Supplementary Figures / Table:

Adenovirally-Induced Polyfunctional T Cells Do Not Necessarily Recognize the Infected Target: Lessons from a Phase I Trial of the AERAS-402 Vaccine

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Affiliations:

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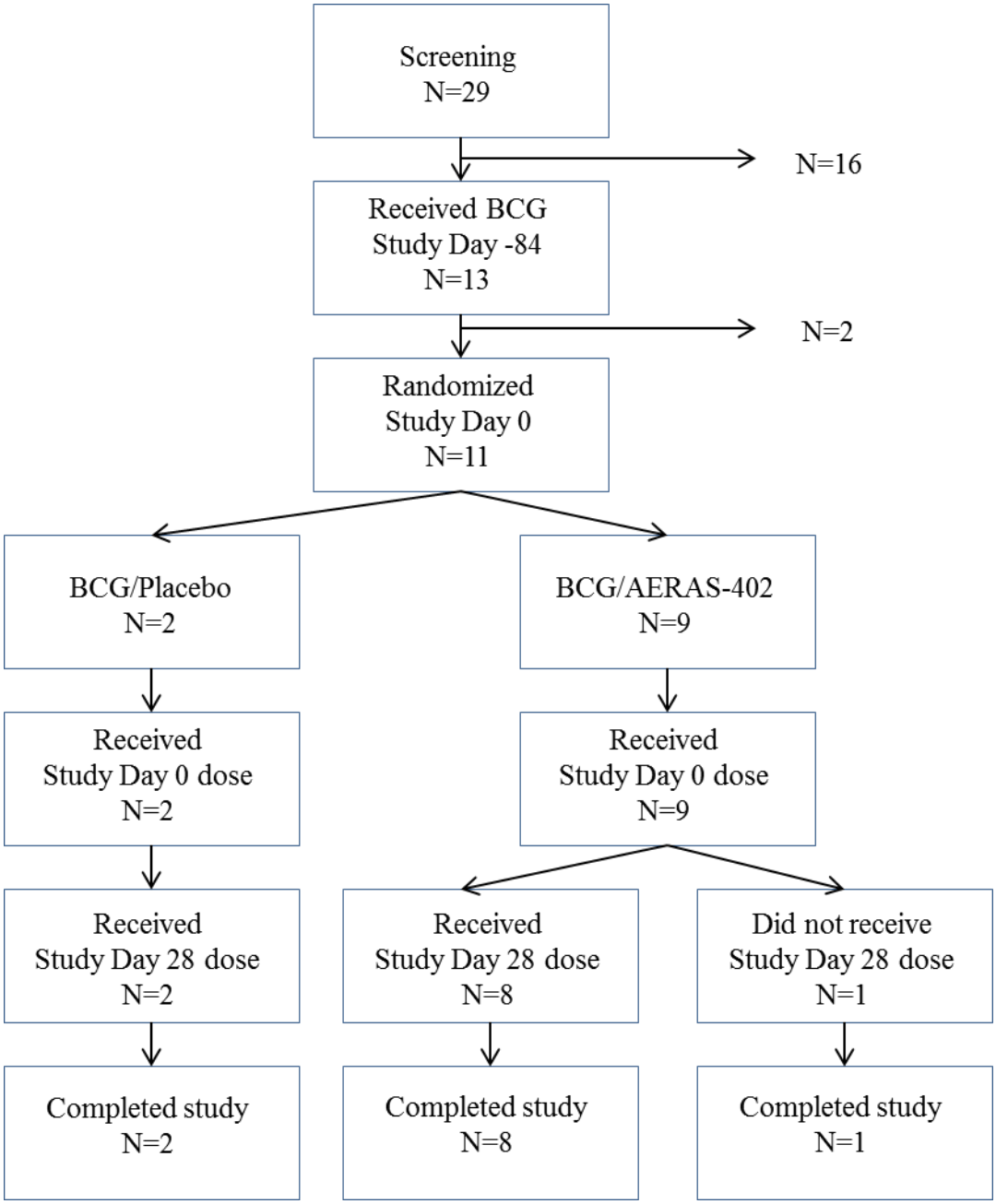
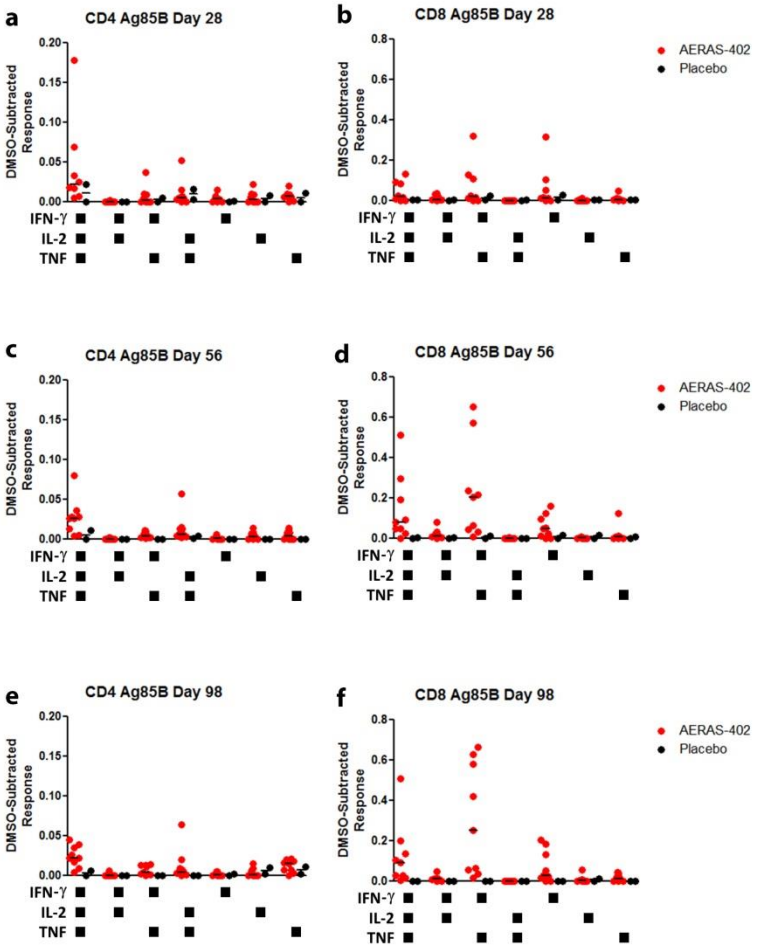
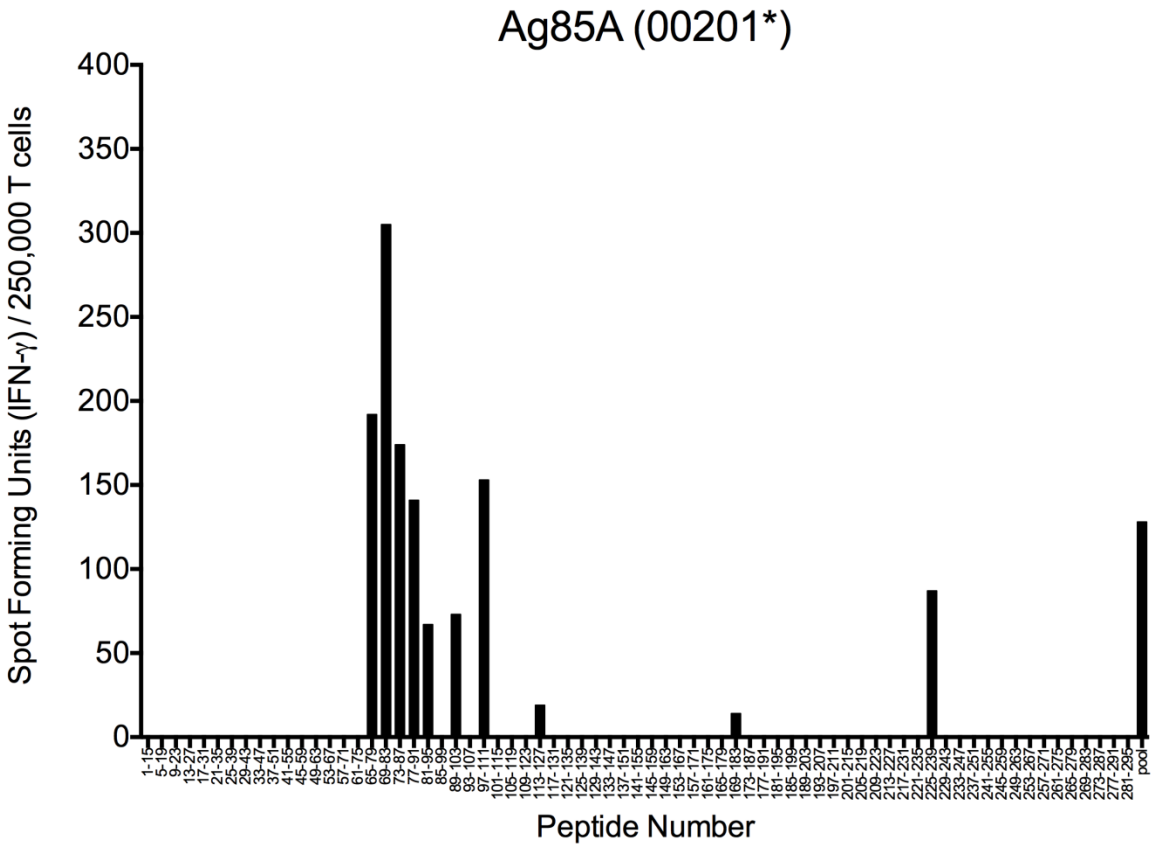


Supplementary Figure S1. Consort Diagram. All participants received BCG on day -84 and 9 participants received AERAS-402 on day 0 and 8 participants received AERAS-402 on day 28. Two participants received placebo on day 0 and day 28.



