Supporting Information

High-Resolution Analysis of Antibodies to Post-Translational Modifications Using Peptide Nanosensor Microarrays

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Supplementary figures

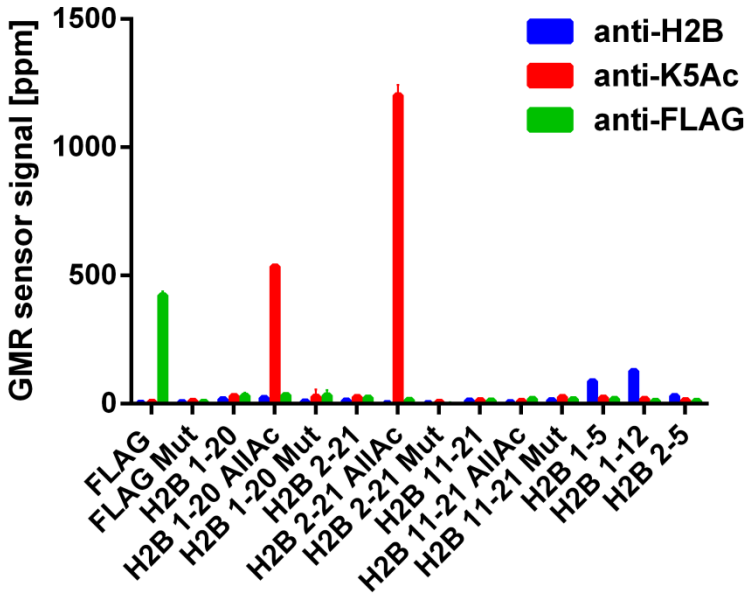

Figure S1. Cross-reactivity tests of anti-H2B, anti-K5Ac, and anti-FLAG. The antibodies were each used to probe separate microarrays that feature 14 peptides. The concentrations of antibodies were 1 µg/mL. Error bars represent the standard deviation of 4 identical sensor signals.

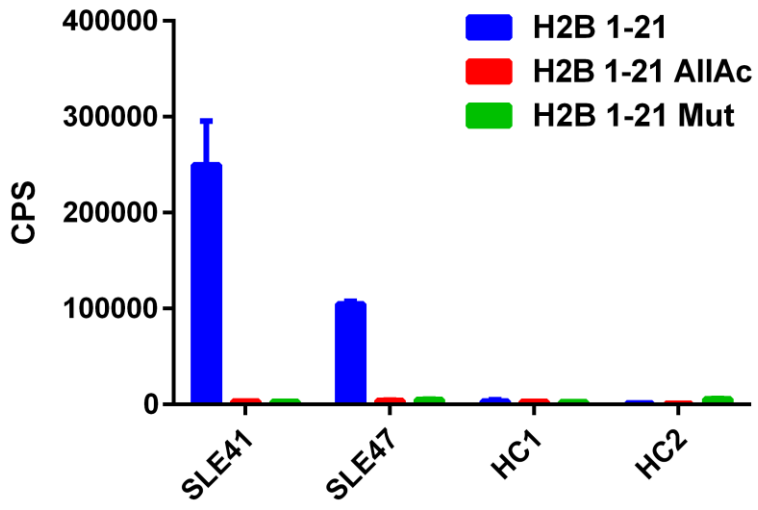

Figure S2. ELISA measurement of serum samples from individuals with SLE and healthy controls. Each sample (SLE41, SLE47, HC1, and HC2) was measured by ELISA, using H2B 1-21 (amino acid sequence: PEPAKSAPAPKKGSKKAVTKA), H2B 1-21 AllAc (PEPAK*SAPAPKK*GSK*KAVTK*A, K*: acetylated lysine), and H2B 1-21 Mut (PEPAASAPAPAAGSAAAVTAA) peptides, respectively. Error bars represent the standard deviation of 2 identical wells.

