Supplemental Information

Metabolomics-based discovery of a metabolite that enhances oligodendrocyte maturation

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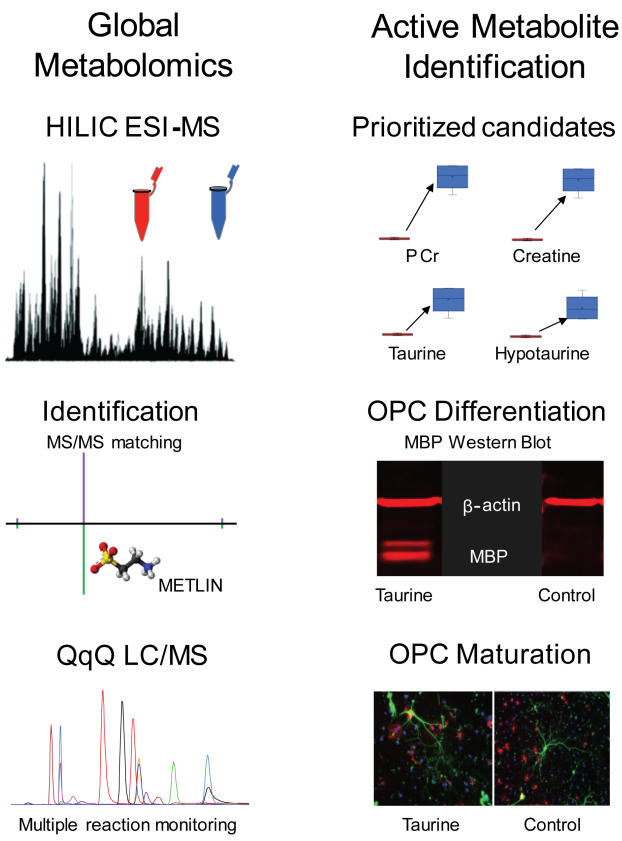
Supplementary Results



Supplementary Figure 1 | Experimental metabolomics discovery workflow. OPCs treated with DMSO and T3 for 6 days were extracted and analyzed using untargeted HILIC/MS profiling in ESI negative mode. LC/MS data acquisition was followed by retention time correction and chromatogram alignment using online XCMS. Metabolite features whose levels significantly changed (fold change > 1.5 and $p < 0.01$) were filtered out and identified by MS/MS matching. The identified metabolites, as well as other key up or down-stream metabolites, which were not identified by global metabolomics analysis, were quantified by more sensitive targeted MRM analysis using authentic compound standards. A list of endogenous metabolites was prioritized for evaluation in the *in vitro* OPC differentiation assay. Impact on differentiation was determined based on MBP expression. *In vitro* OL maturation was evaluated using a co-culture assay involving primary mouse cortical neurons and OPCs.

