# Key regulatory junctions stabilizing the osteoblast phenotype

## Implications for cell and tissue engineering


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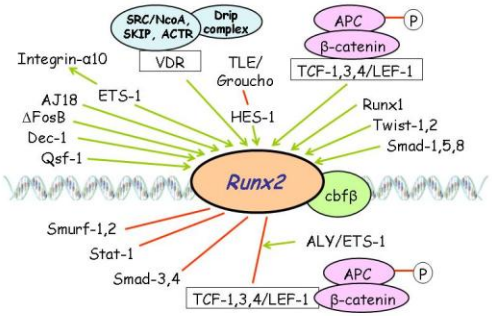
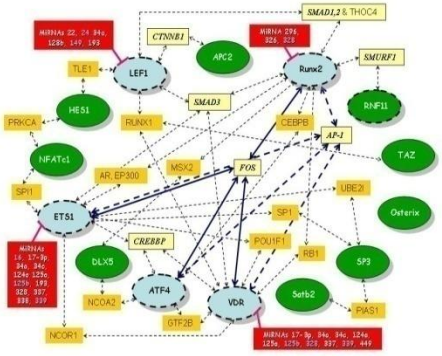
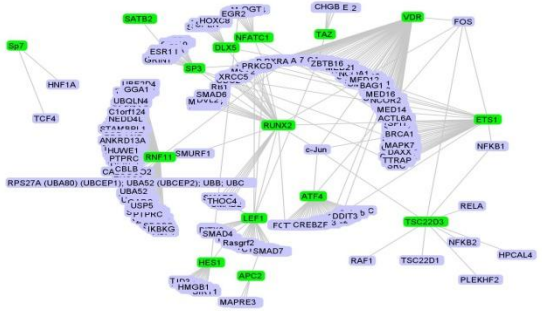
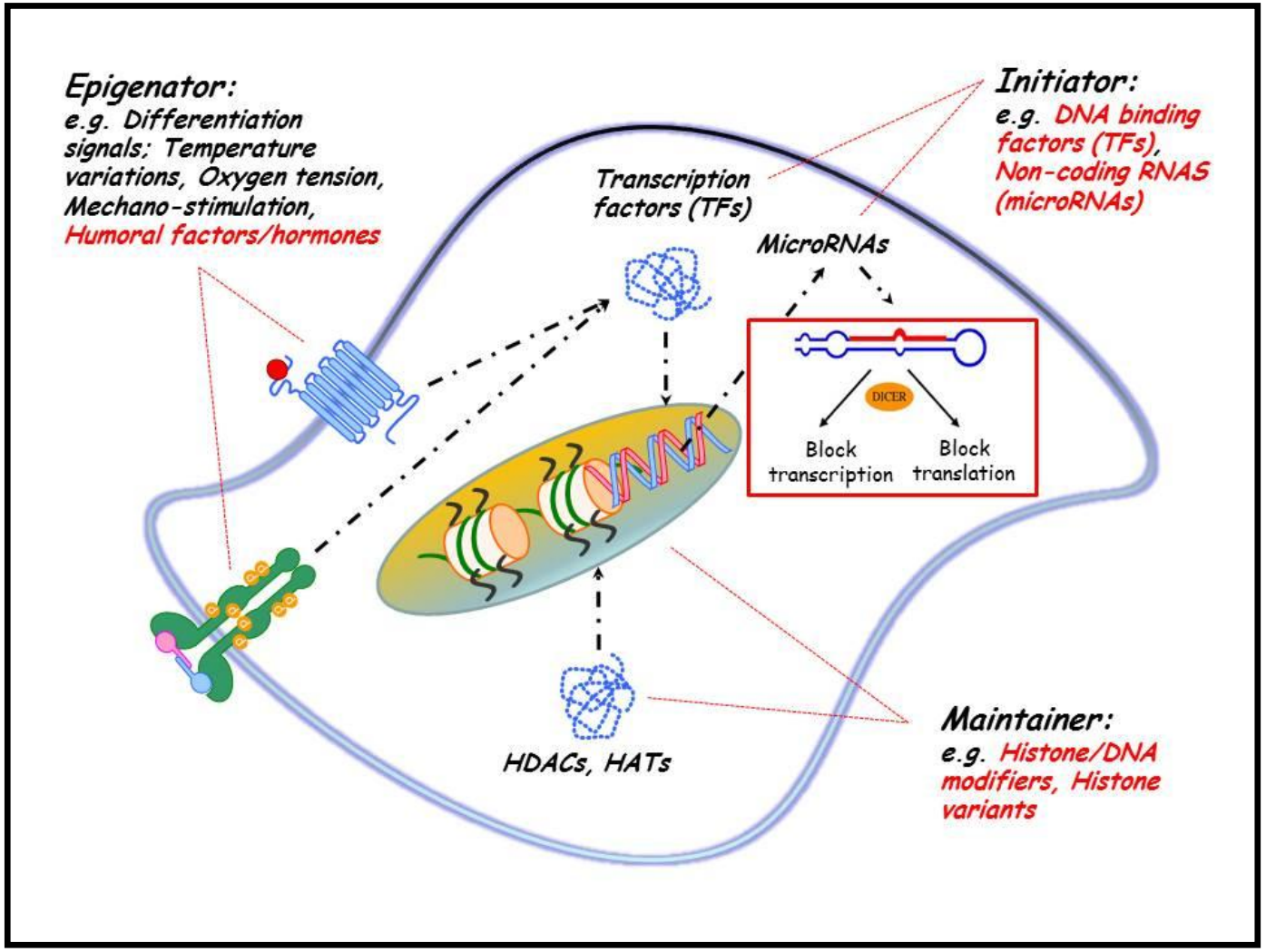


