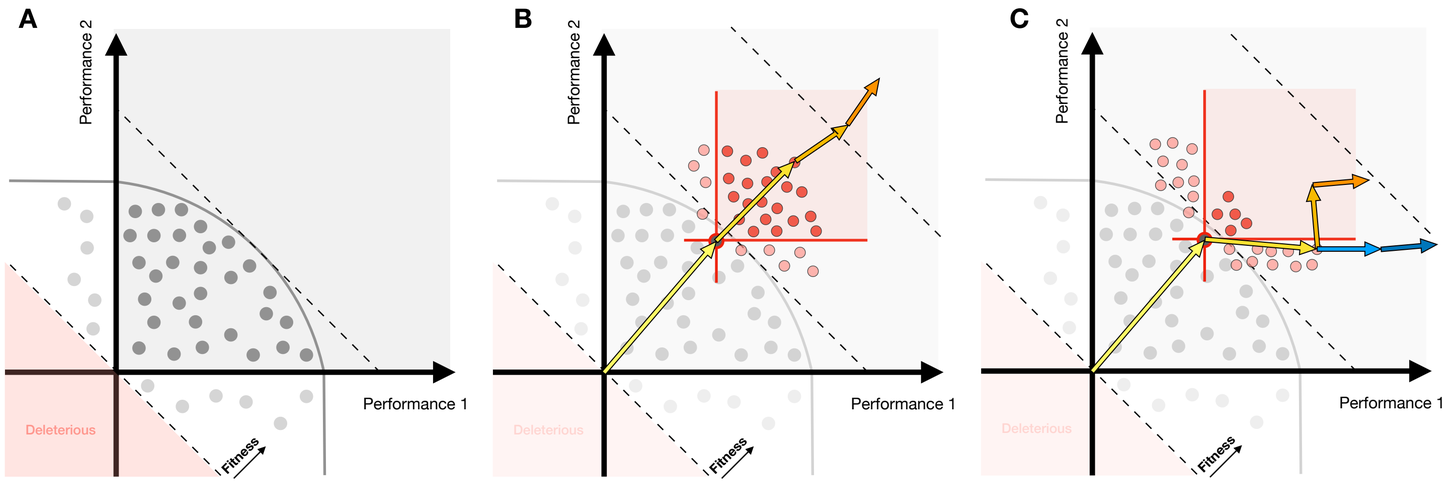
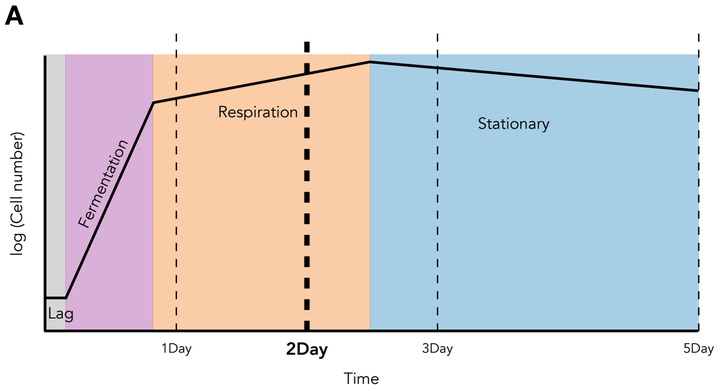
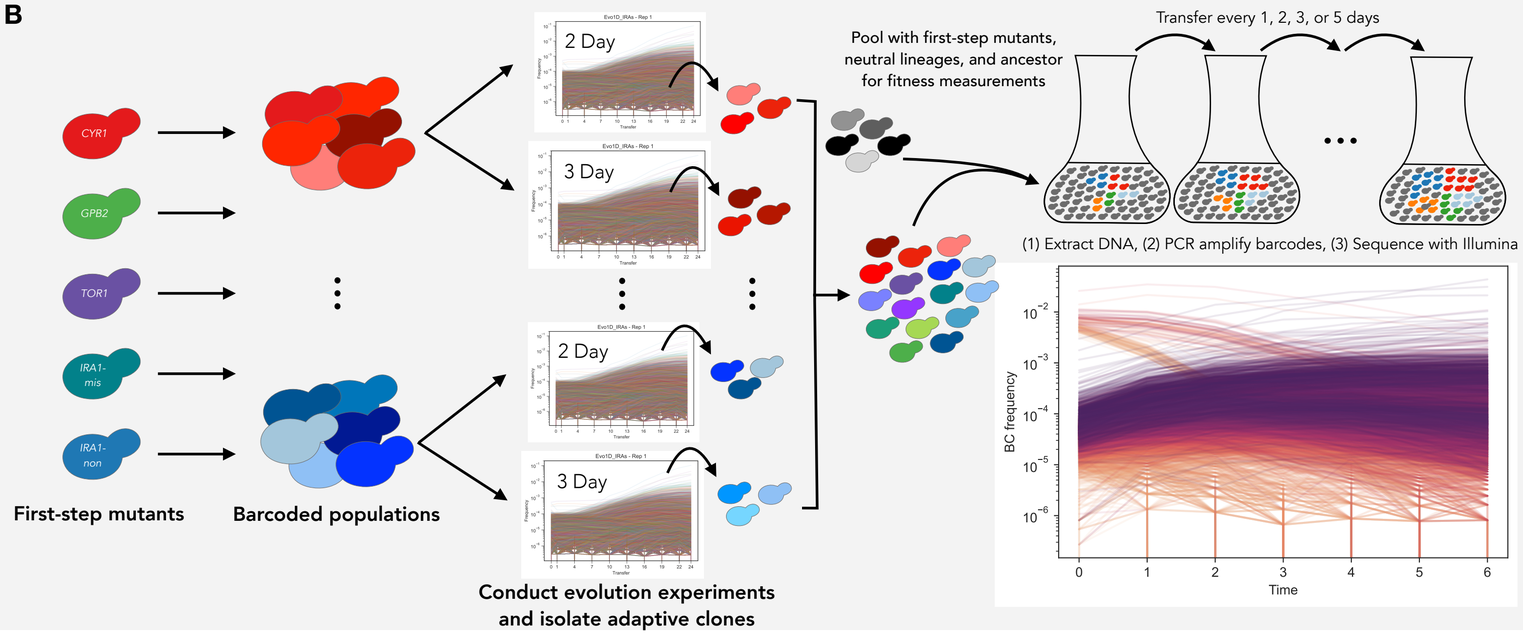
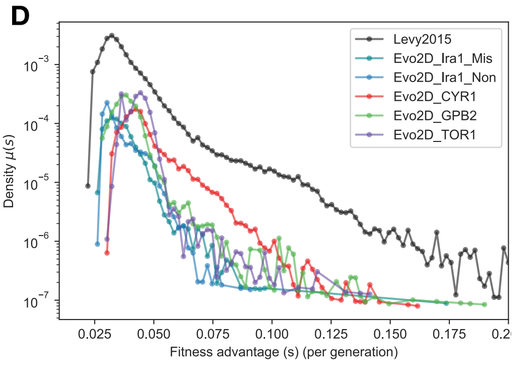

A
Performance 2
Performance 1
Deleterious
Fitness
B
Performance 2
Performance 1
Deleterious
Fitness
C
Performance 2
Performance 1
Deleterious
Fitness

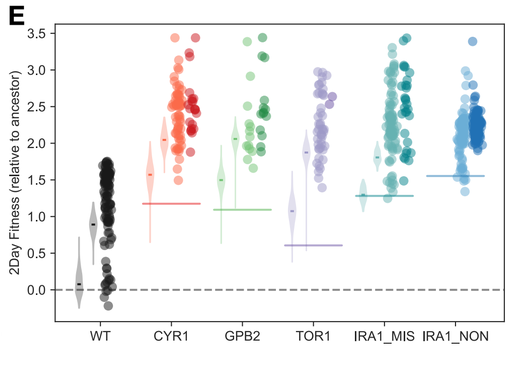


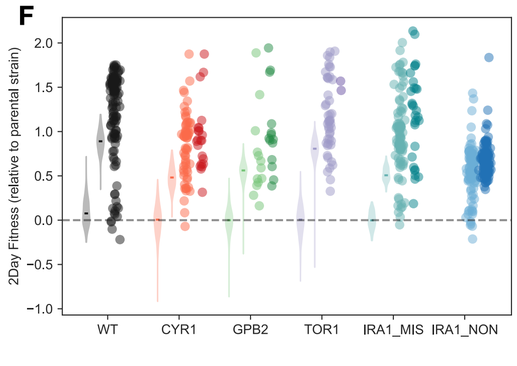
C

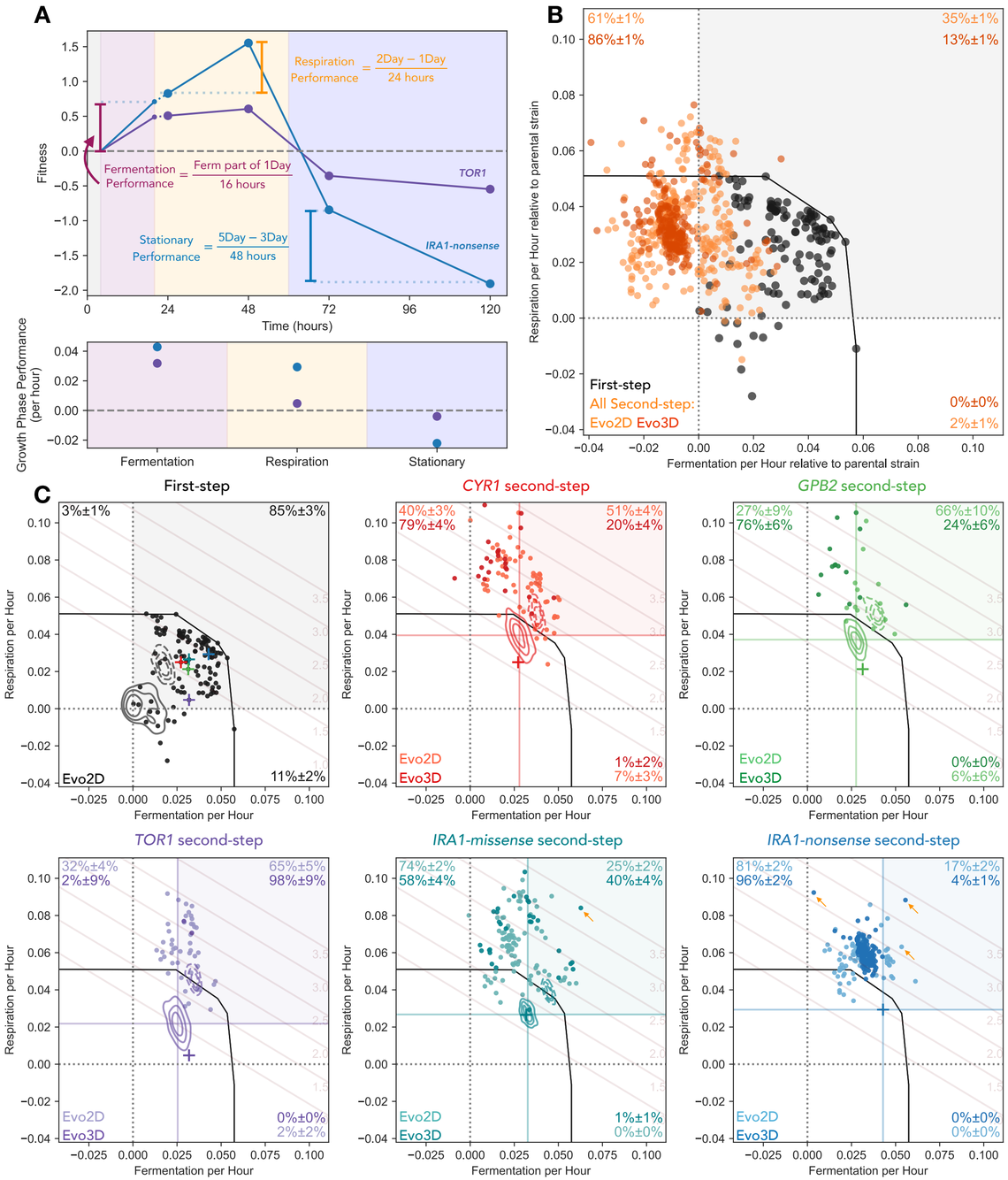
	Parental Genotype	First-step mutation	Evolution Condition	Number of adaptive mutants	Number of diploids	Publication
First Step	WT	-	2Day	121	194	Levy et al. 2015
Second Step	*CYR1*	*cyr1 S917Y*	2Day	68	224	Aggeli et al. 2021
			3Day	20	8	This study
	GPB2	*gpb2 Y282**	2Day	17	228	Aggeli et al. 2021
			3Day	12	20	This study
	TOR1	*tor1 F1712L*	2Day	57	317	Aggeli et al. 2021
			3Day	2	-	This study
	IRA1 missense	*ira1 A1211V*	2Day	90	16	This study
			3Day	28	3	This study
	IRA1 nonsense	*ira1 L1401**	2Day	95	-	This study
			3Day	135	-	This study

