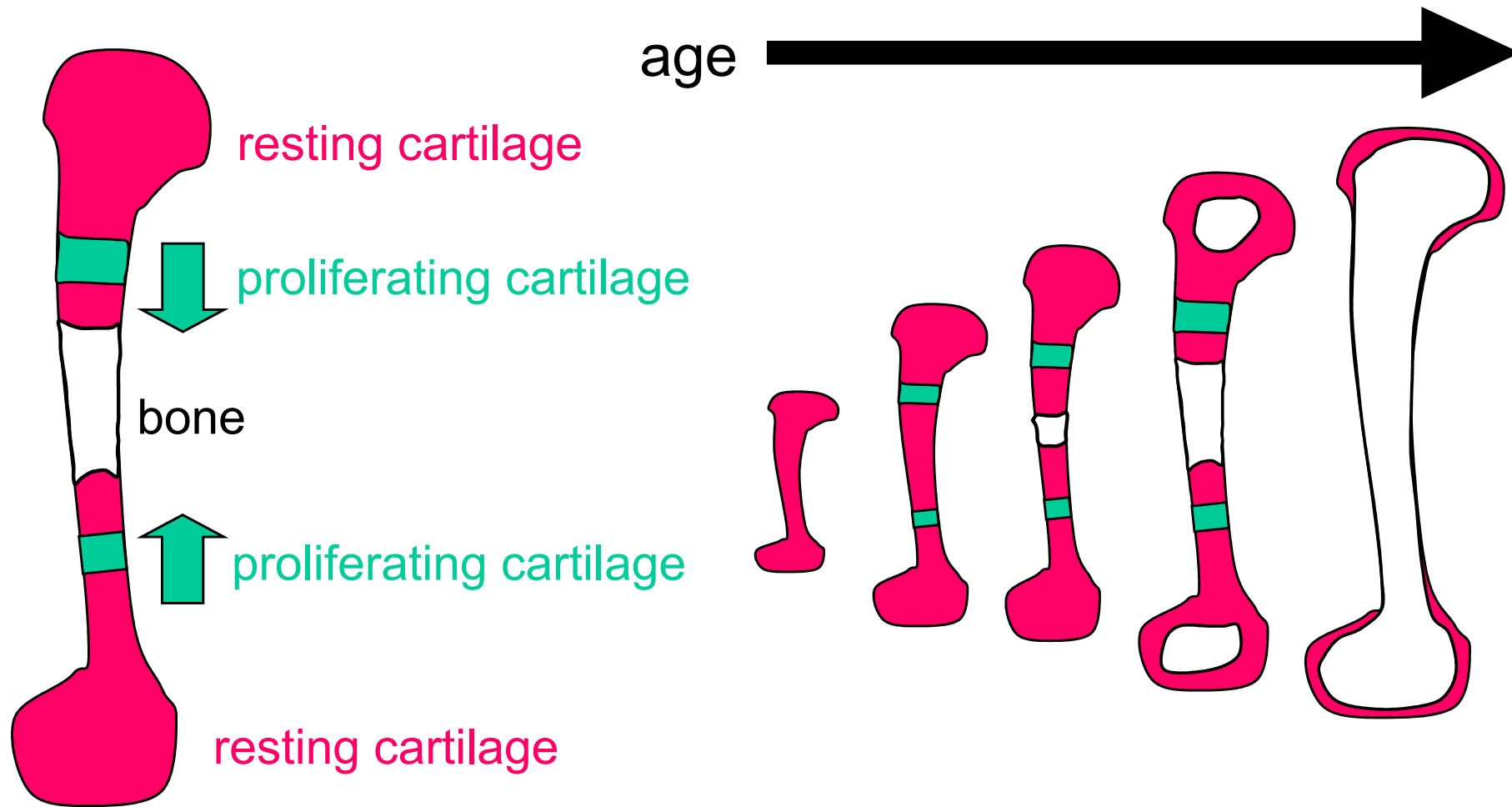
Institute of Experimental Biology, Masaryk University, Brno
Department of Cytokinetics, Institute of Biophysics AVCR, Brno
Cedars-Sinai Medical Center, Los Angeles, USA

Navaznosti

Prednaska navazuje na obecne vymezeni
podstaty bunecneho signalovani

Obecne zakonitosti budou demonstrovany na
priklade FGF signalingu v skeletalni dysplasii

How do the limbs grow?



FGFR3-related skeletal dysplasia

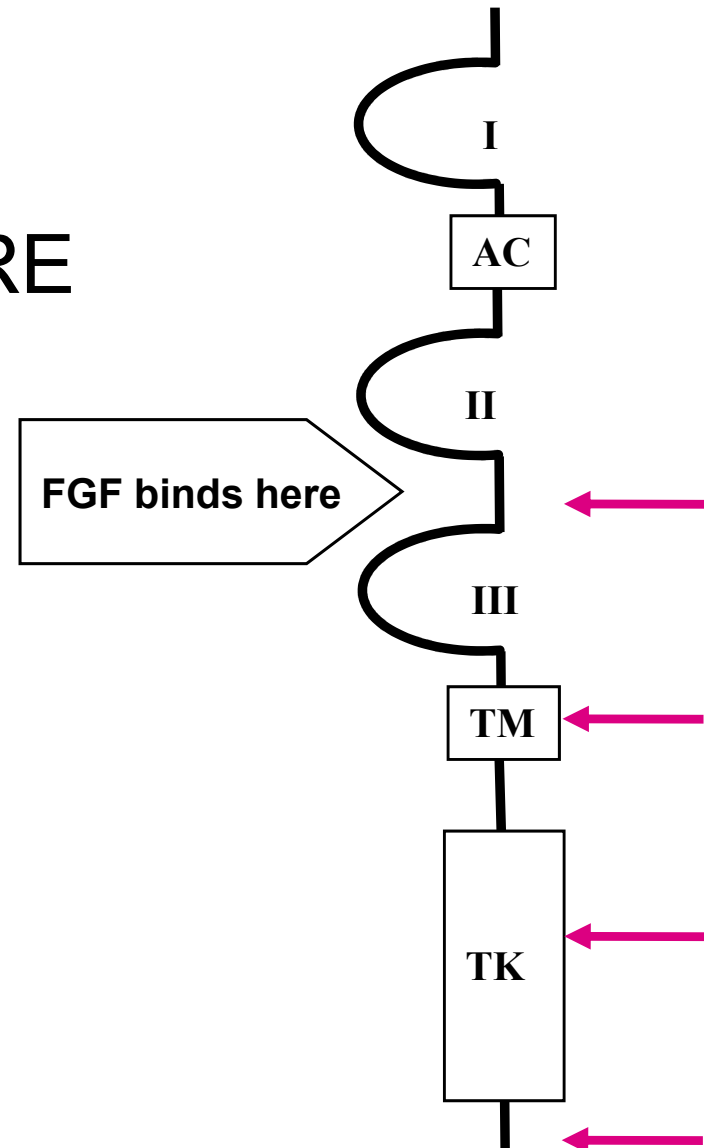


Achondroplasia

FGFR3-related skeletal dysplasia

Hypochondroplasia
Achondroplasia
SADDAN
Thanatophoric Dysplasia

STATURE

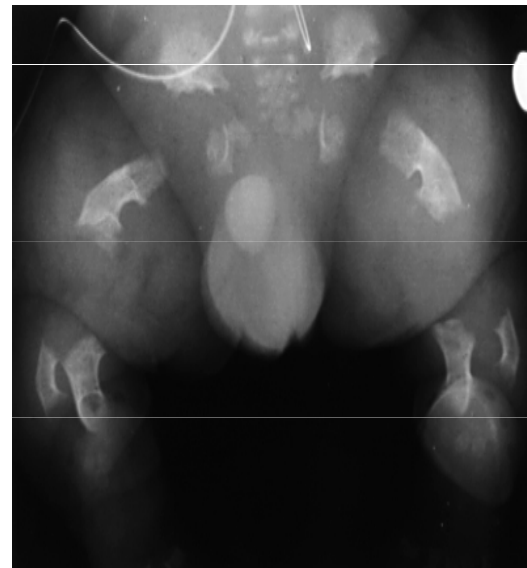
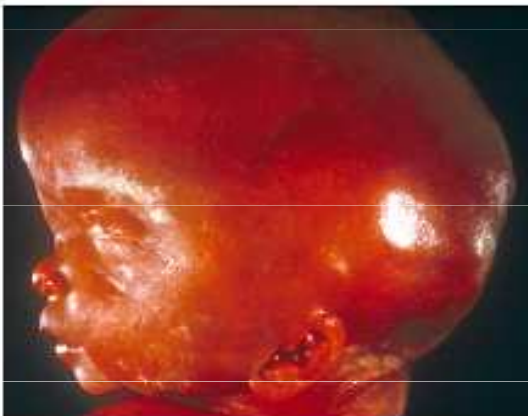


Thanatophoric Dysplasia



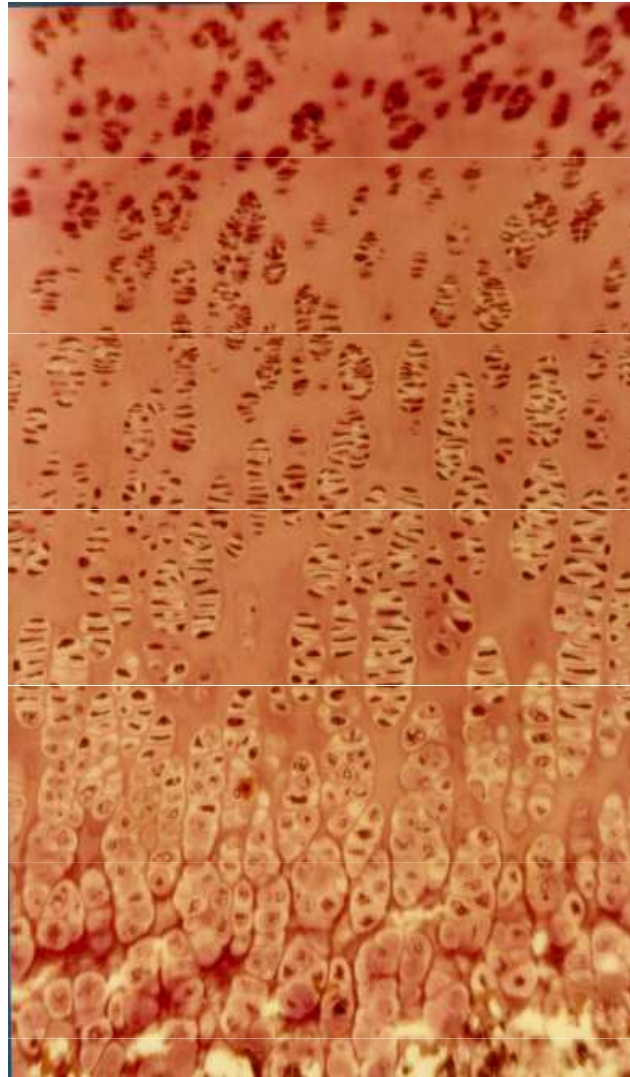
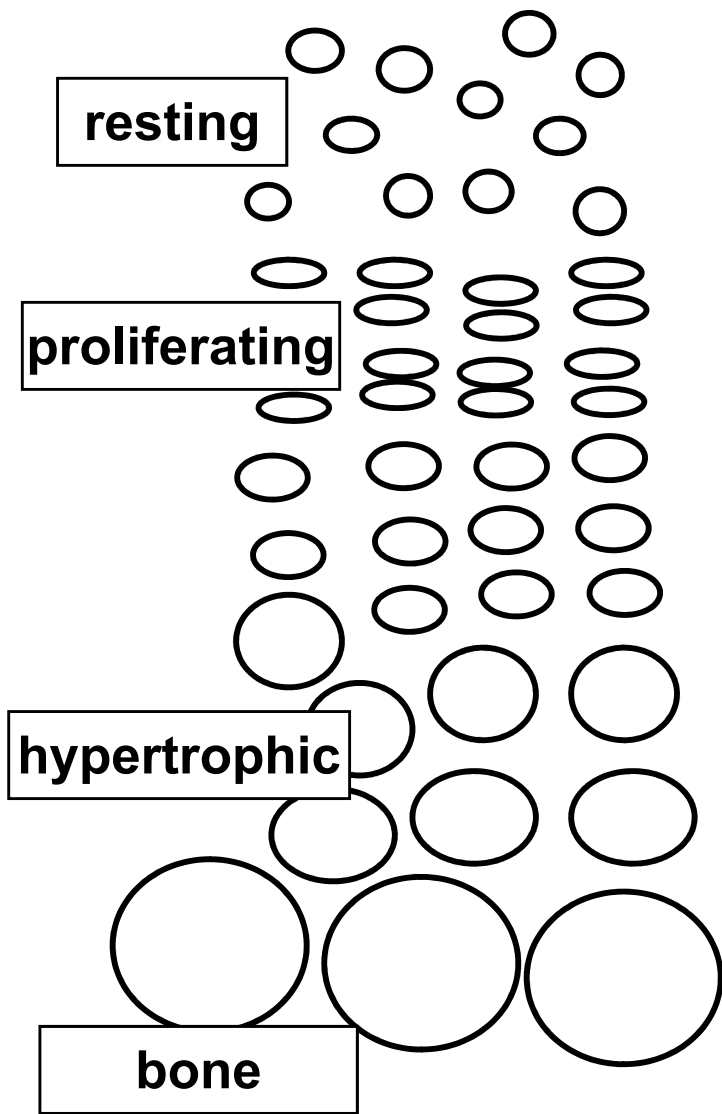
healthy

