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Supplementary Materials for

No evidence for DNA N^6-methyladenine in mammals

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/12/eaay3335/DC1)

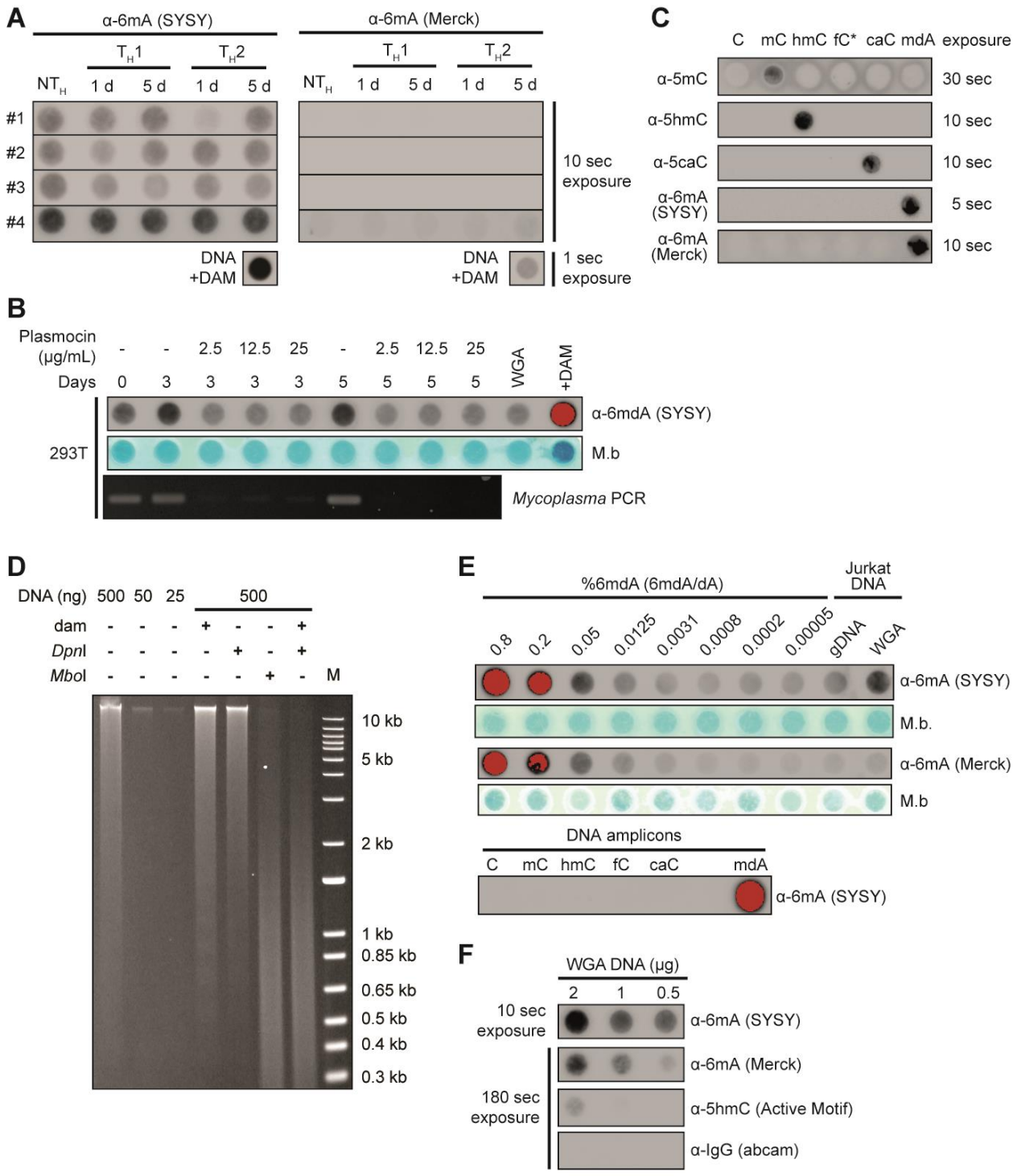

Fig. S1. Antibody detection of 6mdA in mammals is flawed. (**A**) Immuno-dot blot showing relative 6mdA abundance in human CD4+ T-cells during *in vitro* differentiation, for n = 4 biological replicates and n = 2 independent 6mdA antibodies. (**B**) Immuno-dot blot and mycoplasma contamination status showing loss of 6mdA signal upon antibiotic based removal of *Mycoplasma spp*. contamination from cultured 293T cells, n = 1. +DAM, Human CD4+ T-cell DNA treated with bacterial deoxyadenosine methylase (DAM). (**C**) Immuno-dot blot showing

