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Supplemental Information

Early B Cell Factor Activity Controls Developmental and Adaptive Thermogenic Gene Programming in Adipocytes

Anthony R. Angueira, Suzanne N. Shapira, Jeff Ishibashi, Samay Sampat, Jaimarie Sostre-Colón, Matthew J. Emmett, Paul M. Titchenell, Mitchell A. Lazar, Hee-Woong Lim, and Patrick Seale

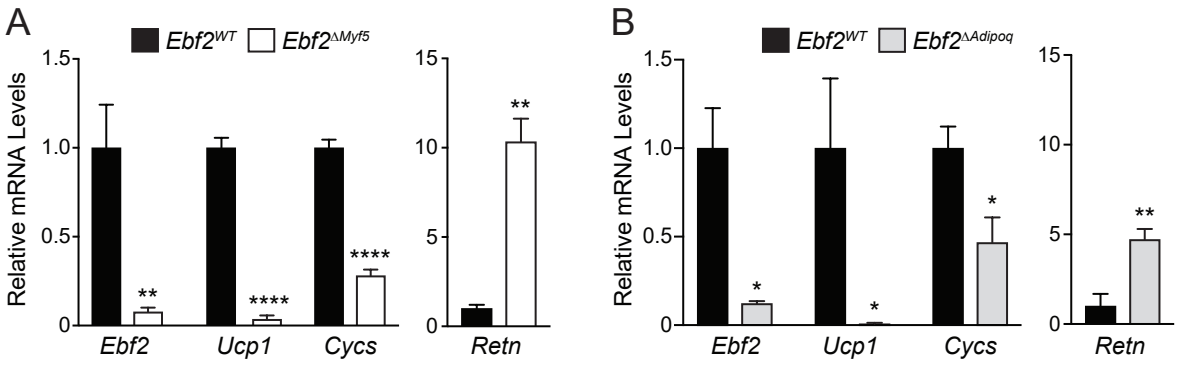

Figure S1. Adipocyte-EBF2 controls thermogenic gene expression during BAT development (Related to Fig.1)

A, B) Relative mRNA levels of *Ebf2*, brown fat genes (*Ucp1*, *Cycs*) and the white adipocyte marker *Retn* in embryonic BAT (E17-E19) from: (**A)** *Ebf2*^{WT} and *Ebf2*$^{\Delta Myf5}$; *and* **(B)** *Ebf2*^{WT} and *Ebf2*$^{\Delta Adipoq}$ mice. (n= 3-6 mice per group; Mean+/-SEM).

