Supplementary Material

Genetic Variants in Genes Involved in Creatine Biosynthesis in Patients with Severe Obesity or Anorexia Nervosa

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Supplementary Table 1: List of primers and screening methods for all investigated genes (*CKB, CKMT1B* and *GATM*).

Gene	Fragment	Forward primer (5' to 3')	Reverse primer (5' to 3')	Fragment length (bp)	Method
CKB	E2/E3	CTGAGTGGTACGCGGGAG	CTCAGCACGTAGTTGGGGTC	658	PCR
	E4/E5	TGACGTCACTGTCCCCGT	GAGAGGGAAAGCGGAGGTAG	1015	PCR
	E6	GGGTTCTCCTGCTGTGAGG	GGACTGATGCTGGGGCAC	378	PCR
	E7/E8	CTGTAGGGGTTTCAGGCAGG	GCAGGCCAAAATAGTTTA	697	PCR
CKMT1B	E2-E7	CATCCTCCCTCCCTGTTC	CTTCTTTGCACTCTCTGTCA	2655	PCR
	E2	CATCCTCCCTCCCTGTTC	TGATTATAGCCCTTCTCTGTT	325	Nested PCR
	E3/E4	CCAAGGTTTACCCTCTTTCTA	GCTGTATAATGACCATGAGTC	739	Nested PCR
	E5-E7	CTTCAATTCCAGTGCTTTCC	TCCTGGGTTGGTTGGTAAA	931	Nested PCR
	E8	TGTCATTCATTCACAGCCAAGTT	TCCTGGGCTCAAGTAATCTGC	457	PCR
	E9	CAGGTTCAGGGCTCTTTCAG	GCCCTCGCAGACCTATCCT	251	PCR
	E10	CTCAGACTGTAGGAAGCAGAT	CCACCATTAGAAGTAACACAGAC	317	PCR
GATM	E1	CACTTCGTCAGAAGTCGCGT	CCGCAGGATCGAGTGAGT	506	PCR
	E2	GTATGGTAGCTGGGGACACG	GCTGAAGGGAAAGCAGTCAG	573	PCR
	E3	GGAGCAAAAGGCCTCAAAAT	TTTGGAGCTTTCCCCTTACA	504	PCR

	E4	GCCTGGCCCTATATAGACCTT	CTCTTTGTGGATCATTTAGAAAAGT	326	PCR
	E5	GCATGTTTTAGTCTAGAACTGAGTAGT	CATTTAGAACCATTAGGAACCATGG	700	PCR
	E6	AGTCTCTGAAACCTGAGAAAAGT	TTGCAAACACAAGTCCCAGC	510	PCR
	E7	CAAAAATGATAGCGGACATGG	GGGCTGAACAAGCTATCTGC	481	PCR
	E8	GAACCACAGGACTCCTCCAA	CAGGCTTACGACCCCTGTTA	344	PCR

Supplementary Table 2: SNPs extracted from the GWAS for BMI or AN in *CKB*, *CKMT1B* and *GATM*, their relationship with the traits and sex-specific effects. This table lists the number of SNPs extracted from the GWAS for BMI (Pulit et al., 2019) and AN (Watson et al., 2019) located in *CKB*, *CKMT1B* and *GATM* and contiguous regions (± 500 kb) as well as significant associations with the respective phenotype. Due to the close proximity of *CKMT1B* and *GATM*, these genes were analysed jointly by examining just one adjacent ± 500 kb region (*; see Table 1). SNPs associated with the investigated traits exhibiting a z score > |3| were classified as sex-dimorphic (Khramtsova et al., 2019). NA: not available.

Gene	Region	BMI GWAS							AN GWAS				
		Total SNPs	Significant SNPs in combined analysis	Significant SNPs in females	Significant SNPs in males	Sex-dimorphic BMI-associated SNPs (z score > |3|)			Total SNPs	Significant SNPs	Sex-dimorphic AN-associated SNPs (z score > |3|)		
						n	Highest z-score	z-score P-value			n	Highest z-score	z-score P-value
CKB	Gene region	24	3	2	0	1	|3.196|	0.0014	5	0	0	*NA*	*NA*
	± 500 kb region	10,967	302	467	0	225	|4.265|	0.00002	3,221	0	0	*NA*	*NA*
CKMT1B*	Gene region	35	0	0	0	0	*NA*	*NA*	8	0	0	*NA*	*NA*
	± 500 kb region	22,211	1	0	0	0	|0.375|	0.71	4,728	0	0	*NA*	*NA*
GATM*	Gene region	256	0	0	0	0	*NA*	*NA*	78	0	0	*NA*	*NA*
	± 500 kb region	22,211	1	0	0	0	|0.375|	0.71	4,728	0	0	*NA*	*NA*

