

Figure S1. In vivo kinetic properties of nucleoside hydrolase activity in Arabidopsis leaf material. Data represent means of two biological replicates. Kinetic constants were determined from Lineweaver-Burk plots as follows: uridine ribohydrolase $K_M = 202,5 \mu\text{M}$, inosine ribohydrolase $K_M = 206,8 \mu\text{M}$, xanthosine ribohydrolase $K_M = 57,9 \mu\text{M}$.

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# -# -
RihA -----MALP-ILLDCDPGHDDAIAIVLALASPELDVKAITSSAGNQ 40
RihB -----MEKRKIIIDCDPGHDDAIAIMMAAKHPAIDLLGITIVAGNQ 41
RihC -----MRLP-IFLDTPGIDDAVAIAAAAFAPPELDLQMLTTVAGNV 40
URH1 MDCGMENCGGISNGDVLGKHEKLIIDTDPGIDDSMAILMAFQTPELEILGLTTVFGNV 59
URH2 -----MAIGDRKKIIDTDPGIDDAMAI FVALNSPEVDVIGLTTIFGNV 44
      : : * * * * * : * * * : : * * *

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RihA TPEKTLRNVLRMLTLLNRTDIPVAGGAVKP--LMRELIADNVHGESGLDGPALPEPTF 97
RihB TLDKTLINGLNVQKLEINVPVYAGMPQP--IMRQQIVADNIHGETGLDGPVFEPLTR 97
RihC SVEKTRRNALQLLHFW-NAEIPLAQGAAVP--LVRAPRDAASVHGESGMAGYDFVEHNR 96
URH1 STQDATRNALLLCEIAGFPDVPVAEGSSEP-LKGGIPRVADFVHGKNGLDVSLPPPSR 117
URH2 YTTLATRNALHLLLEVAGRTDIPVAEGTHKTFLNNTKLRADFVHGKDGGLGNQNFPPPK 103
      : * * : : * : * * * * * : * * * * * : : :

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RihA APQNCTAVELMAKTLRESAEPVTIVSTGPQTNVALLLNHPHLSKRIARIVIMGGAMGL 155
RihB QAESTHAVKYIIDTLMASDGDITLVPVGPLSNIAVAMRMQPAILPKIREIVLMGGAYGT 155
RihC KPLGIAPFLAIRDALMRAPEPVTLVAIGPLTNIALLSQCPECKPYIRRLVIMGGSAGR 154
URH1 KKSEKSAAEFLDEKVEEYYPGEVITLALGPLTNLALAIKRDSSFASKVKKIVILGGAFFS 176
URH2 KPIEKSGPEFLVEQAKLCPGEITVVALGPLTNLALAVQLDPEFSKNVQIIVLGGFAV 162
      : : : * * * * * : * * * * * : : * * * * *

- -+
RihA -GNWTPAAEFNIYVDPEAAEIVFQSGIPVVMAGLDVTHKAQIHVEDTERFRAIGNPVST 214
RihB -GNFTPSAEFNIFADPEAARVFTSGVPLVMMGLDLTNQTVCTPDVIARMERAGGPAGE 214
RihC -GNCTPNAEFNIAADPEAAACVFRSGIEIVMCGLDVTNQAILTDPYLSLTPQLN-RTGK 212
URH1 LGNVNPAAEANIYGDPEAADVFTSGADITVVGINITTTQLKLSDDDLLELGNCKGKHSK 235
URH2 NGNVNPAEANIIFGDPEAADIVFTCGADI IAVGINVTHQVIMTADDKDLASSKGKLAQ 221
      * * * * * * * * * * * * * * * * * * * * * * * * * * * *

