SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1.
A. *P66^{Shc} expression in adipocytes.* P66^{Shc} protein expression in BAT and WAT (upper panel), BAT-derived pre-adipocytes (BAT ADPC; central panel), and WAT-derived pre-adipocytes (WAT ADPC; lower panel). I: insulin treatment; d: days of treatment.
B. *Effects of p66^{Shc} and p66^{Shc}qq expression in Foxo1 localization.* Foxo1 localization in untreated and insulin treated p66^{Shc}-/- BAT pre-adipocytes infected with empty (Vect), p66^{Shc} or p66^{Shc}*qq* retroviral vectors.
C. *Tryglyceride accumulation in WT and p66^{Shc}-/- BAT pre-adipocytes upon treatment with* IBMX/DEX. White light microscopy pictures of Oil red-stained BAT pre-adipocytes from WT and p66^{Shc}-/- mice, untreated or after treatment with IBMX-dexametasone (IBMX-DEX), and their corresponding OD values (this experiment is representative of 3 that gave comparable results).

Supplementary Figure 2.
Gene regulations in WT and p66^{Shc}-/- BAT pre-adipocytes treated with insulin. Venn diagrams representing overlapping gene regulations in WT and p66^{Shc}-/- BAT pre-adipocytes treated with insulin. The table reports the top 20 insulin-regulated genes in each group. In bold are indicated the genes whose analysis is showed in Figure 3C.

Supplementary Figure 3.
A. *Lipid profile and blood levels of insulin and glucose in WT and p66^{Shc}-/- mice.* Plasma levels of free fatty acids, triglycerides, cholesterol, insulin and glucose in WT and p66^{Shc}-/- mice fed standard (SD) and high fat (HF) diet (n=20 mice per group).
B. *Effect of N-acetylcisteine supplementation on weight gain.* Body weight curves of WT 129Sv male mice fed standard or high fat (HF) diets plus or minus 40 mM NAC in the drinking water (n=12 mice per group).

Supplementary Figure 4.
Model of p66^{Shc}-mediated regulation of TG synthesis in adipocytes. The scheme depicts the role of p66^{Shc} in regulating insulin-mediated TG accumulation in adipocytes, and highlights its role in the integration of insulin signaling (right side of the scheme) and mitochondrial respiration (left circle). Availability of carbon sources (Food) is indicated at the top as major determinant of the levels of circulating insulin. Engagement of insulin receptors in adipocytes leads to p66^{Shc} activation (via phosphorylation, as described in the text; vertical red arrow) and its translocation (indicated by the arrowed curve) within the mitochondrial inter membrane space (in pink). There, p66^{Shc} generates H_2O_2, which selectively potentiates insulin signaling (the red arrow indicates the insulin signaling branch regulated by p66^{Shc}-generated H_2O_2: PTEN-PI3K-AKT-Foxo1 pathway, as described in the text) and contributes to gene expression reprogramming by insulin. Regulated expression of selected target genes (UCP1 and others, see text) leads to respiration coupling, inhibition of β-oxidation and TG accumulation. The circled scheme on the left magnifies the electron transfer chain (green arrowed curves refer to electron flow across complexes I to IV) and the redox reaction between p66^{Shc} (in red) and reduced cytochrome *c* (*c*) that catalyzes the partial reduction of O_2 to H_2O_2. Emphasis is given to the fact that production of H_2O_2 by p66^{Shc} depends on availability of respiratory substrates, thus allowing cross-talk between the energetic status of adipocytes and the insulin signaling pathway, via p66^{Shc}.

