

Flow cytometry

Neurons were washed in PBS (Cat.# 14190144, Thermo Fisher) and incubated with Membrane (Cat.# 30093, Biotium) prestain solution, diluted 1:1000 in HBSS, for 5 min at 37 °C. Prestain solution was changed to staining solution and incubated for another 5 min at 37 °C. Cells were dissociated with prewarmed papain (Cat.#LK003176, Worthington) with DNase (Cat.#LS006333, Worthington), Y-27632 and Emricasan for 10 min at 37°C. The reaction was quenched in DMEM/Glutamax (Cat.#10566-016, Thermo Scientific) with 10% fetal bovine serum and collected by centrifugation at 400×g for 5 min. The pellet was resuspended in 4% paraformaldehyde (Cat.# sc-281692, Santa Cruz Biotechnology) and incubated for 10 min at RT on a rolling wheel. Cells were resuspended in DMEM with 10% FBS and spun at 500×g for 5 min after which the cells were incubated in permeabilization buffer (1% BSA, 0.1% saponin in PBS, 10% normal donkey serum (Cat.# AB_2337258, Jackson ImmunoResearch) for 30 min at RT and thereafter labeled with tyrosine hydroxylase antibody or isotype controls (1:400, Cat.# P40101-150, Pel-Freeze and Cat.# AB-105-C R&D respectively) overnight at 4 °C. Cells were washed twice with 2% BSA and 1.5 mM EDTA in PBS and then incubated with diluted Alexa647-conjugated secondary antibody (Cat.# 711-605-152, Jackson ImmunoResearch), for 1 h at RT. Finally, cells were washed as described above with NucBlue Fixed Cell ReadyProbes Reagent (DAPI) (Cat. #R37606, Thermo Scientific) in the second wash and resuspended in wash buffer for flow cytometry analysis on a Beckman Coulter Cytoflex-LX. Initial gating included Membrane-positive cells, thereafter using DAPI positivity in order to exclude duplicates. Finally, the cells were analyzed for the fluorescent intensity of TH using Flow-Jo.