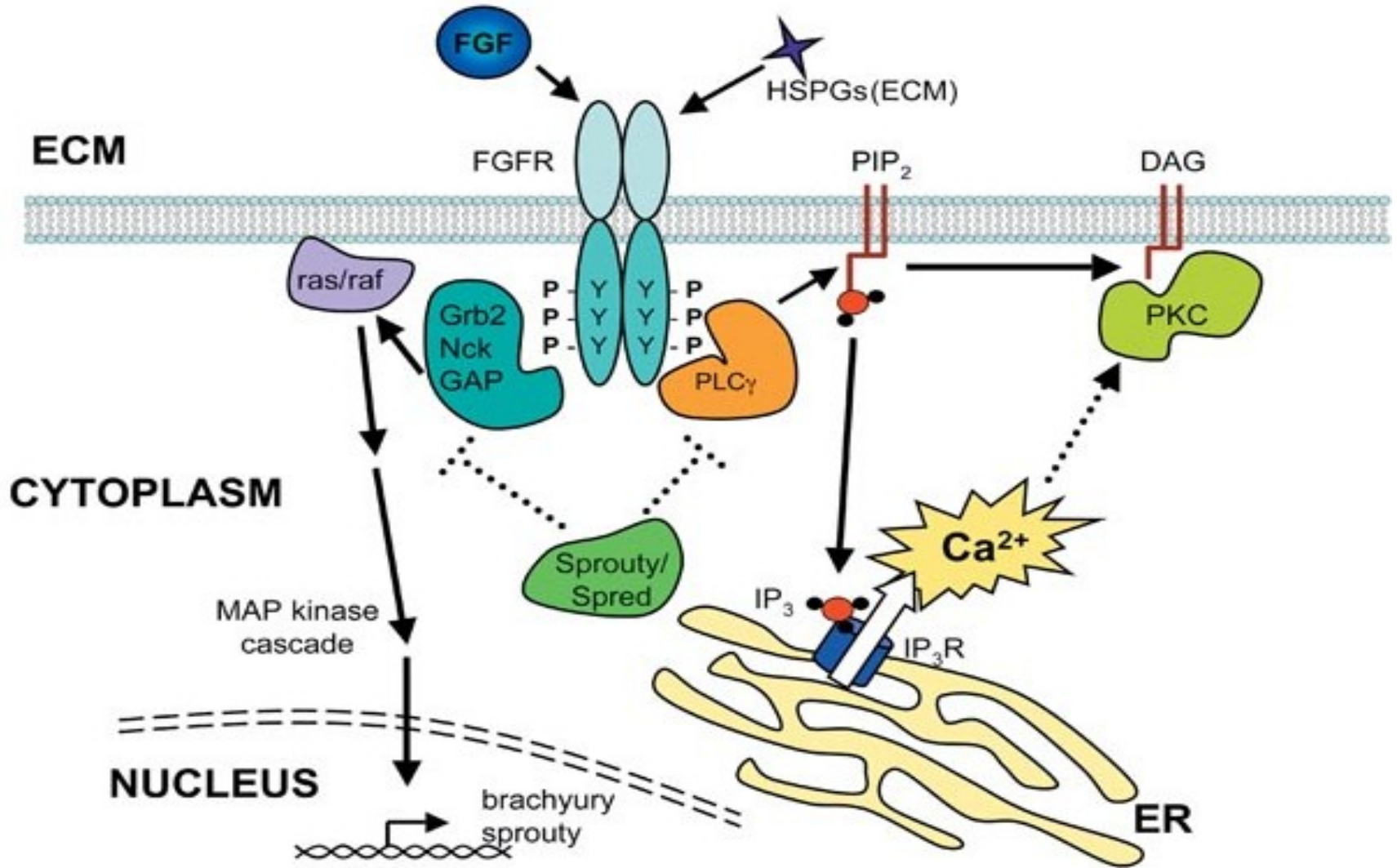


9. MECHANISMS OF DEVELOPMENT I – REGULATION OF LIMB DEVELOPMENT BY FIBROBLAST GROWTH FACTORS (FGF)

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

P. Mohanty-Hejmadi, S. K. Dutta & P. Mahapatra

Department of Zoology, Utkal University, Bhubaneswar 751004, Orissa, India

NIAZI and Saxena¹ 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 species². There have been several subsequent reports³⁻⁷ 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

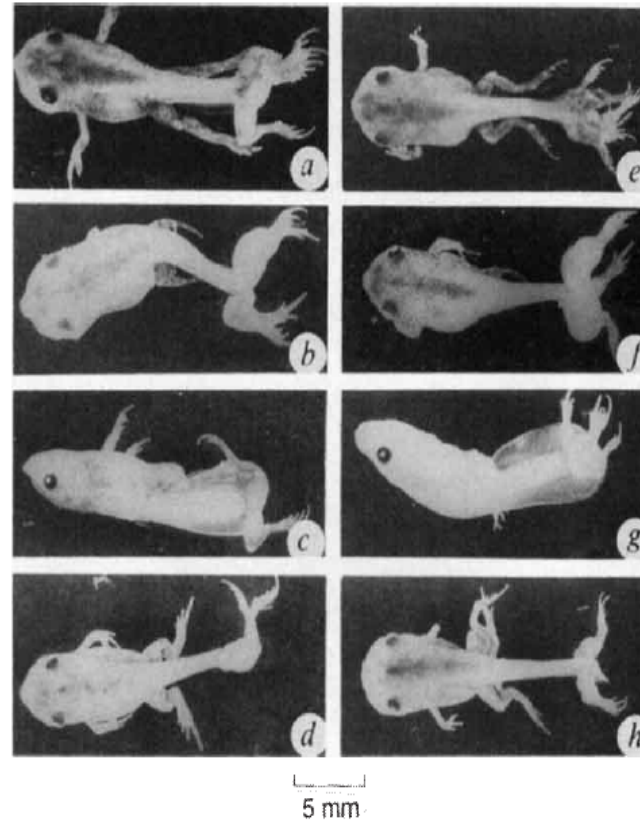
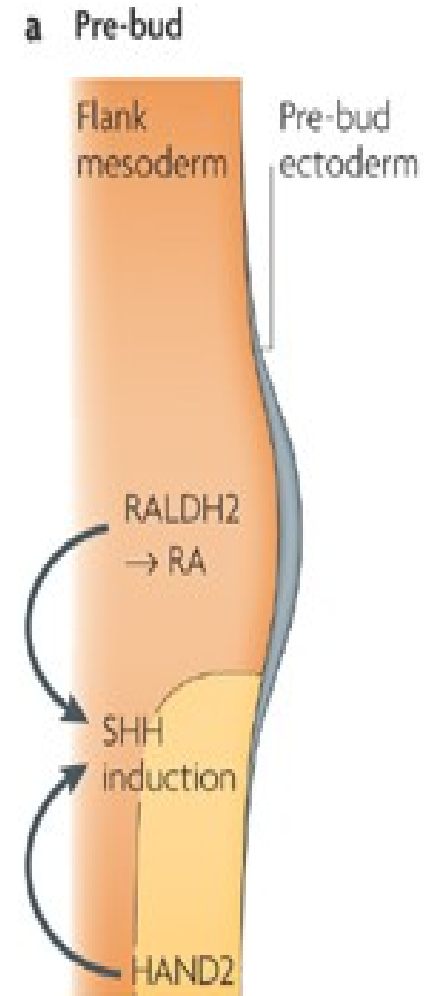


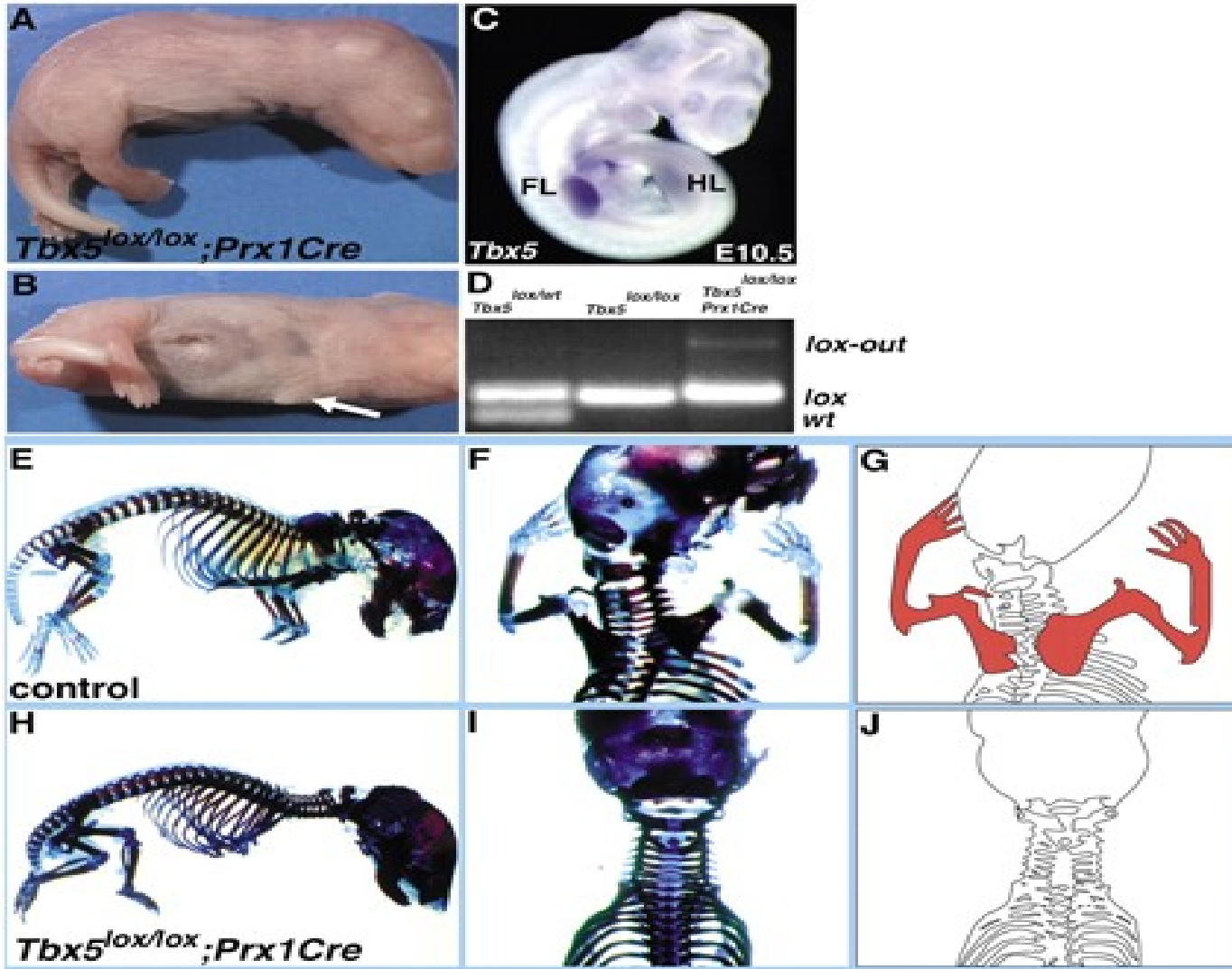
FIG. 1 Effects of vitamin A (10 IU) treatment for various times on limb generation from amputated tail stump. a, Treatment for 24 h, 3 limbs are generated; b, 72 h, 4 limbs; c and d, 96 h, 2 limbs; e, 120 h, 7 limbs; f, 120 h, 3 limbs; g, 144 h, 3 limbs; h, 144 h, 2 limbs, plus an extra pair of limbs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



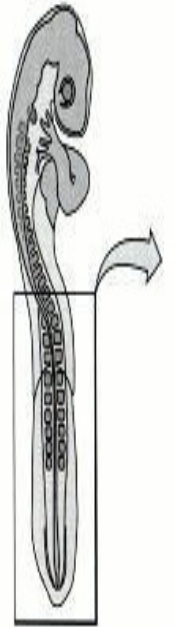
RALDH2- retinaldehyde dehydrogenase 2

.....LATER CAME TBX

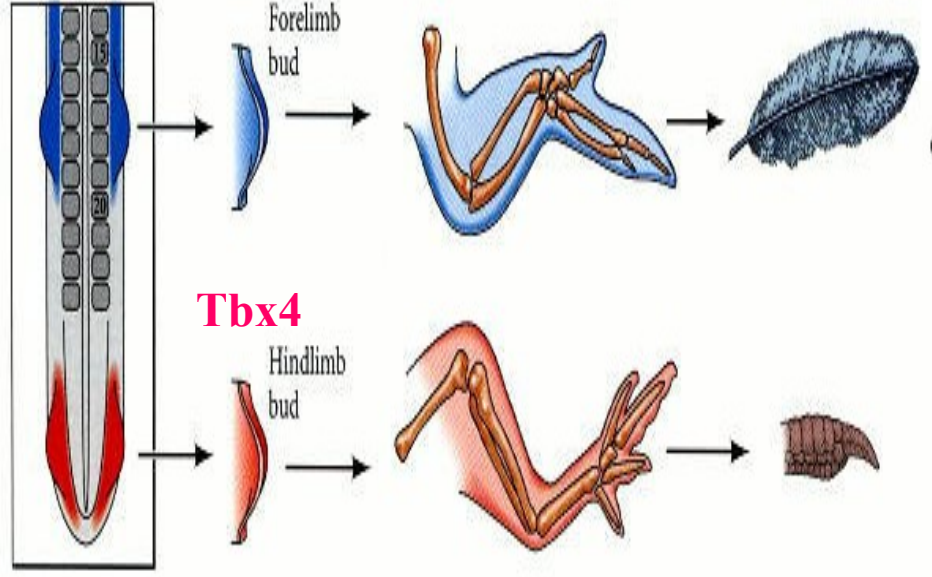
DNA binding domain derived from the prototype gene called transcription factor T. Limb identity factors Tbx4 (hindlimb) and Tbx5 (forelimb)



