Supporting Information

Uckun et al. 10.1073/pnas.0909086107

SI Text

Materials and Methods. Experimental Procedures for Gene Expression Analysis in Syk-transfected U373 Cells. We measured levels of RNA transcripts using the Human Genome U95 Av2 GeneChip microarrays (HG-U9SAv2) from Affymetrix, which interrogates the expression levels of 9670 genes/EST clusters. Total cellular RNA was extracted from a minimum of 10 × 10^6 cells with the use of TriPure isolation reagent (Boehringer Mannheim). DNA was removed from this RNA preparation by DNase treatment (Ambion RNase-Free DNase, Promega). Band then purified by 3 rounds of organic (phenol-chloroform) extraction followed by ethanol precipitation. Further purification of the RNA was achieved by binding to an RNaseasy (Qiagen) column prior to spectrophotometric quantitation for in vitro transcription reaction. First strand cDNA was synthesized with a T7- (dT)_14 primer and second strand cDNA was synthesized with E. coli DNA polymerase I and ligase. The cDNA product was in vitro transcribed and labeled with the Enzo BioArray High Yield RNA labeling kit. The in vitro transcription products were purified on an RNasey spin column prior to fragmentation in buffer containing 40 mM Tris-Acetate (pH 8.1), 100 mM KCl, and 30 mM MgOAC for 35 minutes at 94 °C. RNA processing and hybridization to the U95 Av2 GeneChip oligonucleotide microarray was performed according to the manufacturer’s protocol. The test chips were stained and washed on a fluidics station as per manufacturer’s recommendations. Arrays were scanned with the Genechip System confocal scanner manufactured for Affymetrix by Agilent. Microarray Suite 5.0 software from Affymetrix was used to determine the relative abundance of each gene based on the average difference of intensities. Background, noise, and similarity among the samples. Each human gene in this platform is represented by at least one probe set composed of multiple probe pairs (16–20 pairs). Each probe pair consists of two sets of 25 mer sequences. One set is a perfect match (PM) and the other set has a 13th base mismatch (MM) to serve as an internal control for the signal produced by the perfect match probe. A quantitative Signal metric was used to measure the level of each transcript on the chip (developed by Affymetrix). The algorithm calculates the signal using a one-step Tukey’s Biweight Estimate, which determines a weighted mean that is relatively insensitive to outliers. The estimated real signal is calculated by subtracting the log of the Perfect Match intensity from the stray signal estimate. The mismatch signal is used to estimate the stray signal where appropriate. The probe pair was weighted more strongly to calculate the signal if the signal is closer to the median value. A two-way hierarchical clustering technique was used to organize the expression patterns such that genes in (columns) having similar expression on days 1 and 2 after SYK induction were grouped together and similar treatment groups across all the probe sets were joined together. Using the Affymetrix signal to noise evaluation criteria, we filtered genes that were deemed significantly above background at both days after SYK induction or in both transfected controls for upregulated genes and downregulated genes respectively. Control expression values were calculated by pooling Day 1 and Day 2 untransfected/ uninduced and transfected/uninduced values and the induced values were averaged for transfected/induced SYK-expressing test samples for Day 1 and Day 2. Similar expression values across treatments for each gene and similar treatments across genes were grouped together (JMP Software, SAS). The mean and standard error values of control expression were compared to the SYK induced values using a Students Two-Sample T-test (True Positive Discovery Rates were calculated from performing 39 T-tests). Expression values were analyzed first by joining pairs of genes (in columns) or treatments (in rows) closest in the average expression values (average distance linkage) and then connecting larger groups of probe sets or treatments using the branching structure, whereby larger branch lengths represent larger differences in expression profiles. Six of the 29 interrogated STA3 target genes (viz: sprouty homolog 2/SPRY2, cisteine-rich angiogenic inducer 61/CYR61, SMAD family member 7/SMAD7, zinc finger protein 56/ZFP56, v-fos oncogene homolog/FOS, and reticulon 4/RTN4) belonging to 3 subclusters of genes showed marked upregulation following SYK induction. Four genes showed changes with p-values <0.005 (True positive rate >95%). SPRY2 showed the greatest fold-increase by SYK induction (7-fold, T-test, p = 0.003) and ZFP56 showed the most significant change (p = 0.001). In addition to these 6 genes, 3 additional genes belonging to the same 3 subclusters, namely KLF4 (T-test, p = 0.08), MYC (p = 0.1 and p = 0.11), and JUNB (p = 0.08) genes, also showed a SYK induction-associated increase in expression levels that did not reach statistical significance.

