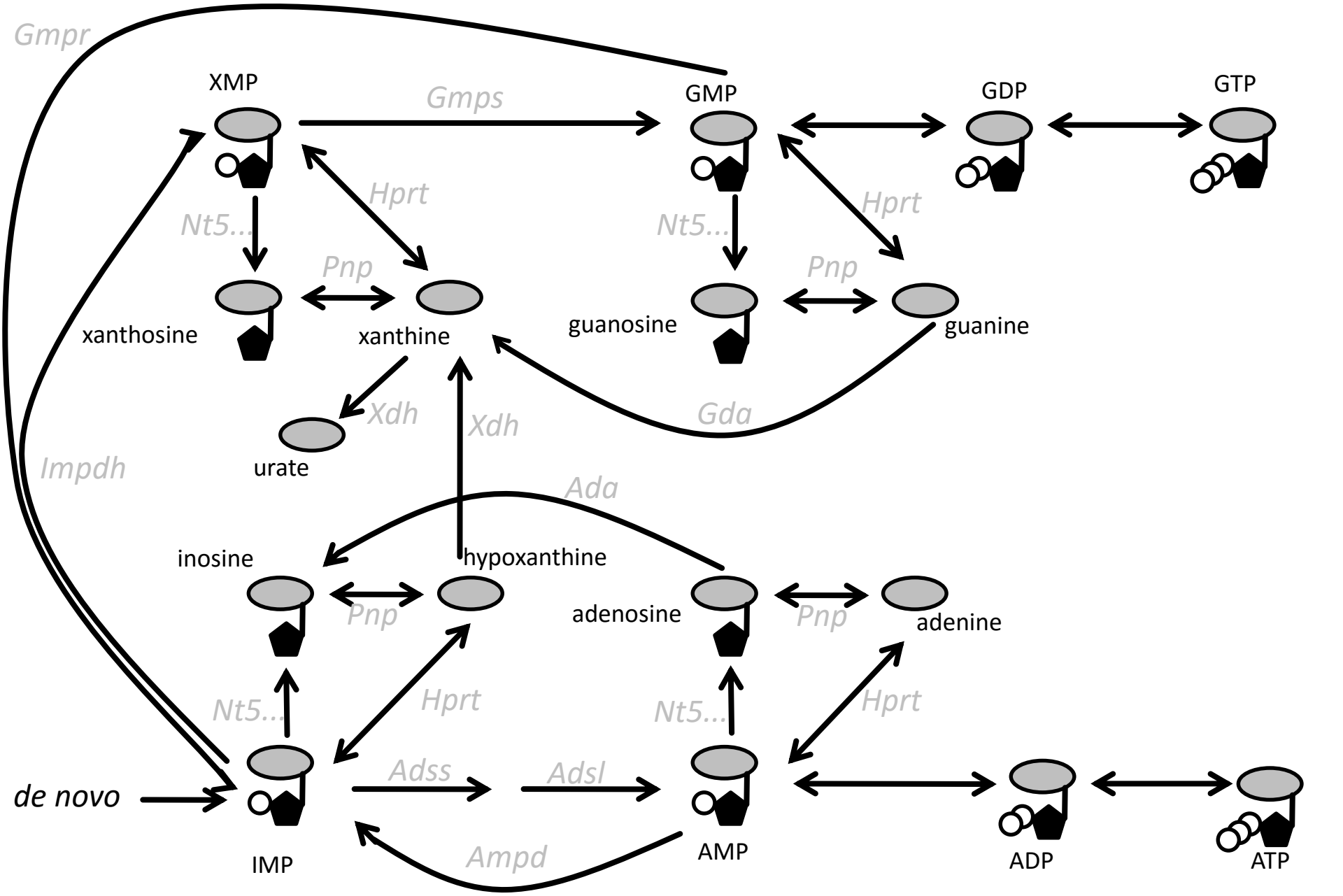
