SUPPORTING INFORMATION

A two-phase model for the non-processive biosynthesis of homogalacturonan polysaccharides by the GAUT1:GAUT7 complex

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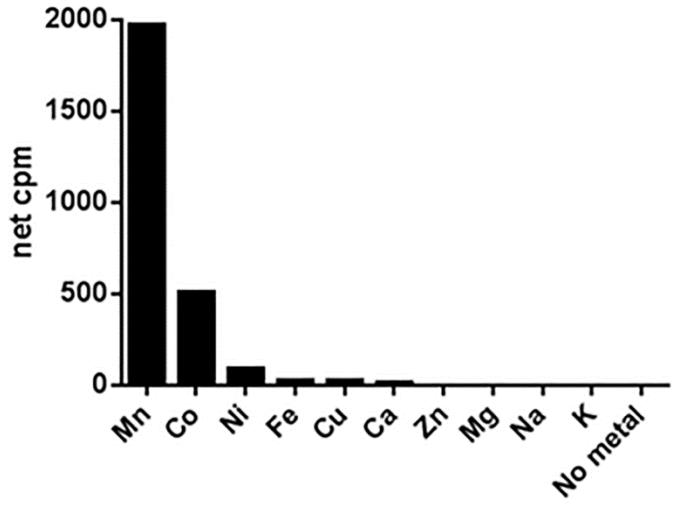

Figure S1. Effect of divalent and monovalent metal ions on the acceptor-dependent HG:GalAT activity of the GAUT1:GAUT7 complex . Metal-depleted GAUT1:GAUT7 was generated by overnight dialysis against HEPES, pH 7.2 buffer containing Chelex-100. After addition of the indicated metals at 0.25 mM in the reaction buffer, GAUT1:GAUT7 was assayed for activity in 5 min reactions containing 100 nM GAUT1:GAUT7, 5 µM UDP-[^{14}C]GalA, 1 mM total UDP-GalA, HEPES buffer pH 7.2, and 0.05% BSA. The activity of all metals other than Mn^{2+} and Co^{2+} yielded less than 5% of the Mn^{2+} activity. Data represent a single experiment. Replicate experiments for Mn^{2+} and Co^{2+} are displayed in Fig. 2C.

