Supporting Information

Characterization of inhibition reveals distinctive properties for human and *Saccharomyces cerevisiae* CRM1

*Alaa Shaikhqasem, Achim Dickmanns, Piotr Neumann and Ralf Ficner**

Department of Molecular Structural Biology, Institute of Microbiology and Genetics, GZMB, Georg-August-University Göttingen, 37077 Göttingen, Germany

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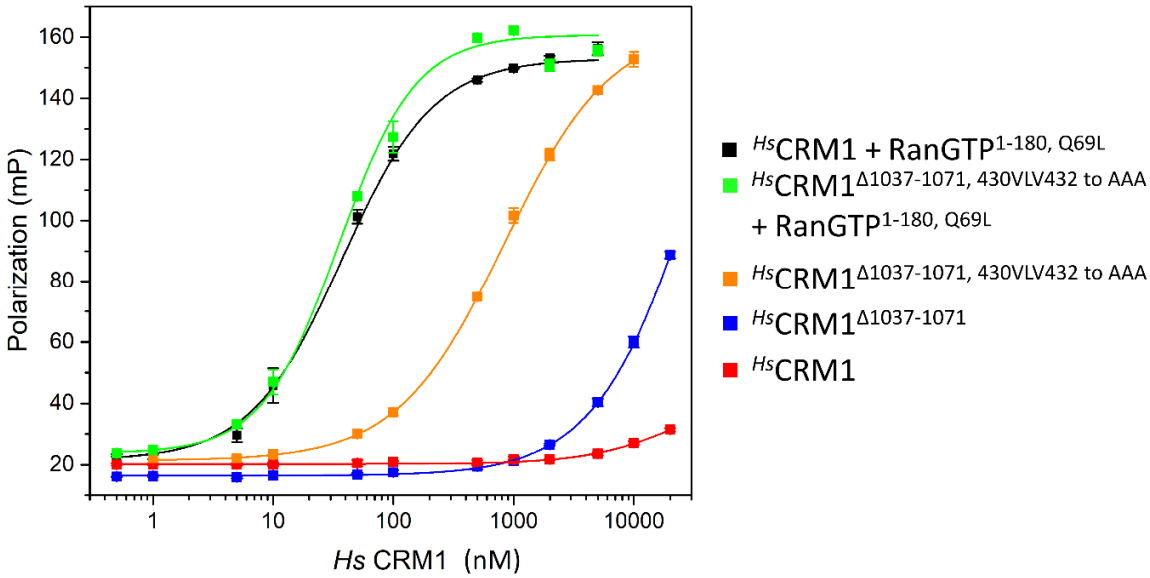

Figure S1. Fluorescently labeled PKI Φ^0Leu NES binding to ^{Hs}CRM1 variants and full-length wild type in the presence or absence of ^{Hs}RanGTP$^{1\text{-}180,\ Q69L}$ by fluorescence polarization binding assay. Wild type ^{Hs}CRM1 as well as the C-terminal helix-acidic loop mutant reveal a very strong binding to the PKI Φ^0Leu NES in the presence of RanGTP. Truncating the C-terminal helix of ^{Hs}CRM1 (Δ1037-1071) increased the affinity to the PKI Φ^0Leu NES in the absence of RanGTP. Furthermore, a combination of C-terminal helix truncation and HEAT9 loop variant (^{430}VLV^{432} mutated to AAA) resulted in a higher increase of ^{Hs}CRM1 affinity to PKI NES in the absence of RanGTP. Error bars illustrate the s.d. for three independent measurements.

