

## Reducing mitochondrial ribosomal gene expression does not alter metabolic health or lifespan in mice | Supplemental File

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### **Figure S1 | Protocol and timeline for metabolic phenotyping of *Mrpl54*<sup>+/-</sup> and WT mouse cohorts**

Metabolic phenotyping in 6-, 18-, and 24-month male and female cohorts began with an assessment of body composition (EchoMRI) followed by 24-48-hour measurement of VO<sub>2</sub>, RER, and ambulatory motion (CLAMS). Each cohort was then assessed using the following tests with 1-2 weeks between each test, in order: blood glucose tolerance (OGTT), intraperitoneal insulin tolerance (iITT), cold challenge (4°C), heart rate and blood pressure monitoring (18M and 24M only), and endurance treadmill, ending with a necropsy and tissue, organ, and plasma collection. Images were created with BioRender.com.

### **Figure S2 | 6M *Mrpl54*<sup>+/-</sup> and WT female body composition as a function of body mass**

No difference in body composition as function of body mass between 6M female *Mrpl54*<sup>+/-</sup> and WT mice. Graphs show mean ± SEM; female WT (orange); and female *Mrpl54*<sup>+/-</sup> (light orange).

### **Figure S3 | Change in *Mrpl54*<sup>+/-</sup> and WT body composition at age 6-, 18-, and 24-months**

Body composition (g) by EchoMRI with increasing age for both male and female *Mrpl54*<sup>+/-</sup> and WT mice. Graphs show mean ± SEM; male WT (blue); male *Mrpl54*<sup>+/-</sup> (light blue); female WT (orange); and female *Mrpl54*<sup>+/-</sup> (light orange). \*\*P ≤ 0.01, \*\*\*P ≤ 0.001, and \*\*\*\*P ≤ 0.0001

### **Figure S4 | Body composition and metabolic testing results in 18M aged male and female mice: *Mrpl54*<sup>+/-</sup> versus WT**

**a)** Body composition (g) by EchoMRI in 18M aged mice showed no difference in total body, fat, or lean mass between either male *Mrpl54*<sup>+/-</sup> (n=11) and WT (n=13) or female *Mrpl54*<sup>+/-</sup> (n=12) and WT (n=12).

**b)** Mean VO<sub>2</sub> (L/hour) by CLAMS and body mass ANCOVA analysis (insets) in 18M aged mice showed no differences for 24-hour, light, or dark periods between either male *Mrpl54*<sup>+/-</sup> (n=11) and WT (n=12) or female *Mrpl54*<sup>+/-</sup> (n=12) and WT (n=12).

**c)** Mean RER by CLAMS in 18M aged mice showed no difference between either male *Mrpl54*<sup>+/-</sup> (n=11) and WT (n=12) or female *Mrpl54*<sup>+/-</sup> (n=12) and WT (n=12).

**d)** Mean ambulatory motion (beam break counts) in 18M aged mice showed no difference between either male *Mrpl54*<sup>+/-</sup> (n=11) and WT (n=12) or female *Mrpl54*<sup>+/-</sup> (n=12) and WT (n=12).

**e)** Mean duration (minutes) on an endurance treadmill for 18M aged mice showed no difference in endurance time between either male *Mrpl54*<sup>+/-</sup> (n=9) and WT (n=11) or female *Mrpl54*<sup>+/-</sup> (n=10) and WT (n=9).

**f)** Mean blood glucose level (mmol/L) over time in response to an oral glucose challenge (OGTT) in 18M aged mice showed no difference between either male *Mrpl54*<sup>+/-</sup> (n=11) and WT (n=12) or female *Mrpl54*<sup>+/-</sup> (n=11) and WT (n=12).

**g)** Mean blood glucose level (mmol/L) and AUC (insets) over time in response to intraperitoneal insulin challenge (iITT) in 18M aged mice showed no differences between either male *Mrpl54*<sup>+/-</sup> (n=12) and WT (n=13) or female *Mrpl54*<sup>+/-</sup> (n=10) and WT (n=11).

**h)** Mean rectal temperature (°C) in response to a 4-hour 4°C cold challenge in 18M aged mice showed no difference between either male *Mrpl54*<sup>+/-</sup> (n=10) and WT (n=9) or female *Mrpl54*<sup>+/-</sup> (n=10) and WT (n=9).

**i)** Mean heart rate (beats/min) and systolic blood pressure (mmHg) in 18M aged mice showed no differences between either male *Mrpl54*<sup>+/-</sup> (n=10) and WT (n=11) or female *Mrpl54*<sup>+/-</sup> (n=10) and WT (n=7). Diastolic blood pressure (mmHg) at 18M was higher in male *Mrpl54*<sup>+/-</sup> compared to WT, with no differences between female *Mrpl54*<sup>+/-</sup> and WT.