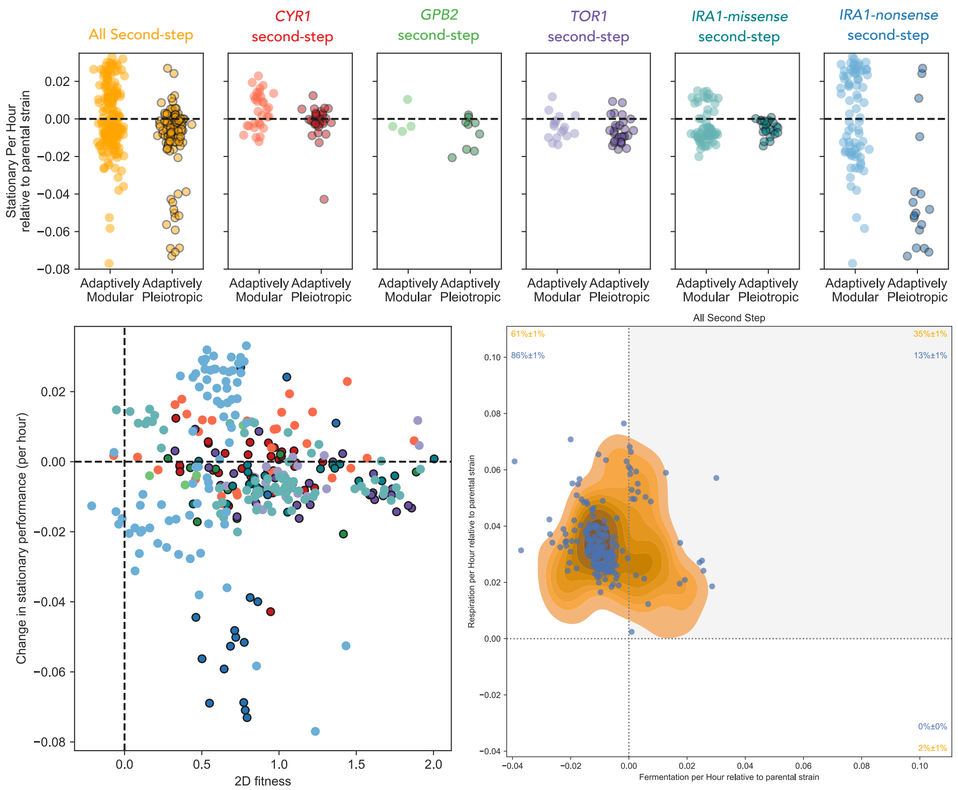


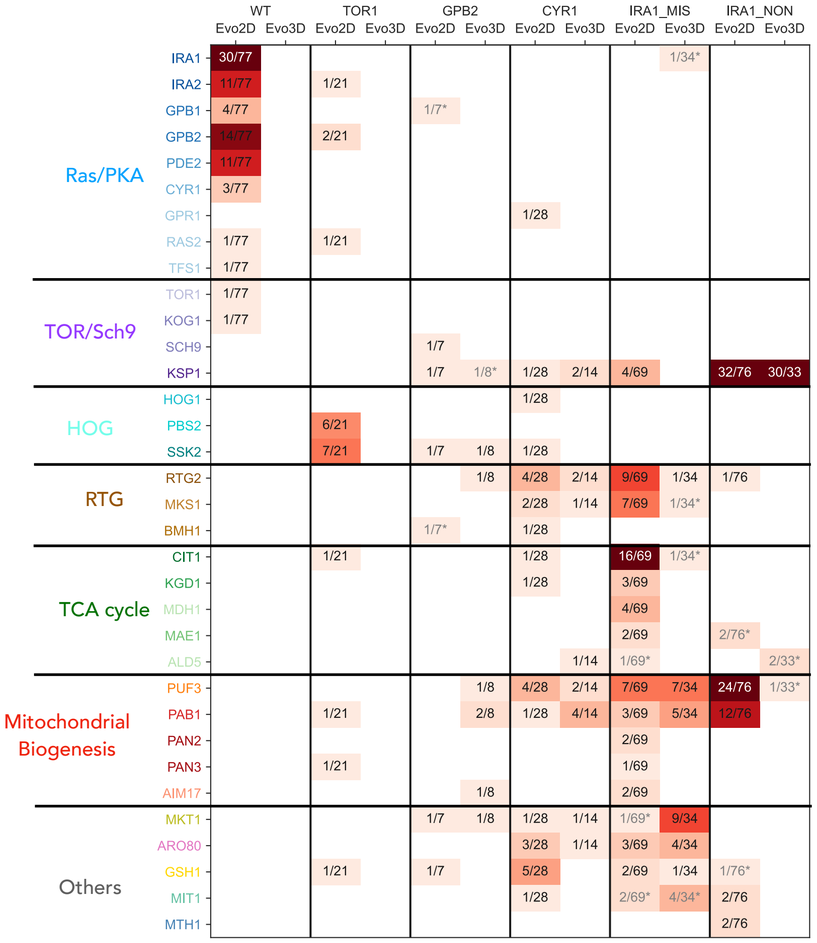


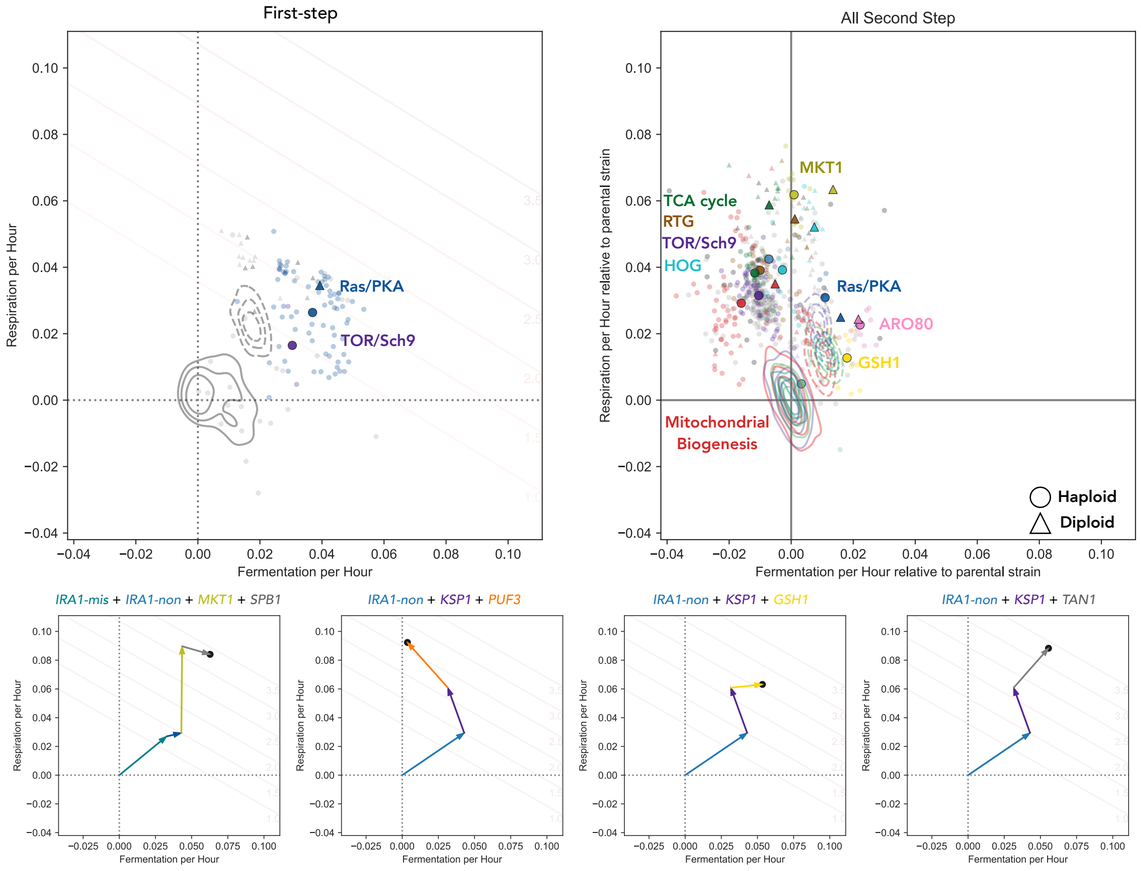




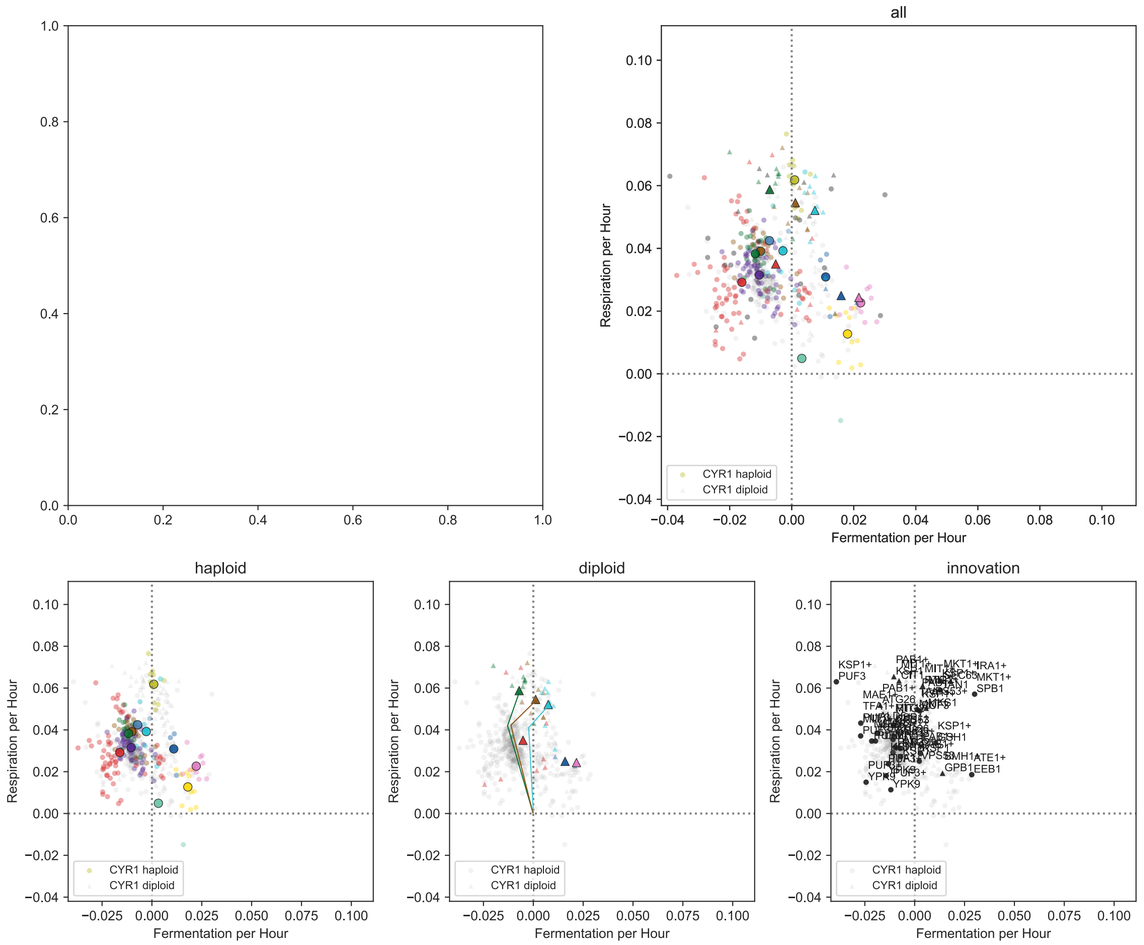

A
Respiration Performance = 2Day − 1Day / 24 hours
Fermentation Performance = Ferm part of 1Day / 16 hours
Stationary Performance = 5Day − 3Day / 48 hours
TOR1
IRA1-nonsense
Fitness
Time (hours)
Growth Phase Performance (per hour)
Fermentation
Respiration
Stationary
B
61%±1%
86%±1%
35%±1%
13%±1%
First-step
All Second-step:
Evo2D Evo3D
0%±0%
2%±1%
Respiration per Hour relative to parental strain
Fermentation per Hour relative to parental strain
C
First-step
3%±1%
85%±3%
Evo2D
11%±2%
CYR1 second-step
40%±3%
79%±4%
51%±4%
20%±4%
Evo2D
Evo3D
1%±2%
7%±3%
GPB2 second-step
27%±9%
76%±6%
66%±10%
24%±6%
Evo2D
Evo3D
0%±0%
6%±6%
TOR1 second-step
32%±4%
2%±9%
65%±5%
98%±9%
Evo2D
Evo3D
0%±0%
2%±2%
IRA1-missense second-step
74%±2%
58%±4%
25%±2%
40%±4%
Evo2D
Evo3D
1%±1%
0%±0%
IRA1-nonsense second-step
81%±2%
96%±2%
17%±2%
4%±1%
Evo2D
Evo3D
0%±0%
0%±0%
Respiration per Hour
Fermentation per Hour

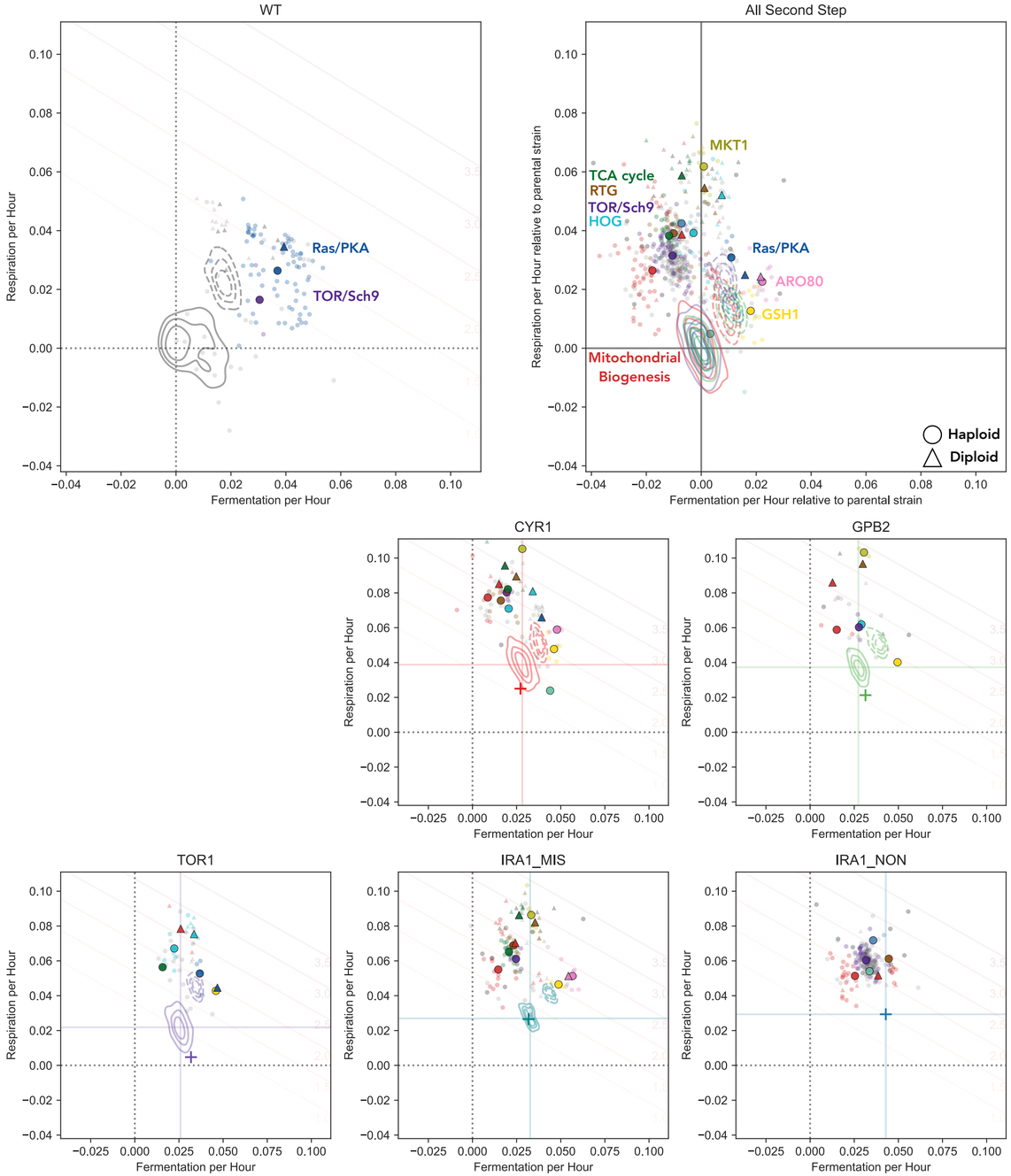

All Second-step
CYR1 second-step
GPB2 second-step
TOR1 second-step
IRA1-missense second-step
IRA1-nonsense second-step