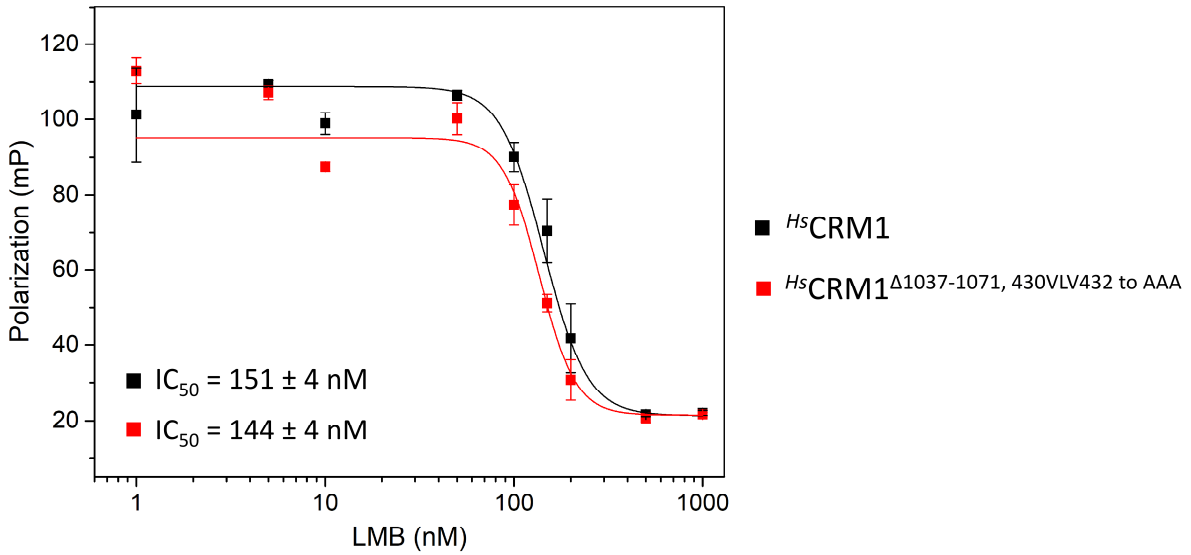

Figure S2. The interaction properties of Leptomycin B (LMB) with the wild type and with the C-terminally truncated HEAT9 loop variant (430VLV432 to AAA) of ^{Hs}CRM1 measured by fluorescence polarization competition assay. The modified CRM1 variant demonstrates a similar inhibition profile as the wild type protein within the same range of half-maximal inhibitory concentration (IC50). Error bars illustrate the s.d. for three independent measurements.

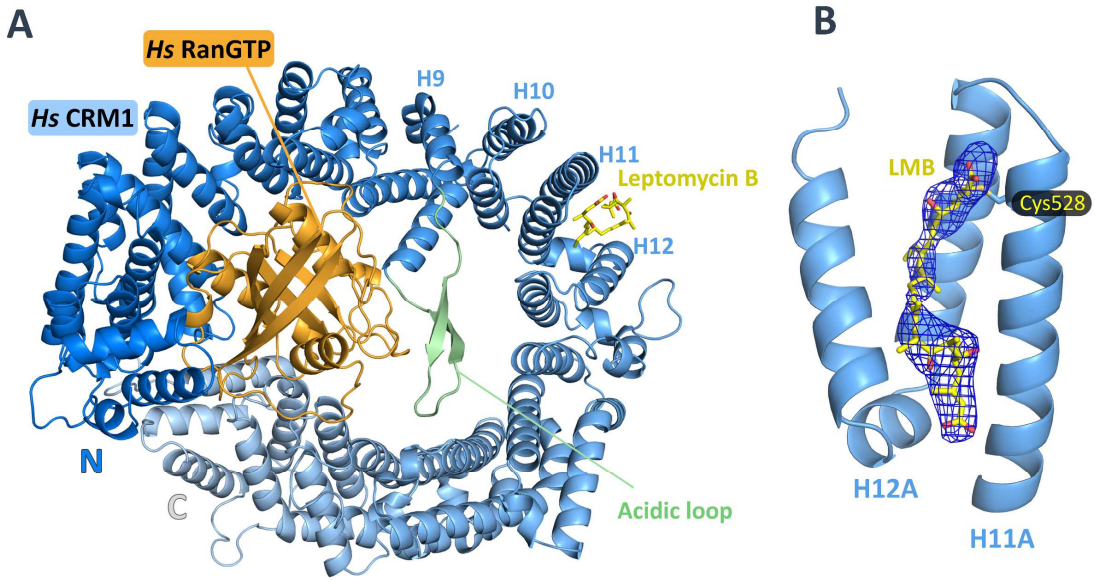

Figure S3. (A) Crystal structure of ^{Hs}CRM1-^{Hs}RanGTP-LMB complex illustrated in cartoon representation. CRM1 is gradient colored from the N-terminus (marine) to the C terminus (white), while Ran is shown in light orange color. The acidic loop adopting the seatbelt conformation is shown in pale green. LMB bound in the nuclear export signal (NES)-binding cleft, formed by HEAT helices 11A and 12A, is shown in stick mode. (B) LMB orientation and localization is defined by a Polder mFo-DFc omit map (blue mesh) contoured at a sigma level 3.0.

