The Role of Enhancers in Genetic and Epigenetic Control of Gene Expression

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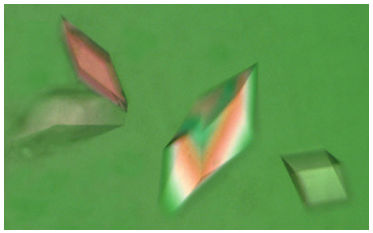
Encode Users Meeting-2016
Stanford University
Stanford, CA
June 8-10, 2016



Acknowledgements

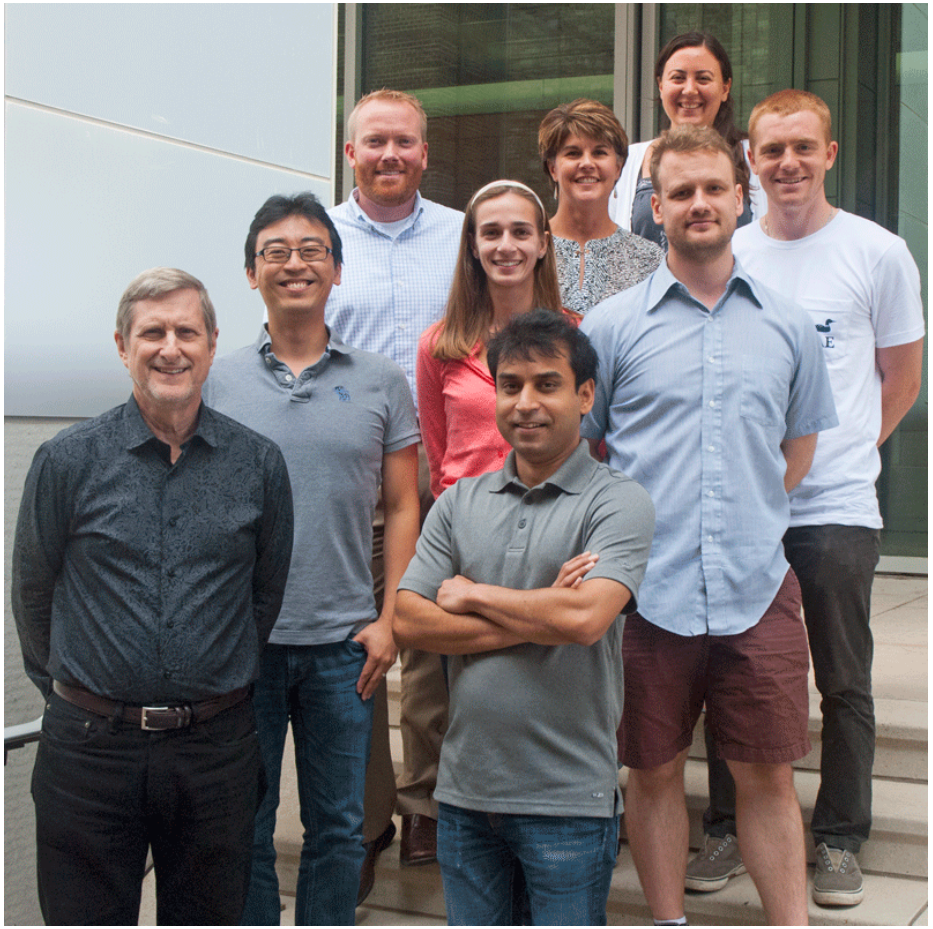
Department of Biochemistry,
University of Wisconsin-Madison

- Mark B. Meyer, Ph.D.
- Seong Min Lee, Ph.D.
- Melda Onal, Ph.D.
- Kathleen A. Bishop, Ph.D.
- Nancy Benkusky
- Hillary St. John, Ph.D.
- Sohel Shamsuzzaman, M.S.
- Alex Carlson

Charles A. O'Brien, Ph.D.,
University of Arkansas for Medical Sciences

Analytical Software/ Tools

- HOMER-Chris Benner (The Salk)
- CRISPR- Zhang Lab (MIT)
- Genome Editing & Engineering at Wisconsin

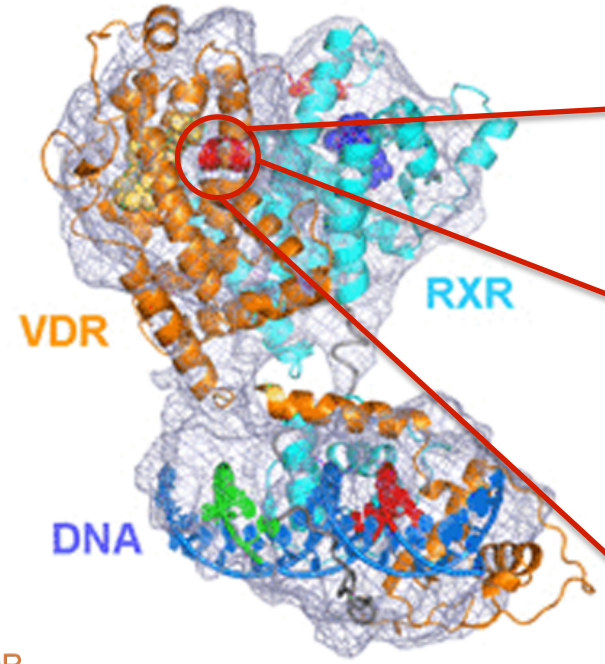


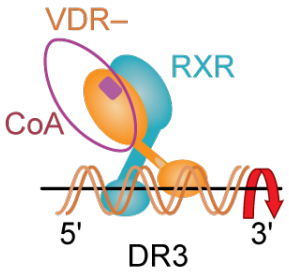
National Institutes of Health: NIDDK and NIAMS

Why Study Enhancers?

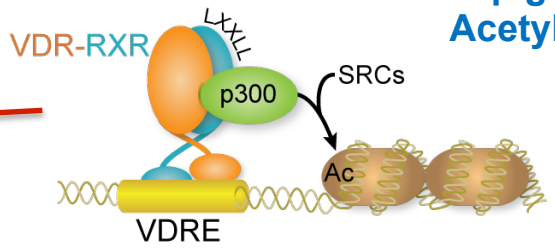
- Enhancers govern cellular phenotype through selective control of gene expression
- Detailed enhancer studies provide relevant insight into basic gene regulatory mechanisms
- Understanding specific enhancer features may reveal roles for SNVs in genome evolution and SNPs in human disease
- Unique enhancer properties could facilitate the development of next generation therapeutics for personalized medicine
- Enhancer/promoter segments of genes can be utilized to create diverse basic as well as clinically relevant animal models

The Vitamin D Receptor (VDR): Basic Functions

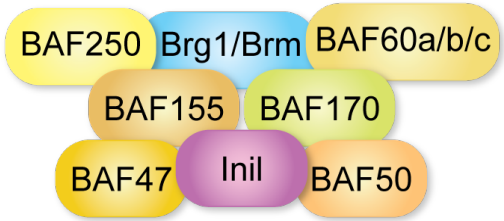




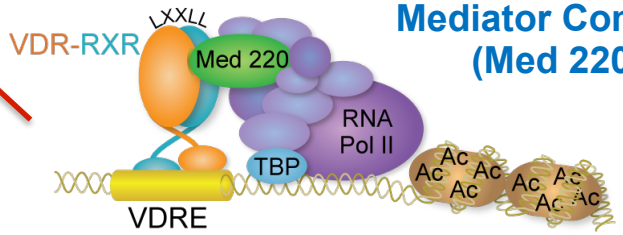
Epigenetic Histone Acetylation Complex (HATs)



Nucleosome Remodeling Complex (SWI/SNF ATPase)

