# Supplementary File 1

# Supplementary Discussion - GTPase kinetics parameters determination, assumptions, and confidence

## Validity of the Michaelis-Menten equation under the experimental conditions used in our GTP cycle experiments.

Michaelis-Menten formalisms have been used for multiple GTPases including Ran[1](#_ENREF_1), Ras[2](#_ENREF_2), or Rap[3](#_ENREF_3).

Historically there have been many attempts to formalize the conditions under which the Michaelis-Menten equation to describe enzyme kinetics are valid (as reviewed by Schnell[4](#_ENREF_4)). These conditions have converged on the steady-state approximation or more generally, on the reactant stationary assumption. The formal condition for steady-state approximation is that t[ES] (the time it takes for the steady-state levels of [ES] complex to accumulate) is substantially shorter than t[S] (the time where [S] changes significantly). The formal condition for reactant stationary assumption is that [S] ≈ [S0] during initial build-up of [ES].

The formal condition for validity of the Michaelis-Menten equation can be expressed as:

, where and , and koff and kon are the rates of [ES] complex formation[5](#_ENREF_5).

The measured dissociation constant, , for the formation of the Ran:GDP:RCC1 complex from Ran:GDP and RCC1, where RCC1 is the human RanGEF is 0.9 μM[6](#_ENREF_6), which is approximately the same as the Km value obtained for the GEF-mediate nucleotide exchange for both *S. cerevisiae* and human Ran. That means than , which means the condition for validity of the Michaelis-Menten equation can be approximated as , and since in all of our GEF experiments both [E0] = 5-20 nM << Km and [E0] << [S0], the conditions holds true for the entire range of [S0] values, both below and above the Km.

As can also be expressed as , and the measured koff of human Ran:GTP and RanGAP from *S. pombe* is estimated to be around 150 s-1, while our measured kcat values range from 1 to 10 s-1, as above, the assumption of steady-state holds true as long as [E0] << Km and [E0] << [S0], which is the case as we used 1-5 nM GAP in all of our experiments.

## Approximating the GTPase cycle as a two-step turnover between the GDP and GTP-bound states.

In this work we approximate the GTPase cycle kinetics with four parameters, the kcat and Km of the GAP-mediated GTP hydrolysis and GEF mediated exchange between GDP and mant-GTP. Although these four parameters do not account for all the molecular details of how different point mutations in Gsp1 could affect the GTPase cycle, we observe that a simple ratio of estimated Michaelis-Menten enzymatic efficiencies (kcat/Km) better explains the genetic interaction data than individual parameters.

**Srm1-mediated nucleotide exchange.**

The biological function of RAN/Gsp1 is dependent on the GTP-loading (activation) of the GTPase by its GEF (Srm1). Unlike other Gsp1-binding proteins, Srm1 has a similar binding affinity to either nucleotide bound conformation (GDP or GTP). The nucleotide exchange reaction includes two intermediate complexes: Srm1 concentration dependent formation of the ternary complex Gsp1:[GDP|GTP]:Srm1 (kon ≈ 5 M-1 s-1 , koff = 55 s-1), followed by complex dissociation to Gsp1:Srm1 (koff ≈ 20 s-1, kon ≈ 1-6 M-1 s-1  ). Under the condition of our experiments, with excess of displacing nucleotide (mant-GTP) and enzymatic amounts of Srm1 the rate of exchange is determined by Gsp1:GDP:Srm1 formation and the koff to Gsp1:Srm1. For wild type RAN, the measured Km of nucleotide exchange measured as a Michaelis-Menten reaction is very similar to the equilibrium dissociation constant for the ternary RAN:GDP:GEF complex (both are ~ 1 μM)[6](#_ENREF_6).

Our data shows strongest effects on the nucleotide exchange kinetics from the increase of the Km (K101R, R108I, R108L, and R108Y **Extended Data Fig. 6**), while R112S and H141R mutants show significant kcat effects (**Extended Data Fig. 6**).

## Potential caveats associated with using the GAP (Rna1) from *S. pombe.*

All of our GAP-mediated GTP hydrolysis kinetics experiments used the wild type and mutant Gsp1 from *S. cerevisiae*, but Rna1 GAP from *S. pombe*. We chose to use the Rna1 ortholog from *S. pombe* as *S. cerevisiae* Rna1 formed soluble aggregates after purification, and *S. pombe* Rna1 was the only RanGAP for which there was a structure in complex with Ran (PDB IDs: 1k5d and 1k5g). While there could be slight differences between the kinetic parameters of *S. pombe* and *S. cerevisiae* GAP Rna1 acting on Gsp1, we do not believe these differences would significantly affect our conclusions, based on the following considerations:

1.) **Sequence conservation between *S. cerevisiae* and *S. pombe* Rna1.** A sequence alignment between *S. cerevisiae*, *S. pombe,* and human GAP proteins shows that all but one interface core residue in the PDB file 1k5d is conserved in sequence between *S. cerevisiae* and *S. pombe* (**Supplementary File 1 Supplementary Fig. 12**). Overall, out of the 1290 Å2 buried by *S. pombe* Rna1 upon interface formation with Ran (PDB ID: 1k5d), 997 Å2 (77%)are buried by residues that are conserved in sequence between *S. pombe* and *S. cerevisiae*, and the sequence identity of the Rna1 interface with Ran/Gsp1 overall is 71% (**Supplementary File 1 Table 1**).

2.) **Comparable kinetic parameters to the human Ran/RanGAP1 pair.** The kinetic parameters for our *S. cerevisiae* Gsp1 and *S. pombe* Rna1 GAP are comparable to the kinetic parameters for the human Ran and human RanGAP1 reported by Klebe *et al.*[6](#_ENREF_6). They estimate a Km of 0.45 μM and kcat of 2.1 s-1 for Ran/RanGAP1 at 25˚C, while our values for the wild type *S. cerevisiae* Gsp1 and *S. pombe* Rna1 at 30˚C are a Km of 0.38 μM and kcat of 9.2 s-1. In addition, it was shown that Rna1 from *S. pombe* can activate the hydrolysis in both human and *S. cerevisiae* Ran/Gsp1 with very similar observed rates of hydrolysis (Fig. 4a in Becker *et al*[7](#_ENREF_7)).