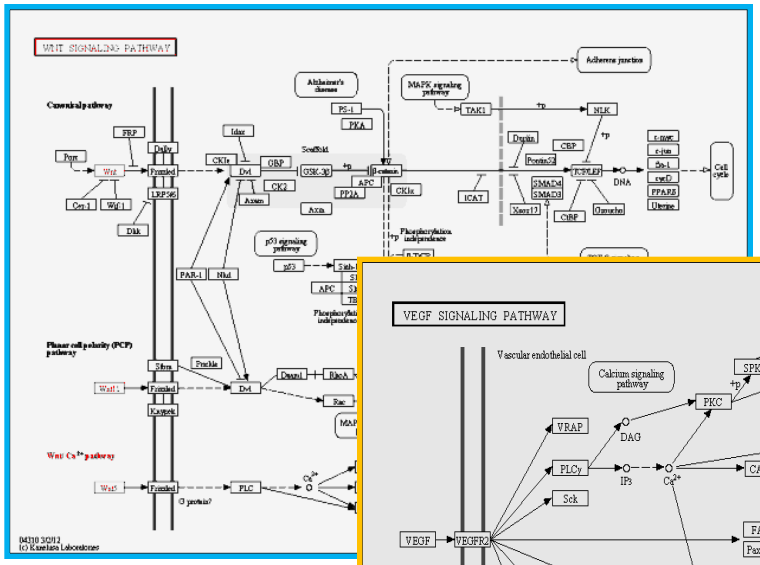



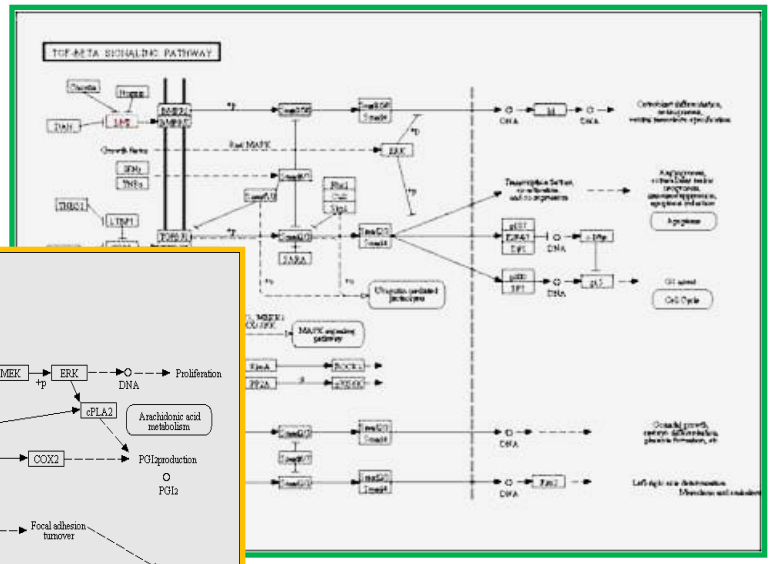



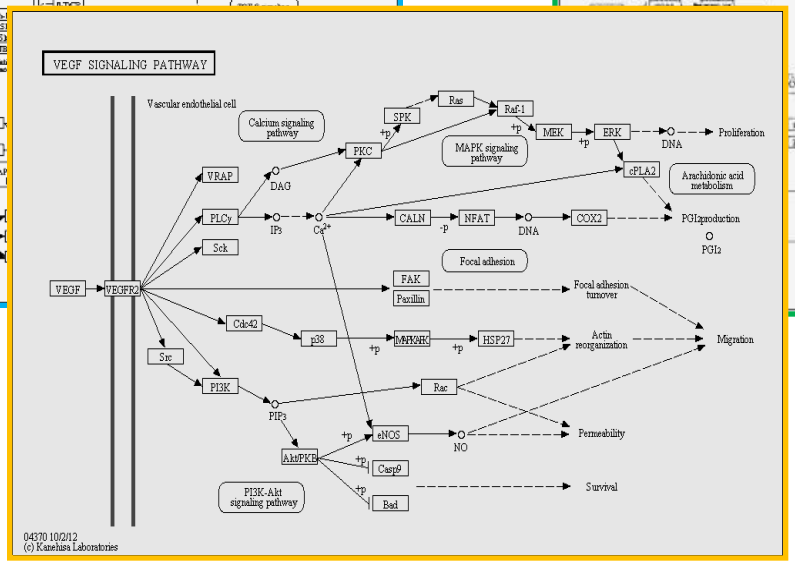
# The «epigenator, initiator, and maintainer» principle of epigenetics on phenotype acquisition and maintenance



