Supplementary Data

Table S1 – List of synthetic RNAs

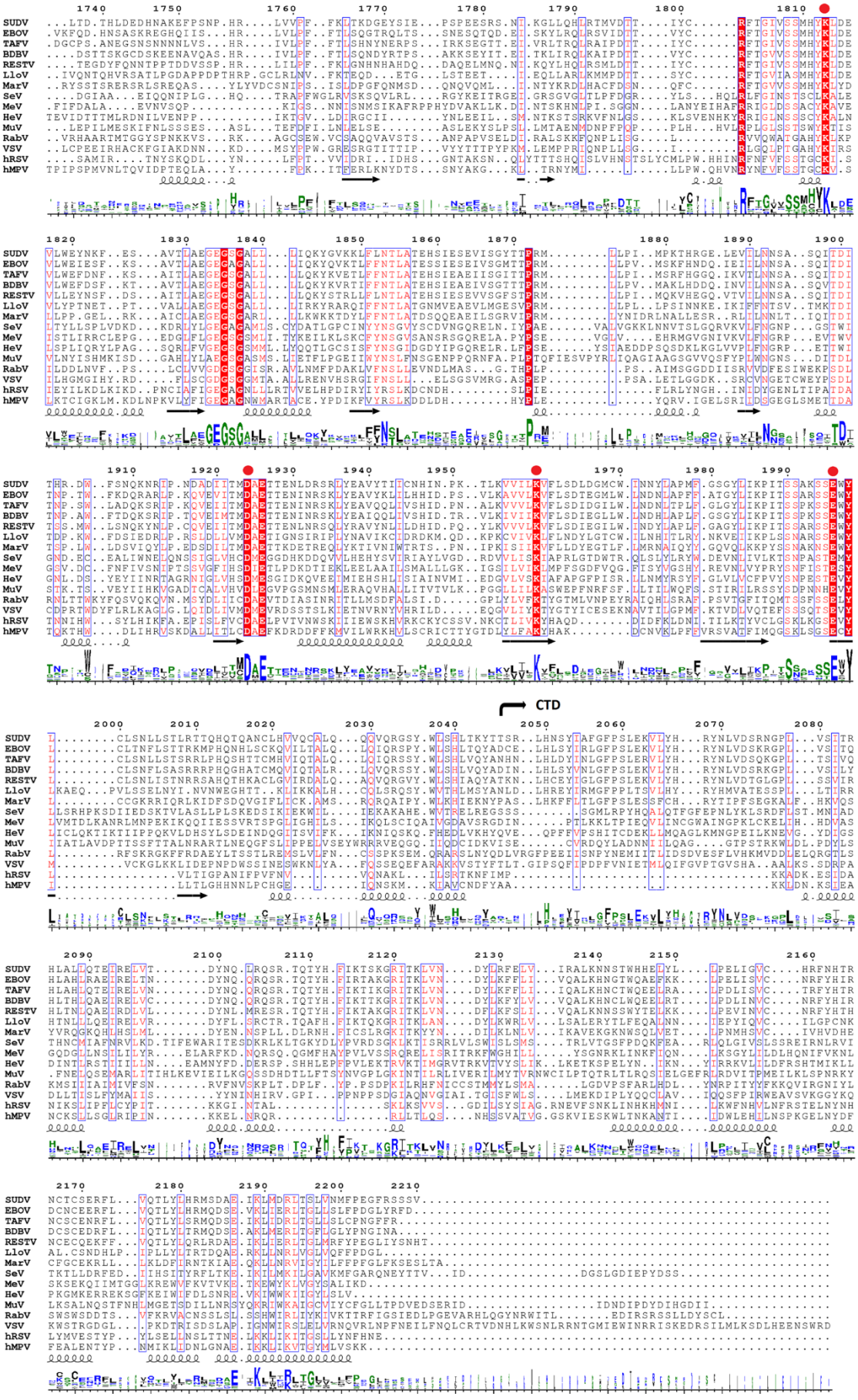
RNA	Sequence	Origin
GpppG-SUDV12	GpppGAUGAAGAUUAAG	chemical synthesis
mGpppG-SUDV12	mGpppGAUGAAGAUUAAG	chemical synthesis
GpppGm-SUDV12	GpppGmAUGAAGAUUAAG	chemical synthesis
mGpppGm-SUDV12	mGpppGmAUGAAGAUUAAG	chemical synthesis
mGpppGmAm-SUDV11	mGpppGmAmUGAAGAUUAAG	chemical synthesis
pG-SUDV12	pGAUGAAGAUUAAG	chemical synthesis
pppG-SUDV12	pppGAUGAAGAUUAAG	chemical synthesis
pppGm-SUDV12	pppGmAUGAAGAUUAAG	chemical synthesis
mGpppGm(Gm)-SUDV12	mGpppGmAUGmAAGmAUUAAGm	chemical synthesis
mGpppGm(Um)-SUDV12	mGpppGmAUmGAAGAUmUmAAG	chemical synthesis
mGpppGm(Am)-SUDV12	mGpppGmAmUGAmAmGAmUUAmAmG	chemical synthesis
GpppG(Am)-SUDV12	GpppGAmUGAmAmGAmUUAmAmG	chemical synthesis
mGpppG(Am)-SUDV12	mGpppGAmUGAmAmGAmUUAmAmG	chemical synthesis
GpppGm(Am)-SUDV12	GpppGmAmUGAmAmGAmUUAmAmG	chemical synthesis
pppG(Am)-SUDV12	GpppGAmUGAmAmGAmUUAmAmG	chemical synthesis
