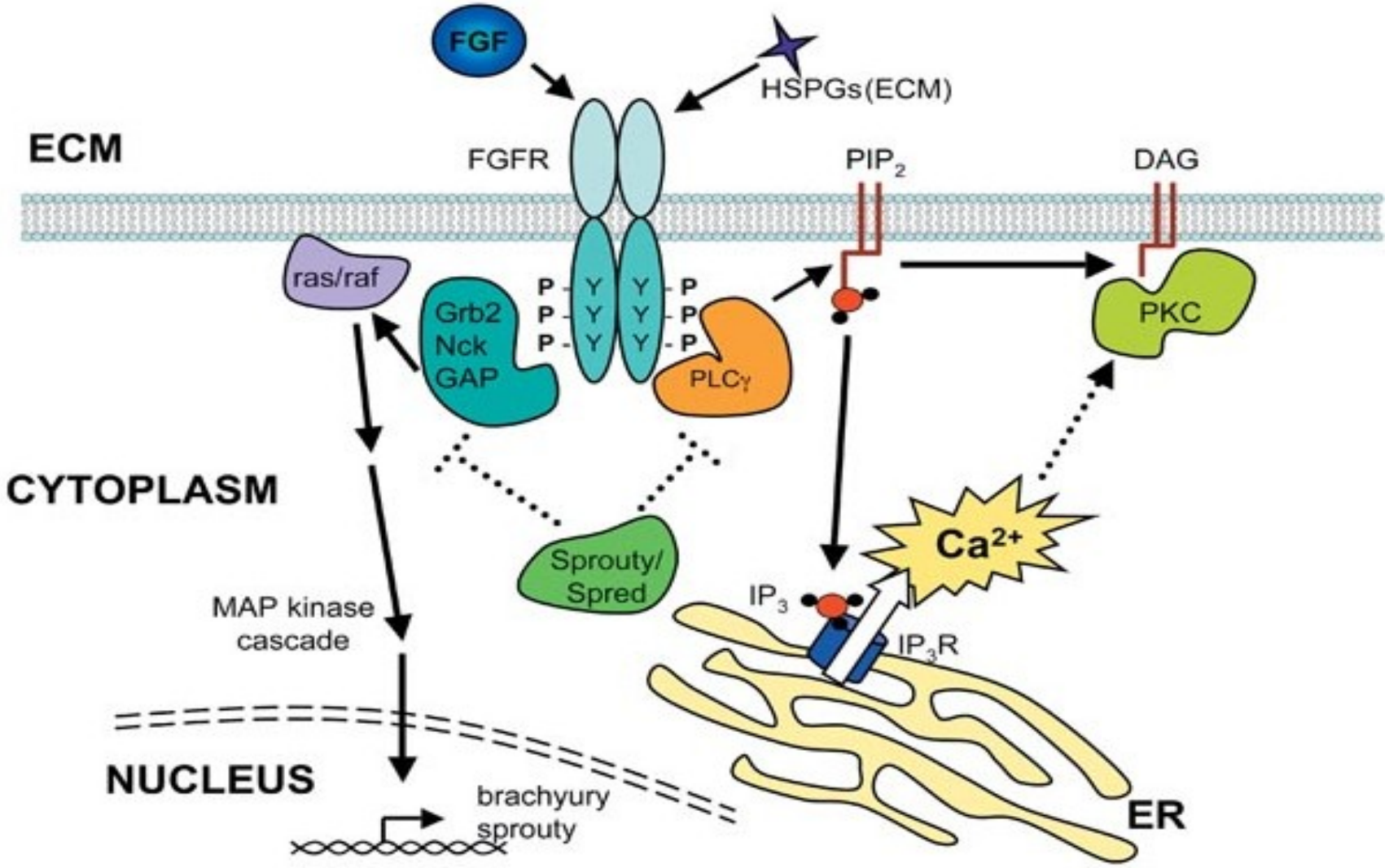
9. MECHANISMS OF DEVELOPMENT I – REGULATION OF LIMB DEVELOPMENT BY FIBROBLAST GROWTH FACTORS (FGF)

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



AT THE BEGINNING THERE WAS VITAMIN A……..

LETTERS TO NATURE

Limbs generated at site of tail amputation in marbled balloon frog after vitamin A treatment

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NIAZI and Saxena[1] first observed that vitamin A has an inhibitory and modifying influence on tail regeneration in *Bufo andersonii* tadpoles. A positive relationship was later found between the inhibiting influence of vitamin A and the developmental stage of the regenerating tail in the same species[2]. There have been several subsequent reports[3–7] on the effects of vitamin A and its derivatives on limb development and regeneration. Thus in regenerating amphibian limbs, application of retinoids produces pattern duplication in the proximodistal and anteroposterior axes of the limb[3,8,9], and local application of retinoic acid to the anterior side of developing chick limbs causes duplications in the anteroposterior axis of limb[10,11]. Here we show that vitamin A can cause limb development when applied to amputated tail stumps of the tadpoles of the marbled balloon frog *Uperodon systoma* (Anura Microhylidae). This is the first report of homeotic transformation mediated through vitamin A in vertebrates.

Following amputation through the middle of the tail at the hind-limb bud stage, tadpoles were exposed to a solution of 10 IU per ml vitamin A palmitate (Arovit, Roche; see Table 1 for details) for 24 h (set I), 48 h (set II), 72 h (set III), 96 h (set

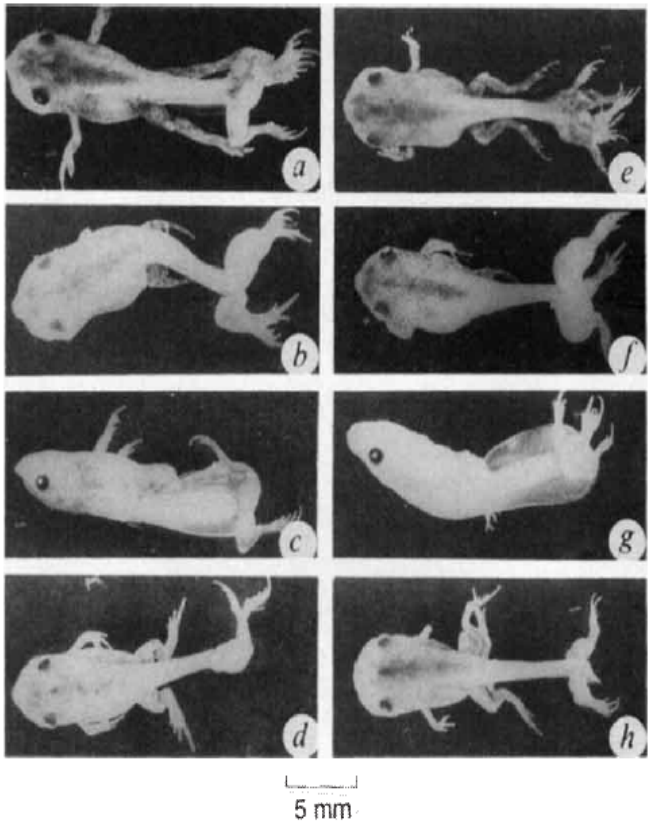
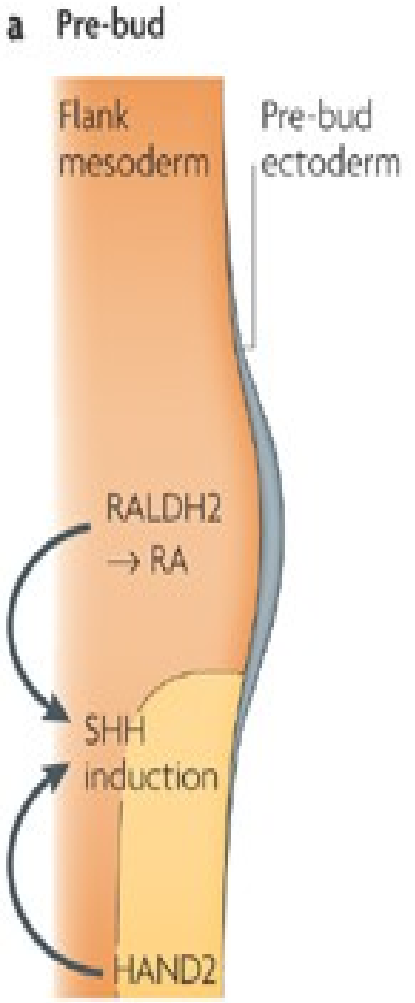


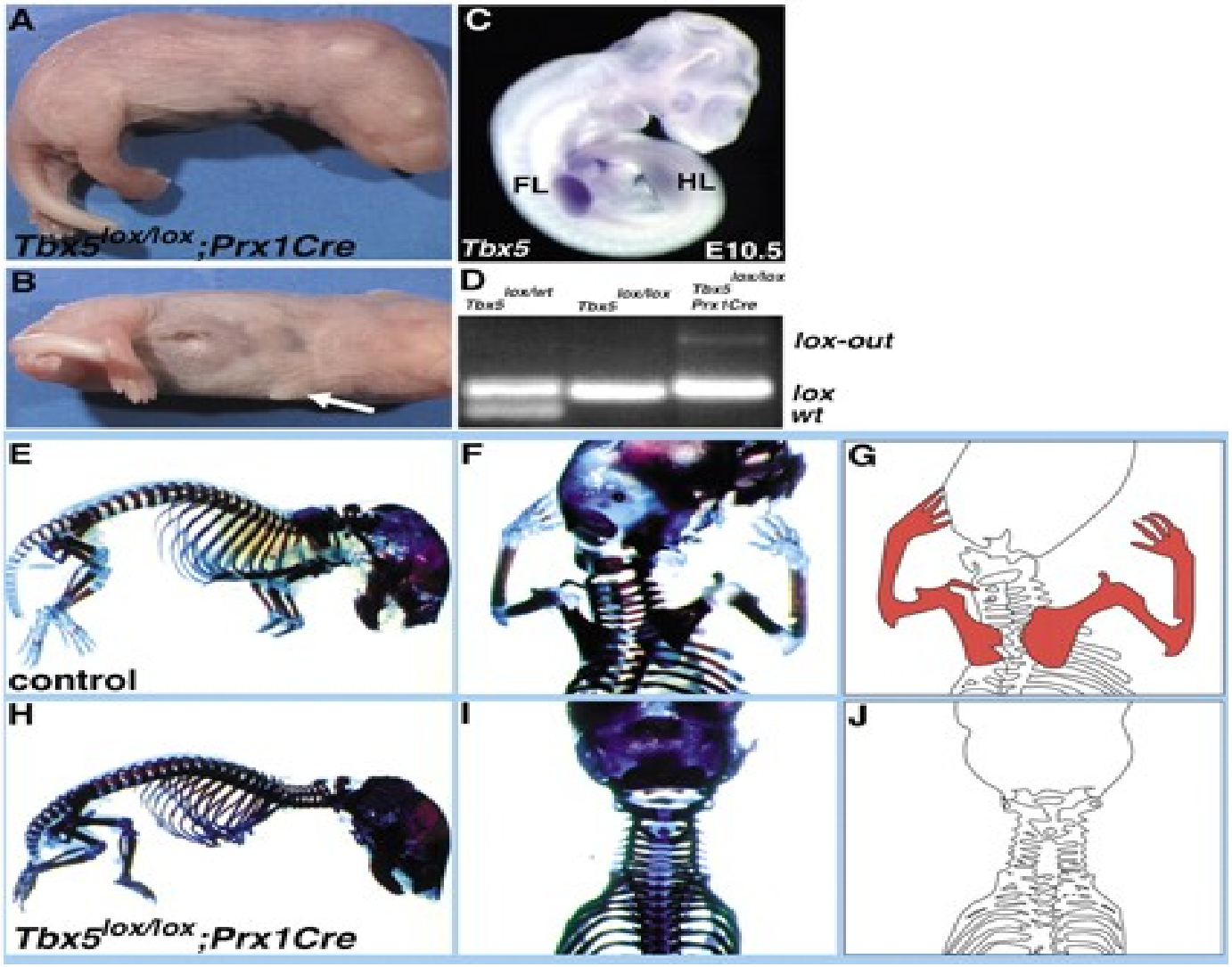
FIG. 1 Effects of vitamin A (10 IU) treatment for various times on limb generation from amputated tail stump. *a*, Treatment for 24 h, 3 limbs are generated; *b*, 72 h, 4 limbs; *c* and *d*, 96 h, 2 limbs; *e*, 120 h, 7 limbs; *f*, 120 h, 3 limbs; *g*, 144 h, 3 limbs; *h*, 144 h, 2 limbs, plus an extra pair of limbs below the original hindlimb.