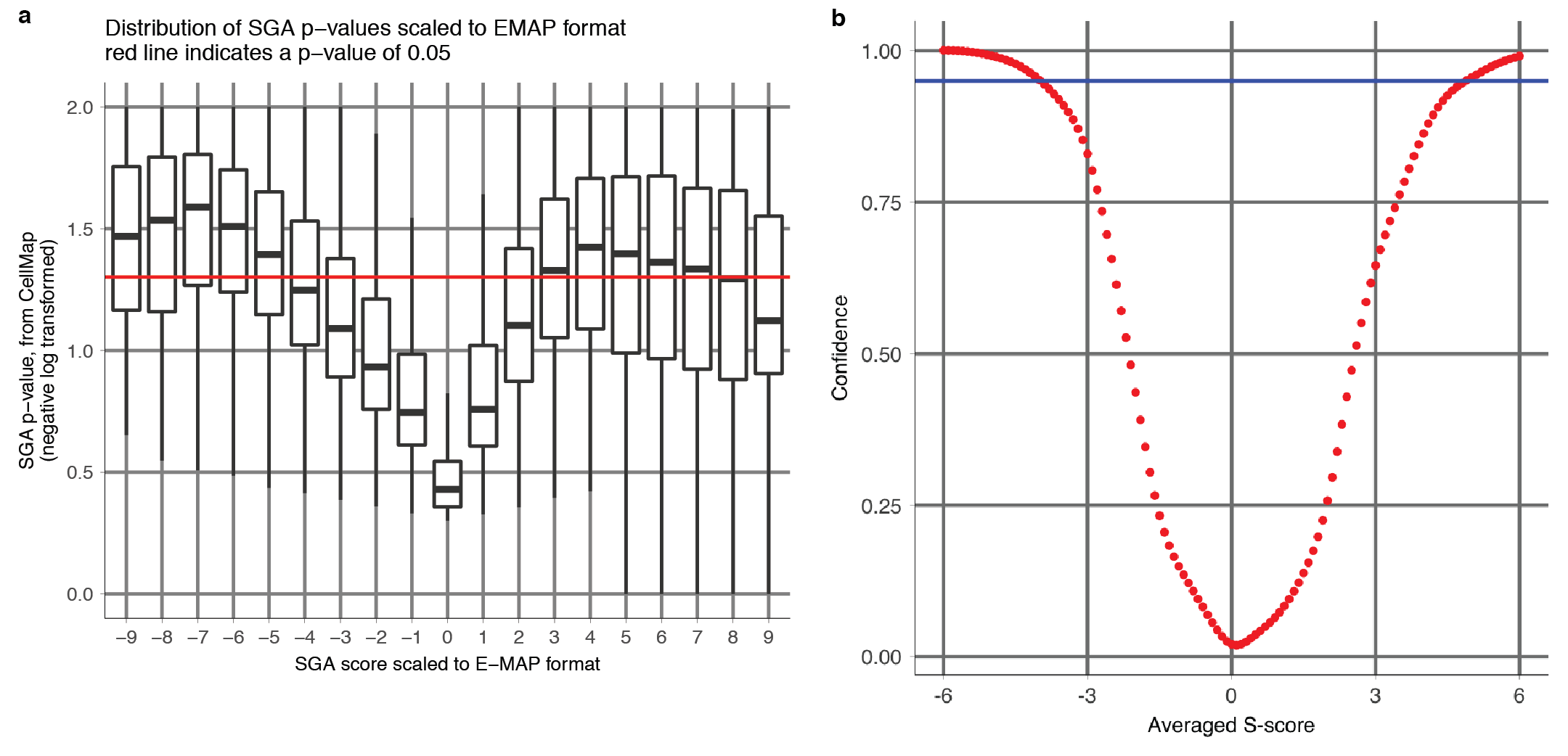
3.) **Our conclusions are based on relative values between the wild-type Gsp1 and its point mutants.** Although we report the absolute values of the kinetics parameters, when we compare the kinetics parameters with the results from genetic interaction profile and AP-MS, we always use the relative parameters as compared to the wild type. Based on the sequence conservation and comparable kinetics described above, we expect the relative ordering of mutants to be similar as well. Importantly, we use the relative kinetic data to group our mutants into three classes. Even in the case of small quantitative differences caused by using the *S. pombe* instead of the *S. cerevisae* Rna1 GAP, we make the assumption that these differences would not significantly affect this grouping.

# Supplementary Figures

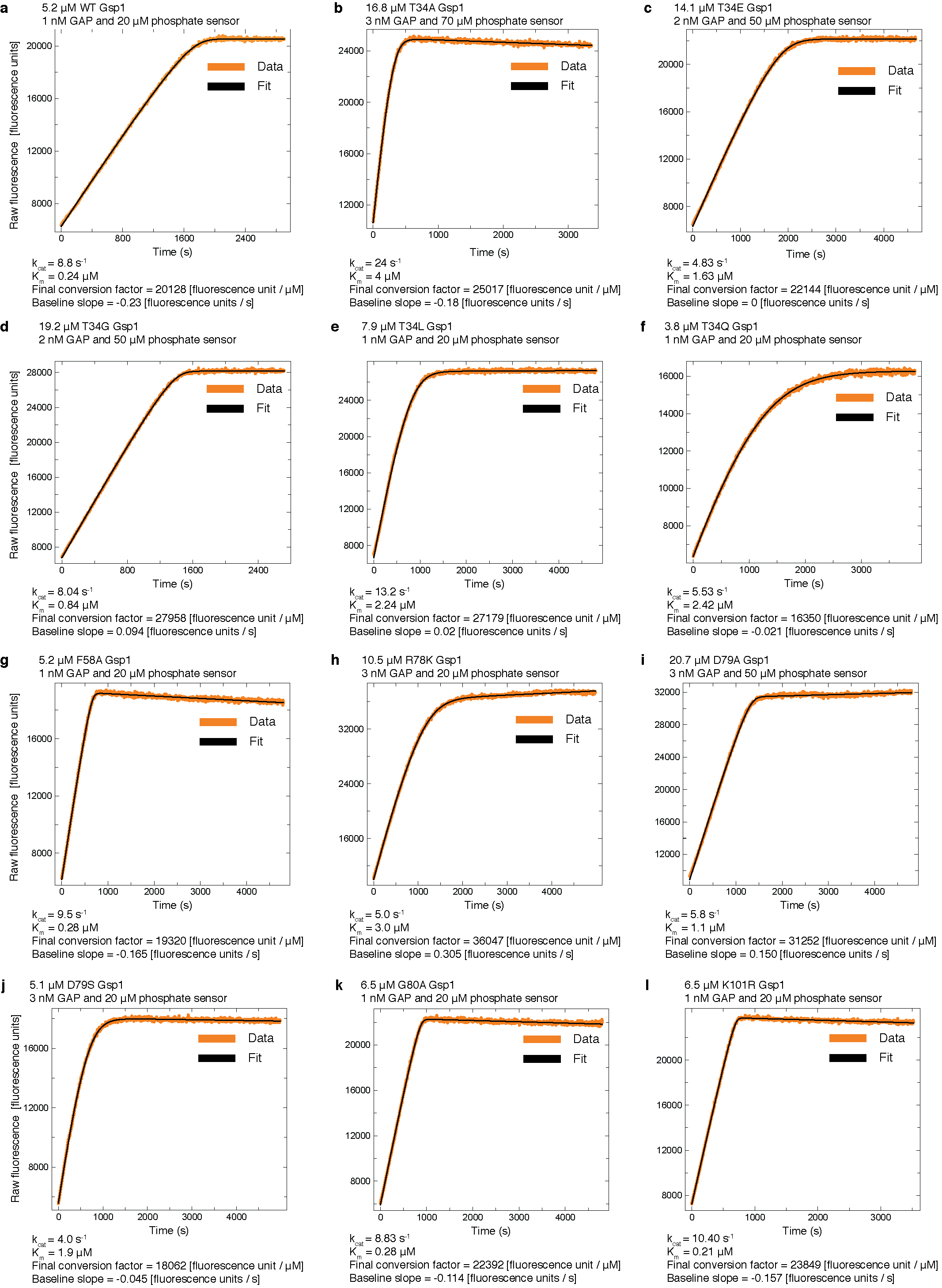
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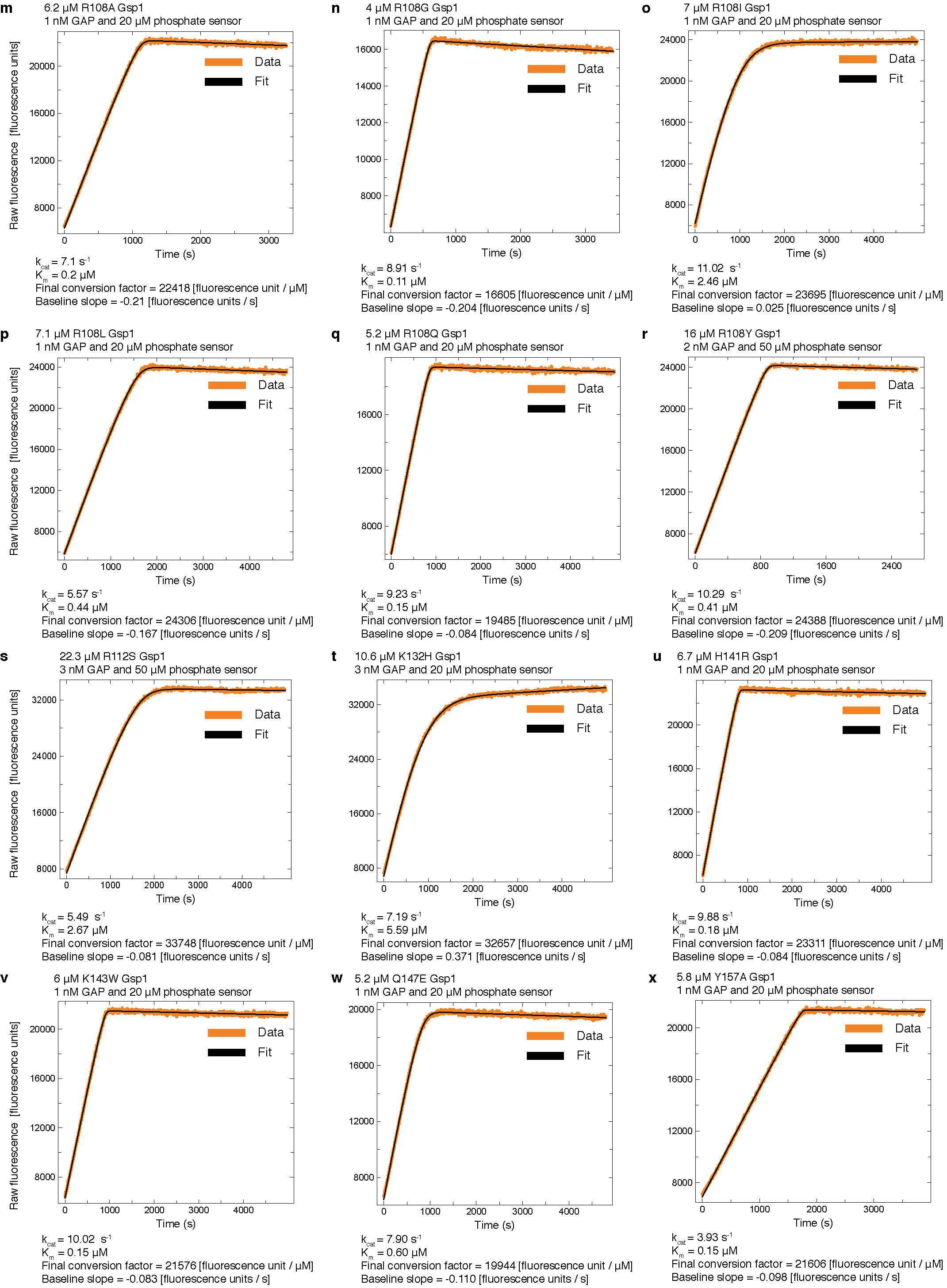
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**Supplementary Figure 1 Cartoon representation of co-complex structures of *S. cerevisiae* Gsp1 with indicated partners (or homologs)**. Srm1 (PDB: 1i2m), Rna1 (PDB: 1k5d), Ntf2 (PDB: 1a2k), Nup1/Nup60 (PDB: 3ch5), Yrb1 (PDB: 3m1i), Yrb2 (PDB: 3wyf), Srp1 (PDB: 1wa5), Kap95 (PDB: 2bku), Crm1 (PDB: 3m1i), Los1 (PDB: 3icq), Pse1(PDB: 3w3z), Kap104 (PDB: 1qbk), Msn5 (PDB: 3a6p), Cse1 (PDB: 1wa5), Mtr10 (PDB: 4ol0). Species and sequence identity to *S. cerevisiae* homologs for these structures are provided in **Supplementary File 1 Table 1**.



