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University Medical Center Rotterdam

# A backbone to systems biology and multifactorial disease: system biology of mineralization, bone formation and osteoporosis

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# Osteoporosis –multifactorial and complex skeletal disease:

- low bone mass
- microarchitectural deterioration of bone tissue

↓

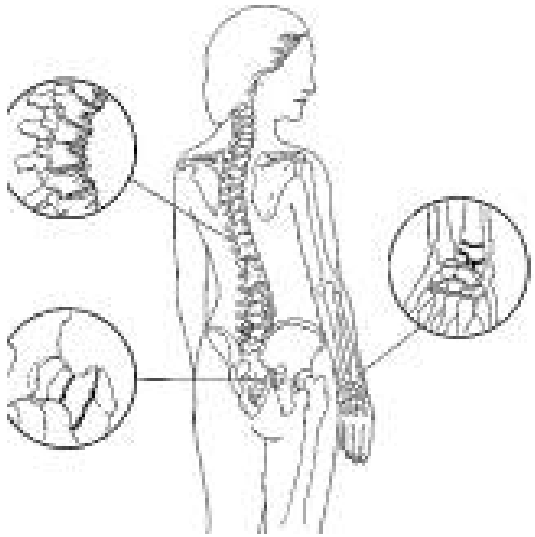
enhanced bone fragility & increase in fracture risk


Electron micrograph of normal bone and osteoporotic bone

# Osteoporosis is a major public health problem in Western-type societies

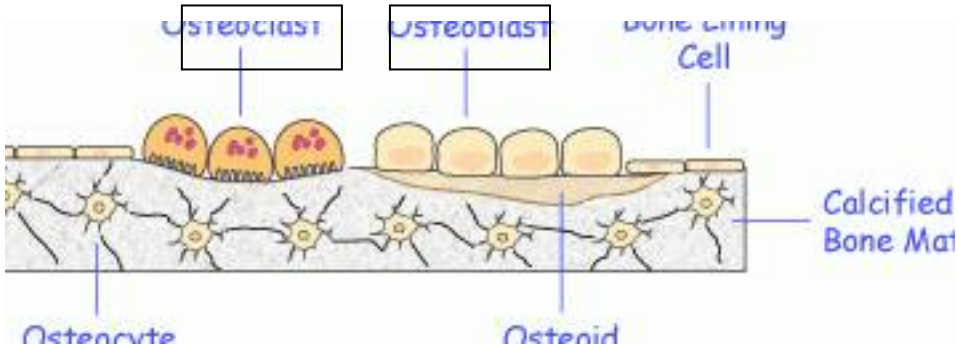
**Prevalence**

- 1 in 3 women and 1 in 12 man over the age of 50 world wide have osteoporosis
- million of fractures annually, mostly involving vertebrae, hip and wrist



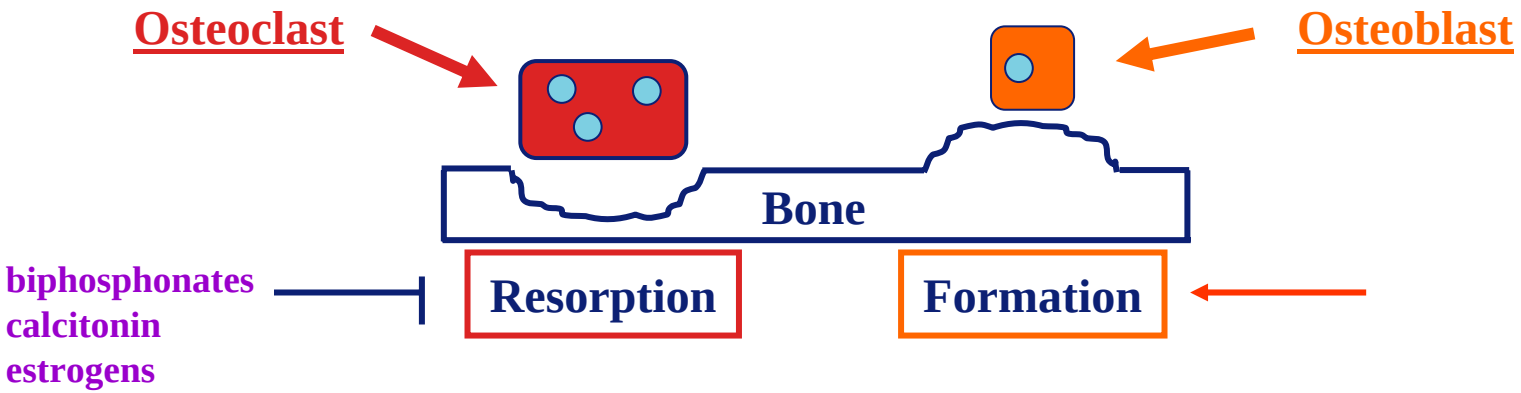
# Bone is composed of:

- osteoblasts - bone forming cells
- osteoclasts - bone resorbing cells
- non-mineral matrix of collagen and non-collagenous proteins
- inorganic mineral salts deposited within the matrix



# Bone Turnover

- The skeleton is metabolically active and constantly remodeling
- Highly-regulated process
- Precise balance between bone break-down (**osteoclasts**) and bone formation (**osteoblasts**)




