

Figure 1. Laminin (LM) enhanced the extent of MIAMI cell neuronal differentiation

Using flow cytometry, we observed that expanding MIAMI cells strongly expressed the integrin (ITG) subunits beta 1, alpha 2, 3 and 5, making both LM and FN suitable molecules for PAM biomimetic surface (isotypes in grey) (A). During neuronal differentiation, the cells acquired a neuronal morphology (B) and LM enhanced neurite formation and resulted in an increased cell length at the end of step 3 (C). The expression of neuronal markers, β 3-Tubulin and NFM (D, E, respectively) were also enhanced at the end of step 3 using a LM substrate, compared to FN or glass as observed by immunocytofluorescence. Cell length results and immunocytofluorescence results are presented as mean length or intensities \pm average deviation. Abbreviation: ICF: immunocytofluorescence.

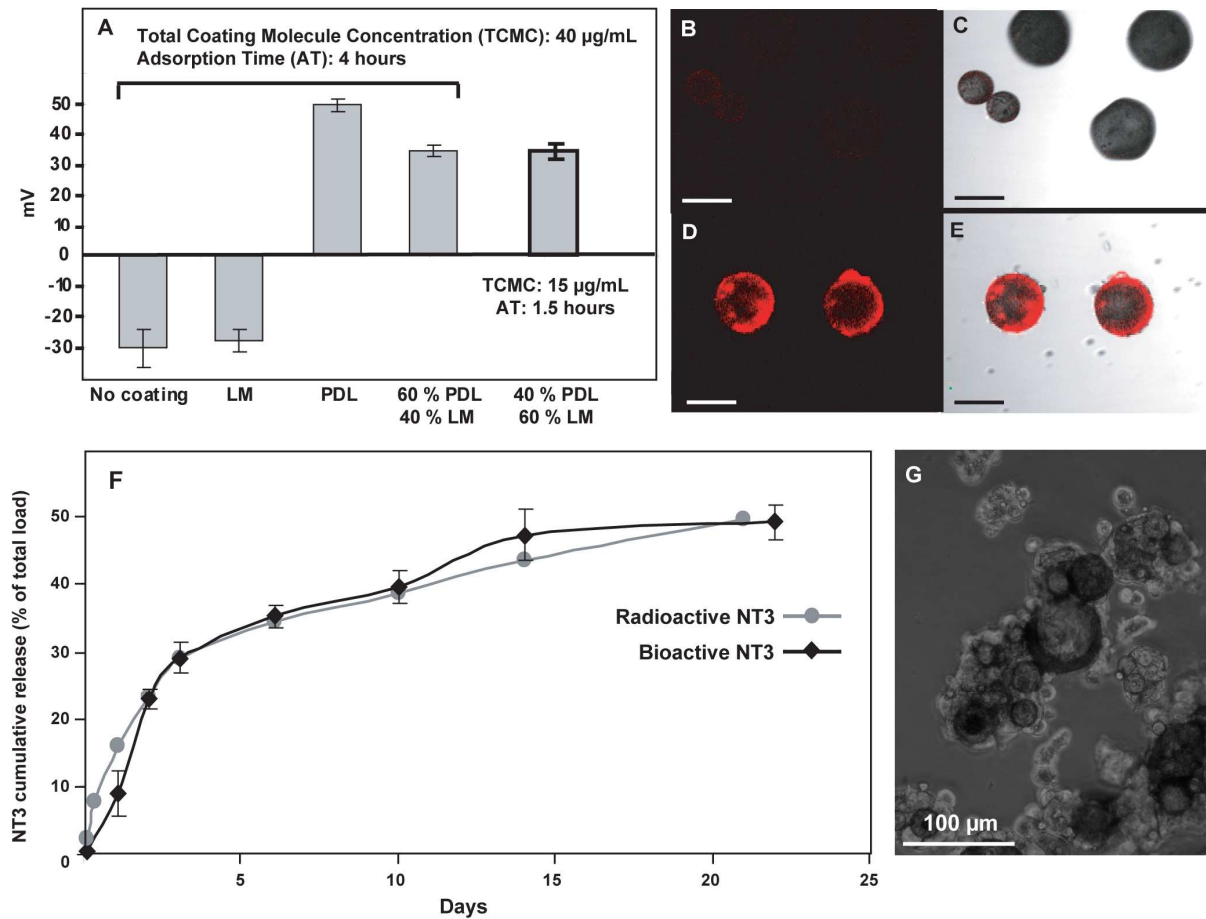


Figure 2. PAM laminin (LM) biomimetic surface & NT3 release *in vitro*

LM induced a negative zeta potential whereas PDL shifted this potential toward positive values, favorable for cell attachment to the surface. PDL/LM-coated PAM formulation was optimized by using a ratio PDL/LM of 40/60 to increase the quantity of adsorbed LM (A). The adsorption time was decreased from 4 to 1.5 hours, and the total molecule concentration used for coating was decreased from 40 µg/mL to 15 µg/mL. No changes in zeta potential were observed using these parameters (A, top right bar). Zeta potential results are presented as average Zeta potential \pm standard deviation. Using anti-LM immunofluorescence, uncoated microspheres (B, C) show no staining whereas PDL/LM-coated microspheres (D, E) stained positive with the anti-LM antibody. NT3 encapsulated within PAMs was released in a bioactive conformation as observed using an *in vitro* bioassay (F). After 22 days *in vitro*, about 50 % of the encapsulated NT3 was released, as observed by radioactivity counting and bioassay, which correspond to approximately 375 ng of NT3 for 0.75 mg PAMs transplanted per animals. Importantly, LM was homogeneously distributed over the PAM surface, which allowed adhesion of 1.5×10^5 cells to 0.75 mg of PAMs prior to transplantation (G). Presented results were obtained from a representative experiment and expressed as average NT3 release \pm standard deviation. C, E: superposition of Nomarsky and fluorescent images. Scale bars: 50 µm. Abbreviations: NT3: neurotrophin 3, PAMs: pharmacologically active microcarriers.

