Ketone body β-hydroxybutyrate is an autophagy-dependent vasodilator

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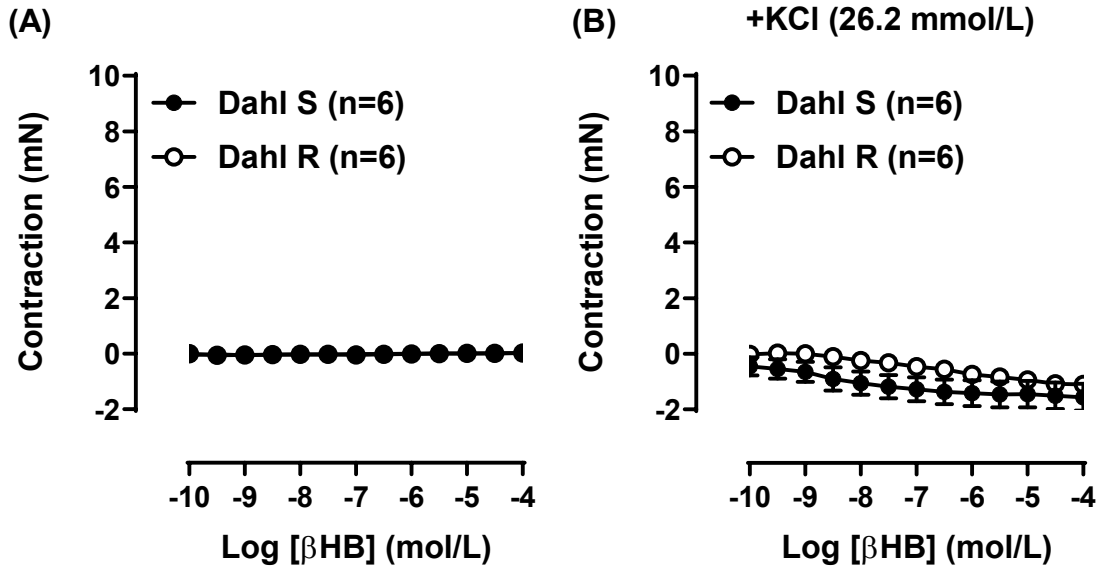
Conflict of interest statement

The authors have declared that no conflict of interest exists.

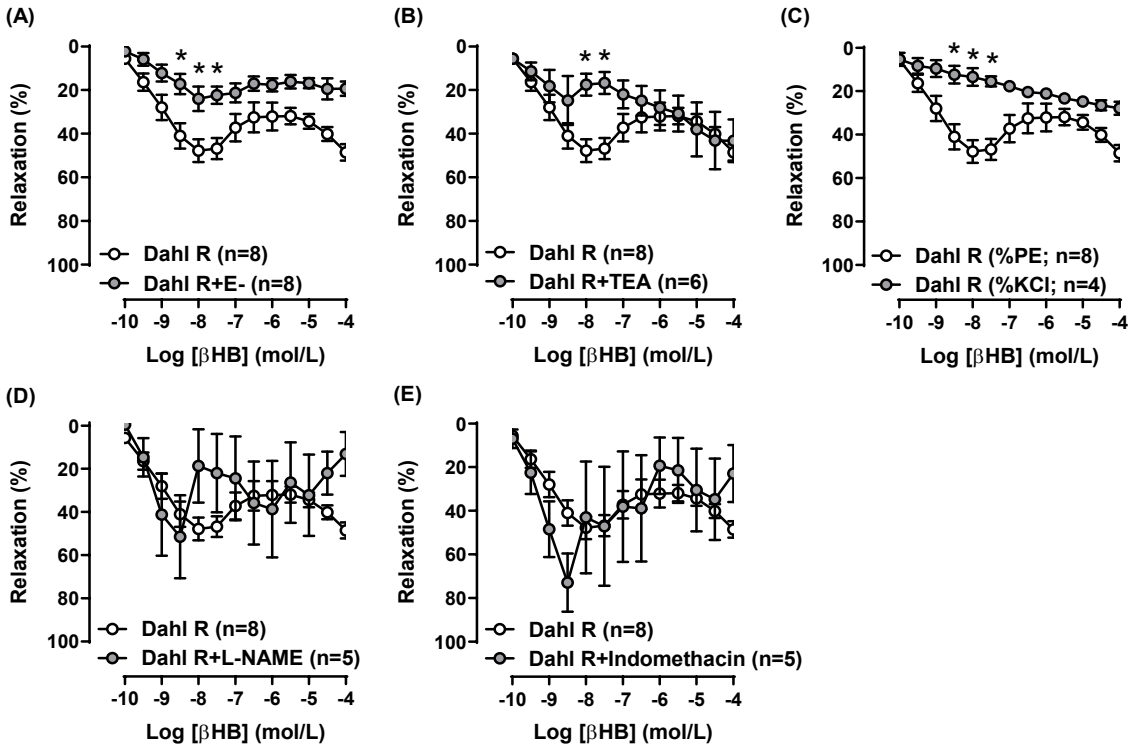
SUPPLEMENTARY FIGURES AND LEGENDS

Supplementary figure 1.



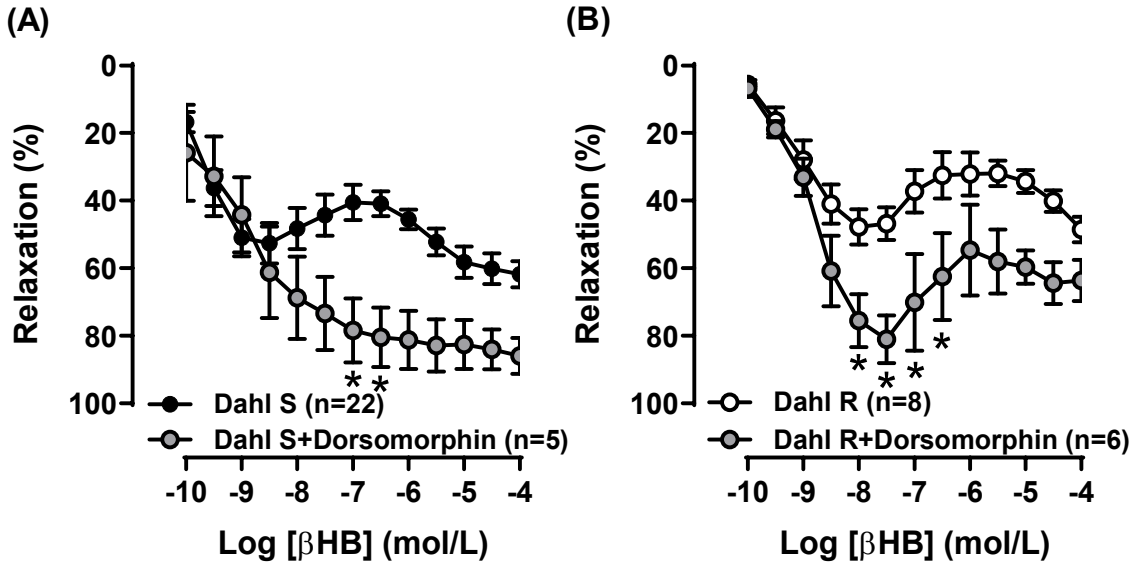
Supplementary figure 1. β-hydroxybutyrate (βHB) does not cause vascular contraction. Contraction concentration-response curves to βHB in mesenteric resistance arteries from Dahl S and Dahl R rats with basal tone (A) and after contraction to a low depolarizing concentration of potassium chloride (KCl, 26.2 mmol/L) (B). Mean ± SEM. n=6.