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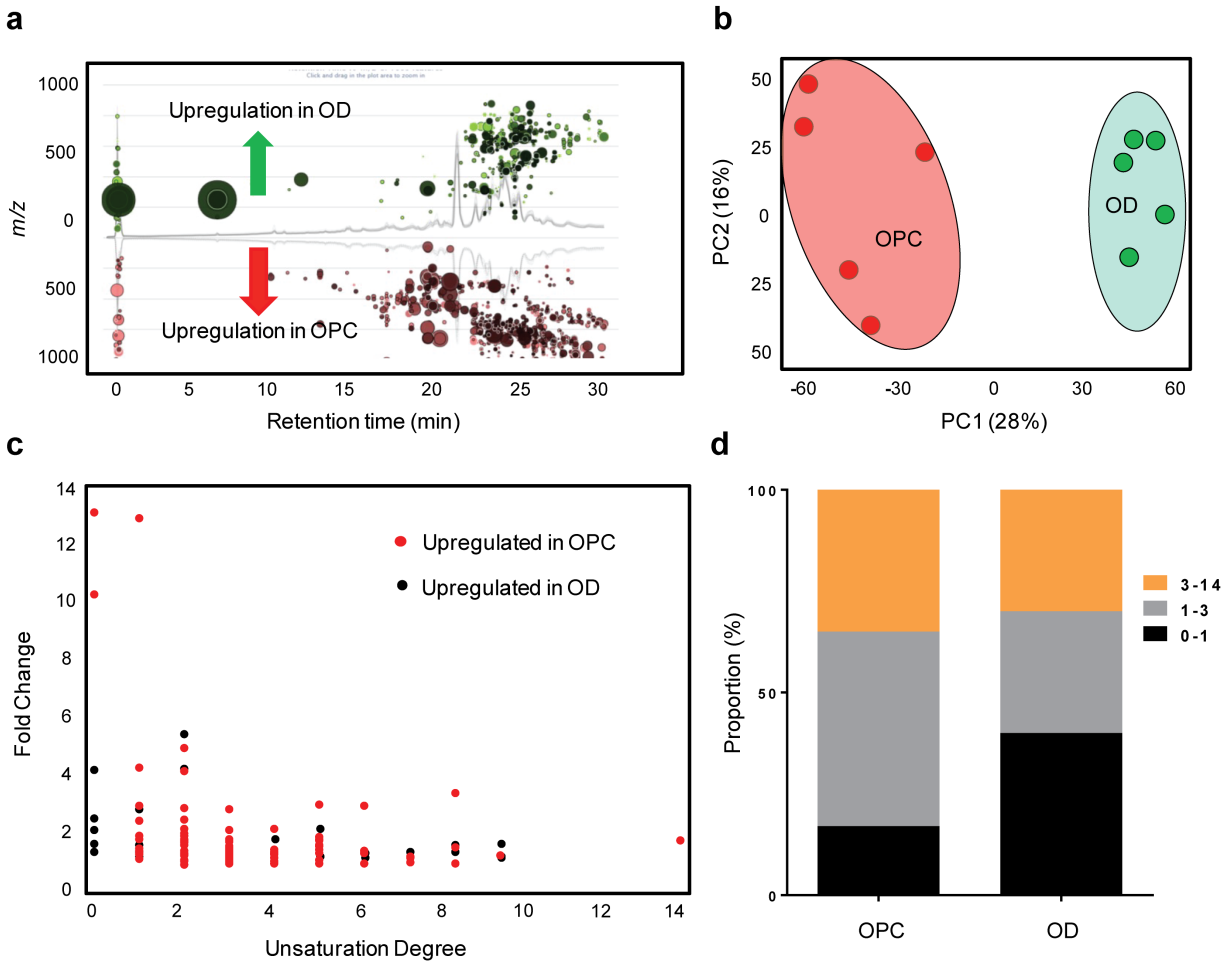

Supplementary Figure 2 | Comparative lipidomics profiling of OPCs treated with DMSO or T3 (1 µM) for 6 days. (**a**) Cloud plot showing dysregulated features between OPCs treated with DMSO and T3 at Day 6. Features were filtered using $p < 0.01$, intensity > 10,000 and fold change > 1.5 (n = 5 replicate cell cultures). (**b**) Principal component analysis scores plots. (**c**) Degree of unsaturation in OPCs. The plot shows the relative fold change of metabolites that are upregulated in OPCs and ODs. (**d**) The proportion distribution of lipids upregulated in OPCs and ODs with different unsaturation degrees (0-1, 1-3, and 3-14).

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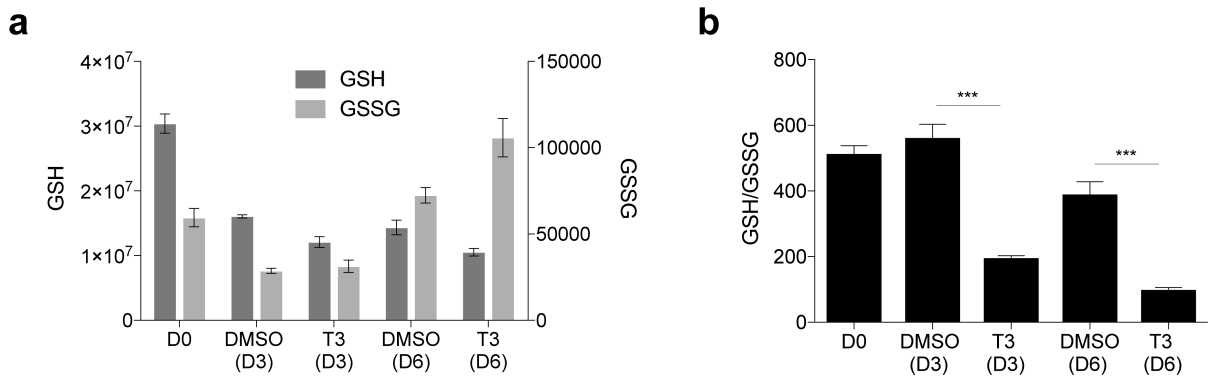

Supplementary Figure 3 | Glutathione (GSH), oxidized glutathione (GSSG), and their GSH/GSSG ratio at Day 0, 3 or 6 for OPC cultures treated with DMSO or T3 (1 μM). Data are mean ±SD (n = 3 replicate cell cultures for each condition). "***" represents p <0.001.

