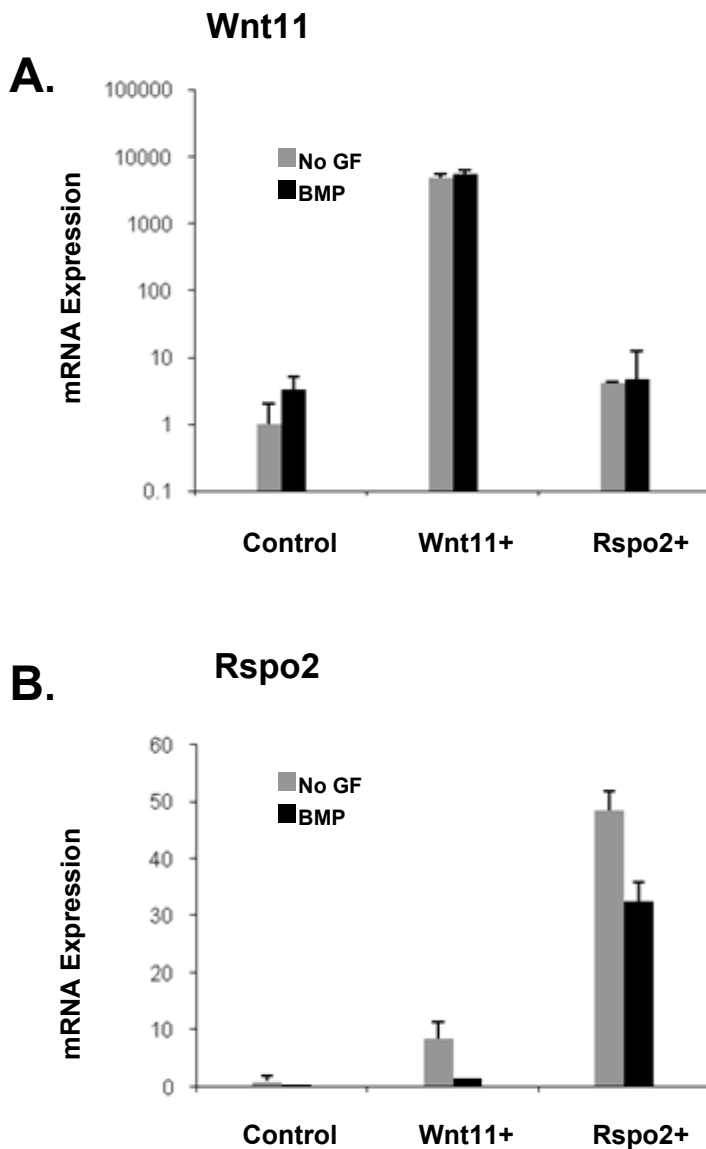


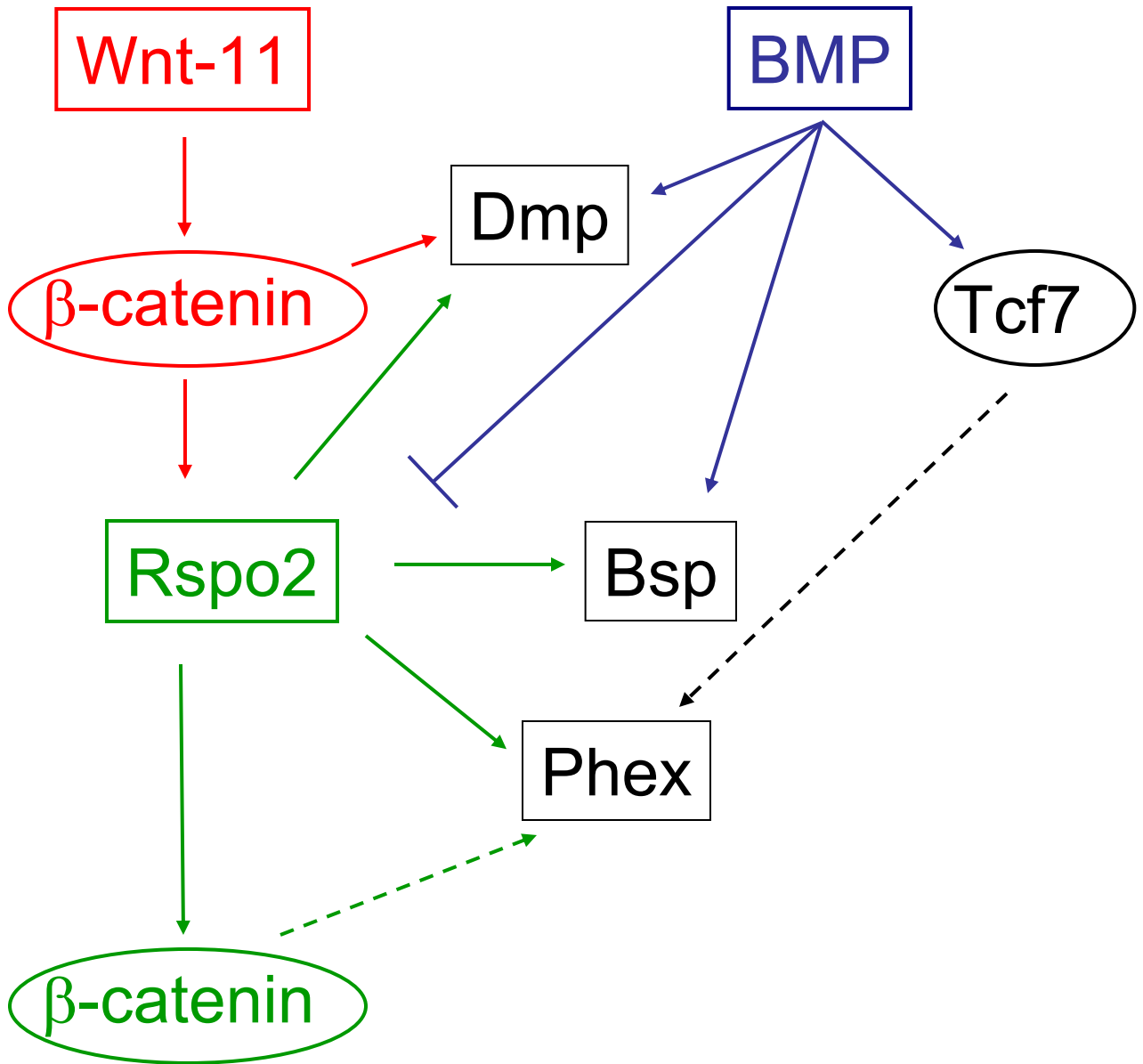
# Friedman et al. supplementary data



**Figure S1. Wnt11 and Rspo2 expression in control and retrovirally infected cells.** MC3T3 control (GFP), Wnt11+, or Rspo2+ cells were placed in osteogenic conditions with or without BMP. The cells were harvested for RNA after nine days of osteo-induction. Gene expression was subsequently analyzed by Q-RT-PCR. **A.** Wnt11. **B.** Rspo2. Expression values represent fold change in gene expression relative to non-BMP treated controls, normalized to beta-actin.

-1.5 kb      cacttttCAAAGttggg  
-1 kb        ggaaaaaCAAAGccaag  
-.9 kb        gtgctaaCAAAGggccc

**Figure S2. Subsequence analysis of the Rspo2 promoter.** Genomatix program was used to analyze a 2 Kb region of the Rspo2 promoter including part of the first exon. Three Tcf/Lef consensus sites were identified.



**Figure S3. Model of Wnt11/Rspo2 mediated osteoblast differentiation.** In this model BMP increases the expression of TCF7, DMP, and BSP and inhibits Rspo2 expression. Wnt11 increases nuclear accumulation of beta-catenin, which is required for increased Rspo2 expression. Coordinated BMP-Wnt signaling, possibly through Tcf7-beta-catenin in addition to other BMP regulated pathways (Smad/MAPK), is required for increased DMP. Rspo2 on the other hand can directly increase DMP, BSP, and Phex gene expression and increase beta-catenin nuclear accumulation. This beta-catenin signaling may partially influence Phex expression, which requires BMP (Tcf7). These results suggest that Phex and BSP are two factors that could be required for the enhanced mineralization promoted by Wnt11 through Rspo2.

