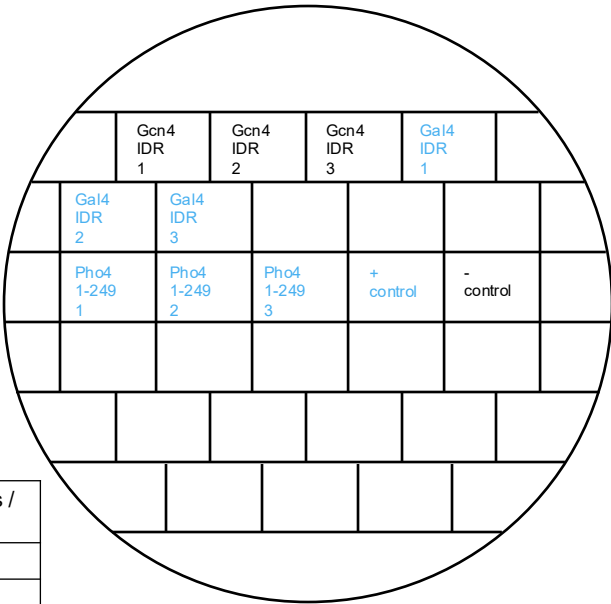
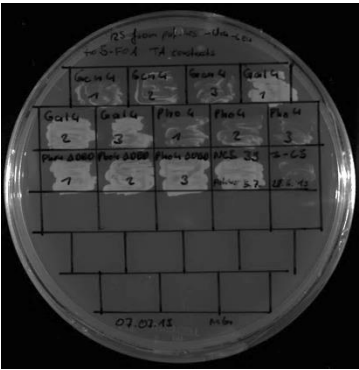


Supplementary Figure S1: Viability of Abf1 constructs assessed by 5-FOA plasmid shuffling assay.

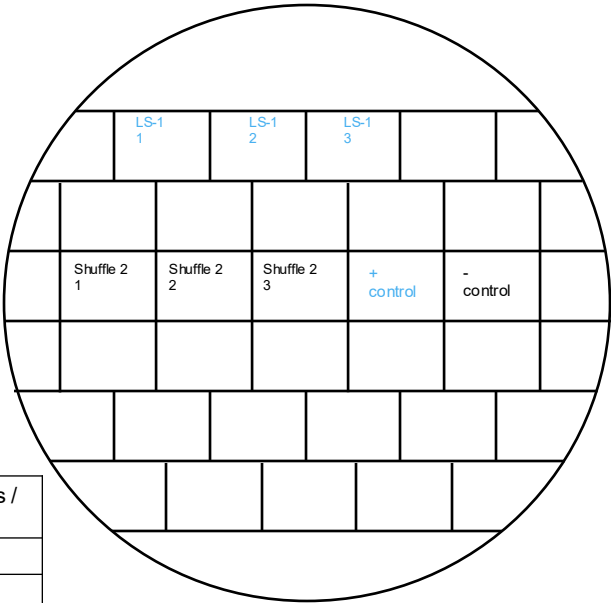
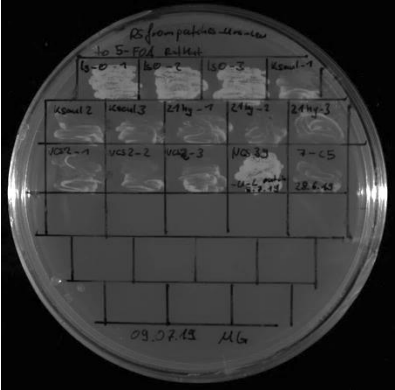
Strains harboring both pRS416-*ABF1* plasmid (*URA3* marker) and pRS315-"*abf1* construct name" (*LEU2* marker) in the strain background with deleted *abf1* chromosomal gene were re-streaked from patches from YNB without uracil and leucine plates onto 5-FOA without leucine plates. Only constructs discussed in the paper are labeled in plate schemes, where names of viable strains are in blue, names of inviable ones in black. Viability on 5-FOA plates was visually scored by comparison to known viable or inviable strains on the same plate. Inviability strains were distinguished from viable strains because they did not show growth of a contiguous patch after at most three days of incubation at 30 °C but only a smeary appearance or at most sparse single colonies, whereas viable strains grew as a contiguously dense patch. If few individual colonies grew instead of a contiguous patch, we tested some of them by colony PCR with the primers listed in Table S8. In all cases, these individual colonies contained plasmids of a wrong size or sequence reflecting recombination between pRS416-*ABF1* and pRS315-"*abf1* construct name".

For each construct, independent clones after transformation were tested. On each slide, a table summarizes the result of the shown plate and in brackets the total result obtained for this construct if all tested clones on all plates were considered. If the same clone showed conflicting results in technical replicates, it was not counted as viable or inviable but included in the total number of tested clones. The final categorization of each construct as viable or inviable followed by the majority of tested clones excluding the clones with ambiguous results in technical replicates.



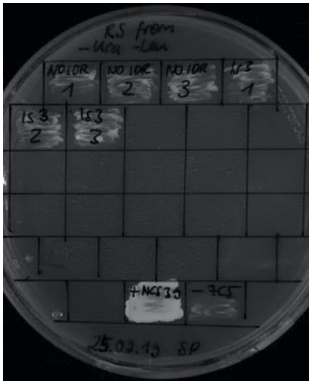
Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
Gcn4 ¹⁷⁻¹⁵⁰	inviable	3 out of 3
Gal4 ⁷⁶⁸⁻⁸⁸¹	viable	3 out of 3
Pho4 ¹⁻²⁴⁹	viable	3 out of 3



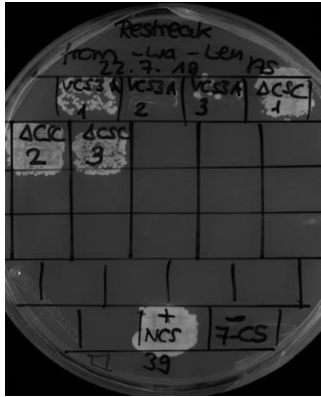
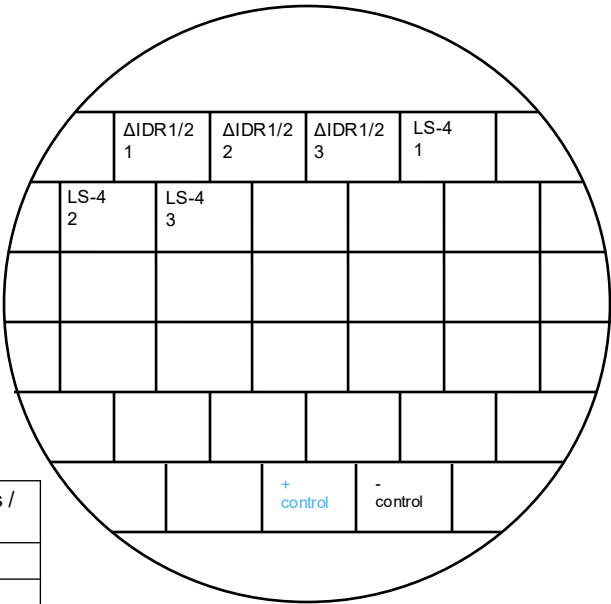
Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
LS-1	viable	3 out of 3
Shuffle 2	inviable	3 out of 3



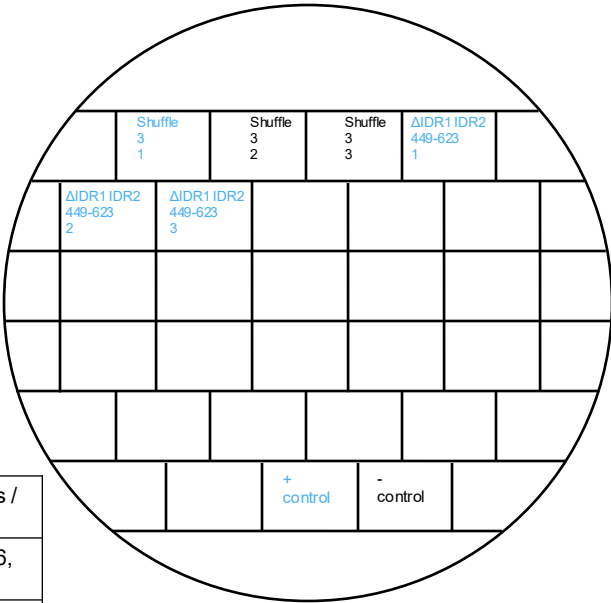
Restreak from YNB –ura –leu to 5-FOA –leu

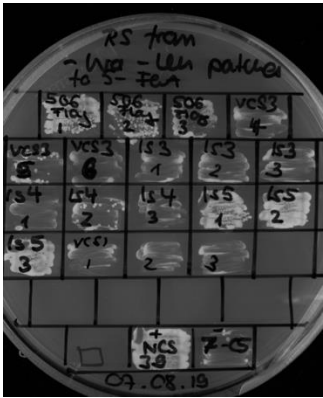
Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
ΔIDR1/2 (NLS-FLAG)	inviable	3 out of 3
LS-4	inviable	3 out of 3



Restreak from YNB –ura –leu to 5-FOA –leu

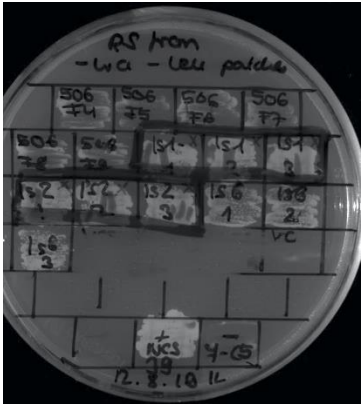
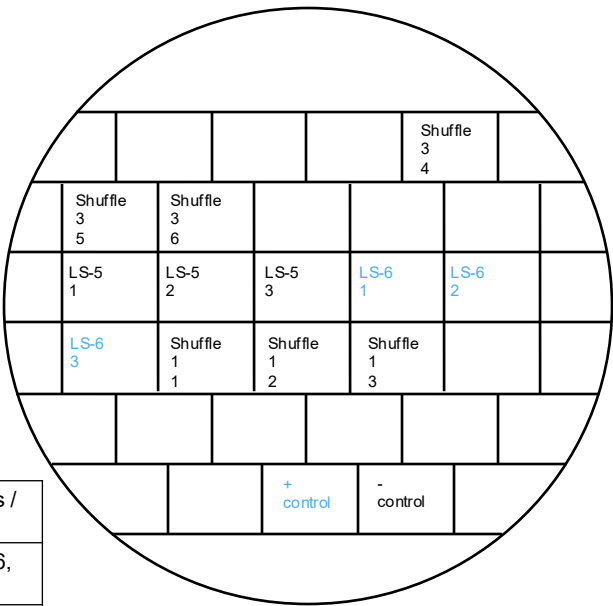
Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
Shuffle 3	inviable	2 out of 3 (in total 5 out of 6, see also slide 5)
ΔIDR1 & IDR2 ⁴⁴⁹⁻⁶²³	viable	3 out of 3





Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
Shuffle 3	inviable	3 out of 3 (in total 5 out of 6, see also slide 4)
LS-5	inviable	3 out of 3
LS-6	viable	3 out of 3
Shuffle 1	inviable	3 out of 3



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
LS-2	viable	3 out of 3 (technical replicates, see also slide 7)
LS-3	viable	3 out of 3 (technical replicates, see also slide 7)
LS-7	viable	3 out of 3 (in total 8 out of 8, see also slide 6)

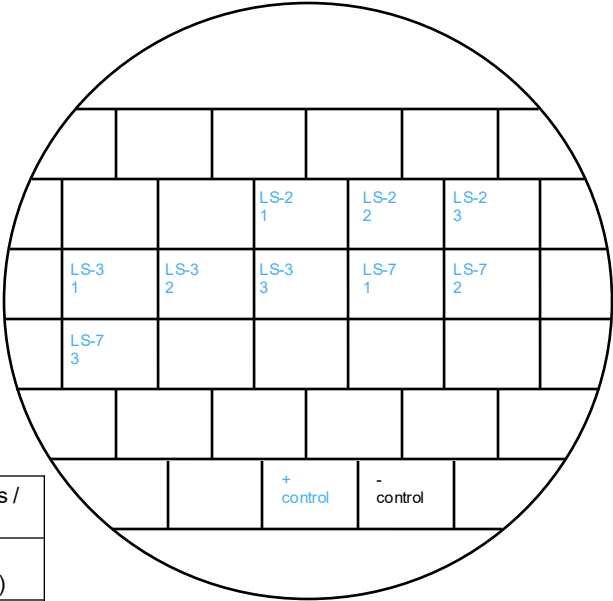
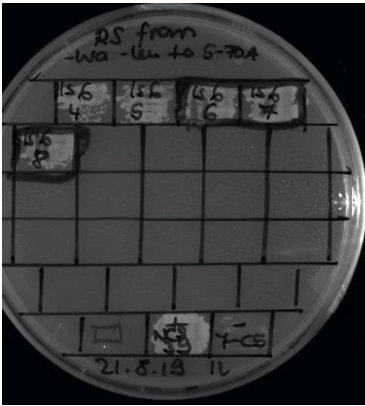


Fig. S1, slide 4



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
LS-7	viable	5 out of 5 (in total 8 out of 8, see also slide 5)

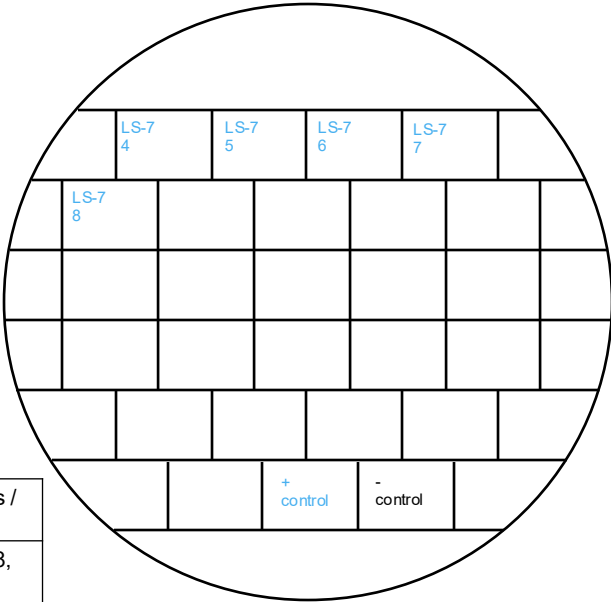
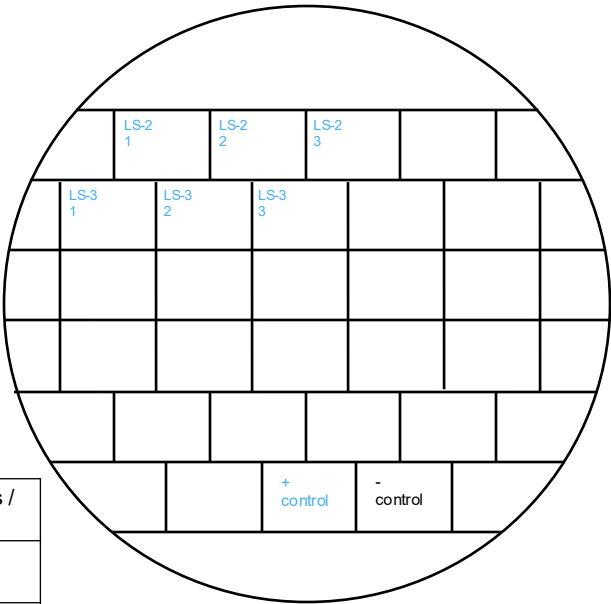
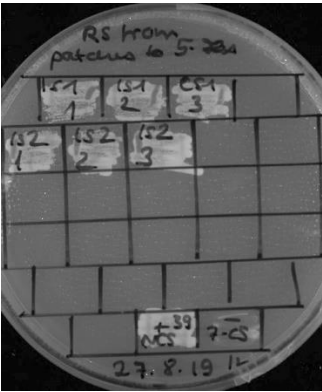
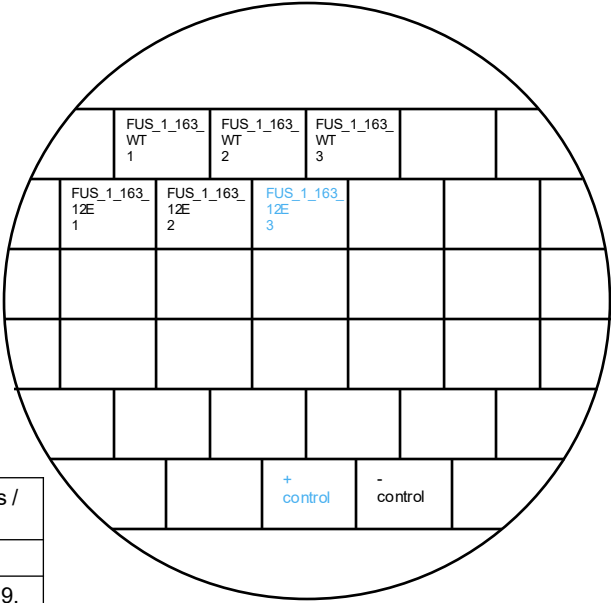
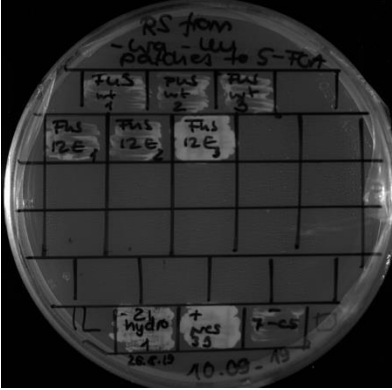


