targets. Experiment numbers are indicated in parentheses. **B)** Loci were sorted by their change in nucleosome occupancy upon glucose depletion. A moving average of the change in Rap1 occupancy upon glucose depletion is plotted on the y-axis. **C)** Intergenic regions were grouped into three categories based on their Rap1 binding affinity (high, intermediate, or low affinity) as measured by PBM[29]. The 306 high-affinity regions had PBM p-values less than 1×10^{-6}, the 288 intermediate affinity regions had PBM p-values between 0.01 and 1×10^{-6}, and the remaining 4945 sites were classified as low-affinity. The average change in Rap1 occupancy (x axis) was determined for each group as a function of the change in nucleosome occupancy (y axis). **D)** Low-glucose Rap1 targets are on a nucleosomal hair-trigger. Nucleosome occupancy in the presence (black) and absence (gray) of glucose is plotted for all yeast intergenic regions. Values are relative to static Rap1 targets in the presence of glucose. Intergenic regions were separated into five groups: static Rap1 targets, low-glucose Rap1 targets, unbound loci containing high-affinity Rap1 sites, unbound loci containing intermediate-affinity Rap1 sites and unbound loci containing low-affinity Rap1 sites.

Figure 6. A "nucleosomal hair-trigger" model for condition-dependent transcription factor binding through coordinated interplay between local chromatin structure and DNA-binding affinity.

See text for details. Note that rather than the typical case of chromatin remodeling proteins acting to remove a nucleosome to effect activation, in this mechanism the enzymes act to position nucleosomes into a repressive configuration. Therefore, the default state of this system is factor binding and gene activation.

Supplemental Figure 1. Rap1 protein and mRNA levels decrease as glucose is depleted.

A) 20 µg of whole-cell extract was separated by a 12% SDS-PAGE gel and probed with anti-

Rap1 antibody. **B)** mRNA levels for Rap1, as measured by microarray[43], decrease upon diauxic shift.

Supplemental Figure 2. Confirmation of ChIP-chip results. A. Representative agarose gel image of ChIP-PCR results, showing results from two genomic regions upstream of *ENB1* and *VHS1*. ChIP (IP) samples are loaded next to their corresponding input (IN). The top band in every lane is the test fragment, and the bottom band is specific to a control (YHR131C). **B.** The average ratio (IP/Input) for Rap1 IPs, during exponential growth, after depletion of glucose (72 hrs), and in a *tup1Δ* background, is plotted with their standard error at 11 loci. The ratio for any site is determined by the (intensity of the test fragment in IP / intensity of the control fragment in IP) / (intensity of the test fragment in input / intensity of the control fragment in input) **C.** The average ratio (IP/Input) for Tup1 IPs is plotted with their standard error at 11 loci. **D.** Two biological replicates of Rap1 ChIPs after glucose depletion (72 hrs) were hybridized directly on the same microarray with Rap1 ChIPs from cells grown in high glucose. The average ratios reported from probes representing the 52 low-glucose Rap1 targets, 262 static Rap1 targets, and telomeric targets is plotted, along with their standard errors.

Supplemental Figure 3. Confirmation of ChIP-chip results at the SGA1 locus.

A. The open reading frames for *SGA1* and *FMC1* are shown. An upstream dubious open reading frame is shaded grey. Arrows indicate direction of transcription. The regions tested by ChIP-PCR (A-D) and the elements on the microarray (1-7) are shown below. **B.** Rap1 IPs during exponential growth (+ glucose) as measured by the microarray (top; 8 replicates) and PCR (bottom; 4 replicates). **C.** Rap1 IPs in low glucose (72 hrs) as measured by the microarray (top; 7 replicates) and PCR (bottom; 4 replicates). **D.** Tup1 IPs during exponential growth (+ glucose) as measured by microarray (top; 8 replicate) and PCR (bottom; 3 replicates). **E.** The enrichment at microarray element 4 is shown for the entire timecourse. **F.** A

