

SUPPLEMENTARY DATA

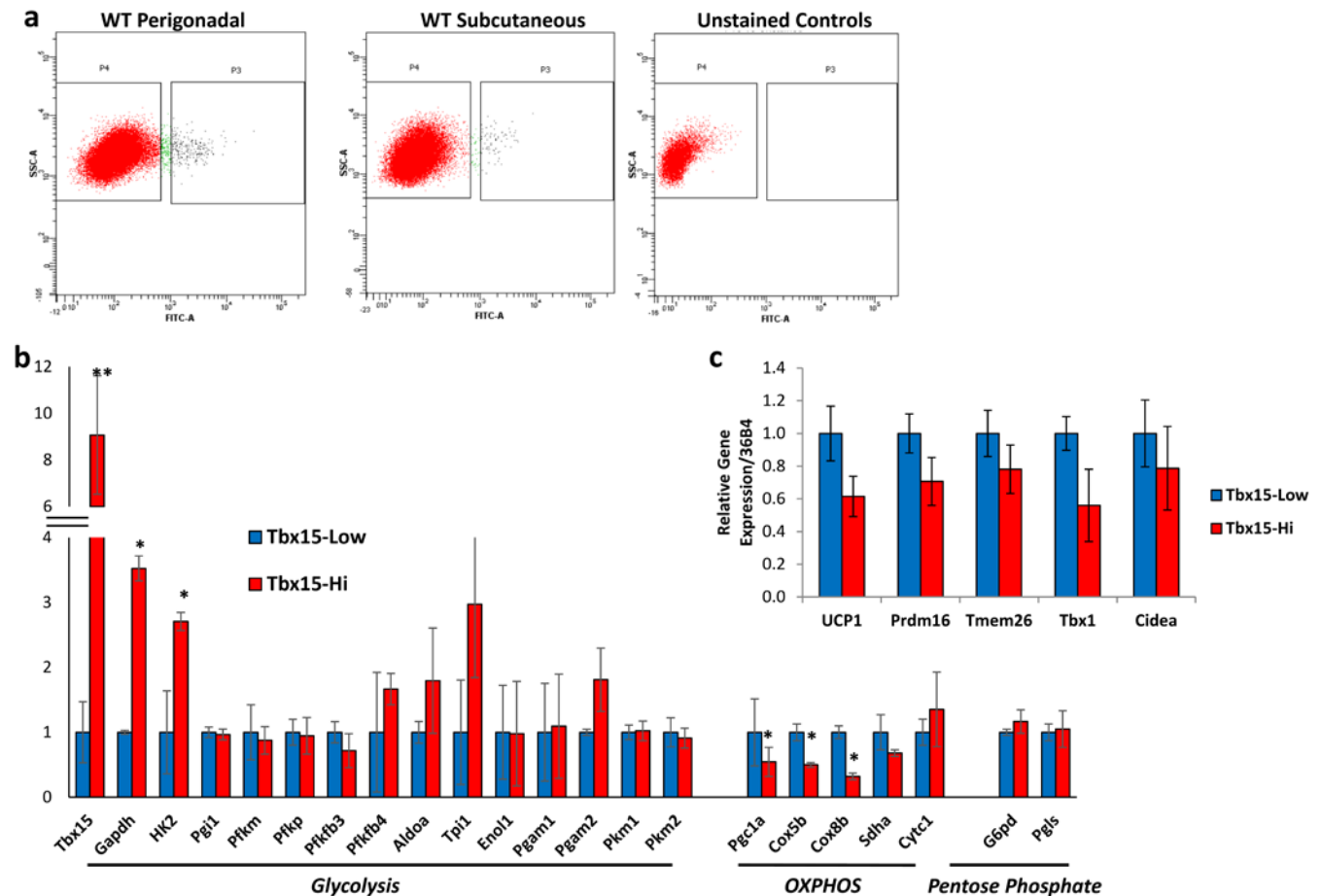
**Supplementary Figure 1. FACS and qPCR controls for  $Tbx15^{Hi}$  and  $Tbx15^{Low}$  adipocytes.**

a) Representative FACS analyses controls. Unstained and LacZ (+) APCs were sorted from WT male mice at 6-8 weeks of age.

