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Targeting B-cell lymphomas with inhibitors of the MALT1 paracaspase

Short title: MALT1 inhibition to target B-cell lymphomas

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Abstract:

The paracaspase MALT1 is an Arg-specific protease that cleaves multiple substrates to promote lymphocyte proliferation and survival. The catalytic activity of MALT1 is normally tightly regulated by antigen receptor triggering, which promotes MALT1 activation by its inducible monoubiquitination-dependent dimerization. Constitutive MALT1 activity is a hallmark of specific subsets of B-cell lymphomas, which are characterized by chromosomal translocations or point mutations that activate MALT1 or its upstream regulators. Recent findings suggest that such lymphomas may be sensitive to treatment with MALT1 inhibitors. Here we review recent progress in the understanding of MALT1 function and regulation, and the development of small molecule MALT1 inhibitors for therapeutic applications.

Introduction

B-cell malignancies can arise from B-cell precursors in the bone marrow, giving rise to B-cell leukemias, or from tissue B-cells, giving rise to localized tumors called B-cell lymphomas. The molecular mechanisms underlying the development of B-cell malignancies have been intensively studied [1,2]. During their development, B-cells undergo multiple gene rearrangements that are required for the generation of a diverse and high affinity antibody repertoire. As a consequence, B-cell malignancies can frequently arise from defective gene rearrangements that lead to oncogenic gene translocations. These can lead to abnormally high expression of translocated proto-oncogenes or to the generation of novel fusion proteins with oncogenic properties. Here, we will focus on lymphoma types with aberrant expression or activity of the proto-oncogene and protease MALT1, and the mechanisms underlying MALT1-dependent cellular transformation. We will also summarize recent progress in the development of techniques to monitor MALT1 activity, which have allowed the identification of first generation MALT1 inhibitors with promising anti-lymphoma effects in preclinical animal studies.

Structure and function of MALT1: scaffold and protease activities

The gene encoding MALT1 was originally discovered and named after a chromosomal translocation associated with an aggressive form of B-cell lymphoma arising from the mucosa-associated lymphoid tissue (MALT) of the gastric mucosa [3]. Subsequent bioinformatic and biochemical approaches have characterized MALT1 as a protein with a central protease-like domain that shares homology with

caspases; MALT1 has therefore also been named paracaspase [4]. Additional structural features of MALT1 are an N-terminal death domain of unknown function, which is followed by two immunoglobulin-like domains, and a C-terminal region containing a third Ig-like domain and a structurally undefined extension [5,6] (**Figure 1A**). These different domains define the molecular function of MALT1, which comprises both scaffold and enzymatic (proteolytic) activities. The N-terminal immunoglobulin domains are required for MALT1's binding to the adaptor protein BCL10 [4,5]. Upon antigen receptor triggering, BCL10 and MALT1 are recruited to the upstream signaling protein CARMA1 [7-9]. The resulting CARMA1-BCL10-MALT1 (CBM) complex promotes the MALT1-dependent recruitment of the ubiquitin ligase TRAF6 via binding sites situated in the C-terminal region [10] and in the second Ig domain of MALT1 [11] (**Figure 1, A** and **B**). This induces the rapid but transient activation of the proliferation-promoting transcription factor NF-κB by the classical (canonical) NF-κB pathway [10,12] (**Figure 1B**). Another consequence of CBM complex formation is the activation of the protease activity of MALT1, which assures long-lasting NF-κB activation by the cleavage of the NF-κB1 inhibitors RelB and A20 (see below) [13-15]. Several other, highly relevant aspects of lymphocyte activation and proliferation are controlled by the cleavage of a growing list of additional MALT1 substrates (**Figure 1B** and **Table 1**). Cleavage of the deubiquitinating enzyme CYLD has been proposed to promote antigen receptor-induced activation of the AP-1 family of transcription factors, and the expression of pro-inflammatory genes such as IL-2 and IL-8 in activated T-lymphocytes [16]. MALT1 further controls gene expression at a post-transcriptional level, by cleaving the RNAse Regnase-1 (also known as MCPIP-1 or Zc3h12a) [17]. MALT1-dependent cleavage of its binding partner BCL10 has been shown to promote lymphocyte adhesion by beta1 integrins [13]. Recently, the protease activity of MALT1 was shown to be required for TCR-induced glutamine uptake and activation of the mTORC1 complex, most likely by the regulation of the amino acid transporter ASCT2, but the substrate responsible for this function remains unknown [18] (**Figure 1B**). Collectively, these findings suggest that MALT1 controls B- and T-cell activation by the cleavage of a multitude of (known and yet to be identified) substrates. Consistent with this idea, mice expressing a catalytically inactive mutant of MALT1 show severe defects in adaptive immune responses [19].

