

Expanded View Figures

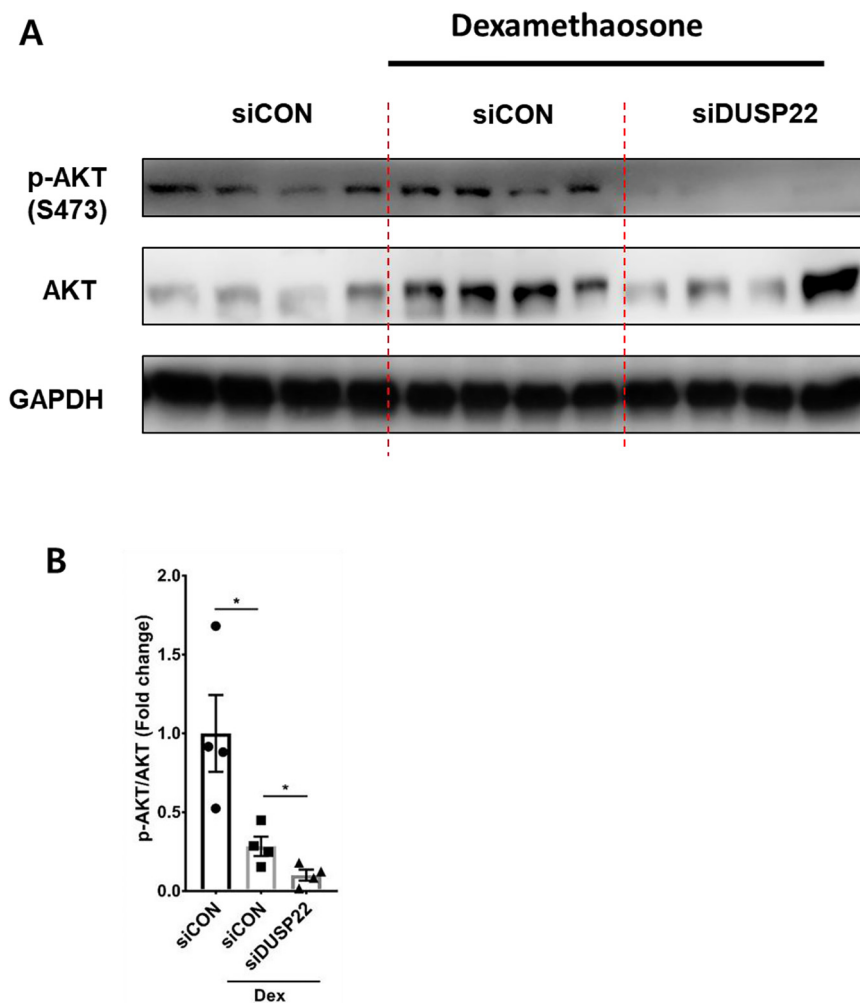


Figure EV1. DUSP22 siRNA reduces the phosphorylation of AKT.

(A, B) Western blot and densitometry analysis of AKT phosphorylation in the Dex-treated tibialis anterior (TA) muscle 3 d after delivery of control or DUSP22 siRNA ($n = 4$) ($p = (\text{siCON} = 0.029, \text{siDUSP22+Dex} = 0.0412)$). GAPDH was used for normalization of expression. * $p < 0.05$ indicate significantly increased or decreased. n represents biological replicates analyzed by Student's t test. Error bars represent the standard error of the mean (SEM). Source data are available online for this figure.

