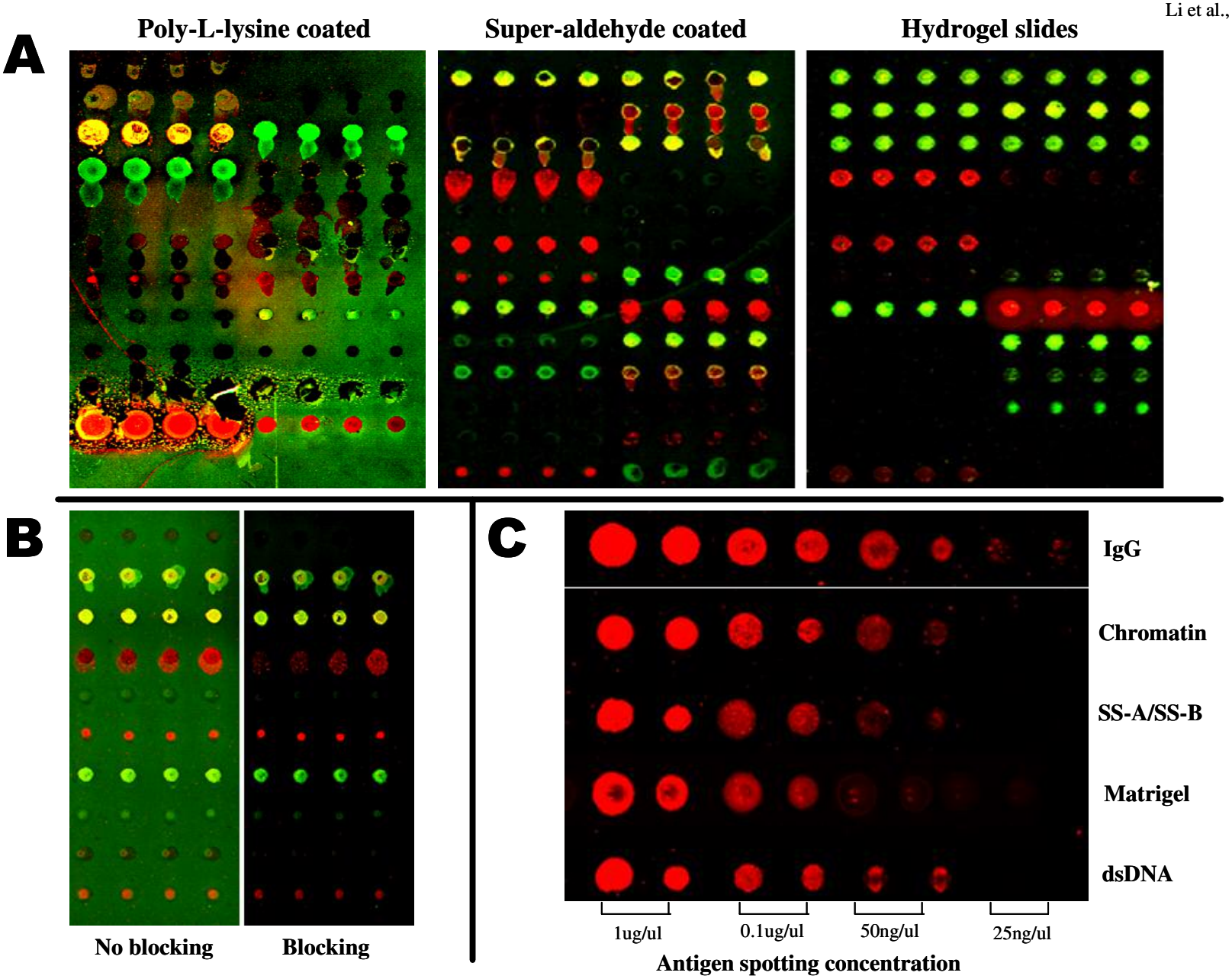
