Supplementary Figures

Fig S1. Injection of hyperpolarizing and depolarizing current are sufficient to regulated TA in isolated *Dmd*^{mdx} cardiomyocytes.

A) Representative action potentials traces of *Dmd*^{mdx} isolated cardiomyocytes in the absence or presence of 1μM isoproterenol (Iso). Hyperpolarizing (20-12 pA) or depolarizing currents (12-8 pA) were injected to maintain the resting membrane potential close to -70 mV (value observed in WT cardiomyocytes) or near - 60 mV (value observed in *Dmd*^{mdx} cardiomyocytes treated with Iso), respectively. Arrow indicates electrical stimulation pulse**. B)** Quantification of TA observed in conditions shown in **(A).** The number in parentheses indicates the *n* value. Comparisons between groups were made using two-way ANOVA plus Tukey post-hoc test, *P<0.05.

Fig S2.

Na^+/Ca^{2+} exchanger (NCX) inhibition does not prevent Iso-induced triggered activity in *Dmd*^{mdx} cardiomyocytes.

(A) Representative action potential traces of *Dmd*^{mdx} isolated cardiomyocytes. Cells were stimulated with 1μM isoproterenol (Iso) in the presence of 10 μM SEA0400 a selective inhibitor of NCX. Arrow indicates electrical stimulation pulse. **B)** Quantification of TA observed in conditions shown in **(A).** The number in parentheses indicates the *n* value. Comparisons between

groups were made using Student´s t-test, *P<0.05 vs Vehicle. **C)** Resting membrane potential of *Dmd*^{mdx} cardiomyocytes. The number in parentheses indicates the *n* value. Comparisons between groups were made using Student´s t-test, *P<0.05 vs vehicle.

Fig S3. WT and in *Dmd*^{mdx} hearts do not show PI uptake after mice were treated with Iso. Representative images of isolated hearts perfused with Tyrode buffer containing 50 μM PI after treatment with Iso (5mg/kg, IP). Cryosections were stained with wheat germ agglutinin (WGA, green) and nuclei were stained blue with DAPI mounting reagent. Representative images of n = 3 per group.

Fig S4. *Dmd*^{mdx}Cx43$^{+/-}$ cardiomyocytes display a lower level of laterally localized Cx43 than *Dmd*^{mdx}.
A) Western blot analysis (top) and quantification (graph) of Cx43 from biotin perfused hearts (biotinylation). Bottom row represents Cx43-immunoblotted samples from heart lysates prior to pulldown (total Cx43). Biotinylated Cx43 levels were expressed as fold change relative to total Cx43 protein levels per sample. The number in parentheses indicates the *n* value. Comparisons between groups were made using two-way ANOVA plus Tukey post-hoc test. *P<0.05 vs Control *Dmd*^{mdx}: Cx43$^{(+/-)}$, †<0.05 vs *Dmd*^{mdx}.

Fig S5. Dystrophic hearts with lower levels of Cx43 prevent the Iso-induced S-nitrosylated levels of Cx43.
A) Top: Western blot detection of Cx43 and Middle: Ponceau staining of samples subjected to the biotin switch assay. Lower: Western blot detection of Cx43 in total cardiac protein lysates. Graph: quantification of 6 independent blots using the ratio of SNO-Cx43/Total. Comparisons between groups were made using two-way ANOVA plus Tukey post-hoc test. *P<0.05 vs WT control, †<0.05 vs WT Iso, **<0.05 vs *Dmd*^{mdx}. **B)** Proximity Ligation assay (PLA) of Cx43 and S-nitrosylation in heart sections of WT, *Dmd*^{mdx} and *Dmd*^{mdx}: Cx43$^{(+/-)}$ mutants. Detection of Wheat germ agglutinin (WGA) (green) and S-nitrosylated Cx43 (Cx43-SNO) (red), respectively. Representative images of n = 4 per group.

Fig S6. Cx43 variants carrying a carboxyterminal truncation or a C271S mutation do not affect gating when co-expressed as heteromeric channel with Cx26S17F. (A) Representative current traces elicited by a voltage pulse from -80 to 0 mV from oocytes expressing heteromeric channels formed by full length, CT truncated Cx43 or Cx43C271S with Cx26S17F. Black and red traces correspond to voltage activated hemichannel currents in the absence or presence of 10 μM DEENO, respectively. Representative traces of n = 9 per group. **(B)** Nitric oxide did not activate homomeric Cx26 and Cx26S17F hemichannels. Representative current traces elicited by a voltage pulse from -80 to 0 mV for an oocyte expressing Cx26 and Cx26S17F. Black and red traces correspond to voltage activated hemichannel currents in the absence or presence of 10 μM DEENO, respectively. Representative traces of n = 6 per group.

Fig. S7. WT, *Dmd*^{mdx} cardiomyocytes display similar K^+ currents. A) Representative current traces before and after application of 100 μM Gap19 and/or 1μM Iso in WT and *Dmd*^{mdx} isolated cardiac cells. K^+ currents were measured using the orange section detected during a ramp protocol.

A negative ramp (from +40 to -120 mV) was used to prevent activating the voltage-gated sodium channel. **B)** Quantification of reversal membrane potential detected in conditions shown in **(A).** The number in parentheses indicates the *n* value.

