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Supplemental Information

Actin Cytoskeletal Disruption following Cryopreservation Alters the Biodistribution of Human Mesenchymal Stromal Cells In Vivo

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Figure S1

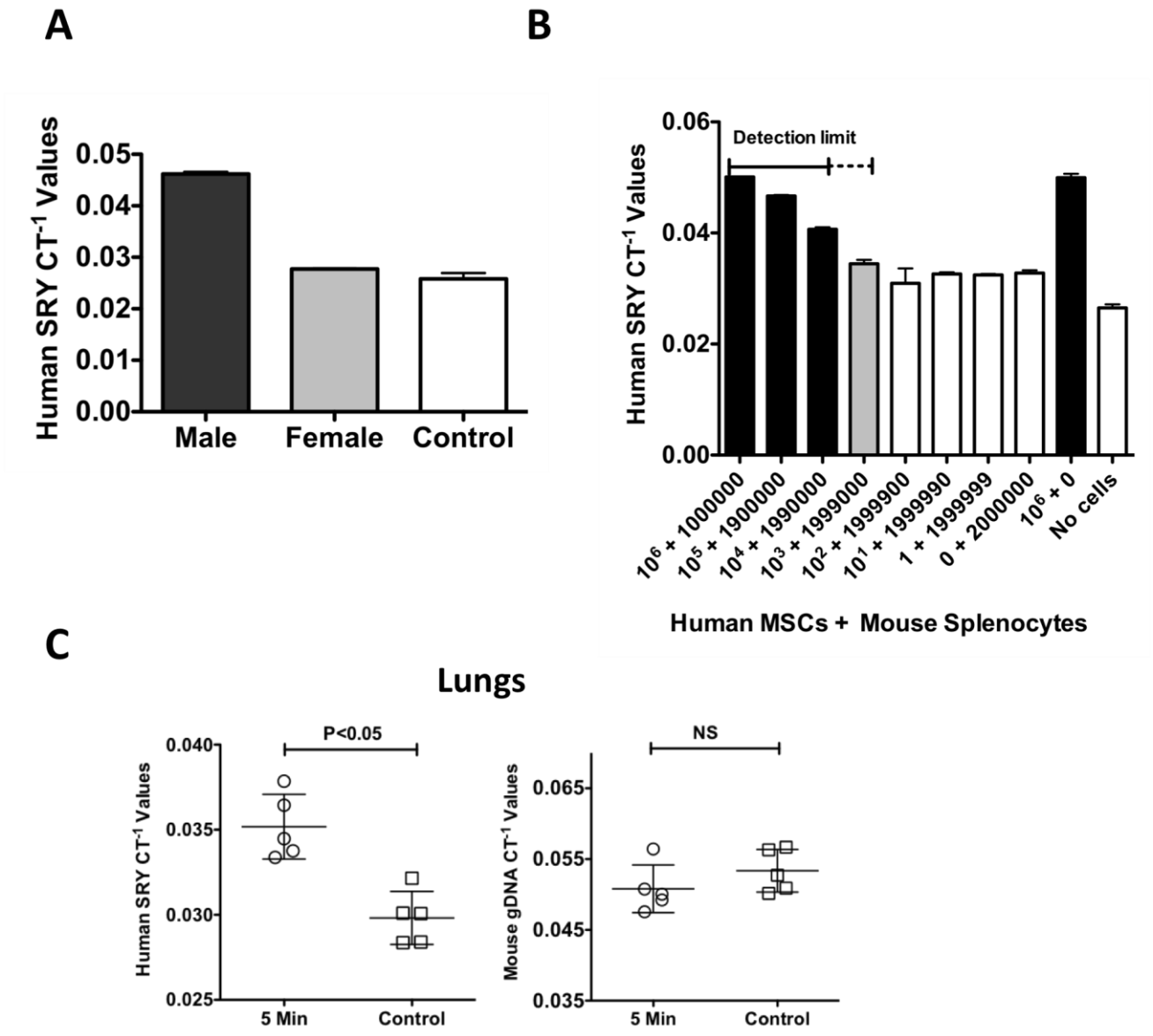

A
Human SRY CT-1 Values
0.05
0.04
0.03
0.02
0.01
0.00
Male
Female
Control
B
Human SRY CT-1 Values
0.06
0.04
0.02
0.00
Detection limit
10^6 + 1000000
10^5 + 1900000
10^4 + 1990000
10^3 + 1999000
10^2 + 1999900
10^1 + 1999990
1 + 1999999
0 + 2000000
10^6 + 0
No cells
Human MSCs + Mouse Splenocytes
C
Lungs
P<0.05
Human SRY CT-1 Values
0.040
0.035
0.030
0.025
5 Min
Control
NS
Mouse gDNA CT-1 Values
0.065
0.055
0.045
0.035
5 Min
Control