+ + +#
RihA IVAELLDFFLEYHKDEKWFVGGAPLHDPCTIAWLLKPELFTSVERWVG VETQKGYTQGM 273
RihB LFSDIMNFTLKTQFENY-GLAGGPVHDATCIGYLINPDGIKTQEMYVEVDVNSGPCYGR 272
RihC MLHALFSHYRSGSMQS----GLRMHDLCAIAWLVRPDLFTLKPCFVAVETQGEFTSGT 266
URH1 LISDMCKFYRDWHVKS-DGVYGVYLHDPVSVFVAVRVDLFTYKKGVVRVETQG-ICVGH 292
URH2 YLCKILDVYYDYHLTAY-EIKGVYLHDPATILAAFLPSLFTYTEGVARVQTSG-ITRGL 278
      . : . . * * * * * : * * * : . * * : . * * * * * *

RihA TVVDYYLGTGNKPN-----ATVMVDVDRQGFVDLLADRLKFYA--- 311
RihB TVCDELGVLGKPN-----TKVGITIDTDWFWGLVEECVRGYIKTH 313
RihC TVVDIDGCLGKPN-----VQVALDLVKGQFQWVAEVLALAS--- 304
URH1 TLMDQGLKRWNGSNPWGYSPTISVAWTVDVEGVLEYVKAKLMKP---- 336
URH2 TLLYNNLKRFEANEWSDKPTVKVAVTVDAPAVVKLIMDRLMES---- 322
      * : : * * * * * * * * * * * * * * *

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Figure S2. Multiple sequence alignment of three ribonucleoside hydrolases RihA, RihB, and RihC of *Escherichia coli* and the two URH isoforms of the nonredundant proteome of *Arabidopsis* using Clustal 2.0.12. Symbols below the alignment are as follows: *, identical residue; :, conserved substitution; ., semiconserved substitution. Symbols above the alignment indicate conserved residues involved in binding: #, Ca²⁺ binding; -, ribose binding; +, base binding.