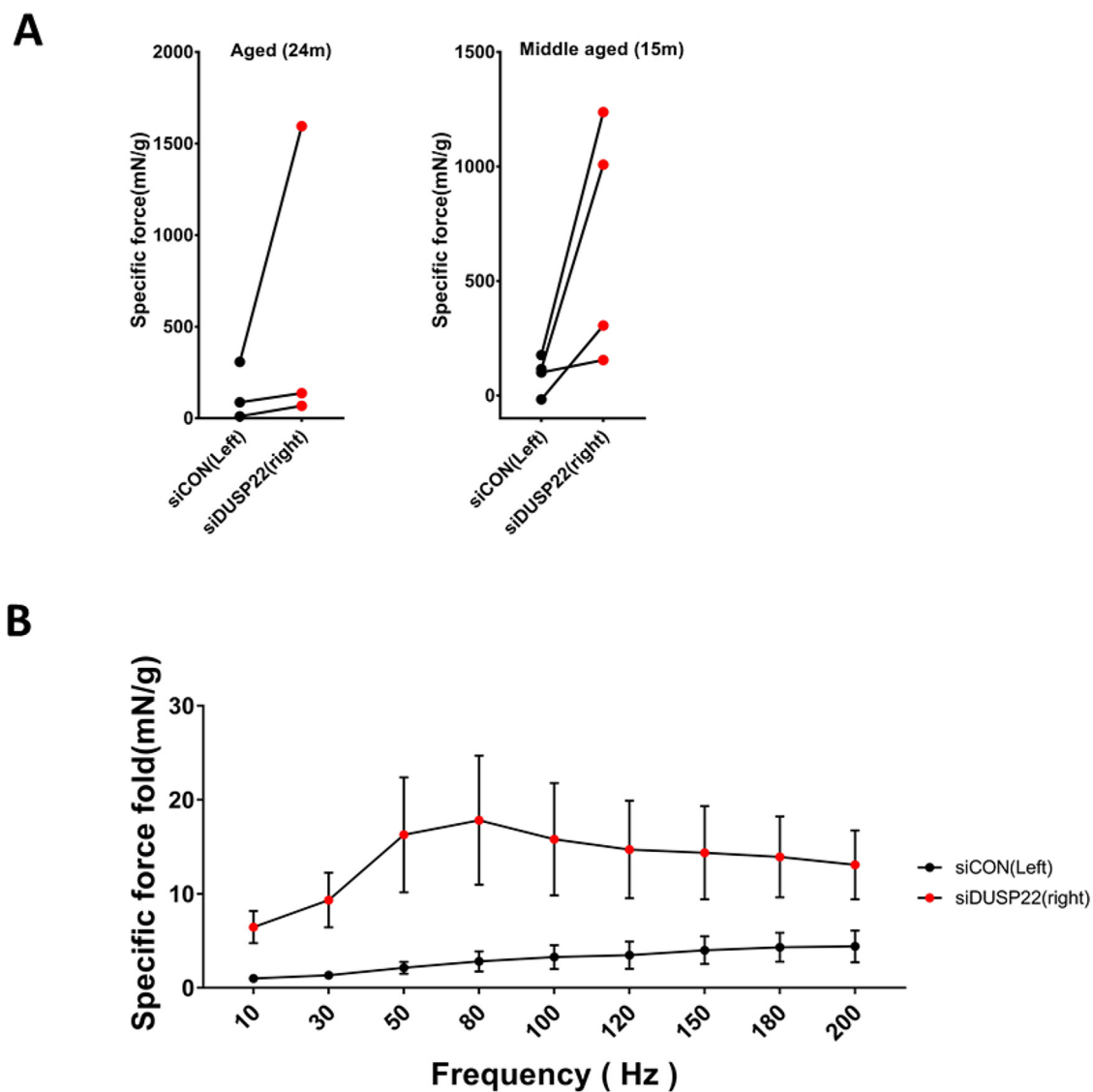
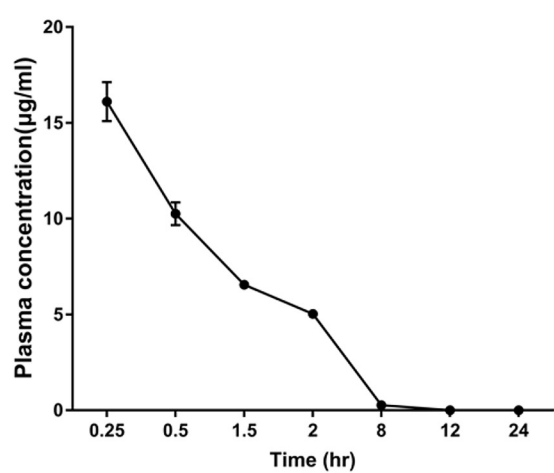


Figure EV2. DUSP22 siRNA enhances tetanic and twitch muscle force in the tibialis anterior muscle.

