

# **Common and rare variants of *WNT16*, *DKK1* and *SOST* and their relationship with bone mineral density**

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**Supplementary Table S1.** Variants that affect the binding of miRNA at least in 3 different miRNA databases (MiRNSP; miRNA-SNP; SNP Function Prediction; MicroSNiPer; miRdSNP; miRTarBase).

Gene	Variant	miRNA	Gain/loss	Log FC #	Adj. p-val #
WNT16	rs17143305	hsa-miR-541	Gain	*	Ns
		hsa-mir4263	Gain	*	Ns
	rs190011371	hsa-miR-383	Loss	-0.527	<b>0.00408</b>
SOST	rs17883310	hsa-miR-1915-3p	Loss	1.58	<b>0.00104</b>
	rs17886183	hsa-miR-5583	Gain	*	Ns
		hsa-miR-98-5p	Loss	*	Ns
	rs75901553	hsa-miR-3190	Gain	*	Ns
		hsa-miR-let-7a (and b.c.d.f.g)	Loss	-1.819	<b>0.007</b>

Gain: Minor allele creates a new binding site for the miRNA

Loss: Minor allele abolish the binding site for the miRNA

# Data from De Ugarte et al. (2015)<sup>1</sup>. Data not shown in the publication.

logFC: Logarithm (Osteoporotic/Non-osteoporotic)

\* logFC < |0.5|; Ns < 0.05

**Supplementary Table S2.** GTEx eQTL data for all the SNVs found in the resequencing of *WNT16*, *DKK1* and *SOST*

SNP	Gene	p value	NES	Tissue
<i>WNT16</i>				
rs17143281	<i>FAM3C</i>	1.3e-11	0.65	Muscle - Skeletal
rs55710688	<i>FAM3C</i>	0.000020	0.20	Skin - Not Sun Exposed (Suprapubic)
		0.000016	0.18	Skin - Sun Exposed (Lower leg)
	<b><i>WNT16</i></b>	0.000019	0.29	Adipose - Subcutaneous
rs2908004	<i>CPED1</i>	0.000029	0.097	Artery - Tibial
	<i>FAM3C</i>	0.000086	-0.15	Skin - Sun Exposed (Lower leg)
rs142005327	<i>FAM3C</i>	5.9e-8	0.22	Skin - Not Sun Exposed (Suprapubic)
		9.5e-7	0.20	Skin - Sun Exposed (Lower leg)
		0.000032	0.13	Nerve - Tibial
	<i>CPED1</i>	0.000029	0.11	Artery - Tibial
rs2707466	<i>FAM3C</i>	0.000050	-0.15	Skin - Sun Exposed (Lower leg)
	<i>CPED1</i>	0.000058	0.094	Artery - Tibial
rs17143305	<i>RP11-3L10.1</i>	7.5e-14	0.87	Testis
	<i>FAM3C</i>	2.9e-29	-0.52	Skin - Sun Exposed (Lower leg)
		5.9e-26	-0.51	Skin - Not Sun Exposed (Suprapubic)
		2.7e-8	-0.21	Nerve - Tibial
		4.6e-8	-0.22	Thyroid
		7.8e-8	-0.27	Stomach
		8.8e-8	-0.34	Esophagus - Mucosa
		1.0e-7	-0.42	Adrenal Gland
		1.0e-7	-0.29	Muscle - Skeletal
		1.1e-7	-0.77	Brain - Spinal cord (cervical c-1)
		4.8e-7	-0.20	Adipose - Subcutaneous
		9.5e-7	-0.31	Brain - Anterior cingulate cortex (BA24)
		0.000028	-0.35	Pancreas
		0.000031	-0.21	Adipose - Visceral (Omentum)
		0.000032	-0.24	Breast - Mammary Tissue
		0.000058	-0.17	Artery - Tibial
	0.000087	-0.18	Esophagus - Muscularis	
	0.000022	-0.28	Brain - Frontal Cortex (BA9)	
	0.000027	-0.30	Brain - Cortex	
<i>DKK1</i>				
rs41281546	<b><i>DKK1</i></b>	8.6e-7	-0.29	Cells - Transformed fibroblasts
rs1569198	<i>PRKG1-AS1</i>	0.000047	0.16	Cells - Transformed fibroblasts
rs74711339	<b><i>DKK1</i></b>	1.3e-7	-0.35	Cells - Transformed fibroblasts
<i>SOST</i>				
rs1237278	<b><i>SOST</i></b>	8.9e-18	-0.26	Artery - Tibial
		3.6e-9	-0.61	Brain - Cortex
		5.4e-9	-0.27	Artery - Aorta
		1.1e-7	-0.43	Artery - Coronary
		0.000024	-0.28	Heart - Atrial Appendage
		0.000013	0.22	Thyroid
rs851058	<i>MPP3</i>	0.000063	-0.17	Cells - Transformed fibroblasts
	<b><i>SOST</i></b>	3.4e-20	-0.28	Artery - Tibial
		1.1e-10	-0.29	Artery - Aorta
		1.2e-8	-0.47	Artery - Coronary
		4.2e-7	-0.35	Heart - Atrial Appendage
rs2023794	<i>DUSP3</i>	0.000069	0.10	Cells - Transformed fibroblasts
	<i>DUSP3</i>	6.0e-7	0.29	Cells - Transformed fibroblasts
	<i>MPP2</i>	0.000022	0.46	Skin - Not Sun Exposed (Suprapubic)
		0.000048	0.50	Skin - Sun Exposed (Lower leg)

