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Supplementary Material

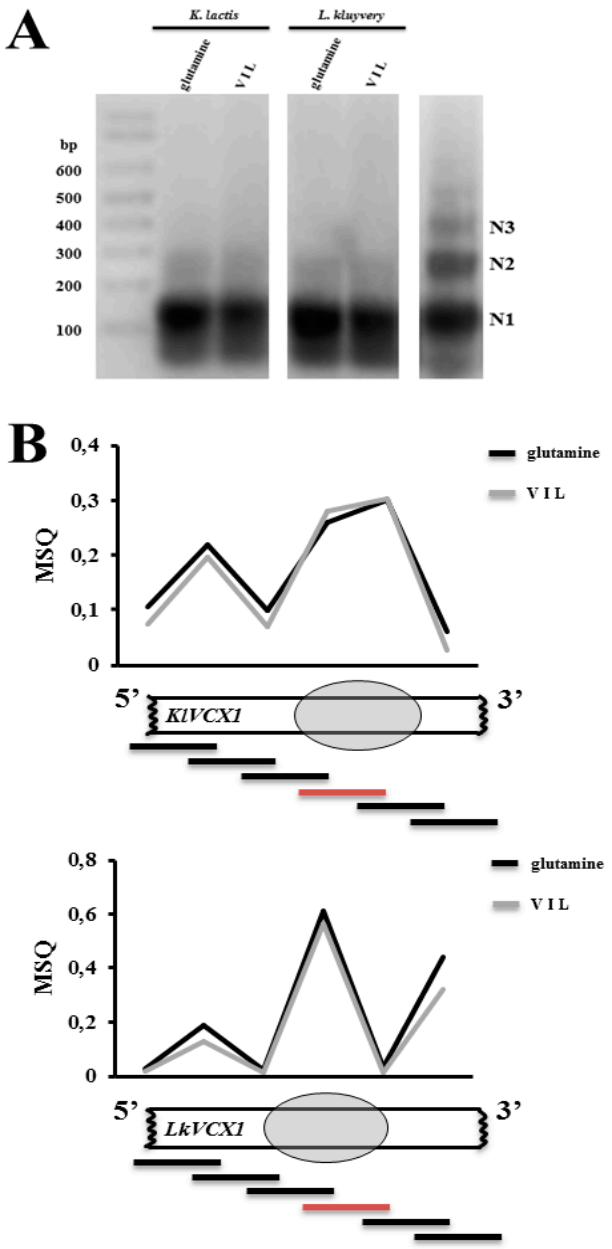

Figure S1. Nucleosome positioning within the *KlVCX1* or *LkVCX1* promoters is equivalent in Gln or VIL. *K. lactis* and *L. kluyveri* wild-type strains were grown on glutamine (Gln) or VIL (OD_{600}=0.5), cultures were cross-linked with formaldehyde (1% final concentration). (**A**) Micrococcal nuclease digestion-generated DNA fragments corresponding to mononucleosomes (N1). (**B**) Isolated DNA was quantified by 6 overlapping amplicons of *KlVCX1* or *LkVCX1* ORF. A well-positioned nucleosome within the *KlVCX1* or *LkVCX1* coding region (gray oval), was observed in the presence of either Gln or VIL as sole nitrogen source, which is shown as mean starting quantity (MSQ). The relative protection of *KlBAT1*or *LkBAT1* nucleosomes (Figs 2B and 2C) was determined respectively using the second peak of *KlVCX1* or *LkVCX1* nucleosomes. Red horizontal line represents the amplicon used to normalize.

Table S1. Deoxyoligonucleotides used for qPCR analysis to compare the transcript levels of the *ScBAT1*, *ScBAT2*, *KlBAT1* and *LkBAT1* genes.