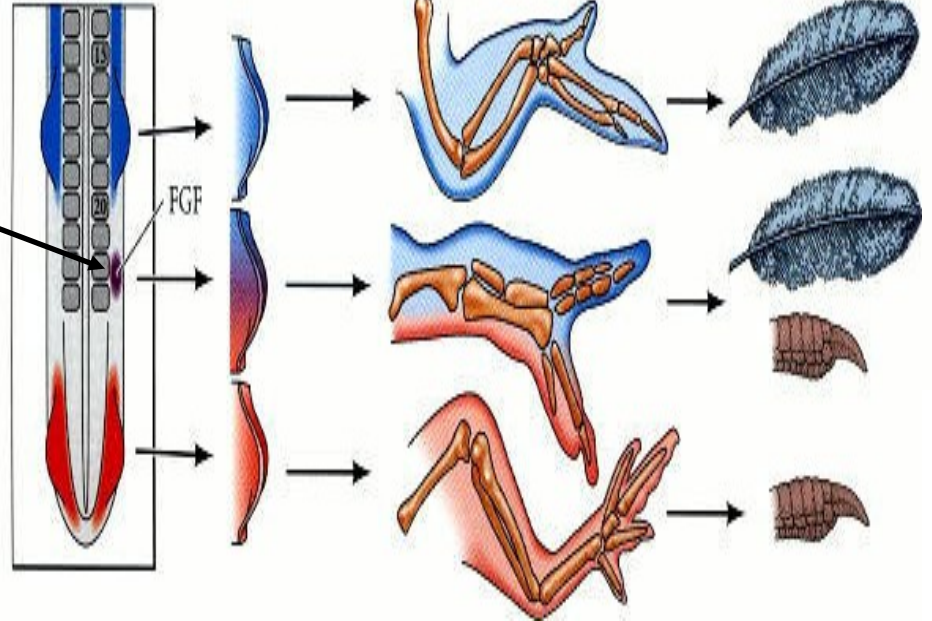
Stage 14/15



(A) Normal

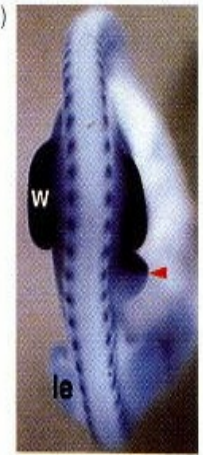


(B) FGF induced

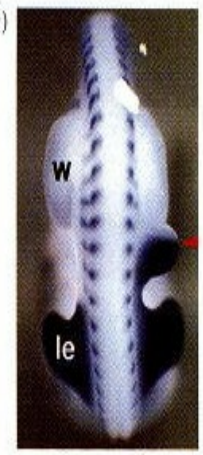


Limb bud induced by implantation of FGF-bead

Tbx5



Tbx4



(E)

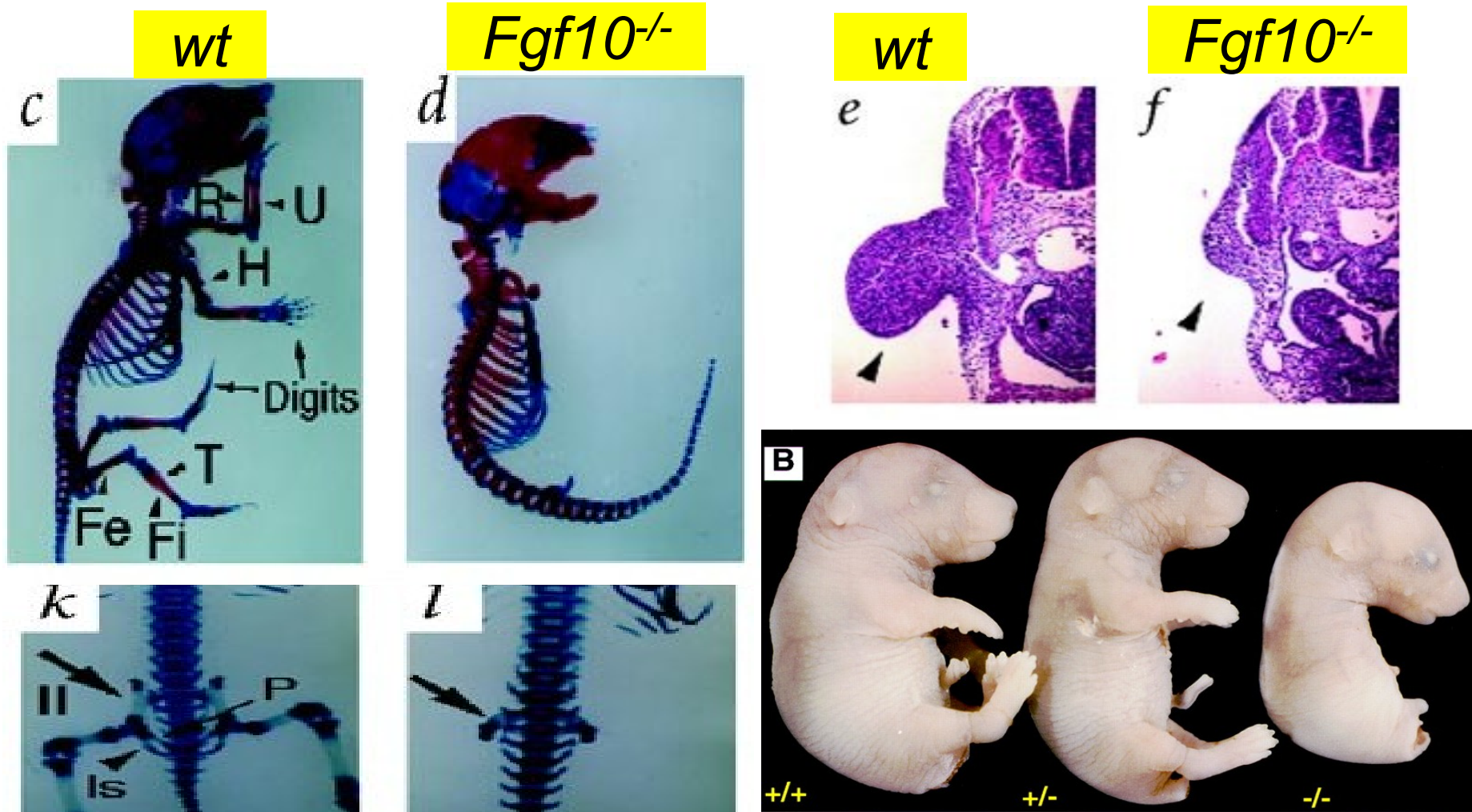


(F)



.....FOLLOWED BY FGF

FGF10 → proliferation in the mesoderm → limb bud growth



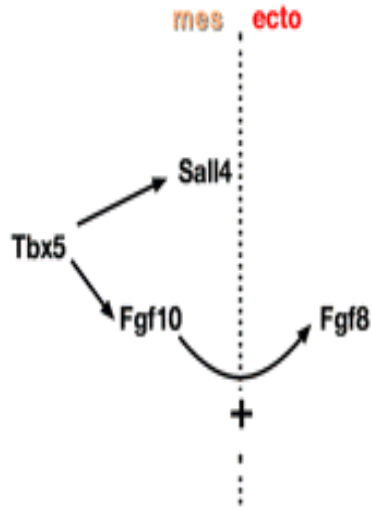
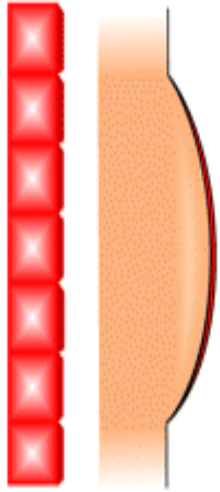
Fgf-10 is required for both limb and lung development and exhibits striking functional similarity to *Drosophila* branchless

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

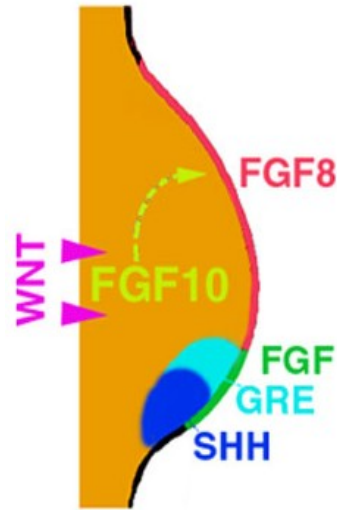
Genes Dev. 1998 12: 3156-3161

Access the most recent version at doi:[10.1101/gad.12.20.3156](https://doi.org/10.1101/gad.12.20.3156)

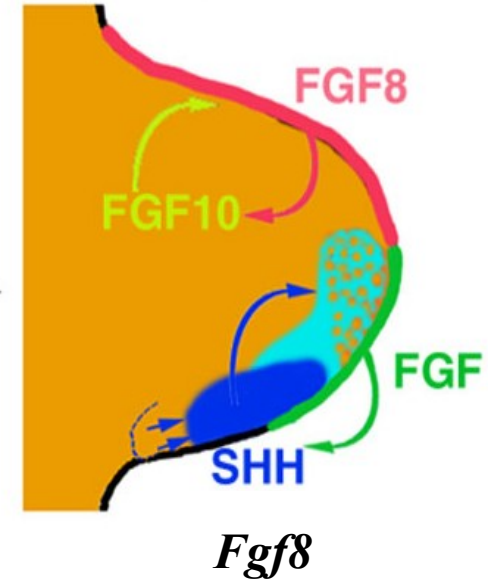
Initiation: *Tbx5* - dependent



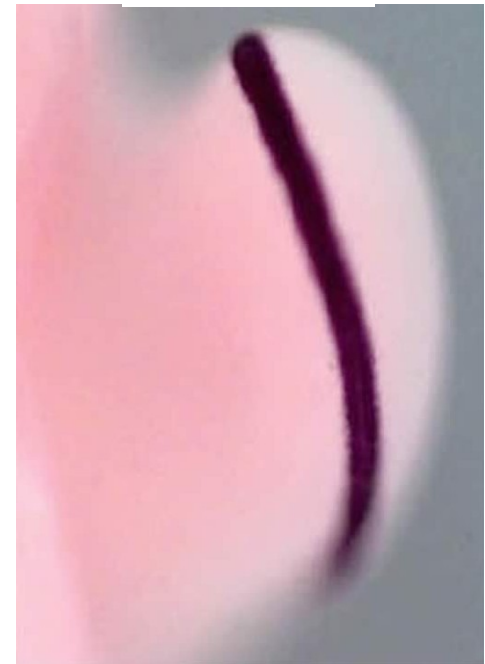
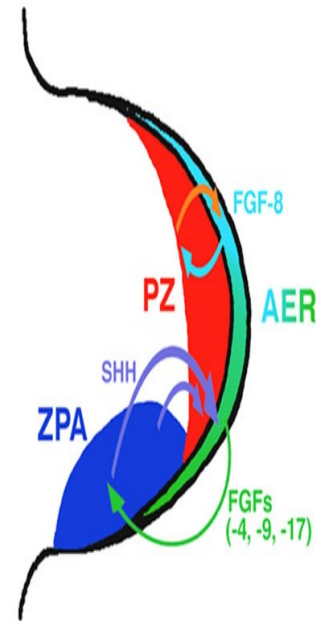
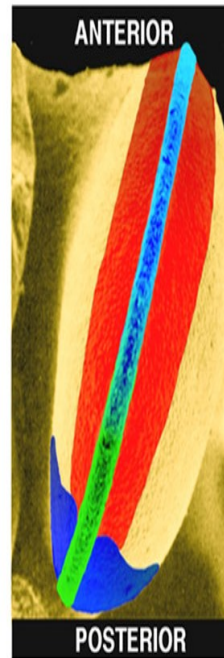
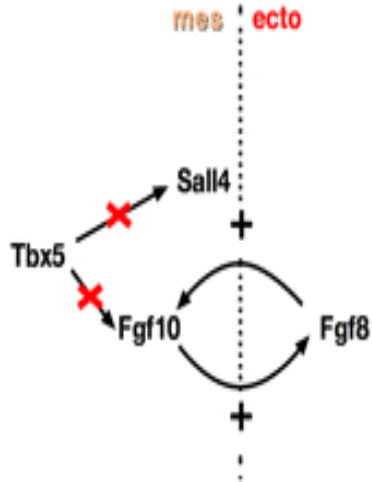
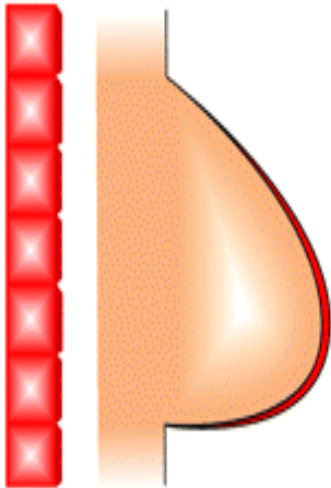
A Induction



B Progression



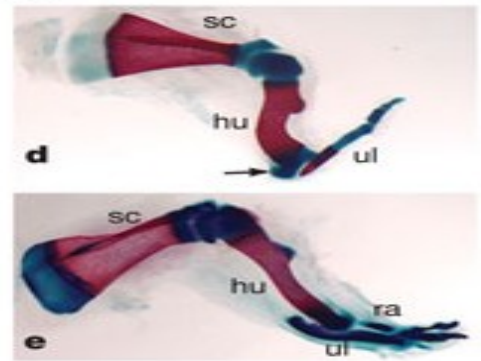
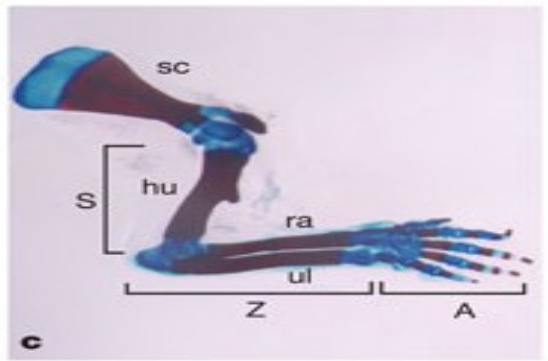
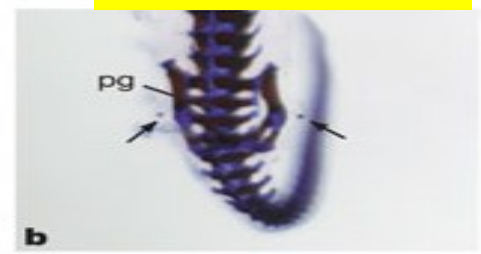
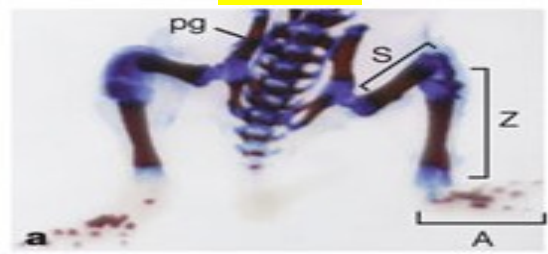
Outgrowth: *Tbx5* - independent



wt

Hindlimb

Fgf8/Fgf4^{-/-}



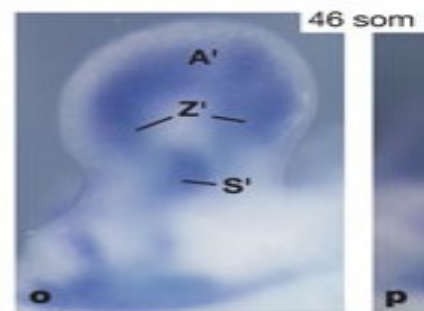
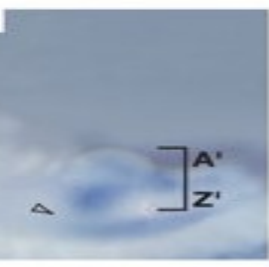
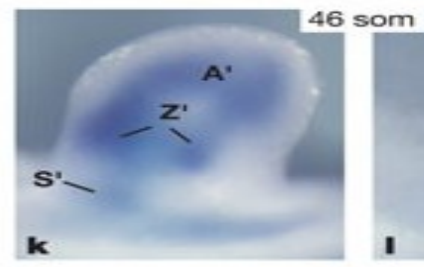
Double KO
No *Fgf8* expression
No *Fgf4* expression