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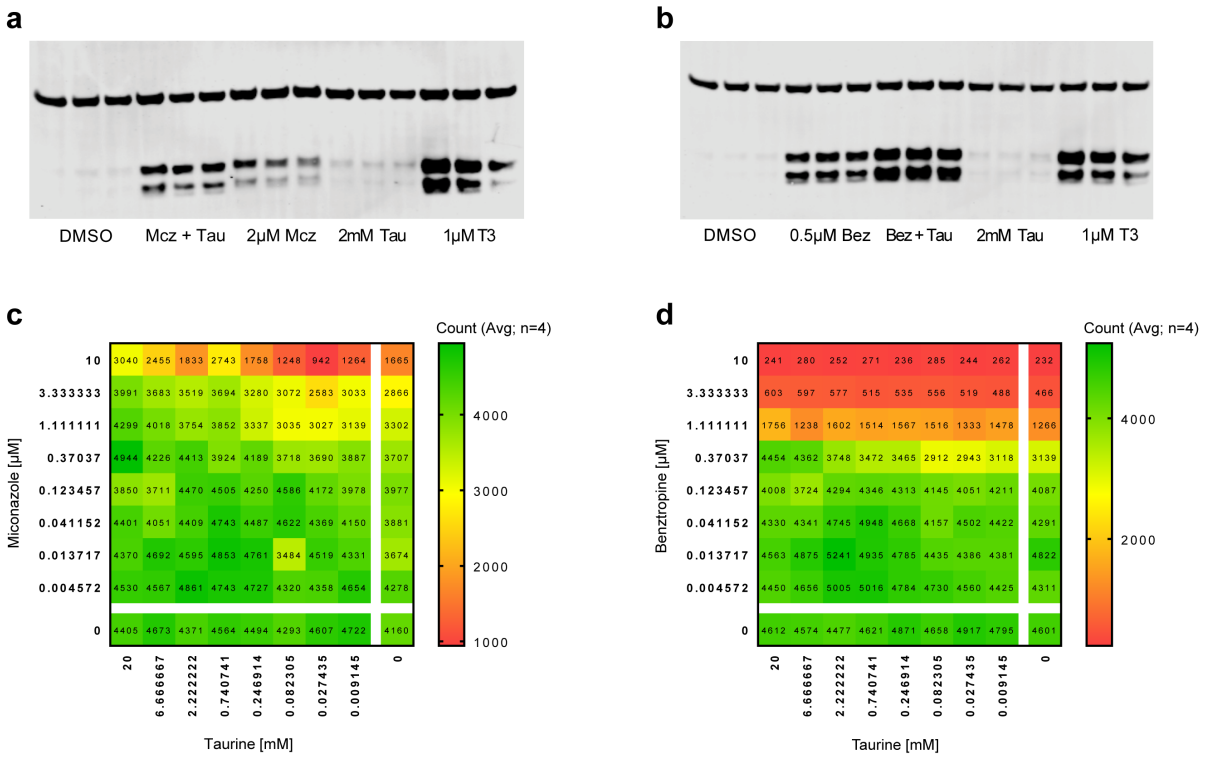

Supplementary Figure 4 | Impact of taurine treatment on OPC differentiation. (**a**) MBP expression following 6 days of differentiation with miconazole (2 µM), miconazole (2 µM) plus taurine (2 mM) or taurine (2 mM) alone. Miconazole treatment initiated at day 0 and taurine treatment initiated at day 2 (n = 3 replicate cell cultures). (**b**) MBP expression following 6 days of differentiation with benztropine (0.5 µM), benztropine (0.5 µM) plus taurine (2 mM) or taurine (2 mM) alone. Benztropine treatment initiated at day 0 and taurine treatment initiated at day 2 (n = 3 replicate cell cultures). "Bez", "Mcz" and "Tau" are the abbreviations for "benztropine", "miconazole" and "taurine"; respectively. (**c and d**) Imaging-based cell counting (n = 4 replicate cell cultures for each condition) of (**c**) Miconazole + taurine and (**d**) Benztropine + taurine-treated OPCs with variable combinations of concentration.