Species	Gene	Primer	Sequence (5′– 3′)	Amplicon Size (bp)
S. cerevisiae	*ScBAT1*	FW. ScB1qPCR	CATCCAAGCCAAGACCAAATG	146
		RV. ScB1qPCR	CACAAGCAGATGGGTCAAGAG	
	ScBAT2	FW. ScB2qPCR	GCTTCAAGGGGTTACCAACA	119
		RV. ScB2qPCR	AGTTCCTTCTTGCCCGTTTT	
	Sc18S	FW. ScB18SCR	ACTTCTTAGAGGGACTATCG	147
		RV. Sc18SqPCR	CAAGATTACCAAGACCTCTC	
K. lactis	*KlBAT1*	FW. KlB1qPCR	TCACTGAAGTCGGTACTATG	117
		RV. KlB1qPCR	CAATGGAGCAGTAACCAATTC	
	Kl18S	FW. KlB18SCR	AACTGGGATGATATGGAAAAGATCTGGC	146
		RV. Kl18SqPCR	TTCTGGAGCTCTGAATCTTTCGTTACC	
L. kluyveri	*LkBAT1*	FW. LkB1qPCR	CTTGTACATCAGACCAACC	148
		RV. LkB1qPCR	GTAATCAGTAGCCTCCAGTT	
	Lk18S	FW. LkB18SCR	AAGCTCGTAGTTGAACTTTGGGTC	150
		RV. Lk18SqPCR	AAGACTTTGATTTCTCGTAAGGTG	

Table S2. Deoxyoligonucleotides used for nucleosome scanning assays in *KlBAT1* locus.

Primer	Sequence (5′– 3′)	Middle of Amplicon (Promoter Coordinate)	5′/3′ end	Size(bp)
1KlB1Fw	CCTGTACCTGAGCCAACGA	-672	-720	96
1KlB1Rv	CACTAGTGTATCGAAACAGGTTG		-624	
2KlB1Fw	AGATGTGGCCGTTTCAATGCA	-631	-682	102
2KlB1Rv	CTGTTCAAACAGGGGAAAACC		-580	
3KlB1Fw	CAACCTGTTTCGATACACTAGTG	-598	-645	95
3KlB1Rv	GAGATGAAGACCGAACCATTG		-550	
4KlB1Fw	GGTTTTCCCCTGTTTGAACAG	-552	-600	97
4KlB1Rv	CAAGGGTTTCACTCACATTGCA		-503	
5KlB1Fw	CAATGGTTCGGTCTTCATCTC	-522	-570	96
5KlB1Rv	TCCCGTCATGCATTGTCACTT		-474	
6KlB1Fw	TGCAATGTGAGTGAAACCCTTG	-478	-524	93
6KlB1Rv	AGGCCTTTCGCAGGCTGAA		-431	
7KlB1Fw	AAGTGACAATGCATGACGGGA	-448	-494	93
7KlB1Rv	ATTGAGAACGCAGGTGAAAGC		-401	
8KlB1Fw	TTCAGCCTGCGAAAGGCCT	-401	-449	96
8KlB1Rv	CACATGCATGACTCACCAAGA		-353	
9KlB1Fw	GCTTTCACCTGCGTTCTCAAT	-375	-421	92
9KlB1Rv	GTAATCTTCGCTCAGGTTCG		-329	
10KlB1Fw	TCTTGGTGAGTCATGCATGTG	-331	-373	85
10KlB1Rv	AGTTTCAATTCCACTTGTACTTGG		-288	
11KlB1Fw	CGAACCTGAGCGAAGATTAC	-303	-349	93
11KlB1Rv	CTGTTGCTTAGTGTTTCCAGTG		-256	
12KlB1Fw	CCAAGTACAAGTGGAATTGAAAC	-267	-313	92
12KlB1Rv	CAGATTGCCGGTTGCATTTC		-221	
13KlB1Fw	CACTGGAAACACTAAGCAACAG	-234	-277	86
13KlB1Rv	GAACCAGGCATCCCACCT		-191	
14KlB1Fw	GAAATGCAACCGGCAATCTG	-195	-240	91
14KlB1Rv	CTCAGTCTATGCGTATTATCGAA		-149	
15KlB1Fw	AGGTGGGATGCCTGGTTC	-149	-209	121
15KlB1Rv	ATGCCCTTCTTATCTGCCTCTT		-88	
16KlB1Fw	TTCGATAATACGCATAGACTGAG	-118	-172	109
16KlB1Rv	CCAAACAAGAACAGAAACAAGAC		-63	
17KlB1Fw	AAGAGGCAGATAAGAAGGGCAT	-59	-109	100
17KlB1Rv	CTTGCCAGGGATGAAATAGCT		-9	
18KlB1Fw	GTCTTGTTTCTGTTCTTGTTTGG	-33	-85	105
18KlB1Rv	CGTAGTCCAATGTAGTTCATGAA		+20	

