Preparation, isolation, and spectroscopic characterization of PGD_3 metabolites
PGD_3 (Cayman Chemicals) was incubated with 0.1 M sodium phosphate buffer, pH 7.4, containing 0.9 % NaCl at a final concentration of 100 μg/ml with shaking at 37°C for varying periods (24 h–144 h) in sealed brown vials flushed with argon. Similar reactions were performed in the presence of 10 % FBS. The reaction mixtures or cell culture media supernatants were acidified with 1 N HCl to pH 3.0 and extracted three times with two volumes of hexane:ethylacetate (50:50). The organic phase was passed over anhydrous sodium sulfate and evaporated under argon. The organic phase was stored in −80°C until further processing. Eicosanoids were separated by reverse phase LC on a Dynamax semi-quantitative C_{18} column (10 × 250 mm) using MeCN: H_2O: acetic acid (70:30:1) at 2 ml/min and the eluate was monitored at 280 nm. The peaks were collected, concentrated using argon and reconstituted in MS-grade methanol for MS/MS and UV spectroscopic analysis. Eicosanoids were analyzed by direct infusion into a triple quadruple mass spectrometer (API 2000, ABI SCIEX) in the negative electrospray ionization mode. The electrospray voltage and ion spray source temperature were set to −4000 V and 300°C, respectively. Nitrogen was used as curtain (12 psi) and nebulizer (15 psi) gas. The declustering, defocusing, and entrance potentials were set at −50 V, −400 V, and −10 V, respectively.

Δ^{12}-PGJ_3 purified by HPLC was used to create a standard calibration curve on the MS operated in multiple reaction-monitoring (MRM) mode for two transitions, 331.5 to 313.5 *m/z* and 331.5 to 269.5 *m/z*. UV spectra of all LC-purified PGD_3 metabolites in methanol were recorded on a Beckman DU7500 Diode Array Spectrophotometer. The molar extinction coefficients for PGJ_2, Δ^{12}-PGJ_2, and 15d-PGJ_2 (all from Cayman Chemicals) were used to calculate the concentrations of PGJ_3, Δ^{12}-PGJ_3, and 15d-PGJ_3, respectively.

Semiquantitative RT-PCR for p53 and β-actin
Semiquantitative-PCR was performed on the cDNA prepared from LSCs using the following primers: p53 5′TTGGATCCATGTTTTGCCAACTGGCC3′ (forward), 5′TTGAATTCAGGCTCCCCTTTCTTGCG 3′ (reverse) and β-actin 5′GCGGGAAATCGTGCGTGCGTGACATT3′ (forward) and 5′GATGGAGTTGAAGGTAGTTTCGTG3′ (reverse). The bands were visualized on an agarose (1 % w/v) gel and evaluated by densitometry.

Apoptosis
The LSCs were diluted in DMEM, resuspended using a 16-gauge needle, and collected by centrifugation. 1×10^5 cells were resuspended in 200 μl of binding buffer (0.1 M HEPES with 1.4 M NaCl, 25 mM $CaCl_2$, pH 7.4). Annexin V FITC (BD Biosciences) was incubated with cells for 15 min on ice followed by flow cytometric analysis.

Cell viability studies
MEL cells were cultured in DMEM containing 10% FBS and treated with various commonly used anti-leukemic drugs such as daunorubicin (DNR), mitoxantrone (MIT), and cytarabine (CYT) at a final concentration 1 μM for 24 h. These compounds were provided by Dr. Thomas Loughran (Penn State Cancer Institute). Nutlin (5 μM; Cayman Chemicals), a p53 activator, was used as a control to demonstrate the lack of activation of p53 and apoptosis in the MEL cells. After 24 h of drug treatment, cell proliferation was measured by MTT assay with CCK-8 kit

from Dojindo Molecular Technologies, Inc. (Gaithersburg, MD). All viability values reported are relative to untreated cells (UT) that was designated to be 100 %. Results represent the mean ± SEM of three independent observations.