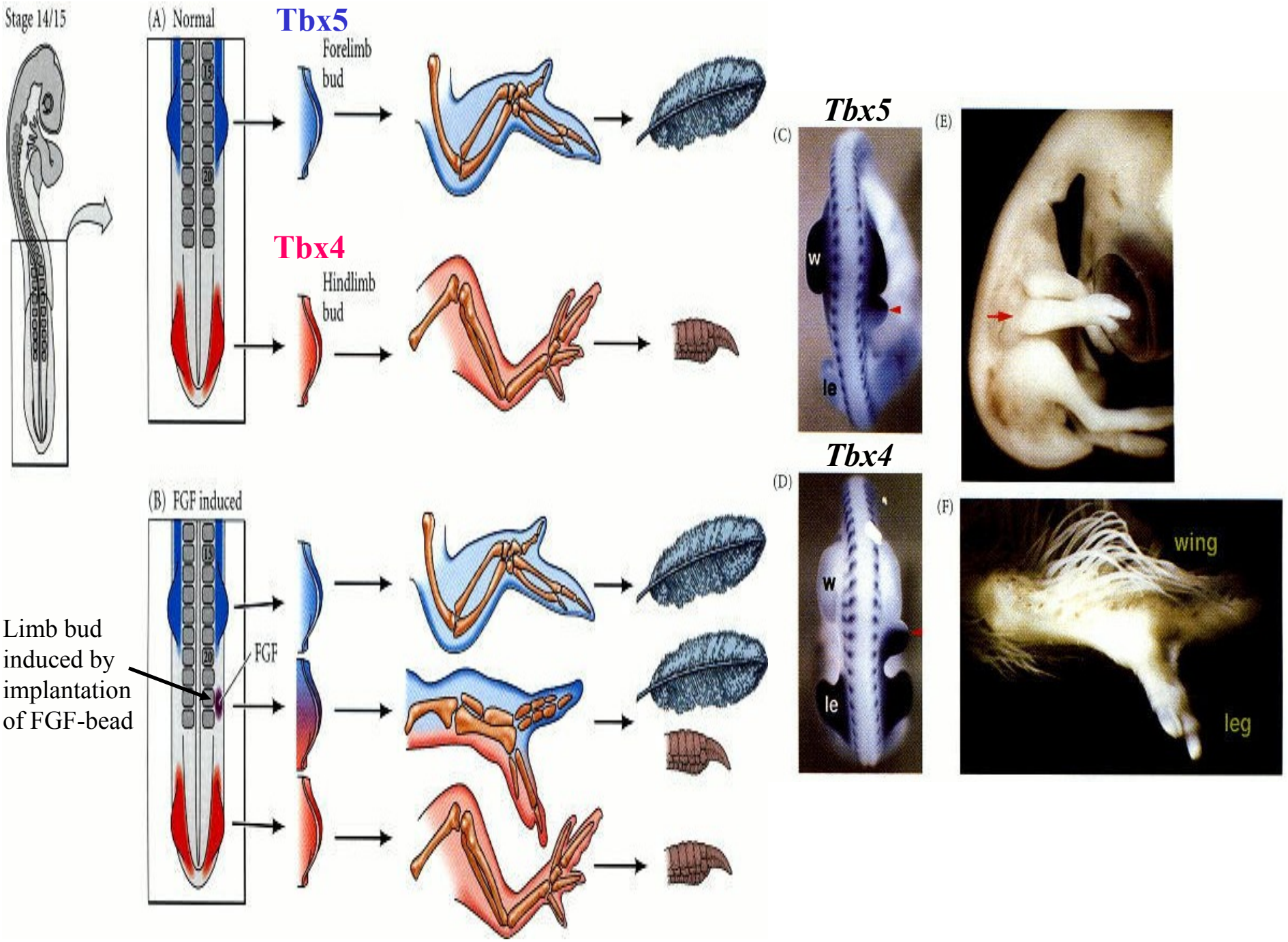


RALDH2- retinaldehyde dehydrogenase 2

……LATER CAME TBX

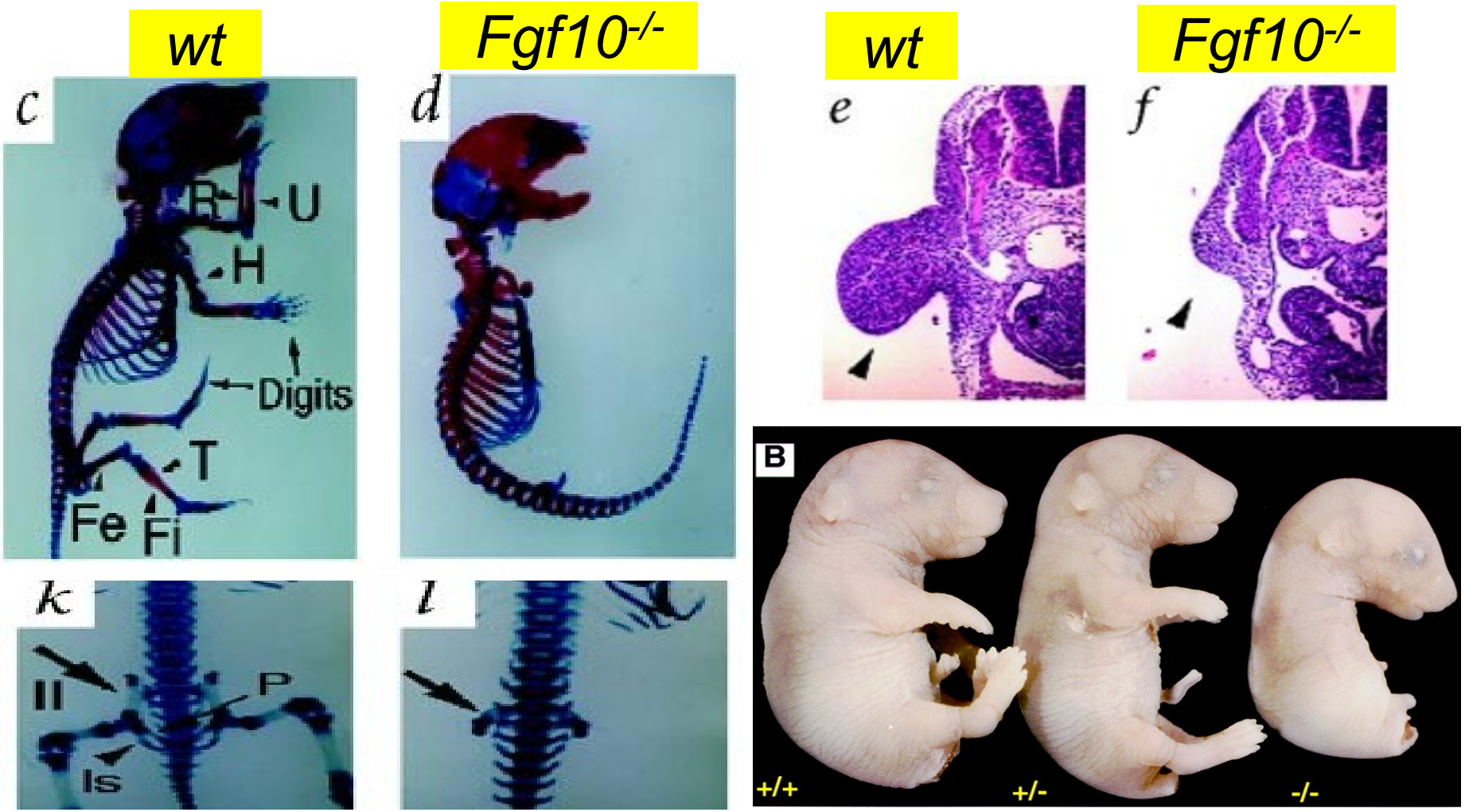
DNA binding domain derived from the prototype gene called transcription factor T. Limb identity factors Tbx4 (hindlimb) and Tbx5 (forelimb)




Stage 14/15
(A) Normal
Tbx5
Forelimb bud
Tbx4
Hindlimb bud
(B) FGF induced
FGF
Limb bud induced by implantation of FGF-bead
Tbx5
(C)
w
le
Tbx4
(D)
w
le
(E)
(F)
wing
leg

……..FOLLOWED BY FGF

FGF10 → proliferation in the mesoderm → limb bud growth

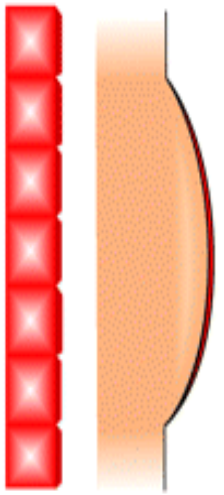


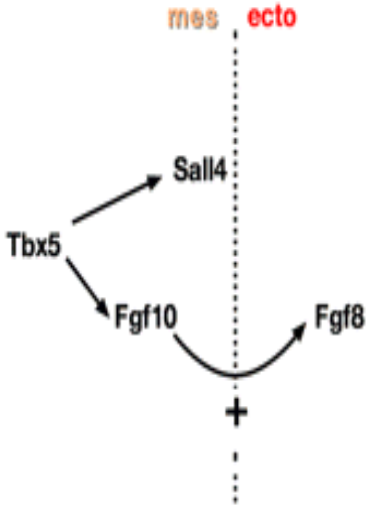
Fgf-10 is required for both limb and lung development and exhibits striking functional similarity to Drosophila branchless

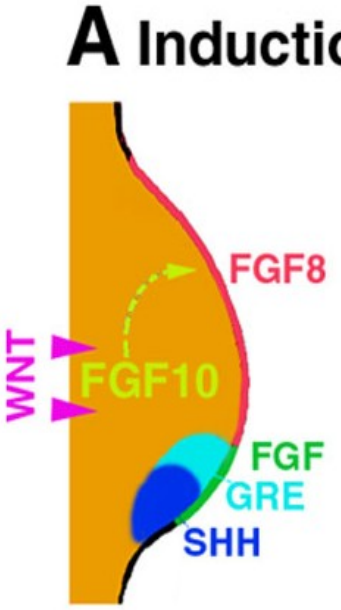
Hosung Min, Dimitry M. Danilenko, Sheila A. Scully, et al.

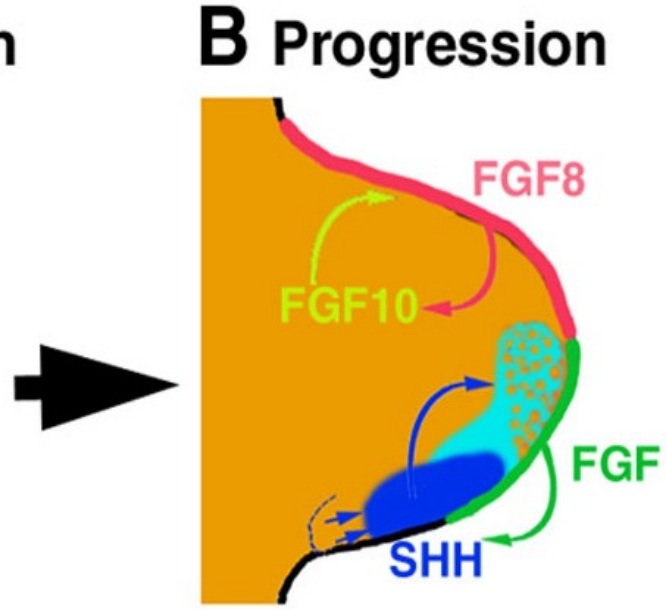
Genes Dev. 1998 12: 3156-3161
Access the most recent version at doi:10.1101/gad.12.20.3156

