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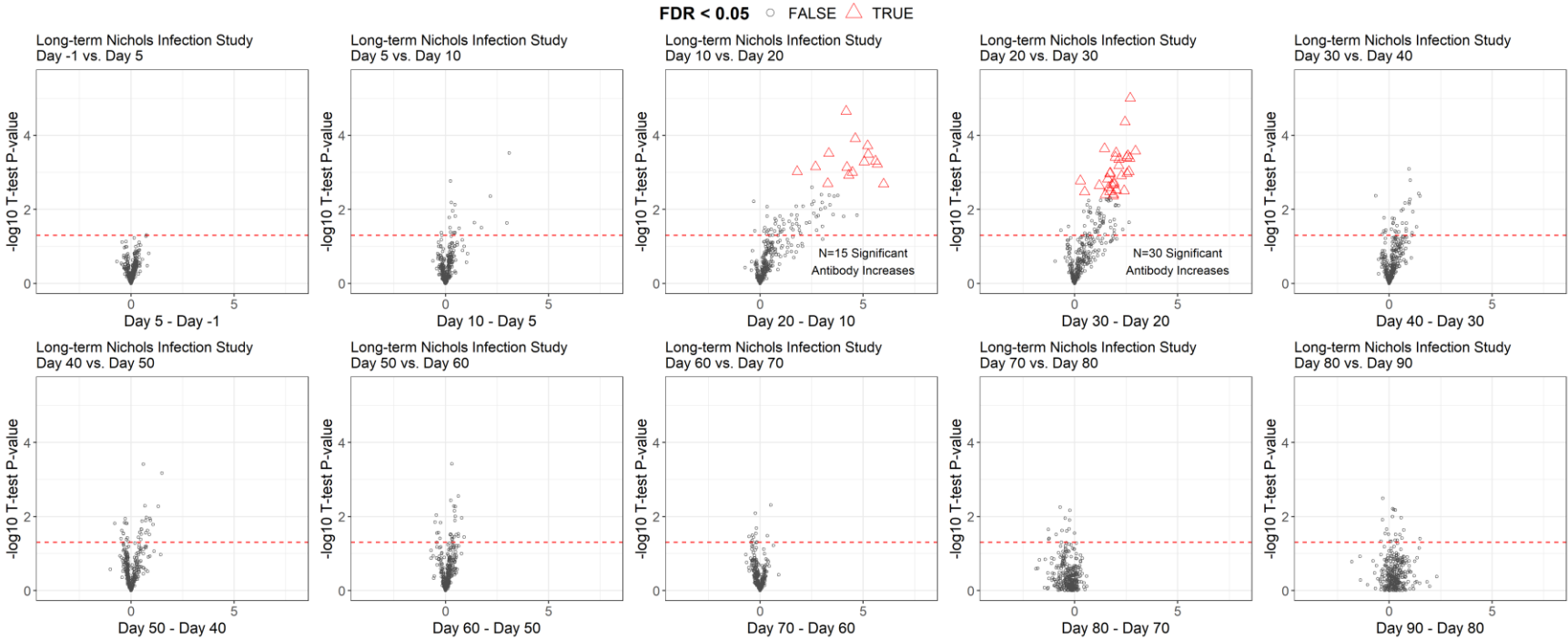
Supplemental information

A novel pan-proteome array for high-throughput profiling of the humoral response to *Treponema pallidum*