MALT1-dependent NF-κB activation and lymphomagenesis

The molecular function of MALT1 in NF-κB activation, and the role of constitutive NF-κB activation in lymphomagenesis have been intensively studied. Heterodimers of the NF-κB1 subtype (p50-RelA or p50-c-Rel dimers) act as major drivers of cellular proliferation and survival as they control the expression of numerous genes that inhibit cell death and actively promote proliferation [20]. MALT1 has been shown to promote NF-κB1 activation by two distinct means that are outlined in **Figure 1B**. As a scaffold protein, MALT1 promotes activation of the IKK complex by the recruitment of the ubiquitin ligase TRAF6 [10], and potentially by the recruitment of the linear ubiquitin chain assembly complex (LUBAC) [21,22]. IKK-mediated phosphorylation of the NF-κB inhibitor IκBα then triggers its proteasomal degradation, and allows NF-κB1 complexes to enter the nucleus and initiate transcription. This so-called canonical, IKK-dependent NF-κB1 activation is rapid but transient, since the transcription and thus the re-synthesis of IκBα is rapidly induced by NF-κB1 as part of a negative feedback mechanism [20]. A second, IKK-independent means by which MALT1 induces NF-κB1 activation is via its protease activity [13-15,23]. Upon its activation, MALT1 cleaves RelB and A20, two proteins that inhibit NF-κB1 activation in lymphocytes [14,15]. In lymphocytes, RelB inhibits canonical NF-κB activation by forming transcriptionally inactive complexes with the NF-κB1 subunit RelA and c-Rel [15,24]. MALT1-dependent RelB cleavage leads to proteasomal degradation of RelB, thereby lowering RelB levels for many hours [15]. MALT1-dependent cleavage of A20, on the other hand, most likely contributes to weaken A20's inhibitory role in NF-κB1 activation [14]. MALT1 also cleaves itself after Arg 149 (**Table 1**), and this autoprocessing controls an essential step of NF-κB1 activation downstream of its nuclear accumulation, by mechanisms that remain to be explored [25]. Collectively, these observations show that the protease activity of MALT1 massively increases the amplitude and duration of the NF-κB1 response.

Lymphomas with constitutive MALT1 activity

In activated lymphocytes, NF-κB activation by MALT1 requires the physical recruitment of MALT1 into a CARMA1-BCL10-MALT1 (CBM) signaling complex that is transiently formed upon triggering of the antigen receptor on the surface of lymphocytes [26]. Recently, B-cell lymphomas with constitutive, antigen-independent MALT1 activity have been described (**Figure 2**) [27-30]. These include diffuse large B-cell lymphoma (DLBCL), with a prevalence of roughly 30-40 % the most common form of lymphoma, mantle cell lymphoma (MCL, frequency 7-10 %) and mucosa-

associated lymphoid tissue lymphoma (MALT, frequency 9 %) (for a recent review, see [31]).

In diffuse large B-cell lymphomas of the activated B-cell (ABC) subtype, several mutations have been described that trigger constitutive MALT1 protease activity. Mutations in the B-cell receptor (BCR)-associated CD79B chain have been associated with chronic BCR signaling [32]. Mutation of a critical tyrosine in the immunoreceptor tyrosine-based activation (ITAM) motif of CD79B inhibits Lyn kinase activity and thus prevents activation-induced down-regulation of the BCR, thereby enhancing CBM complex formation (**Figure 2**) [32]. On the other hand, potentially oncogenic mutations of CARMA1 increase CBM complex formation and thus the activation of downstream signaling pathways [33]. In non-stimulated lymphocytes, the coiled-coil domain of CARMA1 interacts with the CARD domain in an intra-molecular fashion, and thereby keeps CARMA1 in an inactive state [34]. Mutations of the coiled-coil domain, which can be found in roughly 10 % of ABC DLBCLs patient samples, have been shown to favor the active conformation and induce CARMA1 oligomerization and MALT1 protease activity [27,33] (**Figure 2**). The growth of ABC DLBCL cell lines is strongly reduced by interfering with MALT1 upstream regulators such as PKC, CARMA1 and BCL10, but also by MALT1 protease inhibition, indicating a central role for MALT1 protease activity in the maintenance of this aggressive lymphoma subtype [27,28,33,35].

A second type of lymphoma with constitutive MALT1 activity is the MALT lymphoma [3]. Low-grade MALT lymphomas are typically driven by a chronic inflammatory infection with H. pylori, and can be treated by antibiotic treatment. More aggressive, advanced stages of these lymphomas are characterized by antigen-independent constitutive NF-κB activation [3]. A large proportion of these is characterized by the presence of a chromosomal translocation (t(11;18)(q21;q21)) encoding the oncogenic IAP2-MALT1 fusion protein that activates both, NF-κB1 and NF-κB2 (p52-RelB) complexes [4-6,29,36-38] (**Figure 2**). The IAP2-MALT1 fusion recruits TRAF6 to promote canonical, IKK-dependent NF-κB1 activation [11]. In addition, the IAP2-MALT1 is constitutively activated by monoubiquitination [39], and cleaves natural MALT1 substrates such as A20 [14] to promote NF-κB1 activation. Interestingly, the IAP2-MALT1 fusion protein specifically triggers NF-κB2 activation by the cleavage of the Ser/Thr kinase NIK, which is not a natural substrate of MALT1 [29] (**Figure 2**). NIK normally has a very short half-life, but its IAP2-MALT1-dependent cleavage generates a stable NIK fragment, which promotes the activation of the NF-κB2 pathway through phosphorylation and subsequent proteolytic

