

Reciprocal encoding of signal intensity and duration in the glucose-sensing circuit in *Arabidopsis thaliana*

Supplementary Information.

Figure S1 related to Figure 2. Simple reaction kinetic is not able to provide the observed dose-duration reciprocity on a large number of parameter search. A thorough parameter search based on evolutionary algorithm (900,000 trials, see Experimental Procedures) was carried out to fit the simple model (equation shown in Figure 2A) fit into the AtRGS1 internalization data (Figure 1A). **(A)** Heat map of k_1 and k_{-1} over the Top 20000 scored parameter sets (see Material and Method for the scoring function in the parameter fitting algorithm) demonstrated a converged parameter search. Scaled bar is the probability density function (PDF) calculated over the Top 20000 parameter sets. **(B)** Distribution of K calculated based on the Top 20000 parameter sets peaked around $K=0.09$ M (~1.65% [D-glc]). Location of the 10%, 50% and 90% quantile were labeled with a, b, c, respectively. **(C)** Simulation using the top scored parameter set around a, b, or c. None of them showed a satisfactory fitting. **(D)** The Top 50 scored parameter sets did not yield a good fit with the data (top score = 0.8878).

Figure S2 related to Figure 3. Western Blot showing stable AtGPA1 and AGB1 level in the system, independent of D-glucose treatment. Immunoblots probed with the indicated antisera to AtGPA1 (WB:GPA1) or AGB1 (WB:AGB1). See Experimental Procedures for further details.

Figure S3 related to Figure 3. Comparing the one- and two- kinase model and time courses of two-kinase model. This supplementary figure supports Figure 3. **(A)** Box-plot of the scores of the top 50 parameter sets from one-kinase model and two-kinase model ($p < 0.0001$, Mann-Whitney U test). Note that the best score obtained based on the simple kinetic model (Figure 2A and Figure S1) is 0.8878. **(B)** Time courses for each signaling component using the two-kinase model with parameter values ($Md_{2kinase}^{top50}$) shown in Table S3.

Figure S4 related to Figure 3. Glucose-induced AtRGS1-YFP internalization in wild type Col-0, *wnk2* or *wnk5* mutant allele. Four-d-old Arabidopsis seedlings of mutant lines *wnk2-2* and *wnk5-1* and wild type Col-0 were transiently transfected with *Agrobacterium* carrying AtRGS1-YFP (See Experimental Procedures for details of the transient transfection) for 2d before D-glucose treatment at indicated concentration and time: **(A)** 0% D-glc, **(B)** 2% D-glc and **(C)** 6% D-glc. AtRGS1-YFP fluorescence was imaged using confocal microscopy and the percentage of internalization was quantified using ImageJ (see Experimental Procedures).

Figure S5 for Figure 3. Predicted stoichiometry between the dynamics of sugar induced AtRGS1 internalization and active $G\alpha$ accumulation. Simulation presented over 24 h. Circles indicate the fraction of $G\alpha$:GTP in the total pool of $G\alpha$. Squares indicate the fraction of $G\alpha$ transferred from the cycle with AtRGS1 (Figure 3A, the left cycle) into the cycle without AtRGS1 (Figure 3A, the right cycle) by different concentration of D-glucose.