Supplementary Figure S2. Polyfunctional Analysis on Study Days 28, 56 and 98. PBMC and leukapheresis specimens from study days 28 (a, b), 56 (c, d) and 98 (e, f) were thawed, rested overnight, and stimulated for 5-7 hours with DMSO (negative control), SEB (positive control), or the Ag85B peptide pool. Specimens were then stained for viability, phenotypic markers, and intracellular cytokine expression and evaluated by flow cytometry. Data were analyzed using FlowJo software to generate cytokine Boolean gates. Each gate was subjected to DMSO subtraction to remove background and plotted as percent response of CD4 (a, c, e) or CD8 (b, d, f) T cell populations. Each circle represents the response from a single participant. Bars represent the median response for each group. Data is shown for participants immunized with BCG on Study Day -84 and placebo (black circles) and participants vaccinated with BCG on Study Day -84 followed by vaccination with AERAS-402 on Study Days 0 and 28 ($3x10^{10}$ vp; red circles). The gating strategy is shown in Figure 2, panel a.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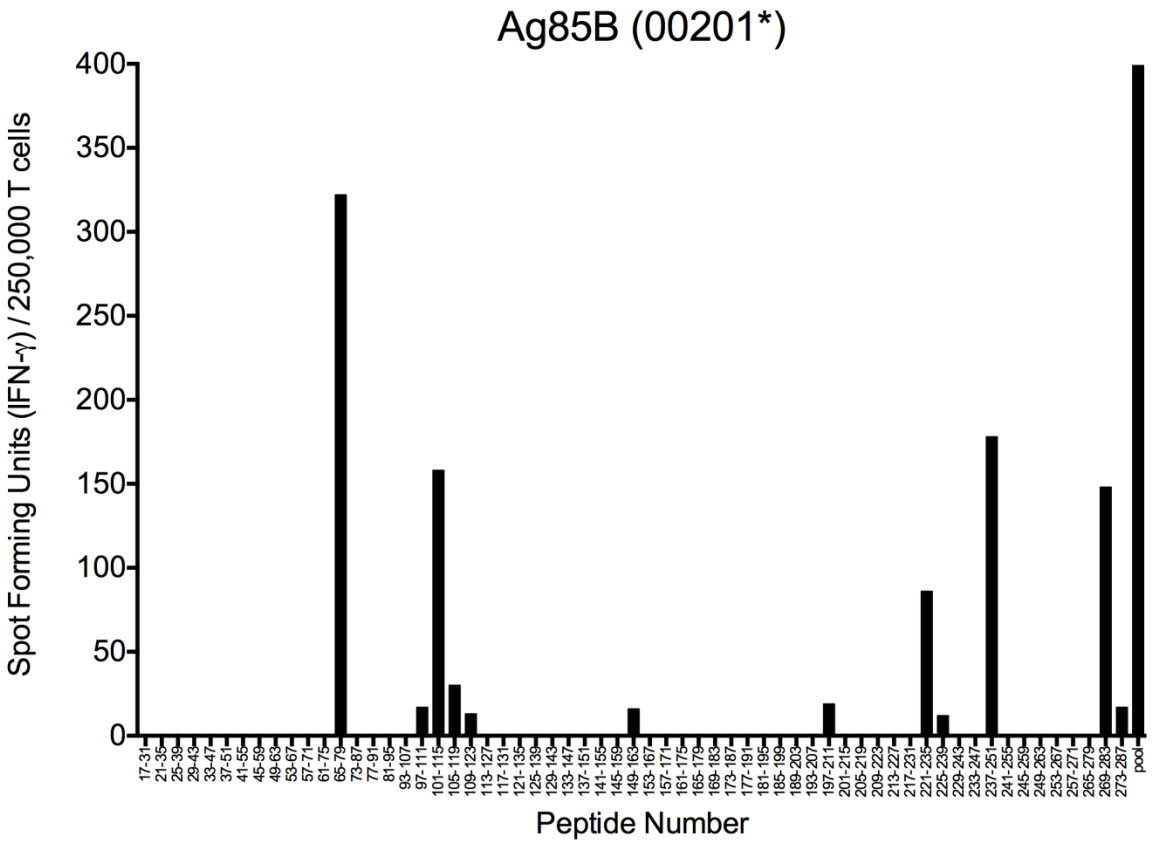
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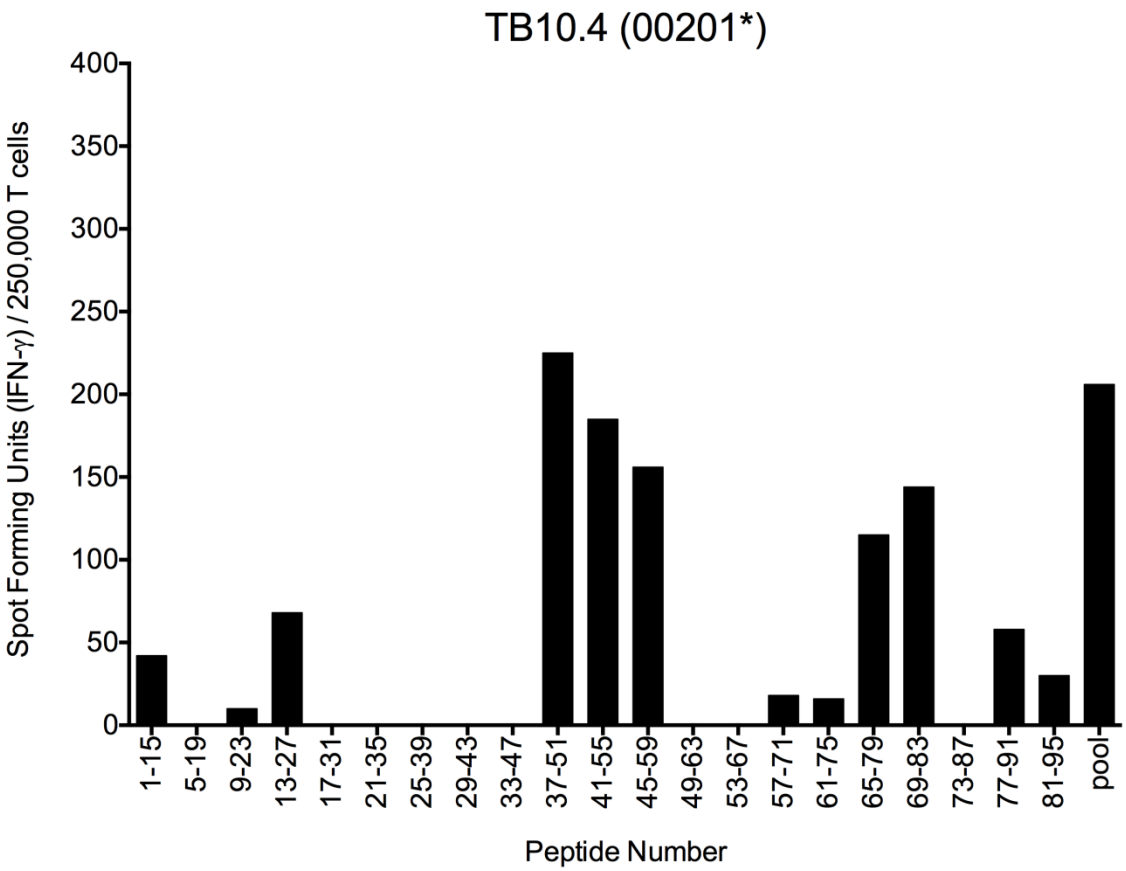
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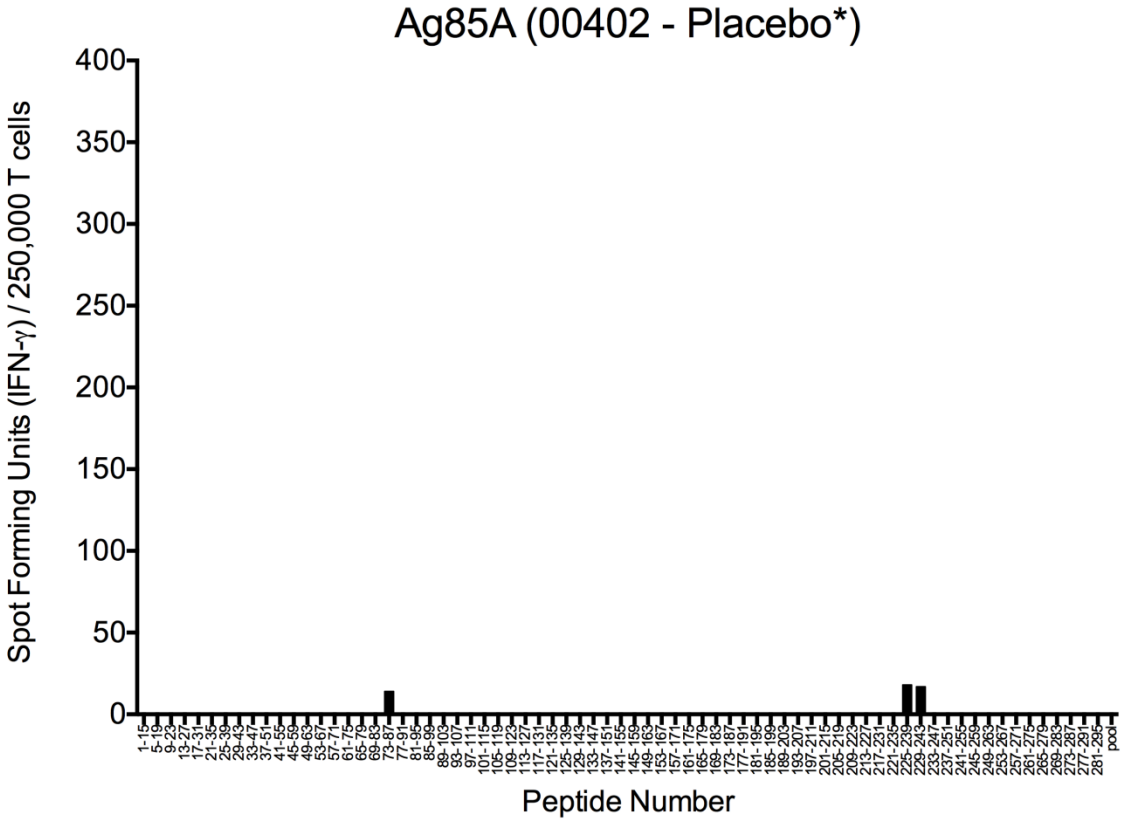
pool
Peptide Number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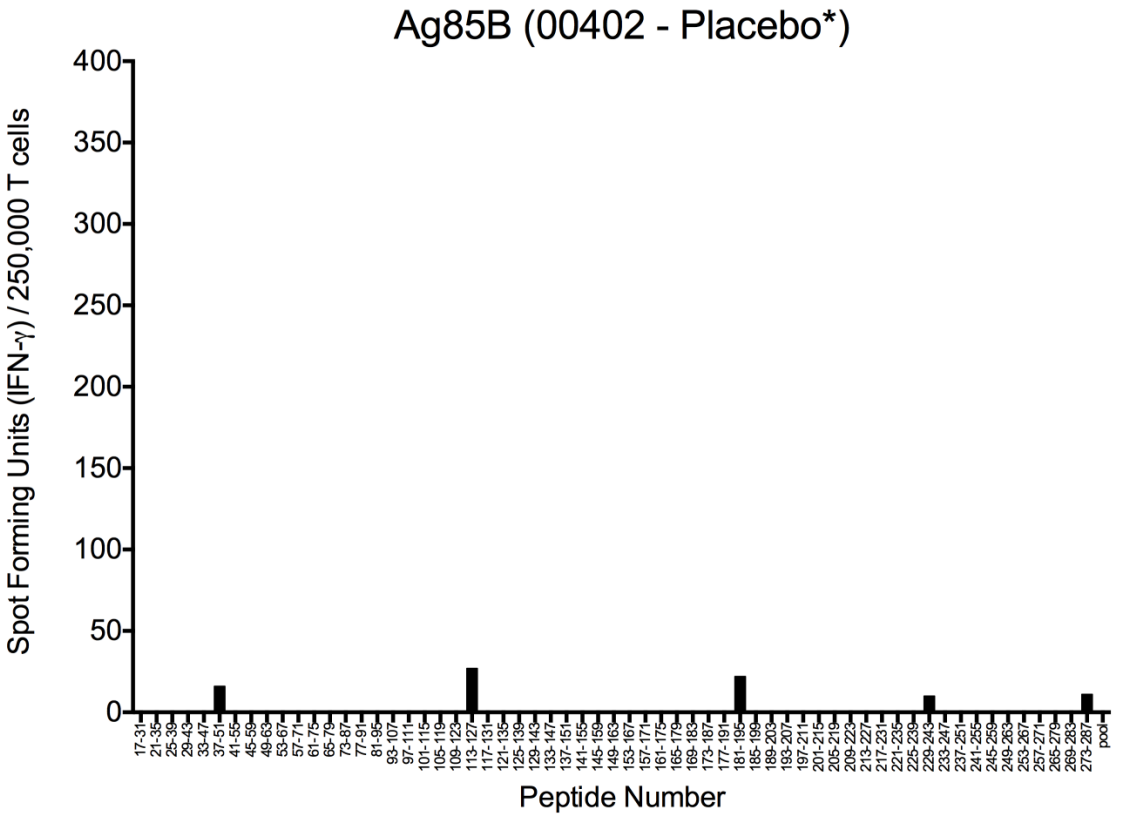
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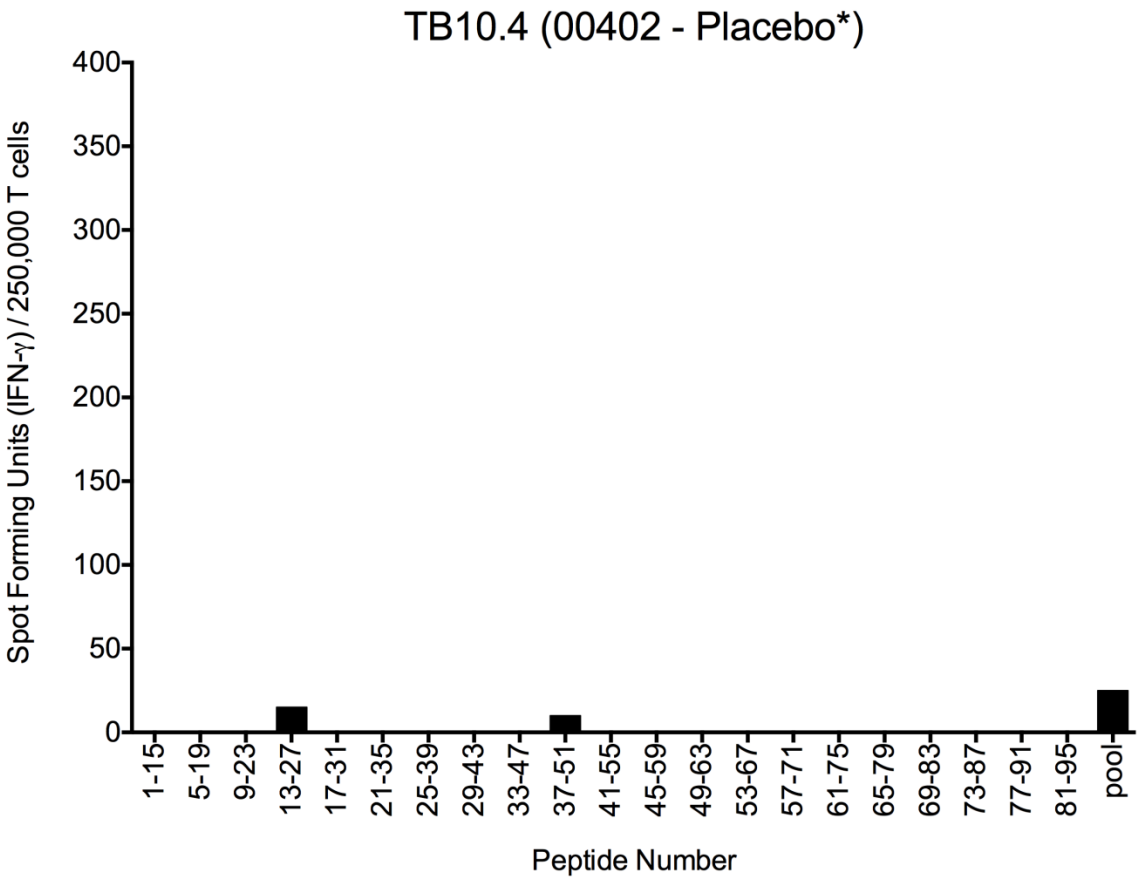
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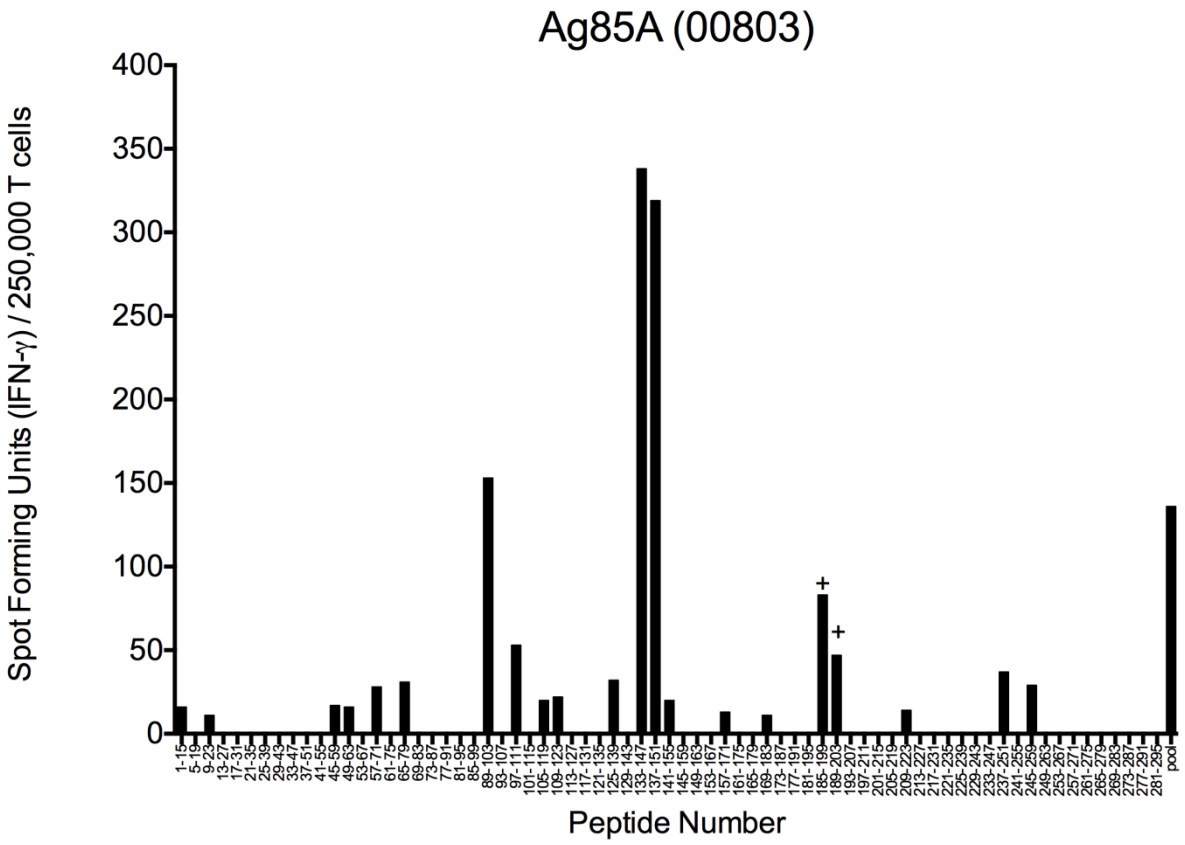
