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## Supplemental information

# Increased SIRT3 combined with PARP inhibition rescues motor function of SBMA mice

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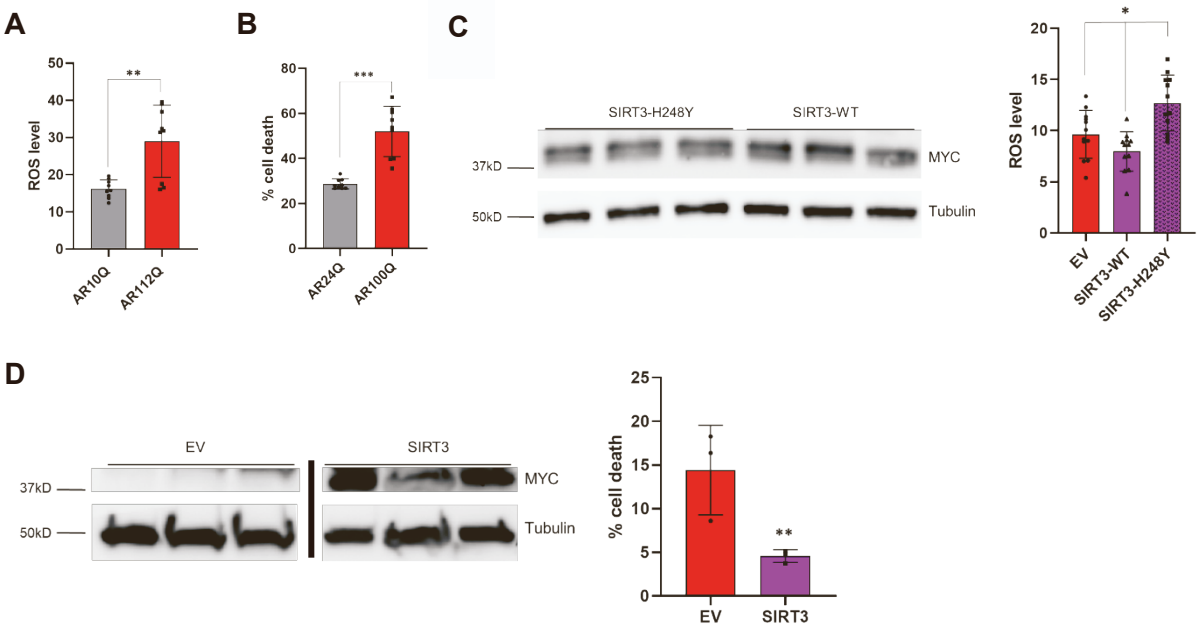
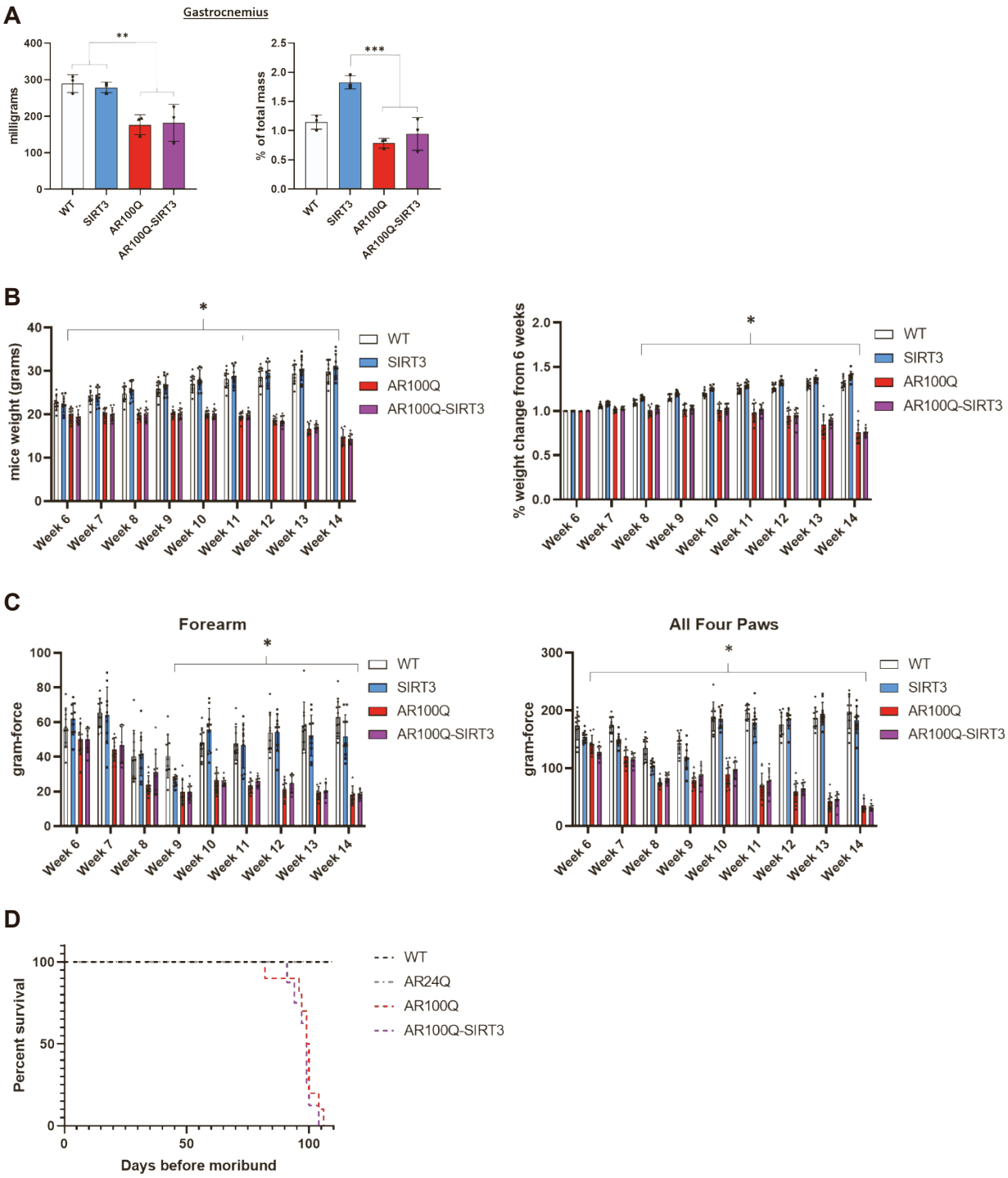
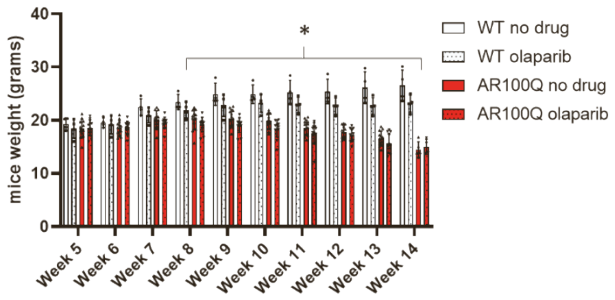


