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Supplemental Information

MED19 Regulates Adipogenesis and Maintenance of White Adipose Tissue Mass by Mediating PPAR γ -Dependent Gene Expression

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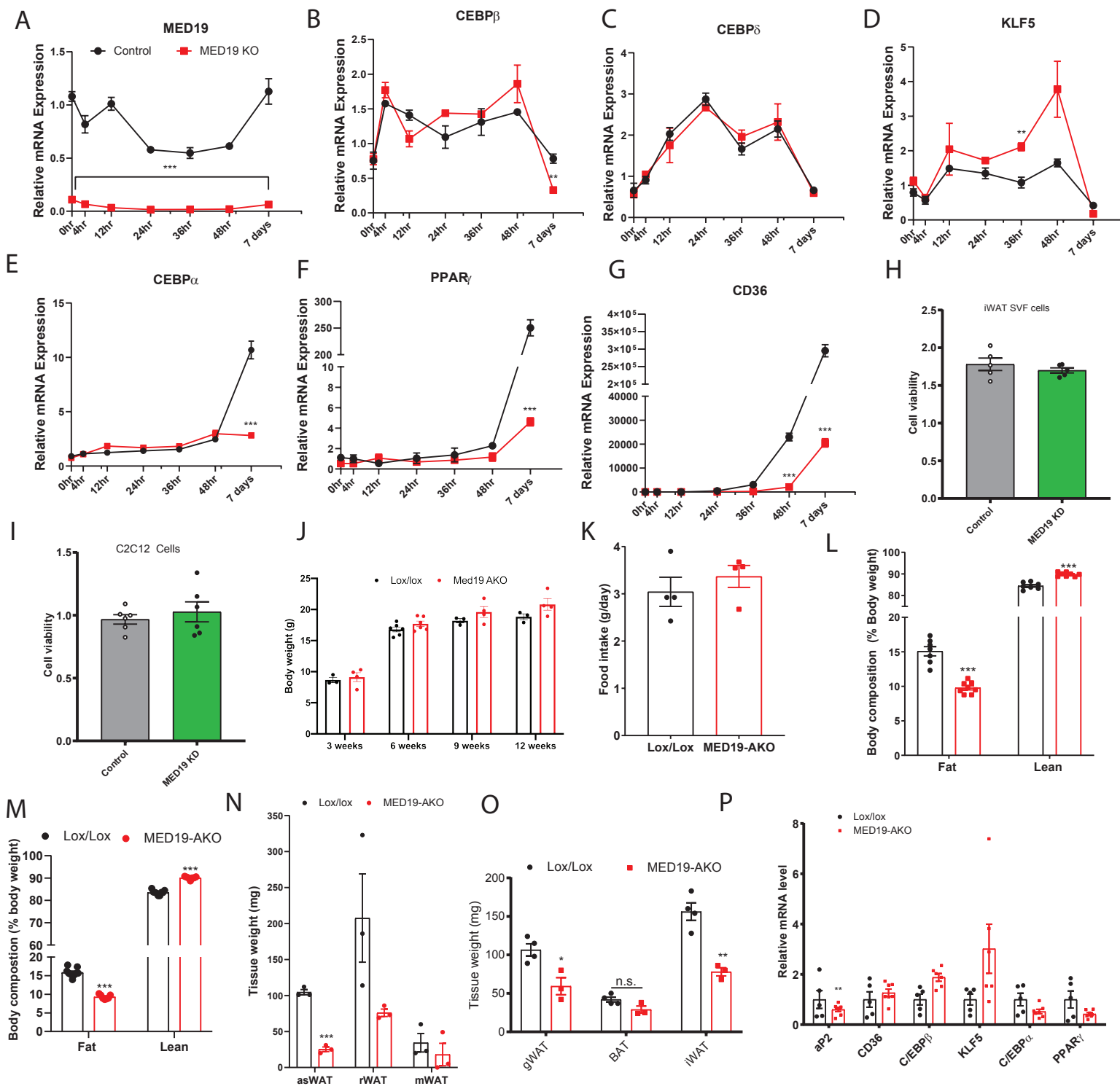