Stationary Per Hour relative to parental strain
0.02
0.00
−0.02
−0.04
−0.06
−0.08
Adaptively Modular
Adaptively Pleiotropic
Change in stationary performance (per hour)
2D fitness
0.0
0.5
1.0
1.5
2.0
All Second Step
61%±1%
35%±1%
86%±1%
13%±1%
0%±0%
2%±1%
Respiration per Hour relative to parental strain
Fermentation per Hour relative to parental strain
0.10
0.08
0.06
0.04
0.02
0.00
−0.02
−0.04

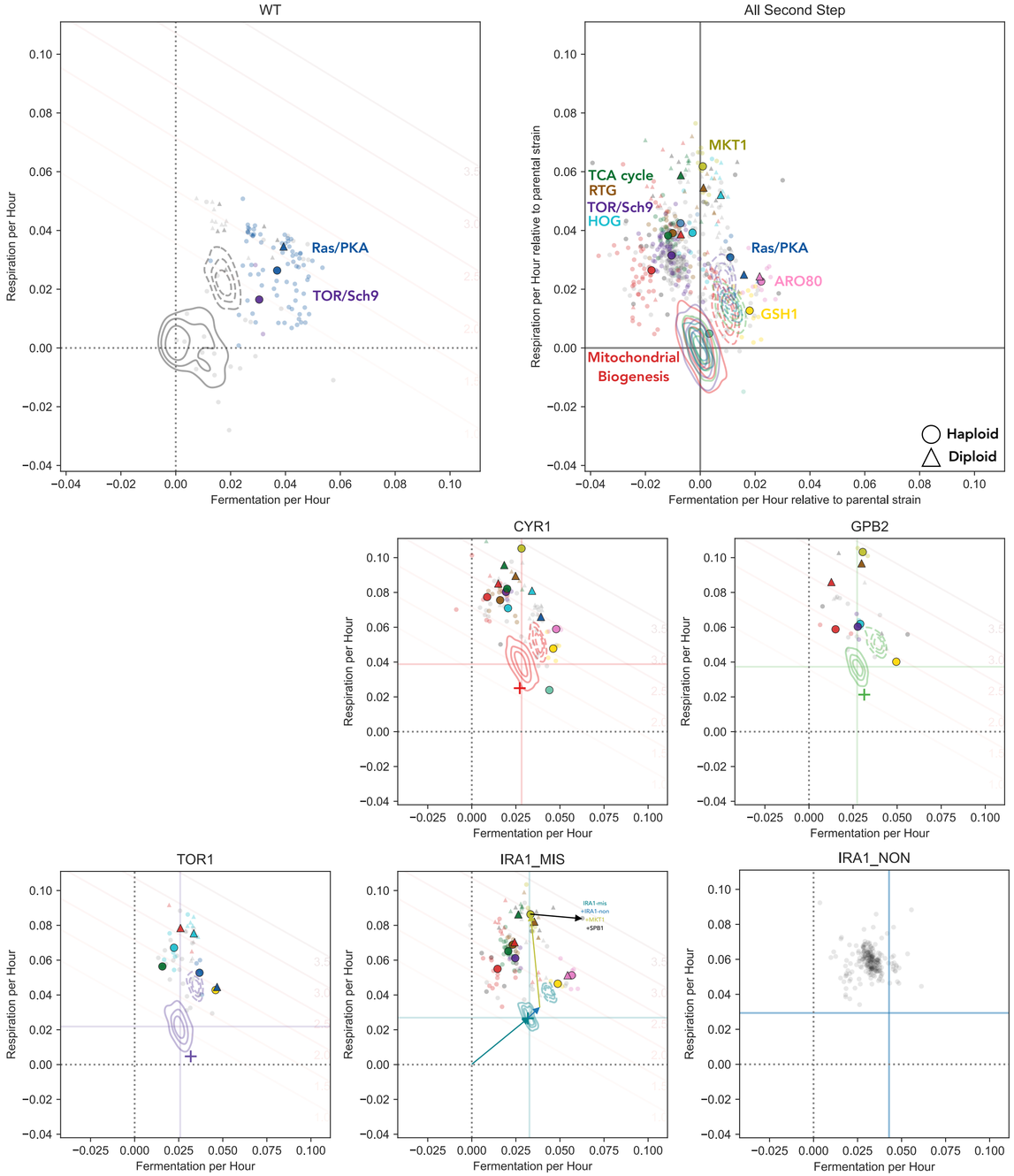

WT
TOR1
GPB2
CYR1
IRA1_MIS
IRA1_NON
Evo2D Evo3D Evo2D Evo3D Evo2D Evo3D Evo2D Evo3D Evo2D Evo3D Evo2D Evo3D
Ras/PKA
IRA1 30/77 1/34*
IRA2 11/77 1/21
GPB1 4/77 1/7*
GPB2 14/77 2/21
PDE2 11/77
CYR1 3/77
GPR1 1/28
RAS2 1/77 1/21
TFS1 1/77
TOR/Sch9
TOR1 1/77
KOG1 1/77
SCH9 1/7
KSP1 1/7 1/8* 1/28 2/14 4/69 32/76 30/33
HOG
HOG1 1/28
PBS2 6/21
SSK2 7/21 1/7 1/8 1/28
RTG
RTG2 1/8 4/28 2/14 9/69 1/34 1/76
MKS1 2/28 1/14 7/69 1/34*
BMH1 1/7* 1/28
TCA cycle
CIT1 1/21 1/28 16/69 1/34*
KGD1 1/28 3/69
MDH1 4/69
MAE1 2/69 2/76*
ALD5 1/14 1/69* 2/33*
Mitochondrial Biogenesis
PUF3 1/8 4/28 2/14 7/69 7/34 24/76 1/33*
PAB1 1/21 2/8 1/28 4/14 3/69 5/34 12/76
PAN2 2/69
PAN3 1/21 1/69
AIM17 1/8 2/69
Others
MKT1 1/7 1/8 1/28 1/14 1/69* 9/34
ARO80 3/28 1/14 3/69 4/34
GSH1 1/21 1/7 5/28 2/69 1/34 1/76*
MIT1 1/28 2/69* 4/34* 2/76
MTH1 2/76