- short long bones
- brachydactyly
- macrocephaly

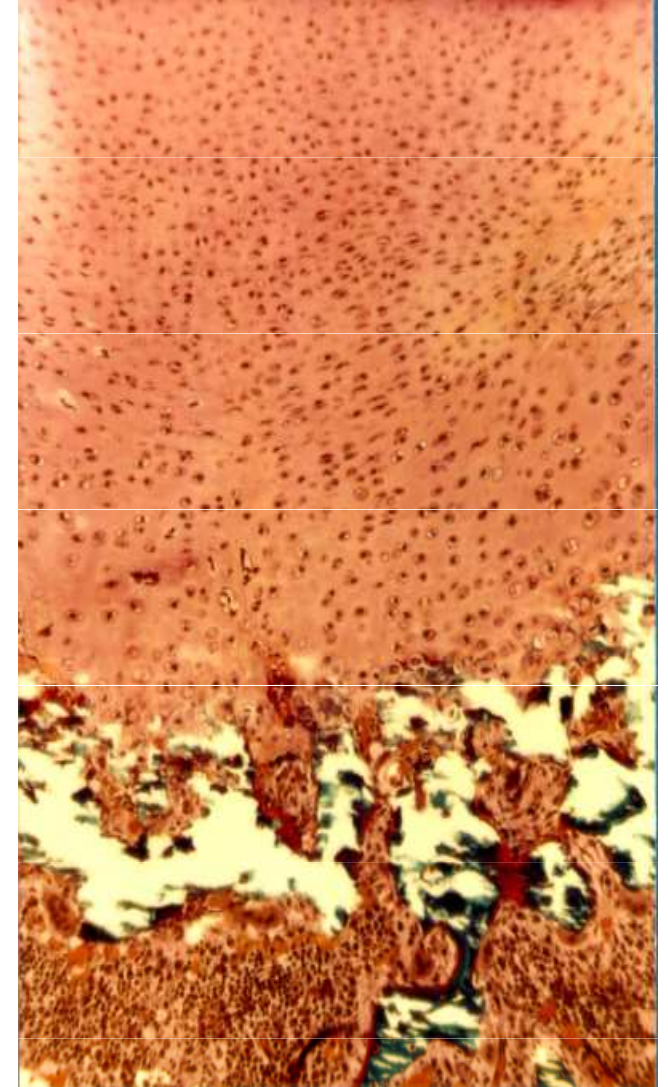


TD

- low nasal bridge
- spinal stenosis
- temporal lobe malformations



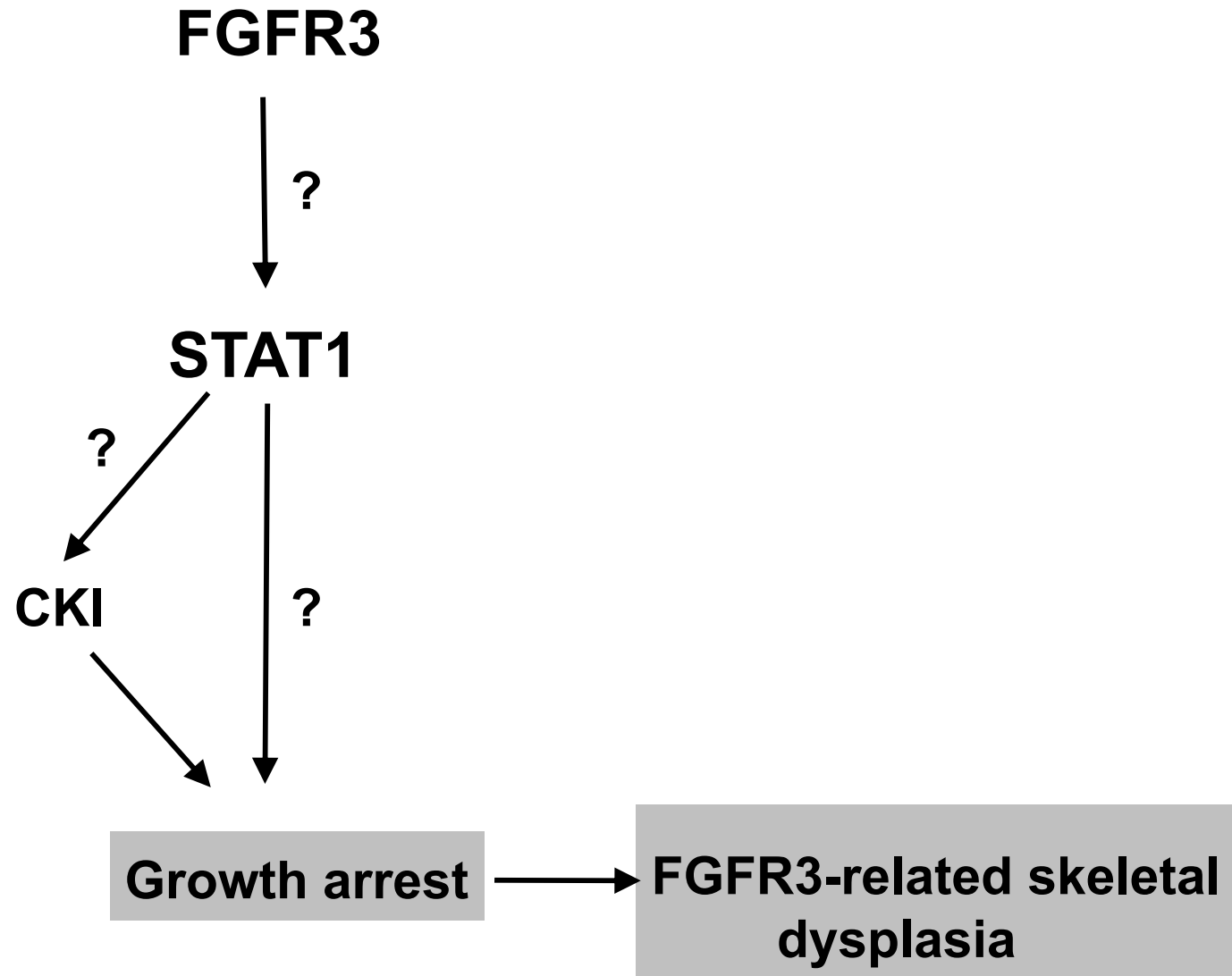
healthy



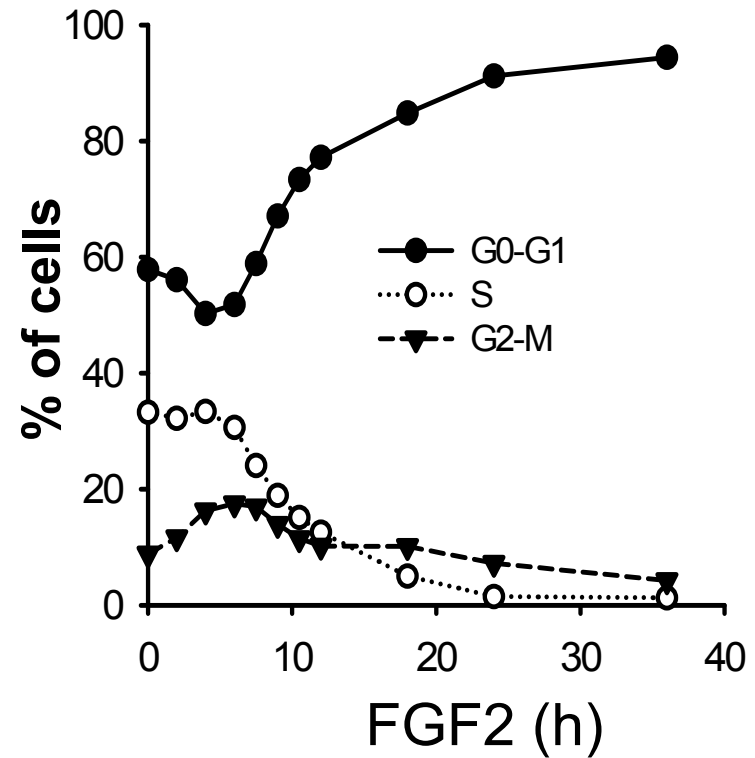
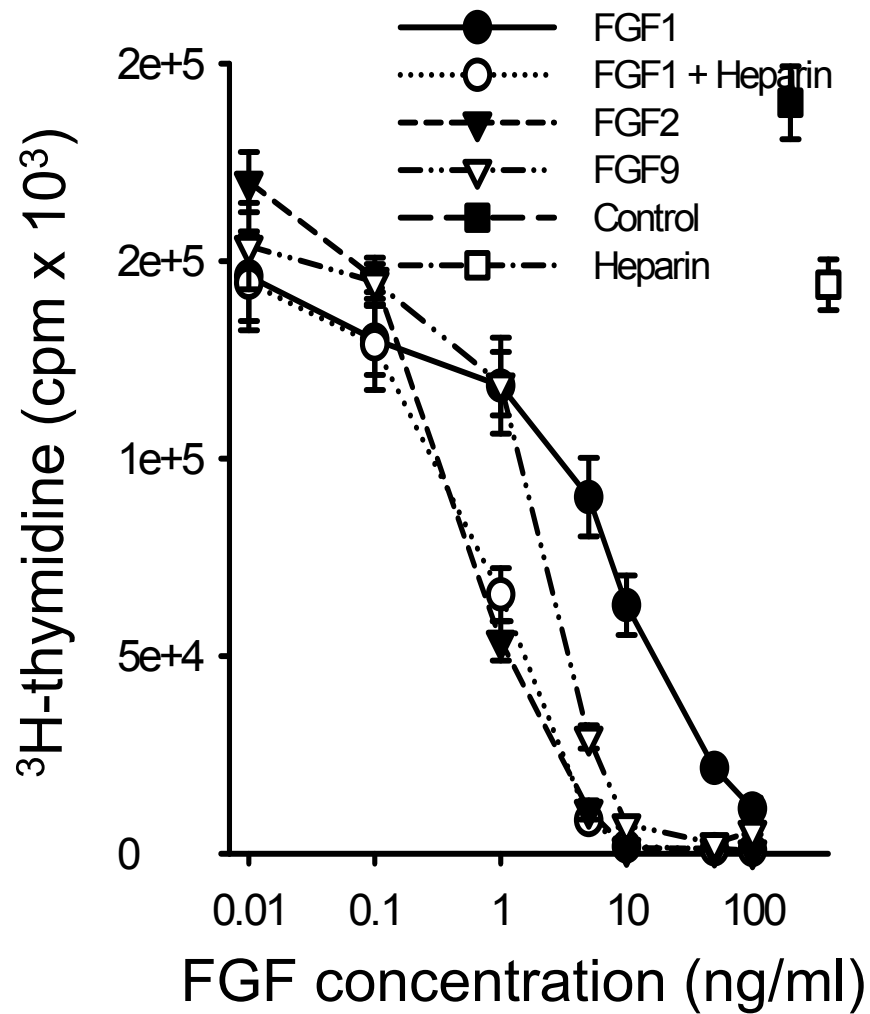
TD

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

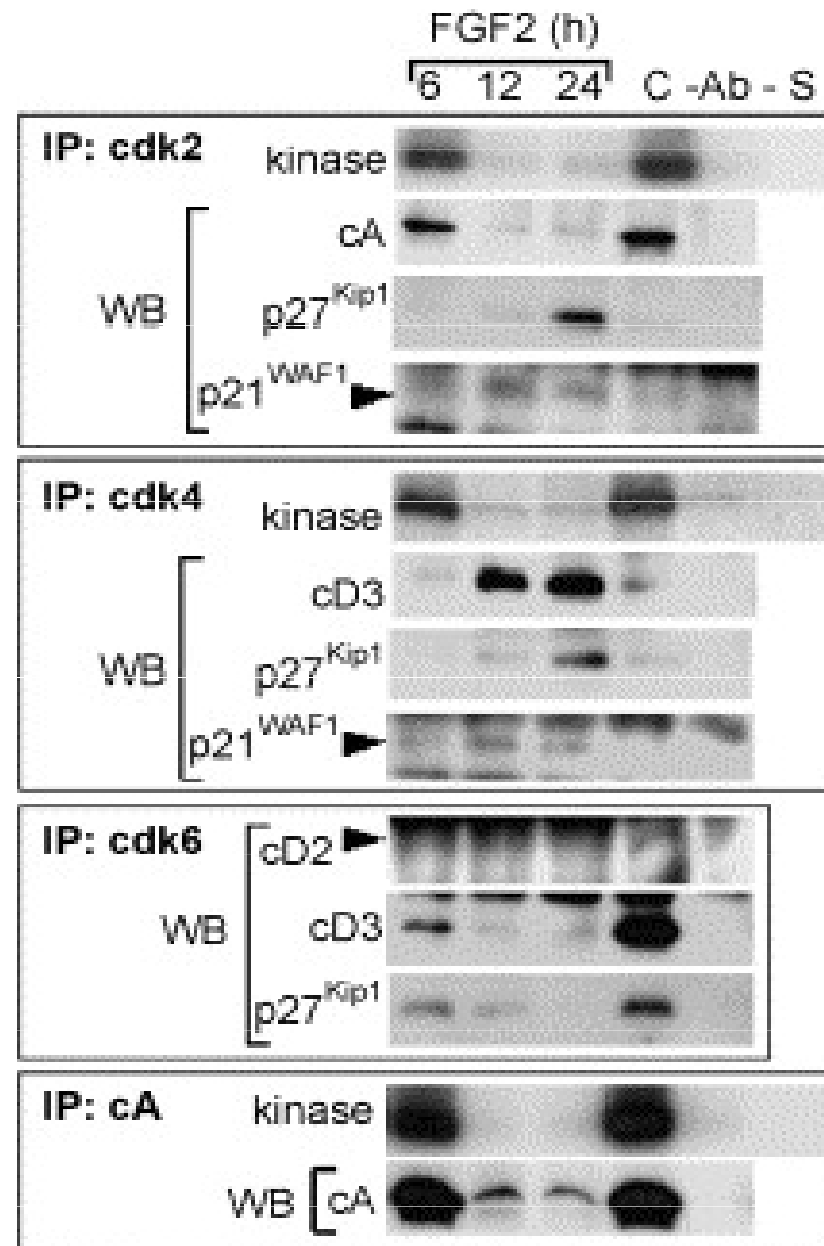
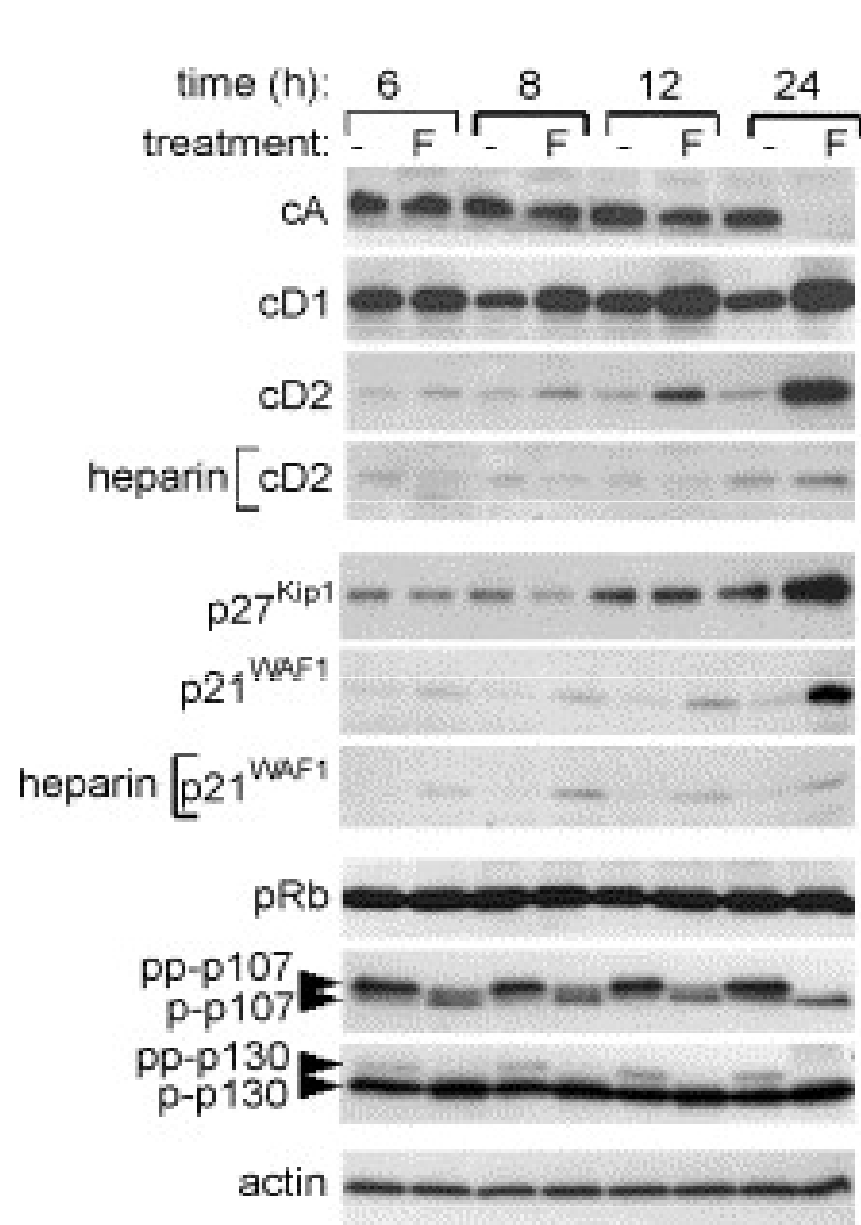
Sahni *et al.*, *Development* 2001, 128, 2119-29.



