SUPPLEMENTARY FIGURES

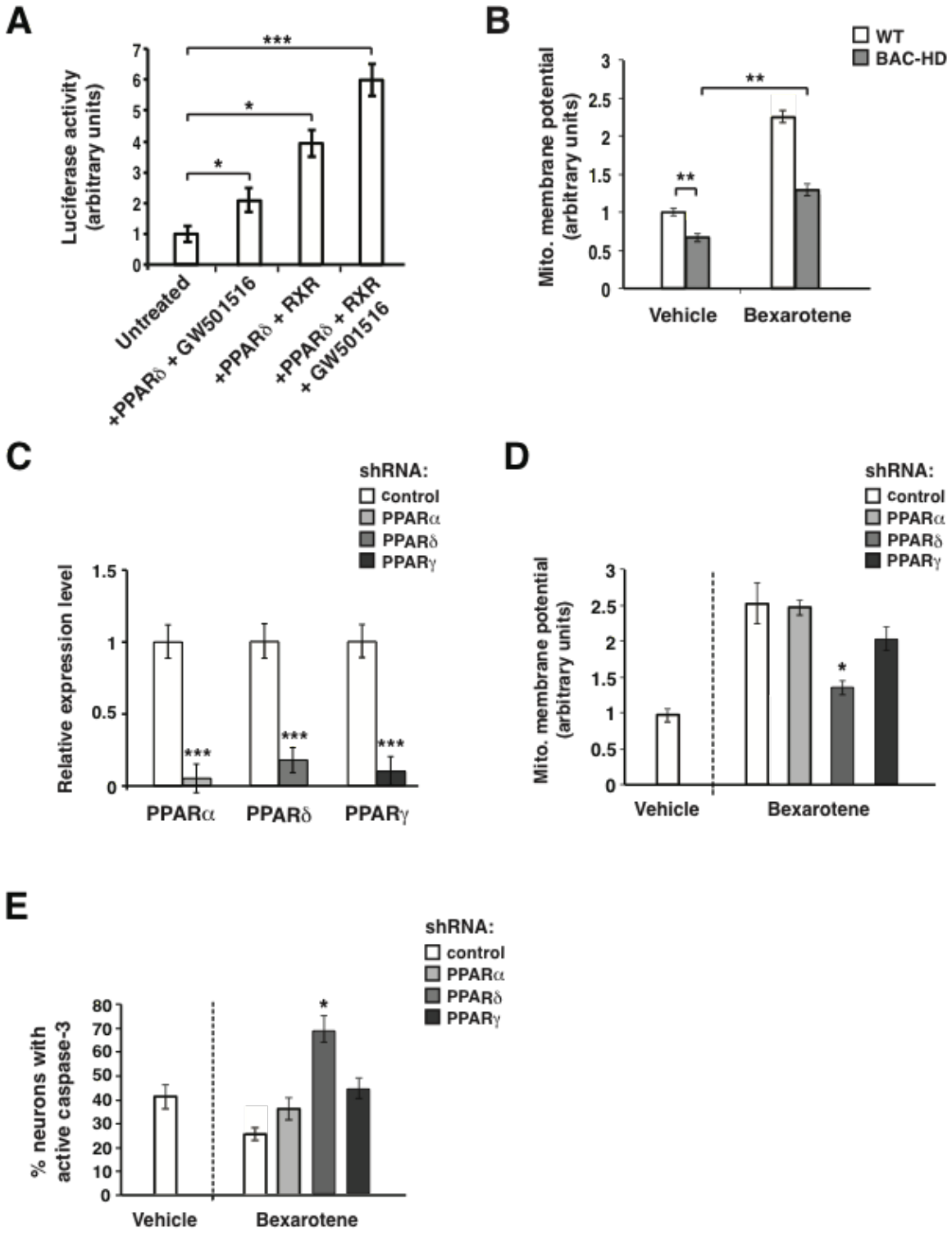

Figure S1. Bexarotene promotes neuroprotection by activating PPARδ.