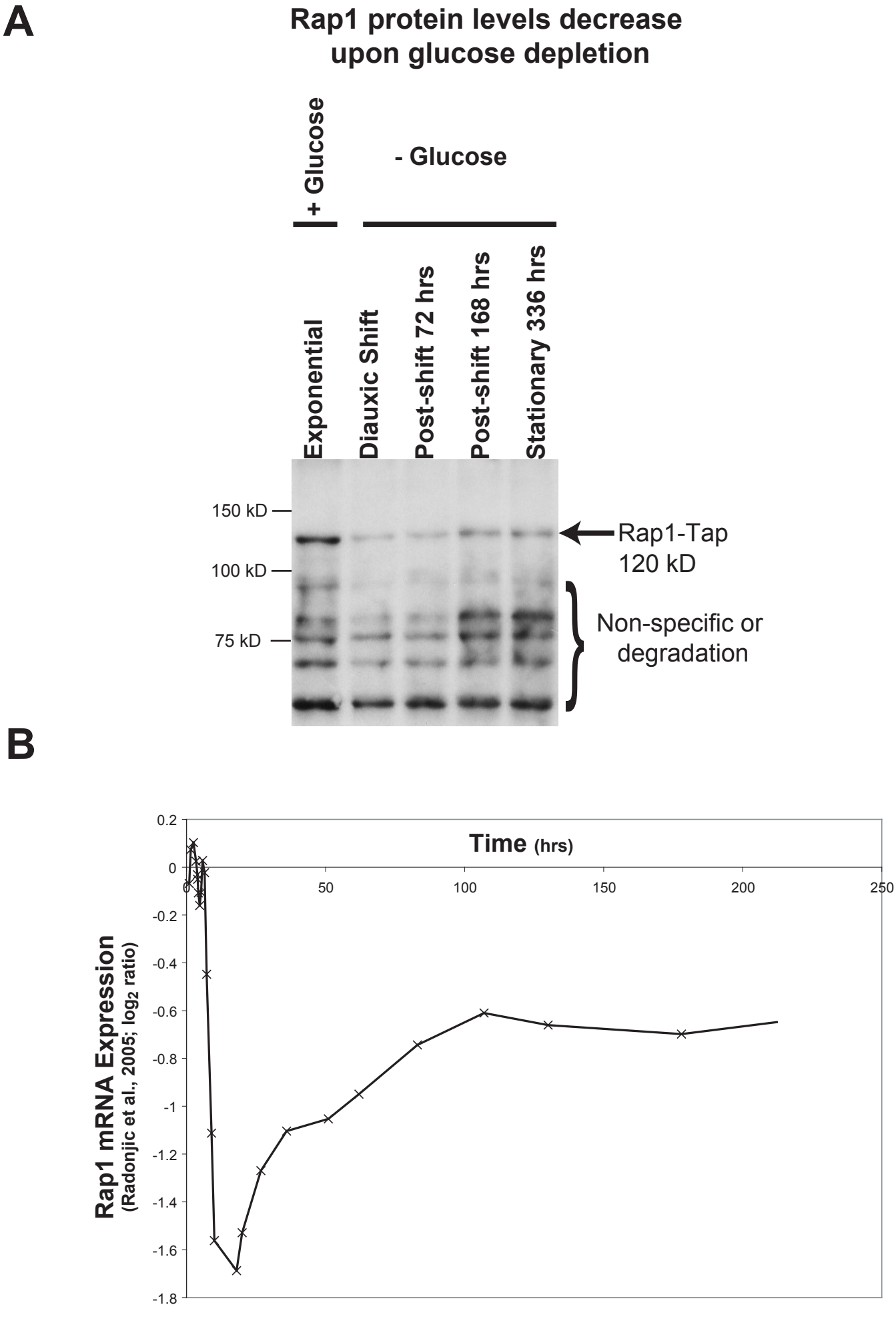
representative gel image for one replicate at each PCR region. IP (IP) samples are loaded next to their corresponding input (IN). The top band in every lane is the test fragment, which is compared to the bottom control band (YHR131C).

Supplemental Figure 4. Computational screens predict the involvement of Tup1-Ssn6 proteins in blocking Rap1p binding. **A.** Schematic representation of two computational screens for effector of Rap1 conditional binding. Screen 1 compares low-glucose targets versus static targets and screen 2 compares low-glucose targets versus all yeast intergenic regions. **B.** Results for the two computational screens listed by Pearson correlation (r) to the classifier variable. The p-value was estimated by 10,000 permutations. The GO biological process is listed for each protein (lower row).

