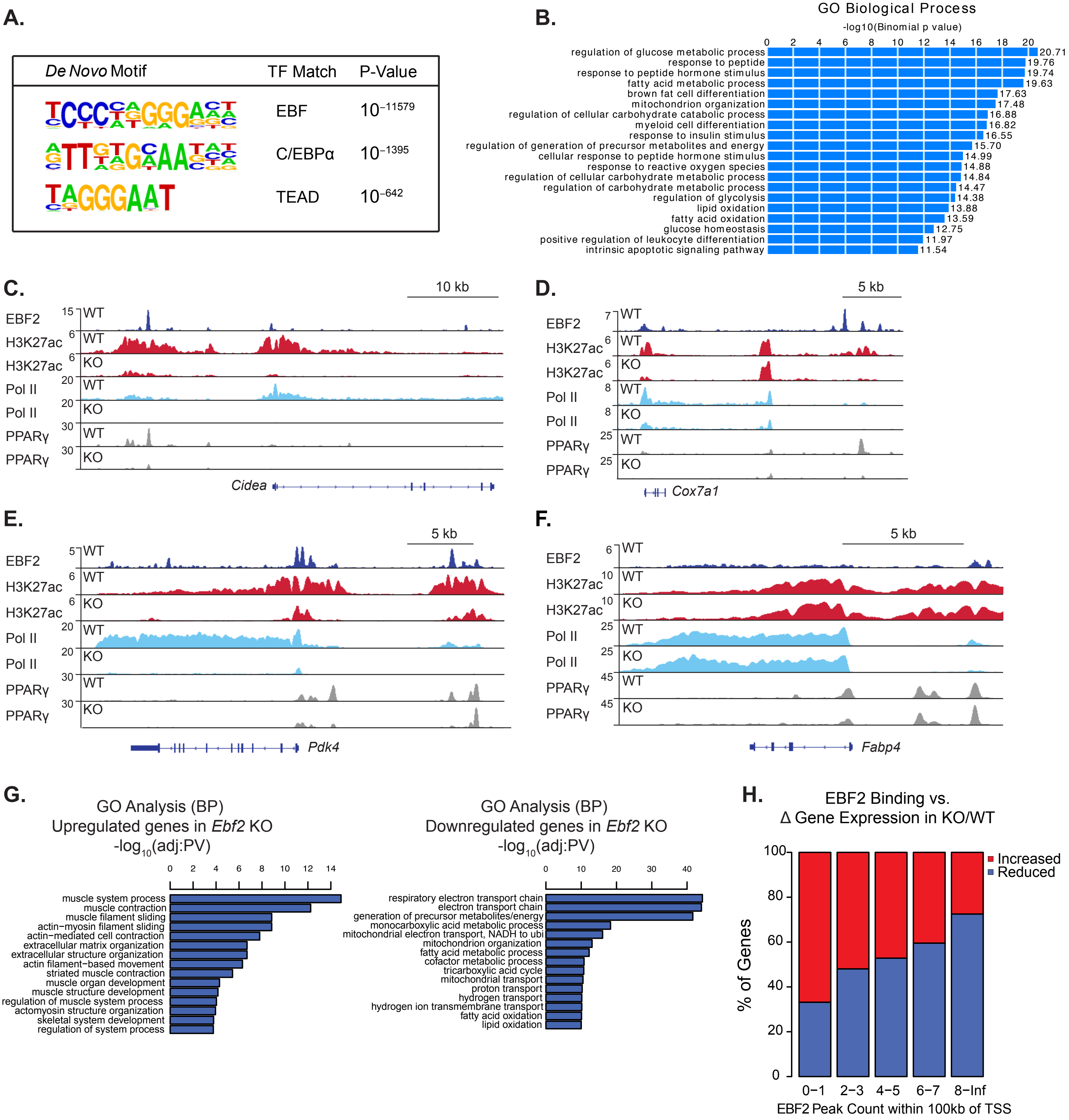
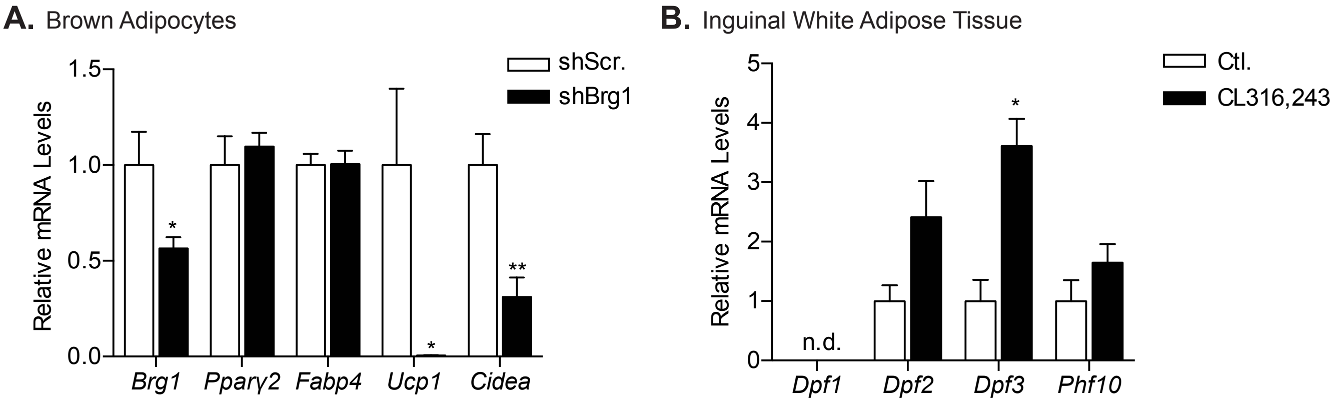
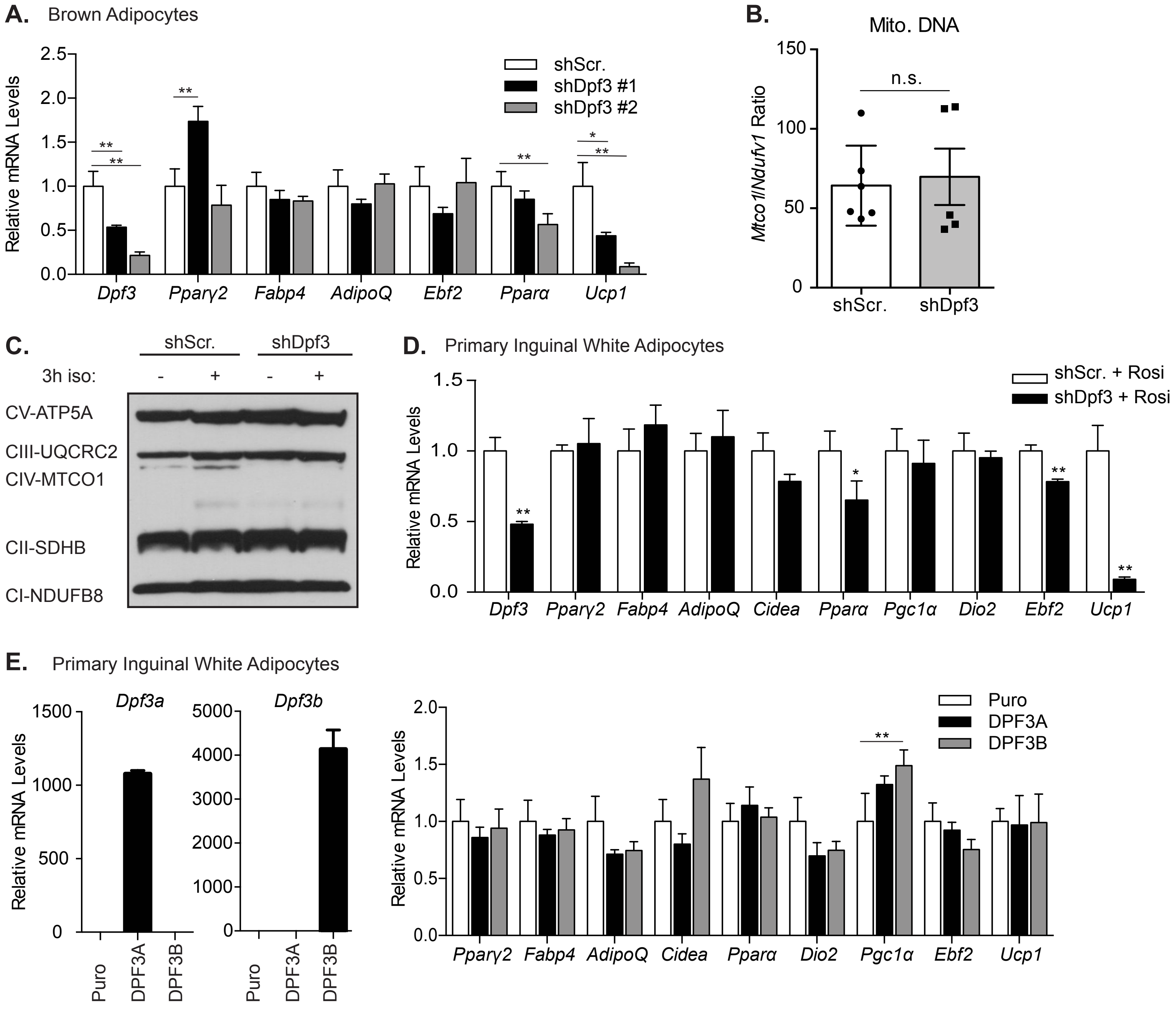
Shapira294405_Figure S1



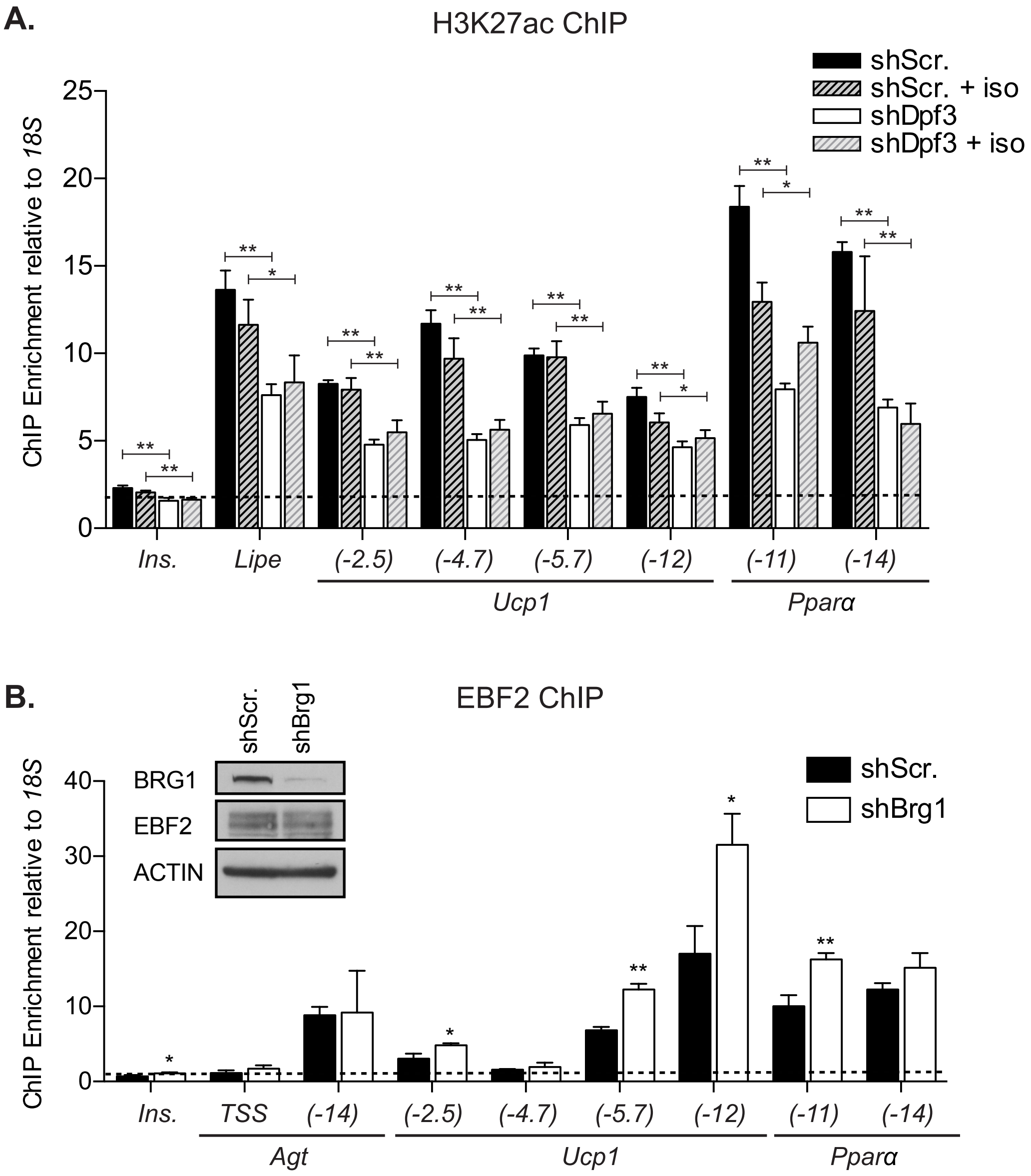
Supplemental Figure 1. EBF2 binds and regulates the chromatin state at brown fat-specific genes. (A) *De novo* motif analysis of EBF2 binding sites. (B) GREAT analysis of the top 5000 genes with nearby EBF2 binding sites in brown adipose tissue. (C) ChIP-seq profiles in reads per million