

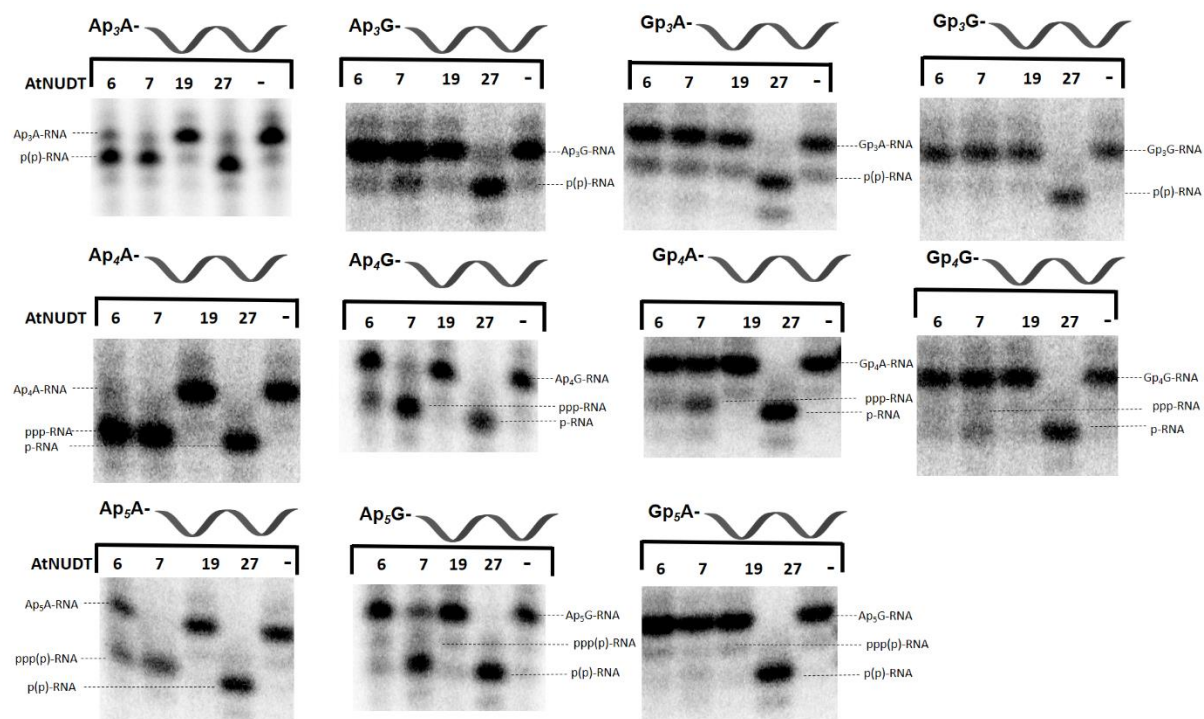
Supplementary information

***Arabidopsis Thaliana* Nudixes have RNA decapping activity**

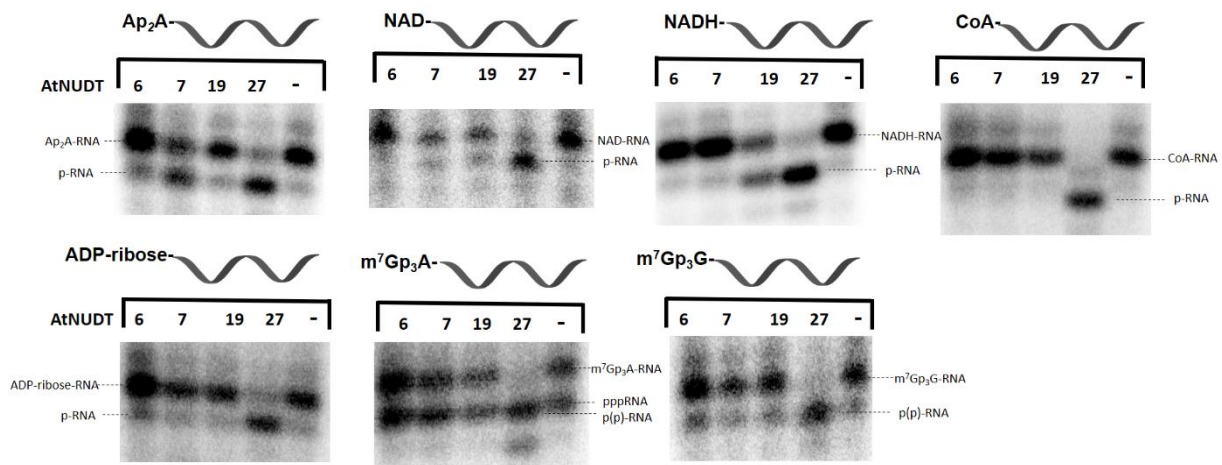
Maria-Bianca Mititelu, Oldřich Hudeček, Agnieszka Gozdek, Roberto Benoni, Ondřej Nešuta, Szymon Krasnodębski, Joanna Kufel and Hana Cahova

Supplementary Table 1: Sequences of DNA templates for *in vitro* RNA transcription.