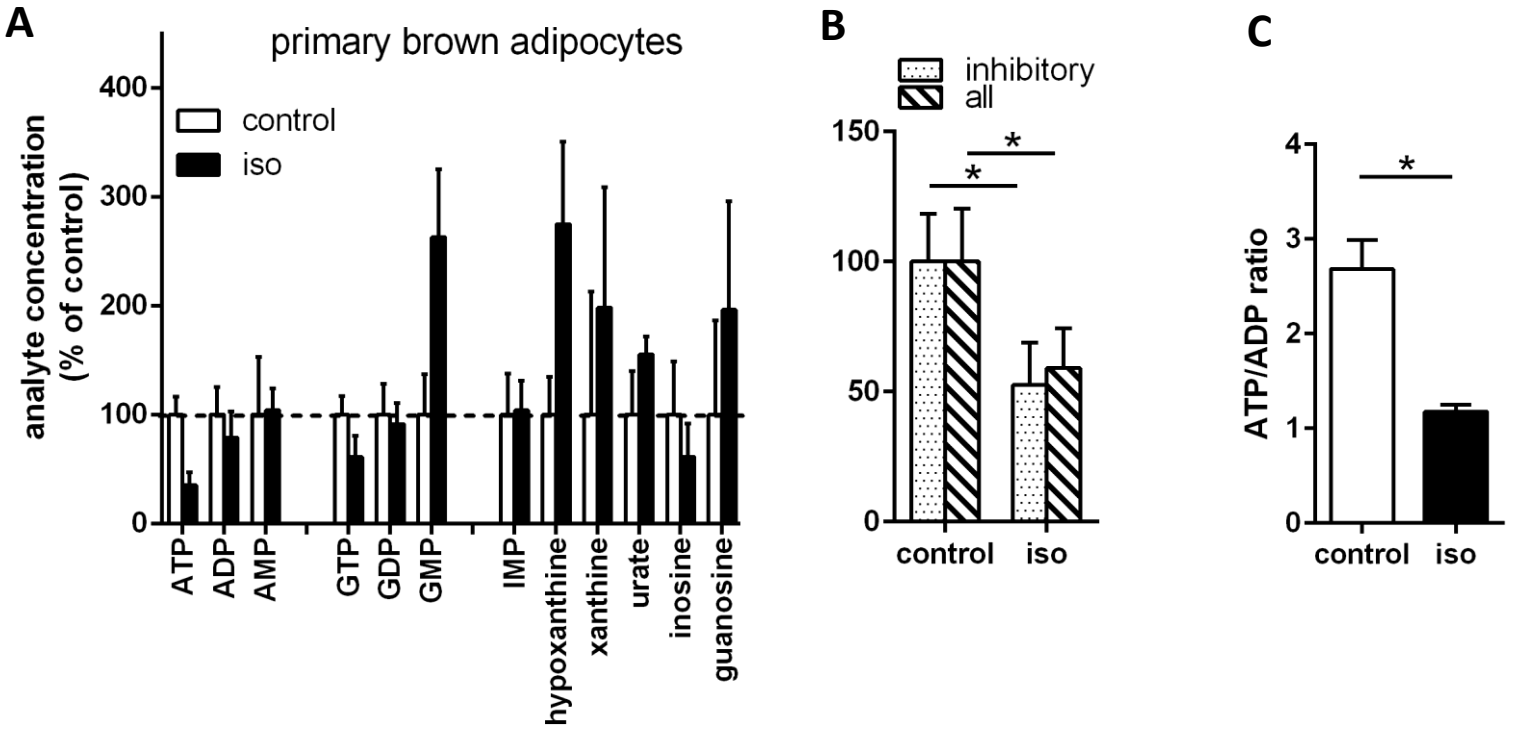
supplemental figure 1