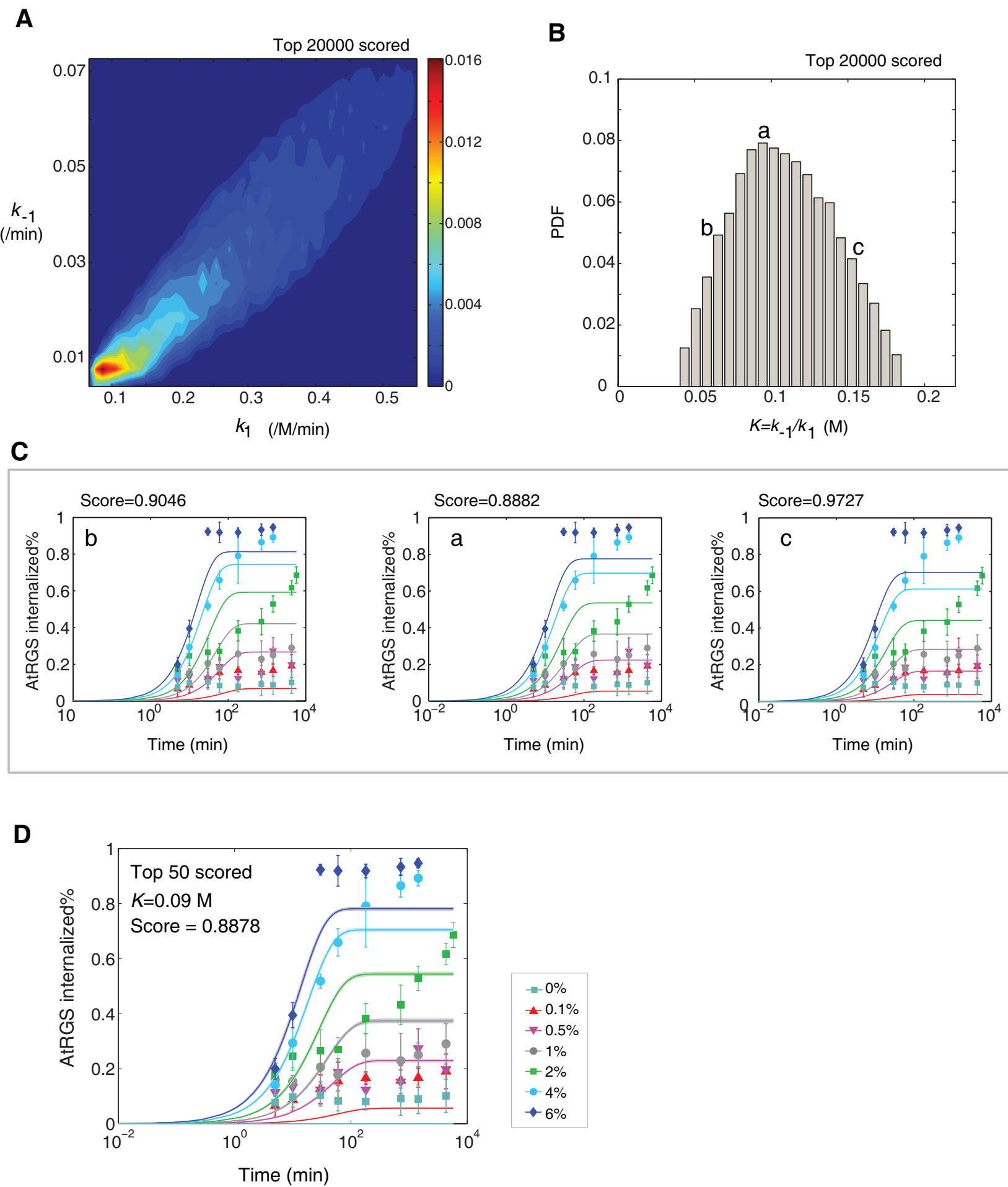
Figure S6 for Figure 1 and 7. Sugar induced AtRGS1-YFP endocytosis in tobacco pavement

cells. View of AtRGS1 endocytosis along the z-stack of the same cell. The middle slice is taken at least 5 μm down from the top slice. Representative images are shown with scale bars of 10 μm .

Table S1. Variables of the quantitative model demonstrated in Figure 3A. Each variable is associated with an ordinary differential equation that captures the rate of change in its abundance over time. Refer to Table S2 for the equations and Table S3 for the corresponding parameter values.

Table S2. Ordinary differential equations of the quantitative model illustrated in Figure 3A. The one-kinase and two-kinase models are specifically noticed in the differential equation of x_{14} .

