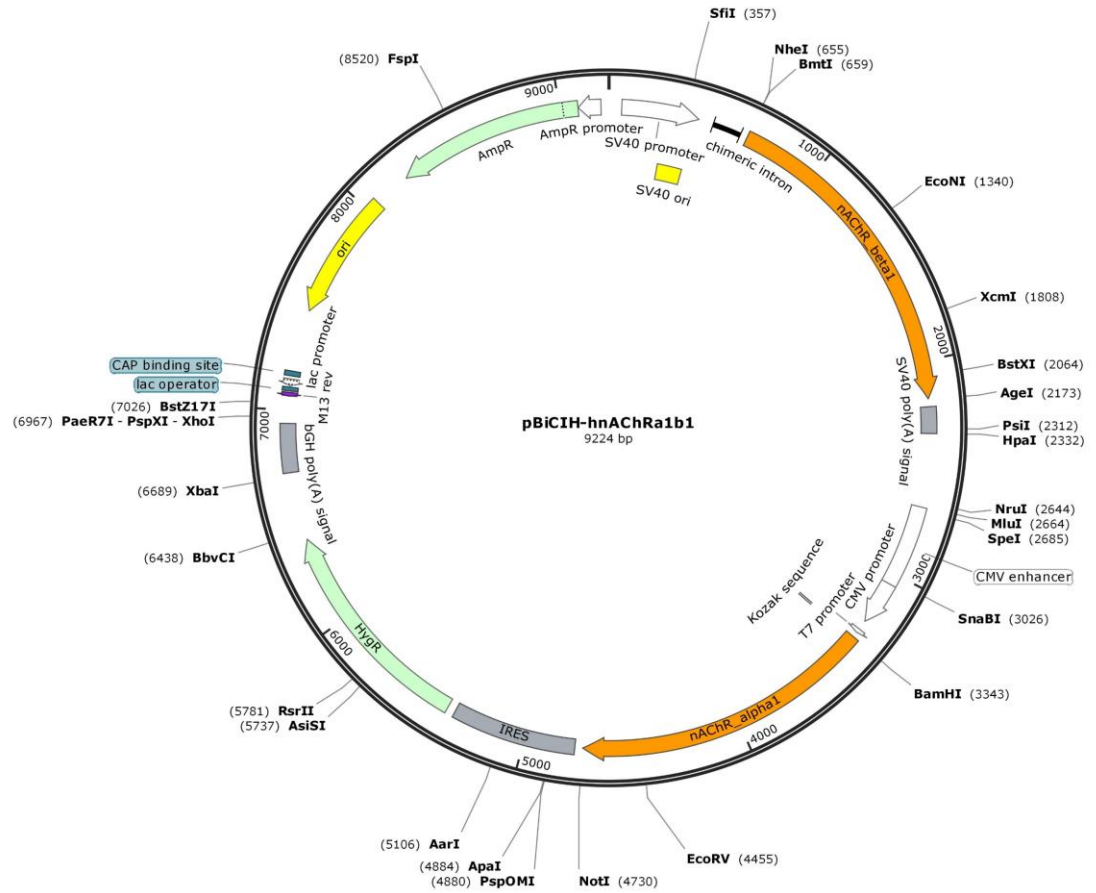


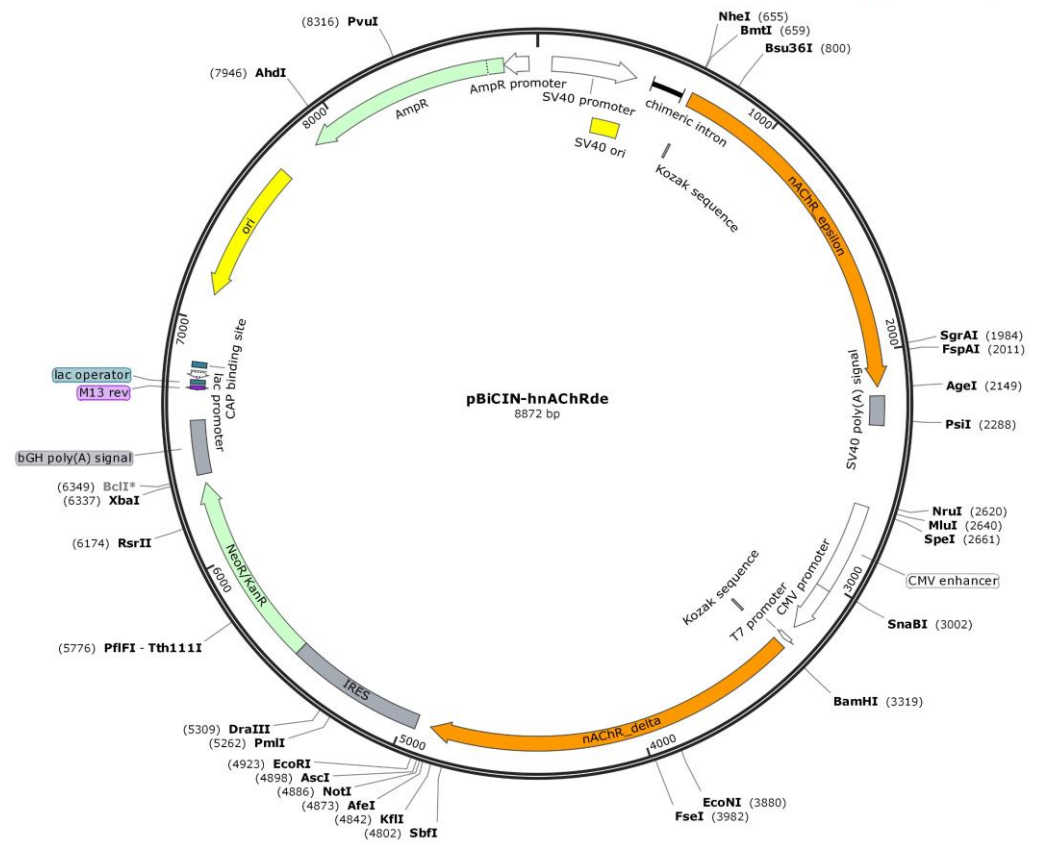
# Supplementary File 3

# S5: Plasmid maps – AChR Subunits

Created with SnapGene®

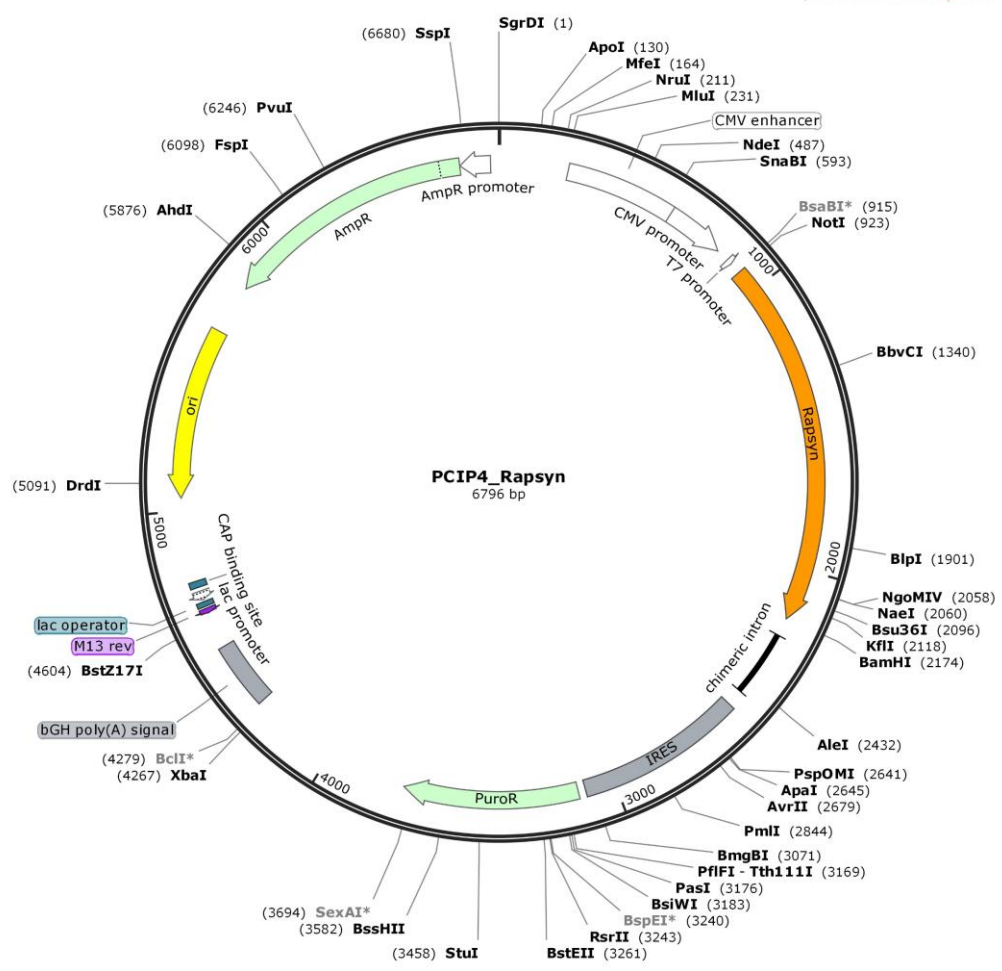


Created with SnapGene®

