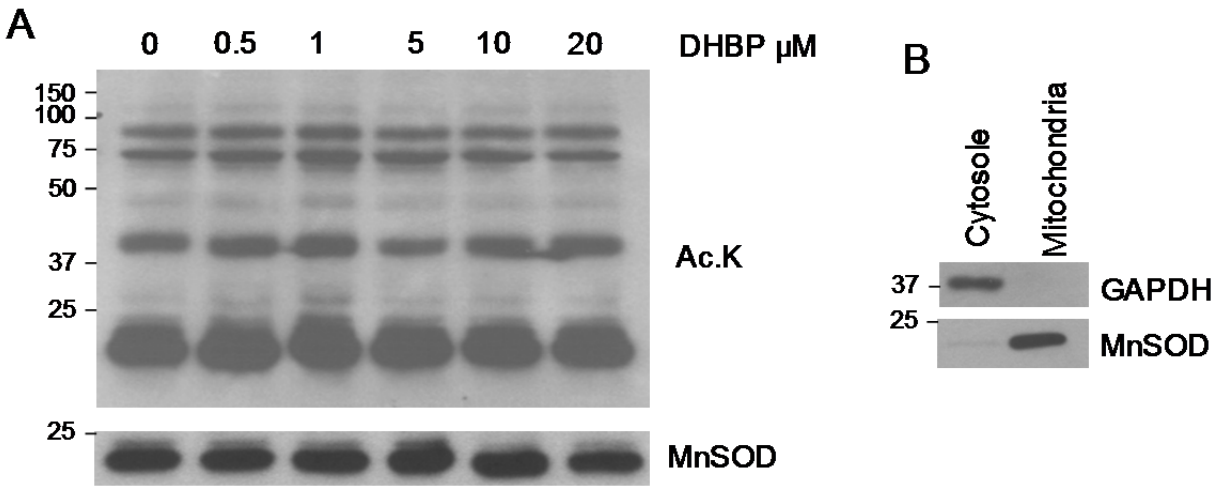
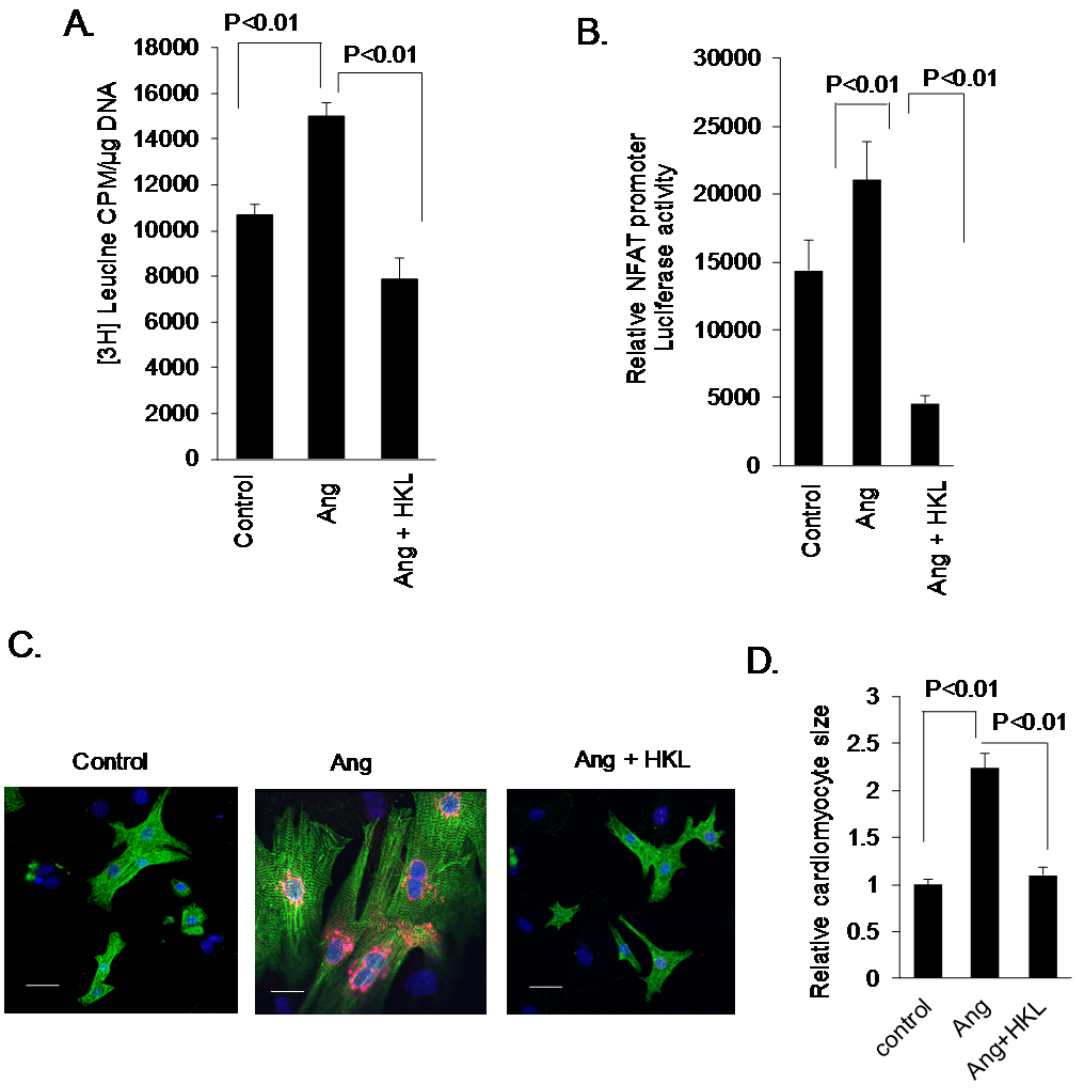
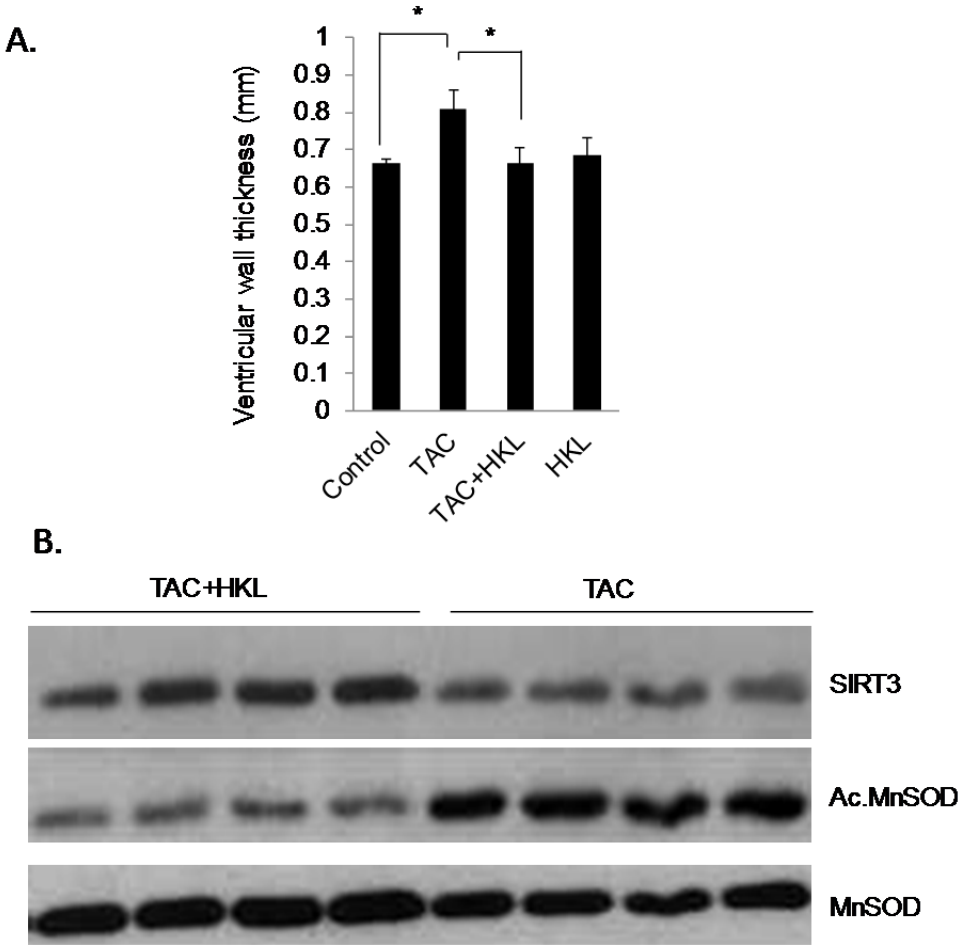
SUPPLEMENTARY FIGURES



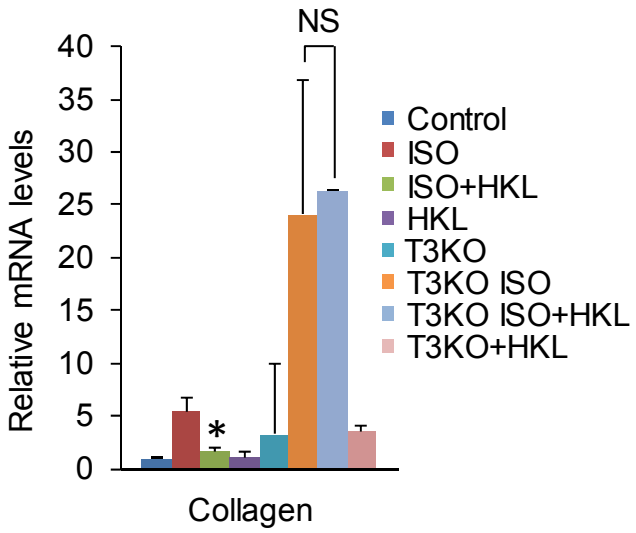
Supplementary Figure 1: Effect of Dihydroxybiphenyl (DHBP) treatment on mitochondrial protein acetylation: (A) Primary cultures of neonatal rat cardiomyocytes were treated with different doses of dihydroxybiphenyl (DHBP) as indicated. Mitochondrial lysate was prepared and analyzed for lysine-acetylation using anti-acetyl lysine antibody (Ac-K). Total MnSOD levels served as loading control. **(B)** Characterization of cytosolic and mitochondrial fractions prepared from cardiomyocytes.



