

Exosomal miR-223 Contributes to Mesenchymal Stem Cell-Elicited Cardioprotection in Polymicrobial Sepsis

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Supplementary information

Supplemental Figure S1

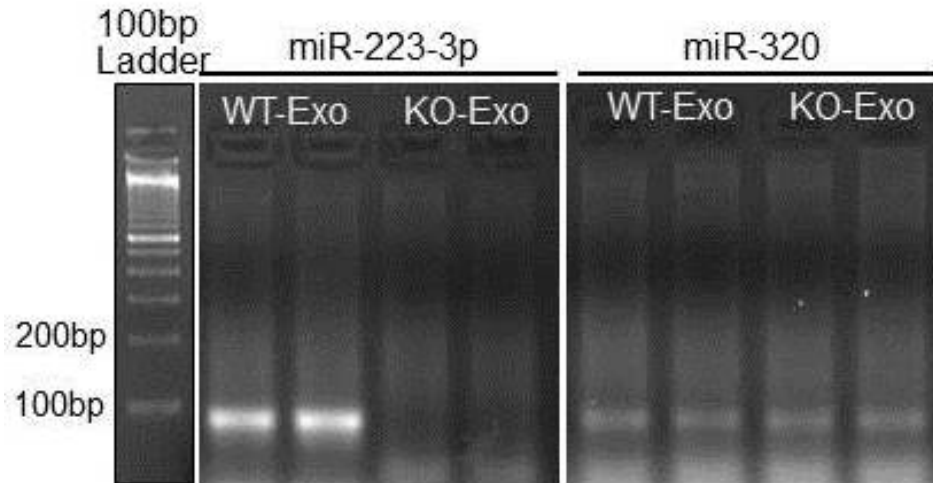


Figure S1: Exosomes were purified from culture supernatants of WT-MSCs and miR-223-KO MSCs, using sucrose gradient ultracentrifugation. Briefly, Cell culture supernatants were centrifuged for 10 min at 300g, 10min at 1200g (2X), 30min at 10000g, 60min at 70000g sequentially. The pellets were re-suspended in 5 ml of 2.6 M sucrose, 20 mM Tris-HCl, pH 7.2, and floated into an overlaid linear sucrose gradient (0.25-2.0 M sucrose, 20 mM Tris-HCl, pH 7.2) in a SW41 tube for 16 h at 100,000g. This yielded a distinct band at the density of ~1.15g/mL, as expected exosome fraction (1.13-1.19 g/mL). We collected this fraction to isolate total RNA for RT-PCR. The results of RT-PCR further confirmed that exosomes derived WT-MSCs (WT-Exo) did contain miR-223, whereas it was not encased in exosomes derived miR-223-KO MSCs (KO-Exo). MiR-320 was used as internal controls.

Supplemental Figure S2

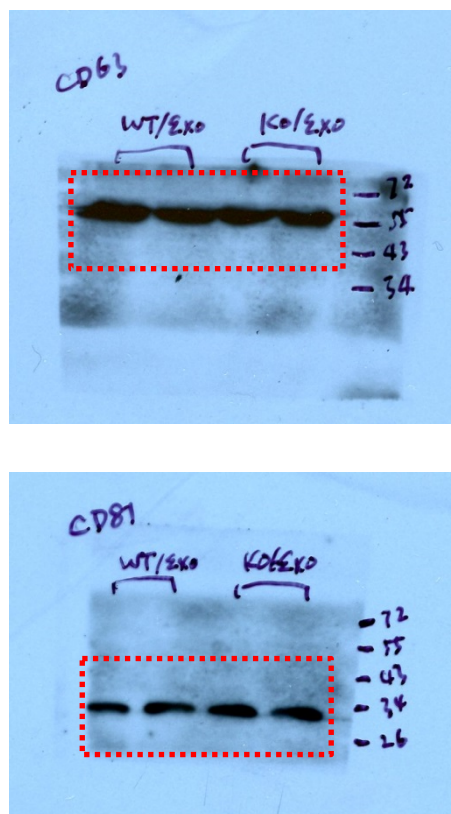


Figure S2: These original membranes were cropped for Figure 5C. The cropping lines are indicated clearly in images.