Transient *Fgf8* expression
Precocious, transient *Fgf4* expression

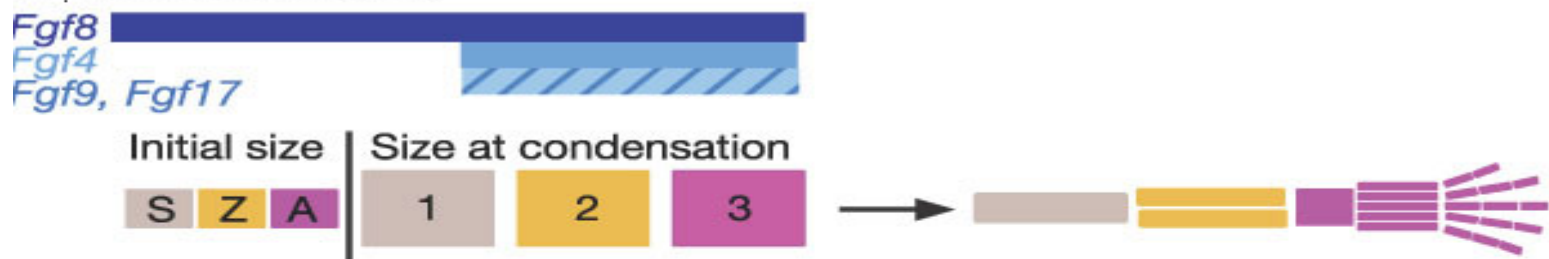
wt

Hindlimb

Fgf8/Fgf4^{-/-}

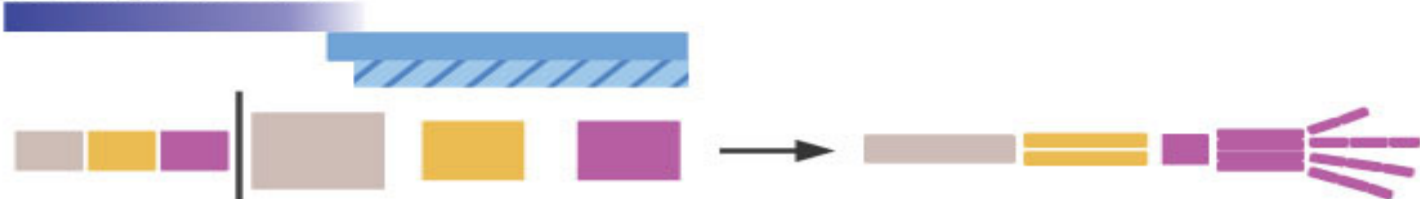


a Normal FL and HL

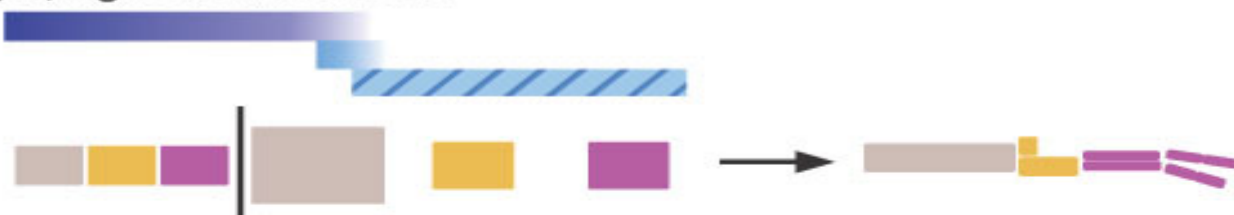


AER KO mutant phenotypes

b *Fgf8* single KO FL



c *Fgf4; Fgf8* double KO FL



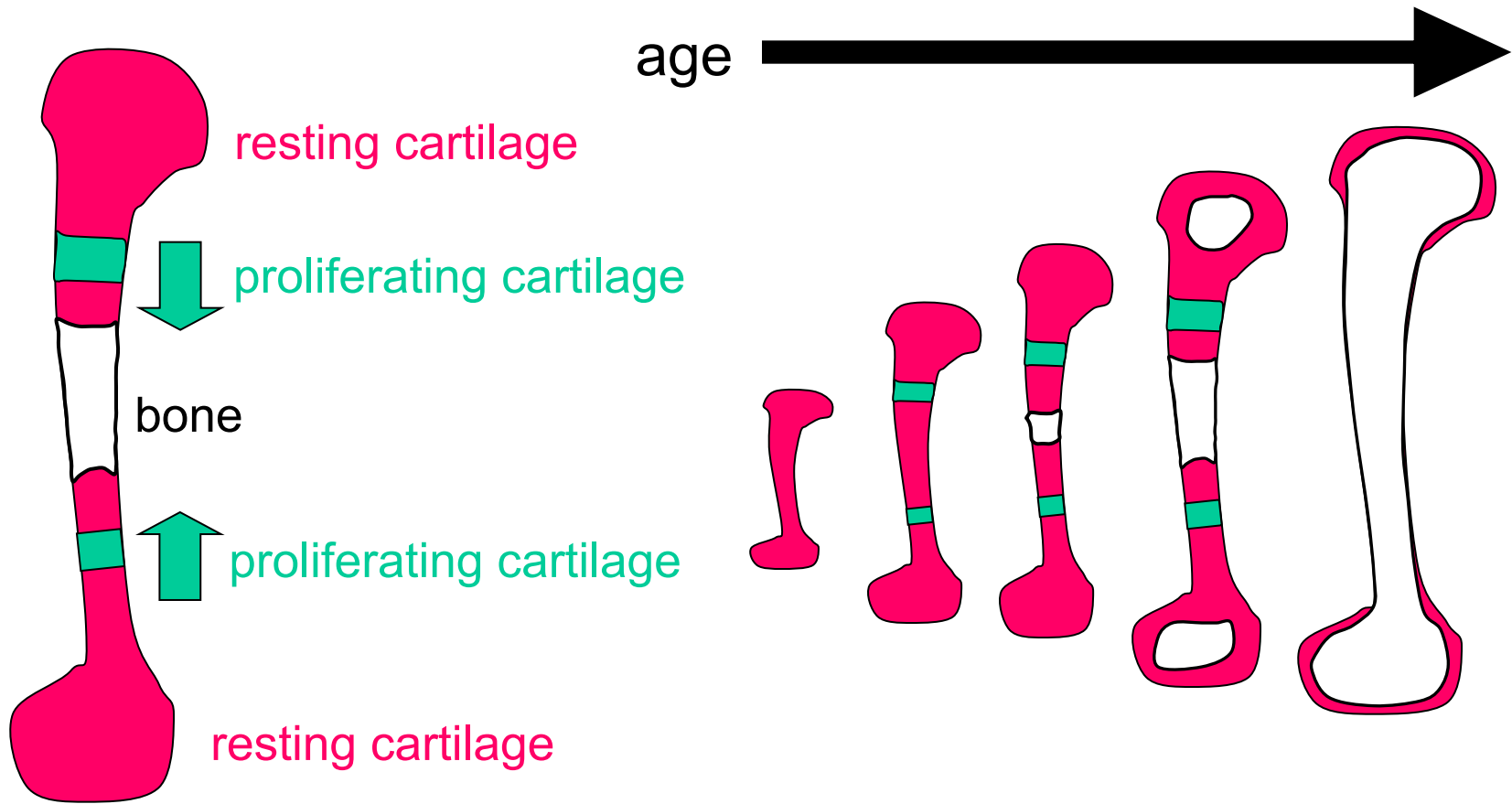
d *Fgf8* single KO HL



e *Fgf4; Fgf8* double KO HL



How do the limbs grow?



FGFR3



Tyrosine Kinase Group

Tyrosine Kinase Like Group

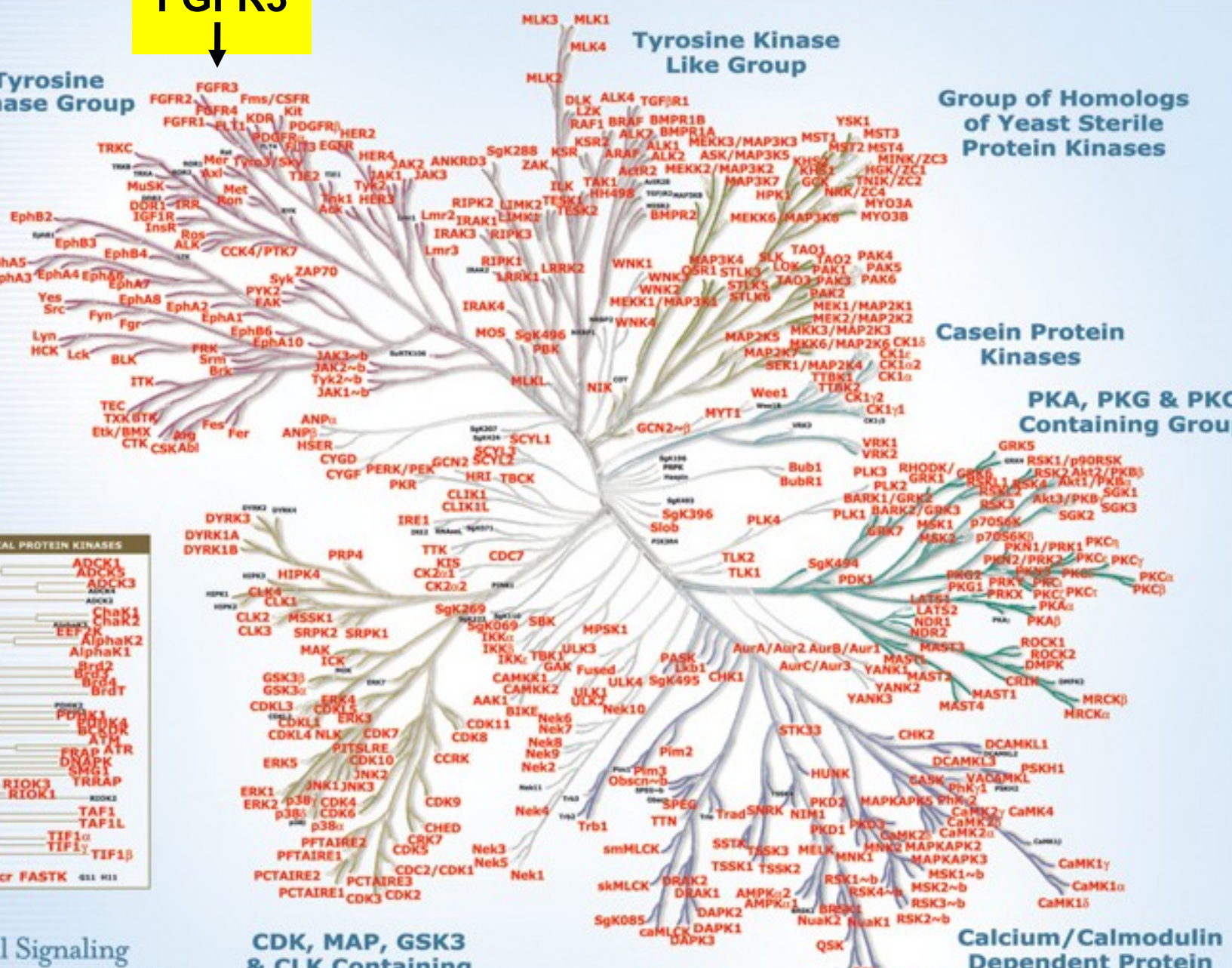
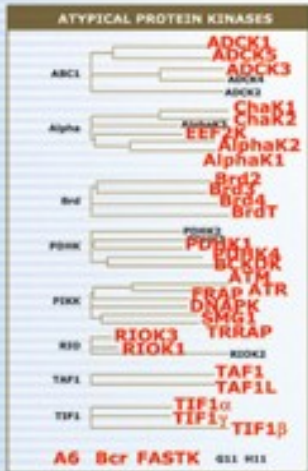
Group of Homologs of Yeast Sterile Protein Kinases

