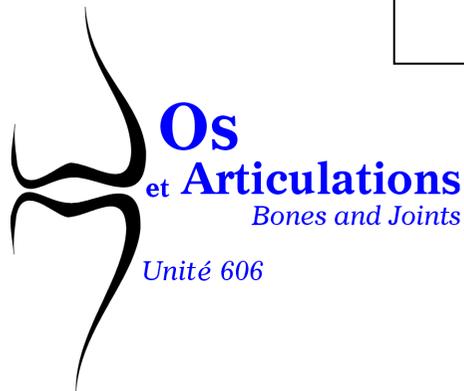




**JPS – JOURNEES PEDAGOGIQUES ET SCIENTIFIQUES
DE L'AE2BM
AMIENS – 8 ET 9 SEPTEMBRE 2011**



Contrôle au niveau cellulaire du remodelage osseux



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**GROUPE HOSPITALIER
LARIBOISIERE - FERNAND VIDAL**

Instituts
thématiques



Inserm

Institut national
de la santé et de la recherche médicale

université
**PARIS
DIDEROT**
PARIS 7

Maintien de la masse osseuse au cours du temps

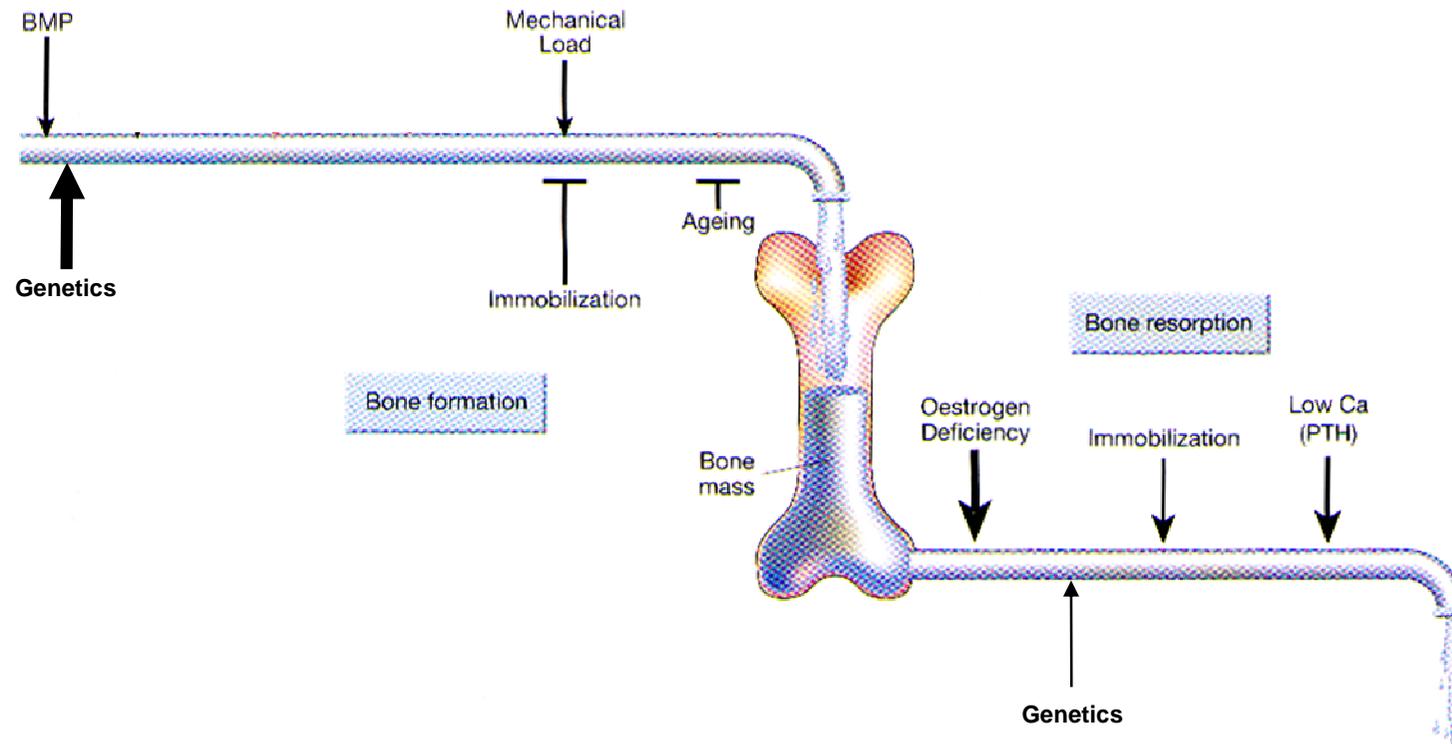
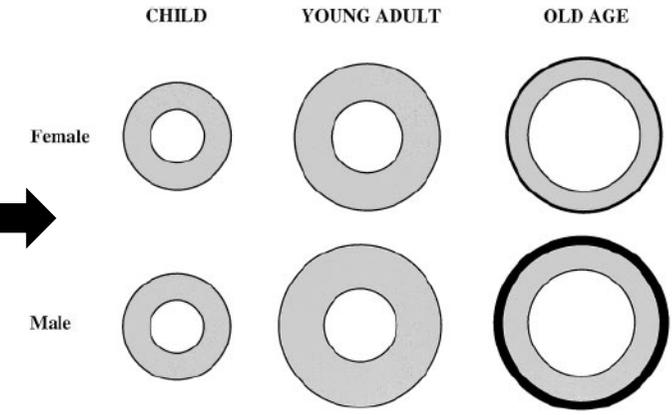
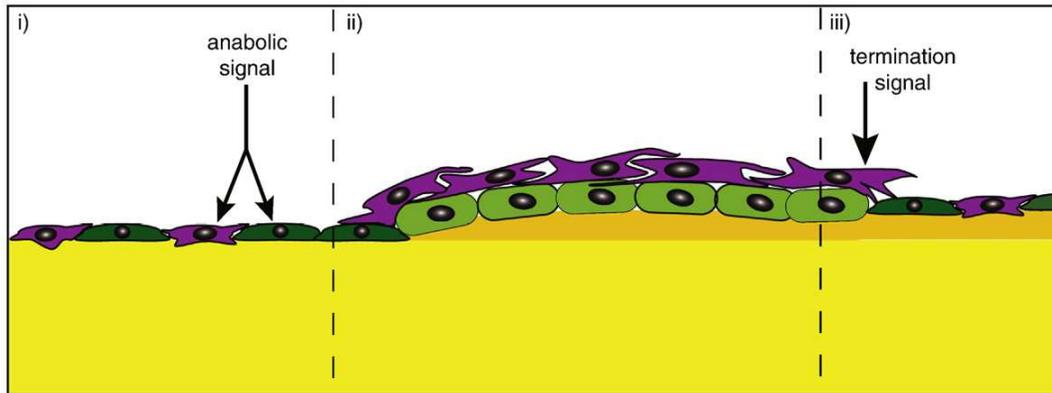


Schéma adapté de Harada et Rodan, 2003

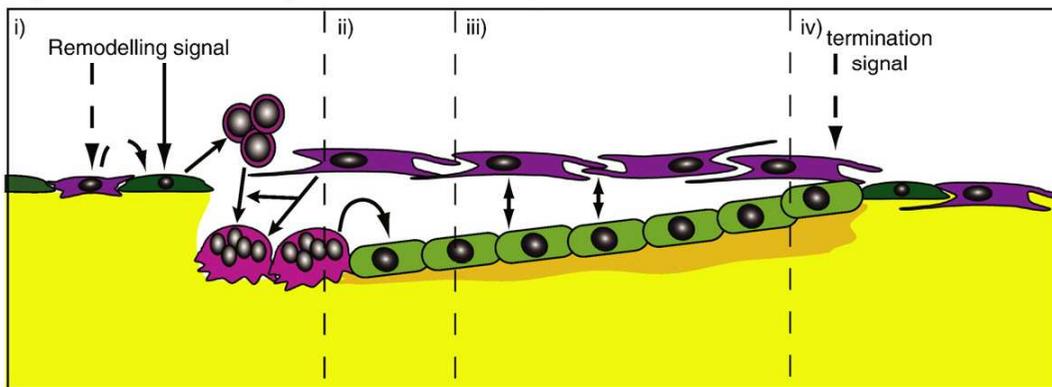
Modelage vs remodelage

A) Bone Modelling



■ Periosteal apposition (since young adulthood)

B) Bone Remodelling



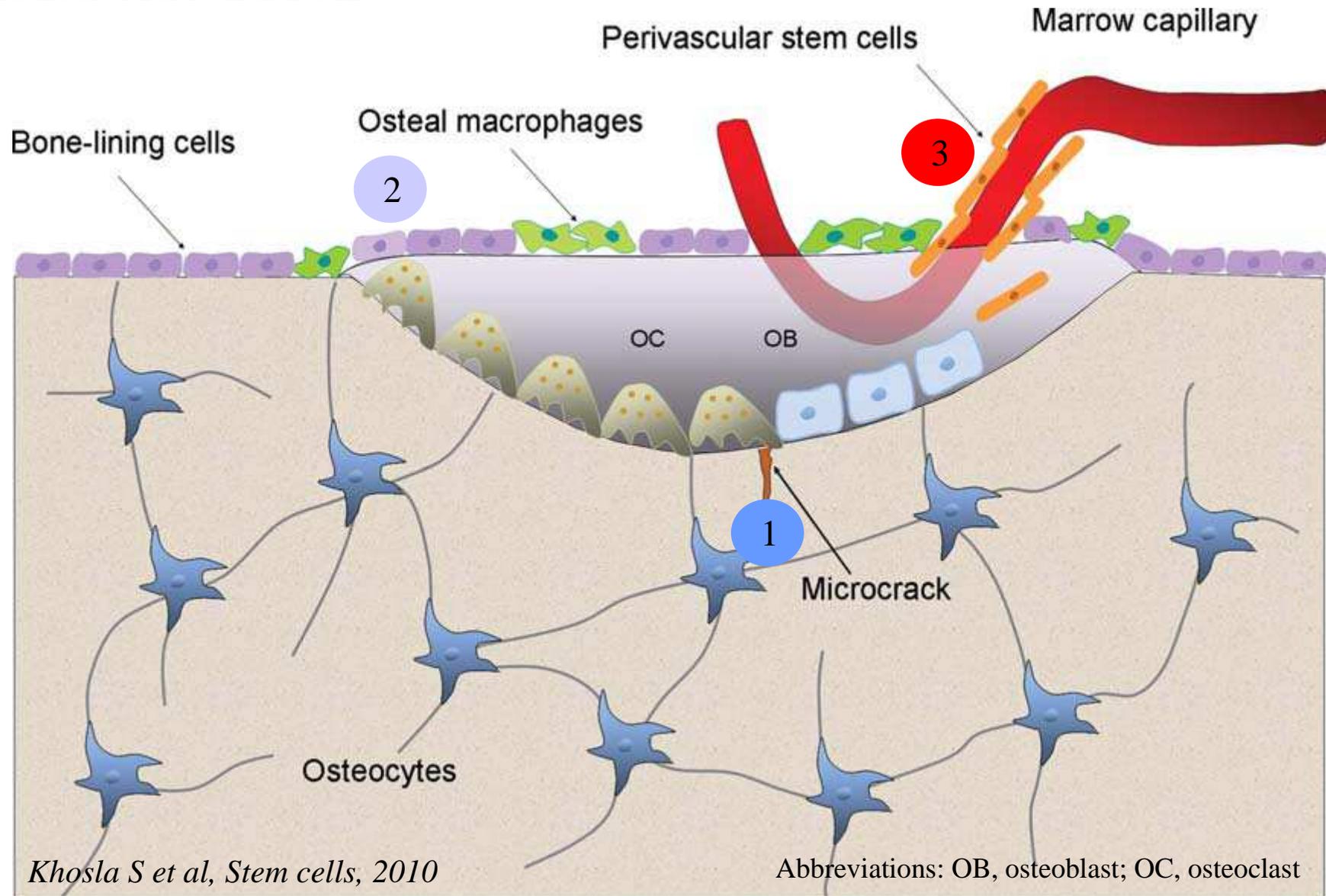
Renouvellement du tissu endommagé ou vieillissant

-  OsteoMac
-  Bone lining cell/immature osteoblast
-  Mature osteoblast
-  Bone
-  New bone
-  Osteoclast precursor
-  Osteoclast

Petit et al, 2008

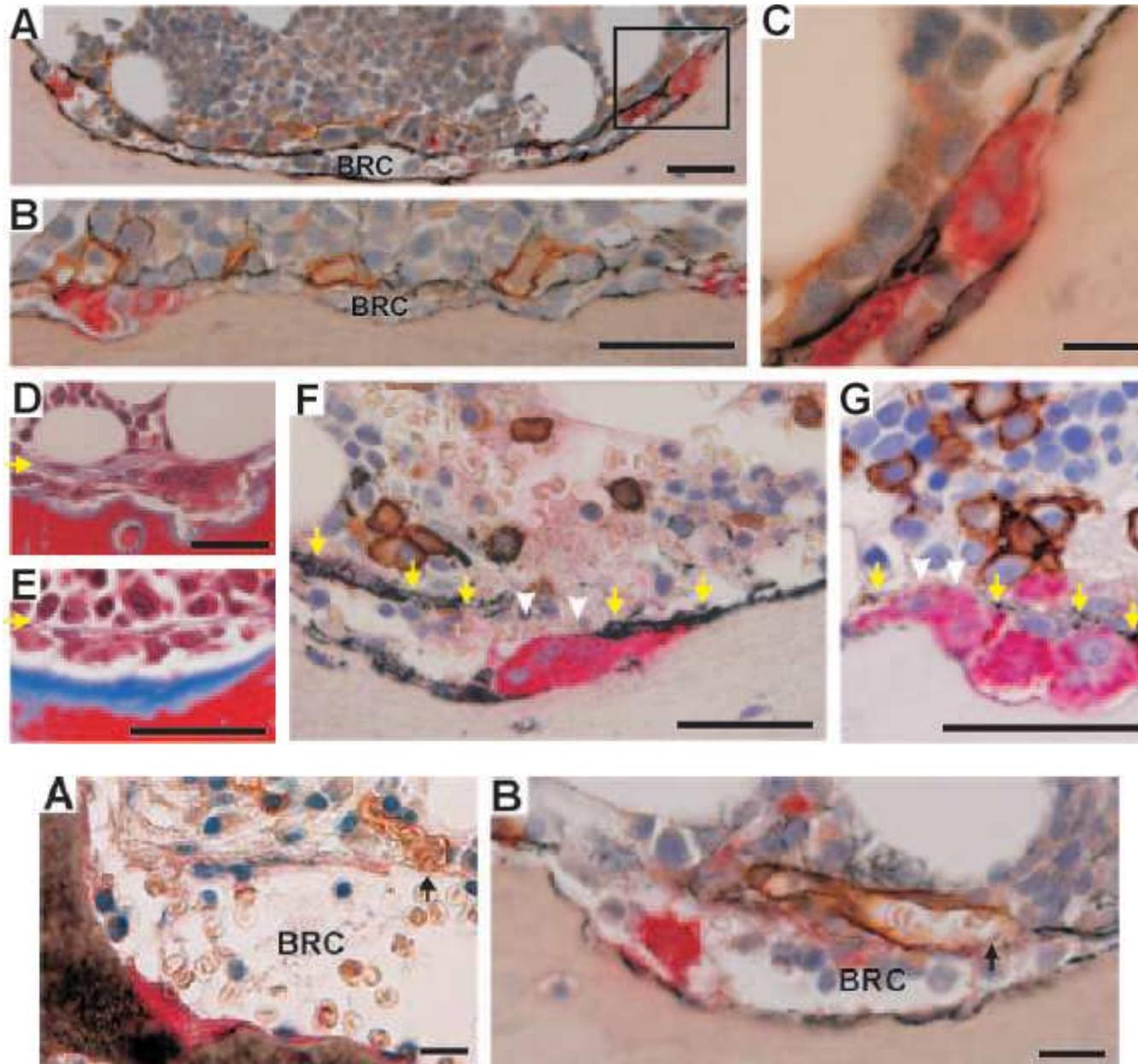
The Bone Remodelling compartment (BRC)