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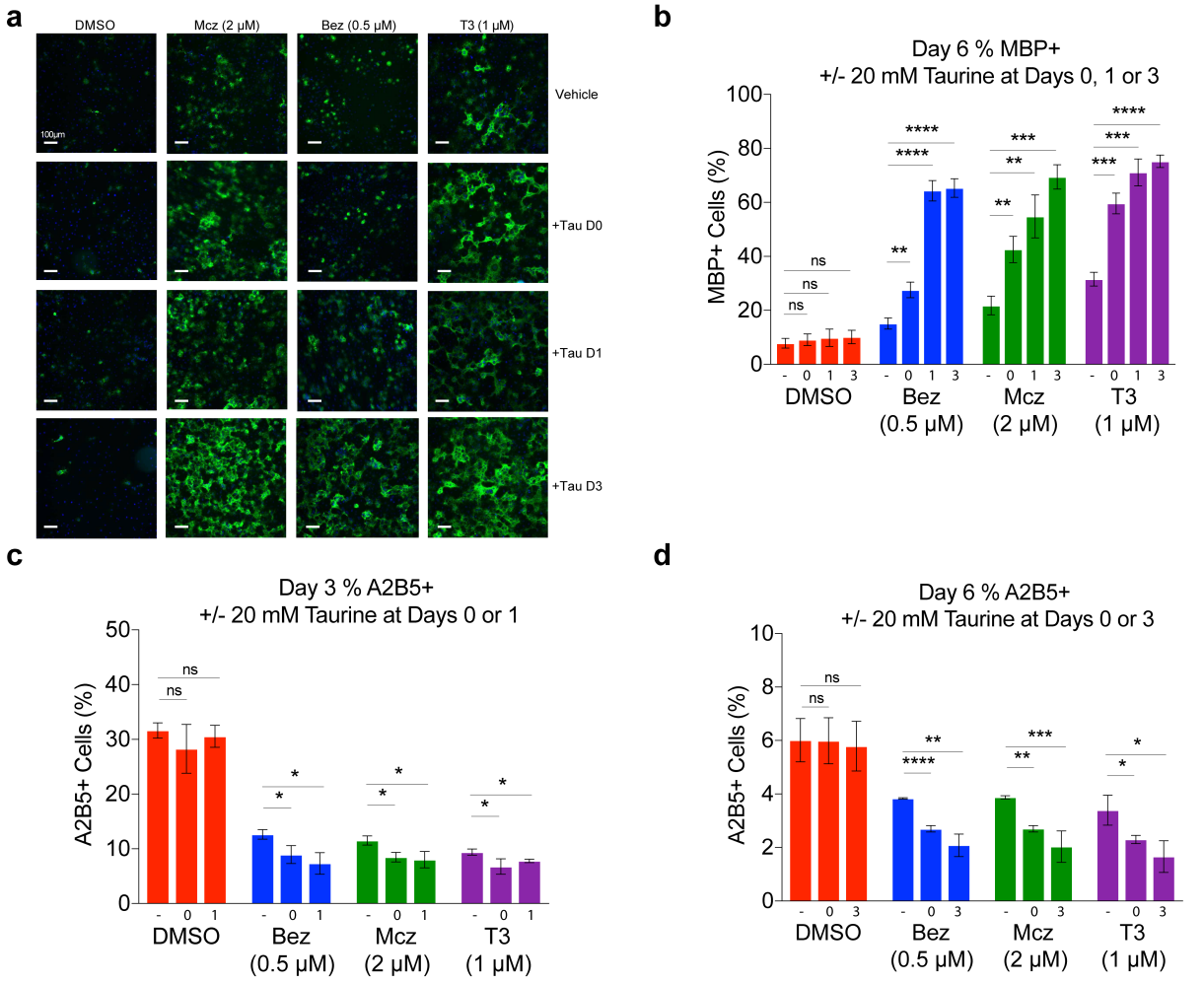