PTK Inhibitors and Other Reagents. SYKINH-61 (1,4-Bis (9-O-dihydroxydiazinyl)phthalazine/hydroquinidine 1,4-phthalazinediyl diether) is a potent and selective chemical inhibitor of SYK (SI Text, Fig. S4 and S5). PCT inhibits SYK as well as JAK1. JANEX-1 (also known as WHI-P131) inhibits JAK3 and EGF-R. Controls included BTK inhibitors CP-1/HI-12 and CP-2/HI-86, CP-3 (COBRA-1), JAK1,2,3 inhibitor AG-490 (Sigma–Aldrich), and the pan-JAK inhibitor Pyridone 6 (Calbiochem).

Establishment of U373 Cells with Ecdysone-Inducible SYK Gene Expression. SYK-deficient U373 cells were transfected with the ecdysone-inducible system regulatory vector, pVgRXR, and with a plND/OS vector containing the cDNA encoding wild-type human syk gene (H-L2824M1) (Invitrogen) using published procedures. plND-syk was linearized with AatII and pVgRXR was linearized with MluI. Ten million U373 cells were transfected with 12 μg of each linearized vector by electroporation using a BioRad GenePulser II Electroporator (240 V/950 μF). Two days after electroporation, cells were reseeded in 150 × 15 mm tissue culture dishes and stably transfected cells were selected with 500 μg/ml G418-sulfate and 500 μg/ml zeocin. Individual clones were screened for the inducible expression of SYK after 24 h treatment with 10 μM ponasterone (Pon-A) (an analogue of ecdysone (Invitrogen) by WBA.

Gene Expression Profiling. We measured levels of RNA transcripts using the Human Genome U95 Av2 GeneChip microarrays (HG-U9SAv2) from Affymetrix that interrogates the expression levels of 9670 genes/EST clusters according to published procedures (SI Text).

B-Lineage Lymphoid Cells. BCL-1 and BCL-2 are EBV-transformed B-lineage lymphoblastoid cell lines. RAMOS and DAUDI are OS-resistant Burkitt’s leukemia/lymphoma cell lines. RS4; 11 is an OS-resistant B-lineage ALL cell line. The establishment and characterization of DT40 cell line, SYK-deficient DT40
clones and its subclones were previously reported (7, 9). We also used primary bone marrow-derived leukemic cells from ProB#4, a previously reported SYK-deficient infant pro-B-cell leukemia patient, and two B-lineage ALL patients who had relapsed after allogeneic SCT with a TBI-containing conditioning regimen, including an 8 yr old patient in third bone marrow relapse and a 21 yr old patient third bone marrow relapse. The secondary use of surplus primary leukemic cells was reviewed by the Parker Hughes Institute Institutional Review Board (IRB) and approved under the exemption category 4 (45 CFR Part 46.101).

Confocal Laser Scanning Microscopy and Apoptosis Assays. Cytotoxicity studies using immunofluorescence staining with antitubulin antibodies and confocal microscopy were performed using standard procedures. A two-color TdT-mediated dUTP nick-end labeling (TUNEL) assay was employed to detect apoptotic nuclei. In some experiments, DNA from supernatants of Triton X-100 lysates analyzed for fragmentation.

Statistical Methods. TUNEL assays were used to determine the percentage of apoptotic cells after various treatments compared to no treatment controls. Mean and standard deviation of pooled values for each treatment across cell types was calculated and significance of the treatment effect was assessed using one-way ANOVA. Each treatment was compared to the control using a Dunnett’s post hoc method with p-values <0.05 deemed significant.