Supplementary figure 2.



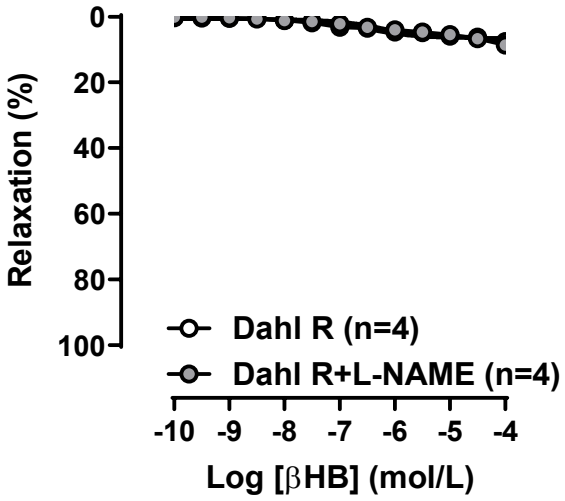
Supplementary figure 2. Concentration-response curves to β-hydroxybutyrate (βHB) in mesenteric resistance arteries from Dahl R rats. Some arteries were endothelium denuded (E-) (A), incubated with tetraethylammonium (TEA, 10 mmol/L) (B), contracted to potassium chloride (KCl, 120 mmol/L), as opposed to phenylephrine (10 µmol/L) (C), incubated with Nω-Nitro-L-arginine methyl ester (L-NAME, 100 µmol/L) (D), or incubated with indomethacin (10 µmol/L) (E). Mean ± SEM. n=4-8. Two-way ANOVA: *p<0.05.

Supplementary figure 3.



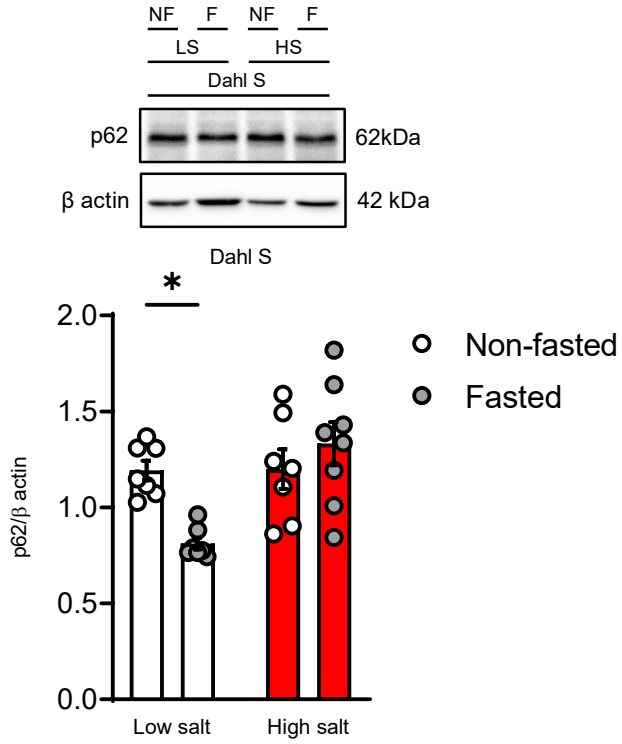
Supplementary figure 3. β-hydroxybutyrate (βHB)-dependent vasodilation is not mediated by AMPKα. Concentration-response curves to βHB in mesenteric resistance arteries from Dahl S (A) and Dahl R (B) rats, with and without the AMPKα inhibitor dorsomorphin (10 μmol/L). Mean ± SEM. n=5-21. Two-way ANOVA: *p<0.05.

Supplementary figure 4.



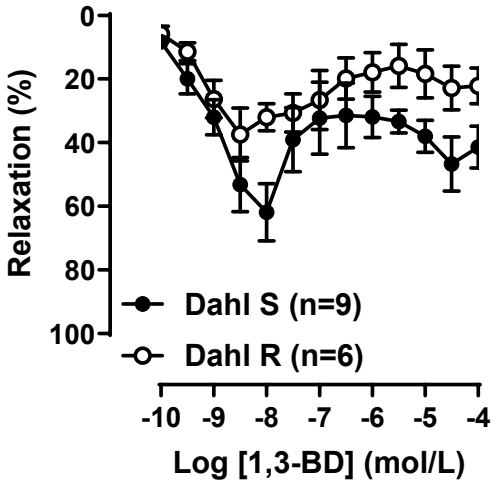
Supplementary figure 4. β-hydroxybutyrate (βHB)-dependent causes minimal nitric oxide-independent vasodilation in aorta. Concentration-response curves to βHB in aortic segments from Dahl R rats, with and without Nω-Nitro-L-arginine methyl ester (L-NAME, 100 μmol/L). Mean ± SEM. n=4.

Supplementary figure 5.



