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Supplemental information

β2-adrenergic receptor signaling regulates metabolic pathways critical to myeloid-derived suppressor cell function within the TME

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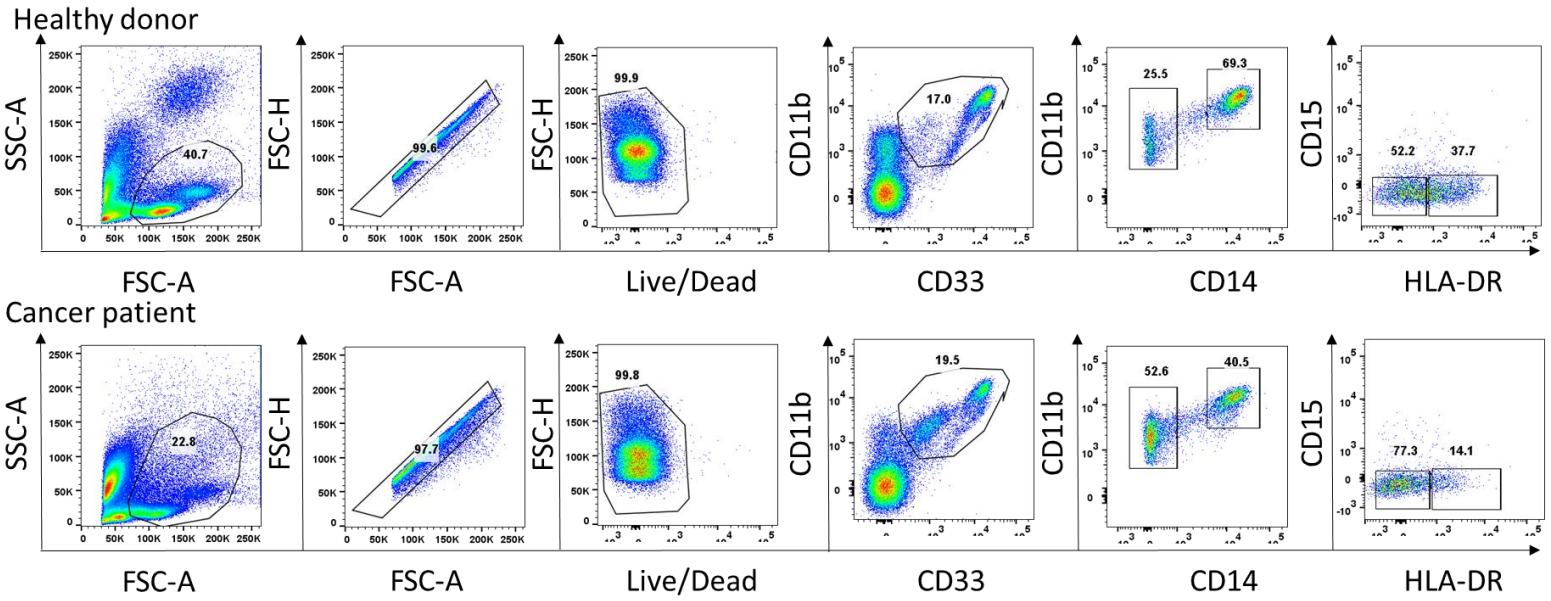
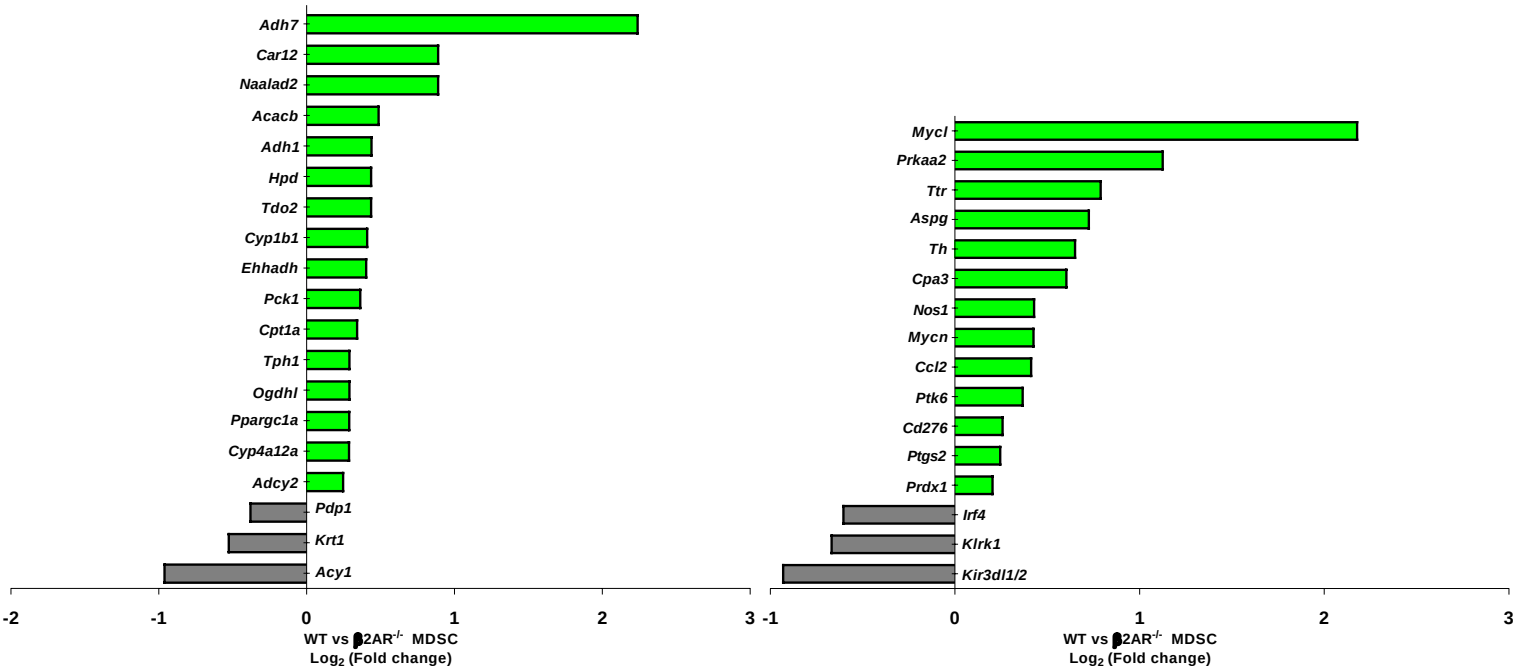
Supplemental Figure 1