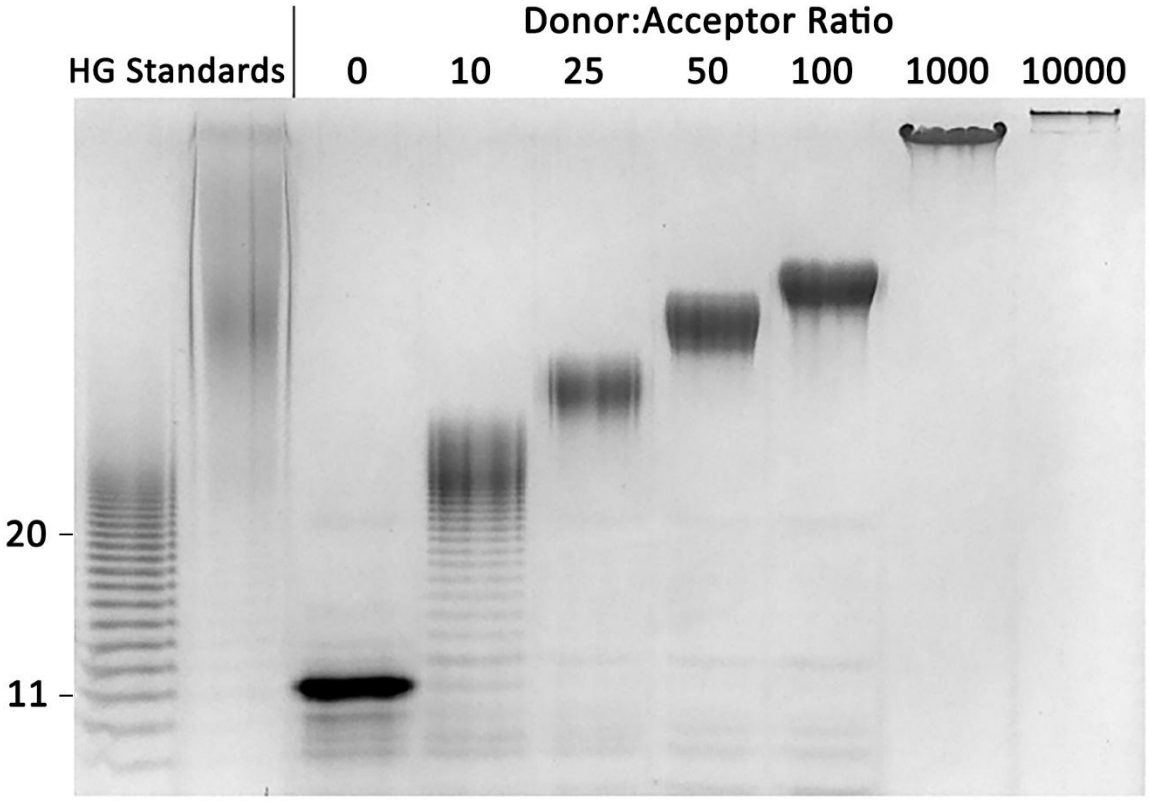

Figure S2. GAUT1:GAUT7 elongates DP11 acceptors in a manner dependent on the available donor:acceptor ratio. Following incubation of GAUT1:GAUT7 with the indicated ratios of UDP-GalA donor to DP11 acceptor for 12 h, samples were loaded into high-percentage PAGE gels stained with alcian blue/silver. Larger products were detected with increasing donor:acceptor ratio. For ratios of 0-100, 25 μM acceptor was incubated with varying amounts of UDP-GalA. For ratios of 1000 and 10000, 1 mM UDP-GalA was incubated with 1 μM and 0.1 μM acceptor, respectively. HG oligosaccharide standards (lane 1) and PGA (lane 2) are described in Fig. 3B.

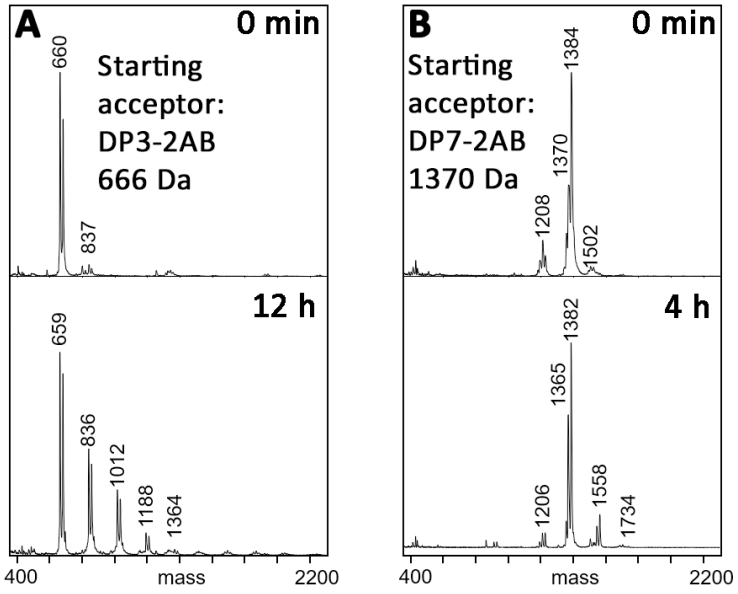

Figure S3. Elongation of DP3 and DP7 acceptors detected by MALDI-TOF MS. After incubation of GAUT1:GAUT7 with 1 mM UDP-GalA and **A**, DP3 acceptor (1 mM) for 12 h or **B**, DP7 acceptor (100 μM) for 4 h, addition of only 1-4 GalA units can be detected by MALDI-TOF MS analysis of 2-AB-labeled HG elongation products. Using either acceptor, longer-chain HG products were not detected after longer reaction incubation times (up to 48 h, data not shown). The series of ions (m/z) with a mass separation of 176 Da is consistent with sequential addition of GalA.

