



24 § equal contribution

25 \* corresponding author: [ianegon@nantes.inserm.fr](mailto:ianegon@nantes.inserm.fr)

26

27 **Key words.** Tregs, tolerance, muscle injury, dystrophin, immunosuppression, knockout rats,  
28 TALEN, nucleases.

29

30 **Acknowledgments.** This work was financially supported by the Région Pays de la Loire  
31 through Biogenouest, and the “TEFOR” project funded by the « Investissements d’Avenir »  
32 French Government program, managed by the French National Research Agency (ANR)  
33 (ANRII-INSB-0014). This work was realized in the context of the Labex IGO project  
34 (n°ANR-11-LABX-0016-01) and the IHU-Cesti project (ANR-10-IBHU-005) which both are  
35 part of the « Investissements d’Avenir » French Government program managed by the ANR.  
36 The IHU-Cesti project is also supported by Nantes Métropole and Région Pays de la Loire.  
37 This work was also realized in the context of the support provided by the Fondation Progreffe.

38

39 **Competing interests:** IA and CG have registered a patent on the use of anti-CD45RC in  
40 Duchenne disease.

41

42 **Abstract.**

43 Duchenne muscular dystrophy (DMD) has as standard pharmacological therapy with  
44 corticosteroids (CS) that decrease inflammation and immune responses present in patients  
45 and animal models. CS have however limited efficacy and important and numerous side  
46 effects. Therefore, there is a need for new anti-inflammatory and pro-tolerogenic treatments  
47 that could replace or decrease doses of CS. We first assessed the status of immune system of  
48 dystrophin-deficient rats (*Dmd<sup>mdx</sup>*) that closely reproduce the phenotype of DMD patients.  
49 *Dmd<sup>mdx</sup>* rats showed increased leukocyte infiltration in skeletal and cardiac muscles,  
50 containing mostly macrophages but also T cells, and increased expression of several  
51 cytokines. Anti-CD45RC Monoclonal antibody (Mab) treatment induced immune tolerance in  
52 models of organ transplantation and GVHD (Graft Versus Host Disease). We observed that  
53 muscles and blood of DMD patients contained T CD4<sup>+</sup> and CD8<sup>+</sup> expressing high levels of  
54 CD45RC<sup>high</sup> cells. Treatment of young *Dmd<sup>mdx</sup>* rats with anti-CD45RC MAb corrected  
55 skeletal muscle strength associated to a depletion of effectors CD45RC<sup>high</sup> T cells with no  
56 obvious side-effects. Prednisolone treatment of *Dmd<sup>mdx</sup>* rats similarly increased skeletal  
57 muscle strength and was also associated to a depletion of effectors CD45RC<sup>high</sup> cells but  
58 resulted in severe weight loss.

59 Overall, *Dmd<sup>mdx</sup>* rats display important immune inflammatory response and thus represent a  
60 useful model to analyze new anti-inflammatory and tolerogenic treatments for DMD. As an  
61 example, a new treatment with anti-CD45RC antibodies improved muscle strength in *Dmd<sup>mdx</sup>*  
62 rats as prednisolone did but without side effects. Anti-CD45RC therapy could complement  
63 other therapies in DMD patients.

64

65

66

67 **Introduction.**

68 Duchenne muscular dystrophy (DMD) is the most common inherited muscle disease. It is  
69 caused by a mutation in the dystrophin gene with a X-chromosomal recessive inheritance that  
70 affects 1 of 3,500 male births <sup>1</sup>. It has a severe prognosis with life expectancy ranging from  
71 the late teens to the mid-30s. Muscle fibers show necrosis and regeneration/degeneration  
72 associated to inflammation with progressive replacement by connective and adipose tissue <sup>1</sup>.

73 The mdx mouse carries a mutation in the *Dmd* gene and is a well-established mouse model of  
74 DMD. Nevertheless, the muscle impairment is rather mild in *mdx* mice compared with DMD  
75 patients indicating that new animal models are required <sup>2</sup>.

76 We previously generated *Dmd*-deficient (*Dmd*<sup>mdx</sup>) rats using TALENs <sup>3</sup>. Forelimb and  
77 hindlimb muscular strength and spontaneous activity were decreased. Skeletal and cardiac  
78 muscles showed necrosis and regeneration of muscle fibers associated to progressive  
79 replacement by fibrotic and adipose tissue. Weak muscle strength and muscle lesions therefor  
80 closely mimic those observed in DMD patients. *Dmd*<sup>mdx</sup> rats represent a useful small animal  
81 model of pre-clinical research for DMD <sup>4</sup>.

82 To date, there is no cure for muscular dystrophies, and despite that gene and cell therapies  
83 will likely bring in the future cure of the disease there is still need for therapies for associated  
84 pathology such as immune responses and inflammation. Immune responses are involved in  
85 the pathophysiology of disease in both DMD patients and *mdx* mice [for a review see <sup>5</sup>].

86 Standard therapy of DMD is based on treatment with corticosteroids (CS), which have been  
87 shown to act at least in part through anti-inflammatory actions and inhibition of CD8<sup>+</sup> T cells  
88 that improve muscle strength in a fraction of patients <sup>5-7</sup>. Apart from its moderate efficacy, CS  
89 treatment is limited by serious systemic side effects, such as short stature, obesity,  
90 psychological symptoms, osteoporosis, diabetes and hypertension <sup>6</sup>. Furthermore, CS through

91 their broad and nonspecific anti-inflammatory effects inhibit inflammatory mechanisms that  
92 promote muscle repair<sup>5</sup>.

93 T effector cells against DMD have been described in patients before and after gene therapy<sup>8-</sup>  
94 <sup>10</sup>. CD4<sup>+</sup> T regulatory cells (Tregs) limit the severity of the disease in *mdx* mice not only  
95 through inhibition of immune responses but also by their tissue repair activity<sup>5, 11, 12</sup>.

96 Thus, inhibition of immune responses and promotion of immune tolerance are potentially  
97 important adjuvants to the therapeutic arsenal to treat DMD patients but these  
98 immunointerventions should at the same time preserve immune responses that promote  
99 muscle regeneration as well as protection against pathogens and cancer cells. Knowledge of  
100 immune responses in DMD patients and animal models are thus important for targeted  
101 immunointerventions associated to other treatments such as gene or cell therapy. Furthermore,  
102 immune responses can also be an obstacle to gene and cell therapy since in both situations  
103 newly produced dystrophin could be recognized as immunogenic and cells expressing it  
104 destroyed<sup>10</sup>. Thus, analyses of immune cells and immunotherapies in *Dmd*<sup>*mdx*</sup> rats could give  
105 potentially important results for development of new treatments for DMD patients.

106 We have described that CD4<sup>+</sup> and CD8<sup>+</sup> Tregs in rats and humans are comprised within  
107 CD45RC<sup>low/-</sup> cells<sup>13, 14</sup>. We have recently shown that treatment with anti-CD45RC  
108 monoclonal antibody (MAb) in a rat model of allograft rejection and in mouse immune  
109 humanized models of graft versus host disease (GVHD) could induce permanent allograft  
110 acceptance and inhibition of GVHD<sup>14</sup>. Anti-CD45RC treatment depleted only T cells that  
111 were CD45RC<sup>high</sup>, i.e. naïve T cells, precursors of Th1 cells and effector memory T cells  
112 including TEMRA cells, whereas CD8<sup>+</sup> or CD4<sup>+</sup> Tregs, both in rats and humans, are  
113 CD45RC<sup>low/-</sup> and thus were spared. Among CD45RC<sup>low/-</sup> cells, CD8<sup>+</sup> and CD4<sup>+</sup> Tregs specific  
114 for donor alloantigens protect from graft rejection. Importantly, immune responses against  
115 third party donors and exogenous antigens were preserved, thus anti-CD45RC antibody

116 treatment does not result in broad immunosuppression but rather specific elimination of T  
117 cells with effector functions and preserved Tregs followed by their activation and expansion  
118 <sup>14</sup>.

119 We thus reasoned that treatment of *Dmd*<sup>mdx</sup> rats with anti-CD45RC MAbs could eliminate  
120 CD45RC<sup>high</sup> effector T cells and enrich CD45RC<sup>low/-</sup> Tregs. The later could then act at the  
121 same time as inhibitors of immune responses and favoring muscle repair and homeostasis. To  
122 the best of our knowledge, treatment with antibodies directed against other cell antigens that  
123 favor immune tolerance in transplantation, GVHD or autoimmune diseases, such as anti-CD3,  
124 -CD28, -CD127 or -CD137, have not been reported in none of the other animal models of  
125 DMD.

126 We first analyzed immune parameters in *Dmd*<sup>mdx</sup> rats and we secondly treated *Dmd*<sup>mdx</sup> rats  
127 with the same anti-CD45RC MAb previously used to induce allograft tolerance in comparison  
128 to the standard of care (i.e. prednisolone). We observed that the skeletal and cardiac muscle of  
129 *Dmd*<sup>mdx</sup> rats showed a leukocyte infiltrate predominantly formed by macrophages and to a  
130 lesser extent by T cells. M2 type macrophages increased with time. Treatment with an anti-  
131 CD45RC depleting MAb resulted in increased muscle strength associated to a decrease in T  
132 cells but not of macrophages. Prednisolone treatment also increased muscle strength and  
133 decreased CD45RC<sup>high</sup> cells but decreased growth of *Dmd*<sup>mdx</sup> rats whereas anti-CD45RC did  
134 not. CD45RC<sup>+</sup> cells are also present in the blood and muscles of DMD patients.

135 Overall, immune responses and inflammation are present in the *Dmd*<sup>mdx</sup> rat muscles and anti-  
136 CD45RC MAb treatment resulted in amelioration of skeletal muscle strength. This is the first  
137 report showing that a treatment with a monoclonal antibody targeting specific T cell  
138 populations results in amelioration of clinical parameters in a pre-clinical model of DMD.

139

140 **Results.**

141 **Increased mononuclear leukocyte infiltration in skeletal muscles of *Dmd<sup>mdx</sup>* rats.**

142 Mononuclear leukocytes in the muscle and spleen of *Dmd<sup>mdx</sup>* rats were analyzed by flow  
143 cytometry using the pan-leukocyte marker CD45 (**Fig 1**). The number of total mononuclear  
144 leukocytes in the muscle of littermate wild type (WT) and *Dmd<sup>mdx</sup>* rats were comparable at 2  
145 weeks of age, but at 4 weeks *Dmd<sup>mdx</sup>* rats showed a sharp increase that was maintained until  
146 week 8 and then decreased at weeks 12 and 14 to values that were still significantly higher  
147 than those observed in littermate WT rats (**Fig. 1A-B**). Granulocytes were rarely observed at  
148 early time points in biopsies stained with Hemalun-Eosin-Saffron (data not shown). Total  
149 leukocyte numbers in the spleen were comparable between WT and *Dmd<sup>mdx</sup>* rats at all-time  
150 points analyzed (**Fig. 1A**). Thus, limb muscles of *Dmd<sup>mdx</sup>* rats showed an anatomical specific  
151 leukocyte infiltrate that indicates the presence of a localized immune/inflammatory response.

152

153 **Presence of macrophages and T cells in skeletal muscle of *Dmd<sup>mdx</sup>* rats as analyzed by**  
154 **cytofluorimetry.**

155 We used flow cytometry analysis to obtain frequencies and absolute numbers, of different  
156 mononuclear leukocyte populations. The analysis of viable CD45<sup>+</sup> mononuclear leukocyte  
157 subpopulations showed that ~90% of muscle infiltrating cells in *Dmd<sup>mdx</sup>* rats were CD68<sup>+</sup> (vs.  
158 ~60% in WT rats), increasing sharply at 4 weeks, maximal at 8 weeks and decreased but were  
159 still higher than WT at 12 and 16 weeks of age and were of higher granularity as shown by  
160 their SSC profile (**Fig. 2A**). In contrast, numbers of spleen CD68<sup>+</sup> macrophages increased  
161 steadily with age and were comparable between *Dmd<sup>mdx</sup>* and WT rats (**Fig. 2A**). Identical  
162 results were obtained with the macrophage marker SIRP $\alpha$  (**Supplementary figure 1**).

163 Analysis of the M2 marker CD163 also showed a similar curve with an increase at 4 weeks,  
164 maintained at 8 weeks and a decrease at 12 and 16 weeks of age with an increase in CD68  
165 expression levels in some animals (**Fig. 2B**). In contrast, the number of CD163<sup>+</sup> macrophages  
166 in spleen increased with age and were comparable between *Dmd<sup>mdx</sup>* rats and WT rats (**Fig.**  
167 **2B**). The ratio of M2:M1 macrophages in muscles of *Dmd<sup>mdx</sup>* rats was comparable at 4 weeks,  
168 increased non significantly at 8 weeks and was significantly higher at 12 and 16 weeks of age,  
169 whereas it was constant in muscles of WT rats (**Fig. 2C**). In the spleen, ratio of M2:M1  
170 macrophages increased with time but was always lower than in muscles and comparable for  
171 both *Dmd<sup>mdx</sup>* and WT rats except at 16 weeks of age in which *Dmd<sup>mdx</sup>* showed a modest but  
172 significant increase vs. WT rats (**Fig. 2C**).

173 Analysis of T cells in muscles showed that total TCR<sup>+</sup>αβ cells (**Fig. 3A-B**) as well as CD4<sup>+</sup>  
174 (**Fig.3 C-D**) and CD8<sup>+</sup> T cells (**Fig. 3E-F**) in *Dmd<sup>mdx</sup>* rats increased sharply at 4 weeks and  
175 then decreased at later time points with significantly higher levels at 4 and 12 weeks vs. WT  
176 animals. In contrast, in the spleen, total TCR<sup>+</sup> cells, CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells for both  
177 *Dmd<sup>mdx</sup>* and WT rats increased steadily to comparable numbers at 8 weeks and remained  
178 stable (**Fig. 3A-F**).

