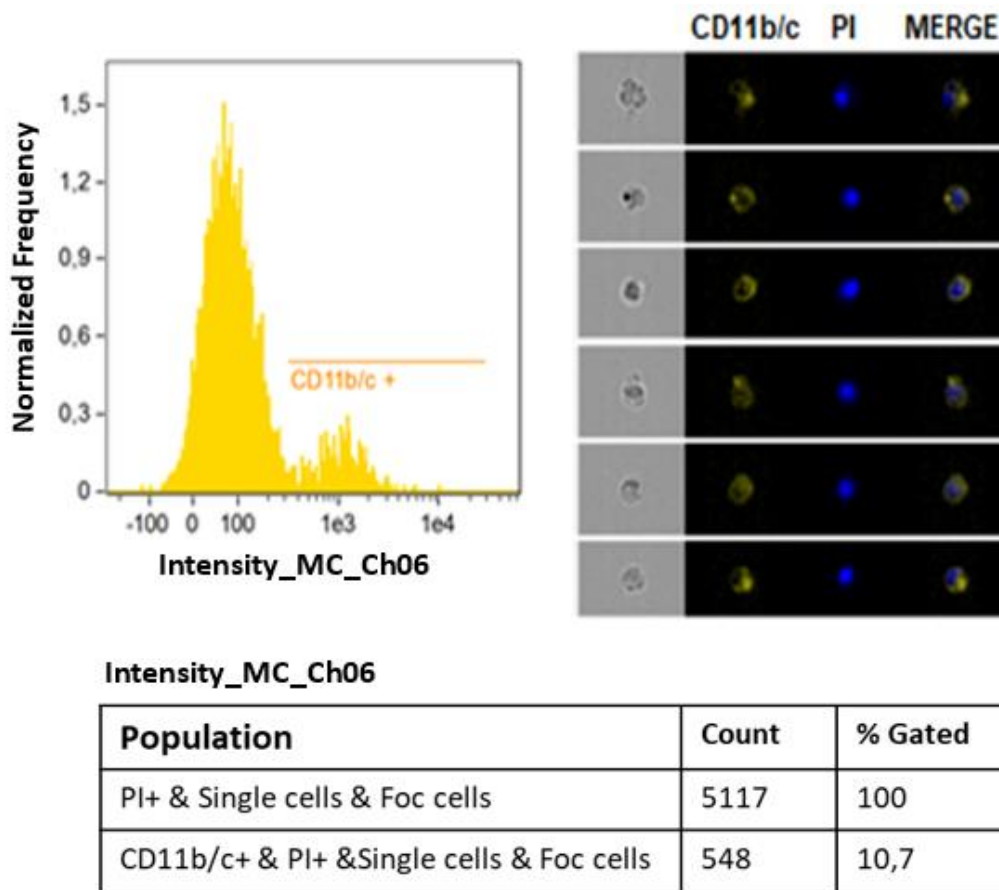


Please cite this paper as:

Souza, E.S., Santos, A.A., Ribeiro-Paz, E.E., Córdoba-Moreno, M., Trevisan, I.L., Caldeira, W., Muxel, S.M., Sousa, K.D. and Markus, R.P. 2022. Melatonin synthesized by activated microglia orchestrates the progression of microglia from a pro-inflammatory to a recovery/repair phenotype. *Melatonin Research*. 5, 1 (Mar. 2022), 55-67. DOI:<https://doi.org/https://doi.org/10.32794/mr112500120>.

SUPPLEMENTARY MATERIAL



Supplementary Fig. 1. Representative histogram and microphotographs obtained by flowsight cytometry.

Histogram of a selected sample showing 10.7% of CD11b/c+ cells gated from total nucleated cells (PI+/CD11b/c+). On the right, microphotographs obtained by flowsight cytometry show protein expression by fluorescence emission of the markers CD11b/c+ (green), PI (blue), and overlapping channels (MERGE). Protein expression of microglial activation markers IBA-1, NOS-2 and ARG-1 were analyzed according to this template in PI+/ CD11b/c+ population.

Table 1. Material, reagents, drugs and antibodies.

