Table S1. Multiplex PCR primers for Luminex® plasmid detection.

Pool 1 PCR Primers

Primer Name	Replicon	Primer Sequence (5' - 3')	Length	Tm	Product Length	Genbank coordinates	Unique
5476	lp36, F	ATTAAAGCACAAATGTATGGCCATTGCTGCATTCT	35	68	361	5294-5654	yes
5477	lp36, R	TGCAGCCAAAAGTAATTTGAAGAATGGACTAAAGA	35	65			no
5472	lp28-4, F	GGGAAATGAGTCAAAAATAAATACCCCAAATGAAA	35	65	437	2598-3034	yes
5473	lp28-4, R	CAGTGTTTGCTTTAAATTTTTTAGGGTGTTCTGGA	35	66			yes
5470	lp28-3, F	GAAGGAGAAGCGGGTGGGCAATAAAATT	28	65	473	12113-12585	yes
5471	lp28-3, R	TGGGCAAAATCTTTTTACTAATAAGCCACTCTTGG	35	65			yes
5484	cp32-7, F	GCGACAAATTCACCATTAAATTTAAAGGGATTCTT	35	65	500	8974-9473	no
5485	cp32-7, R	TTTAGCAAATCCATACATAAATCCAATAAGCCCG	34	65			yes
5492	cp32-3, F	TTTGTTTTGATAATTTCTTGCAAGATTGATGCGAG	35	66	527	26747-27273	yes
5493	cp32-3, R	GGCTCTGCTAGCTTTTCAAGTTCTTCCTCAATATT	35	65			yes
5500	Chromosome, F	ATCTAAGCAATGACAAAACATATTGGGGAACTTGA	35	65	582	147651-148232	yes
5501	Chromosome, R	AAAATGTAAGAACAGCTGAAGAGCTTGGAATGC	33	65			yes
5486	cp32-1, F	CCTAAAATAATAACAATAGCGTCAATTAAGGGCGG	35	65	606	20781-21386	no
5487	cp32-1, R	CCCCAAAAACTTTTCTTTTTTTTGCAACATTTCTA	35	65			yes
5488	lp56, F	CATTTATCCGAAATTTCAGAAGTAACCAAAGCATG	35	65	662	41115-41776	yes
5489	lp56, R	GACATTCAAACTGGGGTTGCAATTGCTATTTT	32	65			yes

Pool 2 PCR Primers

Primer Name	Replicon	Primer Sequence (5' - 3')	Length	Tm	Product Length	Genbank coordinates	Unique
5455	lp54, F	AGCCTTAATAGCATGTAAGCAAAATGTTAGCAGCC	35	66	263	9428-9690	yes
5456	lp54, R	CAAGTGTGGTTTGACCTAGATCGTCAGAAATTGTT	35	66			yes
5482	cp32-9, F	ACCTAAAATAATAACAATAGCGTCAATCAAGGGCG	35	65	401	20831-21231	no
5483	cp32-9, R	GATGTCCTAAGCTGGGATTTGTATCGATTATCACA	35	65			yes
5459	cp9, F	TAGCATGGAGTGCAATAGGACTGGTATTTACTCCG	35	67	285	6355-6639	yes
5460	cp9, R	AGGCCGATGAAGTTGCATTTAAACATTGTAGAG	33	65			yes
5480	cp32-6, F	TTATTTGTGCTATTTTTGCGCTGATAGTTTCTTGC	35	66	521	26276-26803	no
5481	cp32-6, R	TTATTCCAATTGCAGATTCAACTTGACCCTTAAGT	35	65			yes
5496	lp21, F	CCAAATATTTTAACAATAGCAAGTTTGAAAGGCGG	35	65	639	2880-3518	yes
5497	lp21, R	TGAAAAATGAATCTTGCCCTTGACATAACTACCAT	35	65			yes
5474	lp38, F	TTCACTTAATTTAAAATCAAGGGACATGGTTTGGA	35	65	382	19396-19777	yes
5475	lp38, R	TCCTATCCTTTGCCTTTGTCCTCCAGACATATAT	34	65			yes
5601	lp28-1, F	AATTGTCACCTAATTATGGGTTGAGCATTCTCTTG	35	65	278	10643-10920	yes
5602	lp28-1, R	GTCAAGCAATAATGCTGAAAACAAAATGGATGATA	35	65			yes
5536	lp25, F	ATTATTAAAAGCTTTTGGGGCATCATTTTGATTTC	35	65	700	19443-20142	Yes
5537	lp25, R	TCGCTACAAATTTTTCTTTCAAATATTGACTTGGC	35	65			Yes

Pool 3 PCR Primers

Primer Name	Replicon	Primer Sequence (5' - 3')	Length	Tm	Product Length	Genbank coordinates	Unique
5569	lp17	TGTTGTTGTGATTGATCTTCAATTTTATTGATGGC	35	65	552	11051-11602	yes
5570	lp17	GGGCATAAAAGGAACTTTCTTTGGAAATTCTCAAT	35	66			yes
5457	cp26, F	TTACTTAGCATTGCAAATAAAAACGGAATGAAACC	35	69	389	4919-5307	yes
5458	cp26, R	GCGGCTGTTGGGATTGAATAATGTTTAAATATTTT	35	65			yes
5490	cp32-4, F	TGATGTAACTAGTAAAGATTTAGAAGGGGCGGTGA	35	65	415	26918-27332	yes
5491	cp32-4, R	CGTTACTCCAGTTGCAGATTCAGCTTGGTTTT	32	66			yes
5478	cp32-8, F	ACCTAAAATAATAACAATAGCGTCAATCAAGGGCG	35	65	451	20754-21204	no
5479	cp32-8, R	TGCCGTCATTGGAACTATTACACAATTGCTAGTTA	35	66			yes
5468	lp28-2, F	ATGTGACATTTTTGATGTACACAAAGCTTGCATCT	35	65	515	20126-20640	yes
5469	lp28-2, R	CAAAATAGGAATAAGCGACTATTTGTCTGGGGCT	34	66			yes
5609	lp5, F	GTGTTAACTTTAAAATCCCAAACTTTATAATTTTGGGAAA	40	64	738	163-900	yes[a]
5495	lp5, R	CGTTAAAAGTAAACGCACGGCGTATAAACCC	31	66			yes
4596	lp25, F	TTGCTGCCATTTCTCACTTGGTAA	24	68	177	9783-9959	yes
4597	lp25, R	ATAAAAGCGACAGGTTATCGTGCAG	25	67			yes
5466	lp28-1, F	CTTCAACACTTATCAAAAAGTGAAGACCAAGCCAA	35	66	540	590-1129	yes
5467	lp28-1, R	CCCCGATATTTCCTTTGGTGTTTCTTTAGAATTTA	35	65			yes

[a] Primer matches lp21 and lp28-4 at 39/40 positions.

Table S2. ASPE primers for Luminex® plasmid detection.

