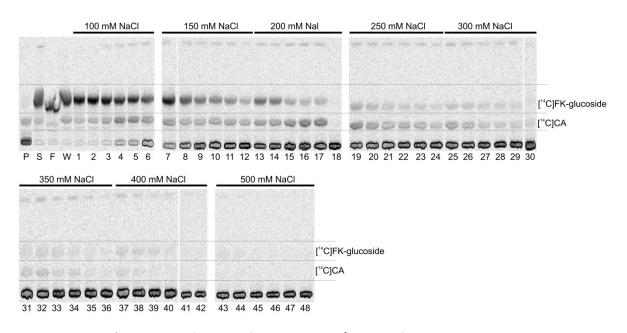
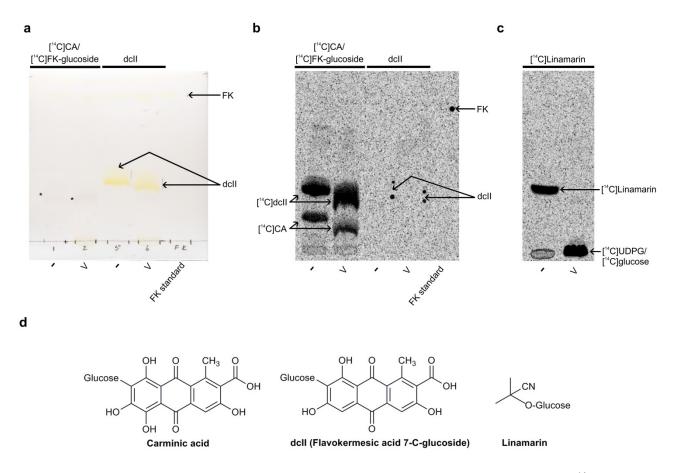


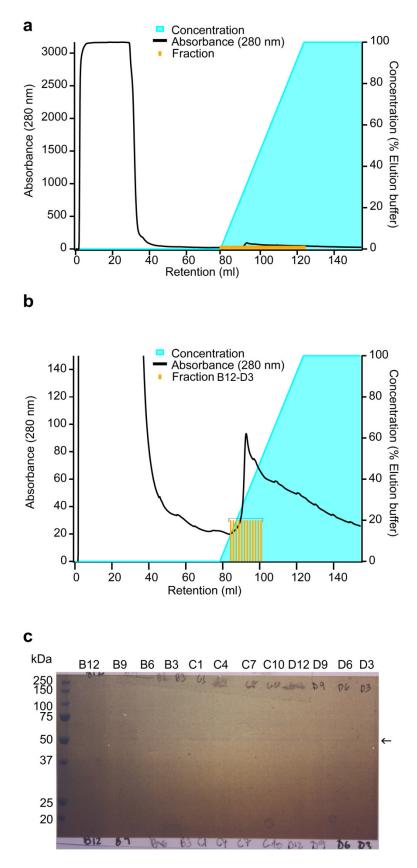
b



**Supplementary Figure 1** | **Partial purification of a** *D. coccus* **FK/KA-specific glucosylation activity** *D. coccus* microsomes were solubilized using reduced Triton X-100 and fractionated by anion-exchange chromatography using Q-Sepharose. Proteins were eluted using a discontinues NaCl gradient from 100 mM-500 mM. Forty-eight eluted fractions were collected together with a pellet (P: Reduced Triton X-100 insoluble protein fraction), a solubilized (S: Reduced Triton X-100 soluble protein fraction), a flow-through (F: Proteins that did not bind to the Q-Sepharose) and a wash fraction (W: Proteins washed off the column with 50 mM NaCl). All protein fractions were tested for glucosylation activity *in vitro* using the flavokermesic acid aglucone and the [<sup>14</sup>C]UDP-glucose donor. **a** Coomassie-stained SDS gels of separated protein fractions. **b** TLC-separated [<sup>14</sup>C] products formed *in vitro* and viewed by phosphorimaging. FK: flavokermesic acid; CA: carminic acid; Asterisk indicates the protein fraction that was analyzed further by proteomics (Fig. 3). The positions of [<sup>14</sup>C]FK-glucoside and [<sup>14</sup>C]CA are indicated. The *in vitro* formation of [<sup>14</sup>C]CA was ascribed to the conversion of kermesic acid that still was bound to the *D. coccus* microsomes despite numerous wash steps during preparation.



