Supplementary Information

Repurposing a bacterial quality control mechanism to enhance enzyme production in living cells

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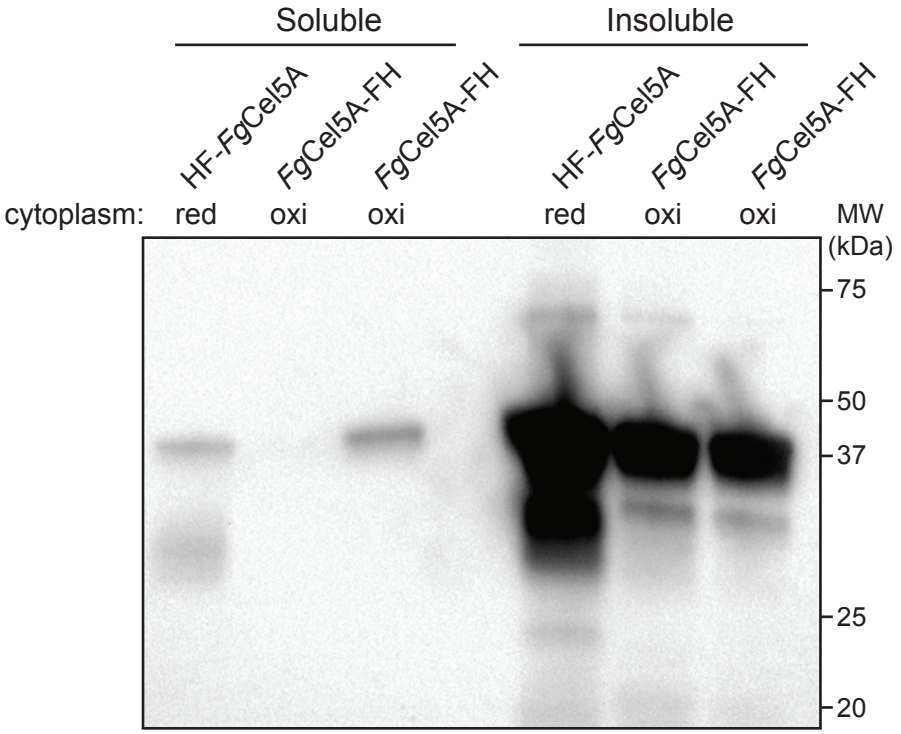
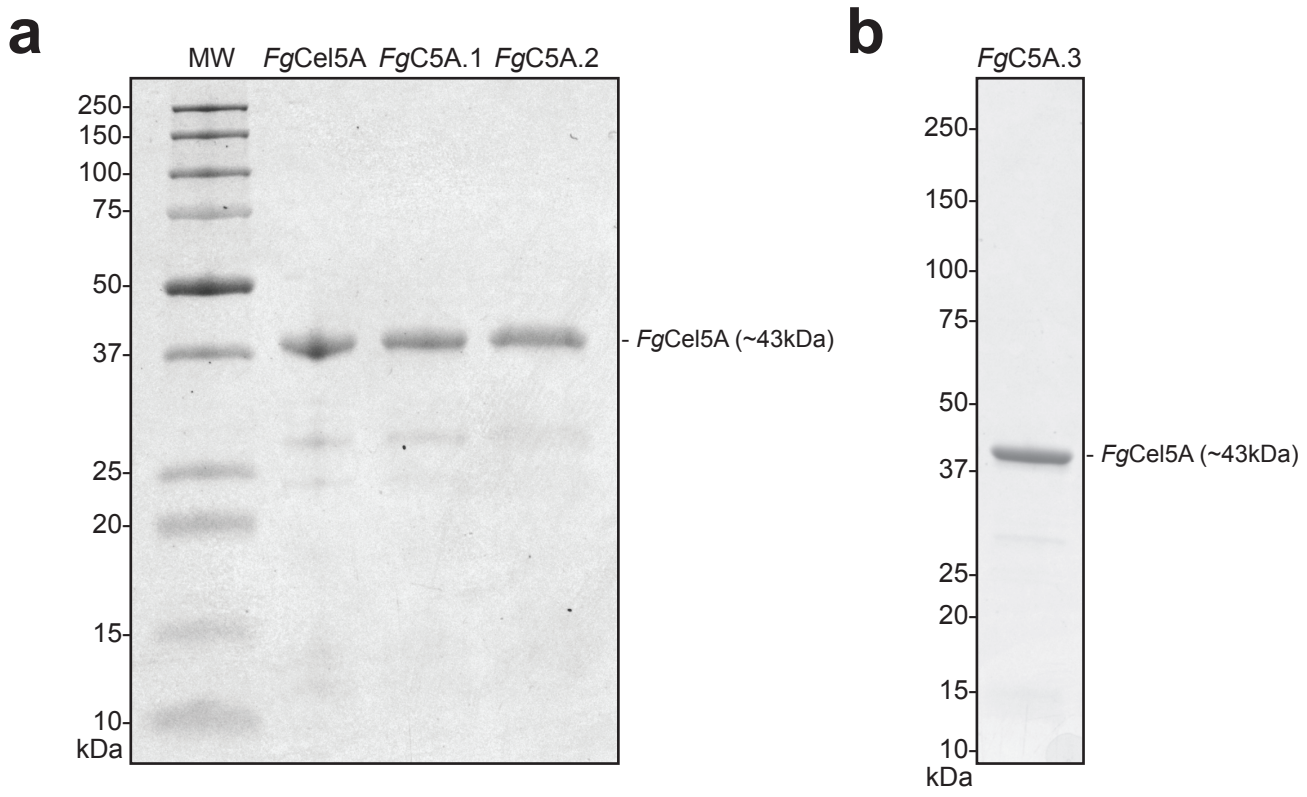
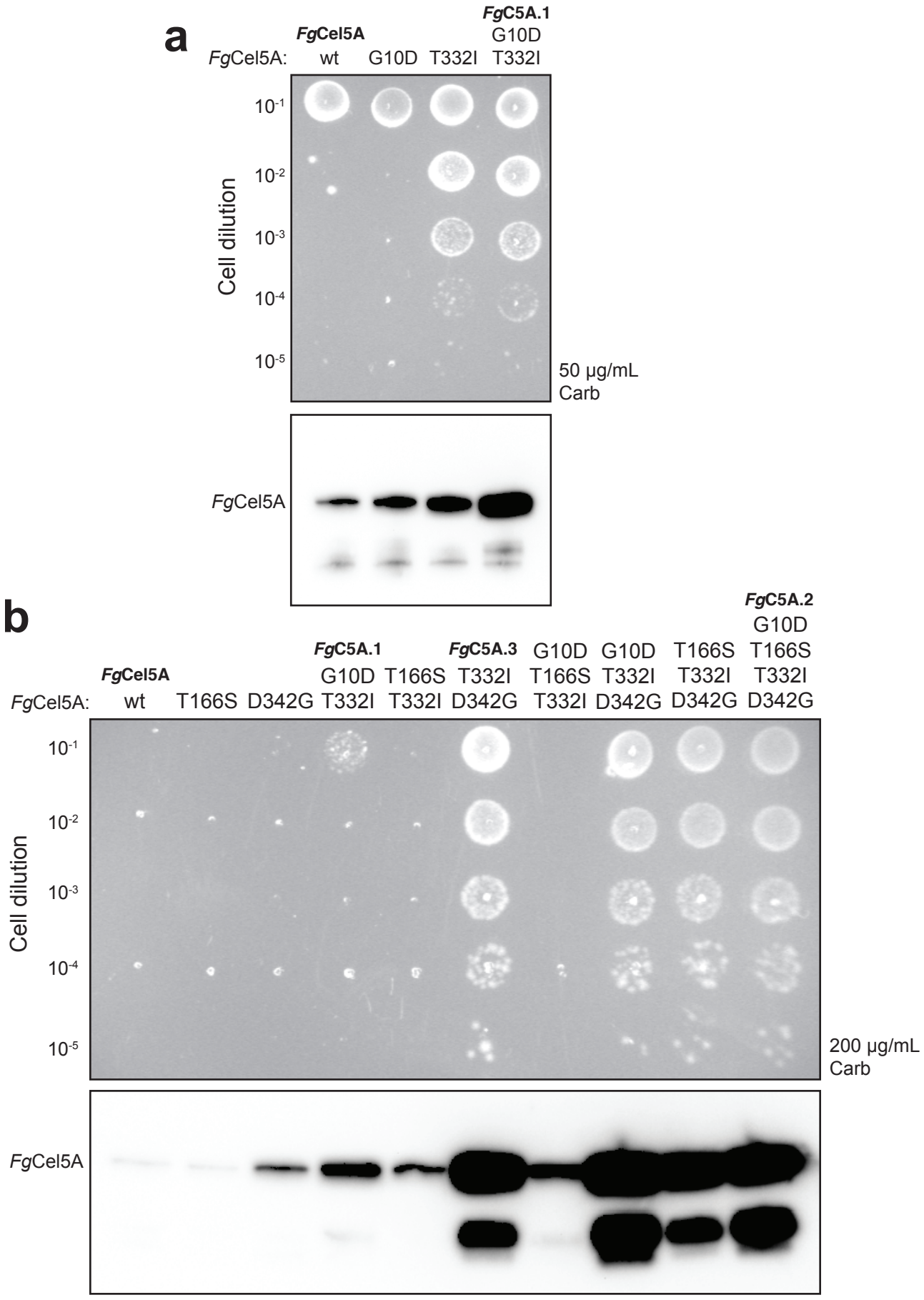


