

## Supplemental Information

### Pairing competitive and topologically distinct regulatory modules enhances patterned gene expression

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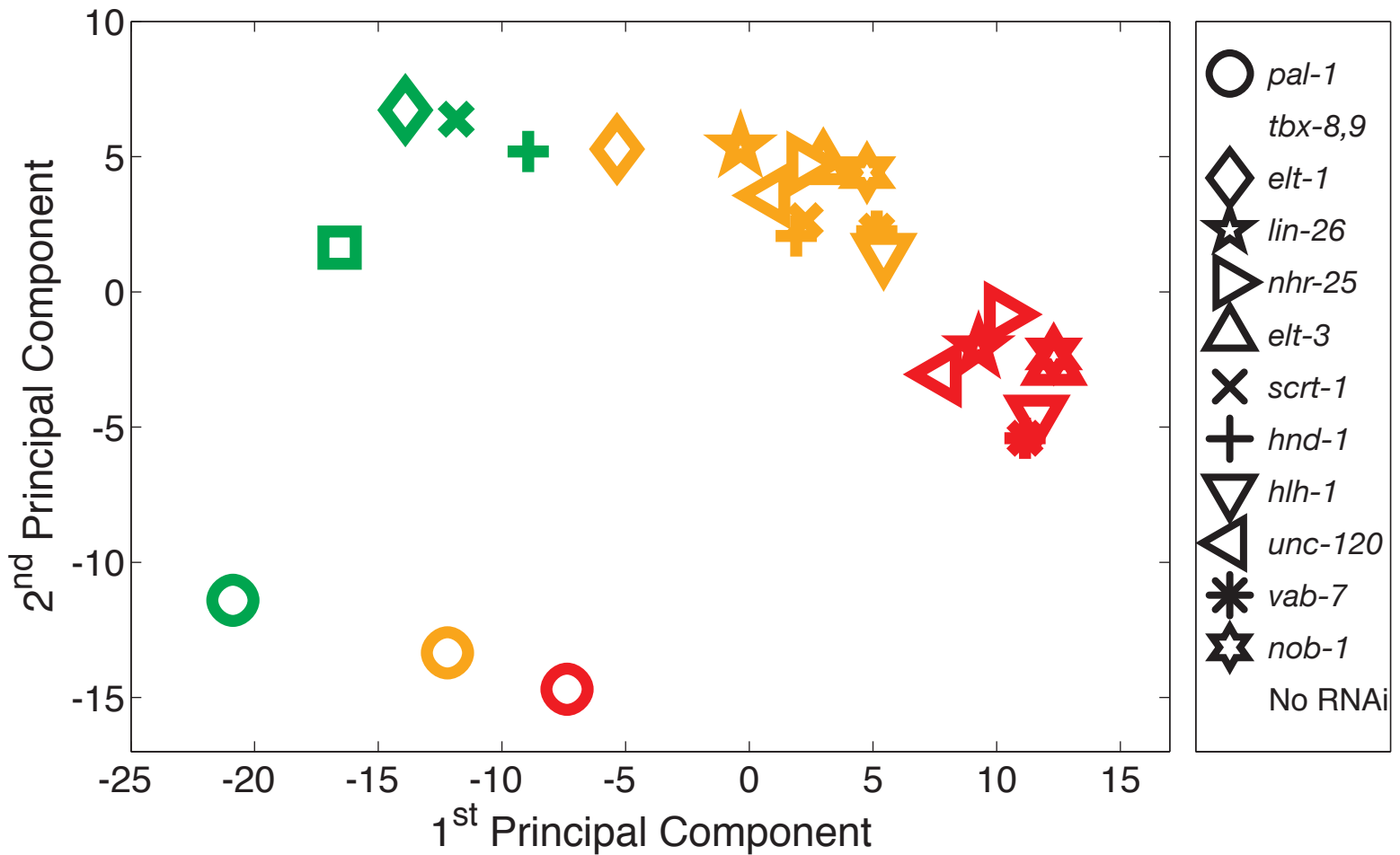
Supplementary Table 1. Perturbation matrix data.

Supplementary Figure 1. Principal components analysis (PCA) of the effect of each TF knockdown at different stages upon C-lineage enriched genes.

Supplementary Figure 2. Reproducibility and strength of yeast-1-hybrid interactions.

