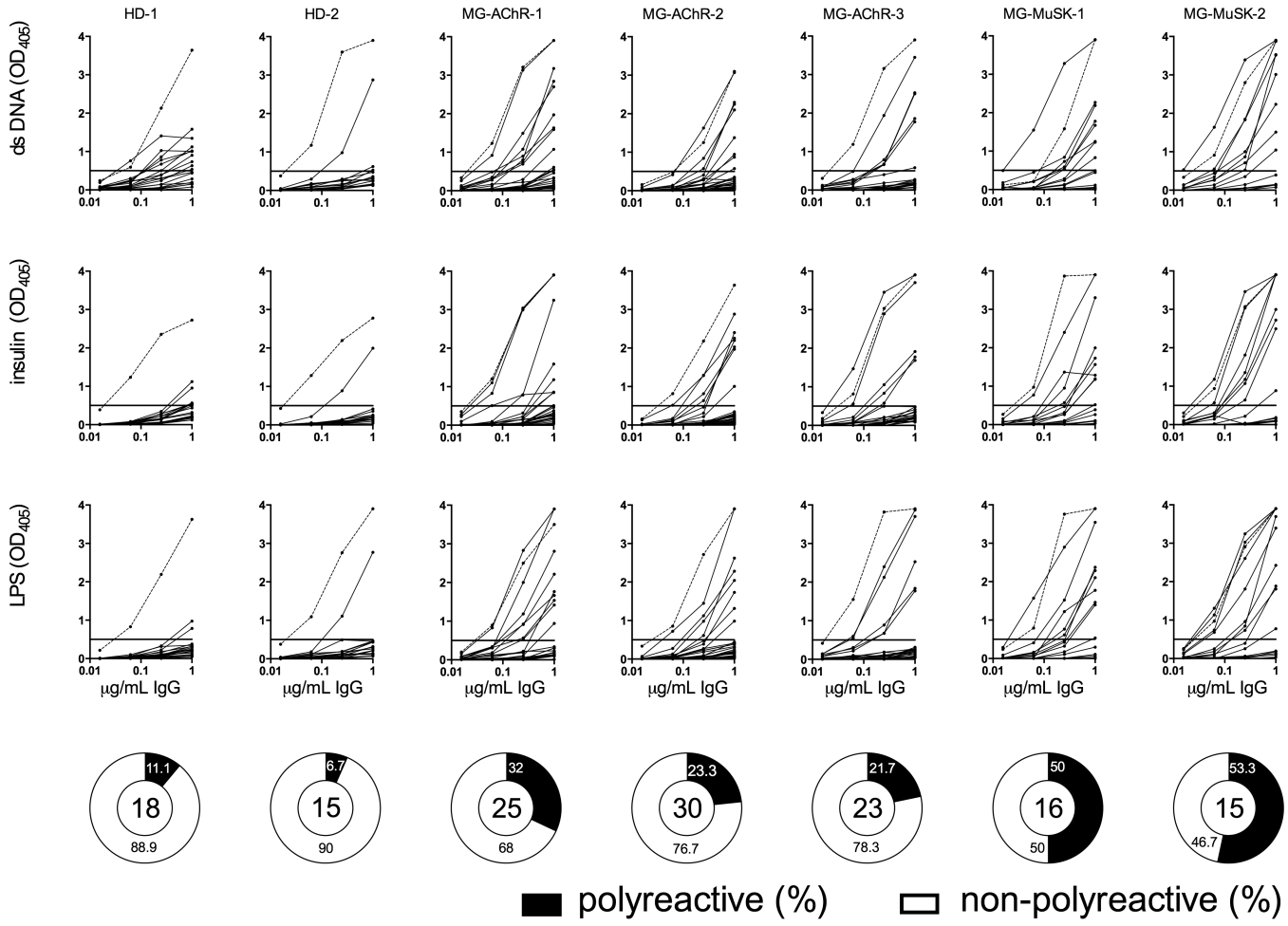
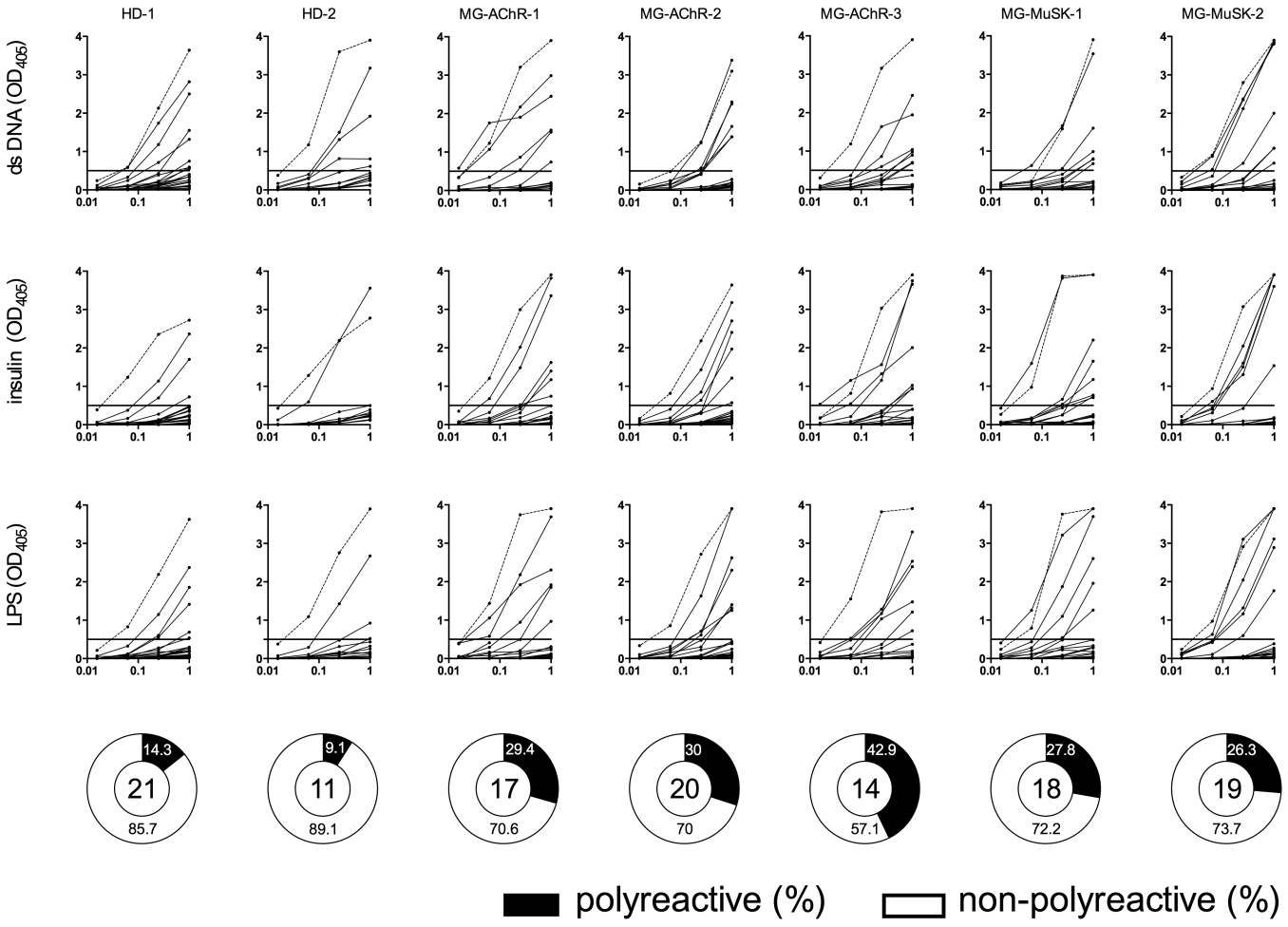
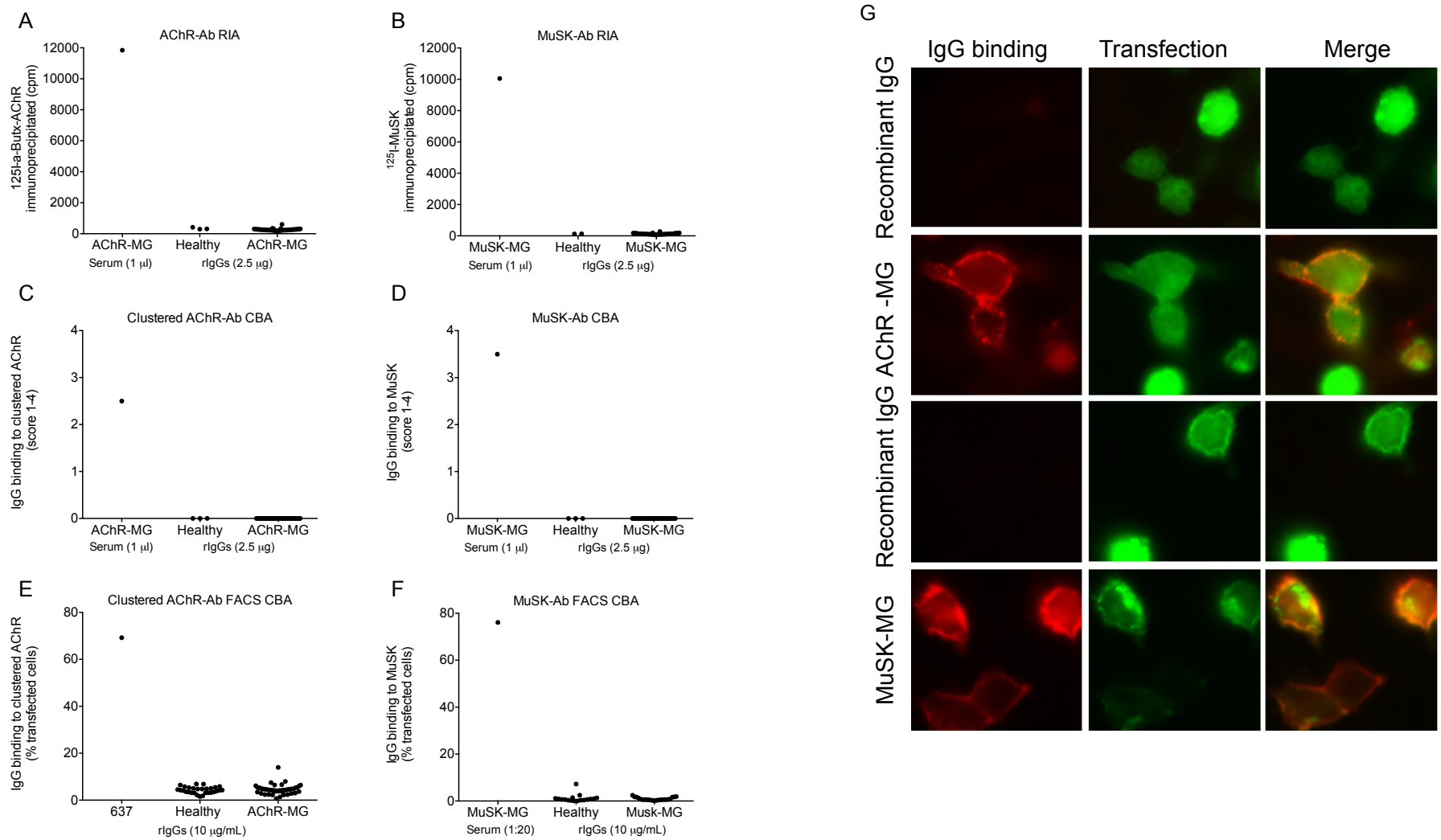
Supplemental Figures



Supplemental Figure 1. The fidelity of the central tolerance checkpoint is compromised in patients with MG: Central tolerance checkpoint polyreactivity ELISAs. The BCR from single new emigrant/transitional B cells ($CD19^{+}CD21^{lo}CD10^{+}IgM^{hi}CD27^{-}$) derived from three AChR MG patients (MG-AChR-1, MG-AChR-2, MG-AChR-3) and two MuSK MG patients (MG-MuSK-1, MG-MuSK-2) were cloned, expressed as recombinant antibodies and then tested for reactivity against dsDNA, insulin and LPS by ELISA. Dotted lines show the positive control, a monoclonal antibody (ED38) cloned from a VpreB+L+ peripheral B cell that is highly poly- and self-reactive. Solid lines show the binding curve of each cloned recombinant antibody. Horizontal lines at 0.5 OD_{405nm} marks the cut-off for positive reactivity. Negative control group includes antibodies from two healthy individuals (HD-1, HD-2). For each individual subject the proportion of polyreactive and non-polyreactive new emigrant B cells is summarized in the pie charts with the total number of tested clones in the center. Black shading indicates the frequency (%) of polyreactive antibodies and white shading indicates the frequency (%) of non-polyreactive antibodies.