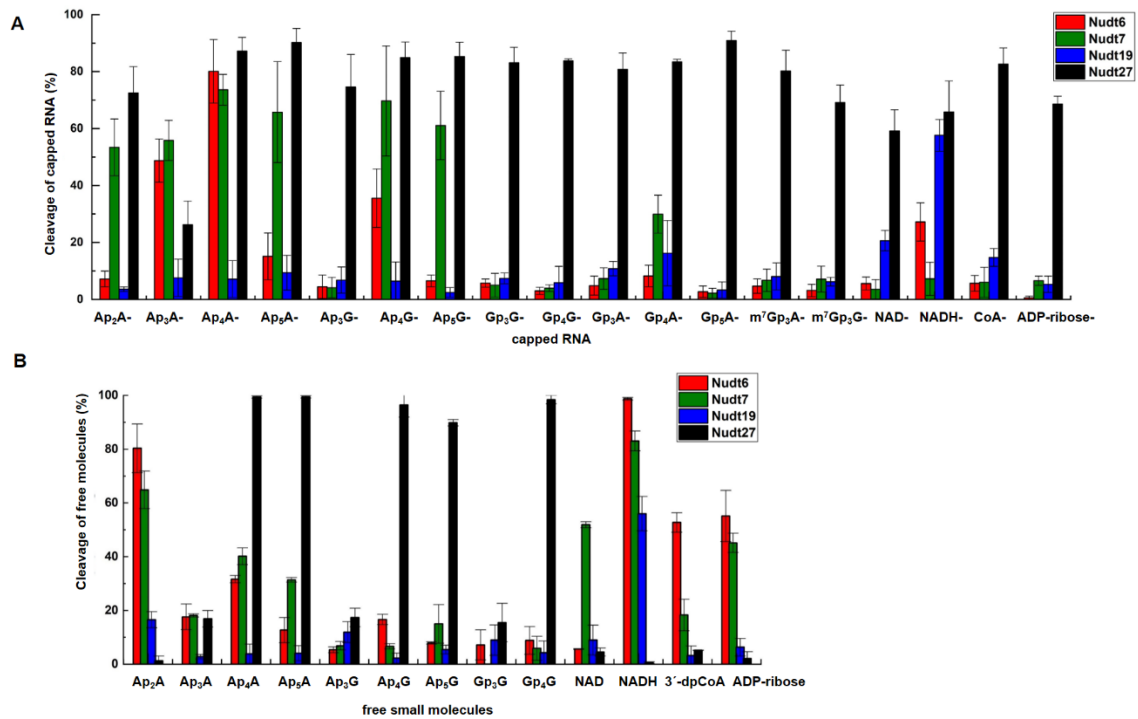
Name	Sequence
35A	5'-CAGTAATACGACTCACTATTAGGGAAGCGGGCATGCGCCAGCCATAGCCGATCA-3'
35G	5'-CAGTAATACGACTCACTATAAGGGAAGCGGGCATGCGCCAGCCATAGCCGATCA-3'



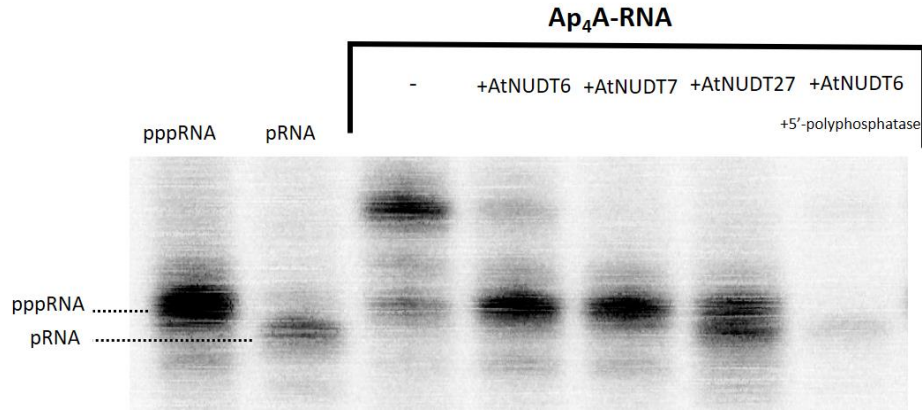
Supplementary Figure 1: Representative examples of PAGE analysis of the RNA decapping activity of AtNUDT6, 7, 19 or 27 on 5'-capped ($Ap_{3-5}A$, $Ap_{3-5}G$, $Gp_{3-5}A$ and $Gp_{3-4}G$) RNA.



