Figure S1. TBT and ROSI-differentiated cells accumulate similar amounts of lipid, but have distinct transcriptomes (Related to Figure 1). Mouse MSCs were differentiated with an adipogenic cocktail (MDI) and vehicle control (0.1% DMSO), ROSI (500 nM), or TBT (50 nM) for 2 weeks. (A) At day 14, MSCs were either fixed for analysis of lipid accumulation or RNA was collected for deep sequencing. (B) Lipid accumulation was determined by normalizing Nile Red relative fluorescence units (RFU) to Hoechst 33342 RFU. 1-way ANOVA, Tukey's multiple comparisons test: **** $p < 0.001$. Data are represented as the mean ± s.e.m. (C-F) Libraries for sequencing were prepared from isolated RNA, sequenced, aligned, counted, quantified, and further analyzed using a bioinformatics pipeline detailed in the Materials and Methods. (C) Normalized counts from 4770 genes with the highest variance above a specified threshold (see Methods) from each replicate were subjected to unbiased hierarchical clustering analysis. (D) Normalized counts from all genes were analyzed using principal component (PC) analysis. (E) Overlap of up- and down-regulated differentially expressed genes (see Methods) in ROSI and TBT replicates as compared to vehicle control (DMSO). (F) Correlation of ROSI and TBT fold-change-over-DMSO values for all genes.
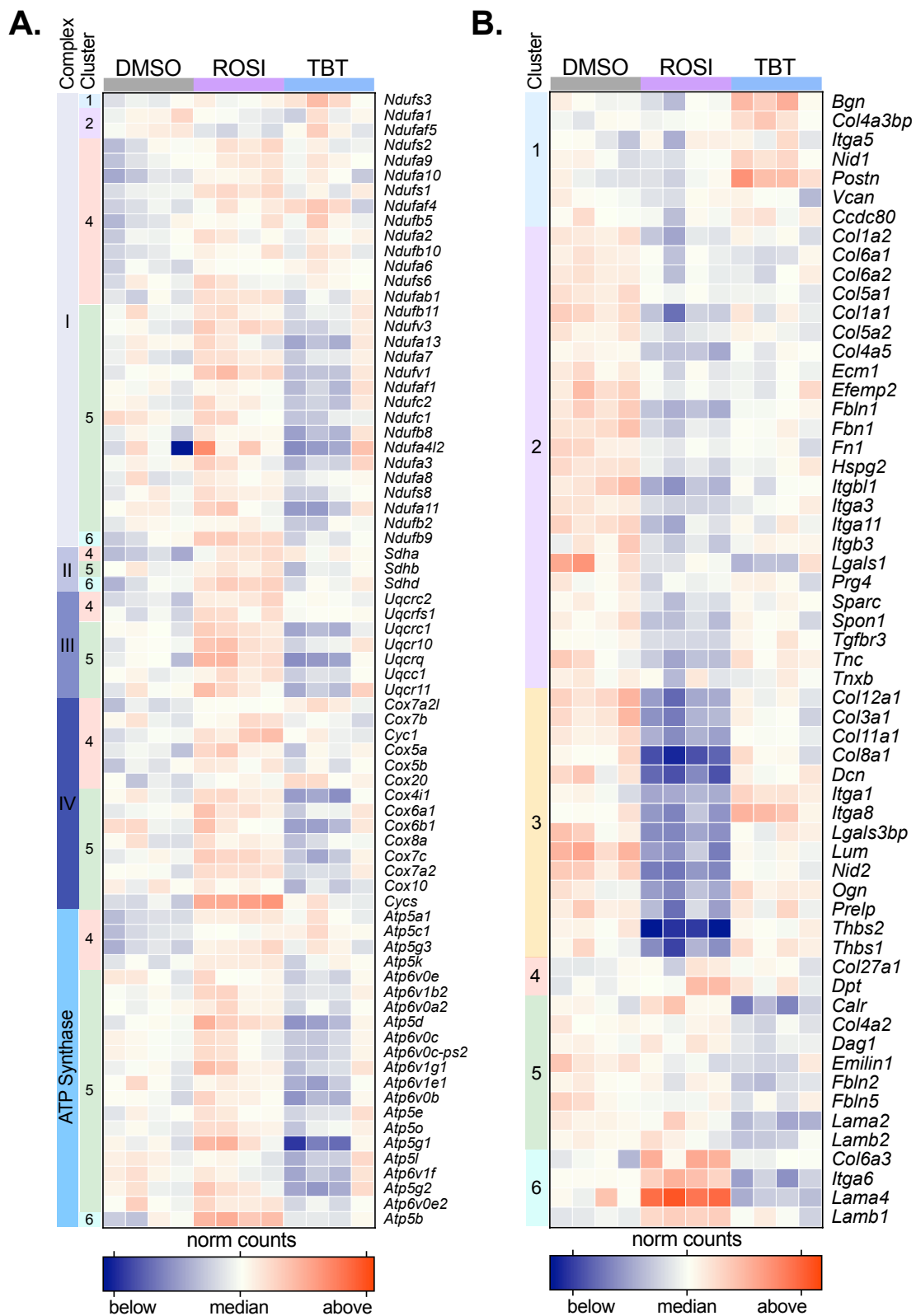
Figure S2. Clustering bias of genes involved in cellular respiration and adipose extracellular matrix (Related to Figure 1). Heatmaps of median-adjusted normalized counts (from Cluster 3.0 / Java TreeView) are presented for (A) nuclear-encoded genes in the electron transport chain (ETC) and (B) extracellular matrix genes known to play a role in adipose tissue (ref 58). (A) Genes are organized by ETC complex; k-means cluster number (see Fig 1A) is also indicated. (B) Genes are organized by k-means cluster number (see Fig 1A).