Supplementary Table 3: Alternative control groups extracted from gnomAD. The alternative control groups were extracted from gnomAD v2.1.1 (Karczewski et al., 2020). The control population for the screening group with severe obesity encompassed all non-Finnish, European individuals of either sex, while the control population for the female patients with anorexia nervosa solely consisted of non-Finnish, European females. The genotype-specific data were calculated on the basis of the allele counts, allele number and number of homozygotes stated in gnomAD for the corresponding SNP. For certain variants, no gnomAD data were available ([a]). Furthermore, if the variant was not detected in the respective screening group (patients with severe obesity or AN), no test was performed ([b]). The AN cohort was exclusively screened for *CKB* ([c]). No phenotypic data regarding the population were available. 11: homozygous wild-type. 12: heterozygous. 22: homozygous mutant allele. MAF: minor allele frequency. *NA*: not available.

Gene	Variant	Amino acid exchange	gnomAD controls for obesity				gnomAD controls for AN			
			11	12	22	MAF	11	12	22	MAF
CKB	rs762206402	Pro112Pro	29506	0	0	0	*NA*[b]	*NA*[b]	*NA*[b]	*NA*[b]
	rs1136165	Arg152Arg	4762	15541	12624	0.38	2006	6617	5520	0.38
	rs200890896	Ala175Ala	*NA*[b]	*NA*[b]	*NA*[b]	*NA*[b]	27053	2	0	0.00004
	rs146047573	Tyr269Cys	64126	10	0	0.00008	*NA*[b]	*NA*[b]	*NA*[b]	*NA*[b]
	rs1803283	Glu364Glu	8473	29704	25749	0.36	3640	12966	11432	0.36
CKMT1B	g.43595061C/T[a]	Thr79Thr	*NA*[a]	*NA*[a]	*NA*[a]	*NA*[a]	*NA*[c]	*NA*[c]	*NA*[c]	*NA*[c]
	g.43595385C/T[a]	Asp120Asp	*NA*[a]	*NA*[a]	*NA*[a]	*NA*[a]	*NA*[c]	*NA*[c]	*NA*[c]	*NA*[c]
	g.43595451G/A[a]	Thr142Thr	*NA*[a]	*NA*[a]	*NA*[a]	*NA*[a]	*NA*[c]	*NA*[c]	*NA*[c]	*NA*[c]
	rs1230355611	Arg184Stop	8644	0	0	0	*NA*[c]	*NA*[c]	*NA*[c]	*NA*[c]
	rs758572075	Val191Ala	9066	31	1	0.002	*NA*[c]	*NA*[c]	*NA*[c]	*NA*[c]
	rs9571	Lys352Lys	64235	41	0	0.0003	*NA*[c]	*NA*[c]	*NA*[c]	*NA*[c]
	rs13234	Ala362Ala	63532	699	6	0.006	*NA*[c]	*NA*[c]	*NA*[c]	*NA*[c]
	rs149544188	Arg399His	63685	840	14	0.007	*NA*[c]	*NA*[c]	*NA*[c]	*NA*[c]
	rs144820945	Gly403Gly	63874	654	5	0.005	*NA*[c]	*NA*[c]	*NA*[c]	*NA*[c]
GATM	rs1288775	Gln110His	34476	25365	4630	0.27	*NA*[c]	*NA*[c]	*NA*[c]	*NA*[c]
	rs146057680	Asp234Val	*NA*[b]	*NA*[b]	*NA*[b]	*NA*[b]	*NA*[c]	*NA*[c]	*NA*[c]	*NA*[c]
	rs773358289	Pro287Ser	*NA*[b]	*NA*[b]	*NA*[b]	*NA*[b]	*NA*[c]	*NA*[c]	*NA*[c]	*NA*[c]
	rs747005297[a]	His292Arg	64530	2	0	0.00002	*NA*[c]	*NA*[c]	*NA*[c]	*NA*[c]
	rs1145086	Leu418Leu	24189	30475	9541	0.39	*NA*[c]	*NA*[c]	*NA*[c]	*NA*[c]

