**Supplemental materials:**

- Supplemental tables 1 and 2
- Supplemental Figures 1-5
- Full Width Western Blots.

| Gene Name	entrez	Description	log2 FC	p-value (adjusted)
Osbpl6	mmu:99031	oxysterol binding protein-like 6	-1.94	2.24E-42
Map3k20	mmu:65964	mitogen-activated protein kinase kinase kinase 20	0.43	1.35E-11
Pde11a	mmu:241489	phosphodiesterase 11A	-7.34	1.53E-07
Ptpn3	mmu:545622	protein tyrosine phosphatase, non-receptor type 3	-0.49	3.31E-03
BC067074	mmu:NA	cDNA sequence BC067074	-1.03	6.60E-03
Wnk4	mmu:69847	WNK lysine deficient protein kinase 4	-0.84	8.31E-03
Adam11	mmu:11488	a disintegrin and metallopeptidase domain 11	-0.69	9.42E-03
Ptp4a2	mmu:19244	protein tyrosine phosphatase 4a2	0.25	1.14E-02
Tm4sf1	mmu:17112	transmembrane 4 superfamily member 1	0.40	1.22E-02
Rapgef4	mmu:56508	Rap guanine nucleotide exchange factor (GEF) 4	-0.51	1.22E-02
Tmem150c	mmu:231503	transmembrane protein 150C	-0.87	1.22E-02
Nlrc5	mmu:434341	NLR family, CARD domain containing 5	-1.39	1.27E-02
Scn4b	mmu:399548	sodium channel, type IV, beta	-0.92	1.65E-02
Cyb5b	mmu:66427	cytochrome b5 type B	0.33	3.30E-02

Supplemental Table 1. Differentially expressed genes between WT sham and N2B KO sham (ACF study).

| Gene Name	entrez	Description	log2 FC	p-value (adjusted)
Osbpl6	mmu:99031	oxysterol binding protein-like 6	-1.99785	1.29E-40
Map3k20	mmu:65964	mitogen-activated protein kinase kinase kinase 20	0.415647	2.47E-10
Pde11a	mmu:241489	phosphodiesterase 11A	-6.95952	7.79E-07
Tmem150c	mmu:231503	transmembrane protein 150C	-1.2525	2.19E-06
Scn4b	mmu:399548	sodium channel, type IV, beta	-1.30484	2.83E-06
Prnp	mmu:19122	prion protein	-0.59073	2.83E-06
Rtn1	mmu:104001	reticulon 1	-1.56903	3.84E-05
Atp8a2	mmu:50769	ATPase, aminophospholipid transporter-like, class I, type 8A, member 2	-1.13203	5.45E-05
Lyz2	mmu:17105	lysozyme 2	-0.69558	5.45E-05
Ano5	mmu:233246	anoctamin 5	-1.85528	0.000104
Malat1	mmu:NA	metastasis associated lung adenocarcinoma transcript 1 (non-coding RNA)	0.836573	0.0002
Flot1	mmu:14251	flotillin 1	-0.38784	0.000268
Pdk1	mmu:228026	pyruvate dehydrogenase kinase, isoenzyme 1	-0.50501	0.000279
Myh7b	mmu:668940	myosin, heavy chain 7B, cardiac muscle, beta	0.989092	0.000321
Mal	mmu:17153	myelin and lymphocyte protein, T cell differentiation protein	-0.66405	0.000457
Gm10603	mmu:NA	predicted gene 10603	3.187213	0.000533
Ucp2	mmu:22228	uncoupling protein 2 (mitochondrial, proton carrier)	1.189522	0.000618
Dyrk2	mmu:69181	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2	0.527205	0.001014
Cpxm2	mmu:55987	carboxypeptidase X 2 (M14 family)	-1.19975	0.001178
Tmem176b	mmu:65963	transmembrane protein 176B	-0.62026	0.003285

