

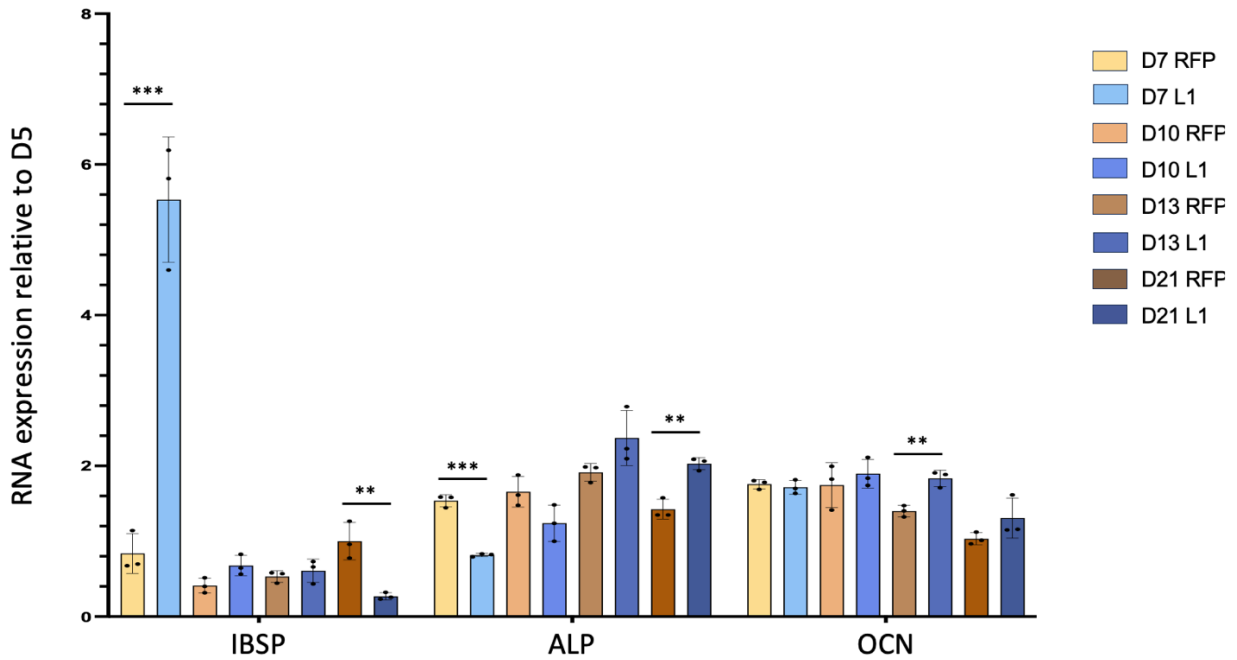
LINE-1 RNA triggers matrix formation in bone cells via a PKR-mediated inflammatory response