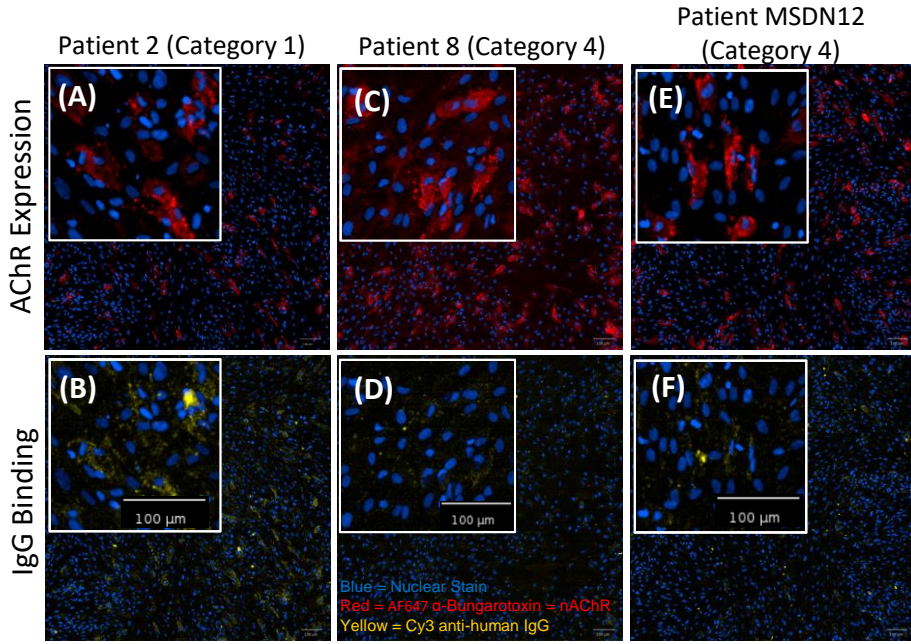


# S6: Plasmid map – Rapsyn

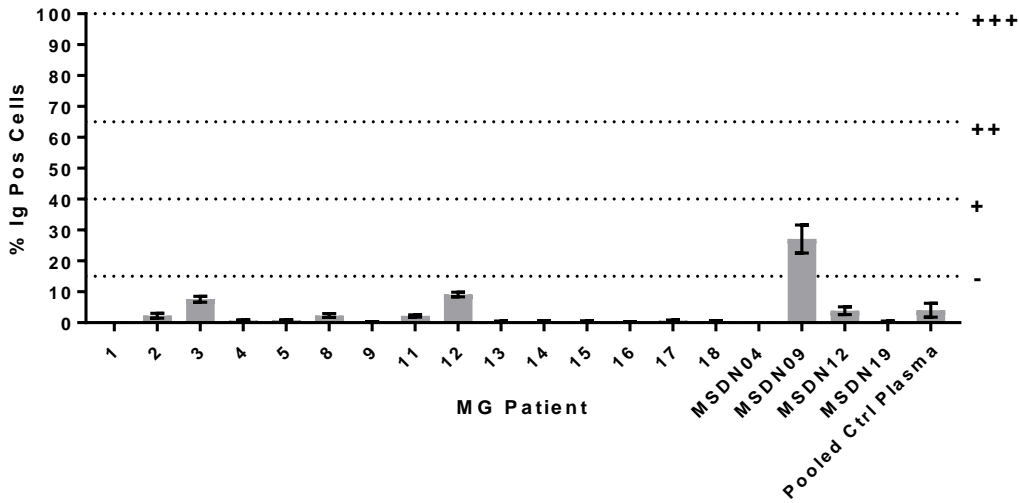
Created with SnapGene®



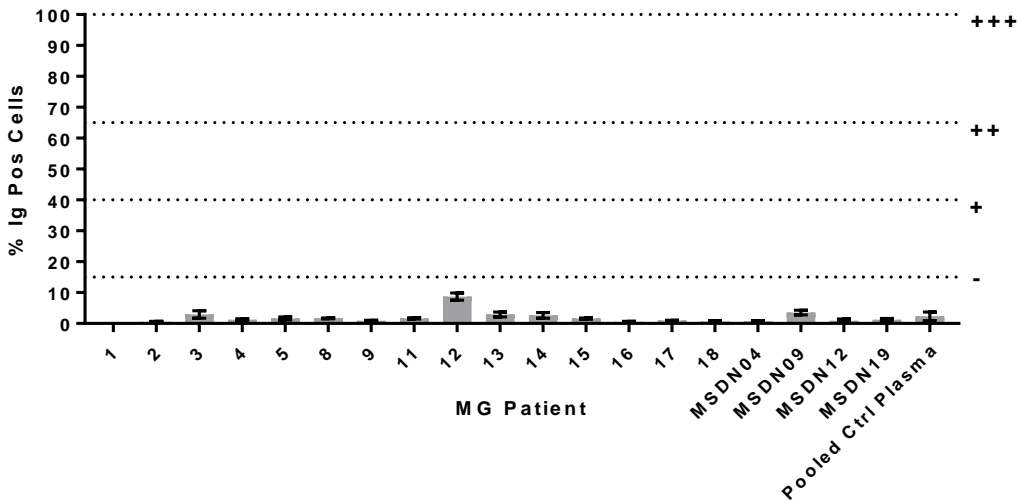
# S7: Additional patients – AChR binding



