Mutations in G protein β subunits promote transformation and kinase inhibitor resistance

Akinori Yoda1, Guillaume Adelmant2,3, Jerome Tamburini1, Bjoern Chapuy1, Nobuaki Shindoh1,4, Yuka Yoda1, Oliver Weigert5, Nadja Kopp1, Shuo-Chieh Wu1, Sunhee S Kim1, Huiyun Liu1, Trevor Tivey1, Amanda L Christie1, Kutlu G Elpek6,7, Joseph Card2, Kira Gritsman1, Jason Gotlib8, Michael W Deininger9, Hideki Makishima10, Shannon J Turley6, Nathalie Javidi-Sharifi11, Jaroslaw P Maciejewski10, Siddhartha Jaiswal12,13, Benjamin L Ebert13,14, Scott J Rodig15, Jeffrey W Tyner11, Jarrod A Marto2,3, David M Weinstock1,14 & Andrew A Lane1

Activating mutations in genes encoding G protein α (Gα) subunits occur in 4–5% of all human cancers1, but oncogenic alterations in Gβ subunits have not been defined. Here we demonstrate that recurrent mutations in the Gβ proteins GNB1 and GNB2 confer cytokine-independent growth and activate canonical G protein signaling. Multiple mutations in GNB1 affect the protein interface that binds Gα subunits as well as downstream effectors and disrupt Gα interactions with the Gβγ dimer. Different mutations in Gβ proteins clustered partly on the basis of lineage; for example, all 11 GNB1 K57 mutations were in myeloid neoplasms, and seven of eight GNB1 I80 mutations were in B cell neoplasms. Expression of patient-derived GNB1 variants in Cdkn2a-deficient mouse bone marrow followed by transplantation resulted in either myeloid or B cell malignancies. In vivo treatment with the dual PI3K-mTOR inhibitor BEZ235 suppressed GNB1-induced signaling and markedly increased survival. In several human tumors, mutations in the gene encoding GNB1 co-occurred with oncogenic kinase alterations, including the BCR-ABL fusion protein, the V617F substitution in JAK2 and the V600K substitution in BRAF. Coexpression of patient-derived GNB1 variants with these mutant kinases resulted in inhibitor resistance in each context. Thus, GNB1 and GNB2 alterations confer transformed and resistance phenotypes across a range of human tumors and may be targetable with inhibitors of G protein signaling.

Numerous somatic mutations are present in less than 5% of cases across multiple tumor types. To extensively catalog mutations in these ‘long-tail’ genes will require sequencing thousands of additional specimens from each tumor subset, a daunting challenge for rare malignancies3. A fraction of mutations in long-tail genes are ‘gain of function’ and may represent tractable therapeutic targets, confer resistance to particular agents or underlie “exceptional responses”4. The timely identification of clinically actionable mutations is particularly pressing, as focused sequencing panels to guide targeted therapeutics are becoming widely used.

To functionally interrogate tumors for gain-of-function alterations, we constructed retroviral cDNA libraries from individual cancers and transduced them into cytokine-dependent cells, such as murine BaF3 cells that express the antiapoptotic protein BCL2 (BaF3-BCL2) or the MYC oncogene (BaF3-MYC)5,6. In this system, oncogenic variants of EGFR, FLT3, RAS and ALK resulting from single nucleotide, insertion or deletion, splice-variant or gene fusion alterations confer cytokine-independent growth5. We then isolated proliferating clones and sequenced the integrated cDNA (Fig. 1a).

We constructed a cDNA library from a patient’s bone marrow infiltrated with blast plasmacytoid dendritic cell neoplasm (BPDCN), an acute leukemia subtype with no obviously targetable driver oncogene7,8, and transduced it into BaF3-BCL2 cells. Three independent clones capable of growth in the absence of IL-3 were isolated. All three harbored GNB1 with a lysine-to-glutamic acid substitution at codon 89 (GNB1 K89E). We confirmed that GNB1 K89E also confers IL-3-independent growth in BaF3-MYC cells (Fig. 1b).

GNB1 encodes a β subunit of heterotrimeric G proteins, which consist of Gα, Gβ and Gγ components that mediate signaling downstream of G protein–coupled receptors9. Upon activation, heterotrimeric G proteins dissociate to form two functional molecules:

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1Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts, USA. 2Department of Cancer Biology and Blais Proteomics Center, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts, USA. 3Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts, USA. 4Drug Discovery Research, Astellas Pharma Inc., Tsukuba, Ibaraki, Japan. 5Department of Medicine III, Campus Grosshadern, Ludwig-Maximilians-University, and Helmholtz Center, Munich, Germany. 6Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts, USA. 7Jounce Therapeutics, Inc., Cambridge, Massachusetts, USA. 8Division of Hematology, Stanford Cancer Institute, Stanford University School of Medicine, Stanford, California, USA. 9Division of Hematology and Hematologic Malignancies, Huntsman Cancer Institute, The University of Utah, Salt Lake City, Utah, USA. 10Department of Translational Hematology and Oncology Research, Taussig Cancer Institute, Cleveland Clinic, Cleveland, Ohio, USA. 11Department of Cell, Developmental and Cancer Biology, Oregon Health and Science University, Knight Cancer Institute, Portland, Oregon, USA. 12Department of Pathology, Massachusetts General Hospital, Boston, Massachusetts, USA. 13Division of Hematology, Brigham and Women’s Hospital, Boston, Massachusetts, USA. 14Broad Institute, Cambridge, Massachusetts, USA. 15Department of Pathology, Brigham and Women’s Hospital, Boston, Massachusetts, USA.