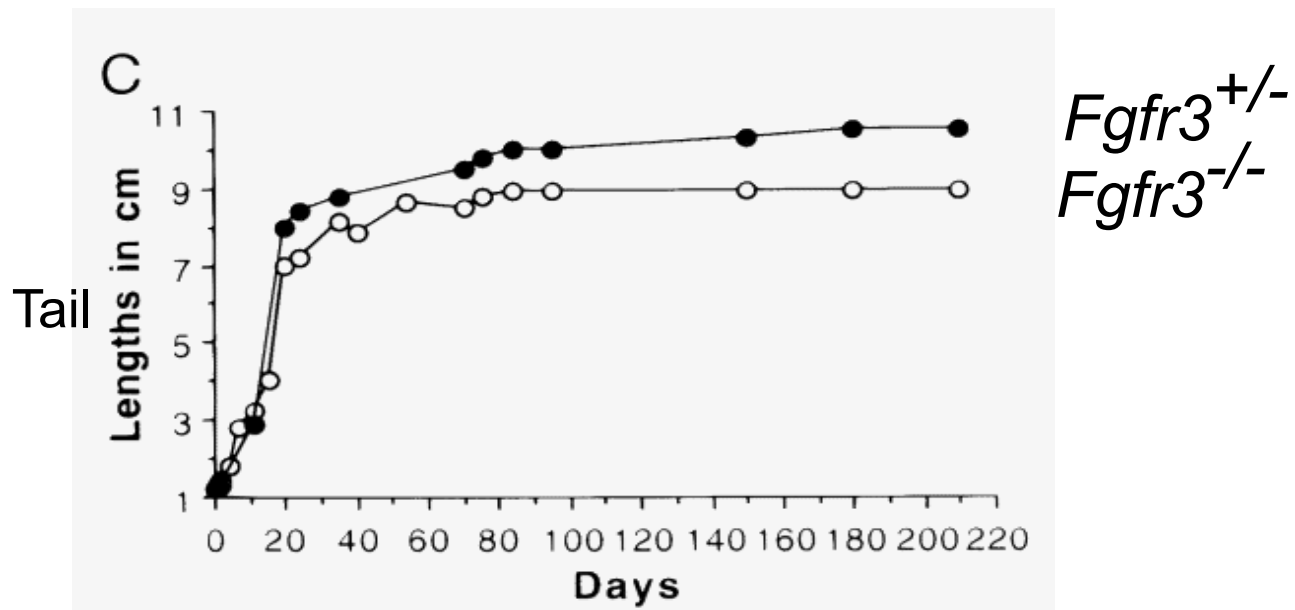
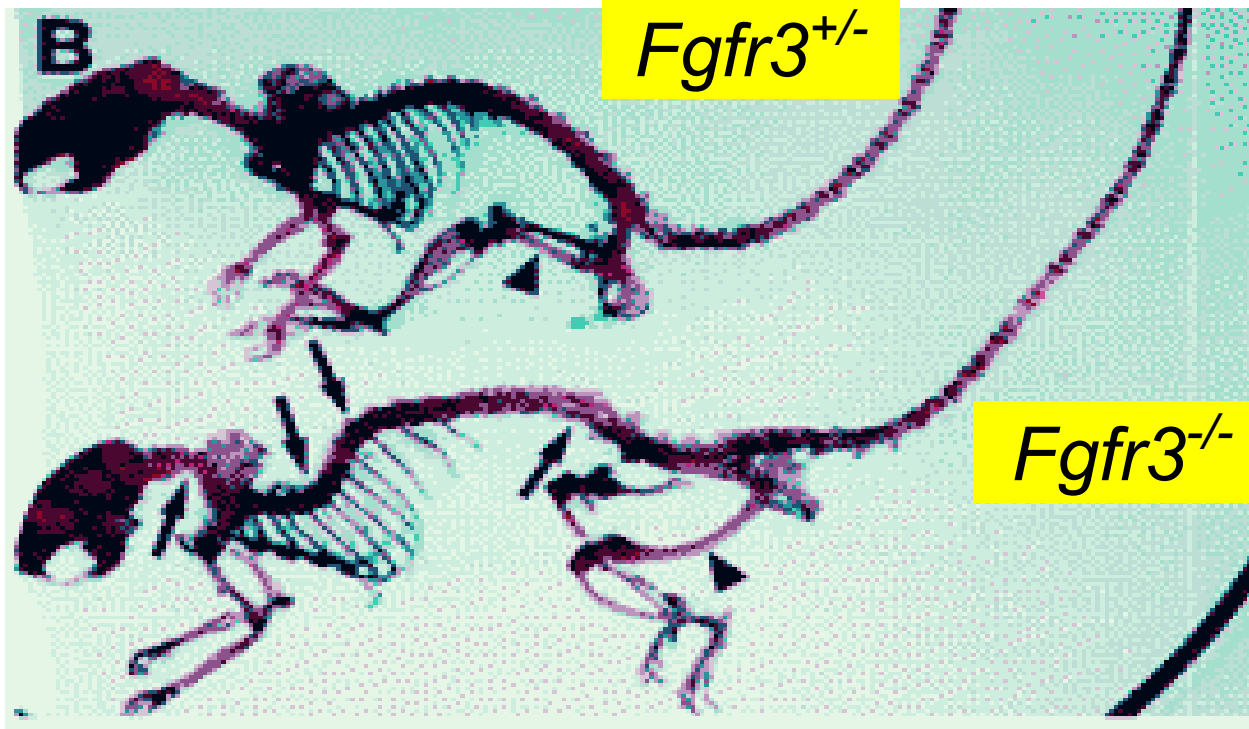
Casein Protein Kinases

PKA, PKG & PKC Containing Group

Calcium/Calmodulin Dependent Protein Kinase Group

CDK, MAP, GSK3 & CLK Containing Group





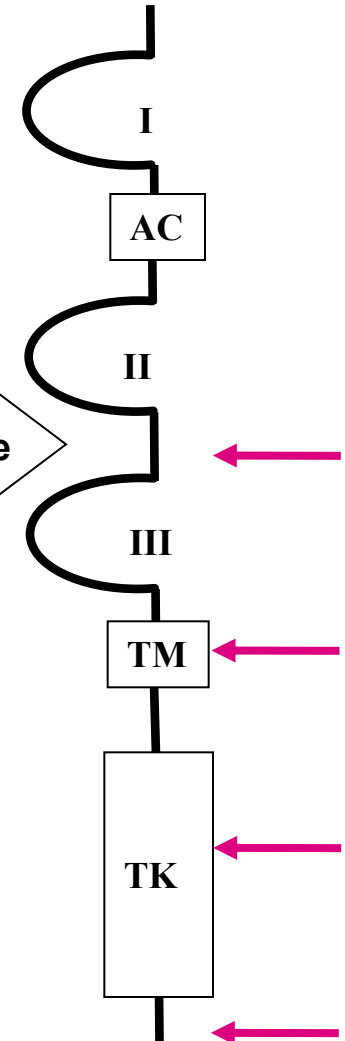
FGFR3-related skeletal dysplasia

Hypochondroplasia
Achondroplasia
SADDAN
Thanatophoric Dysplasia

STATURE



FGF binds here



FGFR3-related skeletal dysplasia



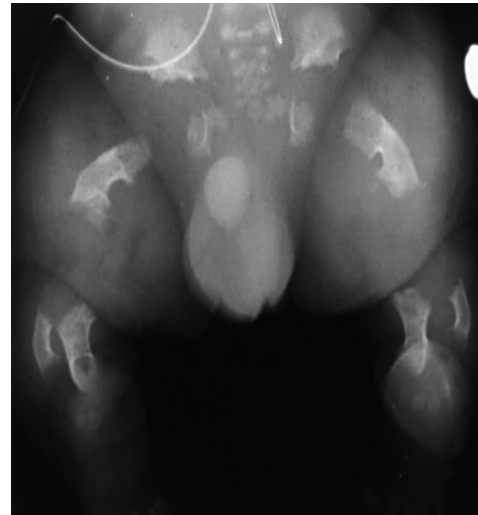
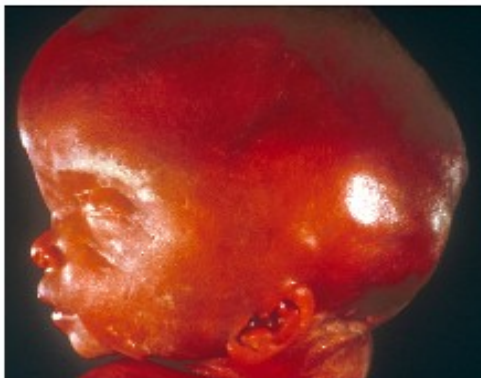
Achondroplasia

Thanatophoric Dysplasia



healthy

- short long bones
- brachydactyly
- macrocephaly



TD

- low nasal bridge
- spinal stenosis
- temporal lobe malformations

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

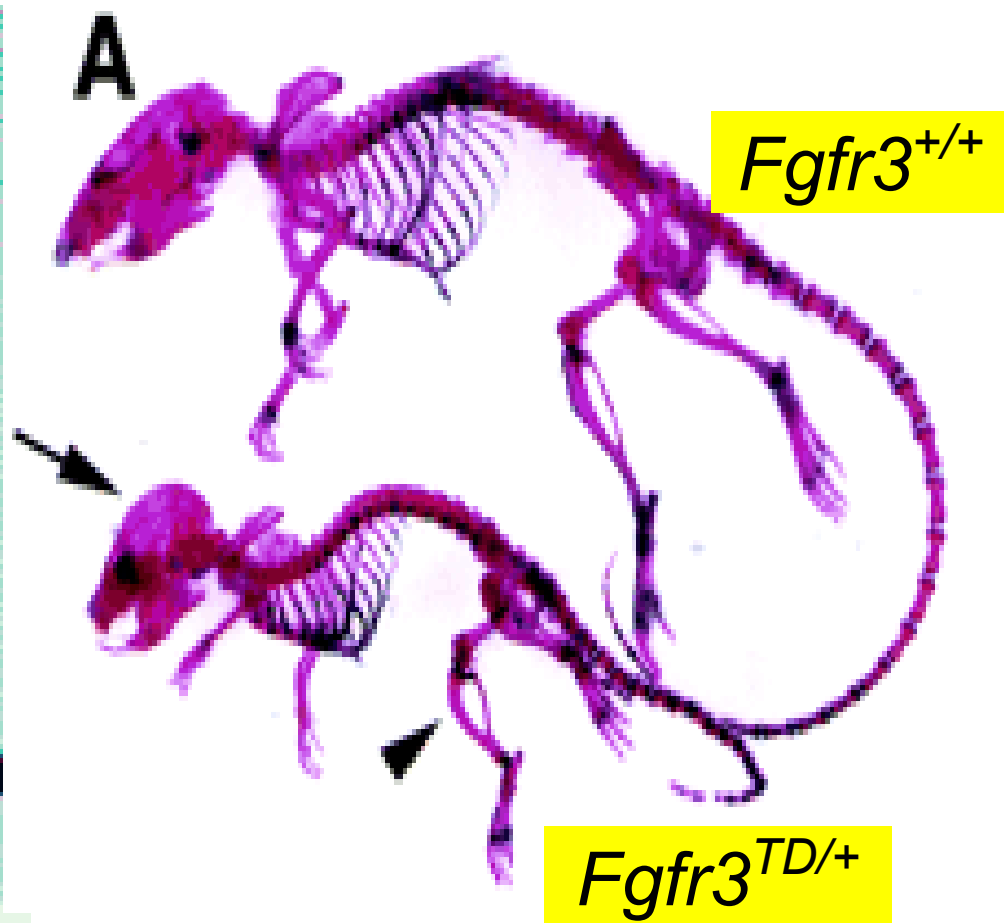
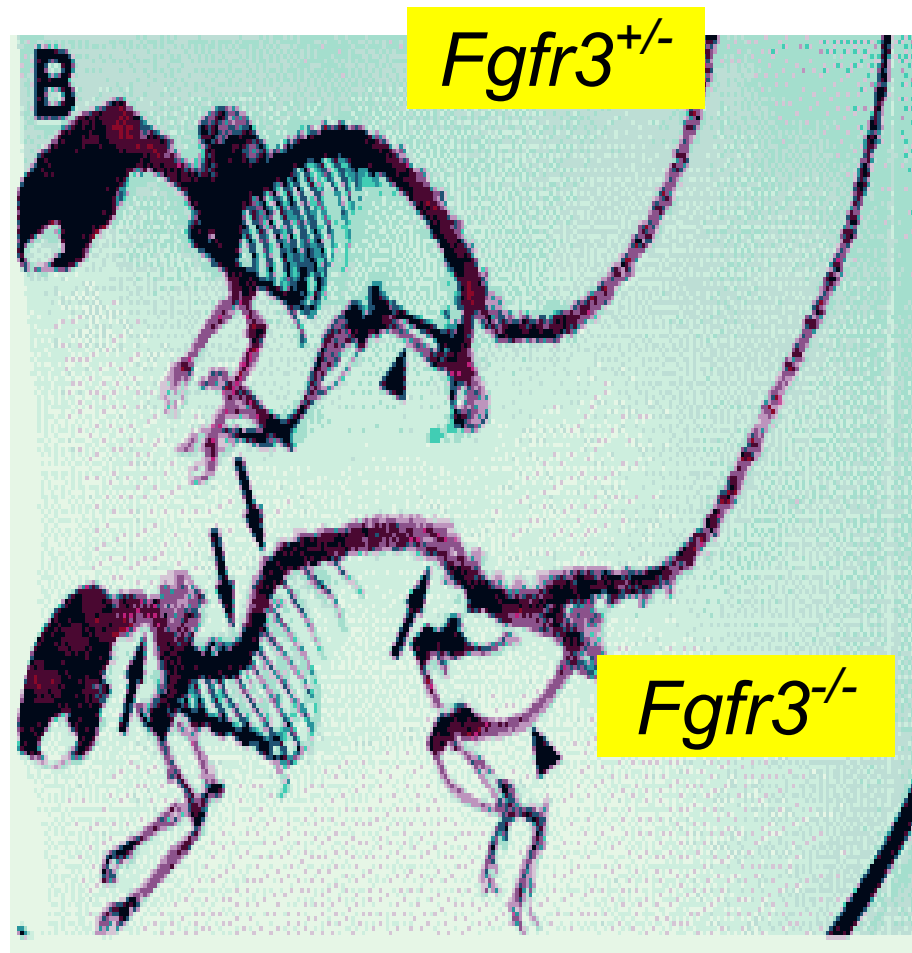
Heidi G. Parker,¹ Bridgett M. VonHoldt,² Pascale Quignon,¹ Elliott H. Margulies,³ Stephanie Shao,¹ Dana S. Mosher,¹ Tyrone C. Spady,¹ Abdel Elkhouloun,¹ Michele Cargill,^{4*} Paul G. Jones,⁵ Cheryl L. Maslen,⁶ Gregory M. Acland,^{7,8} Nathan B. Sutter,⁸ Keiichi Kuroki,⁹ Carlos D. Bustamante,¹⁰ Robert 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).

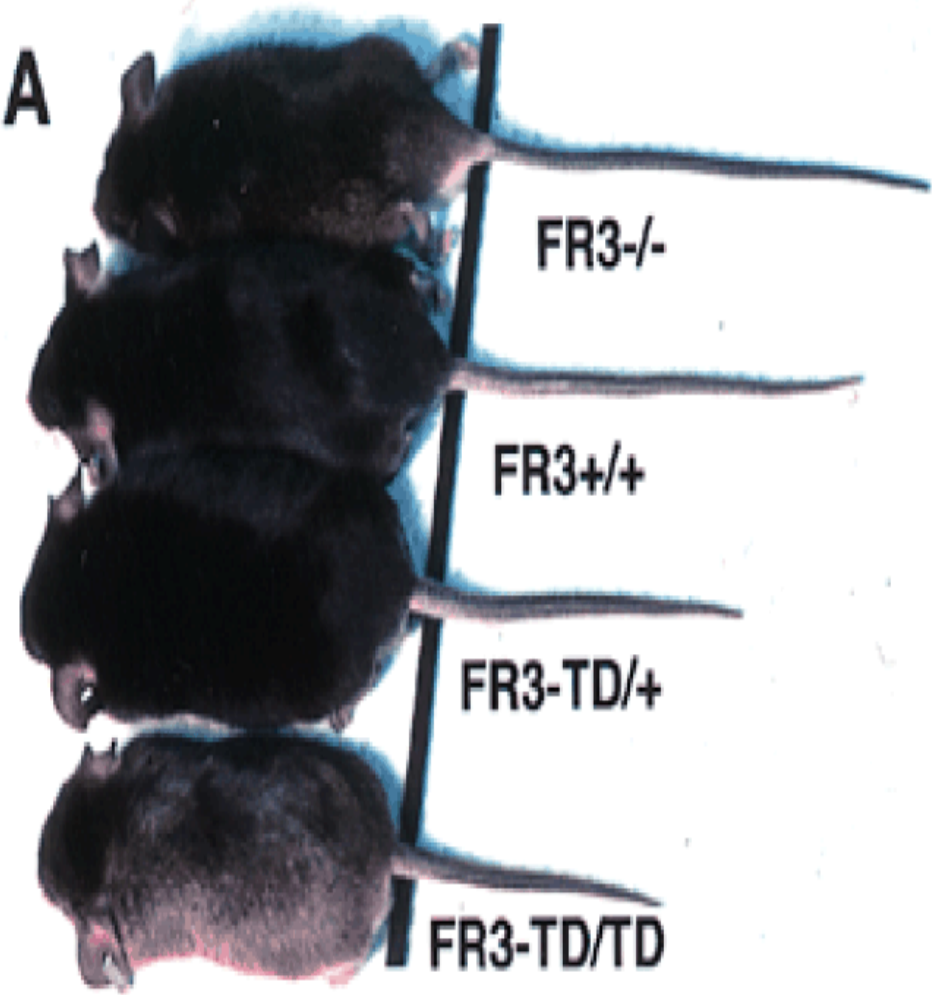
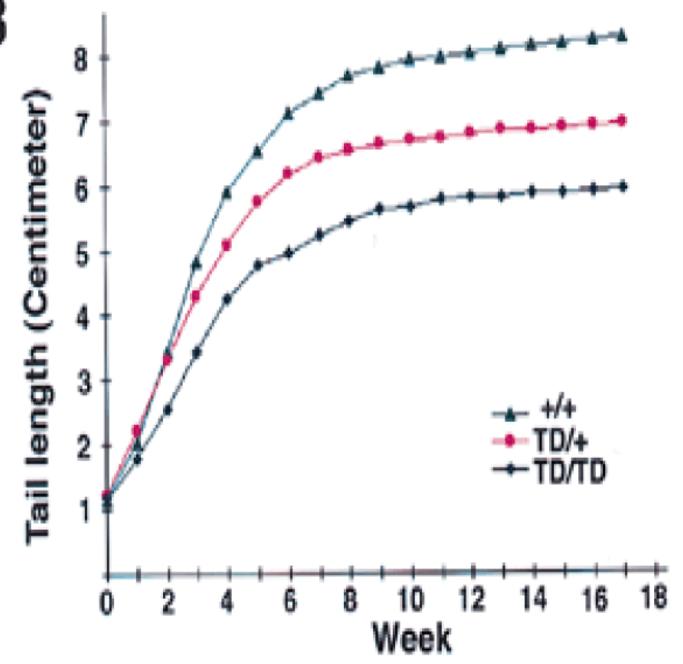
To identify the genetic foundations of breed-defining phenotypes such as canine chondrodysplasia, we developed a multibreed approach for mapping fixed canine traits. A total of 835 dogs from 76 distinct breeds that provided maximal coverage of phenotypic variation were genotyped by using the Affymetrix 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 breed standard (8, 10) and consisted of 111

