**Supplemental Figure S1.** Structural features of AtDIR6.

A, AtDIR6 dimer of the asymmetric unit showing the joint β-sheet between both monomers. B, Representation of the electron density around C-terminal Thr33 of monomer B. C, Structure of the Asn59 paucimannose moieties. D, Structure of the paucimannose sugars connected to Asn123. E, Structure of the paucimannose moiety at Asn59 of monomer B. NAG: *N*-acetyl-glucosamine, FUC: α-Fucose, XYP: β-*D*-Xylopyranose, αMA, α-*D*-Mannose, βMA, β-*D*-Mannose. Density, refined without the moiety atoms, is shown in blue at 1σ. F, Overlay of monomer A (gray) and monomer B (green) showing differences in the active sites. Important monomer B residues that differ from those in monomer A are shown in purple. Red arrows indicate movement of residues.
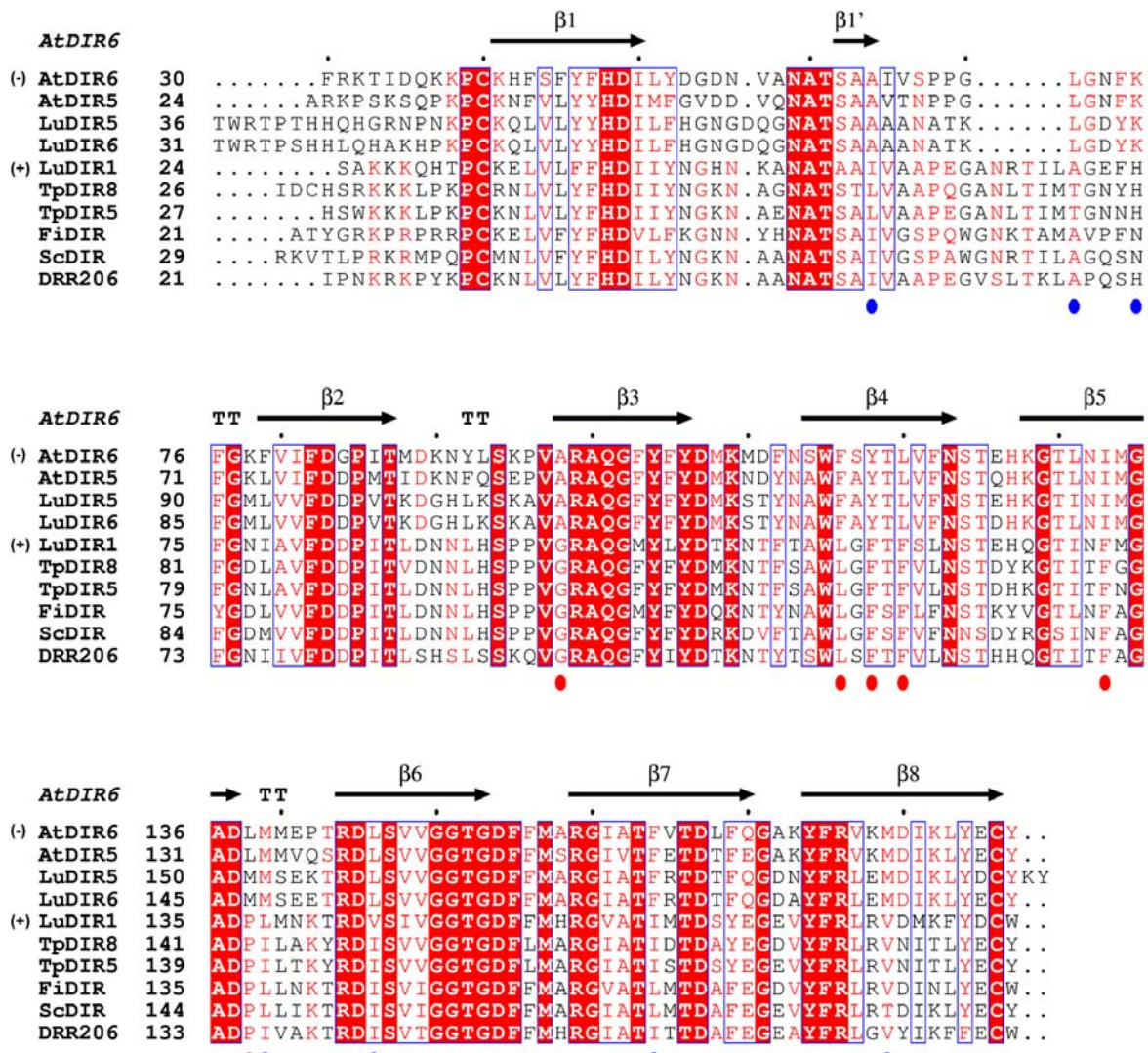
**Supplemental Figure S2.** Sequence of AtDIR6 aligned with those of other (-)- and (+)-DIRs.