Imbalance between bone resorption and bone formation

↓

**osteoporosis**

## *Why osteoblasts?*

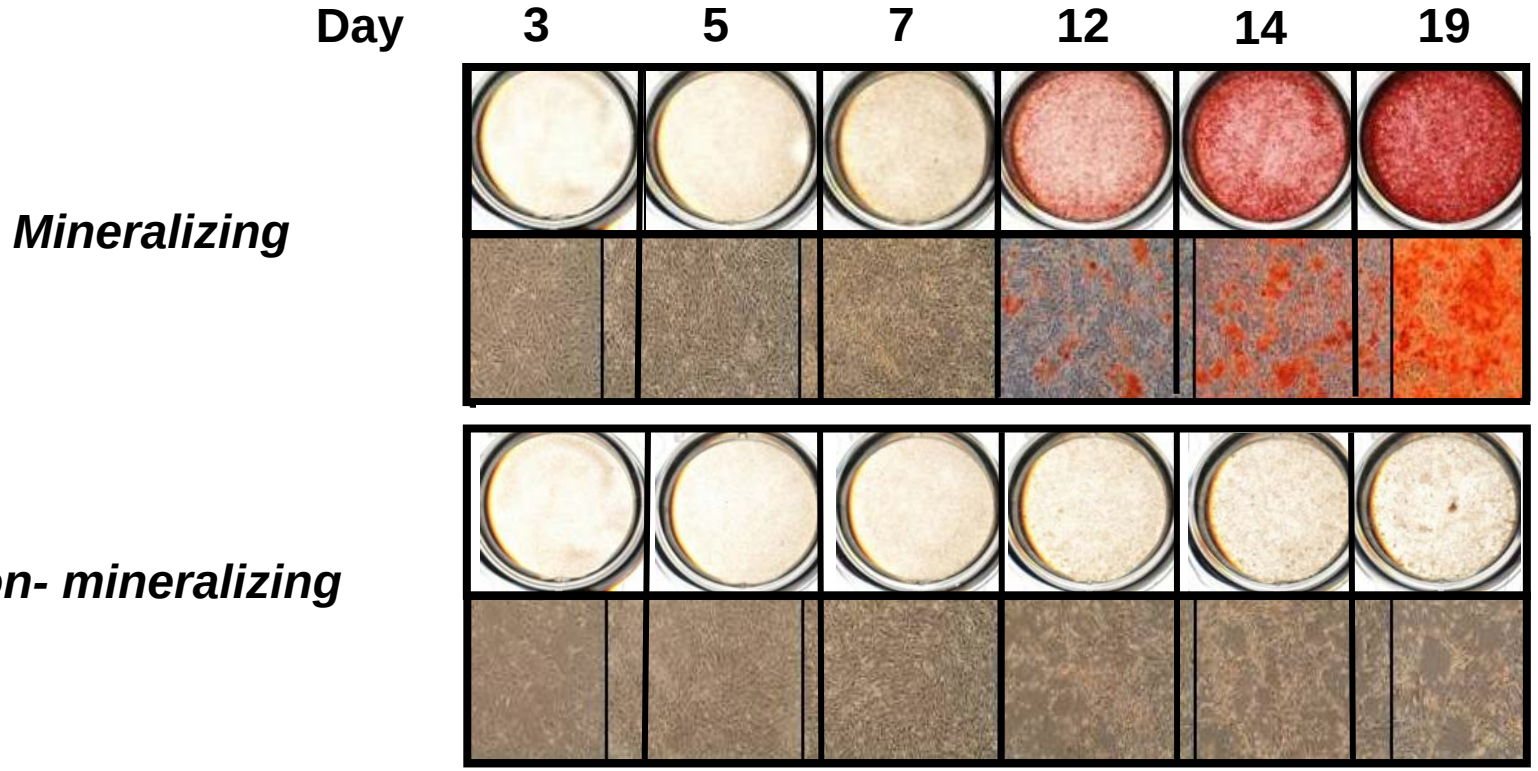
- Osteoblasts play a pivotal role in bone
  - Bone formation
  - Control of bone resorption: osteoclast formation / activity
- Deranged osteoblast differentiation and function is at the basis of the majority of skeletal disorders (osteoporosis)

## The hypothesis of the project:

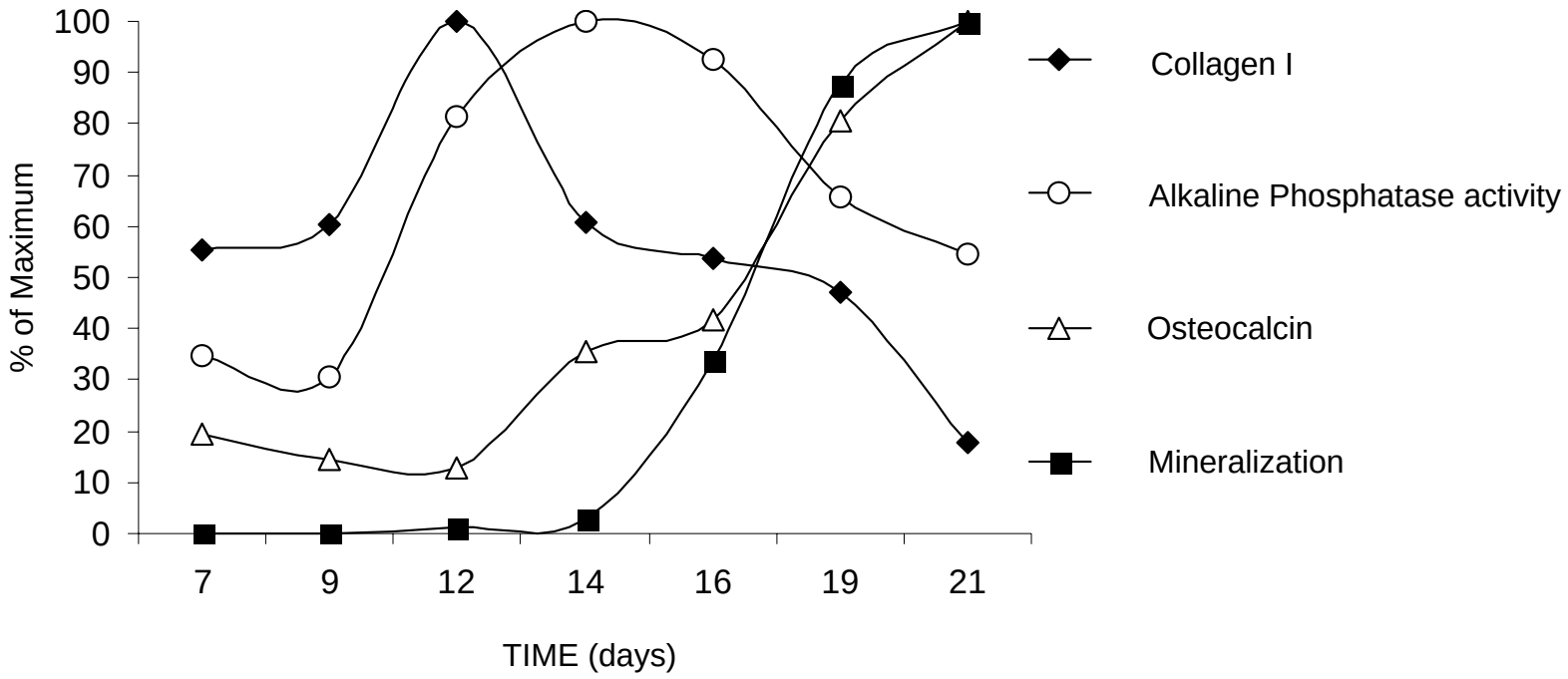
Identification and characterization of genes and molecular networks/cellular processes involved in human osteoblast differentiation and function and implementing this in a system biology approach:

- Identify regulatory networks that control osteoblast differentiation, bone formation and mineralization
- Understand deranged osteoblast function – understand development of osteoporosis
- New diagnostic and prognostic markers for osteoporosis
- Novel therapeutic tools

# In vitro human pre-osteoblast differentiation and bone formation / mineralization model

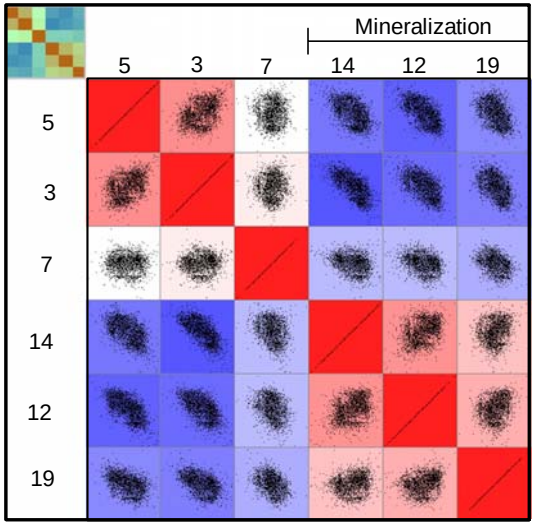


# Collagen type I$\alpha$1, Alkaline phosphatase activity, osteocalcin and mineralization are induced in a time-dependent manner during human osteoblast differentiation



# Affymetrix-based gene expression proffiling during human osteoblast differentiation and mineralization

Identification of Genes, Signaling Pathways, Molecular Networks and Cellu
osteoblast differentiation and mineralization.


*Array-based gene expression patterns are confirmed by patterns assessed by quantitative real time PCR with different sets of RNA*

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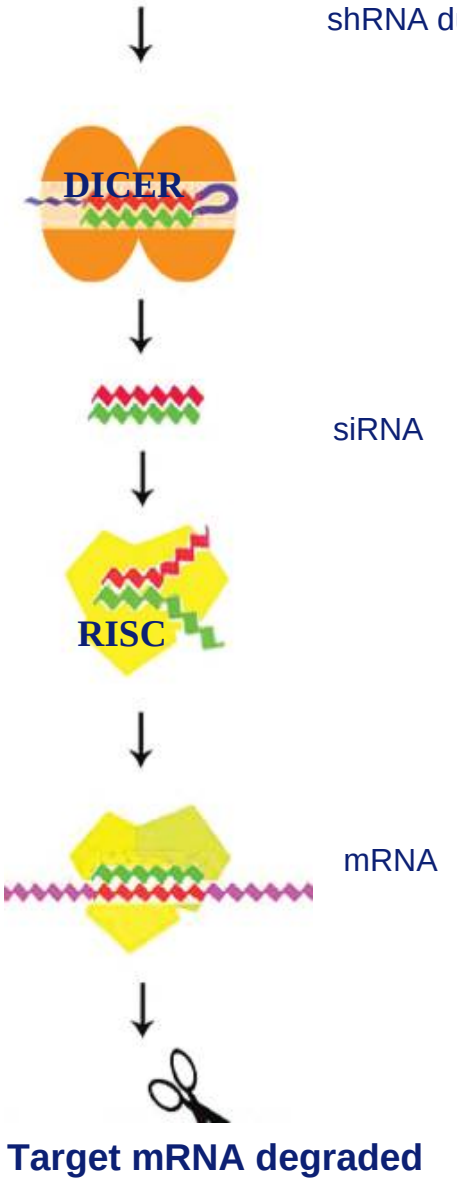
# Summary

- Identified genes that are strongly up- or down-regulated during mineralization
- Identified biological processes and cell components that are overrepresented during mineralization
- Delivered sets of genes, both genes known to be involved in bone metabolism and genes yet unknown to be involved in bone metabolism

- Transcriptional / translational control
- Functional analyses
- Analyse interaction between genes identified / identify functional networks

# Gene functional analysis: RNA interference as a tool for gene silencing




Simplified mechanism:

• Vector-based shRNA are processed by a nuclease called Dicer to form 21-23 bp siRNA duplexes.

•The duplexes then associate with the RNA induced silencing complex (RISC), which contains a helicase that unwinds the duplex.

•Each siRNA strand incorporated RISC is than presented to the cytoplasmic mRNA pool, where it associates with its complementary mRNA strand and, depending on its complementarity, results in either targeted cleavage of the mRNA or translational arrest.

