Supporting Information:

Fourteen Ways to Reroute Cooperative Communication in the Lactose Repressor: Engineering Regulatory Proteins with Alternate Repressive Functions

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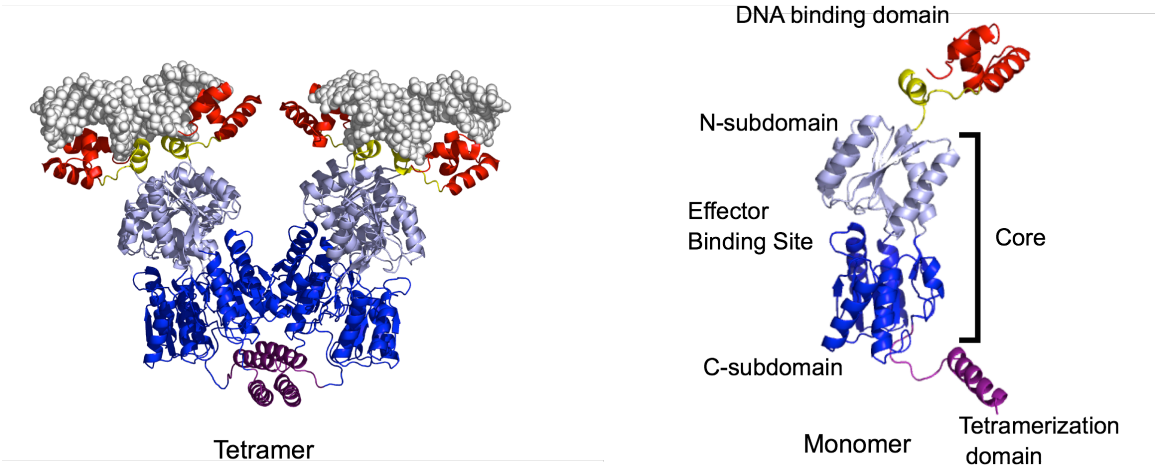

Figure S1: Structure of Tetrameric and Monomeric Lactose Repressor (LacI). The different structural units of LacI are labeled as follows: Helix turn helix motif (red), linker region (yellow), N-subdomain (light blue), C-subdomain (dark blue), and tetramerization domain (purple). The fold of the core domain is highly conserved across the periplasmic binding protein family. The tetrameric LacI is shown binding two stretches of operator DNA (white).

