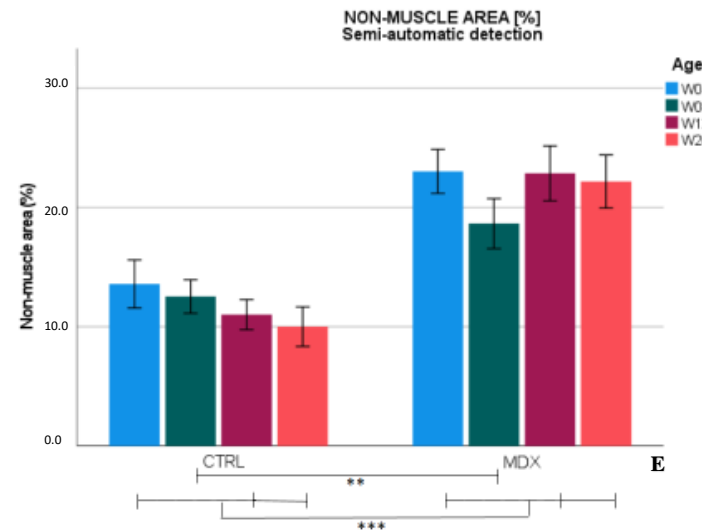
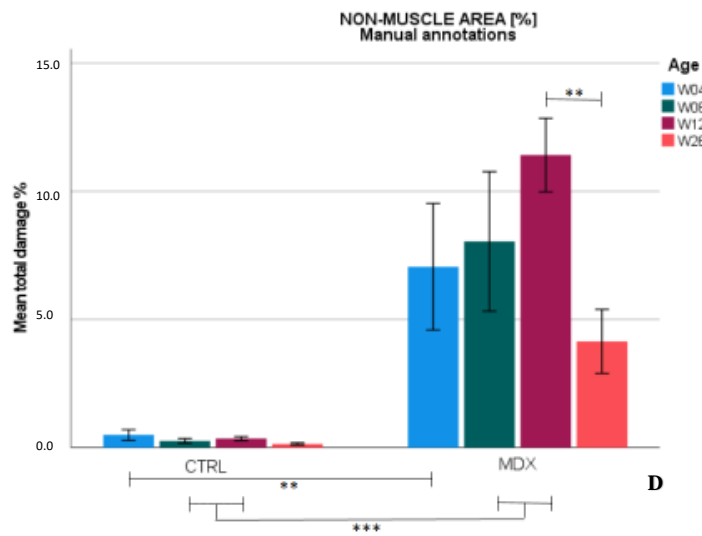
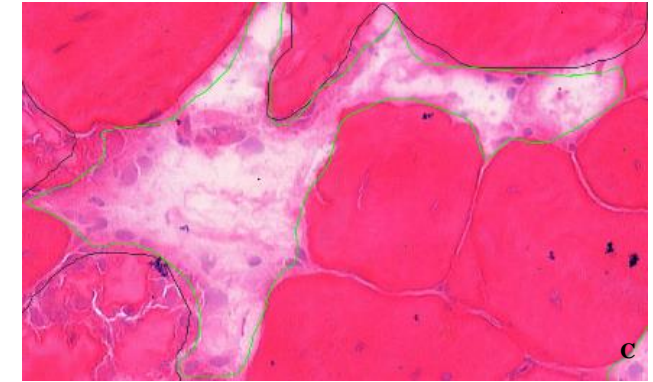
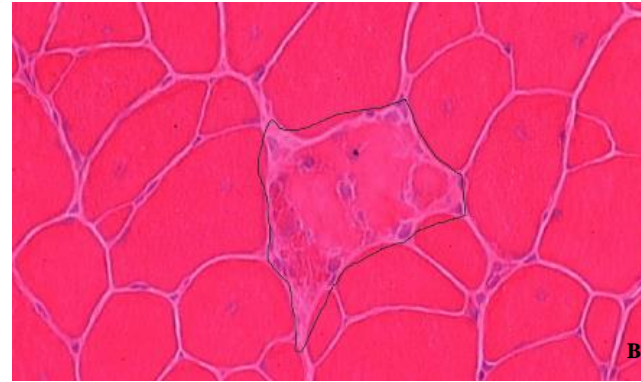
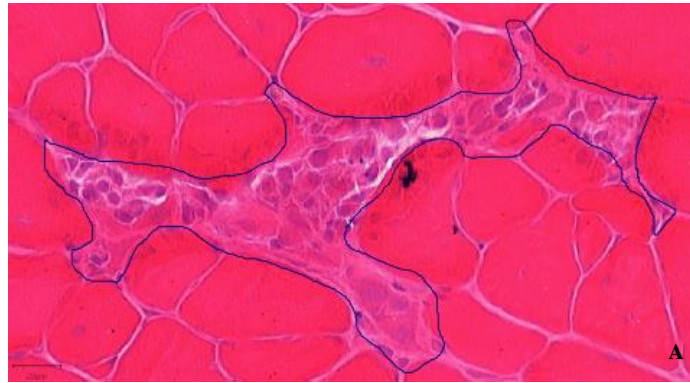