Only full-length sequences are shown. The alignment was generated with ClustalΩ and illustrated using the ESPript webserver (espript.ibcp.fr/). Strictly conserved residues are boxed in red. Those that are conserved in (+)- and (-)-DIRs are highlighted by purple and blue dots, respectively. Red dots indicate residues that are differentially conserved in (+)- and (-)-DIRs. Secondary structure elements of AtDIR6 are shown above the alignment.
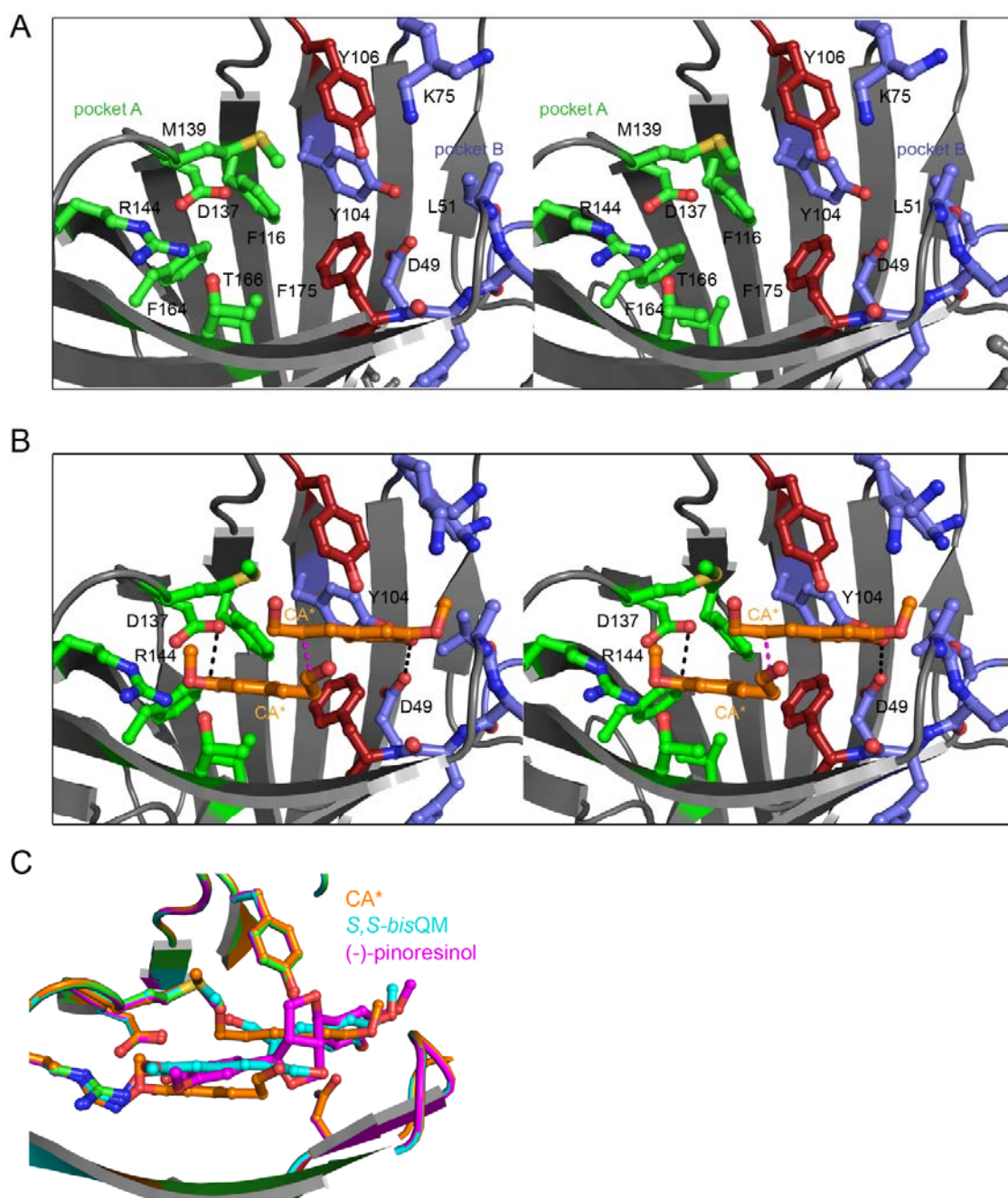
Supplemental Figure S3. Stereo view of Fig. 3 panels *C* and *D*.