# Factors to play with: Signaling molecules

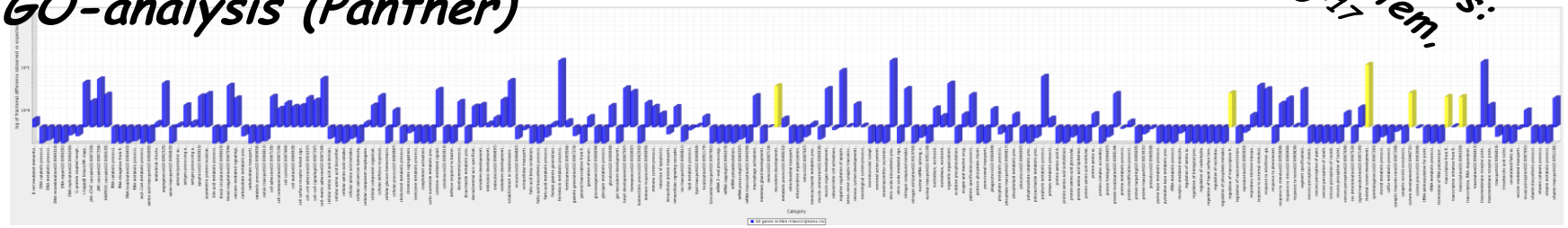


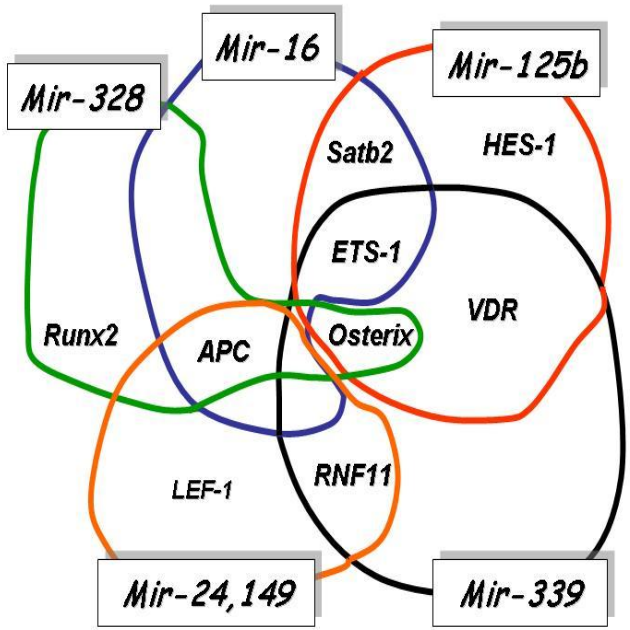

For selected TFs:
Skeletal system,
$p < 10^{-17}$