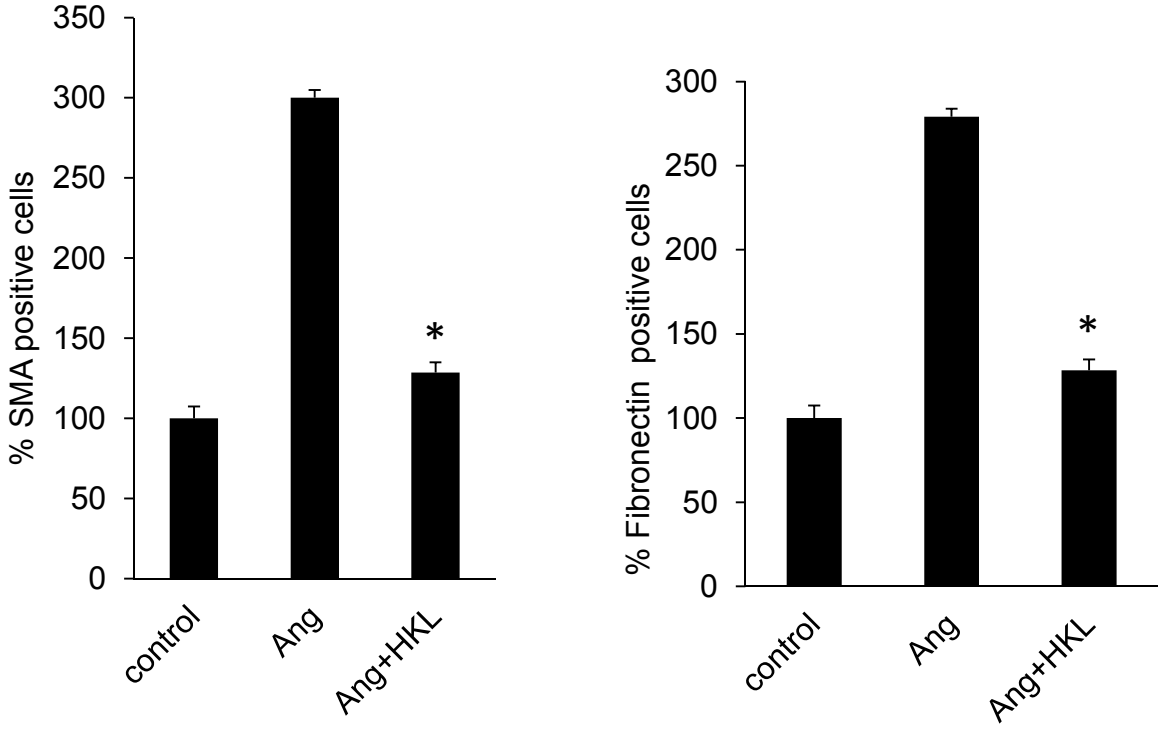
Supplementary Figure 2: HKL blocks Ang-mediated hypertrophy of cardiomyocytes in vitro. (A) Primary cultures of cardiomyocytes labeled with [^{3}H] leucine were treated with 100 nM angiotensin-II (Ang) in the presence or absence of 10µM HKL. Twenty four hrs after treatment cells were harvested and incorporation of [^{3}H] leucine into total cellular proteins was measured. **(B)** Cardiomyocytes were infected with a NFAT-responsive luciferase reporter adenovirus vector. Twelve hours after infection cells were treated with Ang in the presence or absence of HKL for 8 hrs. HKL treatment was given 2 hrs prior to agonist treatment. The luciferase activity was measured using an activity assay kit from Promega, as per manufacturer's protocol. **(C)** Cardiomyocytes were treated with Ang in the presence or absence of 10µM HKL. Cardiomyocytes were identified by α-actinin staining (green) and the release of ANF from nuclei was determined by staining cells for ANF-specific antibody (red). DAPI stain was used to mark the position of nuclei. **(D)** Cardiomyocyte size of α-actinin positive cells was quantified by use of Image J software. Values are expressed as fold change with respect to untreated control. All values are mean ± SE, n=4 independent experiments; Students *t* test.



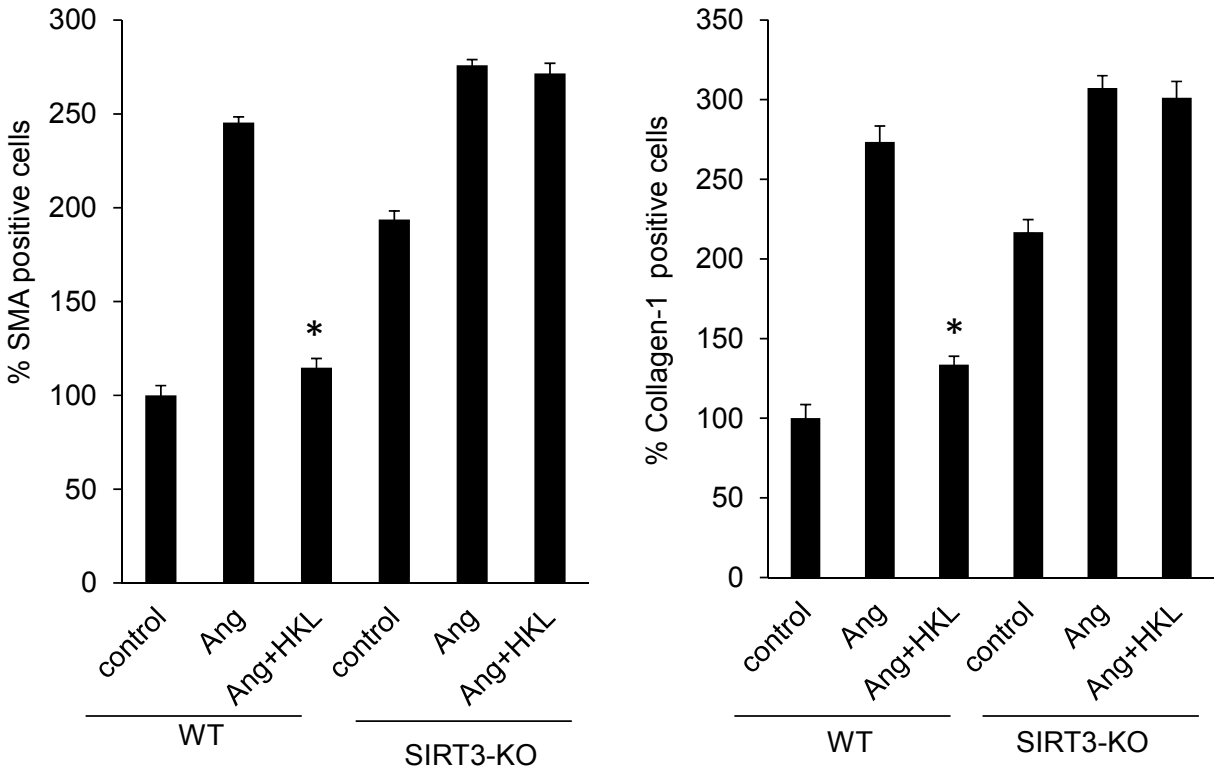
Supplementary Figure 3: Honokiol treatment blocks pressure overload-induced cardiac hypertrophy in mice: Following induction of TAC, mice were treated with HKL (0.2mg/kg/day) daily for 28 days. **(A)** Echocardiographic measurements of LV wall thickness of control, TAC and TAC mice treated with HKL. Mean ± SE, n = 5-8. *P<0.05; ANOVA. **(B)** Heart lysates of TAC mice treated with HKL and TAC alone, were subjected to immunoblotting using indicated antibodies. Results of four mice in each group are shown.