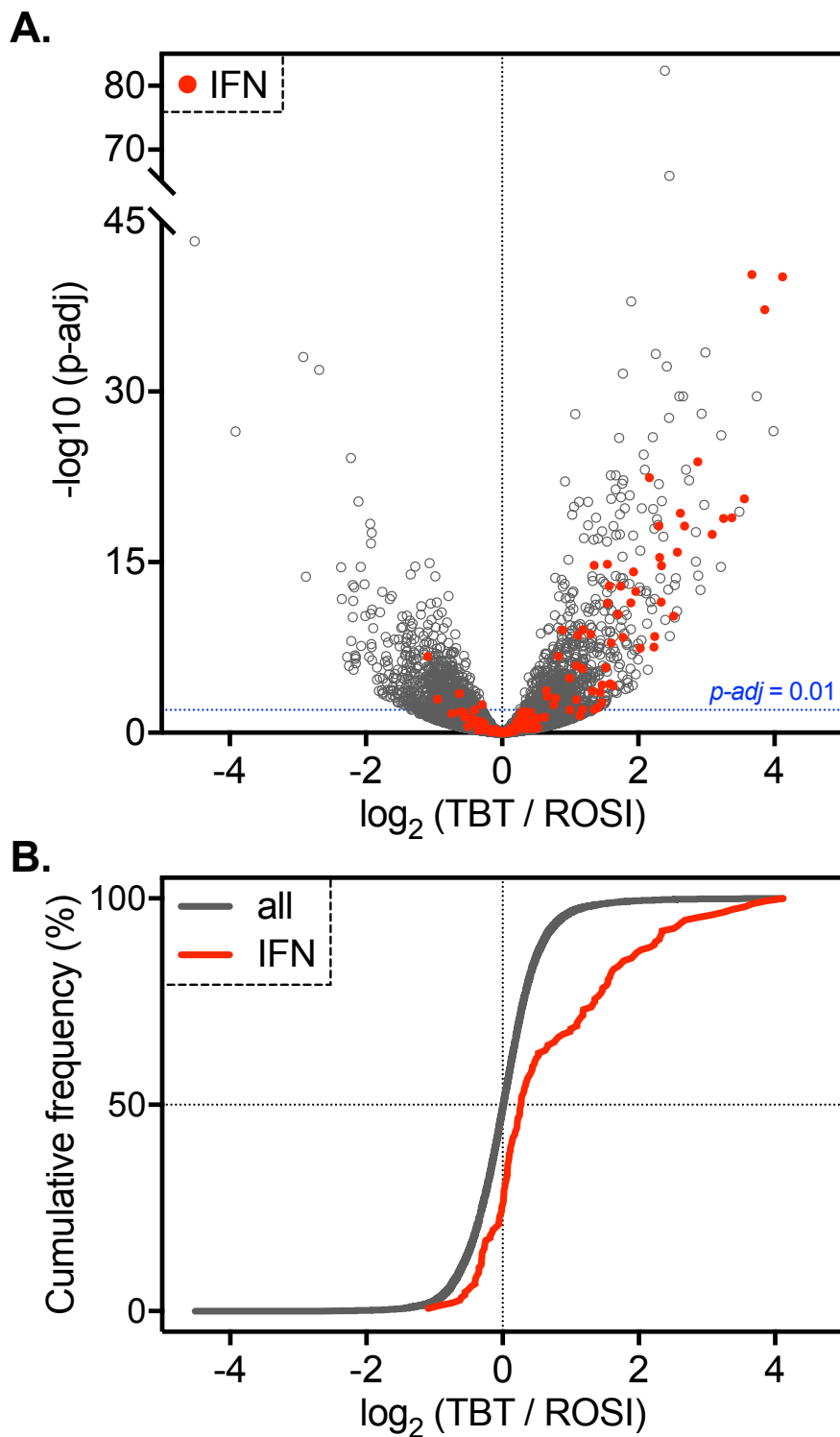
A.