Pool 1 ASPE primers

Primer No.	TAG	Sequence (5'-3')[a]	Replicon	Primer[b] length	Genbank Coordinates	Unique
5523	14	CTACTATACATCTTACTATACTTTTTTTTGGGTACTTTTATCACC	lp36	20/44	5553-5572	Yes
5524	20	CTTTTACAATACTTCAATACAATCAAAATAAATACGGCCCTTTGGACAAGC	lp28-4	27/51	2806-2832	Yes
5558	34	TCATTCATATACATACCAATTCATTAATTTTAATAGTTGGTTGCGACTGGGG	lp28-3	28/52	12160-12187	Yes
5526	52	TCAATCATCTTTATACTTCACAATGGTAGTGCTTTAGGAAATGTCATTGGCA	cp32-7	28/52	9390-9417	Yes
5527	55	TATATACACTTCTCAATAACTAACTCCCACGATAACACACCCGTATTAAAA	cp32-3	27/51	26903-26929	Yes
5555	61	AATCTTACCAATTCATAATCTTCA AGCCTGCGCAATCATTGCCA	Chromosome	20/44	147688-147707	Yes
5529	59	TCATCAATCAATCTTTTTCACTTTAAGCTTGCACACTTTTAGTGAAGAGCCC	cp32-1	28/52	21053-21080	Yes
5530	82	TACATACACTAATAACATACTCATCATATTTGTTCCTTGCATCTTGTTCTGG	lp56	28/52	41395-41422	Yes

Pool 2 ASPE primers

Primer No.	TAG	Sequence (5'-3')[a]	Replicon	Primer[b] length	Genbank Coordinates	Unique
5584	2	CTTTATCAATACATACTACAATCAGAAAAACAGCGTTTCAGTAGATTTGCCT	lp54	28/52	9470-9497	Yes
5585	12	TACACTTTCTTTCTTTCTTTCTTTTATTGATAATTGATAGTGATCCACAGGCCA	cp32-9	30/54	20929-20958	Yes
5586	44	TCATTTACCAATCTTTCTTTATACTCATCATTATCGTTTGCATTAAATCTTTGG	cp9	30/54	6435-6464	Yes
5587	68	TCATAATCTCAACAATCTTTCTTTTTTTTGGAGATTTTAAAAAACCAGAAGCAA	cp32-6	30/54	26405-26434	Yes
5588	77	CAATTAACTACATACAATACATACTGAAAGGGGGAGTTTCTTTTAATAATTGTG	lp21	30/54	3070-3099	Yes
5589	80	CTAACTAACAATAATCTAACTAACCTACGGGGAGCGGCAAGACAACTTT	lp38	25/49	19440-19464	Yes
5603	45	TCATTTCACAATTCAATTACTCAATGCAATATGAATAGGTTTTATTATAGGAATCCG	lp28-1	33/57	10709-10741	Yes
5538	24	TCAATTACCTTTTCAATACAATACAGCCAAAACAAGCTCAAAAGATCCCC	lp25	26/50	19738-19763	Yes

Pool 3 ASPE primers

Primer No.	TAG	Sequence (5'-3')[a]	Replicon	Primer[b] length	Genbank Coordinates	Unique
5571	33	TCAATTACTTCACTTTAATCCTTTTGGATAAATTATGAACCAACTGCAGAAGAA	lp17	30/54	11210-11180	Yes
5550	36	CAATTCATTTCATTCACAATCAATGCTTGGCGCCTGGGCAATTAGATTA	cp26	25/49	5044-5068	Yes
5551	42	CTATCTTCATATTTCACTATAAACTCTCTCCTCTTTTTTCTTTTTTAAGCTCTCTGA	cp32-4	33/57	27257-27225	Yes
5552	47	CTTCTCATTAACTTACTTCATAATTTGGGAAAAGTACGAGTTCAATAATATTTGC	cp32-8	31/55	20792-20822	Yes
5553	51	TCATTTCAATCAATCATCAACAATACATAGACTGATGATCTGGCCTCTTCTTG	lp28-2	29/53	20164-20192	Yes
5554	54	CTTTTTCAATCACTTTCAATTCATCCTCAGAAGACCAAAAGGAATTAGAAATTACG	lp28-1	32/56	636-667	Yes
5556	65	CTTTTCATCAATAATCTTACCTTTCATGCATGCAGAATACTTATTCTTACCGCA	lp5	30/54	306-335	Yes
5590	62	TCAATCATAATCTCATAATCCAATTTTTCATGCTTTGAAACTTTAGCATCTTCA	lp25	30/54	9811-9840	Yes

[a] The xTAG sequence is underlined.

[b] The first number indicates the length of the sequence complementary to the plasmid-specific multiplex PCR product. The second number indicates the length of the entire ASPE primer (xTAG sequence plus primer sequence).

Table S3. Normalized MFI values used to determine replicon presence (positive) or absence (negative).

	Negative (-)	Intermediate	Positive (+)
BB147	≤0.2		>0.2
cp9	≤0.2	>0.2-0.6	>0.6
cp26	≤0.2	>0.2-0.5	>0.5
cp32-1	≤0.2		>0.2
cp32-3	≤0.2		>0.2
cp32-4	≤0.2		>0.2
cp32-6	≤0.15		>0.15
cp32-7	≤0.15		>0.15
cp32-8	≤0.2	>0.2-0.4	>0.4
cp32-9	≤0.2	>0.2-0.5	>0.5
lp5	≤0.2	>0.2-0.4	>0.4
lp17	≤0.2	>0.2-0.4	>0.4
lp21	≤0.15		>0.15
lp25B	≤0.2		>0.2
lp28-1A	≤0.2	>0.2-0.55	>0.55
lp28-2	≤0.2		>0.2
lp28-3	≤0.2	>0.2-0.4	>0.4
lp28-4	≤0.2		>0.2
lp36	≤0.2	>0.2-0.4	>0.4
lp38	≤0.2	>0.2-0.5	>0.5
lp54	≤0.2		>0.2
lp56	≤0.2		>0.2

TABLE S4. Luminex® plasmid analysis assay results for 45 *B. burgdorferi* B31 clones, including 19 passage 5 clones previously characterized by PCR (B31-5A1 through B31-5A19; ref. 41), 25 additional passage 5 clones (B31-2597 through B31-2621), and 3 high passage clones (B31-312 through B31-314).