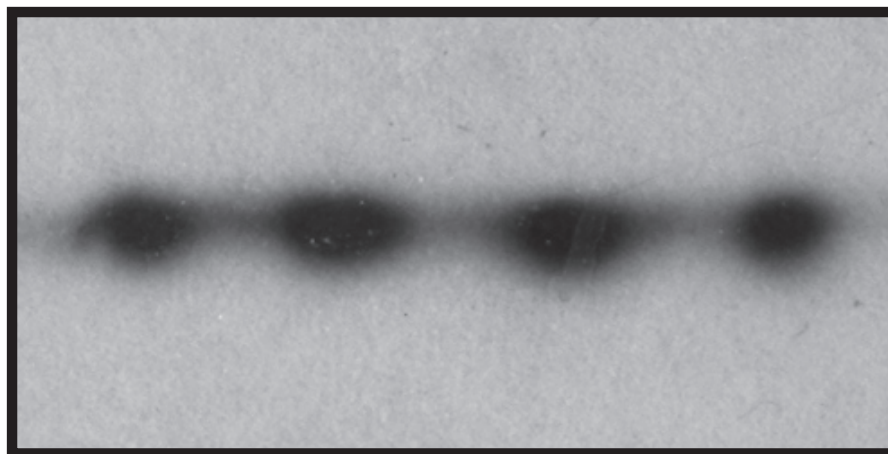
Table S3. Parameter sets used to implement the quantitative model (shown in Figure 3) based on one-kinase ($Md_{1kinase}^{top50}$) or two-kinase ($Md_{2kinase}^{top50}$) model. These values are the median of the top 50 scored parameter sets from large number of parameter search. See Experimental Procedures for the introduction of the algorithm.



