The Protein Phosphatase 2A Regulatory Subunit B56γ Mediates Suppression of T Cell Receptor (TCR)-induced Nuclear Factor-κB (NF-κB) Activity*

Rebecca Breuer, Michael S. Becker, Markus Brechmann, Thomas Mock, Rüdiger Arnold, and Peter H. Krammer

From the Division of Immunogenetics, German Cancer Research Center (Deutsches Krebsforschungszentrum), 69120 Heidelberg, Germany

NF-κB is an important transcription factor in the immune system, and aberrant NF-κB activity contributes to malignant diseases and autoimmunity. In T cells, NF-κB is activated upon TCR stimulation, and signal transduction to NF-κB activation is triggered by a cascade of phosphorylation events. However, fine-tuning and termination of TCR signaling are only partially understood. Phosphatases oppose the role of kinases by removing phosphate moieties. The catalytic activity of the protein phosphatase PP2A has been implicated in the regulation of NF-κB. PP2A acts in trimeric complexes in which the catalytic subunit is promiscuous and the regulatory subunit confers substrate specificity. To understand and eventually target NF-κB-specific PP2A functions it is essential to define the regulatory PP2A subunit involved. So far, the regulatory PP2A subunit that mediates NF-κB suppression in T cells remained undefined. By performing a siRNA screen in Jurkat T cells harboring a NF-κB-responsive luciferase reporter, we identified the PP2A regulatory subunit B56γ as negative regulator of NF-κB in TCR signaling. B56γ was strongly up-regulated upon primary human T cell activation, and B56γ silencing induced increased IkB kinase (IKK) and IkB phosphorylation upon TCR stimulation. B56γ silencing enhanced NF-κB activity, resulting in increased NF-κB target gene expression including the T cell cytokine IL-2. In addition, T cell proliferation was increased upon B56γ silencing. These data help to understand the physiology of PP2A function in T cells and the pathophysiology of diseases involving PP2A and NF-κB.

The transcription factor NF-κB3 plays a key role in the immune system by controlling lymphocyte survival and activation (1). Aberrant NF-κB activity is implicated in lymphoid malignancies and contributes to a variety of autoimmune disorders (2–4). In T cells, NF-κB is activated upon TCR stimulation (5). Proximal TCR signaling induces activation of the protein kinase Cθ (PKCθ), which eventually leads to IKK activation. IKK in turn phosphorylates IkB proteins, which sequester NF-κB in the cytosol. Phosphorylation-induced degradation of IkB proteins allows NF-κB to translocate to the nucleus and activate transcription (6). Whereas activation of NF-κB is well studied, events that terminate NF-κB activity are only partially understood (7).

Prominent examples of negative regulators of TCR-induced NF-κB activity are the two ubiquitin-editing enzymes A20 and cylindromatosis (CYLD). Defects in NF-κB regulation by A20 or CYLD lead to increased NF-κB activity and, consequently, hyperactivation of cells and promotion of lymphoid malignancies (8).

Phosphatases remove activating phosphate groups and, hence, are important regulators of TCR signaling (7, 9). The serine/threonine protein phosphatase 2A (PP2A) is ubiquitously expressed and together with PP1 the most abundant cellular serine/threonine phosphatase (10). PP2A acts in trimeric complexes consisting of a catalytic C subunit, a scaffolding A subunit, and a regulatory B subunit (11). The catalytic activity of PP2A is involved in almost all cellular processes such as various signaling pathways, cell cycle regulation, and migration. Because the catalytic PP2A subunit acts promiscuously with regard to phosphoprotein recognition and dephosphorylation, the regulatory subunits confer substrate specificity and subcellular localization (11). Up until now, four regulatory PP2A B subunit families with up to five family members have been identified, resulting in a plethora of differ-

*This work was supported in part by the Cooperation Program in Cancer Research of the Deutsches Krebsforschungszentrum (DKFZ) and the Israeli Ministry of Science, Culture, and Sport (MOST), the Deutsche Krebshilfe, the Wilhelm Sander Stiftung, and Helmholtz Alliance-Immunotherapy of Cancer Grant HA202.

1 Supported by the José Carreras Leukämie-Stiftung.

2 To whom correspondence should be addressed: Division of Immunogenetics (D030), German Cancer Research Center (DKFZ), Im Neuenheimer Feld 280, 69120 Heidelberg, Germany. Tel.: 49-6221-423718; Fax: 49-6221-411715; E-mail: p.krammer@dkfz.de.