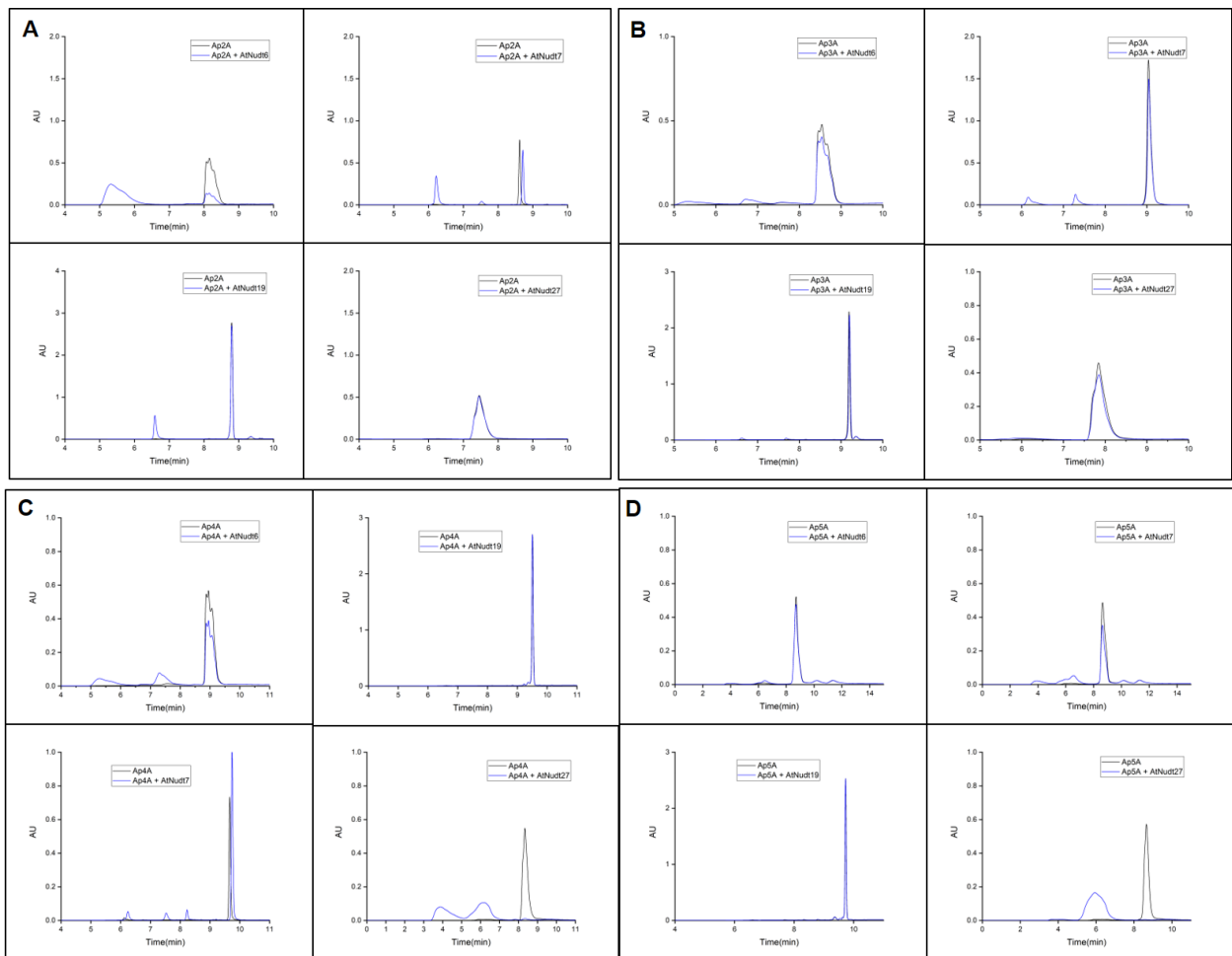
Supplementary Figure 2: Representative examples of PAGE analysis of the RNA decapping activity of AtNUDT6,7,19 or 27 on 5'-capped (Ap_2A , NAD, NADH, CoA, ADP-ribose and $m^7Gp_3(A)G$) RNA.