A, Stereo view of the active site showing important residues of pocket A in green, pocket B in blue, and Tyr106 and Phe175 separating the two pockets in red. D, Stereo view of the potential binding mode of two CA• substrate radicals supported by energy minimization of the manually placed ligands; color code as in (A).
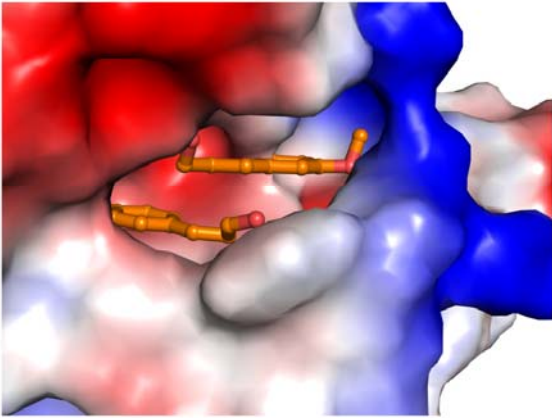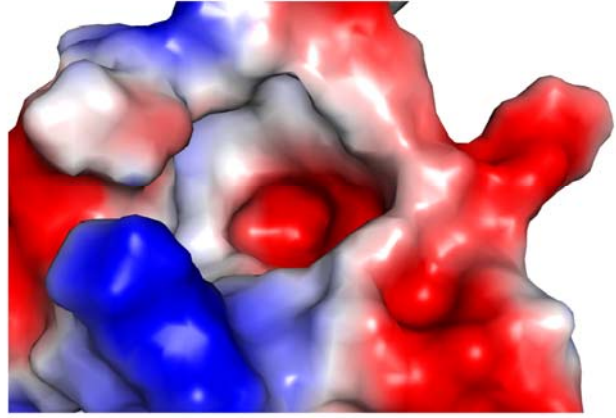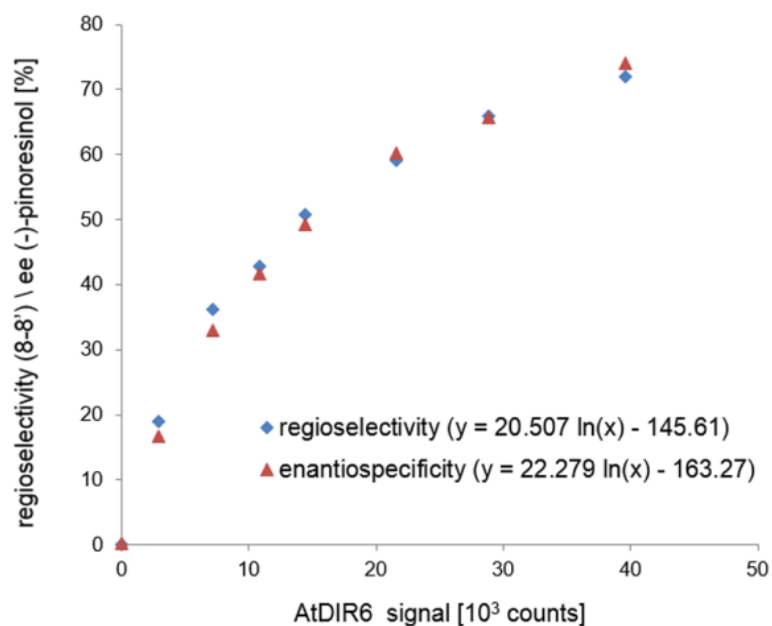
Supplemental Figure S4. Surface representations of AtDIR6 and DRR206 binding cavities.

