Supporting Information

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SI Methods

Western Blot Analysis. RecZFs were analyzed using SDS/PAGE through a NuPAGE Novex 10% Bis-Tris Gel (Invitrogen). Samples for immunoblot analysis were transferred onto a 0.2-μM nitrocellulose membrane and incubated for 2 h in 1× TBS/3% BSA. Antibodies for immunoblotting were diluted to 1:2,000 [conjugated anti-HA horseradish peroxidase antibody (Roche)]. Membranes were washed with 1× TBS and 1× TBS/0.05% Tween20 and were visualized using the enhanced chemiluminescence system (Amersham Biosciences). The internal loading control was a conjugated anti-GAPDH horseradish peroxidase antibody (Sigma).

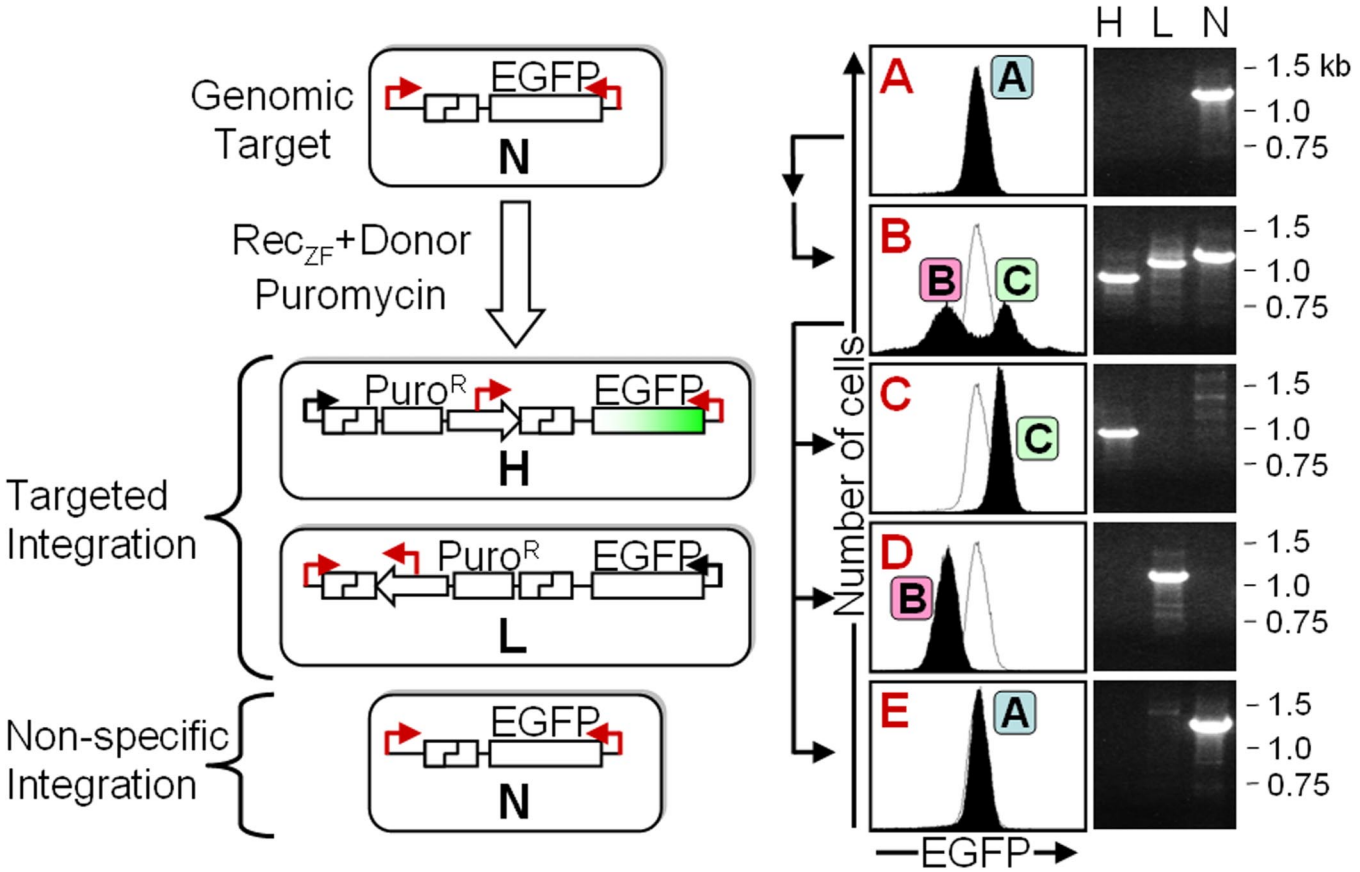

Fig. S1. Clonal analysis of cellular fluorescence and genotype after plasmid integration. (*A*) The basal level of fluorescence from the naïve GinC5 genomic target locus (N). (*B*) Stable integration of plasmid resulted in up- and down-regulation of fluorescence in the bulk population of puromycin-resistant cells. Clones isolated from this bulk population showed higher (*C*), lower (*D*), or unchanged (*E*) levels of EGFP fluorescence relative to that of naïve cells. Genomic PCR confirmed that each phenotype matched the expected genotype at the genomic target locus: EGFP(+) (H; 0.9 kb), EGFP(−) (L; 1.1 kb), and naive (N; 1.2 kb).

Table S1. Hyperactive serine recombinases and their minimal substrates

