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Article

Short Linear Motifs in the spike protein of SARS-CoV-2 variants provide clues into immune hijack and evasion mechanisms of Omicron variant

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Abstract: Short Linear Motifs (SLiMs) are short linear sequences that can mediate protein-protein interaction. Mimicking eukaryotic SLiMs to compete with extra or intracellular binding partners or to sequester host proteins is the crucial strategy of viruses to pervert the host system. The evolved proteins in viruses facilitate minimal protein-protein interactions that significantly affect intracellular signaling networks. Unfortunately, very little information about the SARS-CoV-2 SLiMs is known, especially across the SARS-CoV-2 variants. Through ELM database-based sequence analysis of spike protein from all the major SARS-CoV-2 variants, we identified four overriding SLiMs in the SARS-CoV-2 Omicron variant including LIG_TRFH_1, LIG_REV1ctd_RIR_1, LIG_CaM_NSCaTE_8, and MOD_LATS_1. These SLiMs are highly likely to interfere with various immune functions, interact with host intracellular proteins, regulate cellular pathways, and lubricate viral infection and transmission. These cellular interactions possibly serve as potential therapeutic targets for these variants, and this approach can be further exploited to combat emerging SARS-CoV-2 variants.

Keywords: coronaviruses; SARS-CoV-2; variant; Omicron; SLiMs; spike protein; motifs; covid-19

1. Introduction

SLiMs are short conserved sequences approximately 3-10 amino acids long, with distinct functions that typically lie in the disordered regions of proteins [1]. The characteristic features of the SLiMs are that a single sequence can recognize multiple proteins via a small contacting surface, and any substitution or modification of a single residue significantly modifies its binding affinity to the proteins [2]. Moreover, the short length of the motifs and these factors bring forth an adaptable molecular basis for rapidly evolved proteins of RNA viruses to develop high versatility.

Viruses mimic cellular linear motifs and hijack host cell factors, favoring viral infection. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are single-stranded, positive-sense RNA viruses, and their evolution has been of great concern from the beginning of the pandemic [3][4]. The SARS-CoV-2 spike protein (S-protein) exists as a trimeric protein. It consists of the S1 subunit with the N-terminal domain (NTD), Receptor-binding domain (RBD), subdomains 1 and 2 (SD1 and SD2), and the S2 subunit, with heptad repeat 1 (HR1), the central helix (CH), a connecting domain (CD), heptad repeat 2 (HR2), a transmembrane domain (TM), and the C-terminal part region [5]. The S-protein equips the virus to enter the host cells and is considered the hotspot of virus evolution and the fundamental attribute in developing many vaccines [6].

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Multitudinous factors are involved in the pathogenesis of SARS-CoV-2 infection and are highly complex. In addition, the continuous evolution of SARS-CoV-2, and the mutations specifically in the spike protein, give rise to variants having unique characteristics such as altered transmissibility and severity of the disease. SARS-CoV-2 Omicron variant becomes pronounced, highly transmissible, and is spreading faster than any previous variant [7][8]. Upon infection, viruses trigger a cascade of antiviral responses in the host cells, and they have to develop mechanisms to evade and subvert those antiviral responses. SLiMs in viral proteins manipulate the host cell's essential processes such as cell signaling, cell cycle, DNA damage repair, and immune system [9] to escape from the responses. Therefore, unraveling the system by which viruses modify these processes can promote the expansion of new rational therapies.

We performed an amino acid sequence analysis across the SARS-CoV-2 variants spike protein for potential functional linear motifs using the Eukaryotic Linear Motif (ELM) resource. The principal question was to discover the interference of viral SLiMs with human host networks. In this study, we identified several SLiMs on the spike protein unique to SARS-CoV-2 and the landscape of SLiMs across all the SARS-CoV-2 variants. This could potentially uncover the possible functions of the SLiMs identified in the Omicron variant taking advantage of known host motif-protein interactions assuming the recognized motifs have common interactors in the host and exploiting the pathways for viral entry and replication and dissemination. These findings can be crucial to developing therapeutic intervention strategies that are essential to contain the emerging SARS-CoV-2 variants.

2. Results

2.1. Sequence analysis shows SLiMs unique and common to SARS-CoV-2 variants

We performed sequence analysis to identify SLiMs across the various coronaviruses spike protein sequences (Figure 1A) and found five SLiMs unique to SARS-CoV-2, which include LIG_Integrin_RGD_1, LIG_CaM_IQ_9, MOD_CMANNOS, LIG_GBD_Chelix_1, LIG_AP2alpha_2, and LIG_WW_1 (Supplementary Figure 1A-B). For instance, several studies have shown the presence of LIG_Integrin_RGD_1 in SARS-CoV-2 due to a single amino acid mutation and how this impacts diverse cellular processes, including cell entry [2][1]. These SLiMs should potentially confer SARS-CoV-2 with numerous mechanisms to manipulate the host immune response. Since multiple studies have investigated the occurrence and impact of novel SLiMs associated with SARS-CoV-2 spike protein, this study focused on the SLiMs across the various variants of interest and concern described by the CDC (Centers for Disease Control and Prevention) (Supplementary Figure 1C-D).

With the SARS-CoV-2 Omicron variant containing 34 different mutations compared to the first Wuhan variant (Figure 1B and Supplementary Figure 1D), our sequence analysis for the occurrence of SLiMs identified 92 motifs in the Omicron variant and 88 motifs in both Delta and Wuhan variants (Figure 1C-D). There were 4 SLiMs that were unique to Omicron variant including LIG_TRFH_1, LIG_REV1ctd_RIR_1, LIG_CaM_NSCaTE_8, and MOD_LATS_1 (Figure 1C). The summary of ELM accession data of the identified SLiMs is shown in Table 1. Multiple sequence alignment of the spike protein from various SARS-CoV-2 variants resulted in four sub-groups where Omicron, Delta, Iota, and Kappa variants are closely related clustering into one group (Figure 1E). Overall, this analysis showed both unique and common SLiMs across coronaviruses and across SARS-CoV-2 variants, which would have potential roles in modulating and hijacking the host immune response.