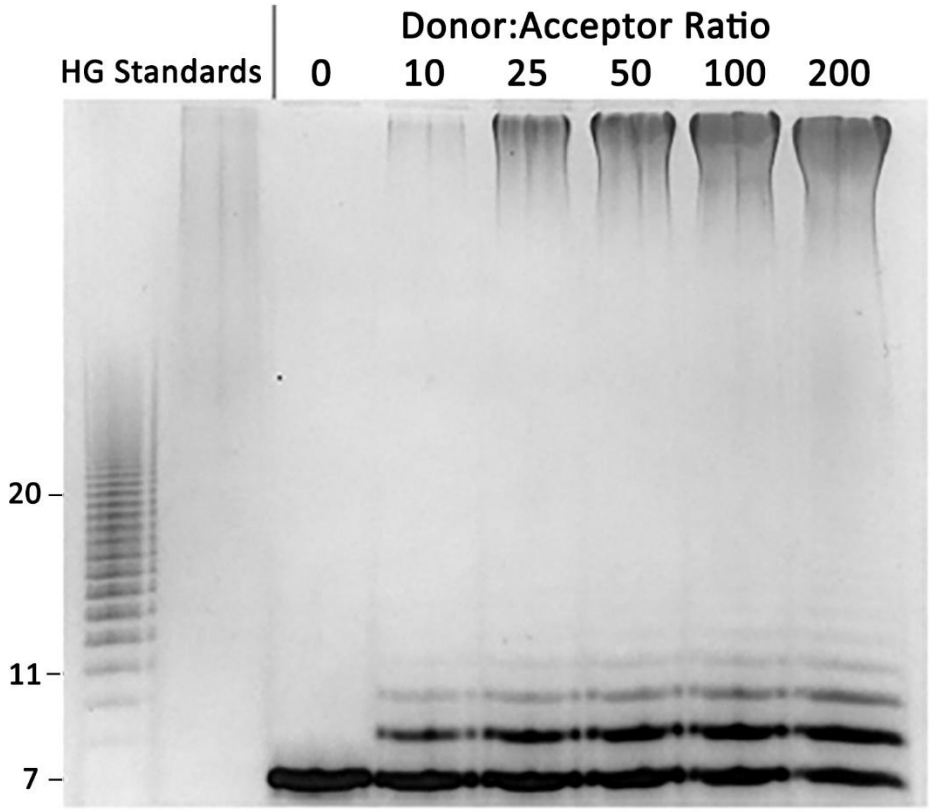

Figure S4. Elongation of DP7 acceptors results in a bimodal product distribution under a donor:acceptor ratio ranging from 10 to 200. Following incubation of GAUT1:GAUT7 with the indicated ratios of UDP-GalA donor to DP11 acceptor for 12 h, samples were loaded into high-percentage PAGE gels stained with alcian blue/silver. High MW products were detected under all donor:acceptor ratios tested. For all reactions, 25 μM DP7 acceptor was incubating with increasing amounts of UDP-GalA. HG oligosaccharide standards (lane 1) and PGA (lane 2) are described in Fig. 3B.

