Supplementary Information

Full Methods

Combinatorial Cloning

All constructs were cloned using a newly developed strategy for combinatorial cloning based on the type IIs restriction enzyme AarI, and adapted from (s*1*) and described in Fig. S1. AarI recognizes a 7bp sequence but cuts 4 bases away from it, leaving a 4-base sticky end. In particular, [N] blocks were cloned in "donor" plasmids flanked by AarI sites with the 4-base sticky ends CCCT (so called "B") and GCGA (so called "C"). [C] blocks were cloned in donor plasmids flanked by AarI sites with sticky ends "C" and TGCG (so called "D"). Combinatorial recombination libraries were created by ligating, in a single step, an [N] and a [C] block into an acceptor plasmid flanked by sticky ends B and D. Single domain duplication libraries were cloned into B-C or C-D acceptor plasmids, gene duplication libraries were cloned into B-D acceptor libraries. All constructs were expressed in yeast, from CEN/ARS plasmids. B-D and B-C acceptors carried a Leu marker, C-D acceptors carried a His marker. In all cases, clones were expressed using a minimal CycI promoter and an AdhI transcription terminator.

Flow cytometry

For flow cytometry experiments we used a W303-derived strain with the following genotype: *MATa, bar1::NatR, far1*Δ*, mfa2::pFUS1-GFP, his3, trp1, leu2, ura3.* Analysis

of pathway-dependent GFP expression by flow cytometry was performed as follows: triplicate cultures were grown to early log phase (OD600=0.05-0.1) in complete synthetic dropout media. At time 0, cultures were treated with 1 μM α-factor (Zymo Research) to activate the pathway. In initial screenings, 50 μL aliquots were taken at time 0 and after 2 hr and were immediately treated with 10 μL of cycloheximide (30 μg/mL), and dispensed into 96-well culture plates. For detailed analysis of pathway activation, aliquots were taken 30 min before addition of α-factor, at time 0, and then every 30 min for 2 hr. In all cases, following incubation at room temperature for 30 min in the dark to allow for GFP fluorophore maturation, plates containing treated cultures were analyzed with a BD LSR-II flow cytometer (BD Biosciences) using a high-throughput sampler. 10,000 cells were counted for each reading, and GFP fluorescence was measured with excitation at 488 nm using a 100 mW Coherent Sapphire laser. Baselines and slopes were averaged for all triplicate cultures and standard deviation were calculated. All experiments were repeated at least twice and baseline and slopes measured in different experiments were found to be in good agreement.

Quantitative Mating Assays

Triplicate cultures of "a-type" (Strain SO992, W303-derived, trp1, leu2, ura3, his3, ADE2 can1 (s*2*)) transformants harboring appropriate plasmids encoding each recombination variant to be tested, were grown at 30°C to mid-log phase (OD600 = 0.5). 1 x10^6 "a-type" cells were mixed with 5 x10^6 "α-type" cells on the surface of a polycarbonate filter and incubated for 3.5 hr at 30°C. Mating efficiency was calculated as described (s*3*), averages from triplicates and standard deviations were calculated.

Growth Assays

Duplicate 5 ml cultures were grown overnight and diluted next morning to O.D = 0.1 in a volume of 30 ml of fresh synthetic drop-out media in 250 ml flasks. Fresh cultures were then incubated at 30°C with shaking at 240RPM, and O.D. were measured every 30 min for about 7 hours. O.D. values were then fitted to a single exponential of the form: $x = x_0 * e^{kt}$, where x is the number of cells at time t, x_0 is the initial number of cells and k is the growth rate. Experiments were repeated twice and found to be in excellent agreement.

Fluorescence Microscopy

All variants analyzed by fluorescence microscopy were labeled N-terminally with GFP. Note that we analyzed all GFP-labeled variants by flow cytometry to confirm that they affected mating pathway response dynamics as their parental non-labeled clones (data not shown). Cultures were grown to early log phase in complete synthetic dropout media. 1 ml samples at OD600=0.1 were sonicated for 2 sec using a Fisher Scientific model 500 sonicator with a 2mm tip at a setting of 11%. 20 μL samples were dispensed in concavalin A-coated wells in 384-well plates and spun down for 1 min at 1500 rpm (500xg). Imaging was performed with an automated inverted Nikon microscope with Perfect Focus using a 60× oil immersion lens (Nikon, Tokyo, Japan). α-factor was added, at time 0, to a final concentration of 1 μM. Images were taken before and 60min after addition of pheromone.

Supplementary Figure 1: Combinatorial cloning strategy based on the Type IIs restriction enzyme AarI.

We developed a strategy to facilitate the simultaneous cloning of multiple fragments in a single step, adapted from a method described by Guet et al. (s*1*). In particular, we used the Type IIs restriction enzyme AarI, which recognizes a 7bp sequence but cuts 4bp away from the recognition site, leaving 4bp sticky ends of the desired sequence. We used this to our advantage, designing unique non-palindromic sticky ends to facilitate the ligation of multiple fragments in a single step. [N] blocks were cloned in "donor" plasmids flanked by AarI sites with the 4-base sticky ends CCCT (so called "B") and GCGA (so called "C"). [C] blocks were cloned in donor plasmids flanked by AarI sites with sticky ends "C" and TGCG (so called "D"). Combinatorial recombination libraries were created by ligating, in a single step, an [N] and a [C] block into an acceptor plasmid flanked by sticky ends B and D. Single domain duplication libraries were cloned into B-C or C-D acceptor plasmids, gene duplication libraries were cloned into B-D acceptor libraries.

Supplementary Figure 2: Random mutagenesis of a duplicated Ste50 does not substantially alter mating pathway response beyond loss of function.

To explore the hypothesis that phenotypic divergence could be mediated by gene duplication followed by sequence divergence by point mutations, we constructed a random point mutation library of Ste50 by error-prone PCR. We chose Ste50 because it is the gene that most often led to phenotypic changes when recombined in our experiments. As shown in panel A, the mutational load is in the range of 2-6 substitutions per gene, with a fairly comparable number of transitions and transversions, all well distributed

along the gene. B, the resulting baseline and slope pairs of the mating response for 50 point mutation variants are shown as green circles. As comparison, we have overlaid as geometric shapes the extent to which ge duplication (black line), domain duplication (orange line), co-expression of all pairs of domain duplications (red line) and domain recombination (blue line) affect the mating pathway response. Our results indicate that about 90% of the analyzed Ste50 point mutants did not alter pathway response, while the remaining 10% led to inhibition of pathway response. This observation is consistent with a model in which point mutations of a duplicated gene most often lead to no change in function (one copy of the duplicated gene is non-functionalized –it losses its function- or both copies are subfunctionalized –in a multi-domain protein the function of one domain is lost). Only very rarely does point mutation leads to neo-functionalization (a new function is acquired by point mutations). In the case of Ste50, mutations that decouple the N-terminal SAM domain from the C-terminal domain, while keeping the SAM domain fully functional, might lead to the inhibition of the mating pathway (as discussed before, the SAM domains of Ste50 and Ste11 might act as dominant negatives).

Supplementary Figure 3: Criteria used to select recombination chimeras for further analysis.

We chose recombination chimeras for detailed analysis according to the following criteria: (i) the (baseline, slope) values of the recombination chimera should differ in a substantial manner from the (baseline, slope) values of the corresponding co-expression pair; and (ii) from those of the WT strain. For that, we first calculated the distance in (baseline, slope) morphospace between corresponding recombination-coexpression pairs

(shown as vectors in morphospace representation in Fig. S3A). We then calculated the distribution of distances (histogram in Fig. S3B) and chose, for further analysis, the recombination variants in the top 20% of the distribution (which follows a power law) according to the Pareto Principle. Fig. S3C shows the distance vectors moved to the origin of coordinates (transformed morphospace); vectors outside of the yellow circle correspond to the top 20% of the distribution. The recombination variants selected are shown in real morphospace in Fig. S3D.

Supplementary Figure 4: Time courses of mating pathway activation measured by flow cytometry for the 10 recombination variants chosen for further analysis.

In all cases, dashed black traces correspond to WT strain and green traces correspond to the specific variant analyzed.

Supplementary Figure 5: The fitness cost of pleiotropic effects could be balanced by gains in mating efficiency.

A, activation of the mating response induces Far1-mediated cell cycle arrest, so high basal activation of the pathway could impair growth (s*4, s5*). In fact, one variant (Ste50 [N]-Ste18 [C]) could not be transformed into cells containing far1, consistent with its high level of basal mating pathway activation. We found that there is no correlation between mating efficiency and growth rate, though we observed a slight correlation between growth rates and baseline levels of pathway activation (data not shown). Interestingly, some clones with growth differences of only 2-3% from WT mate up to ~3 times better than WT. This suggests that the cost in asexual growth likely imposed by

recombination-induced network remodeling might be compensated for by the benefit in mating fitness it confers. B, to further explore this hypothesis, we have simulated the evolutionary dynamics between two strains, WT and Ste5 [N]-Ste11 [C]. WT has a growth-rate advantage of ~3% per generation, whereas Ste5 [N]-Ste11 [C] has a mating advantage of ~3-fold per round of mating. When these two strains are mixed and propagated with rounds of mating every15 generations (Fig. S5B, left panel), WT strain prevails and slowly becomes predominant (yellow trace). In contrast, when cells mate every 10 generations (Fig. S5B, right panel) Ste5 [N]-Ste11 [C] strain prevails and becomes predominant (blue trace). C, the yeast mating pathway shares several proteins with the high osmolarity pathway; thus, variants that alter the mating response could also affect the response to high osmolarity stimuli or lead to inappropriate cross-talk. We tested these possibilities for the ten recombination variants that more substantially affected mating response. In particular, we transformed these variants into a yeast strain carrying a fluorescenct reporter of high osmolarity pathway activation (mCherry controlled by a STL1 promoter) and measured the osmolarity pathway response upon stimulation with 0.4M KCl. As shown in Fig. S5C, variants that substantially affect mating response (as illustrated by the blue contour in morphospace) only marginally affect the response to high osmolarity, with most baseline and slope values clustered near those of the wild type strain. In addition, we observed that none of the analyzed variants led to inappropriate mating to osmolarity or osmolarity to mating cross-talks (data not shown). These results suggest that recombination of domains involved in multiple pathways does not necessarily lead to detectable phenotypic changes in all pathways. In the particular case of the high-osmolarity response, we might hypothesize that the ability

of this pathway to perfectly adapt, as well as the presence of multiple upstream branches might buffer hypothetical effects triggered by the presence of the chimeric proteins.

