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Article

An efficient screening system in yeast to select a hyperactive *piggyBac* transposase for mammalian applications

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Supplementary Materials

Table S1. The sequence of transposases and promoter

Transposases / promoter	Nucleotide sequence
WT PBase	atgggtagttctttagacgatgagcatatcctctctgctcttctgcaaagcgatgacgagcttgttggtgaggattctgacag tgaaatatcagatcacgtaagtgaagatgacgtccagagcgatacagaagaagcgtttatagatgaggtacatgaagtg cagccaacgtcaagcggtagtgaaatattagacgaacaaaatgttattgaacaaccaggttcttcattggcttctaacaga atcttgaccttgccacagaggactattagaggtaagaataaacattgttggtcaacttcaaagtccacgaggcgtagccga gtctctgcactgaacattgtcagatctcaaagaggtccgacgcgtatgtgccgcaatatatatgacccacttttatgcttcaa actatttttttactgatgagataatttcggaaattgtaaaatggacaaatgctgagatatcattgaaacgtcgggaatctatga caggtgctacatttcgtgacacgaatgaagatgaaatctatgctttctttggtattctggtaatgacagcagtgagaaaaga taaccacatgtccacagatgacctctttgatcgatctttgtcaatggtgtacgtctctgtaatgagtcgtgatcgttttgatttttt gatacgatgtcttagaatggatgacaaaagtatacggcccacacttcgagaaaacgatgtatttactcctgttagaaaaat atgggatctctttatccatcagtgcatacaaaattacactccaggggctcatttgaccatagatgaacagttacttggttttag aggacggtgtccgtttaggatgtatatcccaaacaagccaagtaagtatggaataaaaatcctcatgatgtgtgacagtg gtacgaagtatatgataaatggaatgccttatttgggaagaggaacacagaccaacggagtaccactcggtgaatactac gtgaaggagttatcaaagcctgtgcacggtagttgtcgtaatattacgtgtgacaattggttcacctcaatccctttggcaaa aaacttactacaagaaccgtataagttaaccattgtgggaaccgtgcgatcaaacaaacgcgagataccggaagtactga aaaacagtcgctccaggccagtgggaacatcgatgttttgttttgacggacccccttactctcgtctcatataaaccgaagcc agctaagatggtatacttattatcatcttgtgatgaggatgcttctatcaacgaaagtaccggtaaaccgcaaatggttatgt attataatcaaactaaaggcggagtggacacgctagaccaaatgtgttctgtgatgacctgcagtaggaagacgaatag gtggcctatggcattattgtacggaatgataaacattgcctgcataaattcttttattatatacagccataatgtcagtagcaa gggagagaaggttcaaagtcgcaaaaaatttatgagaaacctttacatgagcctgacgtcatcgtttatgcgtaagcgttt agaagctcctactttgaagagatatttgcgcgataatatctctaatattttgccaaatgaagtgcctggtacatcagatgaca gtactgaagagccagtaatgaaaaaacgtacttactgtacttactgcccctctaaaataaggcgaaaggcaaatgcatcgt gcaaaaaatgcaaaaaagttatttgtcgagagcataatattgatatgtgccaaagttgtttctgataa
Optimized hyPBase	atgggcagcagcctggacgacgagcacatcctgagcgccctgctgcagagcgacgacgagctggtgggcgaggaca gcgacagcgaggtgagcgaccacgtgagcgaggacgacgtgcagagcgacaccgaggaggccttcatcgacgaggt gcacgaggtgcagcccaccagcagcggcagcgagatcctggacgagcagaacgtgatcgagcagcccggcagcagc ctggccagcaaccgcatcctgaccctgccccagcgcaccatccgcggcaagaacaagcactgctggagcaccagcaag cccacccgccgcagccgcgtgagcgccctgaacatcgtgcgcagccagcgcggccccacccgcatgtgccgcaacatct acgacccccctgctgtgcttcaagctgttcttcaccgacgagatcatcagcgagatcgtgaagtggaccaacgccgagatca gcctgaagcgccgcgagagcatgaccagcgccaccttccgcgacaccaacgaggacgagatctacgccttcttcggcat cctggtgatgaccgccgtgcgcaaggacaaccacatgagcaccgacgacctgttcgaccgcagcctgagcatggtgtac gtgagcgtgatgagccgcgaccgcttcgacttcctgatccgctgcctgcgcatggacgacaagagcatccgccccaccct gcgcgagaacgacgtgttcacccccgtgcgcaagatctgggacctgttcatccaccagtgcatccagaactacacccccg

