MicroRNA 23a~27a~24-2 cluster regulation of bone formation

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Small non-coding RNA fact sheet

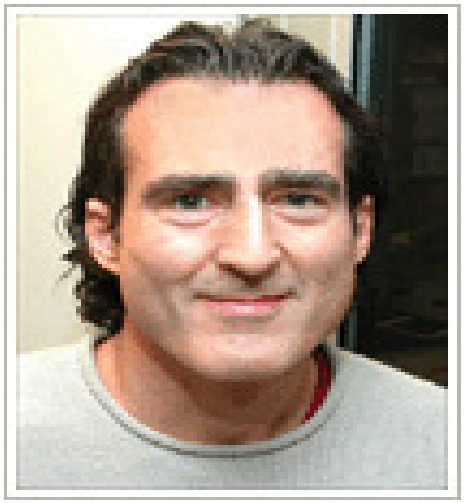
In recent years, numerous studies have documented transcription across 70–90% of the human genome.
2% of the total genome encodes protein-coding genes, suggesting that non-coding RNAs represent most of the human transcriptome.
around 21,000 protein-coding genes, the human transcriptome includes about 9,000 small RNAs, about 10,000–32,000 long non-coding RNAs (lncRNAs) and around 11,000 pseudogenes.
Non-coding RNA generally be divided into several classes based on their size and function:
Transfer RNAs: which are involved in translation of messenger RNAs
MicroRNAs (miRNAs) and small-interfering RNAs (siRNAs), which are implicated in post-transcriptional RNA silencing;
Small nuclear RNAs: which are involved in splicing.
Small nucleolar RNAs: which are implicated in ribosomal RNA modification.
PIWI-interacting RNAs: which are involved in transposon repression
Promoter-associated small RNAs: which may be involved in transcription regulation.
LncRNAs can vary in length from 200 nucleotides to 100 kb, and have been implicated in a diverse range of biological processes.
One of the best-studied and most dramatic examples is *XIST*, a single RNA gene that can recruit chromatin-modifying complexes to inactivate an entire chromosome.