179 The muscles of *Dmd<sup>mdx</sup>* vs. WT rats showed significantly increased levels of Foxp3<sup>+</sup> CD4<sup>+</sup>  
180 Tregs at 4 and 12 weeks (**Fig. 3G-H**). As we previously described<sup>15, 16</sup>, CD8<sup>+</sup> Tregs were  
181 defined as CD8<sup>+</sup>CD45RC<sup>low</sup> T cells and were significantly increased at 4 and 12 weeks (**Fig.**  
182 **3I-J**). In contrast, in spleen, total Foxp3<sup>+</sup>CD4<sup>+</sup> Tregs and CD8<sup>+</sup>CD45RC<sup>low</sup> Tregs of both  
183 *Dmd<sup>mdx</sup>* and WT rats increased comparably at 8 weeks and remained stable (**Fig. 3H and J**).

184 B cells (CD45RA<sup>+</sup> and CD45R<sup>+</sup>) and NK cells (CD161<sup>high</sup>) represented respectively always  
185 <2% and 3% of total muscle leukocytes in both *Dmd<sup>mdx</sup>* and WT rats and were comparable in  
186 spleens of both *Dmd<sup>mdx</sup>* and WT rats (**data not shown**).



187 Thus, the majority of leukocytes in muscles of *Dmd<sup>mdx</sup>* rats were macrophages that reached  
188 maximal levels between 4 and 8 weeks of age and the ratio of M2:M1 increased at 12 and 16  
189 weeks of age. T cells, including CD8<sup>+</sup> and CD4<sup>+</sup> Tregs, showed a similar evolution with  
190 similar proportions of both CD4<sup>+</sup> and CD8<sup>+</sup> cells.

191

192 **Detection of macrophages in cardiac and skeletal muscle of *Dmd<sup>mdx</sup>* rats as analyzed by**  
193 **immunohistology.**

194 We used tissue immunofluorescence to analyze leukocyte populations in cardiac muscle since  
195 flow cytometry analysis required numbers of cells higher than we could routinely obtain from  
196 hearts from WT origin and to confirm the presence in skeletal muscle of leukocytes defined  
197 by flow cytometry. Skeletal and cardiac muscle biopsies at 8 and 12 weeks of age showed the  
198 presence of CD68<sup>+</sup> and CD163<sup>+</sup> macrophages but few CD3<sup>+</sup> cells in connective tissue of both  
199 skeletal and cardiac muscles of *Dmd<sup>mdx</sup>* rats. In comparison, only a few CD68<sup>+</sup> macrophages  
200 were observed sporadically in WT rats (**Fig. 4**). CD163<sup>+</sup> macrophages were notably numerous  
201 in foci of mononuclear cell infiltration in the cardiac muscle. Thus, immunohistology of  
202 skeletal muscles from *Dmd<sup>mdx</sup>* rats confirmed results obtained by flow cytometry and revealed  
203 very similar pattern in cardiac muscle. As previously described<sup>3</sup>, increased fibrosis (**Fig. 4**),  
204 fiber necrosis and regeneration (**data not shown**) are present in skeletal and cardiac muscle of  
205 *Dmd<sup>mdx</sup>* as soon as 4 weeks and more severely at 8 weeks of age. Along with these lesions,  
206 total creatinine kinase (CK) levels in serum, released from damaged muscle fibers, were  
207 comparable at week 2 between *Dmd<sup>mdx</sup>* and WT rats and then increased significantly in  
208 *Dmd<sup>mdx</sup>* rats to reach peak levels between weeks 4 and 8 and decreased at 12 weeks, returning  
209 to normal levels at week 16 (**Supplementary figure 2**).

210 These results indicate that infiltration of muscle by leukocytes was associated to damaged  
211 muscle fibers and elevated CK serum levels.

212

213 **Inflammatory and growth factors in leukocytes infiltrating muscle and serum of *Dmd<sup>mdx</sup>***  
214 **rats.**

215 We used quantitative RT-PCR to analyze mRNA levels of several molecules involved in the  
216 initiation or suppression of immune responses and inflammation, as well as some muscle  
217 trophic factors in isolated mononuclear leukocytes from muscles of *Dmd<sup>mdx</sup>* and WT at 8 and  
218 12 weeks of age (**Fig. 5A**). TNF $\alpha$  expression was particularly and strongly increased in  
219 mononuclear cells from muscles of *Dmd<sup>mdx</sup>* vs. WT at 8 weeks. Similarly, heme oxygenase-1  
220 (HO-1), IFN $\gamma$ , TGF $\beta$ , IL-10 as well as the muscle trophic factor amphiregulin<sup>11</sup> were  
221 significantly increased at 8 and/or 12 weeks (**Fig. 5A**). Arginase and IL-34 were decreased in  
222 mononuclear cells from muscle of *Dmd<sup>mdx</sup>* rats vs. WT rats, at weeks 8 and 12 respectively  
223 (**Fig. 5A**). IL-6 and iNOs were not statistically different in *Dmd<sup>mdx</sup>* vs. WT rats but the former  
224 showed higher numerical levels in *Dmd<sup>mdx</sup>* rats (**Fig. 5A**). Relaxin3 and indoleamine 2,3-  
225 dioxygenase (IDO) were detectable at very low levels without differences among the different  
226 groups of animals (**data not shown**).

227 To further evaluate cytokines in *Dmd<sup>mdx</sup>* rats, we analyzed by using a multiplex assay the  
228 presence of cytokines in the sera of animals at different time points. IL-1beta and IL-10 were  
229 detectable in serum in both *Dmd<sup>mdx</sup>* and WT rats without significant differences between them  
230 at 8, 12 and 16 weeks and as compared to 2 weeks IL-10 was significantly elevated only at 12  
231 weeks (**Fig. 5B**). TNF $\alpha$  and IL-6 levels were undetectable (**data not shown**).

232 Overall, several mediators of inflammation were increased in muscle or serum, such as TNF $\alpha$   
233 and IL-1 $\beta$ , respectively, and several anti-inflammatory molecules, such as HO-1, TGF $\beta$ ,  
234 amphiregulin and IL-10 were increased in muscle, as well as the later also in serum.

235

236 **Treatment with anti-CD45RC MAb depleted CD45RC<sup>high</sup> T cells and improved skeletal**  
237 **muscle strength.**

238 Anti-CD45RC MAb treatment induces organ transplantation tolerance and inhibits GVHD at  
239 least partially mediated by depletion of T CD8<sup>+</sup>CD45RC<sup>high</sup> and CD4<sup>+</sup>CD45RC<sup>high</sup> cells  
240 involved in organ rejection and GVHD and in the organ transplantation model by increased  
241 suppressor activity against donor antigens by CD8<sup>+</sup>CD45RC<sup>low/-</sup> and CD4<sup>+</sup>CD45RC<sup>low/-</sup> Tregs  
242 <sup>14</sup>. Since CD45RC expression levels can differ in different rat strains <sup>17</sup> and have not been  
243 reported in muscle, we first analyzed the distribution of CD45RC<sup>high</sup> and CD45RC<sup>low/-</sup>  
244 leukocytes within different leukocytes subsets in the muscle and spleen of *Dmd<sup>mdx</sup>* and WT  
245 Sprague-Dawley rats.

246 In muscles of *Dmd<sup>mdx</sup>* rats, we observed that, within the CD8<sup>+</sup> T cell population, absolute  
247 numbers of CD45RC<sup>low/-</sup> (**Fig. 3I-J**) and CD45RC<sup>high</sup> cells (**Supplementary figure 3A**)  
248 increased sharply and significantly in *Dmd<sup>mdx</sup>* vs. WT at 4 weeks, remained elevated at 8  
249 weeks and then decreased at 12 weeks to low levels observed in WT rats. Numbers in spleen  
250 of CD45RC<sup>high</sup> and CD45RC<sup>low/-</sup> cells were comparable of both *Dmd<sup>mdx</sup>* and WT rats  
251 (**Supplementary figure 3A and Fig. 3I**).

252 For the TCR<sup>+</sup>CD4<sup>+</sup> cell population absolute numbers of CD45RC<sup>low/-</sup> cells increased  
253 significantly at 4 and 12 weeks in the muscle of *Dmd<sup>mdx</sup>* rats vs. WT and in the spleen  
254 increased progressively without statistical differences (**Supplementary figure 3C-D**).

255 Absolute numbers of CD45RC<sup>high</sup> cells in muscle of *Dmd*<sup>mdx</sup> rats increased but not  
256 significantly vs. WT rats and in the spleen there were no differences between *Dmd*<sup>mdx</sup> and WT  
257 rats (**Supplementary figure 3E-D**).

258 For the non-T cells, which were mostly macrophages, CD45RC<sup>low/-</sup> increased significantly at  
259 4 weeks, remained elevated at 8 weeks and decreased at 12 weeks. (**Supplementary figure**  
260 **3F-G**). TCR<sup>-</sup> CD45RC<sup>high</sup> increased non significantly at 4 and 8 weeks, decreased at weeks 12  
261 and 16 and are statistically higher in *Dmd*<sup>mdx</sup> compared to WT rats (**Supplementary figure**  
262 **3H-G**). In the spleen, TCR<sup>-</sup> cells showed similar proportion of CD45RC<sup>high</sup> and CD45RC<sup>low/-</sup>  
263 cells in *Dmd*<sup>mdx</sup> and WT animals (**Supplementary figure 3F-H**).

264 WT and *Dmd*<sup>mdx</sup> rats were injected with the same anti-CD45RC MAb used in the  
265 transplantation model described above<sup>14</sup> from week 2, since at this time point the leukocyte  
266 infiltration into the muscle has not yet appeared, and every 3.5 days and up to week 12 when  
267 grip force and mononuclear cells from muscle and spleen were analyzed.

268 At 12 weeks of age, treatment with anti-CD45RC MAb significantly depleted  
269 CD8<sup>+</sup>CD45RC<sup>high</sup> T cells in both muscle and spleen of *Dmd*<sup>mdx</sup> and in spleen of WT rats  
270 whereas CD8<sup>+</sup>CD45RC<sup>low/-</sup> T cells were unchanged in both muscle and spleen (**Fig. 6A**).  
271 Numbers of CD4<sup>+</sup>CD45RC<sup>high</sup> T cells in the spleen were decreased but it did not reach  
272 statistical significance (**Fig. 6B**). CD4<sup>+</sup>CD45RC<sup>low/-</sup> (**Fig. 6B**) and FoxP3<sup>+</sup> CD4<sup>+</sup> T cells (data  
273 not shown) were maintained in both muscles and spleen. As in the transplantation models,  
274 other leukocytes that are CD45RC<sup>high</sup> and TCR<sup>-</sup>, such as macrophages and B cells were not  
275 depleted by anti-CD45RC treatment (**Fig. 6C**).

276 At week 12 the animals were analyzed using a grip test. As previously reported<sup>3</sup>, *Dmd*<sup>mdx</sup> rats  
277 had a 30% reduction in forelimb strength compared to WT littermates (**Fig. 6D**). The  
278 treatment with anti-CD45RC MAb significantly improved muscle strength in *Dmd*<sup>mdx</sup> treated  
279 rats vs. *Dmd*<sup>mdx</sup> control animals (**Fig. 6D**). Furthermore, values for *Dmd*<sup>mdx</sup> rats treated with

280 anti-CD45RC MAb were indistinguishable of those of littermate WT controls (**Fig. 6D**),  
281 despite that they showed a significantly lower strength vs. WT animals treated with anti-  
282 CD45RC, but this was due to a slight non-significantly increase in muscle strength of WT  
283 animals treated with anti-CD45RC vs. WT isotype control-treated animals (**Fig. 6D**). The  
284 weight gain curves of *Dmd*<sup>mdx</sup> animals were lower as compared to WT animals and treatment  
285 with anti-CD45RC neither modified this curve (**Fig. 6E**), nor the general aspect of the skeletal  
286 muscle fibrosis (**Supplementary figure 4A-B**) and CK levels in serum (**Supplementary**  
287 **figure 4C**).

288 Thus, anti-CD45RC treatment resulted in increased muscle strength in *Dmd*<sup>mdx</sup> rats and it was  
289 associated to depletion of T CD8<sup>+</sup>CD45RC<sup>high</sup> cells.

290

291 **Treatment with prednisolone improved skeletal muscle strength but had secondary**  
292 **effects.**

293 Since CS are standard treatment in DMD patients <sup>6</sup>, we analyzed the clinical effect of  
294 prednisolone on muscle strength of *Dmd*<sup>mdx</sup> rats as well as in immune cells in skeletal muscle  
295 and spleen of *Dmd*<sup>mdx</sup> rats.

296 Prednisolone-treated rats also showed at 12 weeks of age a significant decrease of  
297 CD8<sup>+</sup>CD45RC<sup>high</sup> T cells in both muscle and spleen of *Dmd*<sup>mdx</sup> rats and in spleen of WT rats  
298 (**Fig. 7A**). CD8<sup>+</sup>CD45RC<sup>low/-</sup> T cells were maintained (**Fig. 7A**). CD4<sup>+</sup>CD45RC<sup>high</sup> T cells  
299 were significantly decreased in spleen but not in muscle (**Fig. 7B**). CD4<sup>+</sup>CD45RC<sup>low</sup> T cells  
300 were also decreased in the spleen but not in the muscle (**Fig. 7B**). Other leukocytes that are  
301 CD45RC<sup>high</sup> and TCR<sup>-</sup>, such as macrophages and B cells were not depleted by prednisolone  
302 treatment (**Fig. 7C**).

