Grimsey et al., http://www.jcb.org/cgi/content/full/jcb.201504007/DC1

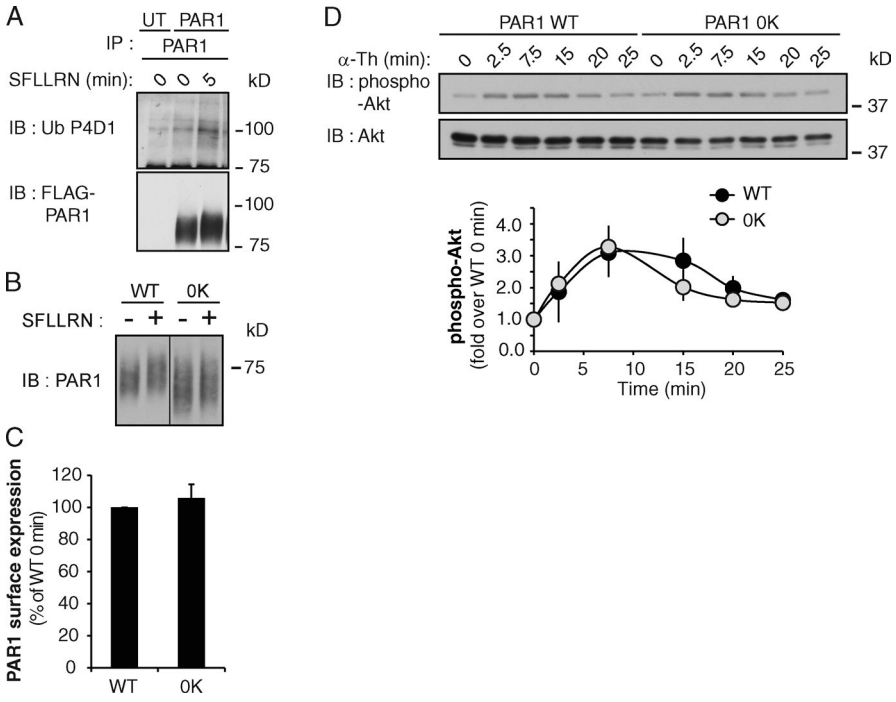

Figure S1. **PAR1 ubiquitination, expression, and Akt signaling.** (A) PAR1 WT or untransfected (UT) HeLa cells were stimulated with 100 µM SFLLRN. Cells were lysed in 1% SDS solution, boiled for 5 min, and immunoprecipitated, and PAR1 ubiquitination was determined. (B) PAR1 WT and 0K mobility on SDS-PAGE after stimulation with 100 µM SFLLRN for 5 min. (C) PAR1 WT and 0K surface expression in HeLa cells. (D) PAR1 WT and 0K HeLa cells were stimulated with 10 nM α-Th, and Akt phosphorylation was determined. The data (mean ± SD [error bars], $n = 3$) were analyzed using a Student's t test.