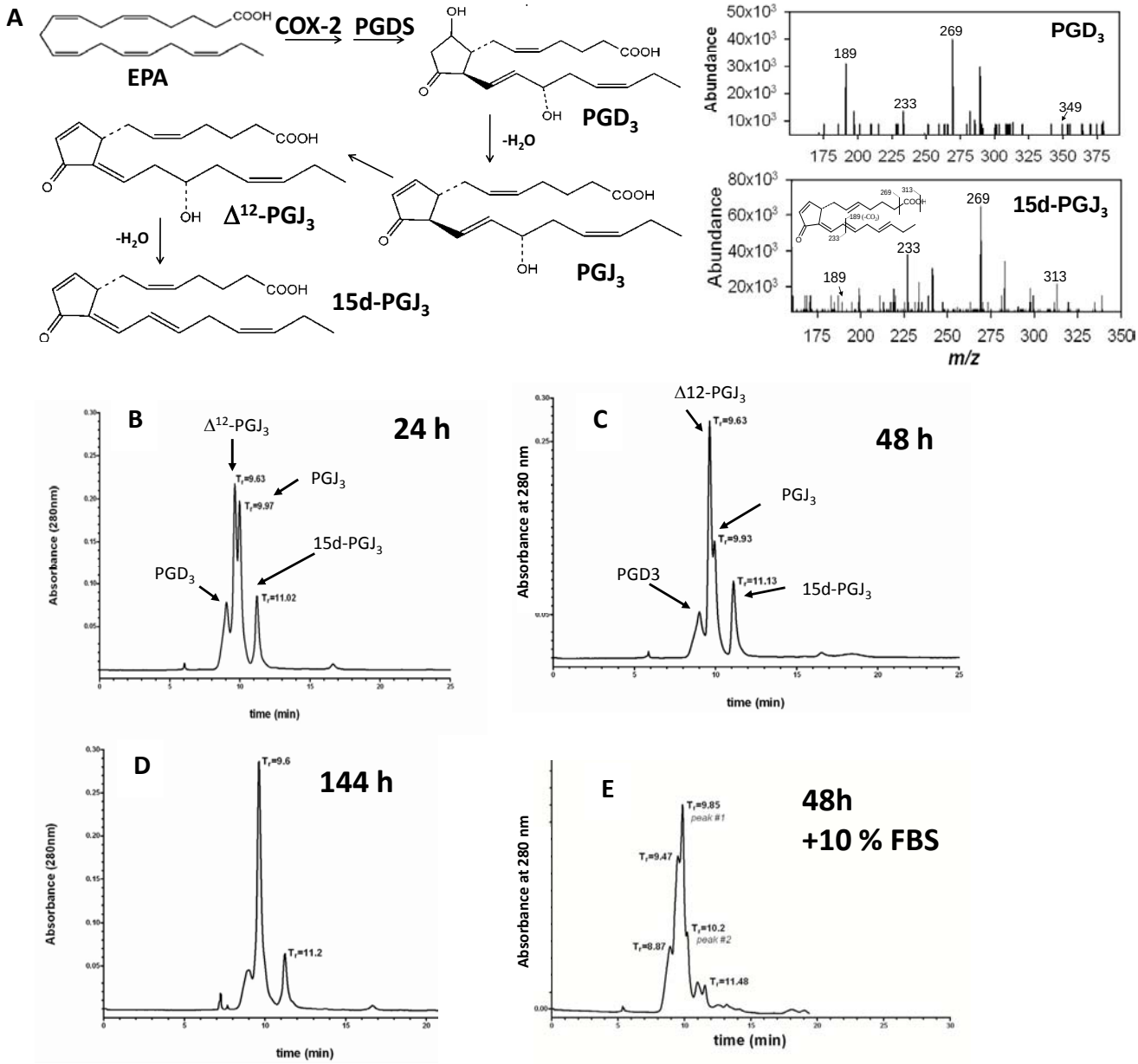

Figure S1. Spontaneous conversion of PGD_3 to PGJ_3, Δ^{12}-PGJ_3, and 15d-PGJ_3 *in vitro*
(A) Schematic showing the pathway of conversion of EPA to CyPGs. Representative MS of PGD_3 and 15d-PGJ_3 are shown. (B, C, D) PGD_3 (from Cayman Chemicals) was incubated with 0.1 M sodium phosphate buffer, pH 7.4, containing 0.9 % NaCl at a final concentration of 100 µg/ml with shaking at 37°C for varying periods (24 h–144 h) in sealed brown vials flushed with argon. (E) PGD_3 was incubated as above in 10 % FBS diluted in phosphate buffered saline for 48 h at 37°C. The PGs were extracted using hexane:ethylacetate (50:50) and the organic phase was concentrated with argon. The eicosanoids were separated by reverse phase LC on a Dynamax semi-quantitative C_{18} column (10 × 250 mm) using MeCN: H_2O: acetic acid (70:30:1) at 2 ml/min and the eluate was monitored at 280 nm. The peaks were collected, concentrated using argon and reconstituted in MS-grade methanol for UV-MS/MS analyses. Representative of N= 8 independent reactions.