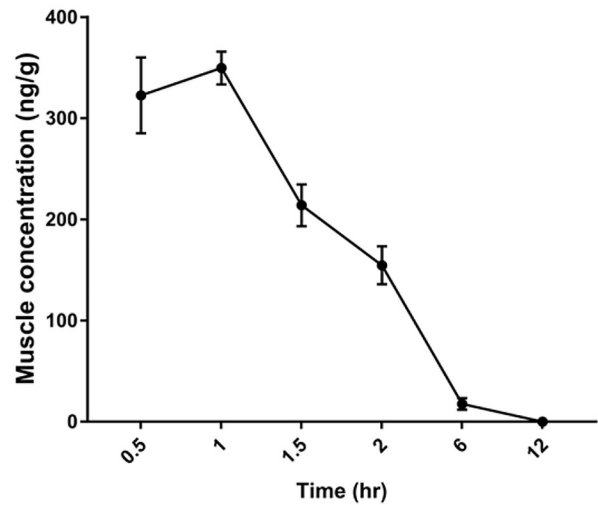
(A) Tetanic muscle contraction measurement in the TA muscle of aged ($n = 3$) ($p = 0.3974$) or middle aged mice ($n = 4$) ($p = 0.0807$) 3 d after the delivery of control or DUSP22 siRNA. (B) Twitch force measure in 15-month-old mice 3 d after the delivery of control or DUSP22 siRNA ($n = 4$) ($p = 0.1054, 0.1677, 0.1964, 0.1858, 0.2118, 0.234, 0.2489, 0.2684, 0.2947$). n represents biological replicates analyzed by Student's t test. Error bars represent the standard error of the mean (SEM). Source data are available online for this figure.

BML-260 detection : Plasma



PK Parameters	Unit	BML-260
		IP
Dose	mg/kg	5.00
AUC _{last}	h*ug/mL	26.14
AUC _{inf}	h*ug/mL	26.70
C _{max}	ug/mL	16.11
T _{max}	h	0.25
V _z /F	L/kg	0.38
Cl/F	L/h/kg	0.19
t _{1/2}	h	1.42

BML-260 detection : Skeletal muscle (tibialis anterior)



PK Parameters	Unit	BML-260
		IP
Dose	mg/kg	5.00
AUC _{last}	h*ug/g	0.73
AUC _{inf}	h*ug/g	0.76
C _{max}	ug/g	0.35
T _{max}	h	1.00
V _z /F	g/kg	11871.11
Cl/F	g/h/kg	6569.42
t _{1/2}	h	1.25

Figure EV3. Pharmacokinetic analysis of BML-260 in 6-week-old male C57BL/6J mice.

Pharmacokinetic analysis of BML-260 in 6-week-old male C57BL/6J mice (5 mg/kg delivered via IP injection) (*n* = 3). BML-260 detection was assessed in the plasma and tibialis anterior muscle. AUC_{last}: Area under the concentration-time curve from time of dosing to the last measurable concentration. AUC_{inf}: Area under the concentration-time curve extrapolated to infinity based on the last measurable concentration. C_{max}: Maximum plasma concentration. T_{max}: Time to reach the maximum plasma concentration. V_z/F: Apparent volume of distribution during the terminal phase. Cl/F: Apparent total body clearance after extravascular administration. t_{1/2}: Terminal half-life. *n* represents biological replicates. Error bars represent the standard error of the mean (SEM). Source data are available online for this figure.