Supplemental Table 2. Differentially expressed genes between WT ACF and N2B KO ACF.
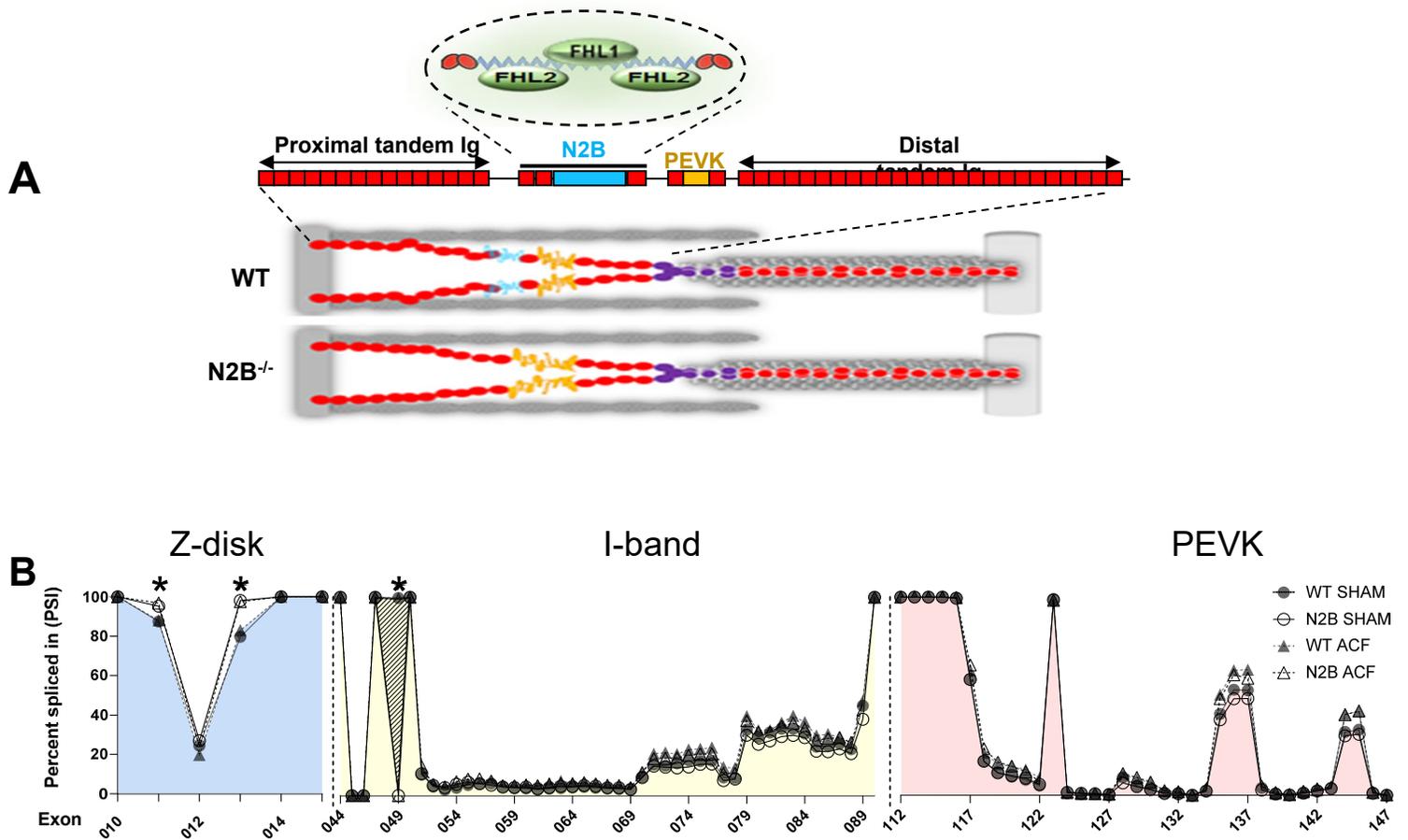
Supplemental Figure 1. A) Schematic of cardiac titin in the half-sarcomere. Upper inset: schematic indicating that the N2B element of titin and that FHL1 and FHL2 co-localize with N2B element. Lower inset: Murine models of WT and N2B element deficient titin. **B)** Analysis of titin exon expression. Titin exon inclusion was analyzed by RNAseq in WT and N2B KO mice after SHAM or ACF surgery. Exons in the Z-disk (blue), I-band (yellow) and PEVK (pink) regions are displayed as percent spliced in (PSI) values. Minor differences between genotypes can be observed in Z-repeats 4 and 6 (exons 11 and 13), where inclusion levels are slightly higher in N2B KO mice. In the I-band and PEVK regions, exon inclusion is slightly elevated after ACF surgery compared to SHAM, but there are no significant differences between genotypes. As expected, exon 49 (N2B exon) is fully spliced out in N2B KO mice (striped area). Asterisks indicate significant differences ($p < 0.05$) in N2B KO vs. WT comparisons. (PSI values outside of shown regions were identical).
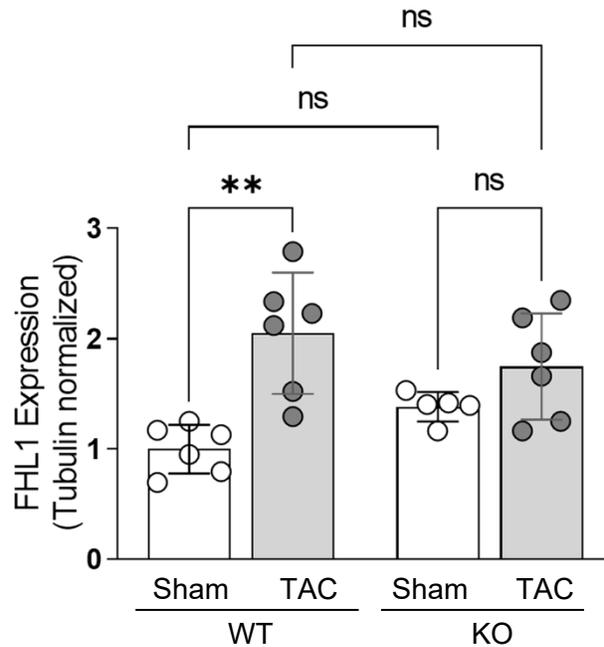