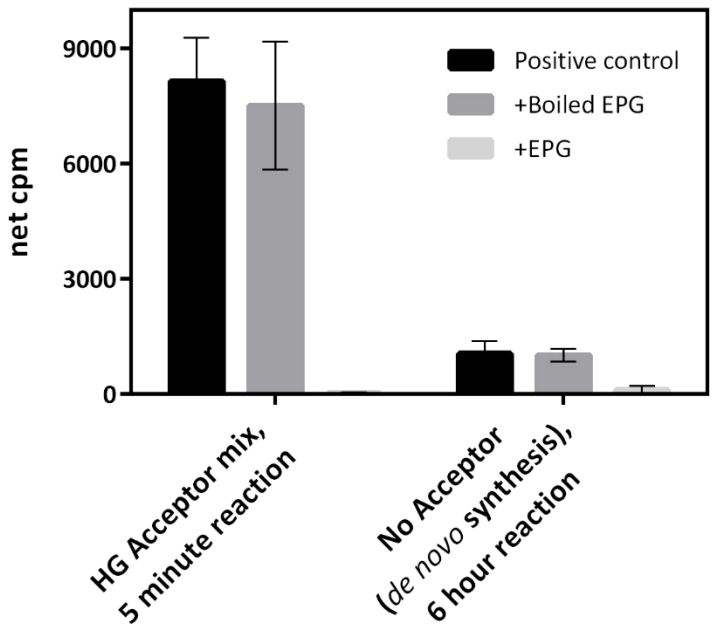

Figure S5. Digestion by endopolygalacturonase (EPG) of HG synthesized by acceptor-dependent elongation and *de novo* synthesis. Acceptor-dependent activity was measured in 5 minute reactions containing 100 nM GAUT1:GAUT7, 5 µM UDP-[^{14}C]GalA and 10 µM HG mix. *De novo* synthesis activity was measured in 6-hour reactions containing 500 nM GAUT1:GAUT7 and 5 µM UDP-[^{14}C]GalA. Following addition of sodium acetate buffer, pH 4.2, samples were incubated for 18 h with H_2O (positive control), 20 mU of endopolygalacturonase, or EPG deactivated by boiling. Error bars represent the standard deviation from three independent experiments.