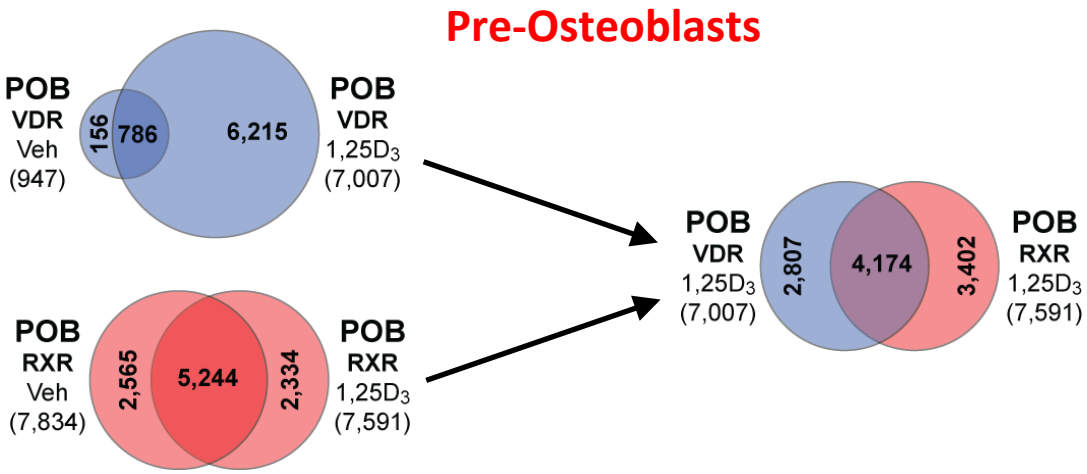


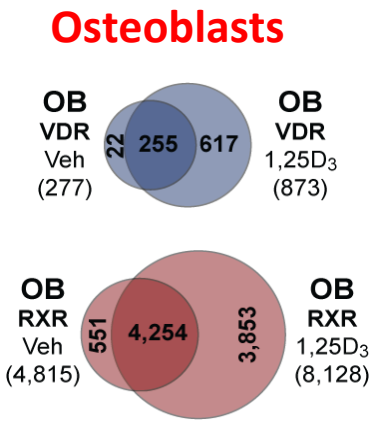
Mediator Complex (Med 220)



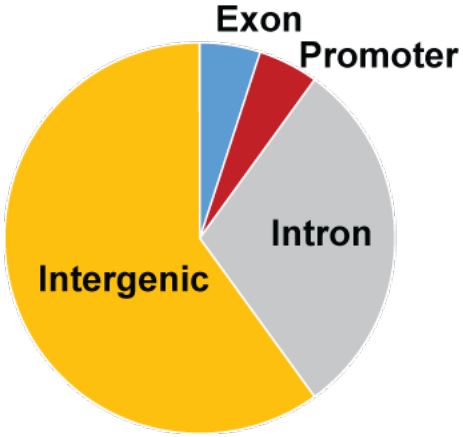
Orlov et al. EMBO J 31: 291 (2011)

Characterization of the VDR Cistrome in Differentiating Osteoblasts



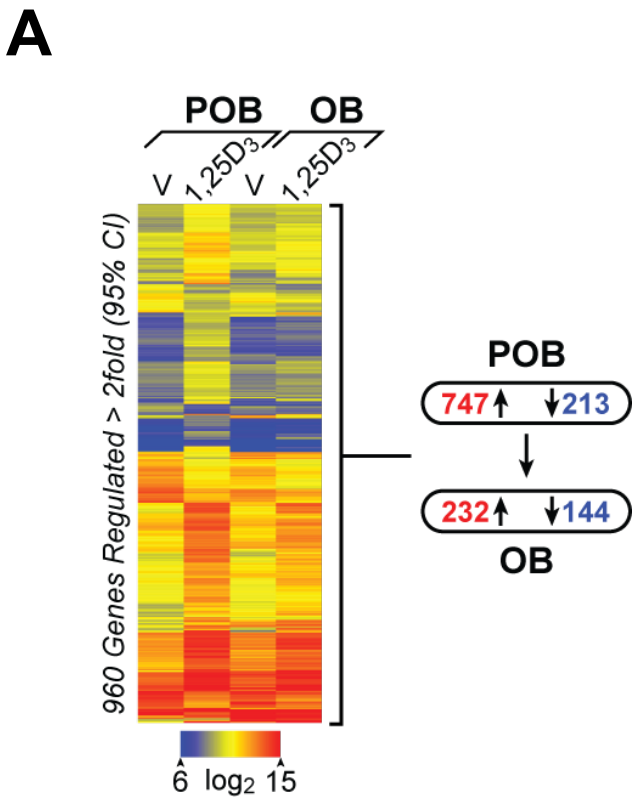


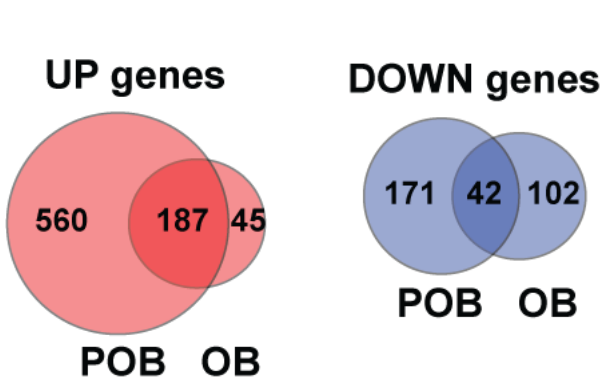
	Rank	TF match	Peak (bkgd)
POB	1	VDR/RXR	20% (1%)
	2	RUNX	34% (11%)
OB	1	VDR/RXR	36% (1%)
	3	RUNX	11% (0.4%)

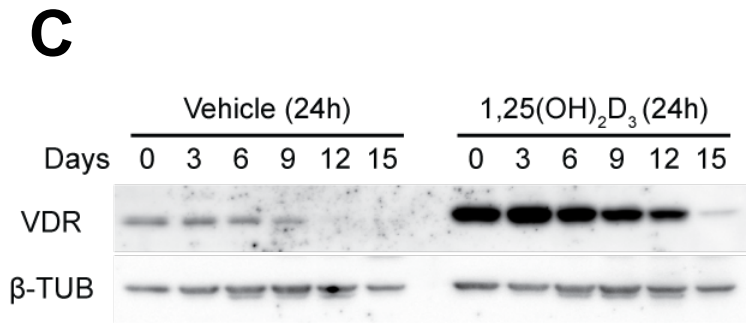


Meyer et al. J Biol Chem 289: 19539 (2014)

Contraction of the 1,25(OH)$_2$D$_3$ Transcriptome After Osteoblast Differentiation

