

Supporting Information
Role of microRNA in CB1 antagonist-mediated regulation of adipose tissue macrophage polarization and chemotaxis during diet-induced obesity

Pegah Mehrpouya-Bahrami, Kathryn Miranda, Narendra P. Singh, Elizabeth E. Zumbun, Mitzi Nagarkatti, and Prakash S. Nagarkatti

Supporting information includes the following:

Figure S1: Gene ontology enrichment mapping reveals SR141716A treatment alters miRs that affect immune response pathways.

Figure S2: miR dysregulation following SR141716A treatment promotes M2-macrophage polarization.

Figure S3: F4/80+ ATM selection efficiency.

Table S1: Body composition before and after SR141716A intervention.

Table S2: Summary of unique miRs that target transcription factors in ATMs.

Table S3: Gene expression profile of Netrin family and its related receptors.

Figure S1.

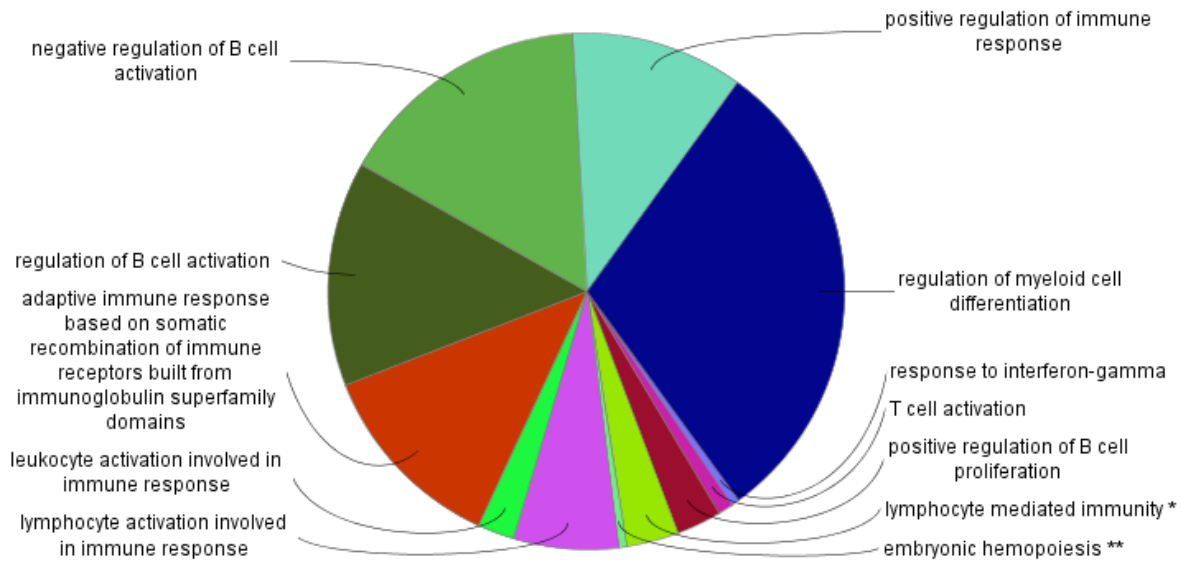


Figure S1: Gene ontology enrichment mapping reveals SR141716A treatment alters miRs that affect immune response pathways. miR target genes in HFD+SR were analyzed for GO enrichment and mapped for GO category: immune system process, using Cytoscape suite with ClueGo and CluePedia plugins. Two-sided hypergeometric statistical analysis was performed with Kappa Score threshold setting of 0.3. Enrichment depletion was calculated based on Benjamini-Hochberg Correlated p-values of <0.05.

Figure S2.

