SUPPLEMENTAL MATERIAL

Paruchuri et al., http://www.jem.org/cgi/content/full/jem.20091240/DC1

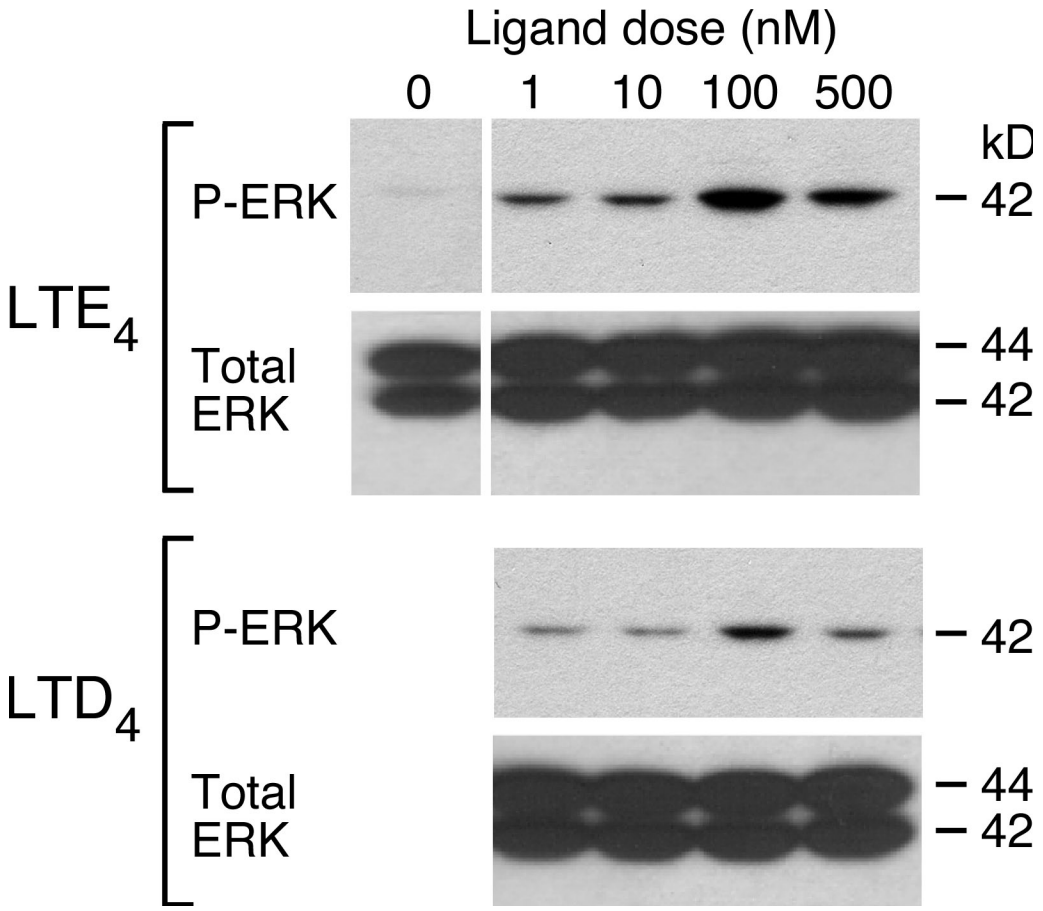

Figure S1. Dose–dependent effects of LTE_4 and LTD_4 on ERK activation in CHO cells. Cells were stably transfected with the human $P2Y_{12}$ construct and stimulated for 15 min with the indicated ligand concentrations. The lanes displayed are from a single autoradiograph from one experiment. Results in a second experiment were similar.

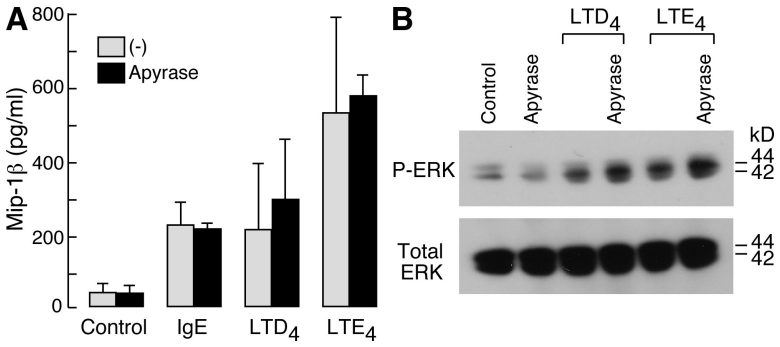

Figure S2. Effect of apyrase treatment on LT-mediated activation of LAD2 cells. (A) LAD2 cells were passively sensitized with human myeloma IgE and stimulated for 6 h with anti-IgE or with 500 nM LTD_4 or LTE_4 in the absence or presence of 10 µM apyrase. Concentrations of MIP-1β were measured by ELISA. (B) LAD2 cells were stimulated for 15 min with LTD_4, 500 nM LTE_4, or buffer (control) in the presence or absence of apyrase. Lysates were resolved by SDS-PAGE and probed with the indicated antibodies against phospho- and total ERK. Results in A are the mean ± 1/2 range from two experiments, whereas B is representative of two experiments.

