Supplemental Material

DJ-1 Protects the Heart Against Ischemia-Reperfusion Injury by Regulating Mitochondrial Fission

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Supplemental Material

Supplemental Figure Legends

Supplemental Figure 1. (**A-B**) Representative immunoblots and densitometric analysis of the cardiac expression of DJ-1 in Cytosolic, Nuclear, and Mitochondrial fractions following 45 minutes of myocardial ischemia and different periods of reperfusion. (**C**) Left Ventricular End Diastolic Diameter (LVEDD), **(D)** Left Ventricular End Systolic Diameter (LVESD), **(E)** Max dP/dT and Min dP/dT and **(F)** Tau measured in mice using echocardiography images and invasive hemodynamics 1 week following myocardial ischemia and reperfusion (1 wk Post). Numbers inside bars indicate sample size. N.D., not detected. Values are means ±SEM. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ vs. WT or Baseline.

Supplemental Figure 2. Relative gene expression of senp1 and senp5 in hearts from WT and DJ-1 KO mice collected following 45 minutes of ischemia and 4 hours of reperfusion. Values are means ±SEM.

Supplemental Figure 3. (A-B) Representative immunoblots and densitometric analysis of dynamin related protein-1 (Drp1) in whole cell fractions of heart homogenates from WT and DJ-1 KO mice following 45 minutes of ischemia and 4 hours of reperfusion. **(C-D)** Representative immunoblots and densitometric analysis of the fusion proteins, mitofusion-1 (Mfn1), mitofusion-2 (Mfn2), and Opa-1, as well as the fission protein, fission-1 (Fis1) in whole cell fractions of heart homogenates from Wild-Type and DJ-1 KO mice. **(E-F)** Representative immunoblots and densitometric analysis of Opa-1, Mfn-1, Mfn-2, and Fis1 in

mitochondrial fractions of heart homogenates from WT and DJ-1 KO mice following 45 minutes of ischemia and 4 hours of reperfusion. Values are means ±SEM. *p<0.05 and ***p<0.001 vs. WT Sham.