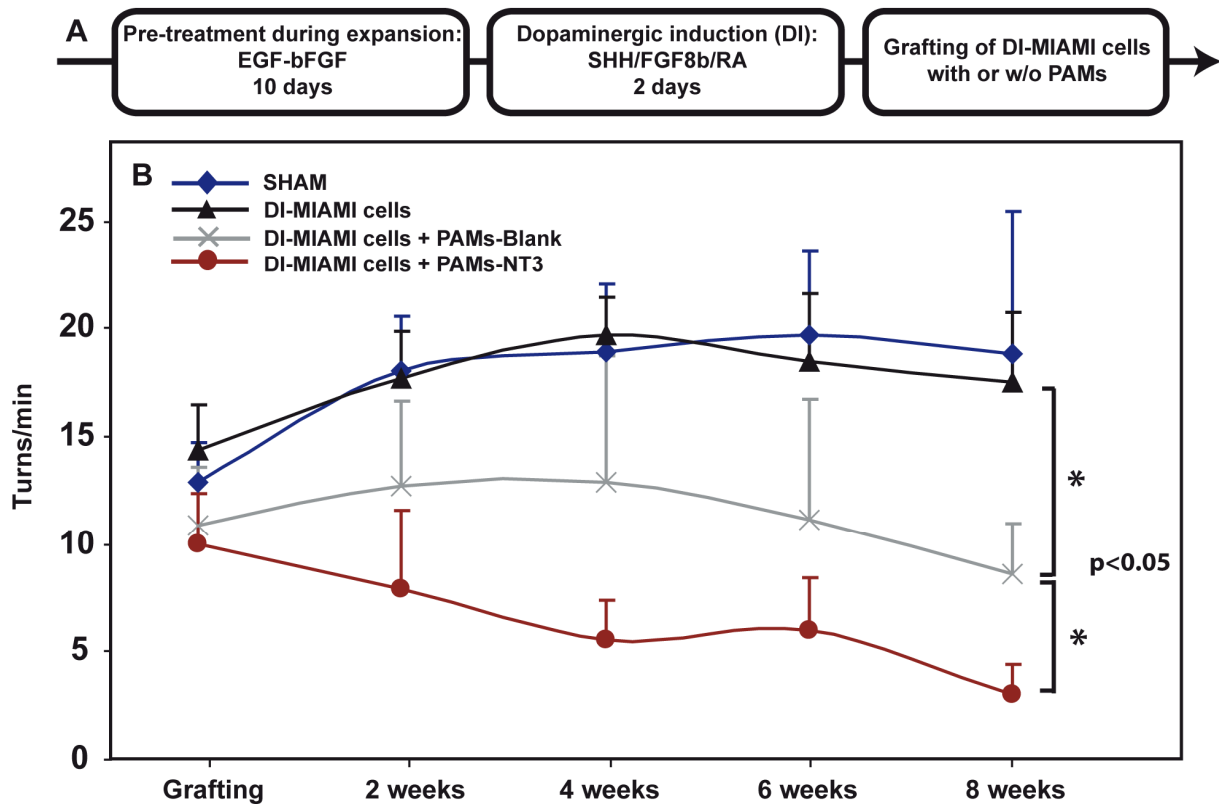


Figure 3. Functional recovery during amphetamine-induced rotational behavior

Before transplantation, cells were pre-treated with EGF-bFGF and further induced toward a dopaminergic phenotype (DI-MIAMI cells) prior to their attachment to PAM biomimetic surface (A). Rats transplanted with medium alone (sham-operated) exhibited a constant increase of their rotational behavior during 6 weeks before stabilization at 8 weeks (B). Rats transplanted with DI-MIAMI cells alone did not exhibit a statistically significant reduced rotational behavior compared to sham-operated rats. However, DI-MIAMI cells adhering to PAMs-NT3 resulted in a constant, and statistically significant, decrease until the end of the experiment. PAMs-Blank also led to an improved rotational behavior, but to a lesser extent compared to PAMs-NT3. Results are presented as the mean rotations of 5-6 rats per group \pm average deviation. Abbreviations: DI-MIAMI: dopaminergic-induced marrow-isolated adult multilineage inducible, NT3: neurotrophin 3, PAMs: pharmacologically active microcarriers.