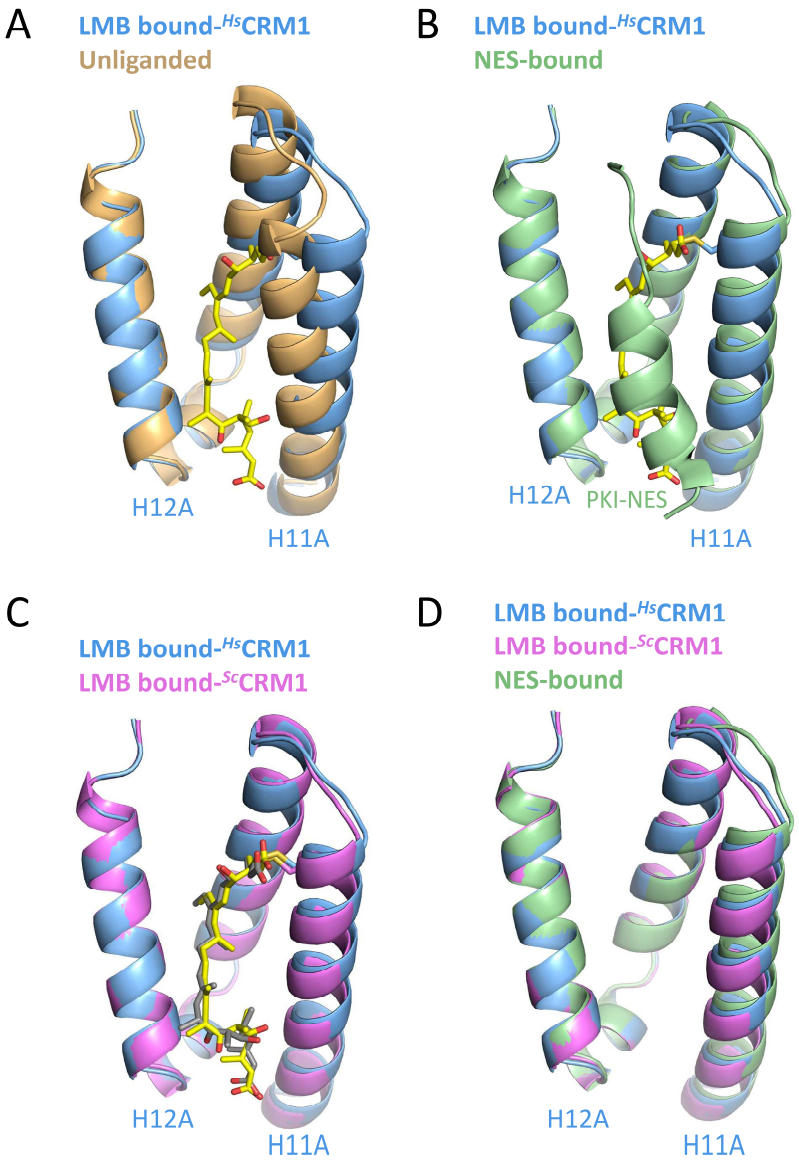

Figure S4. Structural alignment of LMB-bound cleft of ^{Hs}CRM1 with an unliganded cleft, NES-bound cleft, and with LMB-bound cleft of ^{Sc}CRM1. Cartoon representation of LMB-bound cleft of ^{Hs}CRM1 (light blue) superpositioned with the unliganded NES-binding cleft of ^{Sc}CRM1-RanGTP-RanBP1 complex (light orange, PDB ID 3M1I) in (A), with PKI-NES bound ^{Mm}CRM1cleft (pale green, PDB ID 3NBY) in (B), and with LMB-bound cleft of ^{Sc}CRM1 (violet, PDB ID 4HAT) in (C). (D) Superposition of PKI-NES bound cleft with LMB-bound cleft of ^{Hs}CRM1, and LMB-bound cleft of ^{Sc}CRM1. LMB (bound to ^{Hs}CRM1 is colored yellow and to ^{Sc}CRM1 is colored grey) is depicted as sticks in A, B, and C. PKI-NES is shown in cartoon representation in (B). LMB and PKI-NES have been removed in (D).