19KlB1Fw	AGCTATTTCATCCCTGGCAAG	+21	-29	99
19KlB1Rv	TACCTCTTGATGGAATGGAAACT		+70	
20KlB1Fw	TTCATGAACTACATTGGACTACG	+57	+3	107
20KlB1Rv	AAGGACATGGCTCTATAACTTGT		+110	
21KlB1Fw	AGTTTCCATTCCATCAAGAGGTA	+95	+48	93
21KlB1Rv	GACCTTGGTGATTTTAACCTTG		+141	
22KlB1Fw	ACAAGTTATAGAGCCATGTCCTT	+138	+88	99
22KlB1Rv	GACCGAAAACCAACTCATCGT		+187	
23KlB1Fw	CAAGGTTAAAATCACCAAGGTC	+172	+120	104
23KlB1Rv	GTCCATTCGATGGTTAACATATG		+224	
24KlB1Fw	ACGATGAGTTGGTTTTCGGTC	+215	+167	95
24KlB1Rv	ATGGTTTAATCTCTGGGACACC		+262	
25KlB1Fw	CATATGTTAACCATCGAATGGAC	+251	+202	98
25KlB1Rv	GAACACACACGAGGATGGAT		+300	

Table S3. Deoxyoligonucleotides used for nucleosome scanning assays in *LkBAT1* locus.