Fig. S1, slide 5



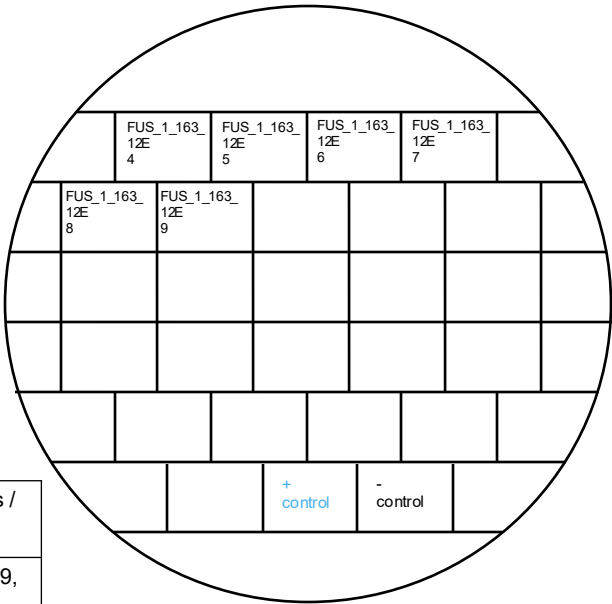
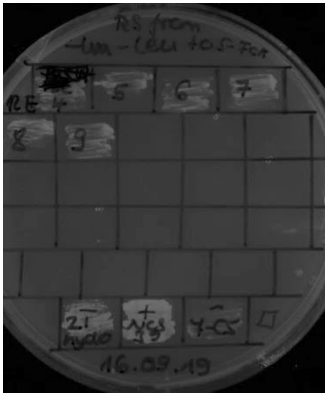
Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
LS-2	viable	3 out of 3 (technical replicates, see also slide 5)
LS-3	viable	3 out of 3 (technical replicates, see also slide 5)



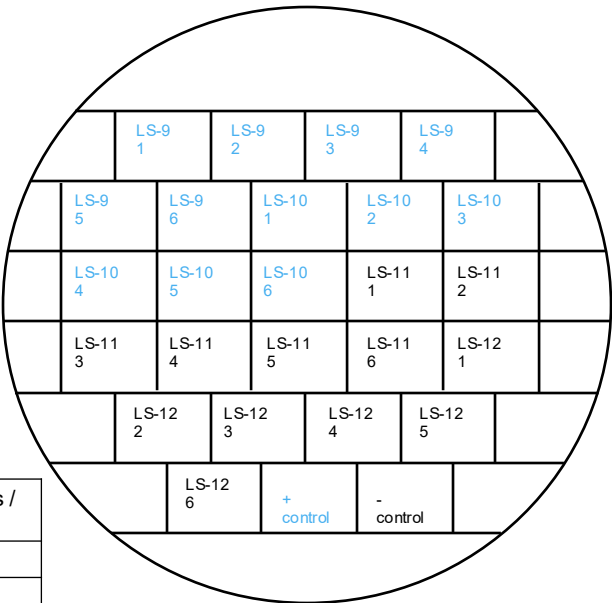
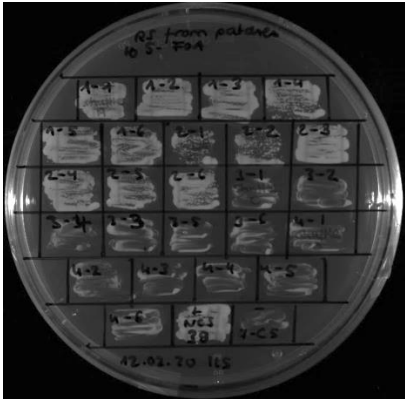
Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Construct	Number of (in)viable clones / total number of clones
FUS ¹⁻¹⁶³	inviability	3 out of 3
FUS ¹⁻¹⁶³ 12E	inviability	2 out of 3 (in total: 8 out of 9, see also slides 8 and 15)



Restreak from YNB –ura –leu to 5-FOA –leu

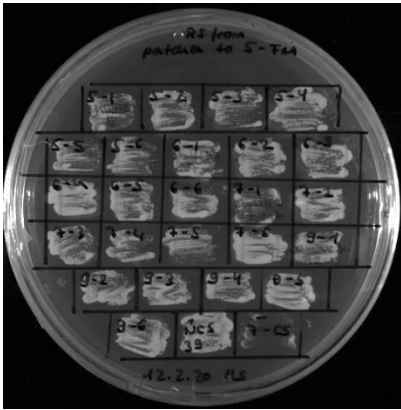
Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
FUS1-16312E	Invisible	6 out of 6 ((in total 8 out of 9, see also slides 7 and 15)



Restreak from YNB –ura –leu to 5-FOA –leu

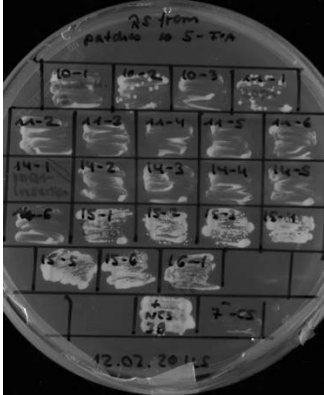
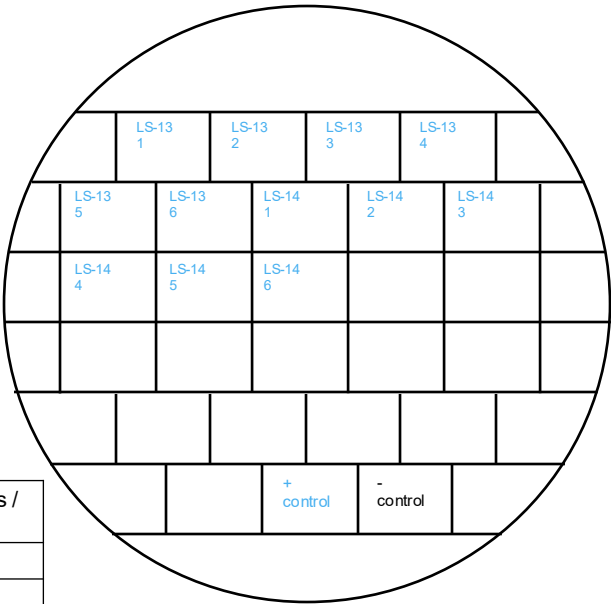
Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
LS-9	viable	6 out of 6
LS-10	viable	6 out of 6
LS-11	invisible	6 out of 6
LS-12	invisible	6 out of 6

Fig. S1, slide 7



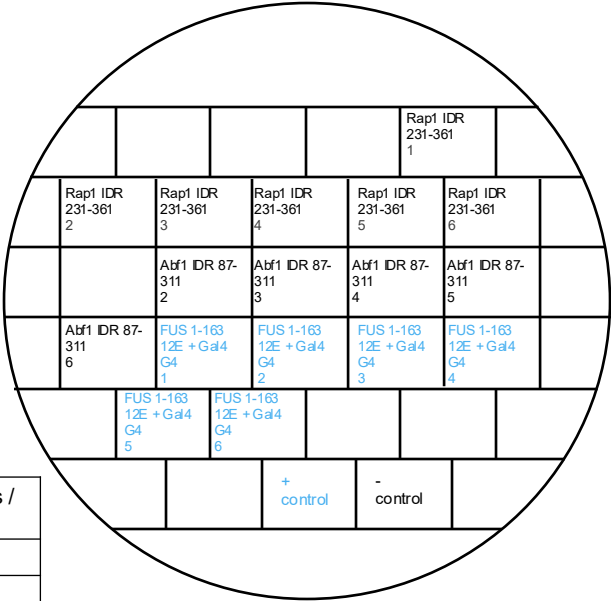
Restreak from YNB –ura –leu to 5-FOA –leu

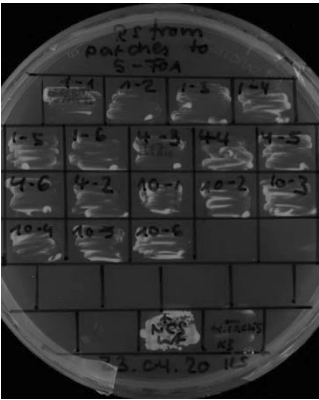
Construct	Construct	Number of (in)viabile clones / total number of clones
LS-13	viable	6 out of 6
LS-14	viable	6 out of 6



Restreak from YNB –ura –leu to 5-FOA –leu

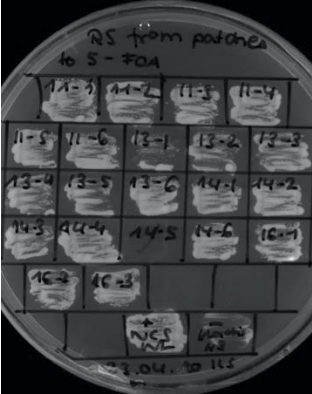
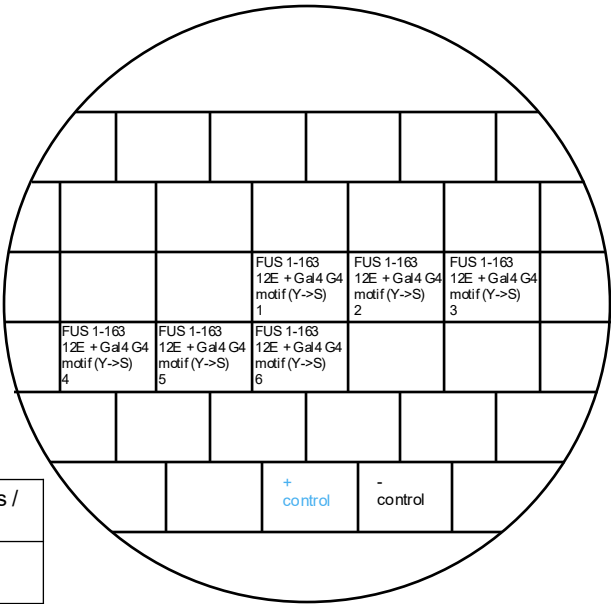
Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
Rap1 ²³¹⁻³⁶¹	inviable	6 out of 6
Abf1 IDR ¹⁸⁷⁻³¹¹	inviable	6 out of 6
FUS1-163 12E + Gal4 ^{G4}	viable	6 out of 6





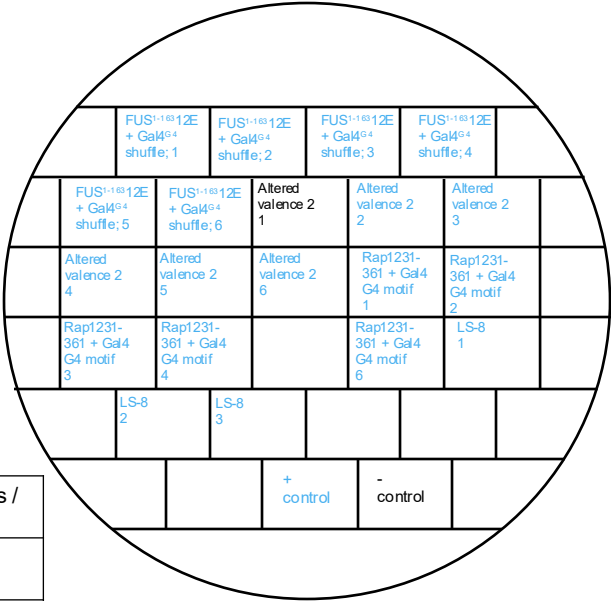
Restreak from YNB –ura –leu to 5-FOA –leu

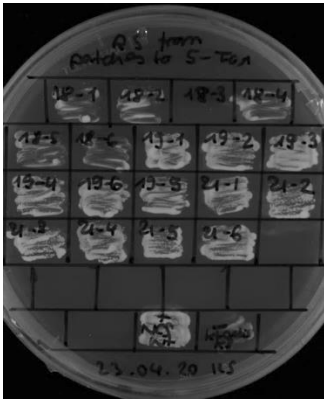
Construct	Construct	Number of (in)viable clones / total number of clones
FUS1-163 12E + Gal4 ^{G4} Y→S in context	inviable	6 out of 6



Restreak from YNB –ura –leu to 5-FOA –leu

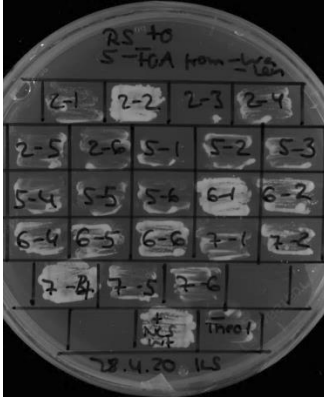
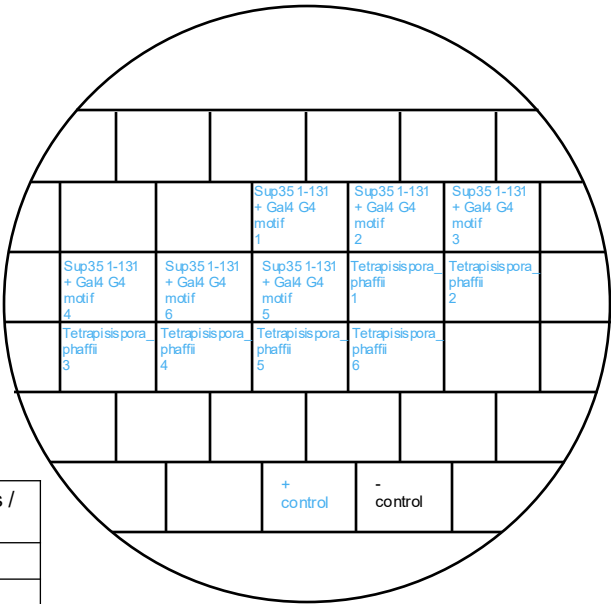
Construct	Construct	Number of (in)viable clones / total number of clones
FUS1-163 12E + Gal4 ^{G4} shuffle	viable	6 out of 6
Altered valence 2	viable	5 out of 6 (in total 5 out of 6, see also slide 23)
Rap1 ²³¹⁻³⁶¹ + Gal4 G4	viable	5 out of 5
LS-8	viable	3 out of 3





Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in) viable clones / total number of clones
Sup35 ¹⁻¹³¹ + Gal4 ^{G4}	viable	6 out of 6
T. phaffii	viable	6 out of 6



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in) viable clones / total number of clones
C. glabrata	in viable	6 out of 6
FUS 1-163 12E + EM	viable	5 out of 5 (in total 6 out of 6, see also slides 12, 23, 24)
FUS 1-163 12E + Abf1 ^{G4}	viable	2 out of 5 (in total 2 out of 2, technical replicate, see also slide 12)