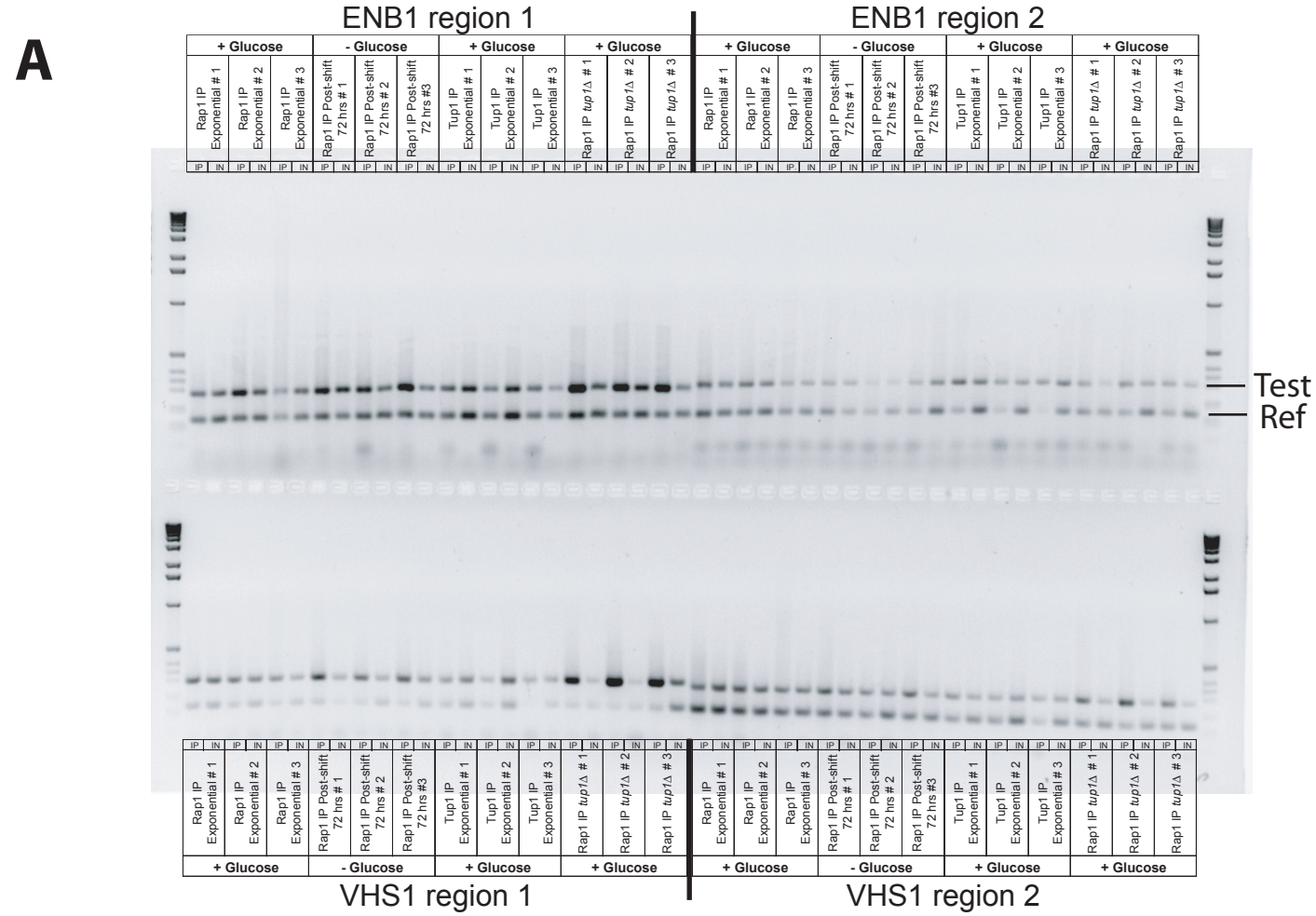
Supplemental Figure 5. Rap1 binding and glycolysis

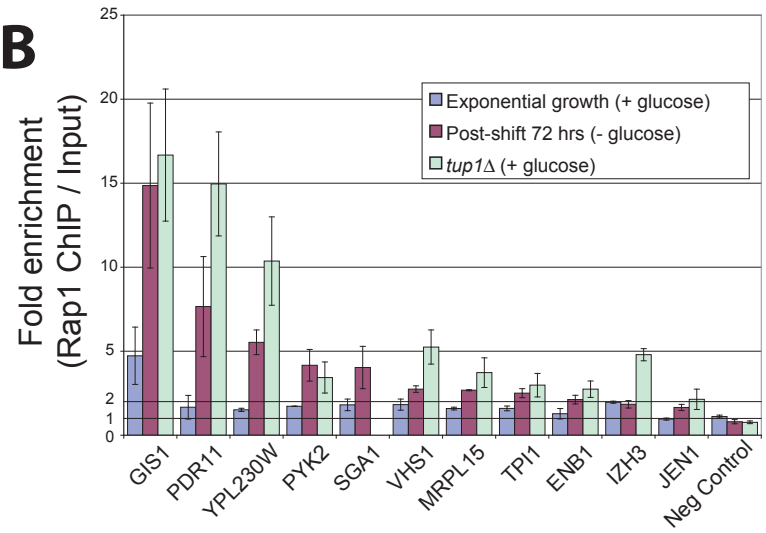
A) Rap1 binds upstream of at least one enzyme involved in each of the eleven steps required to metabolize glucose into ethanol. Static Rap1 targets are shown in red and low-glucose targets are in blue. Arrows indicate the flow of substrate. **B)** During growth in low-glucose, Rap1 binds upstream of genes involved in alternative carbon-source utilization.