**j)** Relative organ weights (% body mass) in 18M aged necropsied mice showed no differences between either male *Mrpl54*<sup>+/-</sup> (n=7) and WT (n=12) or female *Mrpl54*<sup>+/-</sup> (n=10) and WT (n=7). Graphs show mean ± SEM; \*P ≤ 0.05, male WT (blue); male *Mrpl54*<sup>+/-</sup> (light blue); female WT (orange); and female *Mrpl54*<sup>+/-</sup> (light orange).

#### **Figure S5 | 24-month life expectancy in *Mrpl54*<sup>+/-</sup> versus WT males by housing conditions**

**a)** Kaplan Meier survival curves show no difference in 24-month life expectancy (where all mice alive after 24-month are censored) in group-housed males between *Mrpl54*<sup>+/-</sup> (n=42) and WT (n=72).

**b)** Kaplan Meier survival curves show a suggested increase in 24-month life expectancy in individual-housed *Mrpl54*<sup>+/-</sup> (n=39) males compared to WT (n=40).

#### **Figure S6 | Original uncropped western blot images**

**a)** Original uncropped images of western blot (Fig. 5b) of isolated liver mitochondria immunofluorescence with HSP60 antibody (left), protein standard ladder (centre), and stain free gel (right).

**b)** Original uncropped images of western blot (Fig. 6a and 6b) of myoblast protein lysate immunofluorescence with OXPHOS antibody cocktail (top left and right), HSP60 antibody (bottom left), and stain free blot (bottom right).

**c)** Original uncropped images of western blot (Fig. 6a taken from the 4µg loading lanes) of increasing concentration of myoblast protein lysate immunofluorescence with OXPHOS antibody cocktail (left) and stain-free gel (right).