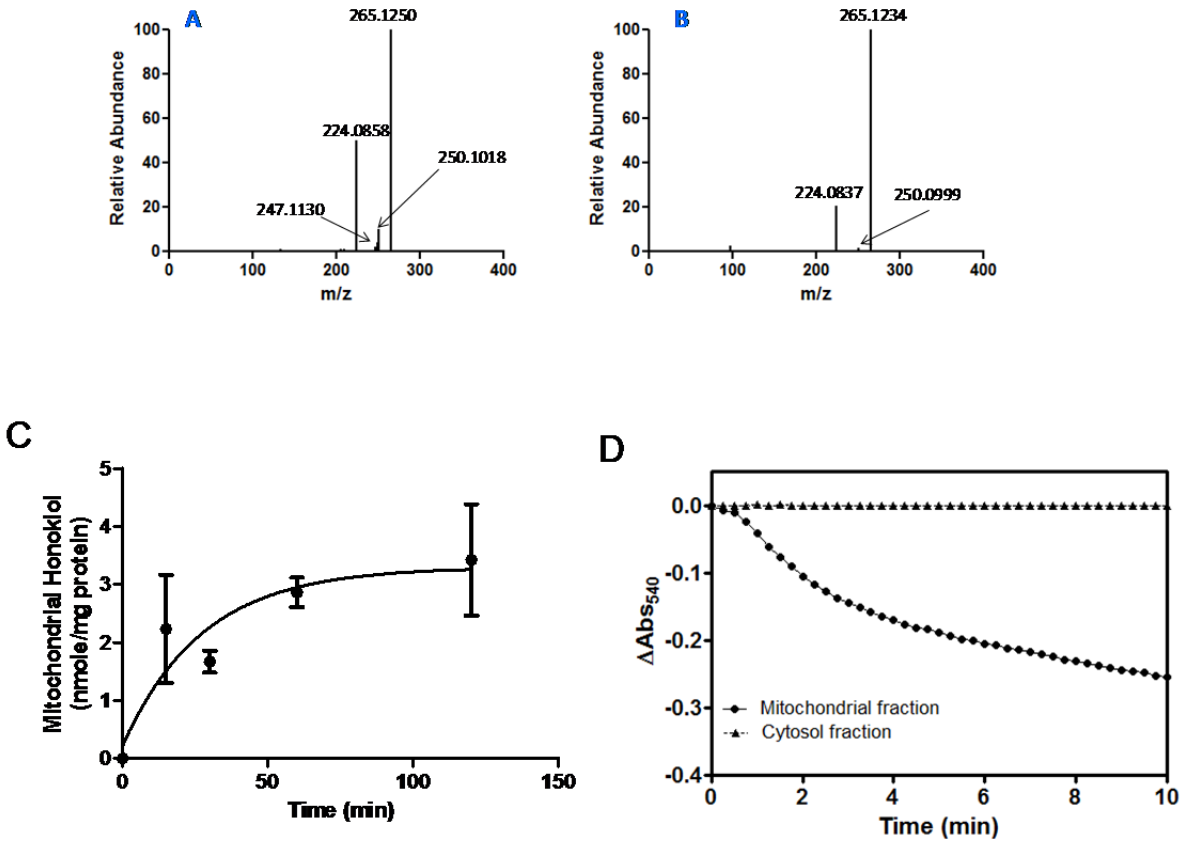
Supplementary Figure 4: HKL treatment blocks ISO-induced cardiac fibrotic response of wild-type, but not SIRT3-KO mice. Collagen-1 mRNA levels were measured in the heart samples of WT and SIRT3KO (T3KO) mice subjected to develop ISO-mediated cardiac hypertrophy in the presence or absence of HKL treatment. Mean ± SE, n = 5; **P* <0.05 compared to ISO., NS, not significant; Students *t* test.



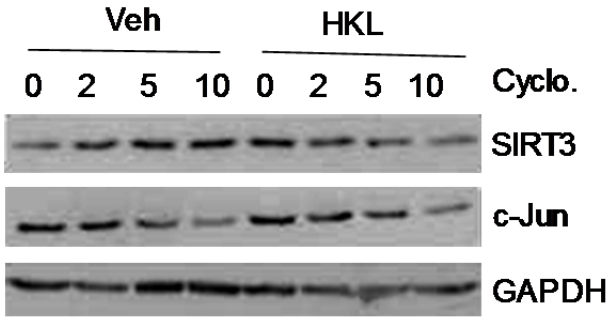
Supplementary Figure 5: Quantification of SMA and fibronectin positive cardiac fibroblasts shown in Fig 6c (main text). Values are mean ± SE, n =3 independent experiments; *P <0.01 compared to angiotensin (Ang) treated group; Students t test.



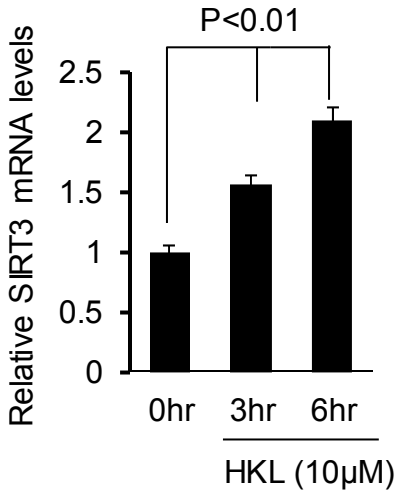
Supplementary Figure 6: Quantification of SMA and collagen-1 positive cardiac fibroblasts shown in Fig 7 (main text). Mean ± SE, n =3 independent experiments; *P<0.01 compared to angiotensin treatment. Students *t* test.



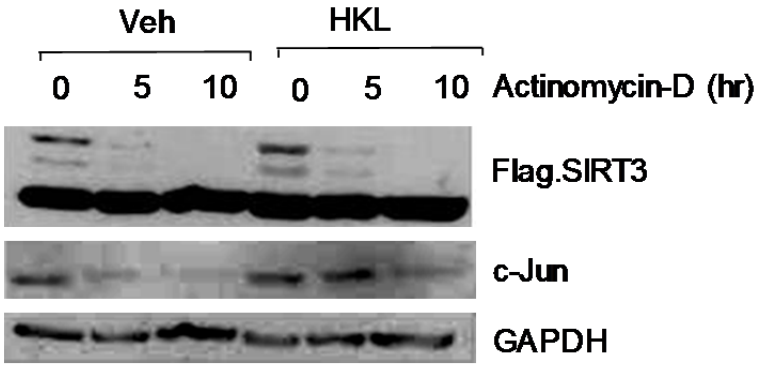
Supplementary Figure 7: Detecting the presence of honokiol inside mitochondria. MELIN database spectra **(A)** and MS2 spectra **(B)** obtained form/z= 265.1234 eluting at 9.6 min in spiked mitochondrial isolate. Major disassociation fragments matched those obtained from the database spectra at a reported CE of 20%, indicating that the m/z corresponded to that of honokiol in the mitochondrial sample. **(C)**. Baseline subtracted mitochondrial honokiol levels were observed to increase with increasing incubation time for the isolated mitochondria, indicating honokiol uptake into the matrix. **(D)** Mitochondrial integrity was evaluated by monitoring absorbance at 540 nm following dosing of 100ug (mitochondrial protein) with 15µL of 2mM $CaCl_2$. Over the 10 minute period, a decrease of 0.3 was observed within the mitochondrial fraction, indicating MPT activation by Ca^{2+}. No similar trends were observed for other cellular fractions or within blank isolation media.



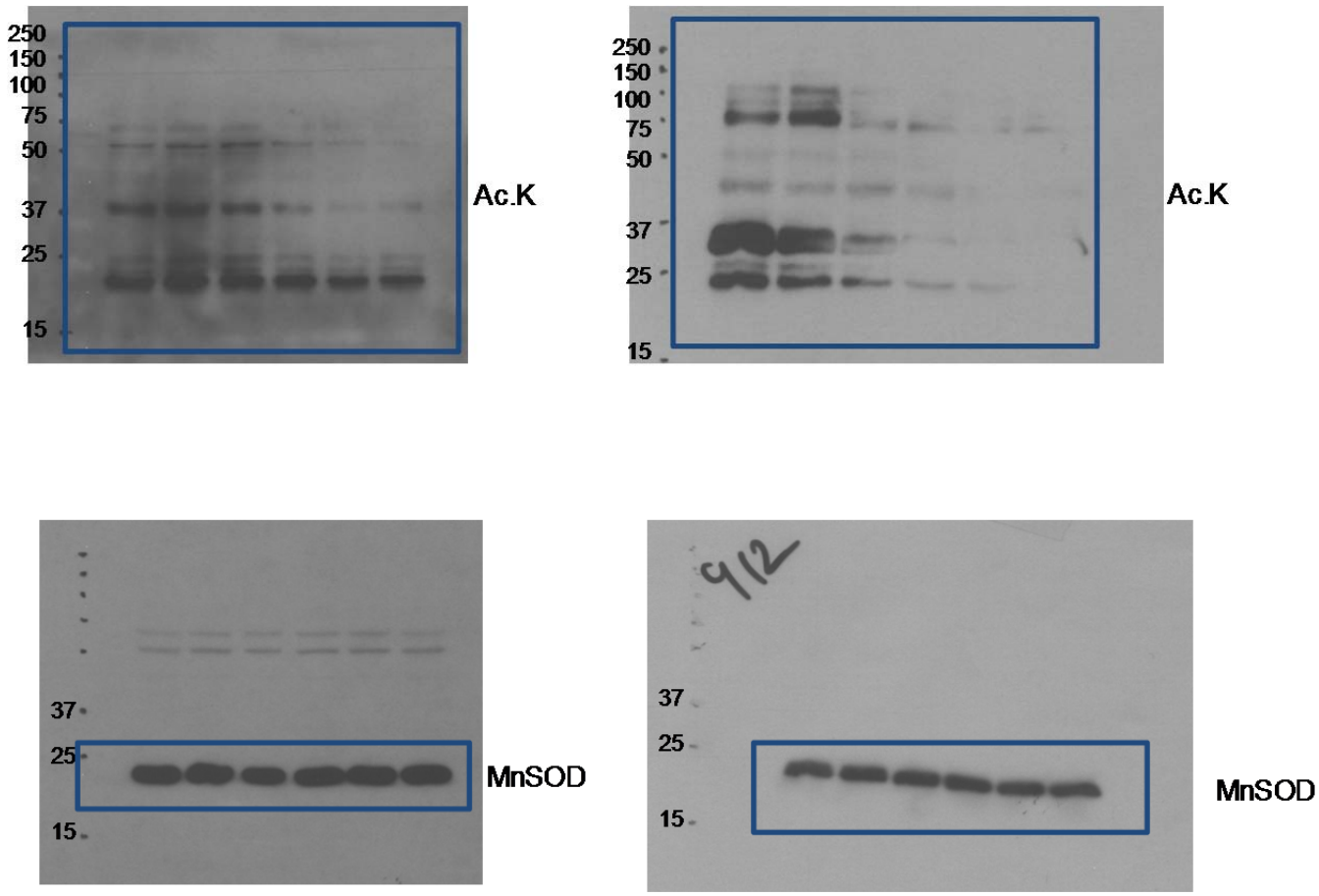
Supplementary Figure 8: Honokiol treatment does not increase SIRT3 protein stability. Following 24hrs of HKL (10μM) treatment, cells were treated with cycloheximide (cyclo) for different time periods (hrs). At the indicated time point cells were harvested and the lysate analyzed by western blotting with indicated antibodies.