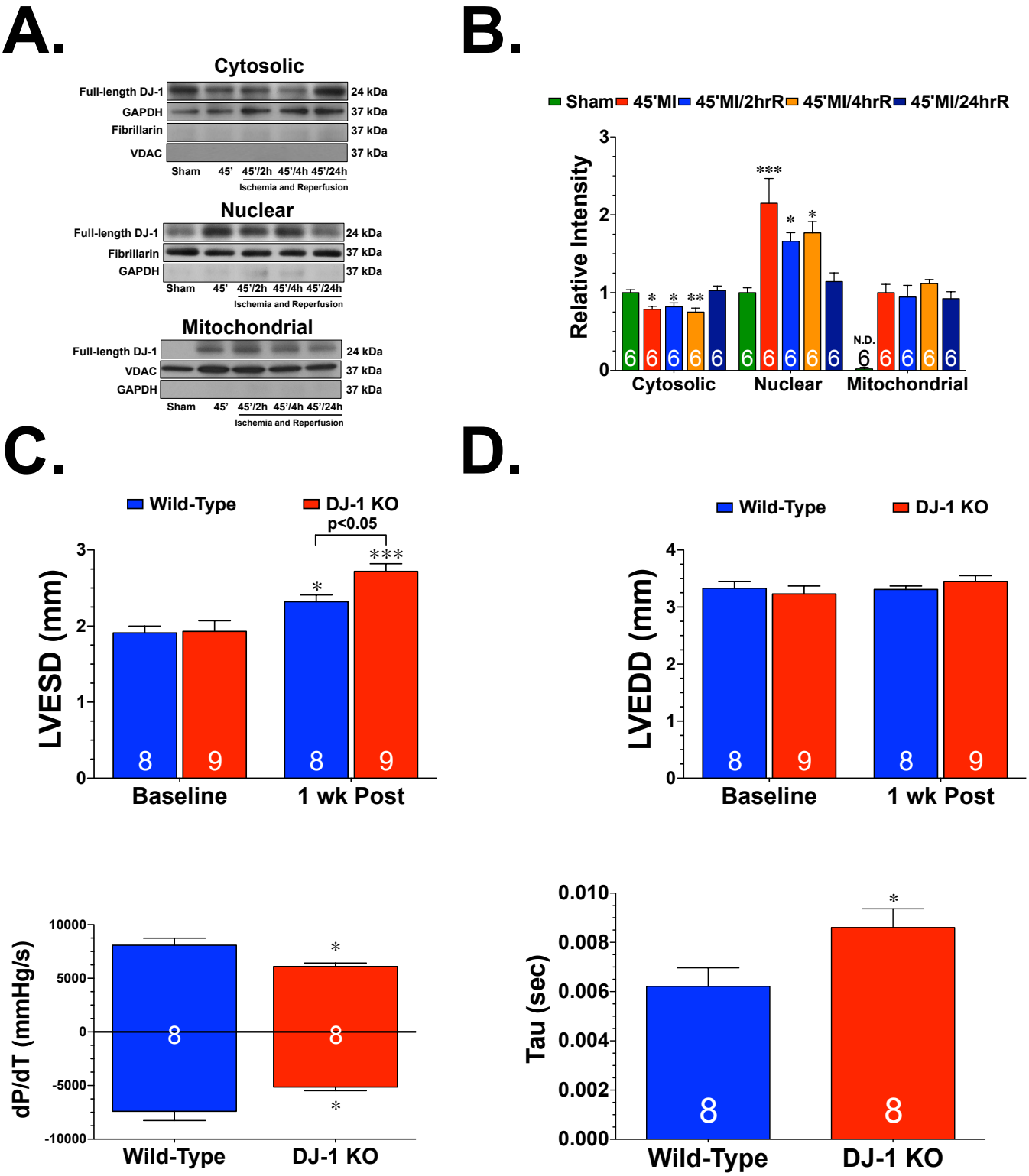
Supplemental Figure 4. (A-B) Representative immunoblots and densitometric analysis of Drp1 in mitochondrial fractions of hearts collected from different groups of WT and DJ-1 KO mice following 45 minutes of ischemia and 4 hours of reperfusion. Mice were administered vehicle (Veh) or the mitochondrial division inhibitor-1 (mdivi-1) 15 minutes prior to the onset of ischemia. **(C)** Myocardial area-at-risk (AAR) as a percentage (%) of total left ventricle (LV) and infarct size (INF) as a percentage of area-at-risk (AAR) and LV, as well as **(D)** circulating Troponin-I levels (ng/mL) were measured in DJ-1 KO mice subjected to 45 minutes of myocardial ischemia and 24 hours of reperfusion. Values are means ±SEM. ***p<0.001 vs. WT Sham.

Supplemental Figure 5. Representative immunoblots and densitometric analysis of **(A-B)** SENP5, (**C-D**) SUMO-1 modified proteins and (**E-F**) SUMO-2/3 modified proteins in samples collected from cells following H/R. Cells were transfected with siRNA-scr, siRNA-DJ1, siRNA-SENP5, or siRNA-DJ1/SENP5. Values are means±SEM of three independent experiments of three biological replicates.

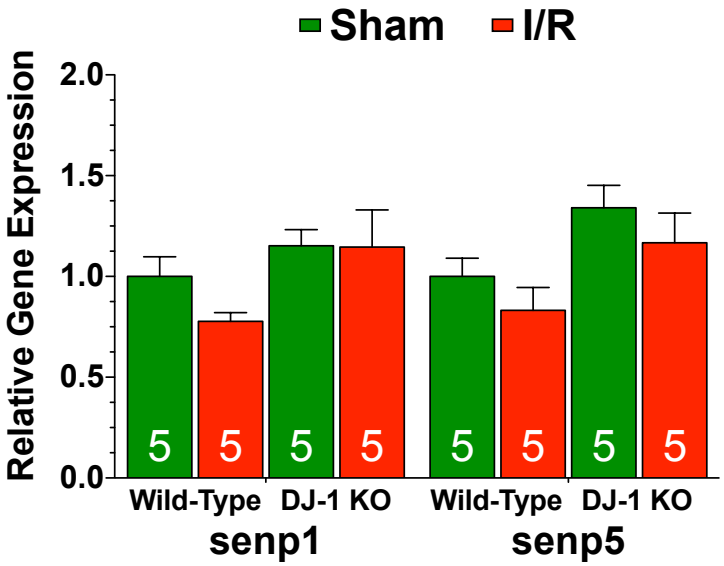
Supplemental Figure 6. (A-C) Representative immunoblots and densitometric analysis of the SUMO status of Drp1 in samples collected from cells following H/R. Cells were transfected with siRNA-scr, siRNA-DJ1, siRNA-SENP5, or

siRNA-DJ1/SENP5. Values are means±SEM of three independent experiments of three biological replicates.

Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3

A.

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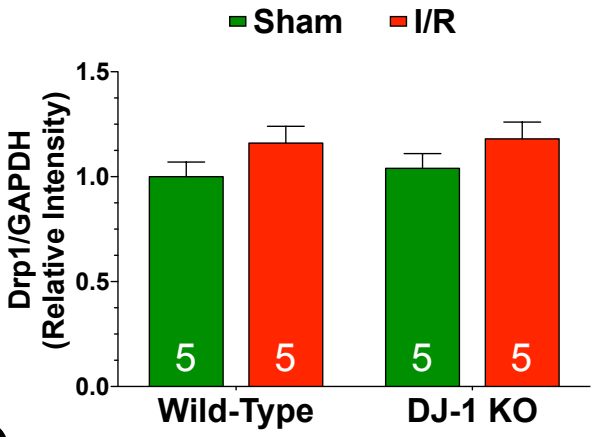

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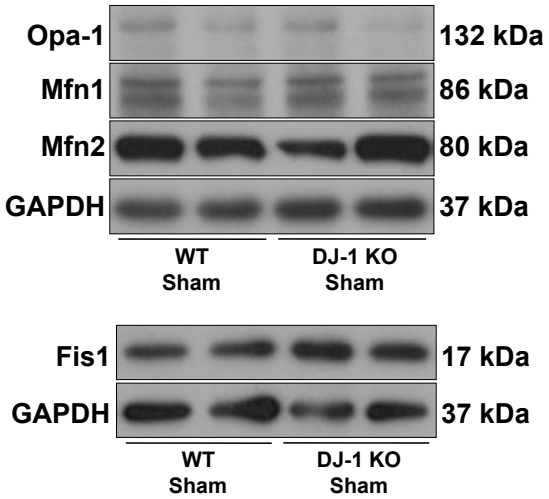

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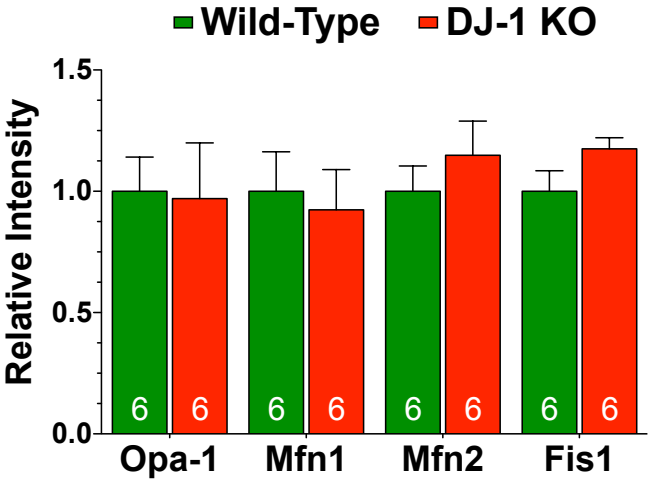

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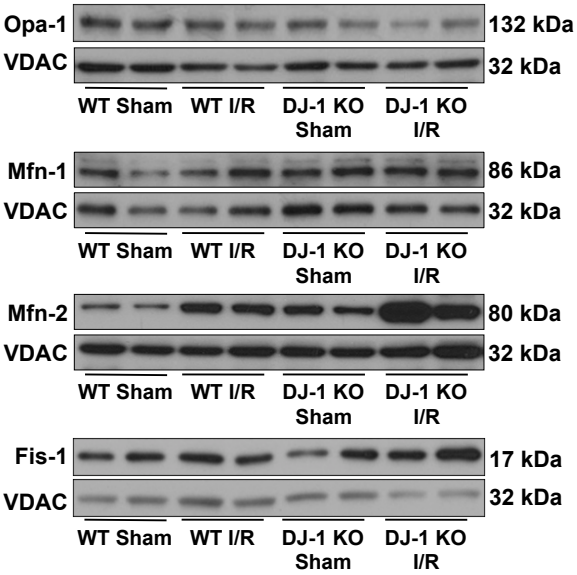