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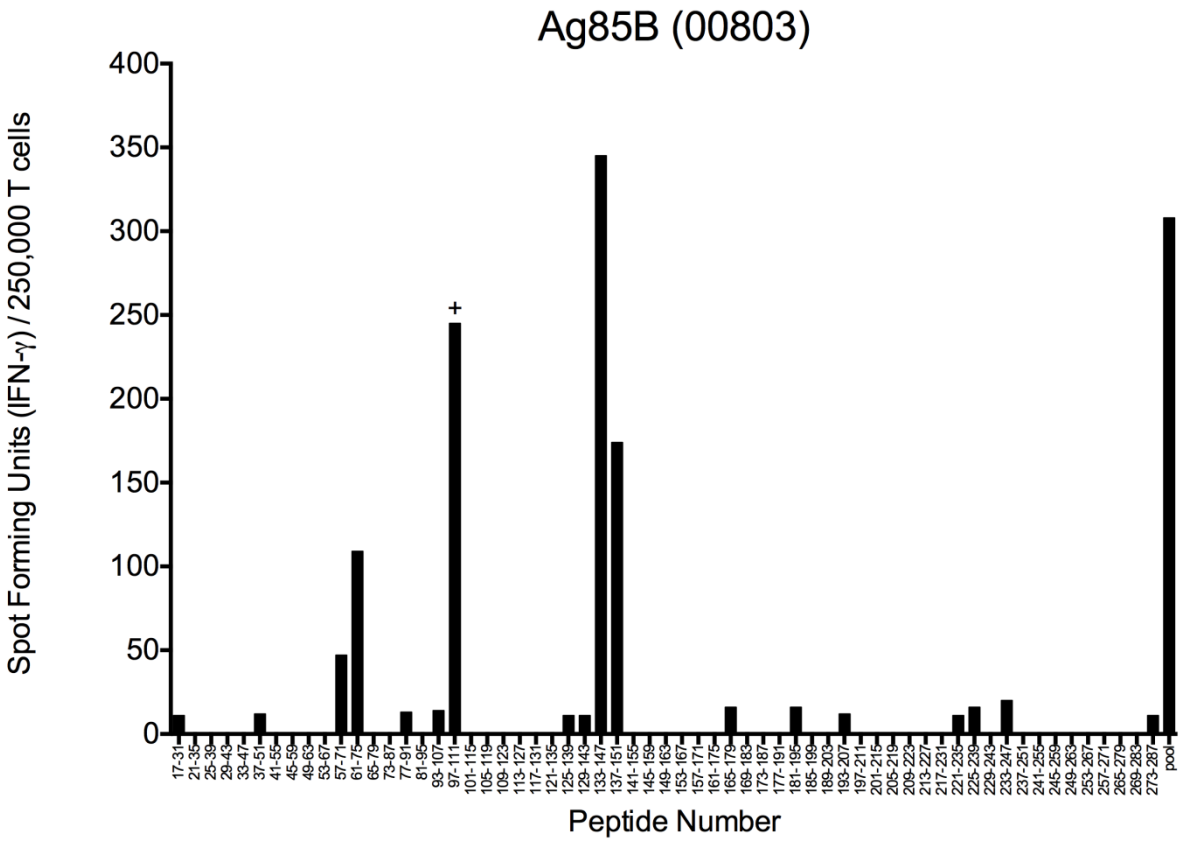
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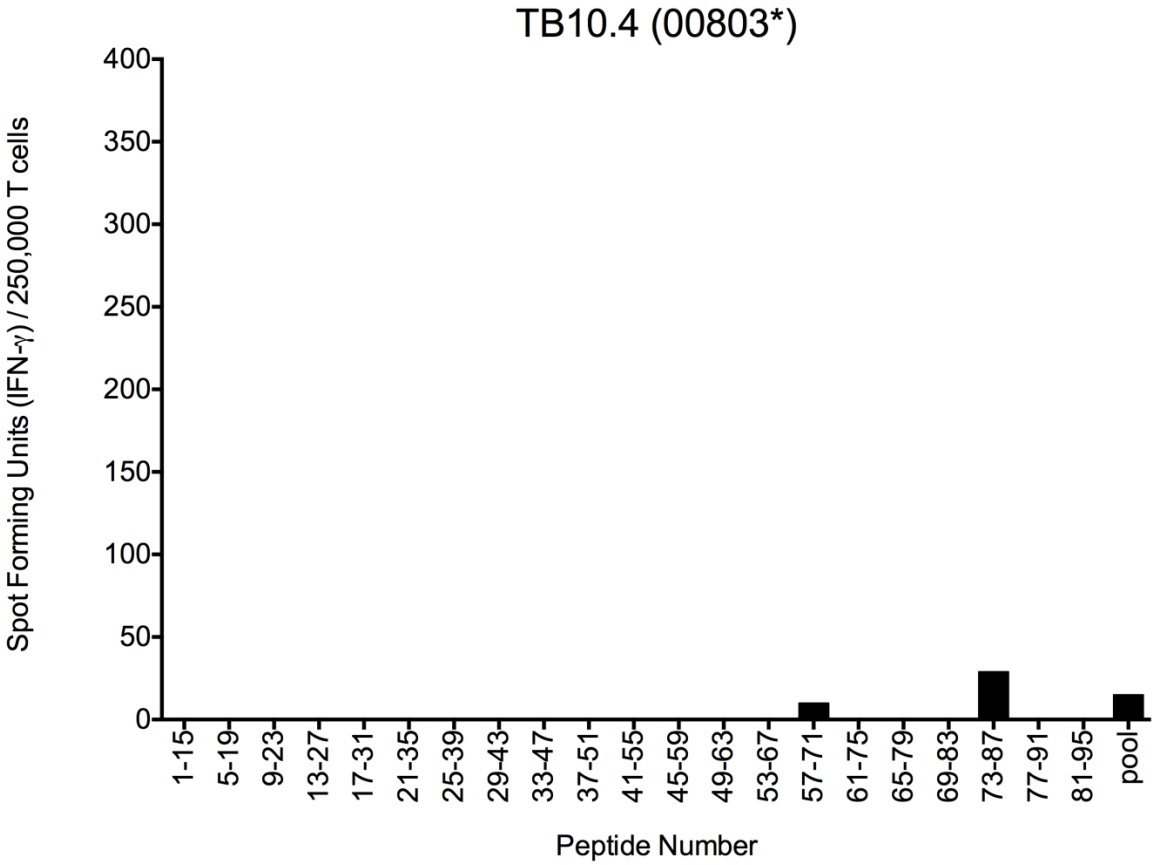
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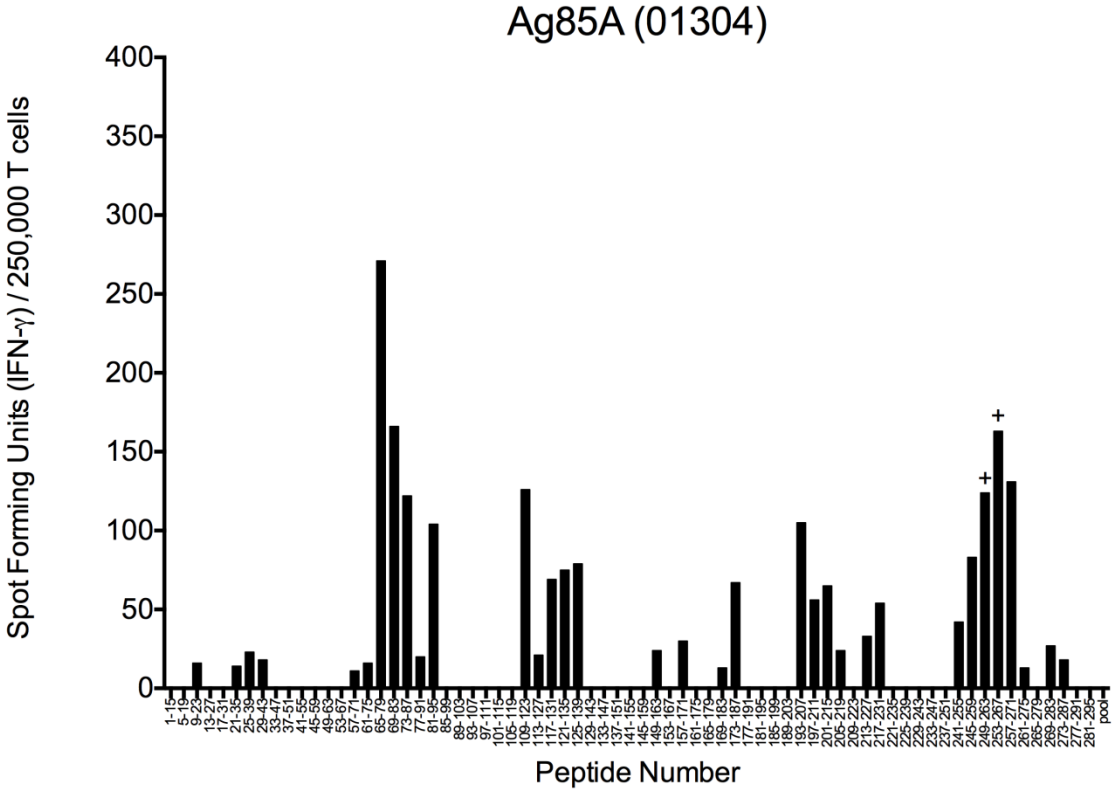
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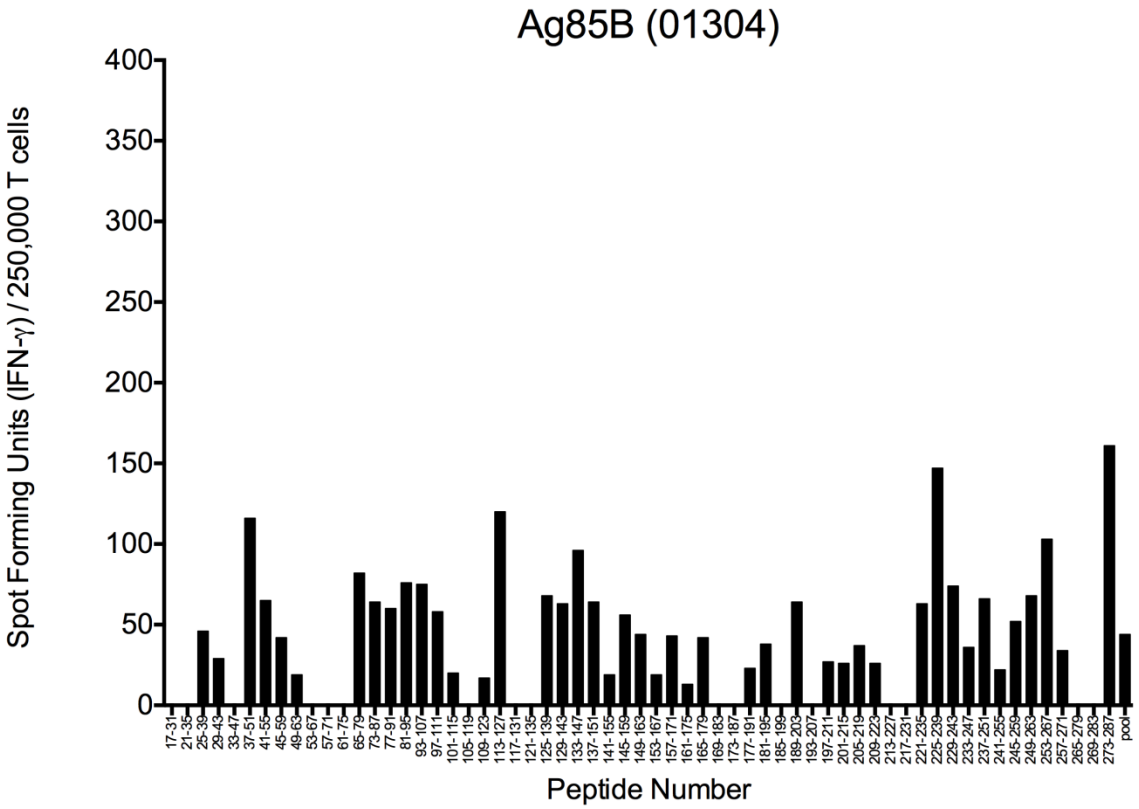
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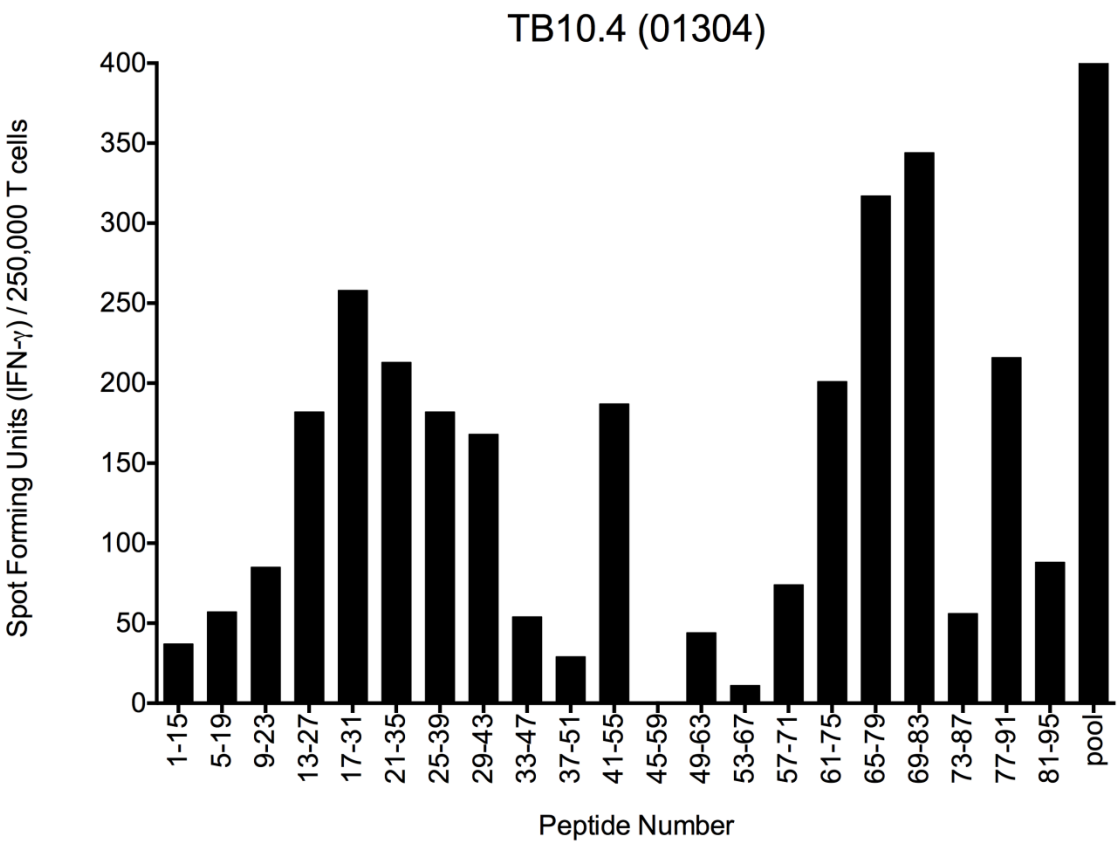
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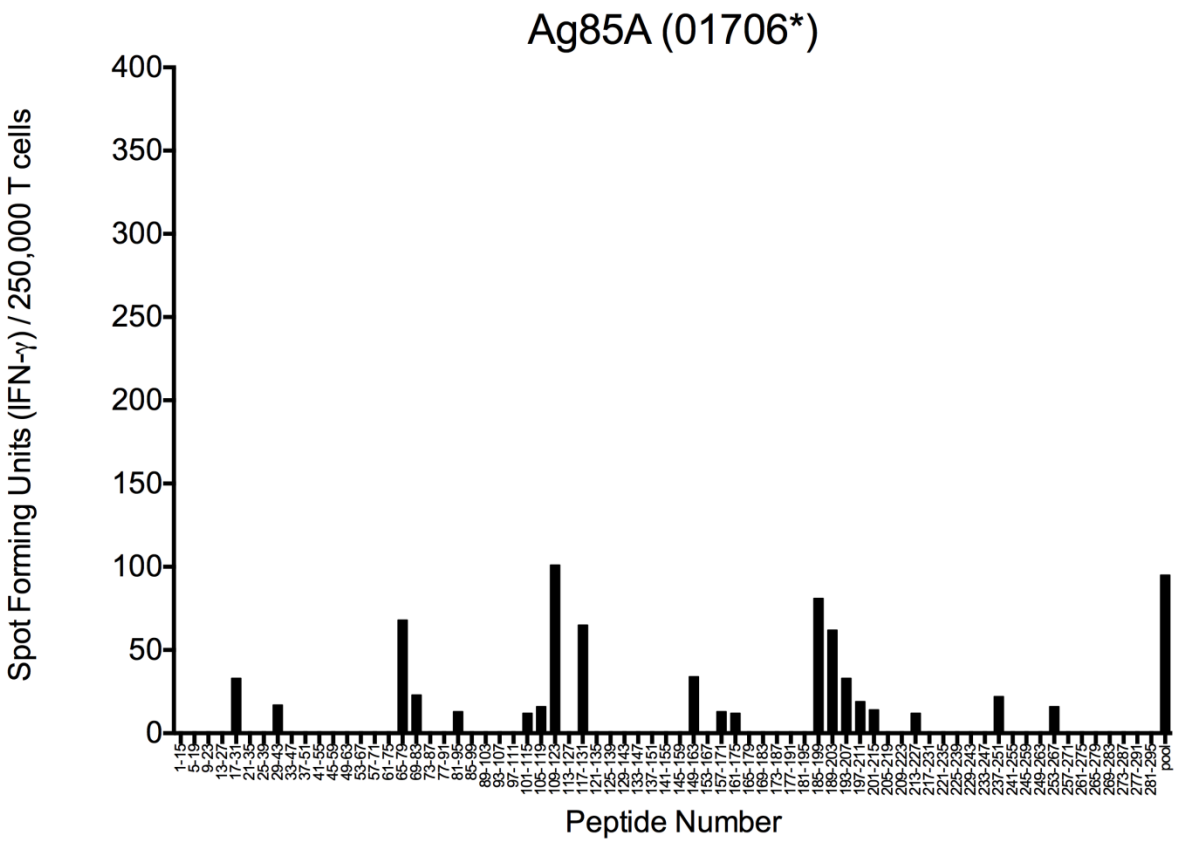
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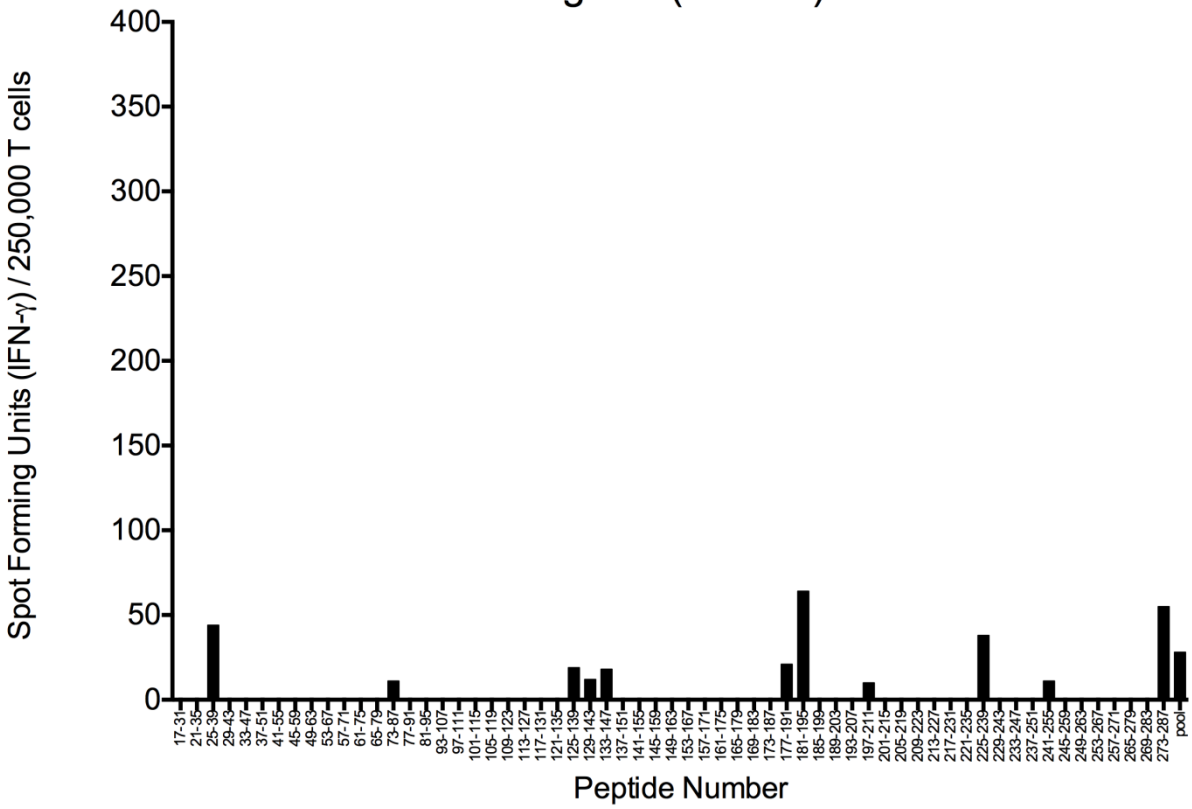
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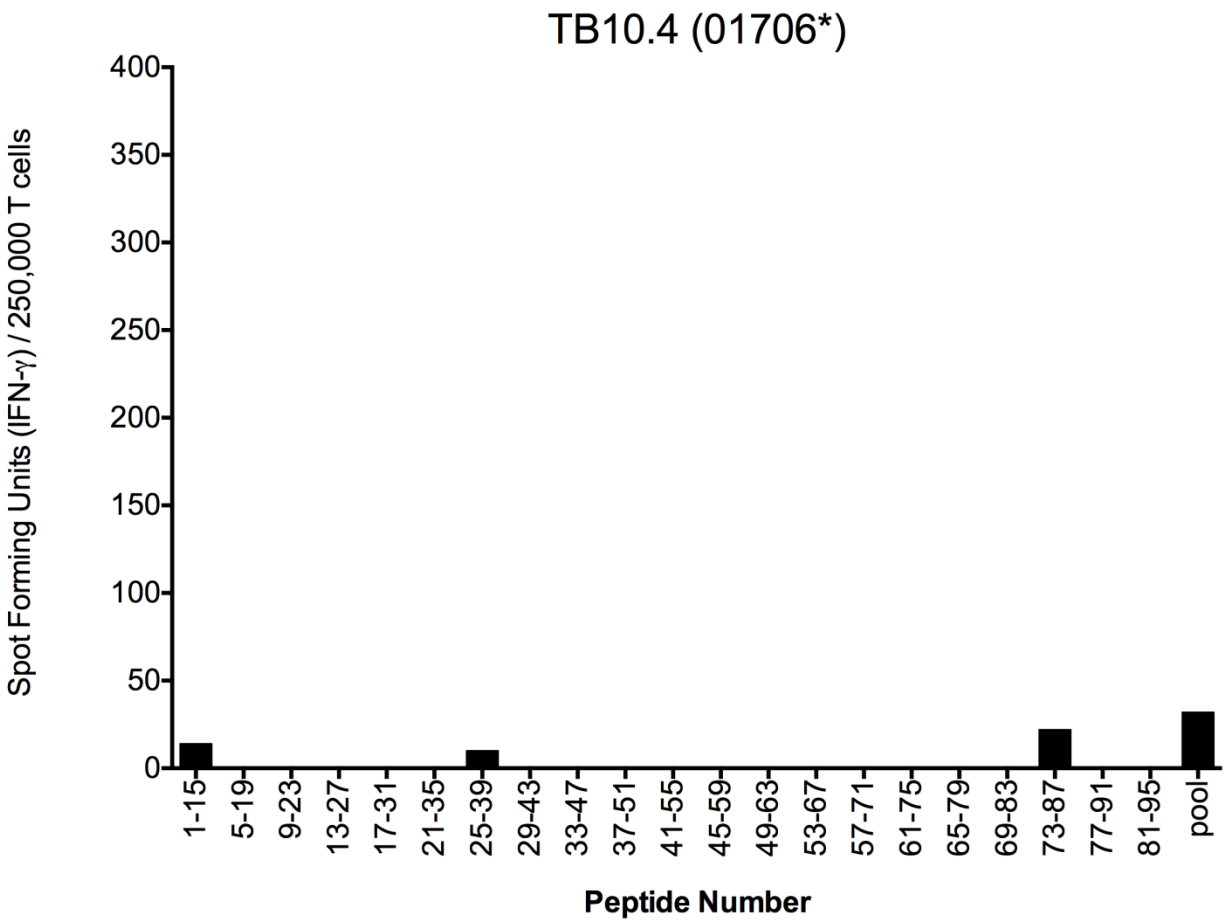
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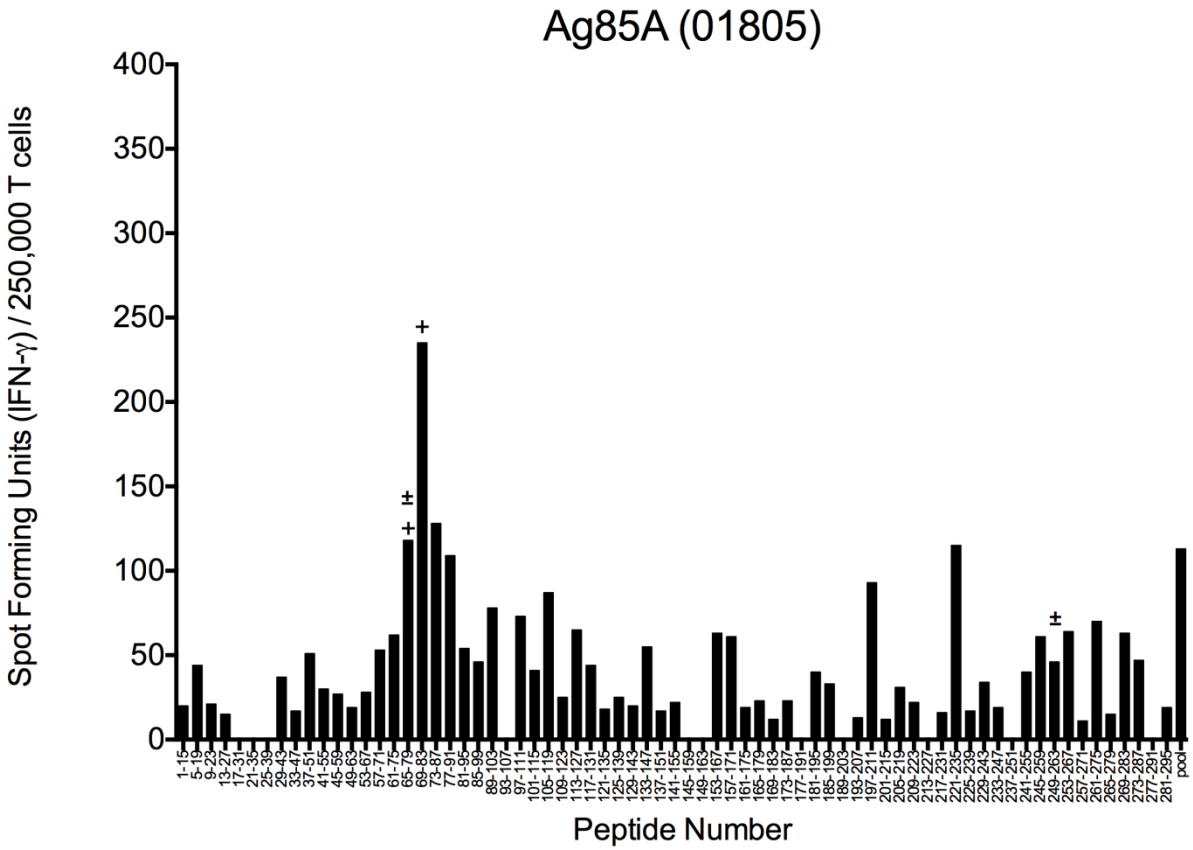