**Supplementary Figure 2** | **Enzymatic verification of** *in vitro*-produced dcll and carminic acid [<sup>14</sup>C] products, formed *in vitro* by incubating *D. coccus* microsomes with the flavokermesic acid aglucone and the [<sup>14</sup>C]UDP-glucose donor, were isolated and either non-treated (-) or treated with viscozyme (V). Non-radioactive dcll and [<sup>14</sup>C]Linamarin, representing a C-glucoside and an O-glucoside, respectively were used as controls for the viscozyme -treatment. **a** TLC plate of separated radioactive and non-radioactive compounds after treatment without or with viscozyme. **b** TLC plate from panel (**a**) viewed by phosphorimaging. **c** [<sup>14</sup>C]Linamarin treated without or with viscozyme followed by TLC-separation and phosphorimaging. **d** Chemical structures of the glucosides treated without or with viscozyme. Asterisk indicates carminic acid that still was bound to the *D. coccus* microsomes despite numerous wash steps during preparation. Likewise, the *in vitro* formation of [<sup>14</sup>C]CA was ascribed to the conversion of kermesic acid that also still was associated to the *D. coccus* microsomes. FK: flavokermesic acid; CA: carminic acid; [<sup>14</sup>C]UDPG: [<sup>14</sup>C]UDP-glucose. Note the slight aberrant product migration of viscozyme-treated samples caused by the increased viscosity from the viscozyme solution.

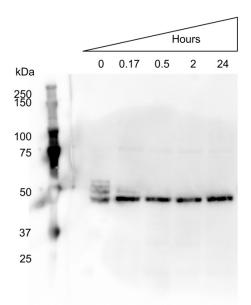


**Supplementary Figure 3** | **Affinity purification of the DcUGT2-Strep protein** Yeast microsomes containing DcUGT2-Strep were solubilized with reduced Triton X-100 and DcUGT2 affinity purified by its Strep-tag II using a *Strep*-Tactin column. **a** The elution profile of DcUGT2-Strep as monitored by the protein absorbance at 280 nm. **b** The elution profile of DcUGT2-Strep as monitored by the protein absorbance at 280 nm. **c** The elution profile of DcUGT2-Strep as monitored at 280 nm. The scale for absorbance at 280 nm has been adjusted compared to (**a**) to easily distinguish the fractions that were analyzed further. **c** Eluted protein fractions corresponding to the fractions indicated in (**b**) were separated on an SDS gel followed by Coomassie-staining. The arrow points to the position of DcUGT2-Strep.

