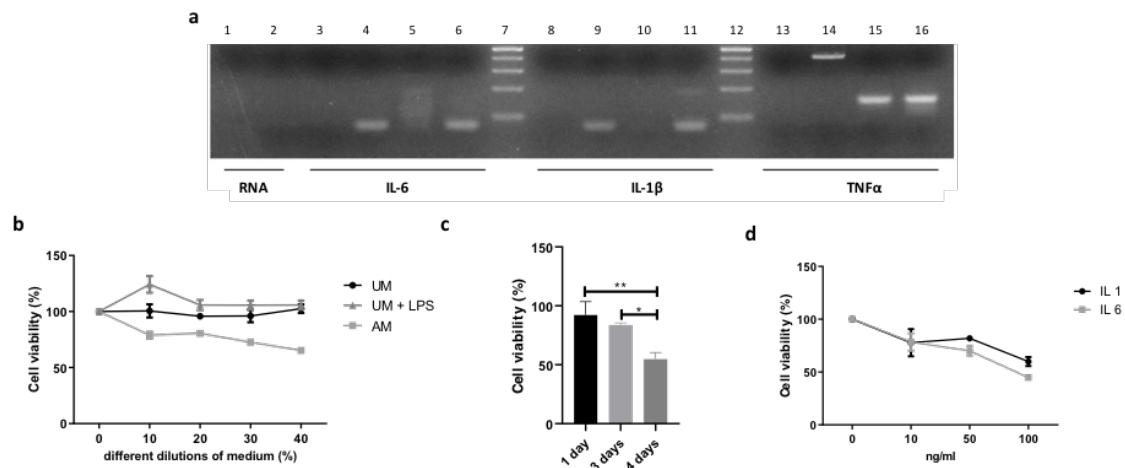


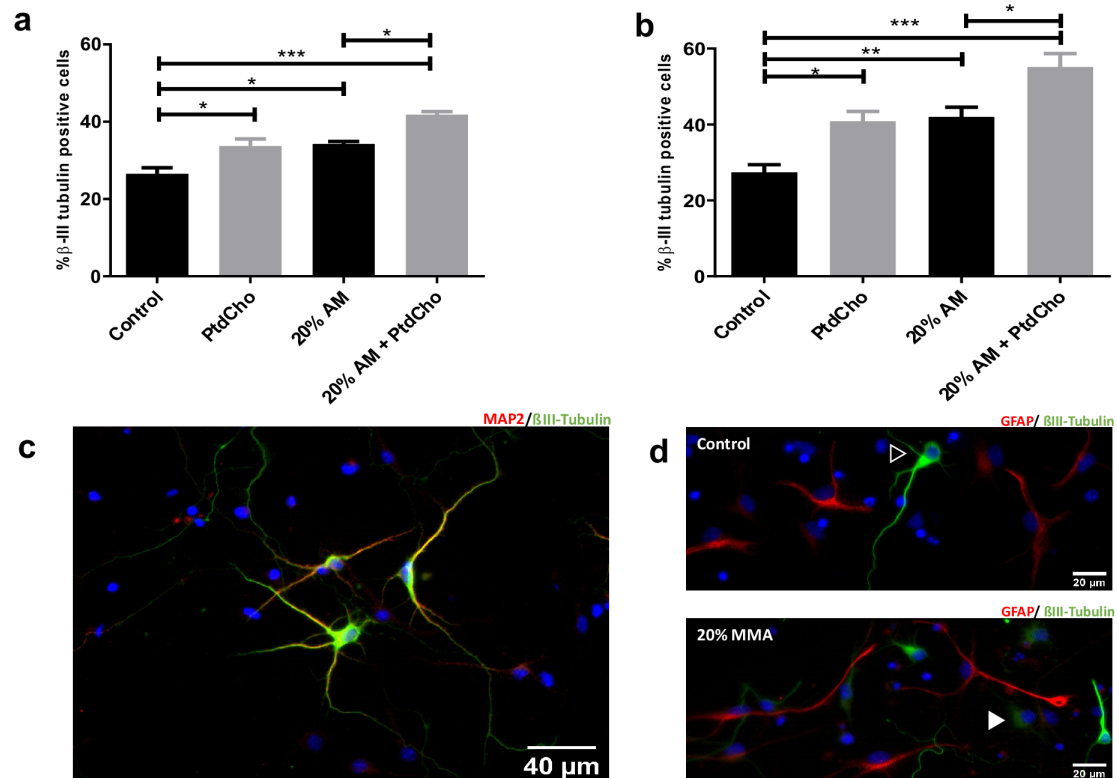
Phosphatidylcholine restores neuronal plasticity of neural stem cells under inflammatory stress