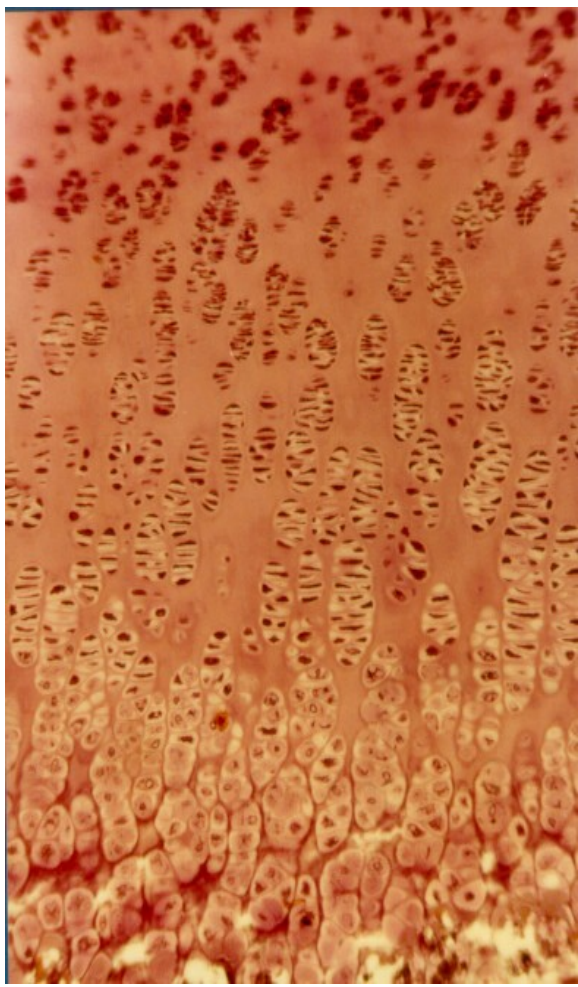
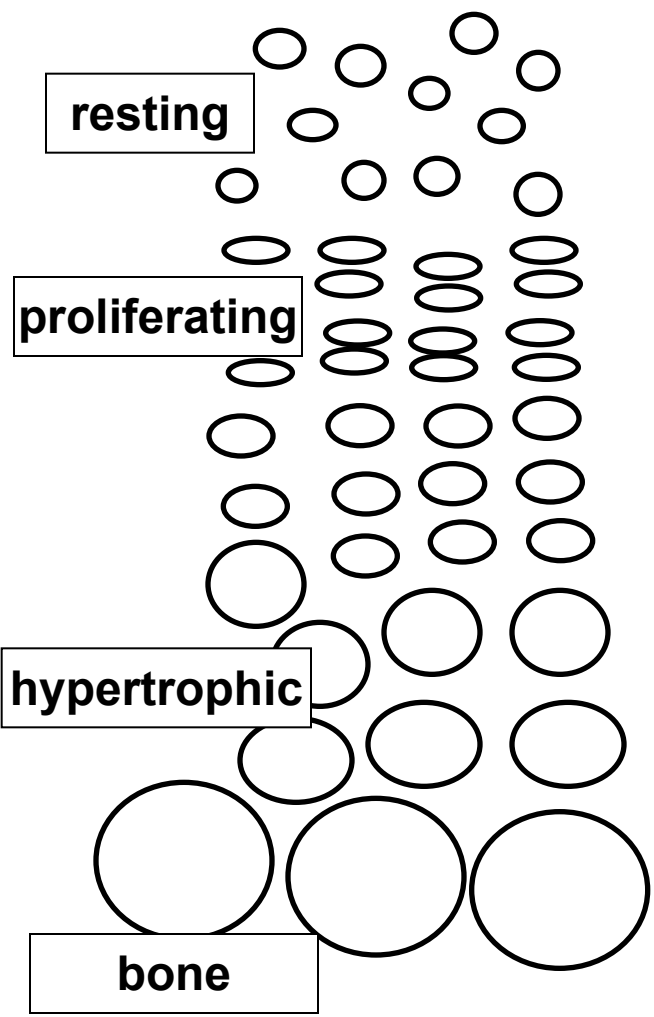




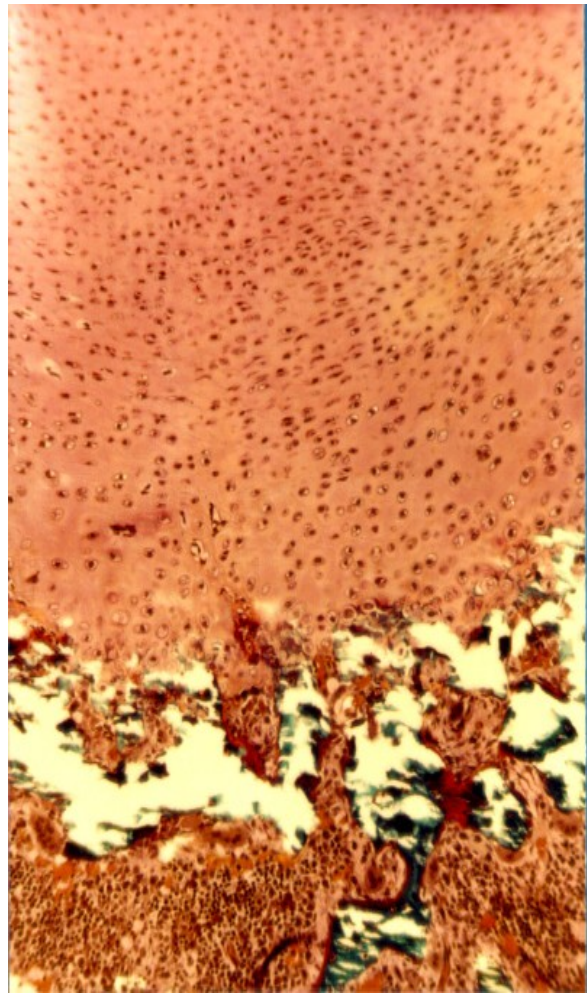
Loss-of-function

vs. Gain-of-function

A**B**

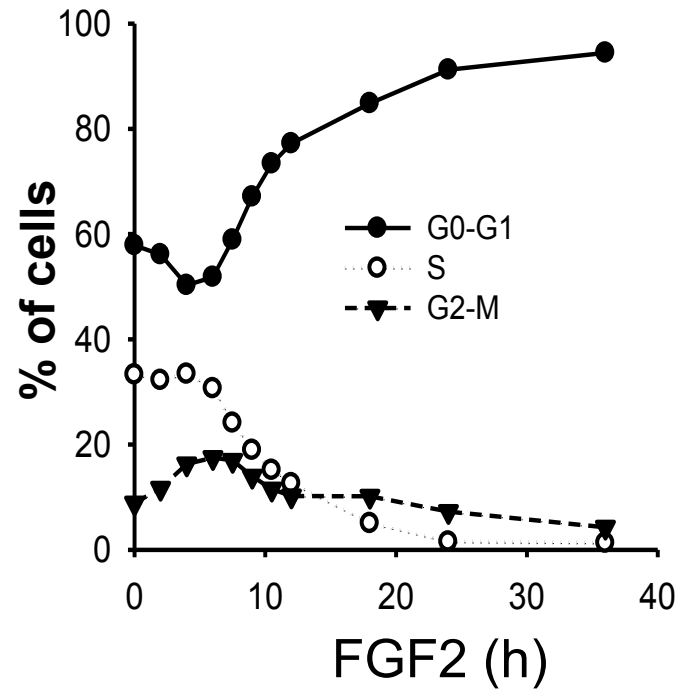
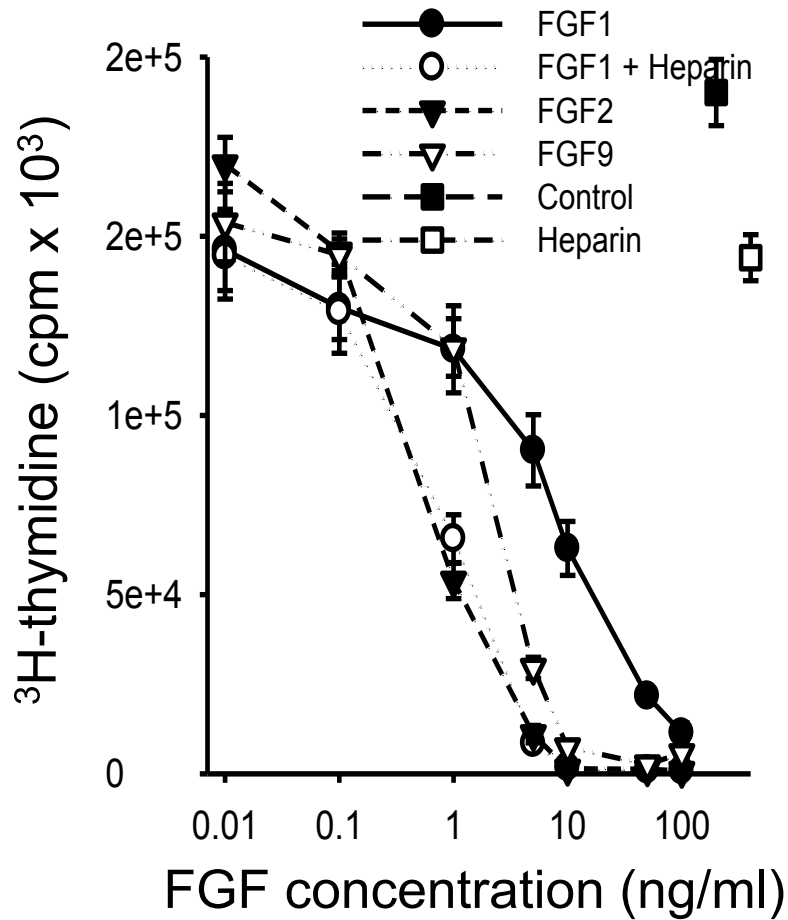