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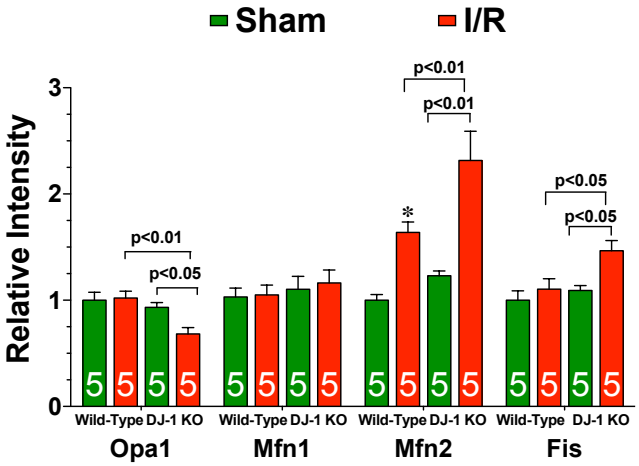
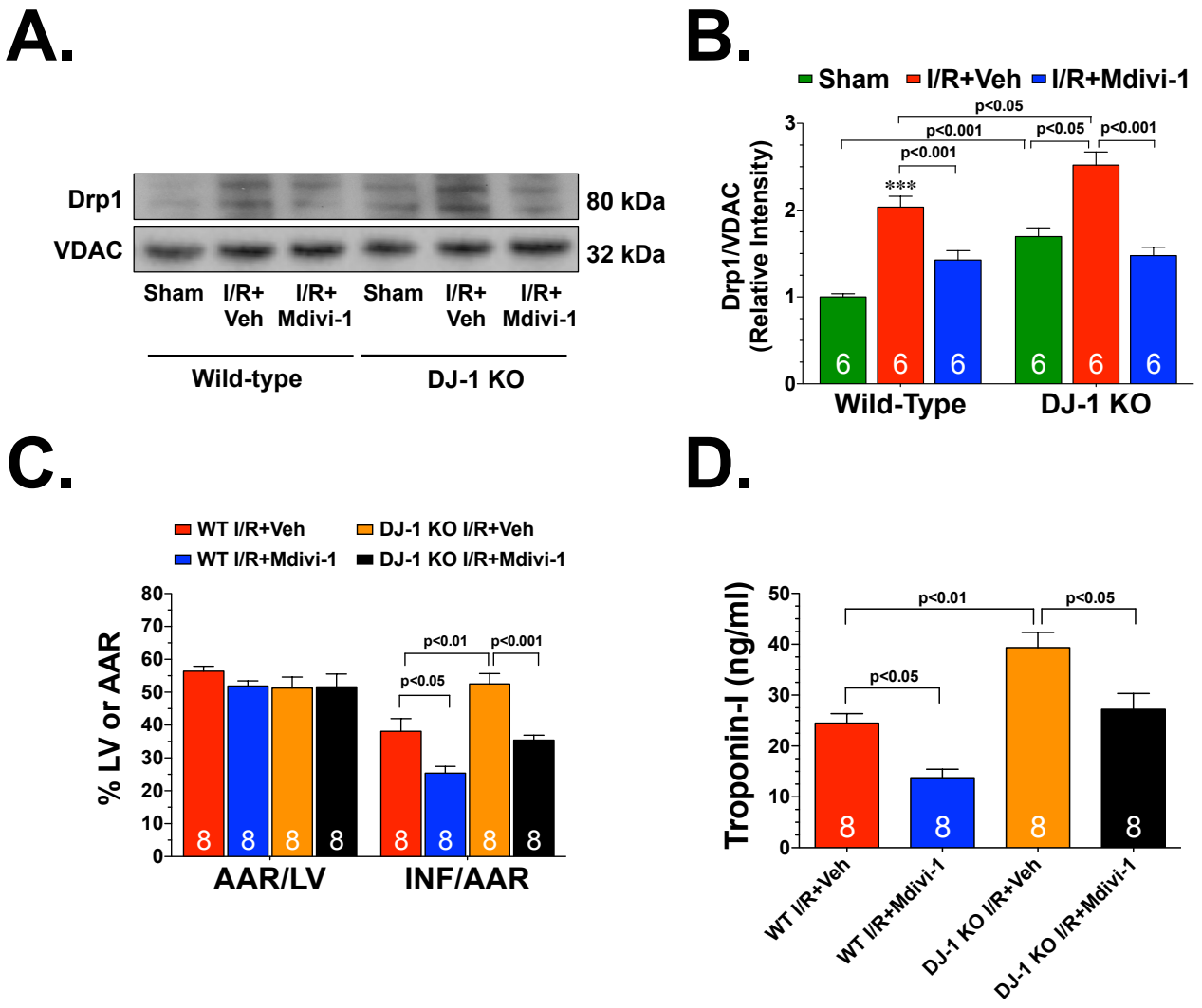
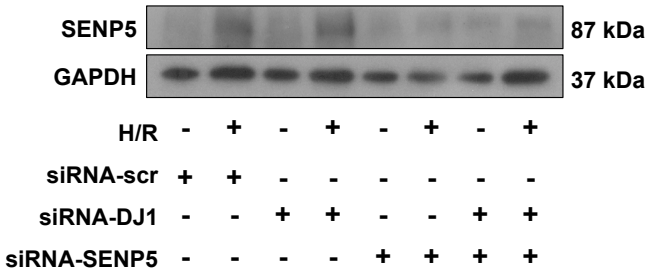