	BB147	cp9	cp26	cp32-1	cp32-3	cp32-4	cp32-6	cp32-7	cp32-8	cp32-9	lp5	lp17	lp21	lp25	lp28-1	lp28-2	lp28-3	lp28-4	lp36	lp38	lp54	lp56
B31-5A1	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-
B31-5A2	+	-	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	-
B31-5A3	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-
B31-5A4	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
B31-5A5	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+
B31-5A6	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
B31-5A7	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	+	+	+	+	+
B31-5A8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
B31-5A9	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-
B31-5A10	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-
B31-5A11	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
B31-5A12	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	+	+	+
B31-5A13	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
B31-5A14	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-
B31-5A15	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
B31-5A16	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-
B31-5A17	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	+	+	+	+	+
B31-5A18	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-
B31-5A19	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
B31-2597	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
B31-2598	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	+	+	+	+	+	+	+
B31-2599	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
B31-2600	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-
B31-2601	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-
B31-2602	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	-	+	+	+	+
B31-2603	+	+	+	+	-	+	+	+	+	+	-	+	+	+	int	+	+	+	+	+	+	+
B31-2604	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
B31-2605	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
B31-2606	+	+	+	+	+	+	+	+	+	+	int	+	+	-	+	+	+	+	+	+	+	+
B31-2607	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
B31-2608	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
B31-2609	+	+	+	+	+	+	+	+	+	+	+	+	+	+	int	+	+	+	+	+	+	-
B31-2610	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
B31-2611	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
B31-2612	+	-	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+
B31-2613	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
B31-2614	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
B31-2615	+	+	+	+	+	+	+	+	+	+	int	+	+	+	-	+	+	+	+	+	+	+
B31-2616	+	+	+	+	+	+	+	+	+	+	int	+	+	+	+	+	+	+	+	+	+	+
B31-2617	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	int
B31-2618	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
B31-2619	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+
B31-2620	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
B31-2621	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	int
B31-312	+	-	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
B31-313	+	-	+	+	+	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
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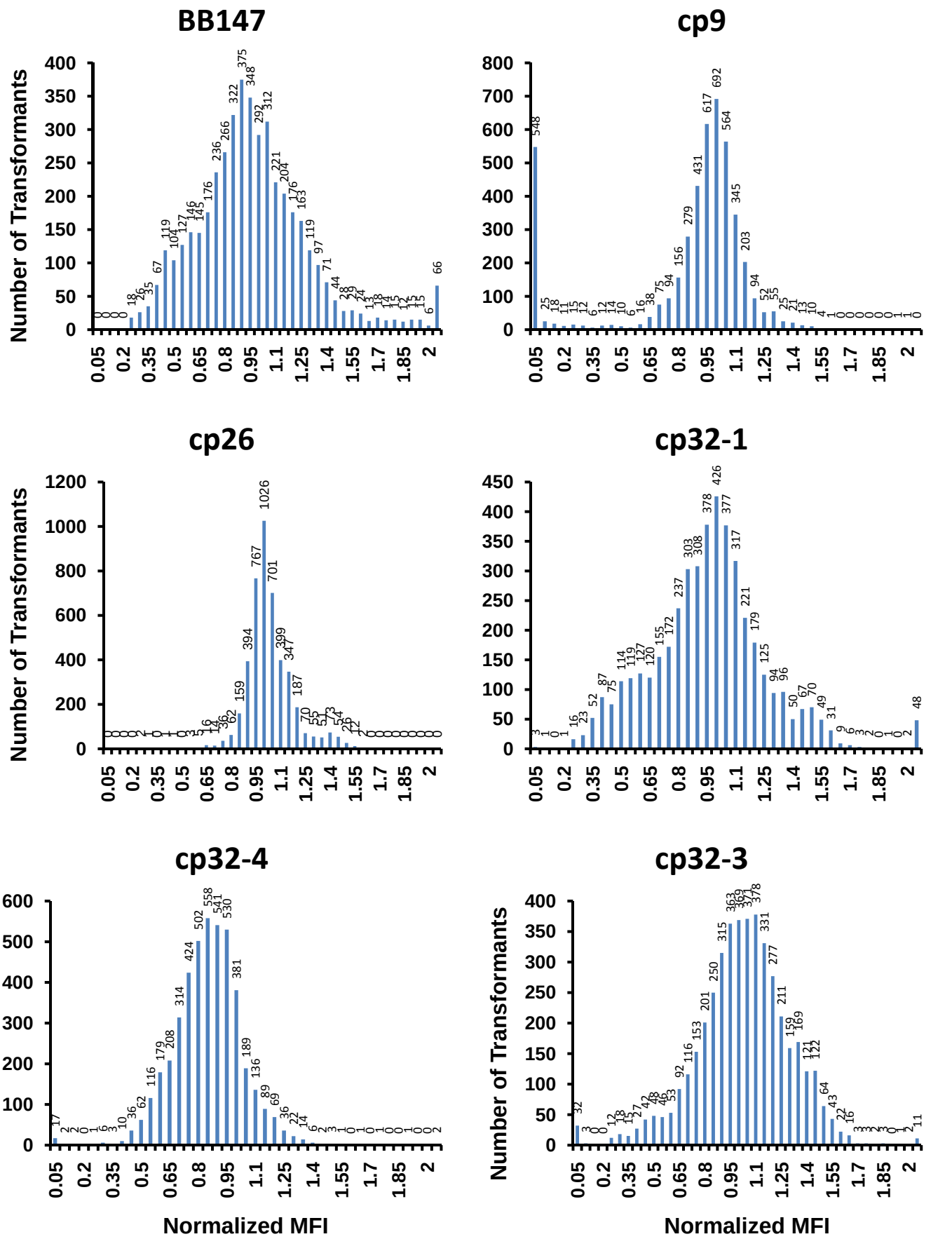
Fig. S1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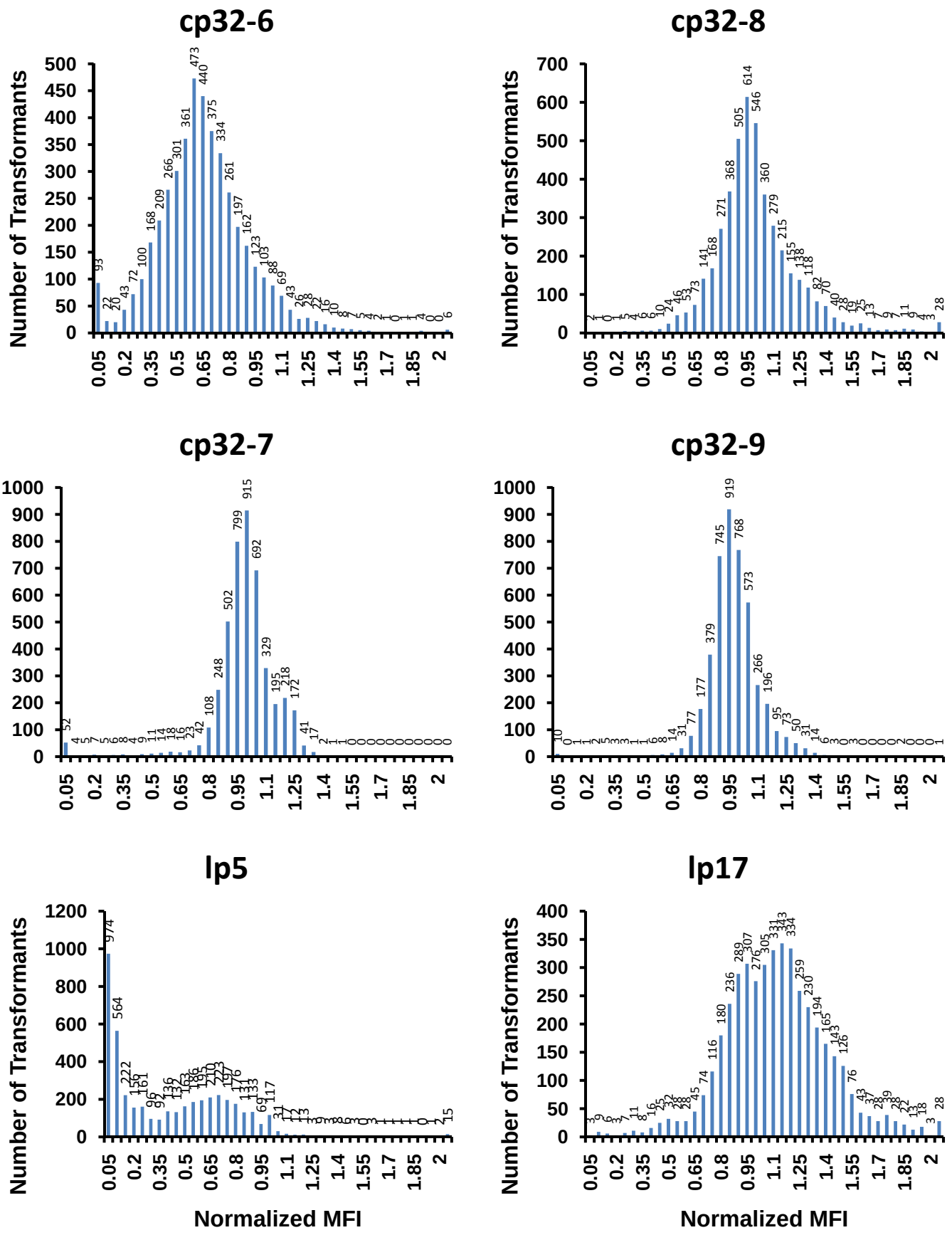
cp26
cp32-1
cp32-4
cp32-3
Number of Transformants
Normalized MFI