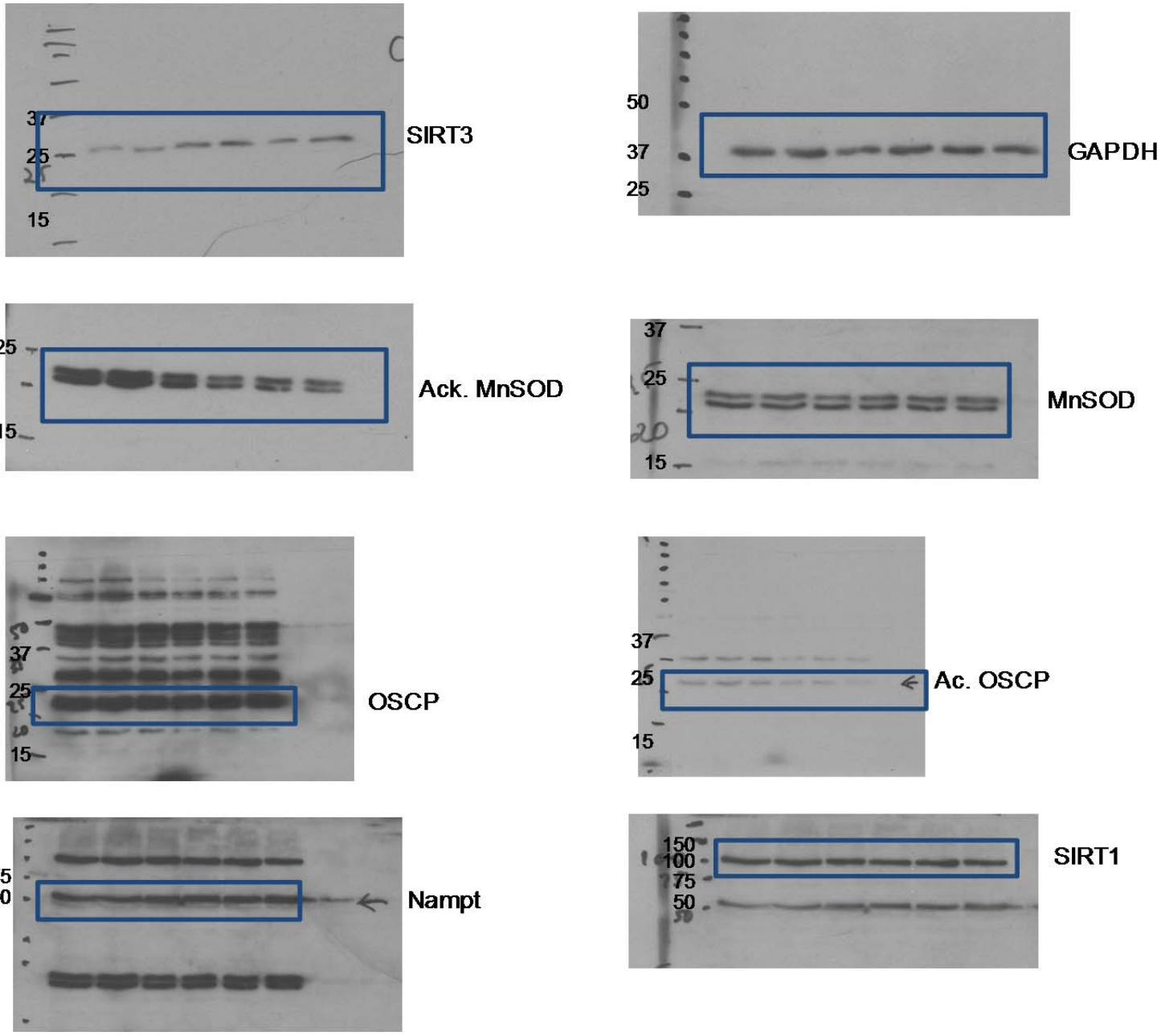
Supplementary Figure 9: Honokiol treatment increases SIRT3 mRNA expression. Cardiomyocytes were treated with 10μM HKL and SIRT3 mRNA levels were measured at 3 and 6 hrs after treatment. Mean ± SE, n = 4 independent experiments; Students *t* test.



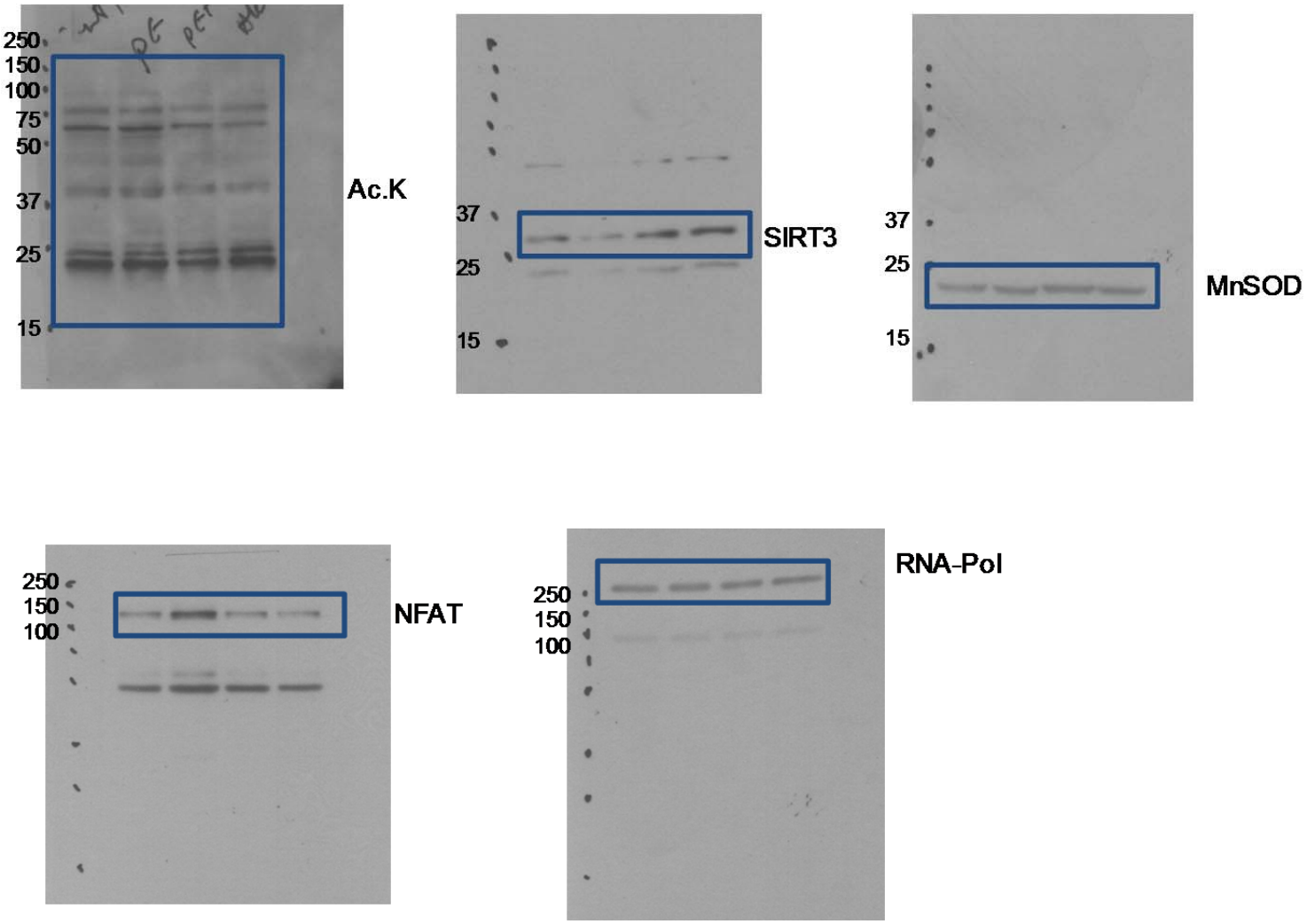
Supplementary Figure 10: Endogenous SIRT3 promoter is needed for HKL-mediated expression of SIRT3. HeLa cells stably expressing Flag.SIRT3 (under CMV promoter) were treated with 10μM HKL for 24hrs; thereafter cells were treated with actinomycin-D for the indicated time points, and then analyzed by western blotting with indicated antibodies.