b) qPCR analysis for  $Tbx15$ , Glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*), Hexokinase 2, glucose-6-phosphate isomerase (*Pgi1*), phosphofructokinase, muscle (*Pfkm*), phosphofructokinase, platelet (*Pfkb*), 6-Phosphofructo-2-Kinase/Fructose-2,6-Biphosphatase 3 and 4 (*Pfkb3*, *Pfkb4*), Aldolase A (*Aldoa*), Triosephosphate isomerase (*Tpi1*), Enolase (*Enol1*),

Pyruvate kinase1 and 2 (*Pkm1* and *Pkm2*), Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*Pgc1a*), Cytochrome C oxidase subunit 5b and 8b (*Cox5b* and *8b*), Cytochrome c (*Cytc*), Succinate Dehydrogenase Complex, Subunit A (*Sdha*), *Glucose-6-phosphate dehydrogenase* (*G6pd*), 6-phosphogluconolactonase (*Pgls*) in RNA isolated from  $Tbx15^{Hi}$  and  $Tbx15^{Low}$  adipocytes after *in vitro* differentiation. Data are shown as mean  $\pm$  SEM of 4 animals per group.

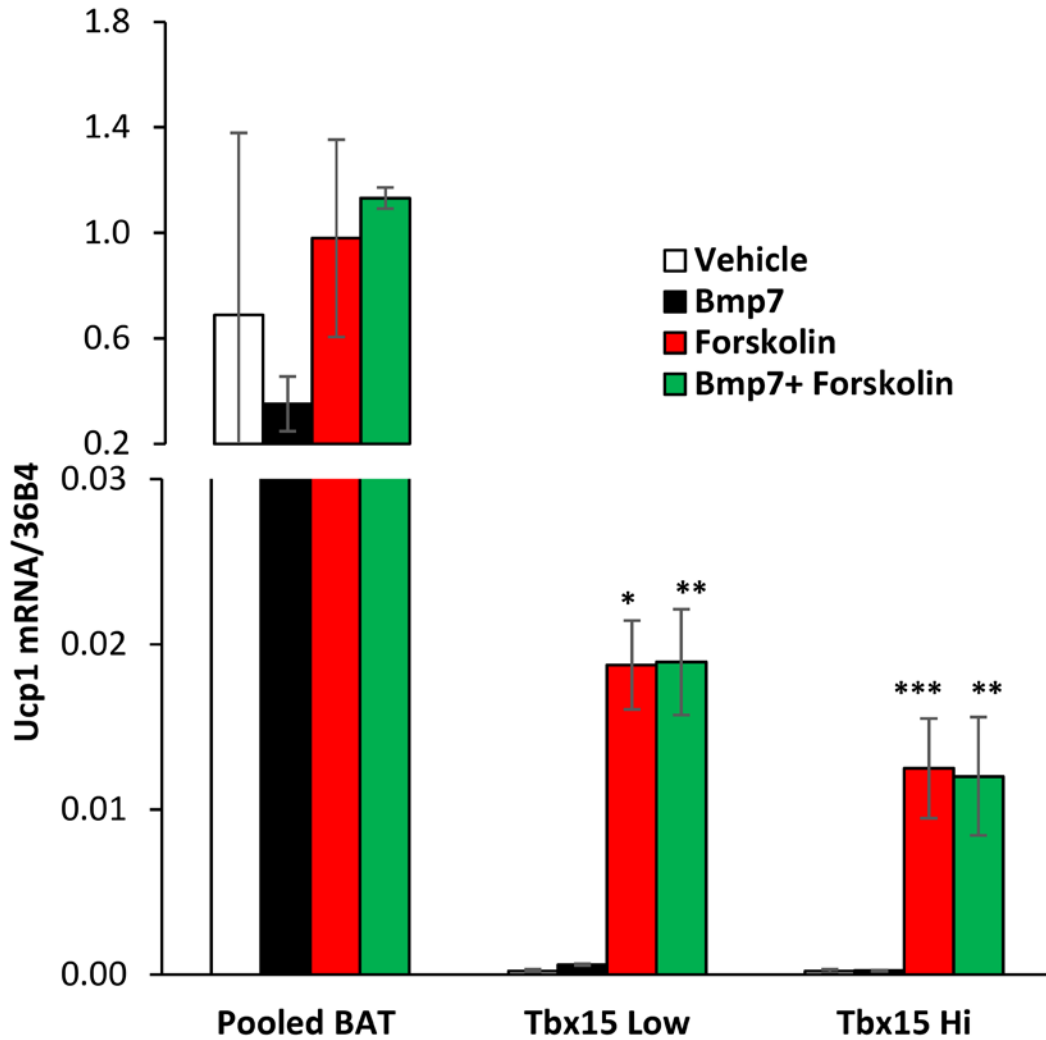
c) qPCR analysis for markers of brown/brite fat markers: *Ucp1*, *Prdm16*, *Tmem26* and *Tbx1*, and *Cidea* in RNA isolated from  $Tbx15^{Hi}$  and  $Tbx15^{Low}$  adipocytes after *in vitro* differentiation. Data are shown as mean  $\pm$  SEM of 4 animals per group.