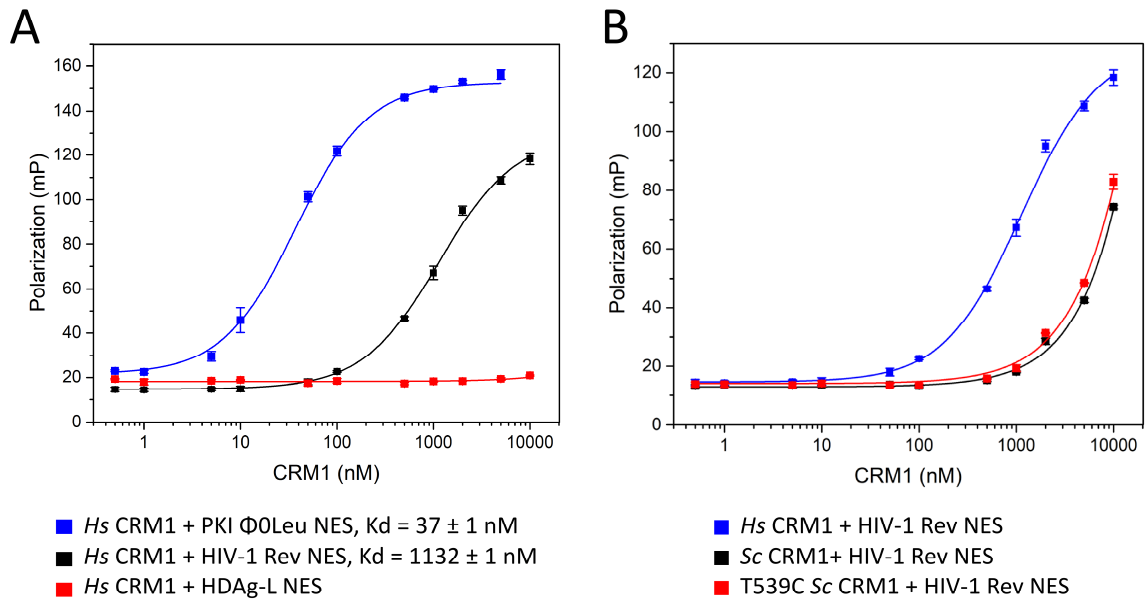

Figure S5. Nuclear export signal peptides binding to CRM1 in the presence of ^{Hs}RanGTP$^{1\text{-}180,\ Q69L}$ as measured by fluorescence polarization binding assay (FP). (A) The binding of ^{Hs}CRM1 to different fluorescently labeled NES peptides. CRM1 was mixed in a concentration range of 0.5 to 5000 nM with Φ^0Leu modified NES peptide from protein kinase A inhibitor protein (PKI Φ^0Leu NES) and in a concentration range of 0.5 to 10000 nM with NES peptide from human immunodeficiency virus type 1 Rev protein (HIV-1 Rev NES) or with the NES peptide from the large delta antigen of hepatitis delta virus (HDAg-L NES). The modified PKI Φ^0Leu NES demonstrates a strong binding towards ^{Hs}CRM1 in the nM range, while the physiological HIV-1 Rev NES demonstrates a weaker binding in the µM range. HDAg-L NES is a CRM1 independent NES and was used as a negative control [1]; the peptide shows no binding towards the human protein under the applied assay conditions. (B) Binding assays of HIV-1 Rev NES with the wild type ^{Hs}CRM1 and ^{Sc}CRM1 as well as with the T539C variant of ^{Sc}CRM1; each mixed in a concentration range of 0.5 to 10000 nM. The wild type and the T539C variant of ^{Sc}CRM1 both demonstrate much weaker binding affinity towards HIV-1 Rev NES compared to the human protein. Error bars illustrate the s.d. for three independent measurements.