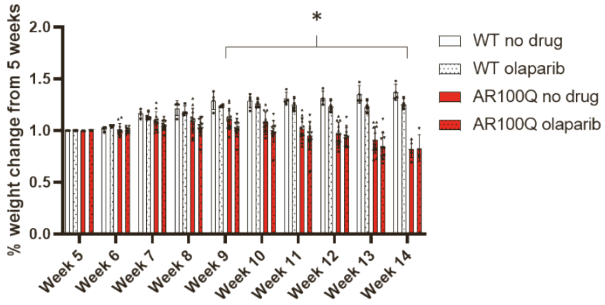

**Supplemental Figure 1, Related to Figure 1. AR112Q PC12 cells have increased ROS. Second set of clonal lines of AR112Q PC12 cells and AR100Q C2C12 cells overexpressing SIRT3, with reduced ROS and death.** **(A)** ROS levels in AR10Q- and AR112Q-expressing PC12 cells (n = 12) after 48 hrs (representative image from 3 experiments)**.** ** = Two-way t-test, p ≤ 0.01. **(B)** Cell death of AR24Q- and AR100Q-expressing C2C12 myotubes (n = 3) after 10 days (representative image from 3 experiments). *** = Two-way t-test, p ≤ 0.001. **(C)** Left, immunoblot of MYC-tagged SIRT3 and tubulin in AR112Q-SIRT3-MYC-overexpressing PC12 cells (n = 3) of clone set 2. Right, ROS levels in AR112Q-SIRT3-MYC-overexpressing PC12 cells (n = 12) of clone set 2 (representative image from 3 experiments)**.** **(D)** Left, immunoblot of MYC-tagged SIRT3, and tubulin in AR100Q-SIRT3-MYC-overexpressing C2C12 myoblasts (n = 3) of clone set 2**.** Line in middle indicates lanes removed from gel. Right, cell death of C2C12 AR100Q-SIRT3-MYC-overexpressing myoblasts (n = 3) of clone set 2 (representative image from 3 experiments)**.** ** = Nested Two-way t-test, p ≤ 0.01. **(A-D)** All statistical analysis show mean ± SD, and used a two-way ANOVA and Tukey's *post-hoc* (* = p ≤ 0.05) unless otherwise noted. AR10Q = non-polyQ-expanded AR, AR112Q = polyQ-expanded AR, AR24Q = non-polyQ-expanded AR, AR100Q = polyQ-expanded AR, EV = empty vector, SIRT3 = SIRT3-myc-his overexpression, SIRT3-H248Y = SIRT3-myc-his overexpression (deacetylase inactive).



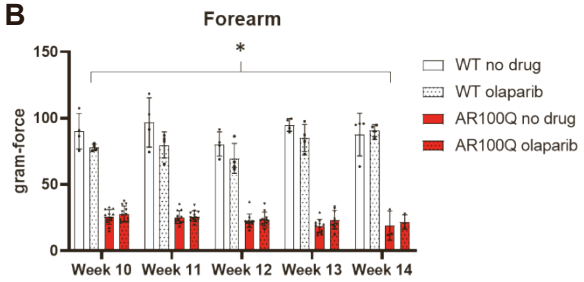

**Supplemental Figure 2, Related to Figure 2. SIRT3 overexpression alone, in AR100Q male mice fails to rescue gastrocnemius mass, total body weight, motor dysfunction and lifespan. (A)** Muscle mass (left) and percent of total mass (right) of gastrocnemius from WT, SIRT3, AR100Q, and AR100Q-SIRT3 male mice (n = 3) at 8 weeks of age. **(B-D)** A behavioral cohort composed of 10 X WT, 10 X SIRT3 only, 10 X AR100Q, and 8 X AR100Q-SIRT3 male mice underwent analysis as indicated in B-D. **(B)** Total body weight (left) and percent weight change (right) starting at 6 weeks of age. * = difference between WT vs. AR100Q and AR100Q-SIRT3. **(C)** Forepaw and all four paws grip strength analysis starting at 6 weeks of age. * = difference between WT vs. AR100Q and AR100Q-SIRT3. **(D)** Kaplan–Meier curve of total days before AR100Q mice became moribund, and were subsequently sacrificed. **(A-D)** All statistical analysis show mean ± SD, and used a two-way ANOVA and Tukey's *post-hoc* (* = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$) unless otherwise noted. WT = wild type, SIRT3 = SIRT3-flag overexpression, AR24Q = non-polyQ-expanded AR, AR100Q = polyQ-expanded AR, AR100Q-SIRT3 = polyQ-expanded AR with SIRT3-flag overexpression.