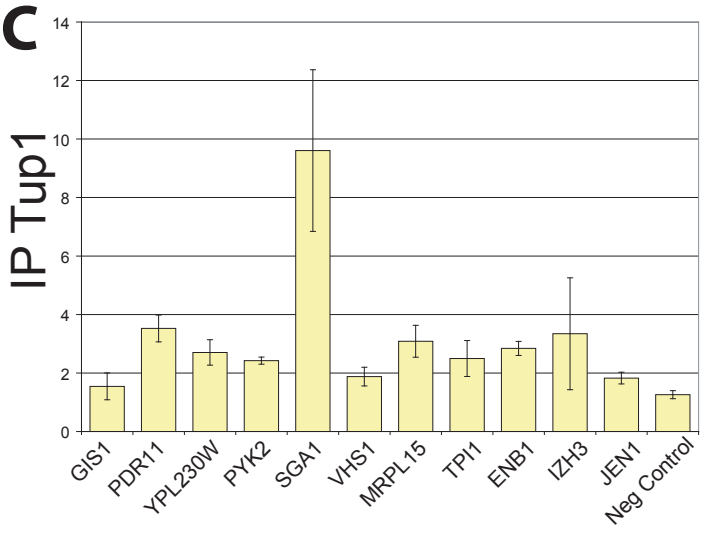
Supplemental Figure 1. Buck and Lieb

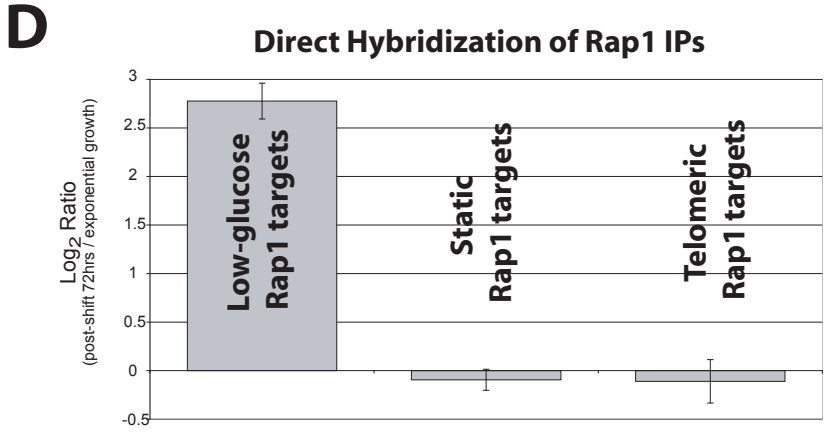


Supplemental Figure 2. Buck and Lieb

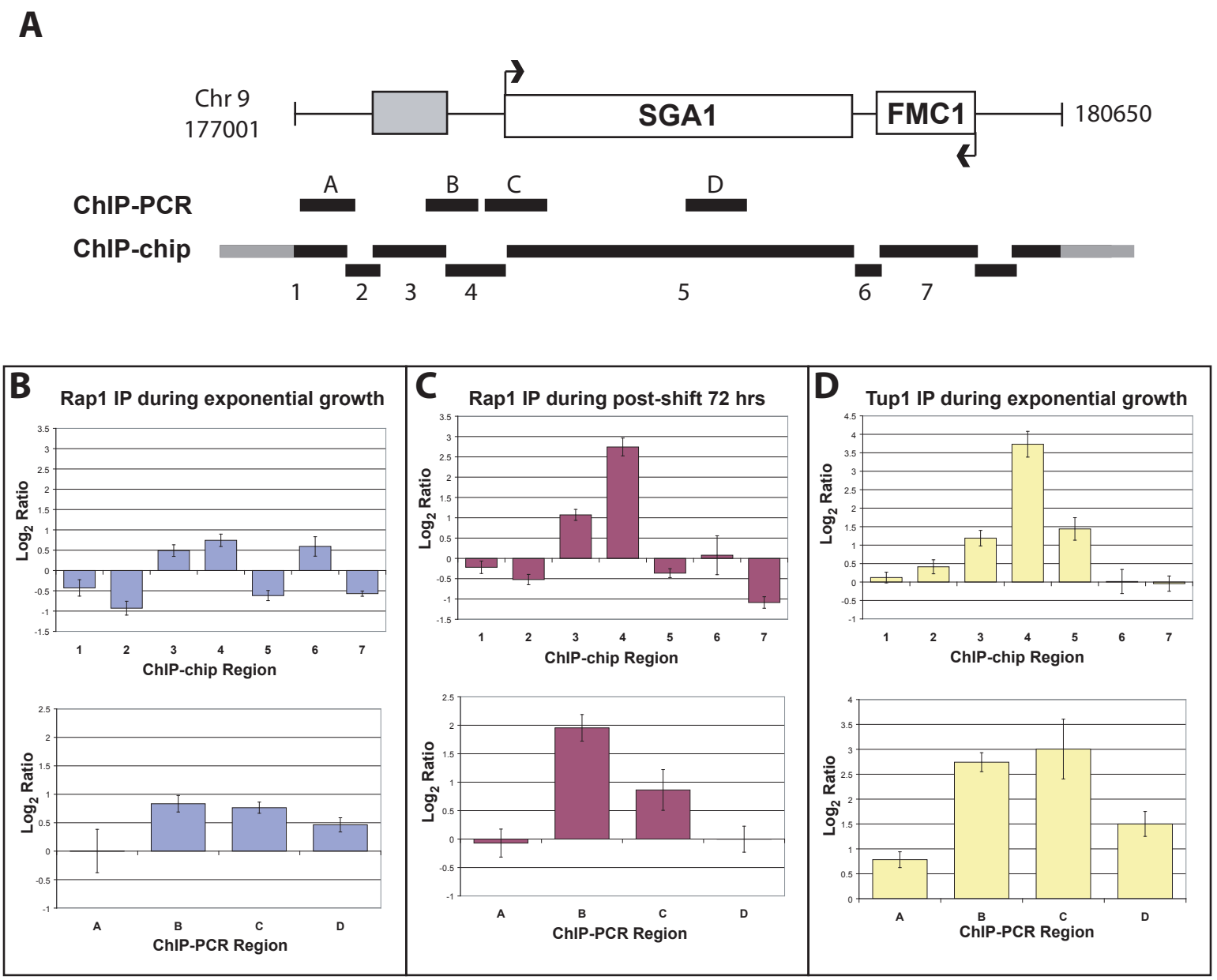


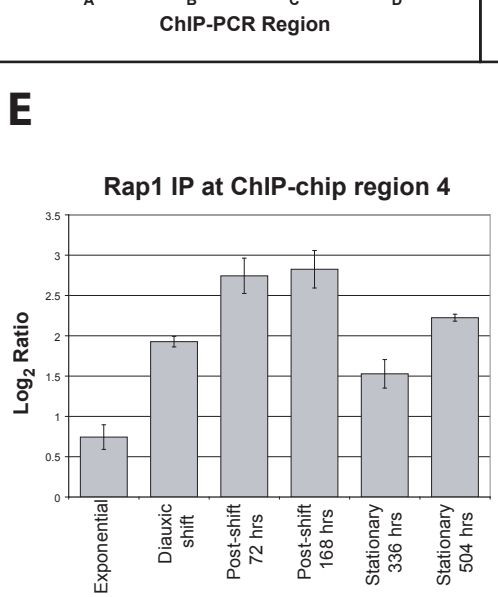


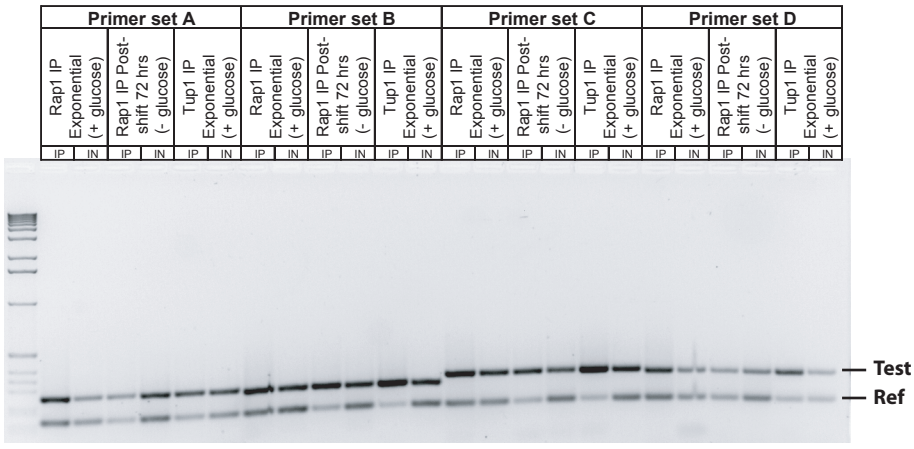




Supplemental Figure 3. Buck and Lieb

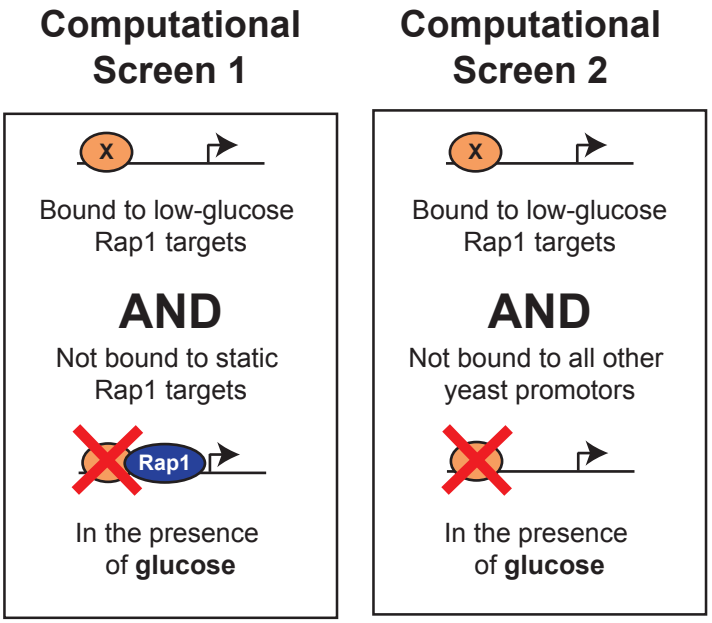






