Supplementary Information

Role of miRNAs shuttled by mesenchymal stem cell-derived small extracellular vesicles in modulating neuroinflammation

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Supplementary Data S1 online (.pdf)

In-depth data analysis of miRNA expressed in IFN-γ-primed and unprimed MSCs identified by microarray analysis

Microarray analysis showed that miR-466q, miR-467g, miR-669c-3p, miR-466m-5p, miR-467f, miR-3082-5p, miR-466i-5p, miR-466i-3p and miR-5126 are significantly dysregulated in IFN-γ-primed MSCs compared to unprimed MSCs.

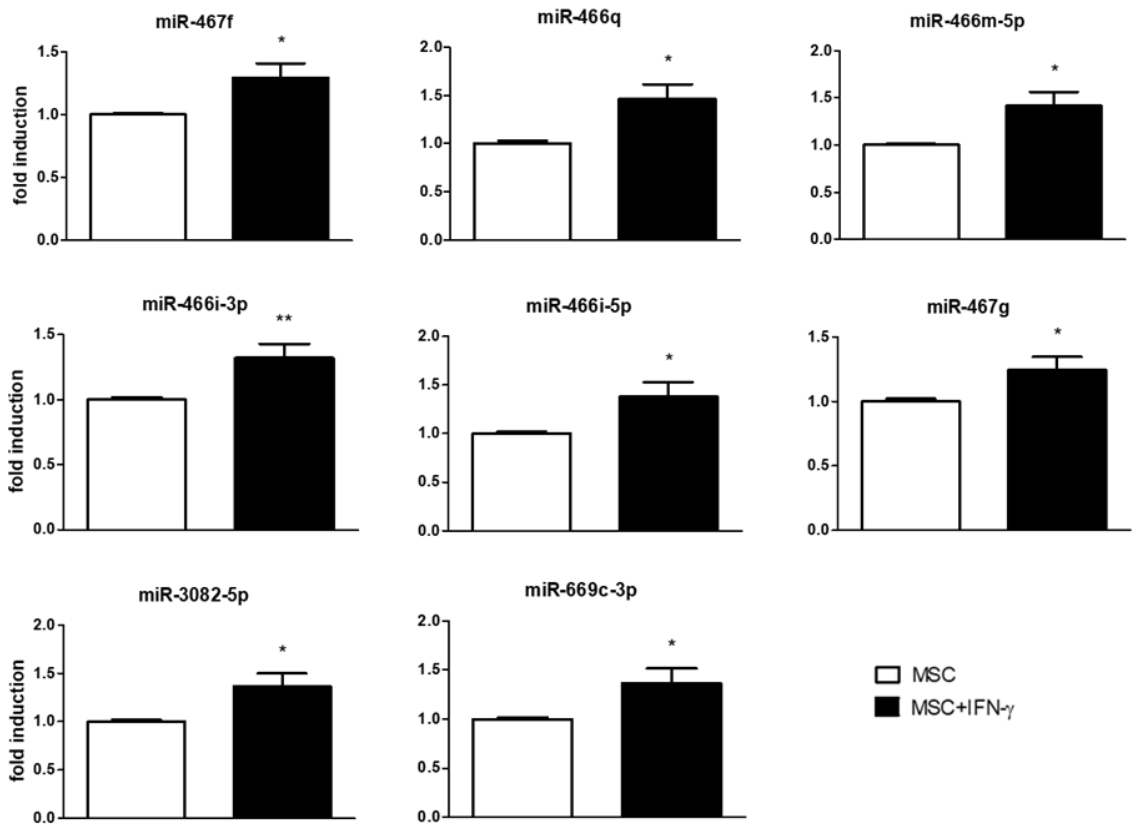
Supplementary data S2 online (.xls)

List of the 1718 target genes predicted for miR-467f through miRWalk database

Supplementary data S3 online (.xls)