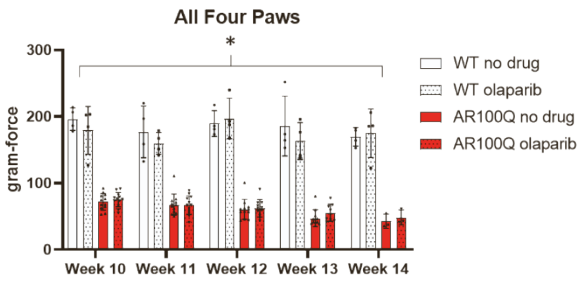
**A**


mice weight (grams)
40
30
20
10
0
*
Week 5
Week 6
Week 7
Week 8
Week 9
Week 10
Week 11
Week 12
Week 13
Week 14
WT no drug
WT olaparib
AR100Q no drug
AR100Q olaparib

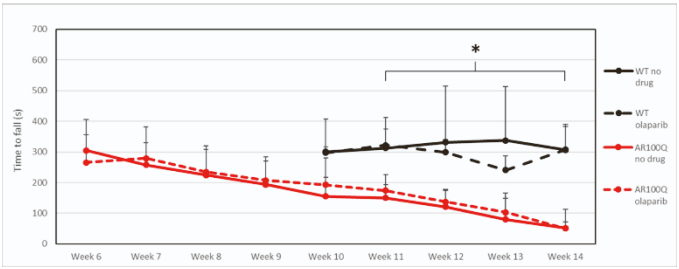

% weight change from 5 weeks
2.0
1.5
1.0
0.5
0.0
*
Week 5
Week 6
Week 7
Week 8
Week 9
Week 10
Week 11
Week 12
Week 13
Week 14
WT no drug
WT olaparib
AR100Q no drug
AR100Q olaparib

