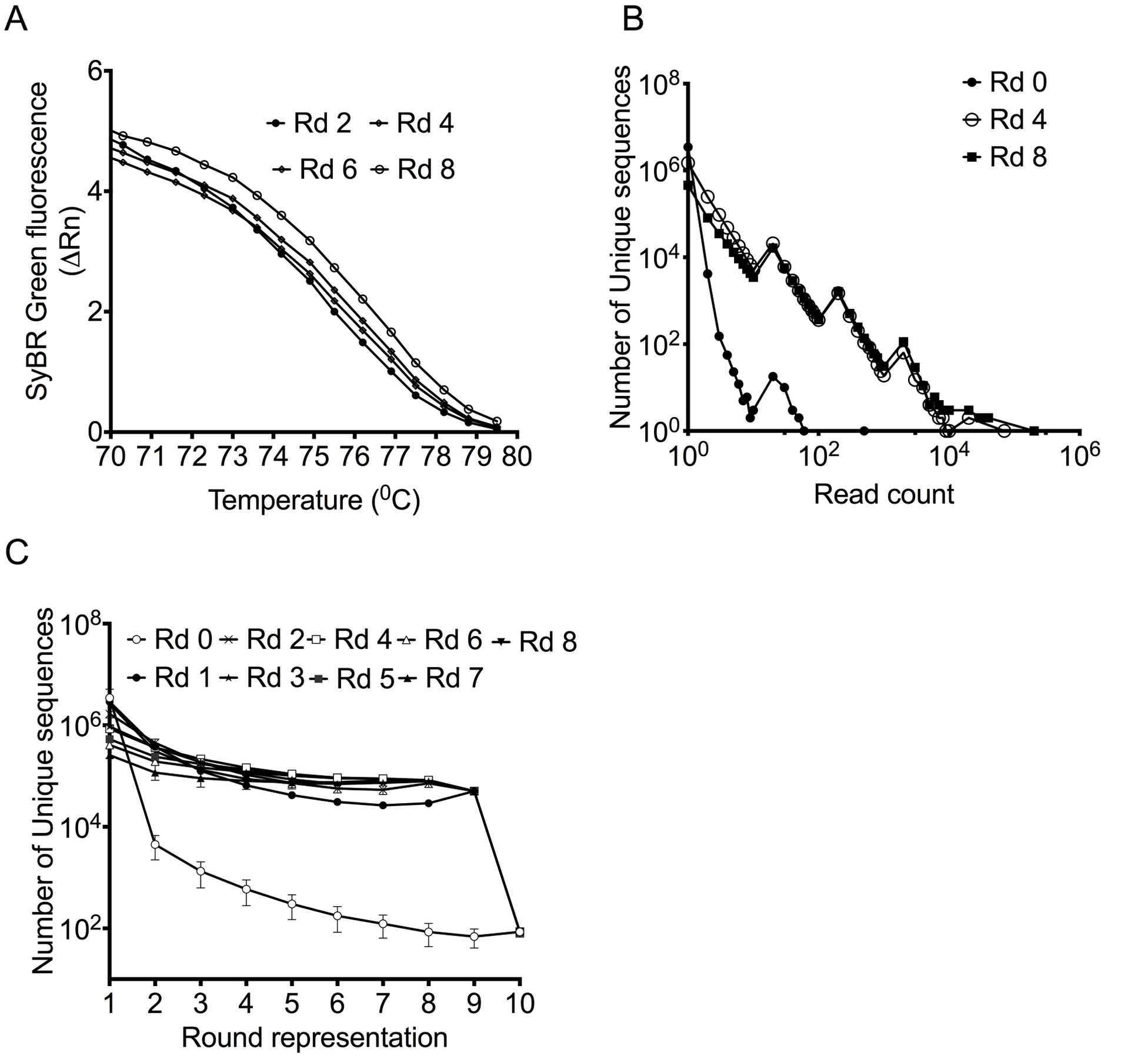
Supplementary Data

Supplementary Figures:

