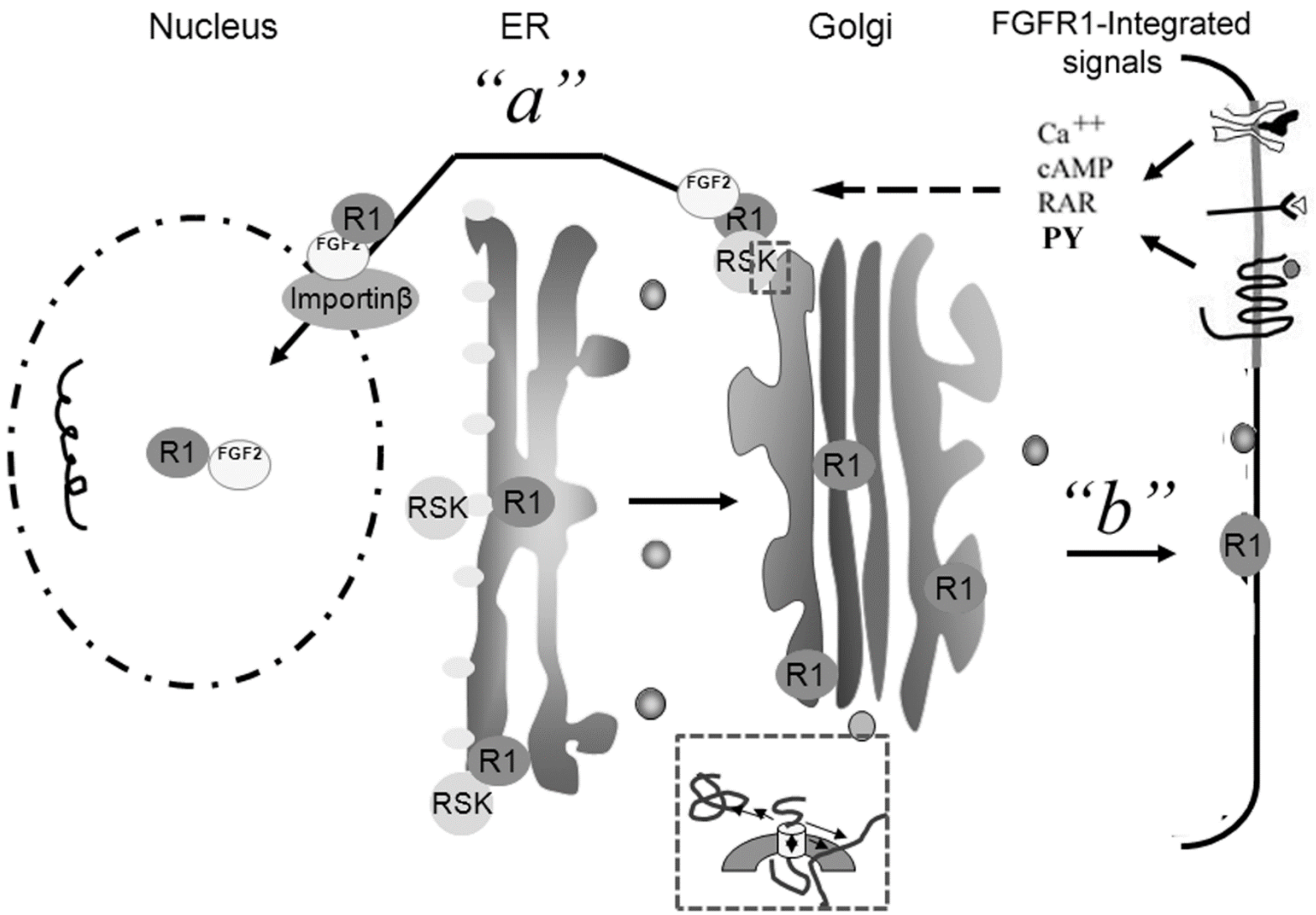
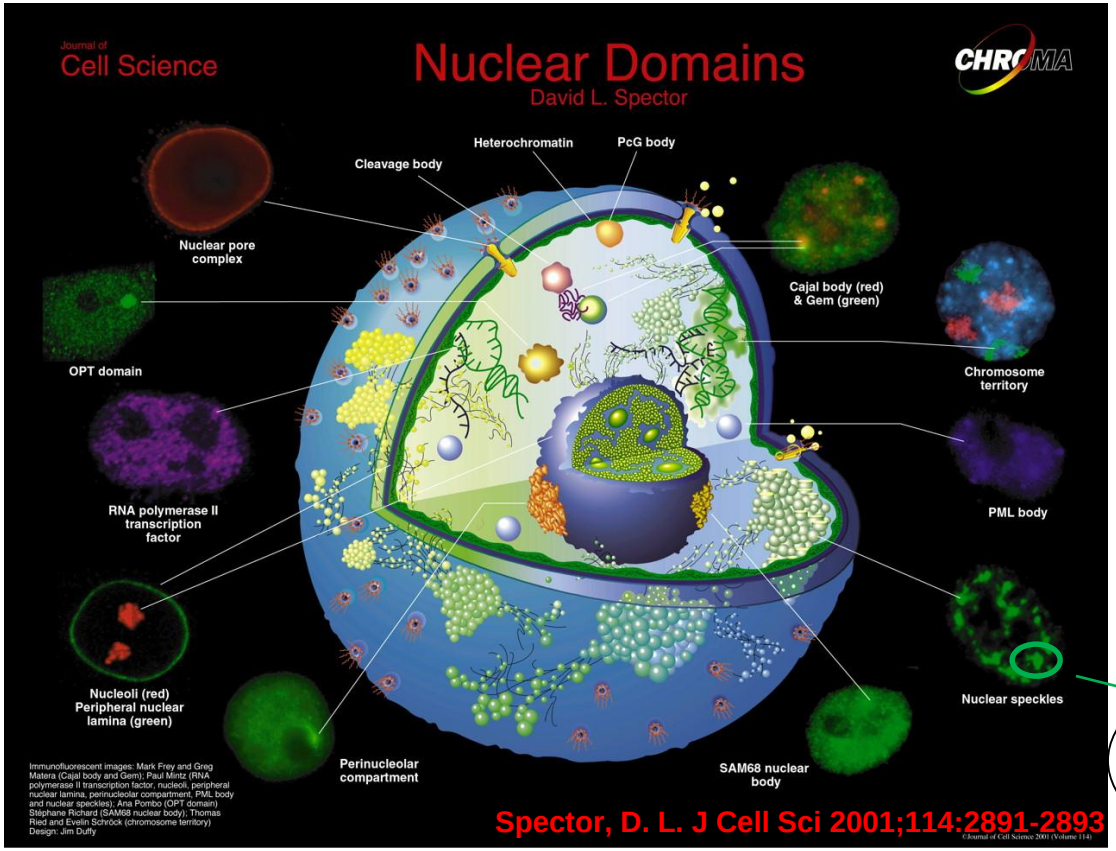
nuclear FGFR1 (R1) trafficking by RSK1,FGF & Importin - Integrative Signaling Mechanism

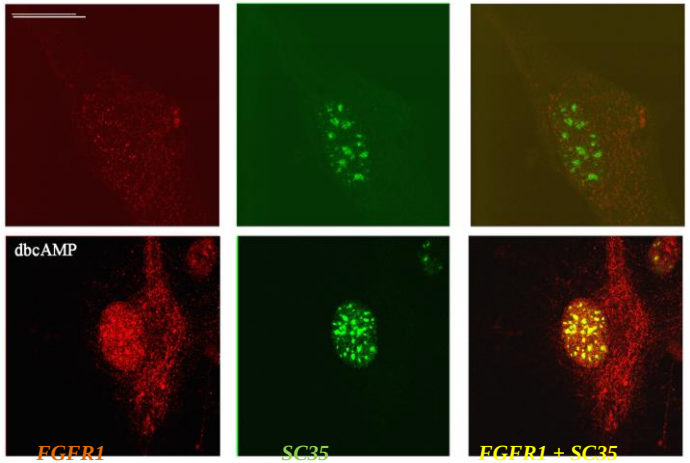


INFS – Global Genome Programmer
Lecture 8

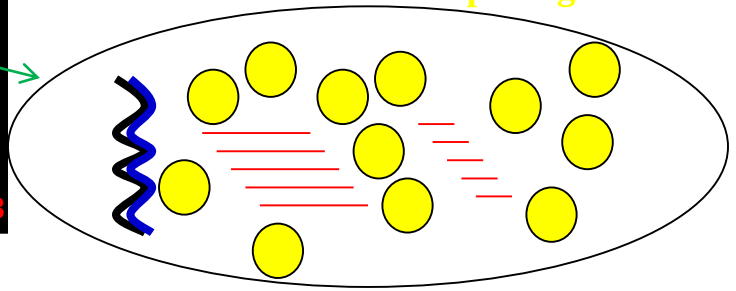
FGFR1 concentrates in Nuclear Bodies (“speckles”)

- The cell nucleus contains nearly 30,00 genes and complex machineries for their replication and expression.
- These processes appear to take place in specialized subnuclear domains (diverse nuclear bodies).

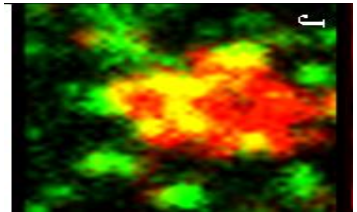




DNA transcription and RNA processing co-occur in nuclear domains rich in splicing factors.



RNA PolII

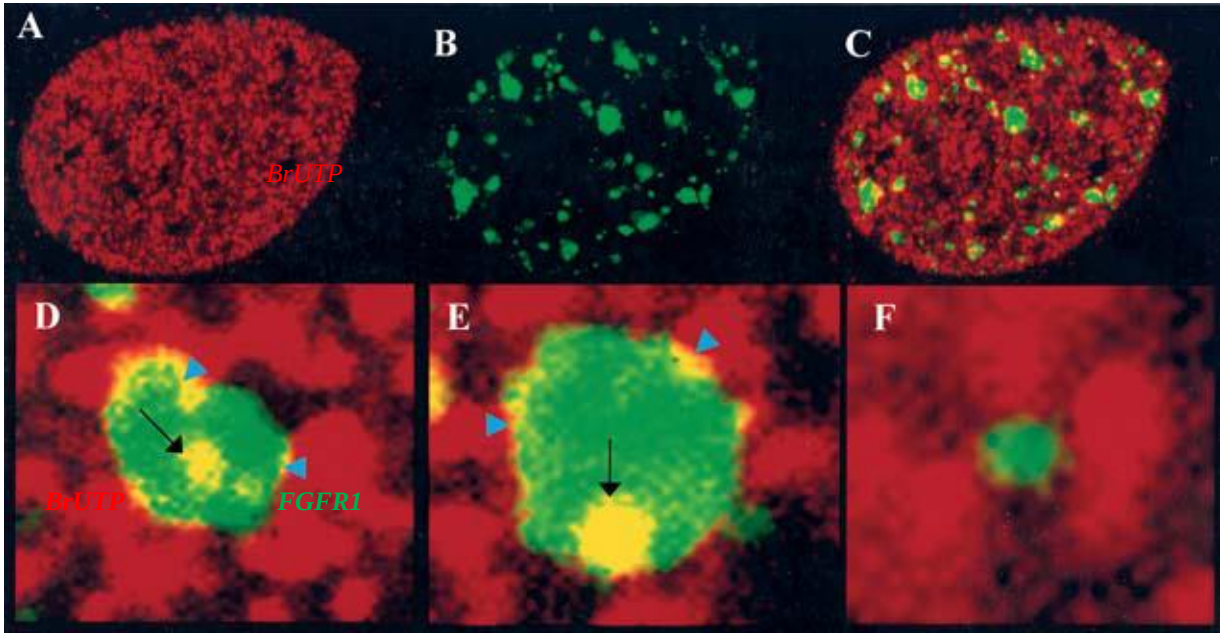


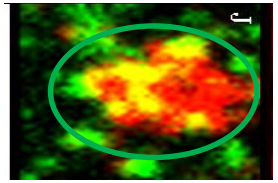
FGFR1

Does nuclear FGFR1 signal directly to genome?

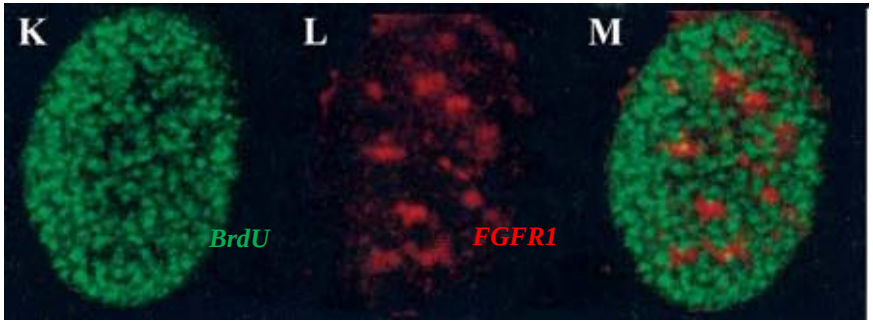
FGFR1 colocalizes with transcription sites:

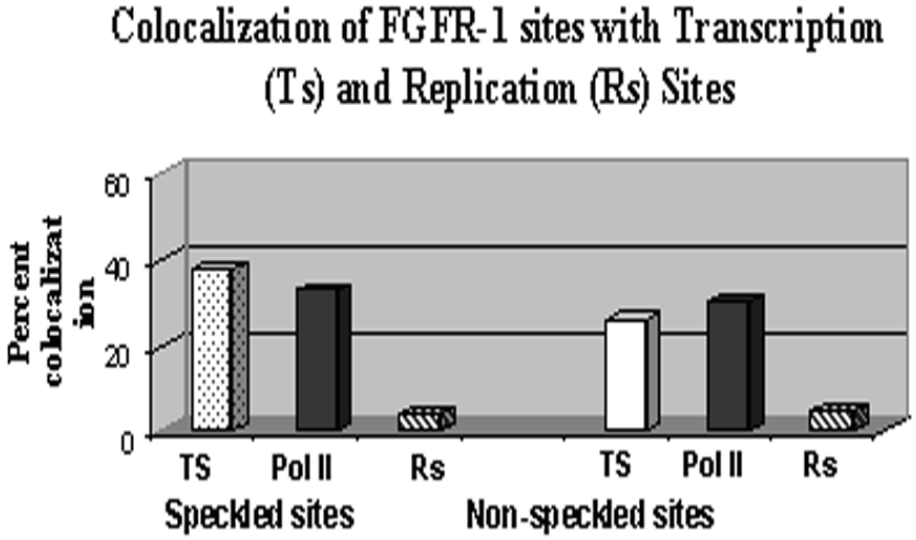
FGFR1, RNA PolII



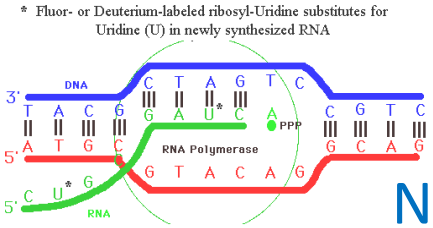


…but not with replication sites:

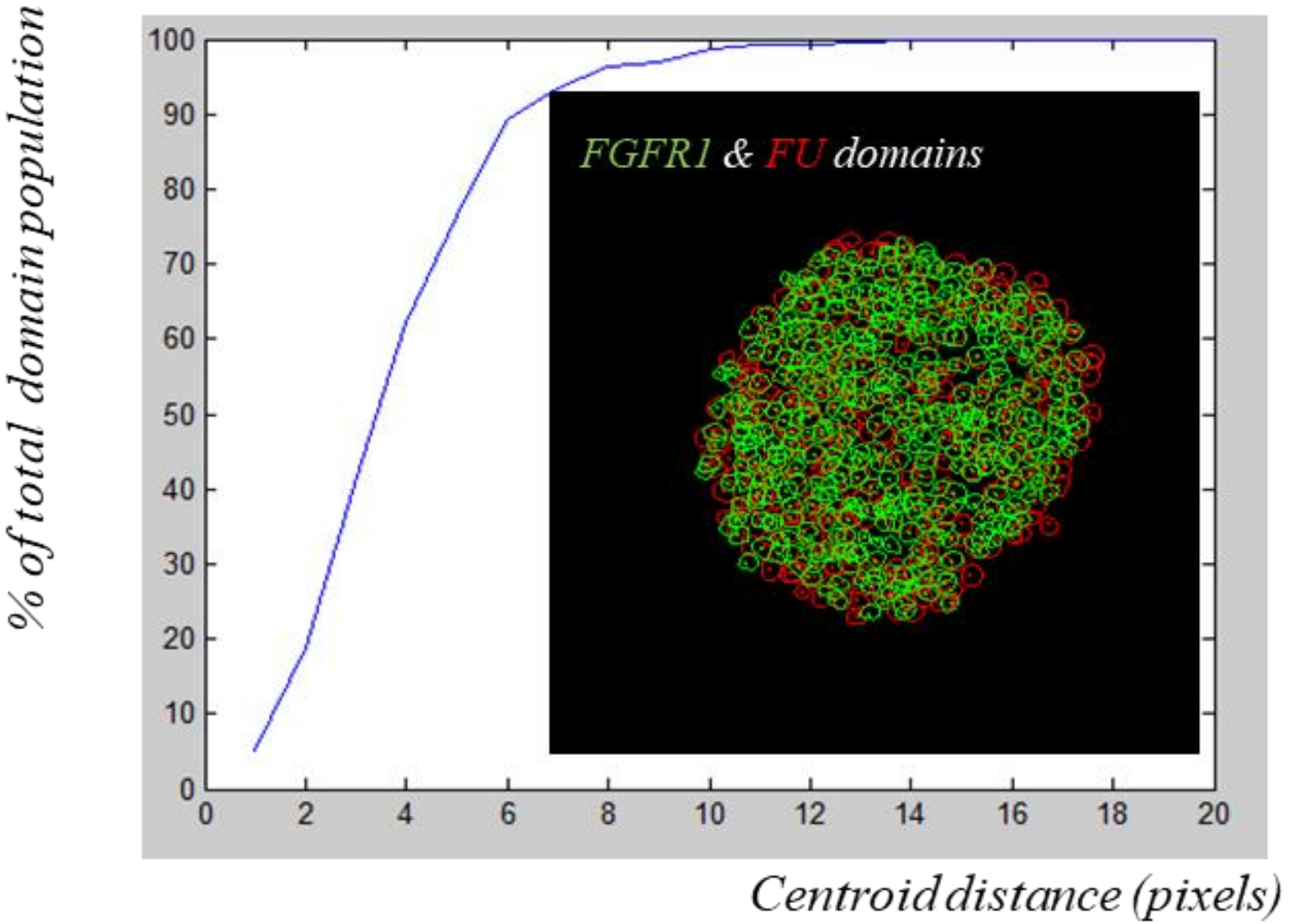




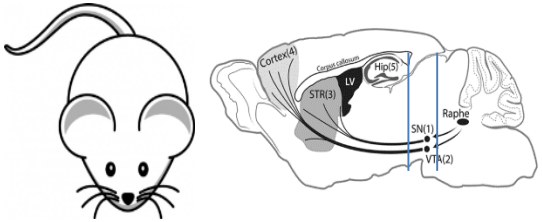
Somanathan, S., E.K. Stachowiak, A.J. Siegel, M.K. Stachowiak, and R. Berezney. 2003. Nuclear matrix bound fibroblast growth factor receptor is associated with splicing factor rich and transcriptionally active nuclear speckles. J Cell Biochem. 90:856-869.

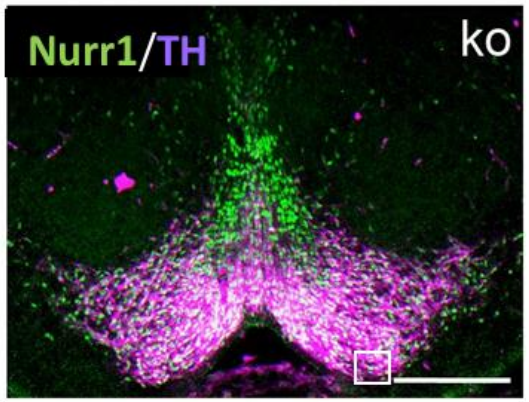


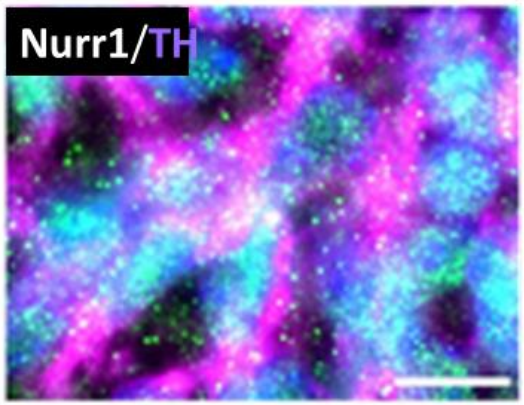
Nuclear FGFR1 colocalizes with RNA transcription sites

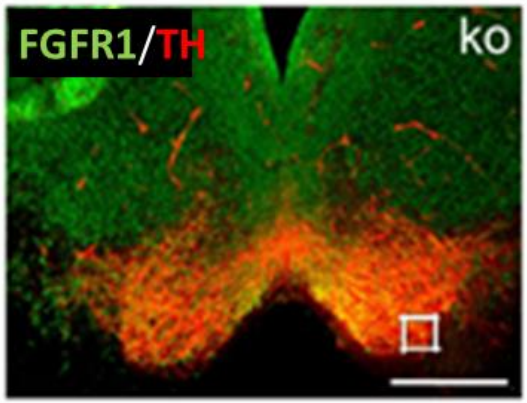


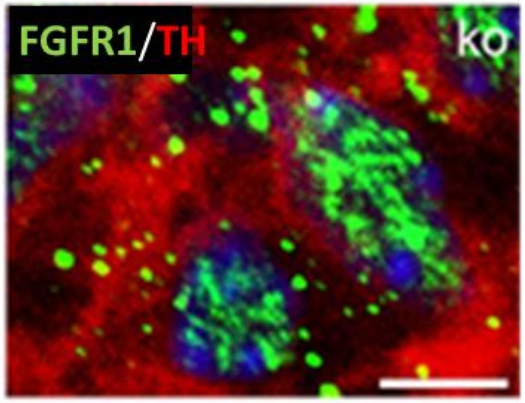
FGFR1 and Nurr1 bind to tyrosine hydroxylase (TH) gene in ventral midbrain





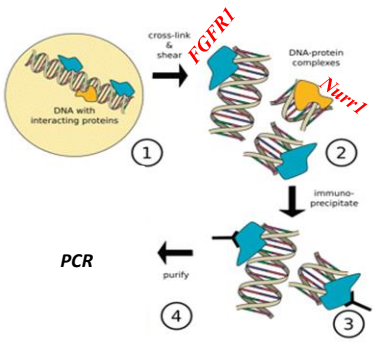


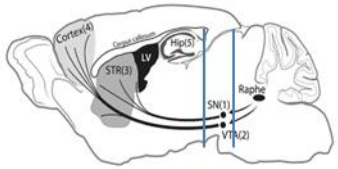


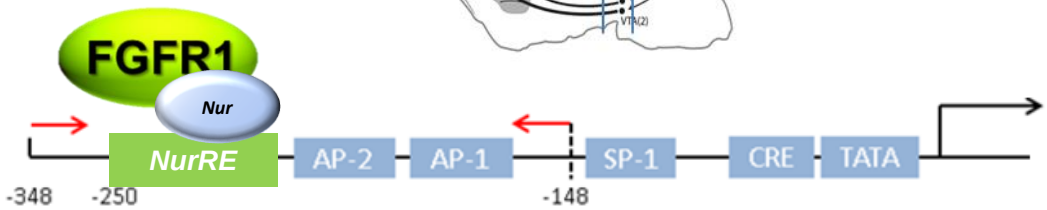


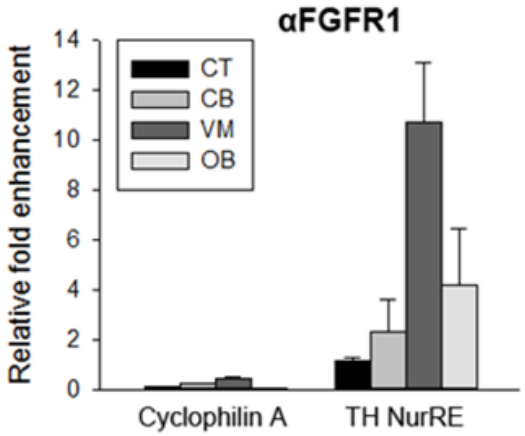
O. Baron, B. Foerthmann, Y-W. Lee, C. Terranova, A. Ratzka, E. K. Stachowiak, C. Grothe, P. Claus and M.K. Stachowiak (2012) Cooperation of nuclear FGFR1 and Nurr1 offers a new interactive mechanism in postmitotic development of mesencephalic dopaminergic neurons. ***J. Biol. Chem****. 287, 19827-19840*

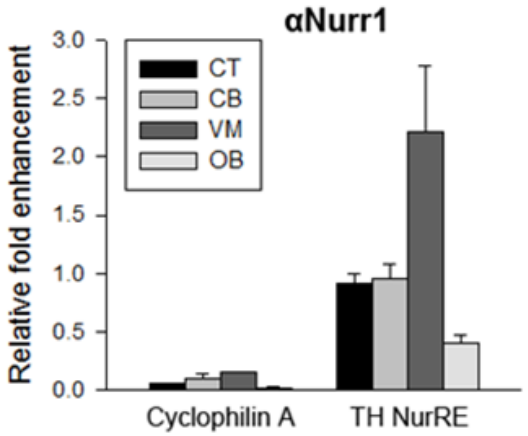
FGFR1 and Nur binding to TH gene in rat brain (ChIP)





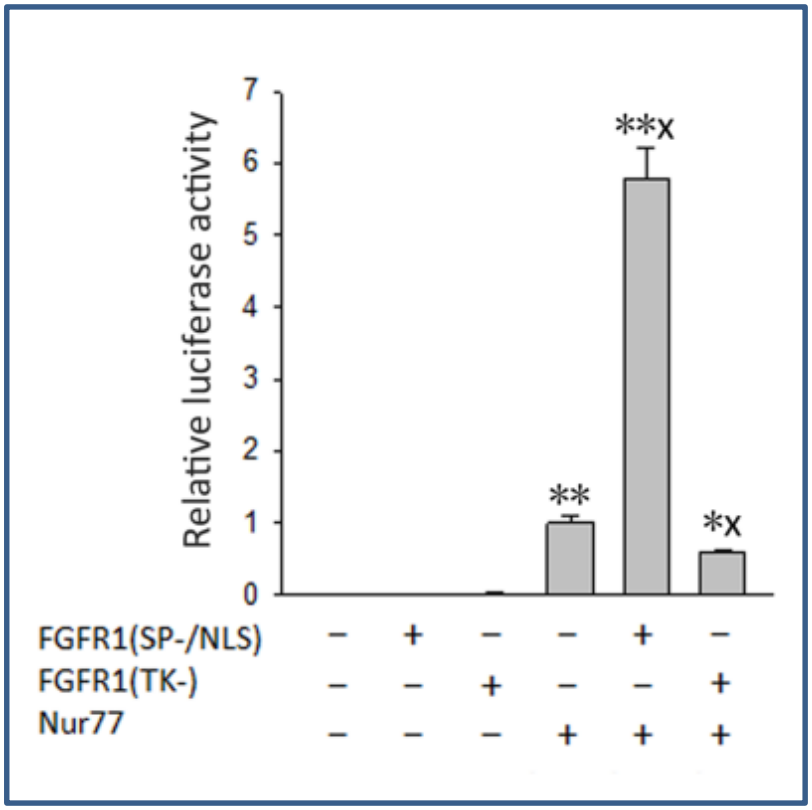


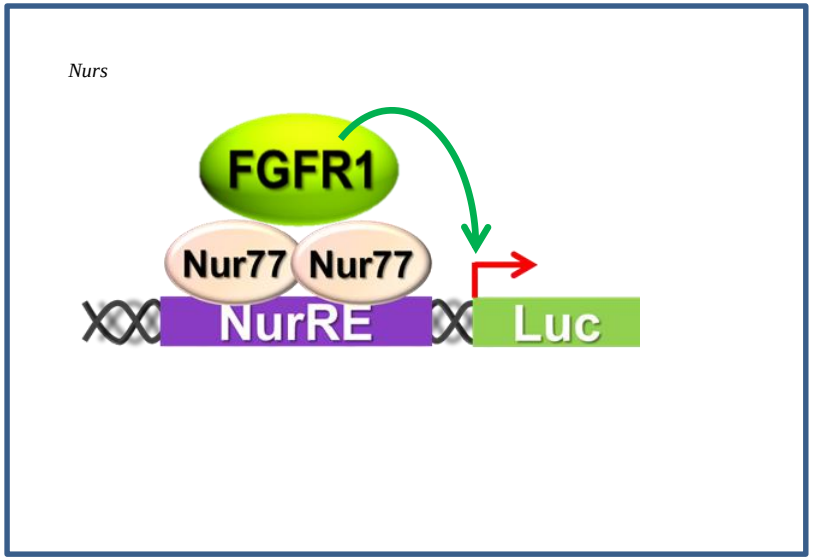




O. Baron, B. Foerthmann, Y-W. Lee, C. Terranova, A. Ratzka, E. K. Stachowiak, C. Grothe, P. Claus and M.K. Stachowiak (2012) Cooperation of nuclear FGFR1 and Nurr1 offers a new interactive mechanism in postmitotic development of mesencephalic dopaminergic neurons.J. Biol. Chem. 287, 19827-19840

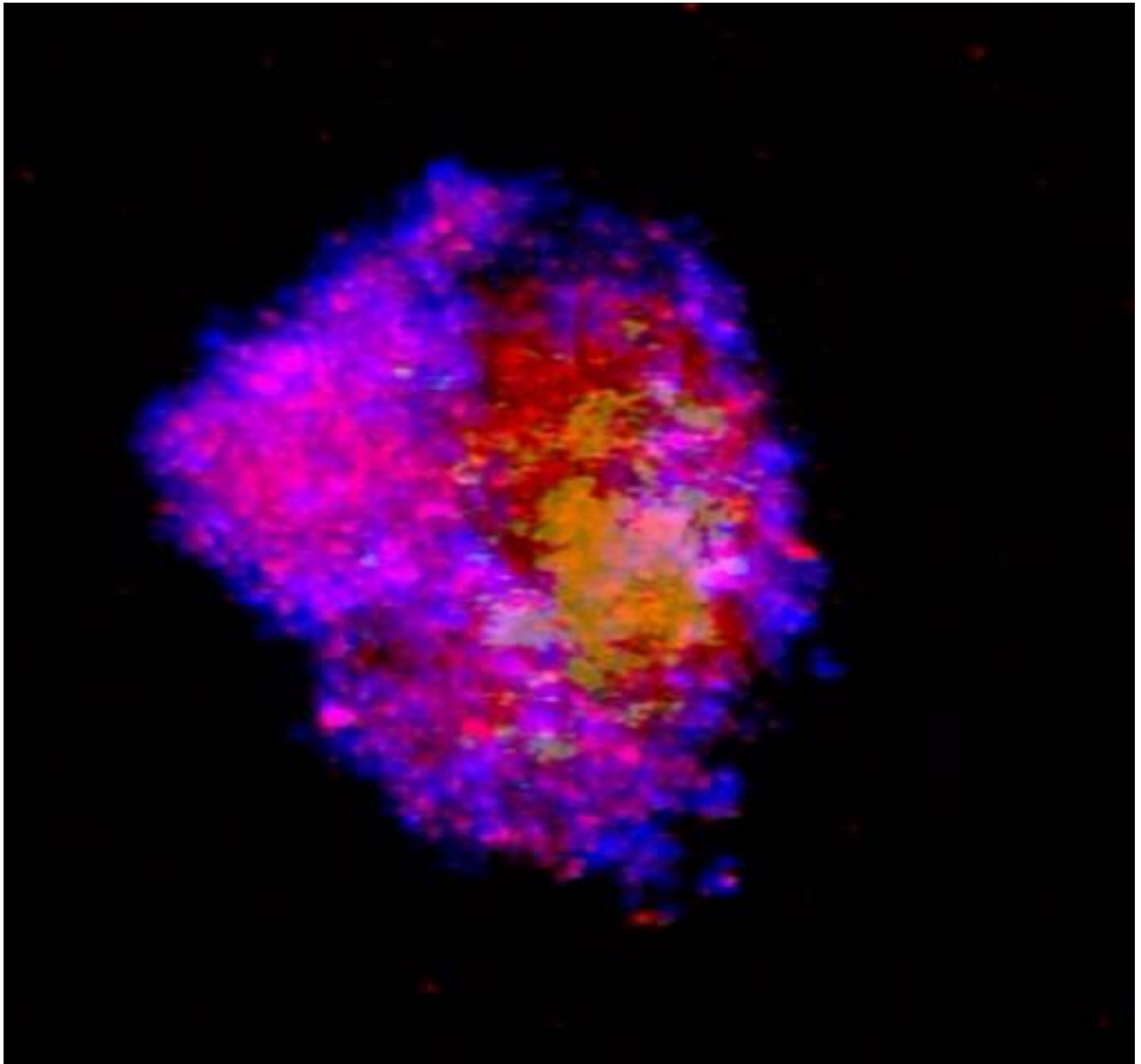
Nuclear FGFR1 Augments the TH gene transcription through NurRE



