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Supplementary Materials for

Epidermal Growth Factor Receptor Is Essential for Toll-Like Receptor 3 Signaling

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Other Supplementary Material for this manuscript includes the following:
(available at www.sciencesignaling.org/cgi/content/full/5/233/ra50/DC1)

Table S1 (Microsoft Excel format). Microarray analysis of TLR3 signaling–mediated gene induction in wild-type and EGFR-expressing MDA-MB-453 cells.

FIGURE S1

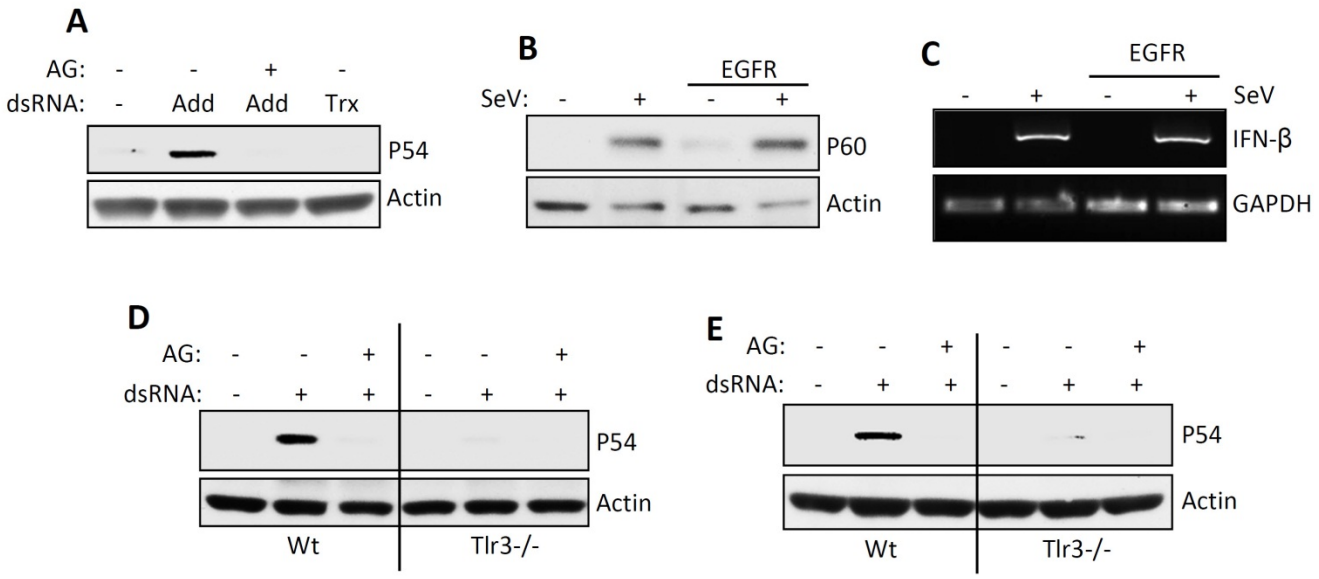

Fig. S1. EGFR is essential for TLR3 signaling, but not RIG-I signaling. (A) IPS-$1^{-/-}$ MEFs were treated (Add) or transfected (Trx) with dsRNA in the absence or the presence of AG1478, and induction of P54 was analyzed by Western Blot. **(B)** Parental or EGFR-reconstituted MDA-MB-453 cells were infected with SeV (at MOI:10), and induction of P60 was analyzed after 8 hours by Western Blot. **(C)** RT-PCR for IFN-β and GAPDH mRNAs from parental or EGFR-reconstituted MDA-MB-453 cells before and after SeV infection (MOI: 10). **(D, E)** Primary BMDMs (D) or BMDCs (E) from WT or $TLR3^{-/-}$ mice were treated with dsRNA in the absence or the presence of AG1478. 6 hours post treatment cell lysates were analyzed for P54 induction by Western blot. Data are representative of at elast two independent experiments.