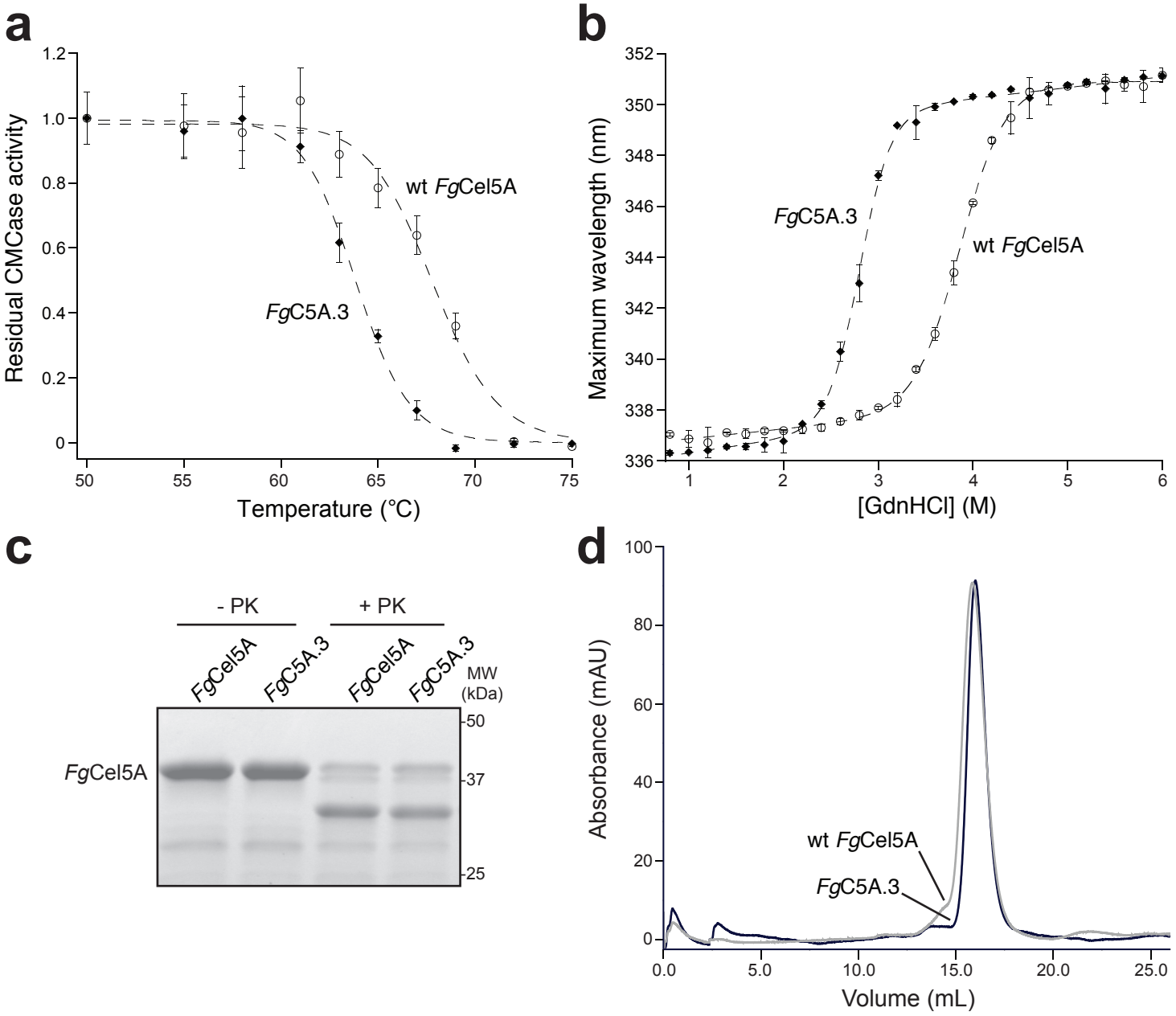
Supplementary Figure S1. Solubility of *Fg*Cel5A in the bacterial cytoplasm. Western blot analysis of soluble and insoluble fractions derived from BL21(DE3) (cytoplasm = reducing; red) or Origami(DE3) (cytoplasm = oxidizing; oxi) cells expressing wt *Fg*Cel5A with N-terminal 6x-His and FLAG tags from pET28a (HF-*Fg*Cel5A) or wt *Fg*Cel5A with C-terminal FLAG and 6x-His tags from pET22b (*Fg*Cel5A-FH). Enzymes were produced in the cytoplasm without the Tat export signal or Bla reporter. Soluble fraction lanes were loaded with 0.5 µg total protein while insoluble fraction lanes contained 0.125 µg total protein. Detection of *Fg*Cel5A was performed using an anti-FLAG antibody. Molecular weight (MW) ladder is marked at right.



