Nuclear export receptor CRM1 recognizes diverse conformations in nuclear export signals

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Abstract

Nuclear export receptor CRM1 binds highly variable nuclear export signals (NESs) in hundreds of different cargoes. Previously we have shown that CRM1 binds NESs in both polypeptide orientations (Fung et al., 2015). Here, we show crystal structures of CRM1 bound to eight additional NESs which reveal diverse conformations that range from loop-like to all-helix, which occupy different extents of the invariant NES-binding groove. Analysis of all NES structures show 5-6 distinct backbone conformations where the only conserved secondary structural element is one turn of helix that binds the central portion of the CRM1 groove. All NESs also participate in main chain hydrogen bonding with human CRM1 Lys568 side chain, which acts as a specificity filter that prevents binding of non-NES peptides. The large conformational range of NES backbones explains the lack of a fixed pattern for its 3-5 hydrophobic anchor residues, which in turn explains the large array of peptide sequences that can function as NESs.

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Introduction

The chromosome region maintenance 1 protein (CRM1) or Exportin-1 (XPO1) binds 8–15 residues-long nuclear export signals (NESs) in hundreds of different protein cargoes (Fornerod et al., 1997; Fukuda et al., 1997; Stade et al., 1997; Ossareh-Nazari et al., 1997; Xu et al., 2012a; Kırlı et al., 2015; Thakar et al., 2013). NES sequences are very diverse but each usually has 4–5 hydrophobic residues (often Leu/Val/Ile/Phe/Met; labeled F0–5) that bind hydrophobic pockets (labeled P0–P4) in a hydrophobic groove formed by HEAT repeats 11 and 12 of CRM1 (9–16). The hydrophobic anchor residues are arranged in many ways, currently described by ten consensus patterns for corresponding NES classes 1a, 1b, 1c, 1d, 2, 3, 1a-R, 1b-R, 1c-R and 1d-R (Figure 1A) (Fung et al., 2015).

Previous structures of CRM1 bound to five different NESs showed virtually identical NES-binding grooves (Fung et al., 2015; Monecke et al., 2009; Dong et al., 2009; Gütter et al., 2010). NESs from Snurportin-1 (SNUPN NES; class 1c) and Protein Kinase A Inhibitor (PKI NES; class 1a) bind CRM1 as α-helix followed by a short β-strand, while the proline-rich NES from HIV-1 REV (Rev NES; class 2) adopts mostly extended conformation (Figure 1B) (Gütter et al., 2010). The majority of CRM1-NES interactions involve NES hydrophobic anchor side chains, with very few polar and main chain interactions. Previously, we studied NESs with the $\Phi_1\Phi_2\Phi_3\Phi_4$ pattern where the $i, i+3, i+7, i+10$ positions suggested a single long amphipathic helix. However, it is perplexing that a long all-helical peptide could fit in the narrow tapering CRM1 groove. Structures of such NESs from kinase RIO2 and cytoplasmic polyadenylation element-binding protein 4 (hRio2 NES, CPEB4 NES) showed that they do not adopt all-helical conformations but unexpectedly adopt helix-strand conformations that bind CRM1 in the opposite or minus (−) polypeptide direction to that of SNUPN NES, PKI NES and Rev NES ((+) NESs) (Fung et al., 2015). hRio2 NES and CPEB4 NES were hence reclassified as class 1a-R NESs (Figure 1A).
Figure 1. Structures of CRM1-bound NESs that match the potentially all-helical class 3 pattern. (A) Current NES sequence patterns (Φ is Leu, Val, Ile, Phe or Met and X is any amino acid). Potential amphipathic α-helices, predicted with hydrophobic patterns of i, i+4, i+7 or i, i+3, i+7 or i, i+3, i+7, i+10, are shaded grey. (B) Structure of PKI NES (F0L) (dark blue, PDB ID: 3NBY), Rev NES (pink, 3NBZ) and CPEB4 NES (yellow, 5DIF) bound to the NES-binding groove of CRM1 (grey surface). NESs are shown in cartoon representations with their Φ side chains shown as sticks. (C) The overall structure of the engineered ScCRM1 (grey)-Ran-GTP (orange)-RanBP1 (purple)-mDia2 NES (pale green) complex. (D) mDia2 NES (pale green), (E) CDC7 NES (green-cyan) and (F) X11L2 NES (forest) bound to the NES-binding groove of ScCRM1 in the engineered ScCRM1-Ran-RanBP1 complex. The X11L2 NES sequence matches the class 3 pattern, but binds CRM1 according to the new hydrophobic pattern F0XX01XXX02XX03XX04 that we termed the class 4 pattern. mDia2 NES is not shown in the leftmost panel of (D) to view the five hydrophobic pockets (P0–P4) of the CRM1 groove. Rightmost panels of (D–F): overlays of 3.0σ positive densities of kick OMIT mFo-DFc maps (calculated with peptides omitted) and final coordinates of the NES peptides. Middle panels of (D–F): black dashes show CRM1-NES hydrogen bonds and polar contacts.