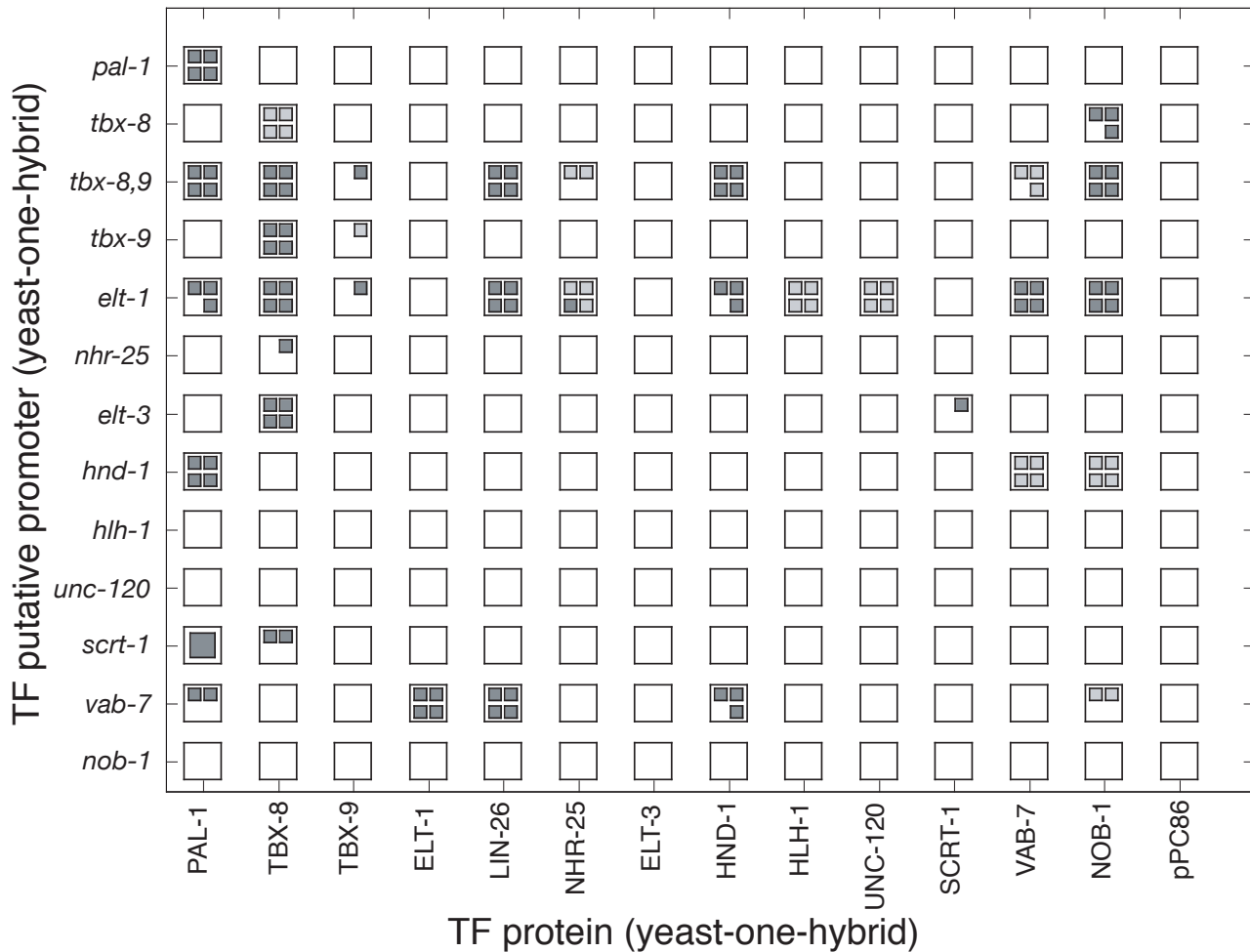
**Supplementary Table 1. Perturbation matrix data**