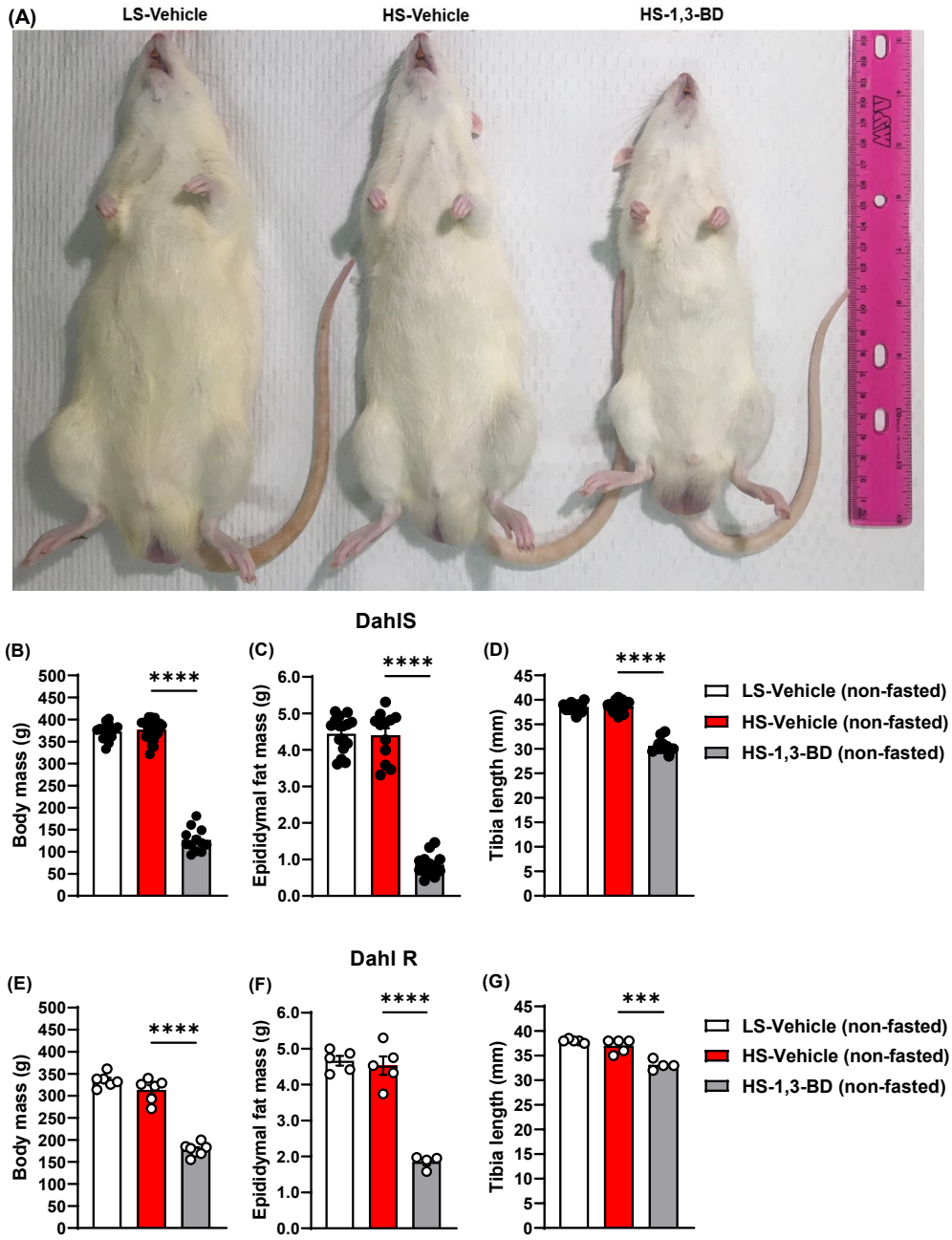
Supplementary figure 5. High salt diet negatively regulates autophagic activity. Protein expression analysis was performed for p62 normalized to β actin in liver biopsies from non-fasted (free access to food) and fasted (24 h) Dahl S rats that had consumed a low or high salt diet for 8 weeks. *Above*, representative images of immunoblots; *below*, densitometric analysis. Mean ± SEM. n=7-8. Two-way ANOVA: *p<0.05.

Supplementary figure 6.



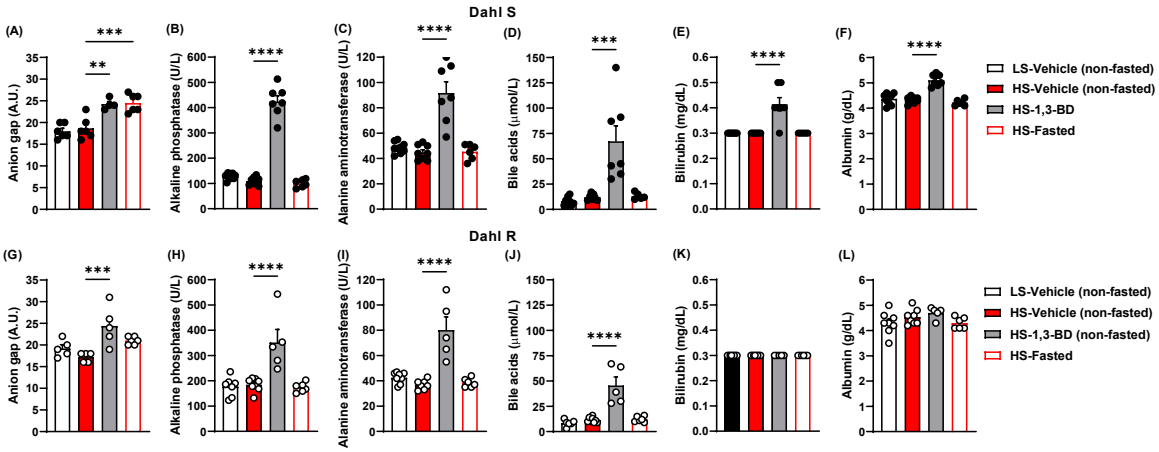
Supplementary figure 6. 1,3-Butanediol (1,3-BD) causes potent vasodilation, similar to β-hydroxybutyrate (βHB). Concentration-response curves to 1,3-BD in mesenteric resistance arteries from Dahl S (A) and Dahl R (B) rats. Mean ± SEM. n=6-9. Two-way ANOVA: *$p<0.05$.

Supplementary figure 7.



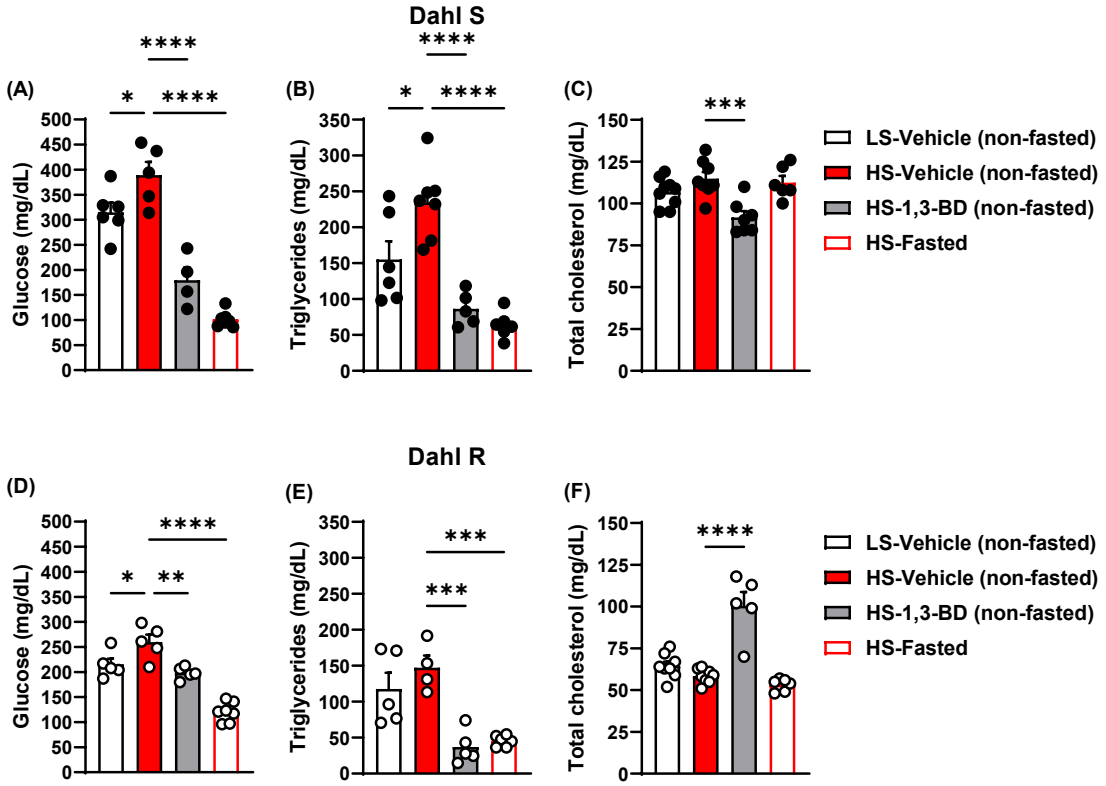
Supplementary figure 7. Reconstitution of β-hydroxybutyrate (βHB) bioavailability with 1,3-butanediol (1,3-BD) stunts growth. Dahl S and Dahl R rats were treated with either a low salt (LS) diet, a high salt (HS) diet, or a HS diet in conjunction with 1,3-BD (20% *v/v*) for eight weeks. Pictured are Dahl R rats after treatment (A). Body mass (B and E), epididymal fat mass (C and F), and tibia length (D and G) were measured in Dahl S and Dahl R rats, respectively. Mean ± SEM. n=4-28. One-way ANOVA: ***p<0.001, ****p<0.0001.