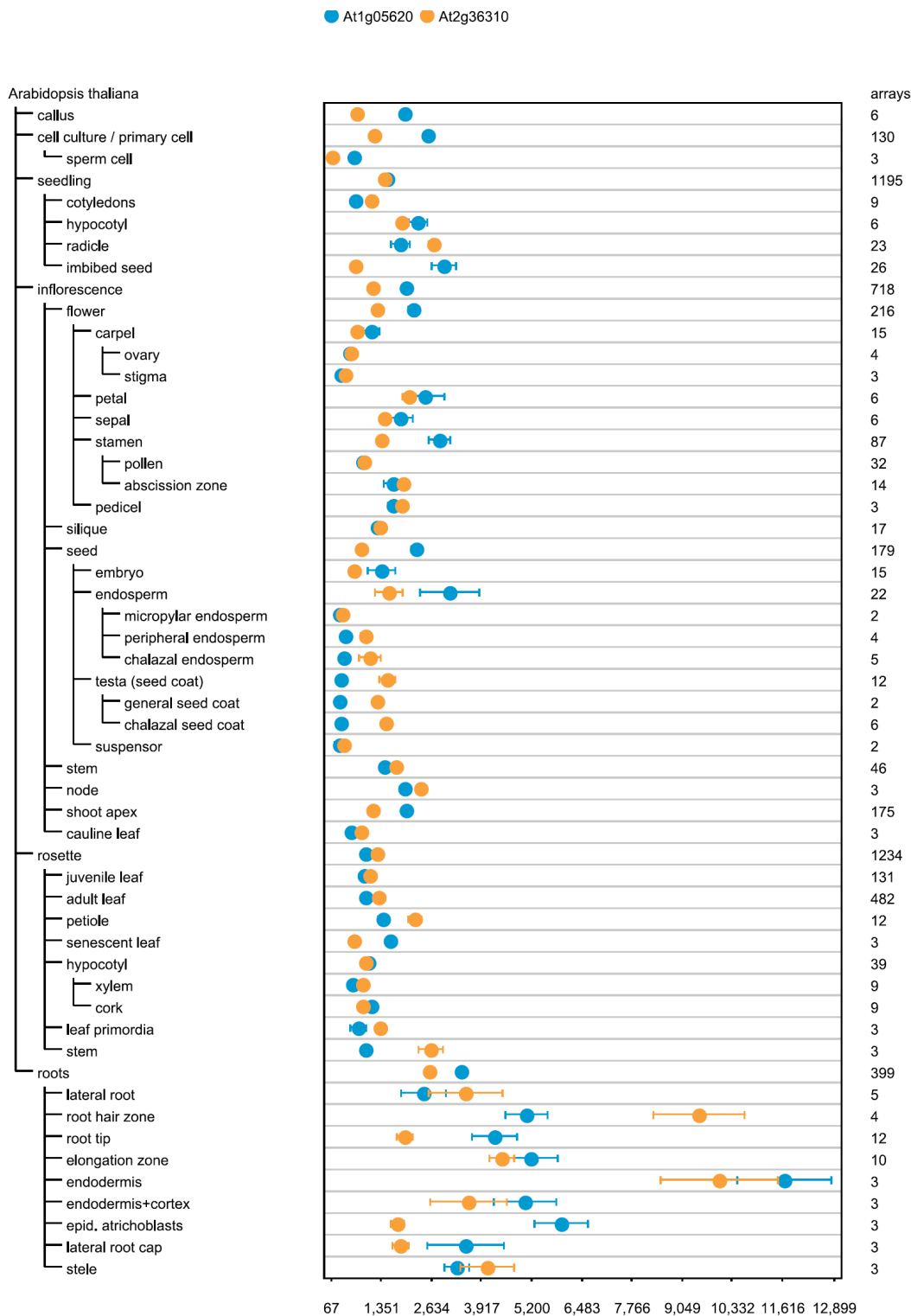


Figure S3. RNA expression of *URH1* (At2g36310) and *URH2* (At1g5620) according to Genevestigator (Zimmermann P, Hirsch-Hoffmann M, Hennig L, Gruissem W. (2004) GENVESTIGATOR: Arabidopsis microarray database and analysis toolbox. Plant Physiol 136: 2621-2632).

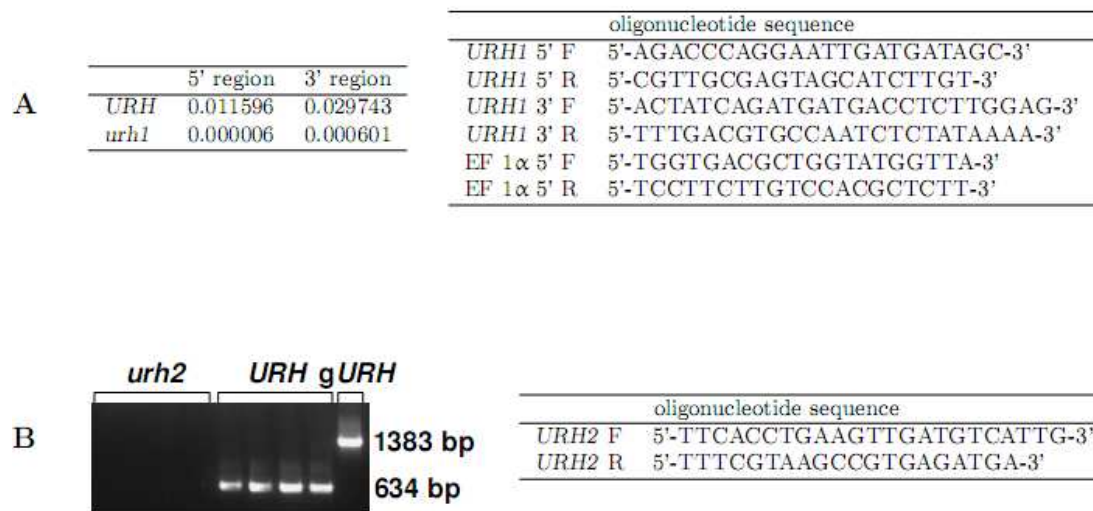


Figure S4. Expression of *URH* genes in *urh* mutants. A, Relative Expression of *URH1* in *urh1* mutant and wildtype Arabidopsis. The transcript levels were determined by quantitative realtime RT-PCR using two gene specific primer pairs in the 3' or 5' part of the transcript. The transcript levels were calculated relative to elongation factor 1 α . Data show means of four biological replicates. B, RT-PCR expression analysis of *URH2* transcript (634bp fragment) in *urh2* mutant and wildtype Arabidopsis (*URH*). RNA was extracted from 4 biological replicates of *urh2* mutant and wildtype Arabidopsis and processed as described in the Methods section. Genomic DNA of wildtype Arabidopsis (*gURH*) was used as control. All plants used in this analysis were six weeks old.

