Cardiomyocyte Specific Deletion of *Sirt1* Gene Sensitizes Myocardium to Ischemia and Reperfusion Injury

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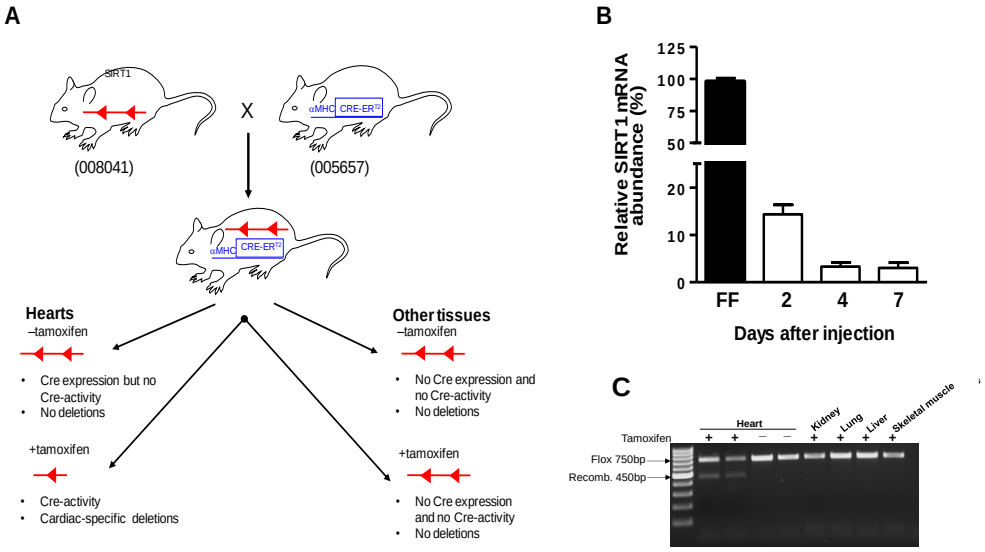
Materials and Methods

icSIRT1 knockout mice generation: The tamoxifen-inducible, cardiac-specific *SIRT1* knockout mice (icSIRT1 KO) were produced as shown below by breeding *SIRT1*$^{flox/flox}$ mice (008041) and CreER^{T2} (005657) mice that were purchased from Jackson Laboratory. Mice will be maintained on a 12 hour light/dark cycle in a controlled environment with water *ad libitum*. Animal procedures will be carried out with protocols approved by the Institutional Animal Care and Use Committee. The primers for genotyping: αMHC-Cre: forward, 5'-CTAGGCCACAGAATTGAAAGATCT-3'; reverse, 5'-GTAGGTGGAAATTCTAGCATCATCC-3'. Transgene: forward, 5'-ATACCGGAGATCATGCAAGC; reverse, 5'-AGGTGGACCTGATCATGGAG-3'. Sirt1: forward, 5'-GGTTGACTTAGGTCTTGTCTG-3'; reverse, 5'-CGTCCCTTGTAATGTTTCCC-3'.



Tamoxifen-inducible disruption of *SIRT1* gene expression in the myocardium. Generation of cardiac-specific SIRT1 KO mice after tamoxifen injection. **(A)** Scheme to achieve cardiac-specific deletions using the CreER^{T2} system. A cardiac-specific promoter (αMHC) drives the expression of a tamoxifen-inducible Cre recombinase (CreER^{T2}). **(B)** *SIRT1* mRNA loss in KO left ventricle (LV). Mice received tamoxifen (5 x 0.08 mg/g i.p.); LV were harvested at the indicated time points (days). *SIRT1* transcript was quantified by RT-qPCR (each group n=3-5). **(C)** PCR analysis of genomic DNA from the hearts of double-heterozygous mice with or without tamoxifen. Genomic DNA was also analyzed from the brain, kidney, lung, liver and skeletal muscle from tamoxifen-treated double-heterozygous mice.

Suppl Table 1. Echocardiographic assessment of cardiac function of young, aged, icSIRT1 KO and aged + AAV-Sirt1 mice under normal physiological and ischemia/reperfusion conditions.

Parameter (unit)	Young		Aged		icSIRT1 KO		Aged + AAV-Sirt1	
	Sham	I45'/R24h	Sham	I45'/R24h	Sham	I45'/R24h	Sham	I45'/R24h
Heart rate (min^{-1})	419.2 ± 26.82	403.8 ± 29.75	363.5 ± 21.19	399.3 ± 7.881	430.5 ± 7.179	472.4 ± 52.66	369.7 ± 20.12	450.0 ± 4.950
IVSd (mm)	0.763 ± 0.042	0.753 ± 0.054	0.838 ± 0.073	0.773 ± 0.046	0.938 ± 0.078	0.805 ± 0.172	0.703 ± 0.092	0.757 ± 0.105
IVSs (mm)	0.912 ± 0.062	0.858 ± 0.060	1.010 ± 0.225	0.977 ± 0.019	1.083 ± 0.075	1.120 ± 0.055	0.993 ± 0.160	0.983 ± 0.039
LVPWd (mm)	0.768 ± 0.027	0.783 ± 0.063	0.880 ± 0.083	0.970 ± 0.075	0.978 ± 0.066	0.885 ± 0.091	0.803 ± 0.049	0.773 ± 0.077
LVPWs (mm)	0.980 ± 0.028	0.945 ± 0.109	1.130 ± 0.107	0.890 ± 0.158	1.125 ± 0.062	0.885 ± 0.194	0.997 ± 0.058	0.712 ± 0.087
LVIDd (mm)	3.560 ± 0.097	3.533 ± 0.221	3.858 ± 0.167	4.073 ± 0.458	3.693 ± 0.140	3.773 ± 0.329	4.287 ± 0.184	4.225 ± 0.250
LVIDs (mm)	2.863 ± 0.081	2.570 ± 0.206	2.898 ± 0.098	3.237 ± 0.309	2.678 ± 0.119	2.968 ± 0.312	3.270 ± 0.090	3.350 ± 0.348
EF (%)	62.47 ± 0.695	56.31 ± 2.569*	57.69 ± 1.743	38.79 ± 1.824*†	60.99 ± 1.166	46.26 ± 1.704*†	53.80 ± 1.490	50.16 ± 2.570*†§
FS (%)	34.36 ± 0.963	26.06 ± 1.214*	29.97 ± 1.137	18.46 ± 0.970*†	29.87 ± 1.715	20.70 ± 1.082*†	27.61 ± 1.014	25.32 ± 1.440*†§
Body weight (g)	26.49 ± 0.415		33.93 ± 0.911		24.33 ± 0.827		32.90 ± 1.331	

Values are means ± SEM, n=5-7, *p<0.05 *vs*. Sham, †p<0.05 *vs*. young I/R, §p<0.05 *vs.* aged I/R.