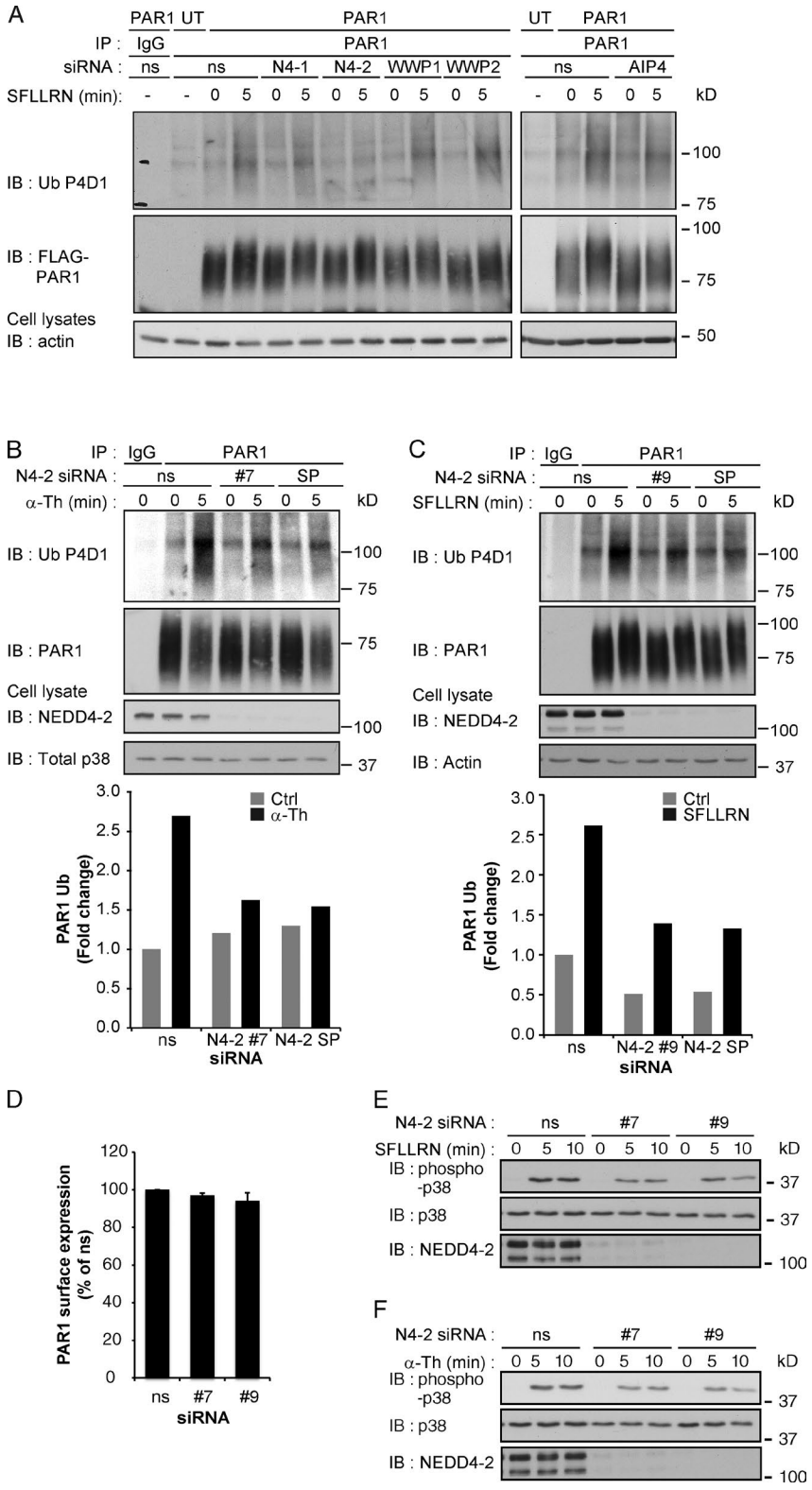

Figure S2. **NEDD4-2 mediates agonist-induced PAR1 ubiquitination.** (A) PAR1 WT or untransfected (UT) HeLa cells were transiently transfected with ns, AIP4, NEDD4-1 (N4-1), NEDD4-2 (N4-2), WWP1, or WWP2 siRNA. Cells were stimulated with 100 µM SFLLRN, lysed, and immunoprecipitated, then ubiquitination of PAR1 was detected. (B) PAR1 HeLa cells were transiently transfected with ns or N4-2 siRNA #7 or N4-2 SMARTpool (SP) siRNA. Cells were stimulated with 10 nM α-Th, lysed, and immunoprecipitated, then ubiquitination of PAR1 was detected. The bar graph shows quantification of PAR1 ubiquitination from a single representative experiment (n = 2). (C) PAR1 HeLa cells were transiently transfected with ns or N4-2 siRNA #9 or SMARTpool (SP) siRNA. Cells were stimulated with 100 µM SFLLRN, lysed, and immunoprecipitated, then ubiquitination of PAR1 was detected. The bar graph shows quantification of PAR1 ubiquitination from a single representative experiment (n = 2). (D) PAR1 surface expression was detected HeLa cells transfected with ns, N4-2 #7, or N4.2 #9 siRNA. The data (mean ± SD [error bars], n = 3) were analyzed using a Student's t test. HeLa cells from D were stimulated with either 100 µM SFLLRN (E) or 10 nM α-Th (F), and phosphorylation of p38 was detected.