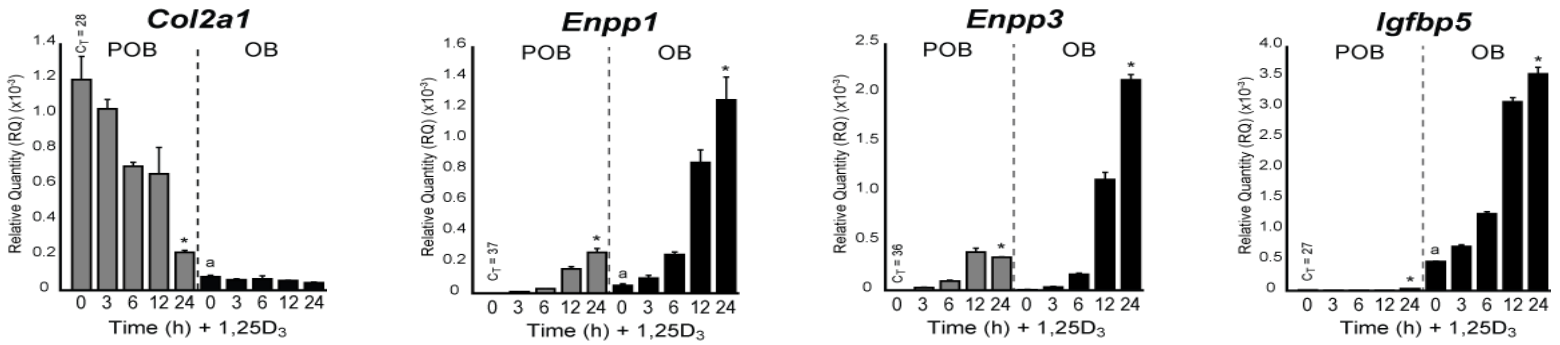


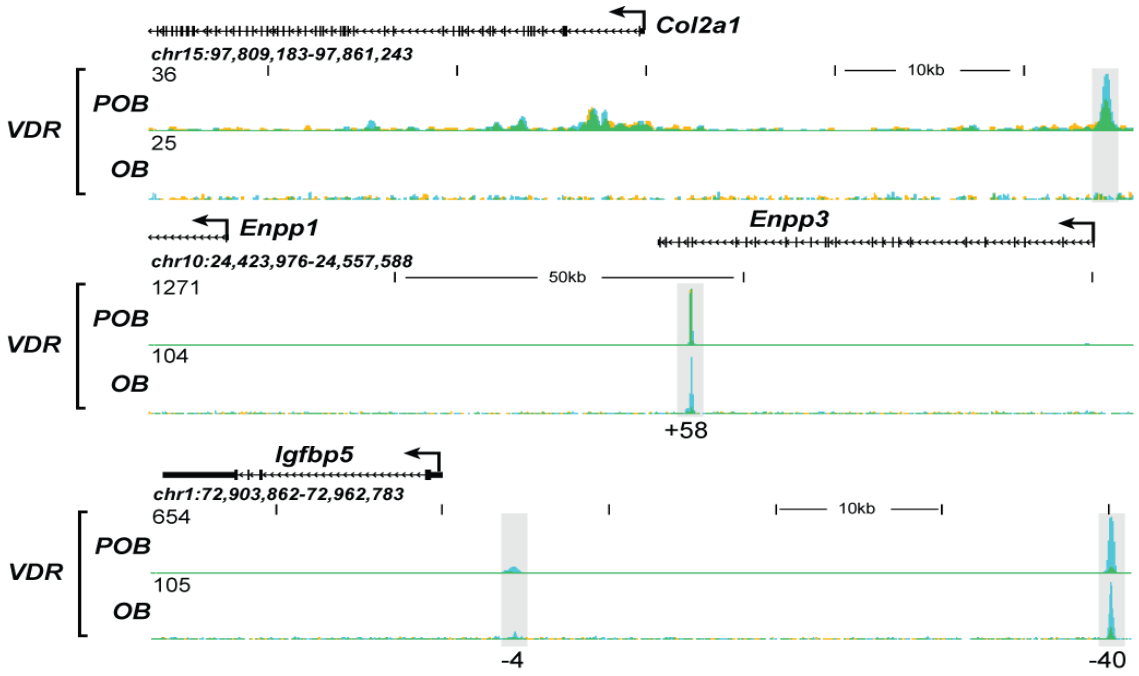




Meyer et al. J Biol Chem 289: 19539 (2014)

Differential Target Gene Responsiveness to $1,25(OH)_2D_3$ Due to Differentiation





HUB Tracks:

Yellow, Vehicle

Blue, $1,25(OH)_2D_3$

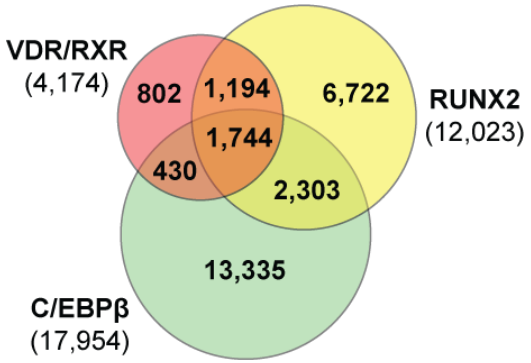
Green, Overlap

Epigenetic Changes in Differentiation

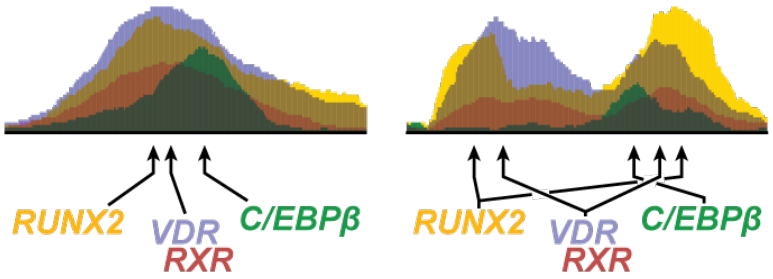
- Enhancers are highlighted by signature histone modifications that are dynamic and include H3K4me1, H3K4me2, H3K9ac and H3K27ac (ENCODE)
- Differentiation/trans-differentiation is characterized by significant changes in histone modification at selected gene loci (ENCODE)
- Changes in histone marks and regulatory factors can contribute to responsivity to secondary regulators such as the vitamin D receptor
- $1,25(OH)_2D_3$ and other hormones provoke changes in histone modification/acetylation and factor binding in a gene-selective manner

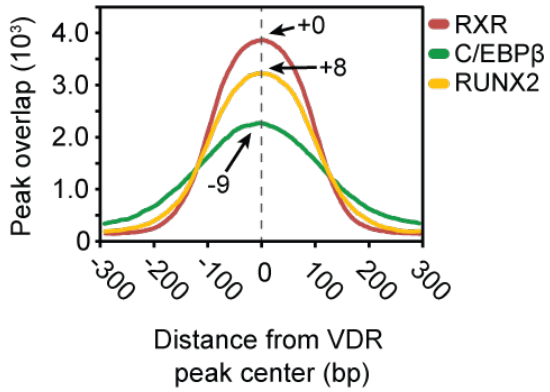
Meyer et al. J Biol Chem 289: 16016 (2014)
St John et al. Mol Endocrinol 28:1150 (2014)
St John et al. Bone 72: 81 (2015)

The Osteoblast Enhancer Complex (OEC): An Example of a Consolidated Enhancer



Venn Part	Rank	TF match	Peak (bkgd)	Motif
VDR/RXR (802)	1	*VDR/RXR*	36% (1%)	
RUNX2 (6,722)	1	*RUNX2*	45% (6%)	
C/EBPβ (13,335)	1	*C/EBPβ*	48% (4%)	
VDR/RXR / RUNX2 / C/EBPβ (1,744 peaks)				
	1	*RUNX2*	50% (12%)	
	2	*C/EBPβ*	24% (8%)	
	5	*VDR/RXR*	7% (2%)	





Meyer et al. J Biol Chem 289: 16016 (2014)
Meyer et al. J Biol Chem 289: 19539 (2014)

Key Features of Enhancers Thus Far

Distal Binding Site Locations: ***Cis*-regulatory modules (CRMs or enhancers) are dispersed across the genome; located in a cell-type specific manner near promoters, but predominantly within introns and distal intergenic regions; frequently located in clusters of elements**

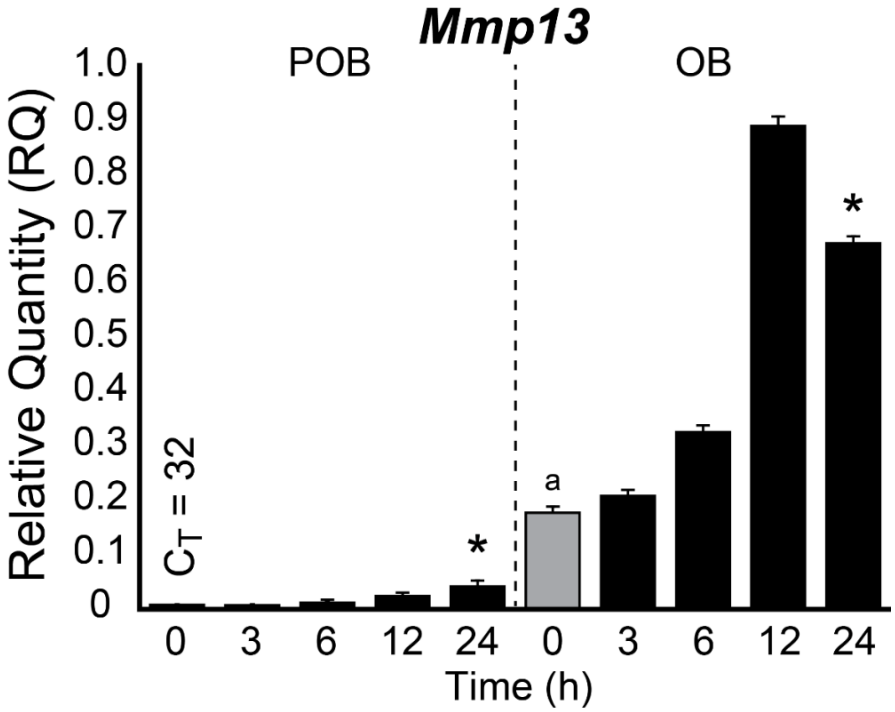
Modular Features: **Enhancers contain binding sites for multiple transcription factors that facilitate both independent or synergistic interaction**