maturation of the NF-κB2 precursor p100 into p52 [29]. This seems to be a unique feature of MALT lymphomas presenting the IAP2-MALT1 fusion, since NF-κB2 is not normally activated by MALT1. The above-described IAP2-MALT1 fusion is present in roughly 50 % of gastrointestinal MALT lymphomas [40,41]. An alternative chromosomal translocation of the MALT1 gene towards the Ig enhancer element, t(14;18)(q32; q21), is prevalent in non-gastrointestinal MALT lymphomas, such as those of the liver, skin, eye and salivary gland [42,43]. This translocation leads to strongly upregulated expression of the translocated MALT1 gene [44], and thereby most likely to oligomerization-induced MALT1 activation. A small proportion of MALT lymphomas with a chromosomal translocation of the BCL10 gene towards the Ig enhancer element, t(1;14)(p22;q32), has also been described [45,46]. In these lymphomas, MALT1 activation is most likely driven by BCL10 overexpression-induced oligomerization of endogenous MALT1 [3,10].

A recently identified third type of lymphoma that frequently presents with constitutive MALT1 activity is mantle cell lymphoma [30]. Two subgroups of MCL have been identified that can be classified by their sensitivity to BCR signaling inhibitors [30]. The BCR-dependent, PKC-sensitive MCLs show constitutive RelB cleavage, indicating deregulated MALT1 protease activity. In contrast, BCR-independent MCLs seem to depend on alternative NF-κB signaling. Thus far, however, it is unclear which genetic alterations underlie MALT1 activation in MCL.

Techniques to monitor MALT1 activity

Several different techniques can be used to assess MALT1 activity; these have been described in detail elsewhere [47]. The detection of cleaved MALT1 substrate fragments in cell or tissue lysates by Western Blot (WB) seems to be the most straightforward way to monitor MALT1 protease activity (**Figure 3A**). However, detection of the cleavage fragments can be difficult for several reasons. First, cleavage may result in only a minimal size shift of the full-length substrate, as described for BCL10, a 233 aa protein that is cleaved after aa 228 [13]. Second, only a minor proportion of the substrate may be cleaved upon MALT1 activation; this is the case for A20 [14]. Third, the cleavage fragment may have a short half-life and therefore be detectable only upon addition of proteasome inhibitors, as described for RelB [15]. Additionally, the amount of protein available from biopsies may be limiting, and the screening of large sample numbers by WB is not convenient. To assess MALT1 activity quantitatively in large sample numbers, fluorescence-based *in vitro* cleavage assays have been established that are amenable to high-throughput

screening [13,14,48]. In these assays, recombinant or immunoprecipitated MALT1 is incubated with an aminomethlycoumarin (amc)-conjugated MALT1 tetrapeptide substrate; proteolytic removal of the peptide from the amc fluorophore will result in a measurable shift in fluorescence emission (**Figure 3B**). A reported optimal substrate in this assay is the LVSR-amc peptide [48], which contains the sequence of the RelB cleavage site [15] (**Table 1**). To screen for MALT1 activity in intact cells, a FRET-based reporter assay has been developed [39]. The reporter contains the fluorescent proteins YFP and CFP, which were fused by a short linker containing a MALT1 cleavage recognition site. The close proximity of YFP and CFP results in FRET, consequently MALT1-mediated cleavage leads to a loss of the FRET signal, which can be quantified by flow cytometry [39] (**Figure 3C**).

Mechanisms of MALT1 protease activation

The molecular events that control MALT1 activation upon antigen receptor triggering are under intense investigation. Crystallographic evidence supports the idea of a major conformational switch between the inactive and the active form of MALT1. The inactive state is characterized by a conformation in which the catalytic dyad between C464 and H415 cannot be formed properly and substrate binding to the recognition site is restricted [49]. The structure of substrate-bound MALT1 (which is thought to mimic the active conformation) clearly differs from the inactive conformation in the position of key elements like the catalytic dyad or the autoinhibitory Ig3 domain [49,50].

The mechanism controlling the switch from the inactive to the active conformation *in vivo* is not well understood, but probably requires a combination of induced proximity, substrate binding and a monoubiquitination-dependent stabilization of the active dimer conformation. Induced proximity, which is also a requirement for the activation of initiator caspases, is most likely achieved by the CARMA1- and BCL10 dependent oligomerization of MALT1, which may occur on cytoplasmic filamentous BCL10 structures [51]. The resulting assembly of MALT1 dimers may allow for a conformational change that can be transmitted by an interaction between E549 in the dimerization interface and the R465 residue that neighbors the active site C464 residue [49,52]. Substrate binding to the active site may induce additional structural rearrangements required for full activation [49]. Interestingly, both MALT1 and caspases undergo autoprocessing, but the purpose and sites of processing are very distinct. MALT1 autoprocessing is not required for its protease activity, and in contrast to caspases, which are processed within the protease domain, MALT1 autoprocessing occurs outside the protease domain, within

the first Ig domain [25]. The only known post-translational modification of MALT1 that is required for MALT1 activation is a monoubiquitination at K644 within the Ig3 domain [39]. Addition of a single ubiquitin moiety is sufficient to activate MALT1 protease activity, and to favor the dimerization of a recombinant purified MALT1 construct *in vitro* [39]. Monoubiquitination may trigger the active conformation of the protease, for example by inactivating the autoinhibitory Ig3 domain, or it may promote an intermolecular interaction between two MALT1 molecules and thereby stabilize active dimers (as proposed in **Figure 3D**).