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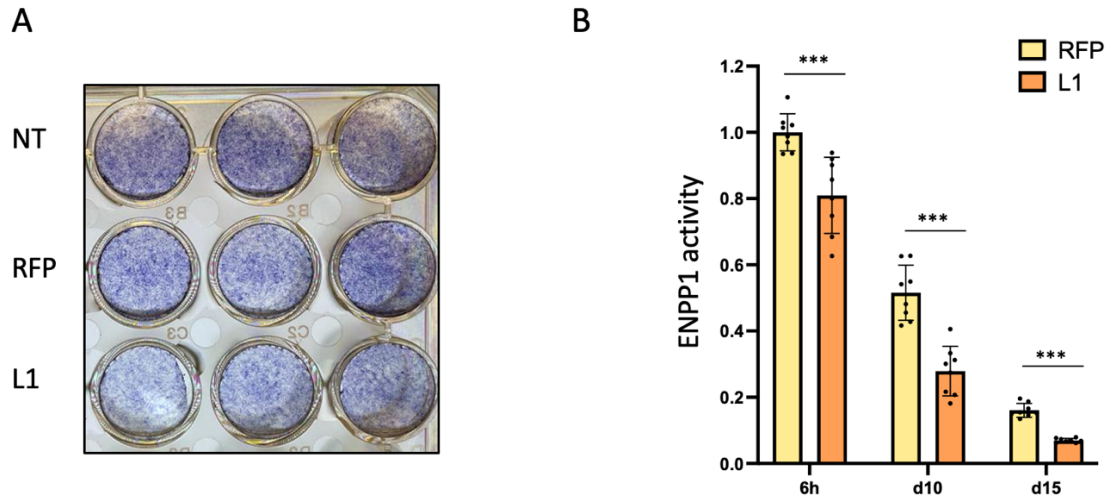
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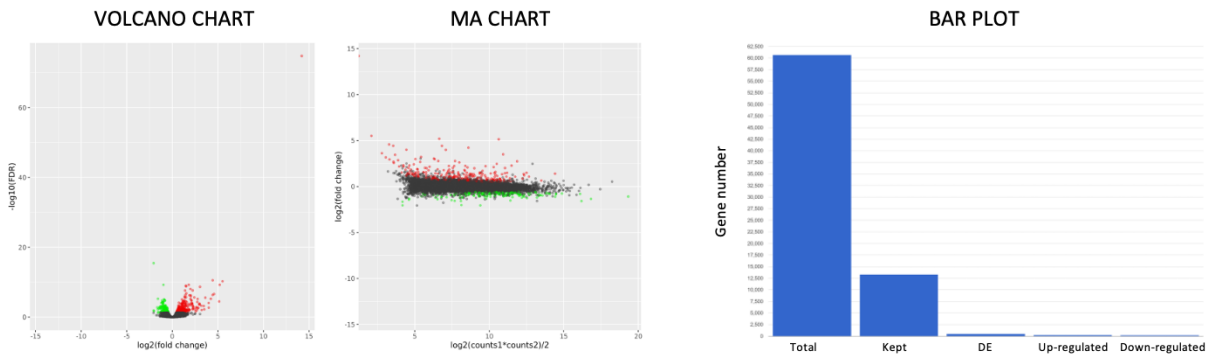
Appendix Figure S1: Timeline expression of late differentiation markers in L1 treated osteoblasts

qRT-PCR of osteogenic genes in RFP and L1 transfected osteoblasts at different time points of osteogenic differentiation. Expression level is normalized on day 5 (not transfected osteoblasts). The graph is shown as mean $\pm$ s.d. of $n = 3$ independent experiments. * $P < 0.05$; ** $P < 0.005$, *** $P < 0.0005$ in Student's t test.

