Supplemental Figure S1

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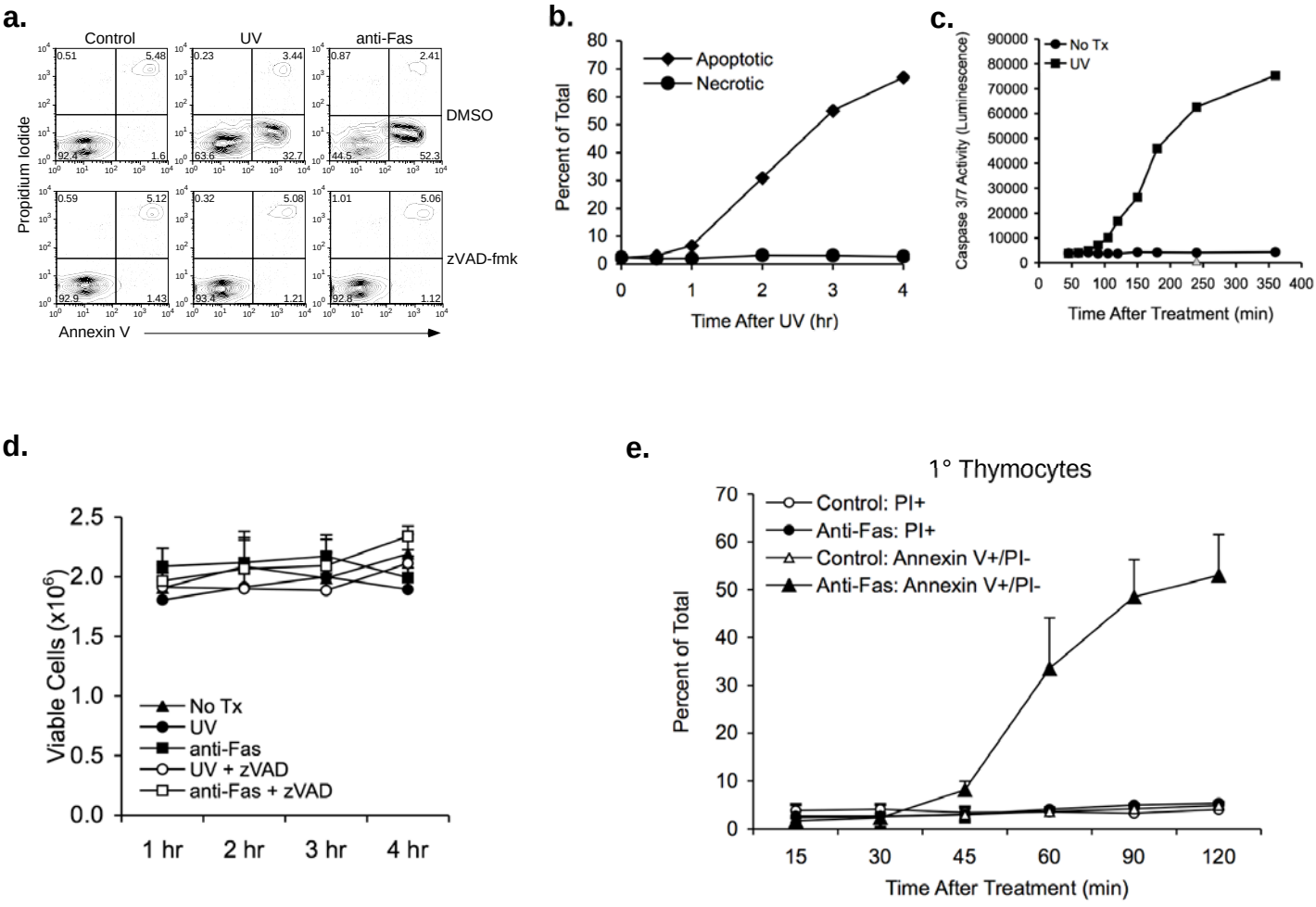
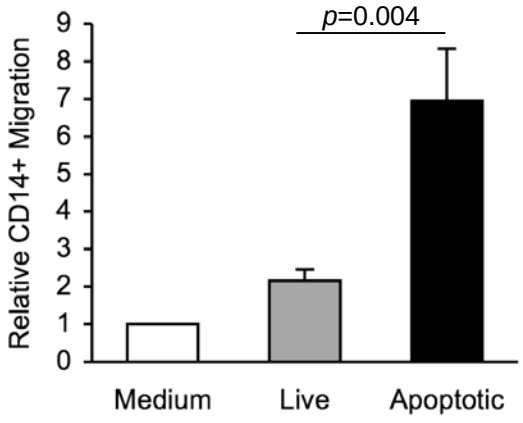