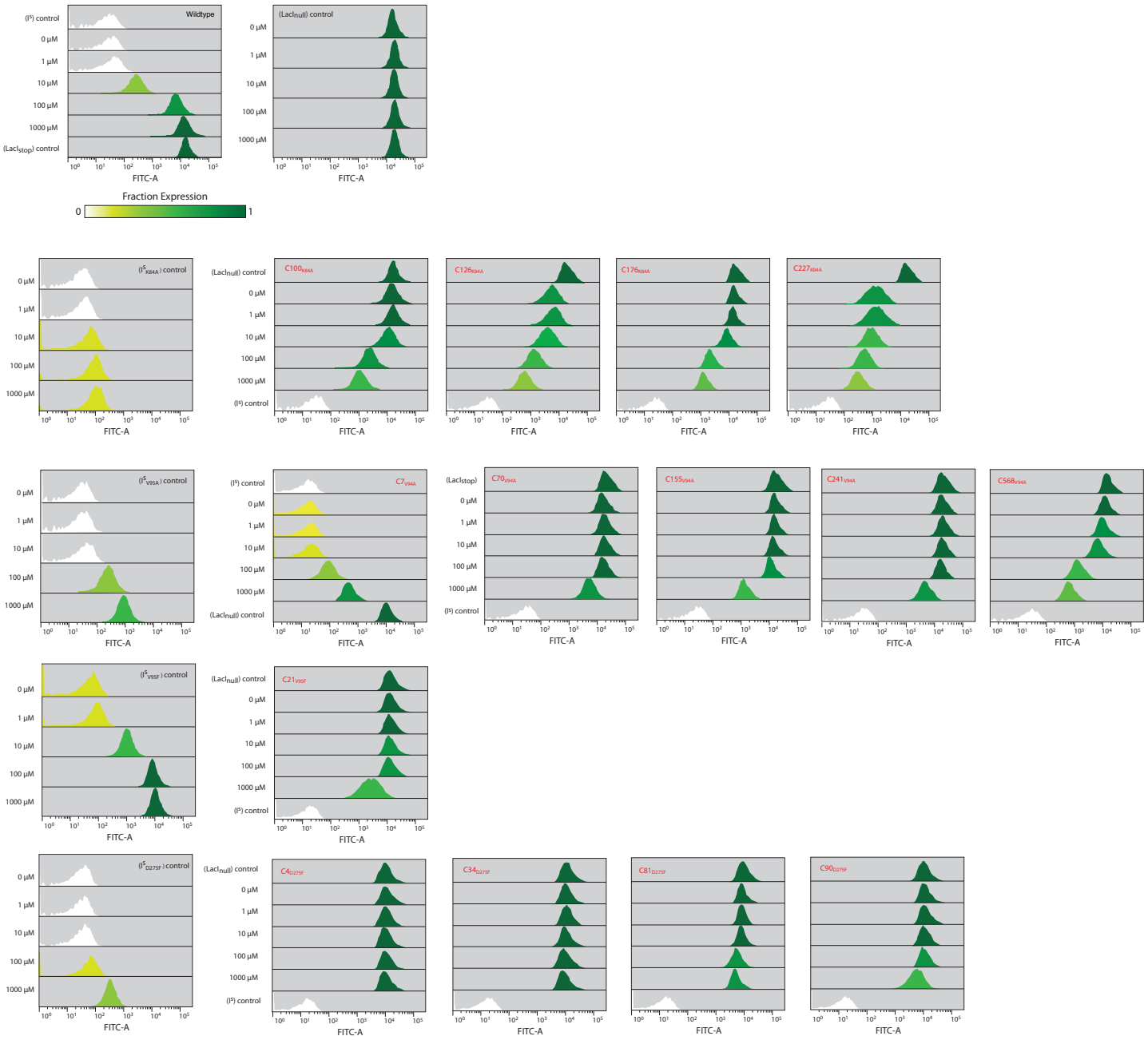

Figure S2: Flow cytometry data for a representative repressor (wildtype) and anti-lacs. Each set of offset histograms shows the level of GFP expression as a function of IPTG. All sets of histograms are appended with expression data for an I^S parent and negative control to show the relative ranges of GFP expression.

Table S1: Function of LacI variants- superfolder GFP expression in solution

Sample	Min Expression	Max Expression	Relative Dynamic Range (%)
WT	23 (0.13%)	14030 (82%)	-
I^s_{K84A}	24.2 (0.14%)	110 (0.65%)	0.62
I^s_{V95A}	74.3 (0.44%)	1003 (5.9%)	6.67
I^s_{V95F}	51.5 (0.30%)	11247 (66%)	80.25
I^s_{D275F}	25.1 (0.15%)	379 (2.2%)	2.50
$C100_{K84A}$	1234 (7.3%)	16544 (97%)	109.56
$C126_{K84A}$	696 (3.3%)	6023 (29%)	31.39
$C176_{K84A}$	1581 (7.6%)	17218 (82%)	90.88
$C227_{K84A}$	373 (1.8%)	1785 (8.5%)	8.18
$C7_{V95A}$	52.4 (0.18%)	1800 (6.1%)	7.23
$C70_{V95A}$	5096 (24%)	15310 (73%)	59.85
$C155_{V95A}$	1565 (7.5%)	19430 (93%)	104.43
$C241_{V95A}$	4894 (23%)	18716 (90%)	81.84
$C568_{V95A}$	816 (4.8%)	15595 (92%)	106.51
$C21_{V95F}$	4011 (26%)	31341 (100%)	90.39
$C81_{D275F}$	18189 (53%)	26263 (87%)	41.53
$C90_{D275F}$	11954 (41%)	28179 (96%)	67.18

