SUPPLEMENTARY INFORMATION

The MTR4 helicase recruits nuclear adaptors of the human RNA exosome using distinct arch-interacting motifs

Lingaraju et al.

Supplementary Figure 1: Vertebrate specific N-terminal insertion in NVL interacts with MTR4

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Supplementary Figure 1 (a) Sequence alignment of N-termini of representative vertebrate and fungal NVL sequences, highlighting the chordate specific insertion (blue dashed box), the W-AIM (red box) and a Nop53 (LFX ϕ D) like region (purple). The sequences were obtained from the NCBI database and aligned using the T-coffee server¹. *Hs* stands for *Homo sapiens, Gg* for *Gallus gallus, Xt* for *Xenopus tropicalis, Dr* for *Danio rerio, Bb* for *Branchiostoma belcheri, Ap* for *Acanthaster planci, Ob* for *Octopus bimaculoides, Lp* for *Limulus polyphemus, Ce* for *Caenorhabditis elegans, Cc* for *Coprinopsis cineria,* Rm for *Rhizopus microsporus, Sc* for *Saccharomyces cerevisiae*, and *Km* for *Kluyveromyces marxianus.* (b) Disorder prediction of human NVL as obtained from the D²P² database². The region of NVL that is commonly predicted to be disordered by multiple algorithms is colored in bright green. (c) Protein coprecipitations by pull down assays. GST tagged MTR4 Δ N or yMtr4 were incubated with the vertebrate specific NVL insertion, Trx-NVL¹⁶⁷⁻²¹⁶ and the N-terminus of Rix7, Trx-Rix7¹⁻²⁰⁶, respectively before co-precipitation with glutathione sepharose beads. A total of 3% of the input (left) and 30% of the eluates (right) were analyzed on 15% SDS-PAGE gels and visualized by staining with coomassie brilliant blue. (d) ITC experiment of MTR4 Δ N with NVL¹⁶⁷⁻²¹⁶. The filled squares show reference corrected titration of NVL¹⁶⁷⁻²¹⁶ into the MTR4 Δ N containing cell. The number of calculated binding sites (N), and dissociation constants (K₀) are shown in the inset.

Supplementary Figure 2: NMR analysis of the MTR4 KOW domain



Supplementary Figure 2 (a) Upper bar chart shows secondary carbon chemical shifts of MTR4 KOW plotted against its primary sequence. The clusters of positive bars >2 represent α -helices and the clusters of negative bars >-2 represent β -strands. The scheme above shows a summary of secondary structure elements as derived from the analysis, and elements are labeled for comparison with panel b. The bottom chart shows a heteronuclear NOE plot of the MTR4 KOW demonstrating the residue-resolved rigidity along the primary sequence. Note that the two prominent dips are located within loops 1 and 2. (b) Secondary structure features of the KOW domain obtained from NMR analysis mapped on to the KOW domain from the crystal structure of MTR4 (PDB 6IEH)³.

Supplementary Figure 3: NVL and Nop53 interact with MTR4 KOW in a similar manner



Supplementary Figure 3 (a) Structural model of MTR4 KOW (PDB 6IEH)³ showing regions of significant backbone chemical shift perturbation (CSPs) upon NVL titration highlighted as red spheres. (b) yMtr4 KOW (PDB 5OOQ) showing significant CSPs (orange spheres) upon yNop53 titration as reported by Falk et al⁴. (c) Protein co-precipitations by pull down assays. GST tagged MTR4 Δ N or the corresponding MTR4 Δ N mutants were incubated with the AIM containing region of human Nop53 (Trx-hNop53 ⁸⁴⁻¹²³) before co-precipitation with glutathione sepharose beads. A total of 1% of the input (left) and 30% of the eluates (right) were analyzed on 15% SDS-PAGE gels and visualized by staining with coomassie brilliant blue. (d) ITC experiment of MTR4 KOW with hNop53 ⁸⁴⁻¹²³. The filled squares show reference corrected titration of hNop53 ⁸⁴⁻¹²³ into the MTR4 KOW containing cell. The number of calculated binding sites (N), and dissociation constants (*K*₀) are shown in the inset.