Supplemental Figure 2: FHL1 expression in pressure-overload N2B KO and WT mice. FHL1 protein expression normalized to β-Tubulin. Analyzed expression data shown as mean ± SD. Statistical significance calculated with two-way ANOVA. FHL1 is significantly elevated in response to TAC (p=0.0004); however, there is no genotype effect (p=0.8). The results of a posthoc test are shown on the figure. Details of statistical analyses are in Supplemental Table 3. Data shown as mean ± SD. **:p<0.01
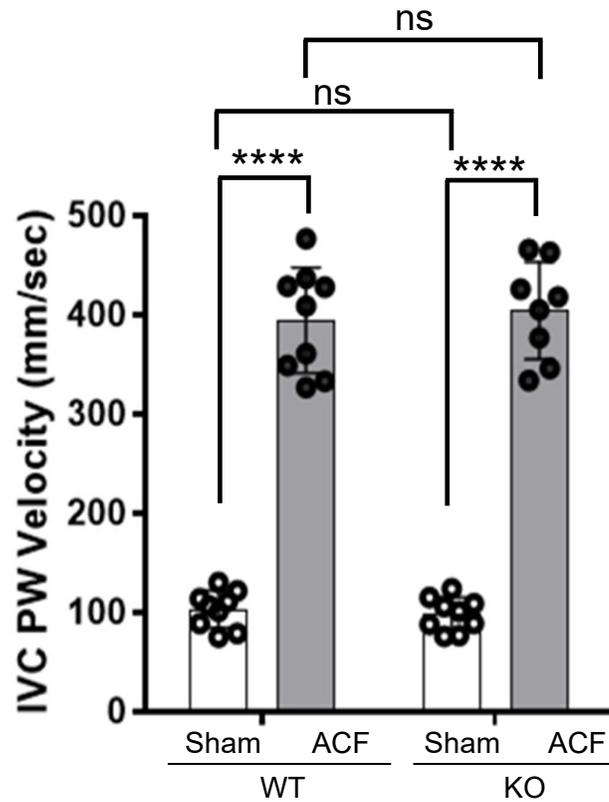
Supplemental Figure 3: Inferior vena cava PW Doppler. PW Doppler peak flow velocity measurements taken just caudal to the fistula site in the inferior vena cava in ACF N2B KO and WT mice. Results are shown as mean ± SD. Statistical significance calculated with two-way ANOVA. Velocity is significantly elevated in response to ACF (p-<0.0001); however, there is no genotype effect (p=0.9). The results of a posthoc tests are shown on the figure. Data shown as mean ± SD. ****:p<0.0001
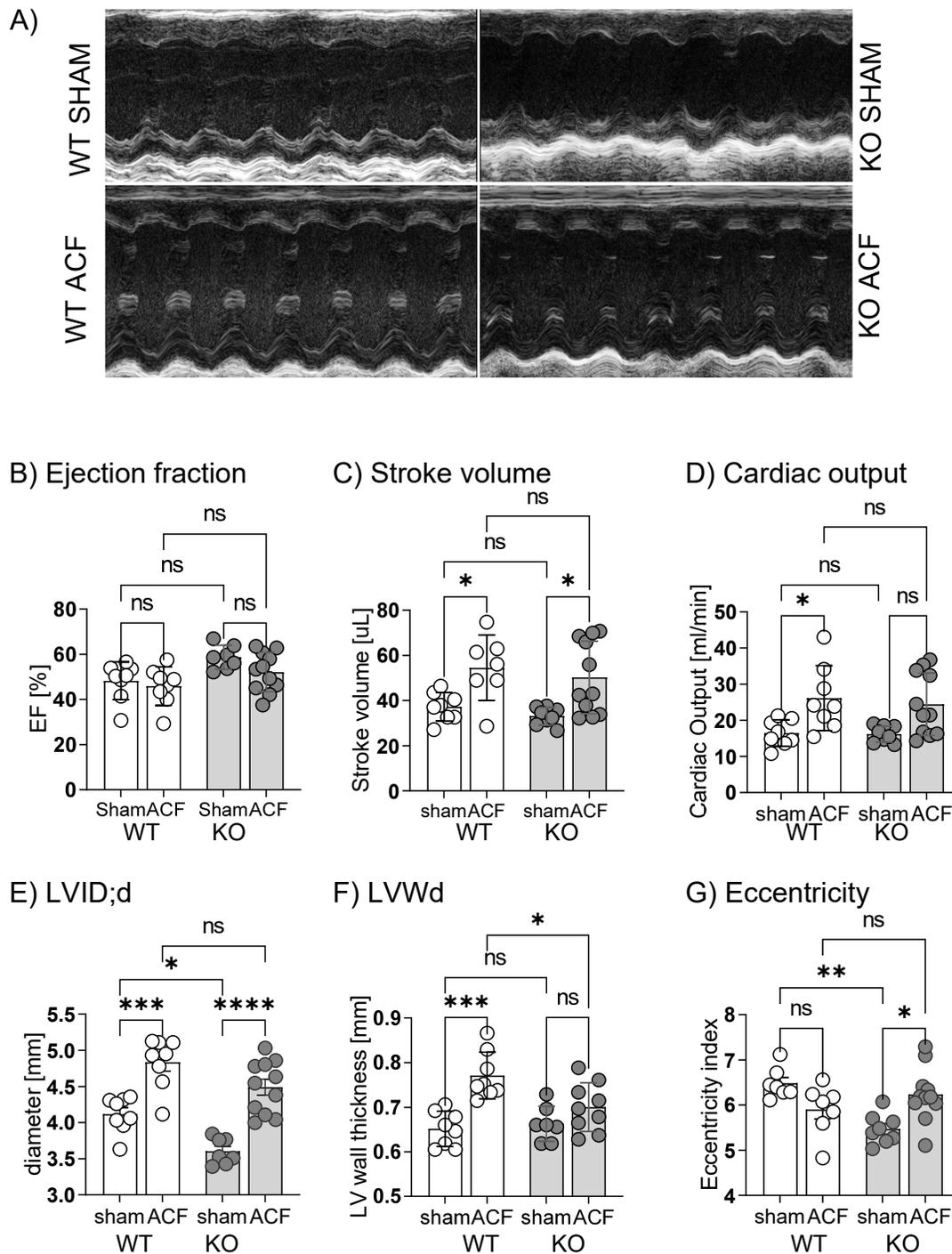
Supplemental Figure 4. Echocardiographic assessment of N2BKO mice 4 weeks after ACF surgery. A) Representative echocardiographic M-mode images of WT-sham, WT-ACF, N2BKO-sham and N2BKO-ACF. ACF does not alter ejection fraction (B) but induces increases stroke volume (C), cardiac output (D), and LV internal diameter in diastole (LVIDd; E) in both WT and N2BKO mice. However, ACF leads to a thickening