Supplementary Figure 6: Dose Response & Cell-to-Cell Variation

Signaling responses are often characterized by both their dynamics of temporal activation, as well as by the specific dose response profile: whereas some pathways follow a graded dose response, others have switch like activation profiles. To explore whether domain recombination could also alter the dose response profile of the mating pathway, we measured pathway response at different concentrations of pheromone for two of the domain recombination variants that most markedly affected the mating pathway temporal response. In particular, yeast strains were stimulated with pheromone and GFP levels were measured after two hours by flow cytometry as described above (Fig. S6A). We found that expression of the domain recombination variant Ste5 [N]-Ste11 [C] shifted the dose response curve towards slightly lower concentrations of pheromone. In addition, we observed that domain recombination could lead to small changes in the variability of the response among cells in a clonal population, measured as the coefficient of variation (CV) of the distribution of the GFP fluorescence signal (Fig. S6B; upper panels depicts histograms of GFP fluorescence before and 2 hs after induction with pheromone, lower panel depicts CV calculated for the GFP fluorescence distributions, values were averaged for two independent experiments). In particular, cells expressing domain recombination variant Ste5 [N]-Ste11 [C] have a wider GFP fluorescence distribution and larger CV values. Taken together, these resutls suggest that

domain recombination might also alter the sensitivity of the mating pathway to pheromone levels.

Supplementary Figure 7: Protein abundance does not correlate with phenotypic effects.

We investigated whether phenotypic effects could simply depend on the abundance of a particular variant. In particular, we labeled the N-termini of seven domain recombination variants and four domain duplication variants with GFP and measured GFP fluorescence levels by flow cytometry as a proxy to estimate protein levels at steady state. We observed that there is no clear correlation between protein abundance and phenotypic effects, though we noticed that single domain variants appeared to be present at higher levels than domain recombination variants.

Supplementary Figure 8: Annotated map of domain architecture and function for the11 mating pathway genes used in this study.

In all cases, targeting/regulatory domains are shown in purple and catalytic domains in orange; whereas [N]-terminal blocks are shown as yellow boxes and [C]-terminal blocks as pink boxes.

Supplementary Figure 9: Putative mechanisms by which the analyzed domain recombination variants might alter mating pathway response.

Supplementary References

s1. C. C. Guet, M. B. Elowitz, W. Hsing, S. Leibler, *Science* **296**, 1466 (May 24, 2002).
s2. S. M. O'Rourke, I. Herskowitz, *Mol Cell Biol* **22**, 4739 (Jul, 2002).
s3. G. F. Sprague, Jr., *Methods Enzymol* **194**, 77 (1991).
s4. M. Peter, A. Gartner, J. Horecka, G. Ammerer, I. Herskowitz, *Cell* **73**, 747 (May 21, 1993).
s5. M. J. Winters, R. E. Lamson, H. Nakanishi, A. M. Neiman, P. M. Pryciak, *Mol Cell* **20**, 21 (Oct 7, 2005).

Supplementary List of Sequences

All constructs were expressed under control of a ~250bp fragment of the constitutive CycI promoter and include the N-t adaptor sequence (present in the vector used for combinatorial cloning):

ATGCTCGAGTCCCTA

Sequences of the different blocks used:

GpaI [C]
GGGTGTACAGTGAGTACGCAAACAATAGGAGACGAAAGTGATCCTTTTCTAC
AGAACAAAAGAGCCAATGATGTCATCGAGCAATCGTTGCAGCTGGAGAAAC
AACGTGACAAGAATGAAATAAAACTGTTACTATTAGGTGCCGGTGAGTCAGG
TAAATCAACGGTTTTAAAACAATTAAAATTATTACATCAAGGCGGTTTCTCCC
ATCAAGAAAGGTTACAGTATGCTCAAGTGATATGGGCAGATGCCATACAATC
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ATGATCCGATCAACAATAAAGATTTGTTTGCATGCAAGAGAATACTGCTAAA
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GATGCTGATAGAAACAACAGTAGTAGAATTAACCTACAGGATATTTGCAAGG
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AATTCAAGGACAAAATAGACGAAATCTTATTCACGAAGACATTGCTAAGGCA
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CTAATGAGTTTCAATTGGAGGGCTCAGCTGCATACTACTTTGATAACATTGAG
AAATTTGCTAGTCCGAATTATGTCTGTACGGATGAAGACATTTTGAAGGGCC
GTATAAAGACTACAGGCATTACAGAAACCGAATTTAACATCGGCTCGTCCAA
ATTCAAGGTTCTCGACGCTGGTGGGCAGCGTTCTGAACGTAAGAAGTGGATT
CATTGTTTCGAAGGAATTACAGCAGTTTTATTTGTTTTAGCAATGAGTGAATA
CGACCAGATGTTGTTTGAGGATGAAAGAGTGAACAGAATGCATGAATCAATA
ATGCTATTTGACACGTTATTGAACTCTAAGTGGTTCAAAGATACACCGTTTAT

TTTGTTTTTAAATAAAATTGATTTGTTCGAGGAAAAGGTAAAAAGCATGCCCA
TAAGAAAGTACTTTCCTGATTACCAGGGACGTGTCGGCGATGCAGAAGCGGG
TCTAAAATATTTTGAGAAGATATTTTTGAGCTTGAATAAGACAAACAAACCA
ATCTACGTGAAACGAACCTGCGCTACCGATACCCAAACTATGAAGTTCGTAT
TGAGTGCAGTCACCGATCTAATCATCCAGCAAAACCTTAAAAAAATTGGTAT
TATATGA

Ste4 [N]
GCAGCACATCAGATGGACTCGATAACGTATTCTAATAATGTCACCCAACAGT
ATATACAACCACAAAGTCTACAGGATATCTCTGCAGTGGAGGATGAAATTCA
AAATAAAATAGAGGCCGCCAGACAAGAGAGTAAACAGCTTCATGCTCAAAT
AAATAAAGCAAAACACAAGATACAAGATGCAAGCTTATTCCAGATGGCCAA
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CAATTTTCAAAGGACATACTTGCTATATTTCTGACATTGAATTTACAGATAAC
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GCTGTGGATCAGACGGGTATACTTACATATGGGATAGCAGATCTCCGTCCGC
TGTACAAAGCTTTTACGTTAACGATAGTGATATTAATGCACTTCGTTTTTTCA
AAGACGGGATGTCGATTGTTGCAGGAAGTGACAATGGTGCGATAAATATGTA
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CAGGTTATCAATAG

Ste18 [C]
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CGAACTTAACAATAAACTGAGGAAAGAACTCAGCCGTGAAAGAATTACTGCT
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CATTACCAGAACTATGGGGCTACCCCGTAGCAGGATCAAATCATTTTATAGA
GGGTTTGAAAAATGCTCAAAAAAATAGCCAAATGTCAAACTCAAATAGTGTT
TGTTGTACGCTTATGTAA

Ste5 [N]
ATGGAAACTCCTACAGACAATATAGTTTCCCCTTTTCACAATTTTGGTAGCTC
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GAGAAGCCCAGTACTCTATCCCCATTGTCAAGAGGAAAAAAATGGACGGAA
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CCCCAAGGGCCGAAAATTTATCAGATAATATACCACCCAAGGTCGCTCCATT
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Ste5 [C]
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Ste50 [N]
ATGGAGGACGGTAAACAGGCCATCAATGAGGGATCAAACGATGCTTCGCCG
GATCTGGACGTGAATGGCACAATATTGATGAATAATGAAGACTTTTCCCAGT
GGTCGGTTGATGATGTGATAACTTGGTGTATATCCACGCTGGAGGTGGAAGA
AACCGATCCATTATGTCAGAGACTGCGAGAAAATGATATTGTAGGAGATCTT
TTGCCGGAATTGTGCTTGCAAGATTGCCAGGACTTGTGTGACGGTGATTTGAA
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Ste50 [C]
TCAAGCTCTTCTCCGATTAACACACATGGAGTGTCCACTACGGTACCTTCTTC
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Ste11 [N]
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Ste11 [C]
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TTTCCCAGATTTTTCTCAAATGCAAGCGATCTTCAAAATAGGCACAAACACGA
CCCCCGAGATACCTTCCTGGGCTACGTCAGAAGGAAAGAATTTCTTAAGAAA
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ATCCATGGCTGGATGCACACATAATTTGA