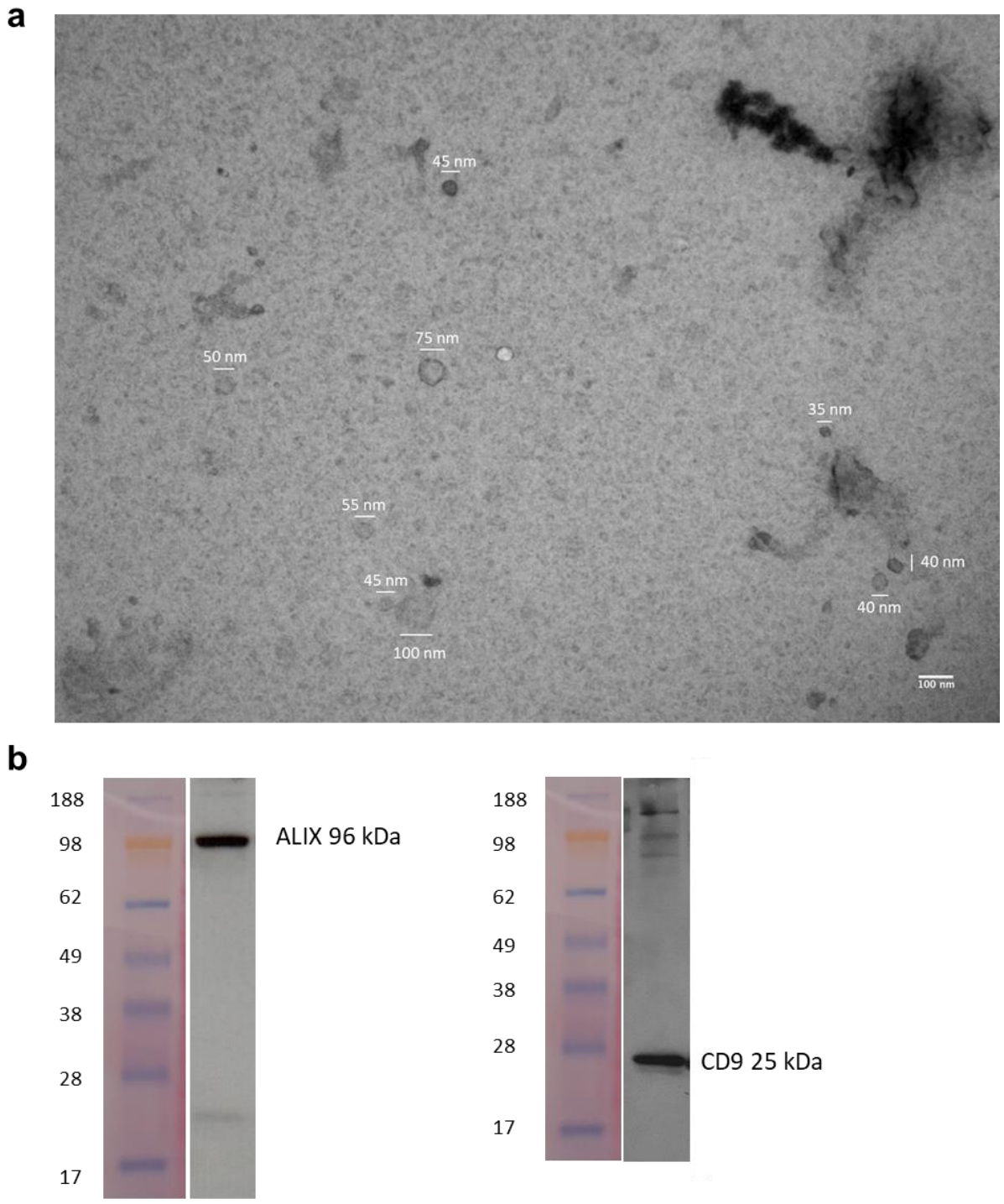
List of the 1157 target genes predicted for miR-466q through miRWalk database

Supplementary Figure S1 online



Supplementary Figure S1 online
Validation of the significantly dysregulated miRNAs in immunomodulatory MSCs by RT-PCR
RT-PCR analysis on MSCs primed or not with IFN-γ confirmed the significant dysregulation of the nine specific miRNAs upon IFN-γ stimulation of MSCs. **P* < 0.05, ***P* < 0.01. Data are presented as mean ± SEM of 3 independent experiments.