Suppl Table 2. *Ex vivo* hemodynamic parameters of cardiac function of young, aged and icSIRT1 KO mice under basal and ischemia/reperfusion conditions.

Parameter (unit)	Young		Aged		icSIRT1 KO	
	Basal	I/R	Basal	I/R	Basal	I/R
Cardiac output (ml/min)	4.342 ± 0.885	3.990 ± 0.691	4.048 ± 0.865	2.190 ± 0.512$^{\#}$†	4.373 ± 0.100	2.449 ± 0.799§†
Coronary flow (ml/min)	2.057 ± 0.079	1.810 ± 0.265	2.093 ± 0.057	1.640 ± 0.286	2.083 ± 0.062	1.603 ± 0.325
Systolic pressure (mmHg)	80.04 ± 12.38	80.95 ± 12.95	75.60 ± 5.387	76.20 ± 4.320	73.10 ± 6.989	67.71 ± 8.133
Diastolic pressure (mmHg)	12.69 ± 4.237	14.98 ± 5.290	30.49 ± 8.705*	39.50 ± 0.479†	32.02 ± 7.062*	31.59 ± 6.897†
Heart weight (wet, g)	0.1429±0.008		0.1828±0.0119		0.1716±0.020	

Values are means ± SEM, n=5, *p<0.05 *vs.* young basal; $^{\#}$p<0.05 *vs.* aged basal; §p<0.05 *vs.* icSIRT1 KO basal; †p<0.05 *vs.* young I/R.

Figure 1A

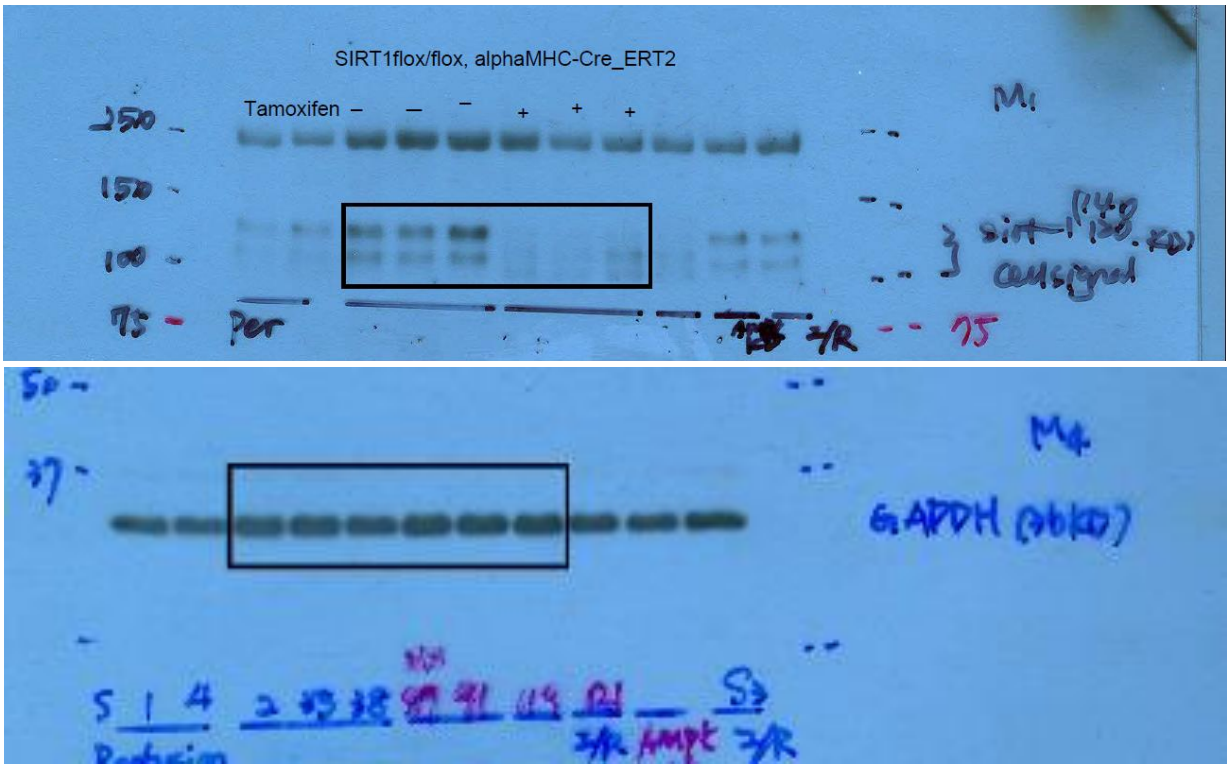
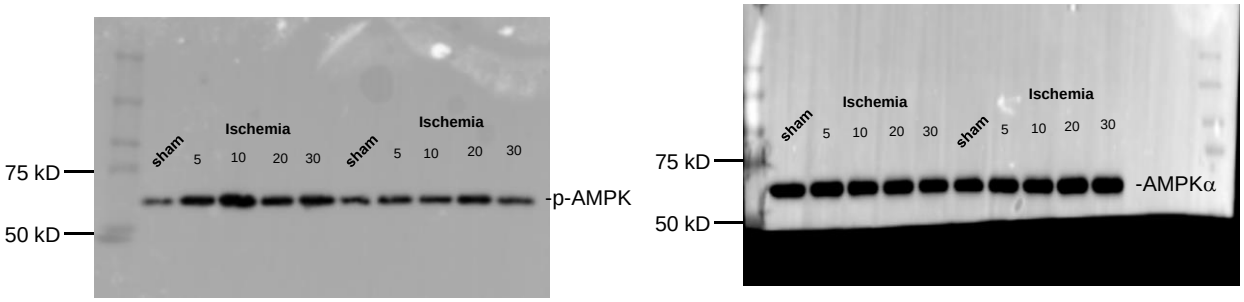


