

**Supplemental data for manuscript:**

**Post B cell depletion autoantibody-producing plasmablasts identified in muscle-specific kinase myasthenia gravis.**

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Table S1. Fractions of B cells (% of total PBMCs) and memory B cells (% of total B cells) in MuSK MG patients pre- and post-rituximab

	Time point	B cells (%)	Memory B cells (%)
MuSK 1 (1 cycle)	Pre-RTX	14	ND
	4 mo post-RTX	0.3	ND
	19 mo post-RTX <sup>A</sup>	11.0	2.3
MuSK 2 (1 cycle)	65 mo post-RTX	3.5	8.2
	76 mo post-RTX <sup>A</sup>	3.6	11.0
	77 mo post-RTX	5.5	8.5
	78 mo post-RTX <sup>A</sup>	13.0	5.4
MuSK 3 (2 cycles Q6mo)	Pre-RTX	16.2	26.2
	3 mo post-1 <sup>st</sup> cycle RTX	<0.1	24.0
	6 mo post-2 <sup>nd</sup> cycle RTX	0.3	23.3
	9 mo post-2 <sup>nd</sup> cycle RTX	0.8	ND
	21 mo post-2 <sup>nd</sup> cycle RTX <sup>A</sup>	1.0	17.9
MuSK 4 (1 cycle)	36 mo post-RTX	14.4	1.2
	72 mo post-RTX	16.0	1.2
	84 mo post-RTX	41.0	1.0

<sup>A</sup>: time point at which experimental sample was obtained, MuSK: muscle-specific tyrosine kinase, ND: not done, PBMC: peripheral blood mononuclear cells, RTX: rituximab,

Table S2. MuSK cell based assay (CBA) results

Sample/subject	Source	$\Delta\%$ positive cells <sup>A</sup>	$\Delta$ MFI <sup>B</sup>
Control	Murine humanized MuSK monoclonal rlg 4A3	96	118554
Control	Human AChR monoclonal rlg 637	2	54
Control	Culture medium	1	57
Control	1% BSA in PBS	1	72
MuSK 1	Serum	<b>95</b>	6769
MuSK 2a	Serum	<b>94</b>	8387
MuSK 2b	Serum	<b>97</b>	11960
MuSK 3	Serum	<b>73</b>	1002
MuSK 4	Serum	1	13
AChR 1	Serum	2	24
AChR 2	Serum	8	48
AChR 3	Serum	11	81
AChR 4	Serum	11	64
AChR 5	Serum	22	102
AChR 6	Serum	0	0
AChR 7	Serum	6	52
AChR 8	Serum	4	31
HD1	Serum	0	11
MuSK 1 <sup>C</sup>	CD27 <sup>+</sup> cell culture	<b>77</b>	558
MuSK 2a <sup>D</sup>	CD27 <sup>+</sup> cell culture	<b>84</b>	1643
MuSK 2b <sup>C</sup>	CD27 <sup>+</sup> cell culture	<b>64</b>	635
MuSK 3 <sup>C</sup>	CD27 <sup>+</sup> cell culture	<b>32</b>	164
MuSK 4 <sup>C,D</sup>	CD27 <sup>+</sup> cell culture	2	29
AChR 1	CD27 <sup>+</sup> cell culture	1	15
AChR 2	CD27 <sup>+</sup> cell culture	0	11
AChR 3	CD27 <sup>+</sup> cell culture	16	94
AChR 4 <sup>C</sup>	CD27 <sup>+</sup> cell culture	0	15
AChR 5 <sup>C</sup>	CD27 <sup>+</sup> cell culture	12	151
AChR 6 <sup>D</sup>	CD27 <sup>+</sup> cell culture	0	109
HD1	CD27 <sup>+</sup> cell culture	1	14
MuSK 1 <sup>C</sup>	Plasmablast culture	<b>47</b>	<b>267</b>
MuSK 2b <sup>D</sup>	Plasmablast culture	<b>30</b>	<b>193</b>
MuSK 3 <sup>C</sup>	Plasmablast culture	<b>11</b>	<b>125</b>
MuSK 4 <sup>C,D</sup>	Plasmablast culture	0	<b>9</b>
AChR 1 <sup>D</sup>	Plasmablast culture	3	55
AChR 2 <sup>D</sup>	Plasmablast culture	2	47
AChR 3 <sup>D</sup>	Plasmablast culture	0	40
HD 1 <sup>D</sup>	Plasmablast culture	2	58