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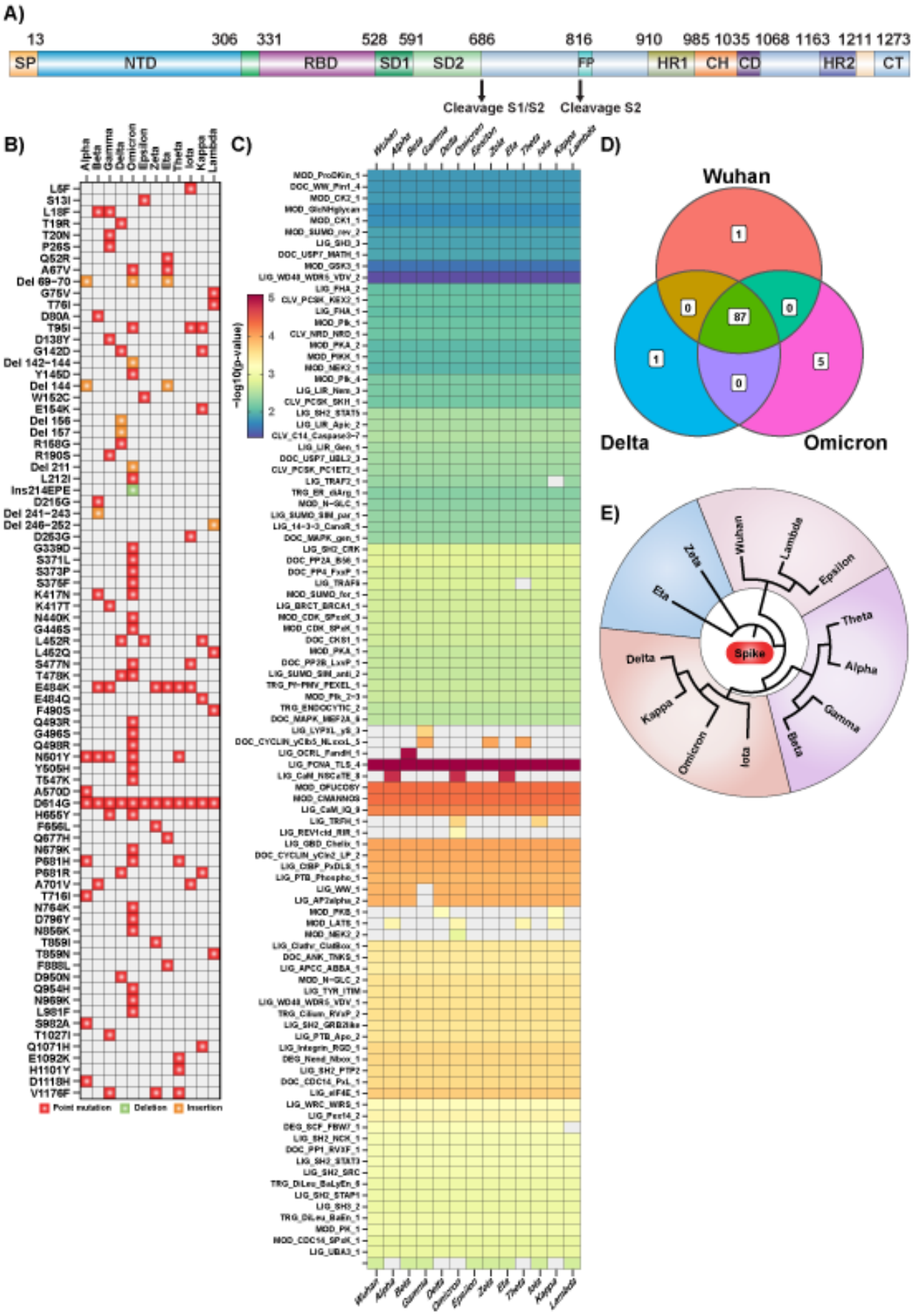

Figure 1: Domain organization and SLiMs in SARS-CoV-2 variants **(A)** Protein domain organization of Spike protein. The S-protein contains the NTD- N-terminal domain, RBD- Receptor binding domain, SD1- Subdomain 1, SD2- Subdomain 2, FP- Fusion peptide, HR1- Heptad repeat1, CH- Central

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helix, CD- Connector domain, HR2- Heptad repeat 2, and CT- Cytoplasmic tail. **(B)** Matrix of mutations on Spike protein across the SARS-CoV-2 variants. **(C)** Heatmap summarizing the identified SLiMs across SARS-CoV-2 variants. **(D)** Venn diagram showing the overlap of SLiMs of Spike protein from Wuhan, Delta, and Omicron variants. **(E)** A circular dendrogram showing the similarity of Spike protein from SARS-CoV-2 variants.

Table 1: List of unique SARS-CoV-2 Omicron SLiMs

2.2. LIG_TRFH_1 motif interaction with shelterin components -TRF2 and TIN2

Viral Motifs	Accession	Instances	Regular expression	Functional site class	Interaction Domain
LIG_CaM_NSCaTE_8	ELME000406	3	W[^P][^P][^P][IL][^P][AGS][AT]	Helical calmodulin binding motifs	EF-hand domain pair
LIG_TRFH_1	ELME000249	3	[FY].L.P	TRFH domain docking motifs	Telomere repeat binding factor
LIG_REV1ctd_RIR_1	ELME000450	10	..FF[^P]{0,2}[KR]{1,2}[^P]{0,4}	RIR motif	DNA repair protein REV1 C-terminal domain
MOD_LATS_1	ELME000334	23	H.[KR]..([ST])[^P]	LATS kinase phosphorylation motif	Protein kinase domain

A study in African green monkey kidney cells (Vero E6) showed that SARS-CoV-2 infection could trigger DNA Damage Response (DDR) and have an impact on telomere stability [10]. The shelterin-associated proteins maintain cellular telomere stability. We recognized the existence of a LIG_TRFH_1 motif (Figure 2A-B) in the SARS-CoV-2 Omicron and the Iota variants. In humans, the telomere repeat factor (TRF)-homology (TRFH) domain interacts with the conserved TRFH-binding-motif (TBM) in the telomere repeat factor 1 (TRF1), telomere repeat factor 2 (TRF2), and TRF1-interacting nuclear protein 2 (TIN2) components of the shelterin complex [11]. Figure 2C represents the structural organization and interaction of human TRF1, TRF2, and TIN2. These proteins and other binding partners of shelterin protect the telomeric DNA sequences (Figure 2D). We hypothesize that the identified viral LIG_TRFH_1 could hijack the cellular TRFH and bind to the TBM motif present in these proteins (Figure 2E). We presume that in Omicron infected cells, this interaction might favor the assembly of the other shelterin components at the viral terminal repeats and thus establish a protective complex that can effectively suppress the cellular DDR, shown in figure 2F. In addition, we assume that this motif could also support maintaining host telomere stability.

