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Supplemental Information

Rapamycin Reverses Metabolic Deficits in Lamin A/C-Deficient Mice

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SUPPLEMENTAL FIGURES

Supplemental Figure S1, related to Figure 1.

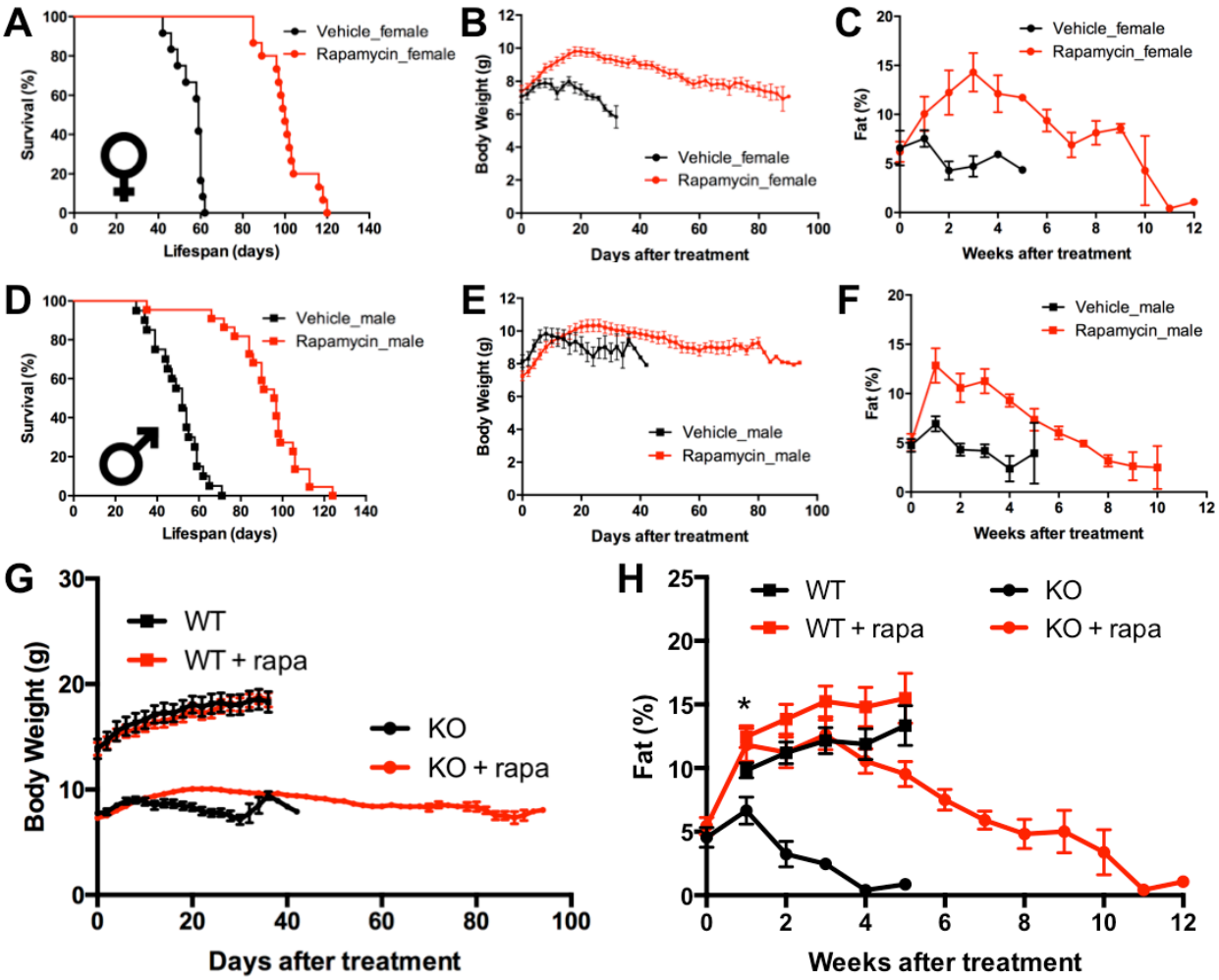

Figure S1. Survivorship and body composition of *Lmna*$^{-/-}$ mice treated with rapamycin, related to Figure 1. (A) Kaplan-Meier plot of female *Lmna*$^{-/-}$ mice that were treated with vehicle (n = 12; black) or 8 mg/kg rapamycin (n = 15; red) every other day via i.p. injection starting at 4 weeks of age and maintained for the remainder of their lifespan. Symbols represent individual mice. (B) Female *Lmna*$^{-/-}$ mice treated with vehicle (started with n = 12; black) or rapamycin (started with n = 16; red) were weighted every other day starting at 4 weeks of age. Symbols represent mean body weight (BW) ± standard error of the mean (SEM) for each treatment. (C) Adiposity (% body fat) was measured weekly [(fat mass/body weight) x 100] from mice treated with vehicle (started with n = 6; black) or rapamycin (started with n = 4; red). Symbols represent mean % body fat ± SEM for each treatment. (D) Kaplan-Meier