pppG(Am)-SUDV22	pppGAmUGAmAmGAmUUAmAmGAmAmCCUUCAmUC	chemical synthesis
GpppG(Am)-SUDV22	GpppGAmUGAmAmGAmUUAmAmGAmAmCCUUCAmUC	chemical synthesis
mGpppG(Am)-SUDV22	mGpppGAmUGAmAmGAmUUAmAmGAmAmCCUUCAmUC	chemical synthesis
GpppGm(Am)-SUDV22	GpppGmAmUGAmAmGAmUUAmAmGAmAmCCUUCAmUC	chemical synthesis
mGpppGm(Am)-SUDV22	mGpppGmAmUGAmAmGAmUUAmAmGAmAmCCUUCAmUC	chemical synthesis
GpppG-hMPV8	GpppGGGACAAGU	chemical synthesis
mGpppG-hMPV8	mGpppGGGACAAGU	chemical synthesis
mGpppGm-hMPV8	mGpppGmGGACAAGU	chemical synthesis
HO-(G)27	HO-GGGGGGGGGGGGGGGGGGGGGGGGGGG	provider (Biomers)
HO-(C)27	HO-CCCCCCCCCCCCCCCCCCCCCCCCCCC	provider (Biomers)
HO-(U)27	HO-UUUUUUUUUUUUUUUUUUUUUUUUUUU	provider (Biomers)
HO-(A)27	HO-AAAAAAAAAAAAAAAAAAAAAAAAAAA	provider (Biomers)
HO-Am(A)26	HO-AmAAAAAAAAAAAAAAAAAAAAAAAAA	provider (Biomers)
HO-AmAm(A)25	HO-AmAmAAAAAAAAAAAAAAAAAAAAAAA	provider (Biomers)
HO-(Am)27	HO-AmAmAmAmAmAmAmAmAmAmAmAmAmAmAmA mAmAmAmAmAmAmAmAm	provider (Biomers)
VP40-31	pppGAUGAAGAUUAAGAAAAAGAGGGAUUUUCUC	*in vitro* transcription

