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Supplemental Information

Blockade of Inflammation and Apoptosis Pathways by siRNA Prolongs Cold Preservation Time and Protects Donor Hearts in a Porcine Model

Jia Wei, Shiyou Chen, Song Xue, Qiangru Zhu, Sha Liu, Li Cui, Xiuguo Hua, and Yongyi Wang

Online Figure 1

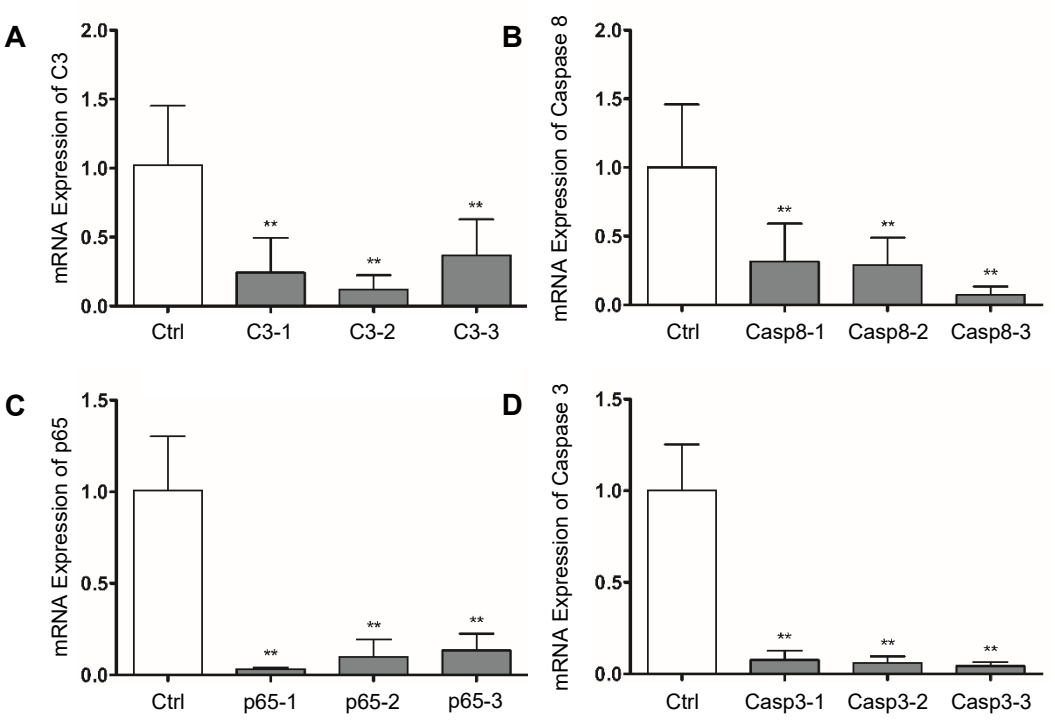

Figure S1 Knockdown efficiency of C3, Caspase 8, p65 and Caspase 3 siRNAs. 12 pairs of siRNAs were designed to target C3, Caspase 8, p65 and Caspase 3, three pairs for each gene. PK 15 and ST cell lines were transfected with each of these 12 pair siRNAs or scramble siRNA (Ctrl) as described in Methods. 48 hour later, cells were harvested to extract total RNA. mRNA expressions of C3 (A), Caspase 8 (B), p65 (C) and Caspase 3 (D) were detected by qPCR. All qPCR results were expressed as $2^{-\triangle\triangle Ct}$ normalized to GAPDH, and relative to the Ctrl (set as 1) . Data shown are the mean ± SEM. **P* < 0.05, ***P* < 0.01 *vs.* Ctrl, n=6.