	gcgcccacctgaccatcgacgagcagctgctgggcttccgcggccgctgccccttccgcgtgtacatccccaacaagccc agcaagtacggcatcaagatcctgatgatgtgcgacagcggcaccaagtacatgatcaacggcatgccctacctgggcc gcggcacccagaccaacggcgtgcccctgggcgagtactacgtgaaggagctgagcaagcccgtgcacggcagctgc cgcaacatcacctgcgacaactggttcaccagcatcccccctggccaagaacctgctgcaggagccctacaagctgaccat cgtgggcaccgtgcgcagcaacaagcgcgagatccccgaggtgctgaagaacagccgcagccgccccgtgggcacca gcatgttctgcttcgacggccccctgaccctggtgagctacaagcccaagcccgccaagatggtgtacctgctgagcagct gcgacgaggacgccagcatcaacgagagcaccggcaagccccagatggtgatgtactacaaccagaccaagggcgg cgtggacaccctggaccagatgtgcagcgtgatgacctgcagccgcaagaccaaccgctggcccatggccctgctgtac ggcatgatcaacatcgcctgcatcaacagcttcatcatctacagccacaacgtgagcagcaagggcgagaaggtgcaga gccgcaagaagttcatgcgcaacctgtacatgggcctgaccagcagcttcatgcgcaagcgcctggaggccccacccct gaagcgctacctgcgcgacaacatcagcaacatcctgcccaaggaggtgcccggcaccagcgacgacagcaccgagg agcccgtgatgaagaagcgcacctactgcacctactgccccagcaagatccgccgcaaggccagcgccagctgcaaga agtgcaagaaggtgatctgccgcgagcacaacatcgacatgtgccagagctgcttctaa
Optimized WT PBase	atgggcagcagcctggacgacgagcacatcctgagcgccctgctgcagagcgacgacgagctggtgggcgaggaca gcgacagcgagatcagcgaccacgtgagcgaggacgacgtgcagagcgacaccgaggaggccttcatcgacgaggt gcacgaggtgcagcccaccagcagcggcagcgagatcctggacgagcagaacgtgatcgagcagcccggcagcagc ctggccagcaaccgcatcctgaccctgccccagcgcaccatccgcggcaagaacaagcactgctggagcaccagcaag agcacccgccgcagccgcgtgagcgccctgaacatcgtgcgcagccagcgcggccccacccgcatgtgccgcaacatct acgacccccctgctgtgcttcaagctgttcttcaccgacgagatcatcagcgagatcgtgaagtggaccaacgccgagatca gcctgaagcgccgcgagagcatgaccggcgccaccttccgcgacaccaacgaggacgagatctacgccttcttcggcat cctggtgatgaccgccgtgcgcaaggacaaccacatgagcaccgacgacctgttcgaccgcagcctgagcatggtgtac gtgagcgtgatgagccgcgaccgcttcgacttcctgatccgctgcctgcgcatggacgacaagagcatccgccccaccct gcgcgagaacgacgtgttcacccccgtgcgcaagatctgggacctgttcatccaccagtgcatccagaactacacccccg gcgcccacctgaccatcgacgagcagctgctgggcttccgcggccgctgccccttccgcatgtacatccccaacaagccc agcaagtacggcatcaagatcctgatgatgtgcgacagcggcaccaagtacatgatcaacggcatgccctacctgggcc gcggcacccagaccaacggcgtgcccctgggcgagtactacgtgaaggagctgagcaagcccgtgcacggcagctgc cgcaacatcacctgcgacaactggttcaccagcatcccccctggccaagaacctgctgcaggagccctacaagctgaccat cgtgggcaccgtgcgcagcaacaagcgcgagatccccgaggtgctgaagaacagccgcagccgccccgtgggcacca gcatgttctgcttcgacggccccctgaccctggtgagctacaagcccaagcccgccaagatggtgtacctgctgagcagct gcgacgaggacgccagcatcaacgagagcaccggcaagccccagatggtgatgtactacaaccagaccaagggcgg cgtggacaccctggaccagatgtgcagcgtgatgacctgcagccgcaagaccaaccgctggcccatggccctgctgtac ggcatgatcaacatcgcctgcatcaacagcttcatcatctacagccacaacgtgagcagcaagggcgagaaggtgcaga gccgcaagaagttcatgcgcaacctgtacatgagcctgaccagcagcttcatgcgcaagcgcctggaggccccacccct gaagcgctacctgcgcgacaacatcagcaacatcctgcccaacgaggtgcccggcaccagcgacgacagcaccgagg agcccgtgatgaagaagcgcacctactgcacctactgccccagcaagatccgccgcaaggccaacgccagctgcaaga agtgcaagaaggtgatctgccgcgagcacaacatcgacatgtgccagagctgcttctaa
GALS promoter	ggaagactctcctccgtgcgtcctcgtcttcaccggtcgcgttcctgaaacgcagatgtgcctcgcgccgcactgctccgaa caataaagattctacaatactagcttttatggttatgaagaggaaaaattggcagtaacctggccccacaaaccttcaaatg aacgaatcaaattaacaaccataggatgataatgcgattagttttttagccttatttctggggtaattaatcagcgaagcgat gattttgatctattaacagatatataaatgcaaaaactgcataaccactttaactaatactttcaacattttcggtttgtattact tcttattcaaatgtaataaaagtatcaacaaaaaattgttaatatacctctatactttaacgtcaaggagaaaaaaccccggga t