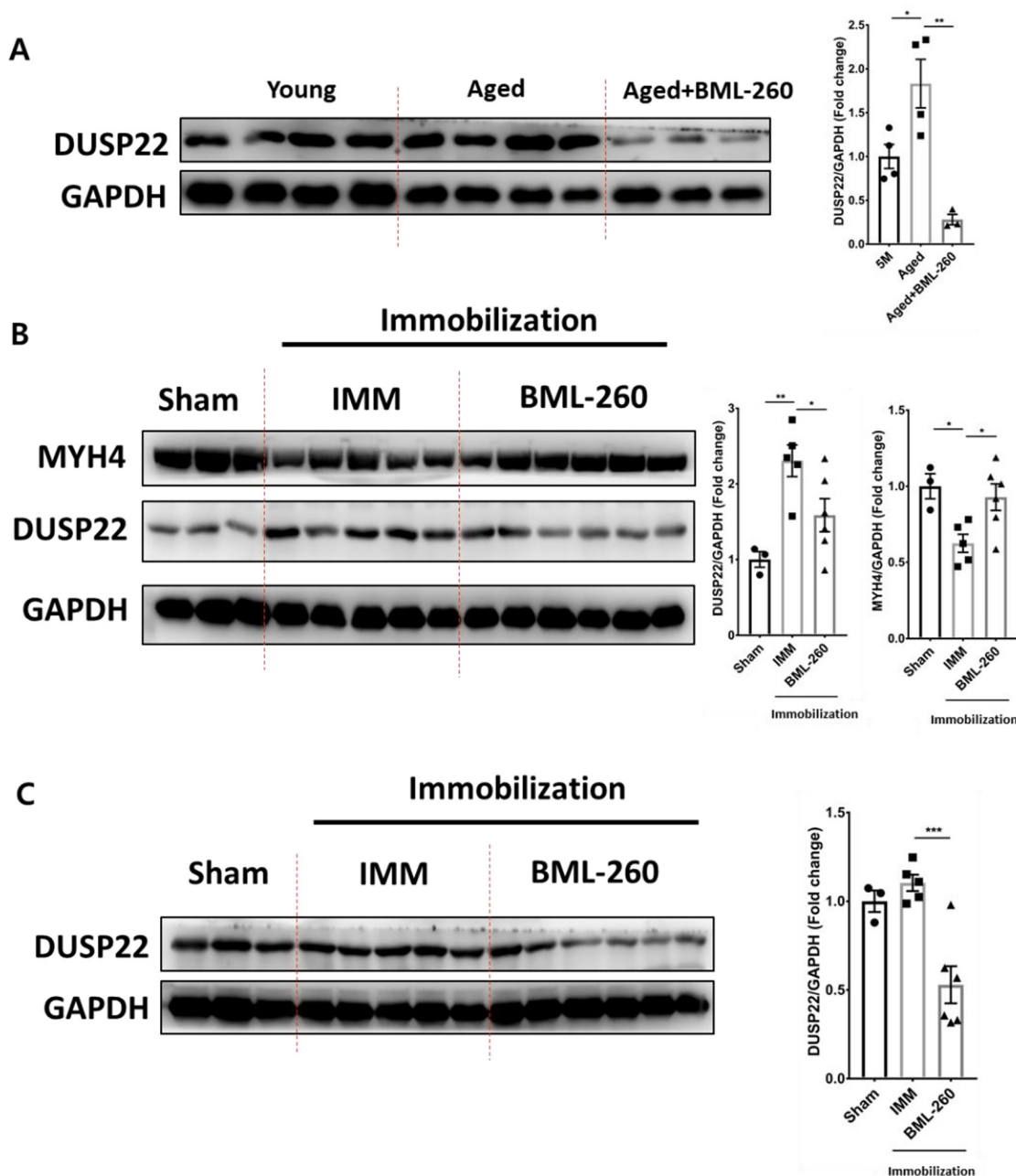


Figure EV4. DUSP22 and MYH4 levels in skeletal muscles of aged or immobilized mice treated with BML-260.

(A) Western blot analysis of DUSP22 expression in the TA of aged mice treated with BML-260 ($n = 4,3$) ($p = (\text{Young} = 0.0362, \text{Aged+BML-260} = 0.0054)$). GAPDH was used for normalization of expression. * $p < 0.05$ and ** $p < 0.01$ indicate significantly increased or decreased. (B, C) Western blot analysis of DUSP22 expression in the TA ($n = 3,5,6$) ($p = (\text{Sham} = 0.0051, \text{BML-260} = 0.0488)$) (B) and gastrocnemius muscle ($n = 3,5,6$) ($p = (\text{Sham} = 0.6845, \text{BML-260} = 0.0008)$) (C) of immobilized (IMM) mice treated with BML-260. For the TA muscle, MYH4 (myosin heavy chain 2B) ($p = (\text{Sham} = 0.0253, \text{BML-260} = 0.0288)$) levels are also shown. GAPDH was used for normalization of expression. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ indicate significantly increased or decreased. n represents biological replicates analyzed by Student's t test. Error bars represent the standard error of the mean (SEM). Source data are available online for this figure.