Figure 2A



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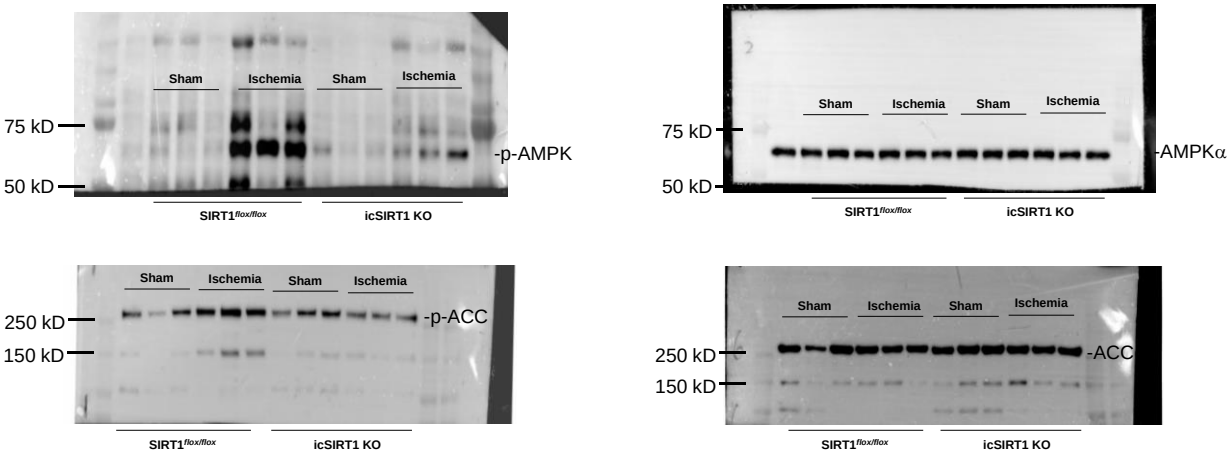

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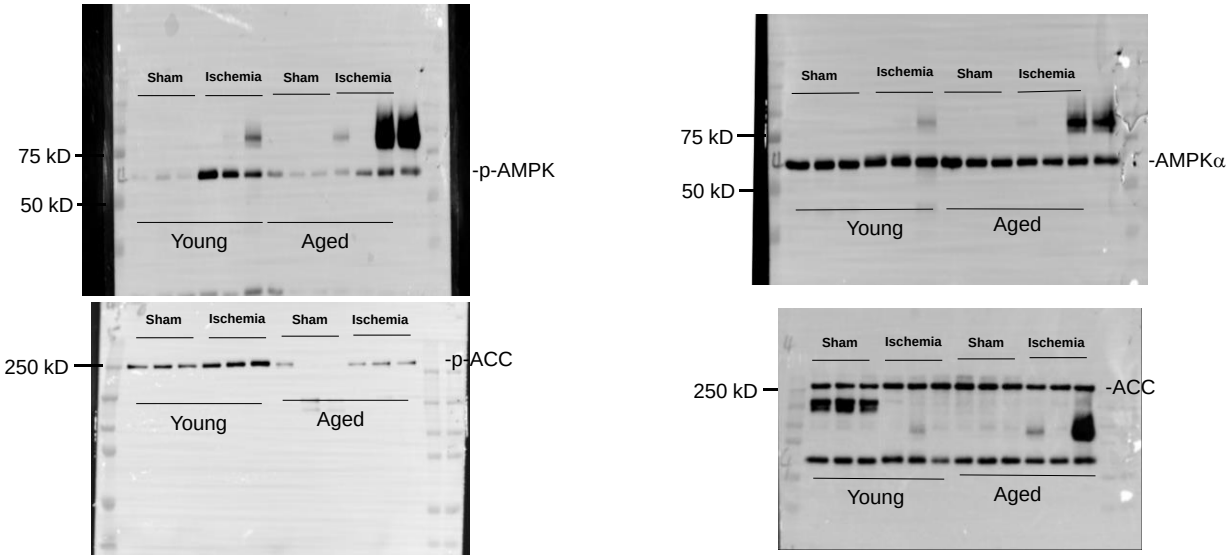