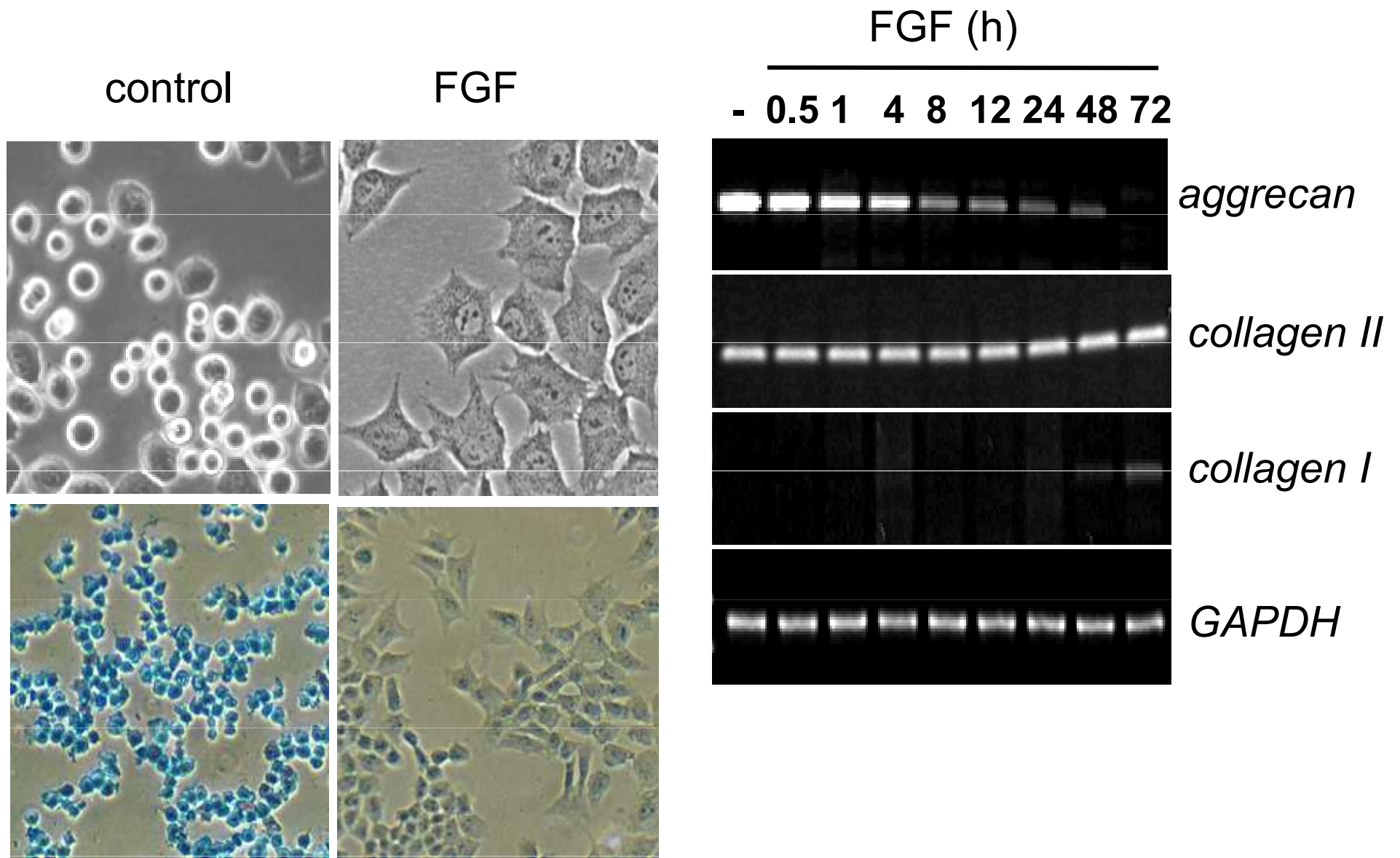
FGF inhibits chondrocyte proliferation by arresting their cell cycle in G1 phase



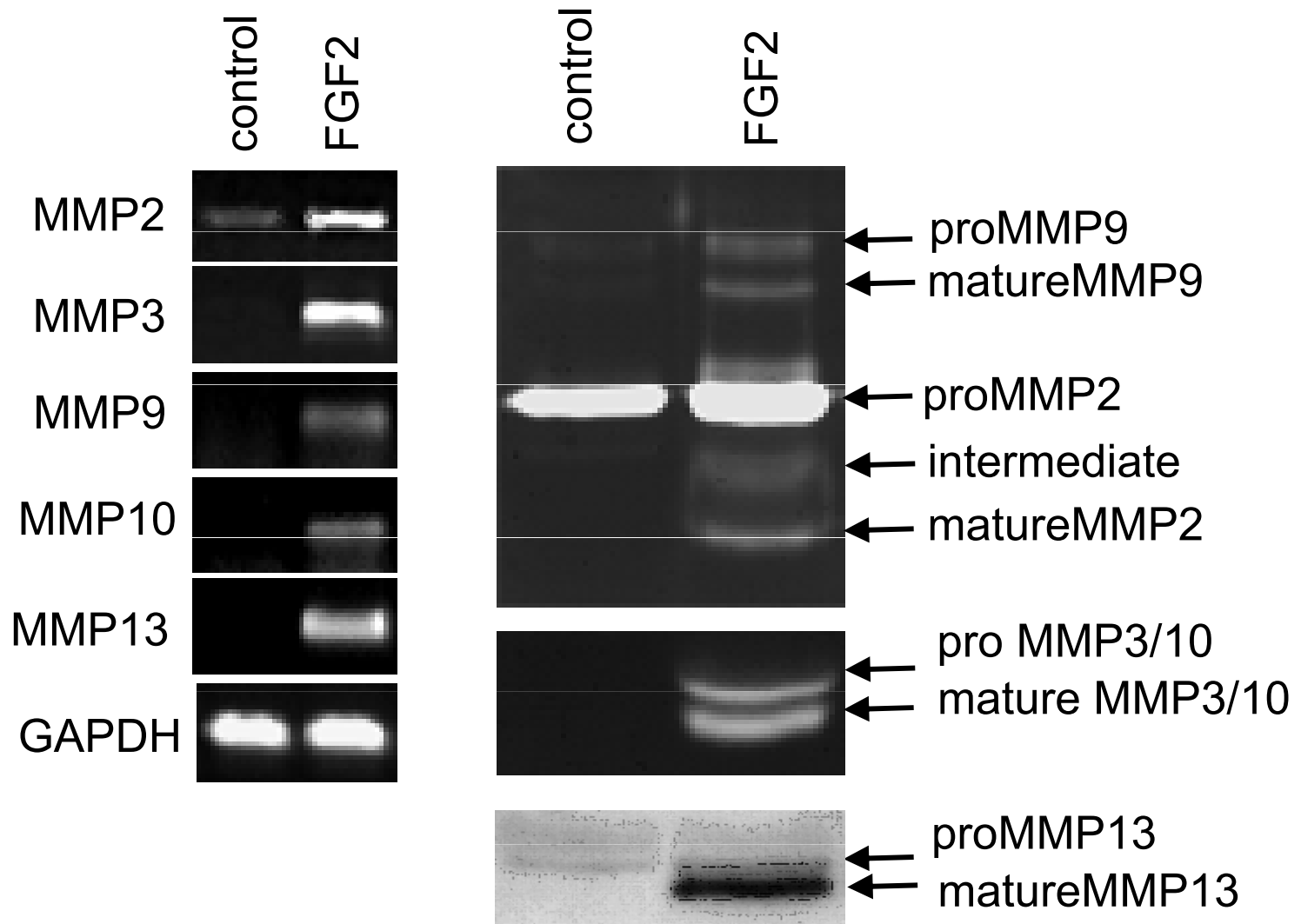
....via inhibition of cdk activity necessary for progression through the G1 phase of a cell cycle



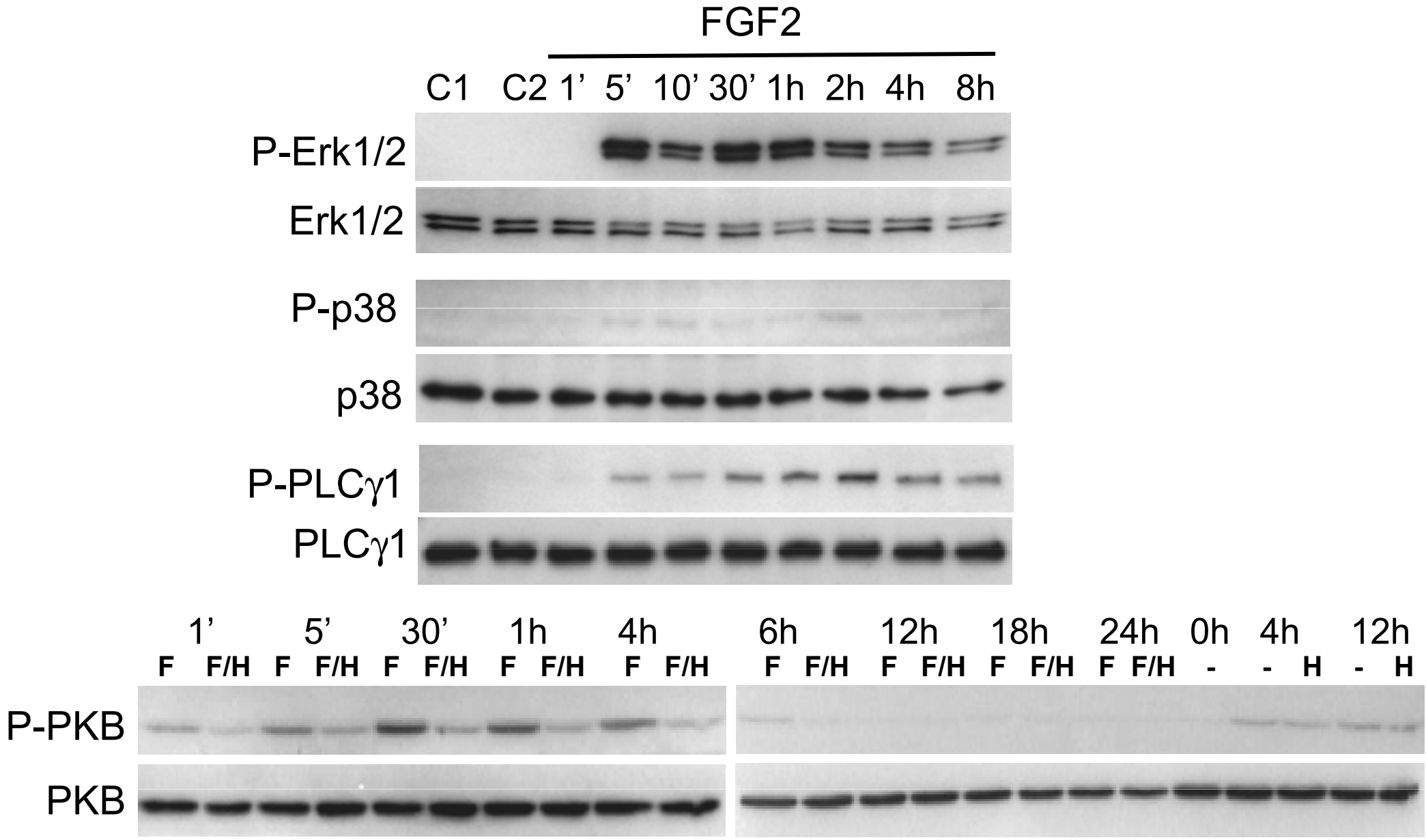
FGF alters the cartilage-like phenotype of chondrocytes



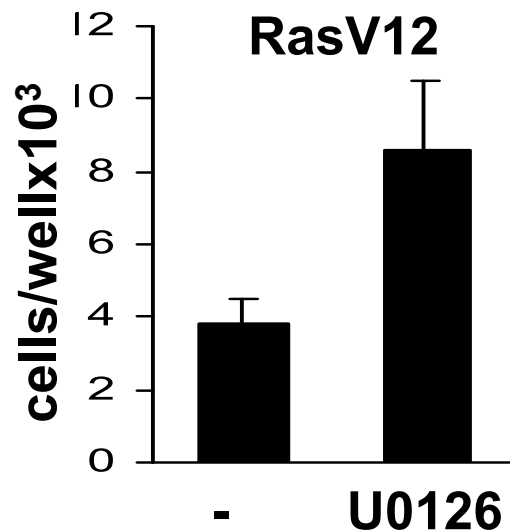
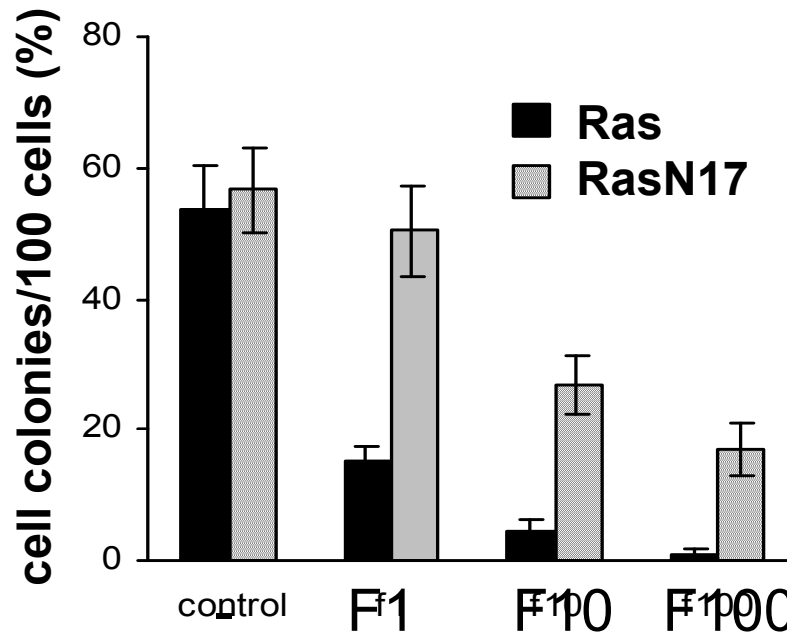
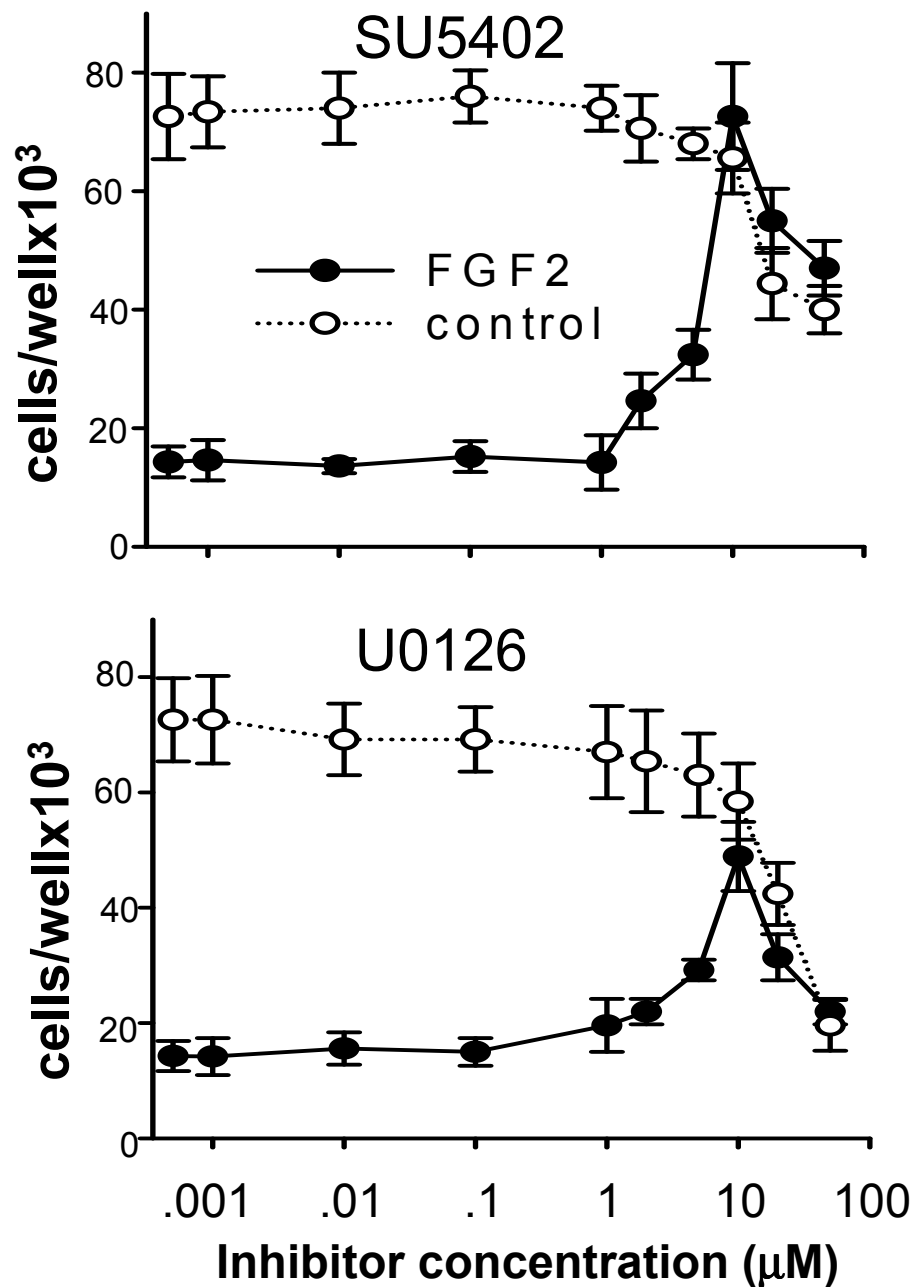
.....via MMP-mediated degradation of extracellular matrix