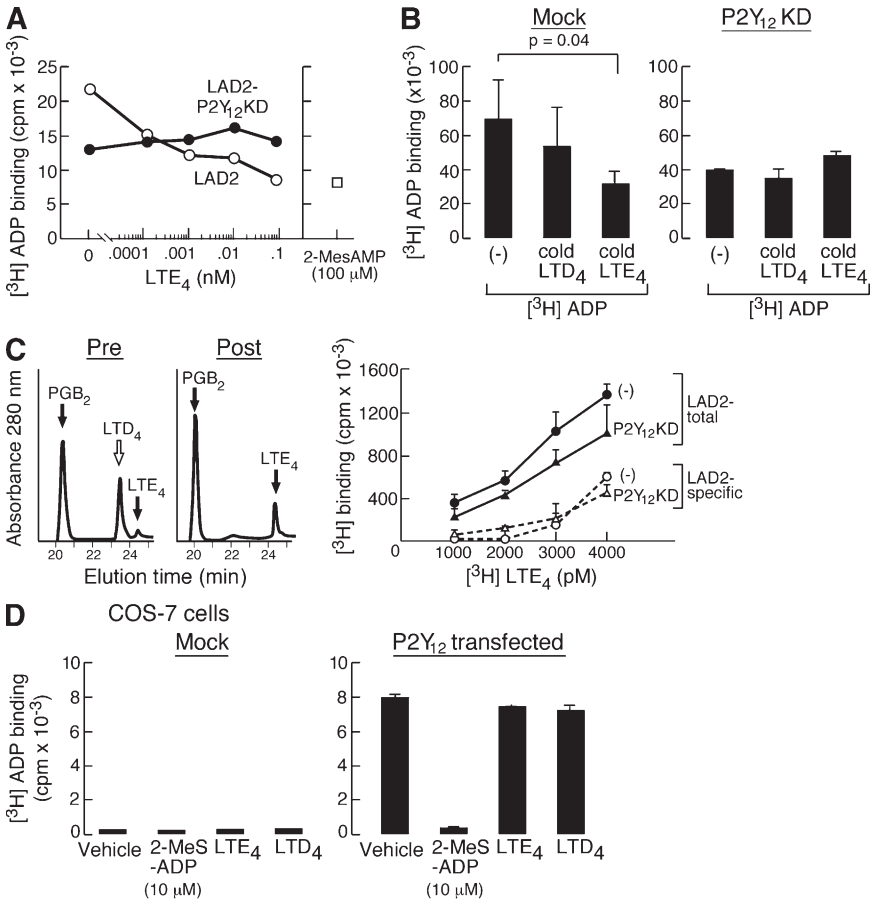

Figure S3. Competitive ligand binding assays. (A) Dose-dependent competition of unlabeled LTE_4 at the indicated doses for binding of 2,000 nM of radiolabeled ADP to the membranes of LAD2 cells with and without shRNA-mediated knockdown of $P2Y_{12}$ receptors in the indicated samples. Specific binding (typically 50–75% of the total) was calculated by subtracting the amount of radiolabel that was insensitive to the selective $P2Y_{12}$ receptor antagonist 2-MesAMP. Nonspecific binding was essentially identical in the membranes with and without $P2Y_{12}$ receptor knockdown. The dose response for the LAD2 membranes is from a single experiment repeated four times with similar results. The binding on the LAD2 cells with $P2Y_{12}$ knocked down was repeated twice. (B) Specific binding of 2,000 nM of radiolabeled ADP to membranes of LAD2 cells. 10 μM of unlabeled LTD_4 and LTE_4 was used as competitors. Results are mean ± SD from three separate experiments. (C) Conversion of [^{3}H]LTD_4 to [^{3}H]LTE_4 as confirmed by HPLC (left). PGB_2 is used as the internal standard for the HPLC, with LTD_4 and LTE_4 eluting at ∼23.5 and 24.3 min, respectively, detected based on absorbance at 280 nm. Binding of [^{3}H]LTE_4 (converted from LTD_4) to membranes of LAD2 cells with and without knockdown of $P2Y_{12}$ receptors (right). Results in a second experiment were similar. (D) Competitive binding of radiolabeled 2-MesADP to COS-7 cells transfected with the human $P2Y_{12}$ construct. Error bars represent ± SD.