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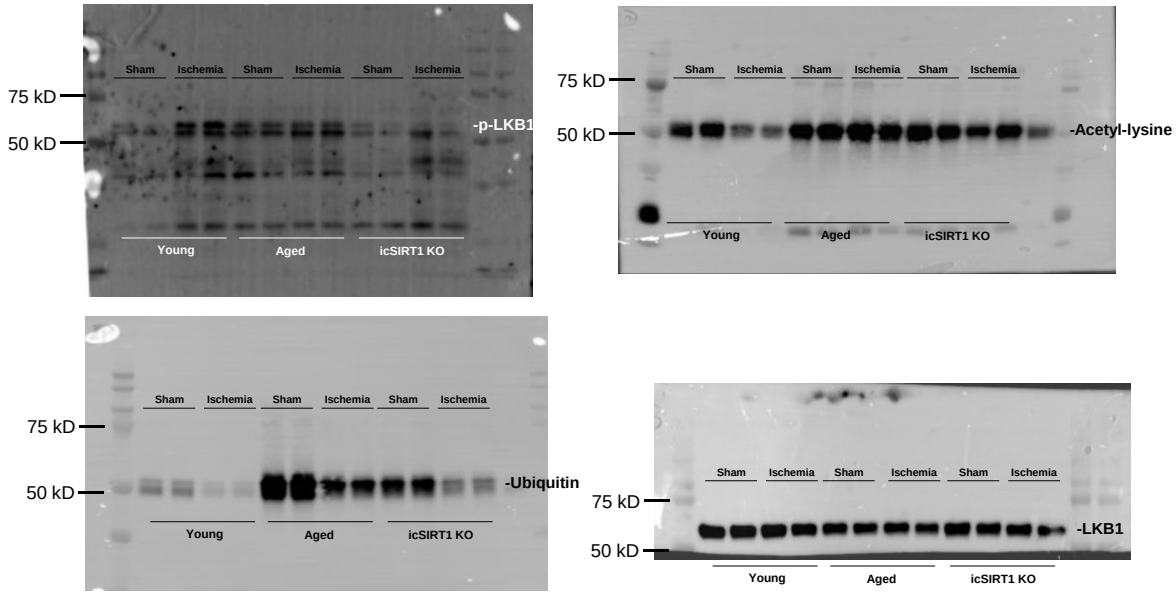
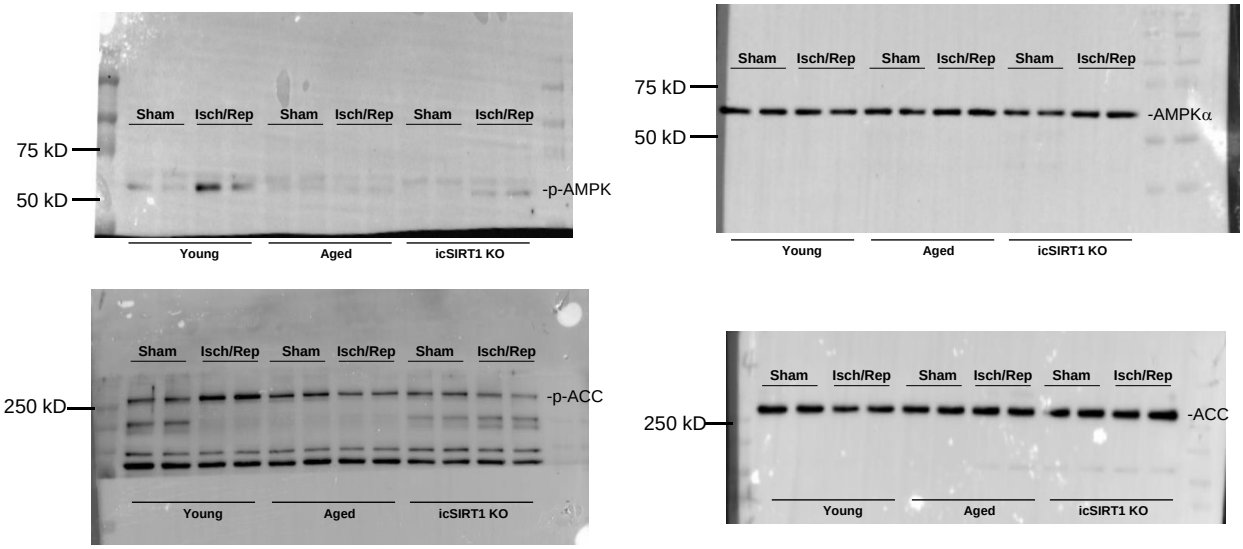
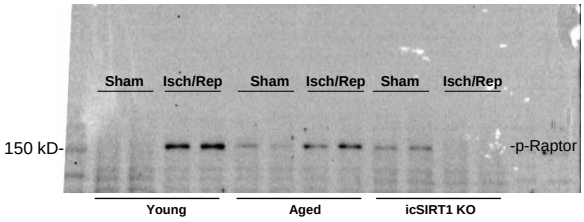


