





## Correction to: EGFR signaling augments TLR4 cell surface expression and function in macrophages via regulation of Rab5a activation

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## **CORRECTION TO: PROTEIN CELL**

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In the original publication the bands in Fig. 1J and Fig. 2B were not visible. The correct versions of Fig. 1J and Fig. 2B are provided in this correction.

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J <u>WT <i>EGF</i>R⁻/⁻</u>		
		P-TLR4
		T-TLR4
		GAPDH
I PS	- + - +	

Figure 1. EGFR activation promotes TLR4 phosphorylation and cell surface expression of TLR4 in response to LPS. (A and B) BMDM were treated with LPS (1 µg/mL) for 6, 12, or 24 h in the presence or absence of pretreatment of PD or TAPI-1. (A) Flow cytometry analysis of cell surface TLR4 intensity in BMDM. (B) Flow cytometry analysis of cell surface TLR4 intensity in BMDM. (C and D) WT (C57BL/6) mice were treated with LPS (10 mg/kg, i.p.). In some groups, mice were pretreated with erlotinib (100 mg/kg, gavage administration) at 30 min prior to LPS i.p. Peritoneal lavage fluids were collected at 24 h after LPS treatment and peritoneal macrophages were identified with F4/80. TLR4 intensity on the surface of peritoneal macrophage was analyzed by flow cytometry. (E and F) BMDM isolated from WT and EGFR<sup>-/-</sup> mice were treated with LPS (1 µg/mL) in vitro for 1 h followed by flow cytometry analysis of cell surface TLR4 intensity. (G and H) WT (C57BL/6) and EGFR-/- mice were treated with LPS (10 mg/kg, i.p.) for 24 h. Peritoneal lavage fluids were collected, and peritoneal macrophages were identified with F4/80. TLR4 intensity on the surface of peritoneal macrophage was analyzed by flow cytometry. (I) Western blot analysis of phosphor-TLR4 in BMDM treated with LPS (1 µg/ mL) for 30 min with or without PD168393 (PD, 10 µmol/L) pretreatment for 30 min. (J) Western blot analysis of phosphor-TLR4 in  $EGFR^{-/-}$  BMDM treated with LPS (1 µg/mL) for 30 min. (K-N) HEK293 cells were transfected with TLR4, MD2, CD14, EGFR, or TLR4 mutant for 48 h, with treatment of LPS (1 µg/mL) for 30 min or 24 h. (K) Diagram of the TLR4 phosphorylation site mutated plasmid. (L) Western blot analysis of the phosphor-TLR4 and phosphor-EGFR in transfected HEK293 treated with LPS for 30 min. (M and N) Flow cytometry analysis of cell surface TLR4 intensity in transfected HEK293 treated with LPS for 24 h. (O and P) BMDM were treated with LPS (1 µg/mL) for 30 min with or without PD168393 (PD) pretreatment for 30 min. (O) Immune-staining of TLR4 and EGFR in BMDM. (P) Coimmunoprecipitation of TLR4 with EGFR in BMDM. (Q) Immunestaining of TLR4 and GM130 in BMDM treated with LPS (1 µg/ mL) for 24 h with or without PD168393 pretreatment for 30 min. (R) Immune-staining of TLR4 and GM130 in EGFR<sup>-/-</sup> BMDM treated with LPS (1 µg/mL) for 24 h. All images and flow cytometric plots are the representatives from at least 4 experiments. The graphs depict mean ± SD of four to six experiments or mice. \*P < 0.05 as compared with control group; †P < 0.05 as compared with the time-matched LPS alone group.



Figure 2. Rab5a-mediated concurrent internalization of TLR4 and EGFR results increased cell surface expression of the receptors. (A and B) BMDM were treated with LPS for 6, 12, or 24 h in the presence or absence of pretreatment of PD168393 (PD) for 30 min. (A) Real time PCR analysis of Rab5a expression. (B) Western blot analysis of Rab5a expression. (C and D) BMDM transfected with si-NC and si-Rab5a for 48 h were treated with LPS (1 µg/mL) for 24 h. Flow cytometry analysis of cell surface TLR4. (E-H) BMDM were treated with LPS (1 µg/ mL) for 1 h or 24 h, with or without clathrin inhibitor chlorpromazine (CPZ 12.5 µmol/L) or PD168393 (PD 10 µmol/L) pretreatment for 30 min. Flow cytometry analysis of cell surface TLR4 at 24 h or after LPS. (I and J) BMDM cells transfected with si-NC or si-Rab5a for 48 h followed by LPS treatment (1 µg/mL) for 1 h. Flow cytometry analysis of cell surface TLR4. (K and L) WT and Rab5a<sup>-/-</sup> BMDM were treated with LPS (1 µg/mL) for 1 h or 24 h. Flow cytometry analysis of cell surface TLR4 at 1 h or 24 h after LPS. (M–O) BMDM were treated with LPS (1 µg/mL) for 1 h with or without PD168393 (PD) pretreatment for 30 min. (M) Immune-staining of TLR4 and EEA1 in BMDM. (N) Immune-staining of TLR4 with Rab5a. (O) Co-immunoprecipitation between TLR4 and Rab5a in BMDM. All flow cytometric plots are the representative from at least 4 experiments. The graphs depict mean ± SD of four to six experiments or mice. \*P < 0.05 as compared with control group; +P < 0.05 as compared with the time-matched LPS alone group.

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