3 The abbreviations used are: NF-κB, nuclear factor-κB; CYLD, cylindromatosis; IKK, IkB kinase; PHA, phytohemagglutinin; PKCθ, protein kinase Cθ; PMA, phorbol 12-myristate 13-acetate; PP2A, protein phosphatase 2A; qPCR, quantitative PCR; TCR, T cell receptor.
ent PP2A holoenzymes (11). To understand specific PP2A function, it is essential to characterize the regulatory subunit involved (12, 13). However, despite its importance in regulation of PP2A, the regulatory PP2A subunit that mediates NF-κB suppression in T cells is not yet defined.

We performed a siRNA screen in Jurkat T cells harboring a NF-κB-responsive luciferase reporter to identify phosphatases involved in TCR-mediated NF-κB signaling (14). Here, we identified and validated the PP2A regulatory subunit B56γ as suppressor of NF-κB in TCR signaling. B56γ was strongly up-regulated upon primary human T cell activation, and B56γ silencing increased IKK and IκBα phosphorylation upon TCR stimulation. In addition, phorbol 12-myristate 13-acetate (PMA)-induced NF-κB activity was suppressed by B56γ, indicating a role of B56γ downstream of PKCθ. B56γ silencing caused enhanced NF-κB activity, which resulted in increased NF-κB target gene expression in primary human T cells. Especially, expression of the important T cell growth factor IL-2 (15) was strongly enhanced on mRNA and protein level, when B56γ expression was silenced. Moreover, T cell proliferation was increased upon B56γ silencing. Thus, B56γ mediates suppression of NF-κB in TCR signaling. These findings contribute to a more detailed understanding of PP2A holoenzyme function in T cell signaling and help to understand the physiology of PP2A cellular function and the pathophysiology of diseases involving PP2A and NF-κB.

**EXPERIMENTAL PROCEDURES**

siRNA Screen and Screen Analysis—The RNAi screening procedure and the screen analysis were performed as described previously (14), except that single replicate siRNA transfections were analyzed separately. This allows for the analysis of the consistency of siRNA phenotypes. The threshold to discriminate hits from background noise was set to a Z score of +1.5 for at least two of three siRNAs transfected per gene in one replicate. To select for consistently scoring candidates, these siRNAs had to score with a Z score above +1.0 in the other replicates.

Cell Culture and Cell Stimulation—Human peripheral T cells were prepared and cultured as described previously (16). Autologous monocytes were isolated from T cell-depleted peripheral blood mononuclear cells and differentiated to dendritic cells as described previously (17). Alternatively, T cell-depleted peripheral blood mononuclear cells were cultured in RPMI 1640 medium with 1% AB-Serum for 1 h, and adherent monocytes were differentiated to dendritic cells accordingly. Jurkat T cells (JE6.1; Sigma) and GLuc-J16 Jurkat T cells (18) were cultured in Iscove’s modified Dulbecco’s medium supplemented with 10% FCS. To induce shRNA expression stably transduced cells were treated with 2 μg/ml doxycycline for 3 days. Jurkat T cells and primary human T cells were stimulated with agonistic soluble α-CD3 (OKT3), α-CD28 (CD82.2; BD Biosciences) and cross-linking goat anti-mouse monoclonal antibodies (Southern Biotechnology) to mimic TCR stimulation. Alternatively, cells were stimulated with PMA (Sigma) or TNFα (D. Männel, University of Regensburg, Germany).

Transfections and Reporter Assays—Jurkat T cells were transfected by electroporation (250 V, 950 microfarads) in 400 ml of Iscove’s modified Dulbecco’s medium using 10–30 μg of plasmid. For NF-κB reporter gene assays, 5 × 105 Jurkat T cells were cotransfected with pGL8XNF-κB-fos and pfos-LacZ as described previously (18). DNA transfection efficiency was normalized to LacZ expression. *Gaussian* activity was measured as described previously (14). For siRNA transfections, Jurkat T cells and primary human T cells were transfected by nucleofection (Amaza; Lonza) according to the manufacturer’s protocol with 1 μM siRNA. 72 h after siRNA transfection cells were used for analysis. Data were statistically analyzed with the two-tailed unpaired t test with Welch’s correction (*, p < 0.05; **, p < 0.01; ***, p < 0.001; ****, p < 0.0001).

siRNA and shRNA Sequences—siRNAs were purchased from Applied Biosystems (siCtrl UUCCGCAAGUGCUACAGTT, siB56γ #1 GCGUUGAGAGCUUACAUCATT, siB56γ #2 GUAAUGUUCUAAACGGAUUTT, siCYLD GAUGUUCUACUUCUAACATT). shRNA vectors were purchased from Open Biosystems. The mature B56γ antisense siRNA sequence was ATCCATTAATTATCTCC.

ELISA—Human IL-2 ELISA was purchased from BD Biosciences and performed according to the manufacturer’s protocol. Data were statistically analyzed with the two-tailed unpaired t test with Welch’s correction (*, p < 0.05; **, p < 0.01; ***, p < 0.001).