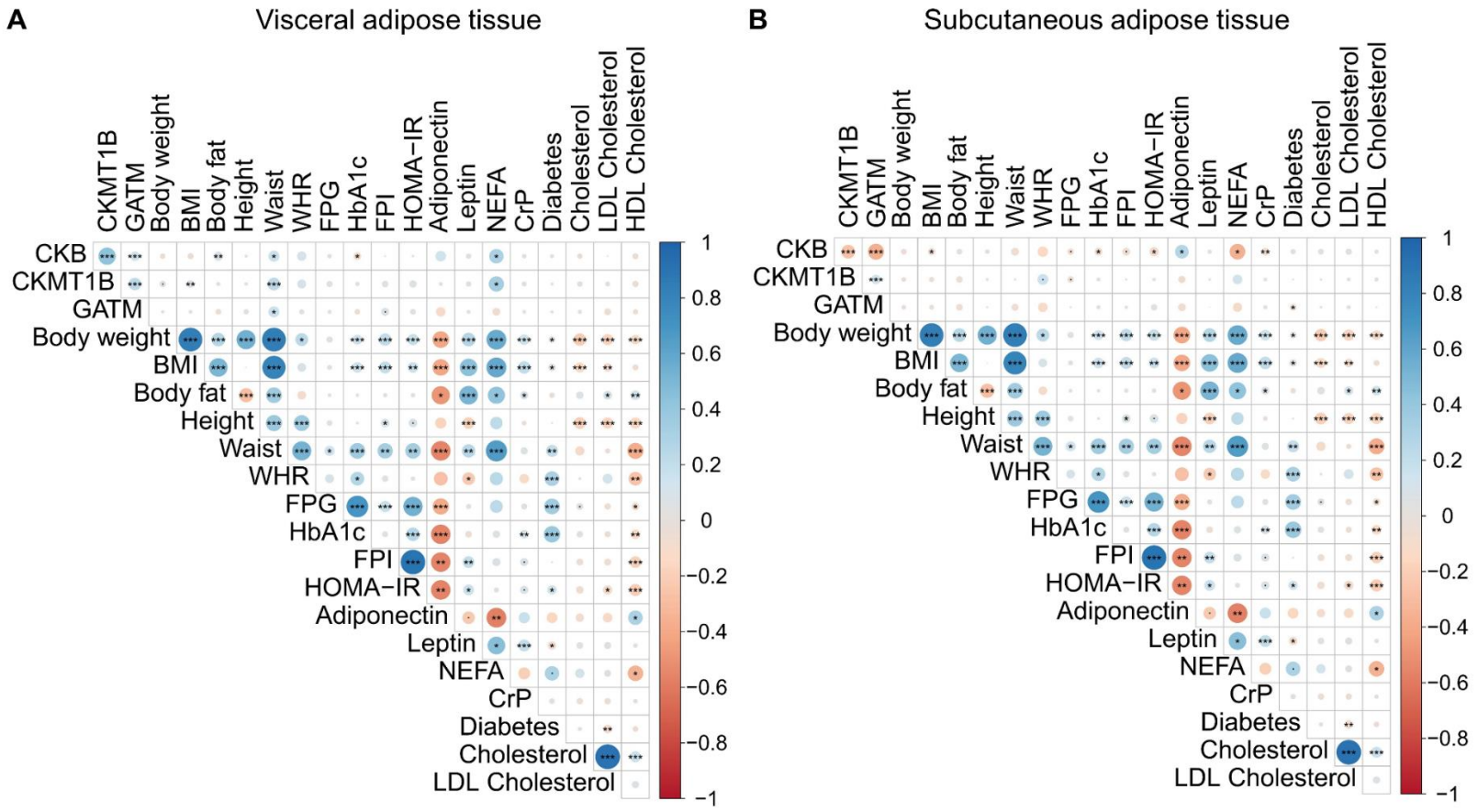
Supplementary Table 4: Statistical association testing of the detected variants and obesity. The two-sided chi-square test or Fisher's exact test was performed with IBM SPSS Statistics (version 28.0.0.0) on the basis of the genotype counts (see Table 3 and Supplementary Table 3). Here, the results of the association testing for the screening and confirmation groups with severe obesity as well as the healthy-lean controls and the alternative gnomAD control cohort (see Tables 2 and Supplementary Table 3) are represented. The minor alleles are given with respect to the forward strand ([a]). If the variant was not detected in the respective study group, no test was performed ([b]; see Table 3). Furthermore, certain variants were not included in gnomAD ([c]; see Supplementary Table 3); thus, no statistical testing could be performed. Nominal associations are shown in bold ($p < 0.05$). The GWAS-derived p-values stated are based on the BMI-GWAS analyzing both sexes combined (Pulit et al., 2019). Again, several variants were not analyzed within this GWAS ([e]). GWAS: genome-wide association study. *NA*: no statistical test performed. p: p-value.

