# METHODS

## Preparation of peanut protein

Crude PE was prepared from defatted raw flour (Golden Peanut Company, Alpharetta, Ga), as previously described.[E1] Briefly, the flour (1:10 wt/vol) was extracted in 10× PBS overnight at 4°C. After centrifugation at 30,000*g* for 60 minutes, the supernatant was filter sterilized, measured for protein concentration by using the BCA method (Pierce), and stored as aliquots at −20°C. Endotoxin levels in PE solutions were less than 0.1 EU/mL, as assessed by using a Chromogenic LAL endotoxin assay kit (GeneScript, Piscataway, NJ).

## Sensitization and intragastric challenge

The experimental protocol for sensitization and challenge to peanut and passive sensitization of C57BL/6 mice with 200 μL of serum (intraperitoneally) derived from peanut-sensitized and challenged BALB/c mice 1 day before oral challenge and 1 hour before the fourth oral challenge was previously described.[E1]

## Pim1 kinase inhibitor and treatment *in vivo*

The small-molecule Pim1 kinase inhibitor (AR460770) was dissolved in citric acid buffer and diluted with saline for *in vivo* study or dissolved in RPMI medium for *in vitro* study. The final concentration of citric acid was less than 0.5%. PE-sensitized and challenged mice received different doses (1, 10, 30, and 100 mg/kg) of the Pim1 kinase inhibitor (PE/PE/AR460-) by means of gavage with a 22-gauge feeding needle (Fisher Scientific) twice a day during the peanut challenge phase. Controls included PE-sensitized and challenged but vehicle (saline)–treated (PE/PE/vehicle) or sham-sensitized but PE-challenged and vehicle-treated (PBS/PE/vehicle) mice.

## Assessment of hypersensitivity reactions

Anaphylactic symptoms were evaluated 30 minutes after the oral challenge, as previously reported[E2]: 0, no symptoms; 1, scratching and rubbing around the nose and head; 2, puffiness around the eyes and mouth, diarrhea, pilar erecti, reduced activity, and/or decreased activity with increased respiratory rate; 3, wheezing, labored respiration, and cyanosis around the mouth and the tail; 4, no activity after prodding or tremor and convulsion; and 5, death. Scoring of symptoms was performed in a blinded manner by an independent observer.

## Histamine levels in plasma

Levels of histamine in plasma were measured with an enzyme immunoassay histamine kit (Beckman Coulter, Fullerton, Calif), as described by the manufacturer. The concentration of histamine was calculated from a standard curve provided by the manufacturer.

## T-cell differentiation and treatment with the Pim1 kinase inhibitor *in vitro*

Differentiation of $T_H1$, $T_H2$, or $T_H17$ cells was performed as previously described with minor changes.[E3,E4] $CD4^+CD45RB^+$ T cells were isolated from naive BALB/c WT mouse spleen cells by using a cell sorter (MoFlo XDP, Beckman Coulter). In the presence of anti-CD3 and anti-CD28 (2 μg/mL) and different concentrations of the Pim1 kinase inhibitor (0, 0.01, 0.1, and 1 μm), isolated naive CD4 T cells were cultured with recombinant mouse (rm) IL-2 (10 ng/mL, R&D Systems, Minneapolis, Minn), rmIL-12 (10 ng/ml, PeproTech, Rocky Hill, NJ), rmIFN-γ (5 ng/mL, PeproTech), and anti–IL-4 mAbs (10 μg/mL, eBioscience) to induce $T_H1$ cell differentiation; with rmIL-2 (10 ng/mL, R&D Systems), rmIL-4 (5 ng/mL, PeproTech), and anti–IFN-γ mAb (10 μg/mL, eBioscience) for differentiation of $T_H2$ cells; and with recombinant human (rh) IL-6 (50 ng/mL, PeproTech), rhTGF-β (2 ng/mL, PeproTech), rmIL-23 (10 ng/mL, PeproTech), anti–IL-4 mAb (10 μg/mL, eBioscience), and anti–IFN-γ mAb (10 μg/mL, eBioscience) for differentiation of $T_H17$ cells. After 6 days of culture, the cells were washed with fresh medium and restimulated with anti-CD3/anti-CD28 for 24 hours for assay of cytokine production and cell proliferation. In some experiments the cells were collected for quantitative RT-PCR and Western blotting after 6 days of culture. The purity of sorted cells was greater than 98%, as determined by using flow cytometry.

## Western blot analysis

Proteins were prepared from jejunal tissue, and cultured cells were lysed as previously described.[E1,E5] Proteins and lysates were resolved by means of SDS-PAGE and transferred to nitrocellulose membranes. Proteins were detected by using antibodies specific for either Runx3 or Pim1 (Santa Cruz Biotechnology), followed by chemiluminescence detection (GE Healthcare, Little Chalfont, United Kingdom).

