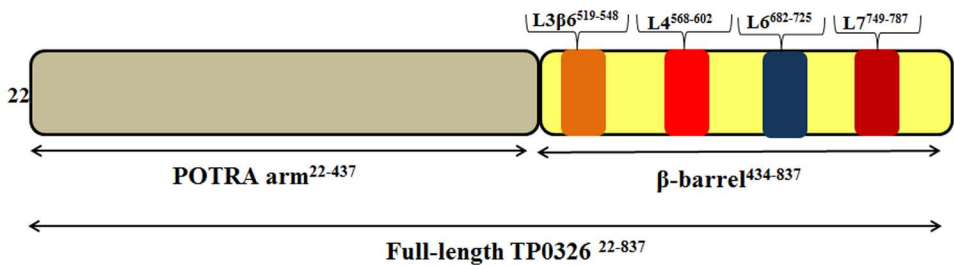
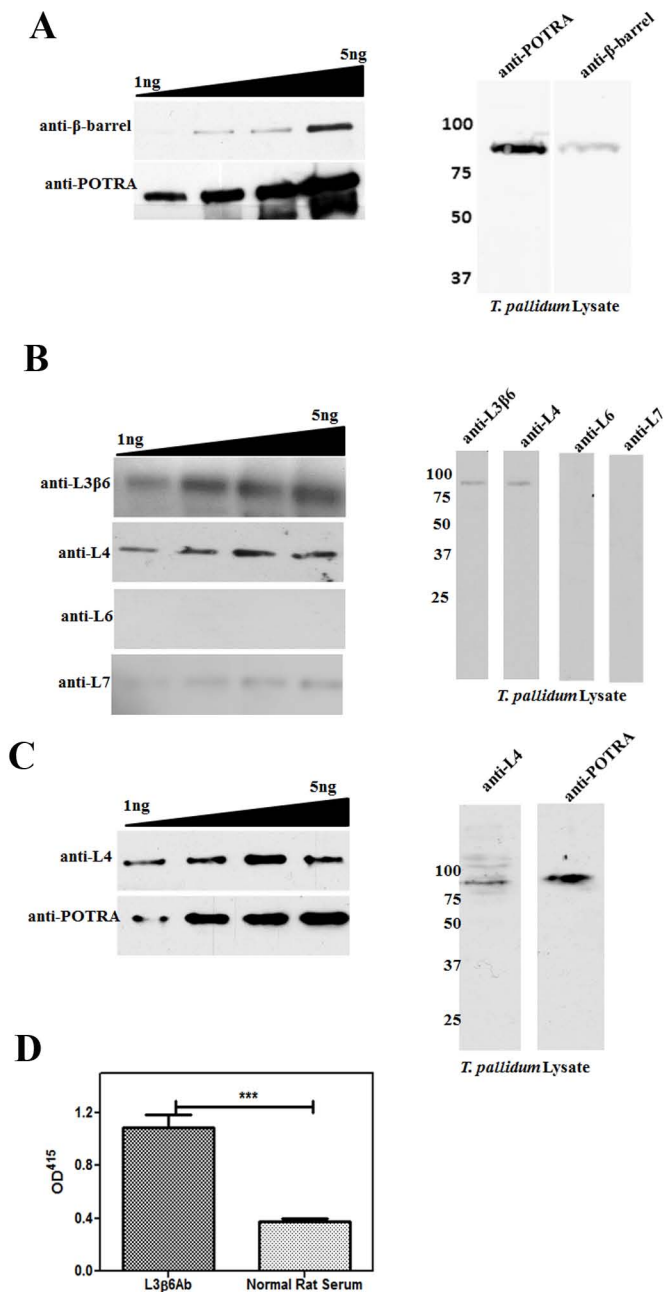