303 Simultaneously, *Dmd*<sup>mdx</sup> rats treated with prednisolone showed significantly increased muscle  
304 strength at 12 weeks to levels identical to those of WT or anti-CD45RC-treated rats (**Fig. 7D**).  
305 Prednisolone-treated *Dmd*<sup>mdx</sup> rats showed marked secondary effects, as shown by a severe  
306 (25%) and significant reduction in growth as compared to WT rats but also to NaCl-treated  
307 *Dmd*<sup>mdx</sup> rats (**Fig. 7E**). Prednisolone had no effect on the growth of WT animals (**Fig. 7E**).  
308 Muscle tissue fibrosis (**Supplementary figure 4A-B**) and CK levels in serum  
309 (**Supplementary figure 4C**) were not modified by prednisolone treatment.

310 Thus, as compared to anti-CD45RC treatment, prednisolone also increased muscle strength  
311 but showed a larger decrease in cell populations including not only CD8<sup>+</sup>CD45RC<sup>high</sup> cells in  
312 muscle and spleen, but also CD4<sup>+</sup>CD45RC<sup>high</sup> and CD4<sup>+</sup>CD45RC<sup>low</sup> cells in spleen and had a  
313 strong negative effect on the growth of *Dmd*<sup>mdx</sup> animals.

314

### 315 **Presence of T CD45RC<sup>high</sup> cells in skeletal muscles and blood of DMD patients.**

316 To further explore the potential of CD45RC as an immunotherapeutic target, we evaluated the  
317 presence of CD45RC<sup>high</sup> cells in peripheral blood in DMD patients. Cytofluorimetry analysis  
318 showed the presence of CD45RC<sup>high</sup> and CD45RC<sup>low/-</sup> among both CD4<sup>+</sup> or CD8<sup>+</sup> T cell  
319 compartments in blood of DMD patients in proportions comparable to those of age-matched  
320 young individuals hospitalized for pathologies not involving the immune system or other  
321 neuromuscular diseases (**Supplementary figure 5**). As for young controls, B and NK cells  
322 from DMD patients were all CD45RC<sup>high</sup> whereas monocytes and PMN were all CD45RC<sup>-</sup>  
323 (**Supplementary figure 6**).

324 Furthermore, the presence of CD45RC brightly positive cells was confirmed in muscle  
325 biopsies from DMD patients and not of normal individuals, as it was the case in muscles of  
326 *Dmd*<sup>mdx</sup> vs. WT animals (**Fig. 8**).

327

328

329 **Discussion.**

330 DMD patients and *mdx* mice show muscle infiltration by different types of leukocytes and  
331 production of a variety of mediators that have been shown to play facilitating or protecting  
332 roles in the evolution of the disease<sup>5</sup>. Not only inflammation and innate immune responses  
333 are present, but also adaptive immune responses including anti-dystrophin T cells and Treg  
334 cells are involved in DMD patients<sup>8,10</sup> and *mdx* mice<sup>5,11,12</sup>. CS are one of the only standard  
335 treatments that DMD patients receive and that prolong ambulation by about 2 years.  
336 Nevertheless, increase muscular strength responses are variable, incomplete and always  
337 associated to serious side effects<sup>6,7</sup>. Despite that the precise mechanisms of action of CS in  
338 DMD patients are ill defined, anti-inflammatory effects are likely important<sup>6,7</sup>. Thus, there  
339 are unmet clinical needs to treat the inflammatory and immune effects caused by dystrophin  
340 deficiency while awaiting for curative gene or stem cell therapies. It is even very likely that  
341 these immunotherapies will be associated to gene and cell therapy to inhibit immune  
342 responses against the vectors, transgene products or antigenic cellular products.

343 The *mdx* mouse is a very useful model but fails to reproduce key symptoms of DMD patients  
344 such as muscular weakness<sup>2</sup>. Thus, although several immunotherapies were successful in *mdx*  
345 mice, such as intravenous immunoglobulin<sup>18</sup>, anti-TNF $\alpha$  antibodies<sup>19</sup>, IL-6 blocking<sup>20</sup>,  
346 tranilast<sup>21</sup>, heme oxygenase-1 (HO-1) inducers<sup>22</sup>, IL-1 receptor antagonist<sup>23</sup> and IL-2  
347 complexes to amplify CD4<sup>+</sup> Tregs<sup>12</sup>, their potential effect in DMD patients is uncertain.  
348 *Dmd*<sup>*mdx*</sup> rats reproduce skeletal and cardiac muscular weakness at early time points and  
349 develop skeletal and cardiac muscle tissue lesions that resemble those observed in DMD  
350 patients<sup>3,4</sup>. In the present manuscript we describe that *Dmd*<sup>*mdx*</sup> rats present mononuclear cells  
351 infiltrating both skeletal and cardiac muscles that appeared early, between 2 and 4 weeks of  
352 age, and that had greatly decreased by 16 weeks of age. The majority of these mononuclear  
353 cells were CD68<sup>+</sup> and SIRP $\alpha$ <sup>+</sup> macrophages and the proportion of M2 CD163<sup>+</sup> increased with



354 time. Macrophages appear early in both *mdx* mice (2 weeks) and DMD patients (2-year-old)  
355 <sup>24</sup>. M2 macrophages have been shown to play protective and regenerative roles in early stage  
356 disease in *mdx* mice <sup>5</sup>. CD4<sup>+</sup> and CD8<sup>+</sup> T cells, including Tregs, were also increased in  
357 muscles of *Dmd*<sup>*mdx*</sup> rats compared to controls. The lesions of muscular fibers, as analyzed by  
358 CK levels in serum, followed the kinetics of leukocyte infiltration, with normal levels at 2  
359 weeks of age and a peak between 4 and 8 weeks of age for a later decrease, possibly reflecting  
360 a more pronounced immune attack at early rather than late time points.

361 Cytokines were produced at increased levels by mononuclear cells from *Dmd*<sup>*mdx*</sup> rats  
362 compared to controls at 8 and/or 12 weeks of age, including IL-1 $\beta$  and TNF $\alpha$ . These  
363 cytokines are increased in DMD patients and *mdx* mice, have been described as potential  
364 immunotherapy targets <sup>25</sup>, since anti-TNF $\alpha$  treatment reduces early muscle damage in *mdx*  
365 mice <sup>19</sup> could be targeted in the future in *Dmd*<sup>*mdx*</sup> rats. Several anti-inflammatory molecules,  
366 such as HO-1, IL-10 and TGF $\beta$ , as well as the muscle trophic factor amphiregulin <sup>11</sup>, were  
367 also produced, likely as a response to inflammation and ongoing immune responses, as  
368 previously described in *mdx* mice and DMD patients <sup>5</sup>. Inhibition of TGF $\beta$  has been shown to  
369 play a dual role since early neutralization in *mdx* mice decreases fibrosis but increases T cell  
370 infiltration and inflammation<sup>26</sup>.

371 We have recently shown that treatment with an anti-CD45RC MAb in a rat model of heart  
372 allograft rejection could induce permanent allograft acceptance <sup>14</sup>. Furthermore, anti-CD45RC  
373 MAb treatment prevented GVHD in immune humanized NSG (NOD *Scid* Gamma) mice <sup>14</sup>.  
374 Anti-CD45RC treatment depleted T cells that were CD45RC<sup>high</sup>, comprising naïve T cells,  
375 precursors of Th1 cells and T effector memory cells including TEMRA cells, whereas CD8<sup>+</sup>  
376 and CD4<sup>+</sup> Tregs both in rats and humans are CD45RC<sup>low/-</sup> <sup>13, 27</sup> and thus were spared. These  
377 CD45RC<sup>low/-</sup> CD8<sup>+</sup> and CD4<sup>+</sup> Tregs that were specific of donor alloantigens could impose

378 allograft tolerance in newly grafted irradiated recipients following adoptive cell transfer.  
379 Importantly, immune responses against third party donors and exogenous antigens were  
380 preserved during treatment with anti-CD45RC, thus depletion of CD45RC<sup>high</sup> cells does not  
381 inhibit all immune responses.

382 We thus reasoned that treatment of *Dmd*<sup>mdx</sup> rats with anti-CD45RC MAbs could eliminate  
383 CD45RC<sup>high</sup> T effector cells and their precursors and enrich CD45RC<sup>low/-</sup> Tregs that could then  
384 act at the same time, not only as inhibitors of immune responses by CD45RC<sup>high</sup>, but also  
385 favor tissue repair and homeostasis by CD45RC<sup>low/-</sup>, such as it has been described for CD4<sup>+</sup>  
386 Tregs both in muscle <sup>11</sup> and adipose tissue <sup>28</sup>. In the present manuscript we show that anti-  
387 CD45RC treatment improved muscle strength to the levels of WT animals and that this was  
388 associated to a depletion of CD8<sup>+</sup> CD45RC<sup>high</sup> T cells at 12 weeks. CD4<sup>+</sup> CD45RC<sup>high</sup> T  
389 effector cells were decreased but not significantly at this time of treatment. Although  
390 CD45RC<sup>low/-</sup> CD8<sup>+</sup> or CD4<sup>+</sup> Tregs were not numerically increased in *Dmd*<sup>mdx</sup> rats this was  
391 also the case in rats that were tolerant to transplanted organs after anti-CD45RC MAb  
392 treatment <sup>14</sup>. Whether their function is increased and play a role in the amelioration of muscle  
393 strength observed in these animals remains to be analyzed in future studies.

394 As for the anti-CD45RC treatment, corticosteroids resulted in a similar increase in muscular  
395 strength that was associated surprisingly to a specific decrease in CD8<sup>+</sup> CD45RC<sup>high</sup> T cells in  
396 muscle but also to a more widespread decrease of CD4<sup>+</sup>CD45RC<sup>high</sup> and CD4<sup>+</sup>CD45RC<sup>low/-</sup>  
397 cells in spleen. This effect has also been observed in DMD patients treated with steroids <sup>5</sup>.  
398 DMD patients treated with corticosteroids showed decreased T cells against dystrophin <sup>10</sup>.

399 The secondary effects of steroids were observed in *Dmd*<sup>mdx</sup> rats, whereas anti-CD45RC  
400 treated animals did not show obvious clinical abnormalities and no weight loss. Since patients  
401 suffer from several important side effects of steroids, anti-CD45RC treatment could result in

402 similar muscle improvement than corticosteroids but without side effects. A potential side  
403 effect of anti-CD45RC treatment could be generalized immunosuppression but we have  
404 already demonstrated that rats treated with anti-CD45RC treatment could mount normal  
405 primary immune responses to new antigens as well as memory immune responses after  
406 secondary immunization <sup>14</sup>.

407 MAbs against CD45RA <sup>25</sup>, CD45RO/B <sup>29</sup> and CD45RB <sup>30</sup> have been used to treat organ  
408 rejection and/or GVHD but none has been used as isolated treatment, neither in animal  
409 models of DMD, nor on animal models of muscle lesions. Even if anti-CD45RA or  
410 antiCD45RO/B could be used in the future, since between 50 to 90% of both of the CD8<sup>+</sup> and  
411 CD4<sup>+</sup> Tregs are CD45RA<sup>high</sup> and CD45RB<sup>high</sup> <sup>14</sup>, the outcome of treatment with anti-CD45RC  
412 is clearly targeting different cell populations and thus distinct and likely more favorable since  
413 it preserves Tregs. Although in mdx mice depletion of total CD4<sup>+</sup> or CD8<sup>+</sup> cells ameliorates  
414 histopathology <sup>31</sup>, none of other tolerizing treatments based on MAbs and used in organ  
415 transplantation, GVHD or autoimmunity, such as anti-CD3, anti-CD127, anti-CD28, have  
416 been previously used in models of DMD and thus the results in the present manuscript could  
417 stimulate the use of these other reagents.

418

419 **Materials and methods.**

420 **Animal experiments and ethical aspects.**

421 *Dmd*<sup>mdx</sup> rats have been previously described<sup>3</sup>. *Dmd*<sup>mdx</sup> and wild-type littermate were raised in  
422 SPF conditions. All the animal care and procedures performed in this study were approved by  
423 the Animal Experimentation Ethics Committee of the Pays de la Loire region, France, in  
424 accordance with the guidelines from the French National Research Council for the Care and  
425 Use of Laboratory Animals (Permit Numbers: CEEA-PdL-10792 and CEEA-PdL-8986). All  
426 efforts were made to minimize suffering. The rats were housed in a controlled environment  
427 (temperature 21±1°C, 12-h light/dark cycle). Blood samples from 2 DMD patients were  
428 obtained as part of their standard care management in the hospital and after obtaining  
429 informed consent from both patients and their parents. Control blood samples were collected  
430 from children who had been admitted in Nantes University Hospital without immune  
431 deficiency. The biocollection used for this analysis is the "pediatrics" collection (Ref: MESR  
432 DC-2011-1399) which is a prospective monocentric collection managed by the University  
433 Hospital of Nantes and approved by the local ethics committee. None of the legal  
434 representatives of the children objected to let them take part in this biocollection. Tissue  
435 samples were obtained from the *Paravertebralis* muscle of four 12 year-old patients (two  
436 DMD patients and two patients free of known muscular disease). Patients were operated at the  
437 Department of Pediatric Surgery of the Centre Hospitalier Universitaire (CHU) de Nantes  
438 (France). They gave written informed consent. All protocols were approved by the Clinical  
439 Research Department of the CHU (Nantes, France), according to the rules of the French  
440 Regulatory Health Authorities (Permit numbers: MESR/DC-2010-1199). Biological sample  
441 bank was constituted in compliance with the national guidelines regarding the use of human  
442 tissue for research (Permit numbers: CPP/29/10).