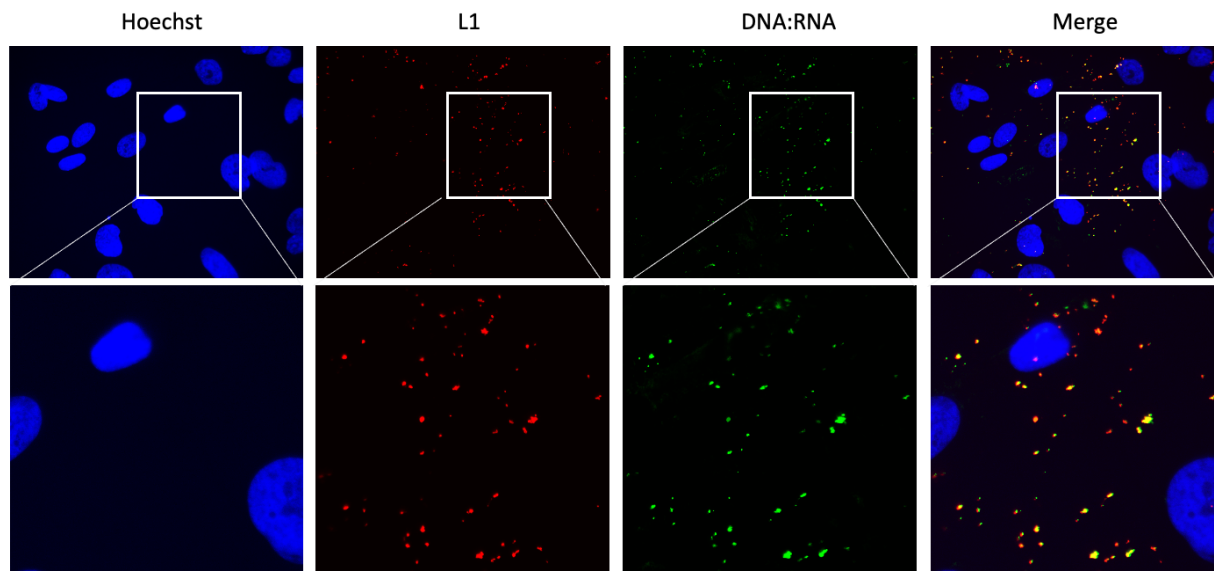


Appendix Figure S2: ALPL and ENPP1 activity in L1 treated osteoblasts

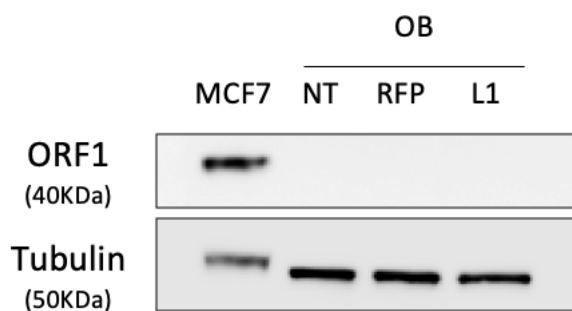
A) Colorimetric ALPL activity assay of osteoblasts 5 days post-L1 transfection. B) ENPP1 activity of osteoblasts 6h, 5 days (d10) and 10 days (d15) after RFP and L1 transfection. The graph is shown as mean $\pm$ s.d. of $n = 3$ independent experiments. *** $P < 0.0005$ in Student's t test.



Appendix Figure S3: Number of DEG between L1 and RFP (RNAseq data)