Figure 5A

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Sham
Isch/Rep
Sham
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-p-Raptor
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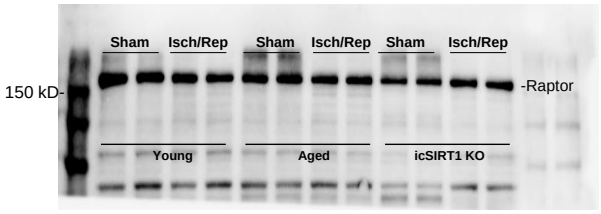
Aged
icSIRT1 KO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Isch/Rep
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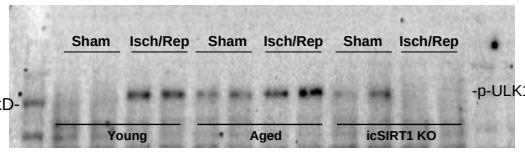
-Raptor
Young
Aged
icSIRT1 KO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Sham
Isch/Rep
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Isch/Rep
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-p-ULK1
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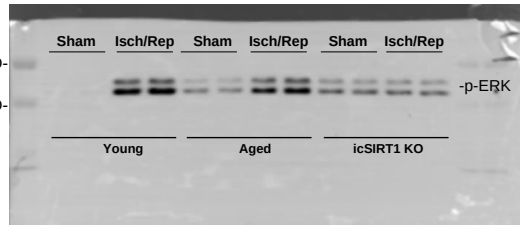
Aged
icSIRT1 KO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Sham
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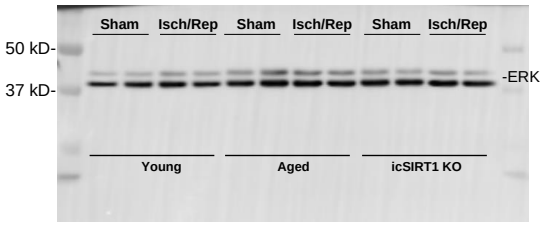
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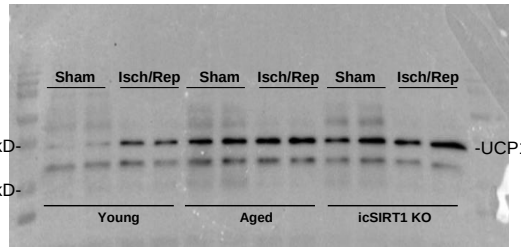
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25 kD-
-UCP1
Young
Aged
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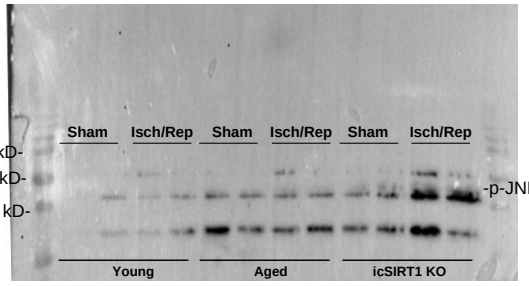
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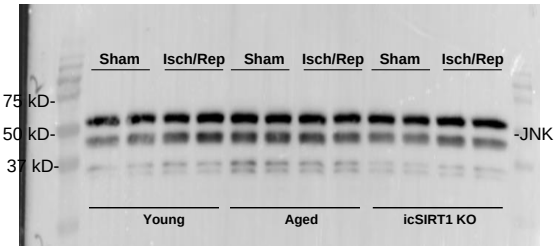
-p-JNK
Young
Aged
icSIRT1 KO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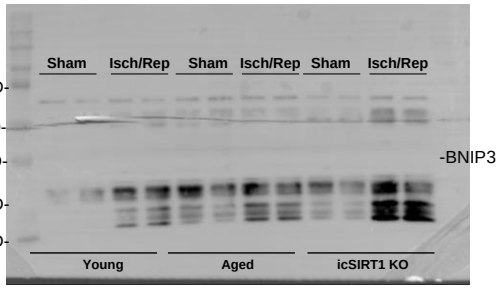
Young
Aged
icSIRT1 KO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Sham
Isch/Rep
Sham
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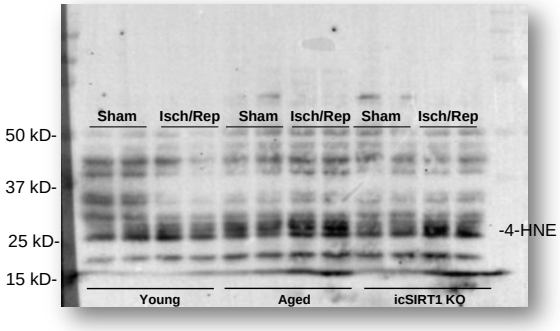
Young
Aged
icSIRT1 KO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Sham
Isch/Rep
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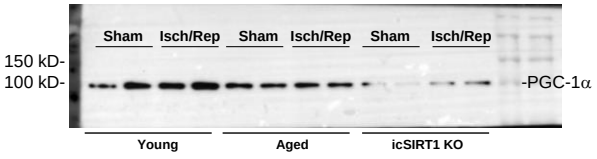
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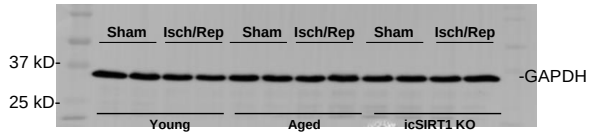
Young
Aged
icSIRT1 KO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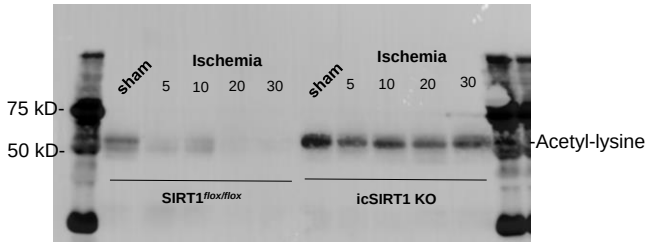
Isch/Rep
Sham
Isch/Rep
Sham
Isch/Rep
37 kD-
25 kD-
-GAPDH
Young
Aged
icSIRT1 KO

Figure 6A



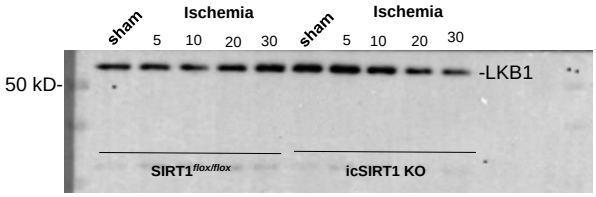
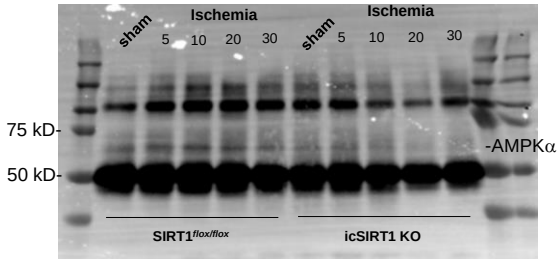
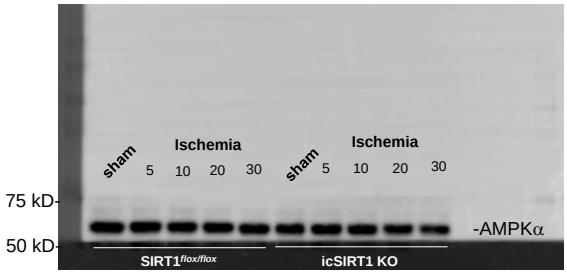
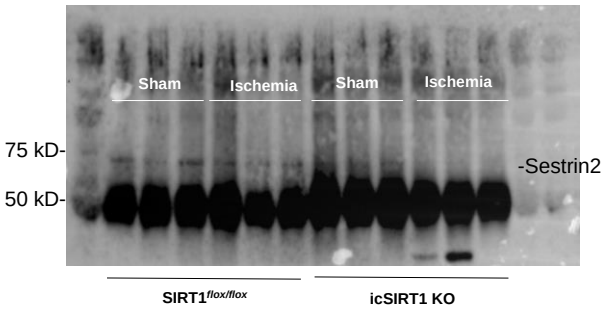