Initiation: *Tbx5* - dependent



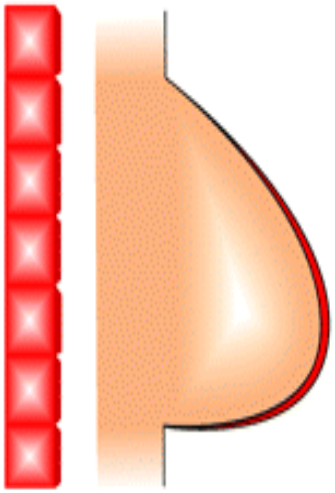


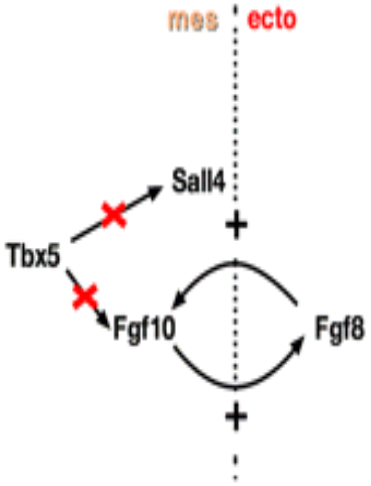


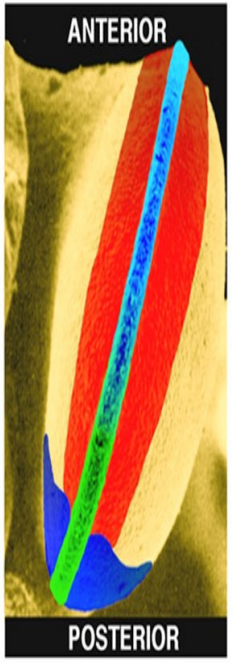


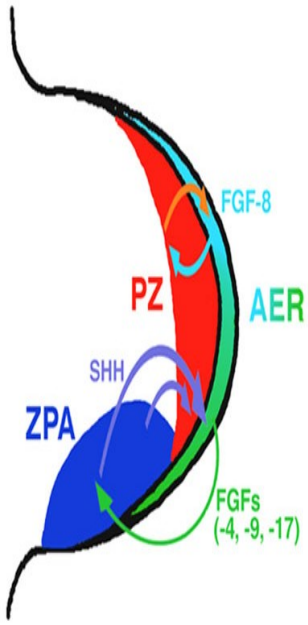
Fgf8

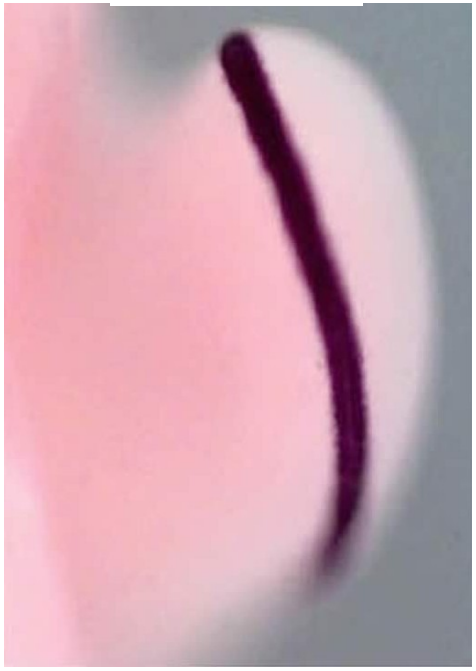
Outgrowth: *Tbx5* - independent







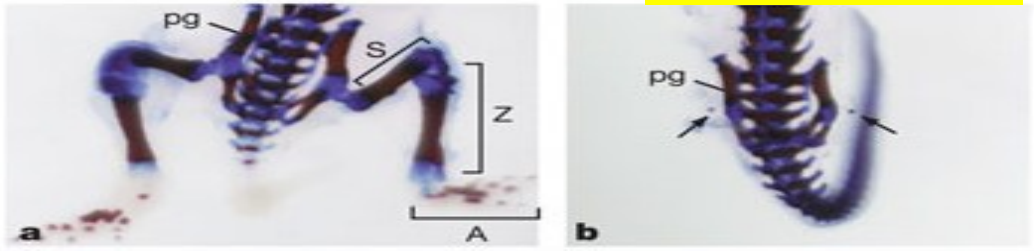




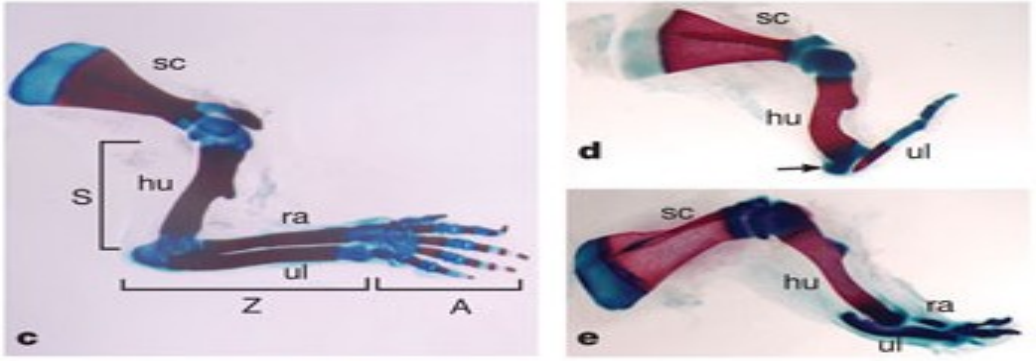
wt

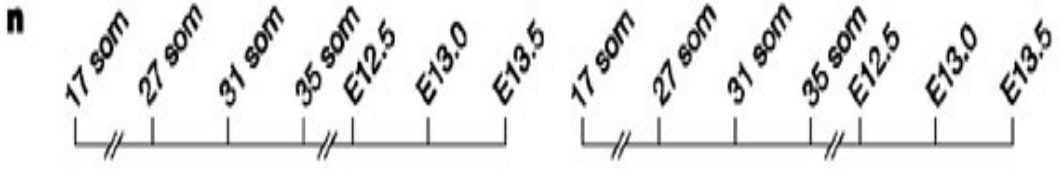
Hindlimb

Fgf8/Fgf4$^{-/-}$



Forelimb





Normal

Fgf8

Fgf4

Fgf8

Fgf4

Cre function

Cre function

Double KO

No *Fgf8* expression

No *Fgf4* expression

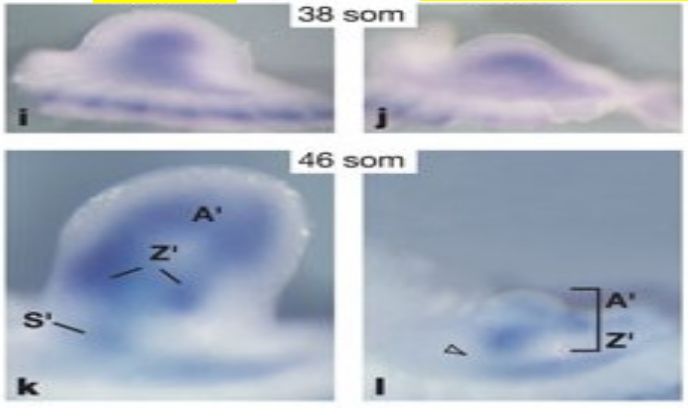
Transient *Fgf8* expression

Precocious, transient *Fgf4* expression

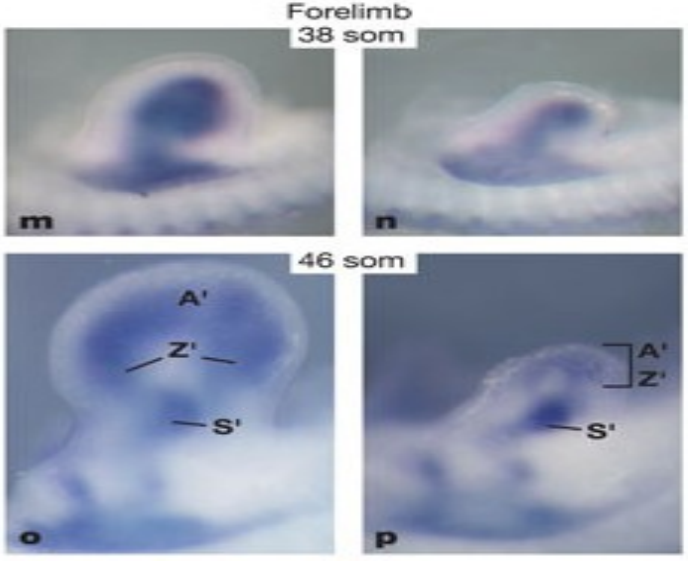
wt

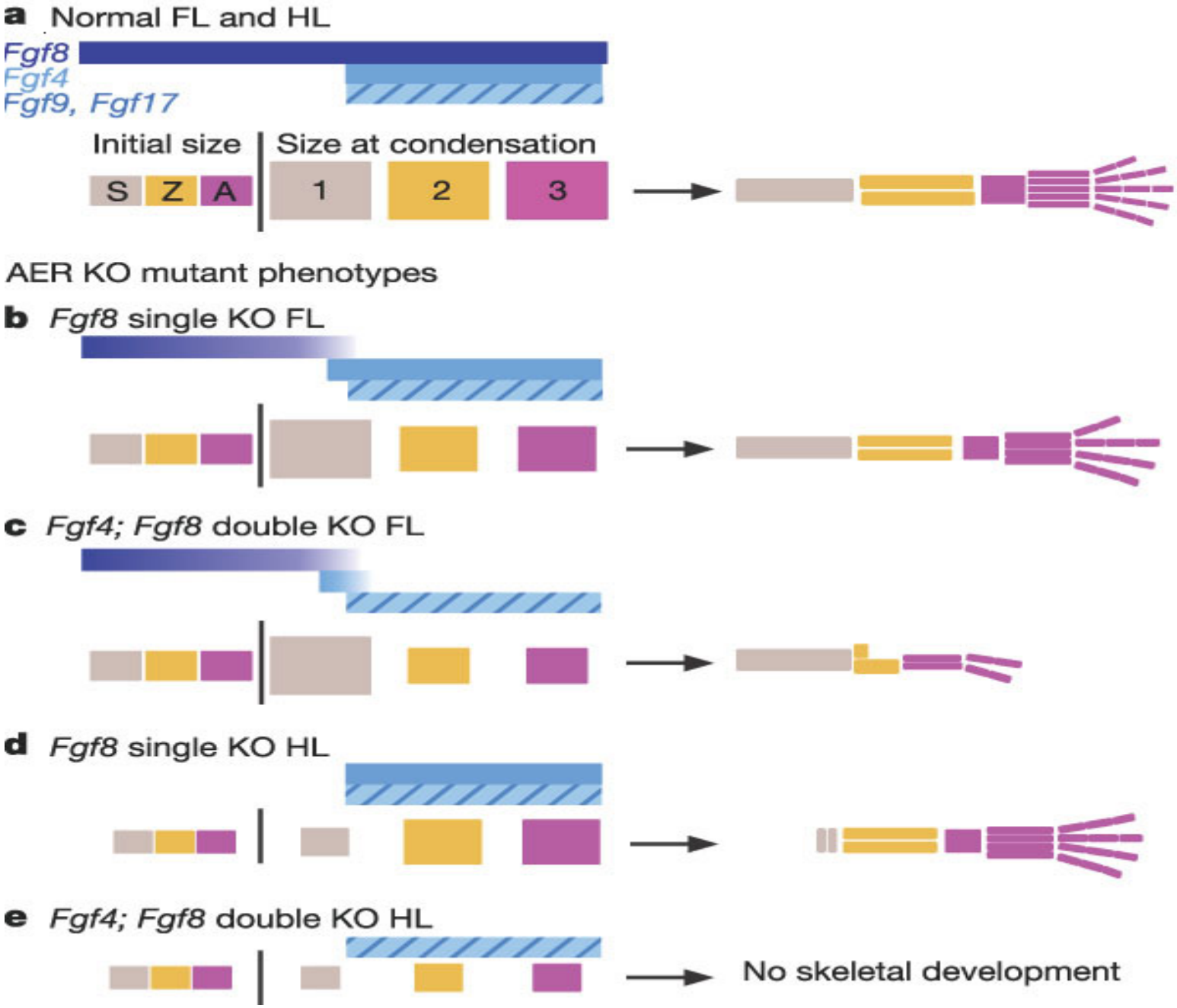
Hindlimb

Fgf8/Fgf4$^{-/-}$

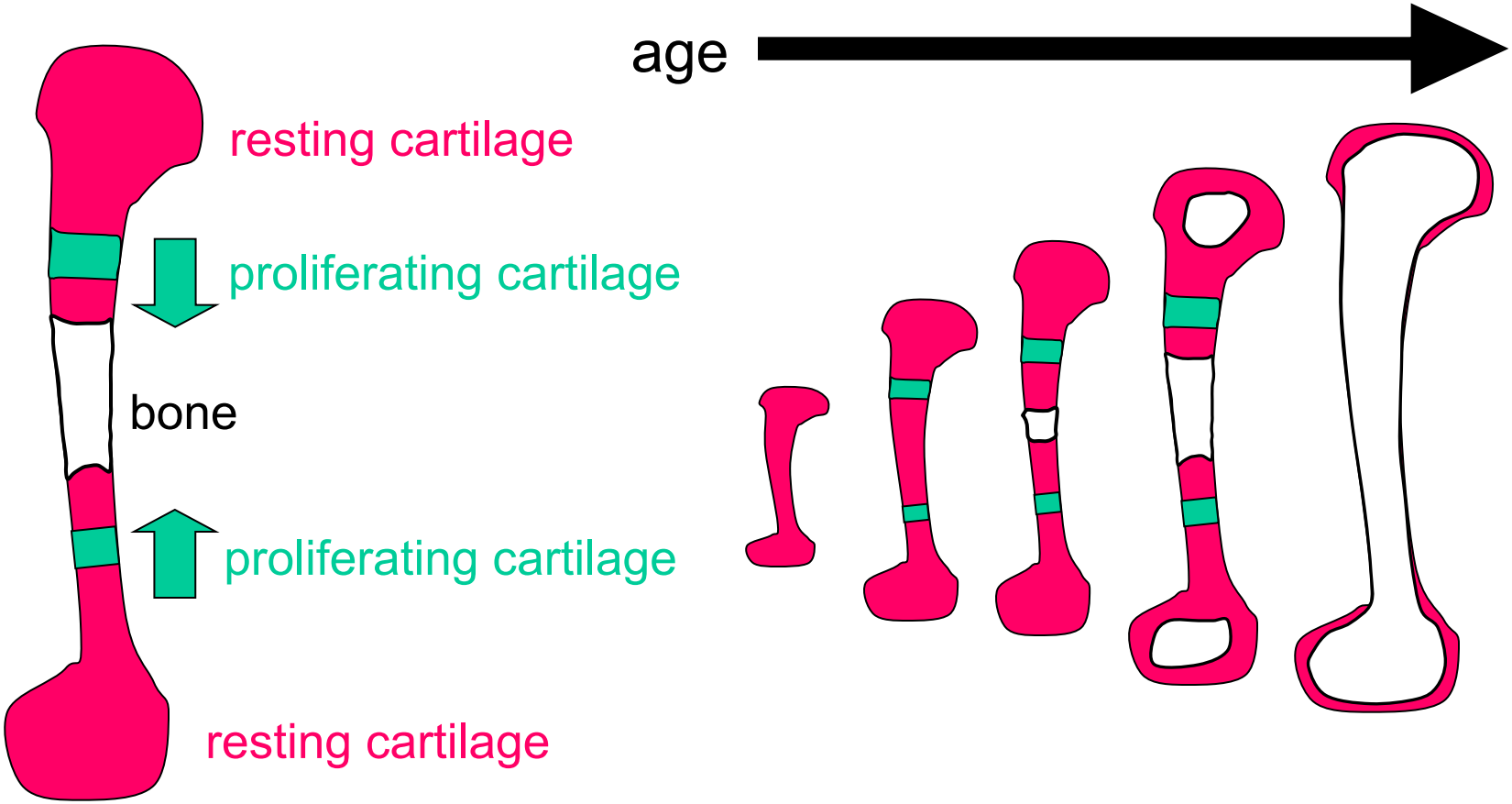


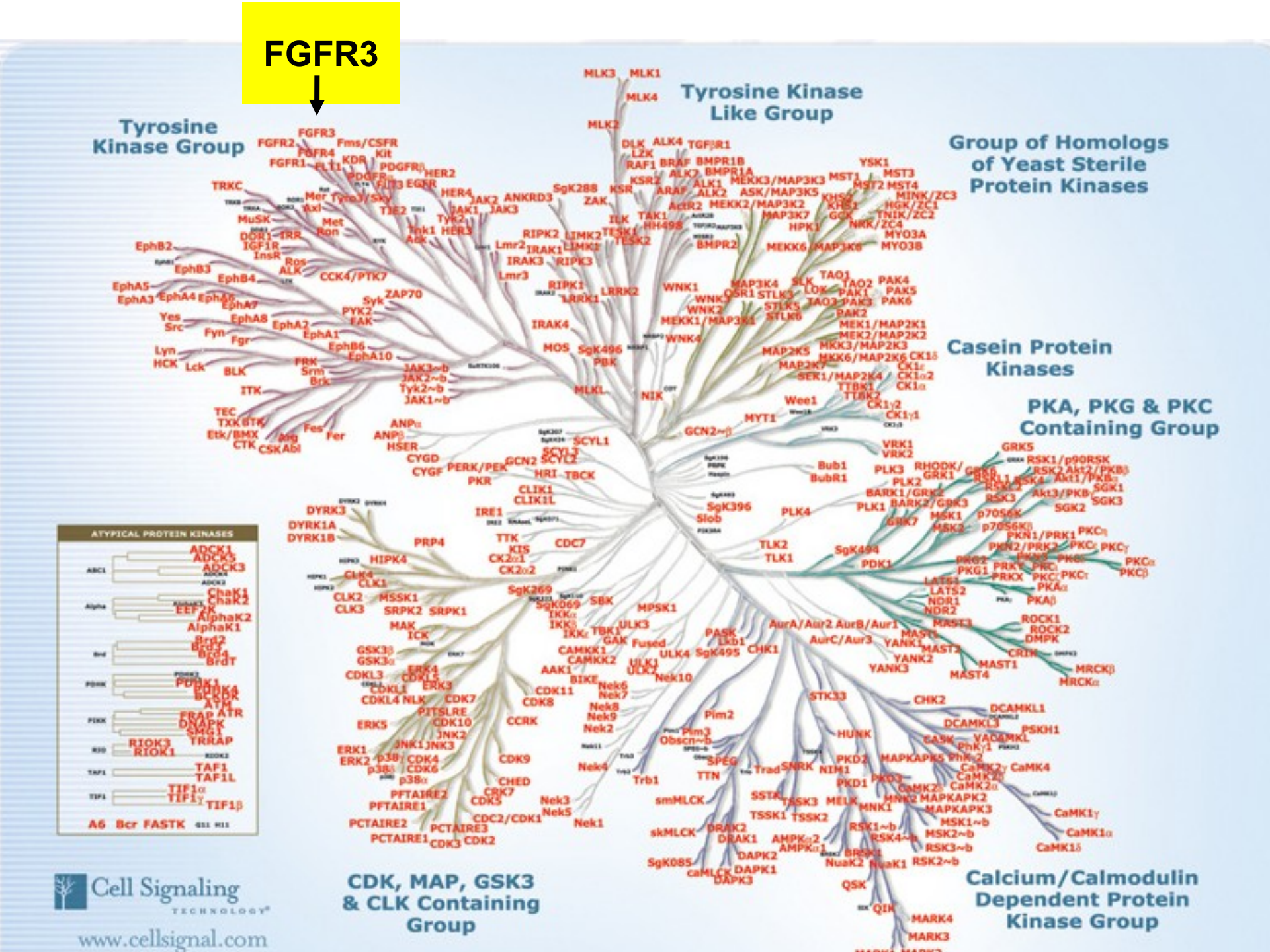
Forelimb

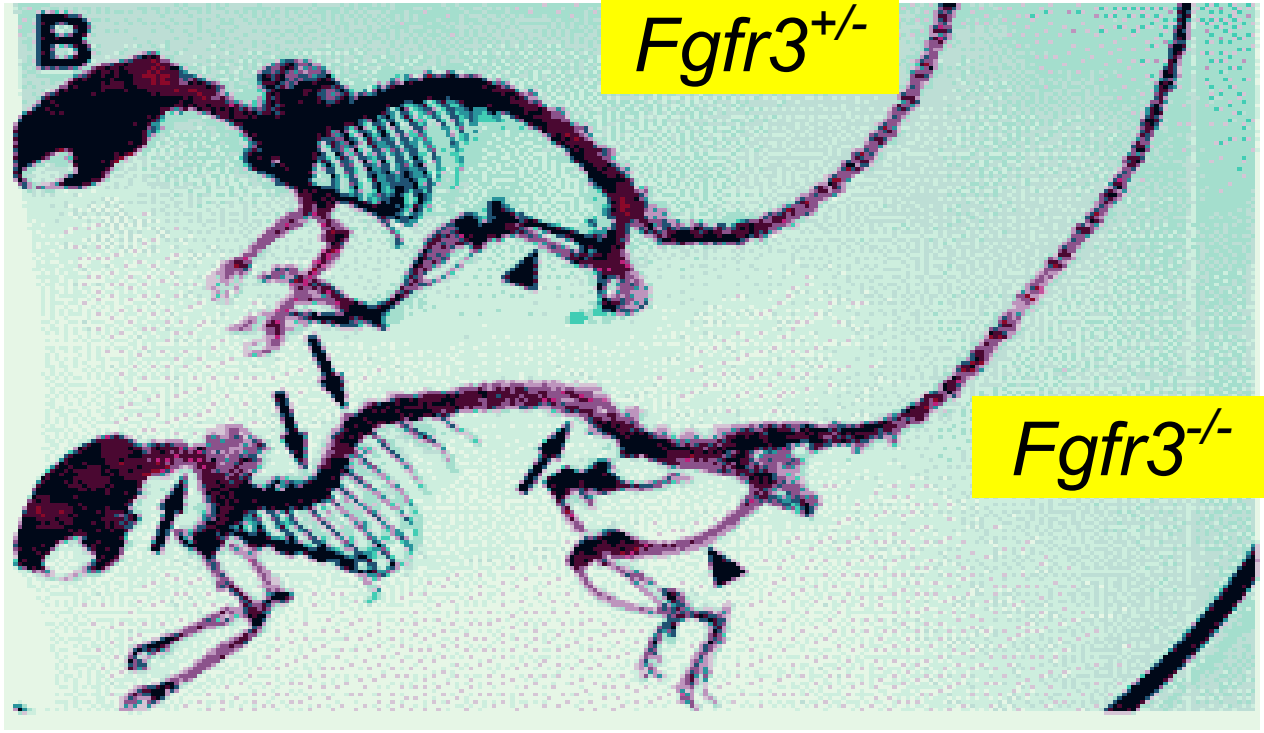


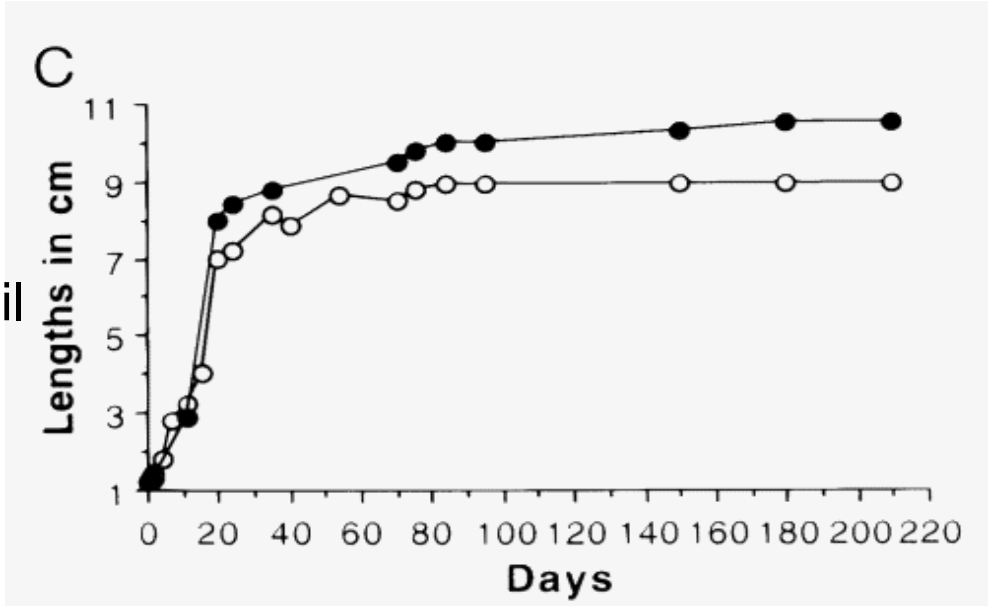

a Normal FL and HL
Fgf8
Fgf4
Fgf9, Fgf17
Initial size
Size at condensation
S
Z
A
1
2
3
AER KO mutant phenotypes
b Fgf8 single KO FL
c Fgf4; Fgf8 double KO FL
d Fgf8 single KO HL
e Fgf4; Fgf8 double KO HL
No skeletal development

