Figure S1

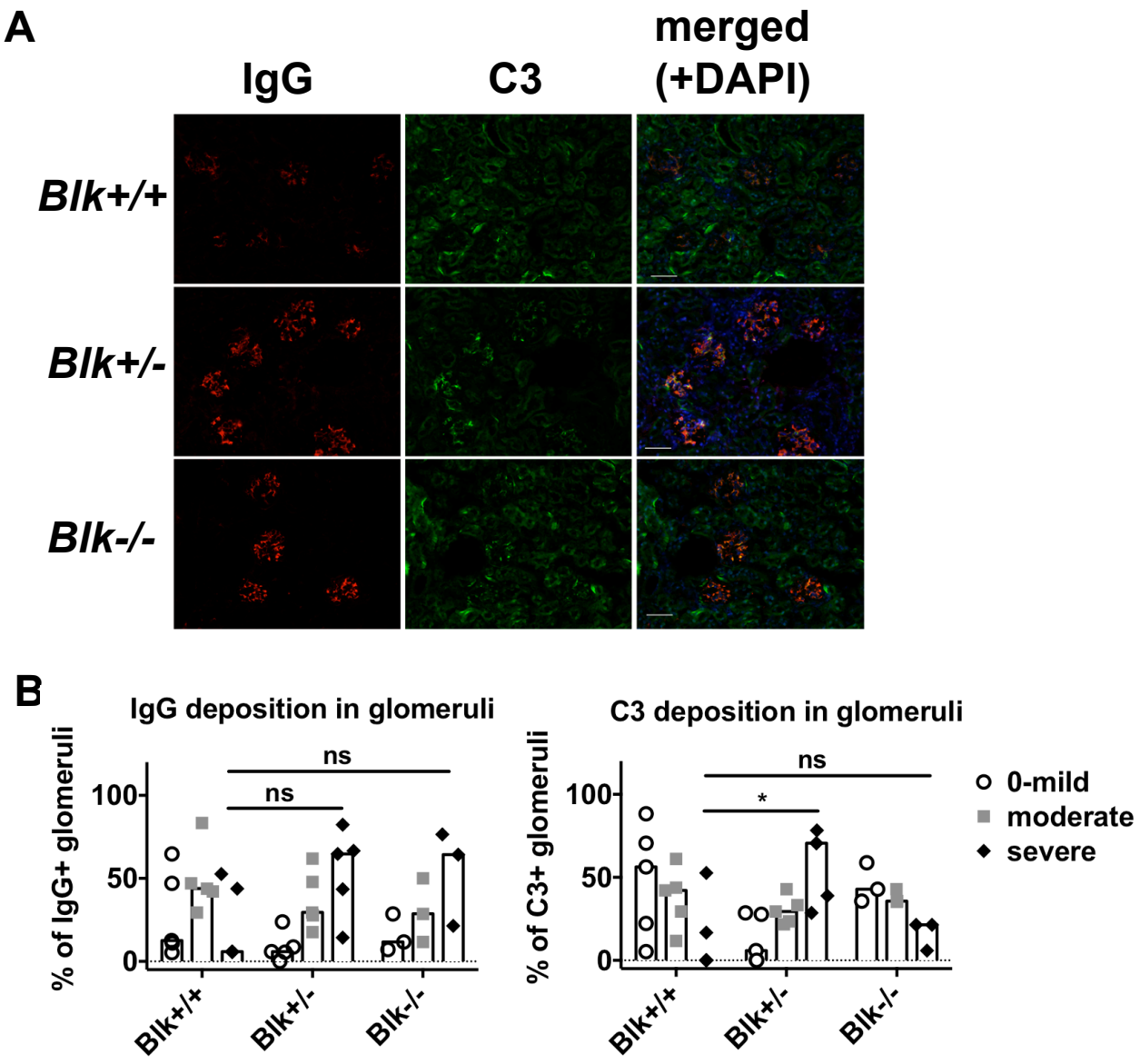

Figure S1. IgG and complement 3 deposition in glomeruli of aged female *Blk+/-* and *Blk-/-* mice. (A) Representative individual and composite images for IgG (Cy3, red), C3 (FITC, green) and nuclei (DAPI, blue) from mice at 49-wk of age. White scale bars in merged images represent 50 μm. **(B)** Individual scores and median percentages of glomeruli exhibiting IgG and C3 deposition from average 20 glomeruli per mouse were graded as zero to mild (open circles), moderate (grey squares) and severe (closed diamonds). *Blk+/-* and *Blk-/-* mice revealed tendencies towards increased IgG and C3 deposition in severe groups of glomeruli. Mice analyzed: *Blk+/+* (n= 5), *Blk+/-* (n= 5), *Blk-/-* (n= 3). Statistical plots are shown as median with Kruskal-Wallis nonparametric test (multiple comparison among *Blk* genotypes; *p≤0.05, ns>0.05).