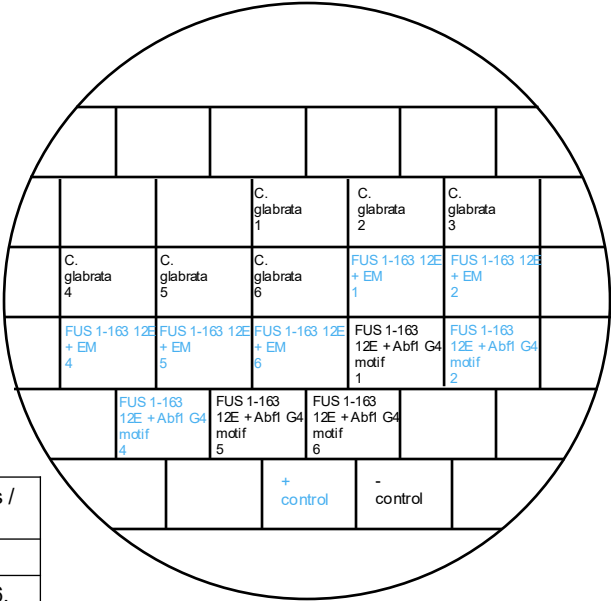
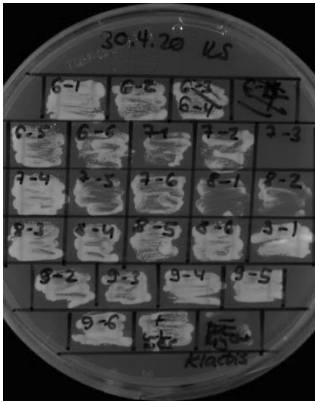
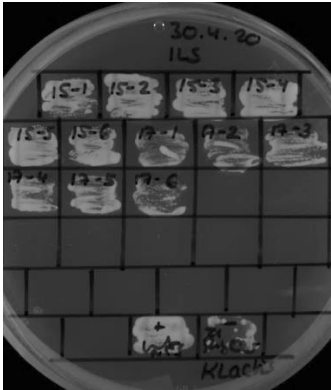
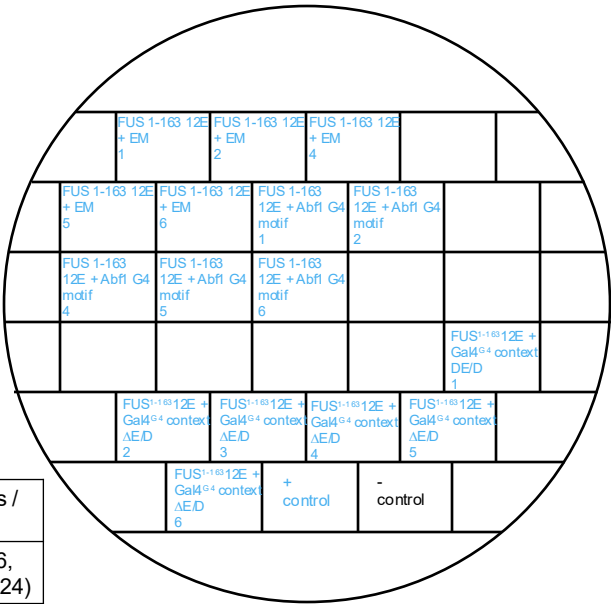


Fig. S1, slide 10



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Construct	Number of (in)viable clones / total number of clones
FUS1-163 12E + EM	viable	5 out of 5 (in total 6 out of 6, see also slides 11, 23 and 24)
FUS1-163 12E + Abf1 ^{G4}	viable	5 out of 5 (in total 2 out of 2, technical replicate, see also slide 11)
FUS1-163 12E + Gal4 ^{G4} context ΔE/D	viable	6 out of 6



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
Altered valence 1	viable	6 out of 6

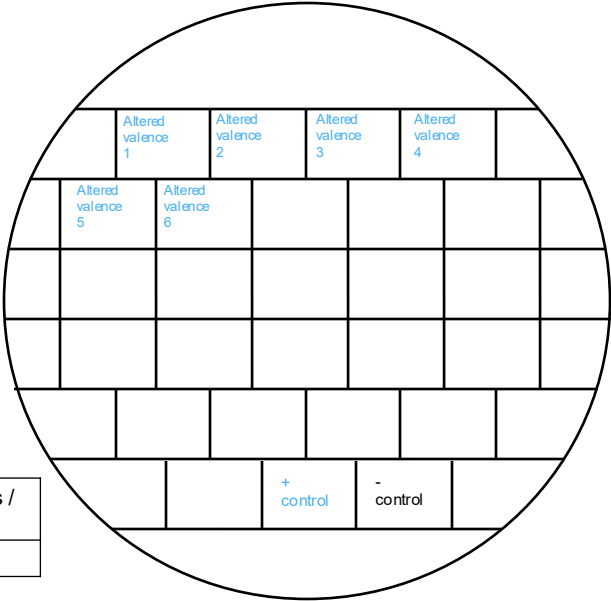
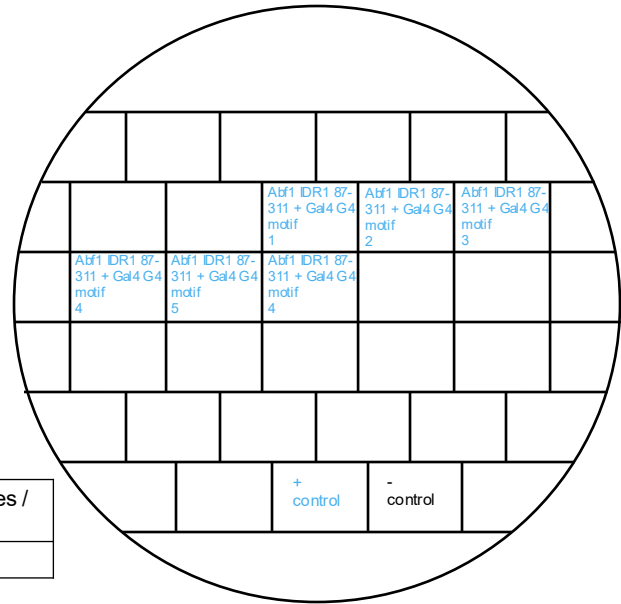
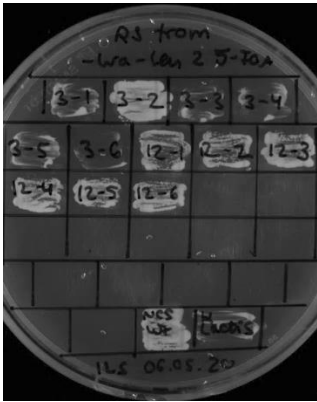
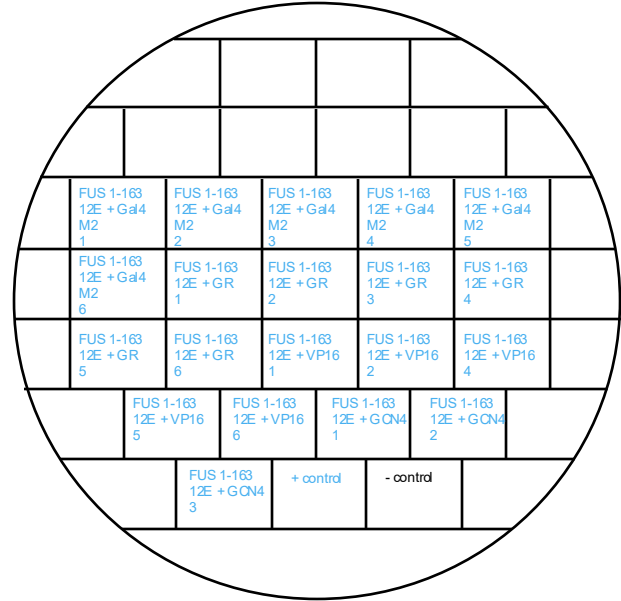
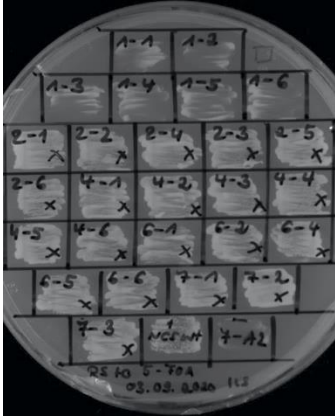


Fig. S1, slide 11



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Construct	Number of (in)viable clones / total number of clones
Abf1 IDR1 ⁸⁷⁻³¹¹ + Gal4 ^{G4}	viable	6 out of 6



Restreak from YNB –ura –leu to 5-FOA –leu

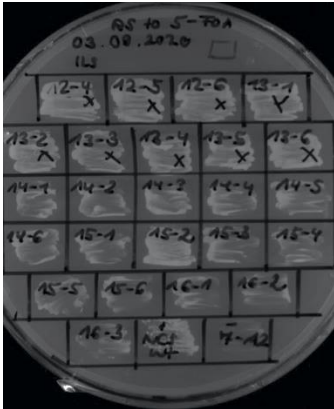
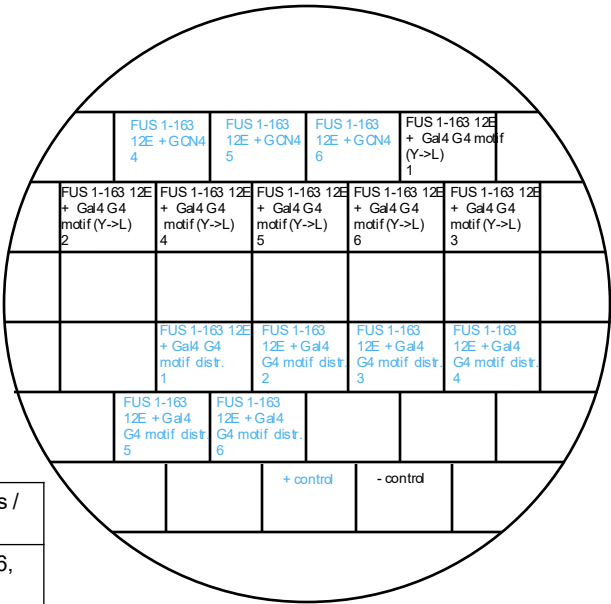
Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
FUS ¹⁻¹⁶³ 12E+ Gal4 ^{M2}	viable	6 out of 6
FUS ¹⁻¹⁶³ 12E+ GR	viable	6 out of 6
FUS ¹⁻¹⁶³ 12E+ VP16	viable	5 out of 5
FUS ¹⁻¹⁶³ 12E+ Gcn4	viable	3 out of 3 (in total 6 out of 6, see also slide 14)

Fig. S1, slide 12



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
FUS¹⁻¹⁶³12E + Gcn4	viable	3 out of 3 (in total 6 out of 6, see also slide 13)
FUS¹⁻¹⁶³12E + Gal4^{G4} Y→L in context	inviable	6 out of 6
FUS¹⁻¹⁶³12E + Gal4^{G4} distr.	viable	6 out of 6



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
FUS¹⁻¹⁶³12E + Gal4^{G4} motif hydro distr.	viable	6 out of 6
Sup35¹⁻¹³¹ + Gal4^{G4} - all acidic residues	inviable	5 out of 6

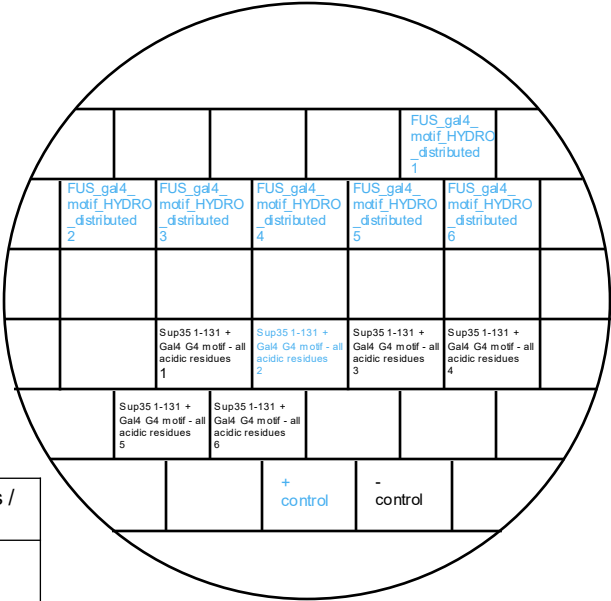
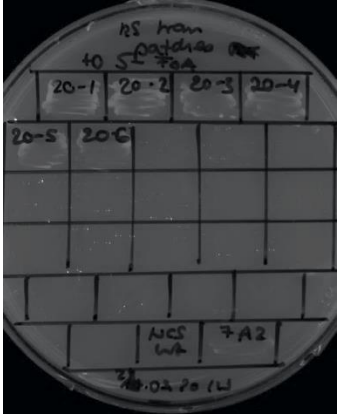
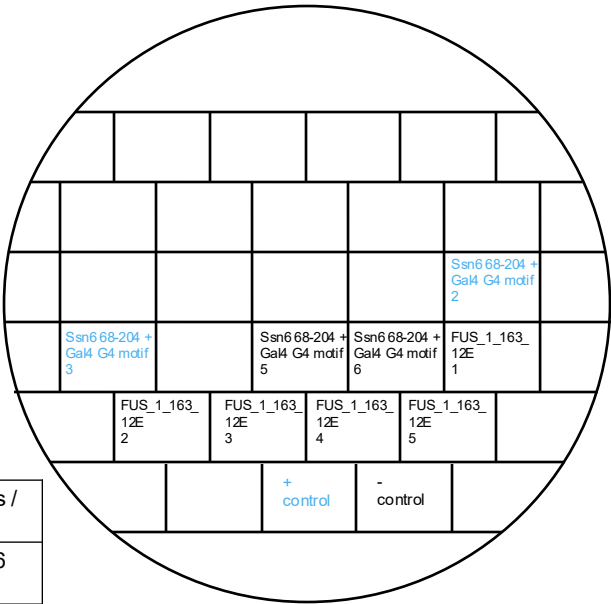


Fig. S1, slide 13



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
Ssn6 ⁶⁸⁻²⁰⁴ + Gal4 ^{G4}	inviable	2 out of 4 (in total 4 out of 6 see plate below)
FUS ¹⁻¹⁶³ 12E	inviable	5 out of 5 (in total 8 out of 9, see also slides 7 and 8)



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
Ssn6 ⁶⁸⁻²⁰⁴ + Gal4 ^{G4}	inviable	6 out of 6 (in total 4 out of 6 see plate above)

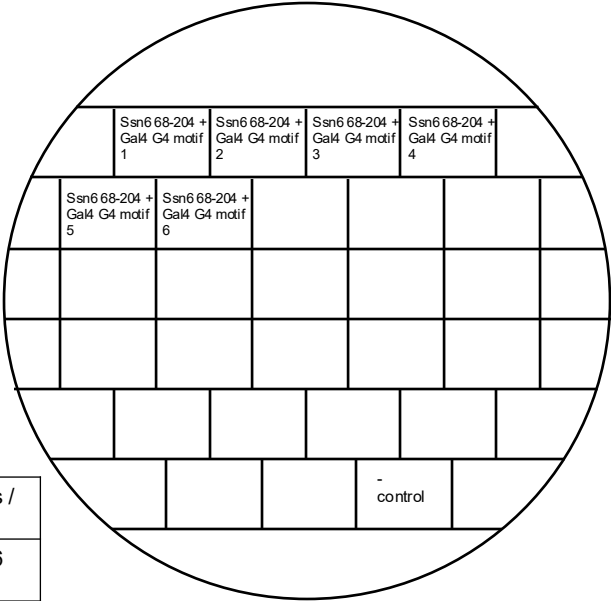
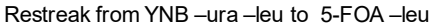
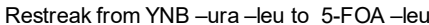
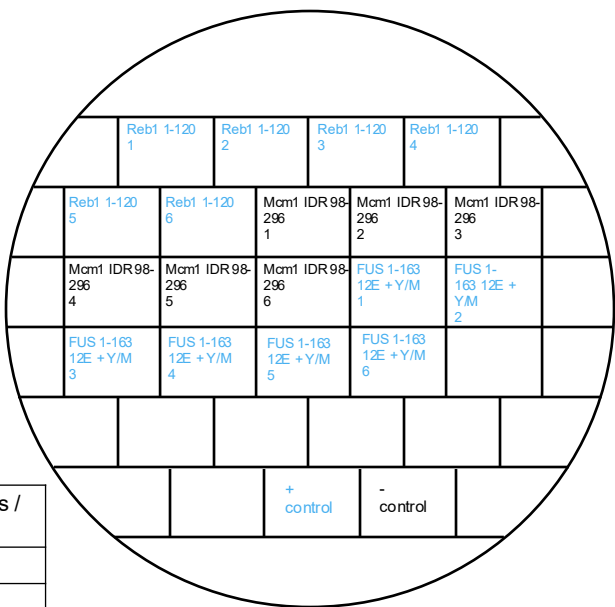