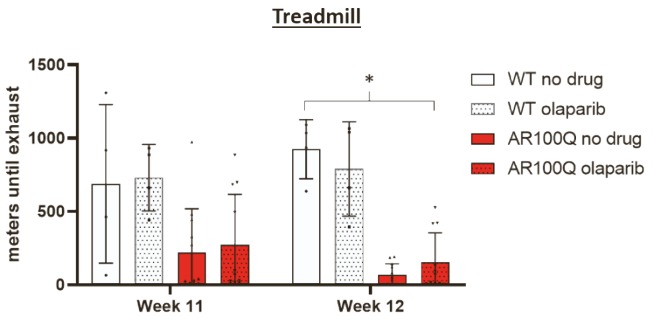
**B**

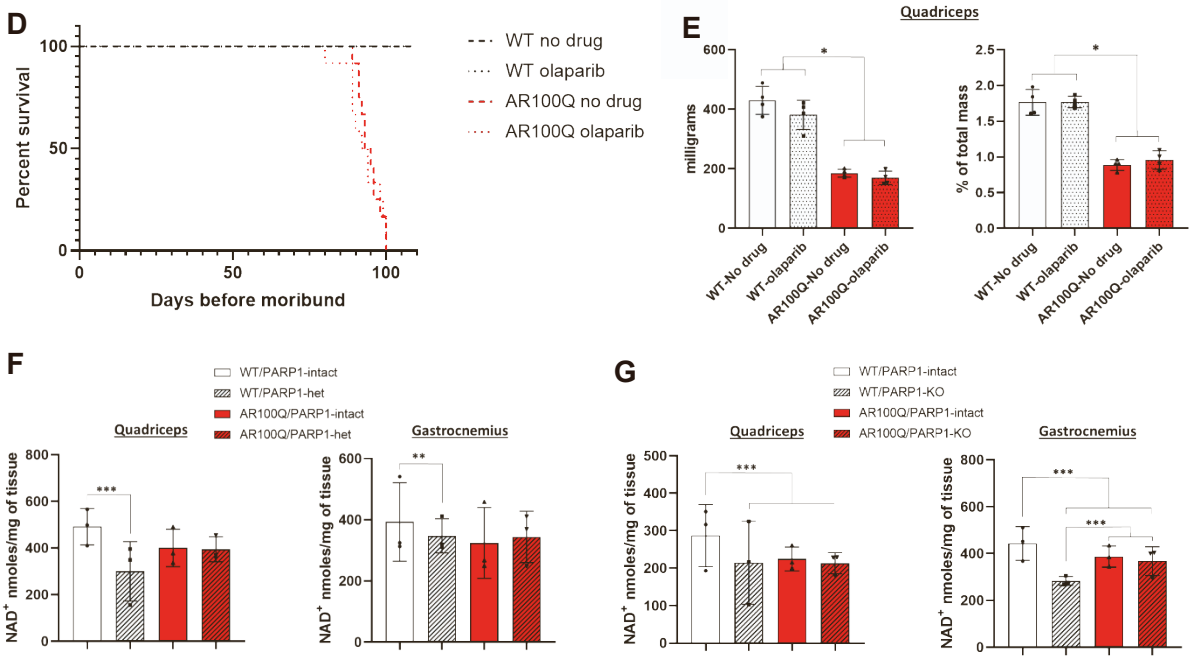

Forearm
gram-force
150
100
50
0
*
Week 10
Week 11
Week 12
Week 13
Week 14
WT no drug
WT olaparib
AR100Q no drug
AR100Q olaparib


All Four Paws
gram-force
300
200
100
0
*
Week 10
Week 11
Week 12
Week 13
Week 14
WT no drug
WT olaparib
AR100Q no drug
AR100Q olaparib