Ste20 [N]
AGCAATGATCCATCTGCTGTATCGGAACTACCAGACAAGGACAGTCTTGATA
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AGCAGTCACTCCAAACATAAATACCAGCAATATAACCCCGGATCATTCTGCT
GACAACACATTTTCTACCATAAATGCGTCCGAGTCAGATCACCAGTTTAATGA
CACTTTACTATCAAAACTGTCGTTAACAGATTCTACAGAAACTATAGAAAAT
AACGCGACAGTGAAGCACCAGCAGCCAGTTGCATCTTCCACAGTAAACTCGA
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CTCTAAACCATCGATGACAACCACGCCAAGACAGATCAATTCAGCTTCCCAT
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TATTCTTCCTCTTCGAAAAAAAGGAAAAGCGGTTCAAATAGTGGTACACTAA
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GGATGATAAAAGGGCCTCATCGTCGTCCAATAATTCTTCCTCATCTTCTATAA
CCACCGCTTTGAGGATATCTACGCCATACAATGCCAAGCATATCCACCATGT
GGGCGTGGACTCCAAGACTGGTGAGTACACAGGTTTGCCGGAGGAATGGGA
AAAATTGTTGACTTCTAGTGGTATTTCCAAAAGAGAACAACAGCAAAACATG
CAAGCAGTCATGGATATTGTCAAATTCTATCAGGATGTCACGGAAACAAACG
GTGAAGATAAA

Ste20 [C]
GAAGATAAAATGTTCAAGACTTTCAACACAACCACAGGATTGCCGGGAAGTC
CTCAAGTTTCAACACCGCCTGCAAACTCATTCAATAAATTTCCTCCGTCGACA
AGTGATTCGCACAATTACGGTTCCAGAACAGGTACACCAATGTCCAATCACG
TCATGTCTCCAACCTTAAATACAGATTCTAGTTCAGCAAACGGGAAATTCATA
CCAAGTAGACCGGCTCCTAAGCCCCCATCTTCTGCGTCCGCTTCAGCTCCAAT
TATAAAATCACCCGTCATGAATTCTGCCGCCAATGTTTCGCCCTTGAAGCAGA
CTCATGCACCTACAACTCCGAACAGGACCAGCCCAAACAGGTCCTCAATATC
AAGAAATGCCACTTTAAAAAAAGAGGAGCAGCCACTACCACCAATACCTCCA
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GAAGCCAGCTCAAGCAAGAAGCTTGTCTAAAGAATTAAATGAGAAAAAGAG
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CATCAATGAGATTCTGGTCATGAAGGGTAGCAAACACCCTAATATAGTTAAT
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TAAGAGCACTGTATTTAATTGCTACAAATGGTACACCCAAGTTAAAGGAACC
CGAGAATCTATCGTCAAGCTTGAAAAAATTCCTTGATTGGTGTTTATGTGTGG
AGCCCGAAGATAGAGCAAGCGCTACGGAATTGCTTCATGATGAATATATCAC
GGAGATAGCTGAAGCCAATTCCTCATTGGCCCCGCTAGTCAAGTTAGCAAGA
TTGAAGAAAGTAGCTGAGAACATGGATGCTGATGAAGATAATGACGACGAT
AACGACAACGAGCATATTAATAAGACAAACAATTGTGACGACAATAACGAT
AGCAAAGAAACCGTAAATTTGGACGTAACTGAAGATGATAAACAAAAGTAA

Cdc42 [N]
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TTCCAGGTTAGCAAGAGAACTGAAAGCAGTAAAATATGTCGAGTGTTCGGCA
CTAACACAACGCGGTTTGAAGAATGTATTCGATGAAGCTATCGTGGCCGCCT
TGGAGCCTCCTGTTATCAAGAAAAGTAAAAAATGTGCAATTTTGTAG

Cdc24 [N]
GCGATCCAAACCCGTTTTGCCTCGGGCACATCTTTATCCGATTTGAAACCAAA
ACCAAGTGCAACTTCCATCTCCATACCCATGCAAAATGTCATGAACAAGCCT
GTCACGGAACAGGACTCACTGTTCCATATATGCGCAAACATCCGGAAAAGAC
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TTAACGATGAGGAGCTTTTCACTATATCCGACGTTTTTGCCAACTCTACTTCC
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CT

Cdc24 [C]
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CAGAAAACCAACACCGACATCAGCCTCAGCAGTCTTCGAAGAAGCATAACG
AGTATGTTAAAATTATCAAGGAATTCGTTGCAACGGAAAGAAAATATGTTCA
CGATTTGGAAATTTTGGATAAATATAGACAGCAGTTATTAGACAGCAATCTA
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CATCAATCTCCGCCTCGAACATAACGGACAACAATGGCAGCCCTCACCACAG
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TTTCAGAAAATTTCAAGAACTCTATTCCAGAATCTTCCATACTCTTCAGGATA
TCATATAATAACAACTCTAATAATACCTCTAGTAGCGAGATCTTCACACTTTT
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ATTTCGAATACACATAATAACAACATTTCACCAATCACCAAGATCAAATATC
AGGACGAAGATGGGGATTTTGTTGTGTTAGGTAGCGATGAAGATTGGAATGT
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TATTGA

Ste7 [N]
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ACCAGGGACAATCTCGAATAGAAGGCCACGTGATGTCTAACATTAATGCAAT
ACAGAATAATAGCAACCTGTTTTTGCGAAGAGGCATAAAAAAAAAACTGACG
TTGGATGCGTTT

Ste7 [C]
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CGCAAAATGAACCTGTTTTAGTTCTGTCTTCTCTATCACAATCCCCGTGTGTAT
CATCATCATCATCTTTGTCCACGCCATGCATTATAGATGCGTACAGTAATAAT
TTCGGATTATCGCCATCATCCACGAATTCTACTCCCTCTACGATTCAGGGATT
GTCCAATATTGCAACACCAGTTGAAAACGAACATTCGATATCACTACCACCTT
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CATAACGATACACCTGATGGCATATTGGATTTGCTGCAACGTATTGTCAACGA
GCCTTCACCAAGATTACCCAAAGACCGTATCTATTCCAAGGAAATGACAGAT
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AAATTTAGACATTGGTGTAGAAAAATAAAATCTAAAATAAAGGAAGACAAG
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GAAAGATCAACCCATTGA

Fus3 [C]
CCAAAGAGAATTGTATACAATATATCCAGTGACTTCCAGTTGAAGTCGTTACT
GGGAGAGGGTGCATACGGTGTGGTATGTTCTGCAACGCATAAGCCCACGGGA
GAAATCGTGGCAATAAAAAAGATCGAACCATTCGATAAGCCTTTGTTCGCAT
TACGTACGCTGCGTGAAATAAAGATCCTGAAGCACTTCAAGCACGAAAATAT
CATAACAATCTTCAACATTCAACGCCCTGACTCGTTCGAAAACTTCAATGAGG
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CAGATGCTGAGTGACGATCATATACAATATTTTATATACCAAACCTTGAGAG
CAGTGAAAGTGCTGCATGGTTCGAACGTCATCCATCGTGATTTAAAGCCCTCC
AACCTTCTCATAAACTCCAACTGTGACTTGAAAGTATGTGATTTCGGTTTAGC
AAGAATCATTGACGAGTCAGCCGCGGACAATTCAGAGCCCACAGGTCAGCA
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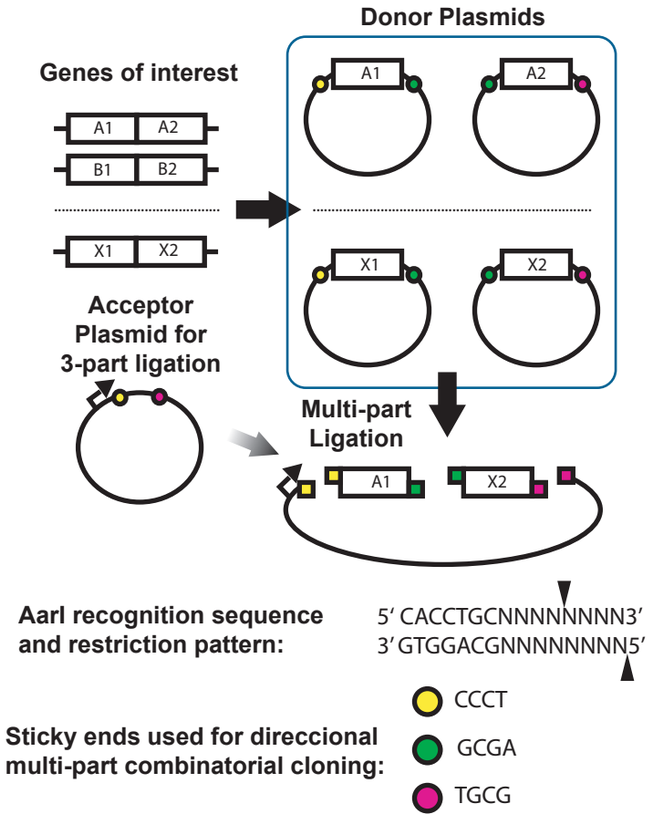
ACTGCTAAGGAGGCACTGGAGCATCCGTATTTGCAAACATACCACGATCCAA
ACGACGAACCTGAAGGCGAACCCATCCCACCCAGCTTCTTCGAGTTTGATCA
CTACAAGGAGGCACTAACGACGAAAGACCTCAAGAAACTCATTTGGAACGA
AATATTTAGTTAG

Supplementary Table 1: Mating Efficiency (relative to WT) of the analyzed recombination variants.