Fig S8. Cx43 hemichannels are involved in the prolongation of APD in WT and dystrophic cardiomyocytes upon isoproterenol treatment. A) Representative action potential traces of WT, Dmd^{mdx} and $Dmd^{mdx}Cx43^{+/-}$ isolated cardiomyocytes. Cells were stimulated with 1μM isoproterenol (Iso) in the absence or presence of Cx43 blockers contained inside the pipette: Gap19 (232ng/μL) and Cx43 CT antibody (abCx43; 2.5ng/μL). Arrows represent electrical stimulation. Note that Dmd^{mdx} isolated cardiomyocytes have extended APD compared to WT and $Dmd^{mdx}Cx43^{+/-}$ isolated cardiac cells. **B)** Quantification of APD observed in **(A)**. The number in parentheses indicates the *n* value. Comparisons between groups were made using two-way ANOVA test plus Tukey post-hoc test. *P<0.05, † <0.05 vs WT isoproterenol.

Fig S9. Nitric oxide canonical pathway (cGMP-PKG) does not mediate TA in isolated cardiomyocytes and Cx43 hemichannels activation.
A) Representative action potentials traces from Dmd^{mdx} isolated cardiomyocytes. Cells were stimulated with 1μM Iso in the absence or presence of ODQ (a highly selective, irreversible, inhibitor of soluble guanylyl cyclase) or KT 5823 (selective protein kinase G inhibitor) contained inside the pipette: ODQ (3 μM) and KT 5823 (1 μM). Arrow indicates electrical stimulation pulse. Note that both inhibitors ODQ and KT 5823 did not affect the TA and the *Vm* induced by Iso stimulation. The number in parentheses indicates the *n* value. Comparisons between groups were made using two-way ANOVA plus Tukey post-hoc test *P<0.05 vs WT. **B)** Representative current traces after application of 10 μM DEENO in an oocyte expressing Cx43 in the absence or presence of intracellular injections of ODQ (3 μM final intracellular concentration) and KT 5823 (1 μM final intracellular concentration). Oocytes were clamped to −80 mV, and square pulses from −80 mV to +90 mV (in 10 mV steps) were then applied for 2s. Normalized currents were obtained from the ratio between recorded current after and before DEENO treatment. Note that soluble guanylyl cyclase and protein kinase G inhibition did not significantly affect NO-induced hemichannel currents in oocytes expressing Cx43.

Fig S10. Full length western blots. (**A**), (**B**), (**C**) and (**D**) biotin Cx43 pull down, biotin N-Cadherin pull down, biotin eNOS pull down and total Cx43 displayed in Fig. 2. (**E**) S-nitrosylated levels of Cx43 and total Cx43 presented (**F**) Fig. 3A. (**G**) and (**H**) S-nitrosylated levels of Cx43 and total Cx43 in human samples showed in Fig. 3D. (**I**) S-nitrosylated levels of Cx43 in oocytes expressing Cx43 upon NO stimulation and (**J**) respectively total Cx43 presented in Fig. 7C. (K) S-nitrosylated levels of Cx4, (L)) total Cx43 showed in Fig. S4 and (M) total Cx43 displayed in Fig. S5. Red lines indicated the western blot cropped showed in each figure previous indicated.

