Supplementary Information

Analysis of *KLHL14* mutations in patients with lymphoid malignancies

Published whole exome and whole genome sequencing data were compiled from the following sources:

BL (1-4)
CLL (5-10)
DLBCL (11-25)
FL (26-32)
MCL (33-36)
MM (37, 38)
MZL (39-46)
Primary_CNS (47-50)
Primary_cutaneous (17, 51)
T_cell_lymphoma (52-72)

Analysis of KLHL14 prevalence within DLBCL gene expression subgroups and genetic subtypes was based on data in ref. (18).

Fig. S1

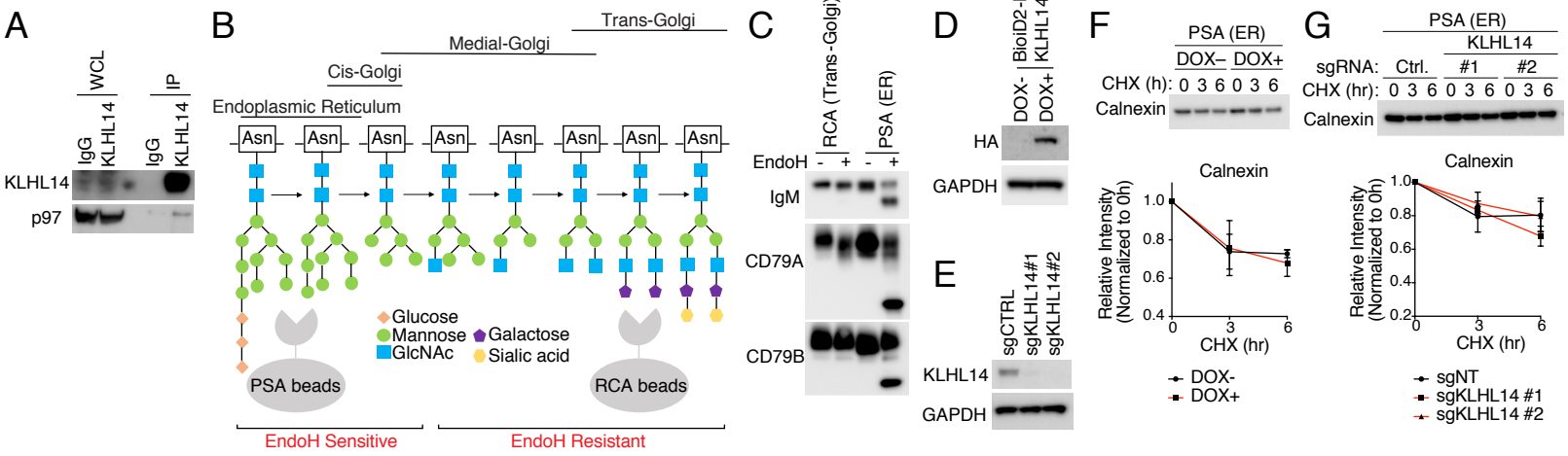

Fig. S1. KLHL14 decreases stability of the immature BCR glycoforms in the ER
(A) Western blot analysis of immunoprecipitated of endogenous KLHL14 in TMD8 cells. IgG antibody immunoprecipitates= negative control: IP, immunoprecipitated; WCL, whole cell lysates. (B) Diagram showing glycan processing in the ER and Golgi and lectin affinity chromatography. (C) Western blot analysis of PSA- and RCA- bound fractions of BCR in TMD8 cells treated with or without EndoH. (D) Western blot analysis of whole cell lysates from tetracycline repressor-expressing TMD8 cells retrovirally transduced with cDNAs encoding BioID2-HA-tagged KLHL14. Cells were treated with DOX (1μg/ml) for 16h. (E) Western blot analysis of whole cell lysates from TMD8-Cas9 lentivirally transduced with sgRNA targeting KLHL14 or a non-targeting control. (F) Top, western blot analysis of PSA-bound fractions of Calnexin in tetracycline repressor-expressing TMD8 cells retrovirally transduced with cDNAs encoding BioID2-HA-tagged KLHL14. Cells were pre-treated with DOX (1μg/ml) for 16h and treated with CHX (50μg/ml) for the indicated time points before fraction. Bottom, quantification of PSA-bound fraction of Calnexin protein levels. (G) Top, western blot analysis of PSA-bound fractions of Calnexin in TMD8-Cas9 cells lentivirally transduced with sgRNA targeting KLHL14 or a non-targeting control. Cells were treated with CHX (50μg/ml) for the indicated time points before fraction. Bottom, quantification of PSA-bound fraction of Calnexin protein levels. Error bars represent SD of triplicates and data are representative of three independent experiments.