Supplementary Figure 1: Gating strategy to identify MDSCs (M-MDSCs) in human samples by flowcytometry. Related to Figure 1.

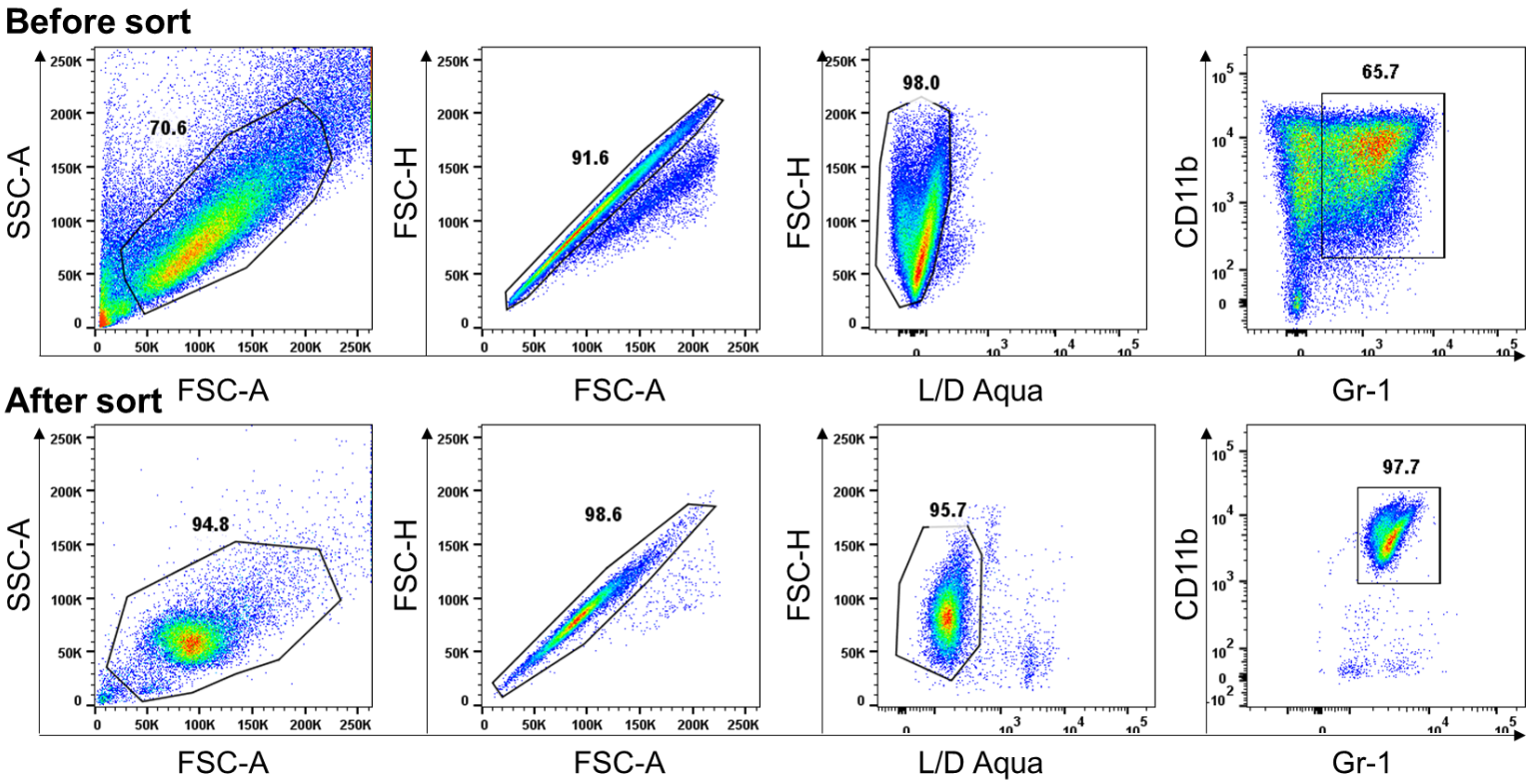
Supplemental Figure 2



Pathway	P-value	Adjusted p-value	Odds Ratio	Combined score
Tryptophan metabolism	1.21E-12	3.65E-10	56.5	1550.55
PPAR signaling pathway	5.15E-06	0.00052	19.94	242.82
Tyrosine metabolism	0.000223	0.005629	25.42	213.78
AMPK signaling pathway	1.9E-06	0.000288	16.14	212.64
Adipocytokine signaling pathway	5.73E-05	0.002169	19.1	186.54
Citrate cycle (TCA cycle)	0.004009	0.03918	21.19	116.93
Fatty acid degradation	0.00957	0.07249	13.56	63.04
Nitrogen metabolism	0.049	0.1954	19.94	60.14
Arachidonic acid metabolism	0.02843	0.1539	7.62	27.12

