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Supplementary Materials for

Redesign of the monomer-monomer interface of Cre recombinase yields an obligate heterotetrameric complex

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Supplemental Methods

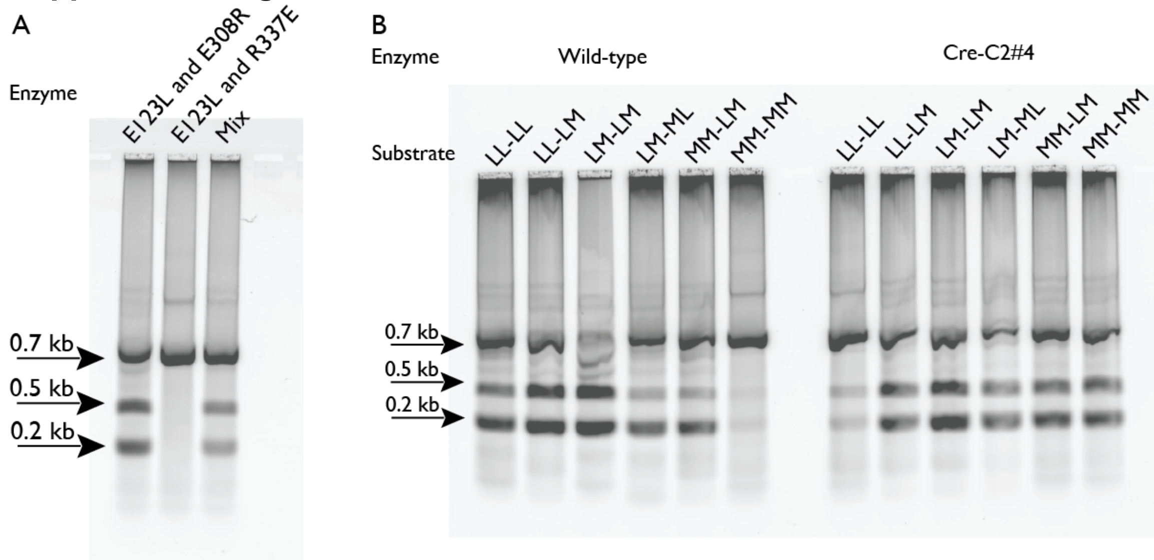
Protein purification of Cre recombinase variants. Proteins were expressed in BL21(DE3) star cells at 25°C using the autoinduction protocol of Studier {Studier, 2005, Protein Expr Purif, 41, 207-234}. The cells were harvested by centrifugation after 48 hours. The cell paste was resuspended in 25mL buffer A (0.7M NaCl, 50mM Tris-HCl pH7.8, 5mM Imidazole), lysed by sonication on ice, and separated from cellular debris by centrifugation. The filtered supernatant was applied to a HisTrap™ HP column (Amersham) and washed with 30mL Buffer A. The column was then washed with 20mL 15% buffer B (0.7M NaCl, 50mM Tris-HCl pH7.8, 500mM Imidazole). Cre was eluted with a linear gradient from 15% buffer B to 100% buffer B, with the elution peak starting at roughly 20% buffer B. Approximately 10mL of the eluted protein was collected and dialyzed overnight at 4°C against 5L dialysis buffer (0.7 M NaCl, 50mM Tris-HCl pH7.8). The protein concentration was then determined by UV absorbance using an extinction coefficient at 280nm of 49 $mM^{-1}cm^{-1}$. The protein retained activity for months when stored at 4° C.

Supplemental Table 1. Cell sorting data from mouse ES cells

		Replicate		
		1st	2nd	3rd
total # of cells sorted		7000	7000	7000
Cre-A1	hbb	414	378	391
	hbb+cmv	3852	3528	3687
	hbb+sp1	3750	3419	3501
Cre-B1	hbb	97	102	85
	hbb+cmv	1237	1258	1120
	hbb+sp1	1150	1080	1202
A1+B1	hbb	1117	1212	1324
	hbb+cmv	5866	6029	6358
	hbb+sp1	5702	6121	5987
Cre-A2	hbb	47	52	41
	hbb+cmv	1127	1116	1052
	hbb+sp1	1053	1002	1119
Cre-B2	hbb	0	0	1
	hbb+cmv	2	2	4
	hbb+sp1	2	1	3
A2+B2	hbb	573	528	607
	hbb+cmv	3180	3409	3698
	hbb+sp1	3221	3336	3593
Cre-A3	hbb	0	0	1
	hbb+cmv	0	1	1
	hbb+sp1	1	0	0
A3+B2	hbb	256	233	284
	hbb+cmv	1598	1652	1701
	hbb+sp1	1503	1527	1606
WT	hbb	372	391	408
	hbb+cmv	3914	4223	4312
	hbb+sp1	3815	3799	4021