healthy

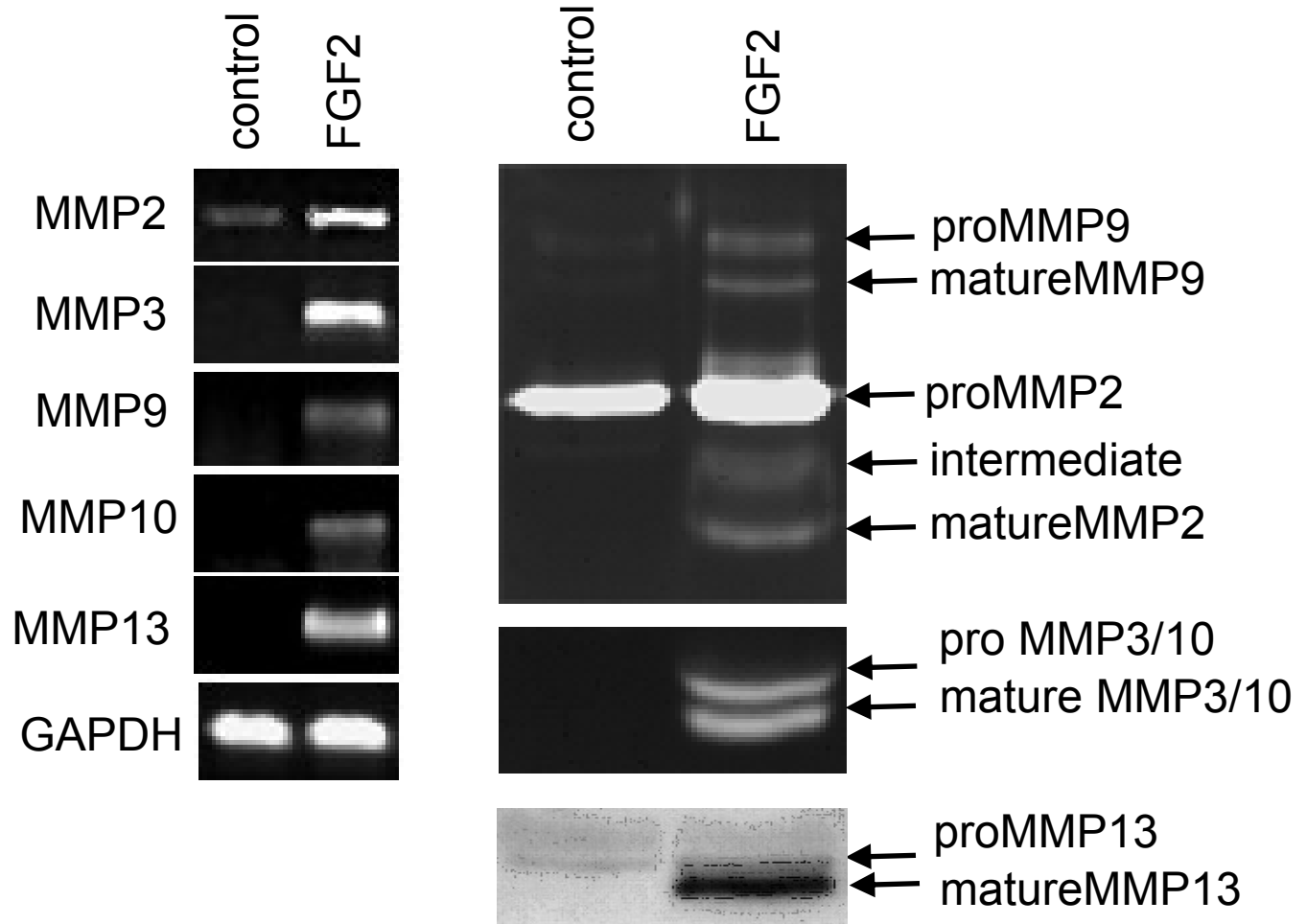


TD

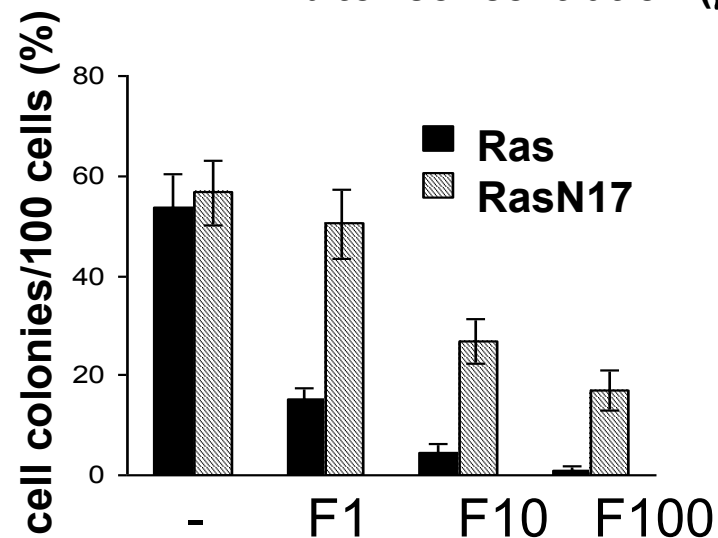
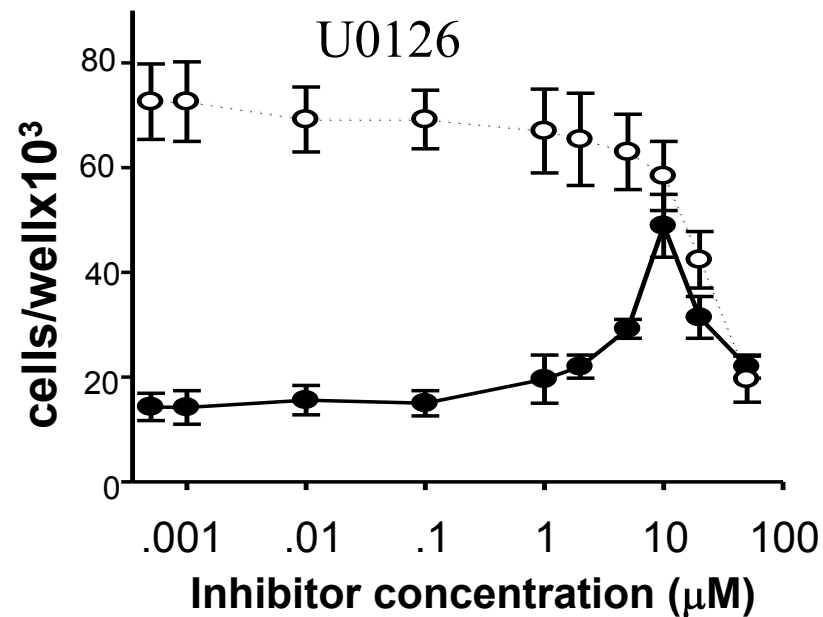
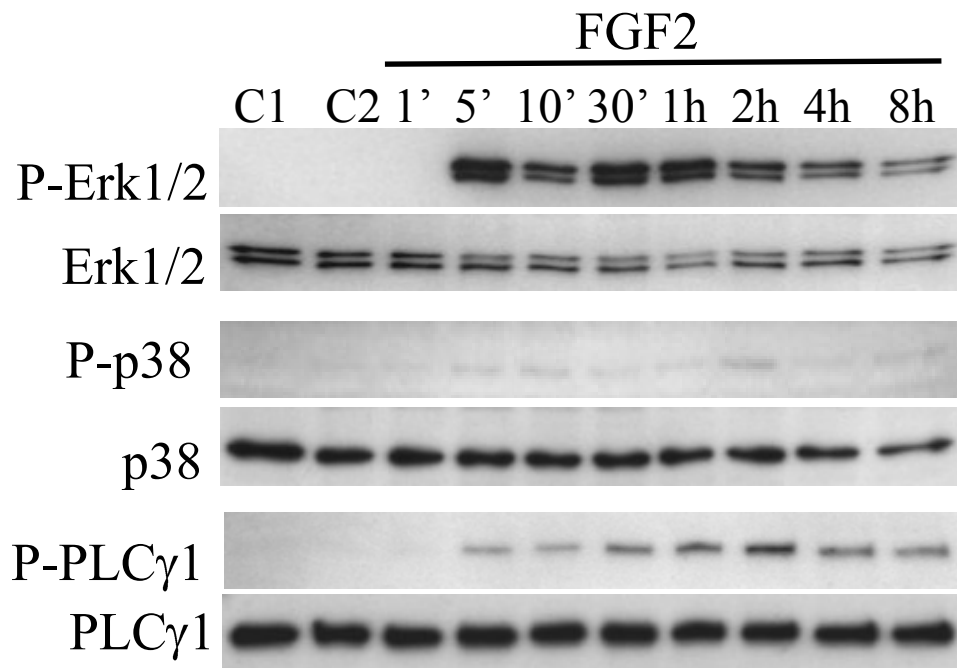
FGFR3 inhibits chondrocyte proliferation by arresting their cell cycle in G1 phase

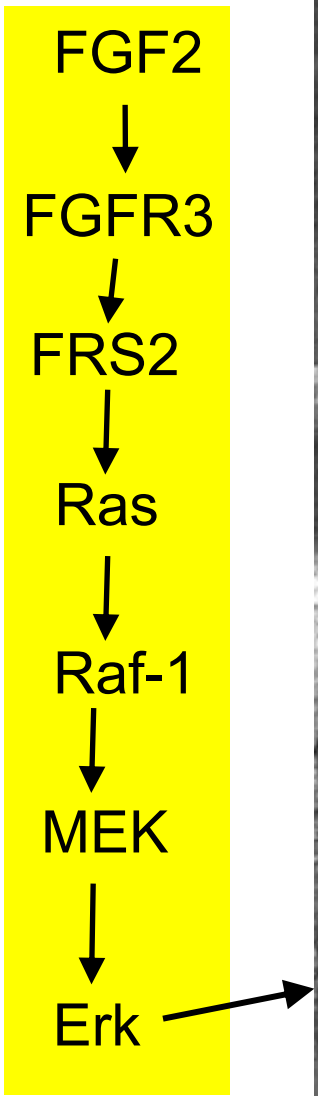


.....via MMP-mediated degradation of extracellular matrix

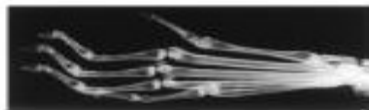


FGFR3 inhibits cartilage growth via Erk pathway



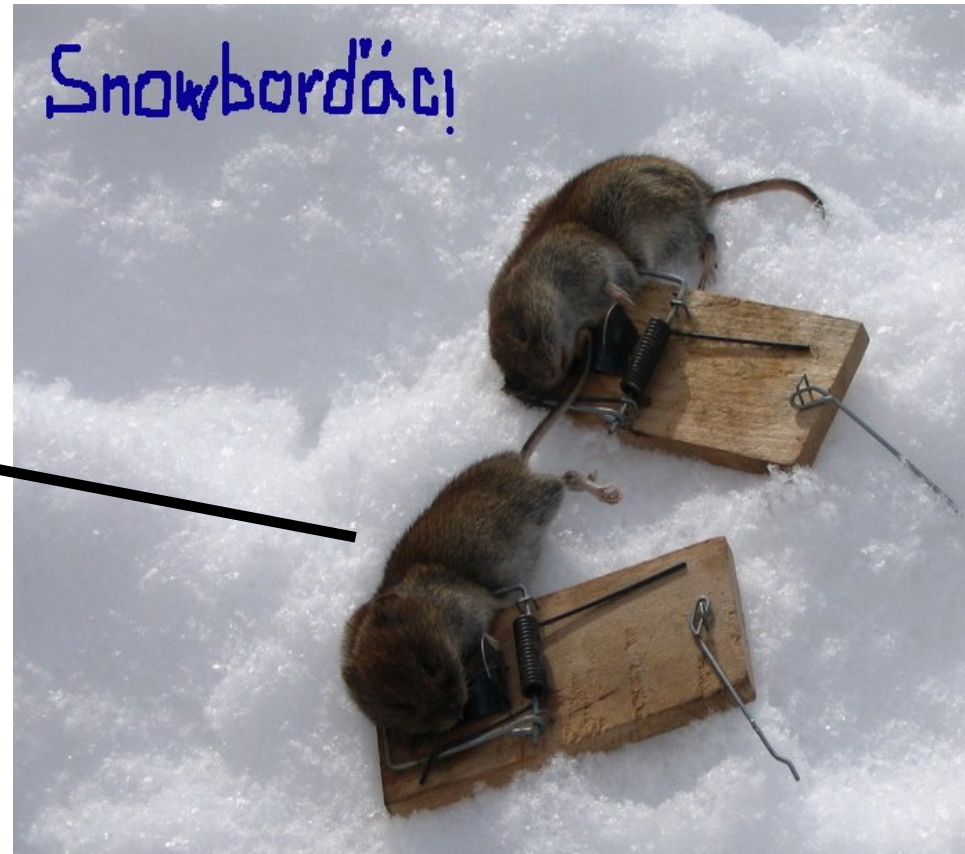


C-type Natriuretic Peptide (CNP) over-expression results in skeleton overgrowth in mice



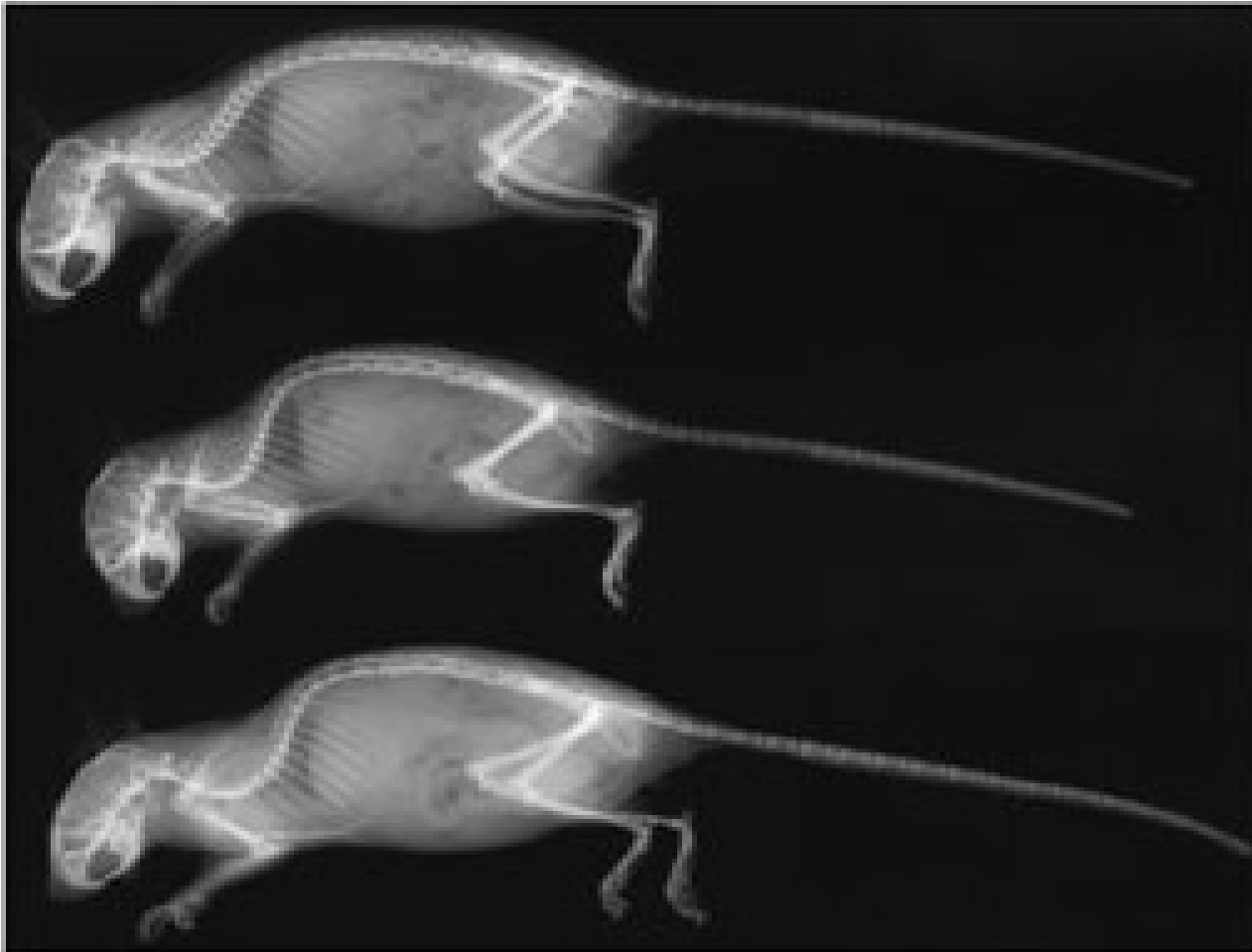
wild-type

CNP ↑



CNP over-expression???