Fig. S1 (cont.)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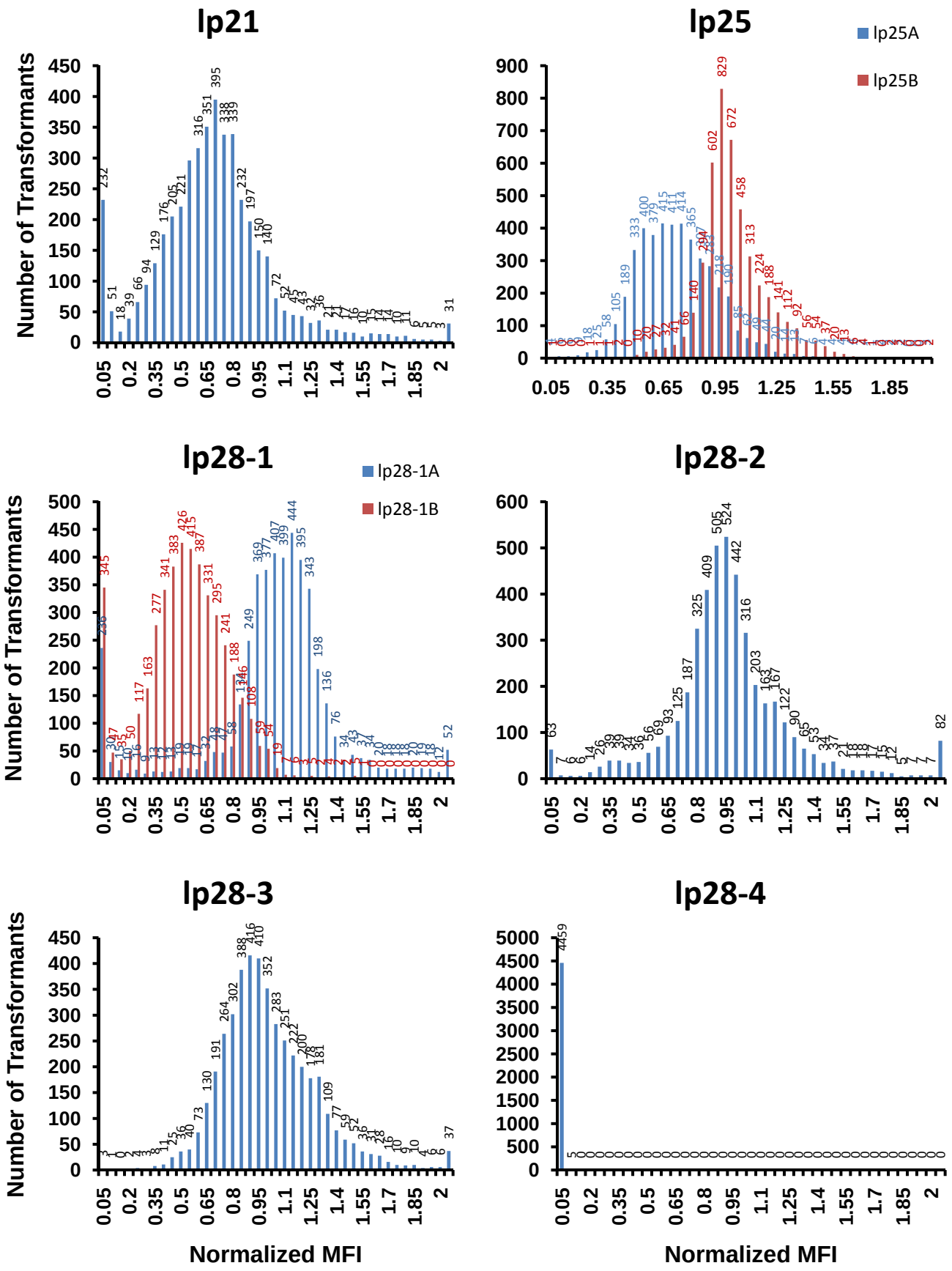
cp32-8
cp32-7
cp32-9
lp5
lp17
Number of Transformants
Normalized MFI

Fig. S1 (cont.)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lp25
lp25A
lp25B
lp28-1
lp28-1A
lp28-1B
lp28-2
lp28-3
lp28-4
Number of Transformants
Normalized MFI

Fig. S1 (cont.)

