Supplementary material 7: Construction of plasmids used in this study. Rnt1p was expressed *in vivo* using the yeast vectors pRS315 (Sikorski and Hieter, 1989), pNS (Ueki *et al.*, 1998), pACT2 (Clonetech Laboratories Inc., Palo Alto, CA), or pGAD and pGBDU (James *et al.*, 1996). The construct pRS315/RNT1 (Abou Elela *et al.*, 1996) was modified by introducing an *XhoI* site upstream of the first AUG in *RNT1* by site-directed mutagenesis (Kunkel, 1985) (primer 5'-CAAGCTTTCTCGAGAATGGGCTC-3'), yielding pRS315/RNT1(2XhoI). pRS315/GFP was produced by inserting a PCR fragment encoding for the Green Fluorescent Protein (GFP) (Carminati and Stearns, 1997) into pRS315/RNT1(2XhoI) digested with *XhoI* in order to express GFP under the control of Rnt1p promoter. pRS315/GFP/RNT1 was generated by inserting a *BamHI-BglII* fragment from AD/RNT1 (Lamontagne *et al.*, 2000) into the pRS315/GFP *BglII* site. The blunt-ended *EcoRI-BglII* fragment from BD/ΔNT2 (Lamontagne *et al.*, 2000) was inserted into the blunt-ended pRS315/GFP *BglII* site to produce pRS315/GFP/ΔNT2. pRS315/GFP/NT2 was constructed by inserting the blunt-ended *EcoRI* fragment from BD/NT2 (Lamontagne *et al.*, 2000) into the blunt-ended pRS315/GFP *BglII* site. pRS315/GFP/ds1 was constructed by inserting the blunt-ended *BamHI-BglII* fragment from BD/ds1 (Lamontagne *et al.*, 2000) into the blunt-ended pRS315/GFP *BglII* site. pRS315/GFP/RNT1-22st was constructed by inserting a *BglII-ApaI* digested PCR fragment encoding for the 22 first amino acids of Rnt1p amplified from AD/RNT1 (primers 5'-TACAAGCTAGATCTGGAGGCTCAAAAGTAGC-3' and 5'-CTATTGG-GGCCCTAACCATTTTCGTTATC-3') into *BglII-ApaI* digested pRS315/GFP. pRS315/GFP/RNT1-82st and pRS315/GFP/RNT1-451ds were cloned as described above using the primer pairs 5'-TACAAGCTAGATCTGGAGGCTCAAAAGTAGC-3' and 5'-

CCTTTTGGGCCCACTCTATCTTAATTGTTAACG-3’, amplifying a fragment encoding for the first 82 amino acids of Rnt1p, or 5’-GAAATTGGAGATCTGGAAGTGAATCTGTGTTAAAAG-3’ and 5’-CGTCTAGGG-CCGTTTCAGCTTGTATCTG-3’, amplifying a fragment encoding for the amino acids 451 to 471 of Rnt1p, respectively. pRS315/GFP/ds4 was constructed by subcloning the *SalI-BglII* fragment from BD/ds4 (Tremblay *et al.*, 2002) into *SalI-BglII* digested pGBDU-C3 in order to change the reading frame, then the *BamHI-BglII* fragment of the resulting subclone was inserted into the *BglII* site of pRS315/GFP. AD/RNT1-K45/I and AD/RNT1-463st were generated by PCR amplification of regions of *RNT1* encoding for the N- and C-terminal domains of the protein under mutagenic conditions (Cadwell and Joyce, 1994). The selected genes were sequenced and single mutations were identified in both cases. pRS315/GFP/RNT1-K45/I was obtained by inserting the *BamHI-BglII* fragment of AD/RNT1-K45/I into the *BglII* site of pRS315/GFP. pRS315/GFP/NT2-K45/I was constructed by inserting the *BglII* digested PCR product encoding for the amino acids 1 to 192 of Rnt1p amplified from AD/RNT1-K45/I (primers 5’-TACAAGCTAGATCTGGAGGCTCAAAAGTAGC-3’ and 5’-TGTTGCAGATCTCTAATCACCAGCCTTTG-3’) into the *BglII* site of pRS315/GFP. pRS315/GFP/ds1-463st was constructed by inserting the blunt-ended *HindIII* fragment of AD/RNT1-463st in the blunt-ended *BglII* site of pRS315/GFP. The *BamHI-BglII* fragment of AD/RNT1-463st was inserted into the *BglII* site of pRS315/GFP to obtain pRS315/GFP/RNT1-463st, and into the *BamHI* site of pGBDU-C3 to produce BD/RNT1-463st. The *BamHI-SalI* fragment of BD/RNT1-463st was inserted into the *BamHI-SalI* sites of pQE31 to create pQE/RNT1-463st. BD/RNT1-D247/R was created

by replacing the *AvrII* fragment of BD/RNT1 (Lamontagne *et al.*, 2000) with an *AvrII* digested PCR insert amplified from BD/RNT1 with a 5' mutagenic oligo (primers 5'-AGACTAGAATTCCTAGGCAGATCGATCTTAAATTCTG-3' and 5'-CCATCATGGTCGACTAAAAGGAACG-3'). BD/RNT1-D247/R was digested with *BamHI-BglII* and inserted into the *BglII* site of pRS315/GFP, producing pRS315/GFP/RNT1-D247/R. The *BamHI-SalI* fragment encoding for the mutated *RNT1* gene from BD/RNT1-D247/R was inserted in the *BamHI-SalI* sites of pQE31 in order to create pQE/RNT1-D247/R. For the localization assay, pNS/RNT1 was generated by inserting a blunt-ended *BamHI-BglII* fragment from AD/RNT1 (Lamontagne *et al.*, 2000) in the blunt-ended *XhoI* site of pNS. AD/ΔNT2 was constructed by inserting a PCR fragment encoding for amino acids 192 to 471 of Rnt1p amplified from AD/RNT1 into the *SmaI* site of pACT2 (primers 5'-ACTGGCAGCTGAATTAAAAACC-3' and 5'-GGAACGTTTCAGCTTG-3'). pNS/ΔNT2 was produced by inserting an *XhoI-NcoI* fragment from AD/ΔNT2 into the *XhoI-NcoI* sites of pNS. pNS/NT2 was generated by inserting the *XhoI-NcoI* fragment from AD/NT2 (Lamontagne *et al.*, 2000) into the pNS *XhoI-NcoI* sites. pNS/ds1 was constructed by inserting a PCR fragment encoding for amino acids 344 to 471 of Rnt1p amplified from AD/RNT1 into the blunt-ended *XhoI* site of pNS (primers 5'-CAAGTTATGCTCGAGAAGACG-3' and 5'-GGAACGTTTCAGCTTG-3').