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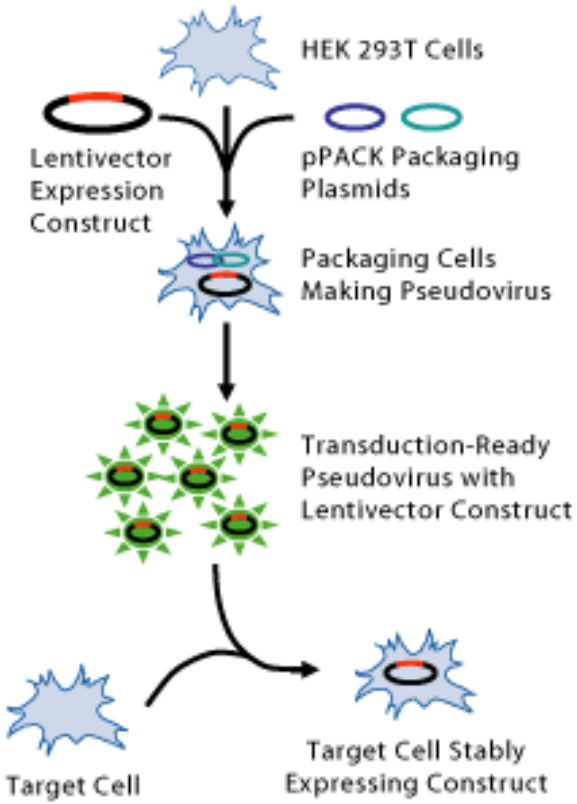
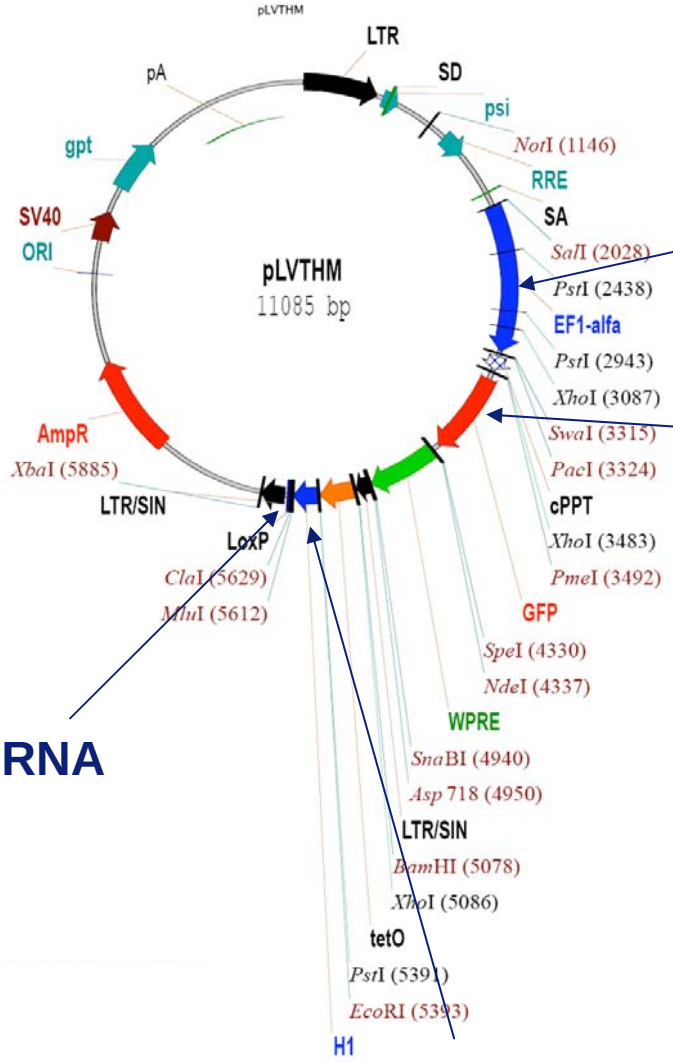
## Lentivector Expression systems




- vectors based on the human immunodeficiency virus 1 backbone
- can stably transduce dividing and non-dividing cells by integrating in their genome and are best suited for long-term gene silencing
- third generation of self-inactivating LV (derived from Trono lab) - 3 or 4 component system
- broad cell tropism
- stable recombinant virus particles

# Diagram of LV vector (pLVTHM)

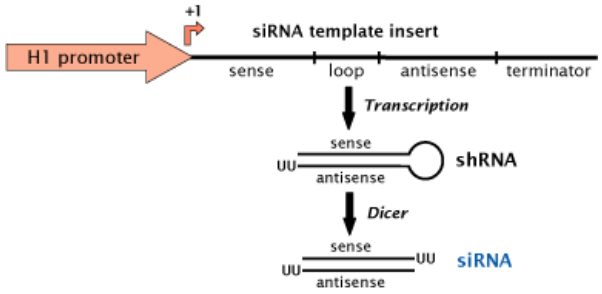



EF1promoter

GFP

shRNA

H1 promoter




**Short hairpin RNA (shRNA)** – A short RNA that contains sense and antisense sequences from a target gene that are connected by a hairpin loop

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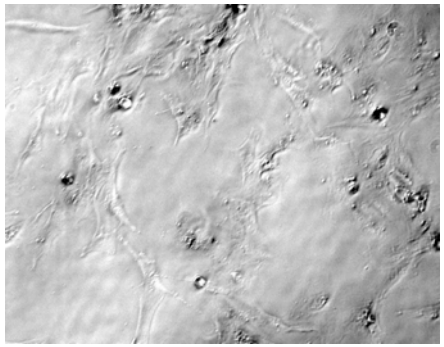
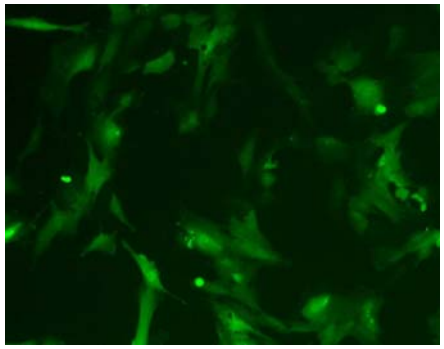
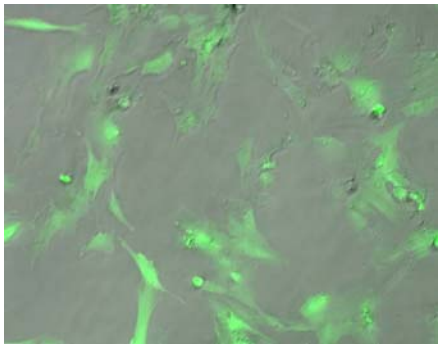
# Human pre-osteoblasts transduced with pGFP-LV 48h after transduction



inverted microscope image

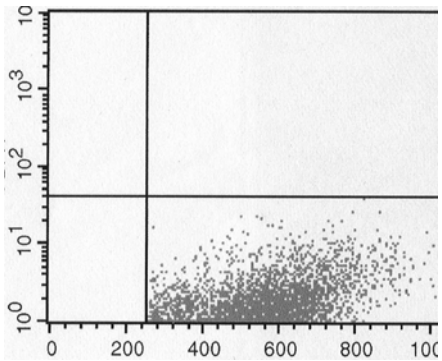


GFP expression

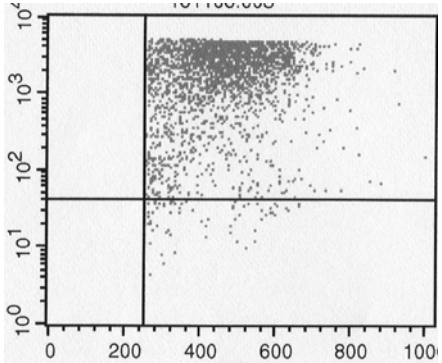


overlay

# Human pre-osteoblasts: GFP expression 21 days after GFP-LV transduction




| Quad | Events | % Gated | % Total |
|---|---|---|---|
| UL | 0 | 0.00 | 0.00 |
| UR | 0 | 0.00 | 0.00 |
| LL | 0 | 0.00 | 0.00 |
| LR | 3750 | 100.00 | 37.50 |




| Quad | Events | % Gated | % Tota |
|---|---|---|---|
| UL | 0 | 0.00 | 0.0( |
| UR | 2733 | 97.43 | 27.3 |
| LL | 0 | 0.00 | 0.0( |
| LR | 72 | 2.57 | 0.7 |