total reads (RPM) for EBF2 (dark blue), H3K27ac (red), RNA Pol II (light blue), and PPARγ (grey) in *Ebf2* wildtype and knockout BAT at *Cidea,* (D) *Cox7a1*, (E) *Pdk4*, and (F) *Fabp4*. (G) Gene ontology analysis of differentially expressed genes in *Ebf2* KO relative to WT brown adipose tissue. (H) Barplot of differentially regulated genes with proximal EBF2 binding sites within a 100kb window around the TSS.



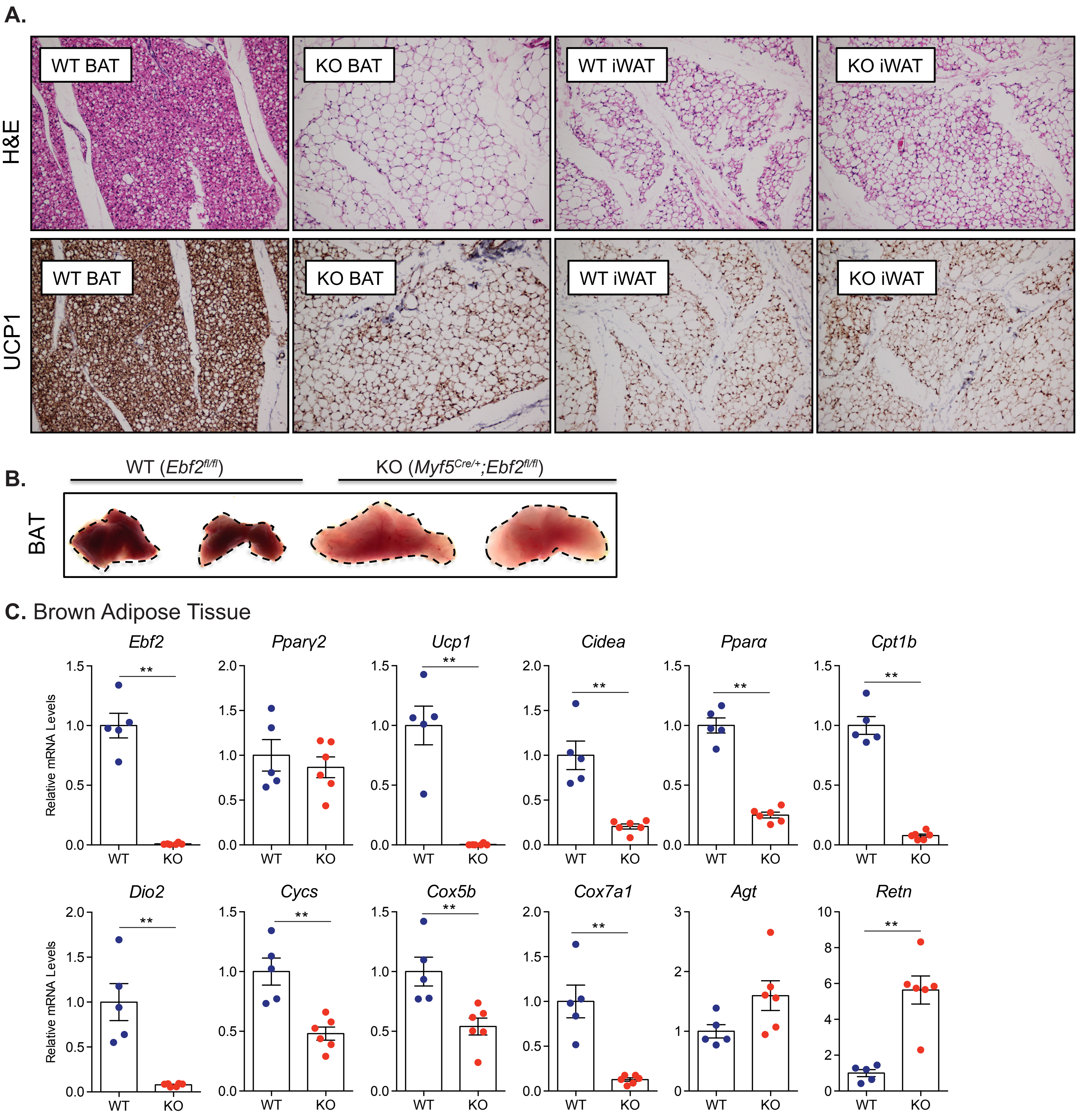