Table S2: % Discrete Differences in the Dynamic Range

IPTG rang	0-1μM	1-10μM	10-100μM	100-1000μM
WT	0.081	1.90	52.94	45.08
$C100_{K84A}$	6.21	-32.12	-61.57	-6.32
$C126_{K84A}$	16.70	-42.30	-44.61	-13.09
$C176_{K84A}$	-6.25	-39.07	-48.04	-6.64
$C227_{K84A}$	9.14	-52.83	-30.76	-16.41
$C7_{V95A}$	-0.31	1.23	17.36	81.41
$C70_{V95A}$	16.31	6.08	-19.50	-80.50
$C155_{V95A}$	-5.016	-5.58	-21.99	-67.41
$C241_{V95A}$	9.30	-8.06	-14.76	-77.18
$C568_{V95A}$	10.41	-14.24	-78.98	-6.78
$C21_{V95F}$	0	0	-6.63	-93.37
$C81_{D275F}$	-10.06	-10.06	-72.12	-7.75
$C90_{D275F}$	0	-2.15	-5.37	-92.48

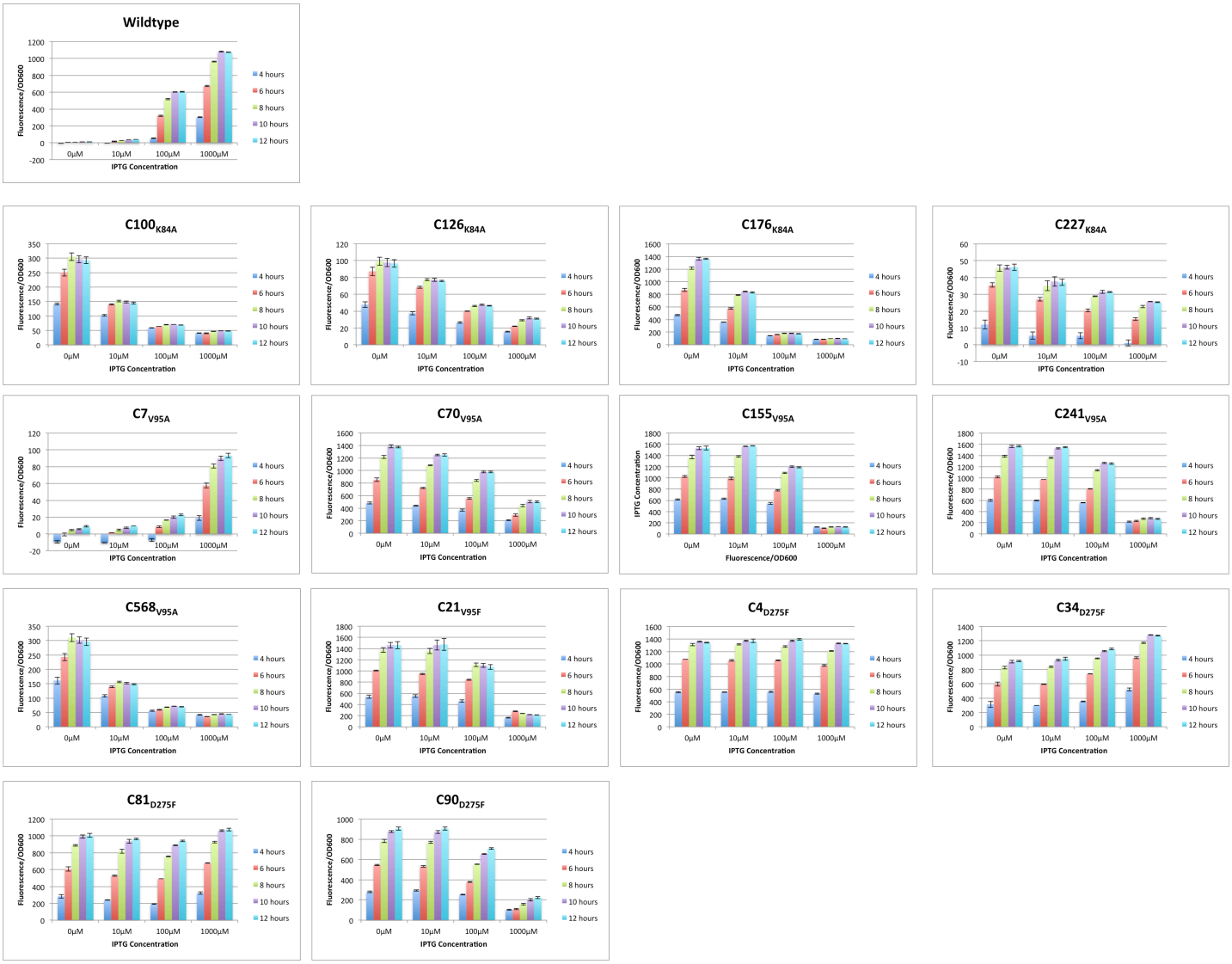

Figure S3: Microplate assay in solution data for a representative repressor (wildtype) and anti-lacs. Each set of histograms shows the level of GFP expression in solution in the presents of IPTG (*i.e.*, at 0,1,10, 100 and 1000μM) at different time points (i.e., 4, 6, 8, 10 and 12 hours).

ADDITIONAL METHODS:

Western Blotting Analysis: For each sample, a fresh 5mL culture of 3.3 cells was grown overnight and then pelleted and resuspended in 50µL of 1X SDS loading dye with 25mM fresh β-mercaptoethanol. The cells were then lysed by being placed in a boiling water bath for 7 minutes. The lysates were centrifuged for 10min and 5µL of supernatant was used for a 1/10X dilution in fresh SDS loading dye. Each sample was loaded onto a Bis-Tris NuPAGE® Novex® gel (Life Technologies), along with a purified wildtype repressor protein control, and then transferred to a 0.2µm nitrocellulose membrane. The blot was then incubated for 1 hour with PBS + 5% milk, then monoclonal anti-LacI antibodies (Millipore, clone 9A5), and then goat anti-mouse HRP conjugate antibodies (Millipore) with triplicate washing with PBS between every step. The blot was then visualized using Amersham ECL Prime Western detection kit (GE) and detected with Image Quant 350 gel docker (GE).