## Intracellular cytokine staining and flow cytometry

Cells from MLNs or differentiated CD4 T cells were labeled with anti-CD3 and anti-CD4 antibodies (eBioscience). For intracellular staining, MLNs or differentiated CD4 T cells were stimulated with 5 ng/mL phorbol 12-myristate 13-acetate and 500 ng/mL ionomycin (Sigma-Aldrich) for 6 hours in the presence of 10 μg/mL brefeldin A (Sigma-Aldrich). After staining for cell-surface markers, cells were fixed with 4% paraformaldehyde in PBS, permeabilized with 0.1% saponin, and stained for intracytoplasmic IL-4, IL-13, IL-17A, IFN-γ, and Runx3 by using antibodies from BD Biosciences and described above (Runx3 antibody). Stained cells were analyzed on a FACSCalibur (BD Biosciences) by using CellQuest software (BD Biosciences).

### REFERENCES

E1. Wang M, Takeda K, Shiraishi Y, Okamoto M, Dakhama A, Joetham A, et al. Peanut-induced intestinal allergy is mediated through a mast cell-IgE-FcepsilonRI-IL-13 pathway. J Allergy Clin Immunol 2010;126:306-16.

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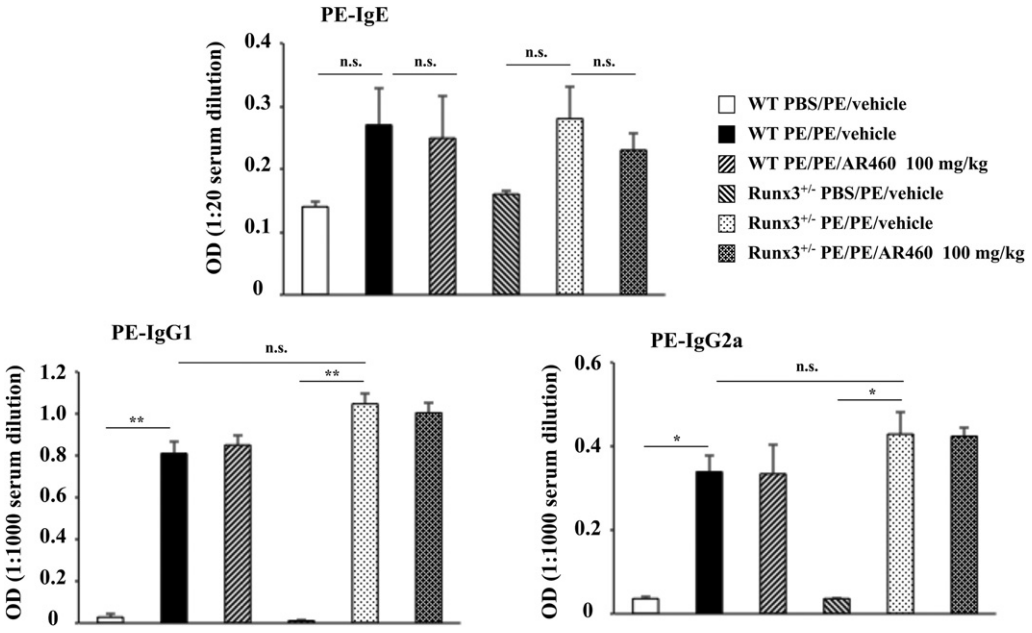


**FIG E1.** Treatment with a Pim1 kinase inhibitor had no effect on serum immunoglobulin production in WT and *Runx3*$^{+/-}$ mice. Serum levels of peanut-specific IgE, $IgG_1$, and $IgG_{2a}$ were assessed by means of ELISA 24 hours after the last challenge and expressed as the optical density of diluted serum, as described in the Methods section. Results were obtained from 3 individual experiments with 4 mice per group. *n.s.*, Not significant; *PBS/PE*, sham sensitized but PE challenged; *PE/PE*, PE sensitized and challenged. $*P < .05$ and $**P < .01$.