FGF2 activates Erk and p38 MAPK, PLC γ and PKB in chondrocytes



.....but only Ras/Erk activity is involved in FGF-induced growth arrest (Krejci *et al.*, *Exp Cell Res* 2004, 295, 152-64)



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.

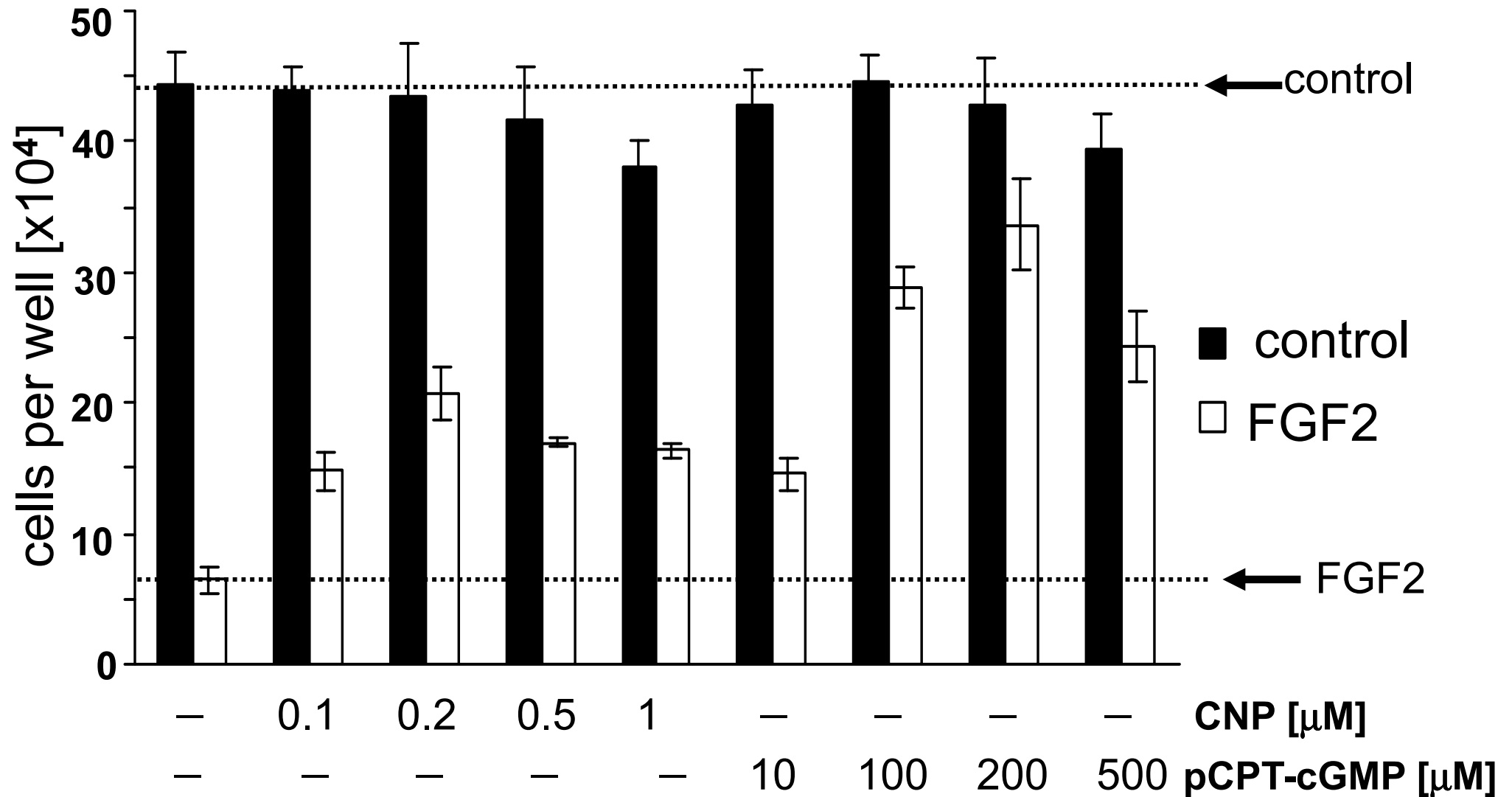
Rauci *et al.*, *J Biol Chem* 2004, 279, 1747-1756.

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

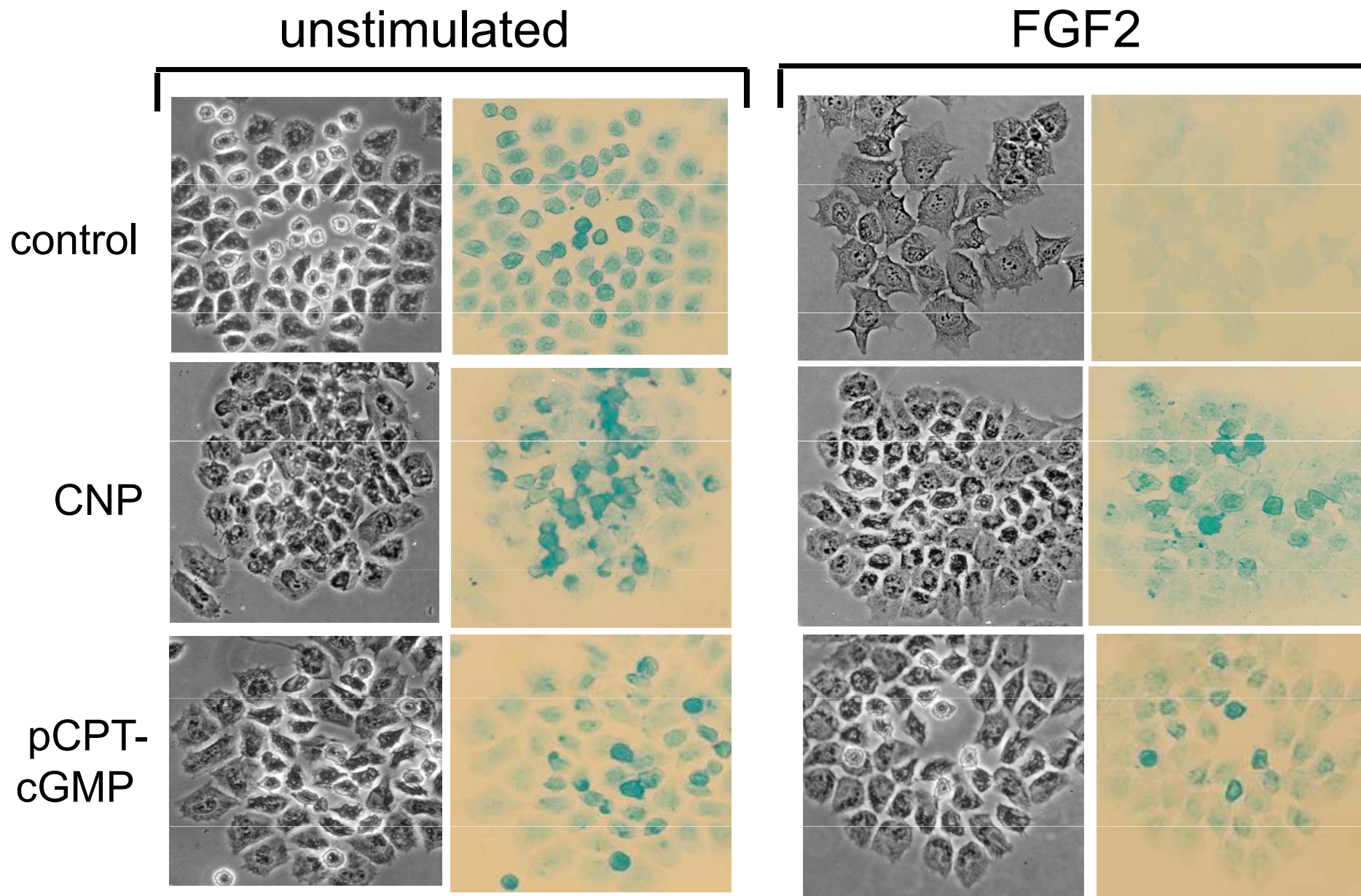
C-type Natriuretic Peptide (CNP) rescues
achondroplastic phenotype in FGFR3-ACH mice.

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

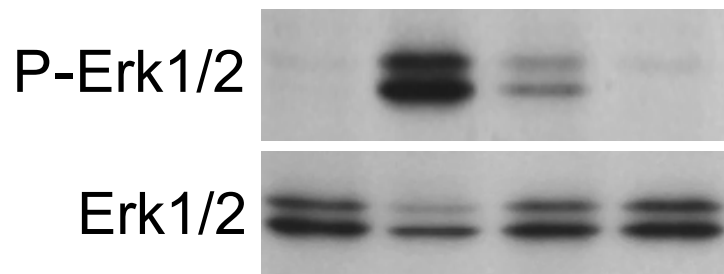
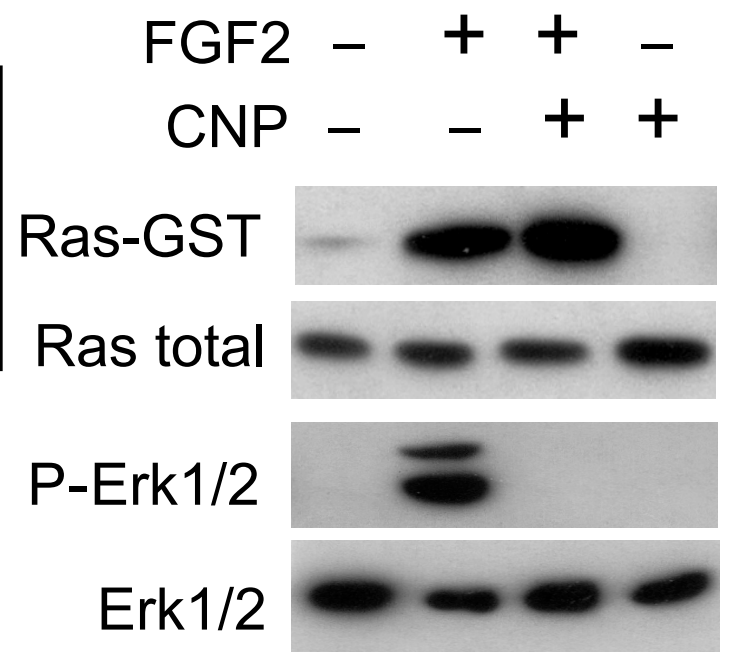
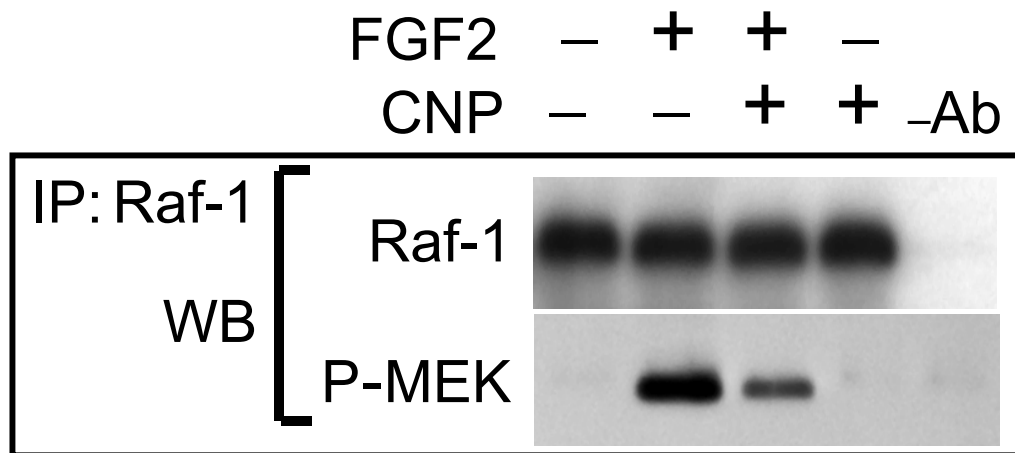
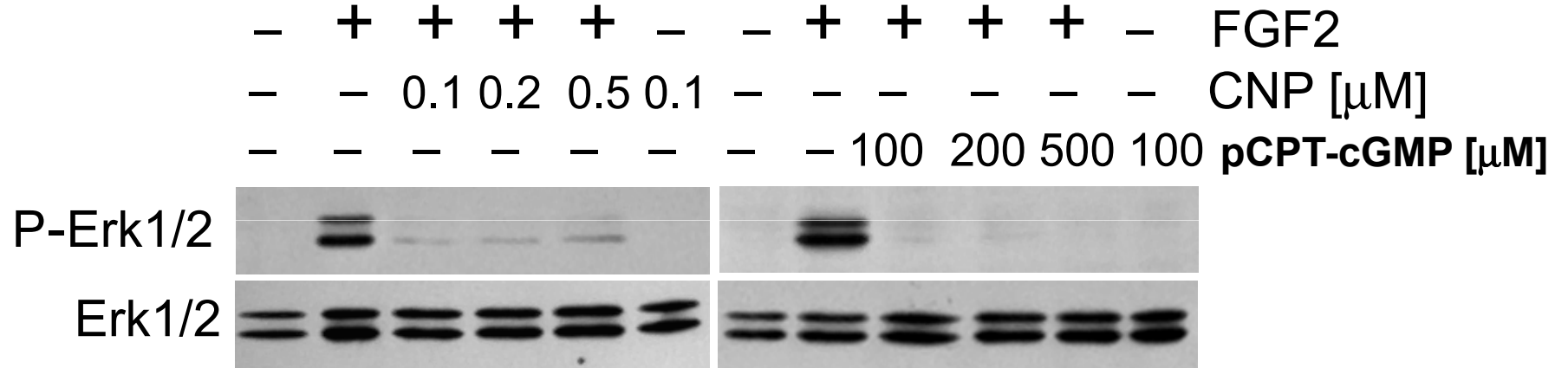
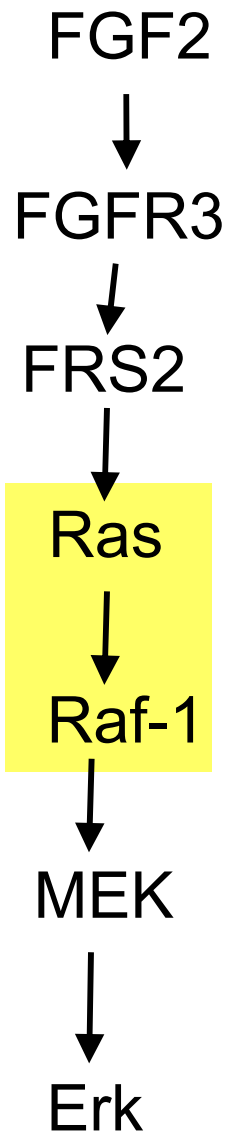
CNP counteracts FGF2-mediated chondrocyte growth arrest through cGMP-dependent pathway



