Supplemental Material

Tributyltin induces a transcriptional response without a brite adipocyte signature in adipocyte models

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Table S1. Mouse primer sequences for reverse transcriptase qPCR.

GENE SYMBOL	FORWARD	REVERSE
Rn18s	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG
B2m	CTGCTACGTAACACAGTTCCACCC	CATGATGCTTGATCACATGTCTCG
Cidea	TGCTCTTCTGTATCGCCCAGT	GCCGTGTTAAGGAATCTGCTG
Elvol3	TCCGCGTTCTCATGTAGGTCT	GGACCTGATGCAACCCTATGA
Fabp4	AGCCCAACATGATCATCAGC	TTTCCATCCCACTTCTGCAC
Plin1	GGGACCTGTGAGTGCTTCC	GTATTGAAGAGCCGGGATCTTTT
Ppara	GCGTACGGCAATGGCTTTAT	GAACGGCTTCCTCAGGTTCTT
Pparg	TGGGTGAAACTCTGGGAGATTC	AATTTCTTGTGAAGTGCTCATAGGC
Ucp1	CTCAGTGGGAGCGACTCTTCA	GGCCTCTGTGGTACACGACAA
Scd1	CCTCCTGCAAGCTCTACACC	CAGCCGAGCCTTGTAAGTTC

Table S2 – Curated genesets related to brite adipogenesis and mitochondrial biogenesis (A) and osteogenesis (B). The table displays the genes used for each curated pathway related to mitochondrial biogenesis or brite fat differentiation and osteogenesis (Accomando et al. 2014; Calderon-Dominguez et al. 2016; Gburcik et al. 2012; Harms and Seale 2013; Hilton et al. 2015; Kajimura et al. 2008; Krings et al. 2012; Lo and Sun 2013; Murholm et al. 2009; Rosell et al. 2014; Spoto et al. 2014; Wang et al. 2007) and osteogenesis (Cappellen et al. 2002; Ji et al. 2000; Saeed and Iqtedar 2015; Schilling et al. 2005; Xiao et al. 2010, 2011; Yao et al. 2008)based on literature review.

Curated Gene Sets	
(a) Brite Adipogenesis and Mitochondrial Biogenesis	**Genes**
Mitochondria	Bckdha, Cabc1, Chchdh10, Coq3, Coq5, Coq9, Cox7a1, Cox8b, Crat, Cyc1, Decr1, Etfdh, Hsha, Idh3a, Letm1, Letmd1, Me3, Plat, Rilp, Sirt3, Ucp1, Uqcr10, Uqcrfs1, Adck3, Cs, Ndufa5, Sdhd, Atp5h, Uqcrc1
Metabolism	Acaa2, Acads, Acadvl, Acc, Acot11, Acot4, Acsf2, Cacb, Coasy, Cyp2b10, Fbp2, Fn3k, Gk, Ldhb, Pank1, Pck1, Pdhb, Pdhx, Pdk2, Pdk4, Pfkb3, Sod2, Acadl, Acox1, Cd36, Acs11, Crat, Slc25a20, Acaa1, Gk, Gpd1, Pnpla2, Mgst3, Gsta4, Cbr3, Ptges2, Nsdhl, Pdha1
Hormone or Vitamin	Cyp2b10, Dio2, Retsat, Rdn16, Irs1, Fgf21, Nrg4, Cnp
Immune or Inflammatory-Related	C8g, Ifnar2, Igsf21, Il15ra, Kng1, Serpina12, Lgals3, Il2, Il4, Il10, Il13, Socs3, Cd163, Adipoq
Transcription Factors and Coregulators	Cebpa, Ebf2, Foxc2, Pparg, Prdm16, Tbx15, Ppargc1a, Sirt1, Ppara, Ppard, Ppargc1b, Sirt3, Ncoa1
Pro-Browning	Cpt1a, Cpt1b, Gata2, Gata3, Klf2, Klf3, Bmp7, Creb1, Dio2, Insr, Mapk14, Nrf1, Wnt5a, Bmp8b, Ptgs2, Elovl3, Slc27a2, Slc27a1, Hoxc9, Shox2, Hoxc8, Hoxc4, Hoxa5, Tfb2m, Ctbp1, Ctbp2, Cidec, Cidea, Tmem26
(b) Osteogenesis	Alpl, Col1a1, Fgf23, Bglap2, Spp1, Postn, Sparc, Bglap, Nfatc1, Ctsk , Tnfsf11, Cybb, Pdgfb, Itgb3, Calcr, Tnfrsf11a, Acp5, Calcr, Mitf, Car2, Mmp9, Sp7, Bmp4 , Fos, Ubd, Mmp13, Runx2, Col1a2, Col2a1, Col11a1, Ibsp, Pdgfa, Itgb1, Smad3, Dmp1, Fgfr2, Tgfb1, Smad1, Mafb, Apoe