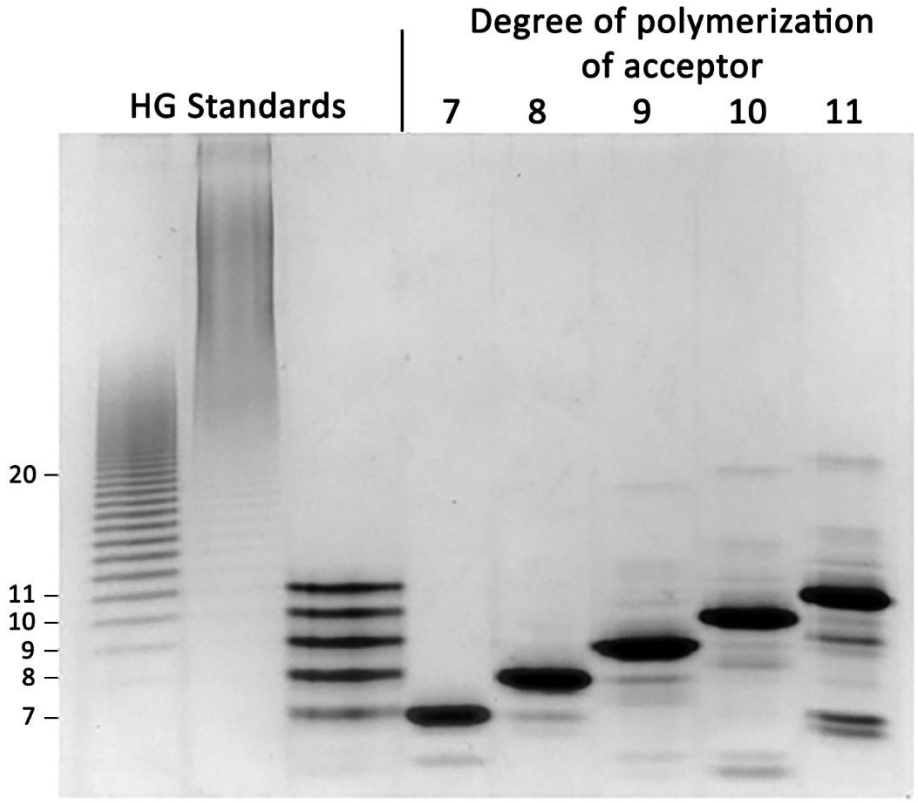

Figure S6 – Boiled enzyme controls, T0 starting acceptors for Fig.6A. As in Fig. 6A, reactions containing 100 nM GAUT1:GAUT7, 1 mM UDP-GalA, and 100 μM acceptor of DP 7-11 were incubated for 12 h. Enzyme was inactivated by pre-boiling prior to the reaction. HG oligosaccharide standards (lane 1) and PGA (lane 2) are described in Fig. 3B. Lane 3 is a mixture of HG DP 7-11 standards, 50 ng each.