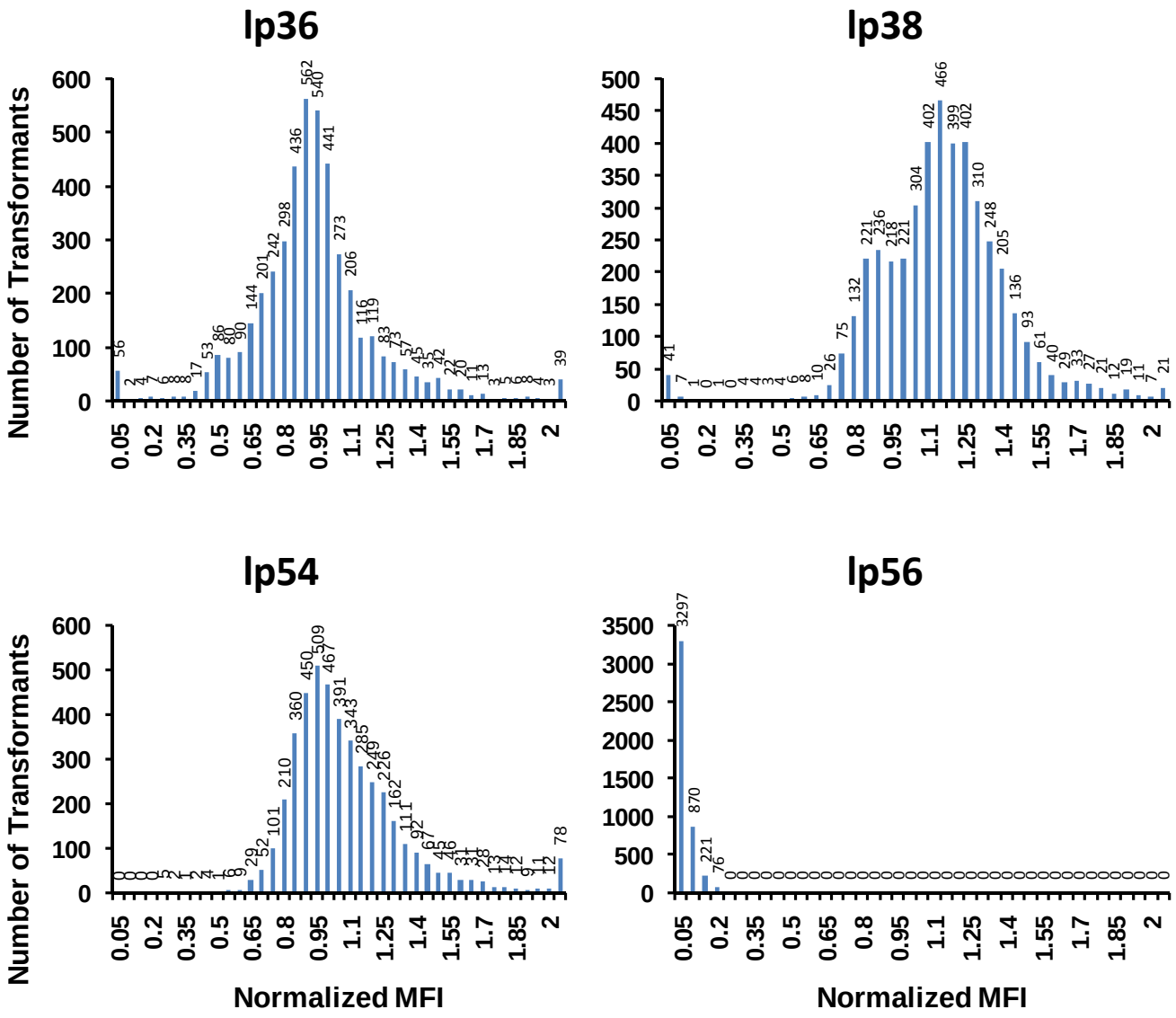
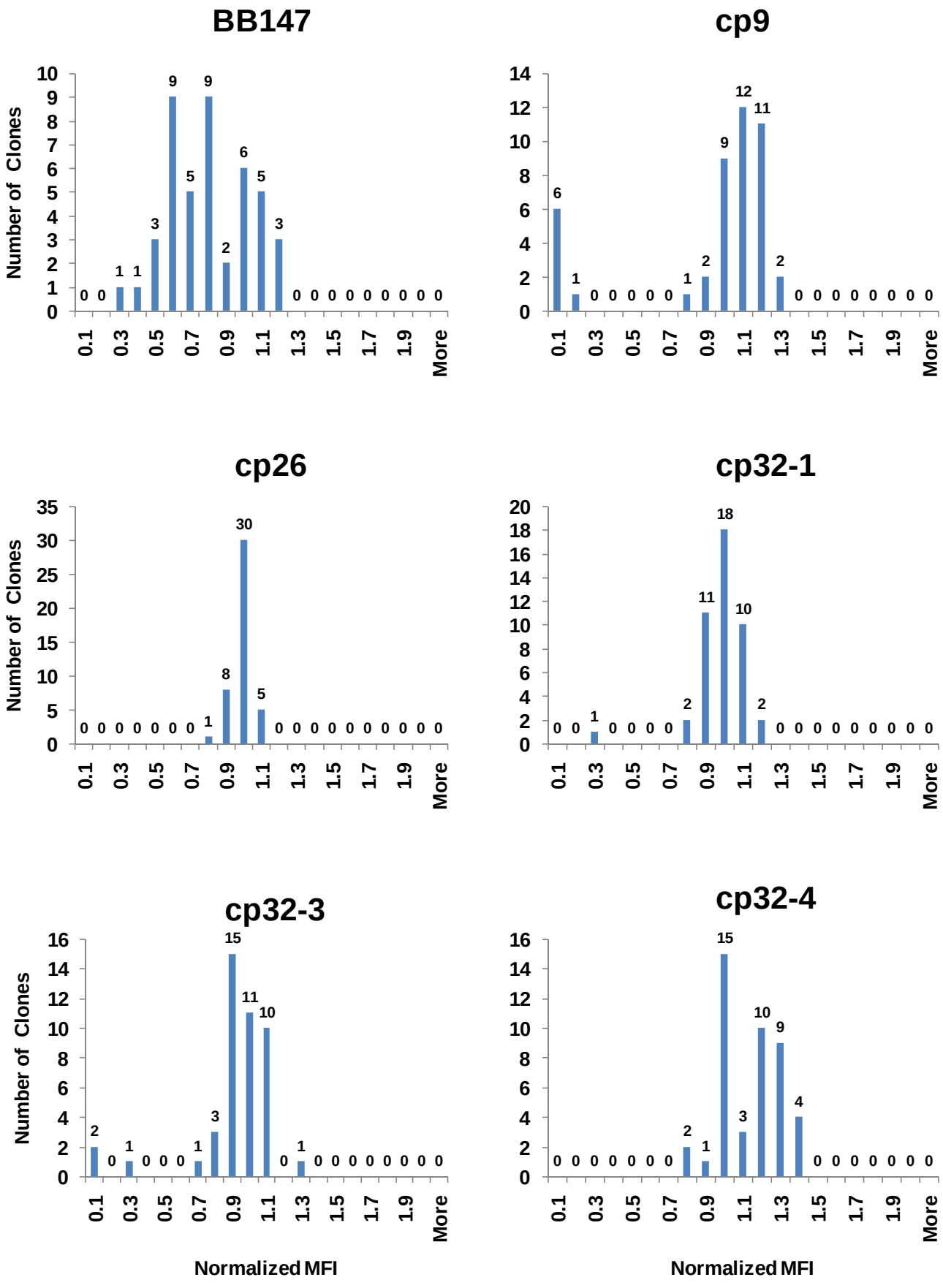


Figure S1. Histograms showing distribution of normalized MFI values for 4464 STM mutants. Each transformant was subjected to the LPAA and the MFI value for each replicon was normalized by dividing by the RPP MFI. Each bar (and the number above it) corresponds to the number of clones with a normalized MFI value in a 0.05 range (e.g. 0-0.05); the last bar represents those clones with normalized MFI values >2.0. MFI values for lp5 were normalized using the median of the five greatest MFI values from each assay plate as described in the text.

