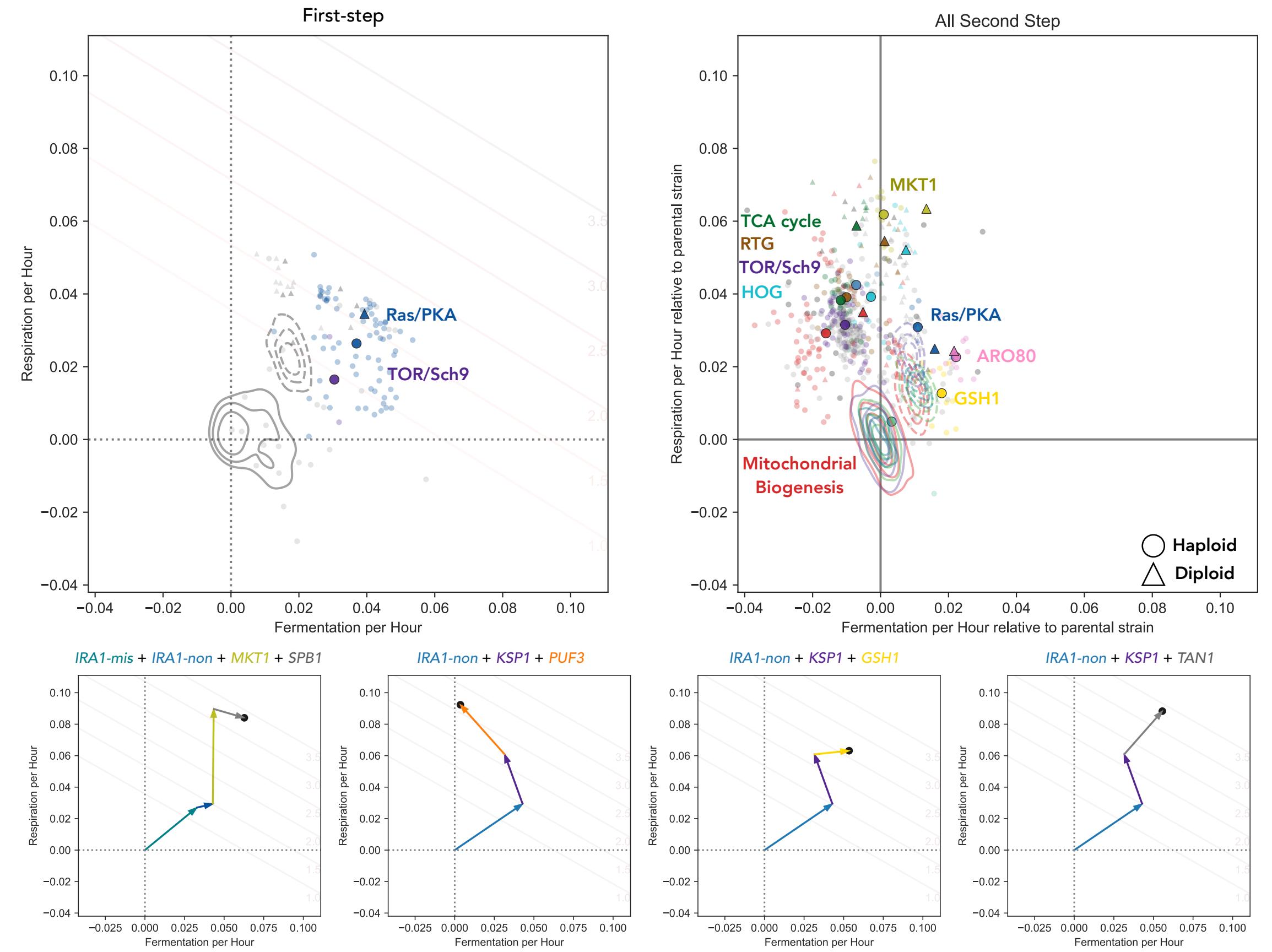
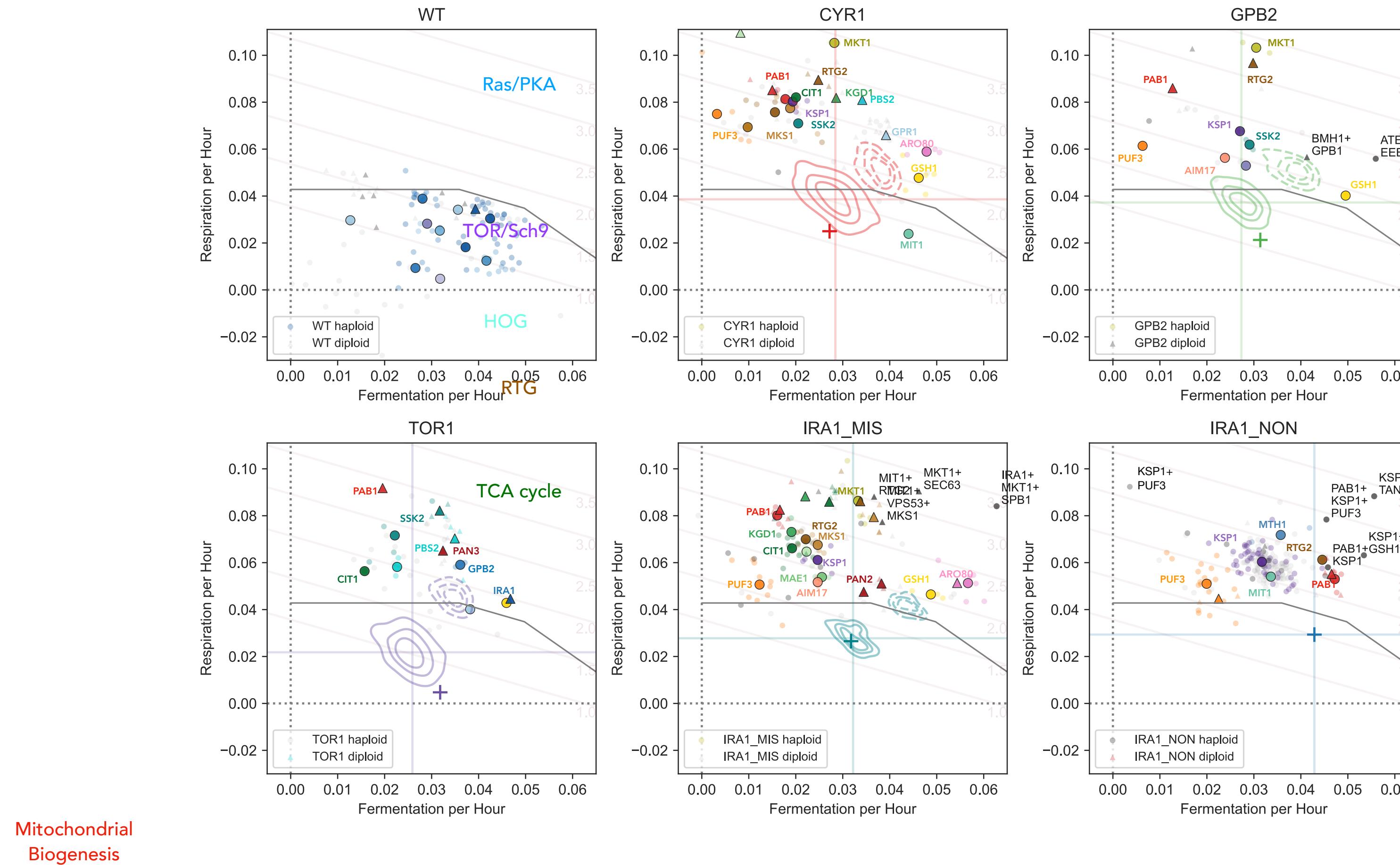


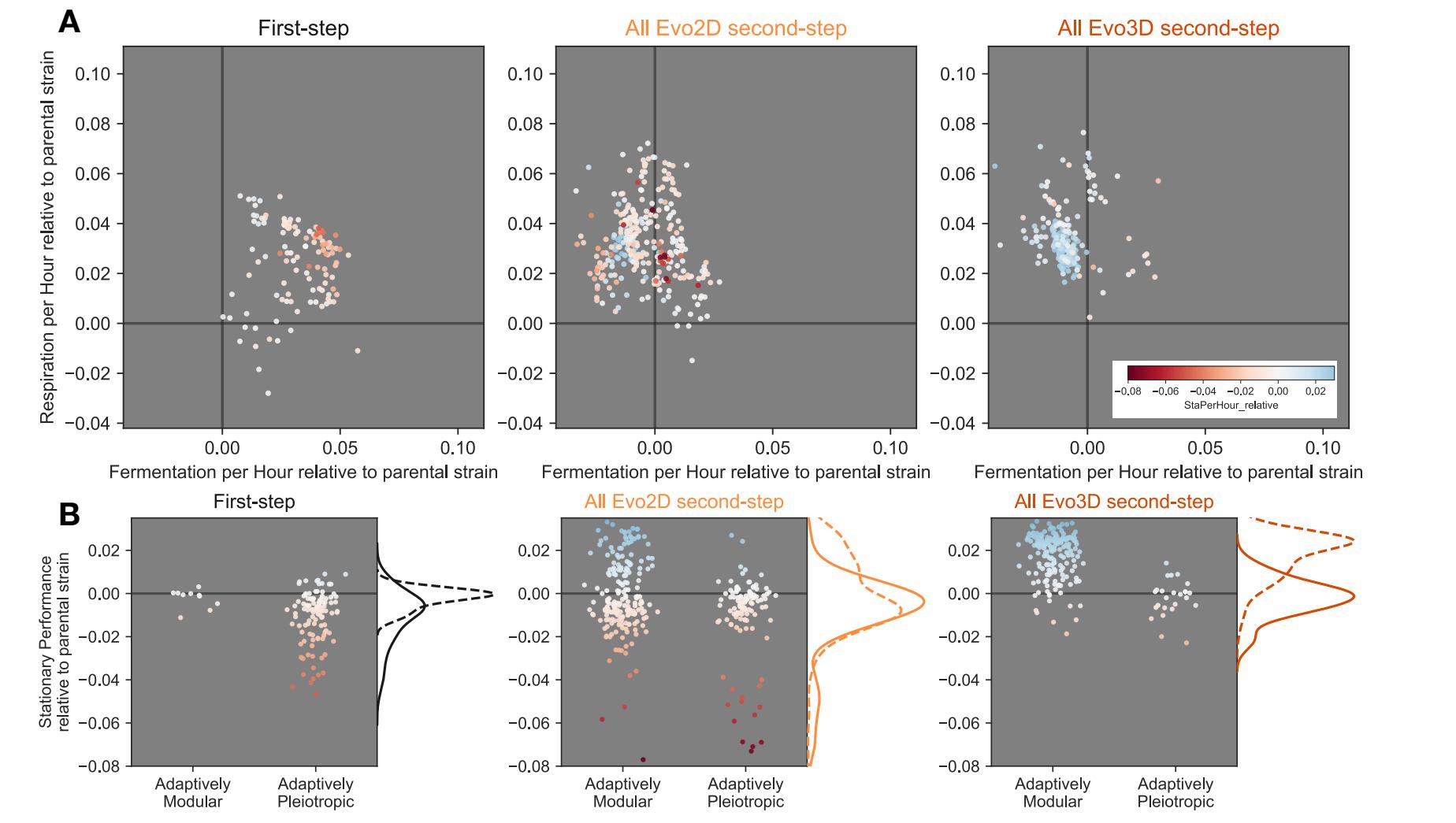
	WT Evo2D	TOR1 Evo2D	TOR1 Evo3D	GPB2 Evo2D	GPB2 Evo3D	CYR1 Evo2D	CYR1 Evo3D	IRA1_MIS Evo2D	IRA1_MIS Evo3D	IRA1_NON Evo2D	IRA1_NON Evo3D
Ras/PKA Pathway	IRA1 30/77							1/30*			
	IRA2 11/77		1/21								
	GPB1 4/77				1/7*						
	GPB2 14/77		2/21								
	PDE2 11/77										
	CYR1 3/77										
	GPR1						1/27				
	RAS2 1/77		1/21								
TOR/Sch9 Pathway	TFS1 1/77										
	TOR1 1/77										
	KOG1 1/77										
	SCH9				1/7						
HOG Pathway	KSP1			1/7	1/8*	1/27	2/14	4/67		32/72	30/33
	HOG1					1/27					
	PBS2 6/21										
RTG Pathway	SSK2 7/21		1/7	1/8	1/27						
	RTG2			1/8	4/27	2/14	9/67	1/30	1/72		
	MKS1 2/27				2/27	1/14	7/67	1/30*			
TCA cycle	BMH1 1/7*			1/27							
	CIT1 1/21				1/27		16/67	1/30			
	KGD1 1/27					3/67					
	MDH1 4/67						2/67				
Regulation of Mitochondrial Biogenesis	MAE1 2/67										
	ALD5 1/14						1/67*				
	PUF3 7/67			1/8	4/27	2/14	7/67	7/30	24/72	1/33*	
	PAB1 1/21			2/8	1/27	4/14	3/67	5/30	12/72		
Others	PAN2 1/21						2/67				
	PAN3 1/21						1/67				
	AIM17 1/8						2/67				
Others	MKT1 1/7		1/7	1/8	1/27	1/14	1/67	9/30			
	GSH1 1/7						2/67	1/30	1/72*		
	ARO80 3/27						3/67	4/30			

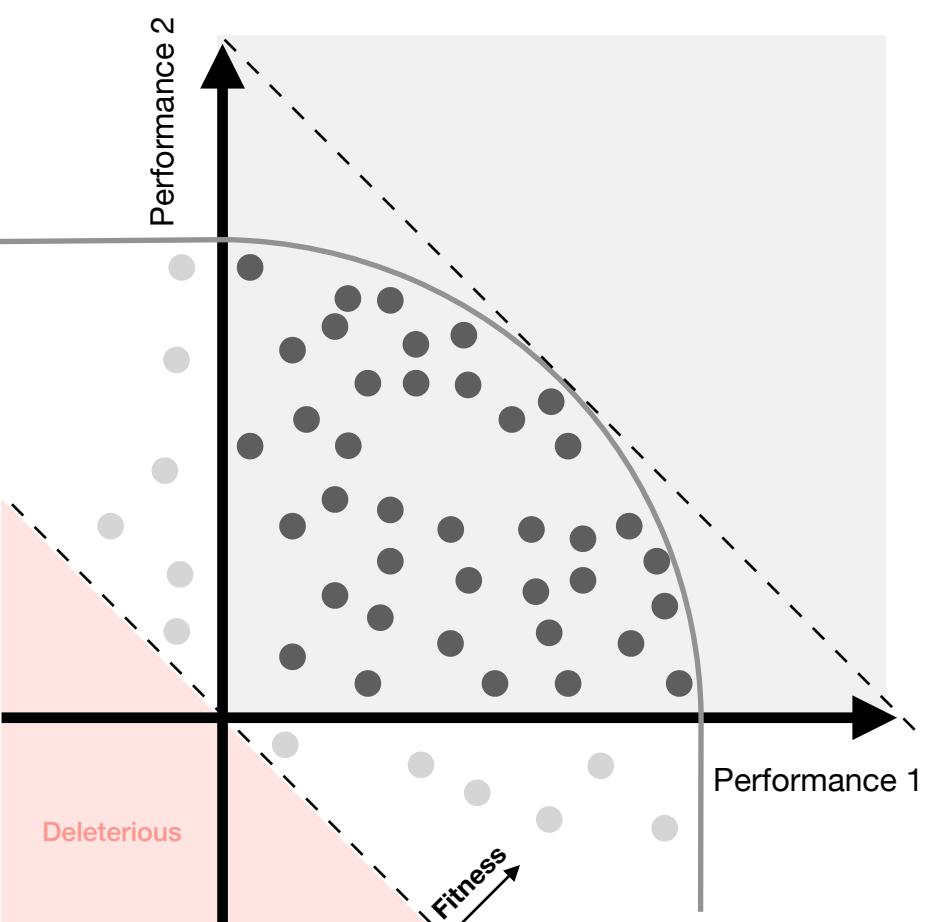
Legend: Loss of function (orange), Modification of function (blue)