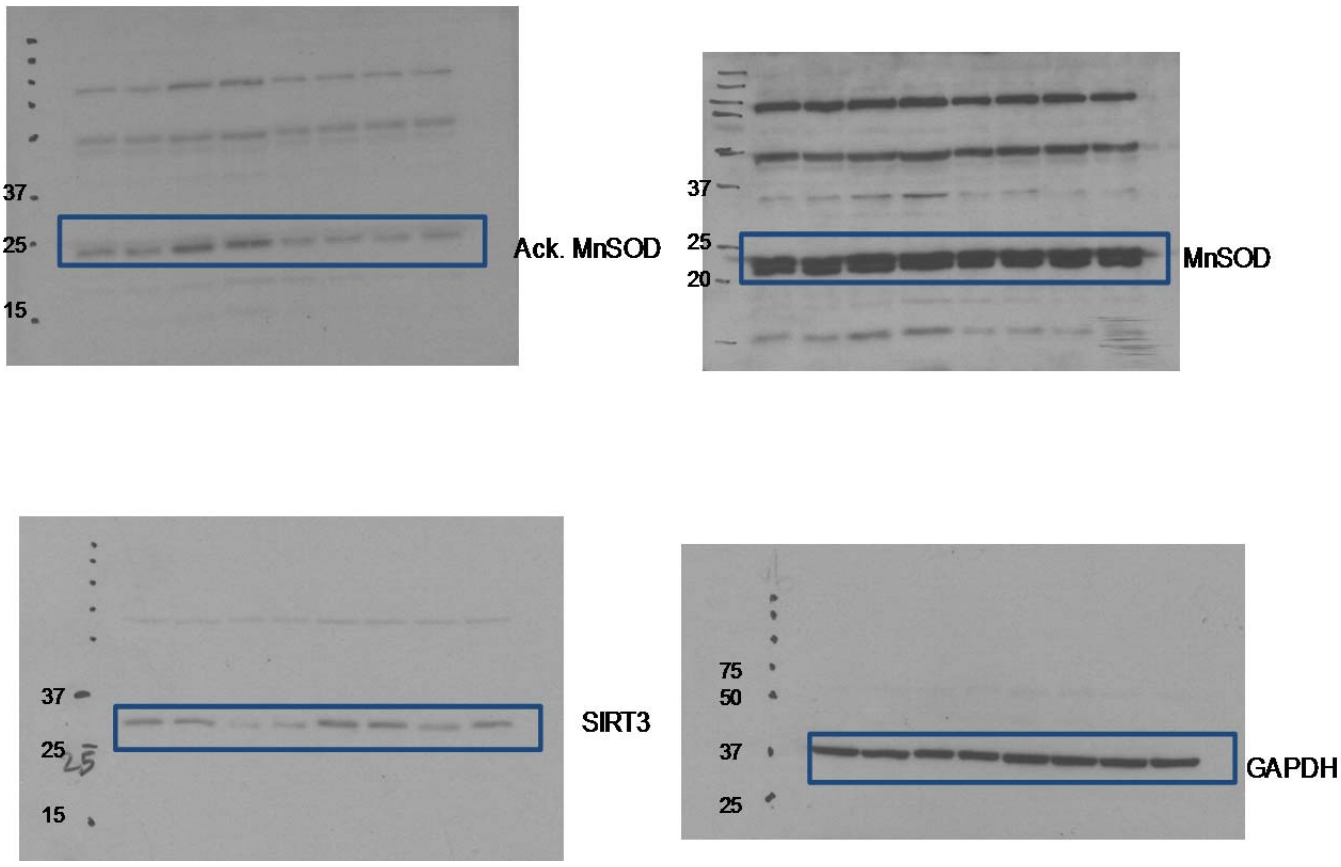
Supplementary Figure 11: Original Blots of Fig. 1A and Fig. 1B



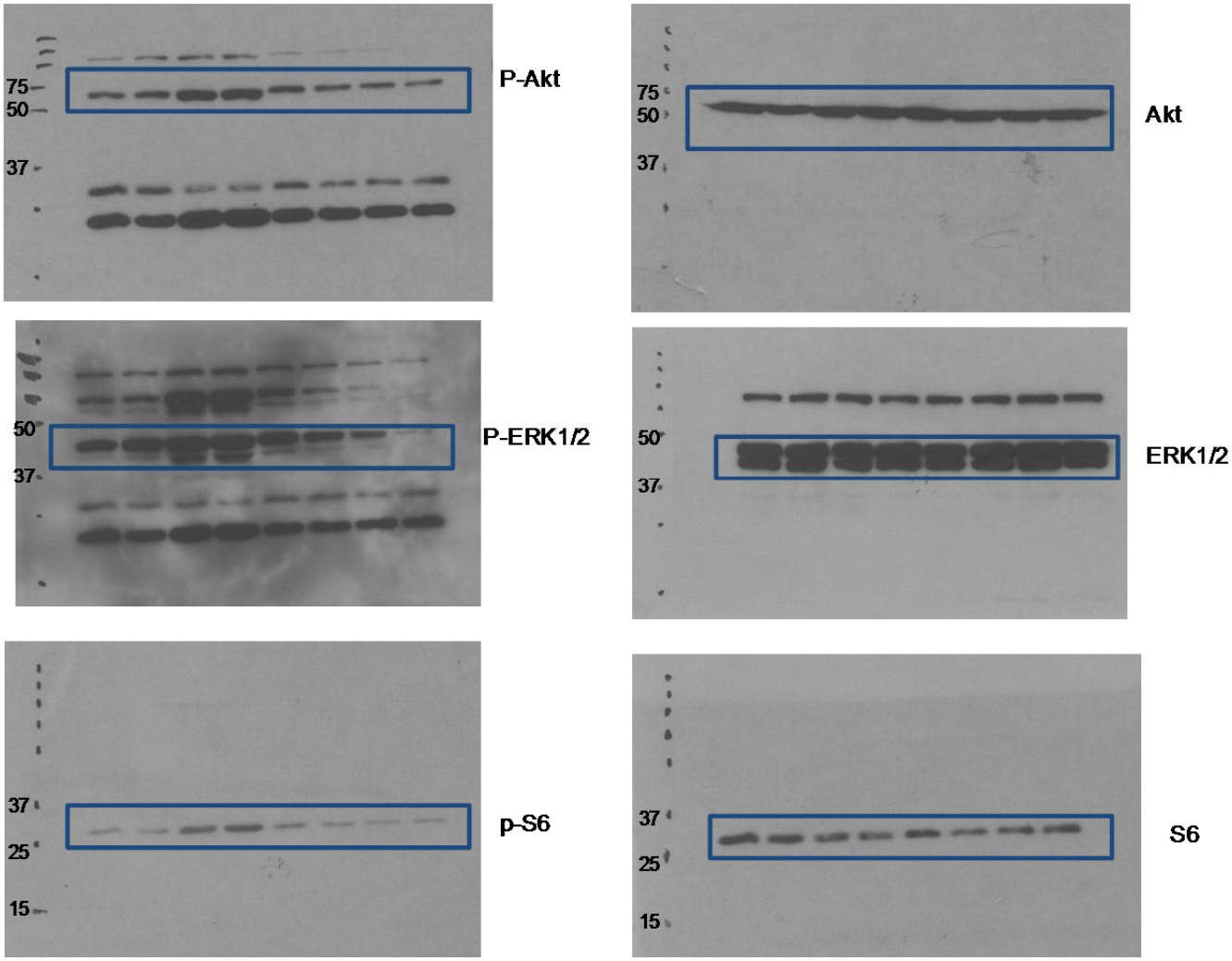
Supplementary Figure 12: Original Blots of Fig. 1C



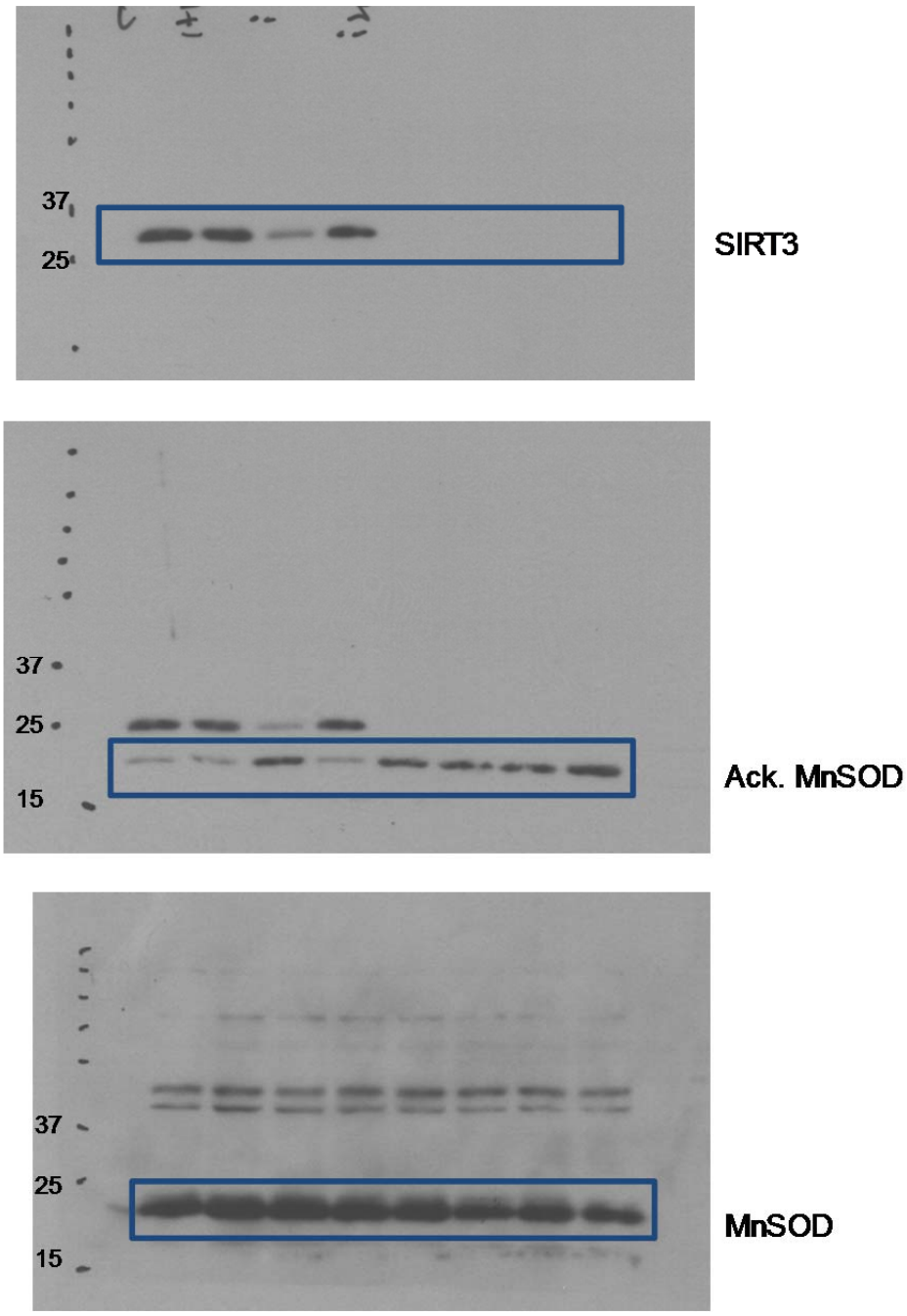
Supplementary Figure 13: Original Blots of Fig. 2