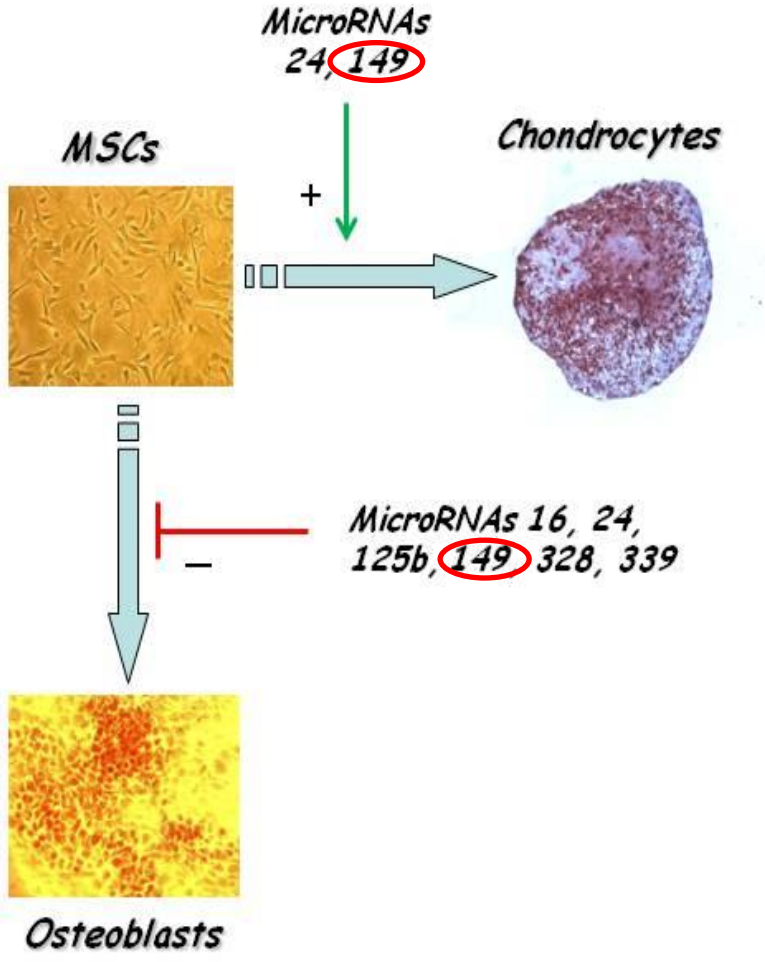
## GO-analysis (Panther)



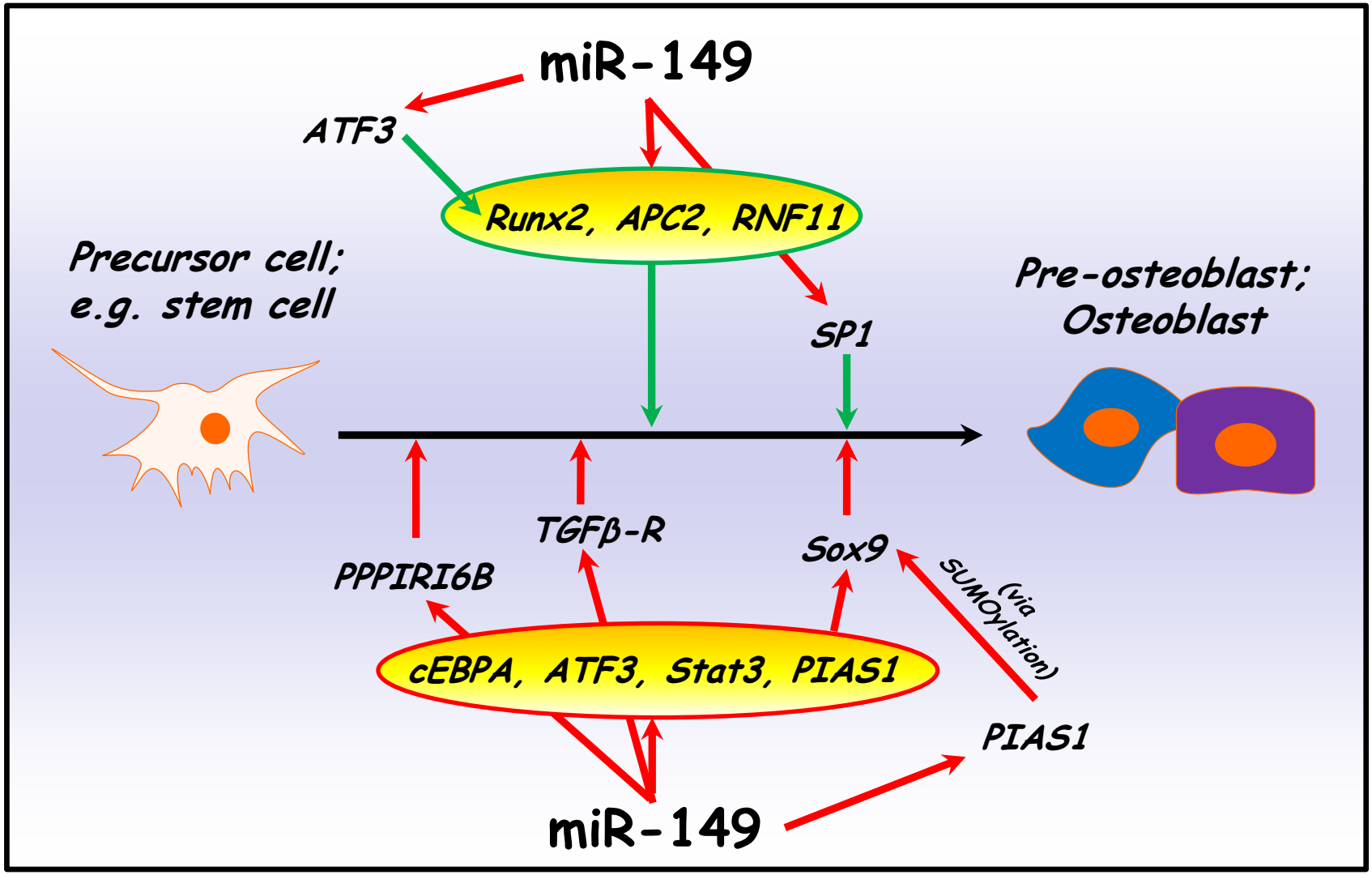
# How the microRNA signature affects differentiation of osteoblasts and chondrocytes from hMSCs

