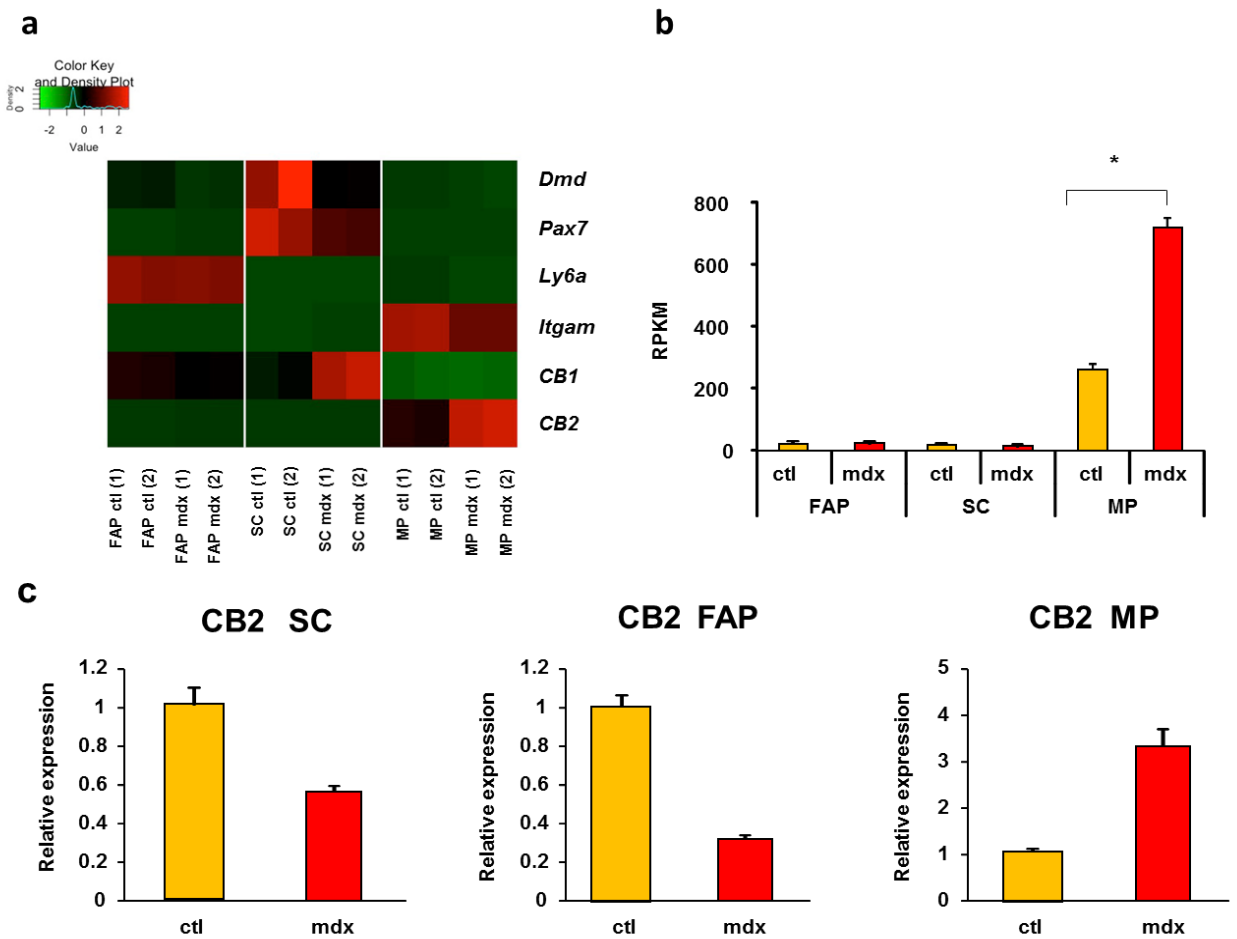


SUPPLEMENTARY INFORMATION

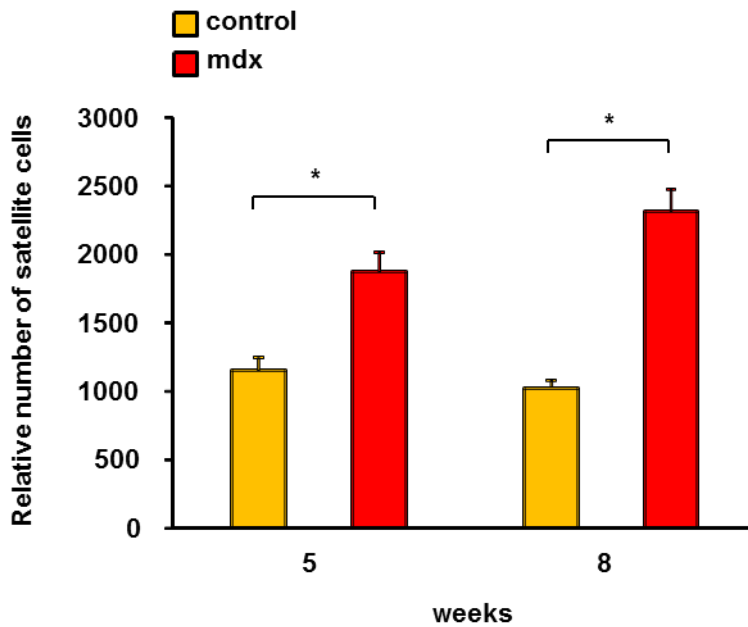
Genetic and pharmacological regulation of the endocannabinoid CB1 receptor in Duchenne Muscular Dystrophy

Authors:

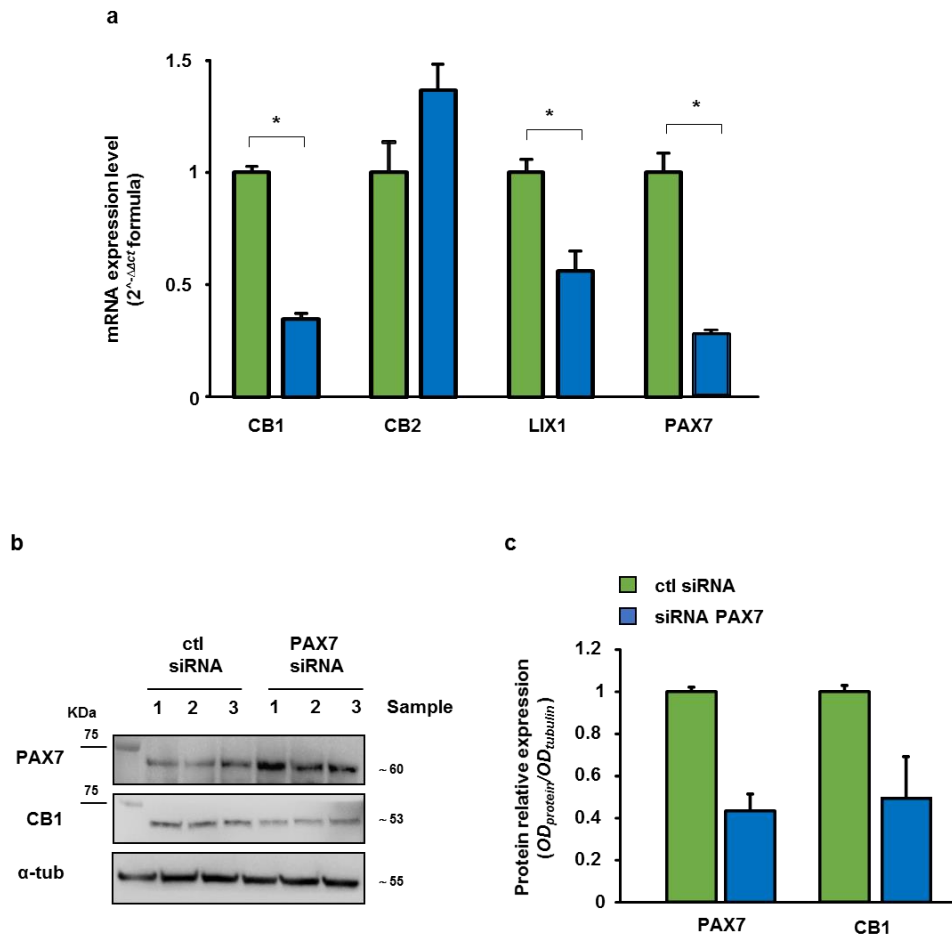
Fabio A. Iannotti, Ester Pagano, Ombretta Guardiola, Simone Adinolfi, Valentina Saccone, Silvia Consalvi, Fabiana Piscitelli, Elisabetta Gazzero, Giuseppe Busetto, Diego Carrella, Raffaele Capasso, Pier Lorenzo Puri, Gabriella Minchiotti and Vincenzo Di Marzo