BMU Basic Multicellular Unit

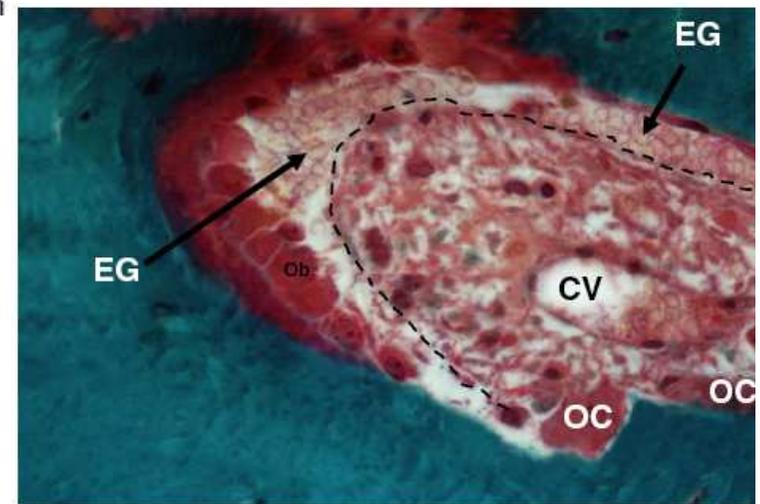
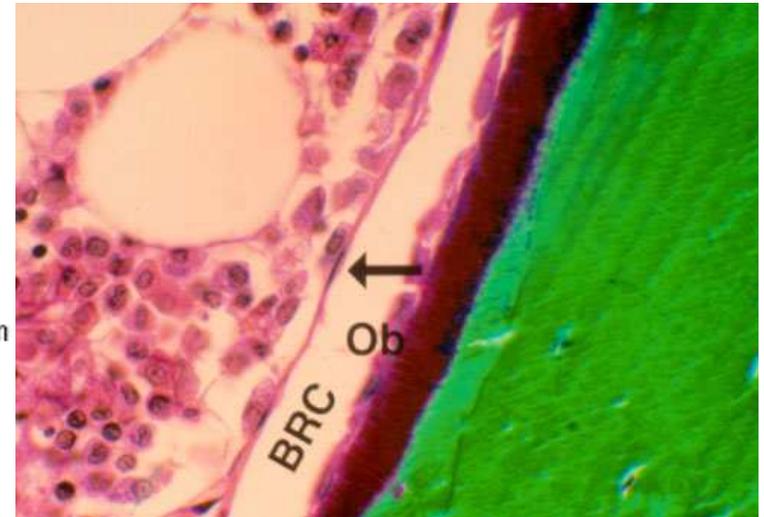
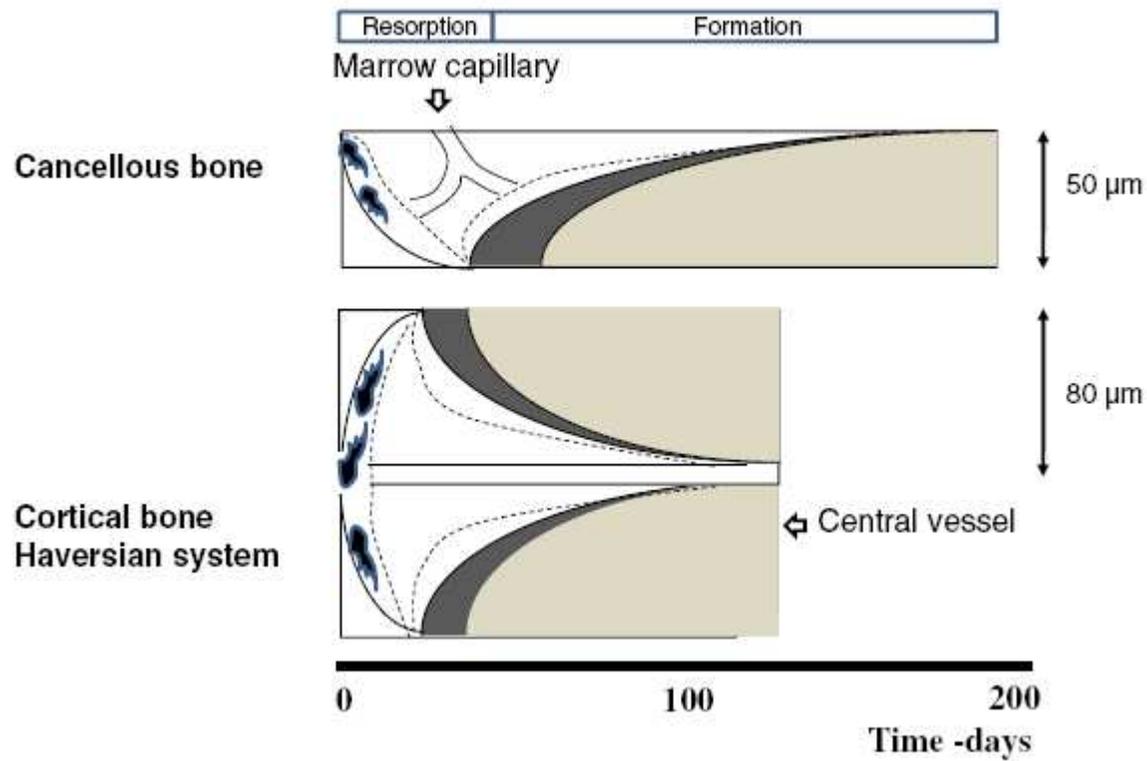


Khosla S et al, Stem cells, 2010

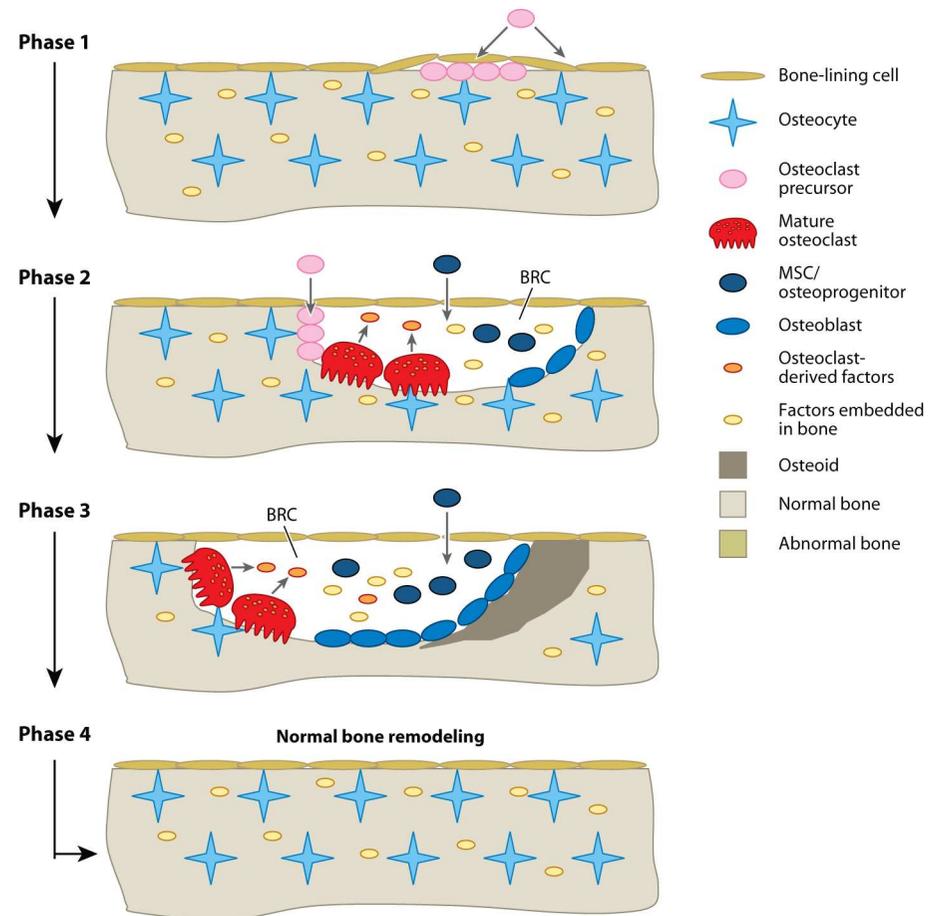
The Bone Remodelling compartment (BRC)



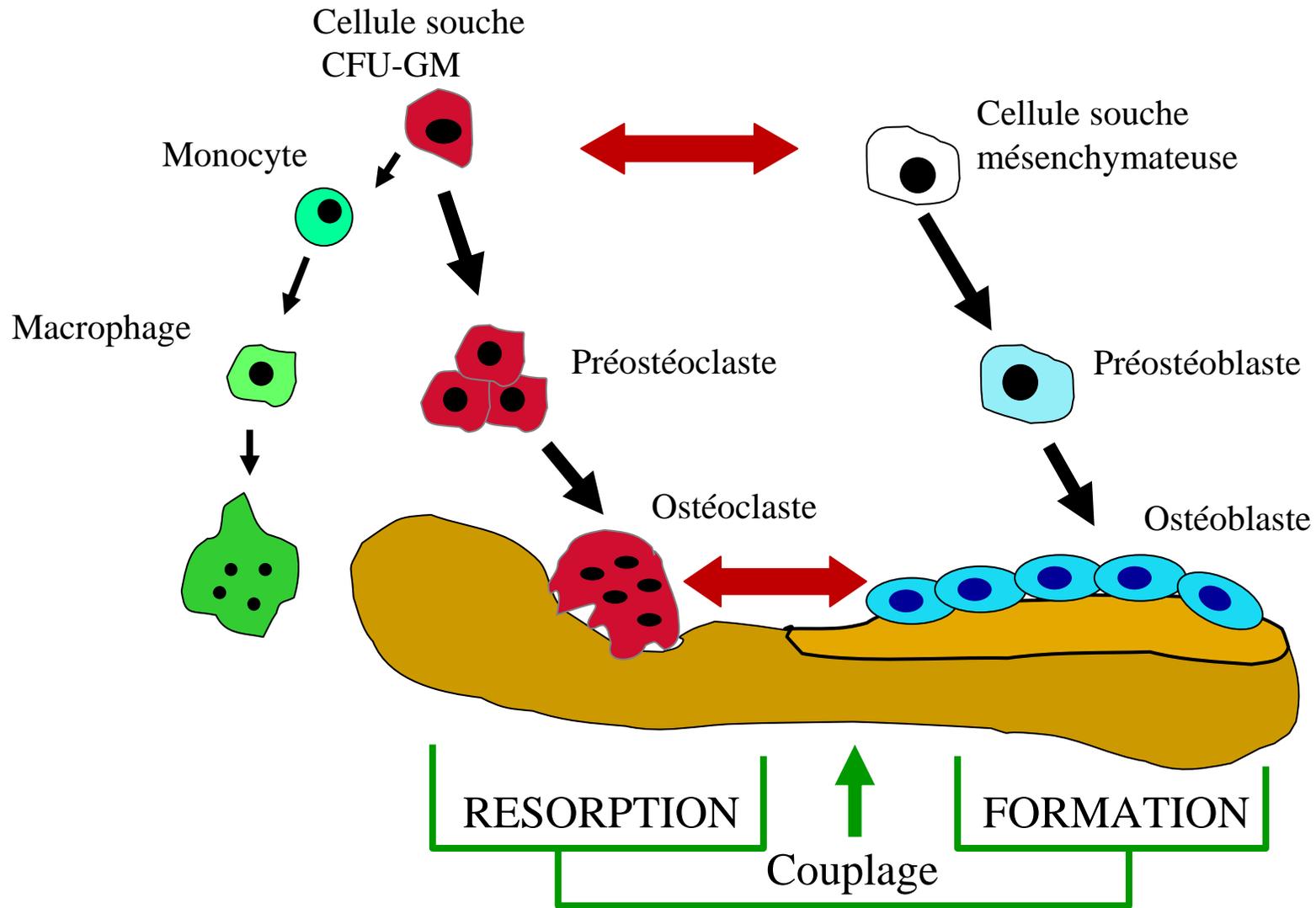
Le remodelage osseux



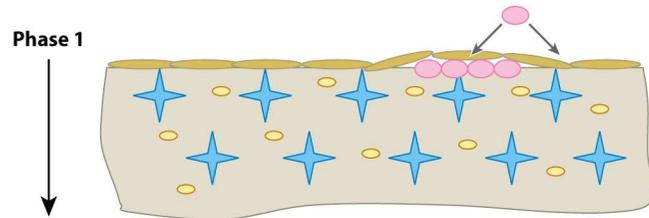
Les phases du remodelage osseux



Le remodelage osseux



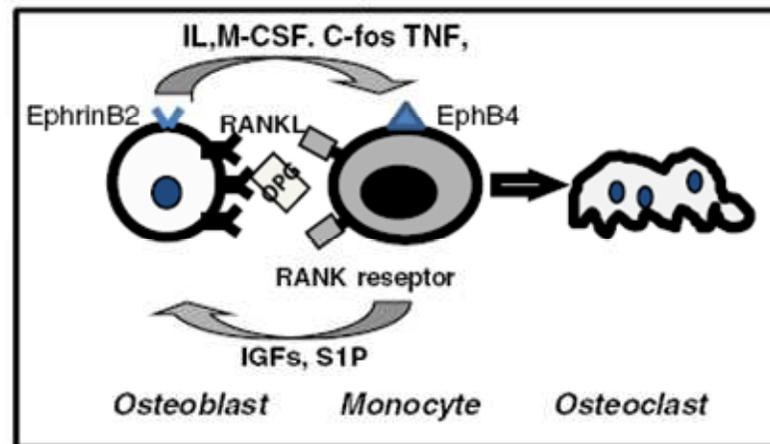
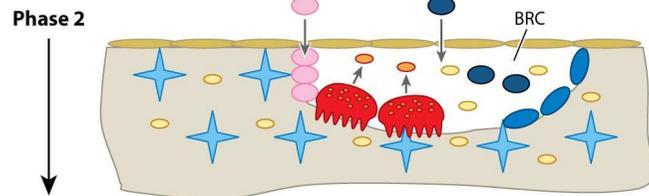
Les phases d'activation et de résorption