specificity of 6mdA antibodies. 10 ng DNA was loaded in each well. *α-fC antibody was not tested. (**D**) Generation of DAM methylated Jurkat cell genomic DNA. *dam*, DNA methyltransferase modifies the adenine residue (N6) in the sequence 5'-GATC-3'. *DpnI*, only cleaves the sequence 5'-GATC-3' when methylated. *MboI* only cleaves the sequence 5'-GATC-3' when not methylated. (**E**) Immuno-dot blot showing relative sensitivity of two 6mdA antibodies. As controls 10 ng of polynucleotide dsDNA fragments (Suppl. Data 2) equivalent to 16 fmol of the modification were loaded onto the dot blot and incubated with antibody against 6mA (SYSY). (**F**) Immuno-dot blot of whole genome amplified Jurkat cell DNA with antibodies specific for 6mdA, 5hmC and a non-specific IgG antibody. (B, E, F) WGA, whole genome amplified CD4+ T-cell DNA.

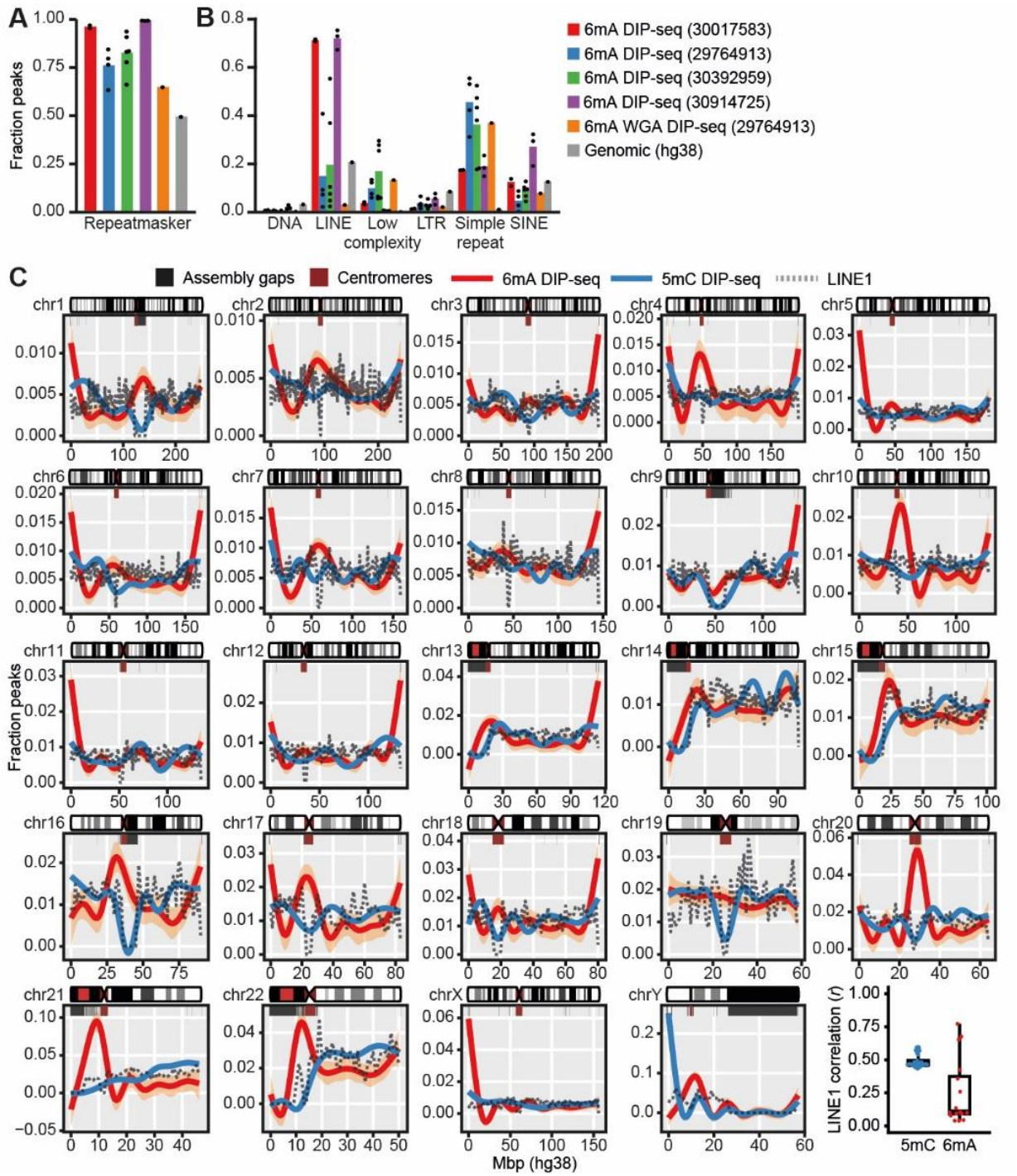

Fig. S2. Extended analysis of repetitive elements and mappability for 6mdA DIP-seq. (**A**) Fraction of enriched DIP-seq peaks located in all Repeatmasker repetitive elements or (**B**) classes for 6mA (n = 16) from multiple studies (n = 4) and expected genomic fractions from hg38. (**C**) Fraction of enriched DIP-seq peaks per Mbp along hg38 chromosomes