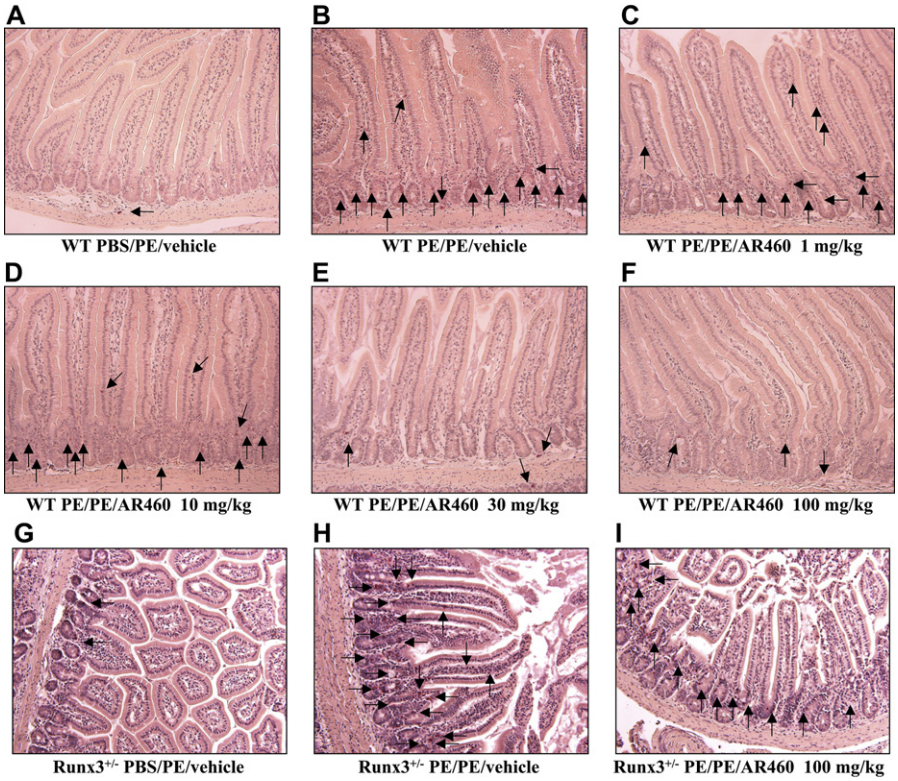


**FIG E2.** Decreased mast cell infiltration in the intestinal walls of PE/PE mice treated with the Pim1 kinase inhibitor. Intestinal mucosal mast cells were quantified in the jejunum by using chloroacetate esterase staining. Representative sections of PBS/PE/vehicle WT mice **(A)**, PE/PE/vehicle WT mice **(B)**, PE/PE/AR460 (1 mg/kg) WT mice **(C)**, PE/PE/AR460 (10 mg/kg) WT mice **(D)**, PE/PE/AR460(30 mg/kg) WT mice **(E)**, PE/PE/AR460 (100 mg/kg) WT mice **(F)**, PBS/PE/vehicle *Runx3*$^{+/-}$ mice **(G)**, PE/PE/vehicle *Runx3*$^{+/-}$ mice **(H)**, and PE/PE/AR460 (100 mg/kg) *Runx3*$^{+/-}$ **(I)** mice are shown (magnification $\times$200). *PBS/PE*, Sham sensitized but PE challenged; *PE/PE*, PE sensitized and challenged.

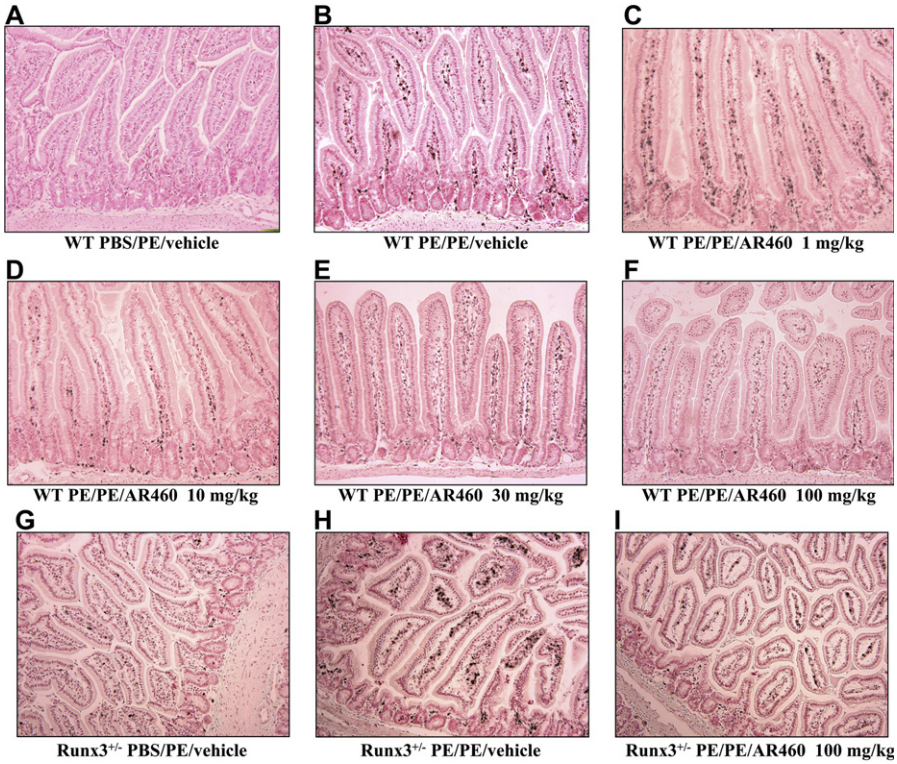


**FIG E3.** Decreased eosinophil accumulation in the intestines of PE/PE mice treated with the Pim1 kinase inhibitor. Eosinophils were identified and numbers were quantitated in the jejunum by staining with anti-MBP antibody. Representative sections of PBS/PE/vehicle WT mice **(A)**, PE/PE/vehicle WT mice **(B)**, PE/PE/AR460 (1 mg/kg) WT mice **(C)**, PE/PE/AR460 (10 mg/kg) WT mice **(D)**, PE/PE/AR460 (30 mg/kg) WT mice **(E)**, PE/PE/AR460 (100 mg/kg) WT mice **(F)**, PBS/PE/vehicle *Runx3*$^{+/-}$ mice **(G)**, PE/PE/vehicle *Runx3*$^{+/-}$ mice **(H)**, and (PE/PE/AR460 (100 mg/kg) *Runx3*$^{+/-}$ mice **(I)** are shown (magnification $\times$200). *PBS/PE*, Sham sensitized but PE challenged; *PE/PE*, PE sensitized and challenged.