Serine recombinase	Hyperactiving mutation(s)	Substrate DNA sequence (20-bp core)
Cin	Y106H	TTCTCTTAAACCAAGGTTTAGGATTG
Gin	Y106H	TTATCCAAAACCTCGGTTTACAGGAA
Hin	Y107H	GTTCTTGAAAACCAAGGTTTTTGATAAA
Sin	I77T	TGTGAAATTTGGGTACACCCTAATCATACA
Tn3	D102Y, E124Q	TGTCTGATAATTTATAATATTTCGAACG

Mutations that release serine recombinases from native regulatory regimes allow them to catalyze unrestricted recombination between minimal dimer binding sites. These hyperactivating mutations are readily selected and have been found for several members (1–5) of the large and diverse serine recombinase family (6).

1. Arnold PH, Blake DG, Grindley ND, Boocock MR, Stark WM (1999) Mutants of Tn3 resolvase which do not require accessory binding sites for recombination activity. *EMBO J* 18:1407–1414.
2. Haykinson MJ, Johnson LM, Soong J, Johnson RC (1996) The Hin dimer interface is critical for Fis-mediated activation of the catalytic steps of site-specific DNA inversion. *Curr Biol* 6:163–177.
3. Klippel A, Cloppenborg K, Kahmann R (1988) Isolation and characterization of unusual gin mutants. *EMBO J* 7:3983–3989.
4. Rowland SJ, Boocock MR, Stark WM (2005) Regulation of Sin recombinase by accessory proteins. *Mol Microbiol* 56:371–382.
5. Haffter P, Bickle TA (1988) Enhancer-independent mutants of the Cin recombinase have a relaxed topological specificity. *EMBO J* 7:3991–3996.
6. Smith MC, Thorpe HM (2002) Diversity in the serine recombinases. *Molecular microbiology* 44:299–307.