Fig. S1

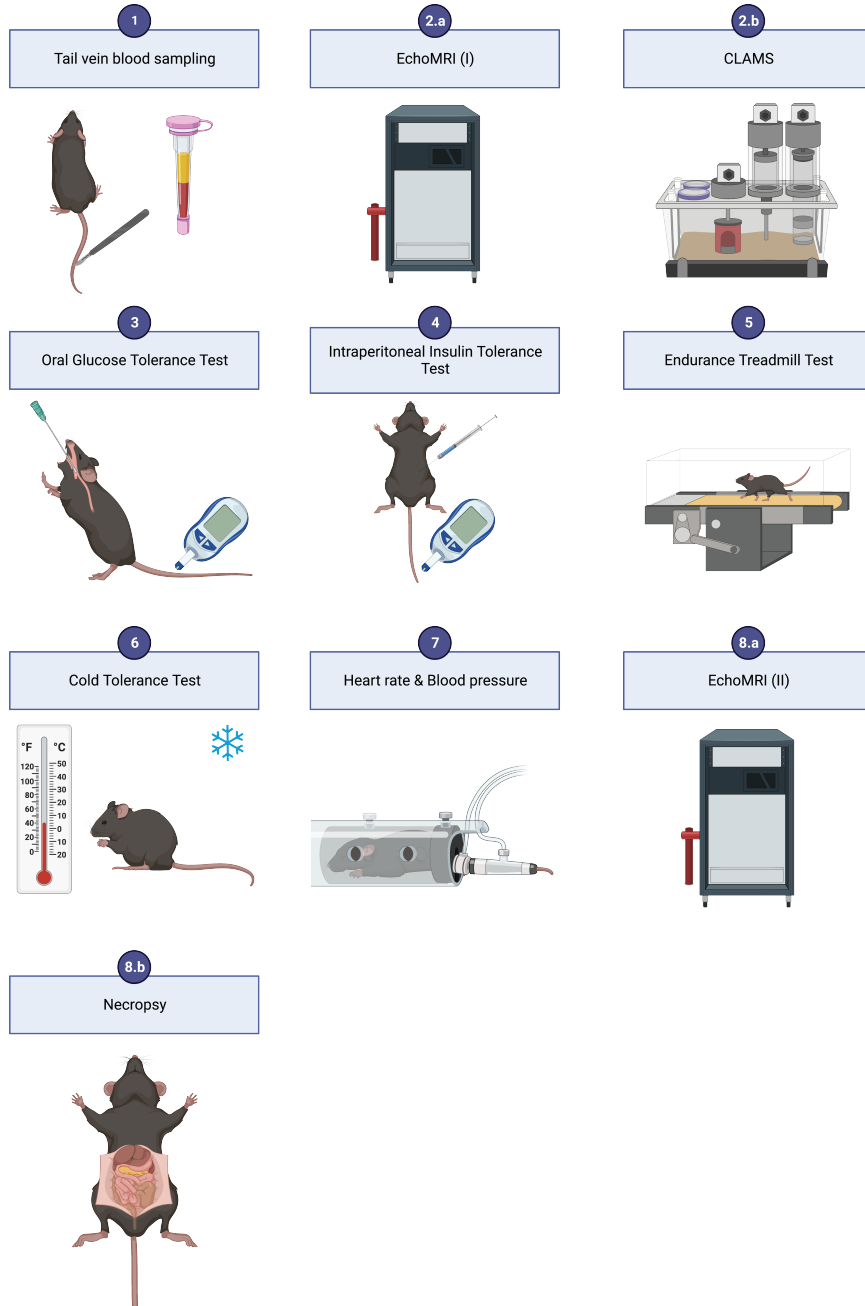


Fig. S2

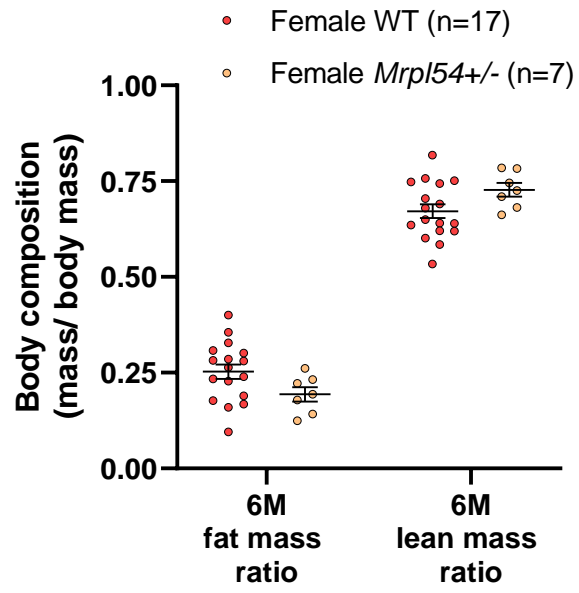


Fig. S3