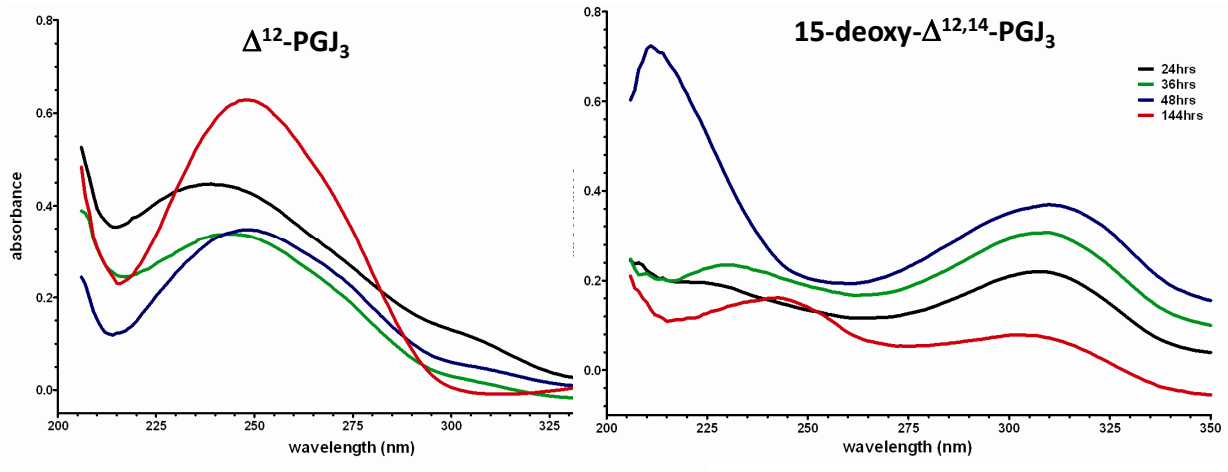

Figure S2. UV-Spectroscopic analysis of Δ^{12}-PGJ_3 and 15d-PGJ_3 as a function of time during conversion

LC-purified Δ^{12}-PGJ_3 and 15d-PGJ_3 at indicated time periods were reconstituted in methanol and analyzed by UV spectroscopy for spectral properties on a Beckman DU7500 Diode Array Spectrophotometer against appropriate solvent background controls. The molar extinction coefficients for PGJ_2, Δ^{12}-PGJ_2, and 15d-PGJ_2 were used to calculate the concentrations of PGJ_3, Δ^{12}-PGJ_3, and 15d-PGJ_3, respectively. Representative of N =3.