- Microfractures, stress mécanique (ostéocytes) : remodelage ciblé
- Modification de l'environnement hormonal (PTH, E2): remodelage non ciblé

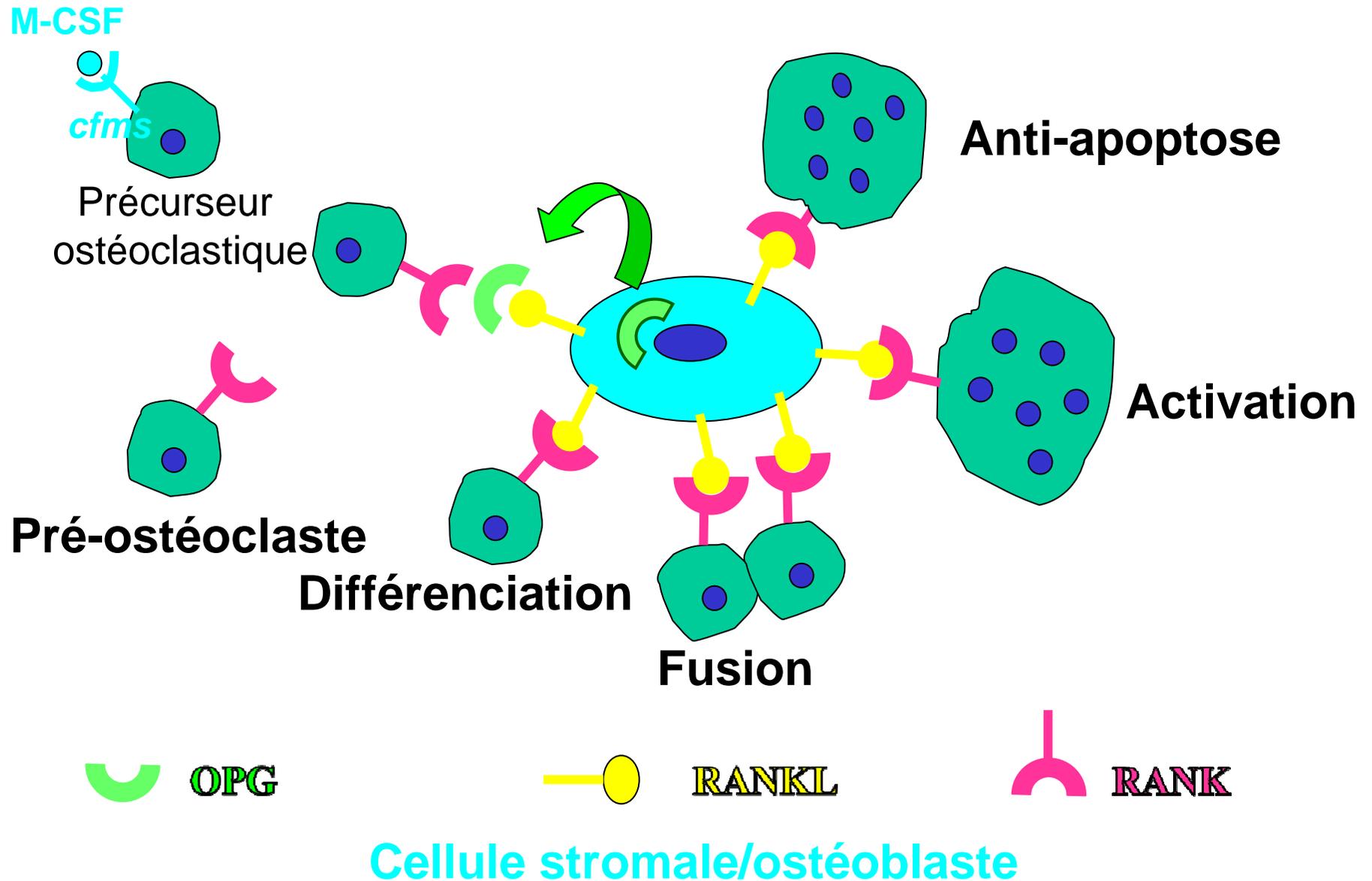
Cellules de la canopée

Antigén	BRC	Vascular endothelium
VEGF	-	+
Von Willebrand Factor	-	+
CD 34	-	+
Alkaline Phosphatase*	+	-
Osteocalcin	+	-
Osteonectin	+	-
IGF 1,2	+	-
TGF β 1,2,3	+	-
bFGF	+	-
OPG	+	-
RANKL	+	-

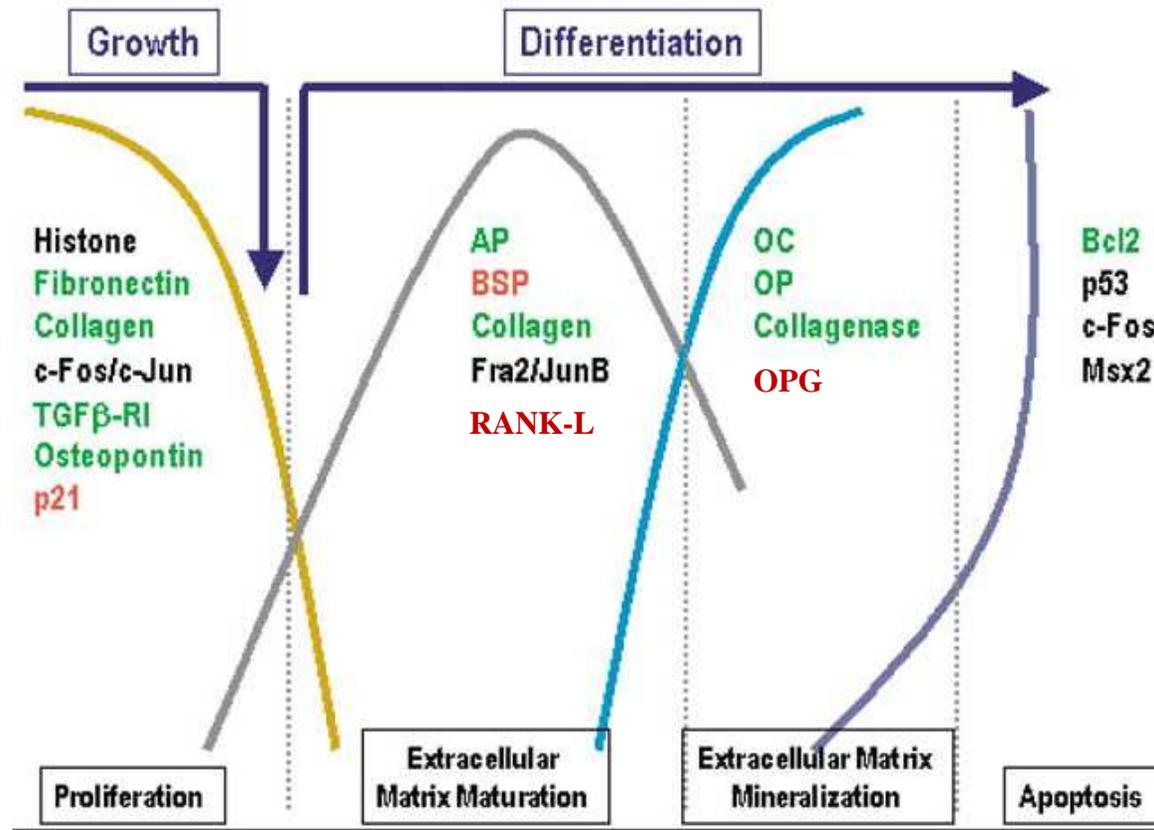


- Bone-lining cell
- Osteocyte
- Osteoclast precursor
- Mature osteoclast
- MSC/osteoprogenitor
- Osteoblast
- Osteoclast-derived factors
- Factors embedded in bone
- Osteoid
- Normal bone
- Abnormal bone