of the LV wall in diastole (LVWd (average of posterior and anterior wall) in WT, but not in N2B KO mice (F), resulting in an increase in eccentricity index (G) in N2B KO-ACF mice. (n= 8, 8, 8, 9 mice for WT-sham, WT-ACF, KO-sham and KO-ACF) Two-way ANOVA, with posthoc test results shown on the figure. * $p \leq 0.05$; ** $p \leq 0.01$; ***$p \leq 0.001$; ****$p \leq 0.0001$. Data shown as mean ± SD. Details of statistical analyses are in Supplemental Table 3.
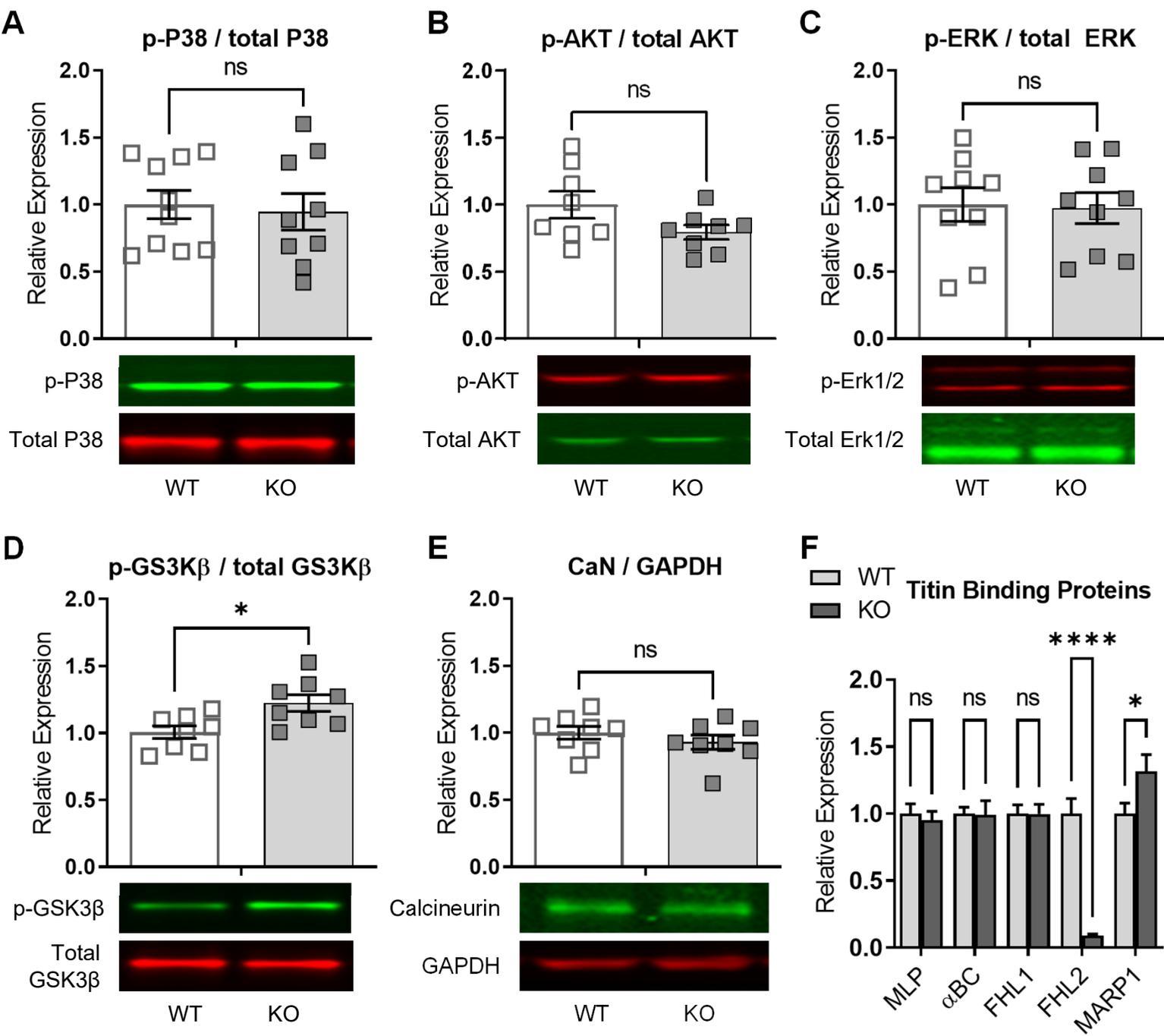
Supplemental Figure 5. Canonical hypertrophy pathways and titin binding proteins under baseline conditions . Western Blot analysis of p-P38/total P38 (A), p-AKT/total AKT (B), p-ERK/total ERK (C), p-GSK3β/total GSK3β (D), and calcineurin/GAPDH (E) in WT and N2B KO mice. Western Blot analysis of titin binding partners (F). Data shown as mean ± SEM. Data shown as mean ± SD. Statistical significance calculated using Student's t-test. * p≤0.05; ****p≤0.0001.
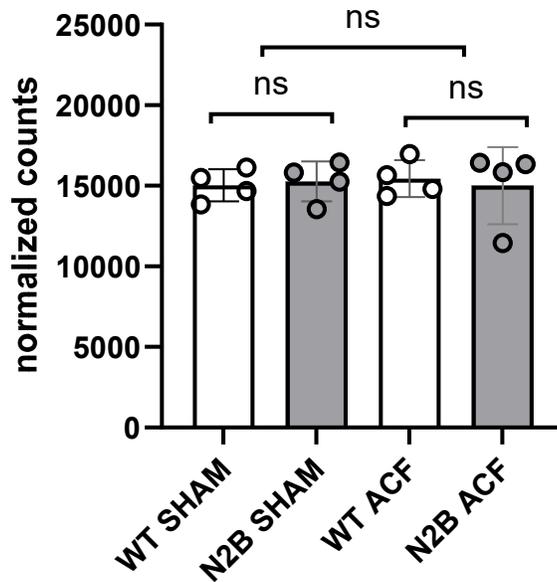
Supplemental Figure 6: Normalized read counts from RNA-seq data for FHL2 were compared between WT and N2B KO samples. Adjusted p-values (padj) based on DESeq2 were not significantly different in SHAM- or ACF genotype comparisons (p=0.93), and neither was the ACF effect between genotypes (p=0.99). Data shown as mean ± SD.