Table S2. Amino acid sequences of the zinc finger-recombinases

RecZF	Amino Acid Sequence
GinC1	MLIGYVRVSTNDQNTDLQRNALVCAGCEQIFEDKLSGTRTDRPGLKRALKRLQKGDTLVVWKLDRLGRSMKHLISLVGELRERGINFRSLTDSIDTSSPMG RFFFYVMGALAEMERELIIERTMAGLAAARNKGRIGGRPPKSGSGEMPYKCPECGKSFSRSDELVRHQRTHTGKKTSGQAGQ
GinC2	MLIGYVRVSTNDQNTDLQRNALVCAGCEQIFEDKLSGTRTDRPGLKRALKRLQKGDTLVVWKLDRLGRSMKHLISLVGELRERGINFRSLTDSIDTSSPMG RFFFYVMGALAEMERELIIERTMAGLAAARNKGRIGGRPPKSGSGEMPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSDPGHLVRHQRT HTGKKTSGQAGQ
GinC3	MLIGYVRVSTNDQNTDLQRNALVCAGCEQIFEDKLSGTRTDRPGLKRALKRLQKGDTLVVWKLDRLGRSMKHLISLVGELRERGINFRSLTDSIDTSSPMG RFFFYVMGALAEMERELIIERTMAGLAAARNKGRIGGRPPKSGSGEMPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSDPGHLVRHQRT HTGEKPYKCPECGKSFSQRAHLERHQRTHTGKKTSGQAGQ
GinC4	MLIGYVRVSTNDQNTDLQRNALVCAGCEQIFEDKLSGTRTDRPGLKRALKRLQKGDTLVVWKLDRLGRSMKHLISLVGELRERGINFRSLTDSIDTSSPMG RFFFYVMGALAEMERELIIERTMAGLAAARNKGRIGGRPPKSGSGEMPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSDPGHLVRHQRT HTGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSRSDDLVRHQRTHTGKKTSGQAGQ
GinC5	MLIGYVRVSTNDQNTDLQRNALVCAGCEQIFEDKLSGTRTDRPGLKRALKRLQKGDTLVVWKLDRLGRSMKHLISLVGELRERGINFRSLTDSIDTSSPMG RFFFYVMGALAEMERELIIERTMAGLAAARNKGRIGGRPPKSGSGEMPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSDPGHLVRHQRT HTGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSRSDDLVRHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGKKTSGQAGQ
GinC6	MLIGYVRVSTNDQNTDLQRNALVCAGCEQIFEDKLSGTRTDRPGLKRALKRLQKGDTLVVWKLDRLGRSMKHLISLVGELRERGINFRSLTDSIDTSSPMG RFFFYVMGALAEMERELIIERTMAGLAAARNKGRIGGRPPKSGSGEMPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSDPGHLVRHQRT HTGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSRSDDLVRHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGK SFSQSGDLRRHQRTHTGKKTSGQAGQ
Tn3C5	MRIFGYARVSTSQQSLDIQIRALKDAGVKANRIFTDKASGSSTDREGLDLLRMKVEEGDVILVKKLDRLSRDTADMIQLIKEFDAQGVAVRFIDDGISTDGY MGQMVVTILSAVAQAERRRILQRTNEGRQEAKLKGIKFGRRRTSGSGEMPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSDPGHLVRHQ RTHTGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSRSDDLVRHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGKKTSGQAGQ

Zinc finger domains (1) were fused to the C-terminus of both Gin and Tn3 hyperactive catalytic domains (2, 3) using a short flexible linker (SGSGEMP) (3). The hyperactive catalytic domains were PCR amplified using a 3′ primer encoding the F1 zinc finger domain (GTG). This fusion product was subsequently digested (AgeI + SpeI) so that 3′ zinc finger domains F2 through F6 (GGC, GGA, GCG, GTG, GCA; each digested XmaI + SpeI) could be iteratively inserted to generate a series of RecZFs of increasing length (Table 1). More details regarding the assembly of zinc finger fusion proteins are available online at www.zincfingertools.org (4).

1. Dreier B, Segal DJ, Barbas CF, III (2000) Insights into the molecular recognition of the 5′-GNN-3′ family of DNA sequences by zinc finger domains. *J Mol Biol* 303:489–502.
2. Klippel A, Cloppenborg K, Kahmann R (1988) Isolation and characterization of unusual gin mutants. *EMBO J* 7:3983–3989.
3. Gordley RM, Smith JD, Graslund T, Barbas CF, III (2007) Evolution of programmable zinc finger-recombinases with activity in human cells. *J Mol Biol* 367:802–813.
4. Mandell JG & Barbas CF, 3rd (2006) Zinc Finger Tools: custom DNA-binding domains for transcription factors and nucleases. *Nucleic Acids Res* 34:W516–W523.