Online Figure 2

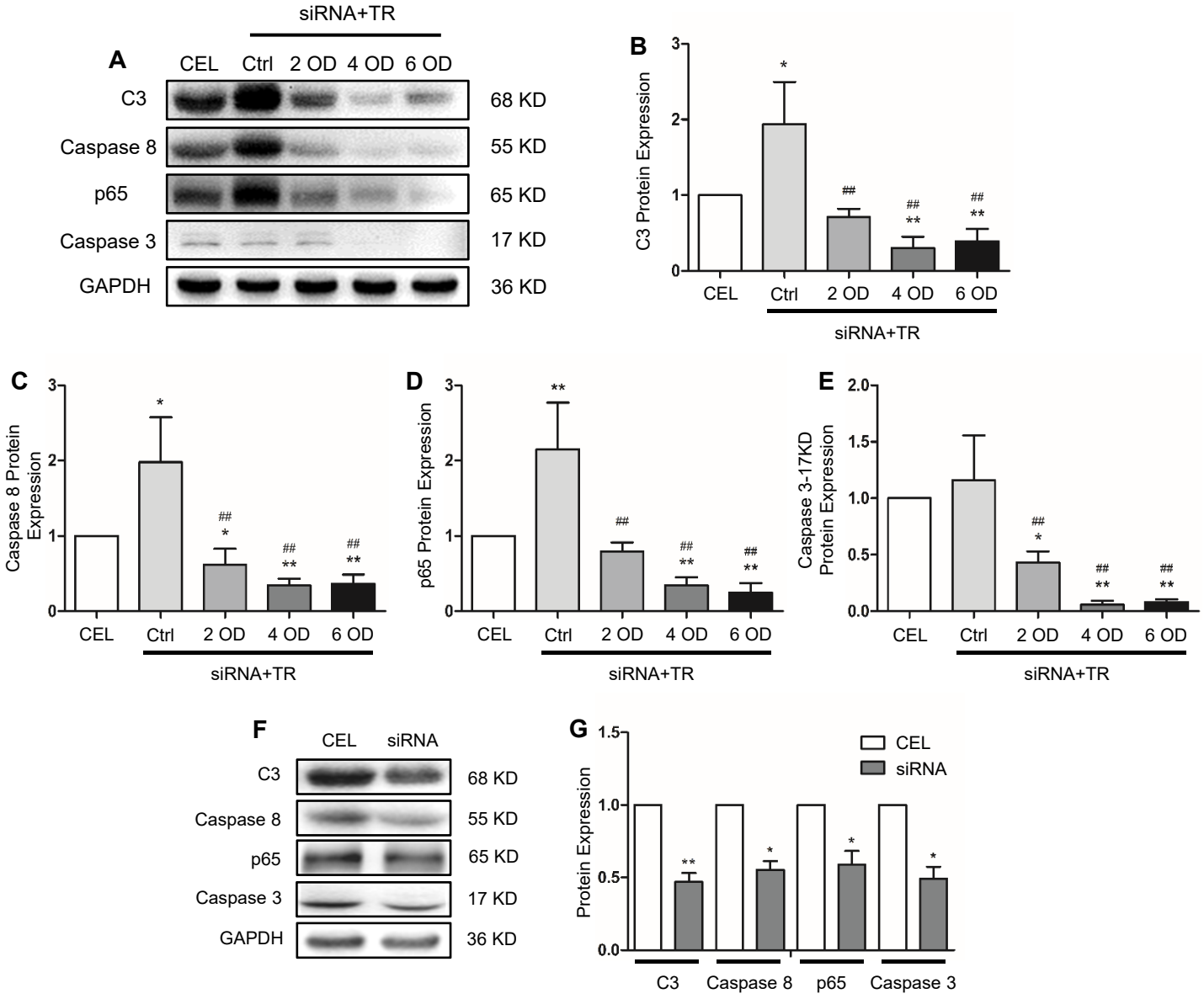

Figure S2 siRNA knockdown efficiency after reperfusion. (A-E) siRNA knockdown efficiency in isolated working hearts. 15 donor hearts were isolated, perfused and preserved using transfection reagent (TR) contained-Celsior solution with 4 OD scramble siRNA (Ctrl) or with 2 OD, 4 OD and 6 OD siRNA, and TR-free Celsior solution (CEL), all hearts were preserved for 12 has indicated as described in Methods. 15 hearts were re-perfused for 3 h, and then the heart tissues were collected, and Complement 3 (C3), Caspase 8, p65 and cleaved Caspase 3 proteins were detected by western blot (A). Each protein shown in A was quantified by normalizing to GAPDH and relative to the CEL group (set as 1) (B-E). (F-G) siRNA knockdown efficiency in transplanted hearts. 6 donor hearts were divided into 2 groups and preserved in Celsior solution (CEL) for 6 h 4 OD siRNAs and TR contained-Celsior solution (siRNA) for 12 h. All hearts were transplanted to the recipients and allowed to beat for 3 h. Then, the heart tissues were collected, and Complement 3 (C3), Caspase 8, p65 and cleaved Caspase 3 proteins were detected by western blot (F). Each protein level shown in F was quantified by normalizing to GAPDH and relative to the CEL group (set as 1) (G). Data shown are mean ± SEM. $*P < 0.05$, $**P < 0.01$ *vs.* CEL; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs.* Ctrl. n=3.