Supplementary figure 8.



Supplementary figure 8. 1,3-Butanediol (1,3-BD) treatment causes metabolic acidosis and hepatotoxicity. Dahl S and Dahl R rats were treated with either a low salt (LS) diet, a high salt (HS) diet, or a HS diet in conjunction with 1,3-BD (20% *v/v*) for eight weeks. Some HS-fed rats from both strains were fasted for 24 h as a positive control. The anion gap (A and G), liver enzymes alkaline phosphatase (B and H) and alanine aminotransferase (C and I), bile acids (D and J), bilirubin (E and K), and albumin (F and L) were measured in serum from Dahl S and Dahl R rats, respectively. Mean ± SEM. n=4-10. One-way ANOVA: **p<0.01, ***p<0.001, ****p<0.0001.

Supplementary figure 9.



Supplementary figure 9. 1,3-Butanediol (1,3-BD) is a caloric restriction mimetic. Dahl S and Dahl R rats were treated with either a low salt (LS) diet, a high salt (HS) diet, or a HS diet in conjunction with 1,3-BD (20% *v/v*) for eight weeks. Some HS-fed rats from both strains were fasted for 24 h as a positive control. Circulating glucose (A and D), triglycerides (B and E), and total cholesterol (C and F) were measured in serum from Dahl S and Dahl R rats, respectively. Mean ± SEM. n=4-9. One-way ANOVA: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

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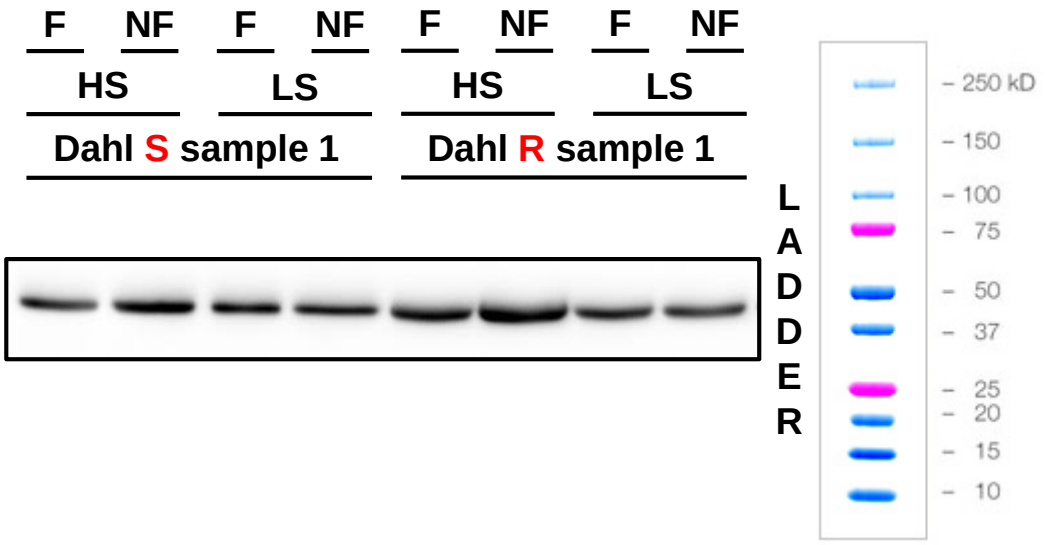
Uncropped Western bots

McCarthy *et al.*

Submission to JCI Insights

How to read the uncropped/unrotated membranes: Set 1 loading

NF: Non-fasting
F: Fasting
LS: Low-salt
HS: High salt

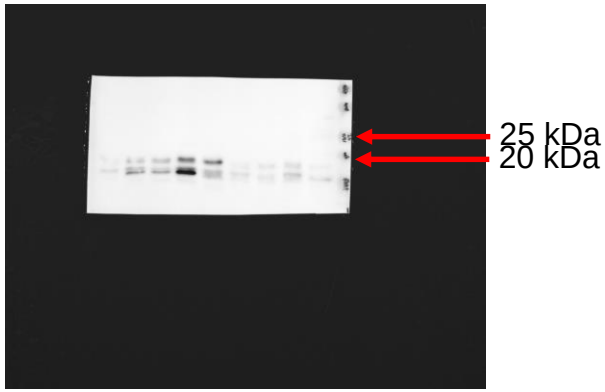


161-0374, Bio-rad (15 µl)

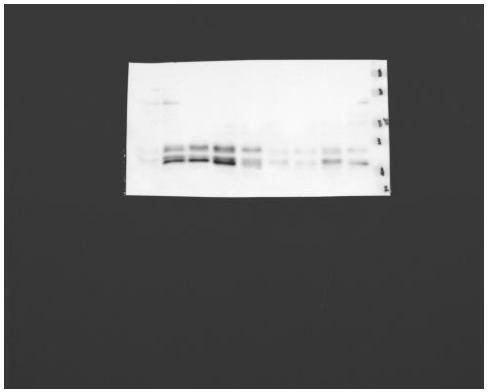
*Dahl S and Dahl R were analyzed independently
**Gel 6 consisted of two sets of Dahl S samples, and no Dahl R