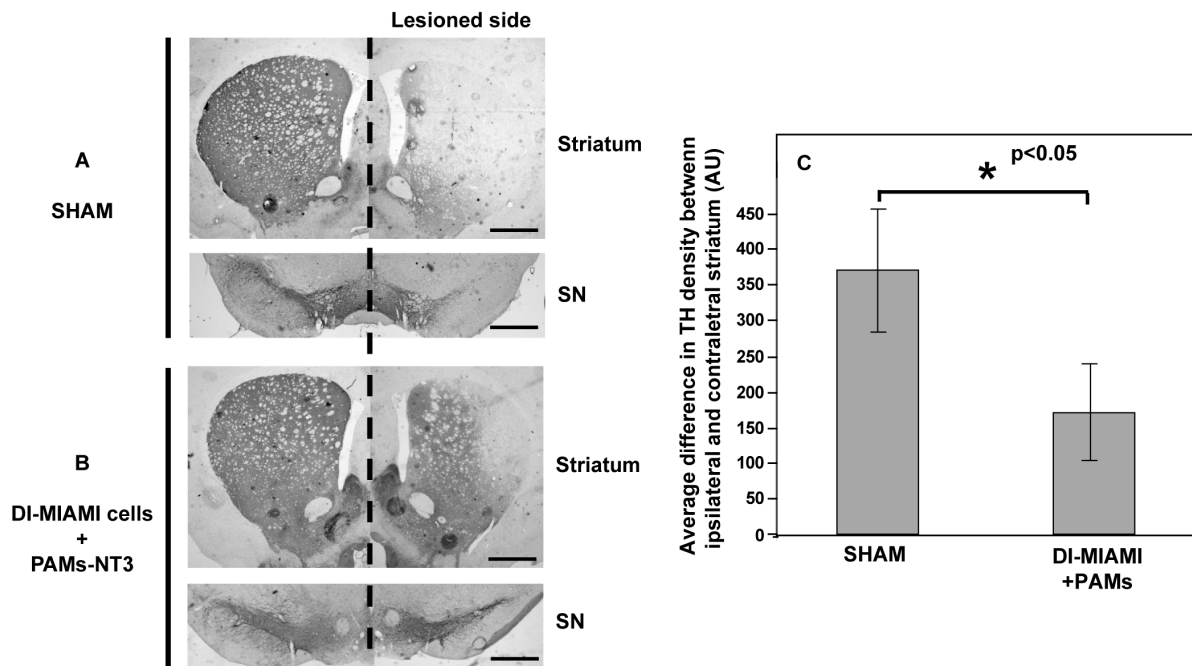


Figure 4. Neuroprotection/repair of nigrostriatal pathway induced by PAMs/DI-MIAMI cell complexes

Eight weeks after transplantation, only a few TH positive fibres were observed in the striatum and a few TH-positive neurons were found in the SN of sham grafted rats (A). Transplantation of DI-MIAMI cells combined to PAMs resulted in an important neuroprotection of the nigrostriatal dopaminergic system, as observed in the striatum and SN (B) and confirmed by densitometric analysis (C). Scale bars: 1 mm. Abbreviations: SN: substantia nigra, DI-MIAMI: dopaminergic-induced marrow-isolated adult multilineage inducible, PAMs: pharmacologically active microcarriers, TH: tyrosine hydroxylase. Densitometric results are presented as mean difference intensities between contralateral and ipsilateral striatum \pm average deviation.