Le système RANK/RANKL/OPG: Couplage ostéoblaste /ostéoclaste



Expression de RANKL et OPG dans l'ostéoblaste



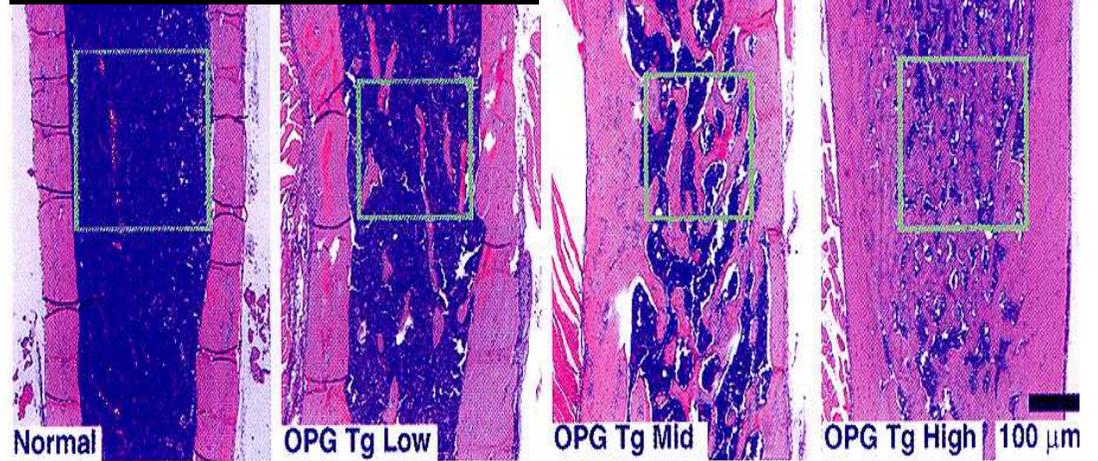
Le système RANK/RANKL/OPG *in vivo*

KO OPG : Ostéoporose



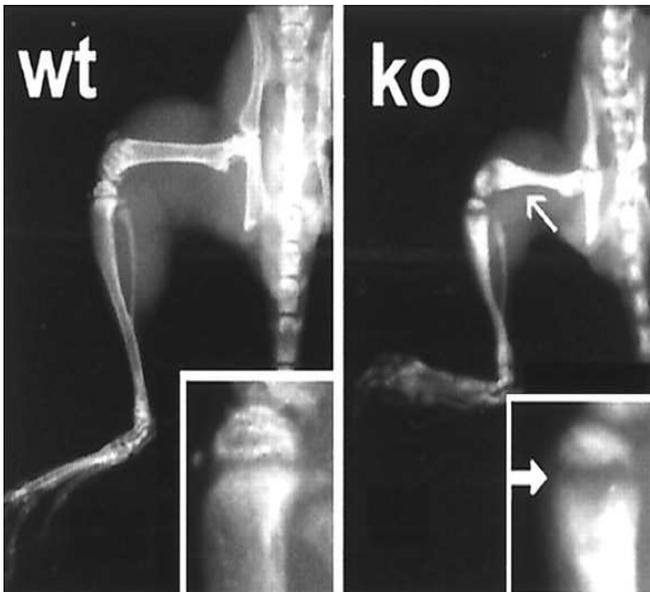
Mizuno et al, 1998

OPG TG : Ostéopétrose



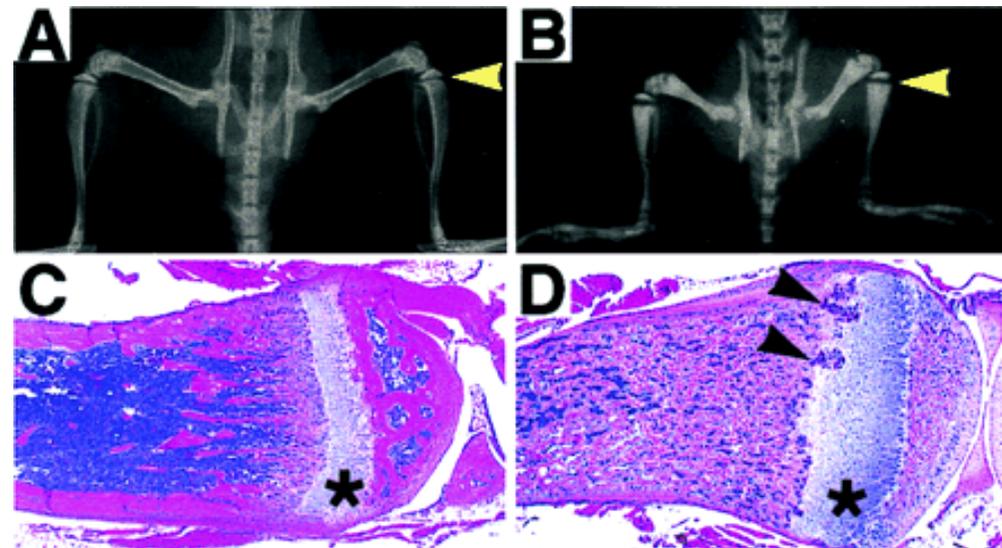
Min et al, 2000

KO RANKL : Ostéopétrose



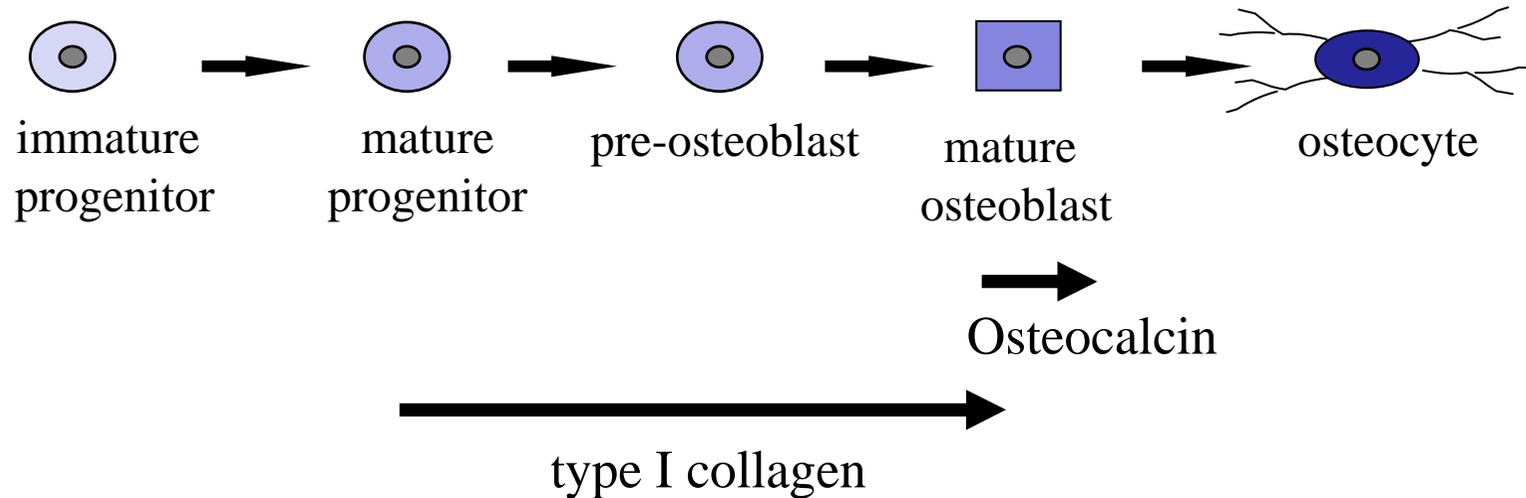
Kong et al. 1999

KO RANK : Ostéopétrose



Hsu et al., 1999

Exemple : Over-expression of Runx2 in osteoblasts



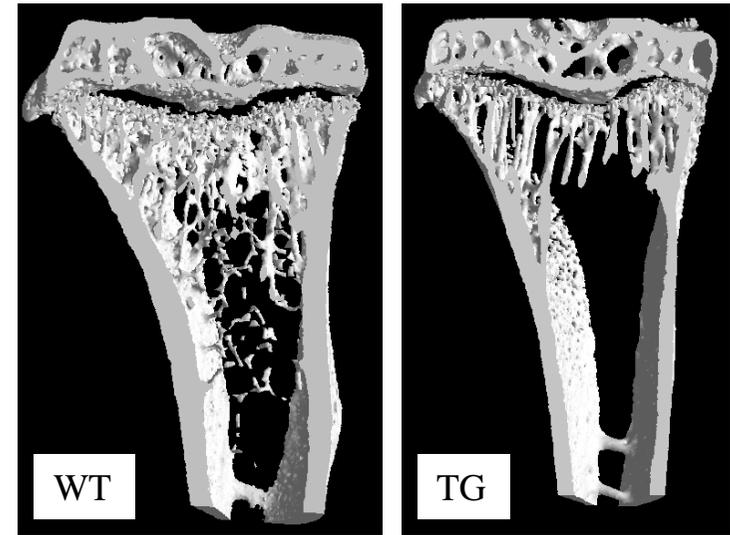
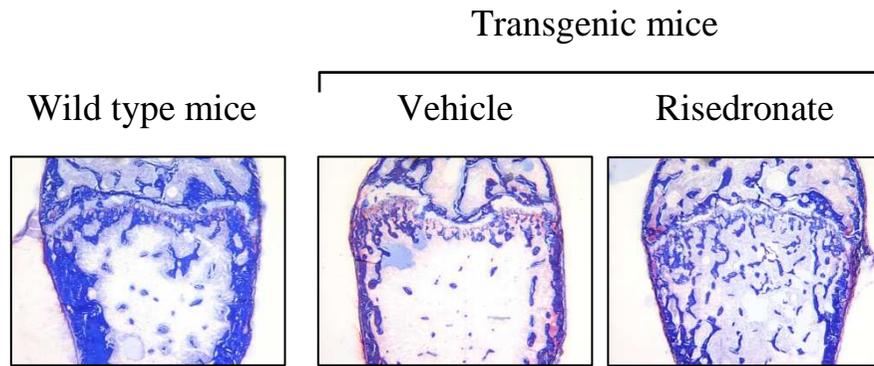
Osteocalcin (*Priemel et al., 1999*)

- **Augmentation de l'activité ostéoblastique**
- **Pas de modification de la résorption**
- **Protection contre la perte osseuse**

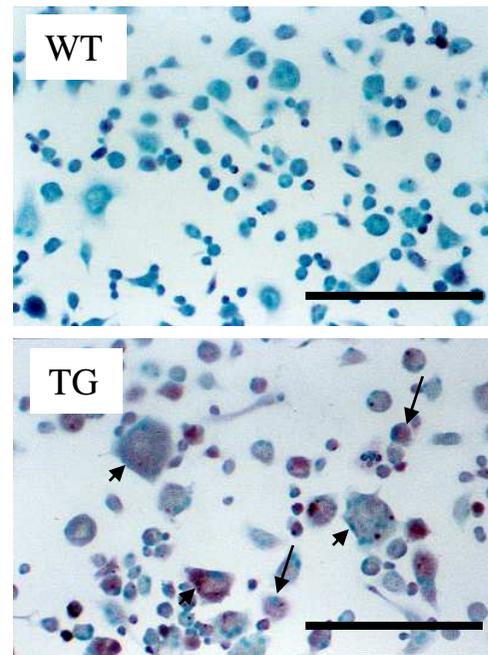
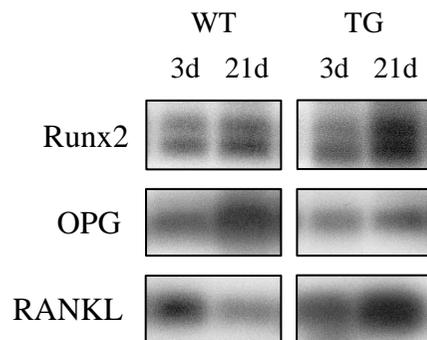
type I collagen (*Geoffroy et al., 2002*)

- **Blocage de la différenciation ostéoblastique**
- **Forte augmentation de la résorption**
- **Perte de la masse osseuse/fractures**

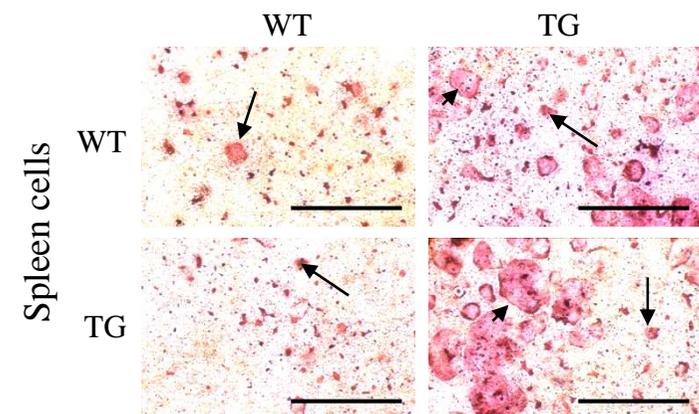
Early over-expression of Runx2 in osteoblasts



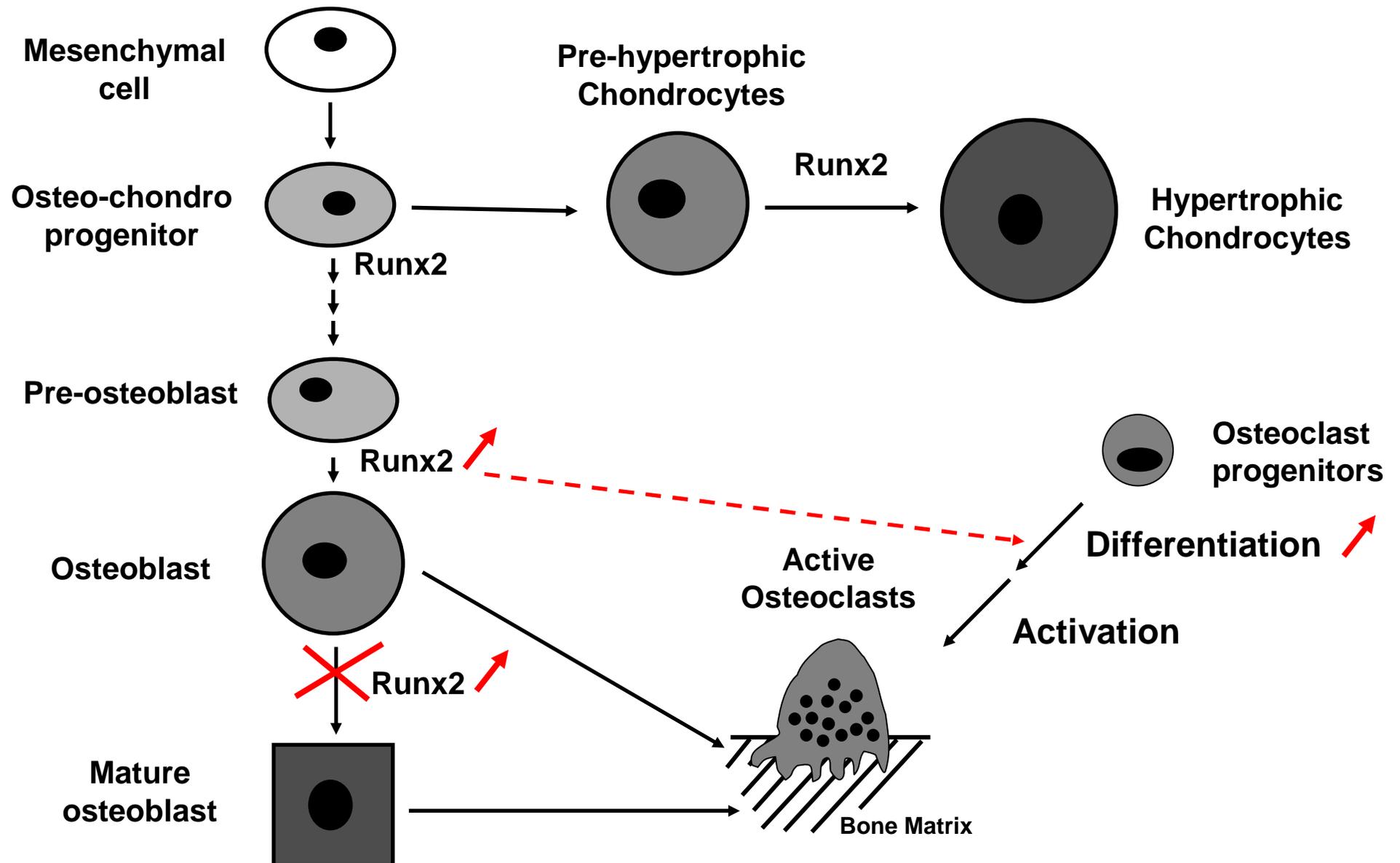
Bone marrow cells



Primary osteoblasts



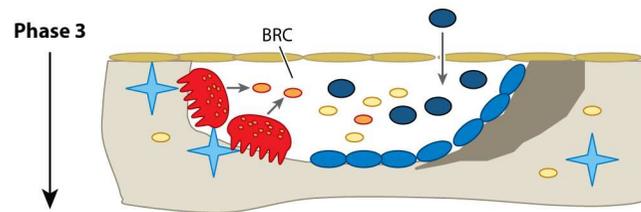
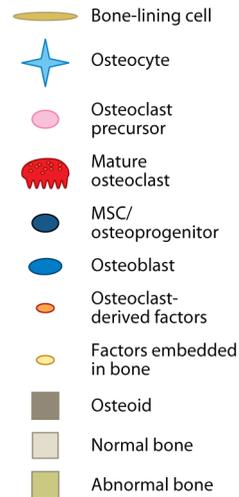
Early over-expression of Runx2 in osteoblasts



La phase de formation

Comment les ostéoblastes sont-ils recrutés sur le site de la résorption?

Comment la quantité de matrice formée est-elle contrôlée?



1. Facteurs de croissance issus de la matrice osseuse résorbée

- **TGF- β 1** ...