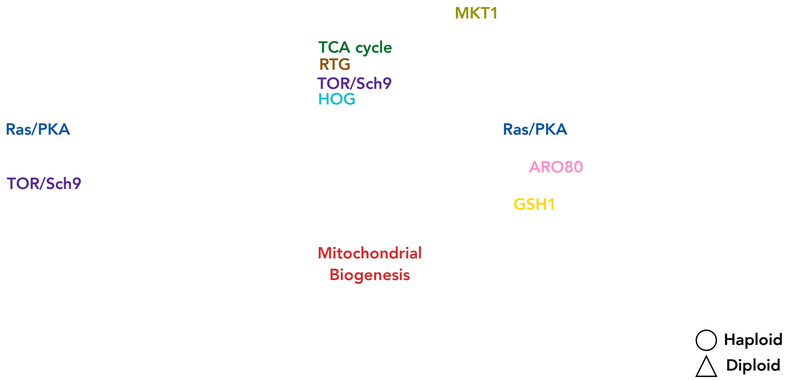

First-step
All Second Step
Respiration per Hour
Fermentation per Hour
Respiration per Hour relative to parental strain
Fermentation per Hour relative to parental strain
Ras/PKA
TOR/Sch9
MKT1
TCA cycle
RTG
TOR/Sch9
HOG
Ras/PKA
ARO80
GSH1
Mitochondrial
Biogenesis
Haploid
Diploid

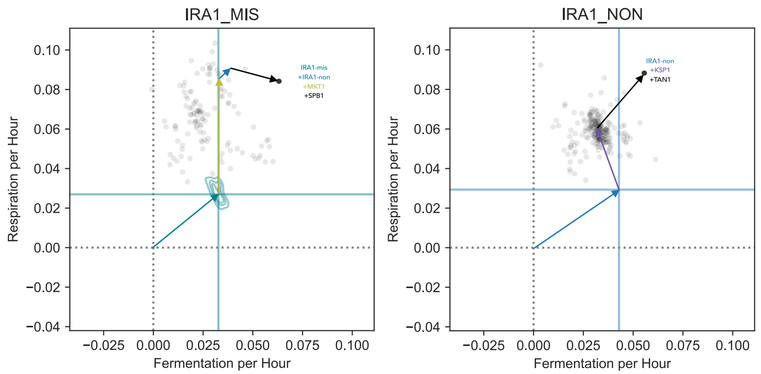
IRA1-mis + IRA1-non + MKT1 + SPB1
IRA1-non + KSP1 + PUF3
IRA1-non + KSP1 + GSH1
IRA1-non + KSP1 + TAN1
Respiration per Hour
Fermentation per Hour

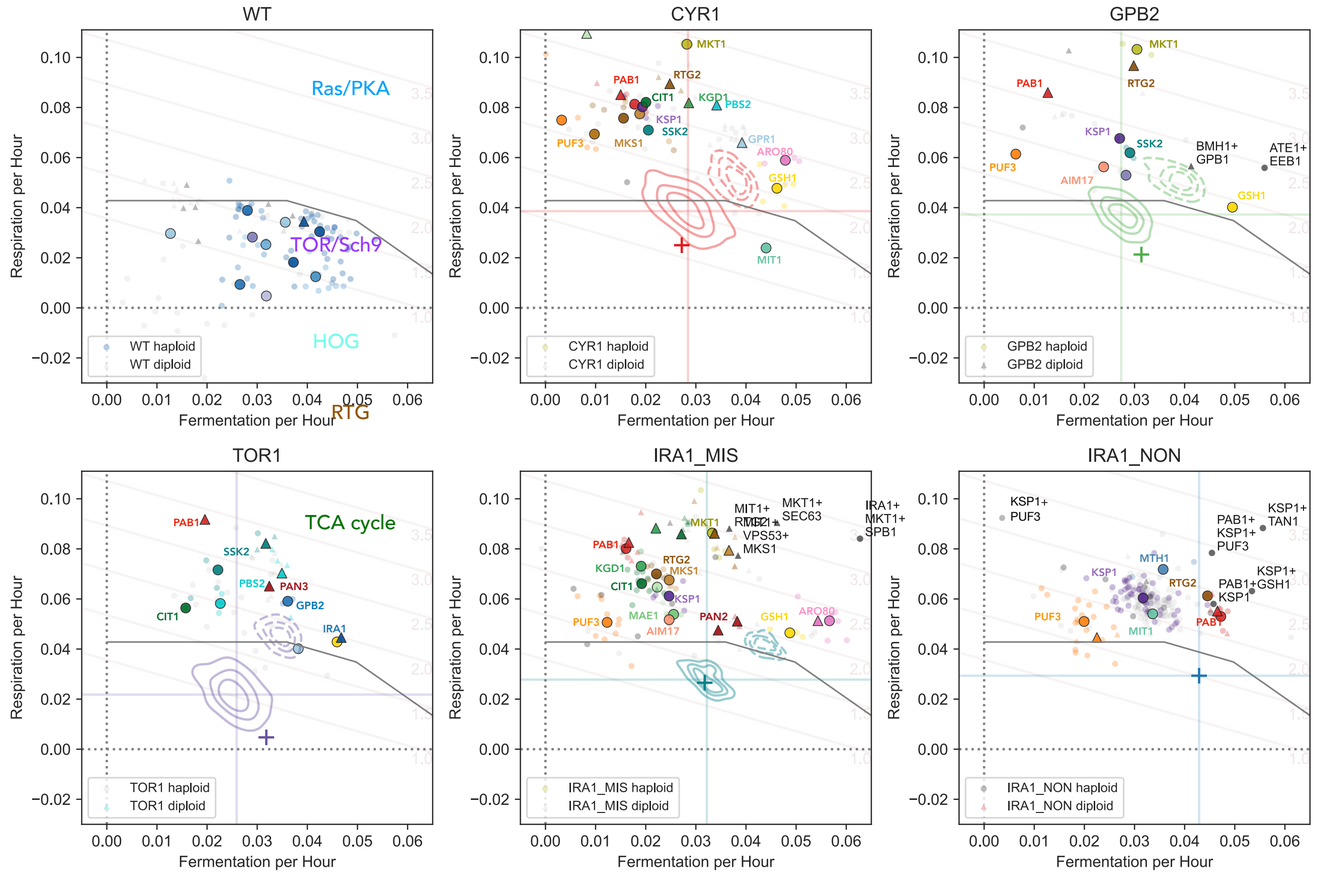

all
haploid
diploid
innovation
Respiration per Hour
Fermentation per Hour
CYR1 haploid
CYR1 diploid
KSP1+
PUF3
MKT1+
IRA1+
SPB1
GPB1
EEB1
YPK9


WT
Ras/PKA
TOR/Sch9
Respiration per Hour
Fermentation per Hour
All Second Step
MKT1
TCA cycle
RTG
TOR/Sch9
HOG
Ras/PKA
ARO80
GSH1
Mitochondrial Biogenesis
Haploid
Diploid
Respiration per Hour relative to parental strain
Fermentation per Hour relative to parental strain
CYR1
GPB2
TOR1
IRA1_MIS
IRA1_NON


WT
All Second Step
CYR1
GPB2
TOR1
IRA1_MIS
IRA1_NON
Respiration per Hour
Fermentation per Hour
Respiration per Hour relative to parental strain
Fermentation per Hour relative to parental strain
Ras/PKA
TOR/Sch9
MKT1
TCA cycle
RTG
TOR/Sch9
HOG
Ras/PKA
ARO80
GSH1
Mitochondrial Biogenesis
Haploid
Diploid
IRA1-mis
+IRA1-non
+MKT1
+SPB1


MKT1
TCA cycle
RTG
TOR/Sch9
HOG
Ras/PKA
Ras/PKA
ARO80
TOR/Sch9
GSH1
Mitochondrial
Biogenesis
Haploid
Diploid

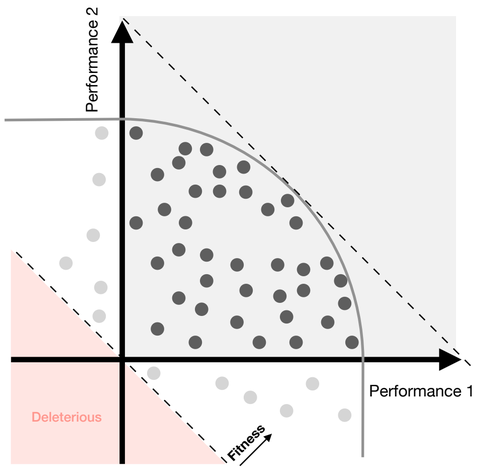

IRA1_MIS
IRA1_NON
Respiration per Hour
Fermentation per Hour
0.10
0.08
0.06
0.04
0.02
0.00
−0.02
−0.04
−0.025
0.000
0.025
0.050
0.075
0.100
IRA1-mis
+IRA1-non
+MKT1
+SPB1
IRA1-non
+KSP1
+TAN1



Mitochondrial Biogenesis

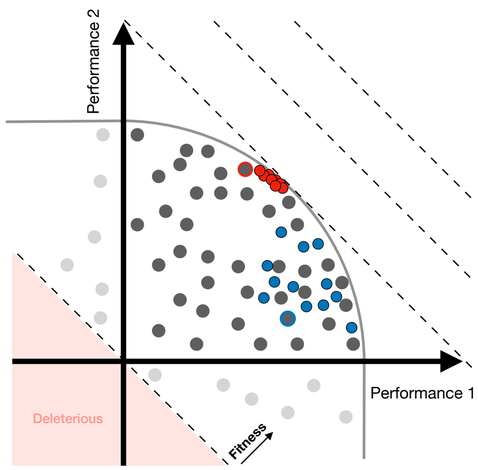
OLD VERSIONS BELOW

A



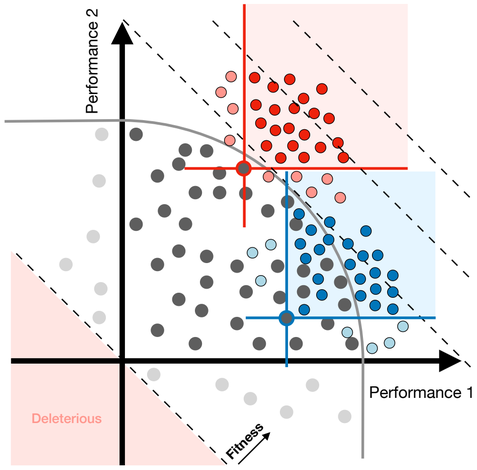
First-step mutations exhibit signs of adaptive pleiotropy and tradeoffs!