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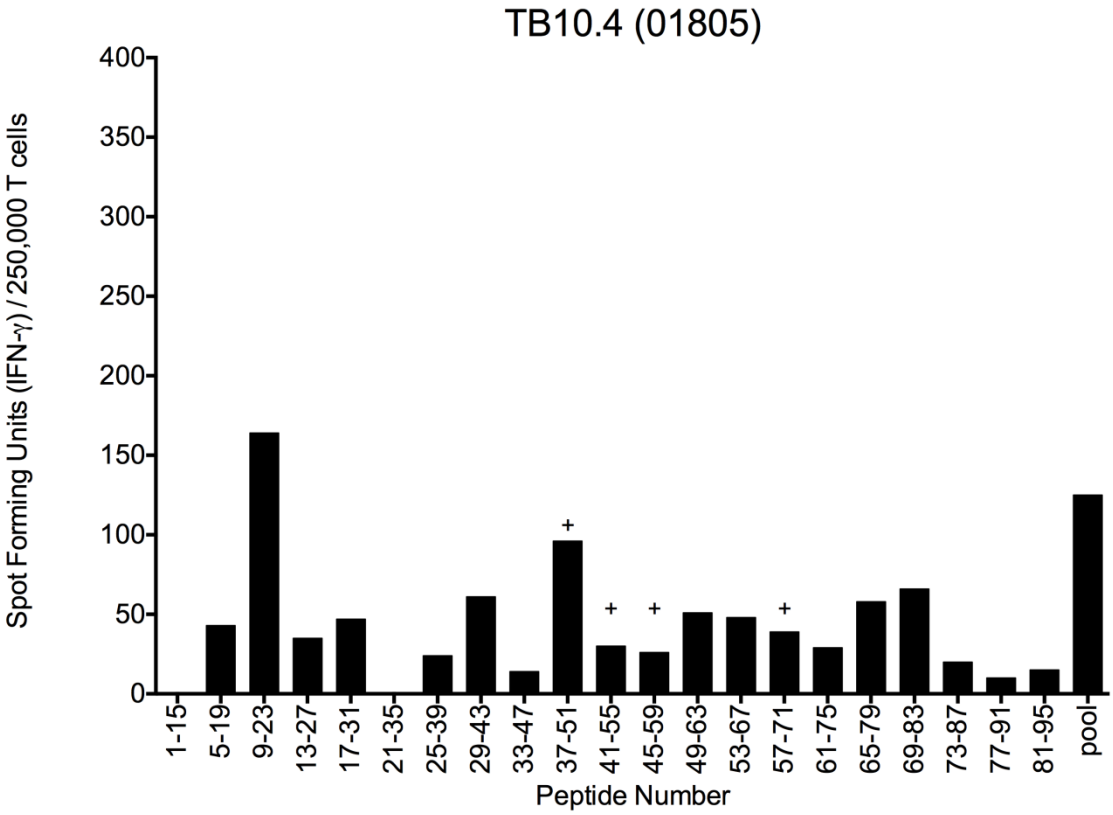