B.

Figure S3. MSCs differentiated in the presence of TBT have highly enriched expression of genes involved in interferon signaling as compared to ROSI (Related to Figure 1). (A) Volcano plot of differential gene expression between TBT- and ROSI-treated MSCs. A manually curated list of genes either directly involved in interferon (IFN) signaling or well-characterized interferon-stimulated genes (ISGs) is highlighted in red (n = 152 genes). (B) Cumulative frequency graph of $\log_2$(fold-change) of TBT versus ROSI samples.
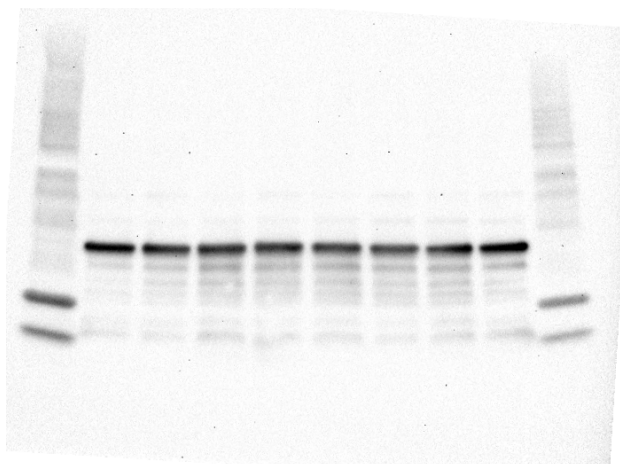
A. pAKT (S473)

Lane: 1 2 3 4 5 6 7 8 9 10
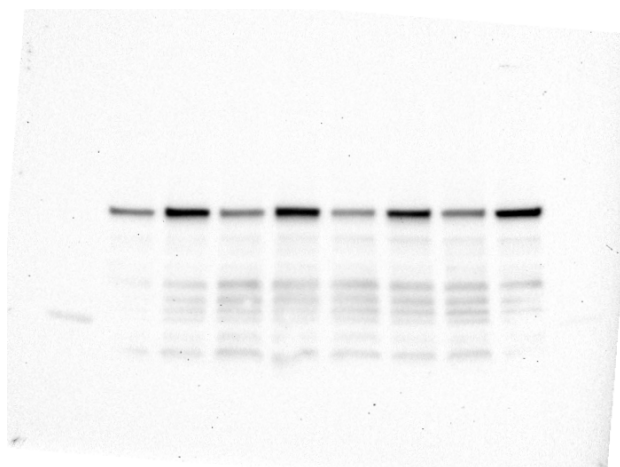
B. pan AKT

Lane: 1 2 3 4 5 6 7 8 9 10
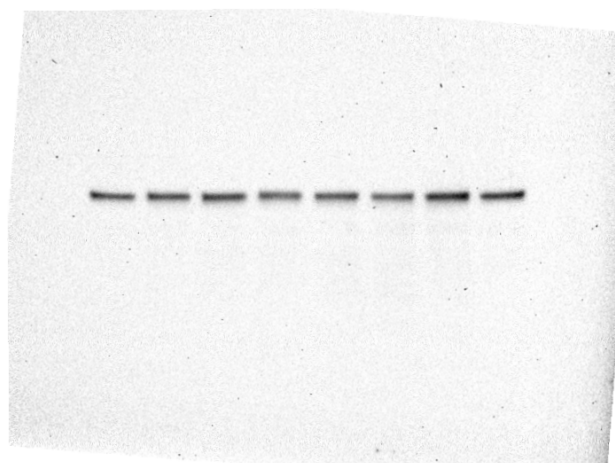
C. GAPDH

Lane: 1 2 3 4 5 6 7 8 9 10

Lane 1: Ladder
Lane 2: DMSO
Lane 3: DMSO + insulin
Lane 4: ROSI
Lane 5: ROSI + insulin
Lane 6: 4204
Lane 7: 4204 + insulin
Lane 8: TBT
Lane 9: TBT + insulin
Lane 10: Ladder

Figure S4. Full-screen images of western blots analysis of insulin signaling proteins (Related to Figure 3D). Western blots were prepared and imaged as described in the Materials and Methods. Raw blot images of (A) phospho-AKT, (B) pan-AKT, and (C) GAPDH. All images are from the same blot that was stripped and reprobed twice (see Methods) in the order presented (A → B → C).