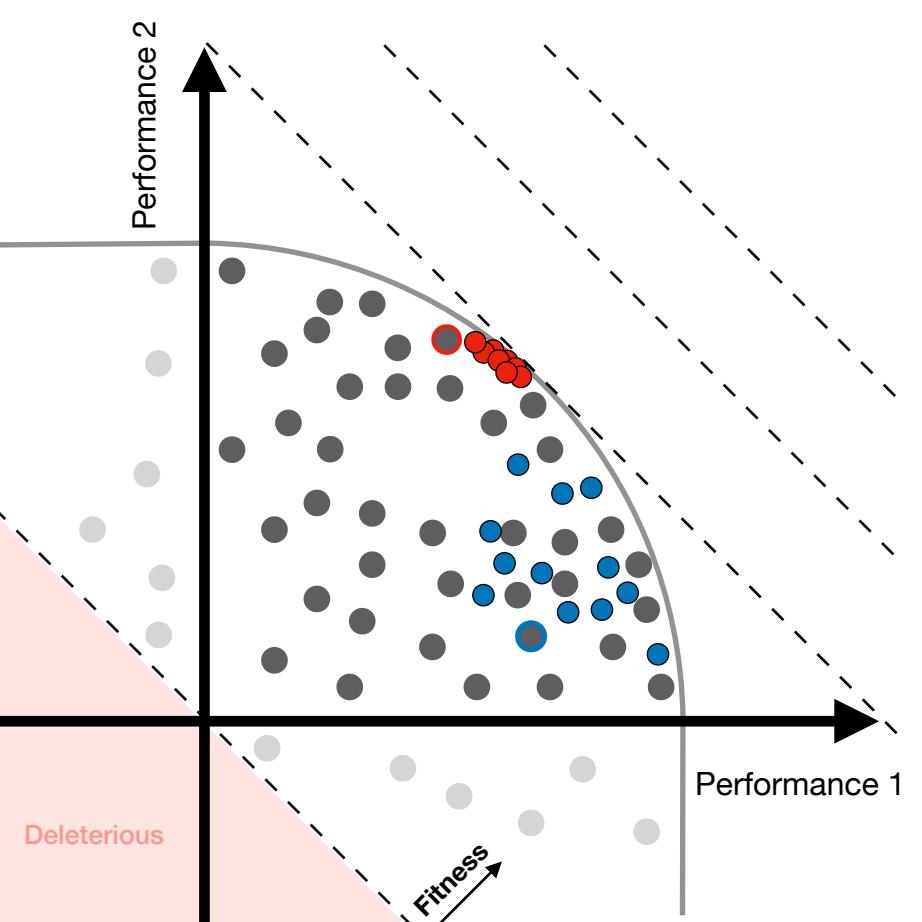


**OLD VERSIONS BELOW**

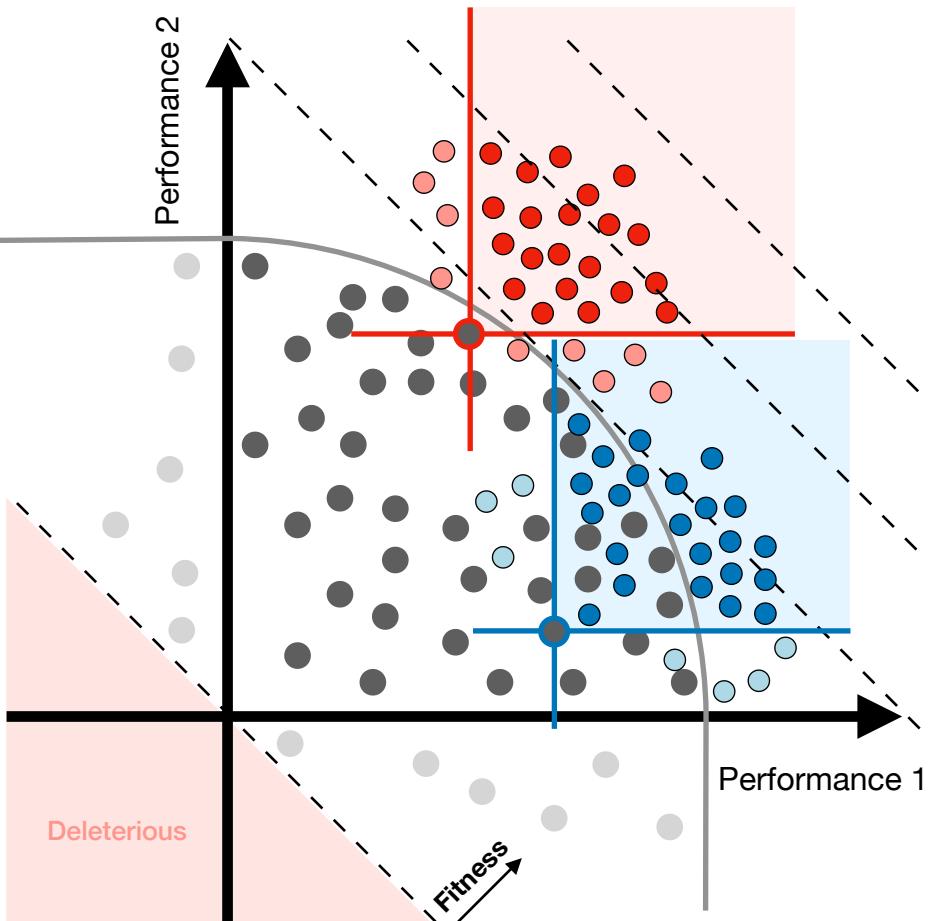


**A**

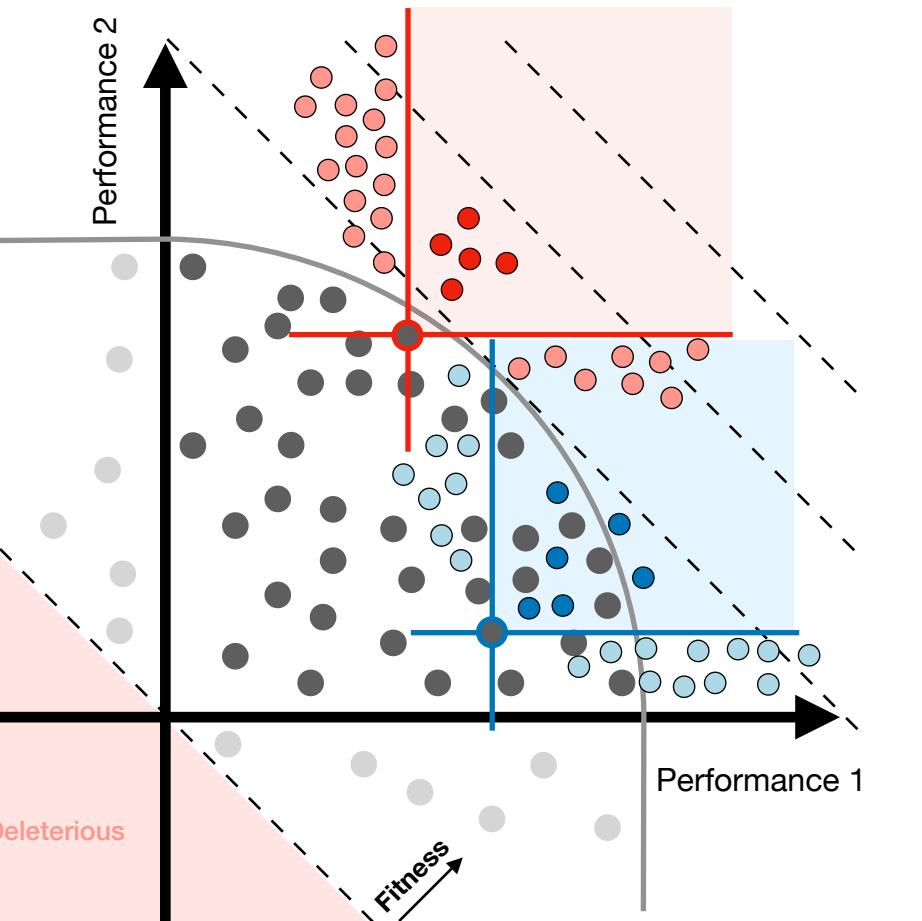
**First-step mutations** exhibit signs of adaptive pleiotropy and tradeoffs!

**B**

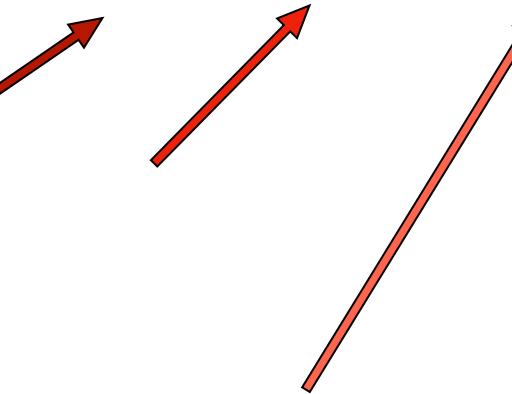
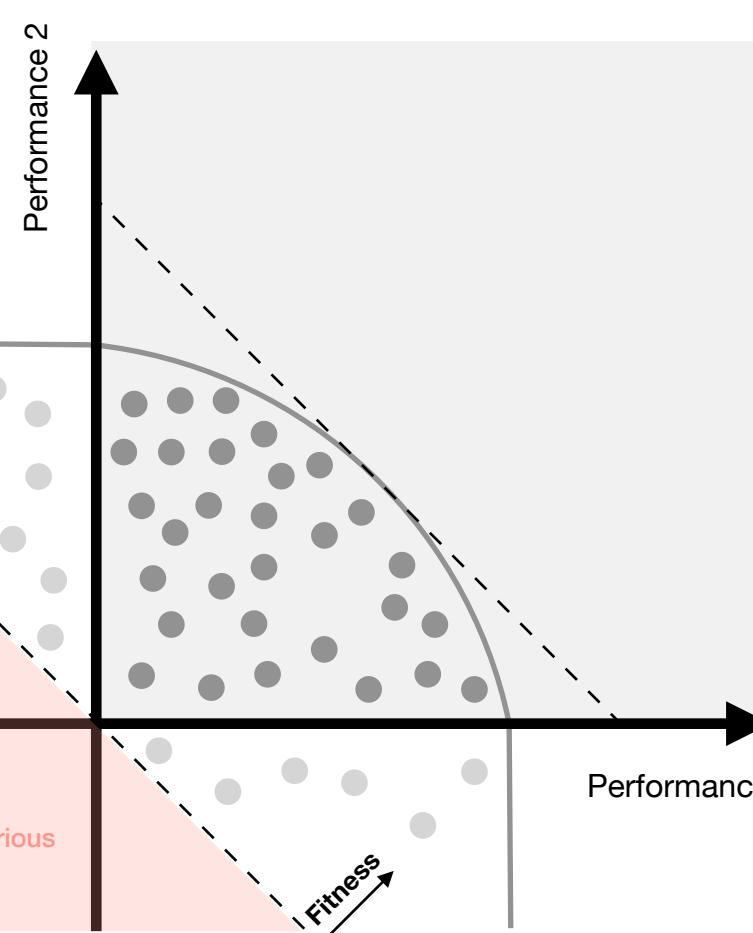
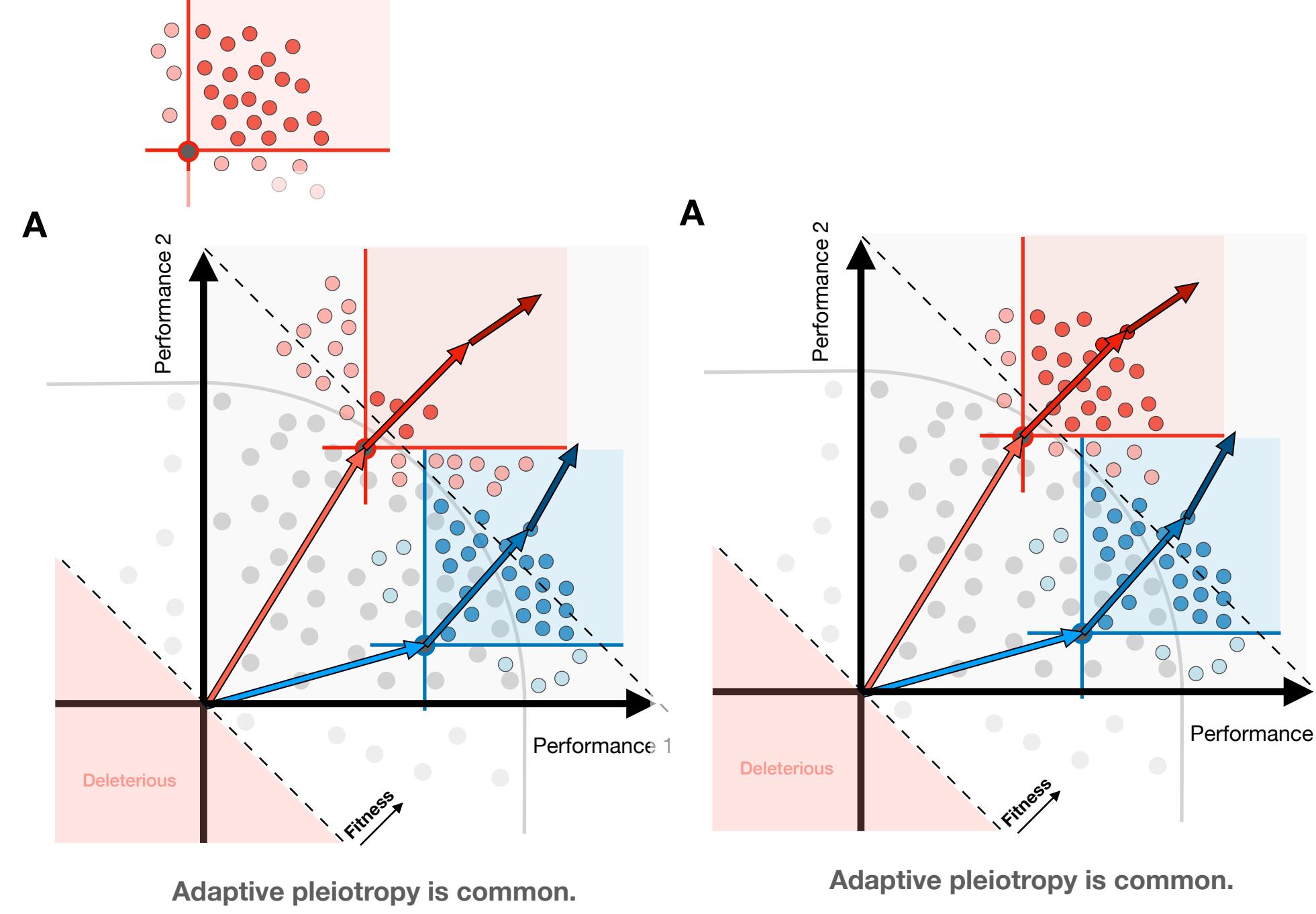
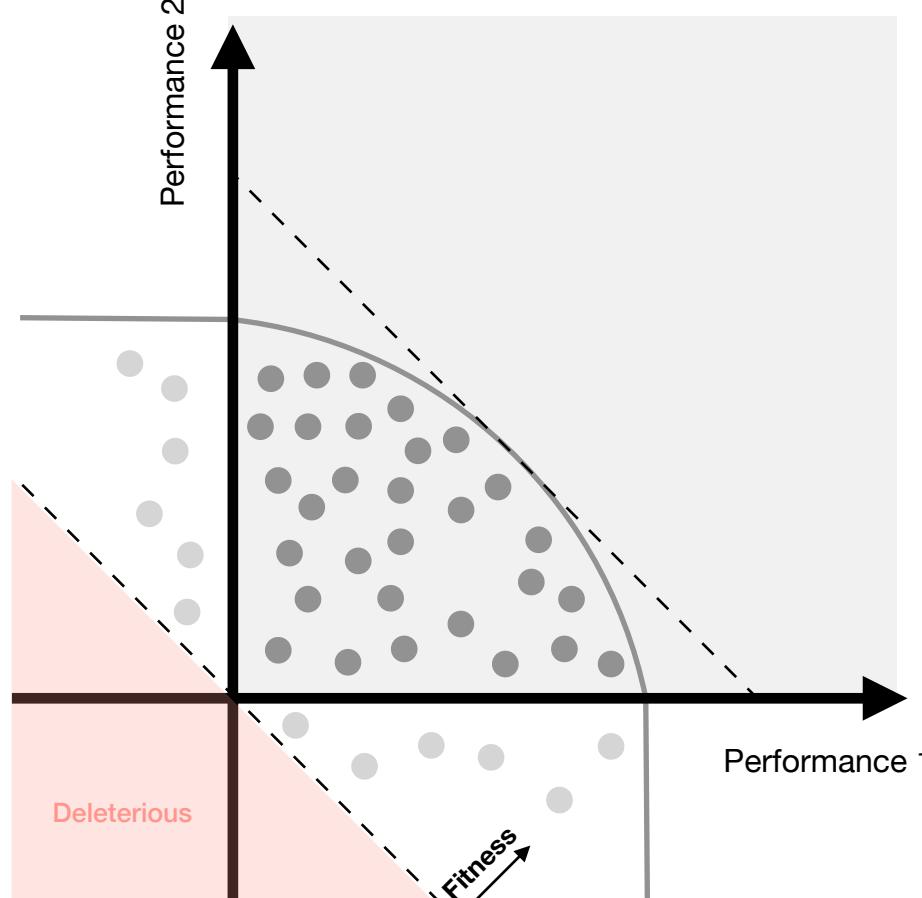
Tradeoffs are driven by functional constraints:  
Second-step mutations cannot improve past these constraints

