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Supplemental information

High-throughput identification of autoantibodies that target the human exoproteome

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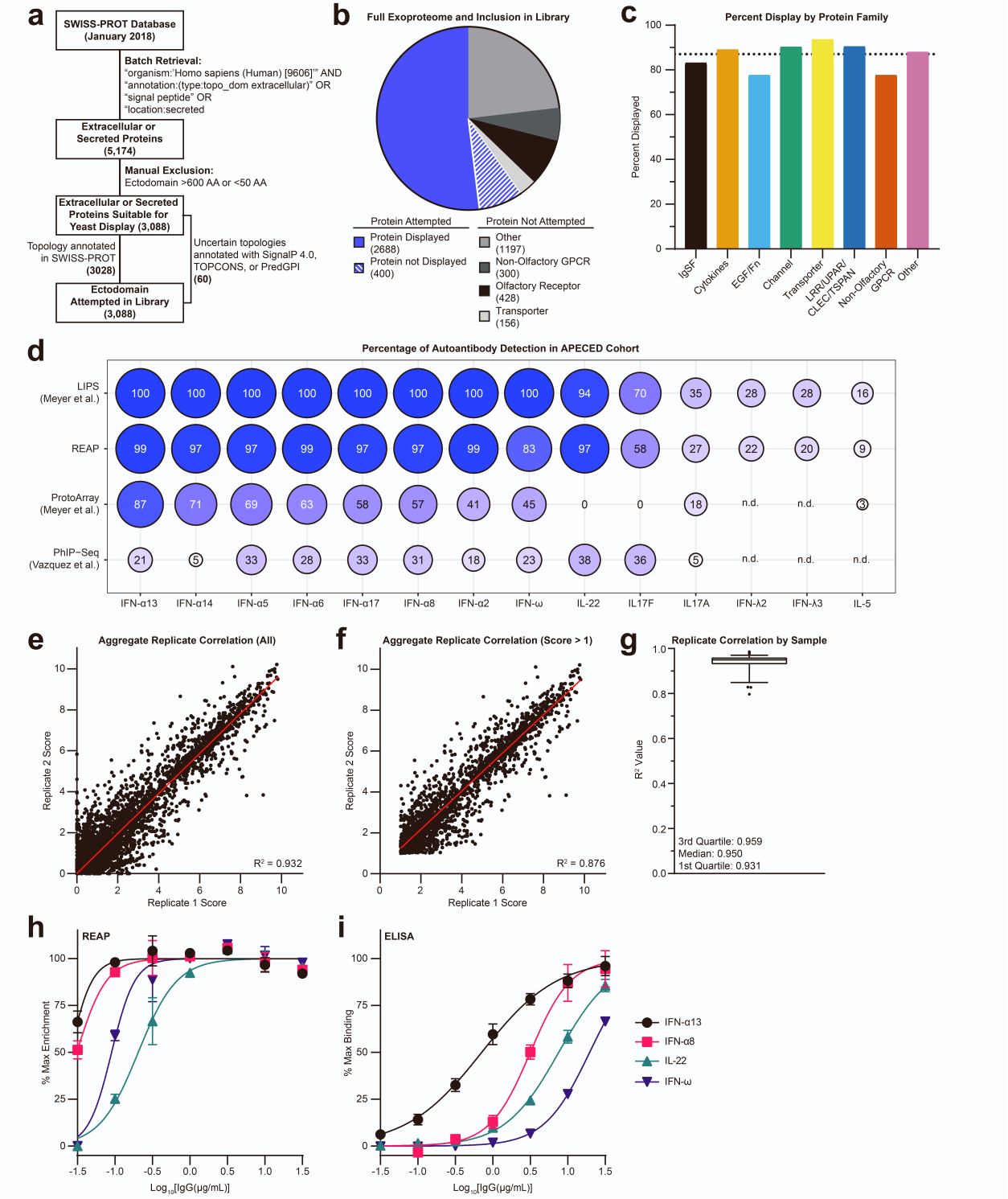