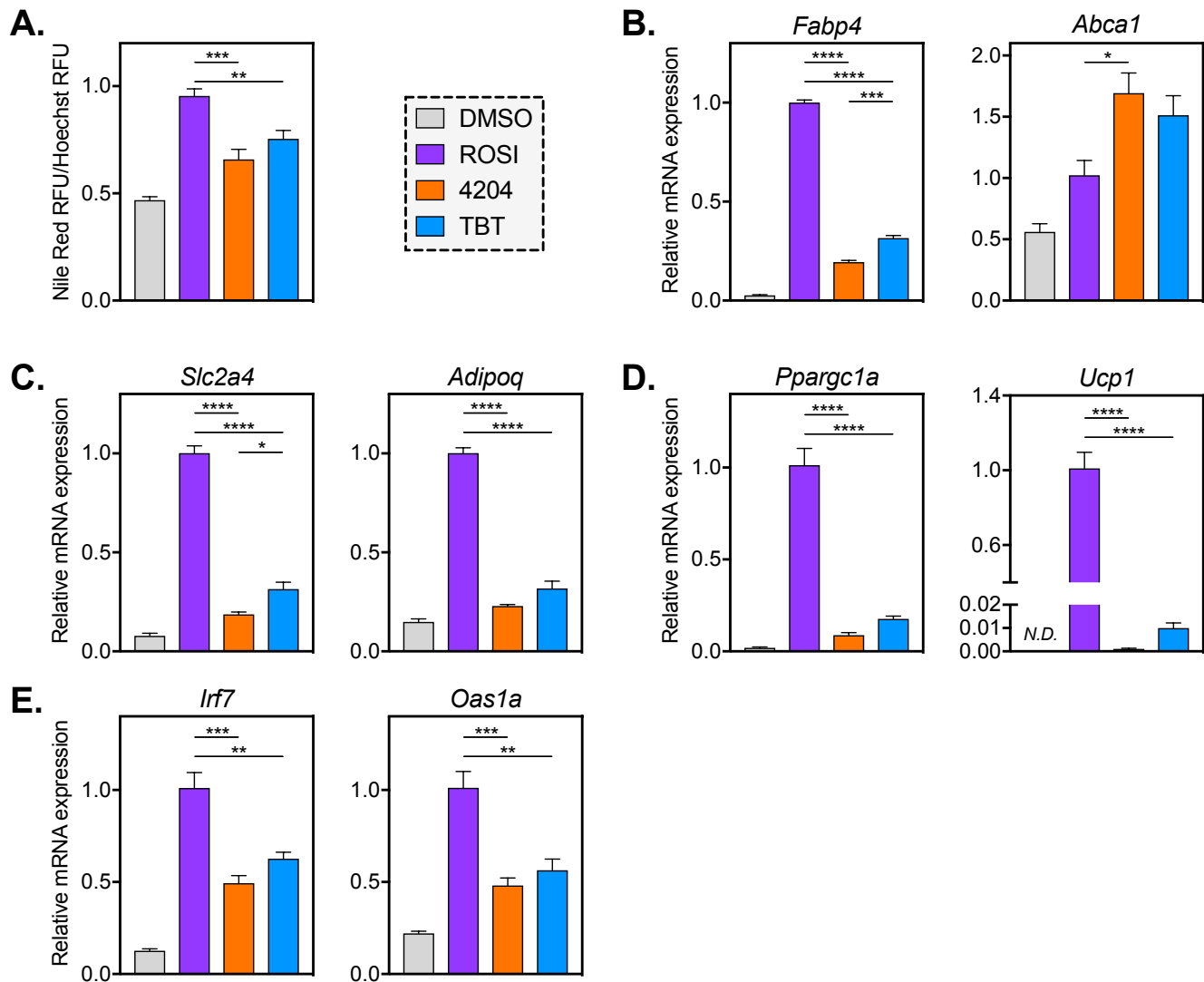
Figure S5. Rexinoid treatment is less adipogenic than ROSI in stromal vascular fraction, but still reveal striking differences in gene expression of BAT markers (Related to Figure 6). SVF was isolated from the inguinal fat depots of female mice and differentiated into adipocytes as described in the Materials and Methods in the presence of T_3 and ROSI (500 nM), 4204 (100 nM), TBT (50 nM), or vehicle control (0.1% DMSO). After 6 days of differentiation, cells were either (A) fixed and stained to assess lipid accumulation or (B-E) lysed for RNA extraction and subsequent analysis of gene expression. (A) Fixed MSCs were stained for neutral lipids (Nile Red) and nuclei (Hoechst 33342). Nile Red fluorescence was normalized to Hoechst for each well. QPCR analysis of (B) canonical PPARγ and RXR targets, (C) markers of adipocyte function, (D) BAT markers, and (E) IFN genes. For ROSI, 4204, and TBT samples: 1-way ANOVA, Tukey's multiple comparisons test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. All data are represented as the mean ± s.e.m. *N.D.* - not detected.