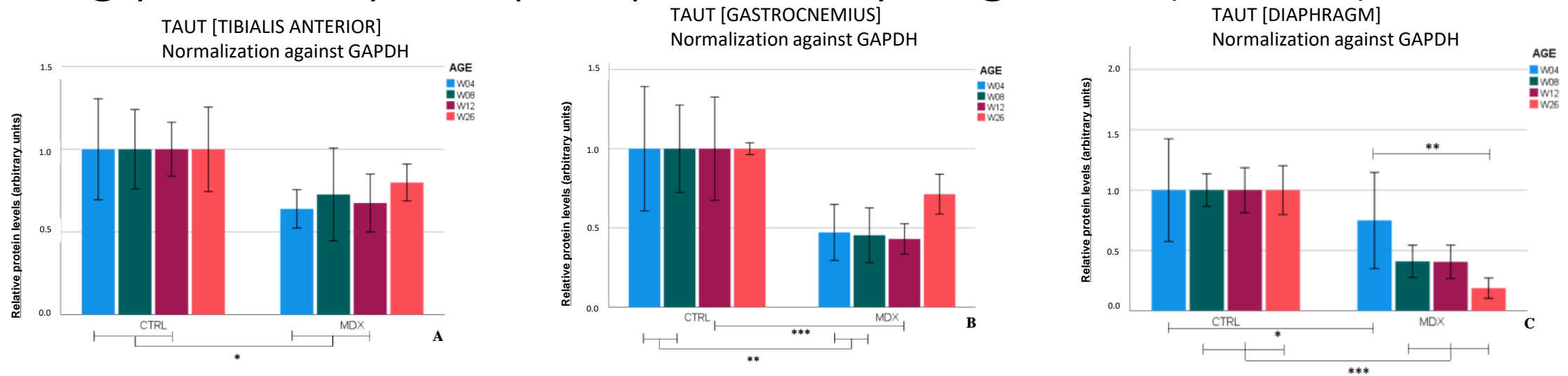
Supplementary files

Figure S1. Assessment of non-muscle area in tibialis anterior of *mdx* and control mice on age 4, 8, 12 and 26 weeks.



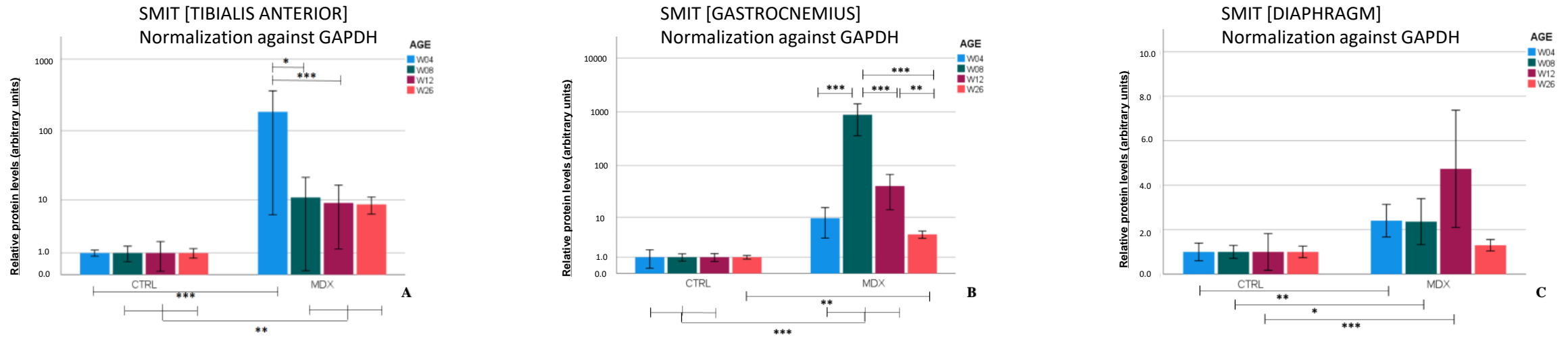
Non-muscle area was defined by (a) infiltrate of immune cells (blue), (b) necrotic cells (grey), and (c) interstitial area with pathological characteristics (green) in the manual annotated version. (d) Representation of mean % non-muscle area (including, necrotic surface, infiltrate area and interstitial space with pathological characteristics) after manual annotation in QuPath. (e) Percentage of non-muscle area according obtained after the colour deconvolution method in QuPath. In total 199 whole muscle sections were analysed. Data is shown as the mean percentage of non-muscle area \pm standard error of the mean and $n = 7-8$. Significant differences are annotated by * ($p \leq 0.05$), ** ($p \leq 0.01$) and *** ($p \leq 0.001$).

