

Here we show that, after fixing the bug, Fig. 4 - 10 (omitting Fig. 8), Supplementary Fig. 5 – 11, and Supplementary Table 4 - 6 remain nearly unchanged. Supplementary Table 3, which includes summarizes the evolutionary constraint on parameters in AND-gated C1-FFLs, is slightly more affected, but not in a manner that affects the main text of the paper. We re-run simulations from the same initial conditions as in Xiong et al.. Because data were processed with the same protocol, legends of figures and tables are simplified or omitted.

Supplementary Table 3. Evolutionary constraint on parameters in AND-gated C1-FFLs

	Signal		TFs		Effector	
	V_n / V_s	M_s / M_n	V_n / V_s	M_s / M_n	V_n / V_s	M_s / M_n
$r_{\text{Act_to_Int}}$	NA	NA	1.25	0.19	8.42	0.13
$r_{\text{mRNA_deg}}$	NA	NA	1.53	0.97	12.9	2.90
$r_{\text{protein_syn}}$	NA	NA	2.00	11.1	53.3	64.2
$r_{\text{protein_deg}}$	NA	NA	1.27	0.30	30.8	15.8
$K_d(0)$	1.46	0.002	0.69	0.01	NA	NA
Locus length	NA	NA	1.27	0.75	1.82	0.69

Results are now calculated from 27 high-fitness replicates (the number was 25 in our original report) that evolved for filtering out a spurious signal, where the signal directly regulates the effector. Calculation details of evolutionary constraint are described in the original table. While most results are qualitatively the same, there are two noticeable differences between the new and the original results. First, the $K_d(0)$ of the signal is now under a mild evolutionary constraint, as its variance ratio is greater than 1. Second, the degradation rate of effector protein is now the second most constrained parameter, while it was the fourth most constrained in the original table.

Supplementary Table 4. Evolutionary constraint on parameters in AND-gated diamonds

	Signal		Fast TFs		Slow TFs		Effector	
	V_n / V_s	M_s / M_n	V_n / V_s	M_s / M_n	V_n / V_s	M_s / M_n	V_n / V_s	M_s / M_n
$r_{\text{Act_to_Int}}$	NA	NA	1.52	0.44	2.00	0.10	9.37	0.10
$r_{\text{mRNA_deg}}$	NA	NA	3.28	6.52	1.09	0.47	12.1	2.12
$r_{\text{protein_syn}}$	NA	NA	3.32	27.3	2.30	22.3	55.9	77.9
$r_{\text{protein_deg}}$	NA	NA	16.6	41.0	0.73	0.37	27.1	17.3
$K_d(0)$	0.71	0.003	0.28	0.003	0.18	0.02	NA	NA
Locus length	NA	NA	3.82	0.72	3.85	0.72	2.04	0.87

Results are now calculated from 18 high-fitness replicates (the number was 18 in our original report) that evolved for filtering out a spurious signal, where the signal indirectly regulates the effector.

Supplementary Table 5. Evolutionary constraint on parameters in isolated AND-gated C1-FFLs

	Signal		Signal-regulated TFs		TF-regulated TFs		Effector	
	V_n / V_s	M_s / M_n	V_n / V_s	M_s / M_n	V_n / V_s	M_s / M_n	V_n / V_s	M_s / M_n
$r_{\text{Act_to_Int}}$	NA	NA	3.10	0.48	1.80	0.15	9.47	0.13
$r_{\text{mRNA_deg}}$	NA	NA	75.1	8.1	1.00	0.46	15.2	1.75
$r_{\text{protein_syn}}$	NA	NA	4.79	35.2	2.82	29.1	128	79.7
$r_{\text{protein_deg}}$	NA	NA	128	42.6	1.46	0.49	25.2	17.8
$K_d(0)$	0.77	0.004	0.37	0.001	0.29	0.03	NA	NA
Locus length	NA	NA	2.63	0.59	2.74	0.59	3.50	0.98

Results are now calculated from 11 high-fitness replicates (the number was 12 in our original report) that evolved for filtering out a spurious signal, where the signal indirectly regulates the effector.