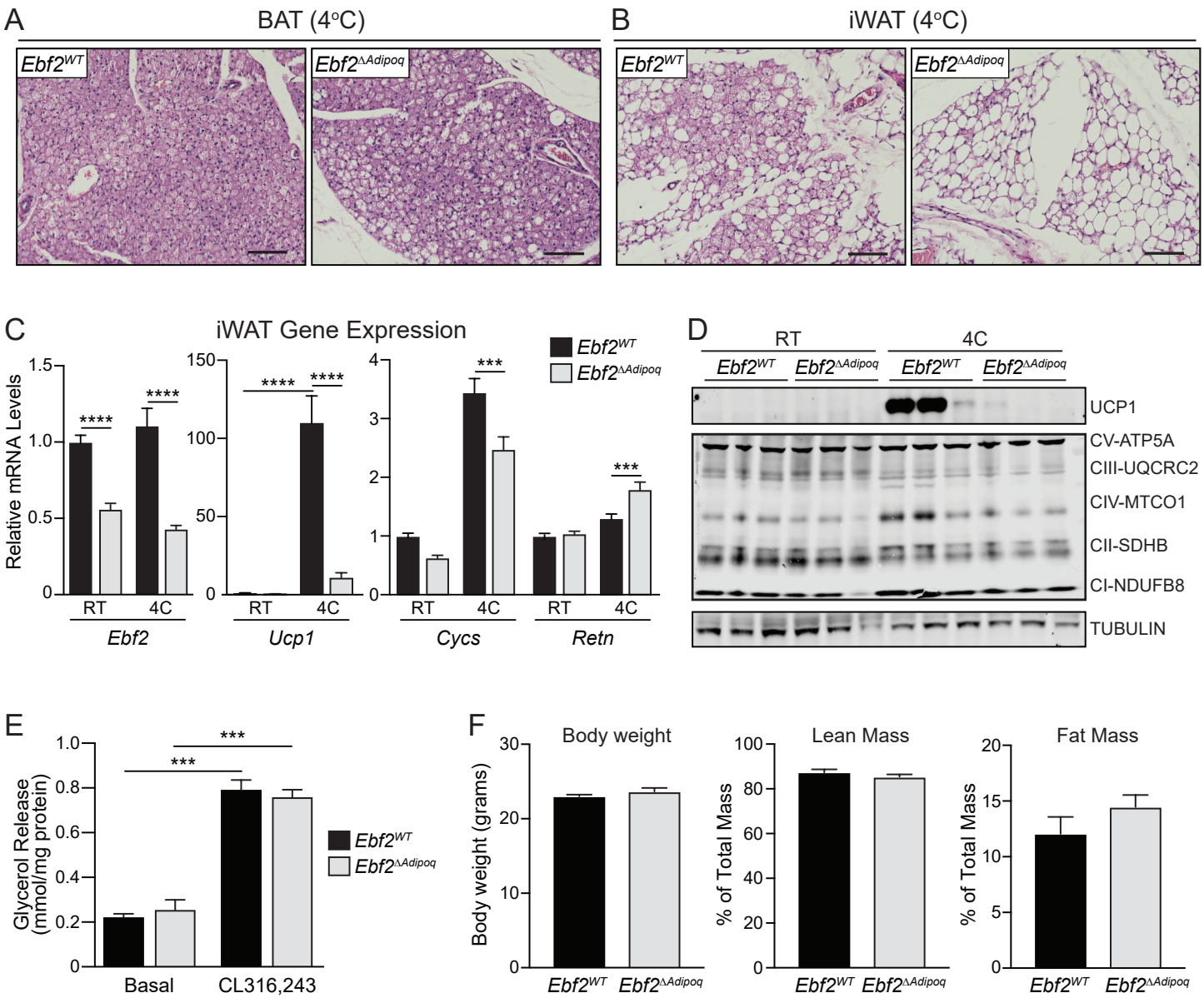

Figure S2. EBF2 is required for cold-induced beiging of inguinal WAT (Related to Fig. 2).

A,B) Hematoxylin and eosin staining of BAT **(A)** and inguinal WAT (iWAT, **B)** from *Ebf2*^{WT} and *Ebf2*$^{\Delta Adipoq}$ mice following exposure to 4°C for 1 week (Scale Bar=100 μm).
C) Relative mRNA levels of *Ebf2*, brown fat genes (*Ucp1*, *Cycs*) and the white adipocyte marker *Retn* in iWAT from *Ebf2*^{WT} and *Ebf2*$^{\Delta Adipoq}$ mice housed at room temperature (RT) or following cold exposure (4°C) for 1 week (n=9-11 mice per group; Mean+/-SEM).
D) Western Blot analysis of UCP1 and mitochondrial respiratory chain components in iWAT from *Ebf2*^{WT} and *Ebf2*$^{\Delta Adipoq}$ mice housed at RT or exposed to 4°C for 1 week.
E) Glycerol release (lipolysis) from eWAT of *Ebf2*^{WT} (n=4) and *Ebf2*$^{\Delta Adipoq}$ (n=6) mice (Mean+/-SEM).
F) Body weight and composition (NMR) of *Ebf2*^{WT} (n=4) and *Ebf2*$^{\Delta Adipoq}$ (n=6) housed at RT (Mean+/-SEM).

