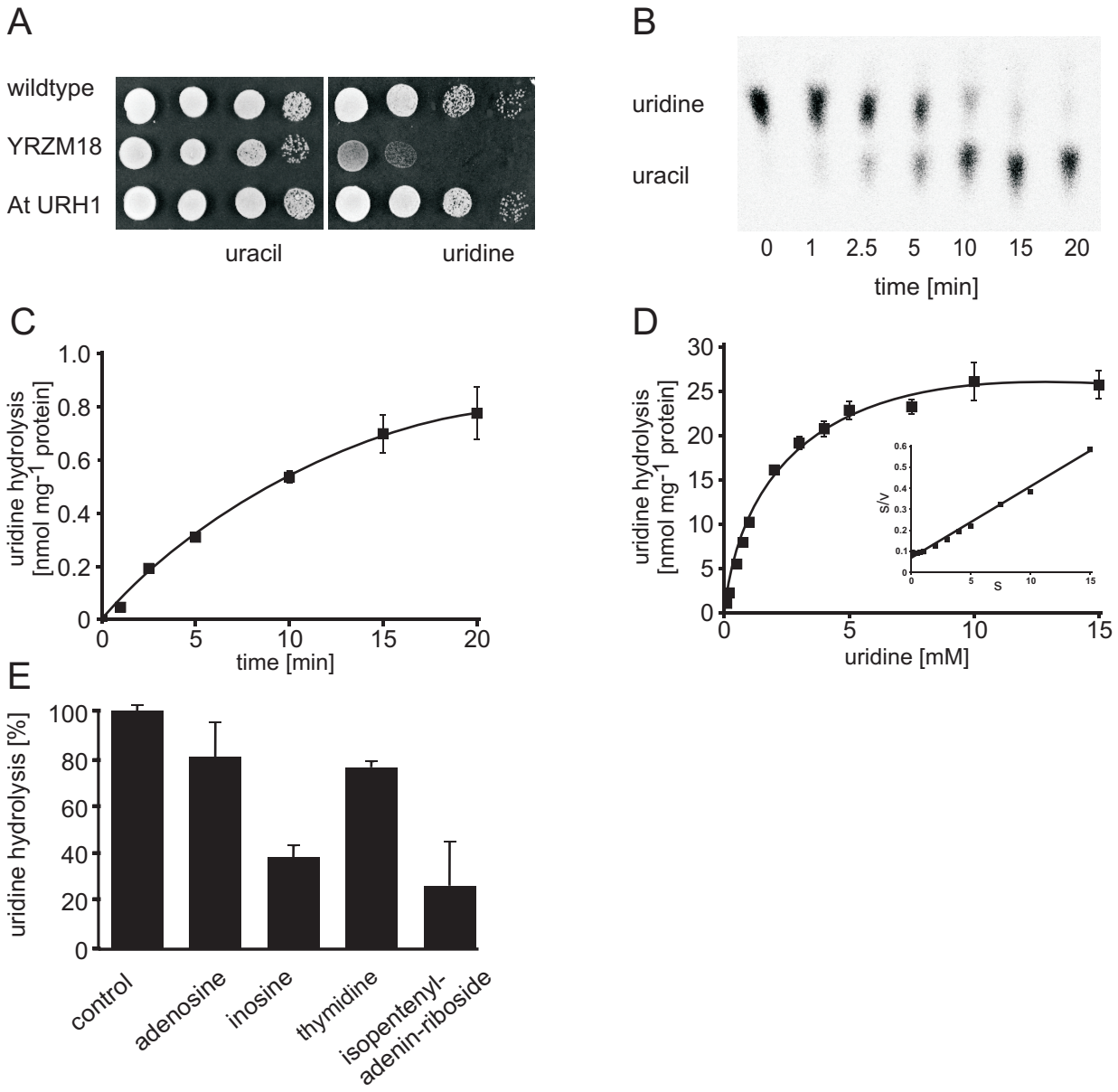
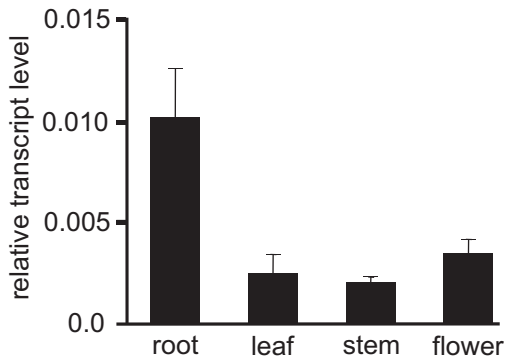
Supplemental Data. Jung et al. (2009). Uridine-Ribohydrolase is a key regulator in the uridine degradation pathway of Arabidopsis