After 21 days in culture efficiency of LV transduction was 97.43%

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## *Summary / Discussion*

- Gene expression profile in direct relation to the human osteoblast differentiation and mineralization.
- Known and novel Genes, Signaling Pathways (canonical pathways), Molecular Networks and Cellular Processes crucial for osteoblast differentiation and bone formation/mineralization.
- Extend the global gene expression by protein and phosphoprotein profiling in relation to osteoblast differentiation, bone formation and mineralization (nano-LC/mass spectrometry).
- Functional characterization of genes in identified networks (ligands agonists/antagonists, inhibitors, RNAi, etc.), with accurate determination of the time dependencies of signal transduction.

Translate biological system into novel computational model, which allows spatio-temporal and quantitative prediction of key regulatory factors involved in osteoblast differentiation

*Ultimately to develop predictive model of human disease*

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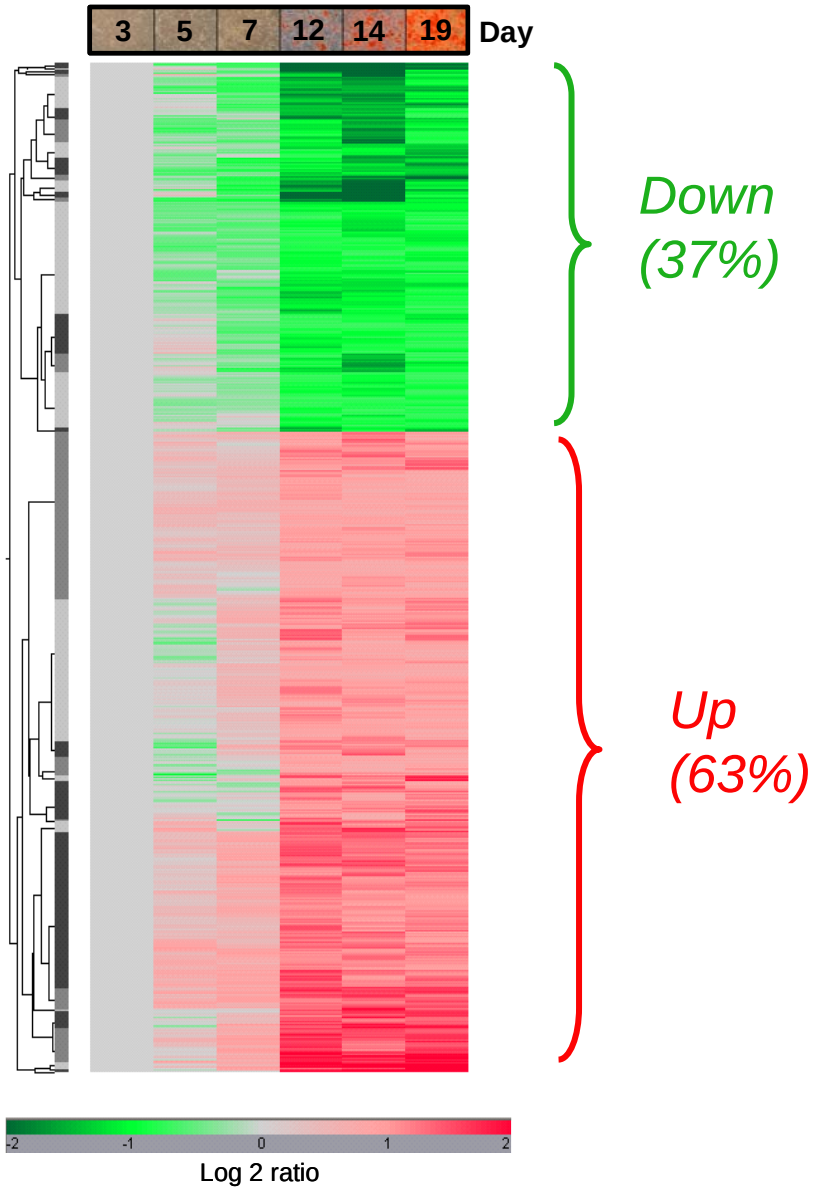
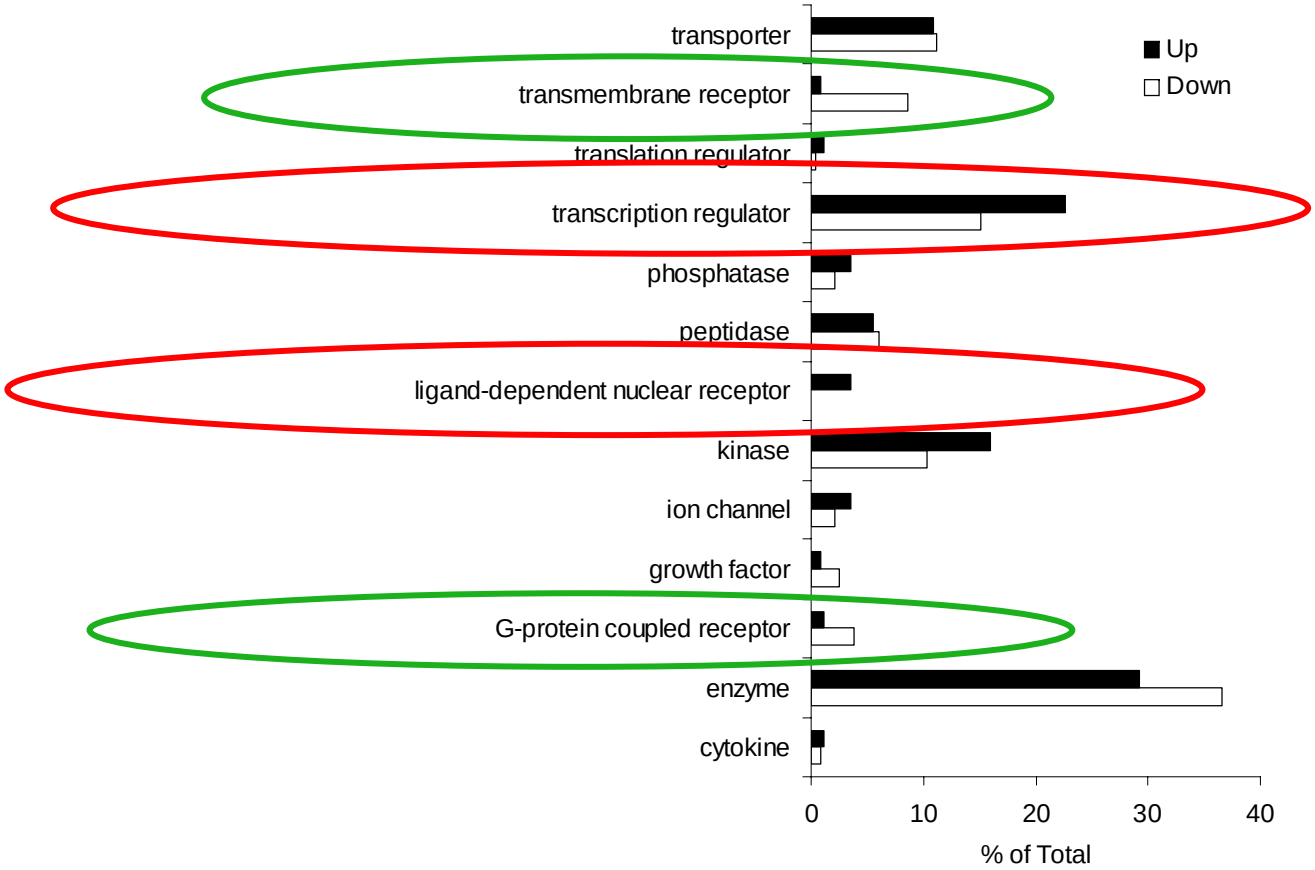
Treescape representation of genes matching the query: 1145 unique genes