Figure S1. Effect of MED19 knockout on adipocyte gene expression and characterization of MED19-AKO mice. Related to Figure 1. (A-G) Gene expression time course of adipogenic genes in MED19-iKO iWAT SVF cells; n=3 each. (H-I) Cell viability in control and MED19 knockdown iWAT SVF cells and C2C12 cells measured using WST1 assay reagent. (J) Body weight of control and MED19-AKO female mice at 3 (n=3 Lox/Lox, 4 MED19-AKO), 6 (n=6 each), 9 (n=3 Lox/Lox, 4 MED19-AKO), and 12wk of age (n=3 Lox/Lox, 4 MED19-AKO). (K) Food intake in MED19-AKO and control male mice; n=4 each. (L) Body composition analysis by EchoMRI of 12wk old male mice; n=7 Lox/Lox, 8 MED19-AKO (M) Body composition analysis by EchoMRI of 12wk old female mice; n=9 Lox/Lox, 7 MED19-AKO. (N) Tissue weight of asWAT, rWAT, and mWAT of 12wk old male MED19-AKO mice; n=3 each. (O) Adipose tissue weights of 3wk old male MED19-AKO mice; n=4 Lox/Lox, 3 MED19-AKO. (P) qPCR analysis of gene expression in iWAT of MED19-AKO and control mice; n=5 Lox/Lox, 7 MED19-AKO. *p < 0.05, **p < 0.005, ***p < 0.0005.

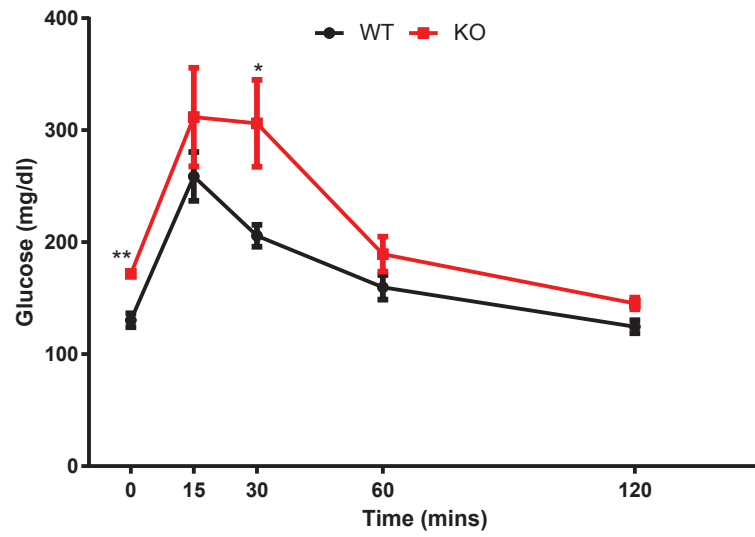


Figure S2. Glucose tolerance test in female mice. Related to Figure 2. Glucose tolerance test in control and MED19-AKO female mice; n=4 Lox/Lox, 3 MED19-AKO. *p < 0.05.

