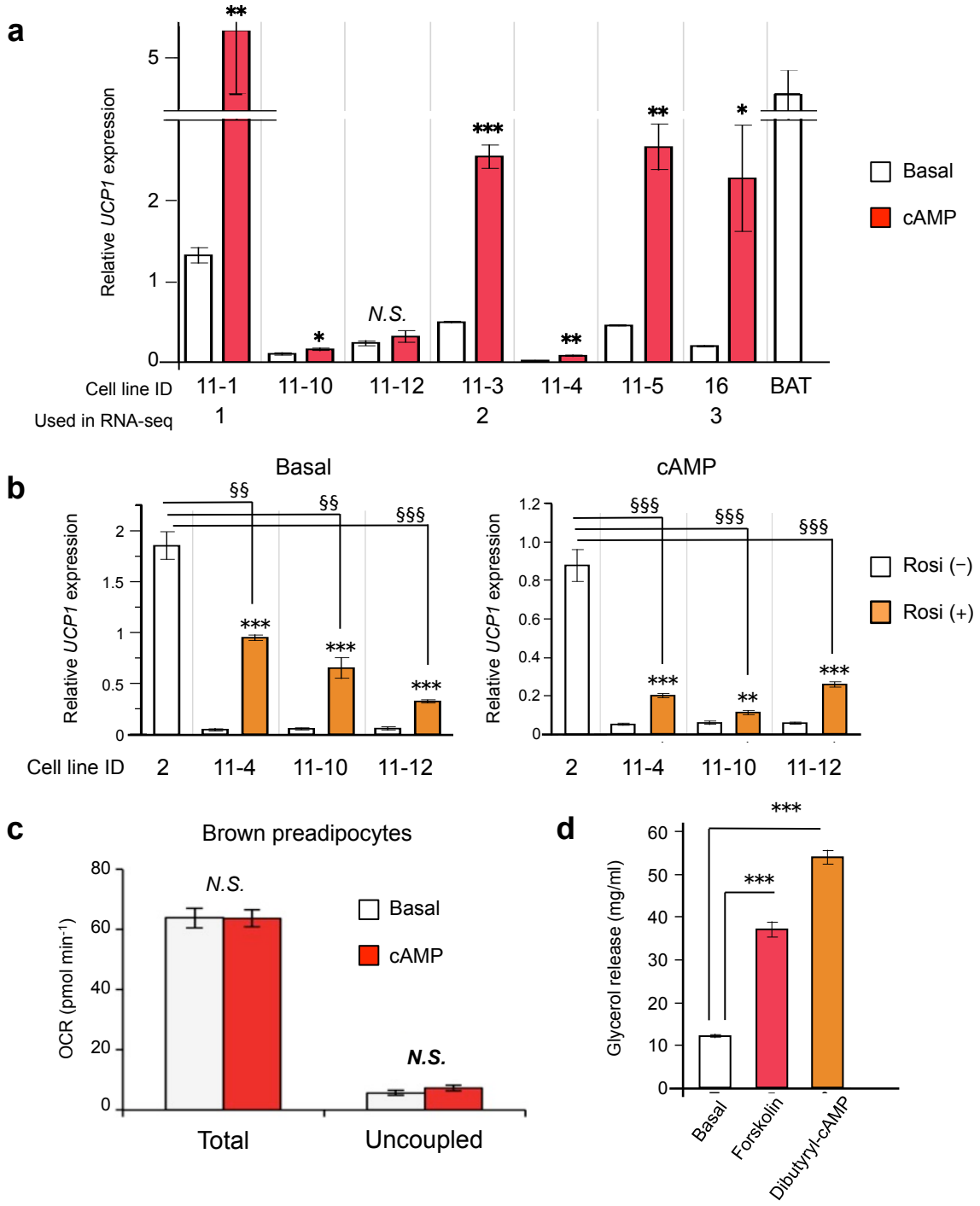
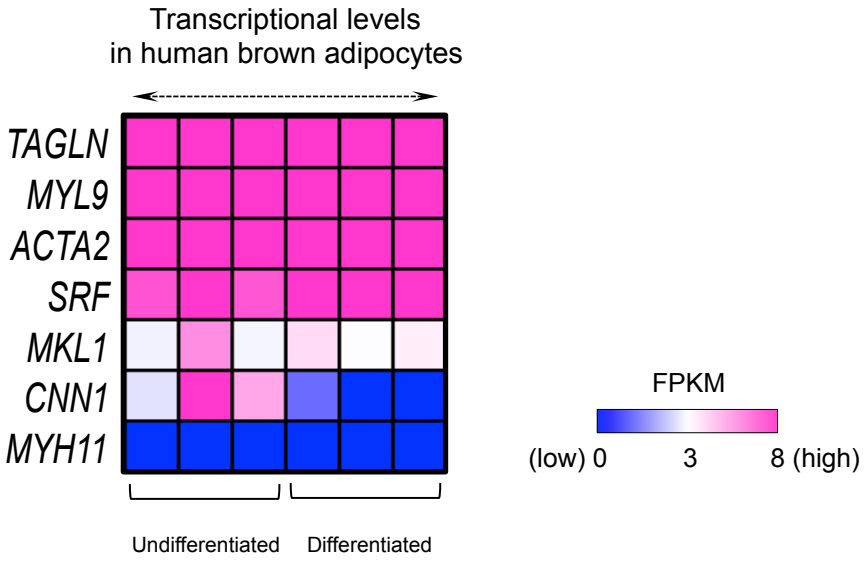