NES: Normalized Effect Size

**Supplementary Table S3.** Minor allele frequency (MAF) from 1000 genomes project: total population (ALL), european population (EUR) and Iberian population in Spain (IBS) and the complete BARCOS cohort frequency.

SNP	Minor allele	ALL	EUR	IBS	BARCOS cohort
<b><i>WNT16</i></b>					
rs55710688	CCCA	0.252	0.233	0.276	0.257
rs2908004	A	0.510	0.443	0.416	0.445
rs142005327	CT	0.257	0.254	0.29	0.28
rs113001389	A	0.004	0	0	0.0007
rs2707466	T	0.503	0.440	0.407	0.463
rs190011371	C	0.001	0	0	0.002
<b><i>DKK1</i></b>					
rs1569198	A	0.682	0.507	0.477	0.468
rs74711339	G	0.019	0.043	0.033	0.057
<b><i>SOST</i></b>					
rs1237278	C	0.436	0.355	0.425	0.392
rs2023794	C	0.074	0.044	0.037	0.056
rs570754792	A	0.0004	0.002	0.005	0.001
rs17882143	T	0.007	0.018	0.033	0.023
rs17883310	A	0.009	0.013	0.023	0.018

**Supplementary Table S4.** Effect size of the associated variants

SNP	Position	Type	Effect size ( $\beta$ and OR)	
			LS-BMD	FN-BMD
<i>WNT16</i>				
rs55710688	p.Met1? g.120965467_120965470dupCCCA	Fs 5'UP	0.018 (0.006, 0.031) 1.019 (1.006, 1.031)	0.009 (0.0001, 0.019) 1.01 (1.0001, 1.019)
rs2908004	p.G72R/p.G82R	M	0.012 (0.0015, 0.023) 1.012 (1.001, 1.023)	
rs142005327	c.346+103_104dupCT c.316+103_104dupCT	I	0.018 (0.006, 0.03) 1.018 (1.006, 1.031)	0.011 (0.002, 0.02) 1.011 (1.002, 1.02)
rs2707466	p.T253I/p.T263I	M	0.014 (0.002, 0.026) 1.014 (1.002, 1.026)	0.01 (0.002, 0.019) 1.01 (1.002, 1.019)
rs3801387 <sup>#</sup>	c.603+2747A>G c.633+2747A>G	I	0.017 (0.005, 0.03) 1.017 (1.005, 1.03)	0.01 (0.0004, 0.019) 1.01 (1.0004, 1.019)
<i>DKK1</i>				
rs1569198	c.548-43A>G	I		0.011 (0.003, 0.019) 1.011 (1.003, 1.019)
<i>SOST</i>				
rs17882143	p.Val10Ile	M	<u>0.041 (0.003, 0.079)</u> <u>1.042 (1.003, 1.082)</u>	