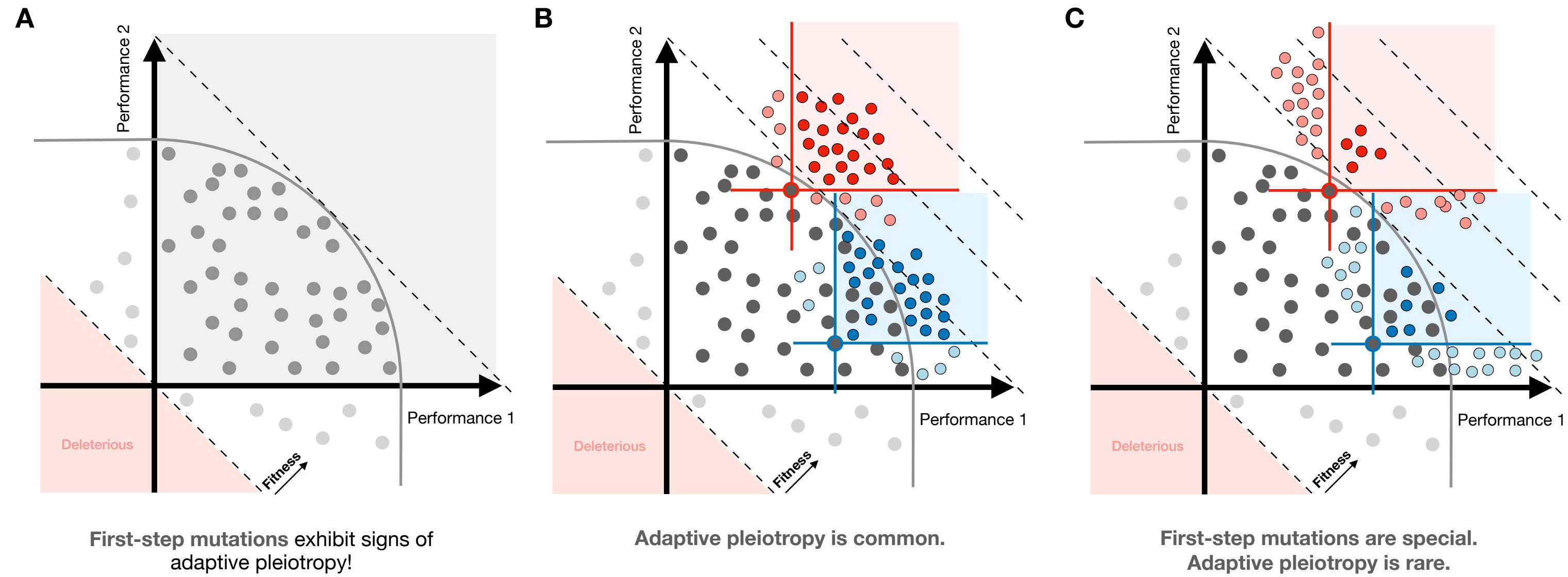
**C**

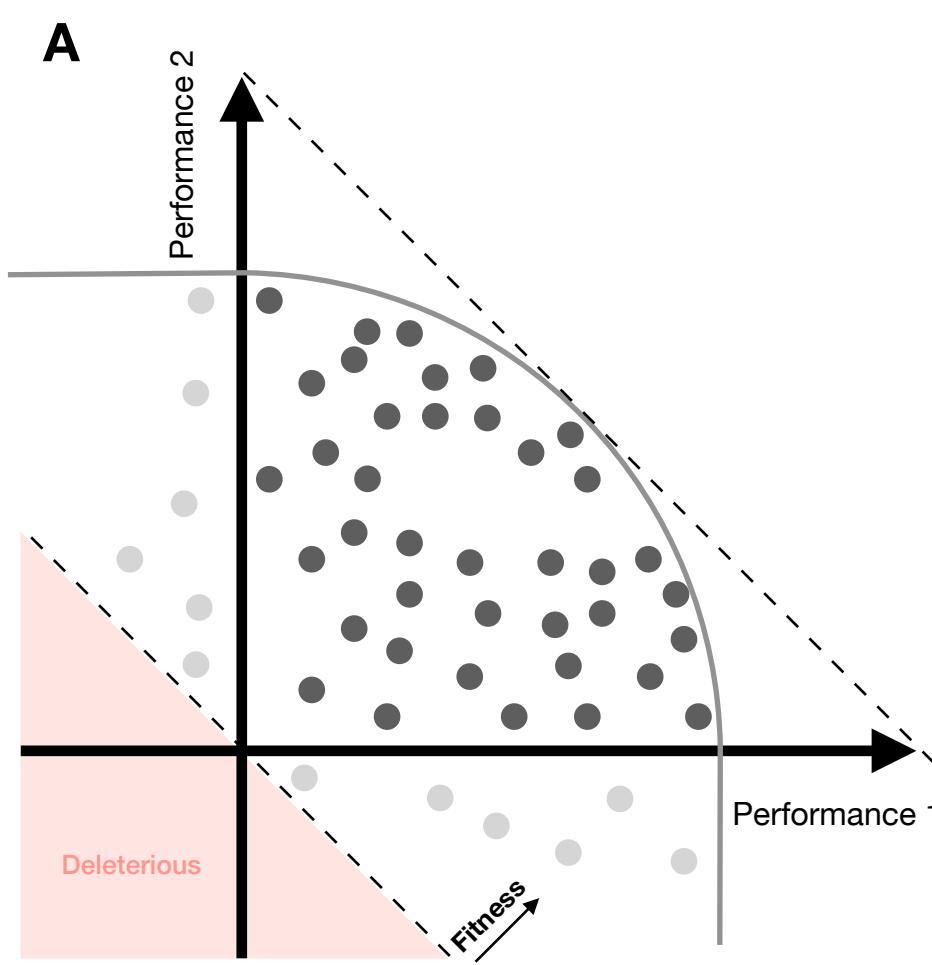
Only "constraint" is mutational distance:  
Second-step adaptive mutants continue to show a pattern of adaptive pleiotropy

**D**

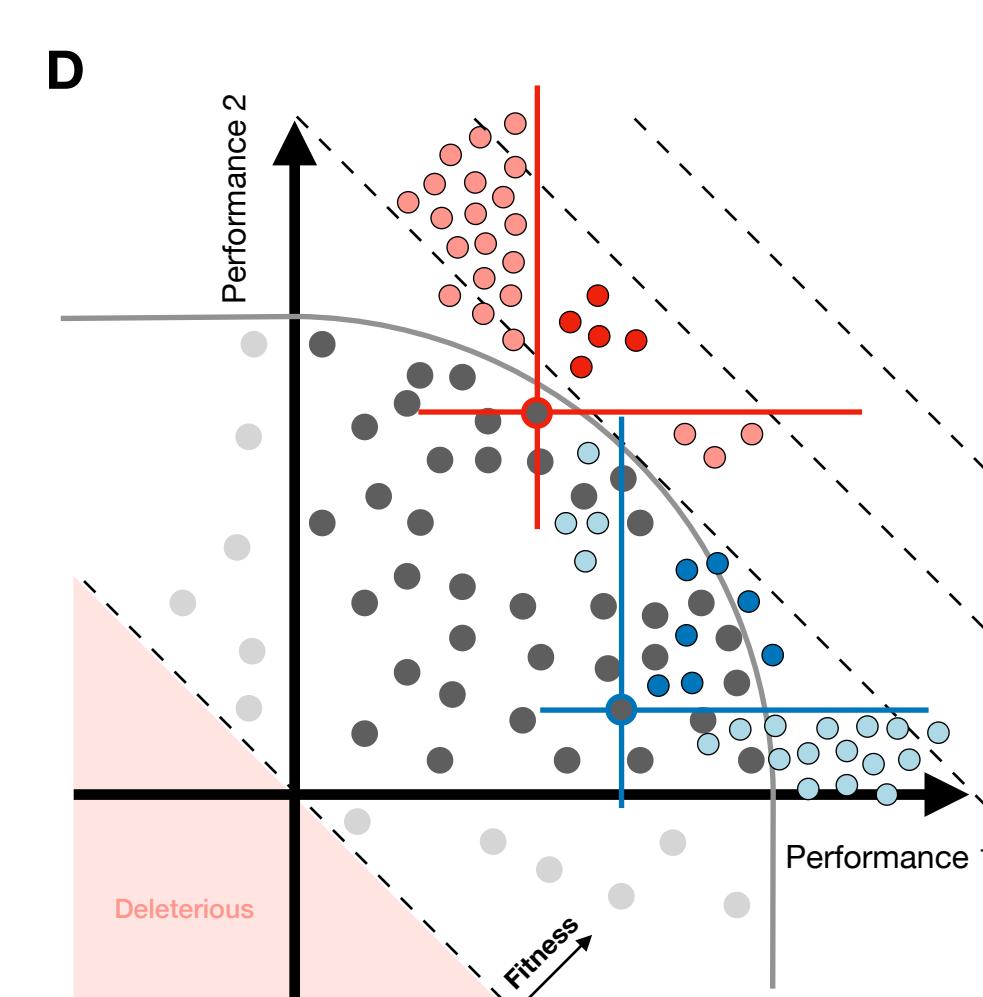
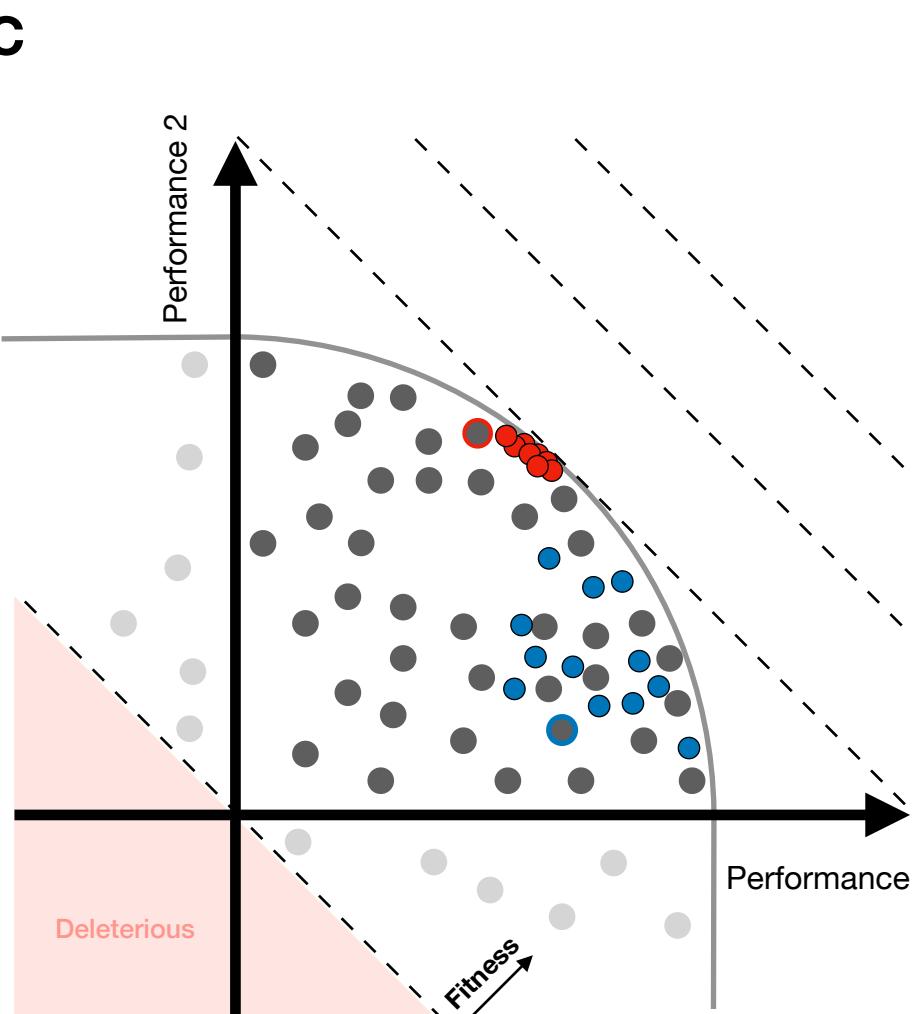
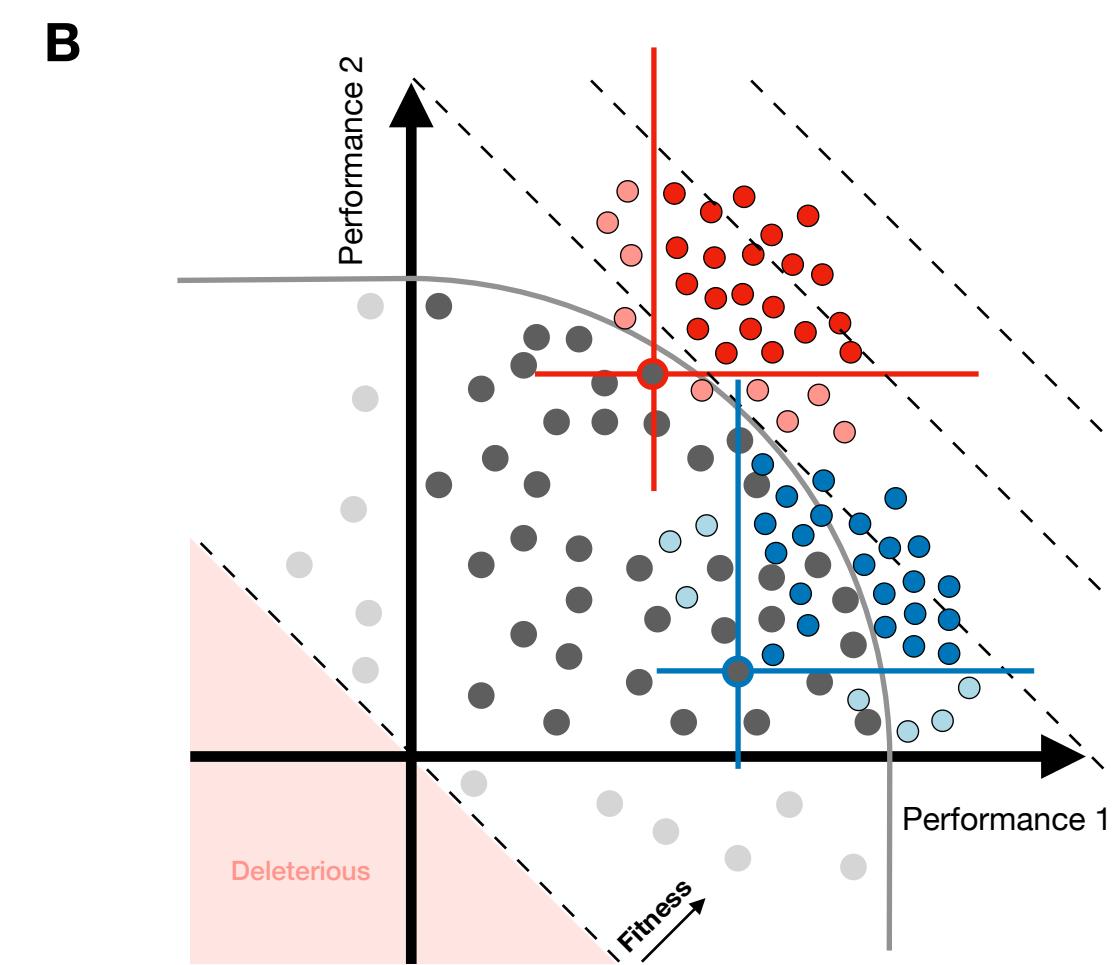
Accessibility of adaptive pleiotropic mutations is reduced:  
Second-step mutations display new patterns of trait improvement