for 6mA (n = 16) or 5mC (n = 45). Bottom right panel indicates the genome-wide correlation with LINE1 elements measured by Pearson correlation per sample. Location of LINE1 elements and assembly gaps obtained from UCSC Repeatmasker and gap track, respectively.

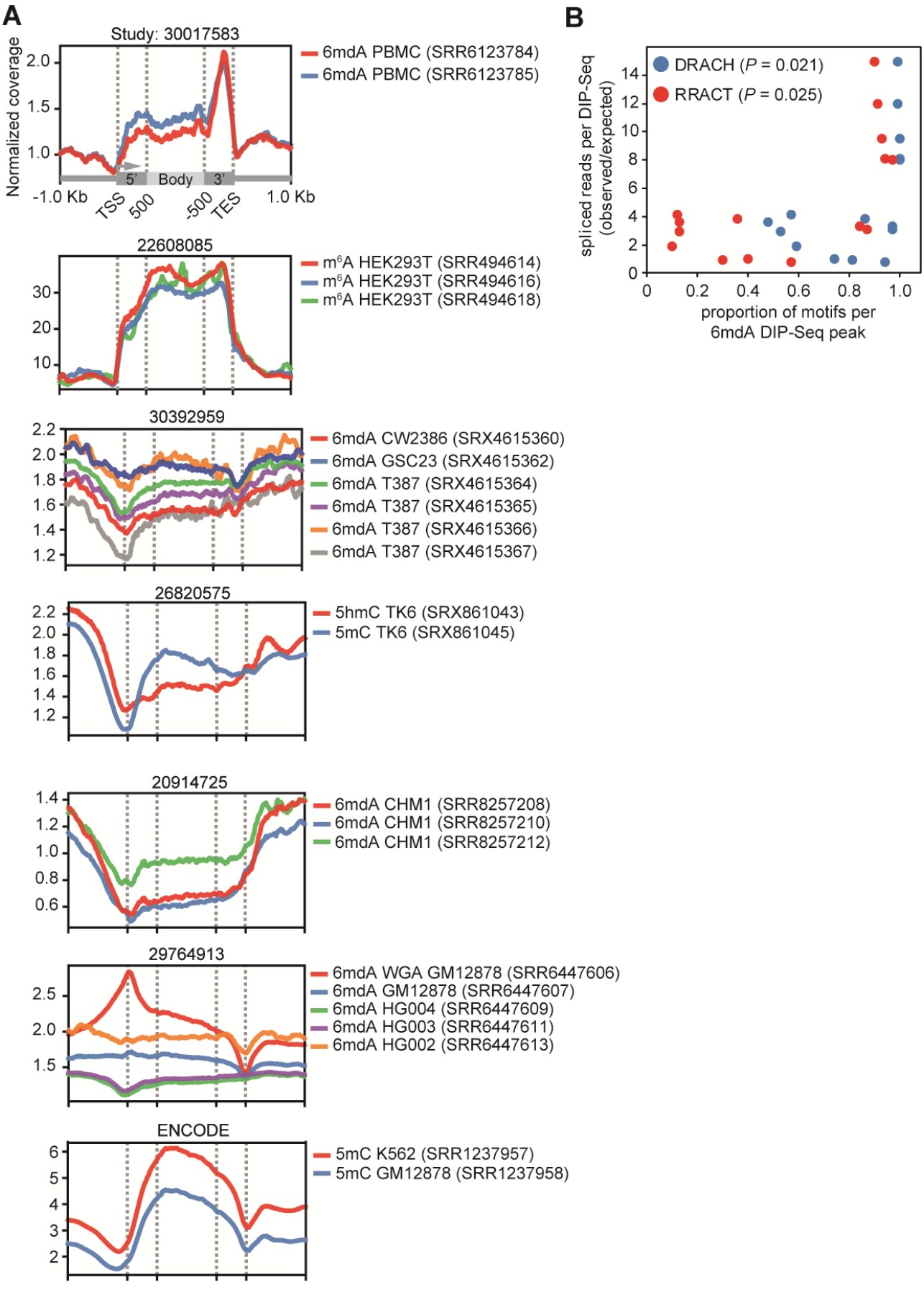
A
Study: 30017583
Normalized coverage
2.0
1.5
1.0
5'
Body
3'
-1.0 Kb
TSS
500
-500
TES
1.0 Kb
6mdA PBMC (SRR6123784)
6mdA PBMC (SRR6123785)
22608085
30
20
10
m6A HEK293T (SRR494614)
m6A HEK293T (SRR494616)
m6A HEK293T (SRR494618)
30392959
2.2
2.0
1.8
1.6
1.4
1.2
6mdA CW2386 (SRX4615360)
6mdA GSC23 (SRX4615362)
6mdA T387 (SRX4615364)
6mdA T387 (SRX4615365)
6mdA T387 (SRX4615366)
6mdA T387 (SRX4615367)
26820575
5hmC TK6 (SRX861043)
5mC TK6 (SRX861045)
20914725
1.4
1.2
1.0
0.8
0.6
6mdA CHM1 (SRR8257208)
6mdA CHM1 (SRR8257210)
6mdA CHM1 (SRR8257212)
29764913
2.5
2.0
1.5
6mdA WGA GM12878 (SRR6447606)
6mdA GM12878 (SRR6447607)
6mdA HG004 (SRR6447609)
6mdA HG003 (SRR6447611)
6mdA HG002 (SRR6447613)
ENCODE
6
5
4
3
2
5mC K562 (SRR1237957)
5mC GM12878 (SRR1237958)
B
DRACH (P = 0.021)
RRACT (P = 0.025)
spliced reads per DIP-Seq
(observed/expected)
14
12
10
8
6
4
2
0
0
0.2
0.4
0.6
0.8
1.0
proportion of motifs per
6mdA DIP-Seq peak

