Supplementary methods

PepArray analysis program

Images of the arrays were recorded with 16-bit depth using an Olympus MVX10 fluorescence microscope equipped with a cooled digital XM10 camera and a motorized Märtzhäuser Wetzlar Tango 2-axis stage. The final images were typically assembled from multiple overlapping images recorded using the Stage Navigator feature in the Olympus Cell-P software package.

Proprietary image analysis software was used for extraction of data from the images. The general strategy was to display the image on a PC-screen and then drag the 4 corners of a deformable quadrangular grid drawn on the screen until they coincided with fluorescent markers in the corners of the array. The number of rows and columns in the drawing grid corresponded to those in the peptide array. The fluorescent corner markers were made during synthesis by incorporation of a peptide with known affinity for the fluorescent secondary antibody. With the grid positioned, a line scan was made of all pixels in the image. During this scan the intensity (8-bit depth) of the individual pixels in the R, G and B channels were recorded together with their x- and y coordinates. The values were accumulated in the corresponding fields in the quadrangular grid and averaged. Each field in the grid represents a synthesis field in the array and the averaged pixel intensity values inside the field represents the signal in that field. The software was designed to handle color images, but since all images in the current work were recorded in greytones, the RGB-values were simply added, i.e. so that a maximum signal of 3 x 255 = 765 AU can be obtained.

The positions of the peptide fields in the array were assigned at random to reduce the influence of local fluctuations in the background signal, and as a further background correction every 9^{th} field was reserved for synthesis of a ”blank” sequence whose signal were subtracted from the surrounding 8 peptide fields to obtain the background corrected values used in the figures and tables in the present work.

Supplementary Figure S1 Schematic drawings of the photolithographic principle. (A) Schematic drawing of the optical setup in the synthesizer. Light from a 365 nm source is collimated and projected onto a digital mirror device. Light reflected from selected mirrors in the digital mirror device is directed into an imaging system that generates an image of the digital mirror device on the synthesis surface. (B) At the outset of each cycle of a solid-phase peptide synthesis strategy, the N-terminal of all the amino acids of the growing peptide chain is protected by a photosensitive protection group, e.g. NPPOC (filled circles, step 1). The photosensitive protection groups are removed by UV-irradiation of predefined array fields in which the peptide chains are subsequently extended with a predefined amino acid protected by a base-labile Fmoc group (filled triangles). With natural peptides, one elongation cycle involves one UV-deprotection step for each of the 20 different amino acids (e.g. steps 2-4 illustrate extension by Glutamic acid, steps 5-7 with Alanine, and steps 8-10 with Valine etc.). At the completion of an elongation cycle, all peptides have been extended by the intended Fmoc amino acid and can be prepared for the next elongation cycle by removal of the Fmoc-groups by piperidine treatment liberating free amino terminals (open circles, step 11) followed by coupling of photosensitive groups to the exposed n-terminal amino groups (step 12).

Supplementary Figure S2. Example of the validation of the specificity of a polyclonal rabbit anti-SNAPC1 antibody using PrEST micro arrays.

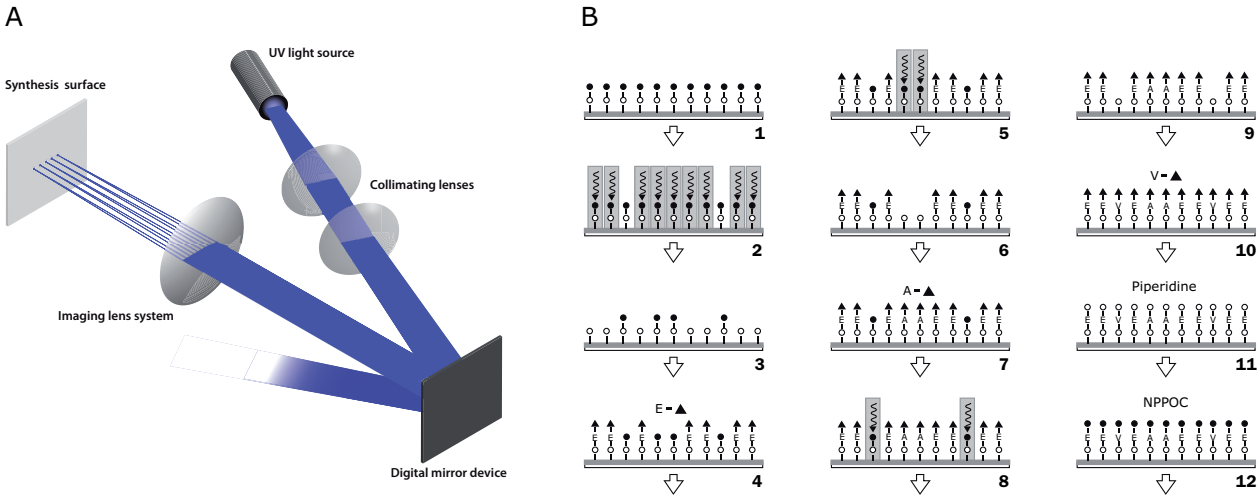
As previously detailed (Nilsson et al., Proteomics, 5(17):4327), antigen microarrays consisting of the target PrEST antigen and 383 different unrelated PrEST antigens were printed and utilized to profile the specificity of the polyclonal anti-SNAPC1 antibody. Bound primary rabbit antibody was detected by a secondary Alexa647-conjugated goat anti-rabbit antibody reagent, incubated, washed and detected in an G2565BA array scanner (Agilent), and images were quantified using image analysis software (GenePix 5.1, Axon Instruments). The fluorescence intensity is shown on the y-axis and the 384 PrEST antigens on the x-axis of the bar graph where the red bars represent signal from the correct antigen.