Supplementary Figure 4: Features of the NVL-MTR4 crystal structure



Supplementary Figure 4 (a) Comparison of the crystal structure of the NVL-MTR4 complex with crystal structure of MTR4 alone and the NRDE-2-MTR4 complex (PDB 6IEG and 6IEH)³. The structures were aligned based on the DExH core region (colored in grey) to represent the difference in orientation of the arch region (colored in light blue) with respect to the DExH core. (b) Zoom-in view of the interactions between MTR4 KOW domain (light blue) and NVL (orange) as displayed in Fig. 3d. The model is overlaid with the refined 2mFo-DFc map (grey mesh) showing the density for NVL and the interacting residues of MTR4. The residues of interest are labeled and the map is contoured at 1.0σ. (c) Zoom in view of MTR4-NVL crystal structure showing the ordered region of NVL (orange). The model is overlaid with the 2mFo-DFc omit map calculated in PHENIX omitting the NVL residues. The map is contoured at 1.0σ. (d) Zoom-in view of the KOW-AIM interfaces in NVL-MTR4, NRDE-2-MTR4 (PDB 6IEH³), Nop53-Mtr4 (PDB 5OOQ⁴) and Trf4-Air2-Mtr4 (PDB 4U4C⁵) crystal structures. KOW domains of the human MTR4 and yeast Mtr4 are colored in light blue and green respectively. NVL, NRDE-2, Nop53 and Air2 are colored in orange, yellow orange, olive and pale orange respectively and the residues of interest are labeled.

Supplementary Figure 5: Features of the NVL-MTR4 crystal structure and structure based mutagenesis



Supplementary Figure 5 (a) Superposition of DExH cores NVL-MTR4 (grey) and Trf4-Air2-Mtr4 (light green; PDB 4U4C⁵) showing that the NVL fragment (orange) from a symmetry related molecule aligns with the Trf4 fragment in Trf4-Air2-Mtr4 structure. NVL and Trf4 are shown in cartoon representation (left panel) and in stick representation (right panel) for clarity. (b) Protein co-precipitations by pull down assays. GST tagged MTR4ΔN was incubated with Trx-NVL¹⁶⁷⁻²¹⁶ WT or Trx-NVL¹⁶⁷⁻²¹⁶ D176A before co-precipitation with glutathione sepharose beads. A total of 1% of the input (left) and 30% of the eluates (right) were analyzed on 12% SDS-PAGE gels and visualized by staining with coomassie brilliant blue.

Supplementary Figure 6: ZCCHC8 harbors both canonical and non-canonical AIMs

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Supplementary Figure 6 (a) Sequence alignment of the N-termini of NVL (orange) and ZCCHC8 (pink), highlighting W-AIM (red box), the respective Nop53-like C-AIM (LFXφD) and ZCCHC8 specific I-AIM (purple boxes). The sequences were obtained from the UniProt database and aligned using the T-coffee server¹. *Hs* stands for *Homo sapiens*, Gg for *Gallus gallus*, *Xt* for *Xenopus tropicalis and*, *Xl* for *Xenopus laevis*. (b) Protein co-precipitations by pull down assays. GST tagged MTR4ΔN was incubated with MBP-ZCCHC8⁹¹⁻²¹¹ or MBP-ZCCHC8¹³⁴⁻²¹¹ before co-precipitation with glutathione sepharose beads. A total of 1% of the input (left) and 30% of the eluates (right) were analyzed on 10% SDS-PAGE gels and visualized by staining with coomassie brilliant blue. (c) Cellular co-IP assay. FLAG-tagged ZCCHC8 constructs (WT, IF-mutant, CTD-deletion) were transiently expressed in cells stably expressing RBM7-LAP. After precipitation of RBM7 taking advantage of the LAP tag, a total of 0.5% of the input (left) and 8.0% of the eluates (right) were analyzed on 4-12% SDS-PAGE gel followed by western blotting. The primary antibody used is indicated below the panel.