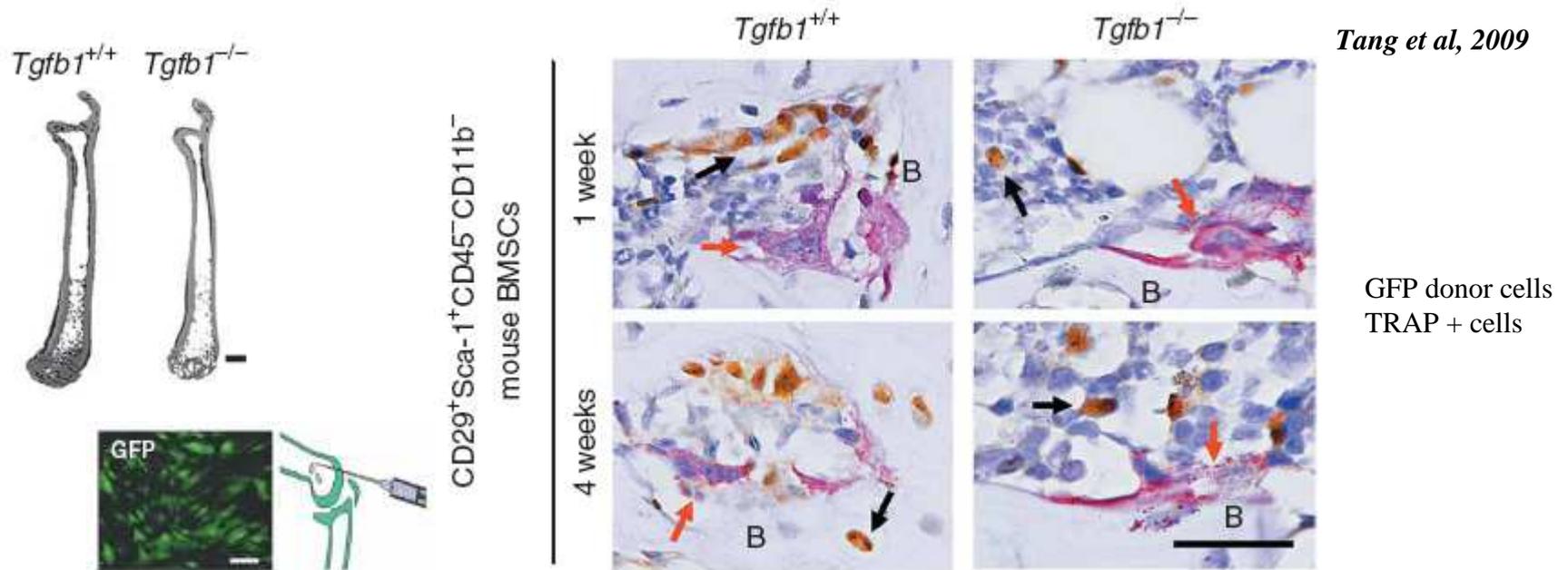
2. Molécules de couplage produits par les ostéoclastes

- **Eph et ephrin**

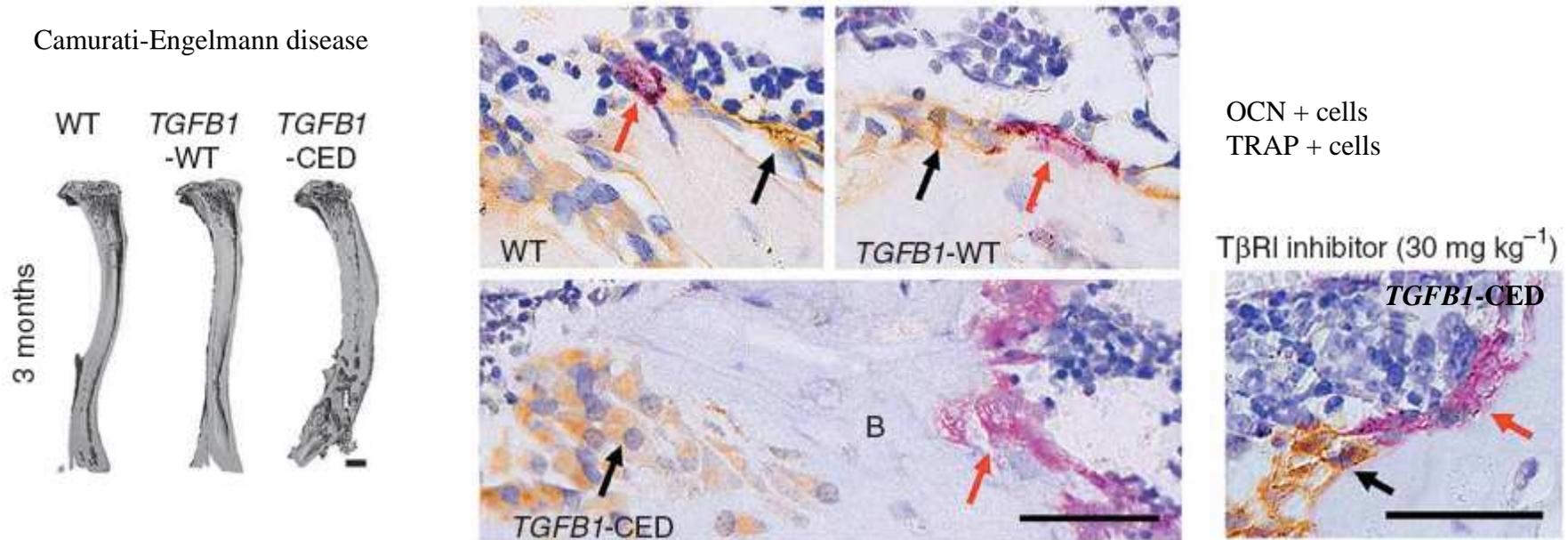
- **SPHK1 (sphingosine kinase 1)**

- **Wnt pathway (Wnt10b, SOST, DKK1)**

Rôle de TGFβ1 dans le couplage OC/OB

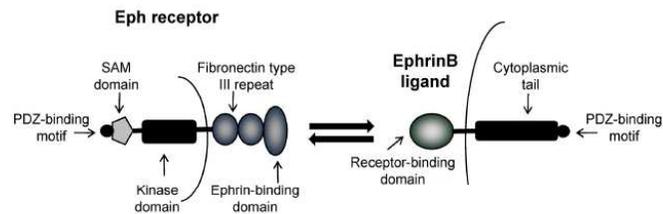
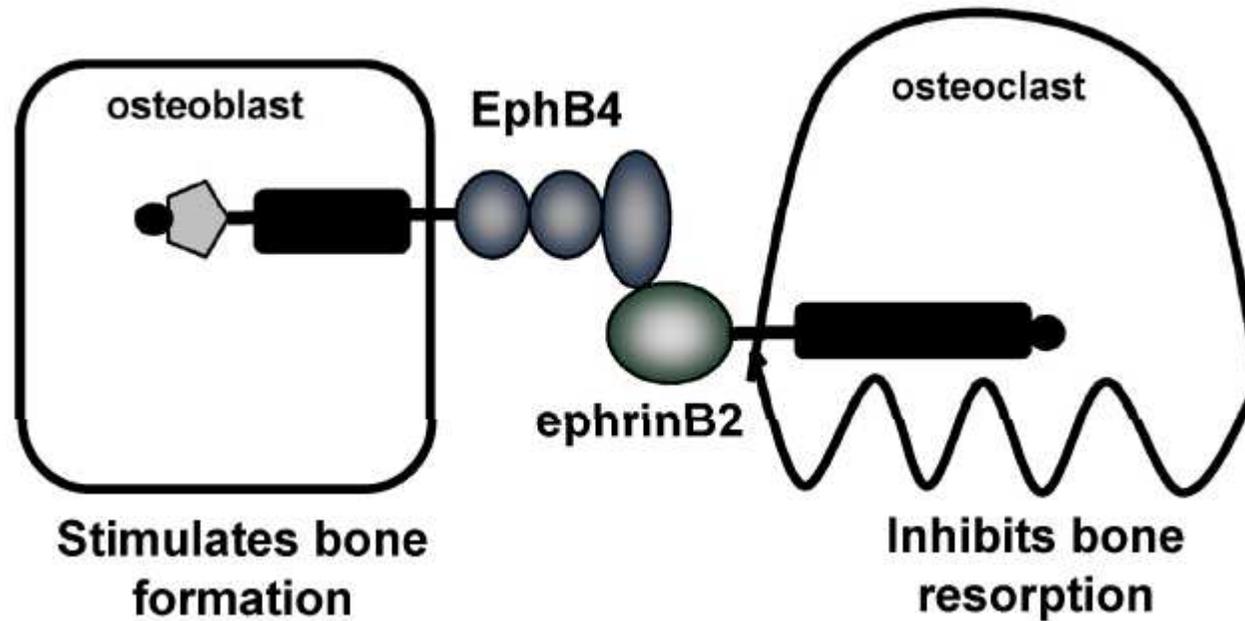


Camurati-Engelmann disease



Le système Eph et ephrin

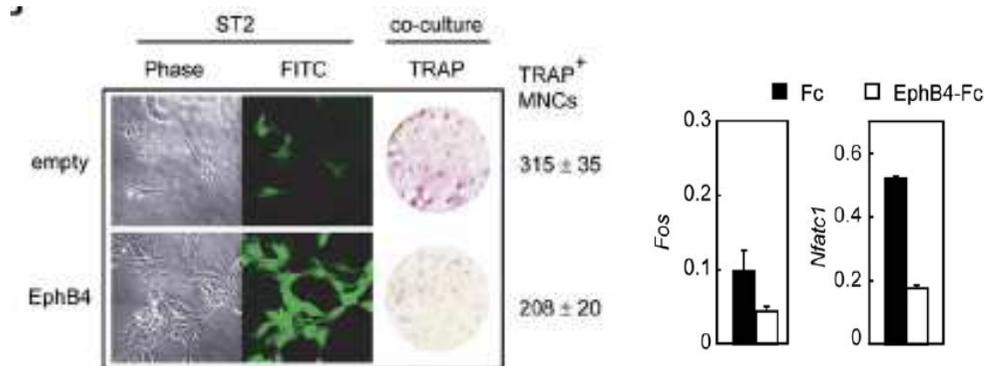
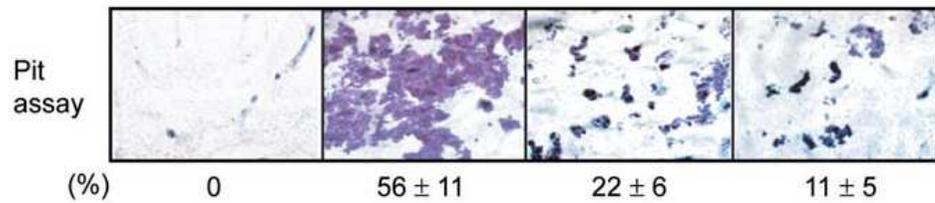
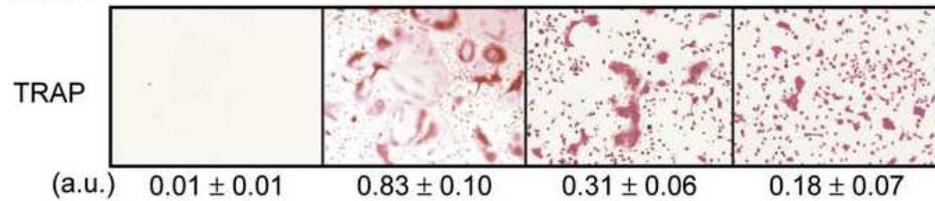
Action bidirectionnelle sur la formation et la résorption osseuse :
Switch de la résorption vers la formation



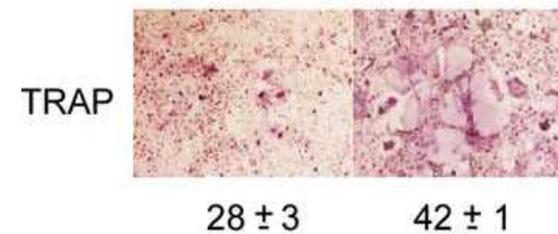
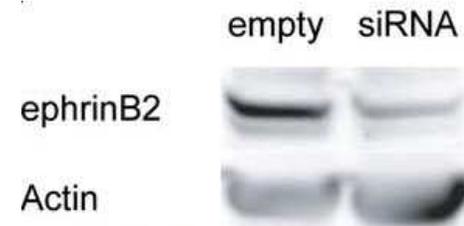
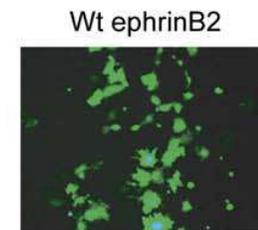
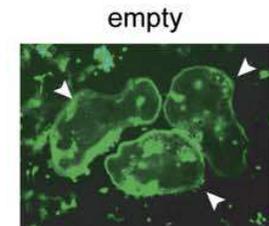
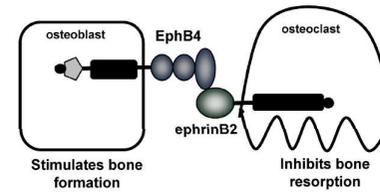
EpHB4 - ephrinB2

Résorption osseuse

EphB4-Fc	0	0	2	4 (µg/ml)
Fc	0	4	0	0 (µg/ml)
RANKL	-	+	+	+



Zhao et al, 2006

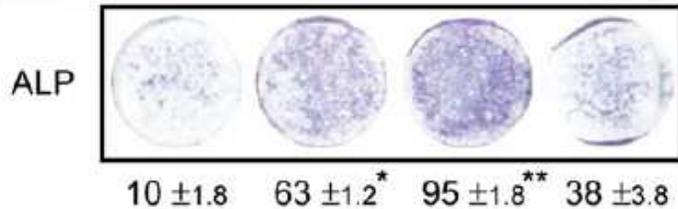


EpHB4 - ephrinB2

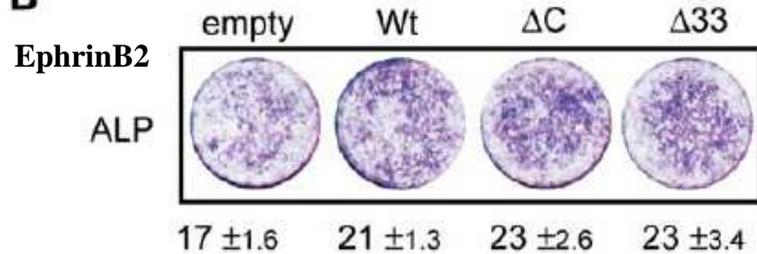
Formation osseuse

A

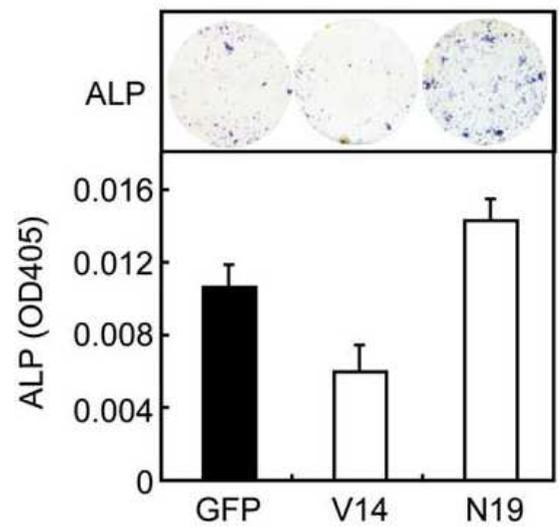
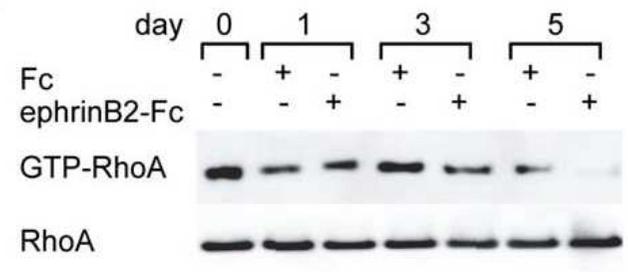
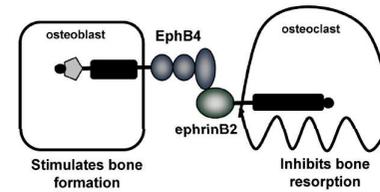
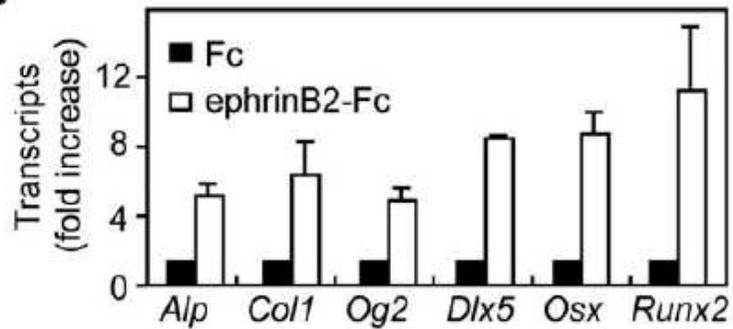
ephrinB2-Fc	-	2	4	2 (µg/ml)
EphB4-Fc	-	-	-	2 (µg/ml)



