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Supplementary Materials for

MerTK signaling in macrophages promotes the synthesis of inflammation resolution mediators by suppressing CaMKII activity

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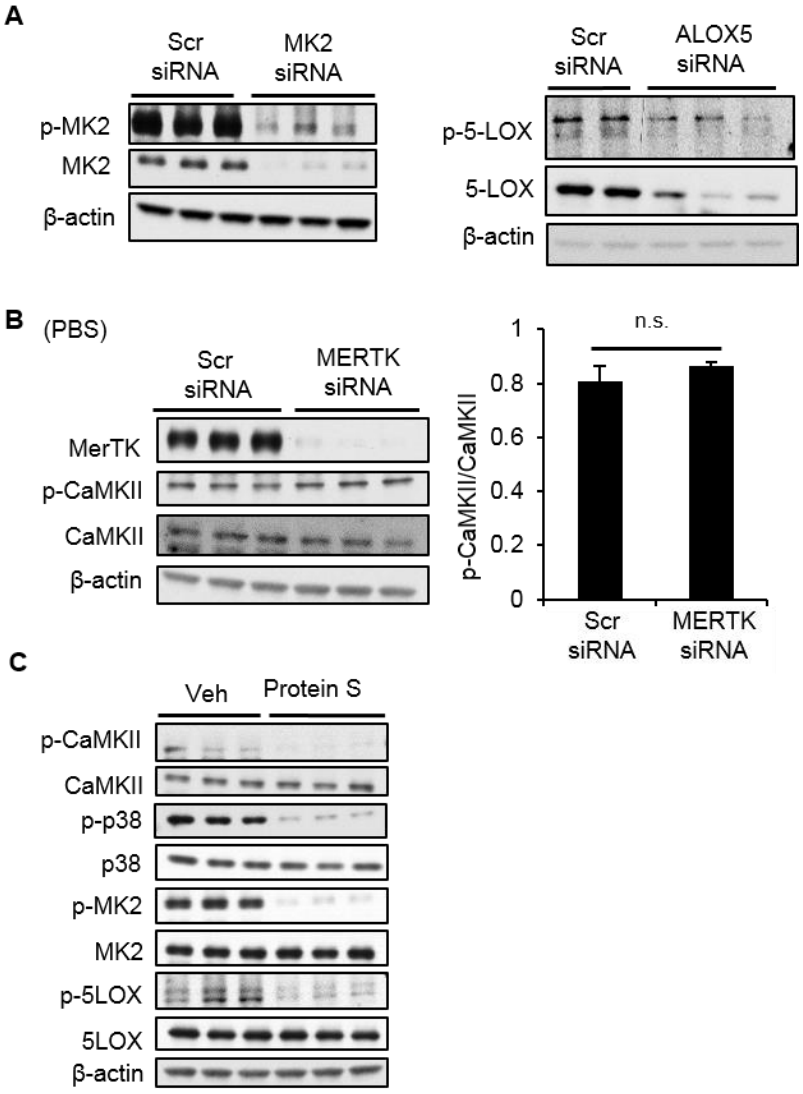

Fig. S1. Validation of MK2 and 5-LOX antibodies with siRNAs; MerTK deletion does not alter CaMKII activity in the absence of Gas6; and protein S can suppress the CaMKII pathway. (**A**) Human macrophages were transfected with scrambled siRNA, MK2-specific siRNA, or ALOX5-specific siRNA. After 72 hours, the cells were lysed and analyzed by Western blotting with antibodies against phospho- (p-) and total MK2 and 5-LOX. β-actin was used as a loading control. Data are from two or three different blood donors. (**B**) Human macrophages were transfected with scrambled siRNA or MERTK siRNA. Left: After 72 hours, cell lysates were analyzed by Western blotting with antibodies against p- and total CaMKII and for β-actin. Right: The ratio of p-CaMKII:total CaMKII was quantified by densitometry. Data are means ± SEM of three different donors. n.s., not significant by two-tailed Student's *t* test. (**C**) Human macrophages were treated with 100 nM protein S or vehicle control. After 7 hours, the cell lysates were analyzed by Western blotting with antibodies against for phosphorylated and total CaMKII, p38, MK2, and 5-LOX; β-actin was used as a loading control. Western blots show data from three different donors.