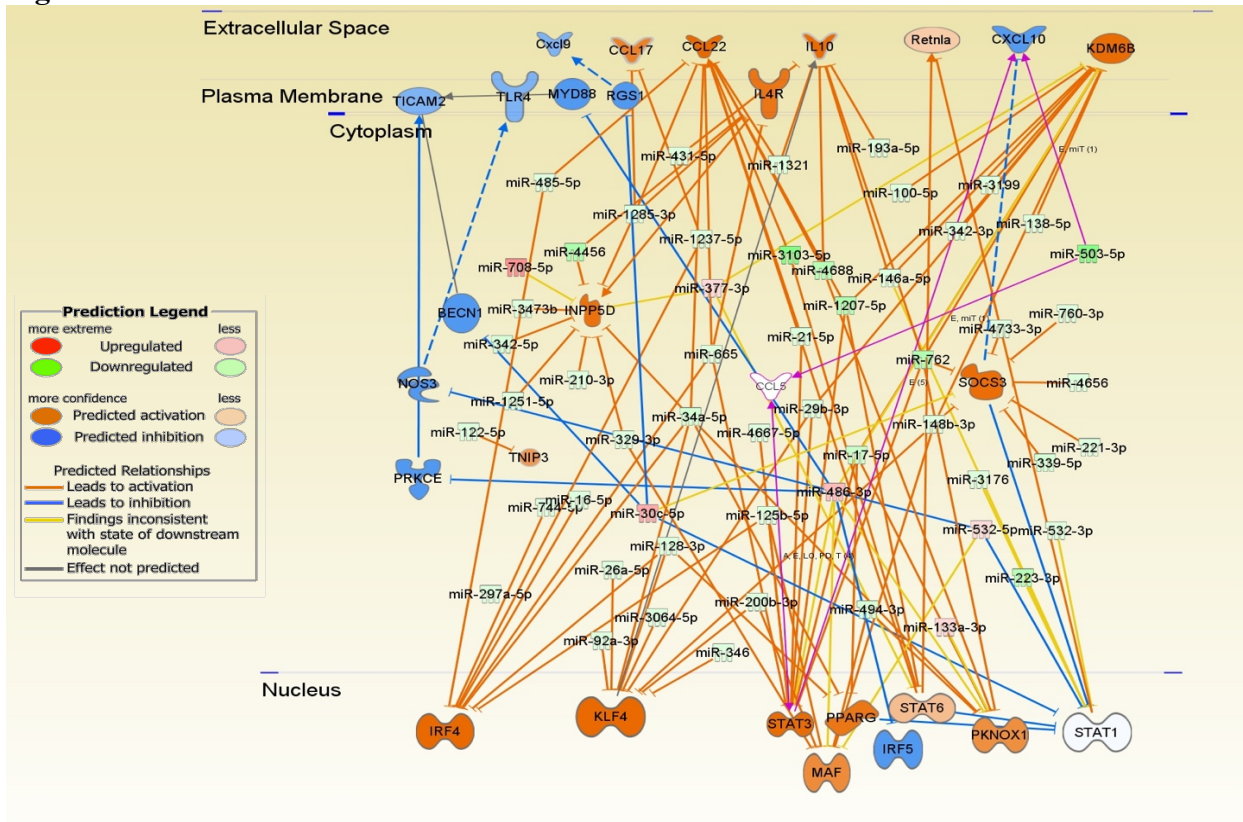


Figure S2: miR dysregulation following SR141716A treatment promotes M2-macrophage polarization. Interactions between the miRs and their target genes were assessed by IPA software (Qiagen). SR141716A treatment changes the microRNA profile, which shifts the adipose tissue macrophage balance to greater anti-inflammatory macrophages (M2, Arginase+). The molecular activity predictor of altered miRs and their associated targets genes demonstrated down-regulation of chemokine repertoire of the pro-inflammatory macrophages M1 (CXCR3 agonists, CXCL9 and CXCL10) and up-regulation of CCR4-agonists CCL17 and CCL22 for M2 macrophages following SR141716A treatment. Treatment with SR141716A led to reduction of free fatty acids in serum, which is an M1 stimuli through TLR4 signaling (data not shown). Stimulation of TLR4 signaling induces activation of the transcription factors NF- κ B (p65 and p50), AP-1, STAT1 and IRF5, which leads to the transcription of M1 genes. Differential expression of miRs following SR141716A treatment correlated with down-regulation of TLR4 signaling pathway and its down-stream transcription factors (STAT1, IRF5, TICAM2 and MyD88). Interestingly, altered miRs following SR141716A treatment triggered extreme predicted activation of the immunosuppressive cytokine IL-10 and its down-stream signaling transcription factors, which are the key factors in polarization of anti-inflammatory M2 macrophages (STAT3 and SOCS3). Furthermore; differential expression of miRs with SR141716A activates more molecules which are involved in M2 macrophage polarization (KLF4, STAT6, IRF4, PPAR γ , and MAF).

Figure S3.

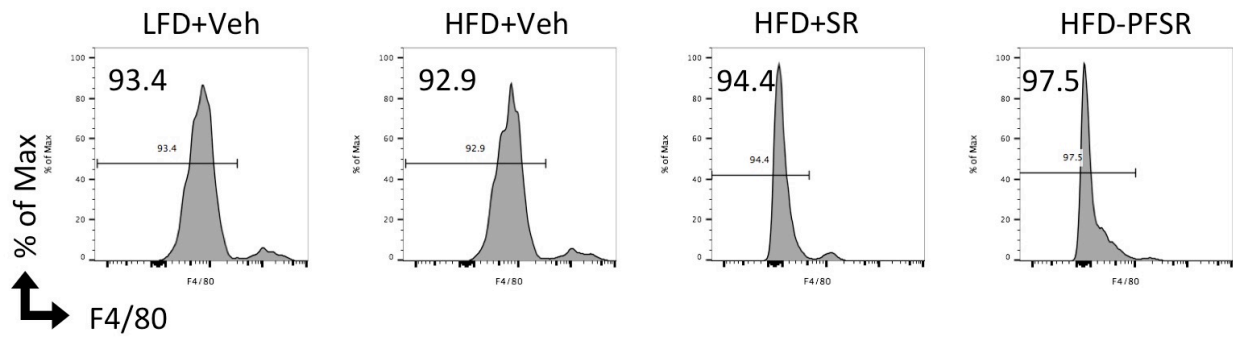


Figure S3: F4/80+ ATM selection efficiency. Flow cytometry was used to assay selection efficiency following Stemcell EasySep selection. Selection efficiency was routinely >90%. Data shown are representative flow cytometry histogram plots of F4/80 expression post-selection.