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





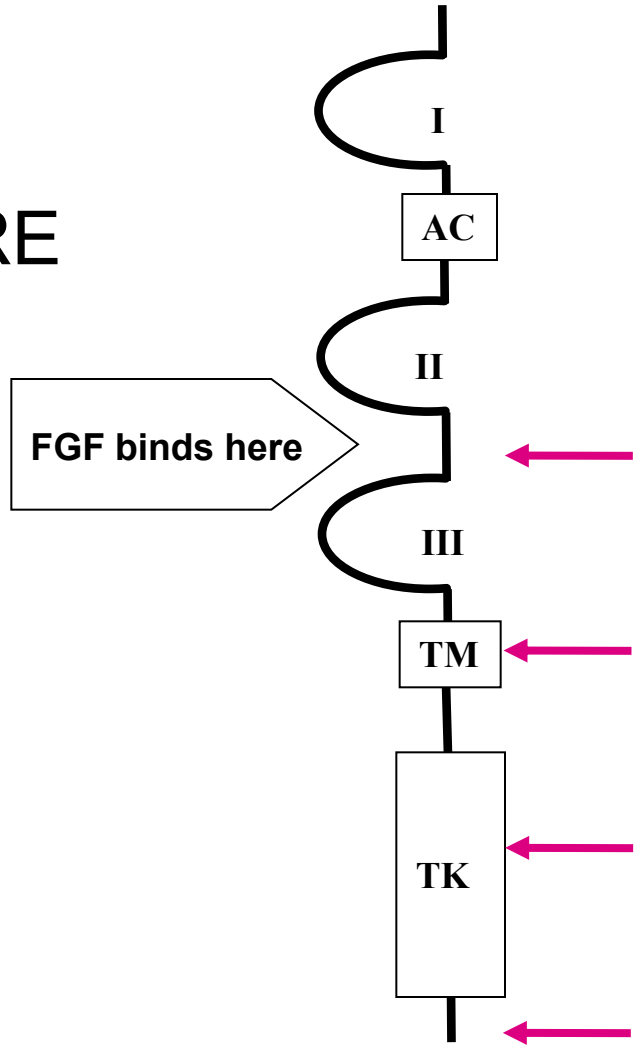
Tail

Fgfr3+/-
Fgfr3-/-

FGFR3-related skeletal dysplasia

STATURE

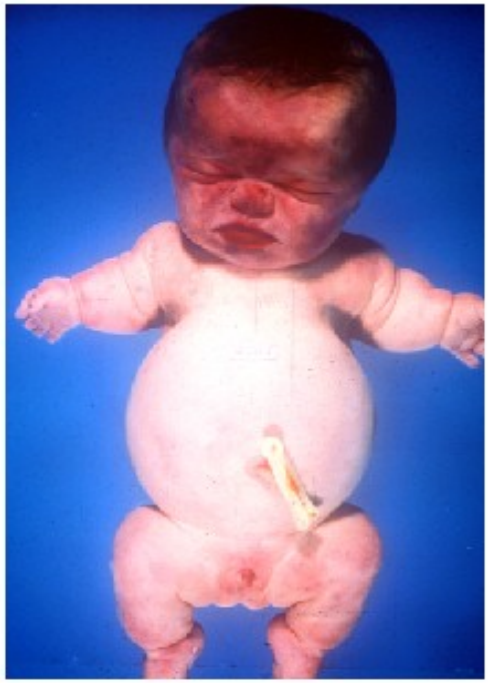
Hypochondroplasia
Achondroplasia
SADDAN
Thanatophoric Dysplasia

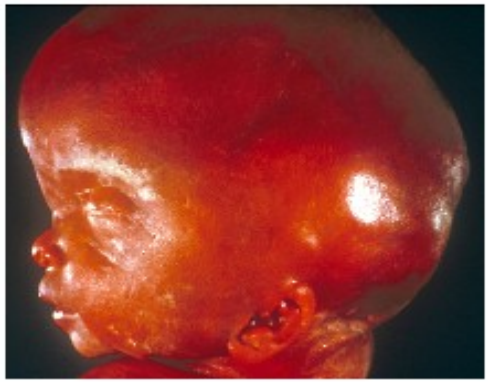


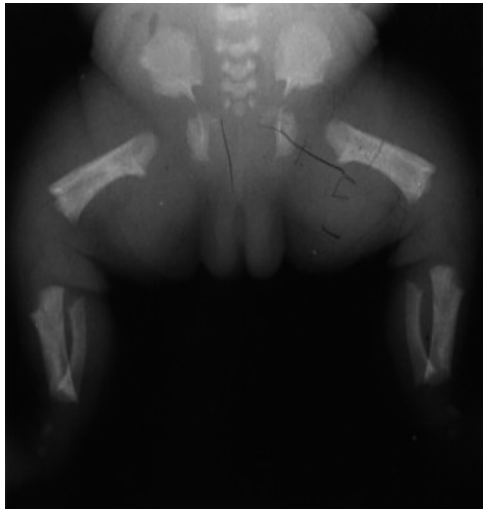
FGFR3-related skeletal dysplasia

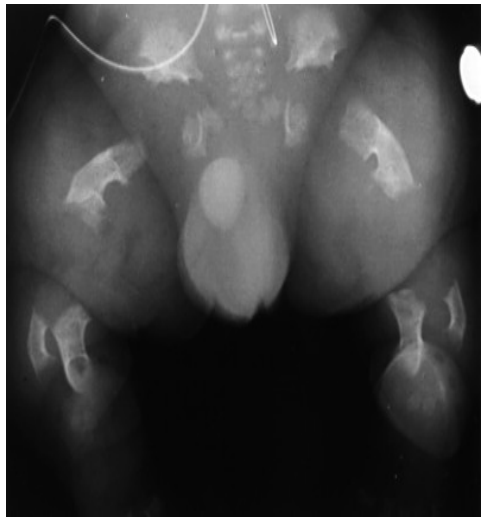
Achondroplasia

Thanatophoric Dysplasia









healthy

TD

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

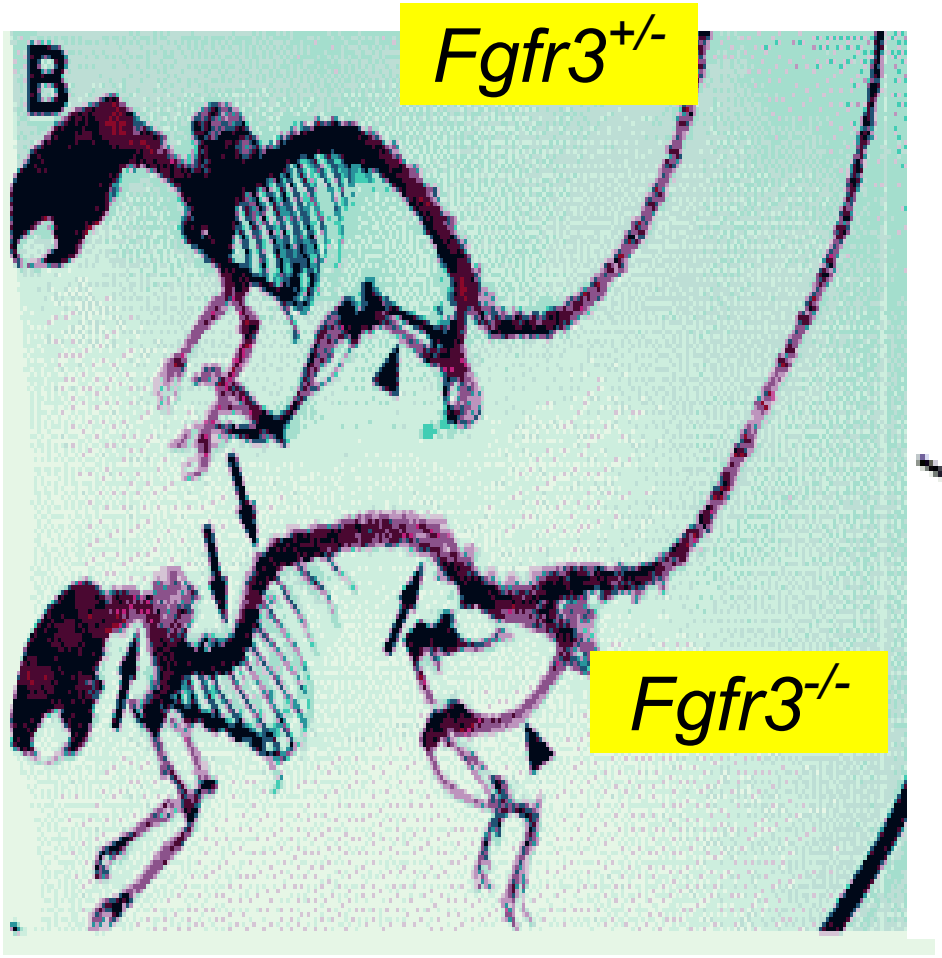
An Expressed *Fgf4* Retrogene Is Associated with Breed-Defining Chondrodysplasia in Domestic Dogs

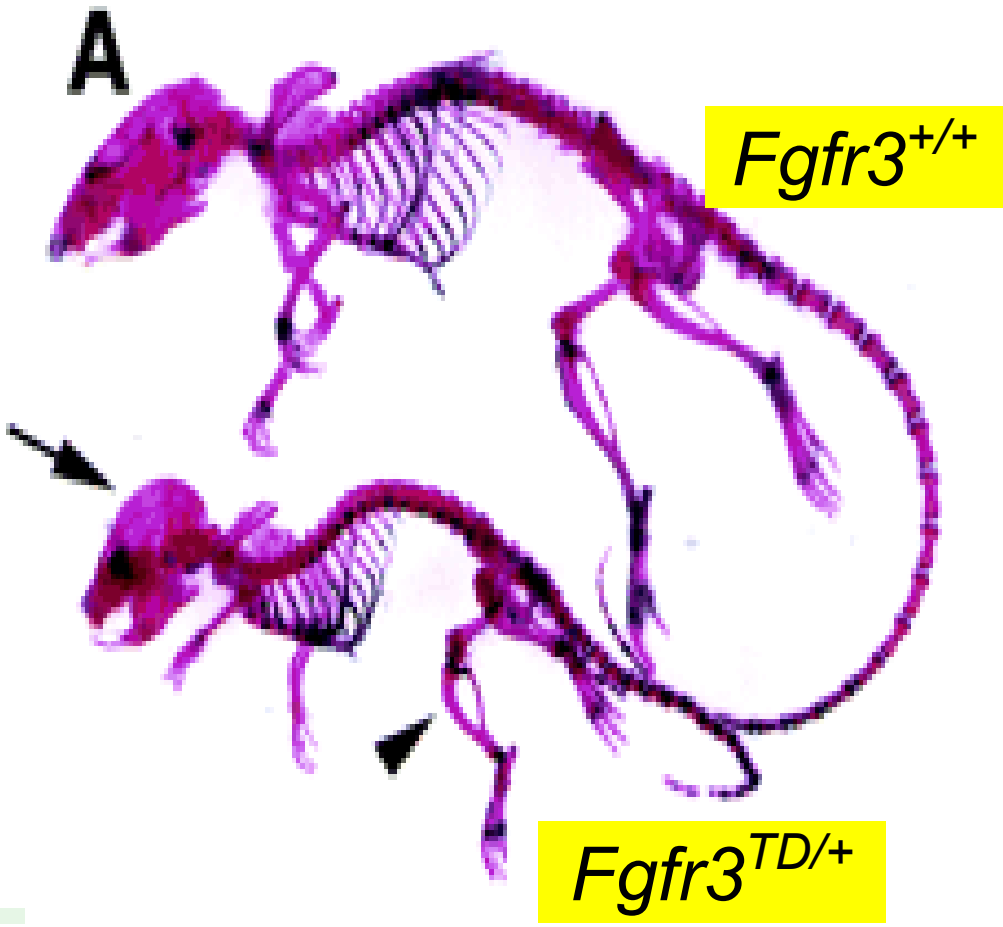
Heidi G. Parker,[1] Bridgett M. VonHoldt,[2] Pascale Quignon,[1] Elliott H. Margulies,[3] Stephanie Shao,[1] Dana S. Mosher,[1] Tyrone C. Spady,[1] Abdel Elkahloun,[1] Michele Cargill,[4]* Paul G. Jones,[5] Cheryl L. Maslen,[6] Gregory M. Acland,[7,8] Nathan B. Sutter,[8] Keiichi Kuroki,[9] Carlos D. Bustamante,[10] Robert K. Wayne,[2] Elaine A. Ostrander[1]†

Retrotransposition of processed mRNAs is a common source of novel sequence acquired during the evolution of genomes. Although the vast majority of retroposed gene copies, or retrogenes, rapidly accumulate debilitating mutations that disrupt the reading frame, a small percentage become new genes that encode functional proteins. By using a multibreed association analysis in the domestic dog, we demonstrate that expression of a recently acquired retrogene encoding fibroblast

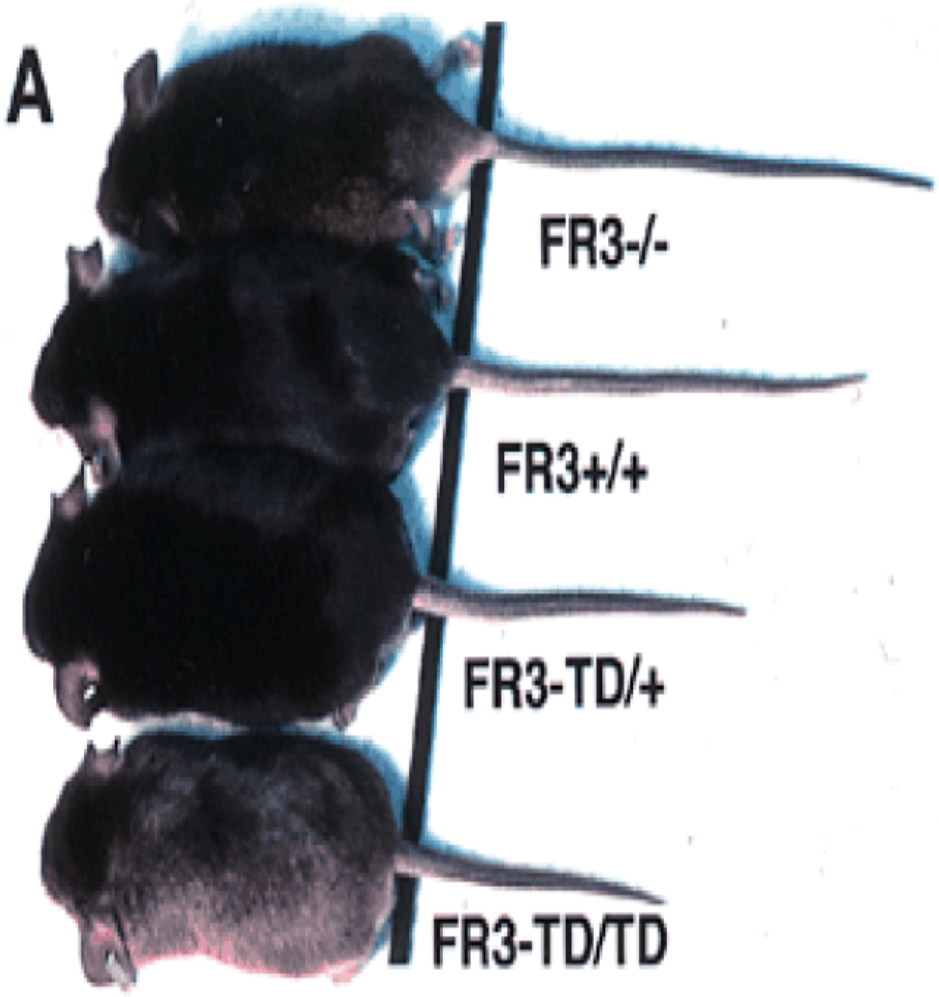
dachshund, Pekingese, and basset hound, where it was found to be dominant and allelic on the basis of arranged crosses (*5*). The phenotype primarily affects the length of the long bones, with growth plates calcifying early in development, thus producing shortened bones with a curved appearance (Fig. 1A) (*6*, *7*).