CNP rescues dwarfism caused by ACH mutation in FGFR3

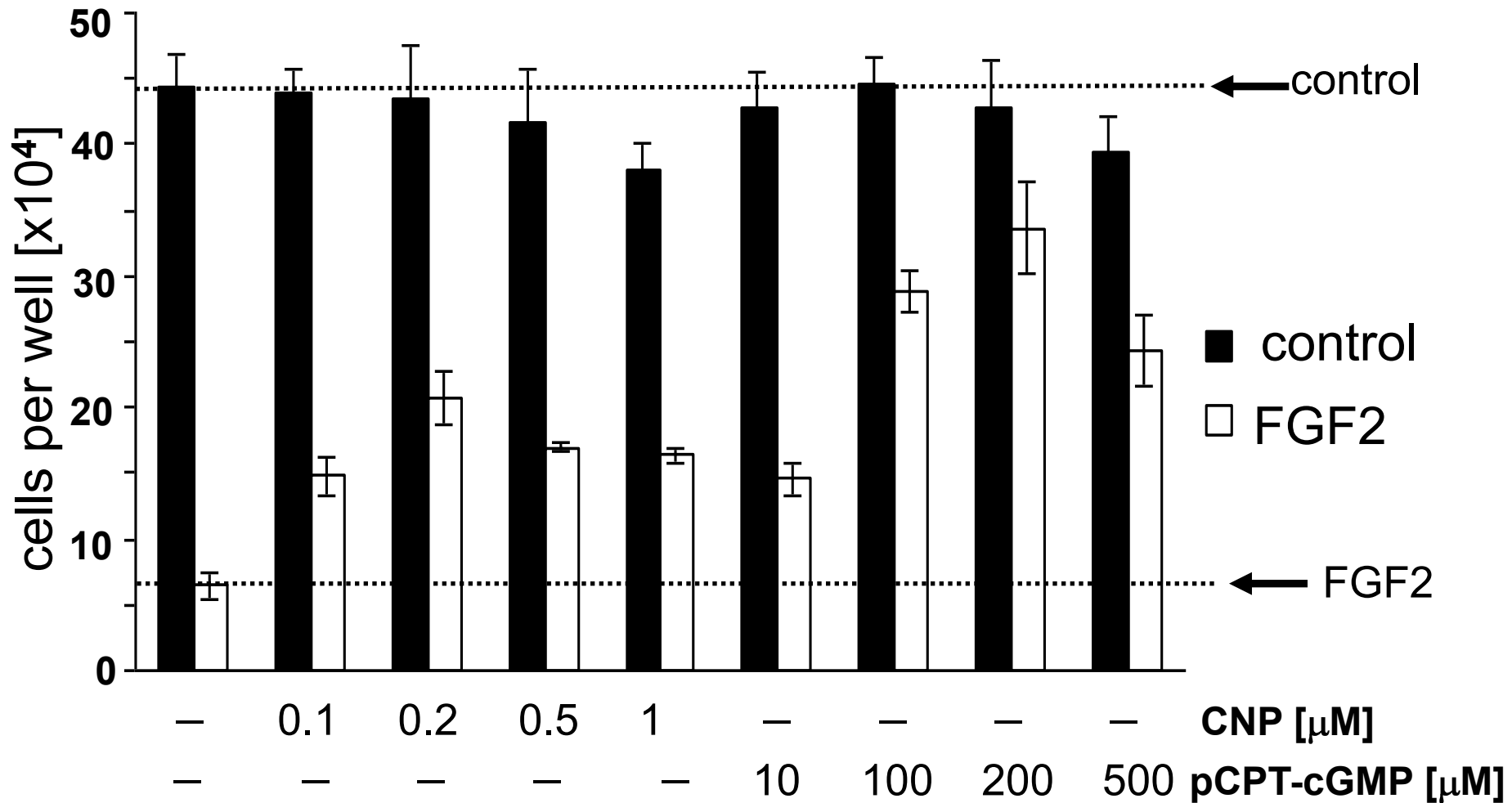


wild-type

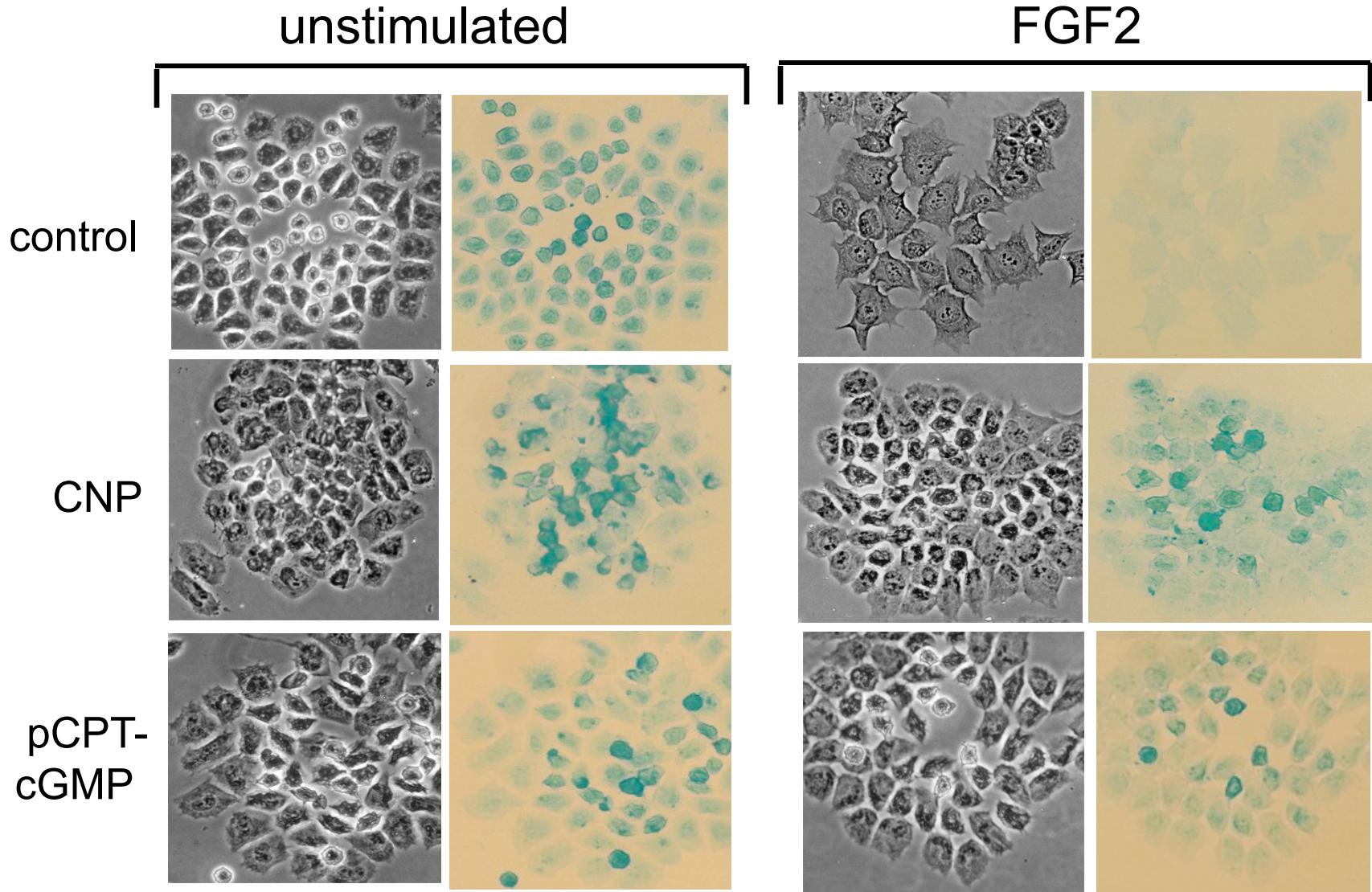
$Fgfr3^{Ach}$

$Fgfr3^{Ach/CNP}$ ↑

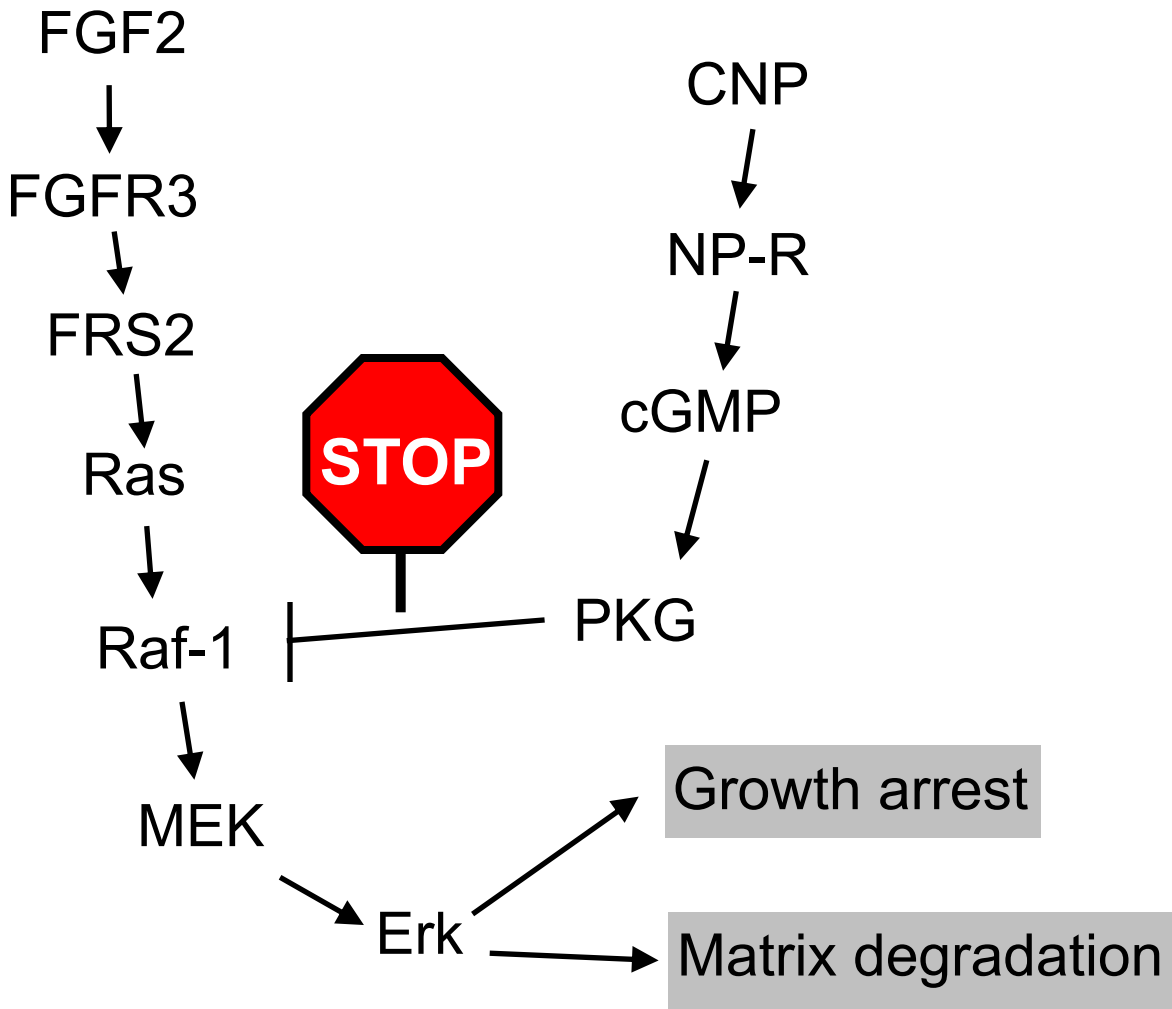
CNP counteracts FGF2-mediated chondrocyte growth arrest through cGMP-dependent pathway



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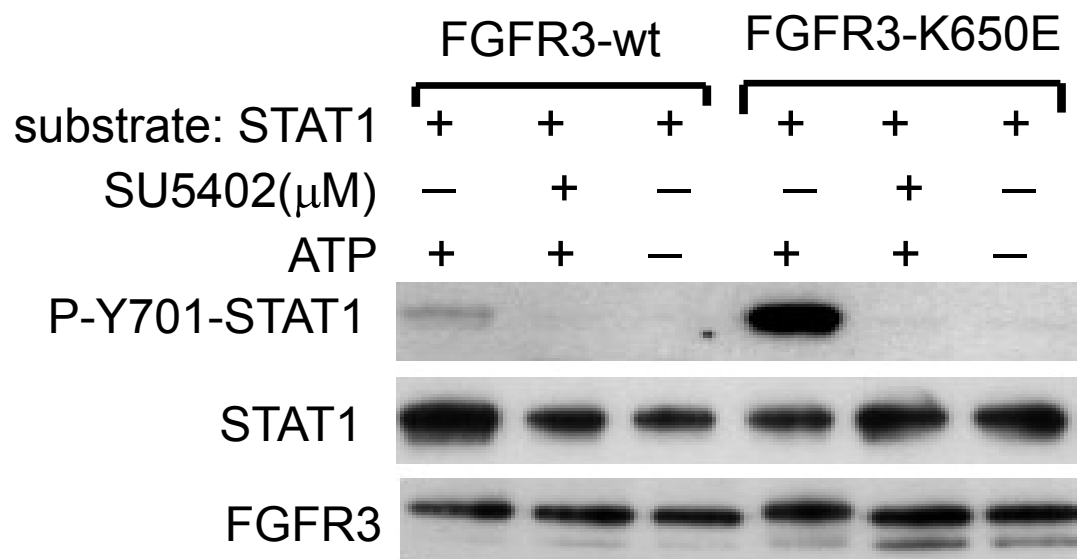
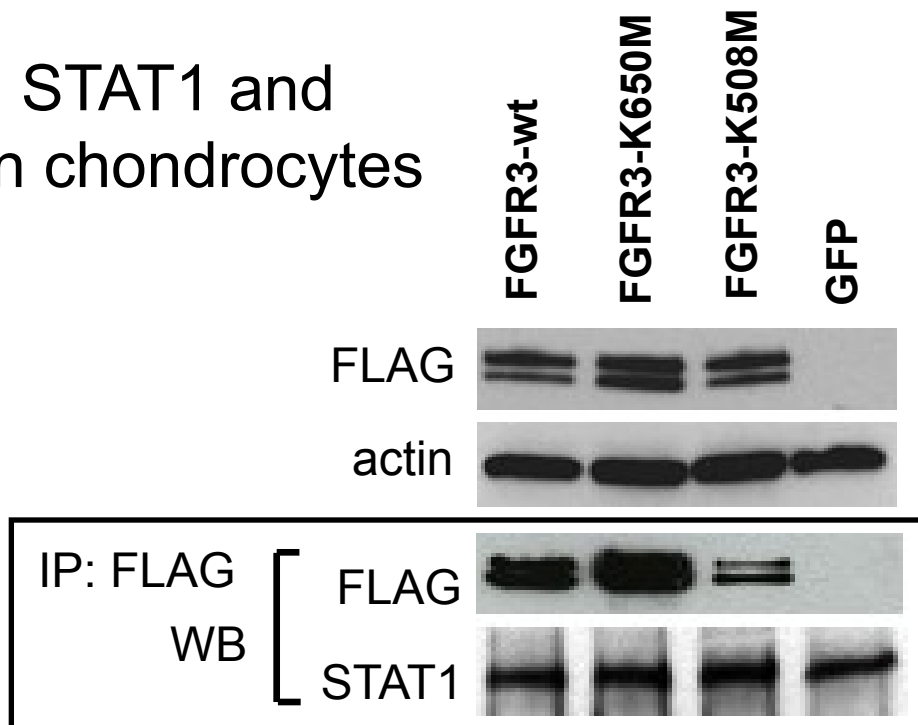
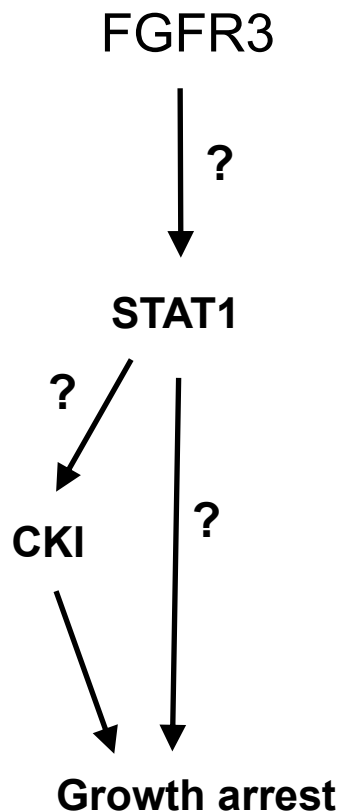
BioMarin Announces Program for BMN-111 for the Treatment of Achondroplasia