Epigenetic Enhancer Signatures: **Defined by dynamically regulated post-translational histone H3 and H4 modifications**

Transcription Factor Cistromes (VDR) are Highly Dynamic: **Cistromes change during cell differentiation, maturation, and disease activation and thus have broad consequential effects on gene expression**

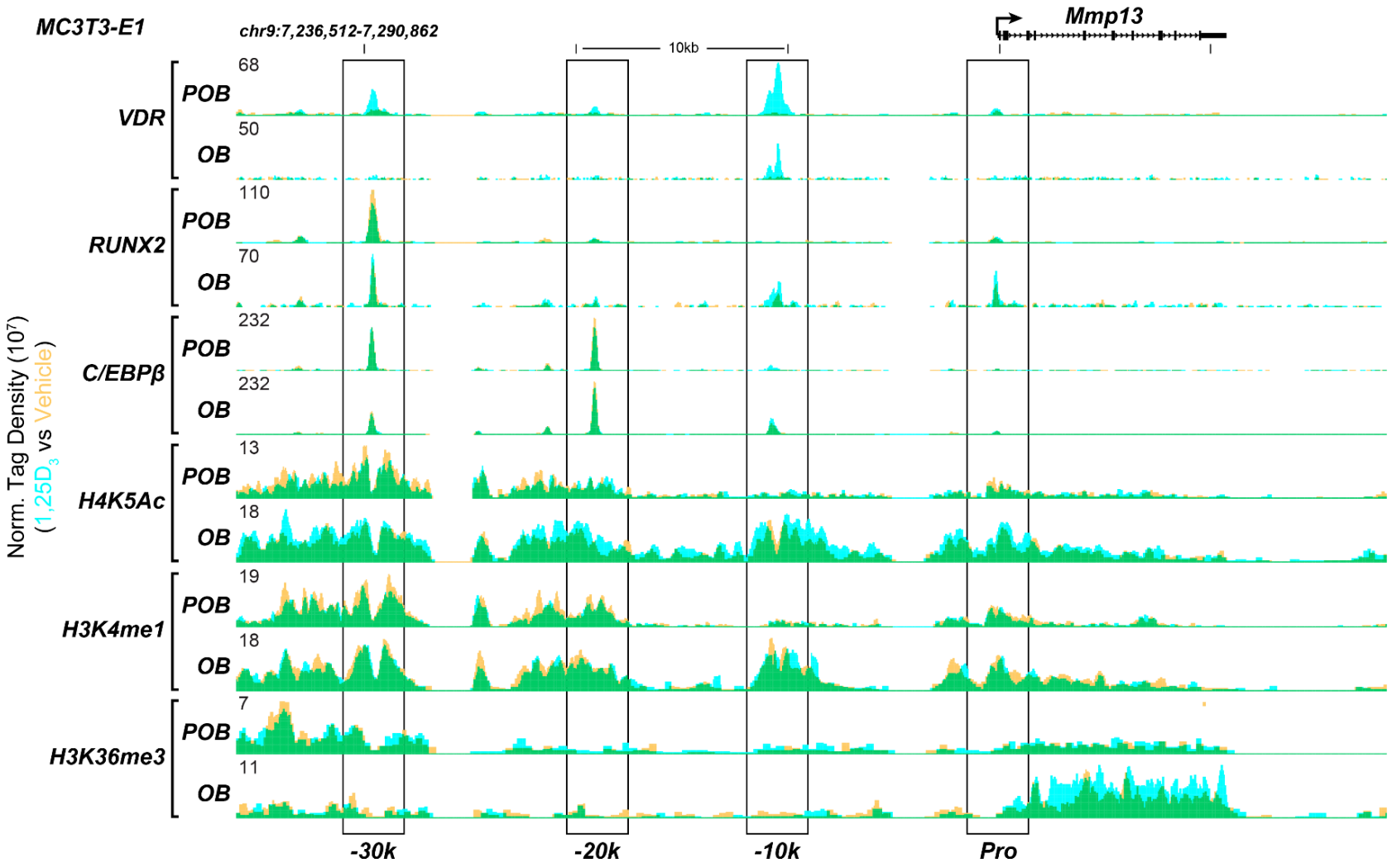
Mmp13 is Regulated by 1,25(OH)$_2$D$_3$ and Differentiation

- Collagenase-3 (Mmp13) degrades extracellular collagens at skeletal sites in bone
- The gene is aberrantly expressed in nearly every cancer or disease with fibrotic complications (breast, prostate, pancreatic, and atherosclerosis)
- *Mmp13* is regulated by a variety of factors including FGF2, PTH, estrogens, 1,25(OH)$_2$D$_3$, and cytokines
- Previous work on regulation has focused almost exclusively on the promoter proximal region of *Mmp13*

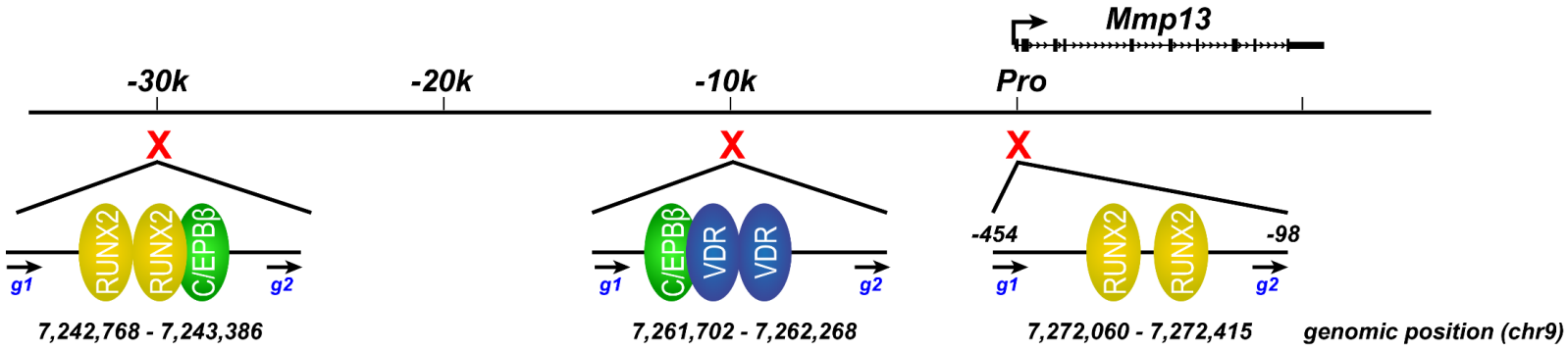


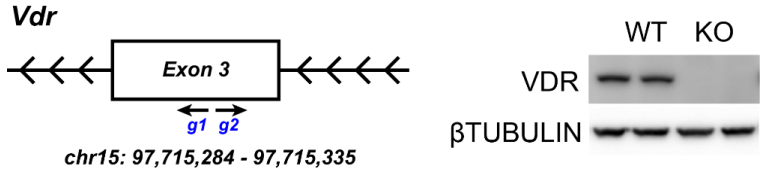
Meyer et al. J Biol Chem 290: 11093 (2015)