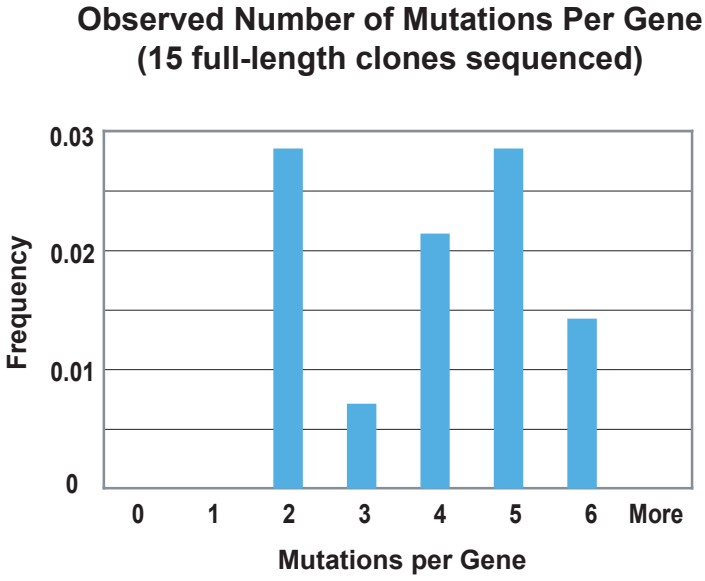
	Relative mating efficiency	Standard deviation
Ste20 [N]-Fus3 [C]	0.09	0.01
Ste50 [N]-Ste20 [C]	0.35	0.02
Ste20 [N]-Ste11 [C]	0.6	0.1
Cdc42 [N]-Ste18 [C]	1.4	0.3
Ste50 [N]-Ste11 [C]	1.7	0.3
Ste4 [N]-Ste5 [C]	1.76	0.01
Ste50 [N]-Cdc24 [C]	1.8	0.2
Ste5 [N]-Ste11 [C]	3.2	0.9
Ste50 [N]-Ste7 [C]	3.30	0.07

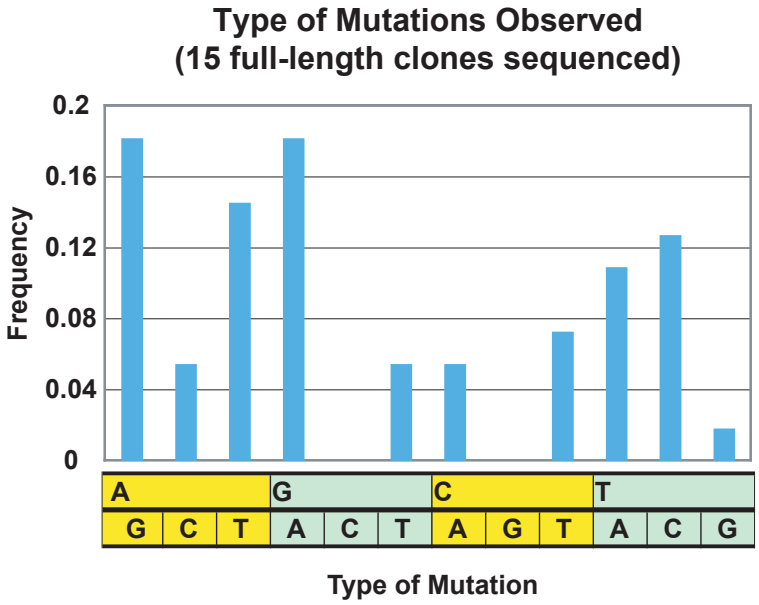
A

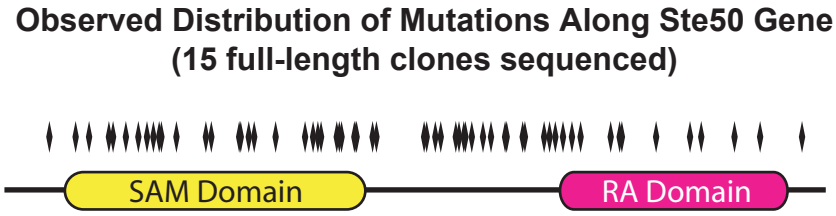
Strategy for Multi-Part Combinatorial Cloning of Domain Recombination Libraries



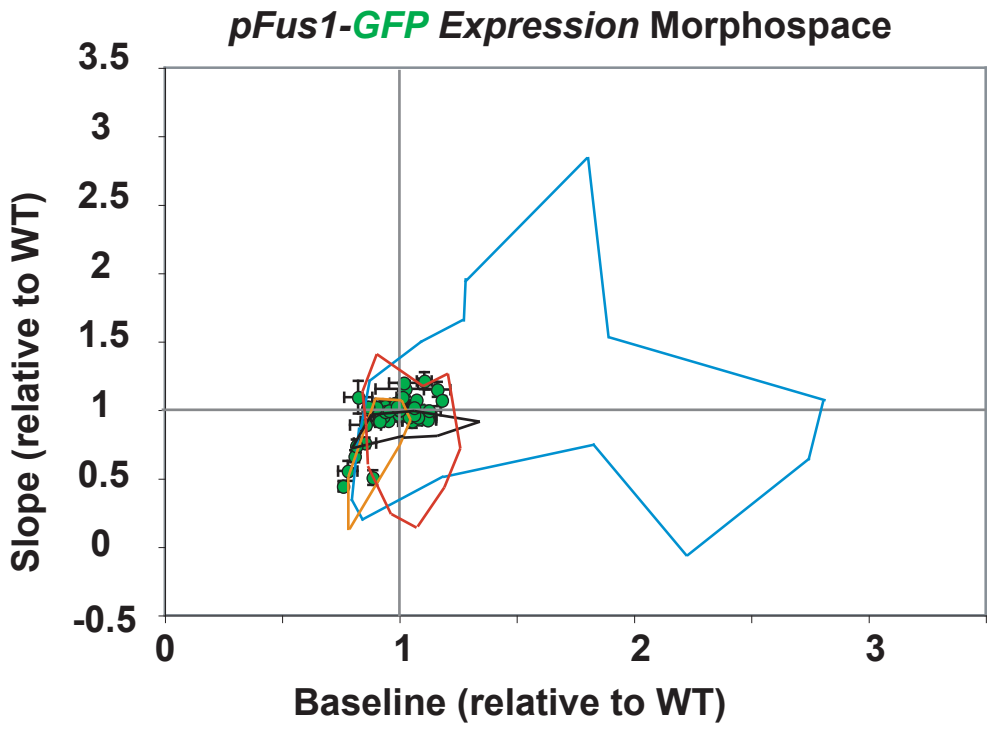
A Characterization of the Random Point Mutation Ste50 Library







B

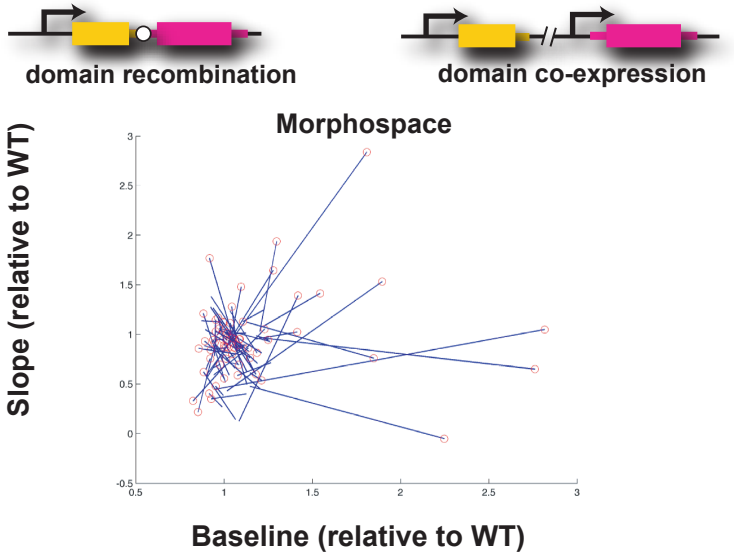


Recombination Chimeras differing from WT and from the corresponding co-expression variants were chosen for further analysis

Supplementary Figure 3

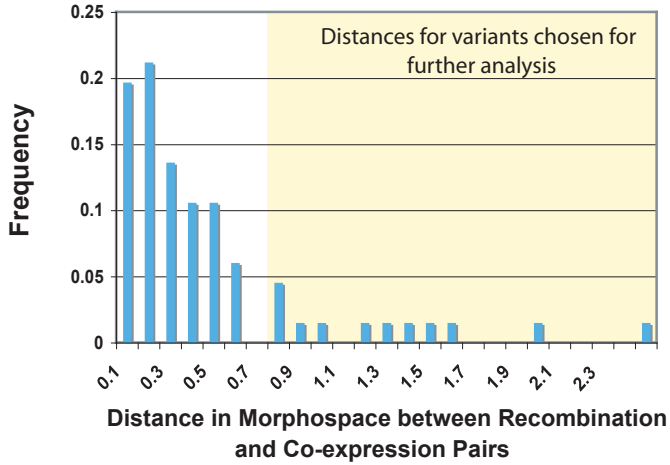
A

Distance in Morphospace between corresponding Recombination and Co-expression Pairs. For every vector, the (slope,baseline) values of the recombination variant is marked with a circle.