Figure S2

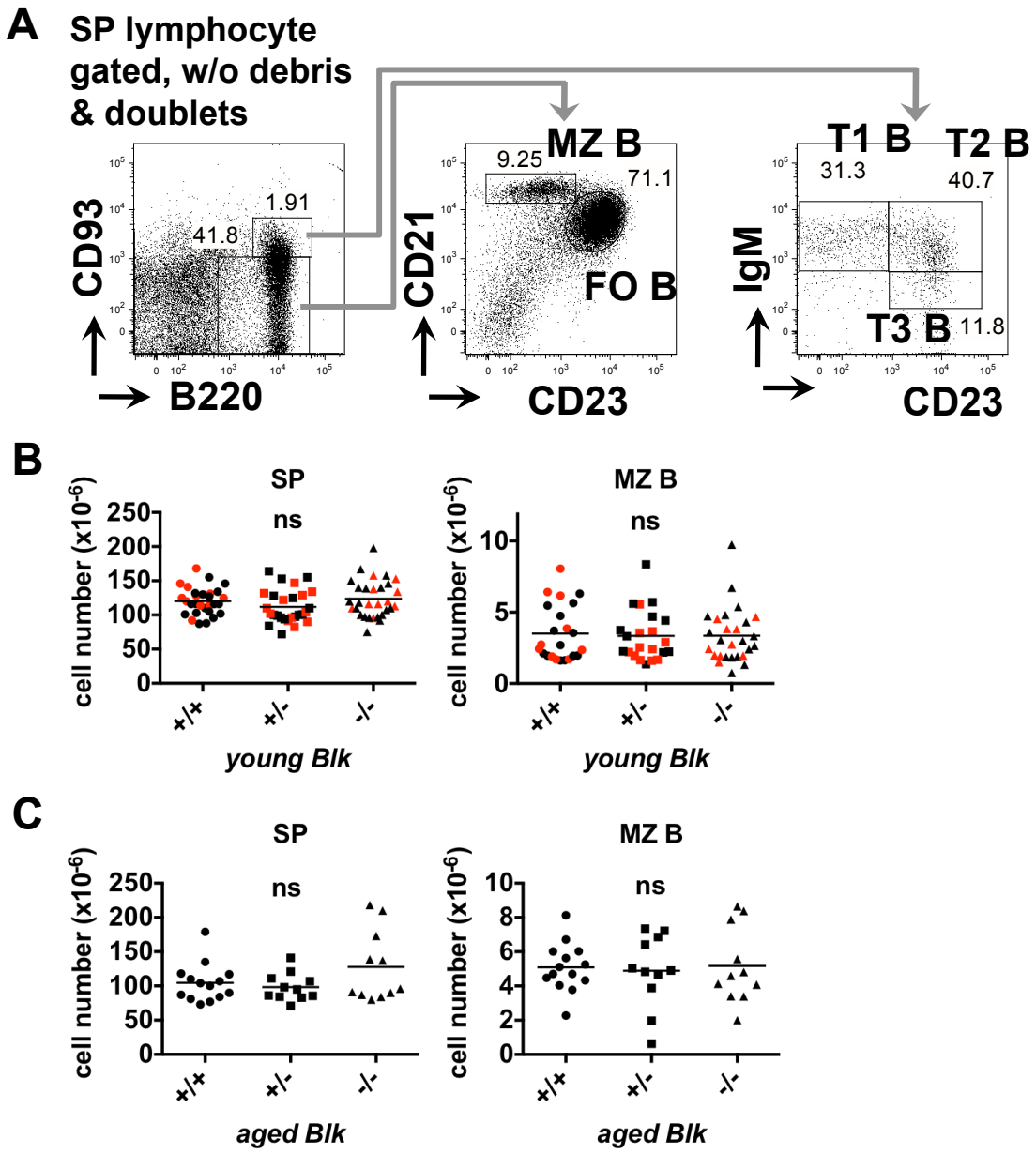

Figure S2. *Blk* genotypes did not affect total numbers of splenocytes and the development of marginal zone B cells of both young and aged mice.
(A) Representative FACS plots showed the gating strategies for marginal zone B (MZ B) and follicular B (FO B), transitional 1, 2, 3 B (T1, T2 and T3 B) cells from total splenocytes discriminated with debris and doublets. **(B & C)** Quantification of total number of splenocytes (SP) and MZ B cells from cohorts of young (8~18-wk-old) (B) and aged (52~62-wk-old) *Blk* mice (C). Males are shown in red filled symbols and females are shown in black filled symbols.