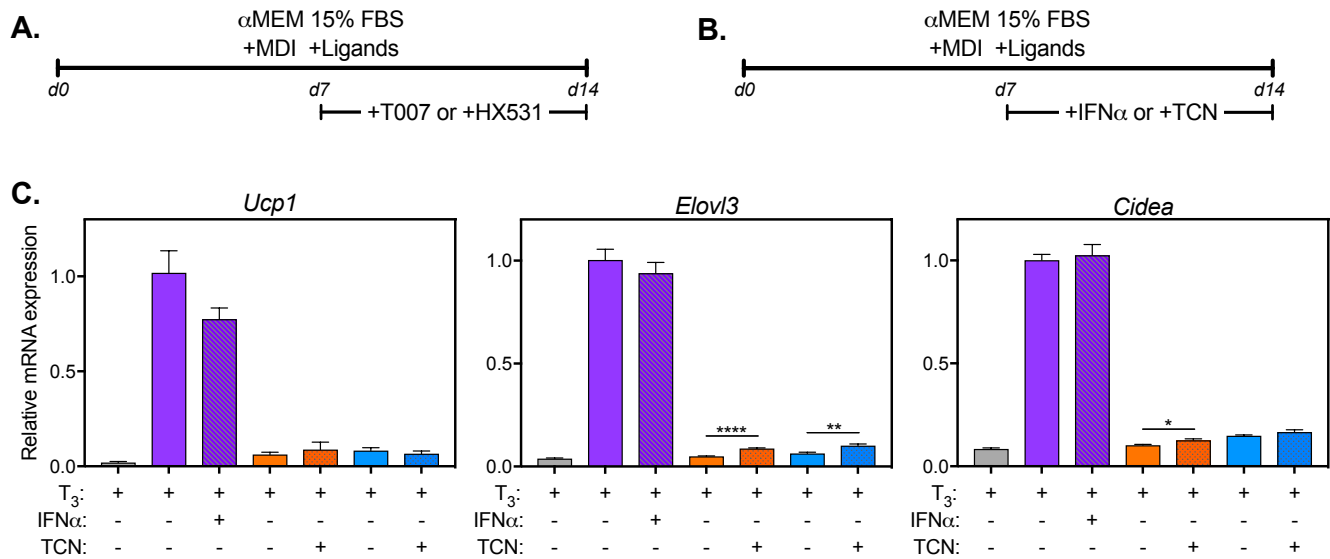
Figure S6. Mechanistic assays to assess the role of PPARγ/RXR or IFN signaling in rexinoid-differentiated MSCs (Related to Figure 7). (A-B) Assay formats for mechanistic studies of differentiating MSCs. MSCs were differentiated into adipocytes in the presence of MDI and ligands (500 nM ROSI, 100 nM 4204, 50 nM TBT, 0.1% DMSO) for 7 days. (A) On day 7 of differentiation, chemical antagonists of PPARγ (T0070907, 1 μM) or RXR (HX531, 10 μM) were added to the culture medium through the end of differentiation. (B) For studies of IFN signaling, mouse recombinant IFNα (1000 U/mL) was added to ROSI samples and the JAK inhibitor TCN (5 μM) was added to 4204/TBT samples. (C) Gene expression of BAT markers in cells treated with modulators of IFN signaling (see panel B). Unpaired t-test, * p < 0.05, ** p < 0.01, **** p < 0.0001. All data are represented as the mean ± s.e.m.