*6 microRNA species specifically block osteoblastogenesis, thereby promoting chondrogenesis, targeting at least 9 transcriptional modulators:*



# *MiR-149 may serve as a switch-MiR yielding either osteoblasts or chondrocytes from stem cells*



# *Experimental approaches:*

1) Identify and challenge **regulatory loops including microRNA species of an osteoblast signature (16, 24, 125b, 149, 328, and 339), as well as 204 and 211, and transcription factors (TFs)** instrumental in «guiding» stem cells to differentiate into osteoblasts, and

2) Test «stabilized» osteoblasts for **resilience towards exposure to cytokines** produced by Th-cells.

# The use of the Mir@nt@n algorithm predicting interactions between transcription factors (TFs) and microRNAs

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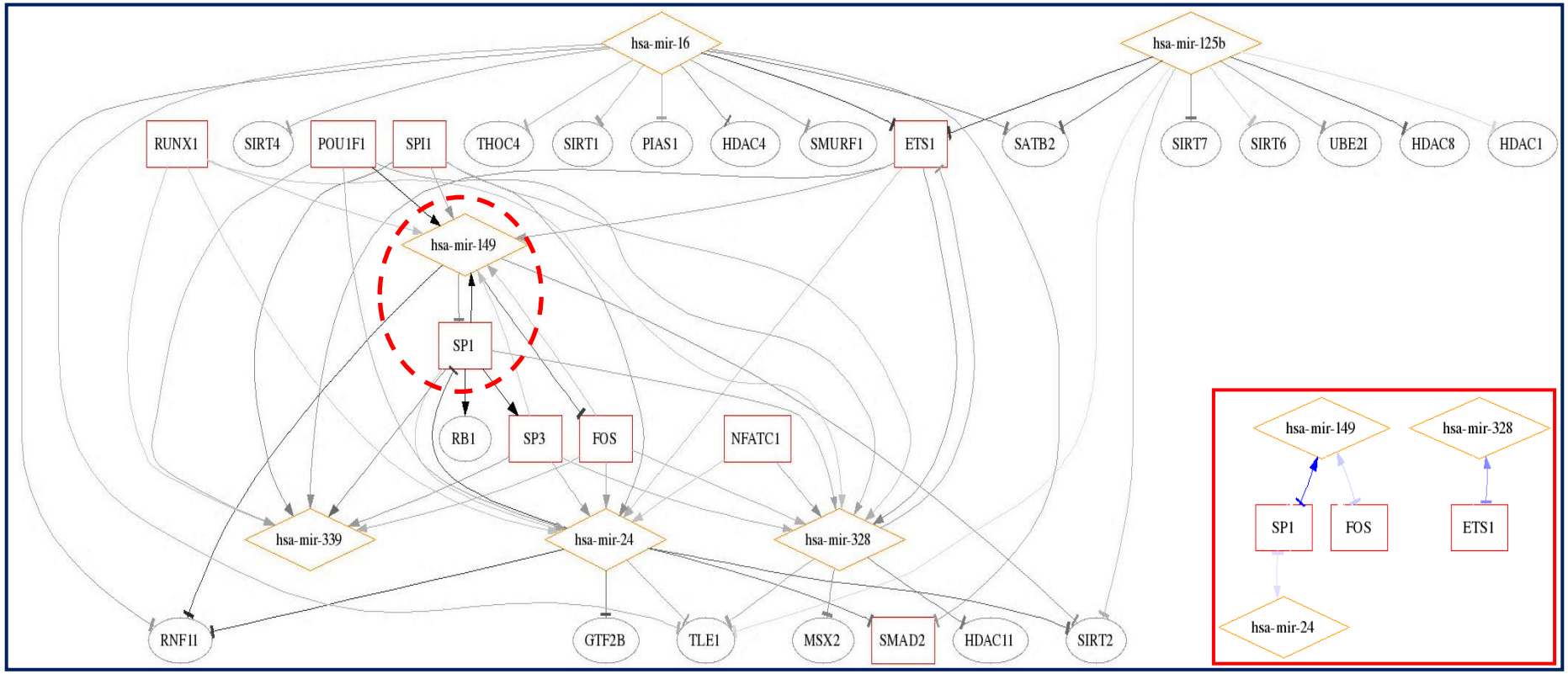
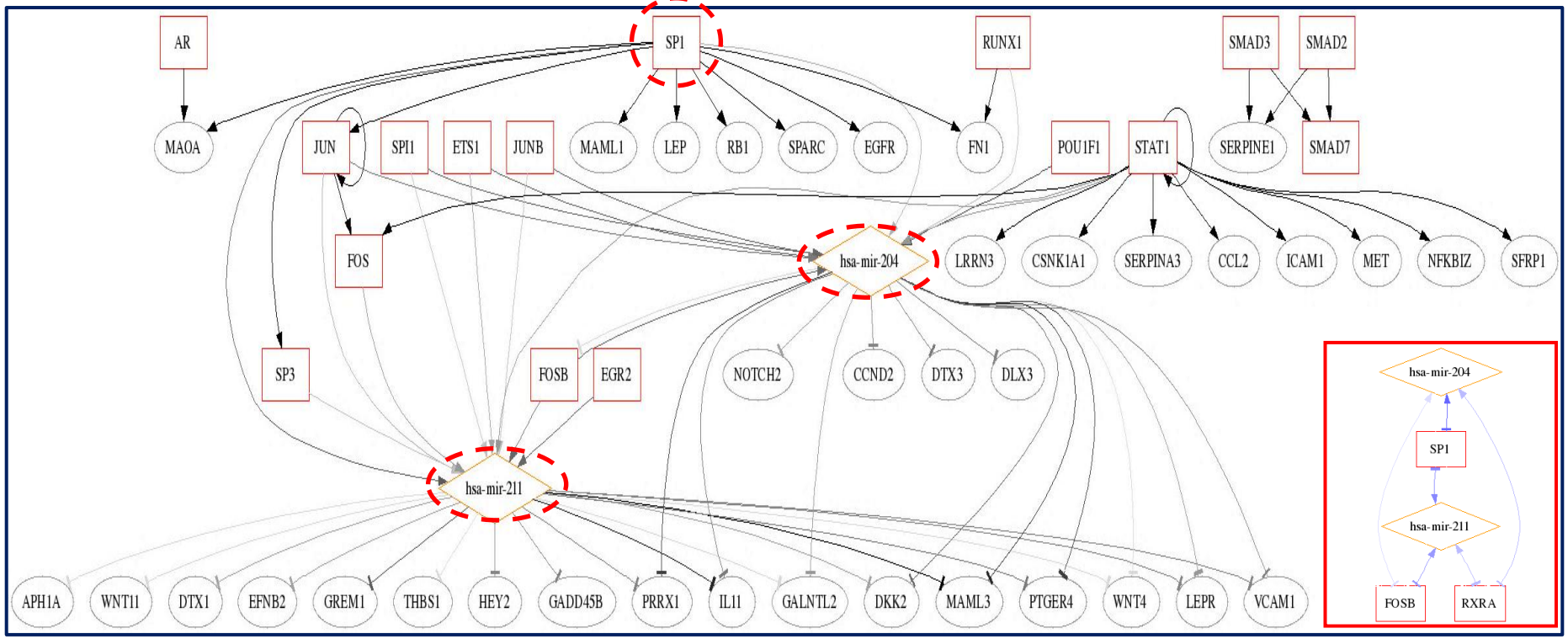
## Osteoblast