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Source data 3. Crystallization conditions of CRM1-NES complexes.
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Figure supplement 1. Binding affinities of NES to CRM1 measured by differential bleaching experiments.
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Figure supplement 2. Modeling strategy for NESs with weak Φ side chain densities.

Figure 1 continued on next page
We do not understand the extent of NES structural diversity nor how NESs with different hydrophobic patterns that presumably reflect different secondary structures all bind to the seemingly invariant and three-dimensionally constrained CRM1 groove. Here, eight new structures of CRM1 bound to diverse NESs show several different and unexpected NES backbone conformations that share only a common one-turn helix element. All NESs also participate in hydrogen bonding with Lys568 of \(^{15}\)CRM1, and mutagenic/structural analysis identifies Lys568 as a selectivity filter that blocks binding of non-NES peptides.

**Results**

**CRM1-bound NESs adopt diverse conformations**

We study three NESs that uniquely match the all-helical class 3 pattern (Φ1XXΦ2XXΦ3XXΦ4). Because most previously studied NESs have substantial helical content, we also study five NESs that match class 2 (Φ1Φ2Φ3Φ4) and class 1b (Φ1Φ2Φ3Φ4) patterns, where the hydrophobic residue positions do not suggest an amphipathic helix (Figure 1A). All eight NESs bind \(^{15}\)CRM1 in the presence of RanGTP with dissociation constants (K\(D\)) of 670 nM-20 μM, and were crystallized bound to the previously described engineered \(^{5}\)CRM1-RanGppNHp-Yrb1p complex (Figure 1—figure supplement 1, Figure 1—source data 1, 2, and 3) (Fung et al., 2015). Details of how the NES peptides were modelled can be found in methods section and in Figure 1—figure supplements 2 and 3. NES-bound CRM1 grooves in the structures (2.1–2.4 Å resolution) resemble the PKI \(^{2}\)NES-bound MmCRM1 groove (Ca/all-atom rmsds 0.5 Å/1.1 Å for 85 groove residues), and all NESs use 4–5 hydrophobic anchor residues to bind P0-P4 hydrophobic pockets of CRM1 (Figures 1D–F and 2) (Güttler et al., 2010).

Class 3 NESs from mouse diaphanous homolog 3 (mDia2 \(^{2}\)NES: 112QSVPEVEALLLRARL1193) and the cell division cycle 7-related protein kinase (CDC7 \(^{2}\)NES: 45QDLRKLCELRMGSSTP367) are indeed all-helix peptides, both forming 3-turn α-helices that occupy only the wide part of the CRM1 groove (Figure 1D,E, Figure 1—figure supplement 4). The last residue of the mDia2 protein (Leu1193) binds the CRM1 P3 pocket leaving the P4 pocket empty. CDC7\(^{2}\)NES is far from the protein C-terminus but structures of longer peptides suggest that CDC7\(^{2}\)NES exits the groove after Met468 or Φ3 (Figure 1—figure supplement 5). The mDia2\(^{2}\)NES and CDC7\(^{2}\)NES sequence patterns should thus be Φ0XXΦ1XXXXΦ2XXΦ3. The Φ4 anchor position is clearly not used in mDia2\(^{2}\)NES and CDC7\(^{2}\)NES even though Φ4 is key for activities of several other NESs (Wen et al., 1995; Meyer et al., 1996; Richards et al., 1996; Scott et al., 2002; Tsukahara and Maru, 2004). The number of Φ anchor residues necessary for CRM1 binding can vary between 3–5 (Figure 1—figure supplement 6). A third class 3-matching NES from beta-amyloid binding protein X11L2 (X11L2\(^{2}\)NES: 55SLSLQELVQQFELPGDVL72) binds differently (Figure 1F, Figure 1—figure supplement 4). \(^{5}\)QELVQQFEAL\(^{5}\) forms a 3-turn α-helix, \(^{6}\)PGDL\(^{7}\) forms a type I β-turn, and X11L2\(^{2}\)NES therefore exhibits a new Φ0XXΦ1XXXXΦ2XXΦ3XXΦ4 (class 4) pattern.

