eTable 1. Clinical characteristics of the MG study cohort

	RIA-dSN MG N=86	AChR-RIA+ MG N=66	MuSK-RIA+ MG N=40
Median age at disease onset (IQR), years	43 (32-55)	59 (39-67)	34.5 (26-51)
Female	46 (53%)	24 (36%)	29 (74%)
Median follow-up (IQR), years	7.7 (1.1-19.4)	4.5 (1.3-11.1)	3.5 (1.5-17)
Sampling under IS therapy	46 (53%)	32 (48%)	24 (60%)
Max. MGFA			
I	34 (40%)	12 (18%)	0
II	29 (34%)	15 (23%)	1 (3%)
III	19 (22%)	23 (35%)	24 (63%)
IV	2 (2%)	9 (14%)	4 (11%)
V	2 (2%)	7 (11%)	9 (24%)
Bulbar involvement at maximum disease severity	25 (29%)	34 (52%)	37 (97%)
Thymoma	1 (1%)	16 (24%)	0
Therapy			
No IS	28 (33%)	12 (18%)	0
CS	33 (38%)	19 (29%)	7 (17%)
CS + ≥ 1 IS	25 (29%)	35 (53%)	33 (83%)
Thymectomy	10 (12%)	26 (39%)	2 (5%)
PIS at last visit[a]			
CSR	9 (10%)	5 (8%)	7 (18%)
PR	12 (14%)	5 (8%)	6 (15%)
MM	25 (29%)	25 (38%)	10 (25%)
Improved	26 (30%)	24 (36%)	15 (38%)
Unchanged/Worsened	14 (16%)	6 (9%)	2 (5%)

AChR= acetylcholine receptor; CS= corticosteroids; CSR= complete stable remission; dSN MG= double seronegative myasthenia gravis; IS=immunosuppressants; IQR=interquartile range; MGFA=myasthenia gravis foundation of America; MM= minimal manifestation; MuSK= muscle tirosin kinase; PIS= post-intervention status; PR=pharmacological remission; RIA= radioimmunoassay.
a: PIS not available for one patient with AChR-RIA+ MG sampled at MG onset.

eTable 2. Clinical characteristics of 16 radioimmunoassay double-seronegative myasthenia gravis patients with antibodies detected only by cell based assays.

Median age at disease onset (IQR), years	34 (27 – 60.5)
Female, n. (%)	7 (44%)
Median follow-up (IQR), years	9 (2.5-18)
Serum sampling under IS therapy	10 (63%)
Antibodies	
Clustered AChR Abs, n. (%)	11 (69%)
MuSK Abs, n. (%)	5 (31%)
MG subgroups	
Juvenile-onset (<18 yrs)	1 (6%)
Early-onset (18-49 yrs)	10 (63%)
Late-onset (50-64 yrs)	3 (19%)
Very late-onset ($\geq$ 65 yrs)	2 (13%)
Autoimmune comorbidities[a]	3 (19%)
Maximum MGFA	
I	8 (50%)
II	4 (25%)
III	4 (25%)
IV	0
V	0
Bulbar involvement at maximum disease severity	3 (19%)

a. Autoimmune diseases: thyroiditis, 2; vitiligo, 1

AChR= acetylcholine receptor; MG= myasthenia gravis; IS=immunosuppressants; IQR=interquartile range; MGFA=myasthenia gravis foundation of America; MuSK= muscle tirosin kinase.

eFigure 1. Serostatus of the seronegative MG cohort.
Results in patients with dSNMG, with each column representing an individual assay and each row representing an individual serum sample. A positive result is defined as weak positive (yellow box) for CBA score=1 and positive (red box) for CBA score between 1.5 and 4. A negative CBA result is displayed as a grey box.
AChR: acetylcholine receptor; F-CBA: fixed cell-based assay; L-CBA: live cell-based assay; MuSK: muscle-specific tyrosine kinase; RIA: radioimmunoassay. RIA-dSN MG: radioimmunoassay double-seronegative myasthenia gravis; SNMG: seronegative myasthenia gravis