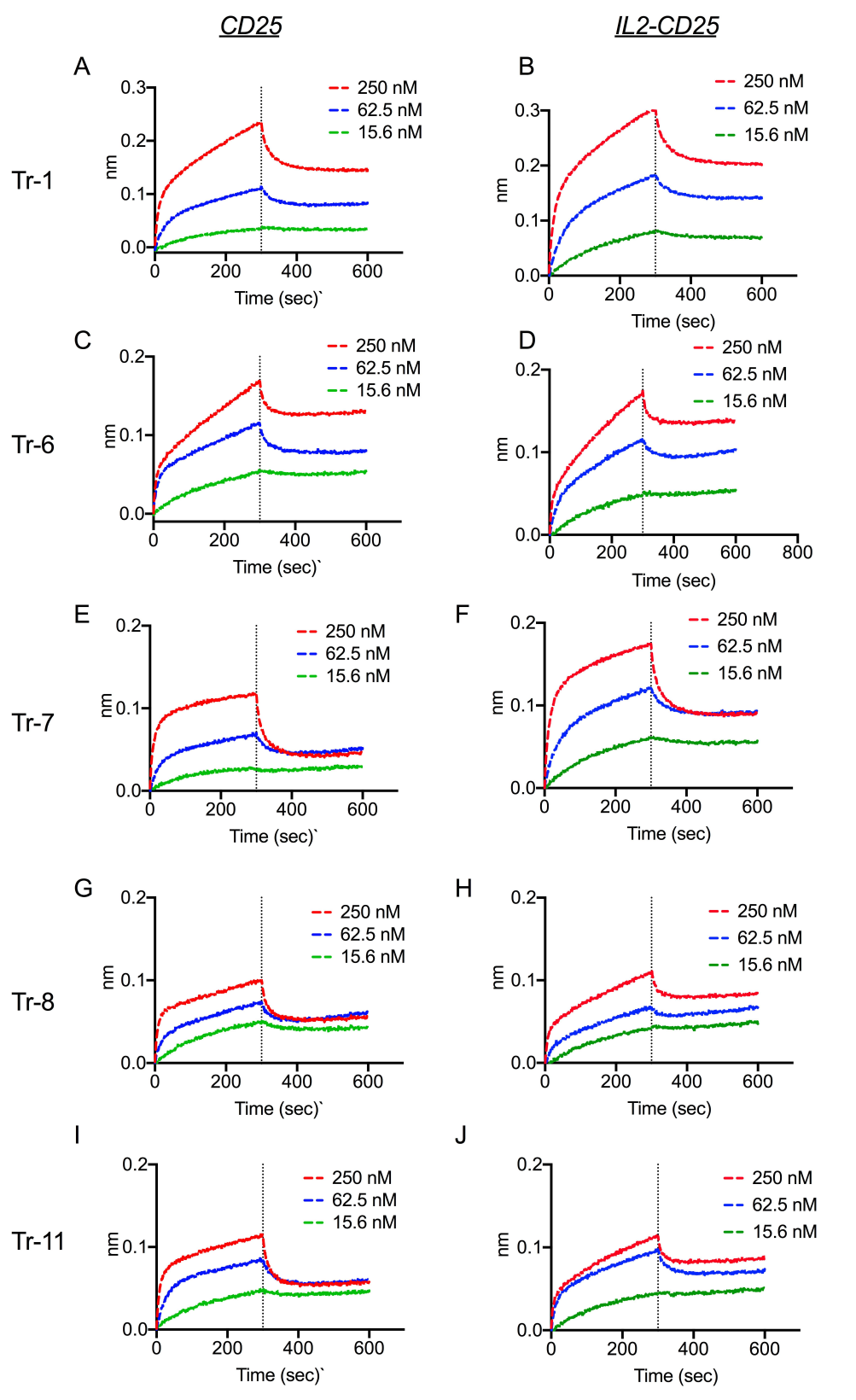
Supplementary Figure S1: Bioinformatics analysis of primary human Treg cell-based SELEX-enriched aptamers