Primer	Sequence (5′– 3′)	Middle of Amplicon (Promoter Coordinate)	5′/3′ end	Size(bp)
1LkB1Fw	CTGGGCTGTAGCTGTTGCA	-646	-689	87
1LkB1Rv	TGGTGTTGCGGGAAGGTGT		-602	
2LkB1Fw	ACTATCCGGTGAAAGGTCAGT	-608	-656	97
2LkB1Rv	TCTACTTGCCTCGGTGTACTT		-559	
3LkB1Fw	ACACCTTCCCGCAACACCA	-574	-620	92
3LkB1Rv	CTTGGAACAGCTGTTTGATGCT		-528	
4LkB1Fw	AAGTACACCGAGGCAAGTAGA	-534	-580	92
4LkB1Rv	ACATGAGTATGGACTCTTGTGAA		-488	
5LkB1Fw	AGCATCAAACAGCTGTTCCAAG	-497	-549	104
5LkB1Rv	TCCGCTGTTGGCCGGCT		-445	
6LkB1Fw	TTCACAAGAGTCCATACTCATGT	-468	-510	85
6LkB1Rv	AGTCGAGCACGATCTGCGA		-425	
7LkB1Fw	AGCCGGCCAACAGCGGA	-425	-469	88
7LkB1Rv	AAACAAGGCTCTTCGTTCGCT		-381	
8LkB1Fw	TCGCAGATCGTGCTCGACT	-377	-443	91
8LkB1Rv	ATGAGTAAGACTGCGTTGAGGT		-352	
9LkB1Fw	AGCGAACGAAGAGCCTTGTTT	-359	-401	85
9LkB1Rv	GTTGTAAGATTTGGTAAGTTGGGA		-316	
10LkB1Fw	ACCTCAACGCAGTCTTACTCAT	-324	-373	99
10LkB1Rv	AAGATGGAATACGAACGGACGA		-274	
11LkB1Fw	TCCCAACTTACCAAATCTTACAAC	-289	-339	101
11LkB1Rv	ACGTGACTCGAGGATAGGCT		-238	
12LkB1Fw	TCGTCCGTTCGTATTCCATCTT	-253	-295	85
12LkB1Rv	TATGCGAGTAACATGCCTGTC		-210	
13LkB1Fw	AGCCTATCCTCGAGTCACGT	-207	-257	100
13LkB1Rv	TTTAGCCGCTACCGGCGTT		-157	
14LkB1Fw	GACAGGCATGTTACTCGCATA	-182	-230	97
14LkB1Rv	CAGGCGACGCGGTCGAT		-133	
15LkB1Fw	AACGCCGGTAGCGGCTAAA	-133	-175	84
15LkB1Rv	TATATCTTGTGACTCACTCGGTT		-91	
16LkB1Fw	ATCGACCGCGTCGCCTG	-102	-149	94
16LkB1Rv	AGGAGCCAAGGATCATTGCTT		-55	
17LkB1Fw	AACCGAGTGAGTCACAAGATATA	-84	-113	88
17LkB1Rv	CAAATCTTTAATATTGGAGAAACGGA		-25	
18LkB1Fw	AAGCAATGATCCTTGGCTCCT	-28	-75	95
18LkB1Rv	TTCAGAGAACTTTTTAGCATTTCGT		+20	
19LkB1Fw	TCCGTTTCTCCAATATTAAAGATTTG	+1	-50	100
19LkB1Rv	CTGGTACTAAATAGACGATTGAG		+50	
20LkB1Fw	ACGAAATGCTAAAAAGTTCTCTGAA	+38	+5	66
20LkB1Rv	GTCCAATGGAGCGGACATAG		+71	
21LkB1Fw	CTCAATCGTCTATTTAGTACCAG	+76	+28	95
21LkB1Rv	CTTGGATGGATTGGTGTTTTTAG		+123	

22LkB1Fw	CTATGTCCGCTCCATTGGAC	+109	+62	94
22LkB1Rv	TTGACCAAAAACCAACTTGTCGT		+156	
23LkB1Fw	CTAAAAACACCAATCCATCCAAG	+147	+100	93
23LkB1Rv	TGGTCCATTCAATGGTCAACATA		+193	
24LkB1Fw	ACGACAAGTTGGTTTTTGGTCAA	+183	+134	98
24LkB1Rv	CATATGGCTTGATTTCTGGAACG		+232	
25LkB1Fw	TATGTTGACCATTGAATGGACCA	+222	+171	99
25LkB1Rv	TAGTGGAAGACGCATGCAGAT		+272	
26LkB1Fw	CGTTCCAGAAATCAAGCCATATG	+255	+210	90
26LkB1Rv	CTTCAAACCTTCAAACAACTCGA		+300	

Table S4. Position and sequence of putative Leu3 binding sites in the promoter regions of genes of the leucine biosynthetic pathway in five yeast species.