Supplementary Figure 1. Isolation of clonal brown adipocytes from adult human BAT.

(a) *UCP1* mRNA expression in differentiated clonal human adipocyte cultures treated with forskolin (cAMP) or vehicle (basal). Biopsied human BAT from the supraclavicular regions as a positive control. n=3 for all groups. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ versus non-treated (basal) cells by one-sided Welch's *t*-test. (**b**) *UCP1* mRNA expression in clonal human adipocyte cultures (as indicated) differentiated with or without rosiglitazone. n=3 for all groups. **$P < 0.01$, ***$P < 0.001$ versus cells without rosiglitazone treatment. §§$P < 0.01$, §§§$P < 0.01$ versus human brown adipocyte culture 2 by one-sided Welch's *t*-test. **(c)** Total and uncoupled cellular respiration in human brown preadipocyte line 2 treated with forskolin (cAMP) or vehicle (basal). OCR represents the rate of oxygen consumption. n=8 for each group. N.S., not significant by one-sided Student's *t*-test. **(d)** Glycerol release in differentiated human brown adipocyte culture 2 in response to cAMP stimuli (forskolin and dibutyryl-cAMP). n=3 for all groups. ***$P < 0.001$ versus non-treated (basal) cells by one-sided Welch's *t*-test. Data are expressed as means ± s.e.m. for all bar graphs.



Supplementary Figure 2. Adult human brown adipocytes express smooth muscle lineage-selective genes.

Expression levels (FPKM, fragments per kilobase of transcript per 10^6 mapped reads) of smooth muscle lineage-selective genes in human brown adipocytes in undifferentiated (left) and differentiated (right) states. n=3 for each group. mRNA expression values were normalized to white adipocytes and visualized in blue (low expression)-white-red (high expression) scheme.