Figure 6B







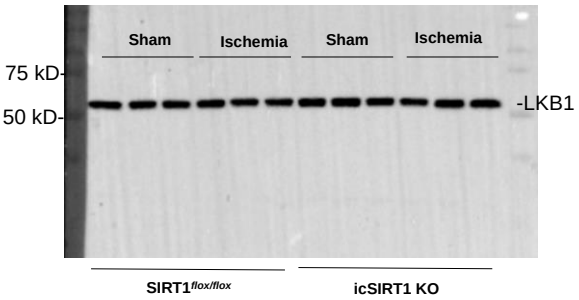
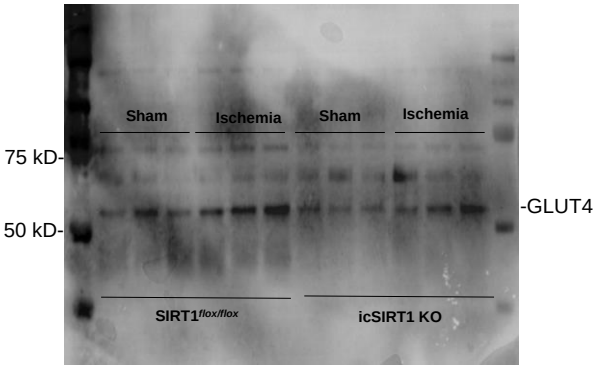
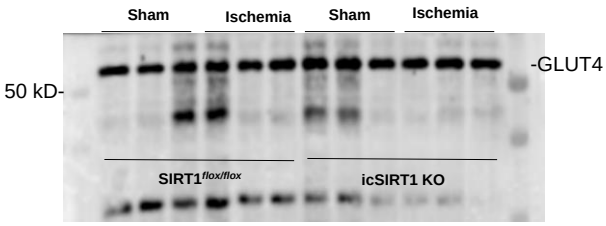


Figure 6C





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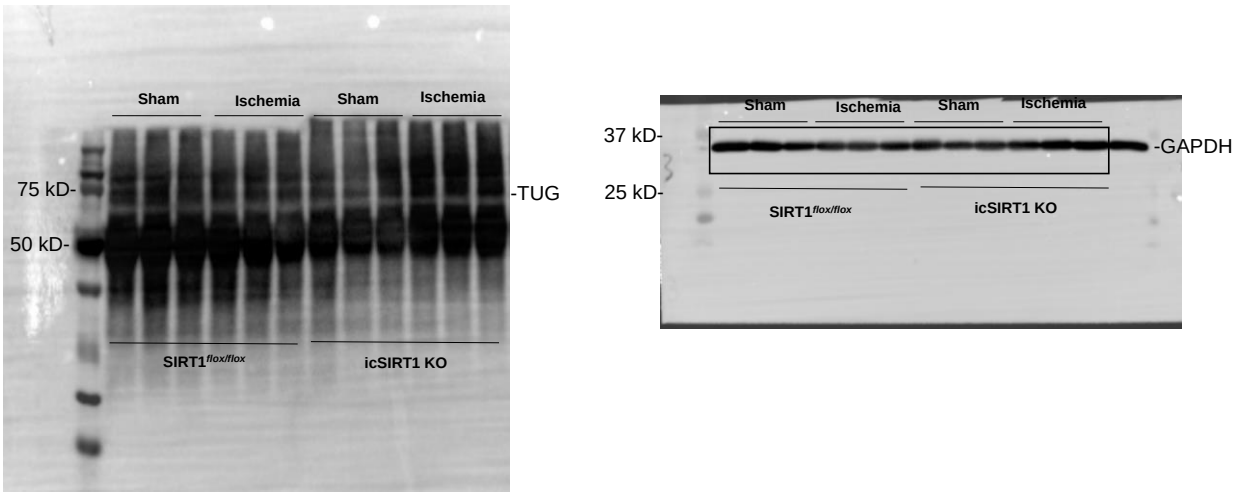

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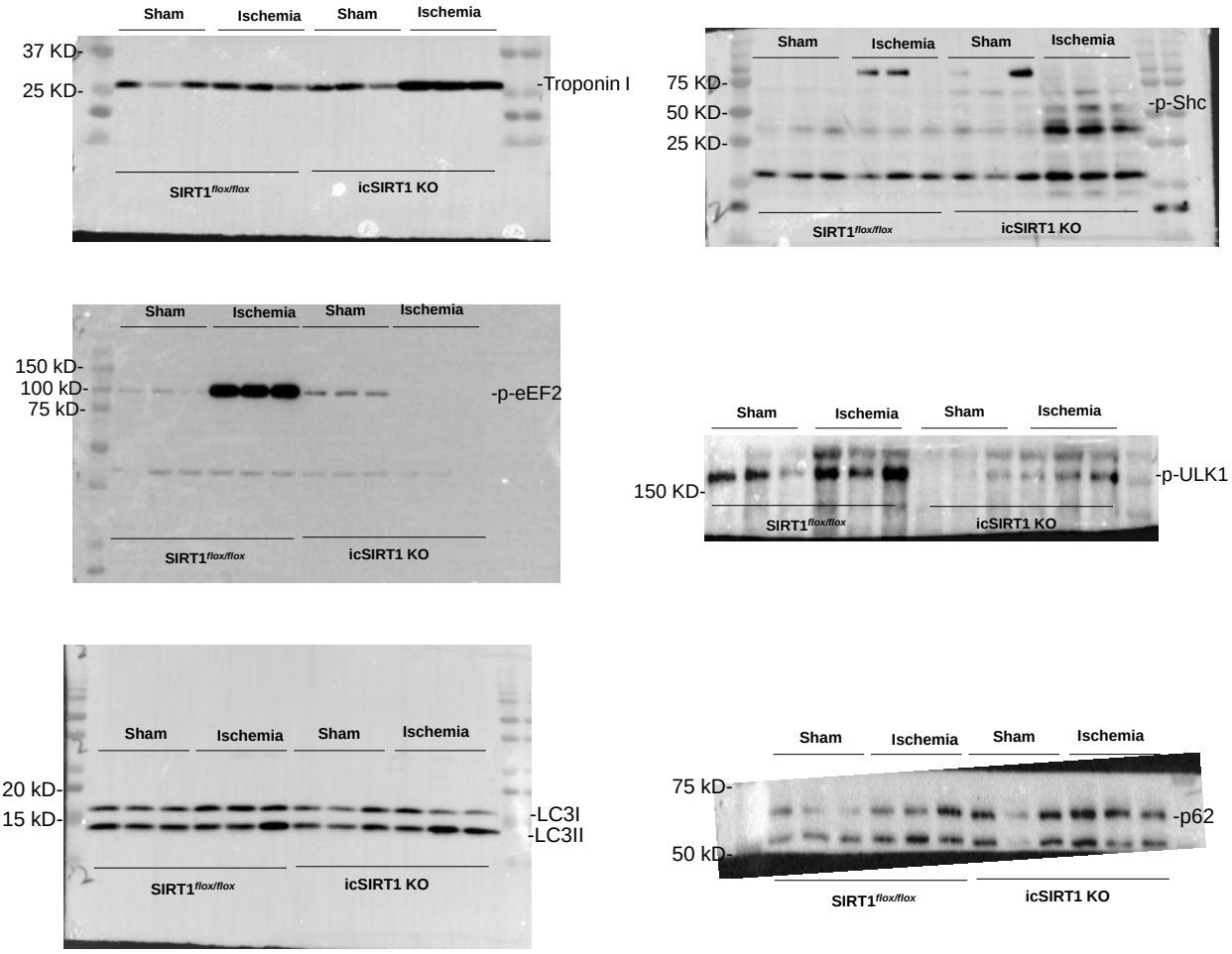