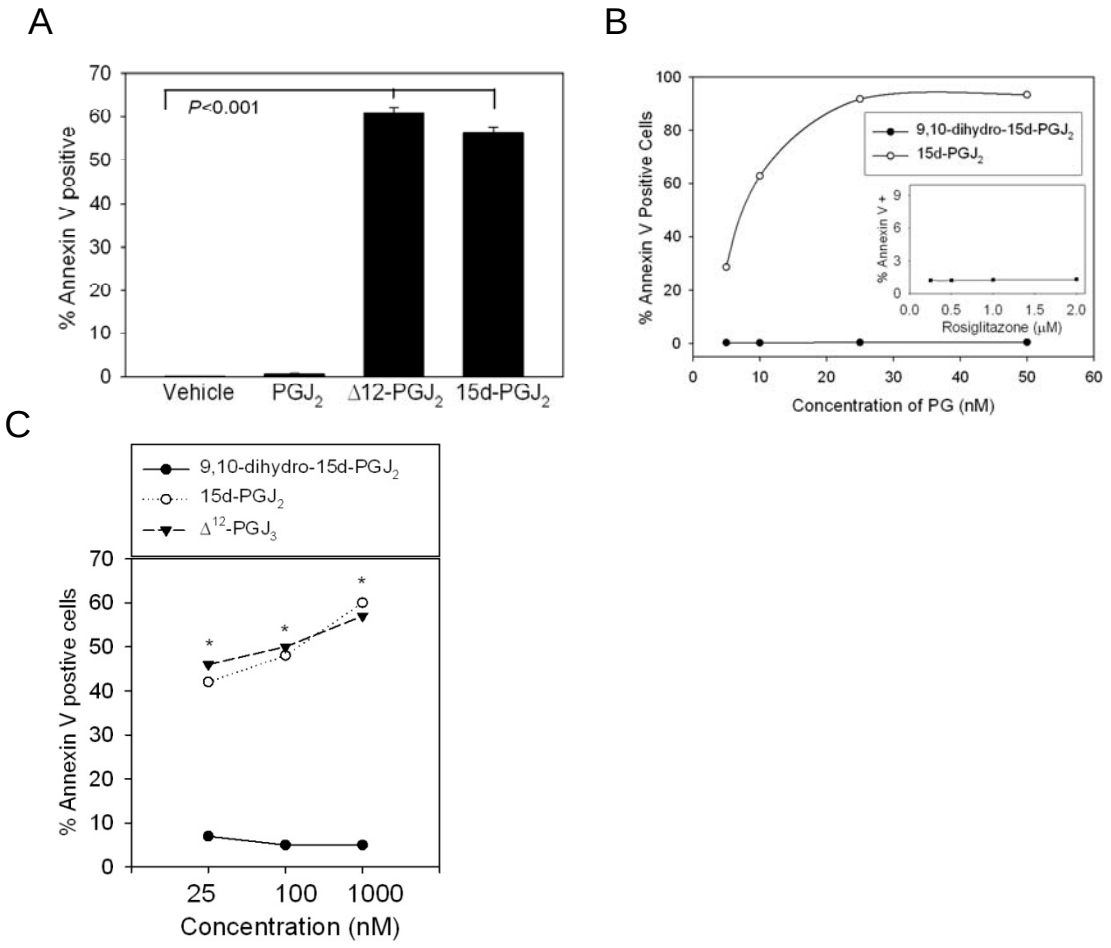

Figure S3. Dose-dependent pro-apoptotic effect of CyPGs on LSCs
(A) Spleen cells from FV-infected mice were sorted for $M34^{+}Sca1^{+}Kit^{+}$ LSCs and incubated with 25 nM of PGJ_2, Δ^{12}-PGJ_2, or 15d-PGJ_2 for 36 h in a methylcellulose stem cell media with 200 ng/ml sonic hedgehog (sHH), 50 ng/ml SCF, and 15 ng/ml BMP4. The cells were stained with annexin V-FITC and analyzed by flow cytometry. Representative of N= 4. Means ± s.e.m. **P*<0.001 compared to vehicle (PBS). (B) Comparison of the proapoptotic function of 9,10-dihydro-15d-PGJ_2 with 15d-PGJ_2. *Inset:* Effect of rosiglitazone on the apoptosis of LSC. Rosiglitazone (0.1–2.0 μM) was incubated with LSC in the culture media for 36 h as described earlier and the cells were subjected to annexin-V staining followed by flow cytometry. (C) Analysis of apoptosis of $BCR\text{-}ABL^{+}Kit^{+}Sca1^{+}$ cells isolated from the spleens of mice transplanted with $BCR\text{-}ABL^{+}$ transduced HSCs after treatment with CyPGs. LSC were treated *ex vivo* with indicated concentrations of each compound for 36 h. Mean ± s.e.m. shown * *P*<0.001.