Supplementary Figure S2 online



Supplementary Figure S2 online

Characterization of s-EV isolated from IFN-γ-primed MSCs through electron microscopy and Western blot

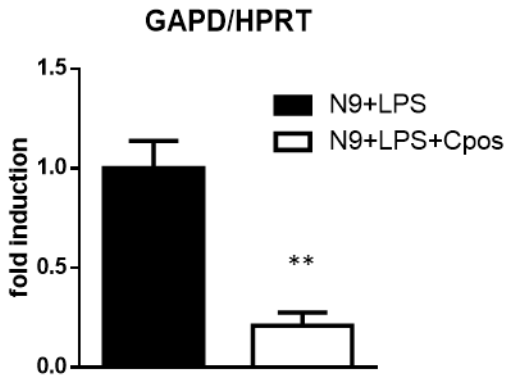
The presence of purified s-EV in the supernatant of IFN-γ-primed MSCs was confirmed by **e**lectron microscopy which identified the presence of nanovesicles with a diameter range of 50-100 nm (representative image in panel **a**) and by western blot analysis showing the expression of the s-EV markers Alix and CD9 (one representative blot is shown in panel **b**)

Supplementary Figure S3 online

a

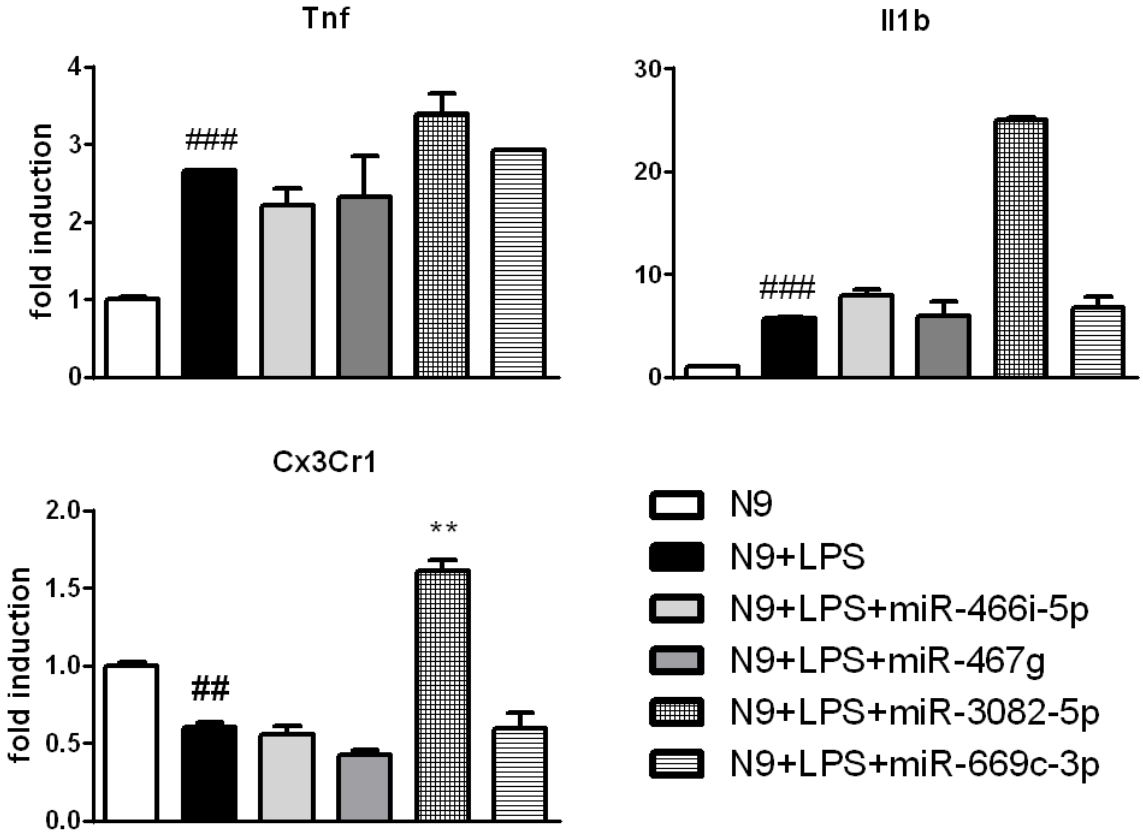
	GAPD cycle (mean)	HPRT cycle (mean)
N9+LPS	21.69	26.71
N9+LPS+Cpos	24.27	27.92