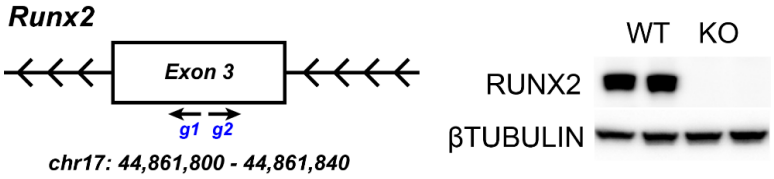
ChIP-Seq Analysis Identifies Distal Upstream Enhancers in the *Mmp13* Locus



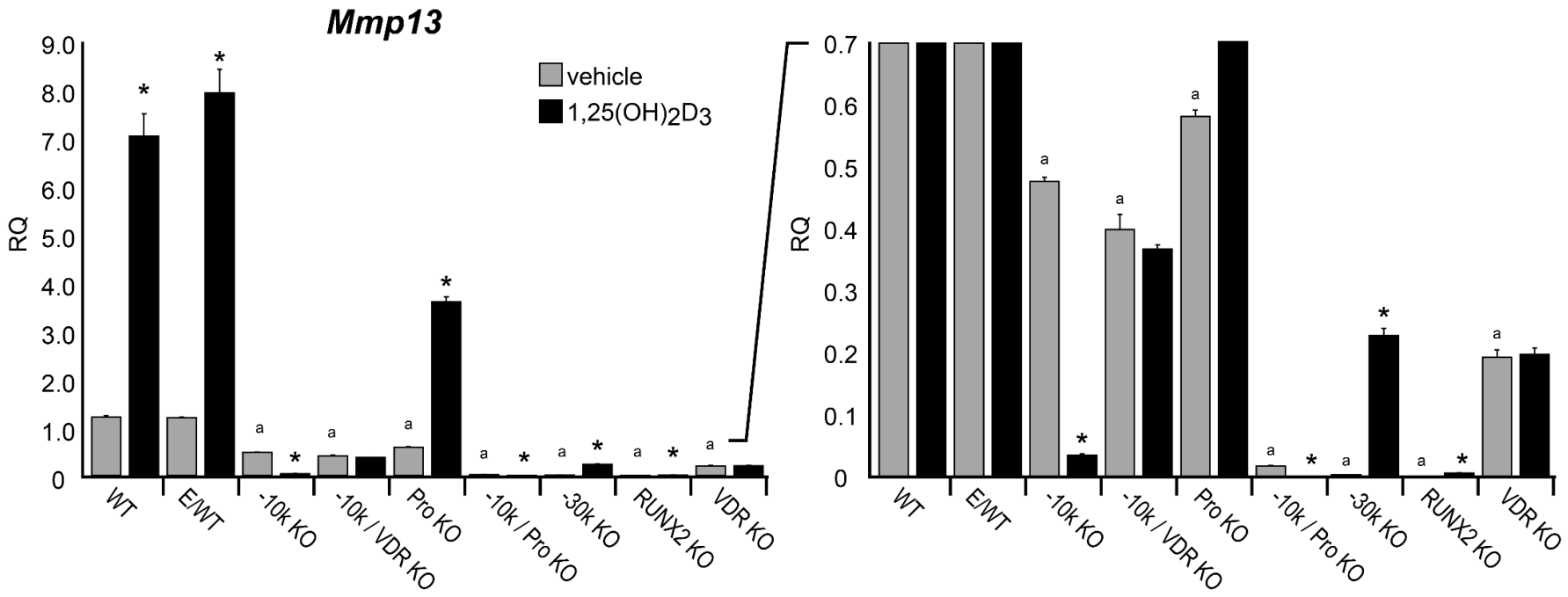
CRISPR/Cas9 Mediated Enhancer and TF Deletion in an Osteoblastic Cell Line





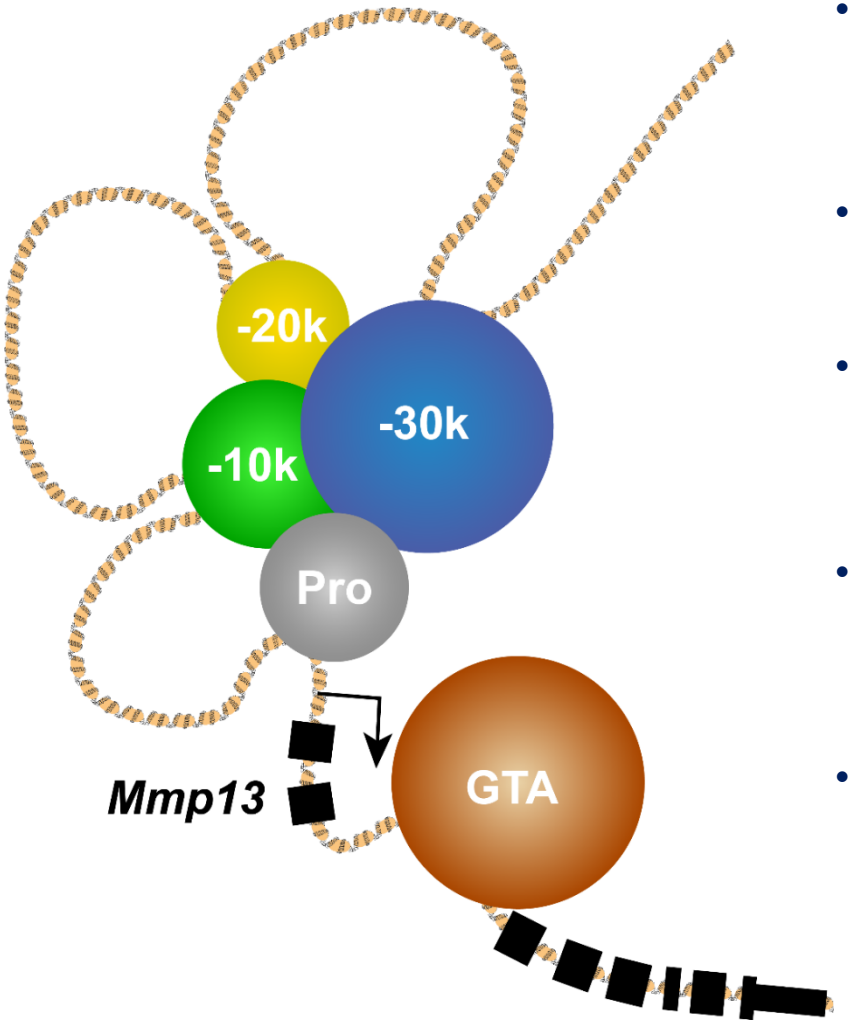


Genome Deletions have Dramatic Effects on Basal *Mmp13* Expression and on $1,25(OH)_2D_3$ Inducibility



- Deletion of the promoter proximal region of *Mmp13* reduces *Mmp13* RNA expression
- Deletion of the -10k *Mmp13* enhancer or VDR reduces basal expression of *Mmp13* RNA and highlights secondary regulation by $1,25(OH)_2D_3$
- Deletion of the -30k *Mmp13* enhancer or RUNX2 eliminates basal expression of *Mmp13* RNA