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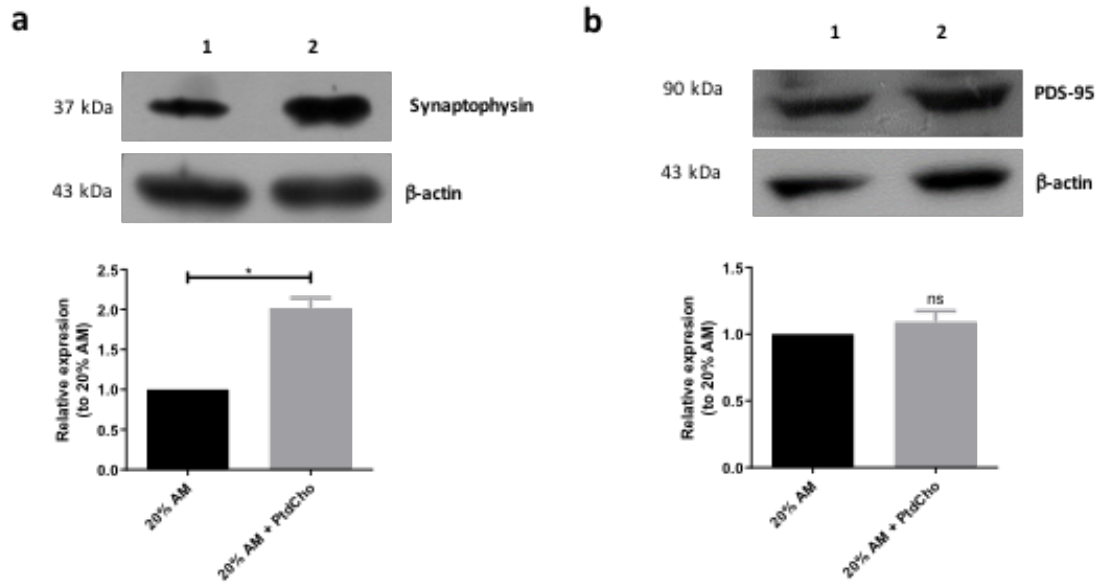


Supplementary figure 1 - Characterization of macrophages-activated media. a)