Unmethylated caps are denoted by ‘Gppp’, N7-methylated caps by ‘^{m}Gppp’, 2’O-methylated residues by ‘X_m’. Full-length sequences can be found in the second column, manufacturing information in the third one.

Table S2 – List of primers

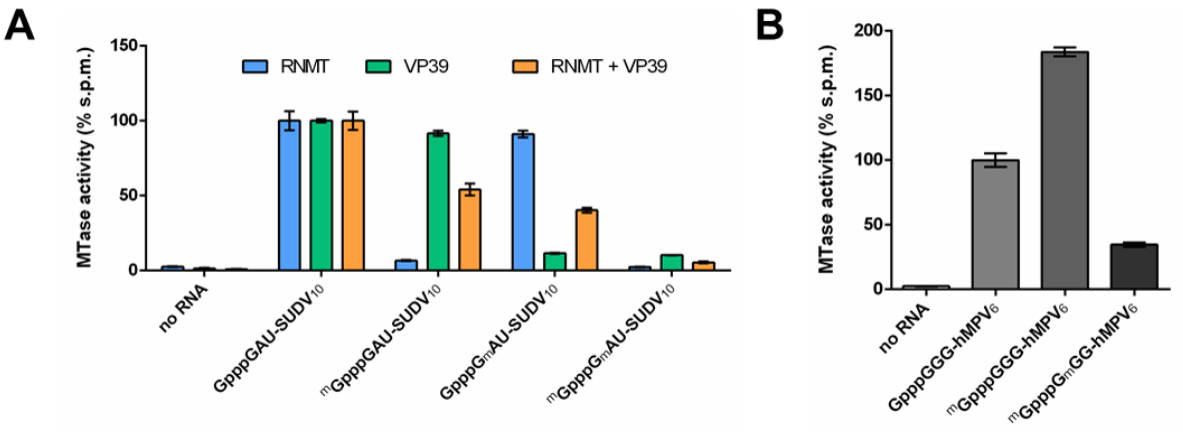
Primers		Sequence
VP40-31	forward	TAATACGACTCACTATA GATGAAGATTAAGAAAAAGAGGGATTTTCTC
	reverse	GAGAAAATCCCTCTTTTTCTTAATCTTCATC TATAGTGAGTCGTATTA

Primers were used to synthetize VP40 sequence-specific RNAs by *in vitro* transcription using the HiScribe T7 High Yield RNA Synthesis Kit (NEB).


CTD

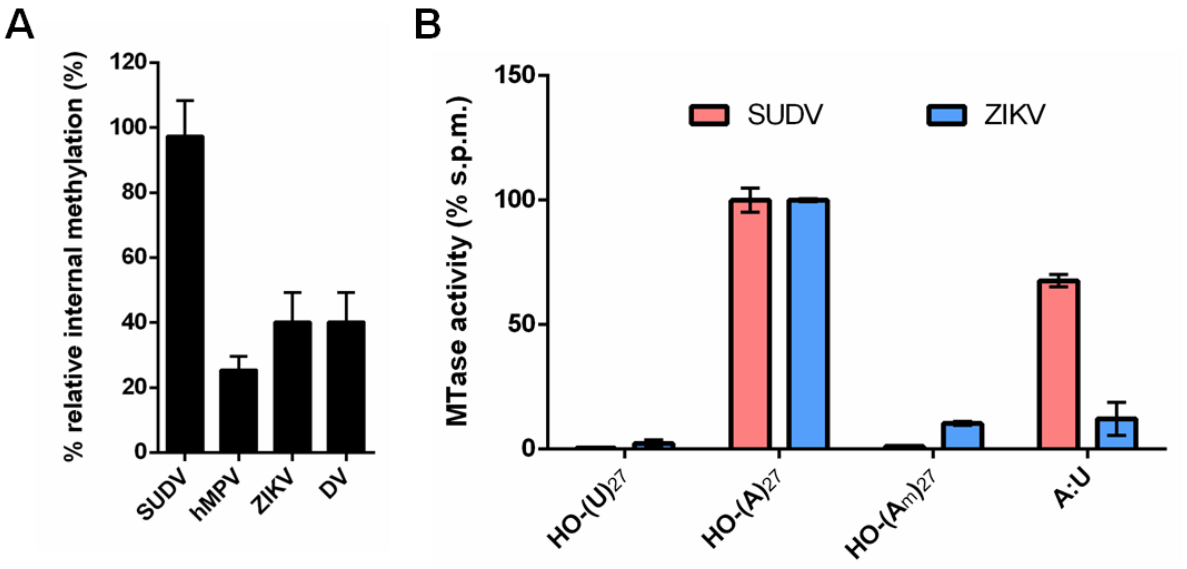
<u>Figure S1</u> – Alignment of mononegavirus MTase+CTD domains.

