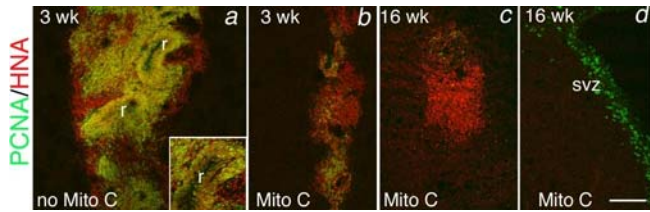


Supplemental Fig. 1. Cyno-1 cells at the end of the differentiation protocol [P2 diff, **(a, b)**] formed large clusters comprised of nestin-positive [green in **(a)**] and β -tubulin (TuJ1)-positive cells (red), located in the surface; 20%–70% of TuJ1-positive cells were TH-positive [green in **(b)**]. After mitomycin C treatment and manual dissociation **(c–e)** cells were replated in expansion medium and fixed 3 days later. Although the percentage of TuJ-positive cells (40%–67%) was similar in both conditions, only 2%–3% of the neurons expressed TH after dissociation. Scale bars = 250 μ m **(a, c)**, 75 μ m **(b, d, e)**. Abbreviation: TH, tyrosine hydroxylase.



Supplemental Fig. 2. In vivo effect of mitomycin C treatment of differentiated Cyno-1 cells before transplantation. In another experiment, differentiated primate Cyno-1 cells were exposed or not to fresh mitomycin C for 90 minutes, 7 days after switching to final differentiation medium (P2 differentiation stage), immediately before harvesting and transplanted into rats. Brains were examined 3 weeks post-transplantation (**a**, **b**). Numerous rosette formations (r) were observed in the grafts from nonmitomycin-treated cells (**a**). Rosettes from differentiated ES cells were highly proliferative: Note that all cells in the rosettes are PCNA-positive, (shown at higher magnification in the inset). Stereological estimation of total graft volume demonstrated a significant reduction in the group receiving mitomycin C treated cells ($t = 3.8$, $p < .05$). Similar reduction (to 25% of untreated) was observed for the neural (NCAM-positive) volume ($t = 5.5$, $p < .01$). Rosette formations in the grafts were almost completely eliminated by mitomycin C treatment (**b**) and the proportion of cells in the grafts expressing PCNA was markedly diminished (**b**). All the grafts contained TH-positive neurons (not shown) at 3 weeks but the number of TH and 5HT-positive neurons was significantly reduced (by 75%) by mitomycin C treatment. (**c**): Sixteen weeks post-transplantation (all cells were treated with mitomycin C in this experiment) few PCNA-positive nuclei ($1,500/\text{mm}^3$, graft volumes $2.32 \pm 0.6 \text{ mm}^3$) were present in the graft core. Numerous PCNA-positive cells were present in the host SVZ (**d**). Scale bar = 120 μm . Abbreviations: ES, embryonic stem; PCNA, proliferating cell nuclear antigen; SVZ, subventricular zone; TH, tyrosine hydroxylase.