# *The use of the Mir@nt@n algorithm predicting interactions between transcription factors (TFs) and microRNAs*

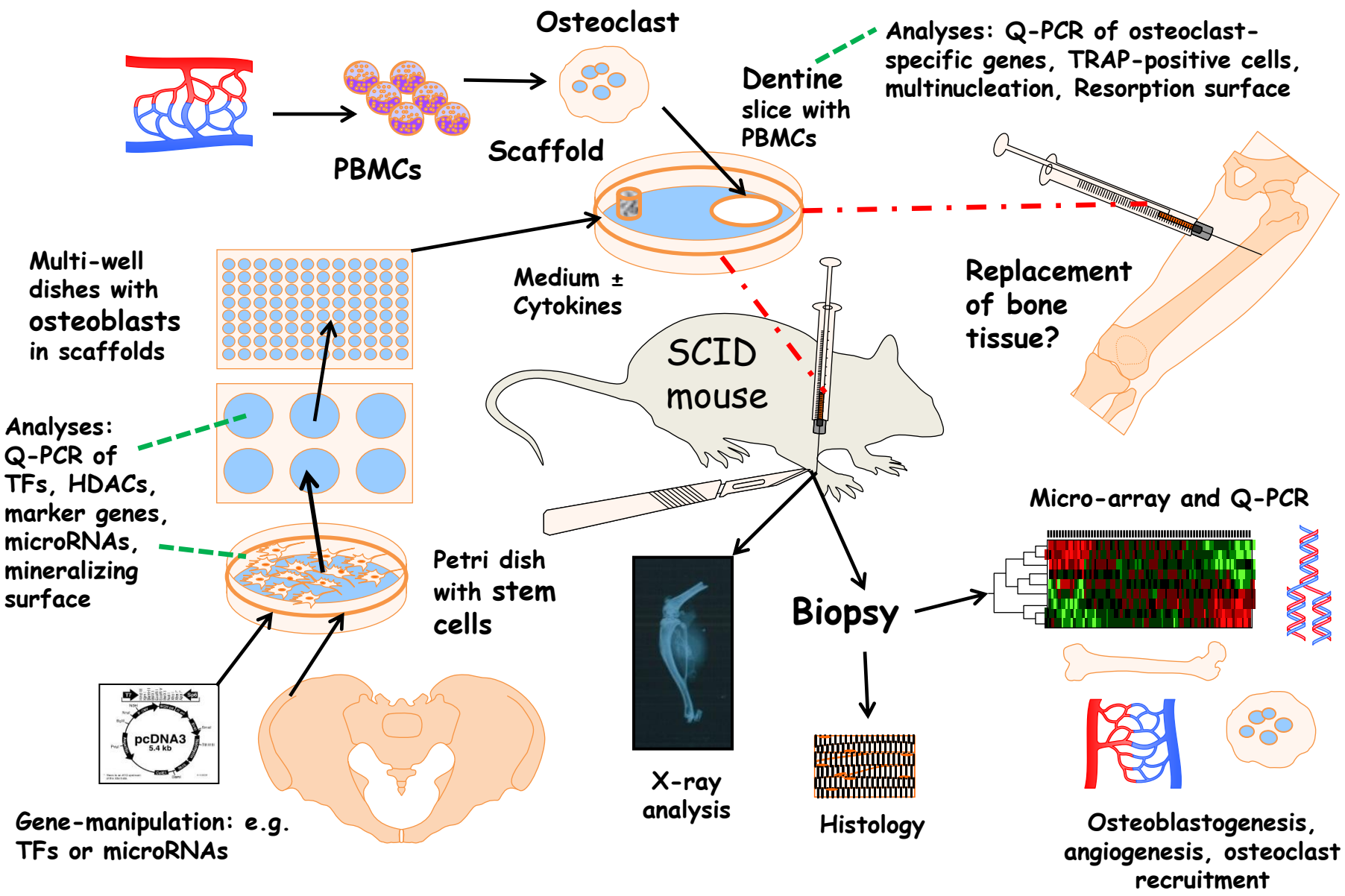
## Osteo-chondro-adipocyte



# *Is SP1 important for Osteoblastogenesis?*

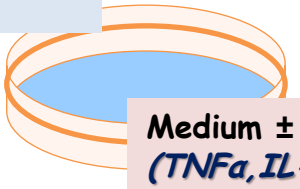
"From a literature search (PubMed) on "SP1 transcription factor and osteoblasts", **SP1** is somehow interfering with the effect of **Runx2**, **SP7** (osterix), **FIAT (inhibitor of ATF4)**, **ETS-like TFs**, MZF1 (myeloid zinc finger), **JUNB**, and also directly affecting the transcription of marker genes like **Col1a1, Col5a1, Col5a3, Col11a2, fibromodulin, osteocalcin, MGP (matrix-gla protein), RANKL, Pit phosphate transporter, Integrin β5, and TGFβ-R1**.

# Outline of interconnected experiments



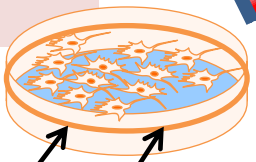
# Outline of key experiments

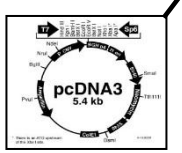
***Analyses conducted:***
Mineralized surface,
Immunohistochemistry,
Q-PCR of microRNAs and
Osteoblast marker mRNAs
and others



**Medium ± Cytokines**
***(TNFa,IL-1, IL-8, and IL-17A)***

**Petri dish with stem cells**






**Gene-manipulation: e.g. of SP1 or microRNAs**

***Stem cells (MSCs or ASCs) are manipulated in terms of either:***

1) SP1-expressing vector or Sh-RNA vs SP1
2) Polycistronic constructs with mir-204/211 or mir-149 and antago-mirs vs same microRNA species

# The effect of SP1 overexpression in engineered osteoblasts

| Parameters | Control = 100% | + Cytokines | + SP1 | + SP1 + Cytokines |
|---|---|---|---|---|
| Runx2 | 100 | 27 | 432 | 324 |
| Collagen1a1 | 100 | 47 | 145 | 133 |
| Osteocalcin | 100 | 23 | 345 | 288 |
| Osterix | 100 | 18 | 182 | 171 |
| Mineralizing «surface» | 100 | 22 | 234 | 198 |