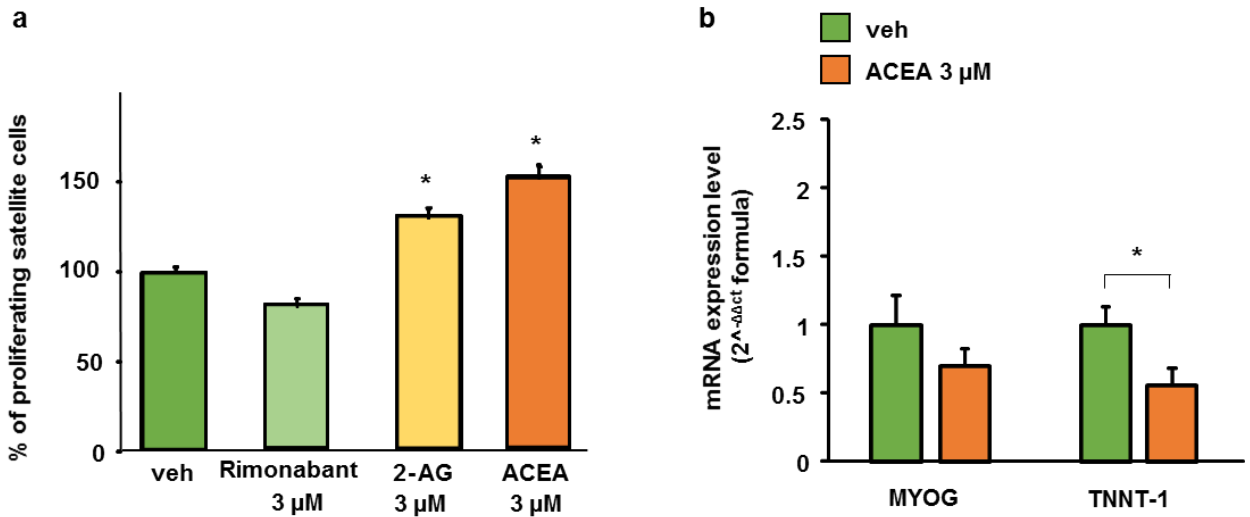
Supplementary Figure 1 Expression of CB2 in skeletal muscle resident cells of mdx mice. **a** Heatmap representation of selected genes obtained from RNA-seq analysis in fibroadipogenic cells (FAP), satellite cells (SC) and macrophages (MP) isolated from 8-weeks old wt (n=4) and mdx (n=4) mice. Red, upregulated; green, downregulated **(B)** Bar Graph showing RPKM (Reads Per Kilobase of transcript per Million mapped reads) normalized values for the *Cnr2* gene obtained from RNA-seq analysis in FAP, SC and MP isolated from 8-weeks old wt and mdx mice. *P ≤ 0.05 vs control animals, determined by Student's t test. **(C)** qPCR validation data.