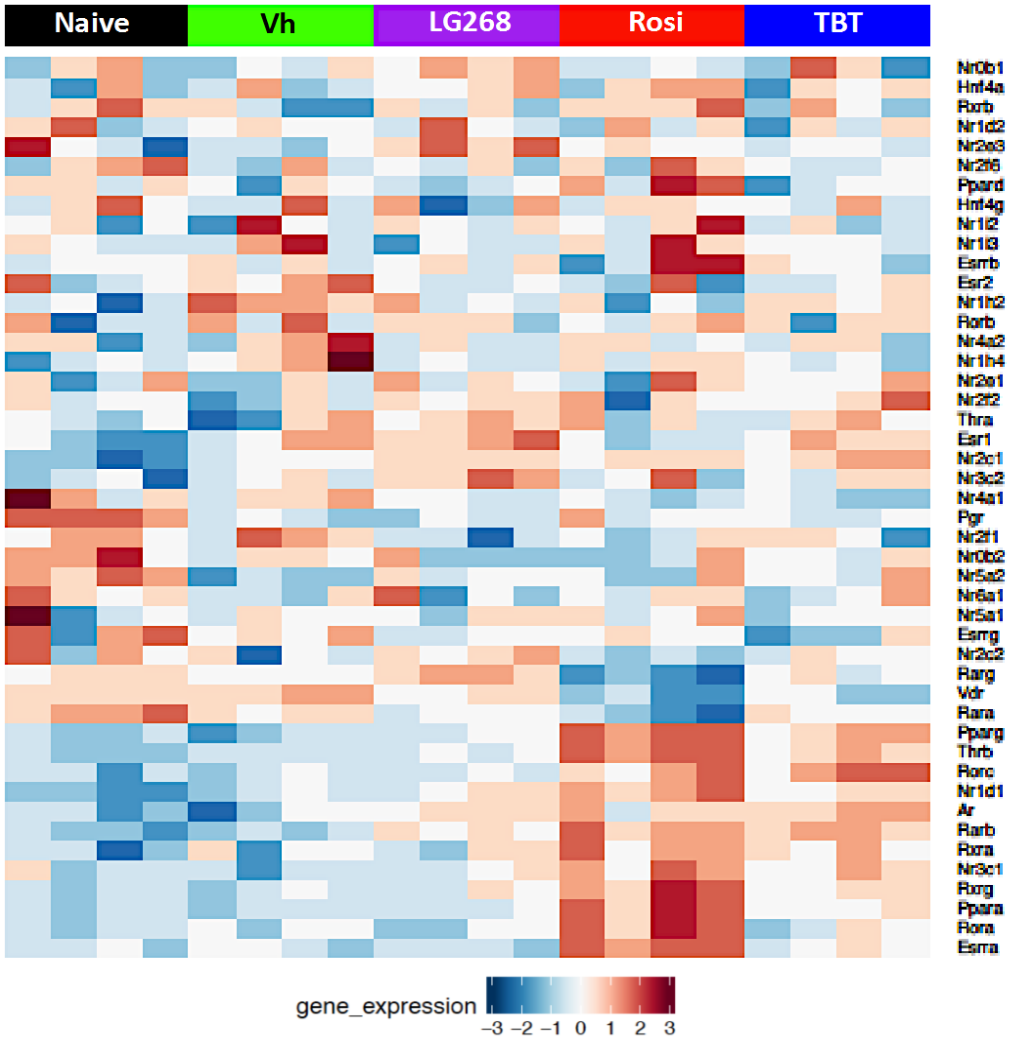


Figure S1 – Differential expression of nuclear receptors by Day 4 of differentiation of BM-MSCs. Primary bone marrow cells were isolated from female, 8 week old, C57BL/6J mice, plated, and allowed to adhere for 7 days. The medium was replaced with basal medium supplemented with osteogenic additives, β-glycerol phosphate, ascorbate, insulin and dexamethasone. Cultures were treated with Vh (DMSO, 0.1%), rosiglitazone (Rosi, 100 nM), LG100268 (LG268, 100 nM) or TBT (80 nM), cultured for 4 days and analyzed for mRNA expression using microarray. The heatmap displays the significant differentially expressed nuclear receptors (fdr<0.05) of 47 identified mouse nuclear receptors from NURSA between the vehicle and chemical-treated BM-MSCs compared to naïve BM-MSCs.