plot of male *Lmna*$^{-/-}$ mice that were treated with vehicle (n = 20; black) or 8 mg/kg rapamycin (n = 22; red). (E) Male *Lmna*$^{-/-}$ mice treated with vehicle (started with n = 20; black) or rapamycin (started with n = 22; red) were weighted every other day starting at 4 weeks of age. (F) Adiposity was measured weekly from mice treated with vehicle (started with n = 11; black) or rapamycin (started with n = 7; red). (G) Mice treated with vehicle (WT, black square, n = 12; KO, black circle, started with n = 32) or rapamycin (WT, red square, n = 13; KO, red circle, started with n = 38) were weighed every other day starting at 4 weeks of age. Symbols represent mean BW ± SEM for each treatment. Data from males and females are combined. (H) Adiposity was measured weekly from mice treated with vehicle (WT, black square, n = 9; KO, black circle, started with n = 8) or rapamycin (WT, red square, n = 9; KO, red circle, started with n = 11). Data from males and females are combined. WT: wild type (*Lmna*$^{+/+}$). KO: knockout (*Lmna*$^{-/-}$). rapa: rapamycin. Symbols represent mean % body fat ± SEM. for each treatment. * $p < 0.05$, WT vs. WT + rapa.

Supplemental Figure S2, related to Figure 1.