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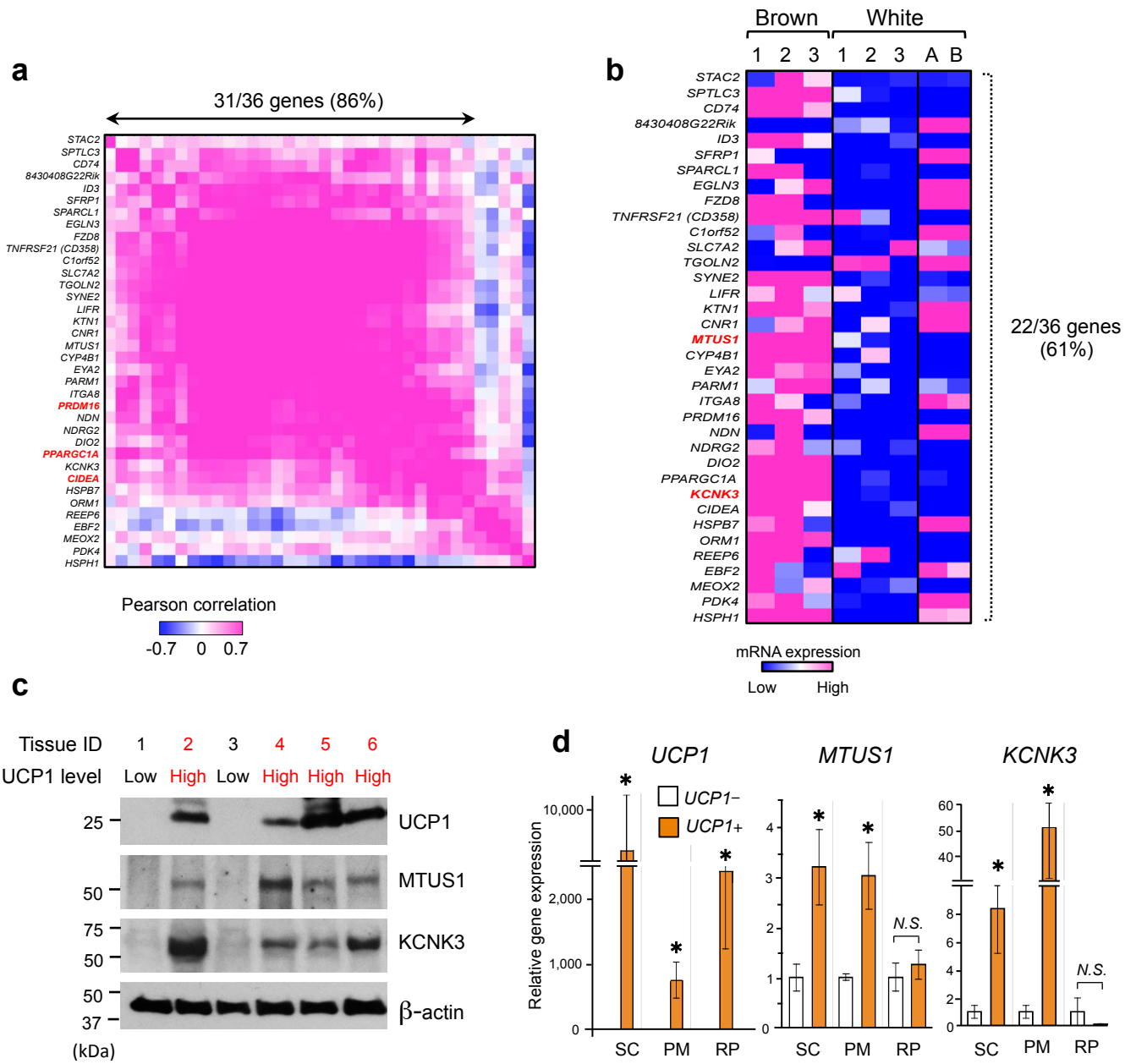
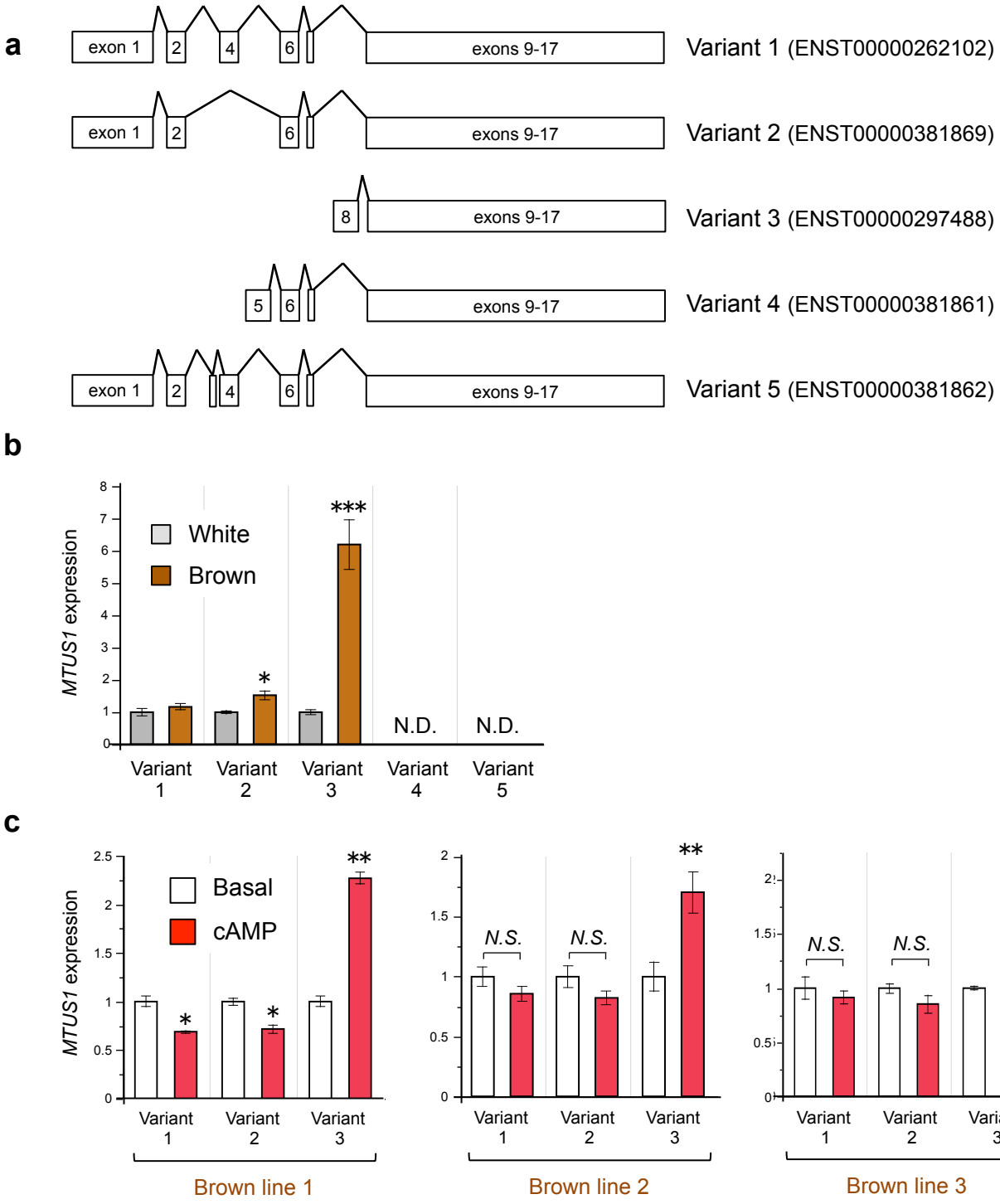