Supplemental Figure 4



Supplemental Figure 5

A.



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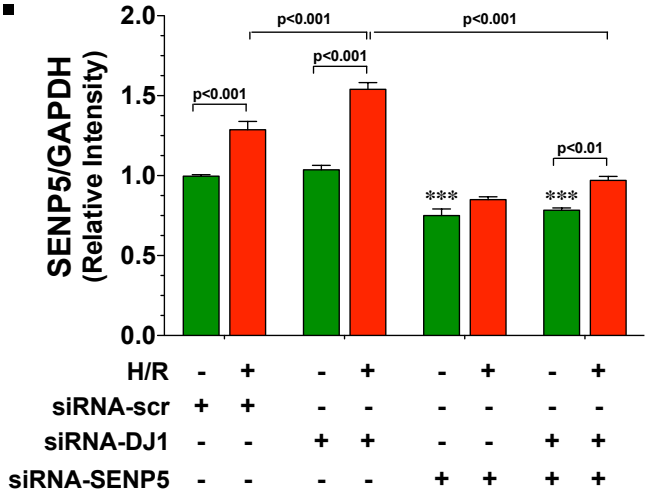

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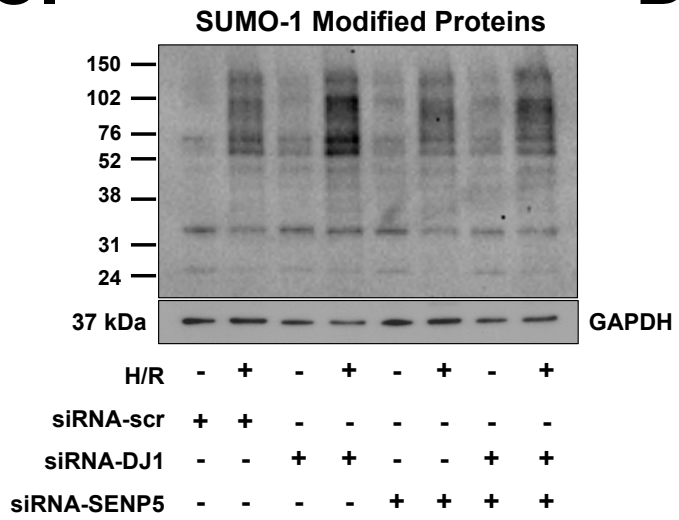

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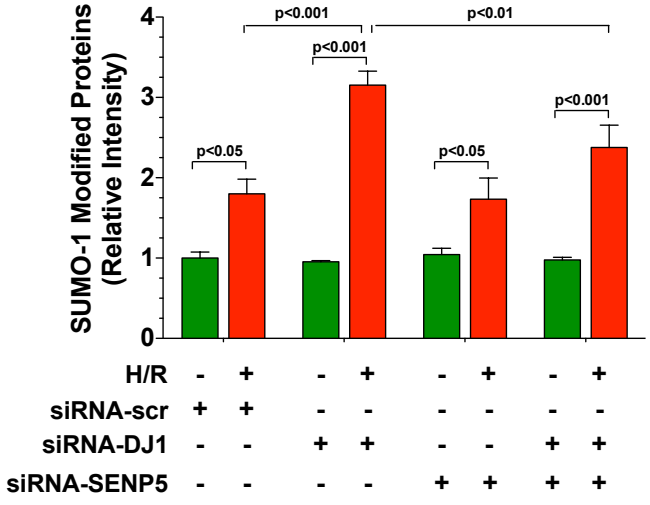