Fig. S1, slide 14



Construct	Result regarding viability on 5-FOA	Number of (in) viable clones / total number of clones
Reb1 ¹⁻¹²⁰ (+PKEEEEGL)	viable	6 out of 6
Mcm1 ⁹⁸⁻²⁹⁶	inviable	6 out of 6
FUS ¹⁻¹⁶³ 12E+ Y/M	viable	6 out of 6



Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
FUS ¹⁻¹⁶³ 12E + TDP-43	viable	6 out of 6
FUS ¹⁻¹⁶³ 12E + TDP-43 Y→S in context	inviable	6 out of 6

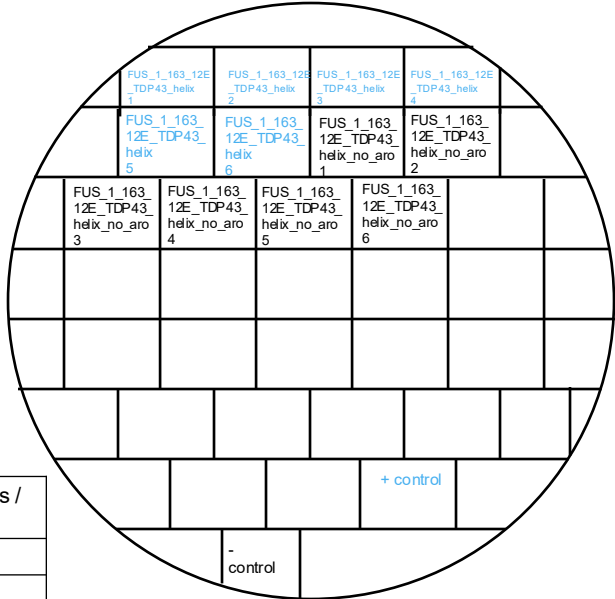
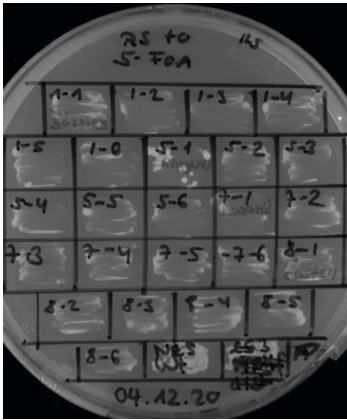
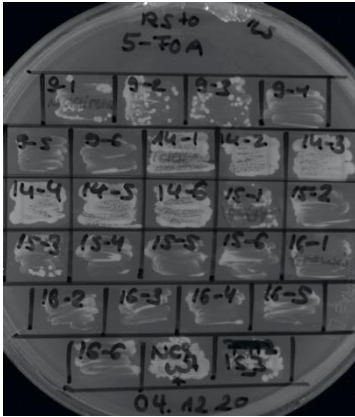
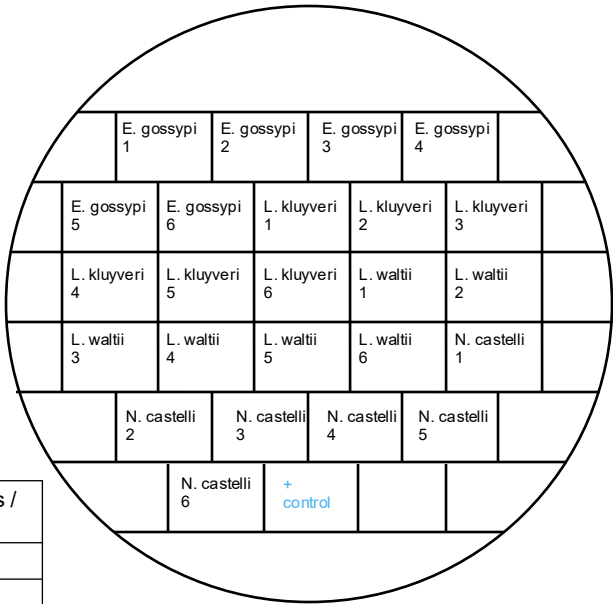


Fig. S1, slide 15



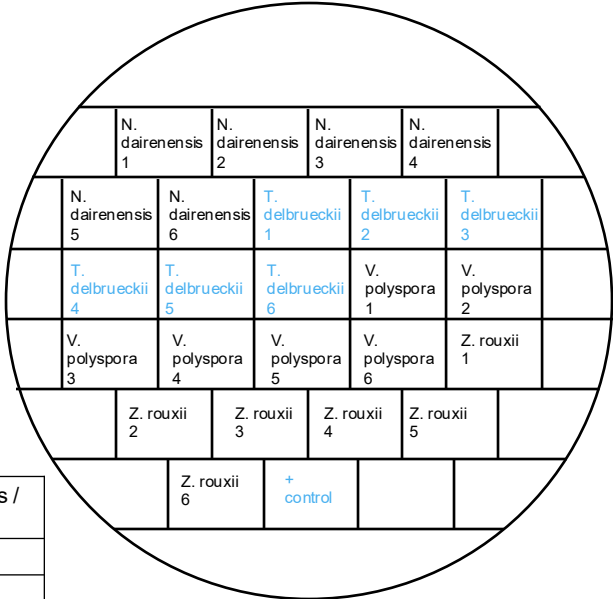
Restreak from YNB –ura –leu to 5-FOA –leu

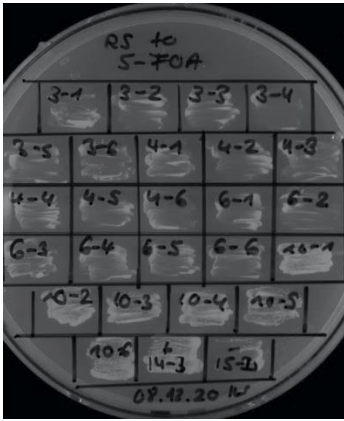
Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
<i>E. gossypi</i>	inviable	6 out of 6
<i>L. kluyveri</i>	inviable	6 out of 6
<i>L. waltii</i>	inviable	6 out of 6
<i>N. castelli</i>	inviable	6 out of 6



Restreak from YNB –ura –leu to 5-FOA –leu

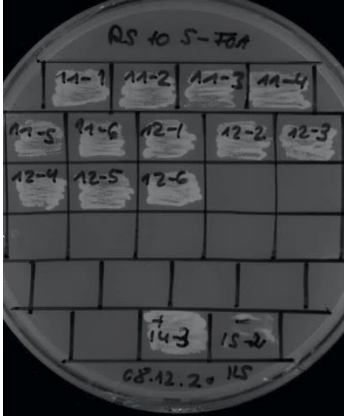
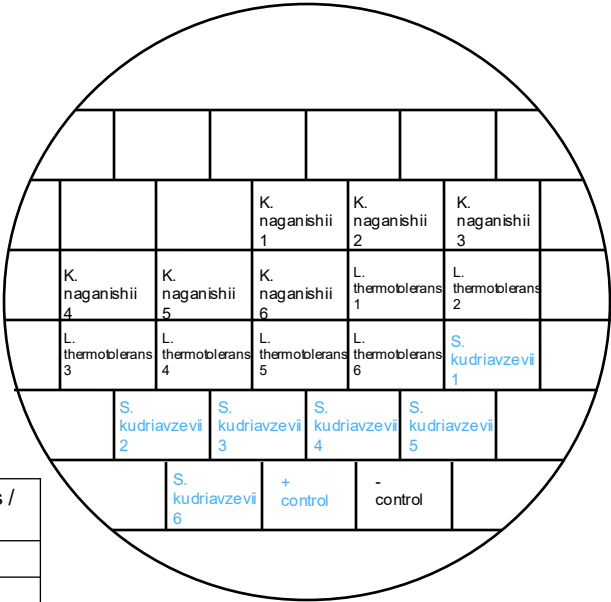
Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
<i>N. dairenensis</i>	inviable	6 out of 6
<i>T. delbrueckii</i>	viable	6 out of 6
<i>V. polyspora</i>	inviable	6 out of 6 (in total 14 out of 14, see also slide 37)
<i>Z. rouxii</i>	inviable	6 out of 6





Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
K. naganishii	inviable	6 out of 6
L. thermotolerans	inviable	6 out of 6
S. kudriavzevii	viable	6 out of 6



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
S. mikatae	viable	6 out of 6
S. uvarum	viable	6 out of 6

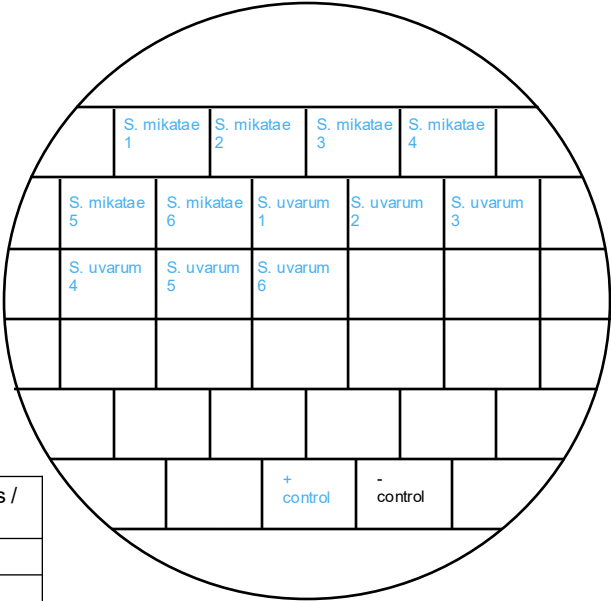
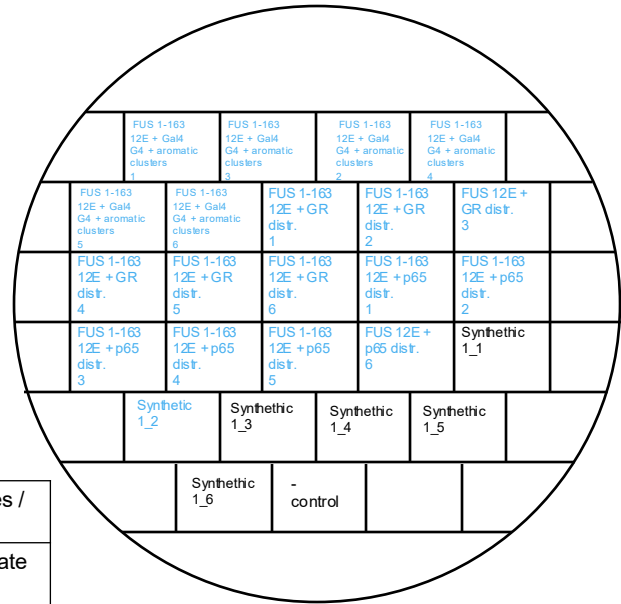
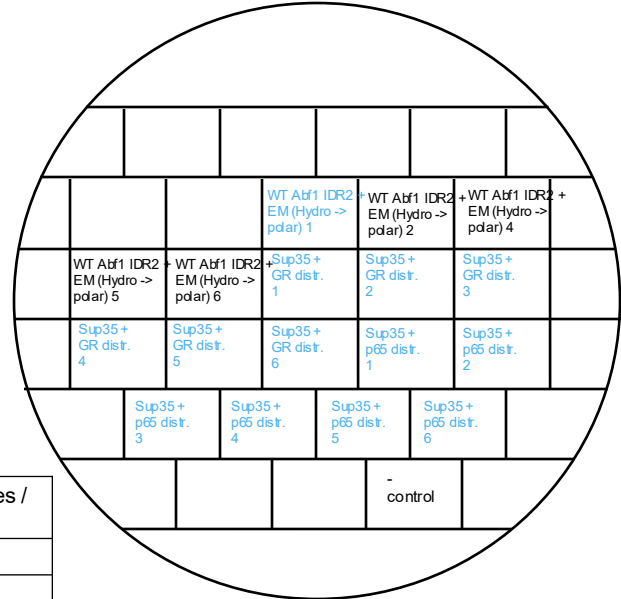
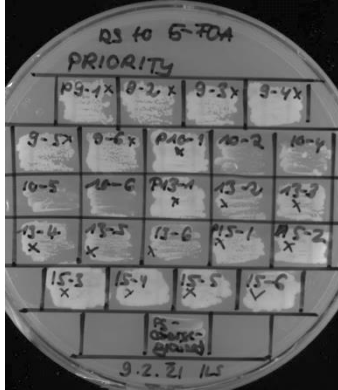


Fig. S1, slide 17



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
FUS1-16312E + Gal4^{G4} aromatic clusters	viable	6 out of 6 (technical replicate see slide 22)
FUS1-16312E + GR distr.	viable	6 out of 6
FUS1-16312E + p65 distr.	viable	6 out of 6
Synthetic 1	inviable	5 out of 6



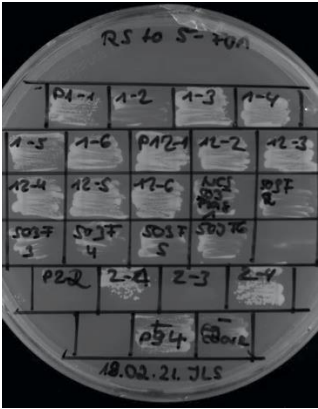
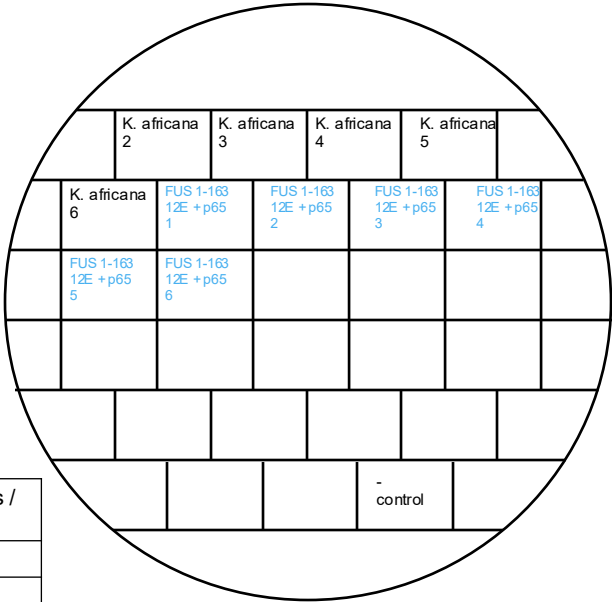
Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
IDR2 WT hydro→polar	inviable	4 out of 5
Sup35¹⁻¹³¹ + GR distr.	viable	6 out of 6
Sup35¹⁻¹³¹ + p65 distr.	viable	6 out of 6



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
K. africana	inviable	5 out of 5
FUS ¹⁻¹⁶³ 12E + p65	viable	6 out of 6



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
FUS ¹⁻¹⁶³ 12E + Gal4 ^{G4} context ΔE/D	viable	5 out of 6
Sup35 ¹⁻¹³¹ + GR	viable	6 out of 6