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

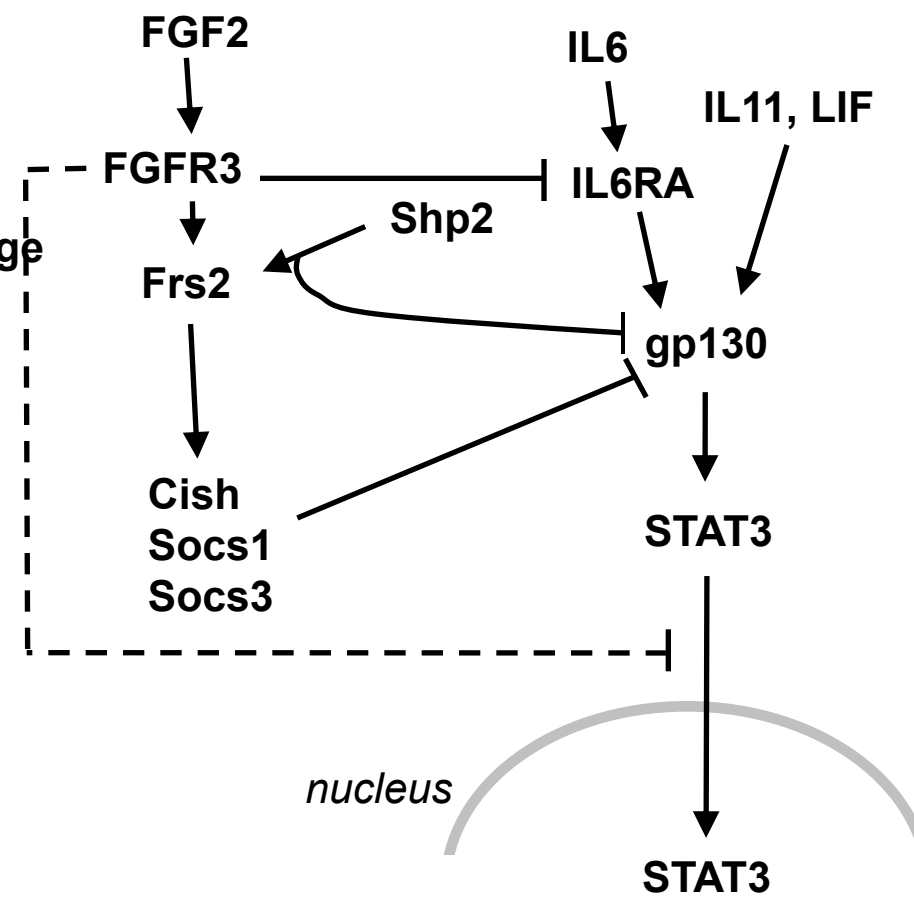
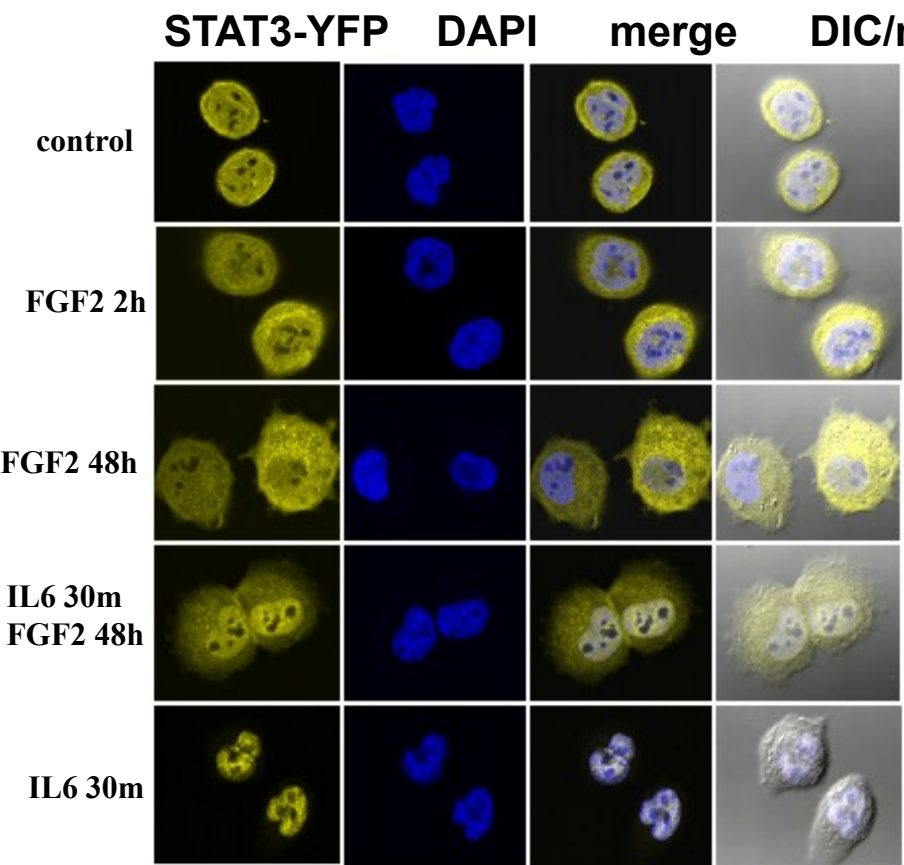
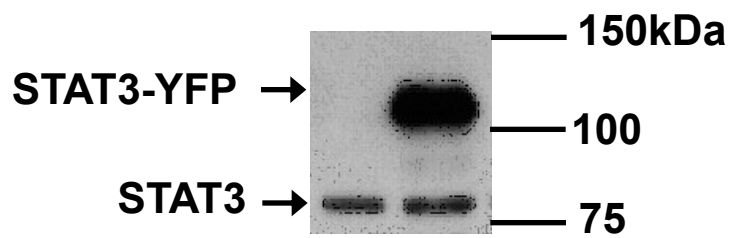
BioMarin Pharmaceutical Inc. (Nasdaq: BMRN) today announced its program for BMN-111, a peptide therapeutic for the treatment of achondroplasia. BioMarin plans to file an IND in the fourth quarter of 2011 and to initiate a Phase 1 clinical trial by the first quarter of 2012.

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.

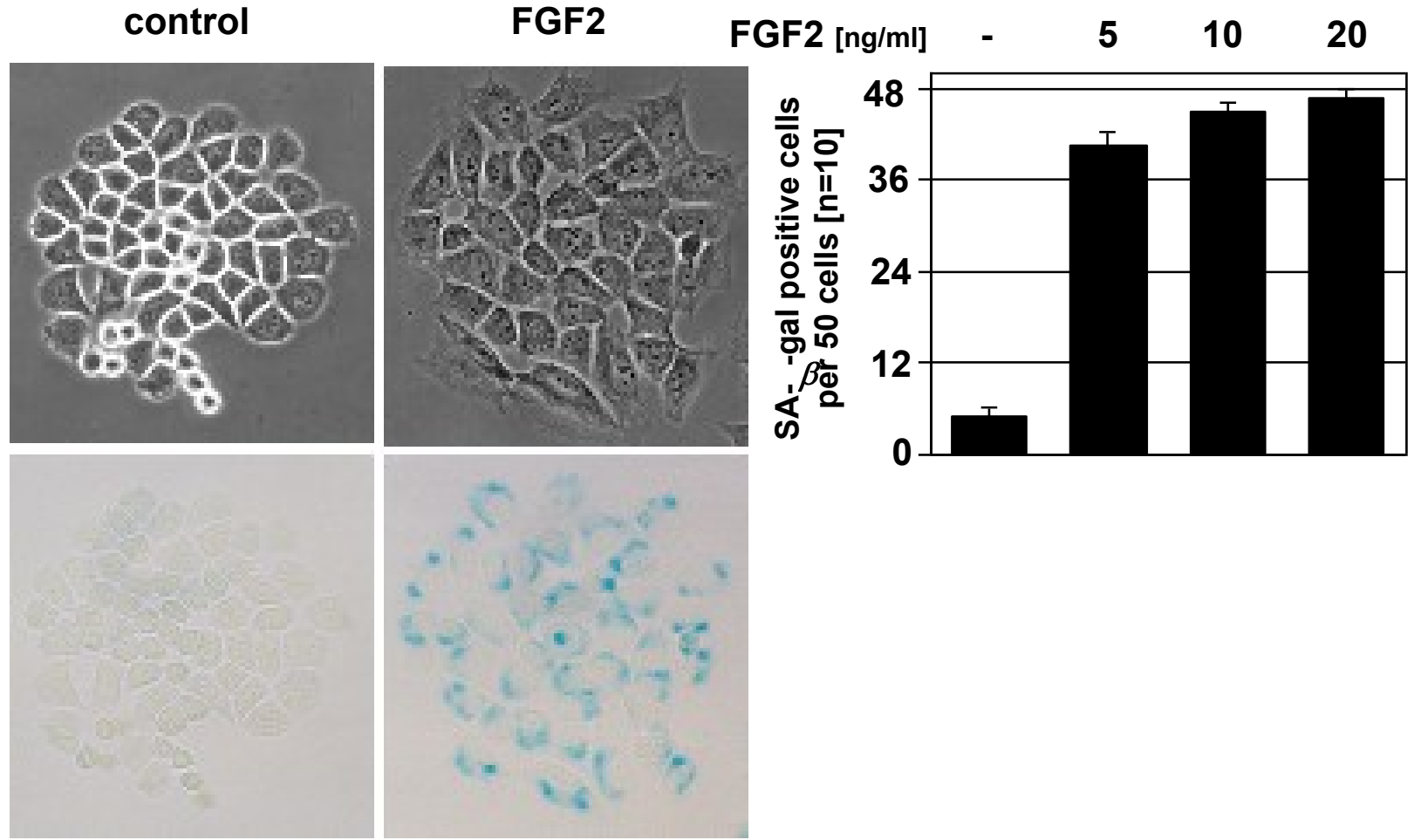
FGFR3 associates with STAT1 and acts as STAT1-kinase in chondrocytes



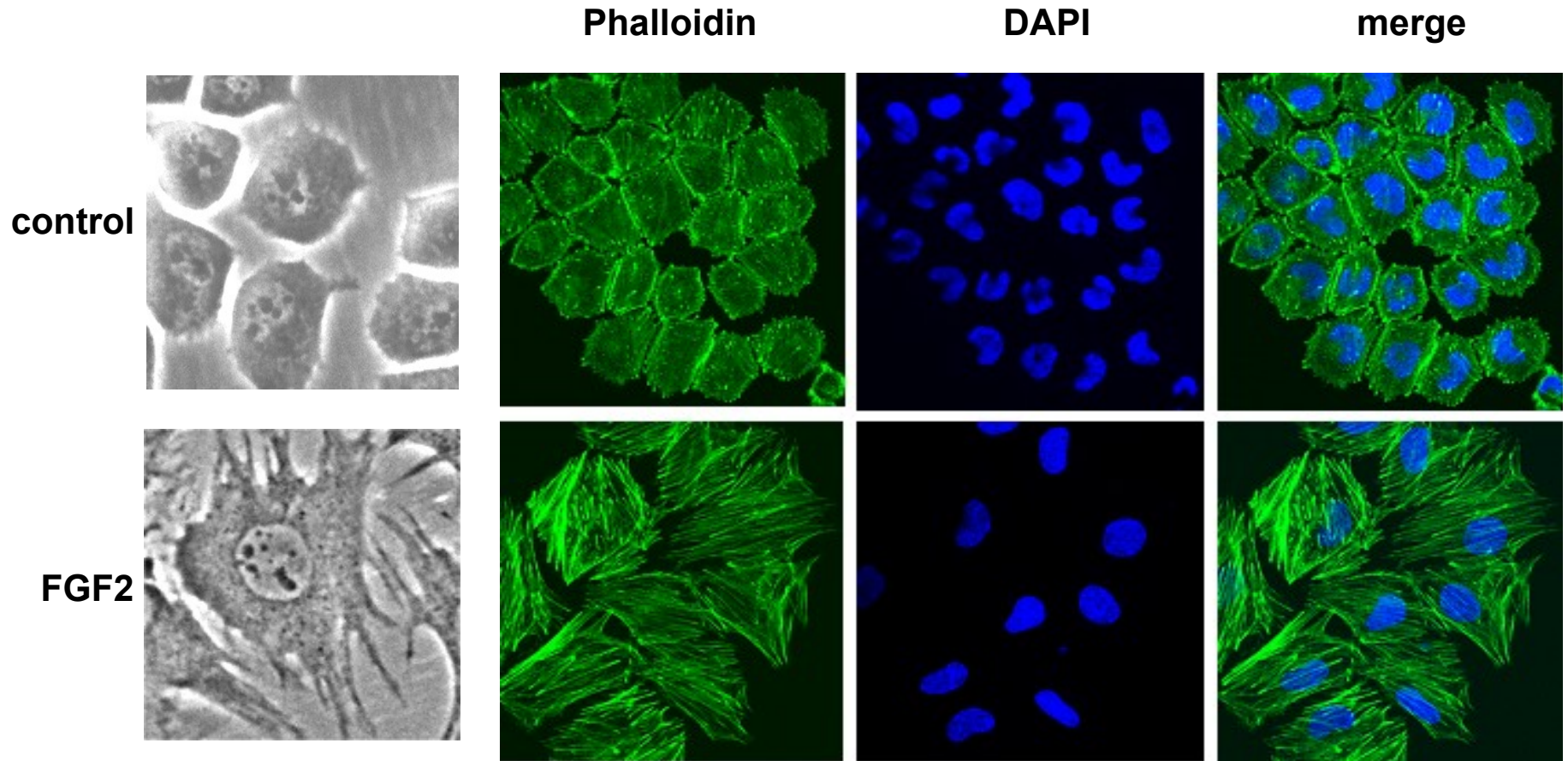
Chronic FGF stimulus inhibits cytokine/STAT signaling in chondrocytes



FGFR3 causes premature senescence in chondrocytes



FGFR3 signals towards the cytoskeleton in chondrocytes



FGFR3 signals via Wnt pathway in chondrocytes

FGF2: C1 15' 1h 3h 6h 12h 24h 48h 72h C2 C3