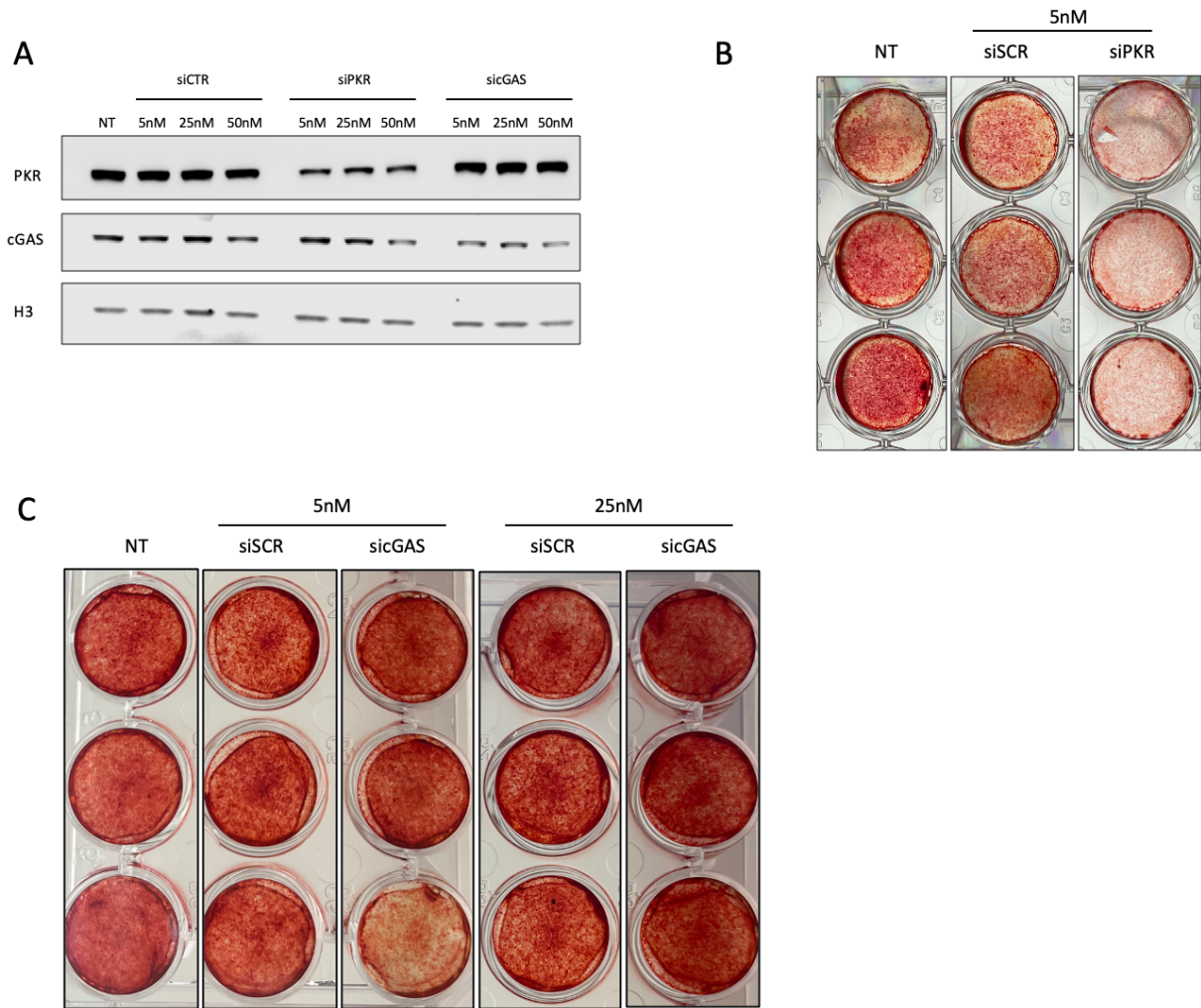


Appendix Figure S4: Colocalization between exogenous L1 RNA and DNA:RNA hybrid IF signal
 Immunofluorescence of DNA:RNA hybrids (green) in osteoblasts 24h after the transfection of cy5-coniugated L1 RNA (red).



Appendix Figure S5: ORF1p expression in L1 transfected cells

Western Blot of ORF1 in untransfected (NT), RFP- and L1-transfected differentiating osteoblasts (OB). Tubulin is used as endogenous calibrator. Human breast cancer cell line (MCF7) extract is used as positive control.



Appendix Figure S6: Effect of PKR and cGAS knockdown on L1 RNA-induced mineralization

A) Western Blot of PKR and cGAS in L1-expressing osteobalsts transfected with three different doses of siRNA. Histone H3 is used as endogenous calibrator. B) Alizarin red staining on L1-expressing osteoblasts transfected with a 5nM pool of PKR targeting siRNAs. C) Alizarin red staining on L1-expressing osteoblasts transfected with a 5nM and 25nM pool of cGAS targeting siRNAs.

Sample	Age	BMI	Sex	FN_TotTScore	group
LDS119A	62.3	32,1144642839595	F	0,85	normal
LDS121A	75.1	29,8287149306521	F	-0,50	normal
LDS122A	81.6	29,6296296296296	F	0,40	normal
LDS124A	52.4	28,7295259628216	F	0,20	normal
DH205A	76.4	26,2487601666336	M	-1,20	osteopenic
DH317A	72.2	24,0187668499002	F	-2,70	osteoporotic
LDS6A	74.0	20,6174477392520	M	-0,65	normal
LDS105A	73.7	26,3979773944081	F	-2,60	osteoporotic
DH318A	67.8	25,5102040816327	F	-2,60	osteoporotic
LDS110A	66.4	30,3757724007630	F	-0,05	normal
LDS3A	75.0	35,1328623076425	M	-0,05	normal
LDS112A	78.5	25,3391251333638	F	-1,40	osteopenic
DH301A	77.7	27,1044021415824	F	-1,90	osteopenic
LDS111A	66.7	28,1359044995409	F	-0,50	normal
LDS106A	53.1	21,1072664359862	F	-1,10	osteopenic
LDS8A	68.4	29,1815509347440	M	1,00	normal
LDS113A	73.6	20,7152052357976	F	-2,30	osteopenic
LDS17A	74.2	24,3227508283679	M	0,45	normal
LDS114A	77.1	28,7403331350387	F	-1,65	osteopenic
LDS4A	74.9	23,9463601532567	M	-0,60	normal
LDS107A	77.5	30,9688581314879	F	0,75	normal
LDS103A	73.2	22,9118356260567	F	-0,85	normal
DH304A	71.4	25,0399187109885	F	-3,00	osteoporotic
LDS9A	66.9	25,6360518617950	M	1,25	normal
LDS115A	63.3	34,7222222222222	F	0,50	normal
DH309A	77.3	25,1038781163435	F	-1,20	osteopenic
LDS118A	69.4	23,1472551213302	F	-3,10	osteoporotic
DH310A	80.5	23,3976124885216	F	-1,80	osteopenic
DH203A	49.9	28,2072728109821	M	-2,60	osteoporotic
LDS128A	89.9	27,0538027739674	F	-1,45	osteopenic
DH315A	63.9	18,2278307855409	F	-2,20	osteopenic
LDS116A	57.6	34,0733811660978	F	0,60	normal
DH303A	80.0	21,8517157272211	F	-2,30	osteopenic
LDS15A	63.6	22,0931700542861	M	-2,60	osteoporotic
LDS1A	59.0	27,2853485703280	M	0,10	normal
DH306A	58.6	29,3788860043791	F	-2,10	osteopenic
DH311A	73.8	16,6888957286003	F	-2,80	osteoporotic
DH308A	69.9	21,8846068166630	F	-0,80	normal
LDS123A	77.4	28,4608265581307	F	-2,50	osteoporotic
LDS16A	88.4	22,4711928245396	M	0,10	normal
LDS14A	75.9	23,4425078599554	M	-0,80	normal
LDS125A	78.3	24,3764172335601	F	-0,90	normal
DH313A	72.1	25,9959123668830	F	-0,30	normal
LDS2A	59.1	27,8547633044988	M	0,90	normal
DH314A	74.6	24,3646292795631	F	-0,80	normal
LDS19A	74.8	25,2831641734658	M	-0,30	normal
LDS109A	44.8	26,1793740810754	F	0,90	normal
LDS13A	73.6	27,2618444341625	M	0,25	normal