Secretion Assay—APC-IL-2 and PE-IFNγ secretion assays were obtained from Miltenyi Biotec and performed according to the manufacturer’s instructions. By catching secreted cytokines on the cell surface with a cytokine-specific antibody that can be coated on cells, the number of cells that secrete this cytokine were detected with a fluorescently labeled cytokine-specific antibody by FACS. This method is especially developed for detection of antigen-specific T cells. To gate on CD4⁺ T cells, cells were costained with anti-CD4-FITC antibody (RPA-T4; BD Biosciences)

T Cell Proliferation Assay—T cell proliferation was measured by [³H]thymidine incorporation as described previously (19). Data were statistically analyzed with the two-tailed unpaired t test with Welch’s correction (**, p < 0.01; ****, p < 0.0001).

Reverse Transcriptase PCR and Quantitative Real-time PCR—Reverse Transcriptase PCR and Quantitative Real-Time PCR were performed as described previously (14).

Lentiviral Transductions—Lentiviral transductions were performed as described previously (14).

Western Blotting—Cells were rinsed in cell lysis buffer (Cell Signaling) supplemented with protease and phosphatase inhibitors (Roche Applied Science). Proteins were resolved by 10% PAGE, transferred to Hybond nitrocellulose membrane (Amer sham Biosciences), and processed according to the manufacturer’s protocol. Quantification was performed using ImageJ software. Horseradish peroxidase (HRP)-conjugated secondary antibodies (Abs) were purchased from Southern Biotechnology Associates. The primary Abs used were anti-B56γ (kind gift from Liu X, University of California, Riverside, CA), anti-PP2A A subunit (6G3; Cell Signaling), anti-PP2A β (1D6; Santa Cruz Biotechnology), anti-HA (3F10; Roche Applied Science), anti-ERK (BD Biosciences), anti-β-actin (Sigma), anti-phospho-ERK (Santa Cruz Biotechnology), anti-IKKβ (Imgenex), anti-IKKα, anti-phos-
**B56γ Suppresses NF-κB in T Cells**

### RESULTS

**B56γ Suppresses TCR- and PMA-induced NF-κB Activation**—We have performed a siRNA screen in Jurkat T cells harboring a NF-κB-responsive luciferase reporter (GLuc-J16 T cells) to identify phosphatases involved in TCR-mediated NF-κB signaling (14). Because not all positive controls scored in the initial screen analysis, we reanalyzed our screen (Fig. 1A). Every gene analyzed in our screen was covered by three independent siRNAs transfected in duplicate. The Z score, a measure of the biological effect of a siRNA reflecting the number of standard deviations from the population mean (20), was applied to replicate transfections separately in our new analysis. To identify PP2A regulatory subunits that mediate suppression of NF-κB in TCR signaling, we specifically analyzed the effects of these subunits included in our new siRNA screen analysis (Fig. 1B). PPP2R25C, the gene encoding B56γ, was the only PP2A regulatory subunit meeting our refined threshold criteria, whereas all other PP2A regulatory subunits did not score at this threshold, indicating a specific role of B56γ in TCR-mediated NF-κB signaling.

Subsequently, we confirmed the results of the reanalysis with NF-κB luciferase reporter gene assays. Knockdown of B56γ by two independent siRNAs increased NF-κB activity upon TCR stimulation compared with control siRNA-treated cells (Fig. 2, A and B). Conversely, overexpression of B56γ decreased NF-κB activity upon TCR stimulation compared with vector control (Fig. 2, C and D). In addition, NF-κB activation by TNFα was decreased by ectopic expression of B56γ compared with vector control (Fig. 2, E). Moreover, activation of NF-κB by PMA, a pharmacological diacylglycerol mimetic, was suppressed upon overexpression of B56γ (Fig. 2, C and F). PMA circumvents proximal TCR signaling and directly activates PKCθ to induce NF-κB activation (21). Hence, B56γ must act on or downstream of PKCθ.

**B56γ Suppresses NF-κB Activation between PKCθ and the IKK Complex**—To further investigate the point of interference of B56γ with NF-κB signaling we analyzed IKK and IκBα phosphorylation upon TCR stimulation in B56γ knockdown cells. Knockdown of B56γ led to increased phosphorylation of the IKK complex 10 min after stimulation compared with control, and this increase lasted up to 45 min of stimulation (Fig. 3). In addition, phosphorylation and degradation of IκBα were elevated in B56γ knockdown cells compared with control cells. Moreover, TCR-induced activation of the MAPK pathway was analyzed. Phosphorylation of ERK was similar in B56γ knockdown cells compared with control cells (Fig. 3). In conclusion, B56γ regulates NF-κB signaling on the level or between PKCθ and IKK complex phosphorylation.