Supplementary Table 6. Summary of mutations that replaced the resident genotype

	Probability that mutation of this type is accepted, given it occurs		Probability that an accepted mutation is of this type	
	First 1,000 evol. steps	Last 1,000 evol. steps	First 1,000 evol. steps	Last 1,000 evol. steps
Substitution	0.34 \pm 0.01	0.35 \pm 0.00	0.178 \pm 0.004	0.210 \pm 0.008
Deletion	0.28 \pm 0.01	0.22 \pm 0.01	0.364 \pm 0.002	0.353 \pm 0.005
Duplication	0.32 \pm 0.01	0.33 \pm 0.01	0.368 \pm 0.003	0.354 \pm 0.005
TF recognition seq.	0.31 \pm 0.01	0.18 \pm 0.02	0.008 \pm 0.000	0.005 \pm 0.001
$r_{\text{Act_to_Int}}$	0.29 \pm 0.01	0.26 \pm 0.01	0.010 \pm 0.000	0.010 \pm 0.001
$r_{\text{mRNA_deg}}$	0.37 \pm 0.02	0.26 \pm 0.02	0.014 \pm 0.001	0.012 \pm 0.001
$r_{\text{protein_syn}}$	0.32 \pm 0.02	0.25 \pm 0.01	0.013 \pm 0.001	0.012 \pm 0.001
$r_{\text{protein_deg}}$	0.34 \pm 0.02	0.26 \pm 0.02	0.014 \pm 0.001	0.011 \pm 0.001
$K_d(0)$	0.30 \pm 0.02	0.17 \pm 0.02	0.006 \pm 0.000	0.004 \pm 0.000
TF identity	0.30 \pm 0.01	0.29 \pm 0.02	0.008 \pm 0.000	0.008 \pm 0.001
Locus length	0.32 \pm 0.01	0.38 \pm 0.01	0.016 \pm 0.001	0.022 \pm 0.002

Results are now calculated from 27 high-fitness replicates (the number was 25 in our original report) that evolved for filtering out a spurious signal, where the signal directly regulates the effector.

Figure 4.

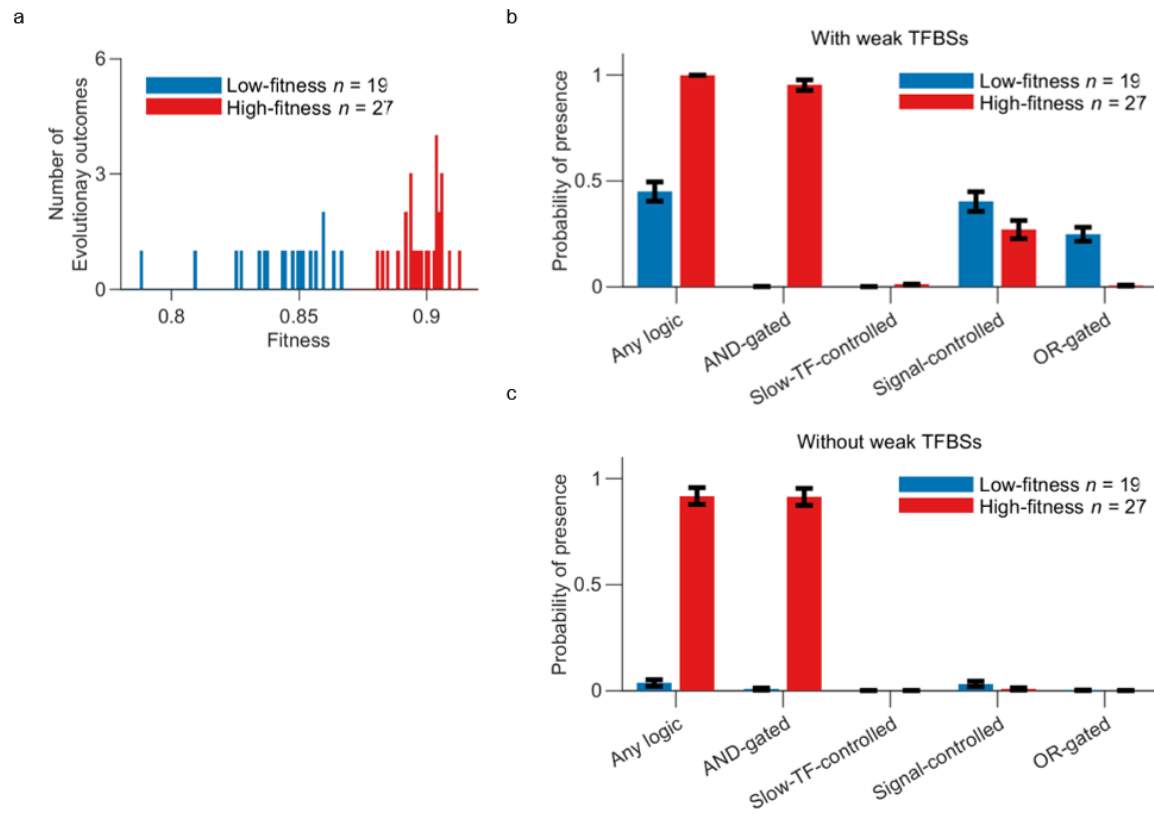
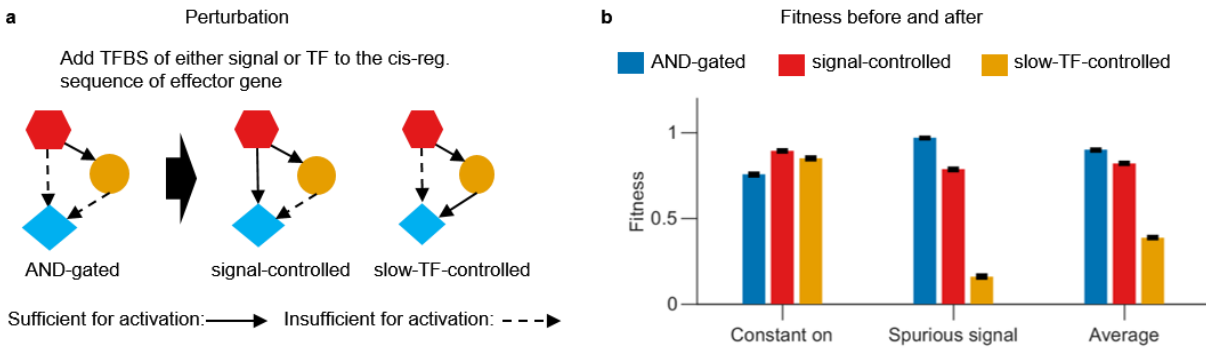