(A) The complexity of the Treg-binding aptamer pools from select rounds of SELEX were tested using a DNA melt assay. Aptamer pools from the later rounds of SELEX demonstrate higher melting temperature than the earlier rounds indicative of enrichment of related sequence and reduced sequence complexity (B) Abundance analysis of the read counts plotted against the unique aptamer sequences from every few rounds of SELEX. Later rounds of SELEX (Rd 4 and Rd 8) show increased abundance (read count) indicative of enrichment of highly homologous Treg-binding sequences than the initial library (Rd 0) (C) Persistence analysis of the round representation of unique aptamer sequences from each round of SELEX (Rounds 1 – 8) as compared to the round representation of aptamer sequences from round 0.


A
SyBR Green fluorescence (ΔRn)
Rd 2
Rd 4
Rd 6
Rd 8
Temperature (0C)
B
Number of Unique sequences
Rd 0
Rd 4
Rd 8
Read count
C
Number of Unique sequences
Rd 0
Rd 2
Rd 4
Rd 6
Rd 8
Rd 1
Rd 3
Rd 5
Rd 7
Round representation

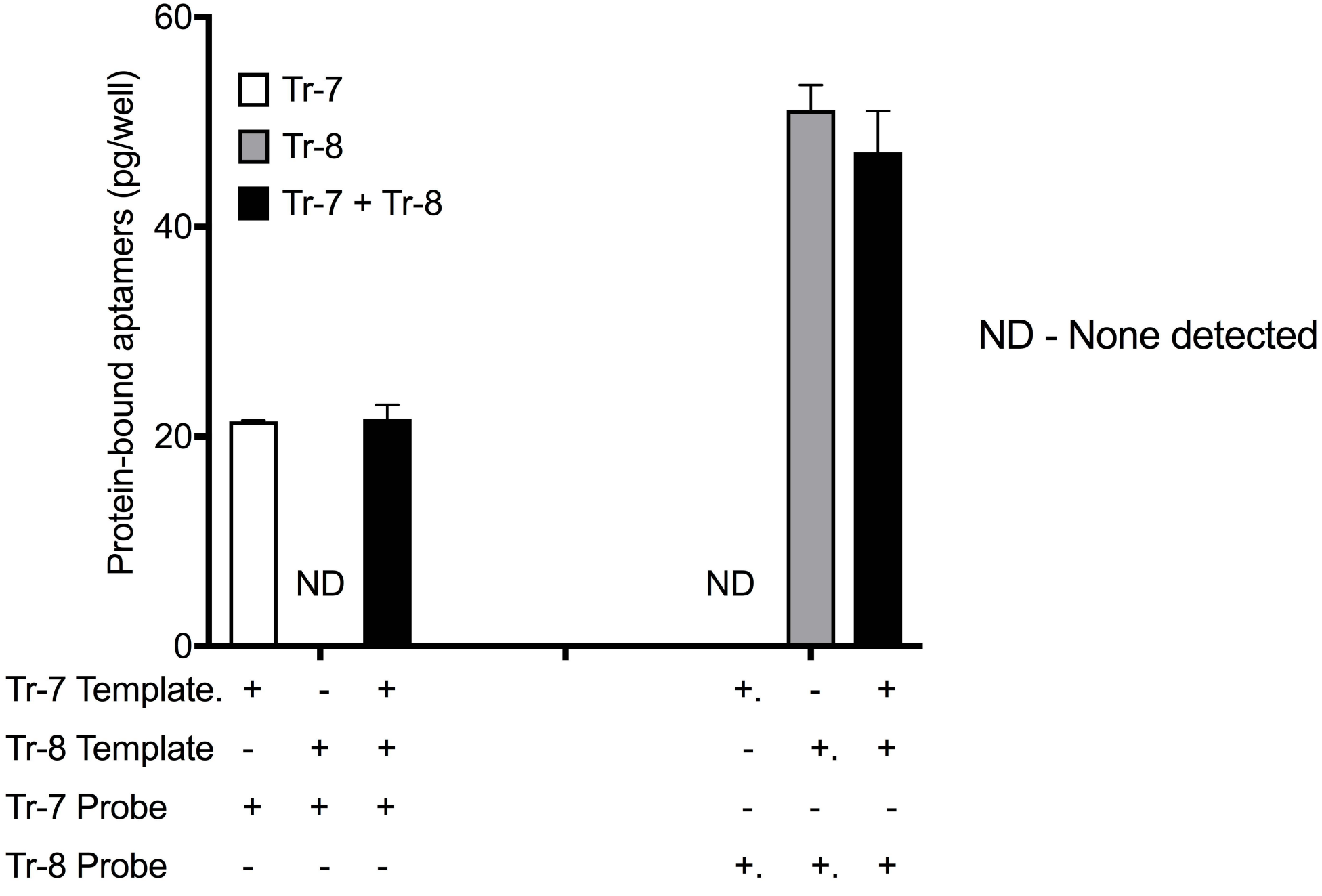
Supplementary Figure S2: Sensorgram of binding kinetics between LIRECAPs and CD25 or IL2-CD25 complex, as measured by biolayer interferometry