Discovery 1: 1993

Victor Ambros

Gary Ruvkum

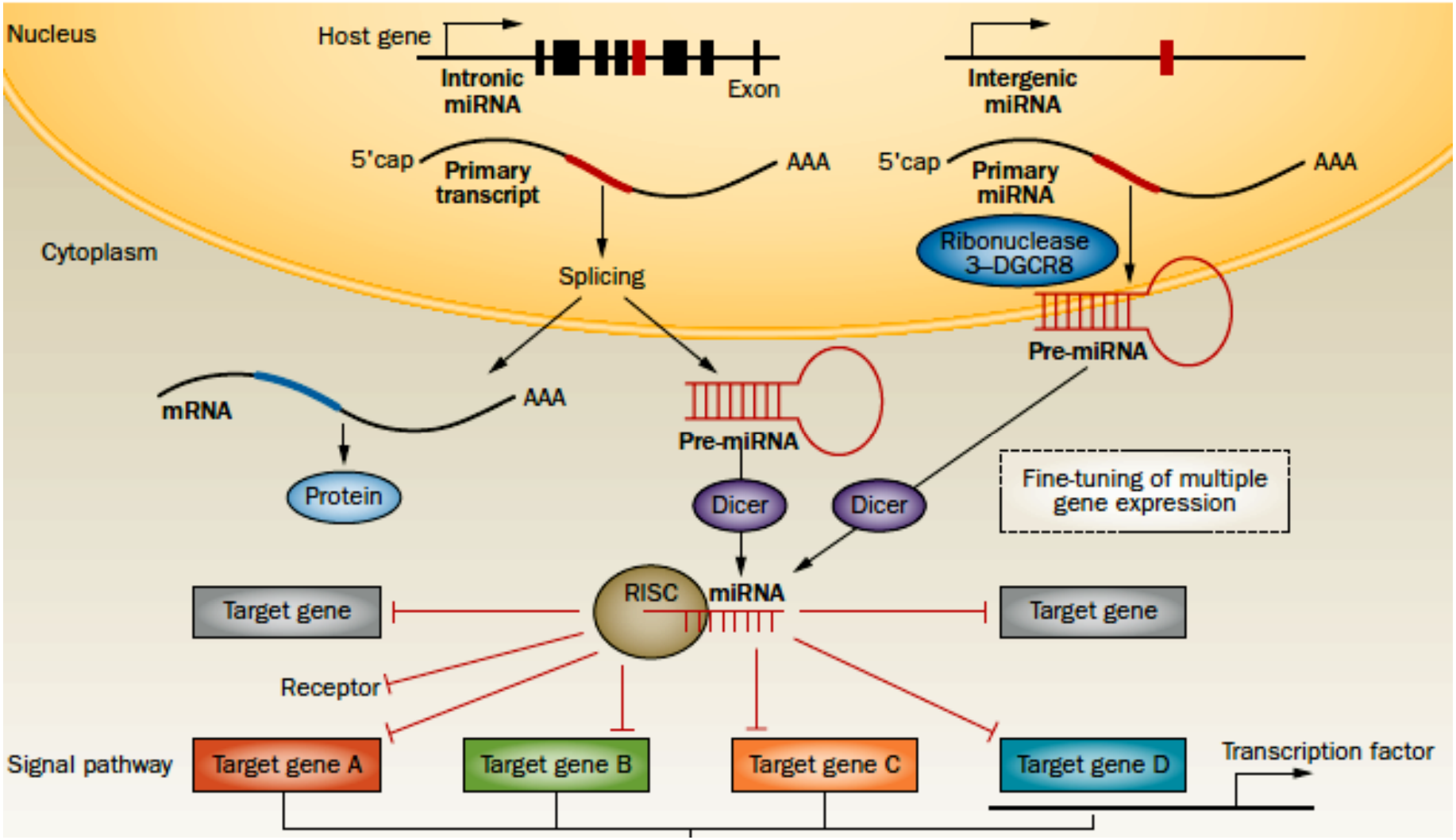
Discovery 2: 1998



Craig Mello

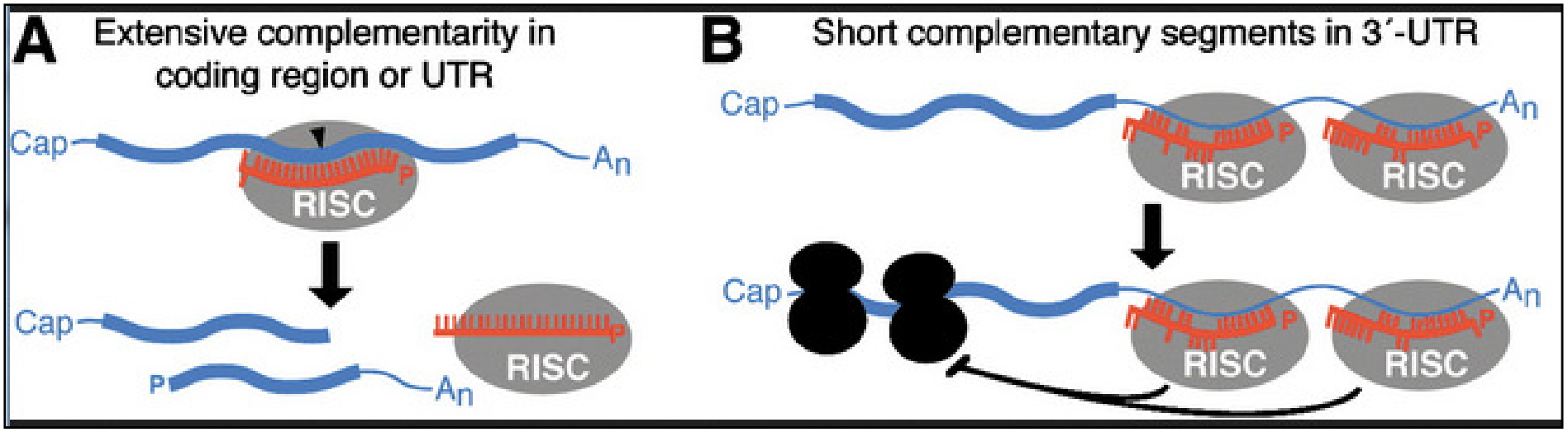
Andrew Fire

Macro view of microRNA Function



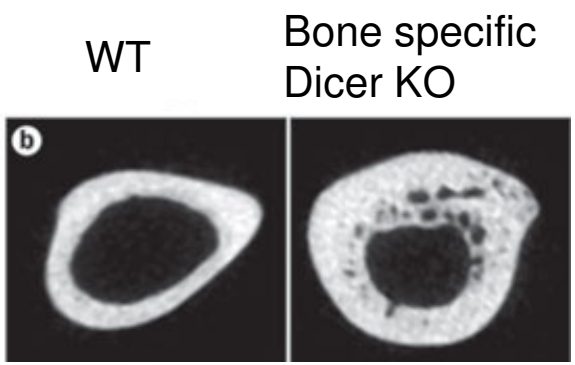
Although miRNA effect on individual target genes is modest, however simultaneous targeting of multiple genes that operate in a regulatory network (represented by target genes A–D) can have synergistic effects on biological functions.

Mechanism of miRNA Function



MicroRNA: Control of Genetic Information

The importance of miRNAs in development and differentiation bone and cartilage has been shown by loss-of-function analyses of Dicer, argonaute-2 and Dgcr8 in mice, which result in embryonic-lethal or severe developmental defects as a consequence of cell cycle arrest and differentiation problems. Furthermore, limb-specific and cartilage-specific deletion of Dicer highlighted the role of miRNAs in the musculoskeletal system: mice with these deletions had smaller limbs or bodies as a consequence of chondrocyte proliferation, accelerated hypertrophic differentiation and subsequent cell death. Osteoclast-specific deletion of Dicer in mice increased bone mass by regulating bone resorption.



Gaur et al. 2010

Loss-of-function of mature miRNAs in this population results in increased bone mass potentially by relieving repression of Runx2 miRNAs (n = 11) and collagen protein levels. Courtesy of J. B. Lian and MQ Hassan, University of Massachusetts Medical School, USA.

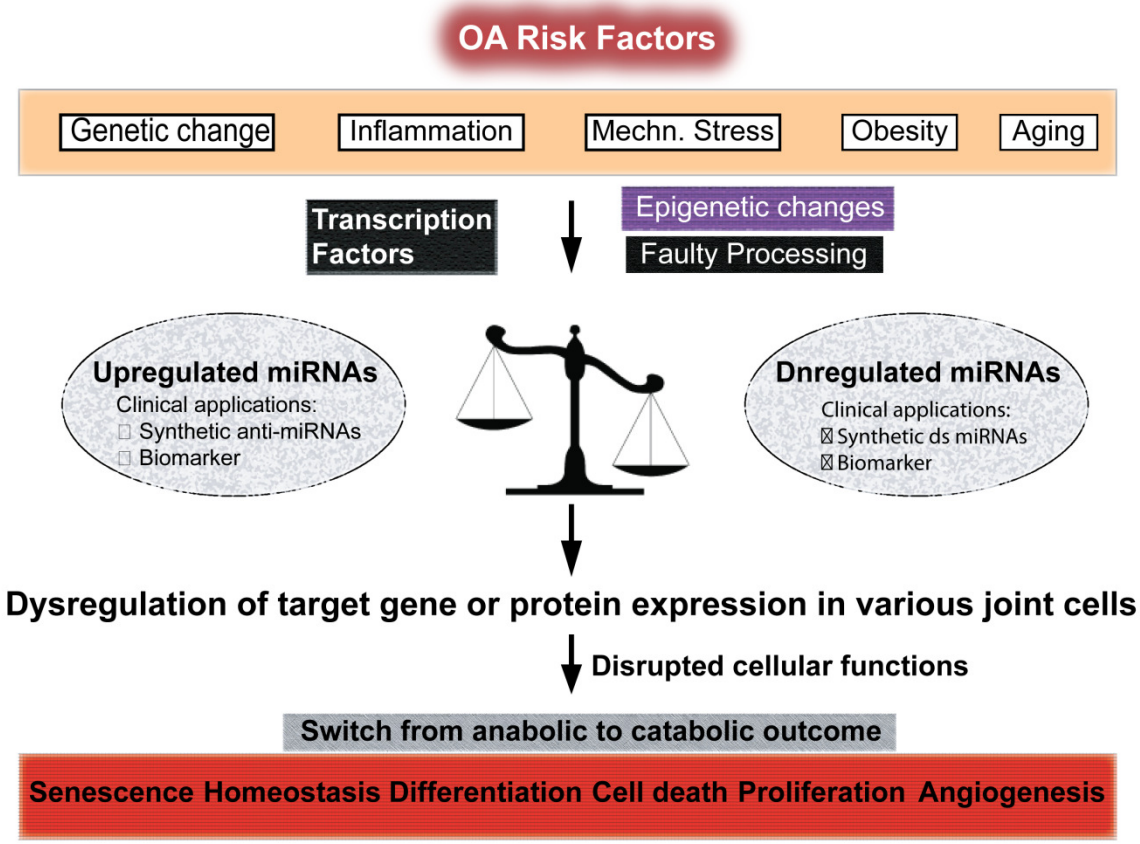
Reviews
Kapinas & Delaney 2011
Taipaleenmaki & Kassem 2012
Lian et al Nature Rev Endo 2012

Osteoarthritis

Definition:
Osteoarthritis (OA), the most common musculoskeletal disorder, is complex, multifaceted, and characterized by degradation of articular cartilage and alterations in other joint tissues.

Overview:
Approximately 40 million Americans were affected by OA as of 2008, a number predicted to increase to 60 million within the next 20 years as a result of population ageing and an increase in life expectancy.