Figure 5.



Perturbation analysis is now applied to 26 of 27 (originally 25 out of 25) high-fitness replicates that evolved for filtering out a spurious signal, where the signal directly regulates the effector.

Figure 6.

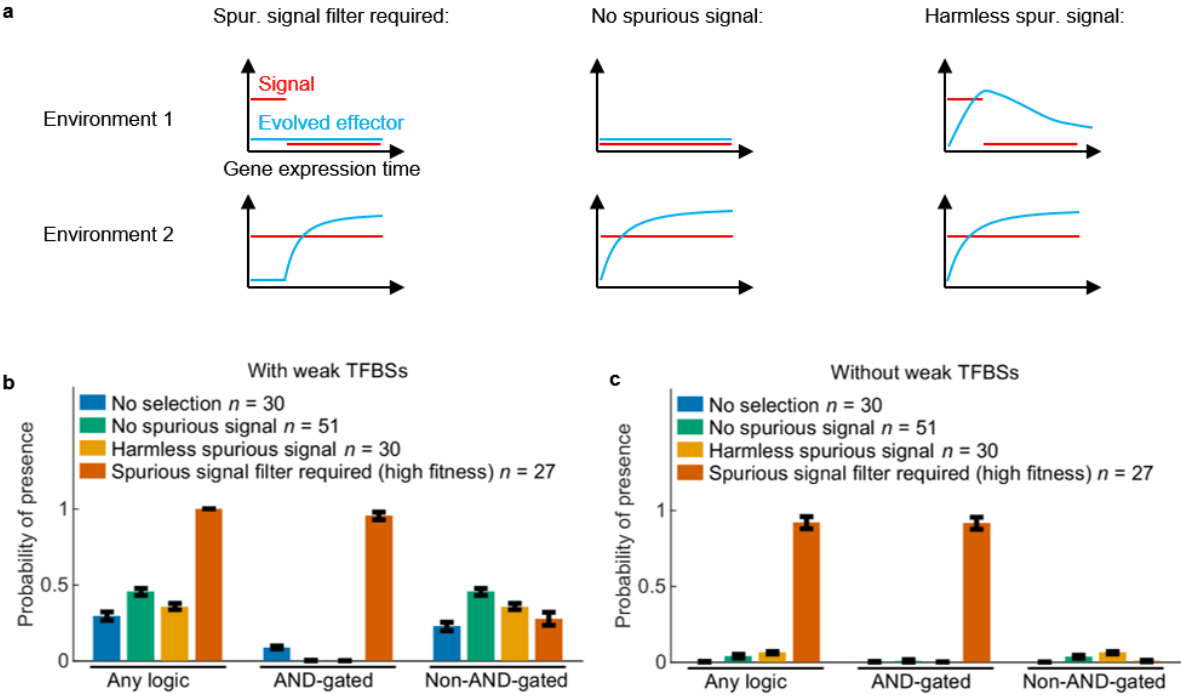


Figure 7.

