

Supplementary Information

Ischemia Reperfusion Injury Provokes Adverse Left Ventricular Remodeling in Dysferlin-Deficient Hearts Through a Pathway That Involves TIRAP Dependent Signaling

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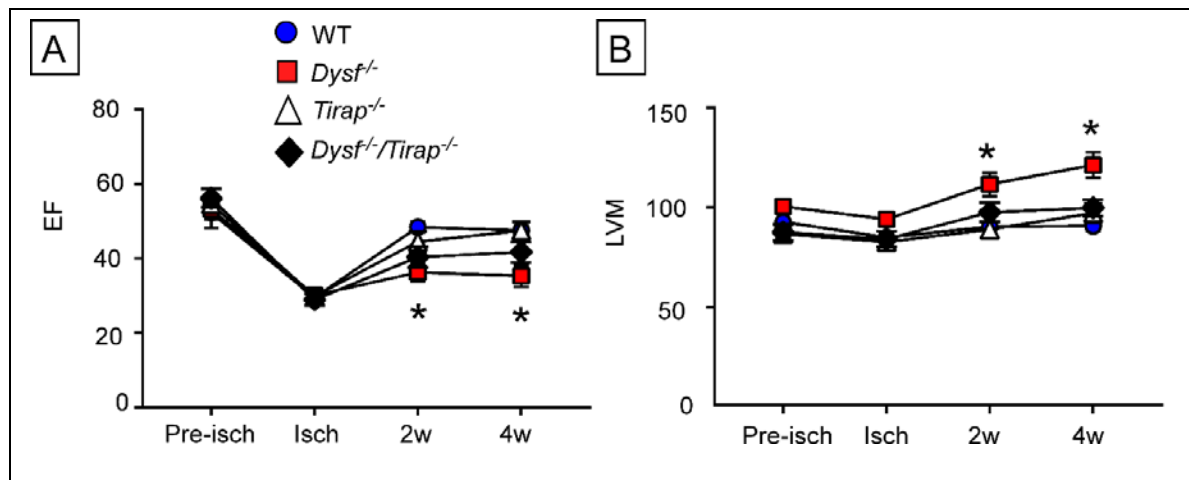
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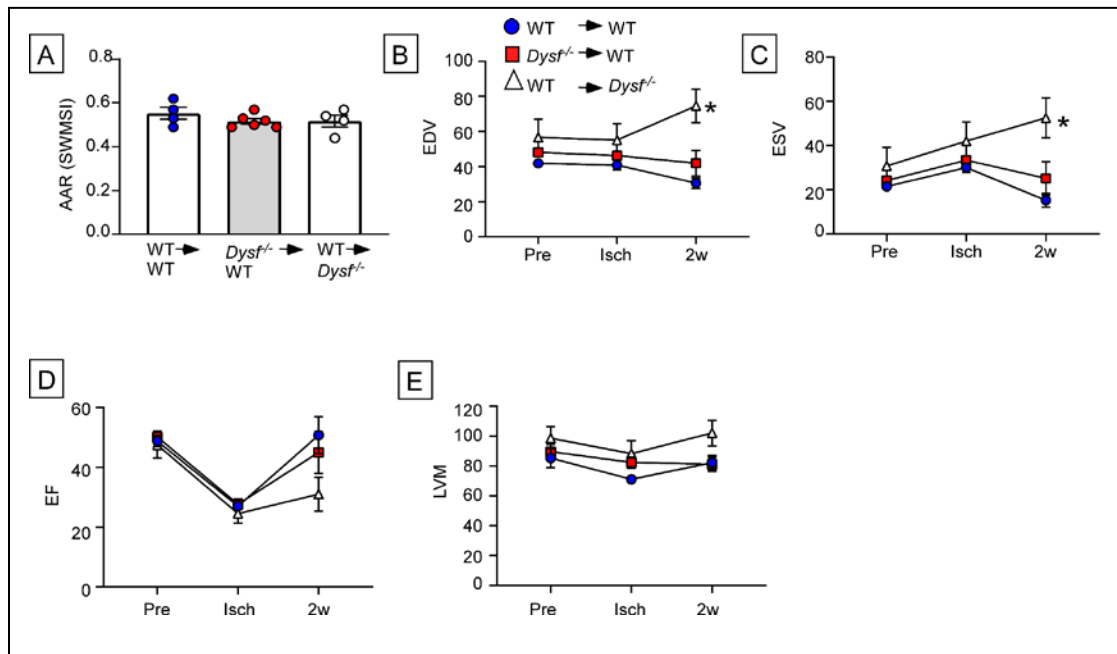
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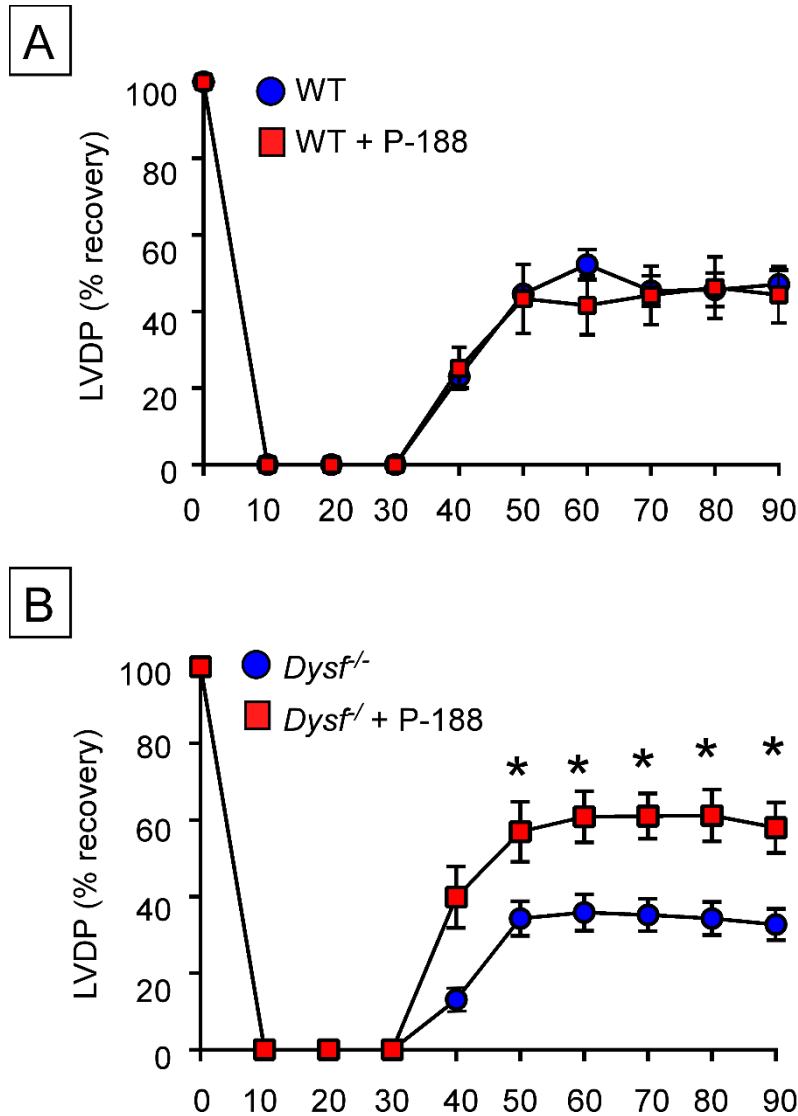
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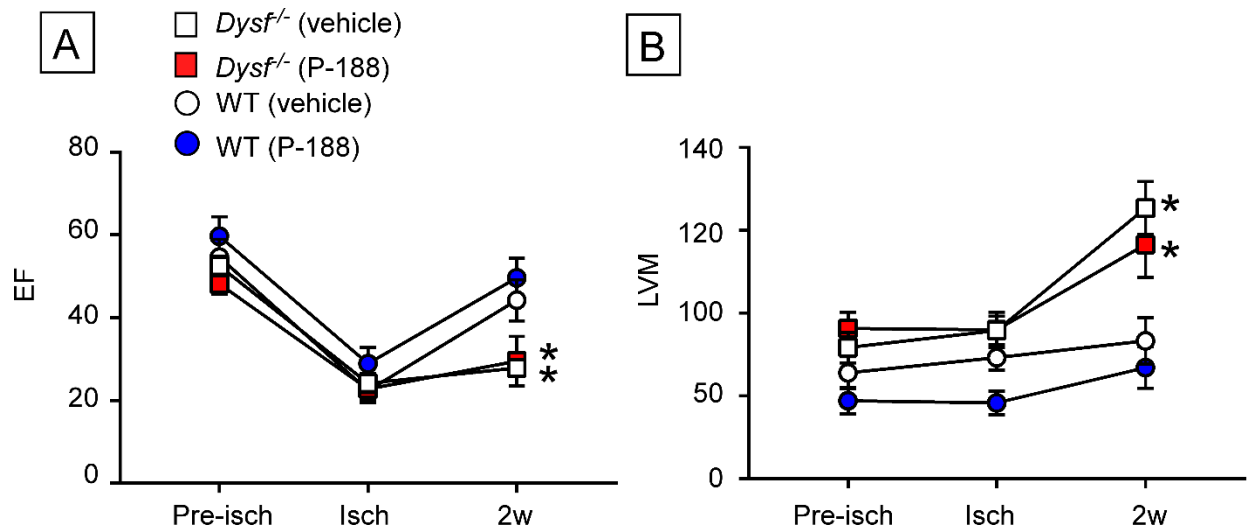
Supplemental Figure S1. Effect of ischemia reperfusion (I/R) injury in WT, *Dysf*^{-/-}, *Tirap*^{-/-} and *Dysf*^{-/-}/*Tirap*^{-/-} mouse hearts. WT (n=11), *Dysf*^{-/-} (n=17), *Tirap*^{-/-} (n=9), *Dysf*^{-/-}/*Tirap*^{-/-} (n=9) mice underwent closed chest ischemia (60 min) followed by 2 to 4 weeks of reperfusion. Mice were imaged by 2-D echocardiography at baseline (pre-ischemia), during the imposition of ischemia, and at 2 and 4 weeks. **(A)** Left ventricular ejection fraction (EF) **(B)** Left ventricular mass (LVM). Data are presented as mean±SEM. (*p<0.05 compared to WT)