Gene	Variant	Minor allele[a]	Study group	Obese vs. healthy-lean controls		Obese vs. gnomAD controls		BMI GWAS	
								Pulit et al., 2019	
				Test	p	Test	p	Tested allele	p
CKB	**rs762206402**	C	**Screening**	Fisher's exact	1	Fisher's exact	**0.006**	*NA*[e]	*NA*[e]
	rs1136165	G	Screening	Chi-square	0.72	Chi-square	0.80	T	$3.9*10^{-15}$
	rs200890896	G	Screening	*NA*[b]	*NA*[b]	*NA*[b]	*NA*[b]	*NA*[e]	*NA*[e]
	rs146047573	C	**Screening**	Fisher's exact	1	Fisher's exact	**0.03**	*NA*[e]	*NA*[e]
			Confirmation	Fisher's exact	1	Fisher's exact	0.17		
			Both	Fisher's exact	1	Fisher's exact	**0.02**		
	rs1803283	C	Screening	Chi-square	0.72	Chi-square	0.57	T	$1.5*10^{-16}$
CKMT1B	g.43595061C/T	T	Screening	Fisher's exact	1	*NA*[c]	*NA*[c]	*NA*[e]	*NA*[e]
	g.43595385C/T	T	Screening	Fisher's exact	1	*NA*[c]	*NA*[c]	*NA*[e]	*NA*[e]
	g.43595451G/A	A	Screening	Fisher's exact	1	*NA*[c]	*NA*[c]	*NA*[e]	*NA*[e]
	rs1230355611	T	Screening	Fisher's exact	1	*NA*[b]	*NA*[b]	*NA*[e]	*NA*[e]
			Confirmation	Fisher's exact	1	Fisher's exact	0.11		
			Both	Fisher's exact	0.45	Fisher's exact	0.13		
	rs758572075	C	Screening	Fisher's exact	1	Chi-square	0.91	*NA*[e]	*NA*[e]
			Confirmation	Fisher's exact	1	Chi-square	0.35		
			Both	Fisher's exact	0.27	Chi-square	0.50		
	rs9571	G	**Screening**	Fisher's exact	1	Fisher's exact	**0.00001**	*NA*[e]	*NA*[e]
	rs13234	C	Screening	Chi-square	0.42	Chi-square	0.13	*NA*[e]	*NA*[e]