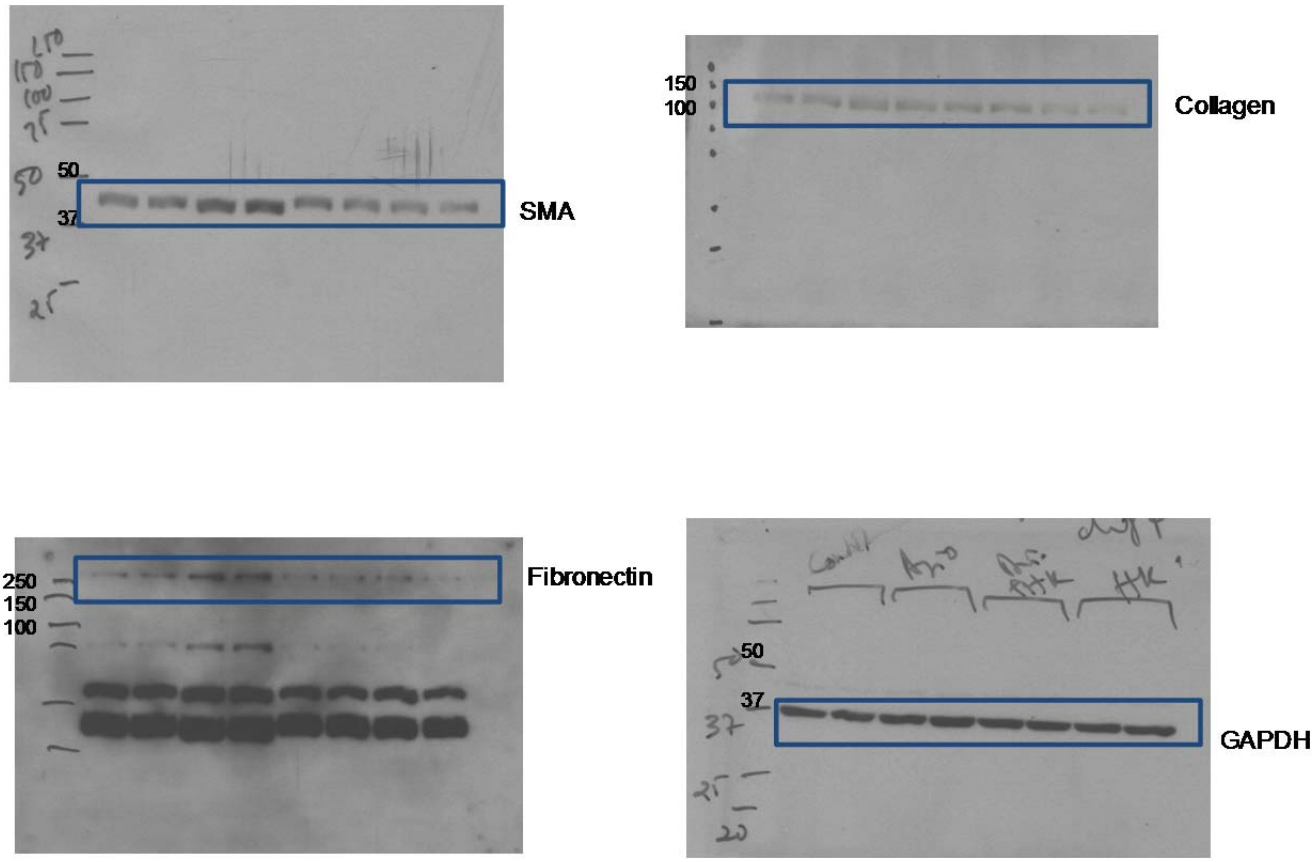
Supplementary Figure 14: Original Blots of Fig. 3



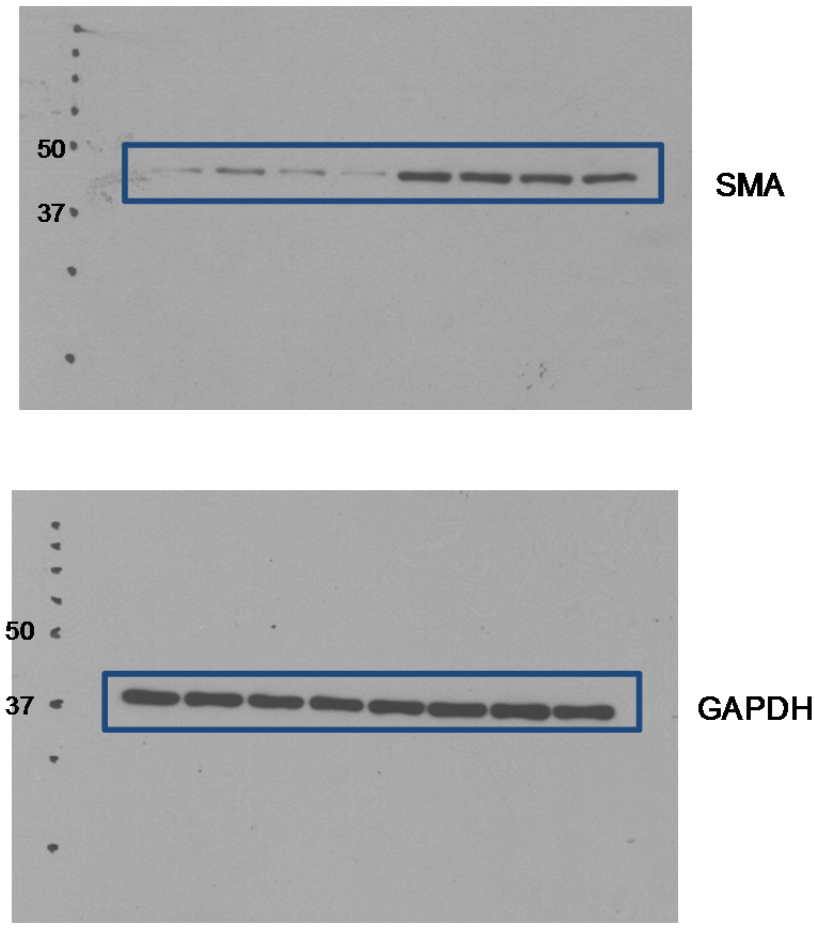
Supplementary Figure 15: Original Blots of Fig. 4