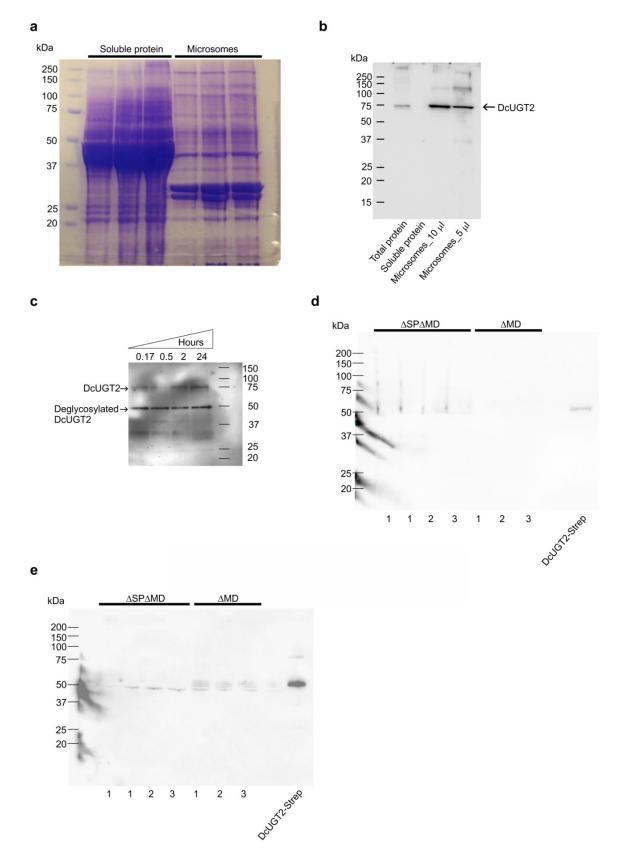
DC_UGT1MHKYLKTSL NIFLFLPVLHSARILGIFPVHGRSL HSINOPTVKF ARGKNVTUSSHFTSKISLFNYKEIVIERGTS- DC_UGT2MEFRLLIALFSV-LMSTSNGAEILGIFPVHGRSL HSINOPTVKF ARGKNVTVFSSFPGKKPVPNYKEIVIERGTS- DC_UGT4	78 77 80 75 79 77 77 74 89 73 79 85 89 85
Dc_UGT1 - SF I DS I SV DDAH - LFNGVQ	155 152 154 154 154 154 154 152 163 152 160 168 166 169
DC_UGTI S LT PATS G T G N D L M I G N P G N P A YW - P V I T S YT - T O M G F G G U Y N T - YH F L R YY F G F Y S L - N N Y M N E VAK K Y F N S	240 238 242 240 241 240 239 243 248 239 244 251 249 254
DC_UGT2 L L FUNT HES VDMPYPLVONCE GG HVKEPG	332 336 335 338 338 338 346 338 346 339 345 343 348
De, UGT1 VN WA DRW YPORDILECKNV FAFISING IFGTMEALYTATPV IIPFLFD GEOGANILVEKGWAIHISYD TLEDKLUSA NEI MNTKYFRAALSGU De, UGT2 NV URKKYFPONDILGRW FPONDILGBW FWG VFSICSAV FGVPN UD PFGYD OFYR ILSFYKEGV VULLOVN DLWK WANN NT VN KYFRAALSGU De, UGT4 KNVL JGTW FPONDILGHPN VK JFITHGGALGUNSAV FGVPN UD PFGYD OFT WIKALKAR GEULOVN DISGUV EKK OFLINNKYFRAALSGU De, UGT5 DKI FISKW MPORDILAHPN VK JFITHGGALGUNSAV FGVPV UD PFGYD OFT WIKALKAR GAUSU De, UGT3 DKI FISKW MPORDILAHPN VK JFITHGGALGUNSAV FGVPV UD PFGYD OFT WIKALKAR GAUSU De, UGT3 DKI FISKW MPORDILAHPN VK JFITHGGALGUNSAV FGVPV UD PFGYD OFT WIKALKAR GAUSU DE, UGT3D, DKI FISKW MPORDILAHPK VK JFITHGGALGUNSAV FGVPV UD PFGYD OFT WIKALKAR GAUSU DE, UGT3D, DKI FISKW MPORDILAHPK VK JFITHGGALGUNSAV FGVPV UD PFGYD OFT WIKALKAR GAUSU DE, UGT3D, DKI FISKW FPORDIFALD VV UFTHGGLUST FEALHOUS VFGVPV UD FFGYD OFT WIKALKAR GAUSU DE, UGT3D, DKI FISKW FPORDIFALD VV UFTHGGLUST FEALHOUS VFGVPV US FFGYD OFT WIKALKAR GAUSU DE, UGT3D, DKI FISKW FPORDIFALD VV UFTHGGLUST FEALHOUS VFGVPV UD FFGYD OFT WIKALKAR GAUSU DM, UGT3S DKI FISKW FPORDILAHPN VK UFTHGGLUST FEALHOUS VFGVPV UD FFGYD OFT WIKALKAR GAUSU DM, UGT3S DKI FISKW FPORDILAHPN VK UFTHGGLUST FEALHOUS VFGVPV UD FFGYD OFT WIKALAGAG DM, UGT3S DKI FISKW FPORDILAHPN VK UFTHGGLUST FEALHOUS VFGVPV UD FFGYD OFT WIKALAGAG DM, UGT3S DKI FISKW FPORDILAHPN VK UFTHGGLUST FEALHOUS VFGVPV UD FFGYD OFT WIKALAGAG DM, UGT3S DKI FISKW FPORDILAHPN VK UFTHGGLUST FEALFOUND VFGVPV UD FFGYD OFT WIKALAGAG DM, UGT3S DKI FISKW FPORDILAHPN VK UFTHGGLUST FEALFOUND VFGVPV UD FFGYD OFL WY KAAAGAG DM, UGT3S DKI FISKW FPORDILAHPN VK UFTHGGLUST FEALFOUND VFGVPV UD FFGYD OFL WY KAAGAG GUD WIKAKAGAG DM, UGT3S DKI FISKW FPORDILAHPN VK UFTHGGLUST FEALFOUND VFGVPV UD FFGYD OFL WY KAAAGAG DM, UGT3S DKI FISKW FPORDILAHPN VK UFTHGGLUST FEALFOUND VFGVPW UD FFGYD OFL WY KAAGAG DKU HKAAGAG DKU HKAKKAKAKAKAKAKAKAKAKAKAKAKAKAKAKAKAKAK	134 132 135 135 138 134 138 134 138 138 148 148 148
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LX2-RGH-X3-VI

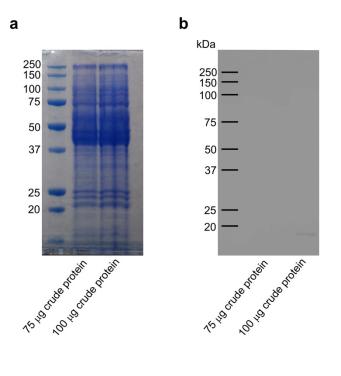
**Supplementary Figure 4** | **Alignment of UGT amino acid sequences** Black and grey indicate identical and similar amino acids, respectively. Multiple sequence alignment was performed with MUSCLE<sup>1</sup> using the MEGA6 software. The alignment is composed of: DcUGT1, DcUGT2, DcUGT4, DcUGT5, DcUGT8 and the partial DcUGT23 from *D. coccus*; the predicted ApUGT2B10 from *A. pisum* (accession no.: XP001948117); BmUGT1 (accession no.: AF324465) from *Bombyx mori*; DmUGT35a (accession no.: AF116555) and DmUGT35b (accession no.: AF116554) from *Drosophila melanogaster*; ZfUGT33A1 (accession no.: GQ915324) from *Zygaena filipendulae*; HsUGT1A6 (accession no.: NM001072), HsUGT1A10 (accession no.: U89508) and HsUGT2B7 (accession no.: NM001074) from *Homo sapiens*. The motifs, "LX2-RGH-X3-VL", "DBR1" and "DBR<sub>2</sub>", are indicated as described in<sup>2,3</sup>. The predicted transmembrane helix (TMH) is given. Important catalytic residues are indicated with an asterisk according to<sup>2</sup>.