(G) % IgM Pos Cells (Background Corrected)

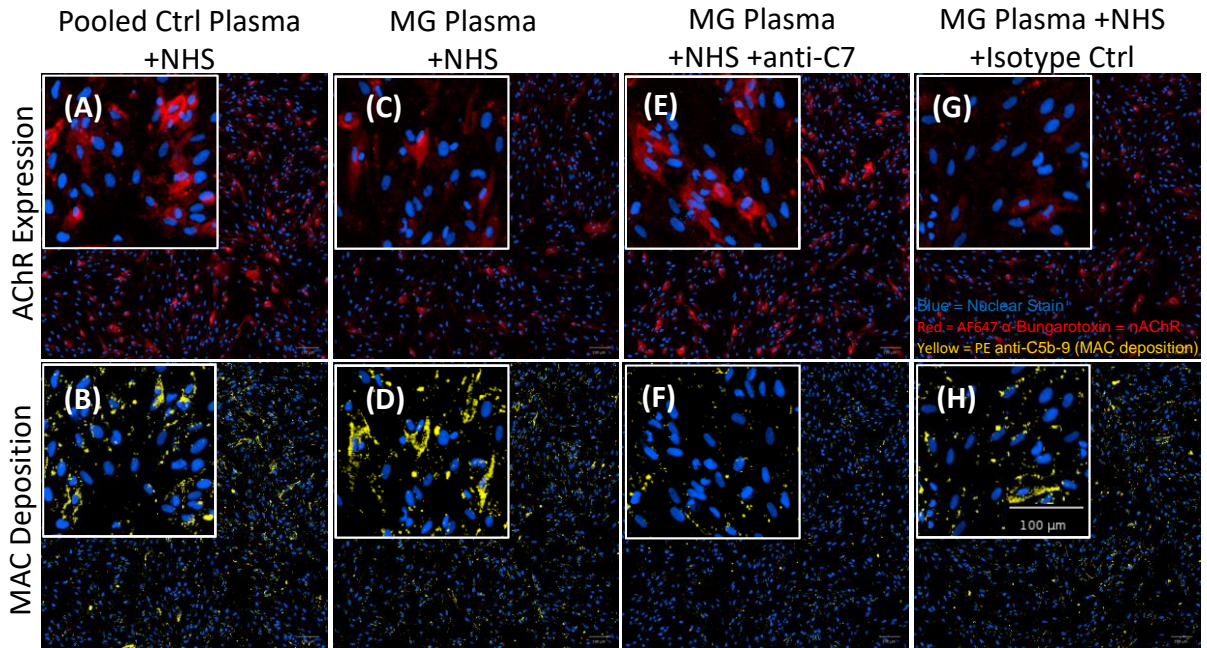


(H) % IgA Pos Cells (Background Corrected)\_For Paper

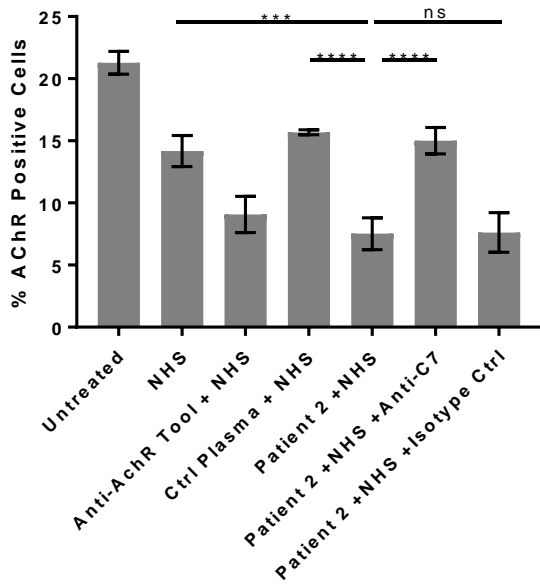


**S7:** Examples of additional patient sample IgG binding to AChR & summary graphs, showing patient IgM and IgA binding to AChR. (A-F) MG patient autoantibody binding pattern to AchR+Rapsyn transfected cells. Representative images from additional patients in categories 1 and 4, showing AChR expression (A, C, E) and patient IgG binding (B, D, F), with nuclear stain in blue, AF647  $\alpha$ -Bungarotoxin in red and Cy3 Anti-Human IgG in yellow. (A-B) MG Patient 2 (Category 1), (C-D) MG Patient 8 (Category 4), (E-F) MG Patient MSDN12 (Category 4). (G-H) Image analysis summary graphs, showing background corrected % IgM Positive cells (G) and % IgA Positive cells (H). The background was corrected by subtracting the fluorescence of the AChR negative cell population from the fluorescence of the AChR positive cell population. The bars represent the Mean $\pm$ SEM, n=3 experiments, except for MG Patient 1 where the bars represent the Mean $\pm$ SD n=2 replicates (n=1 experiment) due to insufficient sample volume to perform additional repeats. Statistical significance was obtained using one-way ANOVA with Dunnet's multiple comparison test, comparing the mean (n=3 experiments) of each plasma sample to the mean of the pooled control plasma sample, except for MG Patient 1 (n=1 experiment, 2 replicates), where statistical significance was obtained using an unpaired t-test, comparing the mean of the two MG patient 1 replicates to the mean of the two pooled control plasma replicates from the same experiment.

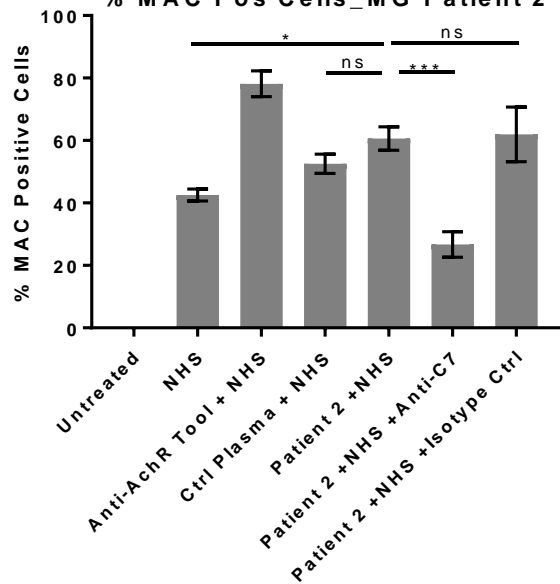
**S8: Additional patients – Category 1, example 2: weaker binding and lower MAC deposition, but blocking C7 effectively inhibits AChR loss (strong complement role in MG)**