Correspondence should be addressed to D.M.W. (dweinstock@partners.org) or A.A.L. (andrew_lane@dfci.harvard.edu).

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Figure 1  Recurrent GNB1 and GNB2 mutations confer cytokine-independent growth. (a) Schematic representation of functional screening using patient-derived cDNA libraries and cytokine-dependent cells. (b) IL-3–independent growth of BaF3-MYC cells expressing wild-type (WT) GNB1, GNB1 K89E or empty vector. *P < 0.05 vs. wild-type; **P < 0.01 vs. wild-type; ***P < 0.01 vs. empty by t-test; error bars represent mean ± s.d. of three replicates. (c) Alterations identified in GNB1 and GNB2 in human cancers. Tumor types are indicated for recurrent mutation sites with three or more missense alterations. AML, acute myelogenous leukemia; aCML, atypical chronic myelogenous leukemia; PV, polycythemia vera; B-ALL, B-cell acute lymphocytic leukemia; CLL, chronic lymphocytic leukemia; FL, follicular lymphoma; DLBCL, diffuse large B cell lymphoma; indel, insertion or deletion. (d) Cell counts of IL-3–independent BaF3-MYC cells expressing GNB1 and GNB2 variants or empty vector 14 d after cytokine withdrawal. Data are relative to wild-type for GNB1 or GNB2. G41* and R214* indicate nonsense mutations. (e) GM-CSF–independent growth of TF-1 cells as in (d). *P < 0.05 and **P < 0.01 vs. wild-type by t-test; error bars represent mean ± s.d. of three replicates.

Figure 2  Mutant Gβ proteins lose interaction with Gα subunits and induce activation of PI3K-AKT-mTOR and MAPK pathways. (a) Molecular representation of a heterotrimeric G protein (blue, Gα; red, Gβ; yellow, Gγ) based on a crystal structure (PDB 1GP2). Corresponding sites of multiple recurrent oncogenic mutations are indicated and side chains of the residues are shown in green. (b) Silver staining of anti-Flag immunoprecipitates (IP) from BaF3-MYC cells expressing wild-type (WT) GNB1 or GNB1 K89E. The band indicated by the arrow was analyzed by mass spectrometry, and the three Gx subunits indicated were identified. (c) Unsupervised clustering analysis of proteins identified in tandem affinity purification–mass spectrometry (TAP-MS) analyses of GNB1 on the basis of their relative association to wild-type and mutant GNB1. Normalized abundance of G protein subunits and PDCL associated with mutant compared to WT GNB1 is shown (red, more binding to mutant than to wild-type; blue, less binding to mutant). (d) Western blotting of anti-Flag IP and whole-cell lysates (WCL) from BaF3-MYC cells expressing Gβ variants. (e) GSEA plots of the indicated sets in TF-1 cells transduced with empty vector isolated after GM-CSF withdrawal (K89E starved, n = 3) compared to the combined data from TF-1 cells transduced with empty vector isolated after GM-CSF withdrawal (n = 3 each; total of n = 9 controls). (f) Heat map of leading edge genes in the AKT gene set from GSEA in TF-1 GNB1 K89E or empty vector-transduced cells with (+) or without (−) cytokine starvation. (g) Western blot analysis of TF-1 cells expressing GNB1 variants or empty vector. p-, phosphorylated.

the GTP-bound Gα monomer and the Gβγ dimer, both of which bind and activate downstream effector proteins. Gain-of-function mutations of Gα have been described in many cancers. However, oncogenic mutations in Gβ have not been explored.
We searched publicly available databases and published reports across all tumor types, and our unpublished sequencing data in hematologic malignancies (Supplementary Table 1) to identify somatic mutations of GNB1 and the closely related family member GNB2. We identified amino acids recurrently altered across multiple tumor types (Fig. 1c and Supplementary Table 1). For example, GNB1 mutations were present in 3 (1.9%) of 157 cases of myelodysplastic syndrome (MDS) or secondary acute myeloid leukemia in one cohort13 and 5 (0.53%) of 944 cases of MDS in another cohort14. Different codon mutations clustered to some extent on the basis of lineage. Most notably, all 11 GNB1 K57 mutations were in myeloid neoplasms compared with one of eight GNB1 180 mutations (P < 0.001 by two-tailed Fisher’s exact test). The remaining 180 mutations were in B cell neoplasms (Fig. 1c). We confirmed that multiple GNB1 variants conferred cytokine-independent growth in IL-3–dependent TF-1 myeloid cells (Fig. 1e).