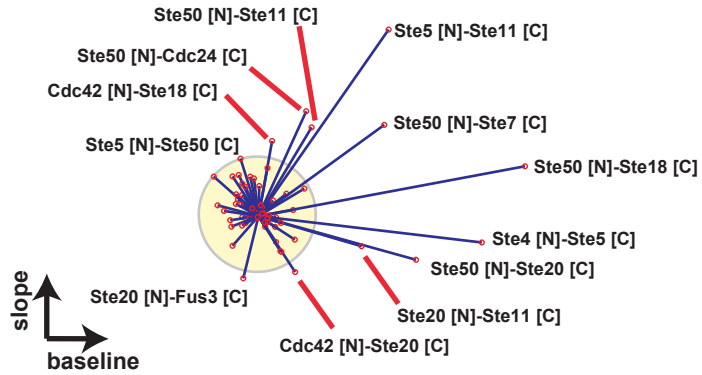
B

Distribution of distances in Morphospace Top 20% of the distribution where chosen for further analysis according to the Pareto Principle.



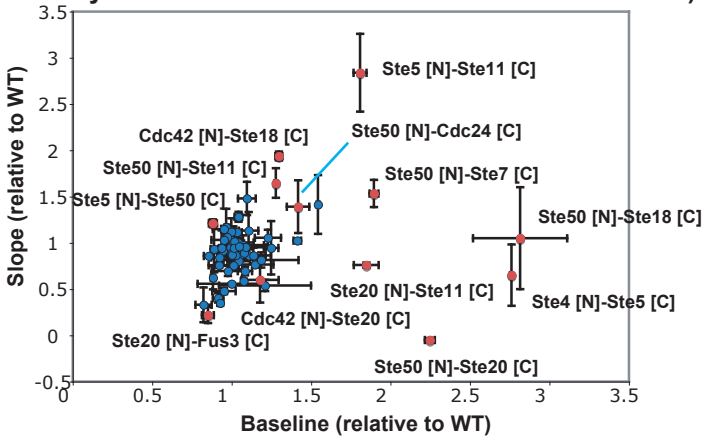
C

Transformed Morphospace (all vectors moved to the origin of coordinates vectors outside of the yellow circle correspond to the top 20% of the distribution shown in the histogram)

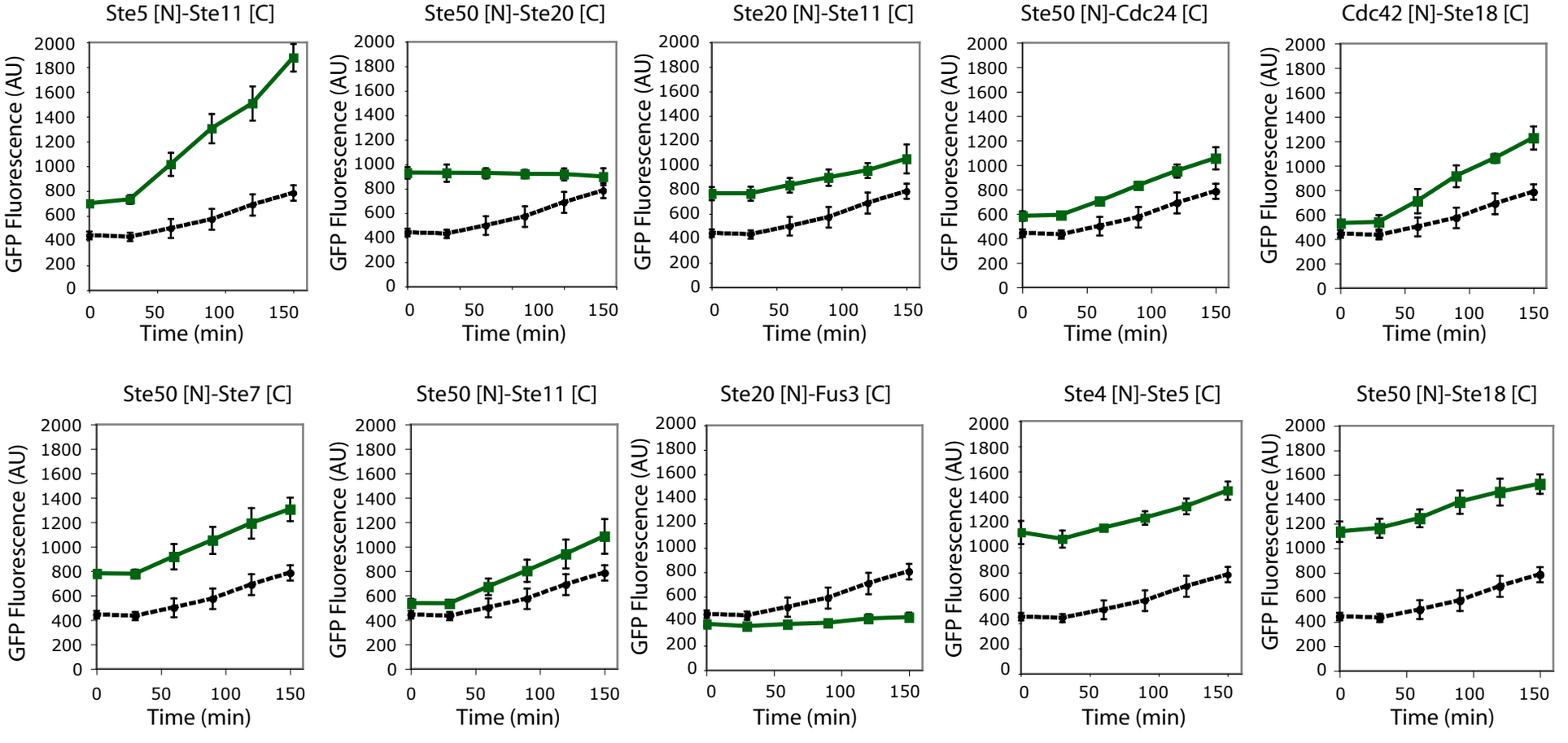


D

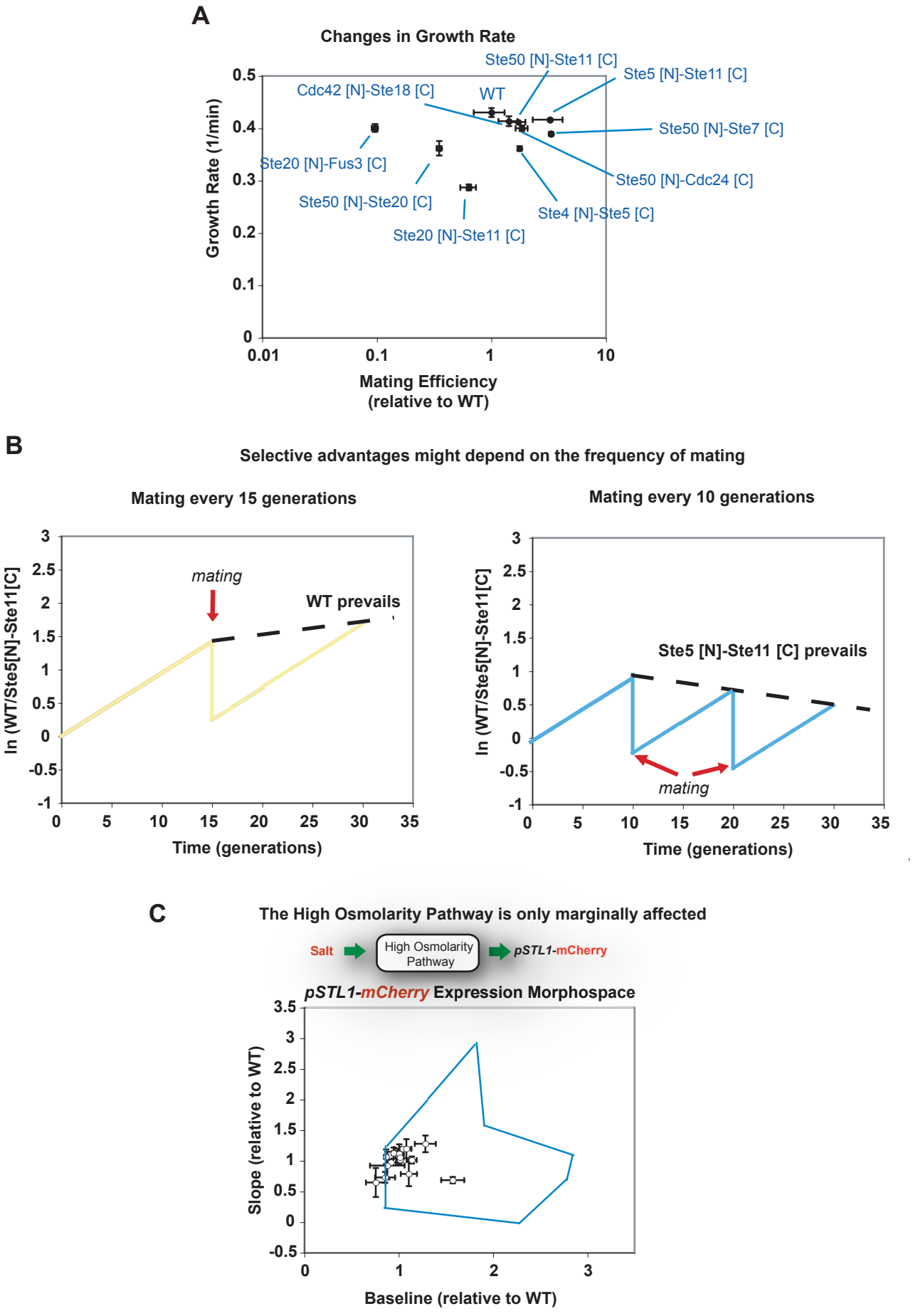
Location on Morphospace of variants chosen for further analysis (Variants Ste5[N]-Ste50[C] and Cdc42[N]-Ste20[C] were not analyzed for they do not differ in a substantial manner form WT)