Supplementary Figure S3. Comparison of epitopes found by the peptide microarray-driven SSA analysis and alternative epitope display approaches.

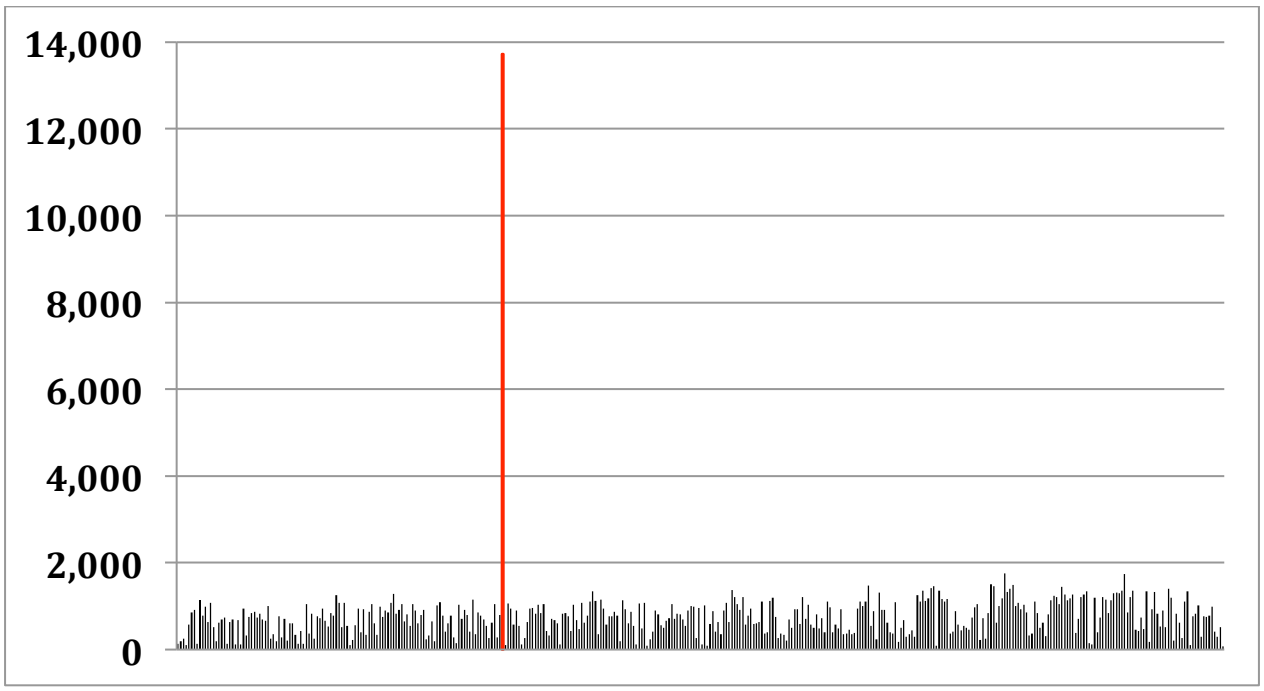
Epitopes were identified by the peptide microarray approach or by bacterial or Luminex display, and the figure shows these epitopes aligned with the PrEST sequence for those 15 of the 22 antibodies where such comparisons were possible. The black line shows the complete PrEST sequence, the red line shows epitopes identified by bacterial display, the blue line shows epitopes

identified by Luminex display, and the green line shows epitopes identified by the peptide microarray approach. Each amino acid could be assigned to whether it belonged to a) both a peptide microarray display defined epitope and a display (bacterial or Luminex) defined epitope; b) to a peptide microarray display defined epitope, but not display (bacterial or Luminex) defined epitope; c) to a display (bacterial or Luminex) display defined epitope, but not a peptide microarray defined epitope: or d) neither. This showed a highly significant correlation between the peptide microarray and display approaches (Chi-square test with Yates correction, $P<0.0001$).

Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3