Figure S1: Exoproteome yeast display library properties related to Figure 1 and Figure 3. a, Flowchart of steps in identification and annotation of extracellular or secreted proteins for inclusion in the library. **b,** Pie chart of all extracellular or secreted proteins identified in **a**. Proteins were not attempted if they had an ectodomain less than 50 amino acids or greater than 600 amino acids. **c,** Percent of library proteins displayed in each protein family. The dotted line represents the aggregate display level in the library. Abbreviations are as follows: immunoglobulin superfamily (IgSF), epidermal growth factor (EGF), fibronectin (Fn), leucine-rich repeat (LRR), urokinase receptor (UPAR), c-type lectin (CLEC), tetraspanin (TSPAN). The cytokine family consists of proteins belonging to tumor necrosis factor,

interferon, interleukin, and growth factor protein families. **d,** Comparison of autoantibody detection frequencies in APS-1 patient cohorts by REAP, LIPS (Meyer et al., 2016), ProtoArray (Meyer et al., 2016), and PhIP-Seq (Vazquez et al., 2020). Frequencies are listed as a percentage inside each circle. Size and color of circles are proportional to detection frequency. For REAP, detection frequency was calculated as in **Figure 3b**. For LIPS and ProtoArray, detection frequencies were provided in the corresponding publication. For PhIP-Seq, detection frequency was calculated based on figures in the corresponding publication. For reactivities labeled n.d., either data was not publicly available or the autoantibody was not tested for in the corresponding assay. **e,** Scatter plot of all REAP reactivities between technical replicates of APS-1 patients screened in **Figure 3**. **f,** Scatter plot of all REAP reactivities with scores greater than 1 between technical replicates of APS-1 patients. **g,** Box plot of sample level R^2 coefficient of determination values from comparisons of all REAP reactivities between technical replicates. R^2 values were calculated individually for each APS-1 patient. Samples below the 5th percentile and above the 95th percentile are depicted as individual points. **h,i,** REAP (**h**) versus ELISA (**i**) dose-response curve comparison for APS-1 autoantibodies against four proteins. REAP data is from a screen conducted using varying concentrations of AIRE.19 IgG. Curves were fit using a sigmoidal 4 parameter logistic curve. For REAP, curves were fit based on Log_2[fold enrichment]. For ELISA, curves were fit based on optical density at 450 nm. Error bars represent standard error of the mean.