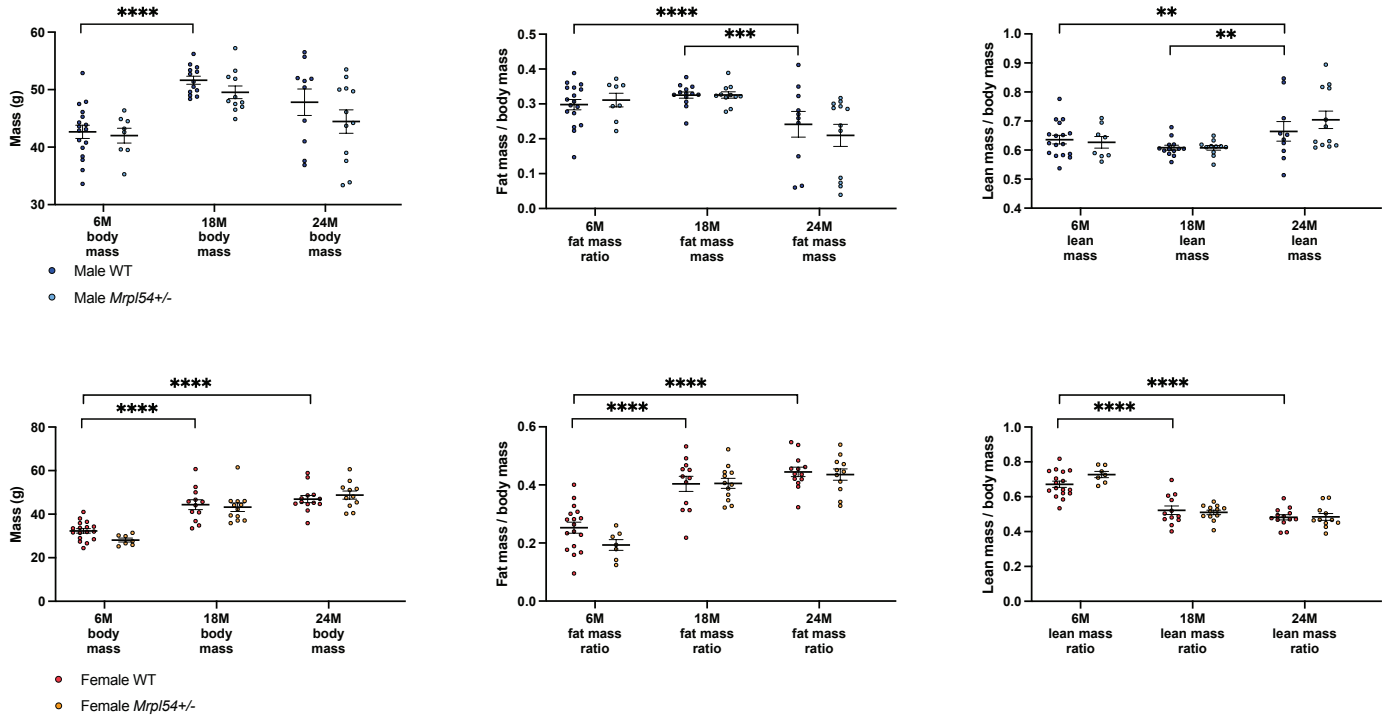


Fig. S4

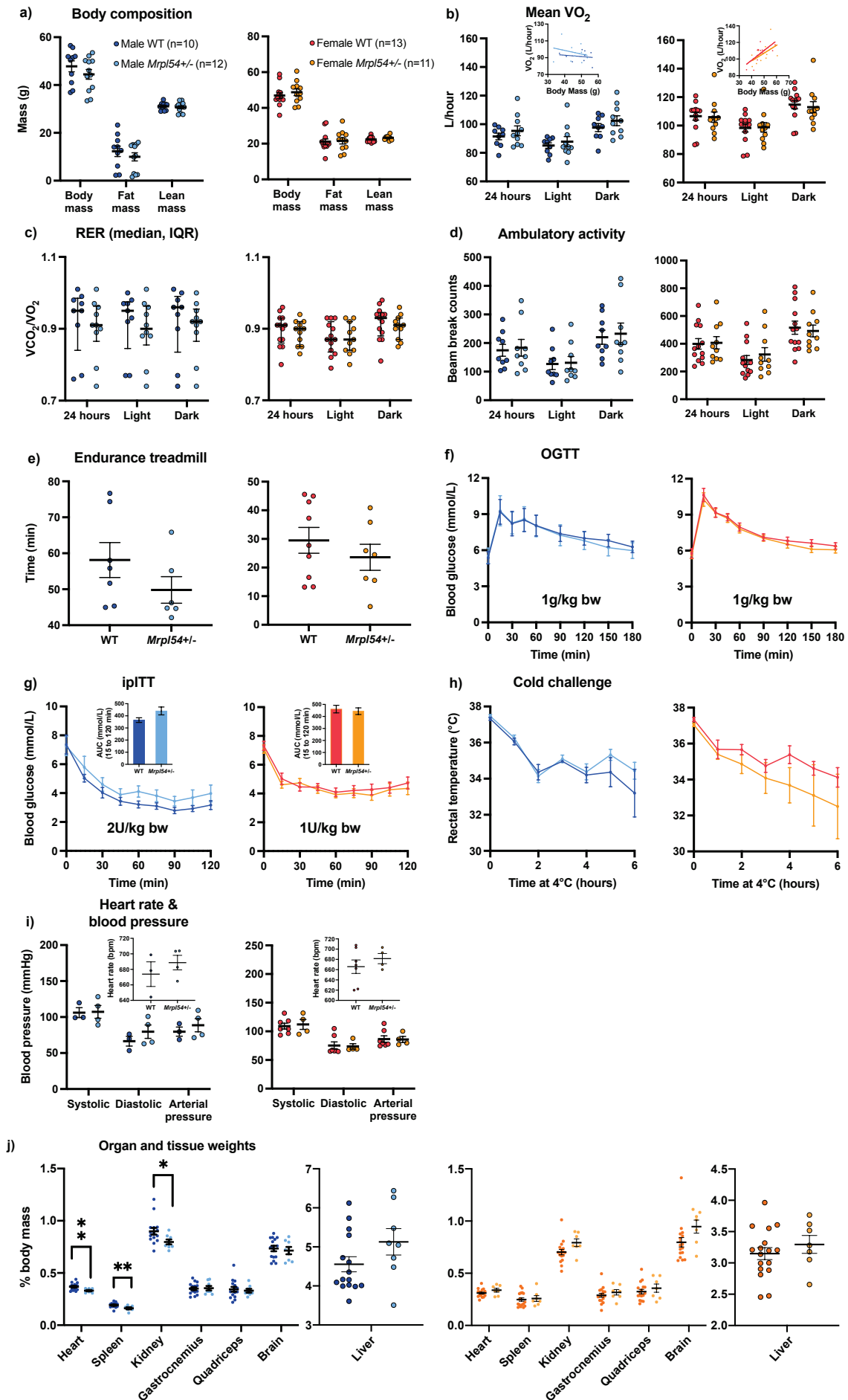


Fig. S5

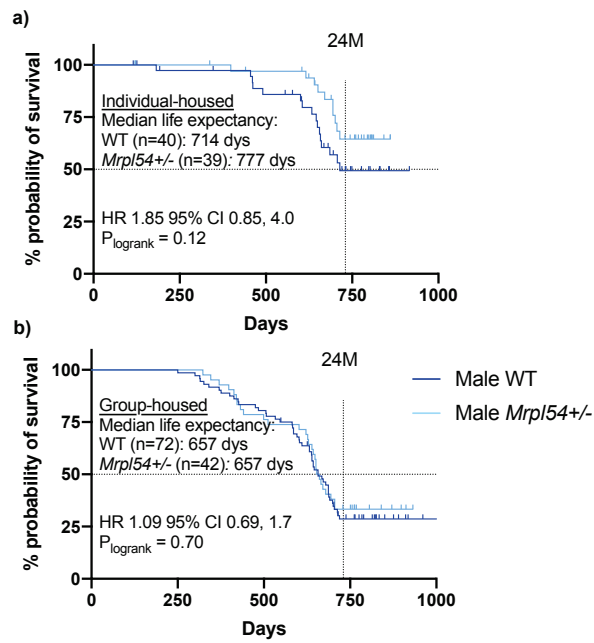
