Supporting Information

Wang et al. 10.1073/pnas.1412685111

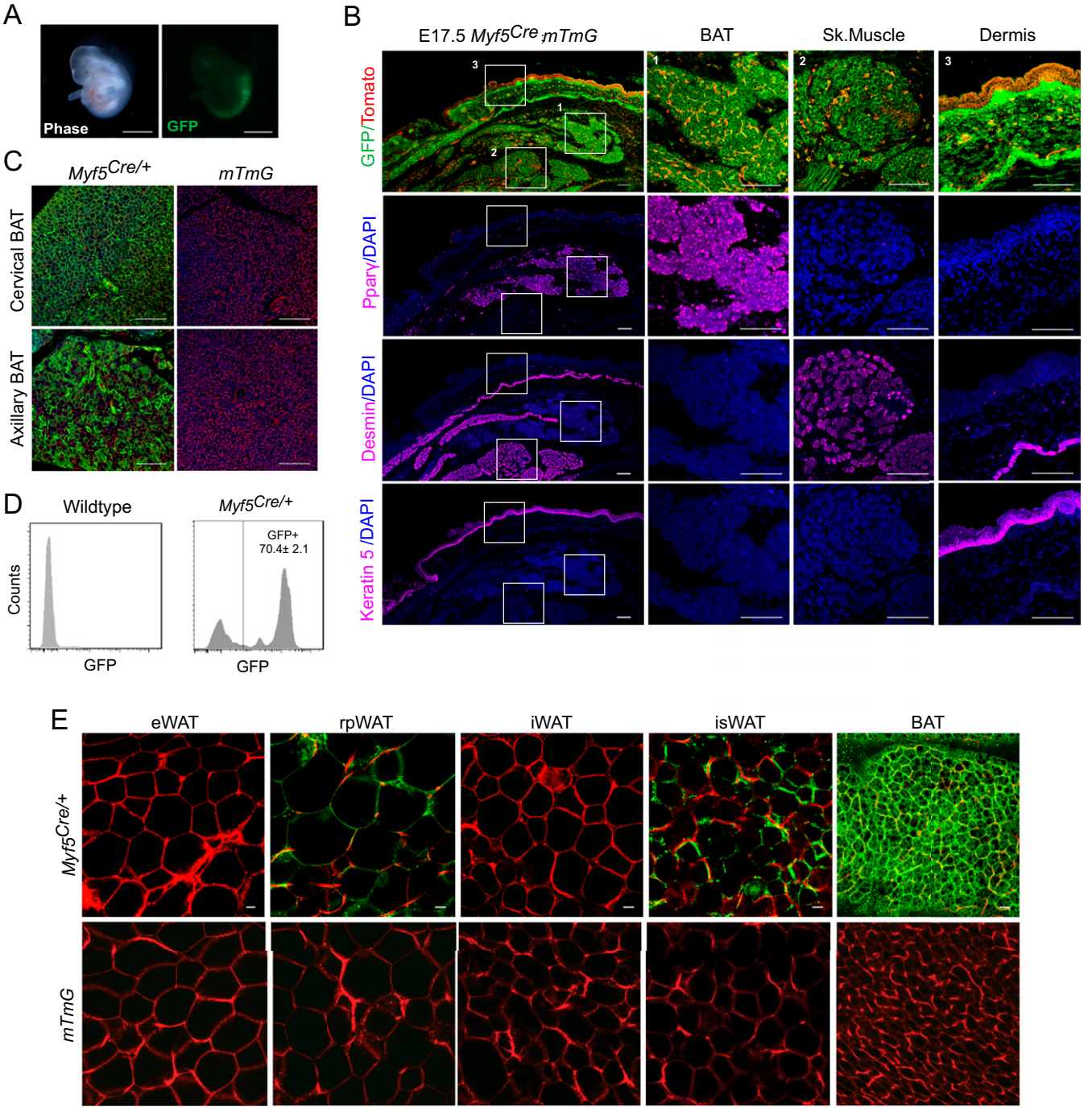

Fig. S1. *Myogenic factor 5* (*Myf5*)^{Cre}-expressing cells develop into brown adipose tissue (BAT) and skeletal muscle and dorsal dermis. (*A*) Embryonic day 9.5 (E9.5) *Myf5*^{Cre}*; mTmG* embryos. (*Left*) Phase contrast. (*Right*) GFP fluorescence. (Scale bar, 1 mm.) (*B*) Immunofluorescence staining of GFP/Tomato, peroxisome proliferator-activated receptor γ (Pparγ) (adipocyte), Desmin (muscle), and Keratin 5 (epidermis) in the interscapular regions of E17.5 *Myf5*^{Cre}*; mTmG* embryos. Enlargements of regions 1, 2, and 3 are shown (*Right*). (Scale bar, 100 μm.) (*C*) GFP and Tomato staining of indicated BAT depots of 4-wk-old *Myf5*^{Cre}*; mTmG* mice. (Scale bar, 100 μm.) (*D*) Flow cytometry analysis of precursor cells (stromal–vascular fraction) from BAT of *Myf5*^{Cre}*; mTmG* mice. Value represents the percent of GFP^{+} cells. $n = 3$ experiments. (*E*) Direct fluorescence analysis of GFP and Tomato expression in several white adipose tissue (WAT) depots (eWAT, epididymal WAT; iWAT, inguinal WAT; isWAT, interscapular WAT; rtWAT, retroperitoneal WAT) and interscapular BAT of *Myf5*^{Cre}*; mTmG* mice. (Scale bar, 20 μm.)

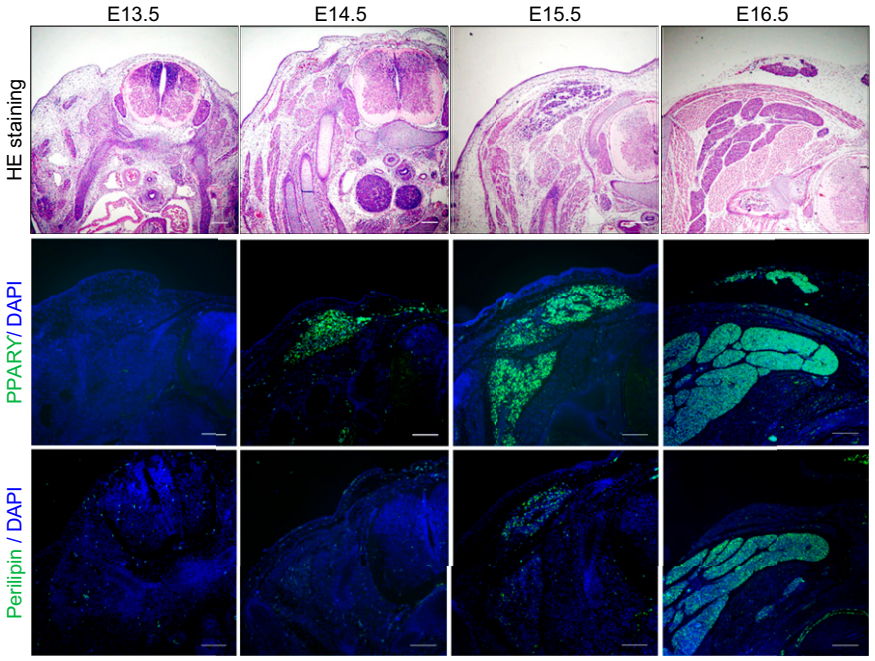

Fig. S2. Brown adipose tissue formation during mouse embryogenesis. H&E staining and immunofluorescence staining of Pparγ and perilipin on serial sections from E13.5 to E16.5 embryos. (Scale bar, 100 μm.)