Full WBs for figures

Phospho P38
41 kDa
Rabbit 700nm
1:1000

Total P38
41 kDa
Mouse 800nm
1:1000
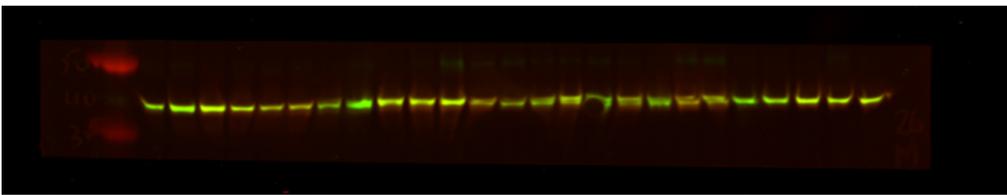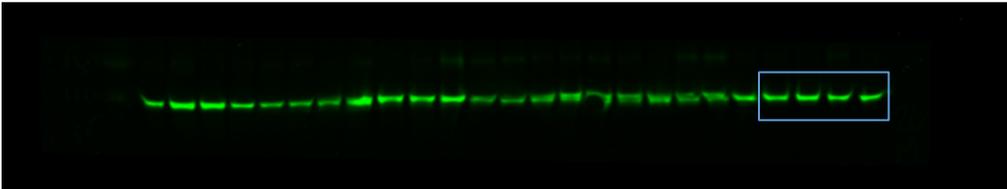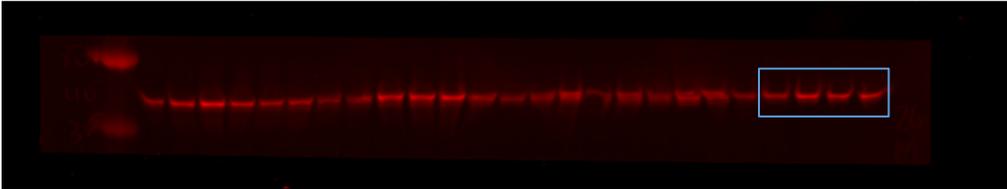
Merge

P38 for Figure 5. Image (A)

Josh-Mat Wblots 2016 Sep-2018 Jan/20180124-0125_N2B western blots

Pan AKT

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 |
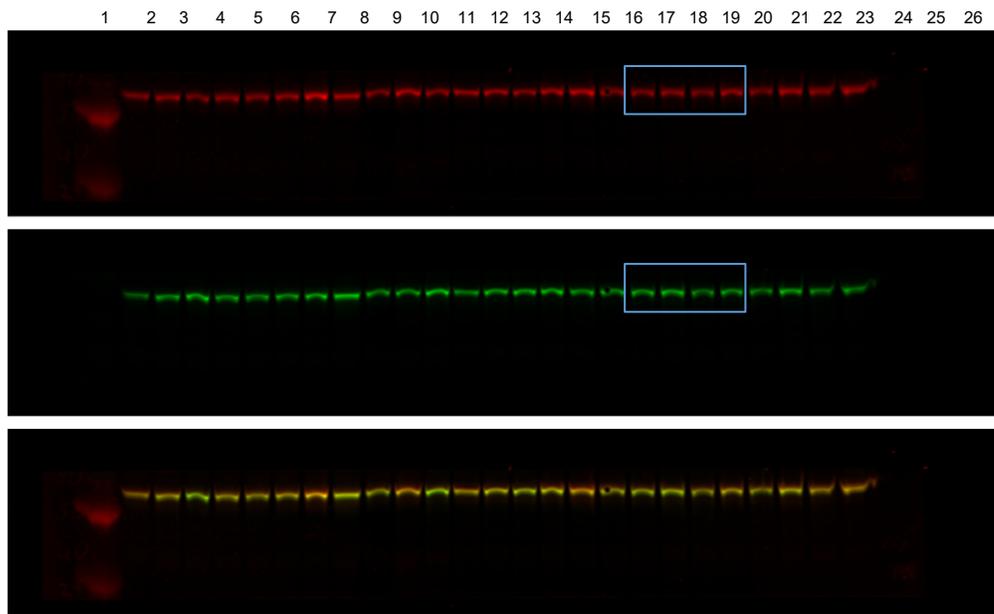
**Phospho AKT
60 kDa
Rabbit 700nm
1:1000**