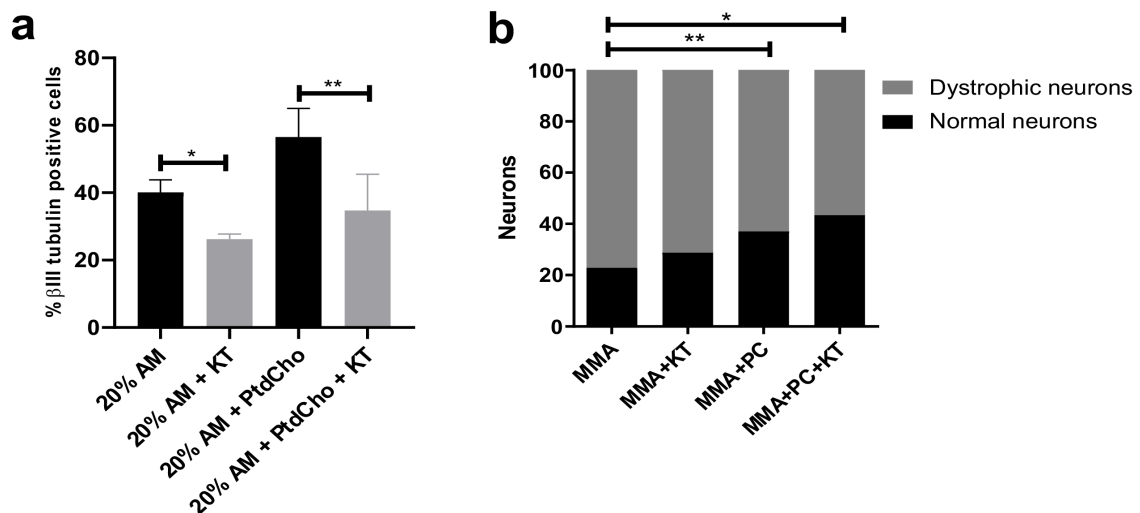
Representative image of the amplification products obtained after the RT-PCR made from total RNA of Raw 264.7 cells grown in DMEM 10% SFB in the presence of 1 μ g/ml LPS for 18 hours (n = 2). -LPS: RNA isolated from unstimulated Raw 264.7 cells and treated with RQ1 DNase was used as a template of the PCR reaction to control for the presence of traces of genomic DNA. +LPS: RNA isolated from LPS-stimulated Raw 264.7 cells and treated with RQ1 DNase was used as a template of the PCR reaction to control for the presence of traces of genomic DNA. C-: PCR water control (negative control). C+: PCR positive control (mouse genomic DNA). -cDNA: PCR product from total cDNA generated from unstimulated Raw 264.7 cells. +cDNA: PCR product from total cDNA generated from LPS-stimulated Raw 264.7 cells. MWM: Molecular weight marker, 100 bp. **b)** Cells were incubated with different dilutions of medium obtained from LPS-stimulated macrophages (AM), medium obtained from macrophages without activation as control medium (UM) or control medium with LPS (UM + LPS) for a period of 4 days, the viability of the NSCs was analysed by MTT assay. **c)** Cells were incubated with 20% of LPS-stimulated macrophages (AM) for the indicated periods of time, and the viability of the NSCs was analysed by MTT assay. Data were presented as mean $\pm$ SEM. **p<0.05. **d)** After incubating the cells with the indicated concentrations of interleukins for a period of 3 days, the viability of the NSCs was analysed by MTT assay.



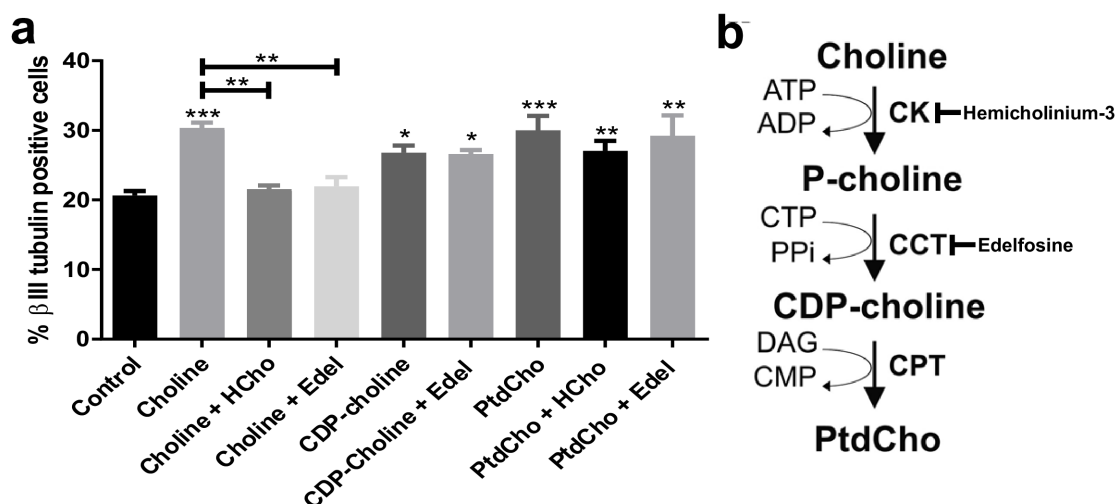
Supplementary figure 2- NSCs differentiation is affected by inflammation and restored by PtdCho. Percentage of β -III Tubulin positive cells analysed by immunocytochemistry coupled to fluorescence microscopy of NSCs exposed to 20% V/V of AM and UM in the presence or in the absence of PC 50 μ M during 1 **(a)** and 2 **(b)** days. Graph is representative of three independent experiments. Data were presented as mean $\pm$ SEM. *** p < 0.001; ** p < 0.01; * p < 0.05. **c)** Representative images of β -III Tubulin and MAP-2 positive neurons incubated during 3 days under control condition (100X). **d)** Representative images of β -III Tubulin and GFAP positive cells incubated during 3 days under control or 20% V/V of AM conditions. Open arrow indicates a normal neuron and white arrow dystrophic neurons.