	AChR-ε F-CBA	AChR-γ F-CBA	MuSK F-CBA	AChR-ε L-CBA	AChR-γ L-CBA	MuSK L-CBA
SNMG-01	Positive	Negative	Negative	Positive	Negative	Negative
SNMG-02	Weak positive	Weak positive	Negative	Positive	Positive	Negative
SNMG-03	Weak positive	Negative	Negative	Positive	Negative	Negative
SNMG-04	Weak positive	Negative	Negative	Positive	Negative	Negative
SNMG-05	Weak positive	Weak positive	Negative	Positive	Positive	Negative
SNMG-06	Weak positive	Negative	Negative	Weak positive	Negative	Negative
SNMG-07	Negative	Negative	Negative	Negative	Weak positive	Negative
SNMG-08	Weak positive	Negative	Negative	Positive	Negative	Negative
SNMG-09	Weak positive	Negative	Negative	Weak positive	Positive	Negative
SNMG-10	Negative	Negative	Negative	Positive	Weak positive	Negative
SNMG-11	Negative	Negative	Negative	Negative	Negative	Negative
SNMG-12	Negative	Negative	Negative	Negative	Negative	Negative
SNMG-13	Negative	Negative	Negative	Negative	Negative	Positive
SNMG-14	Negative	Negative	Weak positive	Negative	Negative	Positive
SNMG-15	Negative	Negative	Negative	Negative	Negative	Positive
SNMG-16	Negative	Negative	Negative	Negative	Negative	Positive
SNMG-17	Negative	Negative	Negative	Negative	Negative	Weak positive
SNMG-18	Negative	Negative	Negative	Negative	Negative	Negative
SNMG-19	Negative	Negative	Negative	Negative	Negative	Negative
SNMG-20	Negative	Negative	Negative	Negative	Negative	Negative
SNMG-21	Negative	Negative	Negative	Negative	Negative	Negative
SNMG-22	Negative	Negative	Negative	Negative	Negative	Negative
SNMG-23	Negative	Negative	Negative	Negative	Negative	Negative
SNMG-24	Negative	Negative	Negative	Negative	Negative	Negative
SNMG-25	Negative	Negative	Negative	Negative	Negative	Negative
SNMG-26	Negative	Negative	Negative	Negative	Negative	Negative
SNMG-27	Negative	Negative	Negative	Negative	Negative	Negative
SNMG-28	Negative	Negative	Negative	Negative	Negative	Negative
SNMG-29	Negative	Negative	Negative	Negative	Negative	Negative

Legend:
- Grey = Negative
- Yellow = Weak positive
- Red = Positive
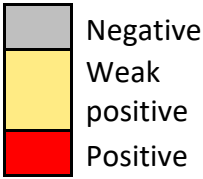
SNMG-30						
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SNMG-80						
SNMG-81						
SNMG-82						
SNMG-83						
SNMG-84						
SNMG-85						
SNMG-86						

Sample	AChR-ε F-CBA	AChR-γ F-CBA	MuSK F-CBA	AChR-ε L-CBA	AChR-γ L-CBA	MuSK L-CBA
ACHR-01	Positive	Weak positive	Negative	Positive	Positive	Negative
ACHR-02	Positive	Weak positive	Negative	Positive	Positive	Negative
ACHR-03	Positive	Positive	Negative	Positive	Positive	Negative
ACHR-04	Positive	Positive	Negative	Positive	Positive	Negative
ACHR-05	Positive	Positive	Negative	Positive	Positive	Negative
ACHR-06	Positive	Weak positive	Negative	Positive	Positive	Negative
ACHR-07	Positive	Negative	Negative	Positive	Weak positive	Negative
ACHR-08	Weak positive	Negative	Negative	Positive	Positive	Negative
ACHR-09	Positive	Weak positive	Negative	Positive	Positive	Negative
ACHR-10	Positive	Weak positive	Negative	Positive	Positive	Negative
ACHR-11	Weak positive	Positive	Negative	Weak positive	Positive	Negative
ACHR-12	Positive	Weak positive	Negative	Positive	Positive	Negative
ACHR-13	Positive	Weak positive	Negative	Positive	Positive	Negative
ACHR-14	Weak positive	Negative	Negative	Weak positive	Weak positive	Negative
ACHR-15	Positive	Weak positive	Negative	Positive	Positive	Negative
ACHR-16	Positive	Weak positive	Negative	Positive	Positive	Negative
ACHR-17	Positive	Weak positive	Negative	Positive	Positive	Negative
ACHR-18	Positive	Positive	Negative	Positive	Positive	Negative
ACHR-19	Positive	Positive	Negative	Positive	Positive	Negative
ACHR-20	Negative	Weak positive	Negative	Negative	Positive	Negative
ACHR-21	Positive	Positive	Negative	Positive	Positive	Negative
ACHR-22	Positive	Weak positive	Negative	Positive	Positive	Negative
ACHR-23	Positive	Positive	Negative	Positive	Positive	Negative
ACHR-24	Positive	Positive	Negative	Positive	Positive	Negative
ACHR-25	Positive	Positive	Negative	Positive	Positive	Negative
ACHR-26	Positive	Positive	Negative	Positive	Positive	Negative
ACHR-27	Positive	Weak positive	Negative	Positive	Positive	Negative
ACHR-28	Positive	Positive	Negative	Positive	Positive	Negative
ACHR-29	Positive	Positive	Negative	Positive	Positive	Negative
ACHR-30	Positive	Positive	Negative	Positive	Weak positive	Negative