Table S1A. BMP Regulated Transcription Factors, ECM, Proteoglycans

Name	Gene Title	Description
<b>Transcription Factors Increased</b>		
Id1	U43884	inhibitor of DNA binding 1
Junb	NM_008416	Jun-B oncogene
Tcf7	AI323642	transcription factor 7, T-cell specific
klf4	BG069413	Kruppel-like factor 4 (gut)
Dlx3	U79738	distal-less homeobox 3
Tcf7	NM_009331	transcription factor 7, T-cell specific
Klf10	NM_013692	Kruppel-like factor 10
Sp7	NM_130458	trans-acting transcription factor 7, Osterix
Tbx2	NM_009324	T-box 2
Rcor2	NM_054048	REST corepressor 2
<b>Transcription Factors Decreased</b>		
Id4	BB121406	inhibitor of DNA binding 4
<b>ECM, Proteoglycans Increased</b>		
Lepre1	NM_019783	leprecan 1
Hapln1	NM_013500	hyaluronan and proteoglycan link protein 1
Cspg4	BB377873	chondroitin sulfate proteoglycan 4
Ibsp	L20232	integrin binding sialoprotein
Col2a1	NM_031163	procollagen, type II, alpha 1
Bglap1	NM_007541	bone gamma carboxyglutamate protein 1
Gpc1	NM_016696	glypican 1
P4ha2	1417149_at	procollagen-proline, 2-oxoglutarate 4- dioxygenase (proline 4-hydroxylase), alpha II polypeptide
Loxl4	NM_053083	lysyl oxidase-like 4
Col11a2	AV242706	procollagen, type XI, alpha 2
<b>ECM, Proteoglycans Decreased</b>		
Otor	AF243504	otoraplin, cartilage-derived retinoic acid sensitive protein
Col8a1	NM_007739	procollagen, type VIII, alpha 1

**Table S1A. BMP regulated expression of transcription factors, ECM, and proteoglycans in pre-osteoblasts.** RNA was harvested at days 3, 6, and 9 after the start of osteo-induction. cRNA samples were hybridized to an Affymetrix 420A 2.0 microarray chip. Only genes showing an expression level  $\geq 400$  with an interquartile range  $>4$  were evaluated. The genes represented in this table showed increased or decreased expression at day 9 in BMP treated cells relative to non-BMP treated GFP controls.

Table S1B. BMP Regulated Receptors and Signal Transduction Molecules

Name	Gene Title	Description
<b>Receptors Increased</b>		
Ly6a	BC002070	lymphocyte antigen 6 complex, locus A, Sca-1
Unc5b	BG065285	unc-5 homolog B ( <i>C. elegans</i> )
Tnfrsf11b	AB013898	tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin)
Lifr	D17444	leukemia inhibitory factor receptor
Bambi	AF153440	BMP and activin membrane-bound inhibitor, homolog ( <i>Xenopus laevis</i> )
Vldlr	NM_013703	very low density lipoprotein receptor
Pthr1	BC013446	parathyroid hormone receptor 1
Vdr	AV290079	vitamin D receptor phosphate regulating gene with homologies to endopeptidases on the X chromosome
Phex	NM_011077	
<b>Receptors Decreased</b>		
Cd34	NM_133654	CD34 antigen
<b>Signal Transduction Increased</b>		
Rasa3	NM_009025	RAS p21 protein activator 3
Rras2	NM_025846	related RAS viral (r-ras) oncogene homolog 2
Rassf5	NM_018750	Ras association (RalGDS/AF-6) domain family 5
Ramp1	NM_016894	receptor (calcitonin) activity modifying protein 1
Plcd1	NM_019676	phospholipase C, delta 1
Rab27b	BB121269	RAB27b, member RAS oncogene family
Jak2	NM_008413	Janus kinase 2
Gpr133	BB548889	G protein-coupled receptor 133
Gpr35	NM_022320	G protein-coupled receptor 35
Trib3	BB508622	tribbles homolog 3 ( <i>Drosophila</i> )
<b>Signal Transduction Decreased</b>		
Rhoj	AF309564	ras homolog gene family, member J
Rgs2	AF215668	regulator of G-protein signaling 2

**Table S1B. BMP regulated expression of receptors and signal transduction molecules in pre-osteoblasts.** RNA was harvested at days 3, 6, and 9 after the start of osteo-induction. cRNA samples were hybridized to an Affymetrix 420A 2.0 microarray chip. Only genes showing an expression level  $\geq 400$  with an interquartile range  $>4$  were evaluated. The genes represented in this table showed increased or decreased expression at day 9 in BMP treated cells relative to non-BMP treated GFP controls.