Supplementary Figure 7: Putative ligand binding phenylalanine in tudor domains is conserved in MTR4 KOW



	I Contraction of the second	*
<i>Hs</i> MTR4 KOW	645	NEG-DDFGWGVVVNFSKKSNVKP685
CeMTR4 KOW	632 R E P K Y I V G F L H A G R L F K V K	5 G D - R D F K W G I L N Q F K K E Q N P D - 671
DmMTR4 KOW	661 T K P Q Y L L P F L Q P G R L V K V A	AGS-QEYDWGIVLNFKKQDQSRK701
ScMtr4 KOW	665 T H P A N A L S F L Q P G R L V E I S	/ N G K D N Y G W G A V V D F A K R I N K R N 706
HsSMN Tudor	82 K K N T A A S L Q Q W K V G D K C S A I W S E D G C I Y P A T	I A S I D F K R E T C <mark>V V V</mark> Y T G 129
HsTDRD3 Tudor	555 M WKPGDECFALYWEDNKFYRAE	/ E A L H S S G M T A V V K F I D 593
HsSPF30 Tudor	65 A	I E E I D E E N G T A A I T <mark>F</mark> A G 110
DmPcl Tudor	339 GAMAPPVAAPSPAVTYALQEDV-FIKCNDGRFYLGT	I I <mark>D</mark> Q T S DQ Y L I R <mark>F</mark> D D 388
<i>Hs</i> MTR4 KOW	686 NSGE-L-DPLYVVEVLLRCSKES-LKNSATE/	A A K P A K P D E <mark>K G</mark> E M Q V V P V L V H L L 736
CeMTR4 KOW	672 DR - N - DQ I Y L C DMM I A I N T E G R F D P T N P A 1	「LVPGFDLPKRRWIRVPMTIDRI721
DmMTR4 KOW	702 NPLK-A-EPSVTIDVLLHVSEAA-AKTGI	ОТ Е <mark>Р</mark> С К Р N Е <mark>Г </mark> С М Е V V Р V А Н Т Ц I 749
ScMtr4 KOW	707 PSAVYTDHESYIVNVVVNTMYIDSPVNLLKPFNPTLPE	5
HsSMN Tudor	130	
HsTDRD3 Tudor	594	
HsSPF30 Tudor	111	
DmPcl Tudor	389	
	*	
<i>Hs</i> MTR4 KOW	737 SAISSVRLYIPKDLRPVDNRQSVLKSIQEVQKRFPDGI	LLDPIDDMGIQD- 787
CeMTR4 KOW	722 TAISAVRLKVPADIDKPDGQMRLDGMMAAATKRFGNQI	PLLDPIQDMEIKTV 773
DmMTR4 KOW	750 TQ I S S I R V Y F P N D L R S A D N R A V L K T I Q E A K K R F P L G P I	VLNPIDDMNIKD- 800
ScMtr4 KOW	768 K S I G N L R L Y M P K D I R A S G Q K E T V G K S L R E V N R F P D G I I	VLDPVKNMKIED- 818
HsSMN Tudor	143 SP I C E V A N N I E Q N A Q E N E N E S Q	VSTD-E 169
HsTDRD3 Tudor	606 K P I	608
HsSPF30 Tudor	123 K P V E E G	128
DmPcl Tudor	400 RKLGGG	40







Supplementary Figure 7 (a) Structural superposition of MTR4 KOW model (light blue) (PDB 6IEH³) with structures of SMN tudor domain (gold) (PDB 1G5V)⁶, TDRD3 tudor domain (turquoise) (PDB 3S6W)⁷, SPF30 tudor domain (smudge green) (PDB 4A4F)⁸ and PcI tudor domain (orange) (PDB 2XK0)⁹. The superpositions were performed in PyMOL graphics system, version 2.2 (Schrödinger LLC). (b) Sequence alignment of representative MTR4 KOW (blue) and tudor (purple) domains, highlighting hydrophobic core residues (brown box) and putative substrate binding residues (red box) of tudor domains. The hydrophobic core resides conserved between KOW and tudor sequences are marked with brown rectangles (•). The residues of interest in MTR4 KOW, Phe677 and Arg743, are marked by green and red asterisks respectively. The sequences were obtained from UniProt database and aligned using the T-coffee server¹. *Hs* stands for *Homo sapiens, Ce* for *Caenorhabditis elegans, Dm* for *Drosphila melanogaster,* and *Sc* for *Saccharomyces cerevisiae*. (c) Cartoon and surface representation of MTR4 KOW (light blue) (PDB 6IEH)³ highlighting the AIM interacting arginine (red) and putative ligand binding phenylalanine (green).