<u>Supplemental Figure S1</u>. Jurkat cells and thymocytes induced to undergo UV- or Fas-mediated apoptosis are primarily apoptotic and not necrotic. a, Jurkat cells induced to undergo apoptosis after UV or anti-Fas treatment were analyzed by flow cytometry for annexin V and propidium iodide (PI) staining at the 4 hr time point after apoptosis induction. The fraction of cells that are apoptotic (positive for annexin V only) versus necrotic (annexin V and propidium iodide positive) are indicated. To verify that the increase in annexin V positive cells arose due to caspase activation, cells were pre-treated with the vehicle control (DMSO) or the pan-caspase inhibitor zVAD-fmk. Note that there are very few necrotic cells and that the population of apoptotic cells is essentially abolished by zVAD-fmk. **b,** Very few necrotic cells are seen under the conditions used for collection of apoptotic cell supernatants. UV irradiated Jurkat cells were analyzed as in **a** at the indicated times. Apoptotic: annexin V+/PI-, Necrotic: PI+. **c,** Caspase 3/7 activation over time in Jurkat cells (as another measure of apoptosis induction) following UV irradiation was measured by luminescent DEVD cleavage assay following UV irradiation. *Triangle* indicates value for zVAD-fmk treated sample at 240 min. **d**, Jurkat cells at $2x10^6$/mL were treated as indicated and cell viability determined by Trypan exclusion on a hemacytometer in triplicate at the indicated times. **e**, Thymocytes treated with anti-Fas for the indicated times and analyzed as in **a** and **b** above indicate no increase in necrotic cells for the duration of the treatment. Error bars indicate s.d.



Supplemental Figure S2. Primary human monocytes migrate to supernatants from apoptotic cells. Supernatants were prepared from untreated (Live) or UV irradiated (Apoptotic) Jurkat cells in RPMI/1% BSA/HEPES and used in a transwell migration assay (3 μm pore size). Monocytes enriched from PBMC of a healthy donor were applied to the upper well and allowed to migrate to the indicated supernatants or medium alone for 2 hr and the migrated CD14+ cells were quantified by flow cytometry (see *Methods*). Mean of triplicate measurements ±s.d from one of two similar experiments is shown.