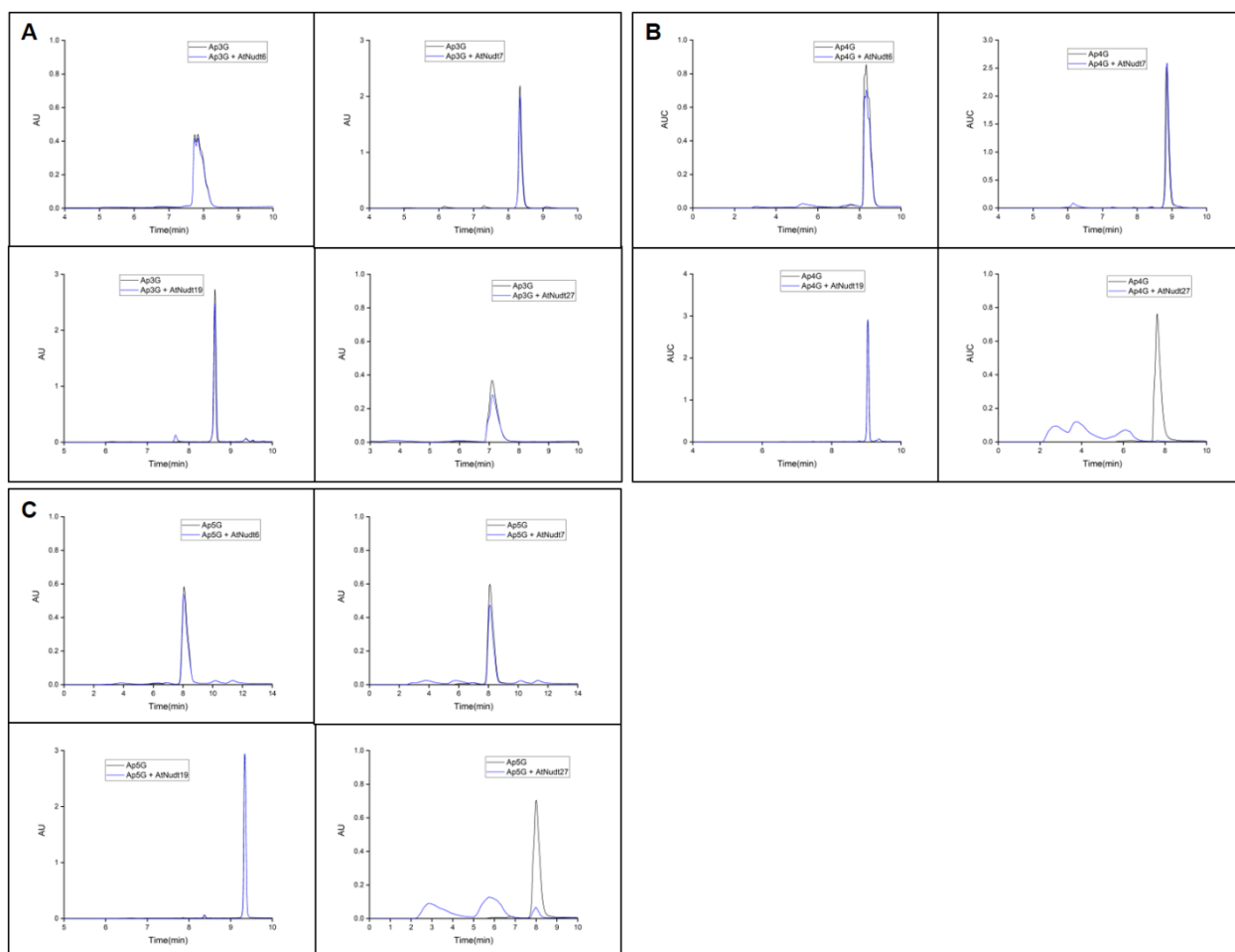
Supplementary Figure 3: A) Cleavage of RNA capped with different small molecules in vitro by AtNUDT6 (500 nM), AtNUDT7 (500 nM), AtNUDT19 (500 nM) and AtNUDT27 (50 nM). Values are in % of cleaved capped RNA and represent a mean of three independent replicates \pm standard deviations. B) Cleavage of different small molecules in vitro by AtNUDT6 (500 nM), AtNUDT7 (500 nM), AtNUDT19 (500 nM), AtNUDT27 (500 nM). Values are in % of cleaved substrate and represent a mean of three independent replicates \pm standard deviations.



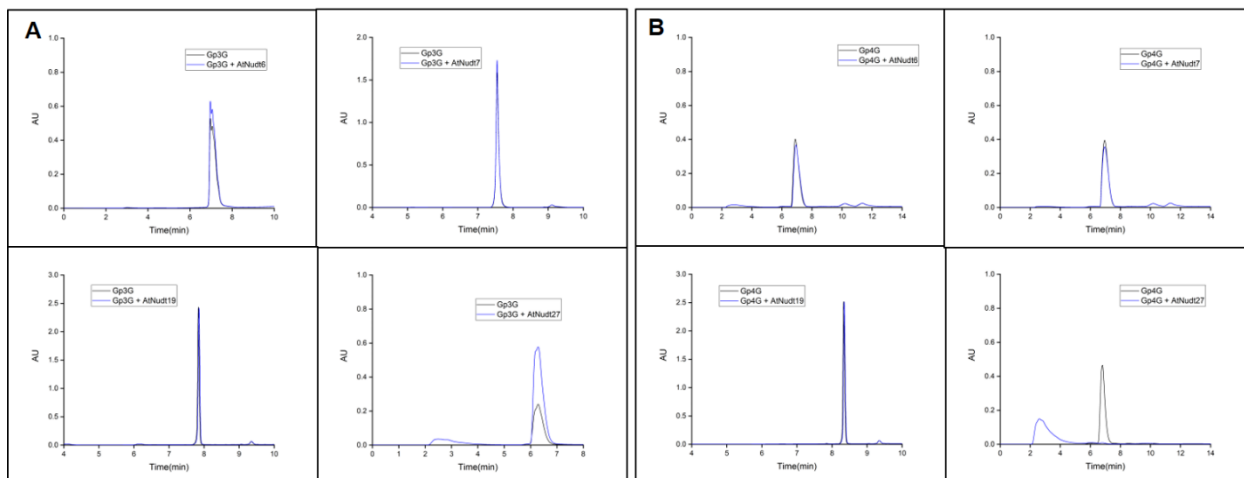
Supplementary Figure 4: Representative example of PAGE analysis of the RNA decapping activity of AtNUDT6,7 or 27 on Ap₄A-RNA. While AtNUDT6 and 7 lead to pppRNA, AtNUDT27 cleavage leads to mixture of pppRNA and pRNA. When 5'-polyphosphatase is added to AtNUDT6 cleaved RNA, pppRNA band disappears and new at level of pRNA is visible.