Figure S3

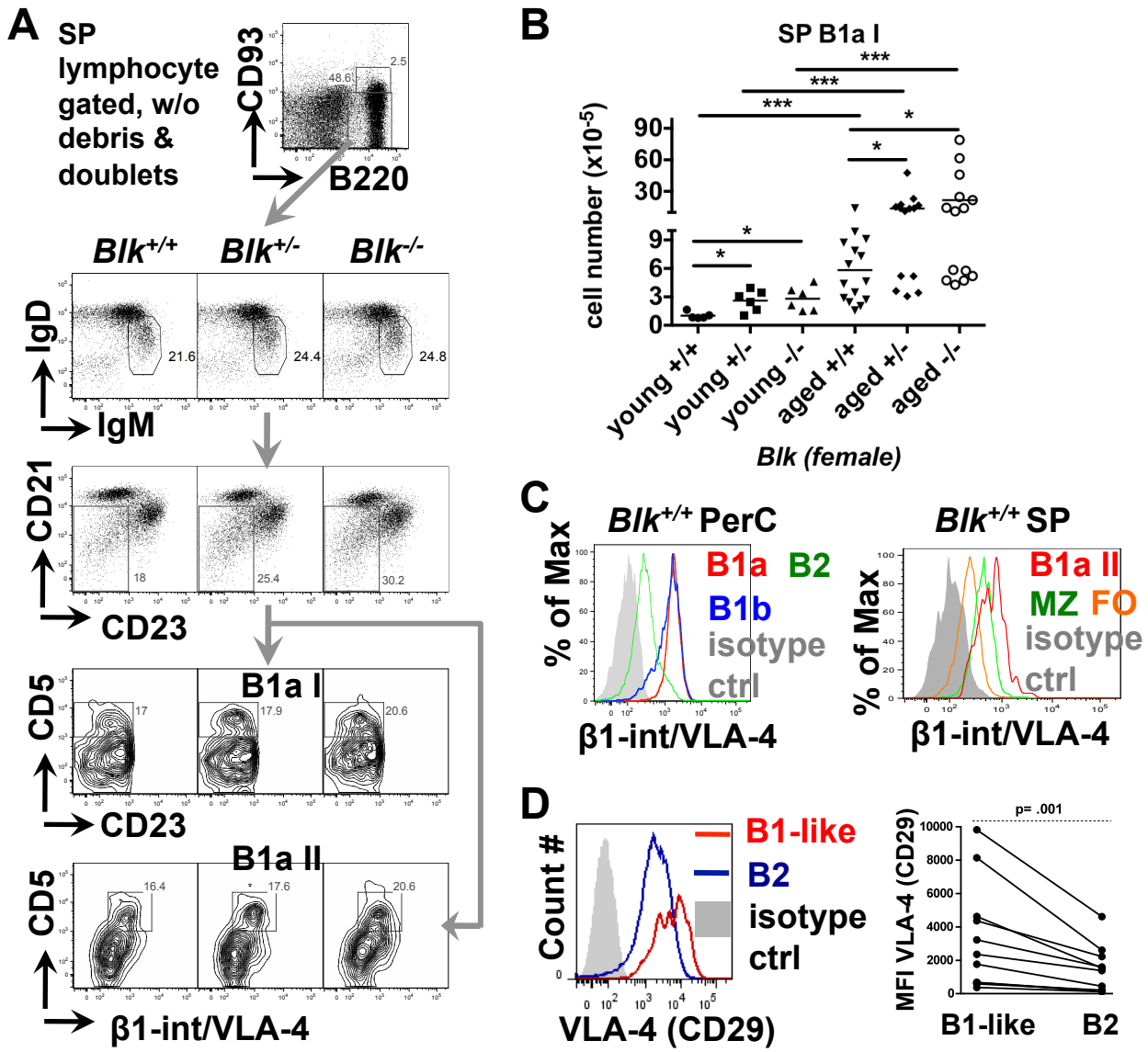

Figure S3. Alternative characterization of splenic B1 cells and VLA-4/β1-integrin is highly expressed in B1/ B1-like cells. (A) Representative FACS plots of young *Blk* mice from the same litter showed the two different gating strategies to characterize splenic B1a cells (B1a I and B1a II) from total splenocytes discriminated with debris and doublets. Number represents the percentage within the gated populations. **(B)** The statistical plot combine the absolute numbers of splenic B1a cells from cohorts of young and aged female *Blk* mice showing a tendency towards increased splenic B1a cells (SP B1a I) in young and aged female *Blk+/-* and *Blk-/-* mice. Aged mice have significantly increased splenic B1a cells compared to young mice. **(C)** Representative FACS overlaid histograms show β1-integrin (β1-int/VLA-4) expression among gated peritoneal (PerC) B1a, B1b and B2 cells of a young *Blk+/+* mouse and splenic (SP) B1a II, follicular (FO), and marginal zone (MZ) B cells of a young *Blk+/+* mouse. The gating strategies for each B subset are shown in Figure 2, Figure S2 and S3A. **(D)** The representative FACS overlaid histogram (left) shows VLA-4 (VLA-4/CD29) expression between gated B1-like and B2 in Figure 5B from human PBMCs. Right panel shows quantification of CD29 in B1-like and B2 cells from 10 healthy donors. Grey filled in (C) and (D) are isotype controls.