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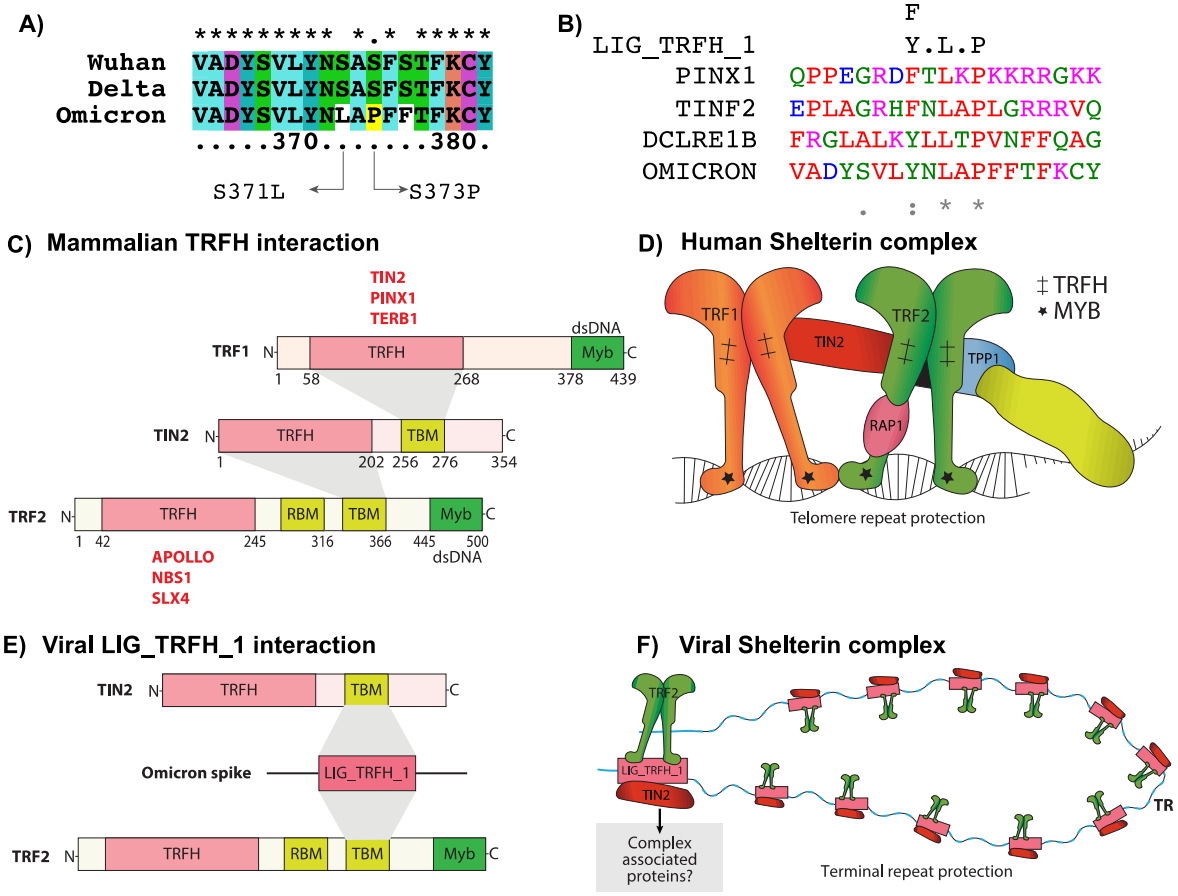

Figure 2: Structure and interaction networks of Shelterin complex associated proteins. **(A)**Snapshot of the multiple sequence alignment of Spike proteins from Wuhan, Delta, and Omicron along with the specific mutations in the Omicron that lead to the emergence of the novel LIG_TRFH_1 motif. **(B)** Snapshot of the multiple sequence alignment of spike protein from Omicron variant along with human proteins that contain this specific motif. **(C)** Domain organization of human TRF1, TRF2, and TIN2. **(D)** Interaction of the human telomere-associated proteins. TIN2 bridges TRF1 and TRF2 that bind to the ds telomeric DNA. **(E)** Omicron SLiM LIG_TRFH_1 interacts with cellular TRF2 and TIN2. **(F)** LIG_TRFH_1 interaction with shelterin proteins protects the viral terminal repeats. Other proteins involved in the protective complex have to be identified. TR-terminal repeats

2.3. LIG_PCNA_TLS_4 and LIG_REV1ctd_RIR_1 motifs are associated with the mutagenic TLS

We located a potential LIG_REV1ctd_RIR_1 motif (Figure 3A-B) exclusively in the spike protein of the SARS-CoV-2 Omicron variant. This SLiM contains a functional RIR motif that is present in the Y-family polymerases that play a central role in translesion DNA synthesis (TLS), a mutagenic branch of cellular DNA damage tolerance [12][13]. RIR motif has been identified in the Y-family DNA polymerases Polη, Polι, and Polκ and is known to interact with the C-terminal domain of Rev1. Rev1 is a crucial member of the eukaryotic Y-polymerases, and its structure is shown in Figure 3C. Furthermore, it was proved that the systematic action of these TLS polymerases is controlled through their interactions with the two scaffolds proteins, the sliding clamp Proliferating cell nuclear antigen (PCNA) and the TLS polymerase Rev1.