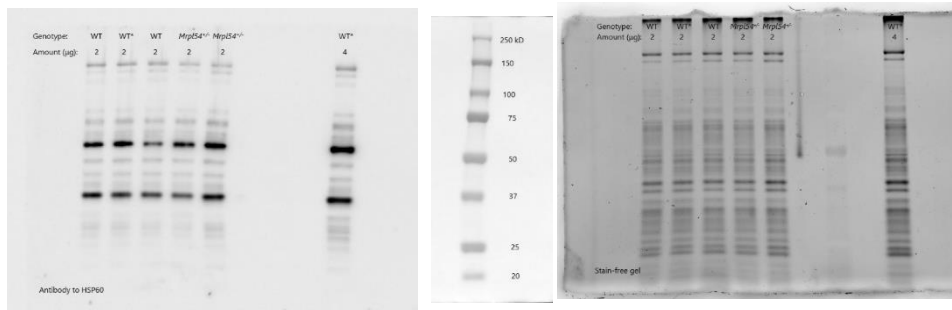


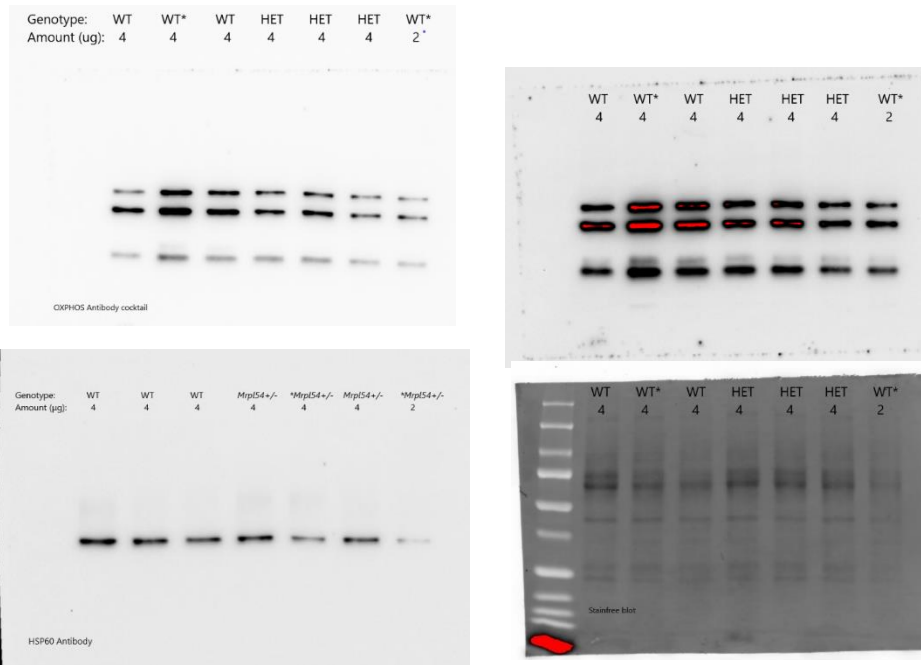


Fig. S6

a)



b)



c)