443

444 **Preparation of muscle and spleen single-cell suspensions.**

445 Muscles of both hindlimbs from WT or *Dmd<sup>mdx</sup>* rats were excised without adipose tissue,  
446 rinsed with PBS and weighed. Muscles were minced, placed in gentleMACS C tubes  
447 (Miltenyi Biotec) with collagenase D (4ml/g of muscle), dissociated using the gentleMACS™  
448 dissociator (gentleMACS program “m\_muscle\_01”) and incubated for two runs of 30 min at  
449 37°C under continuous rotation. After the initial run, undigested muscle was filtrated on a  
450 mesh strainer and the resulting cell suspension was centrifuged and resuspended in PBS FCS  
451 2% 1 mM EDTA. The remaining undigested muscle was further incubated in fresh  
452 collagenase for a new run of 30 min. The debris-free cell suspensions were centrifuged,  
453 resuspended in PBS FCS 2% 1 mM EDTA, and pooled with cells from the first digestion.  
454 Pooled cells were then applied to 15 ml Histopaque 1077 density gradient (Eurobio) and  
455 centrifuged at 1000 x g for 30min. The cells at the interface were collected, washed,  
456 resuspended in PBS FCS 2% 1mM EDTA and counted.

457 Spleen was harvested, perfused with collagenase D, minced and incubated for 15 min at 37°C.  
458 Spleen fragments were then scraped in the presence of PBS FCS 2% 1mM EDTA and  
459 mononuclear cells were recovered using a density gradient (Histopaque 1077, Eurobio). The  
460 cells at the interface were collected, washed, resuspended in PBS FCS 2% 1mM EDTA and  
461 counted.

462

463 **Staining of rat cells for flow cytometry analysis.**

464 Cytofluorimetry analysis was performed as previously described in detail <sup>32</sup>. Briefly, single-  
465 cell suspensions from muscle or spleen were stained with MAbs against the following  
466 antigens : CD45 as a pan leukocyte (clone OX-1), TCRαβ (clone R7/3), CD45RA in B cells  
467 (clone OX33), CD45R/B220 in B cells (clone His24), anti-granulocytes (RP-1 and His48),  
468 CD4 (clone w3/25), CD45RC (clone OX22 or clone OX32), CD25 (clone OX39), CD8 (clone

469 OX8), CD172a/SIRP $\alpha$  (clone OX41), CD161 in NK and myeloid cells (clone 3.2.3), CD163  
470 in macrophages (clone ED2), CD68 for macrophages (clone ED1) and with viability dye  
471 eFluor506 or eFluor450 from eBiosciences to assess cell viability. Analysis was performed on  
472 a BD FACS Verse with FACSuite Software version 1.0.6. Post-acquisition analysis was  
473 performed with FlowJo software.

474

#### 475 **Serum creatinine phosphokinase and cytokine levels.**

476 Blood was collected under anesthesia, serum was isolated and immediately frozen at -20°C.  
477 Total creatinine phosphokinase (CK) activity was determined in the biochemistry department  
478 of Nantes University Hospital.

479 Levels of IL-1 $\beta$ , IL-6, IL-10 and TNF $\alpha$  in the serum of *Dmd*<sup>*mdx*</sup> or WT littermate rats, were  
480 measured by multiplex assay (Luminex technology) (R&D systems) following to the  
481 manufacturer's instructions.

482

#### 483 **Quantitative RT-PCR.**

484 Quantification of mRNA levels was performed as previously described in detail <sup>33</sup>. Briefly,  
485 total RNA extraction has been performed on mononuclear cells from skeletal muscles using  
486 RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. Then quantification  
487 and quality analysis were done on Caliper LabChip GX II (PerkinElmer). RNA with a quality  
488 score between 7 and 10 were retro-transcribed using oligo-dT and M-MLV reverse  
489 transcriptase (Life Technologies). Fast SybrGreen Master Mix 2x was used to performed  
490 qPCR on ViiA 7 (Applied Biosystems) on cDNA in duplicate for each target according to the  
491 manufacturer's instructions. qPCR reaction conditions were 20 seconds at 95°C followed by  
492 40 cycles of 1 second at 95°C, 20 seconds at 60°C and 20 seconds at target melting

493 temperature minus 3°C, ended by a melting curve stage. Calculations were made by DDCT  
494 method. The primers used in this study are listed in **table 1**.

495

496 **Immunohistological analysis and fibrosis quantification.**

497 Immunohistochemistry was performed as previously described in detail <sup>3</sup>. Briefly, tissue  
498 samples of *Biceps femoris* and cardiac ventricular muscles were harvested at 8 and 12 weeks  
499 of age and frozen and 8-µm-thick sectioned for immunofluorescence labelling. Sections were  
500 preliminary fixed with acetone for CD3 labelling and with acetone (30%) in methanol for  
501 CD68, CD163 and CD45RC labelling (10 min, room temperature) and incubated with 0.2%  
502 triton in PBS (10 min, room temperature). Sections were then blocked with 10% goat serum  
503 in PBS and incubated with the primary antibodies. Rabbit polyclonal antibody for CD3  
504 (DakoCytomation, Glostrup, Denmark), mouse monoclonal antibodies for rat CD68, CD163  
505 and CD45RC were used respectively at 1:50; 1:200 and 1:200 (overnight, 4°C). After  
506 washing, goat anti-rabbit and goat anti-mouse antibodies coupled with Alexa 488  
507 (InVitroGen, Carlsbad, CA) were respectively used to reveal CD3 and CD68 primary  
508 antibody (1 h, room temperature). Section were incubated with wheat germ agglutinin Alexa  
509 Fluor 555 conjugate for connective tissue labelling (Molecular Probes, Eugene, OR)  
510 diluted 1:700 in PBS (overnight, 4°C) and nuclei were then labelled with Draq5 (BioStatus  
511 Ltd, Shepshed, UK) diluted at 1:1000 (10min, room temperature). Immunofluorescence  
512 labeling was analyzed with a laser scanning confocal microscope (Zeiss, LSM880, Jena,  
513 Germany).

514 For human muscle, biopsies were obtained from DMD patients undergoing surgery for spinal  
515 deformities and from young individuals undergoing muscle biopsy for other diagnosis. Tissue  
516 was frozen, sectioned and processed as described above for rat tissue using an anti-human  
517 CD45RC MAb (BD Biosciences).

518

519 **Treatment with anti-CD45RC and prednisolone.**

520 WT and *Dmd*<sup>mdx</sup> rats received intraperitoneal injections of the anti-rat CD45RC MAb (clone  
521 OX22, mouse IgG1) or an isotype control MAb (clone 3G8, mouse IgG1) at 1.5 mg/kg, every  
522 3.5 days from week 2 to week 12 of age as previously described <sup>14</sup>. Prednisolone was  
523 administered by daily intraperitoneal injections of 0.5 mg/kg, close to the dose of 1 mg/kg in  
524 mdx mice <sup>34</sup> and 0.75 mg/kg in DMD patients <sup>35</sup> from week 2 to week 12 of age.

525

526 **Grip test.**

527 Grip test was performed as previously described in detail <sup>3</sup>. Rats were placed with their  
528 forepaws on a grid and were gently pulled backward until they released their grip, as  
529 previously described. A grip meter (Bio-GT3, BIOSEB, France), attached to a force  
530 transducer measured the peak force generated.

531

532 **Statistical analysis.**

533 Mann–Whitney t test was used to compare numbers of cells in muscle and spleen of WT vs  
534 *Dmd*<sup>mdx</sup>, CK and cytokine levels in sera.

535 Two-way ANOVA test was used to compare growth curves.

536

537

538



539 **Figure legends.**

540

541 **Figure 1. Number of leukocytes in skeletal muscle and spleen of *Dmd<sup>mdx</sup>* rats.** Hind limb  
542 muscles and spleen were harvested from littermate wild-type (WT) or *Dmd<sup>mdx</sup>* (KO) rats at the  
543 indicated time points of age. Muscles and spleens were digested with collagenase,  
544 mononuclear cells were isolated using a density gradient and analyzed by cytofluorimetry. **A)**  
545 Number of viable CD45<sup>+</sup> cells per gram of muscle (left panel) or whole spleen (right panel) at  
546 different time points. WT, n=4, 5, 7, 7, 9 at 2, 4, 8, 12 and 16 weeks, respectively; *Dmd<sup>mdx</sup>*,  
547 n=3, 6, 10, 11, 16, at 2, 4, 8, 12 and 16 weeks, respectively \* p< 0.05, \*\* p< 0.01, and \*\*\* p<  
548 0.001. **B)** Representative dot-plot analysis of viable SSC CD45<sup>+</sup> mononuclear leukocytes  
549 from muscle (left panel) or spleen (right panel) from animals at 12 weeks of age.

550

551 **Figure 2. Macrophages in skeletal muscle and spleen of *Dmd<sup>mdx</sup>* rats.** Cytofluorimetry of  
552 single-cell suspensions from hind limb muscles or spleen WT or *Dmd<sup>mdx</sup>* (KO) at the indicated  
553 time points of age. **A)** Total number of macrophages CD68<sup>+</sup> cells per gram of muscle (upper  
554 left panel) or of whole spleen (upper right panel). Representative dot plots of macrophages  
555 high granularity using side scatter (SSC<sup>high</sup>) CD68<sup>+</sup> cells after gating on viable (negatively-  
556 stained cells) CD45<sup>+</sup> cells from muscle of WT or *Dmd<sup>mdx</sup>* 12 weeks-old rats (lower panel). **B)**  
557 Total number of viable CD68<sup>+</sup>CD163<sup>+</sup> type 2 macrophages per gram of muscle (upper left  
558 panel) or whole spleen (upper right panel). Representative dot plots of viable CD68<sup>+</sup>CD163<sup>+</sup>  
559 cells from muscle of WT or *Dmd<sup>mdx</sup>* 12 weeks-old rats (lower panels). **C)** Macrophages type 2  
560 (CD68<sup>+</sup>CD163<sup>+</sup>) over type 1 macrophages (CD68<sup>+</sup>CD163<sup>-</sup>) ratios in muscle (left panel) or  
561 spleen (right panel) of WT (black) or *Dmd<sup>mdx</sup>* (grey) rats. n=3, 6, 6, 7 and 8 (at 2,4, 8, 12 and  
562 16 weeks of age, respectively) for *Dmd<sup>mdx</sup>* rats and n= 4, 6, 4, 3 and 4 (at 2,4, 8, 12 and 16

563 weeks of age, respectively) for WT rats. \*  $p < 0.05$ , \*\*\*  $p < 0.01$ . Results were obtained from  
564 several experiments performed using all groups of animals in each experiment.

565

566 **Figure 3. T cells in skeletal muscle and spleen of *Dmd<sup>mdx</sup>* rats.** Hind limb muscles or spleen  
567 from WT or *Dmd<sup>mdx</sup>* (KO) at the indicated time points of age were harvested, collagenase  
568 digested and analyzed by cytofluorimetry. **A)** Total numbers of viable CD45<sup>+</sup>TCR<sup>+</sup> cells per  
569 gram of muscle (left panel) and of total spleen (right panel). **B)** Representative dot plots of  
570 viable CD45<sup>+</sup>TCR<sup>+</sup> cells from muscle of WT or *Dmd<sup>mdx</sup>* 12 weeks-old rats. **C)** Total number  
571 of CD45<sup>+</sup>TCR<sup>+</sup>CD4<sup>+</sup> cells per gram of muscle (left panel) and of total spleen (right panel). **D)**  
572 Representative dot plots of WT or *Dmd<sup>mdx</sup>* 12 weeks-old rat muscle single-cell suspension  
573 showing gating on viable CD45<sup>+</sup>TCR<sup>+</sup>CD4<sup>+</sup> cells. **E)** Total number of TCR<sup>+</sup>CD8<sup>+</sup> cells per  
574 gram of muscle (left panel) and of total spleen (right panel). **F)** Representative dot plots of  
575 WT or *Dmd<sup>mdx</sup>* 12 weeks-old rat muscle single-cell suspension showing gating on viable  
576 CD45<sup>+</sup>TCR<sup>+</sup>CD8<sup>+</sup> cells. **G)** Total number of TCR<sup>+</sup>CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> cells per gram of  
577 muscle (left panel) and whole spleen (right panel). **H)** Representative dot plots of WT or  
578 *Dmd<sup>mdx</sup>* 12 weeks-old rat muscle single-cell suspension showing gating on viable  
579 CD45<sup>+</sup>TCR<sup>+</sup>CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> cells. **I)** Total number of TCR<sup>+</sup>CD8<sup>+</sup>CD45RC<sup>low/-</sup> cells per  
580 gram of muscle (left panel) and whole spleen (right panel). **J)** Representative dot plots of WT  
581 or *Dmd<sup>mdx</sup>* 12 weeks-old rat muscle single-cell suspension showing gating on viable  
582 CD45<sup>+</sup>TCR<sup>+</sup>CD8<sup>+</sup>CD45RC<sup>low/-</sup> cells. n=3, 6, 10, 12 and 4 (at 2, 4, 8, 12 and 16 weeks of age,  
583 respectively) for *Dmd<sup>mdx</sup>* rats and n= 4, 5, 7, 7 and 4 (at 2, 4, 8, 12 and 16 weeks of age  
584 respectively) for WT rats. Results were obtained from several experiments performed using  
585 all groups of animals in each experiment.

586

587 **Figure 4. Immunohistochemical detection of leukocytes in skeletal and cardiac muscle of**  
588 ***Dmd<sup>mdx</sup>* rats.** Skeletal muscle (*Biceps femoris*) and cardiac muscle were harvested at 8 and 12  
589 weeks of age from wild-type (WT) and *Dmd<sup>mdx</sup>* (KO) rats. **A)** Tissue sections were stained  
590 with Draq5 to label nuclei (blue), with wheat germ agglutinin for connective tissue (red) and  
591 with MAbs for detection of cells expressing CD3, CD68 or CD163 (green). Scale bar  
592 identical for all pictures: 100  $\mu$ m.

593

594 **Figure 5. Inflammation markers and growth factors in skeletal muscle of *Dmd<sup>mdx</sup>* rats.**  
595 **A)** Mononuclear cells from skeletal muscles were harvested at 8 and 12 weeks of age from  
596 wild-type (WT) and *Dmd<sup>mdx</sup>* (KO) rats. Total RNA was extracted and mRNA levels for the  
597 indicated molecules were analyzed by quantitative RT-PCR. \*  $p < 0.05$ . **B)** IL1 $\beta$  (left panel)  
598 and IL10 (right panel) levels in the sera of *Dmd<sup>mdx</sup>* (n=11, 3, 10, 5 at 2, 8, 12 and 16 weeks of  
599 age, respectively) or WT (n= 12, 2, 5, 6 at 2, 8, 12 and 16 weeks of age, respectively) rats. \*  
600  $p < 0.05$ .