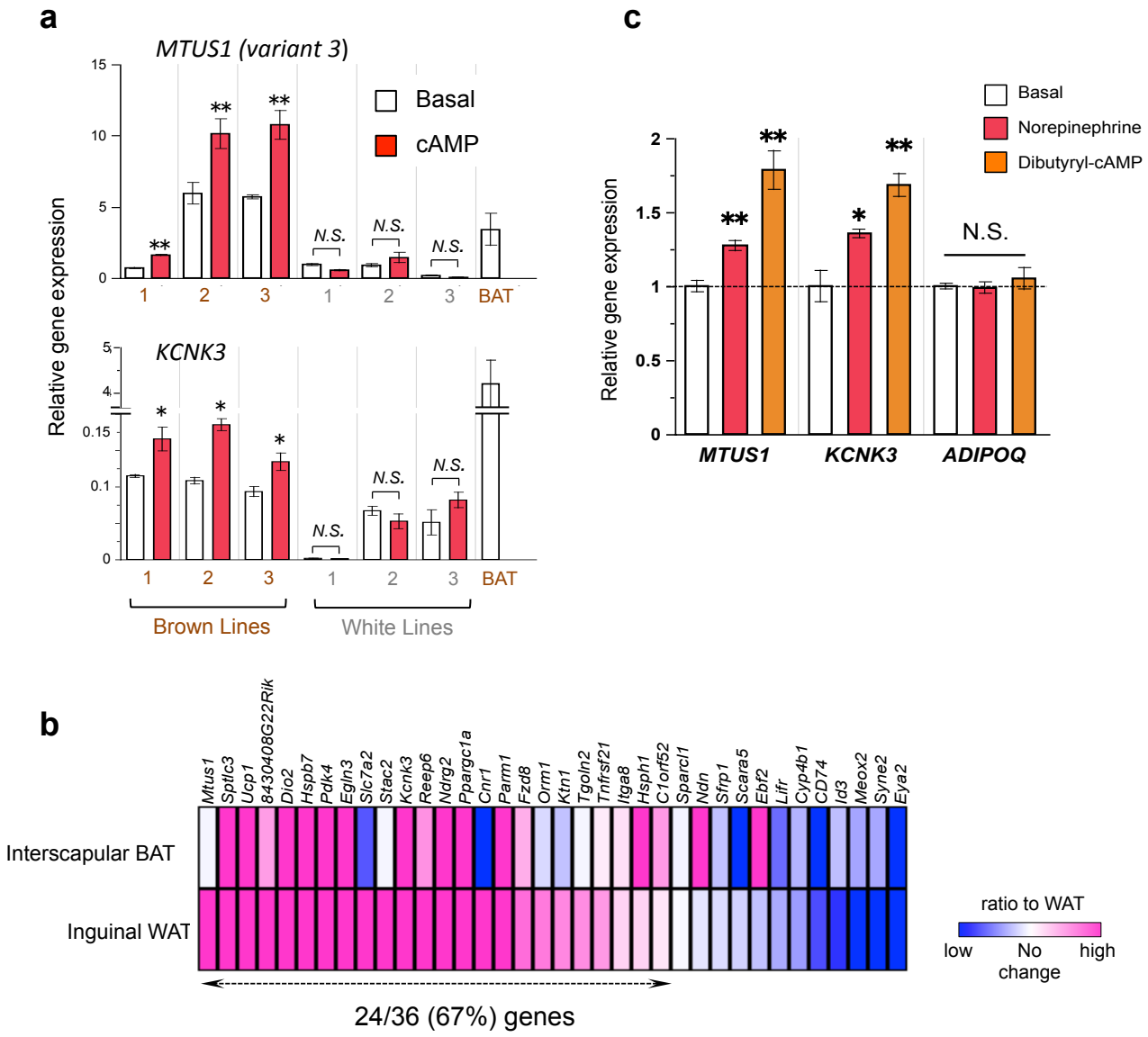
Supplementary Figure 3. Identification of human brown adipocyte markers, *MTUS1* and *KCNK3*.