Figure S2. Relative protein levels of the taurine transporter (TauT) in skeletal muscle and diaphragm of *mdx* and control mice aged 4, 8, 12, and 26 weeks after normalization against glyceraldehyde 3-phosphate dehydrogenase (GAPDH).



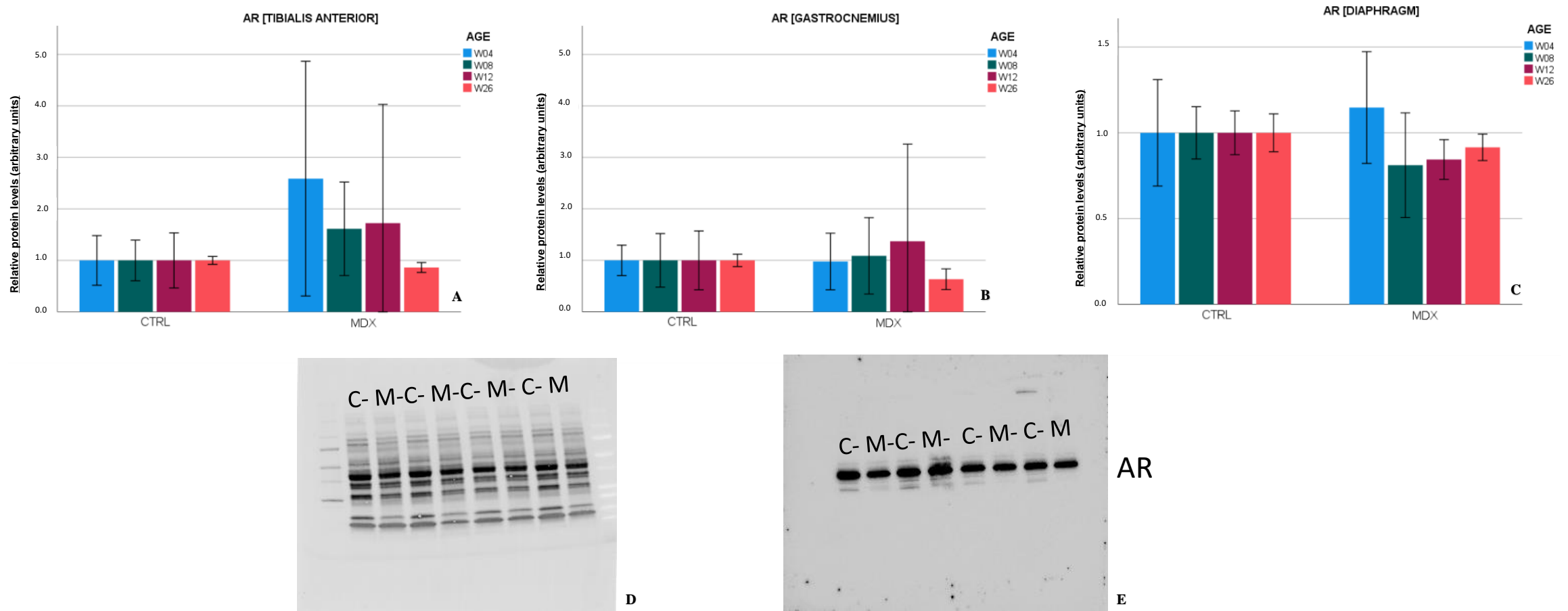
(a) Relative protein levels of TauT in tibialis anterior. **(b)** Relative protein levels of TauT in gastrocnemius. **(c)** Relative protein levels of TauT in diaphragm (n=7-8). Data was normalized against the housekeeping protein GAPDH. Data is shown as mean \pm standard error of the mean (SEM) and n = 7-8. Significant differences are annotated by * ($p \leq 0.05$), ** ($p \leq 0.01$) and *** ($p \leq 0.001$).