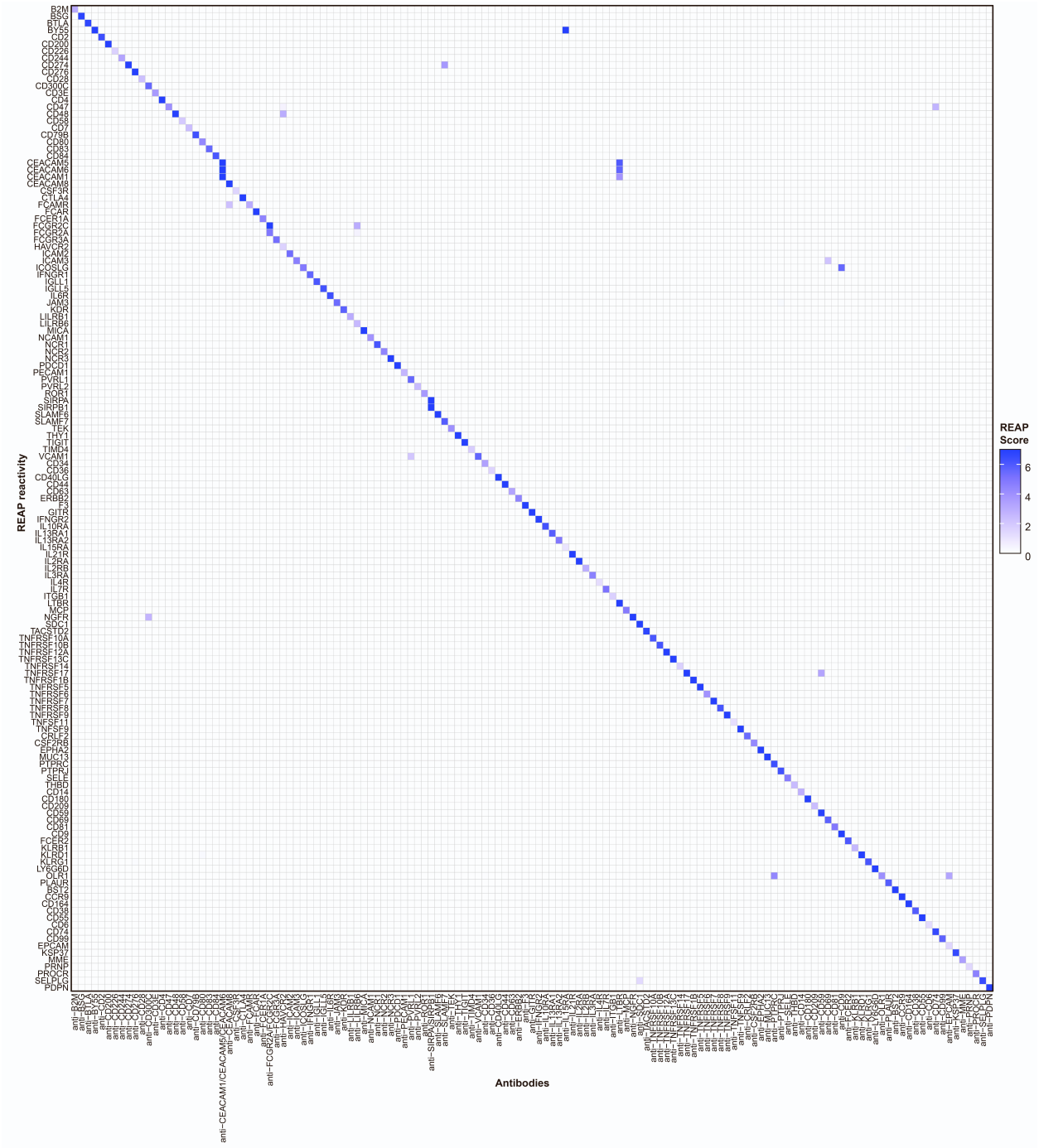


Figure S2: Antibody panel on-target heatmap related to Figure 2. Heatmap of REAP reactivities for known antibody targets from the antibody panel screen in **Figure 2**. Score was artificially capped at 7 to aid visualization.