Table S2. The mutations of candidates

Candidates	Relative activity in yeast	Relative activity in CHO cells	Mutations		
Optimized hyPBase	1	1			
h31-11	9.7	1.19	186, atc→gtc, I186V		
h31-15	10.7	0.66	438, gag→ggg, E438G	458, acc→tcc, T458S	562, gtg→gag, V562E
h31-27	13.6	0.45	4, gct→gtt, A4V 68, agc→ggc, S68G 198, gtg→atg, V198M	30, gag→ggg, E30G 105, aag→agg, K105R 392, ctg→cta, L392L	44, gtg→gcg, V44A 122, ctg→ccg, L122P 567, acc→gcc, T567A
h31-30	14.3	0.73	113, ccc→ctc, P113L	126, cgc→cac, R126H	313, acc→tcc, T313S
h32-20	11.9	0.39	91, cgc→tgc, R91C	296, aac→aaa, N296K	593, cgc→cgt, R593R
h33-14	8	1.23	394, aac→acc, N394T	540, gac→gat, D540D	
h33-23	11.7	0.53	267, cag→caa, Q267Q 602, agc→tgc, S602C	404, atg→acg, I404T 575, aag→gag, K575E	430, tgc→cgc, C430R
h34-7	3	0.77	59, gac→ggc, D59G	423, atg→acg, M423T	461, cag→caa, Q461Q
h34-22	14.6	0.85	71, agc→ggc, S71G	80, atc→gtc, I80V	593, cgc→cgt, R593R
h61-4	9.8	0.93	144, tgc→agc, C144S		
h61-5	7	1.08	156, gag→gtg, E156V	467, acc→tcc, T467S	507, cag→cgg, Q507R
h61-14	8.2	1	551, ccc→ctc, P551L		
h62-9	2.48	0.58	70, ggc→gac, G70D	489, atc→acc, I489T	
h62-14	2.67	0.6	36, agc→ggc, S36G 571, acc→gcc, T571A	367, aac→agc, N367S	518, atg→aag, M518K
h62-16	2.88	1.62	92, atc→aac, I92N 601, cag→cgg, Q601R	119, gtg→gcg, V19A	300, aag→aaa, K300K

