

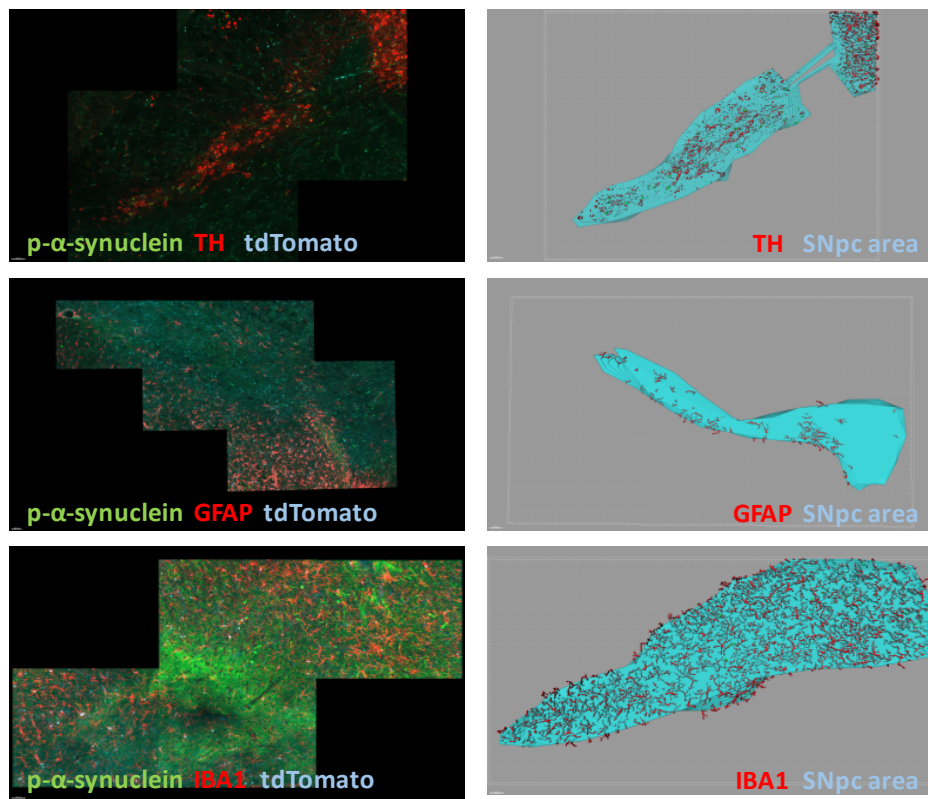
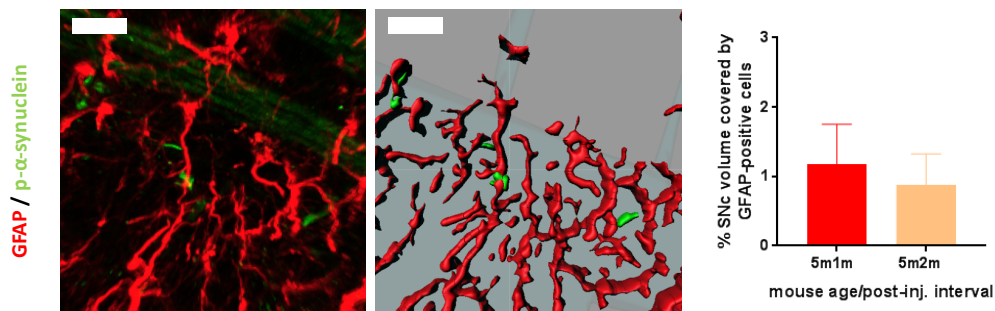
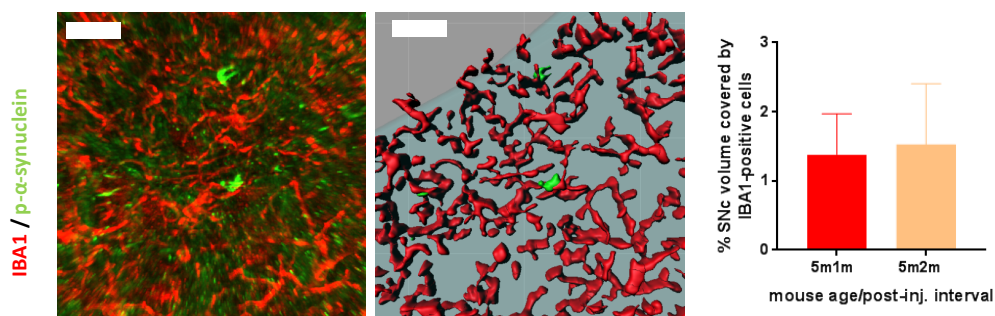
A**B****C**

Figure S4. Increased microglial reactivity but no sign of astrogliosis in SNpc in mice 2 months after PFFs hemilateral injection. Pilot study where post-fixed midbrain coronal sections from two seeded brains harvested for ex vivo FSCV experiments were used. **(A)** Raw data view in IMARIS software for of TH, GFAP and IBA1 (in red, left column) tdTomato reporter (in cyan blue) and phospho-alpha-synuclein (in green) and respective 3d reconstructions of SNpc (right column). Compared with dense IBA1 immunoreactivity within the SNpc, GFAP immunoreactivity was low and limited to the edges of the SNpc. Preliminary quantification of volumes covered by GFAP- **(A)** and IBA1- **(B)** positive signals in the SNpc after PFFs inoculation. We observed a trend of increasing microglial coverage between 1 and 2 m.p.i. in the seeded side, but an opposite, decreasing trend for the percentage area covered by reactive astrocytes. It is very likely that the reactivity of the microglia starts earlier than the astrogliosis in response of alpha-synuclein spreading in the midbrain. Grid and Scalebar 25 μ m.