Supplemental Table . List of constructs and primers

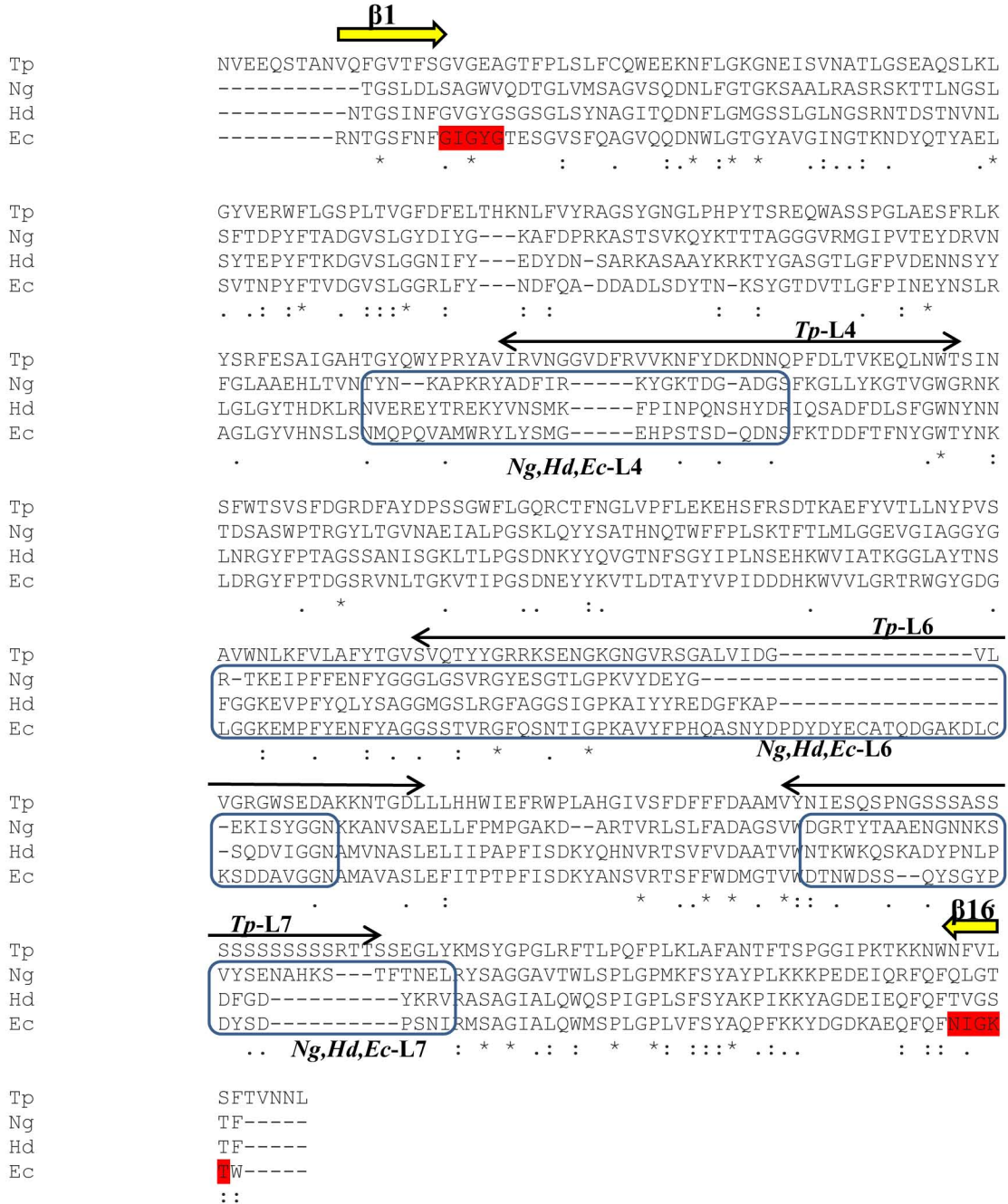
Primer	Description	Sequence
Full length TP0326-F	amplification of <i>TP0326</i>	AAAGCTAGCATGCAGGCAAACGACAATTGGTAC
Full length TP0326-R	amplification of <i>TP0326</i>	GGGAAGCTTCAAATTATTTACCGTGAACGACAACACAA
L3 β 6-F	amplification of <i>L3β6</i>	AAAGGATCCATGGGCAACGGGCTG
L3 β 6-R	amplification of <i>L3β6</i>	CCCCTCGAG GCGGAATACTTGAG
L4-F	amplification of <i>L4</i>	AAAGGATCCATG ATTAGGGTGAAC
L4-R	amplification of <i>L4</i>	CCCCTCGAGCGTCCAAAACGA
L6-F	amplification of <i>L6</i>	AAAGGATCCATGGTTCAAACGTATTAT
L6 ^{Ab} -F	amplification of L6 ^{Ab}	AAAGGATCCATGGGACGGAGGAAAAGCGAA
L6 & L6 ^{Ab} -R	amplification of L6 & L6 ^{Ab}	CCCCTCGAGGTCTCCGGTGTTTTT
L7-F	amplification of <i>L7</i>	AAAGGATCCATGGATGCGGCAATGGTGTAC
L7-R	amplification of <i>L7</i>	CCCCTCGAG CAGTCCTTCAGAGCT
326 ^{Pel} -F	amplification of 326 ^{Pel}	AAAGGATCCCATGCAGGCAAACGACAATTGG
326 ^{Pel} -R	amplification of 326 ^{Pel}	GGGAAGCTTCAAATTATTTACCGTGAACGACAACACAA
POTRA-F	amplification of <i>POTRA</i>	CCCCTAGCATGCAGGCAAACGACAATTGGTAC
POTRA-R	amplification of <i>POTRA</i>	CCCAAGCTTCTGCTCCTCCACATTGAG
β -barrel-F	amplification of <i>β-barrel</i>	AAAGCTAGCATGAACGTTGAAGAACAATCG
β -barrel-R	amplification of <i>β-barrel</i>	TTTAAGCTTCAGATTGTTAACGGTAAA
β -barrel-F1	amplification of <i>β-barrel</i> from Cali-77 and Cali-84	AATGTGGAGGAGCAGTCGACG
β -barrel-R1	amplification of <i>β-barrel</i> from Cali-77 and Cali-84	CTACAAATTATTTACCGTGAAC
L4 ^{Gln593} (+)	Mutagenesis of <i>L4</i>	CAGCCCTTCGACCAGACCGTAAAAGAG
L4 ^{Gln593} (-)	Mutagenesis of <i>L4</i>	TCTTTTACGGTCTGGTCGAAGGGCTG
Skp of <i>E. coli</i> -F	amplification of <i>skp</i>	GATAGCTAGCGCTGACAAAATTGCAATCGTC
Skp of <i>E. coli</i> -R	amplification of <i>skp</i>	GATAAAGCTTTTTAACCTGTTTCAGTACGTCG



