Supplementary Material

Protective role of activated protein C against viral mimetic poly(I:C)-induced inflammation

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Supplementary Figure Legends

Supplementary Fig. S1, related to Fig. 1. Poly(I:C) induces histone extranuclear translocation in endothelial cells. Immortalized HDMECs were stimulated with poly(I:C) (10 µg/mL) for indicated time points (1, 3, 6 h). Cells were fixed and permeabilized before staining of histone H3 with mouse anti-H3 antibody and Alexa Fluor 488-conjugated goat anti-mouse IgG. The nucleus was stained with DAPI. Immunofluorescence images were obtained with confocal microscopy. Arrows indicate extranuclear translocation of histone H3. Scale bar: 20 µm. Data quantitation is shown as means ± standard error from at least 3 independent experiments. **p < 0.01, ***p < 0.001. Ctrl, control.

Supplementary Fig. S2, related to Fig. 2. EPCR and PAR1 are required for APC inhibition of poly(I:C)-induced histone extranuclear translocation in endothelial cells. HDMECs were pretreated with EPCR or PAR1 function-blocking antibodies (15–20 µg/mL for 1h) followed by treatment with APC (20 nM for 3h) before stimulation with poly(I:C) (10 µg/mL for 1h). Cells were fixed and permeabilized, followed by staining for histone H3 with rabbit anti-histone H3 antibody and Alexa Fluor 488-conjugated goat anti-rabbit IgG. DNA was stained with DAPI. Immunofluorescence images were taken by confocal microscopy. Arrows indicate extranuclear translocation of histone H3. Scale bar: 20 µm. Data quantitation is shown as means ± standard error from at least 3 independent experiments. **p < 0.01. Ctrl, control.
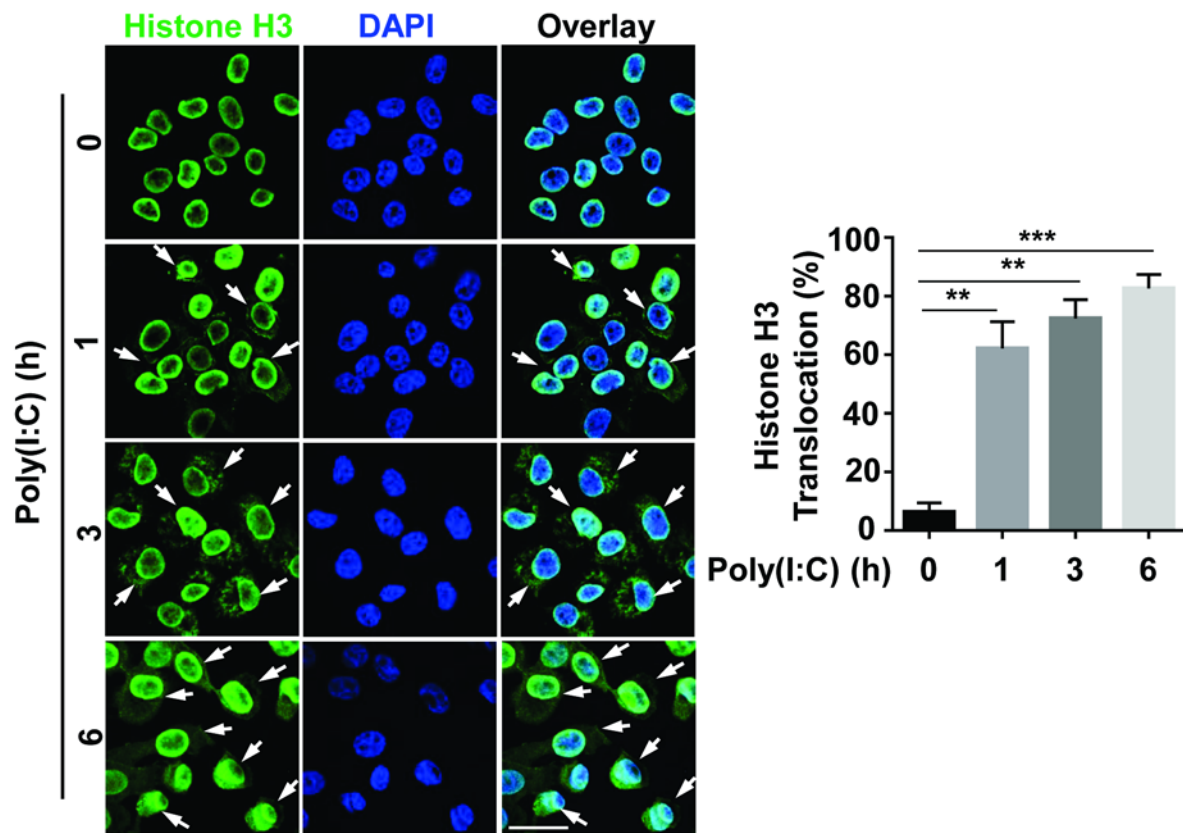
Supplementary Fig. S3, related to Fig. 7. Poly(I:C) elevates integrin Mac-1 expression in neutrophils in mice. Mice were injected i.p. with poly(I:C) (110µg/mouse) for 6 and 24h followed by blood collection and staining for Ly6G-FITC & Mac-1-PE. The cell surface expression of Mac-1 in Ly6G-FITC-positive neutrophil population was measured by the flow cytometry. Representative data were obtained from 3-5 mice per group (n = 3-5).