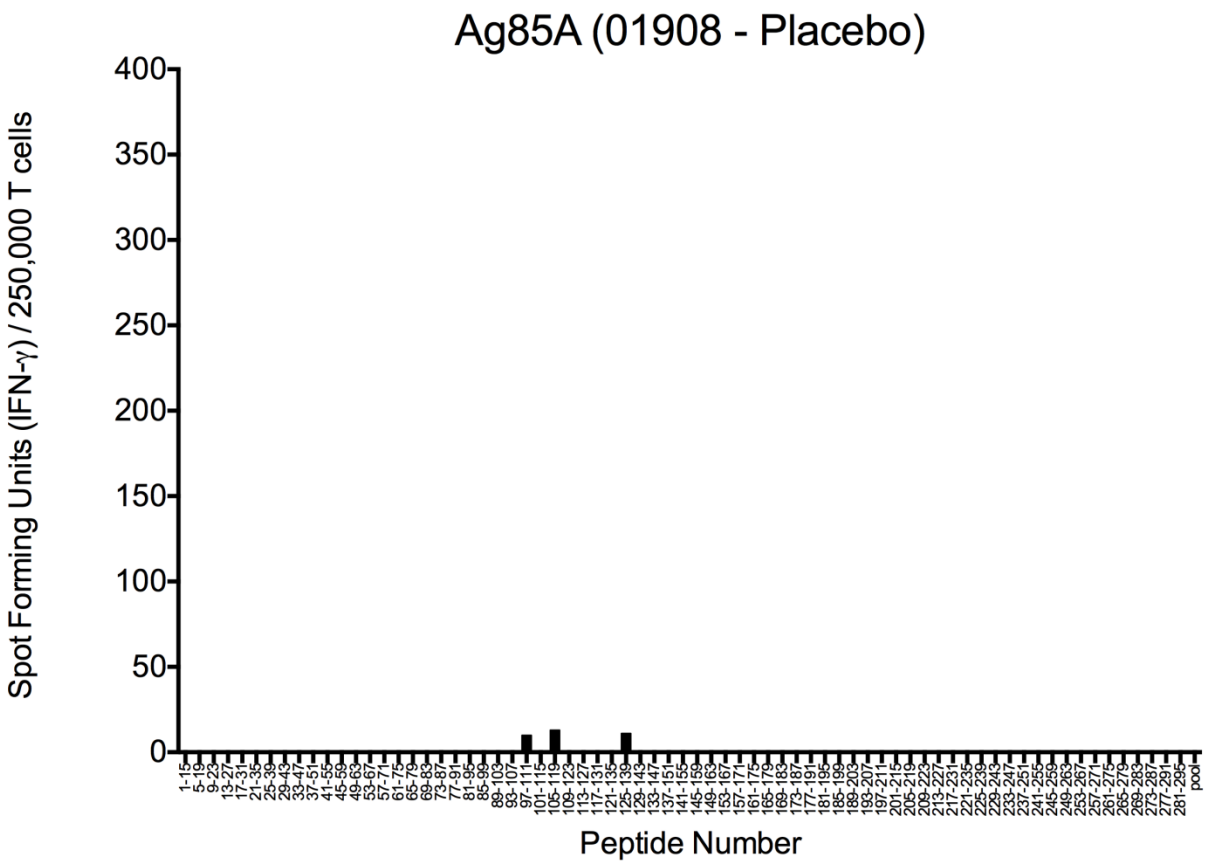
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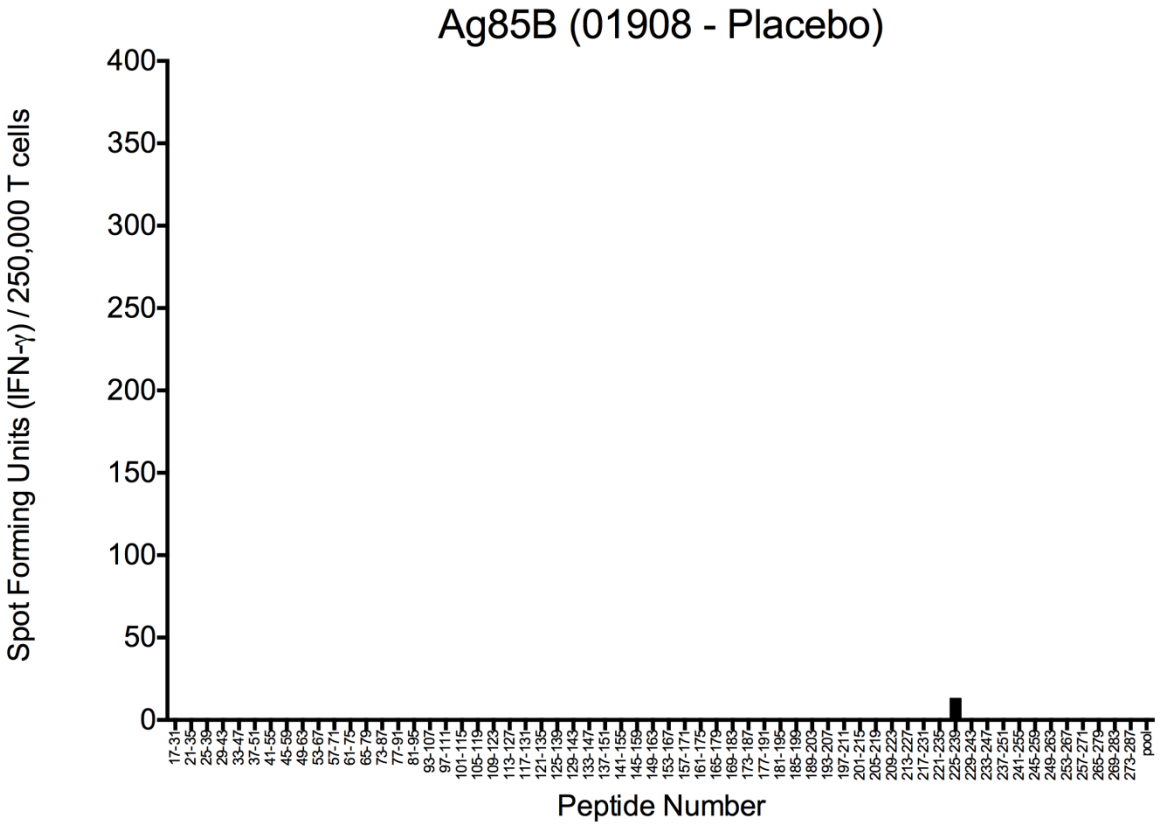
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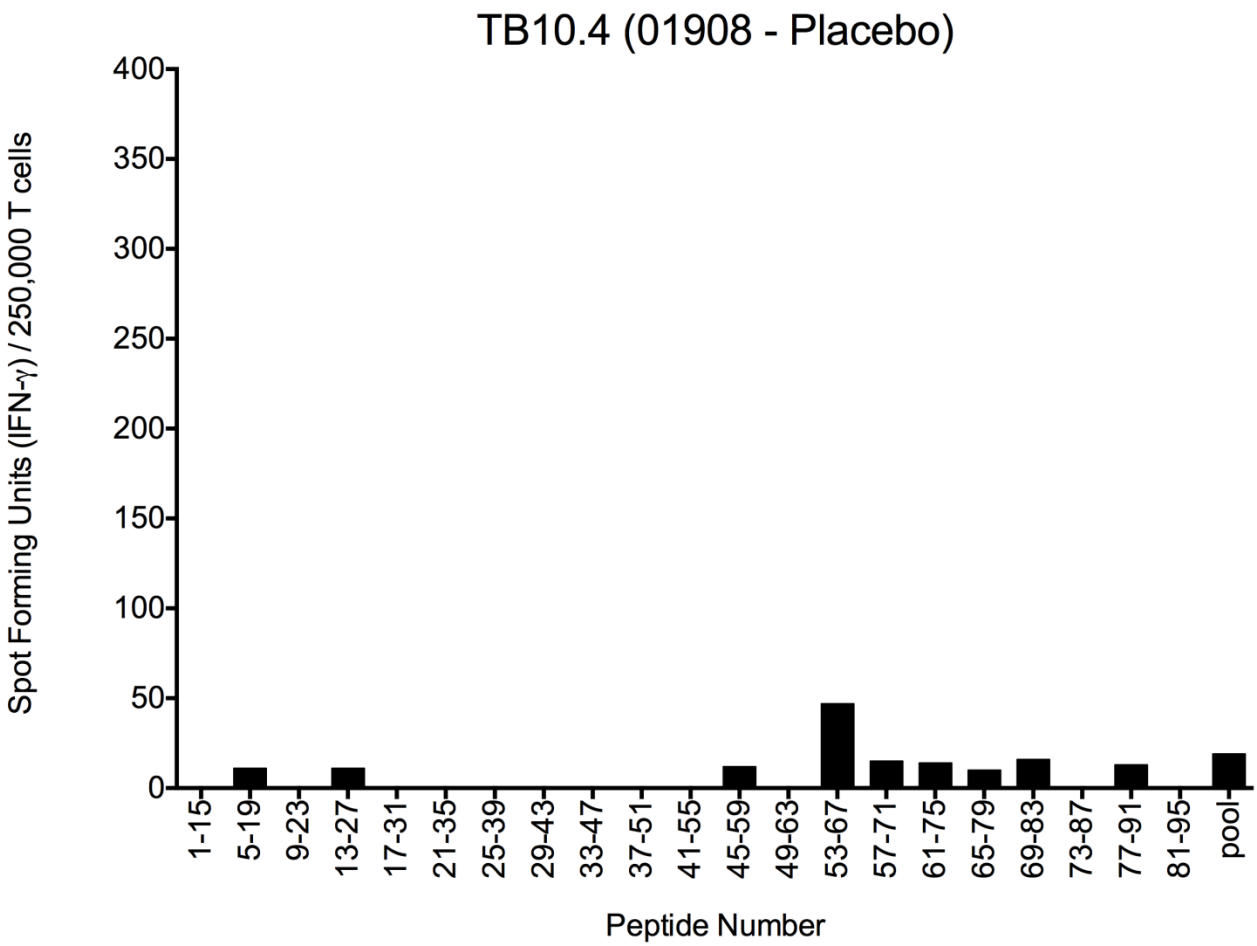
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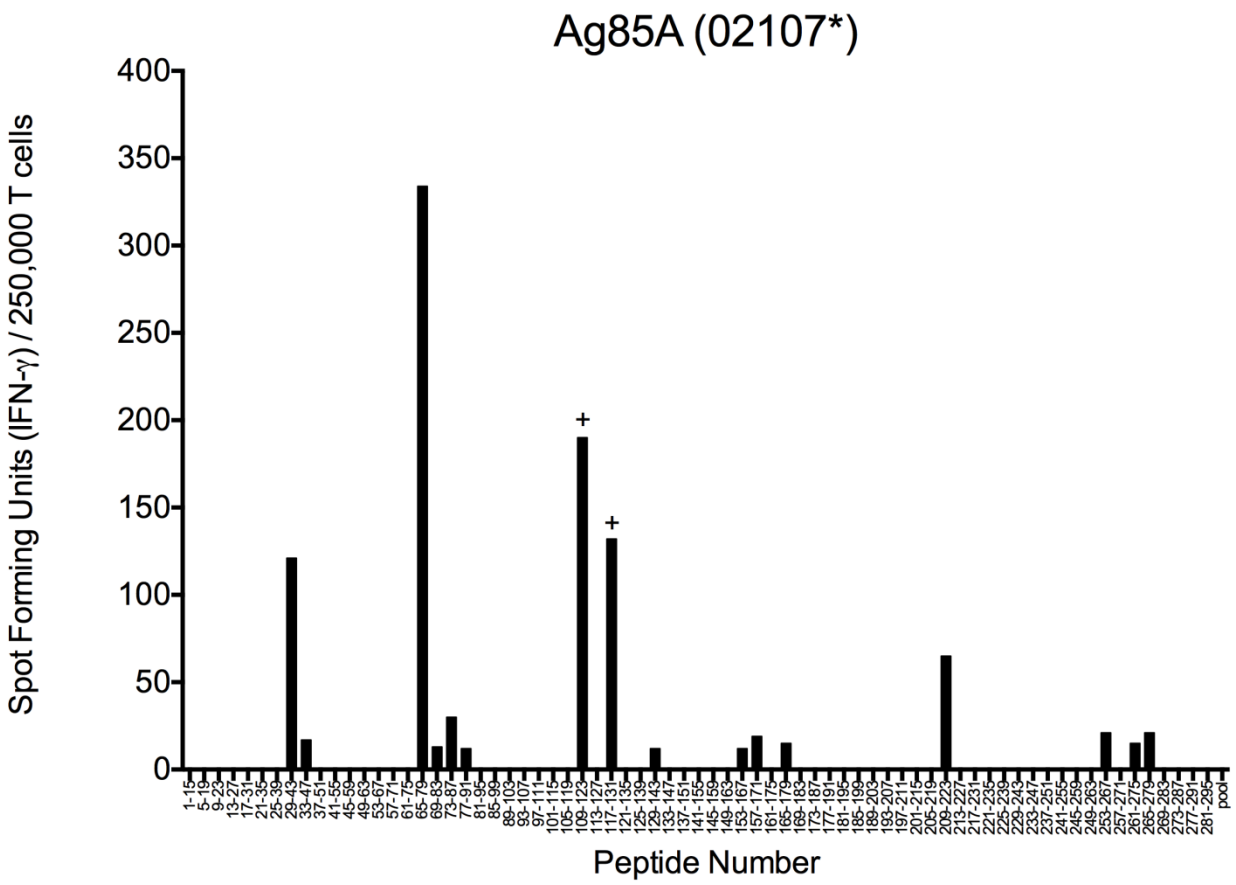
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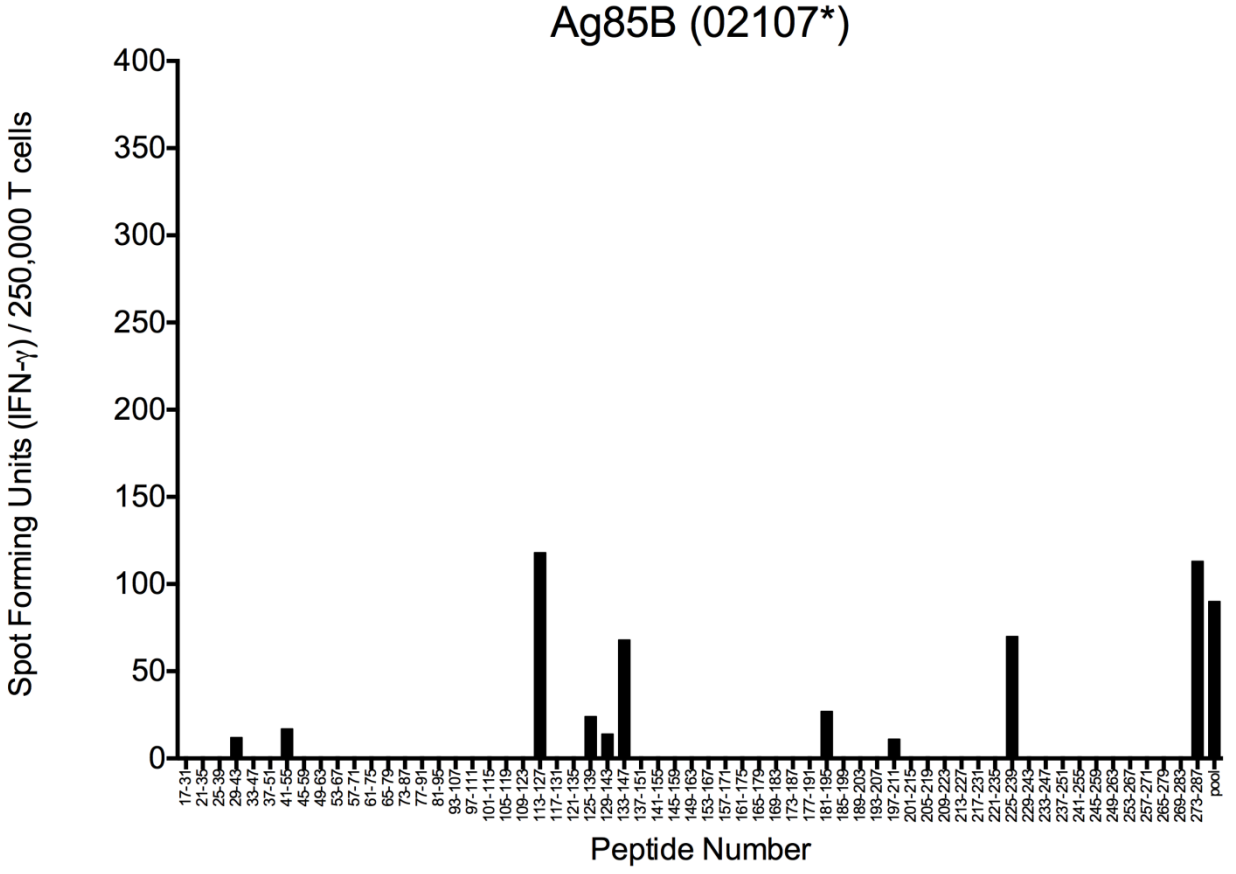
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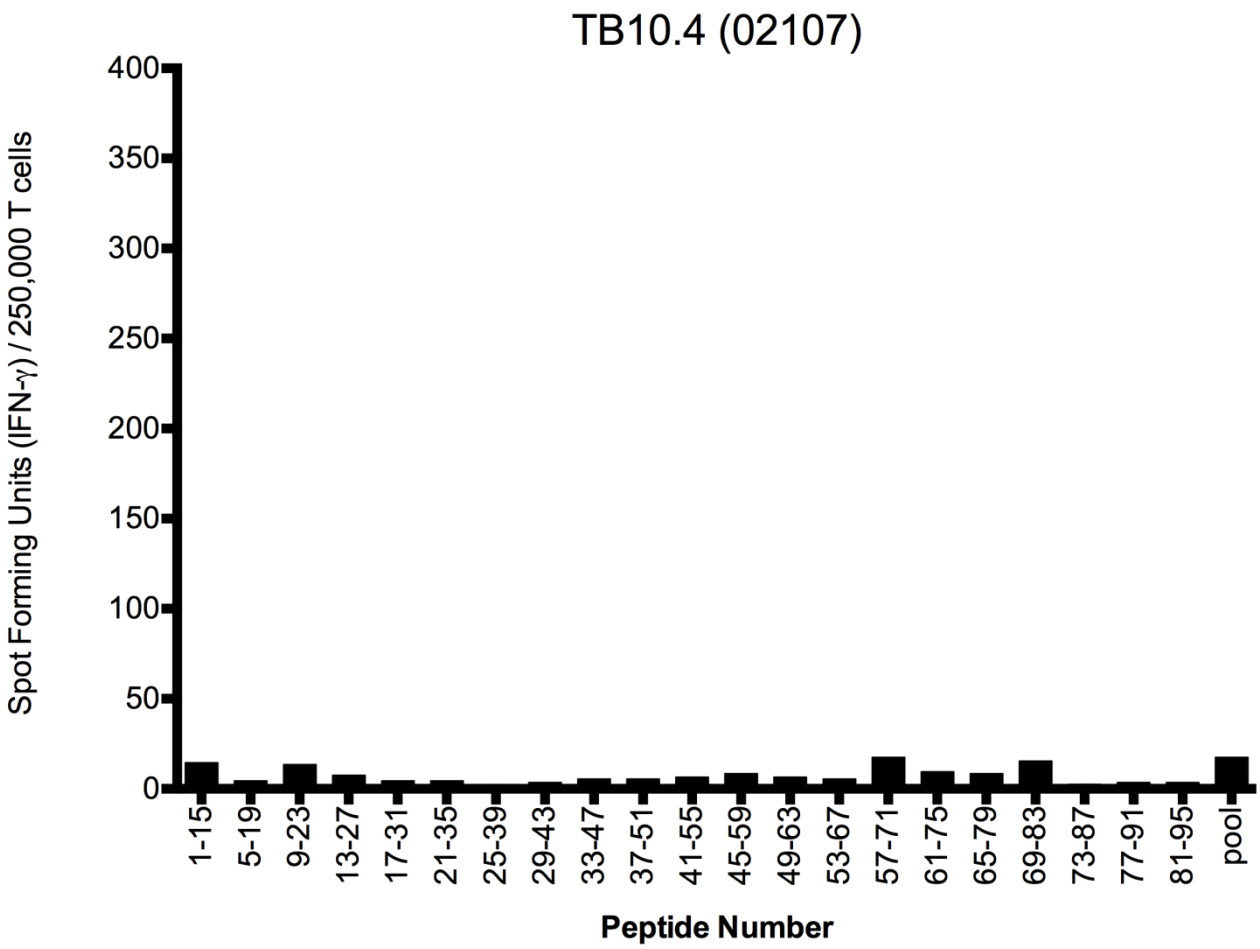
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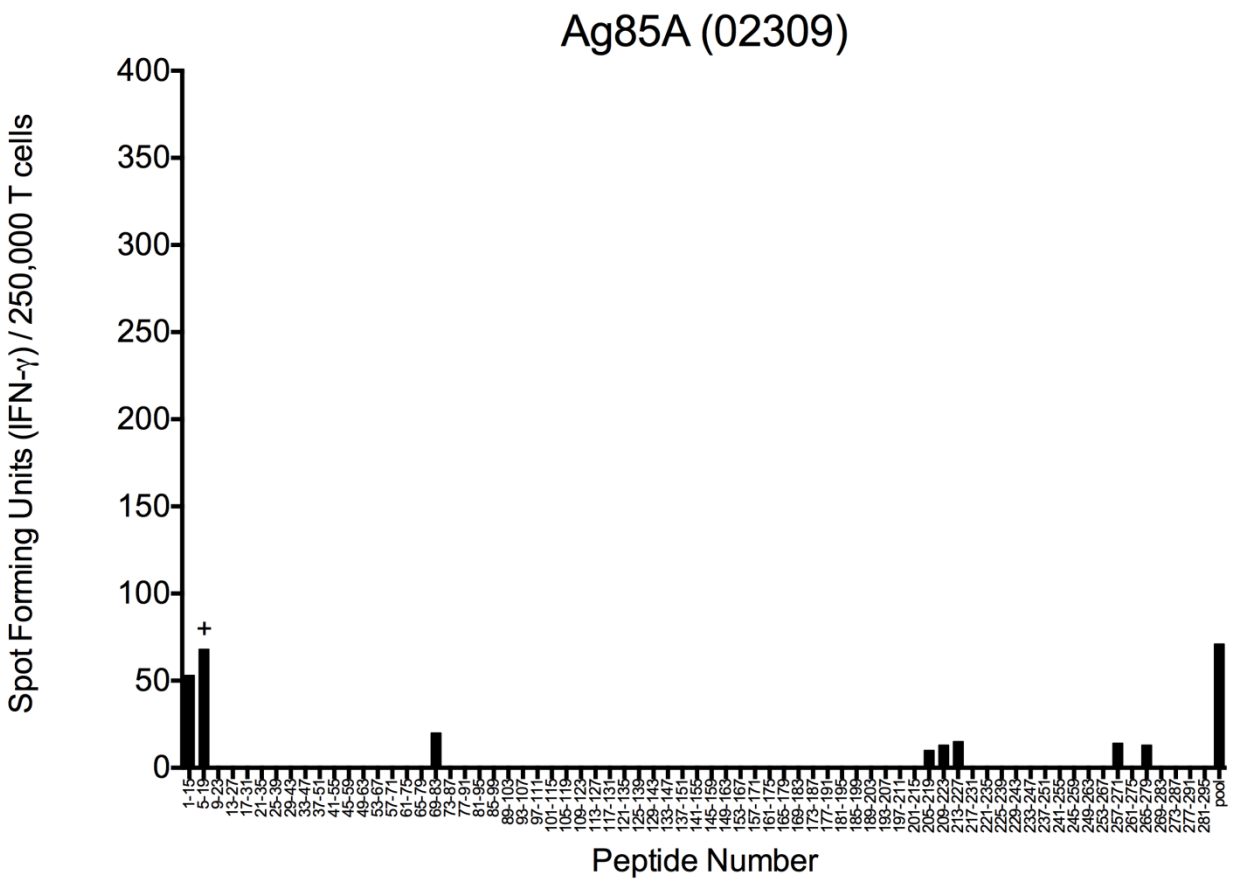
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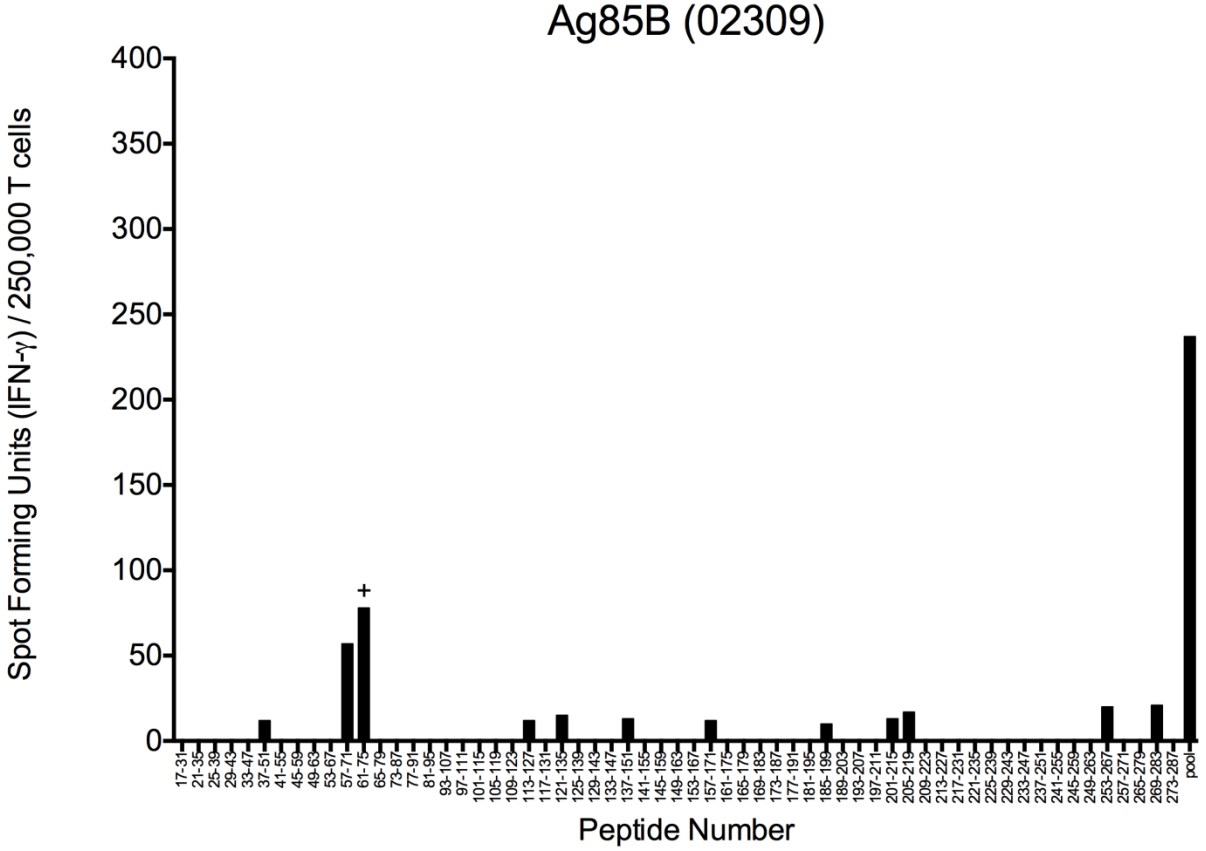
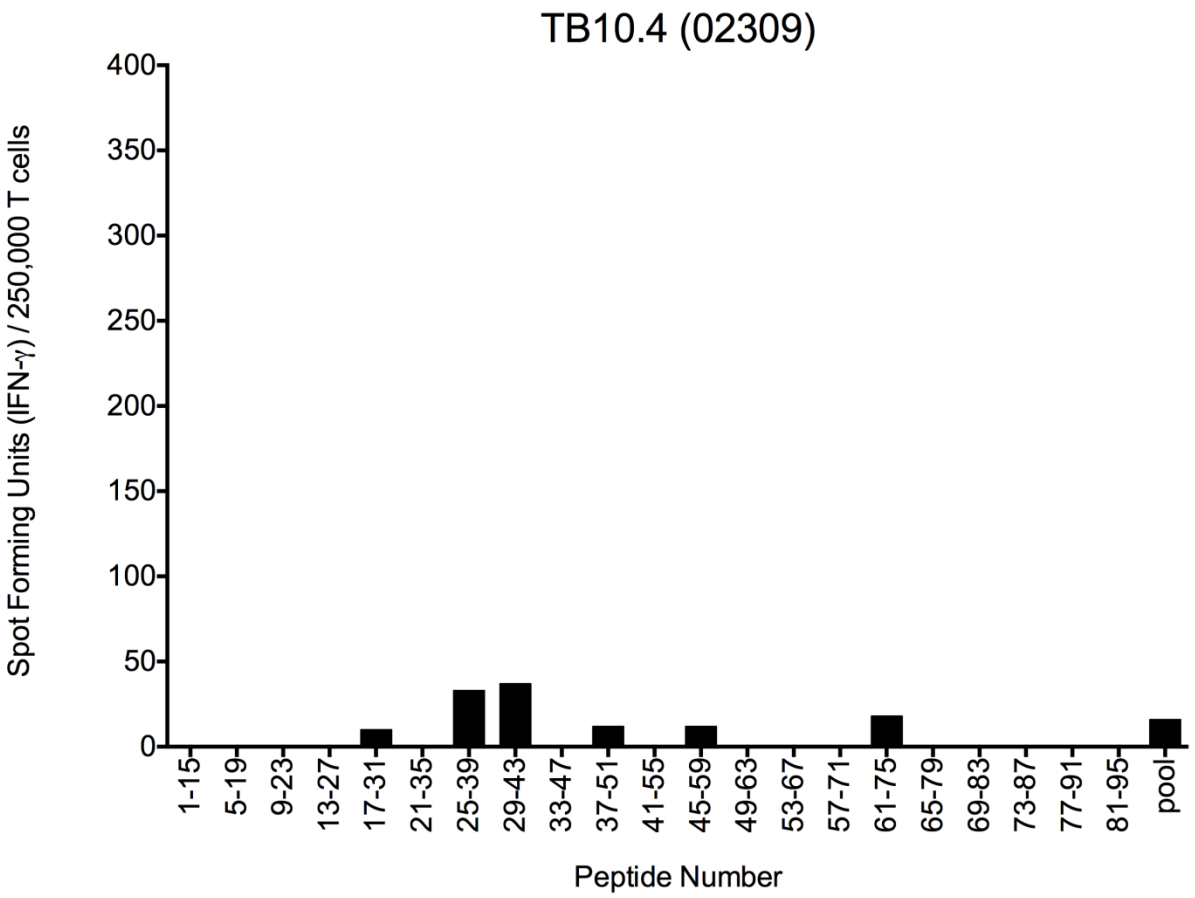
213-227
217-231
221-235
225-239
229-243
233-247
237-251
241-255
245-259
249-263
253-267
257-271
261-275
265-279
269-283
273-287
277-291
281-295
pool
Peptide Number