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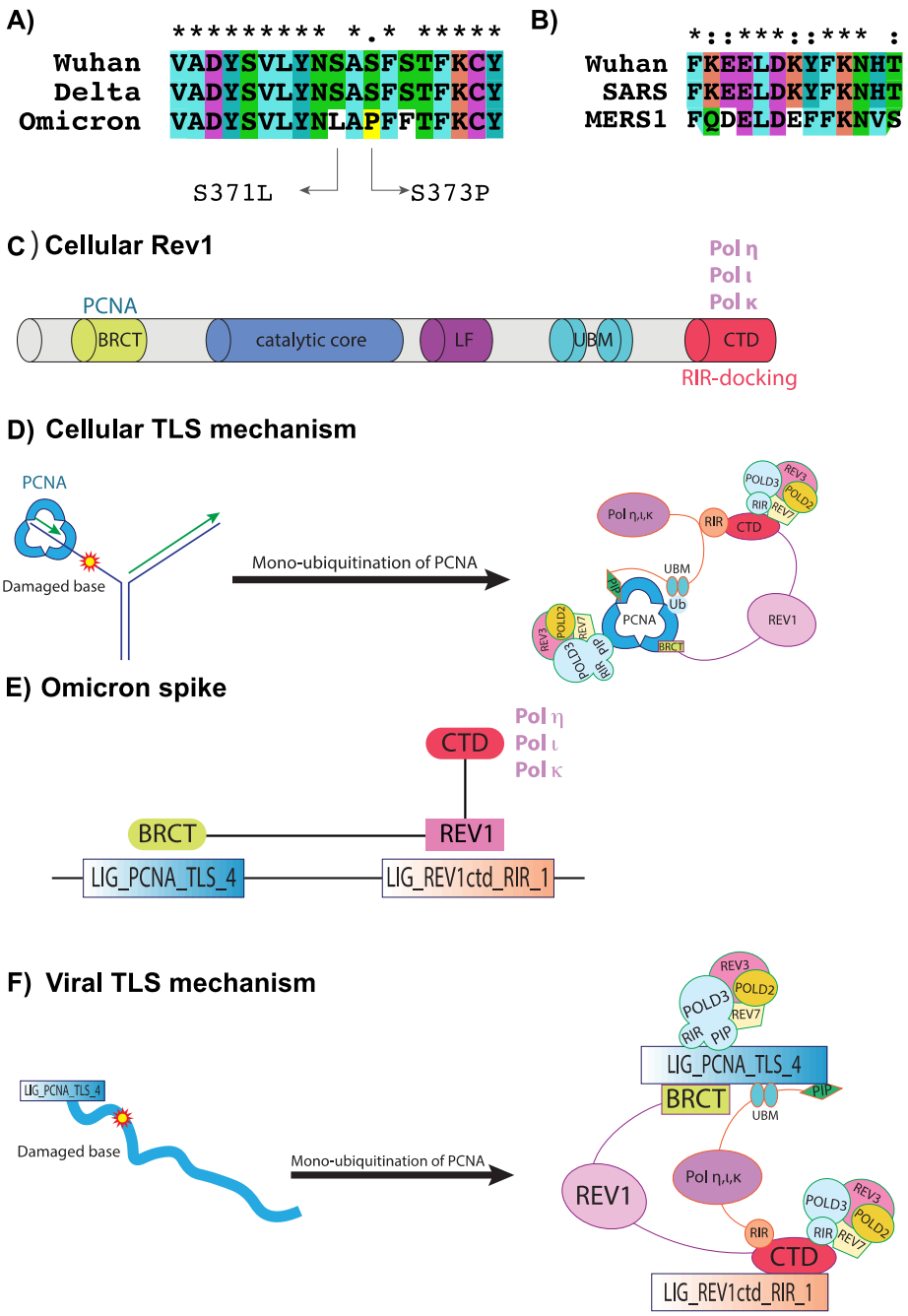

Figure 3: Representation of DNA damage tolerance pathway. **(A-B)** Snapshot of the multiple sequence alignment of Spike proteins from Wuhan, Delta, and Omicron along with the specific mutations in the Omicron that lead to the emergence of the SLiMs. **(C)** Schematic illustration of the domain structure of human Rev1. **(D)** Interactions of human Y-family polymerases in TLS. **(E)** Omicron SLiMs LIG_REV1ctd_RIR_1 and LIG_PCNA_TLS_4 interact with human REV1. **(F)** LIG_REV1ctd_RIR_1 and LIG_PCNA_TLS_4 motifs involvement in the viral TLS.

In addition to the LIG_REV1ctd_RIR_1 motif, we also tracked down a LIG_PCNA_TLS_4 motif, expressed in all the SARS-CoV-2 variants. Mono-ubiquitination of PCNA has been shown to promote TLS machinery and serves as a docking site for key TLS players. Figure 3D depicted the recruitment and interaction of mammalian TLS-associated proteins via PCNA and Rev1. Recently, it was reported that increased ubiquitination in specific regions of PCNA is shown in SARS-CoV-2 infected cells compared to the

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control group, indicating a regulatory mechanism of PCNA during virus infection [14][15]. This can be interpreted as viral infection-induced damage potentially mediating the mono-ubiquitination of PCNA that promotes TLS with increased mutagenesis.

2.4. Ca^{2+}/CaM mediated binding of LIG_CaM_NSCaTE_8 and LIG_CaM_IQ_9 SLiMs

We discovered a pre-eminent motif, LIG_CaM_NSCaTE_8 (Figure 4A), in the Alpha, Eta, and the Omicron variant of SARS-CoV-2. Previously, Ben-Johny *et al*., proposed a blueprint to show the involvement of N-terminal spatial calcium transforming element (NSCaTE) in calcium (Ca^{2+}) /Calmodulin (CaM) mediated regulation of voltage-gated calcium (Ca_V) channels based on an NMR structure [16][17]. In the resting state, CaM interacts with an IQ motif via its C-lobe and is reported that the NSCaTE motif preferentially interacts with an N-lobe of CaM and induces a conformational change (Figure 4C) [18]. The presence of NSCaTE increases the gross channel affinity for Ca^{2+}/CaM over Ca2+-free CaM (apoCaM), providing Ca^{2+} selectivity, and the elimination or donation of the NSCaTE regulates the spatial Ca^{2+} selectivity between global and local profiles.