OA pathogenesis and the putative role of miRNA

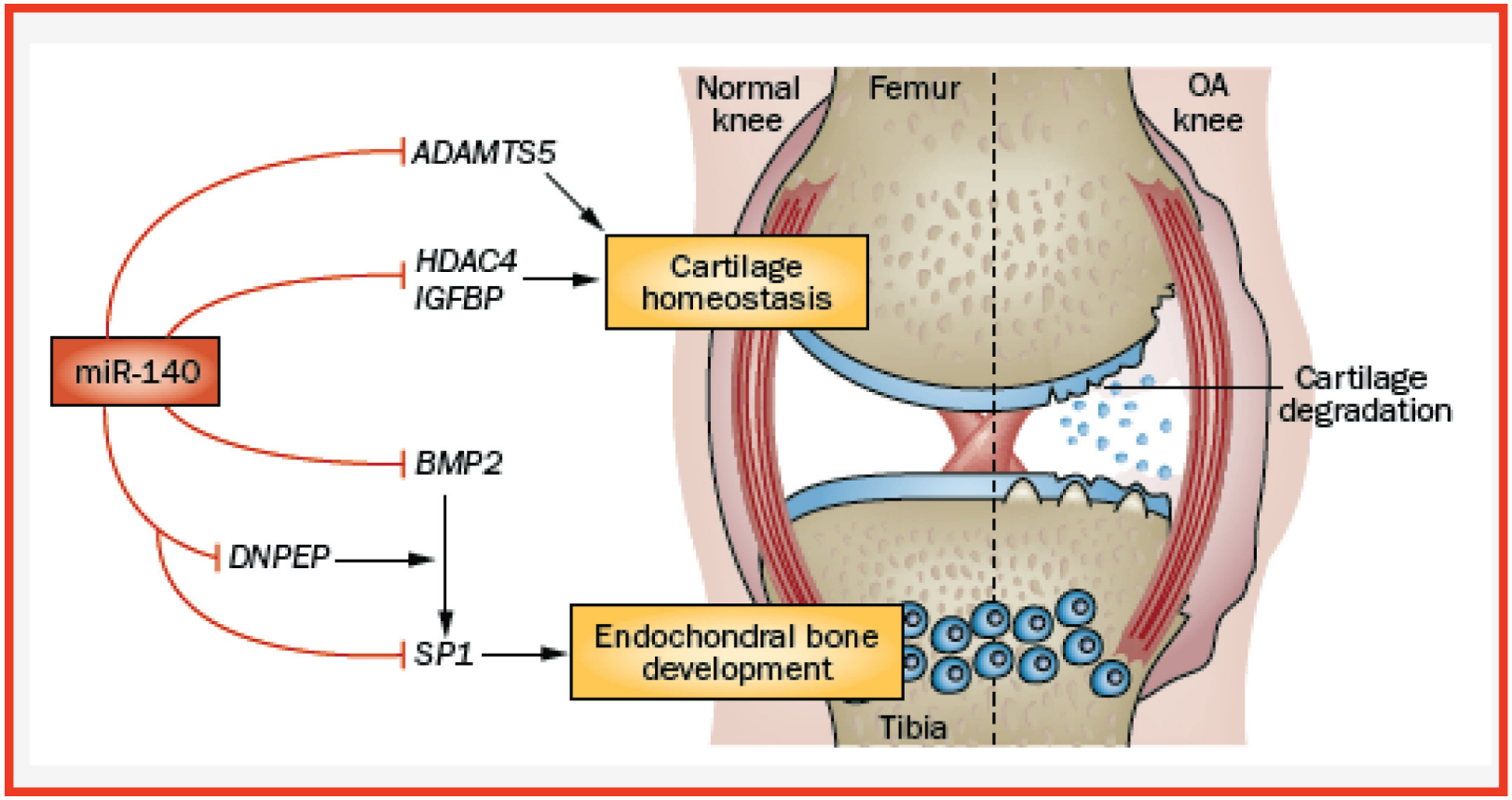


Key points

1. Several pathogenic pathways in OA have been characterized, effective approaches to prevention and treatment of OA are lacking
2. miRNAs are the new players in the gene regulaion, involved in the development of the musculoskeletal system and OA pathology through maintenance of articular chondrocytes
3. miRNAs have a role in transmitting the effects of the main risk factors for OA, such as aging and inflammation, on to cellular homeostasis through their control of multiple target genes
4. Approaches to maintaining or supressing the expression of key miRNAs in OA pathogenesis will lead us to develop new therapeutic & diagnostic targets

Miyaki and Asahara, Nat. Reviews Rheumatology, 2012

miR-140 and Joint health



Possibilities: Understanding novel molecular mechanisms that are involved in the maintenance and destruction of articular cartilage, including extracellular regulators and intracellular signalling mechanisms in joint cells that control cartilage homeostasis, has the potential to identify new therapeutic targets in OA.

Table 1 miRNAs implicated in cartilage health and the development of OA

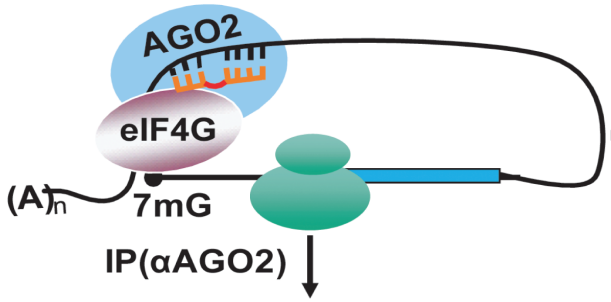
miRNA	Target gene(s)	Function(s) in cartilage	Expression change in OA samples✦
miR-9	MMP13, SIRT1	Homeostasis, ageing	Increased
miR-22	PPARAG, BMP7, SIRT1	Inflammatory response, ageing	Increased
miR-27a miR-27b	MMP13	Inflammatory response, homeostasis	Decreased
miR-34a miR-34b	SIRT1	Apoptosis, ageing	Increased
miR-140	ADAMTS5, IGFBP5 DNPEP, SP1, BMP2 HDAC4	Homeostasis, endochondral bone development	Decreased/increased
miR-145	SOX9	Homeostasis	Not determined
miR-146	TRAF6, IRAK1	Inflammatory response	Increased or decreased dependent on stage of OA
miR-455	SMAD2, ACVR2B, CHRDL1	Chondrocyte differentiation, homeostasis	Increased
miR-675	COL2A1 (indirect effect)	Chondrocyte differentiation, homeostasis	Increased
miR-125b	ADAMTS-4	Chondrocyte differentiation, homeostasis	Decreased

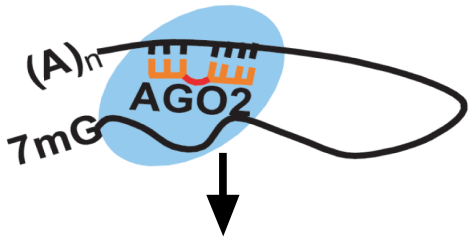
✦ In comparison with healthy people.

MiRNA Silenceosome pulldown by RNA-IP

Argonaute Complex
(Synov. FL/Chondroblast)