Legend: Negative (grey); Weak positive (yellow); Positive (red).
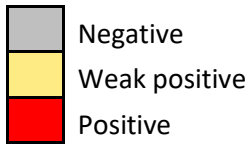
Sample	Col 1	Col 2	Col 3	Col 4	Col 5	Col 6
ACHR-31	red	red	gray	red	red	gray
ACHR-32	yellow	yellow	gray	red	red	gray
ACHR-33	yellow	gray	gray	red	red	gray
ACHR-34	red	red	gray	red	red	gray
ACHR-35	yellow	yellow	gray	red	red	gray
ACHR-36	yellow	gray	gray	red	yellow	gray
ACHR-37	red	red	gray	red	red	gray
ACHR-38	gray	gray	gray	yellow	red	gray
ACHR-39	yellow	gray	gray	red	gray	gray
ACHR-40	red	red	gray	red	yellow	gray
ACHR-41	red	red	gray	red	red	gray
ACHR-42	red	red	gray	red	red	gray
ACHR-43	red	red	gray	red	red	gray
ACHR-44	red	red	gray	red	red	gray
ACHR-45	red	red	gray	red	red	gray
ACHR-46	red	red	gray	red	red	gray
ACHR-47	red	red	gray	red	red	gray
ACHR-48	yellow	red	gray	red	red	gray
ACHR-49	red	red	gray	red	red	gray
ACHR-50	red	red	gray	red	red	gray
ACHR-51	red	red	gray	red	red	gray
ACHR-52	red	yellow	gray	red	yellow	gray
ACHR-53	red	red	gray	red	red	gray
ACHR-54	red	red	gray	red	red	gray
ACHR-55	red	red	gray	red	red	gray
ACHR-56	red	red	gray	red	red	gray
ACHR-57	red	red	gray	red	red	gray
ACHR-58	red	red	gray	red	red	gray
ACHR-59	red	red	gray	red	red	gray
ACHR-60	red	red	gray	red	red	gray
ACHR-61	red	red	gray	red	red	gray
ACHR-62	red	red	gray	red	red	gray
ACHR-63	red	red	gray	red	red	gray
ACHR-64	red	red	gray	red	yellow	gray
ACHR-65	red	red	gray	red	red	gray
ACHR-66	red	red	gray	red	red	gray
MUSK-01	gray	gray	red	gray	gray	red
MUSK-02	gray	gray	red	gray	gray	red
MUSK-03	gray	gray	yellow	gray	gray	red
MUSK-04	gray	gray	red	gray	gray	red
MUSK-05	gray	gray	red	gray	gray	red
MUSK-06	gray	gray	red	gray	gray	red

MUSK-07			red			red
MUSK-08			red			red
MUSK-09			red			red
MUSK-10			red			red
MUSK-11			red			red
MUSK-12			red			red
MUSK-13			red			red
MUSK-14			red			red
MUSK-15			red			red
MUSK-16			red			red
MUSK-17			red			red
MUSK-18			red			red
MUSK-19			red			red
MUSK-20			red			red
MUSK-21			red			red
MUSK-22			red			red
MUSK-23			red			red
MUSK-24			red			red
MUSK-25			yellow			red
MUSK-26			red			red
MUSK-27			red			red
MUSK-28			red			red
MUSK-29			red			red
MUSK-30			red			red
MUSK-31			red			red
MUSK-32			red			red
MUSK-33			red			red
MUSK-34			red			red
MUSK-35			red			red
MUSK-36			red			red
MUSK-37			red			red
MUSK-38			red			red
MUSK-39			red			red
MUSK-40			red			red