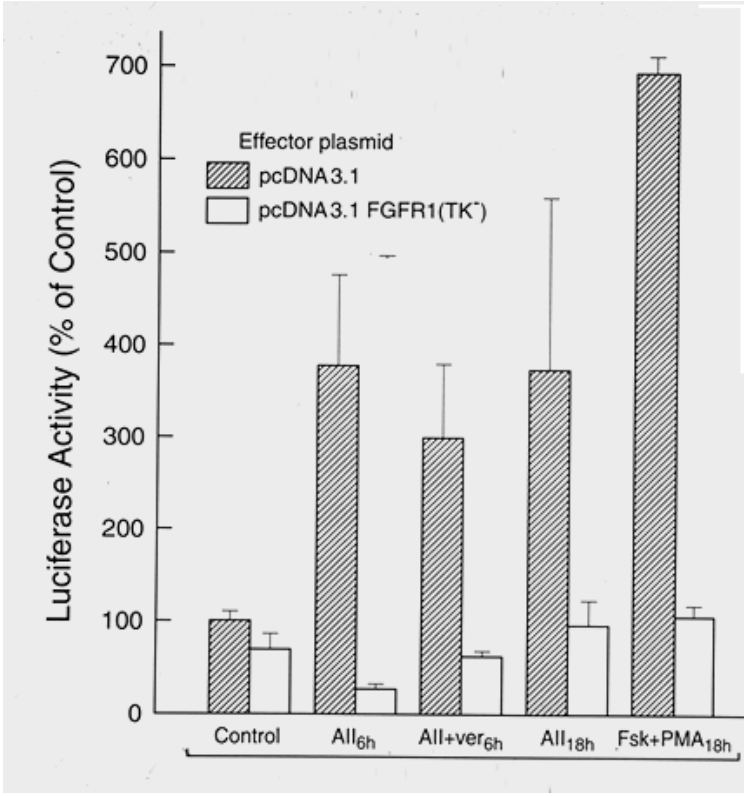


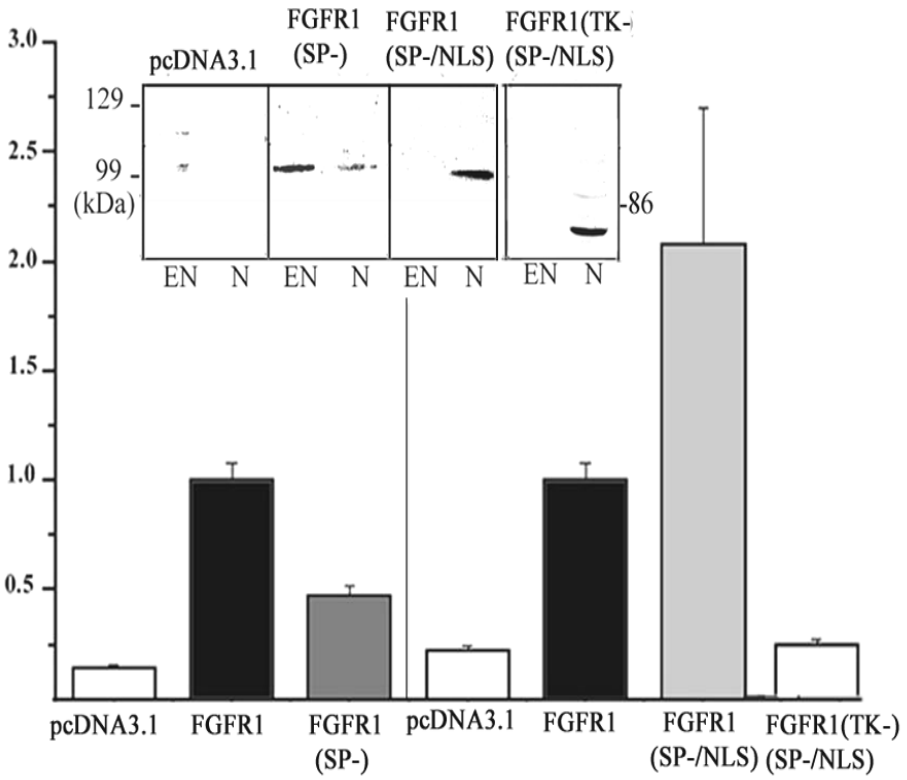
Lee, Y-W., Terranova, C., Birkaya, B., Narla, S., Kehoe, D., Parikh,P., Dong, S., Ratzka, A., Brinkmann, H., Aletta, J., Tzanakakis, E., Stachowiak, E.K., Claus, P., and Stachowiak, M.K. (2012). A novel nuclear FGF Receptor-1 partnership with retinoid and Nur receptors during developmental gene programming of embryonic stem cells J. Cell. Biochem., 113, 2920-2930

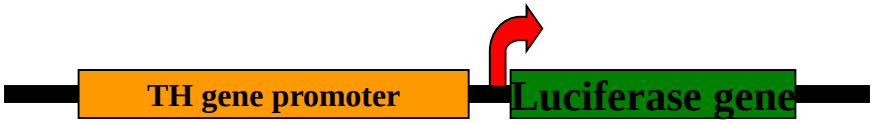
Induction of TH in **Adrenal Medullary Cell** by **Angiotensin II** is accompanied by nuclear accumulation of **FGFR1**



Nuclear FGFR1 is essential and sufficient to stimulate Tyrosine Hydroxylase Gene promoter

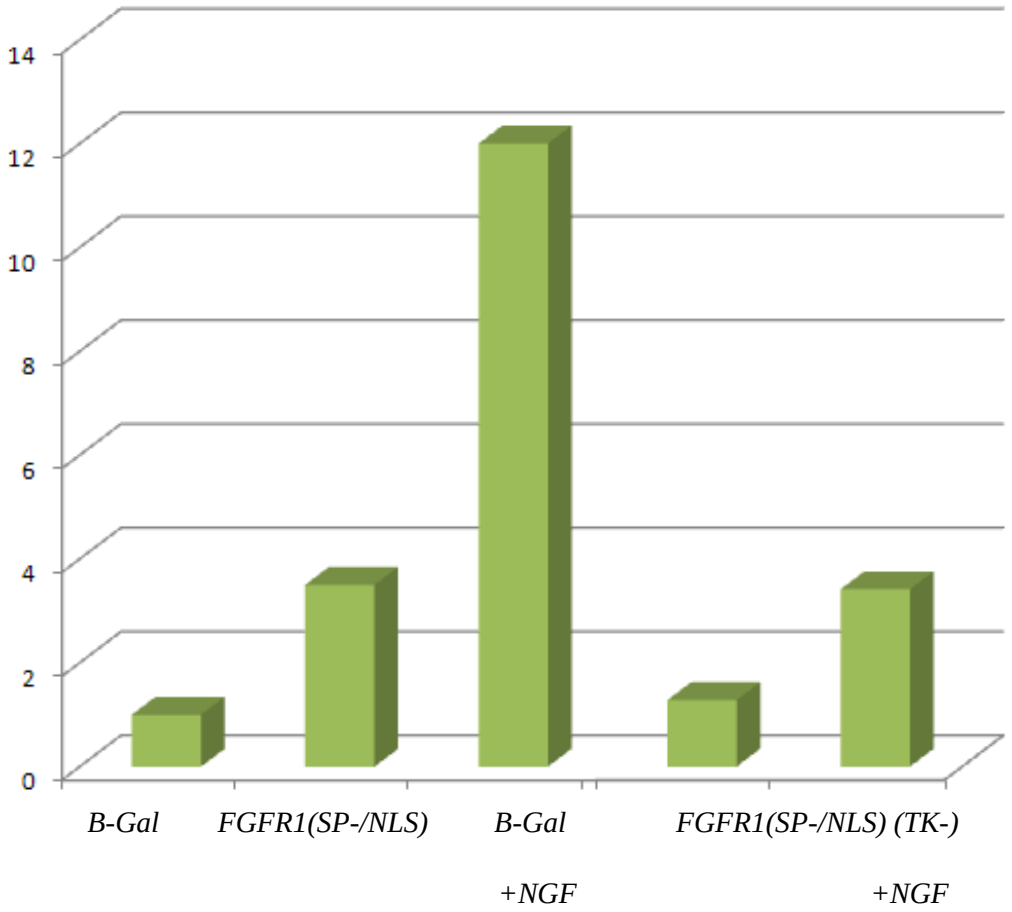




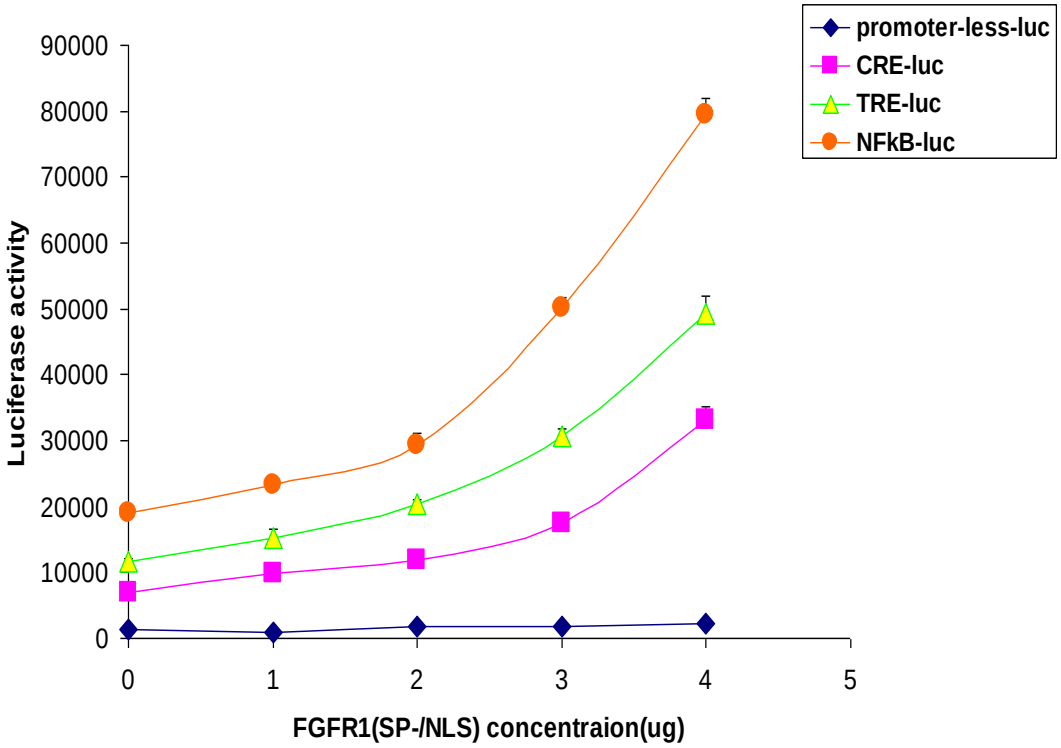


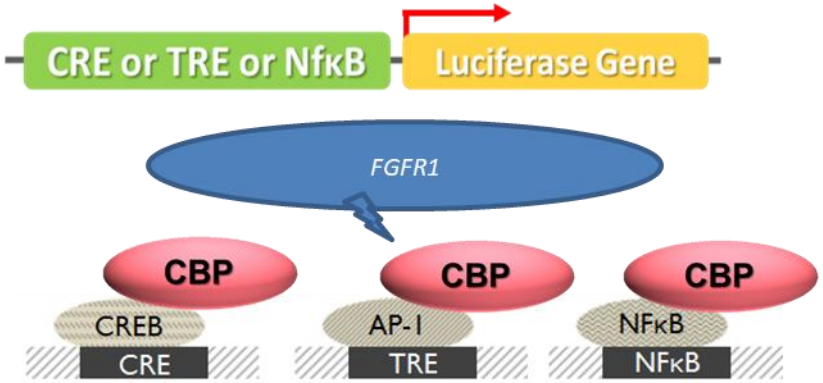
Genes activated by nuclear FGFR1 include: NfL, FGF-2, NSE, TH, DCX, MAP2

Induction of Doublecortin gene by NGF involves nuclear FGFR1



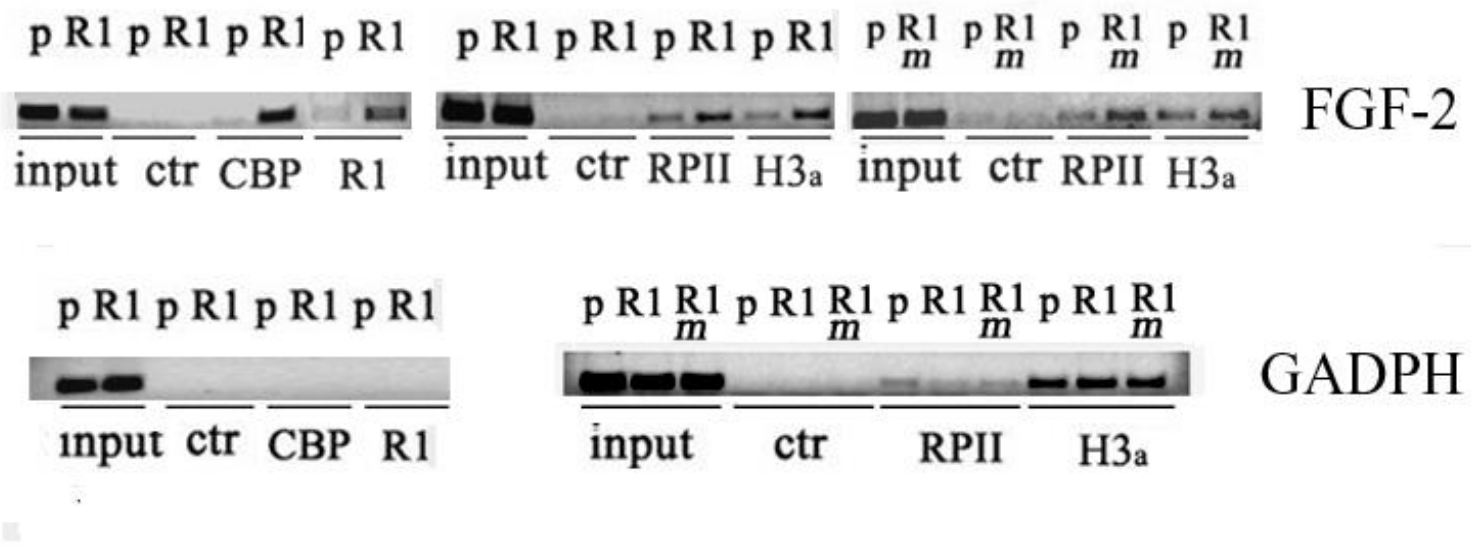
Nuclear FGFR1 Activates CRE and other CREB Binding Protein (CBP) - dependent Enhancers





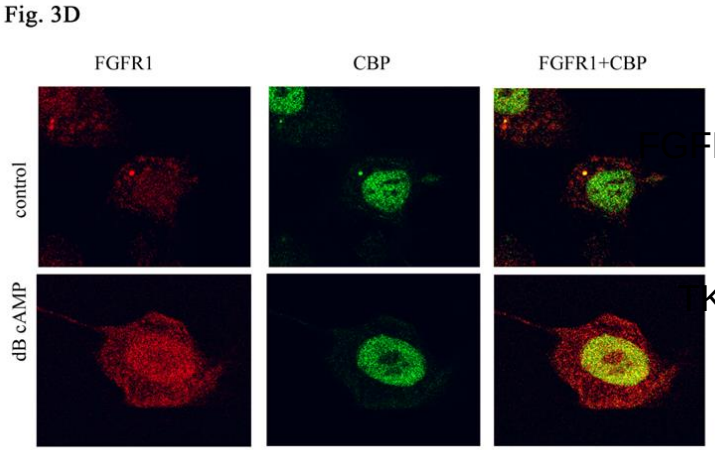
FGFR1 associates with gene promoter, increases binding of CBP, RNA Pol II and acetylation of Histone H3.

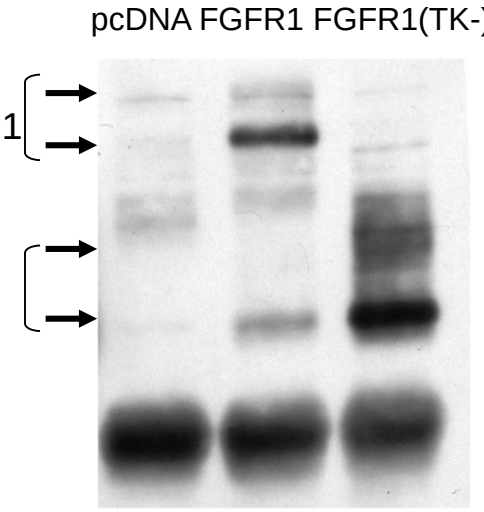
p – pcDNA, - R1 – FGFR1, R1m – kinase inactive R1

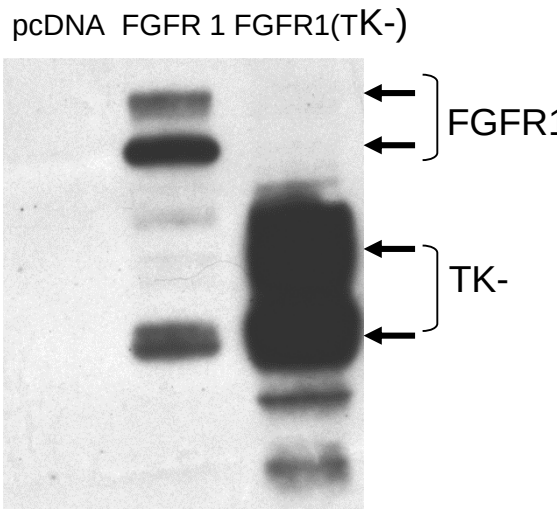


Chromatin immunoprecipitation - FGF-2 and GADPH genes

N-terminal part of nuclear FGFR1 interacts with CREB Binding Protein - CBP



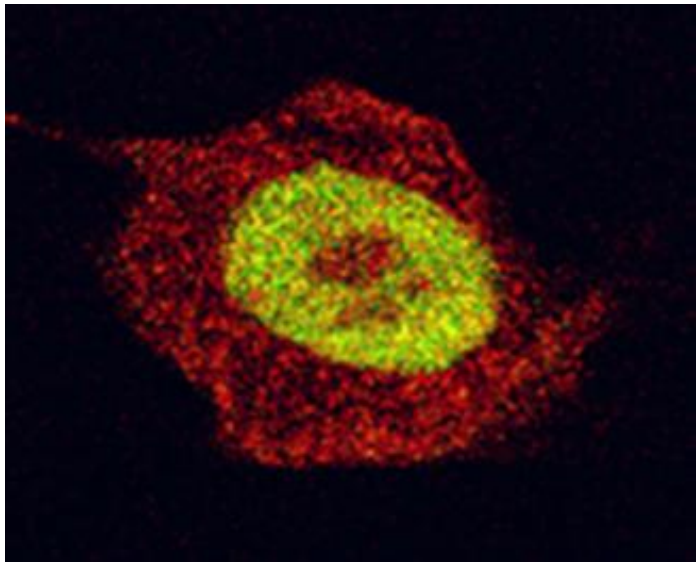




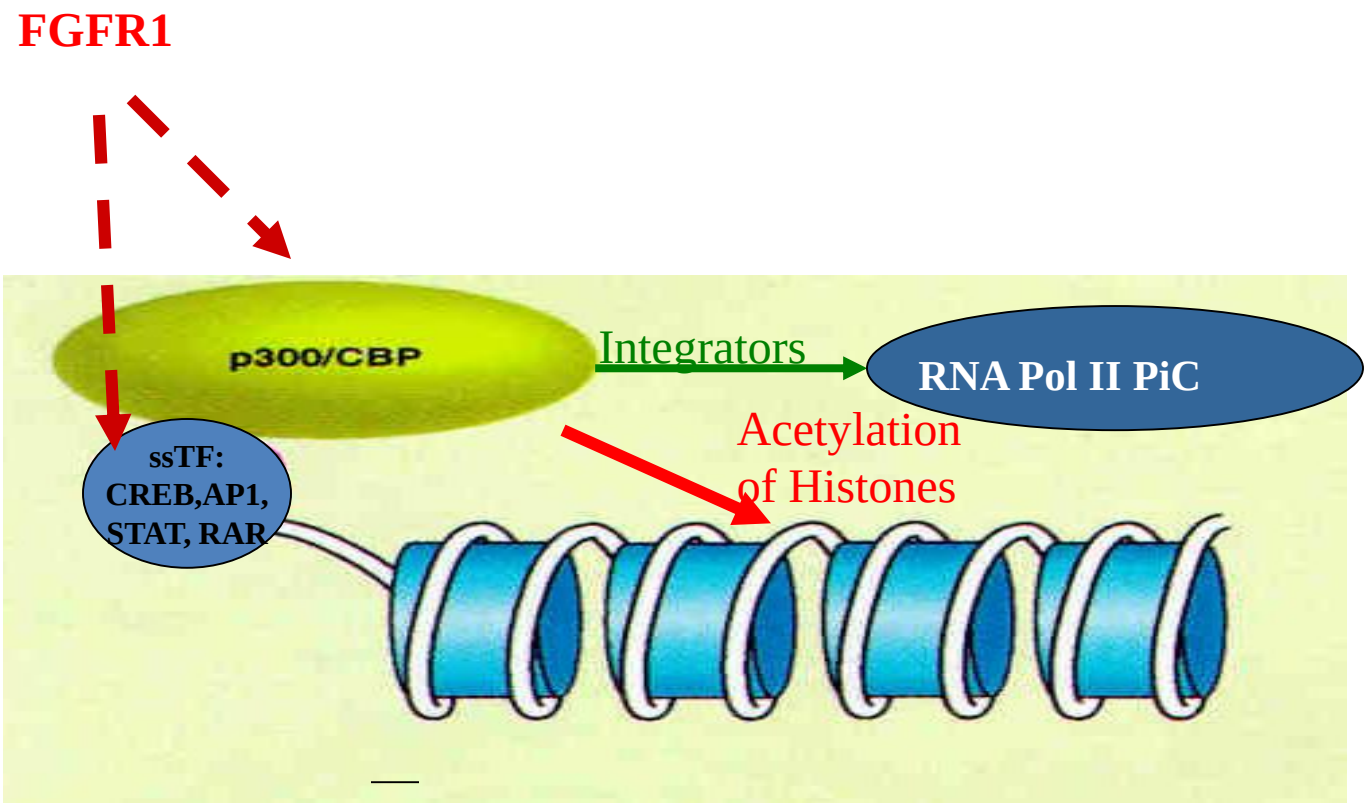
IP---CBP Ab

IB---FGFR1 Ab

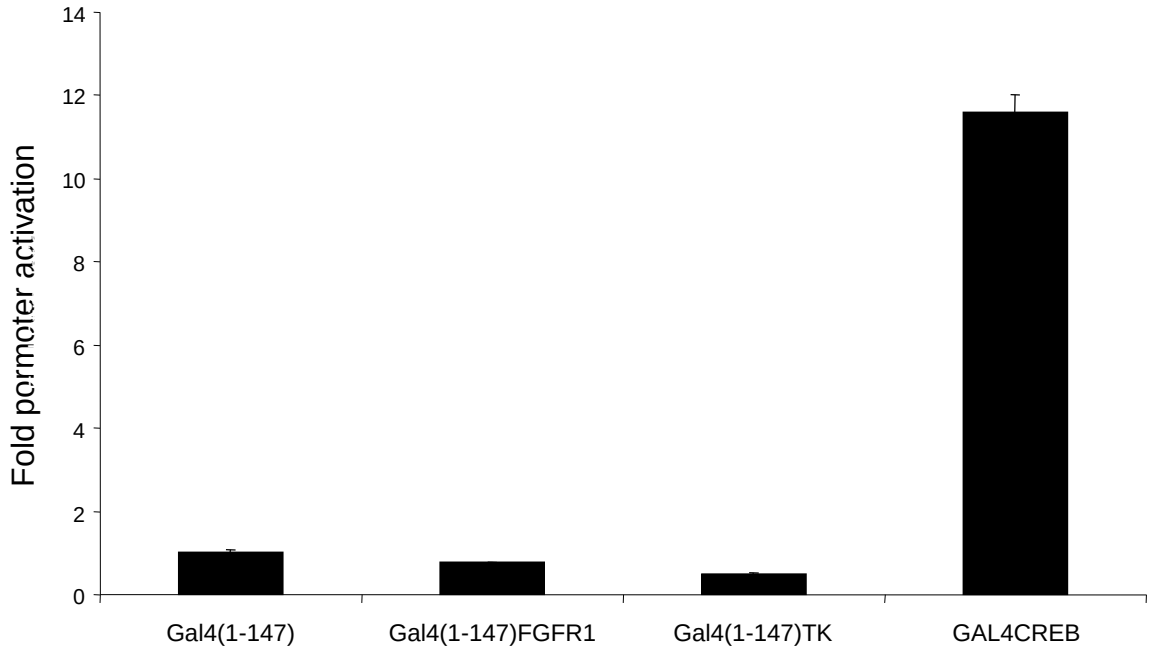
IB---FGFR1



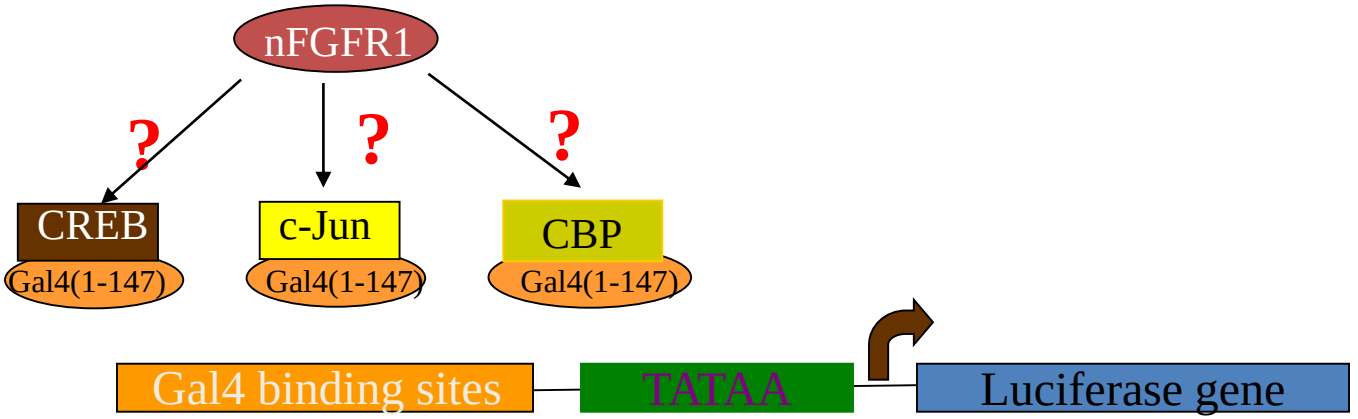
How could nuclear FGFR1 activate transcription::

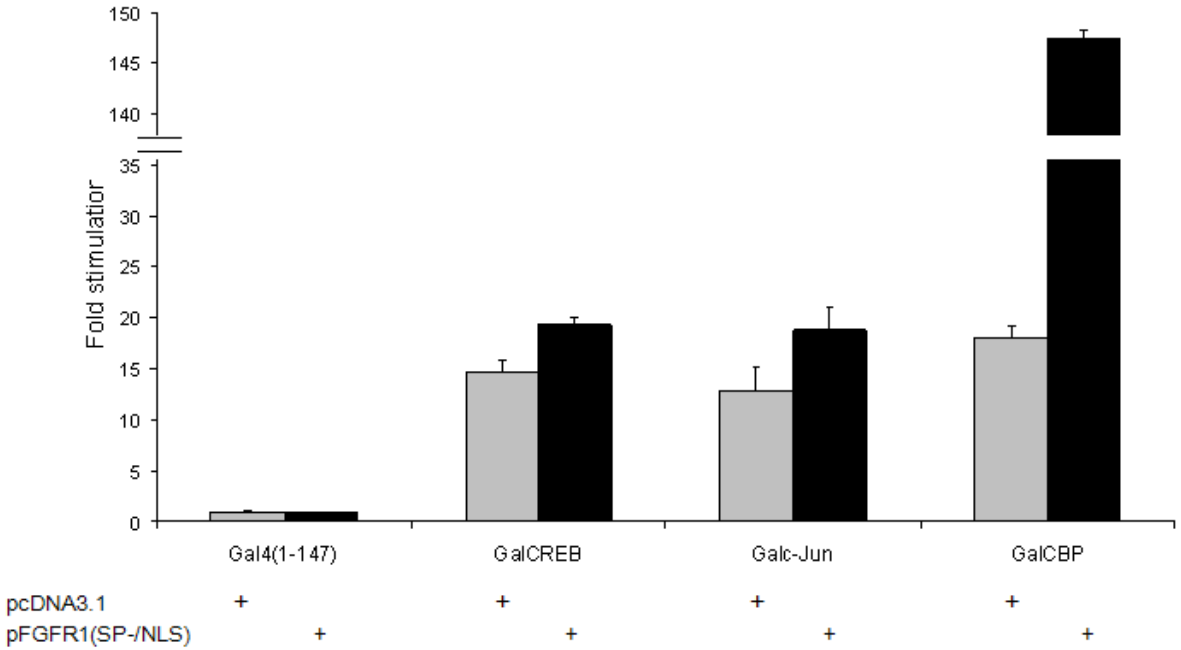


FGFR1 lacks autonomous transactivating function.

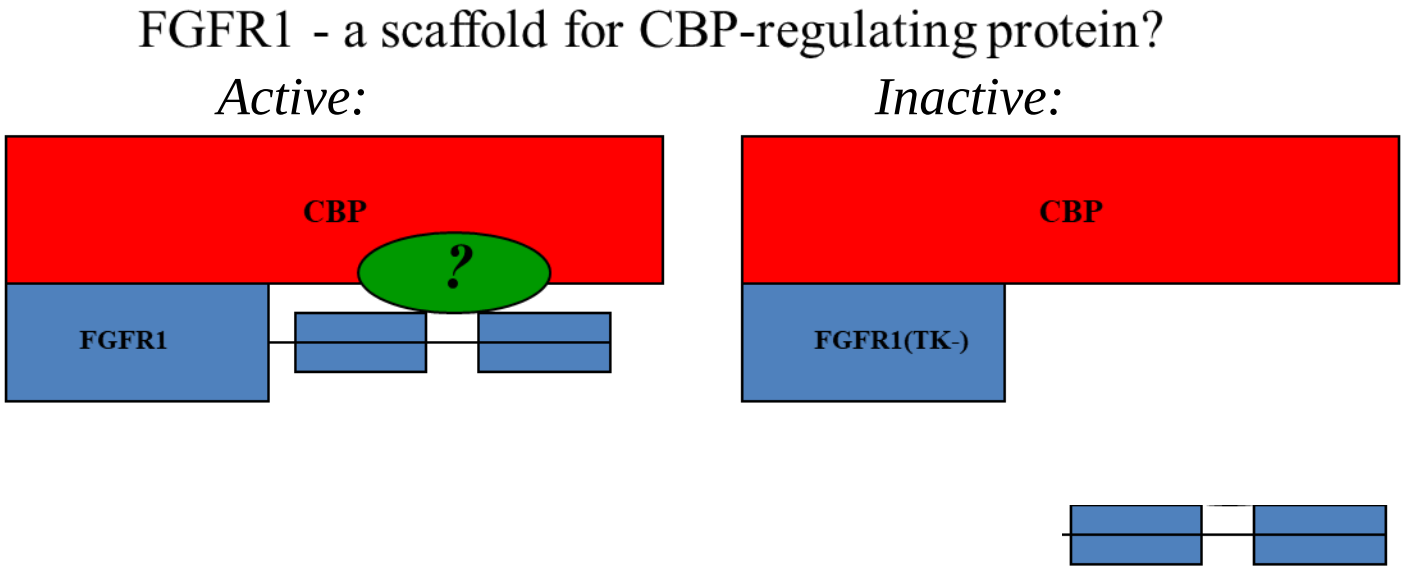


Nuclear FGFR1 increases transcription by via CBP.





Nuclear FGFR1 stimulation of CBP-mediated transcription requires both TK and NT domains but not tyrosine kinase activity.



Yeast 2-hybrid assay

FGFR1 binds to the central region of RSK1

Fig. 1A

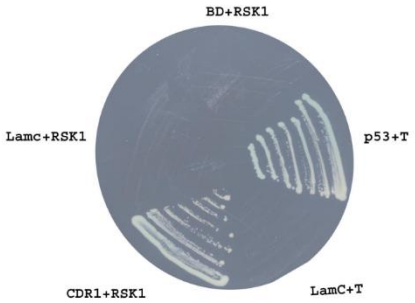
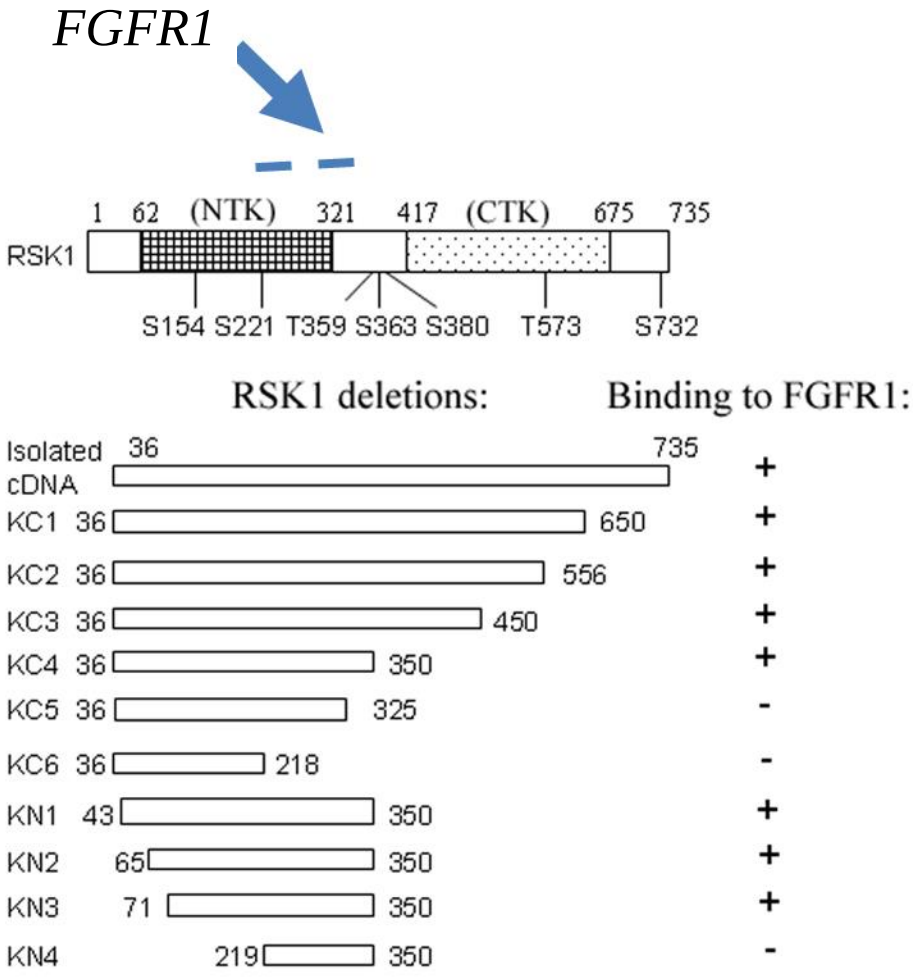


Fig. 1B

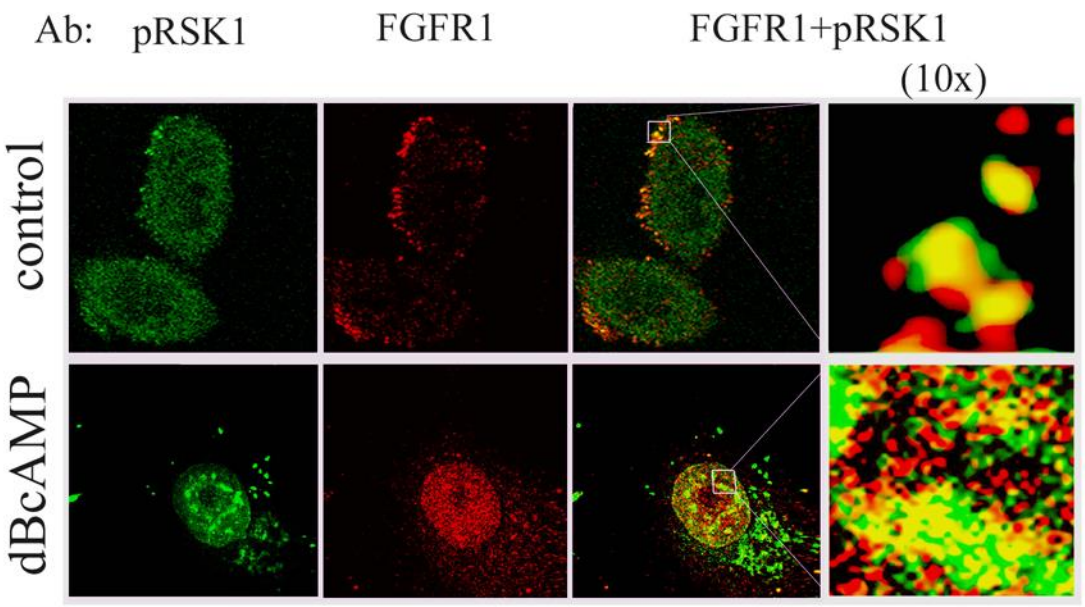


Hu et al., J. Biol. Chem., 279, 29325-29335 (2004);