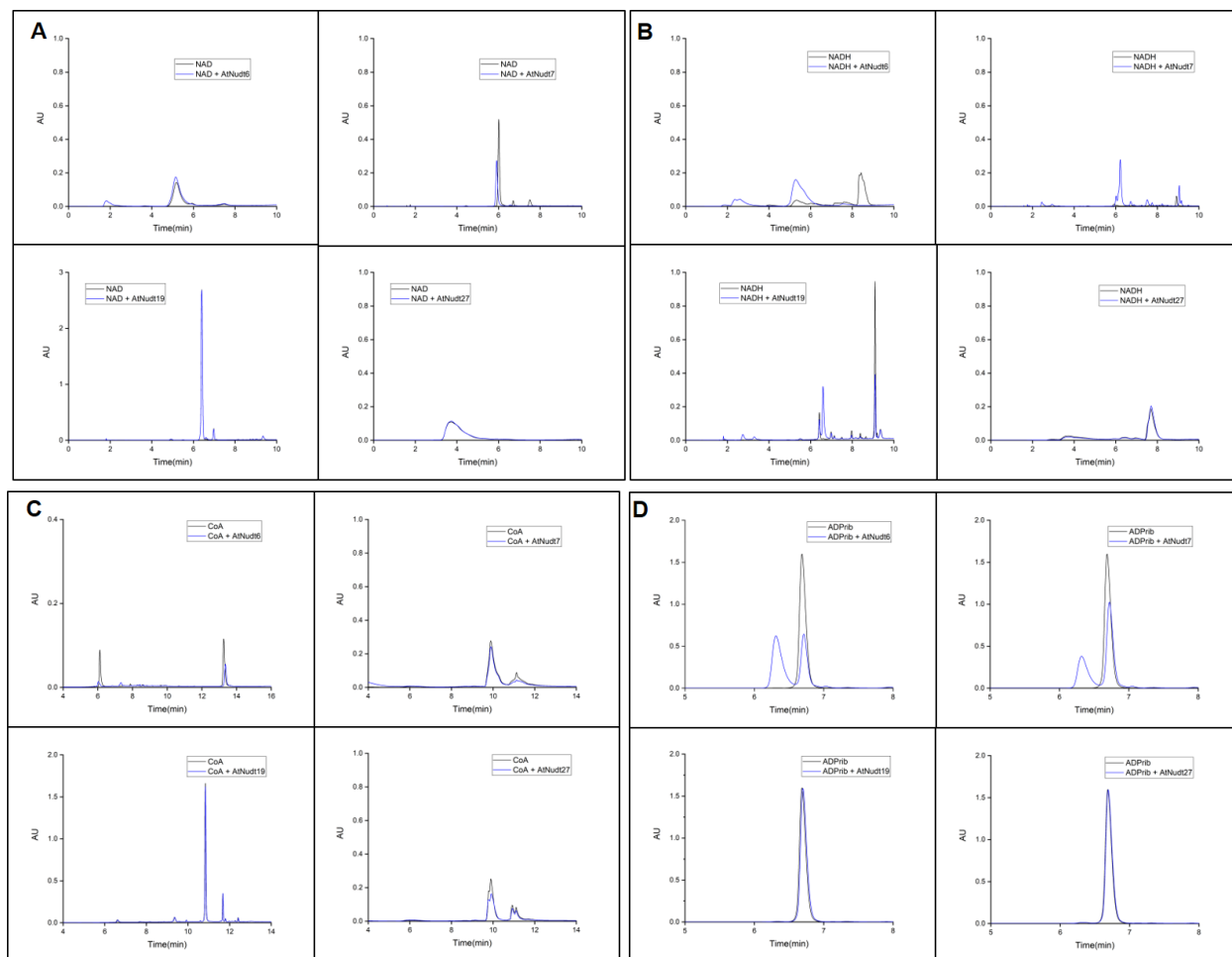
Supplementary Figure 5: HPLC chromatograms of free small molecules before and after incubation with AtNUDT6,7,19 and 27. A) Ap₂A, B) Ap₃A, C) Ap₄A, D) Ap₅A.



