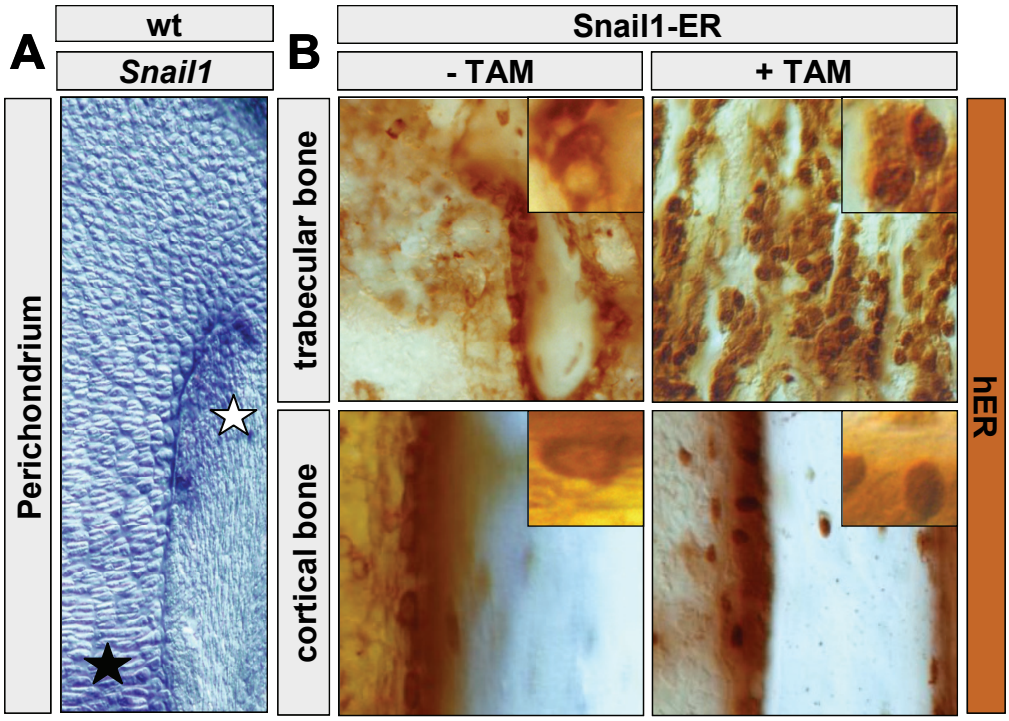
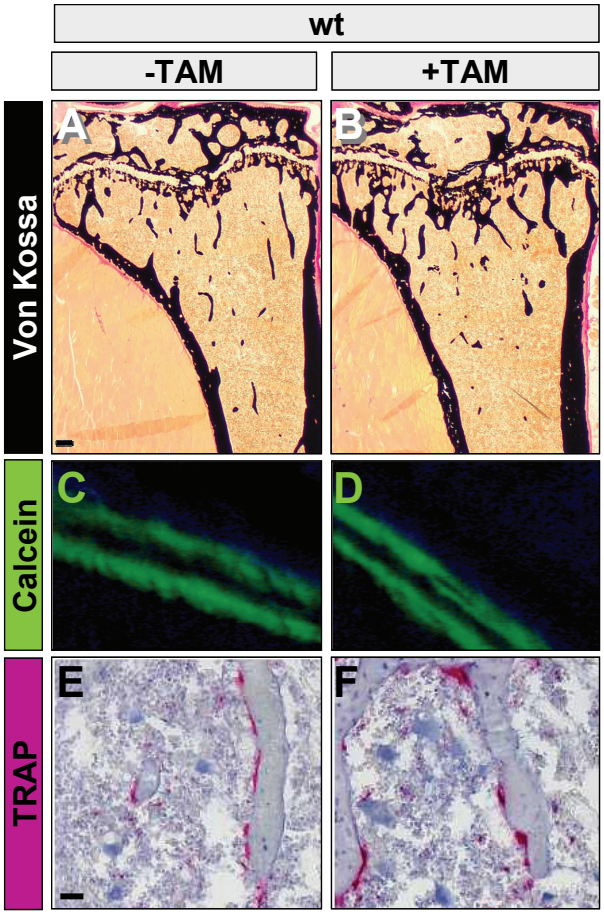
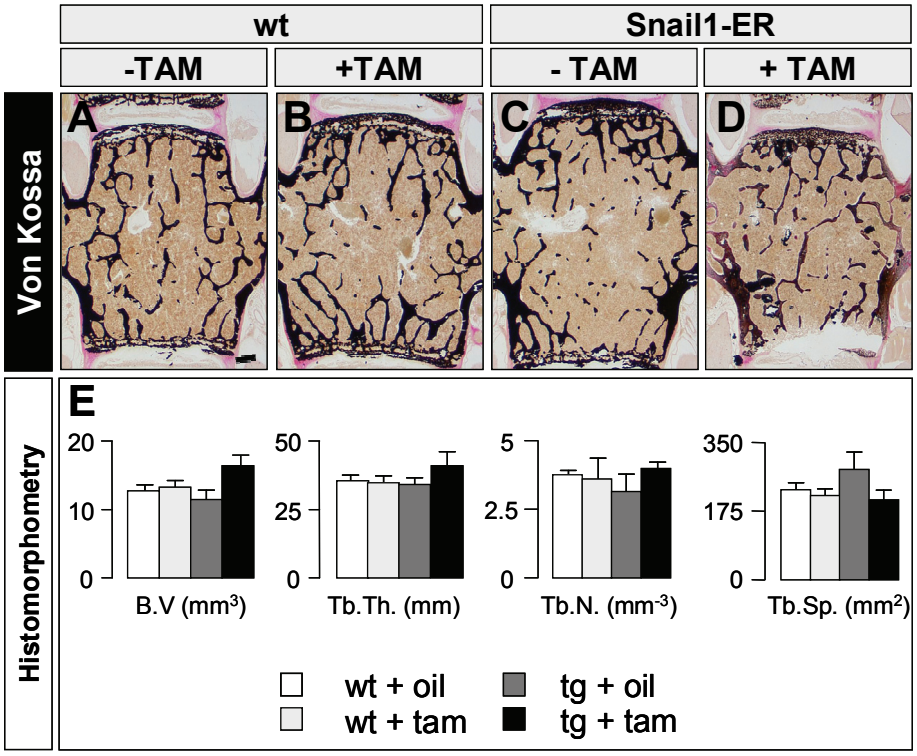


Supplementary Fig. 1. (A) Endogenous *Snail1* expression in the perichondrial area (white star) and in the growth plate (black star; see de Frutos et al., 2007) of the long bones. (B) Subcellular localization of Snail1-ER is regulated by Tamoxifen in transgenic bones. Immunostaining of the Snail1-ER protein in adult bones (12 week-old mice). Note the nuclear translocation after 4 weeks of tamoxifen administration, best assessed in the high power images inserted in each panel.