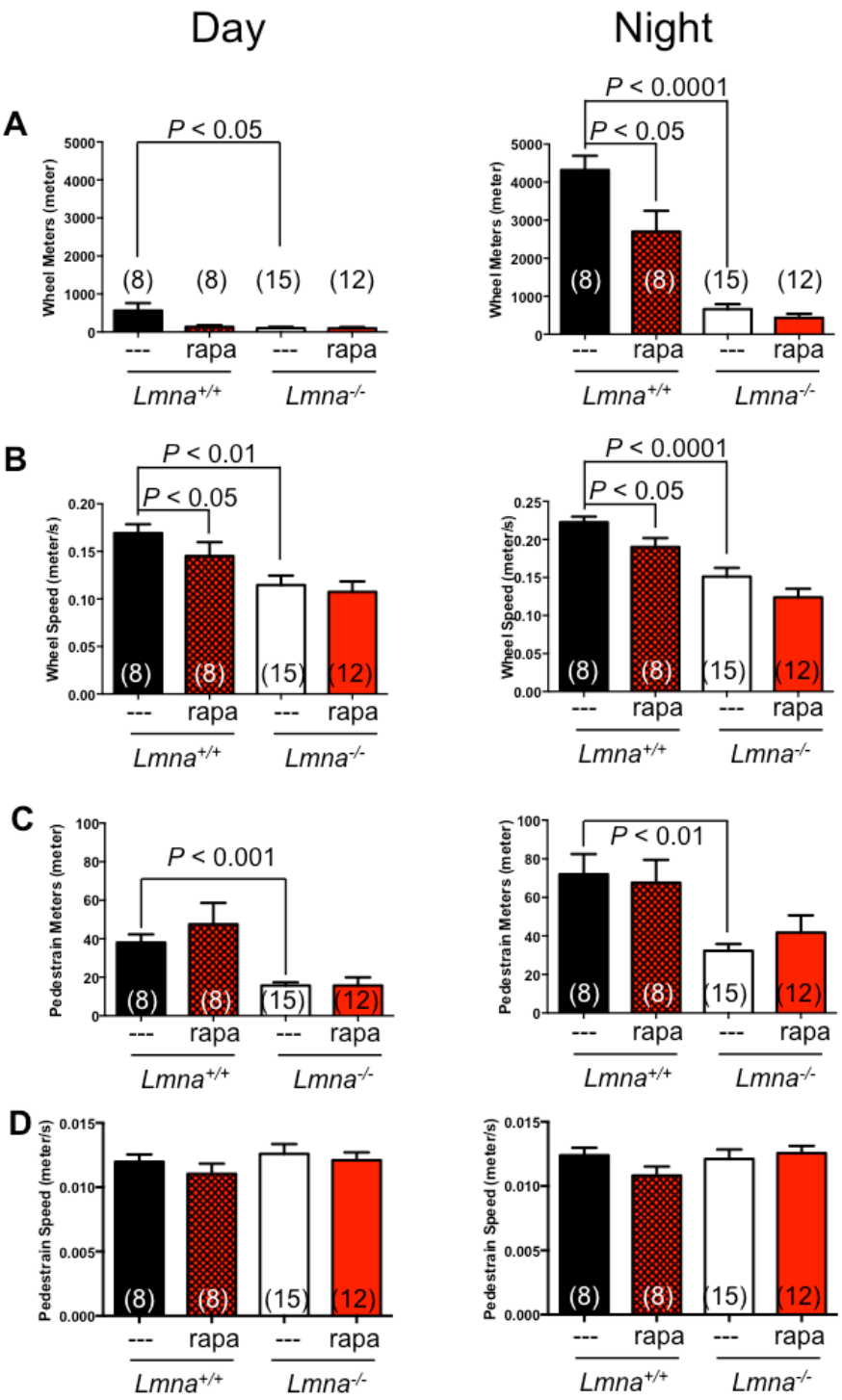

Figure S2. Behavioral activity of *Lmna*$^{+/+}$ and *Lmna*$^{-/-}$ mice treated with or without rapamycin. (A) Wheel meters of mice in the metabolic cage during day and night cycle. (B) Wheel speed of mice in the metabolic cage during day and night cycle. (C) Pedestrian meters of mice in the metabolic cage during day and night cycle. (D) Pedestrian speed of mice in the metabolic cage during day and night cycle. rapa: rapamycin. Each value is mean ± SEM for number of mice indicated in parentheses, and statistical significance was determined by the two-tailed unpaired Student's *t* test.

Supplemental Figure S3, related to Figure 2, 3, and 4.