Hepatic LC3B-I and -II ~19 and 17 kDa

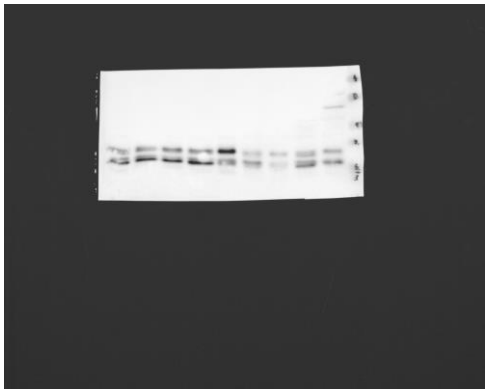
Gel 1



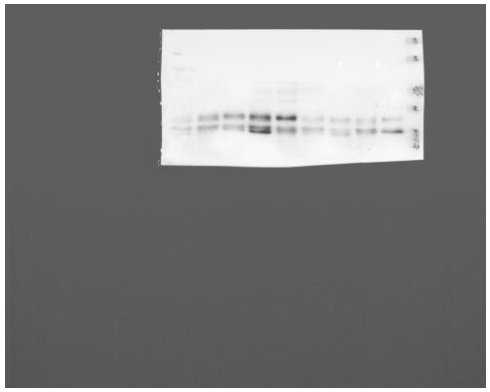
Gel 2



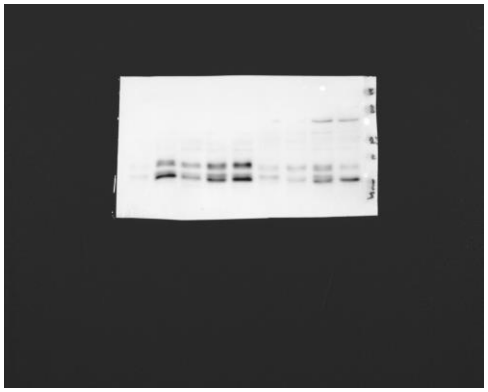
Gel 3



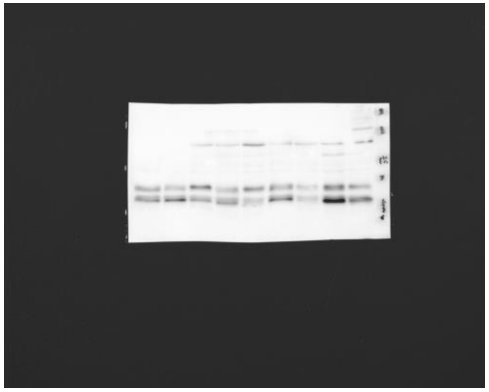
Gel 4



Gel 5



Gel 6

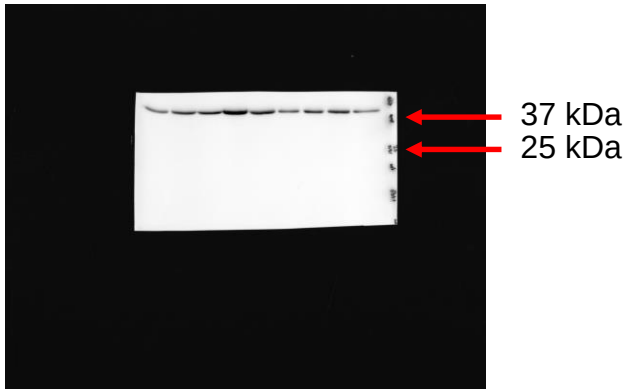


Gels: 12% acrylamide

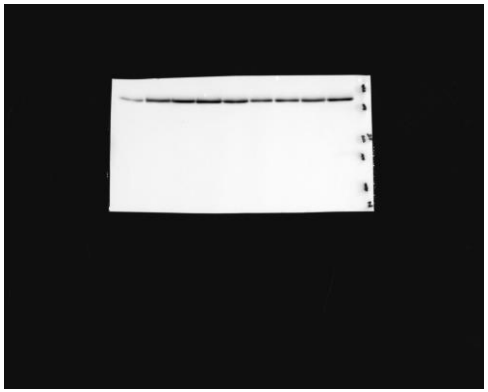
NB600-1384, Novus Biologicals (1:1000)

Hepatic β actin – 42 kDa

Gel 1



Gel 2



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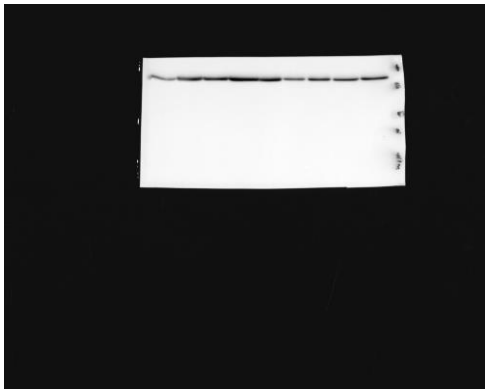
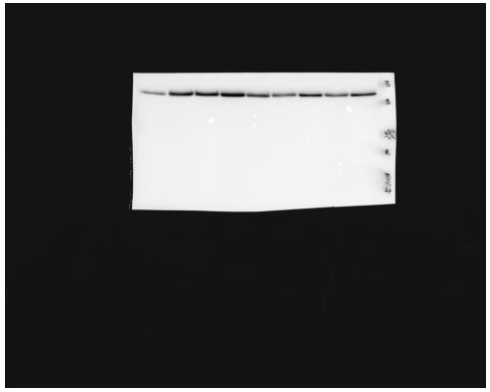
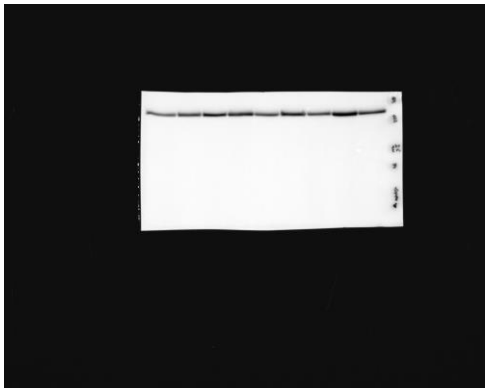