**(I) % AChR Pos Cells\_MG Patient 2**

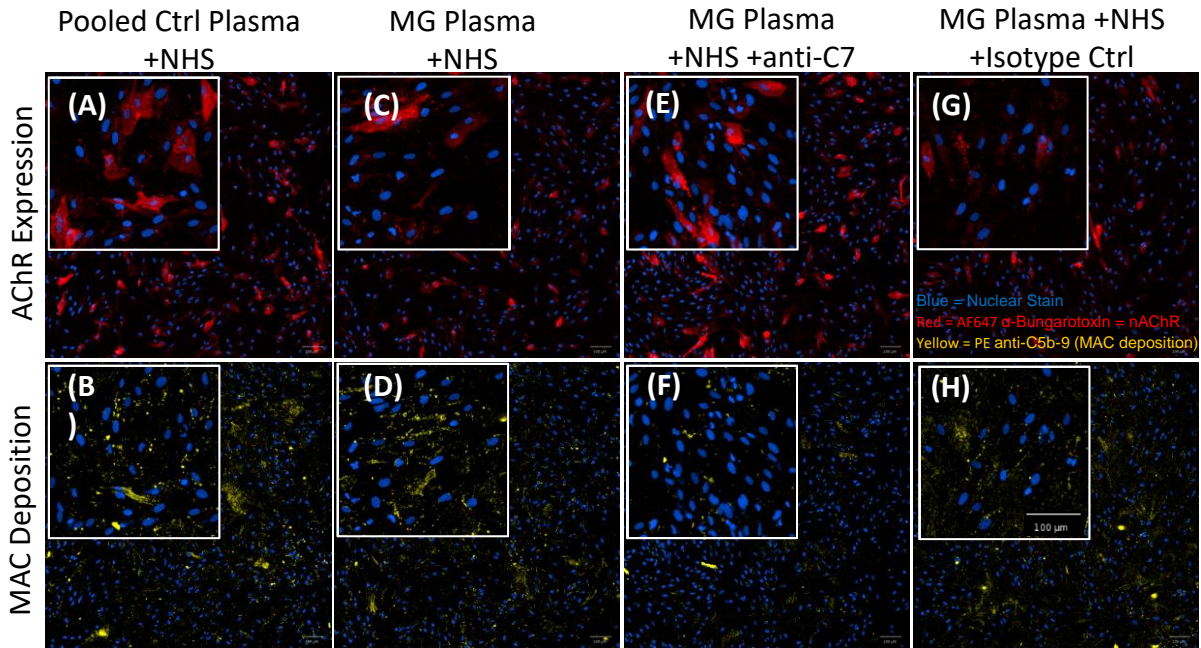


**(J) AChR Pos Cell Population: % MAC Pos Cells\_MG Patient 2**

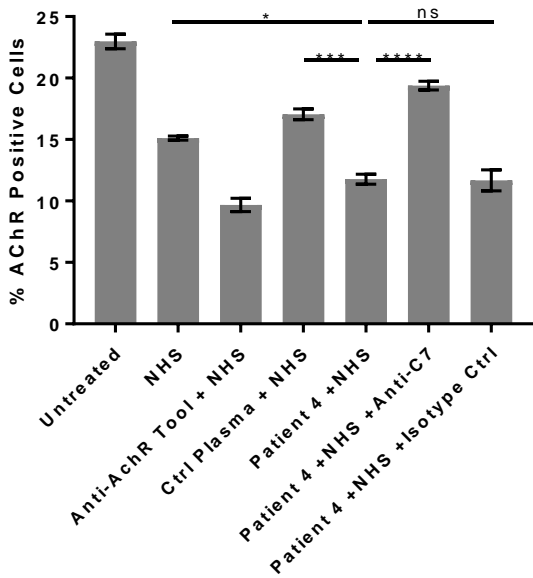


**S8:** Second example of category 1 MG patient plasma tested in the AChR loss and MAC deposition assay. **(A-H)** Representative images from MG patient 2 showing AChR expression **(A, C, E, G)** and MAC deposition **(B, D, F, H)**, with nuclear stain in blue, AF647  $\alpha$ -Bungarotoxin in red and PE anti-C5b-9 in yellow. “NHS” refers to Ig-depleted NHS in this figure. **(A-B)** Pooled control plasma + NHS, **(C-D)** MG Plasma +NHS, **(E-F)** MG Plasma +NHS +anti-C7, **(G-H)** MG Plasma +NHS +Isotype Ctrl. **(I-J)** Image analysis summary graphs for MG patient 2, showing % AChR positive cells **(I)** and % MAC positive cells within the AChR positive cell population **(J)**. The bars represent the Mean $\pm$ SEM, n=3 experiments. Statistical significance was obtained using a repeated measures one-way ANOVA without correction, using Tukey's multiple comparisons test.

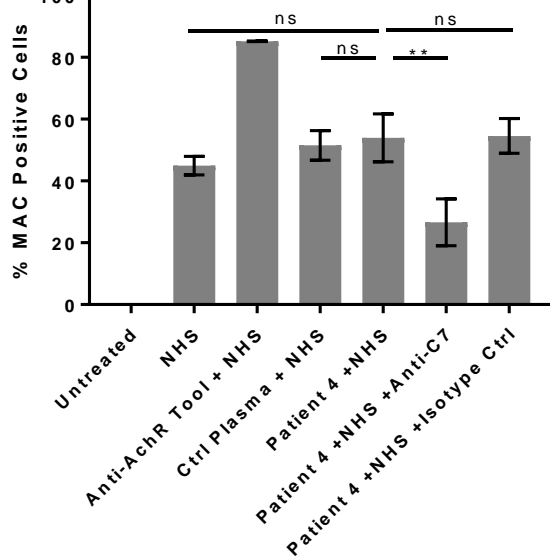
**S9: Additional patients – Category 2 example: undetectable MAC deposition, but blocking C7 still effectively inhibits AChR loss**



**(I) % AChR Pos Cells\_MG Patient 4**



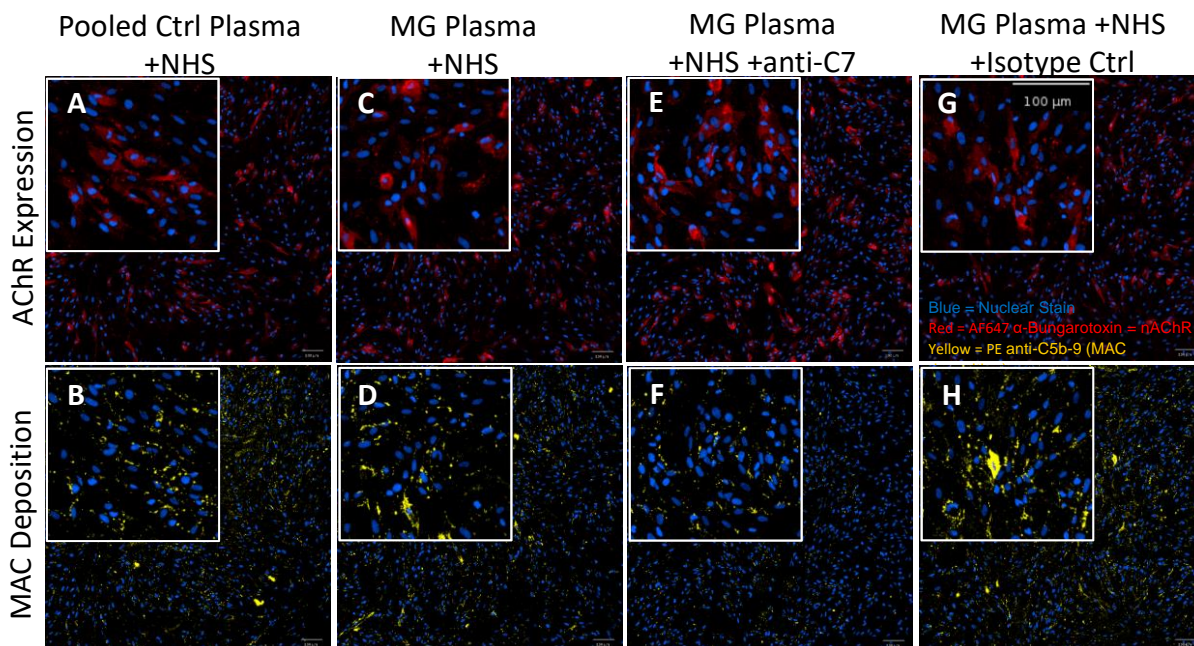
**(J) AChR Pos Cell Population: % MAC Pos Cells\_MG Patient 4**