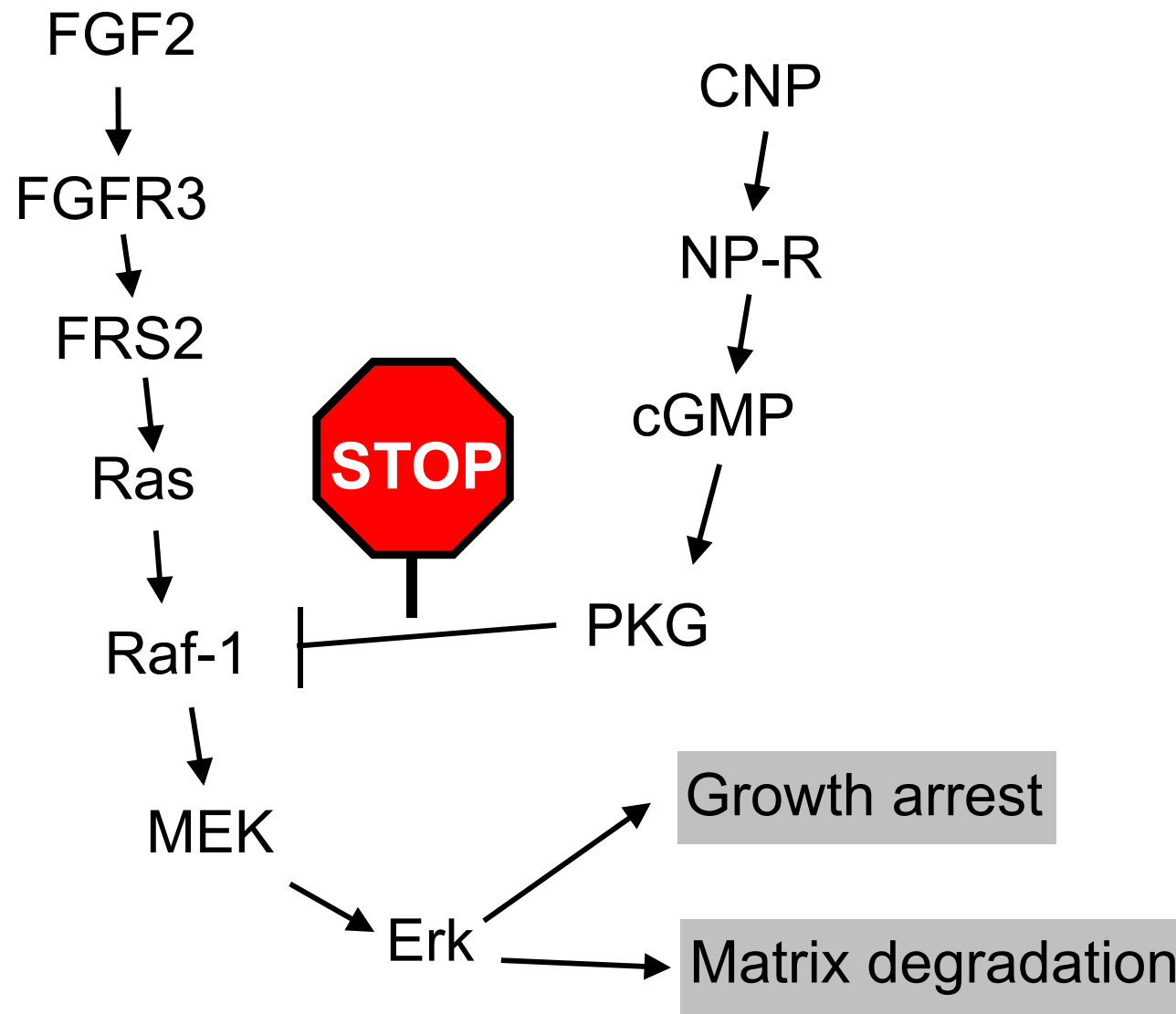
CNP antagonizes FGF2-mediated loss of cartilage extracellular matrix in chondrocytes



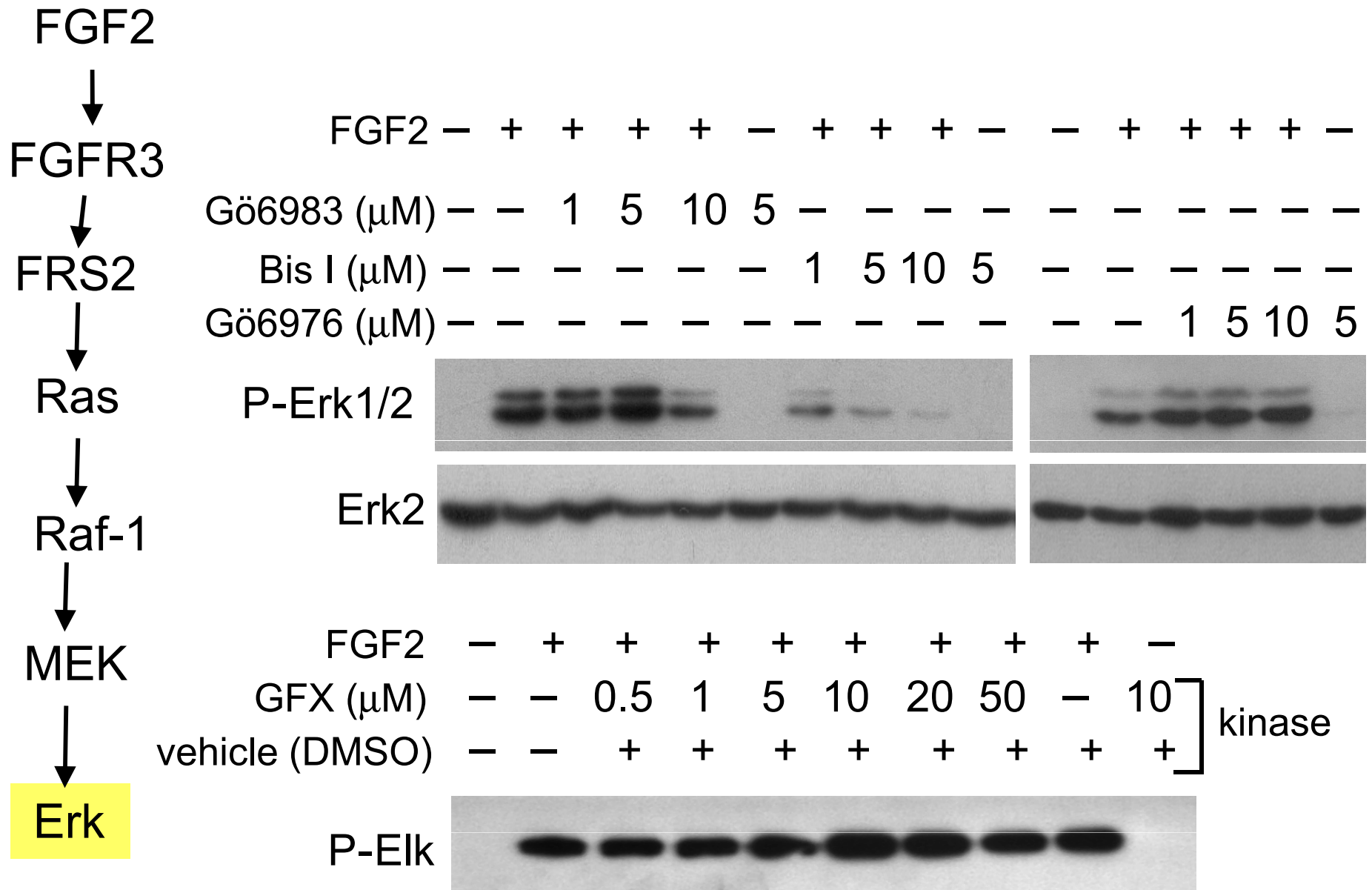
CNP counteracts FGF2-mediated activation of Erk MAP kinase in chondrocytes

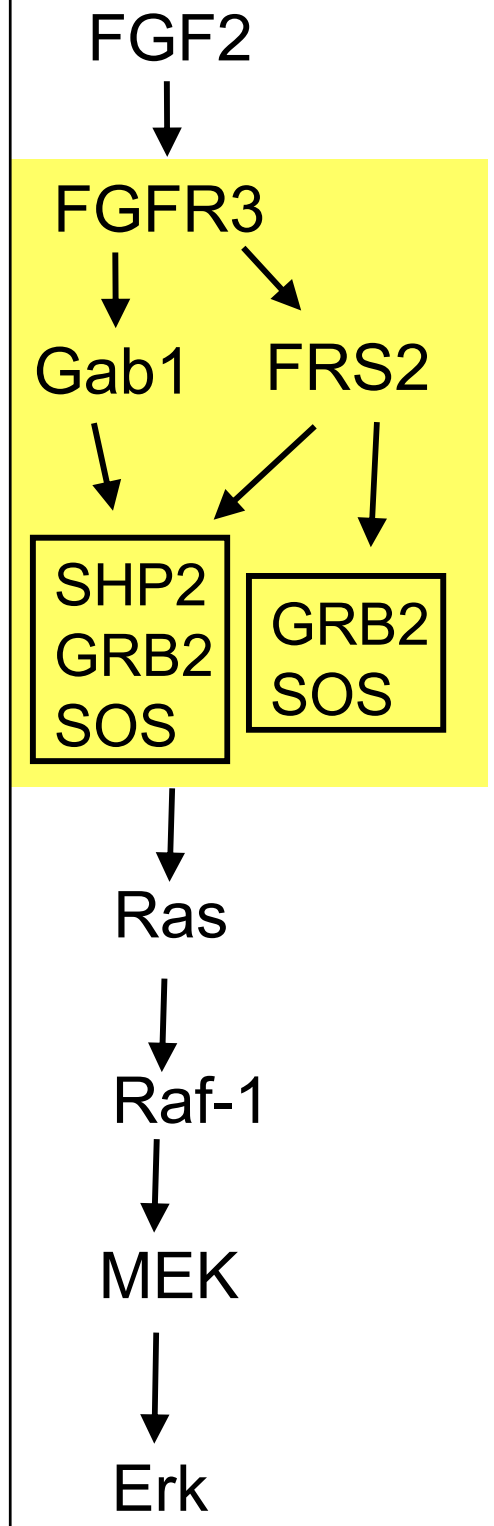


CNP inhibits Erk MAP kinase module at the Raf level



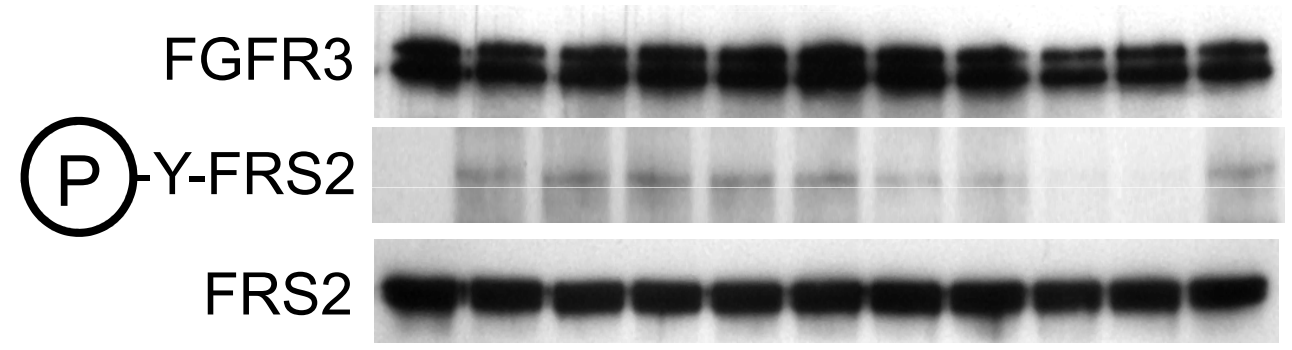
Is protein kinase C (PKC) involved in FGFR3-mediated activation of Erk in chondrocytes?