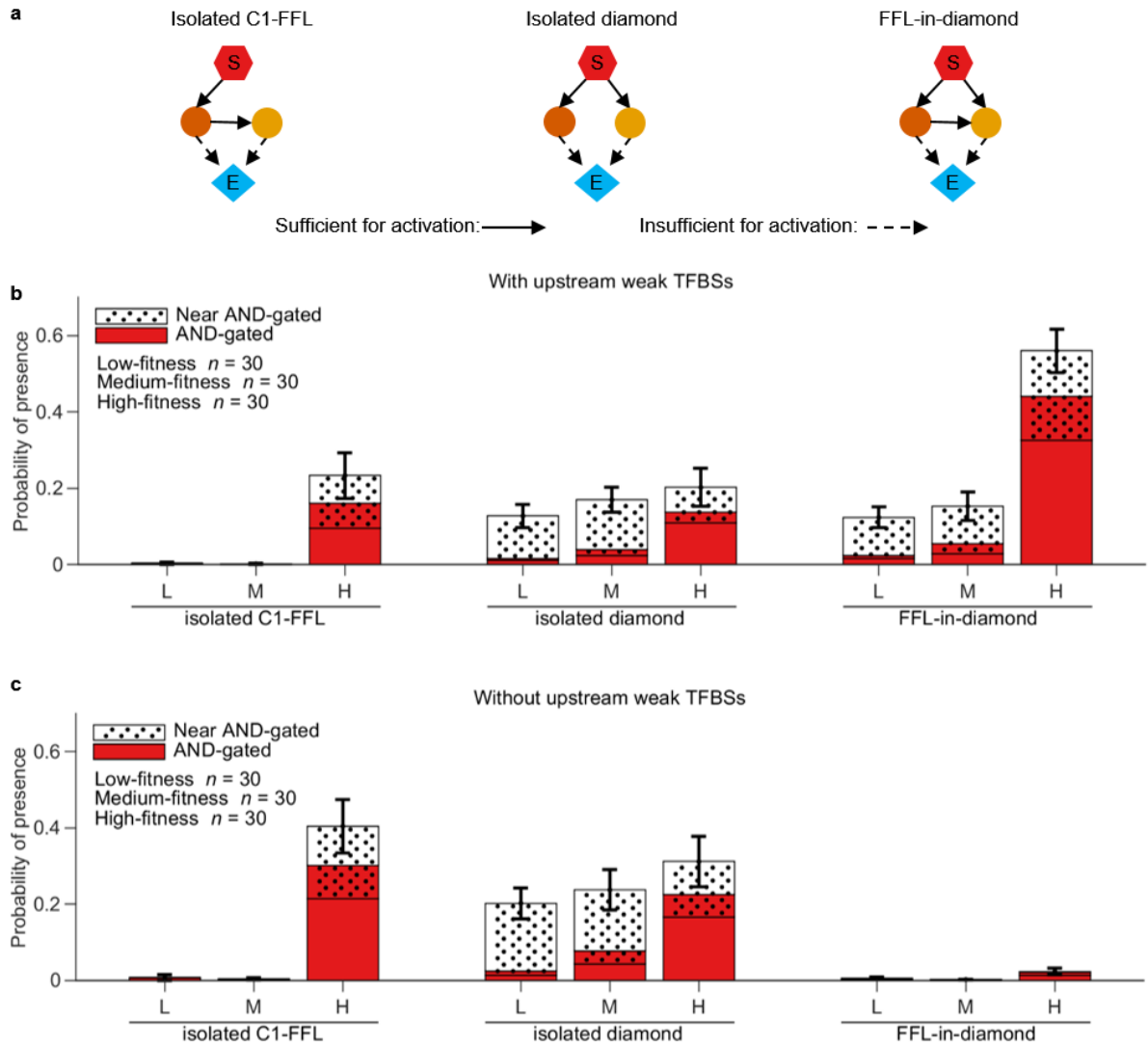
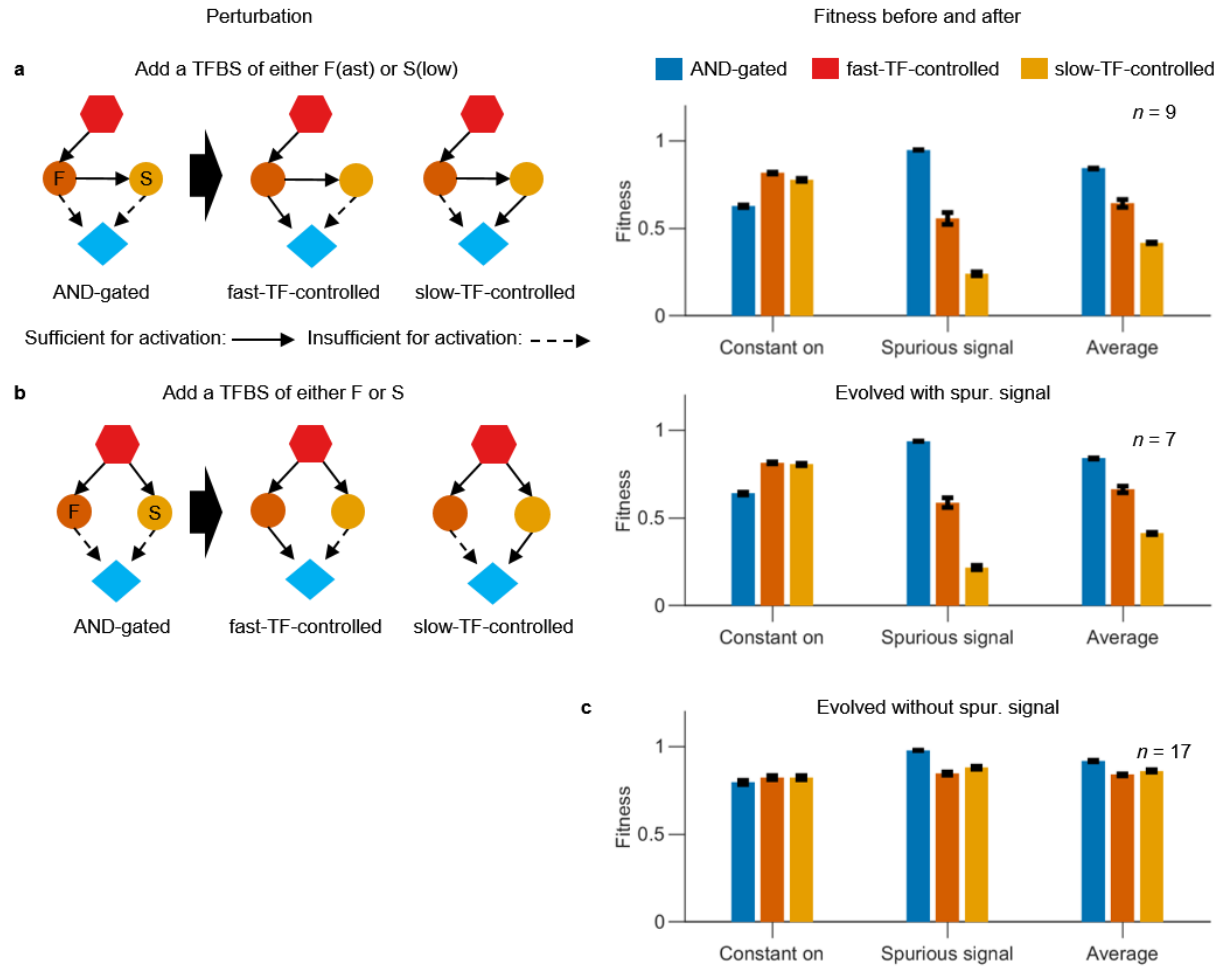