Supplemental figure 2 – Overview of purine metabolism. Metabolite names in black, enzyme names in grey.

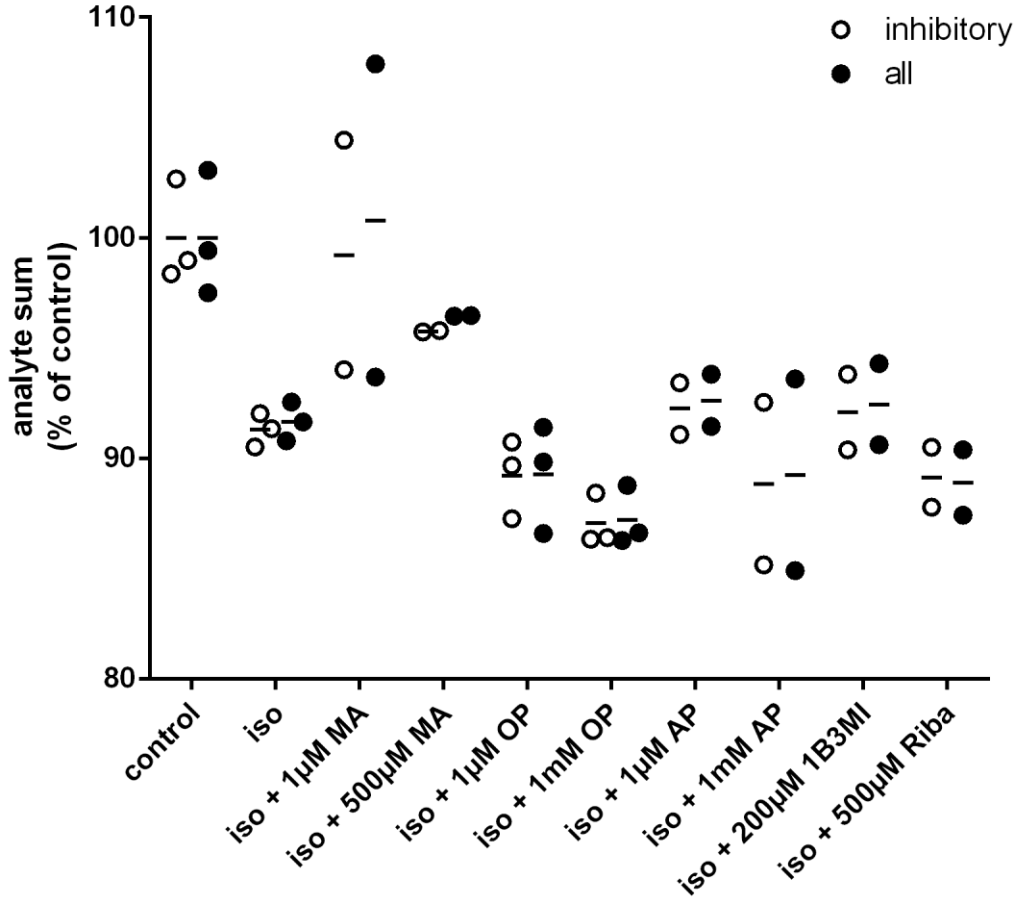


supplemental figure 2



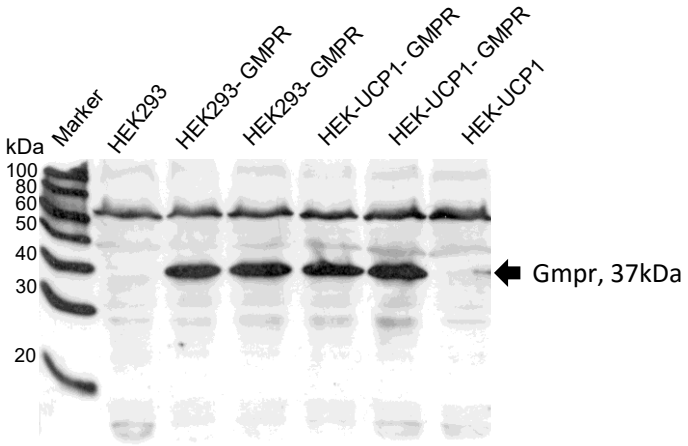
Supplemental figure 2 – Inhibitory purine nucleotides decreased in adrenergically stimulated brown fat cells. A: Cultured primary brown adipocytes were treated with 10µM isoproterenol (iso) for 1h, lysed and analyzed. Purine metabolite concentrations of primary brown adipocyte are depicted as a mean percentage of control ± SD, n=3. **B:** Total molar sum of all purine nucleotides (ATP, ADP, AMP, GTP, GDP, GMP) and of Ucp1 inhibiting nucleotides (ATP, ADP, GTP, GDP) as a mean percentage of control ± SD, n=3. **C:** Ratio between ATP and ADP ± SD, n=3. Stars in B and C indicate a significant difference between control and isoproterenol treated cells, $p < 0.05$.