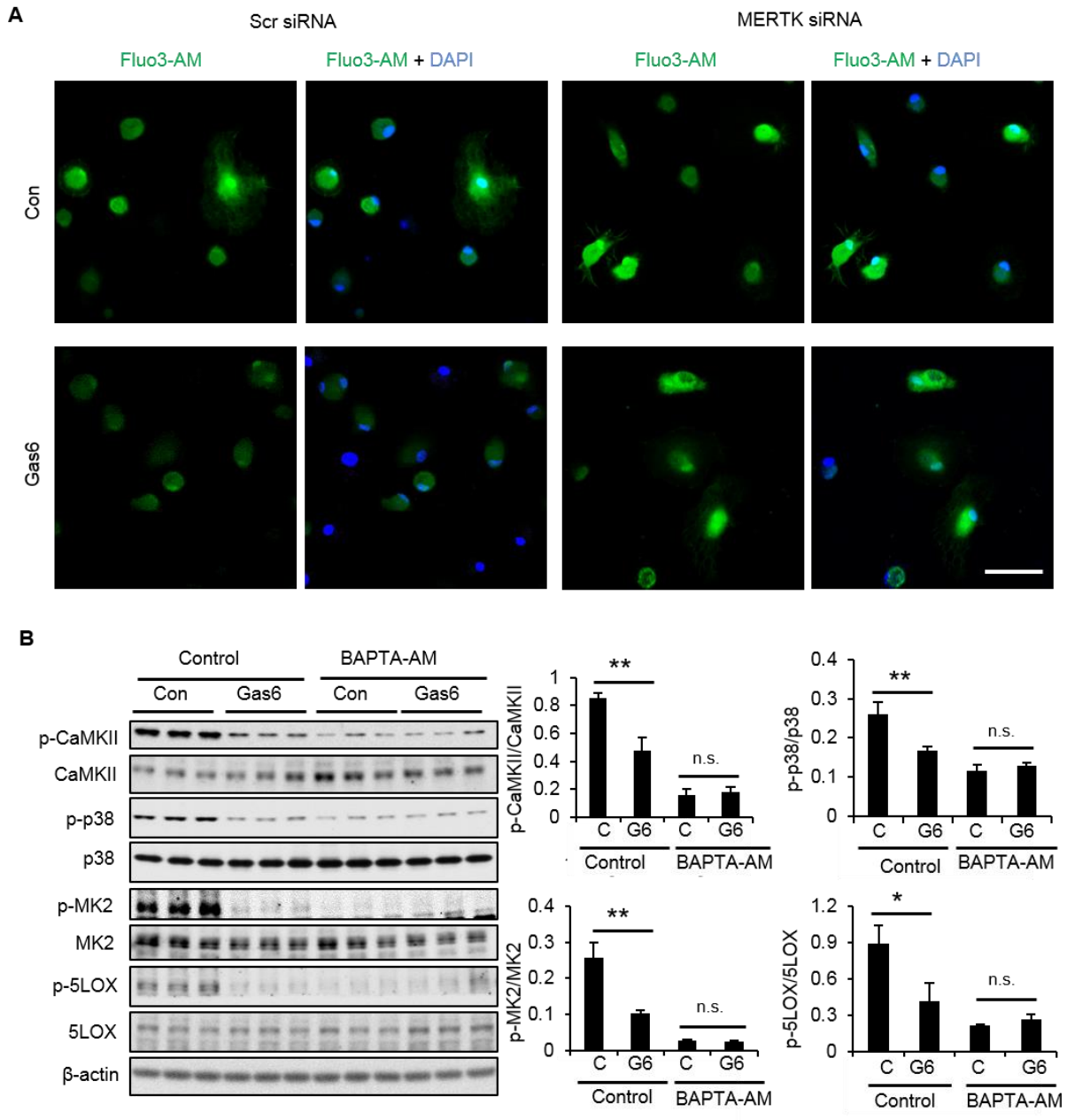

Fig. S2. Gas6 reduces cytosolic Ca^{2+} in a MerTK-dependent manner and BAPTA-AM suppresses CaMKII–p38–MK2–5-LOX signaling. (**A**) Human macrophages were transfected with scrambled siRNA or MERTK-specific siRNA. After 72 hours, the cells were treated with control (Con) or Gas6-conditioned medium for 7 hours, loaded with Fluo3-AM (green), counterstained with DAPI to view nuclei (blue), and viewed by confocal microscopy. Scale bar, 50 μm. (**B**) Macrophages were pre-treated with 5 μM BAPTA-AM for 10 minutes. The cells were then incubated with control or Gas6-conditioned medium for 7 hours. Left: Cell lysates were then analyzed by Western blotting with antibodies against p- and total CaMKII, p38, MK2, and 5-LOX and for β-actin. Right: The ratios of the amounts of the indicated phosphorylated to total proteins were quantified by densitometry. Data are means ± SEM of three different donors. *$P < 0.05$, **$P < 0.01$ *vs.* control medium by one-way ANOVA with post-hoc *t* tests for group comparisons. n.s., not significant.