FIGURE S2

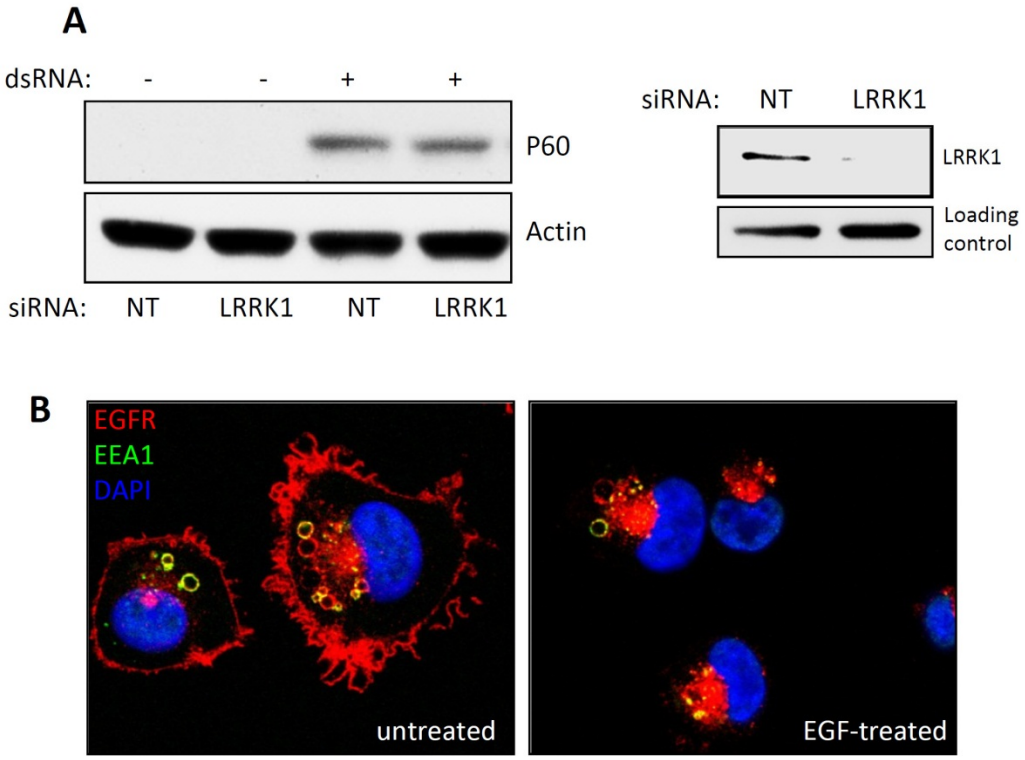

Fig. S2. LRRK1 is not required for TLR3 signaling or the constitutive early endosomal localization of EGFR. HT1080 cells were transfected with non-targeting (NT) or LRRK1 specific siRNA. 48 hours post transfection the cells were split and used for the following experiments. **(A)** Cells were treated with dsRNA, induction of P60 was analyzed by Western blot (left), and LRRK1 knockdown was confirmed by Western blot (right). **(B)** The cells were left untreated or treated with EGF (50 ng/ml) for 30 min, immunolabeled with antibodies against EGFR and EEA1, and analyzed by confocal microscopy. Overlay images are shown. Data are representative of at least two independent experiments.

FIGURE S3

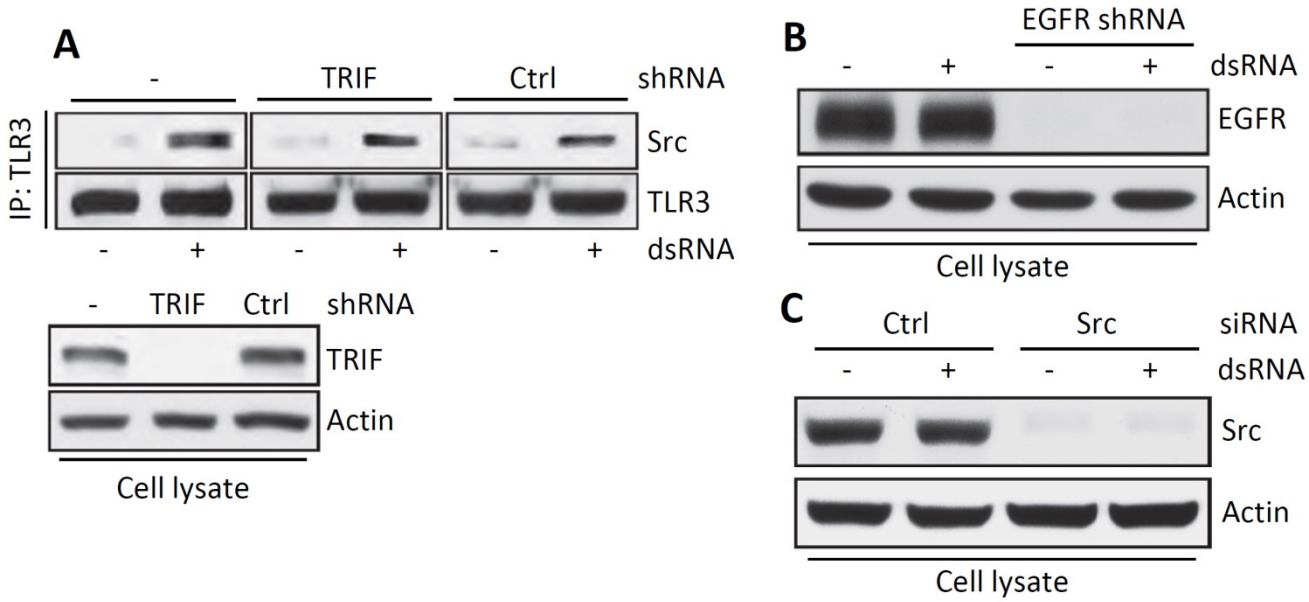

Fig. S3. Src interaction with TLR3 is independent of TRIF, but dependent on EGFR. (A) TRIF knockdown Wt11 cells (as described in Fig. 3D) were treated with dsRNA, cell lysates were immunoprecipitated with anti-Flag antibody, and immunoprecipitates were analyzed for Src by Western blot (upper panel). Cell lysates were analyzed for TRIF and actin by Western blot (lower panel). **(B)** TLR3-reconstituted TLR3$^{-/-}$ MEFs were lentivirally transduced with EGFR-specific shRNA and treated with dsRNA. Cell lysates were analyzed for EGFR and actin by Western Blot. **(C)** Wt11 cells were transfected with Src-specific or a control (ctrl) siRNA. 72 hours post transfection the cell lysates were analyzed for Src and actin by Western Blot. Blots shown here are representative of three independent experiments.