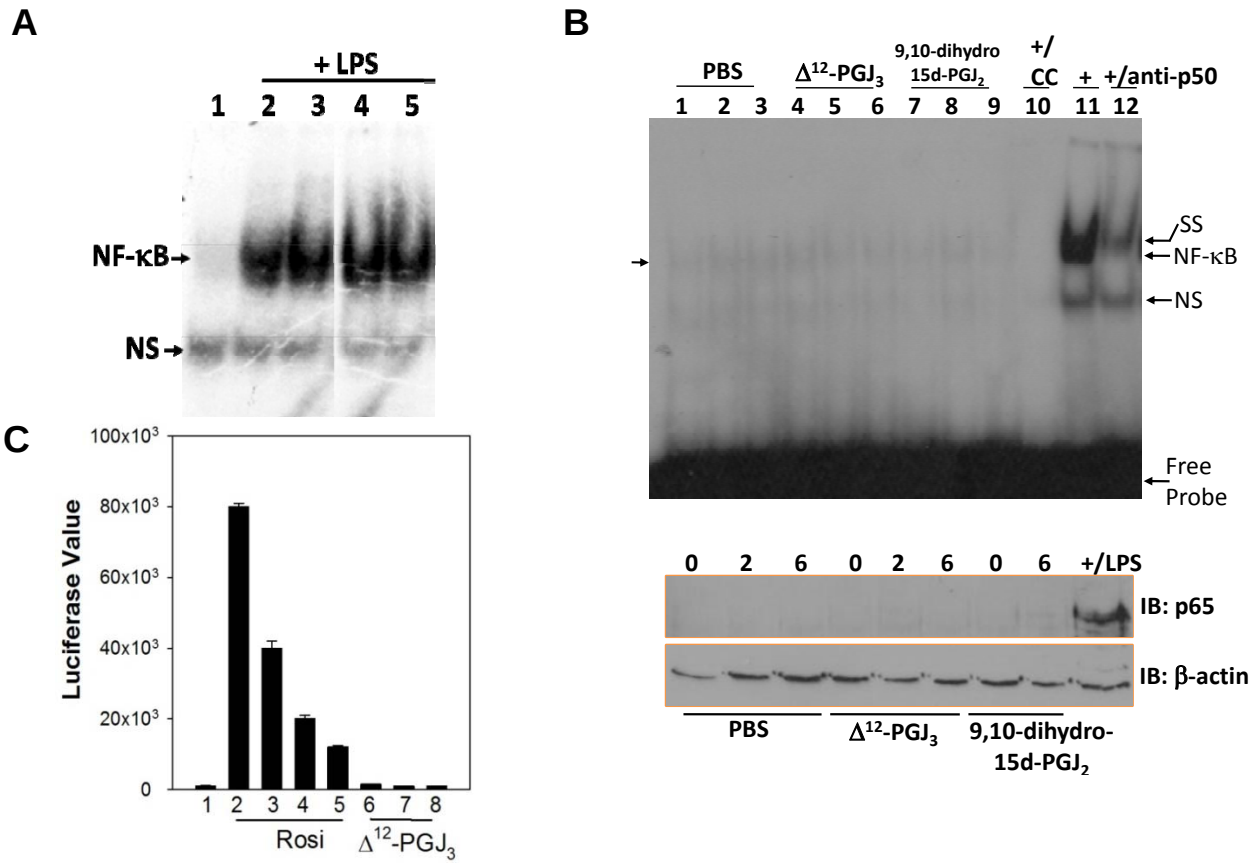

Figure S4. Effect of Δ^{12}-PGJ_3 on NF-κB and PPARγ

(A) Effect of Δ^{12}-PGJ_3 on NF-κB activation. RAW264.7 macrophages were pretreated with DMSO, Δ^{12}-PGJ_3 at 0.25, or 1.0 μM and subsequently stimulated with 100 ng/mL *E.coli* LPS for 4 h. The nuclear extracts were prepared and the binding of NFκB to a ^{32}P-labeled consensus double stranded oligonucleotide probe was examined using gel shift analysis. NS=non-specific band. Lanes 1–5 represent untreated, LPS alone, DMSO+LPS, Δ^{12}-PGJ_3 (0.25 μM) +LPS, and Δ^{12}-PGJ_3 (1 μM) +LPS, respectively. (B) *Top Panel:* BCR-ABL LSCs were sorted from spleens, plated, and treated with PBS (lanes 1–3), 25 nM Δ^{12}-PGJ_3 (lanes 4–6), or 9,10-dihydro 15d-PGJ_2 (lanes 7–9) for 0 (lanes 1, 4, 7), 2 (lanes 2, 5, 8) or 6 (lanes 3, 6, 9) h. The cells were harvested and nuclear extracts were prepared using standard techniques. Ten μg of nuclear protein was used from each sample for the gel-shift reaction. As a positive control for NF-κB, nuclear extract from LPS-treated murine (RAW264.7) macrophages was used (lane 11). Anti-p50 Ab was used with this positive control for a supershift (SS; lane 12), and excess 'cold' probe was used with the positive control as a 'cold competitor' (CC; lane 10). Representative of n=2 shown. NS: Non-specific band. *Bottom Panel:* Western blot of the above mentioned nuclear extracts from BCR-ABL LSCs treated with PBS, Δ^{12}-PGJ_3 or 9,10-dihydro-15d-PGJ_2 for various time periods (0–6 h) probed with anti-p65 and anti–β-actin antibodies. Representative of n= 2 shown. (C) Reporter assay for PPARγ activation. HEK293T cells expressing ligand-binding domain of human PPARγ fused to yeast GAL4 DNA binding domain were transfected with a pGalRE-Luc reporter gene. Lane 1 represents DMSO control, 2–5 represent rosiglitazone at 4, 0.8, 0.16, 0.032 μM, respectively; and 6–8 represent Δ^{12}-PGJ_3 at 1.0, 0.1, 0.01 μM, respectively. Representative of N= 3.