b



Supplementary Figure S3 online
Positive control miRNA, which significantly decreases the mRNA expression of GAPDH, validates efficient transfection
Validation of transfection efficiency was assessed by RT-PCR analysis using a positive control (Cpos), which targets GAPDH expression. **a** cycle threshold and **b** relative quantification over HPRT, as reference gene. $^{**}P < 0.01$. Data are presented as mean ± SEM of 3 independent experiments

Supplementary Figure S4 online

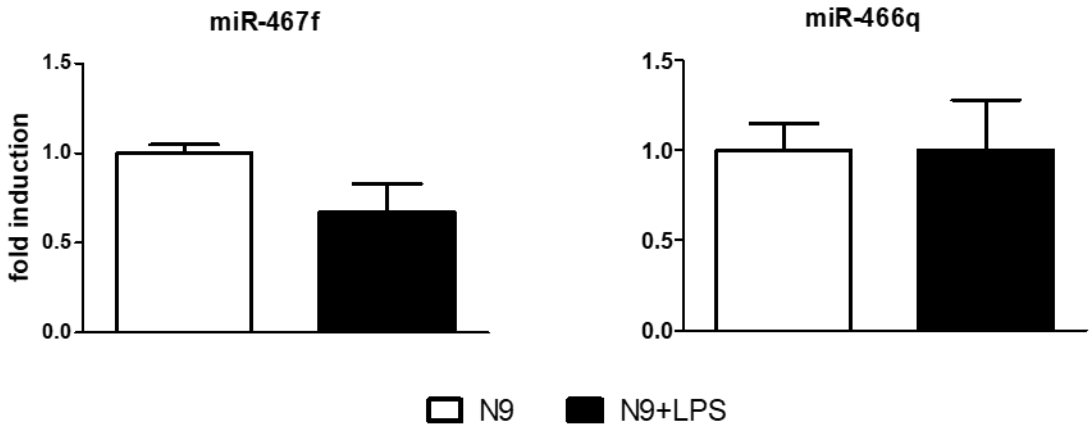


Supplementary Figure S4 online

Expression of Tnf, Il1b and Cx3cr1 by LPS-activated N9 cells transfected with miRNAs that are dysregulated in IFN-γ-primed MSCs but not in their derived s-EV