A) 3X-PPRE luciferase reporter activity in primary cortical neurons from wild-type control mice, co-transfected with Renilla luciferase vector and PPARδ expression vector, and treated with GW501516 (100 nM) and/or transfected with RXR expression vector. PPARδ transactivation is increased by PPARδ agonist treatment or RXR co-expression, and maximally increased by concomitant PPARδ agonist and RXR. **P* < .05, ****P* < .001; t-test. n = 3 biological replicates; n = 3 technical replicates. Results were normalized to untreated neurons at baseline.
B) Mitochondrial membrane potential of primary cortical neurons from WT and BAC-HD mice, treated with vehicle or bexarotene (500 nM), was determined by measuring TMRM fluorescence. ***P* < .01; t-test. n = 3 biological replicates; n = 3 technical replicates. Results were normalized to WT neurons at baseline.
C) Primary cortical neurons from wild-type (WT) mice were transfected with a shRNA vector, as indicated, and the RNA expression level of the indicated PPAR was determined by qRT-PCR analysis. Each PPAR shRNA construct achieved $\geq$ 80% knock-down for the targeted PPAR. ****P* < .001; t-test. n = 3 independent experiments. Results for each PPAR shRNA were normalized to the shRNA empty vector control, which was arbitrarily set to 1.
D) Mitochondrial membrane potential of WT primary cortical neurons, transfected with the indicated shRNA expression vector, and treated with vehicle or bexarotene (500 nM), was determined from the ratio of mitochondrial to cytosolic JC-1 fluorescence. **P* < .05; ANOVA with post-hoc Tukey test. n = 3 biological replicates; n = 3 technical replicates. Results were normalized to vehicle-treated WT neurons.
E) Quantification of active caspase-3 immunostaining of WT primary cortical neurons, transfected with the indicated shRNA expression vector, and treated with vehicle or bexarotene (500 nM). **P* < .05; ANOVA with post-hoc Tukey test. n = 3 biological replicates; 30 – 50 cells were counted / experiment. Error bars = s.e.m.