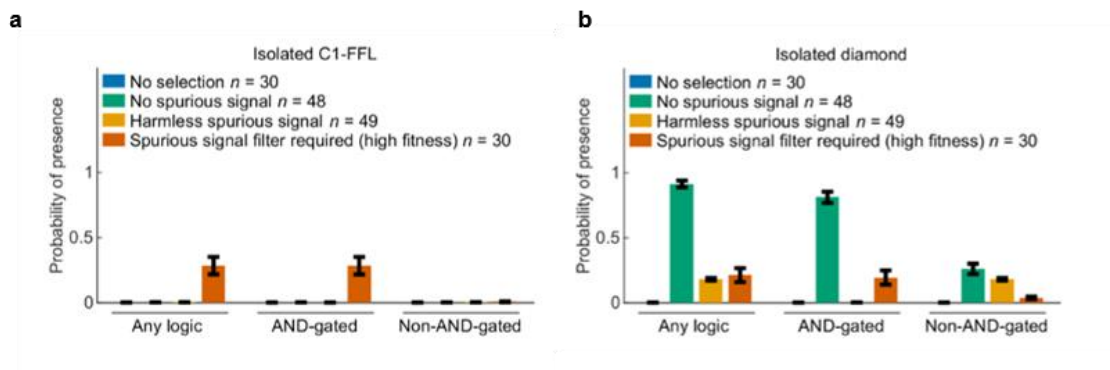