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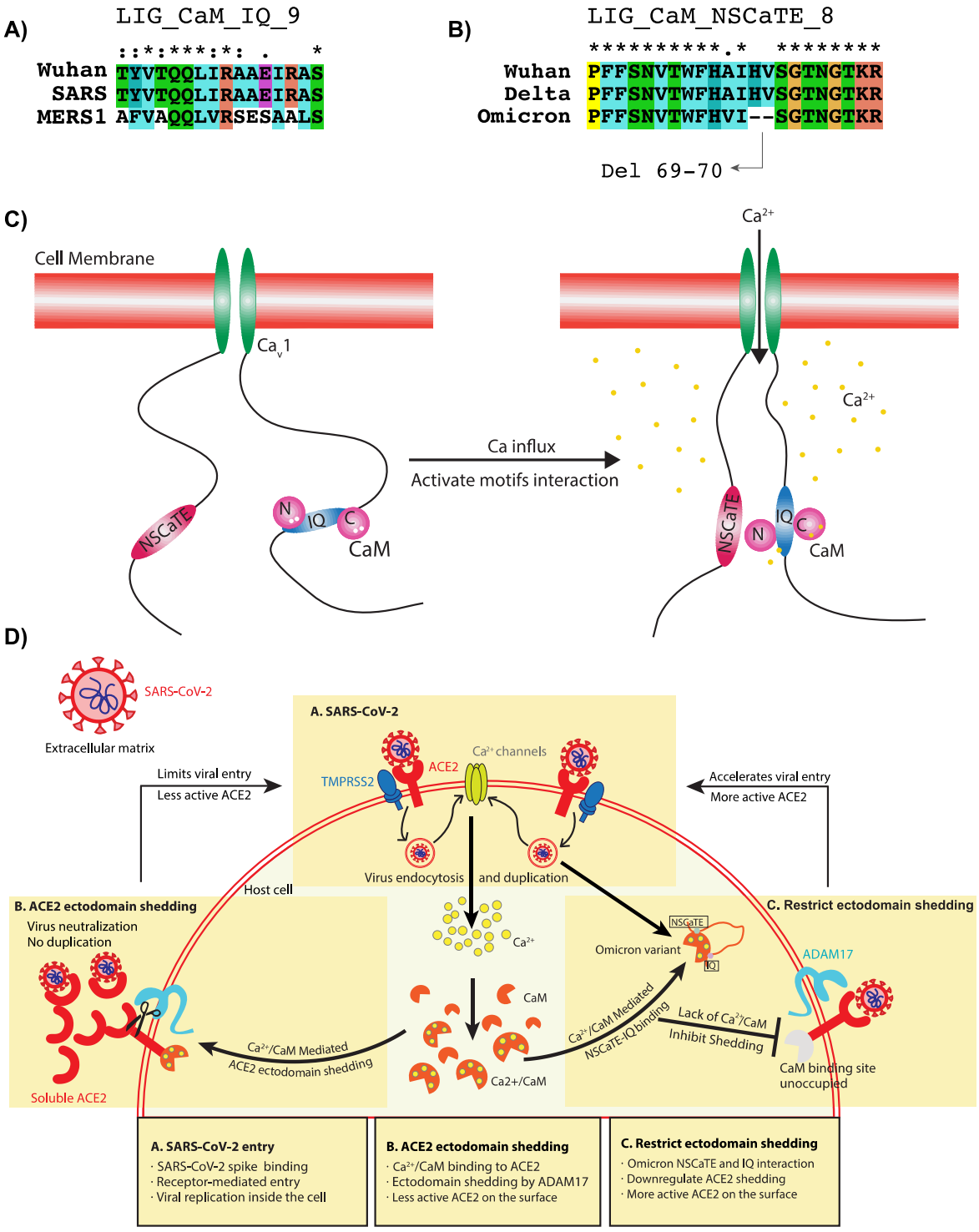

Figure 4: The interaction of NSCaTE and IQ motifs in a Ca^{2+}/CaM mediated manner. Snapshot of the multiple sequence alignment of Spike proteins from **(A)** SARS-CoV-2 (Wuhan), SARS1, and MERS1 viruses; as well as **(B)** Wuhan, Delta, and Omicron along with the specific mutations in the Omicron that lead to the emergence of the SLiMs. **(C)** Ca^{2+} influx facilitates the motif interactions. In the resting state, motifs remain unbound, NSCaTE in the N-terminus, and the IQ motif in the C-terminus. Upon membrane depolarization and Ca^{2+} influx, a Ca^{2+}/CaM complex-mediated interaction of both motifs occurs in the Ca_v1 channel. **(D)** A cartoon representation of the involvement of LIG_CaM_NSCaTE_8 SLiM mediated increased transmissibility in the SARS-CoV-2 Omicron variant**.** The spike glycoprotein on SARS-CoV-2 interacts with the ACE2 to enter the host cells. Viral entry results in an intracellular hike in Ca^{2+} level and hence the Ca^{2+}/CaM complex in the cells. Ca^{2+}/CaM complex-mediated ACE2 catalytic ectodomain shedding by ADAM- 17 generates the soluble form of ACE2. SARS-CoV-2 can bind to the soluble ACE2 as it contains the virus binding site,

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but virus neutralization occurs without an intracellular environment and cannot duplicate. When the Omicron variant enters the cells, Ca^{2+}/CaM mediated binding transpires between the unique Omicron SLiM LIG_CaM_NSCaTE_8 and the LIG_CaM_IQ_9. This process hinders ACE2 ectodomain shedding due to the lack of Ca^{2+}/CaM complex availability for the CaM binding site in the ACE2 cytoplasmic receptor. As a result, more active full-length ACE2 is expressed on the surface for the virus binding.

Interestingly, we have also identified a LIG_CaM_IQ_9 (Figure 4B) motif in all the variants of the SARS-CoV-2. The NSCaTE element can also interact with Ca^{2+}/CaM prebound to an IQ domain peptide, suggesting a possible amino- and carboxyl-termini bridging of the channel [19]. When the two viral motifs LIG_CaM_NSCaTE_8 and LIG_CaM_IQ_9 (Figure 4A-B) come closer in the cytoplasm during Omicron infection, a Ca^{2+}/CaM mediated bridging could occur. A mechanism parallels the Cav1 channels where the NSCaTE element interacts with Ca^{2+}/CaM prebound to an IQ domain peptide (Figure 4D). This process depletes the available free cytoplasmic Ca^{2+}/CaM level and might slow down the Ca^{2+}/CaM associated mechanisms in the virus-infected cells. For instance, Ca^{2+}/CaM mediated ACE2 ectodomain shedding, a rate-limiting step for SARS-CoV-2 entry.

2.5. MOD_LATS_1 intervention in YAP/TAZ localization and Hippo signaling regulation

The MOD_LATS_1 motif (Figure 5A) was observed in the Alpha, Omicron, Theta, and Kappa variants. In cells, YAP/TAZ are the substrates of mammalian large tumor suppressor homolog (LATS) and are the critical effectors of the Hippo pathway. Cellular LASTS1/2 is activated by phosphorylation and directly interacts with and phosphorylates YAP/TAZ, which mediates their localization and function in the hippo signaling pathway [20]. We assume that during Omicron infection, the viral MOD_LATS_1 gets phosphorylated by the cellular LASTS1/2 and thus controls the Hippo pathway (Figure 5B).