**Pan/Total AKT
60 kDa
Mouse 800nm
1:1000**

Merge

AKT for Figure 5. Image (B)

Josh-Mat Wblots 2016 Sep-2018 Jan/20180124-0125_N2B western blots

Erk ½ (MAPK ½)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
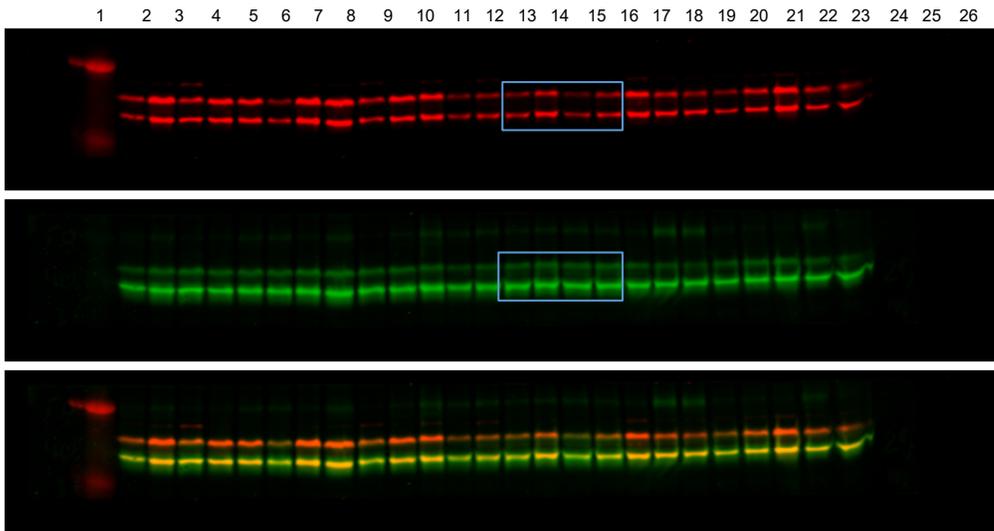
**Phospho ERK
44, 42 kDa
Rabbit 700nm
1:2000**

**Total ERK
44, 42 kDa
Mouse 800nm
1:500**

Merge

ERK for Figure 5. Image (C)

Josh-Mat Wblots 2016 Sep-2018 Jan/20180124-0125_N2B western blots

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26

**Phospho GSK3b
46 kDa
Rabbit 700nm
1:1000**

**Total GSK3b
46 kDa
Mouse 800nm
1:1000**

Merge
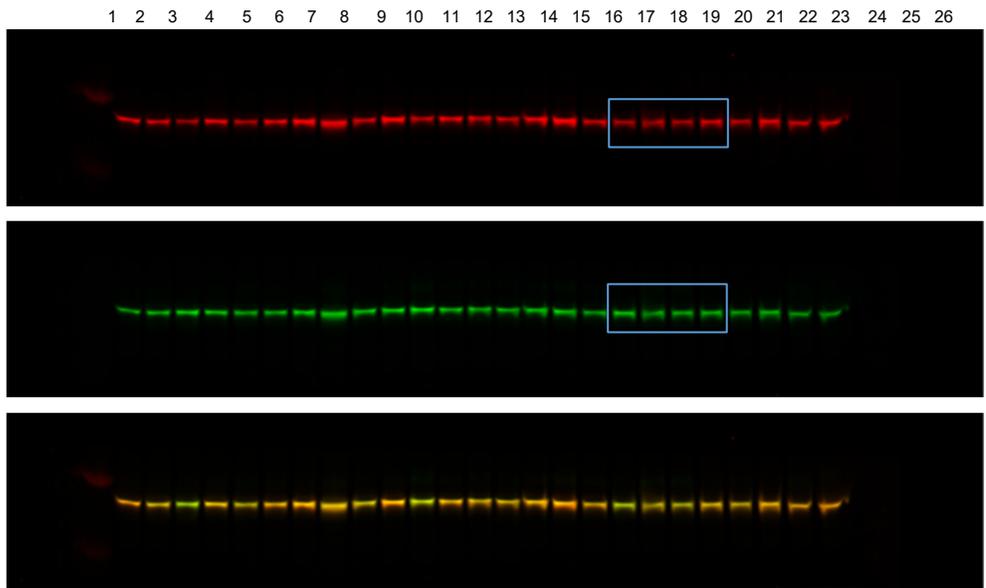
GSK3 beta

GSK3beta for Figure 5. Image (D)