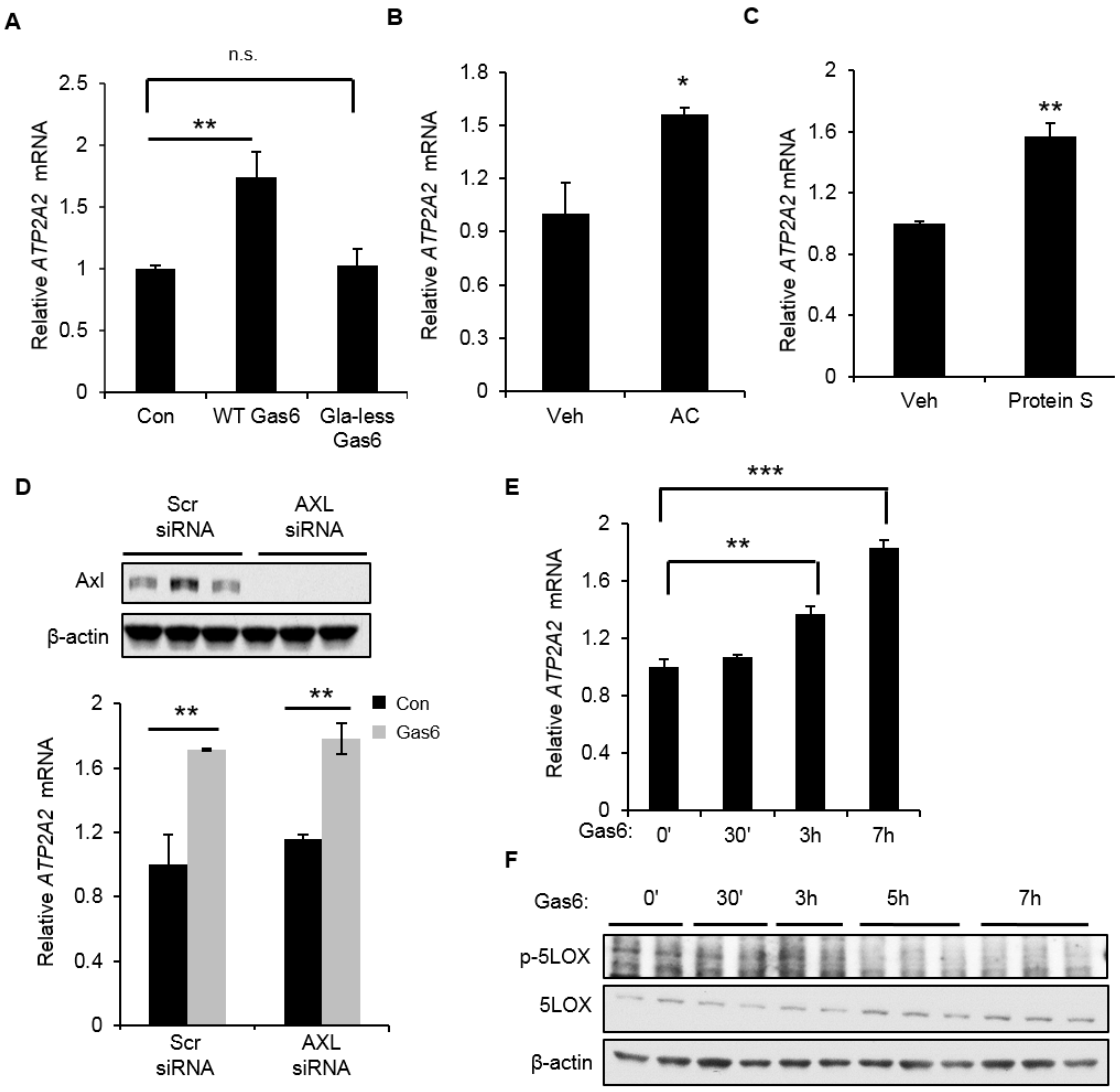

Fig. S3. Further studies related to MerTK ligands and demonstration that Axl does not mediate Gas6-induced *ATP2A2* expression. (**A**) Human macrophages were incubated for 7 hours with control medium or conditioned medium containing 10 nM Gas6 or 10 nM Gla-less Gas6. Cells were then analyzed by real-time qPCR to determine the relative abundance of *ATP2A2* mRNA. Data are means ± SEM of three different donors. $^{**}P < 0.01$ *vs.* control medium by one-way ANOVA with post-hoc *t* tests for group comparisons; n.s., not significant. (**B**) Macrophages were incubated for 7 hours with apoptotic Jurkat cells (AC) at a ratio of 1:5 or vehicle control (Veh) and then analyzed by real-time qPCR to determine the relative abundance of *ATP2A2* mRNA. (**C**) Macrophages were incubated for 7 hours with 100 nM protein S or vehicle control and then analyzed by real-time qPCR to determine the relative abundance of *ATP2A2* mRNA. Data in (B) and (C) are means ± SEM of three different donors. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* vehicle by two-tailed Student's *t* test. (**D**) Macrophages were transfected with scrambled RNA or AXL-specific siRNA. After 72 hours, the cells were lysed and analyzed by Western blotting to detect Axl (top) or incubated with control medium conditioned medium containing10 nM Gas6, followed by measurement of *ATP2A2* mRNA. Data are means ± SEM of three different donors. $^{**}P < 0.01$ *vs.* control medium by one-way ANOVA with post-hoc *t* tests for group comparisons. (**E** and **F**) Macrophages were incubated with Gas6-conditioned medium for the indicated times. Cells were analyzed by real-time qPCR to determine *ATP2A2* mRNA abundance (top) or analyzed by Western blotting for phosphorylated and total 5-LOX. Data in (E) are means ± SEM of three different donors. $^{**}P < 0.01$, $^{***}P < 0.001$ *vs.* time zero by one-way ANOVA with post-hoc *t* tests for group comparisons.