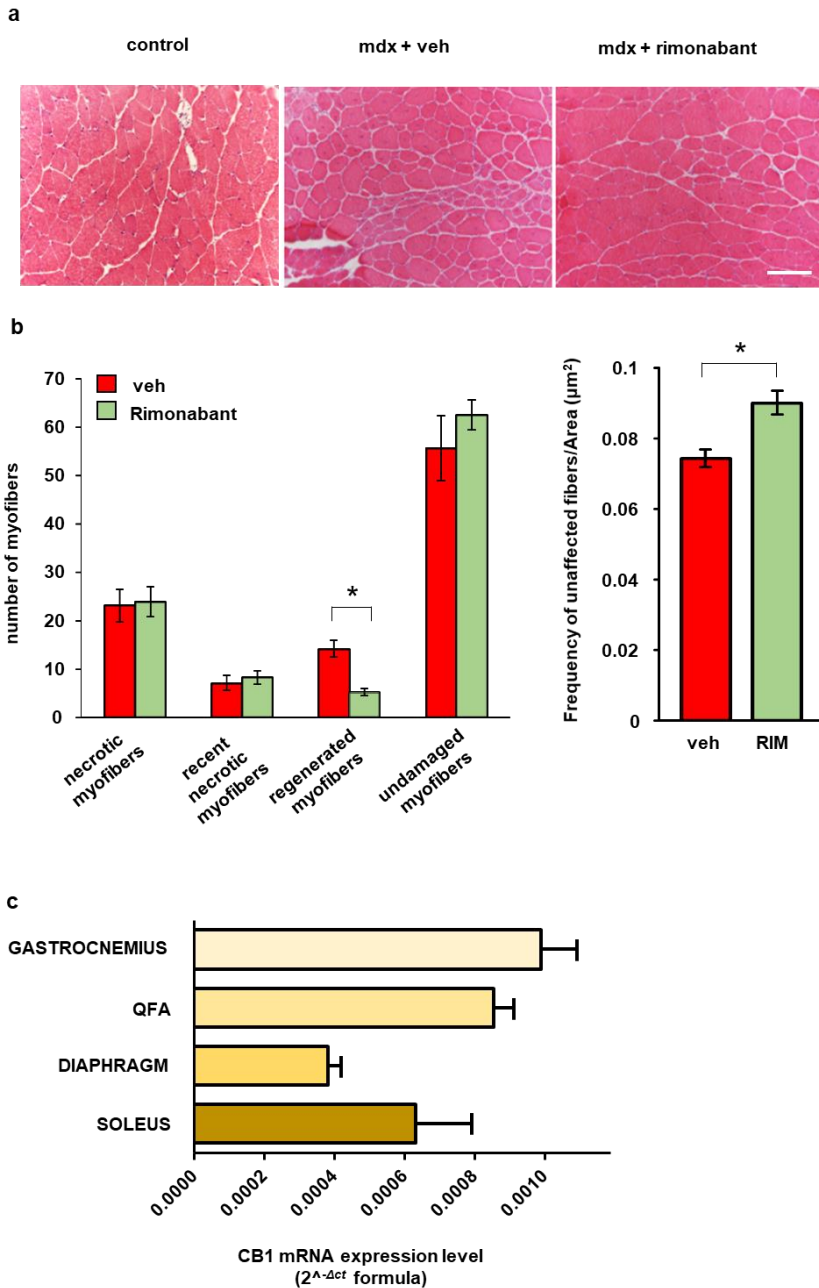
Supplementary Figure 2 Relative number of satellite cells in control and mdx muscles. Bar graph showing the number of satellite cells found in the gastrocnemius muscles of control and mdx mice. Each bar is the mean \pm SEM of four independent determinations. * $P \leq 0.05$ vs control animals, determined by Student's t test.