Supplemental Figure 2. The fidelity of the peripheral tolerance checkpoint is compromised in patients with MG: Peripheral tolerance checkpoint polyreactivity ELISAs. The BCR from single mature naive B cells ($CD19^+CD21^+CD10^-IgM^+CD27^-$) derived from three AChR MG patients (MG-AChR-1, MG-AChR-2, MG-AChR-3) and MuSK MG patients (MG-MuSK-1, MG-MuSK-2) were cloned, expressed as recombinant antibodies and then tested for reactivity against dsDNA, insulin and LPS by ELISA. Dotted lines show the positive control, a monoclonal antibody (ED38) cloned from a VpreB+L+ peripheral B cell that is highly poly- and self-reactive. Solid lines show the binding curve of each cloned recombinant antibody. Horizontal lines at 0.5 marks the cut off OD_{405nm} for positive reactivity. The control group includes antibodies from two healthy individuals (HD-1, HD-2). For each individual subject the frequency (%) of polyreactive and non-polyreactive mature naive B cells is summarized in the pie charts with the total number of tested clones in the center. Black shading indicates the frequency (%) of polyreactive antibodies and white shading indicates the frequency (%) of non-polyreactive antibodies.



Supplemental Figure 3. Mature naive B cell-derived BCRs reactivity toward AChR and MuSK. The BCR from single mature naive B cells ($CD19^+CD21^+CD10^-IgM^+CD27^-$) derived from three AChR MG patients (MG-AChR-1, MG-AChR-2, MG-AChR-3), two MuSK MG patients (MG-MuSK-1, MG-MuSK-2) and two healthy individuals (HD-1, HD-2) were cloned, expressed as recombinant antibodies (rIgG) and then tested for reactivity against AChR or MuSK. A radioimmunoassay (RIA) was first used to evaluate the specificity of the rIgG to AChR **(A)** or MuSK **(B)**. Recombinant antibody (2.5 µg) was diluted to 50 µl in Hartmann's solution and mixed with 1 µl normal human serum and 50 µl of radioactive antigen (either AChR or MuSK from RSR Ltd). These mixtures were incubated overnight at 4°C. Polyreactive goat anti-human Ig (25 µl) was incubated with the mix for 1 hr to allow precipitation. The pellet was centrifuged and washed twice and counted on an automatic gamma counter. Cell-based assays (CBA) were used further evaluate the same recombinant antibodies using transfected human embryonic kidney (HEK) 293T

cells. Transient transfections were established using PEI with the four full-length AChR adult subunits and rapsyn-GFP to allow clustering of the AChR, or full-length MuSK-GFP. Two CBA platforms were used; immunofluorescence (IFA) and flow cytometry. In the IFA approach, cells were incubated with 2.5 µg rIgG in Hartmann's solution, with HEPES buffered Dulbecco's modified Eagle's medium and 1% BSA. Cells were fixed with 4% PFA, washed and incubated with fluorescently labeled goat anti-human IgG. The cells were washed and mounted in anti-fade mounting medium supplemented with DAPI. In the flow cytometry approach cells were incubated with 10 µg/mL of rIgG, washed then incubated with fluorescently labeled goat anti-human IgG. Binding to clustered AChR in the CBAs is shown for the immunofluorescence microscopy **(C)** and flow cytometry **(E)**. Similarly, binding to MuSK in the CBAs is shown for immunofluorescence microscopy **(D)** and flow cytometry **(F)**. Representative images from the immunofluorescence microscopy are shown **(G)** for both the AChR and MuSK CBAs. Positive controls, either serum from MG subjects or a human AChR-specific monoclonal antibody (mAB-637), used in each assay platform are indicated in figure panels. Healthy donor sera were used as negative controls in the flow cytometry CBAs; these samples consistently bound less that 4% of the transfected cells (not shown).