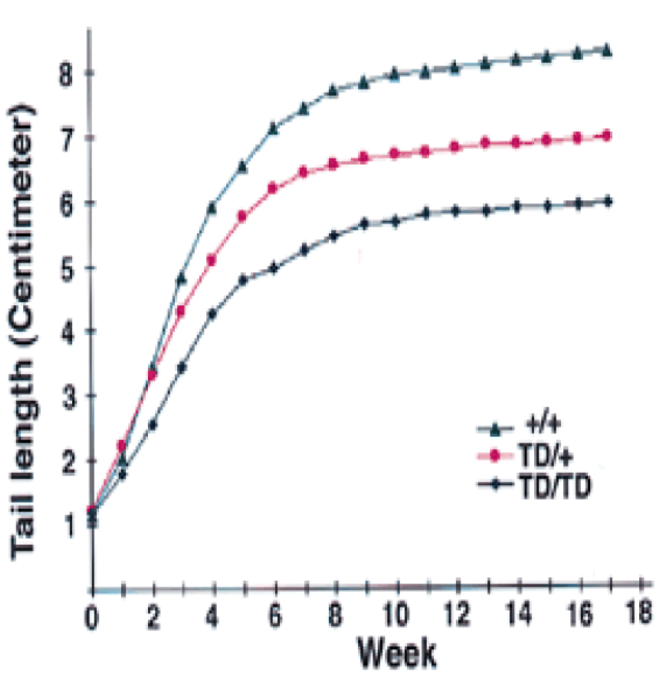
To identify the genetic foundations of breed-defining phenotypes such as canine chondrodysplasia, we developed a multibreed approach for mapping fixed canine traits. A total of 835 dogs from 76 distinct breeds that provided maximal coverage of phenotypic variation were genotyped by using the Affymetrix version 2.0 single-nucleotide polymorphism (SNP) chip (*8*, *9*). Chondrodysplastic breeds, or cases, were defined on the basis of specific morphologic criteria set forth in each

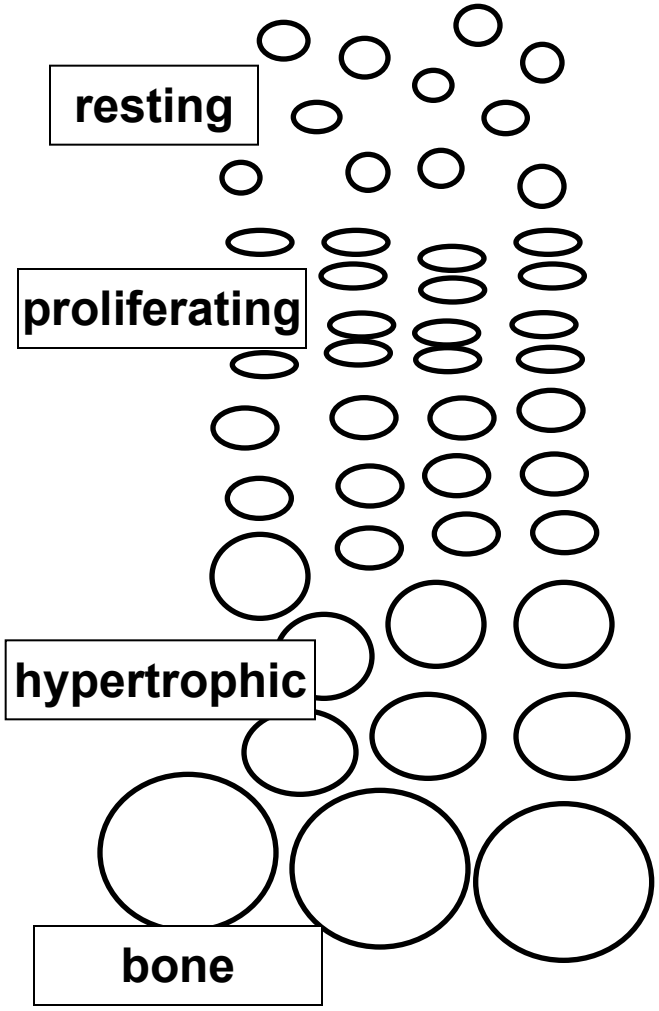


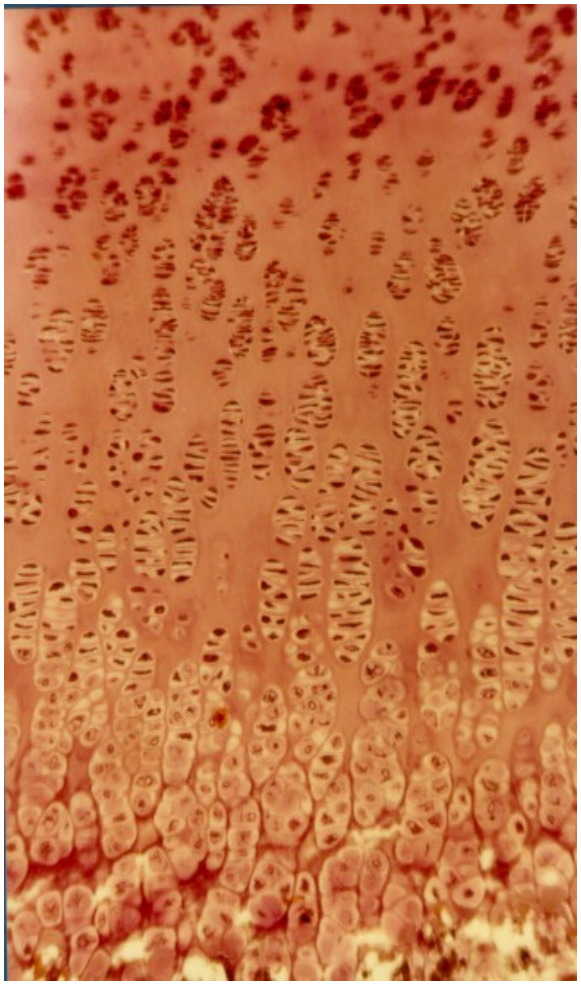


Loss-of-function vs. Gain-of-function

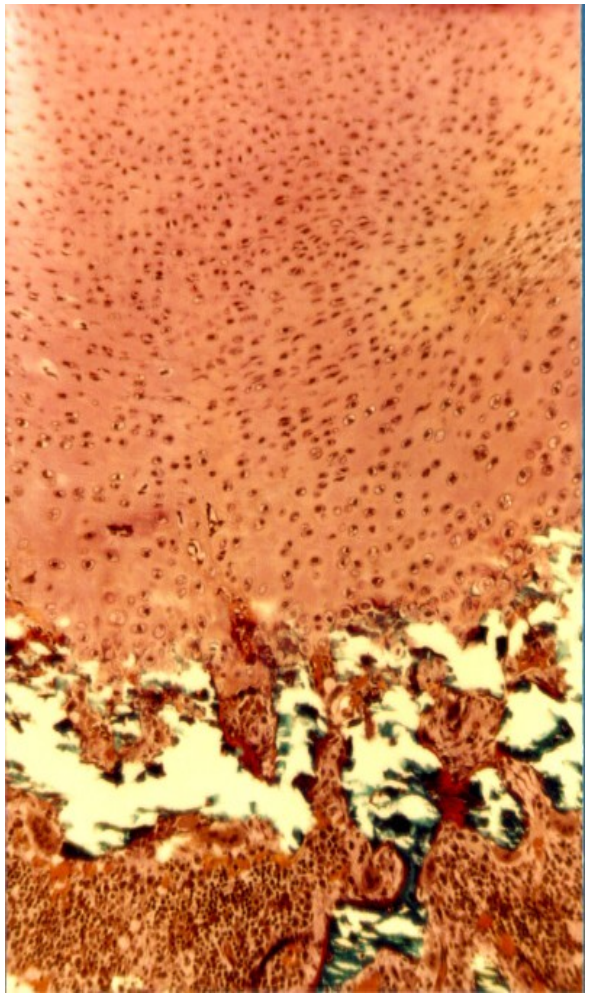

A
FR3-/-
FR3+/+
FR3-TD/+
FR3-TD/TD


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



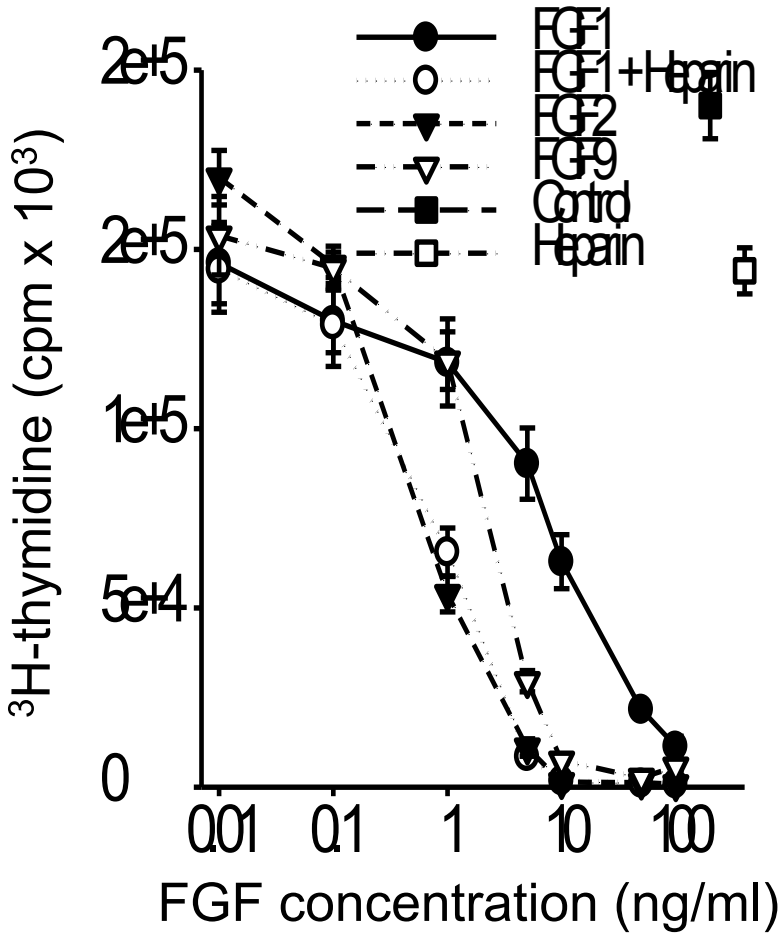


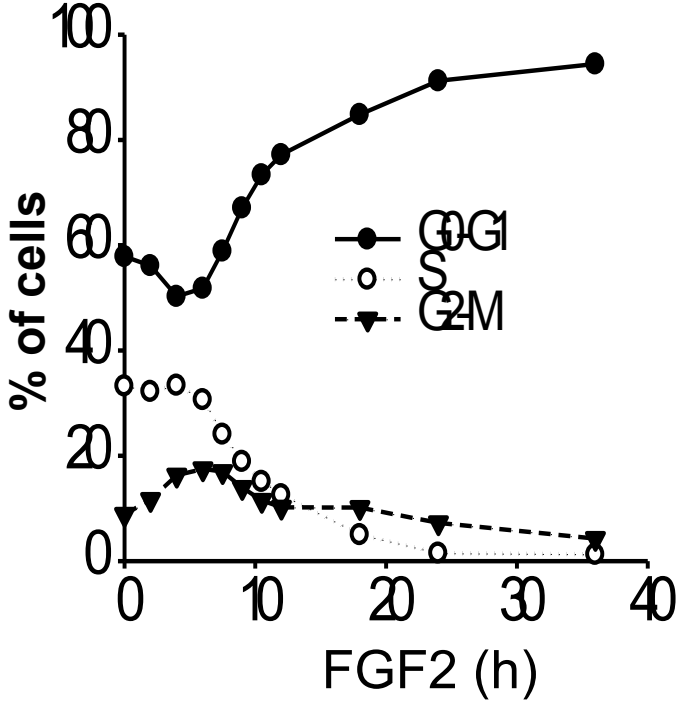
healthy



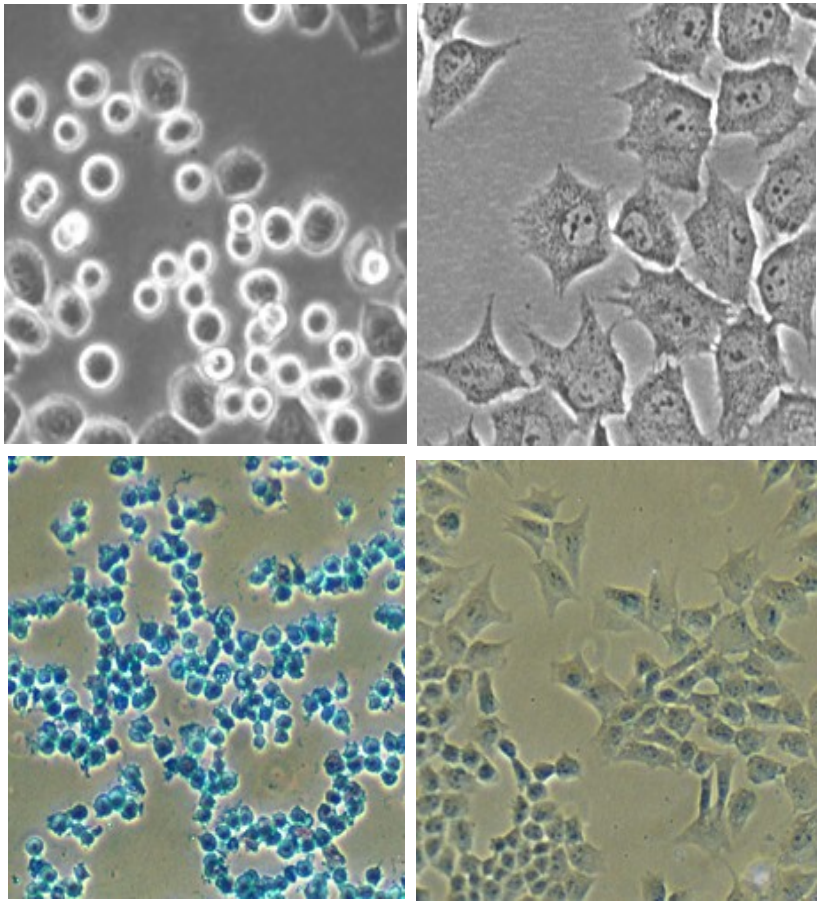
TD

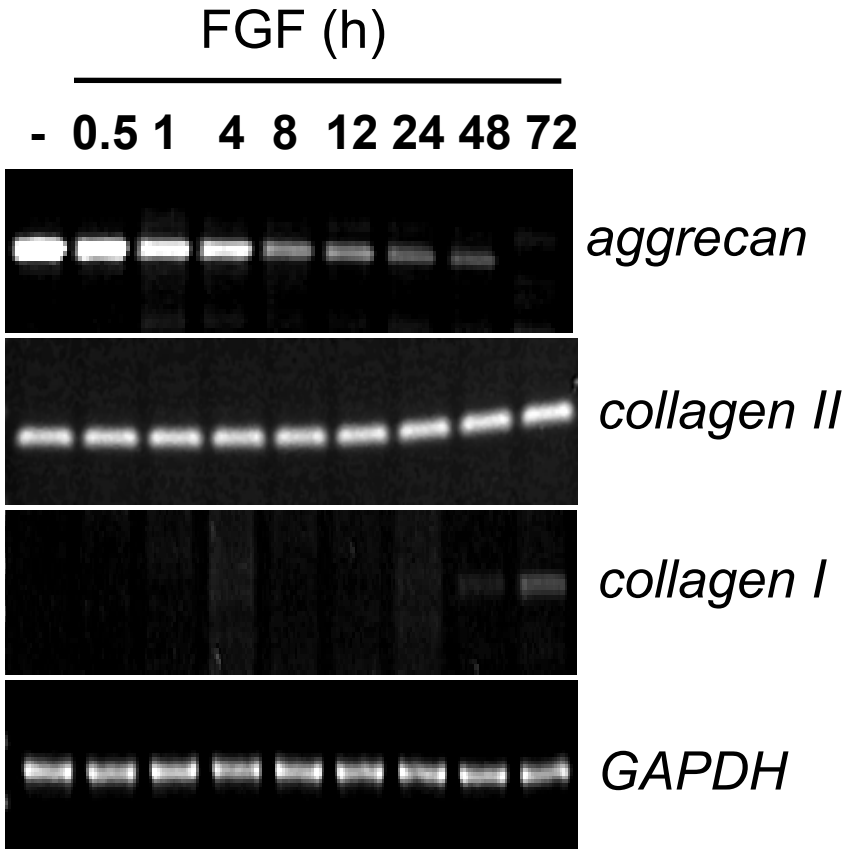
FGFR3 inhibits chondrocyte proliferation by arresting their cell cycle in G1 phase