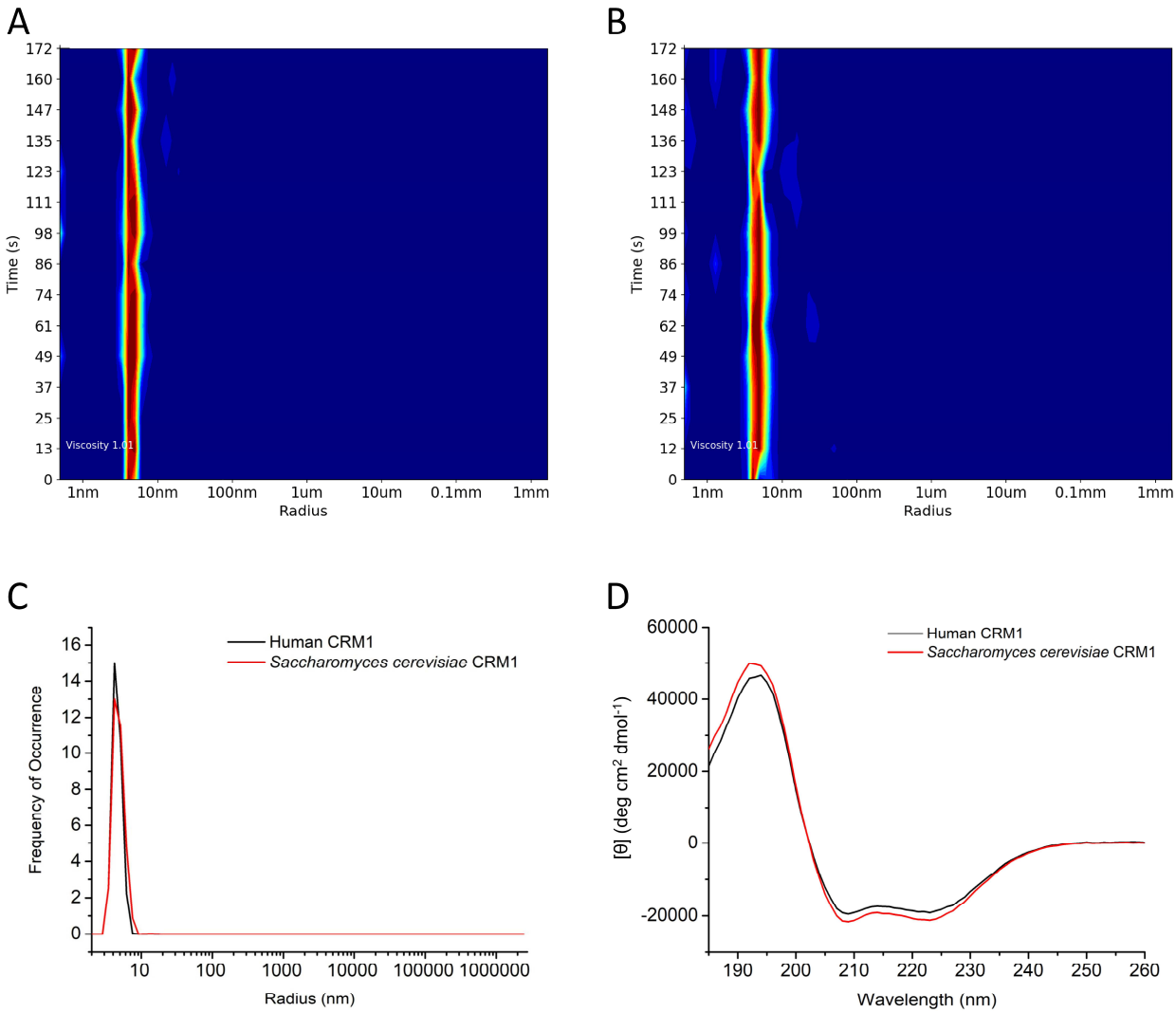

Figure S6. Protein stability and structural integrity of *Saccharomyces cerevisiae* CRM1 compared to human CRM1. (A-C) Dynamic light scattering (DLS) measurements of ^{Hs}CRM1 and ^{Sc}CRM1 in binding assay buffer conditions. DLS radius distribution plot of ^{Hs}CRM1 in (A) and ^{Sc}CRM1 in (B). Plots are shown in the form of signal heat maps (blue = low particle concentration, red = high particle concentration) and confirm that CRM1 from both species is predominantly homogeneous in solution. (C) Schematic representation of particles size distributions of human and yeast CRM1. DLS data reveal that both proteins are homogeneous in solution with an average hydrodynamic radius of approximately 4.6 nm, which excludes the possibility of partial protein denaturation or aggregation under the applied measurements conditions. (D) Circular dichroism spectra of ^{Hs}CRM1 and ^{Sc}CRM1 are very similar which confirms the structural integrity of yeast CRM1 compared to the human protein.

References

(1) Lee, C. H.; Chang, S. C.; Wu, C. H. H.; Chang, M. F. A Novel Chromosome Region Maintenance 1-Independent Nuclear Export Signal of the Large Form of Hepatitis Delta Antigen That Is Required for the Viral Assembly. *J. Biol. Chem.* **2001**, *276* (11), 8142–8148. https://doi.org/10.1074/jbc.M004477200.