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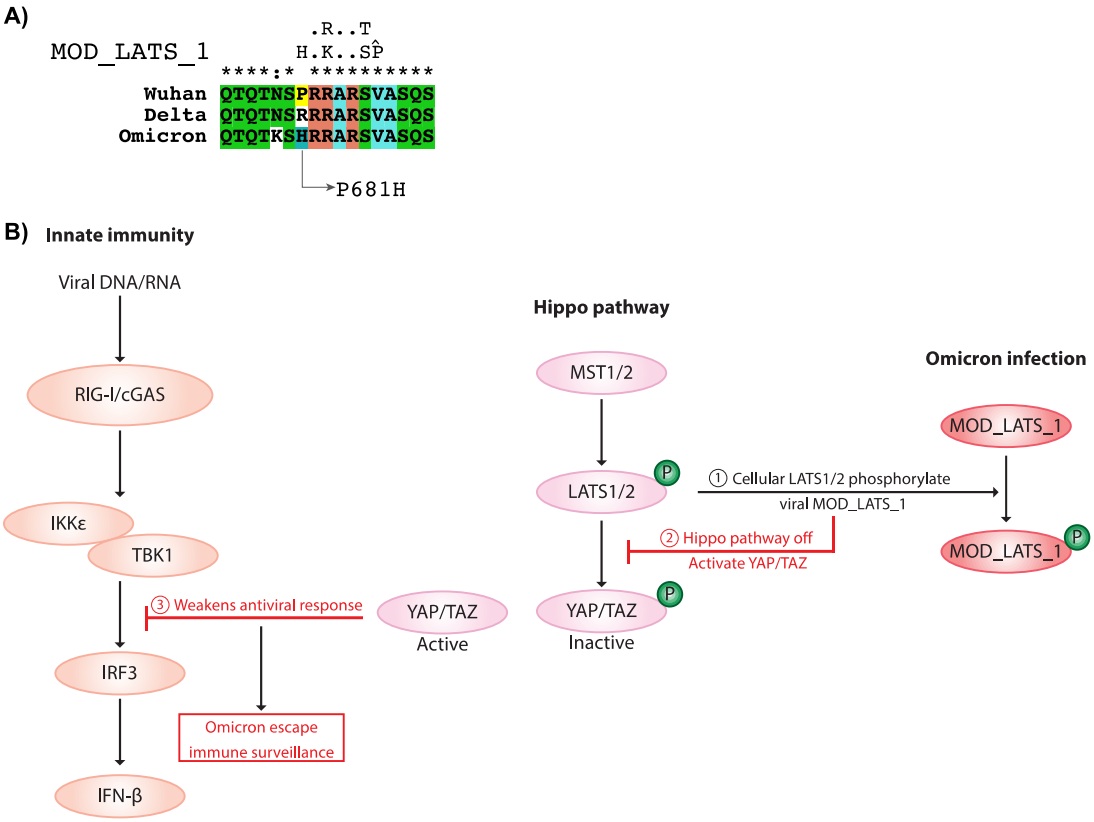

Figure 5: Schematic representation of the Hippo signaling pathway and Omicron MOD_LATS_1 intervention. **(A)** Snapshot of the multiple sequence alignment of Spike proteins from Wuhan, Delta, and Omicron along with the specific mutations in the Omicron that lead to the emergence of the SLiMs. **(B)** Modulation in the hippo signaling pathway during Omicron infection. When the Hippo signaling pathway is active/on, YAP/TAZ proteins get phosphorylated by LATS1/2 kinases and remain in the inactive form. However, during Omicron infection, cellular LATS1/2 kinases phosphorylate viral MOD_LATS_1 leaving active cytoplasmic YAP/TAZ, which can negatively regulate immune response and facilitate Omicron survival.

The expression and the activity of the hippo components serve as an indicator to regulate the magnitude of host antiviral responses. Several studies illustrate that YAP/TAZ is a negative regulator of innate immunity against DNA and RNA viruses [21]. YAP/TAZ abolishes virus-induced activation of TBK1-IKKε and restores viral replication. However, LASTS1/2 mediated Knockout of YAP/TAZ results in an enhanced innate immunity and a reduced viral load [22]. We believe that the MOD_LATS_1 motif could hijack cellular LATS phosphorylation and thus control the Hippo pathway, suggesting YAP/TAZ-related defective antiviral responses. MOD_LATS_1 gets phosphorylated by the cellular LASTS1/2, leaving the active cytoplasmic YAP/TAZ in the infected cells. This activity could weaken the antiviral response, and the viruses escape from the immune surveillance.

3. Discussion

3.1. LIG_TRFH_1 motif maintains viral and cellular terminal repeats from DDR response

Shelterin, a complex of six proteins TRF1, TRF2, POT1, TPP1, TIN2, and Rap1, protects telomeres from various DNA damages (Figure 2D) and prevents end-to-end chromosome damage fusions [23]. Depletion or mutation of any of the components of the shelterin complex triggers telomeric dysfunction and activates DDR pathways [24]. Viral infection can also induce DNA damage and evoke the host DDR, and it communicated

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several studies that the progression of SARS-CoV-2 severity is associated with a decreased telomere length [25][26][27]. In humans, TRFH domains recognize a conserved motif, TBM, present in the shelterin proteins, thereby enabling the integration of communication networks between telomere-associated proteins, DNA- repair, and DNA-damage signaling proteins (de Lange, 2005a). The TBM domain is conserved in the TRF2 and TIN2 components of the shelterin.

We assume analogous to cellular TRFH, the identified viral LIG_TRFH_1 could interact with these two proteins. A possible interaction between LIG_TRFH_1 with TRF2 and TIN2 is presented in Figure 2E. TIN2 is considered the shelterin complex's central hub, which interacts with TRF1, TRF2, and TPP1 and promotes the assembly of an intact shelterin complex, which protects the telomere repeats. TRF2 participates in the t-loop formation and protects telomeres from DDR pathways [28]. Various viruses utilize different mechanisms for genome end-protection, but none of them are fully understood. For instance, Human Herpesvirus 6 (HHV6) and Marek's disease virus (MDV) contain telomere repeat sites in viral genomes that can direct integration of viral genomes toward host telomere facilitated by the TRF1 And TRF2 [29]. We strongly believe that the existence of the LIG_TRFH_1 motif benefits the Omicron variant by potentially hijacking the cellular telomeric factors and mimicking the mechanism by which shelterin suppresses DDR at telomeres.