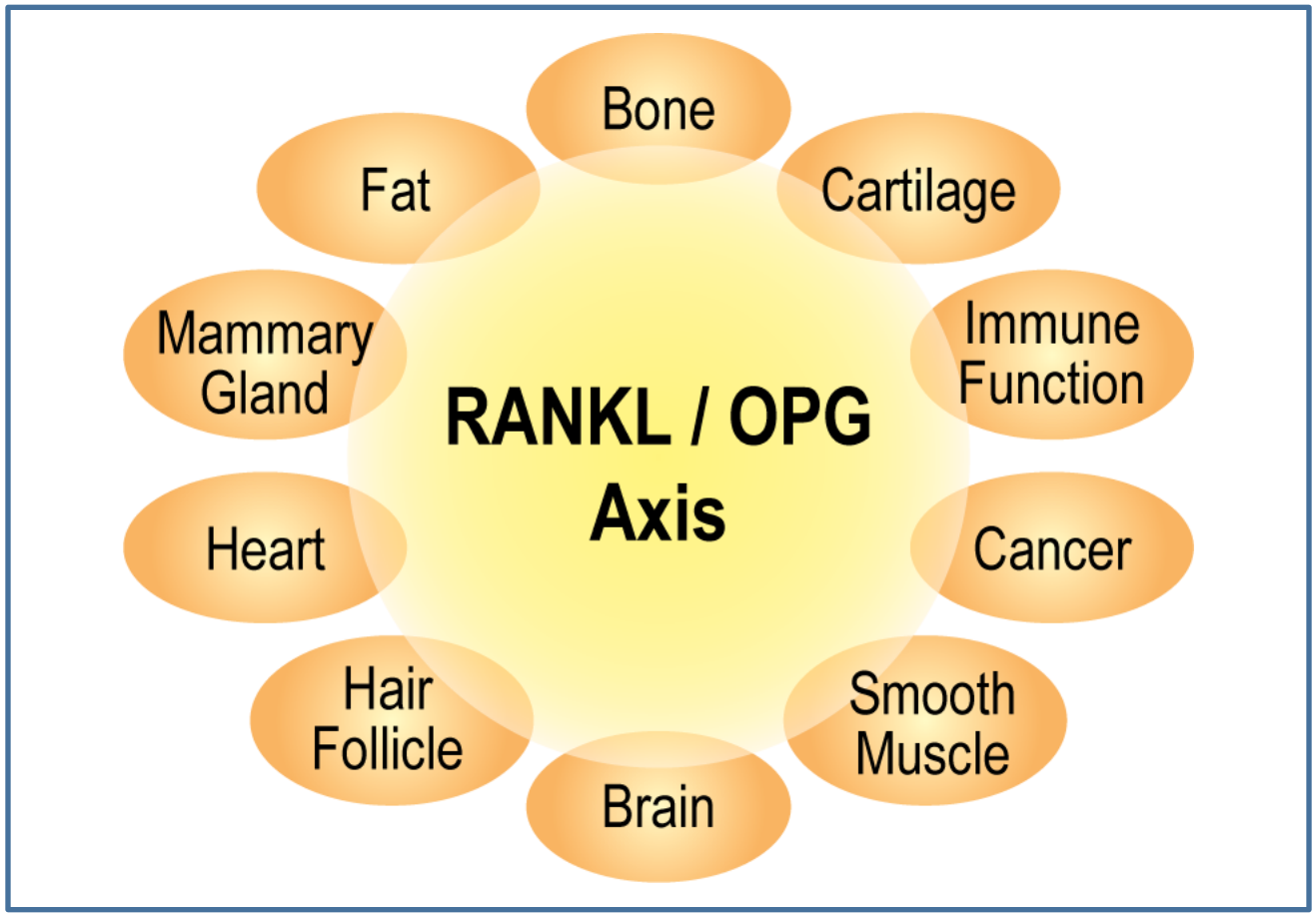
Mmp13 Chromatin Interaction Model



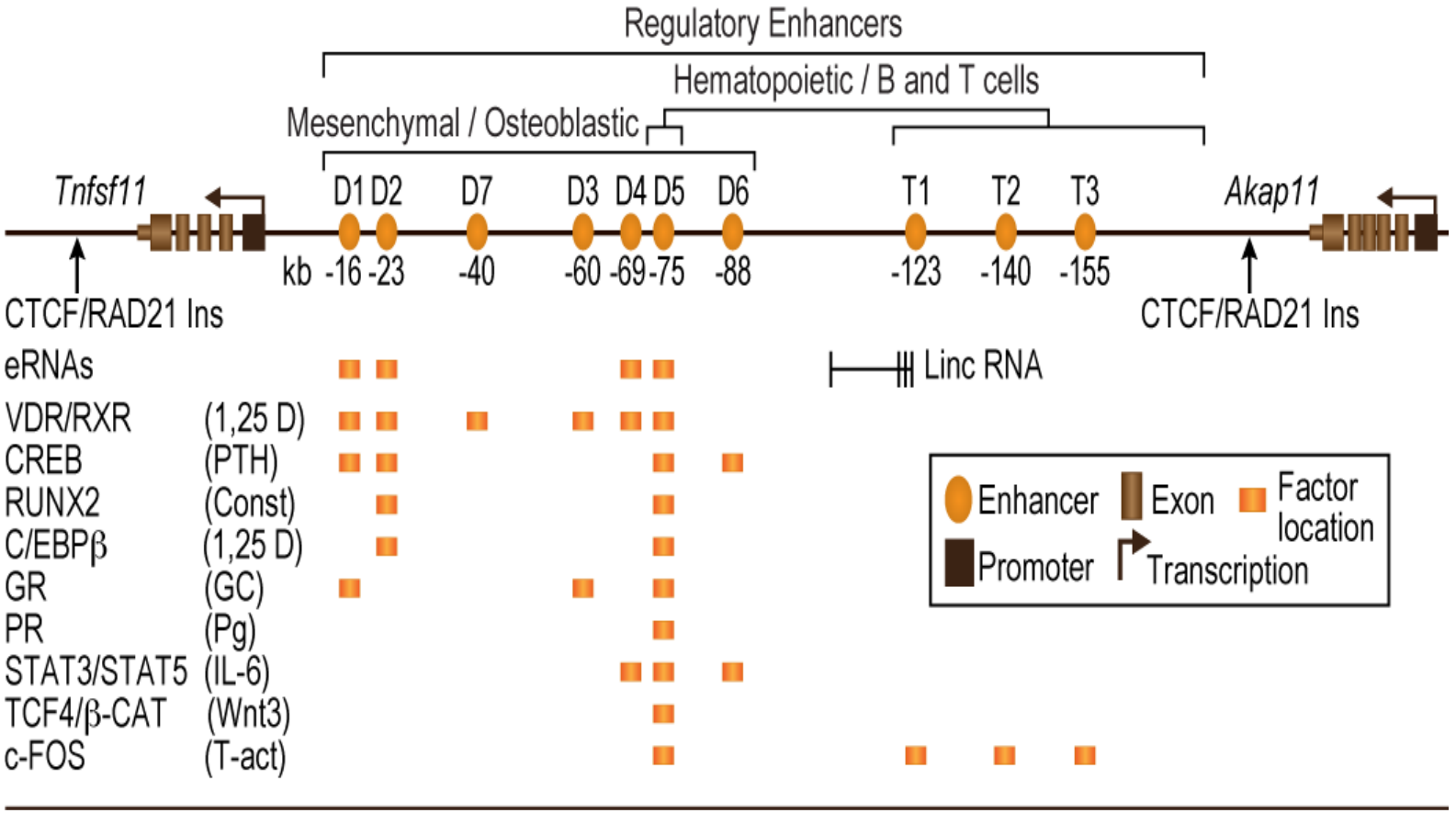
- A dispersed osteoblast enhancer complex at the *Mmp13* locus coalesces at the promoter through chromatin reorganization
- The promoter proximal region is unable to mediate independent regulation
- The -10 kb enhancer mediates hormonal regulation by $1,25(OH)_2D_3$ yet is dominated by the -30 kb enhancer
- The -30 kb region is central to the basal activity of Mmp13 and exhibits hierarchical activity over the remaining enhancers
- Repression by $1,25(OH)_2D_3$ in the absence of the -10 kb enhancer is likely due to independent RUNX2/OSX downregulation by the VDR

Meyer et al. J Biol Chem 290: 11093 (2015)