eFigure 3. Detection of MG antibodies by L-CBA using FACS analysis.
FACS gating and representative examples of Adult/Fetal AChR (SNMG-02, AChR-03) and MuSK
(SNMG-13) detection in MG patients and one control.
AChR: acetylcholine receptor; FACS= fluorescence-activated cell sorting; L-CBA: live cell-based
assay; MG= myasthenia gravis; MuSK: muscle-specific tyrosine kinase.
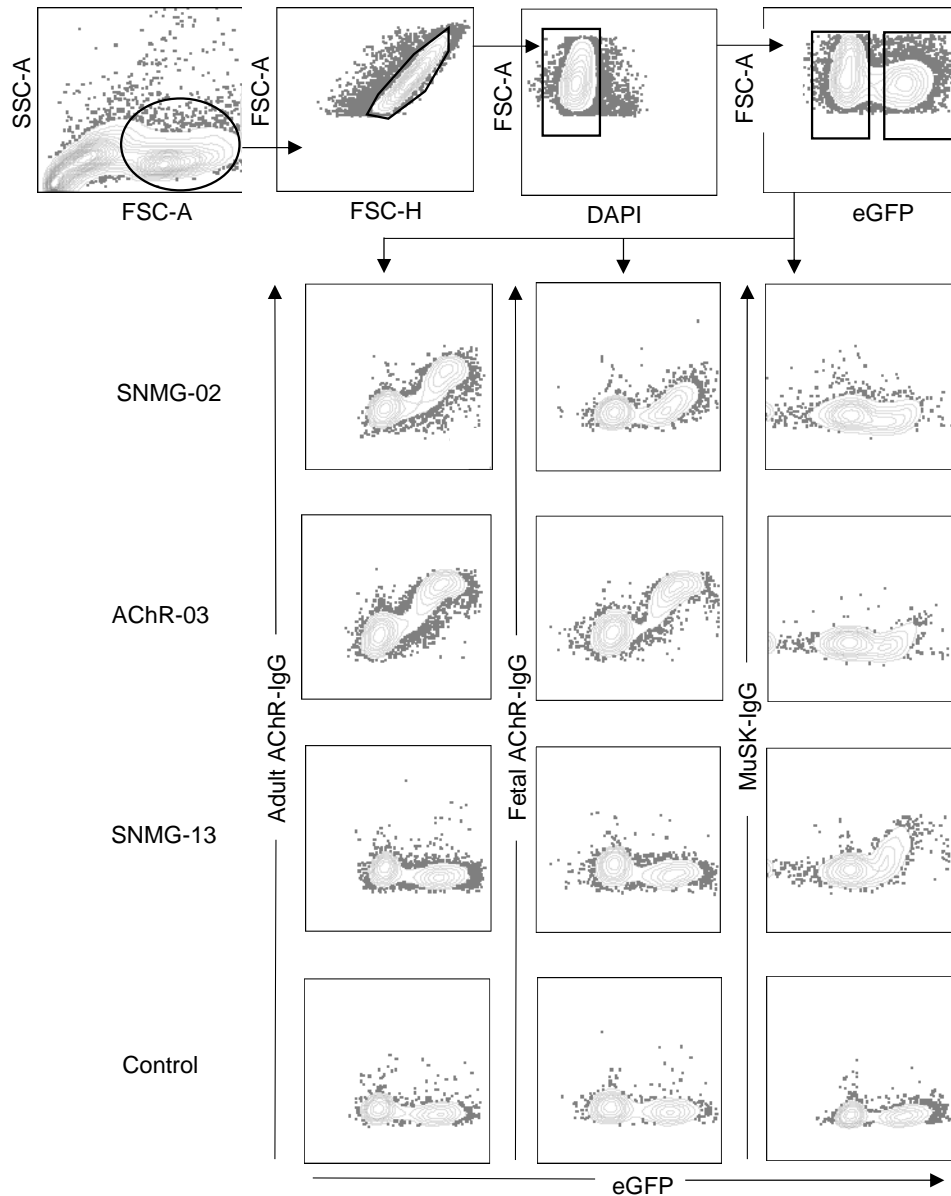
AChR: acetylcholine receptor; DAPI: 4′,6-diamidino-2-phenylindole; eGFP: enhanced-Green
fluorescent protein; FSC: forward scatter; IgG: immunoglobulin G; MuSK= muscle tirosin kinase;
SSC-A: side scatter.