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FIGURE S1

A. Nichols Long-Term Infection Sequential Change in Antibody Levels



B. SS14 Long-Term Infection Sequential Change in Antibody Levels

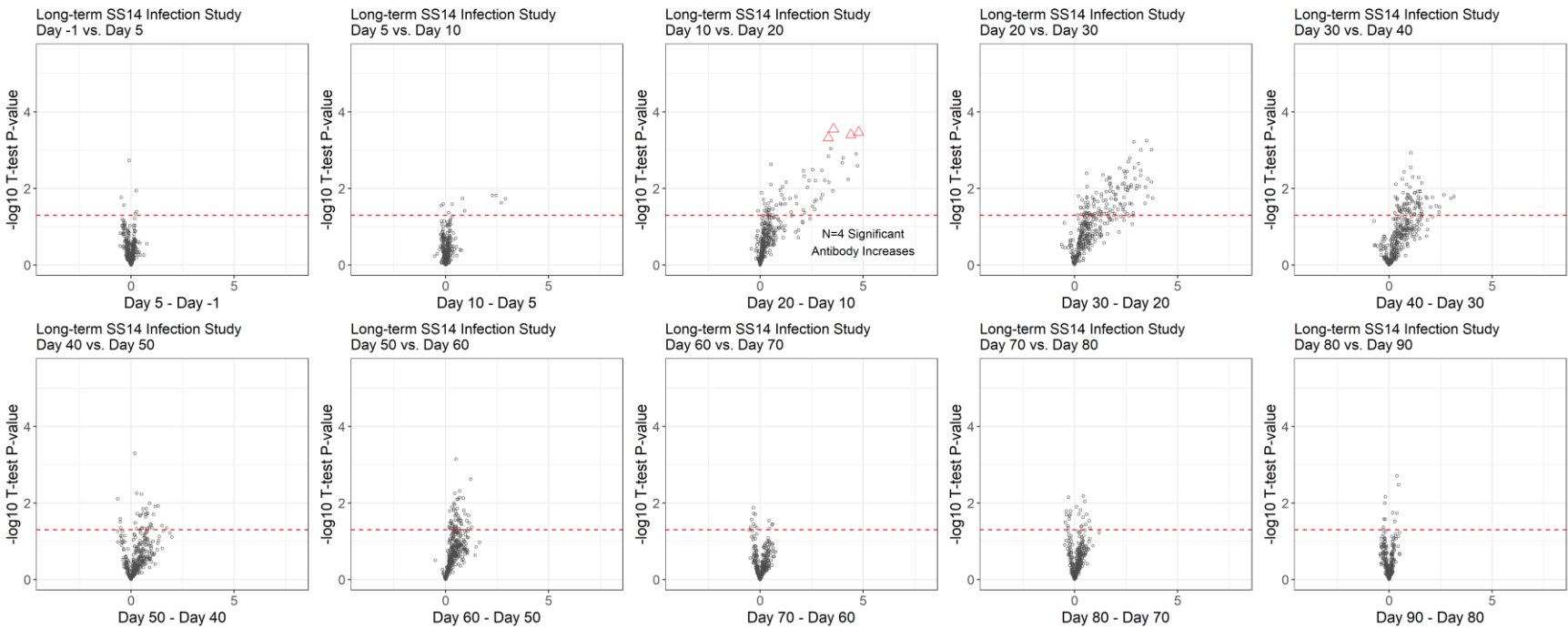
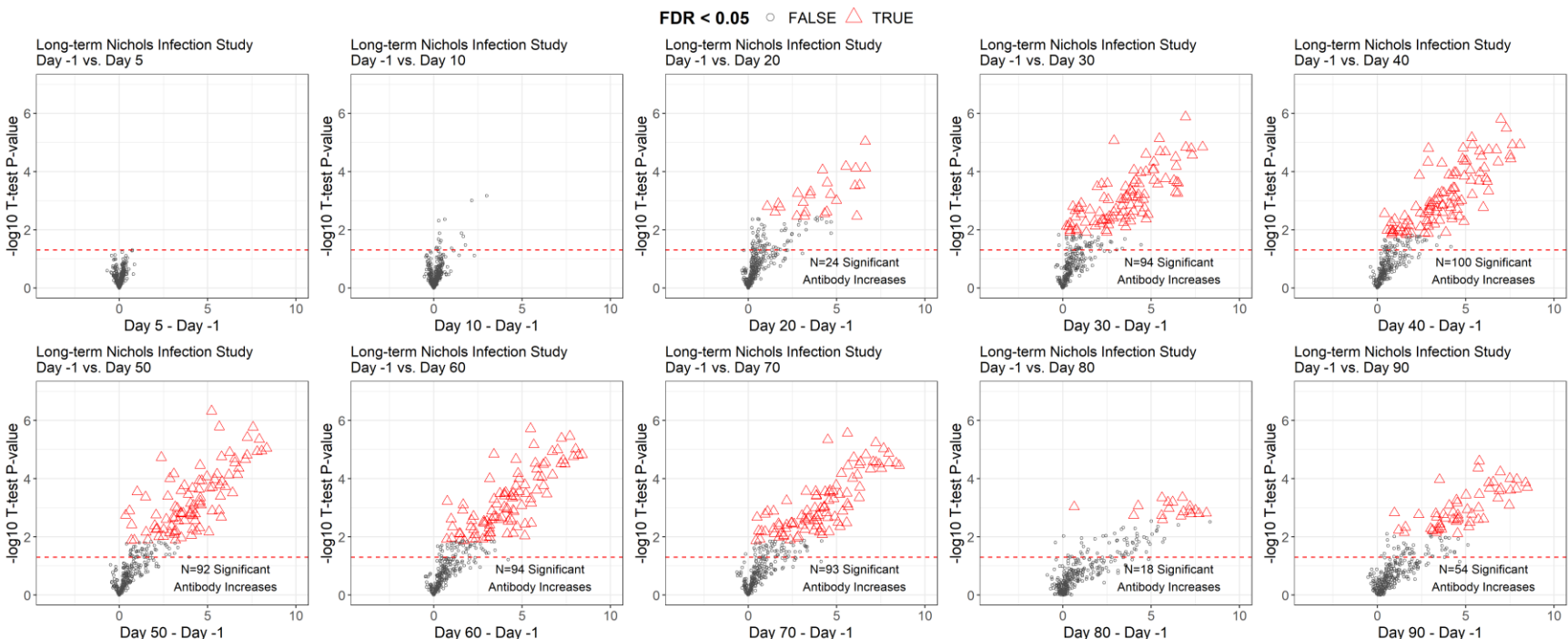
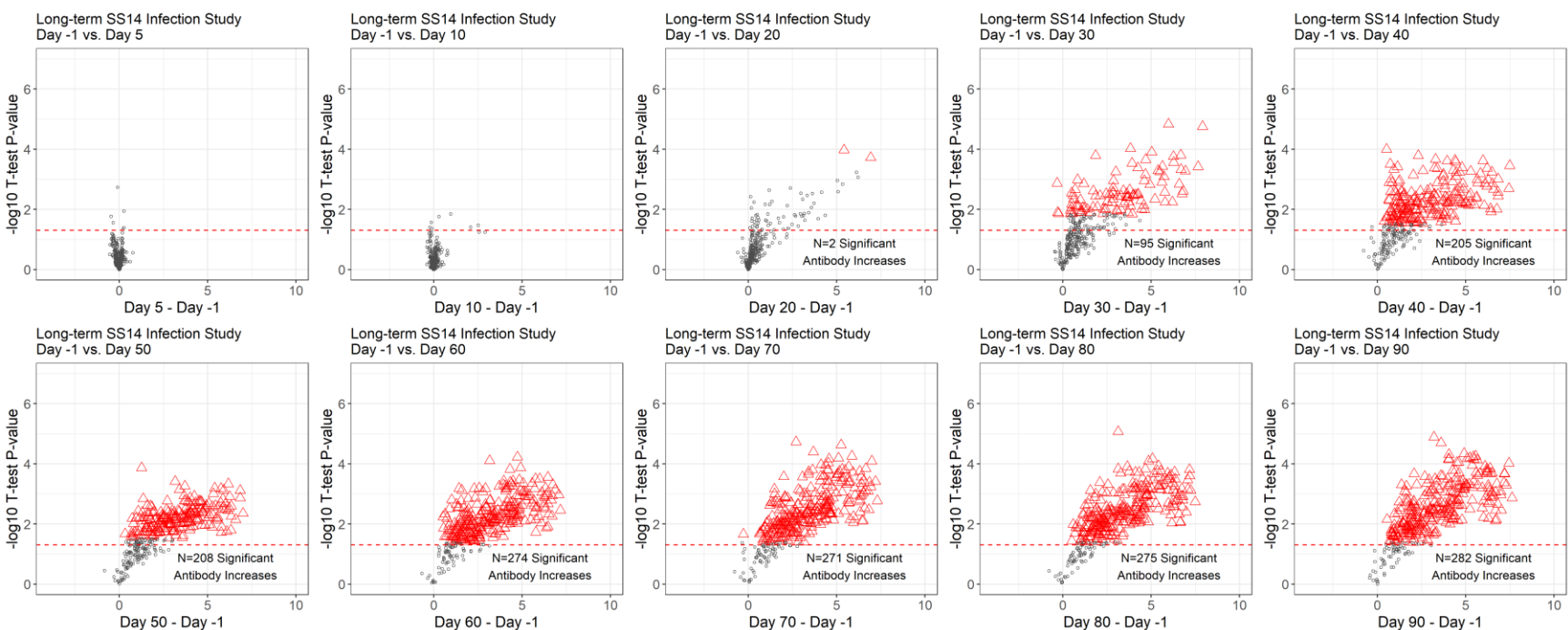