The recurrent mutations affecting residues K57, K78, I80, K89 and M101 are located on the Gβ protein surface that interacts with Gα subunits and downstream effectors15 (Fig. 2a). This is similar to recurrently altered residues in the G protein α subunit SNAS (R201 and Q227) and GNAQ and GNA11 (Q209) that are believed to mediate interactions with Gβγ subunits16. Immunoprecipitation of wild-type and mutant (K89E) Flag epitope–tagged GNB1 (Flag–GBN1) revealed a 40-kDa species specifically associated with the wild-type protein (Fig. 2b). Mass spectrometry analysis of this band detected multiple peptides mapping uniquely to the Gα subunits GNAI2, GNAI3 and GNA11 (Supplementary Table 2). Tandem affinity purification–mass spectrometry analysis using stable isotope-labeled amino acids in culture (SILAC)17 further demonstrated reduced binding of GNB1 K89E, GNB1 I80T and GNB1 K57E to almost all detected Gα subunits but not to Gγ subunits or to the G protein chaperone PDCL18 (Fig. 2c and Supplementary Table 3). This was confirmed by immunoblotting (Fig. 2d). Cell growth promoted by Gβγ mutations was not due to liberation of unbound Gz subunits, because treatment with pertussis toxin, which blocks Gz signaling19,20, did not inhibit growth or ERK phosphorylation in cells harboring GNB1 mutations (Supplementary Fig. 1).

Gβγ activates multiple downstream signaling pathways, including phosphatidylinositol 3-kinase 3-kinase (PI3K) and its downstream signaling proteins AKT and mTOR21, MAP kinase (MAPK)22 and phospholipase Cβ (PLC)23. As expected, gene-expression profiling and gene-set enrichment analysis (GSEA)24 showed that signatures of AKT-mTOR-FOXO3, RAS-ERK and PLC pathways from the Molecular Signatures Database of the Broad Institute (MSigDB) were highly enriched in TF-1 cells expressing GNB1 K89E (Fig. 2e). Leading edge analysis24 identified subsets within each signature that contribute most to the enrichment (Fig. 2f and Supplementary Fig. 2). Increased phosphorylation of the PI3K substrate AKT S473; the MAPK signaling proteins MEK S217 and S221; ERK T202 and Y204; and the mTOR substrate P70S6K T389 AKT S473; MEK S217 and S221; ERK T202 and Y204; and P70S6K T389 was confirmed in cells expressing GNB1 mutants (Fig. 2g). Phosphoproteomics of SILAC-labeled TF-1 cells expressing wild-type GNB1 or GNB1 K89E identified additional sites with increased phosphorylation in cells expressing GNB1 K89E (Supplementary Tables 4 and 5).

To determine whether GNB1 mutants promote transformation in vivo, we performed two separate mouse bone marrow transplantation (BMT) experiments. Pretreatment of BMT donors with 5-fluorouracil (5-FU) preferentially induces myeloid malignancies in this assay; in contrast, transduction of bone marrow from untreated donors favors B cell malignancies25. Loss of the CDKN2A tumor suppressor locus is frequent in BPDCN7 and is recurrent among other malignancies with GNB1 mutations26,27. Recipients of Cdkn2a−/− bone marrow from 5-FU–treated donors transduced with GNB1 K57E, I80T or K89E developed a fatal, transplantable myeloid neoplasm beginning approximately 80 d after transplant (Fig. 3a,b and Supplementary Table 1).
Figure 4 GNB1 and GNB2 mutations confer resistance to kinase inhibitors. (a) Dose response of A375 cells transduced with wild-type (WT) GNB2, GNB2 K78E or empty vector and treated with vemurafenib for 48 h. PC9 cells, which do not harbor a BRAF mutation, were used as a negative control for vemurafenib sensitivity. Data are normalized to DMSO only and to maximal response in empty vector. **P < 0.01, pairwise comparison between GNB mutant and wild-type by t-test; error bars represent mean ± s.d. of three replicates. (b) Western blot analysis of K562 cells transduced with WT GNB1, GNB1 K89E or empty vector and treated with nilotinib (100 nM). * P < 0.05 and **P < 0.01 vs. wild-type; †P < 0.01 vs. empty vector. (c) Dose-response of K562 cells transduced with GNB1, GNB1 K89E or empty vector and treated with nilotinib for 48 h, analyzed as in a. (d) Growth of BaF3-Myc cells transduced with BCR-ABL in combination with WT GNB1, GNB1 K89E or empty vector and cultured in the presence of nilotinib (100 nM). *P < 0.05 and **P < 0.01 vs. wild-type; IP < 0.01 vs. empty by t-test; error bars represent mean ± s.d. of three replicates. (e) Dose-response of SET2 cells transduced with GNB1, GNB1 K89E or empty vector and treated with ruxolitinib for 48 h, analyzed as in a. (f) Growth of SET2 cells transduced with GNB1, GNB1 K89E or empty vector and cultured in the presence of ruxolitinib (1 μM), analyzed as in d. (g) Growth of BaF3-Myc cells transduced with MPL P440L in combination with GNB1, GNB1 K89E or empty vector and cultured in the presence of ruxolitinib (1 μM), analyzed as in d.