Underline and Italics  $\beta$  and OR under dominant model

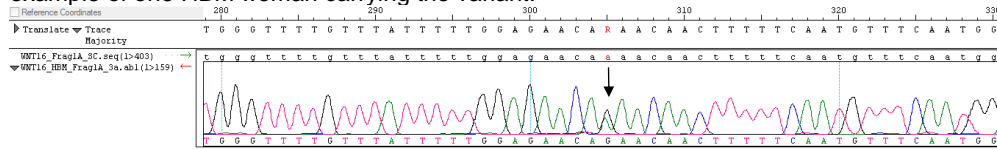
<sup>#</sup> SNP genotyping previously in BARCOS (Estrada *et al.* (2012)<sup>2</sup>).

**Supplementary Table S5.** PCR conditions. Sequence of the primers (5'→3'), annealing temperature (°C), amount of magnesium (mM) and elongation time at 72°C (seconds).

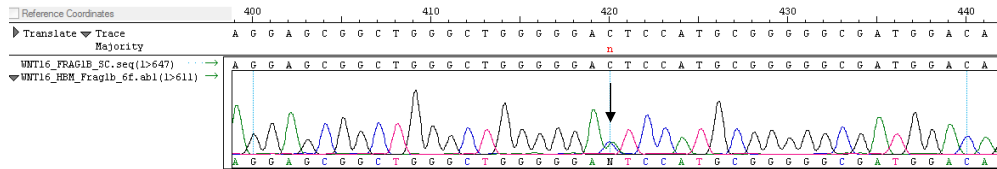
Primers	Sequence (5'→3')	T <sub>m</sub>	Mg <sup>2+</sup> (mM)	Elongation time (s)
WNT16-Frag1a-F	GGTAGCTCCAGTAAGAGATTC	62	2	15
WNT16-Frag1a-R	CAGATTACCGTGTCTTTGGGT			
WNT16-Frag1b-F	CGGAGCCGCTCTCCACCA	62	2	15
WNT16-Frag1b-R	ATTAGGTCACCTCGTCTAAGGG			
WNT16-Frag2-F	ACTTTCAACTGAGGCTGGGG	62	2	15
WNT16-Frag2-R	CTGGAAGTGGGGAGTCAGG			
WNT16-Frag3-F	CTTCCTTTCTAAATATGTAAGTCG	58	2	15
WNT16-Frag3-R	AGGGCTGCCAGTGTGGTT			
WNT16-Frag4-F1	TGGGACAAAAACCAAAGGACG	62	2	15
WNT16-Frag4-R1	TGACCACATGGGTGTTGTAAC			
WNT16-Frag4-F2	AGGATGATCTGCTCTATGTTAAT	62	2	15
WNT16-Frag4-R2	CCCACCATTATTGAGTCCTGT			
DKK1-Frag1-F	GCGCTGATCACAGTCCTTATC	63	2	15
DKK1-Frag1-R	TGCTATAACGCTCGCTGGTA			
DKK1-Frag2-F	GCAGTGGGCAGTAACAGGT	62	2.5	35
DKK1-Frag2-R	TGTATTGAATCATTGAGGGACA			
DKK1-Frag3-F	GAGGAAGTTTGGCTTGTGTTT	62	2	15
DKK1-Frag3-R	CGAAGGAGAAGACAGTAGGAAA			
DKK1-Frag4a-F	GAACCACCTTGTCTTCAAAAATG	63	1.5	20
DKK1-Frag4a-R	TCCAAGAGATCCTTGCGTTC			
DKK1-Frag4b-F	AGGTGCTGCACTGCCTATTT	63	1.5	20
DKK1-Frag4b-R	CCGTATCCTCATTCCAATCAA			
SOST_Frag0a_F	CCGAGTTGGGCAGATCACC	62	1.5	15
SOST_Frag0a_R	TTAATGCAGACGGTCCAGCC			
SOST_Frag0b_F	ACGCGTTCCAGGGATGAATC	62	1.5	15
SOST_Frag0b_R	GGCCAAGGCAGCATTTTCTC			
SOST_Frag1_F	GCTAGAGGAGAAGTCTTGG	62	2	10
SOST_Frag1_R	CCATTCTTCCCCACCTCC			
SOST_Frag2a_F	AGATGTTCAAGGGGCAAAAGC	63	2	30
SOST_Frag2a_R	GGAAGTCGGGCCCACTAG			
SOST_Frag2b_F	GTCACCGAGCTGGTGTGCT	63	2	30
SOST_Frag2b_R	CTCAGGGCCTGGAAGGTCT			
SOST_Frag3a_F	CTCAAGGACTTCGGGACCGA	62	2	15
SOST_Frag3a_R	AAATGAGGGTGGAGGTGG			
SOST_Frag3b_F	AGTCCTGGCTCTGCCACTAA	60	2	15
SOST_Frag3b_R	GGACACATTTCTGCCTAGAAAA			
SOST_Frag3c_F	GGGGGAAAAACTACAAGTGC	62	2	15
SOST_Frag3c_R	TCCTTTCCAAACCCAGACC			
ECR5_F	TCCTTGCCACGGGCCACCAGCTTT	62	2	5
ECR5_R	CCCCCTCATGGCTGGTCTCATTTG			