# Distribution of genes regulated during mineralization by protein family

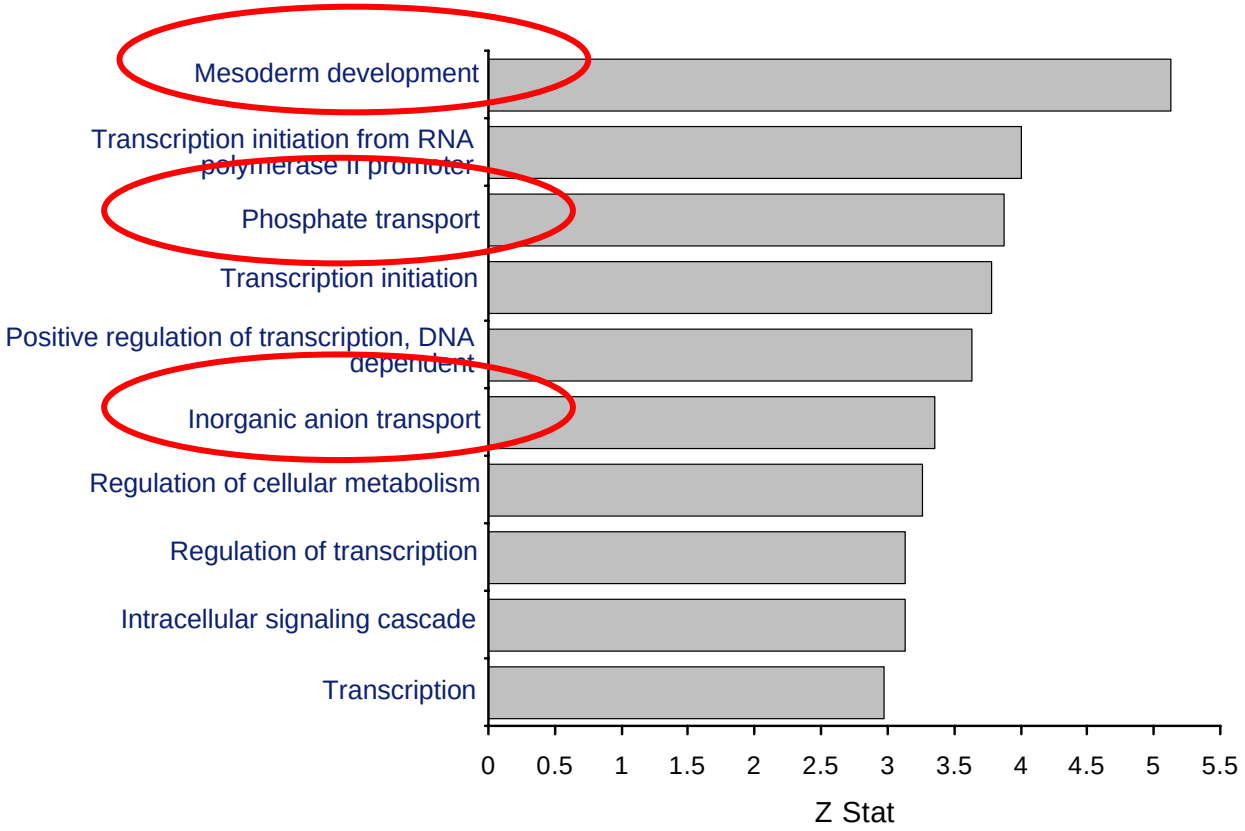
Distribution on basis of genes with known links to protein families.
Still about 45% no link known yet.