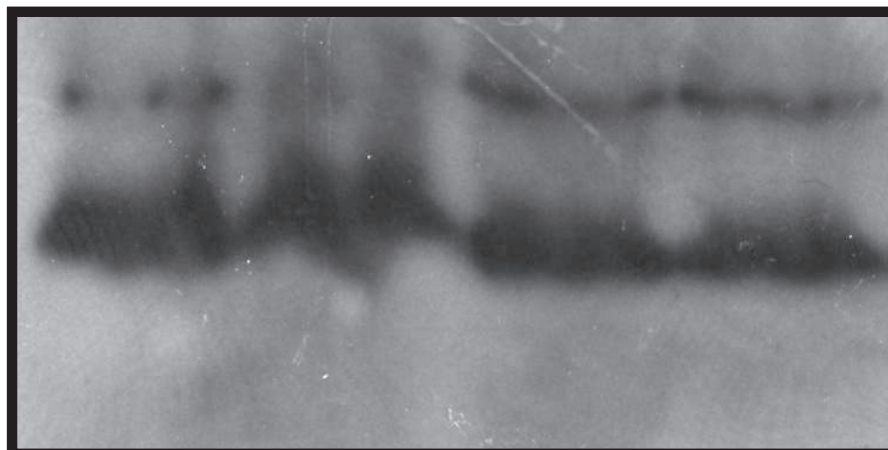
6% D-glucose

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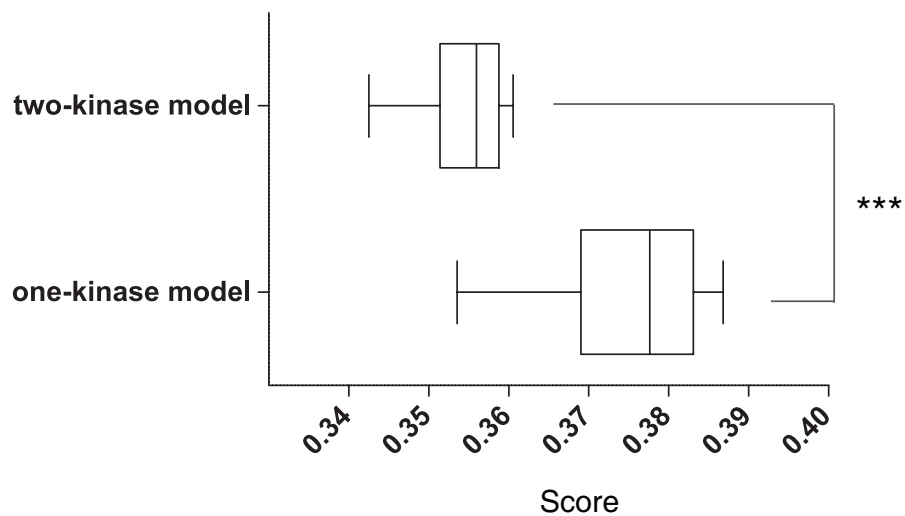