Fig. S1. In vitro interactions between recombinant SYK and recombinant STAT3. (A, B). Far WBA showing dose-dependent physical interactions between purified recombinant SYK and STAT3 proteins. The first protein (SYK in A and STAT3 in B) was loaded at increasing amounts (0.5–5.0 μg/lane). The second protein (STAT3 in A and SYK in Panel B) was used at 2 μg/mL. SYK bound to immobilized STAT3 and STAT3 bound to immobilized SYK in the Far WBA were visualized using specific antibodies and chemiluminescence. (C, D) Kinase assays showing phosphorylation of recombinant STAT3 (Sample 1 = 2.5 μg, Lane 3; Sample 2 = 7.5 μg, Lane 4) by recombinant SYK (2.5 μg). In control kinase reactions, 2.5 μg SYK was used without STAT3 (Lane 1) and 7.5 μg of STAT3 (Lane 2) was used without SYK. (C) Shows the phosphorylation status of SYK and STAT3 proteins when they were added to the kinase reactions alone or in combination. (D) Shows the amount of SYK and STAT3 proteins in each kinase reaction shown in (C), as measured by WBA using a mixture of anti-SYK and anti-STAT3 antibodies. (E) Phosphoamino acid analysis (PAA) of SYK-phosphorylated STAT3 protein showing that phosphorylation is on tyrosine residues. The 32P-labeled STAT3 band in (C, Lane 3) was isolated and subjected to PAA. The positions of ninhydrin-stained phosphoamino acid standards (phosphoserine [S], phosphothreonine [T], and phosphotyrosine [Y]) are indicated with circles.
Fig. S2. In vivo interactions between recombinant SYK and recombinant STAT3. SF21 cells were transfected with PFB-stat3, PFB-syk, or PFB-stat3 + PFB-syk. After 48 hrs, cells were lysed; lysates were immunoprecipitated (IP) with anti-STAT3 or antiSYK antibodies, as indicated. Immune complexes were subjected to WBA with anti-STAT3 [in (A) and (D)], antiSYK [in (B)], or antiphosphotyrosine (APT) [in (C)] antibodies. In Western blots, purified SYK and STAT3 were used as positive controls. Anti-STAT3 WBA of SYK immune complexes (IC) from SF21 cells cotransfected with PFB-stat3 + PFB-syk showed the presence of coimmunoprecipitated STAT3 (A, Lane 2; D, Lane 3). Furthermore, antiSYK WBA of STAT3 IC from the same cells showed the presence of coimmunoprecipitated SYK (B, Lane 2). No STAT3 was detected in SYK IC from SF21 cells transfected with PFB-syk alone (A, Lane 3), and no SYK was detected in STAT3 immune complexes from SF21 cells transfected with PFB-syk alone (B, Lane 3). The APT WBA of the STAT3 IC from SF21 cells transfected with PFB-stat3 showed a negligible level of Y-phosphorylation (C, Lane 1). By comparison, APT WBA of STAT3 IC from SF21 cells cotransfected with PFB-stat3 + PFB-syk showed enhanced Y-phosphorylation of STAT3 (C, Lane 2). SYK IC from the same SF21 cells contained Y-phosphorylated STAT3 (C, Lane 3). The PTK activity of ectopically expressed SYK, as measured by autophosphorylation in the antiphosphotyrosine WBA of SYK IC, was similar in SYK vs. STAT-3 plus SYK transfectants (C, Lanes 3 and 4). STAT3 was expressed at similar levels in STAT3 and STAT3 plus SYK transfectants (C, Lanes 1 and 2). These results provide evidence that STAT3 does not only physically interact with coexpressed SYK in SF21 cells but it also serves as a PTK substrate for SYK. We also sought to determine if the SYK-mediated Y-phosphorylation augments the DNA binding activity of ectopically expressed STAT3 in electrophoretic mobility shift assays (EMSAs). Whole cell extracts were prepared from SF21 cells transfected with PFB-syk alone, SF21 cells transfected with PFB-stat3 alone, as well as from SF21 cells transfected with PFB-syk + PFB-stat3. Whole cell extracts were tested for the presence of active STAT3 by SIE EMSA. The position of the shifted complex of activated STAT3 is indicated with an arrowhead. Extracts from SF21 cells transfected with PFB-syk alone or PFB-stat3 alone did not contain any proteins capable of binding the 32P-labeled STAT-specific m67-SIE probe causing a mobility shift (E, Lanes 