Time Courses of Mating Pathway Activation Measured by Flow Cytometry
In all cases, dashed black trace corresponds to WT strain



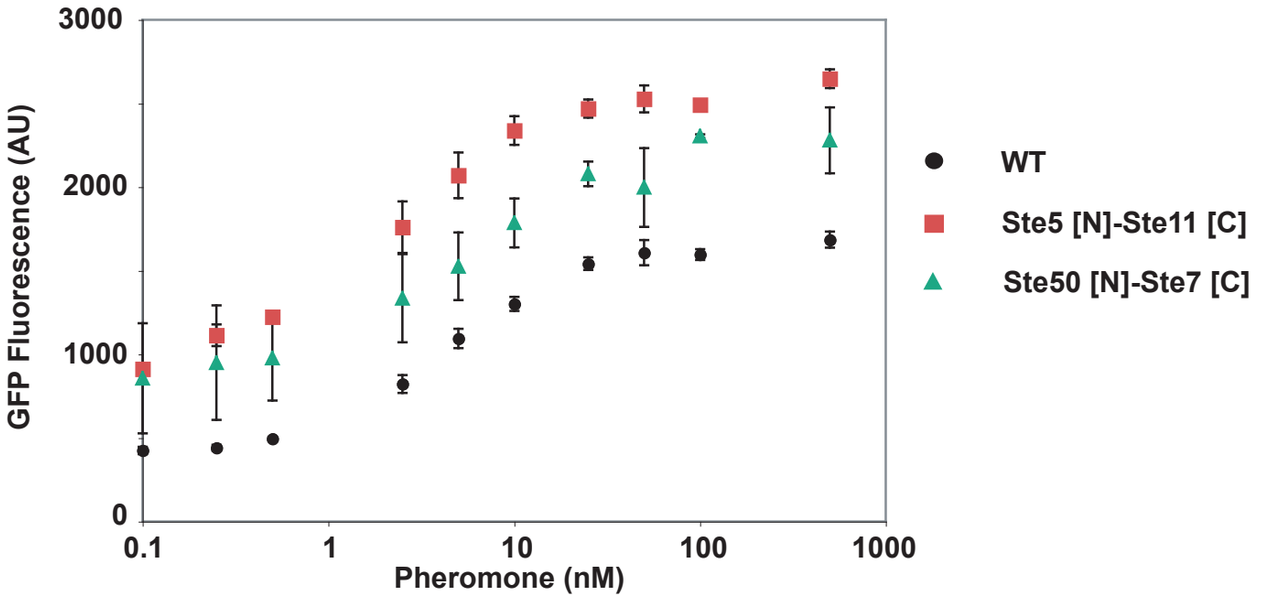
The fitness cost of pleiotropic effects could be balanced by gains in mating efficiency