B



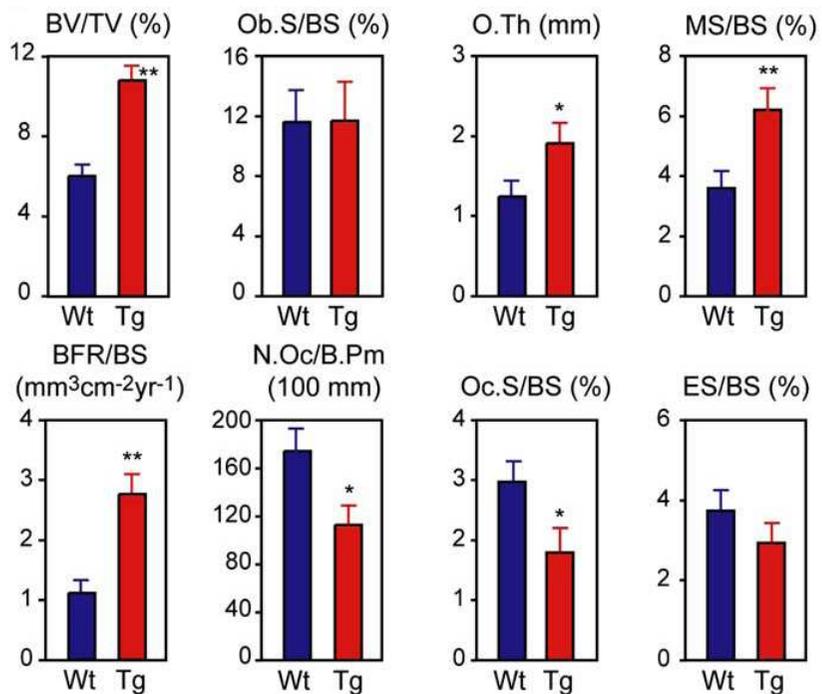
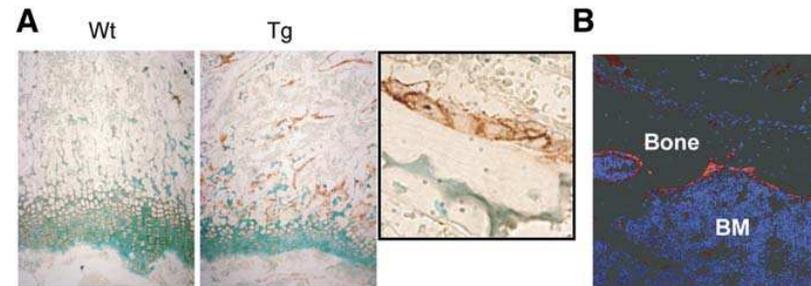
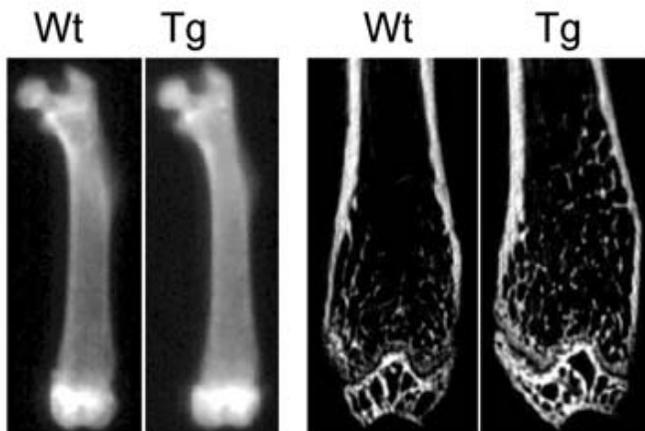
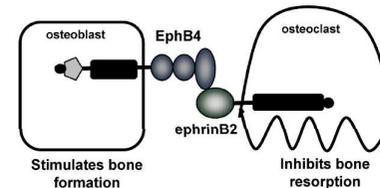
C



V14 RhoA constitutively actif
N19 dominant négatif RhoA

EpHB4 - ephrinB2

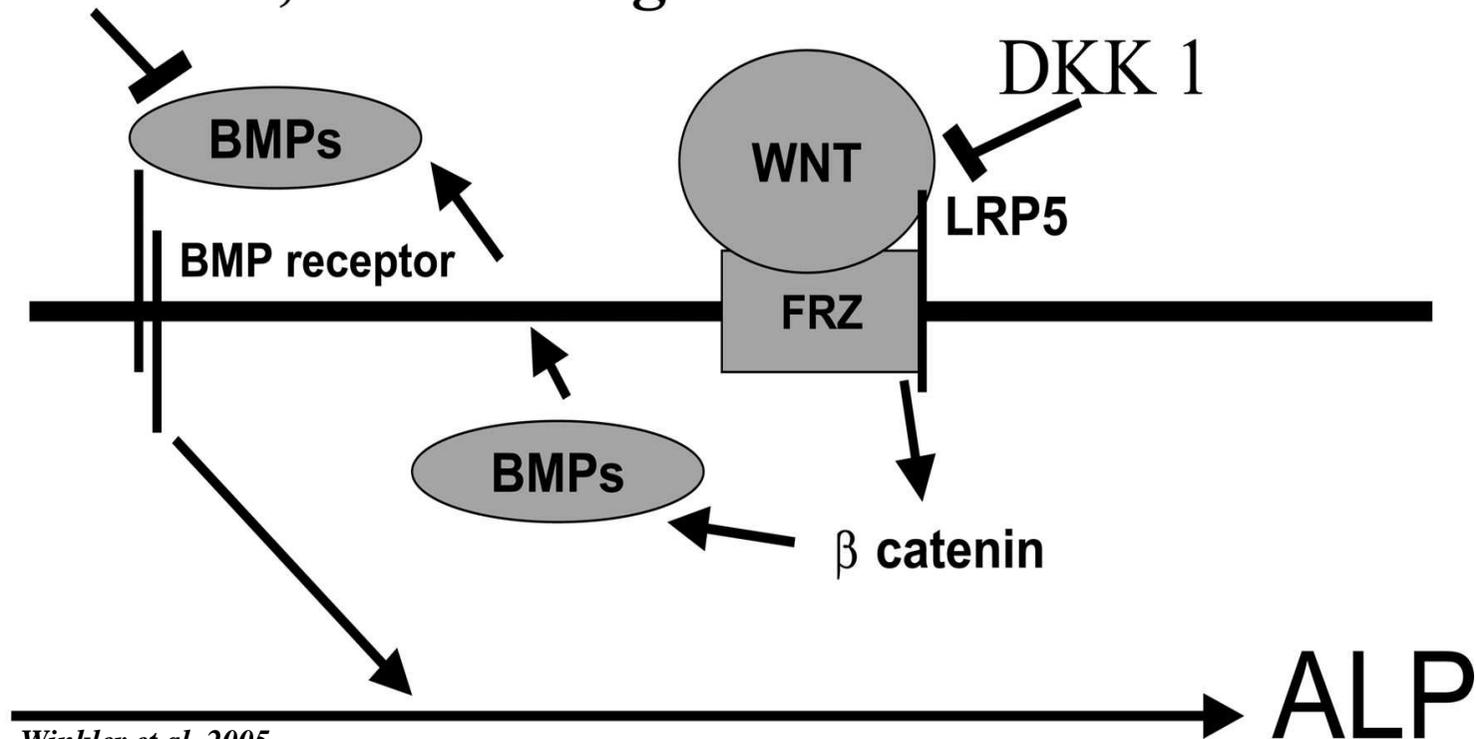
EphB4 transgenic mice show increased bone mass



Zhao et al, 2006

Autres inducteurs ostéoclatiques de l'ostéof ormation

Sclerostin, BMP antagonists

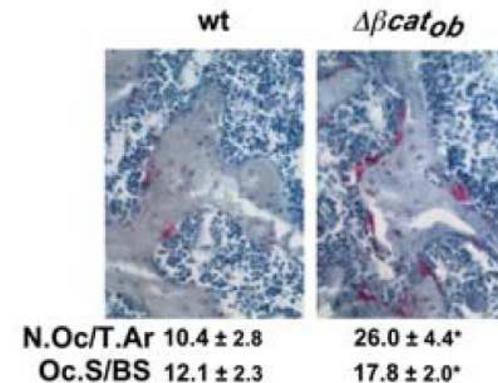
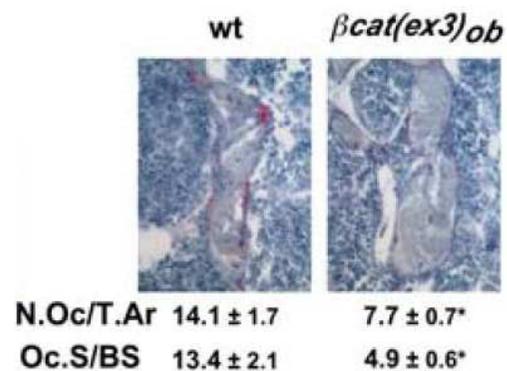
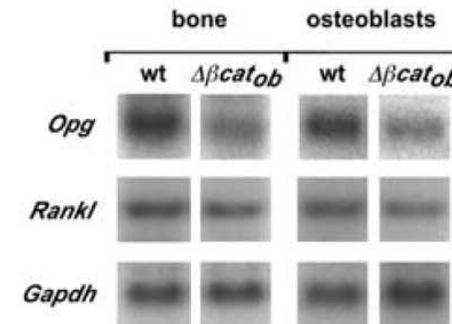
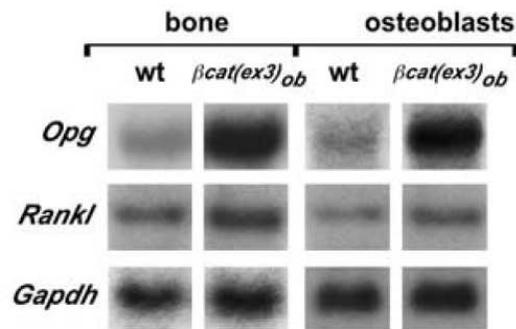


Winkler et al. 2005

Wnt signaling et remodelage osseux

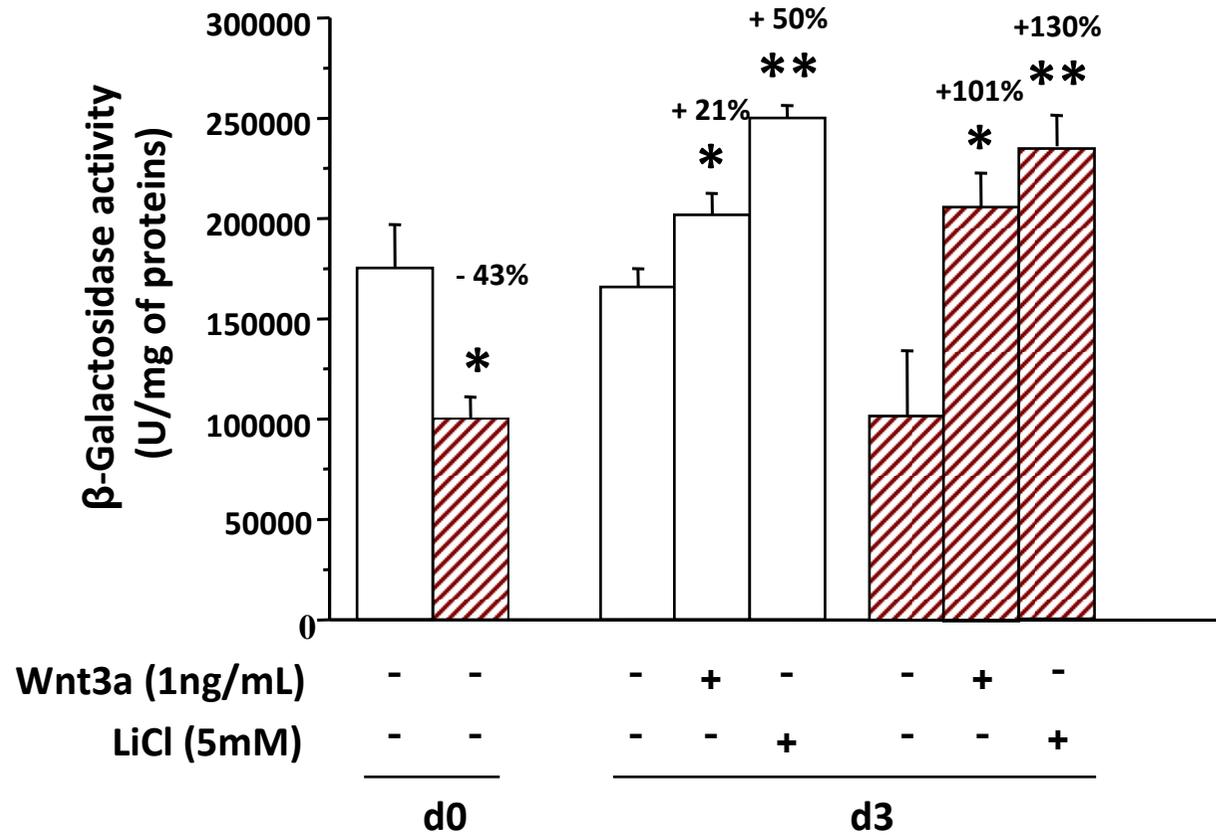
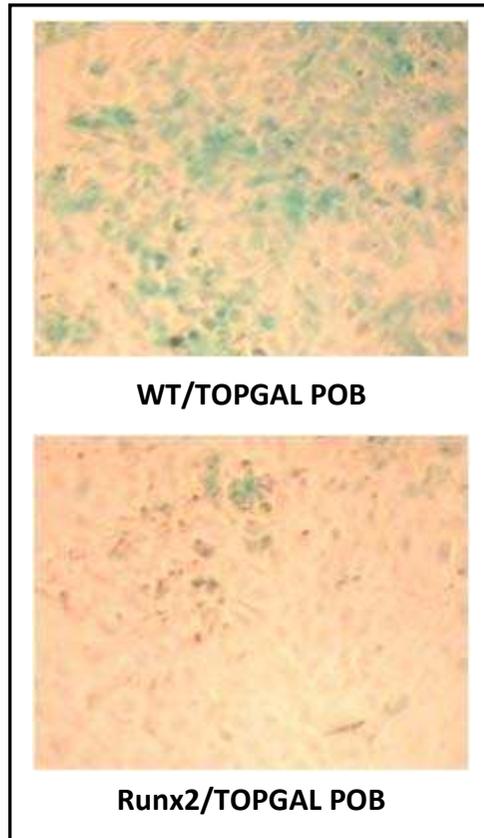
osteoblast-specific *-catenin* gain of function

osteoblast-specific *-catenin* loss of function

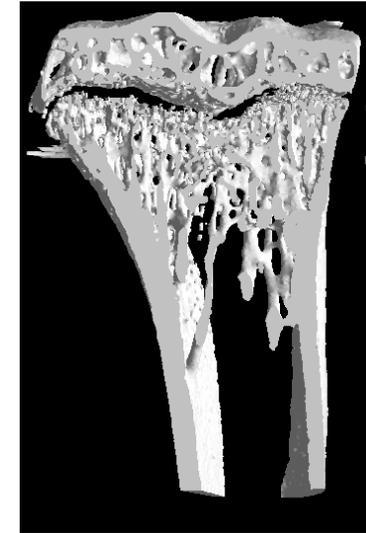
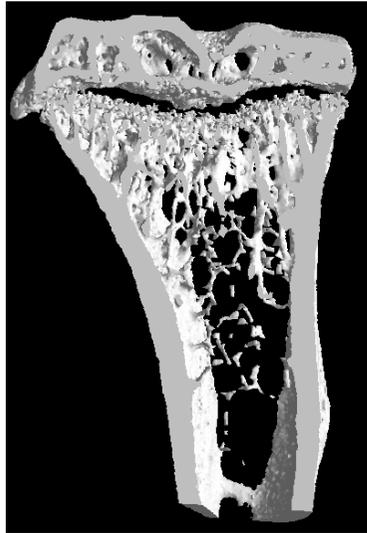