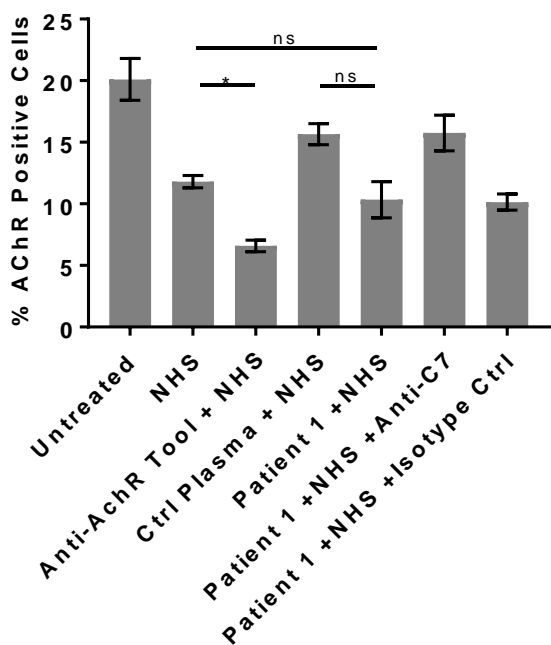


**S9:** Example of category 2 MG patient plasma tested in the AChR loss and MAC deposition assay. **(A-H)** Representative images from MG patient 4 showing AChR expression **(A, C, E, G)** and MAC deposition **(B, D, F, H)**, with nuclear stain in blue, AF647  $\alpha$ -Bungarotoxin in red and PE anti-C5b-9 in yellow. “NHS” refers to Ig-depleted NHS in this figure. **(A-B)** Pooled control plasma + NHS, **(C-D)** MG Plasma +HNS, **(E-F)** MG Plasma +NHS + anti-C7, **(G-H)** MG Plasma +NHS +Isotype Ctrl. **(I-J)** Image analysis summary graphs for MG patient 4, showing % AChR positive cells **(I)** and % MAC positive cells within the AChR positive cell population **(J)**. The bars represent the Mean $\pm$ SEM, n=3 experiments. Statistical significance was obtained using a repeated measures one-way ANOVA without correction, using Tukey's multiple comparisons test.

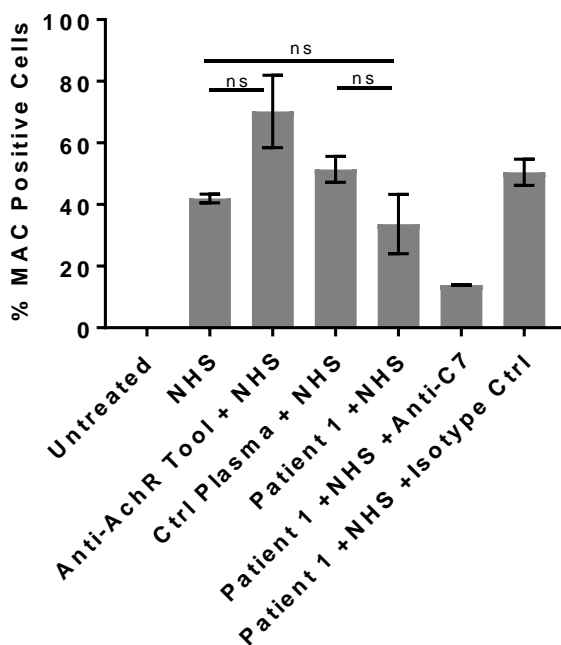
**S10:** Additional patients – Category 3 example: Binding to AChR but undetectable AChR loss and MAC deposition (no complement role - ligand-blocking antibodies?)



(I) % AChR Pos Cells  
MG Patient 1 (Category 3)

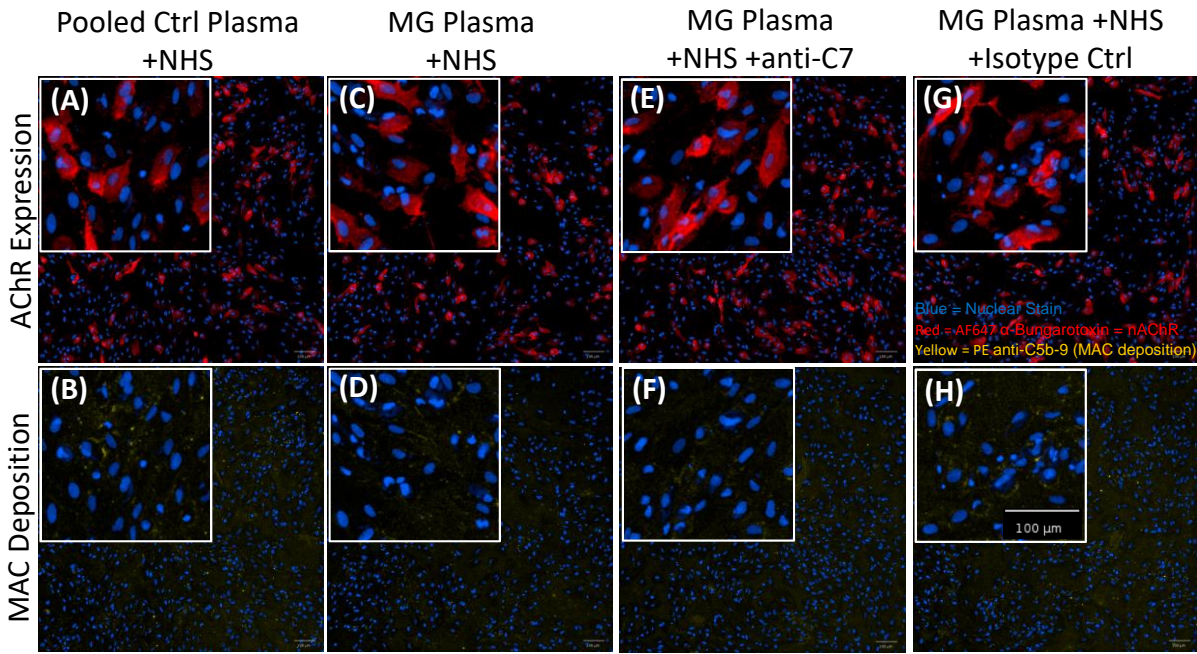


(J) AChR Pos Cell Population:  
% MAC Pos Cells\_MG Patient 1 (Category 3)