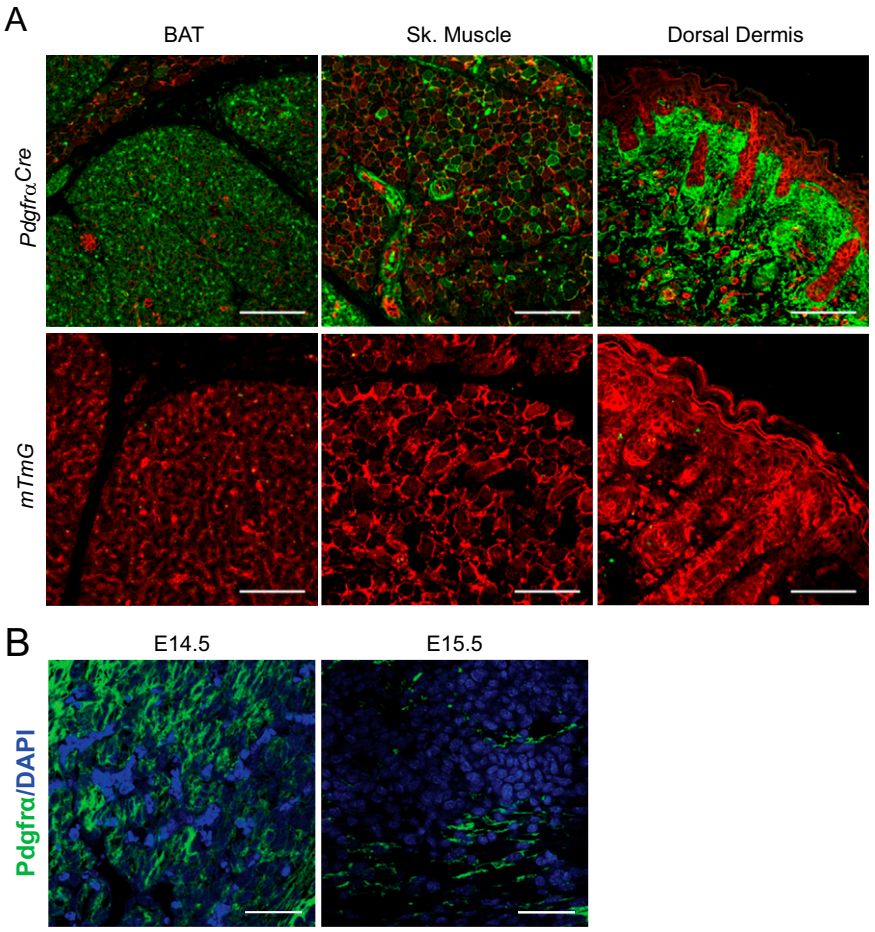

Fig. S3. Platelet-derived growth factor α (Pdgfrα)-expressing cells give rise to brown adipocytes, skeletal muscle, and dorsal dermis. (*A*) Immunofluorescence staining of GFP (green)/Tomato (red) in BAT, skeletal muscle (Sk. muscle), and dorsal dermis of *Pdgfrα*^{Cre}*; mTmG* mice. (Scale bar, 100 μm.) (*B*) Immunofluorescence staining for Pdgfrα (green) in BAT regions of E14.5 and E15.5 embryos. (Scale bar, 50 μm.)