Exemple : Overexpression of Runx2 in osteoblasts

X-Gal staining (day 3)



Exemple : Overexpression of Runx2 in osteoblasts



WT Veh

WT LiCl

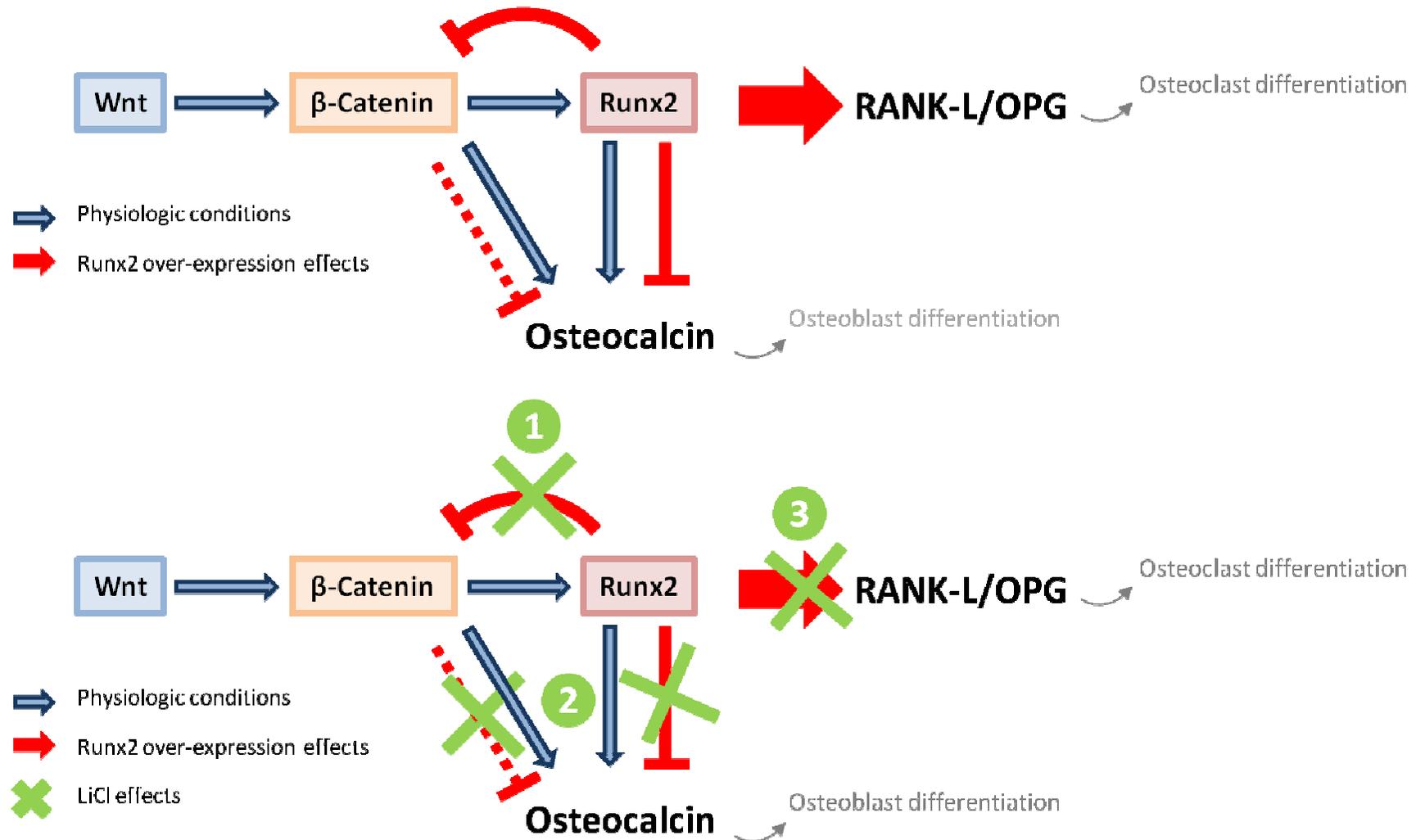
Runx2 Veh

Runx2 LiCl

BV/TV (%)	8.4 ± 0.4	11.5 ± 0.8 #	6.2 ± 0.7 #	10.4 ± 1 **
Tb.N (1/mm)	30.2 ± 1.2	40.5 ± 2.1 #	20.9 ± 2 #	33.2 ± 2.8 **
Tb.Sp (μm)	316.3 ± 14	246.6 ± 21 *	557.5 ± 82 #	315.9 ± 24 **
Tb.Th (μm)	26.6 ± 1	27.8 ± 1.1	30.1 ± 1.7	31.1 ± 1.2

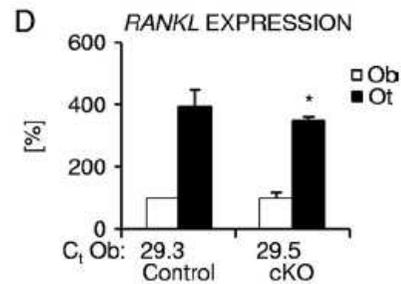
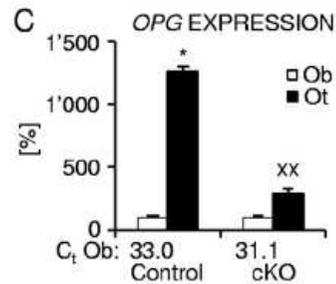
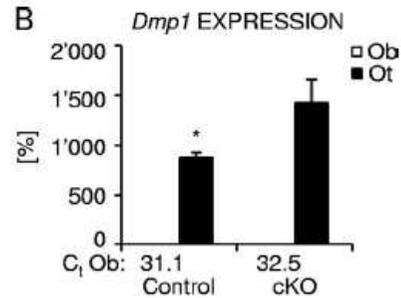
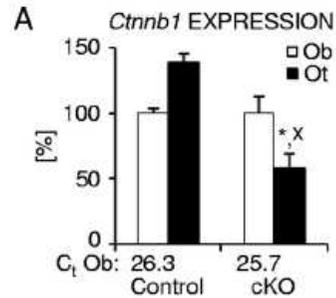
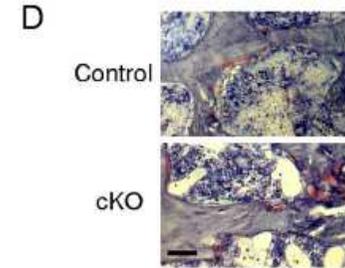
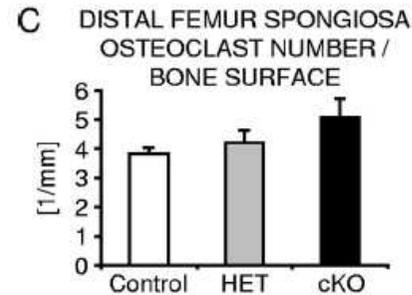
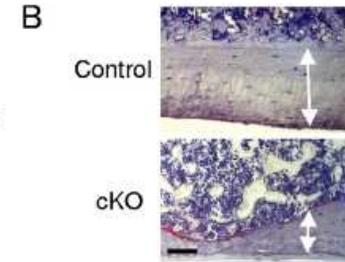
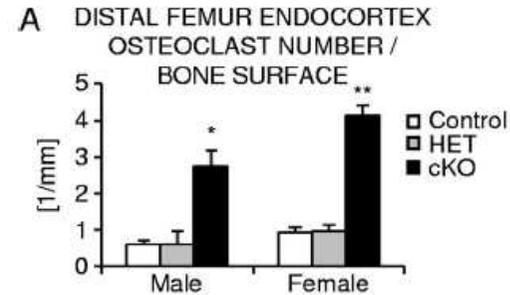
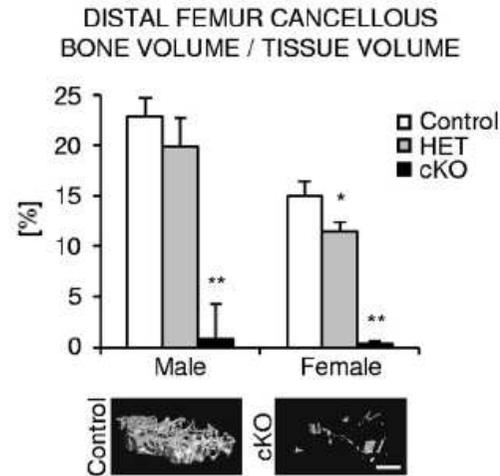
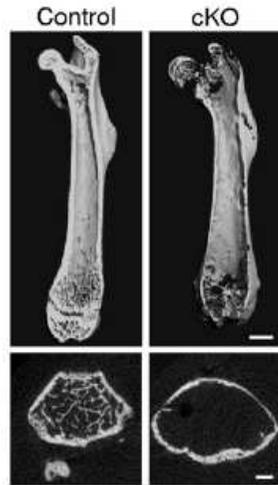
Significantly different from WT Vehicle: * $p < 0.05$; # $p < 0.005$.

Exemple : Overexpression of Runx2 in osteoblasts



Wnt signaling et remodelage osseux

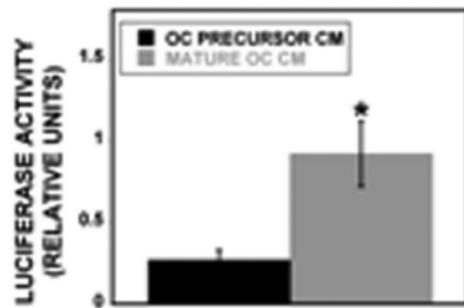
osteocyte-specific *-catenin* cKO mice



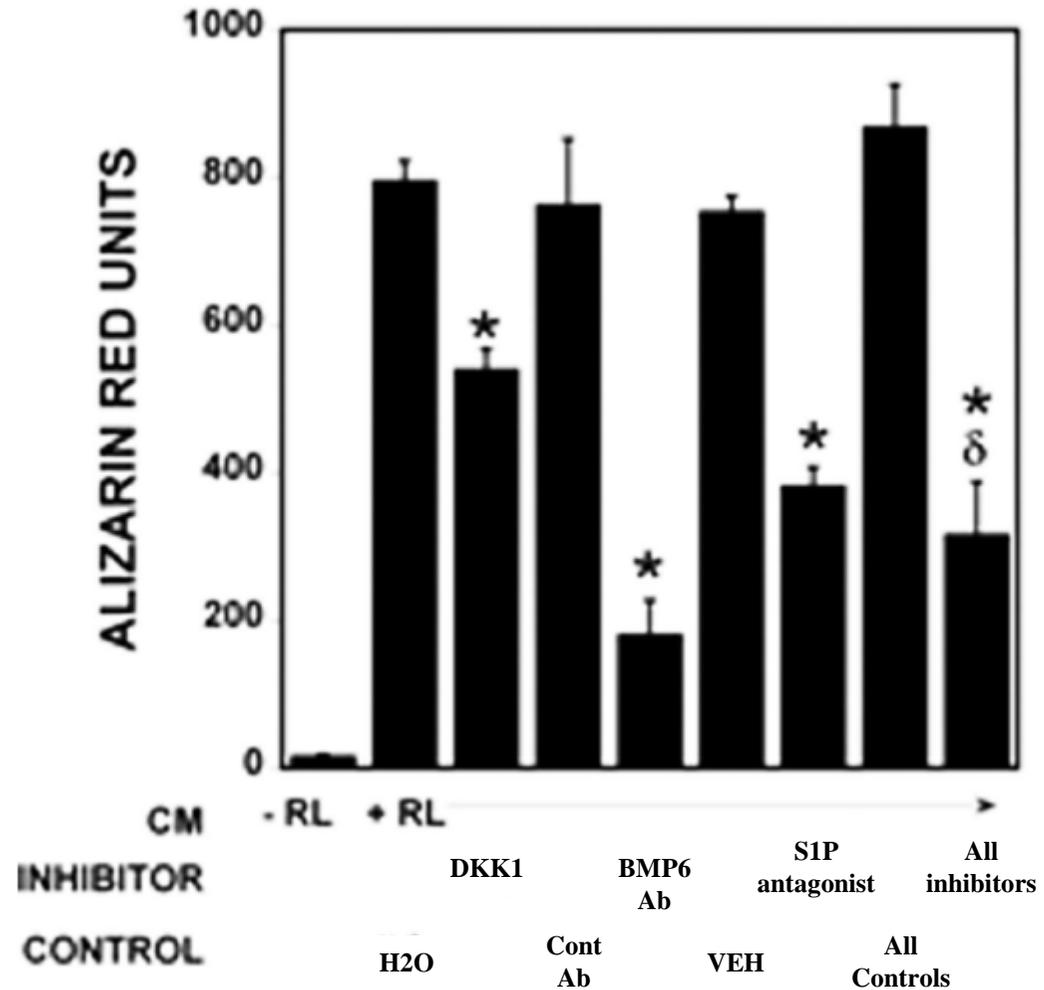
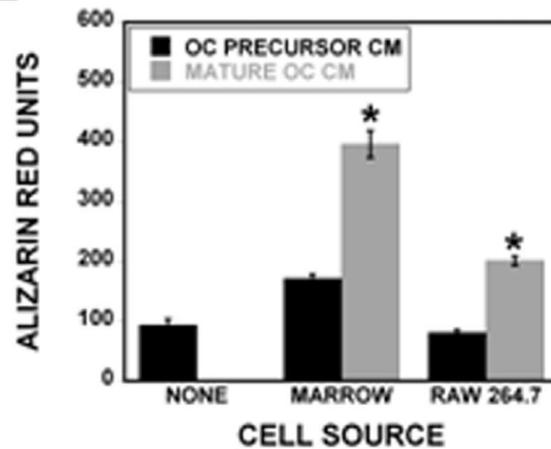
Histomorphometric index ^a	Endocortex	
	Female mice	
	Control	cKO ^c
dLS/BS (%)	72.4 ± 6.0	33.7 ± 6.4**
MS/BS (%)	77.0 ± 5.2	43.3 ± 4.5**
MAR (µm/day)	2.5 ± 0.1	3.5 ± 0.3*
Ob.S/BS (%)	80.0 ± 2.1	67.2 ± 5.5
OS/BS (%)	73.9 ± 2.3	61.7 ± 5.4
Oc.S/BS (%)	2.4 ± 0.4	17.1 ± 1.5**
ES/BS (%)	1.1 ± 0.4	42.6 ± 5.6**