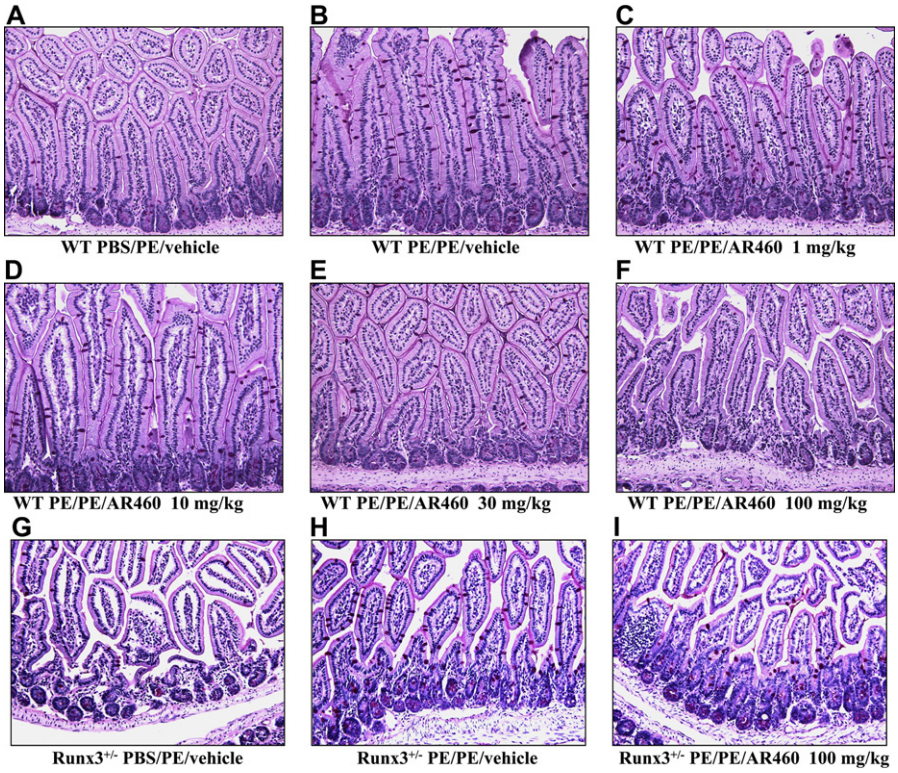


**FIG E4.** Decreased numbers of goblet cells in intestinal epithelia of sensitized and challenged mice treated with the Pim1 kinase inhibitor. Goblet cells were identified by means of periodic acid–Schiff staining 24 hours after the last challenge. Representative sections of PBS/PE/vehicle WT mice **(A)**, PE/PE/vehicle WT mice **(B)**, PE/PE/AR460 (1 mg/kg) WT mice **(C)**, PE/PE/AR460 (10 mg/kg) WT mice **(D)**, PE/PE/AR460 (30 mg/kg) WT mice **(E)**, PE/PE/AR460 (100 mg/kg) WT mice **(F)**, PBS/PE/vehicle *Runx3*$^{+/-}$ mice **(G)**, PE/PE/vehicle *Runx3*$^{+/-}$ mice **(H)**, and PE/PE/AR460 (100 mg/kg) *Runx3*$^{+/-}$ mice **(I)** are shown (magnification $\times$200). *PBS/PE*, Sham sensitized but PE challenged; *PE/PE*, PE sensitized and challenged.