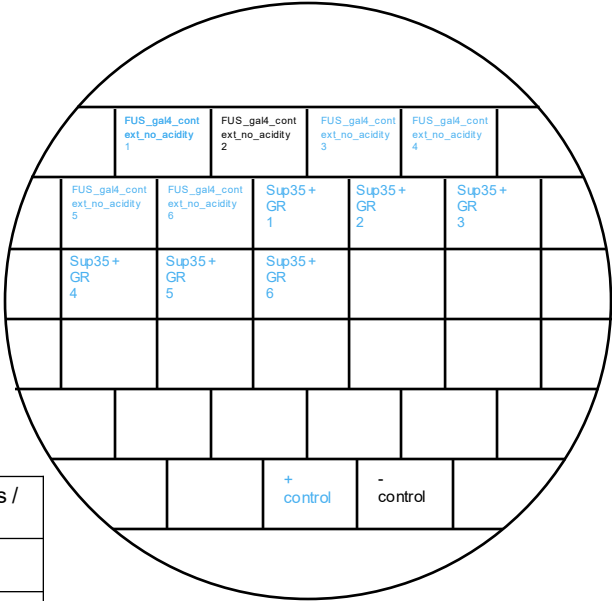
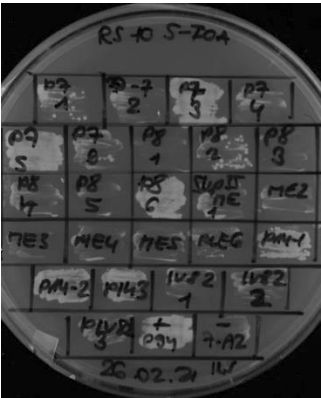
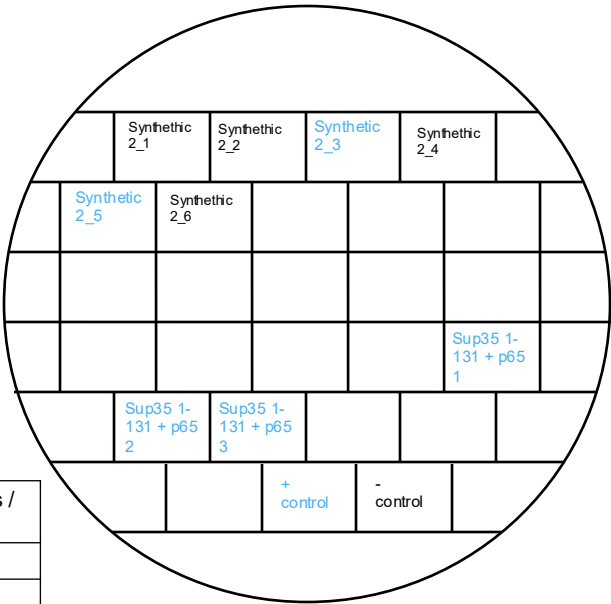


Fig. S1, slide 19



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
Synthetic 2	inviable	4 out of 6
Sup35 ¹⁻¹³¹ + p65	viable	3 out of 3



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
Reb1 ¹⁻⁴²⁰	viable	6 out of 6
T. blattae	inviable	6 out of 6

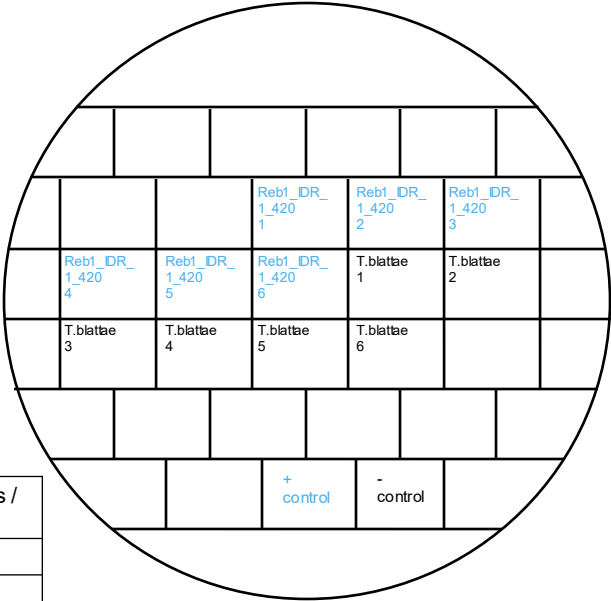
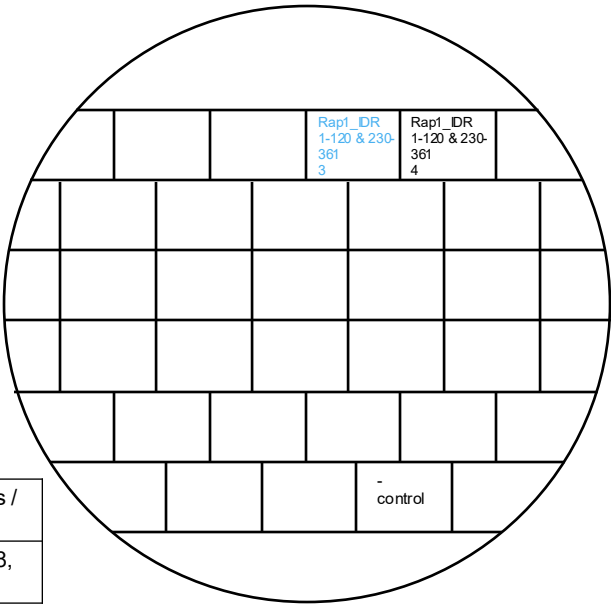
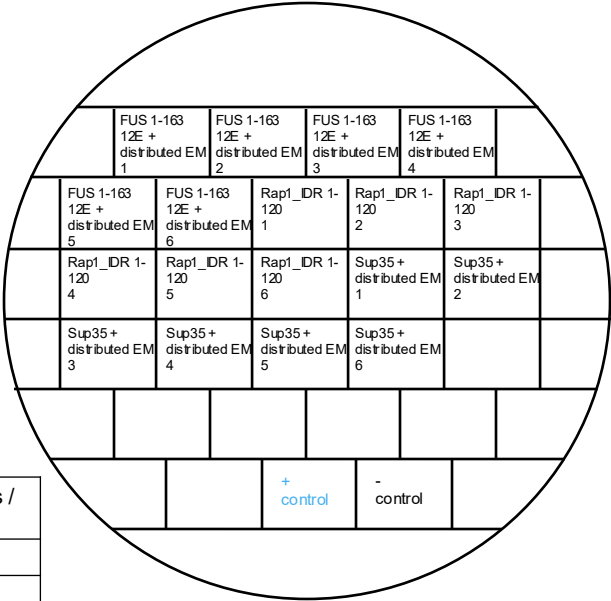
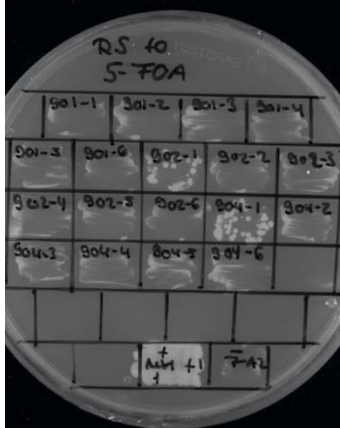


Fig. S1, slide 20



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
Rap1 ^{1-120 & 230-361}	inviabile	1 out of 2 (in total 7 out of 8, see also slide 40)



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
FUS 1-163 12E + EM distr.	inviabile	6 out of 6
Rap1 ¹⁻¹²⁰	inviabile	6 out of 6
Sup35 ¹⁻¹³¹ + EM distr.	inviabile	6 out of 6

Fig. S1, slide 21



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
Altered valence 2	viable	6 out of 6 (in total 5 out of 6, see also slide 10)
FUS ¹⁻¹⁶³ 12E + Gal4 ^{G4} aromatic clusters	viable	6 out of 6 (technical replicate see slide 19)
FUS ¹⁻¹⁶³ 12E + EM	viable	4 out of 4 (in total 6 out of 6, see also slides 11, 12, 24)

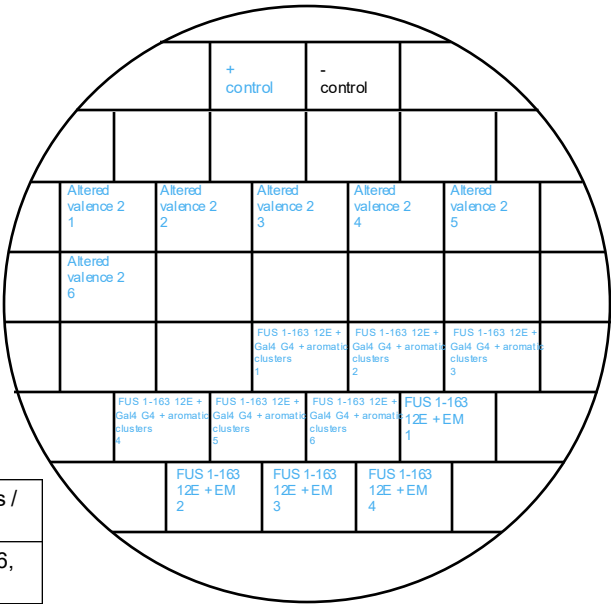
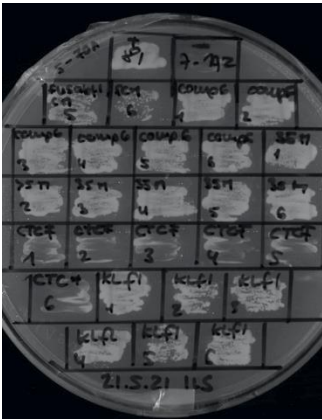
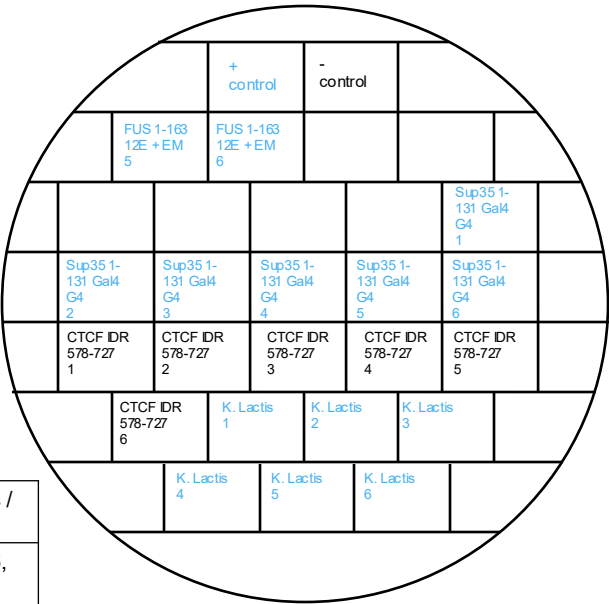


Fig. S1, slide 22



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
FUS1-163 12E + EM	viable	2 out of 2 (in total 6 out of 6, see also slides 11, 12, 23)
Sup35 1-131 + Gal4 ^{G4}	viable	6 out of 6
CTCF ⁵⁷⁸⁻⁷²⁷	inviable	6 out of 6 (technical repliate, see slide 25)
K. Lactis	viable	6 out of 6



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
LS-15	viable	6 out of 6 (in total 14 out of 14 clones, see also slide 37)

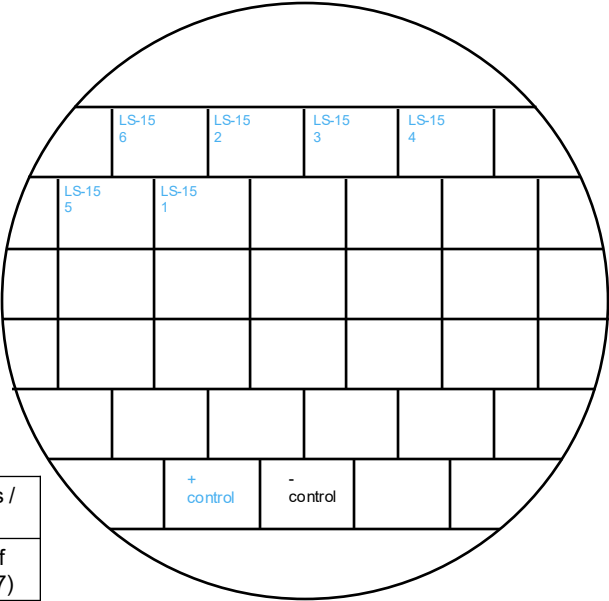
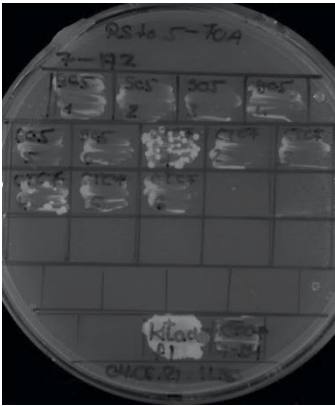
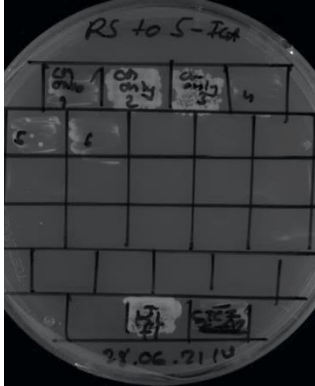
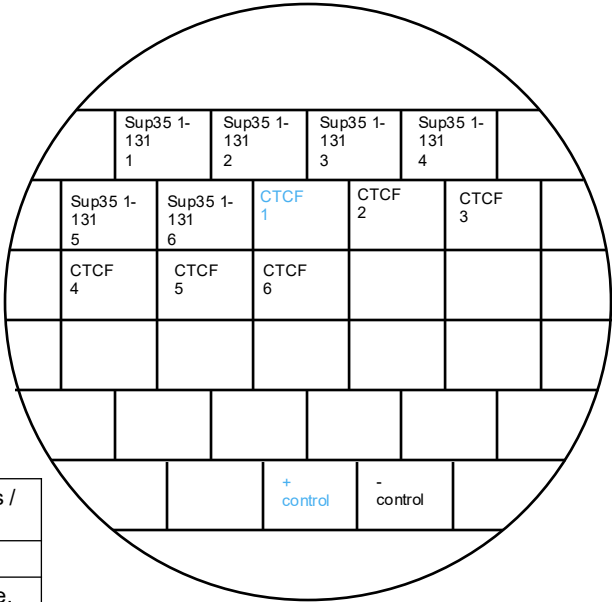


Fig. S1, slide 23



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
Sup35 ¹⁻¹³¹	inviable	6 out of 6
CTCF ⁵⁷⁸⁻⁷²⁷	inviable	5 out of 6 (technical repliate, see slide 24)



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
EM only	inviable	4 out of 6 (in total: 10 out of 12, see also slide 26)

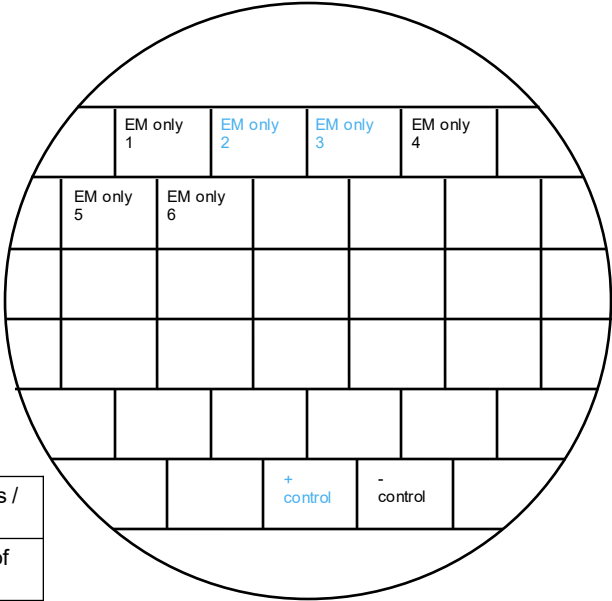
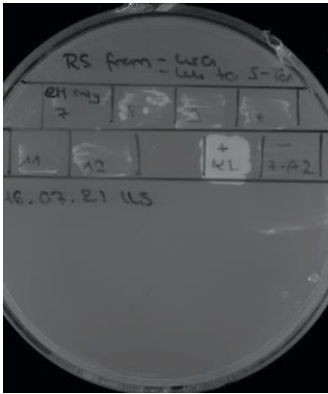
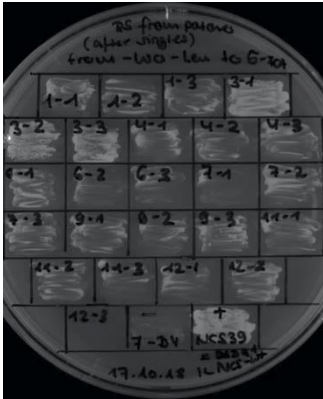
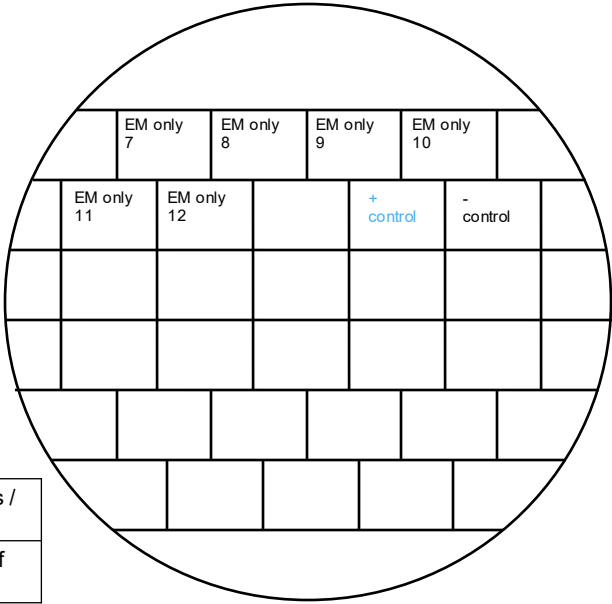