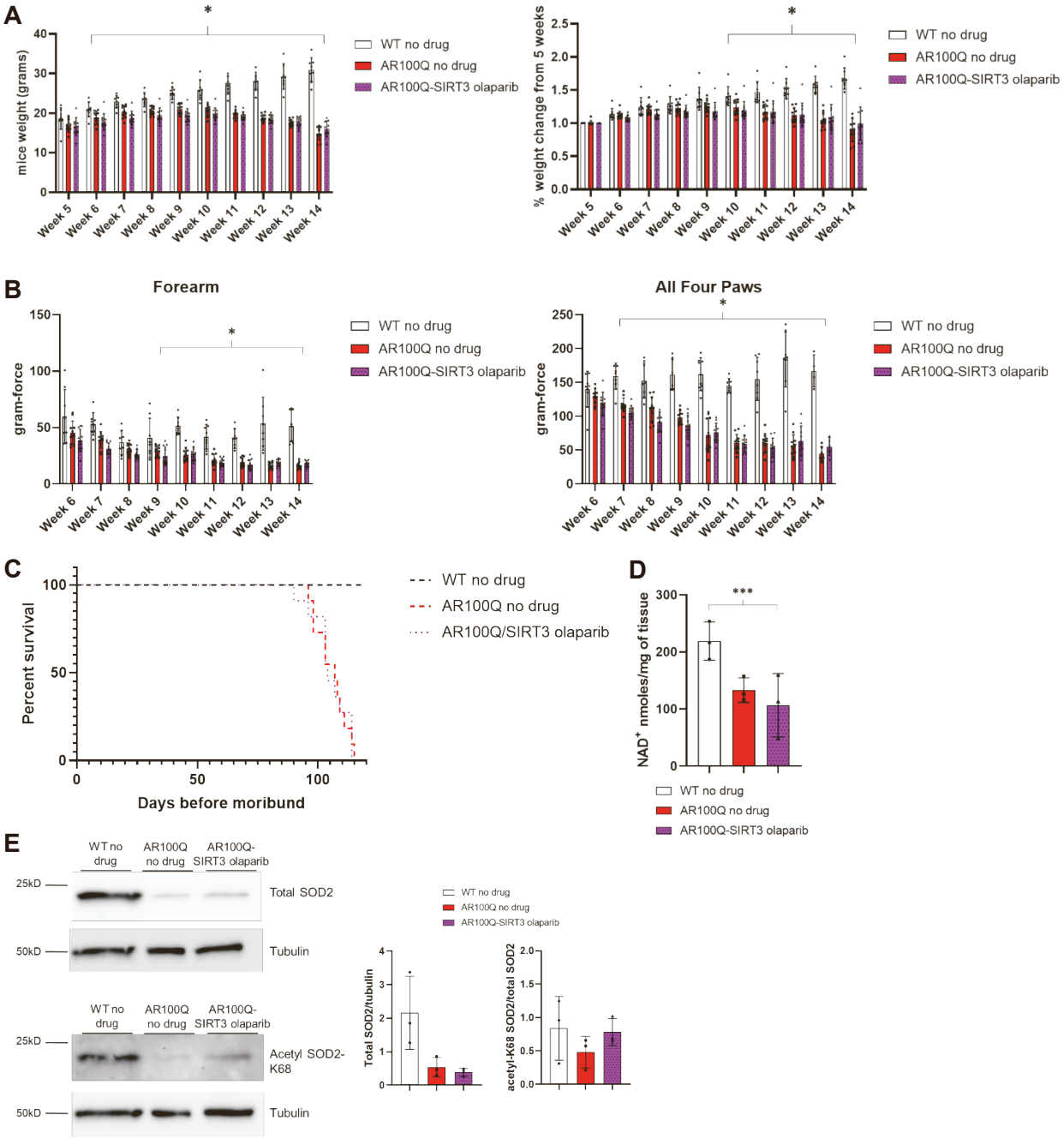
**C**


Rotarod
Time to fall (s)
700
600
500
400
300
200
100
0
*
Week 6
Week 7
Week 8
Week 9
Week 10
Week 11
Week 12
Week 13
Week 14
WT no drug
WT olaparib
AR100Q no drug
AR100Q olaparib

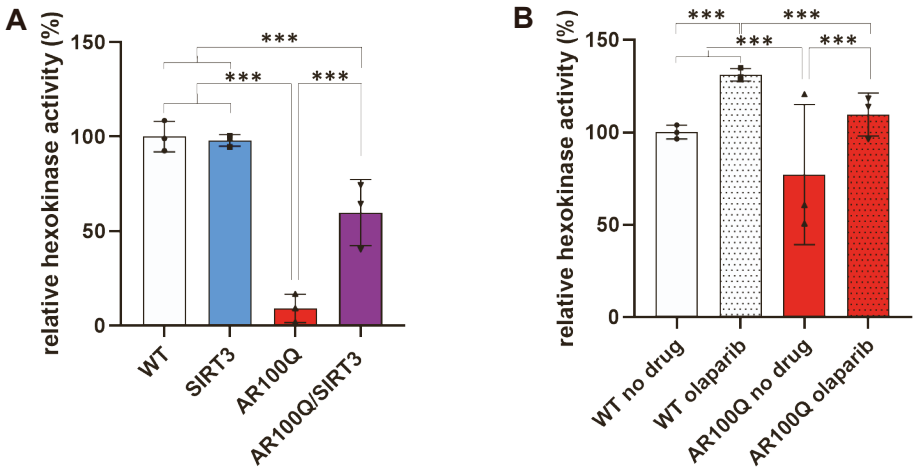

Treadmill
meters until exhaust
1500
1000
500
0
*
Week 11
Week 12
WT no drug
WT olaparib
AR100Q no drug
AR100Q olaparib