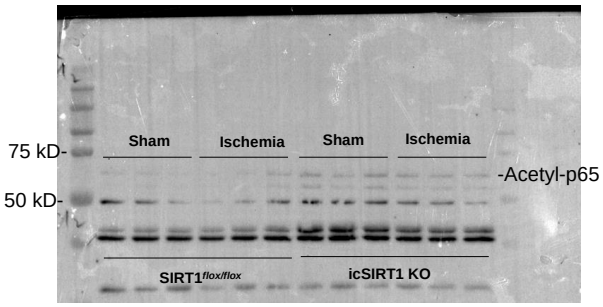
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Ischemia
Sham
Ischemia
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-Acetyl-p65
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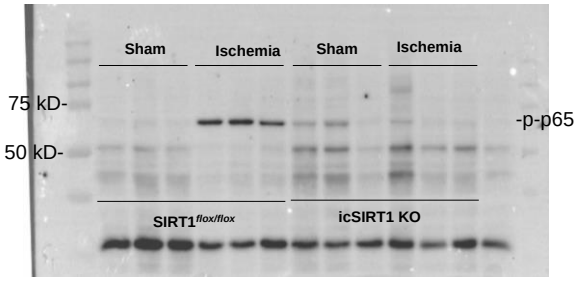
icSIRT1 KO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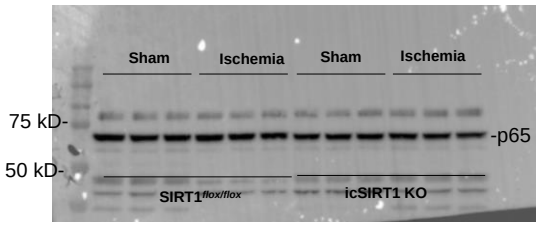
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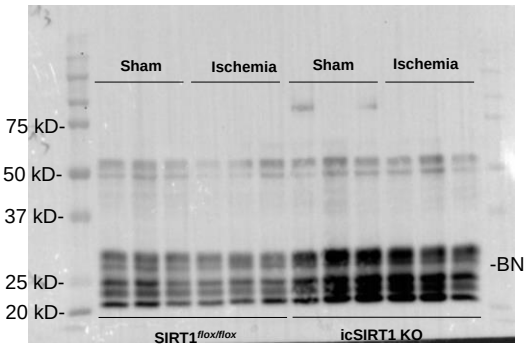
SIRT1flox/flox
icSIRT1 KO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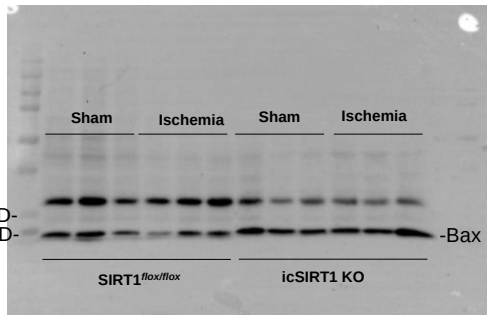
SIRT1flox/flox
icSIRT1 KO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Ischemia
Sham
Ischemia
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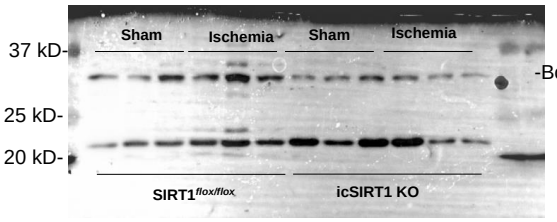
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Sham
Ischemia
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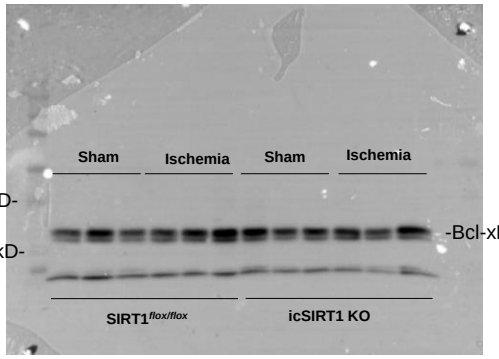
SIRT1flox/flox
icSIRT1 KO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Ischemia
Sham
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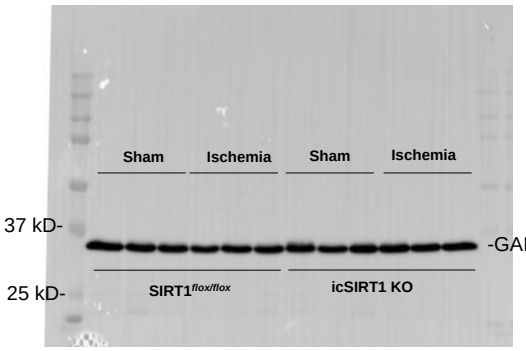
-Bcl-xL
SIRT1flox/flox
icSIRT1 KO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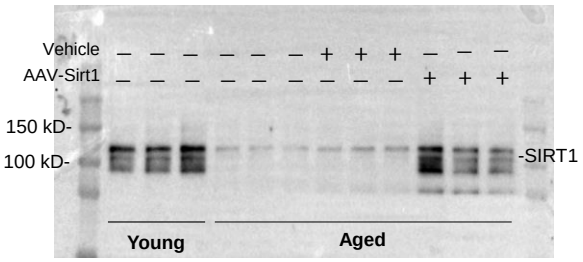
SIRT1flox/flox
icSIRT1 KO