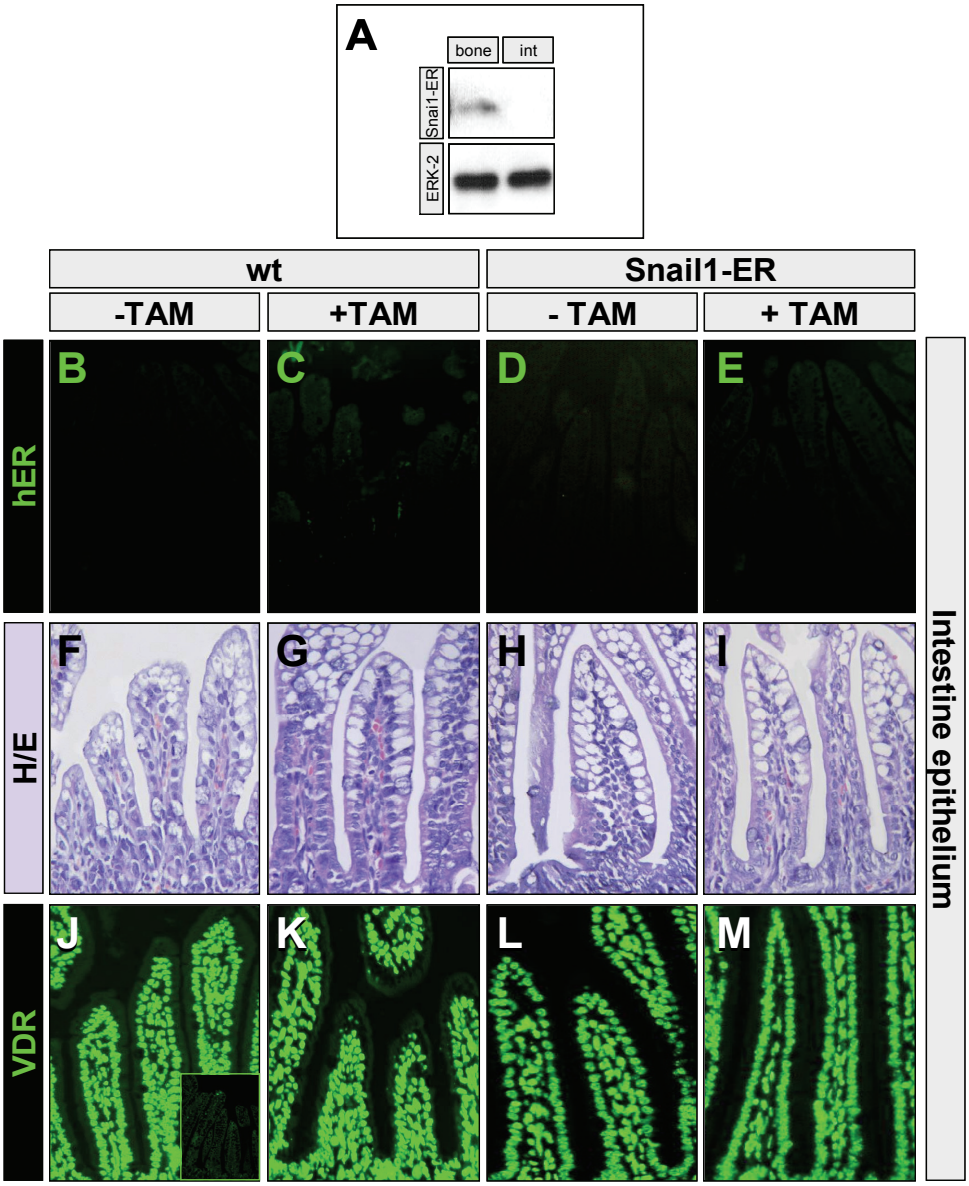
De Frutos, C.A., Vega, S., Manzanares, M., Flores, J.M., Huertas, H., Martinez-Frías, M.L. and Nieto M.A. (2007). Snail1 is a transcriptional effector of FGFR3 signaling during chondrogenesis and achondroplasias. ***Dev. Cell*** *13, 872-883.*



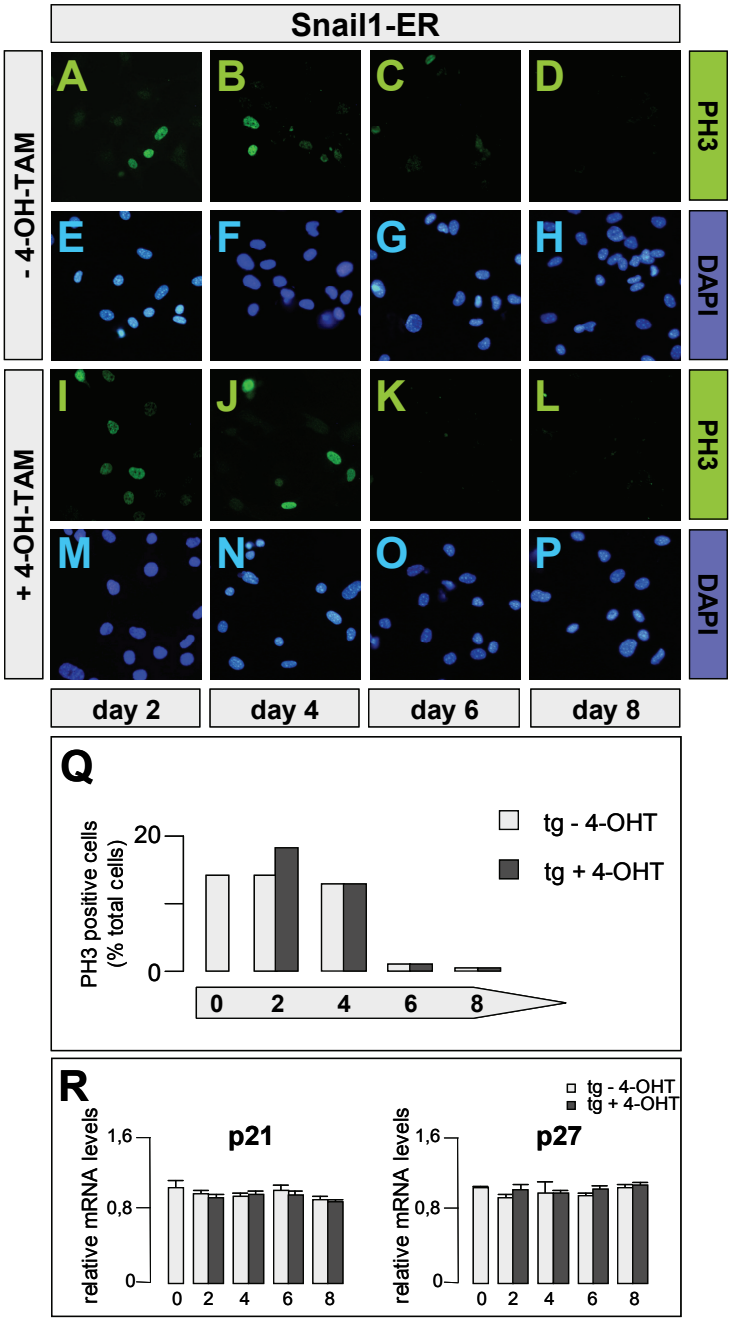
Supplementary Fig. 2. Bone mineralization, cortical thickness, osteoblasts activity and osteoclasts differentiation are not affected by Tamoxifen administration in wild type mice. (A-B) Von Kossa staining in sections of long bones from 16 week-old mice. (C-D) Osteoblasts activity measured by in vivo calcein incorporation into trabecular bone. (E, F) TRAP staining of osteoclasts. Scale bars, 1 mm (A, B) and 100 µm (E, F).