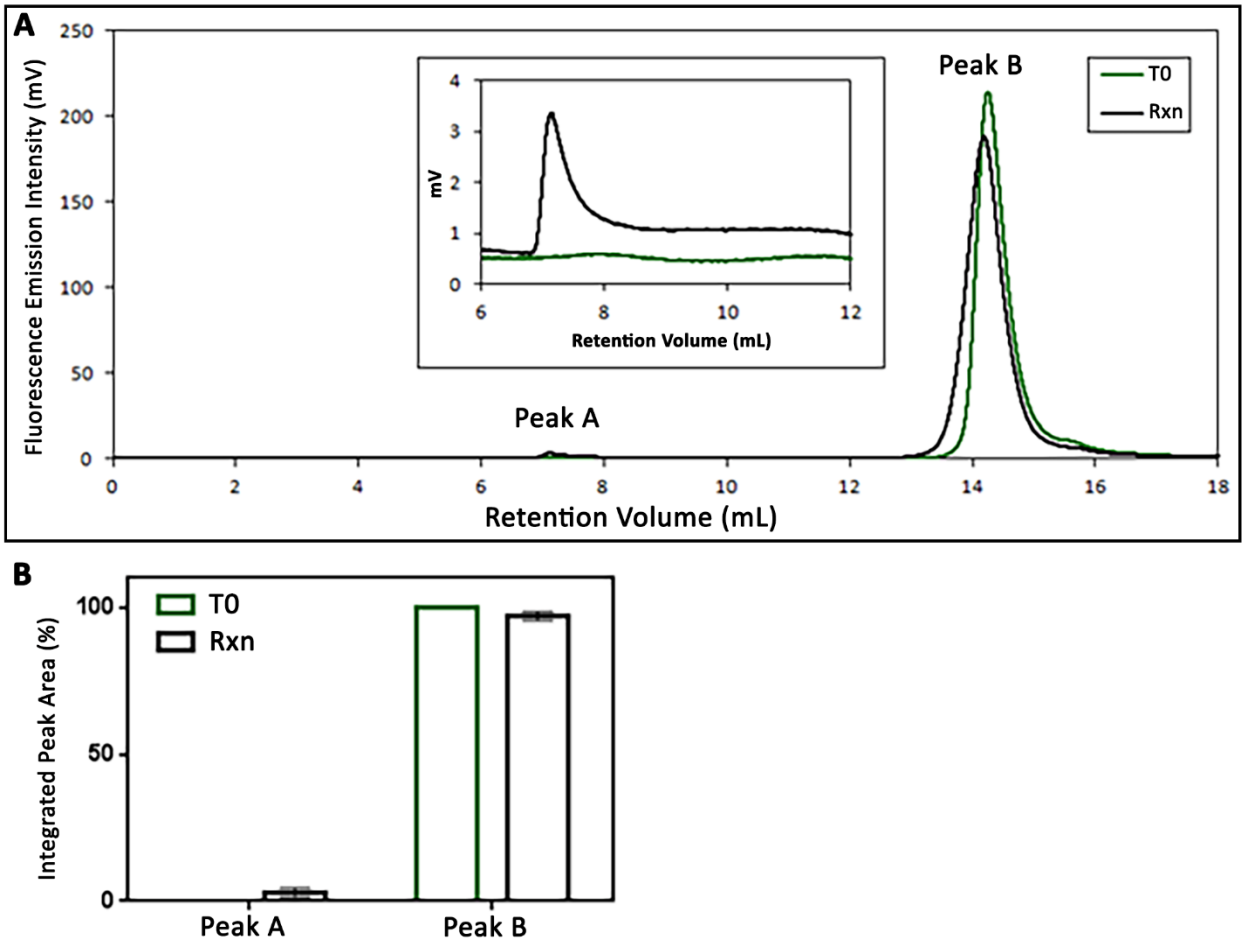

Figure S7. SEC separation with fluorescence detection of DP7-2AB acceptor and high molecular weight products. A, Following a 24 h incubation of GAUT1:GAUT7 with 100 μM DP7-2AB, high molecular weight polysaccharides (Peak A) were measured by size exclusion chromatography with fluorescence detection. The non-symmetrical Peak B contains the unreacted DP7-2AB acceptor and DP8-9 elongation products, as demonstrated in Fig. 5B and Fig. S2. The inset is a magnification of Peak A. GAUT1:GAUT7 was pre-boiled in T0 control samples, representing the unreacted DP7-2AB acceptor (dark green). **B**, Quantitation was performed by integration of peak area using Chromeleon software. Total area in Peak A and Peak B was normalized to 100%. From three replicate experiments, the percentage of integrated peak area in Peak A, representing the proportion of the starting acceptor that was elongated to high MW products, was 2.9%.

Variable substrate	Donor [UDP-GalA] (μM)	Acceptor [HG] (μM)	k_{cat} (s^{-1})	K_M (μM)	k_{cat} / K_M (μM^{-1} s^{-1})	K_i (μM)
UDP-GalA	**5-1000**	**10**	**1.48 ± 0.10**	**219 ± 37.4**	**0.0068**	**-**
UDP-GalA	**5-1000**	**10**	**1.36 ± 0.06**	**231 ± 24.5**	**0.0058**	**-**
UDP-GalA	**5-2000**	**100**	**0.92 ± 0.02**	**151 ± 10.6**	**0.0061**	**-**
UDP-GalA	**5-2000**	**100**	**0.95 ± 0.03**	**136 ± 16.7**	**0.0070**	**-**
HG DP7-23	**1000**	**0.01-50**	**1.99 ± 0.06**	**0.8 ± 0.01**	**2.5**	**40.9 ± 3.6**
HG DP7-23	**1000**	**0.01-50**	**1.63 ± 0.19**	**1.0 ± 0.30**	**1.6**	**32.7 ± 10.6**
HG DP11	**1000**	**0.01-100**	**3.92 ± 0.30**	**1.4 ± 0.2**	**2.8**	**14.6 ± 2.1**
HG DP11	**1000**	**0.01-100**	**4.60 ± 0.80**	**1.8 ± 0.5**	**2.6**	**10.3 ± 3.1**
HG DP7	**1000**	**0.01-100**	**0.61 ± 0.02**	**10 ± 1.4**	**0.061**	**-**
HG DP7	**1000**	**0.01-100**	**0.44± 0.02**	**8.6 ± 2.0**	**0.051**	**-**

Table S1. Results of Michaelis-Menten Kinetics assay experiments of the Arabidopsis heterologously-expressed GAUT1:GAUT7 complex. Kinetic parameters and associated standard deviations (±) were derived from nonlinear regression of assays containing at least 8 measurements. Representative Michaelis-Menten kinetics data are displayed in Fig. 2, D and E, as well as Fig. 4, B and C. Data displayed in the listed figures and in independent replicate experiments are reported in this table.