*Fang et al., **J. Biol. Chem**. – 280(31):28451-62.*

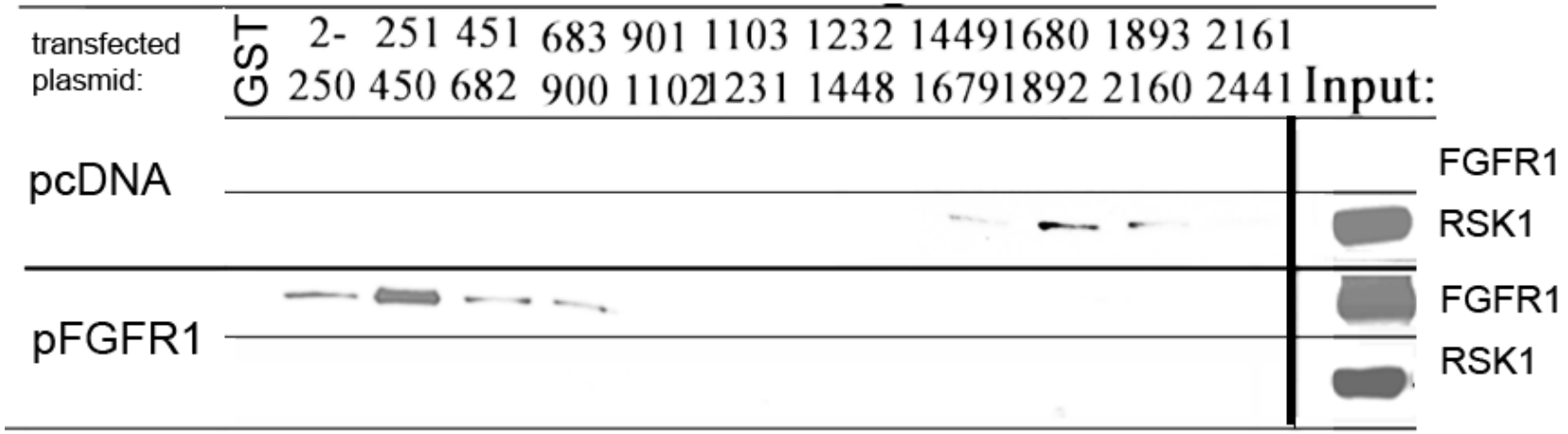
FGFR1-RSK1 Interaction

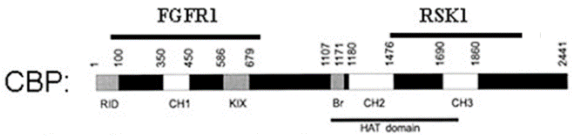
P-RSK1 FGFR1 RSK1-FGFR1

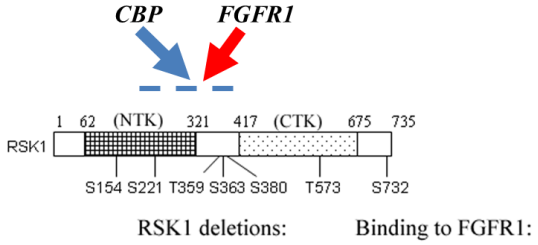


FGFR1 binds to N-terminal region of CBP

GST-CBP (fragments) pull down of transfected FGFR1 or endogenous RSK1

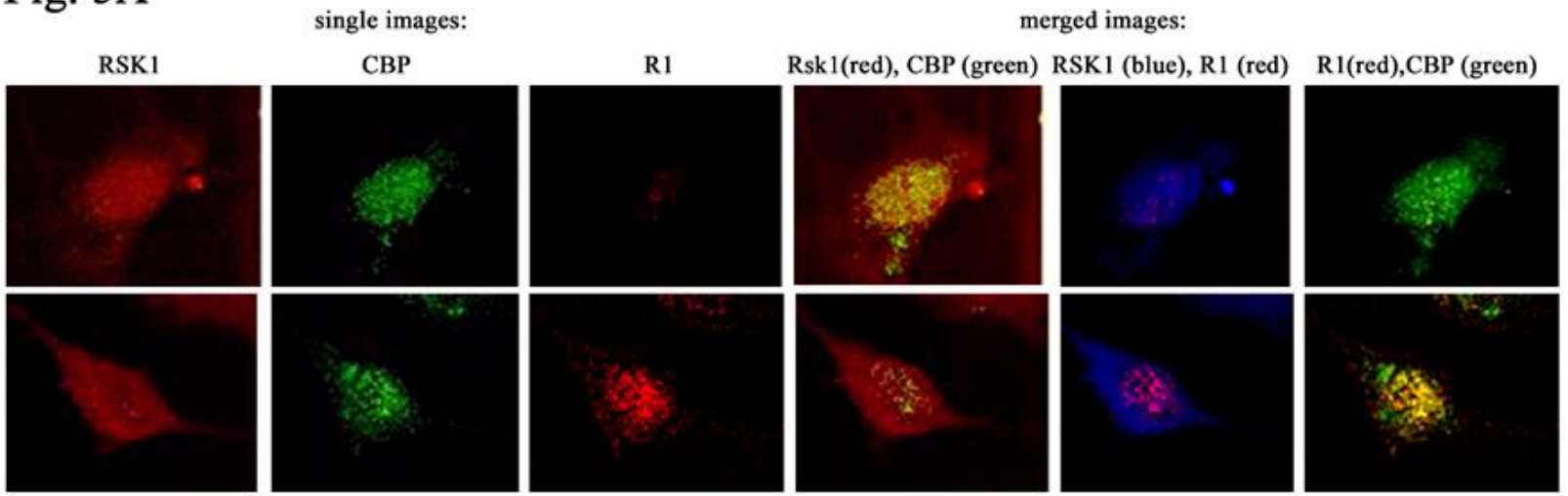




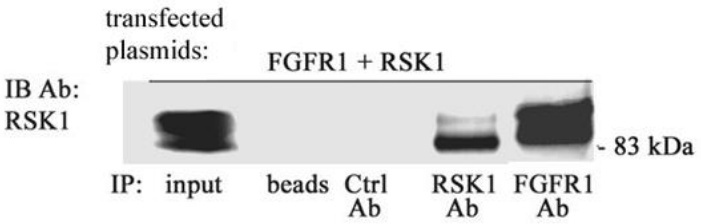


*Fang et al., . **J. Biol. Chem**. – 280(31):28451-62.*

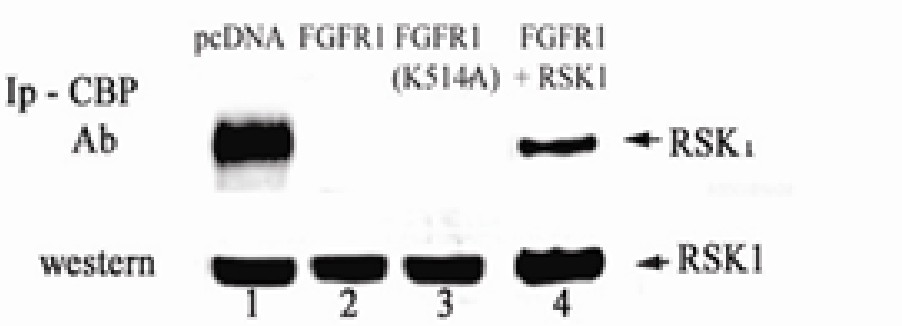
Fig. 5A

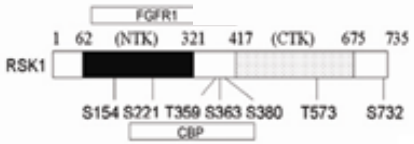


FGFR1 Binds to RSK1:

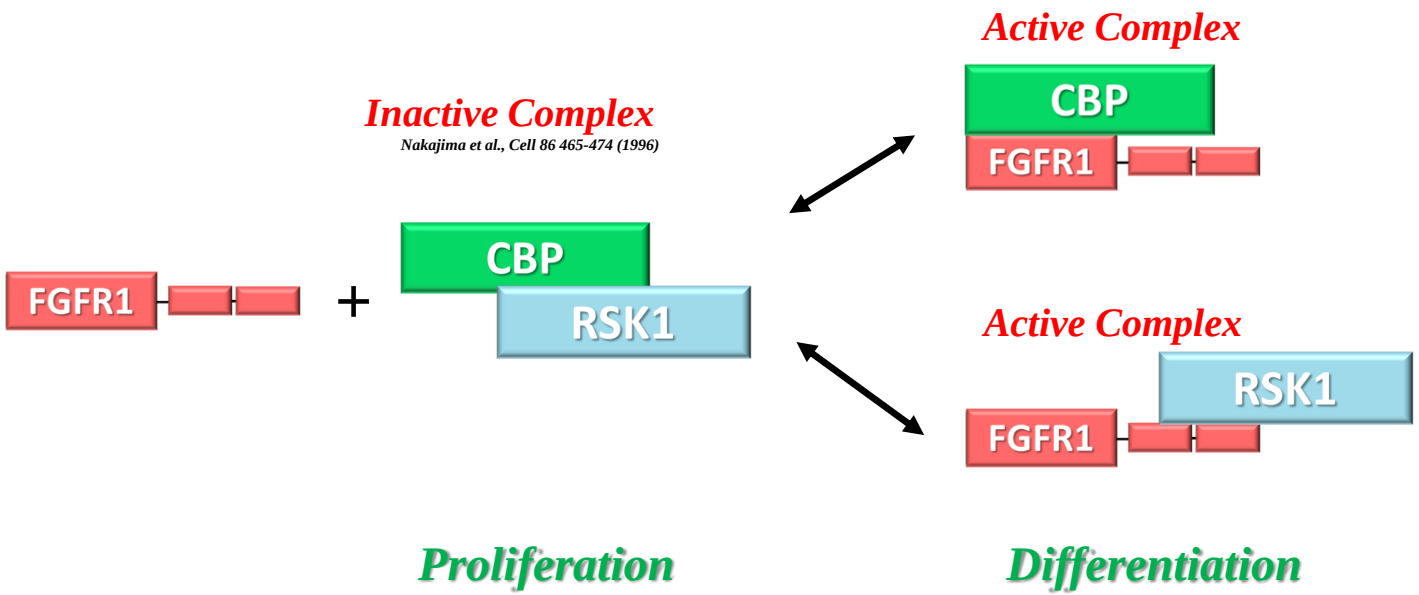


FGFR1 disrupts RSK1-CBP complex:



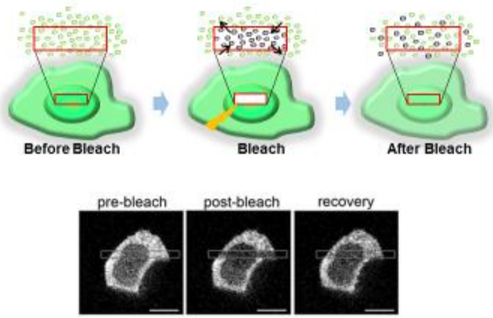


FGFR1 – a molecular switch for CBP-RSK1:

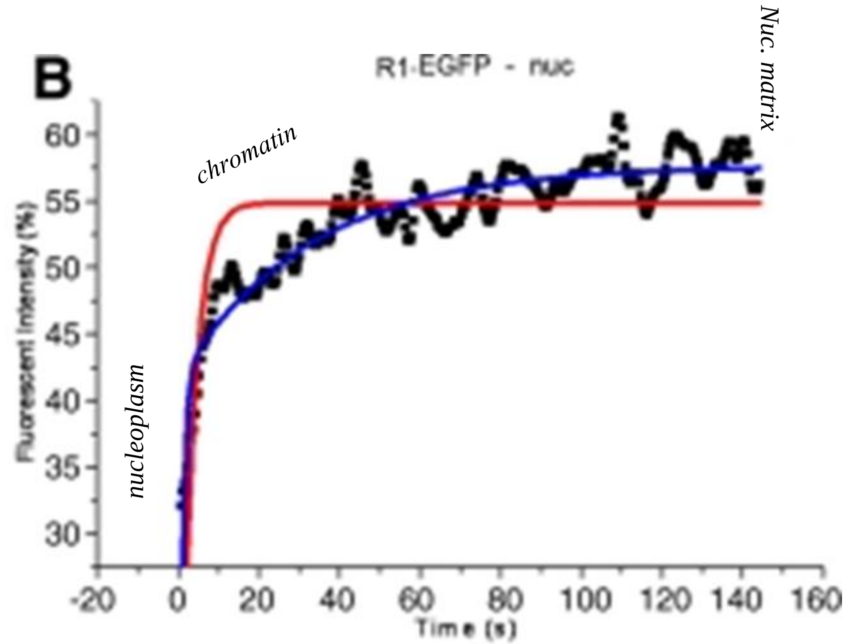


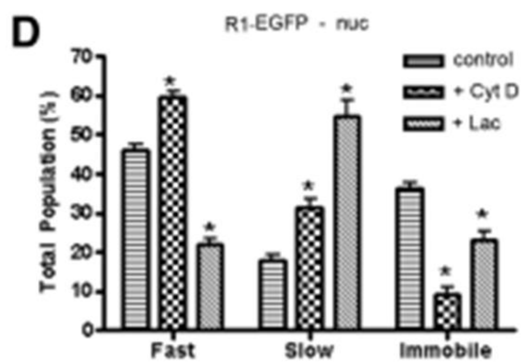
Yafang Hu, Xiaohong Fang, Star M. Duham, Claudia Prada, Ewa K. Stachowiak, Michal K. Stachowiak. (2004) RSK1 is a direct target for the nuclear fibroblast growth factor receptor (FGFR1) – role in FGFR1 signaling. ***J. Biol. Chem****., 279, 29325-29335.*
Fang X, Stachowiak EK, Dubham-Ems S, Klejbor I, Stachowiak MK (2005) Control of CBP signaling by nuclear Fibroblast Growth factor receptor-1 (FGFR1) – a novel mechanism of gene regulation. ***J. Biol. Chem****. – 280(31):28451-62.*

Fluorescence Recovery After Photobleaching (FRAP) of FGFR1-EGFP - kinetic model of gene regulation by nuclear FGFR1



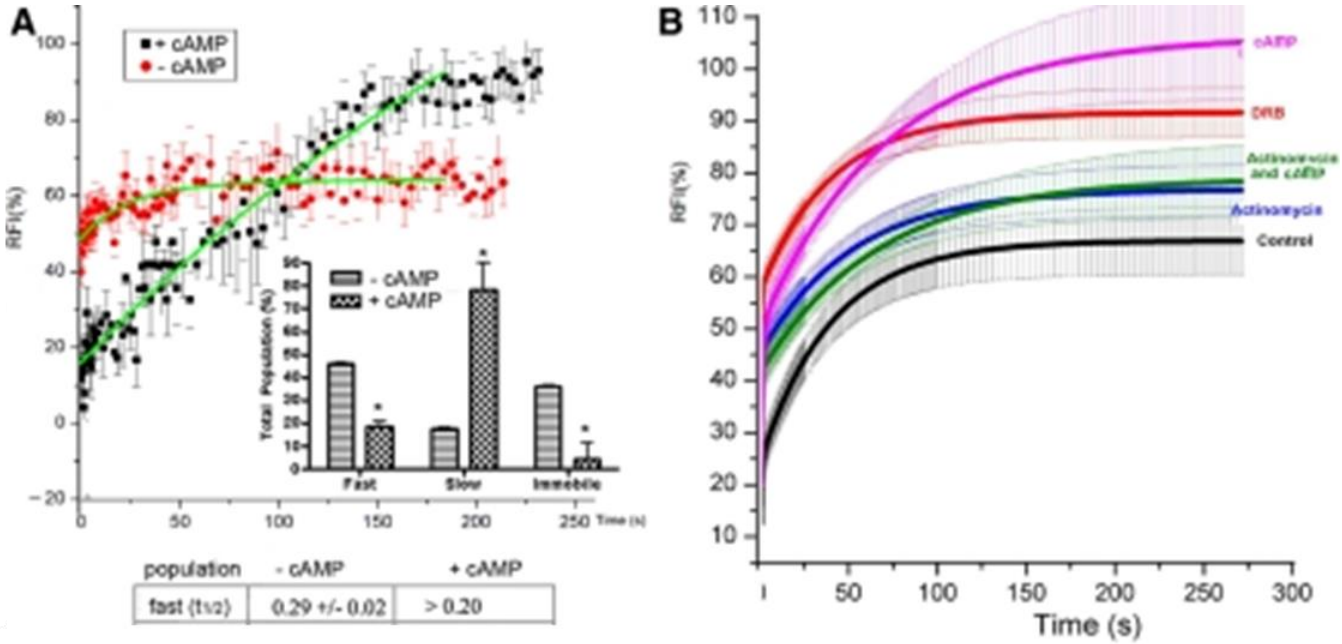
[illegible]
S.M. Dunham, H. Pudavar, P. Prasad, M.K. Stachowiak (2004) J. Physical Chem, 108,10540-10546 S. M. Dunham-Ems, H. Pudavar, J. Myers, P. Prasad, and M. K. Stachowiak. (2006). c. Mol. Biol. Cell 17, 2223-2235.





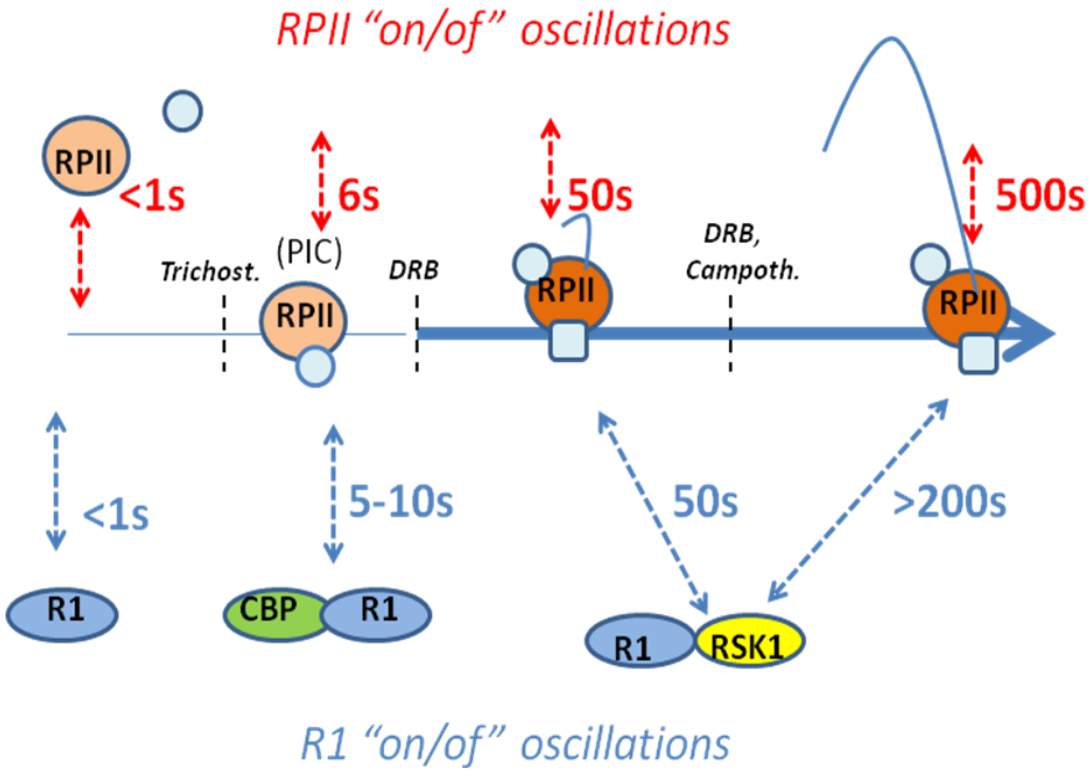
population	control	+ Cyt D	+ Lac
fast ($t_{1/2}$)	0.29 +/- 0.02	0.23 +/- 0.15	1.55 +/- 0.30*
slow ($t_{1/2}$)	24.41 +/- 3.48	50.56 +/- 7.92*	131.43 +/-13.84*

Changes in nFGFR1 mobility during transcription activation and inhibition reflect chromatin-matrix distribution



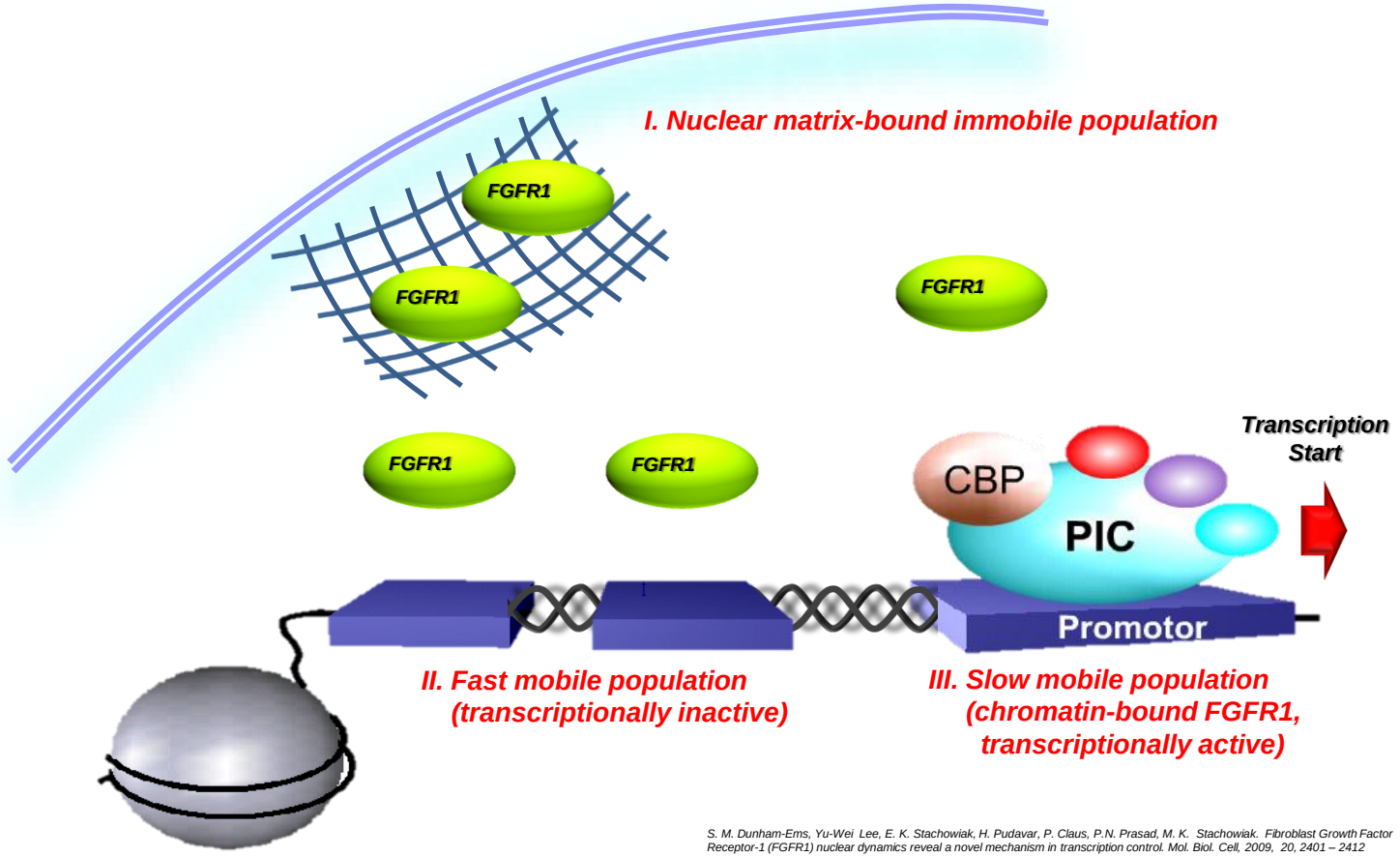
Nuclear FGFR1 movement is affected by its binding partners CBP and RSK1

Transfected plasmids	Fast half-time	Slow half-time	Fast mobility (%)	Slow mobility (%)
R1-EGFP	0.29 ± 0.02	24.41 ± 3.48	46.14 ± 1.57	17.68 ± 1.52
R1-EGFP + CBP	0.55 ± 0.26	133.3 ± 14.91*	25.35 ± 2.22*	75.12 ± 5.53*
R1-EGFP + CBP (451-689)	0.44 ± 0.02	87.45 ± 12.56*	35.25 ± 2.03*	49.02 ± 2.0*
R1-EGFP + CBP (1678-2441)	0.36 ± 0.10	33.71 ± 0.65	38.96 ± 1.78*	0.92 ± 2.89*



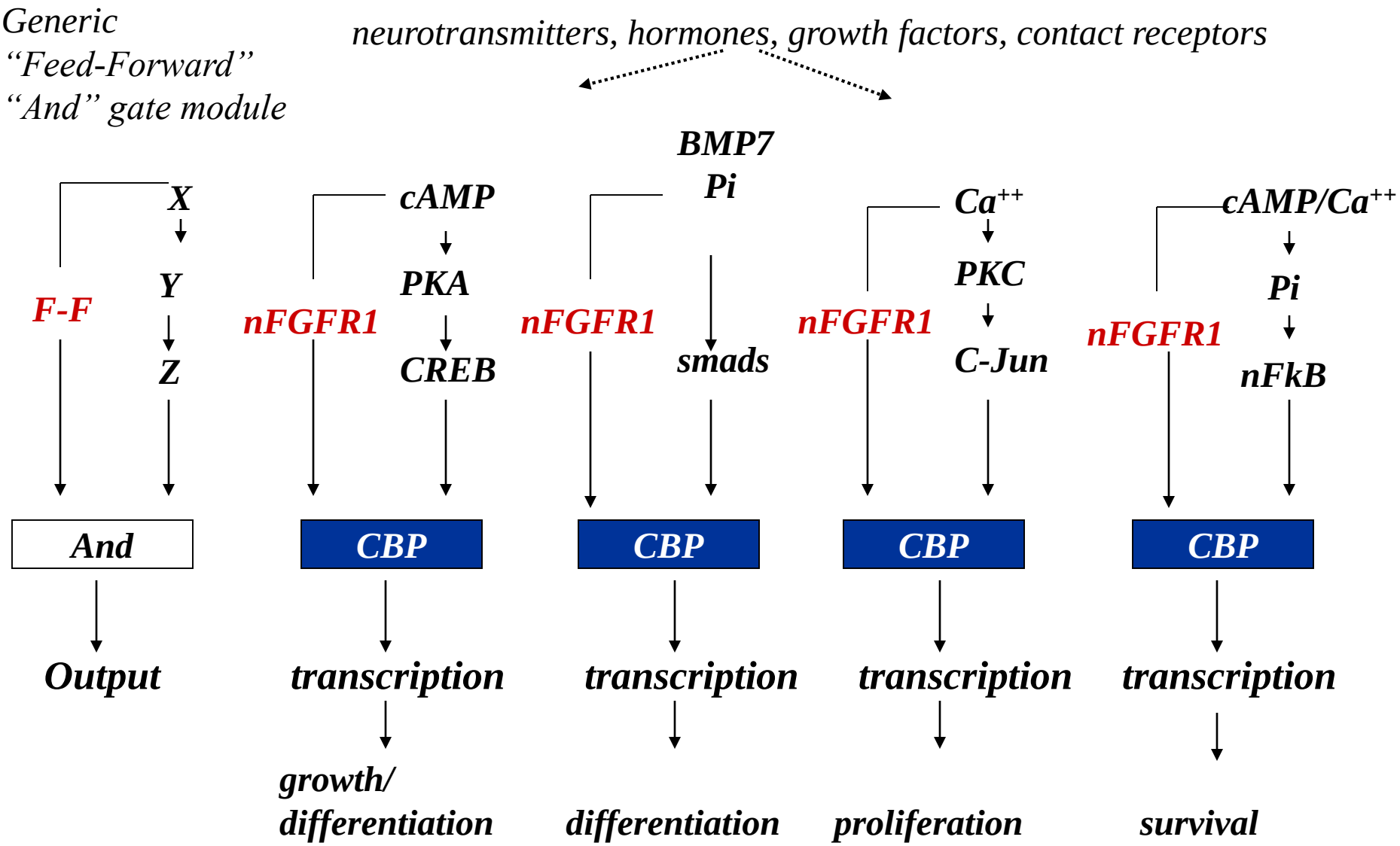
.Fig. 6 . Kinetics of RNA Polymerase II (RPII) "on/off" gene oscillations at different phases of transcription are based on ("PIC"- preinitation complex). Kinetic model of INFS (R1, CBP, RSK1) gene activation is based on our hetherto findings using FRAP R1 kinetics on bulk chromatin, its regulation by CBP and RSK1 and by the demonstrated static interaction among these proteins.

Three Different Population of FGFR1-EGFP in the Nucleus



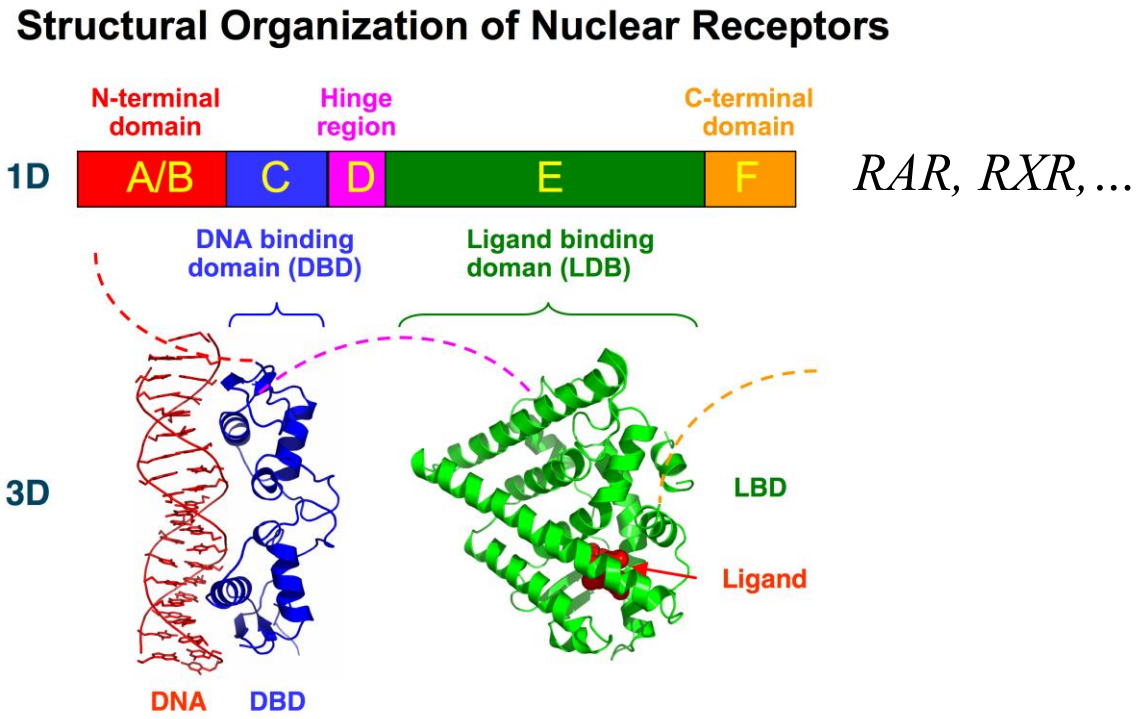
S. M. Dunham-Ems, Yu-Wei Lee, E. K. Stachowiak, H. Pudavar, P. Claus, P.N. Prasad, M. K. Stachowiak. Fibroblast Growth Factor Receptor-1 (FGFR1) nuclear dynamics reveal a novel mechanism in transcription control. Mol. Biol. Cell, 2009, 20, 2401 – 2412

Integrative Nuclear FGFR1 Signaling (INFS) pathway forms common modules that control diverse gene programs.