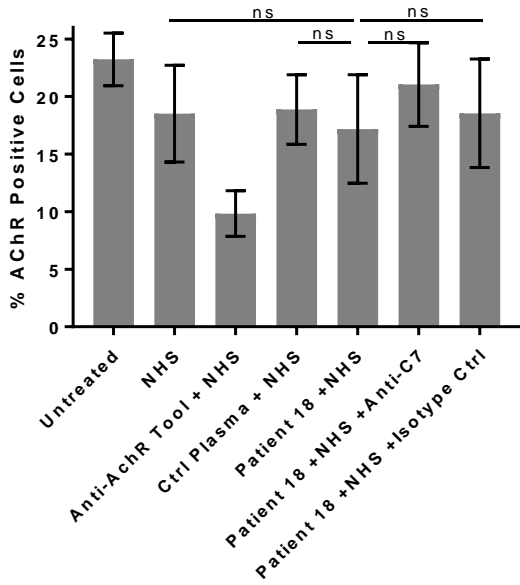


**S10:** Example of category 3 MG patient plasma tested in the AChR loss and MAC deposition assay. **(A-H)** Representative images from MG patient 1 showing AChR expression **(A, C, E, G)** and MAC deposition **(B, D, F, H)**, with nuclear stain in blue, AF647  $\alpha$ -Bungarotoxin in red and PE anti-C5b-9 in yellow. “NHS” refers to Ig-depleted NHS in this figure. **(A-B)** Pooled control plasma + NHS, **(C-D)** MG Plasma +NHS, **(E-F)** MG Plasma +NHS +anti-C7, **(G-H)** MG Plasma +NHS +Isotype Ctrl. **(I-J)** Image analysis summary graphs for MG patient 1, showing % AChR positive cells **(I)** and % MAC positive cells within the AChR positive cell population **(J)**. The bars represent the Mean $\pm$ SD, (n=2 replicates from one experiment). Statistical significance was obtained using unpaired t-test.

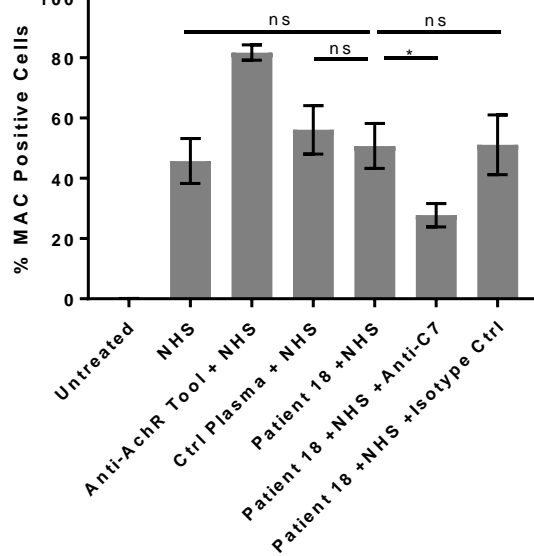
**S11:** Additional patients – Category 4, example 1: moderate anti-AChR titre, no IgG cell binding, no AChR loss, no MAC deposition (no detectable complement role in MG – false positive in ELISA?)



**(I)** % AChR Pos Cells\_MG Patient 18

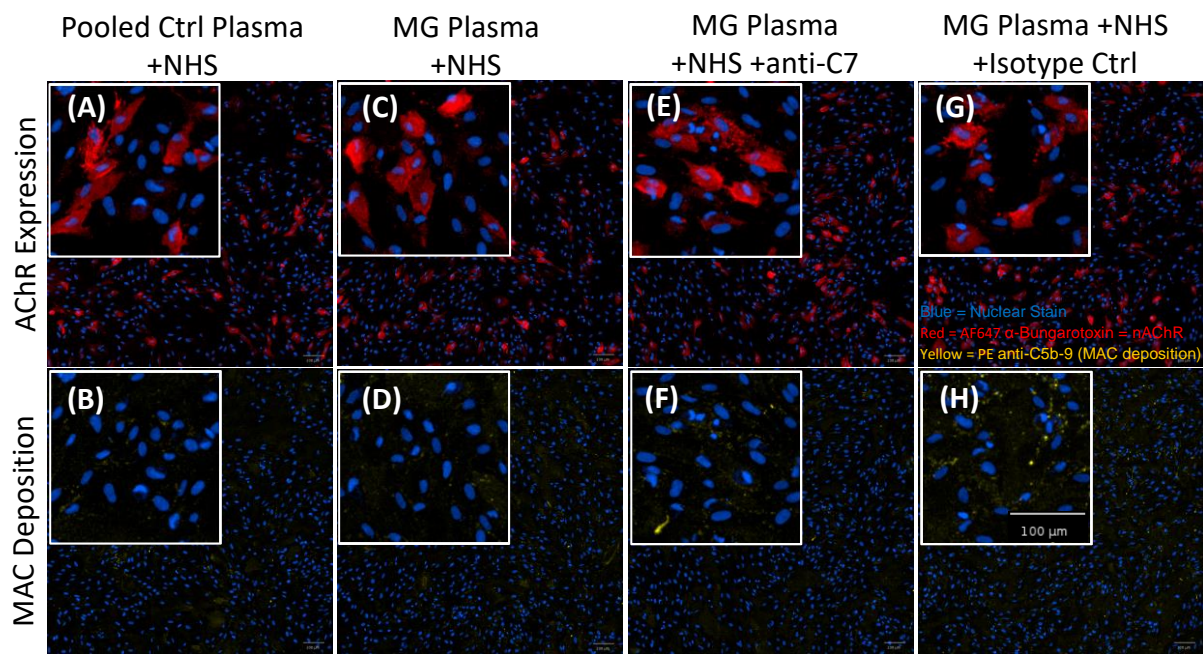


**(J)** AChR Pos Cell Population: % MAC Pos Cells\_MG Patient 18

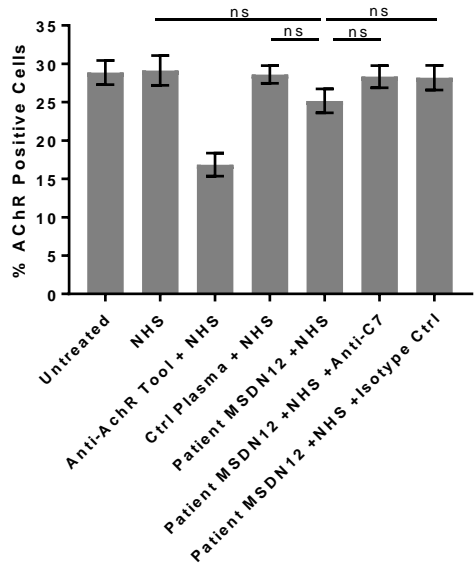


**S11:** First example of category 4 MG patient plasma tested in the AChR loss and MAC deposition assay. **(A-H)** Representative images from MG patient 18 showing AChR expression **(A, C, E, G)** and MAC deposition **(B, D, F, H)**, with nuclear stain in blue, AF647  $\alpha$ -Bungarotoxin in red and PE anti-C5b-9 in yellow. “NHS” refers to Ig-depleted NHS in this figure. **(A-B)** Pooled control plasma + NHS, **(C-D)** MG Plasma +NHS, **(E-F)** MG Plasma +NHS + anti-C7, **(G-H)** MG Plasma +NHS +Isotype Ctrl. **(I-J)** Image analysis summary graphs for MG patient 18, showing % AChR positive cells **(I)** and % MAC positive cells within the AChR positive cell population **(J)**. The bars represent the Mean $\pm$ SEM, n=3 experiments. Statistical significance was obtained using a repeated measures one-way ANOVA without correction, using Tukey's multiple comparisons test.