Fig. S2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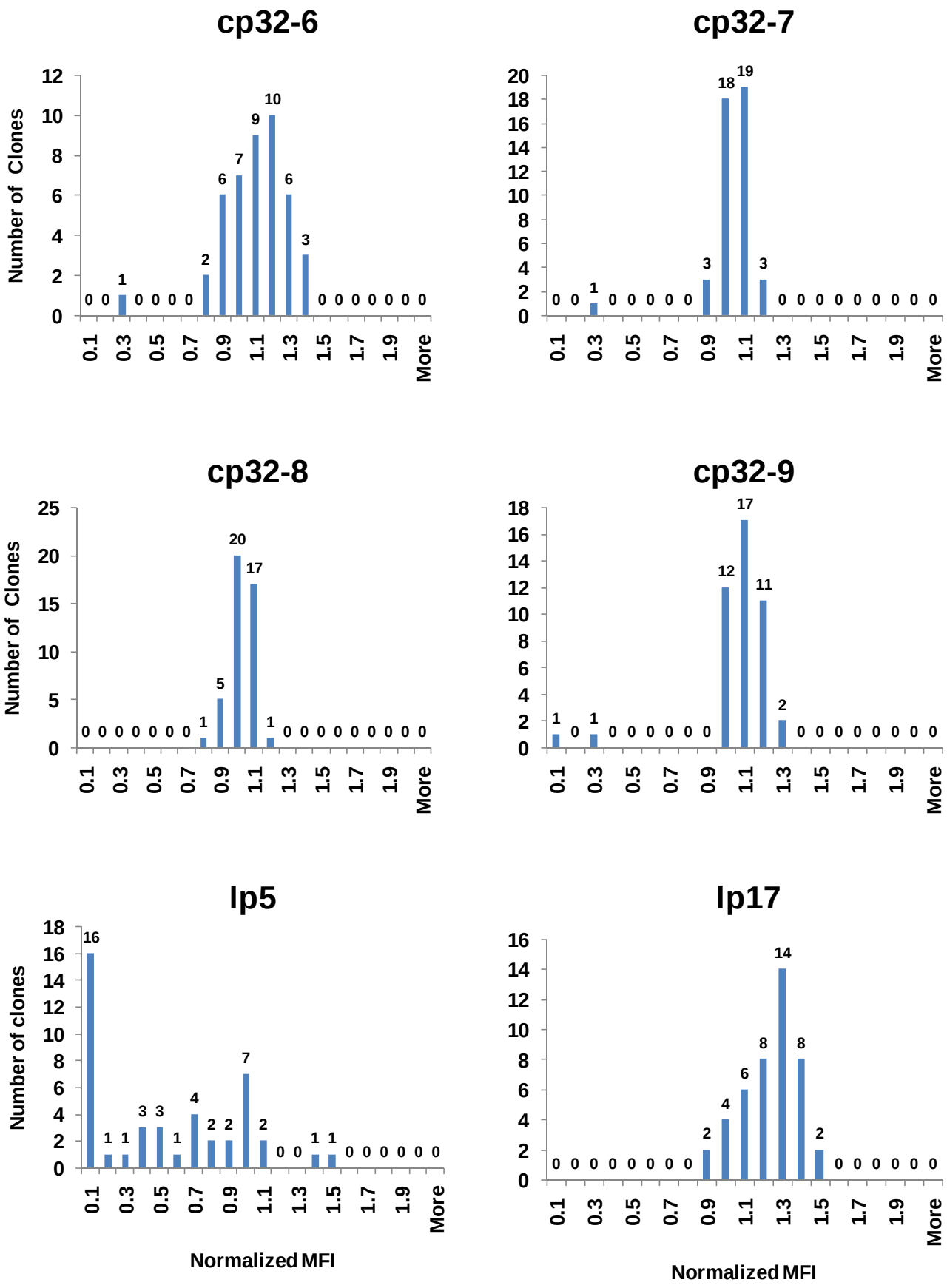
cp9
6 1 0 0 0 0 0 1 2 9 12 11 2 0 0 0 0 0 0 0 0
0.1 0.3 0.5 0.7 0.9 1.1 1.3 1.5 1.7 1.9 More
cp26
Number of Clones
0 0 0 0 0 0 0 1 8 30 5 0 0 0 0 0 0 0 0 0 0
0.1 0.3 0.5 0.7 0.9 1.1 1.3 1.5 1.7 1.9 More
cp32-1
0 0 1 0 0 0 0 2 11 18 10 2 0 0 0 0 0 0 0 0 0
0.1 0.3 0.5 0.7 0.9 1.1 1.3 1.5 1.7 1.9 More
cp32-3
Number of Clones
2 0 1 0 0 0 1 3 15 11 10 0 1 0 0 0 0 0 0 0 0
0.1 0.3 0.5 0.7 0.9 1.1 1.3 1.5 1.7 1.9 More
Normalized MFI
cp32-4
0 0 0 0 0 0 0 2 1 15 3 10 9 4 0 0 0 0 0 0 0
0.1 0.3 0.5 0.7 0.9 1.1 1.3 1.5 1.7 1.9 More
Normalized MFI

Fig. S2 (cont.)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Number of Clones
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0.1 0.3 0.5 0.7 0.9 1.1 1.3 1.5 1.7 1.9 More
cp32-7
0 0 1 0 0 0 0 0 3 18 19 3 0 0 0 0 0 0 0 0
0.1 0.3 0.5 0.7 0.9 1.1 1.3 1.5 1.7 1.9 More
cp32-8
Number of Clones
0 0 0 0 0 0 0 1 5 20 17 1 0 0 0 0 0 0 0 0
0.1 0.3 0.5 0.7 0.9 1.1 1.3 1.5 1.7 1.9 More
cp32-9
1 0 1 0 0 0 0 0 12 17 11 2 0 0 0 0 0 0 0
0.1 0.3 0.5 0.7 0.9 1.1 1.3 1.5 1.7 1.9 More
lp5
Number of clones
16 1 1 3 3 1 4 2 2 7 2 0 0 1 1 0 0 0 0 0 0
0.1 0.3 0.5 0.7 0.9 1.1 1.3 1.5 1.7 1.9 More
Normalized MFI
lp17
0 0 0 0 0 0 0 0 2 4 6 8 14 8 2 0 0 0 0 0 0
0.1 0.3 0.5 0.7 0.9 1.1 1.3 1.5 1.7 1.9 More
Normalized MFI

Fig. S2 (cont.)

