The mutational landscape of Burkitt-like lymphoma with 11q aberration is distinct from that of Burkitt lymphoma

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BRIEF REPORT

The mutational landscape of Burkitt-like lymphoma with 11q aberration is distinct from that of Burkitt lymphoma

RUNNING TITLE

Mutational landscape of Burkitt-like lymphoma with 11q aberration

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KEY POINTS

- *MYC*-negative Burkitt-like lymphoma with 11q aberration harbor a mutational landscape distinct from sporadic BL.
- Bi-allelically inactivation indicates a pathogenic role of the INO80 complex associated *NFRKB* gene in mnBLL,11q.

ABSTRACT

The new provisional lymphoma category Burkitt-like lymphoma with 11q aberration recently described comprises cases similar to Burkitt lymphoma (BL) on morphological, immunophenotypic and gene expression level but lacking the IG-*MYC* translocation. They are characterized by a peculiar imbalance pattern on chromosome 11, but the landscape of mutations is not yet described. Thus, we investigated 15 *MYC*-negative Burkitt-like lymphoma with 11q aberration (mnBLL,11q,) cases by copy number analysis and whole exome sequencing. We refined the regions of 11q-imbalance and identified the INO80 complex-associated gene *NFRKB* as positional candidate in 11q24.3. Next to recurrent gains in 12q13.11-q24.32 and 7q34-qter as well as losses in 13q32.3-q34 we identified 47 genes recurrently affected by protein-changing mutations (each $\geq$3/15 cases). Strikingly, we did not detect recurrent mutations in genes of the ID3-TCF3 axis or SWI/SNF complex that are frequently altered in BL, or in genes frequently mutated in germinal center derived B-cell lymphomas like *KMT2D* or *CREBBP*. An exception is *GNA13* which was mutated in 7/15 cases. We conclude that the genomic landscape of mnBLL,11q, differs from that of BL both at the chromosomal and mutational level. Our findings implicate that mnBLL,11q, is a lymphoma category distinct from Burkitt lymphoma at the molecular level.

INTRODUCTION

Recently, a subgroup of germinal center derived B-cell (GCB) lymphomas has been described that resemble Burkitt lymphoma (BL) with regard to morphology, immunophenotype and gene expression profile but lack the IG-*MYC* translocation typical for BL[1–5]. Instead, these cases are cytogenetically characterized by a peculiar pattern of an 11q aberration consisting of a gain in 11q23.2-23.3 followed by a telomeric loss in 11q24.1-qter. According to the revised 4th edition of the "WHO Classification of Tumours of Haematopoietic and Lymphoid Tissue" these lymphomas have been described as a new provisional entity called "*Burkitt-like lymphoma with 11q aberration*" (abbreviated herein "mnBLL,11q,")[6]. In addition to the 11q aberration, mnBLL,11q, show various secondary imbalances and harbor a more complex karyotype than BL[2].

Recently, the mutational landscape of IG-*MYC* translocated BL has been thoroughly investigated[7–10] and e.g. *MYC, ID3, TP53, SMARCA4 and GNA13* have been identified as recurrently mutated genes. In contrast, mutational analyses of mnBLL,11q, have mainly focused on single genes, showing for example lack of *ID3* mutations in 14 mnBLL,11q, cases[2]. This, together with the lack of IG-*MYC* translocations and the different imbalance patterns suggest that mnBLL,11q, show a genetic make-up quite different from IG-*MYC* translocated BL.

In order to more comprehensively delineate the profile of copy number alterations (CNAs), single nucleotide variants (SNVs) as well as small insertions and deletions (indels) of mnBLL,11q, we performed array-based imbalance mapping and whole exome sequencing (WES) in 15 mnBLL,11q.

METHODS

In this retrospective analysis tumor samples of 15 patients were included that had been diagnosed as BL, atypical BL/BL-like or other aggressive B-cell lymphomas and that lacked an IG-*MYC* translocation. In all cases, the diagnosis of mnBLL,11q, had been considered (refer to Supplemental Methods for detailed description) and an 11q aberration pattern was detected by fluorescence in situ hybridization and imbalance profiling applying the OncoScan™ CNV FFPE assay (12 cases). For three of these cases, the clinical, immunophenotypical and copy number data have been published previously[1,2,5]. DNA extracted from FFPE tissue of all 15 patients was subjected to WES. Refer to the supplemental methods for details.