Synovial fluid miRNA analysis





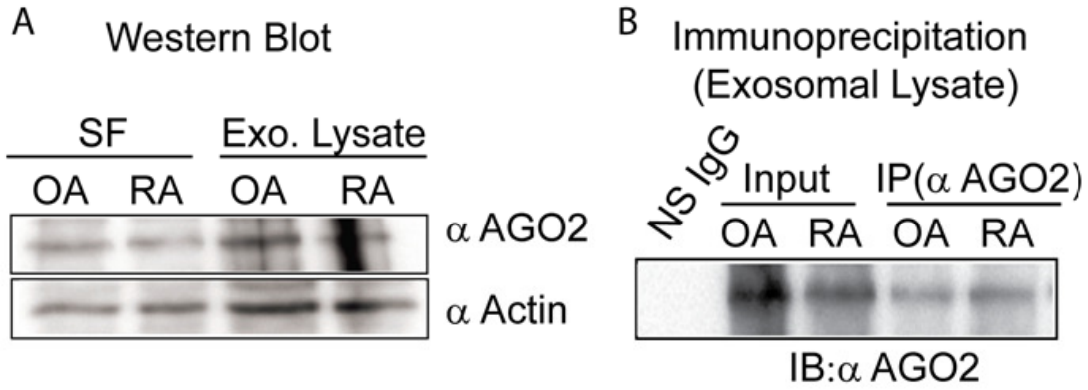
RNA Isolation

mRNA — miRNA

UTR specific primers — RT-QPCR — miR specific primers

3'UTR amplification — miRNA identification

MicroRNAs are present in osteoarthritic synovial fluid



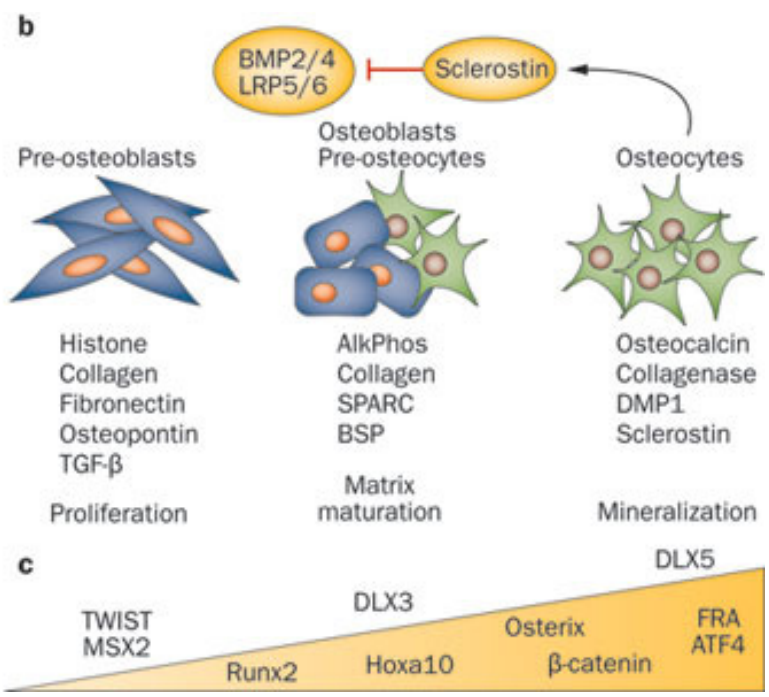
OA = Osteoarthritis synovial fluid
RA = Rheumatoid arthritis synovial fluid

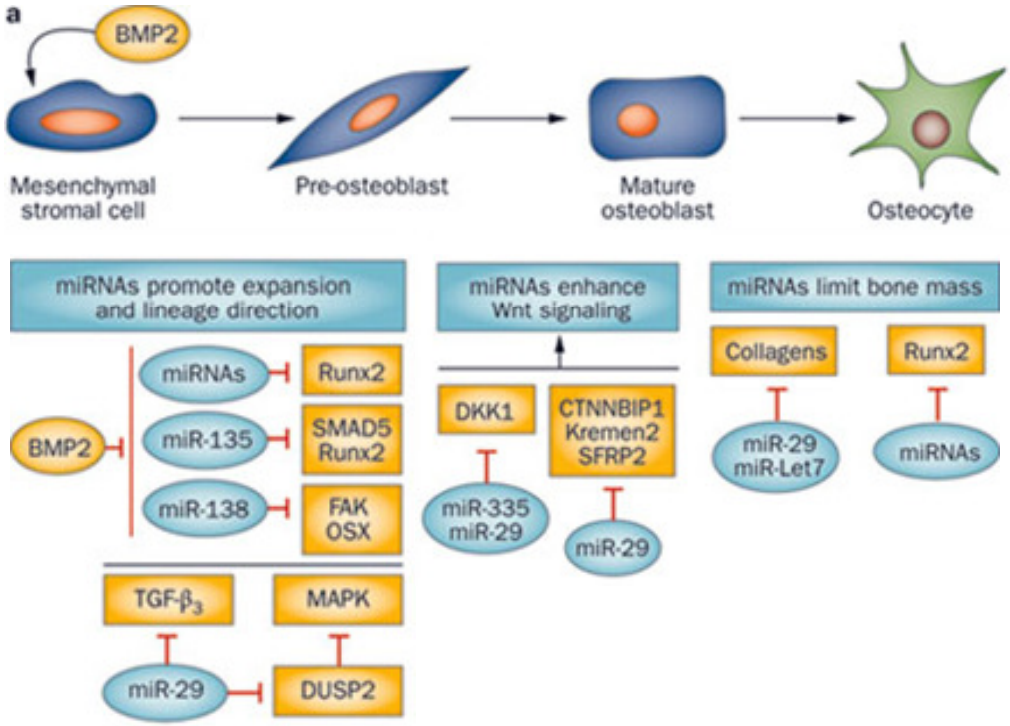
Human miR Finder Array

CT Values

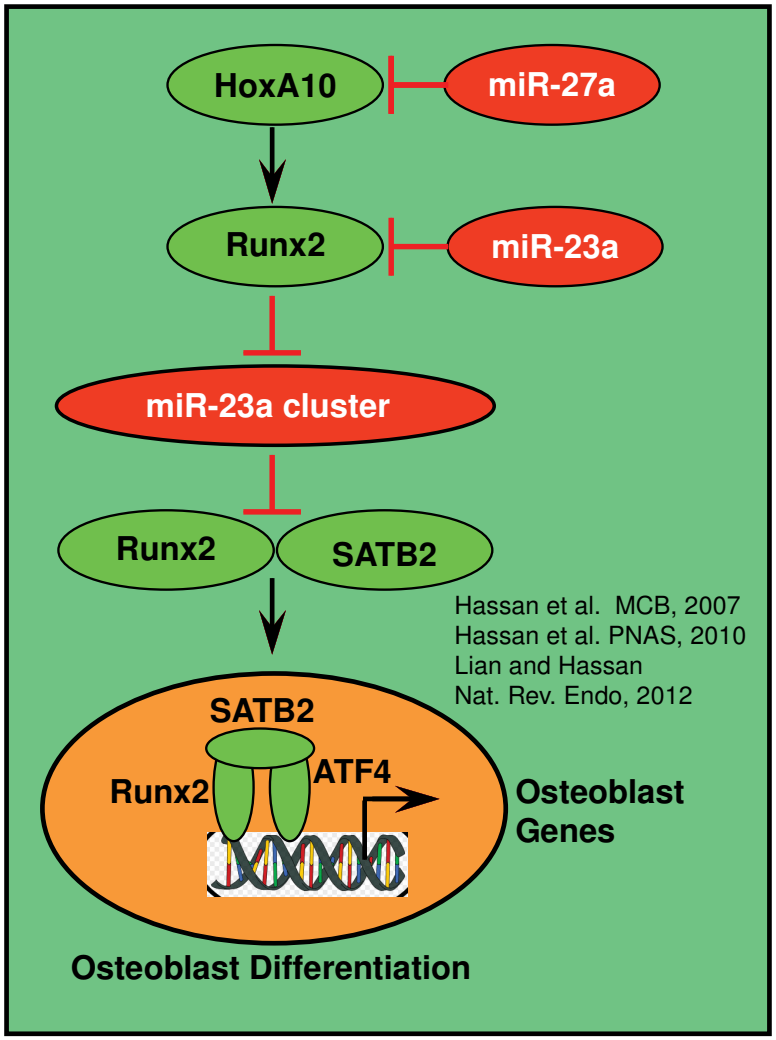
hsa-miR-27a	27.1168
hsa-miR-23a	25.9876
hsa-miR-24-2	29.0437
hsa-miR-101	25.81221
hsa-miR-103a	25.14534
miR-146a	28.00346
hsa-miR-28-5p	26.666
hsa-miR-125a-5p	27.93157
hsa-miR-151-5p	27.01892
hsa-let-7i	24.90489
hsa-miR-302a	27.70409
hsa-miR-140	29.1108
hsa-miR-34c	27.1876
hsa-miR-34b	29.0437
hsa-miR-9	28.12206
hsa-miR-22	24.84534
hsa-miR-145	23.00344