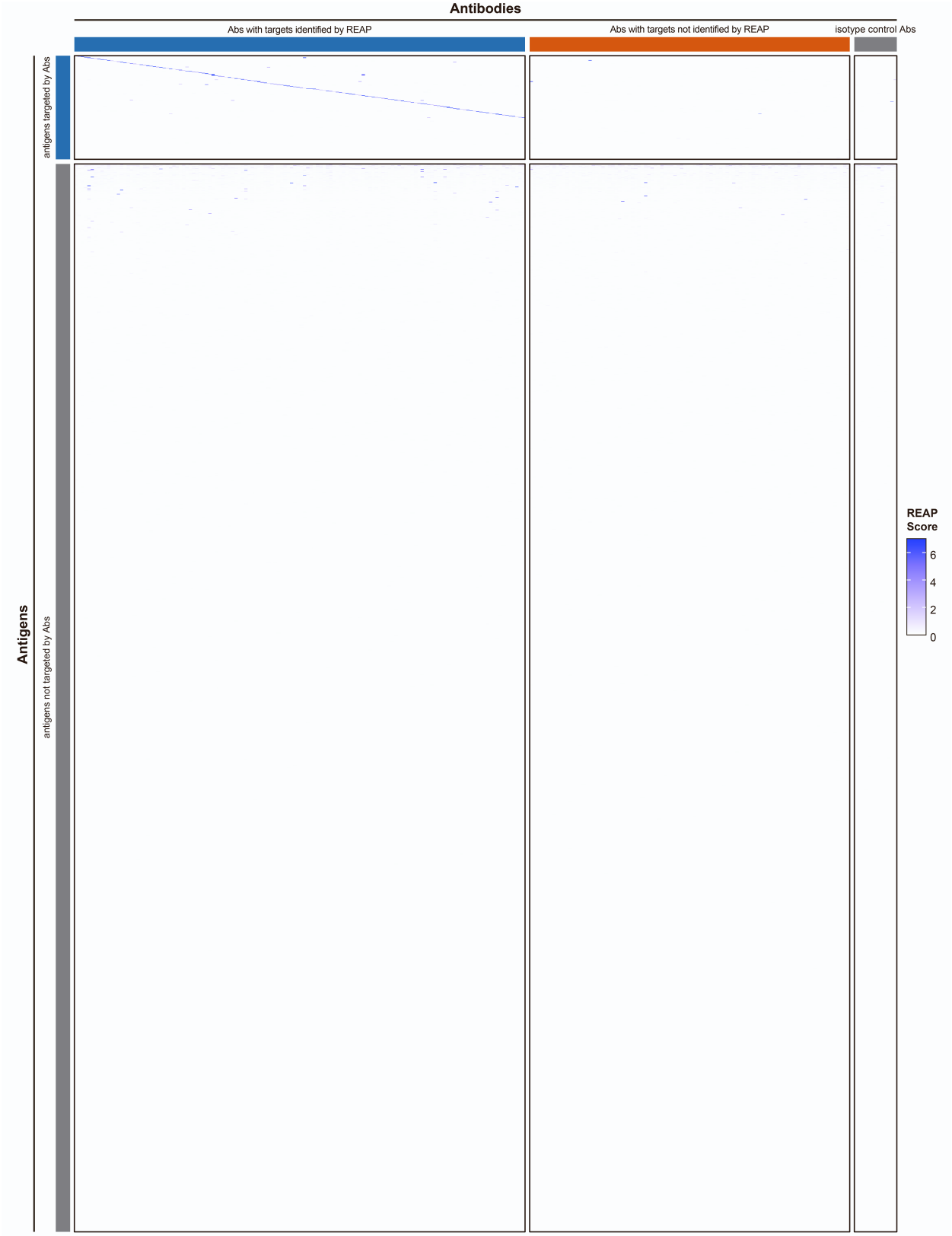
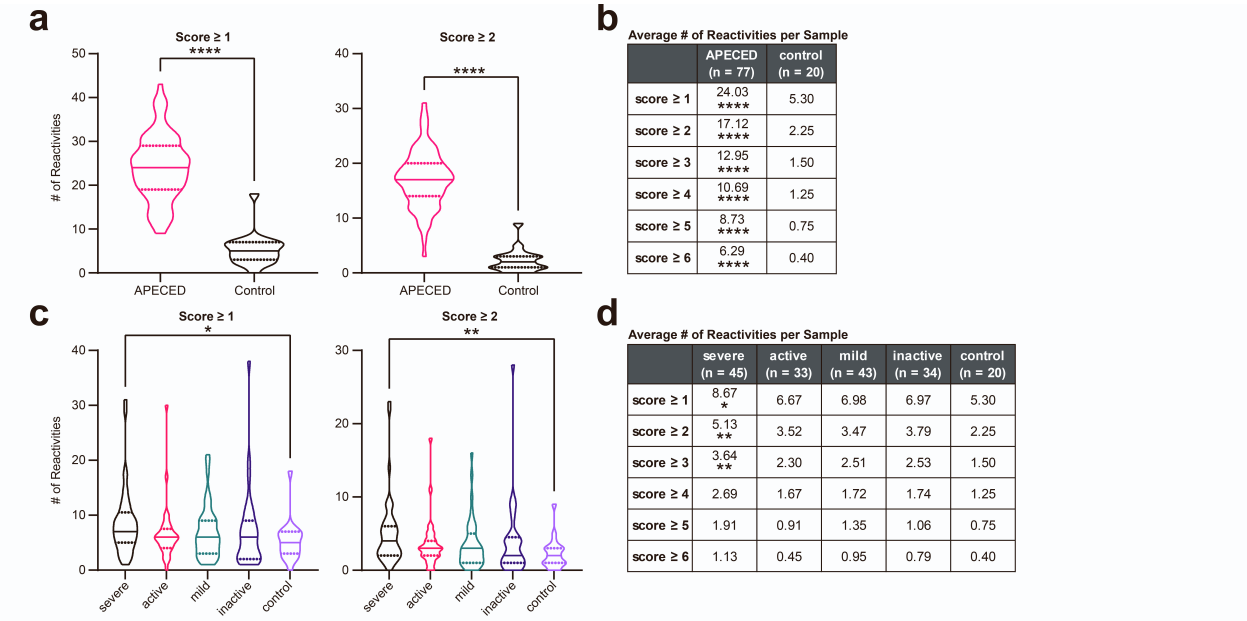


