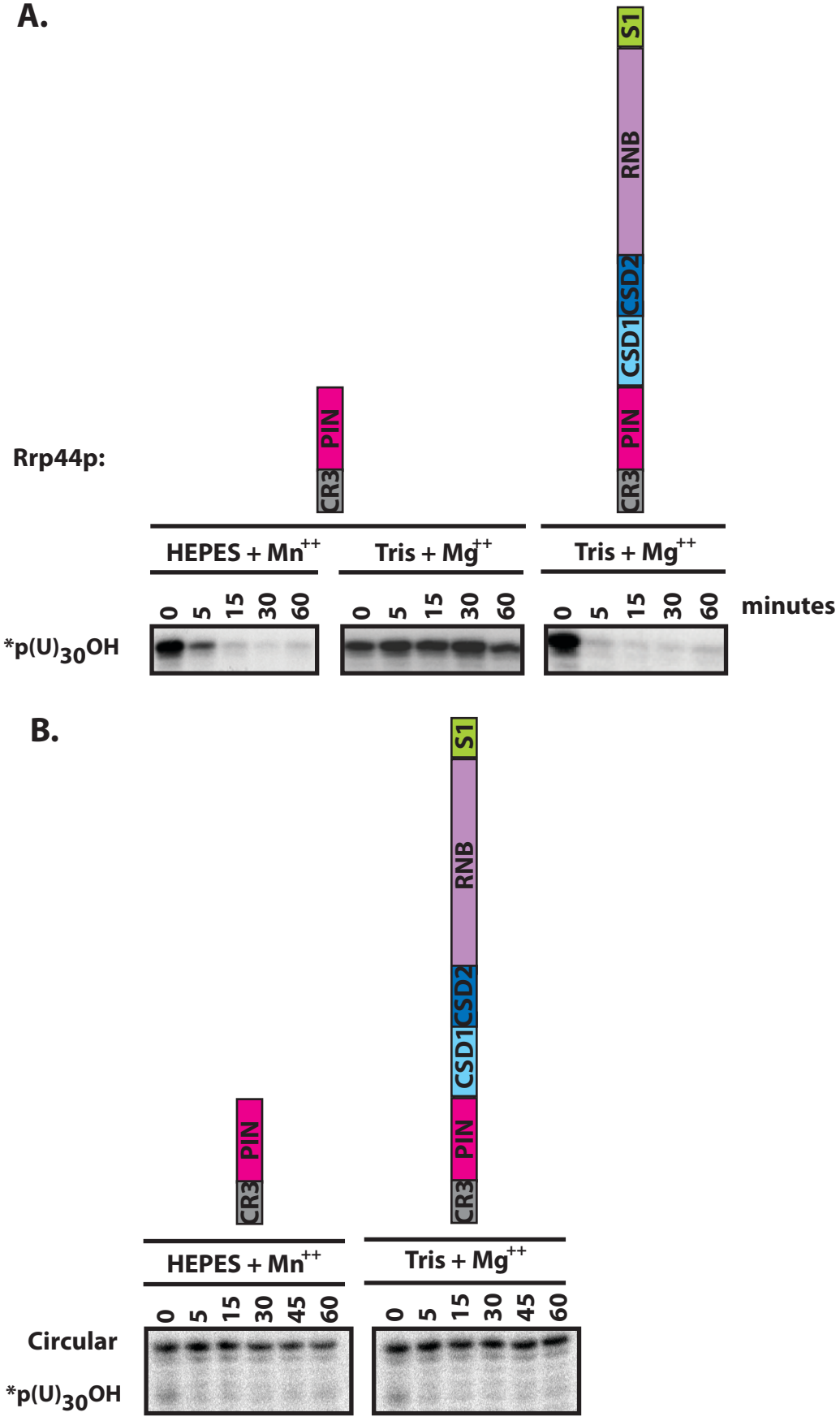
Supplemental figure 1: The PIN domain and RNB domain have similar levels of activity *in vitro*. A. The truncated Rrp44p shows robust ribonuclease activity in HEPES buffer containing Mn^{++} ions (left panel), but is much less active in the Tris buffer containing Mg^{++} ions that was previously used to biochemically characterize Rrp44p (middle panel)[1]. Both the identity of the buffer and the divalent ion contributes to this effect as HEPES + Mg^{++} and Tris + Mn^{++} allow for intermediate activity (data not shown). The activity level of truncated Rrp44p in HEPES/$MnCl_2$ was similar to that of full length Rrp44p in Tris/$MgCl_2$ buffer (compare left to right panels). It has previously been shown that the activity of the full length protein in the Tris/Mg buffer is due to the RNB domain. This strongly suggests that the activity of the PIN domain we detected is robust enough to contribute to overall exosome activity. Furthermore, the observation that the PIN and RNB domains are fully active under different conditions, suggests that one activity or the other might dominate under specific physiological conditions. **B.** The N-terminal region of Rrp44p degrades both linear and circular U_{30} RNA, but prefers the linear substrate (left panel). 40% of the circular substrate was degraded over a 60 minute time course, while 40% degradation of a linear U_{30} substrate under similar conditions typically occurred within 5 minutes. The circular substrate was generated by treating the 5' end labeled substrate with T4 RNA ligase. Three observations confirm the identification of the circular substrate. First, it is only present after T4 RNA ligase treatment and has the mobility expected of a circular substrate. Second, the P^{32} labeling of the circular form is resistant to treatment with alkaline phosphatase, but the linear form is not (data not shown). Third, the full length Rrp44p degrades a linear substrate under

conditions where the RNB exonuclease domain is active, but does not degraded the circular form (right panel).

The indicated recombinant proteins were purified from *E. coli* and incubated with 5' end labeled U_{30} RNA in either 20 mM HEPES pH 7.5, 150 mM NaCl, 3 mM $MnCl_2$, 1 mM DTT or in 10 mM Tris pH 7.5, 75 mM NaCl, 1mM $MgCl_2$, 1 mM DTT.

1. Dziembowski, A., Lorentzen, E., Conti, E. & Seraphin, B. A single subunit, Dis3, is essentially responsible for yeast exosome core activity. *Nat Struct Mol Biol* **14**, 15-22 (2007).


A.
Rrp44p:
CR3
PIN
CR3
PIN
CSD1
CSD2
RNB
S1
HEPES + Mn++
Tris + Mg++
Tris + Mg++
0 5 15 30 60
0 5 15 30 60
0 5 15 30 60
minutes
*p(U)30OH
B.
CR3
PIN
CR3
PIN
CSD1
CSD2
RNB
S1
HEPES + Mn++
Tris + Mg++
0 5 15 30 45 60
0 5 15 30 45 60
Circular
*p(U)30OH