Table S2. Wnt-11 Regulated Transcription Factors, ECM, Proteoglycans

Name	Gene Title	Description
<b>Transcription Factors Increased</b>		
Sox6	AJ010605	SRY-box containing gene 6
Ebf1	BB125261	early B-cell factor 1
<b>ECM, Proteoglycans Increased</b>		
Pcolce2	AF352788	procollagen C-endopeptidase enhancer 2
Hapln1	NM_013500	hyaluronan and proteoglycan link protein 1
Otor	AF243504	otoraplin, cartilage-derived retinoic acid sensitive protein
Dmp1	U65020	dentin matrix protein 1
Phex	U73910	phosphate regulating gene with homologies to endopeptidases on the X chromosome (hypophosphatemia, vitamin D resistant rickets)
<b>Secreted Factors Increased</b>		
Wif1	BC004048	Wnt inhibitory factor 1
Igf1	NM_010512	insulin-like growth factor 1
Rspo2	BG067392	R-spondin 2 homolog ( <i>Xenopus laevis</i> )
Lect1	NM_010701	leukocyte cell derived chemotaxin 1
<b>Receptors Increased</b>		
Ly6a	BC002070	lymphocyte antigen 6 complex, locus A, Sca-1
Vdr	AV290079	vitamin D receptor
Ly6c	NM_010741	lymphocyte antigen 6 complex, locus C
Itga6	BM935811	integrin alpha 6
Phex	NM_011077	phosphate regulating gene with homologies to endopeptidases on the X chromosome
<b>Signal Transduction Increased</b>		
Camk2b	BG862223	calcium/calmodulin-dependent protein kinase II, beta

**Table S2. Wnt11 regulated gene expression.** RNA was harvested at days 3, 6, and 9 after the start of osteo-induction. cRNA samples were hybridized to an Affymetrix 420A 2.0 microarray chip. Only genes showing an expression level  $\geq 400$  with an interquartile range  $>4$  were evaluated. The genes represented in this table showed increased or decreased expression at day 9 in non-BMP treated Wnt11+ cells relative to non-BMP treated controls.

Table S3 A. BMP+Wnt-11 Regulated Transcription Factors, ECM, Proteoglycans, and Secreted Factors

Name	Gene Title	Description
<b>Transcription Factors Increased</b>		
Hmga1	NM_016660	high mobility group AT-hook 1
Egr2	X06746	early growth response 2
Junb	NM_008416	Jun-B oncogene
Foxc2	NM_013519	forkhead box C2
Fosl1	U34245	fos-like antigen 1
Nfatc1	NM_016791	nuclear factor of activated T-cells,
<b>ECM, Proteoglycans Increased</b>		
Dmp1	U65020	dentin matrix protein 1
Tnfrsf25	NM_009398	tumor necrosis factor alpha induced protein 6
Ereg	NM_007950	epiregulin
Otor	AF243504	otoraplin
<b>ECM, Proteoglycans Decreased</b>		
Aspn	NM_025711	asporin
<b>Secreted Factors Increased</b>		
Ctgf	NM_010217	connective tissue growth factor
Il12a	AF128210	interleukin 12a
Adm	NM_009627	adrenomedullin
Bmp8a	NM_007558	bone morphogenetic protein 8a
Rspo2	BG067392	R-spondin 2 homolog ( <i>Xenopus laevis</i> )
<b>Secreted Factors Decreased</b>		
Igfbp5	BF225802	insulin-like growth factor binding protein 5

Table S3 B. BMP+Wnt-11 Regulated Receptors and Signal Transduction Molecules

Name	Gene Title	Description
<b>Receptors Increased</b>		
Ank	NM_020332	progressive ankylosis phosphate regulating gene with homologies to endopeptidases on the X chromosome
Phex	NM_011077	homologies to endopeptidases on the X chromosome
<b>Receptors Decreased</b>		
Ptgfr	NM_008966	prostaglandin F receptor
<b>Signal Transduction Increased</b>		
Rgs4	NM_009062	regulator of G-protein signaling 4
Spry2	BB529691	sprouty homolog 2 ( <i>Drosophila</i> )
Rab27b	BB121269	RAB27b, member RAS oncogene family
Ptgs2	M94967	prostaglandin-endoperoxide synthase 2
Gprc5b	BC020004	G protein-coupled receptor, family C, group 5, member B
Rgs16	U94828	regulator of G-protein signaling 16
<b>Signal Transduction Decreased</b>		
Ddit4	AK017926	DNA-damage-inducible transcript 4/REDD1
Plekha5	1425543_s_at	pleckstrin homology domain containing, family A member 5
Osr1	NM_011859	odd-skipped related 1 ( <i>Drosophila</i> )

**Table S3. Genes showing additive or synergistic changes in expression with Wnt11 and BMP** Microarray analysis was performed and analyzed as previously mentioned. The genes represented in this table showed increased or decreased expression at day 9 in BMP treated Wnt11+ cells relative to BMP treated controls.