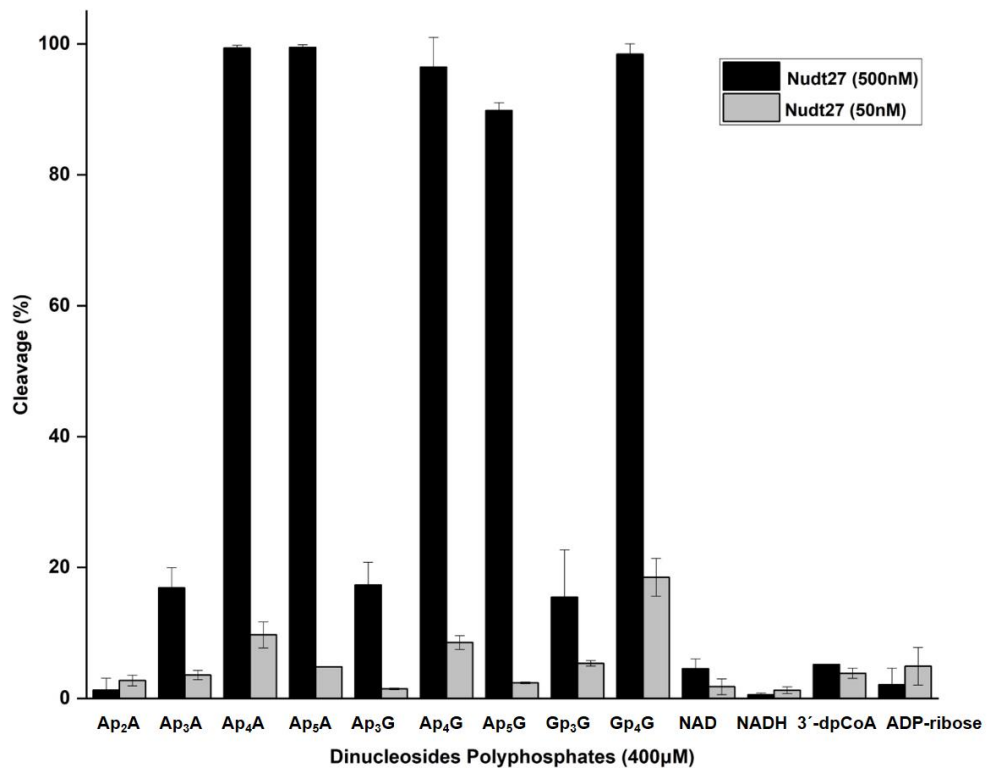
Supplementary Figure 6: HPLC chromatograms of free small molecules before and after incubation with AtNUDT6, 7, 19 and 27. A) Ap₃G, B) Ap₄G, C) Ap₅G.



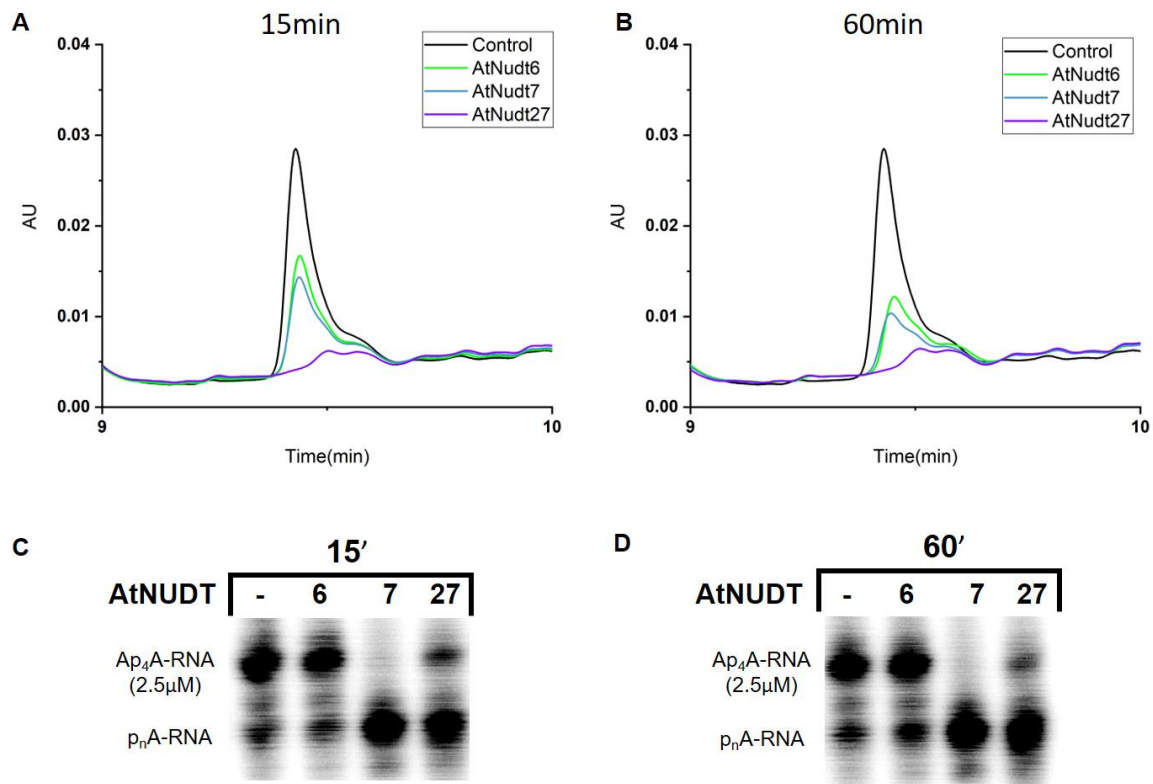
Supplementary Figure 7: HPLC chromatograms of free small molecules before and after incubation with AtNUDT6,7,19 and 27. A) Gp₃G, B) Gp₄G.