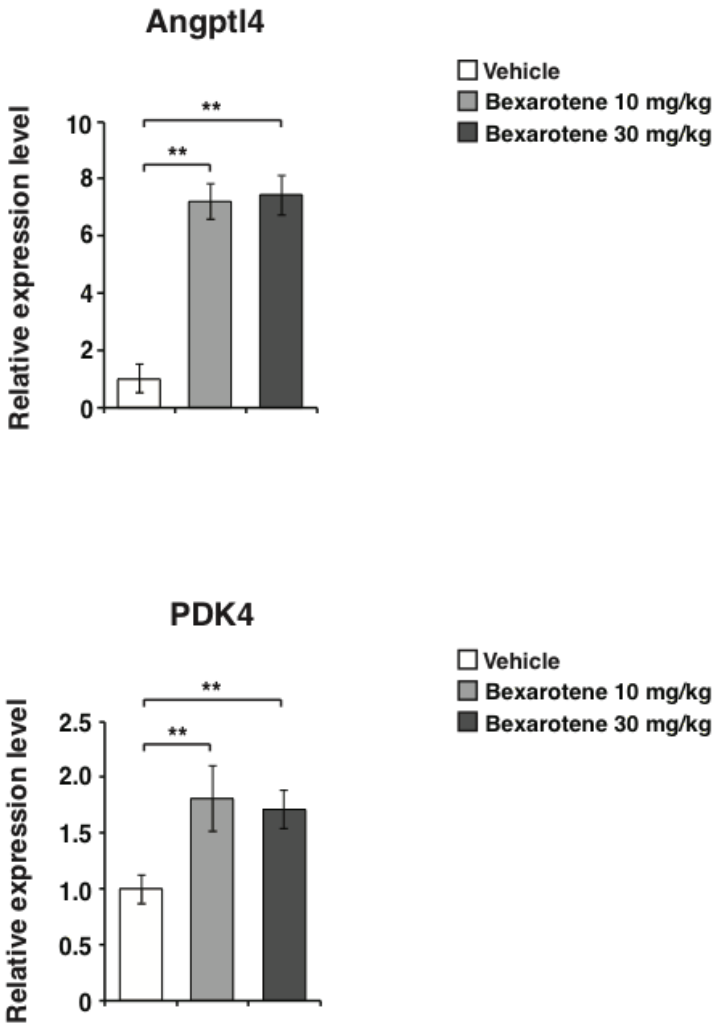

Figure S2. Bexarotene pharmacodynamics analysis of PPARδ target gene activation in CNS yields a suitable dosage and delivery scheme for preclinical trial testing.
Six-week old WT C57BL/6J mice (n = 12 / cohort) received 3 intraperitoneal injections over the course of 5 days, with the indicated compound, and then at one week after initiation of the study, all mice were euthanized. RT-PCR analysis of striatum RNA samples was performed to quantify expression levels of the PPARδ target genes Angptl4 and PDK4. $^{**}P < .01$; ANOVA with post-hoc Tukey test. Results were normalized to vehicle-treated mice, whose expression level was arbitrarily set to 1. Error bars = s.e.m.