Supplementary Fig. 3). Recipients of bone marrow from wild-type donors treated with 5-FU and transduced with GNB1 or GNB1 K89E did not develop any malignancy after 12 months (data not shown). All malignancies from Cdkn2a−/− donors expressed the cell surface markers CD11b, CD11c, CD26, FLT3, CD103 and MHC class II, consistent with a tumor of conventional myeloid dendritic cells (Fig. 3b and Supplementary Fig. 4).

When Cdkn2a−/− donors were not pretreated with 5-FU, recipients of marrow transduced with GNB1 I80T or GNB1 K89E developed B cell acute lymphoblastic leukemia with a significantly shorter latency than recipients of bone marrow transduced with empty vector (Supplementary Fig. 5). All myeloid and lymphoid neoplasms were transplantable, causing fatal disease in secondary recipients with rapid disease progression, causing fatal disease in secondary recipients with rapid disease progression (Fig. 3c and Supplementary Fig. 6a,b). Furthermore, all GNB1-driven neoplasms with evaluable metaphase cytogenetics showed evidence of clonal chromosomal abnormalities (Supplementary Fig. 6c).

To interrogate pathway dependencies in GNB1-transformed cells, we screened a panel of 116 small-molecule inhibitors25 in cells expressing GNB1 K89E. The most potent compounds (with 50% growth inhibition concentration (IC50) < 30 nM) included a MEK inhibitor (GSK-1120212), catalytic site mTOR inhibitors (PP242, INK-128) and dual PI3K-mTOR inhibitors (BEZ235 and PI-103) (Supplementary Table 6). K562 myeloid leukemia cells expressing GNB1 K89E have increased phosphorylation of targets of mTOR (AKT S473, NDRG1 T346, 4E-BP-1 T37 and T46 and P70S6K T389) (Fig. 3c). The dual PI3K-mTOR inhibitor BEZ235 and the catalytic mTOR inhibitor AZD8055 suppressed phosphorylation of these substrates. In contrast, the allosteric mTOR inhibitor rapamycin suppressed only P70S6K T389, and the MEK inhibitor U0126 suppressed only ERK T202 and Y204 phosphorylation (Fig. 3c), which did not differ between K562 cells expressing wild-type GNB1 and those expressing GNB1 K89E.

Because GNB1 K89E activated AKT-mTOR signaling in human leukemia cells, we tested the therapeutic activity of the dual PI3K-mTOR inhibitor BEZ235 in vivo. After transplantation of two independent GNB1 K89E–induced myeloid neoplasms, mice receiving BEZ235 had markedly prolonged survival compared with vehicle-treated mice (Fig. 3d). Treatment with BEZ235 reduced splenomegaly, suppressed PI3K-mTOR signaling and induced apoptosis in neoplastic cells (Fig. 3e and Supplementary Fig. 7). Thus, pharmacologic inhibition of PI3K-mTOR signaling represents a promising strategy for targeting GNB1-mutant tumors in vivo.

A recent screen in melanoma cells harboring activating mutations in the MAPK signaling protein BRAF suggested that activation of G protein–coupled receptors by overexpression confers resistance to the BRAF inhibitor vemurafenib29. We identified GNB2 K78E in a
human melanoma (sample ID: MEL-JWCI-WGS-6) that harbored a V600K substitution in BRAF. Expression of GNB2 K78E in A375 melanoma cells, which harbor BRAF V600E, confirmed relative resistance to vemurafenib compared to wild-type GNB1 (Fig. 4a). These findings raised the possibility that Gβ3 mutations mediate resistance to a range of kinase inhibitors. We detected GNB1 K89E in a patient with BCR-ABL (p210)-rearranged B cell acute lymphoblastic leukemia who had a poor response to ABL inhibitors. The ABL inhibitors imatinib and nilotinib suppressed phosphorylation of the BCR-ABL substrate STAT5 in K562 cells expressing GNB1 K89E but did not suppress AKT-mTOR signaling (Fig. 4b). This was associated with relative resistance to nilotinib compared to wild-type GNB1 (Fig. 4c). In addition, BaF3 cells expressing BCR-ABL with GNB1 K89E, but not with wild-type GNB1, proliferated in the presence of nilotinib (Fig. 4d).

We identified three myeloid neoplasms with co-occurring mutations in GNB1 and MPL (sample IDs: 08-017857, 11-188) or JAK2 (sample ID: 08-021722). Expression of GNB1 K89E but not wild-type GNB1 in SET2 myeloid cells, which harbor V617F substitutions in JAK2, conferred relative resistance to the JAK2 inhibitor ruxolitinib (Fig. 4e). SET2 cells expressing GNB1 K89E, but not wild-type GNB1, proliferated in the presence of ruxolitinib (Fig. 4f). Similarly, BaF3 cells coexpressing MPL P440L with GNB1 K89E, but not with wild-type GNB1, proliferated in the presence of ruxolitinib (Fig. 4g).