RESULTS AND DISCUSSION

We analyzed tumor samples of 15 patients retrospectively diagnosed with mnBLL,11q. The median age at diagnosis was 15.5 (range 4-52) years and the male to female ratio 2.75:1. An underlying immunodeficiency was clinically reported in two of the cases both of which had no

evidence for an EBV infection. The characteristics of the cohort are summarized in Supplemental Table 1.

As a part of the retrospective workup, we analyzed the copy number alterations (CNA) in twelve cases not reported before (excluding the three cases reported in[2]). The median number of CNA in these 12 cases (gains, losses and copy number neutral losses of heterozygosity) was 6.5 (range 3-38), not significantly differing from the findings reported by Salaverria *et* al. using a different array platform (median 12.2, range 2-28)[2]. Considering the 12 novel CNA profiles and those of the three cases previously reported[2], the typical pattern of chromosome 11q-gain/loss was observed in 13/15 mnBLL,11q, cases (Supplemental Figure 1, Supplemental Table 2). In the remaining two cases, in agreement with FISH studies (Supplemental Table 1), only a telomeric loss in 11q24.1-qter was detected without concomitant gain in 11q23. We consider such alterations, which have also recently been reported in one mnBLL,11q, case[2] as a variant 11q aberration. This might point to a more pronounced pathogenic role of the genes in the deleted rather than in the gained region. Besides the changes on chromosome 11, other recurrent imbalances included (partial) trisomy 12 (7/15 cases, minimally gained region in 12q13.11-q24.32), gain in 7q34-qter and loss in 13q32.3-q34 (both 3/15 cases) (Supplemental Figure 1).

Next, we analyzed the mutational profile (SNVs and indels) of mnBLL,11q, using WES. We identified 47 genes showing potentially protein-changing SNVs in $\geq$3/15 of mnBLL,11q, cases (Figure 1A, Supplemental Table 3). Strikingly, the genes recurrently mutated in BL (>15% of BL patients, unpublished data accessible at www.icgc.org and[7,9]) like *MYC, ID3, TCF3, TP53* and *SMARCA4* were not recurrently (>15%) or not at all mutated in any of the mnBLL,11q, cases (Figure 1B, Supplemental Figure 2-3). The notable exceptions were *GNA13* and *DDX3X,* mutated in seven and four of the 15 mnBLL,11q, respectively. The almost complete absence of the BL-associated mutational pattern prompted us to compare the mutational profile of mnBLL,11q, to that of diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL) (unpublished data accessible at www.icgc.org and[11]). Again, we observed only a minor overlap in genes recurrently mutated in both mnBLL,11q, and DLBCL/FL (>15% of cases) including *GNA13*, *TTN* and *EZH2.* Notably, the mutations in *EZH2* affected in all three cases the Y641 mutational hotspot[12]. In contrast, genes like *KMT2D* or *CREBBP* most frequently mutated in GCB-like non-BL, which share with mnBLL,11q, a GCB-like gene expression signature, were not at all mutated in our case series (Supplemental Figure 4-5). Accordingly, our findings show the mutational profile of mnBLL,11q, to be overall distinct from that of BL as well as FL and DLBCL with only few exceptions.

Among the most frequently mutated genes in mnBLL,11q, were *GNA13* and *TTN*, mutated in each 7/15 cases. *GNA13* mutations which frequently occur in GCB-lymphomas like BL[7,9] and GCB-DLBCL[13], lead to a loss of protein function[14]. In line, 5/11 *GNA13* mutations detected in seven mnBLL,11q, (in five mnBLL,11q, cases) were likely deleterious (frameshift, stopgain, stoploss, splice-site), while the remaining six mutations were nonsynonymous mutations located in functional domains of the protein (Supplemental Figure 6A,B). We could verify 9/11 *GNA13* mutations by Sanger sequencing. In line*, in silico* modeling of the *GNA13* mutations confirmed the deleterious nature of the mutations leading to a loss of function or destabilizing the G-protein´s function (Supplemental Table 3, Supplemental Figure 6A,B). Extending the analysis to all genes belonging to the Gα13 signaling pathway, we identified in 8/15 mnBLL,11q, cases mutations in one of the pathway genes (Supplemental Figure 6C) with *GNA13* being the most frequently affected (7/8 cases). Most of the mutations in the other genes co-occurred with the *GNA13* mutations (3/4 cases).