## WNT16

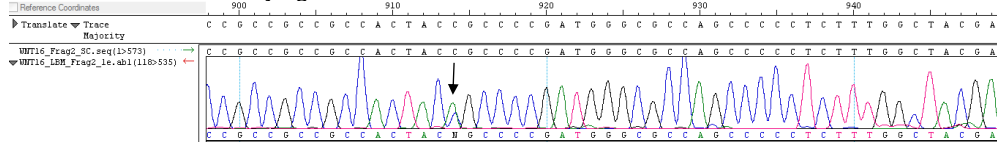
rs4727920; g.120965562A>G; c.65+28A>G (WNT16a) / g.120965562A>G (WNT16b);  
example of one HBM woman carrying the variant.



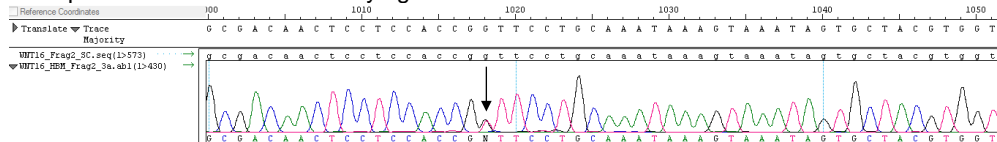
rs201022838; g.120969332C>A; c.66-289C>A (WNT16a) / c.-15C>A (WNT16b); example  
of the only HBM woman carrying the variant.



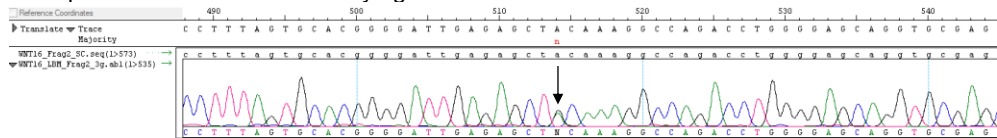
rs17143291; g.120969825C>A; p.Thr90Thr (WNT16a) / p.Thr100Thr (WNT16b); example  
of one LBM woman carrying the variant.



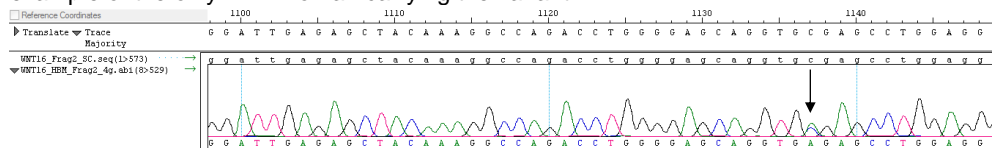
rs147496912; g.120969929G>T; c.316+58G>T (WNT16a) / c.346+58G>T (WNT16b);  
example of one HBM woman carrying the variant.