Figure S2

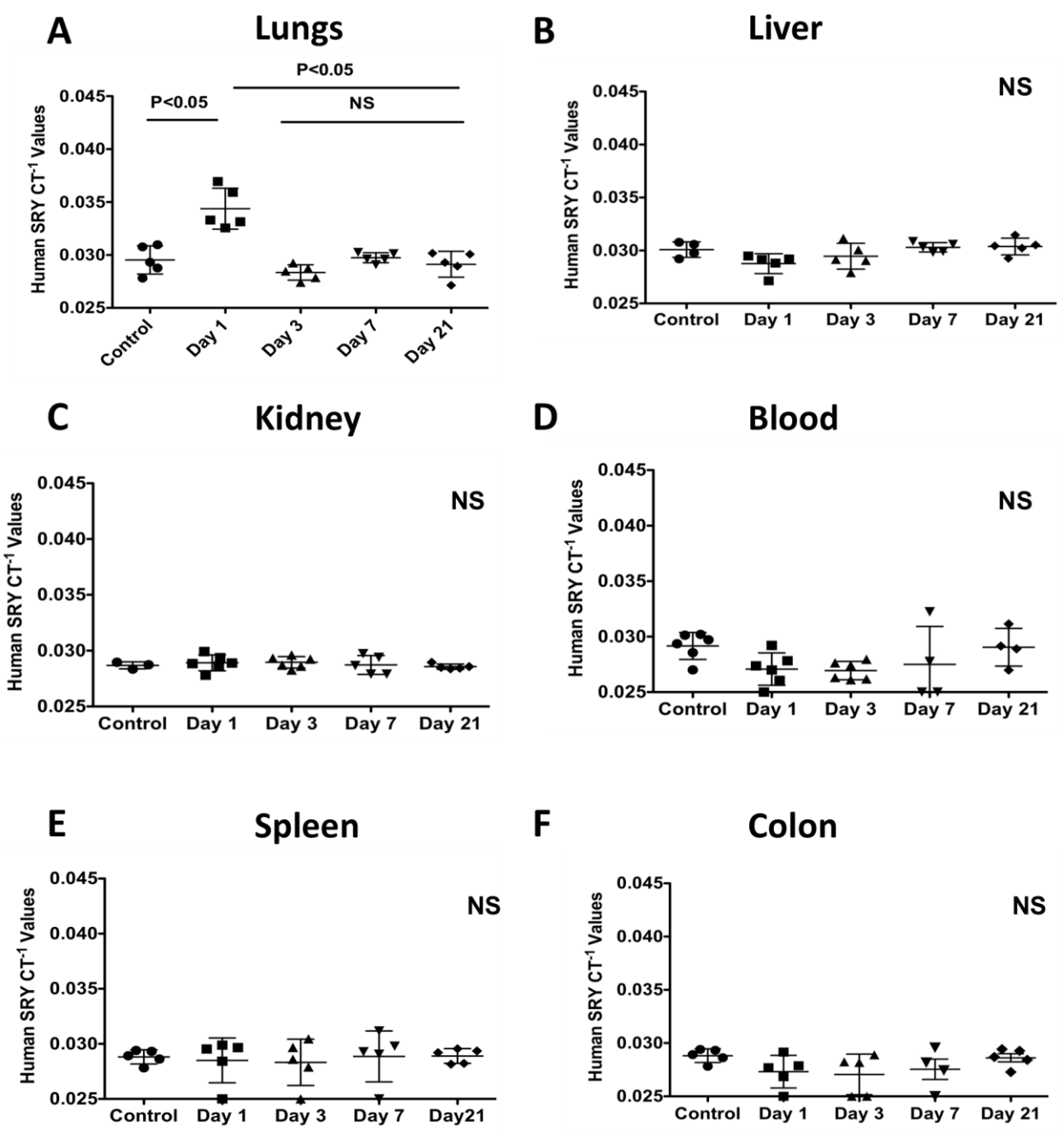

A
Lungs
P<0.05
P<0.05
NS
Human SRY CT-1 Values
0.045
0.040
0.035
0.030
0.025
Control
Day 1
Day 3
Day 7
Day 21
B
Liver
NS
C
Kidney
NS
D
Blood
NS
E
Spleen
NS
Day21
F
Colon
NS