Gel 4



Gel 5

Gel 6

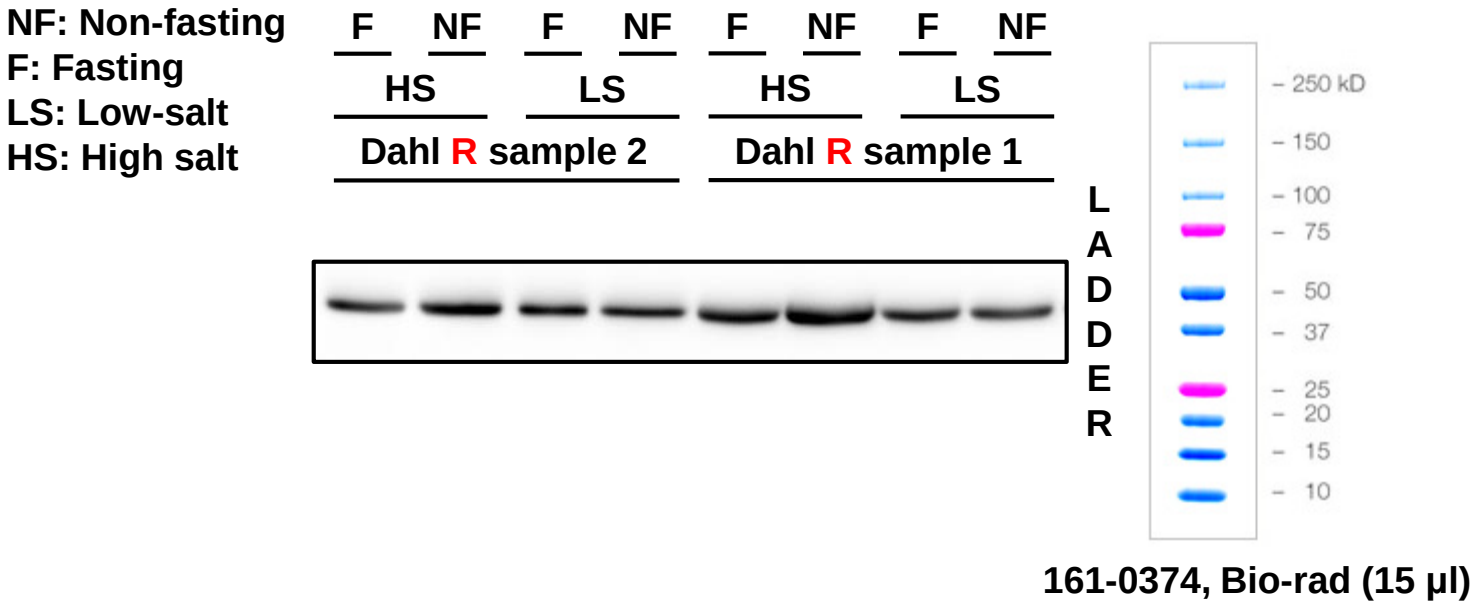


Gels: 12% acrylamide

A3854, Millipore-Sigma (1:5000)

How to read the uncropped/unrotated membranes: Set 2 loading

In set 1, Dahl S samples, for an unknown reason, saturated the images and decreased the pixel density of Dahl R samples. Therefore, in set 2, Dahl R samples were run on their on gels.



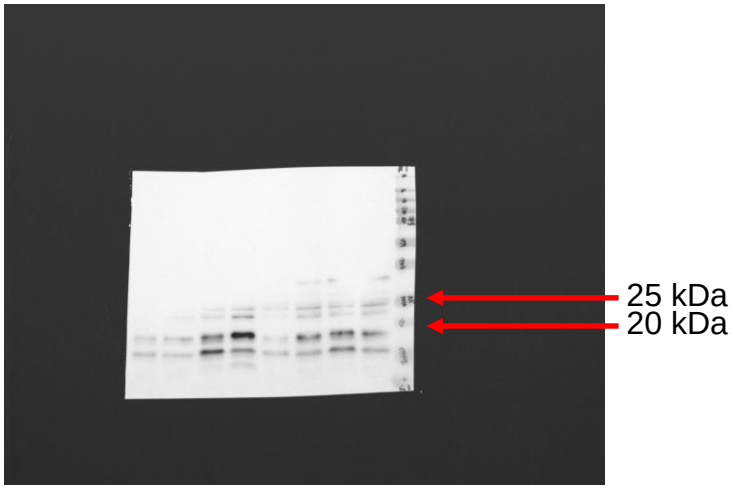
161-0374, Bio-rad (15 µl)

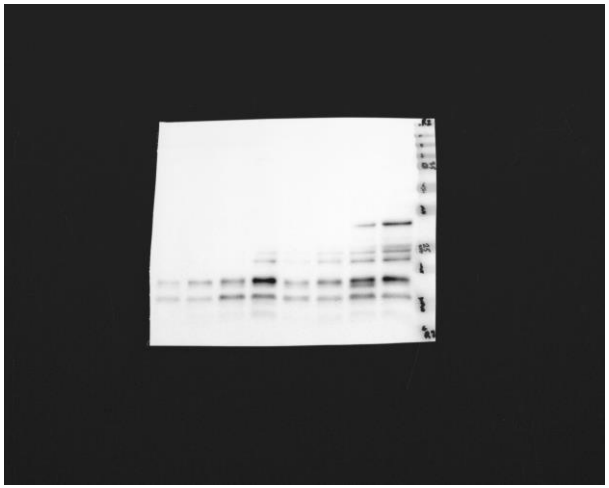
Hepatic LC3B-I and -II ~19 and 17 kDa

Gel 1

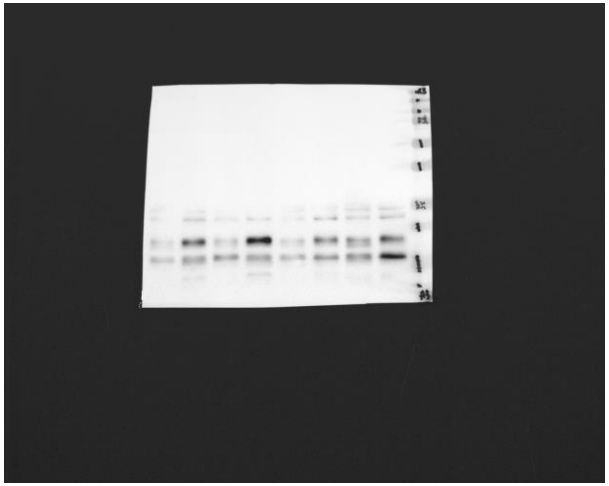
Dahl R

Gel 2

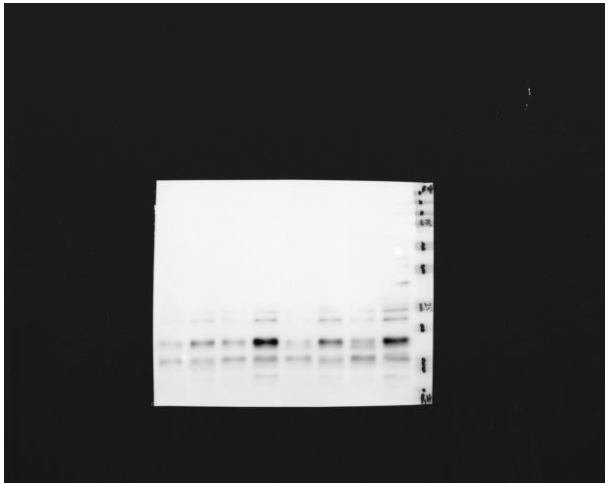




Gel 3



Gel 4



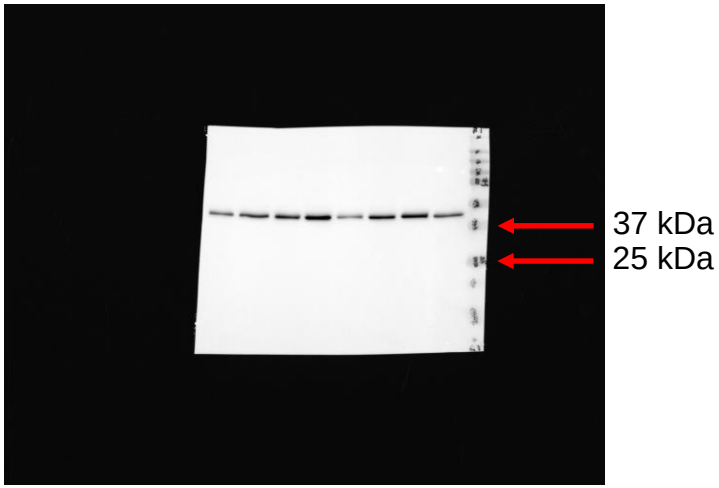
Gels: 12% acrylamide

NB600-1384, Novus Biologicals (1:1000)