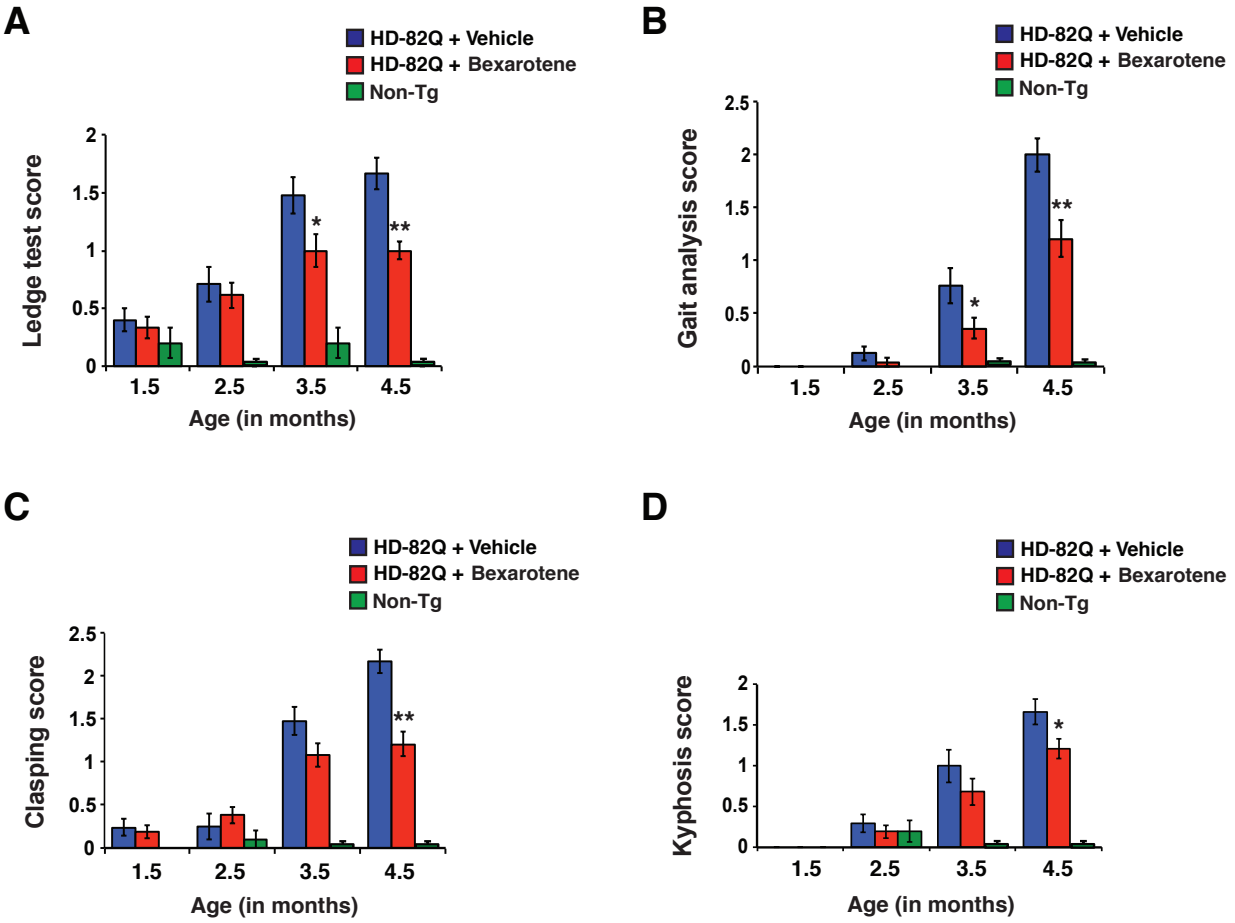

Figure S3. Bexarotene treatment of HD mice ameliorates motor function decline.
A) Quantification of scores from ledge test (0 – 3; where 0 = normal and 3= severe) on cohorts (n = 10 – 19 mice / group) of non-transgenic control mice (Non-Tg), vehicle-treated HD mice, and bexarotene-treated HD mice at monthly intervals, beginning at the initiation of the preclinical trial. **P* < .05, ***P* < .01; ANOVA with post-hoc Tukey test.
B) Quantification of scores from gait analysis (0 – 3; where 0 = normal and 3= severe) on cohorts (n = 10 – 19 mice / group) of non-transgenic control mice (Non-Tg), vehicle-treated HD mice, and bexarotene-treated HD mice at monthly intervals, beginning at the initiation of the preclinical trial. **P* < .05, ***P* < .01; ANOVA with post-hoc Tukey test.
C) Quantification of scores from clasping analysis (0 – 3; where 0 = normal and 3= severe) on cohorts (n = 10 – 19 mice / group) of non-transgenic control mice (Non-Tg), vehicle-treated HD mice, and bexarotene-treated HD mice at monthly intervals, beginning at the initiation of the preclinical trial. ***P* < .01; ANOVA with post-hoc Tukey test.
D) Quantification of kyphosis scores (0 – 3; where 0 = normal and 3= severe) on cohorts (n = 10 – 19 mice / group) of non-transgenic control mice (Non-Tg), vehicle-treated HD mice, and bexarotene-treated HD mice at monthly intervals, beginning at the initiation of the preclinical trial. **P* < .05; ANOVA with post-hoc Tukey test. Error bars = s.e.m.

