

SUPPLEMENTARY INFORMATION

Calcium sensing receptor in adipose tissue: possible association with obesity-related elevated autophagy

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SUPPLEMENTARY FIGURES

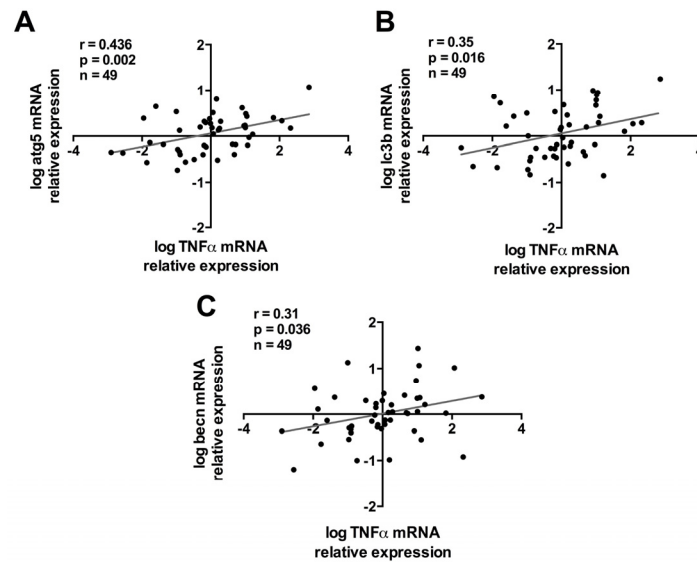


Figure S1. Adipose tissue $\text{TNF-}\alpha$ mRNA content positively correlates with donors' mRNA of autophagy markers atg5, lc3b and becn. All values expressed as log, Pearson correlation coefficient was calculated for the association between $\text{TNF-}\alpha$ mRNA and (A) atg5, (B) lc3b and (C) becn. Each graph depicts the r , and p values, as well as the number of independent donor explants analyzed.

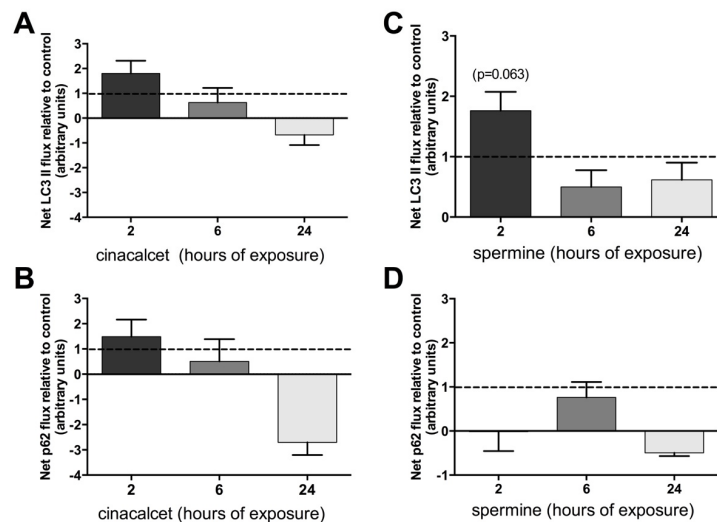


Figure S2. Net flux protein expression for LC3II or p62 was determined after 2, 6 and 24 h of $2\mu\text{M}$ cinacalcet (A and B) or $100\mu\text{M}$ spermine (C and D). LC3 and p62 abundance was normalized by β -actin and showed in representative images in Fig. 3. Differences between vehicle (1) and each treated condition were determined by Wilcoxon signed rank test. p value is presented in each bar. The flux was determined by subtraction of +CQ and -CQ condition in each time of treatment. $n=4$ (A, B and D) and $n=6$ (C) independent experiments.

SUPPLEMENTARY TABLES

Table S1. Anthropometric and biochemical characteristics of adipose tissue donors in the SEM analysis

Variables	median (p25-p75)
Subjects [M/F]	60 [17/43]
Age (years)	40.2 (33.8-47.0)
BMI (kg/m ²)	33.0 (28.9-35.0)
WC (cm)	102.7 (90.8-112.1)
BF (%)	42.6 (35.6-45.3)

M: Male, F: Female, BMI: Body Mass Index, WC: Waist circumference, BF: Body Fat.