TF expression	Stage (number of C cells)	MEAN -no perturbation ( <i>mex-3</i> -/-)	1SD - no perturbation ( <i>mex-3</i> -/-)	<i>pal-1</i>	<i>tbx-8,9</i>	<i>elt-1</i>	<i>lin-26</i>	<i>nhr-25</i>	<i>elt-3</i>	<i>hnd-1</i>	<i>hlh-1</i>	<i>unc-120</i>	<i>scrt-1</i>	<i>vab-7</i>	<i>nob-1</i>
	16C	32C	48C	16C	32C	48C	16C	32C	48C	16C	32C	48C	16C	32C	48C
<i>pal-1</i>	16C	1402	173.3	42	548	1670	NA	NA	NA	1395	NA	NA	1732	NA	NA
	32C	1022	199	50	NA	883	1249	1071	1276	889	926	1178	860	770	1202
	48C	441	167	184	NA	NA	504	603	379	NA	307	517	NA	287	426
<i>tbx-9</i>	16C	210	46.8	128	63	125	NA	NA	NA	223	NA	NA	132	NA	NA
	32C	85	47.5	114	NA	69	120	124	143	87	109	97	93	164	88
	48C	155	22	81	NA	NA	74	103	110	NA	130	141	NA	108	66
<i>elt-1</i>	16C	69	10.6	74	114	31	NA	NA	NA	124	NA	NA	270	NA	NA
	32C	62	15.9	49	NA	16	112	87	131	72	123	96	53	91	129
	48C	72	2	92	NA	NA	121	107	100	NA	107	90	NA	61	112
<i>lin-26</i>	16C	98	8.4	43	60	76	NA	NA	NA	240	NA	NA	149	NA	NA
	32C	256	29.8	192	NA	103	60	268	315	337	506	334	254	352	421
	48C	212	110.2	288	NA	NA	84	262	375	NA	345	274	NA	349	395
<i>nhr-25</i>	16C	21	0.1	11	15	20	NA	NA	NA	28	NA	NA	28	NA	NA
	32C	25	5.8	15	NA	18	32	18	47	32	50	39	43	29	47
	48C	16	1.3	21	NA	NA	32	15	22	NA	18	19	NA	17	24
<i>elt-3</i>	16C	78	10.2	67	41	41	NA	NA	NA	147	NA	NA	77	NA	NA
	32C	162	13.5	60	NA	43	184	294	238	288	465	287	284	304	277
	48C	669	273.2	51	NA	NA	542	657	402	NA	714	716	NA	799	614
<i>hnd-1</i>	16C	42	31	8	17	47	NA	NA	NA	11	NA	NA	65	NA	NA
	32C	73	2.3	13	NA	105	91	99	120	7	67	88	58	80	100
	48C	60	1.5	9	NA	NA	41	47	47	NA	20	30	NA	20	35
<i>hlh-1</i>	16C	65	21.7	3	20	254	NA	NA	NA	216	NA	NA	76	NA	NA
	32C	304	13.7	14	NA	188	244	161	231	158	21	223	171	117	200
	48C	61	3.7	20	NA	NA	138	118	95	NA	26	107	NA	81	98
<i>unc-120</i>	16C	1195	84.7	2340	1299	3828	NA	NA	NA	1136	NA	NA	773	NA	NA
	32C	1090	30.7	2956	NA	1391	1059	725	1261	780	733	685	1581	2808	514
	48C	1246	27.1	1576	NA	NA	347	601	698	NA	1731	930	NA	1197	1326
<i>scrt-1</i>	16C	32	15.9	10	16	11	NA	NA	NA	23	NA	NA	11	NA	NA
	32C	9	0.9	11	NA	7	6	8	9	10	7	8	14	8	7
	48C	14	1.7	13	NA	NA	5	10	5	NA	7	7	NA	7	6
<i>vab-7</i>	16C	61	37.1	5	7	13	NA	NA	NA	20	NA	NA	26	NA	NA
	32C	27	8.9	5	NA	33	34	22	35	45	16	16	9	13	29
	48C	21	1.4	5	NA	NA	33	11	15	NA	21	38	NA	5	12
<i>nob-1</i>	16C	68	8.1	23	45	169	NA	NA	NA	223	NA	NA	100	NA	NA
	32C	230	15.5	70	NA	177	330	337	293	243	408	420	267	177	111
	48C	211	27.7	96	NA	NA	267	187	190	NA	136	246	NA	108	27



**Supplementary Figure 1. Principal components analysis (PCA) of the effect of each TF knockdown at different stages upon C-lineage enriched genes**

A matrix was constructed for the expression intensities of C lineage enriched genes (453 probesets, Baugh et al. 2005a) across the 27 samples indicated in the plot. Principal components analysis was computed on this matrix using the Matlab 'princomp' command and the scores of the first two components are plotted here. The first component (50.4% of the variation) distinguishes developmental time (color), while the second component (18.2% of the variation) appears to distinguish failure of the network by *pal-1*(RNAi) and *tbx-8,9* (RNAi) from the other perturbations.



Supplementary Figure 2. **Reproducibility and strength of yeast-1-hybrid interactions.** For each transcription factor/promoter region pair (see exceptions below), four colonies were patched onto HIS- selective medium or tested for  $\beta$ -gal activity, as appropriate to the reporter used for each promoter region tested (see Experimental Procedures). Each subdivided square presents the results for all four patches, where each subdivision represents a single patch. Dark grey indicates robust growth/ $\beta$ -gal staining, light grey indicates weak growth/staining, and white indicates a lack of growth/staining detectable above background. For several combinations with the *scr-1* promoter (PAL-1, SCRT-1, ELT-1, TBX-8, and UNC-120) yeast were plated onto selective medium immediately after transformation and assayed for growth as colonies. A portion of each transformation was plated onto non-selective medium to confirm viability of cells after transformation. Of the combinations tested in this way, only PAL-1 allowed growth on selective medium.