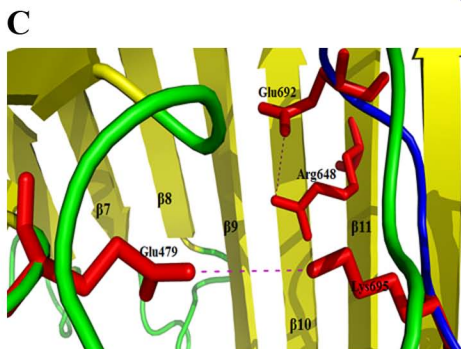
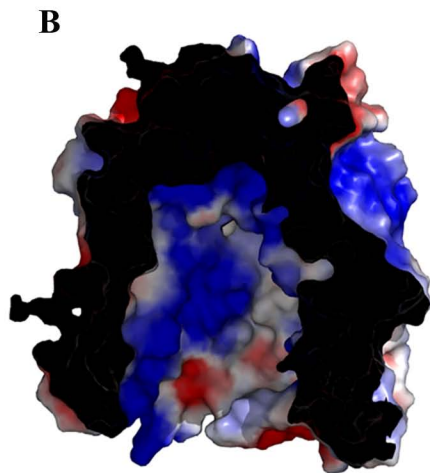
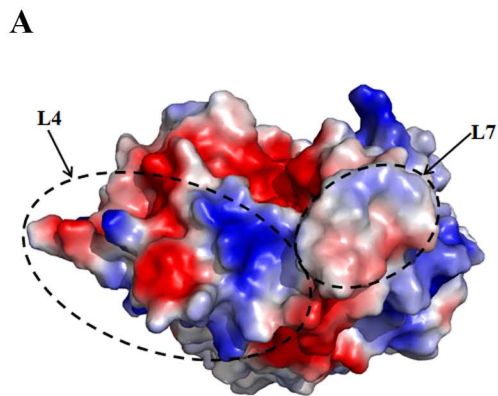
Supplemental Figure 1. Schematic representation of TP0326 recombinant constructs utilized in this study.



Supplemental Figure 2. Sensitivity and specificity of antisera. (A) Immunoblot reactivity of rat anti-β-barrel and -POTRA antisera against graded amounts of recombinant full-length TP0326 and *T. pallidum* whole cell lysates (1×10^8 *Tp* per lane). (B) Immunoblot reactivity of rat anti-L3β6, L4, L6, and -L7 antisera against graded amounts of recombinant β-barrel and *T. pallidum* whole cell lysates (1×10^8 *Tp* per lane). (C) Immunoblot reactivity of rabbit anti-L4 and anti-POTRA against recombinant full-length TP0326 and *T. pallidum* whole cell lysates (1×10^8 *Tp* per lane). (D) Reactivity of rat anti- L3β6 and normal rat antisera against L3 peptide.