The genome-wide analysis demonstrates that SARS-CoV-2 has ultra-conserved 59- and 39-terminal regions, which are shared among betacoronavirus lineage B genomes [30]. Furthermore, it has conserved RNA secondary structures crucial for the replication and transcription of the virus [31] and can also recruit and interact with a range of host and viral protein factors. This region is susceptible or detected by the DNA damage sensing and repair pathways. We presume that during the Omicron infection, the LIG_TRFH_1 can recruit the cellular shelterin proteins to the viral terminal repeats forming a protective complex that can actively suppress the cellular DDR (Figure 2F). Furthermore, we assume that besides evading the host immune attack, the LIG_TRFH_1 SLiM in Omicron might also indirectly favor maintaining the host telomere stability to protect the cells from senescence. Therefore LIG_TRFH_1 could accelerate viral propagation and cellular replication without any hurdle, and thus the participation of the LIG_TRFH_1 motif in these two events might diminish the severity of Omicron infection.

3.2. Generation of highly mutagenic Omicron variant through a unique LIG_REV1ctd_RIR_1 motif

TLS is a mutagenic branch of cellular DNA damage tolerance that enables bypass replication over DNA lesions accomplished by specialized low-fidelity DNA polymerases [12][13]. LIG_REV1ctd_RIR_1 is exclusively present in the Omicron variant and has a functional RIR motif specific to the Y-family polymerases of TLS. The mammalian RIR motif of polymerases η, ι, and κ can bind the C-terminal domain of Rev1 (Rev1-CTD), which in turn interacts with Rev3 and Rev7 subunits of pol ζ (Figure 3D) [32]. In addition, Rev1 has a deoxycytidyl (dCMP) transferase activity and plays a central role in TLS during replication and post-replication DNA repair [33] but lacks polymerase activity. Further, Rev1 recruits inserter and extender polymerases to the lesion site through specific protein-protein binding via its C-terminal domain. It also has an N-terminal BRCT domain by which it interacts with the PCNA.

The interaction between Rev1 and other Y-family DNA polymerases is frail, facilitating rapid and dynamic associations and dissociations among many factors involved in TLS events. Compared to conventional replicative polymerases, the Y-family polymerases have distinct structural and biochemical features that bypass DNA damage. The switch between the replication polymerases and TLS polymerases relies on the post-translational modification status of PCNA. Figure 3E represents the possible participation of LIG_PCNA_TLS_4 and LIG_REV1ctd_RIR_1 motif in TLS. We expect the presence of LIG_PCNA_TLS_4 triggers the initiation of the TLS events in Omicron, and the unique LIG_REV1ctd_RIR_1 interacts with the cellular Rev1, recruits its associated proteins, and

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initiates the mutagenic TLS mechanism. Figure 3F illustrates the involvement of components of TLS in viral mutagenesis.

The large number of mutations observed in the spike protein of SARS-CoV-2 raised severe concerns about the contagious nature of this new virus with little prior immunity. Y-family polymerases have a specific preference for incorporating incorrect nucleotides, and the resulting mutations are sequence-specific. The report shows that Omicron has at least 50 mutations, with 35 of those mutations turning up in the spike protein (Figure 6) compared to the reference strain [34]. We suspect that the two SLiMs, LIG_PCNA_TLS_4, and LIG_REV1ctd_RIR_1, promote mutagenesis in the Omicron infected cells mainly by participating in the crucial protein-protein interactions that regulate the access of translesion DNA polymerases to the primer terminus. Studies indicate that the mutations that emerged in the new SARS-CoV-2 Omicron variant are an essential driver of its increased transmissibility and are supported by our observations.

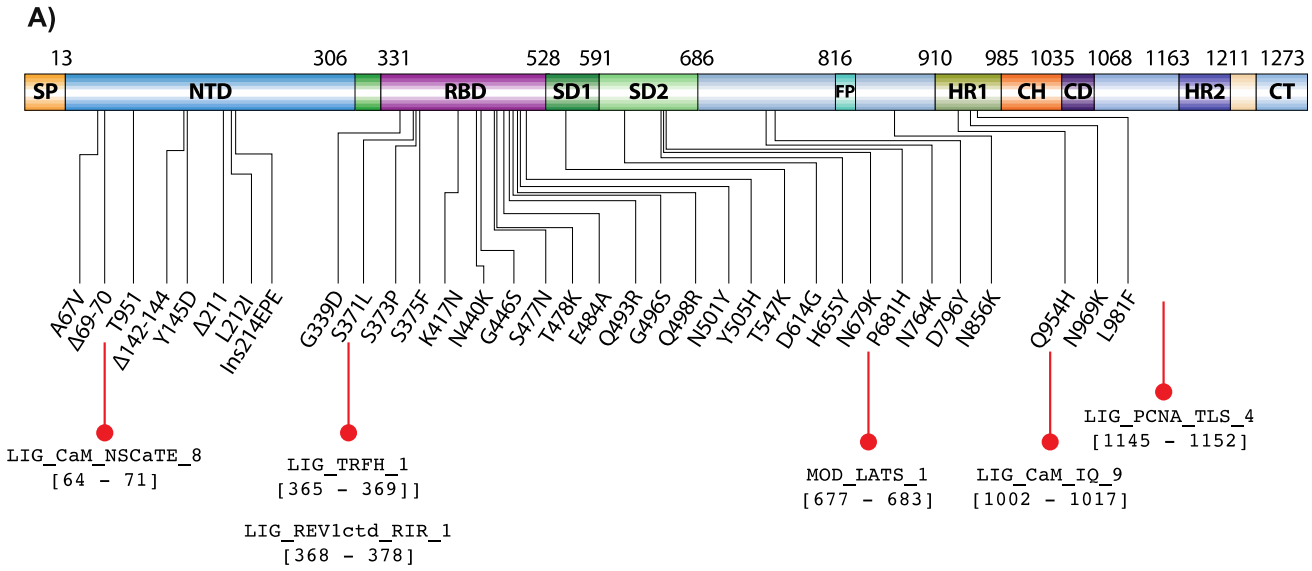

Figure 6: Schematic representation of Omicron spike protein organization and the amino acid mutations. Omicron mutations are shown in a primary structure of SARS-CoV-2 S-protein. Amino acid mutations in SARS-CoV-2 Omicron spike proteins are A67V, Del69-70, T95I, Del142-144, Y145D, Del211, L212I, R214Insertion, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, and L981F. Selected SLiMs introduced due to the mutation in Omicron variants are marked at the bottom of the domain map.