Nuclear FGFR1 in Embryonic Stem Cells
RAR/RXR & Nur partners

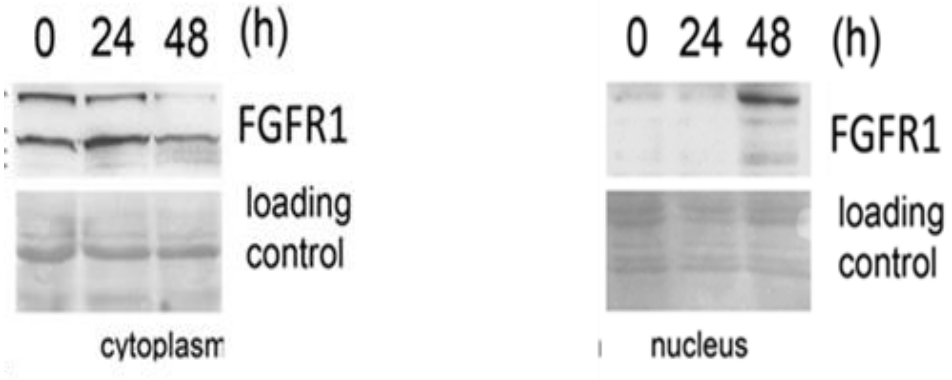
ONTOGENIC REGULATORS - DUAL FUNCTION PROTEINS: RECEPTORS AND TRANSCRIPTIONAL FACTORS



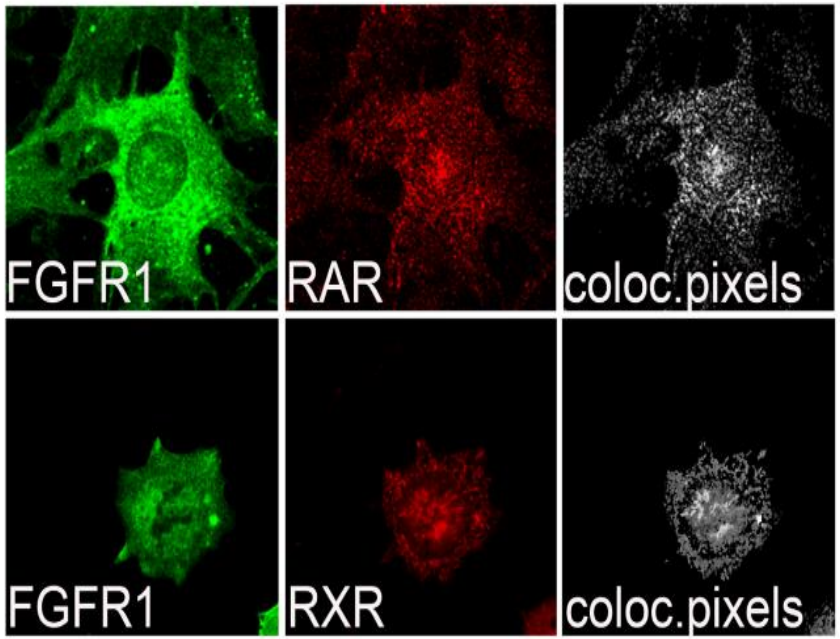
Orphan Nuclear Receptors lack ligand binding domain: Nur77, Nurr1

Interaction of FGFR1 with RXR and RAR:

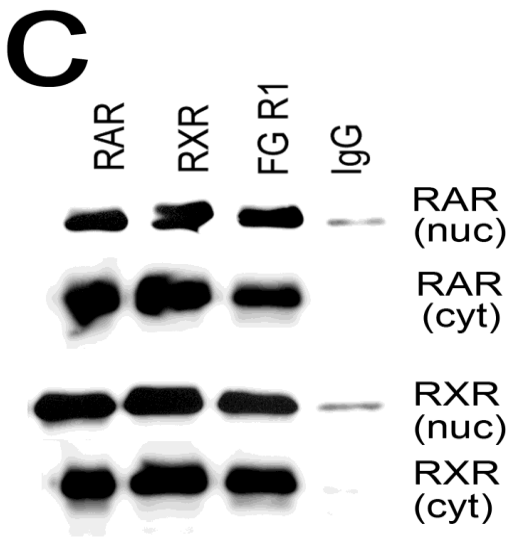
RA:



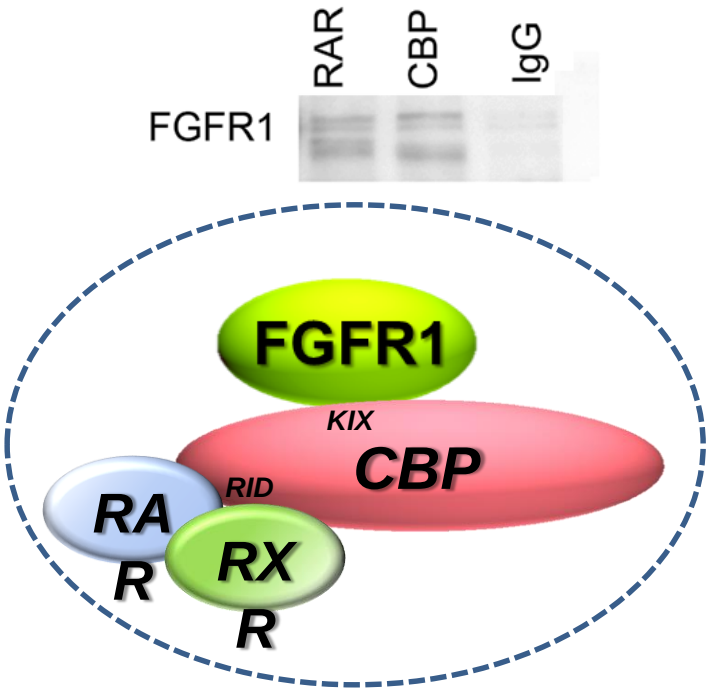
a *Confocal microscopy:*



Coimmunoprecipitation:



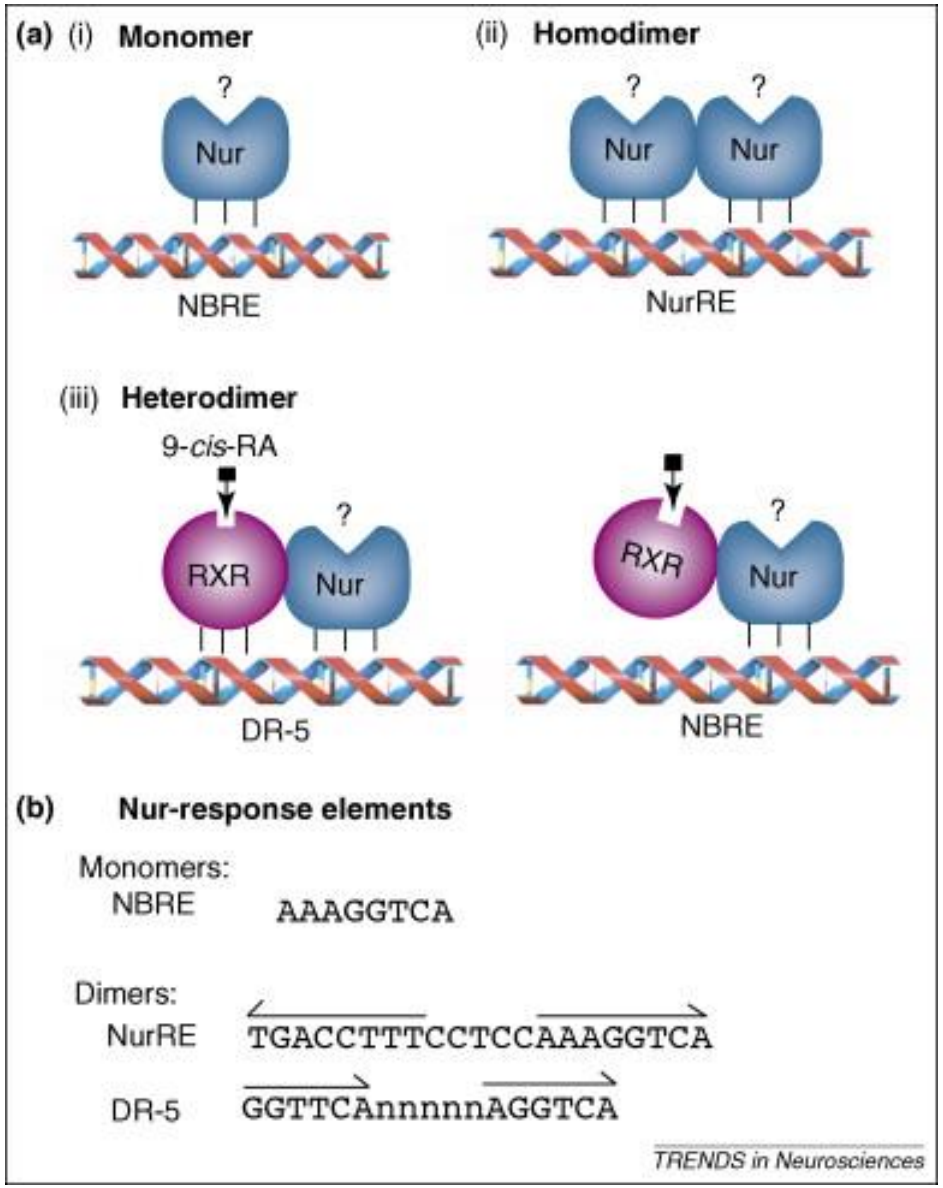
The mutual interaction between nuclear FGFR1 and RAR/RXR may via CBP



co-immunoprecipitation, FRET

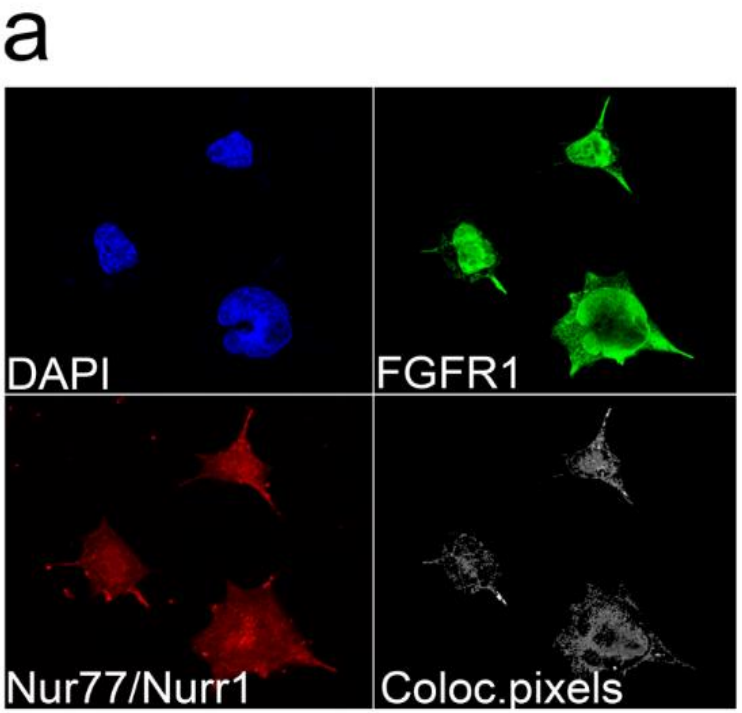
Fang X, Stachowiak EK, Dubham-Ems S, Klejbor I, Stachowiak MK (2005) ***J. Biol. Chem****. – 280(31):28451-62.*
Yafang Hu, Xiaohong Fang, Star M. Duham, Claudia Prada, Ewa K. Stachowiak, Michal K. Stachowiak. (2004) ***J. Biol. Chem****., 279, 29325-29335*
Lee, Y-W., Terranova, C., Birkaya, B., Narla, S., Kehoe, D., Parikh,P., Dong, S., Ratzka, A., Brinkmann, H., Aletta, J., Tzanakakis, E., Stachowiak, E.K., Claus, P., and Stachowiak, M.K. (2012). ***J. Cell. Biochem.,*** *113, 2920-2930*
O. Baron, B. Foerthmann, Y-W. Lee, C. Terranova, A. Ratzka, E. K. Stachowiak, C. Grothe, P. Claus and M.K. Stachowiak (2012). J. Biol. Chem. 287, 19827-19840

Orphan Nuclear Receptors - Nurs

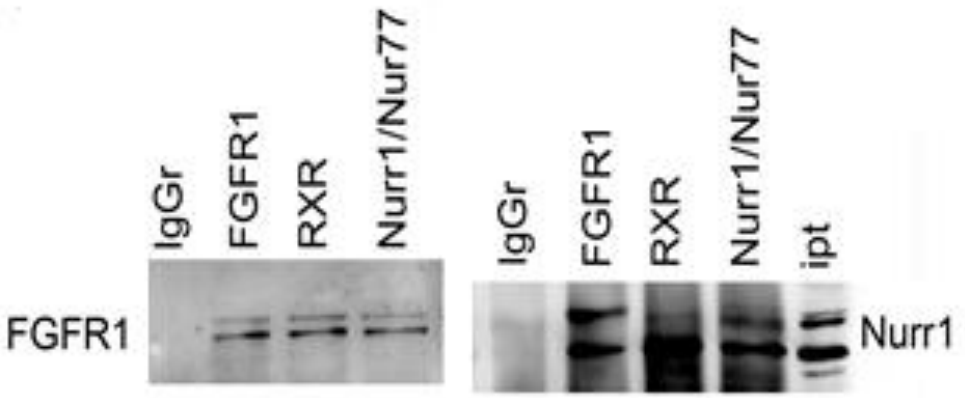


Interaction of FGFR1 with RXR Partners - orphan (Nur) receptors:

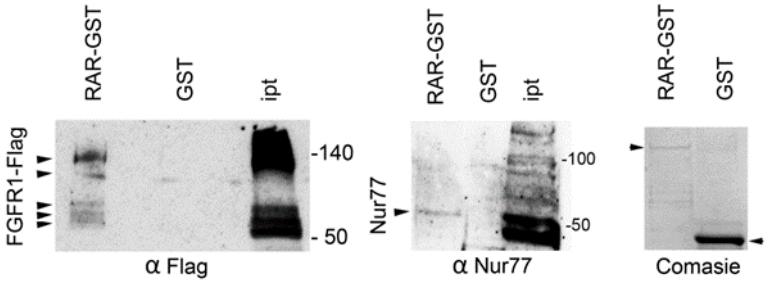
Confocal microscopy:



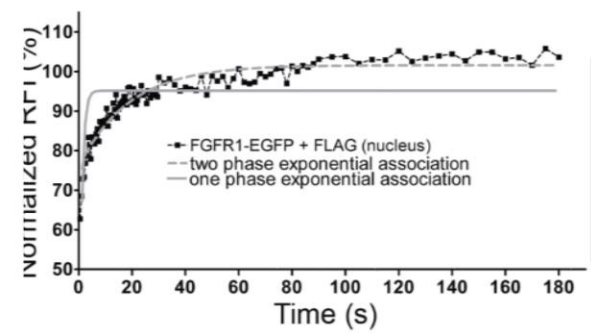
Immunoprecipitation:

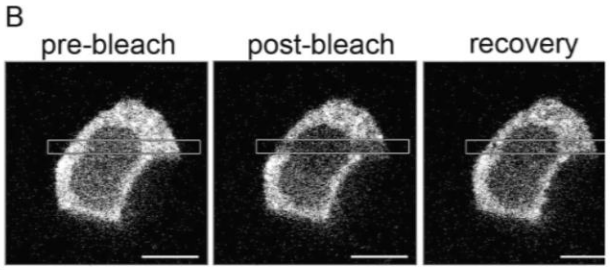


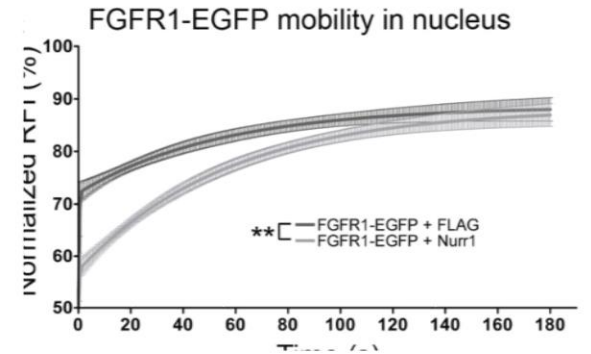
GST –RAR

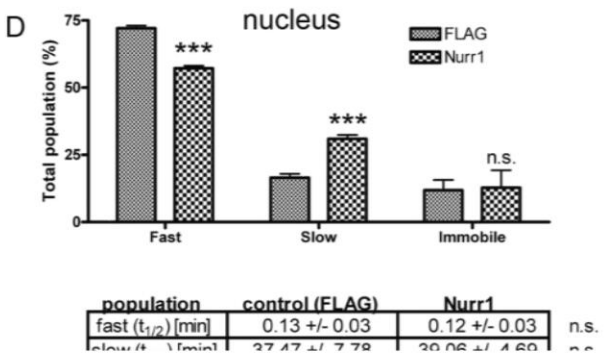


FGFR1 nuclear mobility is reduced by conditions that promote Nur & RXR binding



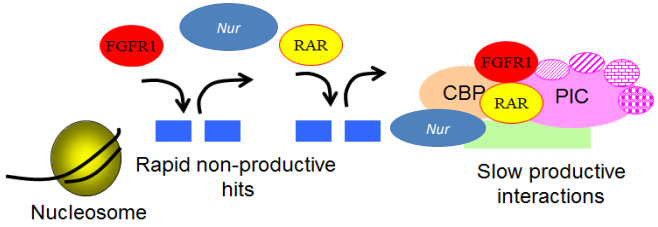






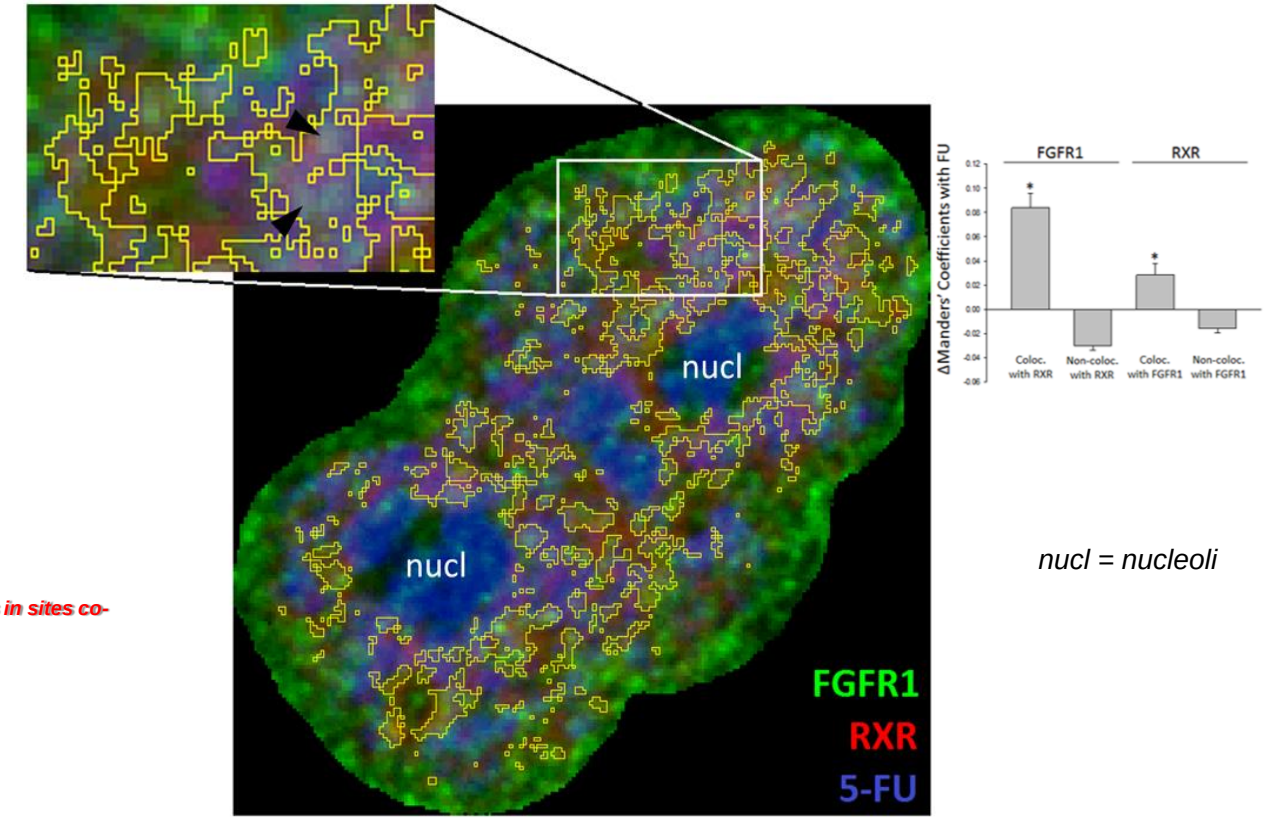
population	control (FLAG)	Nurr1	
fast ($t_{1/2}$) [min]	0.13 +/- 0.03	0.12 +/- 0.03	n.s.

R1-EGFP nuclear population	- RA	+RA
fast ($t_{1/2}$)	0.4 sec	1.0 sec
% population	17%	21%
slow ($t_{1/2}$)	24.8 sec	224.4 sec
% population	16.0%	32.2%
immobile % population	67%	46.8%



FGFR1 & RAR oscillations

Co-distribution of FGFR1, RXR and Transcriptional Domains (5'-FU) in the nucleus

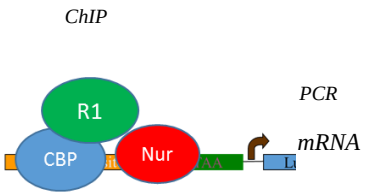


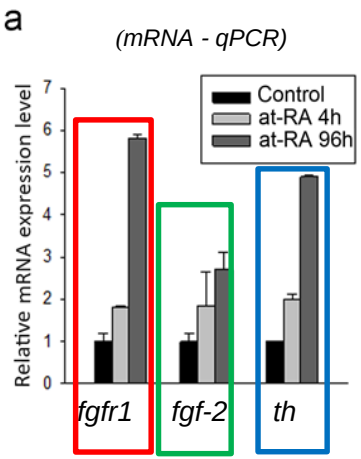
nucl = nucleoli

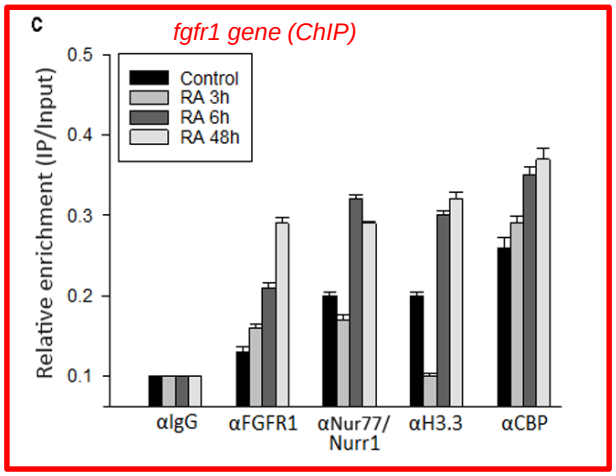
The probability of active transcription increases in sites co-inhabited by both RXR and FGFR1

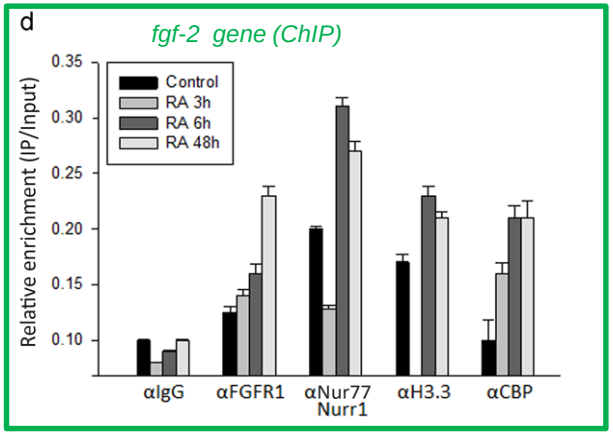
Lee, Y-W., Terranova, C., Birkaya, B., Narla, S., Kehoe, D., Parikh,P., Dong, S., Ratzka, A., Brinkmann, H., Aletta, J., Tzanakakis, E., Stachowiak, E.K., Claus, P., and Stachowiak, M.K. (2012). A novel nuclear FGF Receptor-1 partnership with retinoid and Nur receptors during developmental gene programming of embryonic stem cells J. Cell. Biochem., 113, 2920-2930

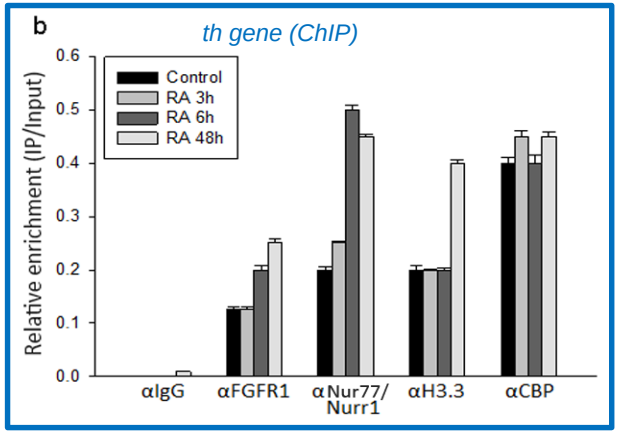
FGFR1 binds to RA-activated genes: fgfr1, fgf-2 & th





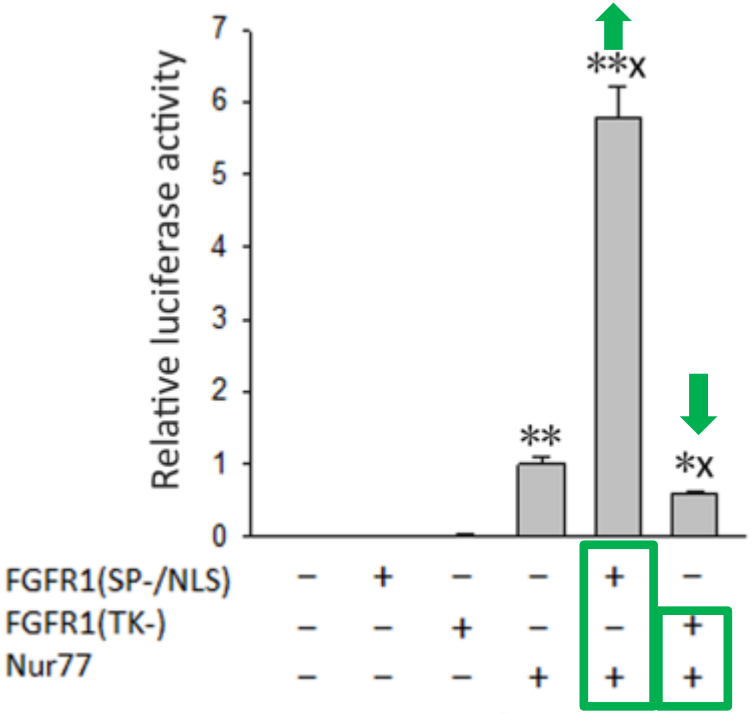


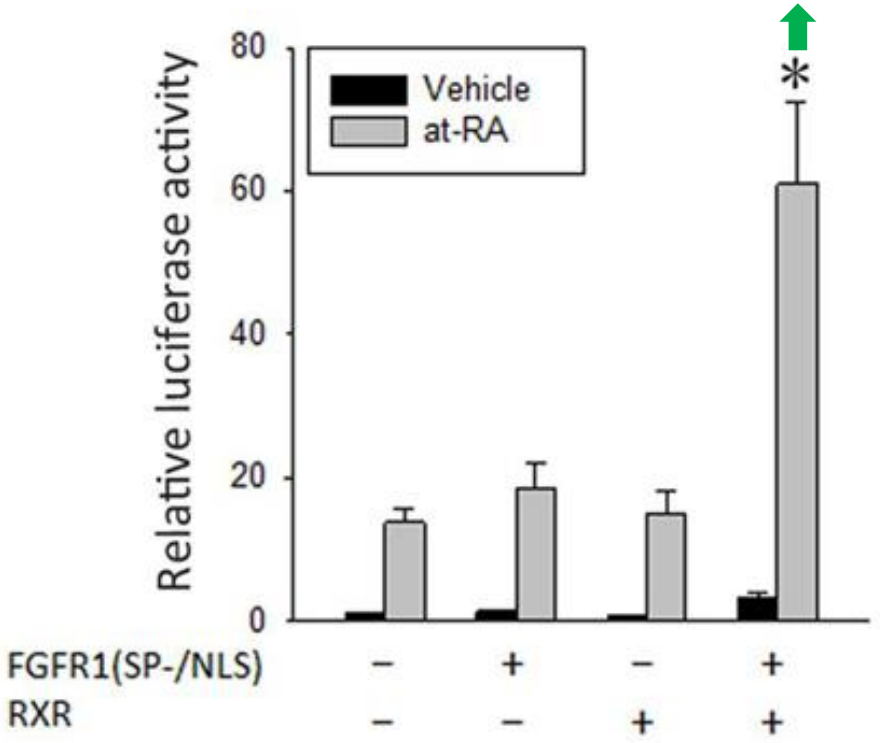


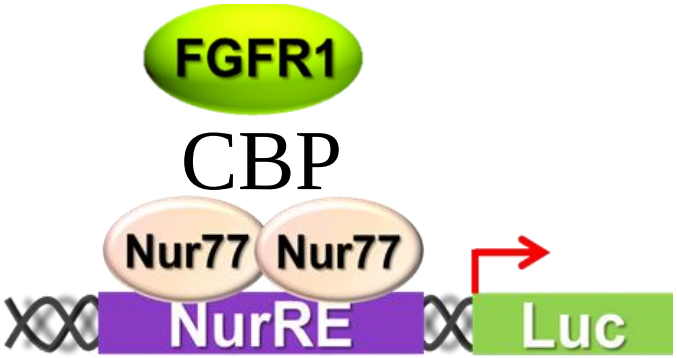


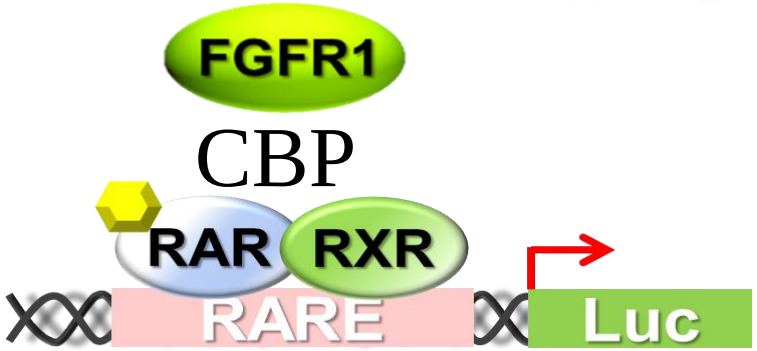
•Lee, et al., **J. Cell. Biochem.,** 113, 2920-2930; **PLOS ONE,** in press (2013).; Baron, et al., **.J Biol. Chem.** 287, 19827-19840

Nuclear FGFR1 activates genomic enhancers ..





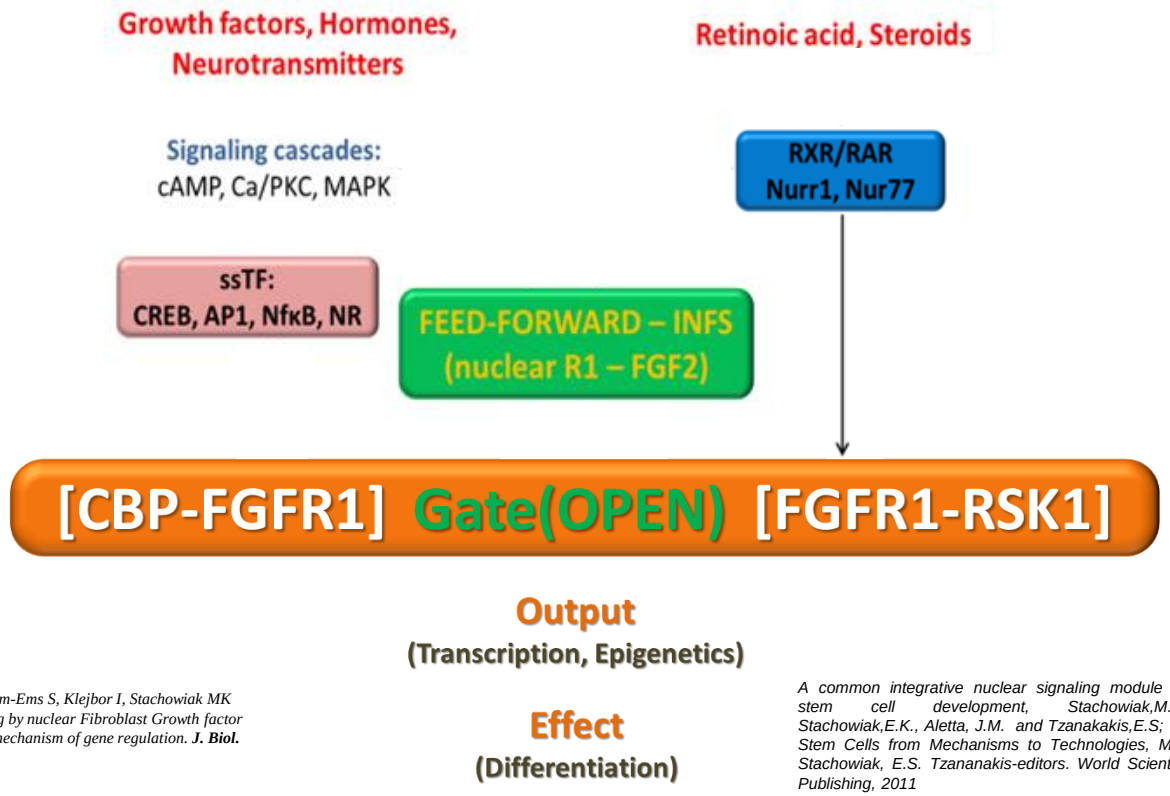




Lee, Y-W., Terranova, C., Birkaya, B., Narla, S., Kehoe, D., Parikh,P., Dong, S., Ratzka, A., Brinkmann, H., Aletta, J., Tzanakakis, E., Stachowiak, E.K., Claus, P., and Stachowiak, M.K. (2012). ***J. Cell. Biochem.,*** *113, 2920-2930; Baron et al., J. Biol. Chem. 2013…..*

Y-W.Lee, E. K. Stachowiak, B. Birkaya, C. Terranova, M. Capacchietti,P. Claus, J. M. Aletta, M.K. StachowiakPLOS ONE, in press (2013).