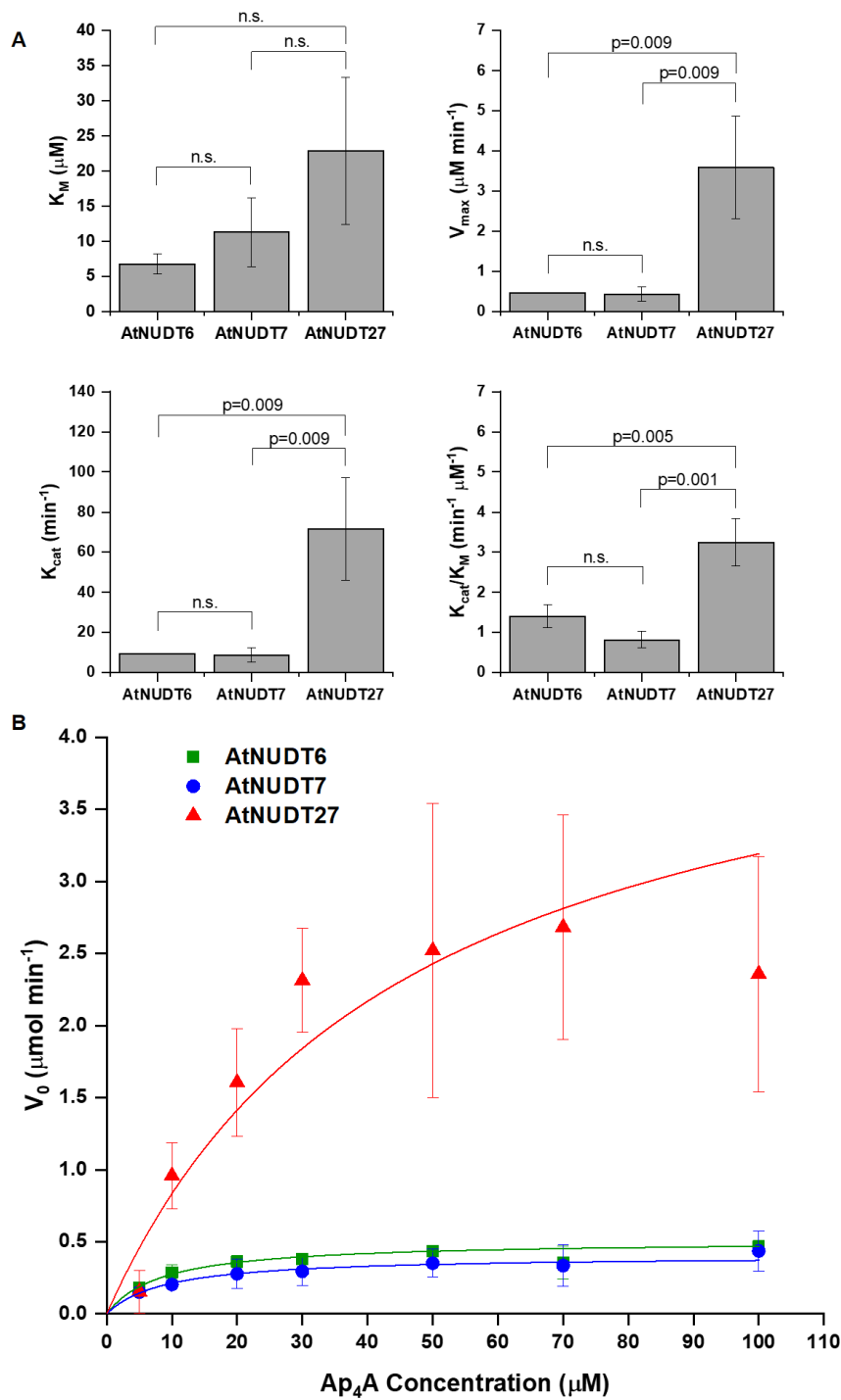
Supplementary Figure 8: HPLC chromatograms of free small molecules before and after incubation with AtNUDT6,7,19 and 27. A) NAD, B) NADH, C) 3' dephosphoCoA, D) ADP-ribose.