B



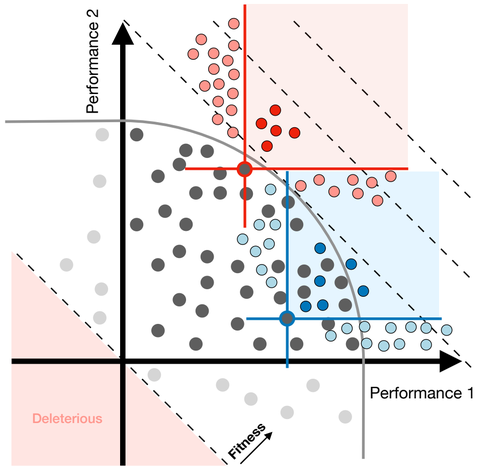
Tradeoffs are driven by functional constraints: Second-step mutations cannot improve past these constraints

C

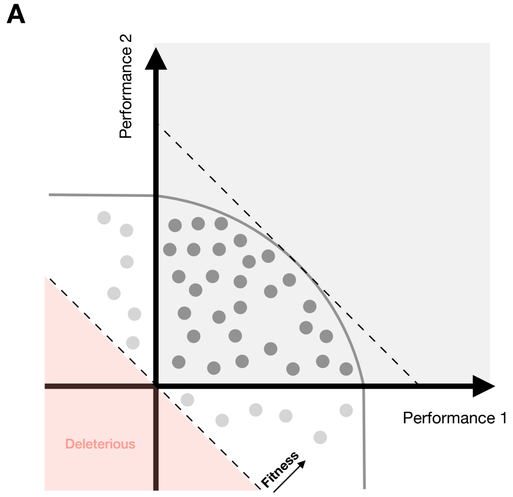


Only “constraint” is mutational distance: Second-step adaptive mutants continue to show a pattern of adaptive pleiotropy

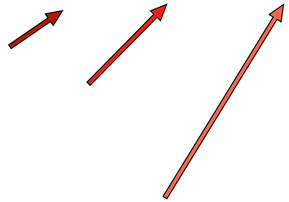
D

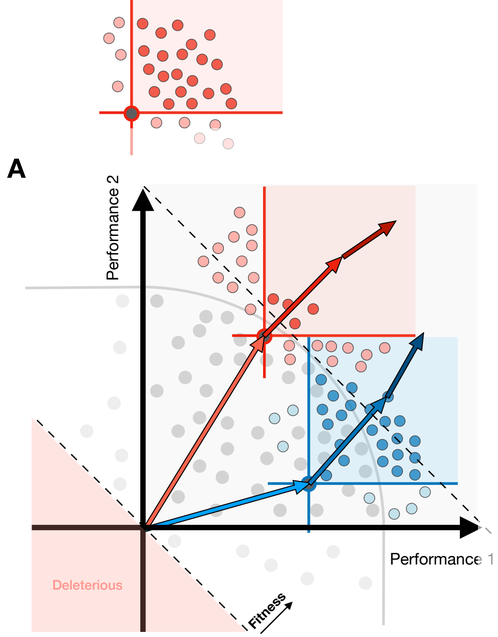


Accessibility of adaptive pleiotropic mutations is reduced: Second-step mutations display new patterns of trait improvement

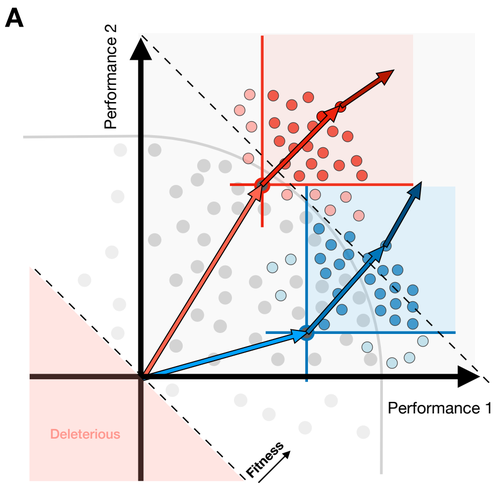


Adaptive pleiotropy is common.

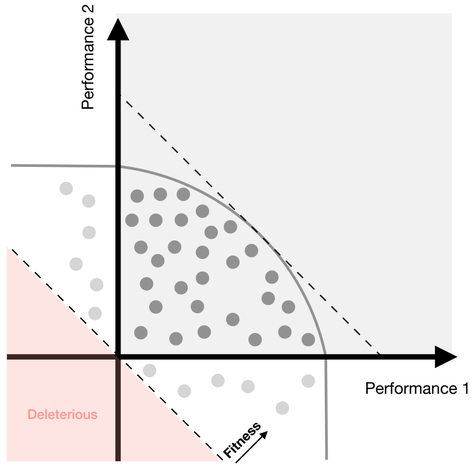


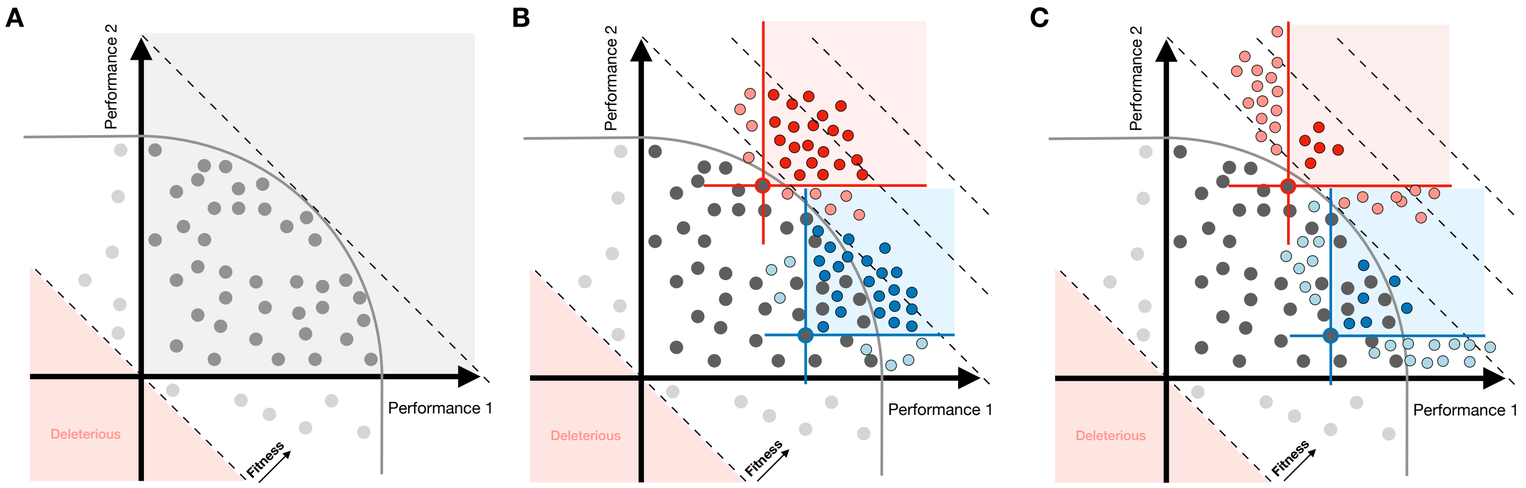


Adaptive pleiotropy is common.



Adaptive pleiotropy is common.

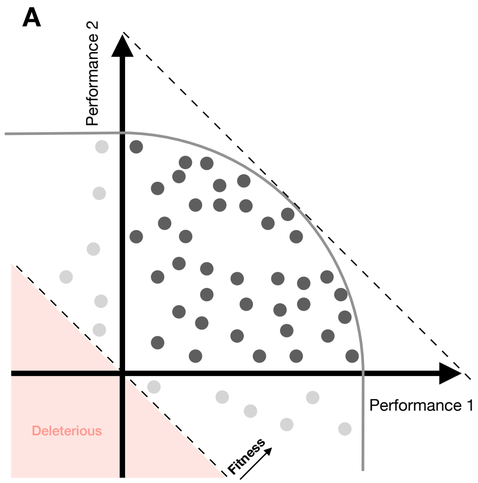


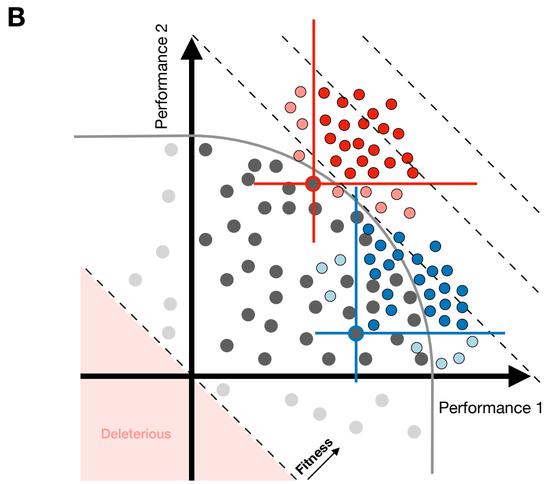


First-step mutations exhibit signs of adaptive pleiotropy!

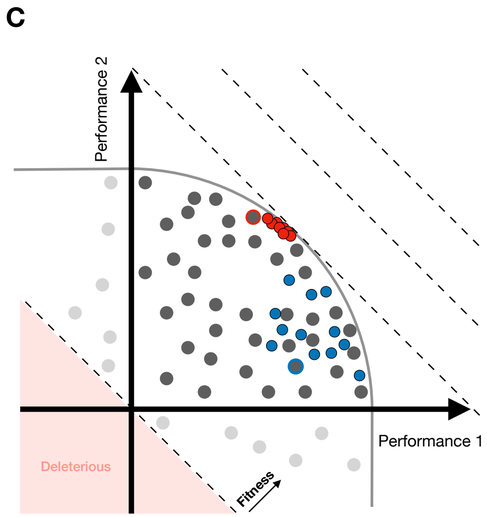
Adaptive pleiotropy is common.

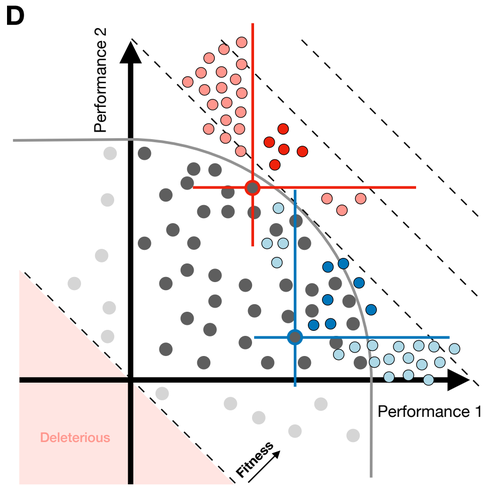
First-step mutations are special. Adaptive pleiotropy is rare.