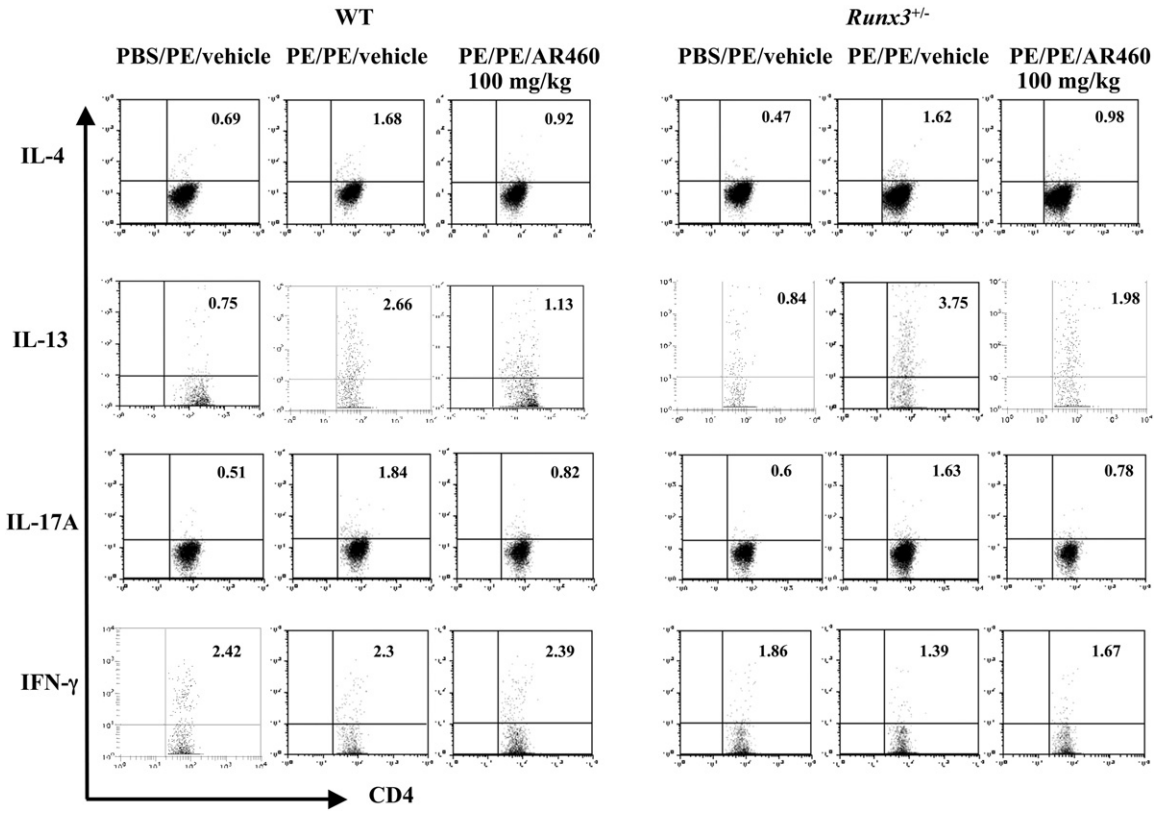


**FIG E5.** Effect of the Pim1 kinase inhibitor on cytokine production. Flow analysis of intracellular cytokine staining of isolated MLNs from WT and *Runx3*$^{+/-}$ mice is shown. Cells were stimulated with phorbol 12-myristate 13-acetate/ionomycin/brefeldin A, as described in the Methods section. Numbers shown in the quadrants represent percentages of the CD4$^{+}$ T lymphocyte–gated cell population, and numbers are representative of 3 independent experiments. *PBS/PE*, Sham sensitized but PE challenged; *PE/PE*, PE sensitized and challenged.

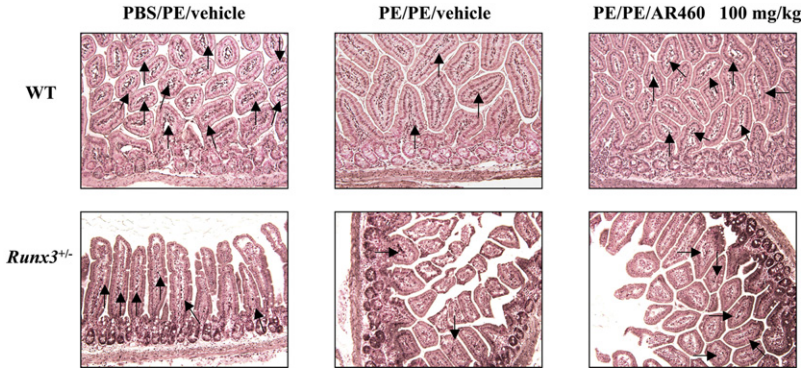


**FIG E6.** Immunohistochemical analysis of Runx3 expression in the jejunal tissues. Representative pictures show Runx3 protein in jejunums of PBS/PE/vehicle WT and *Runx3*$^{+/-}$ mice and PE/PE WT and *Runx3*$^{+/-}$ mice treated with AR460770 or vehicle (magnification ×200). *PBS/PE*, Sham sensitized but PE challenged; *PE/PE*, PE sensitized and challenged.