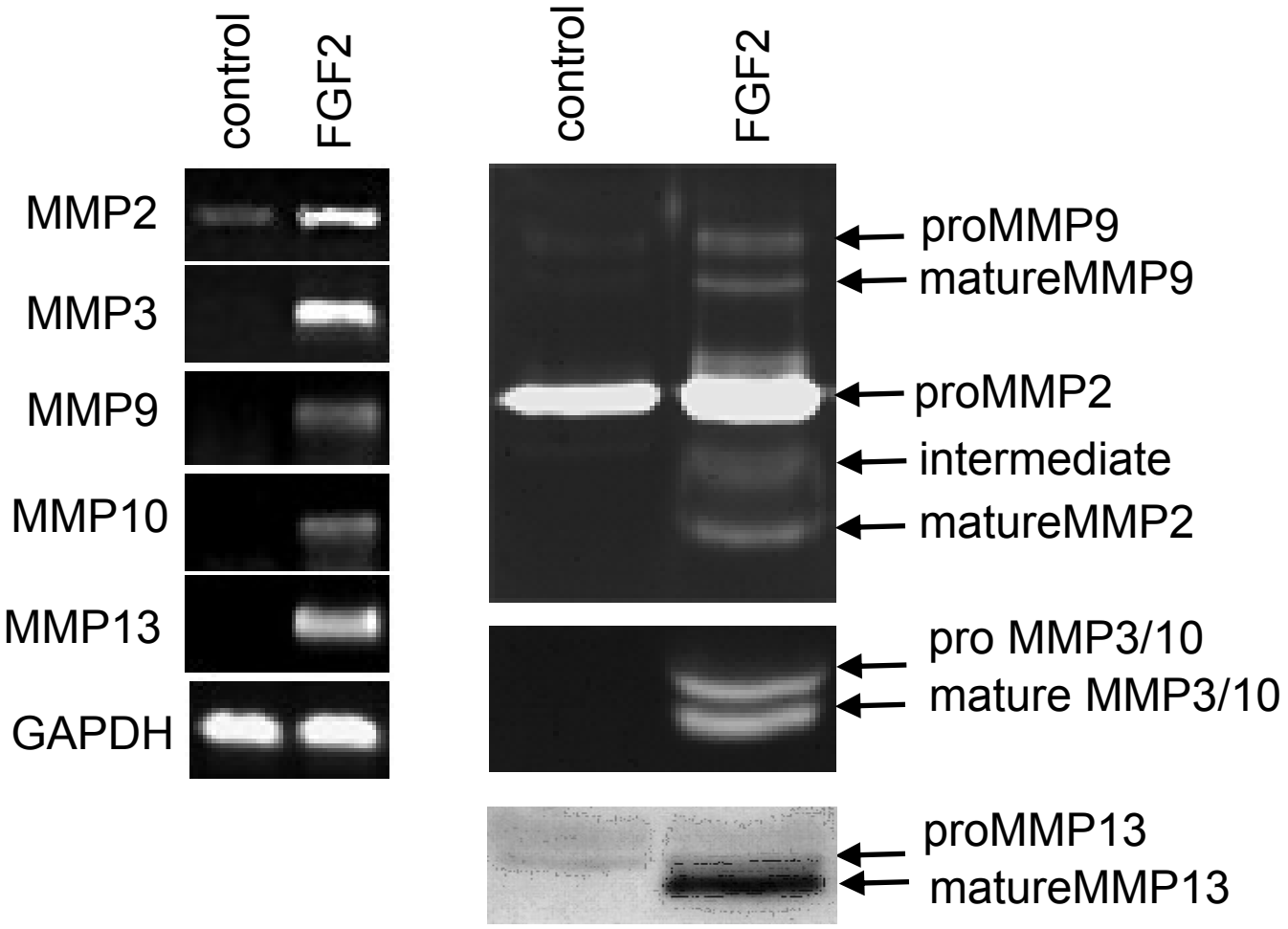


FGFR3 alters the cartilage-like phenotype of chondrocytes

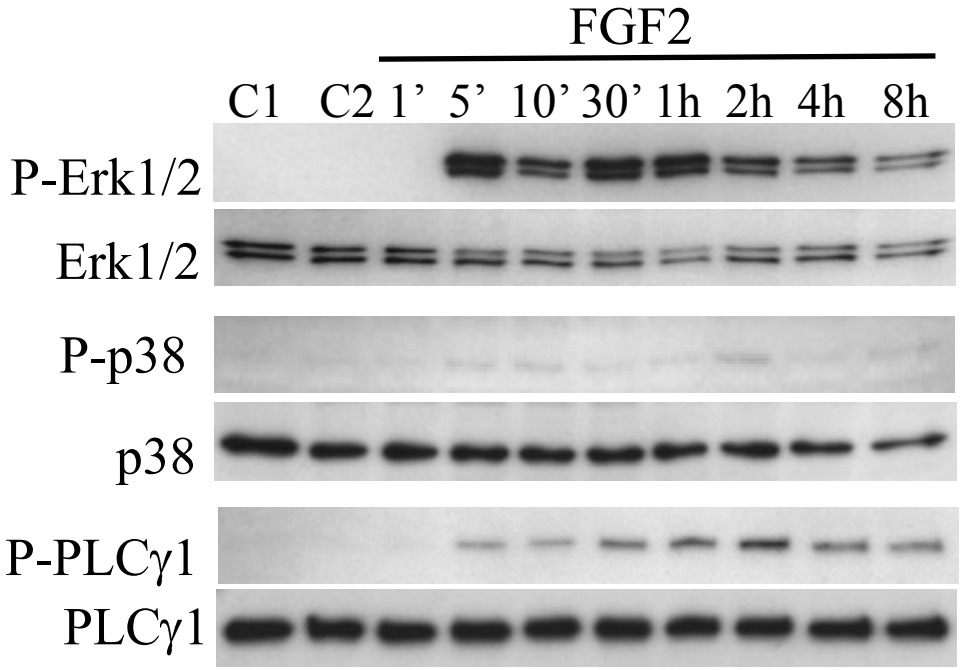


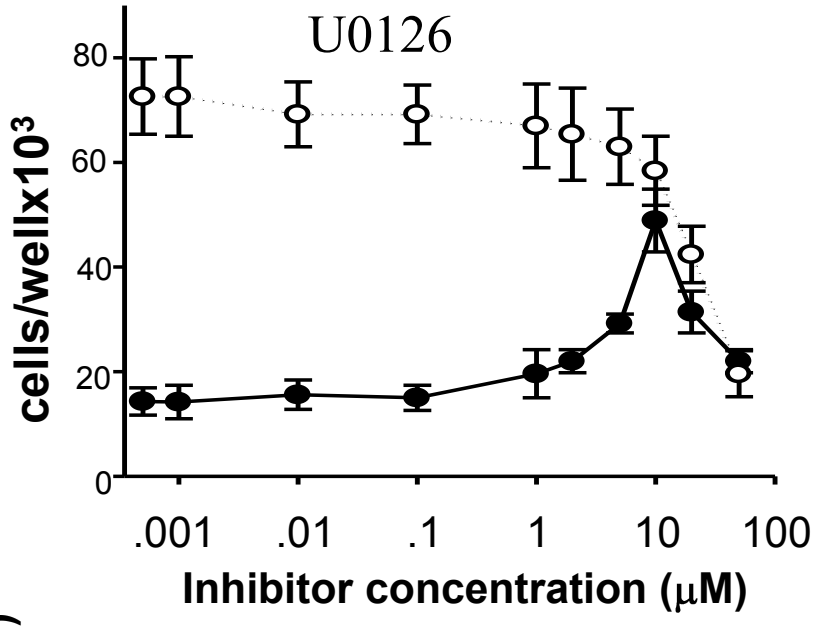


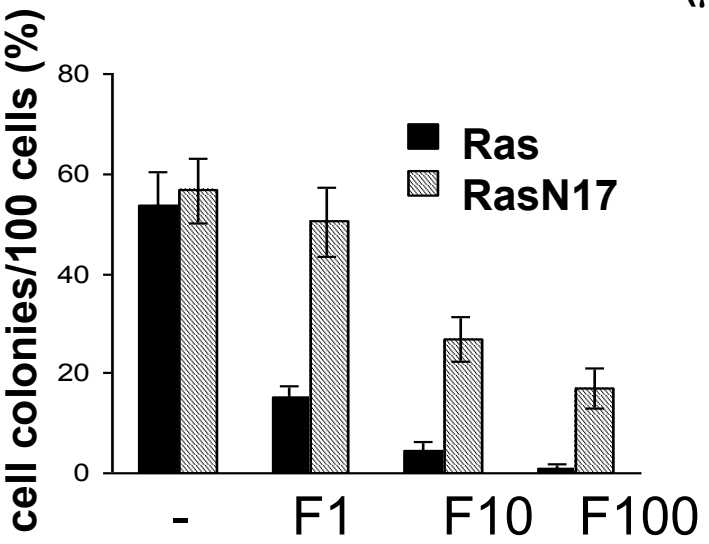
……via MMP-mediated degradation of extracellular matrix



FGFR3 inhibits cartilage growth via Erk pathway







FGF2

↓

FGFR3

↓

FRS2

↓

Ras

↓

Raf-1

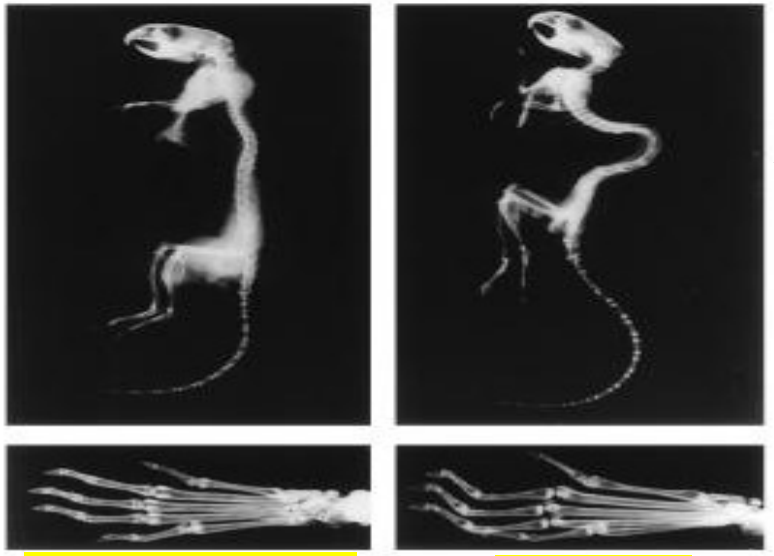
↓

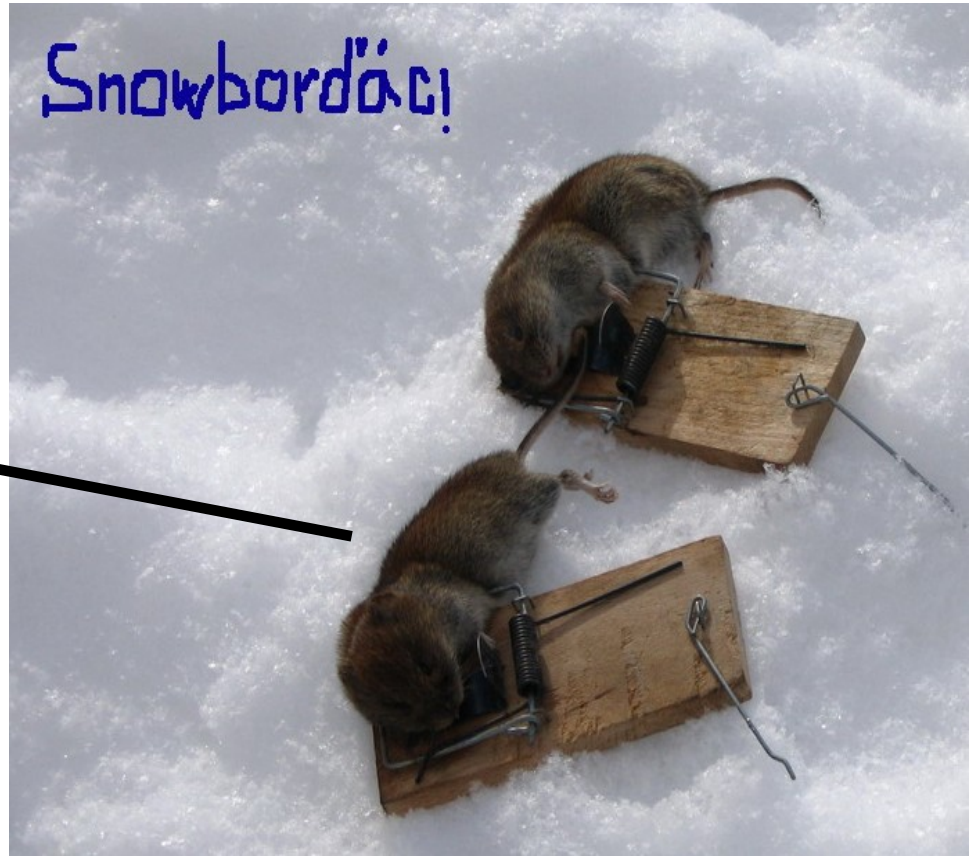
MEK

↓

Erk →

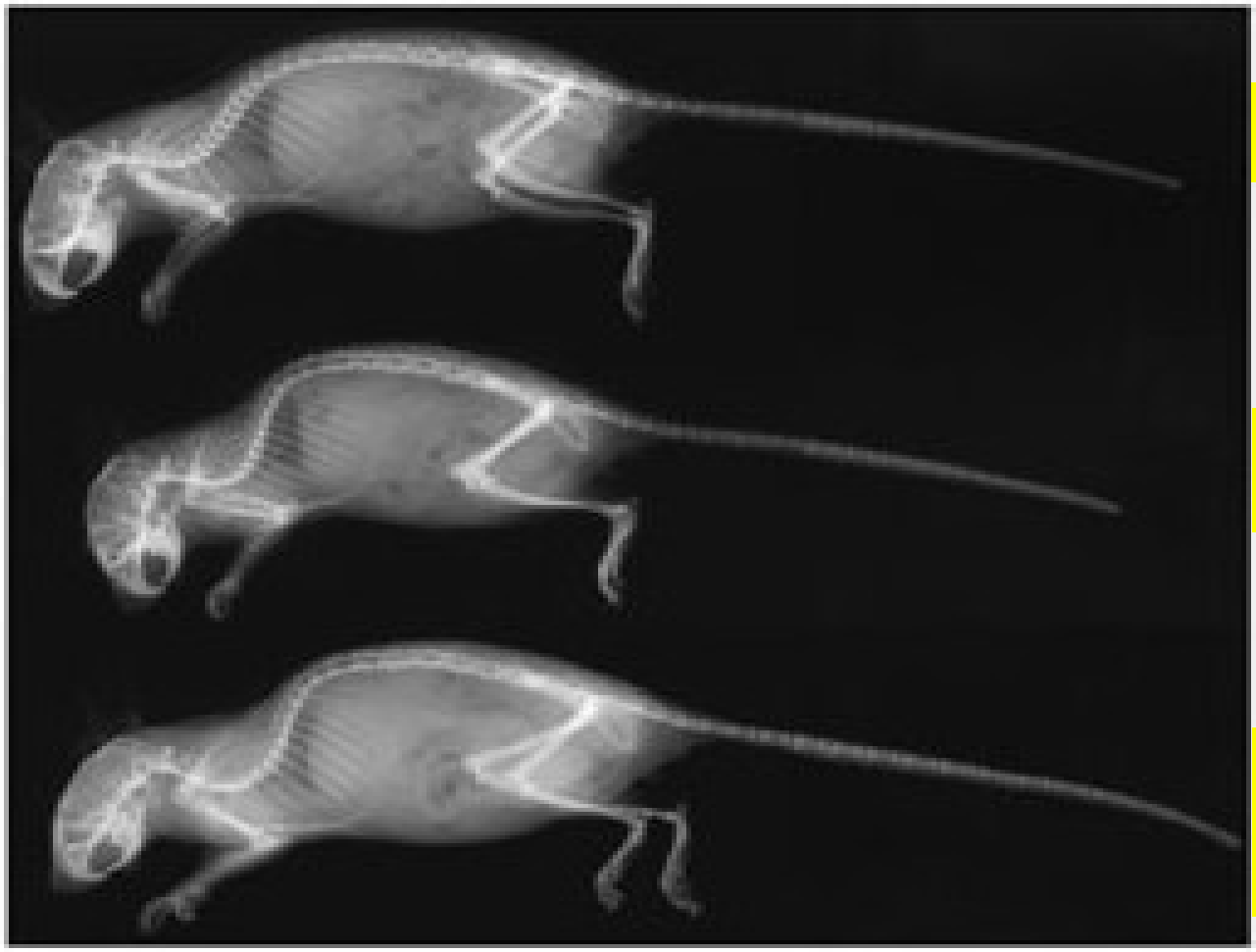
C-type Natriuretic Peptide (CNP) over-expression results in skeleton overgrowth in mice





CNP over-expression???