Figure S1

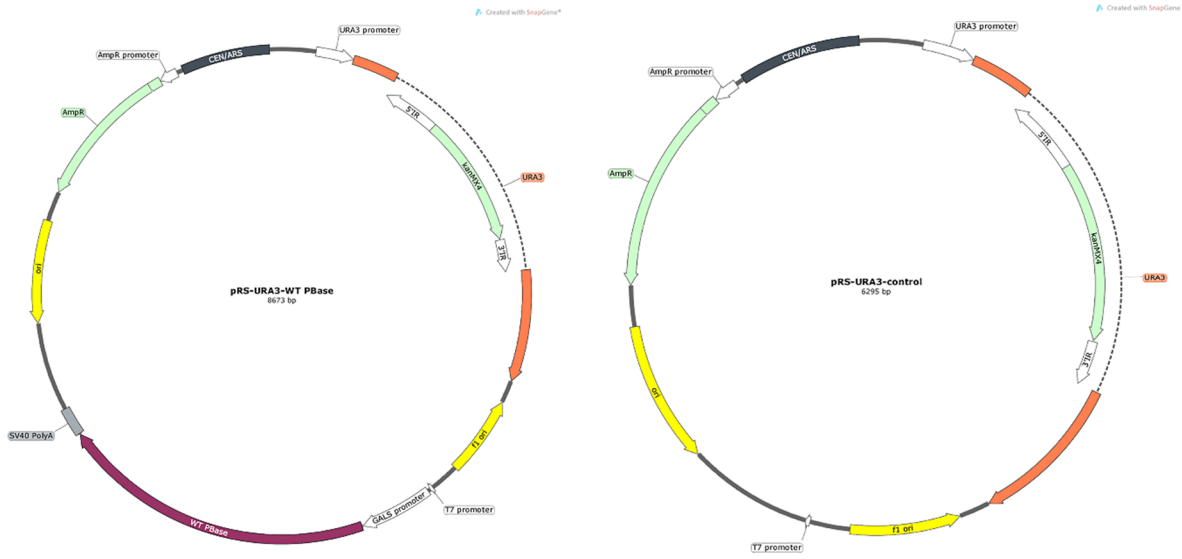

Figure S1. The map of pRS-URA3-WT PBase and pRS-URA3-control. The *ura3* gene was separated by the *piggyBac* transposon at TTAA site, which completely disturbed the normal expression. The *piggyBac* transposase with galactose-inducible GALS promoter was inserted into the multiple cloning site.