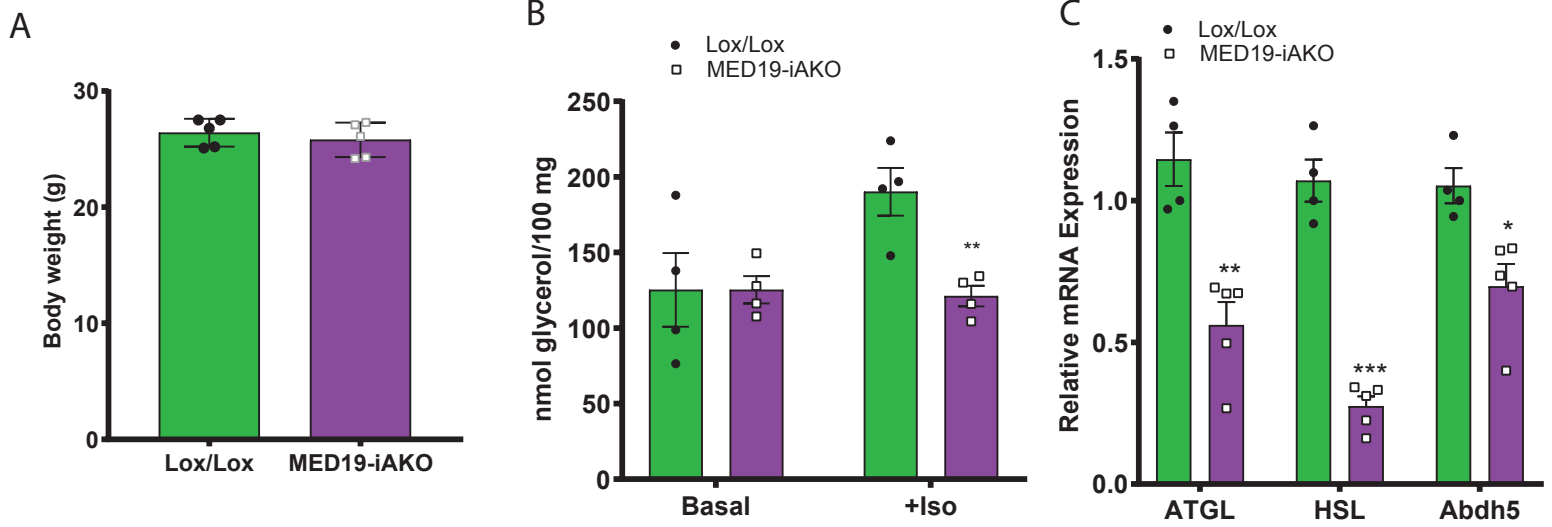


Figure S3. Effect of inducible adipose-specific MED19 KO on body weight and lipolysis. Related to Figure 3. (A) Body weight of MED19-iAKO mice 21 days following TAM injections; n=5 each. (B) Lipolysis assay in iWAT tissue explants from control and MED19-iAKO mice; n=4 each. (C) Expression of lipolysis genes was reduced in iWAT of MED19-iAKO mice; n=5 each. *p < 0.05, **p < 0.005, ***p < 0.0005.