MuSK 1 <sup>c</sup>	MuSK 1-1 rlg	<b>53</b>	771
MuSK 1 <sup>c</sup>	MuSK 1-2 rlg	2	30
MuSK 1 <sup>c</sup>	MuSK 1-3 rlg	7	146
MuSK 1 <sup>c</sup>	MuSK 1-4 rlg	3	138
MuSK 2b	MuSK 2-1 rlg	11	50
MuSK 2b	MuSK 2-2 rlg	1	30
MuSK 2b	MuSK 2-3 rlg	12	55
MuSK 2b	MuSK 2-4 rlg	1	29
MuSK 2b	MuSK 2-5 rlg	3	43
MuSK 2b	MuSK 2-6 rlg	12	68
MuSK 2b	MuSK 2-7 rlg	4	37
MuSK 2b	MuSK 2-8 rlg	18	94
MuSK 2b	MuSK 2-9 rlg	1	24
MuSK 2b	MuSK 2-10 rlg	4	47
MuSK 2b	MuSK 2-11 rlg	1	27
MuSK 2b	MuSK 2-12 rlg	0	22
MuSK 2b	MuSK 2-13 rlg	6	7
MuSK 2b	MuSK 2-14 rlg	0	42
MuSK 2b	MuSK 2-15 rlg	3	23
MuSK 2b	MuSK 2-16 rlg	14	125
MuSK 2b	MuSK 2-17 rlg	3	12
MuSK 2b	MuSK 2-18 rlg	2	19
MuSK 2b	MuSK 2-19 rlg	5	37
MuSK 2b	MuSK 2-20 rlg	12	57
MuSK 2b	MuSK 2-21 rlg	14	80
MuSK 2b	MuSK 2-22 rlg	6	12
MuSK 2b	MuSK 2-23 rlg	20	185
MuSK 2b	MuSK 2-24 rlg	3	15
MuSK 2b	MuSK 2-25 rlg	7	9
MuSK 2b	MuSK 2-26 rlg	11	27
MuSK 2b	MuSK 2-27 rlg	2	20
MuSK 2b	MuSK 2-28 rlg	2	30
MuSK 2b	MuSK 2-29 rlg	13	67
MuSK 2b	MuSK 2-30 rlg	7	10
MuSK 2b	MuSK 2-31 rlg	2	29
MuSK 2b	MuSK 2-32 rlg	10	51
MuSK 2b	MuSK 2-33 rlg	9	47
MuSK 3 <sup>c</sup>	MuSK 3-1 rlg	<b>22</b>	201
MuSK 3 <sup>c</sup>	MuSK 3-2 rlg	13	111
MuSK 3 <sup>c</sup>	MuSK 3-3 rlg	7	170
MuSK 3 <sup>c</sup>	MuSK 3-4 rlg	1	122
MuSK 3 <sup>c</sup>	MuSK 3-5 rlg	7	11

MuSK 3 <sup>c</sup>	MuSK 3-6 rlg	17	124
MuSK 3 <sup>c</sup>	MuSK 3-7 rlg	4	81
MuSK 3 <sup>c</sup>	MuSK 3-8 rlg	9	213
MuSK 3 <sup>c</sup>	MuSK 3-9 rlg	3	10
MuSK 3 <sup>c</sup>	MuSK 3-10 rlg	1	42
MuSK 3 <sup>c</sup>	MuSK 3-11 rlg	2	17
MuSK 3 <sup>c</sup>	MuSK 3-12 rlg	0	14
MuSK 3 <sup>c</sup>	MuSK 3-13 rlg	6	21
MuSK 3 <sup>c</sup>	MuSK 3-14 rlg	1	25
MuSK 3 <sup>c</sup>	MuSK 3-15 rlg	9	8
MuSK 3 <sup>c</sup>	MuSK 3-16 rlg	7	40
MuSK 3 <sup>c</sup>	MuSK 3-17 rlg	0	4
MuSK 3 <sup>c</sup>	MuSK 3-18 rlg	7	13
MuSK 3 <sup>c</sup>	MuSK 3-19 rlg	20	684
MuSK 3 <sup>c</sup>	MuSK 3-20 rlg	<b>32</b>	484
MuSK 3 <sup>c</sup>	MuSK 3-21 rlg	9	13
MuSK 3 <sup>c</sup>	MuSK 3-22 rlg	8	26
MuSK 3 <sup>c</sup>	MuSK 3-23 rlg	9	13
MuSK 3 <sup>c</sup>	MuSK 3-24 rlg	6	54
MuSK 3 <sup>c</sup>	MuSK 3-25 rlg	2	18
MuSK 3 <sup>c</sup>	MuSK 3-26 rlg	3	21
MuSK 3 <sup>c</sup>	MuSK 3-27 rlg	14	190
MuSK 3 <sup>c</sup>	MuSK 3-28 rlg	<b>99</b>	114666
MuSK 3 <sup>c</sup>	MuSK 3-29 rlg	<b>86</b>	2531
MuSK 3 <sup>c</sup>	MuSK 3-30 rlg	<b>82</b>	2842
MuSK 3 <sup>c</sup>	MuSK 3-31 rlg	8	25
MuSK 3 <sup>c</sup>	MuSK 3-32 rlg	0	0
MuSK 3 <sup>c</sup>	MuSK 3-33 rlg	<b>87</b>	27952
MuSK 3 <sup>c</sup>	MuSK 3-34 rlg	3	23
MuSK 3 <sup>c</sup>	MuSK 3-35 rlg	4	21
MuSK 3 <sup>c</sup>	MuSK 3-36 rlg	16	90
MuSK 3 <sup>c</sup>	MuSK 3-37 rlg	0	5
MuSK 3 <sup>c</sup>	MuSK 3-38 rlg	3	21
MuSK 3 <sup>c</sup>	MuSK 3-39 rlg	4	20
MuSK 3 <sup>c</sup>	MuSK 3-40 rlg	12	87
MuSK 3 <sup>c</sup>	MuSK 3-41 rlg	13	75
MuSK 3 <sup>c</sup>	MuSK 3-42 rlg	16	86
MuSK 3 <sup>c</sup>	MuSK 3-43 rlg	7	24
MuSK 3 <sup>c</sup>	MuSK 3-44 rlg	5	9
MuSK 3 <sup>c</sup>	MuSK 3-45 rlg	0	2
AChR 7 <sup>c</sup>	AChR 7-1 rlg	6	27
AChR 7 <sup>c</sup>	AChR 7-2 rlg	2	31