Figure 9.

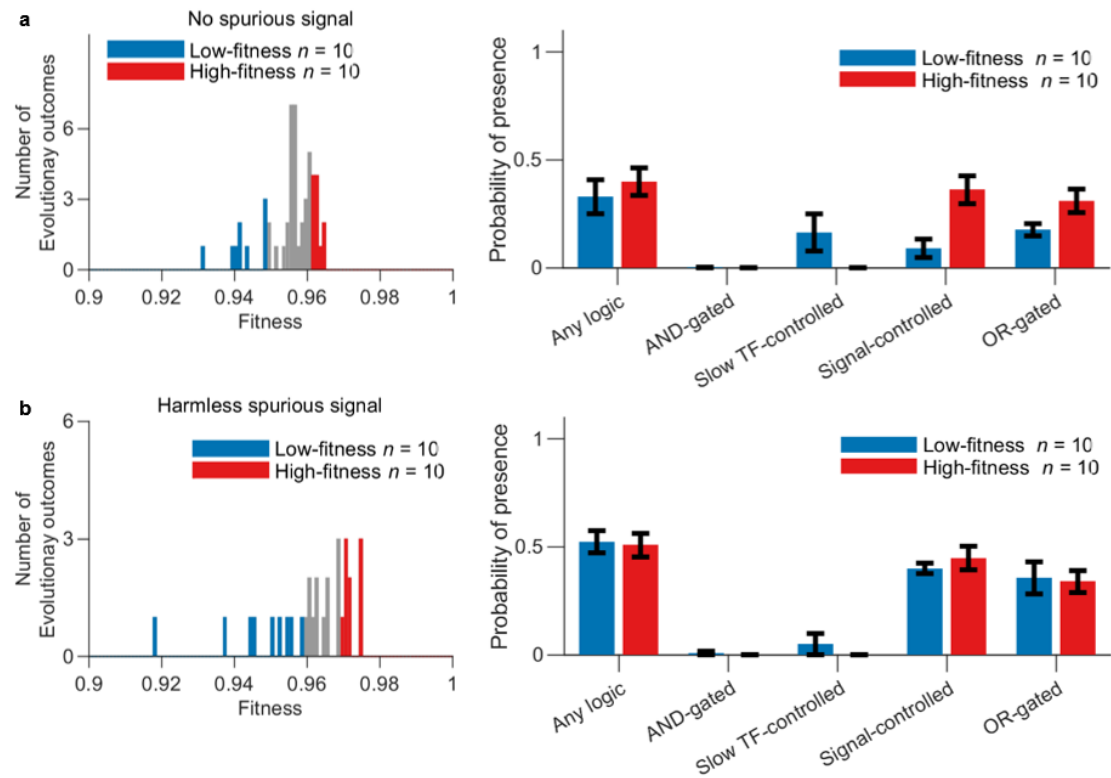


a Perturbation is now applied to 9 of 22 (originally 8 out of 18) high-fitness replicates that evolve AND-gated isolated C1-FFLs. **b** Perturbation is now applied to 7 of 22 (originally 4 out of 26) high-fitness replicates that evolve AND-gated isolated diamonds. **c** Perturbation is now applied to 17 of 35 (originally 15 out of 37) high-fitness replicates that evolve AND-gated isolated diamonds.

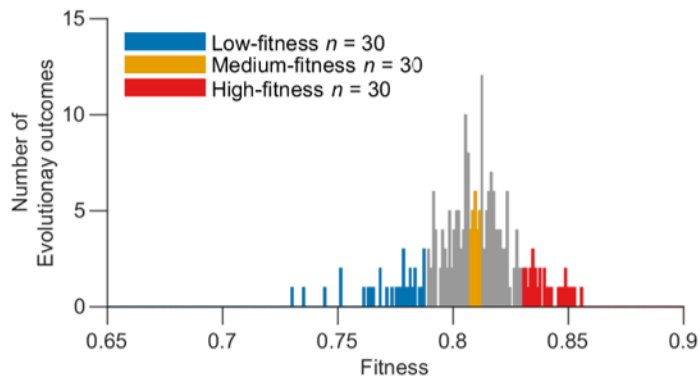
Figure 10.



Supplementary Figure 5.

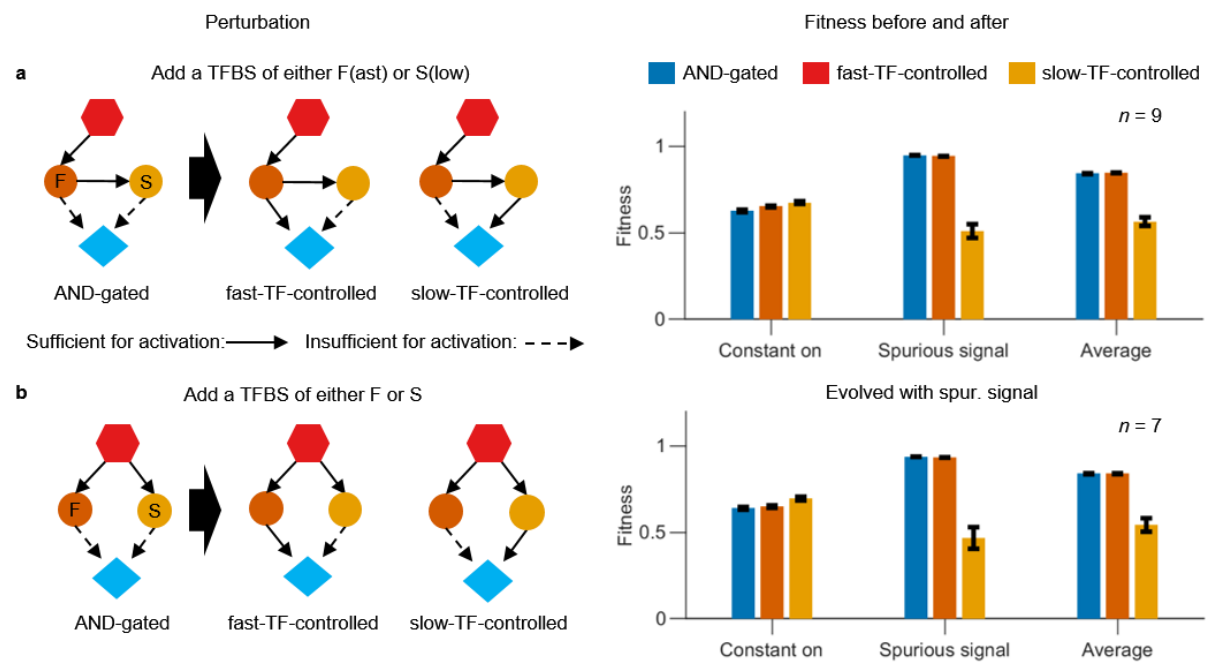


Supplementary Figure 6.