(**a**) Correlation matrix of newly identified human brown adipocyte marker candidates and previously known markers (*PRDM16*, *PPARGC1A*, and *CIDEA* as highlighted by red letters). The color scale shows the Pearson correlation between two given genes in blue (negative correlation)- white (no correlation, 0)-red (positive correlation) scheme. n=23. (**b**) Expression profiles of human brown adipocyte marker gene candidates (as indicated) in three clonal human brown adipocyte cultures 1-3, three clonal human white adipocyte cultures 1-3, and primary human white adipocytes (A and B). The color scale shows z-scored FPKM representing the mRNA level of each gene in blue (low expression)-white-red (high expression) scheme. (**c**) Western blotting for UCP1, MTUS1, and KCNK3 in human supraclavicular biopsies. β-actin was used as a loading control. (**d**) Expression of *MTUS1* and *KCNK3* in UCP1-positive and UCP1-negative adipose tissues from the supraclavicular (SC), posterior mediastinum (PM), and retroperitoneal (RP) depots. SC depots, UCP1-positive tissues (n=6), UCP1-negative tissues (n=2); PM depots, UCP1-positive tissues (n=10), UCP1-negative tissues (n=3); RP depots, UCP1-positive tissues (n=10), UCP1-negative tissues (n=3). **P* <0.05, ***P* <0.01 versus UCP1-negative tissue by one-sided Welch's *t*-test. Data are expressed as means ± s.e.m. for all bar graphs.



Supplementary Figure 4. *MTUS1* variant 3 is highly enriched in brown adipocytes and responsive to cAMP stimulation.

(a) Schematic representation of *MTUS1* transcript variants. Intron–exon structures were downloaded from UniProt. **(b)** Expression of *MTUS1* transcript variants in differentiated human brown and white adipocyte cultures 1-3. n=3 for all groups. **P* <0.05, ****P* <0.001 versus human white adipocytes by one-sided Welch's *t*-test. **(c)** Expression of *MTUS1* isoforms in human brown adipocyte cultures 1-3 at basal and cAMP-stimulated states. n=3 for all groups. **P* <0.05, ***P* <0.01 versus non-treated (basal) cells by one-sided Student's *t*-test. The variance was similar between treated and non-treated cells (*P* = 0.106). Data are expressed as means ± s.e.m. for all bar graphs.



Supplementary Figure 5. Regulation of *MTUS1* and *KCNK3* expression by the cAMP pathway in brown adipocytes.

(a) Expression of *MTUS1* variant 3 and *KCNK3* in differentiated human brown and white adipocyte cultures treated with forskolin (cAMP) or vehcile (basal). n=3 for all groups. **P* <0.05, ***P* <0.01 versus non-treated (basal) cells by one-sided Welch's *t*-test. **(b)** Expression of human brown adipocyte marker candidates (as indicated) in the inguinal WAT of mice treated chronically with CL 316,243 or saline. Averaged mRNA expression values were normalized to white adipose tissue (WAT) of the same depot and visualized in blue (low)–white (no change)–red (high) scheme. mRNA expression in the interscapular BAT is shown as a reference. **(c)** Expression of *MTUS1* and *KCNK3* in differentiated human brown adipocyte culture 2 treated with norepinephrine and a cell-permeable cAMP analog, dibutyryl-cAMP. n=3 for all groups. **P* <0.05, ***P* <0.01 versus non-treated (basal) cells by one-sided Student's *t*-test. The variance was similar between treated and non-treated cells (*P* = 0.427). Data are expressed as means ± s.e.m. all bar graphs.