supplemental figure 3



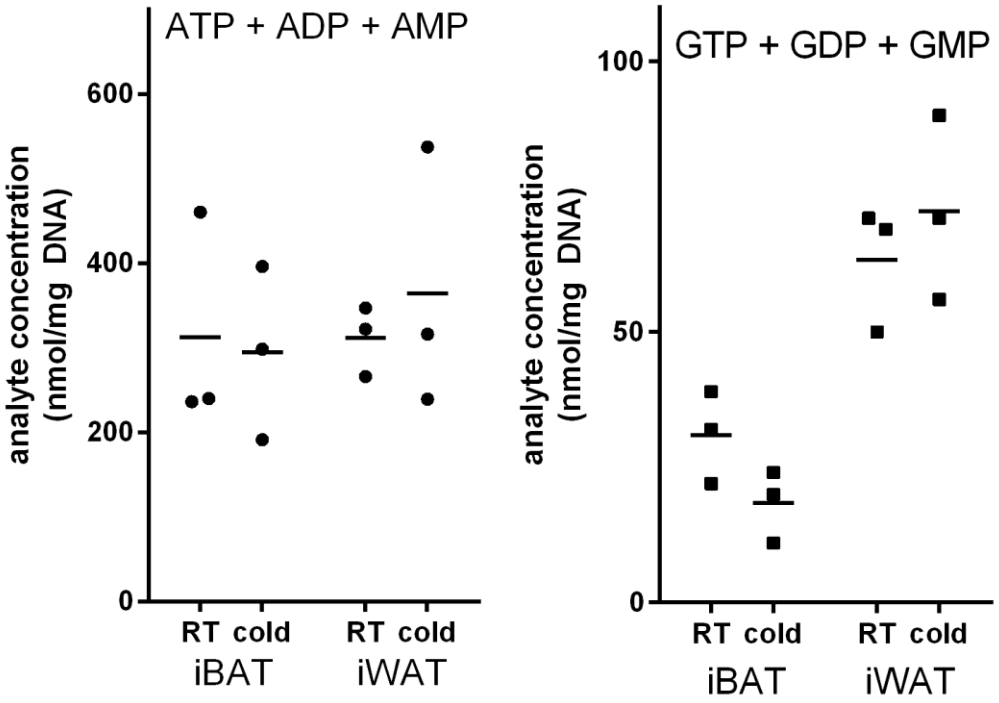
Supplemental figure 3 – Mycophenolic acid infered in isoproterenol-induced decrease of inhibitory and total purine nucleotides . Cultured primary brown adipocytes were treated with 10µM isoproterenol (iso) for 1h in the repsence or absence of inhibitors of purine metabolic enzymes, lysed and analyzed. The total molar sum of all purine nucleotides (ATP, ADP, AMP, GTP, GDP, GMP) and of Ucp1 inhibiting nucleotides (ATP, ADP, GTP, GDP) are depicted as a mean percentage of control ± SD, n=2-3. MA – mycophenolic acid, OP – oxypurinol, AP – allopurinol, 1B3MI – 1-butyl-3-methylimidazole, Riba – Ribavirin.