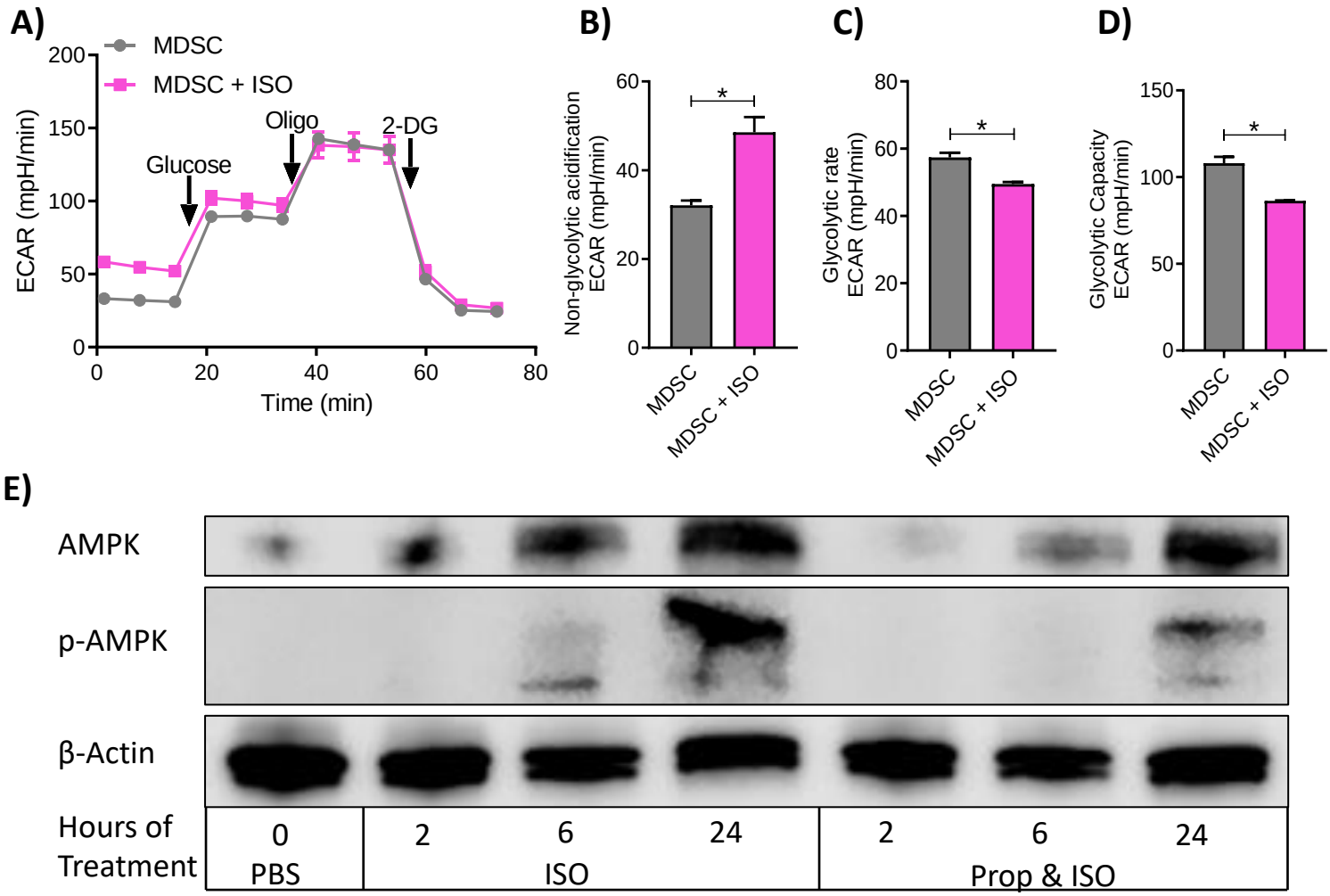
Supplementary Figure 2: β2-AR signaling increases the expression of genes correlated with oxidative phosphorylation and fatty acid oxidation. (A) WT and β2-AR$^{-/-}$ were orthotopically implanted with 4T1 tumor cells. At day 25, WT or β2-AR$^{-/-}$ PMN- MDSCs were sorted by flow cytometry from 4T1 tumors of WT or β2-AR-/- mice (WT or β2-AR-/- MDSCs were pooled from 5 mice per each group) and Nanostring nCounter microarray analysis of WT or β2-AR$^{-/-}$ PMN- MDSCs were performed. (B) The main metabolic pathways related to different genes were evaluated using Enricher website. Related to Figures 2, 3, and 5.