CNP rescues dwarfism caused by ACH mutation in FGFR3

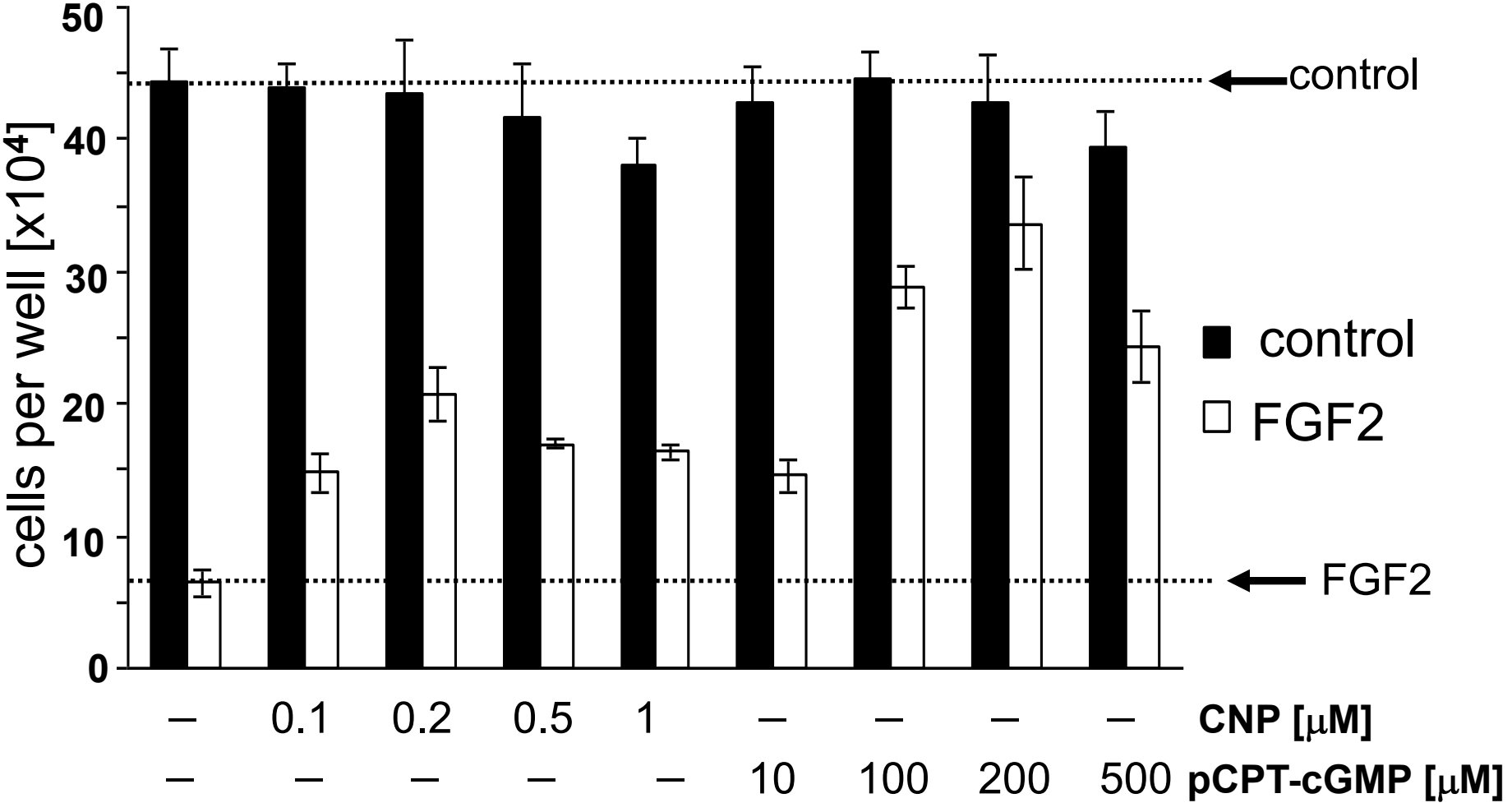


wild-type

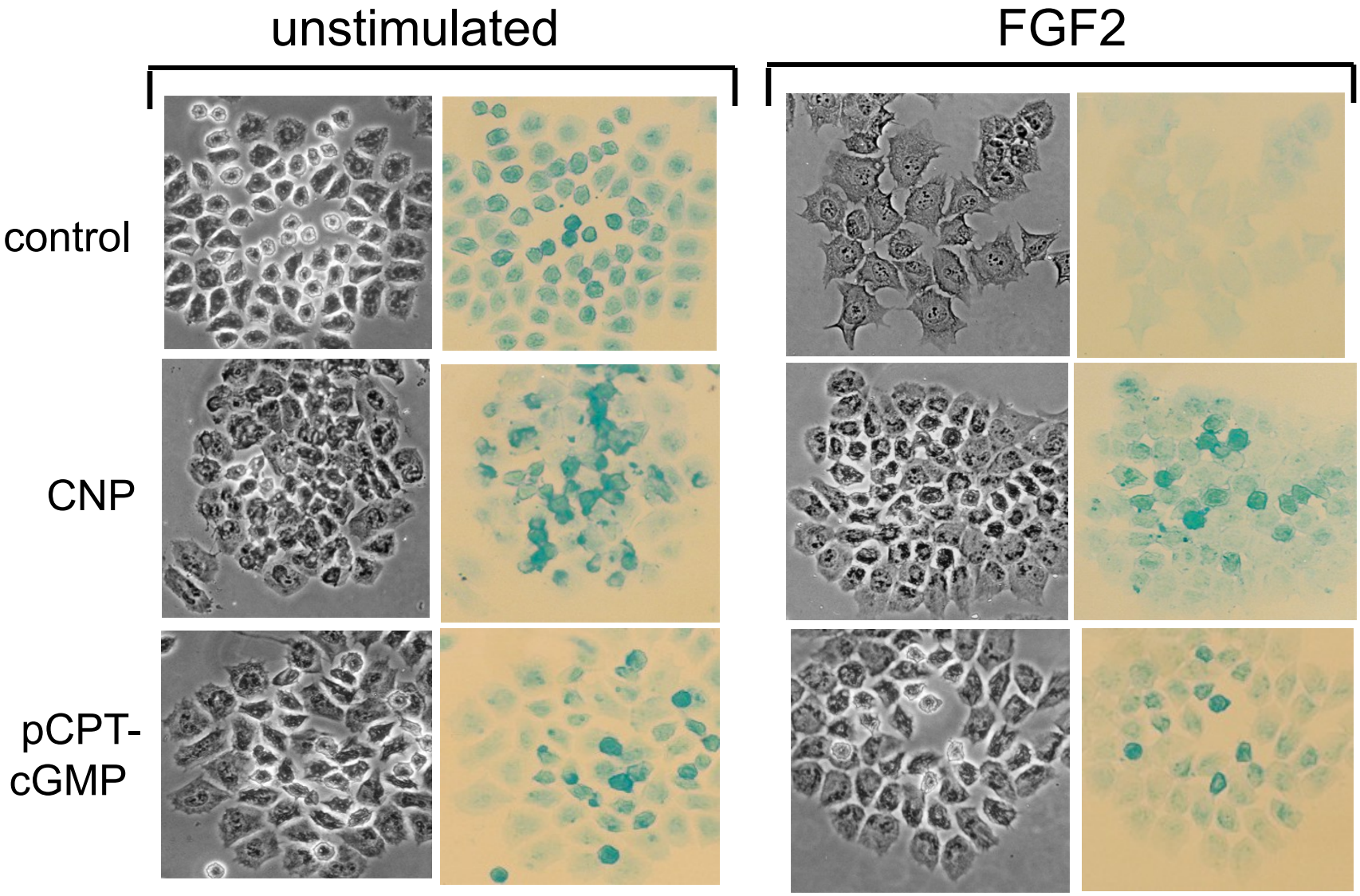
Fgfr3^{Ach}

Fgfr3$^{Ach/CNP}$ ↑

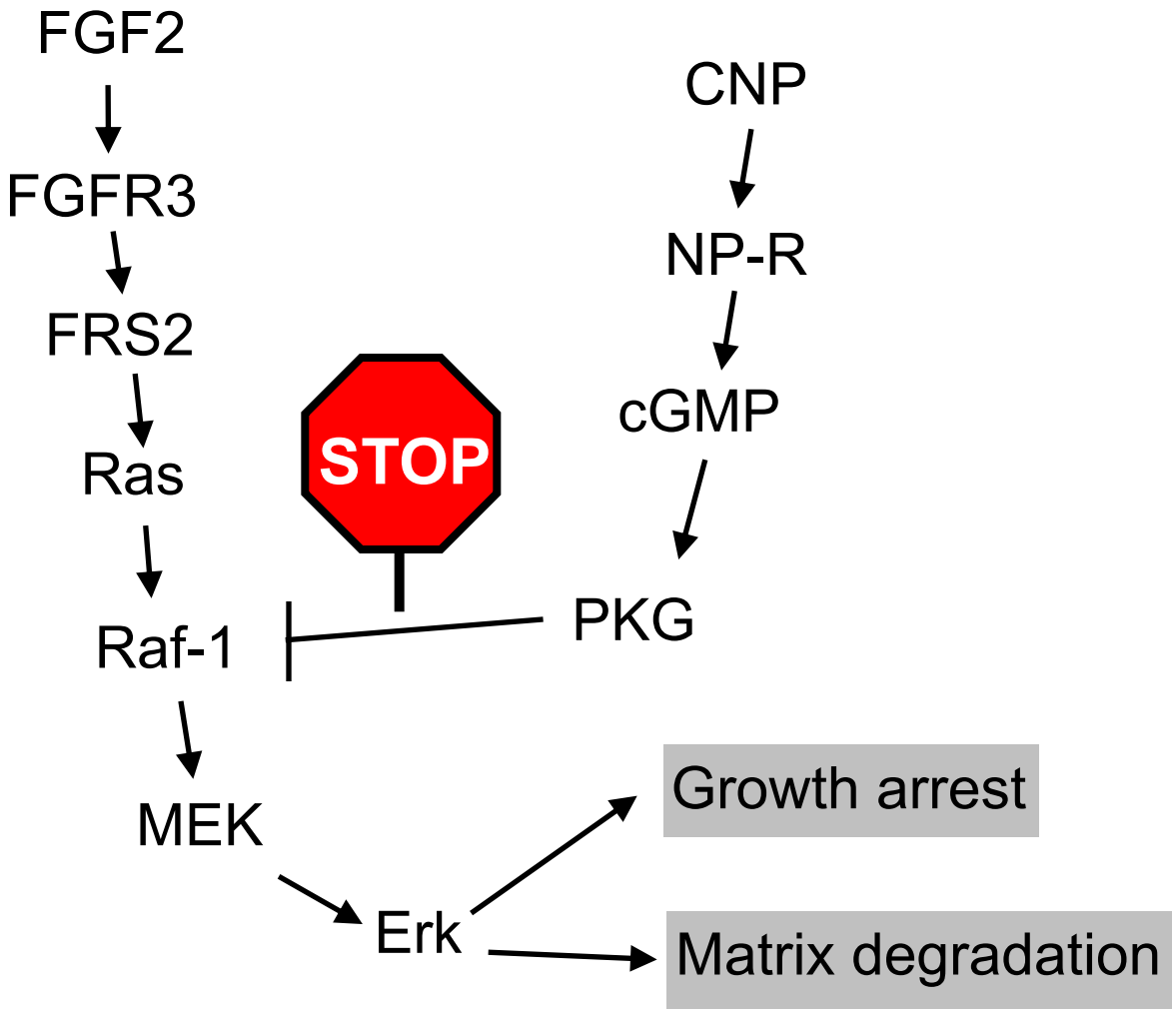
CNP counteracts FGF2-mediated chondrocyte growth arrest through cGMP-dependent pathway



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



CNP inhibits Erk MAP kinase module at the Raf level



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RSS Content

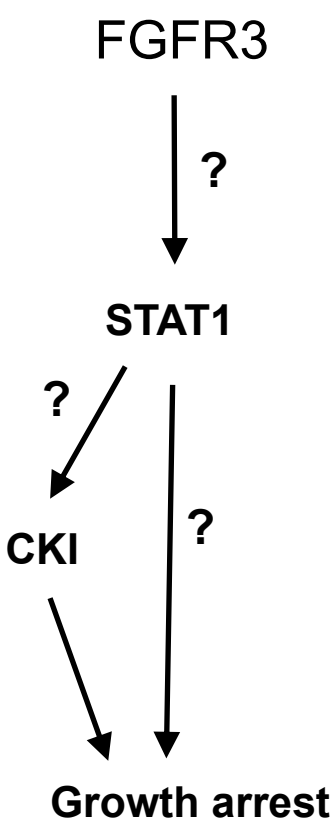
BioMarin Announces Program for BMN-111 for the Treatment of Achondroplasia

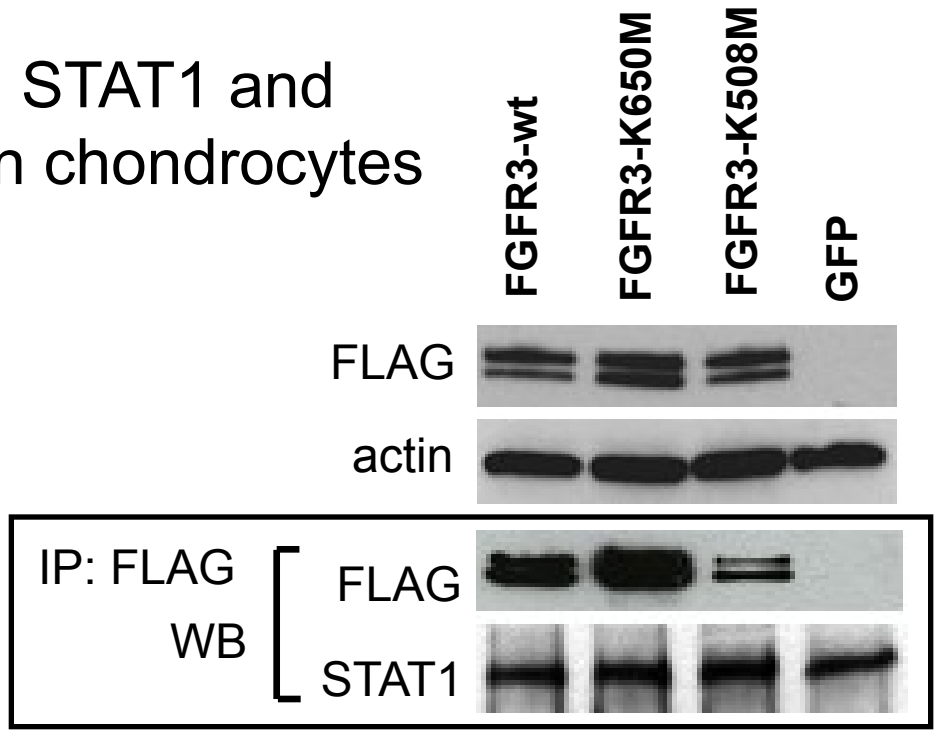
NOVATO, Calif., Oct 19, 2010 /PRNewswire via COMTEX/ --

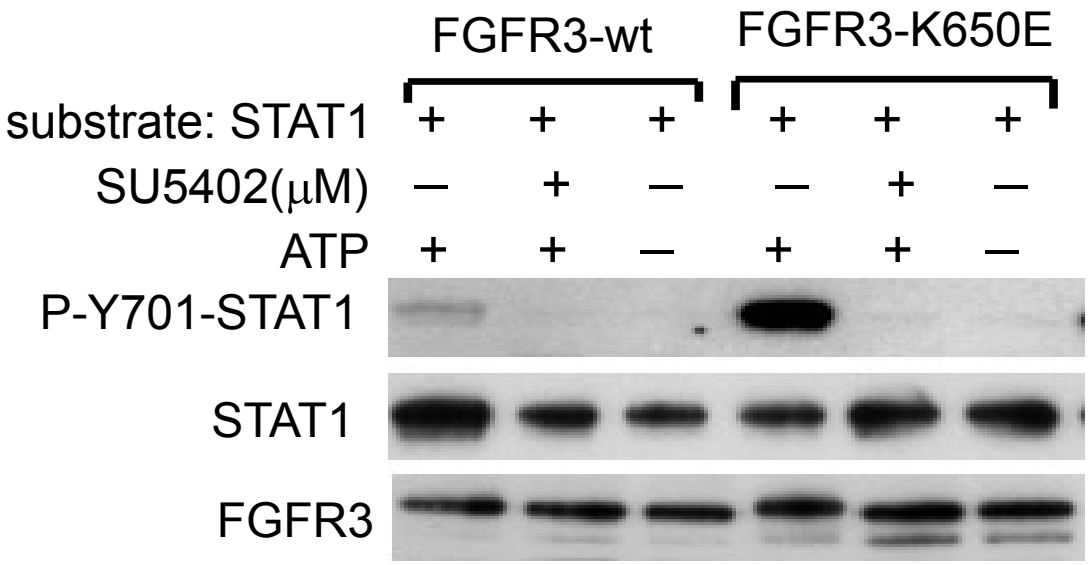
BioMarin Pharmaceutical Inc. (Nasdaq: BMRN) today announced its program for BMN-111, a peptide therapeutic for the treatment of achondroplasia. BioMarin plans to file an IND in the fourth quarter of 2011 and to initiate a Phase 1 clinical trial by the first quarter of 2012.