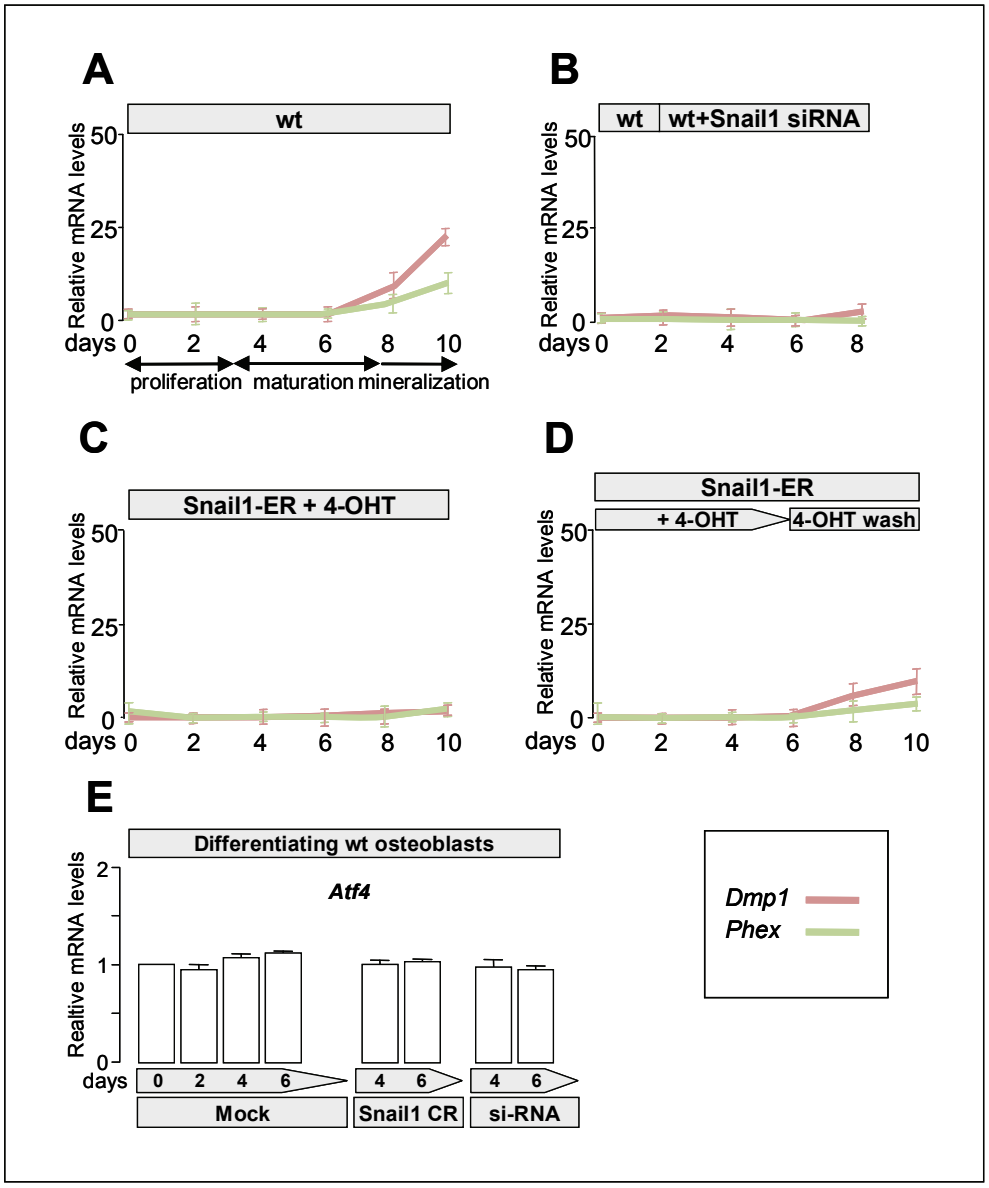
Supplementary Fig. 3. (A-D) Von Kossa staining of vertebrae sections from 16-week-old wild type and Snail1-ER mice (black staining). (E) Histomorphometric analysis (n=5 per condition). Bone volume (B.V.); Trabecular thicknes (Tb. Th.); trabeculae number (Tb.N.) and trabecular spacing (Tb. Sp.). Scale bar, 1mm.