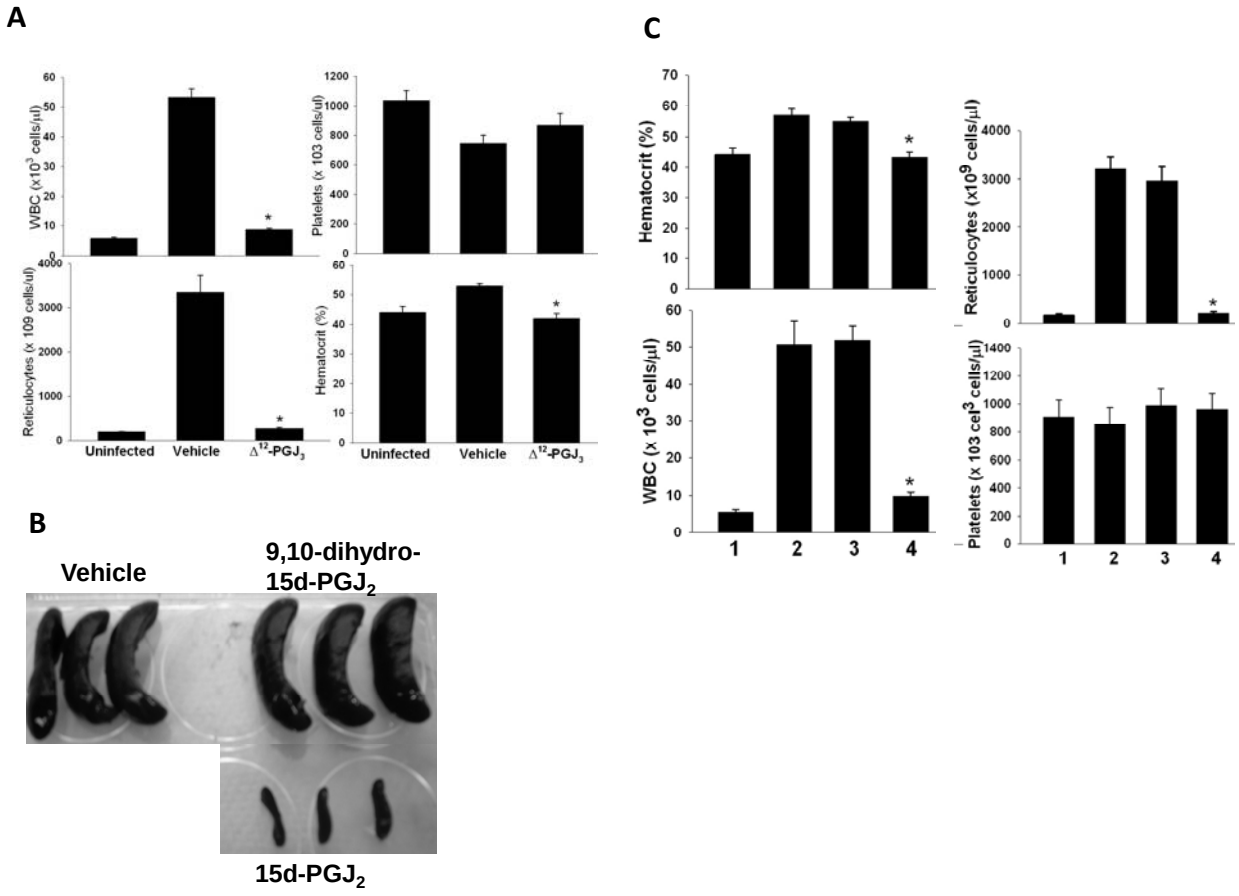

Figure S5. Changes in hematological parameters in FV-infected mice upon treatment with CyPGs

(A) FV-infected Balb/c mice were treated with Δ^{12}-PGJ_3 (0.05 mg/kg) intraperitoneally for 7 d following which the mice were sacrificed and hematological parameters were analyzed on an Advia blood analyzer. FV-infected Δ^{12}-PGJ_3 treated mice were compared to infected vehicle controls. N=5 per group, all data are means ± s.e.m. **P*<0.05. 3 % w/v hydroxypropyl–β-cyclodextrin was used as a vehicle in *in vivo* experiments (B) Spleen sizes of FV-infected mice that were treated with either vehicle, 9,10-dihydro-15d-PGJ_2 (0.05 mg/kg), or 15d-PGJ_2 (0.05 mg/kg); N= 3 pre group. (C) Hematological parameters of Balb/c uninfected, infected, and 15d-PGJ_2 treated mice. Vertical bars 1–4 represent uninfected, infected-vehicle, infected–9,10-dihydro-15d-PGJ_2, and infected–15d-PGJ_2–treated mice, respectively. N= 5 per group. All data are means ± s.e.m. **P*<0.05