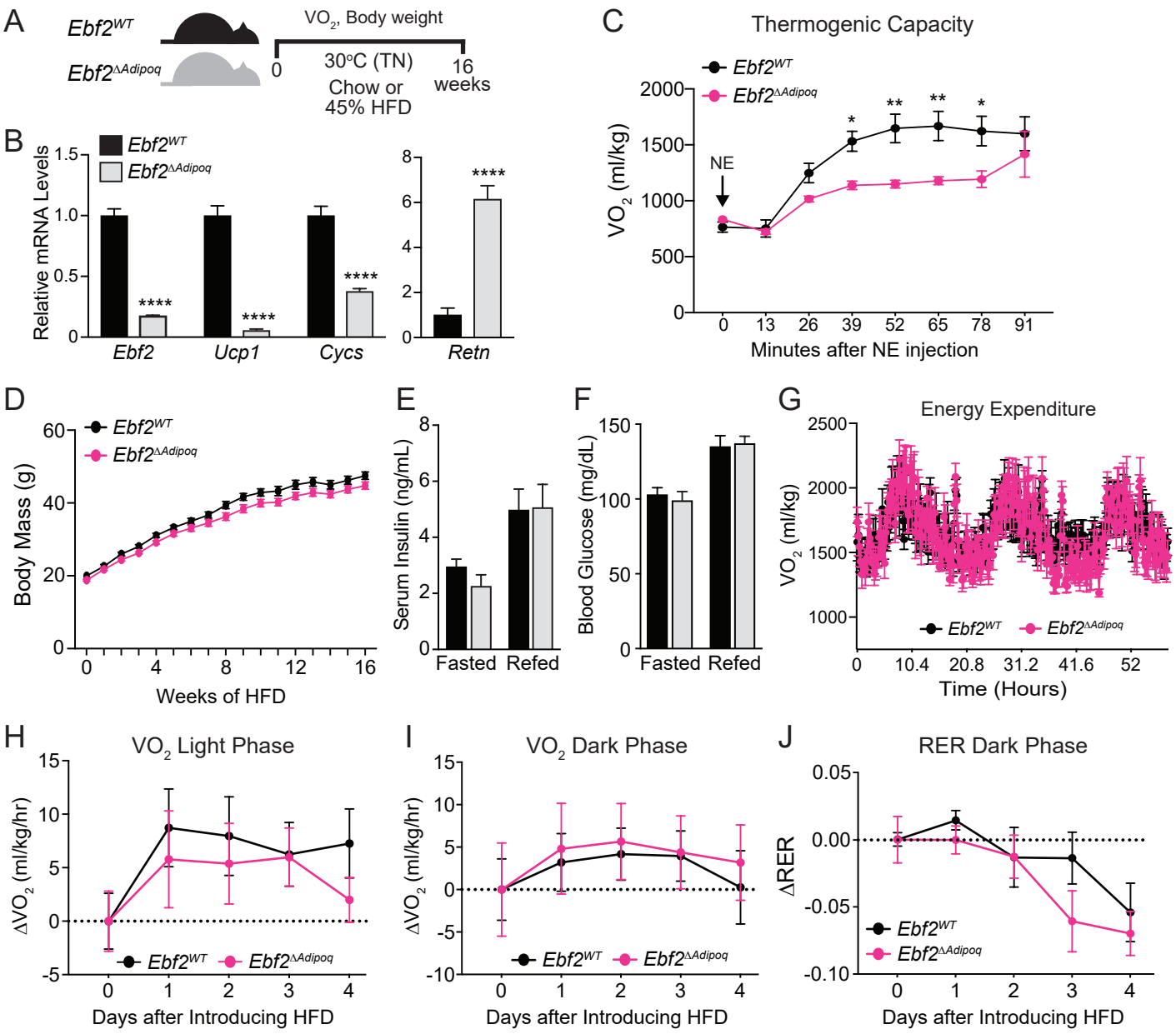

Figure S3. Adipocyte EBF2 is dispensable for high fat diet-induced thermogenesis (Related to Fig. 2).