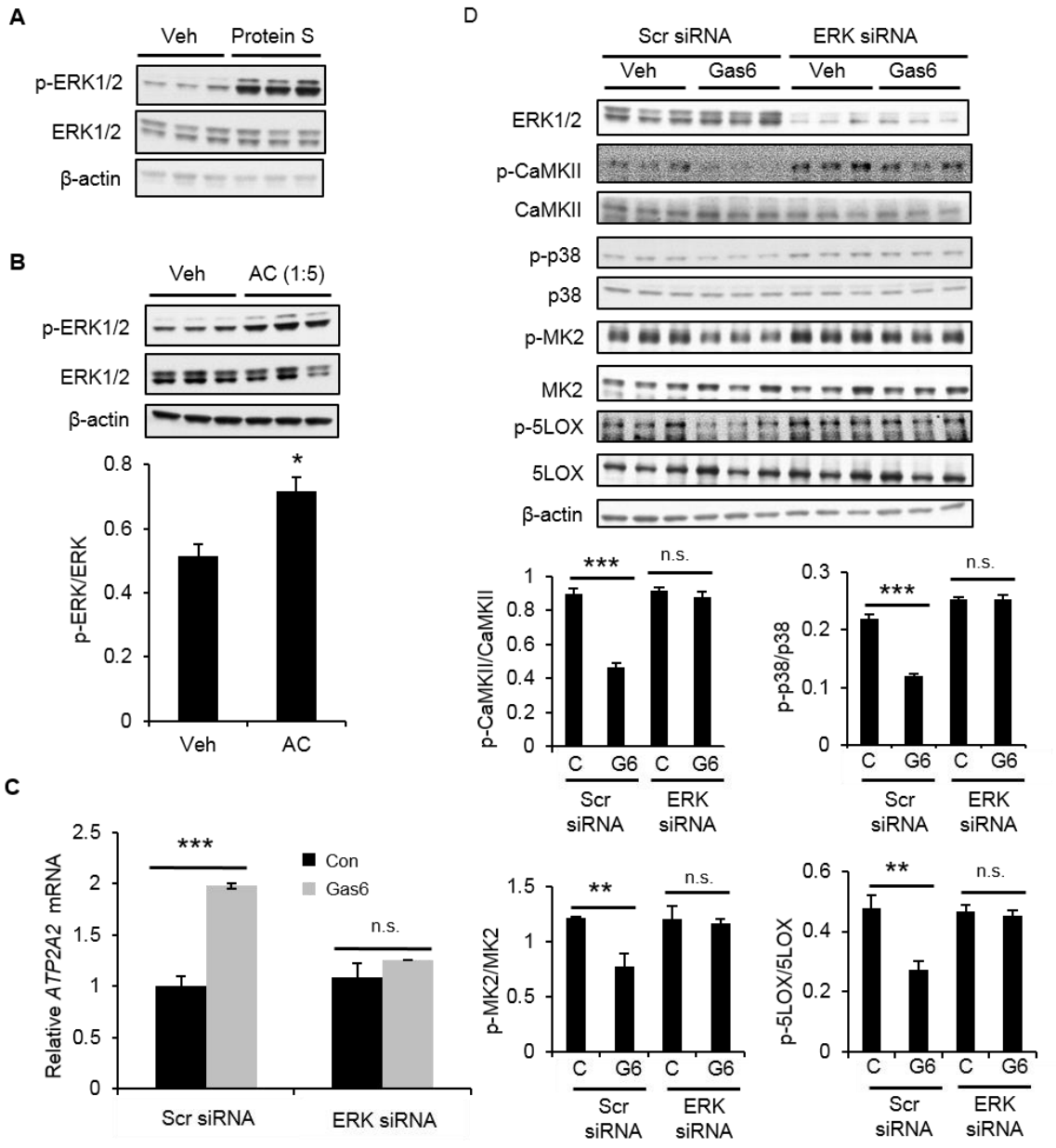

Fig. S4. Protein S and apoptotic cells activate ERK1/2 in macrophages, and ERK1/2 is required for Gas6-mediated suppression of CaMKII–p38–MK2–5-LOX signaling. (**A**) Human macrophages were treated with protein S or vehicle for 30 min. Cells were lysed and analyzed by Western blotting for pERK1/2- and total ERK1/2. Data are from three different donors. (**B**) Macrophages were incubated for 30 min with apoptotic Jurkat cells (AC) at a ratio of 1:5. The ACs were removed by rinsing with cold PBS. Top: The macrophages were lysed and analyzed by Western blotting for p-ERK1/2, ERK1/2, and β-actin. Bottom: The ratio of amounts of p-ERK1/2: total ERK1/2 was quantified by densitometry. Data are means ± SEM of three different donors. **P* < 0.05 *vs.* vehicle by two-tailed Student's *t* test. (**C** and **D**) Macrophages were transfected with scrambled siRNA or ERK-specific siRNA. After 72 hours, the cells were incubated for 7 hours