Development of therapeutic MALT1 inhibitors

The first described cell permeable MALT1 inhibitor was the peptide-based compound VRPR-fmk [13], which was designed based on an optimal tetrapeptide substrate of a protease of the metacaspase family [53], a group of caspase- and paracaspase/MALT1-related proteases expressed in plants, fungi and parasites [4]. Incubation of T cells or cells derived from ABC-DLBCLs with VRPR-fmk efficiently inhibited MALT1 protease activity; inhibitor treatment resulted in reduced IL-2 production or induction of cytotoxicity, respectively [13,27,28]. The recent development of techniques used to assess MALT1 activity [13,14,48] has opened the way to the screening and characterization of small molecule MALT1 inhibitors for therapeutic applications. By the use of the fluorescence-based MALT1 *in vitro* cleavage assay, two groups reported identification of small molecule MALT1 inhibitors from chemical libraries [54,55], which inhibit MALT1 by distinct means (**Figure 3D**). Interestingly, three phenothiazine derivatives proved to function as MALT1 inhibitors [54]. Amongst these were Mepazine and Thioridazine, compounds that have been used in the past as anti-anxiety/anti-psychotic drugs with antagonistic effects on dopamine receptors [56]. The phenothiazine derivatives are reversible, non-competitive MALT1 inhibitors [54], which do not bind the catalytic site but rather an allosteric pocket on MALT1 that is situated between the caspase domain and the Ig3 domain [54,57]. Binding to this hydrophobic pocket most likely prevents the conformational switch of MALT1 into the enzymatically active conformation [57]. In contrast, another newly identified inhibitor, named MI-2, bound to the active site of the protease and acted as a suicide inhibitor [55]. Crystallographic data for the protease domain of MALT1 in its inactive and active form, and bound to competitive or allosteric inhibitors are now available [49,50,57]. Based on these, additional, structure-based inhibitors can likely be developed with the help of *in silico* approaches. MALT1 monoubiquitination on Lysine 644 within the Ig3 domain favors the dimerization of MALT1, most likely via a conformational change that depends on

an intra- or intermolecular ubiquitin-MALT1 interaction [39]; both the unknown ubiquitin ligase as well as the unidentified ubiquitin-MALT1 interaction surfaces may thus represent additional targets for MALT1 inhibition that remain to be explored (**Figure 3D**).

So far, first encouraging results have been obtained for the application of MALT1 inhibitors in the treatment of ABC DLBCL. Phenothiazine derivatives, as well as MI-2, were able to efficiently slow down the growth of a human ABC DLBCL cell line in a mouse xenograft model, demonstrating potential anti-lymphoma activity in this preclinical model. Additional potential fields of applications for MALT1 inhibitors are autoimmune diseases, which are characterized by excessive lymphocyte activation. Indeed, MALT1 inhibition by mepazine was recently shown to efficiently attenuate the onset and progression of experimental autoimmune encephalomyelitis (EAE), a murine model of multiple sclerosis [58]. These findings are consistent with the observation that MALT1-deficient mice and mice expressing a catalytically inactive mutant of MALT1 are fully protected from EAE induction [19,59,60]. Further investigations are needed to optimize the lead substances with respect to inhibitory capacities and to evaluate their clinical safety. If safe and efficient MALT1 inhibitors can be developed, these should be of interest not only for the treatment of ABC DLBCL, but also for MALT and mantle cell lymphomas and for other, yet undiscovered malignancies or inflammatory diseases with constitutive MALT1 activity.

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Figure Legends

Figure 1 : Structure and function of the human paracaspase MALT1.
(A) Domain structure of MALT1, including the death domain (DD), the immunoglobulin-like (Ig) domains and the caspase-like domain. Indicated above are scaffold functions that include domains or motifs required for binding to BCL10 and TRAF6, as well as the presence of multiple C-terminal sites for K63-linked polyubiquitination, which mediate recruitment of NEMO/IKKγ. Indicated below are residues controlling the protease function, including the active site residues (C464 and H415) and the monoubiquitination site (K644) required for MALT1 activation. The position of a recently identified autoprocessing site (R149) is also indicated.
(B) Overview of MALT1-dependent lymphocyte activation via scaffold and protease functions. As a scaffold, MALT1 promotes NF-κB activation via TRAF6 and the IKK complex, which phosphorylates the NF-κB inhibitor IκB to target it for proteasomal degradation. The protease activity of MALT1 controls NF-κB activation, AP-1 activation, mRNA stability, cellular adhesion and mTORC1 activation by the cleavage of various substrates.

Table : MALT1 substrates and the biological consequences of their cleavage.
Table summarizing known MALT1 substrates (with domain structure and cleavage site) and the consequences of their cleavage. Domain structure and cleavage sites are for the human proteins. Abbreviated domain names: cytoskeleton-associated protein-Gly (CAP), caspase recruitment domain (CARD), death domain (DD), immunoglobulin-like (Ig), leucine zipper (LZ), ovarian tumor (OTU), Rel homolgy domain (RHD), transactivation domain (TAD), ubiquitin association (UBA), ubiquitin-specific protease (USP), Zinc Finger (ZF).