Figure S3. Relative protein levels of Sodium/myo-inositol transporter (SMIT) in skeletal muscle and diaphragm of *mdx* and control mice aged 4, 8, 12, and 26 weeks after normalization against GAPDH.



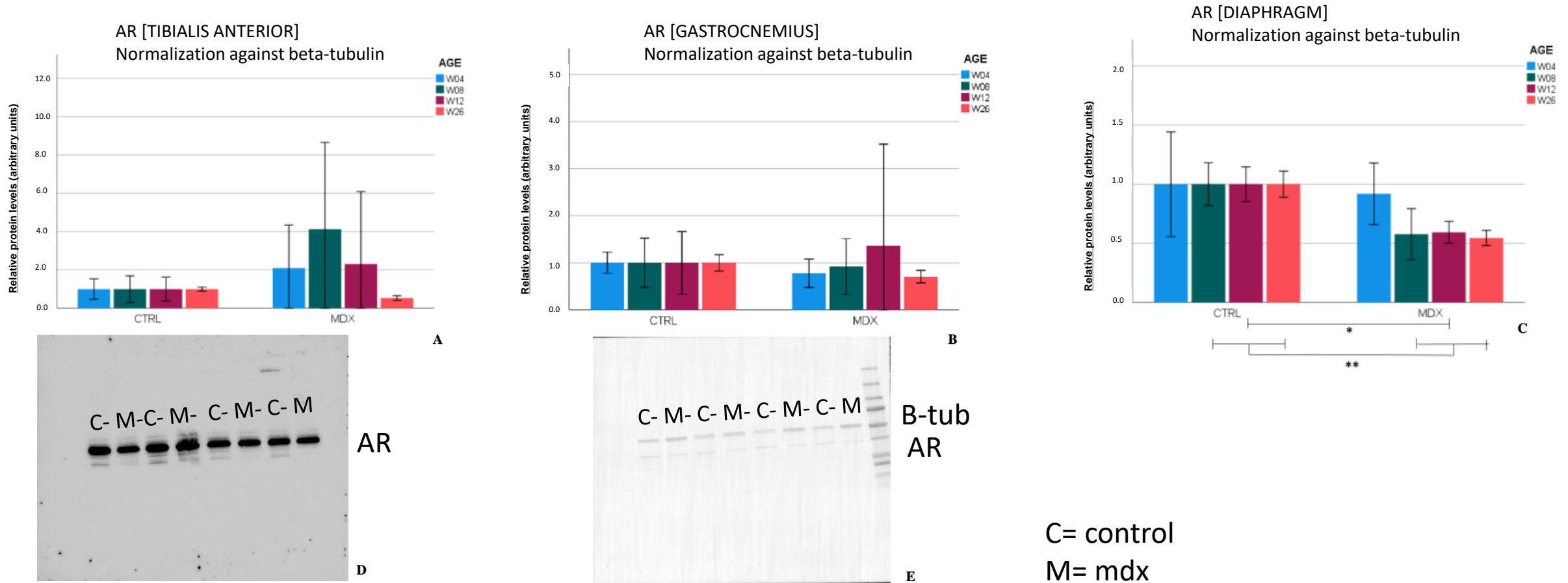
(a) Relative protein levels of SMIT in tibialis anterior. **(b)** Relative protein levels of SMIT in gastrocnemius. **(c)** Relative protein levels of SMIT in diaphragm (n=7-8). Data was normalized against the housekeeping protein GAPDH. Data is shown as mean \pm standard error of the mean (SEM). Significant differences are annotated by * ($p \leq 0.05$), ** ($p \leq 0.01$) and *** ($p \leq 0.001$).

Figure S4. Relative protein levels of aldose reductase (AR) in skeletal muscle and diaphragm of *mdx* and control mice.



Protein levels of AR in mdx mice were reported relative to protein levels in control mice within the same age category. **(a)** Relative protein levels of SMIT in tibialis anterior. **(b)** Relative protein levels of SMIT in gastrocnemius. **(c)** Relative protein levels of SMIT in diaphragm (n=8). Data is shown as mean \pm standard error of the mean (SEM). Significant differences are annotated by * ($p \leq 0.05$), ** ($p \leq 0.01$) and *** ($p \leq 0.001$). Representative image of total protein detected in the diaphragm of 12-week-old mdx and control mice by Stain-Free technology that was used to correct for variation in loading **(d)** and the corresponding image of AR protein imaged by chemiluminescent detection **(e)**.

