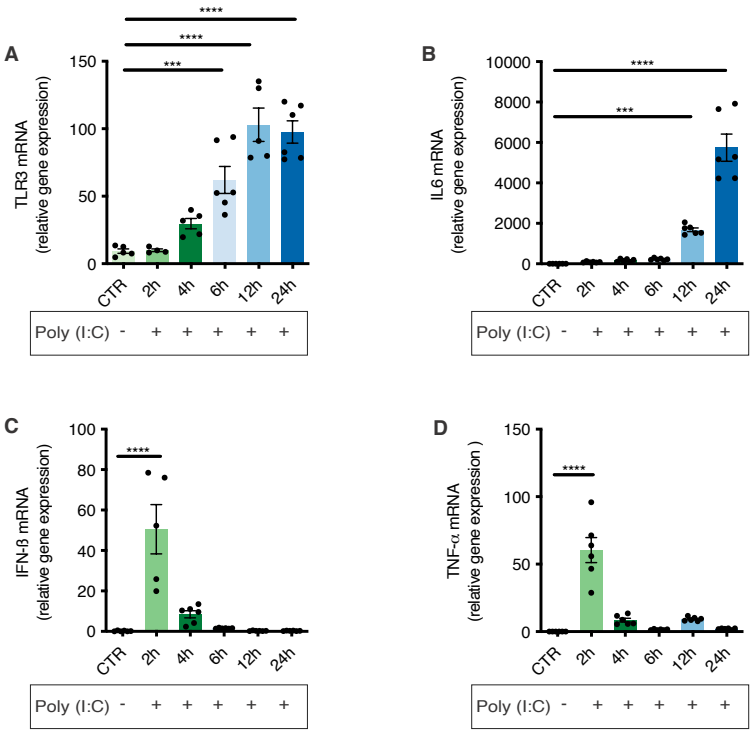
SUPPLEMENTAL MATERIAL

SUPPLEMENTARY FIGURES


A
TLR3 mRNA
(relative gene expression)
150
100
50
0
****
****
***
CTR
2h
4h
6h
12h
24h
Poly (I:C) - + + + + +
B
IL6 mRNA
(relative gene expression)
10000
8000
6000
4000
2000
0
****
***
CTR
2h
4h
6h
12h
24h
Poly (I:C) - + + + + +
C
IFN-ß mRNA
(relative gene expression)
100
80
60
40
20
0
****
CTR
2h
4h
6h
12h
24h
Poly (I:C) - + + + + +
D
TNF-α mRNA
(relative gene expression)
150
100
50
0
****
CTR
2h
4h
6h
12h
24h
Poly (I:C) - + + + + +

Supplemental Figure S1: TLR3 stimulation results in upregulation of IL-6 in neurons

(A) TLR3 regulation after Poly(I:C) treatment. ***p<0.001, ****p<0.0001. n=4-6.

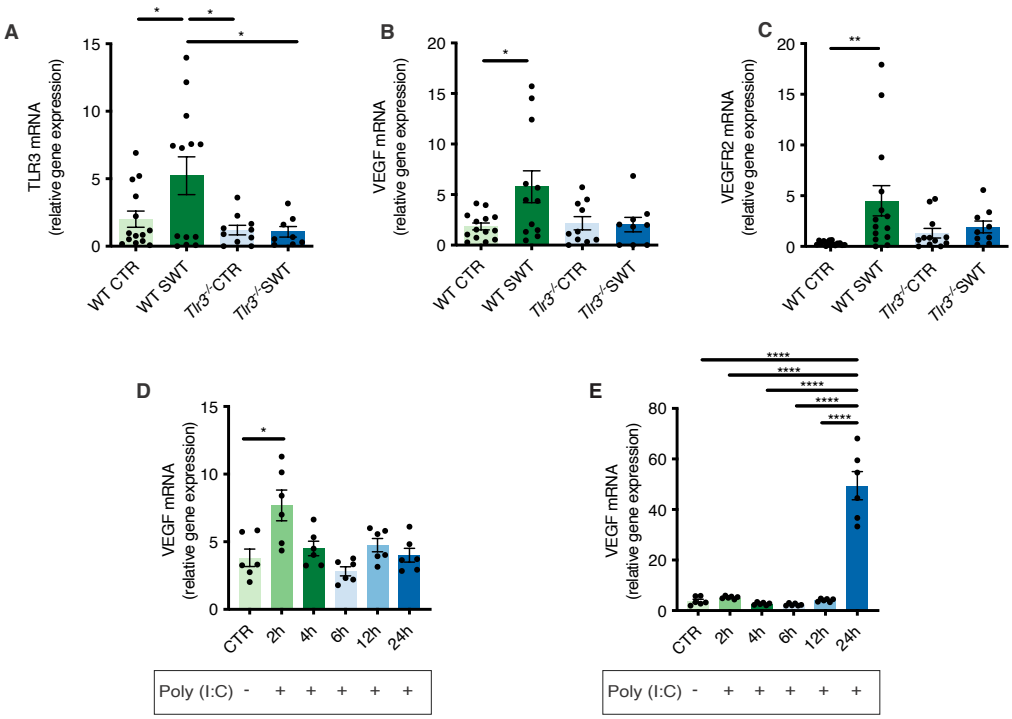
(B) IL-6 regulation after Poly(I:C) treatment. ***p<0.001, ****p<0.0001. n=6.