Fig. S1, slide 24



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
EM only	Invisible	6 out of 6 (in total: 10 out of 12, see also slide 25)



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
NCS-1	invisible	3 out of 3
NCS-3	viable	3 out of 3
NCS-4	invisible	3 out of 3
NCS-6	invisible	3 out of 3
NCS-7	invisible	3 out of 3
NCS-9	invisible	3 out of 3
NCS-11	invisible	3 out of 3
NCS-12	invisible	3 out of 3

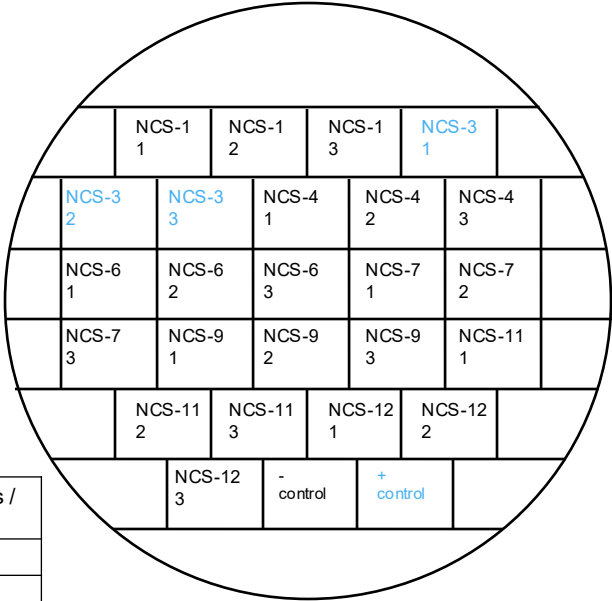
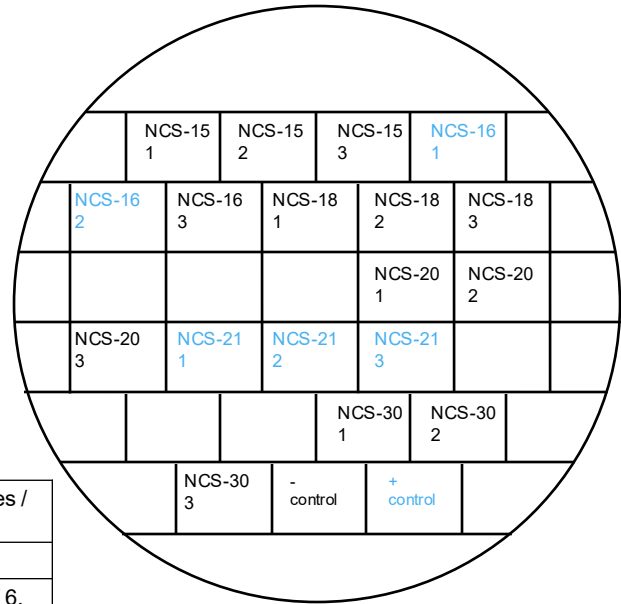
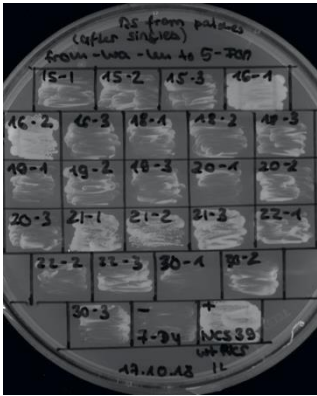
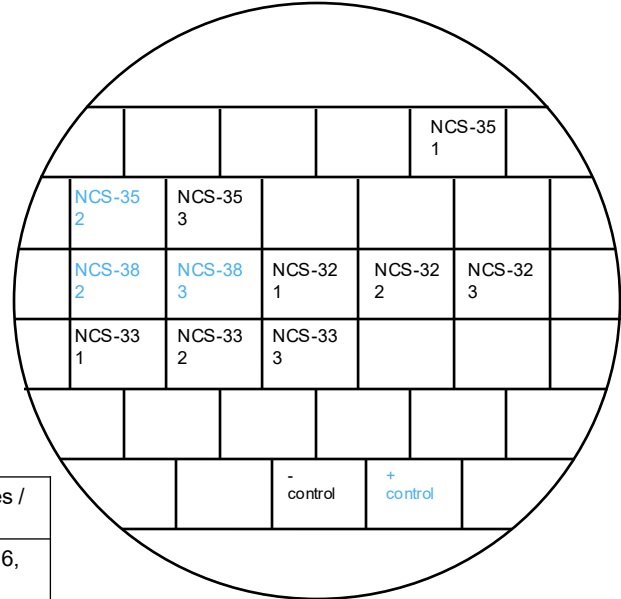
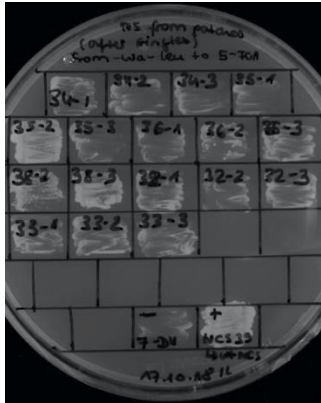


Fig. S1, slide 25



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
NCS-15	inviable	3 out of 3
NCS-16	inviable	1 out of 3 (in total 4 out of 6, see slide 28)
NCS-18	inviable	3 out of 3
NCS-20	inviable	3 out of 3
NCS-21	viable	3 out of 3
NCS-30	inviable	3 out of 3



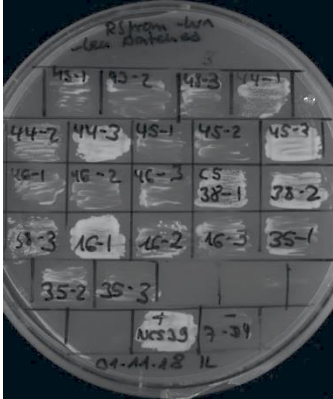
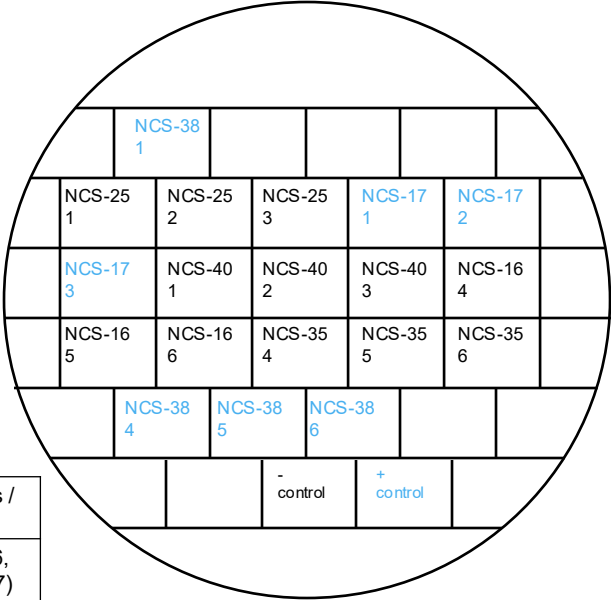
Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
NCS-35	inviable	2 out of 3 (in total 5 out of 6, see also slide 27)
NCS-38	viable	2 out of 2 (in total 5 out of 6, see also slide 27)
NCS-32	inviable	3 out of 3
NCS-33	inviable	3 out of 3



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
NCS-38	viable	4 out of 4 (in total 5 out of 6, see also below and slide 27)
NCS-25	inviable	3 out of 3
NCS-17	viable	3 out of 3
NCS-40	inviable	3 out of 3
NCS-16	inviable	3 out of 3 (in total 4 out of 6, see below and slide 27)
NCS-35	inviable	3 out of 3 (in total 5 out of 6, see also below and slide 27)



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
NCS-43	inviable	3 out of 3
NCS-45	inviable	2 out of 3 (in total 5 out of 6, see also slide 30)
NCS-46	inviable	3 out of 3
NCS-38	viable	2 out of 3 (in total 5 out of 6, see also above and slide 27)
NCS-16	inviable	2 out of 3 (in total 4 out of 6, see above and slide 27)
NCS-35	inviable	3 out of 3 (in total 5 out of 6, see also above and slide 27)

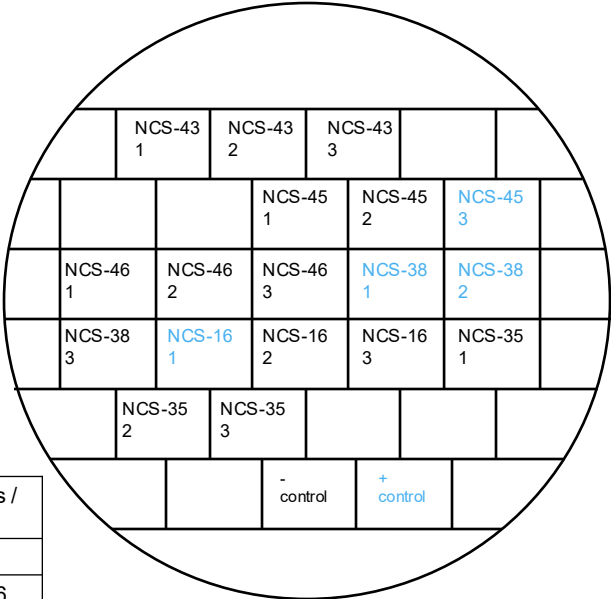
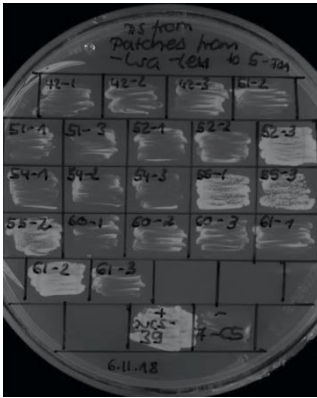
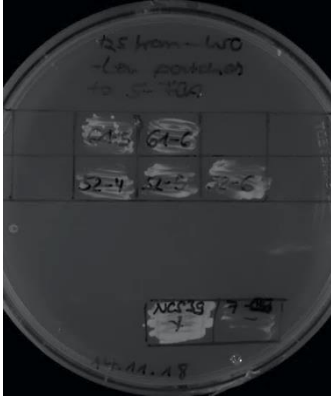
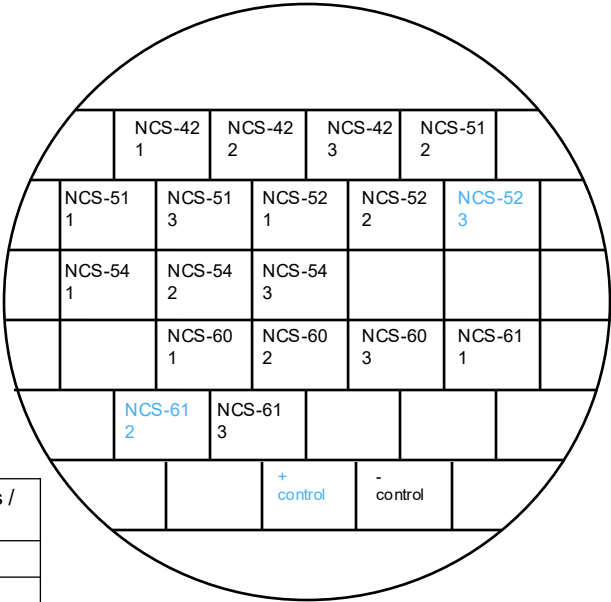


Fig. S1, slide 27



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
NCS-42	inviable	3 out of 3
NCS-51	inviable	3 out of 3
NCS-52	inviable	2 out of 3 (in total 5 out of 6 see also below)
NCS-54	inviable	3 out of 3
NCS-60	inviable	3 out of 3
NCS-61	inviable	2 out of 3 (in total 5 out of 6 see also below)



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
NCS-61	inviable	2 out of 2 (in total 5 out of 6 see also above)
NCS-52	inviable	3 out of 3 (in total 5 out of 6 see also above)

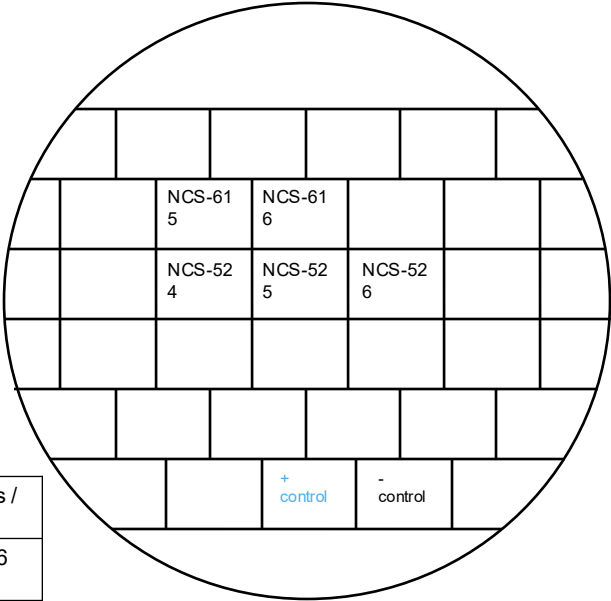
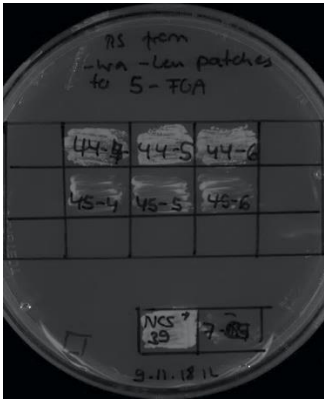
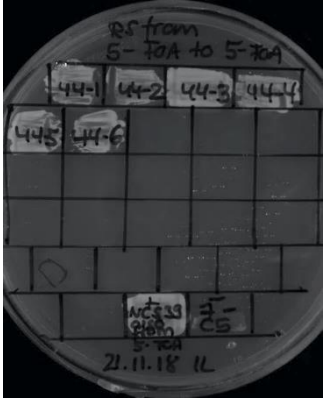
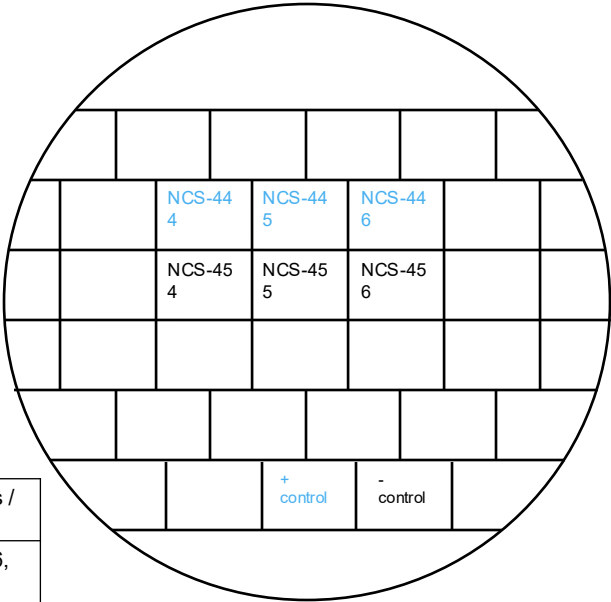