1 and 3). By comparison, a significant mobility shift of the SIE probe was observed in extracts from SF21 cells coexpressing SYK and STAT3 (E, Lane 2). These findings indicate that SYK-mediated Y-phosphorylation of ectopically expressed STAT3 enhances its DNA binding activity. We next searched for a physical association between STAT3 and SYK in human B-lineage lymphoid cells. BCL-1 cells were left untreated or treated with 400 μM PV at 37 °C for 30 min. Cells were lysed using Nonidet P-40, and lysates were immunoprecipitated with either anti-STAT3 (Lanes 1 and 3) or antiSYK (Lanes 2 and 4) antibodies, as indicated (F and G). To detect coimmunoprecipitation of STAT3 and SYK, the immune complexes were resolved by SDS-PAGE and examined for the presence of either SYK (F) or STAT3 (G) by immunoblotting with the corresponding antibody. STAT3 immune complexes contained SYK (F, Lane 3) and SYK immune complexes contained STAT3 (G, Lane 4) in PV-treated BCL-1 cells.
Fig. S3. JAK inhibition does not prevent SYK-mediated activation of STAT3 in an ecdysone-inducible mammalian expression system for SYK. (A) APT WBA of JAK immune complexes immunoprecipitated with a cocktail of antibodies to JAK1, JAK2, and JAK3 from whole cell lysates of syk-transfected, Pon-A treated U373 cells that were cultured in the presence and absence of the pan-JAK inhibitors AG-490 (100 μM) and Pyridone 6 (5 nM) plus the JAK3 inhibitor JANEX-1/WHI-P131 (100 μM). (B) Anti-JAK WBA of JAK IC from the same lysates used in (A). A cocktail of antibodies to JAK1, JAK2, and JAK3 was used for WBA. (C) Anti-phospho-STAT3\textsuperscript{Y705} WBA of STAT3 immune complexes from whole cell lysates of transfected uninduced vs. induced U373 cells that were cultured in the presence of the JAK inhibitor cocktail AG-490 (100 μM) + Pyridone 6 (5 nM) + JANEX-1/WHI-P131 (100 μM). Lysates from syk-transfected U373 cells were prepared before (CON) and at various time points after addition of Pon-A (10 μM) as indicated. Some samples were exposed to PV as well where indicated. (D) Anti-STAT3 WBA of the STAT3 immune complexes shown in (C).
**Fig. S4.** Genetic model of SYK-STAT3 interactions in B-lineage lymphoid cells exposed to oxidative stress. (A–C) Schematic representation of wild-type and mutant syk cDNA constructs (A) and expression of wild-type or mutant SYK in SYK-deficient DT40 lymphoma B-cells (B, C). SYK-deficient (DT40^syk^−) and BTK-deficient (DT40^btk^−) clones of DT40 lymphoma B-cells (DT40^wt^) were previously reported. Mutant syk cDNAs were created by PCR, and cloned into the EcoRI site of the pApuro expression vector. The resulting cDNAs were verified by DNA sequencing. These cDNAs were then transfected into SYK-negative cells by electroporation using gene pulser apparatus (BioRad Laboratories) at 550 V, 25 μF, and selected in the presence of 0.5 μg/mL puromycin. Expression of mutated SYK was assessed by in vitro immune complex KA (B and C, Upper Panels) and WBA (B and C, Lower Panels) using antiSYK antibodies. SYK-deficient DT40 (DT40^syk^−) cells expressing wild-type SYK, catalytic kinase domain mutant (395K to R) of SYK, and C-terminal SH2 domain mutant (28RAR to GAL), and N-terminal SH2 domain mutant (37RQS to GGI) of SYK were designated as DT40^syk^−; rsyk^wt^, DT40^syk^−; rsyk^K−^, DT40^syk^−; rsyk^mSH^2[C/C138]^, and DT40^syk^−; rsyk^mSH^2[N/C138]^, respectively. The PTK activity of SYK immune complexes, as measured by autophosphorylation, was abrogated by the catalytic domain mutation, but was not affected by SH2 domain mutations (C, Upper Panel). Nearly equal amounts of SYK protein were detected by WBA in all of the SYK-deficient DT40 cells stably transfected with wild-type or mutated syk genes (C, Lower Panel) but no SYK protein was detectable in the untransfected SYK-deficient DT40 cells (B and C, Lower Panel). (D) Confocal microscopy showing lack of oxidative stress-induced STAT3 activation and nuclear translocation in SYK-deficient DT40 cells. H2O2-treated SYK-deficient DT40 cells vs. SYK-deficient DT40 cells reconstituted with wild-type SYK were stained with TOTO-3 and phospho-STAT3 antibodies and examined by confocal microscopy for the presence of translocated active phospho-STAT3 protein in the nucleus. We used a BioRad MRC-1024 Laser Scanning Confocal Microscope equipped with a Kr/Ar laser (BioRad) mounted on a Nikon Eclipse E-800 upright microscope equipped for epifluorescence with high numerical aperture objectives (Nikon).