Supplemental Figure 4. Buck and Lieb

A



B

Computational Screen 1

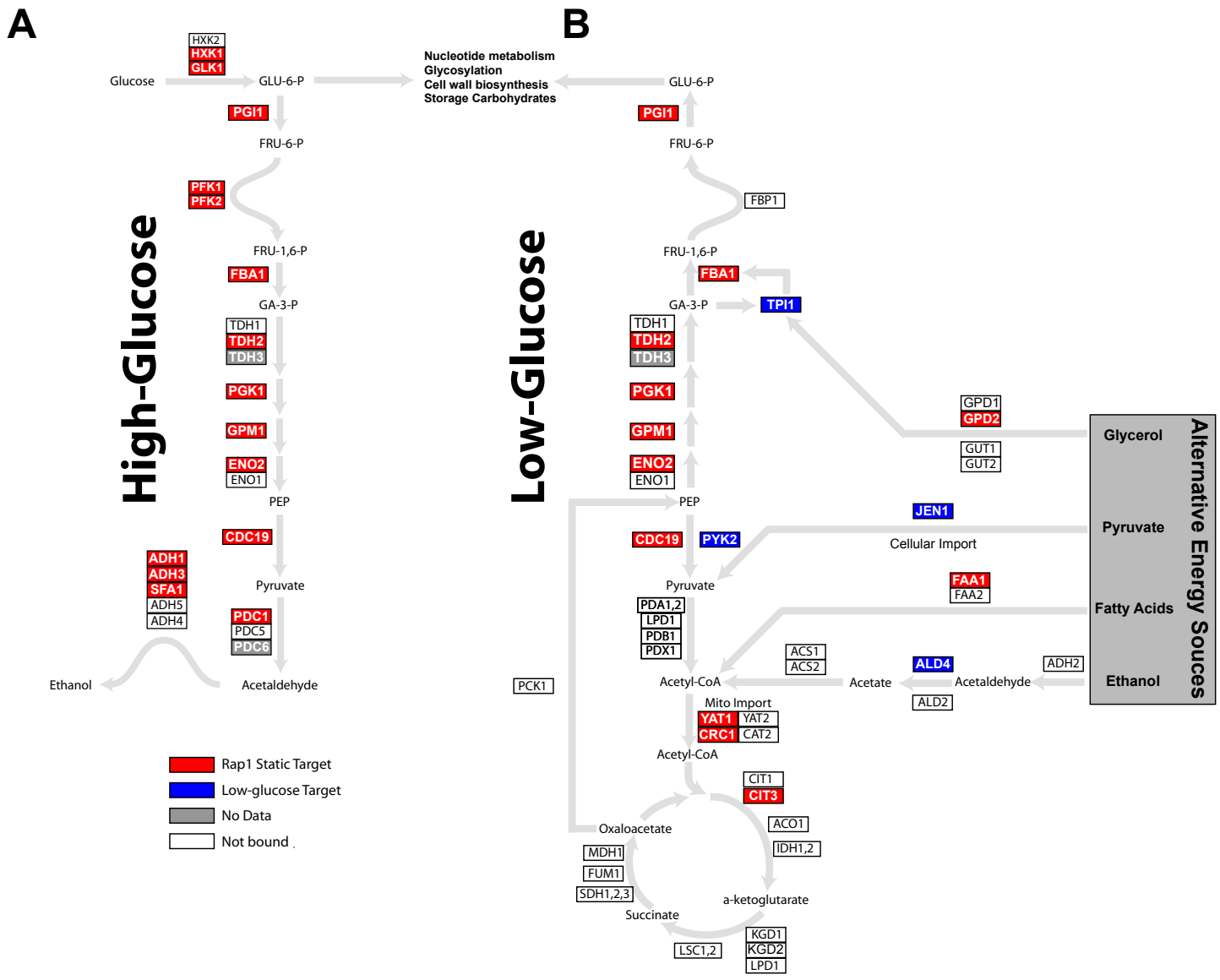
	Description	r
Mig1	Tup1 associated factor Glucose metabolism	0.25 **
Sut1	Tup1 associated factor Sterol transport	0.23 **
Gat1	Regulation of nitrogen utilization	0.23 **
Nrg1	Tup1 associated factor Glucose metabolism	0.23 **
Dot6	Chromatin silencing at telomere	0.22 **

Computational Screen 2

	Description	r
Sut1	Tup1 associated factor Sterol transport	0.14 **
Swi4	G1/S transition of mitotic cell cycle	0.09 **
Nrg1	Tup1 associated factor Glucose metabolism	0.09 **
Phd1	Pseudohyphal growth	0.09 **
Sko1	Tup1 associated factor Negative regulation of transcription	0.07 *

** $p < 0.00001$; * $p < 0.0001$

Supplemental Figure 5. Buck and Lieb



Supplemental Table 1. Experiments performed.