Figure 2 : MALT1 protease activation by genetic aberrations in lymphoma cells
Scheme summarizing oncogenic mutations that promote MALT1 protease activity in ABC DLBCL (upper panels) and MALT lymphoma (lower left panel). CD79 and CARMA1 mutations that were found in ABC DLBCLs activate the protease MALT1 by different mechanisms. Mutations in the ITAM motif of CD79 decrease Lyn kinase activity and thus prevent signal-induced downregulation of the BCR. Coiled-coil mutations in CARMA1 weaken the intramolecular interaction between the coiled-coil and CARD domain and thereby promote the assembly of the CARMA1-BCL10-MALT1 complex. The IAP2-MALT1 fusion protein, which results from a chromosomal

translocation that is frequent in gastric MALT lymphomas, is oligomeric and constitutively active, and can cleave natural MALT1 substrates. In addition, the BIR domains of the fusion protein are able to recruit the kinase NIK, which is then cleaved by the active protease domain. The cleaved NIK fragment is more stable than full length NIK, and activates signaling events leading to NF-κB2 activation. Abbreviated domain names: Caspase recruitment domain (CARD), death domain (DD), immunoglobulin-like (Ig) domain, coiled-coil (CC) domain, membrane associated guanylate kinase (MAGUK) domain, baculoviral IAP repeat (BIR) domain.

Figure 3 : Techniques used to monitor MALT1 activity and strategies for MALT1 inhibition.

(A) The cleavage of MALT1 substrates, such as RelB shown here, leads to the appearance of additional faster migrating cleavage fragments detectable by Western Blot. (B) Incubation of MALT1 with an amc-coupled tetrapeptide substrate allows quantification of protease activity by release of amc, which results in a shift of the fluorescence emission. (C) Intracellular MALT1 activity can be measured by a FRET-based assay, through expression of an YFP-CFP fusion protein containing a MALT1 cleavable linker sequence. (D) Potential strategies for MALT1 inhibition: efficient MALT1 inhibition can be achieved by active site inhibitors, which irreversibly block the active site (such as MI-2), or by allosteric inhibitors that indirectly inhibit MALT1 activity by preventing the switch to the active conformation (such as mepazine). In principle there are additional means of targeting MALT1 activity, including drugs that would affect MALT1 dimerization or MALT1 monoubiquitination. MALT1 monoubiquitination could be targeted by inhibition of the responsible ubiquitin ligase, by blocking the accessibility of the modified lysine residue or by targeting the relevant intra-or intermolecular ubiquitin-MALT1 interaction interface (for simplicity reasons, the model shows only the possibility of an intermolecular ubiquitin-MALT1 interaction that may stabilize the dimer).

References to be highlighted

- Uehata et al.

This study identifies the RNAse Regnase-1 (also known as MCPIP-1) as a substrate of MALT1, and shows that MALT1-dependent Regnase-1 cleavage regulates T-cell activation.

- Pelzer et al.

This study identifies the mechanism of MALT1 activation, which requires its monoubiquitination on Lysine 644, and shows that monoubiquitination of MALT1 is required for the growth of ABC DLBCL cell lines.

•• Nagel et al.

Together with Fontan et al., these studies identify the first small molecule MALT1 inhibitors and show that these inhibit the growth of ABC DLBCL in a xenograft model.

•• Fontan et al.

Together with Nagel et al., these studies identify the first small molecule MALT1 inhibitors and show that these inhibit the growth of ABC DLBCL in a xenograft model.

- Rahal et al.

By screening a panel of 16 anti-cancer drugs, this study identifies subgroups of mantle cell lymphomas with constitutive BCR-driven NF-κB1 activation or BCR-independent NF-κB2 activation, and reveals constitutive MALT1 activity in the BCR-driven subgroup.

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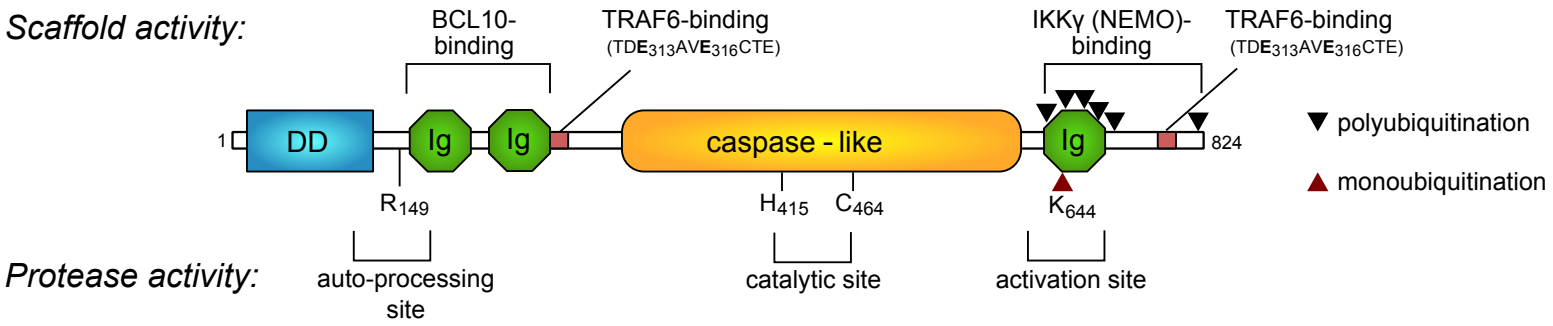
A