Josh-Mat Wblots 2016 Sep-2018 Jan/20180124-0125_N2B western blots
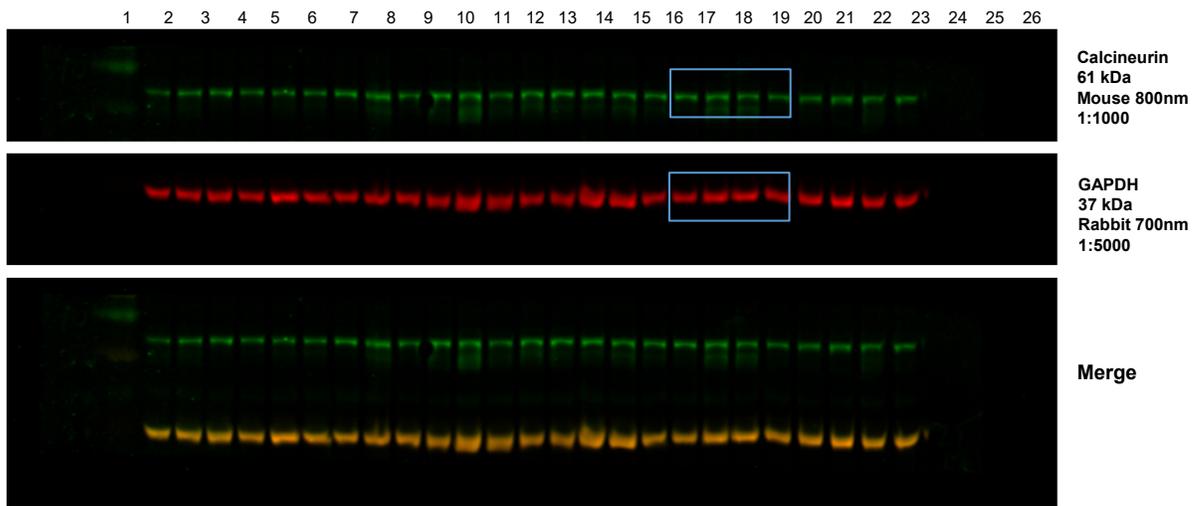
Calcineurin

	Calcineurin 61 kDa Mouse 800nm 1:1000
	GAPDH 37 kDa Rabbit 700nm 1:5000
	Merge

Calcineurin for Figure 5. Image (E)

Josh-Mat Wblots 2016 Sep-2018 Jan/20180124-0125_N2B western blots
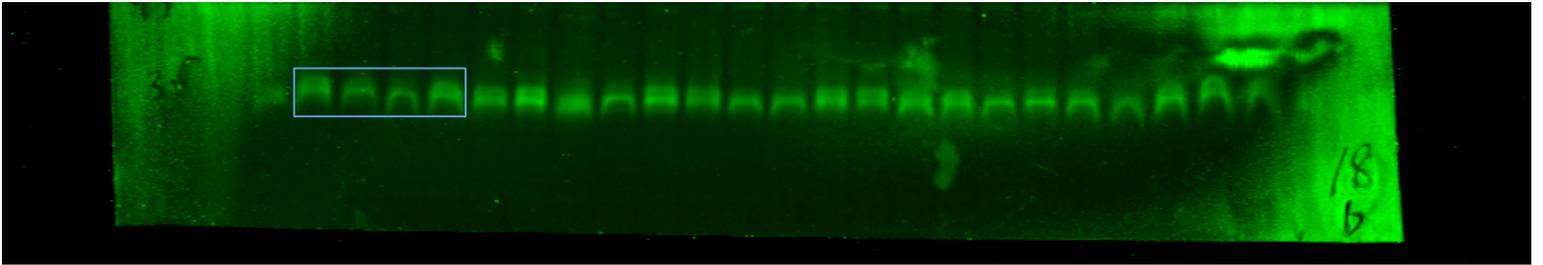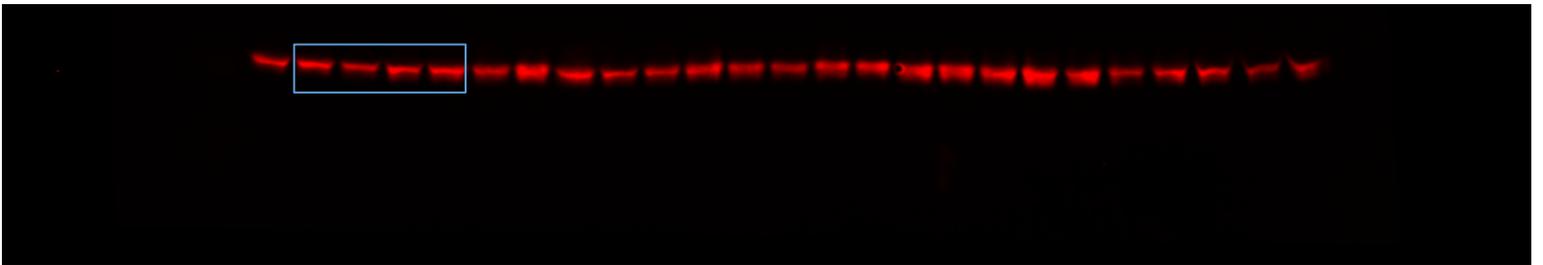
FHL-1

GAPDH

FHL-1 for Figure 6. Image (A)