A) Experimental Paradigm. Adult *Ebf2*^{WT} and *Ebf2*$^{\Delta Adipoq}$ mice were housed at/near thermoneutrality (TN, 30°C) and fed either normal chow or 45% high fat diet (HFD) for 16 weeks.
B) Relative mRNA levels of *Ebf2*, brown fat genes (*Ucp1*, *Cycs*) and the white adipocyte marker *Retn* in BAT from mice in **(A)**. (n=8-13 mice per group; Mean+/-SEM).
C) Oxygen consumption (VO_2) following norepinephrine (NE) injection in *Ebf2*^{WT} and *Ebf2*$^{\Delta Adipoq}$ mice housed at TN and fed a HFD for 10 weeks (n=6 mice per group; Mean+/-SEM).
D) Body weights of *Ebf2*^{WT} (n=11) and *Ebf2*$^{\Delta Adipoq}$ (n=15) mice during HFD feeding at TN (Mean+/-SEM).
E,F) Blood insulin **(E)** and glucose **(F)** levels in *Ebf2*^{WT} (n=8) and *Ebf2*$^{\Delta Adipoq}$ (n=13) mice (fed a HFD for 10 wk) after an overnight fast and following re-feeding for 2 hr (Mean+/-SEM).
G) Basal VO_2 of *Ebf2*^{WT} and *Ebf2*$^{\Delta Adipoq}$ mice following 10 weeks of HFD. (n=6 mice per group; Mean+/-SEM).
H,I) Change in average VO_2 of *Ebf2*^{WT} and *Ebf2*$^{\Delta Adipoq}$ mice in response to introduction of HFD during the light **(H)** and dark phase **(I)**. (n=6 mice per group; Mean+/-SEM)
J) Change in average respiratory exchange ratio (RER) of *Ebf2*^{WT} and *Ebf2*$^{\Delta Adipoq}$ mice following introduction of HFD (during dark phase) (n=6 mice per group; Mean+/-SEM).