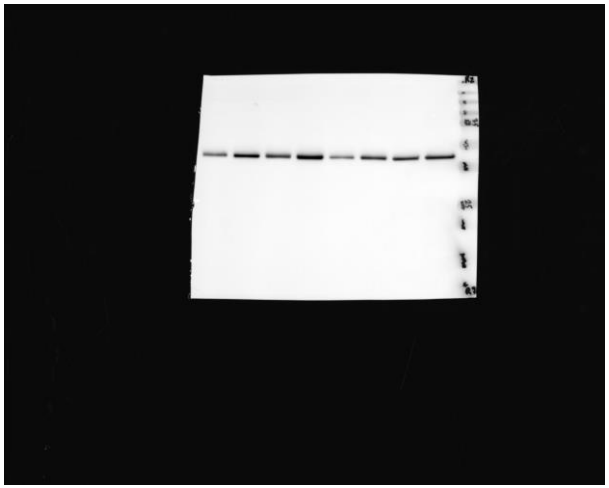
Hepatic β actin– 42 kDa

Gel 1

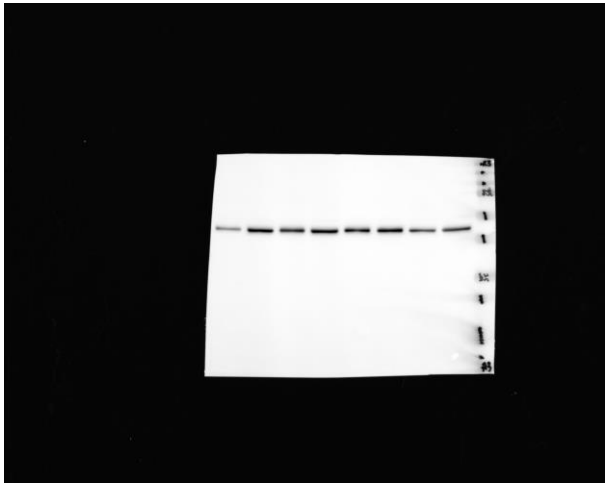
Dahl R



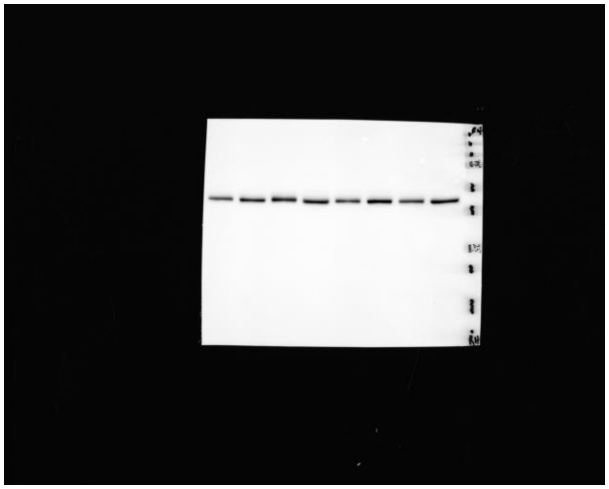
Gel 2



Gel 3



Gel 4

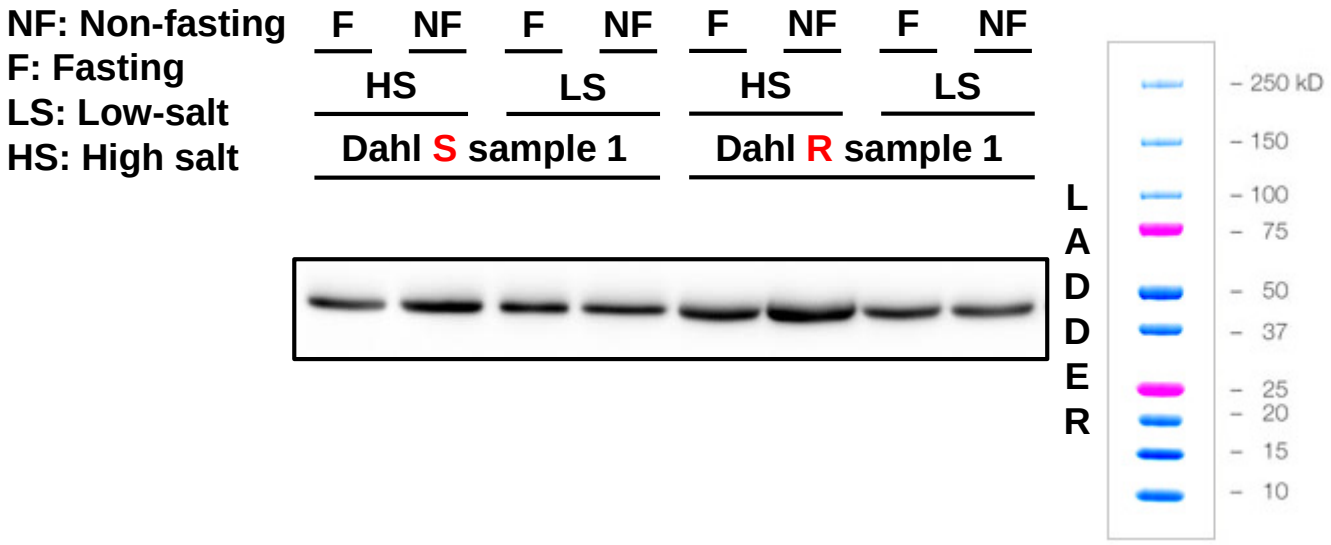


Gels: 12% acrylamide

A3854, Millipore Sigma (1:5000)

How to read the uncropped/unrotated membranes: Set 3 loading

Similar to set 1, Dahl S samples, for an unknown reason, saturated the images and decreased the pixel density of Dahl R samples. Therefore, Dahl R samples were excluded from analysis.