INFS act through Feed-Forward- And-Gate Module



Fang X, Stachowiak EK, Dubham-Ems S, Klejbor I, Stachowiak MK (2005) Control of CBP signaling by nuclear Fibroblast Growth factor receptor-1 (FGFR1) – a novel mechanism of gene regulation. ***J. Biol. Chem****. – 280(31):28451-62.*

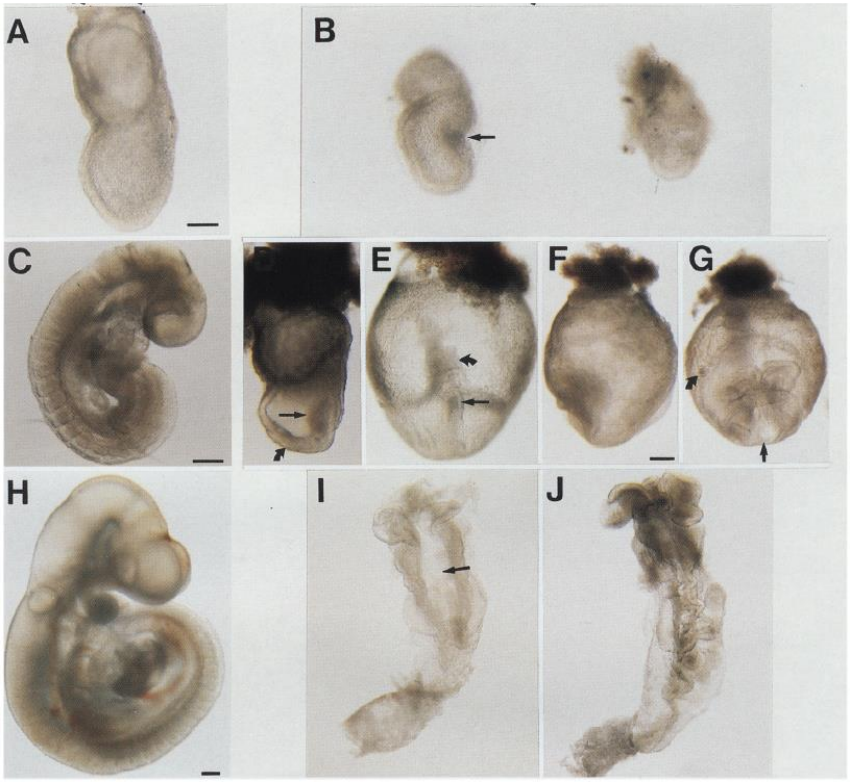
A common integrative nuclear signaling module for stem cell development, Stachowiak,M.K., Stachowiak,E.K., Aletta, J.M. and Tzanakakis,E.S; In: Stem Cells from Mechanisms to Technologies, M.K. Stachowiak, E.S. Tzananakis-editors. World Scientific Publishing, 2011

Global genomics

FGFR1 is indispensable for ontogeny

FGFR1 +/+

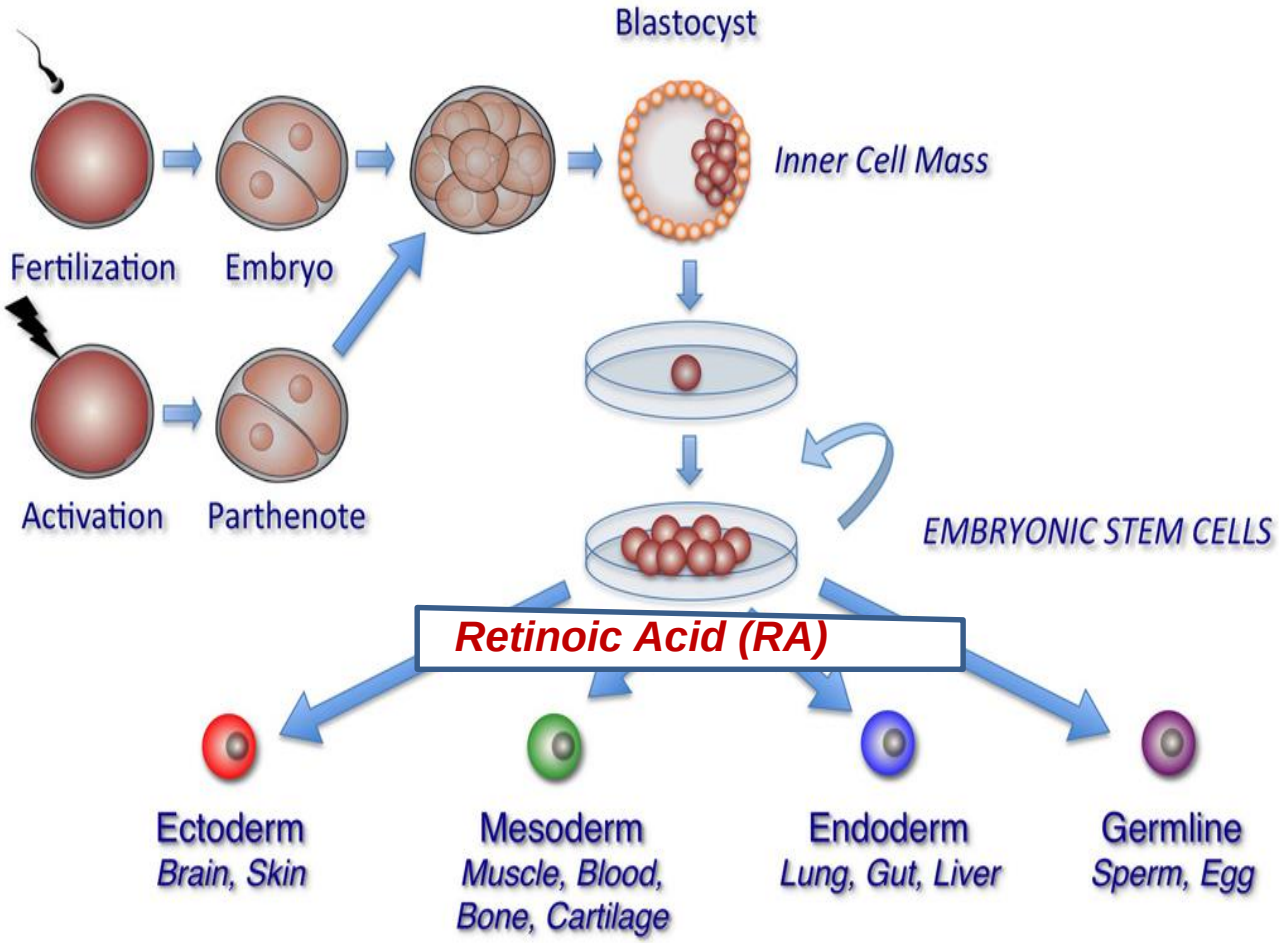
FGFR1 -/-



Masashiro Murakami
&Michael Simons

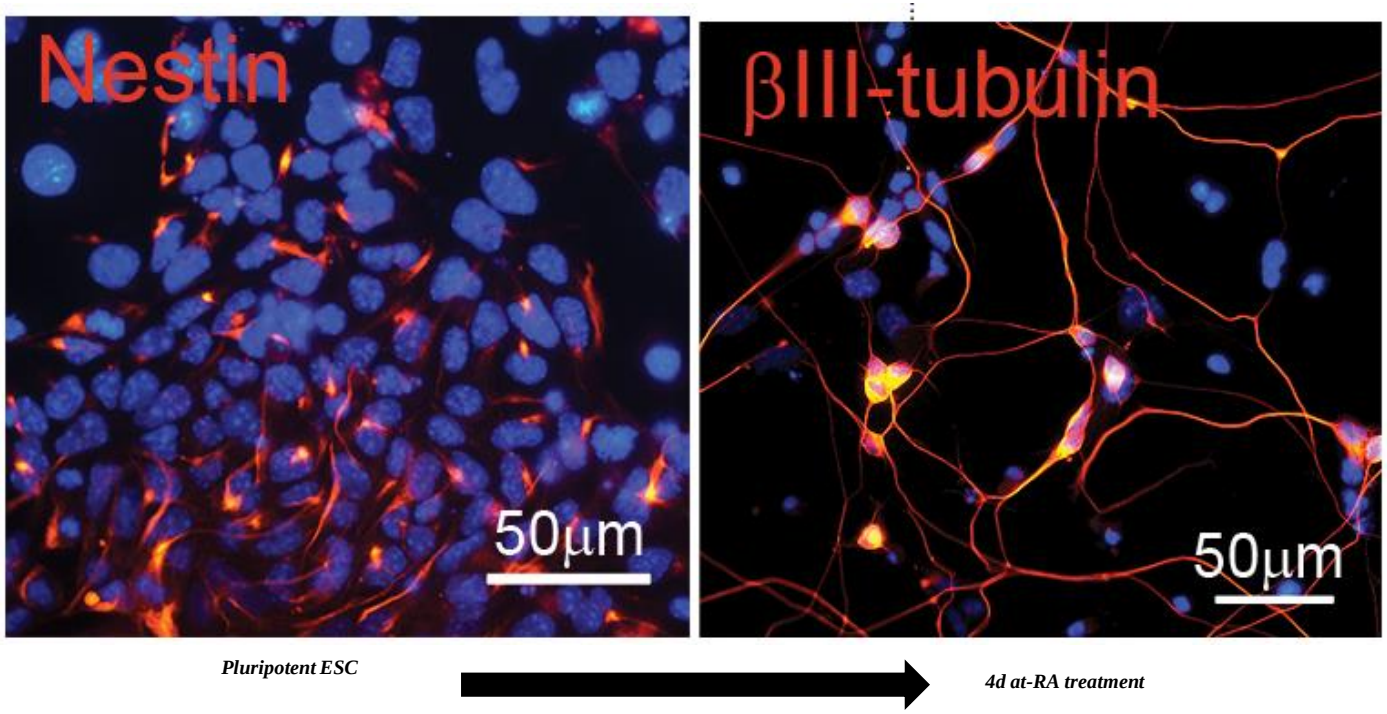
(Gene @ Dev., 8, 3045-57, 1994)

INFS in Pluripotent Embryonic Stem Cells

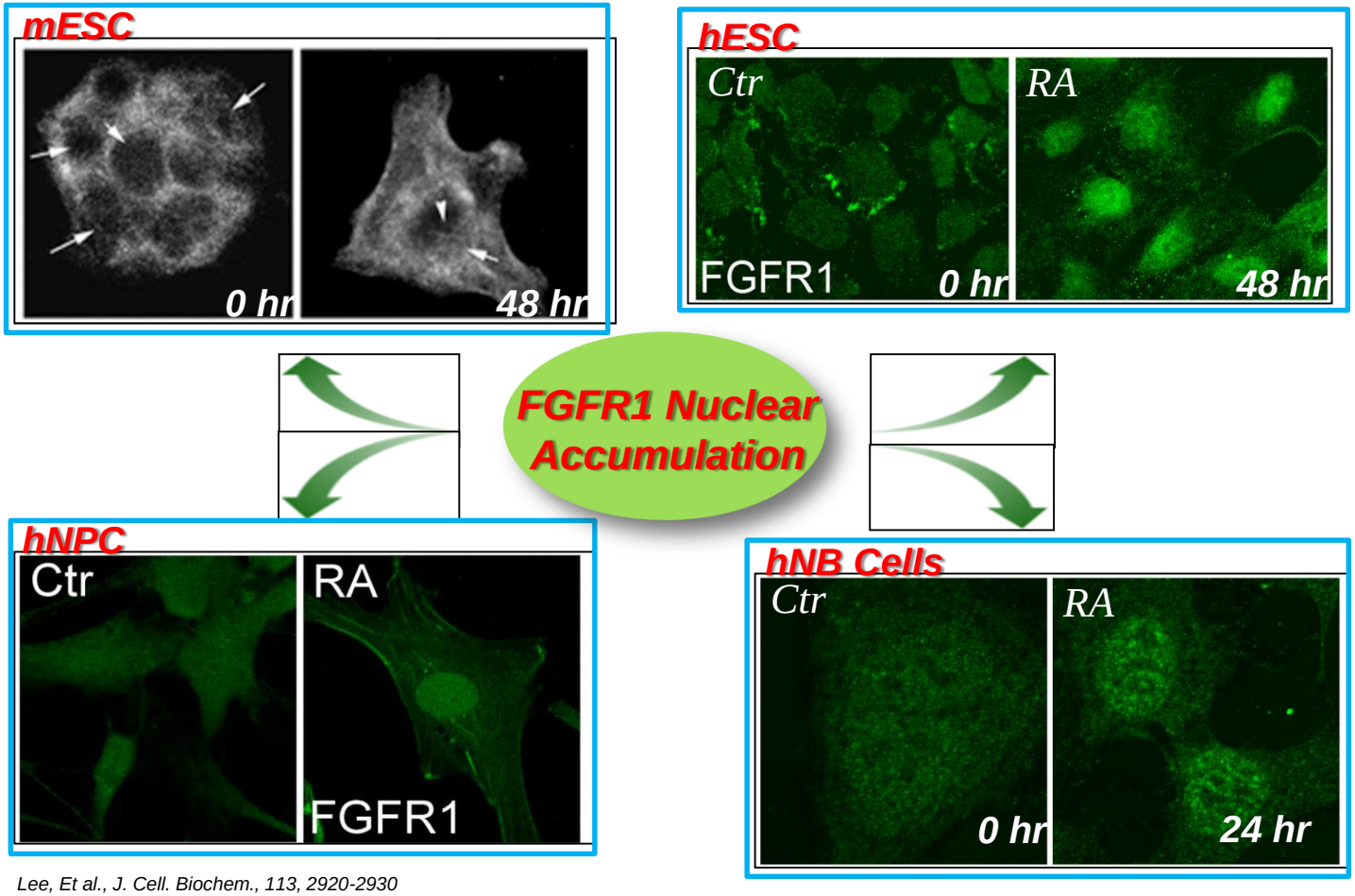


Yabut & Bernstein; Aging Volume 3, No. 5 2005

all-trans Retinoic Acid (RA) induced neuronal differentiation of pluripotent ESC

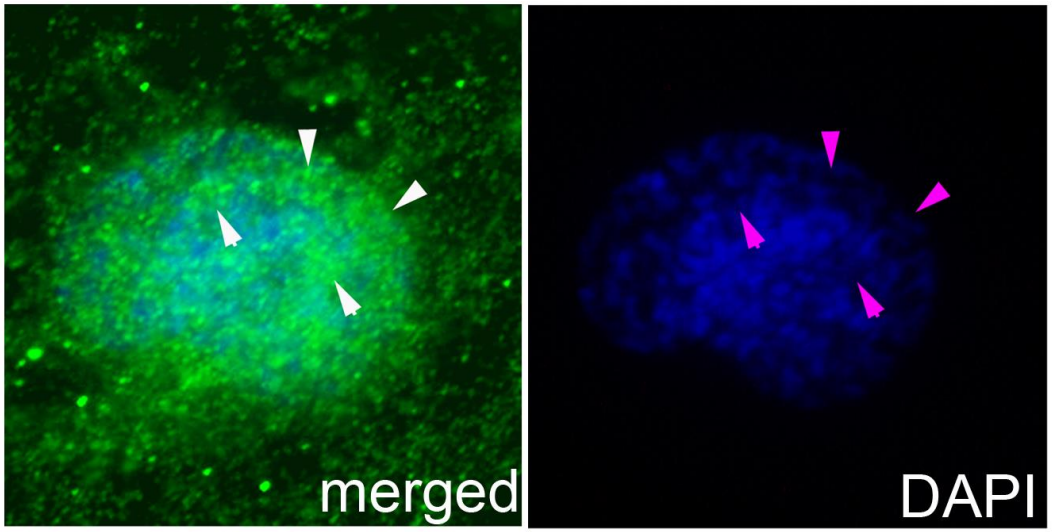


Nuclear Accumulation of FGFR1 is a common response to Retinoic Acid (RA) in Various Cells



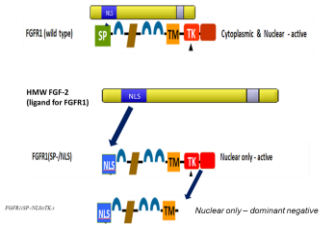
Lee, Et al., J. Cell. Biochem., 113, 2920-2930

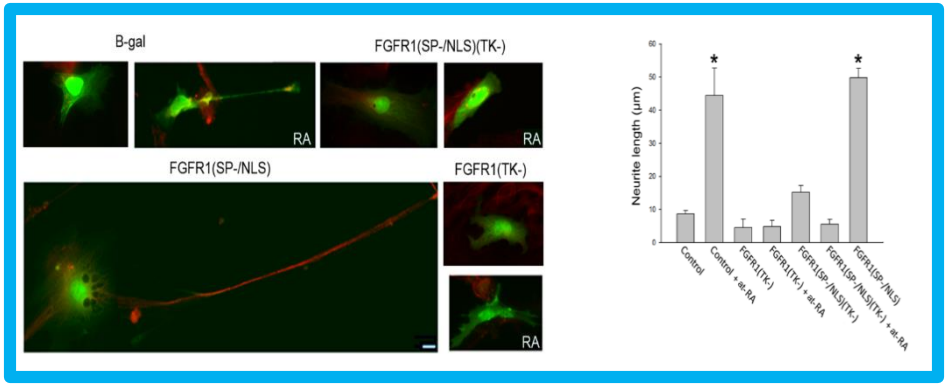
Nuclear FGFR1 is Excluded from Condensed Heterochromatin but enriched in Euchromatin.



Lee, Y-W., Terranova, C., Birkaya, B., Narla, S., Kehoe, D., Parikh,P., Dong, S., Ratzka, A., Brinkmann, H., Aletta, J., Tzanakakis, E., Stachowiak, E.K., Claus, P., and Stachowiak, M.K. (2012). A novel nuclear FGF Receptor-1 partnership with retinoid and Nur receptors during developmental gene programming of embryonic stem cells J. Cell. Biochem., 113, 2920-2930

Nuclear FGFR1 mediates RA-induced neuronal differentiation of pluripotent mESC





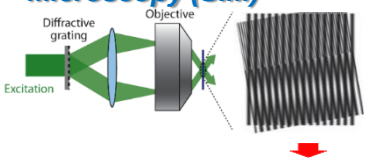
Lee, Y-Wet al., (2012). A novel nuclear FGF Receptor-1 partnership with retinoid and Nur receptors during developmental gene programming of embryonic stem cells J. Cell. Biochem., 113, 2920-2930

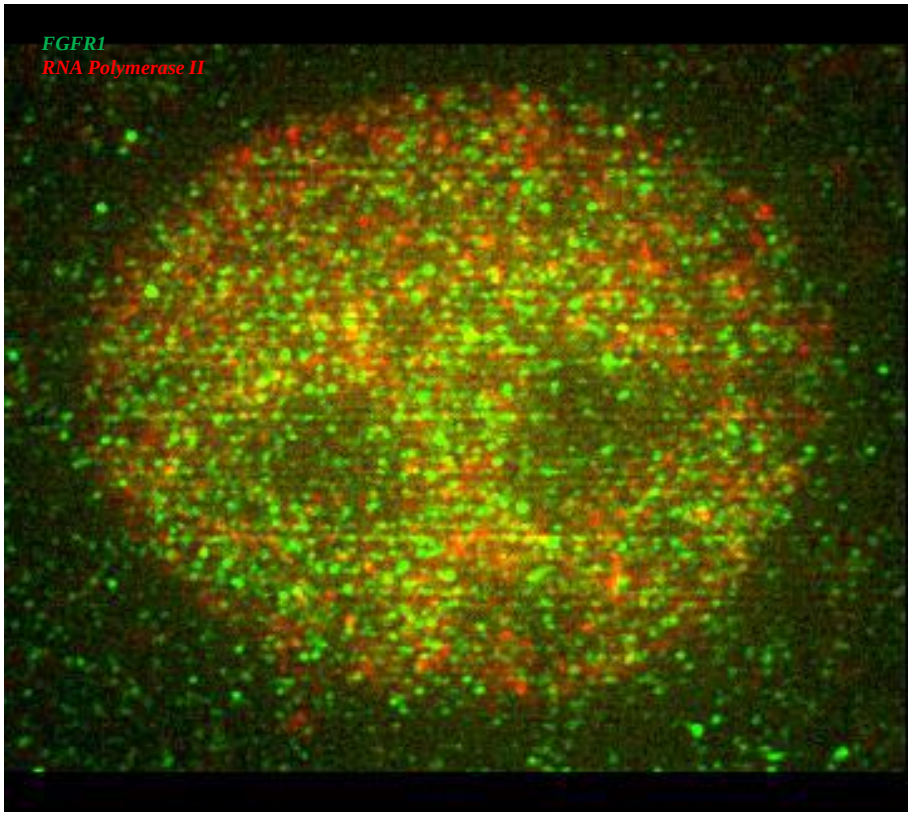
To infinity and beyond…

Buzz Lightyear

FGFR1 localizes within transcription domains

Structured-Illumination Microscopy (SIM)





Hypothesis:

nGFR1 and NR partners mediate neural programing by:

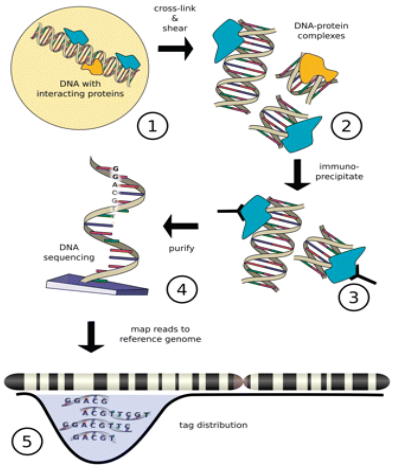
-targeting "master developmental" genes

-targeting multiple gene subsets in diverse development pathways.

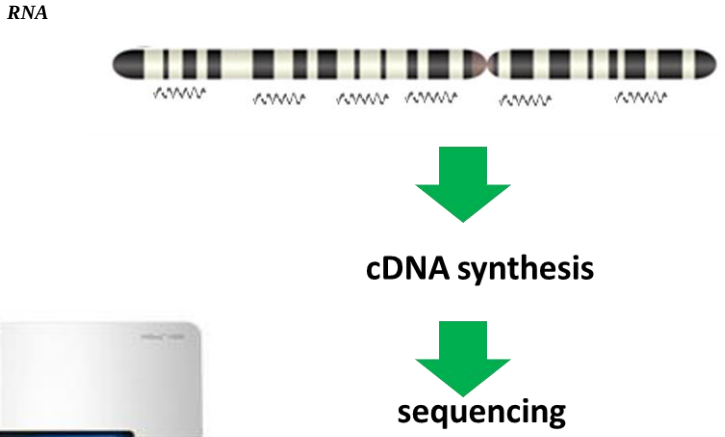
-organizing chromatin topological domains.

Insight into the whole genome !

Cross-linked Chromatin Immunoprecipitation, DNA sequencing (ChIPseq)



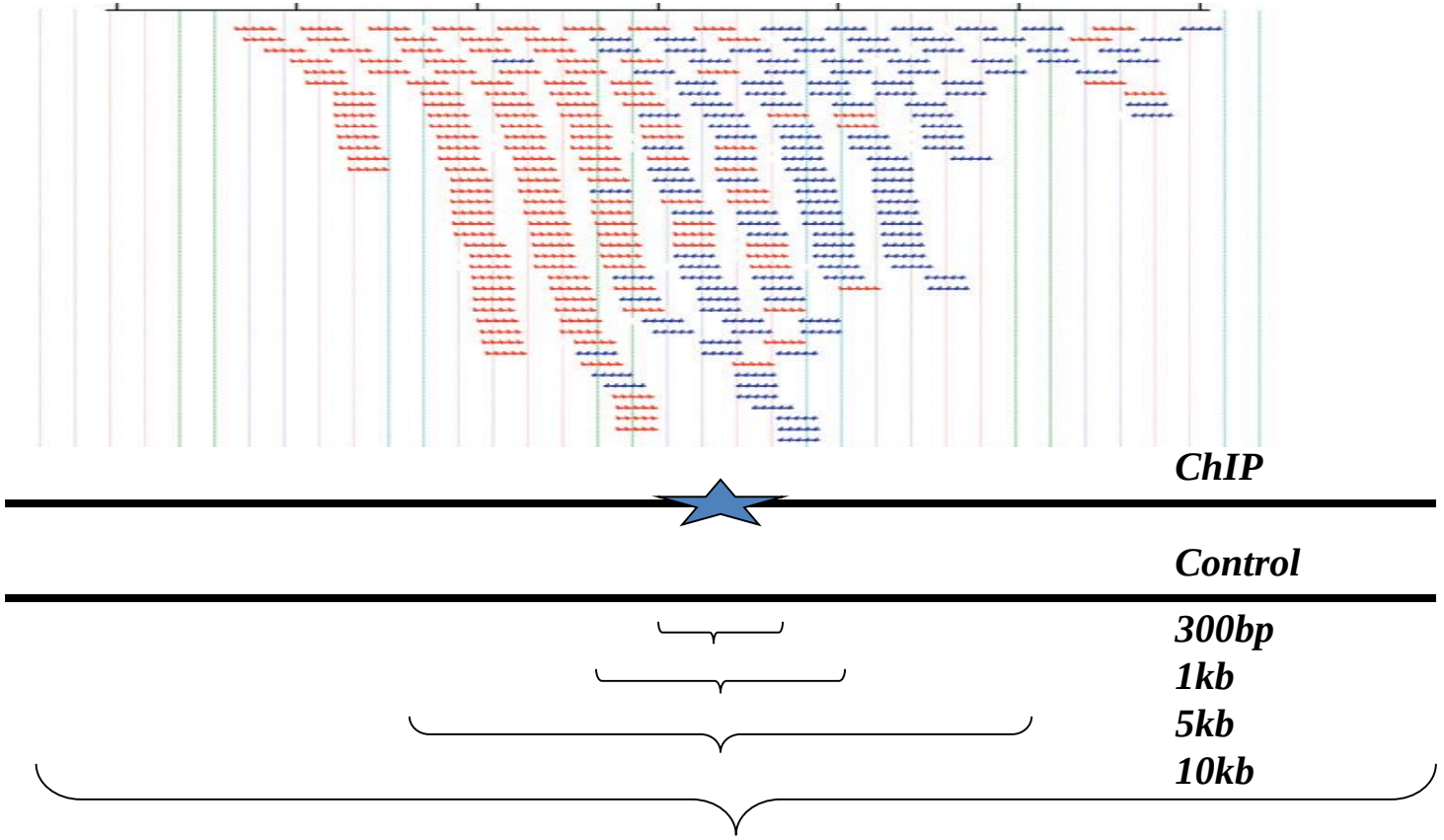
RNA and small RNA sequencing (RNAseq)



Illumina HiSeq 2000

C.Terranova, S. Narla, Yu-Wei Lee, J. Bard, A. Parikh, E.K. Stachowiak, E. S. Tzanakakis, M.J. Buck, B. Birkaya, M.K. Stachowiak, Global developmental gene programing inv
form of Fibroblast Growth Factor Receptor-1 (FGFR1). 2015, PLOS One

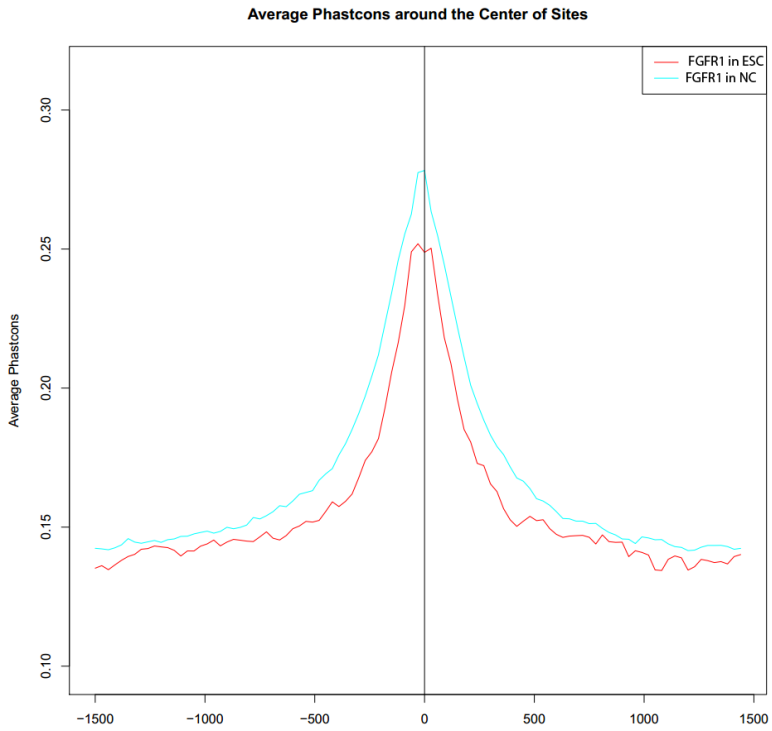
Call FGFR1, RXR and Nur77 peaks (identify binding sites) using Model Based Analysis for ChIP-seq (MACS2)



Zhang et al, Genome Bio, 2008

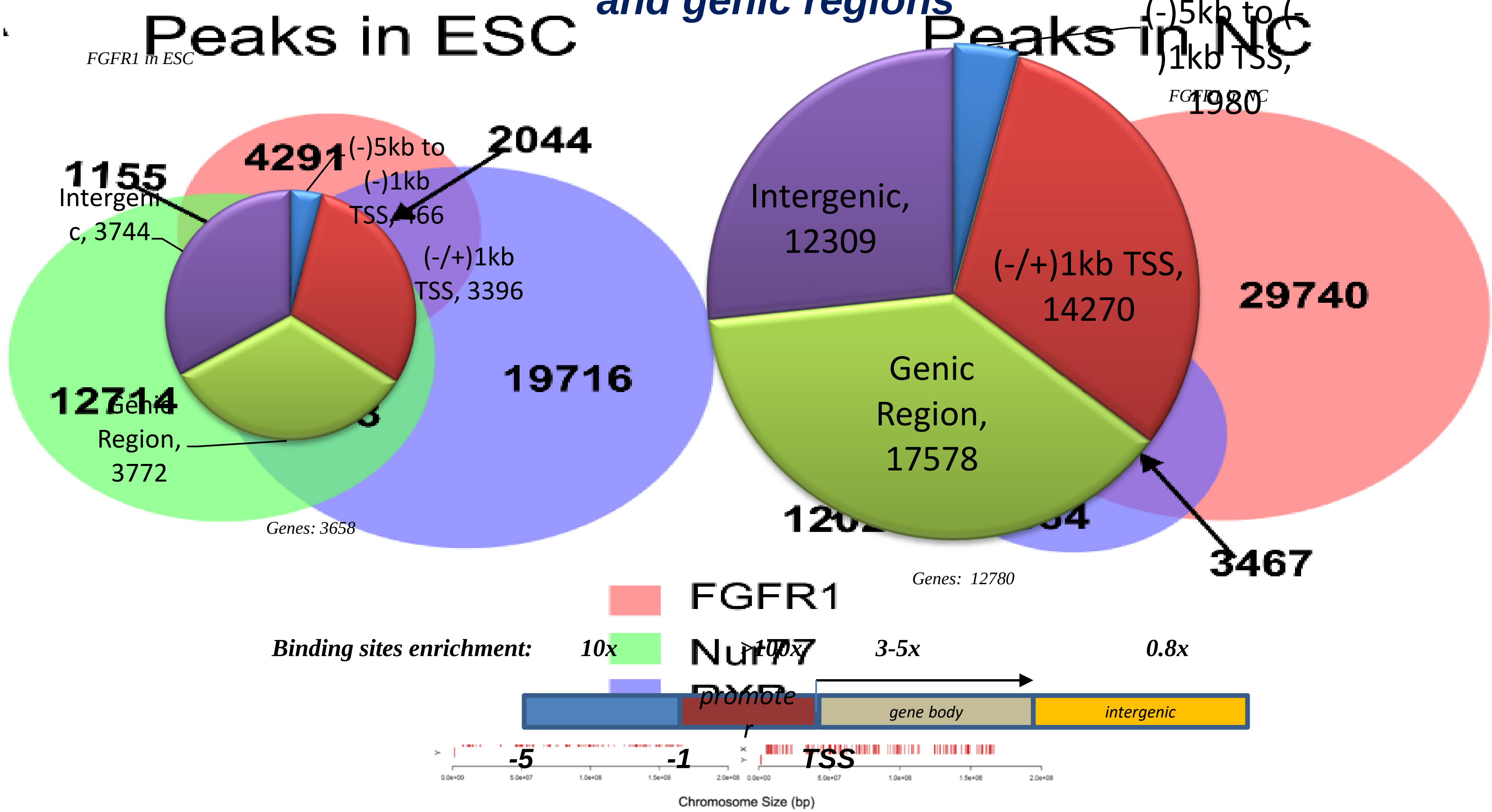

Yes!!!
FGFR1 IS NUCLEAR
and colocalizes with
RXR RAR & Nurr

Sequences of nFGFR1 targeted sites are conserved among different species

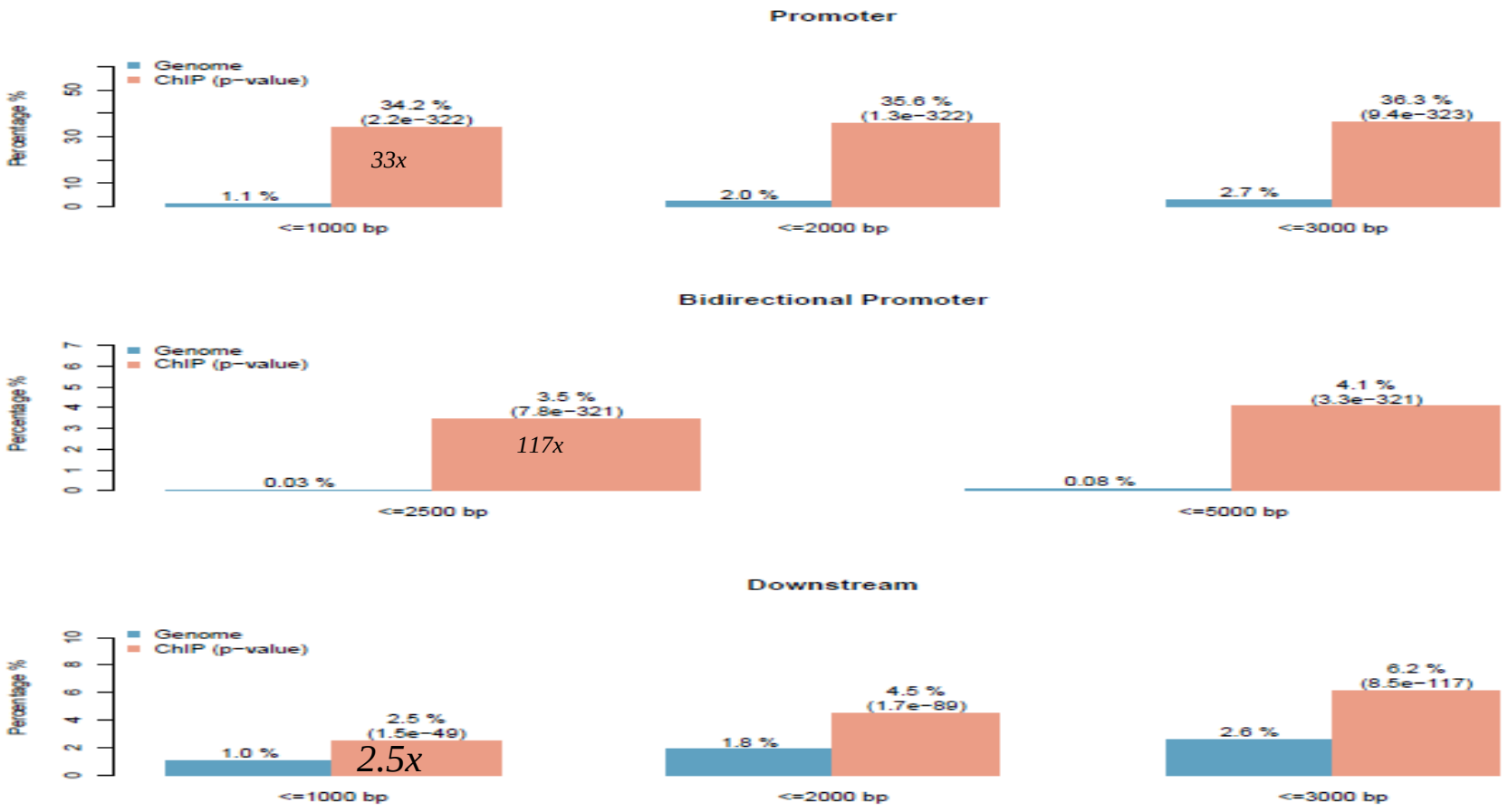


Lessons from ChIPseq:

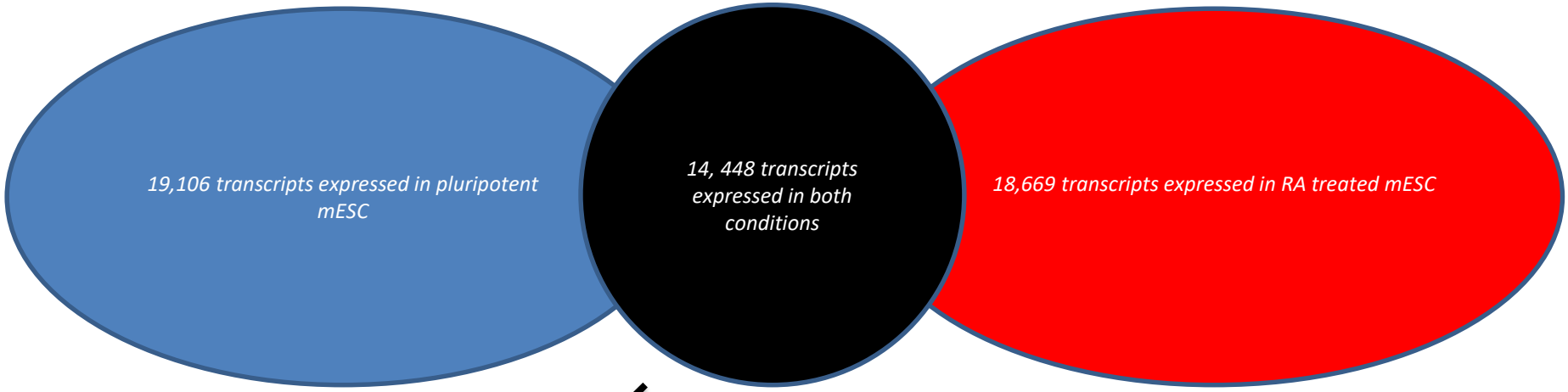
... and genic regions

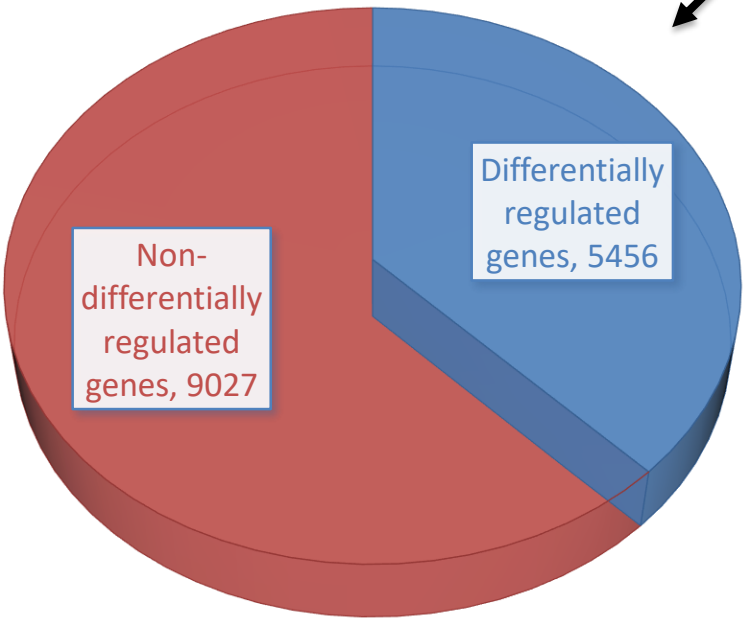


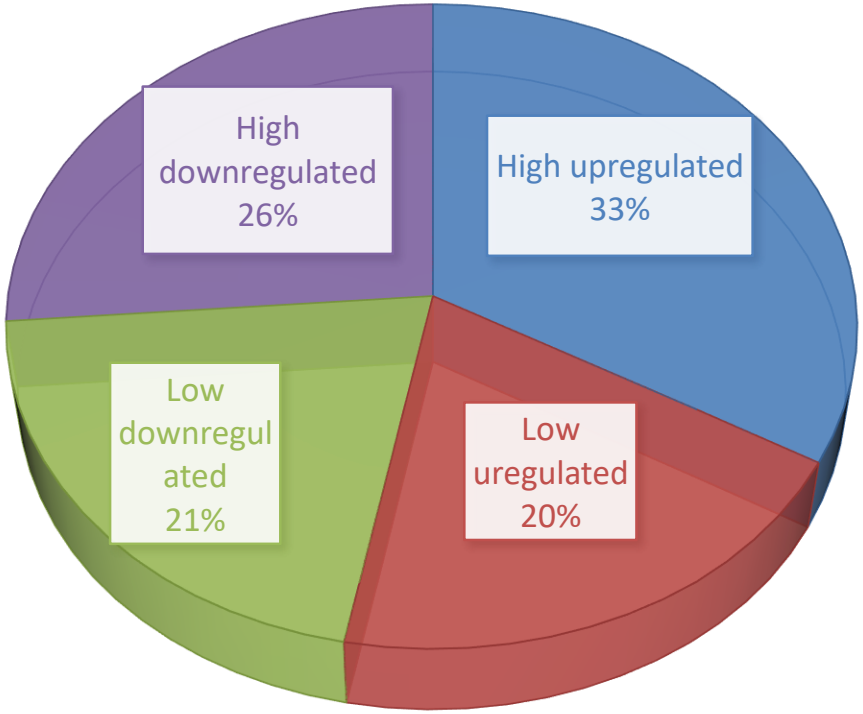
Promoter enrichment of FGFR1 peaks in differentiating mESC (+RA)