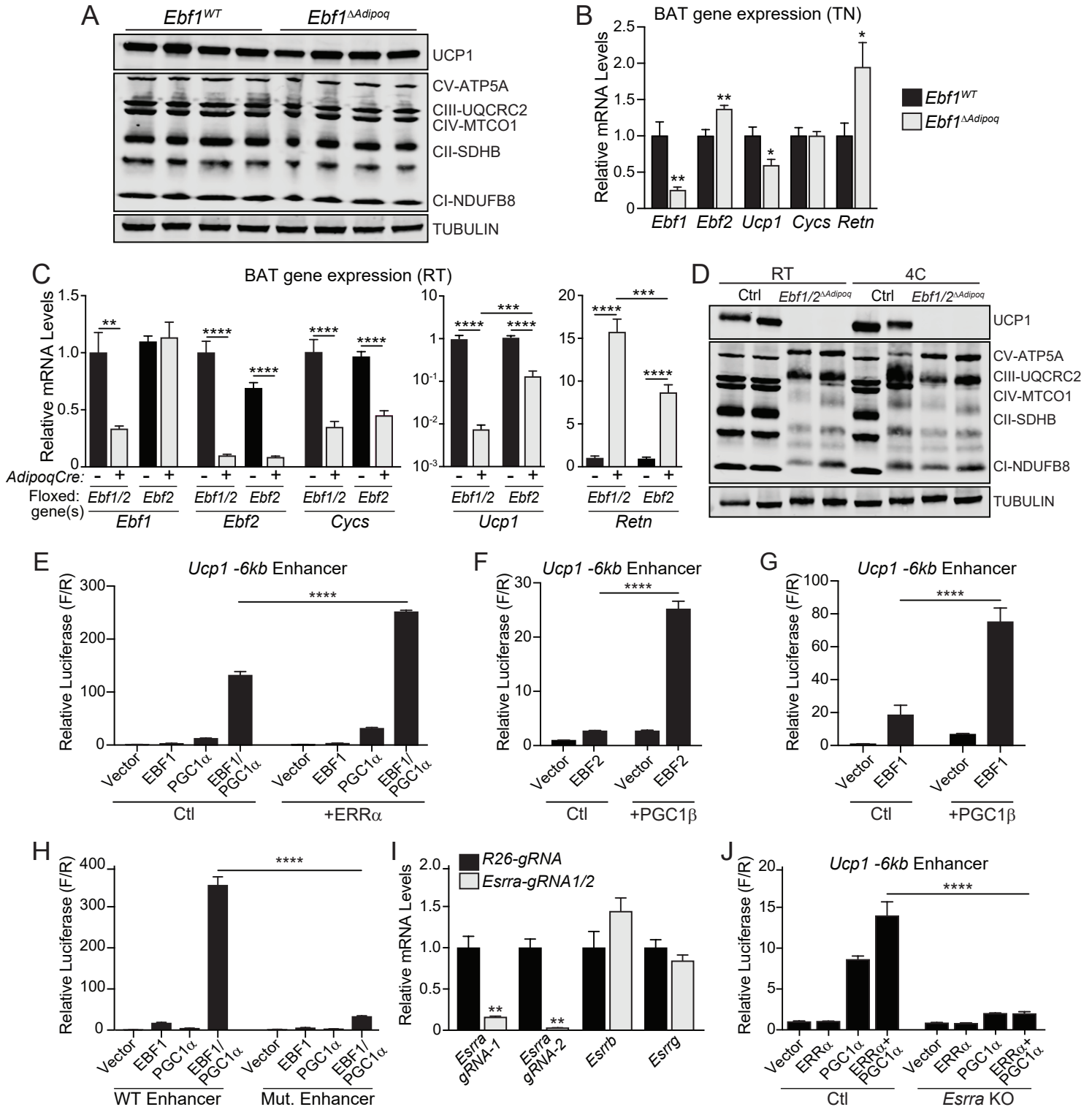

Figure. S4. EBF1 or EBF2 are required for cold-induced BAT recruitment (Related to Figs 3, 4).

A) Western blot analysis of UCP1 and mitochondrial protein levels in BAT from *Ebf1*^{WT} and *Ebf1*$^{\Delta Adipoq}$ mice at room temperature (RT). **B)** mRNA levels of indicated genes in BAT from *Ebf1*^{WT} and *Ebf1*$^{\Delta Adipoq}$ mice housed at 30°C for 1 month. (n=5 mice per group; Mean+/-SEM). **C)** mRNA levels of indicated genes in BAT from *Ebf1/2*^{WT}, *Ebf1/2*$^{\Delta Adipoq}$, *Ebf2*^{WT} and *Ebf2*$^{\Delta Adipoq}$ mice housed at RT (n=3-6 mice per group; Mean+/-SEM). **D)** Western blot analysis of UCP1 and mitochondrial protein levels in BAT from *Ebf1/2*^{WT} and *Ebf1/2*$^{\Delta Adipoq}$ mice housed at RT or 4°C for 1 wk. **E-G)** Activity of the *Ucp1 -6kb* enhancer upon expression of indicated transcription factors. (n=3-6; Mean+/-SEM). **H)** Activity of the wildtype (WT) or Mutant (Mut) *Ucp1 -6kb* enhancer upon expression of EBF1 and PGC1α (n=3; Mean+/-SEM). **I)** mRNA levels of *Essra*, *Essrb* and *Essrg* in NIH3T3 cells following deletion of *Esrra* or *Rosa26* (*R26*) as control (Ctl) using a lentiviral Crispr/Cas9 system. Two gRNAs were designed to target *Esrra* and RT-PCR was used to detect the targeted regions of the transcript (gRNA-1 and -2). (n=3; Mean+/-SEM). **J)** Activity of the *Ucp1 -6kb* enhancer in control (Ctl, *R26-gRNA*) and *Essra* knockout (KO) cells upon expression of ERRα and/or PGC1α. (n=3; Mean+/-SEM).