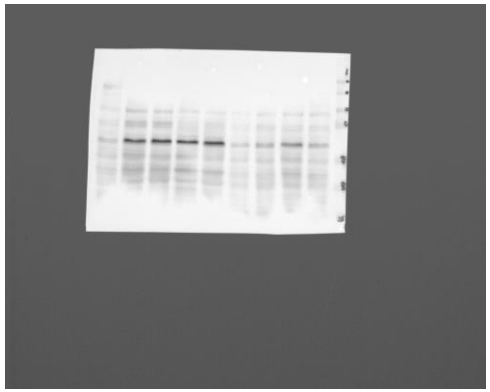
161-0374, Bio-rad (15 µl)

***Dahl S and Dahl R were analyzed independently**

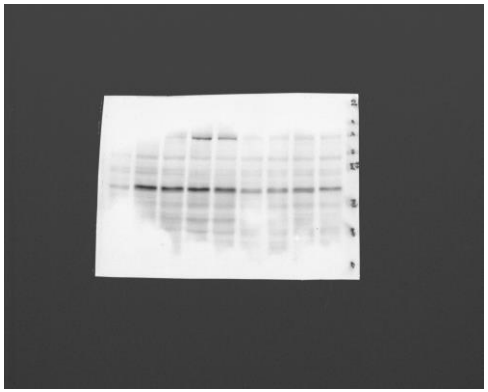
****Gel 6 consisted of two sets of Dahl S samples, and no Dahl R**

p62 – 62 kDa

Gel 1



Gel 2



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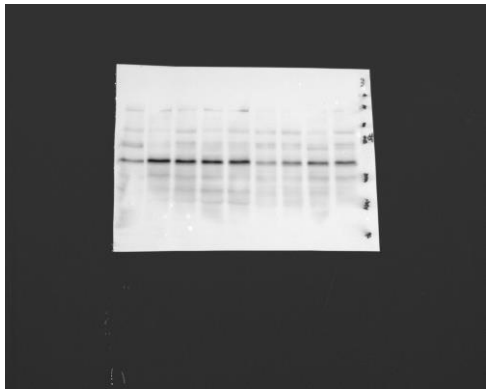
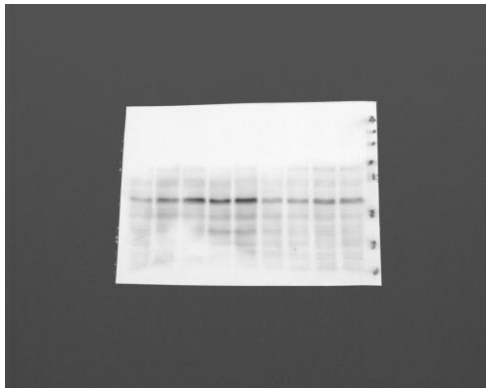
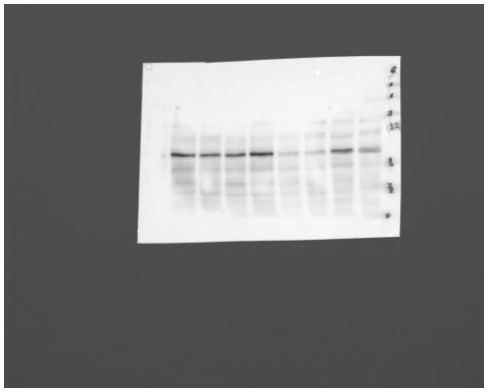


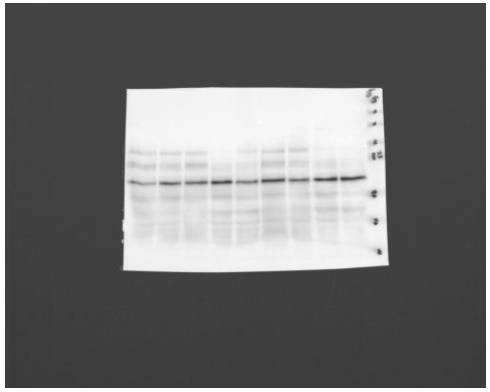
Gel 4



Gel 5

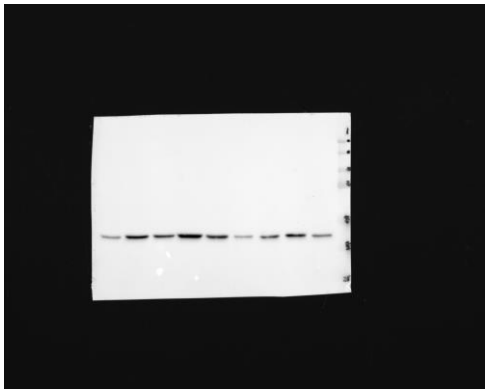


Gel 6

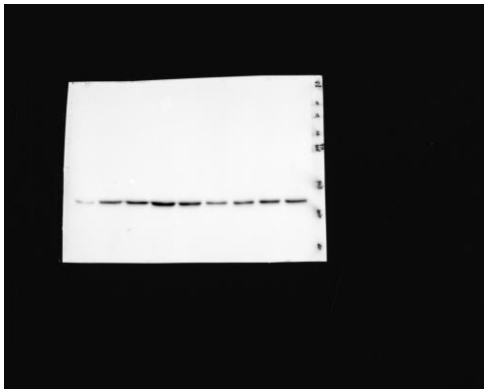


β actin – 42 kDa

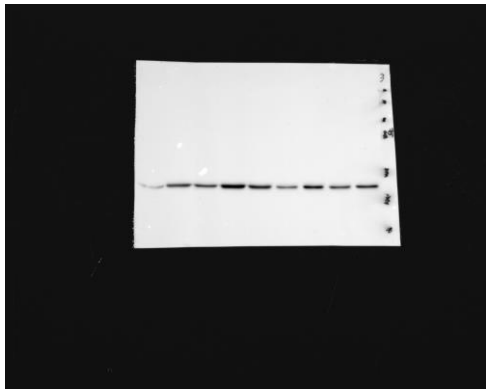
Gel 1



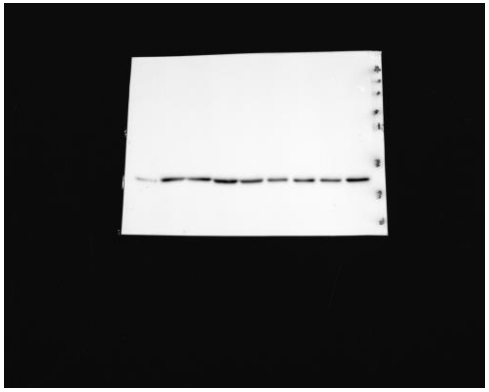
Gel 2



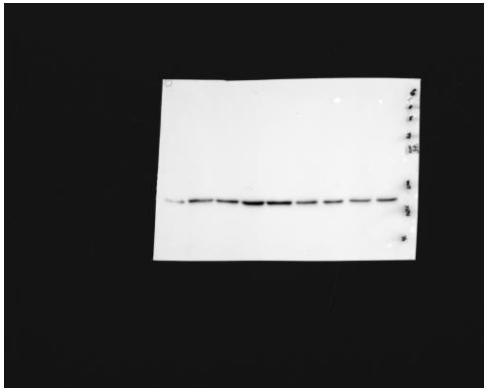
Gel 3



Gel 4



Gel 5



Gel 6