Figure S2

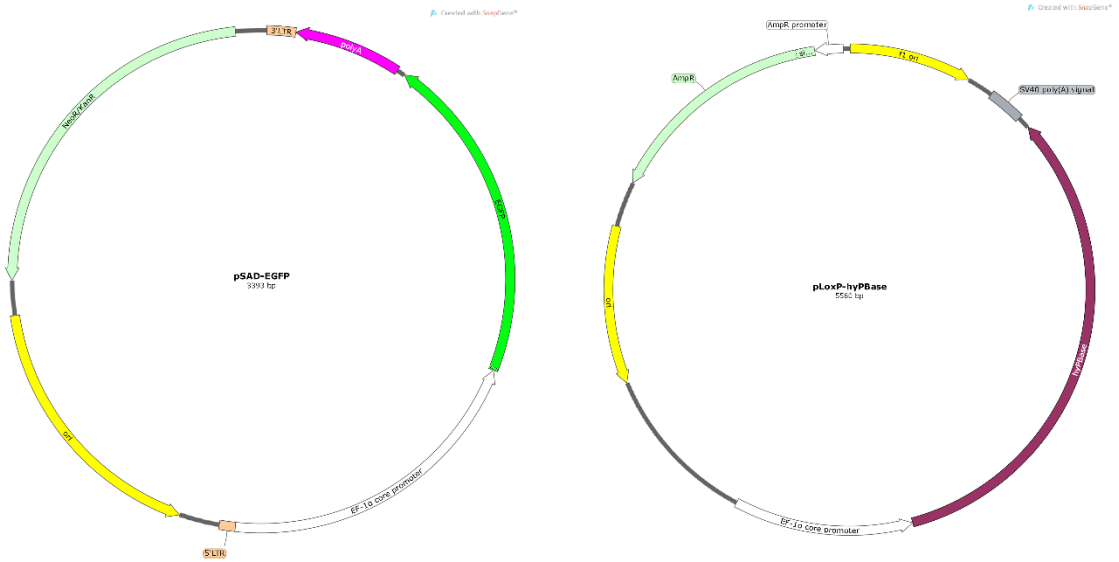

Figure S2. The map of the two-plasmid transposon system in mammalian cells. The transposon plasmid pSAD-EGFP contains the *egfp* gene that can express green fluorescent protein between 5′ LTR and 3′ LTR. The *piggBac* transposase can recognize and combine to the 5′ LTR and 3′ LTR areas, and then removed the transposon. The transposase plasmid contains the transposase optimized hyPBase or its mutants under the EF-1α core promoter.

Figure S3

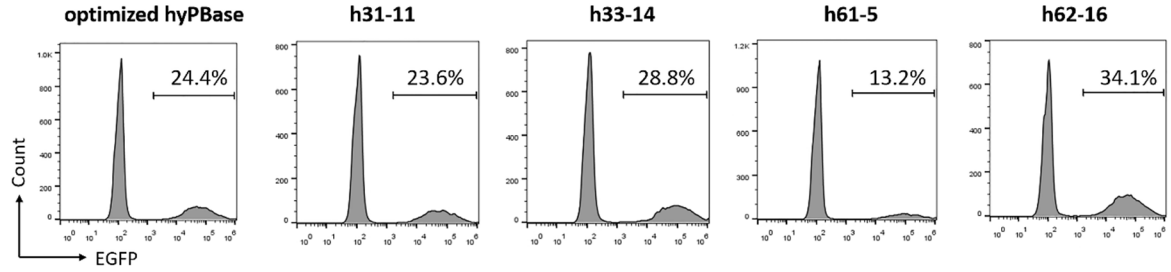

Figure S3. Relative activities of 4 hyperactive *piggyBac* transposase mutants in T Cells. The two-plasmid transposon system (pLoxP-optimized hyPBase/ pLoxP-mutant to express transposase, pSAD-EGFP to express transposon) were electrotransformed into human T cells. After 10 days culture, positive cells which completed gene transposition were measured by flow cytometry. The X-axis represented the intensity of EGFP, and the Y-axis represented the number of cells. The values represented the percent of EGFP cells in each sample.