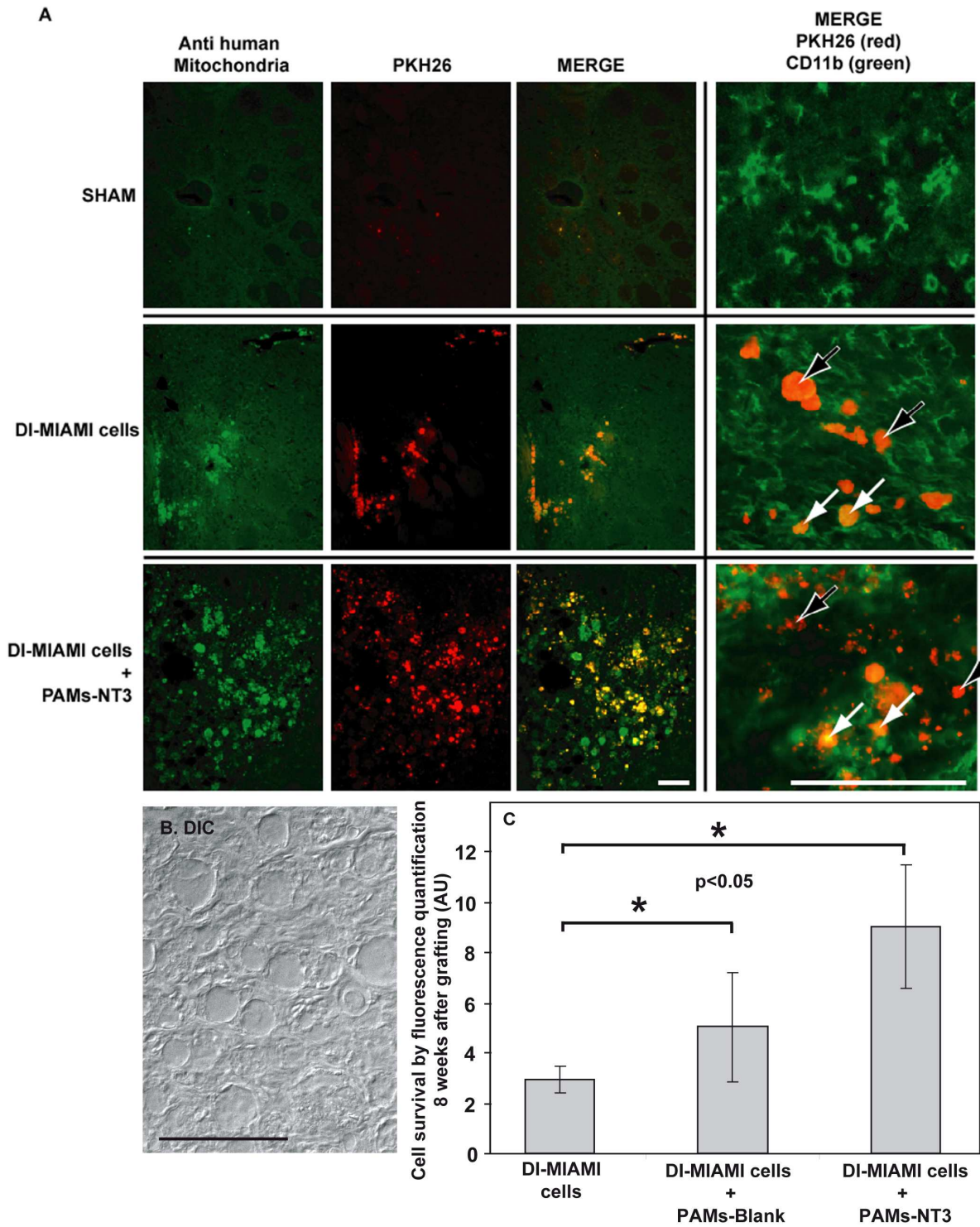


Figure 5. PAMs increased MIAMI cell survival *in vivo*

Colocalization of PKH26 and anti-human mitochondria staining demonstrated that DI-MIAMI cells were still present in the striatum of rats 8 weeks after transplantation (A). There was no strong immune reaction, and most of the PKH26 staining observed did not co-localize with macrophage/microglia (black arrows). White arrows point to engulfed PKH26. DIC: differential interference contrast image showing PAMs at the implantation site 8 weeks after grafting (B). Eight weeks after transplantation, cell survival was almost 2 fold higher in presence of PAMs-Blank, and more than 3 fold higher in presence of PAMs-NT3 (C), as observed by quantification of human mitochondria fluorescent staining intensity. Results are presented as the mean cell survival \pm average deviation. Scale