**B56γ Is Up-regulated upon TCR Stimulation**—To address the function of B56γ in primary human peripheral blood T cells we tested B56γ expression in resting cells and upon T cell activation. Primary human T cells were activated with the mitogen phytohemagglutinin (PHA) and expanded in the presence of IL-2 for up to 6 days. B56γ was expressed at basal levels in the resting state (Fig. 4A). Interestingly, B56γ expression was up-regulated after 1 day of PHA stimulation and remained high during T cell expansion. To test when B56γ was up-regulated...
B56γ Suppresses NF-κB in T Cells

Further kinetics were applied using TCR-agonistic antibodies. B56γ expression was up-regulated after 11 h of TCR stimulation (Fig. 4B). Collectively, B56γ expression is up-regulated upon TCR stimulation, which might point to a role of B56γ in the control of NF-κB activation in activated T cells.

B56γ Suppresses NF-κB Target Gene Expression—To further assess the physiological function of B56γ-mediated NF-κB suppression in primary human T cells, TCR-induced NF-κB target gene transcription was analyzed upon knockdown of B56γ. siRNA-transfected cells were stimulated via the TCR, and NF-κB target gene transcription was analyzed using a qPCR array (Fig. 4, A and B). B56γ-deficient cells exhibited a specific

---

**FIGURE 2.** B56γ suppresses NF-κB activity upon TCR, TNFα, and PMA stimulation. A and B, GLuc-J16 T cells were transfected with nontargeting siRNA (siCtrl) and two independent siRNA oligonucleotides targeting B56γ (siB56γ #1 and #2). After 72 h, B56γ knockdown efficiency was assessed by qPCR (A), and cells were stimulated via the TCR for 5 h with α-CD3 + α-CD28 antibodies or left unstimulated (B). NF-κB activity was measured with a Gaussia luciferase assay. Values were normalized to cell viability using CellTiter-Glo. C–F, Jurkat T cells were transfected with an expression plasmid encoding HA-tagged B56γ or empty vector control. In addition, a NF-κB reporter system was cotransfected to determine NF-κB activity. 48 h after transfection B56γ overexpression was confirmed by Western blotting (C), and cells were stimulated as indicated for 5 h (D–F). NF-κB activity was measured using a reporter gene assay. **, p < 0.01; ***, p < 0.001; ****, p < 0.0001. Error bars, S.D.

---

**FIGURE 3.** B56γ suppresses TCR-induced IKK and IkBa phosphorylation. Jurkat T cells were stably transduced with lentiviral particles containing inducible B56γ shRNA or shRNA control. Transduced cells were selected with puromycin. Inducible B56γ knockdown cells and control cells were treated with doxycycline to induce shRNA expression. After 3 days, cells were stimulated via the TCR with 0.5 µg/ml α-CD3 + α-CD28 antibodies for the indicated time points. Lysates were analyzed using Western blotting. Bands were quantified, and the phospho signal was normalized to the respective total protein signal. The siCtrl 0 time point was set to 1. Numbers below the panels indicate relative quantification of signal intensity. One representative of three donors is shown.

---

**FIGURE 4.** B56γ expression is up-regulated upon T cell stimulation. A, primary human T cells were stimulated for 24 h with PHA and expanded in the presence of exogenous IL-2 for up to 6 days. Samples were taken at the indicated time points, and protein lysates were analyzed by Western blotting. Bands were quantified, and the B56γ signal was normalized to the respective actin signal. The 0 time point was set to 1. Numbers below the panels indicate relative quantification of signal intensity. One representative of five donors is shown. B, primary human T cells were stimulated via the TCR with α-CD3 + α-CD28 antibodies for the indicated time points. Lysates were analyzed by Western blotting. Bands were quantified, and the B56γ signal was normalized to the respective actin signal. The 0 time point was set to 1. Numbers below the panels indicate relative quantification of signal intensity. One representative of three donors is shown.
NF-κB target gene signature compared with control cells with most target genes up-regulated by at least 1.5-fold. After 60 min of stimulation, especially IL-2 and IFNγ transcription were increased upon B56γ knockdown by 2.8- and 2.9-fold, respectively. To substantiate the qPCR array results, we tested IL-2 transcription in more detailed kinetics by conventional qPCR (Fig. 5, A and C). IL-2 transcription was increased by approximately 2-fold. In conclusion, B56γ-mediated NF-κB suppression regulates transcription of several NF-κB target genes including IL-2.