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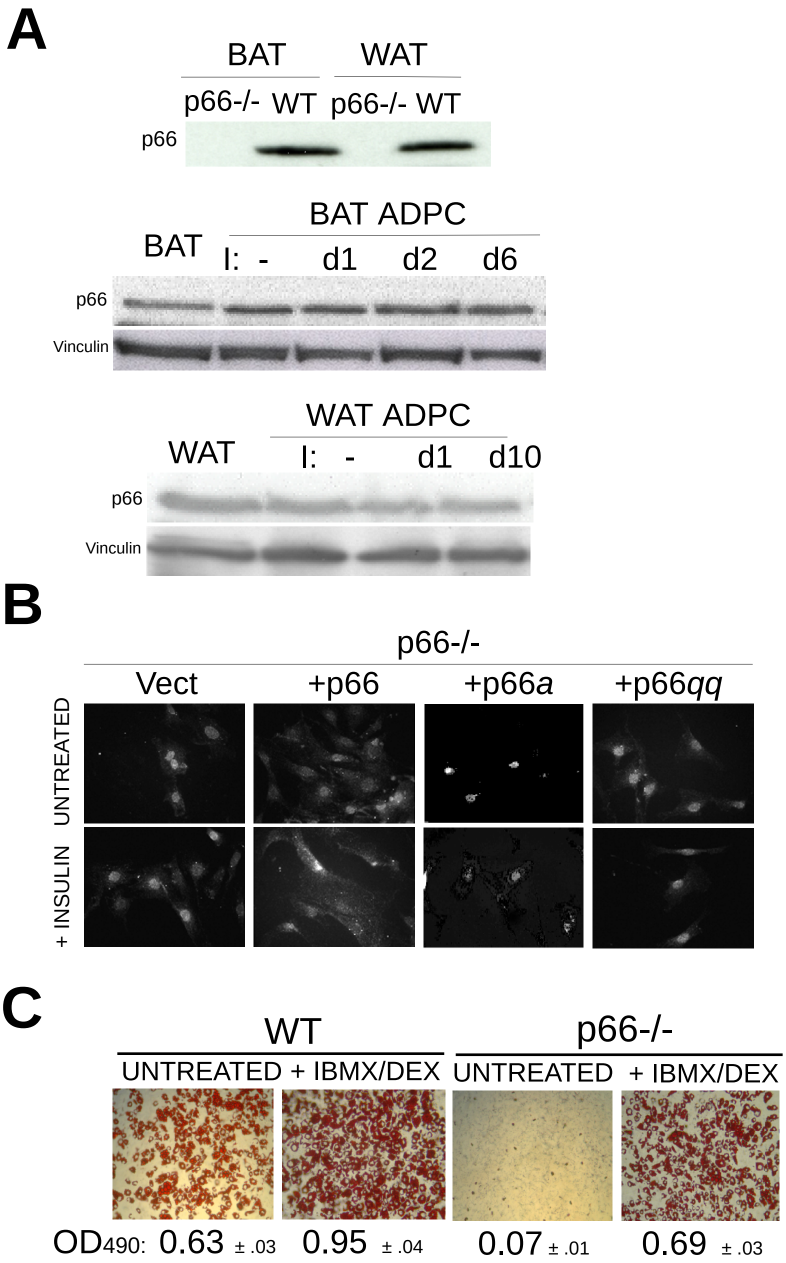
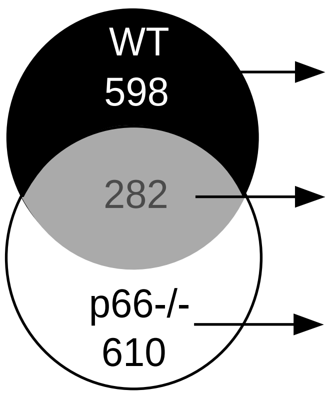
Supplementary Figure 1



Supplementary Figure 2



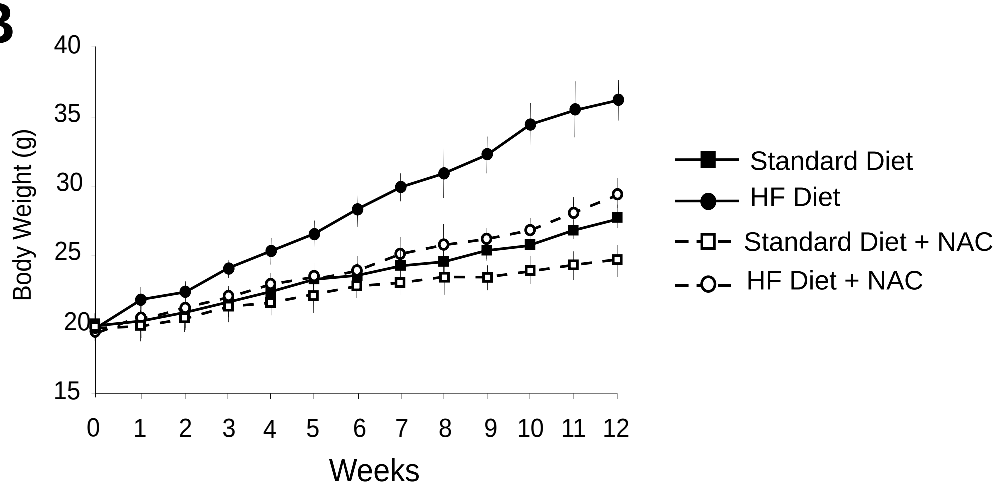
P66-dependent insulin targets	a	Chst2	Cnot2	Copg2as2	Cxcr4
	Foxo	Hbs1l	Homer1	Hrmt1l6	Lztfl1
	Myod1	Ncor1	Nptx1	Parp6	Pkd1l3
	Prkx	Pvr	Scin	Slc16a11	Tnni3k
P66-independent insulin targets	Bsf3	Egr2	**Egr3**	Ereg	Errfi1
	Fosl1	Gm337	Hbegf	Lipl2	Maff
	Obox1	Pla2g1b	**Ptgs2**	Rad51ap1	Rbbp8
	Rgs16	Sema7a	**Pai-1**	Tm7sf3	Zfp606
P66-suppressed insulin regulation	Asb12	Cml2	Creb1	Eya3	Flot2
	Hecw1	Lpp	Mapkap1	Nef3	Nktr
	Pabpc1	Pdcd10	Pde10a	Pkd2l1	Prss2
	Rnf103	Rttn	Stx8	Syk	Tnip 2

Supplementary Figure 3

A

	WT SD	WT HFD	p66-/- SD	p66-/- HFD
Free Fatty Acid (meq/L)	0.57 ± 0.12	1.24 ± 0.32	0.45 ± 0.11	1.11 ± 0.28
Triglycerides (mg/dL)	68 ± 9	143 ± 30	61 ± 6	118 ± 35
Cholesterol (mg/dL)	53 ± 6	112 ± 23	55 ± 8	103 ± 17
Insulin (ng/dL)	1.34 ± 0.22	1.47 ± 0.18	0.93 ± 0.15	0.90 ± 0.12
Glucose (mg/dL)	96 ± 5.4	103 ± 7.9	89 ± 4.5	90 ± 4.3

B



Supplementary Figure 4