Supplemental Figure 2. EBF2 interacts with the BAF complex, which incorporates the subunit DPF3 in brown adipocytes. (A) RT-qPCR analysis of day 7 mature brown adipocytes following shRNA-mediated BRG1 depletion (mean ± SD; n=3, two-sample Student's t-test, * $p < 0.05$, ** $p < 0.01$). (B) RT-qPCR analysis of DPF family member expression in inguinal white adipose following injection with the β3-agonist CL316,243 (mean ± SE; n=3, two-sample Student's t-test, * $p < 0.05$, ** $p < 0.01$).



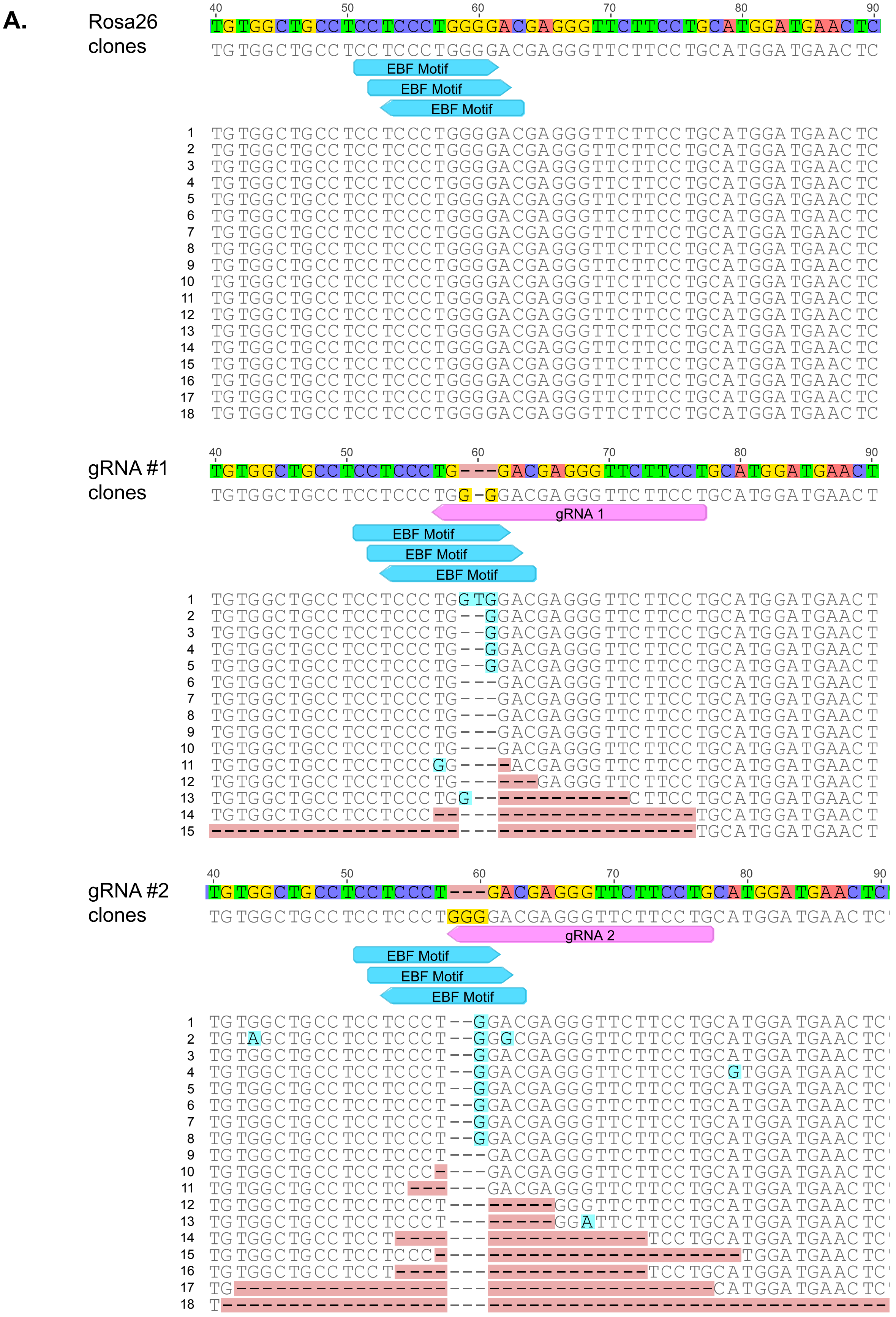
Supplemental Figure 3. DPF3 is required for activation of the brown fat program and mitochondrial function. (A) RT-qPCR analysis of mature brown adipocytes following shRNA-mediated *Dpf3* depletion using two independent shRNAs (n=3). (B) Quantification of mitochondrial DNA content in control and *Dpf3*-depleted cells (n=5-6). (C) Western blot analysis of OXPHOS components in brown adipocytes. (D) RT-qPCR analysis of of day 7 primary inguinal adipocytes differentiated with rosiglitazone following shRNA-mediated *Dpf3* depletion (n=3). (E) RT-qPCR analysis of day 7 primary inguinal adipocytes following retroviral-mediated overexpression of DPF3A or DPF3B (n=3) (all gene expression data show mean ± SD; two-sample Student's t-test, * $p<0.05$, ** $p < 0.01$).



Supplemental Figure 4. DPF3 regulates the chromatin state at brown fat-specific genes. (A) ChIP-qPCR analysis of the H3K27ac profile in brown adipocytes ± isoproterenol (mean ± SD; n=3, two-way ANOVA with Holm-Šídák multiple tests correction comparing shScr. vs. shDpf3 in the basal or stimulated state, * $p < 0.05$, ** $p < 0.01$). (B) EBF2 ChIP-qPCR in BRG1-depleted brown adpocytes (mean ± SD; n=3, two-sample Student’s t-test, * $p < 0.05$, ** $p < 0.01$). Chromatin enrichment is analyzed as percent input recovery and normalized to 18S percent input to produce a fold enrichment. The insulin promoter serves as a negative control.