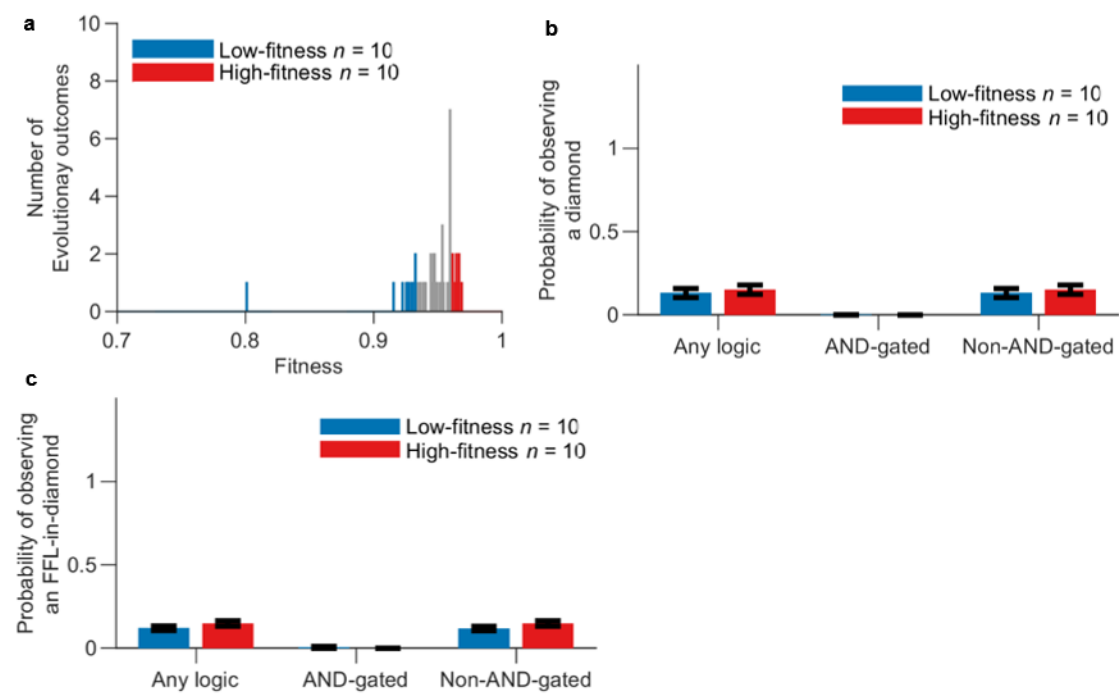


Starting from the same initial conditions, there are now 232 simulation replicates that successfully evolved for 5,1000 evolutionary steps. The number was 238 in Xiong et al. (2019).

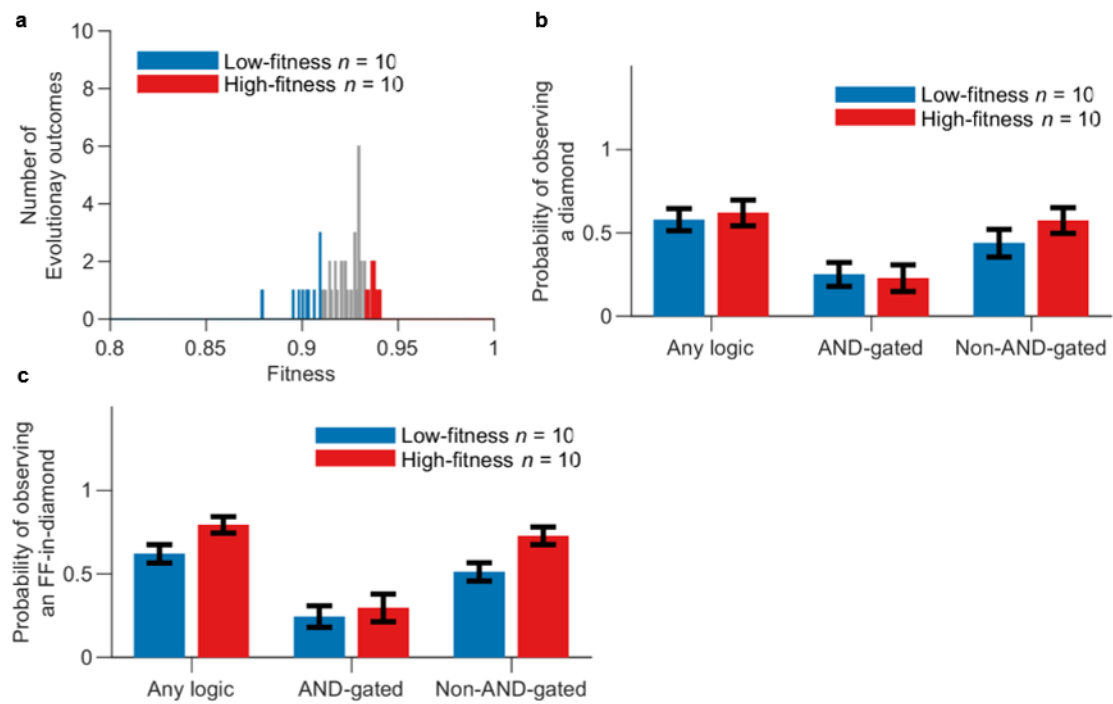
Supplementary Figure 7.