SYKINH-61 (1,4-Bis (9-O-dihydroquinidinyl)phthalazine/hydroquinidine 1,4-phathalazinediyl diether) is a pentapeptide mimic targeting the substrate-binding site of SYK tyrosine kinase. (A) **Chemical structure of SYKINH-61.** Chemical characteristics: (C_{48}H_{55}N_{6}O_{4}; mp: 140–142 °C (lit. 133–135 °C); ¹H NMR (CDCl₃, 300 MHz): δ 8.62 (d, 2H, J = 4.5 Hz), 8.30 (m, 2H), 7.96 (d, 2H, J = 9.0 Hz), 7.90 (m, 2H), 7.54 (d, 2H, J = 2.5 Hz), 7.42 (d, 2H, J = 4.5 Hz), 7.33 (dd, 2H, J = 2.5, 9.0 Hz), 6.95 (d, 2H, J = 6.0 Hz), 3.87 (s, 3H), 3.38 (m, 2H), 2.69 (m, 8H), 1.93 (m, 2H), 1.67 (bs, 2H), 1.57-1.35 (m, 12H), 0.77 (t, 6H, J = 7.0 Hz); ¹³C NMR (CDCl₃, 75 MHz): δ 157.46, 156.31, 147.28, 144.93, 144.58, 132.06, 131.43, 127.30, 122.73, 122.35, 121.75, 118.46, 102.00, 76.25, 60.20, 55.84, 50.84, 49.92, 37.41, 27.29, 26.27, 25.30, 23.51, 11.89. IR (KBr) ν 2931, 2870, 1622, 1508, 1385, 1354 cm⁻¹; LRMS (FAB): 779.2 (M + H)⁺, 471.1, 310.2; HRMS (FAB): calcd for [M + H]/C_{48}H_{55}N_{6}O_{4} 779.4285, found 779.4275). (B) **Model of SYKINH-61 molecule (multicolor) bound to the protein substrate-binding site of SYK kinase domain.** The SYK kinase domain has an active site, which is located in the central region of the protein, dividing the kinase domain into two subdomains—the N-lobe and C-lobe. Phosphorylation of a target tyrosine residue by SYK can occur when ATP binds to the SYK active site (catalytic site), and a protein substrate binds nearby (P-Site). SYKINH-61 is a structurally symmetric molecule that has a unique shape and a size unfit for binding to the ATP binding site of SYK, 686 Å³ volume of the binding pocket within the ATP binding site of EGF receptor kinase, or 530 Å³ volume of JAK kinases. SYKINH-61 was modeled by a docking procedure into the protein substrate-binding region that consists of multiple residues including K565 (Orange), P529 (Purple), and W528 (Red). ATP molecule (Black and White) is situated in a different but nearby site where the ATP triphosphate group binds to D506 through Mg ions (Gray) and the adenine base is sandwiched in a binding cleft near the hinge region (Blue) that links N-lobe with C-lobe of the SYK kinase domain. (C) **SYKINH-61 is a nonpeptide pentapeptide mimic.** The shape of SYKINH-61, which has five individual molecular ring fragments representing functional analogs of five amino acid residues, resembles that of a tyrosine (Y)-containing pentapeptide (GDYEMN), which contains the DYE motif most favored by the protein substrate-binding region (P-Site) of SYK tyrosine kinase. Based on our model, SYKINH-61 is almost completely overlapped in the binding site with a pentapeptide (GDYEMN), which contains a DYE motif most favored, by SYK. The aromatic ring group in the center of the SYKINH-61 molecule takes the position of a Y-residue; the left aromatic ring group and the right aromatic ring group take the position of the backbone of the first residue and that of a glutamate, respectively (C). Similar to the interaction pattern of a peptide substrate with SYK, SYKINH-61 directly interacts with the residues at the end of the A-loop including P529 and the nonterminal side chain atoms of K565. SYKINH-61 has a molecular surface of 670 Å², about half of which are buried by the surrounding protein residues. According to this model, the contacts between SYK and SYKINH-61 are mostly of hydrophobic nature and do not involve any hydrogen bonds.