**Supplementary Figure 2 Comparison of definitions of high confidence S-scores used in our analysis**. **a,** Distribution ofthe SGA scores scaled to the E-MAP S-scores versus their corresponding published p-values from the CellMap **b,** Distribution of the E-MAP S-score averaged from all the individual replicates versus the confidence of the functional genetic interaction reproduced from Collins *et al*[8](#_ENREF_8).

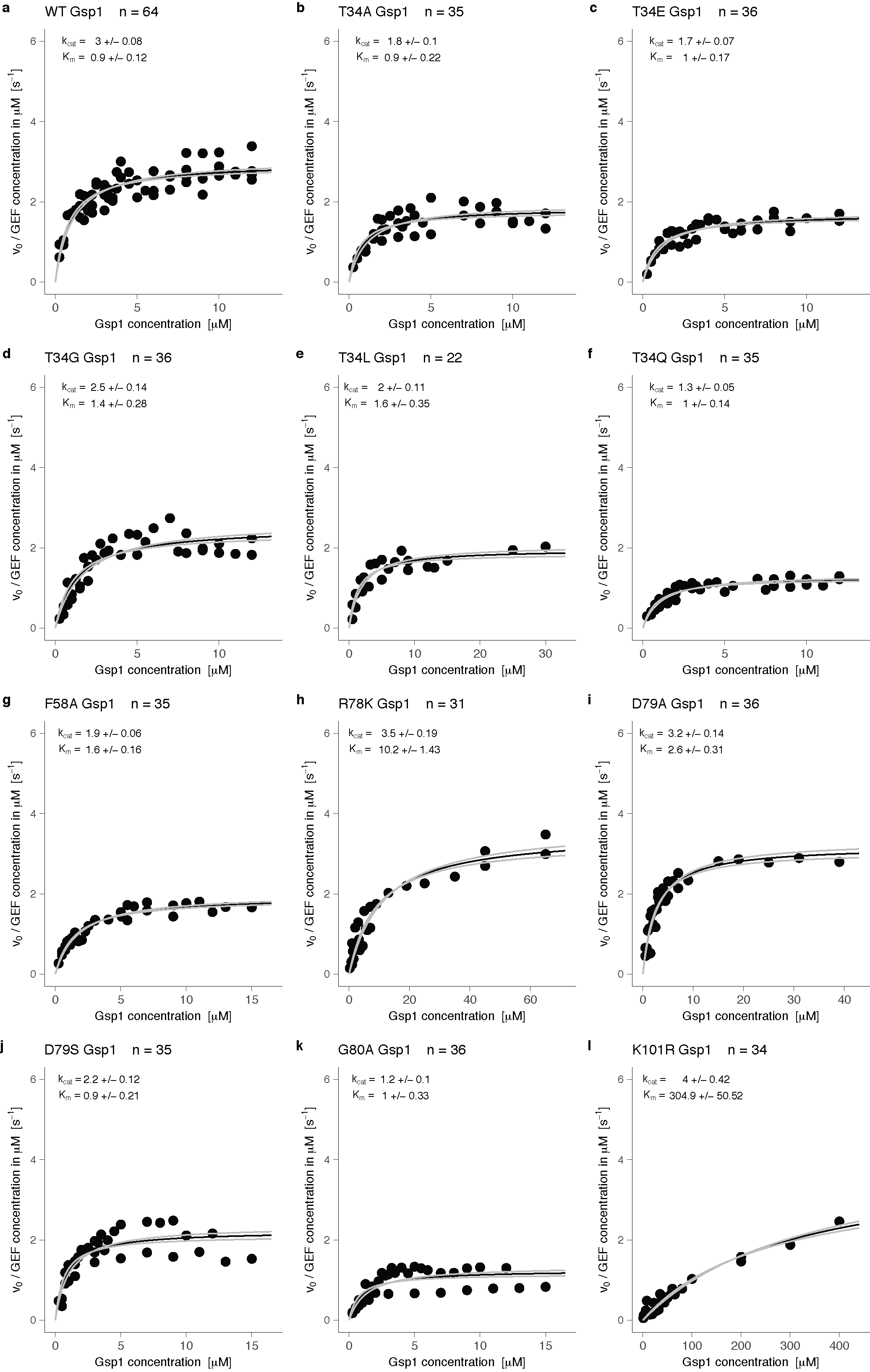


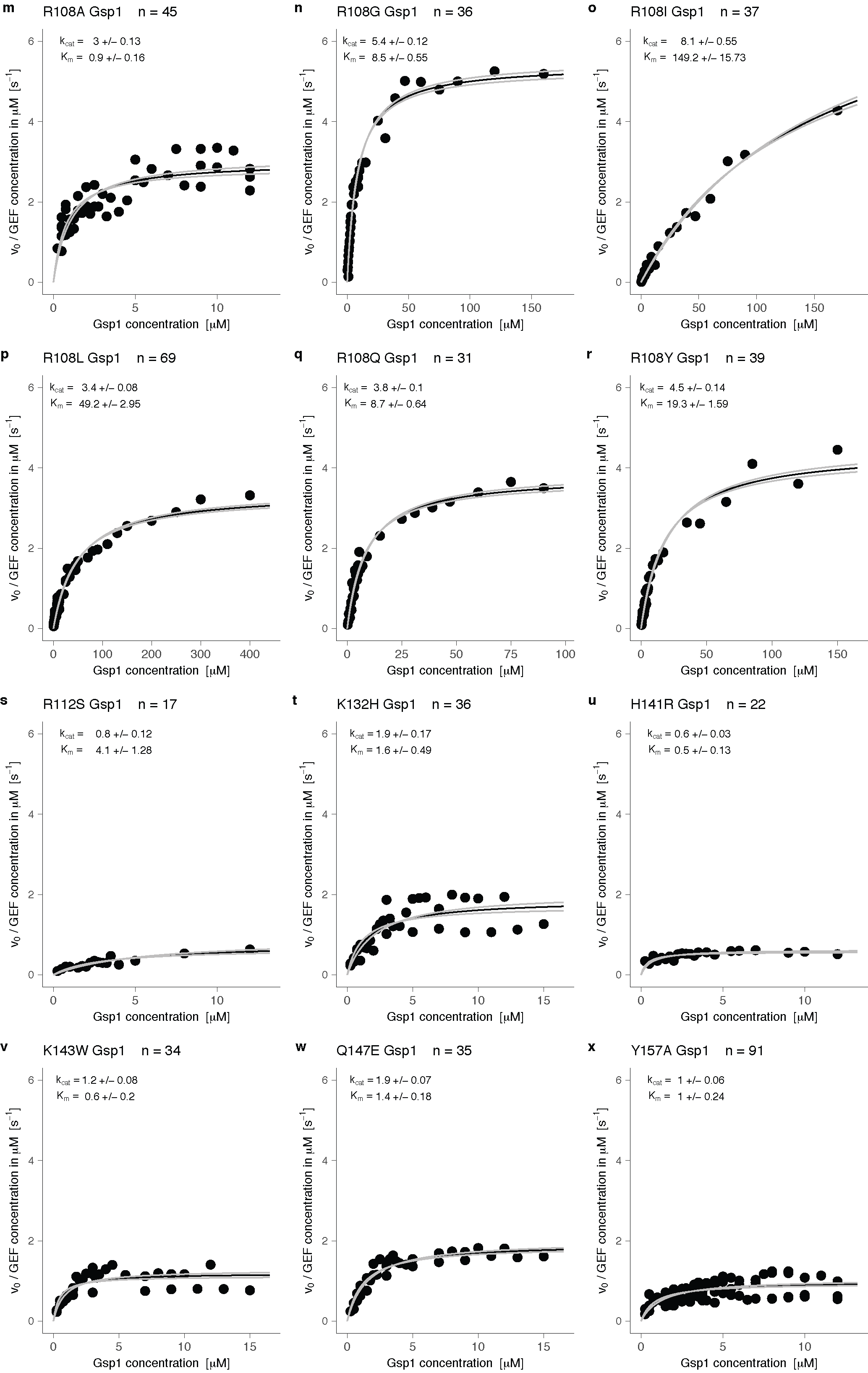
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**Supplementary Figure 3 GAP-mediated GTP hydrolysis** **monitored as fluorescence increase upon binding of released free phosphate to a fluorescent phosphate sensor.** Curves were fit with the integrated Michaelis-Menten equation using the DELA software. Final Michaelis-Menten kinetic parameters (kcat and Km) for each Gsp1 mutant were calculated from three to nine individually fit curves as the ones shown in this figure. **a,** Wild type Gsp1, **b-y,** Gsp1 point mutants.