Supplementary Figure 8 : Arch interacting regions of NVL and ZCCHC8 do not influence MTR4 activity.



Supplementary Figure 8 (a) End point helicase activity assay of MTR4ΔN, MTR4ΔN with KOW-binding regions of NVL and ZCCHC8, and MTR4 with C-terminal domain of ZCCHC8 on RNA duplex substrates. (b) Time course of ATP hydrolysis by MTR4ΔN alone or in presence of RNA and KOW-binding regions of ZCCHC8 (upper panel) and NVL (lower panel). The data show mean (n=3) with standard deviation plotted as error bars. ZCCHC8 C-terminal domain, which is known to stimulate ATPase activity of MTR4¹⁰, is used as positive control (c) Coomassie stained SDS-PAGE gel showing the proteins used in the helicase and ATPase assays.

а



Supplementary Figure 9 (a) Stereo zoom-in view of the interactions between MTR4 KOW domain (light blue) and NVL (orange) as displayed in Fig. 3d. The model is overlaid with the refined 2mFo-DFc map (grey mesh) showing the density for NVL and the interacting residues of MTR4. The residues of interest are labeled and the map is contoured at 1.0 σ . (b) Stereo zoom in view of MTR4-NVL crystal structure showing the ordered region of NVL (orange). The model is overlaid with the 2mFo-DFc omit map calculated in PHENIX omitting the NVL residues. The map is contoured at 1.0 σ .

Supplementary Figure 10: Uncropped gels & blots



Supplementary Figure 10 (a) Fig.1b (b) Fig.2d (c) Fig.3b (d) Fig.3e (e) Fig.4b (f) Fig.4d (g) Fig.5b (h) Fig.5c (i) Fig.5d (j) Supplementary Fig.1c (k) Supplementary Fig.3c (l) Supplementary Fig.5b (m) Supplementary Fig.6b (n) Supplementary Fig.6c (o) Supplementary Fig.8a (p) Supplementary Fig.8c.

Supplementary Table 1: List of primers for generating constructs used in this study.