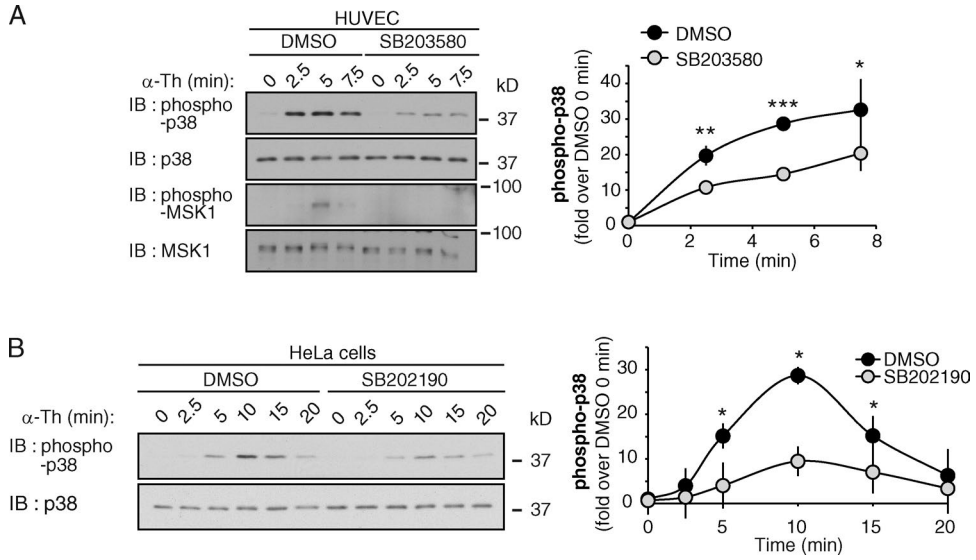

Figure S3. **Thrombin stimulates p38 autophosphorylation in endothelial and HeLa cells.** (A) HUVECs pretreated with DMSO or 5 µM SB203580 for 30 min were stimulated with 10 nM α-Th, and p38 and MSK1 phosphorylation was detected. The data (mean ± SD [error bars], $n = 3$) were analyzed using a Student's t test (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). (B) PAR1 HeLa cells pretreated with DMSO or 50 µM SB202910 for 20 min were stimulated with 10 nM α-Th, and phosphorylation of p38 was determined. The data (mean ± SD [error bars], $n = 3$) were analyzed using a Student's t test (*, $P < 0.05$).