20160928_1wk ACF N2B FHL1-GAPDH

FHL-2
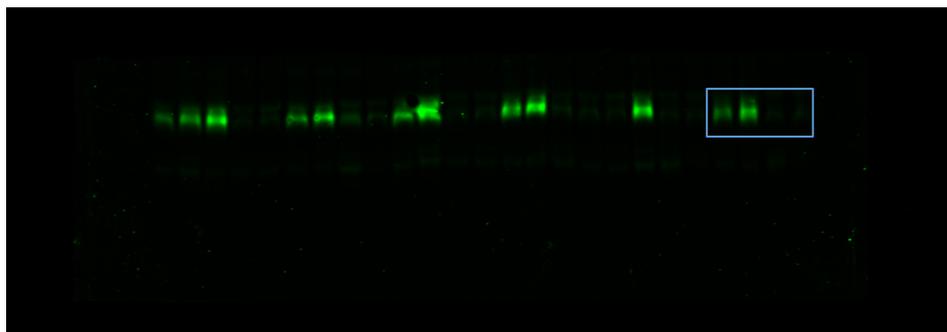
GAPDH
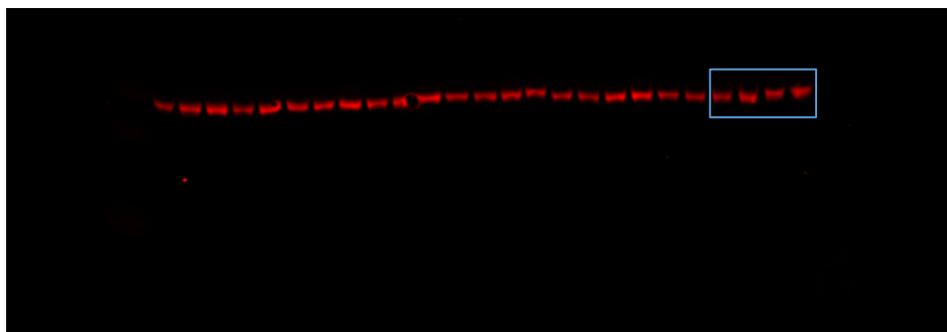
FHL-2 for Figure 6. Image (B)

1wk ACF OWA24 FHL-2 Gapdh

FHL-1
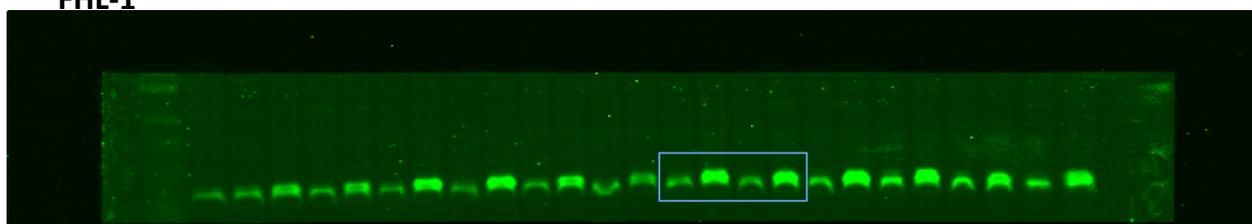
Beta tubulin
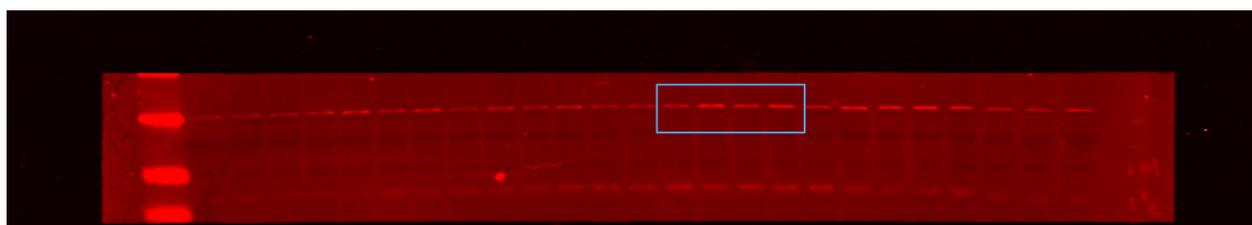

FHL-1 for Figure 7. Image (C)

Blot: 20150428_PN18
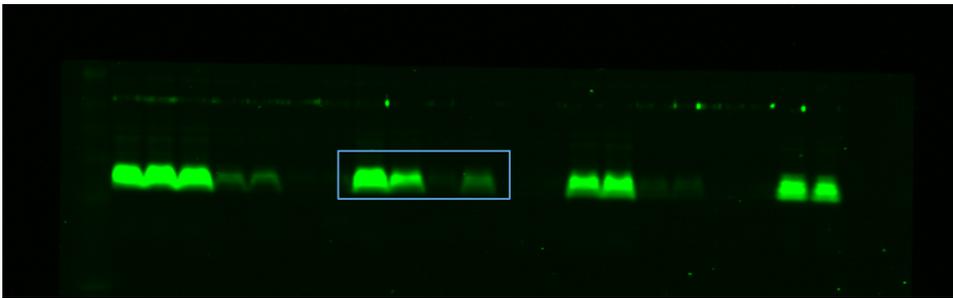
FHL 2

Beta tubulin
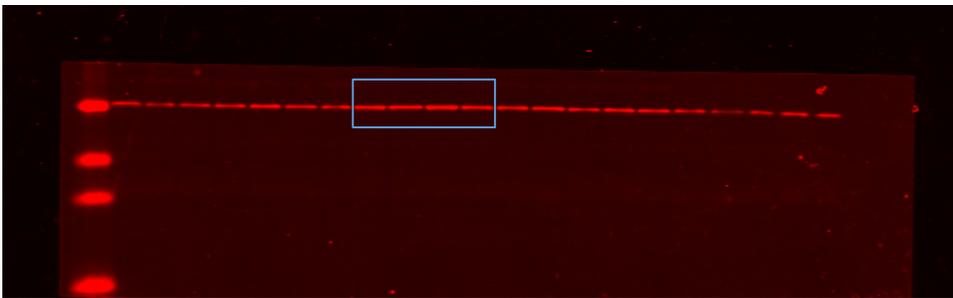
FHL-2 for Figure 7. Image (D)

Blot: 20150602_PN27