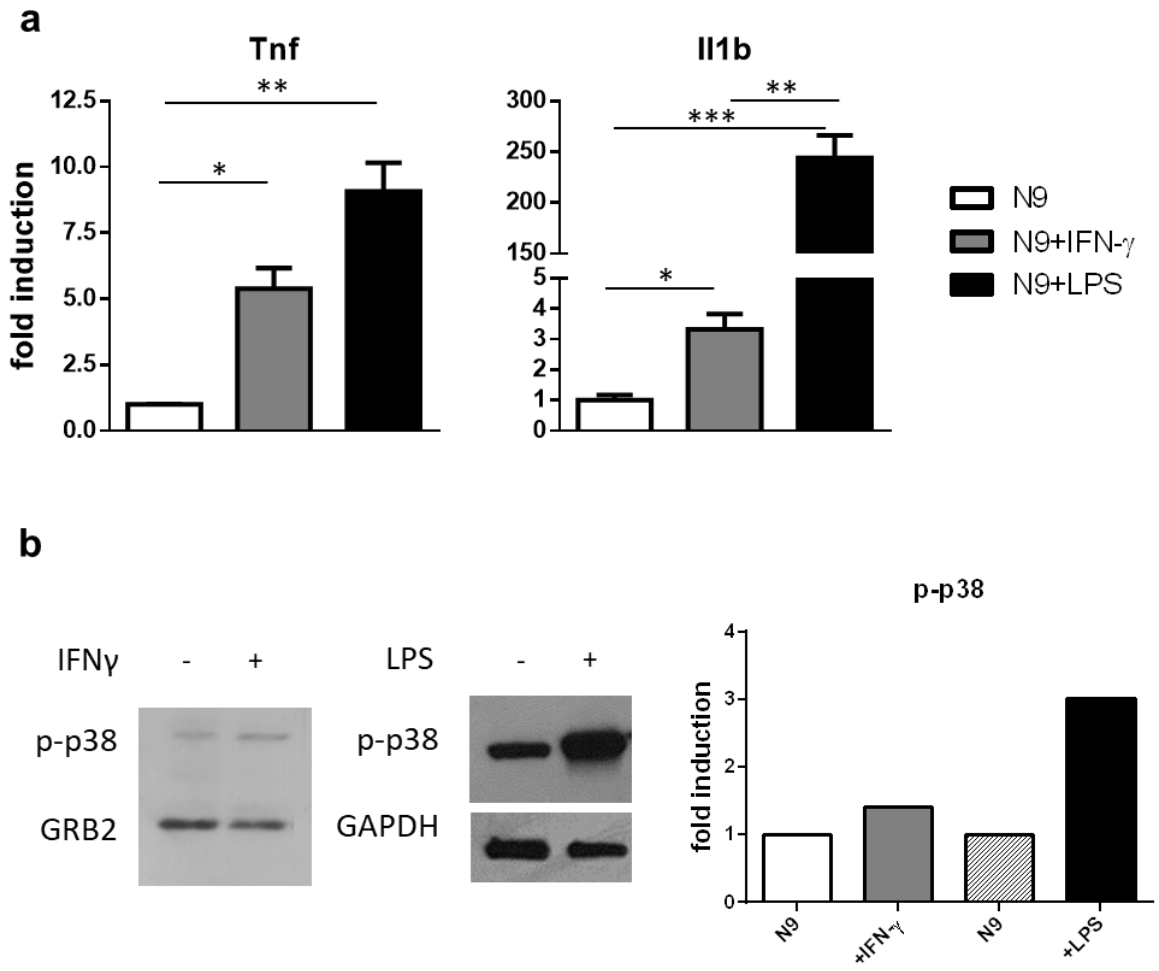
RT-PCR analysis shows that miR-466i-5p, miR-467g, miR-3082-5p, and miR-669c-3p did not affect the expression of Tnf and Il1b in LPS-activated microglia, and that miR-3082-5p induced a significant increase in Cx3cr1 expression in activated N9 cells. ##$P < 0.01$, untreated (N9) vs LPS-activated N9 cells (N9+LPS); ###$P < 0.001$, N9 vs N9+LPS; **$P < 0.01$ N9+LPS vs N9+LPS transfected with specific miRNA (N9+LPS+miRNA). Data are presented as mean ± SEM of 3 independent experiments.

