**ORIGINAL ARTICLE**

**Mechanism of succinate efflux upon reperfusion of the ischemic heart**

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**SUPPLEMENTARY FIGURE LEGENDS**

**Figure S1** Succinate efflux in a porcine MI model only occurs from the ischemic tissue.Pigs were treated as in *Figure 4E*: **s**uccinate is elevated during early reperfusion in the coronary sinus plasma but not jugular vein or aortic root in a pig heart attack model. The LAD was occluded by a balloon catheter for 60 min before removing the occlusion and blood sampled (mean ± S.E.M., n=3). Statistical significance was assessed by two-way ANOVA with Tukey’s post hoc test (\*\**p* <0.01, \*\*\*\**p* <0.0001).

**Figure S2** Succinate is retained in the heart when reperfused with nonspecific transport inhibitors. Succinate levels in hearts after 6 min reperfusion with 1 mM succimer or 1 mM phenylsuccinate (Phsucc) from *Figure 5B* were measured (mean ± S.E.M., n=3-5). Statistical significance was assessed by two-way ANOVA with Dunnett’s post hoc test (\*\*\*\**p* <0.0001 relative to control reperfusion).

**Figure S3** MCT1 inhibition or haploinsufficiency does not affect succinate accumulation.

Hearts were perfused in Langendorff mode and equilibrated with Krebs buffer alone, or for MCTi-pre with 50 µM AR-C141990 for 20 min, before 20 min global no-flow ischemia and snap freezing tissue for succinate quantification by LC-MS/MS (mean ± S.E.M or mean ± range for *MCT1+/-*; WT n=8 (from *Figure 2A*), *MCT1+/-* n=2, MCTi-pre n=4).

**Figure S4** Succinate is retained in the heart when reperfused with MCTi. Succinate levels in hearts after 6 min reperfusion with MCTi from *Figure 6B* were measured (mean ± S.E.M., n=3-5). Statistical significance was assessed by two-way ANOVA with Dunnett’s post hoc test (\*\*\*\**p* <0.0001 relative to control (ctl) reperfusion).

**Figure S5** Succinate retained in the heart after reperfusion in *MCT1+/-* mice is no different from *MCT1+/+* hearts. Succinate levels in *MCT1+/+* and *MCT1+/-* hearts after 6 min reperfusion from *Figure 6C* were measured (mean ± S.E.M., *MCT1+/+* n=7, *MCT+/-* n=5).

**Figure S6** Inhibition of MCT1 with AR-C141990 decreases cardiac ischemia-reperfusion injury. Anesthetized mice were subjected to occlusion of the LAD for 30 min and then the occlusion was removed and the hearts were reperfused for 120 min. The mice were infused IV for 20 min from 5 min before reperfusion with either saline, or saline supplemented with the MCT1i AR-C141990, at a total delivered dose of 1.5 mg/kg body weight. Infarct sizes were determined histologically as a percentage of risk area. Data are mean ± S.E.M., n=5. Statistical significance was assessed by unpaired, two-tailed Student’s t-test where \**p*<0.05.