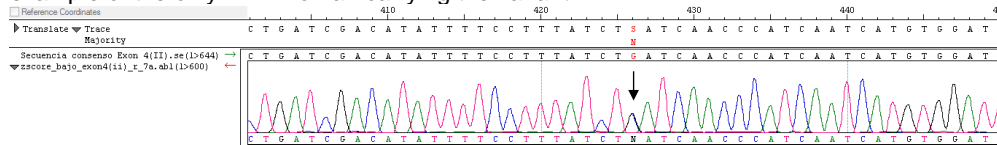
rs140239870; g.120970018A>G; c.316+147A>G (WNT16a) / c.346+147A>G (WNT16b);  
example of one LBM woman carrying the variant.



rs113001389; g.120970045C>A; c.316+174C>A (WNT16a) / c.346+174C>A (WNT16b);  
example of the only LBM woman carrying the variant.



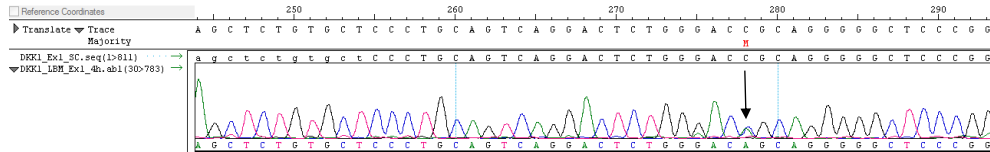
rs190011371; g.120979568G>C; g.120979568G>C (WNT16a) / c.\*169G>C (WNT16b);  
example of the only LBM woman carrying the variant.



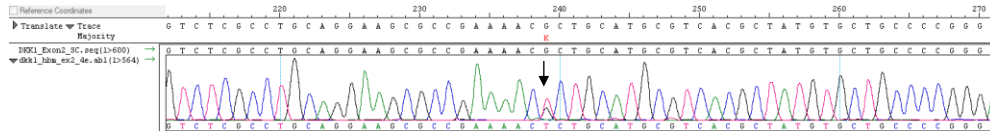
**Supplementary Figure S1.** Examples of DNA sequence chromatograms from various individuals, each containing one of the *WNT16* rare variants described in this paper. In all cases, the variants are in heterozygosis and are signaled by arrows.

## DKK1

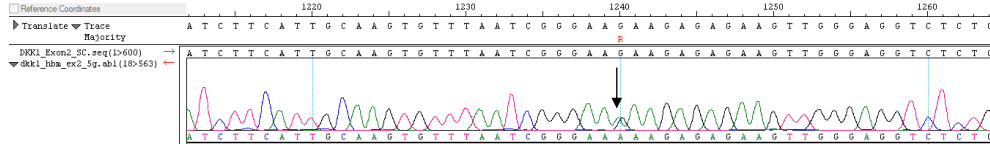
rs540255939; g.54074079C>A; c.-116C>A; example of the only LBM woman carrying the variant.



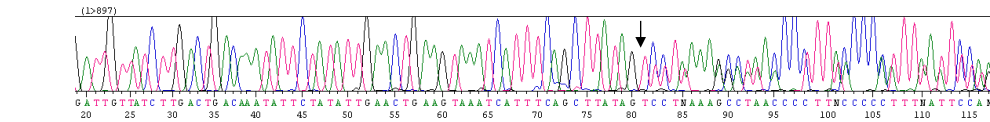
rs149268042; g.54074798G>T; p.Arg120Leu; example of the only HBM woman carrying the variant.



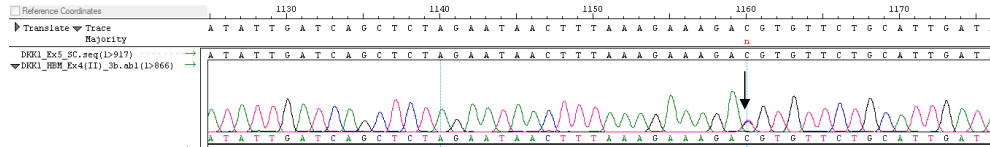
c.406+195G>A; g.54075040G>A; example of the only HBM woman carrying the variant.