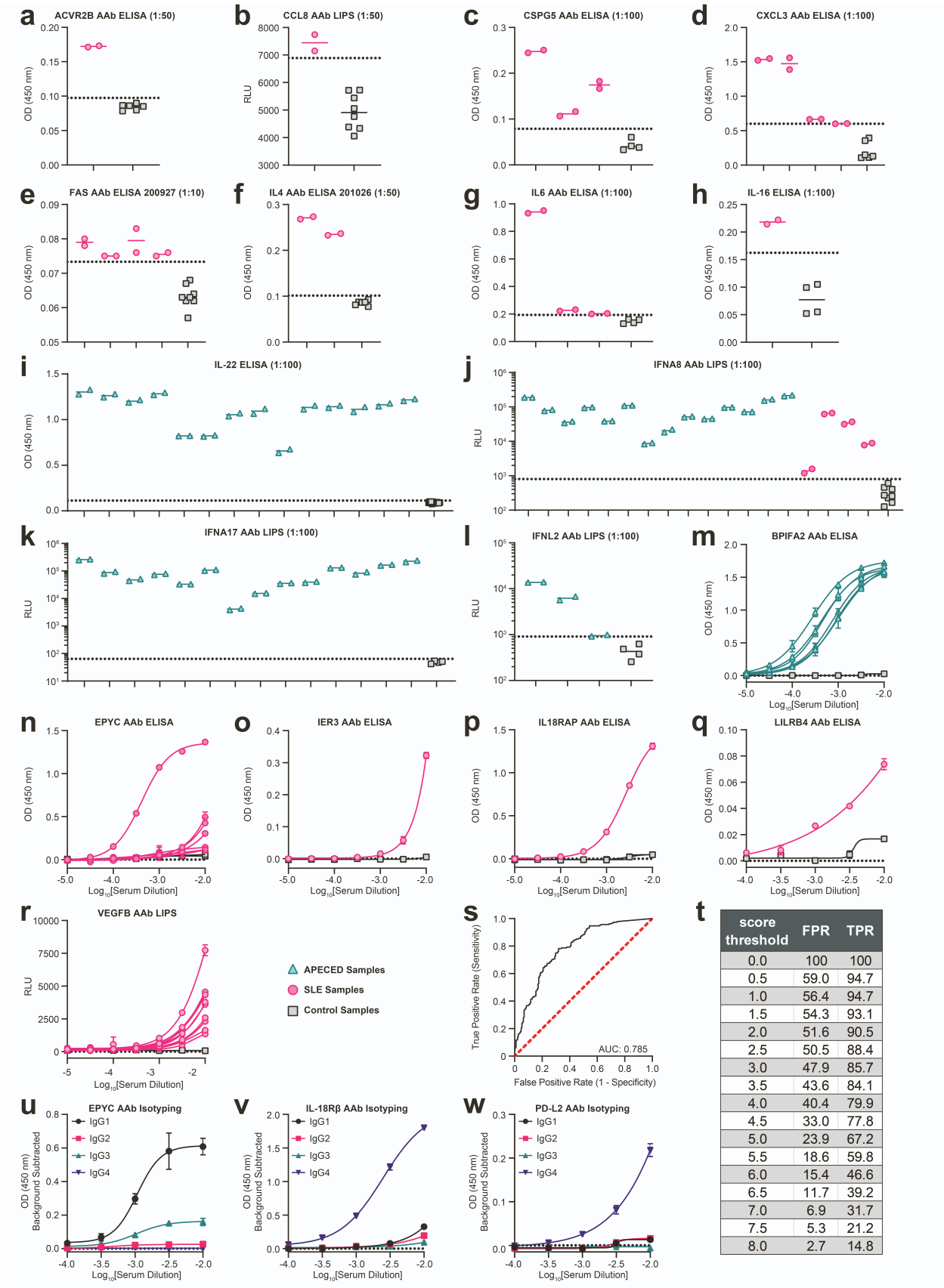
Figure S3: Antibody panel full heatmap related to Figure 2. Heatmap of all REAP reactivities from the antibody panel screen in **Figure 2**. Score was artificially capped at 7 to aid visualization.



