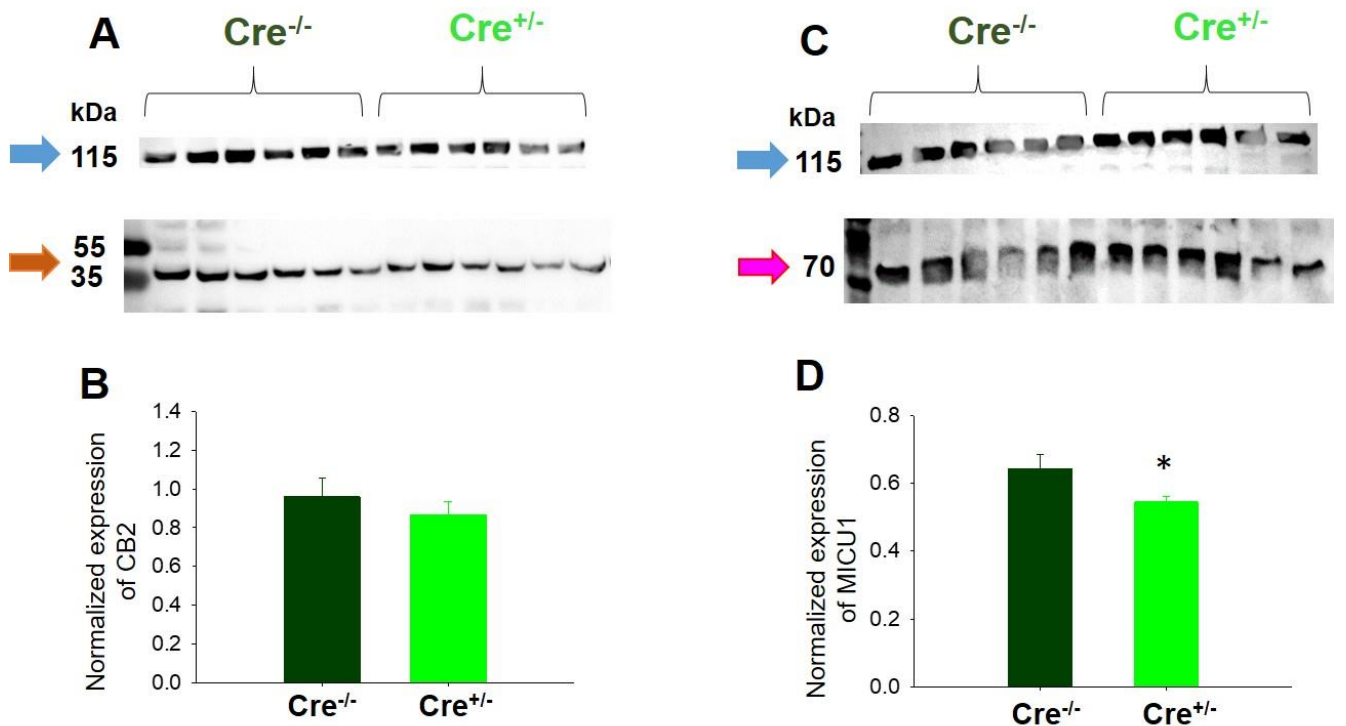


**Figure S1.**  $Ca_{max}$  values during repetitive 100 ms long membrane depolarizations applied from -60 to +30 mV on 21 and 27 FDB cells from  $Cre^{+/-}$  and  $Cre^{-/-}$  mice, respectively, normalized to the maximal change in calcium concentration measured on the given fiber.  $Ca_{max}$  was derived based on Eq. 1 detailed in Methods. Average values were fitted with a Boltzmann function (dashed curves) giving rise to the following parameters for  $Cre^{-/-}$ :  $V_{50} = -10.16$  mV,  $k = 9.15$  and  $Ca_{max} = 0.39 \pm 0.05$   $\mu$ M for  $Cre^{+/-}$ :  $V_{50} = -13.13$  mV,  $k = 8.31$  and  $Ca_{max} = 0.40 \pm 0.04$   $\mu$ M.



**Figure S2.** Western Blot analysis of TA muscle samples. Panels A and C are representative images illustrating the changes of protein expression for CB2 and MICU1 (orange and magenta arrows, 45 and 70 kDa) upon CB1 genetic manipulation and alpha-actinin (blue arrows, 115 kDa) that was used as internal control for normalizing purposes. Panels B and D are

the average normalized protein expression levels in the two samples for CB2 and MICU1, respectively. 6 animals per group were analyzed in at least 3 different independent experiments. \*p<0.05

Nr. of pulses	F/F <sub>0</sub>		Amount of Ca <sup>2+</sup> released (μM)		Peak release flux (mM/s)		Steady flux (mM/s)	
	Cre <sup>-/-</sup>	Cre <sup>+/-</sup>	Cre <sup>-/-</sup>	Cre <sup>+/-</sup>	Cre <sup>-/-</sup>	Cre <sup>+/-</sup>	Cre <sup>-/-</sup>	Cre <sup>+/-</sup>
Nr. of cells	19	26	19	26	19	26	19	26
1	2.63±0.22	2.77±0.20	834.44±62.52	812.74±123.53	10.72±1.57	11.81±1.09	2.83±0.36	3.01±0.22
2	2.48±0.19	2.58±0.15	607.01±57.06	588.84±84.68	5.95±0.83	6.94±0.55	1.80±0.26	2.03±0.15
3	2.33±0.17	2.44±0.14	545.05±51.82	512.57±0.77	5.00±0.75	5.91±0.51	1.51±0.23	1.71±0.14
4	2.20±0.16	2.31±0.12	498.13±47.93	468.42±0.70	4.41±0.67	5.39±0.45	1.33±0.20	1.59±0.13
5	2.08±0.15	2.20±0.11	463.48±45.37	420.34±0.65	3.94±0.61	5.13±0.47	1.27±0.19	1.46±0.11
6	1.99±0.14	2.11±0.10	427.30±42.06	380.15±0.61	3.69±0.58	4.63±0.40	1.18±0.18	1.40±0.12
7	1.91±0.13	2.02±0.10	394.25±39.66	352.62±0.58	3.37±0.55	4.26±0.38	1.11±0.17	1.27±0.11
8	1.84±0.12	1.95±0.09	361.86±37.5	326.58±0.55	3.08±0.51	4.08±0.37	0.99±0.17	1.19±0.10

**Table S1.** Average parameters of the voltage induced calcium transients and calculated release fluxes during tetanic stimulation on FDB muscles.