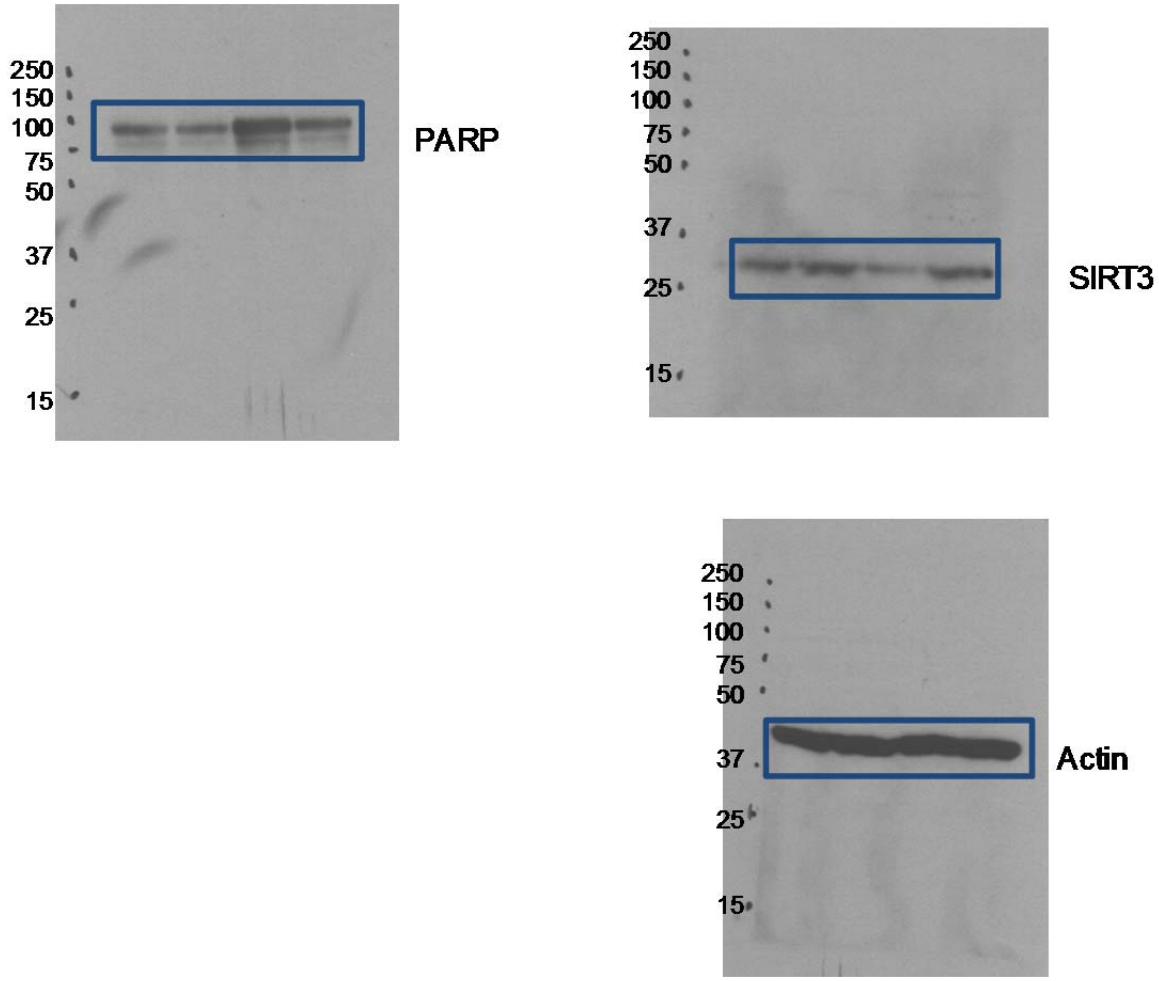
Supplementary Figure 16: Original Blots of Fig. 5



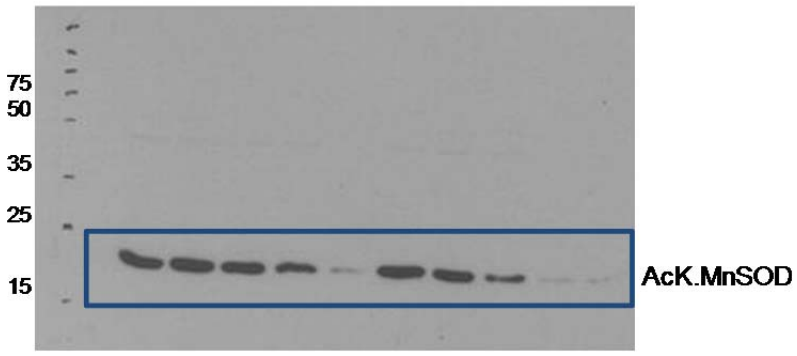
Supplementary Figure 17: Original Blots of Fig. 6

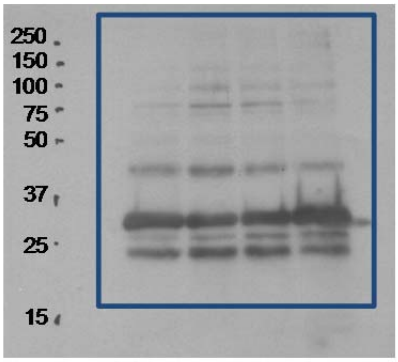


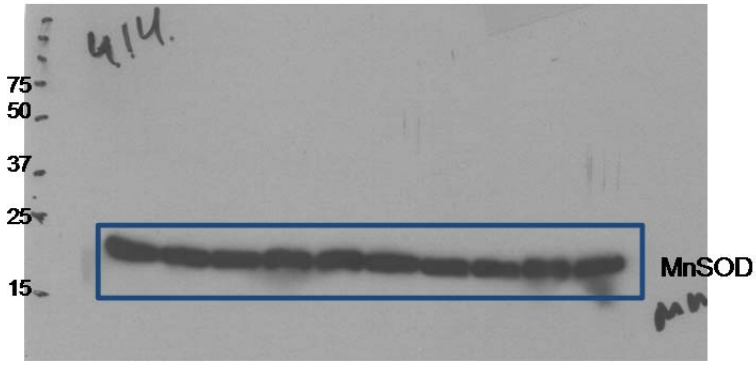
Supplementary Figure 18: Original Blots of Fig. 7

