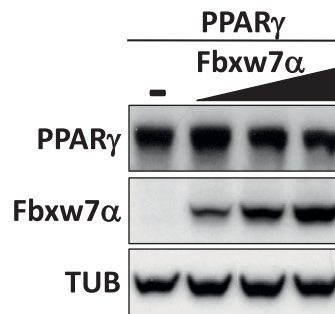
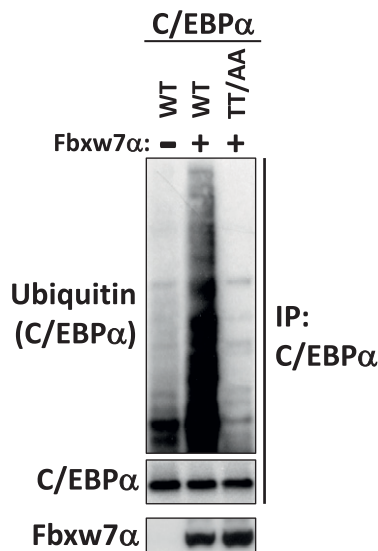


# Supporting Information

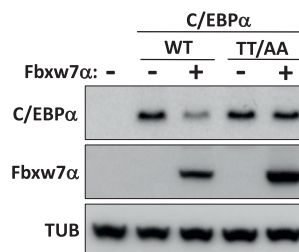
Bengoechea-Alonso and Ericsson 10.1073/pnas.0913367107



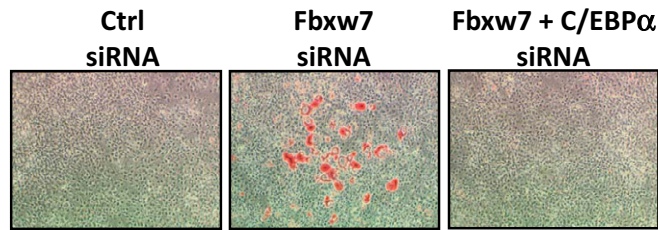
**Fig. S1.** PPAR $\gamma$  is not a substrate for Fbxw7 $\alpha$ . 3T3-L1 cells were transfected with PPAR $\gamma$  in the presence of increasing amounts of Fbxw7 $\alpha$ . The levels of PPAR $\gamma$ , Fbxw7 $\alpha$  and  $\alpha$ -tubulin (TUB) were determined by Western blotting.



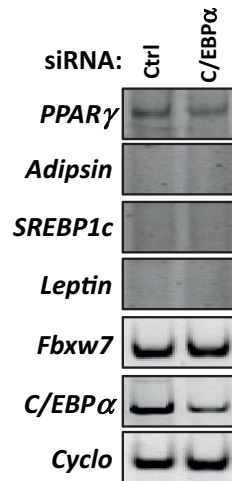
**Fig. S2.** Fbxw7 $\alpha$  enhances the ubiquitination of C/EBP $\alpha$ . HEK293T cells were transfected with C/EBP $\alpha$ , either wild-type or the T222/226A mutant (TT/AA), and HA-ubiquitin in the absence or presence of Fbxw7 $\alpha$ . C/EBP $\alpha$  was immunoprecipitated from total cell extracts, and the ubiquitination of C/EBP $\alpha$  was monitored with anti-HA antibodies. The levels of C/EBP $\alpha$  and Fbxw7 $\alpha$  were determined by Western blotting.



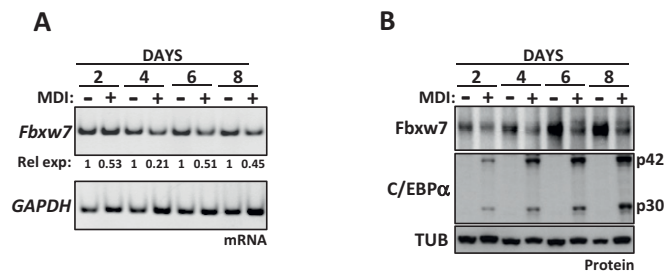
**Fig. S3.** Fbxw7 $\alpha$  is a negative regulator of C/EBP $\alpha$ . 3T3-L1 preadipocytes were transfected with C/EBP $\alpha$ , either wild-type or the T222A/T226A mutant (TT/AA), in the absence or presence of Fbxw7 $\alpha$ . Forty-eight hours after transfection, the levels of C/EBP $\alpha$ , Fbxw7 $\alpha$  and  $\alpha$ -tubulin (TUB) were determined by Western blotting.



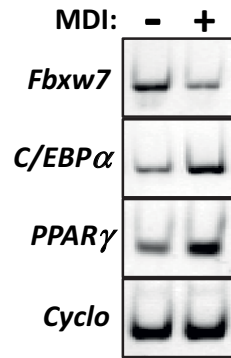
**Fig. 54.** Adipocyte differentiation in *Fbxw7*-deficient cells is dependent on *C/EBPα*. 3T3-L1 preadipocyte cells were transfected with control, *Fbxw7*, or *Fbxw7* plus *C/EBPα* siRNA. Ten days following transfection, cellular lipids were visualized with Oil Red O stain, and the cells were photographed.



**Fig. 55.** Inactivation of *C/EBPα* in 3T3-L1 preadipocytes does not affect the expression of adipocyte markers. 3T3-L1 preadipocytes were transfected with control, *Fbxw7*, or *C/EBPα* siRNA. Ten days following transfection, the mRNA expression of the indicated genes was determined by RT-PCR analysis (Cyclo; cyclophilin).



**Fig. 56.** *Fbxw7* is down-regulated during adipocyte differentiation. (A) 3T3-L1 preadipocytes were left untreated or allowed to differentiate to mature adipocytes for 2, 4, 6, or 8 days. The expression of *Fbxw7* and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was determined by RT-PCR analysis. The expression of *Fbxw7* in the absence of a mixture of methyl-isobutylxanthine, dexamethasone, and insulin (MDI) was set as 1. (B) 3T3-L1 preadipocytes were treated as in A and *Fbxw7* was immunoprecipitated from total cell extracts. The levels of *Fbxw7* in the immunoprecipitates, and *C/EBPα* and  $\alpha$ -tubulin (TUB) in cell extracts were determined by Western blotting.



**Fig. S7.** *Fbxw7* is down-regulated during adipocyte differentiation in human adult stem cells. Adipose-derived adult human stem cells were left untreated or allowed to differentiate to mature adipocytes. The expression of *Fbxw7*, *C/EBPα*, *PPARγ*, and *cyclophilin* (*Cyclo*) was determined by RT-PCR analysis.