Figure S4

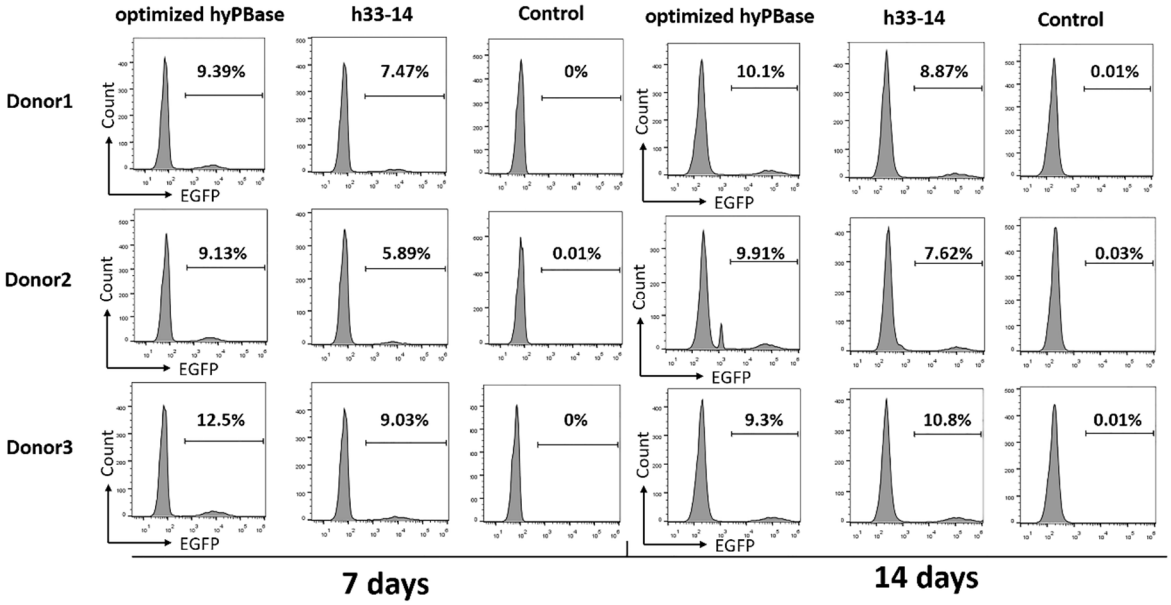

Figure S4. Relative activities of h33-14 in T Cells. The two-plasmid transposon system (pLoxP-optimized hyPBase/ pLoxP- h33-14 to express transposase, pSAD-EGFP to express transposon) were electrotransformed into human T cells. After 7 days and 14 days culture, positive cells which completed gene transposition were measured by flow cytometry. The right were the control cells that transformed into the transposon plasmid pSAD-EGFP only. The X-axis represented the intensity of EGFP, and the Y-axis represented the number of cells. The values represented the percent of EGFP cells in each sample. The experiment was repeated by three groups T cells from 3 different donors.