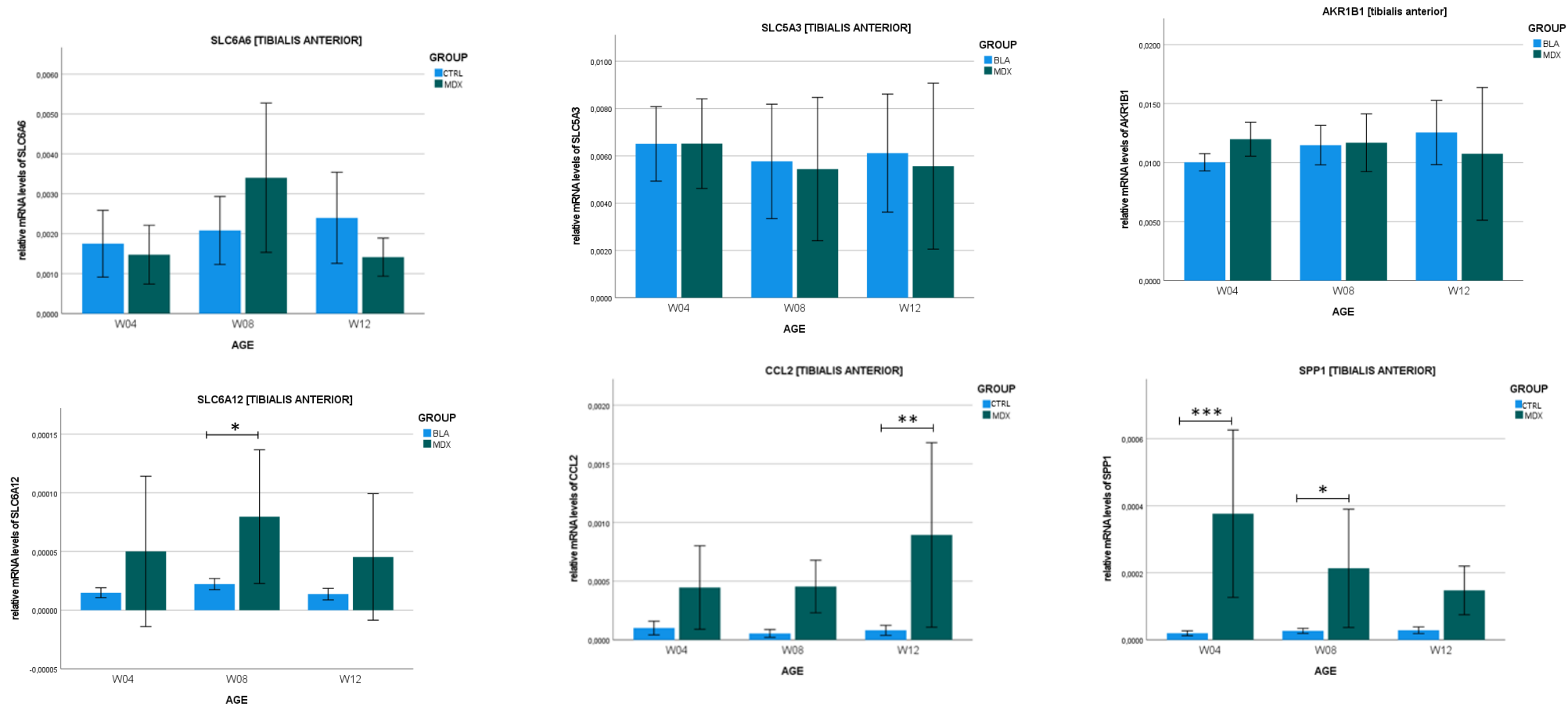
Figure S5. Relative protein levels of aldose reductase in skeletal muscle and diaphragm of *mdx* and control mice normalized against B-tubulin.



Protein levels of AR in *mdx* mice were reported relative to expression in control mice within the same age category.

(a) Relative protein levels of SMIT in tibialis anterior. (b) Relative protein levels of SMIT in gastrocnemius. (c) Relative protein levels of SMIT in diaphragm. Data was normalized against the housekeeping protein B-tubulin. Data is shown as mean \pm standard error of the mean (SEM) and $n = 7-8$. Significant differences are annotated by * ($p \leq 0.05$), ** ($p \leq 0.01$) and *** ($p \leq 0.001$). Representative image of AR protein in the diaphragm of 12-week old mice imaged by chemiluminescent detection (d) and B-tubulin imaged by colorimetric detection, which was used for normalization(e).