Supplemental Figure S3

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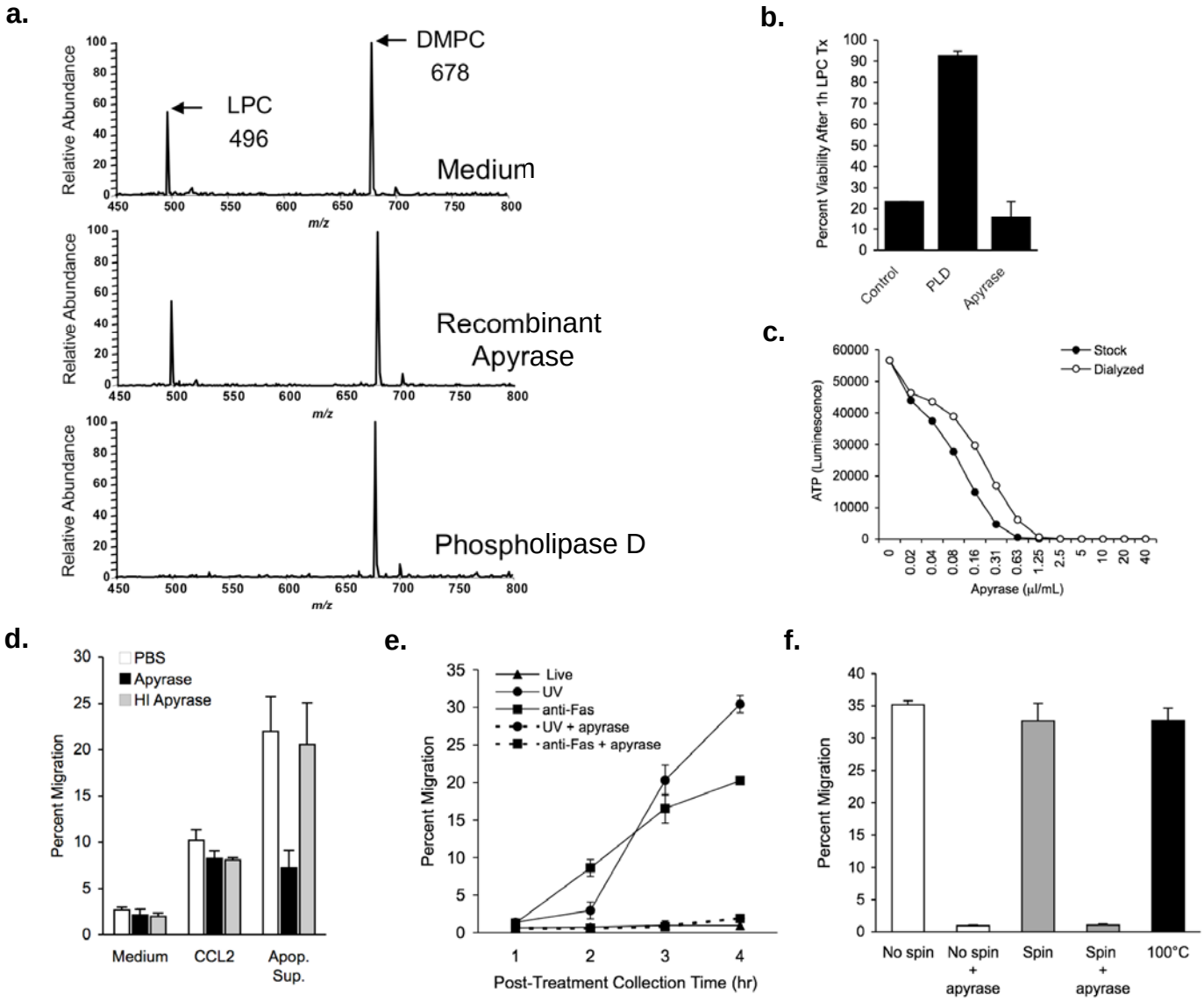
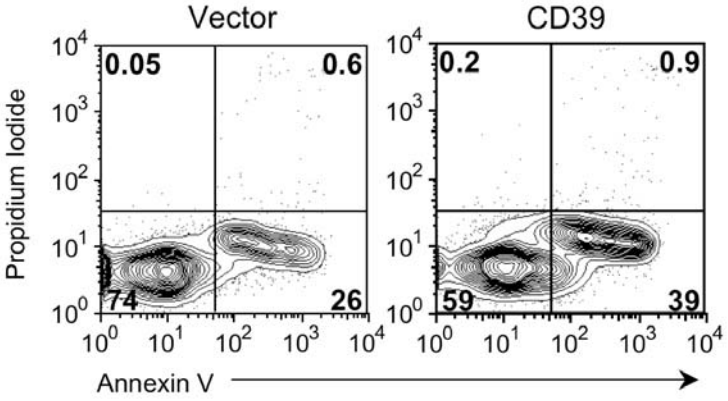


Supplemental Figure S3. Characterization of apyrase-treatment conditions. a, Phospholipase D (PLD), but not recombinant apyrase, degrades LPC. Mass spectroscopic analysis of 250 μg purified lysophosphatidylcholine (LPC) treated with 2.5 units recombinant apyrase or 2.5 units phospholipase D (PLD) for 10 min at 37°C. Peaks for LPC and DMPC (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine) are indicated by arrows. DMPC was added after the reaction as an internal control for lipid extraction. **b,** The degradation of LPC by PLD measured as a reversal of LPC-mediated cytotoxicity. Treatment of THP-1 cells with LPC causes cytotoxicity (within 1hr) that was readily measured as loss of membrane integrity. THP-1 cells were treated with 100 μM LPC in the absence or presence of 0.25 units/mL PLD or 0.025 units/mL apyrase for 1 hr. Cells were counted by hemacytometer and cell viability determined by Trypan exclusion and data plotted as percent viability compared to untreated (no LPC) cells. PLD treatment, but not apyrase, prevented the cytotoxic effects of LPC, indicating degradation of LPC under these assay conditions. **c,** Demonstration of apyrase activity before and after dialysis into PBS. Serial two-fold dilutions of the apyrase preparations were incubated with 500nM of purified ATP for 15 min at room temperature and assayed for ATP activity by a luciferase assay. Stock concentration of apyrase was 50 units/mL. **d,** Heat-inactivation of apyrase prevents the inhibition of migration to apoptotic Jurkat cell supernatants. Transwell migration of THP-1 cells to CCL2 or apoptotic supernatants treated with apyrase or heat-inactivated apyrase (5 min/100°C). **e,** Jurkat cells were induced to undergo apoptosis by UV irradiation or anti-Fas treatment, and supernatants collected at the indicated times. To test the role of nucleotides in stimulating migration in a time course after the induction of apoptosis, supernatants were treated with apyrase 5 min prior to use in the migration assay (broken lines). **f,** Apoptotic Jurkat cell supernatants (UV, 4 hrs) were subjected to high speed centrifugation (100,000 g) for 1.5 hrs and the supernatant fraction was used in a migration assay. Where indicated, apyrase treatment was carried out 5 min prior to use in migration assay. 100°C indicates boiling of supernatants for 5 min prior to use in migration assay. Error bars indicate s.d.