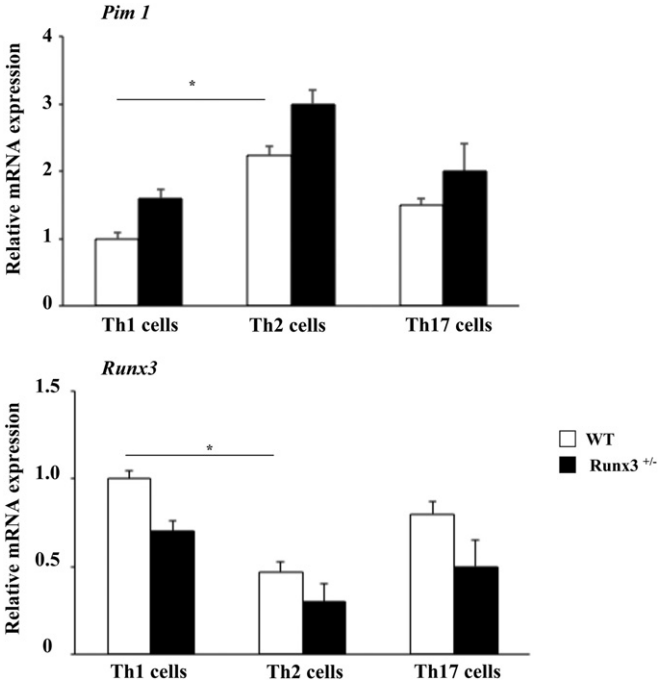


**FIG E7.** *Pim1* and *Runx3* mRNA expression in naive CD4 T cells differentiated *in vitro* into $T_H1$, $T_H2$, or $T_H17$ cells from WT and *Runx3*$^{+/-}$ mice determined using real-time PCR. Data are from 3 independent experiments. **P* < .05.

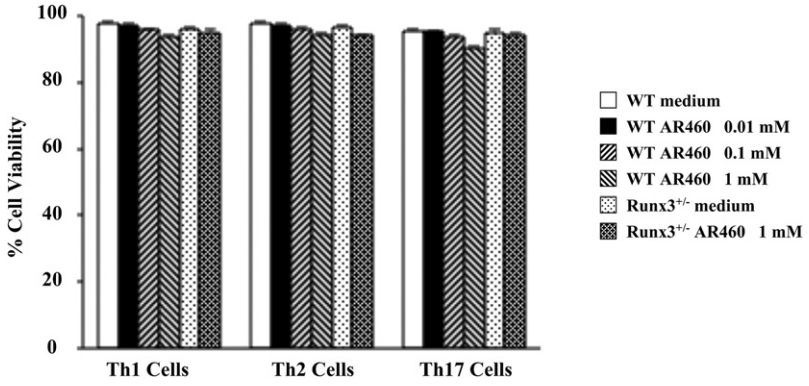


**FIG E8.** Cell viability was determined by means of trypan blue dye staining of polarized $T_H1$, $T_H2$, and $T_H17$ cells cultured in the presence of different concentrations of the inhibitor for 6 days. Data shown are from 3 independent experiments.