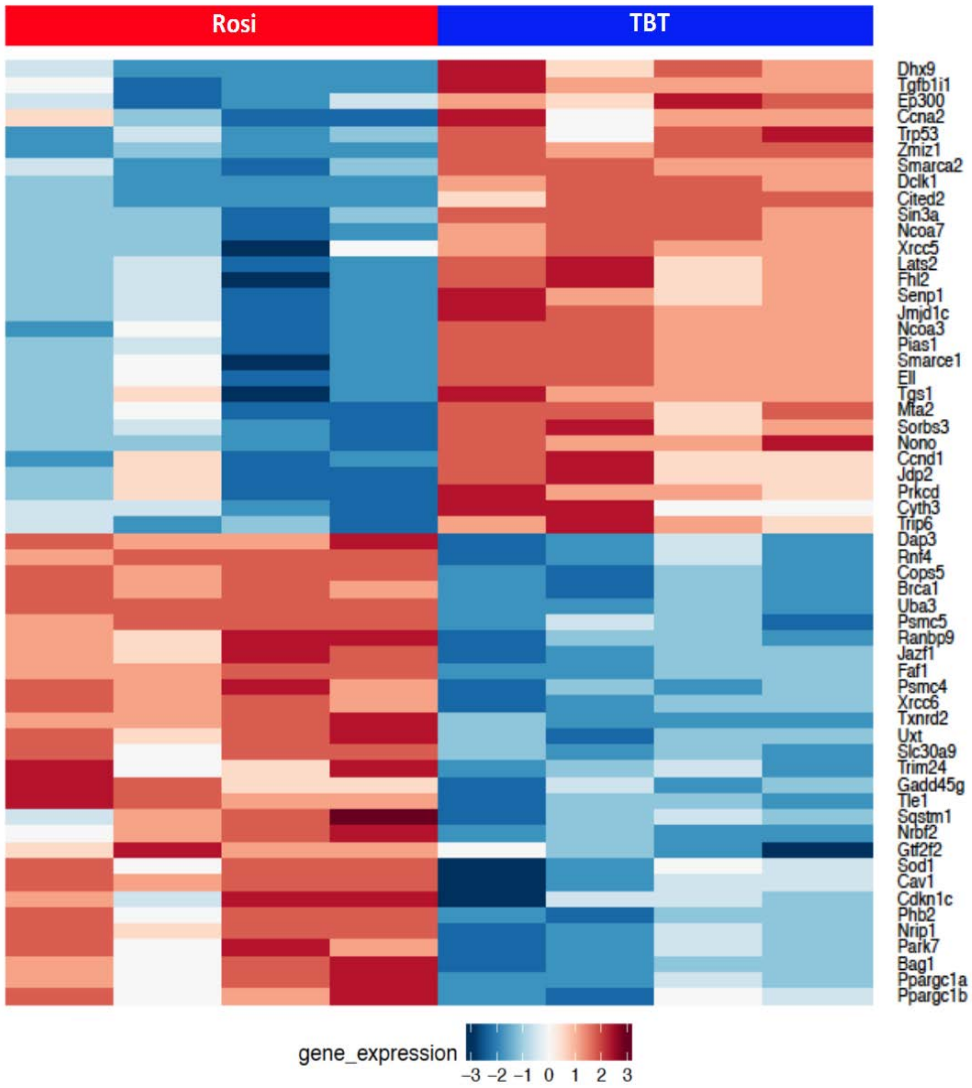


Figure S2 – Differential expression of nuclear receptor coregulators by Day 4 of differentiation between Rosi- and TBT-treated BM-MSCs. Primary bone marrow cells were isolated from female, 8 week old, C57BL/6J mice, plated, and allowed to adhere for 7 days. The medium was replaced with basal medium supplemented with osteogenic additives, β-glycerol phosphate, ascorbate, insulin and dexamethasone. Cultures