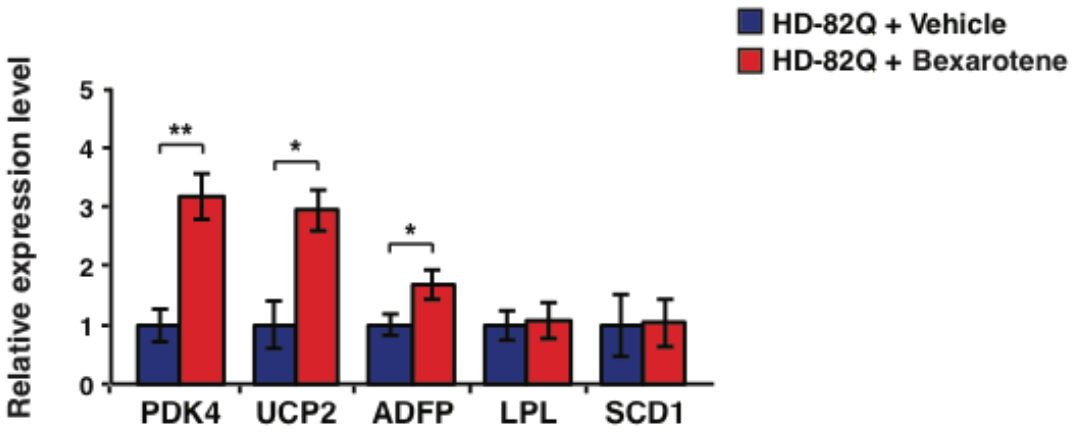

Figure S4. Bexarotene promotes PPARδ activation of target genes in skeletal muscle after just one week of treatment.

RT-PCR analysis of RNA expression of five PPARδ target genes in the quadriceps muscle of 7 week-old vehicle-treated HD mice and bexarotene-treated HD mice, after one week of indicated treatment. *$P < .05$, **$P < .01$; t- test. n = 8 mice / group. Error bars = s.e.m.

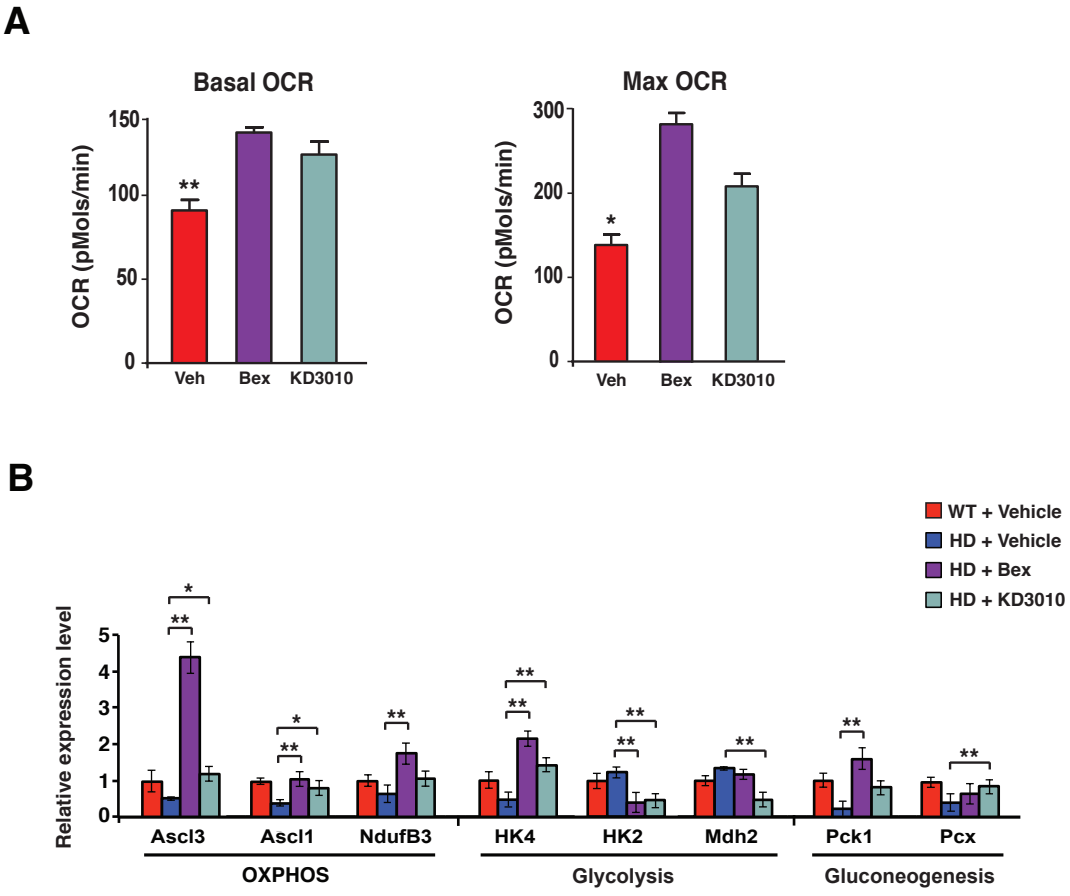

Figure S5. PPARδ activation enhances oxidative function in neurons and restores an oxidative gene expression pattern in the CNS of HD mice.