Primer	Sequence (5'-3')
MTRAN-Fwd	ccaggggcccgactcgatgatttttggaaagaagcccaggatagaagagtc
MTRAN-Rev	cagaccgccaccgactgcttacaagtagaggctggcagcaaacacaatatctctcttg
MTRANAarch-Fwd	gettttttcagttccagaatgttattagctctggctcgggacaggccgttattcagctggatgacc
MTRANAarch-Rev	ggtcatccagctgaataacggcctgtcccgagccagagctaataacattctggaactgaaaaaagc
MTR4 KOW-Fwd	ccaggggcccgactcgatgcacaaaccaaaatactgcttaccttttctac
MTR4 KOW-Rev	
NVL(1-266)-Fwd	
NVL(1-266)-Rev	
NVL(167-216)-Fwd	
NVL (167-216)-Rev	cagacegecacegactoettaateactetecaaaagagaagaatettttgaatee
NVL (167 216) AVEP	
cterm fusion-Fwd	
NVL(167-216) eYFP cterm	ctcgcccttgctcaccatgctgccatcactctccaaaagagaagaatcttttga
fusion-Rev	
MTRAN F677E-Fwd	tgactttggctggggagtagtggtgaatgagtcaaaaaagtcaaatgttaag
MTRAN F677E-Rev	cttaacatttgacttttttgactcattcaccactactccccagccaaagtca
MTRAN R743E-Fwd	gtctttaggaatgtaaagctcaacactgctgatagcagacaggag
MTRAN R743E-Rev	ctcctgtctgctatcagcagtgttgagctttacattcctaaagac
MTRAN for crystallization-	
Fwd	confightere free free free free free free free
NVL(167-216)	gtgtaaagaaagacagtgctttcttgcgcctgtcatgtgagaaaagtaatcc
F186A/D189R-Fwd	
NVL(167-216)	ggattacttttctcacatgacaggcgcaagaaagcactgtctttctt
F186A/D189R-Rev	
NVL(10/-210) W1/3A- Fwd	
NVL(167-216) 1175E-Fwd	ccaggggcccgactcgaaagattctgaaggaggatggtttgaagac
NVL FL-Fwd	taagcagatatcatgaagcccagacctgcag
NVL FL-Rev	tgettageggeegeeggetgagggaeteet
NVL FL W173A/I175E-	ctgccaaagattctgaaggaggagcgtttgcggacaaaaccccaagtgtaaag
Fwd	
NVL FL W173A/I175E-	ctttacacttggggttttgtccgcaaacgctcctccttcagaatctttggcag
Rev	
NVL FL AW-AIM-FWd	
NVL FL ΔW-AIM-Rev	caggtccaagaaaaaactgtctttctttacacttccttcagaatctttggcaggggtcttcaagggaatgg
ZCCHC8(91-211)-Fwd	aagttetgttecagggggeccatggatggacetatattacagattetatteatgaacaatg
ZCCHC8(91-211)-Rev	ccccagaacatcaggttaatggcgttaaacaatgtggctgaagacttgatggtacttg
ZCCHC8(91-211)-eYFP	cateaagtetteageeacattgttggeageatggtgageaagggegag
ZCCHC8(91-211)-eYFP	ctcocccttoctcaccatoctoccaacaatotooctoaaoacttoato
cterm fusion-Rev	etegetettegetettettettettettettettettett
ZCCHC8(91-211)	gaagcggttgccccaatttacgaaggcaagcattagtaaaatacaggacacttcctacaaca
F178A/D181R-Fwd	
ZCCHC8(91-211)	tgttgtaggaagtgtcctgtattttactaatgcttgccttcgtaaattggggcaaccgcttc
F178A/D181R-Rev	
ZCCHC8(91-211) W198A/K202E Rev	gcaaagcaccgtcgttaaacaatgtggctgaagacttgatggtactcgggtatttcagctccttcggaaag
ZCCHC8(91-211)/FL	ttcatoaacaatoctatttcaaaocaatatcatcaagaaagagagagaacototatcaaatttaotaaaaagag
I112R/F115R-Fwd	ttgag
ZCCHC8(91-211)/FL	ctcaaatctttttactaaatttgatacacgttcctctctttctt
I112R/F115R-Rev	
ZCCHC8 FL-Fwd	taagcagatatcatggccgcagaggtgtattt
ZCCHC8 FL-Rev	tgcttagcggccgcttcagaggcctttttgtttttctg

yMtr4-Fwd	ccaggggcccgactcgatggattctactgatctgttcgatgttttcgagg
yMtr4-Rev	cagaccgccaccgactgcttataaatacaaagaaccagcagatacgatatctctatg
Rix7(1-206)-Fwd	ccagggagcagcctcgatggttaaagtaaagtcgaaaaagaactcatt
Rix7(1-206)-Rev	gcaaagcaccggcctcgttaggatttcagagacgaattaggtggagatc
hNop53(84-123)-Fwd	ccaggggcccgactcgatggaaaaactcttcttcgtggacactg
hNop53(84-123)-Rev	cagaccgccaccgactgcttattctcgaggatgaggtcaacc
NVL D176A-Fwd	ccaggggcccgactcgatgaaagattctgaaggaggatggtttattgccaaaac
ZCCHC8(134-211)-Fwd	aagttetgttecagggggcccatgactteetttaatettttgeceeage
ZCCHC8 ∆CTD-Rev	tgcttagcggccgcgctatgaattttagtggccgttg
ZCCHC8 (659-707)-Fwd	ccaggggcccgactcgatgcctatacctgacatgagcaaatttgcaac
ZCCHC8 (659-707)-Rev	cagaccgccaccgactgcttattattcagaggcctttttgtttttctgc

*All primers contain overhangs to facilitate ligation independent cloning

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