Supplementary Figure S2. Purification of *Fg*Cel5A and isolated variants. The wt *Fg*Cel5A enzyme and isolated variants were purified by Ni^{2+}-affinity chromatography, desalted into sodium acetate buffer and concentrated using a centrifugal molecular weight cutoff filter. Protein concentration was determined by BCA assay using a BSA standard. To assess protein purity, a total of 3 µg of purified protein was separated by SDS-PAGE gels and stained with Coomassie blue. Proteins shown include: (a) wt *Fg*Cel5A, *Fg*C5A.1, *Fg*C5A.2, and (b) *Fg*Cel5A.3. Molecular weight (MW) ladder is shown at left.



Supplementary Figure S3. Genetic deconstruction of isolated *Fg*Cel5A variants. Representative spot plating of serially diluted *E. coli* MC4100 cells expressing wt or mutant *Fg*Cel5A enzymes as indicated. Overnight cultures were normalized to an equivalent number of cells, serially diluted in liquid LB, and plated on LB agar supplemented with (a) 50 or (b) 200 µg/mL Carb. Western blot analysis of soluble lysates derived from cells expressing the same wt or mutant *Fg*Cel5A enzymes from pET28a without the Tat export signal or Bla reporter. Each lane was loaded with 3 µg of total protein and blots were probed using anti-FLAG antibody.