Sensorgram obtained from Octet Red96 system showing binding kinetics of (A) Tr-1 to CD25 (B) Tr-1 to IL2-CD25 complex (C) Tr-6 to CD25 (D) Tr-6 to IL2-CD25 complex (E) Tr-7 to CD25 (F) Tr-7 to IL2-CD25 complex (G) Tr-8 to CD25 (H) Tr-8 to IL2-CD25 complex (I) Tr-11 to CD25 and (J) Tr-11 to IL2-CD25 complex. A representative graph from two independent experiments is shown.


CD25
IL2-CD25
A
B
C
D
E
F
G
H
I
J
Tr-1
Tr-6
Tr-7
Tr-8
Tr-11
250 nM
62.5 nM
15.6 nM
nm
Time (sec)`
Time (sec)

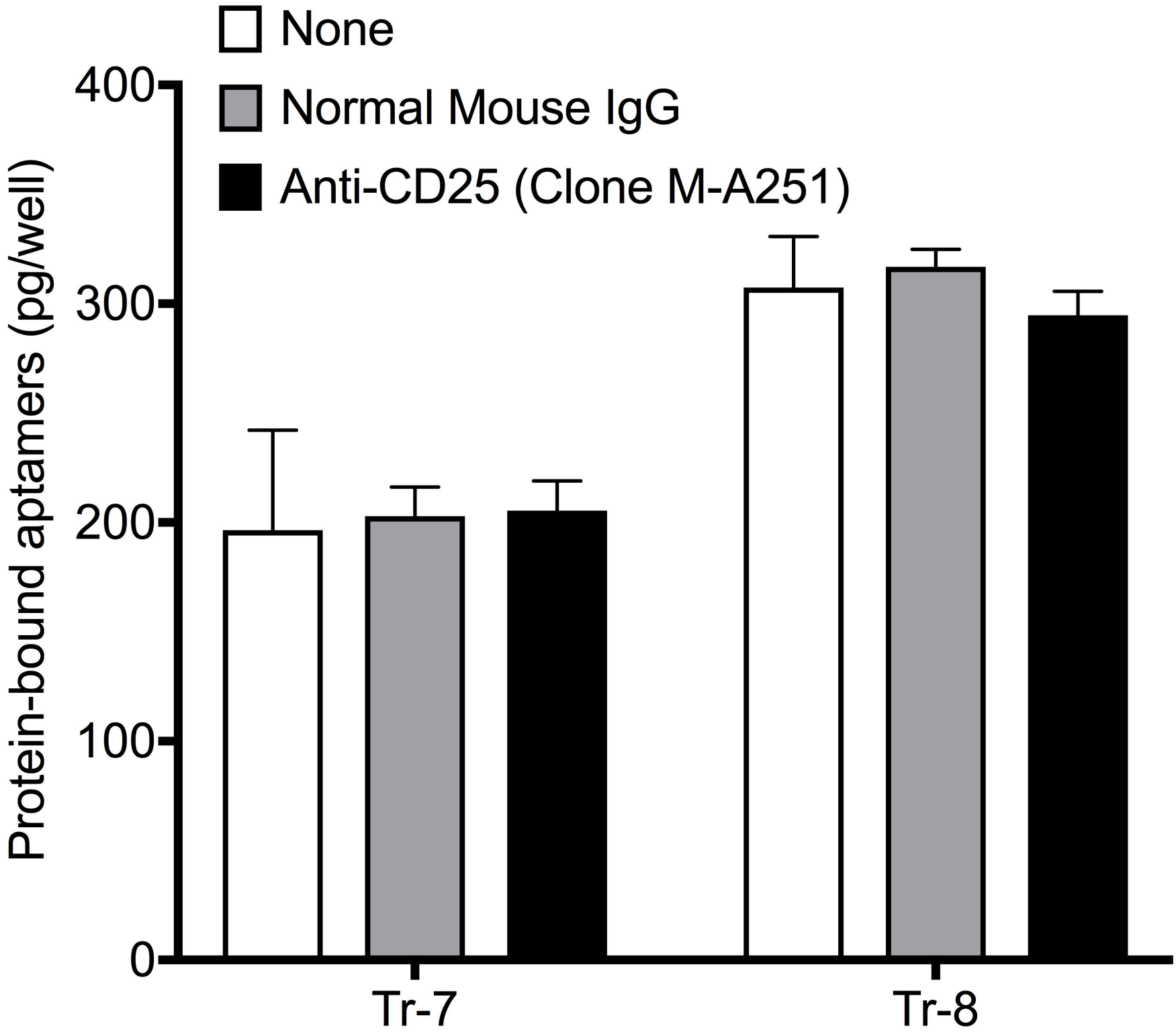
Supplementary Figure S3: Tr-7 and Tr-8 aptamers do not compete with each other for CD25 binding

The ability of anti-CD25 aptamers (Tr-7 and Tr-8) to cross-block binding to CD25 was determined by incubating CD25-coated beads with either of the aptamers alone (Tr-7 or Tr-8) or as equimolar mix (Tr-7 + Tr-8). After washing, bound aptamers were extracted and quantified by RT-qPCR using TaqMan-based probes specific for Tr-7 or Tr-8. No significantly cross-blocking was noted CD25 ($p>0.05$). There was no evidence for cross-identification by the TaqMan probes (e.g. Tr-7 binding by the Tr-8 probe or Tr-8 binding by the Tr-7 probe, marked as ND) highlighting the specificity of the qPCR system. Data showed is the Mean +/- SEM of two independent experiments.


60
40
20
0
Protein-bound aptamers (pg/well)
Tr-7
Tr-8
Tr-7 + Tr-8
ND
ND
ND - None detected
Tr-7 Template. + - + +. - +
Tr-8 Template - + + - +. +
Tr-7 Probe + + + - - -
Tr-8 Probe - - - +. +. +