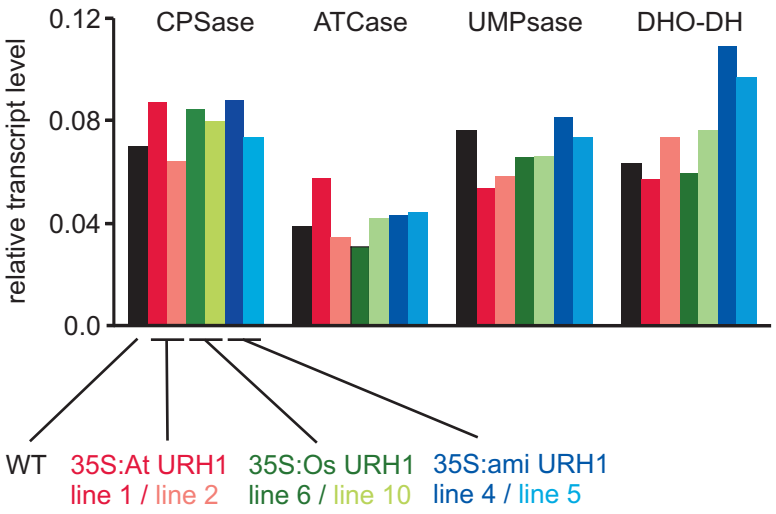
Supplemental Figure 1: Complementation of a yeast mutant and biochemical properties of URH1 synthesized in yeast

(A) Growth of yeast cells on selective minimal agar containing uracil or uridine, respectively as sole pyrimidine source. From left to right serial dilutions of yeast cells (1:10, 1:100, 1:1000, 1:10000) were applied. YRZM18 represents a mutant with deletions in uridine hydrolase (URH1) and uridine kinase (URK1). At URH1 is the complemented YRZM18 yeast strain. (B) Thin layer chromatography (TLC) of the At URH1 nucleosidase reaction products generated by recombinant protein synthesized in *S. cerevisiae*. Reaction mixtures containing the recombinant protein were incubated with 50 µM [^{14}C]-uridine and subsequently subjected to TLC and autoradiography. (C) Time dependent hydrolysis of uridine. Values were calculated from TLC plates with a phosphoimager. (D) Substrate dependence of uridine hydrolysis by At URH1. (E) Inhibition of [^{14}C]-uridine hydrolysis by 10-fold excess of structural analogs. Recombinant URH was incubated for 10 min at 37°C with 500 µM uridine in the absence (control) or presence of effectors. Data given in C to E represent means of three individual experiments ± SE.



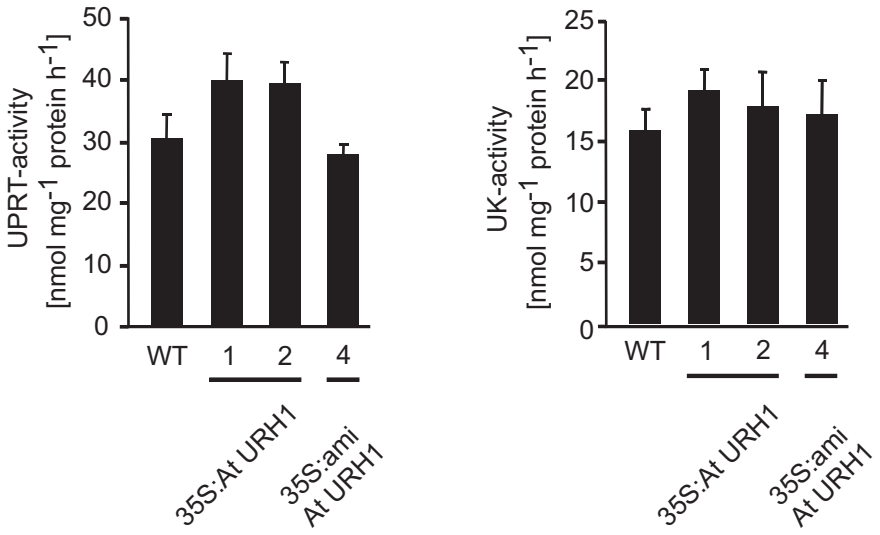
Supplemental Figure 2: Tissue specific expression of URH1 determined by realtime-PCR

The transcript levels were determined by quantitative real-time RT-PCR using gene specific primers. Three independent preparations of total RNA (100 ng) from different Arabidopsis tissues were assayed in duplicate (± SE). The transcript level was calculated relative to elongation factor 1 . For details see Methods section.