In summary, oncocgenic Gβ3 alterations occur in multiple cancers, disrupt GαGβγ interactions, activate canonical signaling downstream of G proteins and confer resistance to targeted kinase inhibitors. We distinguished these functionally relevant mutations from the long catalogs of somatic alterations by cDNA library screening. Like mutations in Gα subunits, different Gβ3 mutations may cluster to some extent within cancer subtypes. Further research is needed to clarify whether differences in signaling downstream of these alleles contribute to context-dependent transformation.

METHODS

Methods and any associated references are available in the online version of the paper.

Accession codes. Gene-expression profiling data have been deposited in Gene Expression Omnibus under accession number GSE60990.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

A.Y., G.A., J.T., B.C., N.S., Y.W., O.K., N.K., S.C.W., S.S.K., H.L., T.T., A.L.C., K.G.E., J.C., N.J.-S. and A.A.L. designed and performed experiments. A.Y., G.A., J.T., B.C., K.G., S.J.E., S.J.R., J.W.T., J.A.M., D.M.W. and A.A.L. analyzed data. J.G., M.W.D., H.M., J.P.M., S.I. and B.L.E. provided essential reagents. A.Y., D.M.W. and A.A.L. wrote the paper.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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1. O’Hare, M. et al. The emerging mutational landscape of G proteins and G-protein-coupled receptors in cancer. Nat. Genet. 41, 412–424 (2013).
2. Garraway, L.A. & Lander, E.S. Lessons from the cancer genome. Cell 153, 17–37 (2013).
3. Lawrence, M.S. et al. Discovery and saturation analysis of cancer genes across 21 human cell lines. Nature 508, 495–501 (2014).
4. Iyer, G. et al. Genome sequencing identifies a basis for everolimus sensitivity. Science 338, 221 (2012).
5. Yoda, A. et al. Functional screening identifies CRLF2 in precursor B-cell acute lymphoblastic leukemia. Proc. Natl. Acad. Sci. USA 107, 252–257 (2010).
6. Shindoh, N. et al. Next-generation cDNA screening for oncogene and resistance phenotypes. PLoS ONE 7, e49201 (2012).
7. Lucioni, M. et al. Twenty-one cases of blastic plasmacytoid dendritic cell neoplasm: focus on biallelic locus 9p21.3 deletion. Blood 118, 4591–4594 (2011).
8. Menezes, J. et al. Exome sequencing reveals novel and recurrent mutations with clinical impact in blastic plasmacytoid dendritic cell neoplasm. Leukemia 28, 1607–1614 (2014).
9. Oldham, W.M. & Hamm, H.E. HeterotrimERIC G protein activation by G-protein-coupled receptors. Nat. Rev. Mol. Cell Biol. 9, 60–71 (2008).
10. Radhika, V. & Dhanasekaran, N. Transforming G proteins. Oncogene 20, 1607–1614 (2001).
11. Van Raamsdonk, C.D. et al. Frequent somatic mutations of GNAQ in uveal melanoma and blue naevi. Nature 457, 599–602 (2009).
12. Vallar, L., Spada, A. & Giannattasio, G. Altered Gαq and adenylate cyclase activity in human GNAQ-deficient pituitary adenomas. Nature 330, 566–568 (1987).
13. Walter, M.J. et al. CRL2 counts: an emerging role of the CRL2 complex in regulating epidermal keratinocytes. Nature 433, 250–257 (2005).
14. Akagi, T. et al. Frequent genomic abnormalities in acute myeloid leukemia/myelodysplastic syndrome. Haematologica 94, 213–223 (2009).
15. Ackerman, E. et al. Variation in CDKN2A at 9p21.3 influences childhood acute lymphoblastic leukemia risk. Leukemia 27, 1275–1282 (2013).
16. Haferlach, T. et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia 28, 241–257 (2014).
17. Ford, C.E. et al. Molecular basis for interactions of G protein βγ subunits with effectors. Science 280, 1271–1274 (1998).
18. Wali, M.A. et al. The structure of the G protein heterotrimer Gαβγ123. Cell 83, 1047–1058 (1995).
19. Adelman, G. et al. DNA ends after the molecular composition and localization of Ku multicomponent complexes. Mol. Cell Proteomics 11, 411–421 (2012).
20. Willardson, B.M. & Tracy, C.M. Chaperone-mediated assembly of G protein subunits. Subcell. Biochem. 63, 131–153 (2012).
21. Hakak, Y. et al. The role of the GPR91 ligand sucindate in hematopoesis. J. Leukoc. Biol. 85, 837–843 (2009).
22. Gupta, S.K. et al. Analysis of the fibroblast transformation potential of GTPase deficient gna12 oncogenes. Mol. Cell. Biol. 12, 190–197 (1992).
23. Stephens, L. et al. A novel phosphoinositide 3 kinase activity in myeloid-derived cells is activated by G protein βγ subunits. Cell 77, 83–93 (1994).
24. Crespo, P., Xu, N., Simonds, W.F. & Gutkind, J.S. Ras-dependent activation of MAP kinase pathways mediated by G protein βγ subunits. Nature 369, 418–420 (1994).
25. Camps, M. et al. Isozyme-selective stimulation of phospholipase C-β2 by G protein βγ subunits. Nature 360, 684–686 (1992).
26. Subramanian, A. et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc. Natl. Acad. Sci. USA 102, 15545–15550 (2005).
27. Krause, D.S., Lazarides, K., von Andrian, U.H. & Van Etten, R.A. Requirement for CD44 in homing and engraftment of BCR-ABL-expressing leukemic stem cells. Nat. Med. 12, 1175–1180 (2006).
28. Agaki, T. et al. Frequent genomic abnormalities in acute myeloid leukemia/myelodysplastic syndrome with normal karyotype. Haematologica 94, 213–223 (2009).
29. Sherborne, A.L. et al. Variation in CDKN2A at 9p21.3 influences childhood acute lymphoblastic leukemia risk. Nat. Genet. 42, 492–494 (2010).
30. Tyner, J.W. et al. Kinase pathway dependence in primary human leukemias determined by rapid inhibitor screening. Cancer Res. 73, 285–296 (2013).
31. Johannessen, C.M. et al. A melanocyte lineage program confers resistance to MAP kinase pathway inhibition. Nature 430, 138–142 (2013).
32. Chapman, P.B. et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N. Engl. J. Med. 364, 2507–2516 (2011).
ONLINE METHODS