(C) TNF-α regulation after Poly(I:C) treatment. ****p<0.0001. n=6.

(D) IFN-β regulation after Poly(I:C) treatment. ****p<0.0001. n=5-6.

Statistical comparison: one-way ANOVA with Tukey post hoc analysis

Abbreviations: CTR=control, SWT=shock wave therapy


A
TLR3 mRNA
(relative gene expression)
WT CTR
WT SWT
Tlr3-/-CTR
Tlr3-/-SWT
B
VEGF mRNA
(relative gene expression)
C
VEGFR2 mRNA
(relative gene expression)
D
VEGF mRNA
(relative gene expression)
CTR
2h
4h
6h
12h
24h
Poly (I:C) - + + + + +
E
VEGF mRNA
(relative gene expression)
Poly (I:C) - + + + + +

Supplemental Figure S2: SWT induces angiogenesis after spinal cord injury

(A) Murine spinal cords were subjected to SCI and subsequent SWT. Spinal cord tissue was harvested and analyzed for gene expression. SWT resulted in upregulation of TLR3 expression in WT mice. *p<0.05. n=8-14

(B) SWT resulted in upregulation of VEGF expression in WT but not *Tlr3*$^{-/-}$ mice. *p<0.05. n=9-14

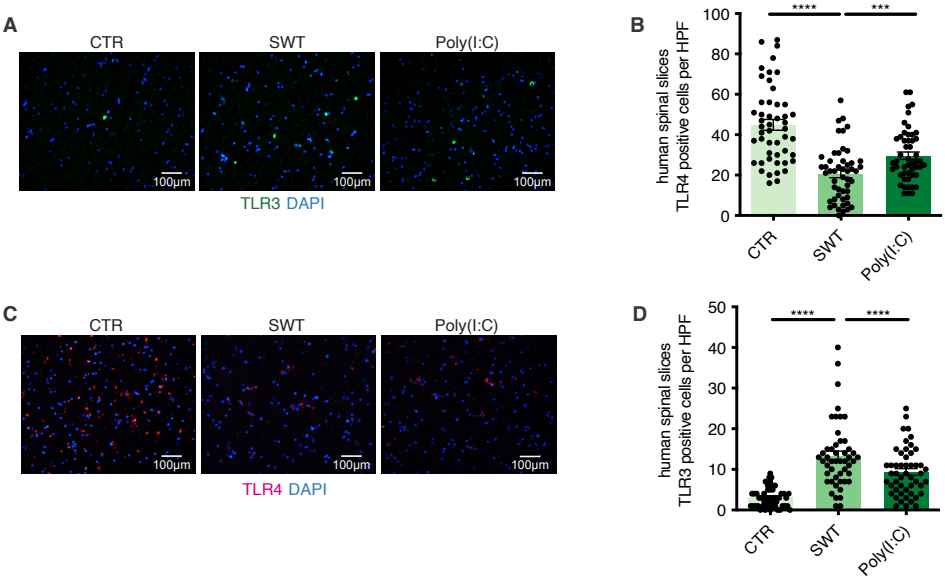
(C) VEGF receptor 2 expression was upregulated after SWT. **p<0.01. n=9-15

(D) SH-SY5Y treated with SWT showed upregulation of VEGF expression. *p<0.05. n=6

(E) SH-SY5Y treated with Poly(I:C) showed upregulation of VEGF expression. *p<0.05. n=6

Statistical comparison: one-way ANOVA with Tukey post hoc analysis

Abbreviations: CTR=control, SWT=shock wave therapy, WT=wild-type


A
CTR
SWT
Poly(I:C)
100µm
TLR3 DAPI
B
human spinal slices
TLR4 positive cells per HPF
****
***
CTR
SWT
Poly(I:C)
C
CTR
SWT
Poly(I:C)
100µm
TLR4 DAPI
D
human spinal slices
TLR3 positive cells per HPF
****
****
CTR
SWT
Poly(I:C)

Supplemental Figure S3: SWT activates TLR3 and downregulates TLR4 in human spinal slices

(A) Immunostaining of TLR3 of human splice cultures treated with SWT or exposed to Poly(I:C).

(B) We found significantly upregulated TLR3 expression in both SWT and Poly(I:C) treated human spinal slices. ****p<0.0001. n=5-6 slices

(C) Immunostaining of TLR4 of human splice cultures treated with SWT or exposed to Poly(I:C).

(D) In parallel, both SWT and Poly (I:C) treatment resulted in down-regulation of TLR4. ****p<0.0001. n=5-6 slices

Statistical comparison: one-way ANOVA with Tukey post hoc analysis

Abbreviations: CTR=control, SWT=shock wave therapy