	APECED (n = 77)	control (n = 20)
score ≥ 1	24.03 ****	5.30
score ≥ 2	17.12 ****	2.25
score ≥ 3	12.95 ****	1.50
score ≥ 4	10.69 ****	1.25
score ≥ 5	8.73 ****	0.75
score ≥ 6	6.29 ****	0.40

	severe (n = 45)	active (n = 33)	mild (n = 43)	inactive (n = 34)	control (n = 20)
score ≥ 1	8.67 *	6.67	6.98	6.97	5.30
score ≥ 2	5.13 **	3.52	3.47	3.79	2.25
score ≥ 3	3.64 **	2.30	2.51	2.53	1.50
score ≥ 4	2.69	1.67	1.72	1.74	1.25
score ≥ 5	1.91	0.91	1.35	1.06	0.75
score ≥ 6	1.13	0.45	0.95	0.79	0.40

Figure S4: Additional APECED and SLE reactivity distributions related to Figure 3 and Figure 4. a, Violin plots of the number of reactivities in APECED and control samples at a score cutoff of 1 or 2. **b,** Mean number of reactivities in APECED and control samples at various score cutoffs, along with indicators of significance. **c,** Violin plots of the number of reactivities in SLE samples stratified by disease severity and control samples at a score cutoff of 1 or 2. **d,** Mean number of reactivities in SLE samples stratified by disease severity and control samples at various score cutoffs. Comparisons were made between each disease severity group and the control group. Significance in **a** and **b** was calculated using a two-sided Mann-Whitney U test. Significance in **c** and **d** was determined using a Kruskal-Wallis test followed by a Dunnett's test. *P ≤ 0.05, **P ≤ 0.01., ****P ≤ 0.0001



t

score threshold	FPR	TPR
0.0	100	100
0.5	59.0	94.7
1.0	56.4	94.7
1.5	54.3	93.1
2.0	51.6	90.5
2.5	50.5	88.4
3.0	47.9	85.7
3.5	43.6	84.1
4.0	40.4	79.9
4.5	33.0	77.8
5.0	23.9	67.2
5.5	18.6	59.8
6.0	15.4	46.6
6.5	11.7	39.2
7.0	6.9	31.7
7.5	5.3	21.2
8.0	2.7	14.8