bars: 100 μ m. Abbreviations: DI-MIAMI: dopaminergic-induced marrow-isolated adult multilineage inducible, PAMs: pharmacologically active microcarriers, NT3: neurotrophin 3, TH: tyrosine hydroxylase.

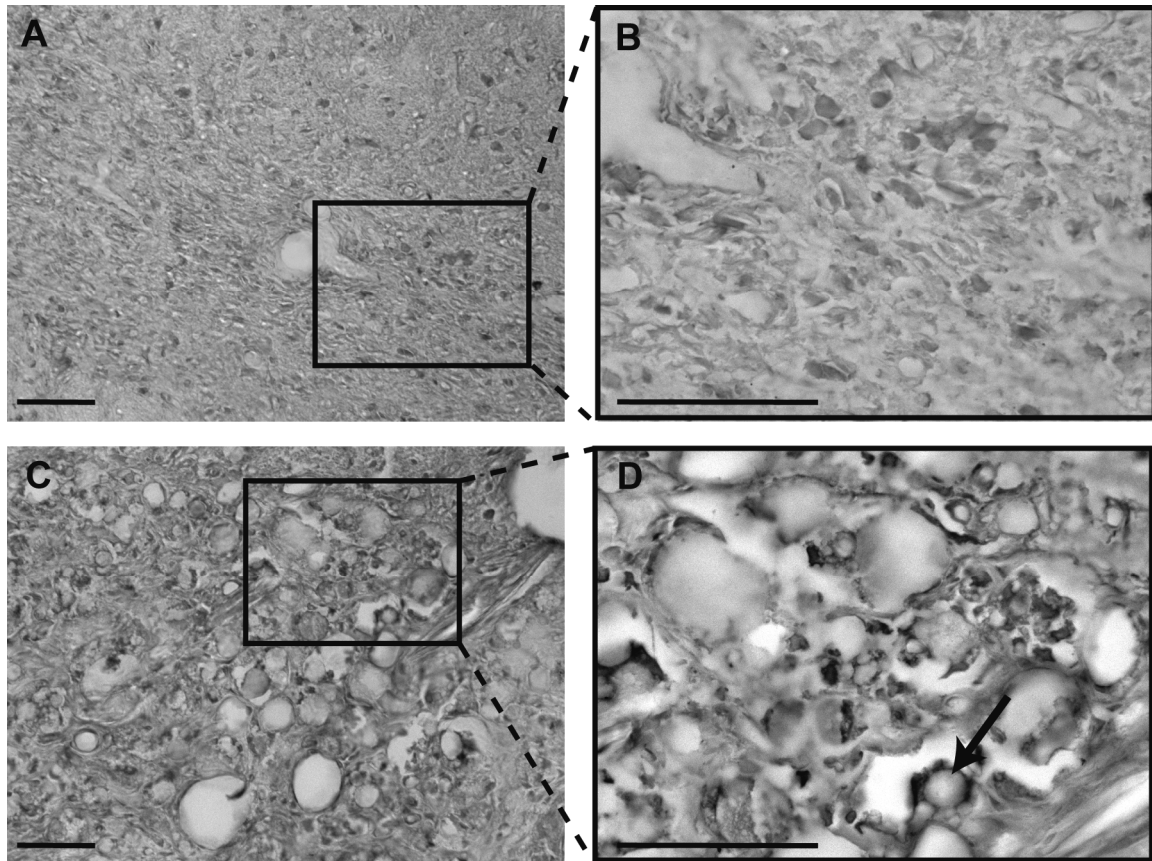


Figure 6. PAMs enhanced the expression of TH by DI-MIAMI cells *in vivo*

Human specific anti-TH immunohistochemistry revealed that DI-MIAMI cells expressed almost no TH after 8 weeks if transplanted alone (A, B). Conversely, grafting of MIAMI cells in combination with PAMs resulted in a higher fraction of human TH positive cells in the striatum after 8 weeks (C, D). Black arrow point to a PAM surrounded by TH positive DI-MIAMI cells. Scale bar: 50 μ m. Abbreviations: DI-MIAMI: dopaminergic-induced marrow-isolated adult multilineage inducible, PAMs: pharmacologically active microcarriers, TH: tyrosine hydroxylase.