3.3. Dynamic switching of cytoplasmic Ca^{2+}/CaM interaction and inhibition of ACE2 ectodomain shedding

SARS-CoV-2 enters the cells by binding to the ACE2 receptors, which comprise an extracellular heavily N-glycosylated N-terminal domain containing the carboxypeptidase site and a short intracellular C-terminal cytoplasmic tail. This protein exists in two forms: a membrane-bound cellular form through which the virus enters the cell and a soluble circulating form [35][36], where SARS-CoV-2 can bind but cannot duplicate because of the unfavorable environment. Hence the circulating ACE2 is presumed to protect from the SARS-CoV-2 infection. Circulating ACE2 is cleaved from the full-length ACE2 on the cell membrane by a disintegrin and metalloproteinase domain-containing protein 17 (ADAM17) and then liberated into the extracellular environment. It has been reported that the calcium signaling pathway is involved in the catalytic ectodomain shedding process of the ACE2 regulated by CaM. A computational analysis of the cytoplasmic domain of

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ACE2 revealed a conserved consensus calmodulin-binding motif and is also shown that calcium-dependent CaM–peptide complex can bind to the cytoplasmic domain of ACE2 and enhance the ADAM17 mediated ectodomain shedding [37][38].

The viral proteins expressed on the surface of the infected cells are capable of perturbing intracellular Ca^{2+} homeostasis in several ways [39]. In the Omicron infected cells, an elevated Ca^{2+} in the cytoplasm expedites a Ca^{2+}/CaM mediated bridging of the two viral motifs LIG_CaM_NSCaTE_8 and LIG_CaM_IQ_9, a mechanism analogous to the CaV1 calcium channels where the NSCaTE element interacts with Ca^{2+}/CaM prebound to an IQ domain peptide [19]. Based on this, we hypothesized the possible involvement of these SLiMs in the shedding processes of ACE2 catalytic ectodomain, which is depicted in figure 4D. The interaction of these motifs could potentially lead to a depletion in the available CaM for ACE2, and the calmodulin-binding motif in the cytosolic tail of ACE2 remains unoccupied. The absence of CaM downregulates the ectodomain shedding and provides full-length ACE2 for virus binding and entry. This process results in an increased active ACE2 expression in the cell surface, and we believe that this could be one of the possible reasons for the high transmissibility of the SARS-CoV-2 Omicron variant. Our finding is further supported by the recent reports that the Omicron infection is ACE2 dependent and that the binding of the Omicron spike to ACE2 is elevated compared to the wild-type virus [40][41].

3.4. Omicron escapes immune surveillance by MOD_LATS_1 SLiM

TANK Binding Kinase 1 (TBK1) is crucial for cytosolic nucleic acid sensing and antiviral defense. The hippo pathway is shown to regulate antiviral defense by modulating the TBK1-mediated control of interferon production. Studies show that Hippo activation abrogates the inhibitory effect of YAP/TAZ on TBK1 and enhances antiviral responses [42]. In mammalian cells, LATS1/2 kinases mediated depletion or deletion of YAP/TAZ relieve TBK1 suppression and boost antiviral responses. In contrast, expression of the transcriptionally inactive YAP dampens cytosolic RNA/DNA sensing and weakens the antiviral defense in cells [43]. Several studies illustrate that YAP/TAZ is a negative regulator of innate immunity against DNA and RNA viruses. YAP/TAZ mainly functions as a transcriptional co-activator, which regulates the transcription of target genes by shuttling between the nucleus and the cytoplasm, thereby affecting cellular immune surveillance against pathogen attacks. In response to the viral nucleic acids, YAP blocks the activation of IRF3 at the dimerization step and limits IFN-β expression and innate antiviral responses to viruses [44].

We firmly believe that Omicron MOD_LATS_1 can easily mimic as a substrate for cellular LATS1 and regulate the hippo pathway's core components, hence restyling the defense mechanism against the SARS-CoV-2 infection. Therefore, we assume that the hippo pathway gets turned off in the Omicron infected cells by diverting the cellular LATS kinase to viral MOD_LATS_1 and phosphorylating it. This step leaves the active YAP/TAZ in the cytoplasm, inhibits TBK1/IRF3, and weakens the antiviral defense mechanism. The likely involvement of MOD_LATS_1 in regulating the hippo pathway is shown in Figure 5B. The abnormal regulation of the Hippo pathway was observed during infection with various viruses such as HBV, HCV, MCV, ZIKV, EBV, KSHV, HPV, and

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MuPyV [45]. For instance, the Zika virus, a member of the *Flaviviridae* family infection, activate an antiviral response and the Hippo pathway, leading to the degradation of the critical proviral factor YAP/TAZ [46], which could regulate immune response and results in reduced ZIKV replication [47]. In this context, the presence of the MOD_LATS_1 motif in the Omicron variant could potentially activate YAP/TAZ activity and subside cellular immunity. This could positively assist in Omicron replication inside cells and strengthen transmission.

4. Materials and Methods

4.1. SARS-CoV-2 protein sequences

We obtained the protein sequence of SARS-CoV-2 Wuhan isolate (Accession number: P0DTC2) and other coronavirus spike protein sequences used in this study from the Uni-Prot database. The mutation information for the SARS-CoV-2 variants was obtained from the CDC website (https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-classifications.html). We developed an in-house python program to generate the spike protein sequence for each variant using the mutation information from CDC.

4.2. Bioinformatics analysis

SLiMs analysis of the spike protein sequences was performed using the online resource ELM prediction tool (http://elm.eu.org/) (Cite PubMed ID: 31680160). Multiple sequence alignment was performed using Clustal Omega (PubMed ID: 28884485), and the alignment tree was plotted using iTOL software (PubMed ID: 33885785). All data formatting, processing, and generation of the various graphs and charts were performed using in-house R scripts (https://www.r-project.org).

5. Conclusions

High transmissibility and less infection are the significant considerations about the SARS-CoV-2 Omicron variant. RNA viruses exhibit a high mutation rate and are equipped with various dynamic strategies that favor their rapid adaptation to the environment. Due to the significant viral evolution, SARS-CoV-2 has a high likelihood of genomic mutations that allow these viruses to accommodate new environments, leading to never-ending long-term threats. We believe that a deep dissection of the interactions between viral and cellular proteins will ultimately lead to a better understanding of the molecular bases of the regulatory networks. Considering molecular mimicry, a common strategy for coronaviruses, we identified four prominent motifs in the SARS-CoV-2 Omicron variant that could mimic and interact with human proteins. Future works have to be done to understand the precise role of these identified SLiMs and thus get a much deeper insight into the SARS-CoV-2 virus in the normal cell. These pieces of information will boost the development of effective therapeutic drugs to fight against SARS-CoV-2 variants.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1: List of identified SLiMs and their overlap in Spike protein from SARS-CoV-2, SARS1, MERS1, and two other human coronaviruses.

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