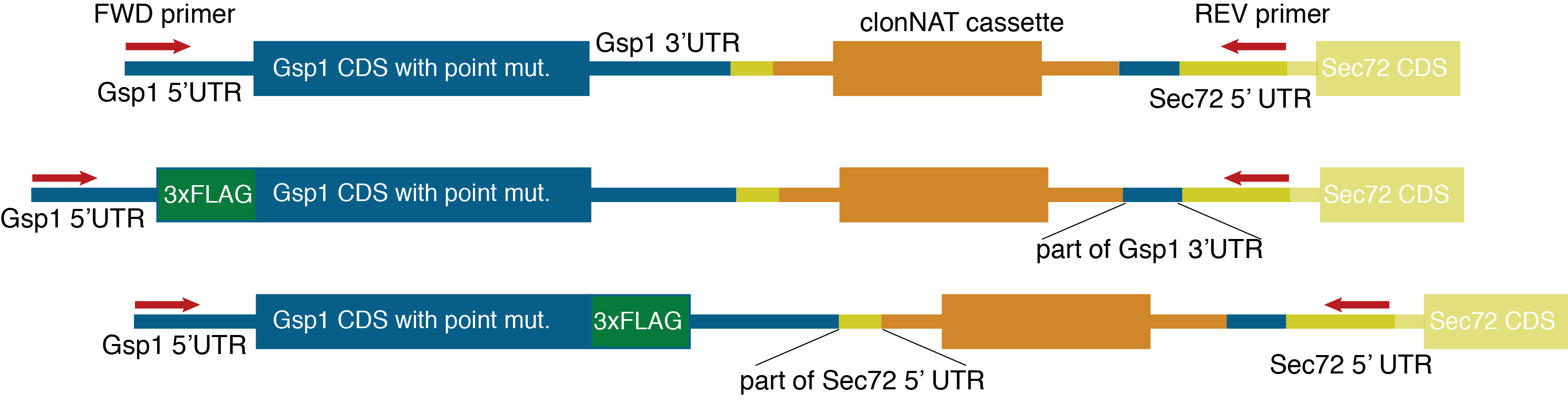


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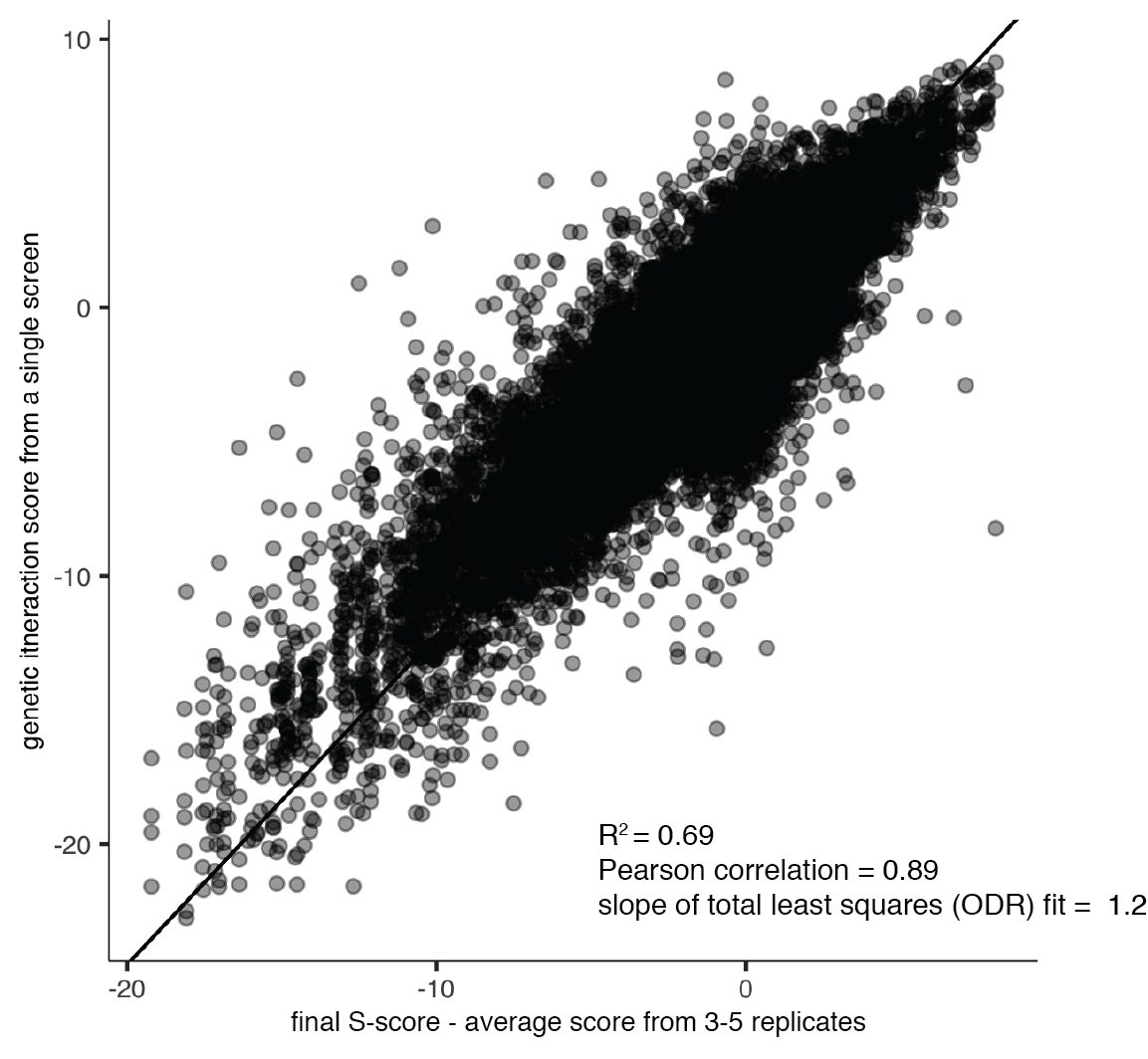
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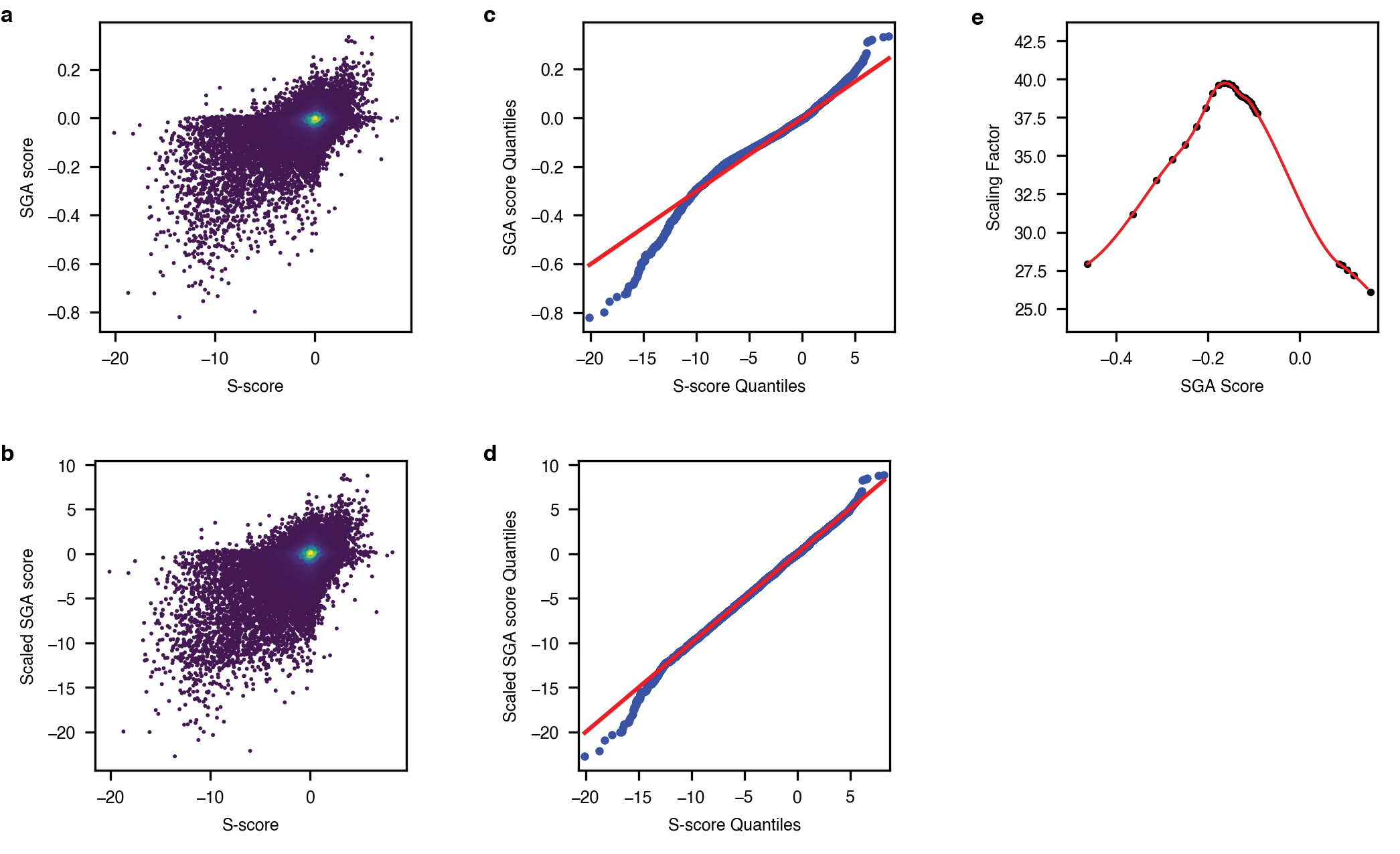
**Supplementary Figure 4 Michaelis-Menten plots for GEF-mediated nucleotide exchange**. Black line represents the Michaelis-Menten fit, and the gray lines represent the plus and minus one standard error of the fit. **a,** Wild type Gsp1. **b-y**, Gsp1 point mutants.