Fig. S1, slide 28



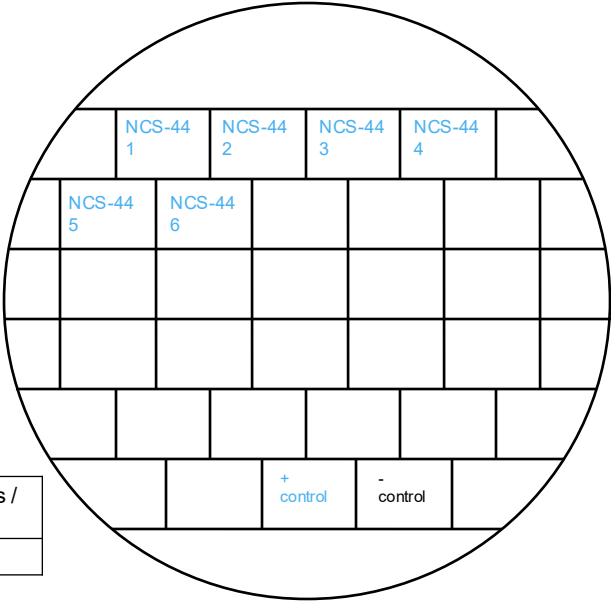
Restreak from YNB –ura –leu to 5-FOA –leu

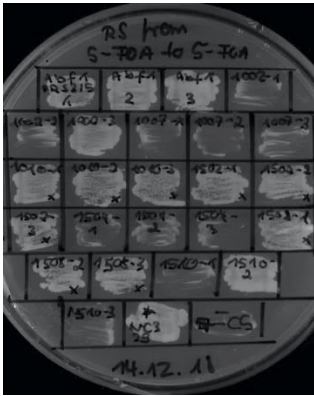
Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
NCS-44	viable	3 out of 3 (in total 6 out of 6, technical replicate see also below)
NCS-45	inviable	3 out of 3 (in total 5 out of 6, see also slide 28)



Restreak from 5-FOA – leu to 5-FOA –leu

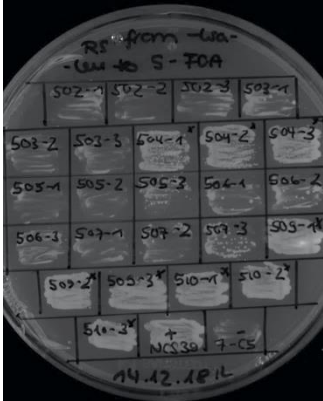
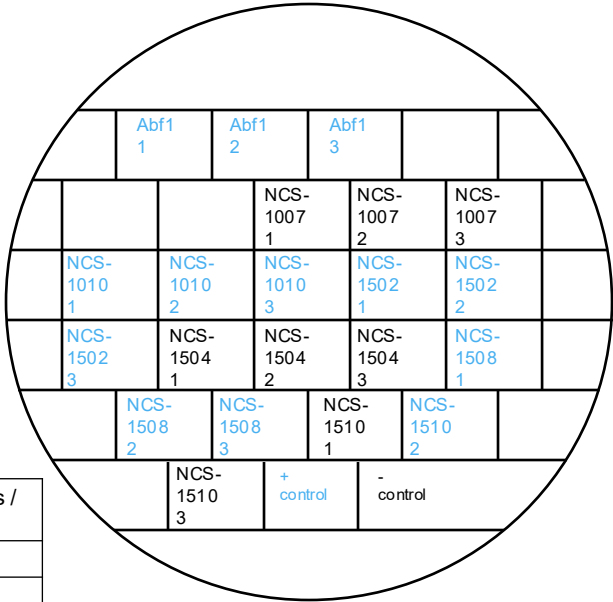
Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
NCS-44	viable	6 out of 6





Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
WT Abf1	viable	3 out of 3
NCS-1007	inviable	3 out of 3
NCS-1010	viable	3 out of 3
NCS-1502	viable	3 out of 3
NCS-1504	inviable	3 out of 3
NCS-1508	viable	3 out of 3
NCS-1510	inviable	2 out of 3 (in total 12 out of 15, see also slides 32 and 33)



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
NCS-502	inviable	3 out of 3
NCS-503	inviable	3 out of 3
NCS-504	viable	3 out of 3
NCS-505	inviable	3 out of 3
NCS-507	inviable	3 out of 3
NCS-509	viable	3 out of 3
NCS-510	viable	3 out of 3

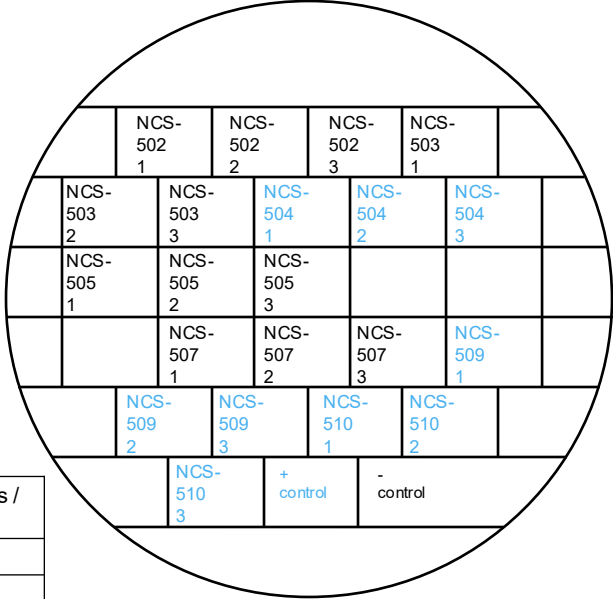
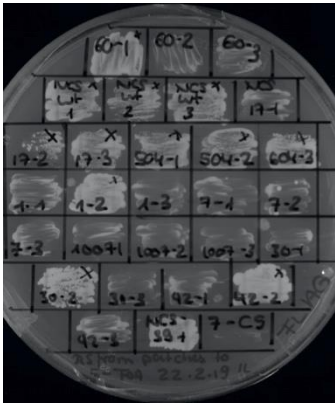
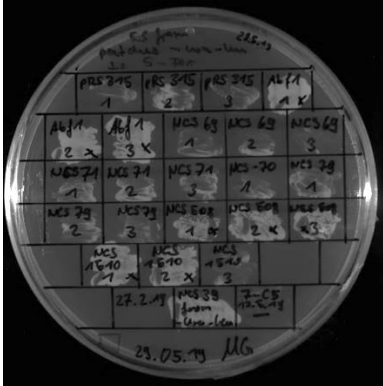
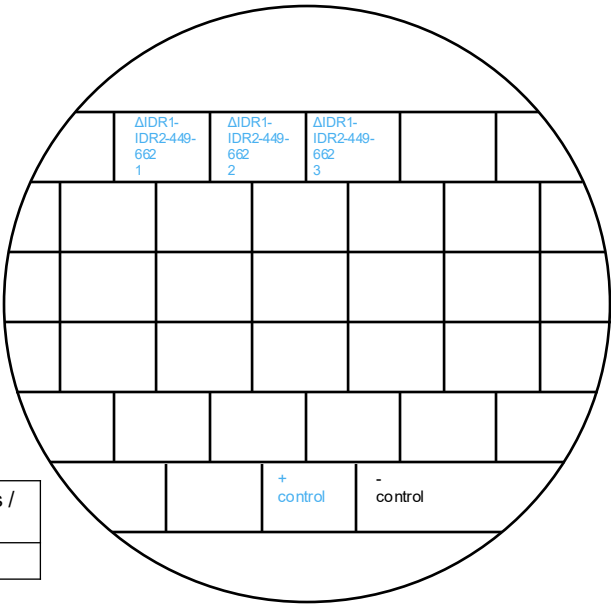


Fig. S1, slide 30



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
ΔIDR1 & IDR2⁴⁴⁹⁻⁶⁶²	viable	3 out of 3



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
pRS315 empty	inviable	3 out of 3
NCS-69	inviable	3 out of 3
NCS-71	inviable	3 out of 3
NCS-70	inviable	1 out of 1 (in total 3 out of 3, see slide 33)
NCS-79	inviable	3 out of 3
NCS-508	viable	3 out of 3
NCS-1510	inviable	1 out of 3 (in total: 12 out of 15, see also slides 31 and 33)

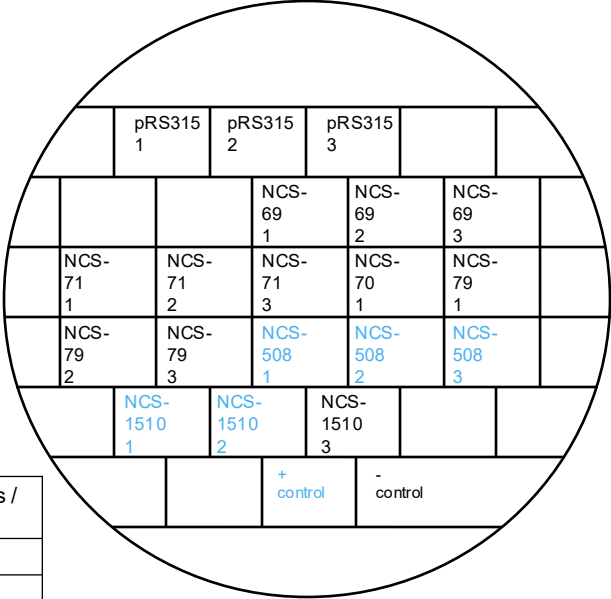
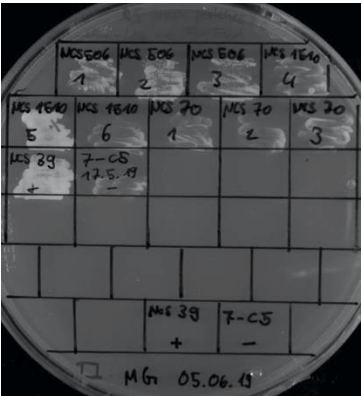
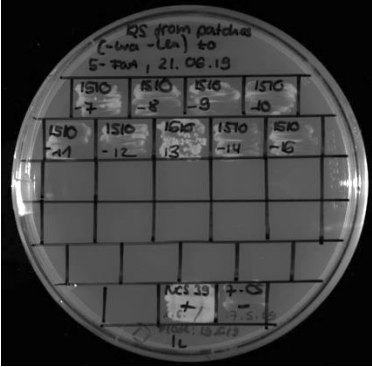
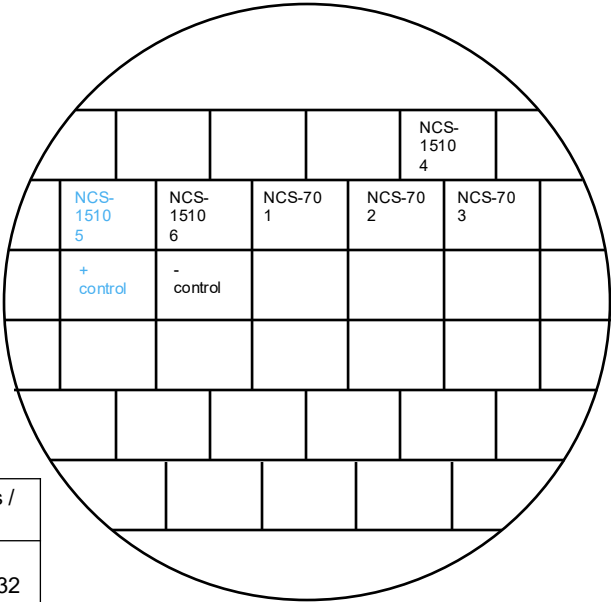


Fig. S1, slide 31



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
NCS-1510	inviable	2 out of 3 (in total 12 out of 15, see also slides 31 and 32 and below)
NCS-70	inviable	3 out of 3 (in total 3 out of 3, see slide 32)



Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
NCS-1510	inviable	9 out of 9 (in total: 12 out of 15, see also slides 31 and 32 and above)

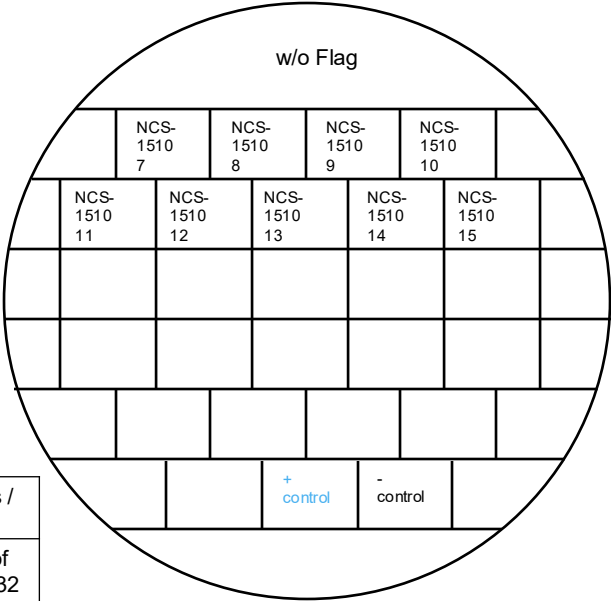
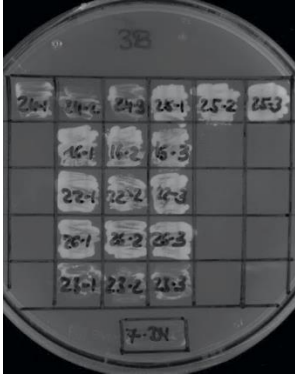
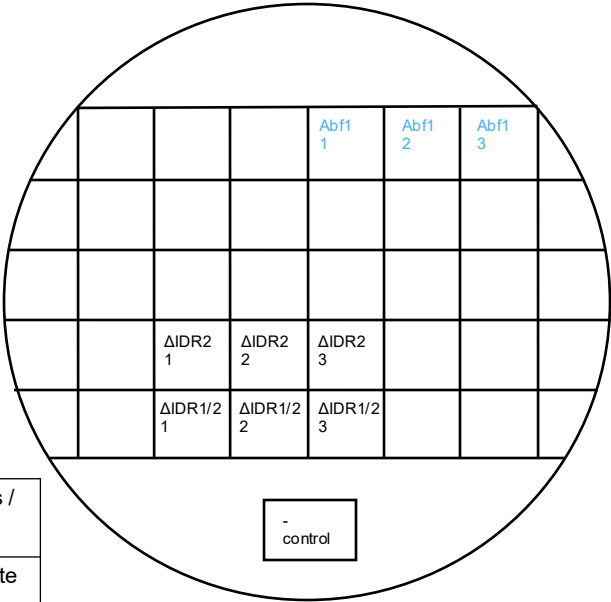


Fig. S1, slide 32



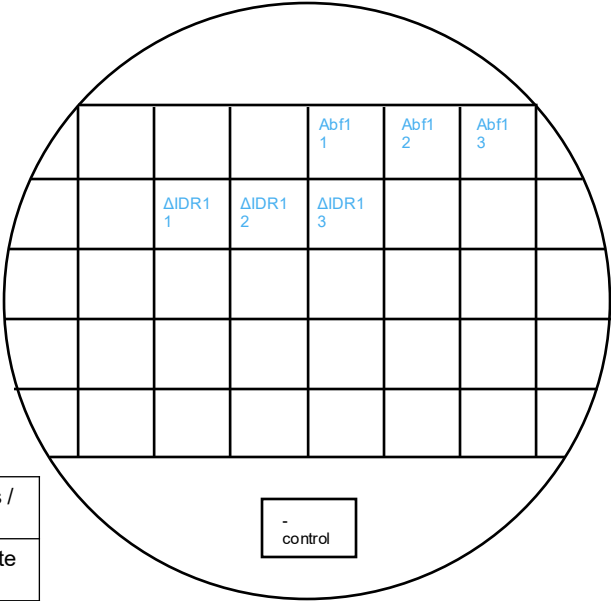
Restreak from YNB –ura –leu to 5-FOA –leu