601

602 **Figure 6. Effect of treatment with anti-CD45RC on lymphoid cell populations, forelimb**  
603 **muscle strength and animal growth.** Hind limb muscles or spleen from WT or *Dmd<sup>mdx</sup>*  
604 (KO) rats were harvested at 12 weeks of age, collagenase digested and analyzed by  
605 cytofluorimetry. **A)** Total numbers of viable CD45<sup>+</sup>TCR<sup>+</sup>CD8<sup>+</sup>CD45RC<sup>high</sup> cells (upper  
606 panels) or viable CD45<sup>+</sup>TCR<sup>+</sup>CD8<sup>+</sup>CD45RC<sup>low/-</sup> (lower panels) cells per gram of skeletal  
607 muscle (left panels) and of total spleen (right panels). **B)** Total numbers of viable  
608 CD45<sup>+</sup>TCR<sup>+</sup>CD4<sup>+</sup>CD45RC<sup>high</sup> cells (upper panels) or viable CD45<sup>+</sup>TCR<sup>+</sup>CD4<sup>+</sup>CD45RC<sup>low/-</sup>  
609 (lower panels) cells per gram of skeletal muscle (left panels) and of total spleen (right panels).  
610 **C)** . Total numbers of viable CD45<sup>+</sup>TCR<sup>-</sup> CD45RC<sup>high</sup> cells per gram of skeletal muscle (left  
611 panels) and of total spleen (right panels). **D)** Muscle strength in *Dmd<sup>mdx</sup>* rats after treatment

612 with an anti-CD45RC MAb. Wild-type (WT) or *Dmd*<sup>mdx</sup> rats received intraperitoneal  
613 injections of the anti-rat CD45RC MAb (clone OX22, 1,5 mg/kg, every 3.5 days) or isotype  
614 control Mab (clone 3G8, 1,5 mg/kg, every 3.5 days) from week 2 to week 12 of age when  
615 muscle strength was analyzed using a grip test. Each point represents a single animal analyzed  
616 in two different experiments. \* p< 0.05. Results were obtained from several experiments  
617 performed using all groups of animals in each experiment. **E)** Weight curves for animal  
618 growth were determined serially. \*\*\*\*p<0.001 between *Dmd*<sup>mdx</sup> and WT rats for both  
619 treatments but no difference between *Dmd*<sup>mdx</sup> rats treated with anti-CD45RC vs. isotype  
620 control.

621

622 **Figure 7. Treatment with prednisolone on lymphoid cell populations and forelimb**  
623 **muscle strength.** Wild-type (WT) or *Dmd*<sup>mdx</sup> (KO) rats received from week 2 of age  
624 intraperitoneal injections of prednisolone (0.5 mg/kg, 5 days per week) or NaCl up to week  
625 12. **A)** Hind limb muscles or spleen from WT or *Dmd*<sup>mdx</sup> were harvested, collagenase  
626 digested and analyzed by cytofluorimetry. Total numbers of viable  
627 CD45<sup>+</sup>TCR<sup>+</sup>CD8<sup>+</sup>CD45RC<sup>high</sup> cells (upper panels) or viable CD45<sup>+</sup>TCR<sup>+</sup>CD8<sup>+</sup>CD45RC<sup>low/-</sup>  
628 (lower panels) cells per gram of muscle (left panels) and of total spleen (right panels). \* p<  
629 0.05. **B)** Total numbers of viable CD45<sup>+</sup>TCR<sup>+</sup>CD4<sup>+</sup>CD45RC<sup>high</sup> cells (upper panels) or viable  
630 CD45<sup>+</sup>TCR<sup>+</sup>CD4<sup>+</sup>CD45RC<sup>low/-</sup> (lower panels) cells per gram of muscle (left panels) and of  
631 total spleen (right panels). **C)** Total numbers of viable CD45<sup>+</sup>TCR<sup>-</sup> CD45RC<sup>high</sup> cells per  
632 gram of skeletal muscle (left panels) and of total spleen (right panels). **D)** Muscle strength  
633 was analyzed using a grip test. Each point represents a single animal analyzed in two different  
634 experiments. \* p< 0.05. Results were obtained from several experiments performed using all  
635 groups of animals in each experiment. **E)** Weight curves for animal growth were determined

636 serially. \*\*<0.01 and \*\*\*\*<0.0001 for *Dmd*<sup>mdx</sup> and WT with NaCl and prednisolone but  
637 importantly \*\*\*p<0.001 between *Dmd*<sup>mdx</sup> rats NaCl vs. prednisolone.

638

639 **Figure 8. CD45RC<sup>+</sup> cells in rat and human dystrophin-deficient skeletal muscles.**

640 Skeletal muscle samples from rat (*Biceps femoris*) and humans (*Paravertebralis*), either from  
641 dystrophin deficient individuals (n=2) or without muscle pathology (n=2). Pictures are  
642 representative images of frozen tissue sections probed with Draq5 to label nuclei and with  
643 anti-rat or human anti-CD45RC MAbs (green).

644

645

646 **Supplementary figure 1. Number of SIRP $\alpha$ <sup>+</sup> macrophages in skeletal muscle and spleen**  
647 **of *Dmd*<sup>mdx</sup> rats.** Hind limb muscles and spleen were harvested from littermate wild-type  
648 (WT) or *Dmd*<sup>mdx</sup> (KO) rats at the indicated time points of age. Muscle and spleen were  
649 digested with collagenase, mononuclear cells were isolated using a density gradient and  
650 analyzed by cytofluorimetry. **A)** Number of viable CD45<sup>+</sup>TCR<sup>-</sup>CD45RA<sup>-</sup>SIRP $\alpha$ <sup>+</sup> cells per  
651 gram of muscle (left panel) or whole spleen (right panel) at different time points. WT, n=4, 5,  
652 7, 7, 9 at 2, 4, 8, 12 and 16 weeks, respectively; *Dmd*<sup>mdx</sup>, n=3, 6, 10, 11, 16 at 2, 4, 8, 12 and  
653 16 weeks, respectively. \*\* p< 0.01, and \*\*\* p< 0.001. **B)** Representative dot-plot analysis of  
654 viable SSC CD45<sup>+</sup>TCR<sup>-</sup>CD45RA<sup>-</sup>SIRP $\alpha$ <sup>+</sup> cells mononuclear leukocytes from muscle (left  
655 panels) or spleen (right panels) from animals at 12 weeks of age.

656

657 **Supplementary figure 2. CK in sera of *Dmd*<sup>mdx</sup> rats.** CK levels were determined  
658 simultaneously in all samples. WT (n=8, 6, 6, 9, 5 at 2, 4, 8, 12 and 16 weeks, respectively),  
659 *Dmd*<sup>mdx</sup> (n=5, 4, 8, 8, 6 at 2, 4, 8, 12 and 16 weeks, respectively). \* p<0.05.

660

661 **Supplementary figure 3. Expression profiles of CD45RC in different mononuclear cell**  
662 **populations.** Hind limb muscles and spleen were harvested from littermate wild-type (WT) or  
663 *Dmd*<sup>mdx</sup> (KO) rats at the indicated time points of age. Muscle and spleen were digested with  
664 collagenase, mononuclear cells were isolated using a density gradient and analyzed by  
665 cytofluorimetry. **A)** Absolute numbers of CD45<sup>+</sup>CD45RA<sup>-</sup>TCR<sup>+</sup>CD8<sup>+</sup>CD45RC<sup>high</sup> cells in  
666 muscle or spleen of WT or *Dmd*<sup>mdx</sup> rats during time. **B)** Representative dot-plot analysis of  
667 viable CD45<sup>+</sup>CD45RA<sup>-</sup>TCR<sup>+</sup>CD8<sup>+</sup>CD45RC<sup>high</sup> or <sup>low/-</sup> mononuclear leukocytes from muscle  
668 (left panels) or spleen (right panels) of WT or *Dmd*<sup>mdx</sup> rats from animals at 12 weeks of age.  
669 **C)** Absolute numbers of CD45<sup>+</sup>CD45RA<sup>-</sup>TCR<sup>+</sup>CD4<sup>+</sup>CD45RC<sup>low/-</sup> cells in muscle of spleen of  
670 WT or *Dmd*<sup>mdx</sup> rats during time. **D)** Representative dot-plot analysis of viable

671 CD45<sup>+</sup>CD45RA<sup>-</sup>TCR<sup>+</sup>CD4<sup>+</sup>CD45RC<sup>high or low/-</sup> mononuclear leukocytes from muscle (left  
672 panels) or spleen (right panels) of WT or *Dmd*<sup>mdx</sup> rats from animals at 12 weeks of age. **E)**  
673 Absolute numbers of CD45<sup>+</sup>CD45RA<sup>-</sup>TCR<sup>+</sup>CD4<sup>+</sup>CD45RC<sup>high</sup> cells in muscle of spleen of WT  
674 or *Dmd*<sup>md</sup> rats during time. **F)** Absolute numbers of CD45<sup>+</sup>TCR<sup>-</sup>CD45RC<sup>low/-</sup> cells in muscle  
675 of spleen of WT or *Dmd*<sup>md</sup> rats during time. **G)** Representative dot-plot analysis of viable  
676 CD45<sup>+</sup>TCR<sup>-</sup>CD45RC<sup>high or low/-</sup> mononuclear leukocytes from muscle (left panels) or spleen  
677 (right panels) from animals at 12 weeks of age. **H)** Absolute numbers of CD45<sup>+</sup>TCR<sup>-</sup>  
678 CD45RC<sup>high</sup> cells in muscle of spleen of WT or *Dmd*<sup>mdx</sup> rats during time.

679

680

681 **Supplementary figure 4. Effects of anti-CD45RC or prednisolone treatments on muscle**

682 **fibrosis and serum CK levels.** Littermate wild-type (WT) or *Dmd*<sup>mdx</sup> (KO) rats were treated

683 with anti-CD45RC or prednisolone since week 2 of age. **A)** *Biceps femoris* muscles were

684 harvested at 12 weeks of age, fixed and paraffin embedded, connective/fibrotic tissue was

685 stained with picosirius for connective tissue and the stained surface was quantified and

686 expressed as the percentage of total area of the tissue analyzed (47 mm<sup>2</sup>). WT isotype control,

687 n=6; WT anti-CD45RC, n=3; KO isotype control, n=6; KO anti-CD45RC, n=3; KO

688 prednisolone, n=6. \* p<0.05 vs. WT controls and anti-CD45RC-treated animals. **B)**

689 Representative picosirius (purple) staining for animals of the indicated group treatments. **C)**

690 (left panel) Sera of *Dmd*<sup>mdx</sup> and WT rats treated with prednisolone or vehicle (NaCl) were

691 collected at 12 weeks of age and CK levels were determined simultaneously in all samples.

692 WT NaCl, n=3; WT prednisolone, n=3; KO NaCl, n=5; KO prednisolone, n=6. (right panel)

693 Sera of *Dmd*<sup>mdx</sup> and WT rats treated with anti CD45RC or isotype control were collected at 4,

694 8 12 and 16 weeks of age and CK levels were determined simultaneously in all samples. WT

695 isotype control (n=12, 4, 11, 4 at 4, 8, 12 and 16 weeks of age, respectively); WT anti

696 CD45RC (n=13, 8, 11, 3 at 4, 8, 12 and 16 weeks of age, respectively); KO isotype control  
697 (n= 6, 4, 14, 3 at 4, 8, 12 and 16 weeks of age, respectively); KO anti CD45RC (n=8, 5, 13, 4  
698 at 4, 8, 12 and 16 weeks of age, respectively).

699

700 **Supplementary figure 5. Cytofluorimetry analyses of CD45RC expression in blood T**  
701 **cells of DMD patients and controls.** Human peripheral blood was drawn, red blood cells  
702 were lysed and white blood cells were incubated with a viability dye and MAbs directly  
703 coupled with the indicated fluorochromes defining CD3, CD4, CD8 and CD45RC or isotype  
704 controls followed by cytofluorimetry analyzes. Ordinate depict reactivity with anti-CD45RC  
705 MAb or isotype control and the boxes define CD45RC<sup>high</sup> or CD45RC<sup>low/-</sup> cells. Abscissa  
706 depict reactivity with anti-CD4 or CD8 MAbs among CD3<sup>+</sup> cells. Controls were young  
707 patients (6-17 years-old) comparable in age to DMD patients and that were hospitalized for  
708 pathologies not involving the immune or the neuromuscular systems.

709

710 **Supplementary figure 6. Cytofluorimetry analyses of CD45RC expression in blood non-**  
711 **T cells of DMD patients and controls.** Human peripheral blood was drawn, red blood cells  
712 were lysed and white blood cells were incubated with a viability dye and MAbs directly  
713 coupled with the indicated fluorochromes defining CD14<sup>+</sup> monocytes, CD19<sup>+</sup> B cells,  
714 CD16+56<sup>+</sup> NK cells and CD45RC<sup>high</sup> cells or isotype controls followed by cytofluorimetry  
715 analyzes. Only one DMD and one control patients are showed as representative example.  
716 Ordinate depict reactivity with anti-CD45RC MAb or isotype control and the boxes define  
717 CD45RC<sup>high</sup> cells. Abscissa depict reactivity with anti-CD14, anti-CD19 or anti-CD16+56  
718 MAbs. Controls were young patients (6-17 years-old) comparable in age to DMD patients and  
719 that were hospitalized for pathologies not involving the immune or the neuromuscular  
720 systems.

