Supplemental Figures – Kwon and Kim et al.

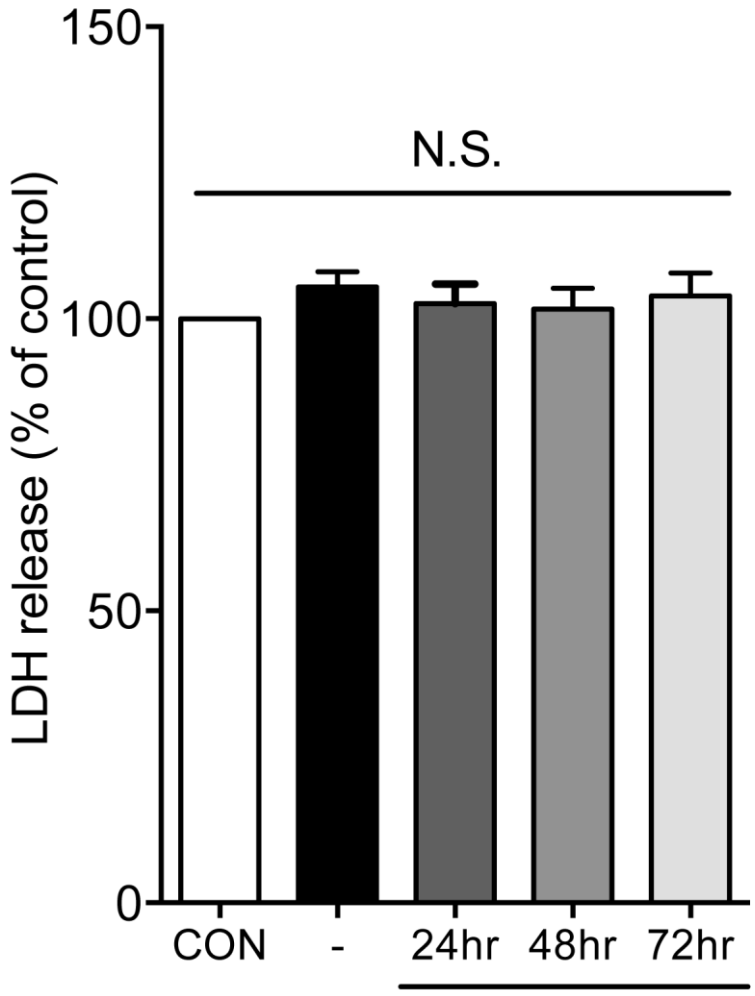


Figure S1. Microglia apoptosis was not examined in all groups

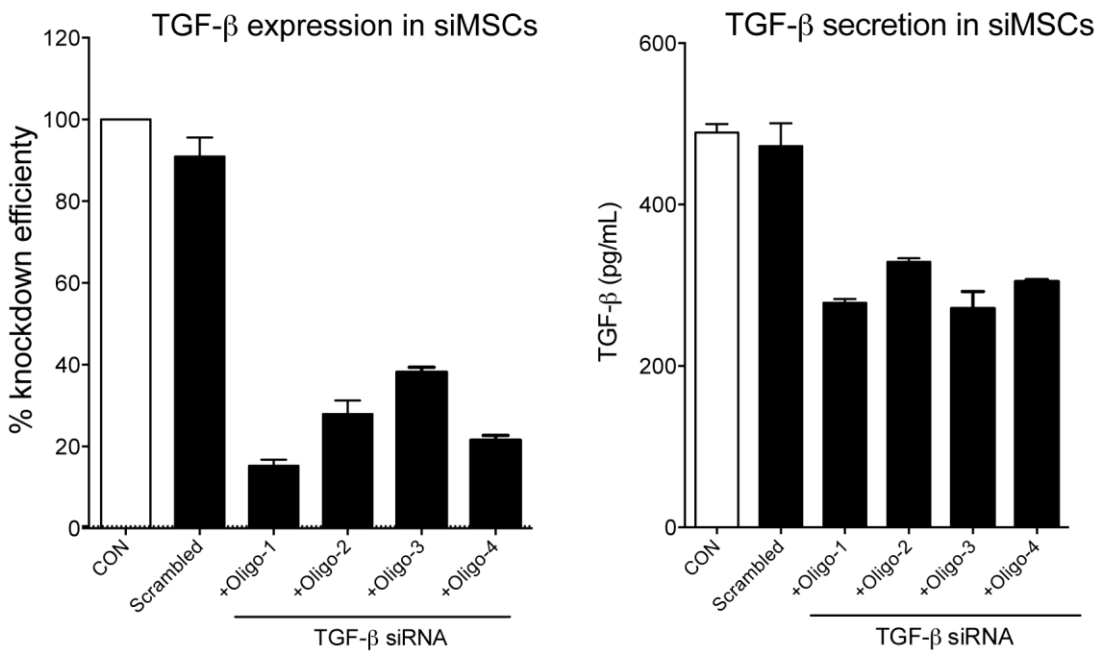
To investigate whether LPS alone or LPS+MSC-CM induce microglia apoptosis, LDH assay was performed according to the manufacturer's instructions. Microglia apoptosis was not examined in all groups. The reported results are based on three independent experiments on separate batches of cells. The data are means ± SEM of three independent experiments.