AChR 7 <sup>c</sup>	AChR 7-3 rlg	2	25
AChR 7 <sup>c</sup>	AChR 7-4 rlg	4	19
AChR 7 <sup>c</sup>	AChR 7-5 rlg	11	60
AChR 7 <sup>c</sup>	AChR 7-6 rlg	0	21
AChR 7 <sup>c</sup>	AChR 7-7 rlg	0	17
AChR 7 <sup>c</sup>	AChR 7-8 rlg	2	51
AChR 7 <sup>c</sup>	AChR 7-9 rlg	13	89
AChR 7 <sup>c</sup>	AChR 7-10 rlg	4	48
AChR 7 <sup>c</sup>	AChR 7-11 rlg	11	59
AChR 7 <sup>c</sup>	AChR 7-12 rlg	7	47
AChR 7 <sup>c</sup>	AChR 7-13 rlg	0	7
AChR 7 <sup>c</sup>	AChR 7-14 rlg	4	34
AChR 7 <sup>c</sup>	AChR 7-15 rlg	3	42
AChR 8 <sup>c</sup>	AChR 8-1 rlg	11	74
AChR 8 <sup>c</sup>	AChR 8-2 rlg	3	42
AChR 8 <sup>c</sup>	AChR 8-3 rlg	2	39
AChR 8 <sup>c</sup>	AChR 8-4 rlg	3	46
AChR 8 <sup>c</sup>	AChR 8-5 rlg	3	45
AChR 8 <sup>c</sup>	AChR 8-6 rlg	11	67
AChR 8 <sup>c</sup>	AChR 8-7 rlg	7	38
AChR 8 <sup>c</sup>	AChR 8-8 rlg	3	34
AChR 8 <sup>c</sup>	AChR 8-9 rlg	3	30
AChR 8 <sup>c</sup>	AChR 8-10 rlg	0	17
AChR 8 <sup>c</sup>	AChR 8-11 rlg	13	67

Bold characters indicate positives, the values of which were greater than the mean + 4SD of the control (AChR, HD1) specimens. Testing of all samples was performed in duplicate; the mean of duplicate experiments is reported. <sup>A</sup>:  $\Delta\%$  positive cells = (%frequency of positive MuSK-GFP-transfected cells/%frequency of MuSK-GFP-transfected cells) – (%frequency of positive GFP-transfected cells/%frequency of GFP-transfected cells), <sup>B</sup>:  $\Delta$ MFI = Alexa Fluor 647 median fluorescence intensity in MuSK-GFP-transfected cells minus Alexa Fluor 647 median fluorescence intensity in GFP-transfected cells, <sup>C</sup>: from previously cryopreserved cells, <sup>D</sup>: specimen was not sufficient to perform duplicate experiments, AChR: acetylcholine receptor, HD: healthy donor, MuSK: muscle-specific tyrosine kinase, rlg: recombinant immunoglobulin.