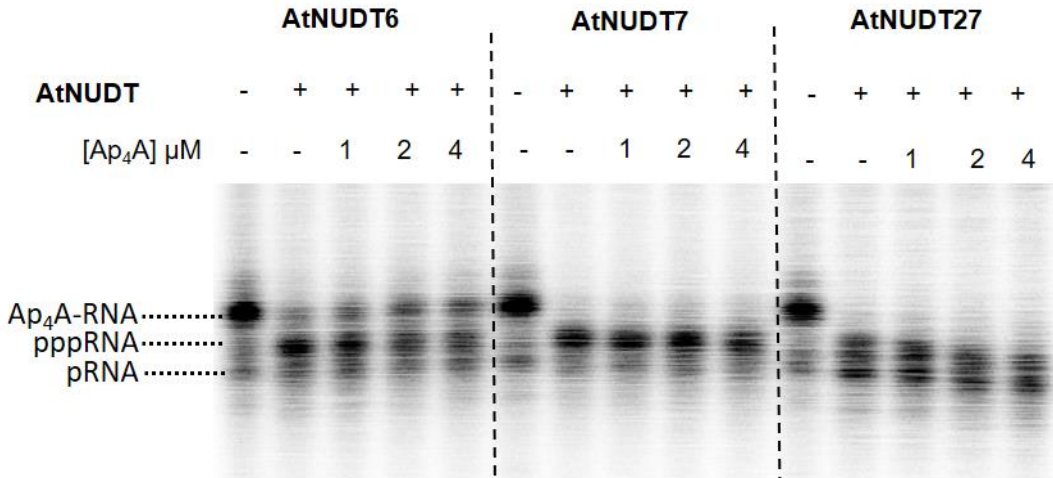
Supplementary Figure 9: Cleavage of various small molecules in vitro by AtNUDT27 at 500 nM and 50 nM concentration. Values are in % of cleaved substrate and represent a mean of three independent replicates \pm standard deviations.



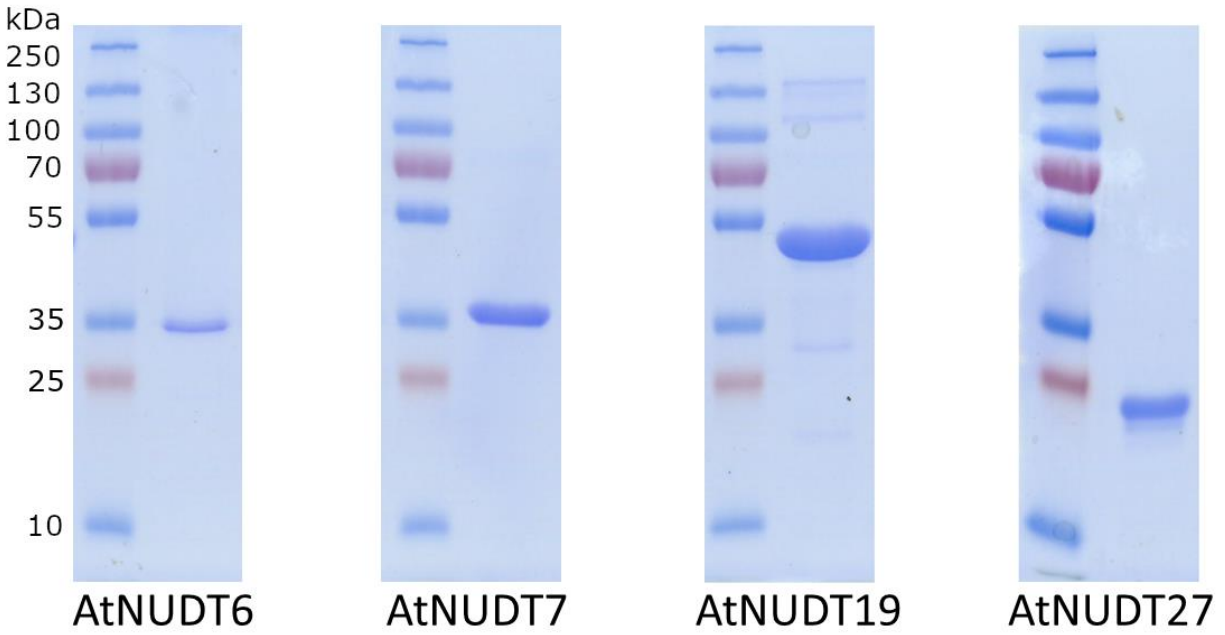
Supplementary Figure 10: HPLC chromatograms of Ap₄A (2.5 μM) before and after incubation with AtNUDT6,7 and 27 (A) 15 min, (B) 60 min. Representative example of PAGE analysis of the RNA decapping activity of AtNUDT6,7 or 27 on Ap₄A-RNA (C) 15 min, (D) 60 min.



Supplementary Figure 11: Kinetic studies. A) Kinetic parameters of AtNUDT6,7 and 27 in reaction with Ap₄A. B) Michaelis-Menten plots of initial velocities obtained for AtNUDT6,7 and 27 in reaction with Ap₄A.



Supplementary Figure 12: Inhibition study. Ap₄A (1, 2 and 4 μM) was added to 1 μM Ap₄A-RNA and cleavage of ³²P labelled RNA by AtNUDT6,7 and 27 was followed by PAGE analysis.



Supplementary Figure 13: SDS-PAGE analysis of the protein samples after size exclusion chromatography: AtNUDT6 - 32 kDa, AtNUDT7 - 37 kDa, AtNUDT19 - 41 kDa, AtNUDT27 - 22 kDa.