A) We measured oxygen consumption rate (OCR) in wild-type primary cortical neurons, treated with vehicle, bexarotene (500 nM), or KD3010 (100 nM), and then quantified basal OCR and maximal OCR. Both bexarotene treatment and KD3010 treatment yielded significant increases in basal OCR and maximal OCR. $*P < .05$, $**P < .01$; ANOVA with post-hoc Tukey test. n = 6 – 7 samples / condition.

B) RT-PCR analysis of RNA expression of PPARδ target genes, representative of different metabolic pathways as indicated, in the striatum of 18 week-old vehicle-treated non-transgenic control mice (WT), vehicle-treated HD mice, bexarotene-treated HD mice, and KD3010-treated HD mice. $*P < .05$, $**P < .01$; ANOVA with post-hoc Tukey test. n = 3 - 6 mice / group.

Error bars = s.e.m.

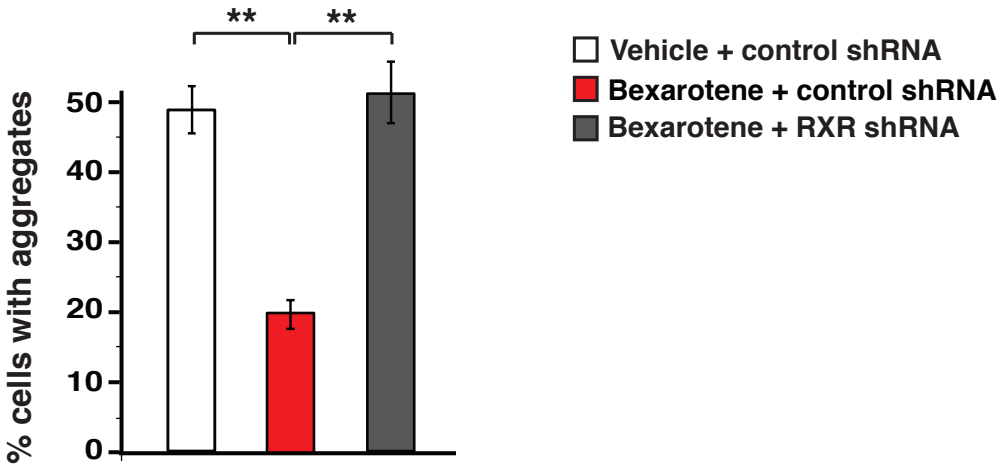

Figure S6. Bexarotene-mediated htt protein aggregate reduction is RXR-dependent. Quantification of Neuro2a cells containing htt protein aggregates, when transfected with a htt-104Q expression vector and the indicated shRNA (control = scrambled shRNA), treated for 24 hrs as indicated (Bexarotene 1 μM), and then exposed to H_2O_2 (25μM, 4 hrs). $^{**}P < .01$; ANOVA with post-hoc Tukey test. n = 30 – 50 cells / sample, 9 samples / condition.