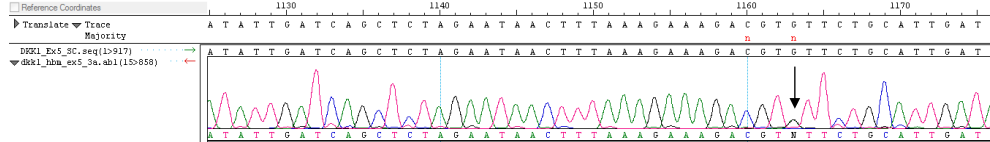
rs200054686; g.54076944delT; c.\*377delT, example of the only LBM woman carrying the variant.



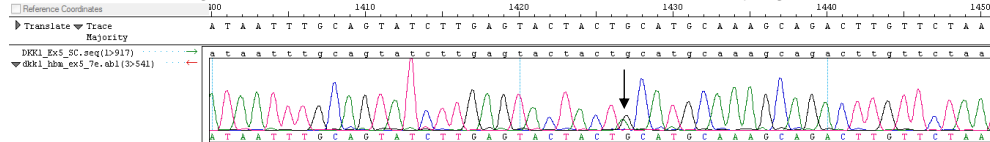
rs953208416; g.54077319C>T; c.\*752C>T, example of the only HBM woman carrying the variant.



rs79759877; g.54077322G>A; c.\*755G>A; example of the only HBM woman carrying the variant.



rs549135224; g.54077585G>A; example of one HBM woman carrying the variant.

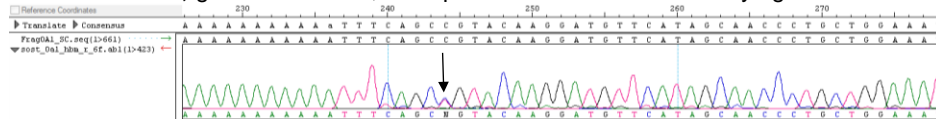


**Supplementary Figure S2.** Examples of DNA sequence chromatograms from various individuals, each containing one of the *DKK1* rare variants described in this paper. In all cases, the variants are in heterozygosis and are signaled by arrows. In the case of rs20054686, which is a heterozygous deletion, the whole chromatogram to the right of it is affected.

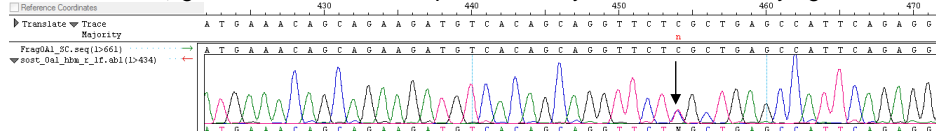


## SOST

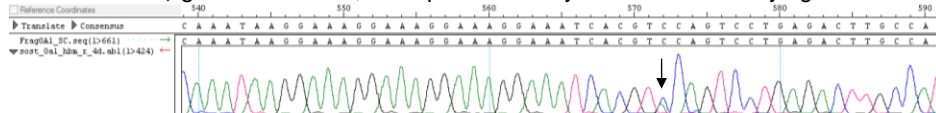
rs184269196; g.41838340G>A; example of one HBM woman carrying the variant.



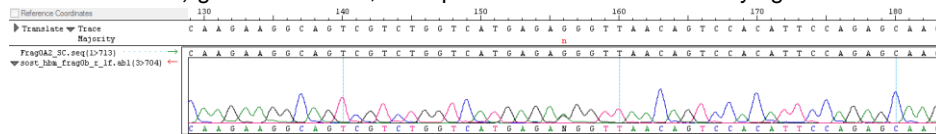
rs79715828; g.41838130G>A; example of the only HBM woman carrying the variant.



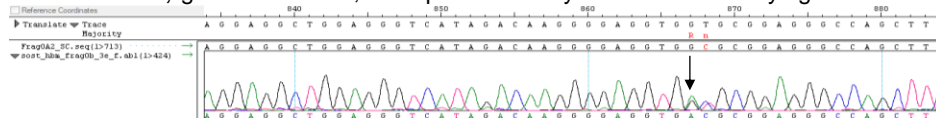
rs74252774; g.41838012G>T; example of the only HBM woman carrying the variant.