**A**



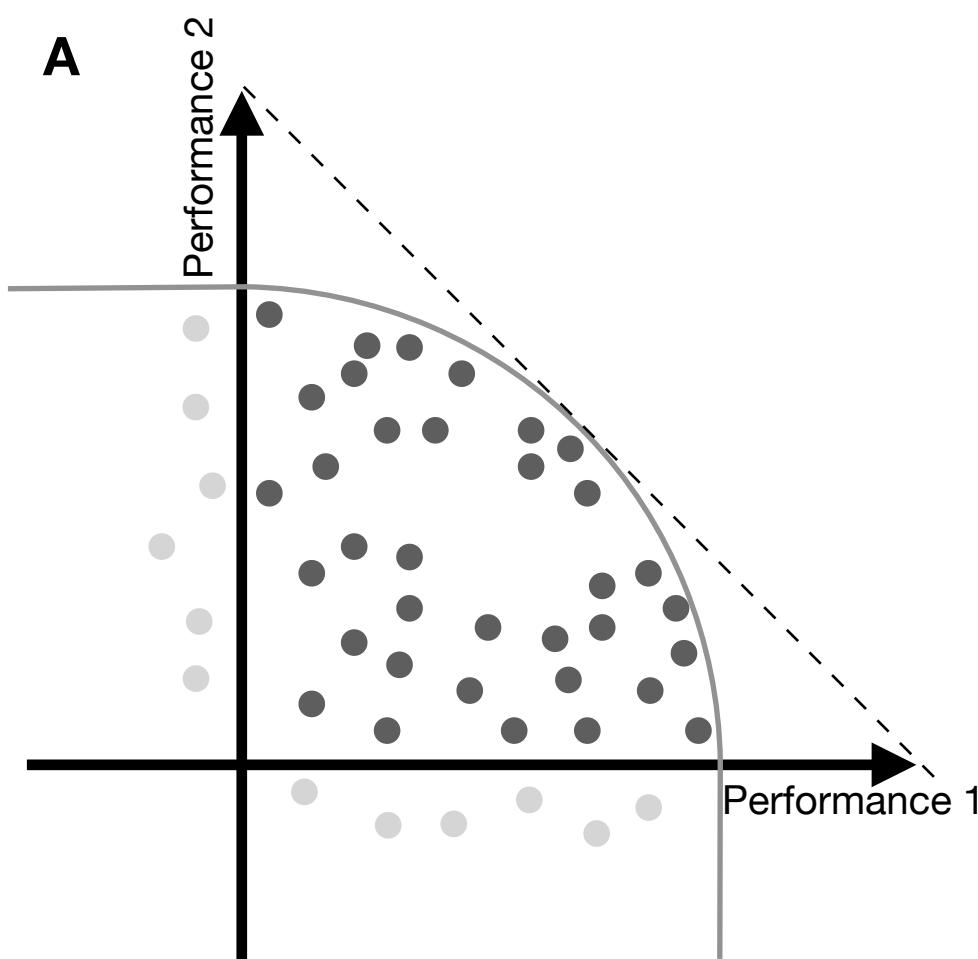


First-step mutations exhibit signs of adaptive pleiotropy (with most improving both performances at once) and tradeoffs (not achieving “perfect” extreme performance)

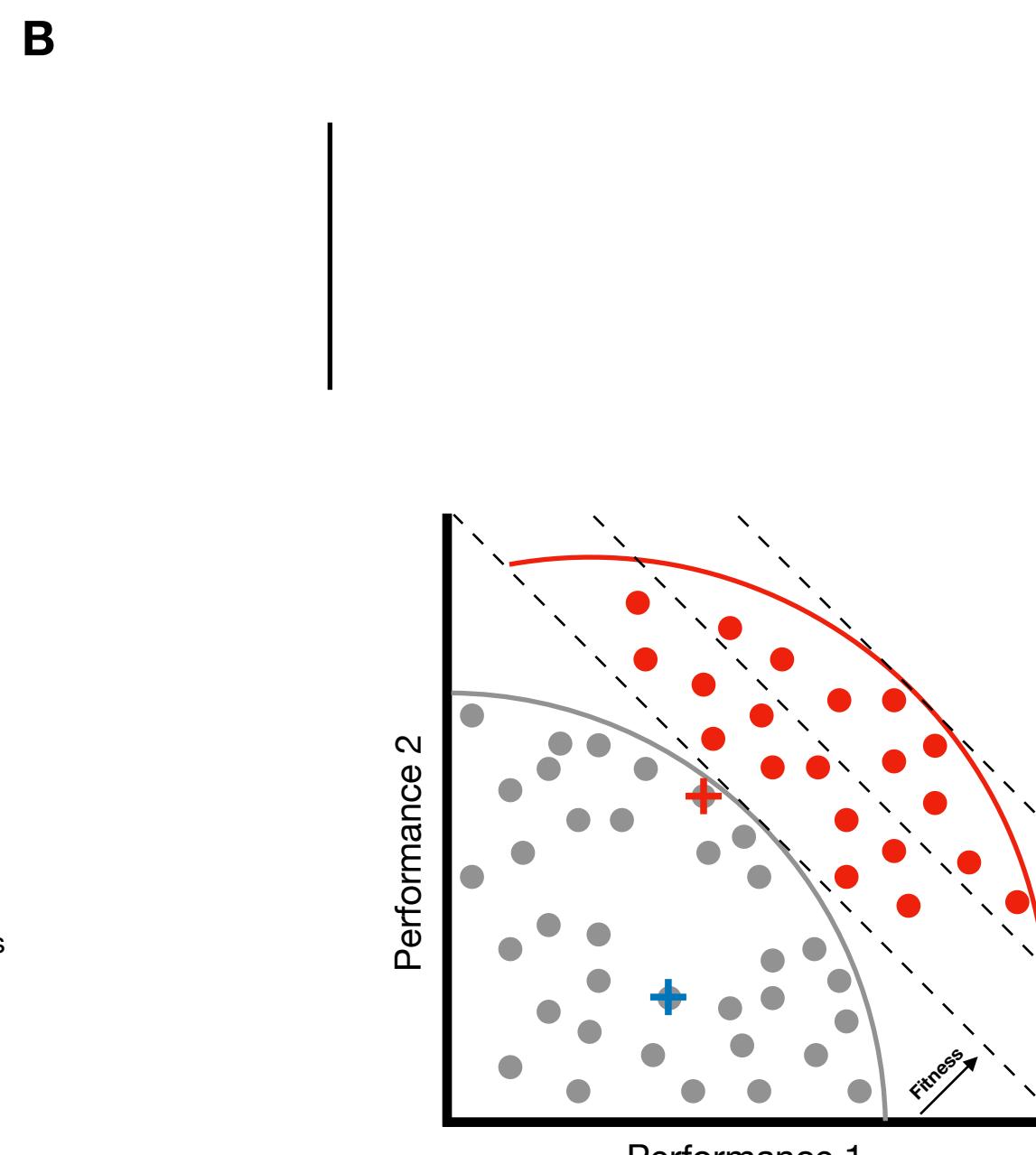


Tradeoffs represent functional/physiological constraints on the improvement of these performances

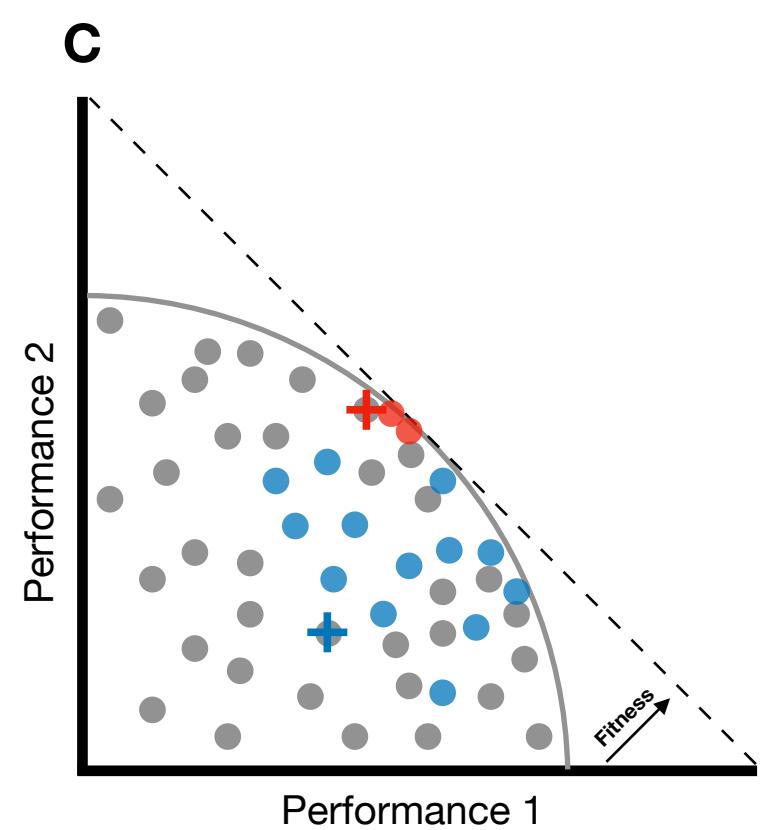
Klein et al., *bioRxiv* (2021); available at <https://doi.org/10.1101/2021.05.10.443311>; this version posted May 10, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a [CC-BY-ND 4.0 International license](https://creativecommons.org/licenses/by-nd/4.0/).



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

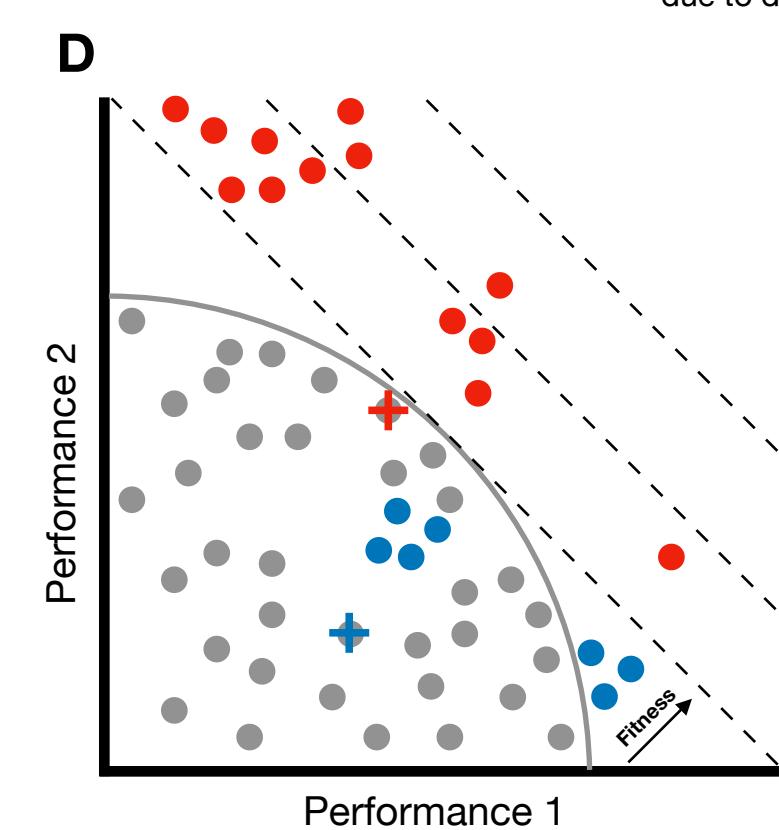


If tradeoffs don't represent any functional or genetic constraints but instead just mutational distance, then further adaptive steps should continue to improve as the first, albeit more slowly due to diminishing returns...