The Diverse Biological Activities of RANKL

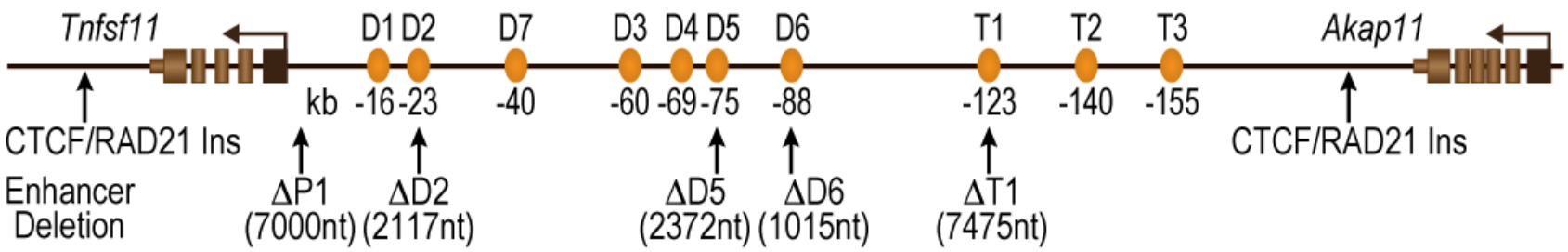


Regulatory Complexity at the *Tnfsf11* (Rankl) Gene Locus Involves Multiple Upstream Distal Enhancers



Pike et al. Bonekey Rep 3: 482 (2014)

Genetic Deletion of *Tnfsf11* (Rankl) Enhancers in the Mouse

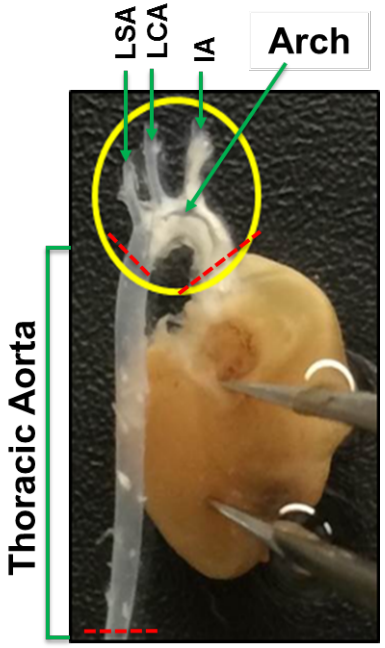


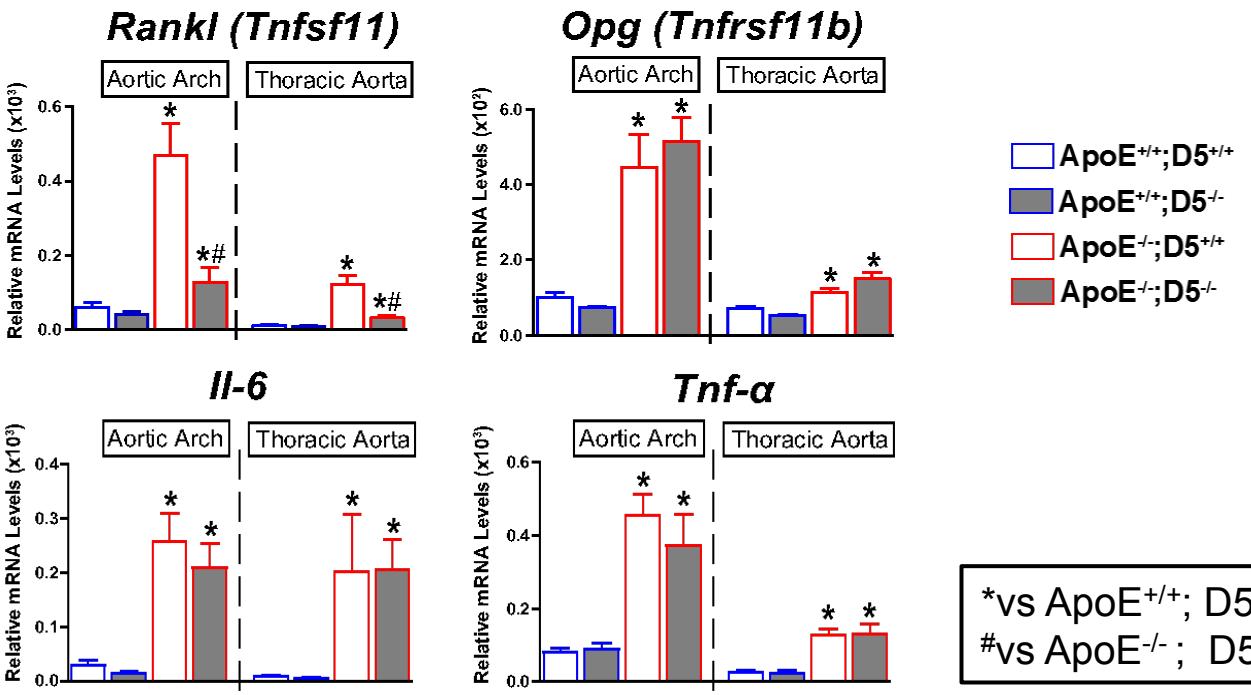
Phenotype

- **Δ RL-P1 (-500 b to -7 kb):** No effect on regulatory expression of Rankl
- **Δ RL-D2:** Reduces expression of Rankl in mesenchymal cells, limits regulation by PTH and induces age-related osteopetrosis
- **Δ RL-D5:** Reduces Rankl expression in mesenchymal and hematopoietic cells, limits regulation by PTH and $1,25(OH)_2D_3$ and induces age-related osteopetrosis
- **Δ RL-D6:** Limits mesenchymal response to inflammatory cytokines with no skeletal phenotype
- **Δ RL-T1:** Prevents Rankl expression in hematopoietic but not skeletal cells

Onal et al. J. Bone Miner. Res. 30: 855 (2015); Onal et al. J. Bone Miner. Res. 31:416 (2016)
Onal et al. Endocrinol. 157:482 (2016)

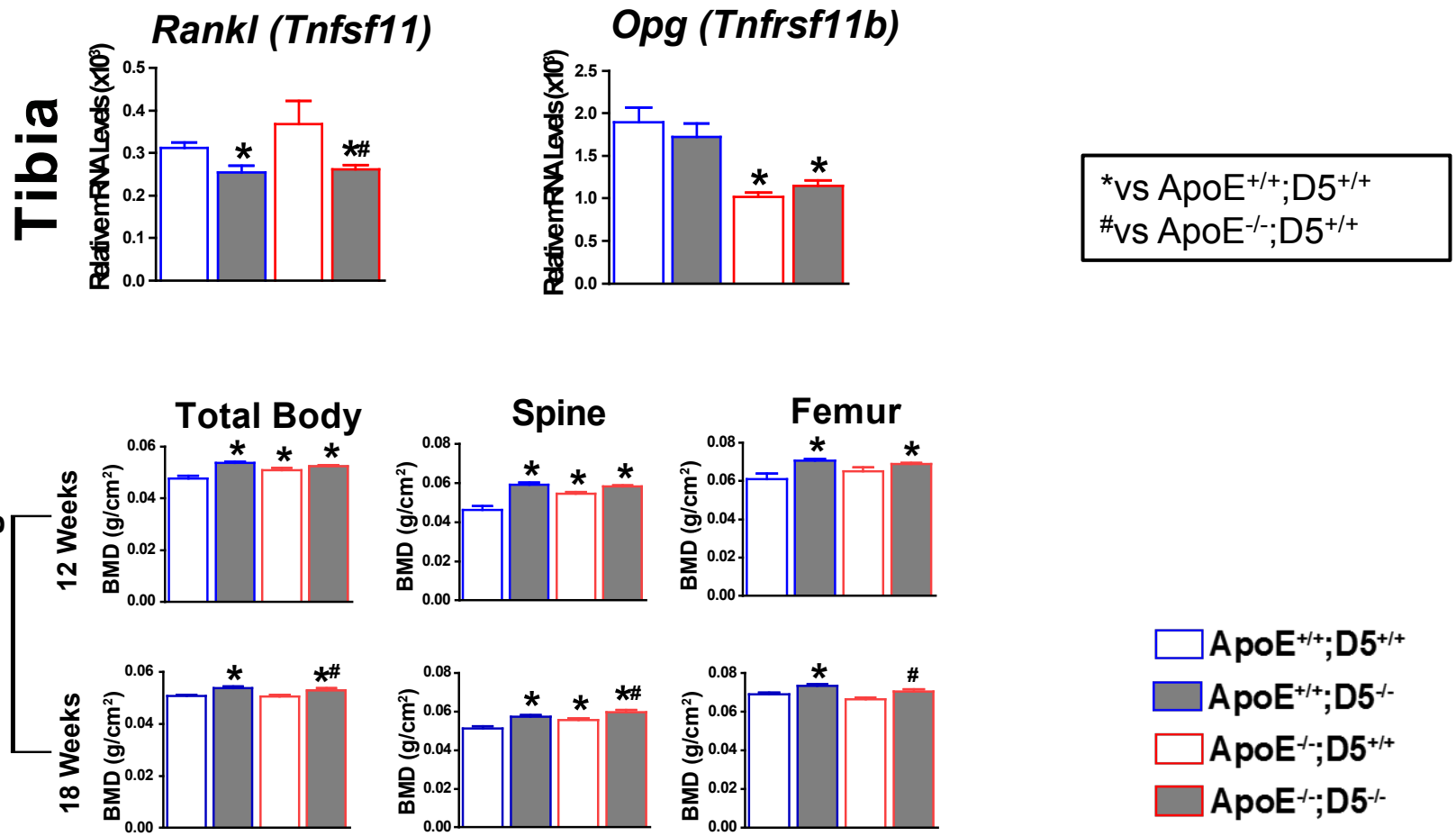
High RANKL Expression in Atherosclerotic Plaques is Compromised in RL-D5 Enhancer Deleted ApoE-null Mice