Figure S6. Gene expression in tibialis anterior of *mdx* and control mice at age 4, 8, and 12. Run 1



Analysis was carried out using Quantstudio, and data was presented as $2^{-\Delta CT}$ mean \pm standard error of the mean (SEM) relative to GAPDH mRNA expression. Significant differences are annotated by * ($p \leq 0.05$), ** ($p \leq 0.01$) and *** ($p \leq 0.001$).

Table S1 : Overview of qPCR data in tibialis anterior of 4-, 8-, and 12- week old control and *mdx* mice.

	AKR1B1		SLC5A3		SLC6A6		SLC6A12		CCL2		SPP1	
Mean value	CTRL W4:	0.010	CTRL 4W	0.007	CTRL 4W	0.002	CTRL 4W	0.00001	CTRL 4W	0.0001	CTRL 4W	0.00002
	MDX W4:	0.012	MDX 4W	0.007	MDX 4W	0.001	MDX 4W	0.00005	MDX 4W	0.0004	MDX 4W	0.00030
	CTRL W8	0.011	CTRL 8W	0.006	CTRL 8W	0.002	CTRL 8W	0.00002	CTRL 8W	0.00005	CTRL 8W	0.000027
	MDX W8	0.012	MDX 8W	0.005	MDX 8W	0.003	MDX 8W	0.00008	MDX 8W	0.00046	MDX 8W	0.000213
P-value	CTRL W12	0.013	CTRL 12W	0.006	CTRL 12W	0.002	CTRL 12W	0.00001	CTRL 12W	0.00008	CTRL 12W	0.000030
	MDX W12	0.011	MDX 12W	0.006	MDX 12W	0.001	MDX 12W	0.00005	MDX 12W	0.00100	MDX 12W	0.000148
	W4	0.345	W4	0.996	W4	0.721	W4	0.236	W4	0.189	<u>W4</u>	<u><0.001</u>
	W8	0.921	W8	0.858	W8	0.091	<u>W8</u>	<u>0.041</u>	W8	0.129	<u>W8</u>	<u>0.046</u>
	W12	0.384	W12	0.763	W12	0.204	W12	0.250	<u>W12</u>	<u>0.003</u>	W12	0.198

	AKR1B1		SLC6A6		SLC6A12		CCL2		SPP1	
Mean value	CTRL W4:	0.02	CTRL W12	0.002	CTRL 8W	0.00001	CTRL W8	0.00006	CTRL W4:	0.00003
	MDX W4:	0.01	MDX W12	0.002	MDX 8W	0.00007	MDX W8	0.00041	MDX W4:	0.00025
						CTRL W12	0.00007	CTRL W8	0.00004	
						MDX W12	0.00075	MDX W8	0.00026	
P-value								CTRL W12	0.00003	
								MDX W12	0.00015	
	W4	0.384	W12	0.462	<u>W8</u>	<u>0.039</u>	W8	0.072	<u>W4</u>	<u>0.034</u>
							<u>W12</u>	<u>0.001</u>	<u>W8</u>	<u>0.035</u>
								<u>W12</u>	<u><0.001</u>	

(a) Results qPCR run 1; performed in triplicates.

(b) Results qPCR confirmational run 2; performed in triplicates. Results that are underlined show significant results in at least 1 run. Results that are in bold and underlines show significant results in both runs.

Table S2 : Overview of protein loading.

		Protein concentration (µg/ml)	Loading (µl)	Loading (µg)
Tibials anterior	Week 4	853,3	20	17
	Week 8	807,8	20	16
	Week 12	925,4	20	18,5
	Week 26	1000	15	15
Gastrocnemius	Week 4	1654,5	15	25
	Week 8	2236,9	15	33,5
	Week 12	2136,9	15	32
	Week 26	1000	15	15
Diaphragm	Week 4	1000	15	15
	Week 8	1000	15	15
	Week 12	1000	15	15
	Week 26	1000	15	15

Table S2: overview of protein loading per gel. Protein concentrations (µg/ml) and loading (µl; µg) are provided in this table. Of note, within one gel the same protein concentration and loading was maintained. Normalization was carried out with stainfree blot technology to correct for variation in loading and data was reported in reference to control values which made comparison between age/tissue possible.