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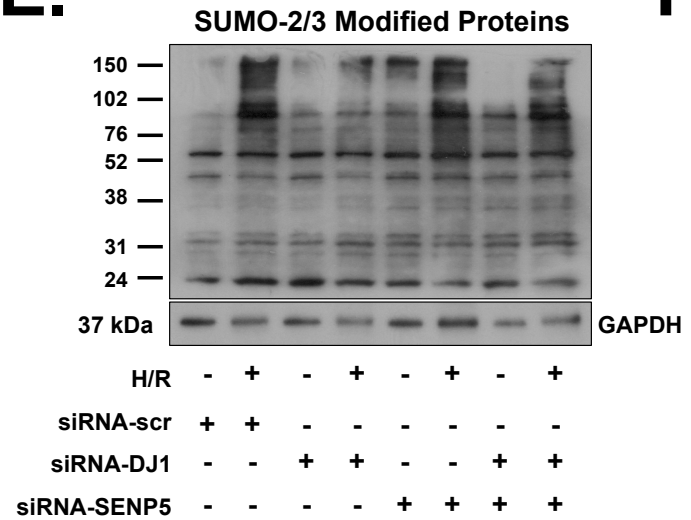

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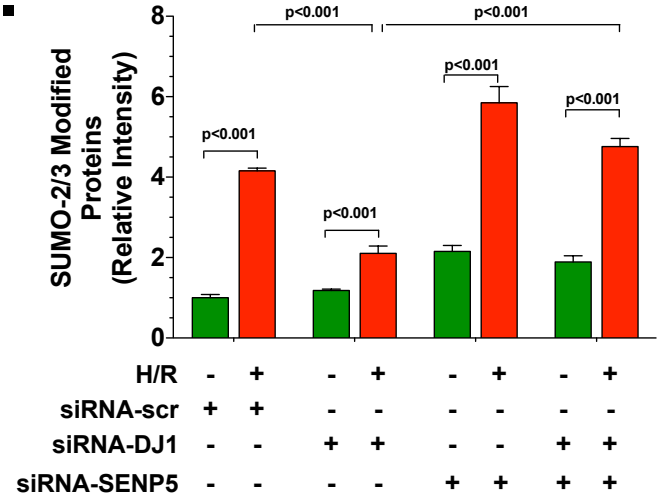
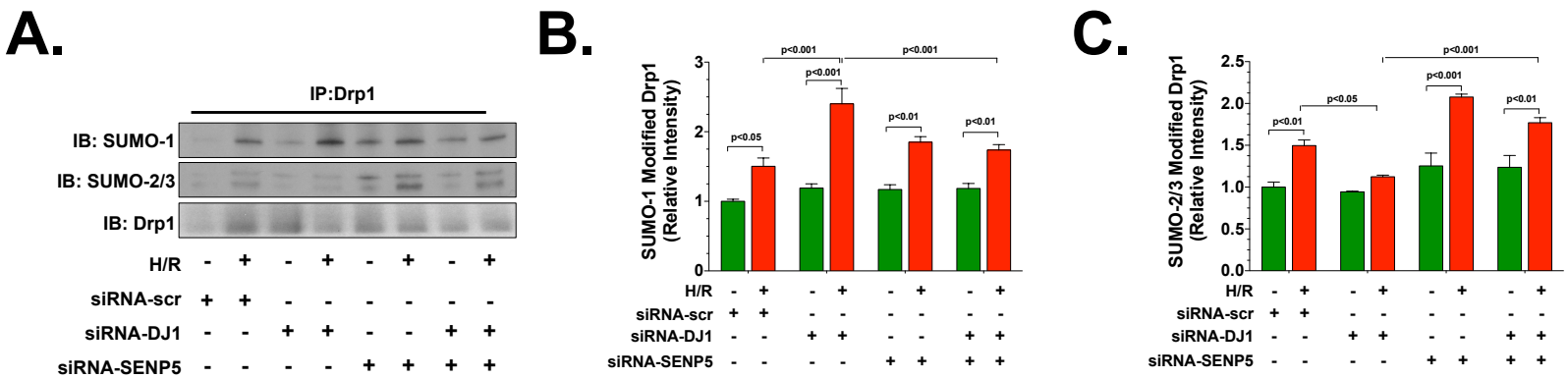
Supplemental Figure 6