were treated with Vh (DMSO, 0.1%), rosiglitazone (Rosi, 100 nM) or TBT (80 nM), cultured for 4 days and analyzed for mRNA expression using microarray. The heatmap displays the significant differentially expressed nuclear receptor coregulators (fdr<0.05) of 280 identified mouse nuclear receptors from NURSA between the Rosi- and TBT-treated BM-MSCs.

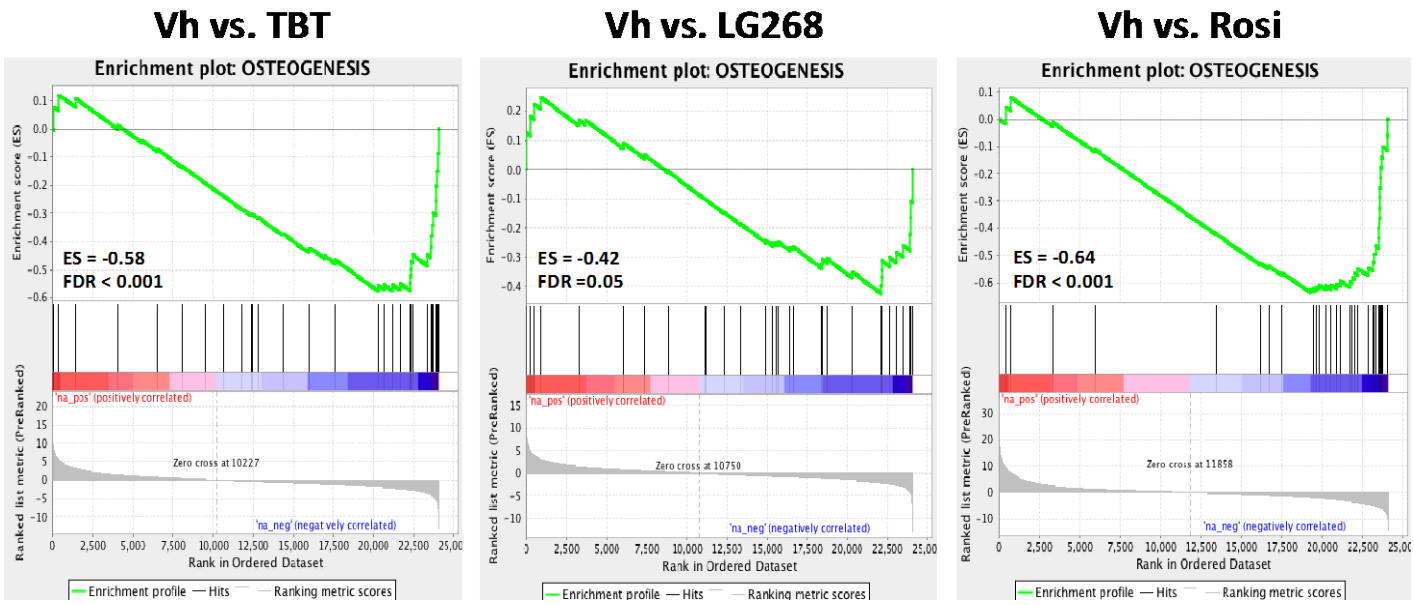
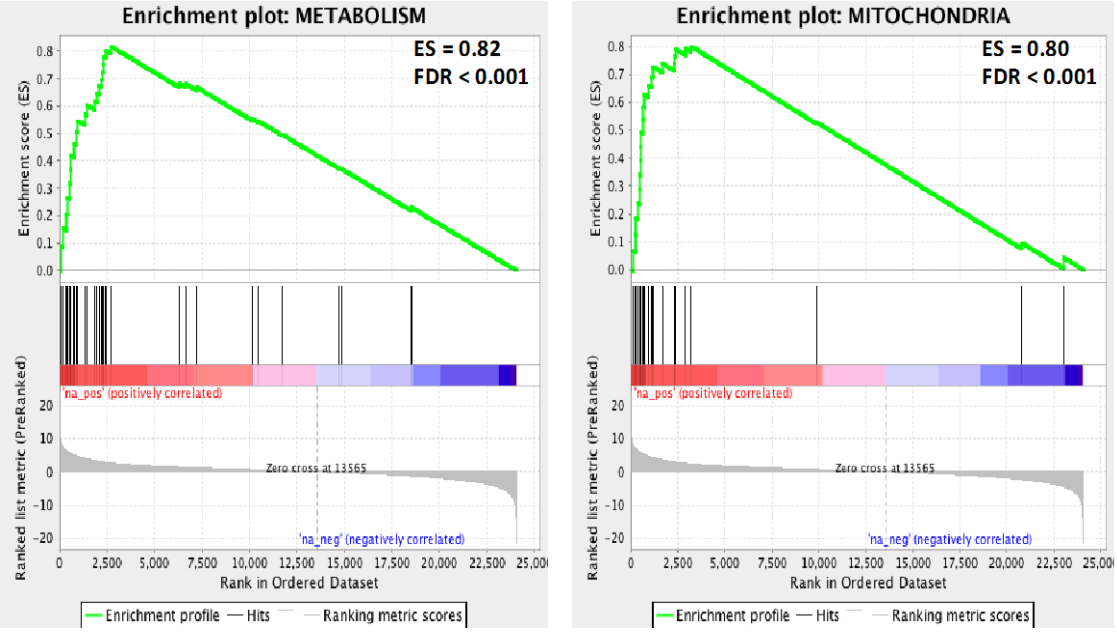