Figure S5: REAP validation and ROC analysis related to Figure 3 and Figure 4. a-l, Single-point ELISAs or LIPS conducted with SLE, APS-1, or control serum to detect autoantibodies against ACVR2B (**a**), CCL8 (**b**), CSPG5 (**c**), CXCL3 (**d**), Fas (**e**), IL-4 (**f**), IL-6 (**g**), IL-16 (**h**), IL-22 (**i**), IFN-α8 (**j**), IFN-α7 (**k**), and IFNL2 (**l**). Serum dilutions are listed in the title of each plot. **m-r,** ELISAs or LIPS conducted with serial dilutions of SLE, APS-1, or control serum to detect autoantibodies against BPIFA2 (**m**), EPYC (**n**), IER3 (**o**), IL18RAP (**p**), LILRB4 (**q**), and VEGF-B (**r**). Dotted lines in **a-l** represent the control average + 3 standard deviations. **s,** Receiver operating characteristic curve of the ability of REAP score to predict validation of a REAP reactivity in an orthogonal assay. A full description of this analysis can be found in the STAR methods. **t,** Table of false positive rate (FPR) and true positive rate (TPR) at various score threshold cutoffs, as determined by the ROC analysis in **s**. **u,** Anti-epiphycan IgG subclass specific ELISA conducted with serial dilutions of serum from the SLE patient with highest titers in **n**. **v,** Anti-IL-18RAcP subclass specific ELISA conducted with serial dilutions of serum from the SLE patient in **p**. **w,** Anti-PD-L2 IgG subclass specific ELISAs conducted with serial dilutions of serum from the SLE patient in **Figure 4f**. All error bars in this figure represent standard deviation. All curves in this figure were fit using a sigmoidal 4 parameter logistic curve.

Table S2: APS-1 patient demographics and clinical characteristics related to Figure 3.

APECED cohort characteristics (n = 77)	Number (%)
Age*	24 (14.4)
Sex (female)	45 (58)
Ethnicity	
White Non-Hispanic	68 (88)
White/Hispanic	5 (7)
AIRE* alleles*	
c.967_979del13	79 (51)
c.769C>T	21 (14)
Clinical manifestations	
Chronic mucocutaneous candidiasis	66 (86)
Adrenal insufficiency	62 (81)
Hypoparathyroidism	63 (82)
Hypothyroidism	18 (23)
Hypogonadism	26 (34)
Autoimmune pneumonitis	28 (36)
Autoimmune hepatitis	25 (33)
Intestinal dysfunction	53 (69)
Exocrine pancreatic insufficiency	1 (1)
Asplenia	10 (13)
Alopecia	26 (34)
Vitiligo	19 (25)
Sjogren's-like syndrome	30 (39)
Autoimmune gastritis	30 (39)
B12 deficiency	20 (26)
Intrinsic factor antibody	24 (31)
Lung-targeted autoantibodies***	
BPIFB1	19 (26)
KCNRG	4 (6)

*Age is represented as mean (standard deviation) in years
**The denominator for *AIRE* mutant alleles is 154
***Data available for 72 patients
AIRE, autoimmune regulator; APECED, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy; BPIFB1, BPI fold containing family B member 1

Table S3: SLE patient and healthy control demographics and clinical characteristics related to Figure 4.

Mean (SD) or as indicated	SLE Cohort (n = 85*)	Healthy Controls (n = 20)
Age, (years)	41.7 (12.6)	37.2 (11)
Sex, N (% female)	76 (89.4)	12 (60)
Ethnicity, N (%)		
Hispanic	22 (26)	3 (15)
Non-Hispanic	35 (41)	8 (40)
African American	28 (33)	9 (45)
Clinical Manifestations, N (%)		
Skin	40 (47.1)	
Mucocutaneous	16 (18.8)	
Musculoskeletal	29 (34.1)	
Renal	20 (23.5)	
Cardiorespiratory	4 (4.7)	
Hematological	7 (8.2)	
Neuropsychiatric	0 (0)	
Serologies, N (%)		
Positive dsDNA	40 (47.1)	
Low complement	34 (40)	
SLEDAI score	6.3 (6.1)	
Medications, any use N (%)		
Prednisone	40 (47.1)	
Hydroxychloroquine	72 (84.7)	
Mycophenolate mofetil	24 (28.2)	
Methotrexate	6 (7.1)	
Azathioprine	4 (4.7)	
Belimumab	6 (7.1)	
Rituximab	4 (4.7)	
Infliximab	1 (1.2)	
Others (cyclophosphamide, rtacrolimus, etc.)	4(4.7)	

Abbreviations: SLEDAI (Systemic Lupus Erythematosus Disease Activity Index).
Prednisone dosing ranges from 5 mg daily to 60 mg daily.
*Complete clinical data was not available for a subset of patients. A total of 106 patients were screened.