FIGURE S2

A. Nichols Long-Term Infection Change in Antibody Levels from Baseline



B. SS14 Long-Term Infection Change in Antibody Levels from Baseline



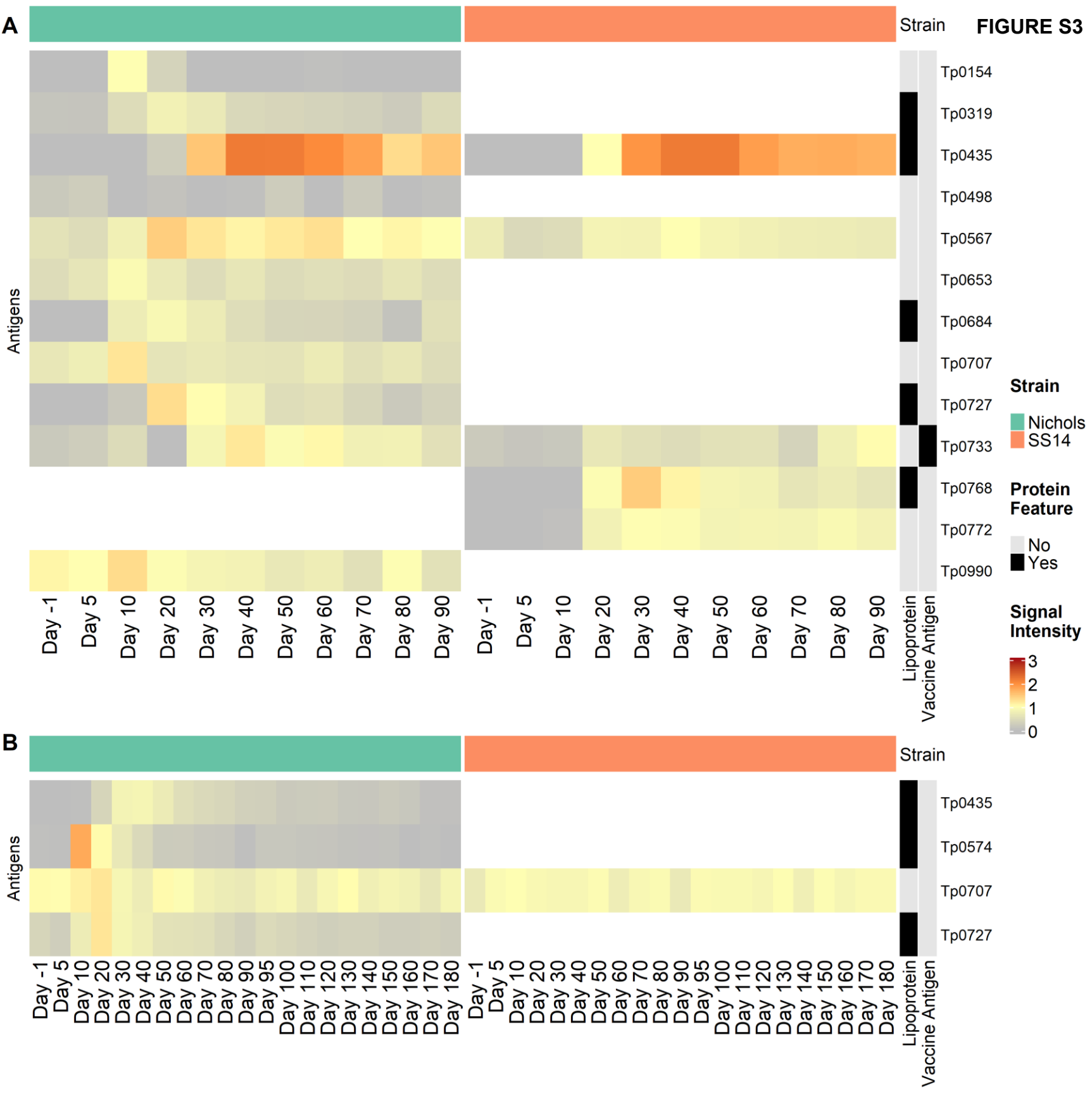


FIGURE S4

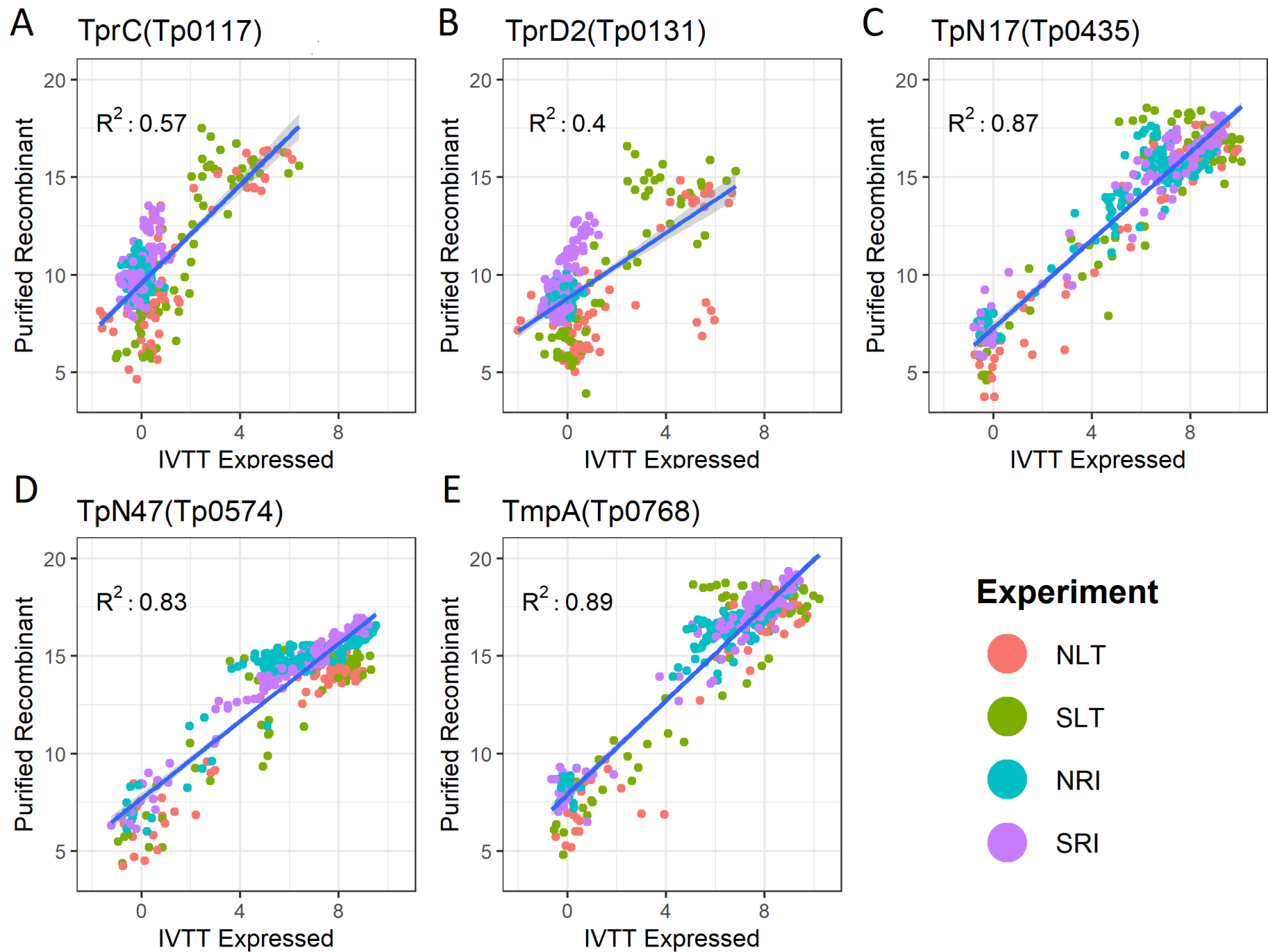


Table S4. Failed cloning

Spot.ID	Gene.ID	Old Gene ID	Printing.ID (Protein.ID)
TPANIC_RS05345	TPANIC_RS05345	TPANIC_0749	DUF2715 domain-containing protein
TPANIC_RS05090	TPANIC_RS05090	TPANIC_1029	possible ATP-dependent RNA helicase
TPANIC_RS04010	TPANIC_RS04010	TPANIC_0810	DNA adenine methyltransferase
TPANIC_RS01705	TPANIC_RS01705	TPANIC_0348	heptaprenyl diphosphate synthase component I domain
TPANIC_RS00135	TPANIC_RS00135	TPANIC_0027	HlyC putative hemolysin
TPANIC_RS03085	TPANIC_RS03085	TPANIC_0624	CorC family transporter
TPANIC_RS04770	TPANIC_RS04770	TPANIC_0968	DUF3798 domain-containing protein
TPANIC_RS00755	TPANIC_RS00755	TPANIC_0147	glycosyl hydrolase family protein
TPANIC_RS03265	TPANIC_RS03265	TPANIC_0660	flgK flagellar hook-associated protein 1
TPANIC_RS00405	TPANIC_RS00405	TPANIC_0083	hypothetical protein
TPANIC_RS01685	TPANIC_RS01685	TPANIC_0344	hypothetical protein
TPANIC_RS01585	TPANIC_RS01585	TPANIC_0324	hypothetical protein
TPANIC_RS04225	TPANIC_RS04225	TPANIC_0854	probable lipoprotein

SUPPLEMENTAL INFORMATION TITLES AND LEGENDS

Figure S1. Longitudinal trajectory of IgG binding levels between sequential time points in long-term (N.LT and S.LT) infected rabbits, related to Figure 2. Volcano plots showing the difference in normalized IgG binding between timepoints for each reactive protein on the array (x-axis) by the