Fig. S6. Potency and selectivity of SYKINH-61 as an inhibitor of SYK kinase. (A and B) SYKINH-61 inhibits recombinant SYK. The enzymatic activity of SYK was determined by measuring its autophosphorylation in a KA in the absence and presence of increasing concentrations of SYKINH-61 vs. the commercially available SYK inhibitor, PCT. The use of recombinant SYK protein in KA have previously been reported. WBA was performed to compare the amount of SYK protein in each KA sample (depicted in B). A 2 hr incubation with nanomolar amounts (1–100 nM) of SYKINH-61 resulted in a marked and concentration-dependent inhibition of SYK kinase activity (A) without affecting the protein integrity of SYK (B). SYK was also inhibited by the ATP site inhibitors WHI-P154 (100 μM) and PCT (25–100 μM) that were included as controls for comparison, albeit at much higher concentrations. (C–I) SYKINH-61 does not inhibit EGF receptor kinase (EGF-R), insulin receptor kinase (IRβ), BTK, HCK, JAK1, JAK2, or JAK3. The enzymatic activity of the indicated PTK was determined by measuring autophosphorylation in a KA after treatment with the indicated concentrations of SYKINH-61. In vitro KA of epidermal growth factor (EGF) receptor kinase IP'd from MDA-MB-231 breast cancer cells, insulin receptor (IRβ) IP'd from HepG2 hepatoma cells, purified recombinant BTK, HCK IP'd from transfected COS7 cells, recombinant JAK1, JAK2, and JAK3 IP'd from Sf21 insect ovary cells transfected with the appropriate baculovirus expression vectors were performed using previously published procedures (reference #s 24, 59, 60). (J and K). SYKINH-61 inhibits native SYK in human B-lineage leukemia cells. NALM-6 human B-lineage leukemia cells were treated with the indicated concentrations of SYKINH-61 for 4 hr at 37 °C. Native SYK was immunoprecipitated from whole cell lysates of NALM-6 cells (50 x 10^6 cells/sample) and its activity was determined by measuring its autophosphorylation (J). Control cells were left untreated or treated with the JAK3/EGF-R inhibitor JANEX-1 at 100 μM. WBA was performed to compare the amount of immunoprecipitated SYK kinase protein in each sample (K).
Fig. S7. Inhibition of SYK prevents oxidative stress-induced STAT3 activation in human B-lineage leukemia/lymphoma cell lines. Nuclear extracts were prepared from BCL-1 and RAMOS cells (A) as well as RS4;11 and DAUDI cells (B) after 30 min treatment with 400 μM PV or 400 μM PV + 100 nM SYKINH-61. The nuclear extracts were preincubated and then the labeled probe was added. In unlabeled competition reactions, 100-fold excess unlabeled homologous SIE or nonhomologous AP-1 probe was added prior to the preincubation. Mobility shifts were determined by electrophoresis as described in Materials and Methods. Following electrophoresis, gels were dried and subjected to autoradiography on film. Controls included treatments with BTK inhibitors CP-1 and CP-2, tubulin depolymerizing CP-3, PCT, and JAK3 inhibitor JANEX-1 at a concentration of 100 μM. Shifted bands are indicated by arrows.
SYK exhibits antiapoptotic activity in DT40 lymphoma B-cells challenged with oxidative stress. Wild-type (WT) and SYK-deficient (SYK−) DT40 cells were exposed to increasing concentrations (2 μM–200 μM) of PV for 30 min, harvested, and DNA from supernatants of Triton X-100 lysates was analyzed for apoptosis-associated ladder-like fragmentation on ethidium bromide-stained 1.2% agarose gels. PV-induced oxidative stress caused apoptosis in SYK− (but not WT) DT40 cells.

Fig. S8. SYK exhibits antiapoptotic activity in DT40 lymphoma B-cells challenged with oxidative stress. Wild-type (WT) and SYK-deficient (SYK−) DT40 cells were exposed to increasing concentrations (2 μM–200 μM) of PV for 30 min, harvested, and DNA from supernatants of Triton X-100 lysates was analyzed for apoptosis-associated ladder-like fragmentation on ethidium bromide-stained 1.2% agarose gels. PV-induced oxidative stress caused apoptosis in SYK− (but not WT) DT40 cells.