a.



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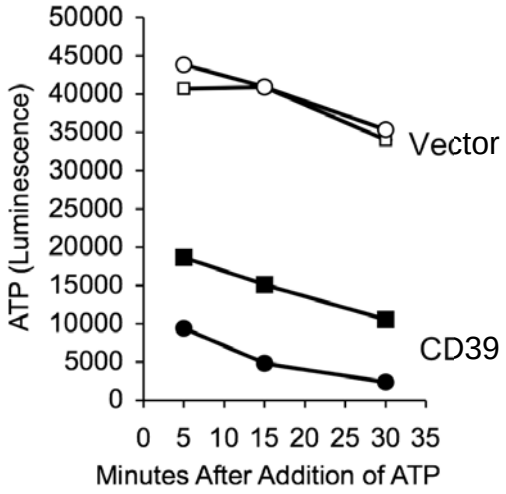
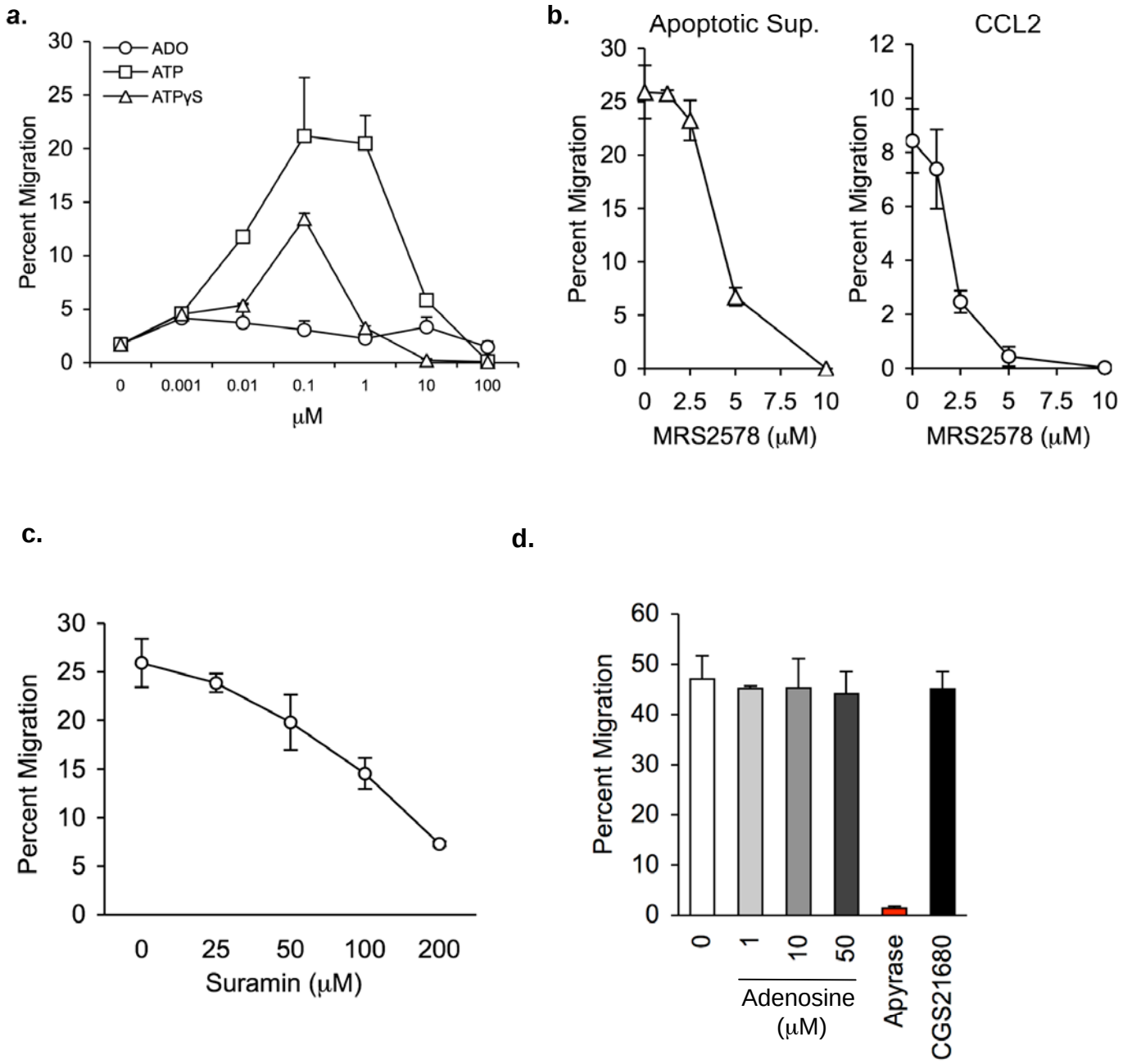