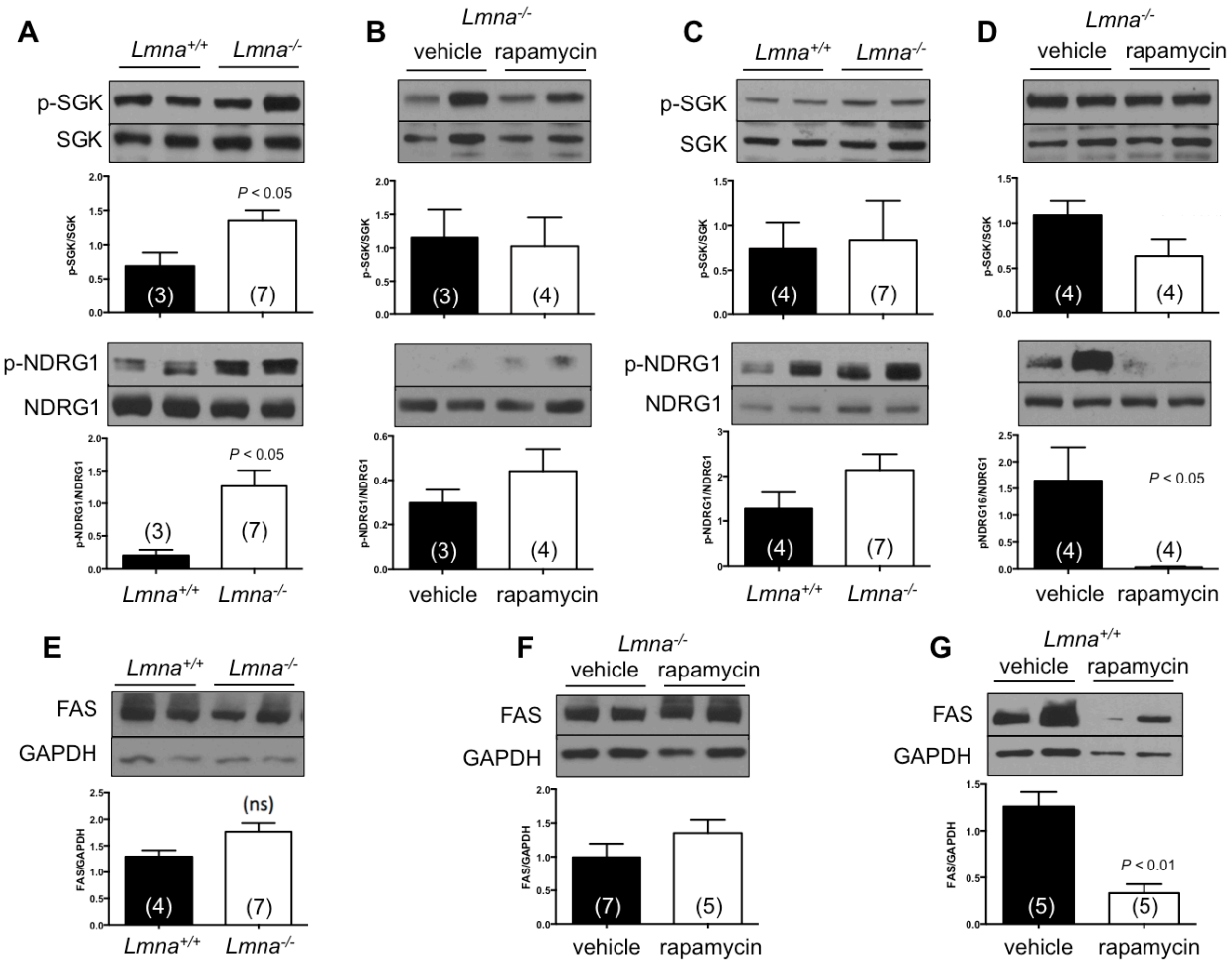

Figure S3. Role of mTORC2 activity and lipogenesis in adipose tissue of *Lmna*$^{-/-}$ mice treated with rapamycin. (A) Western blots of mTORC2 activity, indicated by phosphorylation of SGK and NDRG1, in white adipose tissue (WAT) of *Lmna*$^{+/+}$ and *Lmna*$^{-/-}$ mice. Relative p-SGK (normalized to SGK) and p-NDRG1 levels (normalized to NDRG1) were quantified. (B) Western blots of mTORC2 activity, indicated by phosphorylation of SGK and NDRG1, in WAT of *Lmna*$^{-/-}$ mice treated with or without rapamycin. Relative p-SGK (normalized to SGK) and p-NDRG1 levels (normalized to NDRG1) were quantified. (C) Western blots of mTORC2 activity, indicated by phosphorylation of SGK and NDRG1, in brown adipose tissue (BAT) of *Lmna*$^{+/+}$ and *Lmna*$^{-/-}$ mice. Relative p-SGK (normalized to SGK) and p-NDRG1 levels (normalized to NDRG1) were quantified. (D) Western blots of mTORC2 activity, indicated by phosphorylation of SGK and NDRG1, in BAT of *Lmna*$^{-/-}$ mice treated with or without rapamycin. Relative p-SGK (normalized to SGK) and p-NDRG1 levels (normalized to NDRG1) were quantified. (E) Western blots of lipogenesis, indicated by fatty acid synthase (FAS) level, in subcutaneous fat of *Lmna*$^{+/+}$ and *Lmna*$^{-/-}$ mice. Relative FAS levels (normalized to GAPDH) were quantified. (F) Western blots of lipogenesis, indicated by FAS level, in WAT of *Lmna*$^{-/-}$ mice treated with or without rapamycin. Relative FAS levels (normalized to GAPDH) were quantified. (G) Western blots of lipogenesis, indicated by FAS level, in WAT of *Lmna*$^{+/+}$ mice treated with or without rapamycin. Relative FAS levels (normalized to GAPDH) were quantified. Each value is mean ± SEM for the number of mice indicated in parentheses. *P* values were derived from unpaired two-tailed Student's t-test. ns: no significance.