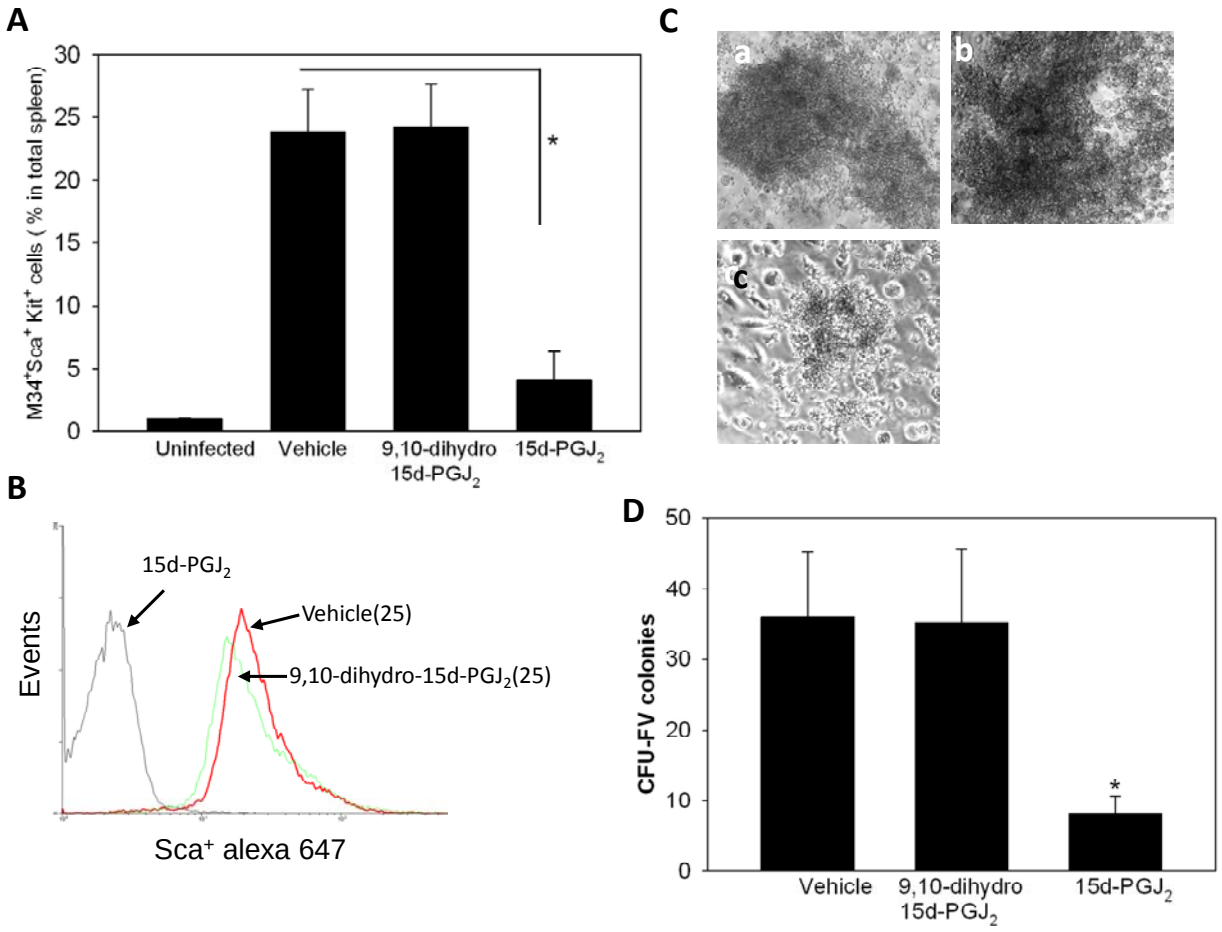

Figure S6. 15d-PGJ_2 eradicates FV-LSC

(A, B) FV-LSC are targeted by 15d-PGJ_2 in the spleen of FV-infected mice. FV-infected mice were treated with 15d-PGJ_2 or 9,10-drihydro-15d-PGJ_2 at 0.05 mg/kg for 7 d. The splenic LSC ($M34^+Sca1^+Kit^+$) cells were analyzed by flow cytometry on day 14 post infection. Uninfected and infected vehicle controls were used for comparison. N= 3; *P<0.05. (C) Splenocytes from infected mice treated with vehicle, 9,10-dihydro-15d-PGJ_2, and 15d-PGJ_2 were plated in methylcellulose media containing FCS without growth factors to examine if treatment of mice with 15d-PGJ_2 or 9,10-drihydro-15d-PGJ_2 affected the formation of CFU-FV colonies, which exhibit factor-independent growth. The colonies were counted 10-14 days after plating. (A, B, C) represent CFU in vehicle-infected, 9,10-dihydro-15d-PGJ_2, and 15d-PGJ_2 treated groups, respectively. (D) shows quantitation of CFU-FV colonies. N= 3 mice per group, *p<0.05