Table S2. Primer sequences for qPCR

mRNA target	Access	Forward primer (5'→ 3')	Reverse primer (5'→ 3')
CaSR	NM_001178065	GATGAGACAGATGCCAGTGC	AAAGAGGGTGAGTGCGATCC
atg5	NM_001286106.1	AACTGAAAGGGAAGCAGAACCA	CCATTTTCAGTGGTGTGCCTTC
atg7	NM_006395.2	CGTTGCCACAGCATCATCTTC	CACTGAGGTTTACCATCCTTGG
becn	NM_003766.4	GGCTGAGAGACTGGATCAGG	CTGCGTCTGGGCATAACG
lc3a	NM_032514.3	CCAGCAAAATCCCGGTGAT	TGGTCCGGGACCAAAAACT
lc3b	NM_022818.4	ACCATGCCGTCGGAGAAG	GGTTGGATGCTGCTCTCGAA
GAPDH	NM_002046	GAAGGTGAAGGTCGGAGTCAAC	CAGAGTTAAAAGCAGCCCTGGT
TNF- α	NM_000594.2	CCAGGCAGTCAGATCATCTTCTC	AGCTGGTTATCTCTCAGCTCCAC

Table S3. Pearson's correlation coefficient for the association between mRNA CaSR versus anthropometric variables

	n	r	p
BMI	49	0.256	0.076
%BF	47	0.458	0.001
WC	42	0.166	0.292

BMI: Body Mass Index, BF: Body Fat, WC: Waist circumference

Table S4. FIT of SEM model

Test	value
χ^2	11.612(11) = 0.39
CFI	0.99
LTI	0.98
RMSEA	0.034[0, 0.156] = 0.501
SRMR	0.06

χ^2 : chi-square, CFI: comparative fit index, LTI: Tucker-Lewis index, RMSEA: root mean square error of approximation, and SRMR: standardized root mean square residual.

Table S5. Coefficients of SEM model

Latent Variables	non-std coeff. $\pm$ standard error	p value	Std. coeff.
<i>Autophagy</i>			
~ atg5	1 $\pm$ 0	NA	0.88
~ atg7	0.72 + 0.22	0.001	0.46
~ lc3a	1.4 + 0.24	0.0000	0.752
~ lc3b	1.34 + 0.31	0.0000	0.597
Regressions	non-std coeff. $\pm$ standard error	p value	Std. coeff.
<i>Autophagy</i> ~ %BF	0.01 + 0.04	0.7731	0.043
TNF- α ~ %BF	0.04 + 0.12	0.7347	0.041
CaSR ~ %BF	0.54 + 0.18	0.0032	0.350
TNF- α ~ <i>Autophagy</i>	1.46 + 0.59	0.0134	0.428
<i>Autophagy</i> ~ CaSR	0.08 + 0.03	0.0040	0.417

Note: ~ means explain for

Table S6. Residuals of SEM model

	atg5	atg7	lc3b	lc3a	TNF-α	CaSR	%BF
atg5	0.000	0.000	0.000	0.000	0.001	-0.001	0.000
atg7	0.000	0.000	-0.001	0.003	0.002	0.007	0.002
lc3b	0.000	-0.001	0.000	0.000	0.000	0.003	-0.001
lc3a	0.000	0.003	0.000	0.000	-0.011	-0.004	-0.002
TNF-α	0.001	0.002	0.000	-0.011	0.000	0.000	0.000
CaSR	-0.001	0.007	0.003	-0.004	0.000	0.000	0.000
%BF	0.000	0.002	-0.001	-0.002	0.000	0.000	0.000

Table S7. Variances of SEM model

Variable	non-std coeff. $\pm$ standard error	p value	Std. coeff.
atg5	0.002 $\pm$ 0.001	0.034	0.23
atg7	0.013 $\pm$ 0.002	0.000	0.79
lc3b	0.01 $\pm$ 0.003	0.000	0.43
lc3a	0.022 $\pm$ 0.005	0.000	0.64
TNF-α	0.065 $\pm$ 0.013	0.000	0.82
CaSR	0.163 $\pm$ 0.022	0.000	0.88
<i>Autophagy</i>	0.006 $\pm$ 0.002	0.000	0.81