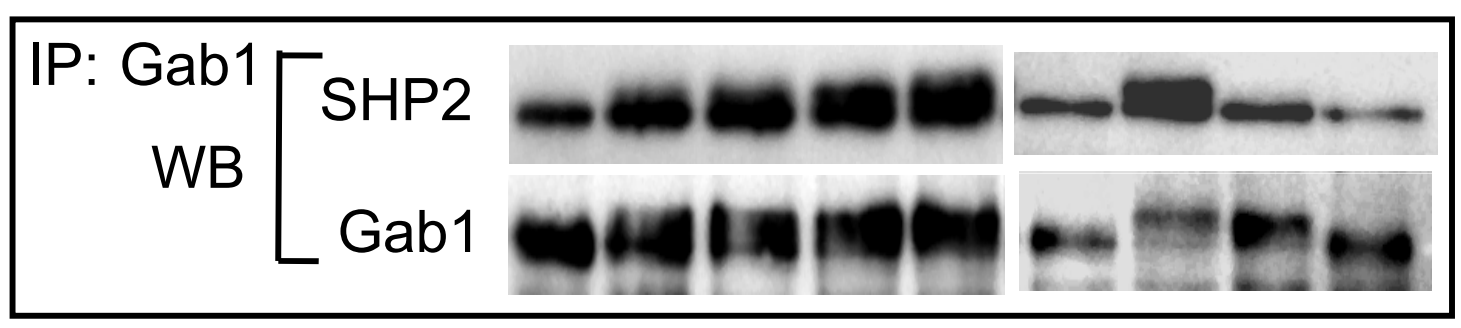
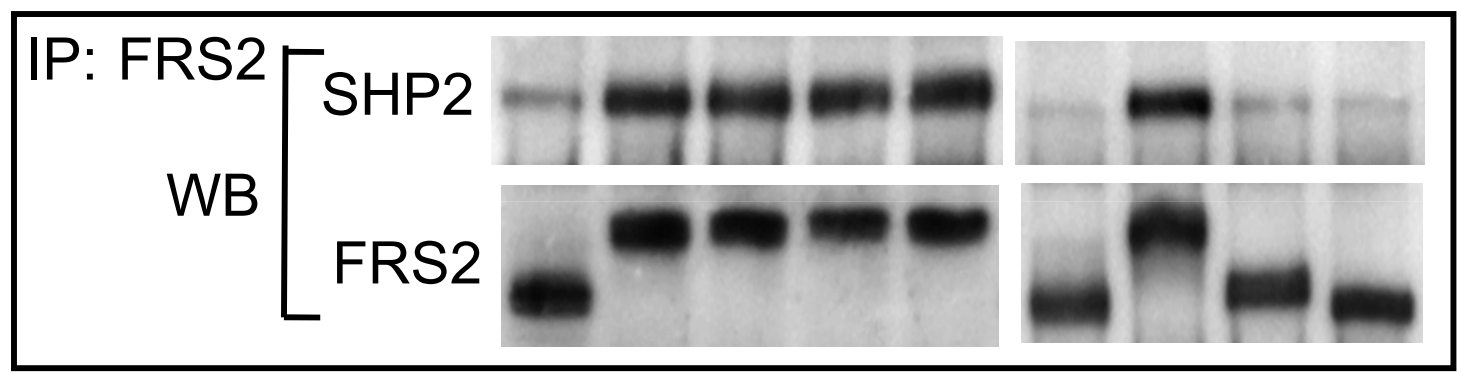


| | | | | | | | | | | | |
|---------------|---|---|---|---|----|----|----|---|----|----|---|
| FGF2 | - | + | + | + | + | + | + | + | + | + | + |
| Bis I (μM) | - | - | 1 | 5 | 10 | 20 | 50 | - | - | - | - |
| SU5402(μM) | - | - | - | - | - | - | - | 1 | 10 | 20 | - |
| vehicle(DMSO) | - | - | + | +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

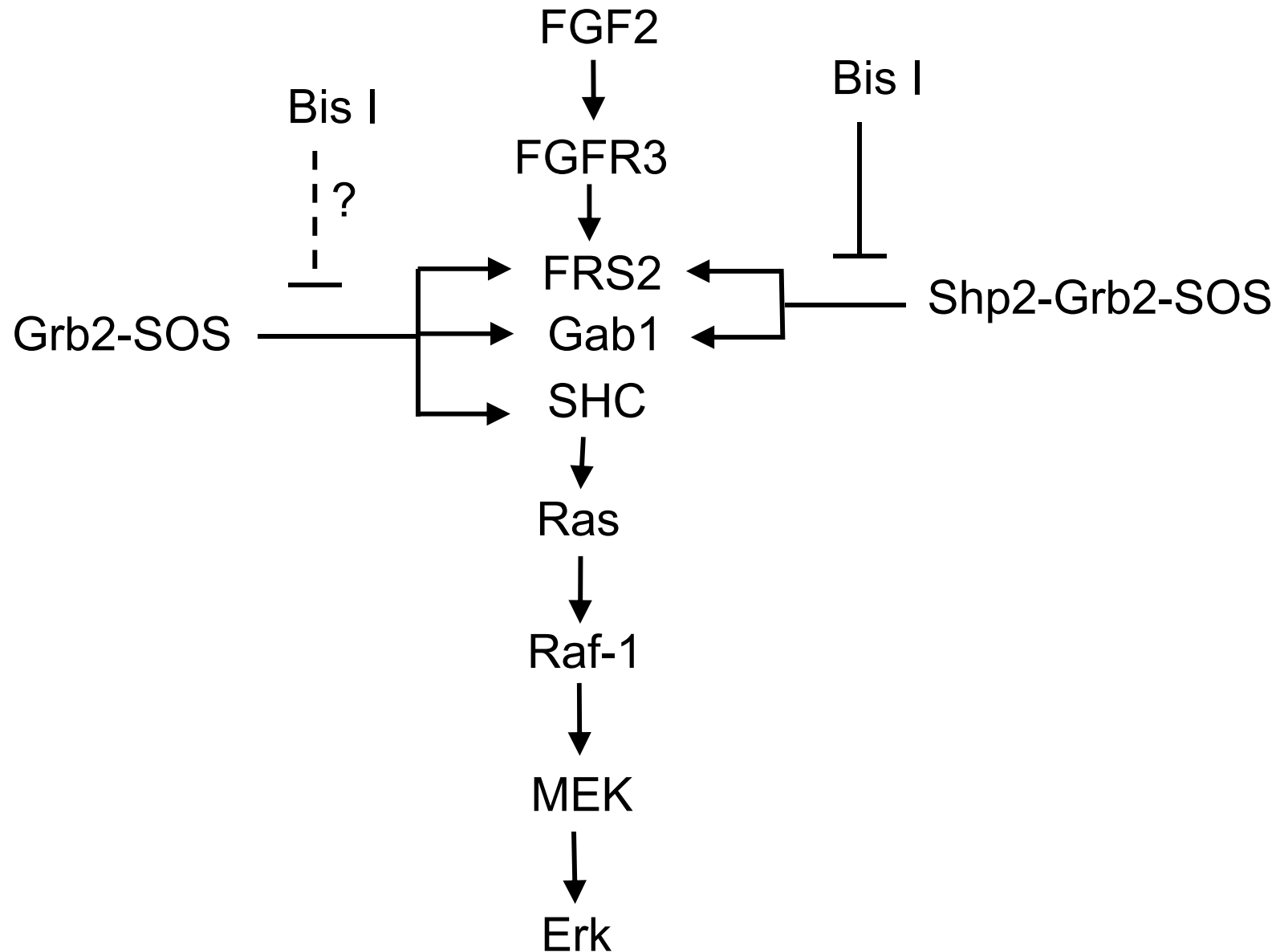
} kinase



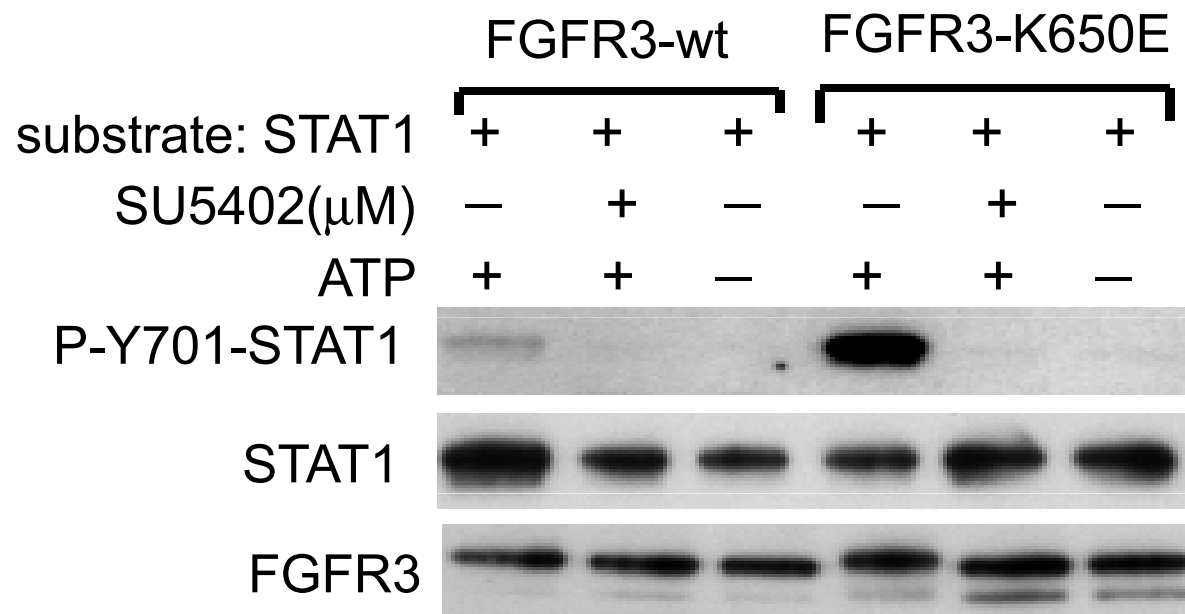
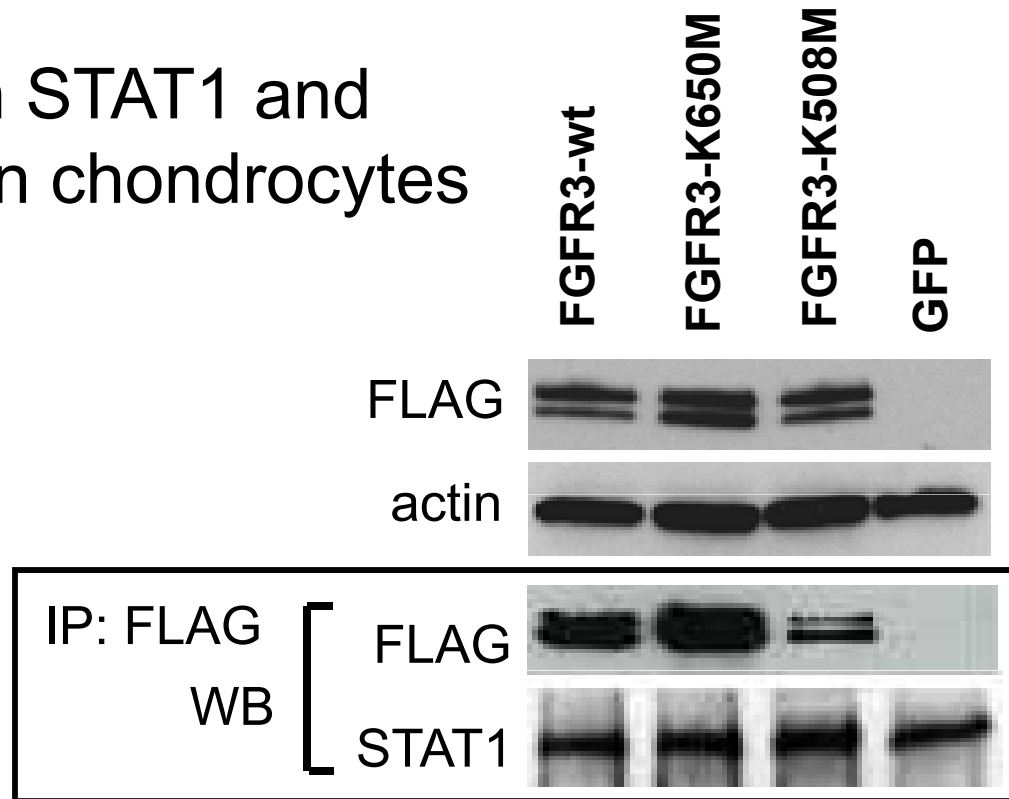
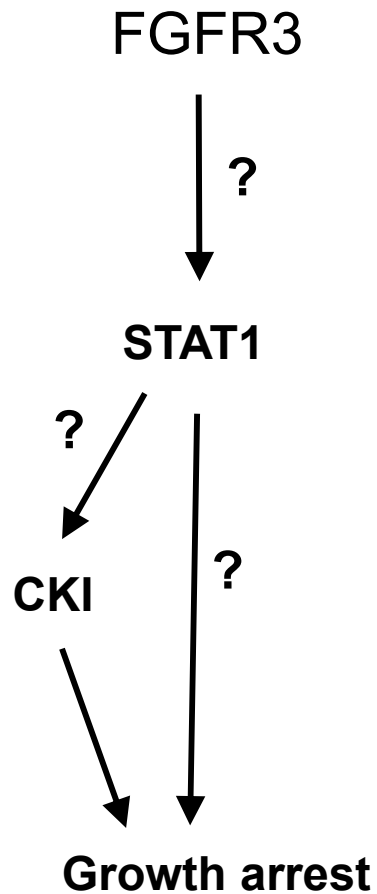
| | | | | | | | | | |
|-------|---|----|-----|-----|----|---|-----|-----|---|
| FGF2 | - | 5' | 10' | 30' | 1h | - | 30' | 30' | - |
| Bis I | - | - | - | - | - | - | - | + | + |



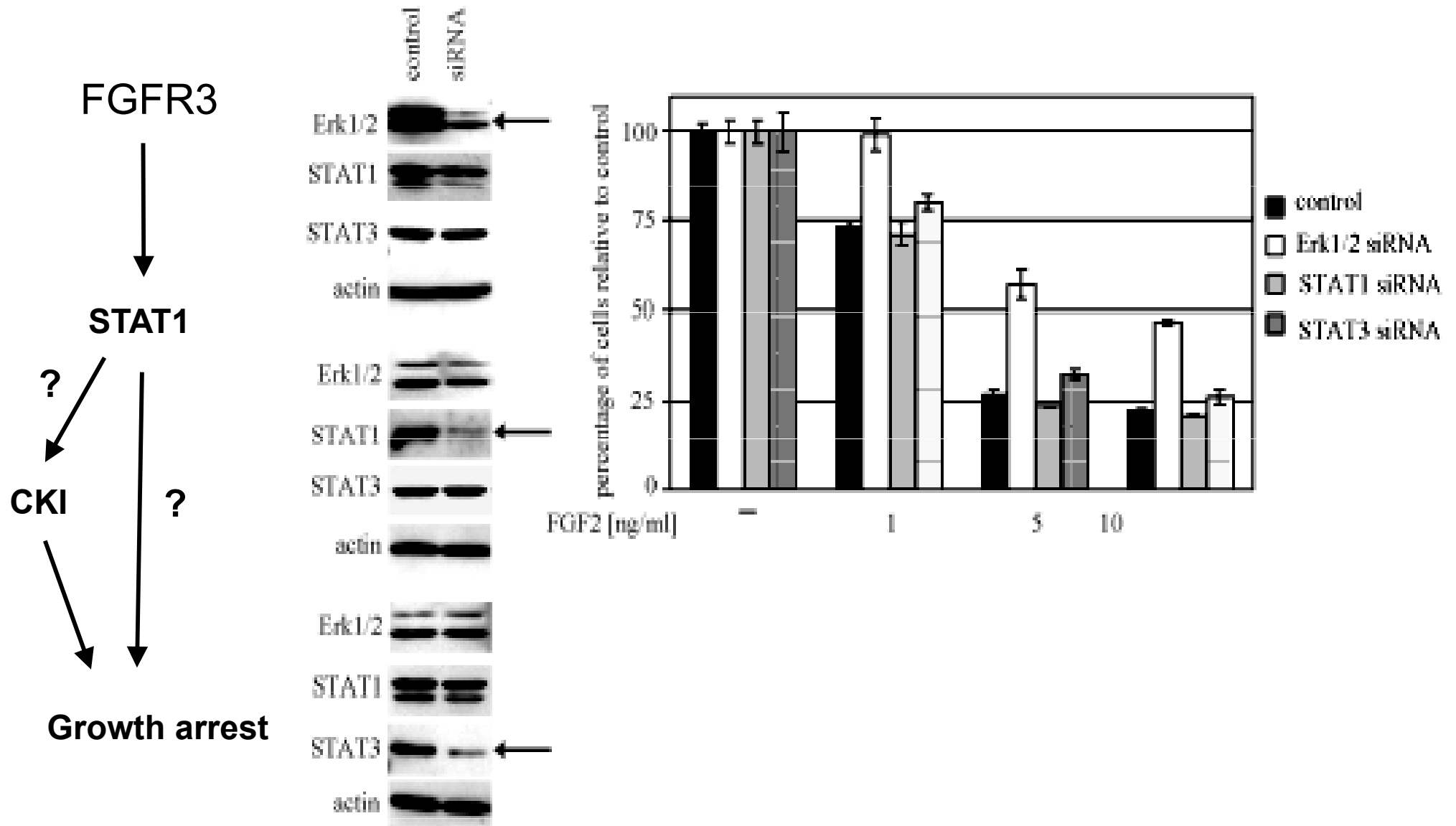
Protein kinase C inhibitor Bisindolylmaleimide I (Bis I) suppresses the FGF2-mediated activation of Erk MAP kinase pathway in chondrocytes by preventing the SHP2 association with FRS2 and Gab1 adaptor proteins