Experiment Number	Array Name	Growth Condition	Protein of interest	Strain	IP Tech
1	PM028I	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 Rap1-Tap	TAP-Tag
2	PM029I	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 Rap1-Tap	TAP-Tag
3	BR046C	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 Rap1-Tap	TAP-Tag
4	BR083C	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 Rap1-Tap	TAP-Tag
5	BR111C	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 Rap1-Tap	TAP-Tag
6	BR136C	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 Rap1-Tap	TAP-Tag
7	BR047C	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 Rap1-Tap	TAP-Tag
8	BR095C	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 Rap1-Tap	TAP-Tag
9	BR107C	YPD 24hrs	Rap1	BY4741 Rap1-Tap	TAP-Tag
10	BR104C	YPD 24hrs	Rap1	BY4741 Rap1-Tap	TAP-Tag
11	PM032I	YPD 24hrs	Rap1	BY4741 Rap1-Tap	TAP-Tag
12	PM118I	YPD 24hrs	Rap1	BY4741 Rap1-Tap	TAP-Tag
13	PM119I	YPD 24hrs	Rap1	BY4741 Rap1-Tap	TAP-Tag
14	BR251C	YPD 24hrs	Rap1	BY4741 Rap1-Tap	TAP-Tag
15	BR089C	YPD 72 hrs	Rap1	BY4741 Rap1-Tap	TAP-Tag
16	PM121I	YPD 72 hrs	Rap1	BY4741 Rap1-Tap	TAP-Tag
17	BR088C	YPD 72 hrs	Rap1	BY4741 Rap1-Tap	TAP-Tag
18	BR206C	YPD 72 hrs	Rap1	BY4741 Rap1-Tap	TAP-Tag
19	PM023I	YPD 72 hrs	Rap1	BY4741 Rap1-Tap	TAP-Tag
20	PM025I	YPD 72 hrs	Rap1	BY4741 Rap1-Tap	TAP-Tag
21	BR145C	YPD 72 hrs	Rap1	BY4741 Rap1-Tap	TAP-Tag
22	BR103C	YPD 168 hrs	Rap1	BY4741 Rap1-Tap	TAP-Tag
23	PM031I	YPD 168 hrs	Rap1	BY4741 Rap1-Tap	TAP-Tag
24	PM120I	YPD 168 hrs	Rap1	BY4741 Rap1-Tap	TAP-Tag
25	BR244C	YPD 168 hrs	Rap1	BY4741 Rap1-Tap	TAP-Tag
26	BR250C	YPD 168 hrs	Rap1	BY4741 Rap1-Tap	TAP-Tag
27	BR102C	YPD 336 hrs	Rap1	BY4741 Rap1-Tap	TAP-Tag
28	PM030I	YPD 336 hrs	Rap1	BY4741 Rap1-Tap	TAP-Tag
29	BR144C	YPD 336 hrs	Rap1	BY4741 Rap1-Tap	TAP-Tag
30	PM073I	YPD 336 hrs	Rap1	BY4741 Rap1-Tap	TAP-Tag
31	PM074I	YPD 504 hrs	Rap1	BY4741 Rap1-Tap	TAP-Tag
32	BR142C	YPD 504 hrs	Rap1	BY4741 Rap1-Tap	TAP-Tag
33	BR108C	YPD Expontential Growth (OD 0.6-1.0)	Mock	BY4741	TAP-Tag
34	BR246C	YPD Expontential Growth (OD 0.6-1.0)	Mock	BY4741	TAP-Tag
35	BR153C	YPD 24hrs	Mock	BY4741	TAP-Tag
36	PM099I	YPD 24hrs	Mock	BY4741	TAP-Tag
37	BR053C	YPD 72 hrs	Mock	BY4741	TAP-Tag
38	PM097I	YPD 72 hrs	Mock	BY4741	TAP-Tag
39	BR151C	YPD 168 hrs	Mock	BY4741	TAP-Tag
40	PM096I	YPD 168 hrs	Mock	BY4741	TAP-Tag
41	GSM050	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741	AB Y-300
42	PM093I	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741	AB Y-300
43	PM122I	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741	AB Y-300
44	PM110C	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 *sut* 1Δ	AB Y-300
45	PM109C	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 *sut* 1Δ	AB Y-300
46	GSM047	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 *sut* 1Δ	AB Y-300
47	PM111I	YPD 24hrs	Rap1	BY4741 *sut* 1Δ	AB Y-300
48	PM112I	YPD 24hrs	Rap1	BY4741 *sut* 1Δ	AB Y-300
49	PM113I	YPD 72 hrs	Rap1	BY4741 *sut* 1Δ	AB Y-300
50	PM114I	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 *mig* 1Δ	AB Y-300
51	PM115I	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 *mig* 1Δ	AB Y-300
52	PM116I	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 *mig* 1Δ	AB Y-300