A, Surface representation of AtDIR6 showing the two manually modelled substrate molecules in the two-lobed binding cavity. B, Surface representation of PsDRR206 with view onto the opening of the active site. Red: negative surface charge, blue: positive.

Supplemental Figure S5. Regio- and enantio-selectivity are tightly linked.

Regioselectivity was analyzed by RP-HPLC as relative increase in pinoresinol formation in presence of AtDIR6 as compared to the undirected control (blue diamonds). Enantioselectivity was analyzed by chiral HPLC and is expressed as enantiomeric excess of (-)- over (+)-pinoresinol (red triangles). Regio- and enantioselectivity show the same increase with increasing amounts of AtDIR6 in the assay. AtDIR6 was quantified by western blot analysis as detailed in the Materials and Methods section.

Table S1. Primers for site-directed mutagenesis. Primer sequences are given in 5' to 3' orientation. Mutated codons are highlighted in bold face and underlined. All primers were obtained from Eurofins Genomics (Ebersberg, Germany).

mutant	target sequence	forward primer	reverse primer
F47A	CACTTCTCGTTCTAT<u>TTC</u>CATGACATCCTCTACGATGG	CACTTCTCGTTCTAT**GCC**CATGACATCCTCTACGATGG	CCATCGTAGAGGATGTCATG**GGC**ATAGAACGAGAAGTG
D49A	CTCGTTCTATTTCCAT<u>GAC</u>ATCCTCTACGATGG	CTCGTTCTATTTCCAT**GCC**ATCCTCTACGATGG	CCATCGTAGAGGAT**GGC**ATGGAAATAGAACGAG
D49N	CTCGTTCTATTTCCAT<u>GAC</u>ATCCTCTACGATGG	CTCGTTCTATTTCCAT**AAC**ATCCTCTACGATGG	CCATCGTAGAGGAT**GTT**ATGGAAATAGAACGAG
K75N	GGACTAGGAAACTTC<u>AAG</u>TTCGGTAAGTTTG	GGACTAGGAAACTTC**AAT**TTCGGTAAGTTTG	CAAACTTACCGA**AAT**TGAAGTTTCCTAGTCC
F82A	GGTAAGTTTGTGATC<u>TTT</u>GATGGGCCCATAACAATGG	GGTAAGTTTGTGATC**GCT**GATGGGCCCATAACAATGG	CCATTGTTATGGGCCCATC**AGC**GATCACAAACTTACC
Y104F	CGCACAAGGCTTC<u>TAT</u>TTCTATGAC	CGCACAAGGCTTC**TTT**TTCTATGAC	GTCATAGAA**AAA**GAAGCCTTGTGCG
Y106F	GGCTTCTATTTC<u>TAT</u>GACATGAAGATGG	GGCTTCTATTTC**TTT**GACATGAAGATGG	CCATCTTCATGTC**AAA**GAAATAGAAGC
S114A	GAAGATGGACTTCAAT<u>TCG</u>TGGTTTTCC	GAAGATGGACTTCAAT**GCG**TGGTTTTCC	GGAAAACCA**CGC**ATTGAAGTCCATCTTC
F116A	GACTTCAATTCGTGG<u>TTT</u>TCCTACACGTTGG	GACTTCAATTCGTGG**GCT**TCCTACACGTTGG	CCAACGTGTAGGA**AGC**CCACGAATTGAAGTC
Y118A	GGACTTCAATTCGTGGTTTTCC<u>CTAC</u>ACGTTGGTGTTTAACTC	GGACTTCAATTCGTGGTTTTCC**GCC**ACGTTGGTGTTTAACTC	GAGTTAAACACCAACGT**GGC**GGAAAACCACGAATTGAAGTCC
D137A	CATAATGGGTGCT<u>GAT</u>TTGATGATGGAGCC	CATAATGGGTGCT**GCT**TTGATGATGGAGCC	GGCTCCATCATCAA**AGC**AGCACCCATTATG
D137N	CATAATGGGTGCT<u>GAT</u>TTGATGATGGAGCC	CATAATGGGTGCT**AAT**TTGATGATGGAGCC	GGCTCCATCATCAA**ATT**AGCACCCATTATG
M139A	GGGTGCTGATTTG<u>ATG</u>ATGGAGCCAACAAG	GGGTGCTGATTTG**GCG**ATGGAGCCAACAAG	CTTGTTGGCTCCAT**CGC**CAAATCAGCACCC