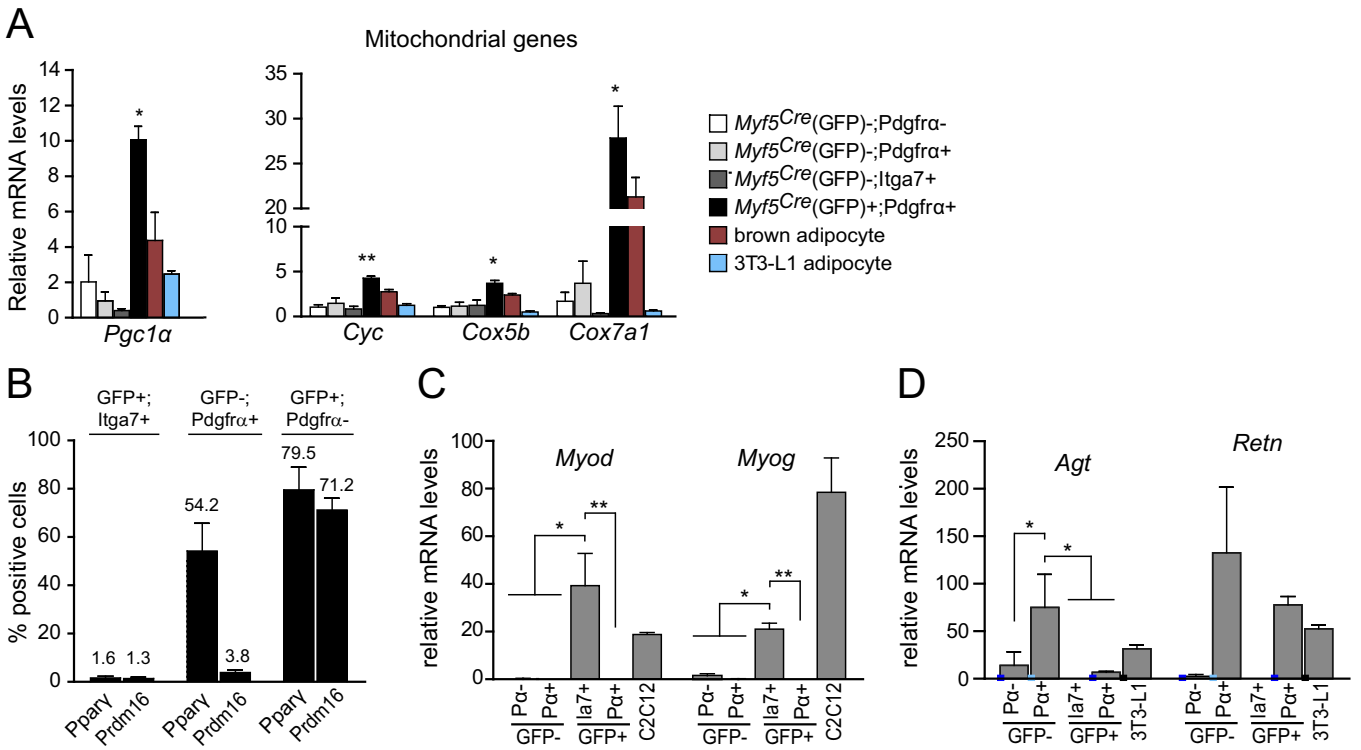

Fig. S4. Brown adipogenic potential of embryonic *Myf5*^{Cre} (GFP)$^{+}$; Pdgfrα^{+} cells. (*A*) mRNA expression levels of *Pgc1α* and mitochondrial genes in differentiated cultures of FACS-purified cells from E14.5 *Myf5*^{Cre}*; mTmG* embryos or cell lines (as indicated). (*B*) Percentage of Pparγ^{+} and PR domain containing 16 (Prdm16)$^{+}$ cells in indicated cell cultures based on immunofluorescence analysis. $n = >45$ fields from three experiments. (*C* and *D*) mRNA levels of muscle (*C*) and white fat (*D*) selective markers in differentiated cultures of sorted cells (as indicated). [Scale bar, 100 μm (*B*) and 20 μm (*D*).]