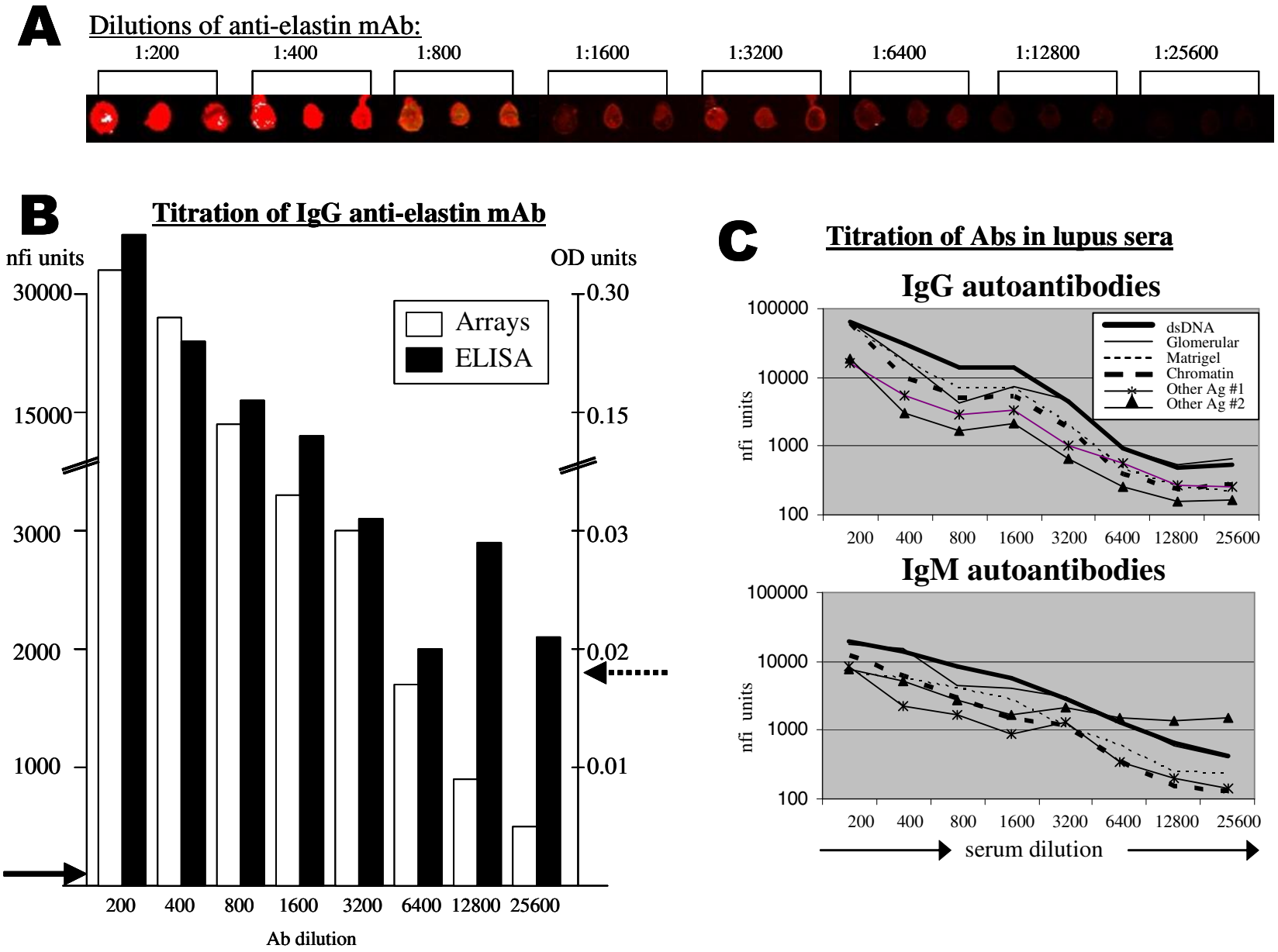