Structures of NESs with non-helical patterns are also informative. The previous structure of Rev\(^{2}\)NES (class 2) suggested that its three prolines may constrain against a helical conformation (Figure 1B) (Güttler et al., 2010). Class 2 NESs in the Mothers against decapentaplegic homolog 4 protein (SMAD4\(^{2}\)NES: 134YERVVSIPGIDLSGL149) and the fragile X mental retardation protein (FMRF\(^{2}\)NES: 423YLKEVQDQLRLRLQ1437) have few to no prolines but still bind CRM1 with mostly loop-like
Figure 2. Structures of NESs with non-helical sequence patterns. (A–E) FMRP NES (light orange), SMAD4 NES (salmon), HDAC5 NES (slate), Pax NES (pale cyan) and FMRP-1b NES (deep teal) bound to the ScCRM1 groove. Black dashes show CRM1-NES hydrogen bonds and polar contacts, and unoccupied CRM1 hydrophobic pockets are labeled. *HDAC5 NES and Pax NES sequences match the class 1b pattern, but both peptides bind CRM1 using Φ residues that match class 1a pattern. Average displacements of each Φ Ca in the eight NESs (including mDia2 NES, CDC2 NES, X11L2 NES in Figure 1) from the equivalent Φ Ca of PKI NES (Φ0L) are 1.3 ± 0.6 (Φ4), 0.8 ± 0.5 (Φ3), 0.7 ± 0.4 (Φ2), 0.9 ± 0.3 (Φ1) and 1.8 ± 0.9 (Φ0) Å.

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Figure supplement 1. Electron densities of the NES peptides.
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Figure supplement 2. Engineering class 1b NESs.
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Structural requirements for an NES

Structures of >13 different CRM1-bound NESs are now available, and may be sorted into five or six groups according to peptide backbone conformations (Figure 3A). Class 1 NESs are helix-strand peptides with either α-helices (class 1a, 1c) or 3₁₀ helices (class 1b). Class 1-R NESs are strand-helix peptides, class 2 NESs are mostly loop-like and class 3 NESs are all-helix peptides. The helix-β-turn X11L2 NES structure revealed a new Φ0XΦ1XΦ02XΦ03XΦ04 (class 4) pattern.

The only common secondary structural element in the NES structures is one turn of NES helix at Φ2XΦ3 (grey box, Figure 3A). This conserved turn of helix is flanked on one side by additional turns of helix (classes 1, 1-R) or by loops (class 2), and on the other side by β-strands (classes 1, 1-R, 2) or β-turn (class 4), or the helix ends as the chain terminates or exits the groove (class 3) (Figure 3A). Dihedral (ψi) angles in the 1-turn of helix gradually increase in progression from helical to β-strand conformations (Figure 3B).
In all (+) NESs, main chain carbonyls of $F_2+1$ and $F_3$ residues in the 1-turn helix element hydrogen bond with the $Sc$ CRM1 Lys579 (or $Mm$ CRM1/ $Hs$ CRM1 Lys568) side chain, much like niche3/4 motifs where carbonyls of residues $i$ and $i+2$ or $i+3$ coordinate a cationic group (Torrance et al., 2009). The $F_3$-Lys579 hydrogen bond is possible only because the $\beta$-strand psi angle turns $F_3$ carbonyl towards Lys579 (Figure 3C). NES helix-Lys579 hydrogen bonds are absent in (-) NESs as backbone carbonyls point in the opposite direction. Here, carbonyls of the N-terminal $\beta$-strand hydrogen bond with Lys579 (Figure 3—figure supplement 1). Therefore, another common structural feature of NESs is hydrogen bonding between NES backbone and $Sc$ CRM1 Lys579 ($Hs$ CRM1 Lys568). Mutations of $Hs$ CRM1 Lys568 impair NES binding. Mutants $Hs$ CRM1(K568A) and $Hs$ CRM1(K568M) bind FITC-PKI NES two to three orders of magnitude weaker than wild type $Hs$ CRM1, supporting the importance of Lys568-NES interactions (Figure 3—figure supplement 2).