Scaffold activity:
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TRAF6-binding (TDE313AVE316CTE)
IKKγ (NEMO)-binding
TRAF6-binding (TDE313AVE316CTE)
1
DD
Ig
Ig
caspase - like
Ig
824
polyubiquitination
monoubiquitination
R149
H415
C464
K644
Protease activity:
auto-processing site
catalytic site
activation site

B

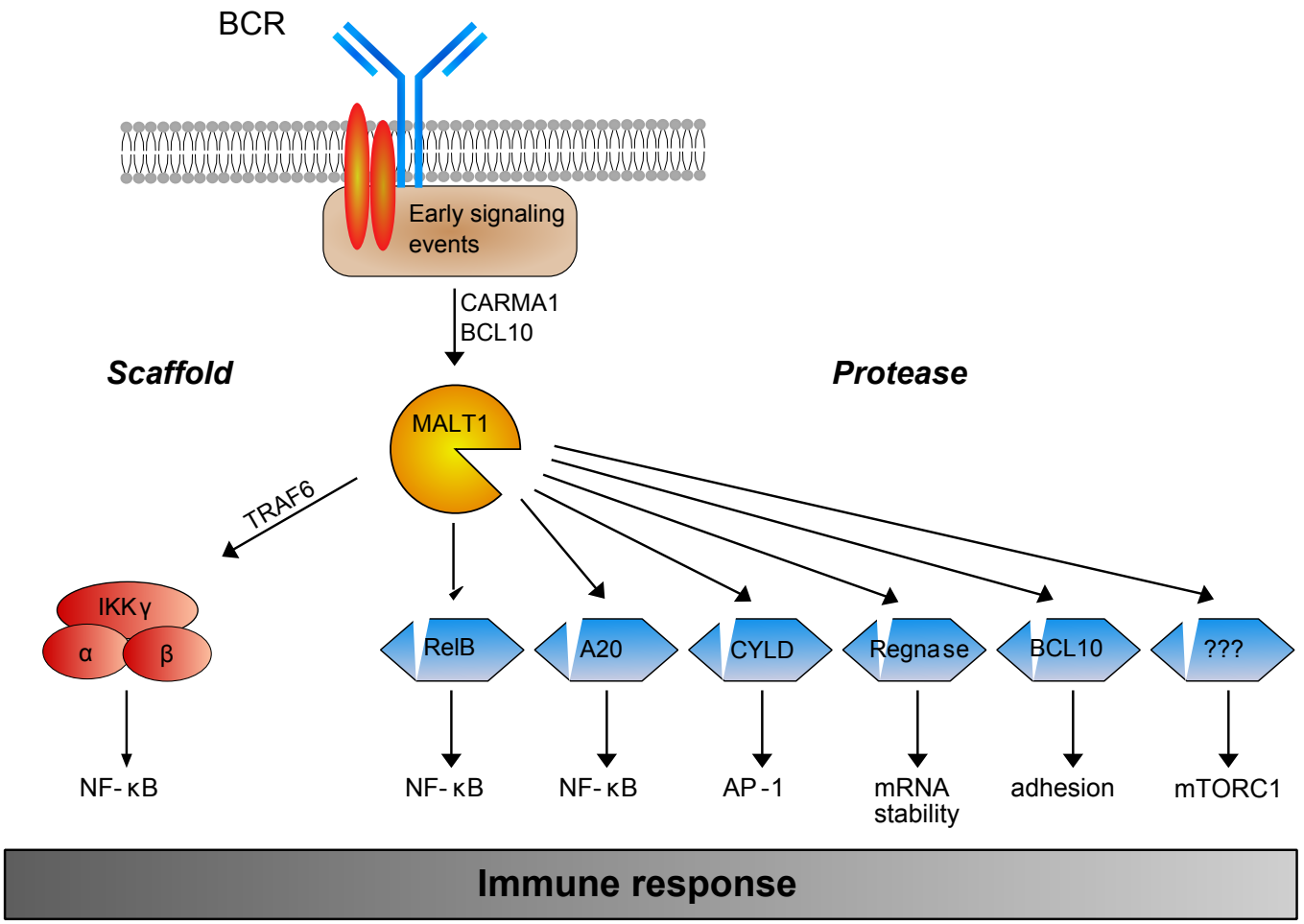

BCR
Early signaling events
CARMA1
BCL10
Scaffold
Protease
MALT1
TRAF6
IKKγ
α
β
RelB
A20
CYLD
Regnase
BCL10
???
NF-κB
NF-κB
NF-κB
AP-1
mRNA stability
adhesion
mTORC1
Immune response