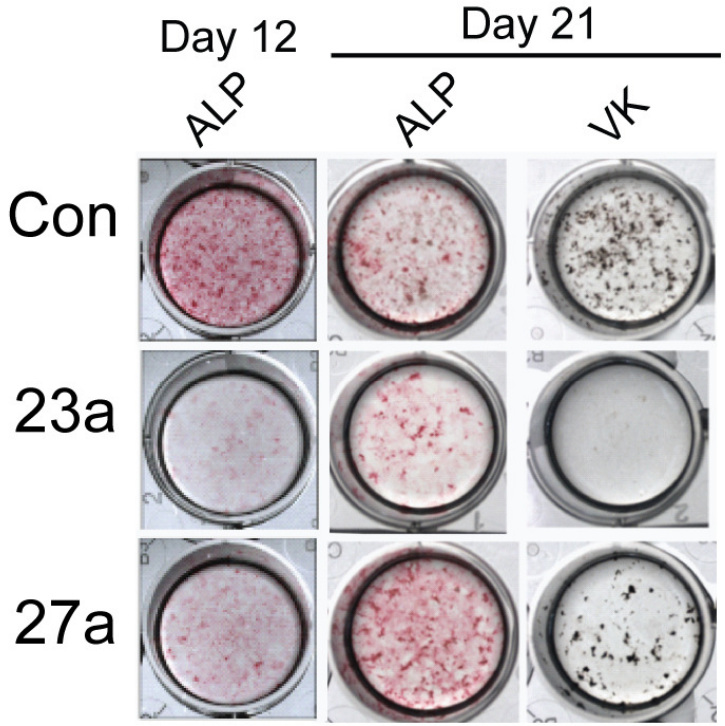
Osteoblast differentiation and effect of microRNAs on osteoblast differentiation



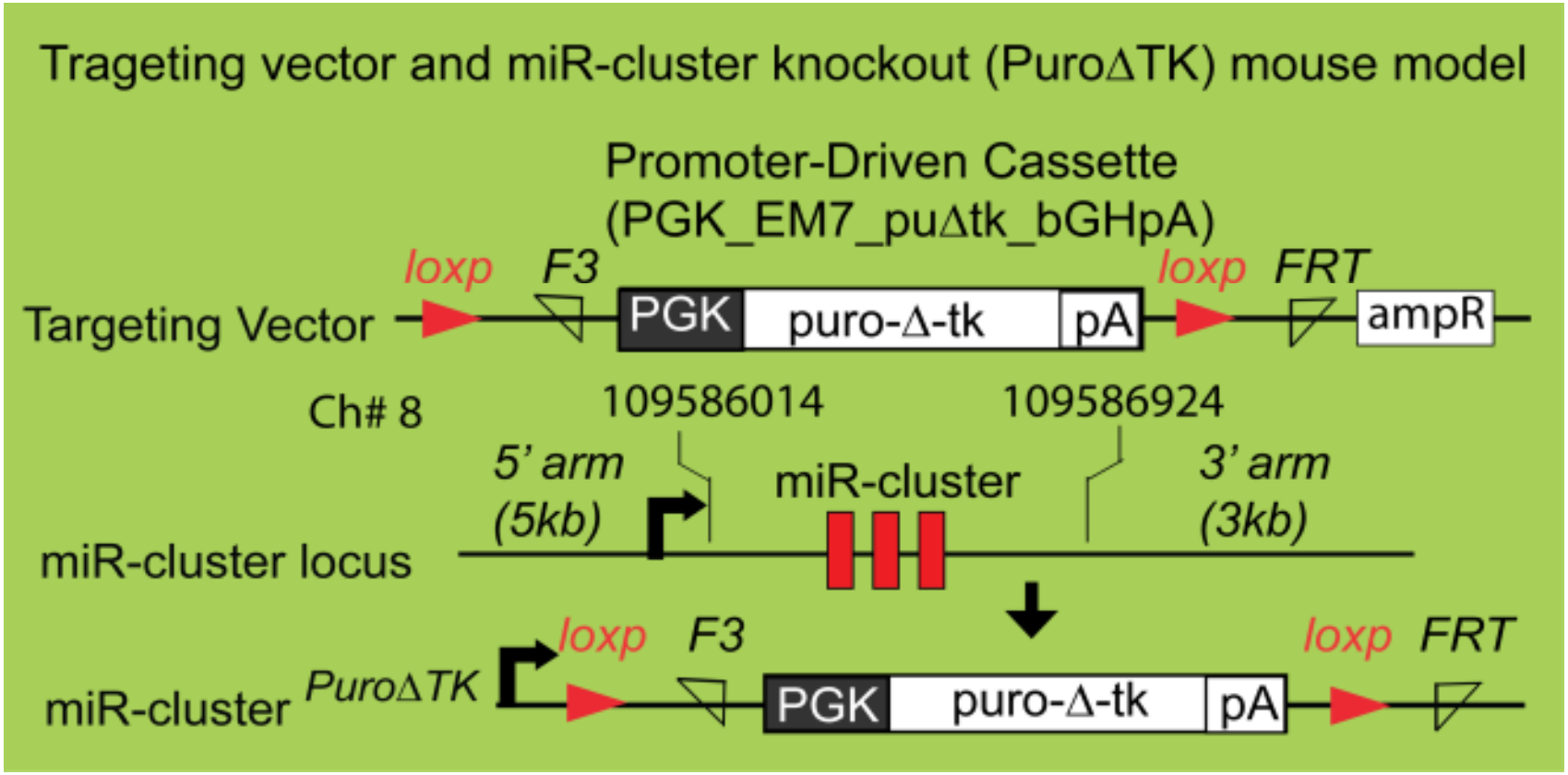


A network connecting Runx2, SATB2 and the miR-23a cluster regulates the osteoblast differentiation program.