721



722 **REFERENCES.**

723

724 [1] Emery AE: The muscular dystrophies. *Lancet* 2002, 359:687-95.

725 [2] Chamberlain JS, Metzger J, Reyes M, Townsend D, Faulkner JA: Dystrophin-deficient  
726 mdx mice display a reduced life span and are susceptible to spontaneous rhabdomyosarcoma.  
727 *FASEB J* 2007, 21:2195-204.

728 [3] Larcher T, Lafoux A, Tesson L, Remy S, Thepenier V, Francois V, Le Guiner C, Goubin  
729 H, Dutilleul M, Guigand L, Toumaniantz G, De Cian A, Boix C, Renaud JB, Cherel Y,  
730 Giovannangeli C, Concordet JP, Anegon I, Huchet C: Characterization of dystrophin deficient  
731 rats: a new model for Duchenne muscular dystrophy. *PLoS ONE* 2014, 9:e110371.

732 [4] Robertson AS, Majchrzak MJ, Smith CM, Gagnon RC, Devidze N, Banks GB, Little SC,  
733 Nabbie F, Bounous DI, DiPiero J, Jacobsen LK, Bristow LJ, Ahlijanian MK, Stimpson SA:  
734 Dramatic elevation in urinary amino terminal titin fragment excretion quantified by  
735 immunoassay in Duchenne muscular dystrophy patients and in dystrophin deficient rodents.  
736 *Neuromuscul Disord* 2017, 27:635-45.

737 [5] Rosenberg AS, Puig M, Nagaraju K, Hoffman EP, Villalta SA, Rao VA, Wakefield LM,  
738 Woodcock J: Immune-mediated pathology in Duchenne muscular dystrophy. *Sci Transl Med*  
739 2015, 7:299rv4.

740 [6] Alman BA: Duchenne muscular dystrophy and steroids: pharmacologic treatment in the  
741 absence of effective gene therapy. *Journal of pediatric orthopedics* 2005, 25:554-6.

742 [7] Gloss D, Moxley RT, 3rd, Ashwal S, Oskoui M: Practice guideline update summary:  
743 Corticosteroid treatment of Duchenne muscular dystrophy: Report of the Guideline

744 Development Subcommittee of the American Academy of Neurology. *Neurology* 2016,  
745 86:465-72.

746 [8] Mendell JR, Campbell K, Rodino-Klapac L, Sahenk Z, Shilling C, Lewis S, Bowles D,  
747 Gray S, Li C, Galloway G, Malik V, Coley B, Clark KR, Li J, Xiao X, Samulski J, McPhee  
748 SW, Samulski RJ, Walker CM: Dystrophin immunity in Duchenne's muscular dystrophy. *N*  
749 *Engl J Med* 2010, 363:1429-37.

750 [9] Gussoni E, Pavlath GK, Miller RG, Panzara MA, Powell M, Blau HM, Steinman L:  
751 Specific T cell receptor gene rearrangements at the site of muscle degeneration in Duchenne  
752 muscular dystrophy. *J Immunol* 1994, 153:4798-805.

753 [10] Flanigan KM, Campbell K, Violette L, Wang W, Gomez AM, Walker CM, Mendell JR:  
754 Anti-dystrophin T cell responses in Duchenne muscular dystrophy: prevalence and a  
755 glucocorticoid treatment effect. *Hum Gene Ther* 2013, 24:797-806.

756 [11] Burzyn D, Kuswanto W, Kolodin D, Shadrach JL, Cerletti M, Jang Y, Sefik E, Tan TG,  
757 Wagers AJ, Benoist C, Mathis D: A special population of regulatory T cells potentiates  
758 muscle repair. *Cell* 2013, 155:1282-95.

759 [12] Villalta SA, Rosenthal W, Martinez L, Kaur A, Sparwasser T, Tidball JG, Margeta M,  
760 Spencer MJ, Bluestone JA: Regulatory T cells suppress muscle inflammation and injury in  
761 muscular dystrophy. *Sci Transl Med* 2014, 6:258ra142.

762 [13] Bezie S, Meistermann D, Boucault L, Kilens S, Zoppi J, Autrusseau E, Donnart A,  
763 Nerriere-Daguin V, Bellier-Waast F, Charpentier E, Duteille F, David L, Anegon I,  
764 Guillonneau C: Ex Vivo Expanded Human Non-Cytotoxic CD8(+)/CD45RC(low/-) Tregs  
765 Efficiently Delay Skin Graft Rejection and GVHD in Humanized Mice. *Front Immunol* 2018,  
766 8:2014.

- 767 [14] Picarda E, Bezie S, Boucault L, Autrusseau E, Kilens S, Meistermann D, Martinet B,  
768 Daguin V, Donnart A, Charpentier E, David L, Anegon I, Guillonneau C: Transient antibody  
769 targeting of CD45RC induces transplant tolerance and potent antigen-specific regulatory T  
770 cells. *JCI insight* 2017, 2:e90088.
- 771 [15] Guillonneau C, Hill M, Hubert FX, Chiffolleau E, Herve C, Li XL, Heslan M, Usal C,  
772 Tesson L, Menoret S, Saoudi A, Le Mauff B, Josien R, Cuturi MC, Anegon I: CD40Ig  
773 treatment results in allograft acceptance mediated by CD8CD45RC T cells, IFN-gamma, and  
774 indoleamine 2,3-dioxygenase. *J Clin Invest* 2007, 117:1096-106.
- 775 [16] Picarda E, Bezie S, Venturi V, Echasserieau K, Merieau E, Delhumeau A, Renaudin K,  
776 Brouard S, Bernardeau K, Anegon I, Guillonneau C: MHC-derived allopeptide activates  
777 TCR-biased CD8+ Tregs and suppresses organ rejection. *J Clin Invest* 2014, 124:2497-512.
- 778 [17] Pedros C, Papapietro O, Colacios C, Casemayou A, Bernard I, Garcia V, Lagrange D,  
779 Mariame B, Andreoletti O, Fournie GJ, Saoudi A: Genetic control of HgCl<sub>2</sub>-induced IgE and  
780 autoimmunity by a 117-kb interval on rat chromosome 9 through CD4 CD45RChigh T cells.  
781 *Genes Immun* 2013, 14:258-67.
- 782 [18] Zschuntzsch J, Zhang Y, Klinker F, Makosch G, Klinge L, Malzahn D, Brinkmeier H,  
783 Liebetanz D, Schmidt J: Treatment with human immunoglobulin G improves the early disease  
784 course in a mouse model of Duchenne muscular dystrophy. *J Neurochem* 2016, 136:351-62.
- 785 [19] Grounds MD, Torrasi J: Anti-TNFalpha (Remicade) therapy protects dystrophic skeletal  
786 muscle from necrosis. *FASEB J* 2004, 18:676-82.
- 787 [20] Pelosi L, Berardinelli MG, De Pasquale L, Nicoletti C, D'Amico A, Carvello F, Moneta  
788 GM, Catizone A, Bertini E, De Benedetti F, Musaro A: Functional and Morphological

789 Improvement of Dystrophic Muscle by Interleukin 6 Receptor Blockade. *EBioMedicine* 2015,  
790 2:285-93.

791 [21] Swiderski K, Todorov M, Gehrig SM, Naim T, Chee A, Stapleton DI, Koopman R,  
792 Lynch GS: Tranilast administration reduces fibrosis and improves fatigue resistance in  
793 muscles of mdx dystrophic mice. *Fibrogenesis & tissue repair* 2014, 7:1.

794 [22] Sun CC, Li SJ, Yang CL, Xue RL, Xi YY, Wang L, Zhao QL, Li DJ: Sulforaphane  
795 Attenuates Muscle Inflammation in Dystrophin-deficient mdx Mice via NF-E2-related Factor  
796 2 (Nrf2)-mediated Inhibition of NF-kappaB Signaling Pathway. *J Biol Chem* 2015,  
797 290:17784-95.

798 [23] Benny Klimek ME, Sali A, Rayavarapu S, Van der Meulen JH, Nagaraju K: Effect of the  
799 IL-1 Receptor Antagonist Kineret(R) on Disease Phenotype in mdx Mice. *PLoS One* 2016,  
800 11:e0155944.

801 [24] Evans NP, Misyak SA, Robertson JL, Bassaganya-Riera J, Grange RW: Immune-  
802 mediated mechanisms potentially regulate the disease time-course of duchenne muscular  
803 dystrophy and provide targets for therapeutic intervention. *PM & R : the journal of injury,*  
804 *function, and rehabilitation* 2009, 1:755-68.

805 [25] Bleakley M, Heimfeld S, Loeb KR, Jones LA, Chaney C, Seropian S, Gooley TA,  
806 Sommermeyer F, Riddell SR, Shlomchik WD: Outcomes of acute leukemia patients  
807 transplanted with naive T cell-depleted stem cell grafts. *J Clin Invest* 2015, 125:2677-89.

808 [26] Andreetta F, Bernasconi P, Baggi F, Ferro P, Oliva L, Arnoldi E, Cornelio F,  
809 Mantegazza R, Confalonieri P: Immunomodulation of TGF-beta 1 in mdx mouse inhibits  
810 connective tissue proliferation in diaphragm but increases inflammatory response:  
811 implications for antifibrotic therapy. *J Neuroimmunol* 2006, 175:77-86.

- 812 [27] Bezie S, Anegon I, Guillonneau C: Advances on CD8+ Tregs and their potential in  
813 transplantation. *Transplantation* 2018.
- 814 [28] Kolodin D, van Panhuys N, Li C, Magnuson AM, Cipolletta D, Miller CM, Wagers A,  
815 Germain RN, Benoist C, Mathis D: Antigen- and cytokine-driven accumulation of regulatory  
816 T cells in visceral adipose tissue of lean mice. *Cell metabolism* 2015, 21:543-57.
- 817 [29] Gregori S, Mangia P, Bacchetta R, Tresoldi E, Kolbinger F, Traversari C, Carballido JM,  
818 de Vries JE, Korthauer U, Roncarolo MG: An anti-CD45RO/RB monoclonal antibody  
819 modulates T cell responses via induction of apoptosis and generation of regulatory T cells. *J*  
820 *Exp Med* 2005, 201:1293-305.
- 821 [30] Lazarovits AI, Poppema S, Zhang Z, Khandaker M, Le Feuvre CE, Singhal SK, Garcia  
822 BM, Ogasa N, Jevnikar AM, White MH, Singh G, Stiller CR, Zhong RZ: Prevention and  
823 reversal of renal allograft rejection by antibody against CD45RB. *Nature* 1996, 380:717-20.
- 824 [31] Spencer MJ, Montecino-Rodriguez E, Dorshkind K, Tidball JG: Helper (CD4(+)) and  
825 cytotoxic (CD8(+)) T cells promote the pathology of dystrophin-deficient muscle. *Clin*  
826 *Immunol* 2001, 98:235-43.
- 827 [32] Menoret S, Fontaniere S, Jantz D, Tesson L, Thinard R, Remy S, Usal C, Ouisse LH,  
828 Fraichard A, Anegon I: Generation of Rag1-knockout immunodeficient rats and mice using  
829 engineered meganucleases. *Faseb J* 2013, 27:703-11.
- 830 [33] Bezie S, Picarda E, Tesson L, Renaudin K, Durand J, Menoret S, Merieau E, Chiffolleau  
831 E, Guillonneau C, Caron L, Anegon I: Fibrinogen-like protein 2/fibroleukin induces long-  
832 term allograft survival in a rat model through regulatory B cells. *PLoS ONE* 2015,  
833 10:e0119686.

834 [34] Fisher I, Abraham D, Bouri K, Hoffman EP, Muntoni F, Morgan J: Prednisolone-induced  
835 changes in dystrophic skeletal muscle. *FASEB J* 2005, 19:834-6.

836 [35] Matthews E, Brassington R, Kuntzer T, Jichi F, Manzur AY: Corticosteroids for the  
837 treatment of Duchenne muscular dystrophy. *Cochrane Database Syst Rev* 2016:CD003725.

838

839

840

841

842 **Table 1**

843 Primer sequences

| Primer name    | Sequence                 | 844 |
|----------------|--------------------------|-----|
| Arg-1 F        | CCA ACTCTTGGGAAGACACCA   |     |
| Arg-1 R        | GTGATGCCCCAGATGACTTT     |     |
| iNOS F         | GACCAA ACTGTGTGCCTGGA    |     |
| iNOS R         | TACTCTGAGGGCTGACACAAGG   |     |
| HO-1 F         | CCACAGCTCGACAGCATGTC     |     |
| HO-1 R         | GTTTCGCTCTATCTCCTCTTCCA  |     |
| IFN $\gamma$ F | AGTGTCATCGAATCGCACCTG    |     |
| IFN $\gamma$ R | TTCTGGTGACAGCTGGTGAAT    |     |
| IL6 F          | GCAAGAGACTTCCAGCCAGTT    |     |
| IL6 R          | CATCATCGCTGTTCATAACAATCA |     |
| TNF $\alpha$ F | CTTCTCATTCTGCTCGTGG      |     |
| TNF $\alpha$ R | GCTACGGGCTTGTCACCTCG     |     |
| TGF $\beta$ F  | CTCAACACCTGCACAGCTCC     |     |
| TGF $\beta$ R  | ACGATCATGTTGGACA ACTGCT  |     |
| IL-10 F        | TGCTATGTTGCCTGCTCTTACTG  |     |
| IL-10R         | TCAAATGCTCCTTGATTCTGG    |     |
| IL-34 F        | CTGGCTGTCCTCTACCCTGA     |     |
| IL-34 R        | TGTCGTGGCAAGATATGGCAA    |     |
| Areg F         | AGATCGCGTTAGCAGCCATAA    |     |
| Areg R         | TCAGCTAGGCTATGGCATGTG    |     |
| rIl-1b Fw2     | ACCTGTCCTGTGTGATGAAAGACG |     |
| rIl-1b Rev2    | CTGCTTGAGAGGTGCTGATG     |     |
| IDO F          | GCTGCCTCCCATTCTGTCTT     |     |
| IDO R          | TGCGATTTCCACCATTAGAGAG   |     |
| Rln3 F         | CTGCGGTCGGGAGTTCATC      |     |
| RIN3 R         | CCAGGTGGTCTGTATTGGCTT    |     |
| rRLN3-Fw2      | GACATCTTGGCCCACGACCCTCT  |     |
| rRLN3-Rev2     | CTCTGCTGCCCCGAACCACTCCG  |     |

Figure 1. Number of leucocytes in skeletal muscles and spleens of *Dmd<sup>mdx</sup>* rats.