Supplementary Fig. S4, related to Fig. 7. Poly(I:C) downregulates thrombomodulin expression in mice. Mice were injected i.p. with poly(I:C) (110µg/mouse) for 24h and liver, kidney and lung tissues were collected, processed and stained with rat anti-thrombomodulin antibody and Alexa Fluor 488-conjugated goat anti-rat IgG. The nucleus was stained with DAPI. Immunofluorescence images were taken by confocal microscopy. Representative images were obtained from 3-5 mice per group (n = 3-5). Scale bar: 50 µm.
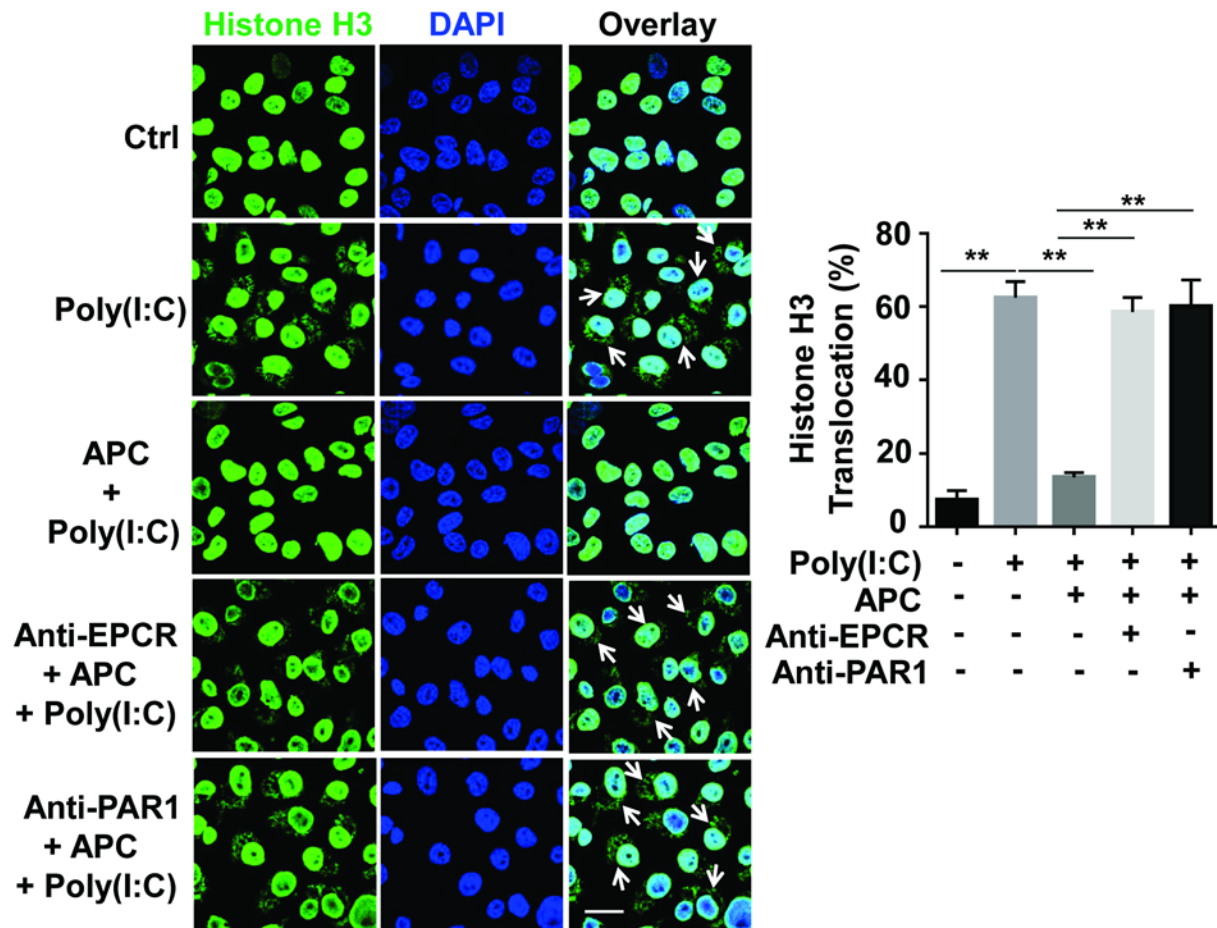
Supplementary Fig. S5, related to Fig. 7. Poly(I:C) does not affect EPCR expression in mice. Mice were injected i.p. with poly(I:C), and lung tissue was harvested for lysis. Tissue lysates were immunoblotted for EPCR and β-actin. The relative expression levels of EPCR are presented.