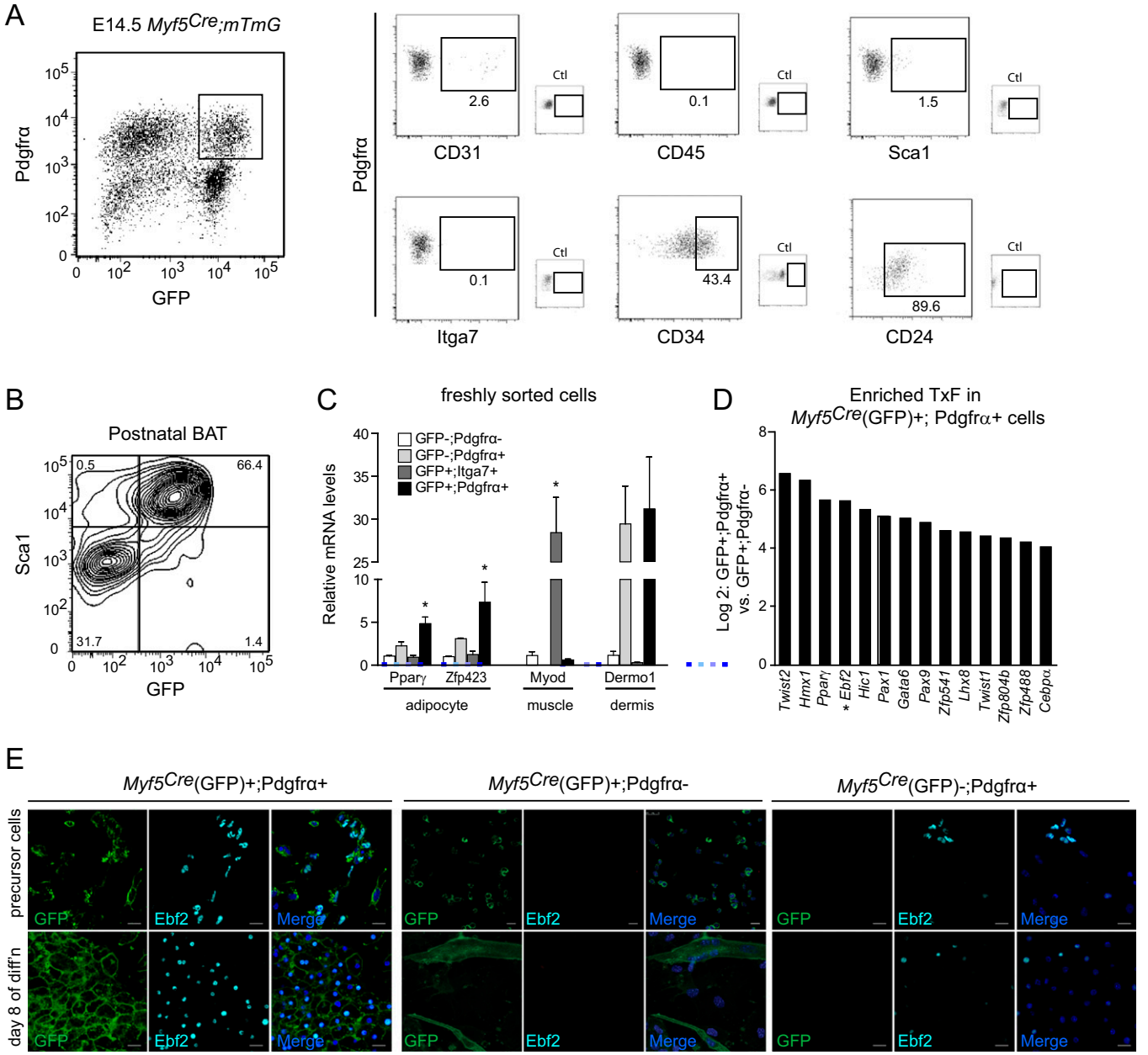

Fig. S5. Molecular analysis of *Myf5*^{Cre} (GFP)$^{+}$; Pdgfrα^{+} precursor cells. (*A*) *Myf5*^{Cre} (GFP)$^{+}$; Pdgfrα^{+} cells were analyzed for their expression of indicated cell surface antigens by flow cytometry. Gates were set based on isotype control staining (*Right*). (*B*) Flow cytometry analysis of Sca1 expression in stromal vascular cells from adult BAT. (*C*) mRNA levels of adipose, muscle and dermis lineage markers in freshly sorted cell fractions (as indicated). Values are mean ± SD, $n = 3$ experiments; *$P < 0.05$. (*D*) Transcriptional factors (TxF) with enriched expression levels (>16-fold) in *Myf5*^{Cre} (GFP)$^{+}$; Pdgfrα^{+} cells relative to *Myf5*^{Cre} (GFP)$^{+}$; Pdgfrα^{-} cells. (*E*) Immunofluorescence analysis of early B cell factor 2 (Ebf2) (cyan) expression in indicated cell fractions before (precursor cells) and after inducing adipocyte differentiation (day 8). (Scale bar, 20 μm.)