	***Saccharomyces cerevisiae* S288c**		***Kluyveromyces lactis* NRRL Y-1140**		***Lachancea kluyveri* NRRL Y-12651**		***Kluyveromyces marxianus* DMKU3-1042**		***Candida auris* B8441**	
Gene / Enzyme	**Gene**	**Coordinates and Sequence**	**Gene**	**Coordinates and Sequence**	**Gene**	**Coordinates and Sequence**	**Gene**	**Coordinates and Sequence**	**Gene**	**Coordinates and Sequence**
ILV2 / Acetolactate synthase (catalitic subunit)	YMR108W	-468 CCGN{4}CGG	KLLA0B12584g	-482 CCGGCCC -566 CCGGTCCC	SAKL0E03740g	-326 CCGGCCC	KLMA_30105	-108 GCAGAAC	B9J08_002629	-441 CCGN{4}CGG -178 CCGN{4}CGG
ILV6 / Acetolactate synthase (regulatory subunit)	YCL009C	-159 CCGTTANCG	KLLA0F12364g	-283 CCGTTTC	SAKL0D08492g	-199 GGGCNGGT -194 CCCGGTTT -824 CCTGTTC	KLMA_30148	-398 GCCGGTTCCG	B9J08_004459	-73 TCGCCGG
ILV5 / Acetohydroxi acid reductoisomerase	YLR355C	-368 GCAGAAC -377 CCGN{4}CGG	KLLA0A02673g	-669 CCGN{4}CGG -820 CCGN{4}CGG	SAKL0B04708g	-432 CCGGCCC -600 GCAGAAC	KLMA_30631	-268 CCGGAAC -391 CCGGAAC	B9J08_003440	-140 CCGN{4}CGG
ILV3 / Dihydroxi acid dehydratase	YJR016C	-853 CCGN{4}CGG -178 GGCCCGGTTCCG	KLLA0F22022g	-193 GCAGAAC -369 CCGN{4}CGG -682 GCCGGTTT -817 CCGN{4}CGG	SAKL0G14454g	-198 CCGN{4}CGG -201 TCGCCGG -489 CCTGTTC	KLMA_40259	-259 CCGN{4}CGG	B9J08_000013	-75 CCGN{4}CGG
LEU4 / Leucine-sensitive α-isopropylmalate synthase	YNL104C	ND	KLLA0F23529g	ND	SAKL0E10472g	ND	KLMA_60226	ND	B9J08_001277	ND

LEU9 / Leucine-resistant α-isopropylmalate synthase	YOR108W	ND	NP		NP		NP		NP	
BAT1 / Mitochondrial branched-chain aminotransferase	YHR208W	-147 CCGN{4}CGG	KLLA0A10307g	-230 GGCAATCTGCC -673 CCGTTTC -697 CCGTTTC	SAKL0B12496g	-171 CCGN{4}CGG -473 CCGN{4}CGG	KLMA_10514	-339 GCAGAAC	B9J08_002453	-295 CCGN{4}CGG
LEU1 / Isopropyl malate isomerase	YGL009C	-217 CCGN{4}CGG -187 CCGN{4}CGG	KLLA0A04961g	-392 CCGN{4}CGG -367 CCGN{4}CGG -763 CCGTTAC	SAKL0H21868g	-822 CCGN{4}CGG -177 CCGN{4}CGG -156 CCGN{4}CGG	KLMA_50154	-269 GCCGGTTT	B9J08_004781	-99 CCGN{4}CGG
LEU2 / α -IPM dehydrogenase	YCL018W	-198 CCGN{4}CGG	KLLA0D04906g	-259 CCGN{4}CGG	SAKL0D08690g	-212 CCGN{4}CGG -190 CCTGTTCC -184 CCGGCCC	KLMA_20483	-334 GCGGAAT -142 GCAGAAC	B9J08_000229	-87 CCGN{4}CGG -99 CCGN{4}CGG
BAT2 / Cytosolic branched-chain aminotransferase	YJR148W	-334 CCGN{4}CGG -366 CCGGAAC -815 TCGCCGG	NP		NP		NP		NP	

Binding sites were found using the YEASTRACT database (http://www.yeastract.com/index.php), selecting the "All *S. cerevisiae* consensus" option and the -1000 bp regions before the start codon. For *S. cerevisiae*, *K. lactis*, *K. marxianus* and *C. auris*, the DNA sequences were downloaded from YGOB database (http://ygob.ucd.ie/). For *L. kluyveri*, the DNA sequences were downloaded from NCBI (https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_000149225.2/). The web sites were accessed on February 2024. *K. marxianus* and *C. auris* were included to extend the analysis to other species phylogenetically close and distant to *K. lactis*. ND, not detected. NP, the gene is not present in the corresponding yeast species.