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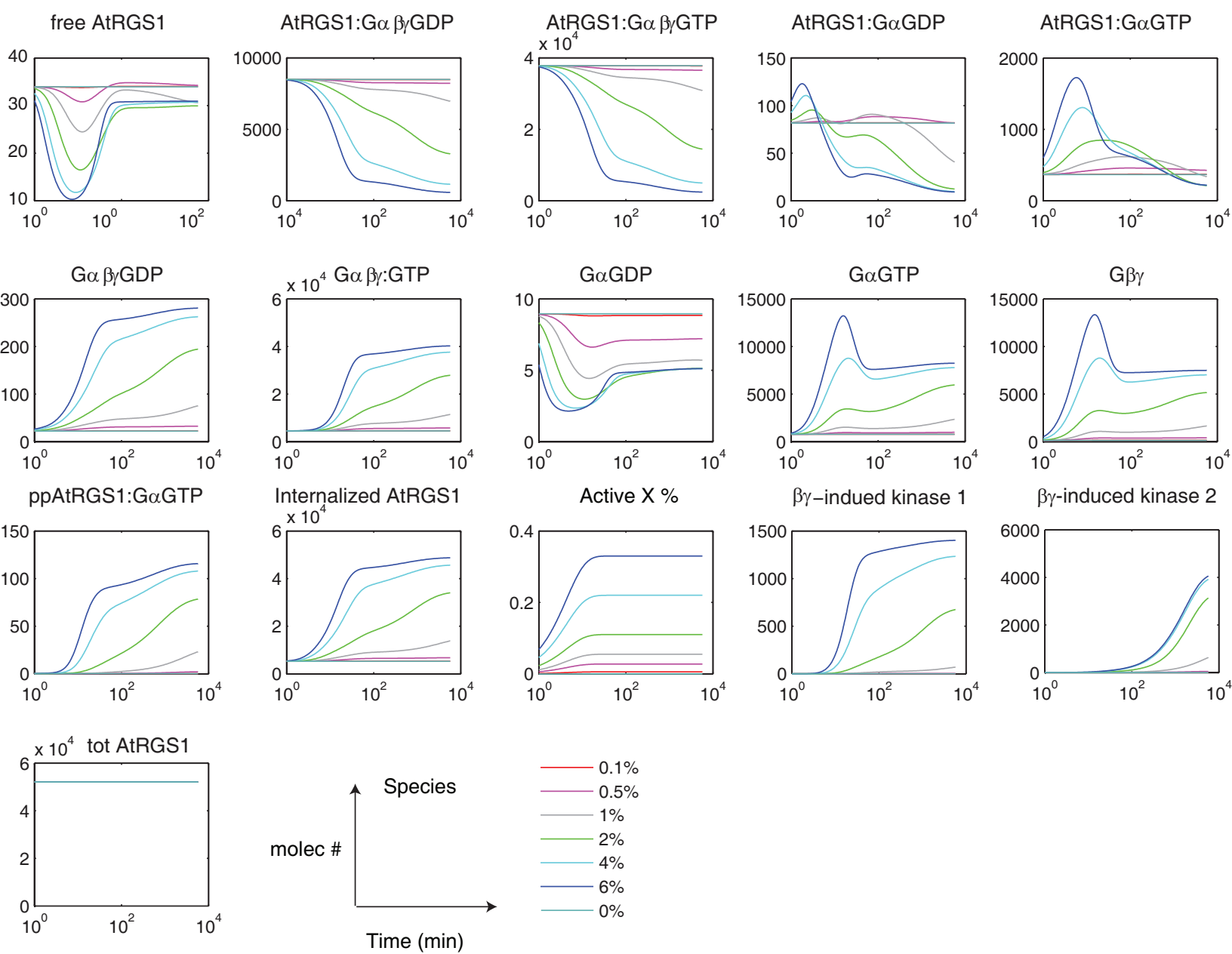
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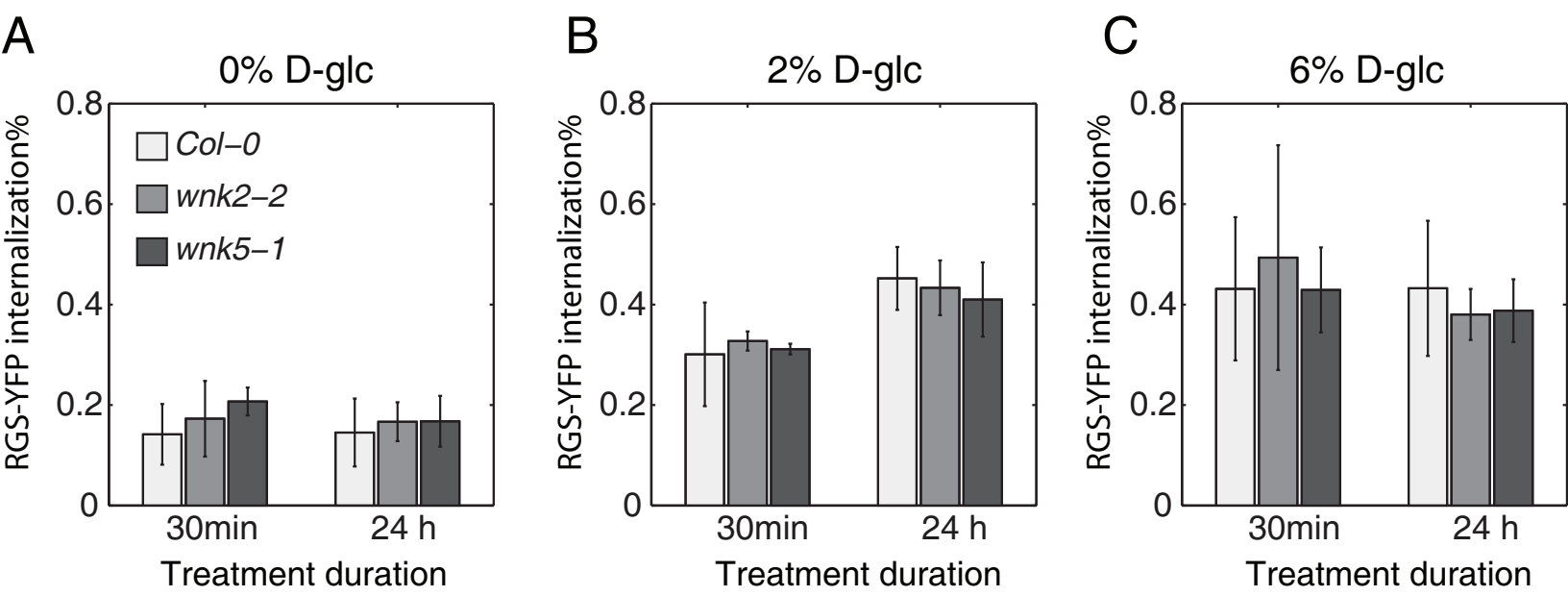