Appendix Table S1: Cohort of femoral bone biopsies

Sample	Age	BMI	Sex	FN_TotTScore	group
i99	80,7	27,2562498	F	-1,10	osteopenic
i100	76,9	19,2708497	F	-3,70	osteoporotic
i86	52,9	21,3040032	F	1,70	normal
i93	62,5	22,7286571	F	-2,80	osteoporotic
i98	65,1	22,9118356	F	1,20	normal
i97	79,0	22,2331151	F	0,60	normal
i66	49,7	24,4039074	F	-0,55	normal
i80	72,2	30,7480666	F	-0,90	normal
i2	82,8	22,2107438	F	-4,10	osteoporotic
i24	51,8	28,7334594	F	-0,10	normal
i40	86,1	20,0350293	F	-3,95	osteoporotic
i67	60,5	21,6622416	F	-0,05	normal
i81	60,2	23,5000000	F	0,30	normal
i6	57,7	16,1941777	F	-3,10	osteoporotic
i59	56,0	24,5000000	F	-0,20	normal
i25	64,4	22,5069252	F	1,90	normal
i12	66,8	21,5598502	F	-2,90	osteoporotic
i38	67,5	23,3217993	F	-1,95	osteopenic
i27	83,0	20,8209399	F	-3,75	osteoporotic
i42	70,4	33,1571023	F	0,50	normal
i65	57,1	29,2011019	F	0,50	normal
i69	66,2	27,5802003	F	0,60	normal
i82	71,1	26,2030738	F	0,20	normal
i10	56,9	23,4935752	F	-2,05	osteopenic
i68	54,9	27,4285714	F	-0,10	normal
i43	69,4	23,2520710	F	-1,60	osteopenic
i28	53,5	20,1408042	F	-0,60	normal
i44	67,3	22,8643755	F	0,60	normal
i57	74,8	27,0144141	F	-2,20	osteopenic
i71	52,6	27,7318641	F	-0,70	normal
i70	71,9	29,0174472	F	-2,70	osteoporotic
i87	55,5	25,8199104	F	-0,10	normal
i19	51,6	19,0617914	F	-2,30	osteopenic
i84	62,5	36,3372093	F	0,95	normal
i78	59,5	23,0915415	F	-1,60	osteopenic
i20	57,2	23,4606914	F	-2,90	osteoporotic
i29	84,1	24,0234375	F	-2,60	osteoporotic
i45	64,3	26,3043392	F	-1,75	osteopenic
i72	53,7	22,8000000	F	-1,40	osteopenic
i90	65,1	24,9035813	F	-0,80	normal
i26	60,8	28,3930211	F	1,55	normal
i89	80,7	26,0633551	F	-0,80	normal
i15	77,9	26,0789715	F	-2,40	osteopenic
i34	54,8	23,3843537	F	1,10	normal
i32	58,4	19,7742023	F	-1,30	osteopenic
i56	59,7	21,3227890	F	0,10	normal
i46	57,2	22,0580691	F	0,30	normal
i60	57,6	22,4913495	F	-1,65	osteopenic
i74	68,8	26,0950917	F	1,70	normal
i91	54,3	24,0776773	F	2,00	normal
i30	61,5	24,4739330	F	-2,60	osteoporotic
i16	73,9	21,8299522	F	-2,20	osteopenic
i75	67,5	24,7474352	F	-2,20	osteopenic
i92	76,7	20,9300000	F	-2,70	osteoporotic
i33	54,6	20,7736572	F	-0,90	normal
i35	59,0	25,8000000	F	0,20	normal
i49	56,6	28,0622837	F	1,75	normal
i62	60,0	20,6350387	F	-2,10	osteopenic
i79	65,9	22,4673868	F	-1,30	osteopenic
i22	81,3	27,2215779	F	-2,75	osteoporotic
i36	54,9	26,8122187	F	-1,40	osteopenic
i76	77,9	19,1326531	F	-1,70	osteopenic
i94	59,6	23,7166718	F	-1,50	osteopenic
i41	68,8	21,5138585	F	-1,40	osteopenic
i50	57,7	22,8373702	F	0,30	normal
i95	70,6	20,6250000	F	0,80	normal
i37	68,3	19,2096838	F	-3,55	osteoporotic
i64	58,1	30,0621020	F	0,10	normal
i55	83,3	25,8853816	F	-2,95	osteoporotic
i47	53,7	18,8277447	F	-2,70	osteoporotic
i21	70,6	18,8581315	F	-3,20	osteoporotic