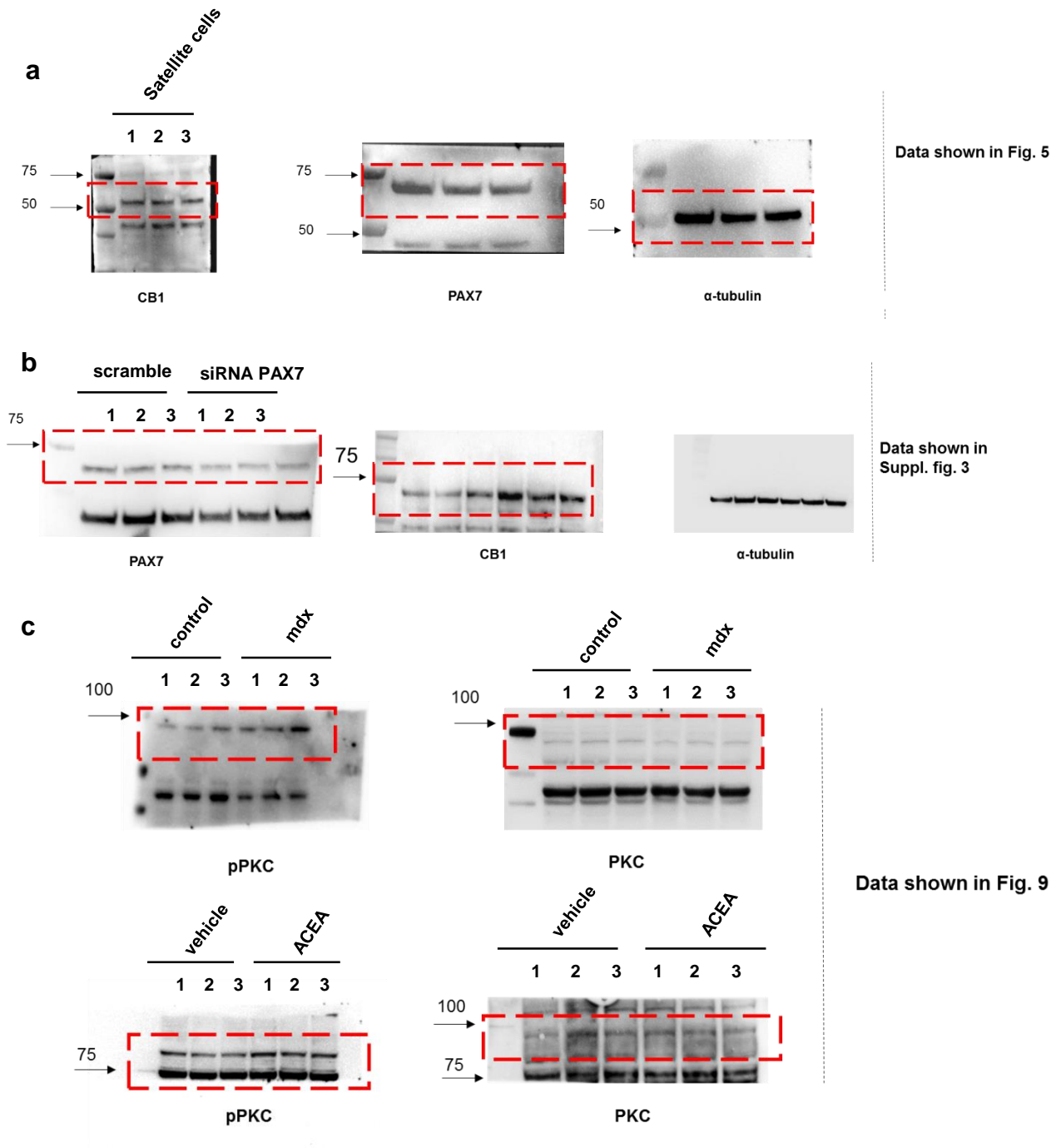
Supplementary Figure 3 CB1 mRNA expression levels in PAX7-silenced satellite cells. **a** The quantification of CB1, CB2, LIX1 and PAX7 transcript levels was performed by quantitative real-time PCR. **b** Representative blots showing the chemiluminescent signal generated by the anti-CB1 or anti-PAX7 antibody in control (scramble) and PAX7-silenced satellite cells. **(c)** Quantification of CB1 and PAX7 protein expression levels normalized to α -tubulin. Each bar is the mean \pm SEM of five separate determinations. * $P \leq 0.05$ vs the control group, determined by Student's t test.



Supplementary Figure 4 Pharmacological role of CB1 in human satellite cells
a Effects of rimonabant (3 μ M), 2-AG (3 μ M) and ACEA ((3 μ M) on satellite cell proliferation (n=4) assessed by incorporation of [3 H] thymidine. **b** Effect of ACEA on satellite cell differentiation. The quantification of myogenin (MYOG) and troponin-1 (TNNT-1) transcripts was performed by quantitative real-time PCR. Each bar is the mean \pm S.E.M. of six separate determinations. *P \leq 0.05 vs the control group, determined by Student's t test.



Supplementary Figure 5 Histological analysis in mdx mice treated with rimonabant. **a** Representative photomicrographs of H&E-stained transverse sections at of quadriceps muscle isolated from wild-type (wt, n=6), mdx mice treated with vehicle (DMSO, n=6) or rimonabant 0.5 mg Kg⁻¹ (n=6) from week 5 to week 7 of age. Scale bars = 100 µm. **b** *Left* The bar graph indicates the number of necrotic, partly regenerated (recently necrotic), fully regenerated and undamaged myofibers in control (DMSO) and rimonabant-treated mdx mice. *Right* The bar graph indicates the number (frequency) of unaffected fiber/area (µm²). **c** mRNA expression levels of CB1 mRNA in the gastrocnemius, soleus, diaphragm and quadriceps muscles of mdx mice (7 weeks) The quantification of transcripts for CB1 was performed by quantitative real-time PCR. Data are expressed as 2^{-ΔCt} formula relative to S16, as described in Materials and Methods. Each bar is the mean ± S.E.M. of at least four separate determinations. *P ≤ 0.05 vs the indicated experimental group, determined by Student's t test.