Online Figure 3

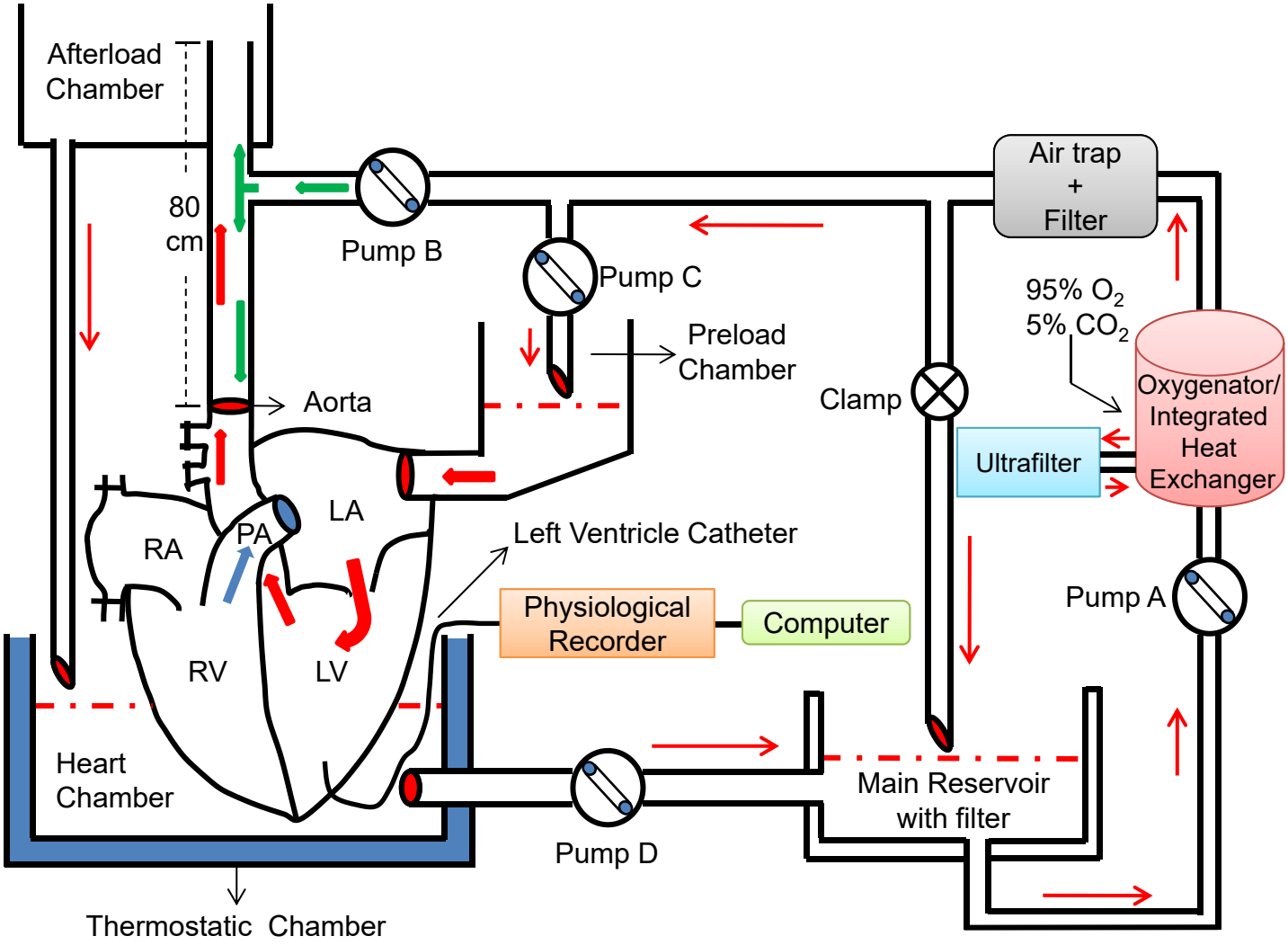

Figure S3 Schematic view of the ex vivo perfusion system. The reperfusion was started in the Langendorff mode, and the perfusate from the main reservoir was pumped (pump A) through an oxygenator-heat exchanger and an air trap. The air bubble-removed oxygenated blood (38 ° C) was then pumped (pump B) into the coronary arteries via the aorta (thick green arrows). The pump C was ceased in this mode. After 10-15 min cardiac stabilization, the system was switched to the working heart mode, in which the oxygenated perfusate was pumped (pump C) into the preload chamber (10-15 mmHg) and pushed into the left atrium (LA). At each atrial systole, the perfusate flowed into the left ventricle and was ejected into the afterload chamber through the aorta (thick red arrows) via a height adjustable soft tube (80 cm long suitable for 60-70 mmHg afterload). The coronary flow went into the coronary sinus, the right atrium (RA) and then the right ventricle (RV), finally being ejected out of the hearts through the pulmonary artery (PA, blue thick arrows). In the working heart mode, pump B was operated to maintain a stable afterload. The catheter that was previously placed into the LV was connected a physiological recorder and a computer to record the LV hemodynamic. The thin red arrows indicate the flow direction.