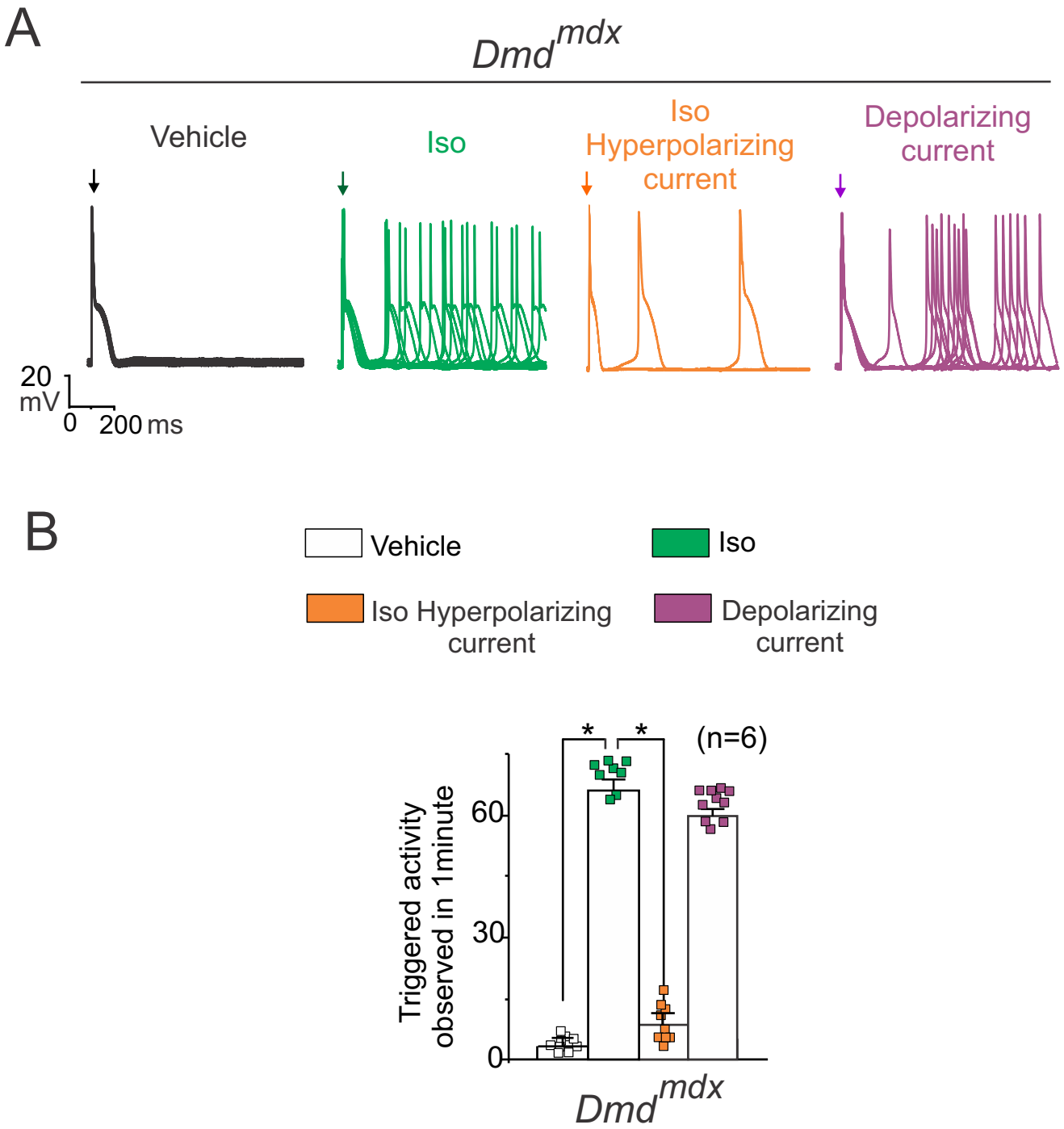

A
Dmd mdx
Vehicle
Iso
Iso
Hyperpolarizing
current
Depolarizing
current
20
mV
0
200 ms
B
Vehicle
Iso
Iso Hyperpolarizing
current
Depolarizing
current
(n=6)
*
*
Triggered activity
observed in 1minute
60
30
0
Dmd mdx

Figure S1

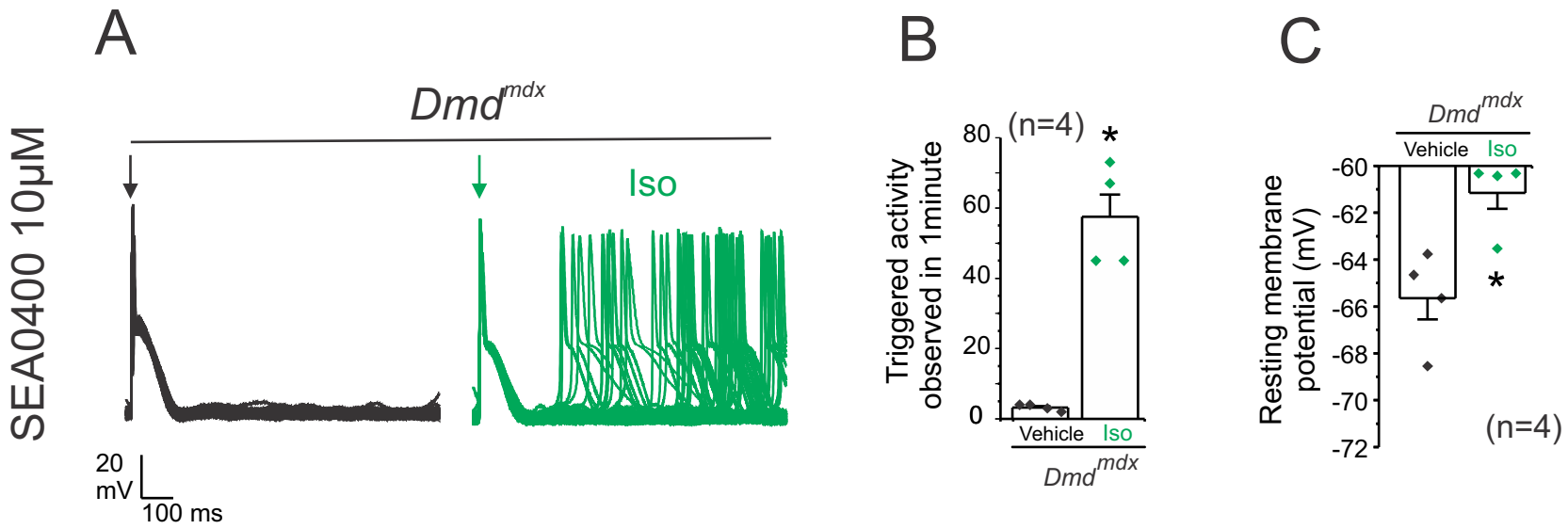

A
Dmd mdx
SEA0400 10µM
Iso
20
mV
100 ms
B
(n=4)
Triggered activity
observed in 1minute
80
60
40
20
0
Vehicle
Iso
Dmd mdx
C
Dmd mdx
Vehicle
Iso
Resting membrane
potential (mV)
-60
-62
-64
-66
-68
-70
-72
(n=4)