Figure S3. GSEA plots of enriched osteogenic genes in TBT-, LG100268-, and rosiglitazone-treated BM-MSCs. Primary bone marrow cells were isolated from female, 8 week old, C57BL/6J mice, plated, and allowed to adhere for 7 days. The medium was replaced with basal medium supplemented with osteogenic additives, β-glycerol phosphate, ascorbate, insulin and dexamethasone. Cultures were treated with Vh (DMSO, 0.1%), rosiglitazone (Rosi, 100 nM), LG100268 (LG268, 100 nM) or TBT (80 nM), cultured for 4 days and analyzed for mRNA expression using microarray. Enrichment analyses were performed for the curated **o**steogenesis pathway geneset (See Table S2).

(Attached as Excel File)

Figure S4 – Top Gene Ontology (GO) terms enriched for each chemical. Primary bone marrow cells were isolated from female, 8 week old, C57BL/6J mice, plated, and allowed to adhere for 7 days. The medium was replaced with basal medium supplemented with osteogenic additives, β-glycerol phosphate, ascorbate, insulin and dexamethasone. Cultures were treated with Vh (DMSO, 0.1%), rosiglitazone (Rosi, 100 nM), LG100268 (LG268, 100 nM) or TBT (80 nM), cultured for 4 days and analyzed for mRNA expression using microarray. The heatmap highlights all the different GO Terms enriched by each chemical exposure on differentiated BM-MSCs (red = upregulation; blue = downregulation; significance = q-value<0.1 and q-value<0.05).