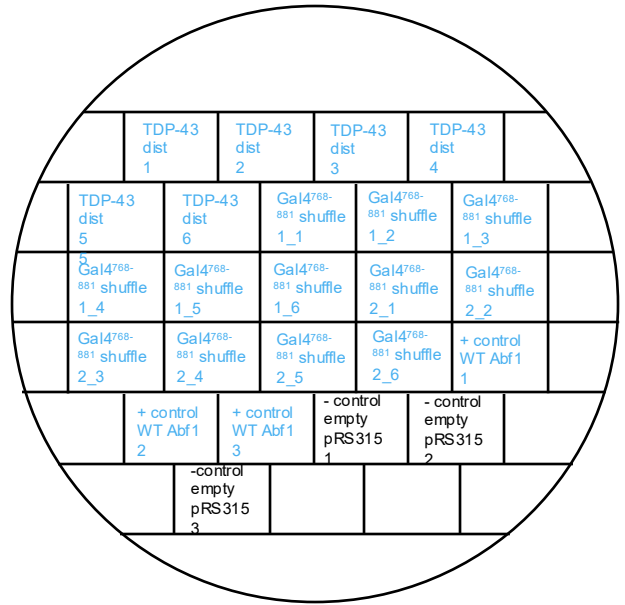
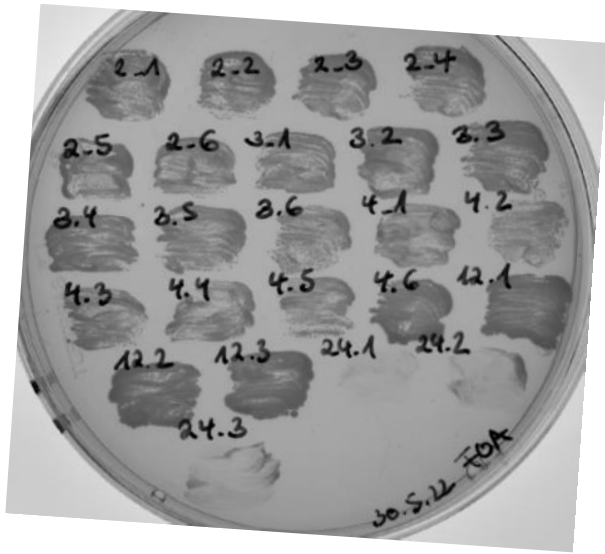
Construct	Result regarding viability on 5-FOA	Number of (in) viable clones / total number of clones
Abf1 (NLS)	viable	3 out of 3 (technical replicate see below)
ΔIDR2	inviable	3 out of 3
ΔIDR1/2	inviable	3 out of 3



Restreak from YNB –ura –leu to 5-FOA –leu

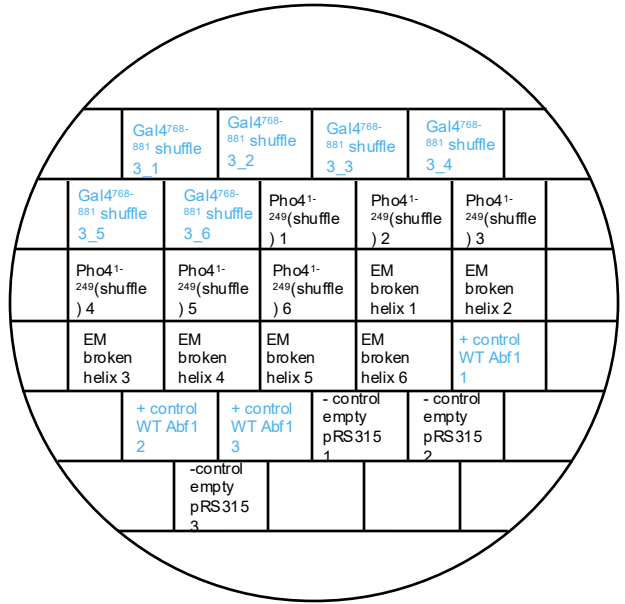
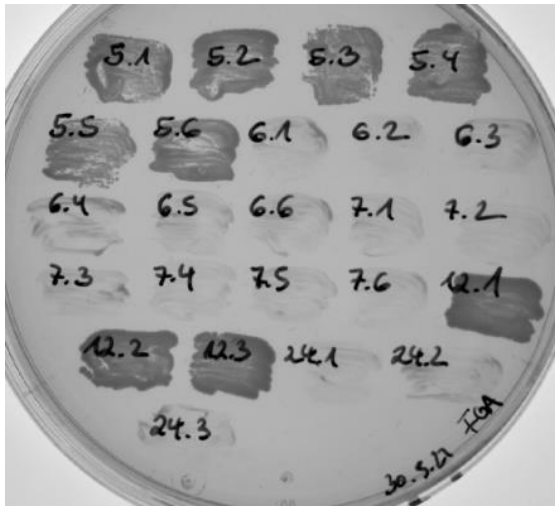
Construct	Result regarding viability on 5-FOA	Number of (in) viable clones / total number of clones
Abf1 (NLS)	viable	3 out of 3 (technical replicate see above)
ΔIDR1	viable	3 out of 3





Restreak from YNB –ura –leu to 5-FOA –leu

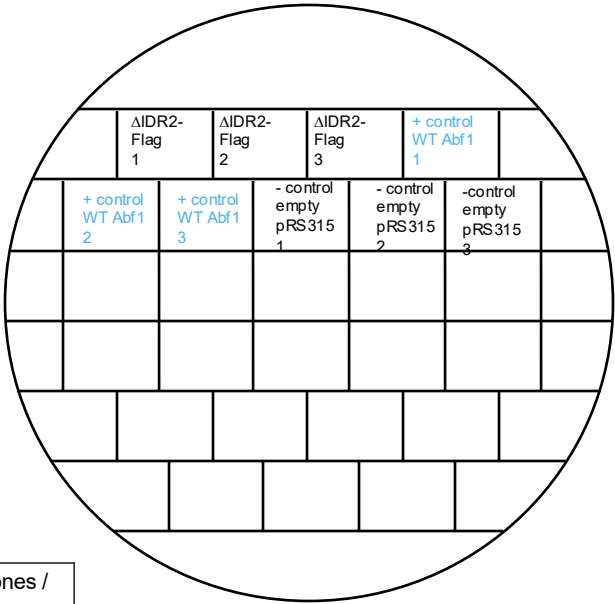
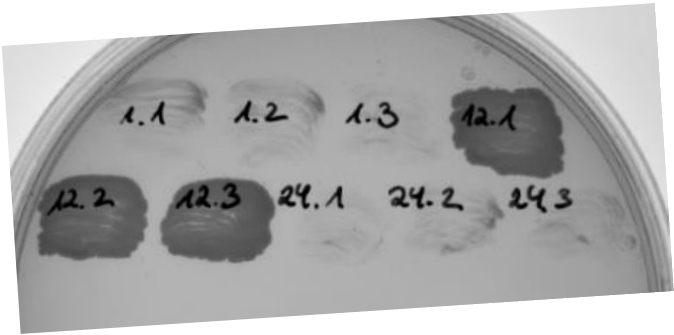
Construct	Result regarding viability on 5-FOA	Number of (in) viable clones / total number of clones
TDP-43 distr.	viable	6 out of 6
Gal4 ⁷⁶⁸⁻⁸⁸¹ shuffle 1	viable	6 out of 6
Gal4 ⁷⁶⁸⁻⁸⁸¹ shuffle 2	viable	6 out of 6



Restreak from YNB –ura –leu to 5-FOA –leu

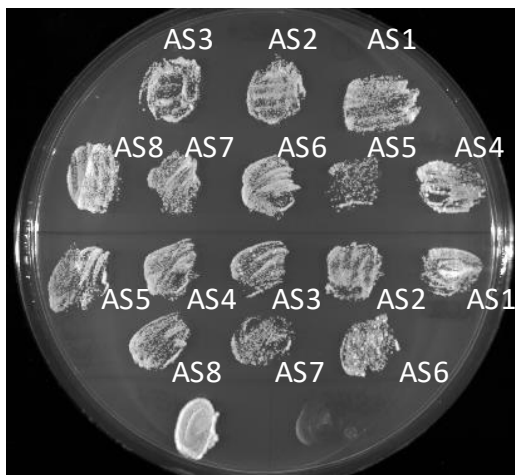
Construct	Result regarding viability on 5-FOA	Number of (in) viable clones / total number of clones
Gal4 ⁷⁶⁸⁻⁸⁸¹ shuffle 3	viable	6 out of 6
Phd4 ¹⁻²⁴⁹ shuffle	inviable	6 out of 6
IDR2 WT broken helix	inviable	6 out of 6

Fig. S1, slide 34



Restreak from YNB -ura -leu to 5-FOA -leu

Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
ΔIDR2-Flag	inviabile	3 out of 3



NCS-506-Flag (clones AS1-AS8)

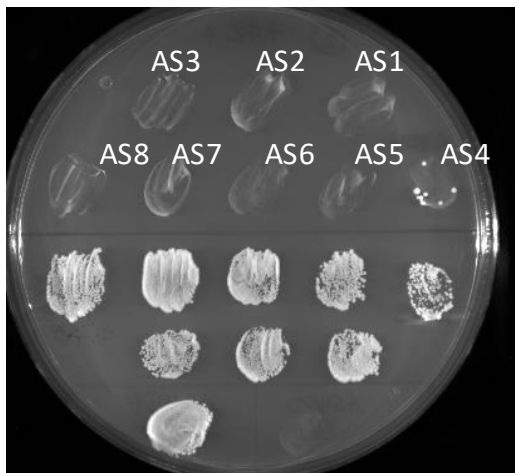
LS-15 (clones AS1-AS8)

+ control WT Abf1

- control empty pRS315

Restreak from YNB -ura -leu to 5-FOA -leu

Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
NCS-506-Flag	viable	8 out of 8
LS-15	viable	8 out of 8 (in total 14 out of 14, see also slide 24)



V. polyspora (clones AS1-AS8)

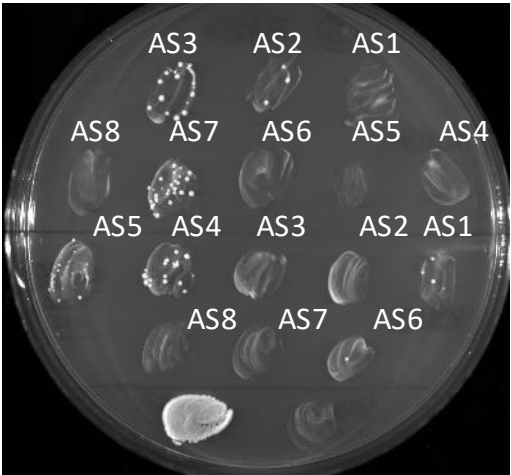
(not part of this study)

+ control WT Abf1

- control empty pRS315

Restreak from YNB -ura -leu to 5-FOA -leu

Construct	Result regarding viability on 5-FOA	Number of (in)viabile clones / total number of clones
V. polyspora	inviable	8 out of 8 (in total 14 out of 14, see also slide 17)



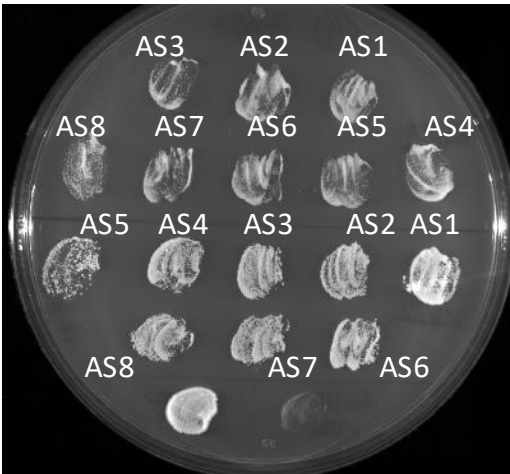
FUS¹⁻¹⁶³12E + Abf1^{G4} distr.
(clones AS1-AS8)

FUS¹⁻¹⁶³12E + EM shuffle
(clones AS1-AS8)

+ control WT Abf1 | - control empty pRS315

Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in) viable clones / total number of clones
FUS ¹⁻¹⁶³ 12E + Abf1 ^{G4} distr.	inviable	8 out of 8 (in total 14 out of 14, see also slide 40)
FUS ¹⁻¹⁶³ 12E + EM shuffle	inviable	8 out of 8 (in total 14 out of 14, see also slide 40)



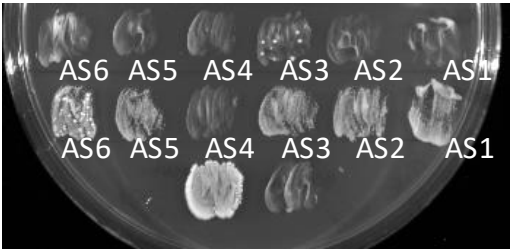
FUS¹⁻¹⁶³12E + Gal4^{G4} all ΔE/D
(clones AS1-AS8)

NCS-21 hydro -> polar
(clones AS1-AS8)

+ control WT Abf1 | - control empty pRS315

Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in) viable clones / total number of clones
FUS ¹⁻¹⁶³ 12E + Gal4 ^{G4} all ΔE/D	viable	8 out of 8
NCS-21 hydro→polar	viable	8 out of 8



Pho4¹⁻²⁴⁹ segmental shuffle (clones AS1-AS6)

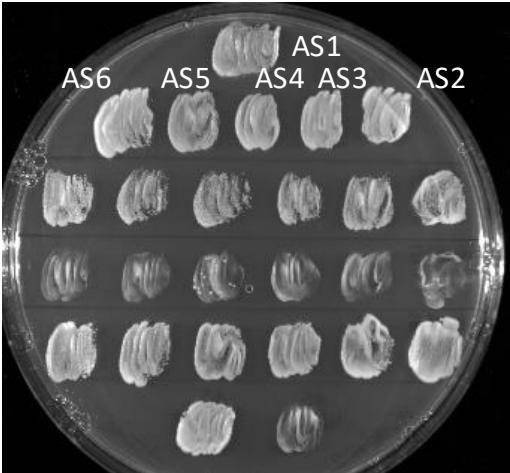
Sup35¹⁻¹³¹ + EM (clones AS1-AS6)

+ control WT Abf1

- control empty pRS315

Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
Pho4 ¹⁻²⁴⁹ segmental shuffle	inviable	6 out of 6
Sup35 ¹⁻¹³¹ + EM	viable	5 out of 6



FUS1-163 12E + Gal4^{G4} distr. II (clones AS1-AS6)

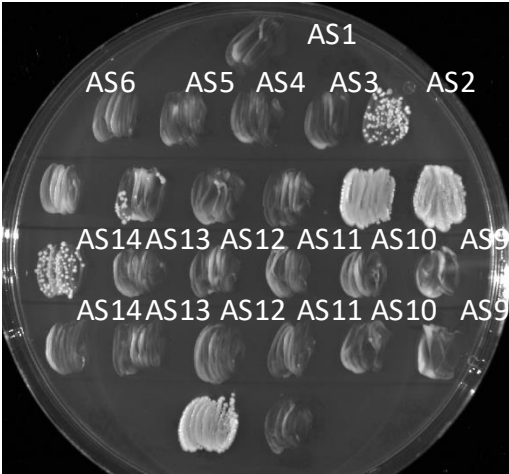
(not part of this study)

+ control WT Abf1

- control empty pRS315

Restreak from YNB –ura –leu to 5-FOA –leu

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
FUS1-163 12E Gal4 ^{G4} distr. II	viable	6 out of 6



Restreak from YNB –ura –leu to 5-FOA –leu

Rap1 IDR¹⁻¹²⁰ & IDR²³⁰⁻³⁶¹ (clones AS1-AS6)

(not part of this study)

FUS¹⁻¹⁶³12E + Abf1^{G4} distr. (clones AS9-AS14)

FUS¹⁻¹⁶³12E + EM shuffle (clones AS9-AS14)

+ control WT Abf1

- control empty pRS315

Construct	Result regarding viability on 5-FOA	Number of (in)viable clones / total number of clones
Rap1 ¹⁻¹²⁰ & 230-361	inviable	6 out of 6 (in total 7 out of 8, see also slide 22)
FUS ¹⁻¹⁶³ 12E + Abf1 ^{G4} distr.	inviable	6 out of 6 (in total 14 out of 14, see also slide 38)
FUS ¹⁻¹⁶³ 12E + EM shuffle	inviable	6 out of 6 (in total 14 out of 14, see also slide 38)