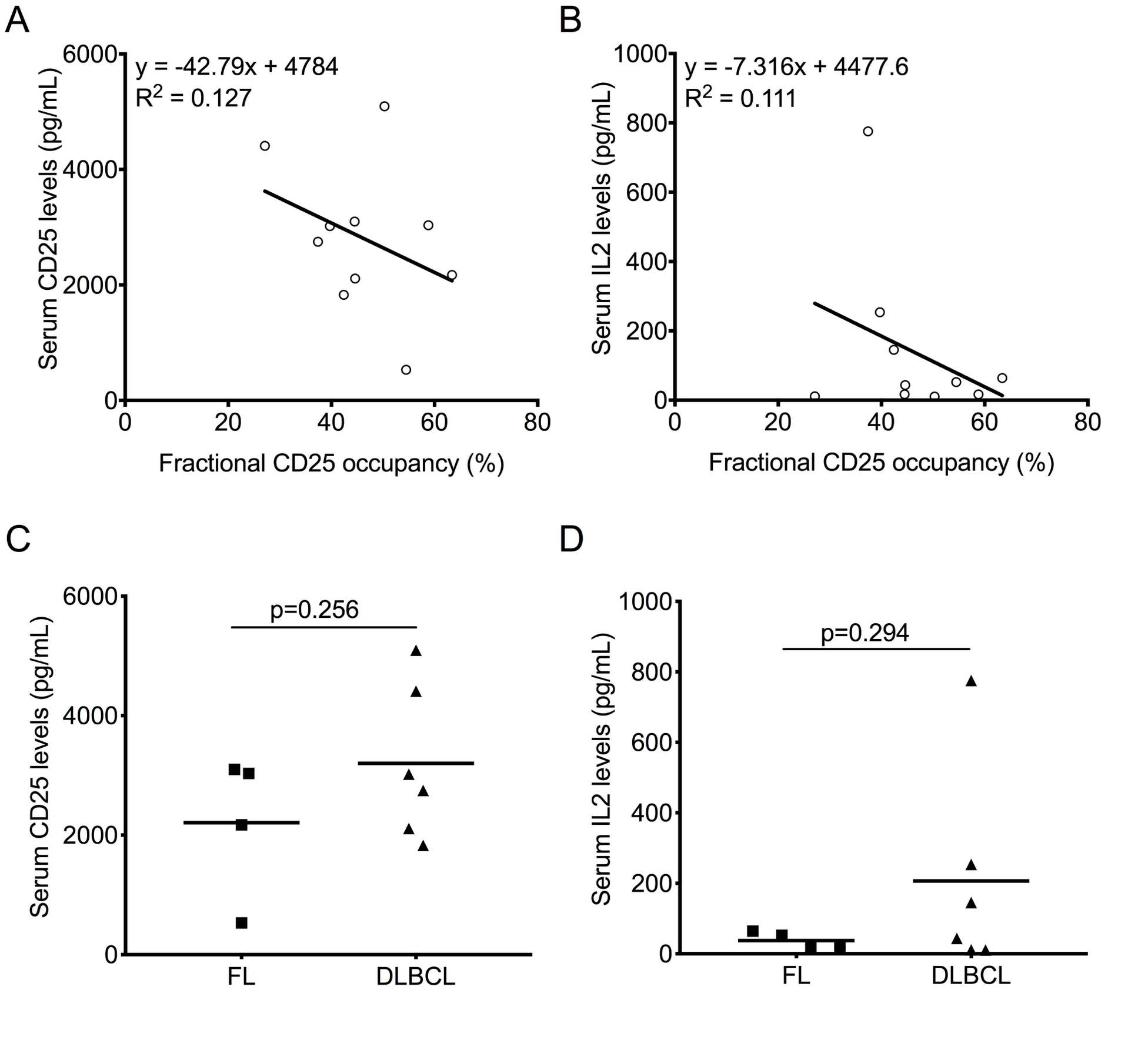
Supplementary Figure S4: Anti-human CD25 mAb (M-A251) does not interfere with the binding of Tr-7 and Tr-8 aptamers to CD25

The effect of anti-human CD25 (Clone M-A251), used for immunoprecipitation of CD25 from serum biospecimens, on the binding of aptamers (Tr-7 and Tr-8) was determined. Dynabeads coated with recombinant CD25 were incubated with anti-CD25 mAb, control murine IgG or buffer for 30 minutes at 37°C and the binding of Tr-7 or Tr-8 to beads was evaluated. M-251 mAb did not significantly alter the binding of either aptamer to CD25 beads. Data showed is a representative plot of two independent experiments.