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**Supplementary Figure 2. Ucp1 expression is not induced in Tbx15<sup>Hi</sup> adipocytes**

qPCR analysis for Ucp1 in RNA isolated from Immortomouse clonal brown, Tbx15<sup>Hi</sup>, and Tbx15<sup>Low</sup> adipocyte cell lines after *in vitro* differentiation. Cells were treated ± 3.3 nM BMP7 for 3 days prior to differentiation and acutely treated ± 10 µM forskolin for 4 hours. Data are shown as mean ± SEM of 3-15 cell lines per group.



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### **Supplementary Figure 3. Seahorse controls for pBABE-Tbx15 adipocytes and analysis of shTbx15 knockdown cell lines.**

a-b) Measurements of extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) in the basal state and after treatment with 125 mM 2-deoxyglucose in pBABE-Empty and pBABE-Tbx15 3T3-L1 adipocytes was determined by calculating the area under the curve (AUC) over three readings. The whole experiment was repeated three times. Data are shown as mean  $\pm$  SEM of 10 cell lines per group and the experiment was repeated three times. Asterisks indicate a significant differences in all panels \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

c) qPCR analysis for *Pfkfb1*, *Pfkfb2*, *Aldoa*, *Aldoc*, *Pgam1*, *Pgam2*, *Pkm2*, *Sdha*, *Cox7b*, *Cox8b*, *G6pd*, and *Pgls* mRNA in pBABE-Empty and pBABE-Tbx15 3T3-L1 cells after adipocyte differentiation. Data are mean  $\pm$  SEM of six independent samples.

d) qPCR analysis for *Tbx15* mRNA was compared between 3T3-L1 cells stably transfected with in shGFP and shTbx15 lentiviral vectors before and after adipocyte differentiation. Western blot analysis for Tbx15 protein in these cell lines was performed prior to differentiation. Data are mean  $\pm$  SEM of triplicate samples, and the experiment was repeated three times.

e) Oil Red O Staining of shGFP and shTbx15 3T3-L1 adipocytes after *in vitro* differentiation. The photographs were taken at 20x magnification. Quantitation of triglycerides from shGFP and shTbx15 adipocytes after *in vitro* differentiation. Data are shown as mean  $\pm$  SEM of 10 replicates per group.

f) qPCR analysis for *Adipoq*, *Ppar $\gamma$* , and *aP2* in RNA isolated from shGFP and shTbx15 adipocytes after six days of *in vitro* differentiation. Data are mean  $\pm$  SEM of triplicate samples, and the experiment was repeated two times.

g) Basal and maximal extracellular acidification rate (ECAR) of shGFP and shTbx15 3T3-L1 adipocytes was determined by calculating the AUC during measurements of basal and maximal ECAR. Maximal ECAR was measured after treatment of adipocytes with 1  $\mu$ M oligomycin. The whole experiment was repeated three times. Data are shown as mean  $\pm$  SEM of 10 cell lines per group and the experiment was repeated three times.

h) Basal and maximal oxygen consumption respiratory rates (OCR) of shGFP and shTbx15 3T3-L1 adipocytes was determined by calculating the AUC during measurements of OCR. Maximal OCR was measured after treatment of adipocytes with 1  $\mu$ M FCCP. Data are shown as mean  $\pm$  SEM of 10 cell lines per group, and the experiment was repeated three times.

i) qPCR analysis for *Pgc1a*, *Cox5b*, *Cox8b*, and *Cox7b*, *Cytc*, *Ndufs1*, *Sdha*, *Gapdh*, *Hk2*, *Pkm1*, *Pkm2*, and *Pdha1* expression from mRNA isolated from shGFP and shTbx15 3T3-L1 adipocytes six days after adipocyte differentiation. Data are mean  $\pm$  SEM of triplicate samples and the experiment was repeated two times.

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