To confirm these results, the induction of IL-2 was analyzed on the protein level. For this purpose, activated primary human T cells were transfected with two independent siRNA oligonucleotides targeting B56γ. siRNAs targeting the known NF-κB suppressor CYLD (8, 22) or nontargeting siRNA were used as controls. Knockdown of B56γ led to an increase in IL-2 secretion for both B56γ siRNA oligonucleotides compared with siRNA control (Fig. 6, A and B). Knockdown of CYLD increased IL-2 secretion to a similar extent. To evaluate the results obtained for all six donors, the -fold increase of IL-2 secretion for knockdown of B56γ and CYLD compared with siRNA control was calculated for both concentrations of TCR-stimulating antibodies (Fig. 6, C and D). The median IL-2 induction was ~2-fold for knockdown of B56γ with two independent siRNA oligonucleotides and for knockdown of CYLD, respectively. In addition, we analyzed IL-2 and IFNγ secretion upon T cell stimulation with staphylococcal enterotoxin B in the presence of autologous dendritic cells using a secretion assay (Fig. 6, E and F). Upon B56γ knockdown, IFNγ- and IL-2-secreting CD4+ T cells were increased compared with control, further substantiating our results.

B56γ Suppresses T Cell Proliferation—To assess whether T cell proliferation was also affected by B56γ-mediated suppression of NF-κB, we performed T cell proliferation assays. siRNA-transfected primary human T cells were stimulated via the TCR, and after 5 days [3H]thymidine incorporation was measured (Fig. 7). Knockdown of B56γ led to an increase in proliferation for both B56γ siRNA oligonucleotides compared with siRNA control (Fig. 7, A and B). The median -fold increase in proliferation of six independent donors comparing B56γ knockdown and control cells was 7.3-fold (Fig. 7C). In conclusion, our data indicate a role of B56γ as suppressor of NF-κB-driven activation in human T cells.

DISCUSSION

NF-κB is an essential transcription factor in immunity, and aberrant regulation of NF-κB contributes to disorders such as cancer and autoimmunity (1–4). Understanding of NF-κB reg-
ulation in physiological and pathophysiological conditions will enable the development of targeted therapies. NF-κB is essential for activation of normal T cells (5). In lymphomas, aberrant NF-κB activity is described to contribute to pathogenesis (23). Activation of NF-κB upon TCR stimulation by phosphorylation is well studied (6). However, termination and fine-tuning of TCR signaling by dephosphorylation are only partially understood (7).

Phosphatases are important negative regulators in signal transduction (11). Serine/threonine phosphatases act in dimeric or trimeric complexes consisting of a promiscuous catalytic subunit, a scaffolding subunit, and a specificity-mediating regulatory subunit (11). The catalytic activity of the serine/threonine phosphatase PP2A is described to be involved in NF-κB regulation (24–28). However, the regulatory subunit that confers specificity to TCR-mediated NF-κB regulation by PP2A was so far undefined.

To our knowledge, this study is the first description of the PP2A regulatory subunit B56 in TCR-mediated NF-κB regulation. We performed a siRNA screen to identify phosphatases involved in TCR signaling (14). B56 was the only PP2A regulatory subunit that scored in this siRNA screen. Both B56 siRNAs scoring in our screen showed a similar strength in NF-κB enhancement, indicating the robustness of our findings. Furthermore, we provide several lines of evidence that the PP2A regulatory subunit B56 mediates suppression of TCR-
induced NF-κB activity. (i) Knockdown of B56γ enhanced TCR-mediated NF-κB activity, and conversely, overexpression of B56γ suppressed NF-κB activity upon TCR triggering. (ii) In primary human T cells B56γ knockdown enhanced TCR-induced NF-κB target gene expression on mRNA and protein levels by ~2-fold. Notably, knockdown of the known negative NF-κB regulator CYLD increased TCR-induced IL-2 secretion to a similar extent. (iii) T cell proliferation was enhanced upon B56γ knockdown. (iv) TCR-induced IKK phosphorylation, IκBα phosphorylation, and IκBα degradation were enhanced upon B56γ knockdown, whereas TCR-induced ERK phosphorylation remained unaffected.

PP2A is described to regulate a plethora of signaling pathways and cellular processes (11). However, PP2A is a trimeric complex consisting of a catalytic C subunit, a scaffolding A subunit, and a regulatory B subunit. The PP2A C and A subunits are represented by two homologous genes in humans, but for the regulatory B subunits a multitude of different unrelated protein families exists (11). The regulatory B subunits confer specificity to the dephosphorylation activity of PP2A and hence are essential to drug a specific PP2A function (12, 13). PP2A regulatory subunits, however, have not been investigated in detail. Several studies link PP2A catalytic activity to regulation of IKK phosphorylation (26, 29, 30). Our results show that B56γ suppresses IKK phosphorylation upon TCR stimulation, indicating a role for B56γ on the level of IKK phosphorylation or upstream. In addition, the PP2A core enzyme, consisting of the catalytic PP2A Cα and scaffolding PP2A Aα subunit, is described to dephosphorylate CARMA1 (CARD-containing membrane-associated guanylate kinase protein 1), a mediator of NF-κB activation in T cells, and to consequently suppress IL-2 secretion upon TCR triggering (24). It is likely that B56γ is the missing regulatory subunit to form the PP2A holoenzyme. B56γ may strengthen phosphatase substrate interaction or modulate phosphatase activity.