Supplementary Figure 5 | Temporal effects of taurine addition on efficacy of drug-induced OPC differentiation. (**a**) Representative time course images of MBP-positive oligodendrocytes on day 6 post-treatment with DMSO, Miconazole (2 μM), Benztropine (0.5 μM), or T3 (1 μM), with or without taurine (20 mM) addition on days 0-3 (MBP, green; DAPI, blue). (**b**) Quantification of MBP-positive OLs from time course study described in (**a**), based on automated image analysis. (**c**) and (**d**) Impact of taurine addition on A2B5-positive immature OPC numbers at various stages of oligodendrocyte differentiation**.** Percentage of A2B5+ nuclei quantified on Day 3 following addition of 20mM taurine on days 0 or 1 (**c**) and quantified on Day 6 following addition of 20 mM taurine on days 0 or 3. Benztropine (0.5 μM), miconazole (2

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μM), and T3 (1 μM) were each added on day 0**.** Data are mean ±SD for the percentage of MBP-positive cells per well (n = 3 replicate cell cultures). "Bez", "Mcz" and "Tau" are the abbreviations for "benztropine", "miconazole" and "taurine"; respectively. "ns", "*", "**", "***", and "****" represent no significance, $p < 0.05$, $p < 0.01$, $p < 0.001$ and $p < 0.0001$; respectively.

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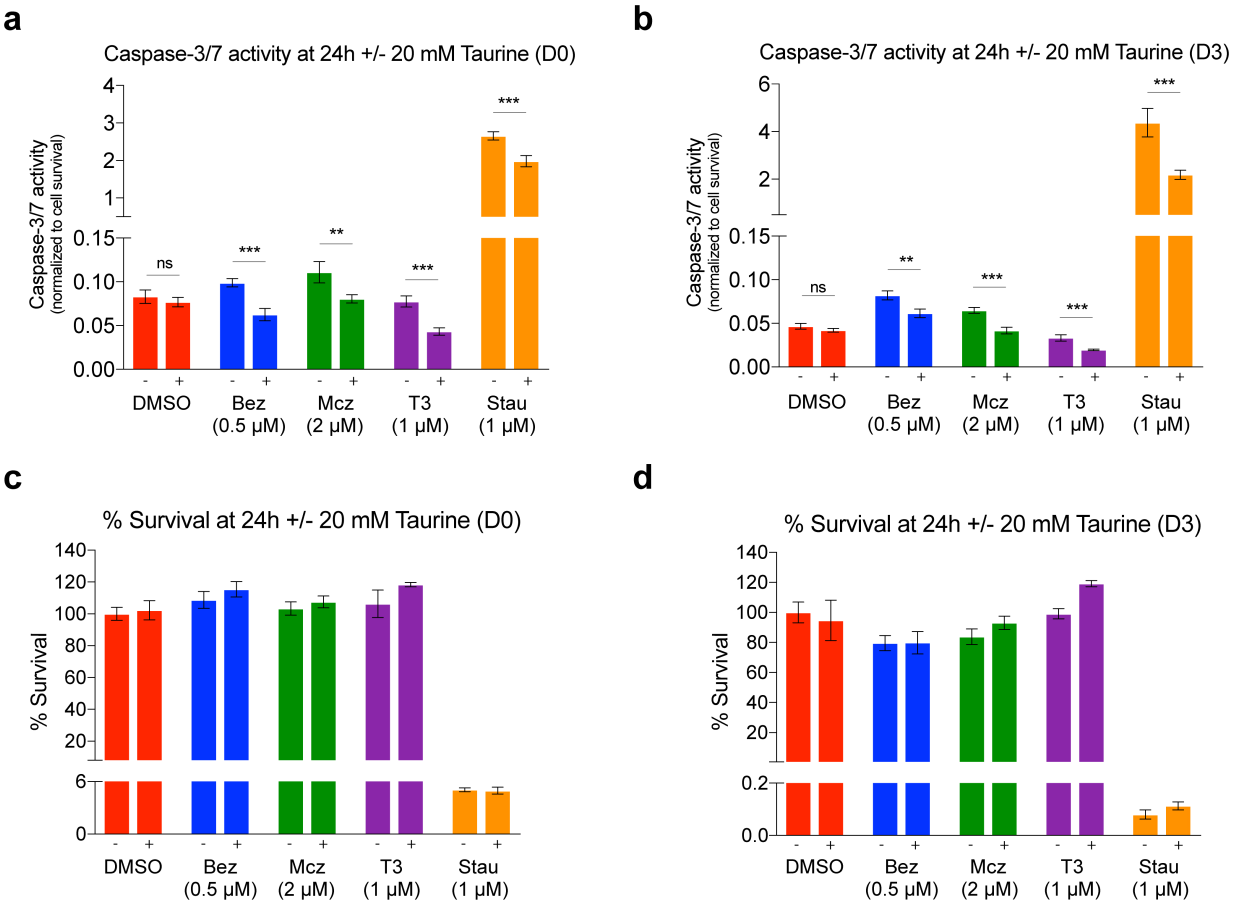