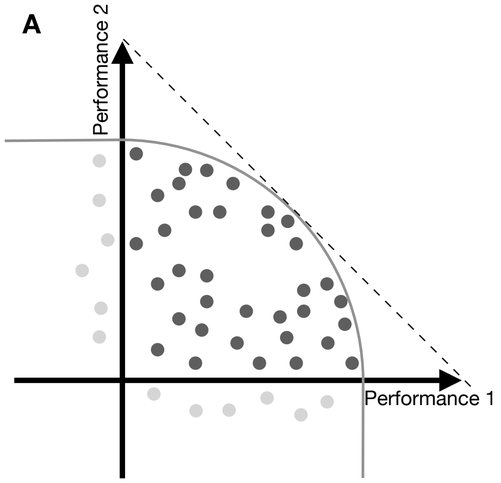
First-step mutations exhibit signs of adaptive pleiotropy (with most improving both performances at once) and tradeoffs (not achieving “perfect” extreme performance





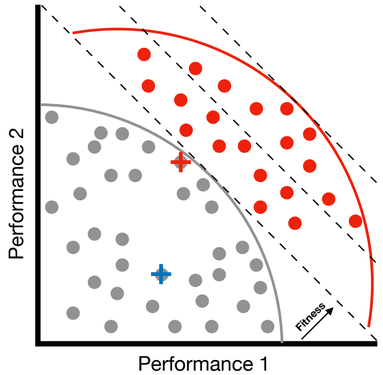
Tradeoffs represent functional/physiological constraints on the improvement of these performances

A



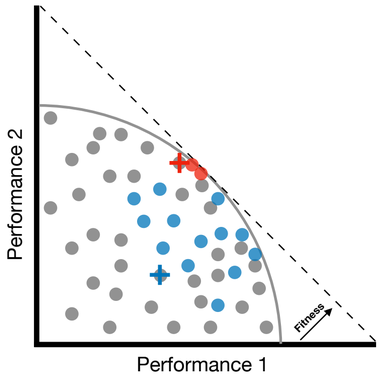
First-step mutations exhibit signs of adaptive pleiotropy (with most improving both performances at once) and tradeoffs (not achieving "perfect" extreme performance

B



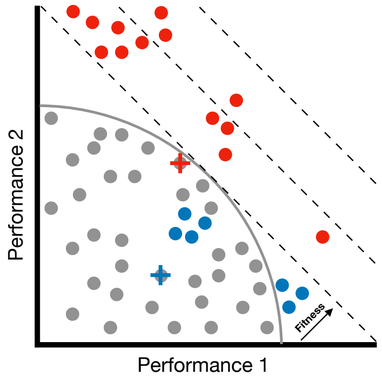
If tradeoffs don't represent any functional or genetic constraints but instead just mutational distance, then further adaptive steps should continue to improve as the first, albeit more slowly due to diminishing returns…

C



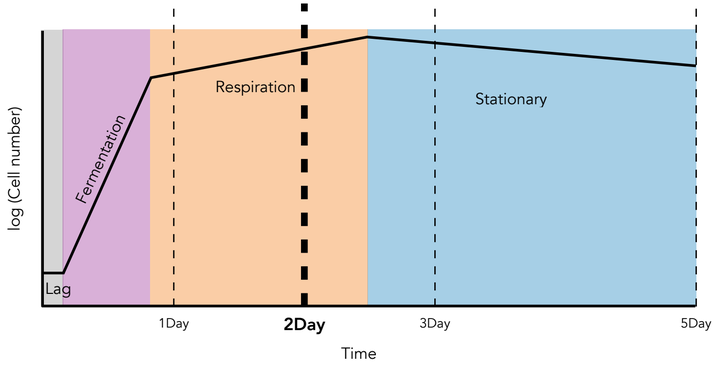
Tradeoffs represent functional/physiological constraints on the improvement of these performances
If true, further steps might move within or along these limits but cannot break through them

D

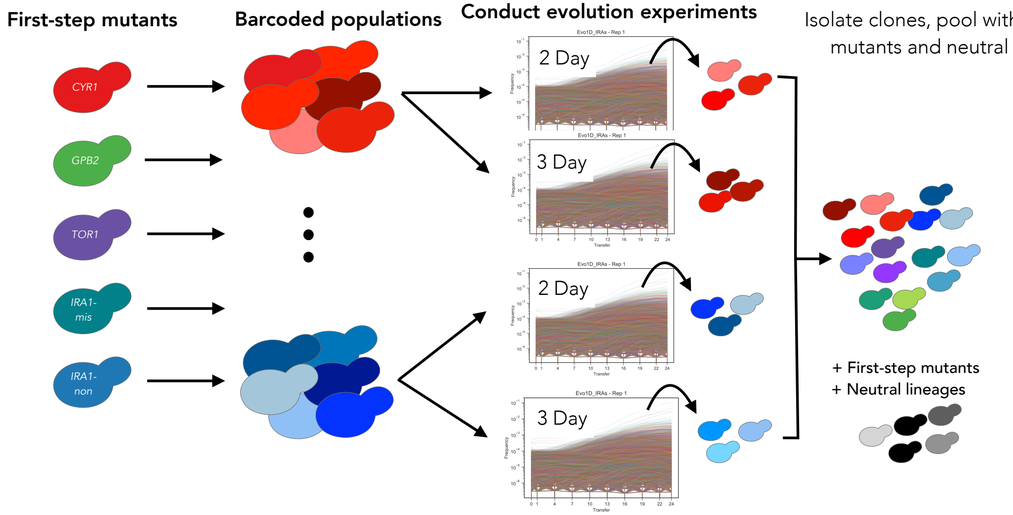


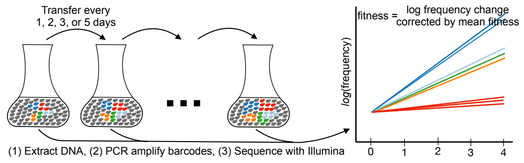
Tradeoffs reflect constraints imposed by genetic wiring, the accessibility of adaptive solutions and their pleiotropic effects.

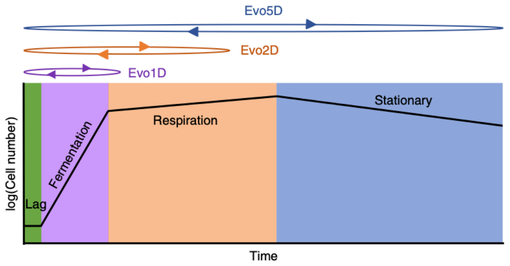
Further adaptive steps may display new patterns of genetic wiring, and these shifts in genetic wiring and pleiotropy may be dependent on the background of the first step



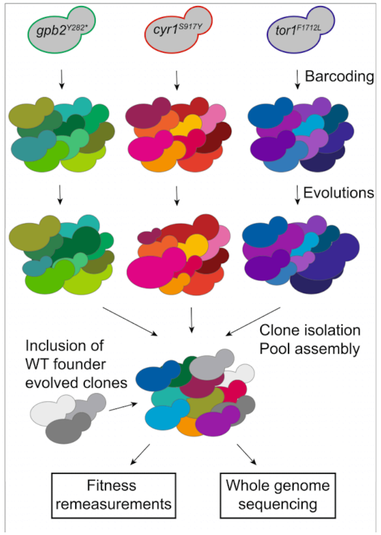
	Parental Genotype	First-step mutation	Evolution Condition	Number of adaptive mutants	Number of diploids	Publication
First Step	WT	-	2Day	121	194	Levy et al. 2015
Second Step	*CYR1*	*cyr1 S917Y*	2Day	68	224	Aggeli et al.
			3Day	20	8	This study
	GPB2	*gpb2 Y282**	2Day	17	228	Aggeli et al.
			3Day	12	20	This study
	TOR1	*tor1 F1712L*	2Day	57	317	Aggeli et al.
			3Day	2	-	This study
	IRA1 missense	*ira1 A1211V*	2Day	90	16	This study
			3Day	28	3	This study
	IRA1 nonsense	*ira1 L1401**	2Day	95	-	This study
			3Day	135	-	This study

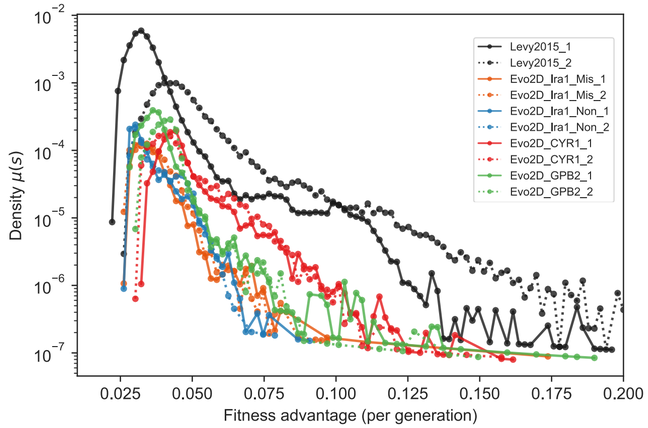


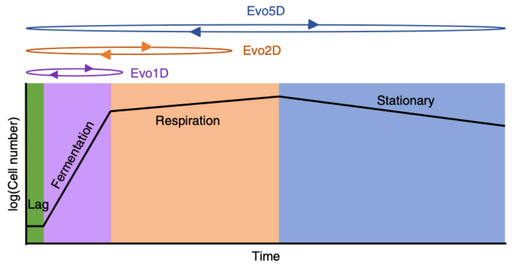


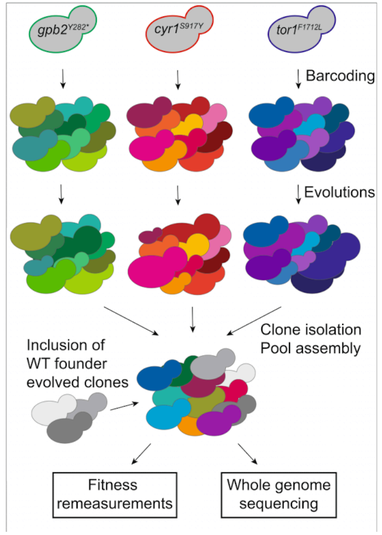


Genotype	Study	Evo2D mutants Included	Number of pure diploids	Number of putative adaptive (excluding pure diploids)
WT	Levy 2015	506	194	121
CYR1	Aggeli 2020	535	224	68
GPB2	Aggeli 2020	1219	228	17
TOR1	Aggeli 2020	726	317	57
IRA1 mis	This study	143	16	90
IRA1 non	This study	95	0	95

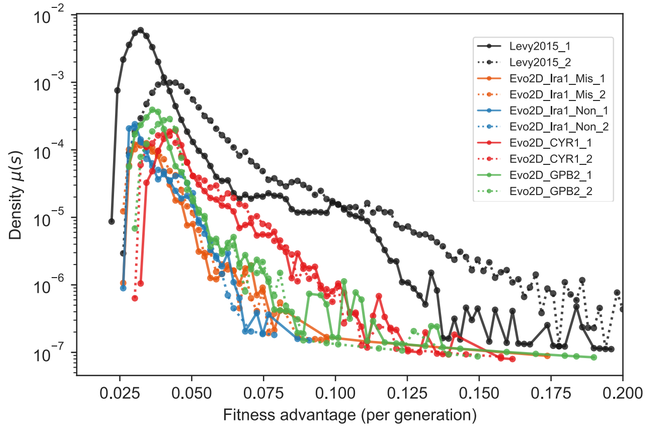




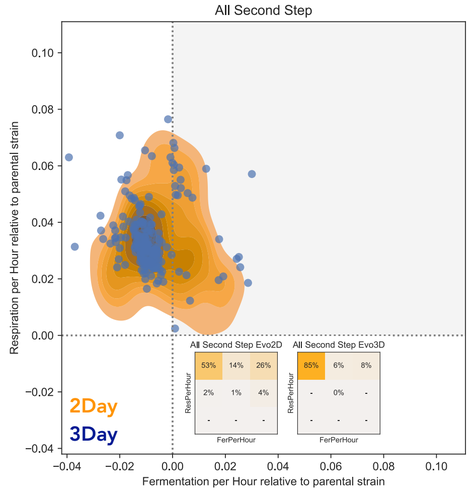


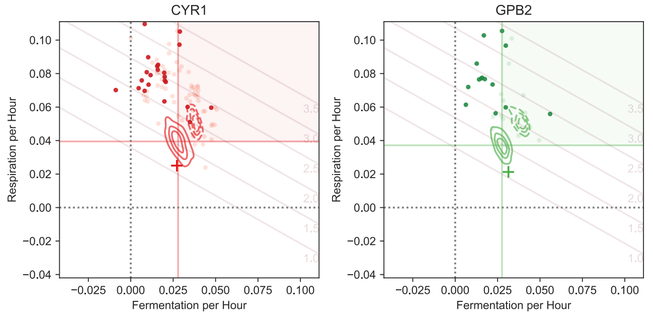


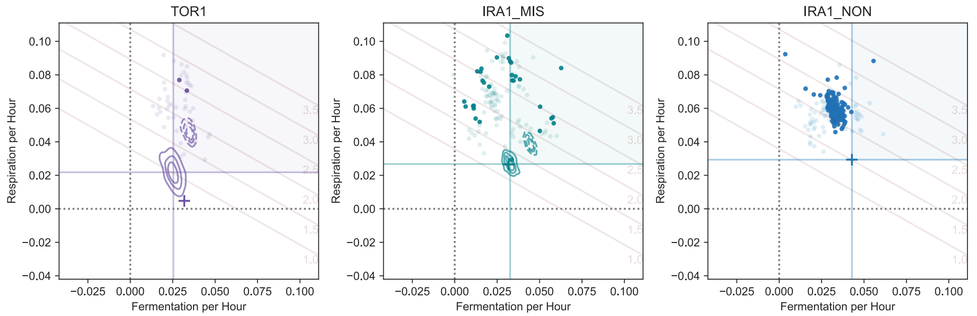
	Evo2D mutants Included	Number of pure diploids	Number of putative adaptive (excluding pure diploids)
WT	506	194	121
CYR1	535	224	68
GPB2	1219	228	17
TOR1	726	317	57
IRA1 mis	143	16	90
IRA1 non	95	0	95