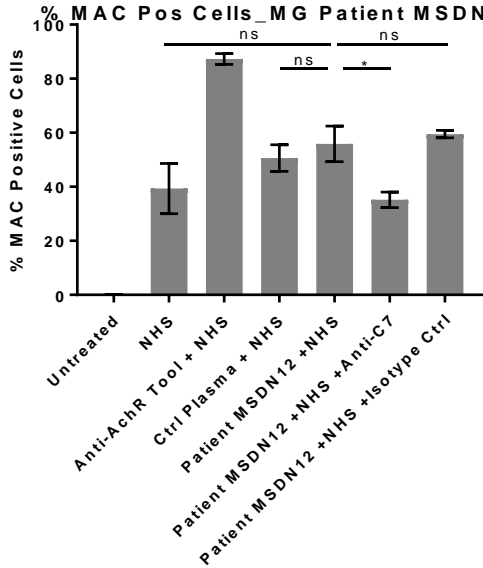
**S12: Additional patients – Category 4, example 2: No complement role in MG (MGFA score II), no anti-AChR Abs detectable by either ELISA or cell binding – potential anti-Musk?**



**(I) % AChR Pos Cells\_MG Patient MSDN12**

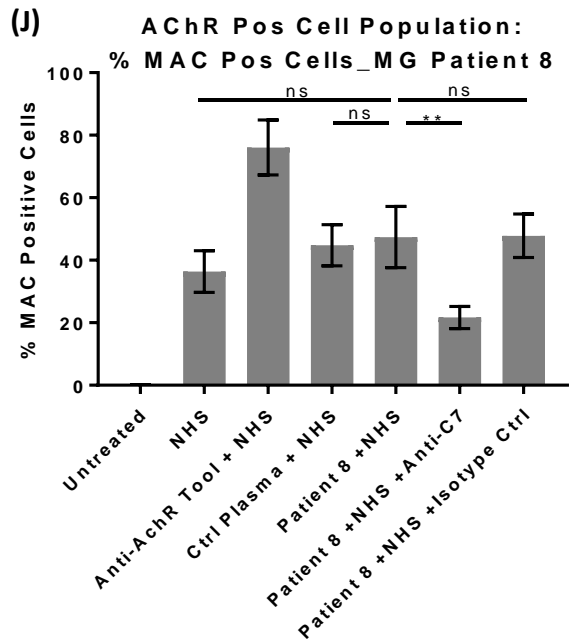
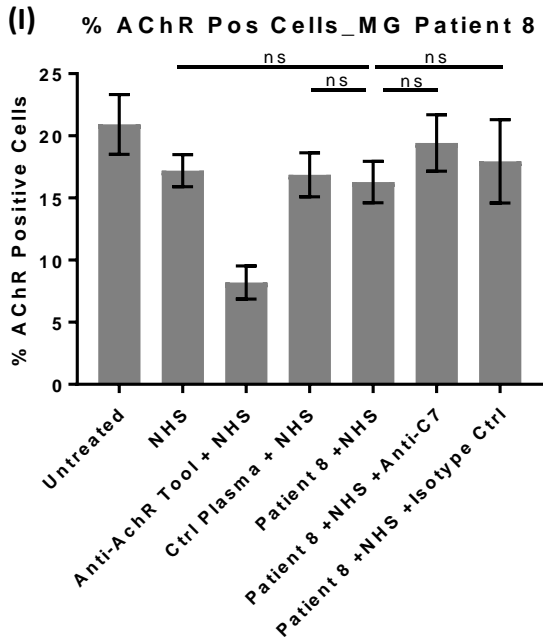
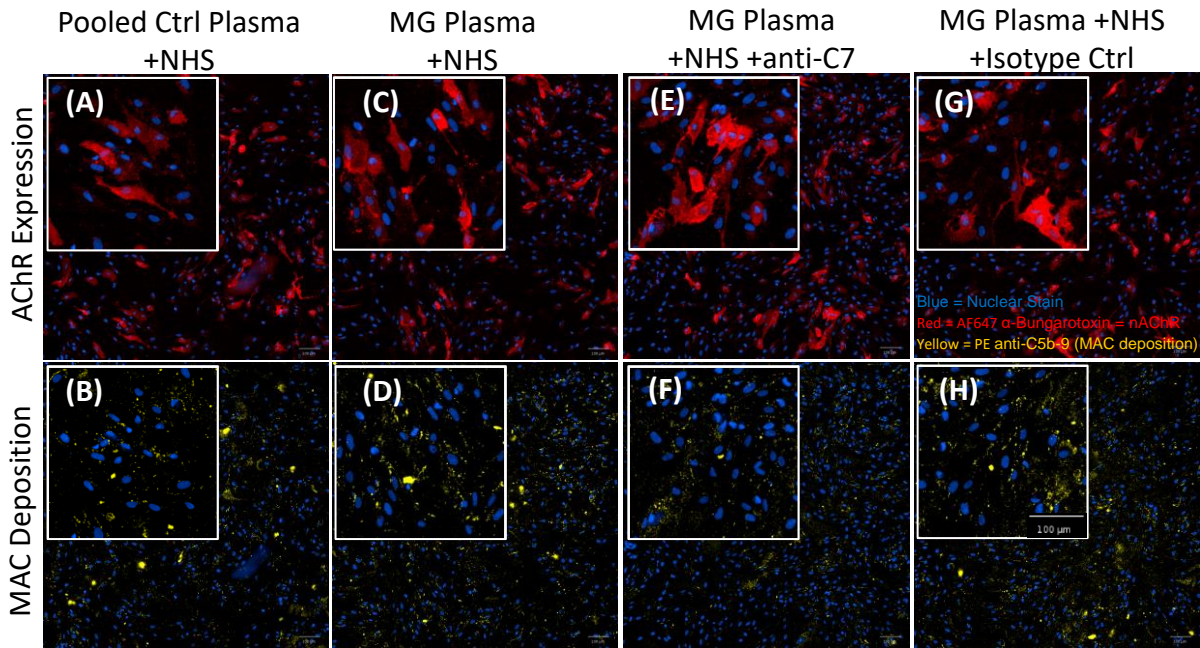


**(J) AChR Pos Cell Population: % MAC Pos Cells\_MG Patient MSDN12**



**S12:** Second example of category 4 MG patient plasma tested in the AChR loss and MAC deposition assay. **(A-H)** Representative images from MG patient MSDN12 showing AChR expression **(A, C, E, G)** and MAC deposition **(B, D, F, H)**, with nuclear stain in blue, AF647  $\alpha$ -Bungarotoxin in red and PE anti-C5b-9 in yellow. “NHS” refers to Ig-depleted NHS in this figure. **(A-B)** Pooled control plasma + NHS, **(C-D)** MG Plasma +NHS, **(E-F)** MG Plasma +NHS + anti-C7, **(G-H)** MG Plasma +NHS +Isotype Ctrl. **(I-J)** Image analysis summary graphs for MG patient MSDN12, showing % AChR positive cells **(I)** and % MAC positive cells within the AChR positive cell population **(J)**. The bars represent the Mean $\pm$ SEM, n=3 experiments. Statistical significance was obtained using a repeated measures one-way ANOVA without correction, using Tukey's multiple comparisons test.