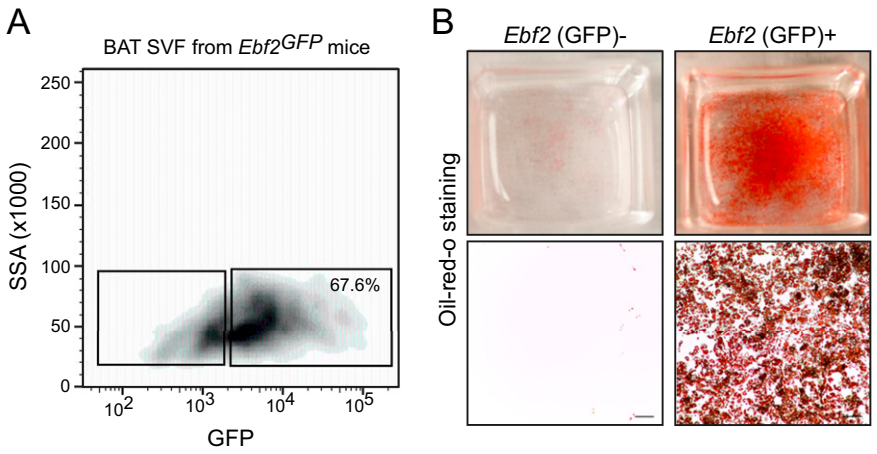

Fig. S6. Ebf2^{+} cells in the stromal vascular fraction of BAT are brown adipogenic precursors. (*A*) Flow cytometry analysis of *Ebf2* (GFP) expression in stromal vascular fraction (SVF) isolated from BAT of *Ebf2*^{GFP} mice. Positive and negative gates were set by analyzing cells from wild-type BAT. Value is the percentage of GFP^{+} cells. (*B*) Sorted cells were induced to undergo adipocyte differentiation followed by Oil Red O staining of lipid droplets. (Scale bar, 100 μm.)