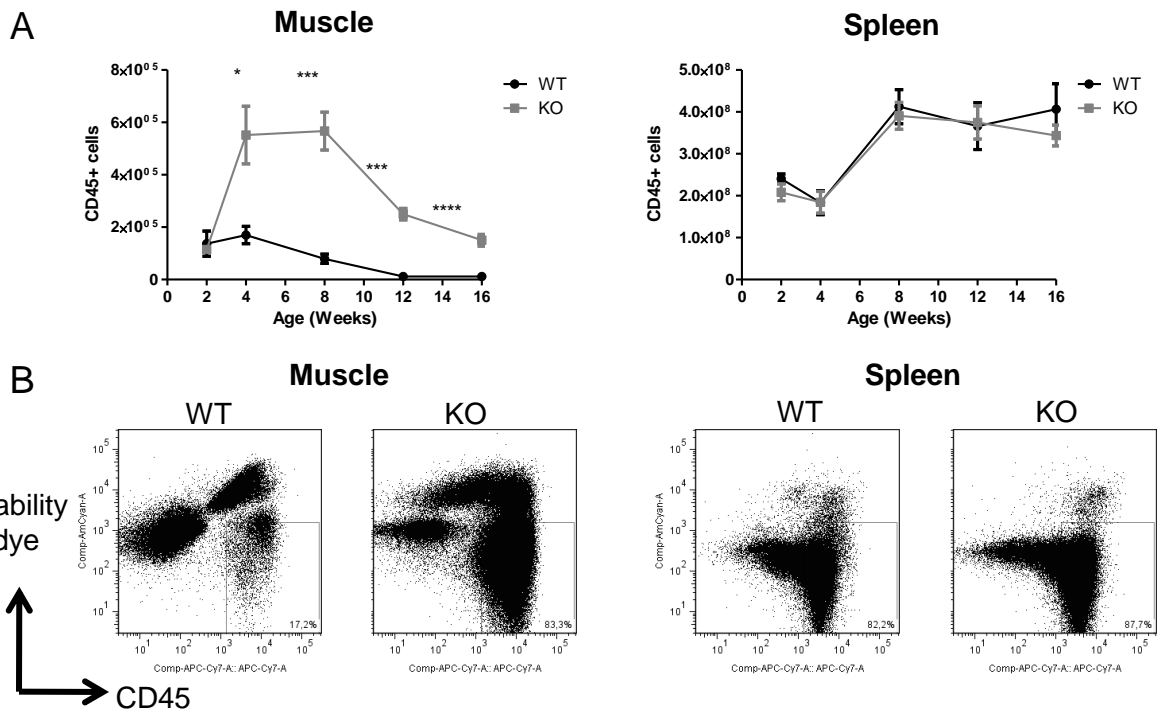




Figure 2. Macrophages in skeletal muscles and spleens of *Dmd<sup>mdx</sup>* rats.

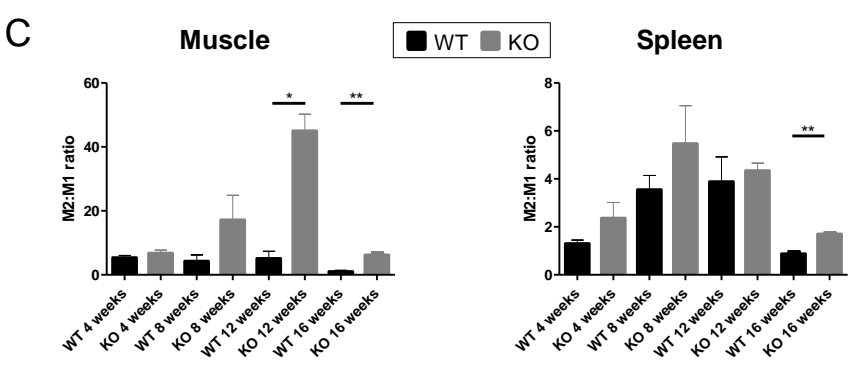
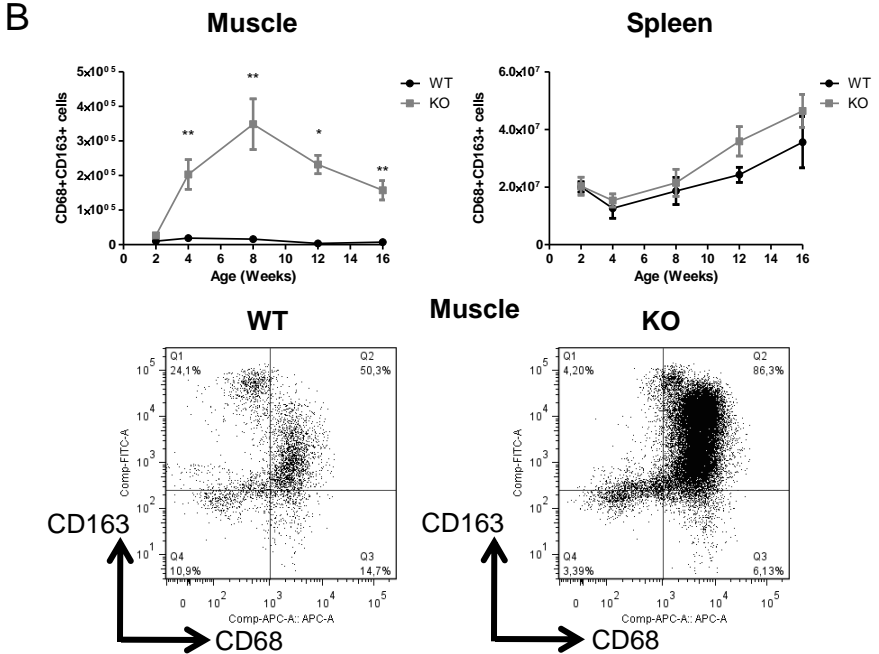
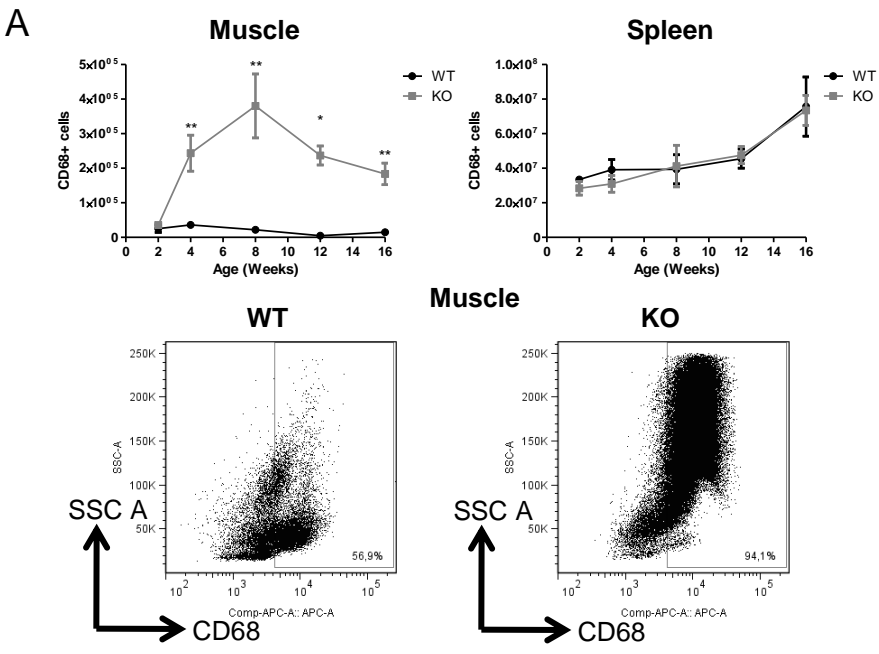


Figure 3. T cells in skeletal muscle and spleens of *Dmd<sup>mdx</sup>* rats.

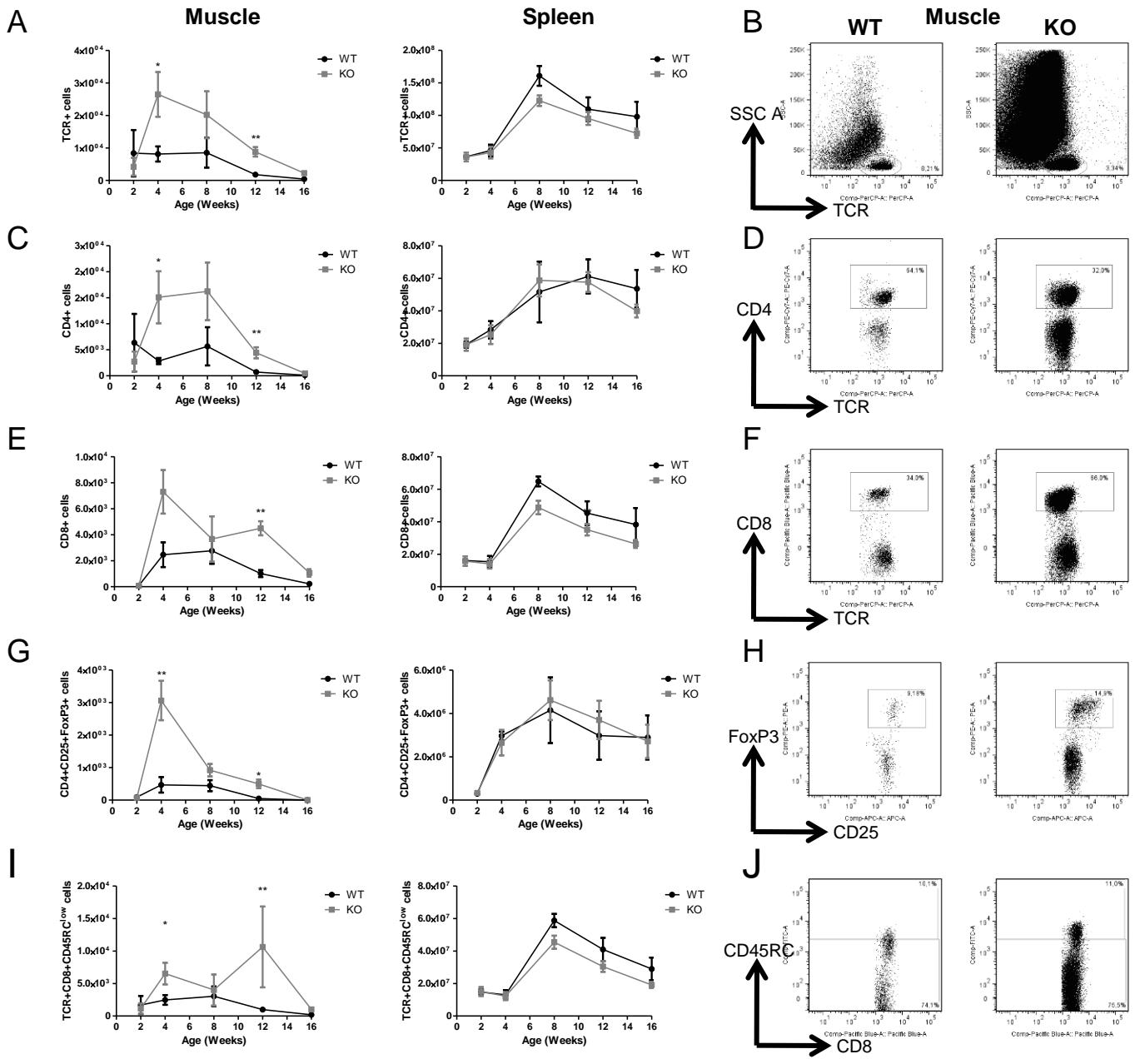


Figure 4. Immunohistochemical detection of leukocytes in skeletal and cardiac muscles of *Dmd<sup>mdx</sup>* rats.

