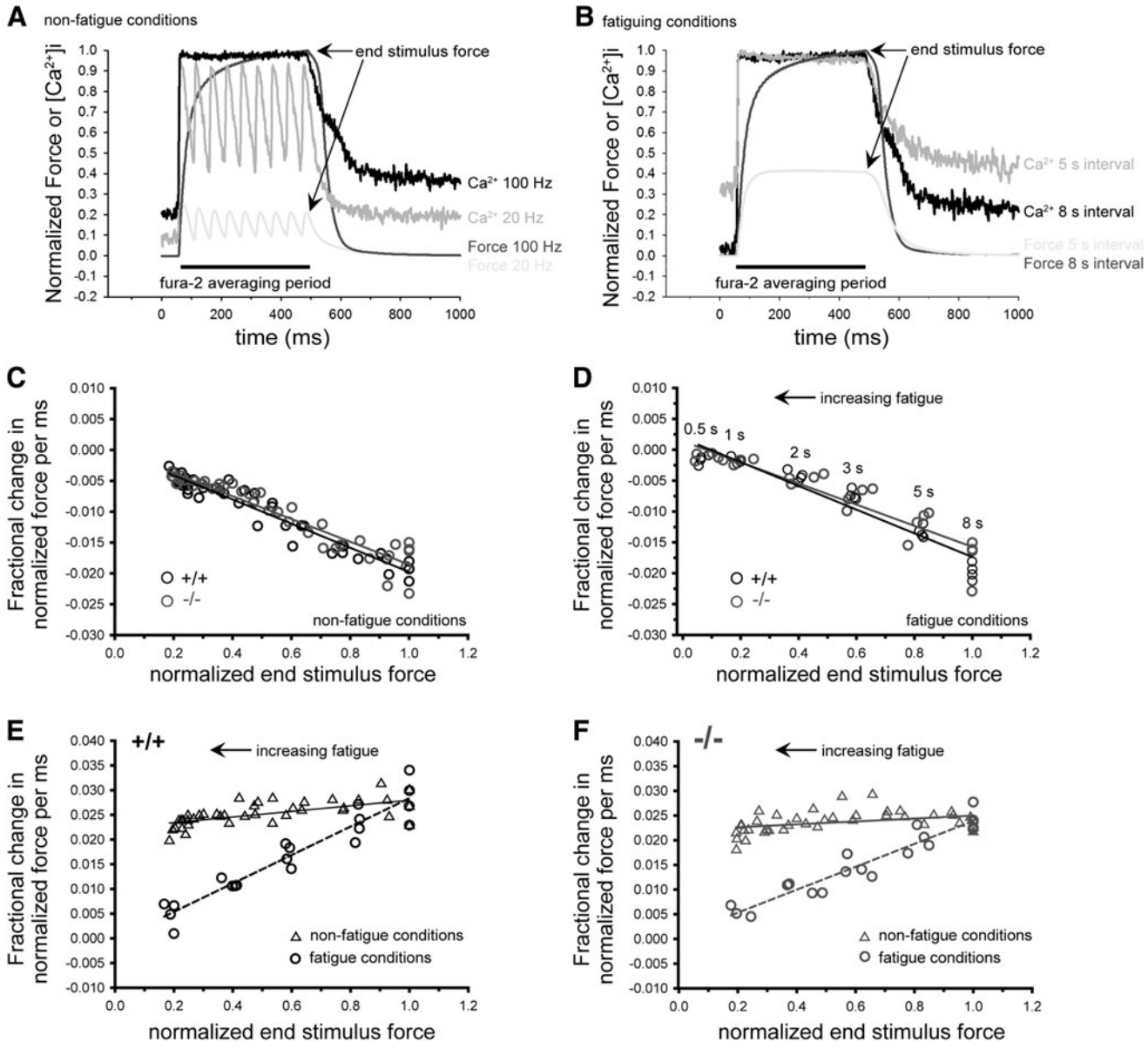
SUPPLEMENTARY FIG. S3. Lumbrical muscle activation and relaxation rates under nonfatiguing and fatiguing conditions. (A) Representative data showing intracellular Ca^{2+} ($[Ca^{2+}]_i$) and force measurements in intact lumbrical myofibers under nonfatiguing conditions *in vitro*. Lumbrical muscles were subjected to 100 consecutive tetanic stimulations every 8 s (nonfatiguing) at stimulation frequencies ranging from 1 to 100 Hz. End stimulus force and $[Ca^{2+}]_i$ were simultaneously recorded, and the mean values of the 100 traces are shown. Force and $[Ca^{2+}]_i$ data at 20 and 100 Hz are shown as examples. The Ca^{2+} indicator fura 2-AM was used to measure $[Ca^{2+}]_I$, and fura 2-AM emissions were collected and averaged over a 500 ms period from individual traces. **(B)** Representative data showing fluxes in $[Ca^{2+}]_i$ and force in lumbrical muscles under fatiguing conditions induced by shortening the time (stimulus interval) between consecutive tetanic stimulations. Lumbrical muscles were subjected to 100 maximal 100 Hz stimulus bursts that were spaced at 0.5, 1, 2, 3, 5, and 8 s intervals. Stimulus intervals between 0.5 and 5 s caused muscle fatigue, whereas an 8 s interval did not cause fatigue. Representative data of end stimulus force and $[Ca^{2+}]_i$ using 5 (fatiguing) and 8 s (nonfatiguing) stimulus intervals are shown. Under fatiguing conditions, force dropped substantially and $[Ca^{2+}]_i$ was slower to return to baseline levels. To obtain additional evidence that loss of GSNOR had no impact on lumbrical fatigue resistance, we determined the maximal muscle relaxation and activation (force development) rates that may decrease under fatiguing conditions. The maximal rate of activation is defined as the fractional change in normalized force produced per ms-slope or first derivative of the upward stroke of the force curve. The maximal rate of relaxation is defined as the fractional change in normalized force produced per ms-slope or first derivative of the downward stroke of the force curve. **(C)** We tested whether GSNOR deficiency impacted maximal rate of relaxation at stimulation frequencies ranging from 1 to 100 Hz under nonfatiguing and fatiguing conditions. The slope of the best fit line represents maximal muscle relaxation rate. Loss of GSNOR did not significantly impact relaxation rates (wild type: $1.96 \pm 0.9\ \mu s^{-1}$, $r^2 = 0.93$; GSNOR null: $1.85 \pm 1\ \mu s^{-1}$, $r^2 = 