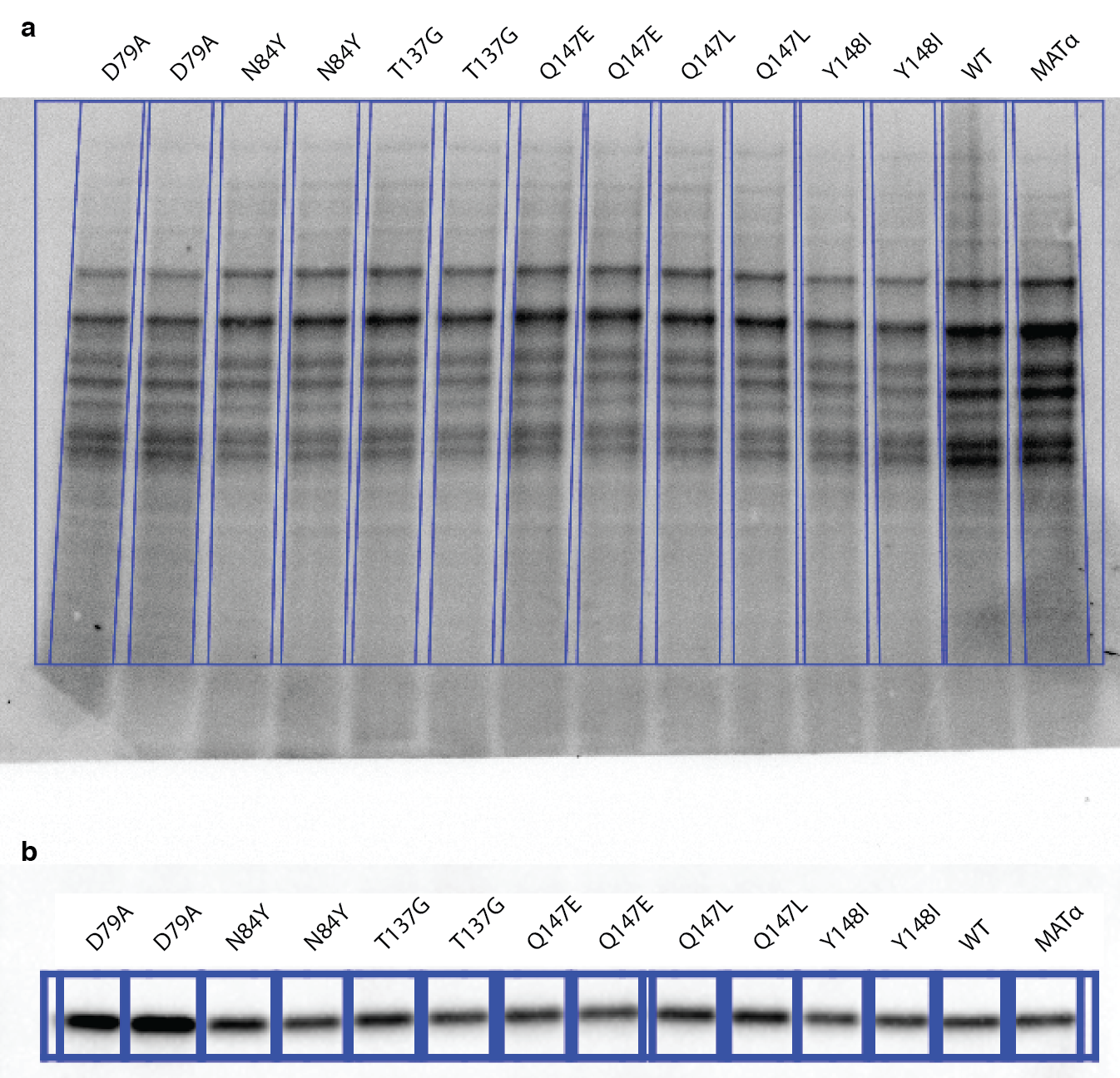
**Supplementary Figure 5 Schematic of genomically integrated *GSP1* constructs**. For E-MAP experiments, wild type or mutant GSP1 cassettes including the clonNAT resistance cassette were integrated into the MATα strain. For AP-MS the constructs also included either an N-terminal or a C-terminal 3xFLAG tag (M**DYKDHDGDYKDHDIDYKDDDDK**GGGGA and GGGGA**DYKDHDGDYKDHDIDYKDDDDK**, respectively).