Figure 7A



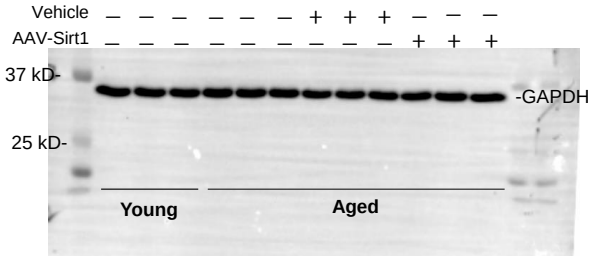
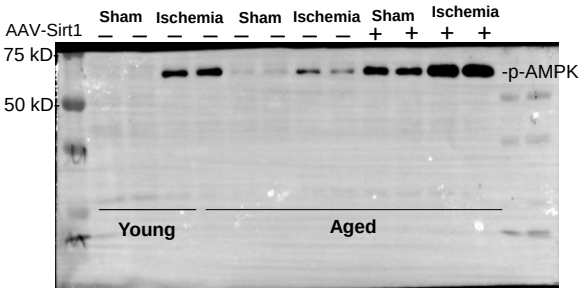
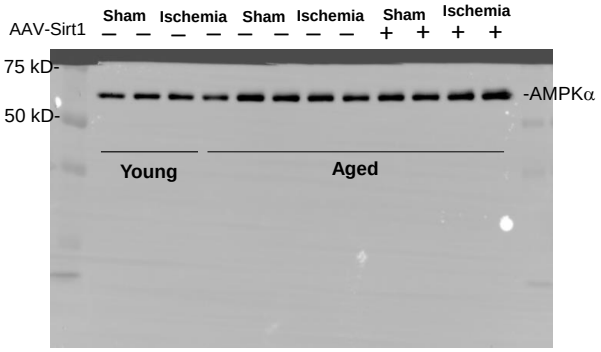


Figure 7B





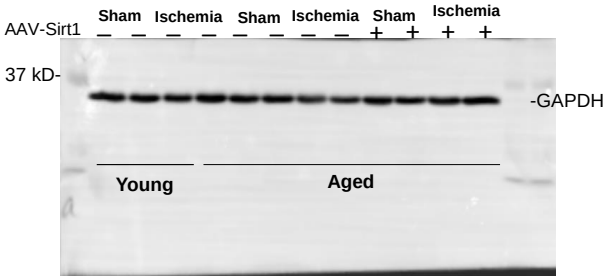
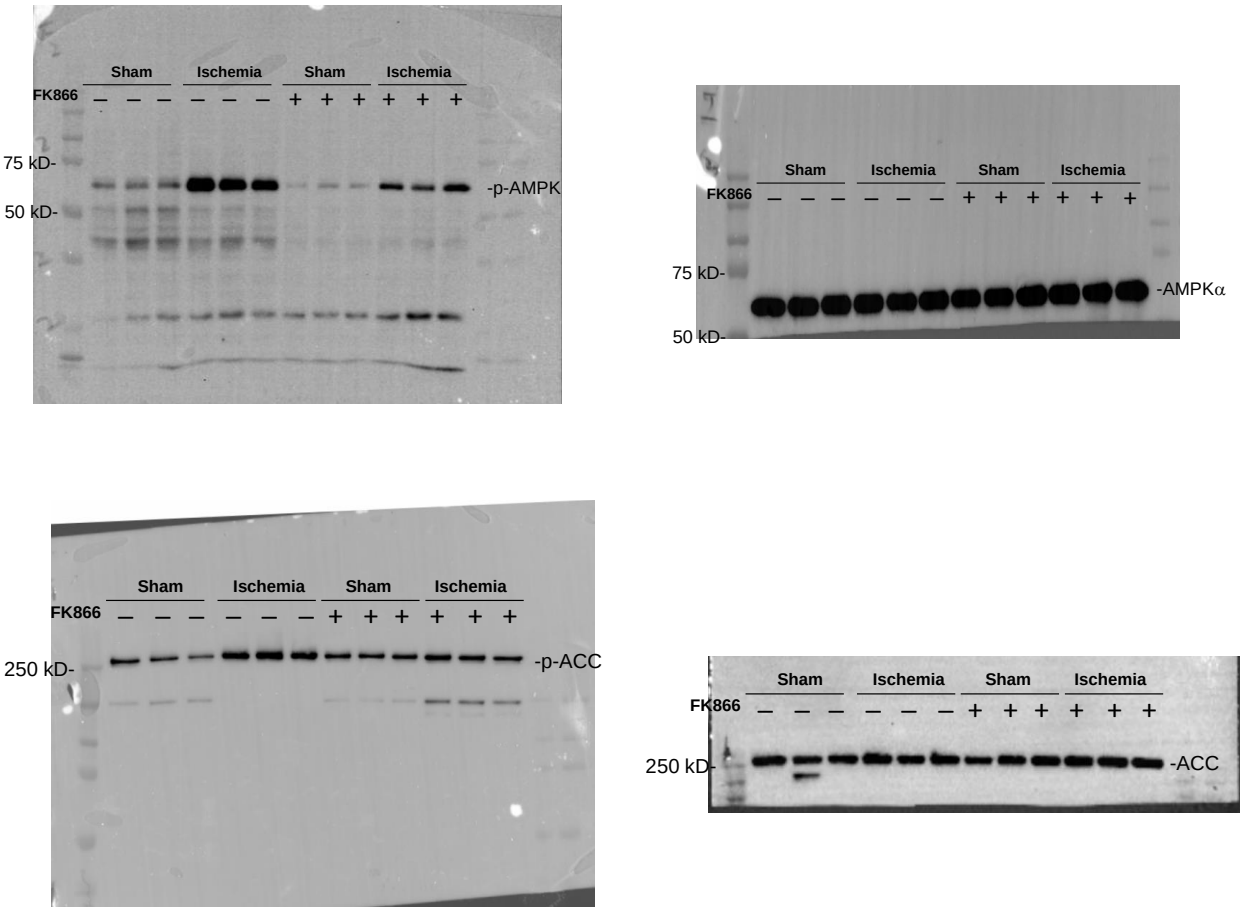


Figure 8D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