Appendix Table S2: Cohort of iliac bone biopsies

PRIMER NAME	FW SEQUENCE	REV SEQUENCE
L1 5'UTR-ORF1	GAATGATTTTGACGAGCTGAGAGAA	GTCCTCCCGTAGCTCAGAGTAATT
RPL13A	GAAAGCCAAGATCCACTACC	TGGGTCTTGAGGACCTCTGT
CXCL3	CGCCCAAACCGAAGTCATAG	GCTCCCCTTGTTCAGTATCTTTT
CXCL8	GAGAGTGATTGAGAGTGGACCAC	CACAACCCTCTGCACCCAGTTT
CCL20	TGCTGTACCAAGAGTTTGCTC	CGCACACAGACAACTTTTCTTT
CXCL6	AGAGCTGCGTTGCACTTGTT	GCAGTTTACCAATCGTTTTGGGG
CCL3	AGTTCTCTGCATCACTTGCTG	CGGCTTCGCTTGGTTAGGAA
CCL2	CAGCCAGATGCAATCAATGCC	TGGAATCCTGAACCCACTTCT
CXCL5	AGCTGCGTTGCGTTTGTTTAC	TGGCGAACACTTGCAAGATTAC
CXCL10	GTGGCATTCAAGGAGTACCTC	TGATGGCCTTCGATTCTGGATT
PTGS2	TAAAGTGCATTGTACCCGGAC	TTTGTAGCCATAGTCAGCATTGT
TLR2	ATCCTCCAATCAGGCTTCTCT	GGACAGGTCAAGGCTTTTTTACA
CXCL2	GGCAGAAAGCTTGCTCAACCC	CTCCTTCAGGAACAGCCACCAA
CXCL1	AGCTTGCCTCAATCCTGCATCC	TCCTTCAGGAACAGCCACCACT
BMP2	ACTACCAGAAACGAGTGGGAA	GCATCTGTTCTGGAAAACCT
RUNX2	TCAACGATCTGAGATTGTGGG	GGGGAGGATTTGTGAAGACGG
MSX2	CACCCTGAGGAAACACAAGAC	TGCACGCTCTGAATGGAG
ALPL	ACTGGTACTCAGACAACGAGAT	ACGTCAATGTCCTGATGTTATG
OCN	GGCGTACCTGTATCAATGG	GTGGTCAGCCAACCTCGTCA
IBSP	CACTGGAGCCAATGCAGAAGA	TGGTGGGGTTGTAGGTTCAAA

Appendix Table S3: qPCR primers used in this study