In summary, an active NES (1) can use many different backbone conformations to present 3–5 hydrophobic anchor residues into 3–5 CRM1 hydrophobic pockets (P0 and/or P4 are sometimes not used), (2) has one turn of helix with helix-strand transition that binds the central portion of the CRM1 groove and (3) has backbone conformation that can hydrogen bond with Lys568 of $Hs$ CRM1.

$Hs$ CRM1 Lys568 is a selectivity filter for NES recognition

What then are CRM1 groove features that selectively recognize the key NES features? Arrangement of hydrophobic pockets in the groove likely selects NESs with suitably placed $F$ residues. Groove shape, tapering and most constricted at $Sc$ CRM1 Lys579 ($Hs$ CRM1 Lys568), likely selects for NES.
helices that transition to strands or NES helices that end (Figure 3C). Is groove-constricting \( ^{\text{H}} \text{CRM1} \) Lys568 perhaps key for differentiating active from false positive NES sequences? We tested mutants \( ^{\text{H}} \text{CRM1}(\text{K568A}) \) and \( ^{\text{H}} \text{CRM1}(\text{K568M}) \) for interactions with three already identified false positive NESs that match NES consensus but do not bind CRM1: peptides from Hexokinase-2 (Hxk2\text{pep}; \text{18DVPKELMQGQNEEKIFTV}\text{36}, class 3 match), Deformed Epidermal Autoregulatory Factor 1 homolog (DEAF1\text{pep}; \text{452SWLYLEEMVNSLINTAQI}\text{469}, class 1a-R match) and COMM domain-containing protein 1 (COMM1\text{pep}; \text{173KTLSEVESISSLQP}\text{193}, class 3 match) (Figure 4A) (Xu et al., 2012c, 2015). Wild type \( ^{\text{H}} \text{CRM1} \) does not bind the peptides but \( ^{\text{H}} \text{CRM1}(\text{K568A}) \) binds Hxk2\text{pep} and DEAF1\text{pep}, and \( ^{\text{H}} \text{CRM1}(\text{K568M}) \) binds DEAF1\text{pep} but not Hxk2\text{pep}, suggesting that Lys568 is important in filtering out false positive NESs (Figure 4A).

Both \( ^{\text{Sc}} \text{CRM1}(\text{K579A}) \)-bound Hxk2\text{pep} and DEAF1\text{pep} are all-helix peptides (Figure 4B, C, Figure 4—source data 1). The fourth turn of the Hxk2\text{pep} helix packs into hydrophobic space widened by removal and rearrangement of the Lys579 and Glu582 side chains, respectively (Figure 4B). The 2.5-turn α-helix of DEAF1\text{pep} binds in the (−) direction and is slightly longer than helices in true (−) NESs (Figure 4C). Superpositions of Hxk2\text{pep} and DEAF1\text{pep} onto wild type CRM1 grooves show the fourth turn of the Hxk2\text{pep} helix and the N-terminus of the DEAF1\text{pep} helix clashing with \( ^{\text{Sc}} \text{CRM1} \) Lys579/\( ^{\text{H}} \text{CRM1} \) Lys568 side chains (Figure 4B, C, Figure 4—source supplement 2). The rest of the mutant \( ^{\text{Sc}} \text{CRM1}(\text{K579A}) \) groove is highly similar to the wild type groove. Therefore, the key feature of the wild type groove that prevents Hxk2\text{pep} and DEAF1\text{pep} binding is Lys568, which is not only a critical hydrogen bond donor for binding NESs, but its long side chain also blocks binding of sequences that do not meet NES structural requirements.

**Discussions**

Class 1a, 1b, 1c, 2, 3, 4 and 1a-R NES structures show 5–6 distinct backbone conformations that match their respective hydrophobic sequence patterns. We can infer structures of remaining NES classes: class 1d NESs \( (\Phi\text{1XX\Phi}2\text{XX\Phi}3\text{X}\Phi4) \) are likely α-helix-strand and other (−) NES classes are likely the reverse of their (+) counterparts. Symmetrical class 2, 3 and 4 patterns are identical in both (+)/(−) directions but (−) class 3 and 4 NESs, lacking β-strands to hydrogen bond with \( ^{\text{H}} \text{CRM1} \) Lys568, may not be ideal NESs.