Table S1: Body composition before and after SR141716A intervention.

Week of Diet	Week 12	Week 16
<i>Fat Mass (Grams)</i>		
LFD+Veh	6.7±0.49 ^a	7.94±0.38 ^{b@}
HFD+Veh	22.1±0.78 ^a	23.8±0.69 ^{b#}
HFD+SR	22.86±0.16 ^a	13.4±0.74 ^{b&}
HFD-PFSR	22.12±0.29 ^a	21.9±.92 b [#]
<i>Lean Mass (Grams)</i>		
LFD+Veh	19.71±0.37 ^a	20.3±0.62 ^{a@}
HFD+Veh	22.8±0.39 ^a	22.9±0.11 ^{a#}
HFD+SR	21.09±0.75 ^a	20.08±0.29 ^{a#}
HFD-PFSR	21.7±0.28 ^a	19.8±0.36 ^{a#}
<i>Body Fat %</i>		
LFD+Veh	25.369±0.78 ^a	28.11±0.44 ^{b@}
HFD+Veh	49.22±0.49 ^a	50.96±0.359 ^{b#}
HFD+SR	52.01±0.36 ^a	40.02±0.38 ^{b&}
HFD-PFSR	50.47±0.92 ^a	52.51±0.79 ^{b#}

Table S1. SR141716A causes significant shrinkage of fat mass. Body composition including fat mass, lean mass and fat percentage was assessed at baseline and after 4 weeks of treatment by DEXA. Values not sharing superscripted letters (a,b) differ significantly over time ($p < 0.05$). Values not sharing superscripted symbols (#, @, &) differ significantly among different treated groups ($p < 0.05$).

Table S2: Summary of unique miRs that target transcription factors in ATMs.

miRs	Log2 FC	Target	Relevance	Algorithm
mir-20b-5p	-2.6	PPARg	Differentially expressed in M1 and M2. PPARg ↓ = ↑ M1 and ↓ M2 ∴with treatment, PPARG ↑	IPA
miR-466f-5p	-4.7	KLF4	↑ in M2 and ↓ in M1, ∴with treatment, KLF4 ↑	mirmap/db
miR-466f-1	-2.5			mirmap
miR-25-3p				mirmap/db
miR-324-5p	-3.9	LCN2	LCN2 ↓ (k.o.)= ↑ M1 and ↓ M2 ∴with treatment, LCN2 ↑	mirmap
miR-1892	-3.8	SIRT1	SIRT1 ↓ (k.o.)= ↑ M1 and ↓ M2 ∴with treatment, SIRT1 ↑	mirmap
miR-669b-5p	-1.5			mirmap
miR-132-3p	-3.7			mirwalk
mir-20b-5p	-2.6	STAT3	Switch to M2 w. ↑ Stat3 ∴with treatment, Stat3 ↑	mirmap/db
miR-346-3p	-3.2			mirmap
miR-7030-5p	-4.3			mirmap/db
miR-466j-5p	-2.8	STAT6	Switch to M2 assoc w. ↑ Stat6 ∴with treatment, Stat6 ↑	mirmap
miR-466c-5p	-3			mirmap
miR-466f-1	-2.5			mirmap
miR-324-5p	-3.9			mirmap

Table S2. This table lists macrophage polarization-associated transcription factors that are targeted by dysregulated miRs following SR141716A treatment. The miRs, target genes, downstream effect, log₂ fold change values, and applied algorithm are listed.

Table S3: Gene expression profile of Netrin family and its related receptors

Full name	RefSeq#	Gene Symbol	Fold Change HFD+SR vs. HFD+Vehicle
Netrin 3	NM_010947	Ntn3	-1.0333
Netrin 4	NM_021320	Ntn4	-1.1058
Netrin G1	NM_030669	Ntng1	-2.0009
Netrin G2	NM_133501	Ntng2	-2.1862
Deleted in colorectal carcinoma	NM_007831	Dcc	-1.0703
Neogenin	NM_008684	Neo1	-1.7731
Unc-5 homolog A	NM_153131	Unc5a	-1.0039
Unc-5 homolog C	NM_009472	Unc5c	0.7627
Unc-5 homolog D	NM_153135	Unc5d	-1.1118
Adenosine A2a receptor	NM_009630	Adora2a	-3.9071
Adenosine A2b receptor	NM_007413	Adora2b	1.5548

Table S3. This table lists gene expression profile of several Netrin family genes and its related receptors.