Alignment of the MTase+CTD domain of *Sudan ebolavirus* (SUDV; accession code YP_138527.1) with those of other members of the *Filoviridae* family [*Zaire ebolavirus* (EBOV; AAG40171.1), *Tai Forest ebolavirus* (TAFV; ALT19766.1), *Bundibugyo ebolavirus* (BDBV; YP_003815440.1), *Reston ebolavirus* (RESTV; APA16576.1), *Marburg marburgvirus* (MARV; P35262) and *Lloviu cuevavirus* (LLOV; YP_004928143.1)], as well as members of the *Paramyxoviridae* family [Sendai virus (SeV; P06447), Measles virus (MeV; BAB60955.1), Hendra virus (HeV; O89344) and Mumps virus (Muv; P30929)], the *Rhabdoviridae* family [Rabies virus (RabV; ABZ81226.1), vesicular stomatitis virus (VSV; ABP01784.1)] and the *Pneumoviridae* family [human respiratory syncytial virus (hRSV; AAX23996.1), human metapneumovirus (hMPV; AGJ74101.1)]. The alignment was generated using T-Coffee[68] and MAFFT[69] software, taking into account available structural information (PDB codes 4UCI for hMPV, 5A22 for VSV). It was visualised using ESPript 3x[70]. WebLogo[71] was used to illustrate degrees of conservation of amino-acids (shown underneath the alignment). The numbering on top of the alignment indicates amino-acid positions in the *Sudan ebolavirus* sequence, the red dots highlight the K-D-K-E motif, the bent arrow indicates the approximate start of the CTD. Spirals and arrows underneath the alignment indicate positions of helices and β-strands in the hMPV structure.



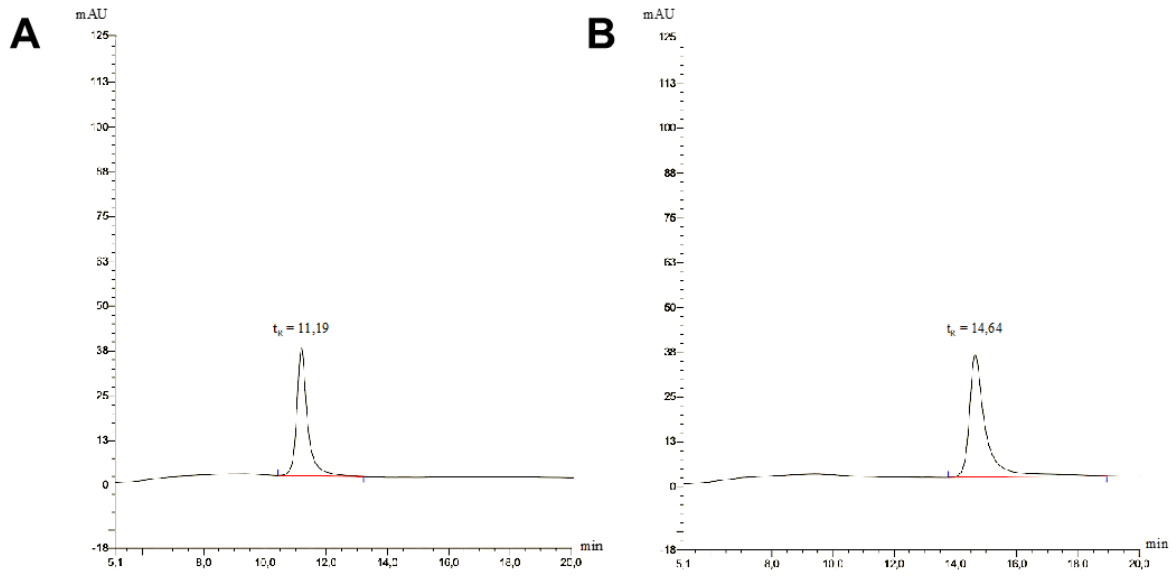
<u>Figure S2</u> - Cap MTases reveal specific activity on the cap structure.