Supplementary Figure 6

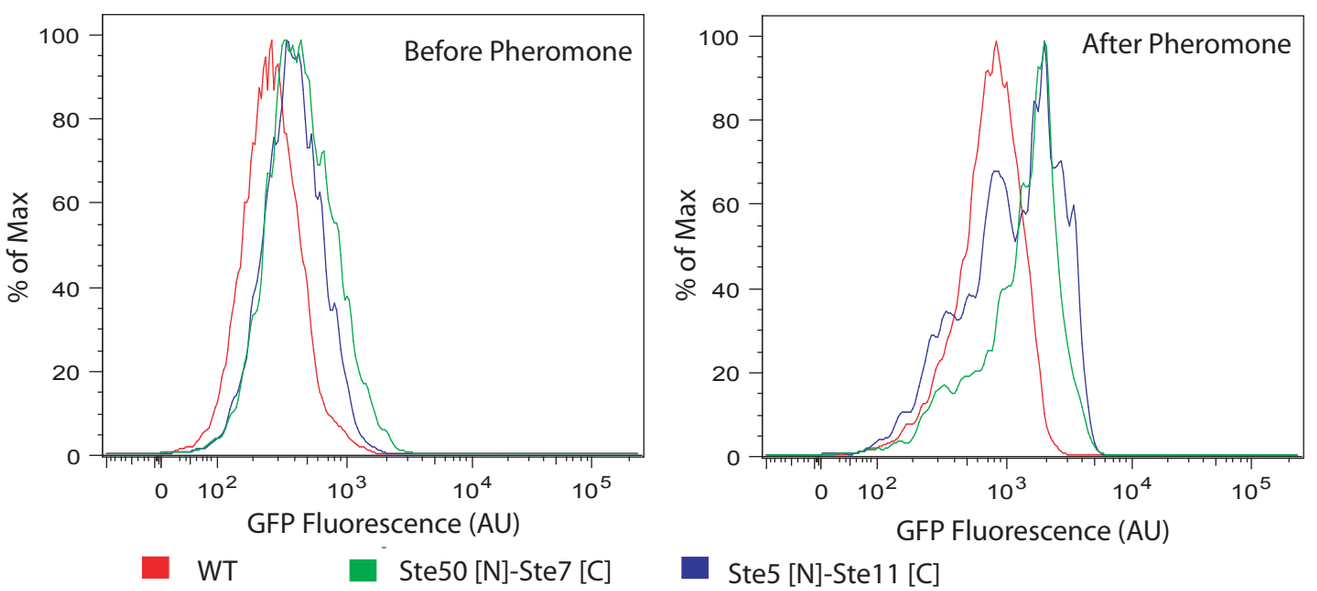
A

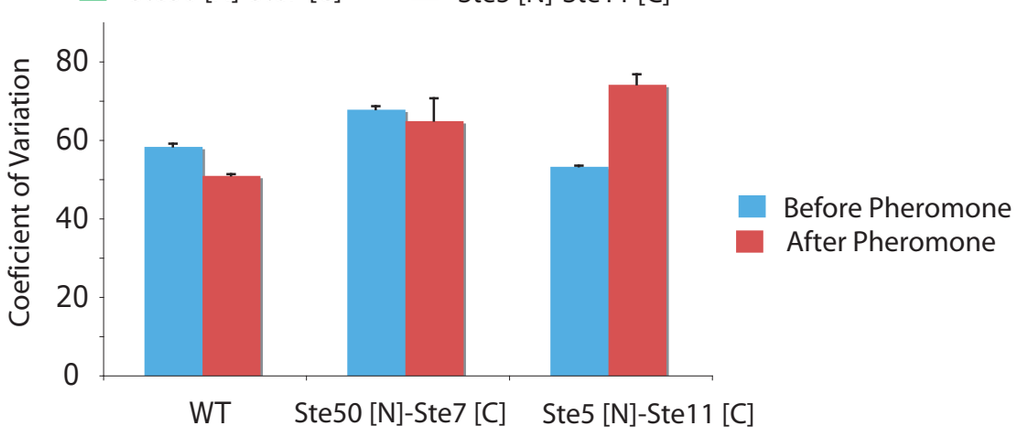
Pheromone Dose Response Curves



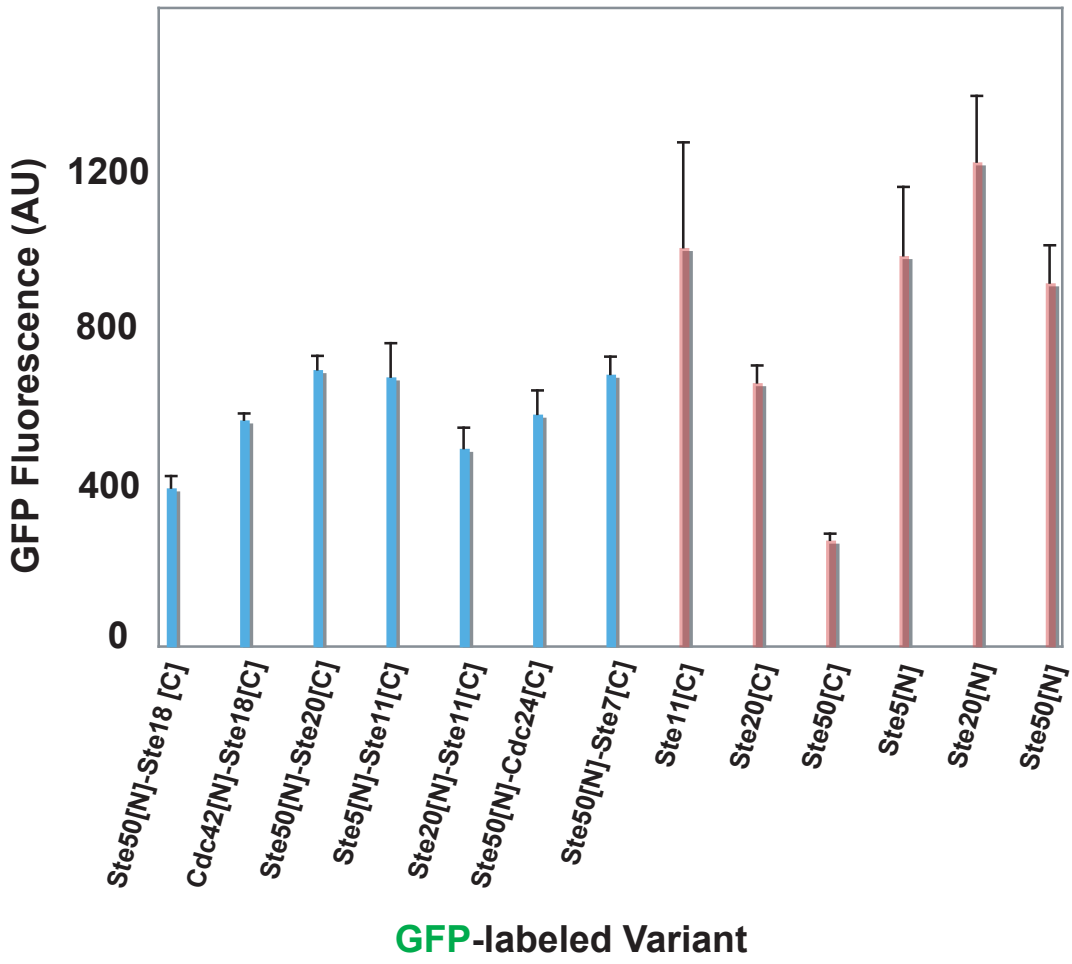
B

Analysis of Cell-to-Cell Variability in Pahtway Response

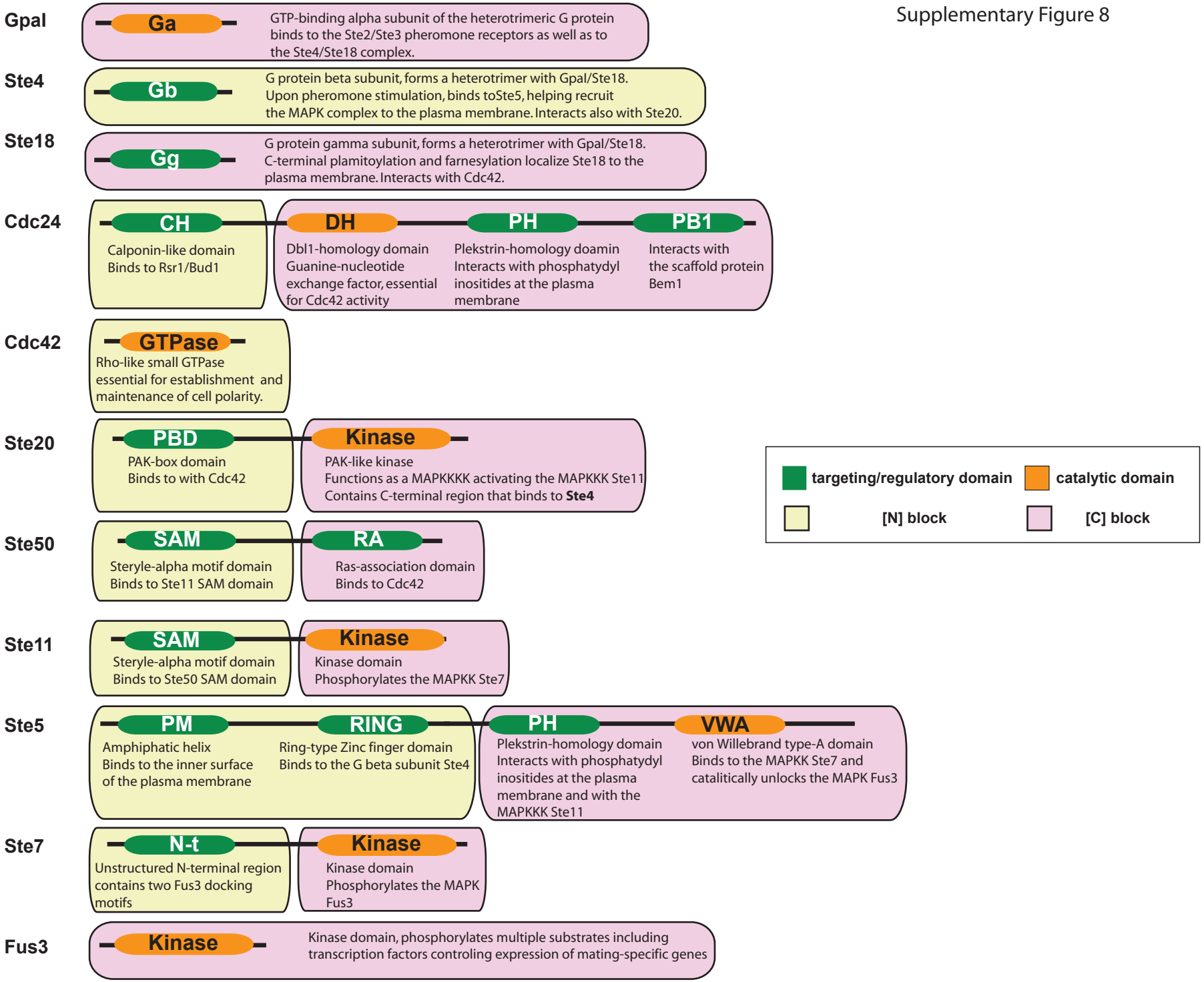




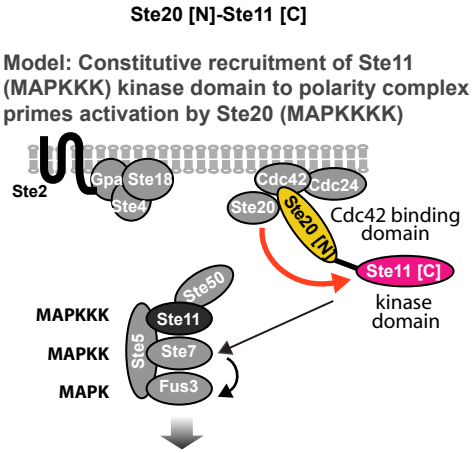
There is no correlation between protein abundance and effects on mating pathway response



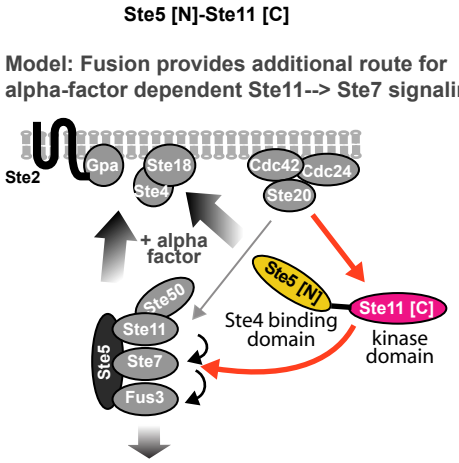
Supplementary Figure 8



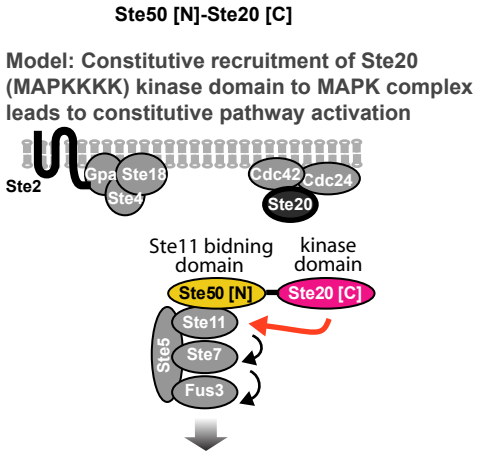
A


Ste20 [N]-Ste11 [C]
Model: Constitutive recruitment of Ste11 (MAPKKK) kinase domain to polarity complex primes activation by Ste20 (MAPKKKK)
Ste2
Gpa
Ste18
Ste4
Cdc42
Cdc24
Ste20
Ste20 [N]
Cdc42 binding domain
Ste11 [C]
kinase domain
Ste50
Ste11
Ste7
Fus3
Ste5
MAPKKK
MAPKK
MAPK

B


Ste5 [N]-Ste11 [C]
Model: Fusion provides additional route for alpha-factor dependent Ste11--> Ste7 signaling
Ste2
Gpa
Ste18
Ste4
Cdc42
Cdc24
Ste20
+ alpha factor
Ste5 [N]
Ste11 [C]
Ste4 binding domain
kinase domain
Ste50
Ste11
Ste7
Fus3
Ste5

C


Ste50 [N]-Ste20 [C]
Model: Constitutive recruitment of Ste20 (MAPKKKK) kinase domain to MAPK complex leads to constitutive pathway activation
Ste2
Gpa
Ste18
Ste4
Cdc42
Cdc24
Ste20
Ste11 bidning domain
kinase domain
Ste50 [N]
Ste20 [C]
Ste11
Ste7
Fus3
Ste5

List of Plasmids used in this study

Library Type	Clone #	Clone Name	Promoter	Clone Identity	
Full length					
	1	Cdc24	CycI	Cdc24	
	2	cdc42Y	CycI	cdc42	
	3	Fus3P	CycI	Fus3	
	4	gpaIP	CycI	gpaI	
	5	Ste11	CycI	Ste11	
	6	Ste18P	CycI	Ste18	
	7	Ste20	CycI	Ste20	
	8	Ste4Y	CycI	Ste4	
	9	Ste5	CycI	Ste5	
	10	Ste50	CycI	Ste50	
	11	Ste7	CycI	Ste7	
BLOCKS	1	Cdc24P	CycI	Cdc24[C]	
	2	Cdc24Y	CycI	Cdc24[N]	
	3	Ste11P	CycI	Ste11[C]	
	4	Ste11Y	CycI	Ste11[N]	
	5	Ste20P	CycI	Ste20[C]	
	6	Ste20Y	CycI	Ste20[N]	
	7	Ste50P	CycI	Ste50[C]	
	8	Ste50Y	CycI	Ste50[N]	
	9	Ste5P	CycI	Ste5[C]	
	10	Ste5Y	CycI	Ste5[N]	
	11	Ste7P	CycI	Ste7[C]	
	12	Ste7Y	CycI	Ste7[N]	
Domain Recombination	1	C10	CycI	Ste7[N]	Ste5[C]
	2	C11	CycI	Cdc42[N]	Ste5[C]
	3	C3	CycI	Ste4[N]	Ste5[C]
	4	C4	CycI	Ste20[N]	Ste5[C]
	5	C7	CycI	Ste50[N]	Ste5[C]