Supplemental Figure 3



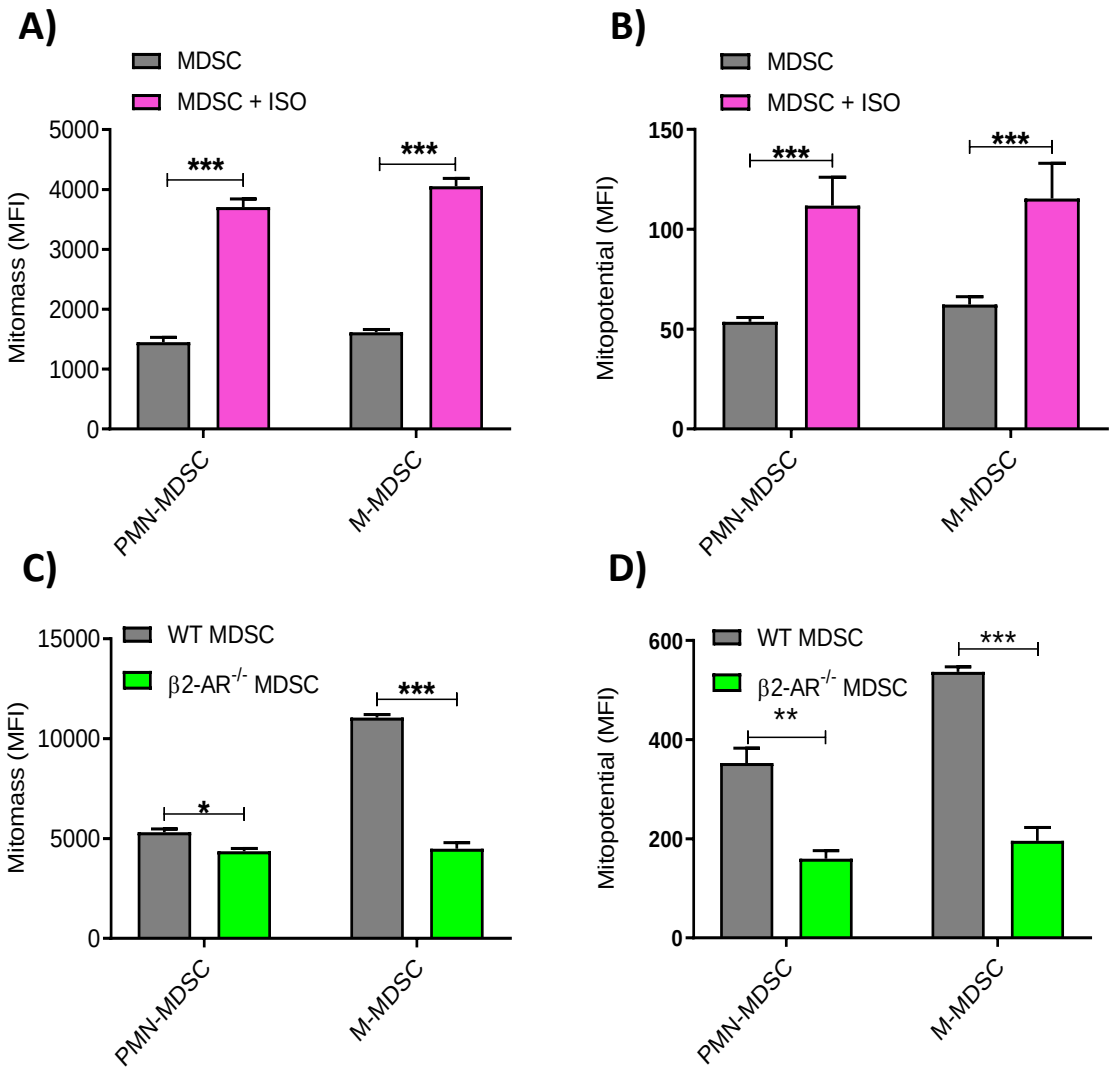
Supplementary Figure 3: Flowcytometric analysis of bone marrow derived MDSCs *in vitro*. Bone marrow cells were cultured with GM-CSF and IL-6 in presence or absence of isoproterenol for 6 days and MDSCs phenotype were analyzed by flowcytometry. Related to Figure 2.