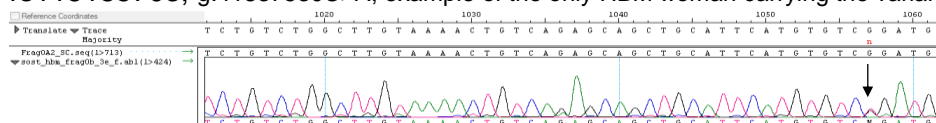
rs567865956; g.41837786C>T; example of one HBM woman carrying the variant.



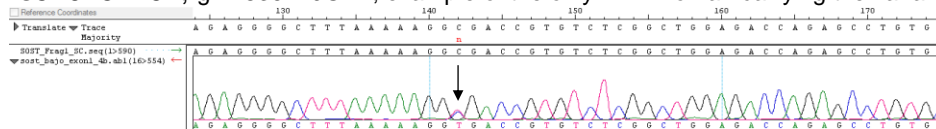
rs61105240; g.41837720C>T; example of the only HBM woman carrying the variant.



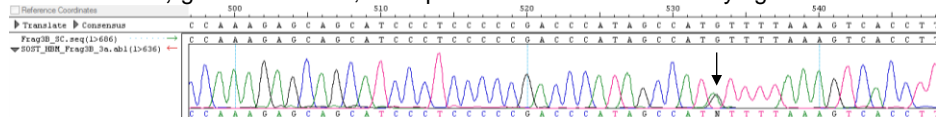
rs115185703; g.41837530C>A; example of the only HBM woman carrying the variant.



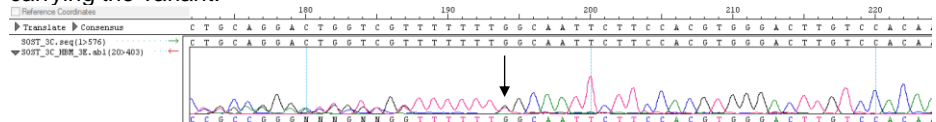
rs570754792; g.41836179G>A; example of the only LBM woman carrying the variant.



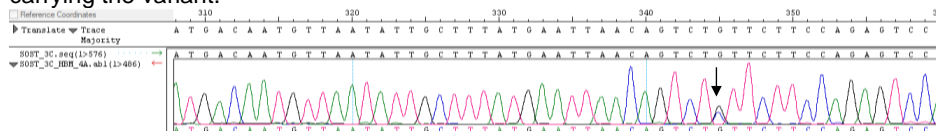
rs17886183; g.41831706C>T; example of one HBM woman carrying the variant.