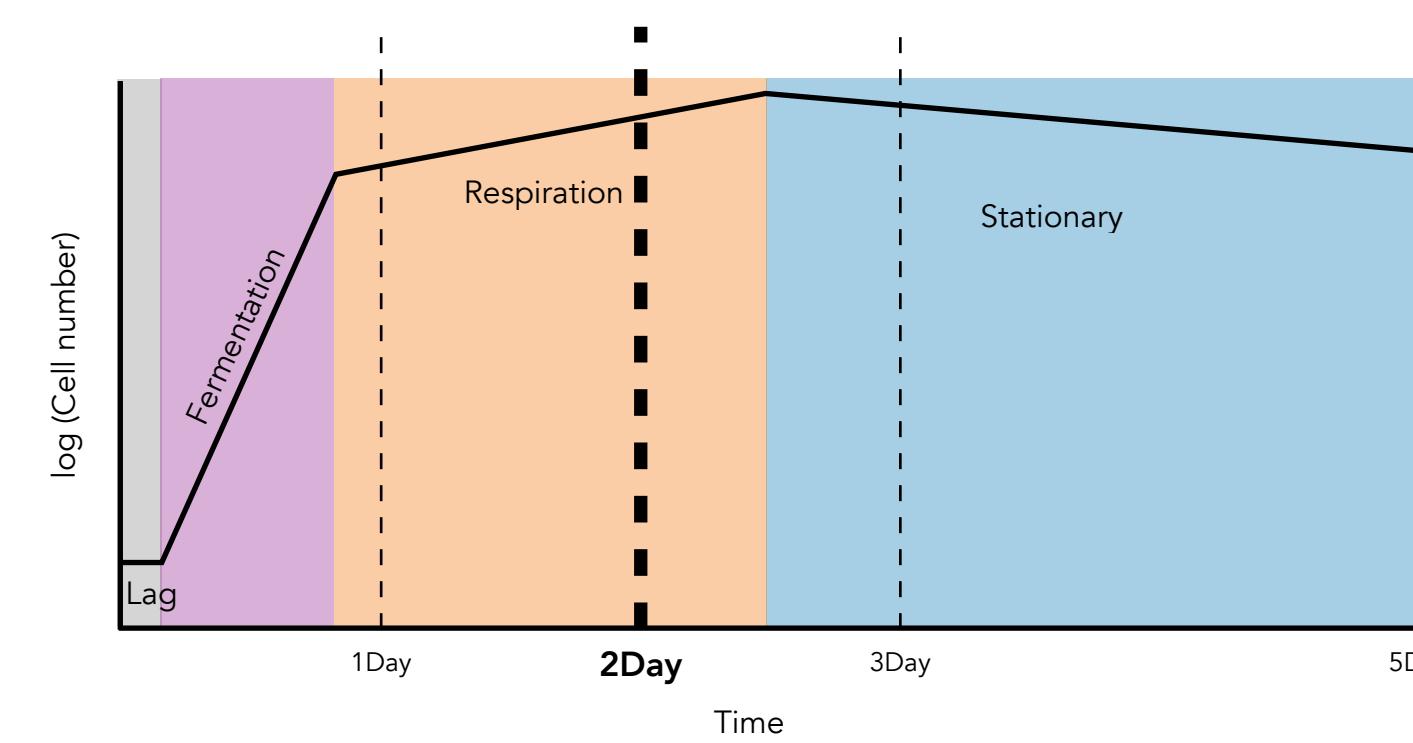
Tradeoffs represent functional/physiological constraints on the improvement of these performances

If true, further steps might move within or along these limits but cannot break through them

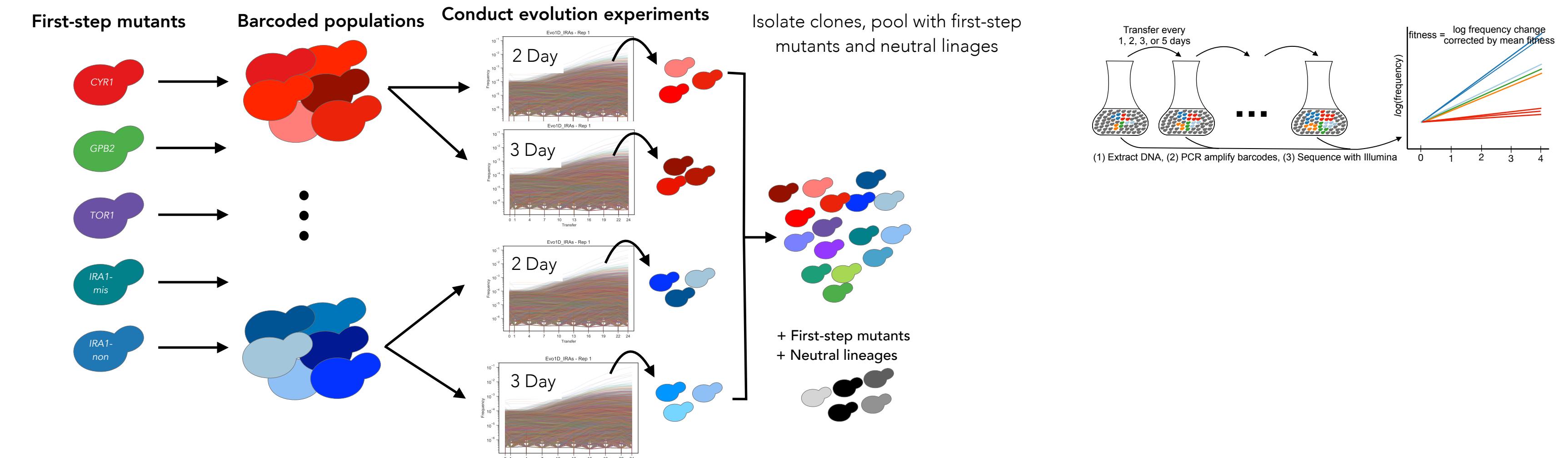


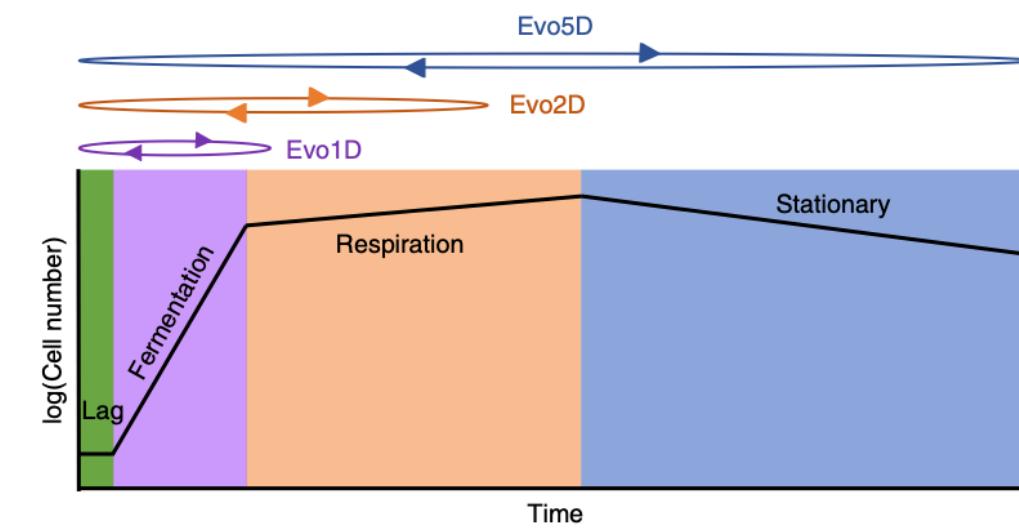
Tradeoffs reflect constraints imposed by genetic wiring, the accessibility of adaptive solutions and their pleiotropic effects.

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

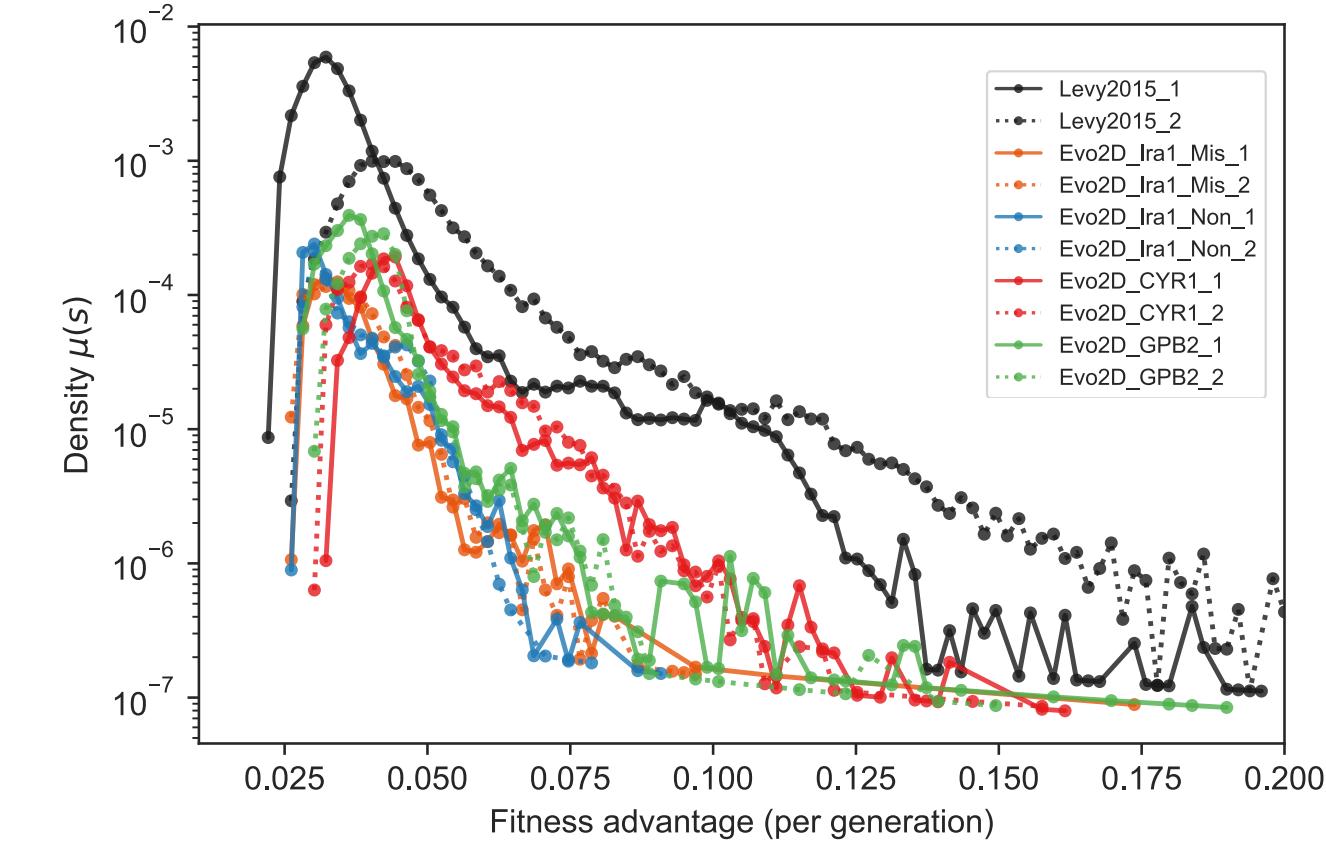
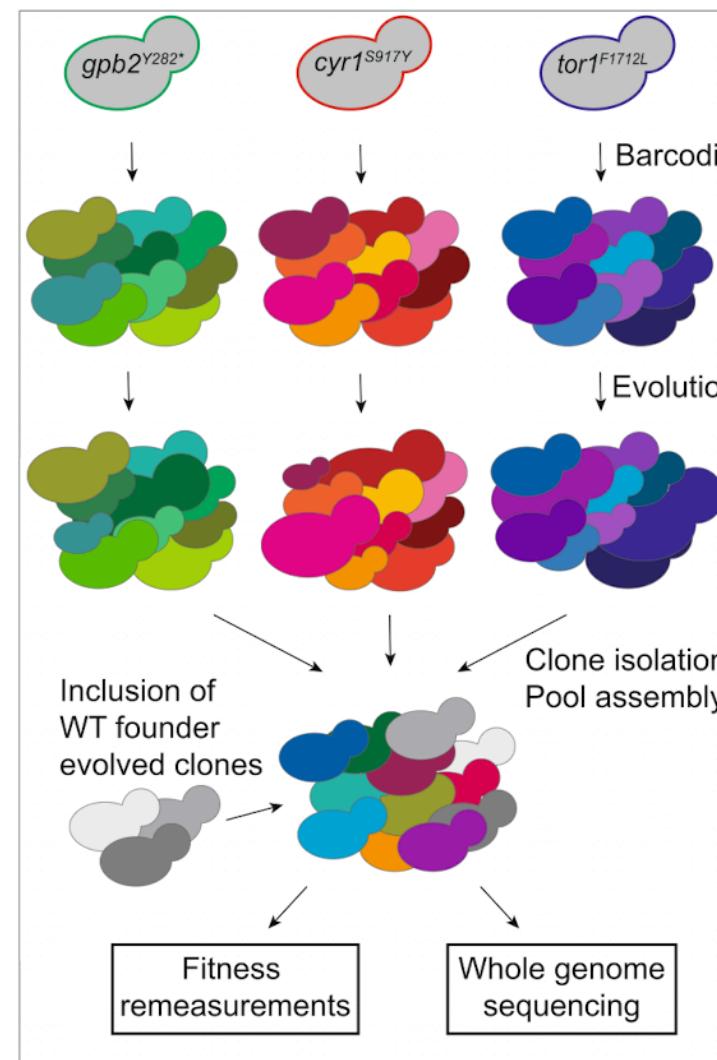