Dataset S1. Global Proteome SILAC enrichment ratio
Differential protein expression upon KLHL14 expression (averaged normalized SILAC ratios (KLHL14/empty vector)) identified by quantitative mass spectrometry in TMD8 cells

Dataset S2. Ubiquitinome SILAC enrichment ratio
Differentially ubiquitinated proteins upon KLHL14 expression (averaged normalized SILAC ratios (KLHL14/empty vector)) identified by di-glycine remnant quantitative mass spectrometry in TMD8 cells

Dataset S3. Brunello sgRNA library normalized read counts
Normalized gRNA sequencing read counts from the genome-scale CRISPR-Cas9 screen in DMSO- or ibrutinib-treated KLHL14 wild-type (WT) and KLHL14 knockout (KO) TMD8 cells

Dataset S4. Ibrutinib synergy score
Average fold-change in the log2-transformed normalized gRNA sequencing read counts from Dataset S3

Dataset S5. DGE for RNA-Seq
The RNA-seq digital gene expression values in DMSO- or ibrutinib-treated KLHL14 wild-type (WT) and KLHL14 knockout (KO) TMD8 cells

SI References

1. Grande BM*, et al.* (2019) Genome-wide discovery of somatic coding and noncoding mutations in pediatric endemic and sporadic Burkitt lymphoma. *Blood* 133(12):1313-1324.
2. Bouska A*, et al.* (2017) Adult high-grade B-cell lymphoma with Burkitt lymphoma signature: genomic features and potential therapeutic targets. *Blood* 130(16):1819-1831.
3. Love C*, et al.* (2012) The genetic landscape of mutations in Burkitt lymphoma. *Nat Genet* 44(12):1321-1325.
4. Richter J*, et al.* (2012) Recurrent mutation of the ID3 gene in Burkitt lymphoma identified by integrated genome, exome and transcriptome sequencing. *Nat Genet* 44(12):1316-1320.
5. Amin NA*, et al.* (2016) A Quantitative Analysis of Subclonal and Clonal Gene Mutations before and after Therapy in Chronic Lymphocytic Leukemia. *Clin Cancer Res* 22(17):4525-4535.
6. Ljungstrom V*, et al.* (2016) Whole-exome sequencing in relapsing chronic lymphocytic leukemia: clinical impact of recurrent RPS15 mutations. *Blood* 127(8):1007-1016.
7. Landau DA*, et al.* (2015) Mutations driving CLL and their evolution in progression and relapse. *Nature* 526(7574):525-530.
8. Puente XS*, et al.* (2015) Non-coding recurrent mutations in chronic lymphocytic leukaemia. *Nature* 526(7574):519-524.
9. Quesada V*, et al.* (2011) Exome sequencing identifies recurrent mutations of the splicing factor SF3B1 gene in chronic lymphocytic leukemia. *Nat Genet* 44(1):47-52.
10. Wang L*, et al.* (2011) SF3B1 and other novel cancer genes in chronic lymphocytic leukemia. *N Engl J Med* 365(26):2497-2506.
11. Arthur SE*, et al.* (2018) Genome-wide discovery of somatic regulatory variants in diffuse large B-cell lymphoma. *Nat Commun* 9(1):4001.
12. Dobashi A*, et al.* (2018) TP53 and OSBPL10 alterations in diffuse large B-cell lymphoma: prognostic markers identified via exome analysis of cases with extreme prognosis. *Oncotarget* 9(28):19555-19568.
13. Greenawalt DM*, et al.* (2017) Comparative analysis of primary versus relapse/refractory DLBCL identifies shifts in mutation spectrum. *Oncotarget* 8(59):99237-99244.
14. Chapuy B*, et al.* (2016) Diffuse large B-cell lymphoma patient-derived xenograft models capture the molecular and biological heterogeneity of the disease. *Blood* 127(18):2203-2213.
15. de Miranda NF*, et al.* (2014) Exome sequencing reveals novel mutation targets in diffuse large B-cell lymphomas derived from Chinese patients. *Blood* 124(16):2544-2553.
16. Lohr JG*, et al.* (2012) Discovery and prioritization of somatic mutations in diffuse large B-cell lymphoma (DLBCL) by whole-exome sequencing. *Proc Natl Acad Sci U S A* 109(10):3879-3884.
17. Zhou XA*, et al.* (2018) Genomic Analyses Identify Recurrent Alterations in Immune Evasion Genes in Diffuse Large B-Cell Lymphoma, Leg Type. *J Invest Dermatol* 138(11):2365-2376.