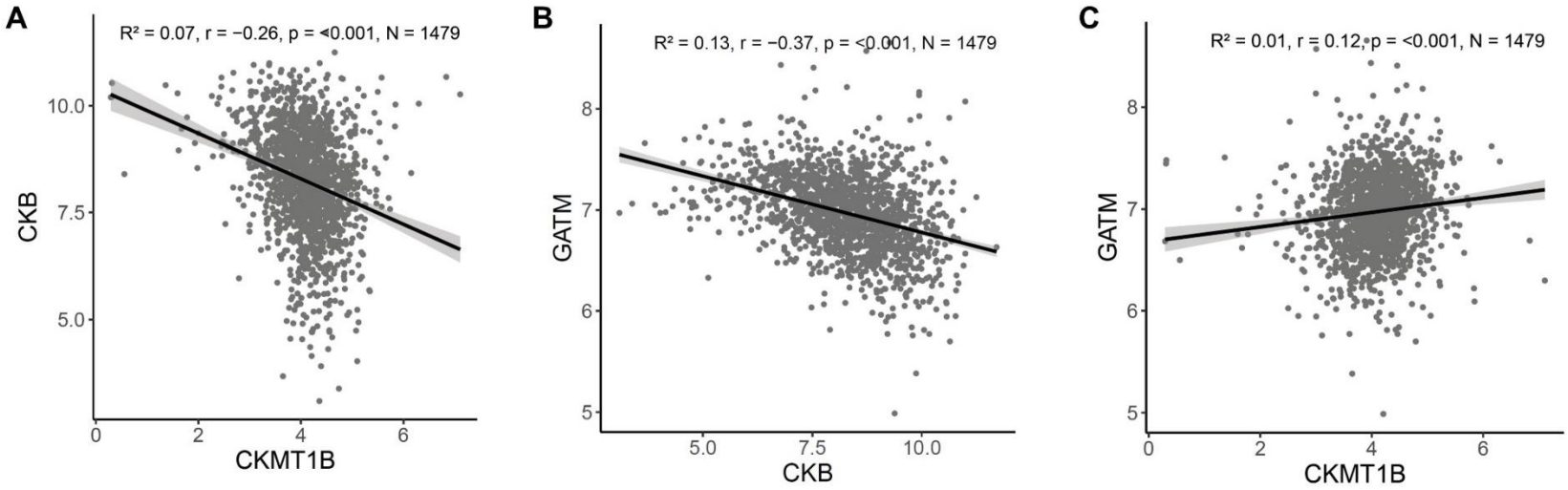
	rs149544188	A	Screening	Fisher's exact	1	Chi-square	0.93	A	0.21
			Confirmation	Fisher's exact	0.73	Chi-square	0.60		
			Both	Fisher's exact	1	Chi-square	0.56		
	rs144820945	G	Screening	Fisher's exact	1	Chi-square	0.33	G	0.28
GATM	rs1288775	A	Screening	Chi-square	0.88	Chi-square	0.75	A	0.007
	rs146057680	C	Screening	Fisher's exact	1	*NA*[b]	*NA*[b]	T	0.41
	rs773358289	A	Screening	Fisher's exact	1	*NA*[b]	*NA*[b]	*NA*[e]	*NA*[e]
	rs747005297	C	**Screening**	Fisher's exact	1	Fisher's exact	**0.009**	*NA*[e]	*NA*[e]
	rs1145086	T	Screening	Chi-square	0.99	Chi-square	0.88	A	0.001

Supplementary Table 5: Statistical association testing of *CKB*-located variants and anorexia nervosa. Here, the chi-square test or Fisher's exact test was performed using IBM SPSS Statistics (version 28.0.0.0) on the basis of the genotype counts (see Table 3 and Supplementary Table 3). Here, only the results of the association testing for anorexia nervosa in all study groups investigated for this phenotype (see Table 3 and Supplementary Table 3) are represented. Nominally significant p-values are shown in bold ($p < 0.05$). The minor alleles are given with respect to the forward strand ([a]). If the variant was not detected in the AN study group, no test was performed ([b]). The presented p-value was extracted from the latest GWAS for AN (Watson et al., 2019). Again, several variants were not analyzed in this GWAS ([c]). p: p-value. *NA*: no statistical test performed.