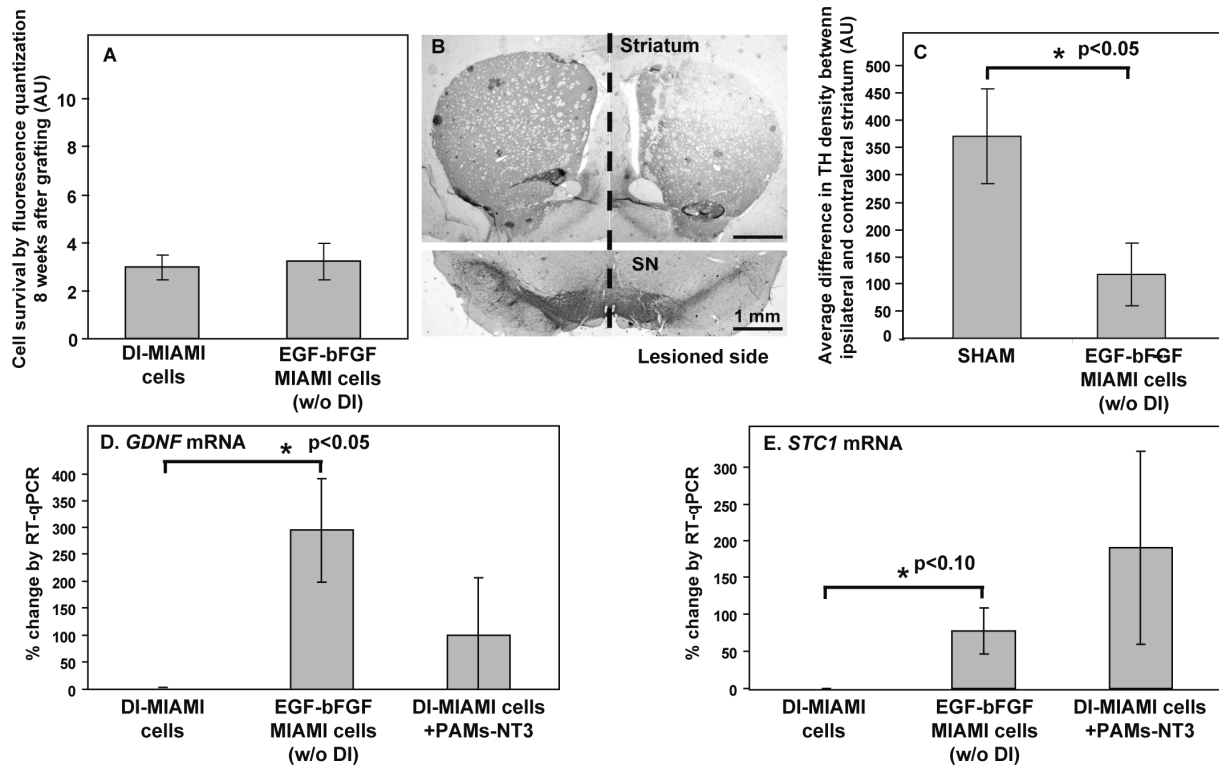


Figure 7. Effects of EGF-bFGF pre-treated MIAMI cells, without dopaminergic induction

Similarly to DI-MIAMI cells, MIAMI cells only pre-treated with EGF-bFGF (without DI) and transplanted without PAMs poorly survived. Despite their poor survival, these cells induced a functional recovery, correlated with a high density of TH fibres in the striatum (B, C). This could be explained by their differential secretion of molecules such as *GDNF* and *Stanniocalcin 1*, as observed by RT-qPCR (D, E). Noteworthy, adhesion onto PAMs-NT3 tended to increase the secretion profile of DI-MIAMI cells (D, E, top right bar). TH density results are presented as mean differences \pm average deviation calculated from 3 slides taken in 3 different rats for each group. RT-qPCR results were obtained from three different experiments and presented as the average % change of mRNA expression \pm average deviation. Abbreviations: DI: dopaminergic induction, TH: tyrosine hydroxylase, *STC1*: *Stanniocalcin 1*.