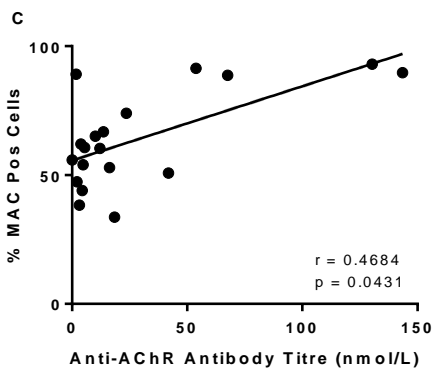
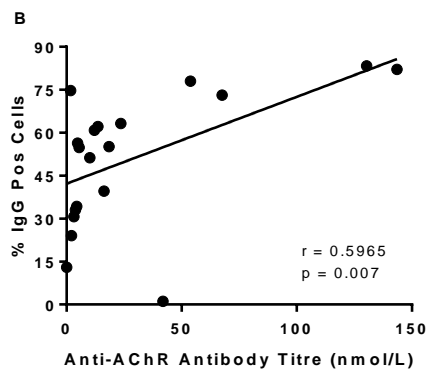
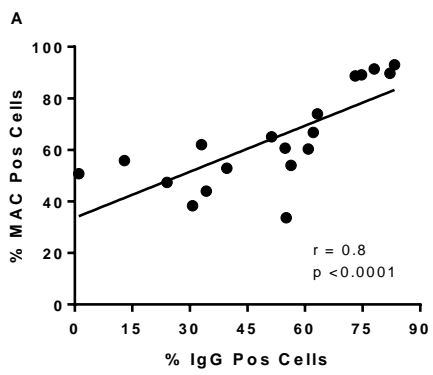
**S13:** Additional patients – Category 4, example 3: low anti-AChR titre, low IgG cell binding, no AChR loss, no MAC deposition (below assay detection limits?)





**S13:** Third example of category 4 MG patient plasma tested in the AChR loss and MAC deposition assay. **(A-H)** Representative images from MG patient 8 showing AChR expression **(A, C, E, G)** and MAC deposition **(B, D, F, H)**, with nuclear stain in blue, AF647  $\alpha$ -Bungarotoxin in red and PE anti-C5b-9 in yellow. “NHS” refers to Ig-depleted NHS in this figure. **(A-B)** Pooled control plasma + NHS, **(C-D)** MG Plasma +NHS, **(E-F)** MG Plasma +NHS + anti-C7, **(G-H)** MG Plasma +NHS +Isotype Ctrl. **(I-J)** Image analysis summary graphs for MG patient 8, showing % AChR positive cells **(I)** and % MAC positive cells within the AChR positive cell population **(J)**. The bars represent the Mean $\pm$ SEM, n=3 experiments. Statistical significance was obtained using a repeated measures one-way ANOVA without correction, using Tukey's multiple comparisons test.

# S14: Correlations between ELISA anti-AChR titres, IgG cell binding to AChR and MAC deposition



**S14:** Correlation between the following three data sets: IgG cell binding to AChR vs. MAC deposition on the AChR positive cells (**A**); IgG cell binding to AChR vs. ELISA anti-AChR titres (**B**); ELISA anti-AChR titres vs. MAC deposition on the AChR positive cells (**C**). A two-tailed, non-parametric Spearman correlation with 95 % confidence interval was calculated and the mean values from each pair of data sets being compared were plotted in in GraphPad Prism v7.05

**Table S3: Patient details and anti-AChR autoantibody titre**

Patient No	Donor ID	Age	Sex	MGFA classification	Anti-Acetylcholine Receptor Antibody (Datasheet)	Anti-Acetylcholine Receptor Antibody (In-House Data)
					nmol/L	nmol/L
1	2032293716	66 year(s) 7 month(s) 29 day(s)	F	Not provided	18,40	Not Tested
2	2032304683	50 year(s) 8 month(s) 23 day(s)	F	Not provided	9,60	5.43
3	2032338964	79 year(s) 6 month(s) 17 day(s)	F	Not provided	270	143.49
4	2032332689	66 year(s) 11 month(s) 29 day(s)	M	Not provided	9.4	4.80
5	2032331318	80 year(s) 4 month(s) 1 day(s)	M	Not provided	3.8	16.28
8	2033025476	87 year(s) 11 month(s) 4 day(s)	M	Not provided	0,56	2.18
9	2033008247	85 year(s) 11 month(s) 15 day(s)	M	Not provided	4,00	3.21
11	2032989947	72 year(s) 9 month(s) 12 day(s)	M	Not provided	6,80	4.37
12	2033030028	53 year(s) 11 month(s) 15 day(s)	F	Not provided	7,40	10.13
13	2032982232	72 year(s) 1 month(s) 29 day(s)	M	Not provided	10,80	3.80
14	2032997019	61 year(s) 10 month(s) 22 day(s)	M	Not provided	13,00	13.65
15	2032972675	57 year(s) 1 month(s) 16 day(s)	M	Not provided	18,50	12.09
16	2032992447	86 year(s) 10 month(s) 23 day(s)	M	Not provided	40,40	23.60
17	2033005700	57 year(s) 3 month(s) 8 day(s)	F	Not provided	62,00	67.52
18	2032974599	40 year(s) 0 month(s) 26 day(s)	F	Not provided	90,50	41.89
MSDN04		58	F	IV	Not provided	130.32
MSDN09		54	M	IV	Not provided	1.78
MSDN12		21	M	II	Not provided	<Detection Range
MSDN19		75	F	IIB	Not provided	53.84
Pooled Ctrl Plasma		Not Known	Not known	N/A	N/A	<Detection Range

# Table S4: Correlation Results

	<b>% IgG Pos Cells vs. % MAC Pos Cells</b>	<b>% IgG Pos Cells vs. Anti-AChR Antibody Titre (nmol/L)</b>	<b>% MAC Pos Cells vs. Anti-AChR Antibody Titre (nmol/L)</b>
<b>r (rs, rho)</b>	0.8	0.5965	0.4684
<b>95% confidence interval</b>	0.5329 to 0.9221	0.1812 to 0.8312	0.003569 to 0.7668
<b>P value (two-tailed)</b>	<0.0001	0.007	0.0431
<b>P value summary</b>	****	**	*
<b>Exact or approximate P value?</b>	Approximate	Approximate	Approximate
<b>Significant? (alpha = 0.05)</b>	Yes	Yes	Yes
<b>Number of XY Pairs</b>	19	19	19