**Supplemental Figure 3, Related to Figure 3. Olaparib alone fails to rescue weight loss, motor dysfunction, and lifespan of AR100Q male mice. (A-D)** A behavioral cohort composed of 4 X WT-No drug, 4 X WT-olaparib, 11 X AR100Q-No drug, and 11 X AR100Q-olaparib male mice underwent analysis as indicated. **(A)** Weight (left) and percent weight change (right) from 5 weeks analysis starting at 5 weeks of age. * = difference between WT vs. AR100Q. **(B)** Forepaw and all four paws grip strength analysis starting at 10 weeks of age. * = difference between WT vs. AR100Q. **(C)** Top, accelerating rotarod analysis starting at 6 weeks of age; * = difference between WT vs. AR100Q. Bottom, accelerating treadmill analysis at 11 and 12 weeks of age. **(D)** Kaplan–Meier curve of total days before AR100Q mice became moribund, and were subsequently sacrificed. **(E)** Muscle mass (left) and percent of total mass (right) of quadriceps from WT and AR100Q male mice (n = 3) at 8 weeks of age fed no-drug compounded food or fed olaparib-compounded food. **(F)** Level of $NAD^+$ in quadriceps (left) and gastrocnemius (right) from WT and AR100Q male mice (n = 3) at 8 weeks of age with intact PARP-1 or with one allele of PARP-1 lost (PARP-1 het = heterozygous for PARP-1 gene). **(G)** Level of $NAD^+$ in quadriceps (left) and gastrocnemius (right) from WT and AR100Q male mice (n = 3) at 8 weeks of age with intact PARP-1 or with PARP-1 fully knocked out (PARP-1 KO = PARP-1 gene knock out). **(A-G)** All statistical analysis show mean ± SD, and used a two-way ANOVA and Tukey's *post-hoc* (* = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$) unless otherwise noted. WT = wild type, AR100Q = polyQ-expanded AR, olaparib = PARP inhibitor (1150 grams of olaparib in 1 kg of feed).




**Supplemental Figure 4, Related to Figure 4. Treating AR100Q-SIRT3 male mice with olaparib did not rescue weight loss, rotarod function, grip strength, or lifespan. (A-C)** A behavioral cohort composed of 8 X WT no-drug, 11 X AR100Q no-drug, and 11 X AR100Q-SIRT3 olaparib treated male mice or their muscle underwent analysis as indicated. **(A)** Weight (left) and percent weight change (right) from 5 weeks analysis starting at 5 weeks of age. * = difference between WT vs. AR100Q and AR100Q-SIRT3. **(B)** Forepaw and all four paws grip strength analysis starting at 6 weeks of age. * = difference between WT vs. AR100Q. **(C)** Kaplan–Meier curve of total days before AR100Q mice become moribund, and was subsequently sacrificed. **(D)** Level of $NAD^+$ in quadriceps dissected from WT, AR100Q, and AR100Q-SIRT3 male mice (n = 3) at 8 weeks of fed no-drug compounded food or fed olaparib-compounded food. **(E)** Left, immunoblots of total and acetylated (acetyl-K68) SOD2 and tubulin in quadriceps muscle of WT, AR100Q, and AR100Q-SIRT3 male mice (n = 3) at 8 weeks of age fed no-drug compounded food or fed olaparib-compounded food. Right, densitometry analysis of total SOD2 and acetyl-K68/total SOD2 after each were normalized to tubulin loading control. **(A-E)** All statistical analysis show mean ± SD, and used a two-way ANOVA and Tukey's *post-hoc* (* = $p \leq 0.05$). WT = wild type, AR100Q = polyQ-expanded AR, AR100Q-SIRT3 = polyQ-expanded AR with SIRT3-flag overexpression, olaparib = PARP inhibitor (1150 grams of olaparib in 1 kg of feed).




**Supplemental Figure 5, Related to Figure 6. Overexpressing SIRT3 alone or inhibiting PARPs with olaparib alone increases hexokinase activity in quadriceps of AR100Q mice. (A)** Levels of hexokinase in quadriceps from WT, SIRT3, AR100Q, and AR100Q-SIRT3 male mice (n = 3) at 11 weeks of age. **(B)** Levels of Hexokinase in quadriceps from WT, and AR100Q male mice (n = 3) at 11 weeks of age fed no-drug compounded or olaparib-compounded food. All statistical analysis show mean ± SD, and used a two-way ANOVA and Tukey's *post-hoc* (*** = $p \leq 0.001$). WT = wild type, SIRT3 = SIRT3-flag overexpression, AR100Q = polyQ-expanded AR, AR100Q-SIRT3 = polyQ-expanded AR with SIRT3-flag overexpression, olaparib = PARP inhibitor (1150 grams of olaparib in 1 kg of feed).