The role of *TTN* mutations in mnBLL,11q, is unclear. It needs to be considered that several other large and/or late replicating genes are contained within the list of recurrently mutated genes in mnBLL,11q, besides *TTN* (Figure 1A). Such genes are mutated in several cancer types (refer to the Supplemental Results and Discussion) but their pathogenic role in tumor development is unclear[15]. Modeling of the *TTN* mutations confirmed the mutations to be rather non-oncogenic and hence, constitute most likely passenger variants (Supplemental Table 3).

Next, we asked whether genes in the minimal regions of gain and loss were targeted by mutations in addition to imbalances. 3/45 genes (*PCSK7, DSCAML1*, *TMEM25*) mapping to the minimal region of gain were mutated in each only one mnBLL,11q, case. With regard to genes located in the minimally deleted region, we confirmed previous findings of recurrent mutations in *ETS1* (2/15 cases, including one published mutation[2]) and detected recurrent mutations in *NFRKB* (4/15) which were verified by Sanger sequencing (Supplemental Table 3). Interestingly, 3/4 *NFRKB* mutations were stopgain mutations and accordingly *NFRKB* function is supposed to be bi-allelically lost in these three cases, i.e. by deletion of one allele and mutation of the other. *In silico* modeling of the mutations further supported the deleterious character of the mutations (Figure 2, Supplemental Table 3, Supplemental Results and Discussion). In line, NFRKB expression was described to be significantly lower in mnBLL,11q, compared to BL based on expression array analysis ($p<0.007$, Supplemental Figure 7)[2]. NFRKB encodes a nuclear factor related to the kappaB binding protein, belonging to the INO80 chromatin remodeling complex[16] which plays a role in transcriptional regulation[17]. Extending the analysis to all genes of the INO80 complex, showed mutations in 5/15 mnBLL,11q, cases. Given the absence of recurrent mutations in genes of the SWI/SNF chromatin remodeling complex in mnBLL,11q, it is intriguing

to speculate that mutations in the INO80 complex in mnBLL,11q, have a comparable function as the mutations in the SWI/SNF complex in BL.

Finally, besides *NFRKB* only *MACF1*, *UBE2A* and *DST,* each mutated in 3/15 mnBLL,11q, have been reported to be differentially expressed in comparison to BL[2].

Taken together, our data clearly show that besides the chromosomal translocation and imbalance patterns also the mutational profile of mnBLL,11q, is strikingly different from BL and non-BL. Our findings suggest a role of *GNA13* in the pathogenesis of mnBLL,11q, and identify the INO80 complex-associated gene *NFRKB* as a candidate gene in the deleted region in 11q24.3. Moreover, our findings support the recognition of mnBLL,11q, as an entity distinct from *MYC*-positive BL but also from other aggressive GCB-lymphomas like DLBCL. Finally, the observations presented, particularly the lack of a BL-specific mutation pattern, combined with lack of an IG-*MYC* fusion might aid in the differential diagnostic process distinguishing IG-*MYC* translocated BL from mnBLL,11q.

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AUTHORSHIP CONTRIBUTIONS

B.B., H.H., L.L., G.O., G.R., ES.J., R.A-Y. and W.K. provided tumor samples and clinical data. S.B. and I.N. performed cytogenetic analysis. J.A., H.T. and P.N. performed WES. J.S., K.K., D.H. and M.S. analyzed the WES data. CW.K. performed expression analysis. F.R. and RB.R. modeled the functional impact of the mutations. R.W. analyzed the OncoScan data and interpreted the WES data. R.S. designed the study and coordinated the project. R.W., J.S. and R.S. interpreted the data and wrote the manuscript. All authors read and approved the final manuscript.

DISCLOSURE OF CONFLICTS OF INTEREST

R.S. received a speaker´s honorary from Roche. The other authors declare no conflict of interest.