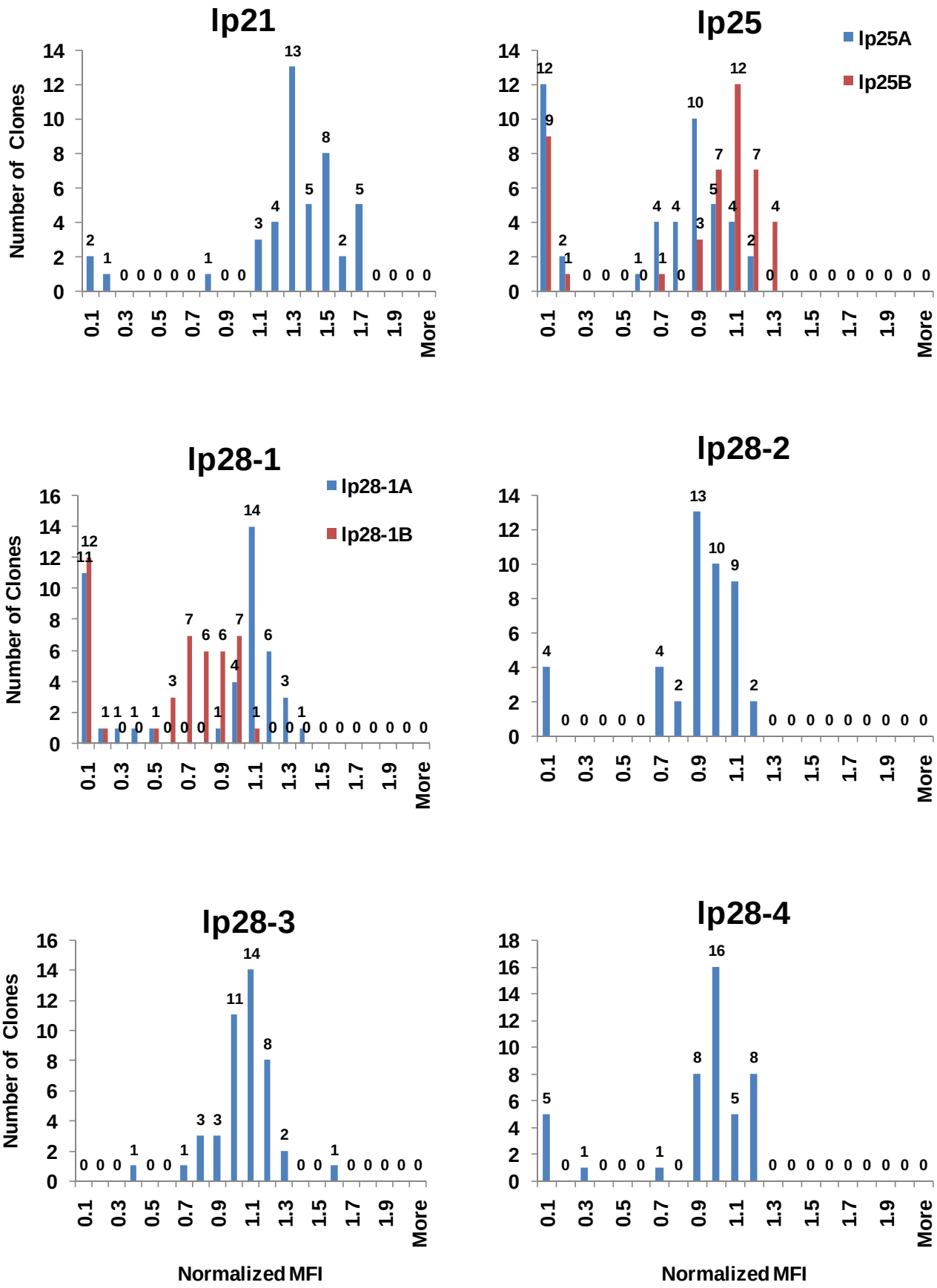
Ip21
Number of Clones
0.1 0.3 0.5 0.7 0.9 1.1 1.3 1.5 1.7 1.9 More
2 1 0 0 0 0 0 1 0 0 3 4 13 5 8 2 5 0 0 0 0
Ip25
Ip25A
Ip25B
12 9 2 1 0 0 0 0 1 0 4 1 4 0 10 3 5 7 4 12 2 7 0 4 0 0 0 0 0 0 0 0
Ip28-1
Ip28-1A
Ip28-1B
12 11 1 1 0 1 0 1 0 0 3 0 0 7 1 6 6 4 7 14 1 0 6 0 3 1 0 0 0 0 0 0 0 0
Ip28-2
4 0 0 0 0 0 4 2 13 10 9 2 0 0 0 0 0 0 0 0 0
Ip28-3
0 0 0 1 0 0 1 3 3 11 14 8 2 0 0 1 0 0 0 0 0
Ip28-4
5 0 1 0 0 0 1 0 8 16 5 8 0 0 0 0 0 0 0 0 0
Normalized MFI
Normalized MFI

Fig. S2 (cont.)