Cell culture and reagents. A375 (ATCC), BaF3 (RIKEN), K562 (ATCC), SET-2 (DSMZ) and TF-1 (ATCC) cells were cultured according to manuals from distributors. In this study we used BaF3 stably expressing cMyc (pMXs-neo-hMyc) if not specified in the text. Construction of plasmids by PCR cloning and retroviral infections were performed as previously described31.

Retroviral cDNA library screening. Detailed procedure was described previously. Briefly, mRNA was isolated from viably frozen bone marrow specimens containing ≥ 90% BPDNC tumor cells using the TRIZol and FastTrack MAG mRNA Isolation Kit (Life Technologies), according to the manufacturer's instructions. cDNA libraries were assembled using the CloneMiner II cDNA Library Construction Kit (Life Technologies) and destination vector (pMSCVpuroATT), according to the manufacturer's instructions. Retrovirus was packaged in 293T cells by cotransfection with pEcoPack vector. Viral supernatant was applied to BaF3 expressing hBCL2 (BaF3-BCL2) cells in the presence of ml3. Puromycin was added to the media 2 d after infection. On day 4 after infection, cells were plated into a 96-well plate with puromycin containing media without ml3. After 10–28 d, viable colonies were isolated. Integrated cDNAs were amplified by PCR and sequenced. Integrated cDNAs were also cloned into pMSCVpuro and packaged into retrovirus that was used to confirm the ability of mutant alleles to transform BaF3 or TF-1 cells to growth factor independence.

Western blot analysis. Sample preparation of whole cell lysates and immune-precipitants, SDS-PAGE, membrane transfer and blotting were performed as previously31. Antibodies to phosphorylated (phospho-) ERK T202/Y204 (4370), ERK (4695, 9107), phospho-AKT S473 (4060, 9271), AKT (4961), phospho-NDRG1 T346 (5482), NDRG1 (9408), phospho-E-BP1 T37/46 (2855), E-BP1 (9452), phospho-P70S6K T389 (9234), P70S6K (9202),-phospho-CRB/ABL (phospho-c-Ab Y245, 5300), phospho-STAT5 Y694 (4322), caspase-3 (9665), and β-actin (4967) were purchased from Cell Signaling. Antibodies to GNA12 (sc-13534), GNA13 (sc-726), GNAQ/11 (sc-392) and GNA13 (sc-410) were purchased from Santa Cruz Biotechnology. Antibody to Flag (F3165) was purchased to confirm the ability of mutant alleles to transform BaF3 or TF-1 cells to growth factor independence.

Proliferation assays. Proliferation was measured manually by cell counting every 2–7 d, starting with a concentration of 10^5 cells ml^{-1}. Cells were washed twice before plating to remove residual IL-3. Serum-reduced media were also used in the assay.

SILAC labeling and protein purification for proteomic analysis. SILAC labeling reagents (89982 and 89990) were purchased from Thermo Scientific and cells were labeled according to the manufacturer's instructions. cDNA libraries were assembled using the CloneMiner II cDNA Library Construction Kit (Life Technologies) and destination vector (pMSCVpuroATT), according to the manufacturer's instructions. Retrovirus was packaged in 293T cells by cotransfection with pEcoPack vector. Viral supernatant was applied to BaF3 expressing hBCL2 (BaF3-BCL2) cells in the presence of ml3. Puromycin was added to the media 2 d after infection. On day 4 after infection, cells were plated into a 96-well plate with puromycin containing media without ml3. After 10–28 d, viable colonies were isolated. Integrated cDNAs were amplified by PCR and sequenced. Integrated cDNAs were also cloned into pMSCVpuro and packaged into retrovirus that was used to confirm the ability of mutant alleles to transform BaF3 or TF-1 cells to growth factor independence.