A
Poly-L-lysine coated
Super-aldehyde coated
Hydrogel slides
B
No blocking
Blocking
C
IgG
Chromatin
SS-A/SS-B
Matrigel
dsDNA
1ug/ul
0.1ug/ul
50ng/ul
25ng/ul
Antigen spotting concentration


A
Dilutions of anti-elastin mAb:
1:200
1:400
1:800
1:1600
1:3200
1:6400
1:12800
1:25600
B
Titration of IgG anti-elastin mAb
nfi units
OD units
30000
15000
3000
2000
1000
0.30
0.15
0.03
0.02
0.01
Arrays
ELISA
200
400
800
1600
3200
6400
12800
25600
Ab dilution
C
Titration of Abs in lupus sera
IgG autoantibodies
IgM autoantibodies
nfi units
100000
10000
1000
100
dsDNA
Glomerular
Matrigel
Chromatin
Other Ag #1
Other Ag #2
200
400
800
1600
3200
6400
12800
25600
serum dilution

Supplementary Data

Figure S1. Optimal slide chemistry and coating antigen concentrations for the glomerular proteome arrays.

A: A spectrum of glomerular/GBM antigens was spotted onto poly-L-lysine, super-aldehyde, or polyacrylamide coated "Hydrogel" slides. A pooled cocktail of NZM2410-derived IgM/IgG mAbs specific for dsDNA and/or total glomerular lysate (12) was applied to these different slides. The slides were then developed with Cy5-coupled goat anti-mouse IgG and Cy3-labeled goat anti-mouse IgM Abs, and scanned.

B: Shown are the scanned images using antigen-coated Hydrogel slides, with or without "blocking" using 0.1% Tween 20 and 0.5% BSA in PBS. After blocking, a pooled cocktail of NZM2410-derived IgM/IgG mAbs specific for dsDNA and/or total glomerular lysate (12), was applied, and developed as described above.

C: Different concentrations of antigens were coated onto Hydrogel slides and blocked. Serum from a B6.*Sle1.lpr* mouse, seropositive for anti-dsDNA and anti-glomerular Abs, was then added and developed using Cy5-labeled goat anti-mouse IgG Abs. Depicted are representative titration profiles against a couple of nuclear/glomerular antigens. The optimal coating concentration for most of the antigens was 1 ug/ml, as described in Methods.

Figure S2. Assessing the sensitivity of the glomerular proteome arrays.

A: Indicated are the reactivity intensities of serial dilutions of a commercial IgG mAb (stock = 1 mg/ml) specific for elastin. Similar titration curves were obtained using anti-collagen IV, anti-vimentin, anti-myosin, anti-fibrinogen IV, and anti-hemocyanin mAbs (data not shown).

B: The above titration experiment was also carried out in parallel using a conventional ELISA methodology. Indicated are the nfi units using the arrays (left axis, white bars), and the ELISA ODs (right axis, black bars), corresponding to different dilutions of the anti-elastin mAb. Arrowed on the respective axes are the background reactivities on the arrays or the ELISA assays, corresponding to the observed levels of binding to buffer alone, or irrelevant control antigens (e.g., ovalbumin or BSA). Shown titration study is representative of 2 independent experiments.

C: Serum from a seropositive B6.*Sle1.lpr* mouse was serially diluted, and applied

to the glomerular proteome arrays. Depicted are respective IgG (top) and IgM (bottom) reactivities (expressed as nfi units) to dsDNA, total glomerular lysate, Matrigel, chromatin, and 2 additional antigens (indicated as Ag#1 and Ag#2) to which this particular serum demonstrated strong reactivity. Ag#1 and Ag#2 were hemocyanin and myosin, respectively, for the IgG Abs, and fibrinogen IV and entactin, respectively, for the IgM Abs. Shown titration study is representative of 2 independent experiments.