with control or Gas6-conditioned medium. The cells were then assayed by real-time qPCR to determine relative *ATP2A2* mRNA abundance (C) or by Western blotting analysis for the indicated phosphorylated and total proteins (D). Bottom: The ratios of the amounts of the indicated proteins were quantified by densitometry. Data are means ± SEM of three different donors. ***P* < 0.01, ****P* < 0.001 *vs.* control medium by one-way ANOVA with post-hoc *t* tests for group comparisons; n.s., not significant.

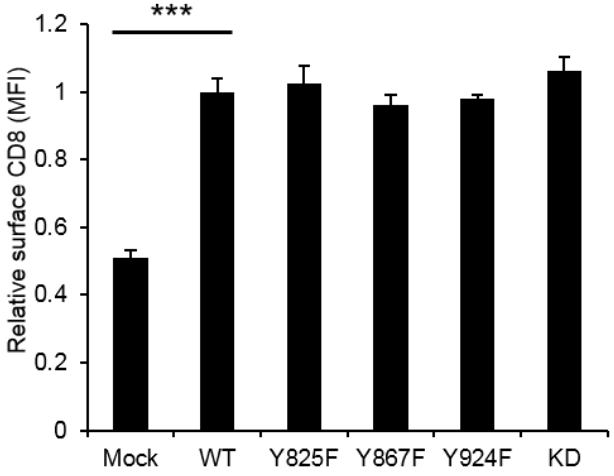

Fig. S5. Detection of the cell surface expression of CDMer proteins in transfected HEK 293 cells. The cell surface expression of CDMer in the same groups of transfected cells described in Fig. 4A was assessed by flow cytometry with an anti-CD8 antibody. Data are means ± SEM of 3 experiments. ***$P < 0.001$ *vs.* cells subjected to mock transfection without plasmid by one-way ANOVA with post-hoc *t* tests for group comparisons.