Figure 1

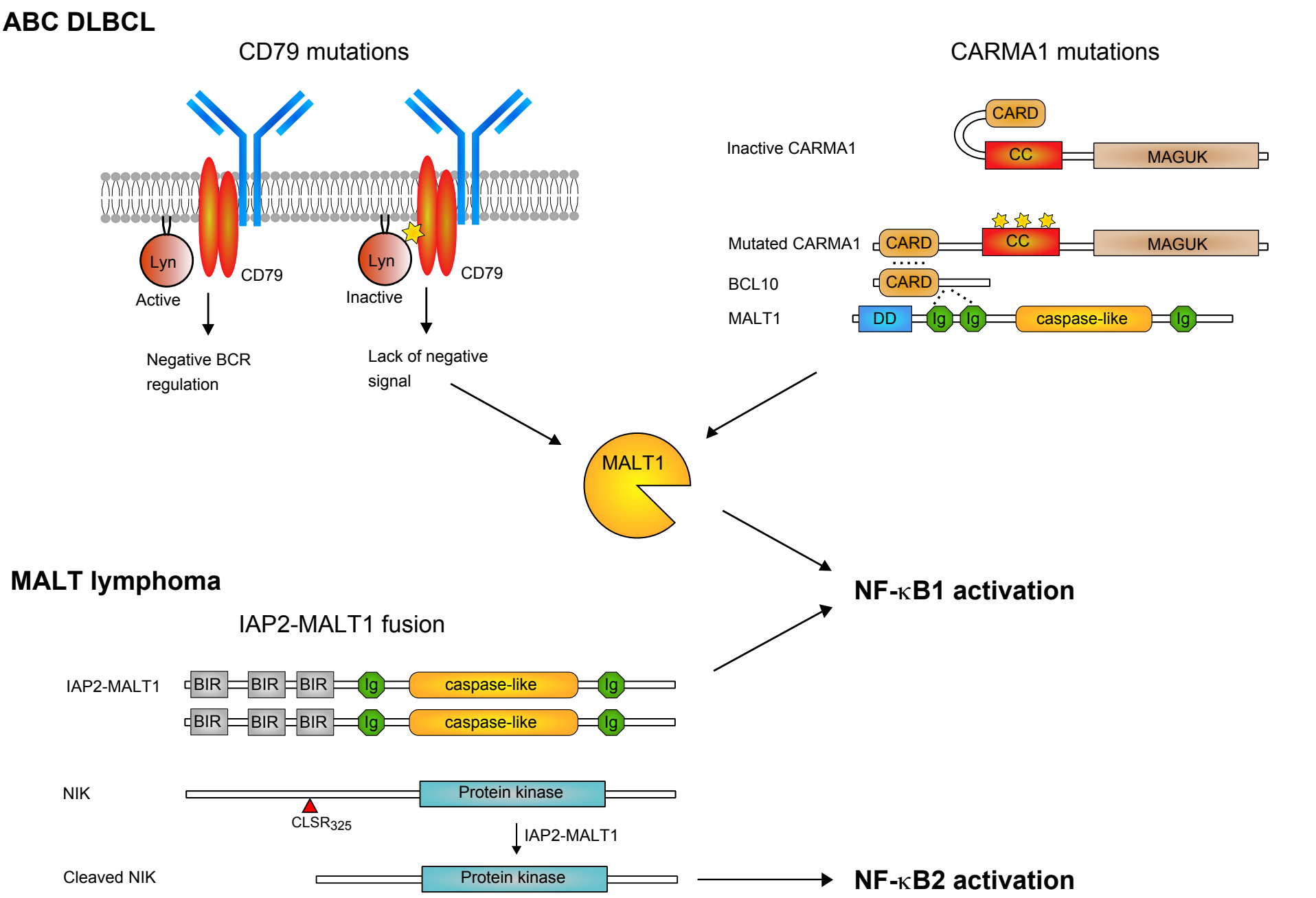

ABC DLBCL
CD79 mutations
CARMA1 mutations
Lyn
CD79
Active
Inactive
Negative BCR regulation
Lack of negative signal
Inactive CARMA1
CARD
CC
MAGUK
Mutated CARMA1
BCL10
MALT1
DD
Ig
caspase-like
MALT1
NF-κB1 activation
MALT lymphoma
IAP2-MALT1 fusion
IAP2-MALT1
BIR
NIK
Protein kinase
CLSR325
Cleaved NIK
NF-κB2 activation