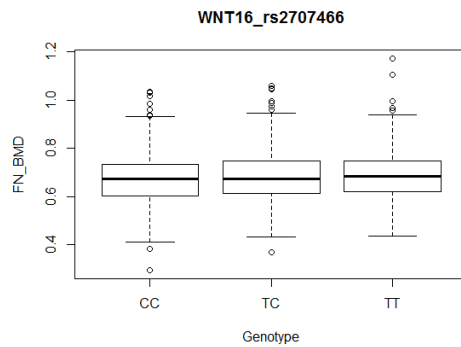
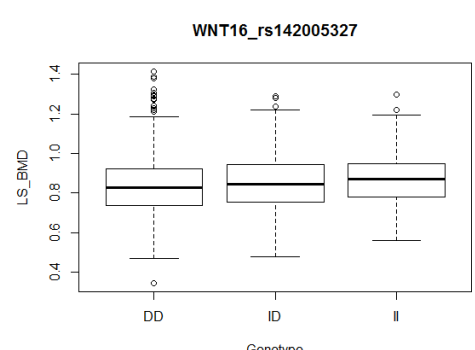
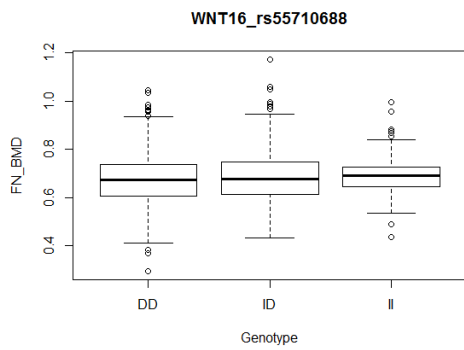
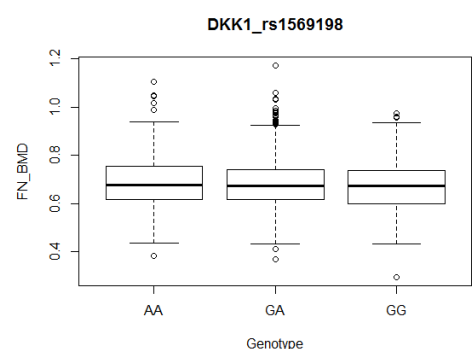
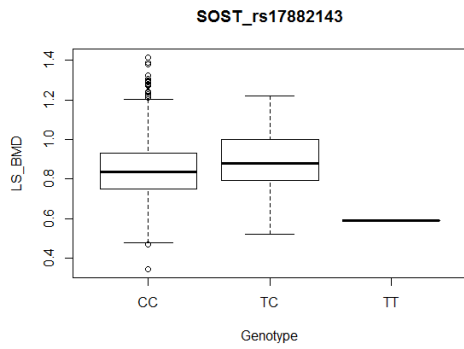
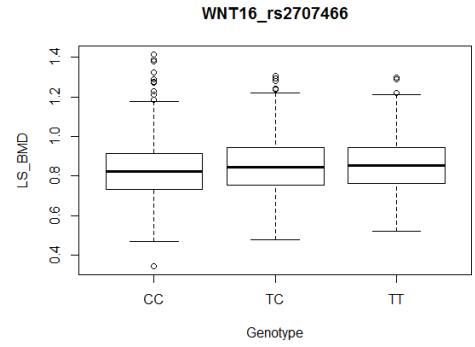
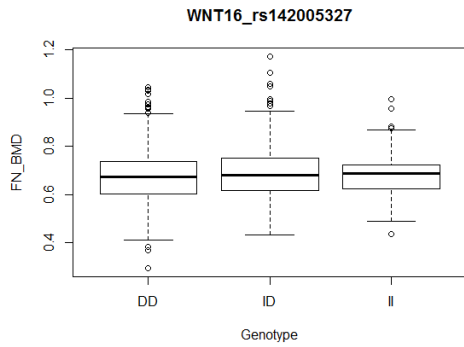
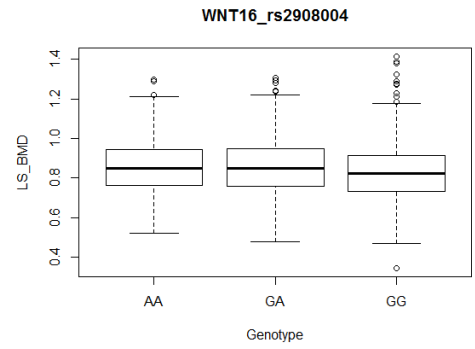
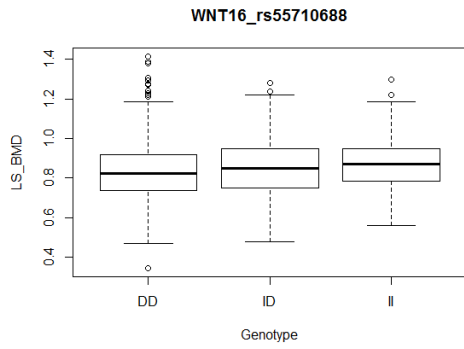
rs17885979; g.41831307delC; c.\*1404delG; example of the only HBM woman carrying the variant.



rs566556646; g.41831153C>G; c.\*1557G>C; example of the only HBM woman carrying the variant.



**Supplementary Figure S3.** Examples of DNA sequence chromatograms from various individuals, each containing one of the *SOST* rare variants described in this paper. In all cases, the variants are in heterozygosis and are signaled by arrows. In the case of rs17885979, which is a heterozygous deletion, the whole chromatogram to the right of it is affected.



**Supplementary Figure S4.** Boxplots for the nominally significant associated SNPs rs55710688, rs2908004, rs142005327, rs2707466, rs17882143, rs1569198 with LS-BMD or FN-BMD.

## References

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