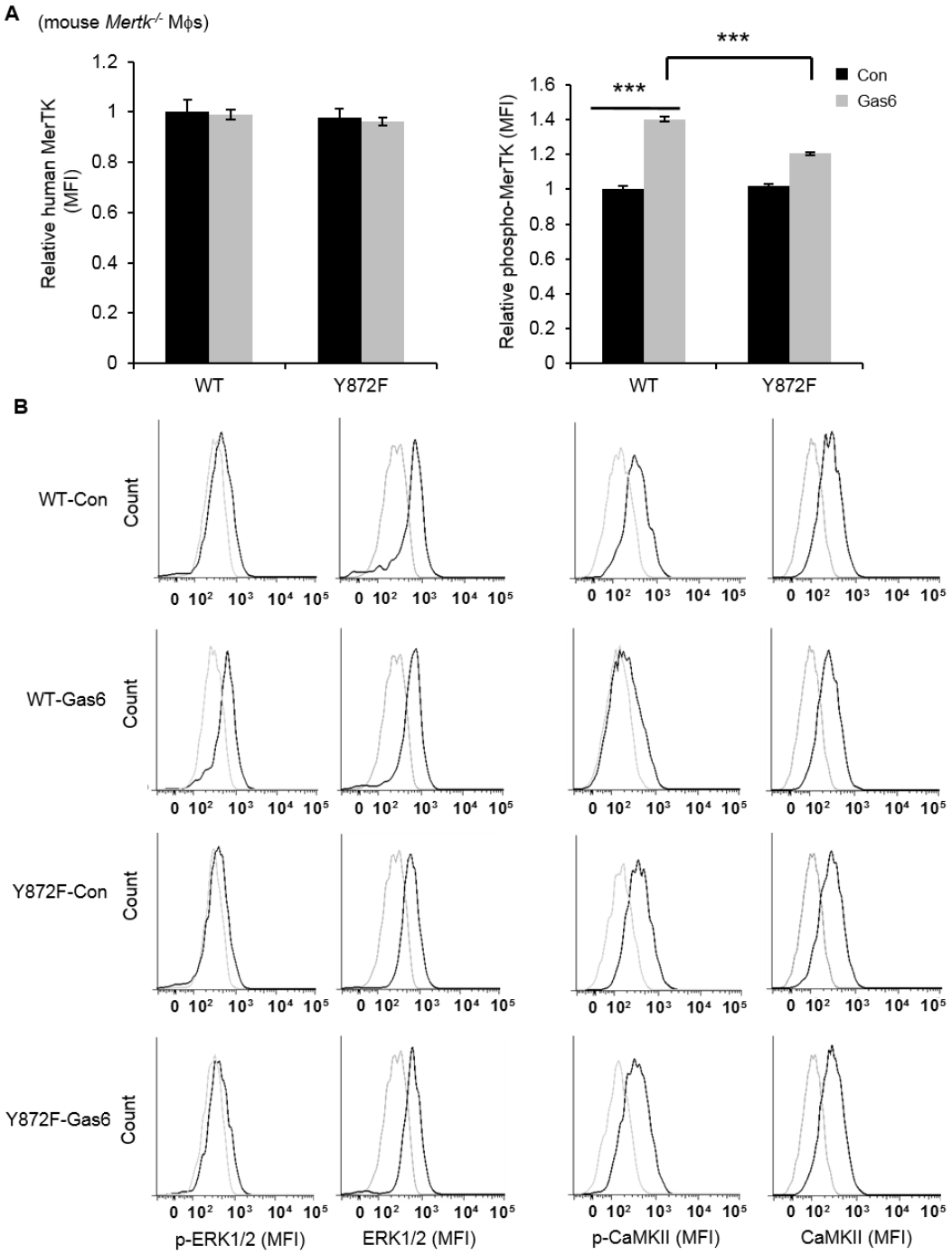

Fig. S6. Tyr^{872} in the cytoplasmic tail of human MerTK is required for the activation of ERK1/2 and CaMKII. (**A**) Representative flow diagrams for the experiment shown in Fig. 4B. Gray lines indicate the control IgG signal (background), and black lines indicate the signals for anti-p-ERK1/2, anti-total ERK1/2, anti-p-CaMKII, and anti-total CaMKII, as indicated. (**B**) *Mertk*$^{-/-}$ mouse macrophages transduced with retroviruses expressing human WT and Y872F MerTK from the experiment in Fig. 4B were assayed by flow cytometry to determine the cell surface expression of MerTK and the abundance of intracellular p-MerTK. Data are means ± SEM of three different mice. ***$P < 0.001$ *vs.* control medium–treated WT-expressing cells or Gas6-treated WT-expressing cells by one-way ANOVA with post-hoc *t* tests for group comparisons.