None
Normal Mouse IgG
Anti-CD25 (Clone M-A251)
Protein-bound aptamers (pg/well)
400
300
200
100
0
Tr-7
Tr-8

Supplementary Figure S5: Correlation between total levels of soluble CD25, IL2 and IL2-occupied CD25 complexes in serum obtained from lymphoma subjects

(A) Correlation between soluble CD25 and fractional occupancy of soluble CD25 in the serum of lymphoma subjects (includes both FL and DLBCL) (n=10). (B) Correlation between IL2 and fractional occupancy of CD25 by IL2 in serum of lymphoma subjects (n=10) (C) Comparison of levels of soluble CD25 in serum is subjects with FL (n=4) or DLBCL (n=6) (D) Comparison of IL2 in serum of subjects with FL (n=4) or DLBCL (n=6)


A
y = -42.79x + 4784
R2 = 0.127
Serum CD25 levels (pg/mL)
Fractional CD25 occupancy (%)
B
y = -7.316x + 4477.6
R2 = 0.111
Serum IL2 levels (pg/mL)
Fractional CD25 occupancy (%)
C
p=0.256
Serum CD25 levels (pg/mL)
FL
DLBCL
D
p=0.294
Serum IL2 levels (pg/mL)
FL
DLBCL

Supplementary methods:

DNA melt assay:

The change in the complexity of aptamer pools was monitored after select rounds of enrichment using a DNA melt assay[20]. 20 pmoles of PCR DNA generated from the Treg-bound RNA pool after SELEX (Rounds 2, 4, 6 and 8) were individually mixed with equal amounts of 2X SYBR green PCR mix (Promega, USA). Samples were then subjected to a standard DNA melt curve analysis (95°C to 25°C, over 20 min ramp time). SYBR green fluorescence was plotted against the temperature and the melt curve was generated. The complexity of nucleotide sequences in the aptamer library progressively decreased after each round of SELEX due to enrichment and increase in the copy number of Treg-specific sequences that results in a shift in the overall melting curve towards higher Tm (shift to right).

Supplementary tables:

Supplementary Table S1: Levels of soluble CD25, IL2 and fraction of IL2-occupied CD25 in serum of lymphoma patients (n=10):

Patient ID	*Lymphoma subtype*	*IL2 (pg/mL)*	*Soluble CD25 (pg/mL)*	*Soluble CD25 occupancy (%)*
MF375	FL	64.16	2172.64	63.4
MF410	FL	17.17	3099.27	44.5
MF504	DLBCL	10.67	5094.89	50.3
MF822	DLBCL	253.61	3019.71	39.7
MF1110	DLBCL	43.53	2109.96	44.6
MF1109	FL	16.77	3033.55	58.8
MF1257	DLBCL	775.72	2748.44	37.4
MF1362	FL	52.3	530.81	54.5
MF1999	DLBCL	11.09	4409.04	27.1
MF2070	DLBCL	145.43	1828.79	42.4

Supplementary Table S2: Binding conditions used for Treg cell-based SELEX:

Cell-based SELEX was performed using 1.5 nmoles of aptamers. The DNA template and the primer sequences for transcribing RNA aptamer library are as follows:

DNA Template: 5'- TCGGGCGAGTCGTCTG - N20 - CCGCATCGTCCTCCC -3';

Forward primer: 5'- TAATACGACTCACTATAGGGAGGACGATGCGG -3'

Reverse: 5'- TCGGGCGAGTCGTCTG -3'

Transcribed RNA aptamer library was folded at 2 μM concentration in AIM-V (BSA) medium by step-wise incubation at 95°C for 5 minutes, 65°C for 10 minutes, followed by 37°C for 20 minutes. Folded aptamers were further diluted to 100 nM concentration before SELEX. All binding and washing steps were done at 37°C.