(**A**) RNMT (human N7 MTase) and VP39 (vaccinia virus 2'O MTase) activity on a set of capped, synthetic SUDV sequence-specific RNAs (13mers), with methylated residues at different positions (Gppp: cap, ^{m}Gppp: N7-methylated cap, G_m: 2'O-methylated residue). Groups have been normalized to the highest activity measured for HO-$(A)_{27}$ (n=3). Data represent normalized mean ± standard deviation. (**B**) hMPV MTase activity measurement on a set of synthetic hMPV sequence-specific RNAs (13mers) with methylated residues at different positions (Gppp: cap, ^{m}Gppp: N7-methylated cap, G_m: 2'O-methylated residue) mimicking 5' cap structures. Groups have been normalized versus unmethylated control (n=6). Data represent normalized mean ± standard deviation.



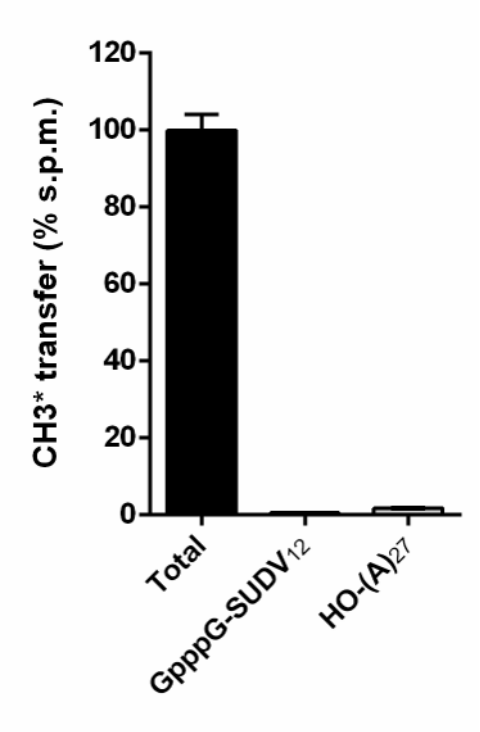
<u>Figure S3</u> – Internal 2'O methyltransferase activity is a specific feature of SUDV MTase.

(**A**) Ratios of methyltransferase activities on cap-1 RNAs ($^{m}GpppX_{m}$-RNA) over those on capped but unmethylated RNAs (GpppX-RNA) for MTase domains of *Sudan ebolavirus* (SUDV), *Human metapneumovirus* (hMPV), *Zika flavivrus* (ZIKV) and *Dengue flavivirus* (DV). RNA sequences were virus-specific. Values represent mean ratio ± standard deviation. (**B**) Comparison of MTase activity profiles of SUDV and ZIKV MTase domains on different synthetic homopolymeric RNAs and a equimolar mix of HO-$(A)_{27}$ and HO-$(U)_{27}$ (A:U). Groups have been normalized versus highest signal measured for HO-$(A)_{27}$ (n=3). Data represent normalized mean ± standard deviation.



<u>Figure S4</u> - HPLC controls for adenosine and 2'O-methylated adenosine identification.

(**A**) HPLC profile of HO-$(A)_{27}$ treated with snake venom phosphodiesterase and alkaline phosphatase. The adenosine (2'OH-A) control eluted after 11.2 min under the conditions of the experiment. (**B**) HPLC profile of HO-$(A)_{27}$ digested with snake venom phosphodiesterase and alkaline phosphatase. The 2'O-methylated adenosine (2'OCH_3-A) control eluted after 14.6 min.



