Human electronegative low-density lipoprotein modulates cardiac repolarization via LOX-1-mediated alteration of sarcolemmal ion channels

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Supplementary Figures

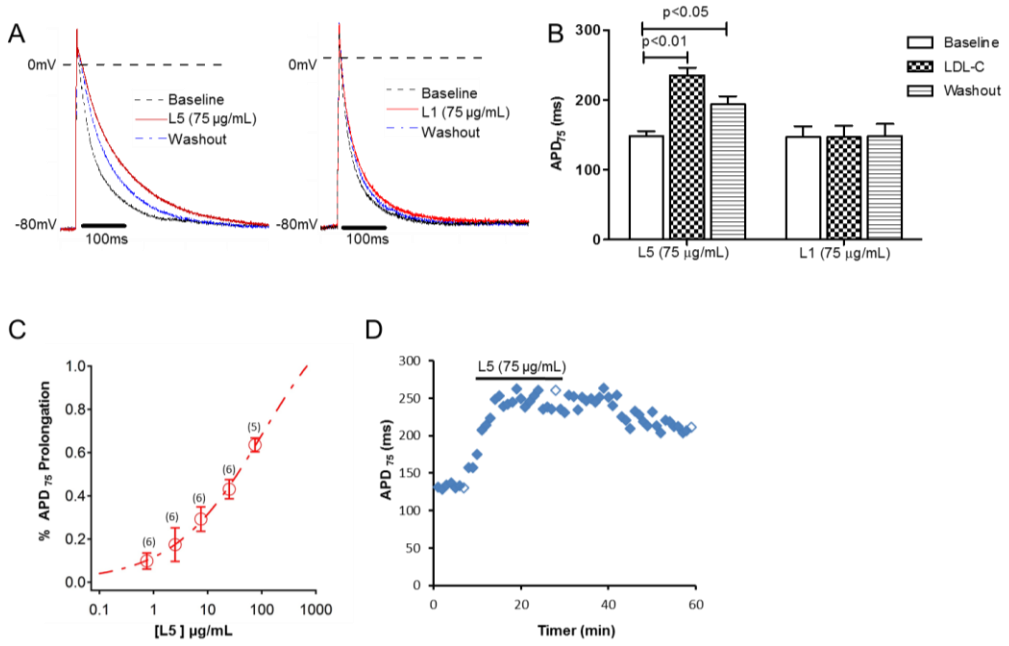

Figure S1. Prolonged action potential duration (APD) in H9c2 cells treated with L5. A, Representative action potential traces of H9c2 cells at baseline, during perfusion with 75 μg/mL L1 or L5, or after 30 min washout. **B,** Perfusion with L5 directly prolonged the APD of H9c2 cells, whereas perfusion with L1 did not. The effects of L5 were only partially reversible. n=8 per group. **C,** L5 acutely prolonged APD in a dose-dependent manner. The prolongation percentage (%) was calculated with respect to the baseline value. When the data were fitted to the Hill Equation, the half-maximal inhibitory concentration (IC50) was 54.24 ± 13.2 μg/mL, and the Hill coefficient was 0.62 ± 0.009. The n number of each concentration was shown in quotes. **D:** Time-course analysis of the effect of L5 (75 μg/mL) on ADP showed that L5 prolonged APD within 5 min.