Supplemental Figure S4, related to Figure 1 and 5.

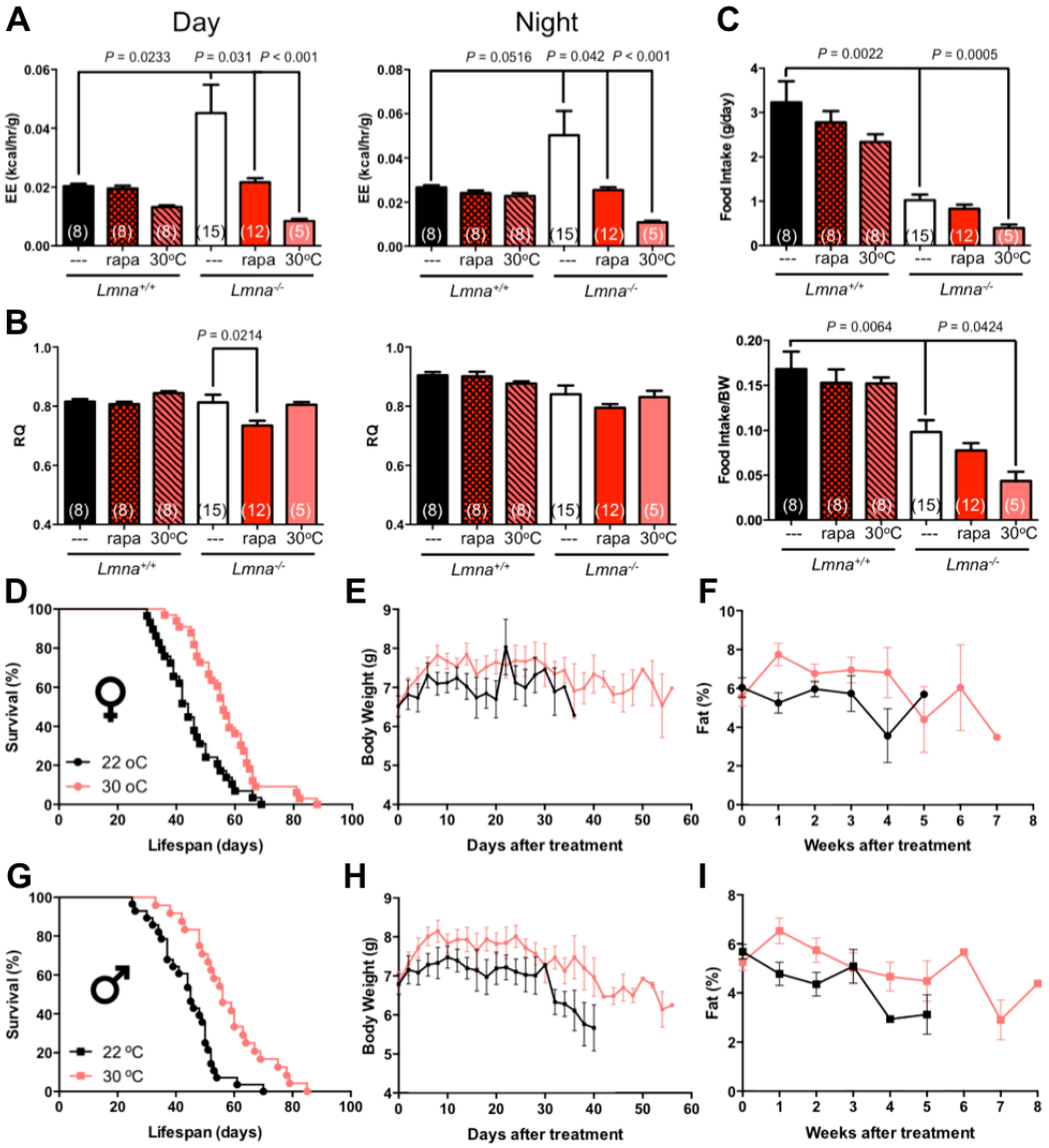

Figure S4. Higher housing temperature increased the survival of *Lmna*$^{-/-}$ mice, related to Figure 1 and 5. (A) Energy expenditure (EE) of *Lmna*$^{+/+}$ and *Lmna*$^{-/-}$ mice treated with rapamycin (rapa) or 30 ^{o}C during day and night cycle. (B) Respiratory quotient (RQ) of *Lmna*$^{+/+}$ and *Lmna*$^{-/-}$ mice treated with rapamycin or 30 ^{o}C during day and night cycle. (C) Food intake and food intake normalized with body weight (BW) of *Lmna*$^{+/+}$ and *Lmna*$^{-/-}$ mice treated with rapamycin or 30 ^{o}C. Each value is mean ± SEM for the number of mice indicated in parentheses, and statistical significance was determined by the two-tailed unpaired Student's *t* test. (D) Kaplan-Meier plot of female *Lmna*$^{-/-}$ mice that were treated at 22 ^{o}C (n = 28; black) or 30 ^{o}C (n = 24; pink) starting at 4 weeks of age and maintained for the remainder of their lifespan. Symbols represent individual mice. (E) Female *Lmna*$^{-/-}$ mice treated at 22 ^{o}C (started with n = 28; black) or 30 ^{o}C (started with n = 24; pink) were weighed every other day starting at 4 weeks of age. Symbols represent mean BW ± SEM for each treatment. (F) Adiposity (% body fat) was measured weekly [(fat mass/BW) x 100] from female *Lmna*$^{-/-}$ mice treated at 22 ^{o}C (started with n = 29; black) or 30 ^{o}C (started with n = 24; pink). Symbols represent mean % body fat ± SEM. (G) Kaplan-Meier plot of male *Lmna*$^{-/-}$ mice that were treated at 22 ^{o}C (n = 29; black) or 30 ^{o}C (n = 33; pink) starting at 4 weeks of age. (H) Male *Lmna*$^{-/-}$ mice treated at 22 ^{o}C (started with n = 29; black) or 30 ^{o}C (started with n = 32; pink) were weighted every other day starting at 4 weeks of age. (I) Adiposity was measured weekly from male *Lmna*$^{-/-}$ mice treated at 22 ^{o}C (started with n = 29; black) or 30 ^{o}C (started with n = 31; pink).

Supplemental Figure S5.