Figure S3

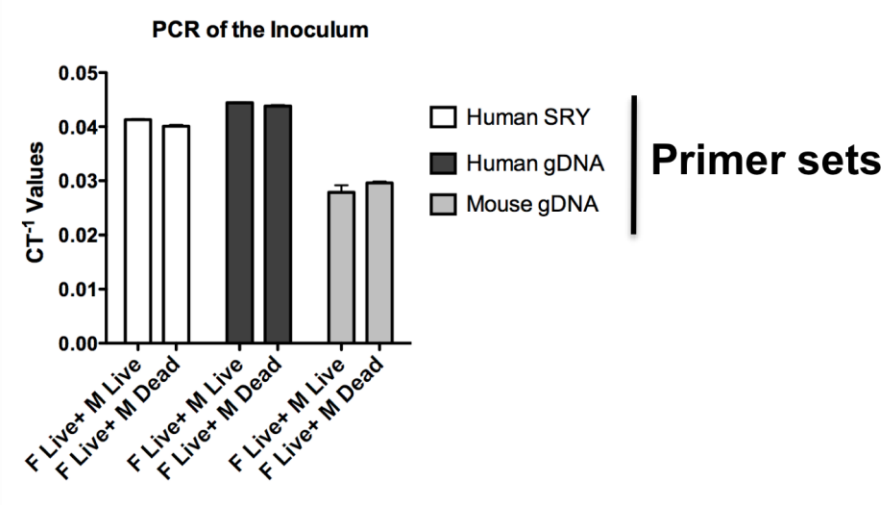
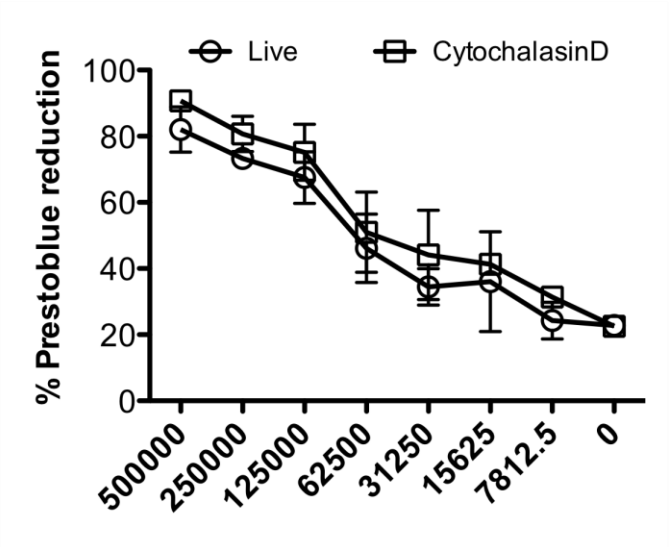


Figure S4



Supplementary Figure legends

Figure S1: Specificity, sensitivity and feasibility of detecting male human MSCs in mixtures of C57BL/B6 mice cells. (A) Genomic DNA was prepared from MSCs derived from female and male individuals and were subjected to SRY genomic DNA real time PCR. (B) Human MSCs and mouse splenocytes were mixed *in vitro* at the indicated ratios and were subjected to genomic DNA extraction, SRY realtime PCR. (C) $1X10^6$ human male MSCs were intravenously injected

into C57BL/B6 mice via tail vein. Five minutes post infusion, the animals were sacrificed and the lung tissues were excised and subject to genomic DNA extraction and Human SRY and Mouse genomic DNA real time PCR. Individual CT^{-1} values, as well as, cumulative mean±SD are shown from two independent experiments (n=3/2 animals per group) Significance was considered at $P<0.05$ based upon two tail T-test.

Figure S2. **Biodistribution of human MSCs in C57BL/B6 mice.** $1X10^6$ human male MSCs were intravenously injected into C57BL/B6 mice via tail vein. The animals were sacrificed at the indicated time points and the organs were collected for genomic DNA extraction and SRY realtime PCR amplification. The organs subjected to SRY realtime PCR analysis were, (A) Lung, (B) Liver, (C) Kidney, (D) Blood, (D) Spleen and (F) Colon. N= 4-5 animals per time point. Significance was considered at $P<0.05$ based upon 1-way ANOVA-Tukey's Multiple Comparison Test.

Figure S3. **SRY can be detected in dead male MSCs.** Human MSCs from a male donor which were either live or heat killed, were mixed with live MSCs derived from a female donor at a ratio of 1:1. The mixed cells were subjected for genomic DNA extraction and realtime PCR was performed for human SRY, human gDNA and mouse gDNA amplification. N=2 independent replicates.

Figure S4. **Effect of 2mM Cytochalasin D on MSC viability.** MSCs were treated with 2mM Cytochalasin D for 2hours. Subsequently, they were trypsinized and seeded onto 96 well plates along with control MSCs. The cells were incubated with presto blue dye for 3 hours. The reduction of presto blue was calculated as described in the materials and methods (PrestoBlue® Cell Viability Reagent, Life technologies). N= 2 independent replicates of two unique MSC donors.