Figure S2

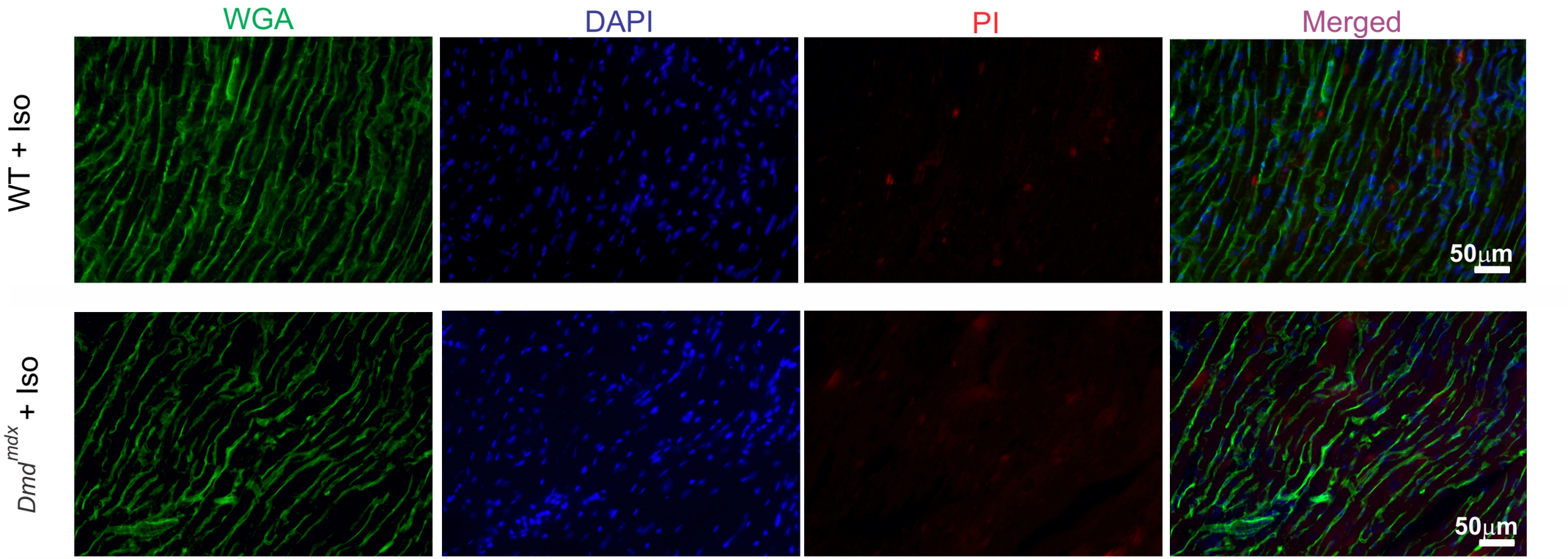


Figure S3

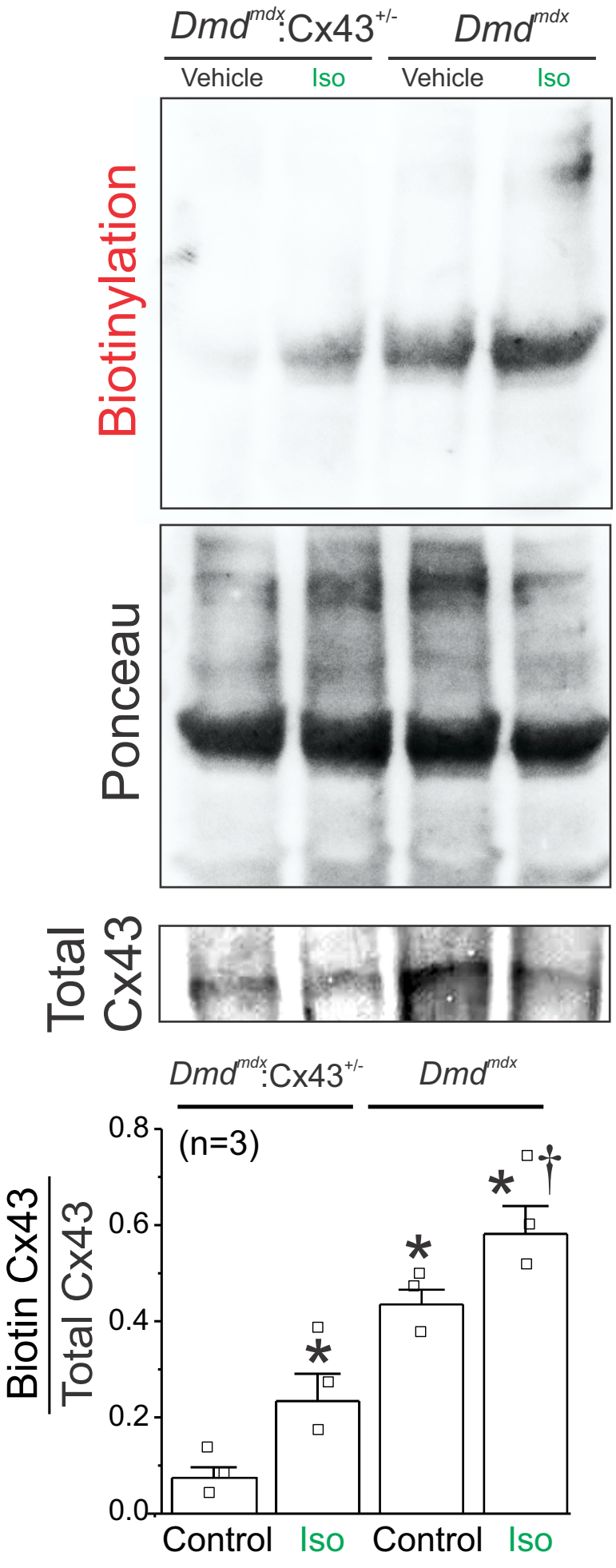
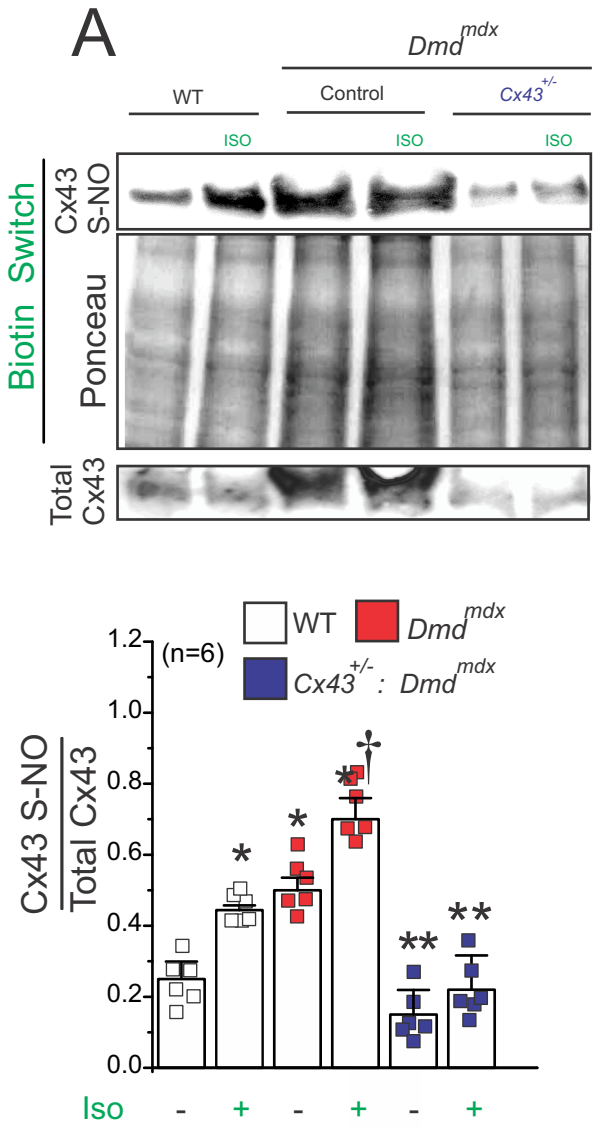