Online Figure 4

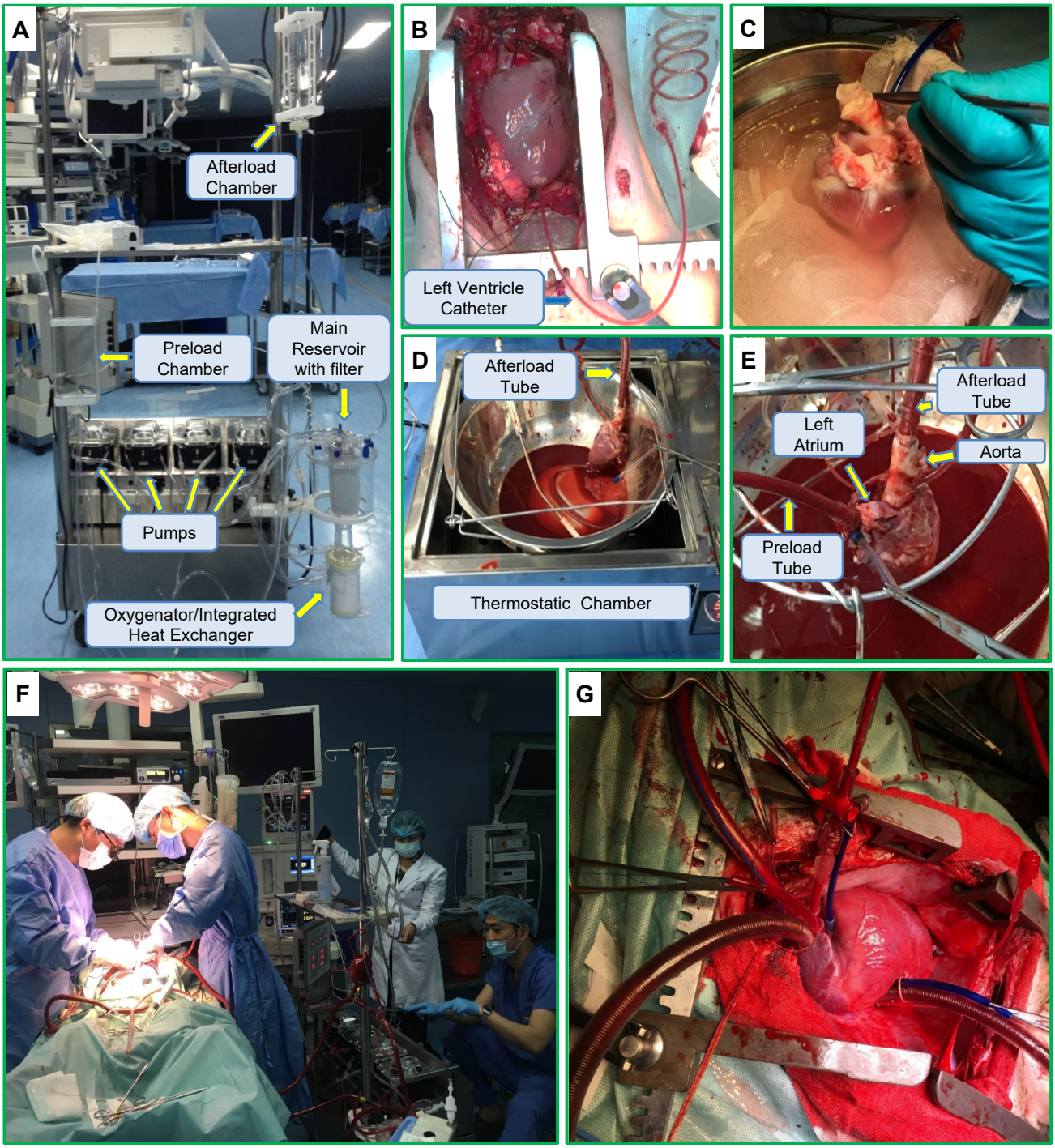

Figure S4 *Ex vivo* perfusion and transplantation photos. (A) *Ex vivo* perfusion system. (B) Exposure of the donor heart and placement of a left ventricle catheter via the apex to record the hemodynamic data. (C) Isolation and cold preservation of the donor heart. (D) The *ex vivo* reperfusion was initiated on langendorff mode after preservation. (E) The reperfusion was switched to working heart mode. (F) Cardiopulmonary bypass and transplantation. (G) *In vivo* reperfusion of the donor heart.