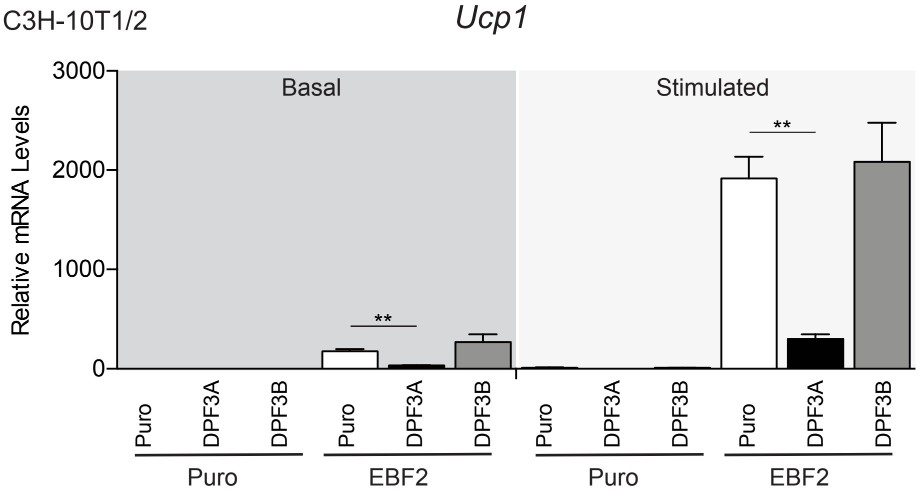


Supplemental Figure 5. EBF2 transcriptionally regulates *Dpf3* expression (A) H&E staining and UCP1 immnohistochemical staining of representative sections from the BAT and iWAT of 12-week-old WT (*Ebf2*$^{fl/fl}$) and KO (*Myf5*$^{Cre/+}$;*Ebf2*$^{fl/fl}$) mice. (B) Gross morphlogy of brown adipose tissue from WT and KO mice. (C) Gene expression analysis of common, brown fat-specific, mitochondrial, and white fat-specific genes in WT and KO BAT (mean ± SEM; n=5-6, two-sample Student's t-test, ** $p < 0.01$)



Supplemental Figure 6. EBF2 directly regulates *Dpf3* expression via an intronic enhancer. (A) TA cloning and sequencing showing mutations induced following CRISPR-Cas9 editing of the EBF motif in the *Dpf3* +20k enhancer.

A. C3H-10T1/2



Supplemental Figure 7. Critical role for histone binding activity of DPF3 in brown adipocytes.
(A) RT-qPCR analysis of differentiated C3H-10T1/2 cells under basal or isoproterenol-stimulated conditions. (mean ± SD; n=3, two-sample Student's t-test, ** $p < 0.01$).

Table S1. Primers used for RT-qPCR, ChIP-qPCR, and FAIRE-qPCR analysis

Gene	**Forward**	**Reverse**
Actl6a	CGTTCTCAGGGAGAGGAGTC	CAACTTCATCTCCGCCGTACA
Actl6b	TACAGCAAGGCATCGTCAAG	CTTGGCTGCAATCATGTAAGGA
Adiponectin	GCACTGGCAAGTTCTACTGCAA	GTAGGTGAAGAGAACGGCCTTGT
Agt	AAGACCCTGCATGATCAGCTC	CTTCCTGCCTCATTCAGCATC
Arid1a	TCCTCAGTCAACCAGCAGTT	ACTGAGTTGCTCCTGCTCAT
Arid1b	GGCAGATCCCAGGGCAG	CCTCCTGGGTATGGGCTG
Arid2	TCCGGGACTTCACAGATGAAA	TCTTTAACAATCTGCGCCCAC
Brg1	AAGAAGCAGAAGAAACGTGGG	CACTGCTGCTGTCTTTGTACT
Brm	CTCGCGAGCAAGTGTCA	ACTAGGCCCCAGAATTGGTC
Cidea	TGCTCTTCTGTATCGCCCAGT	GCCGTGTTAAGGAATCTGCTG
Cox5b	GCTGCATCTGTGAAGAGGACAAC	CAGCTTGTAATGGGTTCCACAGT
Cox7a1	CAGCGTCATGGTCAGTCTGT	AGAAAACCGTGTGGCAGAGA
Cpt1b	GCTCATTTCCGGGACAAAGG	TTGGTACAGGAACGCACAGT
Cycs	GCAAGCATAAGACTGGACCAAA	TTGTTGGCATCTGTGTAAGAGAATC