PRODUCT	SUPPLIER	CATALOG #
Culture PLATES AND SLIDES		
cell strainer	BD Falcon®, New Jersey, USA	352360
75 cm ² Cell culture Flask	Nest Biotech Co., Ltda, Jiangsu, China.	708011
24-well plates	Nest Biotech Co., Ltda, Jiangsu, China.	702011
8-well chamber slides	Millicell® EZ slide, Merck KGaA, Darmstadt, Germany	PEZGS0816
CHEMICALS, MEDIUM		
Ethanol		
Sodium Chloride (NaCl)	Labsynth, São Paulo, BR	C1060.01.AH
Potassium Chloride (KCl)	Merck KGaA, Darmstadt, Germany,	1049361000
Anhydrous disodium hydrogen phosphate (Na ₂ HPO ₄)	Merck KGaA, Darmstadt, Germany,	1065861000
Potassium dihydrogen phosphate (KH ₂ PO ₄)	Merck KGaA, Darmstadt, Germany,	1048771000
bovine serum albumin BSA -	Merck KGaA, Darmstadt, Germany,	9048-46-8
neural tissue dissociation kit	Mytilenyi Bergisch Gladbach, Germany	130-094802
paraformaldehyde, PFA	Labsynth, São Paulo, BR	P1005.01.AH
Triton-X	AMRESCO®, VWR Life Sciences, Pensilvania, USA	97062-208
Acetone	Labsynth, São Paulo, BR	A1017.01.BJ
Methanol	Labsynth, São Paulo, BR	A1085.01.BJ
Dulbecco's modified Eagle's medium DMEM	Gibco, Thermo Fisher Scientific, Inc., Waltham, MA, USA	12100-046
penicillin/streptomycin, pen/strep	Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA	15070-063
fetal bovine serum, FBS	Gibco, Thermo Fisher Scientific, Inc., Waltham, MA, USA	10437028
DRUGS		
Trypan blue	Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA	15250-061
Lipopolysaccharides from Escherichia coli, LPS	O127:B8, Sigma Aldrich, St Louis, MO, USA	97-473-0
Minocycline hydrochloride	Sigma Aldrich, St Louis, MO, USA	13614-98-7
Melatonin	Sigma Aldrich, St Louis, MO, USA	M5250
Luzindole	Sigma Aldrich, St Louis, MO, USA	117946-91-5
ANTIBODIES AND FLUORESCENT MARKERS		
Mounting Medium with DAPI - Aqueous, Fluoroshield	Abcam, Cambridge, England	Ab104139
Propidium Iodid, PI	Molecular probes, Invitrogen, Oregon, USA	P3566
anti-CD11b FITC	BD Biosciences, New Jersey, USA	554982
anti-GFAP AF488	Biologend, San Diego, CA, USA	644704
Anti-CD11b/c PEcy7 conjugated	Biologend, San Diego, CA, USA	201818
Anti-IBA-1	Immunny, Campinas, SP, Brazil	IM0565
Anti-NOS2	Santa Cruz Biotechnology, TX, USA	SC651
Anti-ARG-1	Biologend, San Diego, CA, USA	678862
Donkey Anti-Rabbit PerCP-Cy5.5	Santa Cruz Biotechnology, TX, USA	SC45106
Goat Anti-Mouse PEcy7	Biologend, San Diego, CA, USA	405315
Zymosan A from Saccharomyces cerevisiae	Sigma Aldrich, St Louis, MO, USA	58856-93-2
Zymosan conjugated with Alexa Fluor 488	Thermo Fisher Scientific, Inc., Waltham, MA, USA	Z23373
EQUIPMENT AND SOFTWARES Zeiss Microscopy, Jena, Germany		
Amnis® FlowSight® flow cytometry	Luminex, Austin, TEXAS, USA	
IDEAS® software	Luminex, Austin, TEXAS, USA	
LSM 880 confocal microscope Axio Observer	Zeiss Microscopy, Jena, Germany	
LSM 510 confocal microscope	Zeiss Microscopy, Jena, Germany	
GraphPad Prism 9.0 software	Graphpad, San Diego, California, USA	