Figure S5

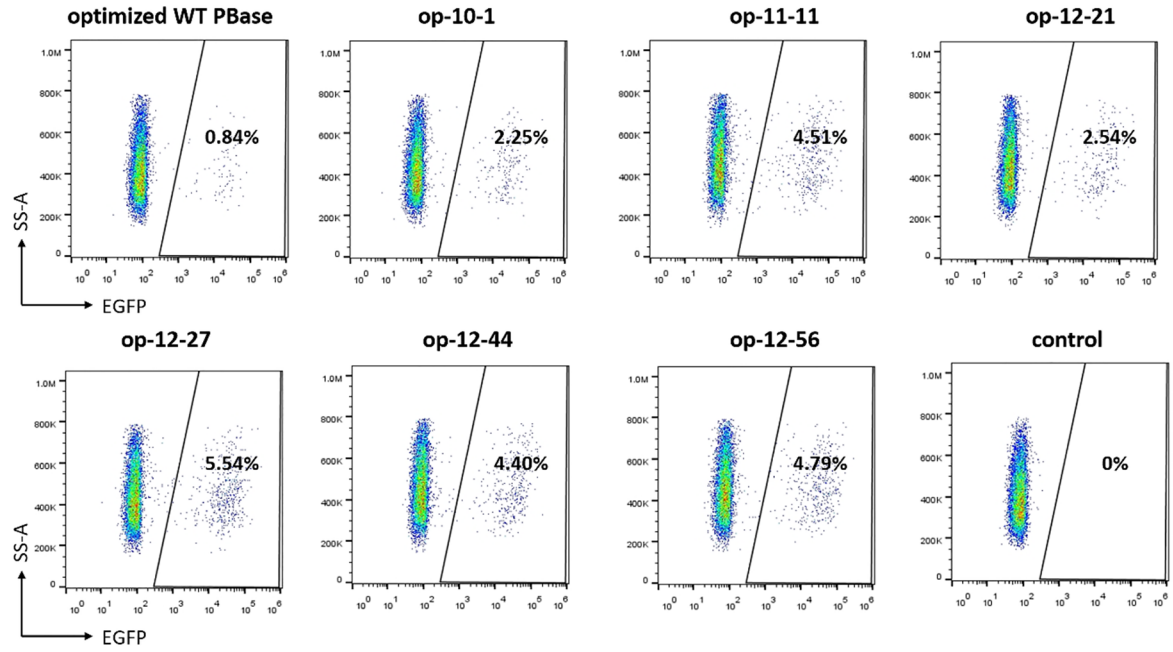

Figure S5. Relative activities of 6 mutants of optimized WT PBase in T Cells. The two-plasmid transposon system (pLoxP-optimized WT PBase/ pLoxP-mutant to express transposase, pSAD-EGFP to express transposon) were electrotransformed into human T cells. After 10 days culture, positive cells which completed gene transposition were measured by flow cytometry. The last was the control cells that transformed into the transposon plasmid

pSAD-EGFP only. The X-axis represented the intensity of EGFP, and the Y-axis represented the SS-A of cells. The values represented the percent of EGFP cells in each sample.

Figure S6

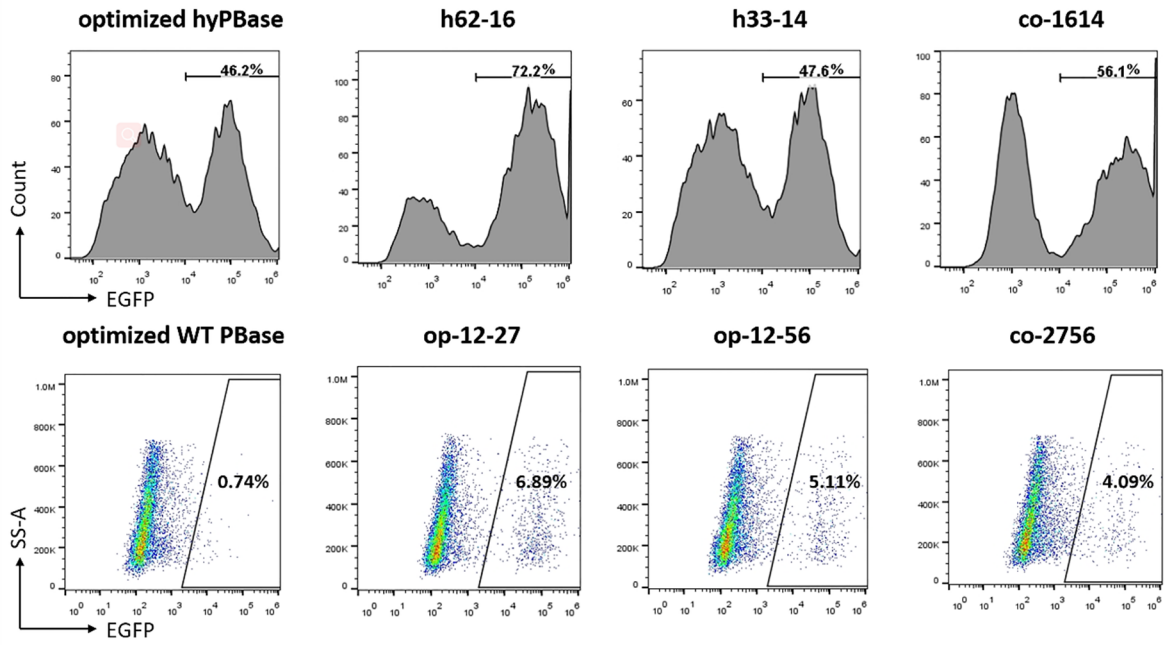

Figure S6. Relative activities of the optimized hyPBase-combined mutant (upper) and the optimized WT PBase-combined mutant (lower) in CHO Cells. The two-plasmid transposon system were electrotransformed into CHO cells. After 14 days culture, positive cells which completed gene transposition were measured by flow cytometry. The mutant co-1614 is the mutant that integrated the mutations of h33-14 and h62-16 into one sequence. The mutant co-2756 is the mutant that integrated the mutations of op12-27 and op12-56 into one sequence. The X-axis represented the intensity of EGFP, and the Y-axis represented the number of cells (upper) or the SS-A of cells (lower). The values represented the percent of EGFP cells in each sample.