Structures of many diverse NES sequences suggest how one unchanging peptide-bound CRM1 groove can recognize up to a thousand different peptides. Dependence of 3–5 hydrophobic residues in 8–15 residues-long NES arises from the substantial binding energy of anchor hydrophobic side chains interacting with 3–5 CRM1 hydrophobic pockets. However, lack of contact with NES backbone allows anchor side chains to be presented in many conformations including both N- to C-terminal orientations, explaining broad specificity defined by highly variable spacings between anchors. Interestingly, NES conformation is not entirely restrained, as CRM1 groove constriction imposes either exit/termination of the NES chain or its continuation in extended configuration. Solutions for the broadly specific NES recognition contrast with those of analogous systems. MHC I and II proteins, each recognizing at least hundreds of different peptide antigens, use many peptide main chain contacts for affinity with only a few supplementary peptide side chain interactions (Zhang et al., 1998; Madden, 1995). The result here is a conformational selection of particular lengths of extended peptides binding in conserved N- to C-terminal orientation, with little sequence restriction. The Calmodulin-helical peptide system is yet another contrast, as the binding domain uses its flexible fold to adapt to various helical ligands (Tidow and Nissen, 2013; Hoeflisch and Ikura, 2002).

CRM1-NES structures expanded the six NES patterns derived from peptide library studies to the eleven patterns shown in Figures 1A and 3A. The ever-expanding set of NES patterns suggests that no fixed hydrophobic pattern likely describes the NES. Furthermore, only ~50% of consensus-matching previously reported NESs that were tested actually bound CRM1, contributing to the inefficiency of available NES predictors (with precision of 50% at 20% recall rate) (Xu et al., 2012b, 2015; Kosugi et al., 2014; Fu et al., 2011). The many available NES structures, diversity of NES conformations and the structurally conserved one-turn helix NES element revealed here will enable development of structure- rather than sequence-based NES predictors (Raveh et al., 2011; Schindler et al., 2015; Trelet et al., 2013; Yan et al., 2016). There is a need to identify many more CRM1 cargoes as apoptosis of different cancer cells upon CRM1 inhibition by the drug Selinexor (in clinical trials for a variety of cancers) and other inhibitors (Parikh et al., 2014; Mendonca et al., 2015).
Figure 4. HsCRM1 Lys568 is a selectivity filter. (A) False positive NES sequences with Φ residues of consensus matches underlined. Pull-down binding assay of ~5 μg immobilized GST-NESs and 7.5 μM ScRanGTP with 2.5 μM of either wild type HsCRM1 or mutant HsCRM1(K568A) or HsCRM1(K568M) in 200 μL reactions. (C–D) Structures of Hxk2pp (pink) (C) and DEAF1pp (purple) (D) bound to ScCRM1(K579A). Left panels, peptides were removed to show the surface of the mutant ScCRM1(K579A) groove. Middle panels, peptides bound to the mutant ScCRM1(K579A). Right panels, CRM1(K579A)-bound Hxk2pp and DEAF1pp superimposed onto the PKI NES-bound MmCRM1 groove (3NBY; CRM1 H12A helices were aligned; PKI NES not shown) to show steric clash of the Hxk2pp and DEAF1pp peptides with the MmCRM1 Lys568 side chain.

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Figure supplement 1. Electron densities of the HXK2pp and DEAF1pp false positive NES peptides bound to the ScCRM1(K579A) mutant.
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Figure supplement 2. Additional overlays of Hxk2pp and DEAF1pp onto NES-bound wild type CRM1 grooves.
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Finally, we find that the $^{14}$C CRM1 Lys568 side chain acts as a filter that physically selects for NESs with helices that transition to strands or end at the narrow part of the CRM1 groove. Interestingly, Lys568 interacts electrostatically with $^{14}$C CRM1 Glu571, which is mutated to glycine or lysine in chronic lymphocytic leukemia and lymphomas with poor prognosis (Puente et al., 2011; Jardin et al., 2016; Camus et al., 2016). Disease mutations will abolish Glu571-Lys568 contacts and possibly affect NES binding and selectivity.