**Supplementary Figure 5** | **Deglycosylation of truncated DcUGT2** $\Delta$ **MD-Strep protein** Yeast microsomes containing DcUGT2 $\Delta$ MD-Strep were deglycosylated with glycanases for different periods of time: 0 min (0); 10 min (0.17); 30 min (0.5); 2 h (2) and 24 h (24). Proteins were separated on an SDS gel followed by western blotting using an anti-Strep antibody. Note that the anti-strep antibody reacts with the marker protein bands.



**Supplementary Figure 6** | **Uncropped gel or blot images** Images displayed in cropped versions in the article. **a** Uncropped image of Fig. 2a: Coomassie-stained SDS gel of separated microsomal/soluble protein from *D. coccus*. **b** Uncropped image of Fig. 8a: Non-treated total protein, soluble protein and microsomes from *D. coccus*. Two different amounts of microsomes were loaded. **c** Uncropped image of Fig. 8b: *D. coccus* microsomes treated with glycanases for 10 min (0.17); 30 min (0.5); 2 h (2) and 24 h (24). **d** Uncropped image of Fig. 9a: Soluble protein from three individual *S. cerevisiae* transformants expressing either  $\Delta SP$ -DcUGT2 $\Delta$ MD-Strep ( $\Delta$ SP $\Delta$ MD) or DcUGT2 $\Delta$ MD-Strep ( $\Delta$ MD) were analyzed by western blotting. Transformant 1 expressing  $\Delta SP$ -DcUGT2 $\Delta$ MD-Strep was loaded twice. Yeast microsomes, containing the full-length DcUGT2-Strep, were used as an additional size marker control. **e** Uncropped image of Fig. 9b: Microsomal protein from three individual *S. cerevisiae* transformants expressing either  $\Delta SP$ -DcUGT2 $\Delta$ MD-Strep ( $\Delta$ MD) were analyzed by western blotting. Transformant 1 expressing  $\Delta SP$ -DcUGT2 $\Delta$ MD-Strep ( $\Delta$ MD) were analyzed by western blotting. Strep ( $\Delta$ SP $\Delta$ MD) or DcUGT2 $\Delta$ MD-Strep ( $\Delta$ MD) were analyzed by western blotting. Transformant 1 expressing an additional size marker control. **e** Uncropped image of Fig. 9b: Microsomal protein from three individual *S. cerevisiae* transformants expressing either  $\Delta SP$ -DcUGT2 $\Delta$ MD-Strep ( $\Delta$ MD) were analyzed by western blotting. Transformant 1 expressing  $\Delta SP$ -DcUGT2 $\Delta$ MD-Strep ( $\Delta$ MD) were analyzed by western blotting. Transformant 1 expressing  $\Delta SP$ -DcUGT2 $\Delta$ MD-Strep ( $\Delta$ MD) were analyzed by western blotting. Transformant 1 expressing  $\Delta SP$ -DcUGT2 $\Delta$ MD-Strep ( $\Delta$ MD) were analyzed by western blotting. Transformant 1 expressing  $\Delta SP$ -DcUGT2 $\Delta$ MD-Strep was loaded twice. Yeast microsomes, containing the full-length DcUGT2-Strep, were used as an additional size marker control.



**Supplementary Figure 7** |**Rabbit pre-immune serum test** The pre-immune serum of the rabbit, used to produce anti-DcUGT2 antibodies, was tested for cross-reactivity towards a crude *D. coccus* protein extract. The test was carried out on two amounts (75 μg and 100 μg) of crude protein. **a** Coomassie-stained SDS gel of separated crude *D. coccus* proteins. **b** Western blot analysis of crude *D. coccus* proteins probed with rabbit pre-immune serum (1:1000 dilution), followed by incubation with secondary HRP-conjugated antibodies and chemiluminescence detection.

## **Supplementary References**

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- 3 Senay, C. *et al.* The importance of cysteine 126 in the human liver UDP-glucuronosyltransferase UGT1A6. *Biochim. Biophys. Acta, Protein Struct. Mol. Enzymol.* **1597**, 90-96 (2002).