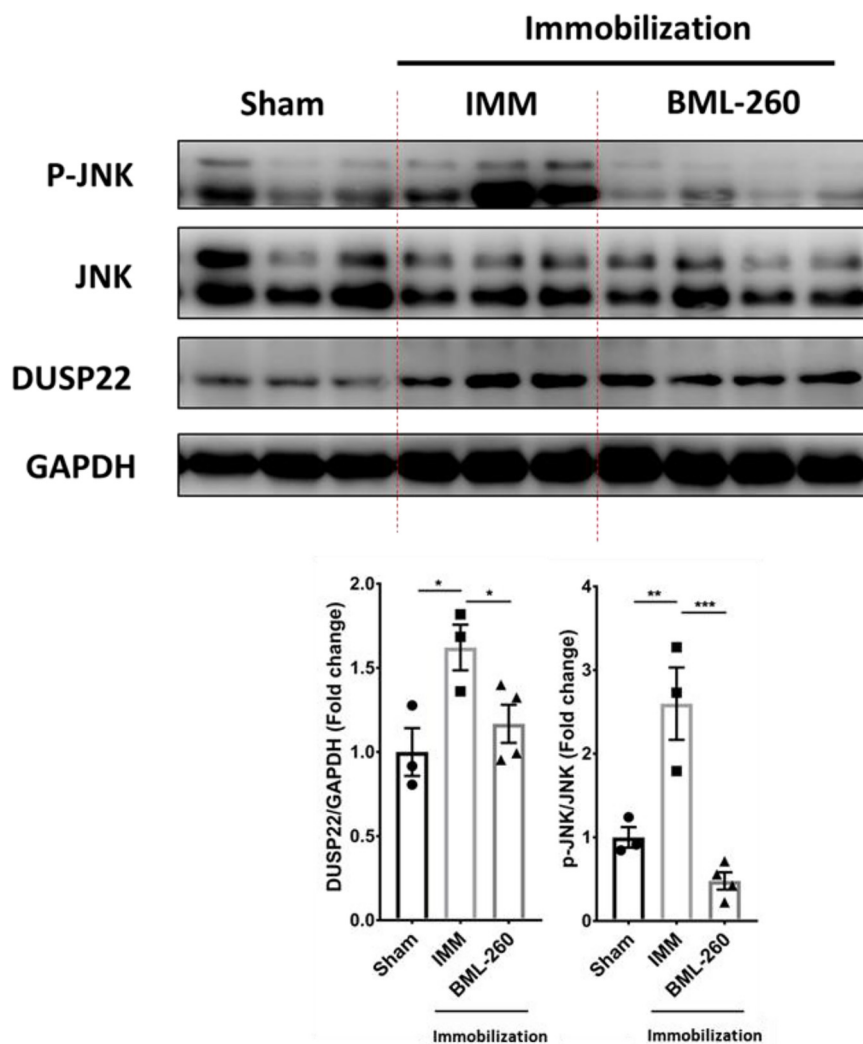


Figure EV5. DUSP22, JNK and phosphorylated JNK levels in the tibialis anterior of immobilized mice treated with BML-260.

Western blot analysis of DUSP22 ($p = (\text{Sham} = 0.0341, \text{BML-260} = 0.0494)$), JNK and phosphorylated JNK (JNK-P) ($p = (\text{Sham} = 0.0052, \text{BML-260} = 0.0007)$) levels in the TA muscle of immobilized mice ($n = 3,4$). GAPDH was used for normalization of expression. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ indicate significantly increased or decreased. n represents biological replicates analyzed by Student's t test. Error bars represent the standard error of the mean (SEM). Source data are available online for this figure.