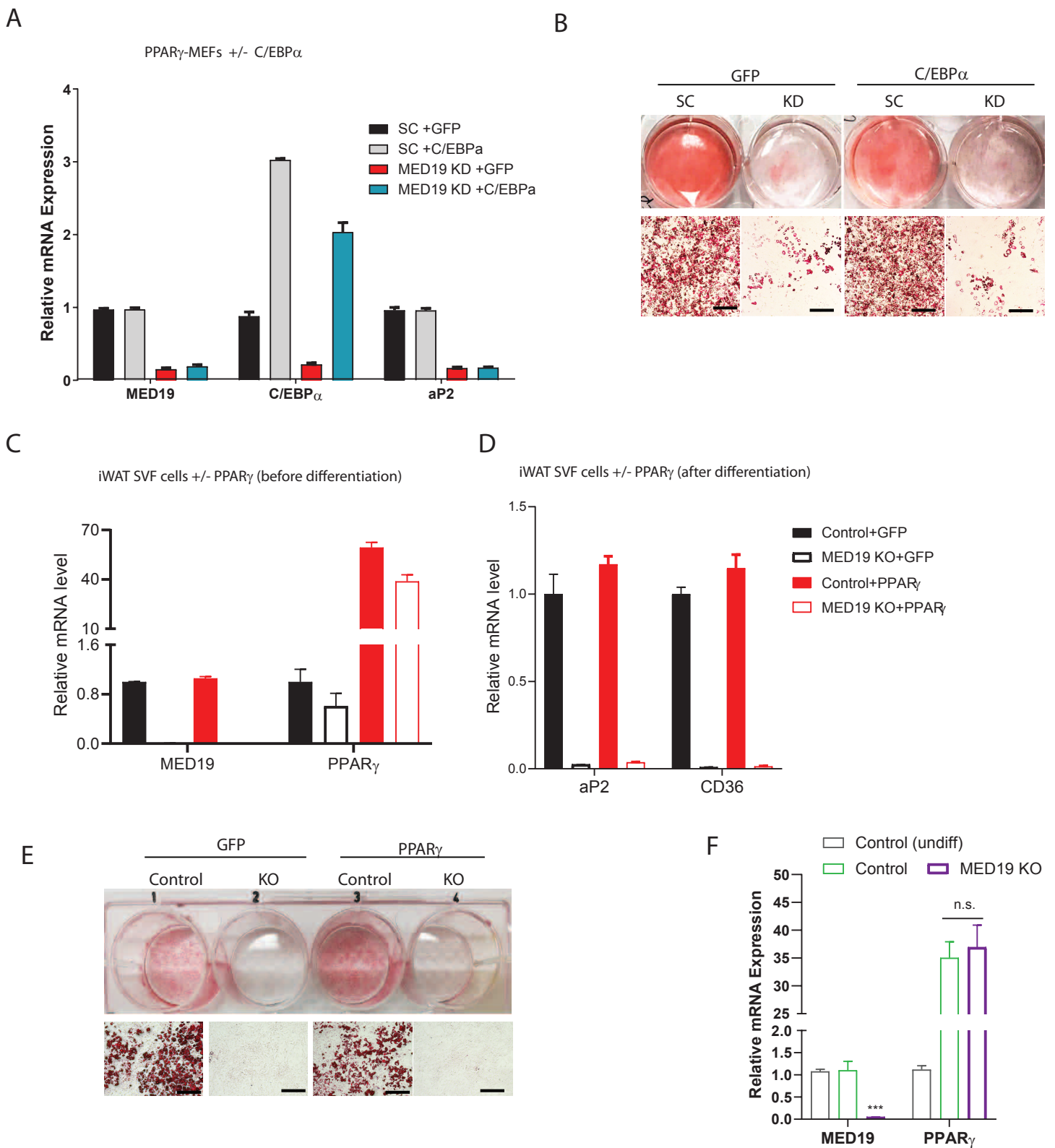


Figure S4. PPAR γ and/or C/EBP α overexpression does not rescue adipogenesis in MED19 depleted cells. Related to Figure 4. (A) Gene expression analysis in differentiated PPAR γ 2-MEFs overexpressing GFP or C/EBP α and treated with scrambled or MED19 shRNA; n=3 each. **(B)** Oil red O staining of the cells in panel A. Scale bar=300 μ m. **(C-D)** Gene expression analysis before **(C)** and after **(D)** differentiation of MED19-iKO cells overexpressing PPAR γ and treated with vehicle or 4-hydroxy-tamoxifen to induce MED19 KO; n=2 each. **(E)** Oil red O staining in the cells from panel D. Scale bar=300 μ m. **(F)** Gene expression analysis of MED19-iKO cells under the same conditions used in the PLA assay in Figure 4J-K; n=3 each. *p < 0.05, **p < 0.005, ***p < 0.0005.

Supplemental Table 1: Oligonucleotide sequences. Related to STAR Methods.

Target	Fwd (5' to 3')	Rev (3' to 5')	Reference
L32	TTCCTGGTCCACAATGTCAA	GGCTTTTCGGTTCTTAGAGGA	This paper
Cidea	CAGTGATTTAAGAGACGCGG	TCTGCAATCCCATGAATGTC	This paper
Med19	AGTGGCCCCTTCTACCTGAT	TAGGCCTGCTCCAGGTTGTA	This paper
aP2	GCGTGGAATTCGATGAAATCA	CCCGCCATCTAGGGTTATGA	This paper
C/EBP α	CCAAGAAGTCCGGTGGACAAG	TTGTTTGGCTTTATCTCGGC	This paper
KLF5	AGCGACGTATCCACTTCTGC	CAGGTGCACTTGTAGGGCTT	This paper
Krox20	TTGACCAGATGAACGGAGTG	CAGGTGCACTTGTAGGGCTT	This paper
MyoD	TACAGTGGCGACTCAGATGC	GAGATGCGCTCCACTATGCT	This paper
MyoG	CTACAGGCCTTGCTCAGCTC	ACGATGGACGTAAGGGAGTG	This paper
Desmin	GTGGATGCAGCCACTCTAGC	TTAGCCGCGATGGTCTCATAAC	This paper
Myf5	CAGCCCCACCTCCAACCTG	GGGACCAGACAGGGCTGTTA	This paper
CD36	GCGACATGATTAATGGCACA	CCTGCAAATGTCAGAGGAAA	This paper
PRDM16	CAGAGGTGTCATCCAGGAG	ACGGATGTACTTGAGCCAGC	This paper
Pgc-1 α	TGTAGCGACCAATCGGAAAT	TGAGGACCGCTAGCAAGTTT	This paper
Ebf2	GCTGCGGGAACCGGAACGAGA	ACACGACCTGGAACCGCCTCA	This paper
Lipe	ACGCTACACAAAGGCTGCTT	TCTCGTTGCGTTTGTAGTGC	This paper
Agpat2	GAGCCTTCTACTTGGCCATCCA	TTGATTGTTTCCTGAGGTGAAGAGC	This paper
Retn	AAGAACCCTTTCATTTCCCCTCC	GTCCAGCAATTTAAGCCAATGTT	This paper
ATGL	CCAACGCCACTCACATCTAC	GCCTCCTTGGACACCTCAAT	This paper
Abdh5	TGGTGTCCCACATCTACATCA	CAGCGTCCATATTCTGTTTCCA	This paper
FABP4-PPRE	AA TGTCAGGCA TCTGGGAAC	GACAAAGGCAGAAA TGCACA	Lefterova et al., 2010
CD36 PPRE	CCAACGGAAGTGA TTTGAGC	TTGCTGCTACACTCCAGCAT	Lefterova et al., 2010
Retn-ChIP	AGATTCTATTGAGTCATTCACA	AAGGGGCCTGTCCCTTTGGA	Rajakumari et al., 2013
Lipe-ChIP	TGGGTCATAGTTGGCTAGGG	CAGTGGGGACATGGTAAGT	Rajakumari et al., 2013
Agpat2-PPRE	AGTCCTCAGACCACCTGACC	TCCAGTCCTGTCTTCTCCA	Villanueva et al., 2011