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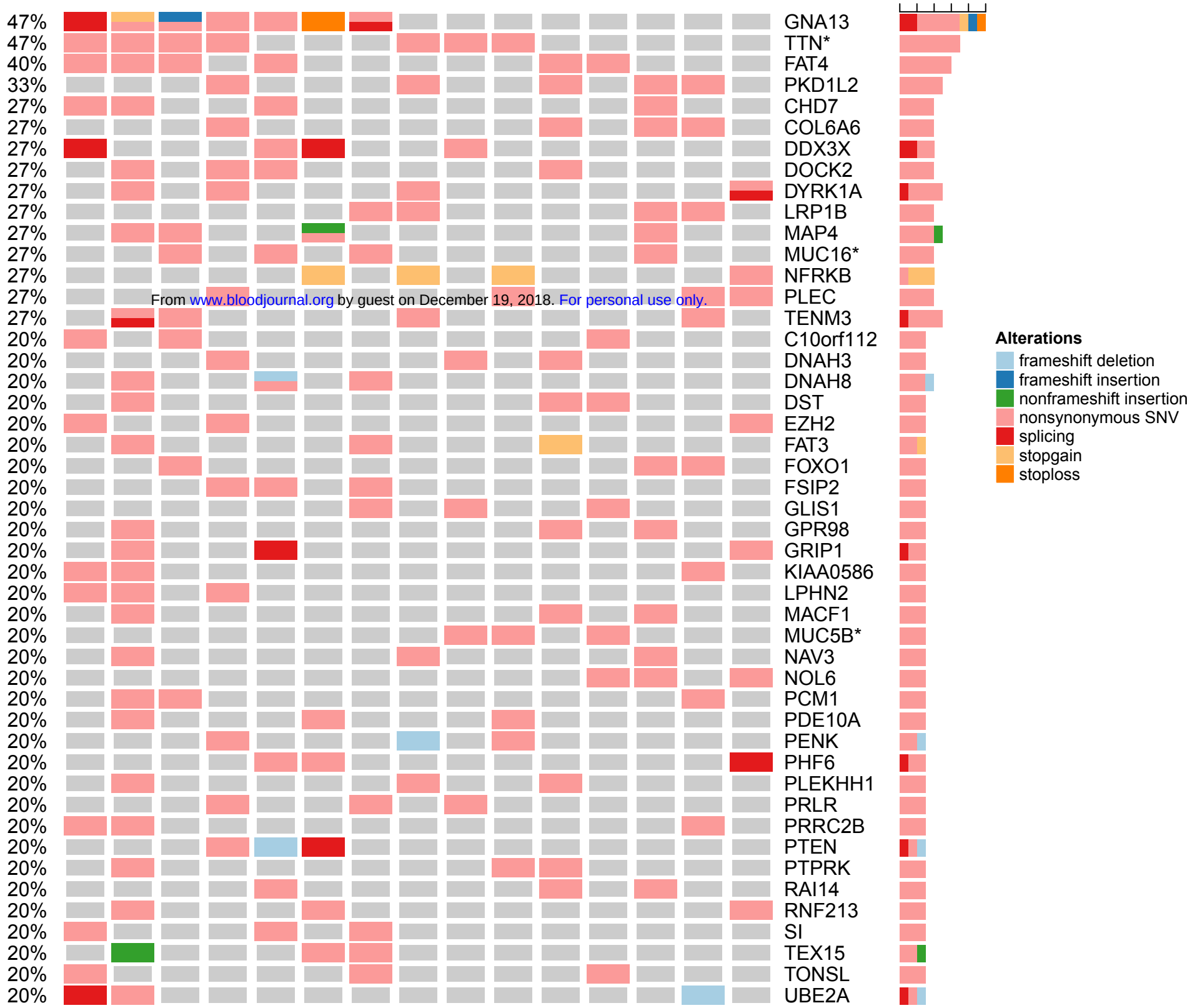
FIGURE LEGEND

Figure 1: Mutational landscape of mnBLL,11q, showing recurrent *GNA13* mutations.
(A) Depicted are the potentially protein-changing SNVs and indels. Columns encode samples and rows different genes. Different mutation types are color-coded in the oncoprint, where different types of mutation can coexist in one sample. The * indicate that mutations within this genes are considered as dubious hits as reported by Lawrence *et* al. [15]. (B) Overall, the mutational profile differs between the two lymphoma entities, and only few genes are frequently mutated in both including *GNA13* and *DDX3X*. Included are those genes which are mutated in ≥4/15 mnBLL,11q, cases and the 13 genes recurrently mutated in >6/39 (>15%) BL cases (median age at diagnosis 8 (range 2-18) years) based on unpublished data from whole genome sequencing accessible at www.icgc.org.