| OPG/Rank-L ratio | 100 | 435 | 27 | 321 |
| PPARγ | 100 | 534 | 28 | 43 |
| HSL | 100 | 385 | 35 | 58 |
| Oil-Red-O «surface» | 100 | 689 | 36 | 32 |
| Mir-149 | 100 | 546 | 34 | 75 |
| Mir-328 | 100 | 465 | 37 | 58 |

# The effect of mir-204/211 suppression in engineered osteoblasts

| Parameters | Control = 100% | + Cytokines | + Antago-mirs | + Antago-mirs + Cytokines |
|---|---|---|---|---|
| Runx2 | 100 | 25 | 389 | 319 |
| Collagen1a1 | 100 | 36 | 319 | 272 |
| Osteocalcin | 100 | 27 | 321 | 251 |
| Osterix | 100 | 31 | 301 | 199 |
| Mineralizing «surface» | 100 | 47 | 247 | 167 |
| OPG/Rank-L ratio | 100 | 378 | 21 | 271 |
| PPARγ | 100 | 444 | 19 | 39 |
| HSL | 100 | 417 | 27 | 47 |
| Oil-Red-O «surface» | 100 | 571 | 27 | 41 |
| Mir-149 | 100 | 449 | 31 | 69 |
| Mir-328 | 100 | 577 | 41 | 48 |

# Summary and Conclusion

1) *The use of bioinformatics (the Mir@nt@n algorithm) backed by PubMed searches yields interesting paradigm shifts as to which of many TFs are the better markers for cell phenotypes (SP1 instead of Runx2; Osterix = SP7) characterizing osteoblasts?*

2) *Manipulating members of regulatory loops encompassing TFs and microRNAs makes it easier to either disrupt or reinforce the stability of a certain phenotype (e.g. enhance osteoblast resilience against exposure to imflammation).*

3) *Identifying regulatory loops encompassing microRNAs and TFs may thus be important/mandatory for the success of cell engineering/replacement cell therapy.*

*To be commended for their scientific and technical support and never-ceasing enthusiasm:*
*INSERM U844, Montpellier, France*
*&*
*Université Montpellier 1, enabling me to work within the U844 as a guest professor for 3 years*

*Thank you for your attention!*