Autres inducteurs OC de l'ostéof ormation

RAW264.7 precursor (-RL) and multinucleated and RAW 264.7-derived osteoclast (+RL) conditioned media (CM)

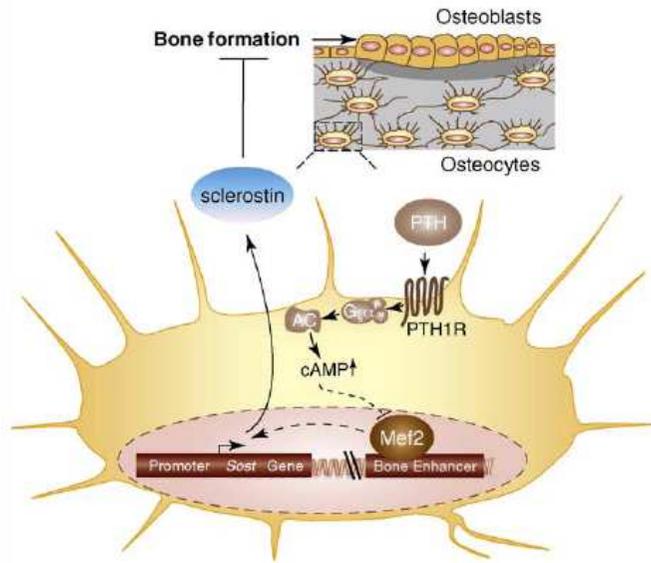


TOP-Flash

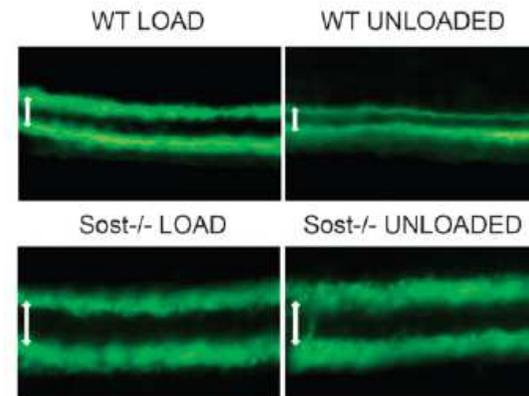
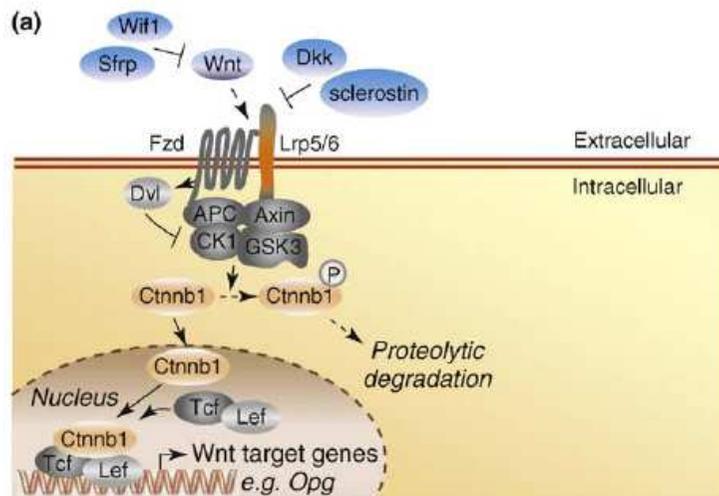
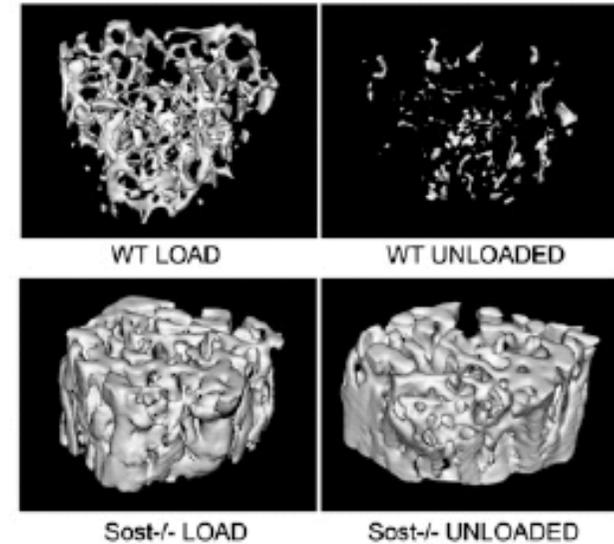
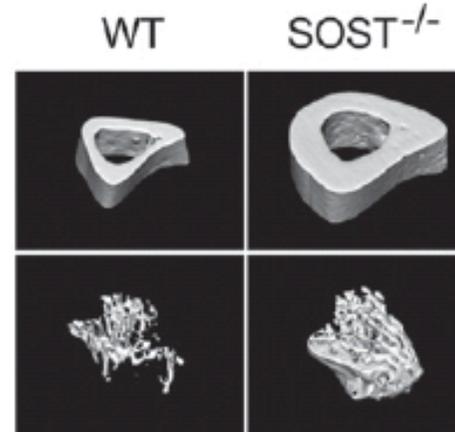


Sclerostine et remodelage osseux

SOST cKO mice

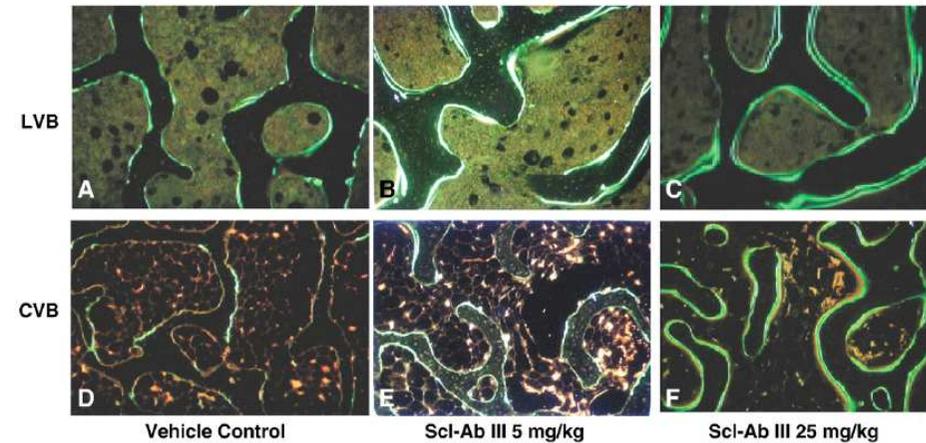
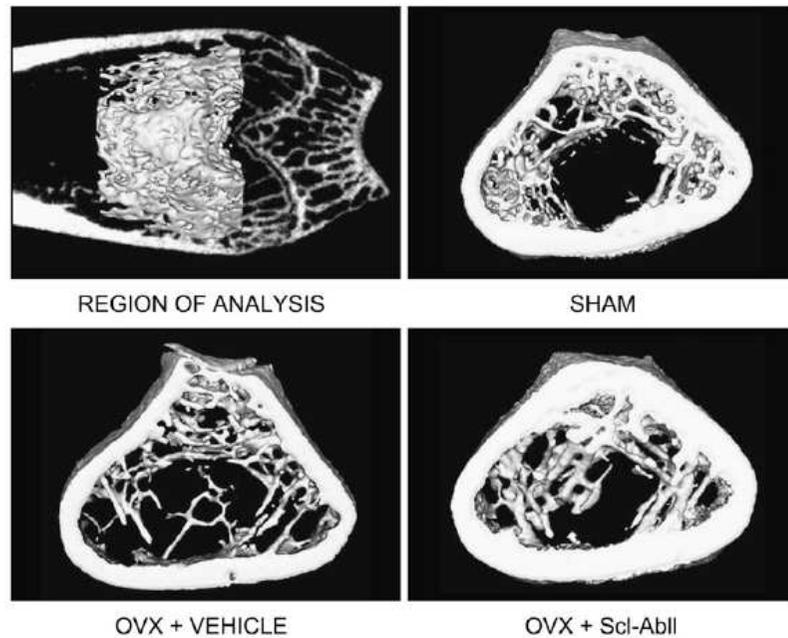


TRENDS in Endocrinology & Metabolism



Lin et al, 2010

Sclerostine et remodelage osseux



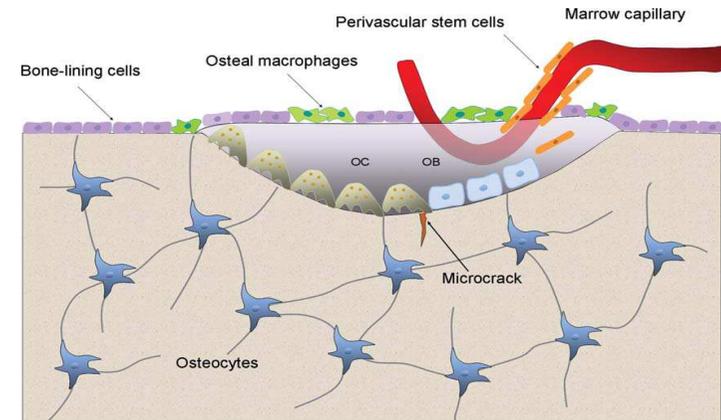
Tian et al, 2010

Li et al, 2009

cancellous bone histomorphometry variables from red marrow LVB and yellow marrow CVB.

Parameters	CVB				LVB			
	Basal (n=7)	Vehicle (n=8)	5 mg/kg (n=8)	25 mg/kg (n=8)	Basal (n=8)	Vehicle (n=8)	5 mg/kg (n=9)	25 mg/kg (n=9)
BV/TV	25.7 ± 4.1	23.8 ± 3.5	29.4 ± 4.1	37.5 ± 6.5 ^{ab}	25.1 ± 4.1	24.4 ± 3.7	31.9 ± 7.9 ^{ab}	45.2 ± 4.6 ^{ab}
Tb.Th	50.8 ± 6.0	45.7 ± 6.8	54.5 ± 10.5	65.3 ± 7.0 ^{ab}	65.1 ± 11.1	62.2 ± 7.2	91.6 ± 6.8 ^{ab}	119.4 ± 17.7 ^{ab}
Tb.N	5.1 ± 0.7	5.2 ± 0.4	5.5 ± 0.7	5.7 ± 0.6	3.9 ± 0.3	3.9 ± 0.6	3.5 ± 0.2	3.8 ± 0.4
Tb.Sp	149.8 ± 24.7	147.4 ± 15.3	130.5 ± 16.7	111.2 ± 21.9 ^{ab}	195.4 ± 22.5	196.2 ± 35.7	197.1 ± 20.5	144.5 ± 18.7 ^{ab}
Fat cell volume	~100 ± 0	~100 ± 0	~100 ± 0	~100 ± 0	3.8 ± 2.2	5.5 ± 3.6	4.4 ± 1.8	3.1 ± 1.5
MS/BS	5.3 ± 4.8	7.0 ± 3.3	22.2 ± 16.3 ^{ab}	47.5 ± 13.2 ^{ab}	25.9 ± 8.6	23.7 ± 6.3	59.6 ± 5.7 ^{ab}	78.7 ± 4.1 ^{ab}
MAR	0.4 ± 0.1	0.4 ± 0.1	0.6 ± 0.1 ^{ab}	0.6 ± 0.0 ^{ab}	0.7 ± 0.1	0.6 ± 0.1	0.9 ± 0.0 ^{ab}	1.0 ± 0.1 ^{ab}
BFR/BS	1.9 ± 1.4	2.9 ± 1.4	12.6 ± 9.6 ^{ab}	30.2 ± 8.2 ^{ab}	16.9 ± 6.3	14.7 ± 5.1	54.2 ± 4.0 ^{ab}	79.0 ± 6.6 ^{ab}
Er.S/BS	1.3 ± 0.5	1.4 ± 0.3	1.2 ± 0.4	1.0 ± 0.3	3.6 ± 0.7	4.1 ± 0.8	1.7 ± 0.3 ^{ab}	0.7 ± 0.2 ^{ab}

Rôle des « OsteoMacs » au cours du remodelage



- **Détection des signaux d'induction**
- **Induction de l'expression par les ostéoblastes de RANKL et CSF-1**
- **Facilitation de la prolifération des cellules bordantes et la formation de la canopée**
- **Régulation de la fonction des ostéoblastes**
- **Régulation des phases tardives de la minéralisation**

Résumé

Le remodelage osseux implique :

- **Couplage entre ostéoblastes et ostéoclastes parfaitement régulé**
- **Régulation par des hormones et par des molécules sécrétées par les ostéocytes en réponses aux stress mécaniques**
- **Formation d'entité spécialisées les BRC et la formation de la canopée permettant de créer un environnement favorable au couplage OB/OC et la régulation des activités cellulaires**