Dmd^mdx:Cx43^+/-
Dmd^mdx
Vehicle
Iso
Vehicle
Iso
Biotinylation
Ponceau
Total Cx43
Dmd^mdx:Cx43^+/-
Dmd^mdx
(n=3)
Biotin Cx43 / Total Cx43
0.8
0.6
0.4
0.2
0.0
Control
Iso
Control
Iso

Figure S4


A
Dmd mdx
WT
Control
Cx43 +/-
ISO
ISO
ISO
Biotin Switch
Cx43 S-NO
Ponceau
Total Cx43
WT
Dmd mdx
Cx43 +/- : Dmd mdx
(n=6)
Cx43 S-NO / Total Cx43
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Iso - + - + - +

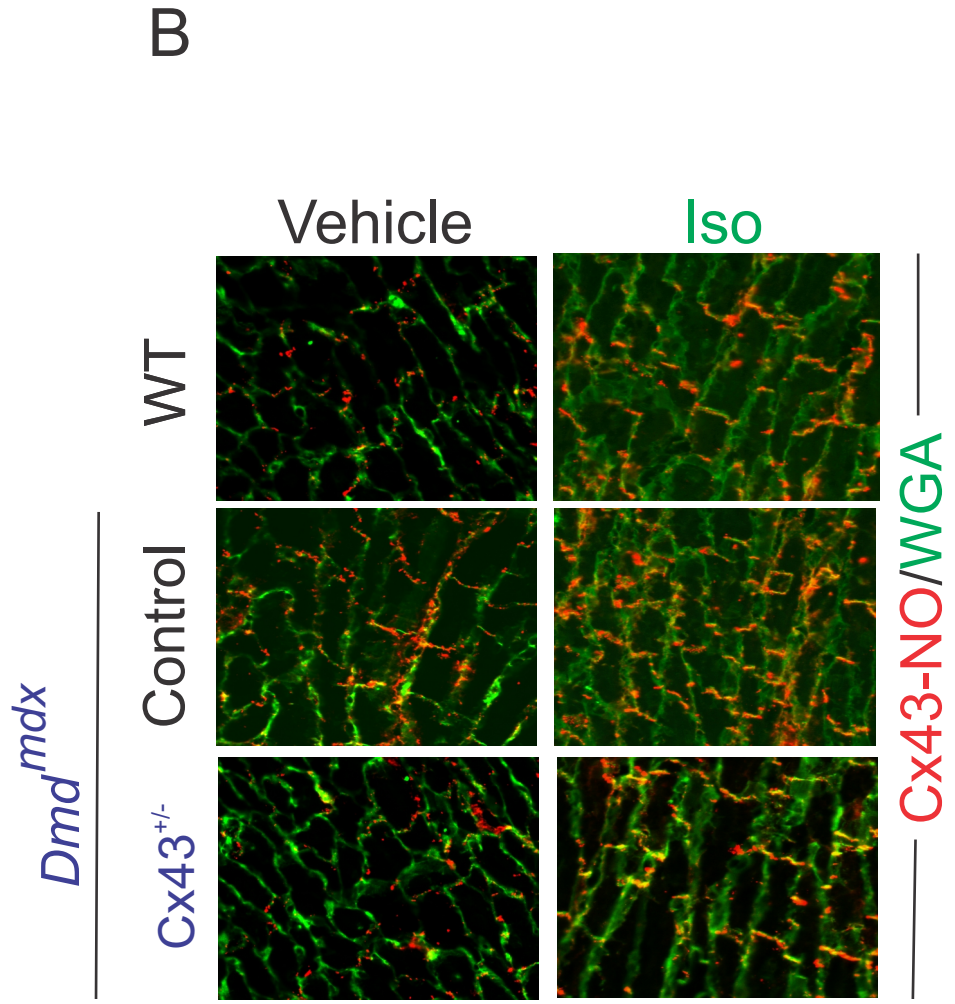

B
Vehicle
Iso
WT
Control
Cx43 +/-
Dmd mdx
Cx43-NO/WGA

Figure S5

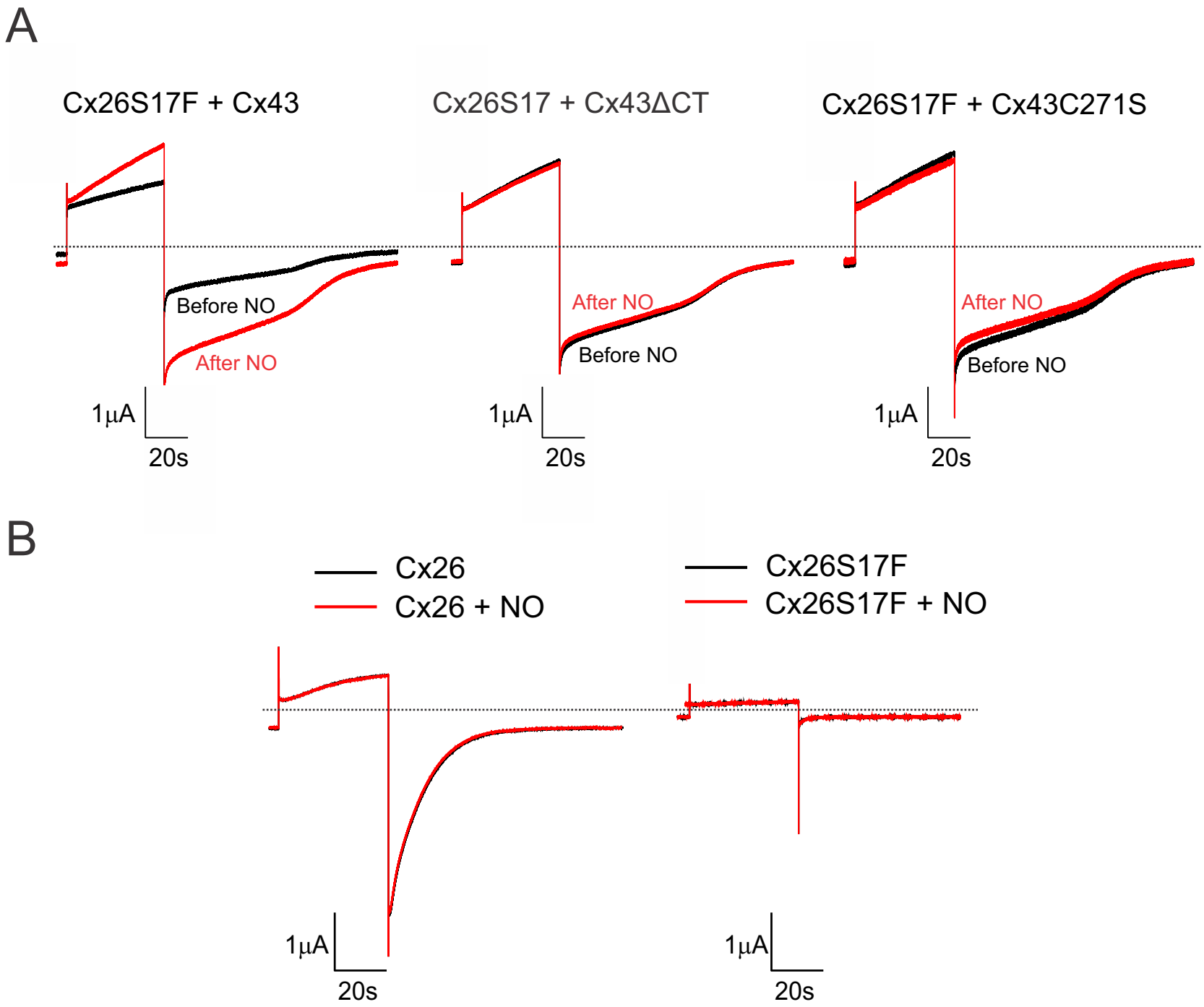

A
Cx26S17F + Cx43
Cx26S17 + Cx43ΔCT
Cx26S17F + Cx43C271S
Before NO
After NO
After NO
Before NO
After NO
Before NO
1μA
20s
1μA
20s
1μA
20s
B
Cx26
Cx26 + NO
Cx26S17F
Cx26S17F + NO
1μA
20s
1μA
20s