Supplementary Figure 6 | Comparative effects of taurine treatment on apoptosis in OPCs and premyelinating OLs. Activity of the apoptosis indicator Caspase-3/7 is shown 24h after addition of 20 mM taurine on (**a**) Day 0 (D0) or (**b**) Day 3 (D3). Benztropine (0.5 µM), miconazole (2 µM), and T3 (1 µM) were each added on day 0. (**c**) and (**d**) Parallel quantitative analysis of cell survival for treatments described in (**a**) and (**b**). "Bez", "Mcz" and "Stau" are the abbreviations for "benztropine", "miconazole" and "staurosporine"; respectively. Data are mean ±SD (n = 3 replicate cell cultures). "ns", "**", and "***" represent no significance, $p < 0.01$, and $p < 0.001$; respectively.

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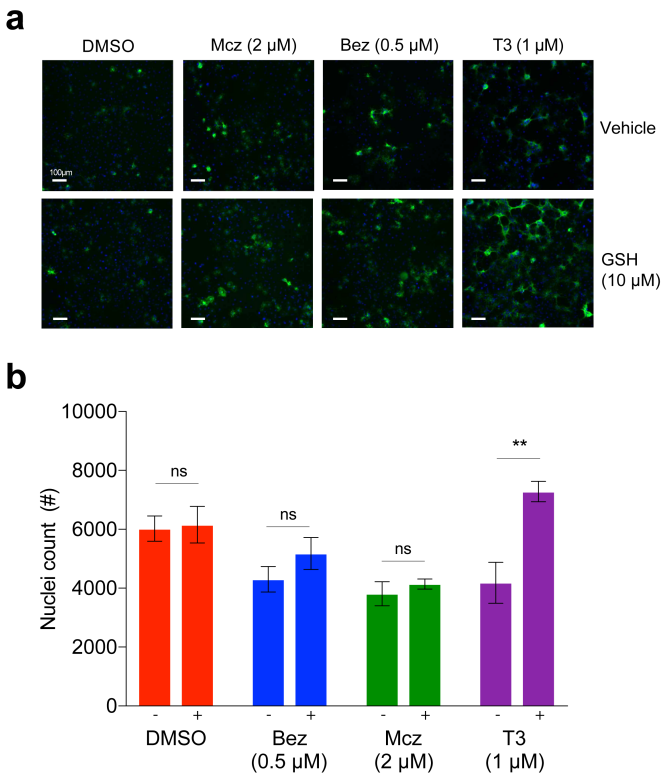

Supplementary Figure 7 | Impact of alternative reducing agents on drug-induced OPC differentiation. (**a**) Representative images of MBP expression following 6 days of treatment with Benztropine (0.5 µM), miconazole (2 µM) or T3 (1 µM) in the presence or absence of reduced glutathione (GSH, 10 µM) (MBP, green; DAPI, blue). (**b**) Parallel quantitative imaging-based analysis of cell survival for treatments described in (**a**). Data are mean ±SD (n = 3 replicate cell cultures). "Bez" and "Mcz" are the abbreviations for "benztropine" and "miconazole"; respectively. "ns" and "**" represent no significance and $p < 0.01$; respectively.

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Supplementary Figure 8 | Binary global metabolomics analysis of taurine co-treatment. (**a**) Binary global metabolomics analysis of the impact of taurine (20 mM) supplementation on pre-myelinating OLs (harvested on day 3, D3) and OLs (harvested on day 6, D6) differentiated using Benztropine (0.5 μM) (**a**) or Miconazole (2 μM). Drug and taurine treatment initiated on day 0 (**b**). Numbers represent fold changes of metabolites, with values smaller than one suggesting down-regulation and values larger than one suggesting up-regulation. White indicates non-detected (i.e., below limit of detection). Darker circles represent a p-value < 0.05.