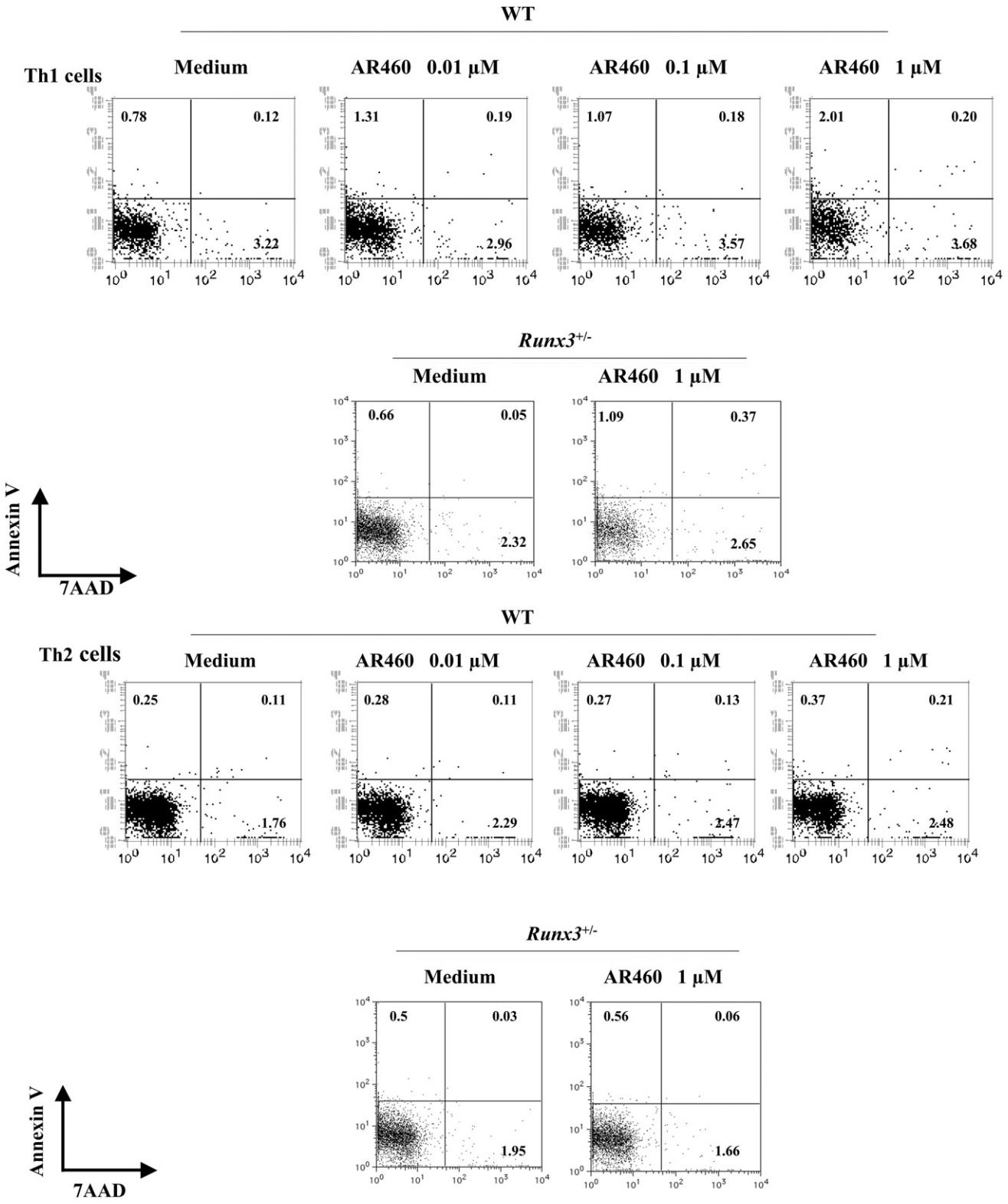


**FIG E9.** Cell apoptosis was determined by means of flow cytometry with Annexin V and 7AAD dual staining of polarized $T_H1$, $T_H2$, and $T_H17$ cells cultured in the presence of different concentrations of inhibitor for 6 days. Numbers indicate percentages of cells in each quadrant and are representative of 3 independent experiments.