A



B





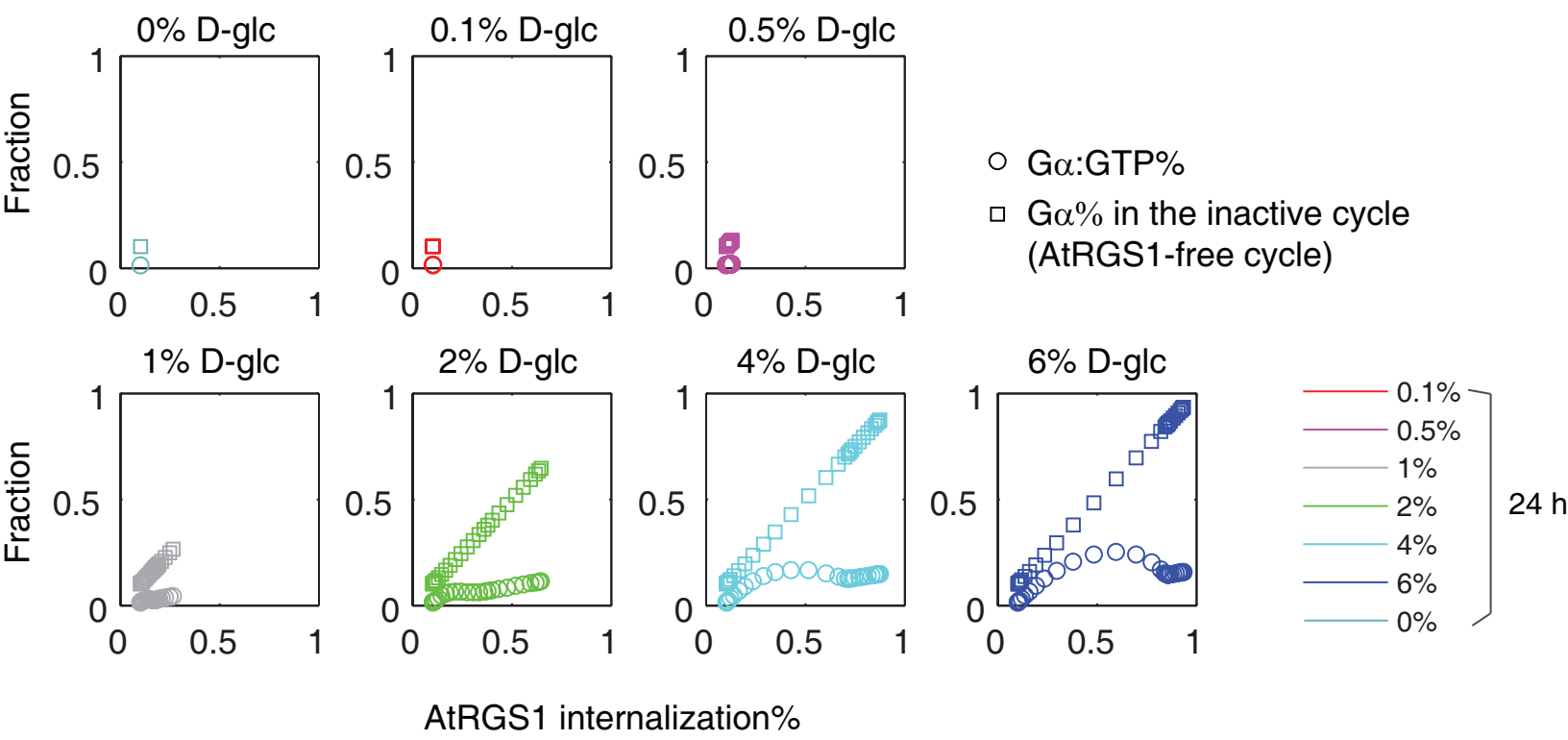


Figure S6