inverse \log_{10} P-value of paired Student's T-tests. In each plot, the horizontal red dashed line represents an unadjusted P-value of 0.05, and all values above the red line are below 0.05. After adjustment for the false discovery rate, antibody responses that remained statistically significant were highlighted as red triangles. **(A)** Sequential time point differences (Day -1 vs day 5; day 5 vs day 10, day 10 vs day 20 and so on) for long-term Nichols-infected animals. **(B)** Sequential time point differences for long-term SS14-infected animals.

Figure S2. Longitudinal trajectory of IgG binding levels versus baseline in long-term (N.LT and S.LT) infected rabbits, related to Figure 3. Volcano plots showing the difference in normalized IgG binding of each post-infection time point versus the pre-infection (Day -1) time point for each reactive protein on the array (x-axis) by the inverse \log_{10} P-value of paired Student's T-tests. In each plot, the horizontal red dashed line represents an unadjusted P-value of 0.05, and all values above the red line are below 0.05. After adjustment for the false discovery rate, antibody responses that remained statistically significant were highlighted as red triangles. **(A)** Sequential time point differences for long-term Nichols-infected animals vs day -1. **(B)** Sequential time point differences for long-term SS14-infected animals vs day -1.

Figure S3. IgM antibody binding profile in LT and RI rabbit groups, related to STAR METHODS-Array probing. **(A)** Heatmap of the 13 total antigens overall recognized in long-term Nichols-infected animals (left side, green header), and SS14-infected rabbits (right side, orange header) over the 90-day infection period. Only the *T. pallidum* proteins with significant immune reactions, or at least 4 out of 6 rabbits that responded to the protein in either strain group, were included in the heatmap. The category of each protein as a lipoprotein or possible vaccine candidate is shown in black and grey at the right of the heatmap. **(B)** Heatmap of the four antigens overall recognized in Nichols-infected animals (left side, green header) and SS14-infected

rabbits (right side, orange header) over the 180-day infection/treatment/re-infection experiment. Treatment occurred at day 30 post-inoculation, and reinfection with the homologous strain occurred 60 days post-treatment. Only the *T. pallidum* proteins with significant immune reactions, or at least four out of six rabbits that responded to the protein in either strain group, were included in the heatmap. The category of each protein (lipoprotein or a vaccine candidate) is shown in black and grey at the right of the heatmap.

Figure S4. Correlation between serum reactivity of IVTT expressed protein and control recombinant proteins expressed in *E. coli*, related to STAR METHODS-Array probing. (A-E)

Pearson correlations between signal to IVTT-expressed and purified recombinant protein TprC (Tp0117), the SS14-specific TprD2 (Tp0131) antigen, Tpn17 (Tp0435), Tpn47 (Tp0574), and TmpA (Tp0768). R values are reported within individual panels. Rabbit groups are color-coded.

Table S4. Failed cloning, related to STAR METHODS - Array construction.