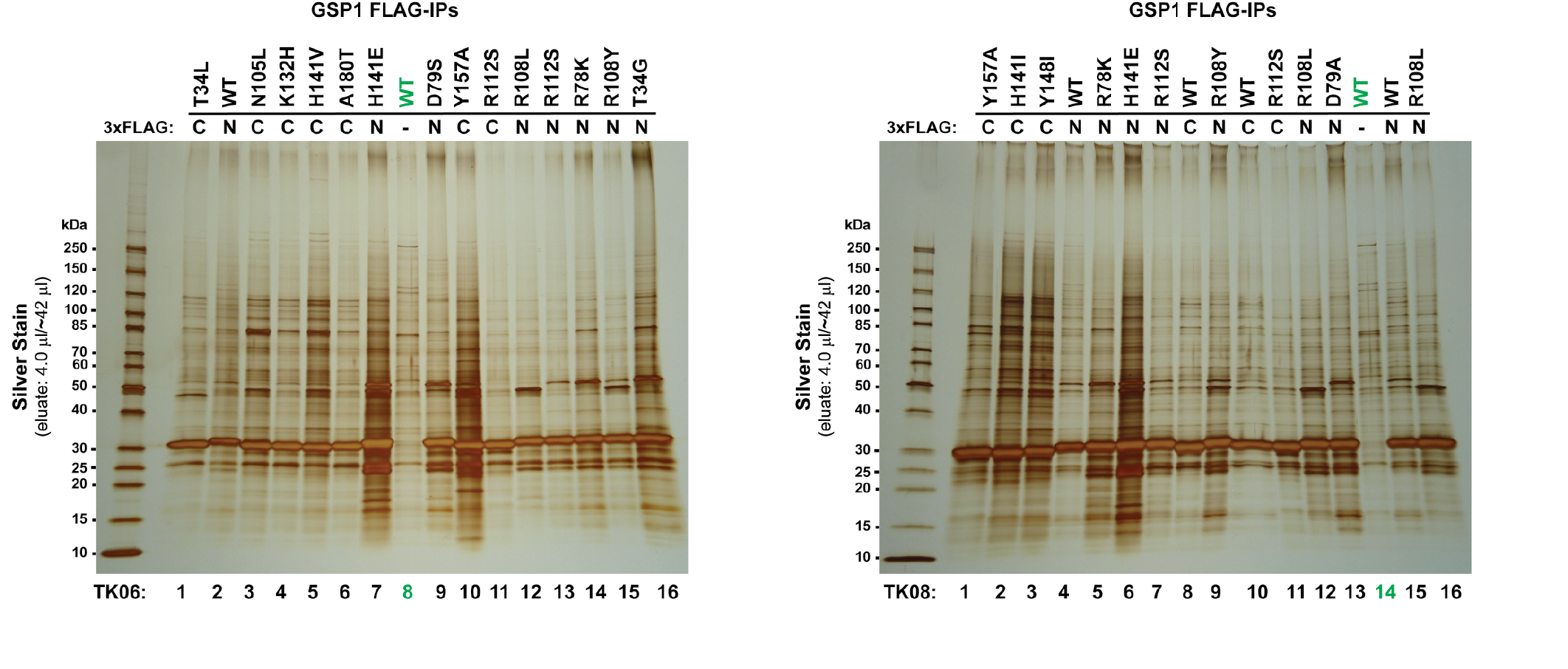
**Supplementary Figure 6 Reproducibility of *GSP1* point mutant E-MAP screens**, represented as a linear relationship between the genetic interaction S-score from a single E-MAP experiment and the final average S-score based on three or more replicates. The linear fit was calculated using the odregress function from the pracma R package.



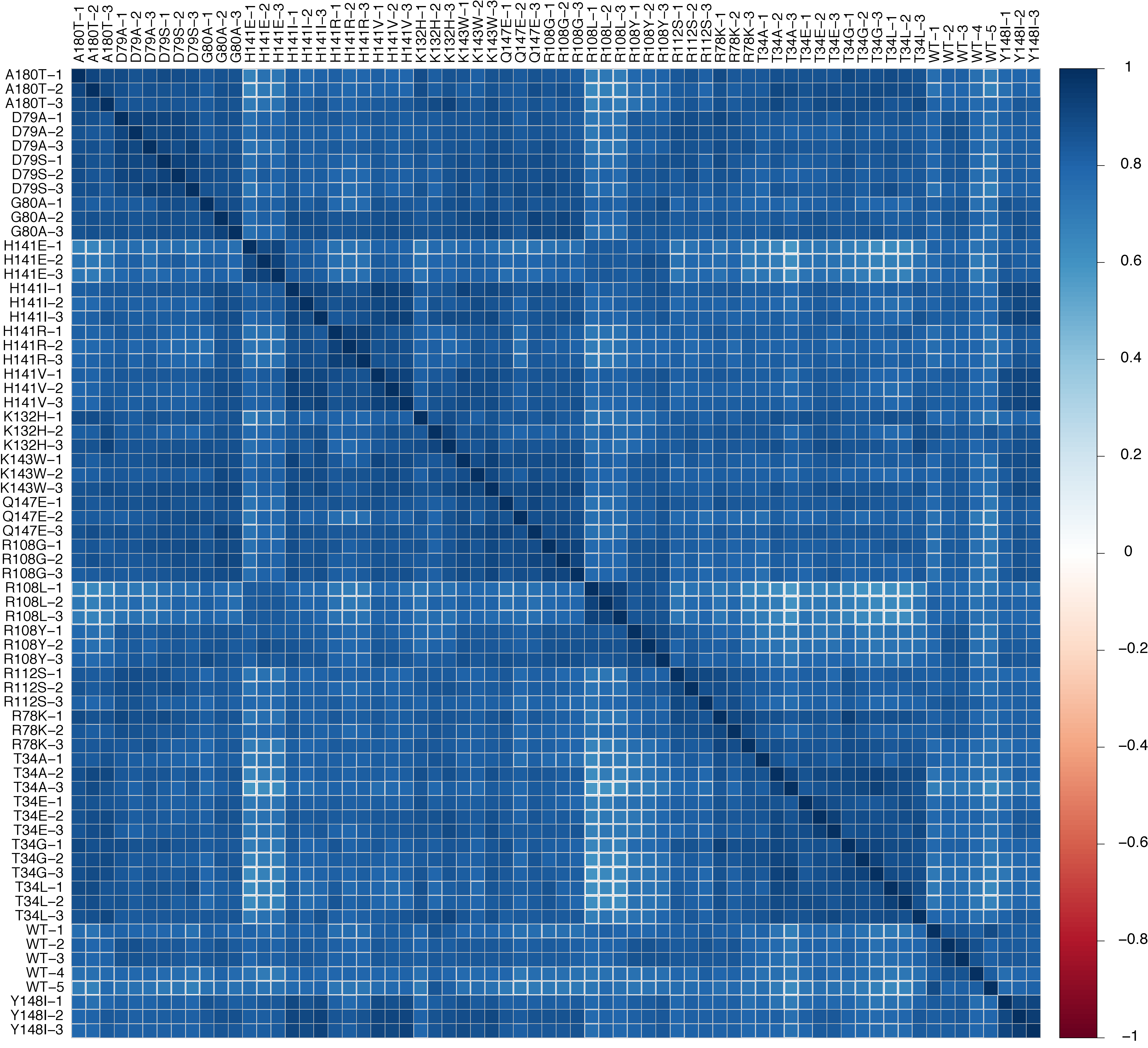
**Supplementary Figure 7 Non-linear scaling of SGA data from the Cell Map to E-MAP format.** **a,** Distribution of S-scores from the chromatin biology E-MAP dataset and the SGA score from the CellMap dataset. **b,** Distribution of S-scores from the chromatin biology E-MAP dataset and the *scaled* SGA score from the CellMap dataset. **c,** Quantile-quantile plot showing the distribution of genetic interaction scores from the CellMap and E-MAP chromatin biology datasets. **d,** Quantile-quantile plot showing the distribution of genetic interaction scores from the CellMap and E-MAP chromatin biology datasets after the CellMap dataset was scaled. **e,** The scaling function applied to the CellMap data. Red curve is the fitted spline of the scaling factors between the E-MAP S-scores and the SGA scores. Black dots represent the individual bins.