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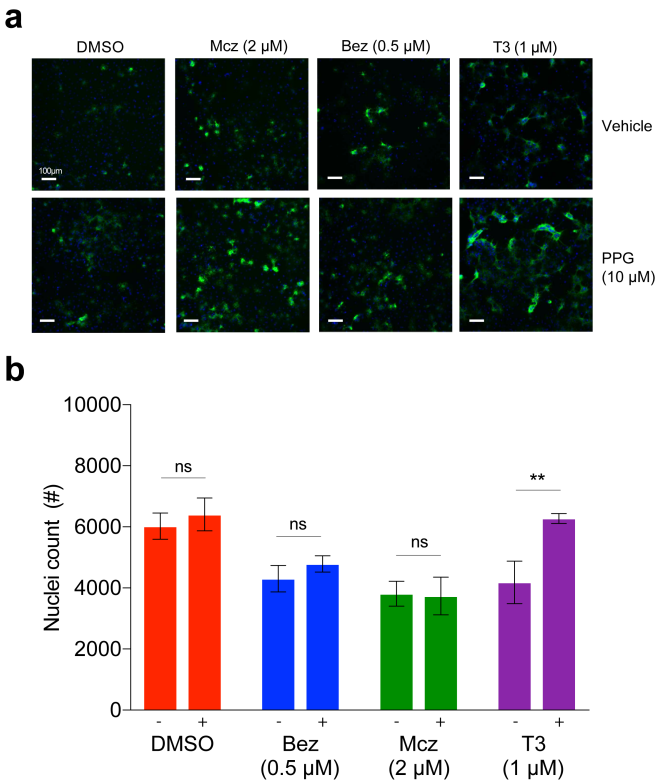

Supplementary Figure 9 | Impact of propargyl glycine (PPG) on drug-induced OPC differentiation. (**a**) Representative images of MBP expression following 6 days of treatment with Benztropine (0.5 μM), miconazole (2 μM), and T3 (1 μM) in the absence or presence of PPG (10 μM) (MBP, green; DAPI, blue). Drug and PPG treatment initiated on day 0. Note, for comparison, images for vehicle treatment are identical to those used in Supplementary Figure 7a. (**b**) Parallel quantitative imaging-based analysis of cell survival for treatments described in (**a**), in the absence (-) or presence (+) of PPG (10 μM). Data are mean ±SD (n = 3 replicate cell cultures). "Bez" and "Mcz" are the abbreviations for "benztropine" and "miconazole"; respectively. "ns" and "**" represent no significance, and $p < 0.01$; respectively.

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Supplementary Table 1 | The MRM transition of targeted metabolites in this study.

Compound Name	Precursor Ion	Qualifier	Quantifier	Dwell (ms)	Fragmentor (V)	Collision Energy (V)
Cysteine sulfate	202.0	NA	73.9	30	380	26
Ascorbic acid	177.0	140.9	95.0	30	380	2
Cysteate	170.0	106.1	123.9	30	380	14
Taurocyamine	168.0	59.0	125.9	30	380	14
3-Sulfino-L-alanine	154.0	74.0	44.2	30	380	10
Methionine	150.1	NA	61.0	30	380	14
Homocysteine	136.0	56.1	90.1	30	380	10
Creatine	132.1	44.1	90.0	30	380	10
Taurine	126.0	NA	107.9	30	380	10
L-Cysteine	122.0	58.9	75.9	30	380	10
Creatinine	114.1	43.1	44.1	30	380	14
hypotaurine	110.0	48.0	91.9	30	380	6
Serine	106.1	42.1	60.1	30	380	10
Cysteamine	78.0	NA	61.0	30	380	14
Glycine	76.0	31.1	30.1	30	380	38
GSSG	613.2	355.0	483.9	30	380	18
GSH	308.1	76.0	84.0	30	380	46
L-Cystine	241.0	120.0	152.0	30	380	10
L-Cystathionine	223.1	88.0	134.0	30	380	10
Creatine-phosphorous	212.0	114.0	90.1	30	380	10

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