Figure 2

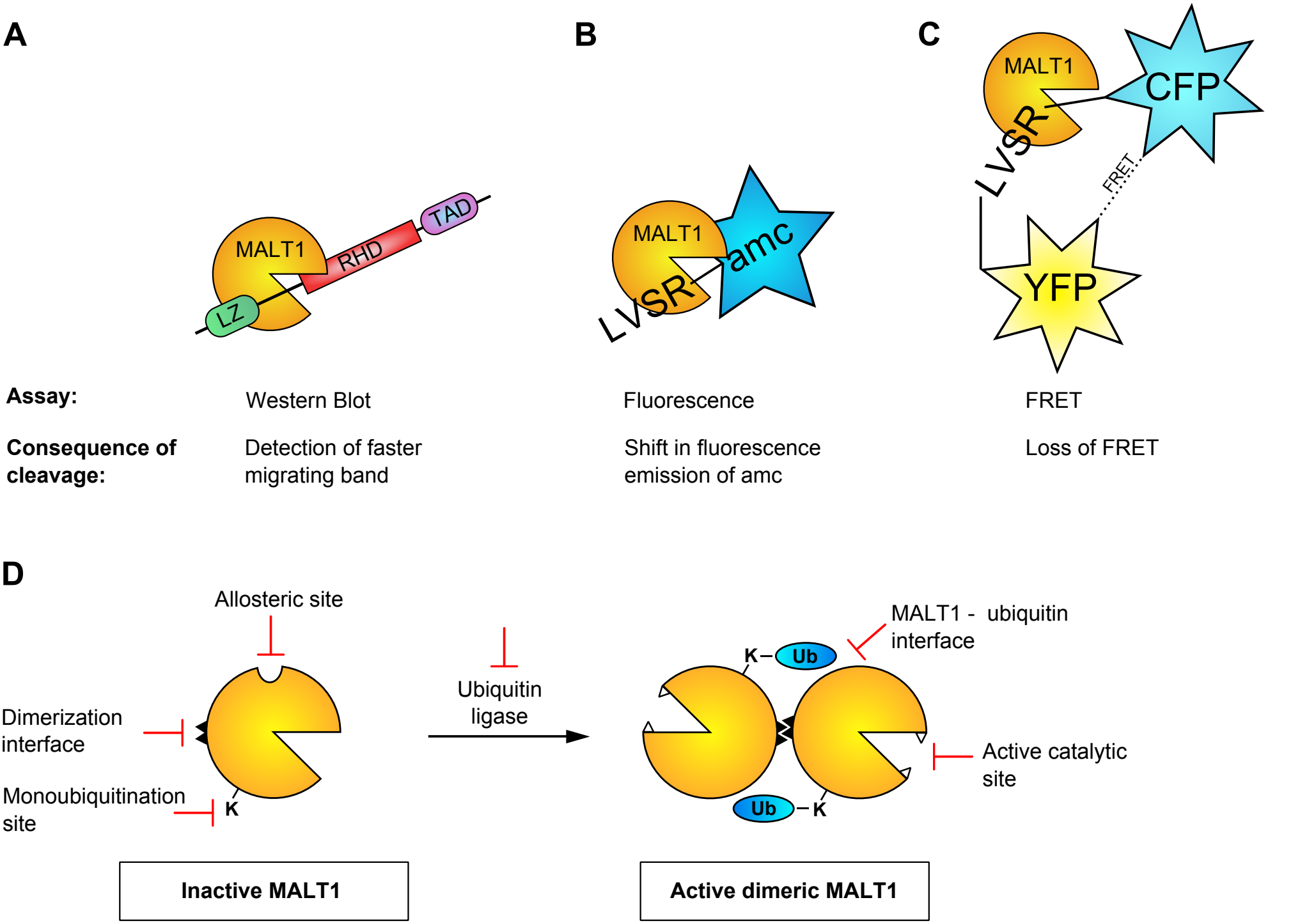

A
MALT1
RHD
TAD
LZ
B
MALT1
amc
LVSR
C
MALT1
CFP
LVSR
FRET
YFP
Assay:
Western Blot
Fluorescence
FRET
Consequence of cleavage:
Detection of faster migrating band
Shift in fluorescence emission of amc
Loss of FRET
D
Allosteric site
Dimerization interface
Monoubiquitination site
K
Ubiquitin ligase
MALT1 - ubiquitin interface
K
Ub
Active catalytic site
Ub
K
Inactive MALT1
Active dimeric MALT1

Figure 3

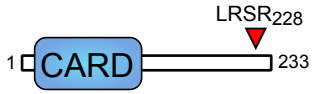
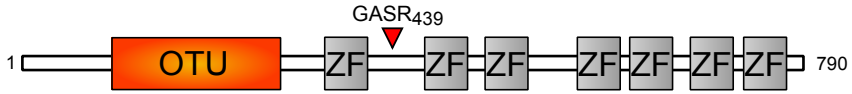
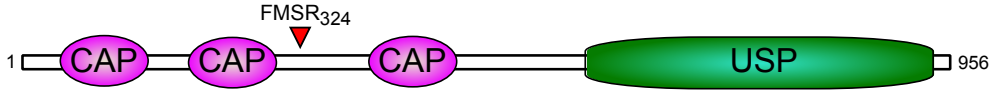
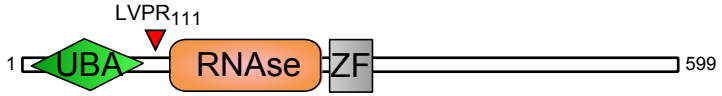
Substrate	*Protein domain structure*	*Effect*	*Reference*
BCL10		Adhesion	Rebeaud et al., 2008
A20		NF-κB activation	Coornaert et al., 2008
CYLD		AP-1 activation	Staal et al., 2011
RelB		NF-κB activation	Hailfinger et al., 2011
Regnase-1		mRNA stabilization	Uehata et al., 2013
MALT1		NF-κB activation	Baens et al., 2014

Table 1