Supplemental Figure S3

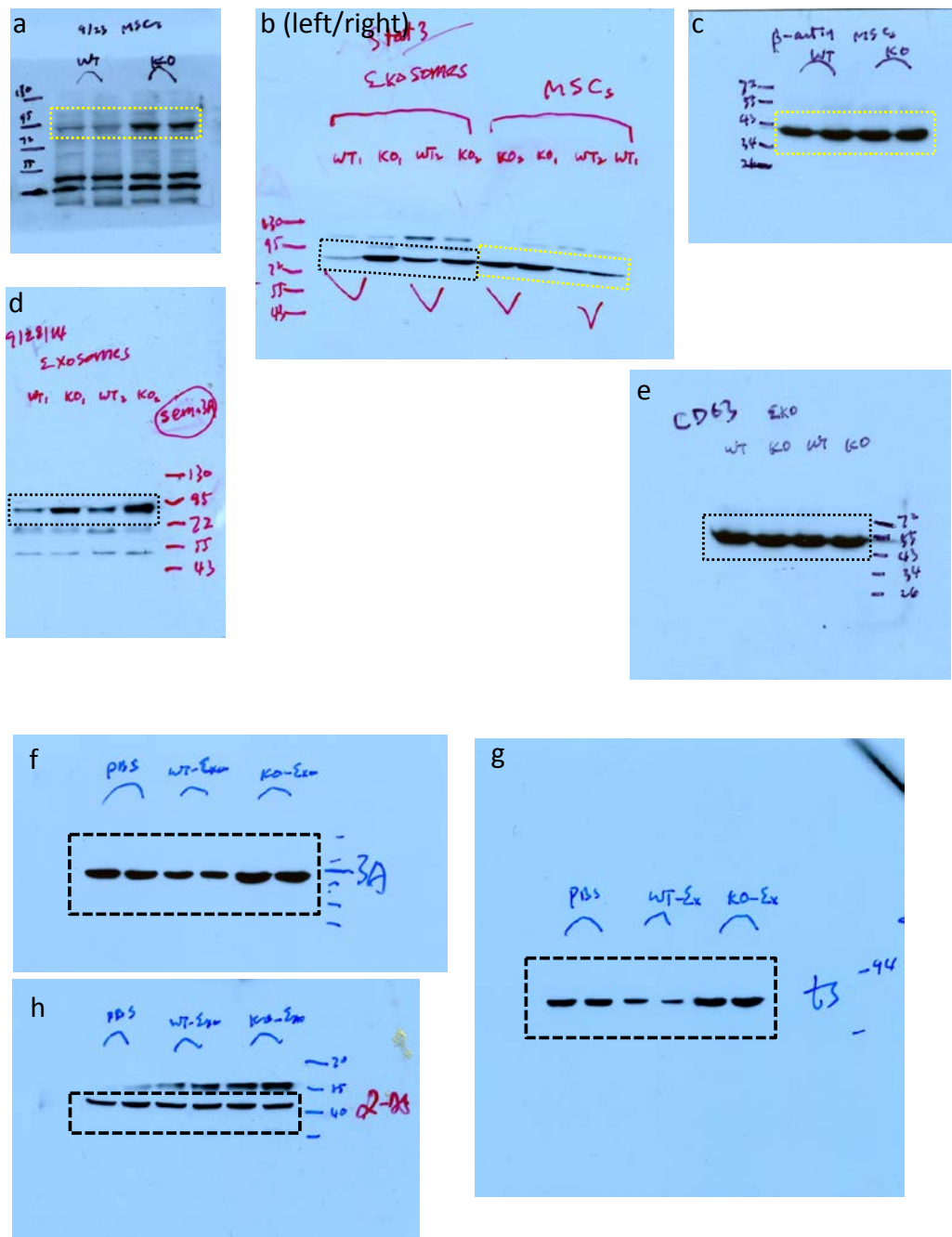


Figure S3: These original membranes a, b-right and c were cropped for Figure 7B; d, b-left and e for Figure 7D; f, g and h for Figure 7I. The cropping lines are indicated clearly in images. The molecular weight (MW) of Sema3A is predicted to be 95kDa. The MW of Stat3 is predicted to be 86kDa. The MW of actin is approximately 42kDa.