Fig. S3. Extended analysis of 6mdA DIP-seq read splicing and genic location. (**A**) Metagene signal over gene bodies normalized to 1x depth (reads per genome coverage, RPGC) for DIP-seq 6mA (n = 16), 5hmC (n = 1), 5mC (n = 3) and RNA RIP-seq m^6A (n = 3). 5', 5' UTR; 3', 3' UTR; Body, Gene body; TSS, Transcription start site; TES, transcription end site. (**B**) Comparison of consensus motif frequency in 6mdA peaks and the proportion of read splicing in 6mdA DIP-seq data sets. P-value of Spearman's correlation (*rho*) is shown.

Table S1. SMRT-seq read coverage in studies of 6mdA in mammalian DNA.

Study	Species	Sample	Coverage	PMID
Zhu et al.	Human	HG002	18.2	29764913
Zhu et al.	Human	HG003	17.2	29764913
Zhu et al.	Human	HG004	16.3	29764913
Zhu et al.	Human	WGA	39.4	29764913
Wu et al.	Mouse	WT	>30*	27027282
Wu et al.	Mouse	KO	>30*	27027282
Pacini et al.	Human	AK1	101	30914725
Pacini et al.	Human	CHM1	54	30914725
Xiao et al.	Human	HX1	103	30017583

*not genome wide

Table S2. Primers used to detect *Mycoplasma* species. Fw, forward primer, Rv, reverse primer

Primer	Sequence	Species	Product size (bp)
(Fw) Myco 5-1	CGC CTG AGT AGT ACG TTC GC	*M. fermentans*, *M. bovis*	518
(Fw) Myco 5-2	CGC CTG AGT AGT ACG TAC GC	*A. laidlawii*	525
(Fw) Myco 5-2a	TGC CTG AGT AGT ACA TTC GC	*M. gallisepticum*	504
(Fw) Myco 5-2b	TGC CTG GGT AGT ACA TTC GC	*Ureaplasma spp.*	504
(Fw) Myco 5-5	CGC CTG GGT AGT ACA TTC GC	*M. pirum*	504
(Fw) Myco 5-6	CGC CTG AGT AGT AGT CTC GC	*M. arginini*, *M. hominis*, *M. hyorhinis*, *M. orale*, *M. pneumoniae*	520 522 518 520 517
(Rv) Myco 3-1	GCG GTG TGT ACA AGA CCC GA	*M. arginini*, *M. bovis*, *M. fermentans*, *M. gallisepticum*, *M. hominis*, *M. orale*, *M. pirum*, *Ureaplasma spp.*	
(Rv) Myco 3-2	GCG GTG TGT ACA AAA CCC GA	*M. hyorhinis*, *M. pneumoniae*	
(Rv) Myco 3-3	CGG TGT GTA CAA ACC CCG A	*A. laidlawii*	

Other Supplementary Materials

Data S1. Summary of analyzed datasets and their relationship to figures.
Data S2. Summary of amplicons used in dot blots.