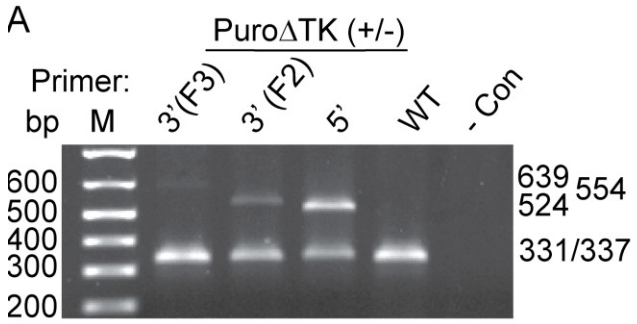


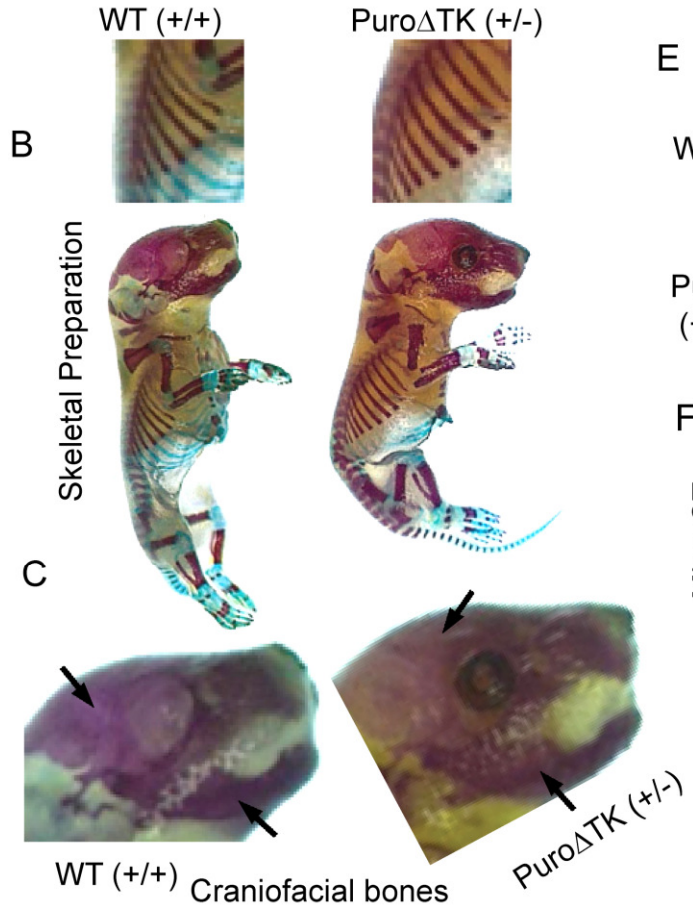


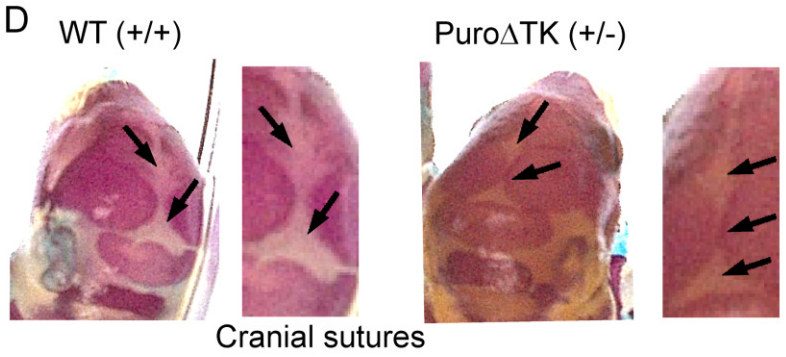
In vivo deletion of the miR-23a cluster: The PuroΔTK primary mouse model

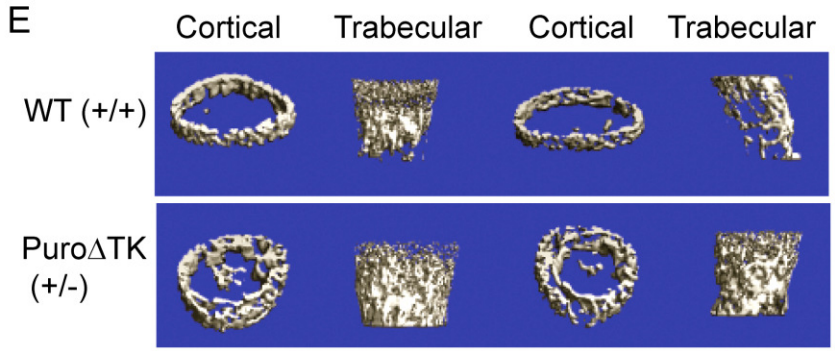


MiR-23a Cluster global Knockout Displayed High Bone Mass









F

MicroCT

	WT (+/+)	PuroΔTK (+/-)
BV/TV	0.1077	0.1731
Cortical bone volume	603.022	624.626
Apparent mineral density (mgAA/ccm)	86.9306	162.8
Tb.Th	0.017	0.019
TB.Sp	0.2516	0.12

Values =average of cortical and trabecular measurements (n=4)