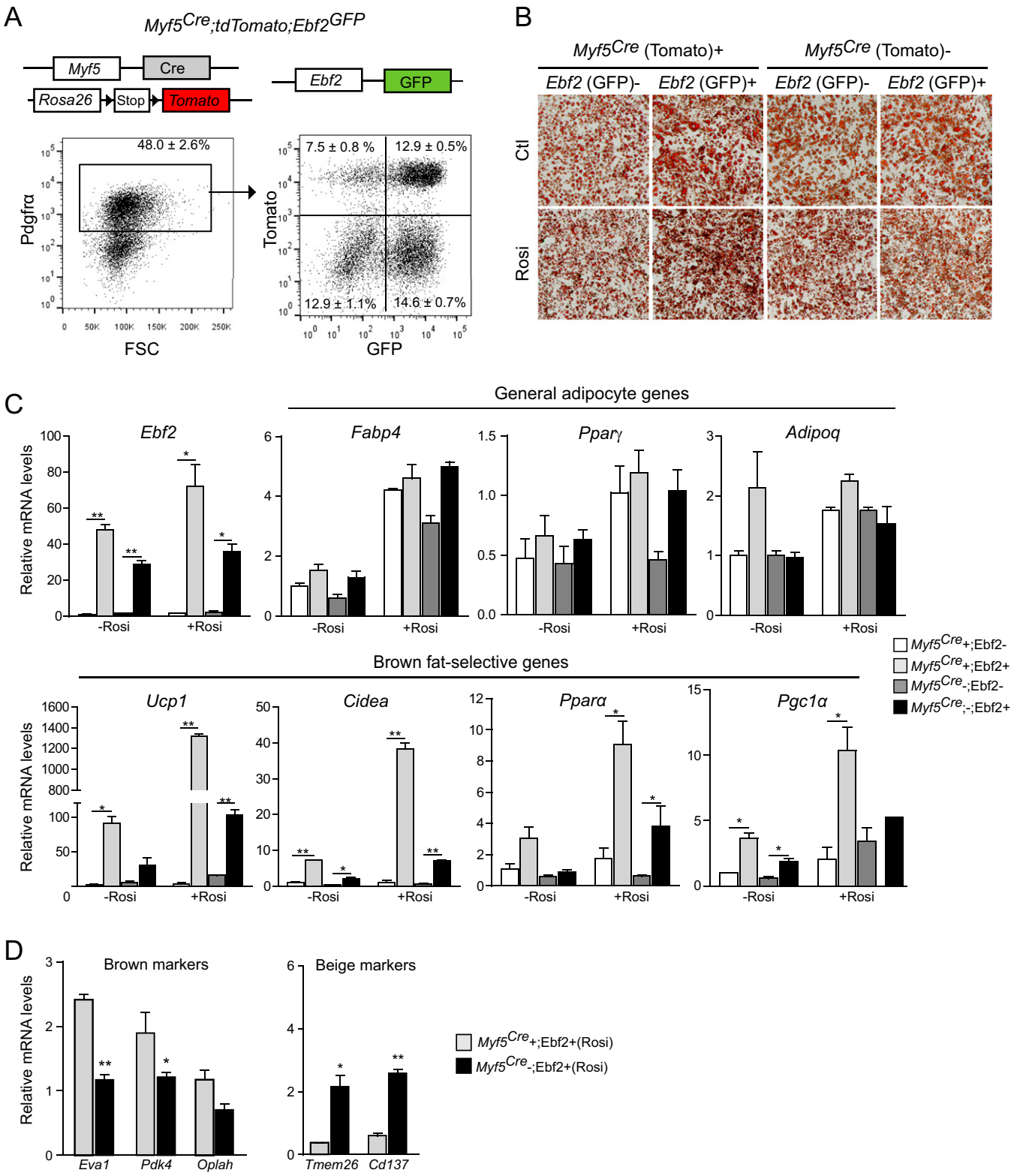

Fig. S7. *Ebf2*-expression marks precursor cells with brown and beige adipogenic potential. (*A*) Fibroblasts from E14.5 *Myf5*^{Cre}*; tdTomato; Ebf2*^{GFP} embryos were fractionated based on their expression of GFP, Pdgfrα, and tdTomato. The percentage of each cell fraction is reported as mean ± SD, $n = 3$ experiments. (*B*) Oil Red O staining of indicated cell cultures after adipocyte differentiation in the presence or absence of 0.5 mM rosiglitazone (Rosi). (*C*) mRNA levels of *Ebf2*, general adipogenic genes and brown-fat–selective genes in cultures from *B*. (*D*) mRNA levels of brown (*Left*) and beige (*Right*) fat markers in indicated cell cultures. Expression values are mean ± SD, $n = 3$ experiments; $^{*}P < 0.05$, $^{**}P < 0.01$.