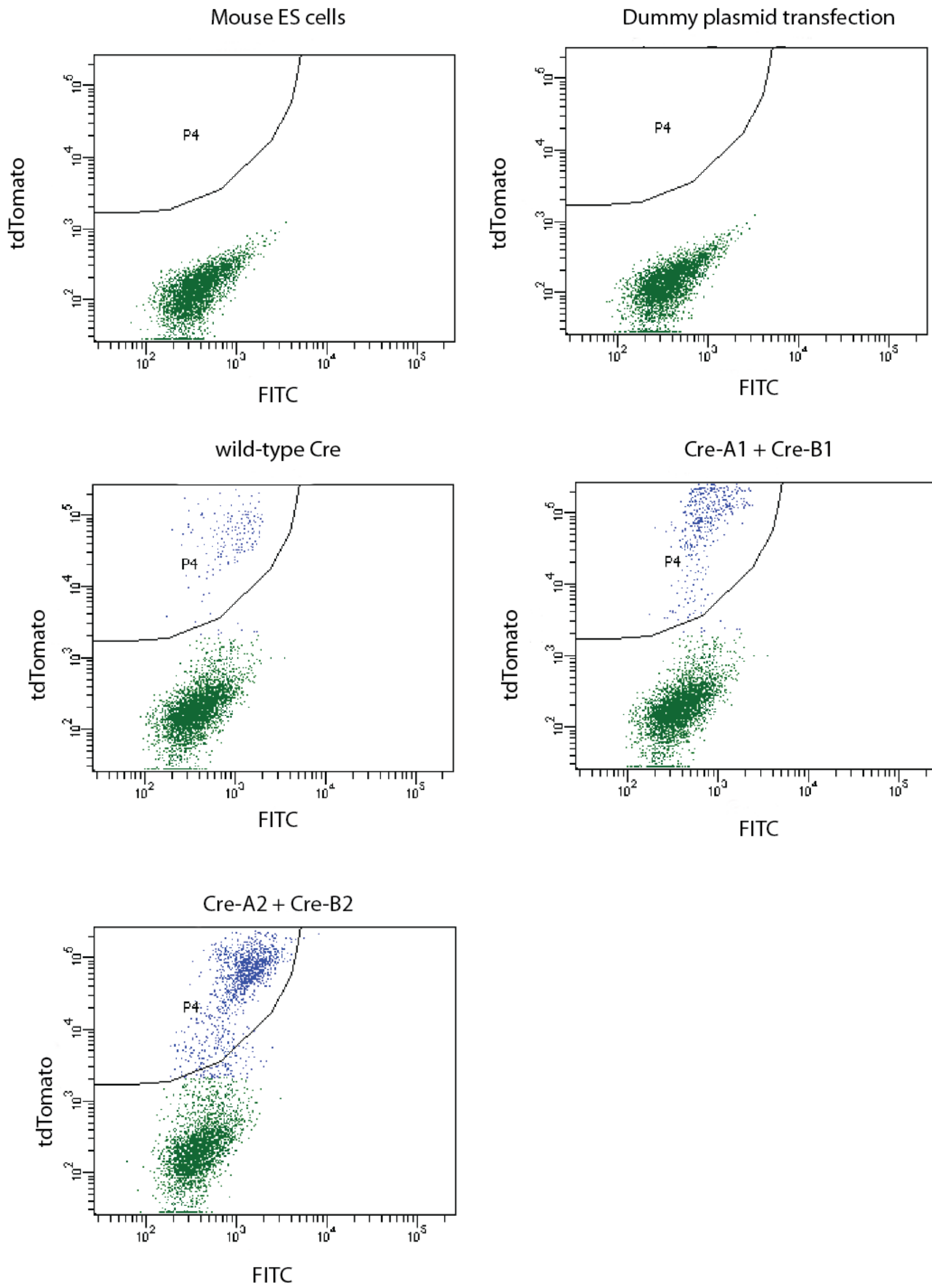
Plasmids with the hbb minimal promoter alone or with either the cmv and sp1 enhancers driving different cre variants were co-transfected into Ai14 mouse embryonic stem (ES) cells containing a reporter cassette with tdTomato preceded by a floxed stop codon. The same total amount of DNA was used for all transfections, and 3 independent transfections were performed for each Cre variant. The number of tdTomato positive cells was measured by flow cytometry.

Supplemental Figure 1.



Supplemental Figure 1 Legend. (A) *In vitro* assay results for Cre mutant pairs lacking computationally designed mutations. Linear DNA substrate (0.7 kb) with direct loxP repeats was incubated with Cre mutants. Lane 1: Cre-E123L/E308R; lane 2: Cre-E123L/R337E; lane 3: A 1:1 mixture of above two Cre mutants. The E123L/E308R mutations are insufficient to eliminate activity in this monomer, indicating that additional mutations are necessary to achieve the goal of obligate heterotetramers. (B) *In vitro* assay results for Cre proteins with wild-type monomer-monomer interfaces. Wild-type Cre and Cre-C2#4 were assayed for recombination activity against six loxP/M7 hybrid RT sites. The left panel: wild-type Cre recombined robustly on all six RT sites except for all M7 site. The right panel: Cre-C2#4 recombined all six RT sites, although with diminished activity with increased number of loxP half-sites.

Supplemental Figure 2.



Supplemental Figure 2 Legend. Representative raw data from flow sorting experiments. Each point shows the fluorescence in the red channel (tdTomato)

versus green channel (FITC). The cell-only and dummy plasmid experiments exhibit roughly identical autofluorescence. The gating for identifying RFP-positive is the region of each plot labeled ‘P4’.