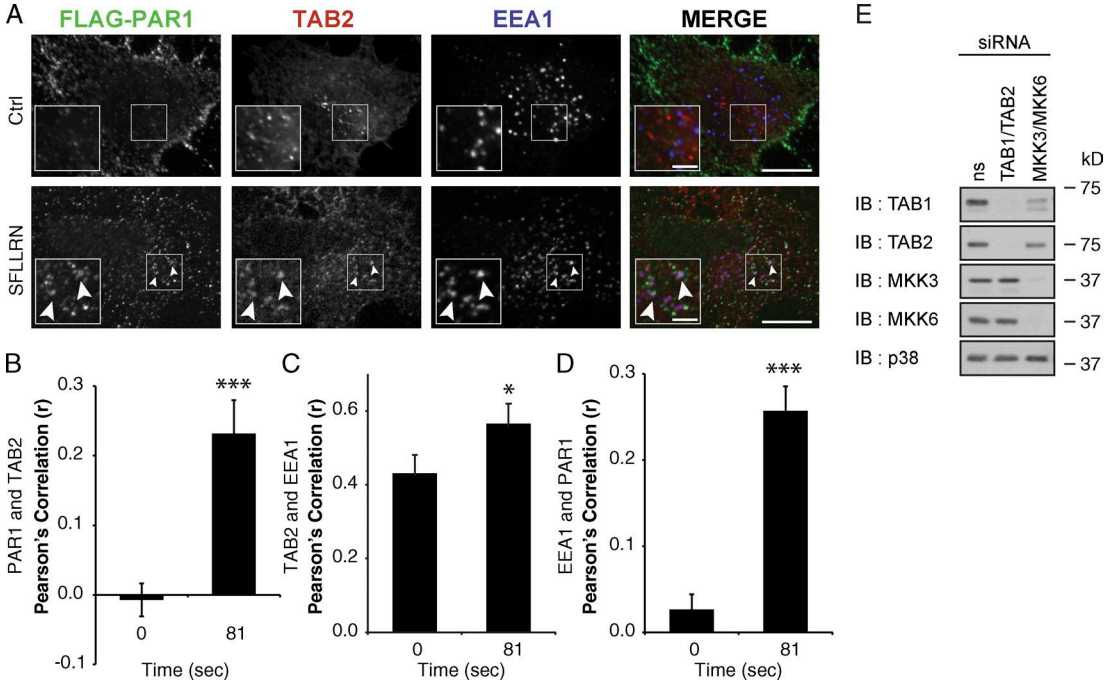

Figure S4. **Colocalization of PAR1, TAB2, and EEA1 and TAB1 expression in MKK3/MKK6-deficient HeLa cells.** (A) PAR1 and TAB2 WT tdTomato coexpressed in HeLa cells were stimulated with 100 µM SFLLRN for 81 s. Images are of fixed cells. Arrowheads show PAR1 WT, TAB2 WT, and EEA1-containing punctae. Insets are magnifications of the boxed areas showing PAR1 WT, TAB2 WT, and EEA1 colocalization punctae (arrowheads) in the merged image. Bars: (main panels) 10 µm; (insets) 2.5 µm. (B–D) The data (mean ± SD [error bars], $n = 12$) from three independent experiments represent Pearson's correlation coefficients (r) that were calculated for PAR1 versus TAB2, TAB2 versus EEA1, EEA1 versus PAR1, and control versus agonist-stimulated and analyzed using a Student's t test (*, $P < 0.05$; ***, $P < 0.001$). (E) PAR1 HeLa cells were transfected with ns, TAB1–TAB2, or MKK3/MKK6 siRNAs. Cells were lysed and expression of TAB1, TAB2, MKK3, MKK6, and p38 was detected.