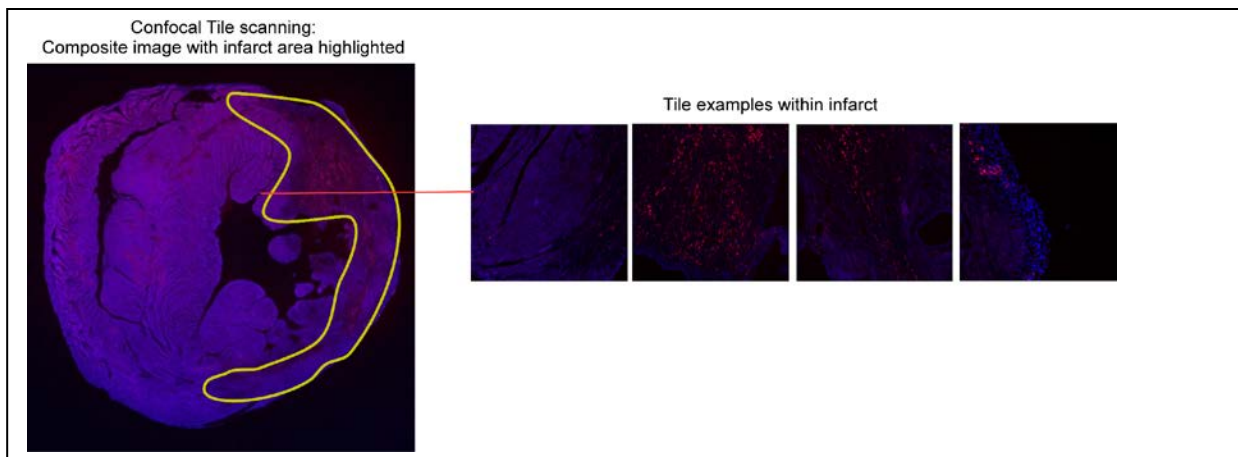
Supplemental Figure S2. Effect of I/R injury in chimeric WT mice. WT mice that underwent bone marrow reconstitution with WT bone marrow (WT → WT) or *Dysf*^{-/-} bone marrow (*Dysf*^{-/-} → WT) and *Dysf*^{-/-} mice that underwent bone marrow reconstitution with WT bone marrow (WT → *Dysf*^{-/-}) were subjected to closed chest ischemia (60 min) followed by 2 weeks of reperfusion. Mice were imaged by 2-D echocardiography at baseline (pre-ischemia), during ischemia, and at 2 weeks (n= 4-6/group). **(A)** Area-at-risk (AAR [SWMSI]), **(B)** Left ventricular end-diastolic volume (EDV), **(C)** Left ventricular end-systolic volume (ESV), **(D)** Left ventricular ejection fraction (EF), **(E)** Left ventricular mass (LVM), Data are presented as mean ± SEM. (* p < 0.05 compared to WT→WT)



Supplemental Figure S3. Effect of Poloxamer 188 (P188) on recovery of LV function in WT and *Dysf*^{-/-} mice following I/R injury ex vivo. Hearts from WT and *Dysf*^{-/-} mice were subjected to 30 min ischemia followed by reperfusion for 60 minutes ex vivo using a Langendorff apparatus. Hearts were perfused with P188 (10 μ M) or diluent during baseline and during reperfusion. Recovery of LV function was determined by measuring the percent of LV developed pressure (LVDP) after I/R injury. **(A)** Effect of P188 on LVDP in WT mice following I/R injury (n=5-8 per group), **(B)** Effect of P188 on LVDP in *Dysf*^{-/-} mice following I/R injury (n=4-7 per group). Data are presented as mean $\pm$ SEM. (*p<0.05 compared to WT).



Supplemental Figure S4. Effect of Poloxamer 188 (P188) on left ventricular ejection fraction and left ventricular mass in WT and *Dysf*^{-/-} mice following I/R injury. WT and *Dysf*^{-/-} mice, subjected to closed chest ischemia (60 min) or a sham procedure followed by 2 weeks of reperfusion, were treated with P188 or an equal volume of diluent immediately following reperfusion, 6h later and then daily for 14 days. Mice were imaged by 2-D echocardiography at baseline (pre-ischemia), during ischemia and at 2 weeks after reperfusion (n = 5-6/group). **(A)** Left ventricular ejection fraction (EF), **(B)** Left ventricular mass (LVM). Data are presented as mean±SEM. (*p<0.05 compared to WT)



Supplemental Figure S5. Confocal tile scanning for enumerating CD68+ and Ly6G+ cells within the infarct zone. 10X tile scanning was performed at the mid-papillary level using confocal microscopy. Images (~70-130 per section) were stitched together using the Axiovision software to form whole mid-papillary section images. Individual sequential tiles are shown to the right of the composite image to demonstrate how CD68+ and Ly6G+ cells were enumerated within the infarct zone.