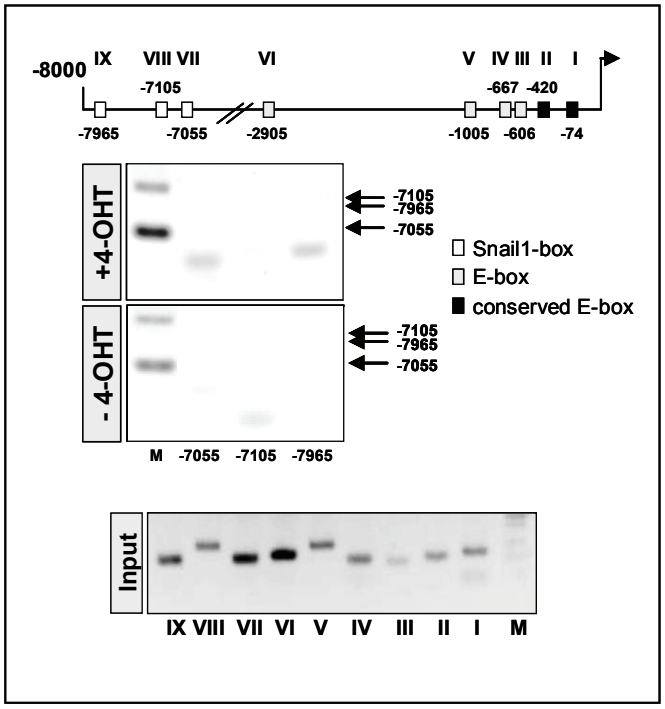
Supplementary Fig. 4. (A) Western blot and (B-E) immunohistochemical analysis confirms that no transgenic protein (hER) is present in the intestine. Thus, tamoxifen administration does not have any impact on tissue morphology (F-I) or VDR expression (J-M). The inset in (J) shows a negative control for the VDR antibody. Int, intestine



Supplementary Fig. 5. Snail1 activation does not affect the proliferation in osteoblasts in culture. (A-D and I-L) Phospho-histone 3 immunofluorescence (PH3) shows the mitotic transgenic osteoblasts during *in vitro* differentiation in the presence or in the absence of 4-OH-TAM. (E-H and M-P) Cells were counterstained with DAPI to reveal their nuclei. (Q) Quantification of PH3-positive cells. (R) Real time PCR shows no variations in the mRNA levels of p21 and p27, indicating that contrary to its action in chondrocytes, Snail1 does not activate p21 in osteoblasts.



Supplementary Fig. 6. Snail1 is necessary for osteoblast differentiation in culture. (A, B) Relative mRNA levels of *Dmp1* and *Phex* in wild type and transgenic mesenchymal cells during their differentiation to osteoblasts. (C, D) Snail1 activation inhibits *Dmp1* and *Phex* expression in cultured osteoblasts. This effect is reversible, as assessed by the onset of their expression when 4-OHT is washed out. *Dmp1* (light brown) and *Phex* (light green). (E) the expression of *Atf4* is not affected by Snail1 activation.



Supplementary Fig. 7. Snail1 does not bind to its perfect match E-boxes (GCAGGTG; Cano et al., 2000) located from 7 to 8Kb upstream of the *Runx2* gene coding region. These Snail1 E-boxes are not conserved in the human promoter. The arrows on the right indicate the positions of the predicted amplified fragments. Input material was tested for each primer set (boxes I to IX).