Analysis using Ingenuity
(http://www.ingenuity.com)

# Gene Ontology analyses (www.geneontology.org)

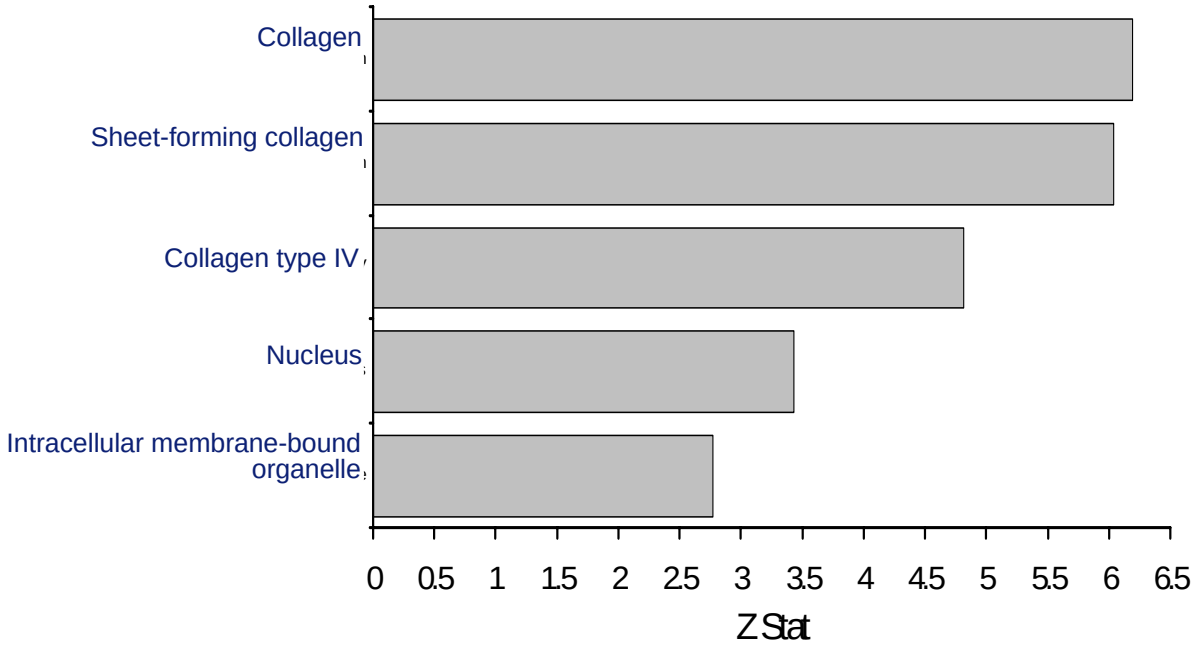
*Biological process*




Ontology Traverser, C. Shaw, Baylor College of Medicine, Houston, USA

# Gene Ontology analyses (www.geneontology.org)

*Cell Component*

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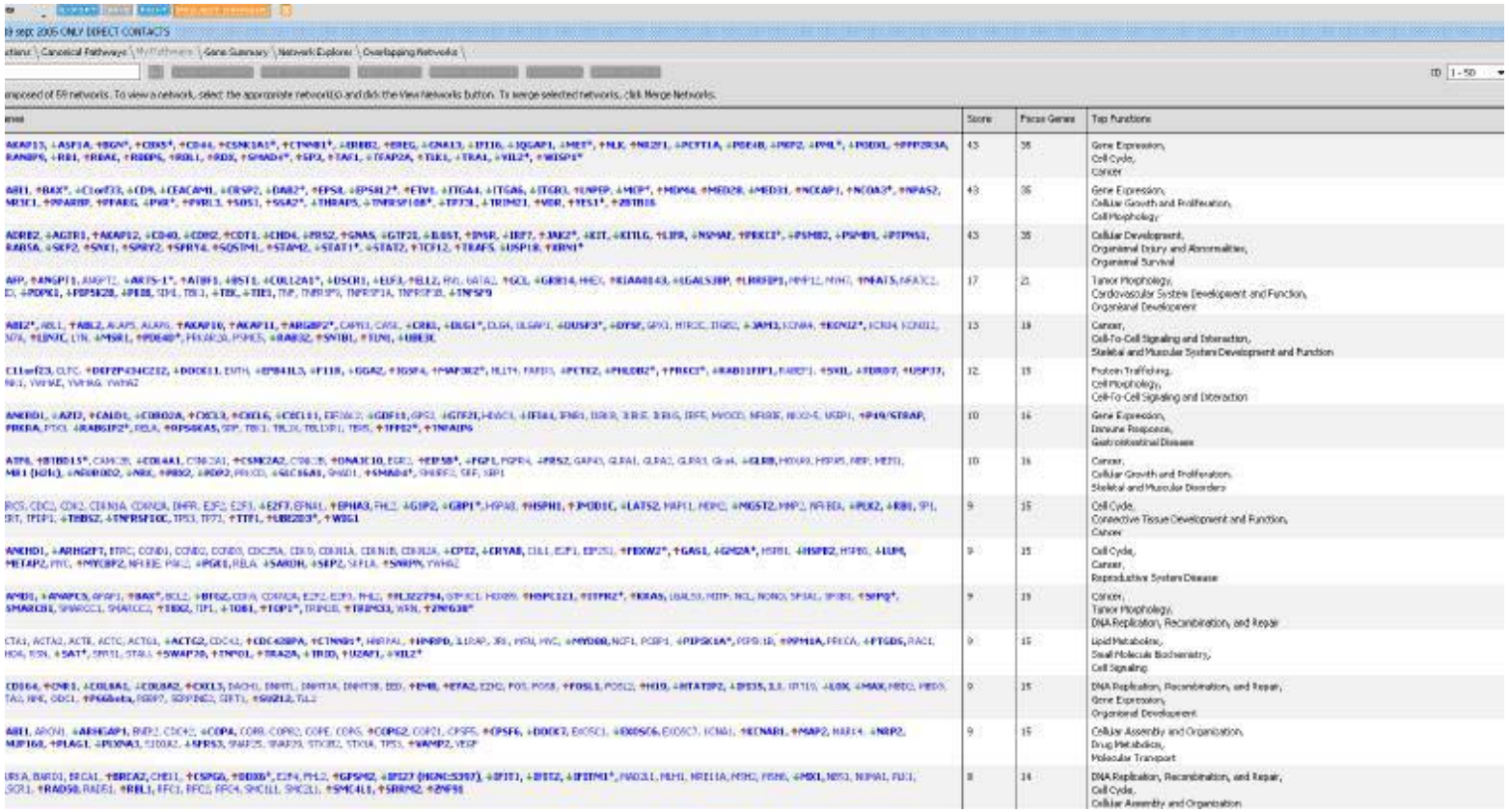
# 1145 unique genes: 1093 gene IDs mapped to the **Ingenuity Pathways Knowledge Database**