Figure S4

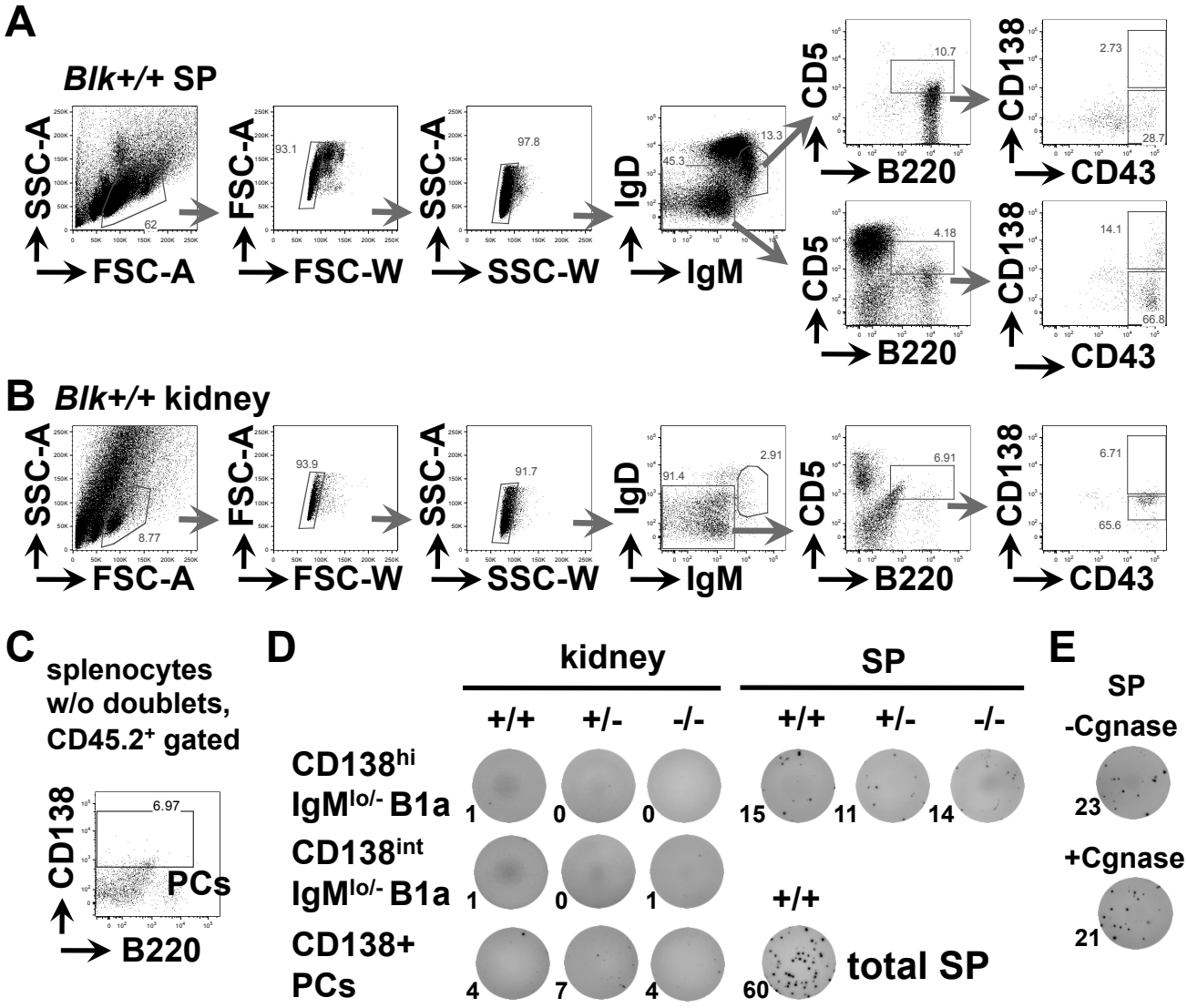

Figure S4. Sorting strategies CD138^{+} B1a cells from the spleen and kidney and poor IgG secretion by antibody-secreting cells in kidneys of aged female *Blk* mice. (A-C) Sorting strategies of splenic CD138^{+} B1a cells (A), class-switched B1a cells (B) and plasma cells (PCs) (C) from kidneys. **(D)** Representative IgG ELISPOT data from triplicates out of 2 independent experiments demonstrated class-switched (IgM$^{lo/-}$) CD138^{hi} and CD138^{int} B1a cells from kidneys (200 sort-purified cells/well). Both CD138^{hi} IgM$^{lo/-}$ B1a cells sorted from spleens (500 sort-purified cells/well) and CD138^{+} plasma cells (PCs) sorted from kidneys (1000 sort-purified cells/ well) were positive controls of IgG-secreting cells. *Blk*+/+ splenocytes (2.5x10^{4}/well) were, on the contrary, the other technical positive controls of the ELISPOT assay, respectively. The number represents counted spots within the well. **(E)** Representative ELISPOT data from triplicates proved that collagenase-treatment does not affect IgG secretion from total splenocytes (SP). Collagenase treatment did not interfere IgG secretion from antibody-secreting cells. The number represents counted spots within the well (2.5x10^{4} splenocytes/well).