<u>Supplemental Figure S4</u>. Characterization of stable transfectants expressing human ecto-apyrase CD39. **a**, Induction of apoptosis in Jurkat cells stably transfected with vector control or human CD39 4 hrs after UV irradiation as assessed by flow cytometry for annexin V and propidium iodide staining. **b,** Demonstration of increased ecto-nucleotidase activity by CD39 overexpressing Jurkat cells. Ectoapyrase activity of two clones of each cell type in **a** were determined by incubation with exogenously added ATP (80 nM) and incubated at 37°C. At the indicated times after addition of ATP, supernatants were collected and ATP levels determined.



<u>Supplemental Figure S5</u>. ATP, but not adenosine induces THP-1 migration, and the dose-dependent inhibition of migration by suramin. a, THP-1 migration to the indicated concentrations of adenosine (ADO), ATP, or non-hydrolyzable ATP (ATPγS) was assessed. **b,** THP-1 cells were pretreated with $P2Y_6$ antagonist MRS2578 at the indicated concentrations for 30 min prior to use in migration assay to apoptotic supernatants (*left*) or 50ng/mL CCL2 (*right*). The inhibition due to MRS2578 was not considered specific since it inhibited migration to both the apoptotic cell supernatants and CCL2. **c,** THP-1 cells were pretreated with the indicated doses of suramin for 30 min prior to migration toward apoptotic supernatants. **d,** The indicated concentration of adenosine was added to apoptotic supernatants prior to use in THP-1 migration assay. Apyrase pretreatment of the same supernatants was included as a control. Also THP-1 cells were pretreated with the A2a agonist CGS21680 (0.1 μM) for 30 min prior to use in migration assay. Error bars indicate s.d.