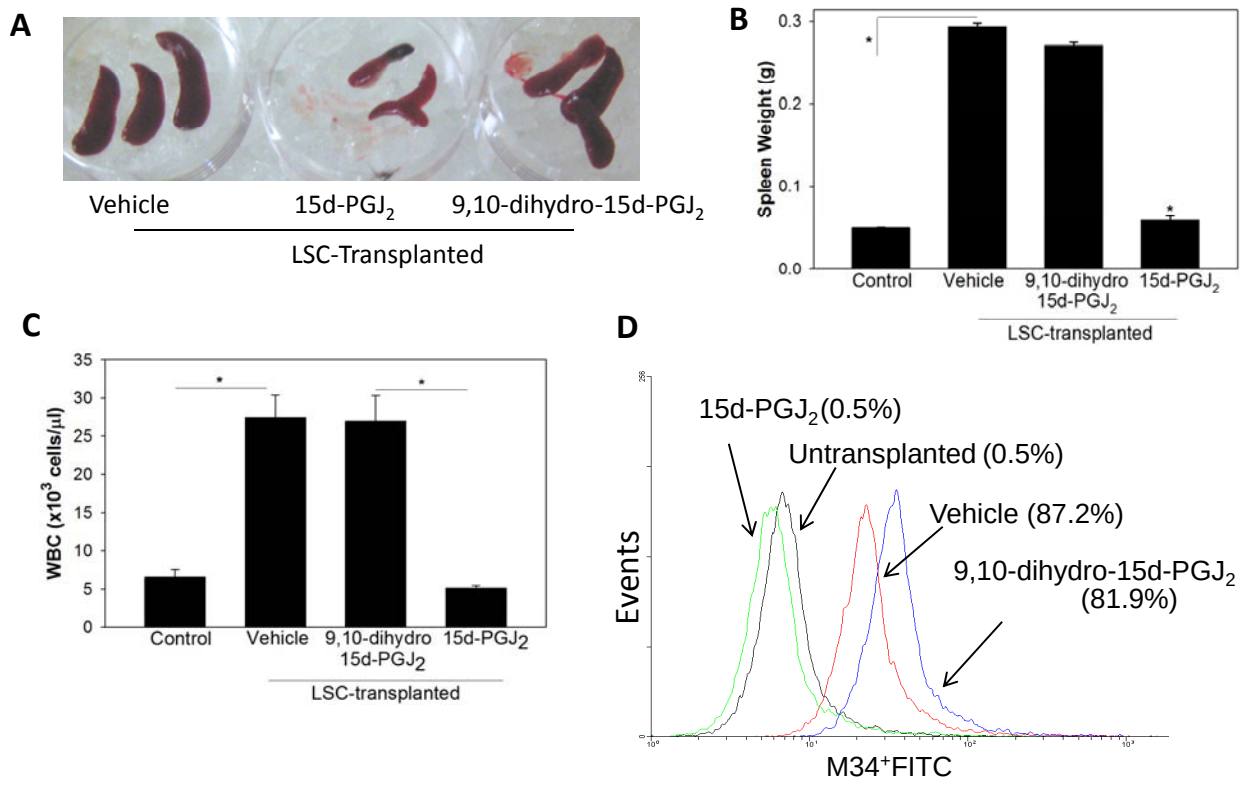

Figure S7. 15d-PGJ_2 eradicates FV-LSC in $Stk^{-/-}$ mice transplanted with *in vitro* expanded FV-LSCs

LSCs sorted from the spleens of FV-infected mice were transplanted into $Stk^{-/-}$ mice (on a Balb/c background). After 6 weeks such mice were treated daily for 1 week with vehicle (hydroxypropyl–β-cyclodextrin), 15d-PGJ_2 (0.05 mg/kg), or 9,10-dihydro-15d-PGJ_2 (0.05 mg/kg) by intraperitoneal injection. The mice were sacrificed 51 days post LSC transplantation for analysis. (A) Spleens of mice comparing splenomegaly in vehicle, 15d-PGJ_2, or 9,10-dihydro-15d-PGJ_2 treated transplanted mice (B) Spleen weight compared to control (untransplanted mice) after treatment. (C) WBC counts in the peripheral blood of the mice after treatment. (D) Flow cytometric analysis of the spleen of untransplanted and LSC transplanted mice after treatment. All data are mean ± s.e.m. * $p<0.05$ compared to control or 9,10-dihydro-15d-PGJ_2 treated groups. N= 5 per group.

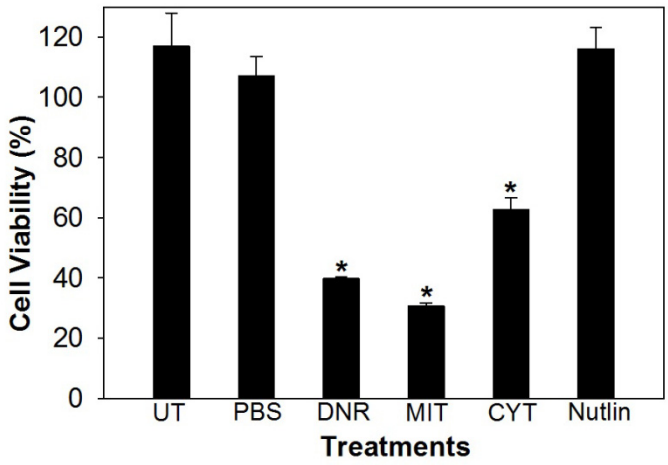

Figure S8. **Activation of apoptosis of MEL cells by anti-leukemic drugs**
MEL cells were cultured in DMEM containing 10% FBS and treated with various commonly used anti-leukemic drugs such as daunorubicin (DNR), mitoxantrone (MIT), and cytarabine (CYT) at a final concentration 1 μM for 24 h. Nutlin (5 μM), a p53 activator, was used as a control to demonstrate the lack of activation of p53 and apoptosis in the MEL cells. After 24 h of drug treatment, cell proliferation was measured by MTT assay with CCK-8 kit from Dojindo Molecular Technologies, Inc. (Gaithersburg, MD). All viability values are relative to untreated cells (UT) that was designated to be 100 %. Results represent the mean ± SEM of three independent observations.