Sample preparation of whole cell lysates and immune-precipitants, SDS-PAGE, membrane transfer and blotting were performed as previously31. Antibodies to phosphorylated (phospho-) ERK T202/Y204 (4370), ERK (4695, 9107), phospho-AKT S473 (4060, 9271), AKT (4961), phospho-NDRG1 T346 (5482), NDRG1 (9408), phospho-E-BP1 T37/46 (2855), E-BP1 (9452), phospho-P70S6K T389 (9234), P70S6K (9202), phospho-CRB/ABL (phospho-c-Ab Y245, 5300), phospho-STAT5 Y694 (4322), caspase-3 (9665), and β-actin (4967) were purchased from Cell Signaling. Antibodies to GNA12 (sc-13534), GNA13 (sc-726), GNAQ/11 (sc-392) and GNA13 (sc-410) were purchased from Santa Cruz Biotechnology. Antibody to Flag (F3165) was purchased to confirm the ability of mutant alleles to transform BaF3 or TF-1 cells to growth factor independence.

Proliferation assays. Proliferation was measured manually by cell counting every 2–7 d, starting with a concentration of 10^5 cells ml^{-1}. Cells were washed twice before plating to remove residual IL-3. Serum-reduced media (50% RPMI, 50% Opti-MEM, 1% FCS and 50 µM 2-mercaptoethanol) was used in the assay.

Inhibitor assays. Cells were plated at a density of 10^5 cells ml^{-1} in 96-well plates (100 µl per well) with drugs or vehicle (DMSO). MTT assay (Sigma) was performed following the above protocol.

Sample processing for phosphoproteomic analysis. Combined SILAC-encoded cell pellets were solubilized by repeated pipetting using in ten volumes of 7.2 M guanidine HCl in 0.1 M ammonium bicarbonate. Insoluble material was pelleted for 10 min at 10,000 x g, and the protein concentration of the supernatants quantified by bicinchoninic acid assay. Next, 400 µg of soluble proteins were transferred to a tube and cysteine residues were reduced and alkylated as described for the TAP samples. The concentration of guanidine HCl was lowered to 720 mM by adding nine volumes of 100 M ammonium bicarbonate. Proteins were digested overnight at 37 °C using 5 µg of trypsin. An additional 20 µg of trypsin was added the following morning and incubated for another 4h at 37 °C. The resulting tryptic peptide solutions were acidified by adding TFA to a final concentration of 1% and incubated at 37 °C for 30 min to cleave RapiGest, followed by centrifugation. Peptides were desalted by C18 solid phase extraction followed by strong cation exchange (SCX), both performed in batch-mode format. Eluted peptides were concentrated by vacuum centrifugation, and reconstituted with 20 µl of 0.1% TFA.

Liquid chromatograph–tandem mass spectrometry (LC-MS/MS) analysis, tryptic peptides. Tryptic peptides derived from the gel band and from the TAP samples were analyzed by LC-MS/MS on an LTQ-Orbitrap-XL mass spectrometer (Thermo, Waltham, MA) equipped with a Digital PicoView electrospray source platform (New Objective, Woburn, MA). The instrument was operated in data-dependent mode, in which the eight most abundant ions in each MS scan were selected (isolation width = 2.8 Da, threshold = 20,000) for MS/MS by collisionally activated dissociation (CAD) (35% normalized collision energy). Dynamic exclusion was enabled with a repeat count of 1 and exclusion duration of 30 s. ESI voltage was set to 2.2 kV.

Sample processing for phosphoproteomic analysis. Combined SILAC-encoded cell pellets were solubilized by repeated pipetting using in ten volumes of 7.2 M guanidine HCl in 0.1 M ammonium bicarbonate. Insoluble material was pelleted for 10 min at 10,000 x g, and the protein concentration of the supernatants quantified by bicinchoninic acid assay. Next, 400 µg of soluble proteins were transferred to a tube and cysteine residues were reduced and alkylated as described for the TAP samples. The concentration of guanidine HCl was lowered to 720 mM by adding nine volumes of 100 M ammonium bicarbonate. Proteins were digested overnight at 37 °C using 20 µg of trypsin. An additional 20 µg of trypsin was added the following morning and incubated for another 4h at 37 °C. The resulting tryptic peptide solutions were acidified by adding TFA to a final concentration of 1% and desalted on a 100 mg Waters C18 solid phase extraction plate. Peptides were eluted with 600 µl of 80% acetonitrile and 0.1% TFA. Phosphopeptides were enriched using magnetic Fe-NTA agarose beads (300 µl of a 5% bead suspension) as described32. The enrichment was done for 30 min at room temperature with end-over-end rotation. After removing the supernatant, beads were washed three times with 400 µl 80% acetonitrile and 0.1% TFA and one time with 400 µl of 0.01% acetic acid. Phosphopeptides were eluted for 5 min at room temperature with 50 µl of 0.75 M ammonium hydroxide containing 100 mM EDTA. The beads were washed once with 50 µl of water, and this wash was combined with the eluate. Phosphopeptides were concentrated to 10 µl by vacuum centrifugation. Ammonium formate (pH 10) was added to yield a final concentration of 20 M. Two independent biological replicates were analyzed following the above protocol.