Materials and methods

Crystallization of CRM1-Ran-RanBP1-NES complexes

CRM1 ($^{13}$CRM1 residues 1–1058, $^{337}$DLTVK$^{541}$ to GLCEQ, V441D), Yrb1p (yeast RanBP1 residues 62–201), human Ran full length and various NESs were expressed and purified as described in Fung et al. (Fung et al., 2015). CRM1 (K579A) mutant was expressed in pGex-TEV and purified like CRM1. Crystallization, data collection and processing, and solving of the structures were also performed in the same manner as previously described. X-ray/stereochemistry weight and X-ray/ADP weight were both optimized by phenix.refine in PHENIX (RRID:SCR_014224).

NES peptides are modeled according to the positive difference density (2mFo-DFc map) at the binding groove after refinement of the CRM1-Ran-RanBP1 model. In all structures, there are good electron densities for the NES main chain and directions of side chain density in the helical portion of the peptides allow unambiguous determination that they are all oriented in the positive (+) NES orientation. Side chain assignments of the NES peptides are guided by (1) densities of $\Phi$ side chains that point into the binding groove, (2) densities for long non-$\Phi$ side chains such as arginine, phenylalanine and methionine and (3) physical considerations such as steric clashes.

For example, to model the bound mDia2NES peptide (sequence: GGSY$^{1179}$SVPEVEALLARLA L$^{1192}$), we made use of the obvious electron densities (mFo-DFc map) for long side chains to guide sequence assignment. There is a strong side chain density suitable for an arginine side chain on the peptide (white dashed circle in Figure 1—figure supplement 2A). There are only two arginine residues in the mDia2NES peptide, Arg1189 and Arg1191. If the long side chain density is assigned to Arg1189, then Arg1191 would end up pointing into the binding groove – a very energetically unfavorable and unlikely situation. Furthermore, Ala1188 would end up in the P2 pocket of CRM1 where there is an obvious density for a longer hydrophobic side chain (left panel, Figure 1—figure supplement 2A). On the other hand, when Arg1191 is assigned to the long and continuous side chain density (adjacent to helix H12A of CRM1), the remaining side chains in the NES end up in positions that are consistent the electron densities.

For the FMRP-1bNES (sequence: $^{1}$GGS-YLKEVDQLRALERLQID$^{20}$), there are no obvious long side chain densities that could help with modeling. There are however obvious densities for several side chains in the first two turn of the NES helix. These side chain densities are consistent with two possible sequence assignments: $^{5}$KLEYDQRL$^{14}$ or the more C-terminal $^{11}$LRAERLQID$^{20}$. We tested modeling of FMRP-1bNES by refining both peptide models and by testing a mutant peptide that should distinguish between the two models. Ten cycles of PHENIX refinement of the $^{5}$KLEYDQRL$^{14}$ model resulted in positive and negative difference densities (mFo-DFc map) at several NES side chains, which suggested an incorrect assignment (left panels, Figure 1—figure supplement 2B). In contrast, different densities are absent when the $^{11}$LRAERLQID$^{20}$ model is refined (right panels, Figure 1—figure supplement 2B). The final FMRP-1bNES structure was therefore modelled as $^{11}$LRAERLQID$^{20}$. The sequence assignment of FMRP-1bNES was also tested using a mutant FMRP-1bNES that has the sequence KLEYDVQLR. If the NES is $^{5}$KLEYDQRL$^{14}$, FMRP-1bNES mutant YLKEDQLRALER should bind well to CRM1. However, if $^{11}$LRAERLQID$^{20}$ is the FMRP-1bNES, mutant YLKEDQLRALER should not bind CRM1 as the C-terminal half of the NES or $^{17}$LQID$^{20}$ which includes $\Phi 3$ and $\Phi 4$ is missing. Results in Figure 1—figure supplement 3 show that FMRP-1bNES
mutant YLKEVDQLRALER does not bind CRM1, providing further support that the NES is indeed 
\[^{11}\text{LRA}^{20}\text{LERQID}\] as currently assigned.

**NES activity assays**
Pull-down binding assays, in vivo NES activity assay and differential bleaching experiments for deter-
mining binding affinities were all performed the same way as described in Fung et al. (2015). The 
data were analyzed in PALMIST (Scheuermann et al., 2016) and plotted with GUSSI (Brautigam, 2015).