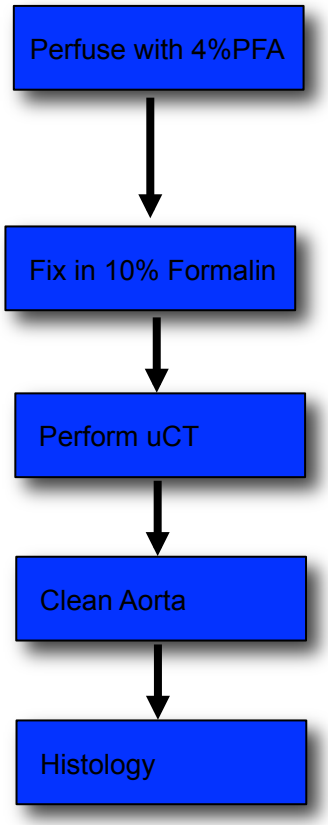


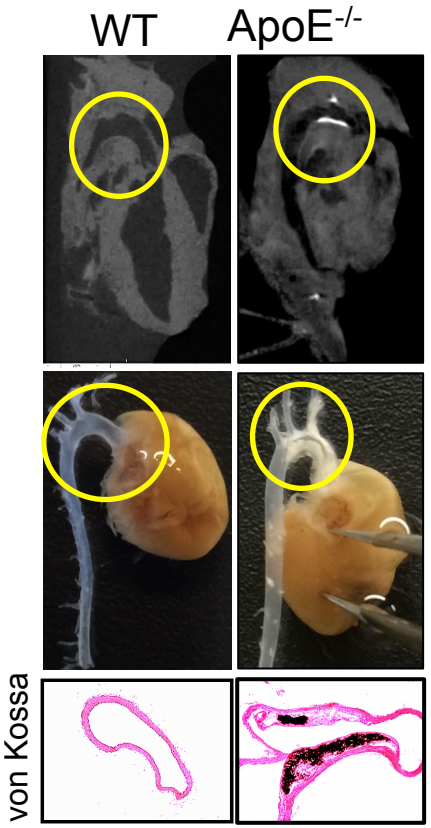
Shamsuzzaman et al. 2016

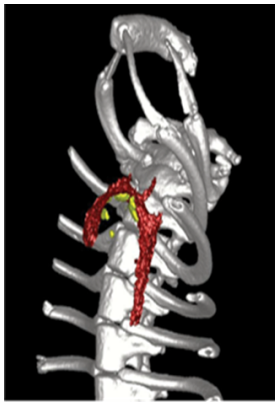
Deletion of the RANKL RL-D5 Enhancer Induces Osteopetrosis in Mice



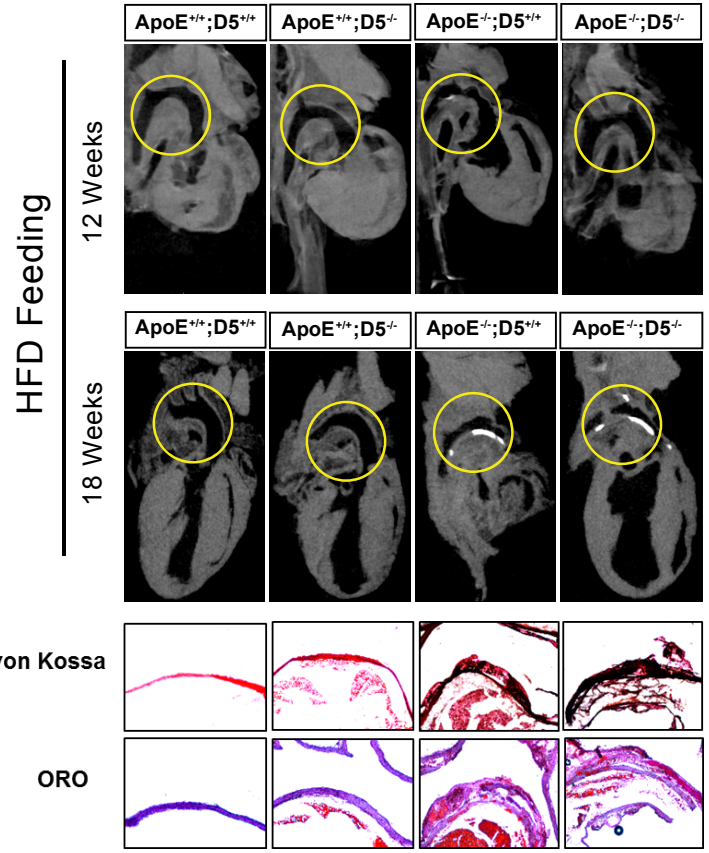
Analysis of Atherosclerotic Plaques by µCT







Reduced RANKL Expression in the Atherosclerotic Plaques of RL-D5 Enhancer Deleted Mice Delays the Progression of Calcification



	Genotype	Frequency of Calcification				Presence of Fatty Streak			
		ApoE+/+;D5+/+	ApoE+/+;D5-/-	ApoE-/-;D5+/+	ApoE-/-;D5-/-	ApoE+/+;D5+/+	ApoE+/+;D5-/-	ApoE-/-;D5+/+	ApoE-/-;D5-/-
HFD Feeding	12 Weeks	0% (0/10)	0% (0/8)	75% (6/8)	12.5% (1/8)	0% (0/10)	0% (0/8)	100% (8/8)	100% (8/8)
	18 Weeks	0% (0/10)	0% (0/7)	100% (8/8)	100% (8/8)	0% (0/10)	0% (0/7)	100% (8/8)	100% (8/8)

CONCLUSION

RANKL plays a significant role in atherosclerotic plaque calcification, perhaps by promoting bone formation

So What Have We learned About Enhancers?

- Located distal to, yet interact collectively at promoters
- Integrate multiple incoming signals at genes through modular and often hierarchical mechanisms
- Are highly dynamic during differentiation and disease
- Retain temporal, tissue- and hormone-specific expression properties in vivo
- Are active in disease settings, often in a unexpected manner
- Provide the mechanistic environment for the selective activity of SNPs that cause gene mis-expression
- May represent highly selective approaches for therapeutic targets