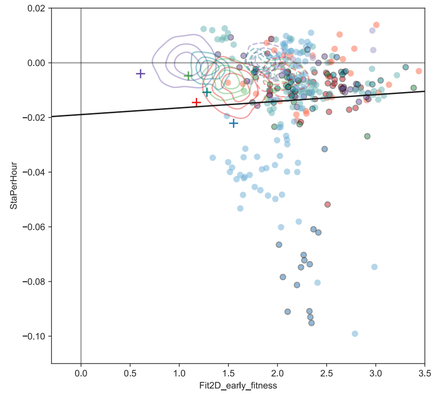


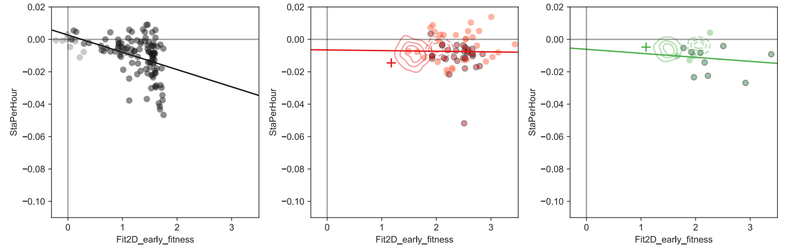
	Evo3D mutants	Number of pure diploids	Number of putative adaptive mutants
CYR1	35	8	20
GPB2	34	20	12
TOR1	2	-	2
IRA1 mis	32	3	28
IRA1 non	135	0	135