Supplemental Figure 3. Sequence alignment of the β -barrel domains of *T. pallidum* (Tp), *N. gonorrhoeae* (Ng), *H. ducreyi* (Hd) and *E. coli* (Ec) BamA. The multiple sequence alignment was performed using the ClustalW server (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>). Identical positions are indicated by asterisks, while strongly homologous residues are denoted by colons. Weakly homologous residues are marked by single dots. The β -1 and β -16 strands of TP0326 β -barrel are indicated by yellow arrows. The residues essential for lateral opening of *E. coli* BamA are highlighted in red. The L4, L6 and L7 loops of TP0326 are depicted by black lines, whereas the corresponding loops in *Ng*, *Hd*, and *EC* BamAs are within the blue box.



Supplemental Figure 4. Electrostatic potential of the TP0326 β -barrel viewed from the extracellular face (A) and inside the β -barrel (B). (C) Salt bridges (dashed lines) predicted by ModWeb to stabilize L6 to L3 and $\beta 11$.

Supplemental Figure 5A

Nichols 1 MLKKASAFLLIASCVCMSLAWAQANDNWEYEGKPI SAISFEGLEYIARGOLDTIFSOYKGQK
SS14 1 MLKKASAFLLIASCVCMSLAWAQANDNWEYEGKPI SAISFEGLEYIARGOLDTIFSOYKGQK
Dal-1 1 MLKKASAFLLIASCVCMSLAWAQANDNWEYEGKPI SAISFEGLEYIARGOLDTIFSOYKGQK
Chicago 1 MLKKASAFLLIASCVCMSLAWAQANDNWEYEGKPI SAISFEGLEYIARGOLDTIFSOYKGQK
Mexico 1 MLKKASAFLLIASCVCMSLAWAQANDNWEYEGKPI SAISFEGLEYIARGOLDTIFSOYKGQK

Nichols 61 WTYEYLYLEILQKVYDLEYFSEVSPKAVPTDPEYQYVMLQFTVKERPSVKGIKMVGNSQIR
SS14 61 WTYEYLYLEILQKVYDLEYFSEVSPKAVPTDPEYQYVMLQFTVKERPSVKGIKMVGNSQIR
Dal-1 61 WTYEYLYLEILQKVYDLEYFSEVSPKAVPTDPEYQYVMLQFTVKERPSVKGIKMVGNSQIR
Chicago 61 WTYEYLYLEILQKVYDLEYFSEVSPKAVPTDPEYQYVMLQFTVKERPSVKGIKMVGNSQIR
Mexico 61 WTYEYLYLEILQKVYDLEYFSEVSPKAVPTDPEYQYVMLQFTVKERPSVKGIKMVGNSQIR

Nichols 121 SGDLLSKILLKKGDIYNEVKMKVDQESLRRHYLDQGYAAVKISCEAKTEAGGVVVFQFTIQ
SS14 121 SGDLLSKILLKKGDIYNEVKMKVDQESLRRHYLDQGYAAVKISCEAKTEAGGVVVFQFTIQ
Dal-1 121 SGDLLSKILLKKGDIYNEVKMKVDQESLRRHYLDQGYAAVKISCEAKTEAGGVVVFQFTIQ
Chicago 121 SGDLLSKILLKKGDIYNEVKMKVDQESLRRHYLDQGYAAVKISCEAKTEAGGVVVFQFTIQ
Mexico 121 SGDLLSKILLKKGDIYNEVKMKVDQESLRRHYLDQGYAAVKISCEAKTEAGGVVVFQFTIQ