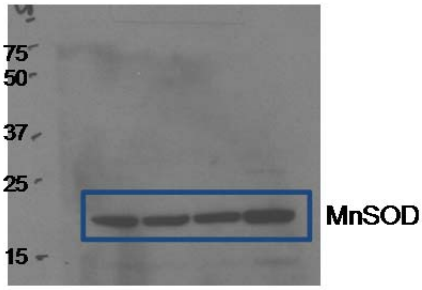


Supplementary Figure 19: Original Blots of Fig. 8

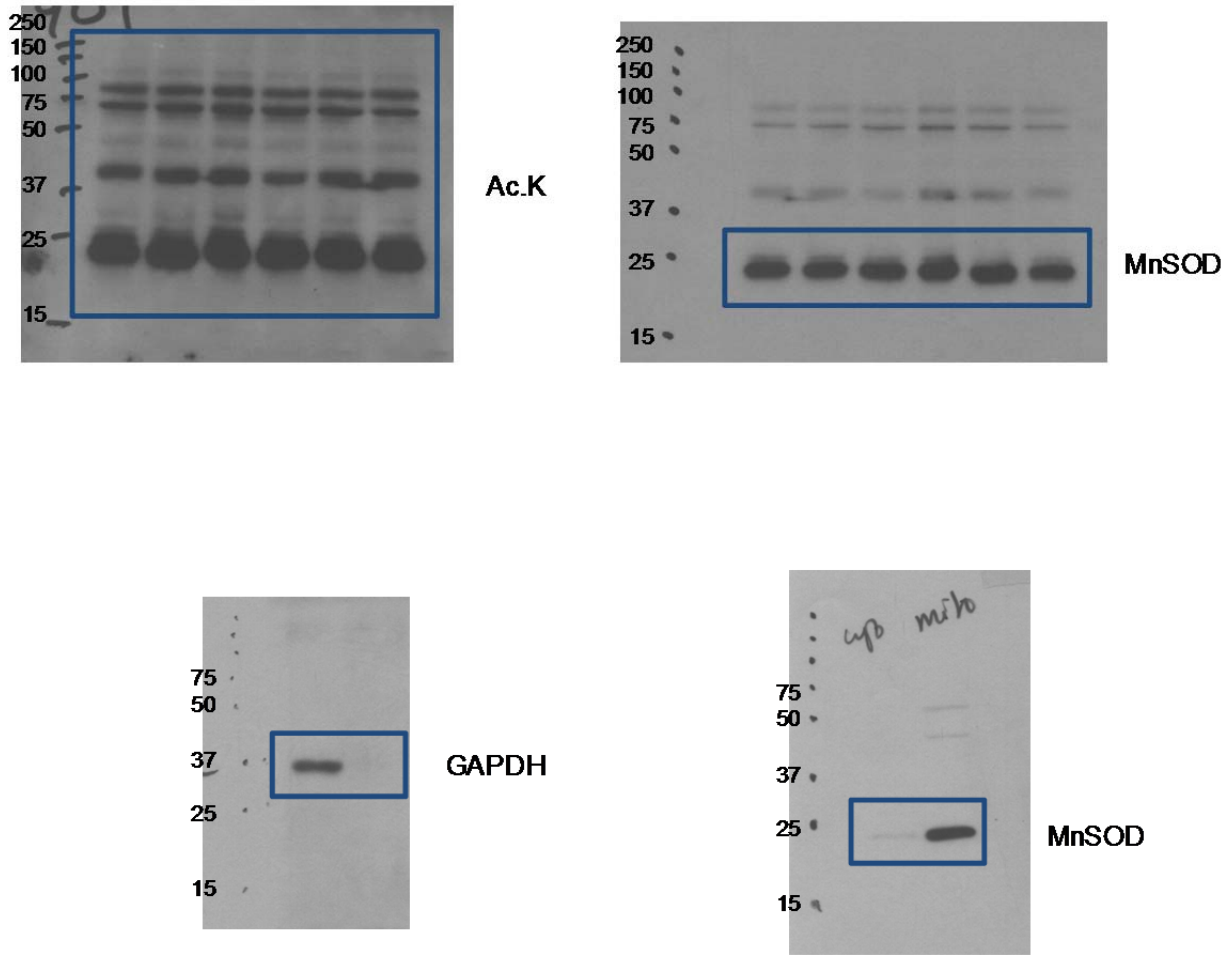




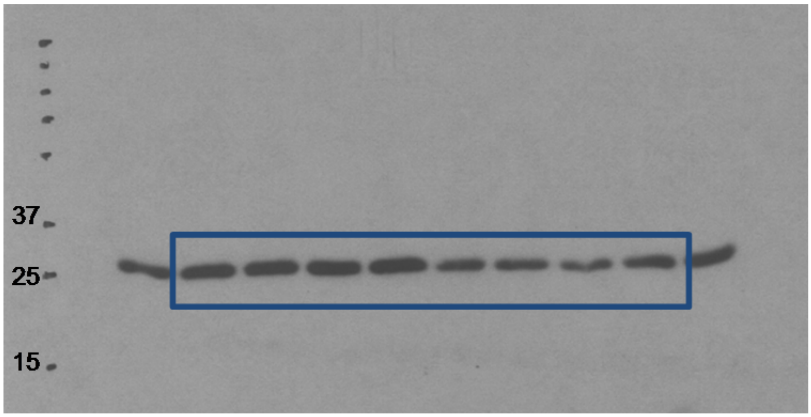


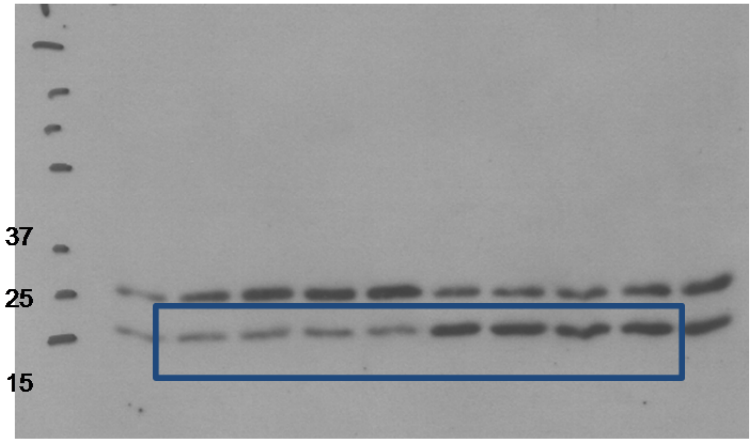


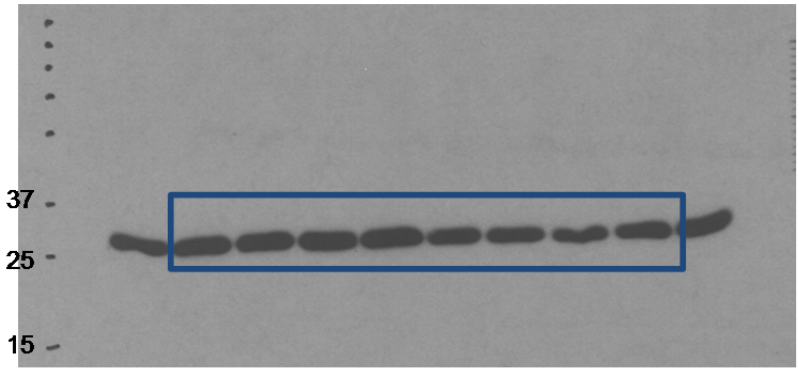
Supplementary Figure 20: Original Blots of Fig. 9.



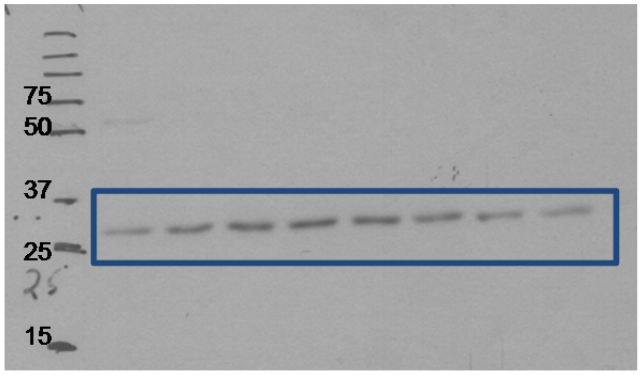
Supplementary Figure 21: Original Blots of Suppl. Fig. 1

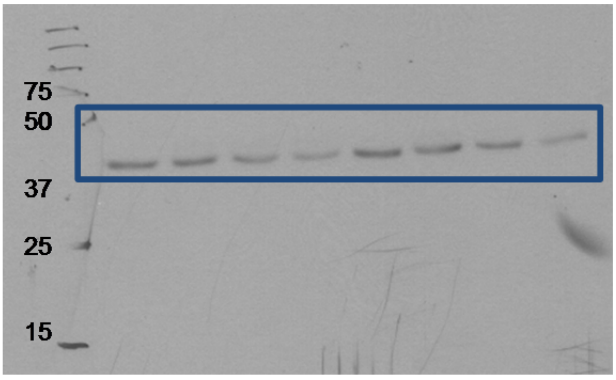


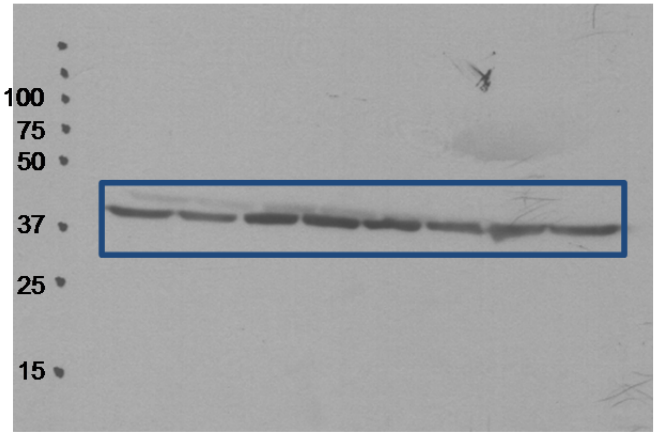




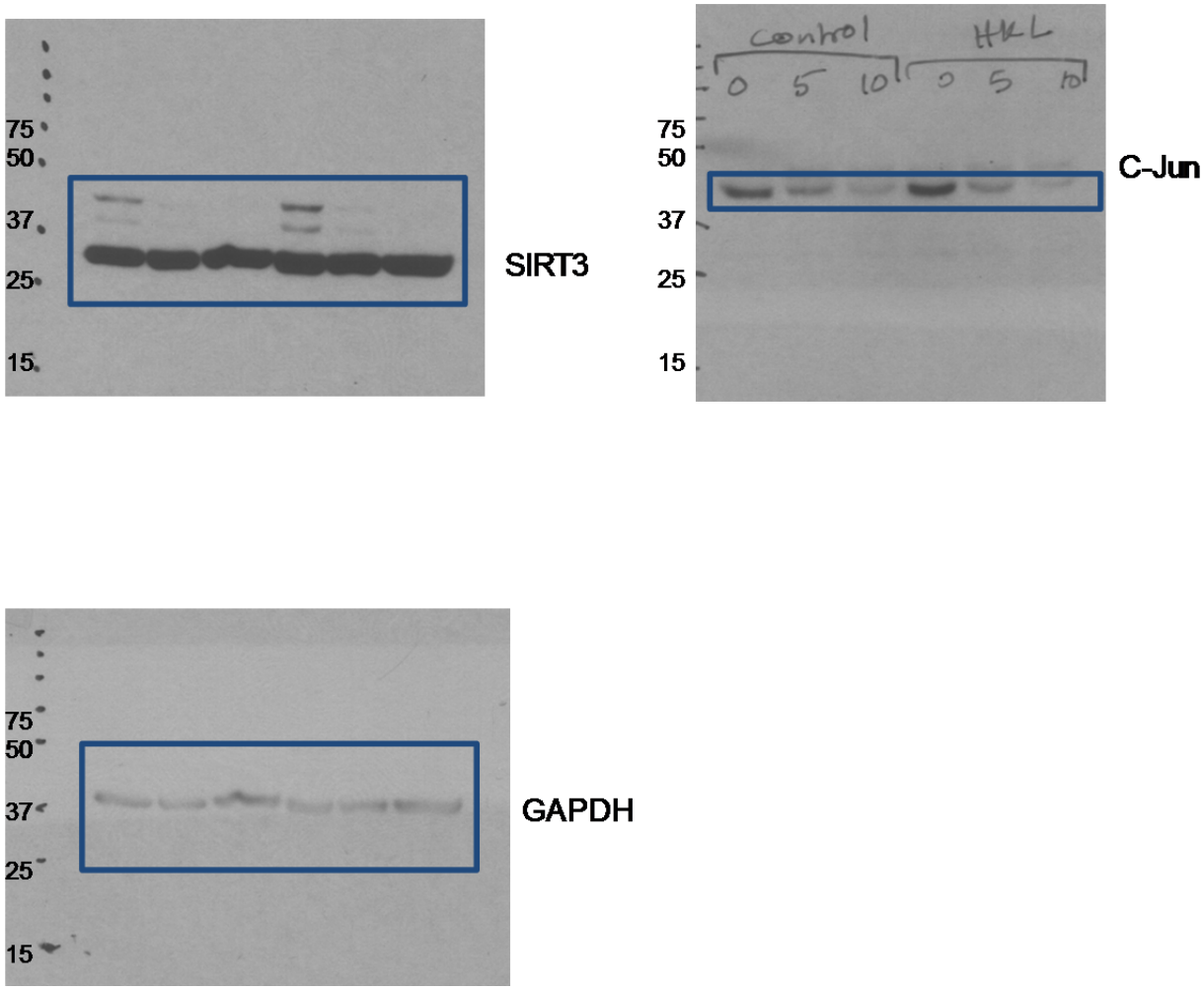
Supplementary Figure 22: Original Blots of Suppl. Fig. 3







Supplementary Figure 23: Original Blots of Suppl. Fig. 8



Supplementary Figure 24: Original Blots of Suppl. Fig. 10