M140A	GGGTGCTGATTTGATGAT GG AGCCAACAAG	GGGTGCTGATTTGATG**GCG**GA GCCAACAAG	CTTGTTGGCTC**CGC**CATCAAATC AGCACCC
R144M	GGAGCCAACA<u>AGA</u>GATCTAT CGG	GGAGCCAACA**ATG**GATCTATC GG	CCGATAGATC**CAT**TGTTGGCTC C
F164L	GGCTCGTGGGATCGCTACC<u>T TC</u>GTGACTGATTTATTTC	GGCTCGTGGGATCGCTACC**TT G**GTGACTGATTTATTTC	GAAATAAATCAGTCAC**CAA**GGT AGCGATCCCACGAGCC
T166V	CGCTACCTTCGTG<u>ACT</u>GATTT ATTTCAAGG	CGCTACCTTCGTG**GTT**GATTTA TTTCAAGG	CCTTGAAATAAATC**AAC**CACGA AGGTAGCG
F169A	CGTGACTGATTTA<u>TTT</u>CAAGG GGCTAAG	CGTGACTGATTTA**GCT**CAAGG GGCTAAG	CTTAGCCCCTTG**AGC**TAAATCA GTCACG
A172D	GACTGATTTATTTCAAGGGG<u>G CT</u>AAGTATTTCCGAG	GACTGATTTATTTCAAGGG**GA T**AAGTATTTCCGAG	CTCGGAAATACTT**ATC**CCCTTGA AATAAATCAGTC
F175W	GGGGCTAAGTAT<u>TTC</u>CGAGT TAAGATGG	GGGGCTAAGTAT**TGG**CGAGTT AAGATGG	CCATCTTAACTCG**CCA**ATACTTA GCCCC
V177W	GTATTTCCGA<u>GTT</u>AAGATGG ATATTAAG	GTATTTCCGA**TGG**AAGATGGA TATTAAGC	GCTTAATATCCATCTT**CCA**TCGG AAATAC
M179A	GTATTTCCGAGTTAAG<u>ATG</u>G ATATTAAGCTCTATG	GTATTTCCGAGTTAAG**GCG**GA TATTAAGCTCTATG	CATAGAGCTTAATATC**CGC**CTTA ACTCGGAAATAC
PLL-motiv	GGGTGCTGAT<u>TTGATGATG</u>G AGCCAACAAG	GGGTGCTGAT**CCGTTGTTG**GA GCCAACAAG	CTTGTTGGCTC**CAACAACGG**AT CAGCACCC