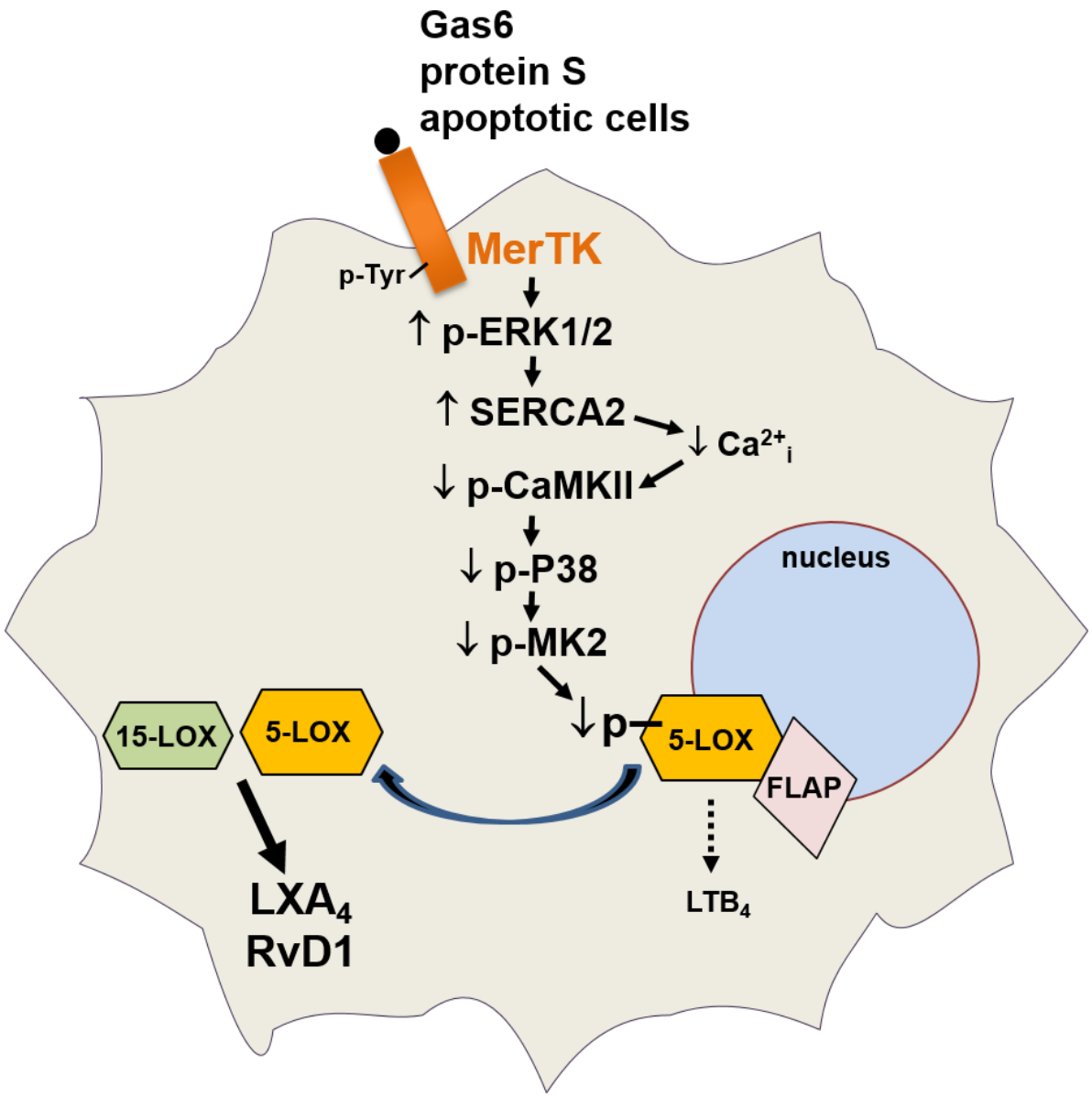

Fig. S7. Summary scheme of MerTK-mediated resolution signaling. MerTK activation by Gas6, protein S, or apoptotic cells, through a process requiring certain tyrosine residues in the cytoplasmic tail of MerTK, activates ERK, which is followed by the induction of *ATP2A2* expression. The decrease in the concentration of intracellular Ca^{2+} (Ca^{2+}_i) caused by the increased SERCA abundance suppresses CaMKII activity, which then reduces the activities of the kinases p38 and MK2. The decrease in MK2 activity (i) reduces the abundance of nuclear 5-LOX, which together with 5-LOX–activating protein (FLAP) catalyzes the formation of LTB_4; and (ii) increases the abundance of nonphosphorylated, cytoplasmic 5-LOX, which, in concert with 15-LOX (12/15-LOX in mice), leads to an increase in the abundance of LXA_4, RvD1, or both. Thus, MerTK activation increases the ratio of LXA_4/RvD1 to LTB_4, which favors resolution over inflammation.