18. Schmitz R, *et al.* (2018) Genetics and Pathogenesis of Diffuse Large B-Cell Lymphoma. *N Engl J Med* 378(15):1396-1407.
19. Reddy A, *et al.* (2017) Genetic and Functional Drivers of Diffuse Large B Cell Lymphoma. *Cell* 171(2):481-494 e415.
20. Park HY, *et al.* (2016) Whole-exome and transcriptome sequencing of refractory diffuse large B-cell lymphoma. *Oncotarget* 7(52):86433-86445.
21. Morin RD, *et al.* (2016) Genetic Landscapes of Relapsed and Refractory Diffuse Large B-Cell Lymphomas. *Clin Cancer Res* 22(9):2290-2300.
22. Mareschal S, *et al.* (2016) Whole exome sequencing of relapsed/refractory patients expands the repertoire of somatic mutations in diffuse large B-cell lymphoma. *Genes Chromosomes Cancer* 55(3):251-267.
23. Morin RD, *et al.* (2013) Mutational and structural analysis of diffuse large B-cell lymphoma using whole-genome sequencing. *Blood* 122(7):1256-1265.
24. Pasqualucci L, *et al.* (2011) Analysis of the coding genome of diffuse large B-cell lymphoma. *Nat Genet* 43(9):830-837.
25. Chapuy B, *et al.* (2018) Molecular subtypes of diffuse large B cell lymphoma are associated with distinct pathogenic mechanisms and outcomes. *Nat Med* 24(5):679-690.
26. Hellmuth JC, *et al.* (2018) Duodenal-type and nodal follicular lymphomas differ by their immune microenvironment rather than their mutation profiles. *Blood* 132(16):1695-1702.
27. Tsukamoto T, *et al.* (2017) High-risk follicular lymphomas harbour more somatic mutations including those in the AID-motif. *Sci Rep* 7(1):14039.
28. Zamo A, *et al.* (2018) Differences between BCL2-break positive and negative follicular lymphoma unraveled by whole-exome sequencing. *Leukemia* 32(3):685-693.
29. Bouska A, *et al.* (2017) Combined copy number and mutation analysis identifies oncogenic pathways associated with transformation of follicular lymphoma. *Leukemia* 31(1):83-91.
30. Green MR, *et al.* (2015) Mutations in early follicular lymphoma progenitors are associated with suppressed antigen presentation. *Proc Natl Acad Sci U S A* 112(10):E1116-1125.
31. Pasqualucci L, *et al.* (2014) Genetics of follicular lymphoma transformation. *Cell reports* 6(1):130-140.
32. Green MR, *et al.* (2013) Hierarchy in somatic mutations arising during genomic evolution and progression of follicular lymphoma. *Blood* 121(9):1604-1611.
33. Agarwal R, *et al.* (2019) Dynamic molecular monitoring reveals that SWI-SNF mutations mediate resistance to ibrutinib plus venetoclax in mantle cell lymphoma. *Nat Med* 25(1):119-129.
34. Wu C, *et al.* (2016) Genetic heterogeneity in primary and relapsed mantle cell lymphomas: Impact of recurrent CARD11 mutations. *Oncotarget* 7(25):38180-38190.
35. Zhang J, *et al.* (2014) The genomic landscape of mantle cell lymphoma is related to the epigenetically determined chromatin state of normal B cells. *Blood* 123(19):2988-2996.
36. Bea S, *et al.* (2013) Landscape of somatic mutations and clonal evolution in mantle cell lymphoma. *Proc Natl Acad Sci U S A* 110(45):18250-18255.