Figure S6

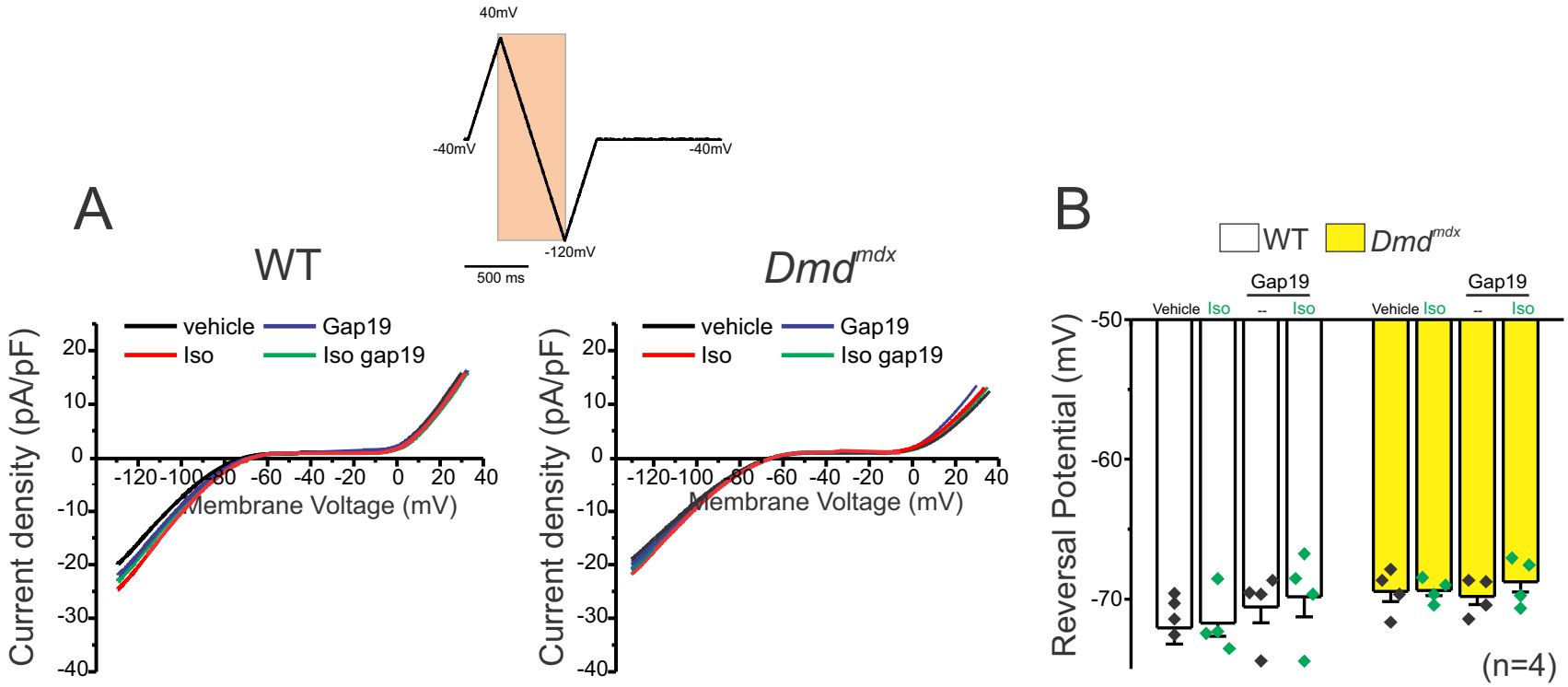

A
WT
Dmd mdx
40mV
-40mV
-40mV
-120mV
500 ms
vehicle
Gap19
Iso
Iso gap19
Current density (pA/pF)
Membrane Voltage (mV)
B
WT
Dmd mdx
Gap19
Vehicle
Iso
--
Iso
Reversal Potential (mV)
(n=4)