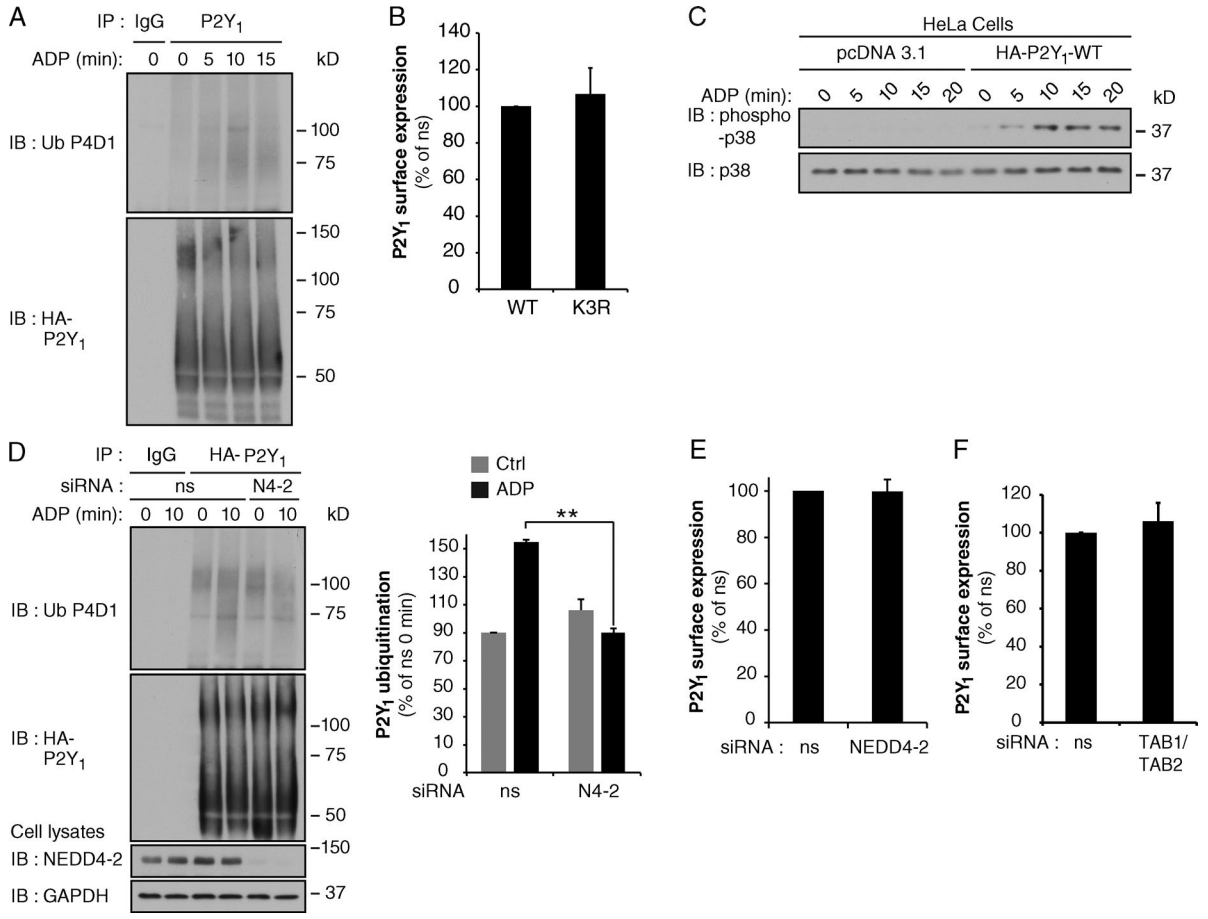

Figure S5. **$P2Y_1$ receptor ubiquitination, expression, and signaling in HeLa cells.** (A) HA-$P2Y_1$ HeLa cells were stimulated with 10 µM ADP and immunoprecipitated, then $P2Y_1$ receptor ubiquitination was determined. (B) $P2Y_1$ WT and ubiquitin-deficient K3R mutant cell surface expression in HeLa cells was determined. The data (mean ± SD [error bars], $n = 3$) were analyzed using a Student's *t* test. (C) HeLa cells transfected with pcDNA3.0 or HA-P2Y1 were stimulated with 10 µM ADP, and p38 phosphorylation was determined. (D) HA-$P2Y_1$ HeLa cells transfected with ns or NEDD4-2 (N4-2) siRNA were stimulated with 10 µM ADP. $P2Y_1$ receptor was immunoprecipitated and ubiquitination was determined. The data (mean ± SD [error bars], $n = 3$) were analyzed using a Student's *t* test (**, $P < 0.01$). (E) $P2Y_1$ WT surface expression was determined in HeLa cells transfected with ns or NEDD4-2 siRNA. The data (mean ± SD [error bars], $n = 3$) were analyzed using a Student's *t* test. (F) $P2Y_1$ WT surface expression was determined in HeLa cells transfected with ns or TAB1–TAB2 siRNAs. The data (mean ± SD [error bars], $n = 3$) were analyzed using a Student's *t* test.