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SS14 181 EGKQTVVSRIQFKGNKAFTE SVLKKV LSTQEARFLTSGVFKENALEADKAAVHSSYYAERG
Dal-1 181 EGKQTVVSRIQFKGNKAFTE SVLKKV LSTQEARFLTSGVFKENALEADKAAVHSSYYAERG
Chicago 181 EGKQTVVSRIQFKGNKAFTE SVLKKV LSTQEARFLTSGVFKENALEADKAAVHSSYYAERG
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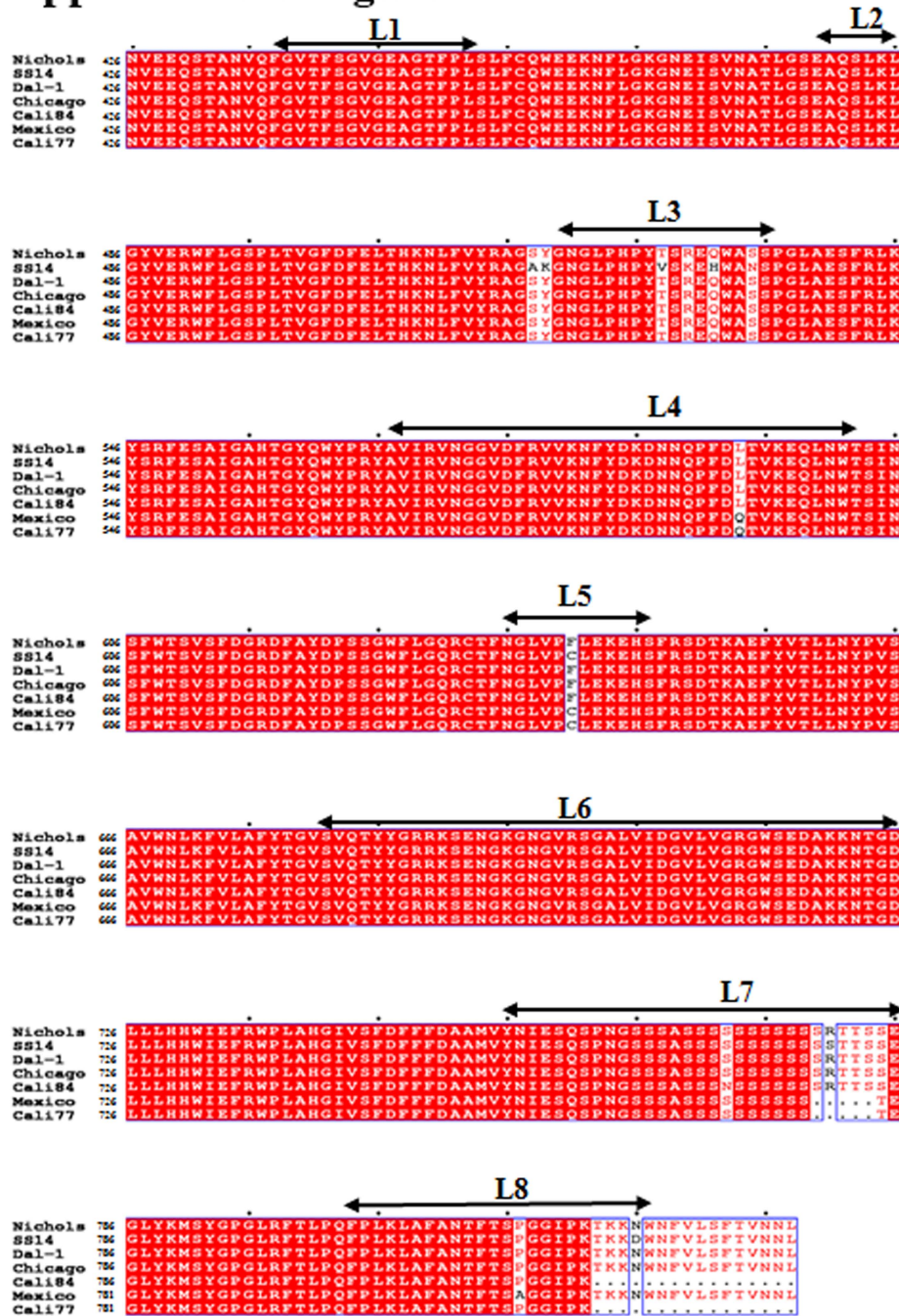
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Dal-1 241 YIDARVEGVAKTVDKKTDASRNLVTLTYTVVEGEQYRYGGVTIVGNQIFSTEELQAKIRL
Chicago 241 YIDARVEGVAKTVDKKTDASRNLVTLTYTVVEGEQYRYGGVTIVGNQIFSTEELQAKIRL
Mexico 241 YIDARVEGVAKTVDKKTDASRNLVTLTYTVVEGEQYRYGGVTIVGNQIFSTEELQAKIRL