Supplementary Fig. S6, related to Fig. 8. EPCR and PAR1 are required for APC inhibition of poly(I:C)-induced permeability in endothelial cells. HDMECs were pretreated with EPCR or PAR1 function-blocking antibodies (15–20 µg/mL for 1h) followed by treatment with APC (20 nM for 3h) before stimulation with poly(I:C) (10 µg/mL for 4h). The amount of Evans blue dye that leaked into the lower chamber in the Trans-well assay plates was measured. Results are shown as means ± standard error from at least 3 independent experiments. $*p < 0.05$, $**p < 0.01$.
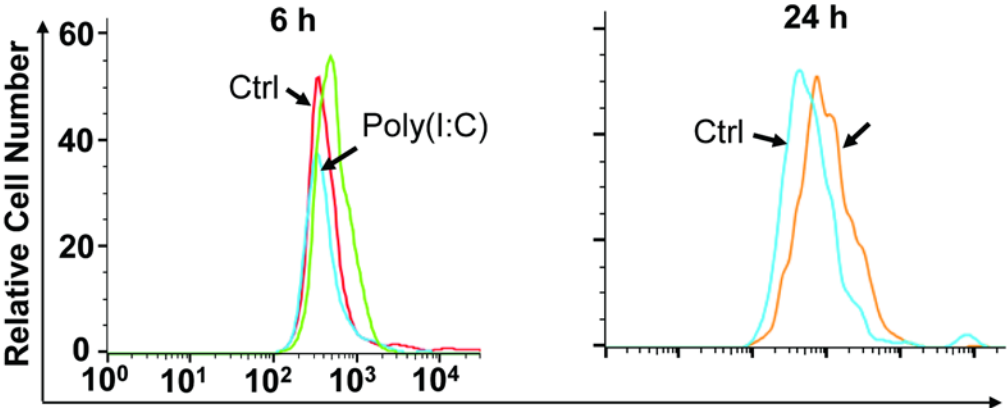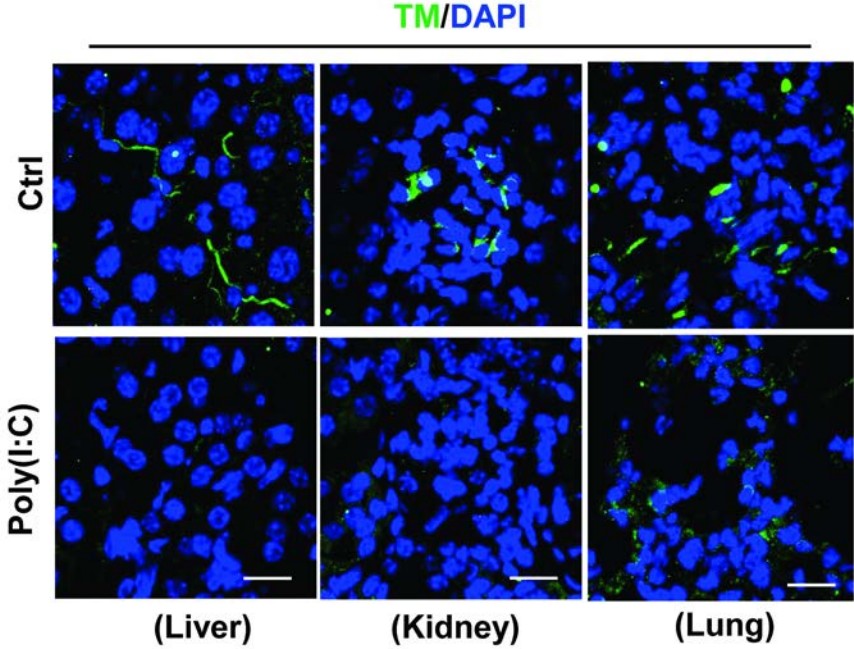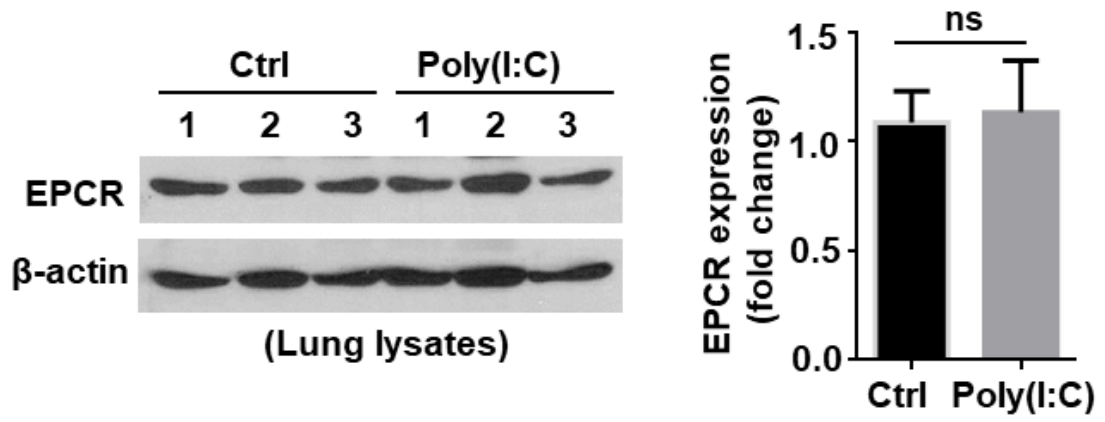
Supplementary Figure S1, related to Fig. 1

Supplementary Figure S2, related to Fig. 2

Supplementary Figure S3, related to Fig. 7

Supplementary Figure S4, related to Fig. 7

(Liver) (Kidney) (Lung)

Supplementary Figure S5, related to Fig. 7

Supplementary Figure S6, related to Fig. 8

Poly(I:C)	-	+	+	+	+
APC	-	-	+	+	+
Anti-EPCR	-	-	-	+	-
Anti-PAR1	-	-	-	-	+

(HDMEC)