Supplementary figure 3- Synaptophysin and PSD-95 levels under inflammation and PtdCho treatment. Representative image of Western Blot showing Synaptophysin (a) and PSD-95 (b) levels in NSCs exposed to 20% V/V of AM in the presence or in the absence of PtdCho (50 μ M) during 3 days. β -actin was used as control loading. The gels/blots displayed here are cropped, and without high-contrast (overexposure). The full-length gels and blots are included in a Supplementary Information file. Densitometric analysis, *p < 0.05, ns: no statistical significance (Student's T-test).



Supplementary figure 4 - PtdCho effects and the PKA/CREB signalling pathway under inflammatory conditions (AM). **a)** Percentage of β -III Tubulin positive cells analysed by immunocytochemistry coupled to fluorescence microscopy of NSCs exposed to 20% V/V of AM in the presence or in the absence of PC 50 μ M and PKA inhibitor (KT5720 (10 μ M)) after 72 hours. Cells were incubated during 30 min with the PKA inhibitor prior to liposomes addition. Graph is representative of three independent experiments. Data were presented as mean $\pm$ SEM. *** p < 0.001; ** p < 0.01; * p < 0.05. **b)** Number of normal and dystrophic neurons under inflammatory conditions in the presence or in the absence of PtdCho or KT5720, ** p < 0.05 *** p < 0.001 (Student's T-test).



Supplementary figure 5 – Effect of choline, CDP-choline and PtdCho on neuronal differentiation. **a)** Percentage of β -III Tubulin positive cells analysed by immunocytochemistry coupled to fluorescence microscopy of NSCs treated during 24 hours with choline (50 μ M), CDP-choline (50 μ M), PtdCho (50 μ M) in the presence or in the absence of the indicated inhibitors hemicholineum-3 (HCho, 50 μ M) for choline kinase and Edelfosine (Edel, 5 μ M) for phosphocholine cytidyltransferase α . Cells were incubated during 30 min with the inhibitors prior to liposomes addition. Graph represents the percentage of neuronal differentiation measured in three independent experiments. Data were presented as mean $\pm$ SEM. *** p < 0.001; ** p < 0.01; * p < 0.05. **b)** Schematic representation of the Kennedy pathway for PtdCho biosynthesis.

Supplementary Movie: Real-time observation of calcium influx after KCl. Cells were loaded with the Ca^{2+} -sensitive fluorescence indicator Fluo-3/AM and depolarized with high-KCl (100 mM) in Ringer solution. Fluorescence was measured every 0.25 s for a total of 12 s with confocal laser system (Zeiss LSM 880). Assay condition are indicated in the video.