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Supplemental Information

Piconewton Mechanical Forces Promote Neurite Growth

Vittoria Raffa, Francesca Falcone, Sara De Vincentiis, Alessandro Falconieri, Maria P. Calatayud, Gerardo F. Goya, and Alfred Cuschieri

Supplementary Materials

Table S1. Set of genes known to be expressed in NGF differentiated PC12 cells

Dclk1	ENSRNOG00000032922
Sik1	ENSRNOG00000001189
Serpine1	ENSRNOG00000001414
Vgf	ENSRNOG00000001416
Rgs2	ENSRNOG00000003687
Arl4a	ENSRNOG00000004282
Rdh10	ENSRNOG00000006681
Ptgs1	ENSRNOG00000007415
Mmp13	ENSRNOG00000008478
Apha2	ENSRNOG00000009222
Ripply2	ENSRNOG00000010004
Tph1	ENSRNOG00000011672
F3	ENSRNOG00000011800
Sgk1	ENSRNOG00000011815
Tmem95	ENSRNOG00000015669
Mafb	ENSRNOG00000016037
Fzd4	ENSRNOG00000016848
Egr3	ENSRNOG00000017828
Hbegf	ENSRNOG00000018646
PVR	ENSRNOG00000019202
Fosl1	ENSRNOG00000020552
Sprr1a	ENSRNOG00000024028
Sprr1b	ENSRNOG00000031144
Mmp3	ENSRNOG00000032626
Mmp10	ENSRNOG00000032832
Csrnp1	ENSRNOG00000033433
Kdm6b	ENSRNOG00000037613
Plaur	ENSRNOG00000037931
Prss22	ENSRNOG00000039698
Arc	ENSRNOG00000043465
Fosb	ENSRNOG00000046667
Ifrd1	ENSRNOG00000050997
Cited2	ENSRNOG00000056940

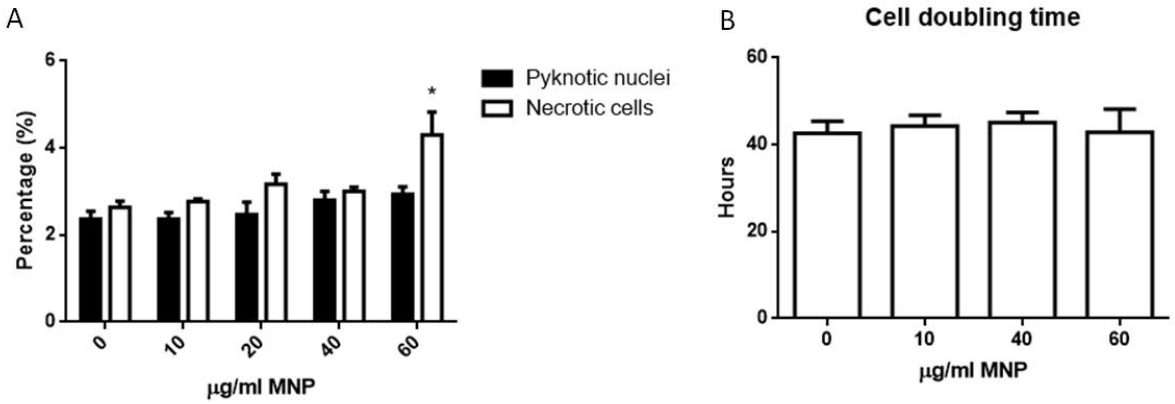

Fig. S1. PC12 cells were incubated by adding MNPs to the cell growth medium at the final concentration of 0, 10, 20, 40 or 60 µg ml^{-1} for 72 h. No difference was found in the fraction of pyknotic cells and in cell doubling time at any dose tested. Additionally, the percentage of necrotic cells was always below the 5%, being significantly different from the control only at the highest concentration tested. A) Necrosis was calculated as percentage of PI positive cells. 1-way ANOVA test followed by Bonferroni correction, n=3, p=0.01. Pyknotic nuclei were evaluated by DAPI staining. ANOVA test followed by Bonferroni correction, n=3, p=0.23. B) Cell doubling time. n=3, 1-way ANOVA test, p=0.95.

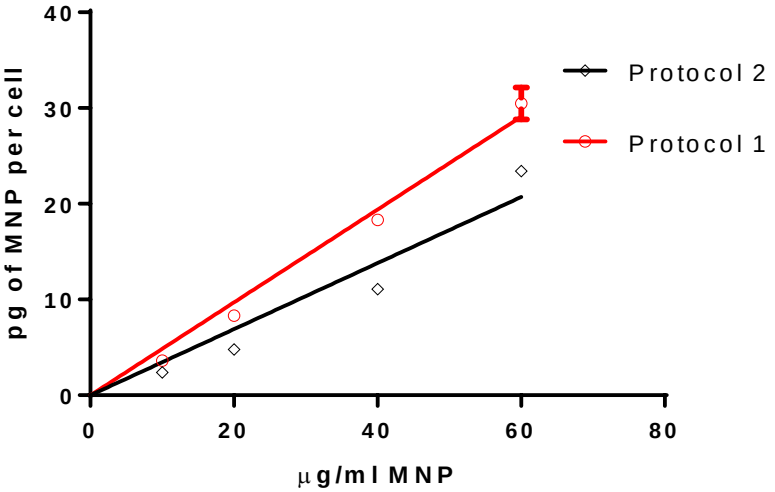

Fig. S2. MNP cell labelling. Protocol 1: cells cultured for 96 h in differentiation medium supplemented with MNPs. N=6, linear regression analysis, R^2=0.94. Protocol 2: cells cultured for 48 h in cell growth medium supplemented with MNPs and then 96 hours in differentiation medium. N=6, linear regression analysis, R^2=0.99.

Table S2. Neurite number per cell. N=100. Kruskal Wallis test followed by HDS correction.

	M^-MNP^-	**M^+MNP^-**	**M^-MNP^+**	**M^+MNP^+**	**P value**
3.4 pg MNP, 72 h stretch	2.34±0.12	2.27±0.11	2.270±0.11	2.47±0.13	0.765
4.8 pg MNP, 72 h stretch	2.36±0.10	2.42±0.12	2.30±0.11	2.58±0.12	0.490
3.4 pg MNP, 120 h stretch	3.12±0.12	2.97±0.12	3.22±0.13	3.65±0.13$^{*,\#\#}$	0.001
NGF 2 h	2.02±0.10	2.34±0.12	2.14±0.11	2.45±0.11*	0.027