BMN-111, for the treatment of achondroplasia, is an analog of C-type Natriuretic Peptide (CNP), a small cyclic peptide that is a positive regulator of bone growth. It is produced and has a receptor in the growth plate, and along with the fibroblast growth factor receptor 3 (FGFR3), regulates normal bone growth. In addition to short stature, there are complications in achondroplasia that are related to bone compression (e.g. foramen magnum narrowing, spinal stenosis, upper respiratory narrowing) of nervous tissues or other tissues.

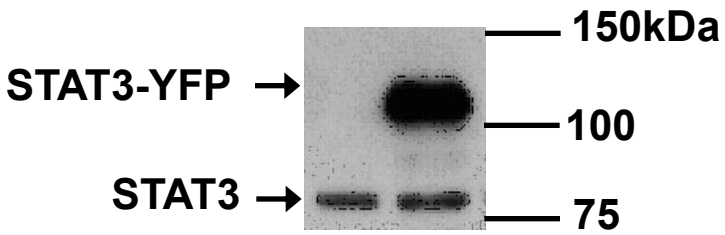
FGFR3 associates with STAT1 and acts as STAT1-kinase in chondrocytes

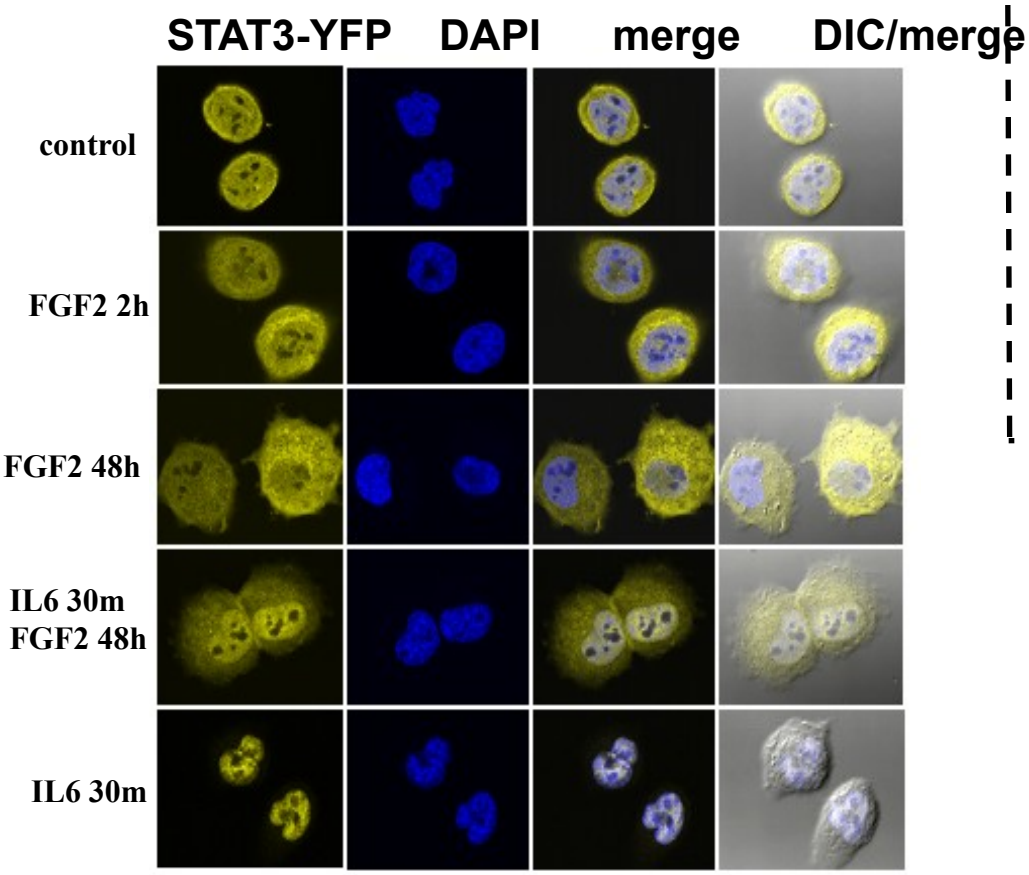


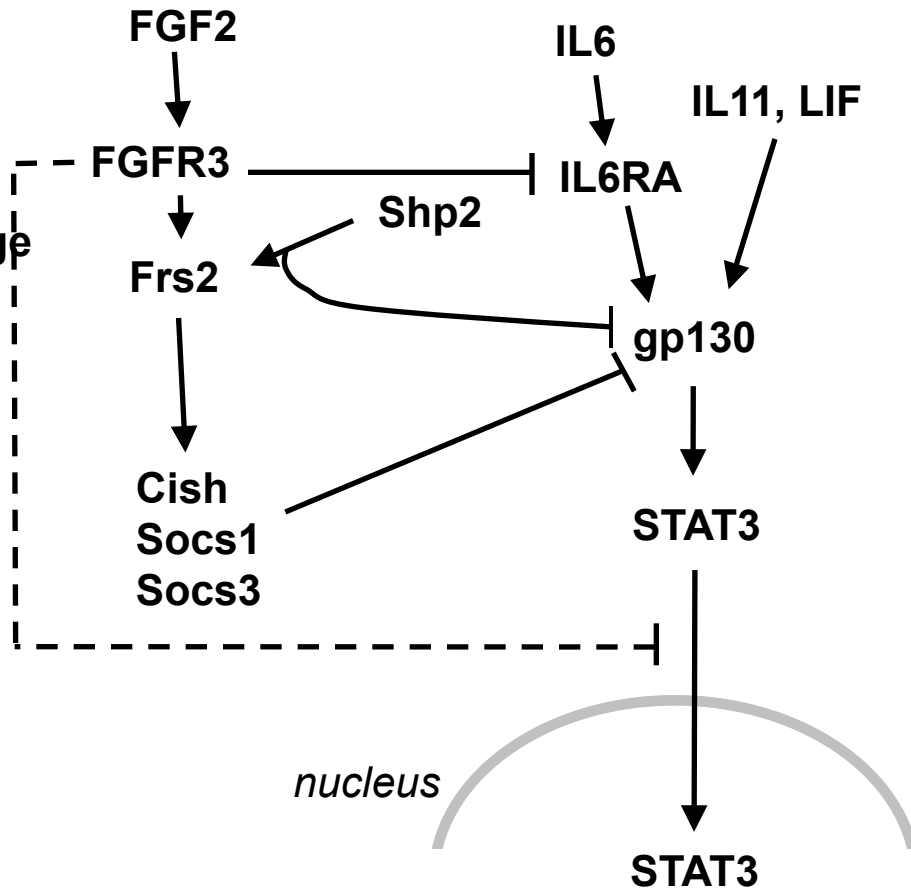




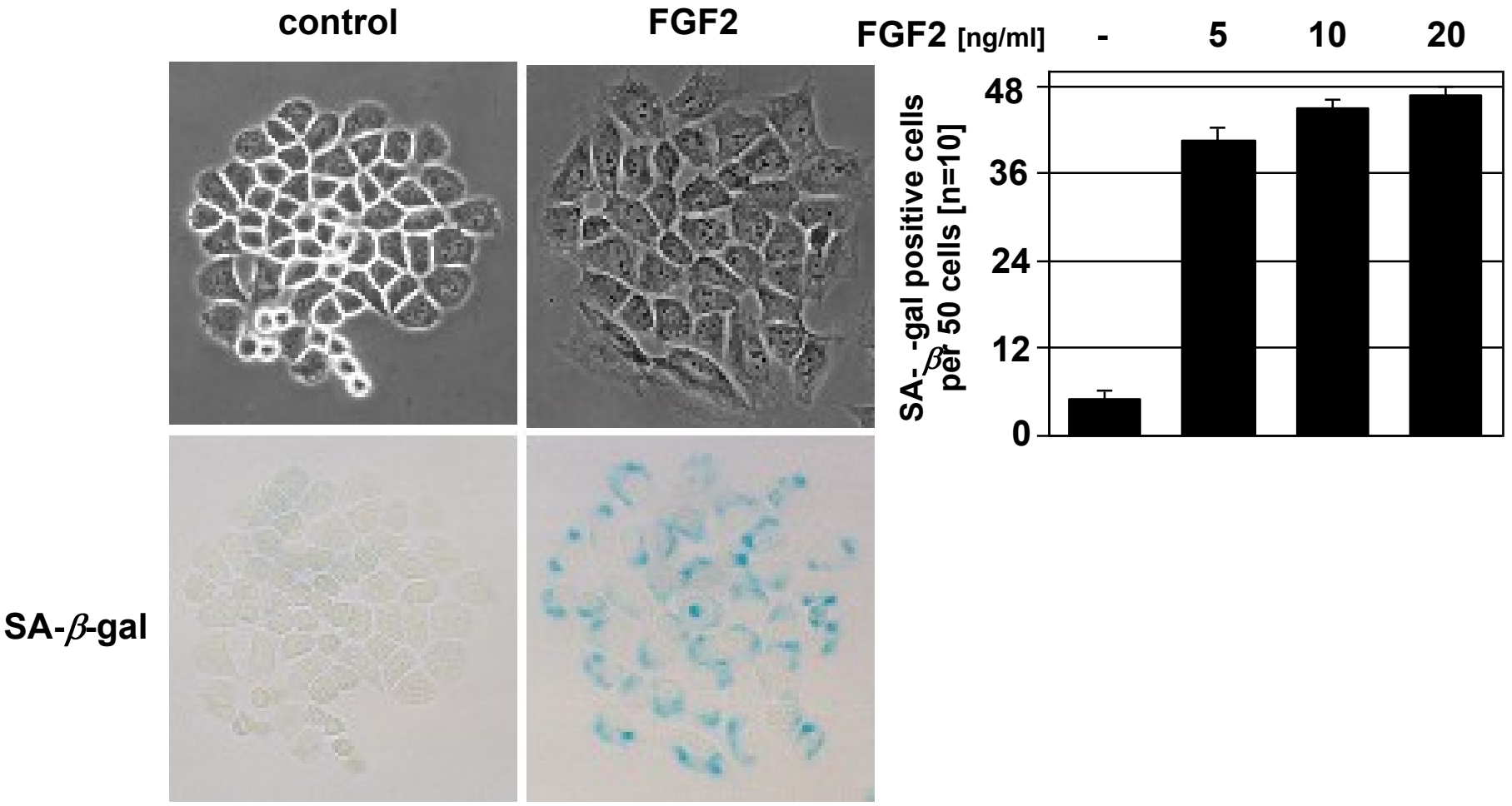
Chronic FGF stimulus inhibits cytokine/STAT signaling in chondrocytes



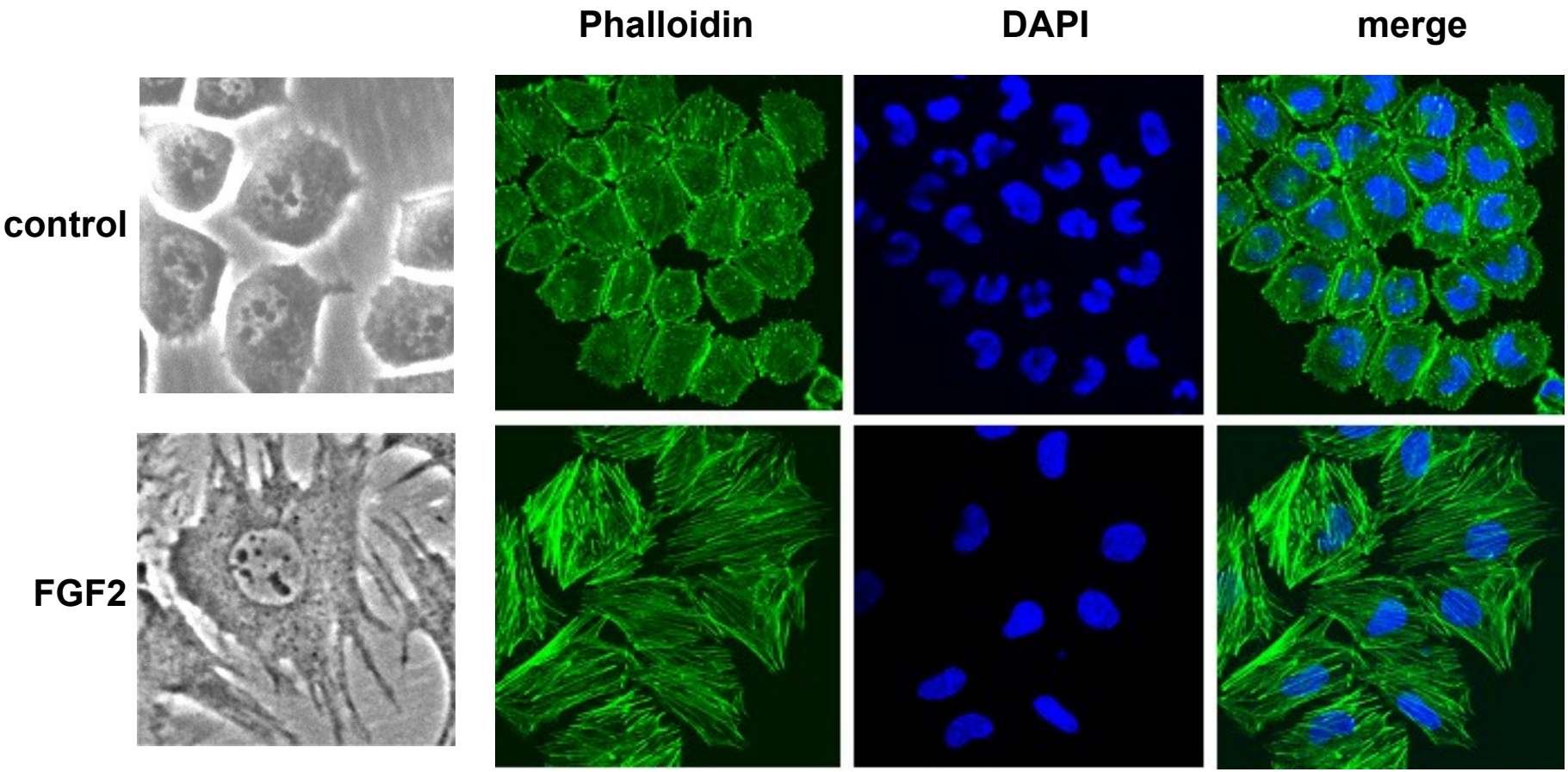




FGFR3 causes premature senescence in chondrocytes



FGFR3 signals towards the cytoskeleton in chondrocytes



FGFR3 signals via Wnt pathway in chondrocytes