Supplemental Figure 3: Expression level of pyrimidine *de novo* synthesis genes in URH mutants

The transcript levels of Carbamoylphosphate synthase (CPSase); Aspartate transcarbamoylase (ATCase); Dihydroorotate dehydrogenase (DHODH) and UMP-synthase (UMPSase) were determined by quantitative real-time RT-PCR using gene specific primers. Three independent preparations of total RNA (100 ng) from different Arabidopsis tissues were assayed in duplicate (standard error below 10%). The transcript level was calculated relative to elongation factor 1 . For details see Methods section. 35S:At URH1, plants expressing At URH1 under control of CaMV35S promoter; 35S:amiRNA, mutants expressing a microRNA construct directed against At URH1 under control of CaMV35S promoter. numbers indicate individual mutant lines.



Supplemental Figure 4: Activities of salvage pathway enzymes.

Activities of uridine kinase (UK) and uracil phosphoribosyltransferase (UPRT) were determined from 20 days old soil grown seedlings as given in Methods. (A) UK activities (B) UPRT activities. 35S:At URH1, plants expressing At URH1 under control of CaMV35S promoter; 35S:amiRNA, mutants expressing a microRNA construct directed against At URH1 under control of CaMV35S promoter. numbers indicate individual mutant lines. All experiments were repeated at least three times showing similar results.

Supplemental Table. 1: Primers used for cloning and realtime (RT) PCR

RT-PCR Primers

RT_At URH1_for	5´-GGTGGGATCTCAAACGGTG-3´
RT_At URH1_rev	5´-CTCAGCTGCACTTTTCTCAG-3´
RT_Os URH1_for	5´-AACCATATTTGGCAACACCACC-3´
RT_Os URH1_rev	5´-ATTTGCTTCAGCAGCTGGGC-3´
RT_amiRNA_for	5´-GTTTCAAACACCGGAGCTG-3´
RT_amiRNA_rev	5´-TCGGAACTTCCTTCTGCAA-3´
RT_*Ef1α*for	5´-GAGACCAAGTACTACTGCAC-3´
RT_*Ef1α*rev	5´-GTTGGTCCCTTGTACCAGTCAAG-3´
RT_CPSaseLSU_for	5'-GTGGCGAATGGTCAGATCC-3'
RT_CPSaseLSU_rev	5'-AAGAAGTCCTGAAGAGCGGTC-3'
RT_ATCase_for	5'-TTACGAAGCAGCTCGTGGG-3'
RT_ATCase_rev	5'-CAAGCAGTAGCTTTAGAAGAGC-3'
RT_DHO-DH_for	5'-TCTCCACCAAGATGTTGAGAGA-3'
RT_DHO-DH_rev	5'-GGATCGACTTGAAGCCATCC-3'
RT-UMPSase_for	5'-CAGACGCTCATTCTGATTTTGTG-3'
RT-UMPSase_rev	5'-TTTATGATTCCCCGTCCCACG-3'

Primers for URH expression in Yeast and *E. coli*

URH1yeastfor	5'-CAAGGGGTTTTAACTGCAGTGAGAAAACG-3'
URH1yeastrev	5'-CGTGCTCTGGAA-TTCATCTTTTATGGC-3
URH1colifor	5'-GGATTGTCATATGGATTGTGG-3'
URH1colirev	5'-CTCATGAGCTCTGGCTTCATC-3'

Primers for Overexpression constructs

Oex_AtURH1for	5´-TTTCTCGAGATGGATTGTGGTATGGAG-3´
Oex_AtURH1rev	5´-TTTAAGCTTTTATGGCTTCATCAGCTTTGC-3´
Oex_OsURH1for	5´-GAGTTAGTTTTGGCTCGAGTTGTGATG-3´
Oex_OsURH1rev	5´-CACAAGAAATAGGTCTAGAATCGCTCATGG-3´

Primers for URH1-GUS construct

URH1gusfor	5'-ATAACCACCCTT-GTCTAGAATCATTTTACG-3'
URH1gusrev	5'-ACAATCCATCCCGGGAAAGA-TAATTAAACG-3'

Primers for GFP-At URH1 construct

GFP_AtURH1for	5′-CTGAGAGTTTCTAGAGGTTTTAAT-3′
GFP_AtURH1rev	5′-AAACATCATCTCGAGTGGCTTCAT-3′

Primers used for At URH amiRNA

Primer A	5′-CACCCTGCAAGGCGATTAAGTTGGGTAAC-3′
Primer B	5′-GCGGATAACAATTTCACACAGGAAACAG-3′
I miR-s URH1	5′-gaTCACACAAGAGTAAAGCGCTGtctctcttttgtattcc-3′
II miR-a URH1	5′-gaCAGCGCTTTACTCTTGTGTGAtcaaagagaatcaatga-3′
III miR*s URH1	5′-gaCAACGCTTTACTCATGTGTGTtcacaggtcgtgatatg-3′
IV miR*a URH1	5′-gaACACACATGAGTAAAGCGTTGtctacatatatattcct-3′