Nichols 301 KRGAIMNMVAFEQGFQALADAYFENGYTSNYLNKEHRDTAEKTL SFKITVVERERSHVE
SS14 301 KRGAIMNMVAFEQGFQALADAYFENGYTSNYLNKEHRDTAEKTL SFKITVVERERSHVE
Dal-1 301 KRGAIMNMVAFEQGFQALADAYFENGYTSNYLNKEHRDTAEKTL SFKITVVERERSHVE
Chicago 301 KRGAIMNMVAFEQGFQALADAYFENGYTSNYLNKEHRDTAEKTL SFKITVVERERSHVE
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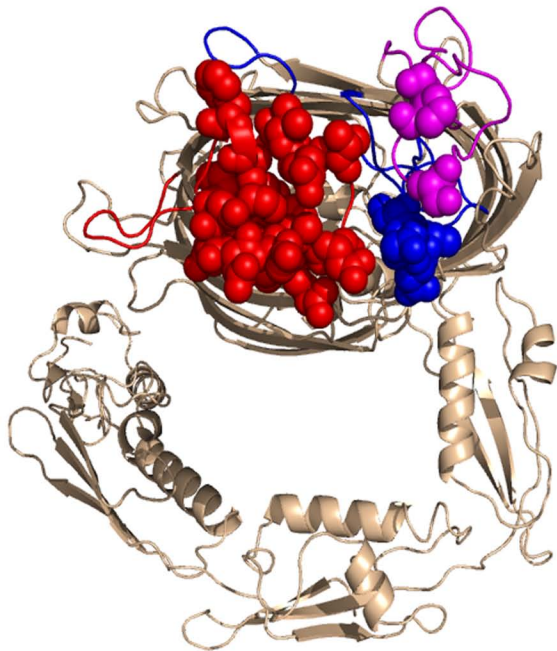
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Dal-1 361 HIIKGTKNTKDEVILREMLLKP GDVFSKSKFTDSLRLNLFNLYRFSSLVDPVRPGSEQDL
Chicago 361 HIIKGTKNTKDEVILREMLLKP GDVFSKSKFTDSLRLNLFNLYRFSSLVDPVRPGSEQDL
Mexico 361 HIIKGTKNTKDEVILREMLLKP GDVFSKSKFTDSLRLNLFNLYRFSSLVDPVRPGSEQDL

Nichols 421 VDIIIL
SS14 421 VDIIIL
Dal-1 421 VDIIIL
Chicago 421 VDIIIL
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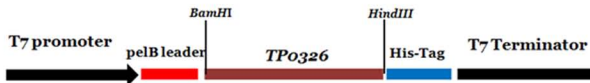
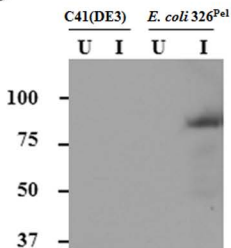
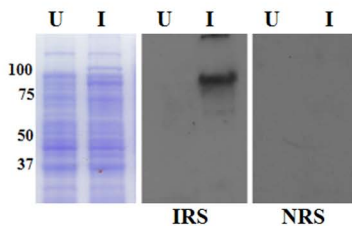
Supplemental Figure 5B



Supplemental Figure 5. Multiple sequence alignment of POTRA arm (A) and β -barrel (B) sequences from geographically diverse *T. pallidum* strains. Identical residues are highlighted in red. Accession numbers: Nichols (AAC65313); Chicago (ADD72452); SS14 (ACD70752); Dallas-1 (ABW94728); Mexico A (ABW94735), Cali84 (KP713716), Cali77 (KP713715).



Supplemental Figure 6. Predicted conformational B cell epitopes in TP0326 are shown as spheres. The predicted three large extracellular loops L4, L6 and L7 are shown in red, blue and magenta, respectively.

A**B****C**

Supplemental Figure 7. Construction and expression of 326^{pel} in *E. coli*. (A) Orientation of the 326^{pel} construct in pET26b. Shown are the positions of PelB leader sequence, hexahistidine-tag and restriction sites used for cloning. (B) Immunoblot analysis of un-induced (U) and IPTG-induced (I) C41(DE3) or *E. coli* 326^{pel} cells with rat anti-POTRA antiserum. (C) Immunoblot analysis of un-induced (U) and IPTG-induced (I) *E. coli* 326^{pel} with C41(DE3)-adsorbed IRS and NRS. In panels B and C, molecular mass standards (kDa) are indicated on the right.