supplemental figure 4



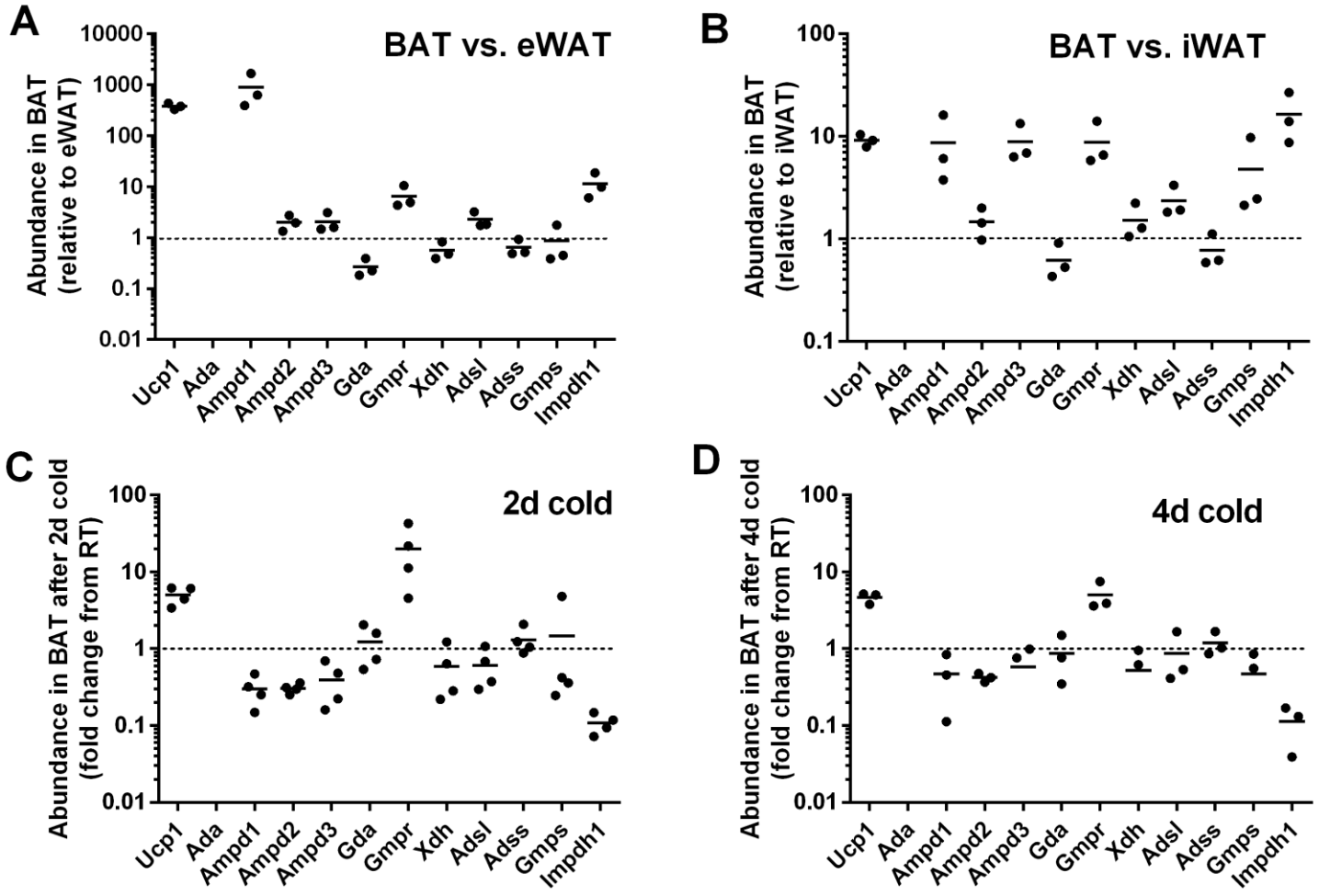
Supplemental figure 4 – Forced expression of Gmpr in HEK293 cells led to expression of Gmpr protein. We transiently transfected HEK293 cells or HEK293 cells expression Ucp1 with 10µg of a Gmpr expression construct. Cells were harvested and subjected to Western Blot analysis. The expected band size is indicated. The upper band in non-specific.

supplemental figure 5



Supplemental figure 5 – Purine pool sizes in vivo. We harvested interscapular brown adipose tissue (iBAT) and inguinal white adipose tissue (iWAT) from mice kept at room temperature (RT) or exposed to 5°C for 24 hours (cold). We analyzed nucleotide metabolite concentrations. Individual metabolites (e.g. ATP) levels varied enormously between biological replicates. Assuming the source of this variability to be very rapid post mortem changes mostly in phosphorylation state, we added amounts of ATP+ADP+AMP and GTP+GDP+GMP as a surrogate measure of the respective pool sizes.

supplemental figure 6



Supplemental figure 6 – Expression of purine metabolism genes in brown fat. We analyzed a publicly available transcriptomic dataset (GEO accession GSE63031; by Q Hao and HB Hansen, Copenhagen, Denmark) generated by deep-sequencing of transcripts isolated from murine adipose tissues. **A:** Brown adipose tissue (BAT) transcript abundance relative to epididymal white adipose tissue (eWAT). **B:** BAT transcript abundance relative to inguinal white adipose tissue (iWAT). **C:** Fold change in BAT transcript abundance after 2 days of cold exposure. **D:** Fold change in BAT transcript abundance after 4 days of cold exposure.