Cano, A., Pérez, M. A., Rodrigo, I., Locascio, A., Blanco, M. J., Del Barrio, M. G., Portillo, F. and Nieto, M. A. (2000). The transcription factor Snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression. ***Nature Cell Biol.*** *2, 76-83*

Supplementray Table I. Oligonucleotides

	GENE	SEQUENCE (5' to 3')	fragment size (bp)
mice genotyping		ACGATAAGCTCGAGCCATCTGC	
		ACCGAGATGATGTAGCCAGCAG	450
RT-PCR	*Gapdh*	CAAAGTGGAGATTGTTGCCATC	
		CACCACCTTCTTGATGTCATC	709
	Snail 1	AGCTGGCCAGGCTCTCGGTG	
		TAGCAGGGTCAGCGAGGGCC	391
Q-RT-PCR	*Alkaline Phosphatase*	GAGAGGTCCAGGCAACTTCCA	
		GGAATAAAGGCAGAGCCAGGAAT	102
	Bglap1/Osteocalcin	GGCTGGAAGACCGCCTACAAA	
		CCCGGAGAGCCAAAG	100
	Cbfa1/Runx2	GGCCGGGAATGATGAGAACTAC	
		CGCTCCGGCCCACAAAT	101
	Collagen1a1	GGAGAGAGCATGACCGATGGA	
		GGTGGACATTAGGCGAGGAA	101
	Cyp27b1	TACCTGAGCCAGGTGCTCTT	
		GGCTGTCTTCCGAATGGTTA	102
	Dmp1	CCACAGACACCACACCGTCC	
		TGTCTGCCTCATCCTCACTG	100
	Gapdh	CTGAGCAAGAGAGGCCCTATCC	
		CTCCCTAGGCCCCTCCTGTT	104
	Ibsp/Sialoprotein	ACCACACCCCAAGCACAGACT	
		TCGTCGCTTTCCTTCACTTTTG	102
	p21	AGGAGCCAGGCCAAGATGGT	
		GCTTTGACACCCACGGTATTCA	100
	p27	AGAACTAACCCGGGACTTGG	
		CCAGGGGCTTATGATTCTGA	102
	Phex	GATTTCCTGCTGGAGAGCTG	
		TGTAAATTCATGGCCCACAA	100
	Tnfsf11/Rankl	CAACATTTGCTTTCGGCATCAT	
		AACTTGGGATTTTGATGCTGGTTT	102
	Tnfrsf11b/Osteoprotegerin	AAACACACGGACTGCAGCACAT	
		CACTTTTGCGTGGCTTCTCTGT	100
	Snail 1	CCACACTGGTGAGAAGCCATTC	
		TCTTCACATCCGAGTGGGTTTG	101
	Spp1/Osteopontin	TGATTTGCTTTTGCCTGTTTGG	
		AGGATCTGGGTGCAGGCTGTA	102
	Vdr	CAAGGACAACCGGCGACACT	
		TTACGCTGCACCTCCTCATCTG	102
ChIP assay	*Runx2 E-box I*	AAAGAGGGAGGGAAGAGAGCAA	
		CGAATGAAGCATTCACACAATCC	150
	Runx2 E-box II	TGGCAGAAAGGAAAAGCCTTA	
		GCCTTCCTGGCATTCAGAA	126
	Runx2 E-box III	CTTGCAAGTGATACAATCCCAA	
		CCTCCTCCCTTTCCTTCATTAT	103
	Runx2 E-box IV	CCAAGCTTAGGAAGACAAGCAA	
		TCAACTGAGTGTGTGGCGTT	105
	Runx2 E-box V	CGTGGCGGCTCTTACAATAAA	
		TCACTGTCCACGCTGATGAAA	100
	Runx2 E-box VI	GCATTTGCTTACTATCCTATAGCAAC	
		CTGTGCCAGTGTGATCTTTATC	120
	Runx2 E-box VII	CATTCTAGAATGATCCAACTTAATGG	
		TTACAGGAATAGATTGGGTTAGAATTAG	104
	Runx2 E-box VIII	ATCTGCATGATTGGTTTGAAC	
		CCATTAAGTTCCATCATTCTAGAATG	112
	Runx2 E-box IX	CATGACTACAACTCATTCTTGCCTC	
		AGCCAGTAAATGAATACATGTGTCTG	100
	VDR E-box I	TAGGAGAGAGGACGCAACTCC	
		CGCTGCAGGGAGCCGTTCTCT	101
	VDR E-box II	TGGCAGAAAGGAAAAGCCTTA	
		AGAGAACGGCTCCCTGCAGCG	100
	VDR E-box III	GATCCGTGATGTAGCCACCAC	
		GTGCAGTGGTTGATTCCAAGT	100