**Accession codes**
Structures and crystallographic data have been deposited at the PDB: 5UWI (CRM1-HDAC5\textsuperscript{NES}), 
5UWH (CRM1-Pax\textsuperscript{NES}), 5UWO (CRM1-FMRP-1b\textsuperscript{NES}), 5UWJ (CRM1-FMRP\textsuperscript{NES}), 5UWU (CRM1- 
SMAD4\textsuperscript{NES}), 5UWP (CRM1-mDia2\textsuperscript{NES}), 5UWQ (CRM1-CDC7\textsuperscript{NES}), 5UWR (CRM1-CDC7\textsuperscript{NES} ext), 5UWS 
(CRM1-X11L2\textsuperscript{NES}), 5UWT (CRM1(K579A)-Hxxk\textsuperscript{2PP}), 5UWW (CRM1(K579A)-DEAF1\textsuperscript{2PP}).

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| Ho Yee Joyce Fung, Yuh Min Chook | 2017 | Crystal Structure of HDAC5 NES Peptide in complex with CRM1-Ran-RanBP1         | http://www.rcsb.org/pdb/explore/explore.do?structureId=5UWI                  | Publicly available at the RCSB Protein Data Bank (accession no. 5UWI) |
| Ho Yee Joyce Fung, Yuh Min Chook | 2017 | Crystal Structure of Paxillin NES Peptide in complex with CRM1-Ran-RanBP1    | www.rcsb.org/pdb/explore/explore.do?structureId=5UWH                        | Publicly available at the RCSB Protein Data Bank (accession no. 5UWH) |
| Ho Yee Joyce Fung, Yuh Min Chook | 2017 | Crystal Structure of Engineered FMRP-1b NES Peptide in complex with CRM1-Ran-RanBP1 | www.rcsb.org/pdb/explore/explore.do?structureId=5UWO                      | Publicly available at the RCSB Protein Data Bank (accession no. 5UWO) |
| Ho Yee Joyce Fung, Yuh Min Chook | 2017 | Crystal Structure of FMRP NES Peptide in complex with CRM1-Ran-RanBP1        | www.rcsb.org/pdb/explore/explore.do?structureId=5UWJ                      | Publicly available at the RCSB Protein Data Bank (accession no. 5UWJ) |
| Ho Yee Joyce Fung, Yuh Min Chook | 2017 | Crystal Structure of SMAD4 NES Peptide in complex with CRM1-Ran-RanBP1       | www.rcsb.org/pdb/explore/explore.do?structureId=5UWW                       | Publicly available at the RCSB Protein Data Bank (accession no. 5UWW) |
| Ho Yee Joyce Fung, Yuh Min Chook | 2017 | Crystal Structure of mDia2 NES Peptide in complex with CRM1-Ran-RanBP1       | www.rcsb.org/pdb/explore/explore.do?structureId=5UWP                       | Publicly available at the RCSB Protein Data Bank (accession no. 5UWP) |
| Ho Yee Joyce Fung, Yuh Min Chook | 2017 | Crystal Structure of CDC7 NES Peptide in complex with CRM1-Ran-RanBP1        | www.rcsb.org/pdb/explore/explore.do?structureId=5UWQ                       | Publicly available at the RCSB Protein Data Bank (accession no. 5UWQ) |
| Ho Yee Joyce Fung, Yuh Min Chook | 2017 | Crystal Structure of CDC7 NES Peptide (extended) in complex with CRM1-Ran-RanBP1 | www.rcsb.org/pdb/explore/explore.do?structureId=5UWR                       | Publicly available at the RCSB Protein Data Bank (accession no. 5UWR) |
| Ho Yee Joyce Fung, Yuh Min Chook | 2017 | Crystal Structure of X11L2 NES Peptide in complex with CRM1-Ran-RanBP1       | www.rcsb.org/pdb/explore/explore.do?structureId=5UWS                       | Publicly available at the RCSB Protein Data Bank (accession no. 5UWS) |
| Ho Yee Joyce Fung, Yuh Min Chook | 2017 | Crystal Structure of Hxk2 Peptide in complex with CRM1 K579A mutant-Ran-RanBP1 | www.rcsb.org/pdb/explore/explore.do?structureId=5UWT                       | Publicly available at the RCSB Protein Data Bank (accession no. 5UWT) |
| Ho Yee Joyce Fung, Yuh Min Chook | 2017 | Crystal Structure of DEAF1 Peptide in complex with CRM1 K579A mutant-Ran-RanBP1 | www.rcsb.org/pdb/explore/explore.do?structureId=5UWW                       | Publicly available at the RCSB Protein Data Bank (accession no. 5UWW) |

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