Figure S7

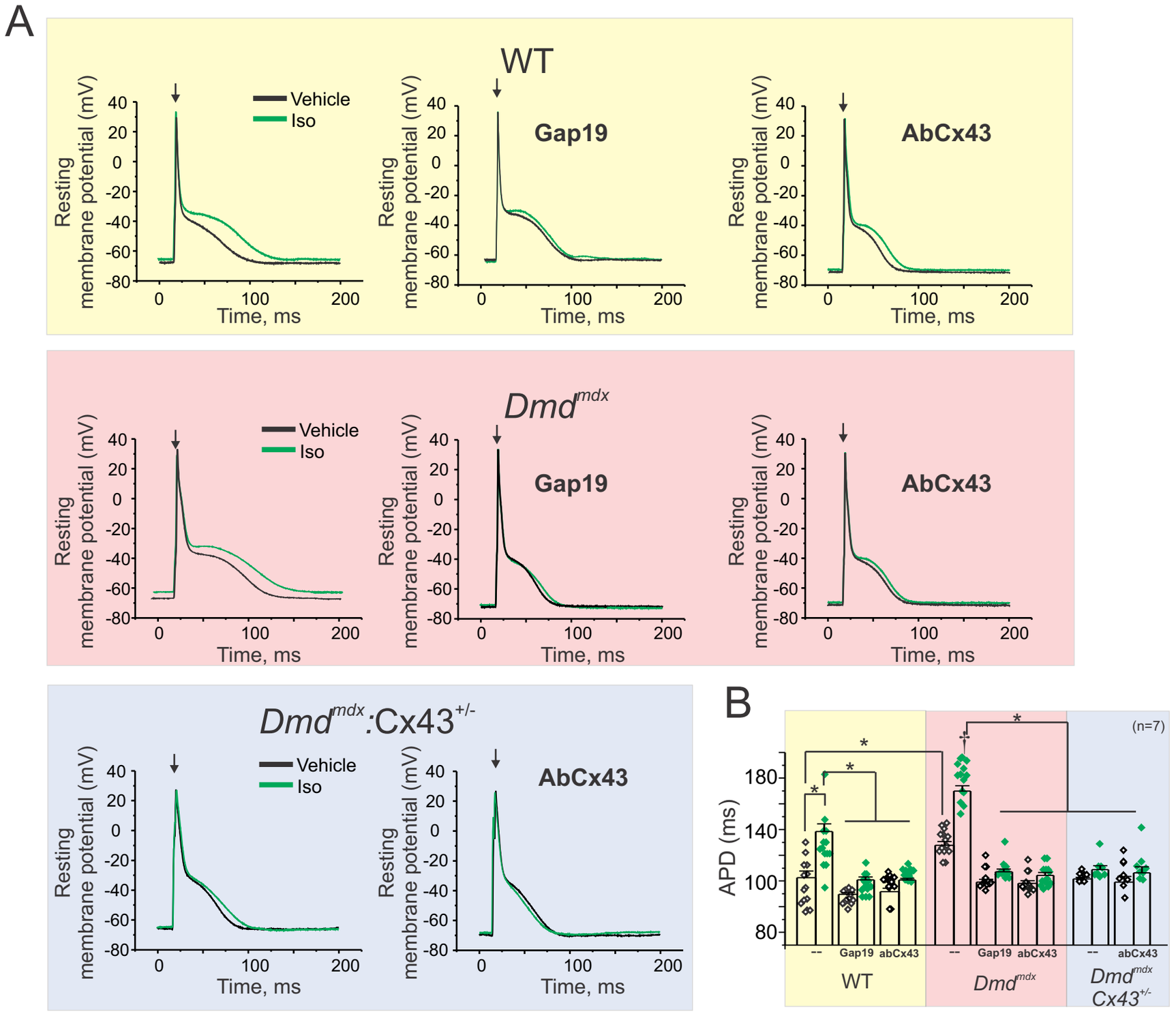

A
WT
Vehicle
Iso
Gap19
AbCx43
Resting membrane potential (mV)
Time, ms
Dmd^mdx
Dmd^mdx:Cx43^+/-
B
APD (ms)
(n=7)
--
Gap19
abCx43
WT
Dmd^mdx
Dmd^mdx Cx43^+/-

Figure S8

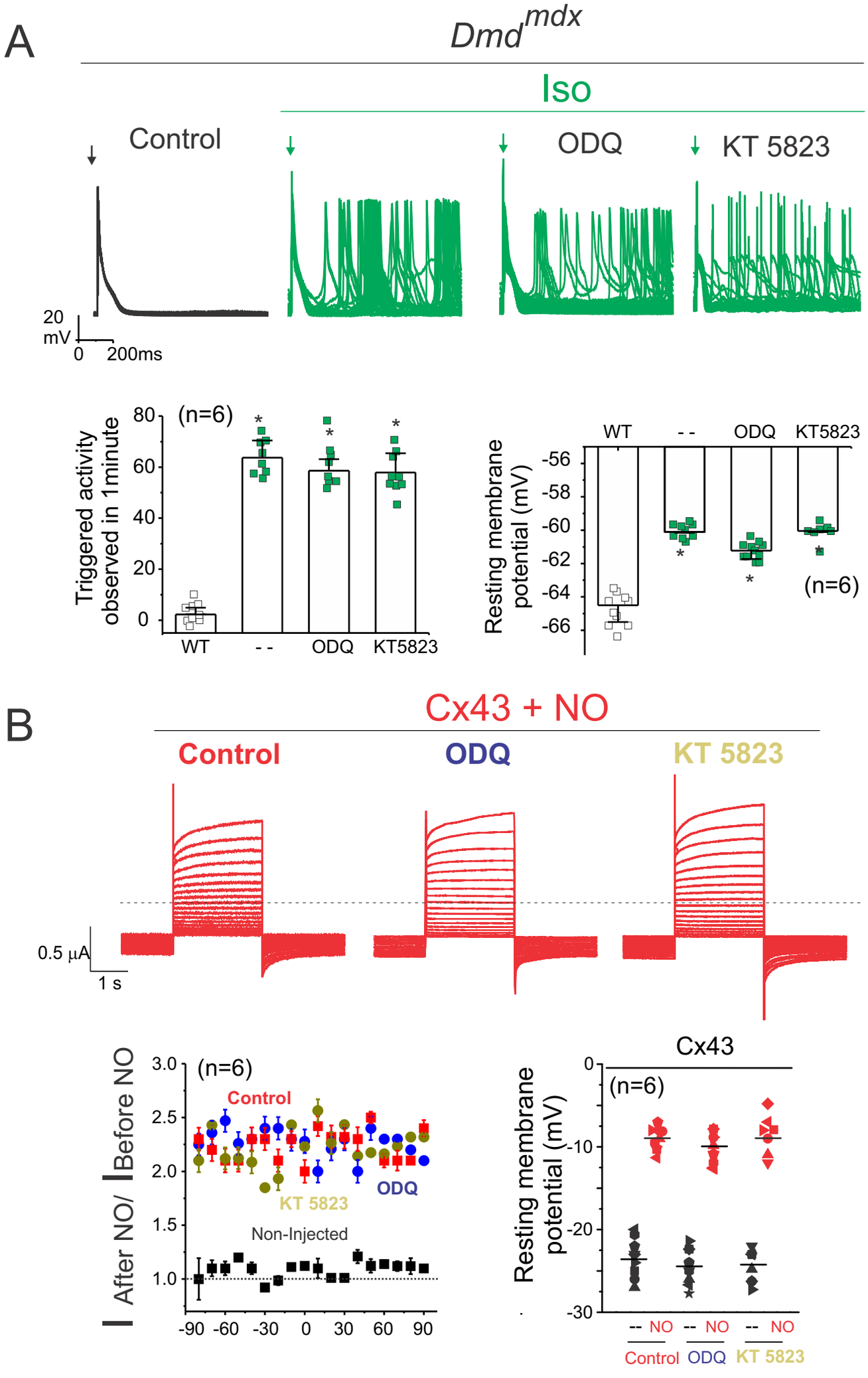

A
Dmd mdx
Iso
Control
ODQ
KT 5823
20 mV
0
200ms
Triggered activity observed in 1minute
(n=6)
WT
- -
ODQ
KT5823
Resting membrane potential (mV)
(n=6)
B
Cx43 + NO
Control
ODQ
KT 5823
0.5 µA
1 s
I After NO/ I Before NO
(n=6)
Control
ODQ
KT 5823
Non-Injected
Cx43
(n=6)
Resting membrane potential (mV)
-- NO
Control
ODQ
KT 5823

Figure S9