53	BR190C	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 *nrg* 1Δ	AB Y-300
54	GSM254	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 *nrg* 1Δ	AB Y-300
55	GSM049	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 *nrg* 1Δ	AB Y-300
56	GSM216	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 *sko* 1Δ	AB Y-300
57	GSM217	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 *sko* 1Δ	AB Y-300
58	GSM226	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 *sko* 1Δ	AB Y-300
59	GSM099	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 *dot6* Δ	AB Y-300
60	BR061C	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 *dot6* Δ	AB Y-300
61	GSM101	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 *dot6* Δ	AB Y-300
62	GSM195	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 *dot6* Δ	AB Y-300
63	GSM230	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 *gat1* Δ	AB Y-300
64	GSM098	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 *gat1* Δ	AB Y-300
65	GSM048	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 *gat1* Δ	AB Y-300
66	BR101C	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 *tup1* Δ	AB Y-300
67	BR198C	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 *tup1* Δ	AB Y-300
68	GSM196	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 *tup1* Δ	AB Y-300
69	GSM201	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 *tup1* Δ	AB Y-300
70	BR195C	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 *tup1* Δ	AB Y-300
71	GSM148	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 *isw2* Δ	AB Y-300
72	GSM149	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 *isw2* Δ	AB Y-300
73	GSM100	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 *isw2* Δ	AB Y-300
74	GSM151	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 *hda1* Δ	AB Y-300
75	GSM198	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 *hda1* Δ	AB Y-300
76	BR062C	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 *hda1* Δ	AB Y-300
77	GSM102	YPD Expontential Growth (OD 0.6-1.0)	Rap1	BY4741 *hda1* Δ	AB Y-300
78	GSM152	YPD Expontential Growth (OD 0.6-1.0)	Tup1	BY4741 Tup1-Tap	TAP-Tag
79	GSM154	YPD Expontential Growth (OD 0.6-1.0)	Tup1	BY4741 Tup1-Tap	TAP-Tag
80	BR063C	YPD Expontential Growth (OD 0.6-1.0)	Tup1	BY4741 Tup1-Tap	TAP-Tag
81	GSM155	YPD Expontential Growth (OD 0.6-1.0)	Tup1	BY4741 Tup1-Tap	TAP-Tag
82	YOI-N-174	YPD Expontential Growth (OD 0.6-1.0)	Tup1	BY4741 Tup1-Tap	TAP-Tag
83	YOI-N-185	YPD Expontential Growth (OD 0.6-1.0)	Tup1	BY4741 Tup1-Tap	TAP-Tag
84	YOI-N-183	YPD Expontential Growth (OD 0.6-1.0)	Tup1	BY4741 Tup1-Tap	TAP-Tag
85	YOI-N-191	YPD Expontential Growth (OD 0.6-1.0)	Tup1	BY4741 Tup1-Tap	TAP-Tag
86	BR060C	YPD 72 hrs	Tup1	BY4741 Tup1-Tap	TAP-Tag
87	GSM104	YPD 72 hrs	Tup1	BY4741 Tup1-Tap	TAP-Tag
88	YOI-N-173	YPD 72 hrs	Tup1	BY4741 Tup1-Tap	TAP-Tag
89	GSM146	YPD 72 hrs	Tup1	BY4741 Tup1-Tap	TAP-Tag
90	YOI-N-219	YPD 72 hrs	Tup1	BY4741 Tup1-Tap	TAP-Tag
91	YOI-N-220	YPD 72 hrs	Tup1	BY4741 Tup1-Tap	TAP-Tag
92	YOI-N-192	YPD Expontential Growth (OD 0.6-1.0)	Histone H3	BY4741	AB H3
93	YOI-N-134	YPD Expontential Growth (OD 0.6-1.0)	Histone H3	BY4741	AB H3
94	YOI-N-202	YPD Expontential Growth (OD 0.6-1.0)	Histone H3	BY4741	AB H3
95	YOI-N-193	YPD 72 hrs	Histone H3	BY4741	AB H3
96	YOI-N-138	YPD 72 hrs	Histone H3	BY4741	AB H3
97	YOI-N-201	YPD 72 hrs	Histone H3	BY4741	AB H3
98	YOI-N-136	YP EtOH Expontential Growth (OD 0.6-1.0)	Rap1	BY4741	AB Y-300
99	YOI-N-117	YP EtOH Expontential Growth (OD 0.6-1.0)	Rap1	BY4741	AB Y-300
100	YOI-N-141	YP EtOH Expontential Growth (OD 0.6-1.0)	Rap1	BY4741	AB Y-300
101	YOI-N-119	YP EtOH Expontential Growth (OD 0.6-1.0)	Rap1	BY4741	AB Y-300
102	BR059C	YPD Expontential Growth (OD 0.6-1.0)	Sut1	BY4741 Sut1-Tap	TAP-Tag
103	YOI-N-089	YPD Expontential Growth (OD 0.6-1.0)	Sut1	BY4741 Sut1-Tap	TAP-Tag
104	YOI-N-075	YPD Expontential Growth (OD 0.6-1.0)	Sut1	BY4741 Sut1-Tap	TAP-Tag
105	YOI-N-200	YPD Expontential Growth (OD 0.6-1.0)	Sut1	BY4741 Sut1-Tap	TAP-Tag
106	BR100C	YPD 72 hrs	Sut1	BY4741 Sut1-Tap	TAP-Tag
107	JDL_g_024_I	YPD Expontential Growth (OD 0.6-1.0)	Rap1	S288C	AB

108	JDL_g_109_I	YPD Expontential Growth (OD 0.6-1.0)	Rap1	S288C	AB
109	JDL_g_124_I	YPD Expontential Growth (OD 0.6-1.0)	Rap1	S288C	AB
110	JDL_g_128_I	YPD Expontential Growth (OD 0.6-1.0)	Rap1	S288C	AB
111	JDL_g_132_I	YPD Expontential Growth (OD 0.6-1.0)	Rap1	S288C	AB
112	JDL_g_084_I	YPD 24hrs	Rap1	S288C	AB
113	JDL_g_095_I	YPD 24hrs	Rap1	S288C	AB
114	JDL_g_093_I	YPD 72 hrs	Rap1	S288C	AB
115	JDL_g_096_I	YPD 72 hrs	Rap1	S288C	AB
116	JDL_g_125_I	YPD 336 hrs	Rap1	S288C	AB
117	JDL_g_126_I	YPD 336 hrs	Rap1	S288C	AB