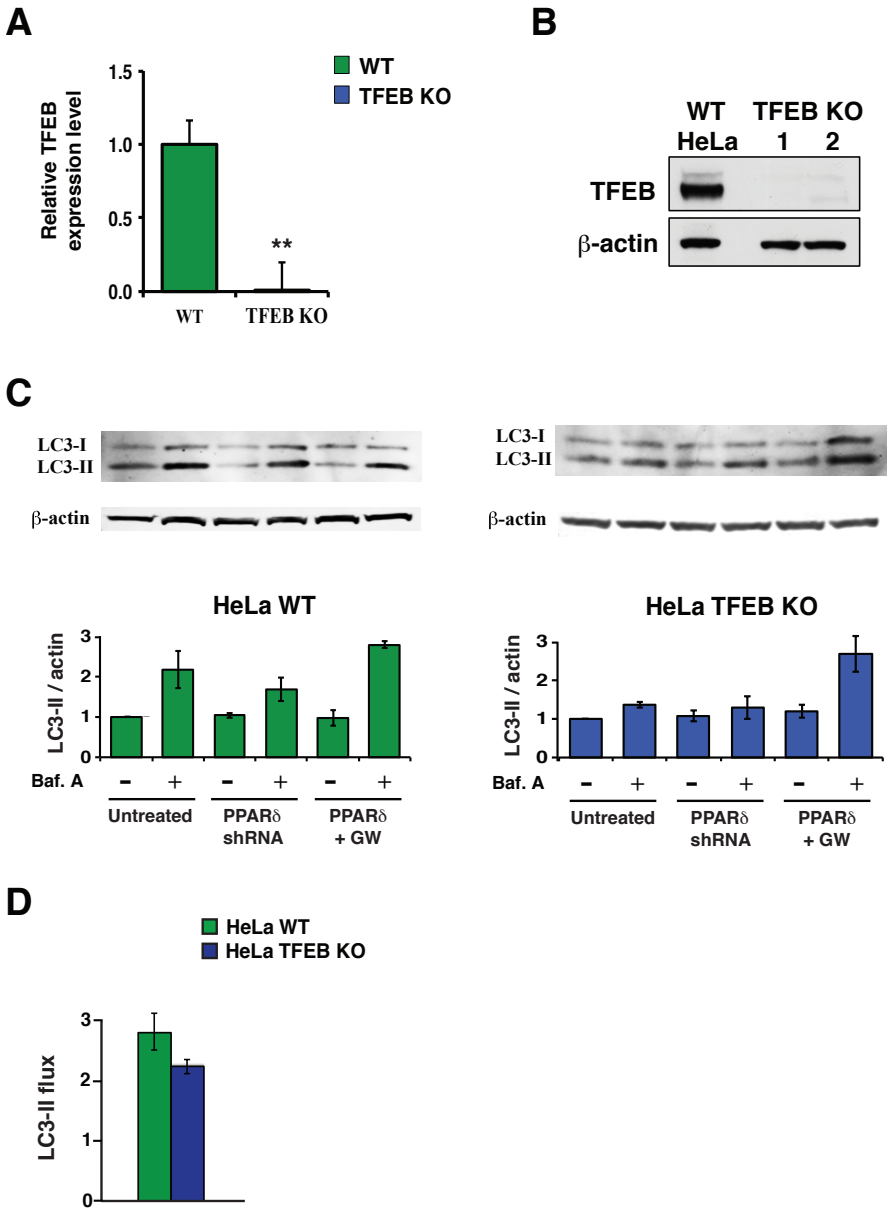

Figure S7. PPARδ activation of autophagy does not require TFEB.
A) Validation of TFEB knock-out status in HeLa cells by qRT-PCR analysis. $^{**}P < .01$; t-test. n = 3 independent experiments. Results were normalized to normal (WT) HeLa cells.
B) Validation of TFEB knock-out status in HeLa cells for two different lines by immunoblot analysis. β-actin served as a loading control.
C) LC3 immunoblot analysis of normal (WT) HeLa cells and TFEB knock-out HeLa cells (line 1 from panel B) cultured in normal media in the presence or absence of bafilomycin, and transfected with a PPARδ shRNA vector, or a PPARδ expression vector and treated with GW501516 (100 nM), as indicated. β-actin served as a loading control.
D) Densitometry analysis of LC3 immunoblotting in (C) for autophagy flux quantification reveals that autophagy flux is similar in normal HeLa cells and TFEB knock-out HeLa cells when PPARδ activation is modulated. n = 3 independent experiments.