Moreover, B56γ suppressed PMA-induced NF-κB activity. PMA circumvents proximal TCR signaling and directly activates PKCθ (21). Hence, B56γ must act downstream or on the level of PKCθ to suppress NF-κB (Fig. 8). Besides TCR triggering and PMA treatment, TNFα stimulation also induces NF-κB activation in T cells (6). TNFα-induced NF-κB activity was also suppressed by B56γ in T cells. In line with these findings, B56γ was reported to mediate dephosphorylation of TRAF2 (TNF receptor-associated factor 2) upon TNFα stimulation in an astrocyte cell line (26). TRAF2 is a mediator of TNFα-induced NF-κB activation that is not relevant in TCR signaling (6, 31). This indicates a dual role of B56γ in NF-κB regulation, suppressing both TNFα- and TCR-mediated NF-κB activation by distinct mechanisms.

FIGURE 7. B56γ suppresses TCR-induced proliferation. Primary human T cells were activated with PHA and IL-2 for 3 days. Afterward cells were transfected with nontargeting siRNA (Ctrl) or two independent siRNA oligonucleotides targeting B56γ. A, 72 h after transfection, knockdown was confirmed by Western blotting. B, 72 h after transfection, cells were stimulated for 5 days via the TCR with indicated concentrations of α-CD3 + α-CD28 antibodies or left unstimulated. T cell proliferation was measured using [3H]thymidine incorporation. Values represent mean, and error bars show S.D. C, six independent donors were analyzed as in A and B, and the increase in proliferation upon B56γ knockdown was calculated relative to siCtrl. Horizontal lines represent the median. **, p < 0.01; ****, p < 0.0001.
We have shown enhanced expression of B56γ upon activation of primary human T cells. This may imply a potential role of B56γ in differentiation of these cells. In line with our findings, two other B56 family members, B56β and B56δ, were shown to be up-regulated during differentiation of a neuron-like cell line (32). In addition, also other modulators of NF-κB are reported to be up-regulated upon T cell activation (14, 33, 34).

NF-κB is relevant in many malignancies. Especially in lymphomas and leukemias, regulators of NF-κB are mutated or differentially expressed (14, 35–37). Besides a general connection between PP2A and cancer, B56γ is implicated as a tumor suppressor in different settings (12). B56γ is described to be down-regulated in human melanoma and in several lung cancer cell lines (38, 39). Moreover, deregulation of several oncogenic signaling pathways upon depletion of B56γ, including Akt and Wnt signaling, have been reported (12). In addition, the tumor suppressor p53 was described to be activated by B56γ-containing PP2A. Importantly, NF-κB and p53 fulfill opposing roles in tumorigenesis, acting as protooncogene and tumor suppressor, respectively (3). B56γ not only activates p53 and regulates Akt and Wnt signaling, our findings, that B56γ, in addition, suppresses NF-κB add further weight to the tumor suppressive role of B56γ and make it an interesting target for cancer therapy.

Acknowledgments—We thank H. Weyd for critical discussion; A. Schrader, T. Schmenger, and U. Matiba for excellent technical assistance; and X. Liu (University of California, Riverside, CA) for kindly providing the B56γ antibody.