Tetramer and Dimer IPTG Sensitivity Screening: Sensitivity screens were conducted by spot plating 5 colonies of each sample onto 4 different screening conditions containing S-Gal with 0, 1, 10, or 100µM IPTG and then incubating for 4 hours at 37°C. As in the directed evolution screens, each sensitivity screen was accompanied by colonies with the wildtype repressor, non-inducible variant, and negative control variant. The possible phenotypes were binned into 4 categories consisting of colorless, light grey, partially black, and pure black. On every plate, the non-inducible and negative controls set the standards for the colorless and pure black phenotypes, respectively. Every sample colony was scored relative to the controls at 24 hours and 48 hours after spot plating. The final phenotype for a given sample, time, and IPTG concentration was determined by averaging the scores across all colonies for that sample. Using 4 different scoring bins, each sample's phenotype was generally consistent across colonies on a given plate and between multiple screens. The dimer versions of each sample were generated by truncating the C-terminal tetramerization domain helix with a premature stop codon 11 amino acids before the end of the gene [1]. The sequences confirmed and each dimer sample was screened using the same method as above.

Fluorescence Time Course Microplate Assays*:* Overnight bacterial cultures were grown as described in the "Growth and Flow Cytometry" section of the Methods. The cultures were diluted 100-fold in M9 media containing either 0, 10, 100, or 1000µM IPTG. Each sample was aliquoted in triplicate in a black, 96-well plate (Perkin Elmer). The plate was covered with a Breathe-Easier sealing membrane (Diversified Biotech) and grown in a 37°C shaker. After 4 hours, GFP fluorescence (ex: 485/20 nm, em: 528/20 nm) and optical density (OD600) were measured in regular time intervals using a Synergy HT plate reader (BioTek). The fluorescence values were normalized to the optical density and then averaged among all replicates.

1. Chen, J., Matthew, K. S., and Culard, K. S. J. B. C. (1992) Deletion of Lactose Repressor Carboxyl-terminal Domain Affects Tetramer Formation conditions, 13843-13850.