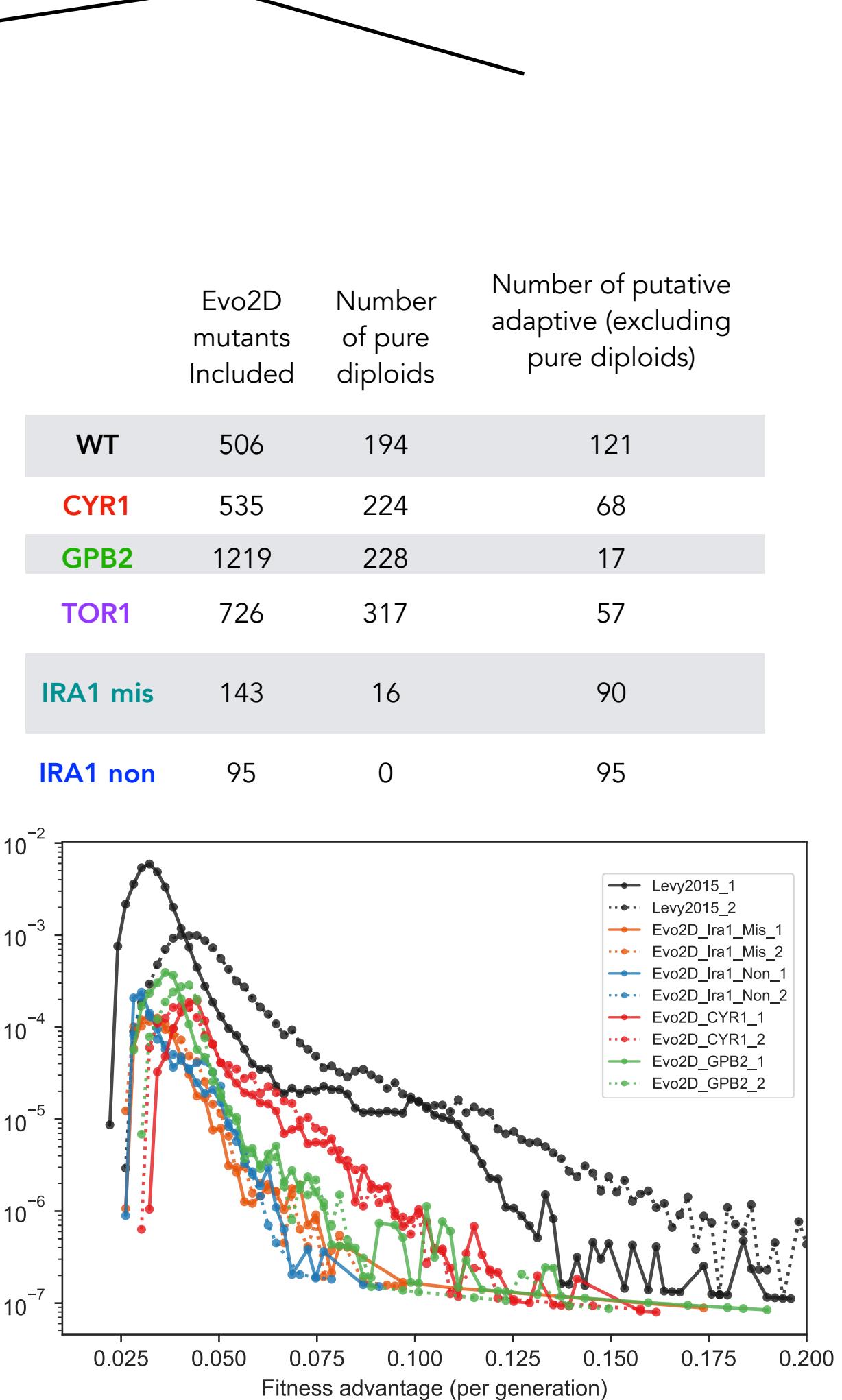
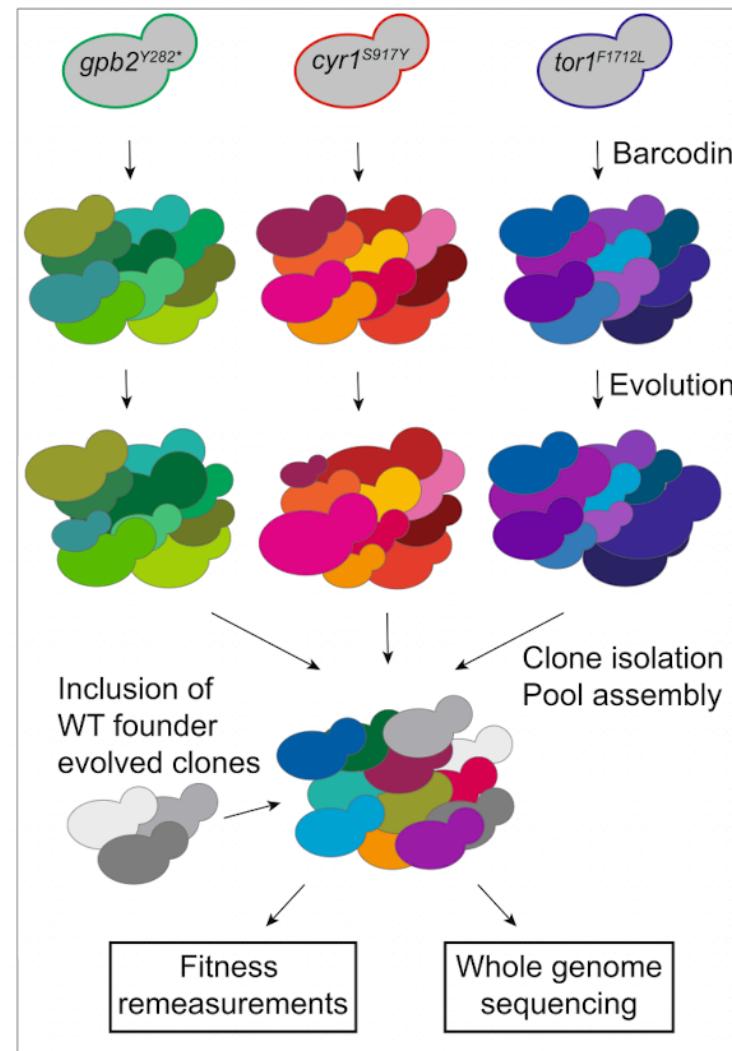
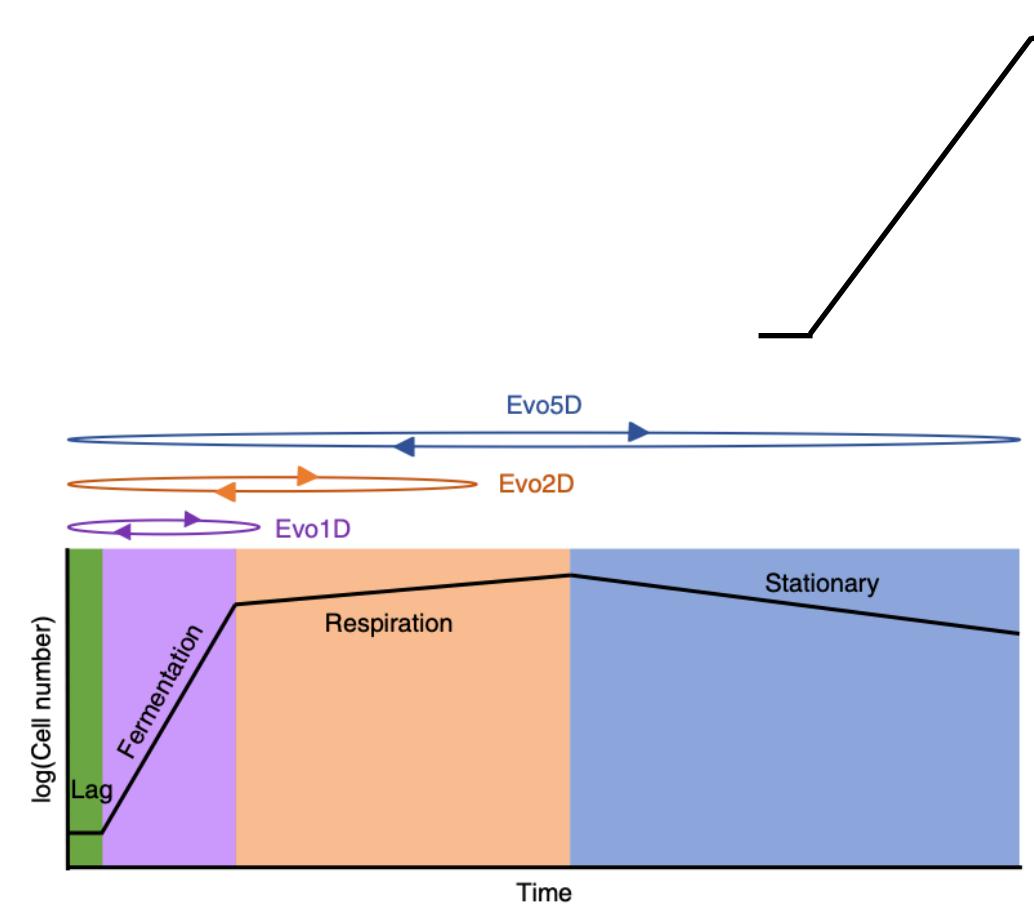
	Parental Genotype	First-step mutation	Evolution Condition	Number of adaptive mutants	Number of diploids	Publication
First Step	WT	-	2Day	121	194	Levy et al. 2015
Second Step	<b>CYR1</b>	<i>cyr1 S917Y</i>	2Day	68	224	Aggeli et al.
			3Day	20	8	This study
Second Step	<b>GPB2</b>	<i>gpb2 Y282*</i>	2Day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

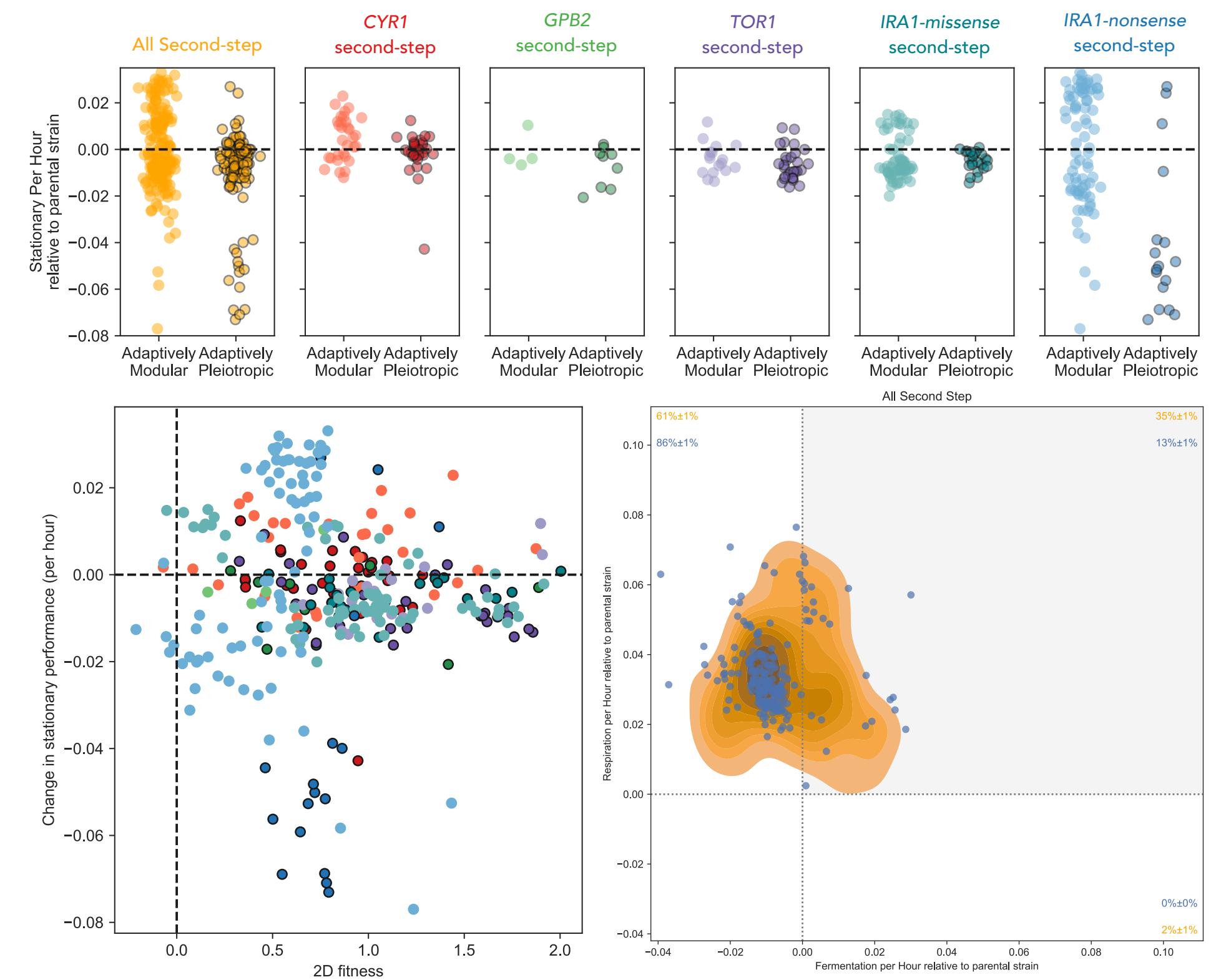




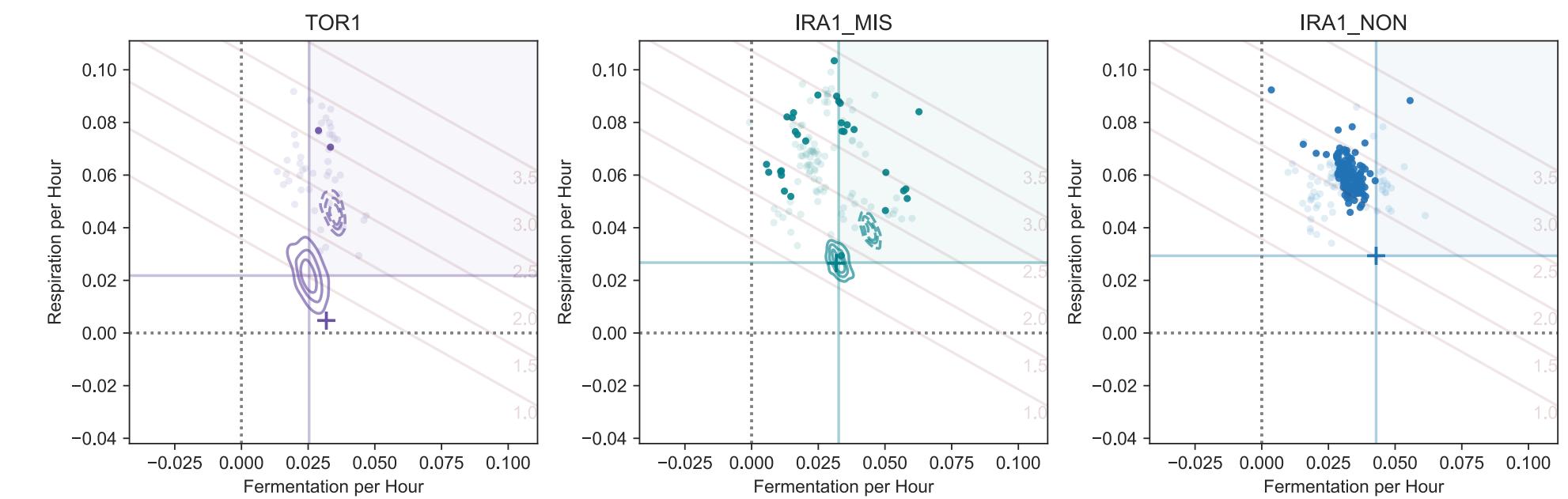
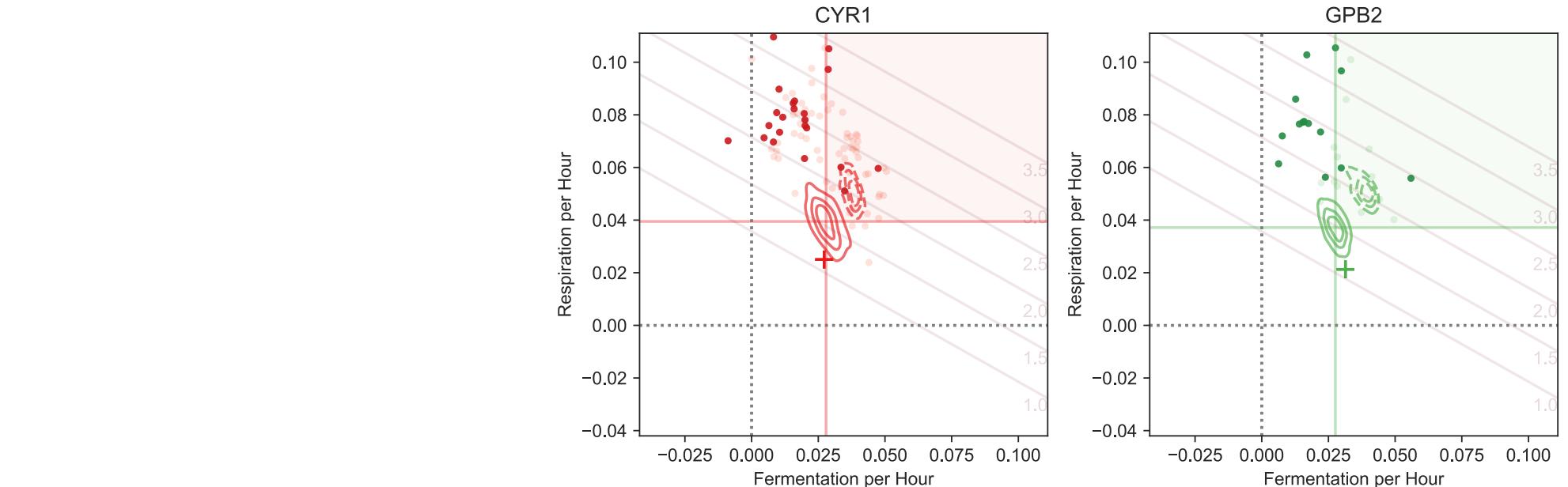
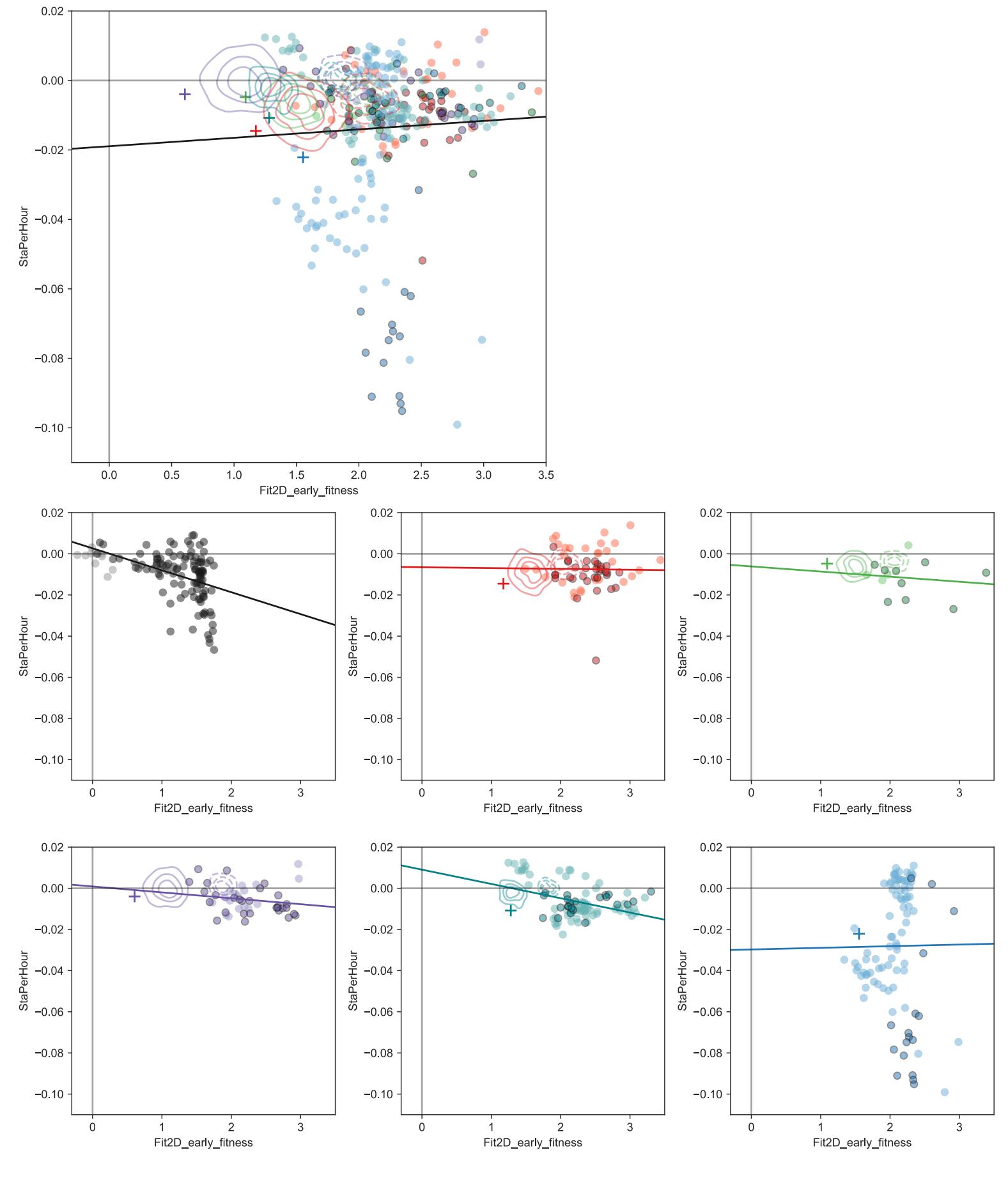
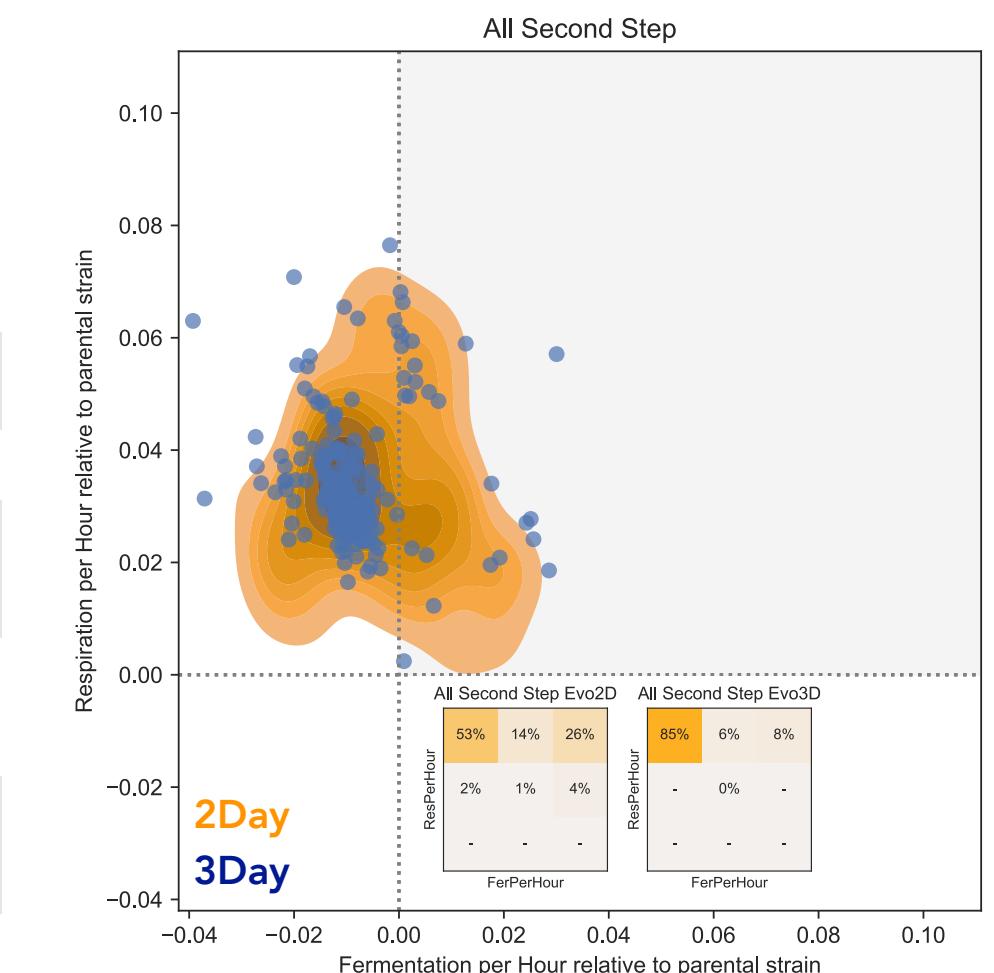
Genotype	Study	Evo2D mutants Included	Number of pure diploids	Number of putative adaptive (excluding pure diploids)
WT	Levy 2015	506	194	121
CYR1	Aggeli 2020	535	224	68
GPB2	Aggeli 2020	1219	228	17
TOR1	Aggeli 2020	726	317	57
IRA1 mis	This study	143	16	90
IRA1 non	This study	95	0	95