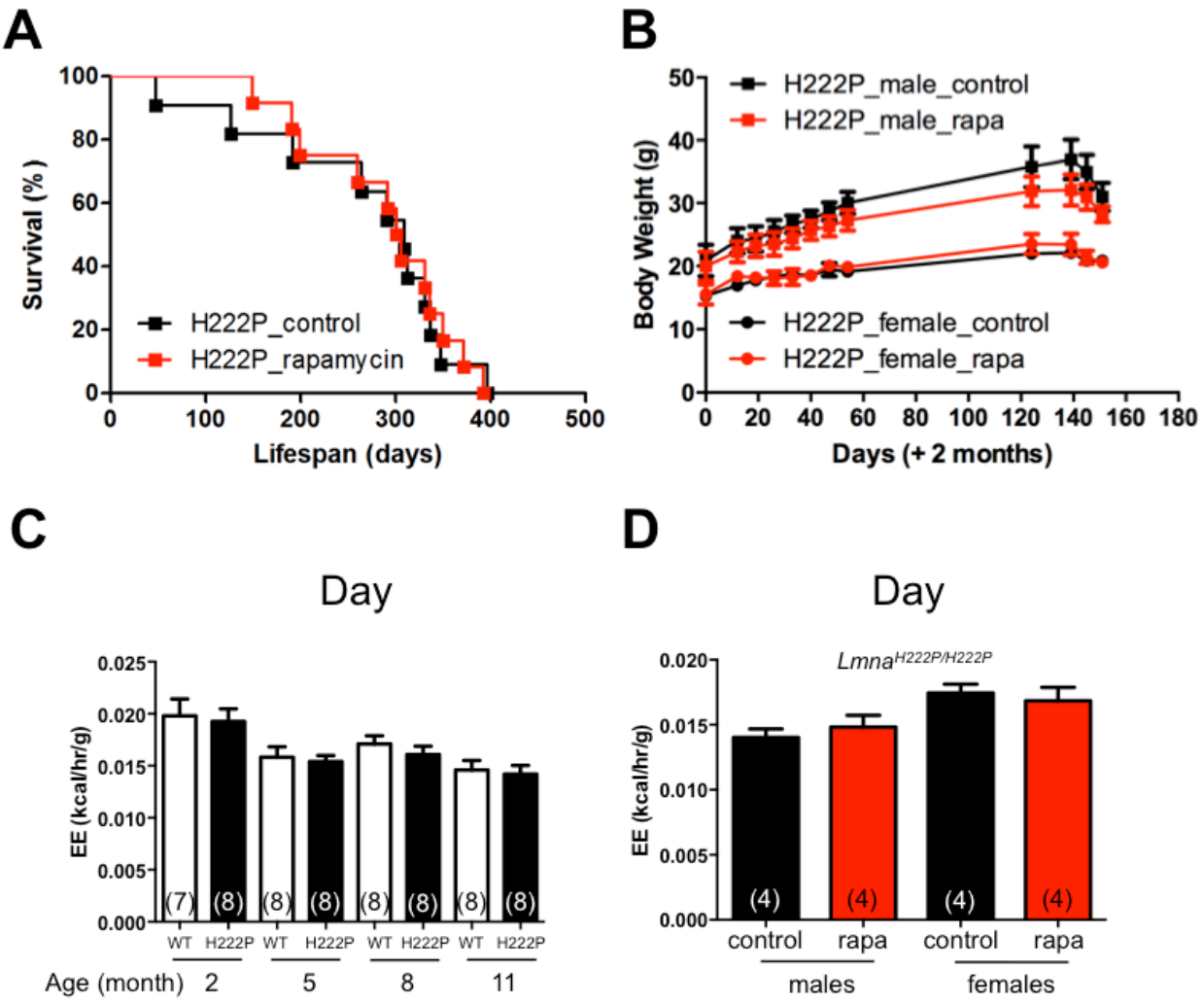

Figure S5. Rapamycin does not enhance the survival of *Lmna*$^{H222P/H222P}$ mice. (A) Kaplan-Meier plot of *Lmna*$^{H222P/H222P}$ mice that were treated with control chow (n = 11; black) or rapamycin chow (n = 12; red) starting at 2 months of age and maintained for the remainder of their lifespan. Symbols represent individual mice. Data from males and females are combined. (B) Body weight (BW) from multiple age points did not show a difference between control (black) and rapamycin (red) treated *Lmna*$^{H222P/H222P}$ mice. Symbols represent mean BW ± SEM for each treatment. (C) Energy expenditure (EE) is no different between *Lmna*$^{H222P/H222P}$ (H222P) mice and wild type (WT; *Lmna*$^{+/+}$) mice with age. (D) Five months of rapamycin (rapa) treatment started in 2 months old of *Lmna*$^{H222P/H222P}$ mice did not affect the EE. Each value is mean ± SEM for the number of mice indicated in parentheses.