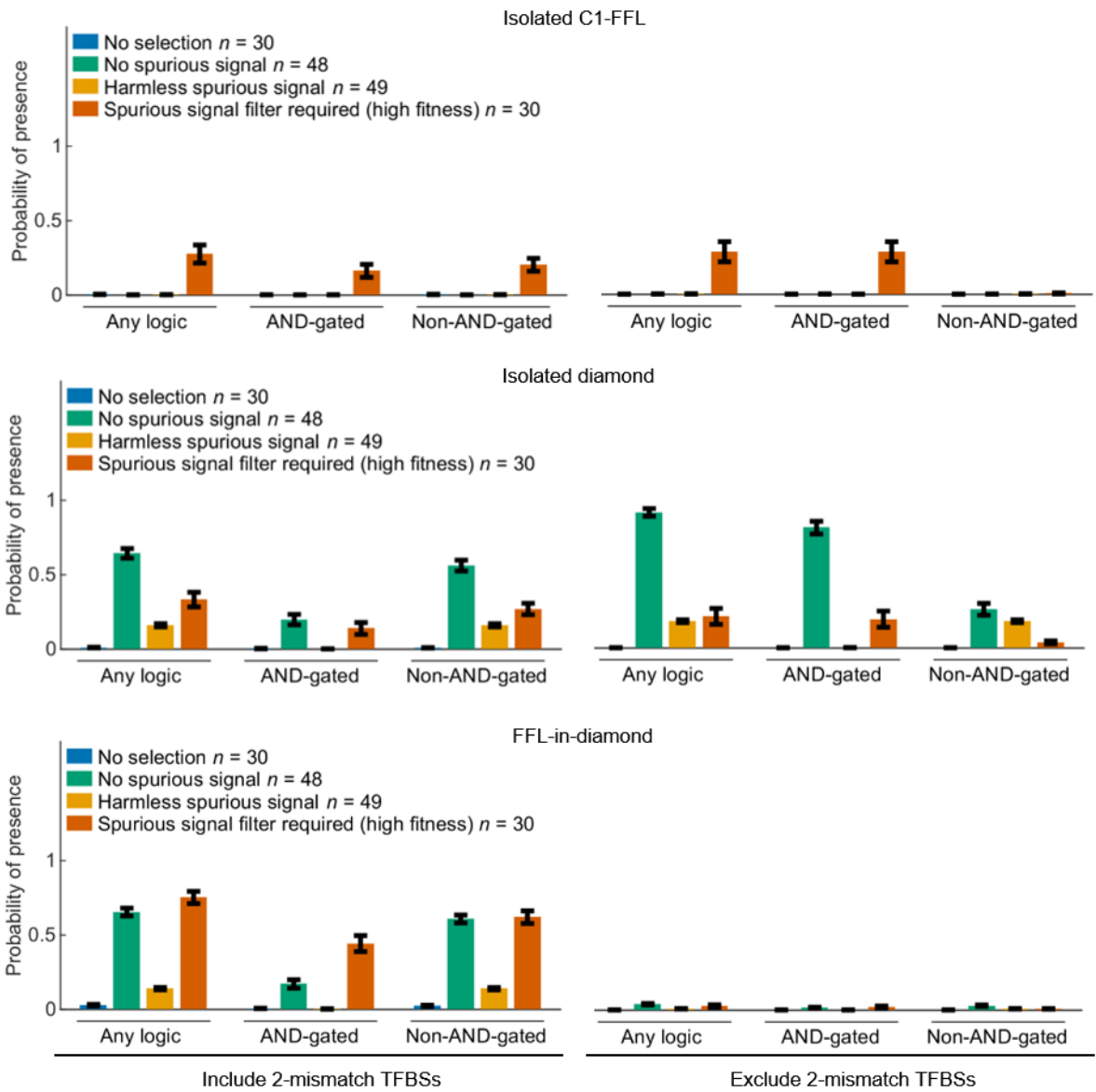
Supplementary Figure 8.



Supplementary Figure 9.



Supplementary Figure 10.



Supplementary Figure 11.