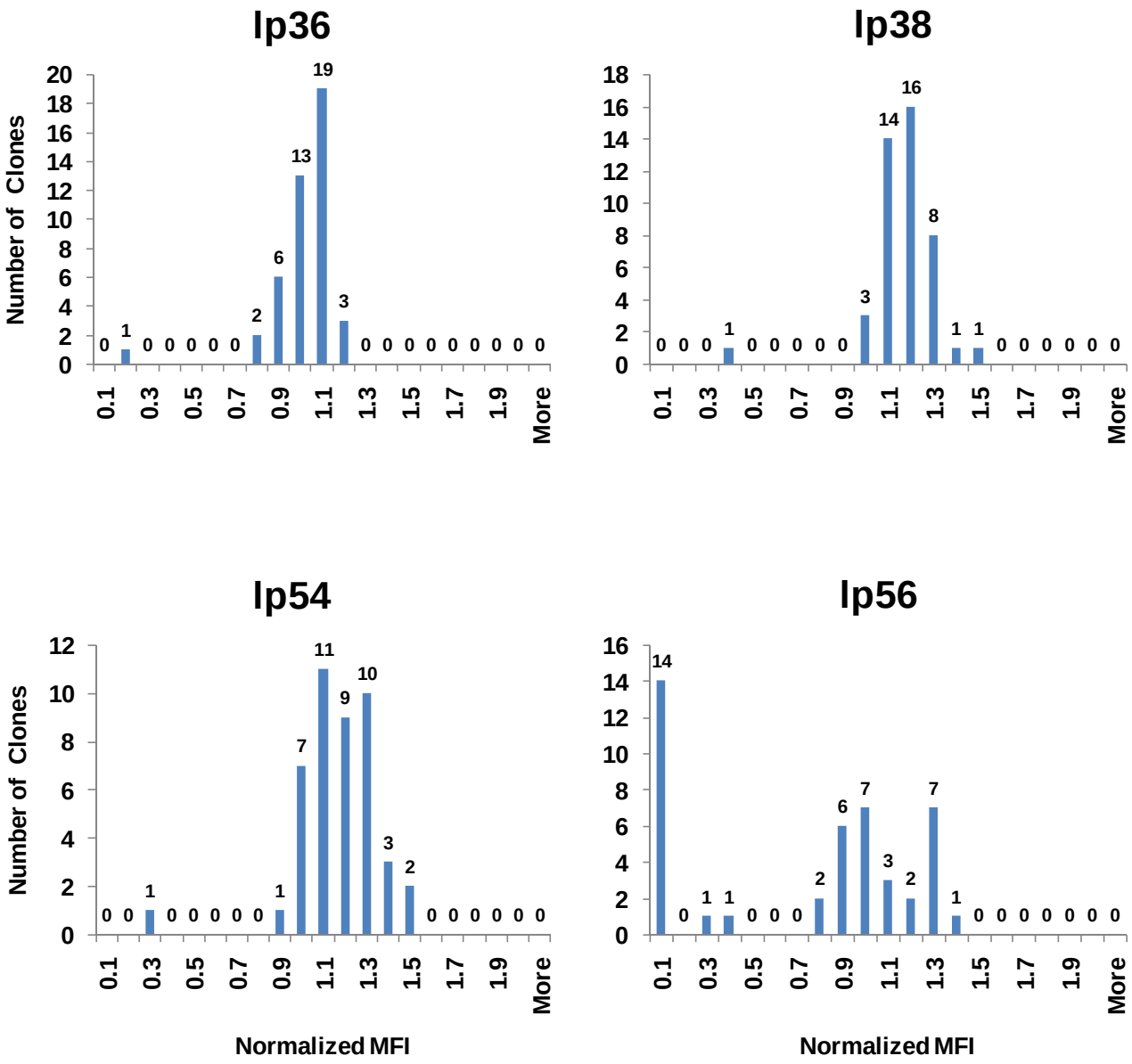

Figure S2. Histograms showing distribution of normalized MFI values for 44 B31 clones. Each clone was subjected to the LPAA and the MFI value for each replicon was normalized by dividing by the MFI value for the RPP. Each bar (and the number above it) corresponds to the number of clones with a normalized MFI value in a 0.1 range (e.g. 0-0.1). MFI values for lp5 were normalized using the median of the five greatest MFI values from each assay plate as described in the text.

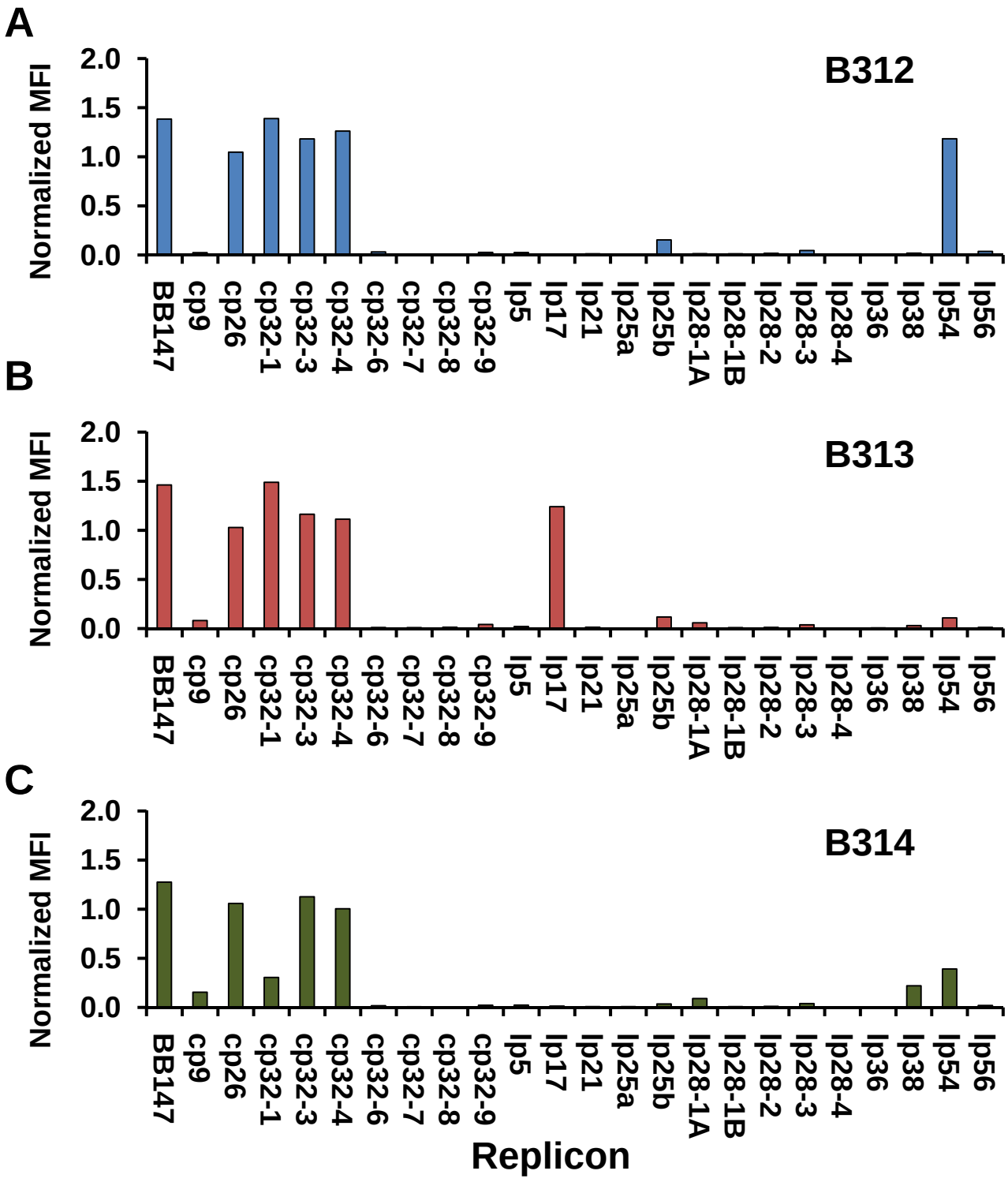

Figure S3. Plasmid content analysis of high passage *B. burgdorferi* strains using the Luminex® Assay. A scraping of frozen stock of the indicated strains were used as template for Luminex® plasmid analysis. Median fluorescence intensity (MFI) obtained for each *B. burgdorferi* replicon was normalized by dividing culture MFI values by RPP MFI values. (A) B312 (B) B313 (C) B314.