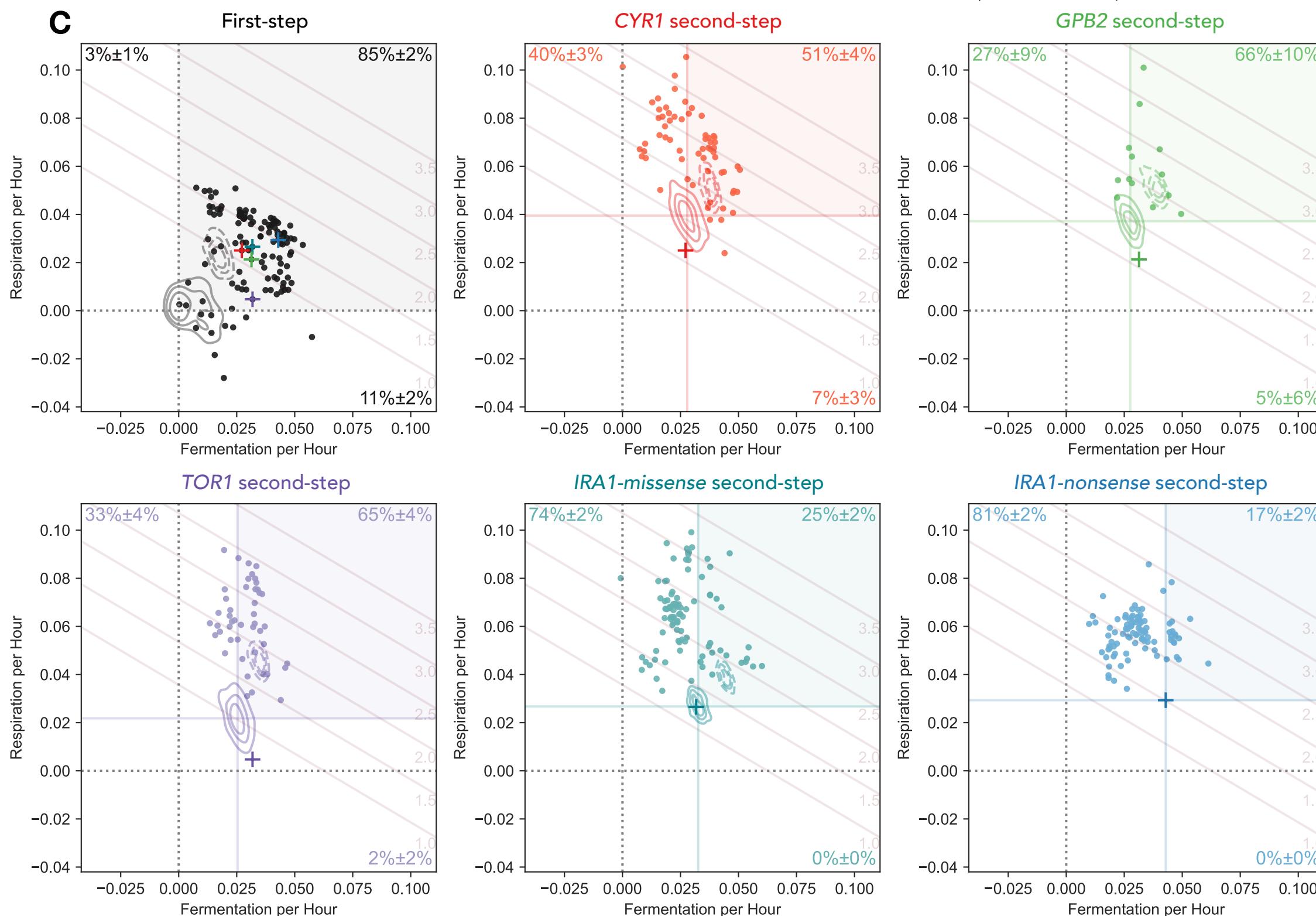
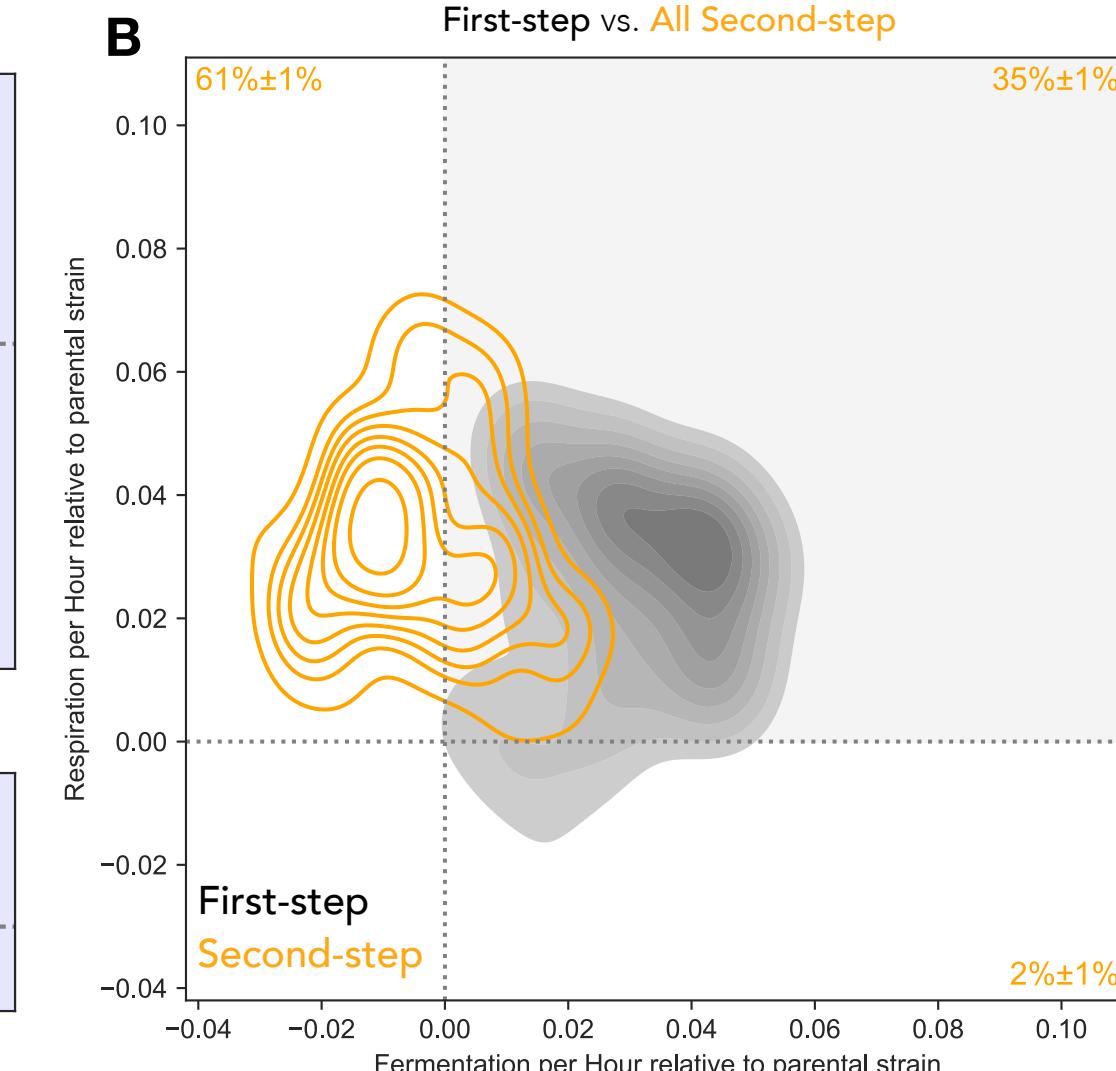
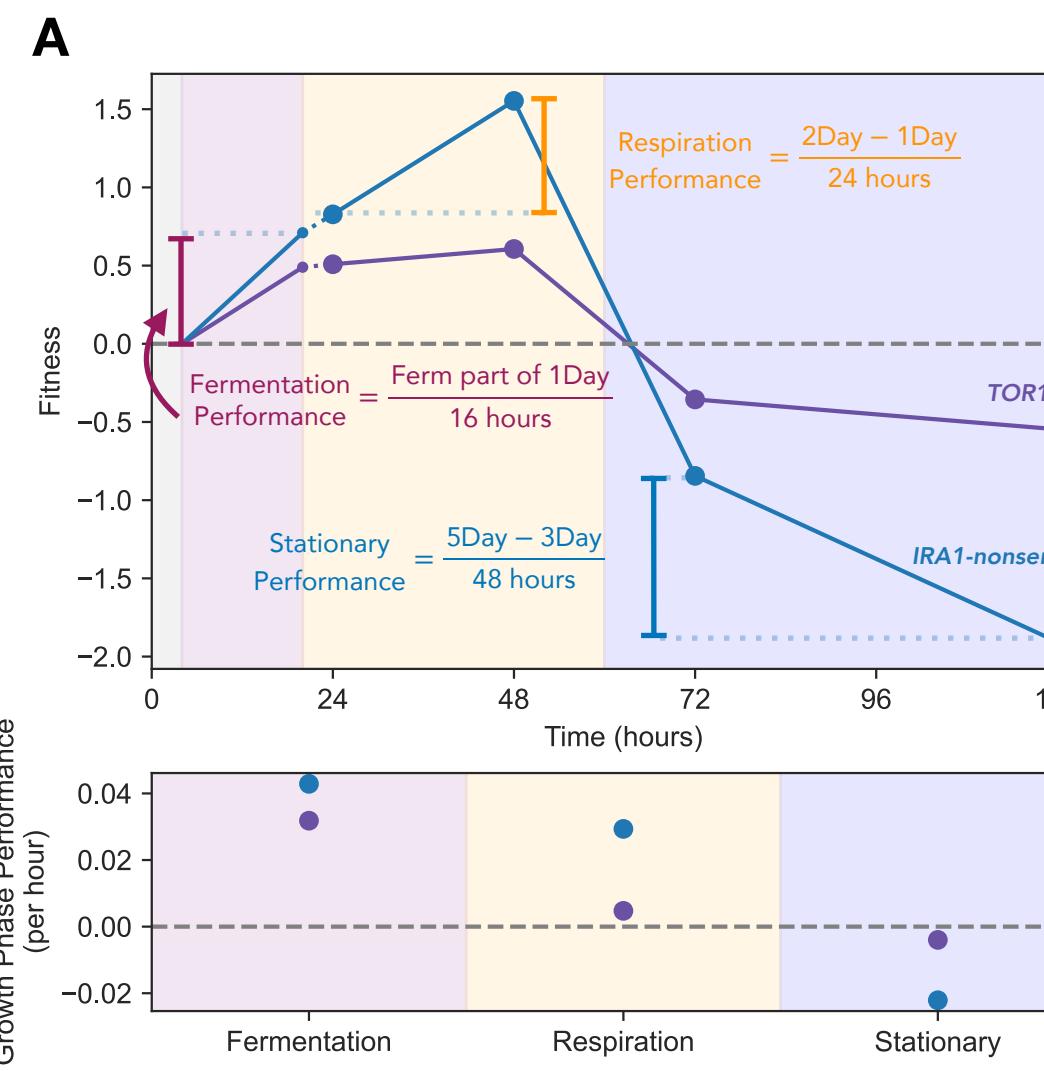