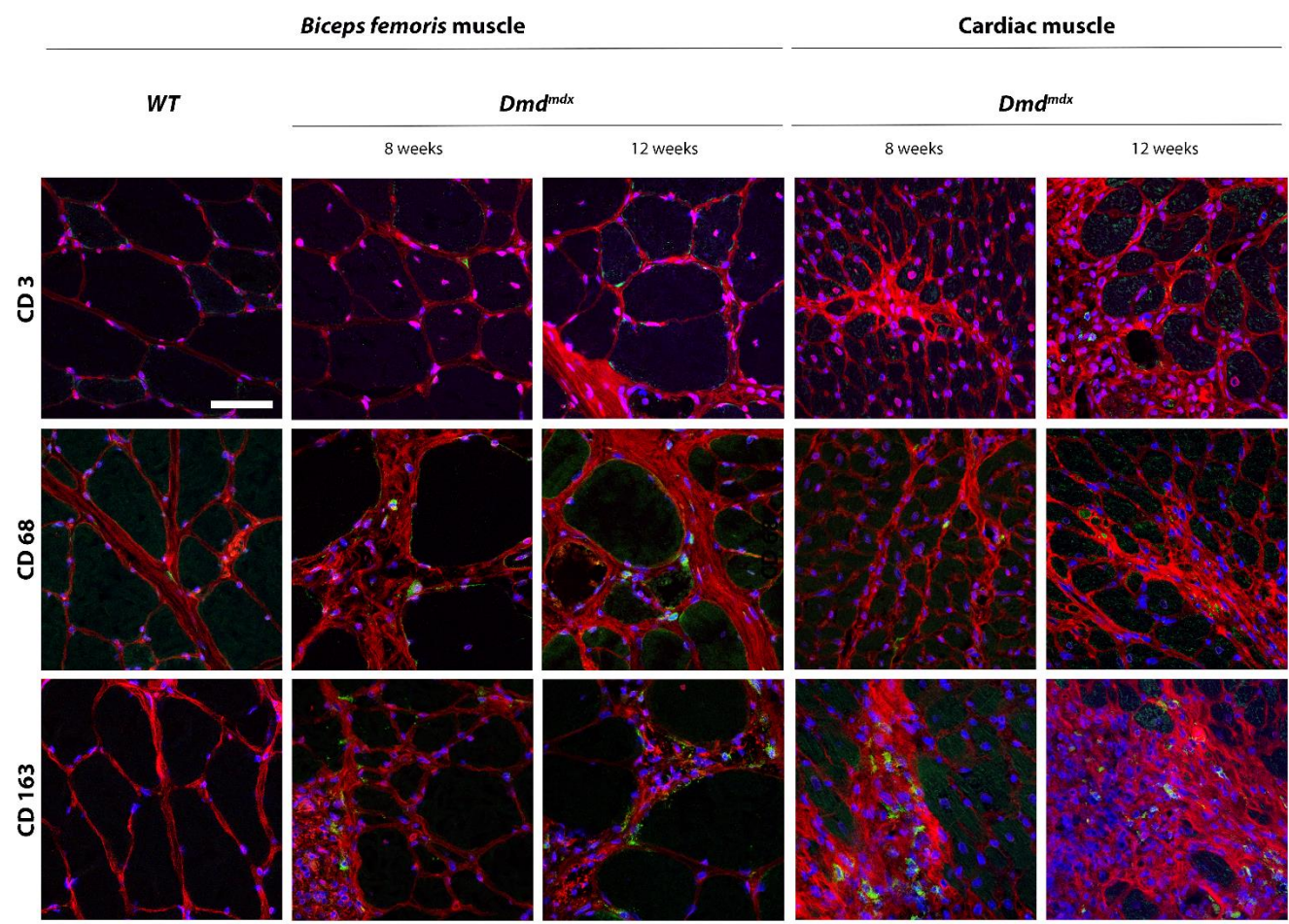
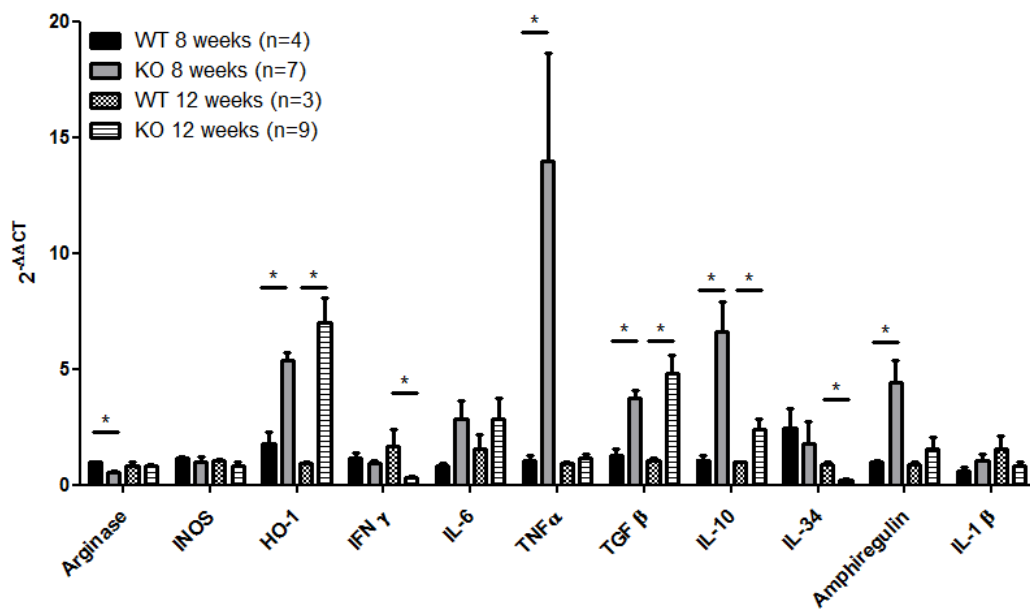


Figure 5. Inflammation markers and growth factors in skeletal muscles of *Dmd<sup>mdx</sup>* rats.

A



B

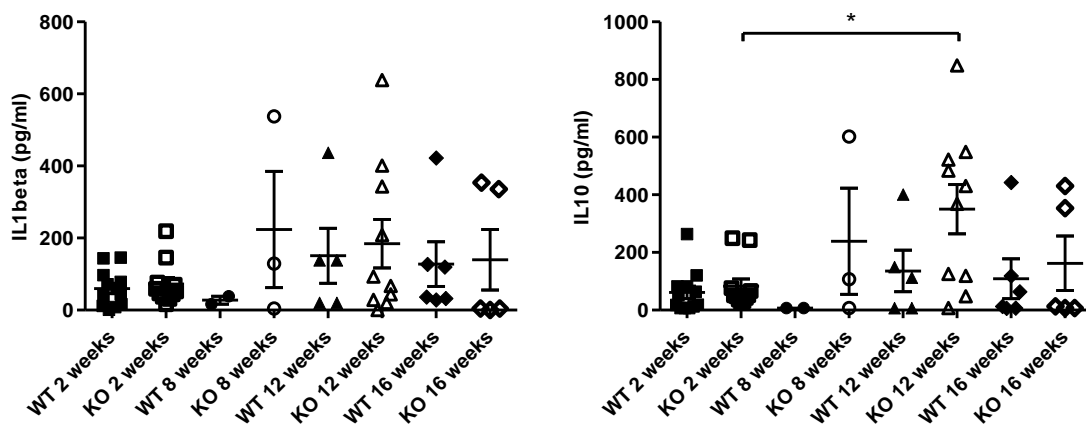


Figure 6. Effect of treatment with anti-CD45RC on lymphoid cell populations, forelimb muscle strength and animal growth.

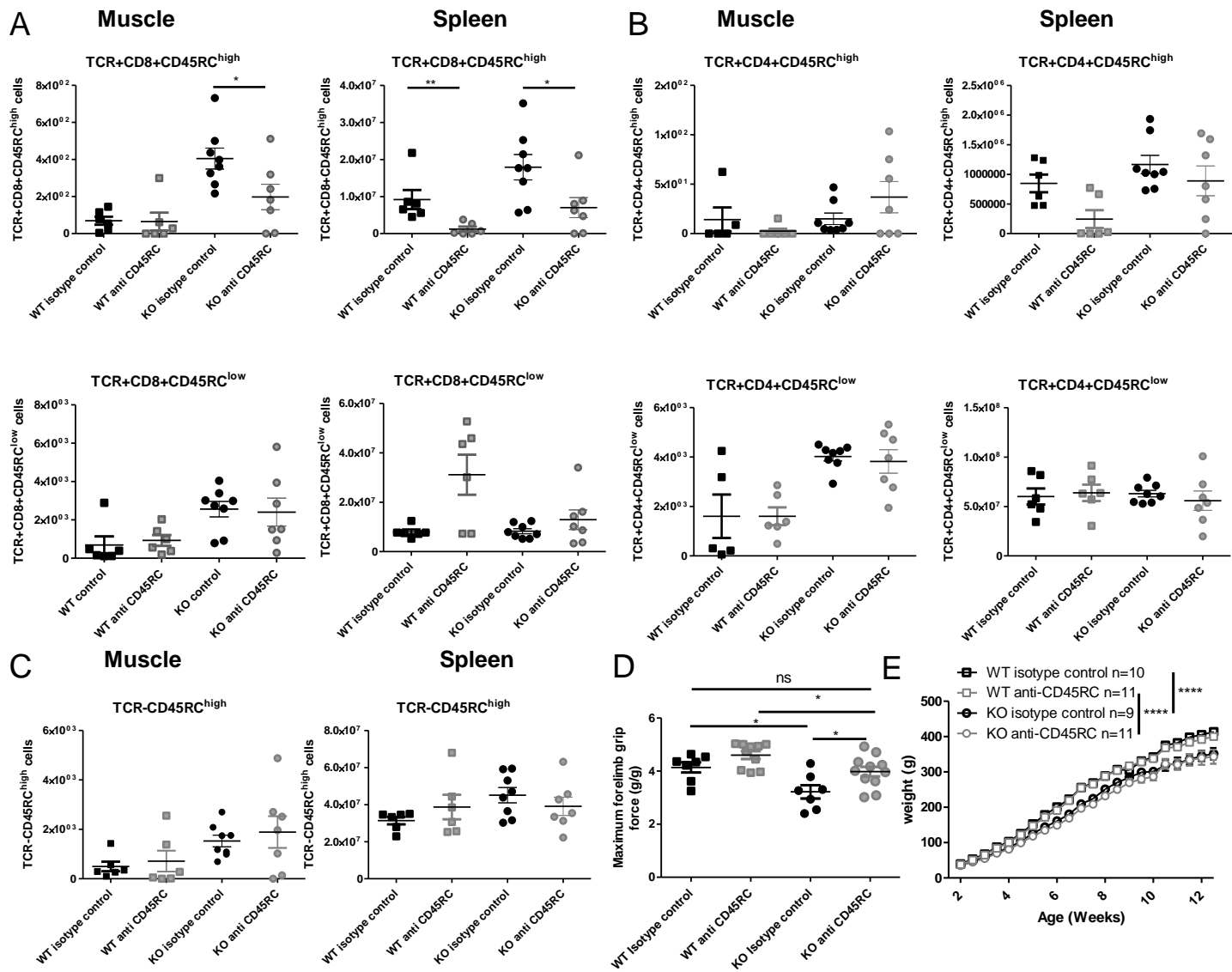


Figure 7. Treatment with prednisolone on lymphoid cell populations and forelimb muscle strength.

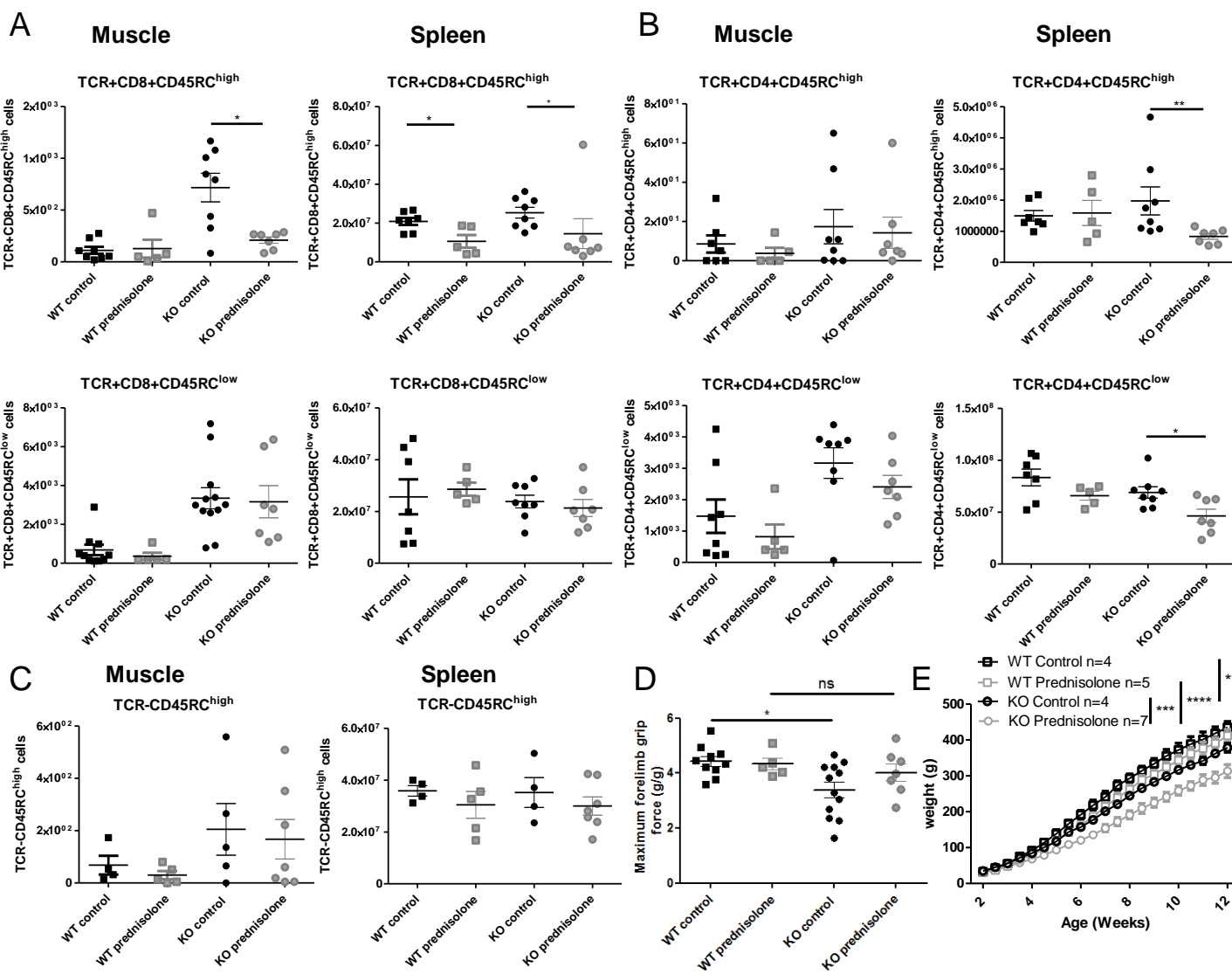


Figure 8. CD45RC<sup>+</sup> cells in rat and human dystrophin-deficient skeletal muscles.