Supplementary Figure S4. Biophysical characterization. (a) Thermal stability of wt *Fg*Cel5A (open circle) and *Fg*C5A.3 (closed diamond). Enzymes were incubated at indicated temperatures for 30 min prior to standard reaction with CMC. Residual CMCase activity was calculated by normalizing activity to that at 50°C. Error bars represent propagated error from the standard deviation of three samples. The data is fit using a Hill equation to calculate the T_M where half of the residual activity remains. (b) Thermodynamic stability of wt *Fg*Cel5A (open circle) and *Fg*C5A.3 (closed diamond). Unfolding of the proteins was monitored by tryptophan (280 nm excitation) emission peak shift at increasing GdnHCl concentrations. Denaturation occurred for 1 h at 25°C. Fluorescence emission spectra were recorded at 1-nm intervals between 320-370 nm after excitation at 280 nm using a scan rate of 10 nm per second using a fluorometer (QuantaMaster PTI); slit width was set at ± 3 nm. Maximum peak emission was determined through curve fitting the emission spectra from 320 nm to 370 nm with a third-order Taylor series expansion around the maximum wavelength as described previously (Monsellier and Bedouelle, 2005 *Protein Eng Des Sel*). Fluorescence maxima were plotted as a function of denaturant and directly fit using a six-parameter fit described elsewhere (Pace, 1990 *Trends Biotechnol*) to determine the Gibb's free energy for each protein. All curve fitting was done using KaleidaGraph. Error bars are the standard deviation of three samples. (c) Protease susceptibility of wt *Fg*Cel5A and *Fg*C5A.3. Samples were treated with 10 ng/µL Proteinase K for 5 min at 37°C (+PK) and compared to untreated controls (-PK). Reactions were quenched by the addition of 5 µM phenylmethanesulfonyl fluoride (PMSF). Proteolytic degradation was visualized by separating proteins on an SDS-PAGE gel and staining the gel with Coomassie blue. (d) Oligomerization of wt *Fg*Cel5A (light grey) and *Fg*C5A.3 (dark grey). Absorbance profile was created by separating 100 µg purified enzymes by size exclusion chromatography using a Superdex 75 column.

Supplementary Table S1. SAXS parameters, structural parameters and fitting software.

Data-collection parameters	
Instrument:	MacCHESS (Cornell)
Beam line:	G1 hutch
Detector:	Dual Pilatus 100K-S SAXS/WAXS
Exposure time (s):	2
Concentration range (mg/mL):	40-0.1
q Range[1] ($Å^{-1}$):	0.0172-0.2612
Buffer:	50 mM sodium acetate (pH 5)
Structural parameters[1]	
I(0) (cm^{-1}) [from (P(r))]:	0.520 ± 0.010
R_g (Å) [from (P(r))]:	30.69 ± 0.73
I(0) (cm^{-1}) [from Guinier fit]:	0.531 ± 0.005
R_g (Å) [from Guinier fit]:	30.63 ± 3.58
D_{max} (Å)	106
Fitting error[1,2]	
χ^2:	0.110
Software employed	
Data averaging and subtraction:	RAW
Data processing:	Primus (ATSAS)
Envelope generation:	DAMMIF
3-D representation:	PyMol

[1]For a 1.28 mg/mL sample of *Fg*C5A.3.
[2]From the DAMMIF fit.

Supplementary Table S2. Library focusing by Tat-based QC selection

Selective pressure		Percentage of selected library members[1]		Selectivity[2]
	[Carb] (µg/mL)	Highly active	Active	$CFU_{neutral}/CFU$
No	0	4.4	37.4	-
Neutral	25	9.9	44.0	1
Moderate[3]	50	6.9	42.5	4
High	100	3.3	9.8	>10

[1]CMCase activity of solubility-focused clones was determined by clearance halos on LB agar supplemented with CMC. Highly active was defined as having similar or greater clearance halos than wt *Fg*Cel5A on at least two of three plates. Active was defined as having clearance above background but less than wt *Fg*Cel5A on at least two of three plates.
[2]Library focusing was determined by normalizing the CFUs at the given selective pressure with the CFUs at neutral selective pressure; a value could not be obtained for the case of no selective pressure due to the plasmid-encoded marker being used to provide resistance.
[3]Selective pressure used earlier in the paper to isolate *Fg*C5A.1.