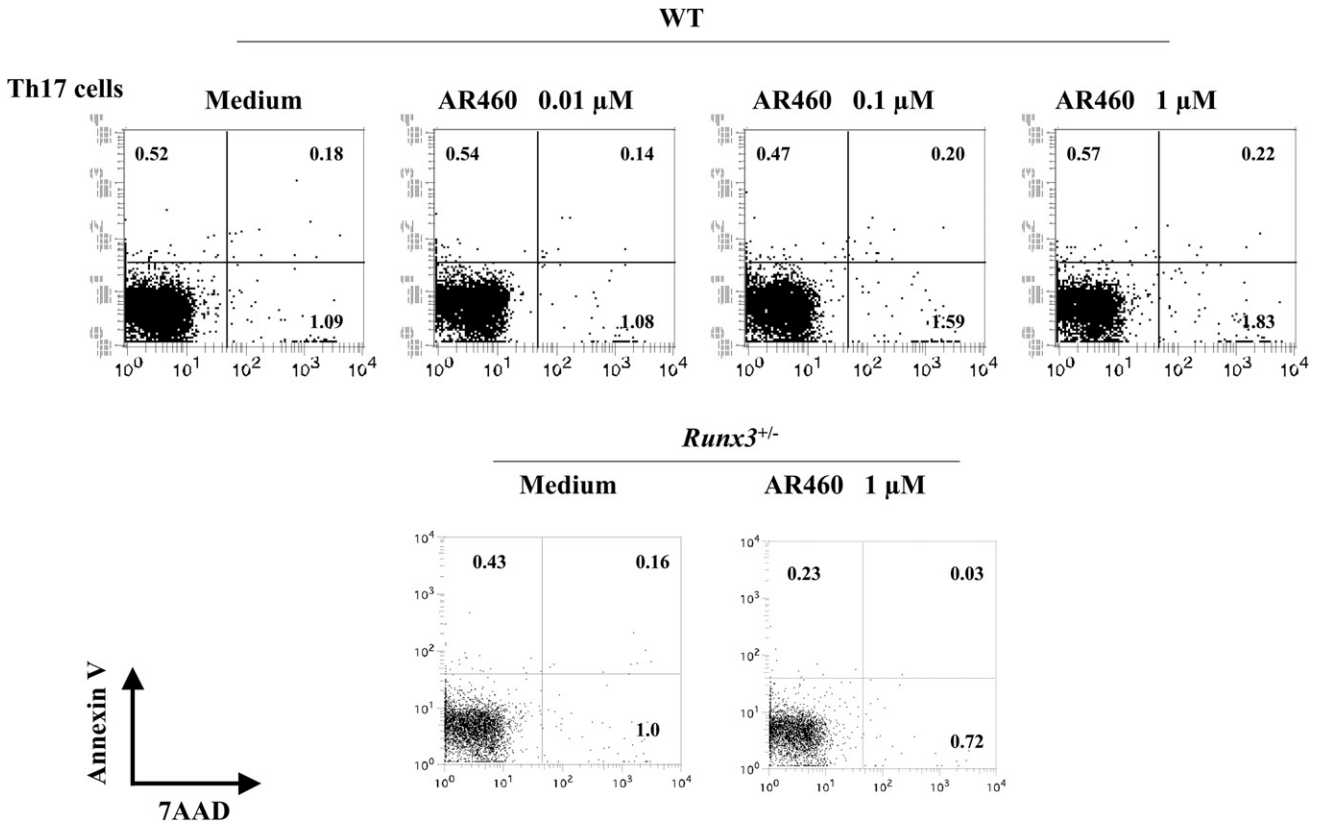


**FIG E9.** *(Continued)*

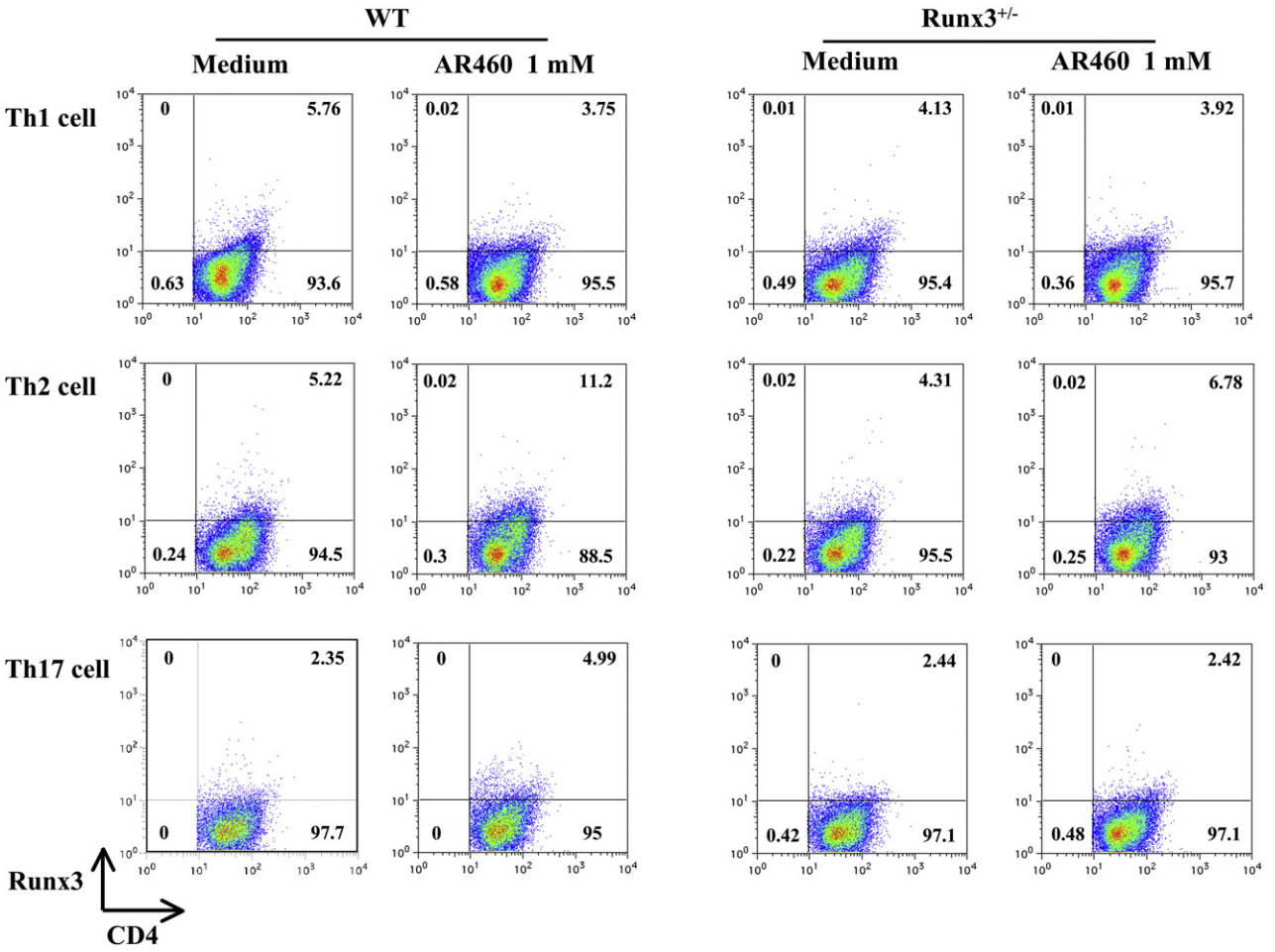


**FIG E10.** Effect of the Pim1 kinase inhibitor on Runx3 protein levels in the polarized $T_H1$, $T_H2$, and $T_H17$ cells determined by intracellular staining. Biotinylated rabbit anti-human Runx3 polyclonal antibody was prepared as described in the Methods section. Cells were stimulated with phorbol 12-myristate 13-acetate/ionomycin/brefeldin A, as described in the Methods section. Numbers shown in the quadrants represent percentages of the $CD4^+$ T lymphocyte–gated cell population, and numbers are representative of 3 independent experiments.