REFERENCES
1. Hayden, M. S., West, A. P., and Ghosh, S. (2006) NF-κB and the immune response. Oncogene 25, 6758–6780
2. Chaturvedi, M. M., Sung, B., Yadav, V. R., Kannappan, R., and Aggarwal, B. B. (2011) NF-κB addiction and its role in cancer: “one size does not fit all.” Oncogene 30, 1615–1630
3. Perkins, N. D. (2012) The diverse and complex roles of NF-κB subunits in cancer. Nat. Rev. Cancer 12, 121–132
4. Sun, S. C., Chang, J. H., and Jin, J. (2013) Regulation of nuclear factor-κB in autoimmunity. Trends Immunol. 34, 282–289
5. Smith-Garvin, J. E., Koretzky, G. A., and Jordan, M. S. (2009) T cell activation. Annu. Rev. Immunol. 27, 591–619
6. Hayden, M. S., and Ghosh, S. (2004) Signaling to NF-κB. Genes Dev. 18, 2195–2224
7. Ruland, J. (2011) Return to homeostasis: down-regulation of NF-κB responses. Nat. Immunol. 12, 709–714
8. Harhaj, E. W., and Dixit, V. M. (2012) Regulation of NF-κB by deubiquitinas. Immunol. Rev. 246, 107–124
9. Acuto, O., Di Bartolo, V., and Michel, F. (2008) Tailoring T-cell receptor signals by proximal negative feedback mechanisms. Nat. Rev. Immunol. 8, 699–712
10. Virshup, D. M., and Shenolikar, S. (2009) From promiscuity to precision: protein phosphatases get a makeover. Mol. Cell 33, 537–545
11. Janssens, V., and Goris, J. (2001) Protein phosphatase 2A: a highly regulated family of serine/threonine phosphatases implicated in cell growth and signalling. Biochem. J. 354, 417–439
12. Westermarck, J., and Hahn, W. C. (2008) Multiple pathways regulated by the tumor suppressor PP2A in transformation. Trends Mol. Med. 14, 152–160
13. Lambrecht, C., Haesen, D., Sents, W., Ivanova, E., and Janssens, V. (2013) Structure, regulation, and pharmacological modulation of PP2A phosphatases. Methods Mol. Biol. 1053, 283–305
14. Brechmann, M., Mock, T., Nickles, D., Kiessling, M., Weit, N., Breuer, R., Müller, W., Wabnitz, G., Frey, F., Nicolay, J. P., Booken, N., Samstag, Y., Klemke, C. D., Herling, M., Boutou, M., Krammer, P. H., and Arnold, R. (2012) A PP4 holoenzyme balances physiological and oncogenic nuclear factor-κB signaling in T lymphocytes. Immunity 37, 697–708
15. Boyman, O., and Sprent, J. (2012) The role of interleukin-2 during homeostasis and activation of the immune system. Nat. Rev. Immunol. 12, 180–190
**B56γ Suppresses NF-κB in T Cells**

16. Peter, M. E., Kischkel, F. C., Scheuerpflug, C. G., Medema, J. P., Debatin, K. M., and Krammer, P. H. (1997) Resistance of cultured peripheral T cells towards activation-induced cell death involves a lack of recruitment of FLICE (MACH/caspase 8) to the CD95 death-inducing signaling complex. *J. Immunol.* 159, 1207–1212

17. Weyd, H., Abeler-Dörner, L., Linke, B., Mahr, A., Jahndel, V., Pfirang, S., Schnölzer, M., Falk, C. S., and Krammer, P. H. (2013) Annexin A1 on the surface of early apoptotic cells suppresses CD8⁺ T cell immunity. *PloS One* 8, e62449

18. Arnold, R., Liou, J., Drexler, H. C., Weiss, A., and Kiefer, F. (2001) Caspase-mediated cleavage of hematopoietic progenitor kinase 1 (HPK1) converts an activator of NFκB into an inhibitor of NFκB. *J. Biol. Chem.* 276, 14675–14684

19. Schmidt, A., Oberle, N., Weiss, E. M., Vobis, D., Frischbutter, S., Baumgras, R., Falk, C. S., Haag, M., Brügger, B., Lin, H., Mayr, G. W., Reichardt, P., Gunzer, M., Suri-Payer, E., and Krammer, P. H. (2011) Human regulatory T cells rapidly suppress T cell receptor-induced Ca²⁺, NF-κB, and NFAT signaling in conventional T cells. *Sci. Signal.* 4, ra90

20. Birmingham, A., Selfors, L. M., Forster, T., Wrobel, D., Kennedy, C. J., Shanks, E., Santoyo, J., J., S. Santoyo-Lopez, J., and C. Shamu, C. E. (2009) Statistical methods for analysis of high-throughput RNA interference screens. *Nat. Methods* 6, 569–575

21. Truneh, A., Albert, F., Golstein, P., and Schmitt-Verhulst, A. M. (1985) Resistance of cultured peripheral T cells towards activation-induced cell death involves a lack of recruitment of FLICE (MACH/caspase 8) to the CD95 death-inducing signaling complex. *Euro. J. Immunol.* 27, 1207–1212

22. Kovalenko, A., Chable-Bessia, C., Cantarella, G., Israël, A., Wallach, D., Jost, P. J., and Ruland, J. (2007) Aberrant NF-

23. Jost, P. J., and Ruland, J. (2007) Aberrant NF-

24. Eitelhuber, A. C., Warth, S., Schimmack, G., Düwel, M., Hadian, K., Demski, K., Beisker, W., Shinohara, H., Kurosaki, T., Heissmeyer, V., and Krappmann, D. (2011) Dephosphorylation of Carma1 by PP2A negatively regulates NFκB signalling by deubiquitination. *Nature* 421, 801–805

25. Jost, P. J., and Ruland, J. (2007) Aberrant NFκB signalling in lymphoma: mechanisms, consequences, and therapeutic implications. *Blood* 109, 2700–2707