Supplementary Figure 6: Uncropped representative immunoblots shown from at least three independent experiments with anti-CB1, anti-PAX7, anti-PKC and anti pPKC antibodies obtained from satellite cells or skeletal muscle tissues from vehicle or ACEA treated mdx mice .

Supplementary Table 1: Endocannabinoid 2-AG levels in primary myoblasts isolated from DMD donors.

Patient:	Vol of growth media (GM)	2-AG levels normalized to vol. of GM (pmol ml ⁻¹)	Amount of total lipid extract (mg)	2-AG levels normalized to lipid extract (pmol mg ⁻¹)
Healthy 1	12	1.44	1.08	15.01
Healthy 2	12	0.78	1.43	5.99
Healthy 3	12	0.99	1.10	7.54
D1	12	1.09	1.20	9.75
D2	12	1.20	1.24	9.86
D5	12	1.44	1.12	16.29
D6	12	1.00	0.96	12.96
D8	12	1.06	1.31	10.64
D9	12	1.05	1.17	10.87
D10	12	1.97	1.30	17.91

Supplementary Table 2: Clinical indices of DMD patients

Patient:	Sex:	Age:	Biopsy diagnosis:
Donor 1 (D1)	Male	7 years 1 month	Exons 50-55 Deletion
Donor 2 (D2)	Male	1 years 7 months	Exons 45-51 Duplication
Donor 3 (D3)	Male	0 years 7 months	Exon 19 Puntiform mutation
Donor 4 (D4)	Male	1 years 10 months	Exon 51 Deletion
Donor 5 (D5)	Male	4 years 10 months	Exon 50 Deletion
Donor 6 (D6)	Male	6 years 6 months	Exon 45 Deletion
Donor 7 (D7)	Male	3 years 4 months	Exon 45 Deletion
Donor 8 (D8)	Male	3 years 11 months	Exon 25 Stop mutation
Donor 9 (D9)	Male	4 years 9 months	Exons 19-24 Deletion

Supplementary Table 3: List of primers used in qPCR analysis

Gene	FORWARD Sequence (5'->3')	REVERSE Sequence (5'->3')
Hum CB1	TCTGTTTCATCGTGATGC	CTTGGCTAACCTAATGTC
Murine CB1	GGGCACCTTCACGGTTCTG	GTGGAAGTCAACAAAGCTGTAGA
Murine troponin-T (Tnnt-1)	CTGTGGTGCCTCCTTTGATTC	TGCGGTCTTTTAGTGCAATGAG
Murine myogenin (Myog)	ATGGAGCTGTATGAGACATCCC	TTACACACCTTACATGCCCCAC
Hum troponin-T (TNNT-1)	TGATCCCGCCAAAGATCC	TCTTCCGCTGCTCGAAATGTA
Hum myogenin (MYOG)	GGGGAAAACACTCCTGCCTGTC	AGGCGCTCGATGTACTGGAT
Murine MyHC	AGGCGGACCTACTGTAAC	AGAGATGGAGAAGATGTGG
Murine neoMyHC	GTCACGCAATGCAGAAGAGA	CAGGTCCTTACCCTGCTGTT
Murine TNF α	GCGGCCACAGAAAACACTC	CTCCCAACTGGTCAAGGCATC
Murine IL6 receptor	AGCGACACTGGGGACTTA	ACAGCCTTCGTGGTTGGAG
Hum PAX7	AGCACTGTGCCCTCAGTGAGTT	TCCGACTGCGTCGCTGCTTG
Murine PAX7	CTCAGTGAGTTCGATTAGCCG	AGACGGTTCCTTTGTCCG
Murine TGF β	CTTCAATACGTCAGACATTCGGG	GTAACGCCAGGAATTGTTGCTA
Reg. 2	TCAACCCAAGGCCACTTAGG	GGTTCAGTCCTCAGCTCCAG
Reg. 3	AGAGTGTCTGGCCTTCTCA	CTTGCTCCCGTATTCCAAGA
Reg. 4	ACCTTACGACTCGACTGGAC	CGCAGGAATTAGGGGACATG
Hum S16	TCGGACGCAAGAAGACAGCGA	AGCGTGCGGGCTCAATCAT
murine S16	CTGGAGCCTGTTTTGCTTCTG	TGAGATGGACTGTCGGATGG
TBP	CTGGAATTGTACCGCAGCTT	TCCTGTGCACACCATTTTTTC