37. Guo G, *et al.* (2018) Genomic discovery and clonal tracking in multiple myeloma by cell-free DNA sequencing. *Leukemia* 32(8):1838-1841.
38. Bolli N, *et al.* (2014) Heterogeneity of genomic evolution and mutational profiles in multiple myeloma. *Nat Commun* 5:2997.
39. Hyeon J, *et al.* (2018) Targeted deep sequencing of gastric marginal zone lymphoma identified alterations of TRAF3 and TNFAIP3 that were mutually exclusive for MALT1 rearrangement. *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc* 31(9):1418-1428.
40. Pillonel V, *et al.* (2018) High-throughput sequencing of nodal marginal zone lymphomas identifies recurrent BRAF mutations. *Leukemia* 32(11):2412-2426.
41. Spina V, *et al.* (2016) The genetics of nodal marginal zone lymphoma. *Blood* 128(10):1362-1373.
42. Clipson A, *et al.* (2015) KLF2 mutation is the most frequent somatic change in splenic marginal zone lymphoma and identifies a subset with distinct genotype. *Leukemia* 29(5):1177-1185.
43. Parry M, *et al.* (2013) Whole exome sequencing identifies novel recurrently mutated genes in patients with splenic marginal zone lymphoma. *PLoS One* 8(12):e83244.
44. Martinez N, *et al.* (2014) Whole-exome sequencing in splenic marginal zone lymphoma reveals mutations in genes involved in marginal zone differentiation. *Leukemia* 28(6):1334-1340.
45. Kiel MJ, *et al.* (2012) Whole-genome sequencing identifies recurrent somatic NOTCH2 mutations in splenic marginal zone lymphoma. *J Exp Med* 209(9):1553-1565.
46. Rossi D, *et al.* (2012) The coding genome of splenic marginal zone lymphoma: activation of NOTCH2 and other pathways regulating marginal zone development. *J Exp Med* 209(9):1537-1551.
47. Chapuy B, *et al.* (2016) Targetable genetic features of primary testicular and primary central nervous system lymphomas. *Blood* 127(7):869-881.
48. Braggio E, *et al.* (2015) Genome-Wide Analysis Uncovers Novel Recurrent Alterations in Primary Central Nervous System Lymphomas. *Clin Cancer Res* 21(17):3986-3994.
49. Vater I, *et al.* (2015) The mutational pattern of primary lymphoma of the central nervous system determined by whole-exome sequencing. *Leukemia* 29(3):677-685.
50. Bruno A, *et al.* (2014) Mutational analysis of primary central nervous system lymphoma. *Oncotarget* 5(13):5065-5075.
51. Mareschal S, *et al.* (2017) Identification of Somatic Mutations in Primary Cutaneous Diffuse Large B-Cell Lymphoma, Leg Type by Massive Parallel Sequencing. *J Invest Dermatol* 137(9):1984-1994.
52. Farmanbar A, *et al.* (2018) Mutational Intratumor Heterogeneity is a Complex and Early Event in the Development of Adult T-cell Leukemia/Lymphoma. *Neoplasia* 20(9):883-893.
53. Dufva O, *et al.* (2018) Aggressive natural killer-cell leukemia mutational landscape and drug profiling highlight JAK-STAT signaling as therapeutic target. *Nat Commun* 9(1):1567.
54. Jallades L, *et al.* (2017) Exome sequencing identifies recurrent BCOR alterations and the absence of KLF2, TNFAIP3 and MYD88 mutations in splenic diffuse red pulp small B-cell lymphoma. *Haematologica* 102(10):1758-1766.