0.9$). **(D)** Because fatigue may slow maximal relaxation rates, we tested whether loss of GSNOR slowed lumbrical muscle relaxation rate by measuring the rate of force change as a function of end stimulus force produced at specific stimulus intervals from 0.5 (very fatiguing) to 8 s (no fatiguing). The slope of the best fit line represents maximal muscle relaxation rate. Wild-type lumbrical muscles exhibited a similar rate of relaxation ($1.92 \pm 1.2\ \mu s^{-1}$, $r^2 = 0.92$) to GSNOR null muscles ($1.71 \pm 1.5\ \mu s^{-1}$, $r^2 = 0.86$). **(E)** Skeletal muscle fatigue may also slow the rate of muscle activation and force development. To determine whether GSNOR deficiency slowed muscle activation, we first determined the maximal rate of activation of lumbrical muscles in wild-type muscle under nonfatiguing and fatiguing conditions as described earlier for maximal relaxation rate. As expected, the maximal rate of force development was unaffected in wild-type muscle under nonfatiguing conditions ($5.7 \pm 0.6\ \mu s^{-1}$, $r^2 = 0.4$), but it was significantly reduced under fatiguing conditions ($28.7 \pm 2.2\ \mu s^{-1}$, $r^2 = 0.9$). **(F)** The impact of GSNOR depletion on maximal rate of activation of lumbrical muscle contraction. GSNOR null lumbrical muscles exhibited a similar maximal rate of activation to wild-type muscles under both nonfatiguing ($2.9 \pm 1.1\ \mu s^{-1}$, $r^2 = 0.15$) and fatiguing ($23.3 \pm 1.9\ \mu s^{-1}$, $r^2 = 0.9$) conditions, providing additional evidence that the absence of GSNOR has no impact on lumbrical muscle fatigue resistance *in vitro*. $n = \geq 4$ for all groups.


A
non-fatigue conditions
Normalized Force or [Ca2+]i
end stimulus force
Ca2+ 100 Hz
Ca2+ 20 Hz
Force 100 Hz
Force 20 Hz
fura-2 averaging period
time (ms)
B
fatiguing conditions
Normalized Force or [Ca2+]i
end stimulus force
Ca2+ 5 s interval
Ca2+ 8 s interval
Force 5 s interval
Force 8 s interval
fura-2 averaging period
time (ms)
C
Fractional change in normalized force per ms
+/+
-/-
non-fatigue conditions
normalized end stimulus force
D
increasing fatigue
0.5 s
1 s
2 s
3 s
5 s
8 s
Fractional change in normalized force per ms
+/+
-/-
fatigue conditions
normalized end stimulus force
E
+/+
increasing fatigue
Fractional change in normalized force per ms
non-fatigue conditions
fatigue conditions
normalized end stimulus force
F
-/-
increasing fatigue
Fractional change in normalized force per ms
non-fatigue conditions
fatigue conditions
normalized end stimulus force