Ag85B (02309)
Spot Forming Units (IFN-γ) / 250,000 T cells
400
350
300
250
200
150
100
50
0
+
17-31
21-35
25-39
29-43
33-47
37-51
41-55
45-59
49-63
53-67
57-71
61-75
65-79
73-87
77-91
81-95
93-107
97-111
101-115
105-119
109-123
113-127
117-131
121-135
125-139
129-143
133-147
137-151
141-155
145-159
149-163
153-167
157-171
161-175
165-179
169-183
173-187
177-191
181-195
185-199
189-203
193-207
197-211
201-215
205-219
209-223
213-227
217-231
221-235
225-239
229-243
233-247
237-251
241-255
245-259
249-263
253-267
257-271
265-279
269-283
273-287
pool
Peptide Number


TB10.4 (02309)
Spot Forming Units (IFN-γ) / 250,000 T cells
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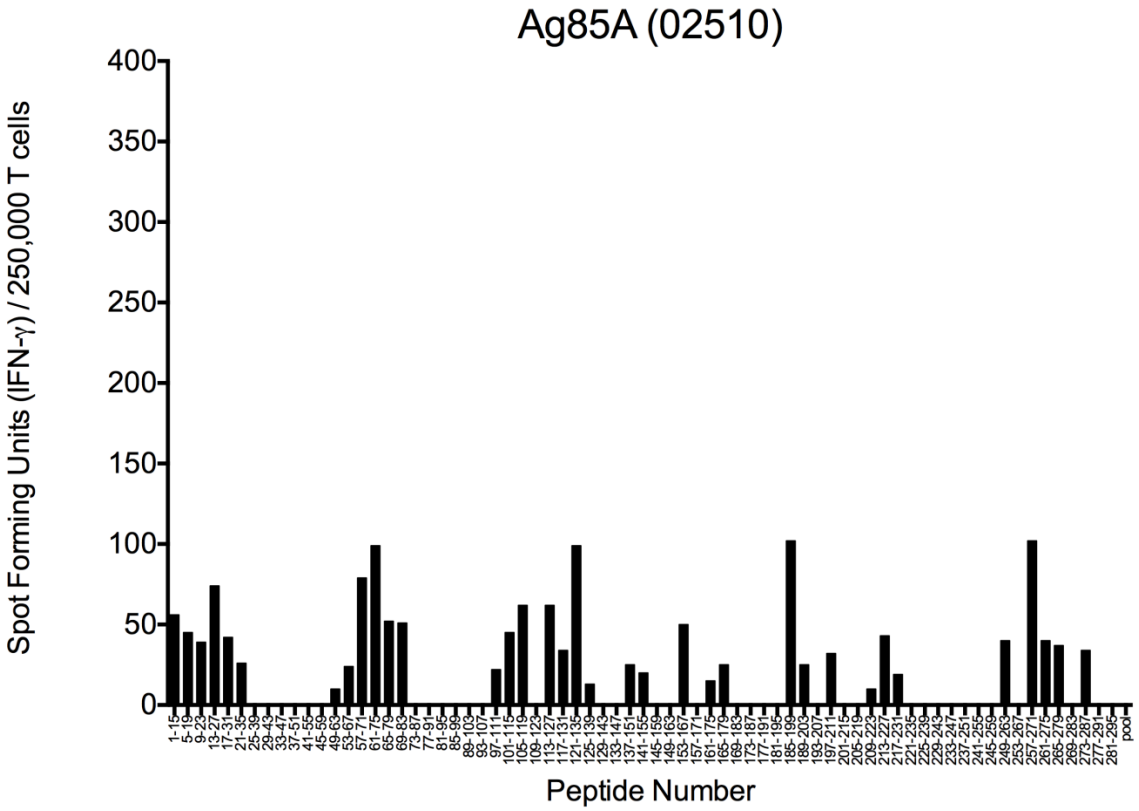
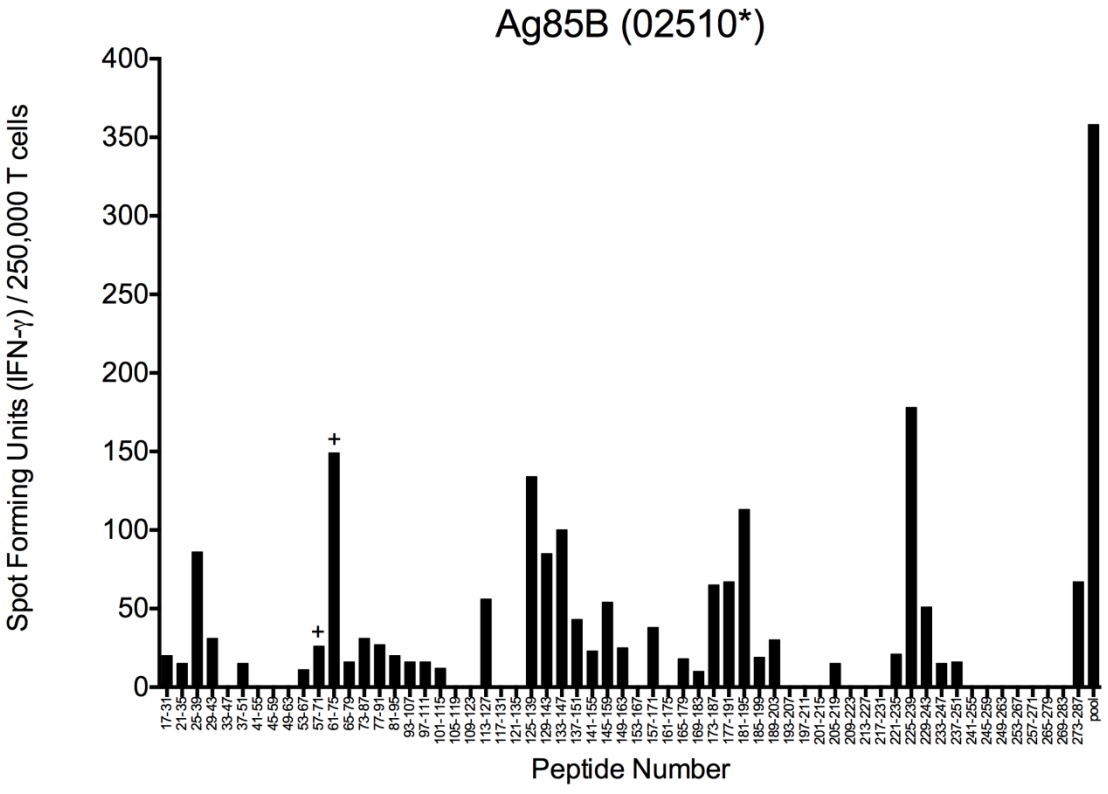
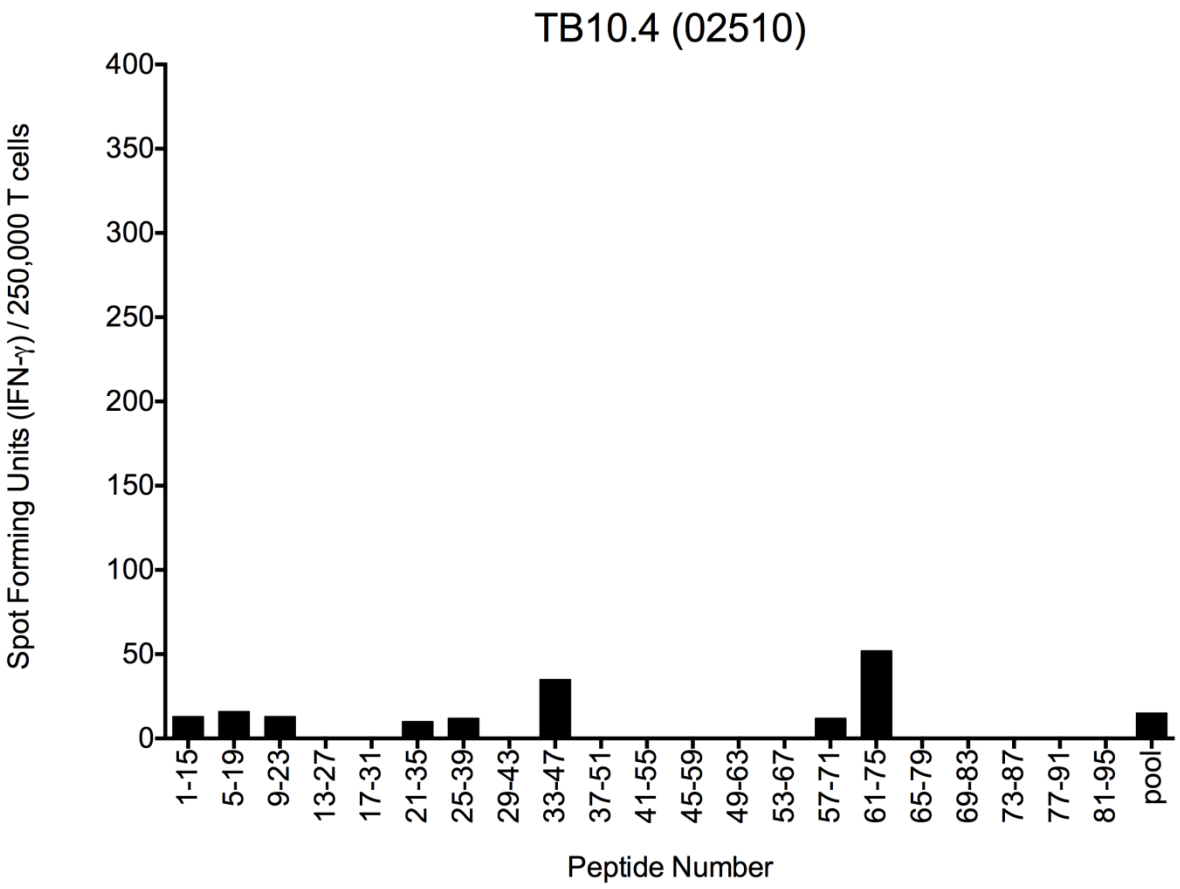
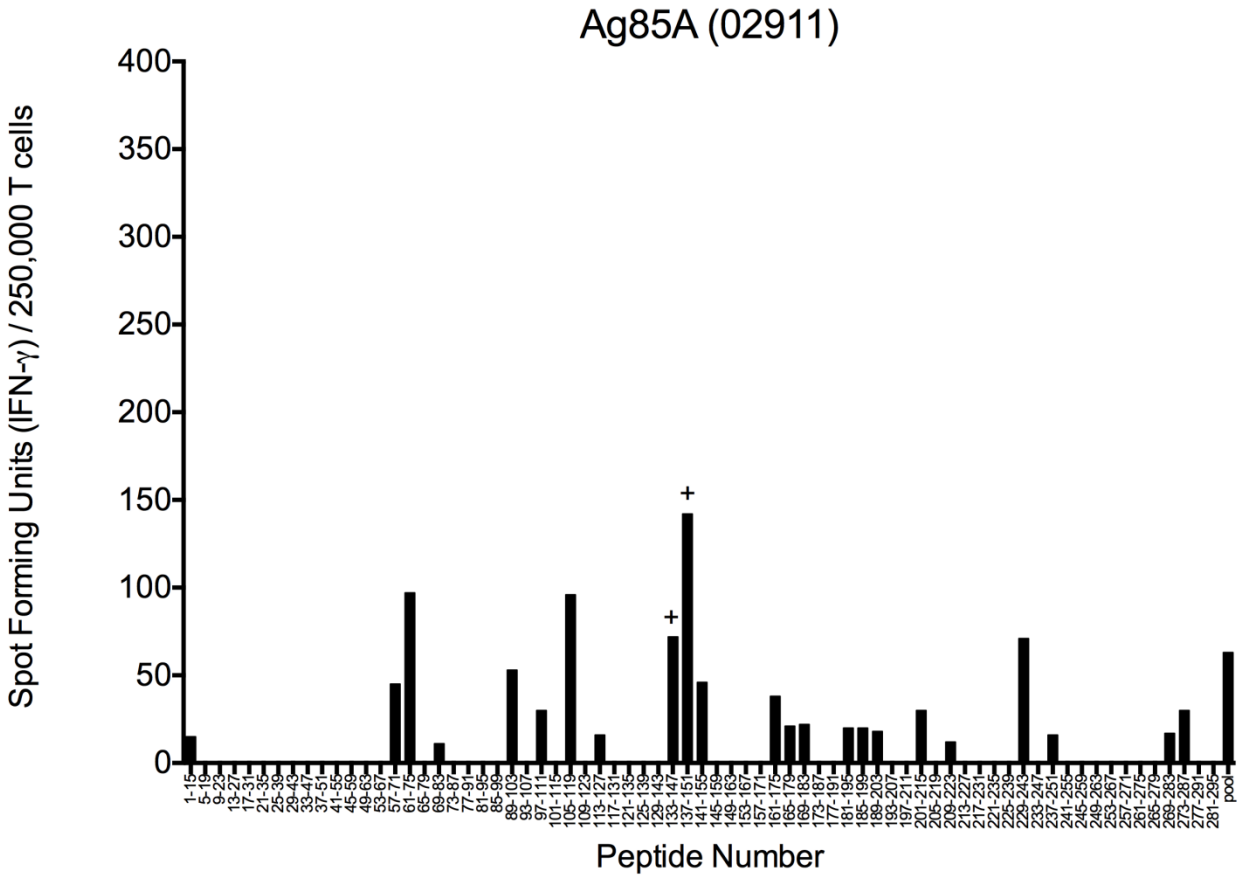
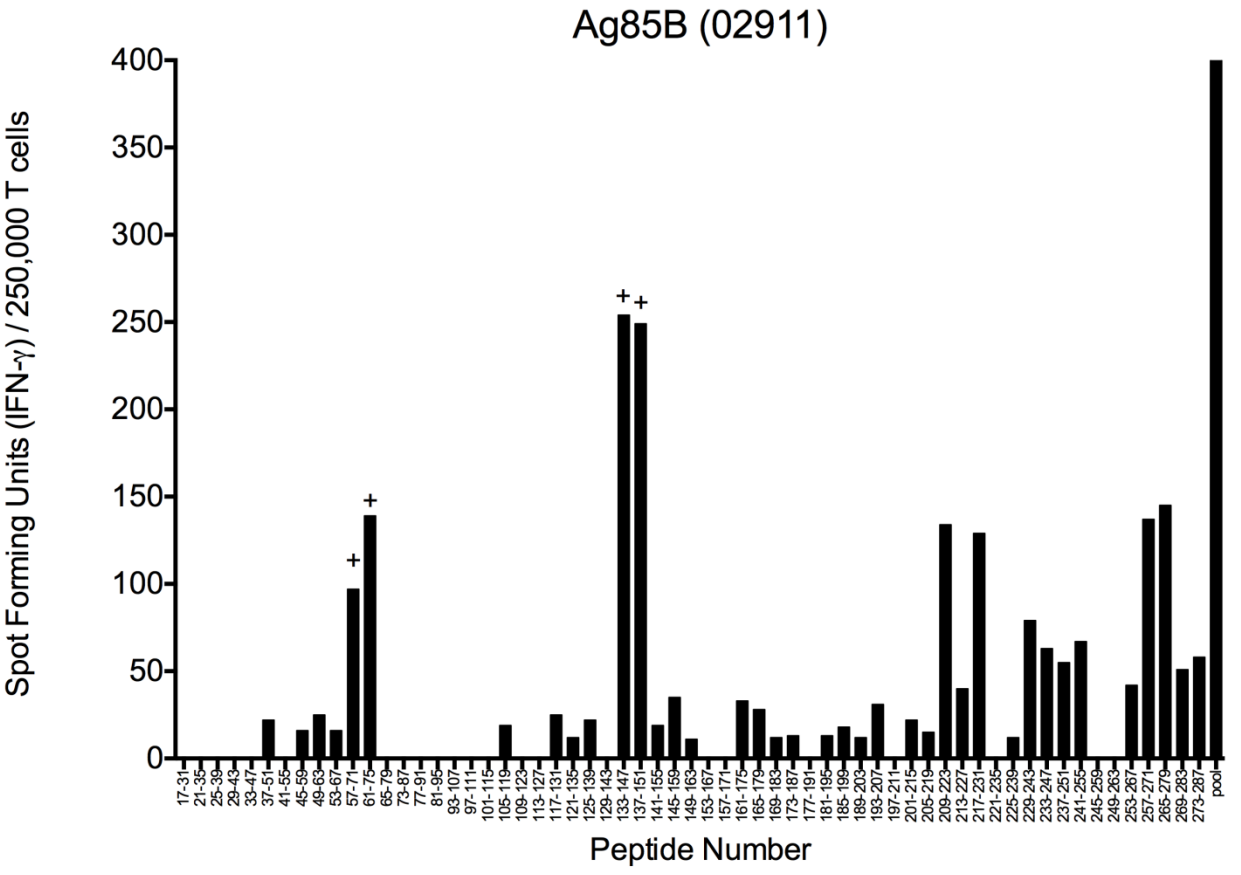
350
300
250
200
150
100
50
0
1-15
5-19
9-23
13-27
17-31
21-35
25-39
29-43
33-47
37-51
41-55
45-59
49-63
53-67
57-71
61-75
65-79
69-83
73-87
77-91
81-95
pool
Peptide Number