RNA-seq: differential analysis of gene expression in pluripotent and RA treated mESC

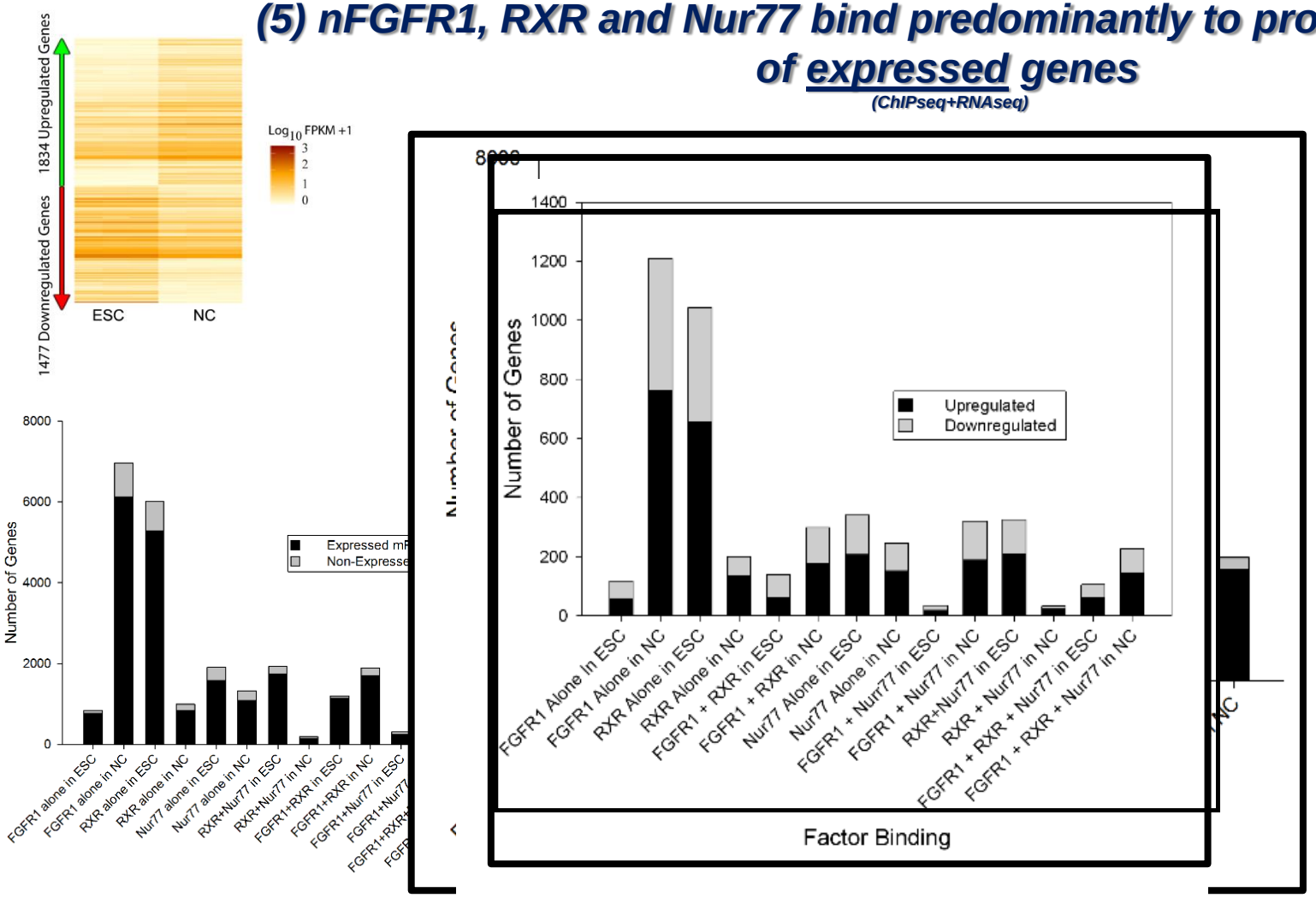




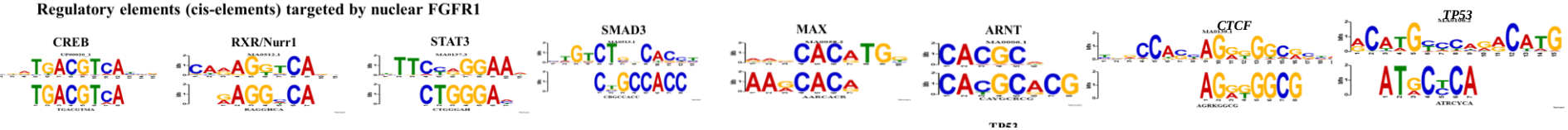


(5) nFGFR1, RXR and Nur77 bind predominantly to proximal promoters of expressed genes

(ChIPseq+RNAseq)

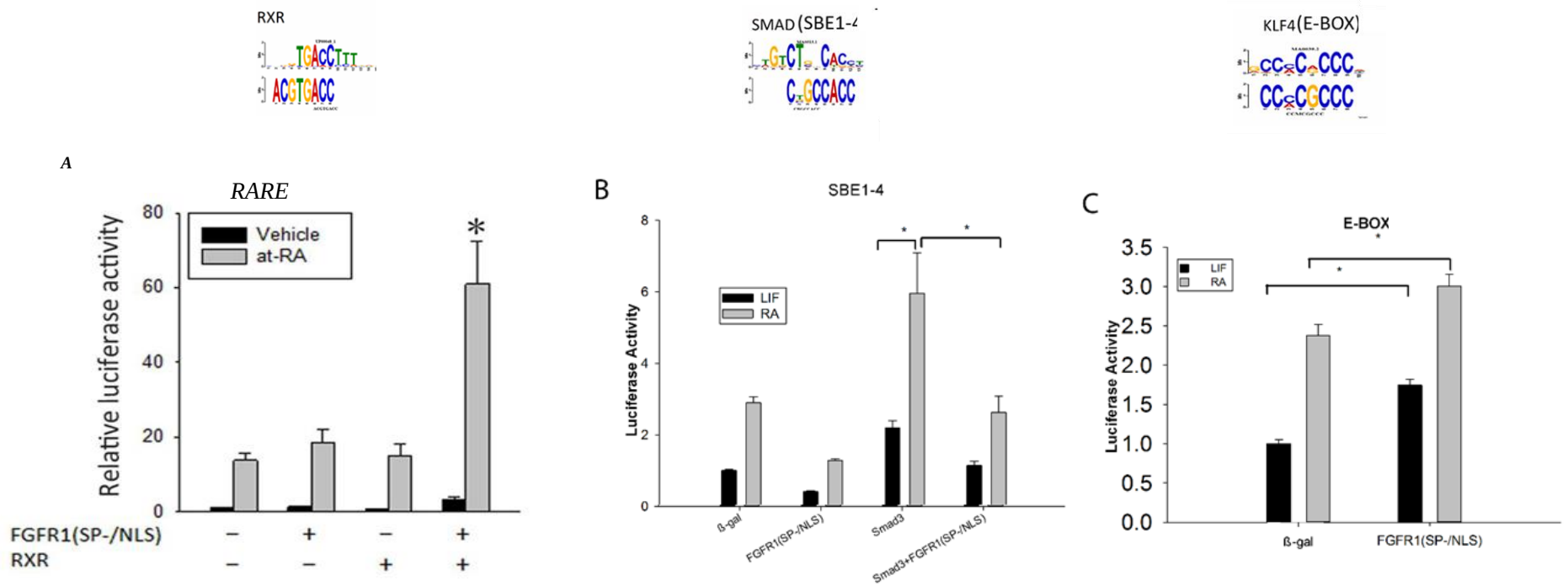


Regulatory elements (cis-elements) targeted by nuclear FGFR1



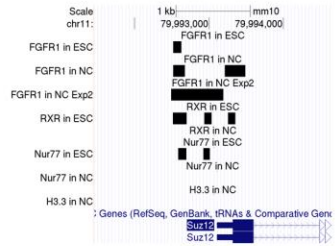
Factor	MOTIF (BOLD are unique to factor)
FGFR1	**ARNT**, ATF1, CTCF, **ERG/ELK4, KLF4,** MAX, MZF1, NRF1, Nurr1, **Pou2f3, Pou5f1:Sox2,** RARα, RFX1, RXRα, **SMAD**, Sox8, SP1, STAT, **TCF3**, **TP53,** YY1, **ZBTB33,** ZFP161
RXR	ATF1, CTCF, **Irx4**, mycn, MZF1, Nurr1, Nr1h3:RXRα, **Pitx2, Pou3f3, PPARG,** Prrx2, RARα, RFX1, RXRα, Sox8, SP1, YY1, **ZEB1**, ZFP161
Nur77	ATF1, CTCF, **Hic1**, MAX, mycn, NRF1, Nurr1, **Pax6**, Prrx2, **Six6**, SP1, STAT, RXRα, YY1, ZFP161

Nuclear FGFR1 activates RARE, E-Box and inhibits SBE

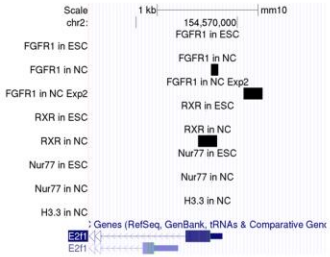


FGFR1 binding to several pluripotency genes in differentiating NC

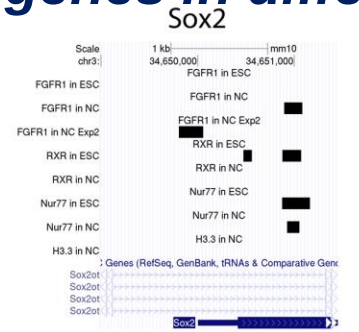
Suz12



E2f1

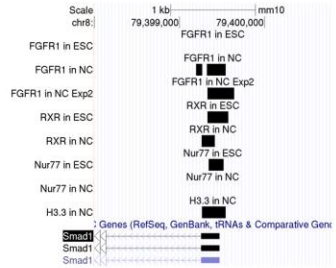


Sox2

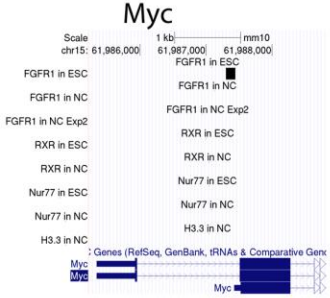


Klf4

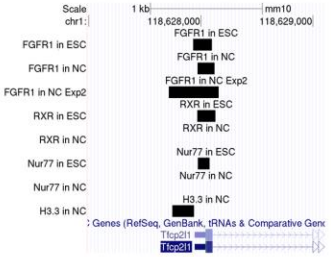
Smad1



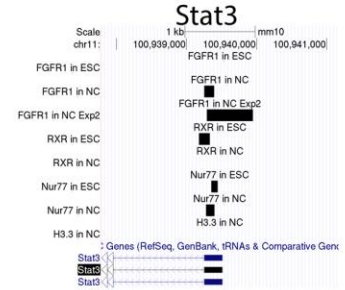
Myc



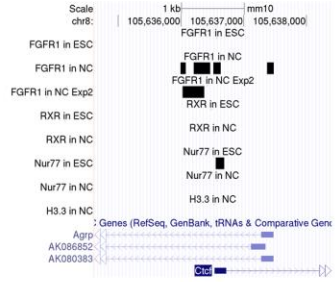
Tfcp2l1



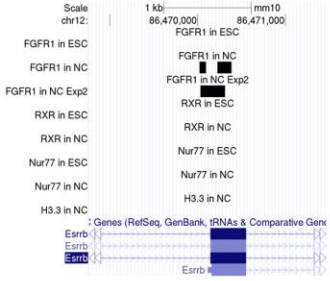
Stat3



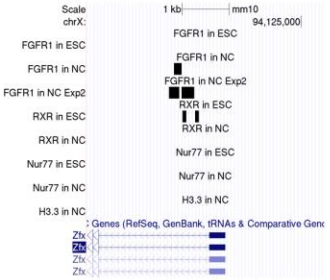
CTCF



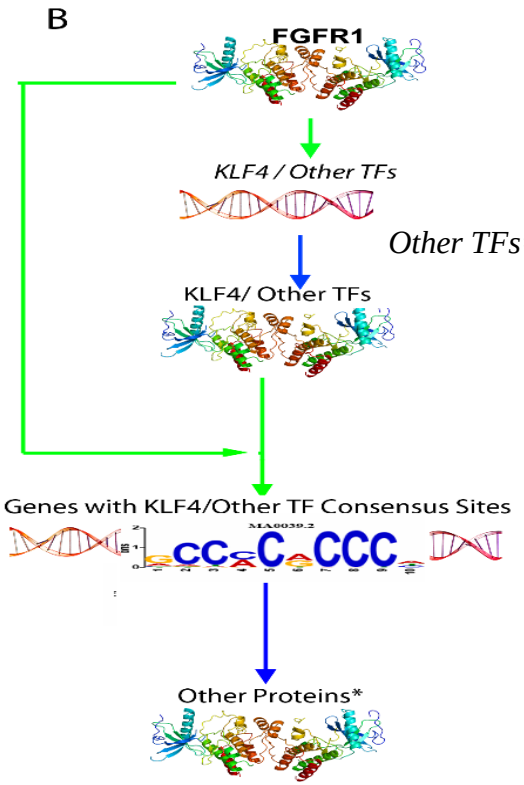
Esrrb



ZFX



nFGFR1 2-levels of gene control



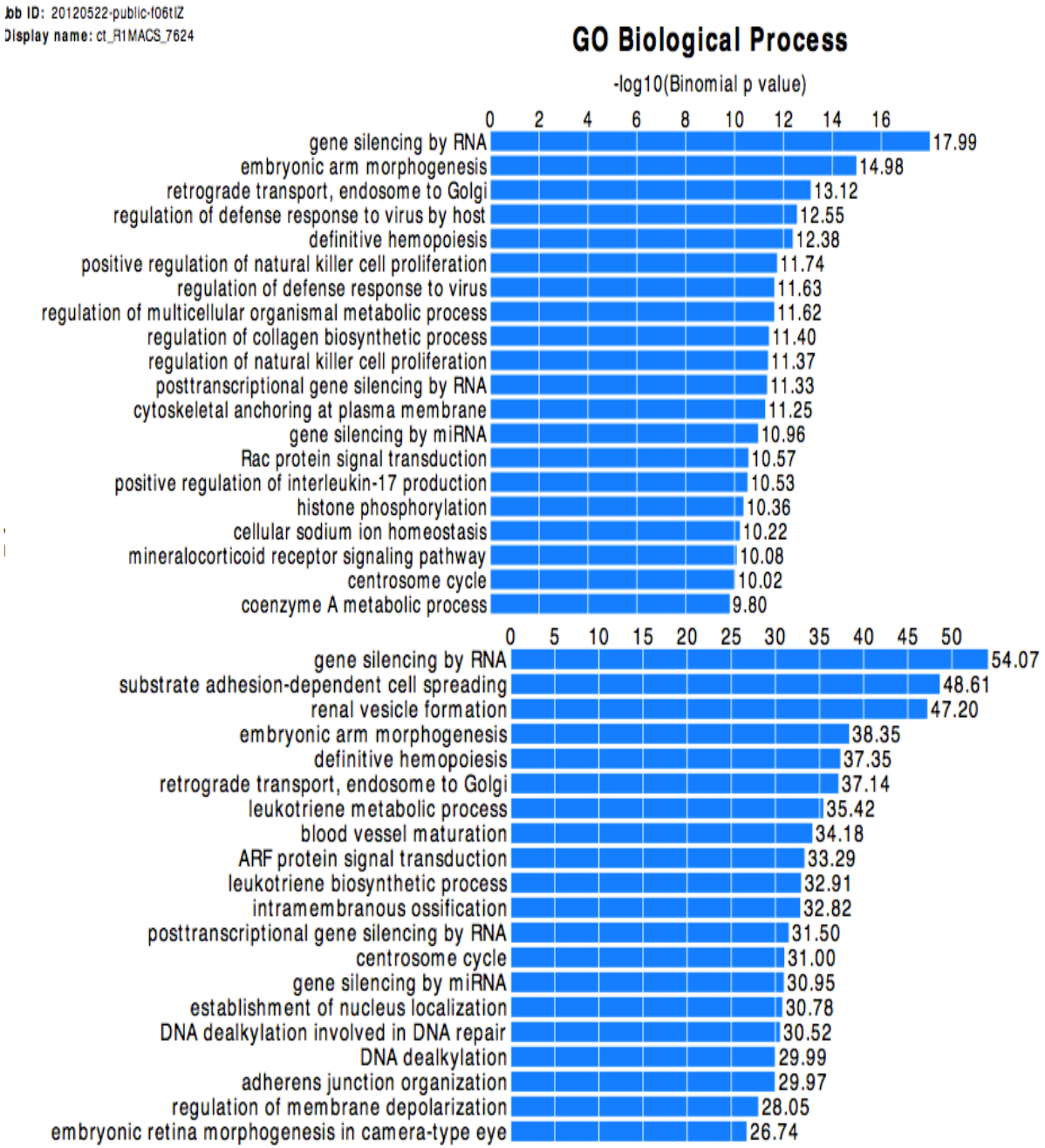
Other TFs=TP52, SMAD, CTCF, MYC, OCT4, SOX2 and STAT3

Global developmental gene programing involves a nuclear form of Fibroblast Growth Factor Receptor-1 (FGFR1). 2015, PLOS One, in press

FGFR1-binding gene ontology

- *RA*

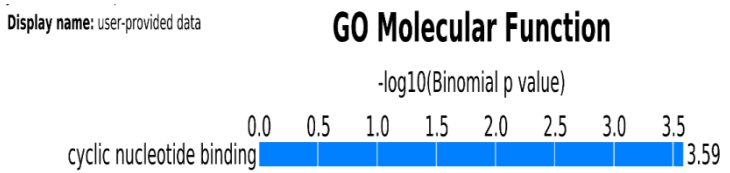
- *+RA*

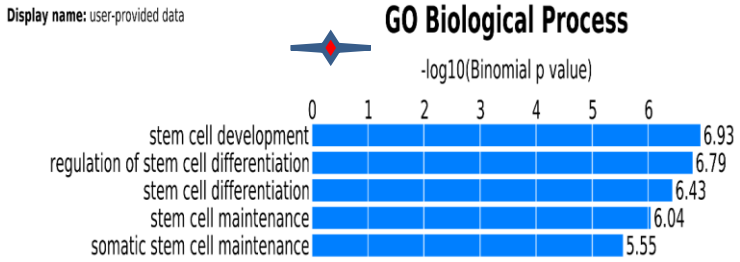


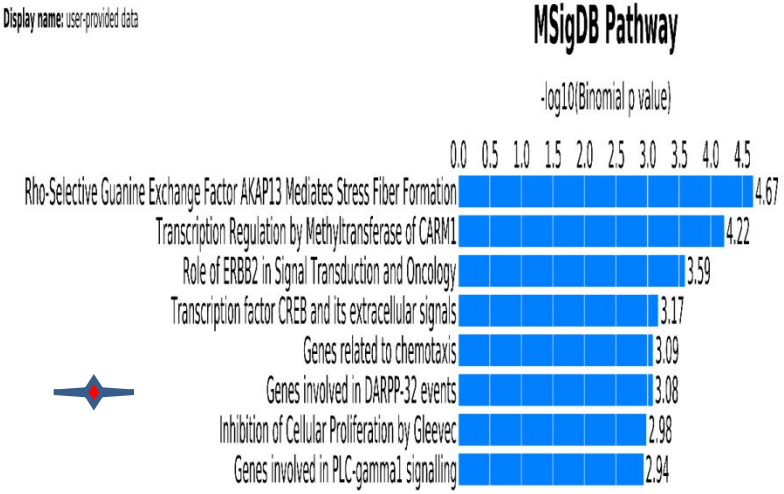
GREAT Gene Ontology based on FGFR1 binding sites

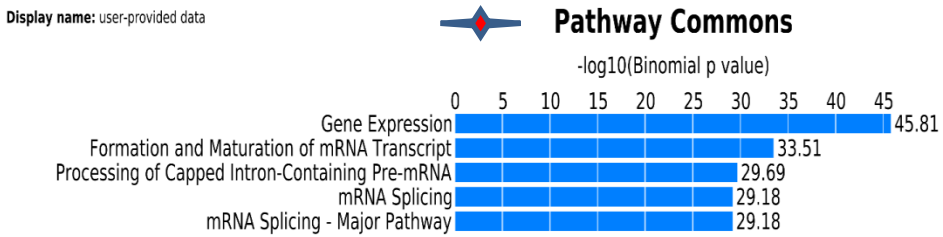
Pluripotent mESC

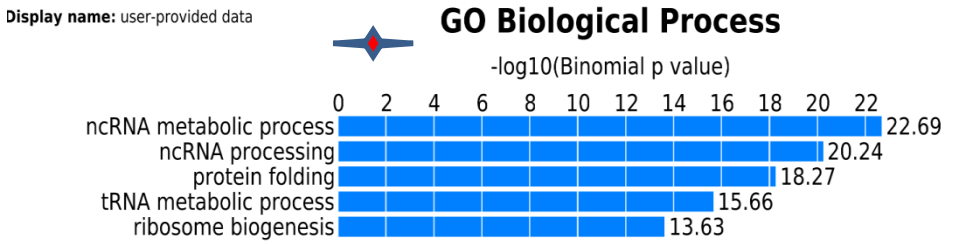
48h at-RA treated mESC

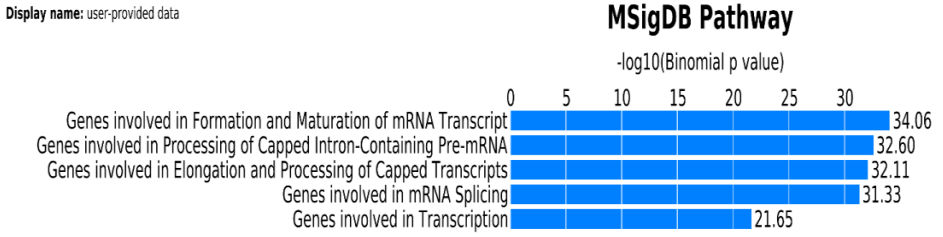


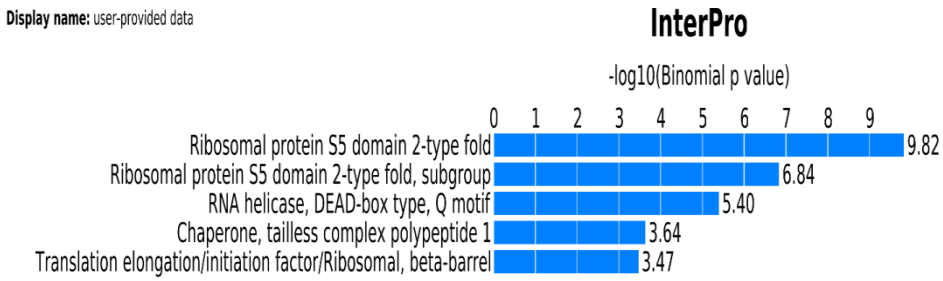












Ingenuity Pathway Analysis (IPA)
Enriched pathways:

FGFR1

-Pluripotency
-Axonal guidance
-Wnt/β-catenin
-ESC pluripotency
-Cardiogenesis
-Osteonegesis
-cancer
- Hair and Skin Development and Function

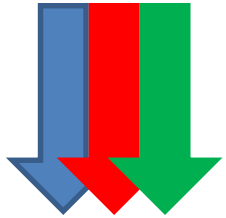
FGFR1
+RXR

--Pluripotency
- Nervous system developmnet
-Axonal guidance
-Wnt/β-catenin
-ESC pluripotency
-Cardiogenesis
-Basal cells carcinoma
-myogenic developmnet

FGFR1
+Nur

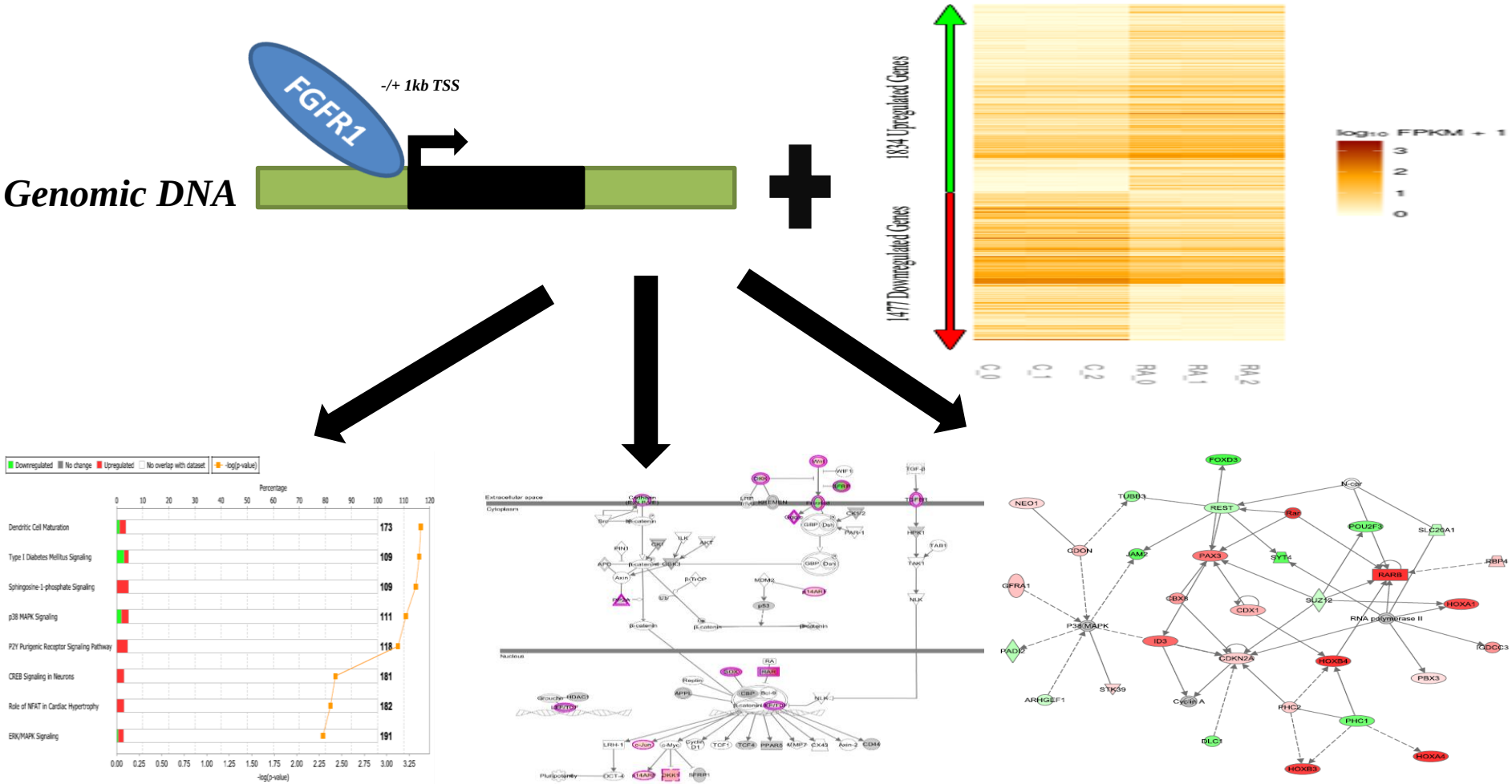
-Axonal guidance
-Wnt/β-catenin
-ESC pluripotency
-Nitric Ox. Signaling
-Dopamine-DARP signaling
-G protein receptor signaling

FGFR1
+RXR
+Nur



Metabolism:
-glucose
-glycogene
-folate
-histidine

Ingenuity Pathway Analysis (IPA): Combination of ChIP- and RNA-seq

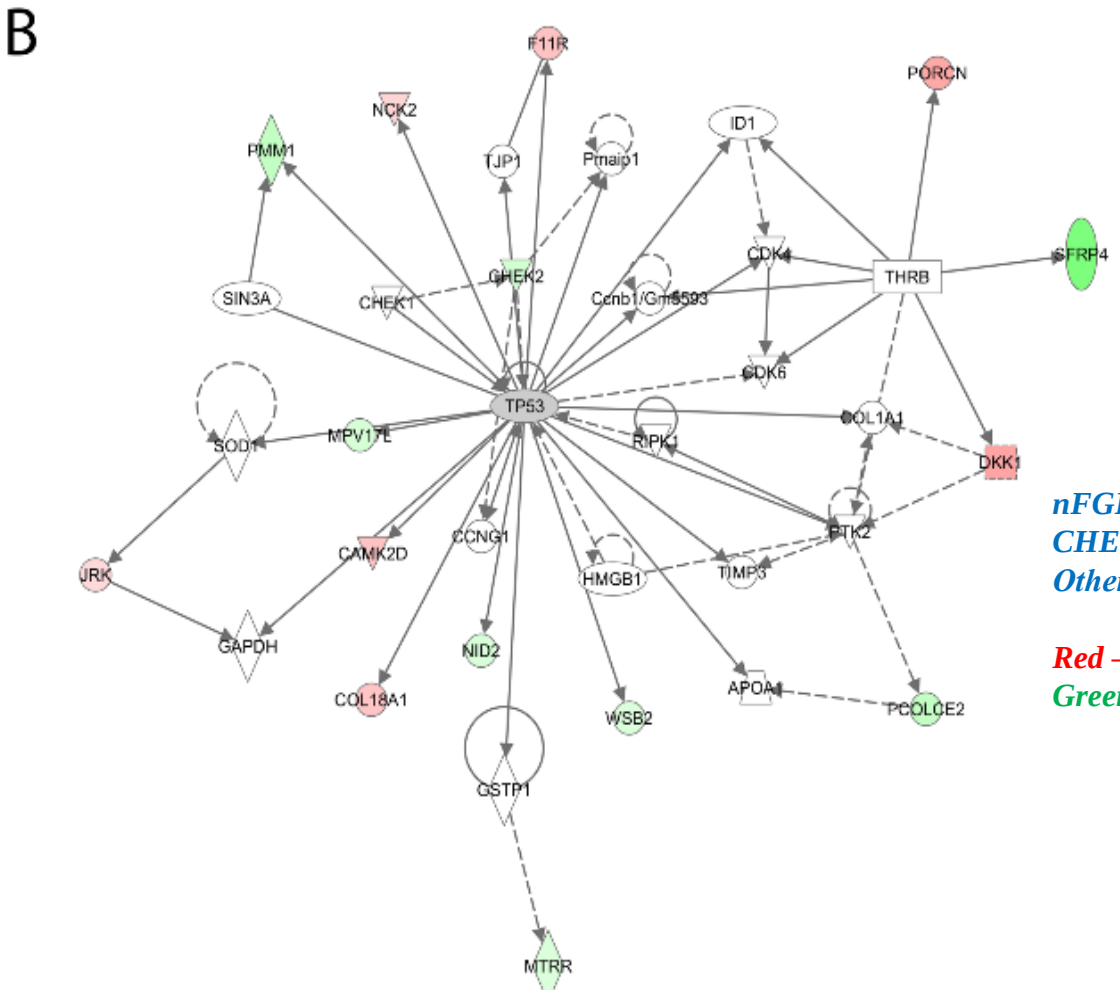


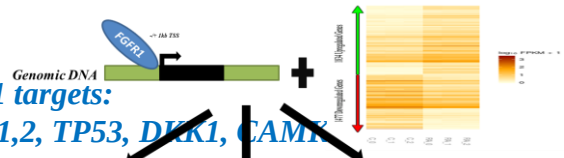
Wnt

Organ developmnet

Ingenuity Pathway Analysis (IPA): Combination of ChIP- and RNA-seq nFGFR1 alone targets regulated genes of cell proliferation and survival pathways

Top network controlling cell proliferation and survival:

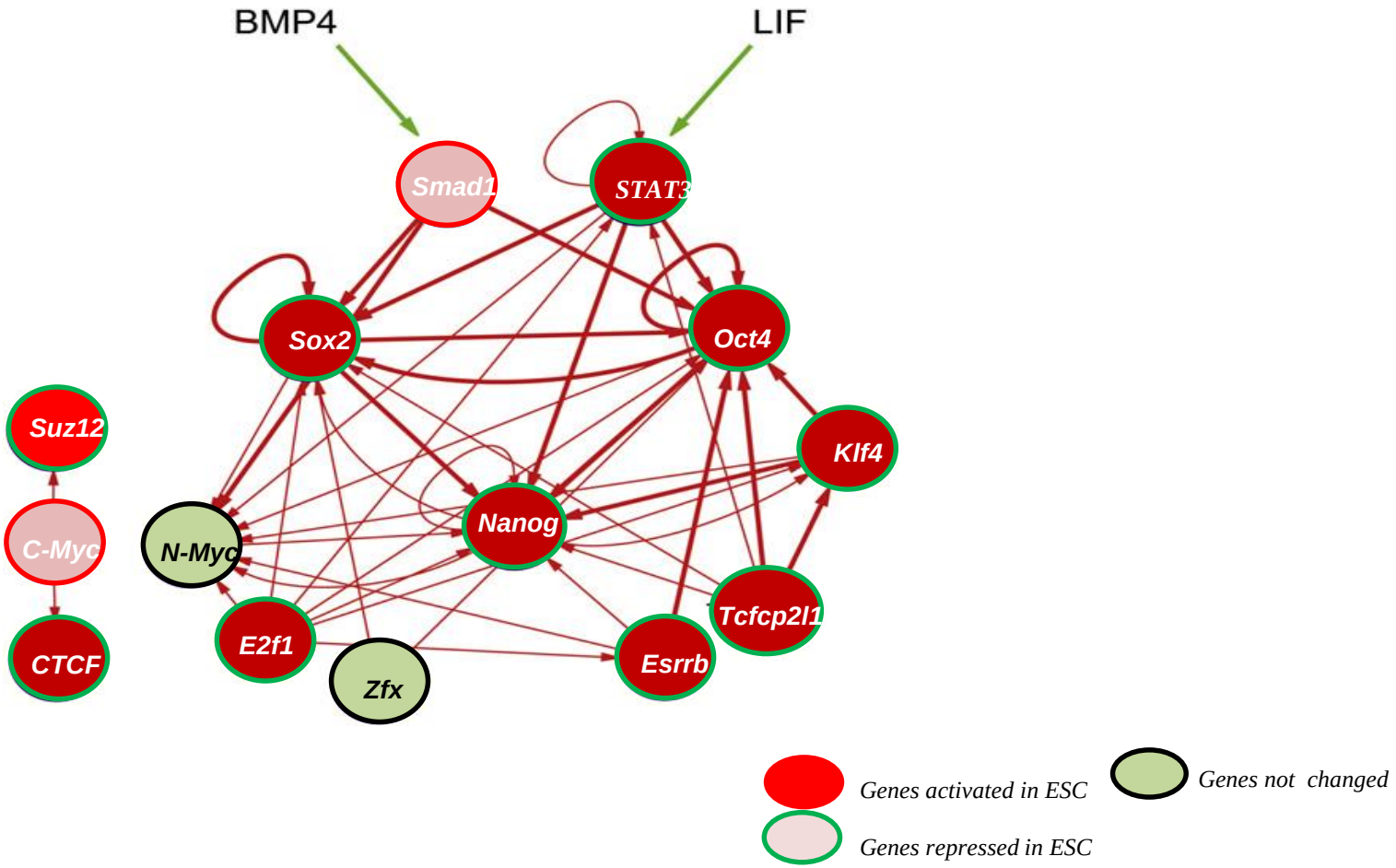




nFGFR1 targets:
CHECK1,2, TP53, DKK1, CAMK
Other: BRCA1, fos, jun, myc, akt, …

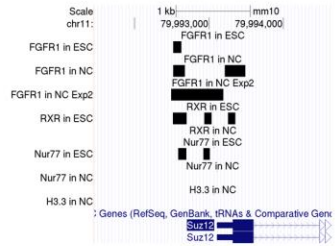
Red – activated genes
Green – inhibited genes

Active pluripotency network in ESC

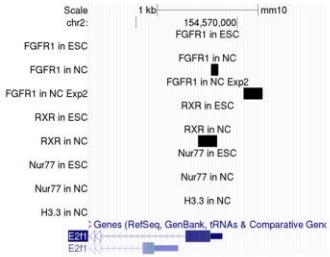


FGFR1 binding to several pluripotency genes in differentiating NC

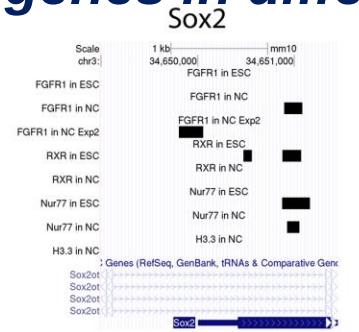
Suz12



E2f1

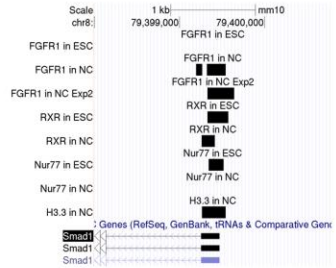


Sox2

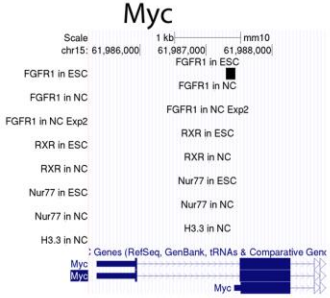


Klf4

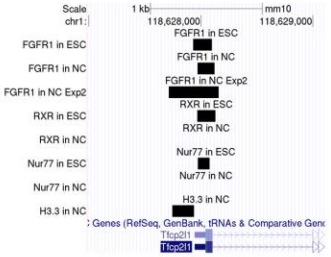
Smad1



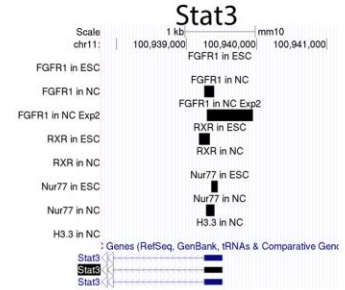
Myc



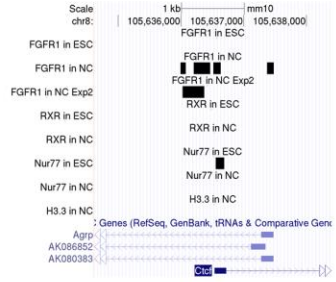
Tfcp2l1



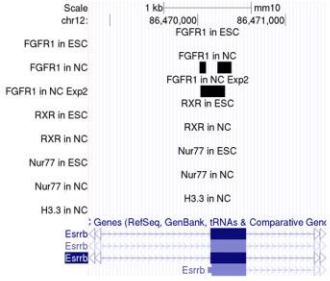
Stat3



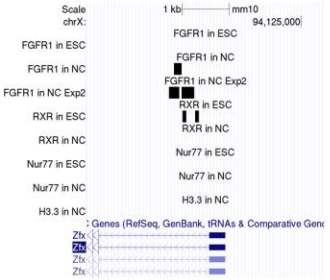
CTCF



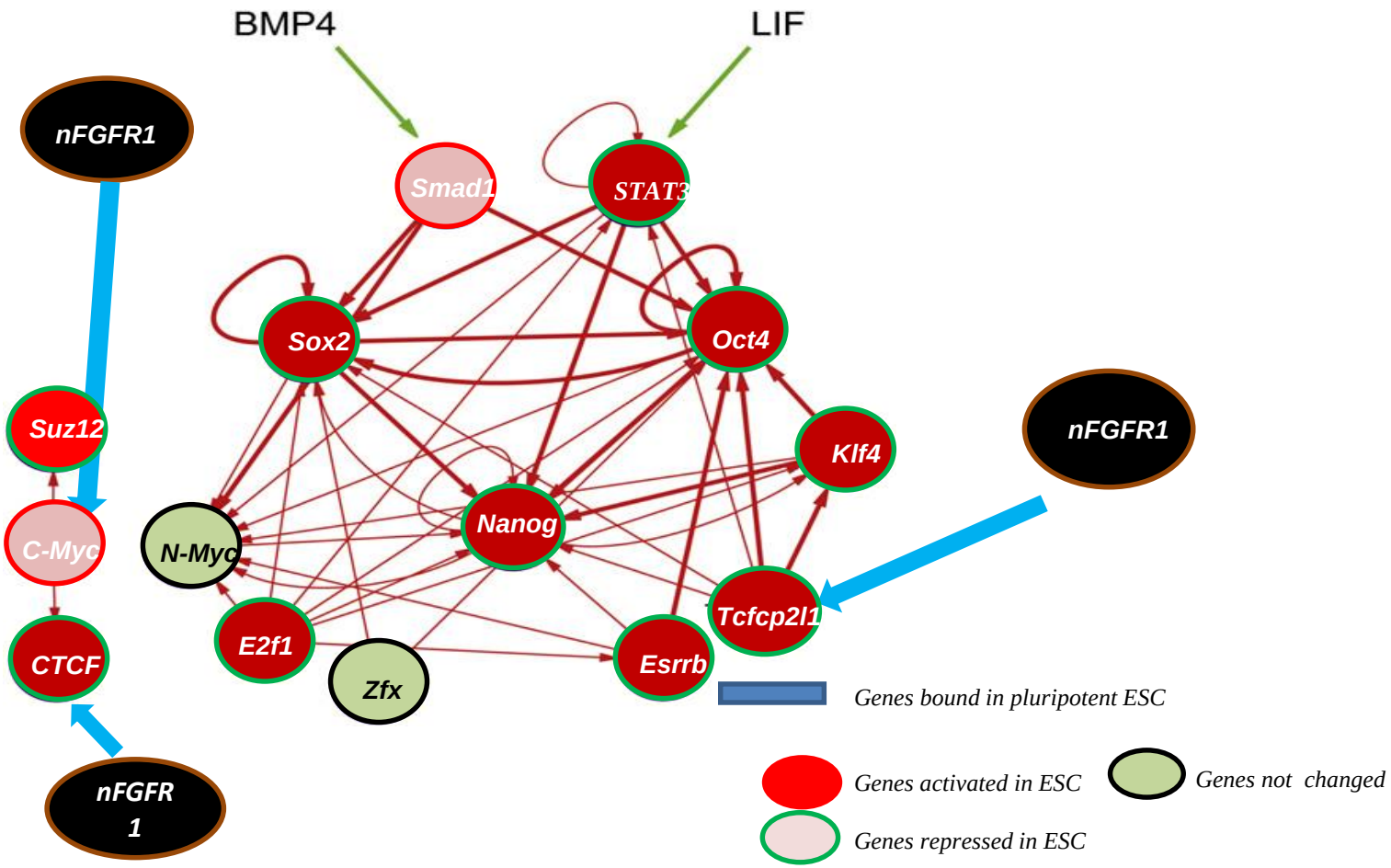
Esrrb



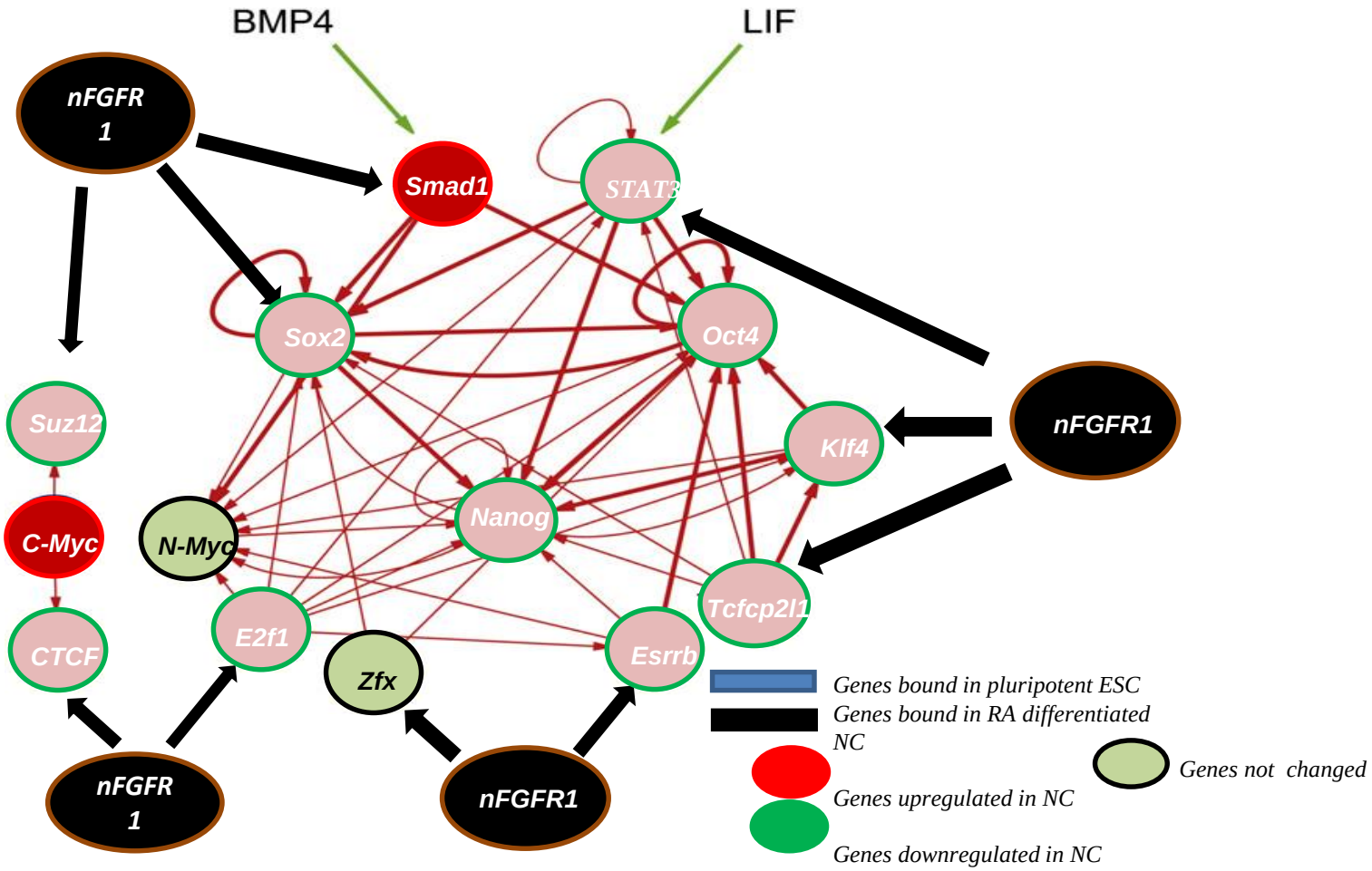
ZFX



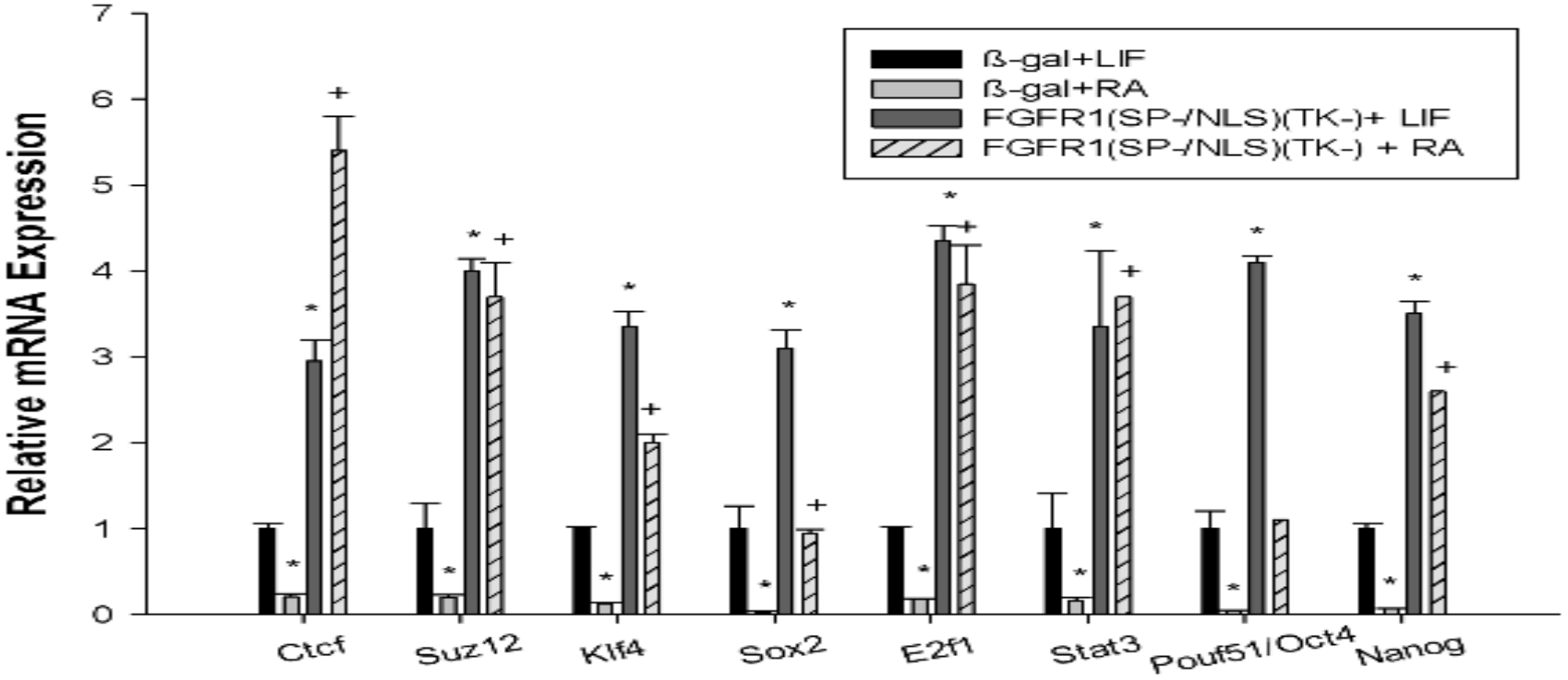
Active pluripotency network in ESC - nFGFR1 targets few genes



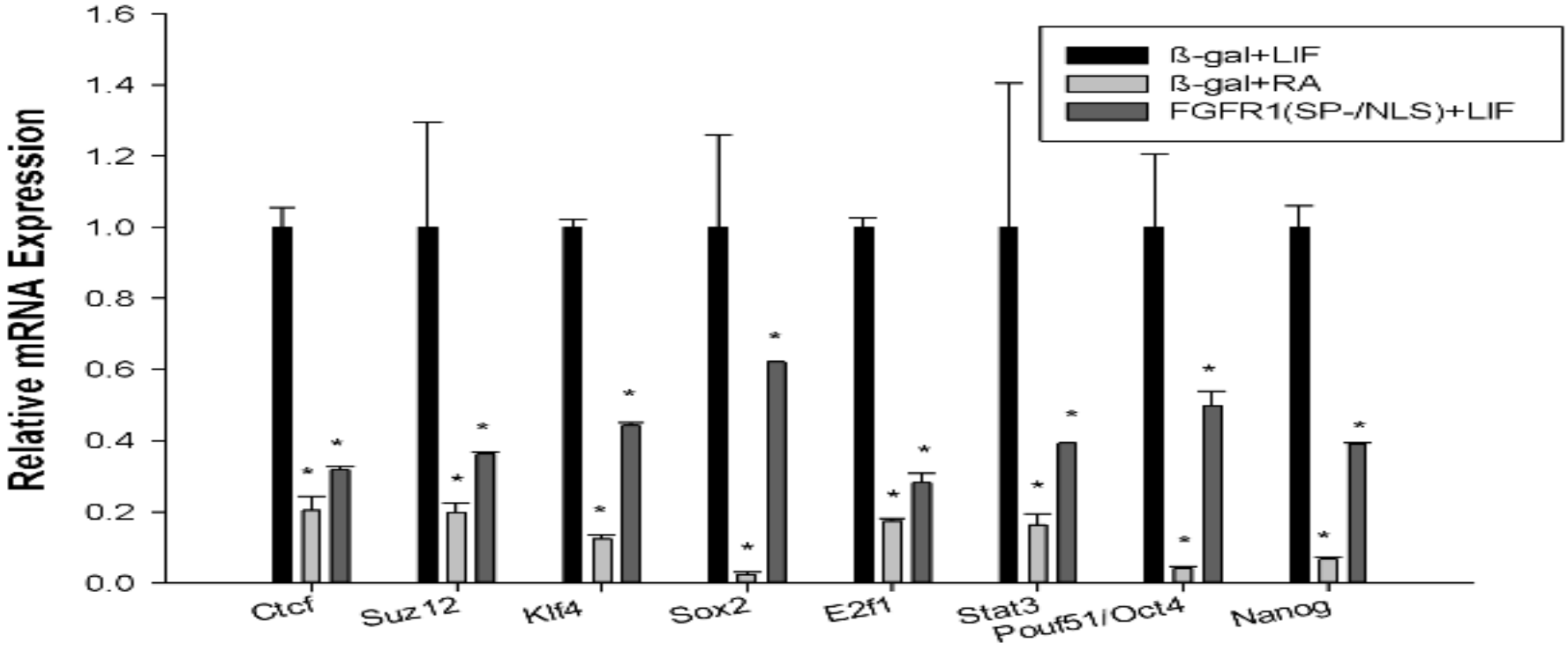
nFGFR1 turns off pluripotency network in differentiated ESC (NC)



nFGFR1 represses pluripotency genes during neuronal programing

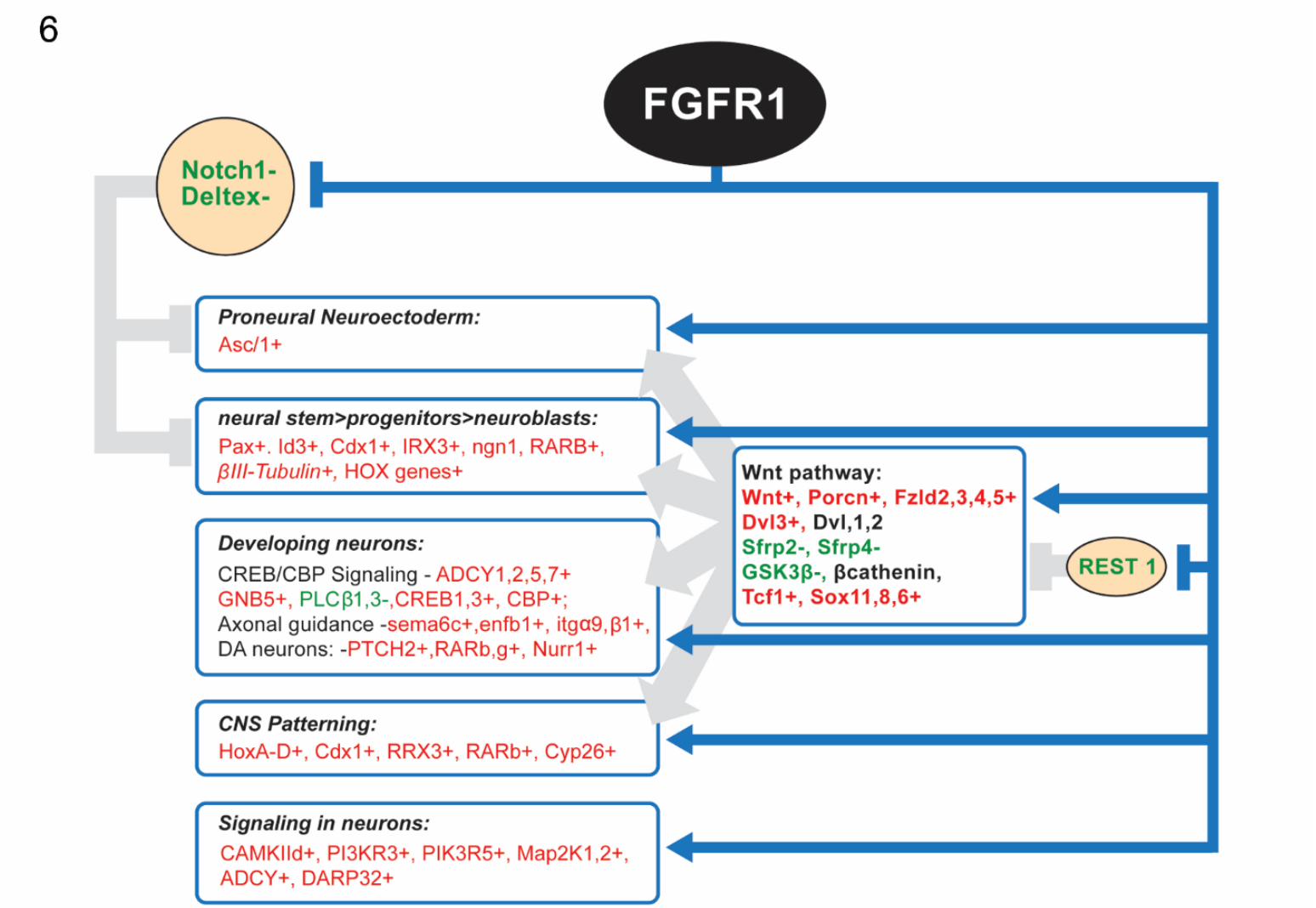


Nuclear active FGFR1 (NLS) downregulates core pluripotency genes in the absence of RA



nFGFR1 controls multiple stages in neural development

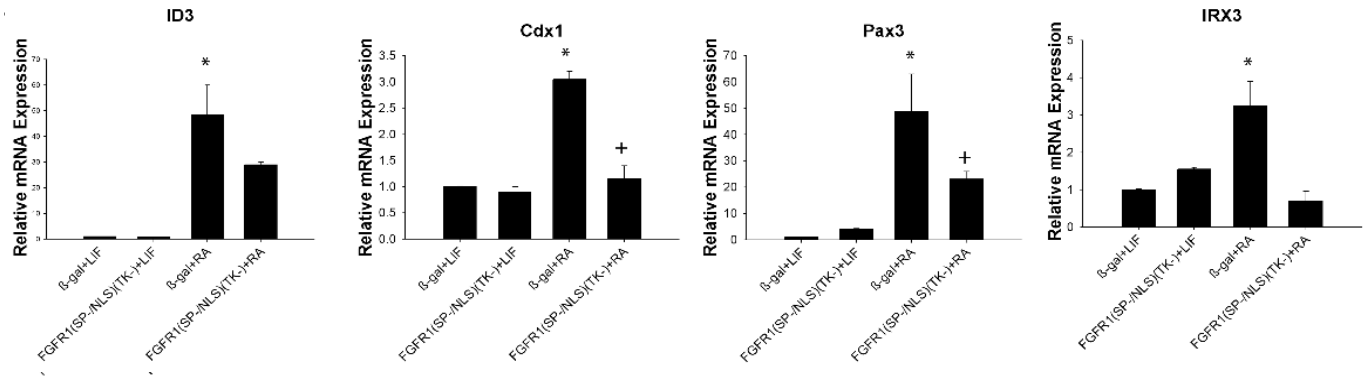
((Terranova et al., 2015) and linked database).



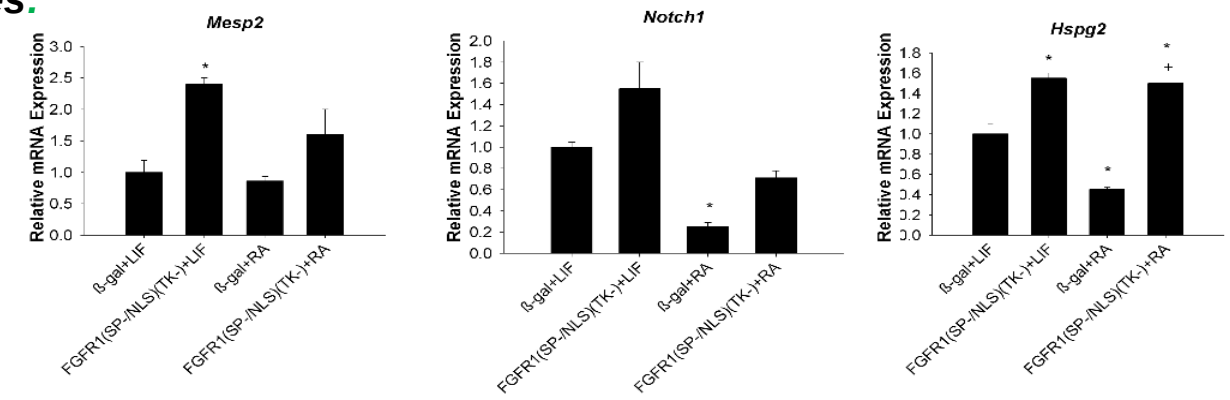
Decisions – brains or muscles?

nFGFR1 upregulates neuronal and downregulates mesodermal genes:

Neuronal genes:
Id3
Cdx1,
Pax3
IRX3
Res,Pouf3
Foxd3

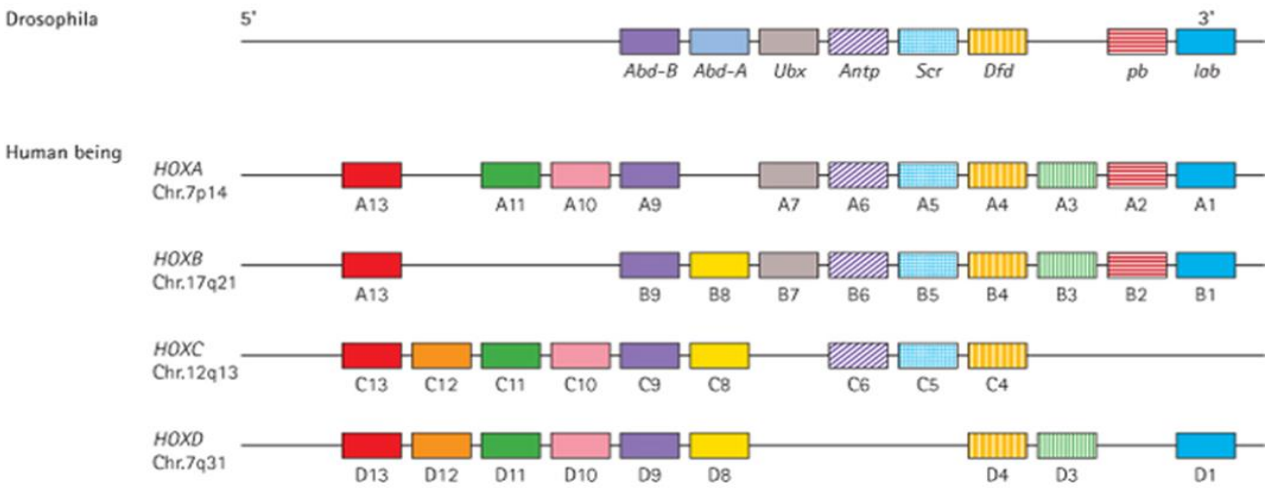


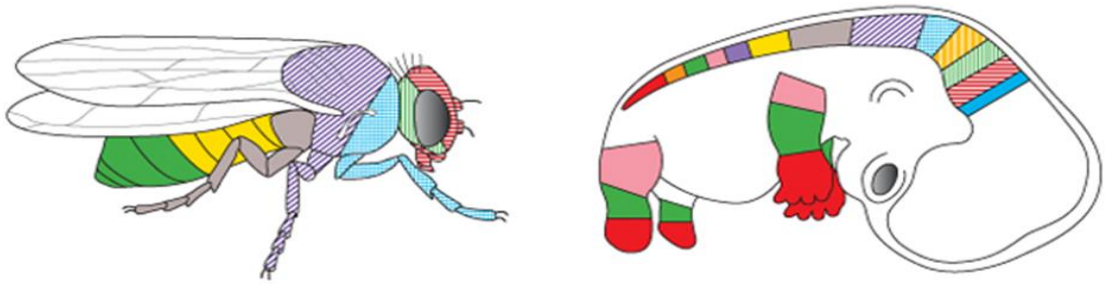
Mesodermal genes:
Mesp2
Ntoch1
Hespg2 (Perlecan)
DUSP6
lfng,
Porcn
Ndk1
FGF23/FGF23



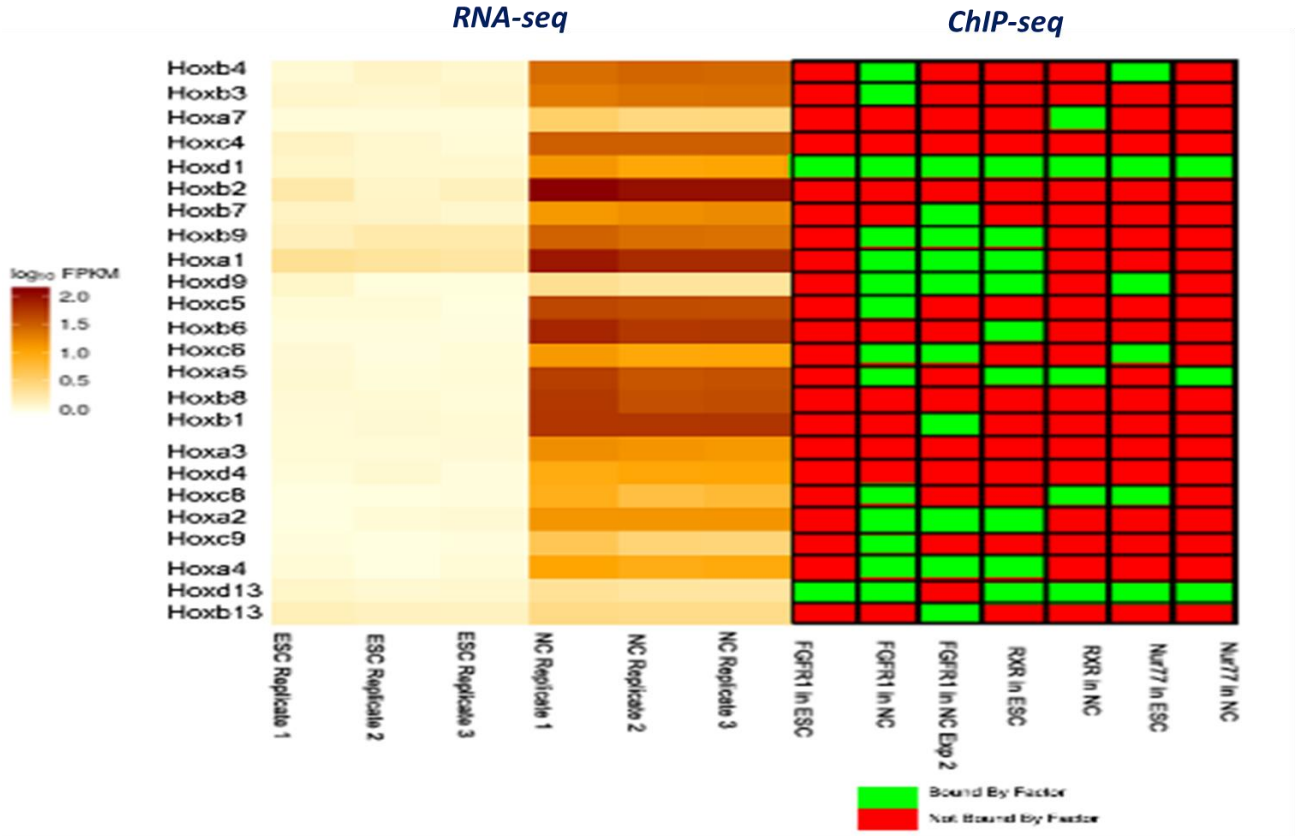
Building body parts and axes

Hox genes - architects of body axes, segmentation, limbs, nervous system, musculature and skeletal system