Supplemental Figure S6

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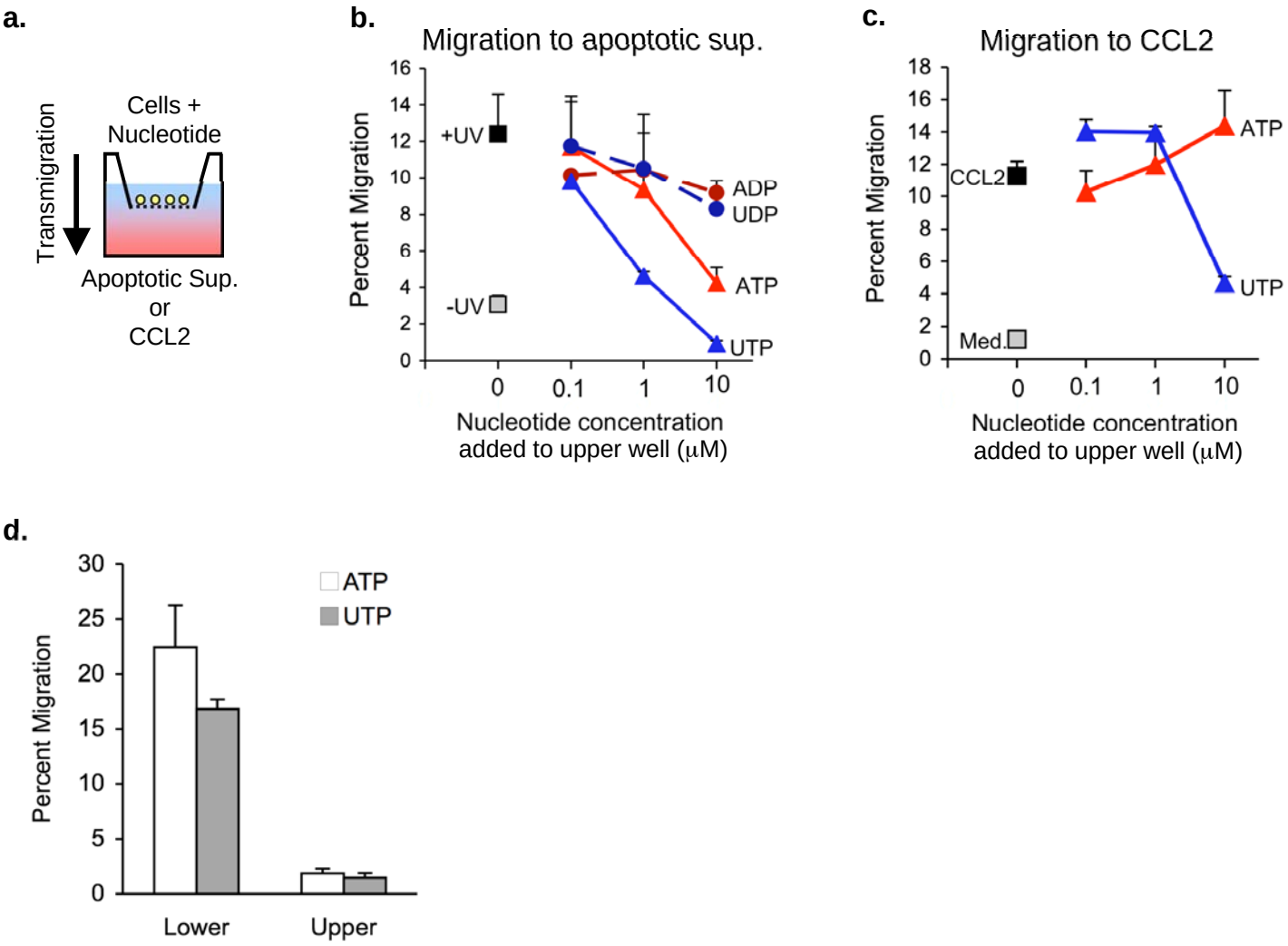
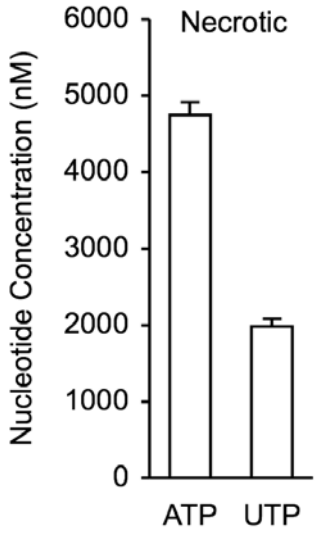
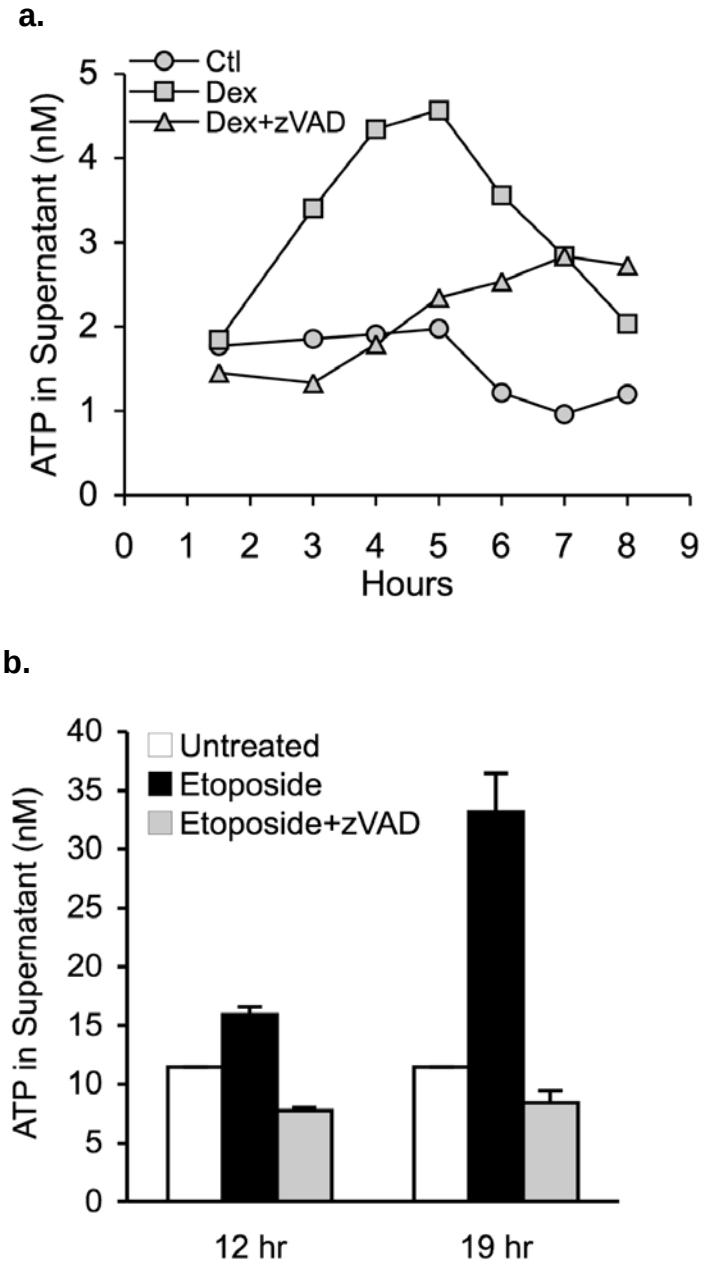