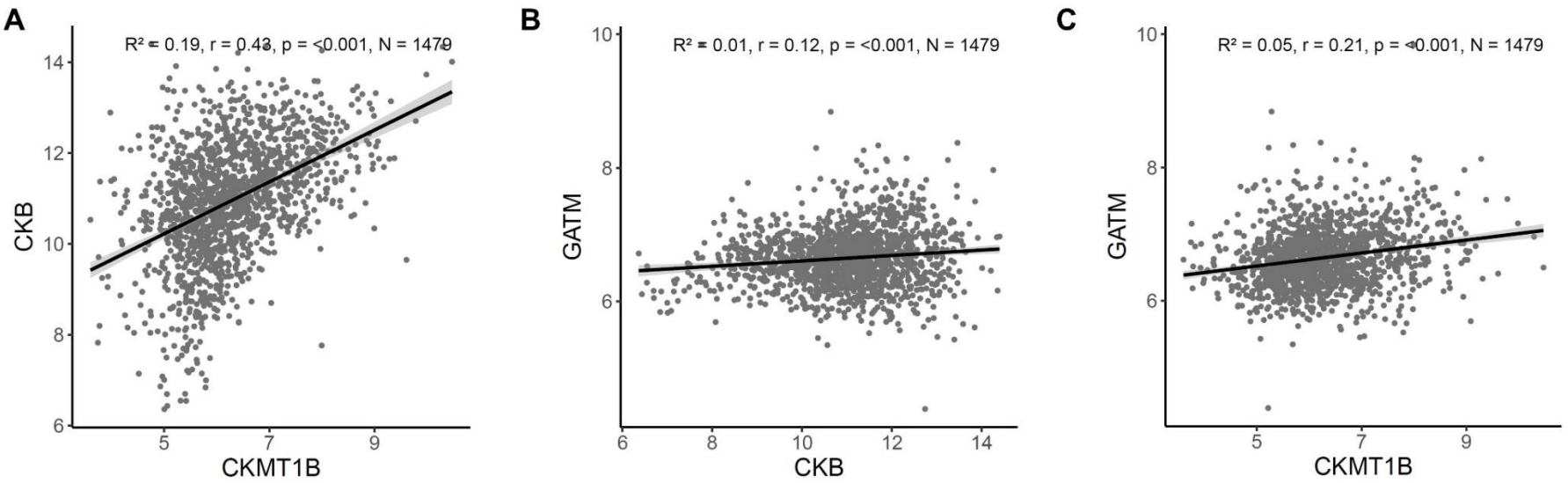
Variant	Alternative allele	AN vs. healthy-lean		AN vs. gnomAD controls		AN GWAS	
		Test	p	Test	p	Watson et al., 2019	
						Tested allele	p
rs762206402	C	*NA*[b]	*NA*[b]	*NA*[b]	*NA*[b]	*NA*[c]	*NA*[c]
rs1136165	C	Chi-square	0.63	Chi-square	0.97	G	0.07
rs200890896	G	Fisher's exact	1	Fisher's exact	**0.02**	*NA*[c]	*NA*[c]
rs146047573	C	*NA*[b]	*NA*[b]	*NA*[b]	*NA*[b]	*NA*[c]	*NA*[c]
rs1803283	C	Chi-square	0.87	Chi-square	0.94	C	0.34