Anti-miR-cluster inducible single copy transgenic mouse

A

5'- ATCACATCGCCAGGGACTTACC *TTCCTGTCAG* GGAAATCCCTGGCAATGTGA CTGAGCTCTGCCA

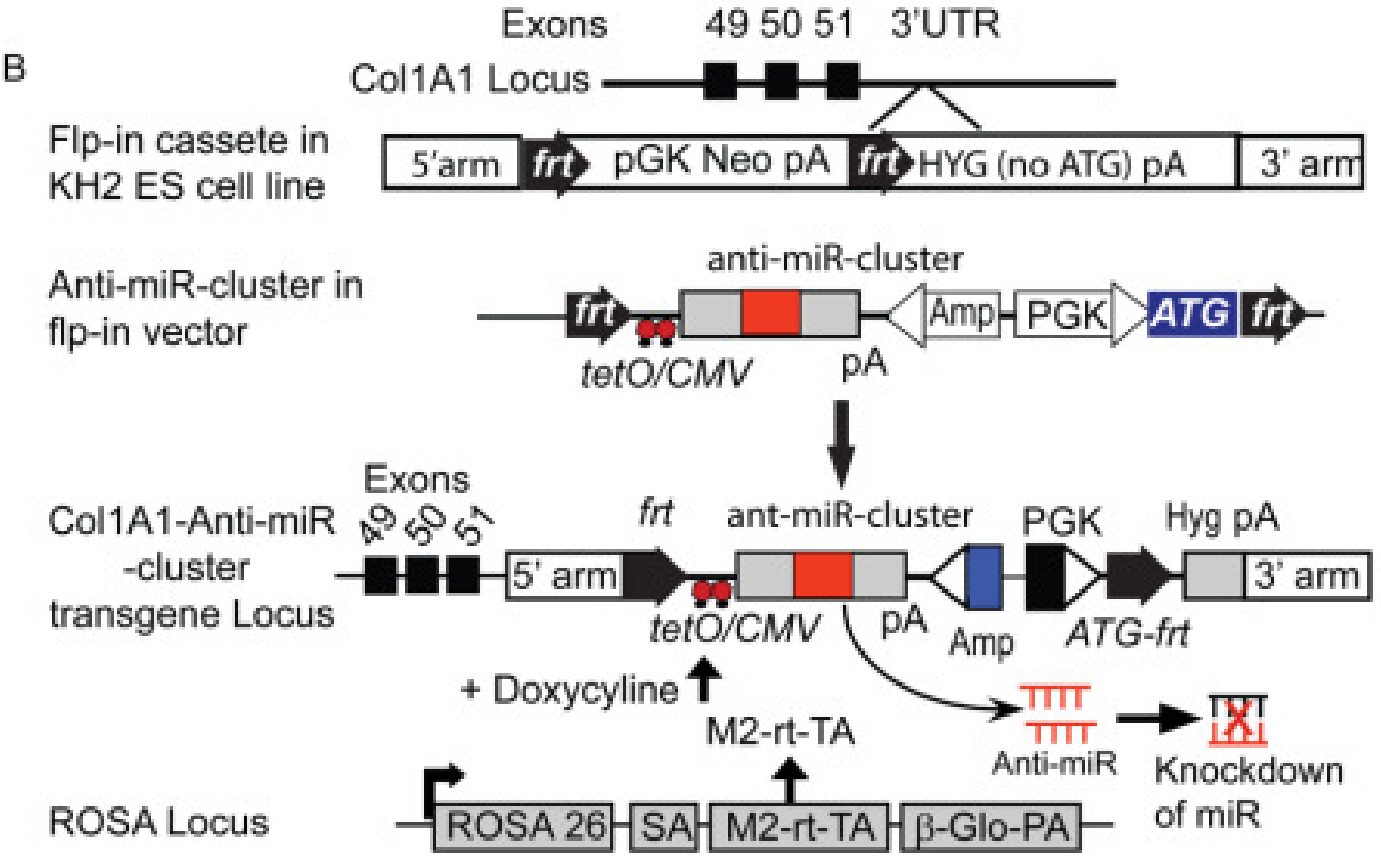
CCGAGGATGCTGCCCGGGGGATCCGTTCACAGCGGCTAAGTCCCACC *TTCCTGTCAG* GCGGAACT

TAGCCACTGTGA CGGGGGTGGCAGAGAGGCCCCGAAGCCTGTGCCTGGCGATCCGTGGCTCAATTCAG

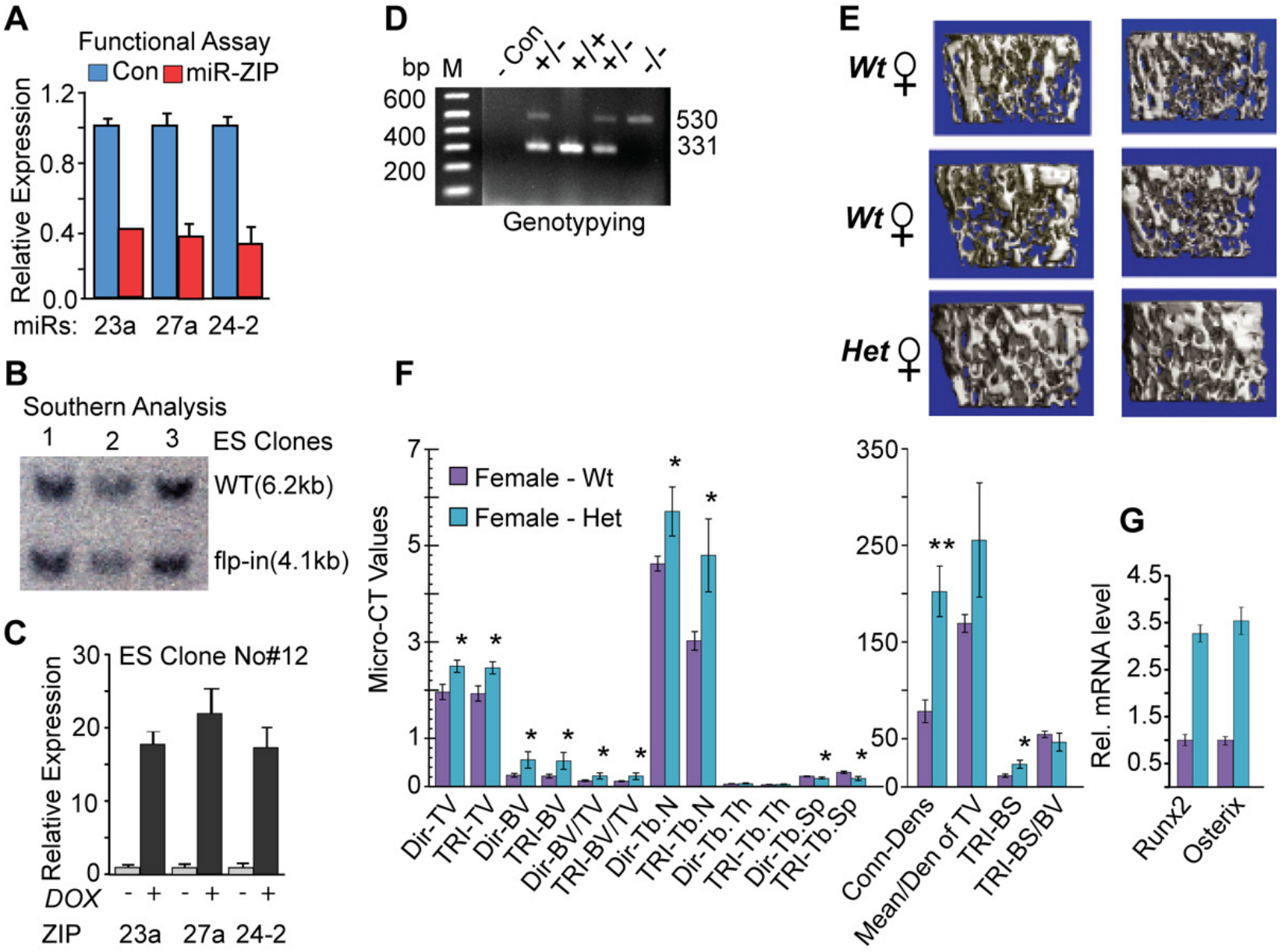
CAGGCACCGC *TTCCTGTCAG* CTGTTCCTGCTGAACTGAGCCA TTTTTT-3'