Rounds	Binding medium	[RNA]	Preclearing ($CD4^+CD25^{neg}$ Teff cells)	Binding ($CD4^+CD25^{high}$ Treg cells)
Rounds 1 to 4	AIM-V (BSA)	1.5 nmoles (100 nM)	15 min, once	30 min
Rounds 5 to 8	AIM-V (BSA)	1.5 nmoles (100 nM)	15 min, twice	30 min

Supplementary Table S3: CD25-binding aptamers identified in this study:

Sequence of control (C-248) and CD25-binding RNA aptamers identified in this study is given below. The nucleotides corresponding to the N20 region is italicized and underlined.

C-248	GGGAGGACGAUGCGG*CACACCGAAAUGUCCCGACU*CAGACGACUCGCCCGA
Tr-1	GGGAGGACGAUGCGG*UCCUGUCGUCUGUUCGUCCC*CAGACGACUCGCCCGA
Tr-6	GGGAGGACGAUGCGG*CGUUUCCUCUGGUUCGUCCC*CAGACGACUCGCCCGA
Tr-7	GGGAGGACGAUGCGG*UGAGUCGUUCCCUUCGUCCC*CAGACGACUCGCCCGA
Tr-8	GGGAGGACGAUGCGG*GCCGUUGUUGUGUGCCGCCC*CAGACGACUCGCCCGA
Tr-11	GGGAGGACGAUGCGG*AUUCUGGUUACUGGCCGCCC*CAGACGACUCGCCCGA

Supplementary Table S4: Assay conditions for biolayer interferometry:

Biolayer interferometry was used to measure aptamer-target binding kinetics. Following assay conditions were used and the data was collected at 30°C.

Steps	***Buffer***	***Time***
Equilibrium	1X binding buffer	180 secs
Loading	1 μM biotinylated aptamers in 1X binding buffer	600 secs
Blocking	5 ug/mL Biocytin	300 secs
Washing	1X Binding buffer	300 secs
Baseline	1X Binding buffer	60 secs
Association	Four-fold serial dilution of target proteins in 1X Binding buffer (250 nM, 62.5 nM and 15.6 nM)	300 secs
Dissociation	1X Binding buffer	300 secs

Supplementary Table S5: Primers and probes used in the probe-based RT-qPCR to quantify IL2 occupancy:

	Sequences
Forward primer	5'-TATAGGGAGGACGATGCGG-3'
Reverse primer	5'-TCGGGCGAGTCGTCTG-3'
Tr-1 probe	5'-/FAM/TCTGGGGAC/ZEN/GAACAGACGACAGGA/IBFQ/-3'
Tr-6 probe	5' /HEX/TCTGGGGAC/ZEN/GAACCAGAGGAAAC/IBFQ/-3'
Tr-7 probe	5'-/FAM/TCTGGGGAC/ZEN/GAAGGGAACGACTCA/IBFQ/-3'
Tr-8 probe	5'-/TET/TCTGGGGCG/ZEN/GCACACAACAACGGC/IBFQ/-3'

Supplementary Table S6: Increase in copy number of top enriched RNA aptamers (per million unique reads) in select rounds during Treg cell-based SELEX

SELEX rounds	*Tr-1*	*Tr-2*	*Tr-3*	*Tr-5*	*Tr-6*	*Tr-7*	*Tr-8*	*Tr-9*	*Tr-10*	*Tr-11*	*Tr-12*
Rd 0	0.36	0.14	0.07	0.07	0.07	0.07	0.21	ND*	ND	0.07	ND
Rd 2	309	243	908	333	167	220	219	173	21	311	216
Rd 4	10733	2048	2184	1472	1631	1104	1278	1119	805	964	1020
Rd 8	22995	9963	3106	2427	4832	4332	4017	3246	5282	1828	1827

* ND – Not detectable