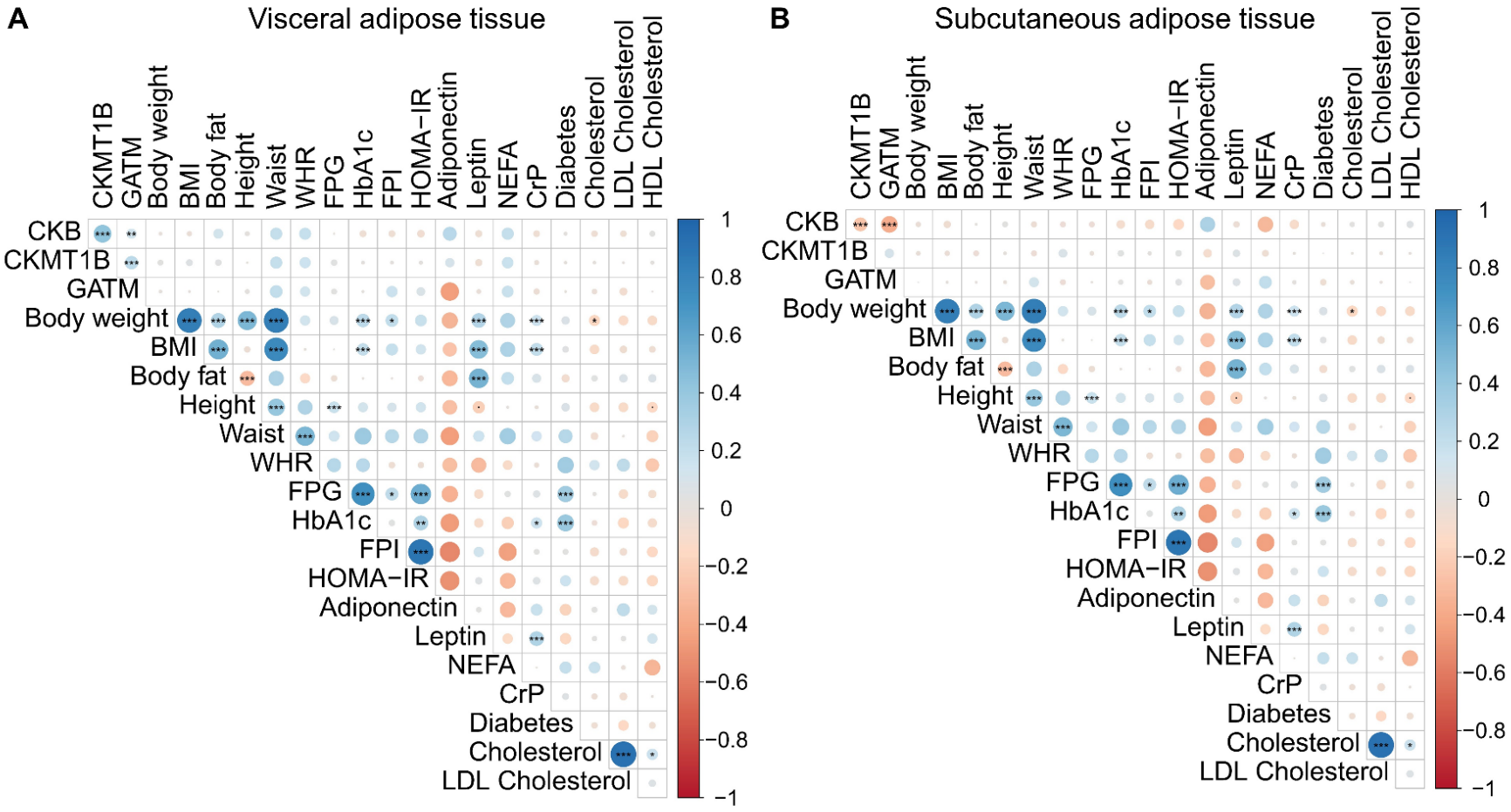
Supplementary Figure 1: Unadjusted correlations of *CKB*, *CKMT1B*, and *GATM* in visceral and subcutaneous fat. The presented data are based on RNA-sequencing data of 1,479 subjects of the Leipzig Obesity Biobank. For each individual, data for the visceral (A) and subcutaneous (B) adipose were available. The correlations were calculated with Pearson's correlation coefficient. P-values are not adjusted for multiple comparisons. Positive correlations are shown in blue, while negative correlations are represented in red. The size of the dot refers to the degree of correlation. p <0.001 (***), p <0.01 (**), and p <0.05 (*). BMI: body mass index. CrP: c-reactive protein. FPI: fasting plasma insulin. FPG: fasting plasma glucose. HbA1C: hemoglobin A1C. HDL: high-density lipoprotein. HOMA-IR: homeostatic model assessment for insulin resistance. LDL: low-density lipoprotein. NEFA: nonesterified fatty acids. WHR: waist-to-hip ratio.



Supplementary Figure 2: Multiple testing adjusted gene correlations in the subcutaneous adipose tissue. Correlations between the three genes of interest are shown. These correlations were calculated with Pearson's correlation coefficient. P-values are adjusted for multiple inference.