6	C8	CycI	Cdc24[N]	Ste5[C]
7	C9	CycI	Ste11[N]	Ste5[C]
8	D1	CycI	Ste5[N]	Ste50[C]
9	D10	CycI	Ste7[N]	Ste50[C]
10	D11	CycI	Cdc42[N]	Ste50[C]
11	D3	CycI	Ste4[N]	Ste50[C]
12	d4	CycI	Ste20[N]	Ste50[C]
13	d8	CycI	Cdc24[N]	Ste50[C]
14	D9	CycI	Ste11[N]	Ste50[C]
15	E1	CycI	Ste5[N]	Ste18[C]
16	E10	CycI	Ste7[N]	Ste18[C]
17	E11	CycI	Cdc42[N]	Ste18[C]
18	E3	CycI	Ste4[N]	Ste18[C]
19	E4	CycI	Ste20[N]	Ste18[C]
20	E7	CycI	Ste50[N]	Ste18[C]
21	E8	CycI	Cdc24[N]	Ste18[C]
22	E9	CycI	Ste11[N]	Ste18[C]
23	f1	CycI	Ste5[N]	Ste20[C]
24	f10	CycI	Ste7[N]	Ste20[C]
25	f11	CycI	Cdc42[N]	Ste20[C]
26	f3	CycI	Ste4[N]	Ste20[C]
27	f7	CycI	Ste50[N]	Ste20[C]
28	f8	CycI	Cdc24[N]	Ste20[C]
29	f9	CycI	Ste11[N]	Ste20[C]
30	g1	CycI	Ste5[N]	Ste11[C]
31	g10	CycI	Ste7[N]	Ste11[C]
32	g11	CycI	Cdc42[N]	Ste11[C]
33	g3	CycI	Ste4[N]	Ste11[C]
34	g4	CycI	Ste20[N]	Ste11[C]
35	g7	CycI	Ste50[N]	Ste11[C]
36	g8	CycI	Cdc24[N]	Ste11[C]
37	h1	CycI	Ste5[N]	Fus3[C]
38	h10	CycI	Ste7[N]	Fus3[C]
39	h11	CycI	Cdc42[N]	Fus3[C]
40	h3	CycI	Ste4[N]	Fus3[C]
41	h4	CycI	Ste20[N]	Fus3[C]

42	h7	CycI	Ste50[N]	Fus3[C]	
43	h8	CycI	Cdc24[N]	Fus3[C]	
44	h9	CycI	Ste11[N]	Fus3[C]	
45	j1	CycI	Ste5[N]	Cdc24[C]	
46	j10	CycI	Ste7[N]	Cdc24[C]	
47	j11	CycI	Cdc42[N]	Cdc24[C]	
48	j3	CycI	Ste4[N]	Cdc24[C]	
49	j4	CycI	Ste20[N]	Cdc24[C]	
50	j7	CycI	Ste50[N]	Cdc24[C]	
51	j9	CycI	Ste11[N]	Cdc24[C]	
52	k1	CycI	Ste5[N]	Ste7[C]	
53	k11	CycI	Cdc42[N]	Ste7[C]	
54	k3	CycI	Ste4[N]	Ste7[C]	
55	k4	CycI	Ste20[N]	Ste7[C]	
56	k7	CycI	Ste50[N]	Ste7[C]	
57	k8	CycI	Cdc24[N]	Ste7[C]	
58	k9	CycI	Ste11[N]	Ste7[C]	
59	l1	CycI	Ste5[N]	Gpa[C]	
60	l10	CycI	Ste7[N]	Gpa[C]	
61	l11	CycI	Cdc42[N]	Gpa[C]	
62	l3	CycI	Ste4[N]	Gpa[C]	
63	l4	CycI	Ste20[N]	Gpa[C]	
64	l7	CycI	Ste50[N]	Gpa[C]	
65	l8	CycI	Cdc24[N]	Gpa[C]	
66	l9	CycI	Ste11[N]	Gpa[C]	

Co-expression of unlinked domains

1	C10	CycI	Ste7[N]	CycI	Ste5[C]
2	C11	CycI	Cdc42[N]	CycI	Ste5[C]
3	C3	CycI	Ste4[N]	CycI	Ste5[C]
4	C4	CycI	Ste20[N]	CycI	Ste5[C]
5	C7	CycI	Ste50[N]	CycI	Ste5[C]
6	C8	CycI	Cdc24[N]	CycI	Ste5[C]
7	C9	CycI	Ste11[N]	CycI	Ste5[C]
8	D1	CycI	Ste5[N]	CycI	Ste50[C]
9	D10	CycI	Ste7[N]	CycI	Ste50[C]
10	D11	CycI	Cdc42[N]	CycI	Ste50[C]

11	D3	CycI	Ste4[N]	CycI	Ste50[C]
12	d4	CycI	Ste20[N]	CycI	Ste50[C]
13	d8	CycI	Cdc24[N]	CycI	Ste50[C]
14	D9	CycI	Ste11[N]	CycI	Ste50[C]
15	E1	CycI	Ste5[N]	CycI	Ste18[C]
16	E10	CycI	Ste7[N]	CycI	Ste18[C]
17	E11	CycI	Cdc42[N]	CycI	Ste18[C]
18	E3	CycI	Ste4[N]	CycI	Ste18[C]
19	E4	CycI	Ste20[N]	CycI	Ste18[C]
20	E7	CycI	Ste50[N]	CycI	Ste18[C]
21	E8	CycI	Cdc24[N]	CycI	Ste18[C]
22	E9	CycI	Ste11[N]	CycI	Ste18[C]
23	f1	CycI	Ste5[N]	CycI	Ste20[C]
24	f10	CycI	Ste7[N]	CycI	Ste20[C]
25	f11	CycI	Cdc42[N]	CycI	Ste20[C]
26	f3	CycI	Ste4[N]	CycI	Ste20[C]
27	f7	CycI	Ste50[N]	CycI	Ste20[C]
28	f8	CycI	Cdc24[N]	CycI	Ste20[C]
29	f9	CycI	Ste11[N]	CycI	Ste20[C]
30	g1	CycI	Ste5[N]	CycI	Ste11[C]
31	g10	CycI	Ste7[N]	CycI	Ste11[C]
32	g11	CycI	Cdc42[N]	CycI	Ste11[C]
33	g3	CycI	Ste4[N]	CycI	Ste11[C]
34	g4	CycI	Ste20[N]	CycI	Ste11[C]
35	g7	CycI	Ste50[N]	CycI	Ste11[C]
36	g8	CycI	Cdc24[N]	CycI	Ste11[C]
37	h1	CycI	Ste5[N]	CycI	Fus3[C]
38	h10	CycI	Ste7[N]	CycI	Fus3[C]
39	h11	CycI	Cdc42[N]	CycI	Fus3[C]
40	h3	CycI	Ste4[N]	CycI	Fus3[C]
41	h4	CycI	Ste20[N]	CycI	Fus3[C]
42	h7	CycI	Ste50[N]	CycI	Fus3[C]
43	h8	CycI	Cdc24[N]	CycI	Fus3[C]
44	h9	CycI	Ste11[N]	CycI	Fus3[C]
45	j1	CycI	Ste5[N]	CycI	Cdc24[C]
46	j10	CycI	Ste7[N]	CycI	Cdc24[C]

47	j11	CycI	Cdc42[N]	CycI	Cdc24[C]
48	j3	CycI	Ste4[N]	CycI	Cdc24[C]
49	j4	CycI	Ste20[N]	CycI	Cdc24[C]
50	j7	CycI	Ste50[N]	CycI	Cdc24[C]
51	j9	CycI	Ste11[N]	CycI	Cdc24[C]
52	k1	CycI	Ste5[N]	CycI	Ste7[C]
53	k11	CycI	Cdc42[N]	CycI	Ste7[C]
54	k3	CycI	Ste4[N]	CycI	Ste7[C]
55	k4	CycI	Ste20[N]	CycI	Ste7[C]
56	k7	CycI	Ste50[N]	CycI	Ste7[C]
57	k8	CycI	Cdc24[N]	CycI	Ste7[C]
58	k9	CycI	Ste11[N]	CycI	Ste7[C]
59	l1	CycI	Ste5[N]	CycI	Gpa[C]
60	l10	CycI	Ste7[N]	CycI	Gpa[C]
61	l11	CycI	Cdc42[N]	CycI	Gpa[C]
62	l3	CycI	Ste4[N]	CycI	Gpa[C]
63	l4	CycI	Ste20[N]	CycI	Gpa[C]
64	l7	CycI	Ste50[N]	CycI	Gpa[C]
65	l8	CycI	Cdc24[N]	CycI	Gpa[C]
66	l9	CycI	Ste11[N]	CycI	Gpa[C]