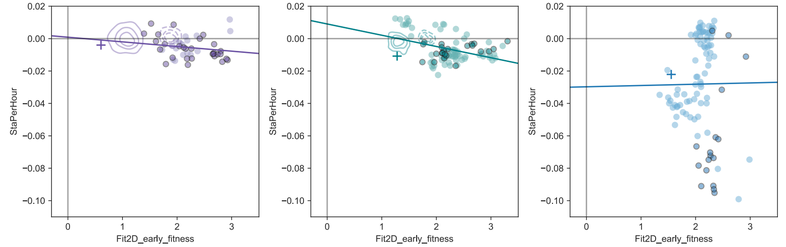


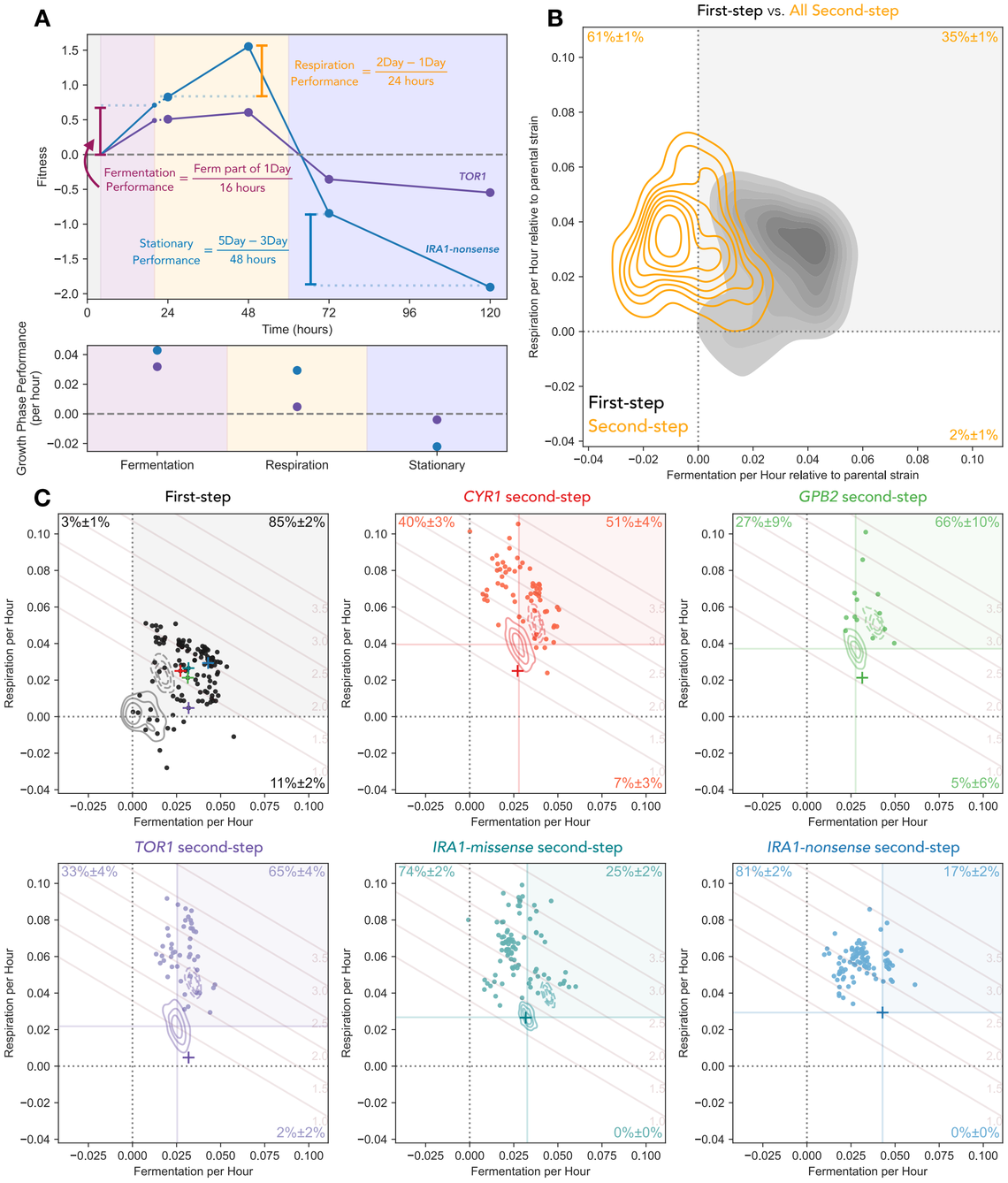


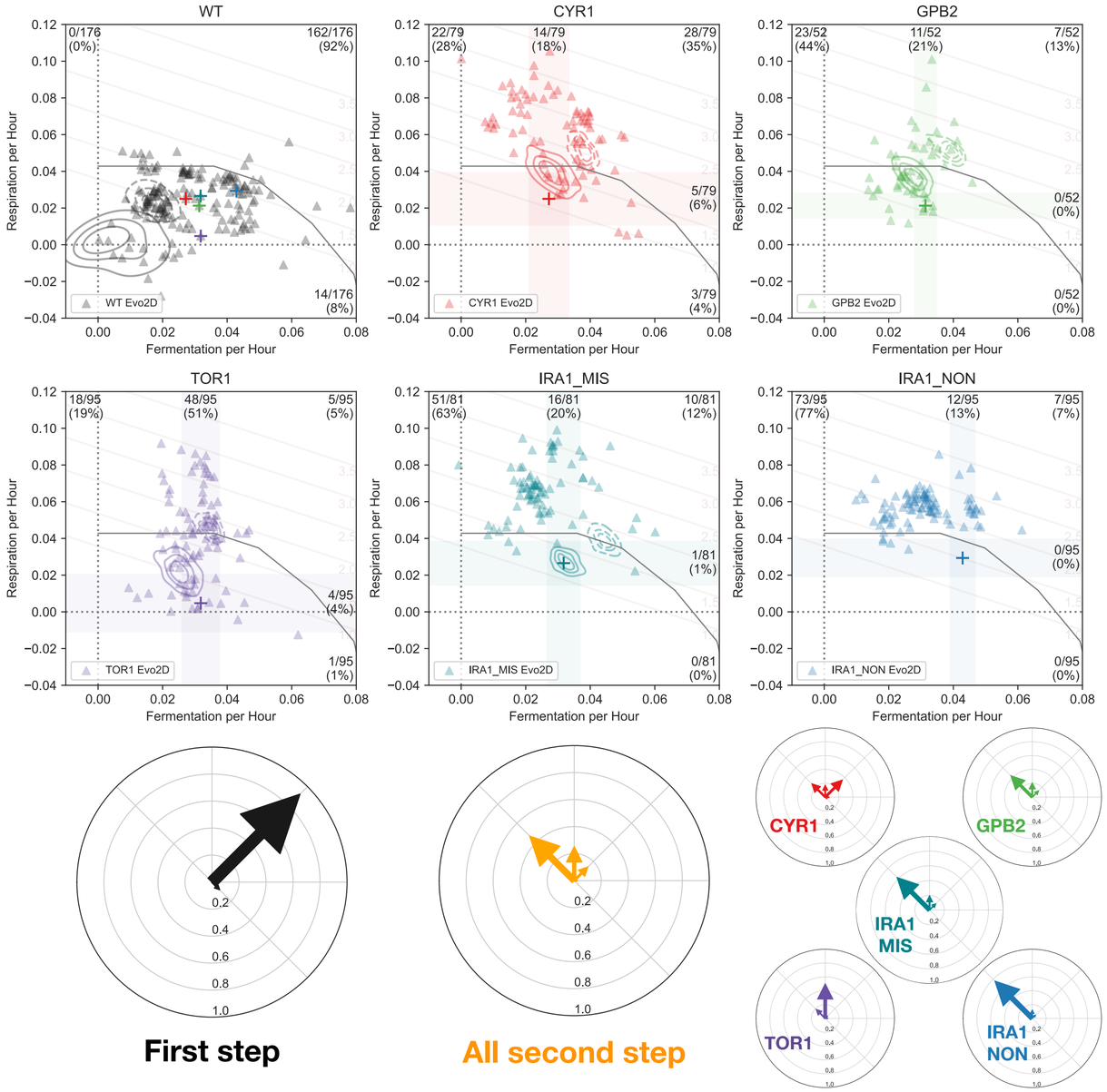






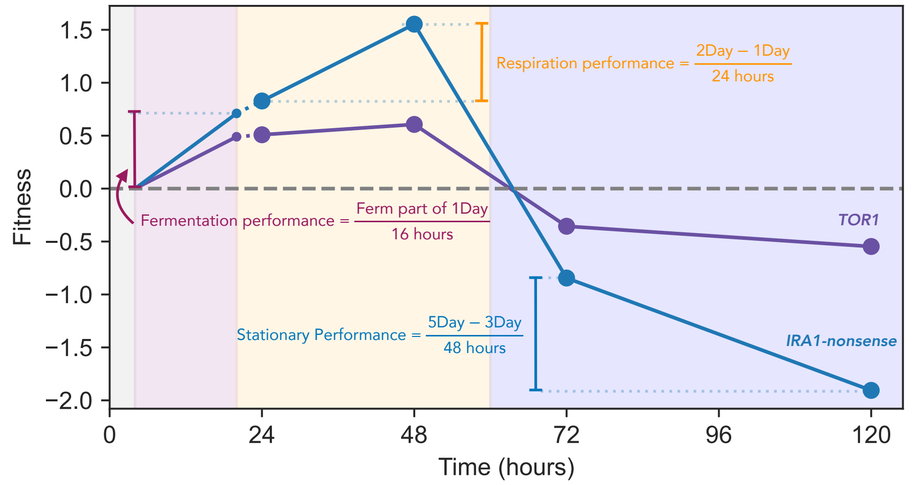


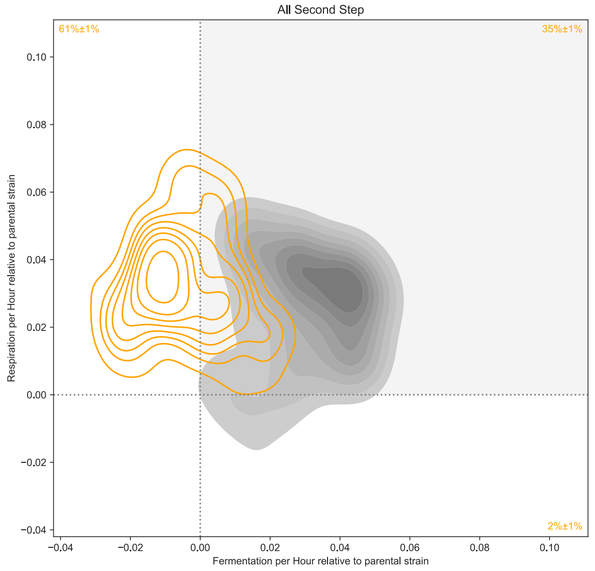

A
Respiration Performance = 2Day − 1Day / 24 hours
Fermentation Performance = Ferm part of 1Day / 16 hours
Stationary Performance = 5Day − 3Day / 48 hours
TOR1
IRA1-nonsense
Fitness
Time (hours)
Growth Phase Performance (per hour)
Fermentation
Respiration
Stationary
B
First-step vs. All Second-step
61%±1%
35%±1%
First-step
Second-step
2%±1%
Respiration per Hour relative to parental strain
Fermentation per Hour relative to parental strain
C
First-step
3%±1%
85%±2%
11%±2%
CYR1 second-step
40%±3%
51%±4%
7%±3%
GPB2 second-step
27%±9%
66%±10%
5%±6%
TOR1 second-step
33%±4%
65%±4%
2%±2%
IRA1-missense second-step
74%±2%
25%±2%
0%±0%
IRA1-nonsense second-step
81%±2%
17%±2%
0%±0%
Respiration per Hour
Fermentation per Hour

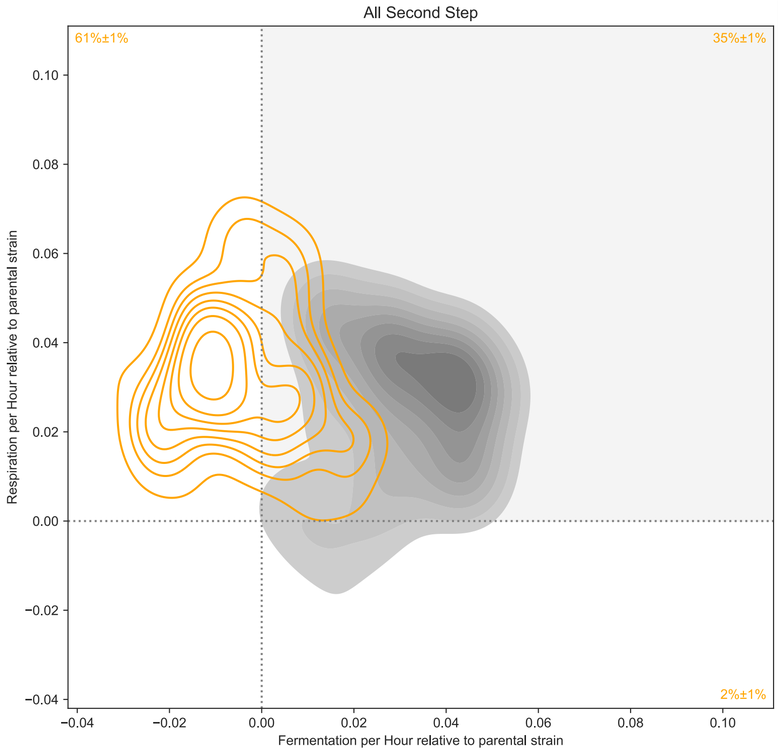


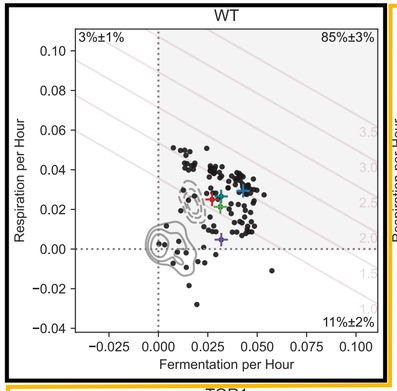
First step

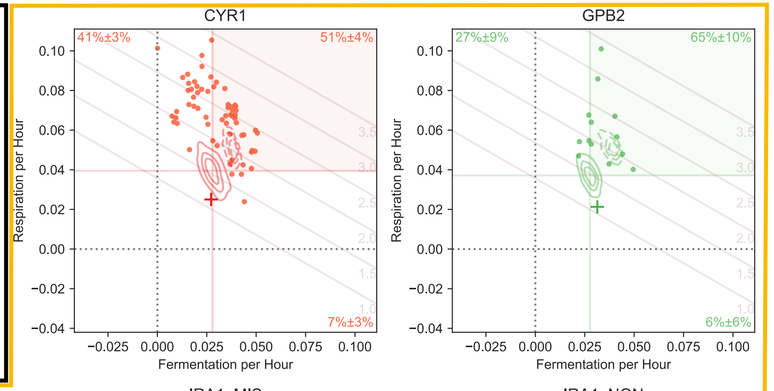
All second step

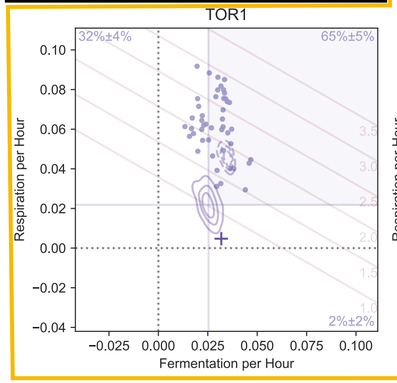

Respiration performance = 2Day − 1Day / 24 hours
Fermentation performance = Ferm part of 1Day / 16 hours
Stationary Performance = 5Day − 3Day / 48 hours
TOR1
IRA1-nonsense
Fitness
Time (hours)

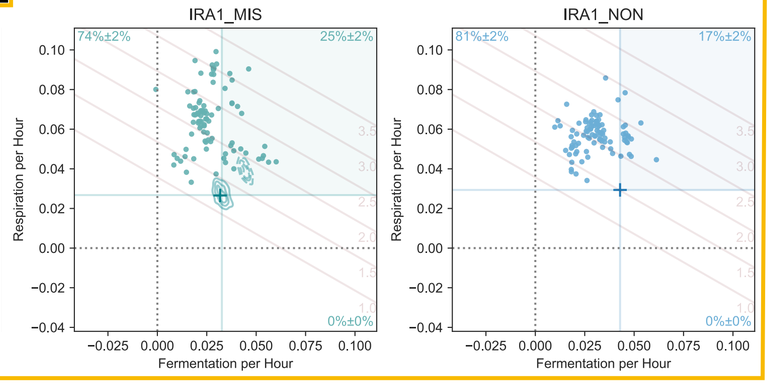

All Second Step
61%±1%
35%±1%
2%±1%
Respiration per Hour relative to parental strain
Fermentation per Hour relative to parental strain

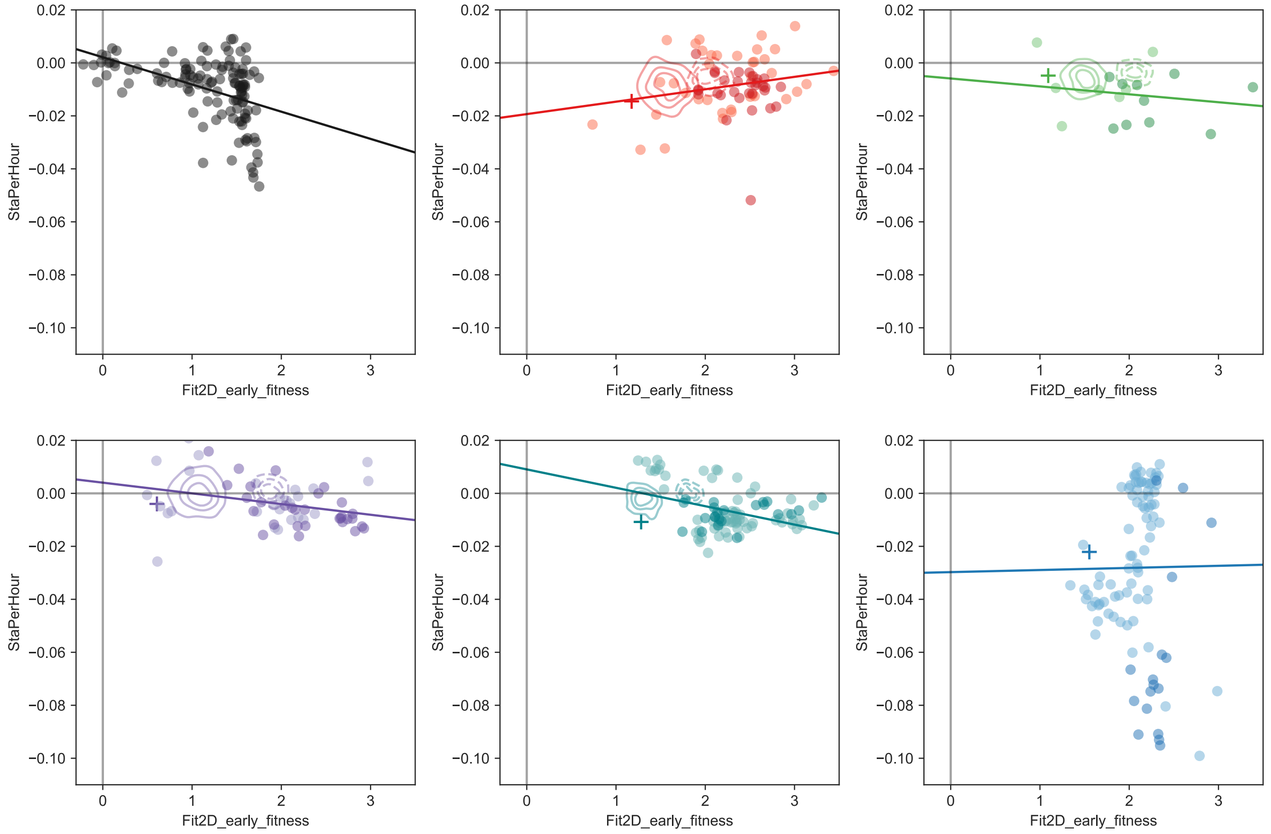

All Second Step
61%±1%
35%±1%
2%±1%
Respiration per Hour relative to parental strain
Fermentation per Hour relative to parental strain


WT
3%±1%
85%±3%
11%±2%
Respiration per Hour
Fermentation per Hour


CYR1
41%±3%
51%±4%
7%±3%
GPB2
27%±9%
65%±10%
6%±6%
Respiration per Hour
Fermentation per Hour


TOR1
32%±4%
65%±5%
2%±2%
Respiration per Hour
Fermentation per Hour


IRA1_MIS
74%±2%
25%±2%
0%±0%
IRA1_NON
81%±2%
17%±2%
0%±0%
Respiration per Hour
Fermentation per Hour


StaPerHour
Fit2D_early_fitness
0.02
0.00
−0.02
−0.04
−0.06
−0.08
−0.10
0
1
2
3