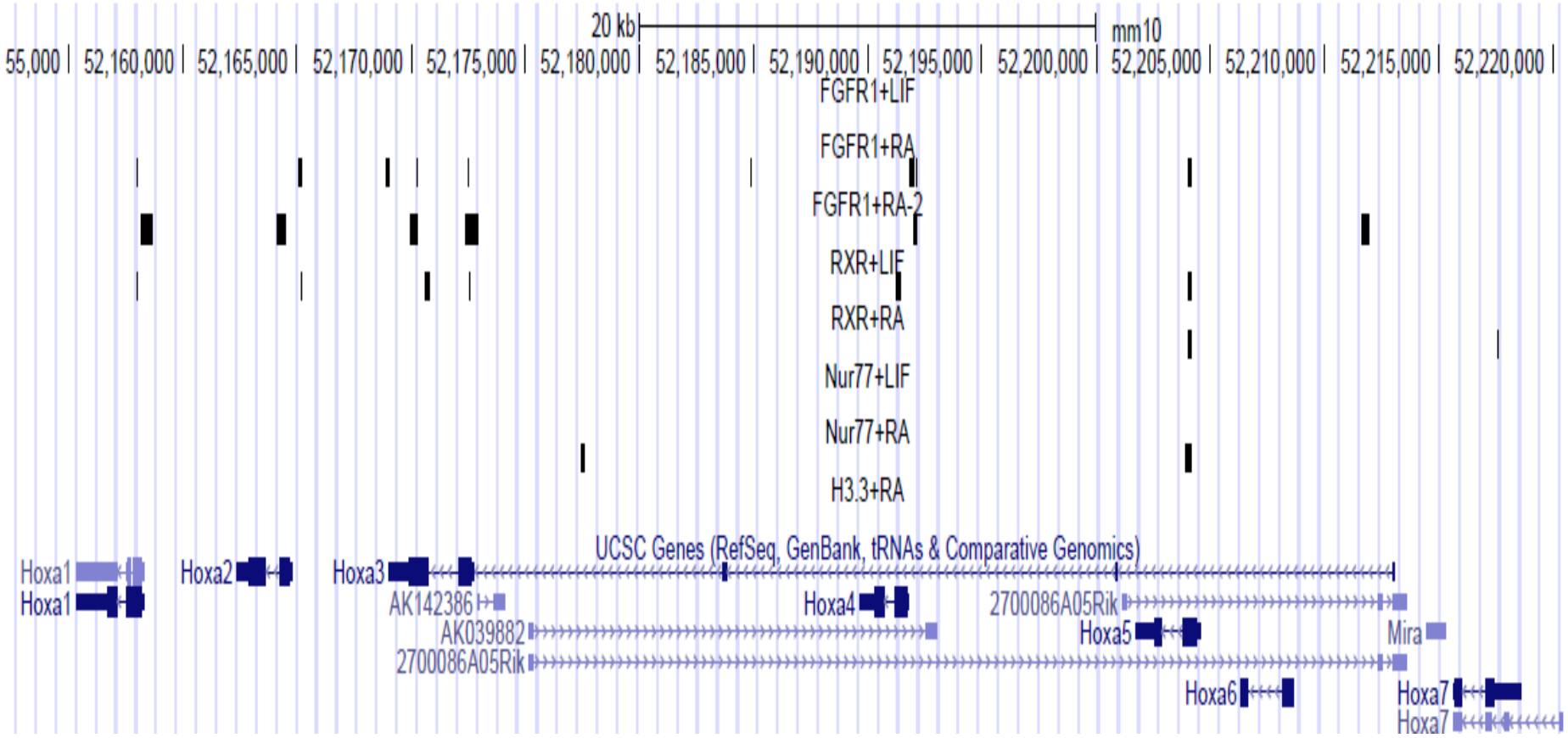




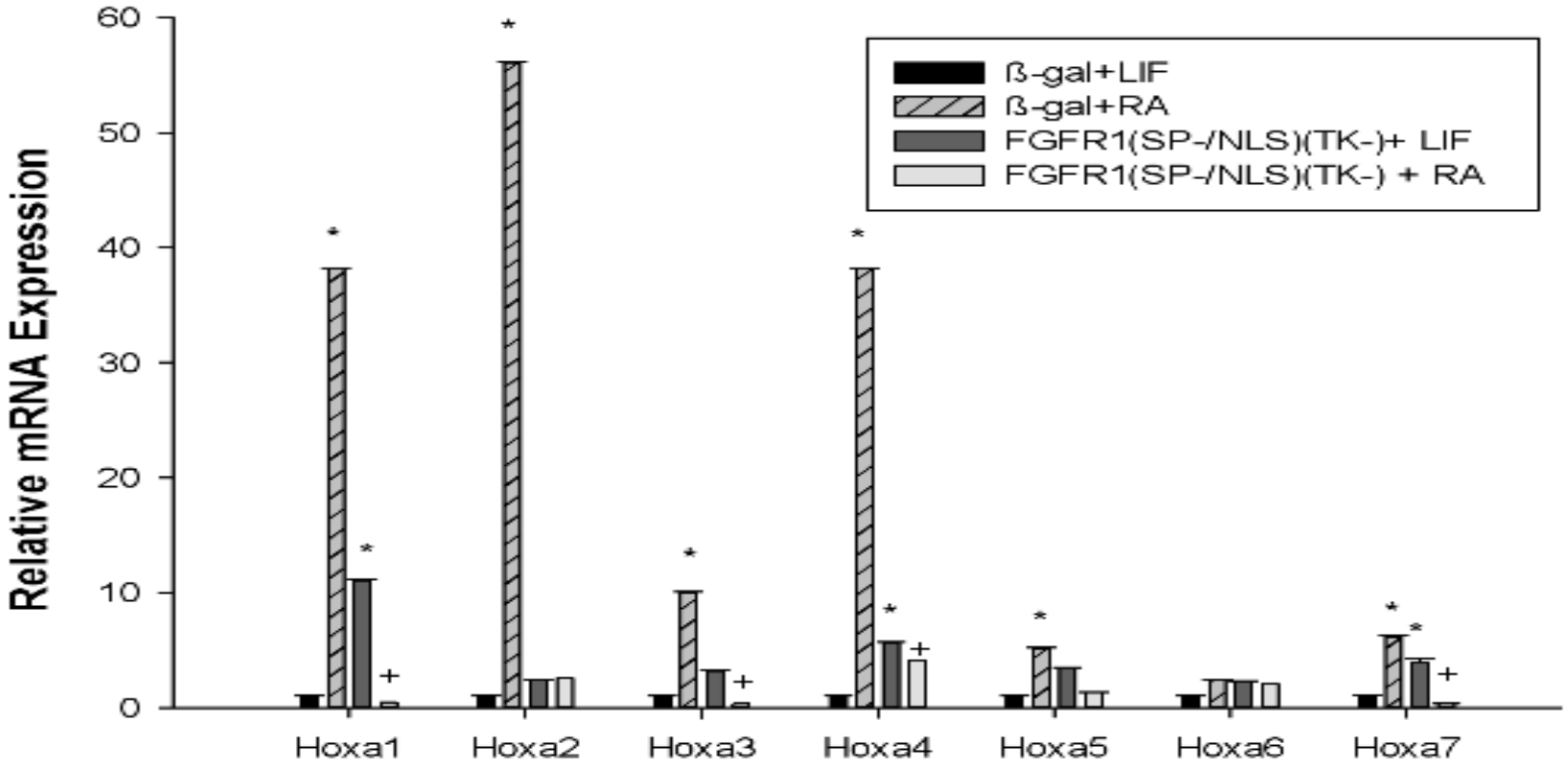
Nuclear FGFR1, RXR and Nur77 binding and gene activities in the Hox clusters in ESC and NC



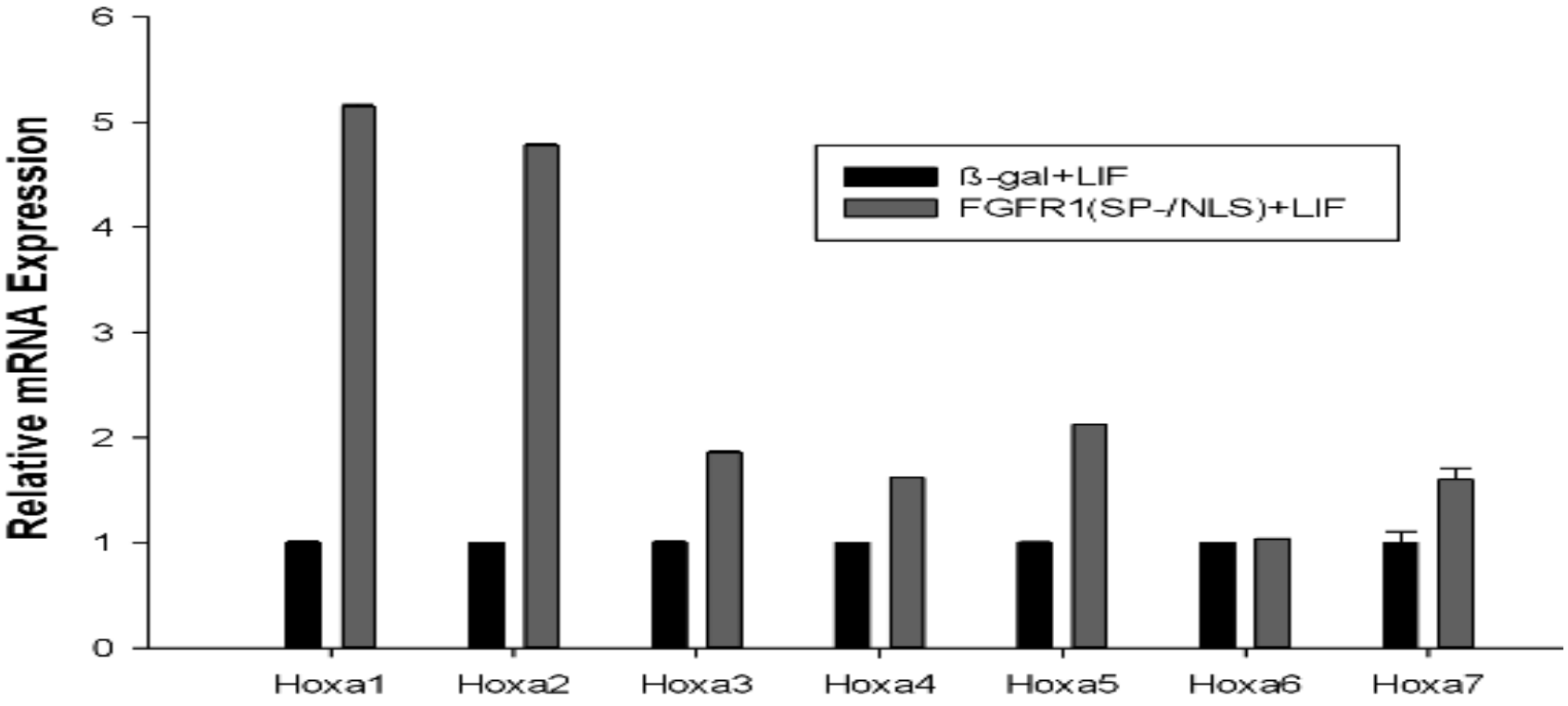
Nuclear FGFR1 binding to 3' HoxA genes in NC



Dominant negative nuclear FGFR1 (NLSTK-) blocks RA upregulation of core 3' Hoxa genes



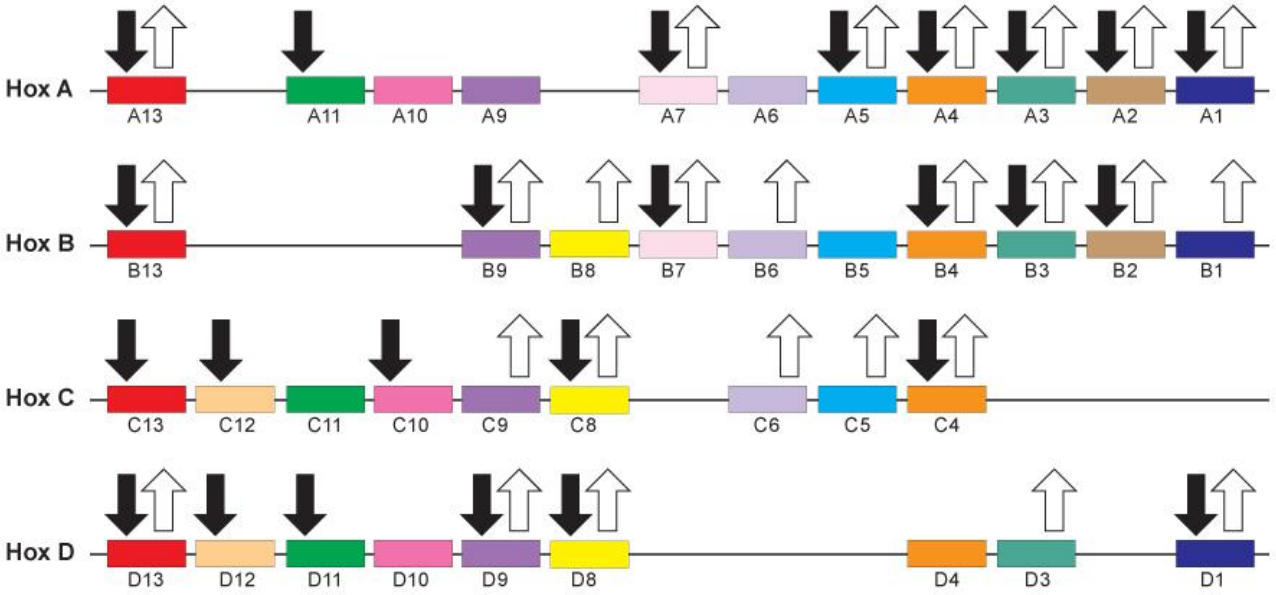
Nuclear active nuclear FGFR1 (NLS) upregulates core 3' Hoxa genes in the absence of RA



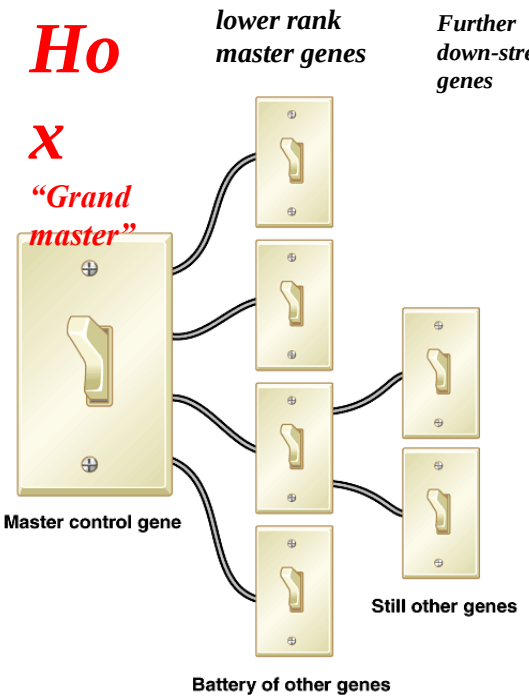
nFGFR1 directly upregulates Homeobox (Hox) genes in NC

nFGFR1 binding to promoter

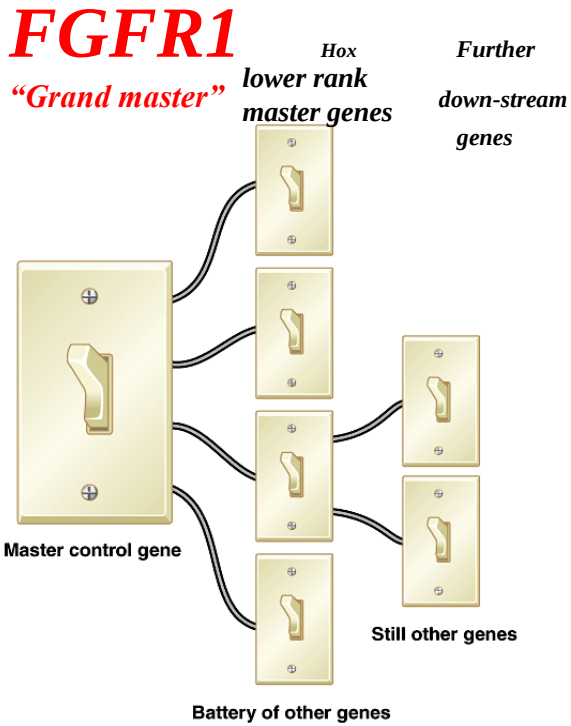
Upregulation of Hox mRNA



Change in the Hierarchy of Master Genes



Old

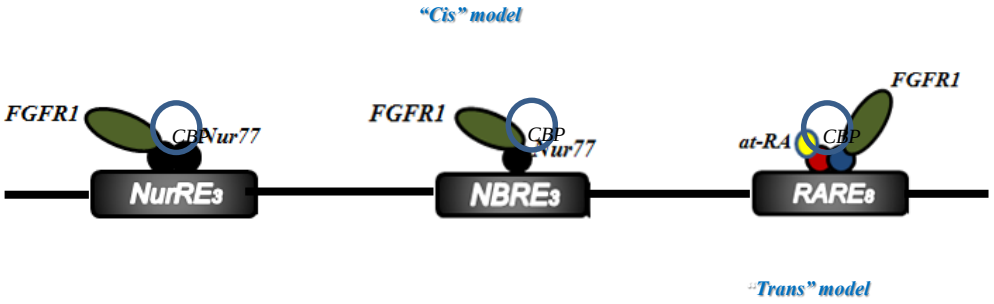


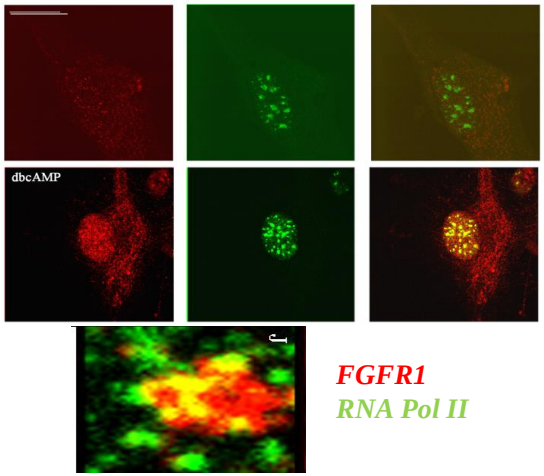
New

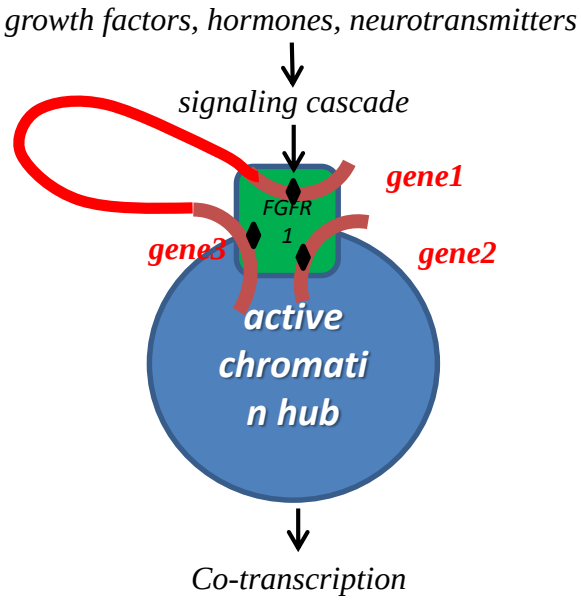
3C
Chromatin Structure

Brandon Decker

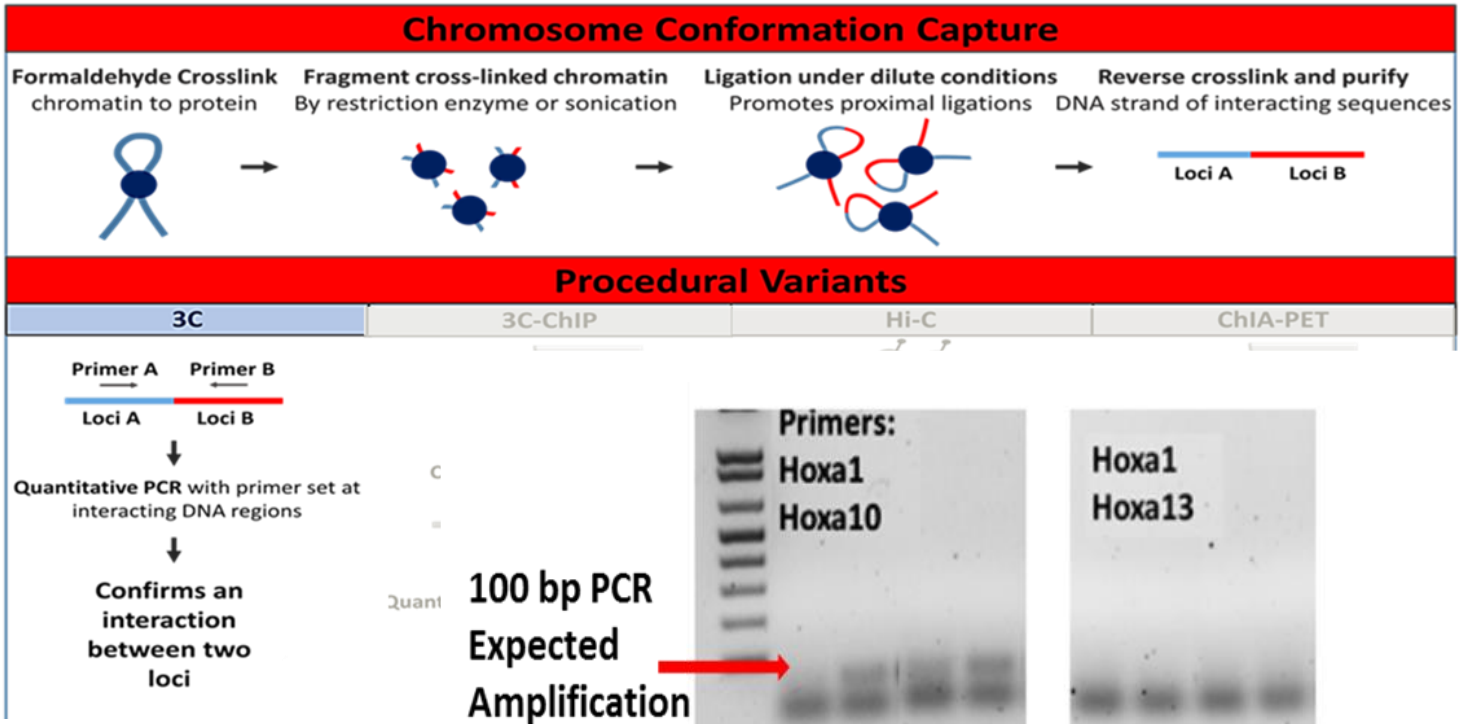
How to coordinate regulation of the thousands of genes?



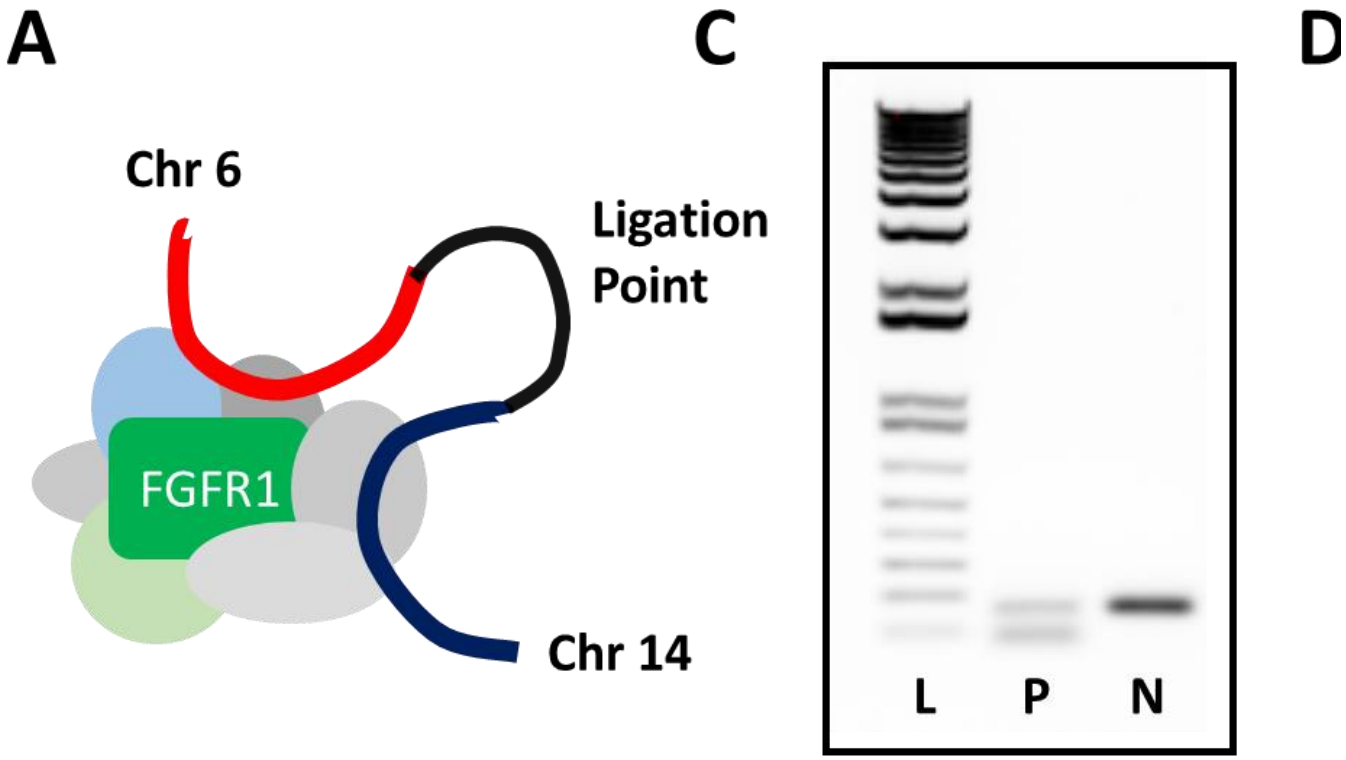




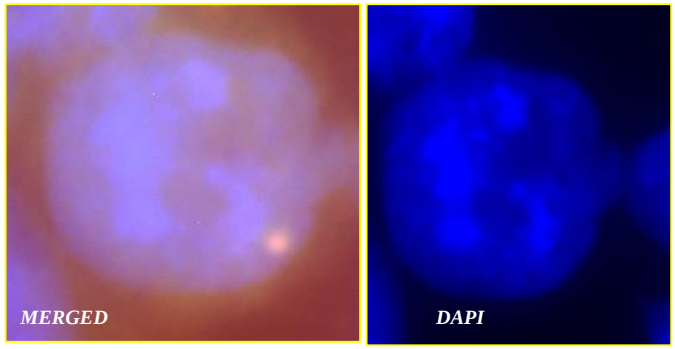
Chromatin Interaction Analysis Approaches – 3C

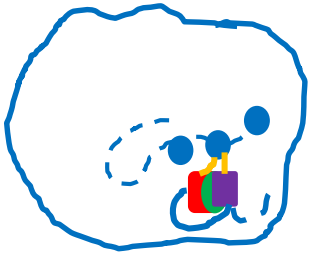


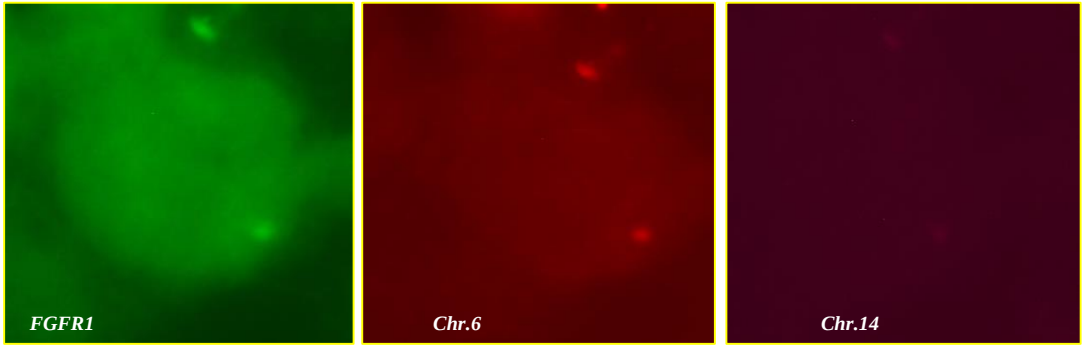
Chromatin Conformation Capture – 3C



In Situ Hybridization/Immunocytochemistry verifies interchromosomal interactions involving nFGFR1







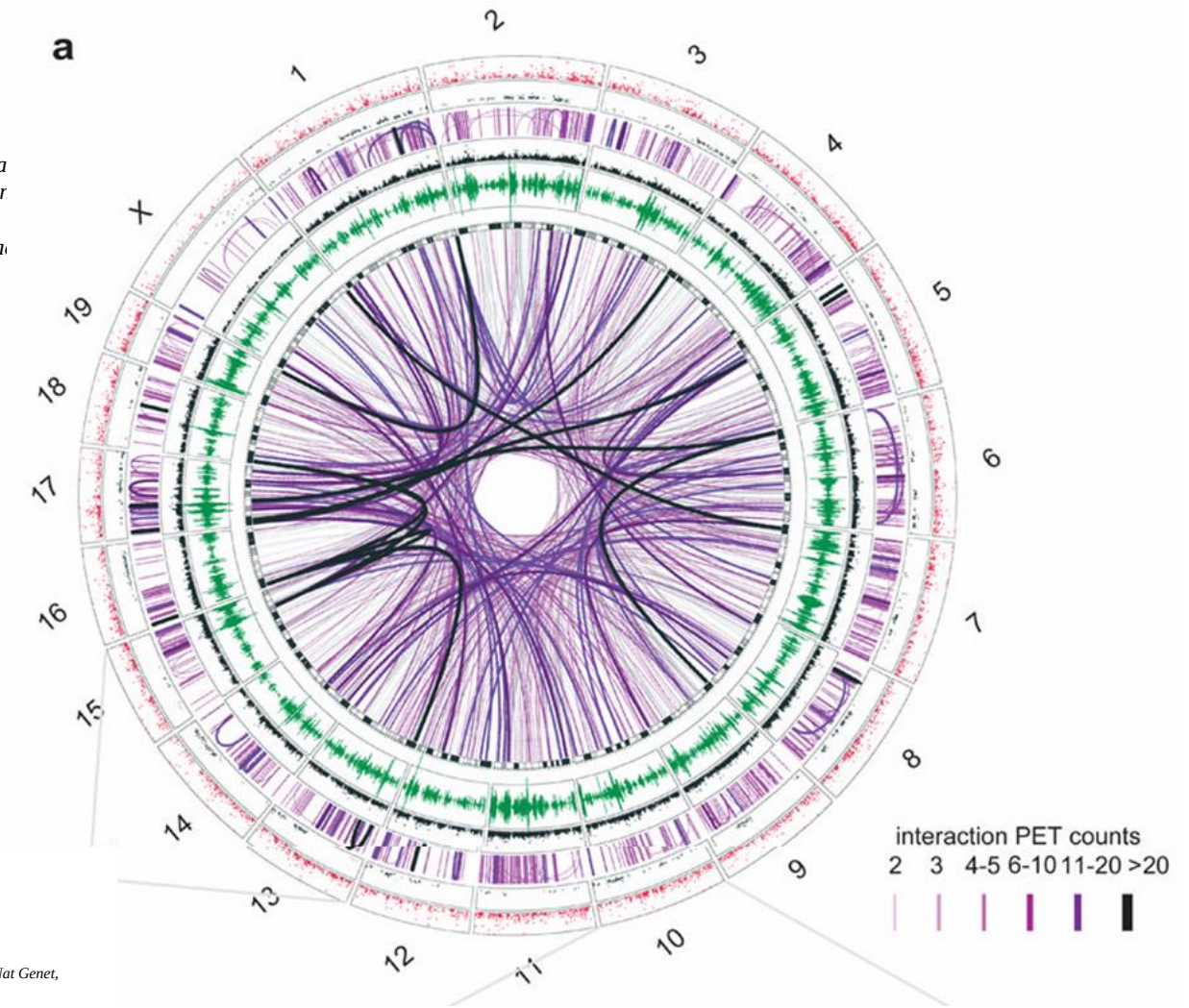
RA #14

Chromosome 6-- 5' Alexa Fluor 546

chromosome6-- 14' Alexa Fluor 546

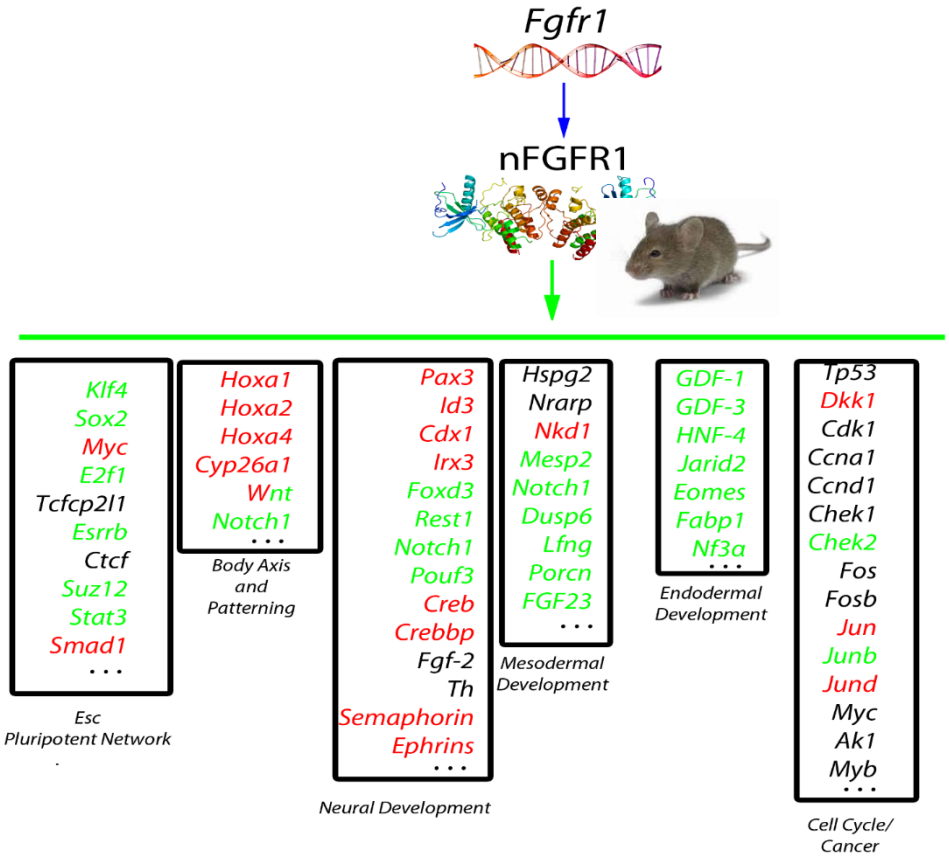
FGFR1 –Alexa 488

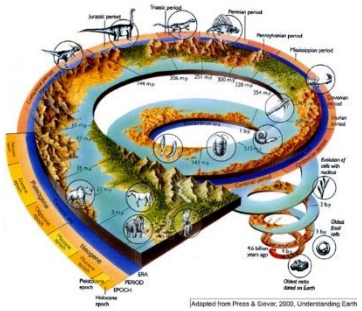
Track I : ***ChIP-seq p300*** *– genome-wide p300 binding*
Track II: ***ChIP-seq LAD*** *– genome-wide LAD binding*
Track III: ***ChIA-PET CTCF*** *– Intrachromosomal intera*
Track IV: ***ChIP-seq CTCF*** *– genome-wide CTCF bindir*
Track V: ***RNA-seq*** *– Genome-wide gene expression*
Track VI: ***ChIA-PET CTCF*** *– Interchromosomal intera*



Handoko, L., et al., CTCF-mediated functional chromatin interactome in pluripotent cells. Nat Genet, 2011. 43(7): p. 630-8.

Evidence based theory of ontogeny – Nuclear FGFR1 a new type master regulator of ancient and new genes





Global developmental gene programing involves a nuclear form of Fibroblast Growth Factor Receptor-1 (FGFR1). 2015, PLOS One

New Theory of Ontogeny

The End