Supplemental Figure 4



Supplementary Figure 4) β-AR signaling in MDSCs decreases glycolysis, activates AMPK signaling. (A-D) MDSCs were derived from bone marrow in the presence of GM-CSF and IL-6, with or without isoproterenol (ISO). A-D) MDSC Glycolysis was measured using a Seahorse Extracellular Flux Analyzer (Arrows indicate when reagents were added: (1) glucose; (2) oligomycin; and (3) 2-DG. B) Non-glycolytic acidification rate. C) Glycolytic rate. D) Glycolytic capacity. E) PMN-MDSCs were sorted from bone marrow of 4T1 tumor bearing mice and treated with PBS, ISO or propranolol plus ISO. Levels of AMPK and phospho-AMPK were measured by western blot. These data are presented as mean ± SD from three biological replicates in all graphs, and the students T test was used to analyze statistical significance between two groups. In all panels * $P<0.05$. A P value less than 0.05 was considered significant. Related to Figure 2.

Supplemental Figure 5



Supplementary Figure 5: β2-AR signaling increases mitochondrial mass and mitochondrial potential in PMN-MDSCs. (A) MDSCs were derived from bone marrow in presence of GM-CSF and IL-6 with or without ISO and mitochondrial mass (Mitomass) and mitochondrial potential (Mitopotential) were measured using flowcytometry. WT and β2-AR$^{-/-}$ were orthotopically implanted with 4T1 tumor cells. At day 25, the level of Mitomass and Mitopotential were evaluated in MDSCs using flowcytometry (gated on live CD45+, CD11b+ and Ly6G+ for PMN-MDSC or Ly6C+ for M-MDSCs). The data are presented as mean ± SD from three biological replicates in all graphs and the students T test was used to analyze statistical significance between two groups. In all panels * P<0.05, ** P<0.01 and ***P<0.001. A P value less than 0.05 was considered significant. Related to Figure 2.

Supplemental Table 1

Subject	Buffy Coat Straws	Age at Collection	Race	Morphology	Stage Group Path	Stage Group Clin	Collection Year	ER
PT-00301521	8	49	White	Infiltrating duct carcinoma, NOS	99	3B	2014	Negative
PT-00189487	7	62	White	Infiltrating duct carcinoma, NOS	3A	3A	2007	Negative
PT-00080162	7	64	White	Infiltrating duct carcinoma, NOS	3C	2A	2009	Negative
PT-00389106	8	78	White	Infiltrating duct carcinoma, NOS	NA	3B	2018	Negative
PT-00195101	4	83	White	Infiltrating duct carcinoma, NOS	3C	3A	2007	Negative
PT-00248044	7	49	White	Non-Patient - Family Member			2009	
PT-00248210	7	63	White	Non-Patient - Family Member			2009	
PT-00249151	7	60	White	Non-Patient - Family Member			2009	
PT-00249263	5	78	White	Non-Patient - Community Volunteer			2010	
PT-00251287	8	83	White	Non-Patient - Community Volunteer			2012	

Supplemental Table 1: Healthy individuals and patient characteristics. Related to Figure 1.