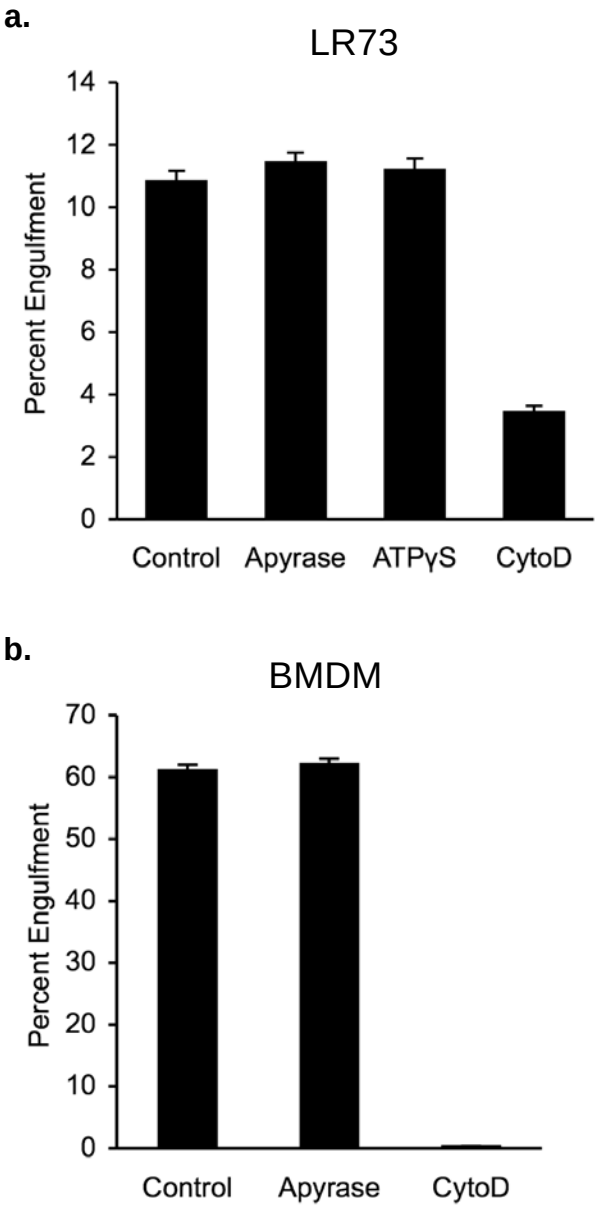
<u>Supplemental Figure S6</u>. Effect of adding nucleotides to the upper well on monocyte migration.
a, Schematic of the experiments in **b** and **c**. The indicated concentrations of nucleotides were added to the upper well along with THP-1 cells and migration was assessed toward apoptotic supernatants (shown in **b**) or CCL2 (shown in **c**) placed in the lower chamber. **d,** ATP or UTP does not induce chemokinesis or random migration of monocytes. 100nM ATP or UTP was added to the lower chamber only or the upper chamber only (THP-1 cells in the upper chamber in all cases) in a transwell migration assay. Error bars indicate s.d.