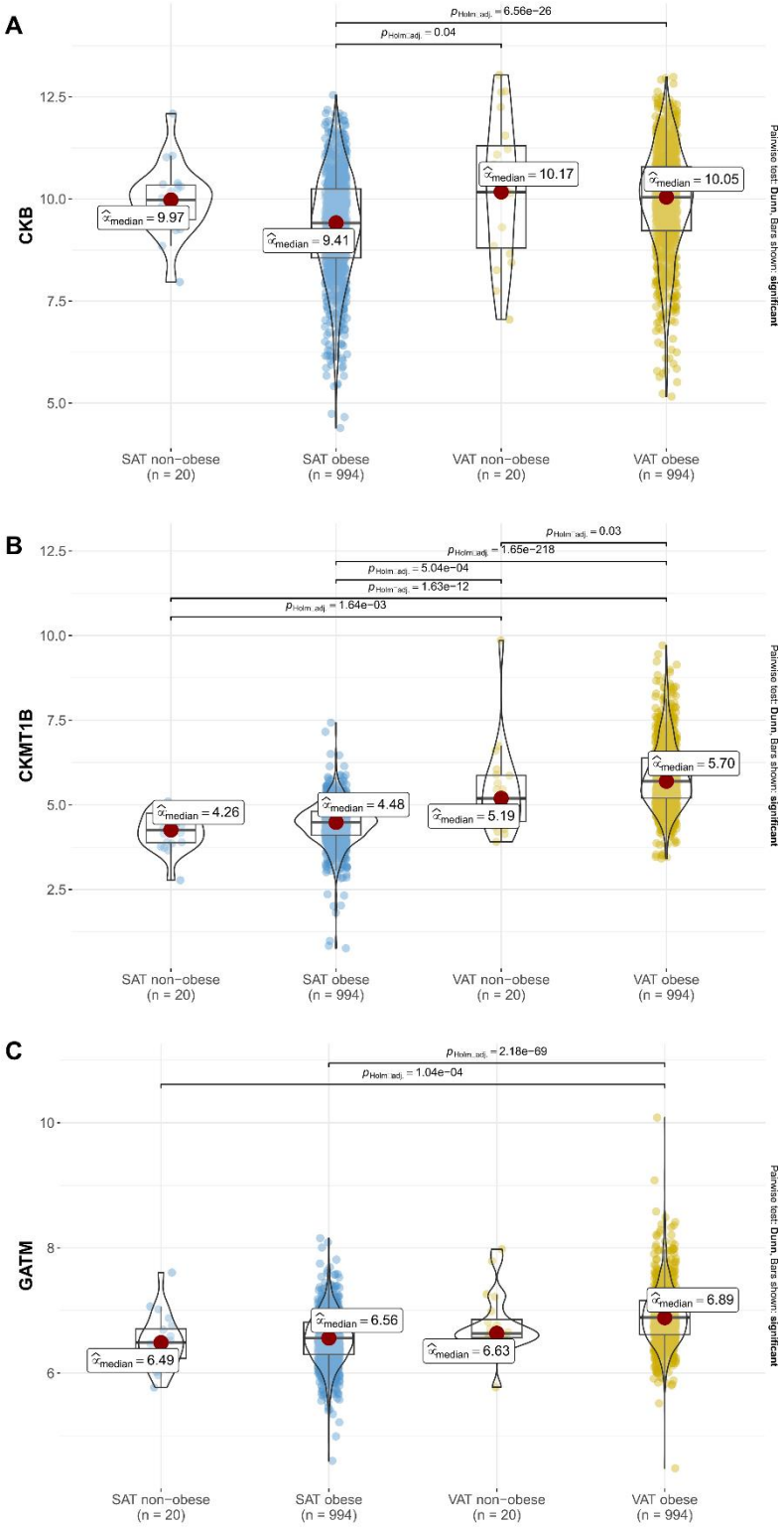


Supplementary Figure 3: Multiple testing adjusted gene correlations in visceral adipose tissue. Correlations between the three genes of interest are shown. These correlations were calculated with Pearson's correlation coefficient. P-values are adjusted for multiple inference.



Supplementary Figure 4: Adjusted correlations of *CKB*, *CKMT1B* and *GATM* in VAT and SAT in participants under 50 years of age. The present data are based on RNA-sequencing data of the Leipzig Obesity Biobank. Here, exclusively participants under the age of 50 were investigated to prevent a post-menopausal bias (n = 1,014). For each included participant, data for the visceral (A) and subcutaneous (B) adipose tissue was analysed. Correlations were calculated with Pearson's correlation coefficient and p-values were adjusted for multiple comparisons using the Hommel's method (Hommel, 1988). Positive correlations are shown in blue. Negative correlations are represented in red. The size of the dots refer to the degree of correlation. p <0.001 (***), p <0.01 (**), and p <0.05 (*). BMI: body mass index. CrP: c-reactive

protein. FPI: fasting plasma insulin. FPG: fasting plasma glucose. HbA1C: hemoglobin A1C. HDL: high-density lipoprotein. HOMA-IR: homeostatic model assessment for insulin resistance. LDL: low-density lipoprotein. NEFA: nonesterified fatty acids. WHR: waist-to-hip ratio.



Supplementary Figure 5: Gene expression of *CKB*, *CKMT1B* and *GATM* in probands with and without obesity under the age of 50. Here, the gene expression of *CKB* (A), *CKMT1B* (B) and *GATM* (C) are shown. The underlying data are based on probands with and without obesity under the age of

50 years included in the Leipzig Obesity Biobank (n = 1,014). Since the data was not always normally distributed, the non-parametric Kruskal-Wallis one-way ANOVA was performed. The respective effect size was calculated as epsilon square, while pairwise comparisons were performed with Dunn's test. P-values were adjusted based on Hommel's method (Hommel, 1988). SAT: subcutaneous adipose tissue. VAT: visceral adipose tissue.

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