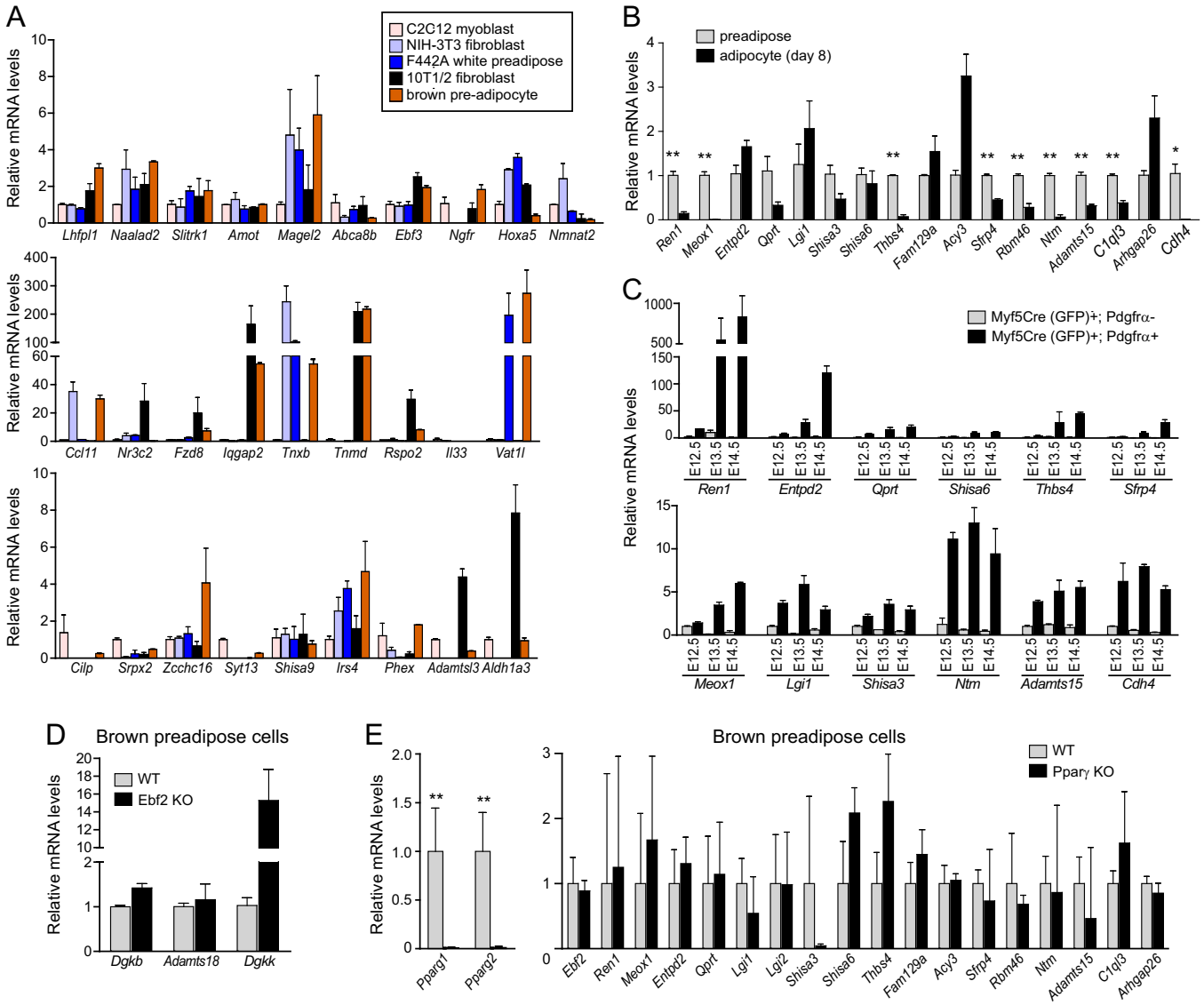

Fig. S8. Ebf2 regulates the gene expression profile of brown preadipose cells. (*A*) mRNA levels of brown preadipose signature genes in cell lines (as indicated). (*B*) mRNA levels of signature genes before and after adipocyte differentiation in an immortalized brown fat cell line. (*C*) mRNA levels of signature genes in sorted $Myf5^{Cre}$ (GFP)$^{+}$; Pdfgrα^{-} and $Myf5^{Cre}$ (GFP)$^{+}$; Pdfgrα^{+} cells from the dorsal region of mouse embryos at different stages. (*D*) mRNA levels of *Dgkb*, *Adamts18*, and *Dgkk* in wild-type (WT) and Ebf2 knockout (KO) brown preadipose cells. (*E*) mRNA levels of signature genes in WT and Pparγ KO brown preadipose cells. All expression data are mean ± SD, $n = 3$–7; $^{*}P < 0.05$, $^{**}P < 0.01$.