LC-MS/MS analysis, phosphopeptides. Enriched phosphopeptides were analyzed by multidimensional LC-MS/MS33 on an LTQ-velos mass spectrometer. The spectrometer was operated in data-dependent mode, in which the top ten most abundant ions in each MS scan were subjected to alternating CAD.
known signaling pathways downstream of G proteins (lethally irradiated (5.5 Gy × two doses), and then transplanted with 10⁶ donor gene chip in the Dana-Farber Microarray Core (http://macf-web.dfci.harvard.edu/). Small molecule, or mutant GNB1 were interrogated against a panel containing graded effects of peptides from the reverse database with a false discovery rate (FDR) greater than 1%. SILAC intensities for the light and heavy isotopes of each precursor were extracted from raw LC/MS data using custom Python scripts in the multiplierz environment. When necessary (i.e., for gel band analysis), a rapid peptide matching algorithm was used to identify peptide sequences mapping uniquely to a single entry in Entrez Gene. The phosphorylation sites identified across the two biological replicates were matched to the corresponding protein entry in Uniprot.

Clustering analysis. The relative log₁₀ intensities of each protein in the GNB1 wild-type (WT) and GNB1 mutants (K89E, 180T or K57E) TAP samples were calculated after combining the intensities of their constituent peptides. In the case of GNB1 itself, the intensity of peptides overlapping the mutated site was not included in the ratio calculation. The ratio of wild-type to mutant GNB1 in each TAP was used to normalize the ratio of all interacting proteins. Unsupervised clustering analysis of the normalized relative abundance of G protein subunits and PDCL between the mutant and wild-type GNB1 was performed in R using the heatmap2 package. GNB2, which was only detected by a peptide shared with GNB1, was excluded from this analysis.

Gene-expression profiling and GSEA. Total RNA was extracted in biological triplicates using Trizol from stably transfected TF-1 cells (empty vector control vs. K89E) with or without 12 h cytokine starvation. The quality was assessed using a bioanalyzer and subsequently profiled on an Affymetrix Human Gene ST 1.0 microarray with or without 12 h cytokine starvation. The quality was assessed using a bioanalyzer and subsequently profiled on an Affymetrix Human Gene ST 1.0 bioanalyzer and subsequently profiled on an Affymetrix Human Gene ST 1.0 microarray with or without 12 h cytokine starvation. The quality was assessed using a bioanalyzer and subsequently profiled on an Affymetrix Human Gene ST 1.0 microarray with or without 12 h cytokine starvation. The quality was assessed using a bioanalyzer and subsequently profiled on an Affymetrix Human Gene ST 1.0 microarray with or without 12 h cytokine starvation. The quality was assessed using a bioanalyzer and subsequently profiled on an Affymetrix Human Gene ST 1.0 microarray with or without 12 h cytokine starvation. The quality was assessed using a bioanalyzer and subsequently profiled on an Affymetrix Human Gene ST 1.0 microarray with or without 12 h cytokine starvation. The quality was assessed using a bioanalyzer and subsequently profiled on an Affymetrix Human Gene ST 1.0 microarray with or without 12 h cytokine starvation. The quality was assessed using a bioanalyzer and subsequently profiled on an Affymetrix Human Gene ST 1.0 microarray with or without 12 h cytokine starvation.
31. van Bodegom, D. et al. Differences in signaling through the B-cell leukemia oncoprotein CRLF2 in response to TSLP and through mutant JAK2. *Blood* **120**, 2853–2863 (2012).

32. Ficarro, S.B. et al. Magnetic bead processor for rapid evaluation and optimization of parameters for phosphopeptide enrichment. *Anal. Chem.* **81**, 4566–4575 (2009).

33. Ficarro, S.B. et al. Online nanoflow multidimensional fractionation for high-efficiency phosphopeptide analysis. *Mol. Cell Proteomics* **10**, O111.011064 (2011).

34. Askenazi, M., Parikh, J.R. & Marto, J.A. mzAPI: a new strategy for efficiently sharing mass spectrometry data. *Nat. Methods* **6**, 240–241 (2009).

35. Parikh, J.R. et al. multiplierz: an extensible API based desktop environment for proteomics data analysis. *BMC Bioinformatics* **10**, 364 (2009).

36. Askenazi, M., Marto, J.A. & Linial, M. The complete peptide dictionary—a metaproteomics resource. *Proteomics* **10**, 4306–4310 (2010).

37. Chapuy, B. et al. Discovery and characterization of super-enhancer-associated dependencies in diffuse large B cell lymphoma. *Cancer Cell* **24**, 777–790 (2013).

38. Weigert, O. et al. Genetic resistance to JAK2 enzymatic inhibitors is overcome by HSP90 inhibition. *J. Exp. Med.* **209**, 259–273 (2012).

39. Elpek, K.G. et al. Lymphoid organ-resident dendritic cells exhibit unique transcriptional fingerprints based on subset and site. *PLoS ONE* **6**, e23921 (2011).