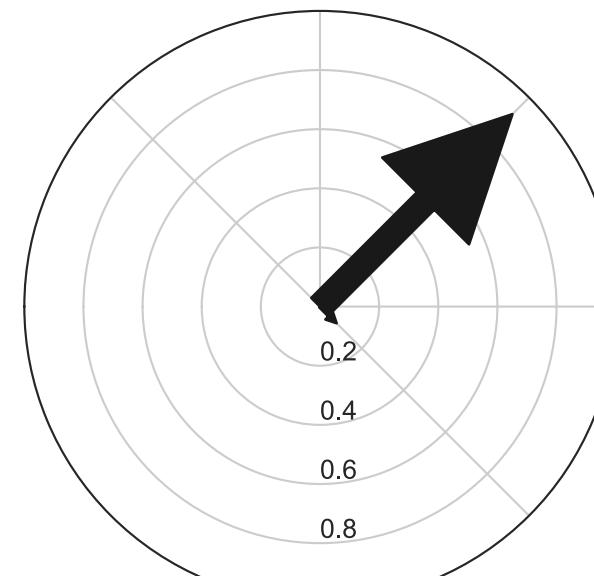
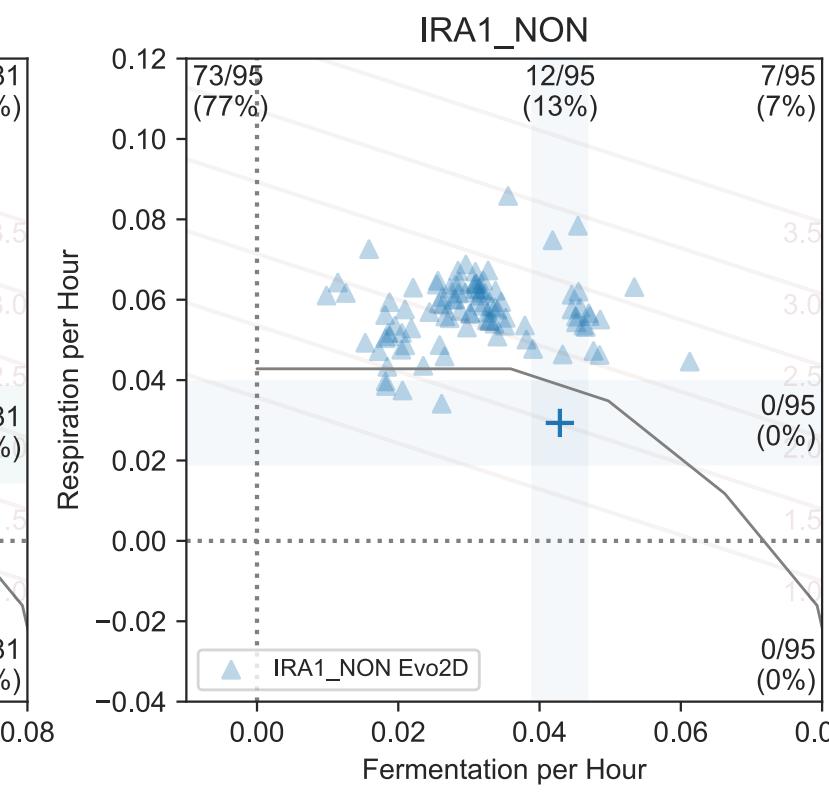
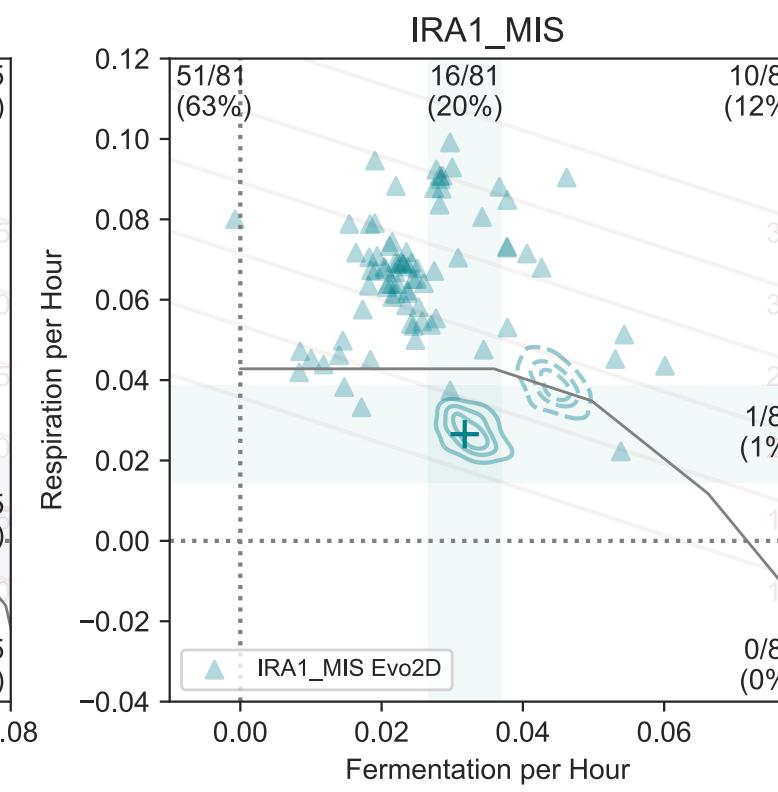
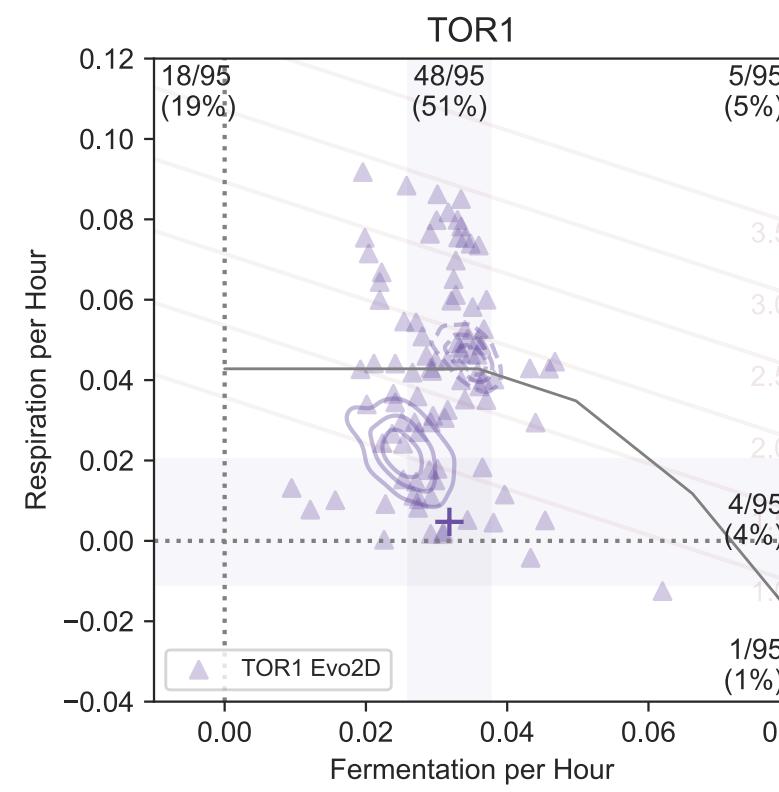
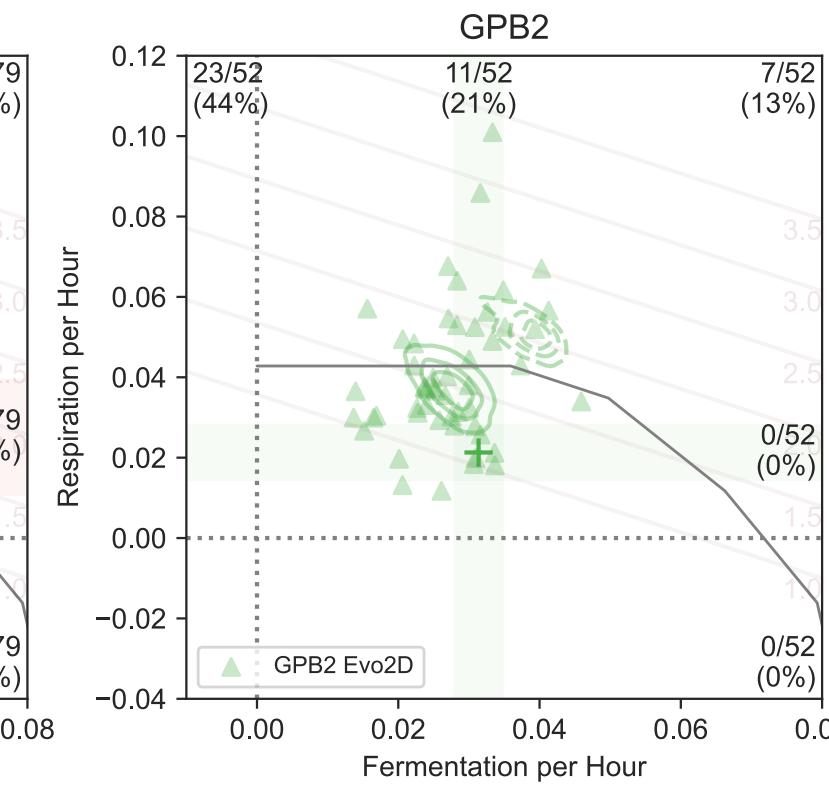
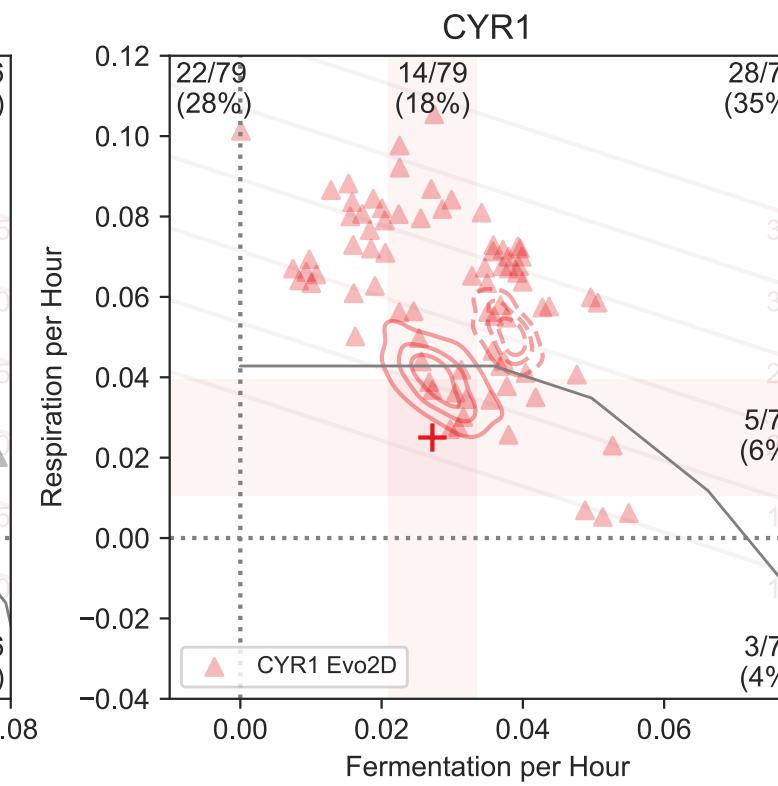
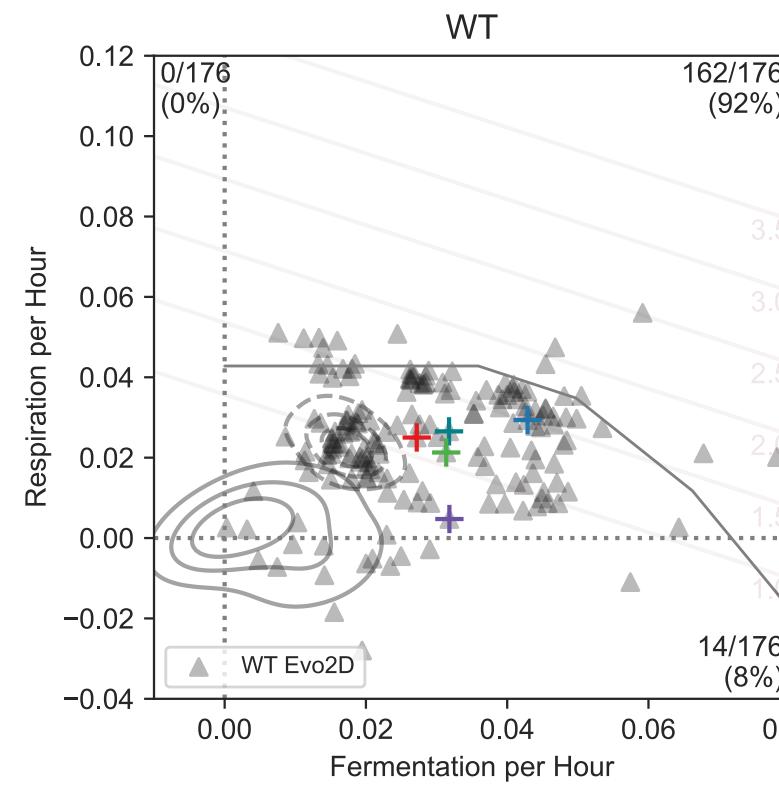




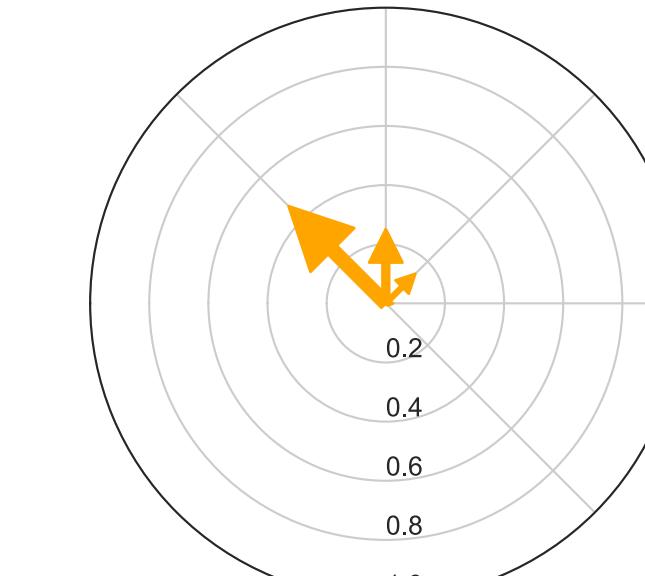
	Evo3D mutants	Number of pure diploids	Number of putative adaptive mutants
<b>CYR1</b>	35	8	20
<b>GPB2</b>	34	20	12
<b>TOR1</b>	2	-	2
<b>IRA1 mis</b>	32	3	28
<b>IRA1 non</b>	135	0	135







**First step**



**All second step**