a) Rosiglitazone vs TBT – BM-MSCs



b) Rosiglitazone vs TBT – 3T3 L1 cells

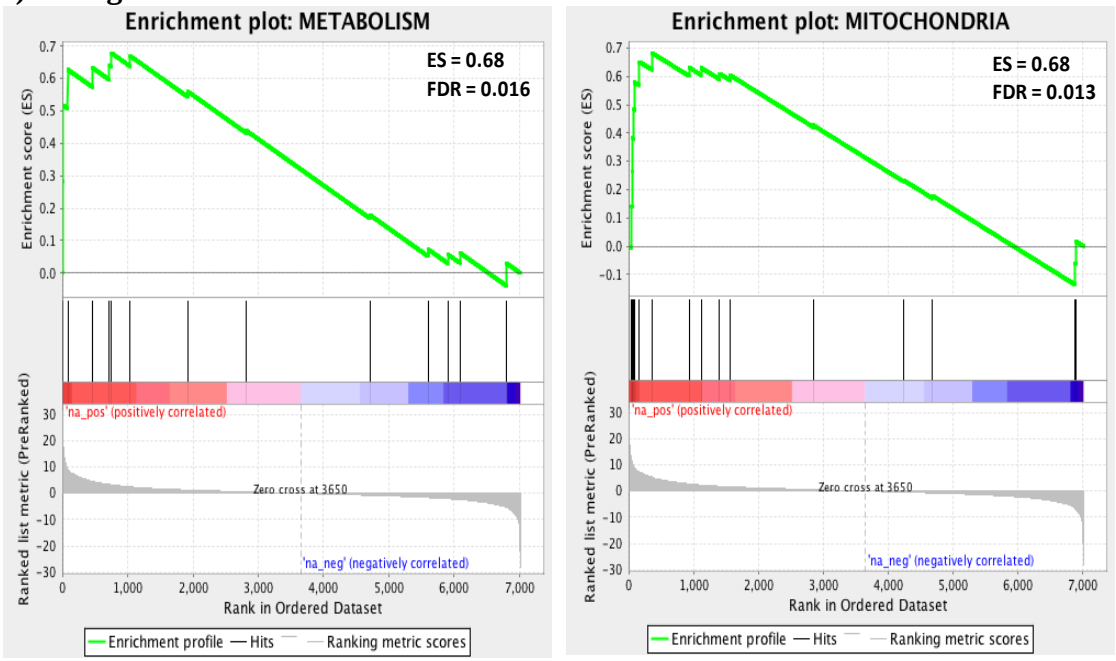


Figure S5. GSEA plots of rosiglitazone- versus TBT- enriched metabolism- and mitochondria-related gene sets (a) in BM-MSCs and (b) in 3T3 L1 cells. Primary bone marrow cells were isolated, differentiated and treated as described in Figure 2. Data for 3T3 L1 cells are from a publically available data set (GSE53004). Enrichment analyses were performed for the curated metabolism and mitochondria pathway genesets (see Table S2).

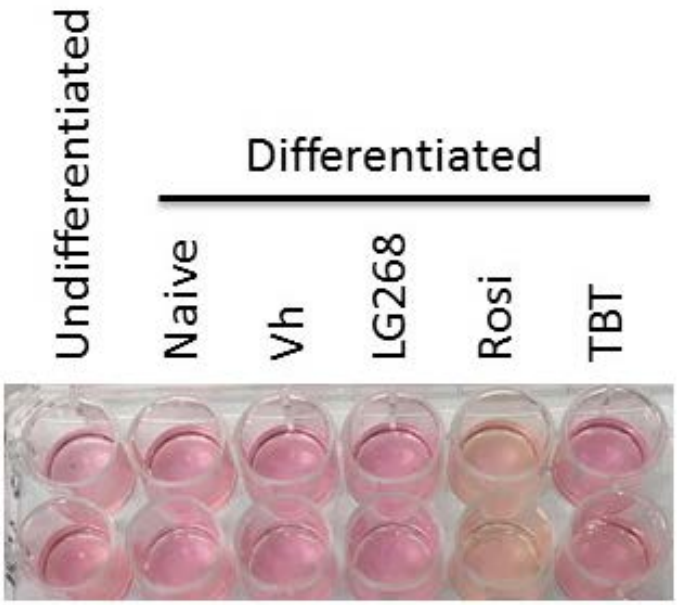


Figure S6. 3T3 L1 cells cultured with LG268, Rosi and TBT during differentiation. Cells were plated and then incubated for 4 days. Differentiation and dosing were carried out as described in Figure 6. Pictures were taken prior to mitochondrial biogenesis/activity analyses. Representative image.

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