Dio2	CAGTGTGGTGCACGTCTCCAATC	TGAACCAAAGTTGACCACCAG
Dpf1	AGAACCCGCTCAAGTCCCTT	GGTCTGCGAGTCGAGGAAAG
Dpf2	GGGAAGTCCAAGAGTAAGGGT	CGCTTTCGAGGTATGCTTTTG
Dpf3	GAGTACCACACTGGAAGCCT	TCCCTTCTTCTACGTTTTCATCA
Ebf2	GCTGCGGGAACCGGAACGAGA	ACACGACCTGGAACCGCCTCA
Fabp4	ACACCGAGATTTCCTTCAAACTG	CCATCTAGGGTTATGATGCTCTTCA
Pbrm	CCTATACACCCCCACAGTCTAC	CAGCTCTCATTTCACTGCTGA
Phf10	TGACCTCCTTCAAGCGGAAA	CGCAATGCTGTTAAACCCAGT
Pgc1α	CCCTGCCATTGTTAAGACC	TGCTGCTGTTCCTGTTTTC
Pparα	GCGTACGGCAATGGCTTTAT	GAACGGCTTCCTCAGGTTCTT
Pparγ2	GCATGGTGCCTTCGCTGA	TGGCATCTCTGTGTCAACCATG
Retn	CTGTCCAGTCTATCCTTGCACAC	CAAAGGCACAGCAGTCTTGA
Smarcb1	AAACCTAACACTAAGGATCATGGA	TGATGGACACAGCCTTGTACT
Smarcc1	CAACCAGGTCAAATACCAGGC	CACTCCCAGTAGGGTGAATGT
Smarcc2	AACAGCCCAGATTCAGACAGA	TGAGGGTCCTTTCTTAGCGTT
Smarcd1	CAAAATCGAAATCACAATGCAAAGA	GAGATCCATGTAGGCCTGTGA
Smarcd2	CCCAGAGTCTCAGGCATACAT	GTTTTCGCTTTTGCGTCAGAG
Smarcd3	GAGCCGTGATCTCAAGGTGAT	GCTGCTGGATCTTACAGTAGAA
Smarce1	CGGGACAAAGGGAAGCGAAG	GGGGCATAAGATGGTCTTTTTGA
Tbp	GAAGCTGCGGTACAATTCCAG	CCCCTTGTACCCTTCACCAAT
Ucp1	ACTGCCACACCTCCAGTCATT	CTTTGCCTCACTCAGGATTGG
Mtco1 (Mt.)	TGCTAGCCGCAGGCATTAC	GGGTGCCCAAAGAATCAGAAC
Ndufv1 (Mt.)	CTTCCCCACTGGCCTCAAG	CCAAAACCCAGTGATCCAGC

ChIP/FAIRE	**Forward**	**Reverse**
18S	AGTCCCTGCCCTTTGTACACA	CGATCCGAGGGCCTCACT
Ins1	GGACCCACAAGTGGAACAAC	GTGCAGCACTGATCCACAAT
Agt (TSS)	CTTGGTCAAGCCTGGATTCTC	CCAACCTAGACAAGCACAGCTATC
Agt (-14kb)	GCAGGCAGGCATCCACGTCTT	CAGTGAGGAGCACCGGTCTGG

Hbb	CAGGGAGAAATATGCTTGTCATCA	GTGAGCAGATTGGCCCTTACC
Lipe	TGGGTCATAGTTGGCTAGGG	CAGTGGGGACATGGGTAAGT
Ucp1 (-2.5kb)	CAAATGGTGACCGGGTGCCCT	GGGTGACTGACCCTCTGTGACG
Ucp1 (-4.7kb)	CCCCACTGCCTGTCACGTTCA	GAAGCTGCCGAATGGTGCGTC
Ucp1 (-5.7kb)	ACCACACCATTTGGAGCCTGAC	TGAGTTTGCAGGGAGGATGGGC
Ucp1 (-12kb)	GTCACCAAGCCTTCCTCCGCA	GTGAGCTGGTGGTGGTCAGGG
Pparα (-11kb)	AAGAGCATGGGACAGTGGCCG	TGGCCAGCTGAAGGTCACCAC
Pparα (-14kb)	CCTGCCCCATAGGCAGTATGGTC	ACAGGGGCAGAAGCCAAGCTG