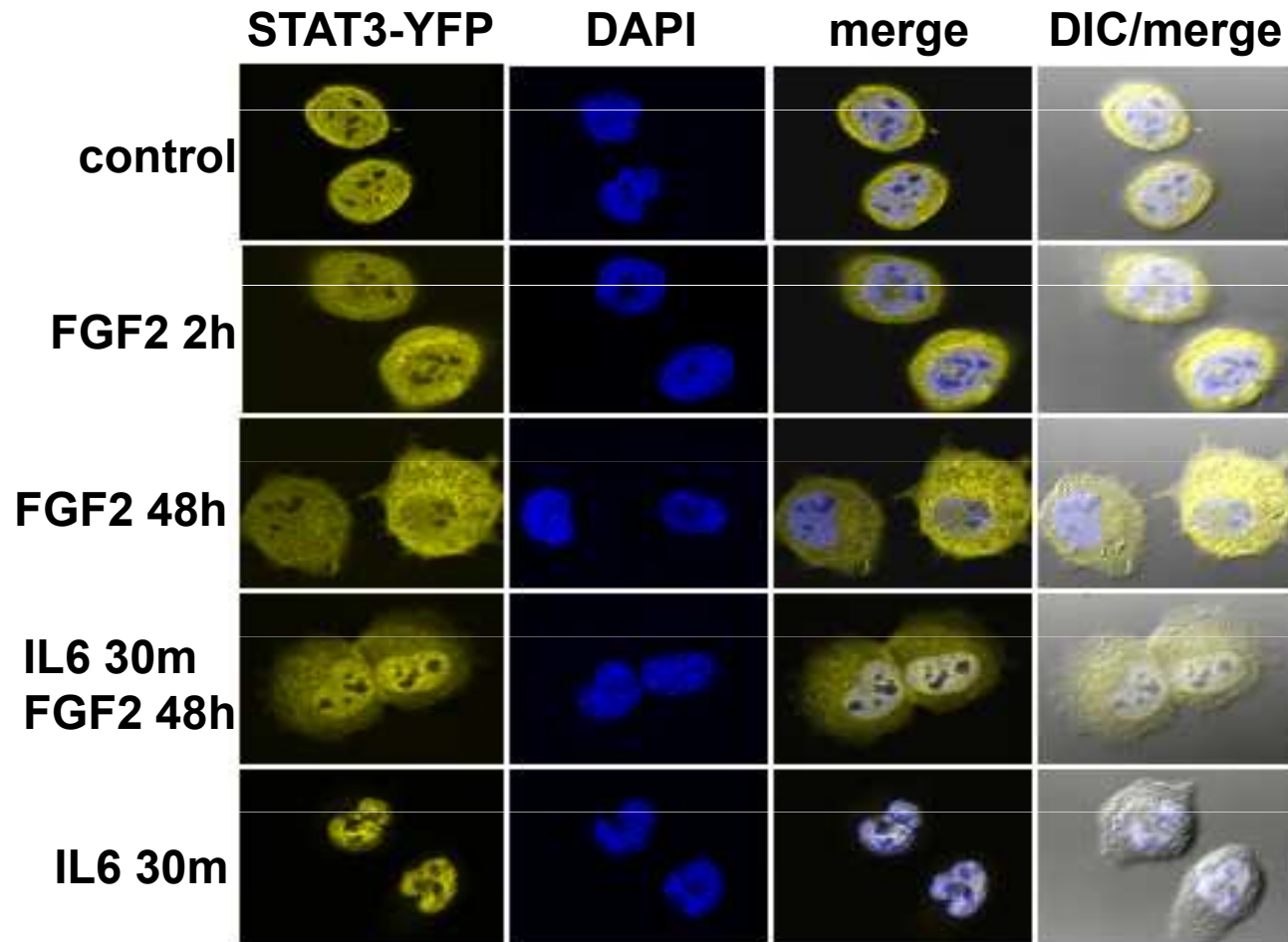
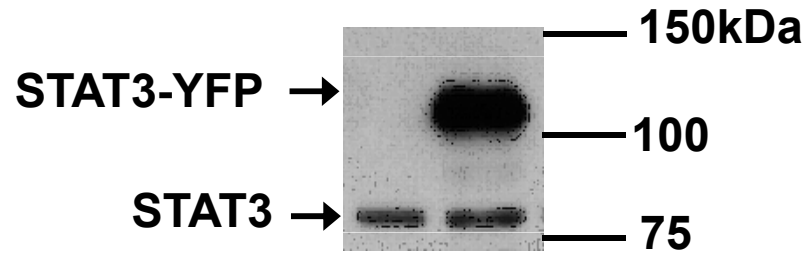
FGFR3 associates with STAT1 and acts as STAT1-kinase in chondrocytes



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



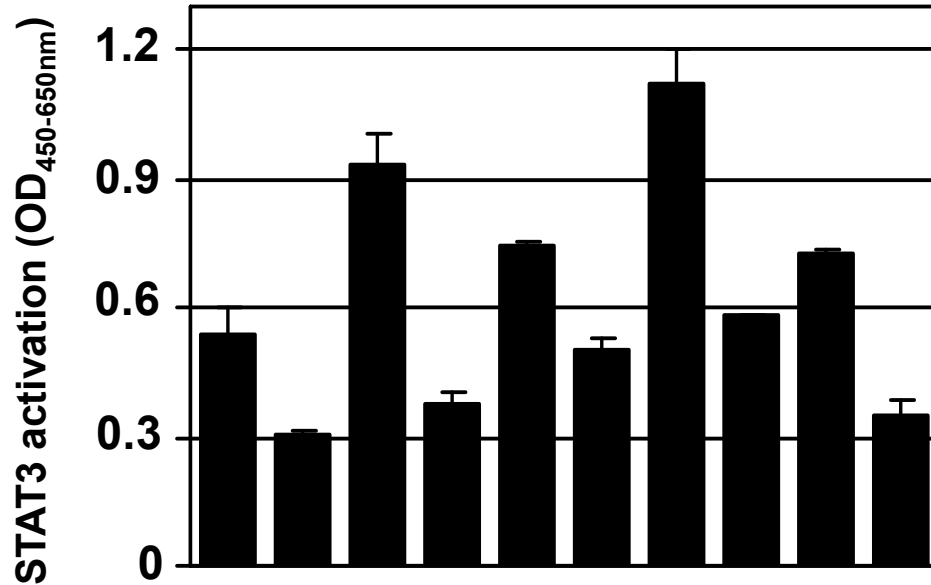
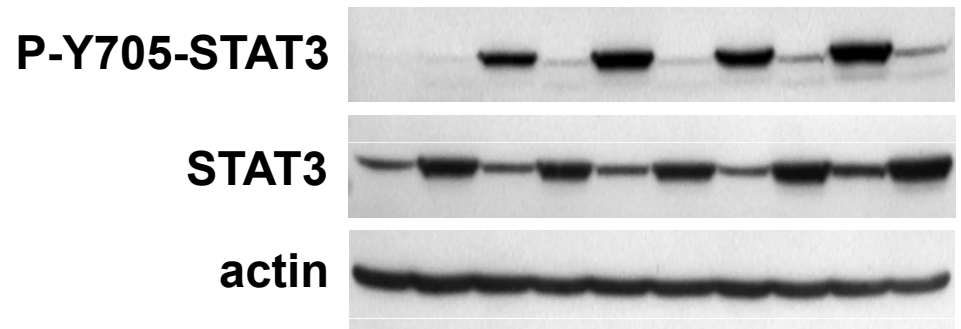
Chronic FGF stimulus inhibits cytokine/STAT signaling in chondrocytes



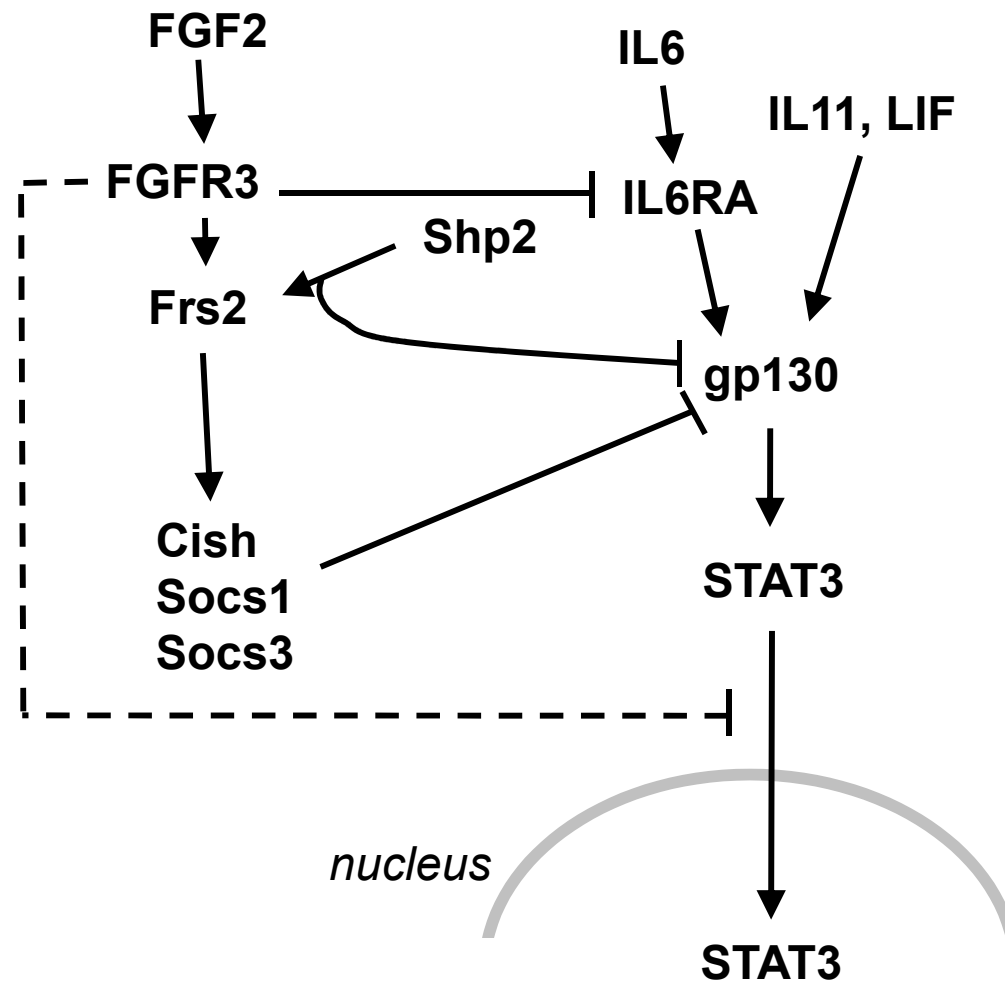
Chronic FGF stimulus inhibits cytokine/STAT signaling in chondrocytes

| | | | | | | | | | | |
|--------------|---|---|---|---|---|---|---|---|---|---|
| FGF2 (72h) | - | + | - | + | - | + | - | + | - | + |
| IL6 | - | - | + | + | - | - | - | - | - | - |
| IL11 | - | - | - | - | + | + | - | - | - | - |
| LIF | - | - | - | - | - | - | + | + | - | - |
| IFN γ | - | - | - | - | - | - | - | - | + | + |