<u>Figure S5</u>– Evaluation of methyl transfer by MTase assay on different RNA substrates.

Total radioactivity (Total) corresponds to the radioactivity of 2 µM of radioactive SAM (SAM*) in the reaction mix volume used in the assays. The methyl transfer was measured in the presence of 2 µM of SAM* onto two RNAs (at 10 µM concentrations): a synthetic, SUDV sequence-specific, 13-mer RNA with a cap (**G**pppG), and a 27-mer polyadenosine (HO-(A_{27})). Groups have been normalized versus « Total » control (n=3). Data represent normalized mean ± standard deviation.

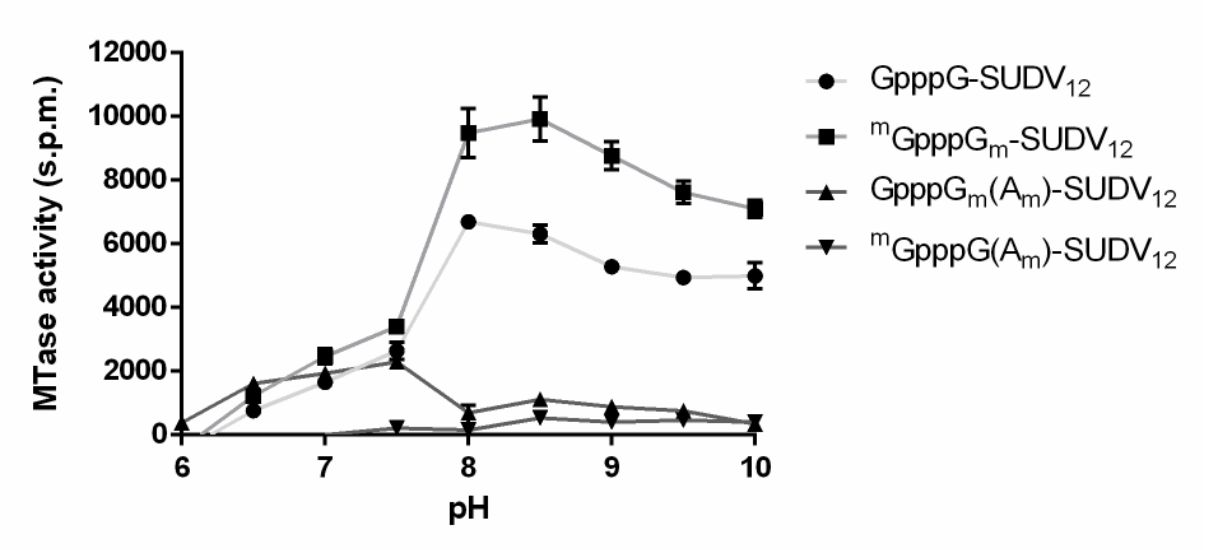


Figure S6 - N7 and 2'O MTase reactions have a different optimal pH.

Sudan ebolavirus (SUDV) L protein methyltransferase domain (MTase) flanked by the C-terminal domain (CTD) methyltransferase activity evaluation at different pHs (from 6.0 to 10.0) on a set of synthetic SUDV sequence-specific RNAs (13-mers) as indicated in the figure.

Supplementary References

68. Notredame, C., Higgins, D. G. & Heringa, J. T-Coffee: A novel method for fast and accurate multiple sequence alignment. *J. Mol. Biol.* **302,** 205–217 (2000).

69. Katoh, K., Misawa, K., Kuma, K. & Miyata, T. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* **30,** 3059–3066 (2002).

70. Robert, X. & Gouet, P. Deciphering key features in protein structures with the new ENDscript server. *Nucleic Acids Res.* **42,** W320-324 (2014).

71. Crooks, G. E., Hon, G., Chandonia, J.-M. & Brenner, S. E. WebLogo: a sequence logo generator. *Genome Res.* **14,** 1188–1190 (2004).