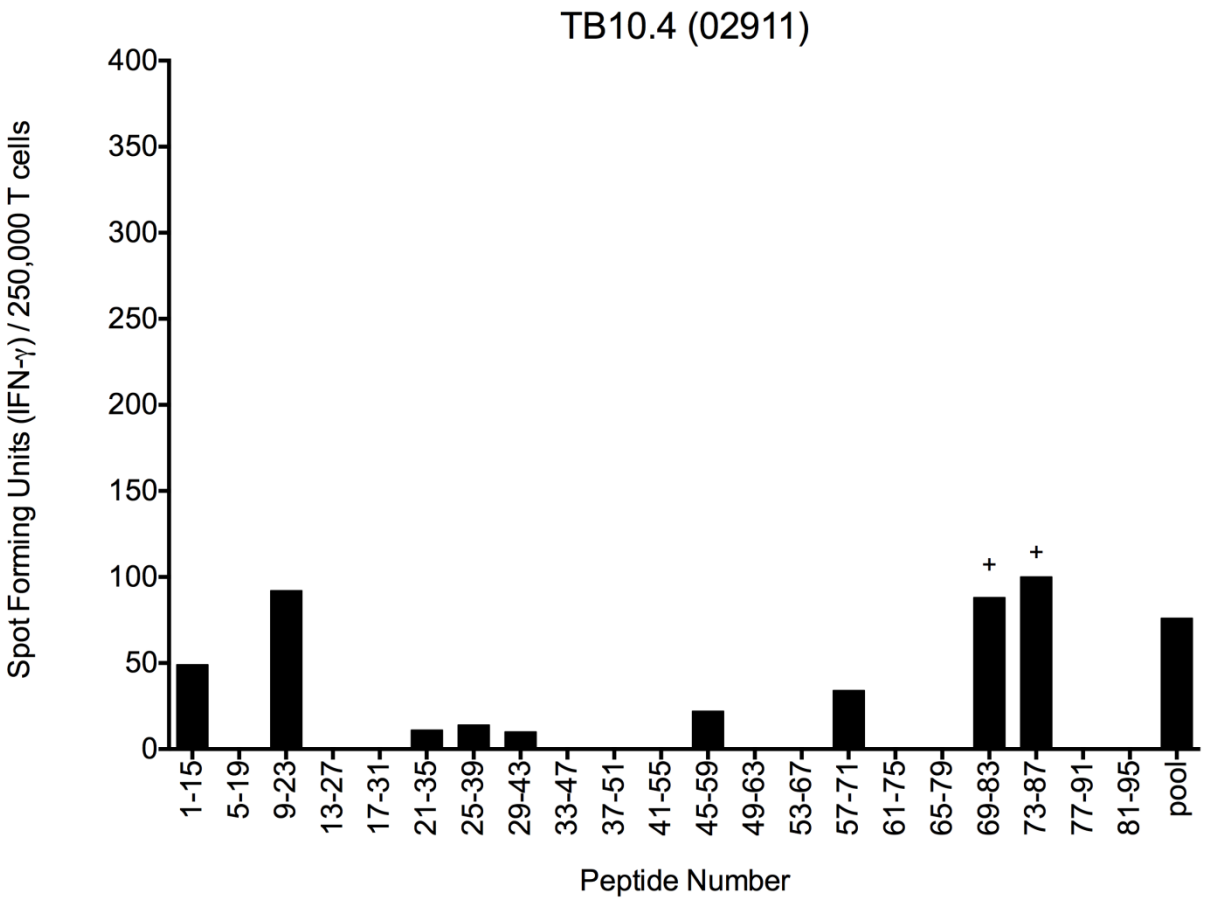

Ag85A (02510)
Spot Forming Units (IFN-γ) / 250,000 T cells
Peptide Number


Ag85B (02510*)
Spot Forming Units (IFN-γ) / 250,000 T cells
Peptide Number

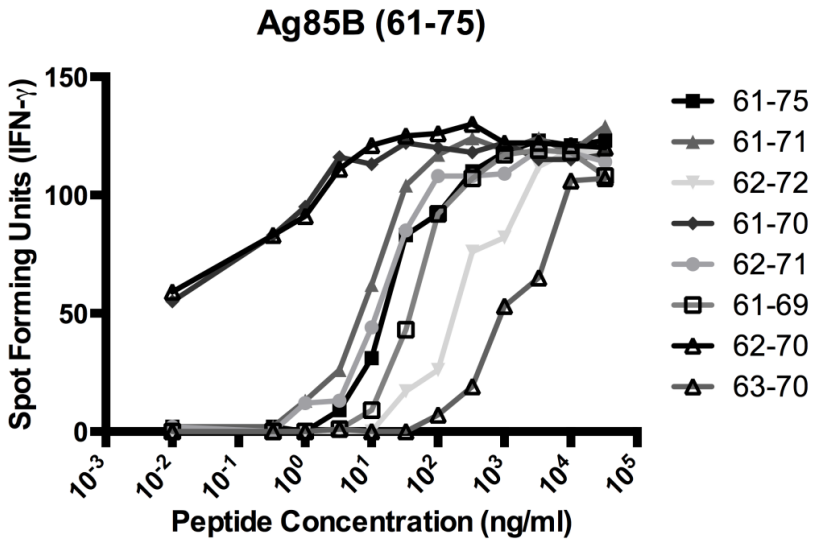

TB10.4 (02510)
Spot Forming Units (IFN-γ) / 250,000 T cells
400
350
300
250
200
150
100
50
0
1-15
5-19
9-23
13-27
17-31
21-35
25-39
29-43
33-47
37-51
41-55
45-59
49-63
53-67
57-71
61-75
65-79
69-83
73-87
77-91
81-95
pool
Peptide Number


Ag85A (02911)
Spot Forming Units (IFN-γ) / 250,000 T cells
Peptide Number

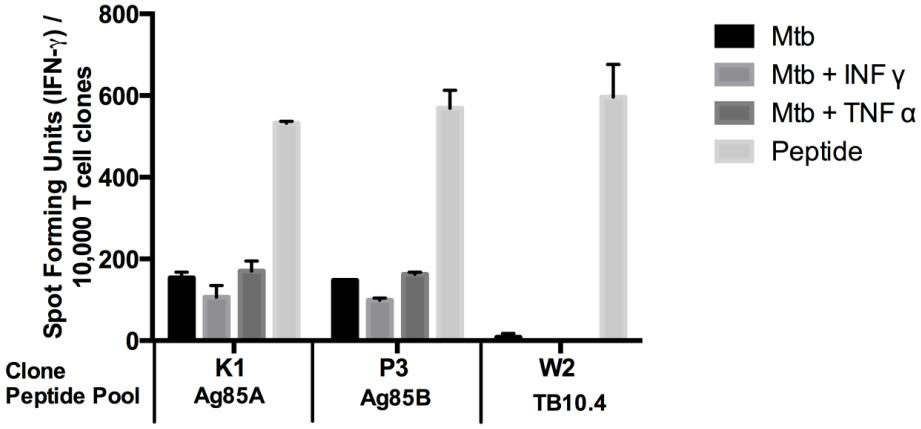

Ag85B (02911)
Spot Forming Units (IFN-γ) / 250,000 T cells
Peptide Number



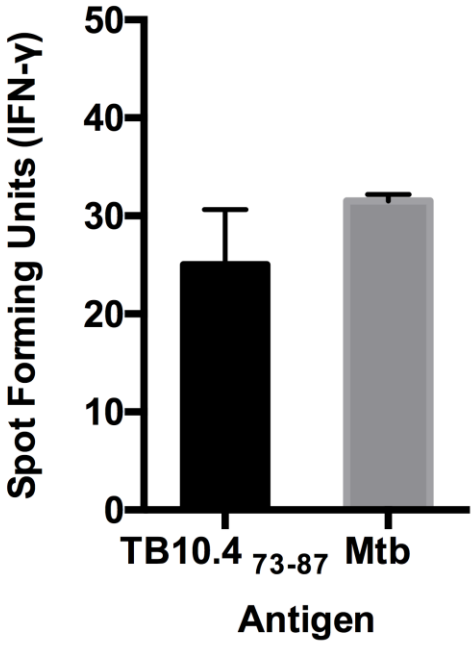
Supplementary Figure S3. Frequency of *ex vivo* CD8$^+$ specific responses to 15mer peptides in AERAS-402. To map epitopes recognized by CD8$^+$ T cells to AERAS-402, a participant's *ex vivo* response was determined by IFN-γ ELISPOT in response to all 15mers for the three antigens contained in AERAS-402 (Ag85A, Ag85B, TB10.4) and then with the peptide pool. CD8$^+$ T cells were positively selected from PBMC using magnetic beads (Stemcell Technologies) such that >97% of the cell population were CD8$^+$ T cells. These CD8$^+$ T cells were used as a source of responder T cells and tested in duplicate at a cell concentration of 250,000 cells per well. Autologous DC (20,000 cells/well) were used as APC and peptide pools (5 ug/ml, final concentration of each peptide) were added to the assay. Where sufficient PBMC was not available to make DC, CD4 depleted cells were used (CD8/others*). The antigen is followed by the participant identification in parentheses. Stable CD8$^+$ T cell clones that were derived are illustrated (+) and also summarized in Table 4. For participant 01805, clone A4-2(4) $Ag85B_{65-79}$ and clone A6-2(0) $Ag85B_{249-263}$, the Ag85a illustration (±) denotes that we did not have sufficient PBMC to perform ex-vivo deconvolution of the Ag85B peptide pool.



Supplementary Figure S4. Minimal Epitope Titration for Antigen 85B$_{61-75}$. To map the minimal epitope, autologous LCL (20,000 cells/well) were pulsed with peptide at the concentrations indicated and co-cultured with T cells (1000 cells/well). IFN-γ was assessed by ELISPOT after 18 h co-culture. Each point represents the mean of duplicate determinations. Participant 02911 is shown as a representative example.



Supplementary Figure S5. Treatment with TNFα and IFN-γ does not increase recognition of Mtb by T cell clones. 10,000 T cell clones were incubated in the presence of 0.5ng/ml IL-2 overnight in an ELISPOT with autologous DC pulsed with 5ug/ml peptide or DC infected (MOI 30:1) with Mtb that had been incubated with IFN-γ, TNF-α or no cytokine. Participant 02911 is shown as a representative example. Error bars represent the standard deviation of the mean of duplicate determinations.



Supplementary Figure S6. A CD8$^+$ T cell clone generated from participant with LTBI (D603W4 TB10.4 $_{73-87}$) using same cloning method recognizes Mtb-infected DC. 10,000 T cell clones were incubated in the presence of 0.5ng/ml IL-2 overnight in an ELISPOT with autologous DC pulsed with 5ug/ml peptide TB10.4 (aa 73-87; SSTHEANTMAMMARD) or DC infected (MOI 30:1) with Mtb. Error bars represent the standard deviation of the mean of duplicate determinations.

Evaluation	Screen (45 Days)	Study Day											
		-84	-77	-56	-14	0	7	14	28	35	42	56	98
Visit window*	NA	NA	±1	±3	±7	±7	±1	±3	±3	±1	±3	±3	±7
Written informed consent	x												
Assess eligibility criteria	x												
Verify eligibility criteria		x				x			x				
Medical history[h]	x	x	x	x	x	x							
Physical examination[h]	x	x	x	x	x	x							
Serum β-hCG[f]	x												
Vital signs	x	x[c]				x[c]			x[c]				
Urine illicit drug screen	x												
ECG	x												
QuantiFERON	x												x
Hepatitis B, C	x												
HIV-1	x												x
Urinalysis	x[a]					x[a]	x		x[a]	x		x	
Serum chemistry[b]	x[a]					x[a]	x		x[a]	x		x	
CBC, differential, platelets	x[a]					x[a]	x		x[a]	x		x	
Urine β-hCG[f]		x				x			x				
AERAS-402 neutralization						x						x	
HLA						x							
Immunology: ICS**, ELISPOT		x			x				x			x	
Leukapheresis					x								x[d]
BCG administration		**x**											
AERAS-402/placebo administration						**x**			**x**				
Adverse events (incl con meds)						x	x	x	x	x	x	x	
Serious adverse events (incl con meds)						x	x	x	x	x	x	x	x[e]
Site(s) of injection examination		x	x	x		x	x	x	x	x	x	x	
Phlebotomy by visit, mL[g]	17	40			40	19	7		47	7		54	5
Cumulative phlebotomy volume	17	57			97	116	123		170	177		231	236

NA=Not applicable

*Windows for Study Days -77, -56, -14 and Study Day 0 are based on the date of the Study Day -84 visit. Windows for Study Days 7, 14, 28 and 98 are based on the date of the Study Day 0 visit. Windows for Study Days 35, 42 and 56 are based on the date of the Study Day 28 visit.

** ICS was also performed on day 98.

a. These screening evaluations were done no earlier than 7 days prior to BCG administration on Study Day -84 and 3 days prior to study vaccine administration on Study Day 0 and Study Day 28.
b. Serum chemistries = ALT, AST, total bilirubin, ALP and creatinine.
c. Vital signs (blood pressure, heart rate, oral temperature) obtained pre-vaccination, and 30±5 and 60±5 minutes post-vaccination.
d. Study Day 98 leukapheresis was performed any time after the Study Day 56 visit but no later than the Study Day 98 visit.
e. Follow-up for serious adverse events is continued by 6-monthly telephone contact as part of the Registry Protocol.
f. For females of child-bearing potential only. Serum β-hCG was done no earlier than 7 days prior to Study Day -84. Urine β-hCG was done on Study Day -84 prior to BCG administration and on Study Day 0 and Study Day 28 prior to study vaccine administration.
g. Phlebotomy volumes were based on the following estimates: Serum β-hCG: 1 mL, QuantiFERON: 3 mL, Hepatitis B/C: 4 mL, HIV-1: 2 mL, Chemistry: 2 mL, CBC: 5 mL, AERAS-402 neutralization: 7 mL, HLA: 5 mL, ICS: 24 mL, ELISPOT: 16 mL.
h. Update medical history and physical examination from Study Days -84 through Study Day 0.

Note: The amount of blood required for this study is within WHO guidelines for blood donation.

Supplementary Table S1. Complete Summary Schedule of Participant Evaluations