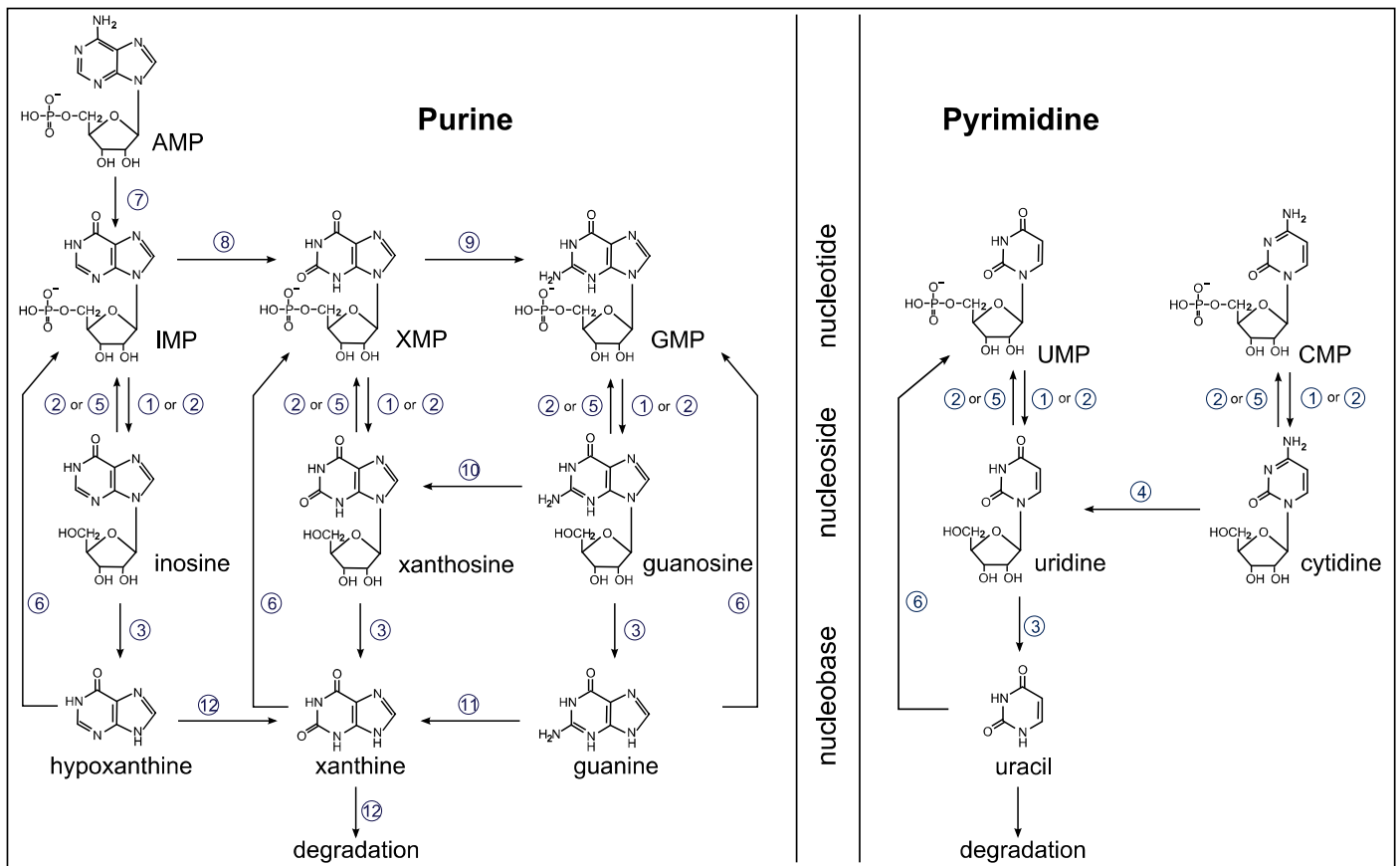


Figure S5. Nucleoside and nucleobase salvage pathways for purines and pyrimidines. Enzymes are numbered and general enzyme nomenclature is as follows: 1) NMP specific 5' nucleotidase; 2) nucleoside phosphotransferase; 3) **nucleoside hydrolase**; 4) cytidine deaminase; 5) nucleoside kinase; 6) nucleobase phosphoribosyl transferase; 7) AMP deaminase; 8) IMP dehydrogenase; 9) GMP synthase; 10) guanosine deaminase; 11) guanine deaminase; 12) xanthine dehydrogenase

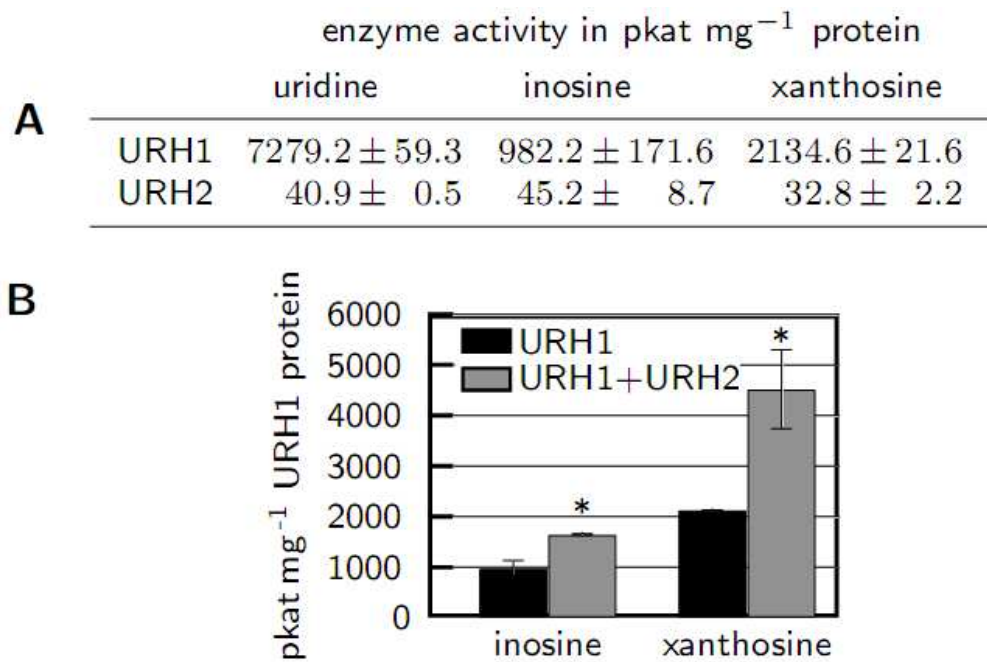


Figure S6. Nucleoside hydrolase activity of recombinant URH proteins. A, Total activity of nucleoside to nucleobase conversion after over-expression in *E. coli* and non-denaturing purification of recombinant URH1 and URH2 proteins from the soluble extracts. Activity measurements were performed from desalted eluate fractions with the respective nucleoside at 200 μ M. Data are expressed in pkat per mg protein after Ni-NTA agarose purification and show means \pm SD ($n = 3$). B, Total activity of purified recombinant URH1 and a mixture of equal amounts of recombinant URH1 and URH2 from desalted eluate fractions. Data show means \pm SD ($n = 3$). Significant differences ($P < 0.05$) between URH1 and URH1 + URH2 using unpaired two-tailed t -tests are marked with *.