FIGURE S4

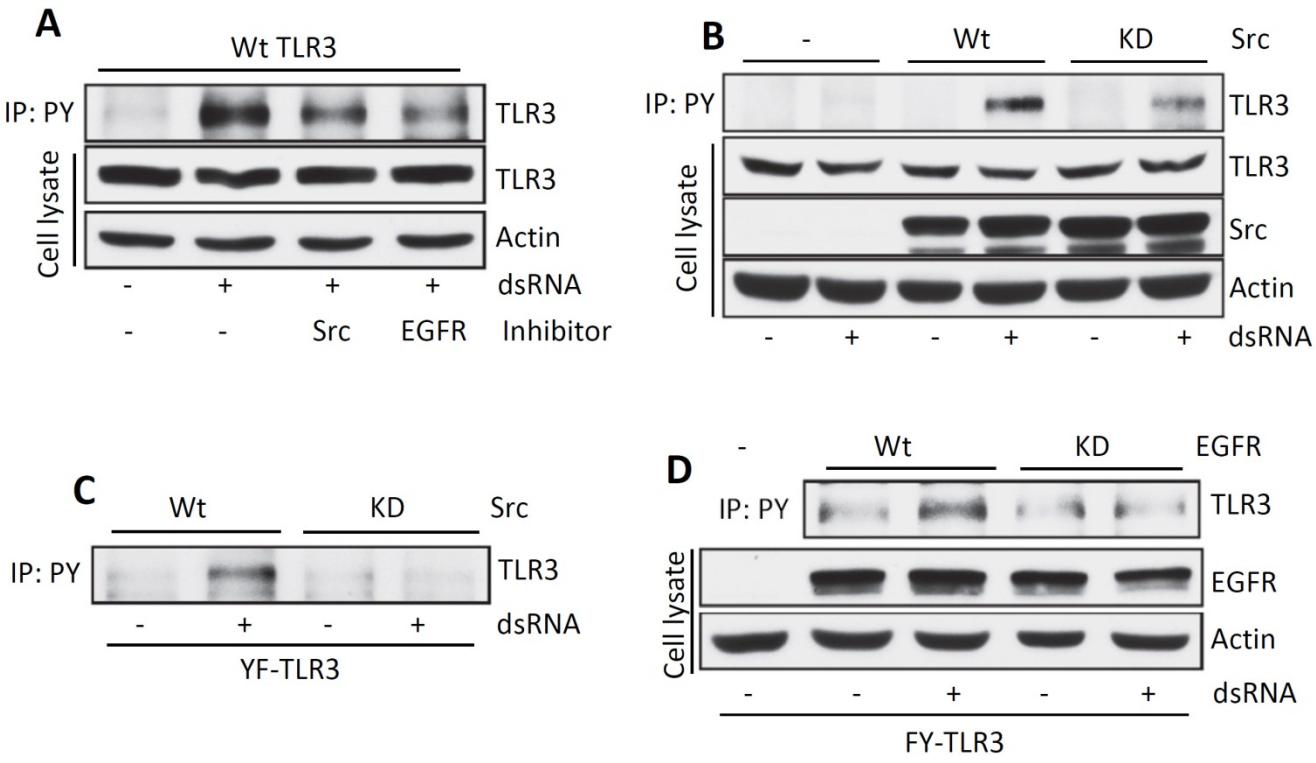

Fig. S4. The protein tyrosine kinase activities of Src and EGFR are required for phosphorylation of Tyr^{759} and Tyr^{858} in TLR3. (A) Wt11 cells were treated with dsRNA in the absence or the presence of Src inhibitor (PP2) or EGFR inhibitor (AG1478). Anti-P-Tyr (PY) immunoprecipitates were analyzed for TLR3 by Western Blot. Cell lysates were analyzed for TLR3 (Flag) and actin by Western Blot. **(B)** TLR3-expressing SYF$^{-/-}$ MEFs reconstituted with WT or kinase dead (KD) Src and treated with dsRNA. Anti-P-Tyr (PY) immunoprecipitates were analyzed for TLR3 by Western blot. Cell lysates were analyzed for TLR3 (Flag), Src and actin by Western Blot. **(C)** YF-TLR3-expressing SYF$^{-/-}$ MEFs reconstituted with WT or Kinase dead (KD) Src, were treated with dsRNA, anti-P-Tyr (PY) immunoprecipitates were analyzed for TLR3 by Western blot. **(D)** FY-TLR3-expressing 453 cells reconstituted with WT or kinase dead (KD) EGFR (K721R) were treated with dsRNA. Anti-P-Tyr (PY) immunoprecipitates were analyzed for TLR3 by Western Blot. Cell lysates were analyzed for EGFR and actin by Western blot. Blots shown here are representative of at least two independent experiments.