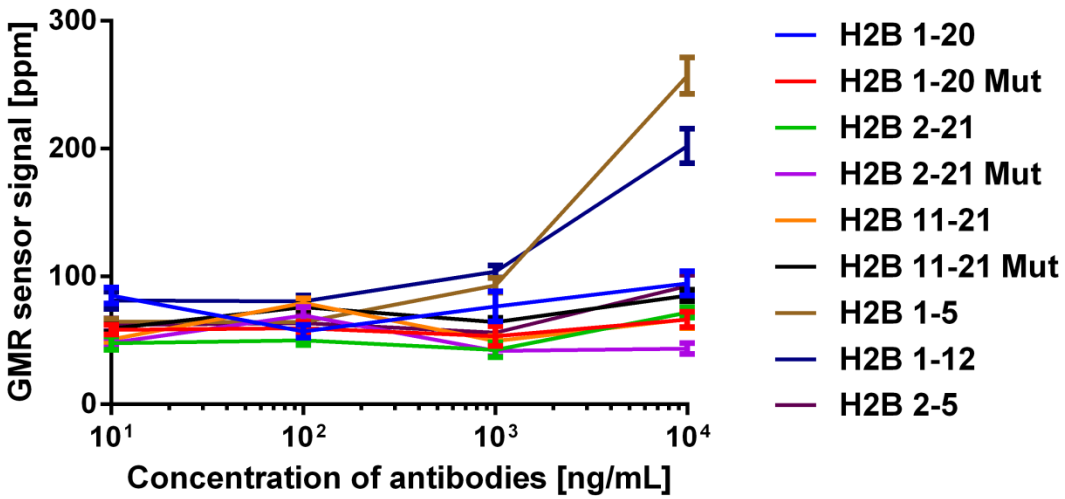

Figure S3. Titration curves of anti-H2B antibody. Anti-H2B antibody was used to probe GMR peptide microarrays with 9 H2B peptides immobilized on the nanosensors. This commercial antibody was found to bind to shorter peptides of H2B terminal tail (H2B 1-5 or H2B 1-12), but not to H2B 1-20. In addition, the comparison between H2B 1-5 and H2B 2-5 showed that the binding requires the N-terminal proline residue. Error bars represent the standard deviation of 4 identical sensor signals.

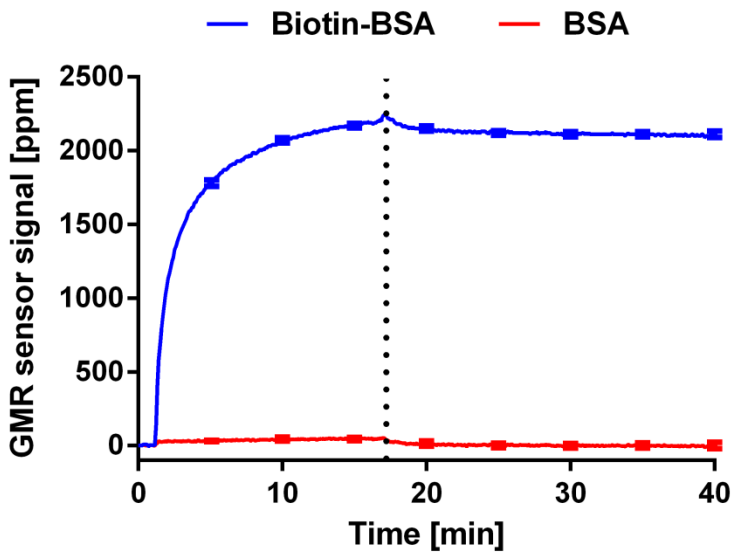

Figure S4. No effect of glycine-HCl pH 2.0 regeneration solution on biotin-streptavidin interaction. Biotinylated BSA was immobilized on the nanosensors of a chip and streptavidin-coated magnetic nanoparticles were added. After sufficient amounts of MNPs were bound to the sensor, glycine-HCl pH 2.0 was added to the chip at ~ 17 min as indicated by the dotted line.