Figure S2. Concentration of TGF-β and CX3CL1 in MSC-conditioned media

	A: MSC-CM	B: Culture medium for MSC	MSC production (A-B)
TGF-β (pg/mL)	1747±29.325	718±36.8	1029 (mean value)
CX3CL1 (pg/mL)	15.94343±0.14849	not detected	15.94343 (mean value)

Figure S3. Concentration of TGF-β and CX3CL1

Groups	TGF-β (pg/mL)	Cx3CL1 (pg/mL)
Microglia culture medium	798.98±12.5	not detected
Con (microglia alone)	975.12±45	not detected
LPS (100ng/mL)	828.91±21.5	not detected
LPS+MSC-CM	1744.9±95.8	13.955±1.4
LPS+MSC-CM+TGF-βR inhibitor	1457.15±7.5	10.955±1.2
LPS+MSC-CM+CX3CL1 antibody	1677.3±21.3	not detected
LPS+MSC-CM+TGF-βR inhibitor+CX3CL1 antibody	1382.1±45.1	not detected



S4. TGF-β1 siRNA transfection reduced the TGF-β1 expression and secretion in siMSCs

To reduced TGF-β1 expression and secretion in MSCs, MSCs were stably transfected with FlexiTube small interfering RNA (siRNA) for rat TGF-β1 or negative control siRNA using HiPerFect Transfection Reagent. The siRNA transfection was performed in serum-free medium following to manufacture's indication. The supernatant and siMSCs were separated and collected 48h after transfection for ELISA and qPCR analysis. All siRNAs were tested for mRNA knockdowns by real-time PCR. Most of siRNAs reduced about 50%-80% of mRNA expression and about 50% of TGF-β1 secretion. The most efficient target sequence for RNA interference was selected among four sequences provided by Qiagen. The data are means ± SEM of three independent experiments.

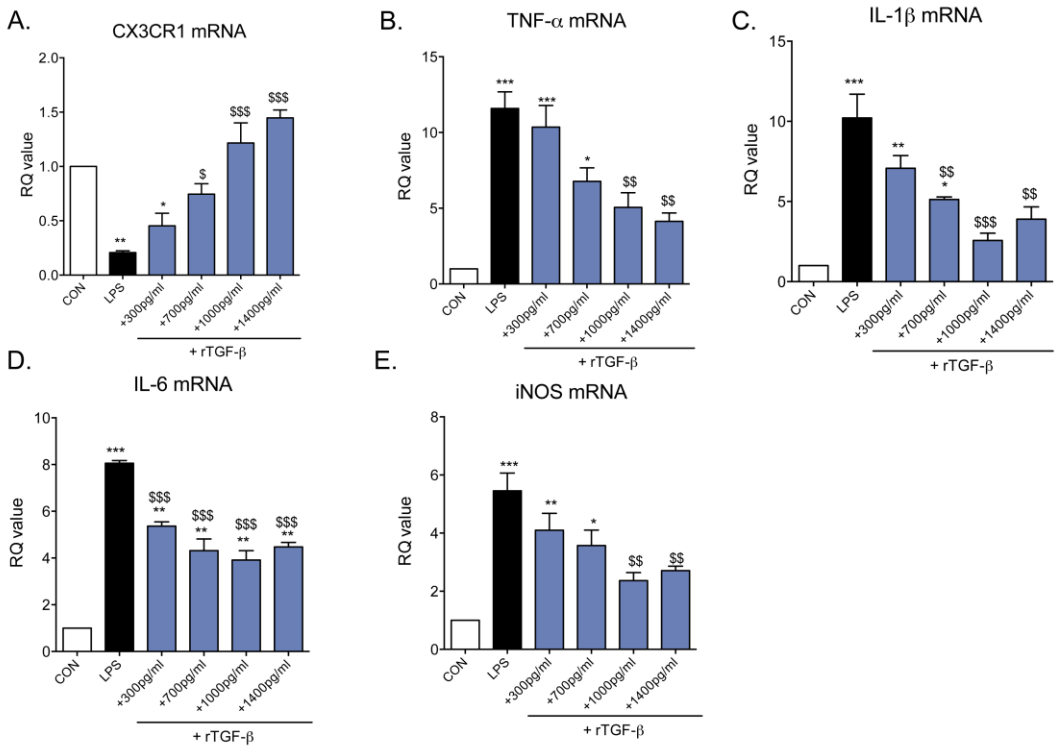


Figure. S5. The dose-dependent effect of recombinant TGF-β on microglia functional properties in LPS-stimulated microglia

Various dose (300, 700, 1000, 1400 pg/mL) of recombinant TGF-β (rTGF-β) was treated in LPS-stimulated microglia to investigate whether TGF-β releasing capacity in MSCs is related with microglia functional properties. In LPS-stimulated microglia, rTGF-β rescued the reduced CX3CR1 expression (A) and inhibited the increased gene expression of TNF-α (B), IL-1β (C), IL-6 (D), and iNOS (E) in dose-dependent manners. 1000pg/mL of rTGF-β dosage showed same effect with MSC-CM in LPS-stimulated microglia. The 1000pg/mL of concentration was similar with TGF-β concentration in MSC released. The data are means ± SEM of three independent experiments. $^{*}p<0.05$, $^{**}p<0.01$ $^{***}p<0.001$ compared with control (CON), $^{\$}p<0.05$, $^{\$\$}p<0.01$, $^{\$\$\$}p<0.001$ compared with LPS. $^{+}P<0.05$, $^{+++}P<0.001$ compared between two groups.