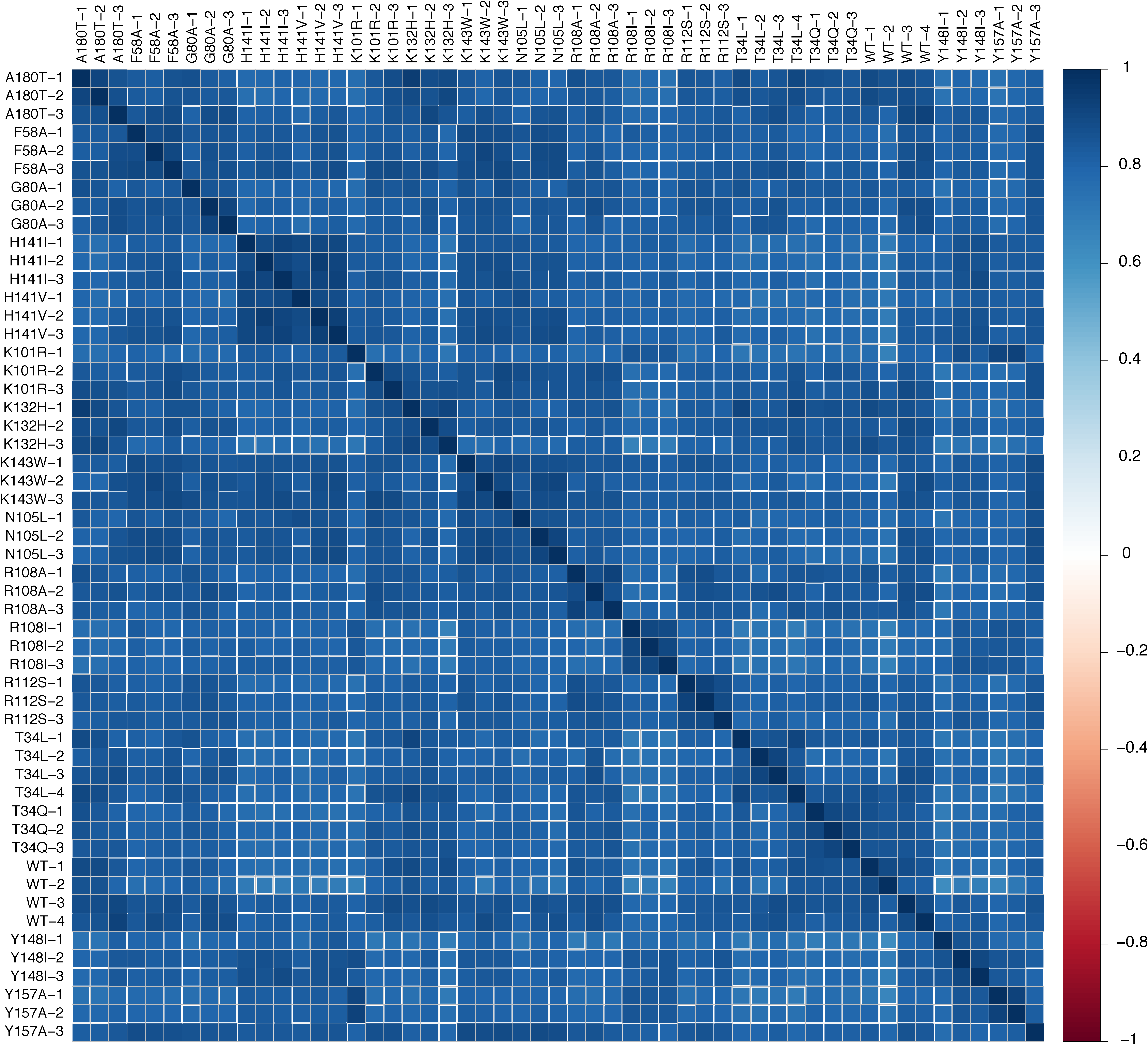
**Supplementary Figure 8 Example data for Gsp1 protein expression estimation by Western blot.** A, Total protein staining. b, Western blot of wild type Gsp1 and its mutants with anti-RAN antibody.