*List of networks*

- *Score: indicating significance of network*
- *Number of Focus genes: genes matching the query that are known to interact with other proteins.*
- *Top functions indicate the processes these genes have been shown to be involved in.*



| Score | Focus Genes | Top Functions |
|---|---|---|
| 43 | 38 | Gene Expression, Cell Cycle, Cancer |
| 43 | 38 | Gene Expression, Cellular Growth and Proliferation, Cell Morphology |
| 43 | 38 | Cellular Development, Organismal Injury and Abnormalities, Organismal Survival |
| 17 | 21 | Tumor Morphology, Cardiovascular System Development and Function, Organismal Development |
| 15 | 19 | Cancer, Cell-To-Cell Signaling and Interaction, Skeletal and Muscular System Development and Function |
| 12 | 15 | Protein Trafficking, Cell Morphology, Cell-To-Cell Signaling and Interaction |
| 10 | 16 | Gene Expression, Immune Response, Gastrointestinal Disease |
| 10 | 16 | Cancer, Cellular Growth and Proliferation, Skeletal and Muscular Disorders |
| 9 | 15 | Cell Cycle, Connective Tissue Development and Function, Cancer |
| 9 | 15 | Cell Cycle, Cancer, Reproductive System Disease |
| 9 | 15 | Cancer, Tumor Morphology, DNA Replication, Recombination, and Repair |
| 9 | 15 | Lipid Metabolism, Small Molecule Biochemistry, Cell Signaling |
| 9 | 15 | DNA Replication, Recombination, and Repair, Gene Expression, Organismal Development |
| 9 | 15 | Cellular Assembly and Organization, Drug Metabolism, Molecular Transport |
| 8 | 14 | DNA Replication, Recombination, and Repair, Cell Cycle, Cellular Assembly and Organization |

Analysis using Ingenuity (http://www.ingenuity.com)

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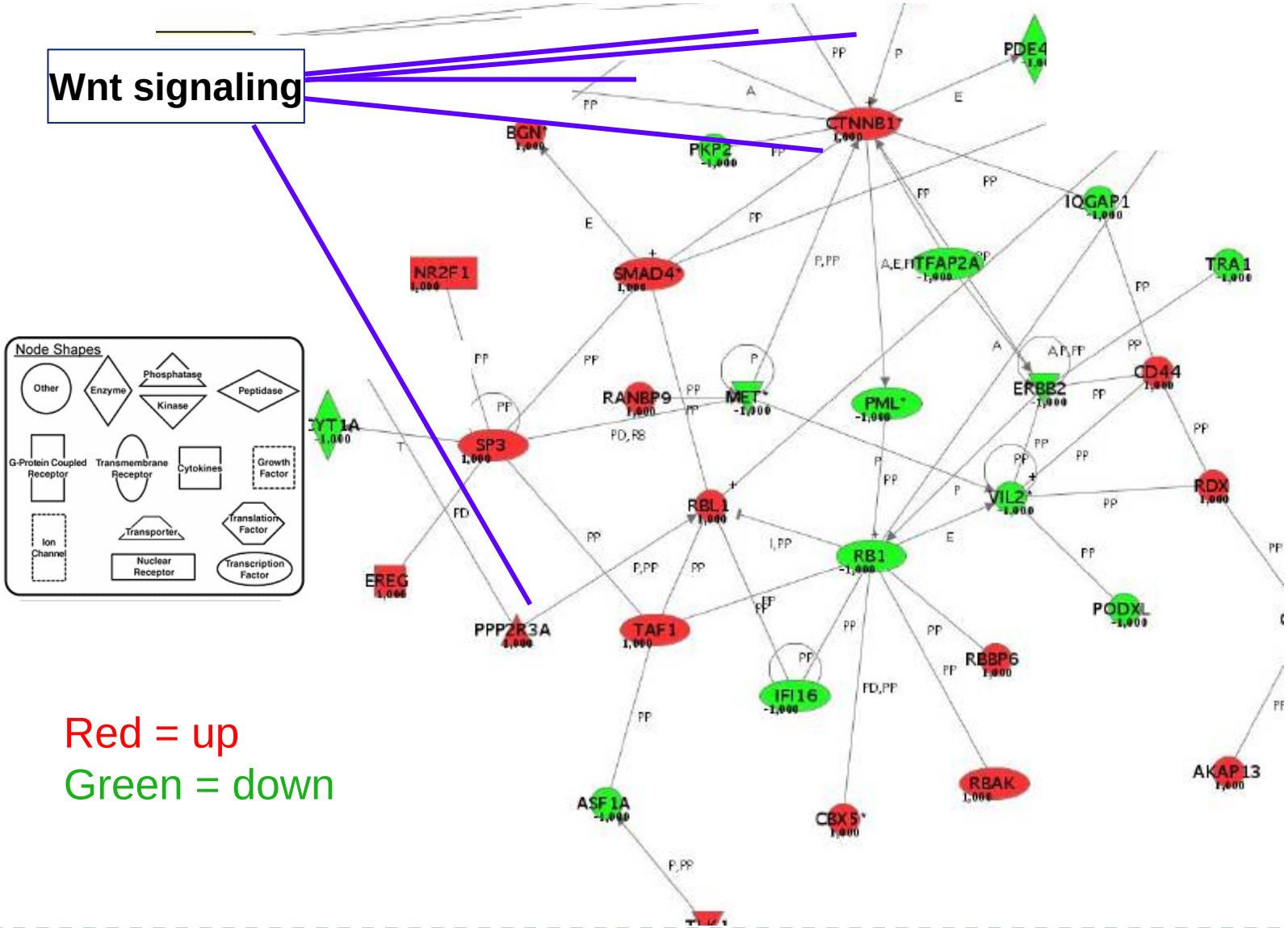
# Ingenuity Pathways Knowledge Database

Graphical display of one of the Top Networks as identified using the Ingenuity Pathways Knowledge Database

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