Supplementary Figure S5 online



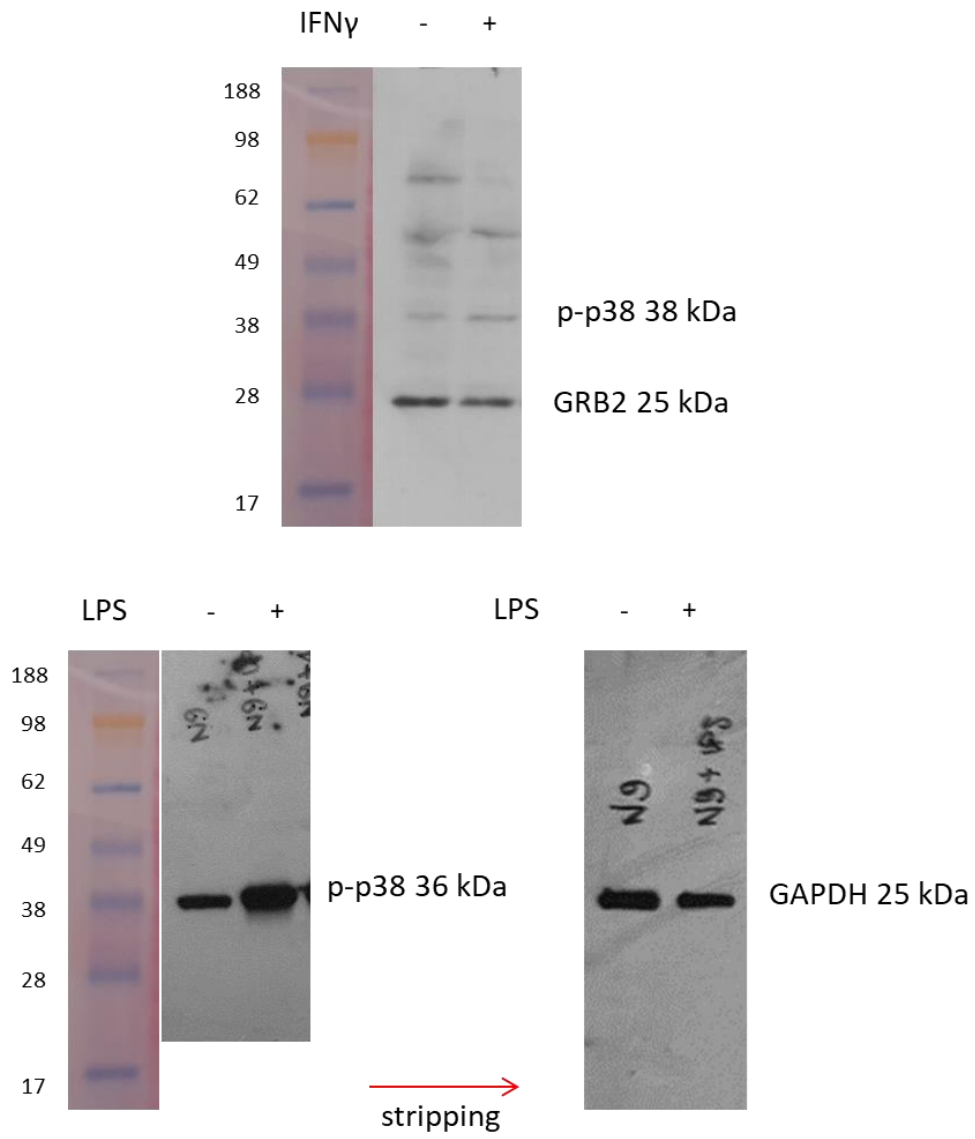
Supplementary Figure S5 online
Expression of miR-467f and miR-466q in microglia cell line activated or not with LPS
RT-PCR results demonstrated that N9 microglia line cells express both miR-467f and miR-466q at basal level, and that miRNA expression did not increase upon LPS-activation of the cells. Data are presented as mean ± SEM of 3 independent experiments

Supplementary Figure S6 online



Supplementary Figure S6 online
LPS is a stronger more consistent activator of N9 cells that IFN-γ
a Analysis of Tnf and Il1b mRNA expression by RT-PCR and **b** quantification of p-p38 by Western blotting performed in N9 cells stimulated or not with IFN-γ (100 ng/ml) or LPS (1 μg/ml) for 24h showed a stronger activation of N9 cell upon LPS activation in comparison to IFN-γ. *$P < 0.05$, **$P < 0.01$, *** $P < 0.001$. Data are presented as mean ± SEM of 3 independent experiments (a) and as fold induction of 1 experiment (b).

Supplementary Figure S7 online



Supplementary Figure S7 online
Unedited gels of Supplementary Figure S6 online