55. da Silva Almeida AC*, et al.* (2015) The mutational landscape of cutaneous T cell lymphoma and Sezary syndrome. *Nat Genet* 47(12):1465-1470.
56. Kataoka K*, et al.* (2015) Integrated molecular analysis of adult T cell leukemia/lymphoma. *Nat Genet* 47(11):1304-1315.
57. Jiang L*, et al.* (2015) Exome sequencing identifies somatic mutations of DDX3X in natural killer/T-cell lymphoma. *Nat Genet* 47(9):1061-1066.
58. Choi J*, et al.* (2015) Genomic landscape of cutaneous T cell lymphoma. *Nat Genet* 47(9):1011-1019.
59. Crescenzo R*, et al.* (2015) Convergent mutations and kinase fusions lead to oncogenic STAT3 activation in anaplastic large cell lymphoma. *Cancer Cell* 27(4):516-532.
60. Moffitt AB*, et al.* (2017) Enteropathy-associated T cell lymphoma subtypes are characterized by loss of function of SETD2. *J Exp Med* 214(5):1371-1386.
61. McKinney M*, et al.* (2017) The Genetic Basis of Hepatosplenic T-cell Lymphoma. *Cancer Discov* 7(4):369-379.
62. Kiel MJ*, et al.* (2015) Genomic analyses reveal recurrent mutations in epigenetic modifiers and the JAK-STAT pathway in Sezary syndrome. *Nat Commun* 6:8470.
63. McGirt LY*, et al.* (2015) Whole-genome sequencing reveals oncogenic mutations in mycosis fungoides. *Blood* 126(4):508-519.
64. Kiel MJ*, et al.* (2014) Integrated genomic sequencing reveals mutational landscape of T-cell prolymphocytic leukemia. *Blood* 124(9):1460-1472.
65. Palomero T*, et al.* (2014) Recurrent mutations in epigenetic regulators, RHOA and FYN kinase in peripheral T cell lymphomas. *Nat Genet* 46(2):166-170.
66. Koo GC*, et al.* (2012) Janus kinase 3-activating mutations identified in natural killer/T-cell lymphoma. *Cancer Discov* 2(7):591-597.
67. Song TL*, et al.* (2018) Oncogenic activation of the STAT3 pathway drives PD-L1 expression in natural killer/T-cell lymphoma. *Blood* 132(11):1146-1158.
68. Woollard WJ*, et al.* (2016) Candidate driver genes involved in genome maintenance and DNA repair in Sezary syndrome. *Blood* 127(26):3387-3397.
69. Wang L*, et al.* (2015) Genomic profiling of Sezary syndrome identifies alterations of key T cell signaling and differentiation genes. *Nat Genet* 47(12):1426-1434.
70. Ungewickell A*, et al.* (2015) Genomic analysis of mycosis fungoides and Sezary syndrome identifies recurrent alterations in TNFR2. *Nat Genet* 47(9):1056-1060.
71. Yoo HY*, et al.* (2014) A recurrent inactivating mutation in RHOA GTPase in angioimmunoblastic T cell lymphoma. *Nat Genet* 46(4):371-375.
72. Sakata-Yanagimoto M*, et al.* (2014) Somatic RHOA mutation in angioimmunoblastic T cell lymphoma. *Nat Genet* 46(2):171-175.