Supplemental Figure S7. Supernatants from necrotic cells contain more ATP than UTP. Jurkat cells ($2x10^6$/mL) were treated with 0.01% Triton X-100 to induce necrosis for 5 min at 37°C prior to collection of cell-free supernatants. Samples were analyzed for ATP and UTP as described in the *Methods* section. Data shown are mean ±s.d. of triplicate measurements.

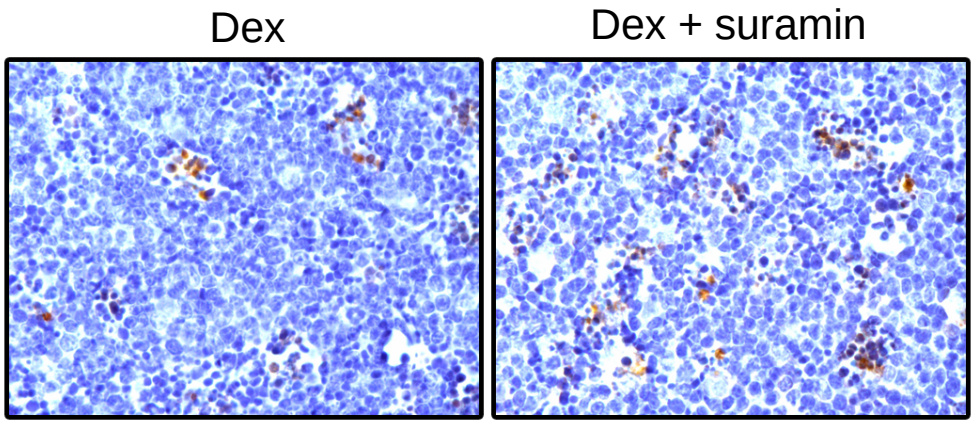


Supplemental Figure S8. Thymocytes and lung epithelial cells induced to undergo apoptosis by Dex or etoposide release ATP. a, Supernatants from thymocytes treated with 50 μM dexamethasone (Dex) for the indicated times were analyzed for the levels of ATP present. This release was abolished by inhibition of caspase activity (zVAD). **b,** BEAS human lung epithelial cells were plated at $1x10^6$/mL and allowed to adhere overnight. Cells were treated with 60 μM etoposide in the absence or presence of zVAD-fmk for the times indicated and cell-free supernatants were assayed for ATP. Error bars indicate s.d.



Supplemental Figure S9. Apyrase treatment does not inhibit apoptotic cell engulfment *in vitro*. Addition of apyrase does not significantly affect engulfment of apoptotic thymocytes by LR73 cells (**a**) or bone marrow-derived macrophage (**b**). Cells were preincubated with apyrase (0.025 units/mL), ATPγS (1 μM), or cytochalasin D (5 μM, as positive control for inhibition of engulfment) for 10 min prior to addition of fluorescently labeled apoptotic thymocytes. Cells were incubated for 1 hr (BMDM) or 2 hr (LR73) and engulfment was assessed by flow cytometry. Error bars indicate s.d.

a.



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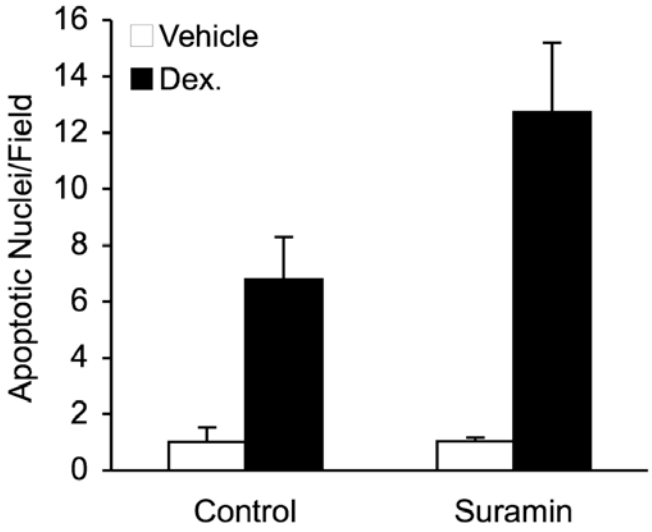

<u>Supplemental Figure S10</u>. Increase in the number of apoptotic nuclei in the thymi of mice treated with Dex+suramin compared to Dex alone. **a,** Representative anti-ssDNA (Apostain) staining of sections from thymi of mice treated for 6 hr with Dex alone or Dex+suramin (20x magnification). **b,** Quantification of apoptotic nuclei (brown) in the sections from **a**. Three mice per group were used and 20-32 fields per section were counted. Error bars indicate s.d.