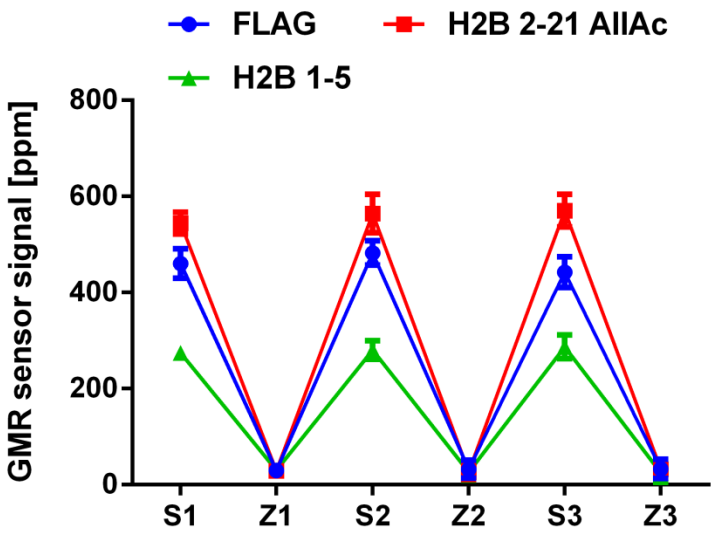

Figure S5. Regeneration efficiency testing with GMR nanosensor microarrays. GMR microarrays were used to measure repeated cycles of an antibody-containing sample (anti-FLAG, 1 µg/mL; anti-K5Ac, 0.5 µg/mL; and anti-H2B, 5 µg/mL), followed by measurement with no antibodies, with regeneration between measurements. "S" indicates the plateau signal from the antibody measurements. "Z" indicates the plateau signal from the measurement on no antibodies (buffer solution was incubated instead of the sample, and secondary antibodies were added). 1 hour regeneration was performed between "S" and "Z", and the chip was treated with glycine-HCl pH 2.0 for 10 min between "Z" and "S".

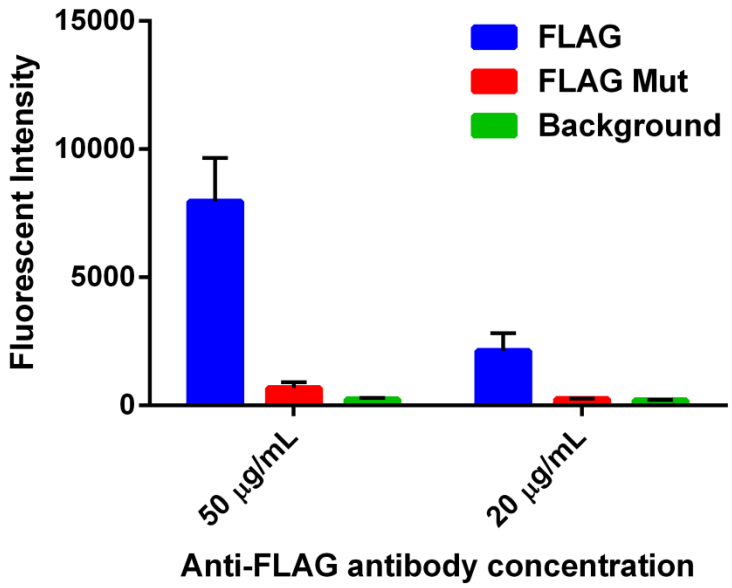

Figure S6. Fluorescence measurement of anti-FLAG antibody with *in-situ* synthesized peptide GMR nanosensor microarrays. The microarrays were probed with two different concentrations of FITC-tagged anti-FLAG antibodies (M2 clone, 50 and 20 µg/mL). Fluorescent images were taken over the FLAG- and FLAG Mut-synthesized areas. More than 100 points over each area were selected to calculate averaged fluorescent intensity and standard deviations (error bars). Background represents the area where no peptide was synthesized.