26. Eitelhuber, A. C., Warth, S., Schimmack, G., Düwel, M., Hadian, K., Demski, K., Beisker, W., Shinohara, H., Kurosaki, T., Heissmeyer, V., and Krappmann, D. (2011) Dephosphorylation of Carma1 by PP2A negatively regulates NFκB signalling by deubiquitination. *Nature* 421, 801–805

27. Yang, J., Fan, G. H., Wadzinski, B. E., Sakurai, H., and Richmond, A. (2001) Protein phosphatase 2A interacts with and directly dephosphorylates RelA. *J. Biol. Chem.* 276, 47828–47833

28. Li, S., Wang, L., Berman, M. A., Zhang, Y., and Dorf, M. E. (2006) RNAi screen in mouse astrocytes identifies phosphatases that regulate NF-κB signaling. *Mol. Cell* 24, 497–509

29. Sun, S. C., Magirwar, S. B., and Harhaj, E. (1995) Activation of NF-κB by phosphatase inhibits the phosphorylation of 1κBα at phosphatase 2A-sensitive sites. *J. Biol. Chem.* 270, 18347–18351

30. Rieckmann, P., Thévenin, C., and Kehrl, J. H. (1992) Okadaic acid is a potent inducer of AP-1, NF-κB, and tumor necrosis factor-α in human B lymphocytes. *Biochem. Biophys. Res. Commun.* 187, 51–57

31. Fu, D. X., Kuo, Y. L., Liu, B. Y., Yeang, K. T., and Giam, C. Z. (2003) Human T-lymphotropic virus type I tax activates IκB kinase by inhibiting IκB kinase-associated serine/threonine-protein phosphatase 2A. *J. Biol. Chem.* 278, 1487–1493

32. McCright, B., Rivers, A. M., Audlin, S., and Virshup, D. M. (1996) The B56 family of protein phosphatase 2A (PP2A) regulatory subunits encodes differentiation-induced phosphoproteins that target PP2A to both nucleus and cytoplasm. *J. Biol. Chem.* 271, 22081–22089

33. Autschbach, F., Palou, E., Mechtcheri, G., Rohr, C., Pirotto, F., Gasser, N., Otto, H. F., Schraven, B., and Gaya, A. (1999) Expression of the membrane protein tyrosine phosphatase CD148 in human tissues. *Tissue Antigens* 54, 485–498

34. Cohen, S., Dadi, H., Shao, E., Sharfe, N., and Roifman, C. M. (1999) Cloning and characterization of a lymphoid-specific, inducible human protein tyrosine phosphatase, LyP. *Blood* 93, 2013–2024

35. Agati, T., Motegi, M., Tamura, A., Suzuki, R., Hosokawa, Y., Suzuki, H., Ota, H., Nakamura, S., Morishima, Y., Taniwaki, M., and Seto, M. (1999) A novel gene, *MALT1* at 18q21, is involved in t(11;18) (q21;q21) found in low-grade B-cell lymphoma of mucosa-associated lymphoid tissue. *Oncogene* 18, 5785–5794

36. Bignell, G. R., Warren, W., Seal, S., Takahashi, M., Rapley, E., Barfoot, R., Green, H., Brown, C., Biggs, P. J., Lakhan, S. R., Jones, C., Hansen, J., Blair, E., Hofmann, B., Siebert, R., Turner, G., Evans, D. G., Schrander-Stumpel, C., Beemer, F. A., van Deenen, L. H. M., Halley, D., Delpech, B., Cleve-land, M. G., Leigh, I., Leisti, J., and Rasmussen, S. (2000) Identification of the familial cylindromatosis tumour-suppressor gene. *Nat. Genet.* 25, 160–165

37. Morgan, J. A., Yin, Y., Borowsky, A. D., Kuo, F., Nourmand, N., Koontz, J. I., Reynolds, C., Sorensen, L., Griffith, C. A., Graeme-Cook, F., Harris, N. L., Weisenburger, D., Pinkus, G. S., Fletcher, I. A., and Sklar, J. (1999) Breakpoints of the (11;18)(q21;q21) found in low-grade B-cell lymphoma of mucosa-associ-47ed lymphoid tissue (MALT) lymphoma lie within or near the previously undescribed gene MALT1 in chromosome 18. *Cancer Res.* 59, 6205–6213

38. Deichmann, M., Polychronidis, M., Wacker, J., Thome, M., and Näher, H. (2001) The protein phosphatase 2A subunit By gene is identified to be differentially expressed in malignant melanomas by subtractive suppression hybridization. *Melanoma Res.* 11, 577–585

39. Chen, W., Possmato, R., Campbell, K. T., Plattner, C. A., Pallas, D. C., and Hahn, W. C. (2004) Identification of specific PP2A complexes involved in human cell transformation. *Cancer Cell* 5, 127–136