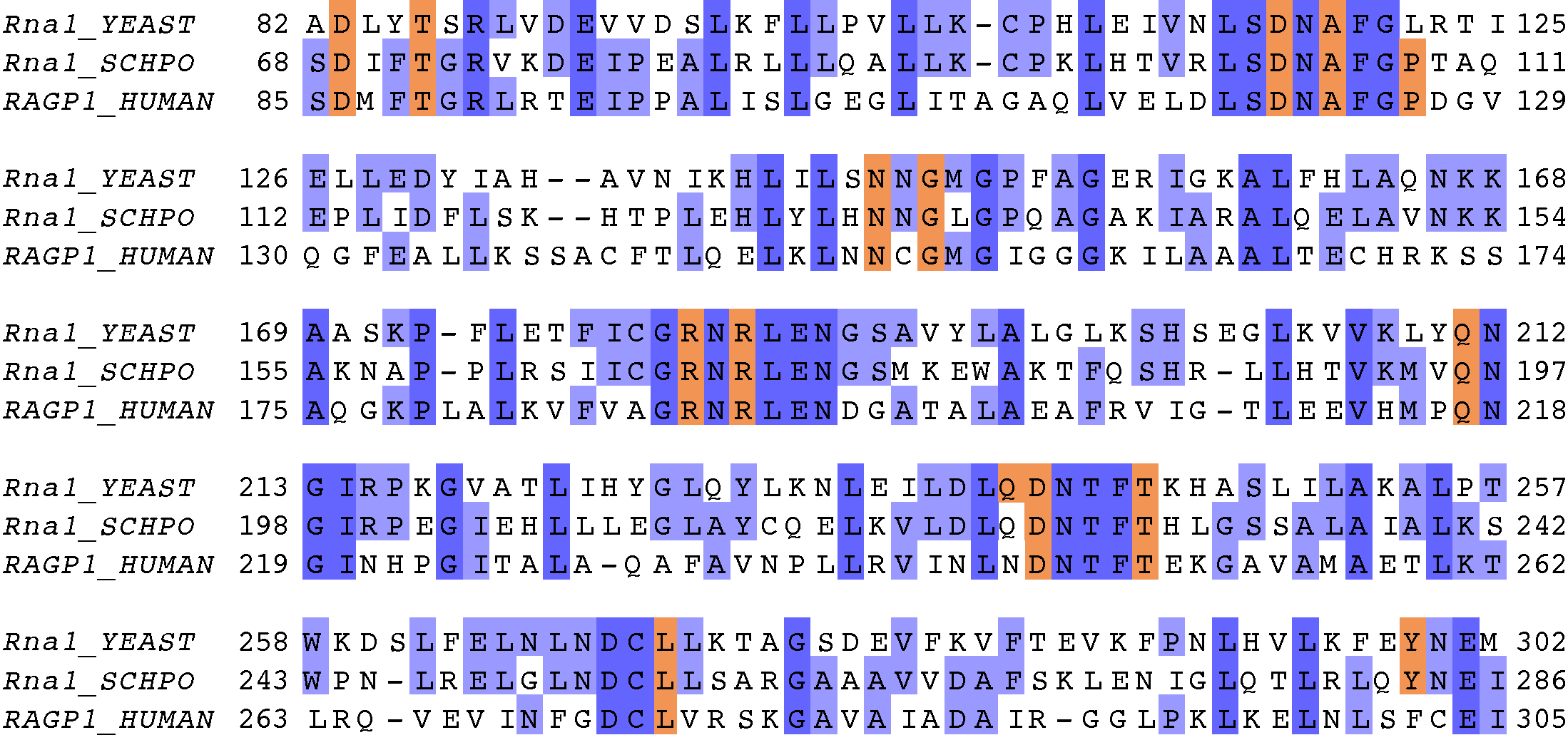
**Supplementary Figure 9 Silver stain gels after FLAG immunoprecipitation of amino or carboxy terminally 3xFLAG tagged genomically integrated Gsp1.** The strongest band at approximatelly 30 kDa corresponds to tagged Gsp1. Untagged wild type Gsp1 (lanes 8 and 14 in the left and right gel, respectively) were used as negative control for mass spectrometry analysis.



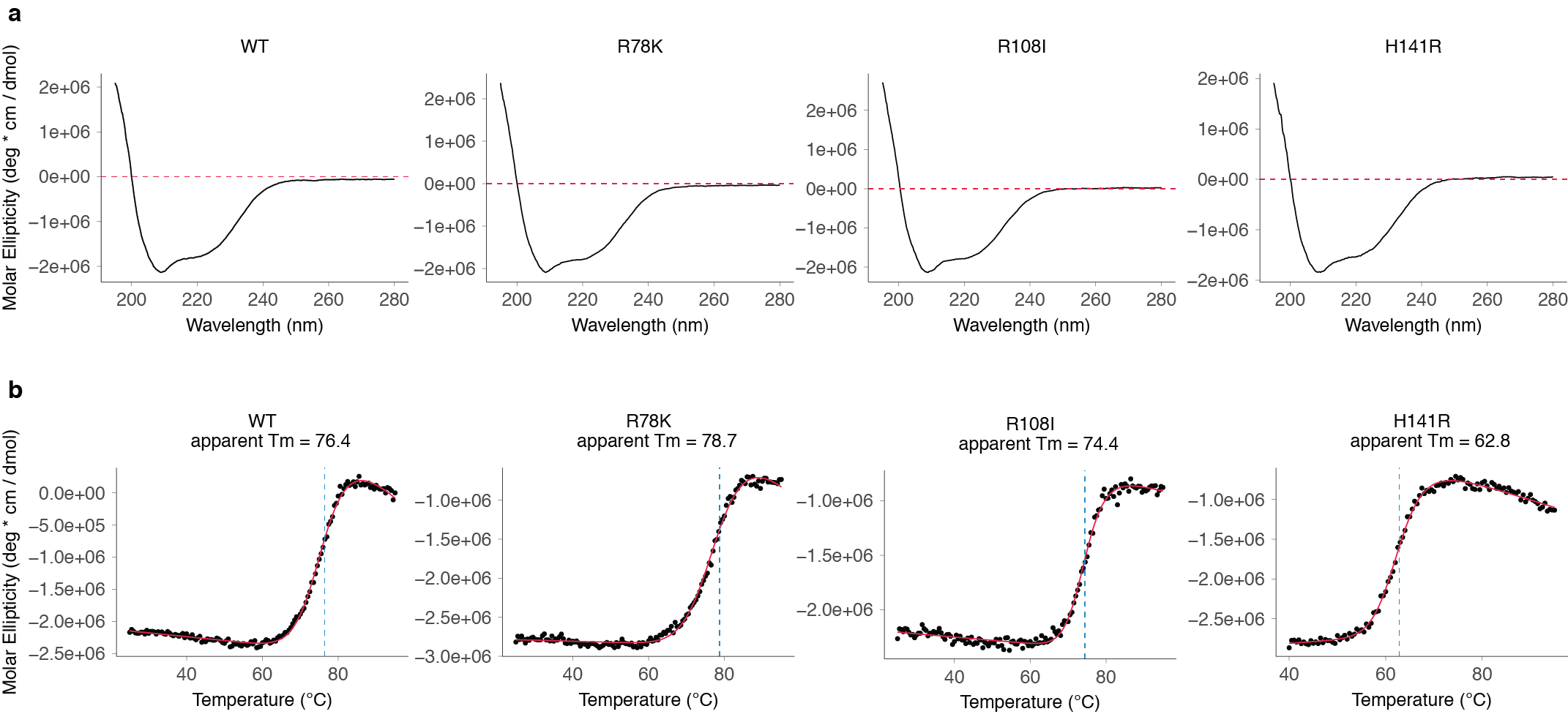
**Supplementary Figure 10 Clustering of individual AP-MS replicates** **based on correlations between protein abundance before the final scoring.** Data shown are for amino-terminally FLAG tagged wild type and Gsp1 mutants.



**Supplementary Figure 11 Clustering of individual AP-MS replicates** **based on correlations between protein abundance before the final scoring.** Data show are for carboxy-terminally FLAG tagged wild type and Gsp1 mutants.



**Supplementary Figure 12 Multiple sequence alignment between Rna1 from *S. cerevisiae* (*Rna1\_YEAST*) and *S. pombe* (*Rna1\_SCHPO*), as well as human RanGAP (*RAGP1\_HUMAN,*** excluding the C-terminal SUMO conjugation domainwhich is absent in Fungi).Overall sequence identity between *S. cerevisiae* and *S. pombe* Rna1 is 39%, with 53% sequence similarity.Interface core residues (based on the X-ray crystal structure between *S. pombe* Rna1 and mammalian Ran, PDB ID: 1k5d) are highlighted in orange. All residues except Pro108 in *S. pombe* Rna1, which corresponds to Leu122 in *S. cerevisiae* Rna1 are conserved in sequence between *S. cerevisiae* and *S. pombe* Rna1.



**Supplementary Figure 12 Circular dichroism (CD) data** for wild type Gsp1 and select mutants. **a,** CD spectra. **b,** Irreversible temperature melts.



**Supplementary Figure 13 HPLC reverse phase chromatograms** **of a GTP/GDP mix (top) and that of a purified and GTP loaded wild type Gsp1 (bottom).**

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**Supplementary Figure 14 Accuracy estimation for determining the kinetic parameters of GAP-mediated GTP hydrolysis from individual time courses spanning [S] > Km to [S] << Km fit with an accurate solution of the integrated Michaelis Menten (IMM) equation.** Each time course was simulated using the experimentally determined parameters determined from the fitted IMM model, with added Gaussian noise similar to the experimental fluorescence signal noise. The deviation from the mean is plotted against a ratio of initial substrate (Gsp1:GTP) concentration and the experimentally determined Km. Deviation from the mean is reported either as standard deviation or , where N = 100 simulations, and simulation\_param and experimental\_param are experimental and simulated kcat, Km, and kcat/Km, respectively. Here, simulated refers to the average of the fitted values for the simulated data sets.

# References

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# Supplementary Tables