Loop *Anti-miR-23a* *Loop* *Anti-miR-27a* *Loop* *Anti-miR-24-2*

B



MiR-23a Cluster Knockdown Displayed High Bone Mass



High-throughput RNA sequencing and targets for miR-23a cluster

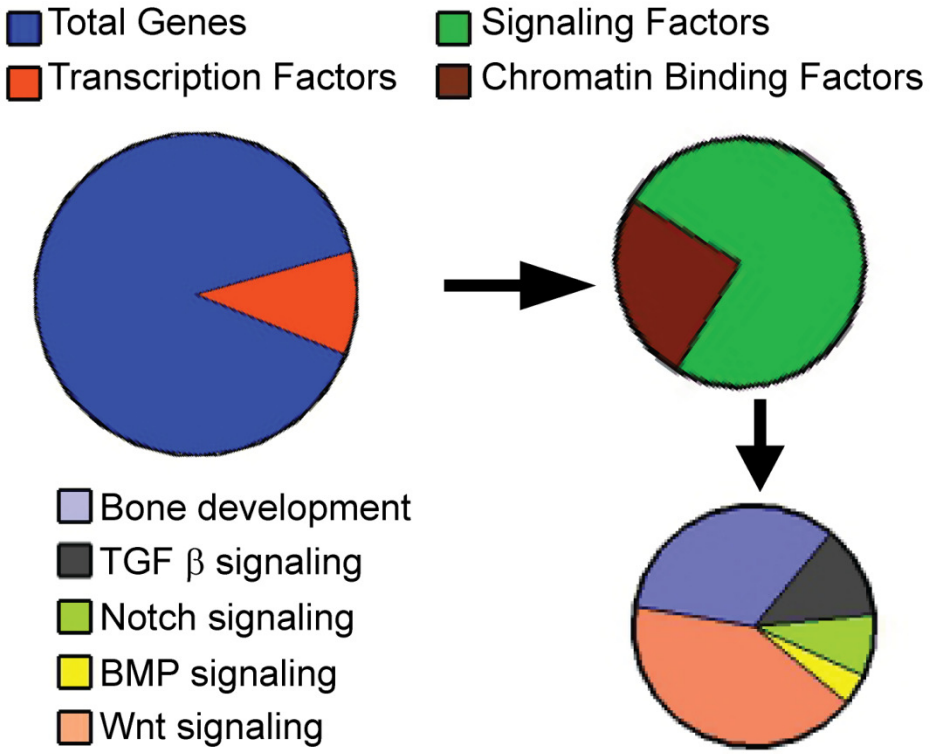
A

Significantly Changed Genes

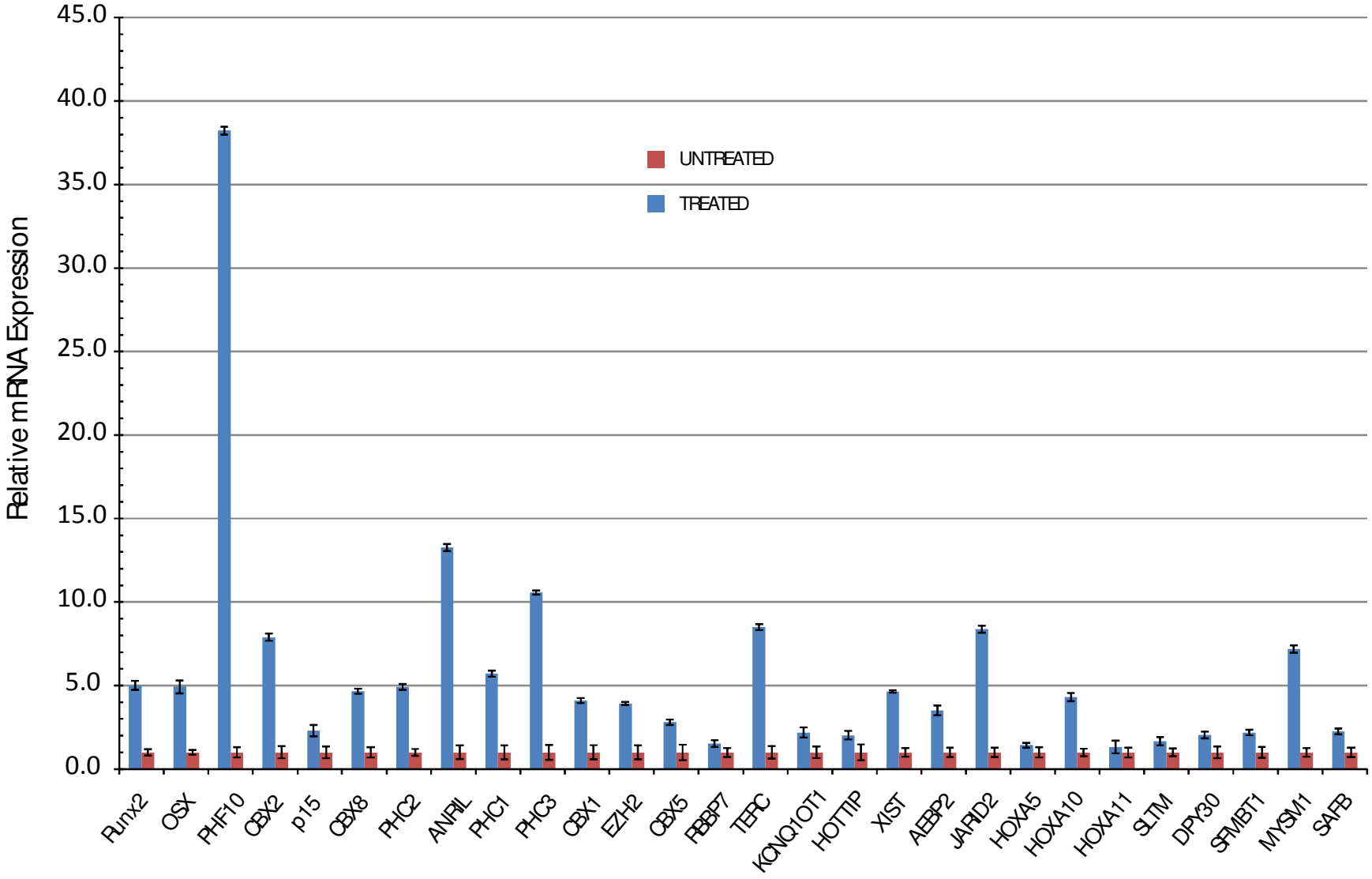
RNA Seq	2 fold
Total Genes	2705
Transcription Factors	277
Chromatin Binding	54
Skeletal Development	39
Wnt Pathway	40
TGF-B Pathway	14
Notch	5
BMP Pathway	2

B

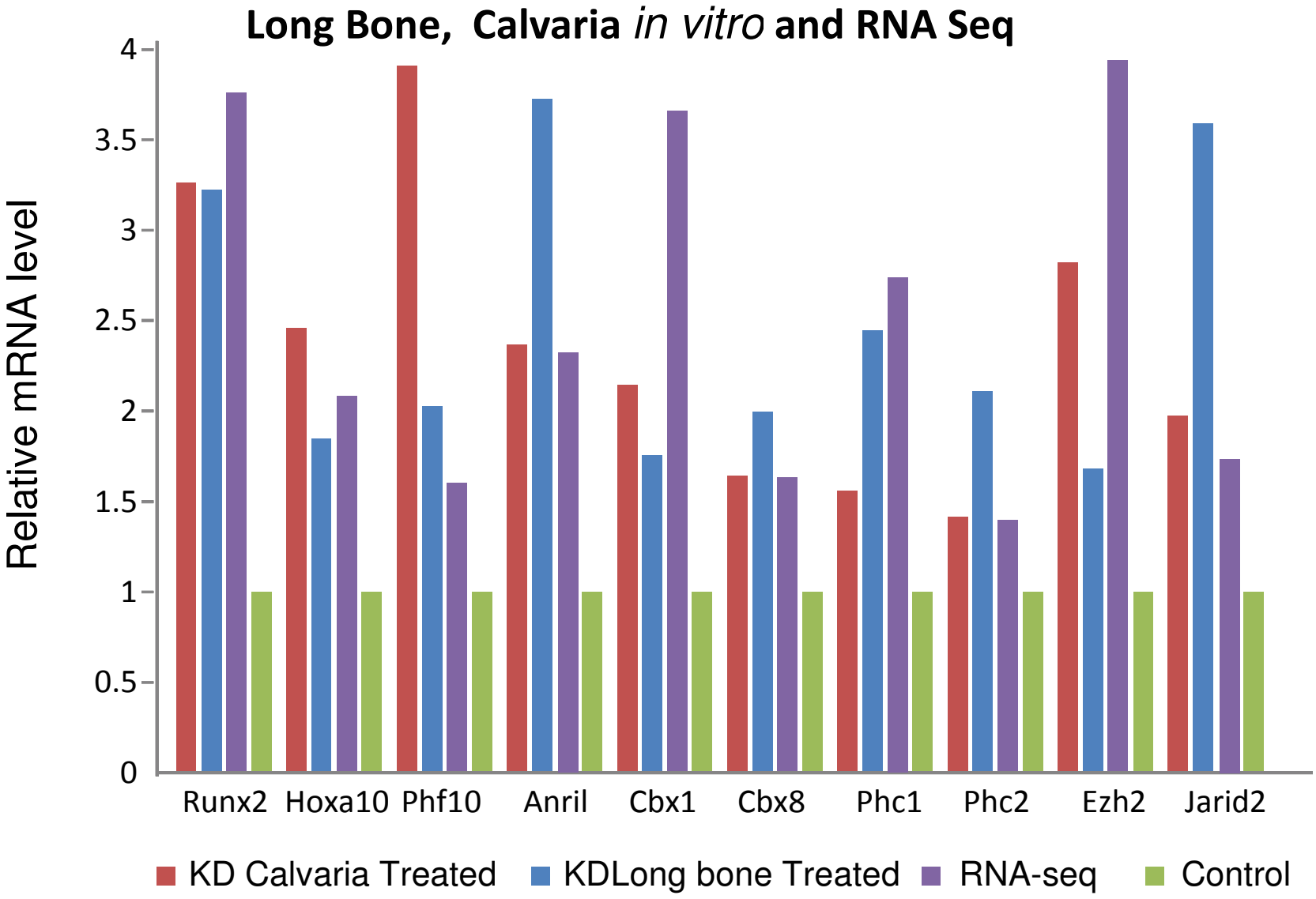
Proportions of the factors within RNA-Seq



Key chromatin binding factors analysis in miR-23a cluster knock down het mice



Key factors confirmed by three different analysis



Conclusion:

1. MiR-23a cluster knockdown mice have high bone mass phenotypes.
2. MiR-23a cluster deregulates osteoblast growth and inhibits osteoblast differentiation *in vitro* and *in vivo.*
3. MiR-23a cluster regulates the expression of the chromatin remodeling factors, crucial for bone formation and development.
4. This tiny biologically processed RNA represses gene expression and represents a power approach for treating skeletal disorders including osteoporosis and osteoarthritis.

Sincere Thanks to…..

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