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

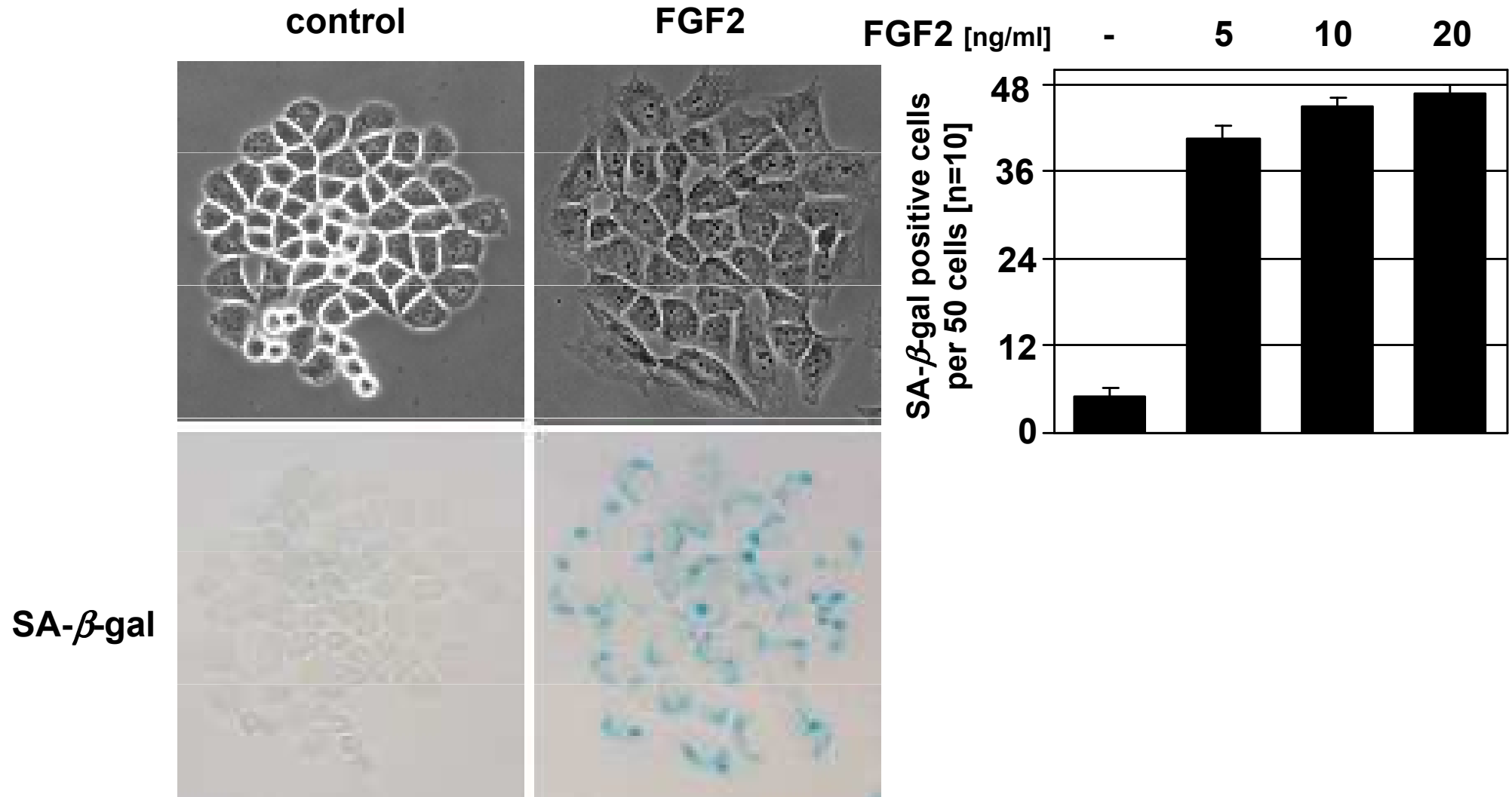


Chronic FGF stimulus inhibits cytokine/STAT signaling in chondrocytes

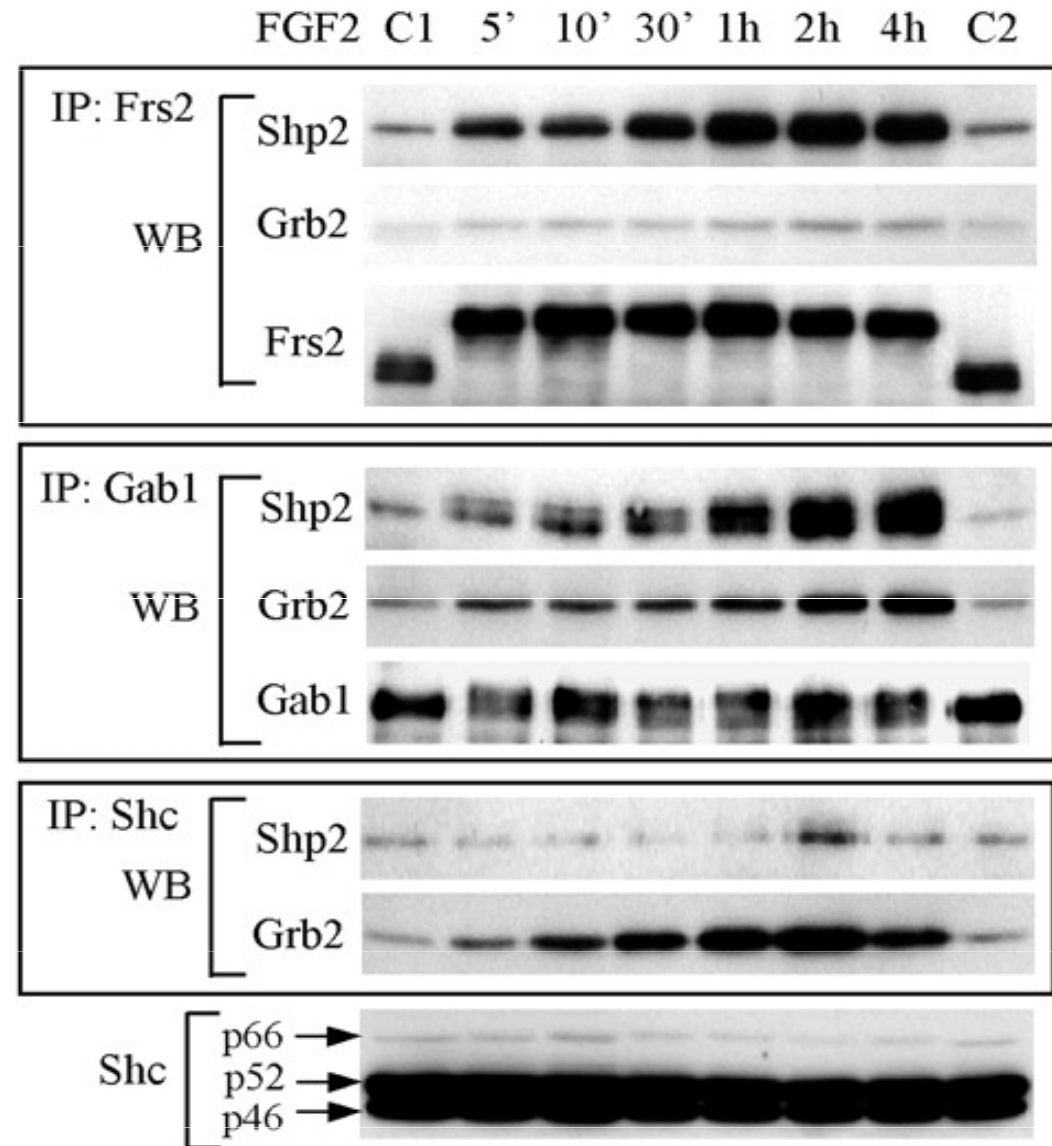
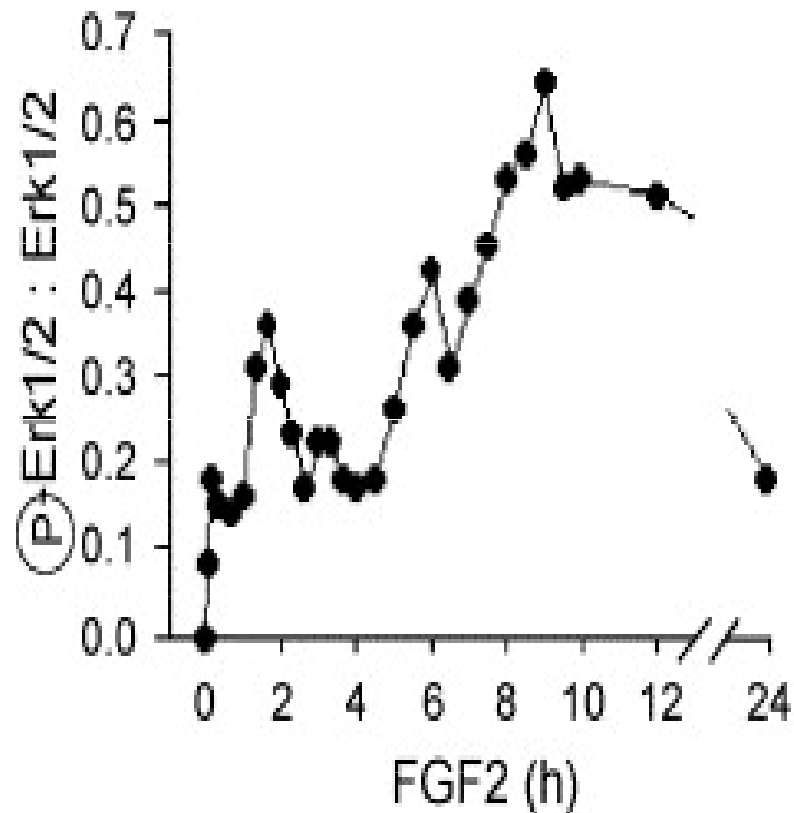


Krejci *et al.*, manuscript in preparation

FGF2 causes premature senescence in chondrocytes

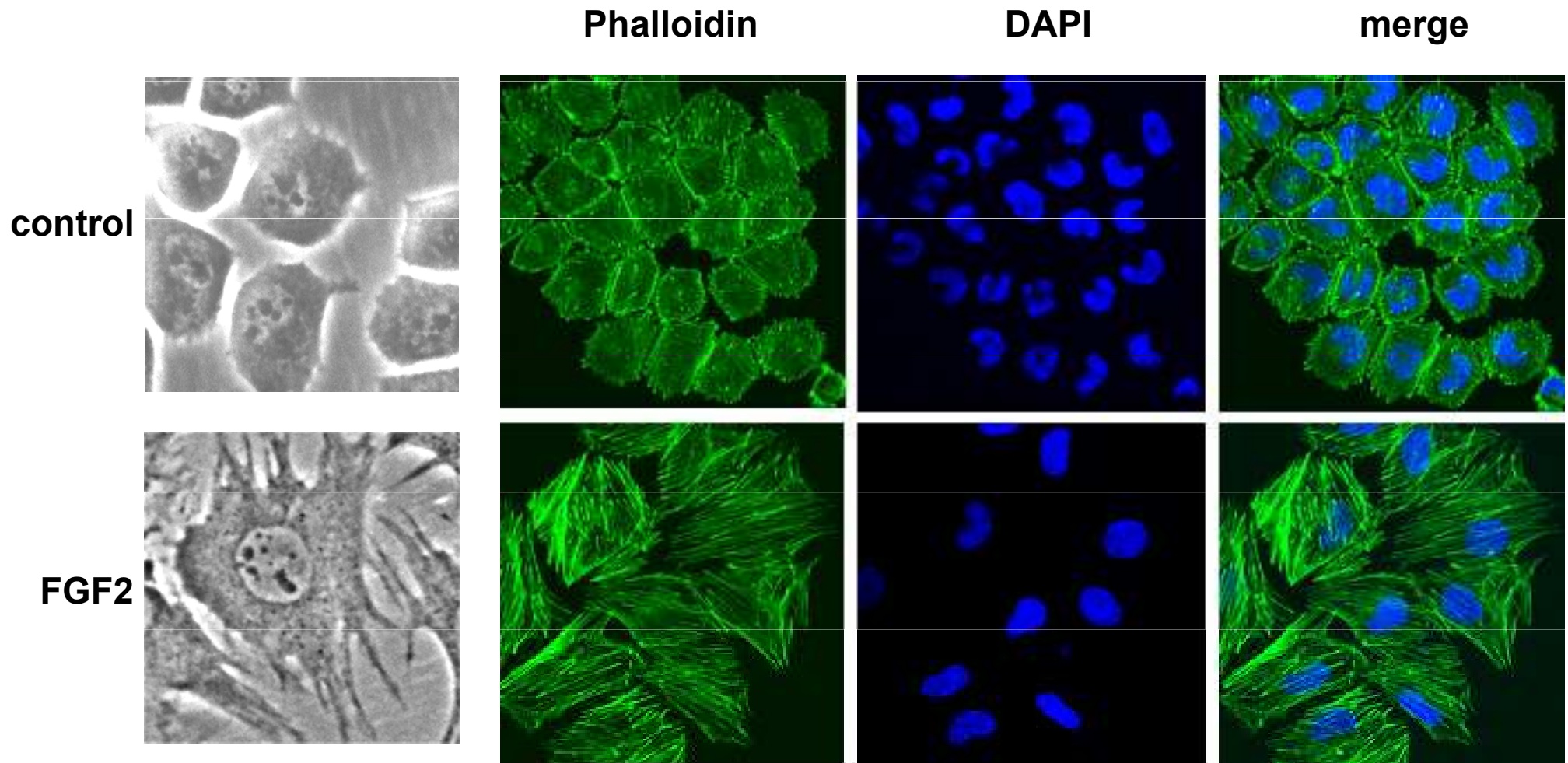


FGFR3 recruits multiple adapter proteins to activate Ras/Erk signaling pathway

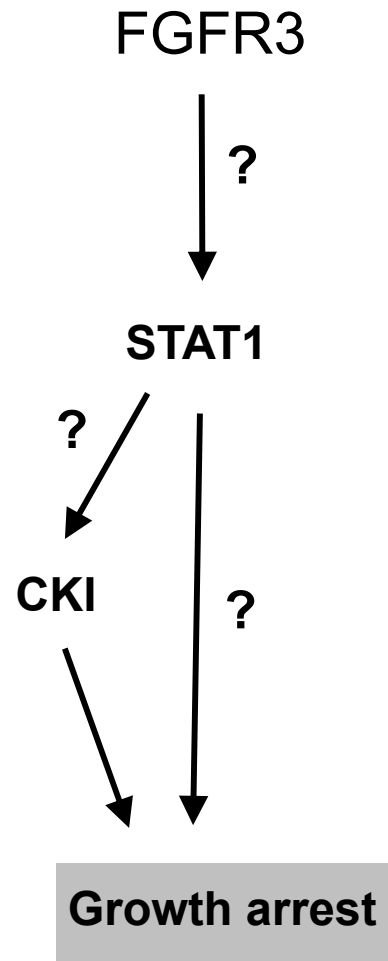


Krejci *et al.*, manuscript in preparation

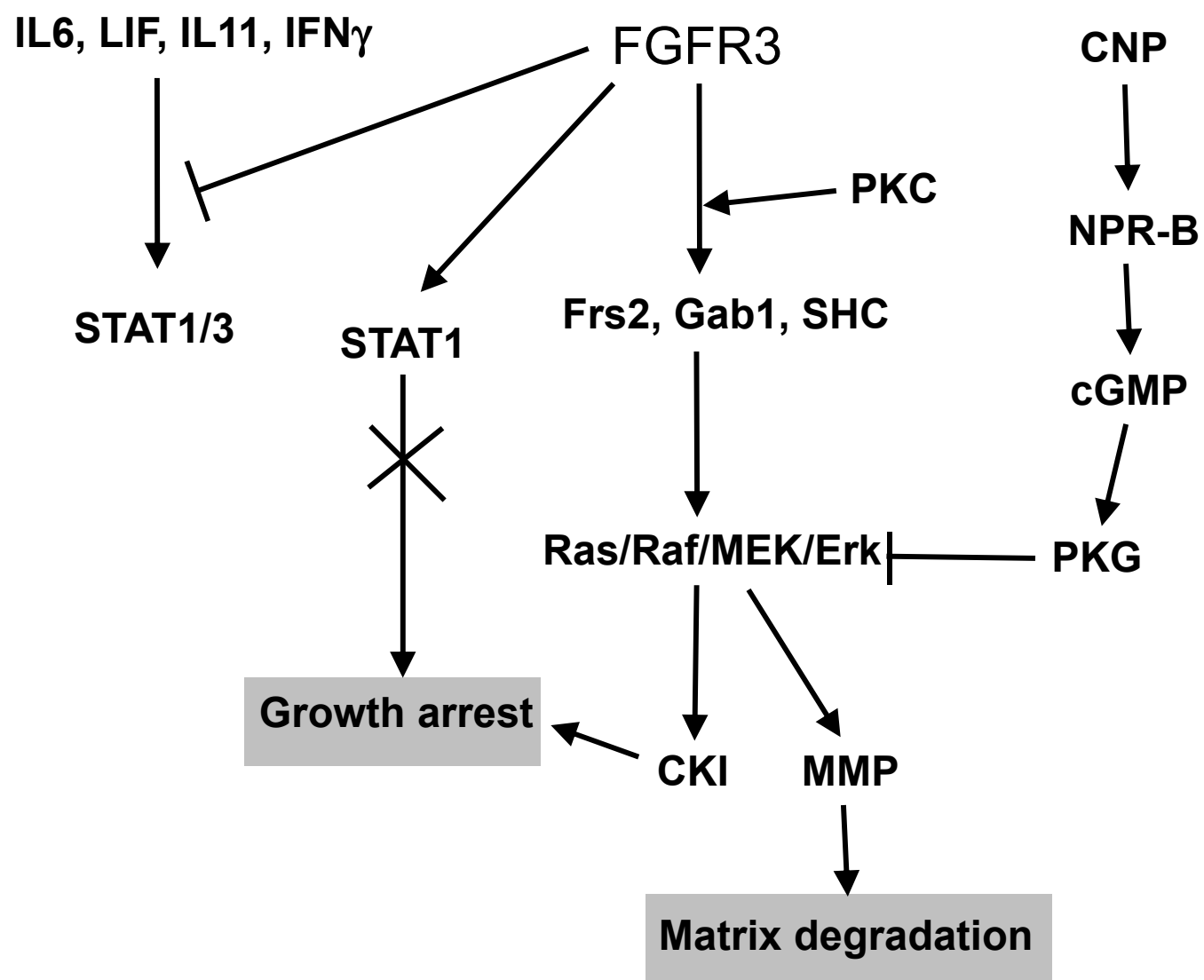
FGF2 signals towards the cytoskeleton in chondrocytes



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