Figure 2: Modeling of NFRKB mutations. *NFRKB* mutations (red lollipops) annotated on protein primary sequence with additional information regarding post-translational modifications (PTMs) and domain composition. 3D structural model showing the deubiquitinating enzyme binding domain (PDB ID: 4UF5), the winged domain (PDB ID: 3U21) and transcriptional regulation domain (by homology; PDB ID: 3BY6). The two stop gain mutations R290* and K322* are predicted to delete the protein region responsible for mediating interactions with transcriptional regulators and/or DNA while leaving the N-terminal region deputed to the interaction with deubiquitnating enzymes. The R174C mutation is predicted to perturb adjacent phosphosites, e.g. S176 and is located in a motif predicted by ELM (elm.eu.org) to be either phosphorylation site of GSK3 and MAP kinase or docking site for USP7 or the Pin1 WW domain. The C-terminal deletion by Q1103* is predicted to have detrimental consequences, as it is rich in phosphorylation sites (e.g. CK1 and GSK3) or recognition sites for FHA and WDR5.

A



B

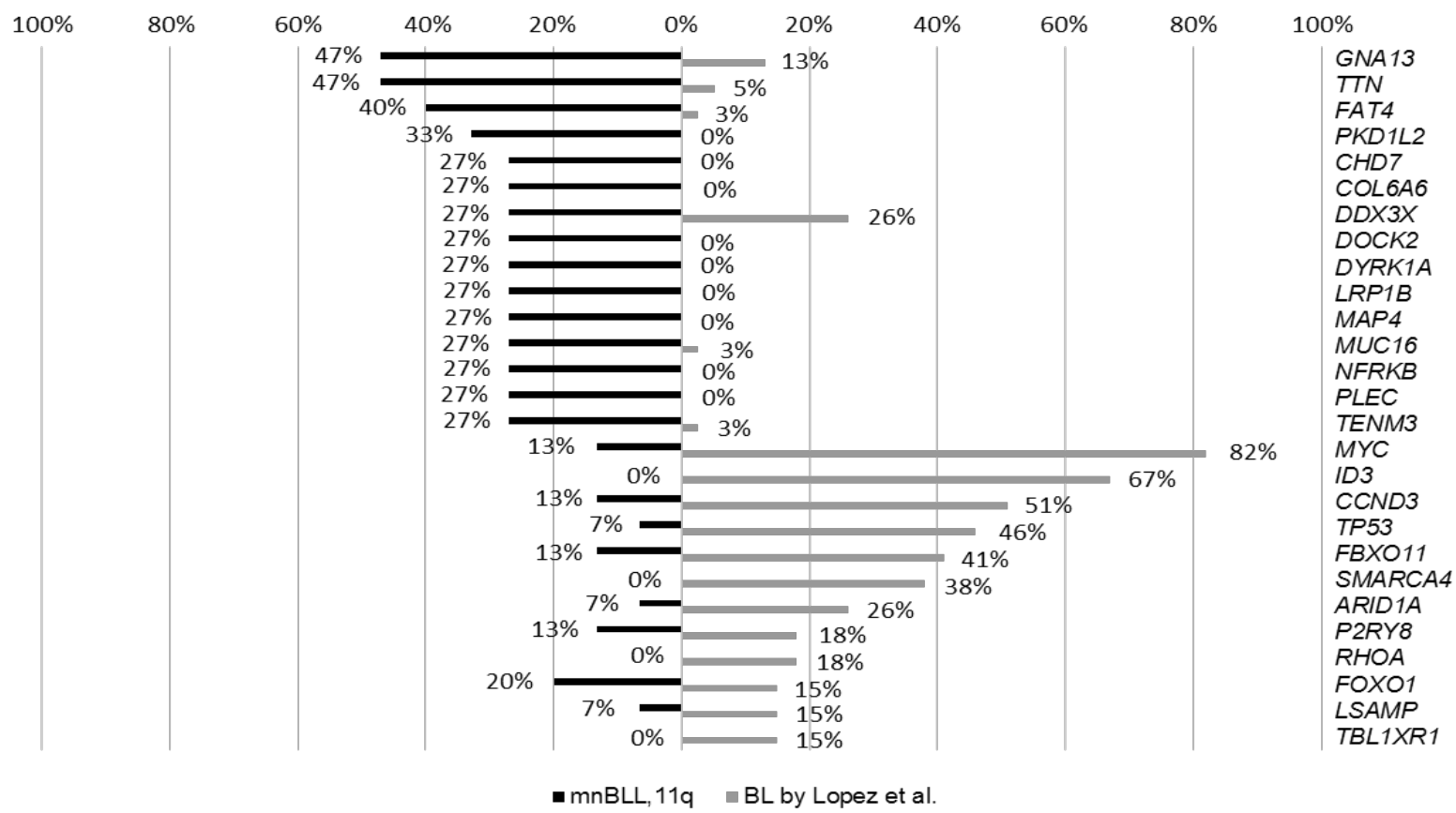
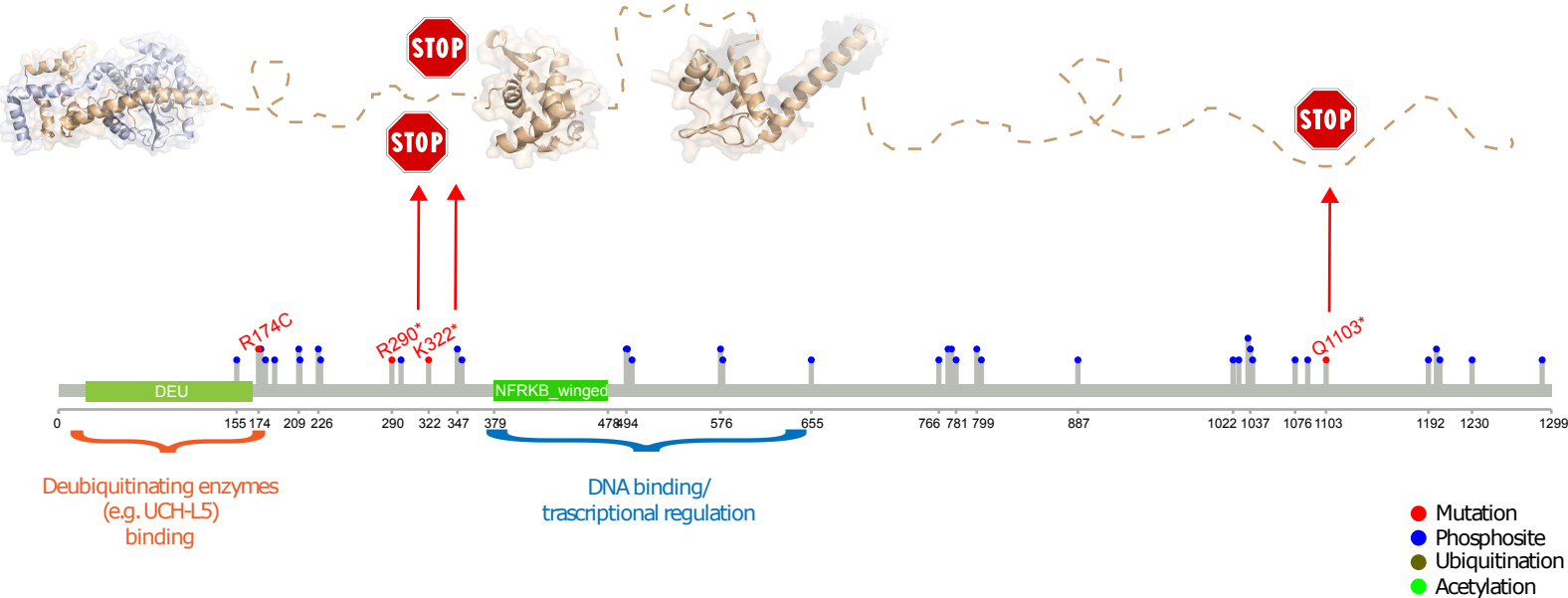


Figure 1


STOP
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R290*
K322*
Q1103*
DEU
NFRKB_winged
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322 347
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478 494
576
655
766 781 799
887
1022 1037
1076 1103
1192
1230
1299
Deubiquitinating enzymes (e.g. UCH-L5) binding
DNA binding/ trascriptional regulation
Mutation
Phosphosite
Ubiquitination
Acetylation

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