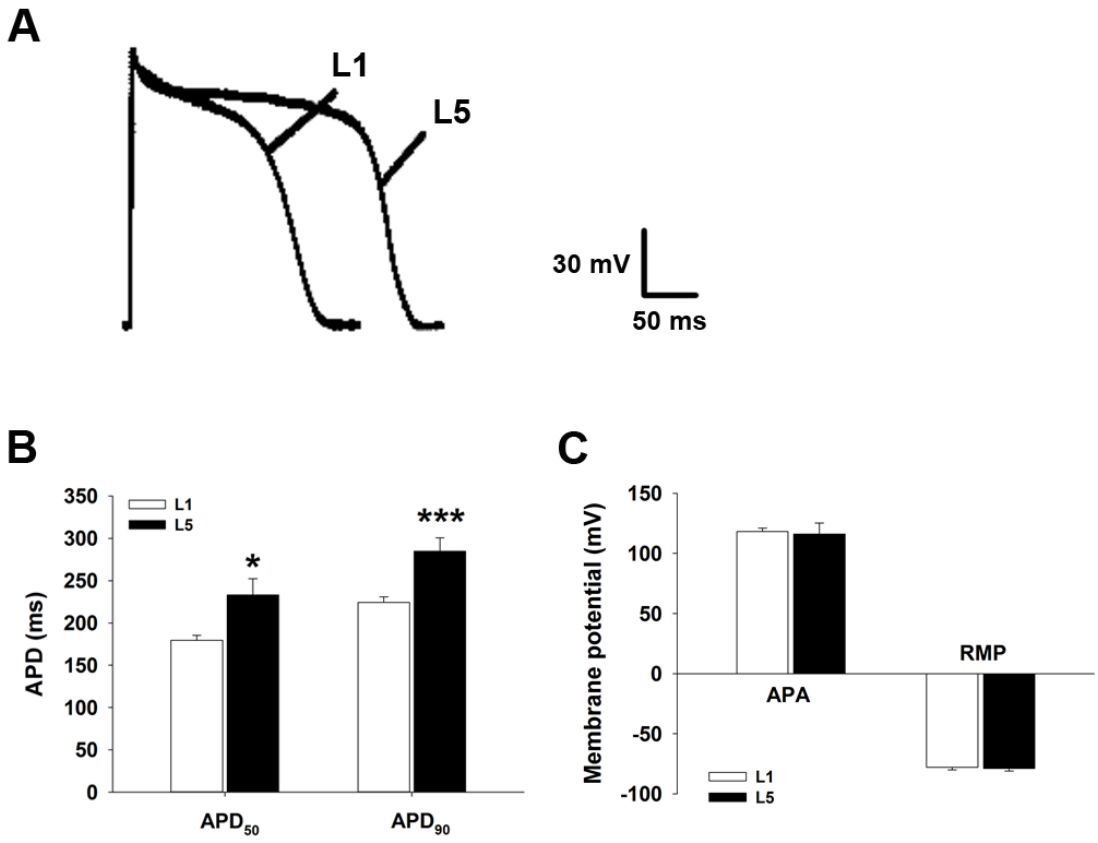

Figure S2. L5-induced prolongation of action potential duration (APD) in ventricular myocytes isolated from guinea pigs. A, Superimposed representative action potential traces were recorded from guinea pig cardiomyocytes treated with 30 μg/mL L5 or L1. **B,** Comparison of the action potential duration at 50% (APD_{50}) and 90% (APD_{90}) repolarization between 2 groups of cardiomyocytes. **C,** Comparison of the action potential amplitude (APA) and the resting membrane potential (RMP) between 2 groups of cardiomyocytes. n=4 per group. **P*<0.05 and ****P*<0.01 vs. L1-treated cardiomyocytes.

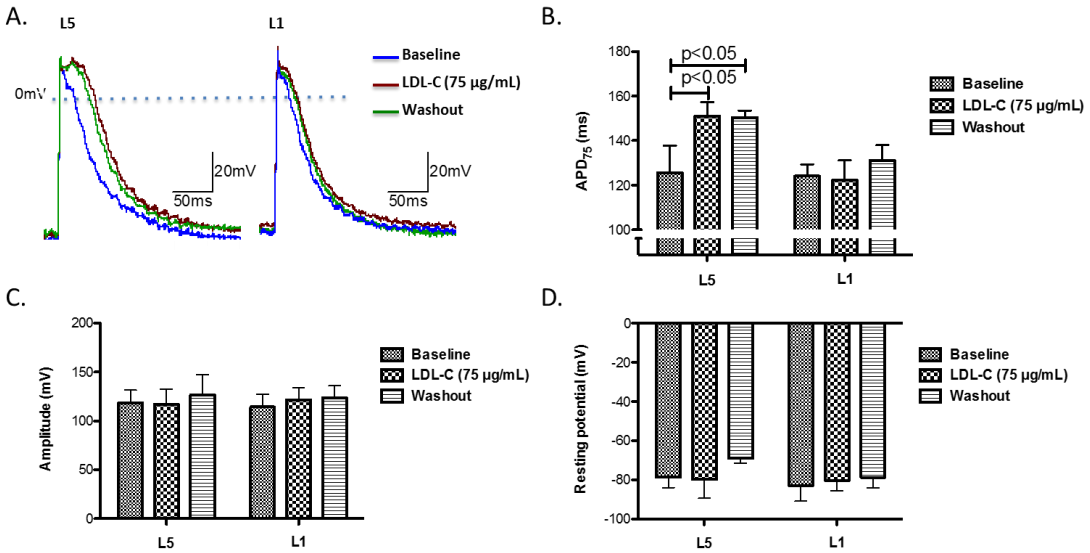

Figure S3. L5-induced prolongation of action potential duration (APD) in myocytes isolated from sheep. A, Representative action potential traces were recorded from sheep myocytes at baseline, during perfusion with 75 µg/mL L5 or L1, and after washout. The direct effects of L5 or L1 perfusion on **(B)** APD, **(C)** amplitude, and **(D)** resting potential in sheep myocytes. n=6 per group.

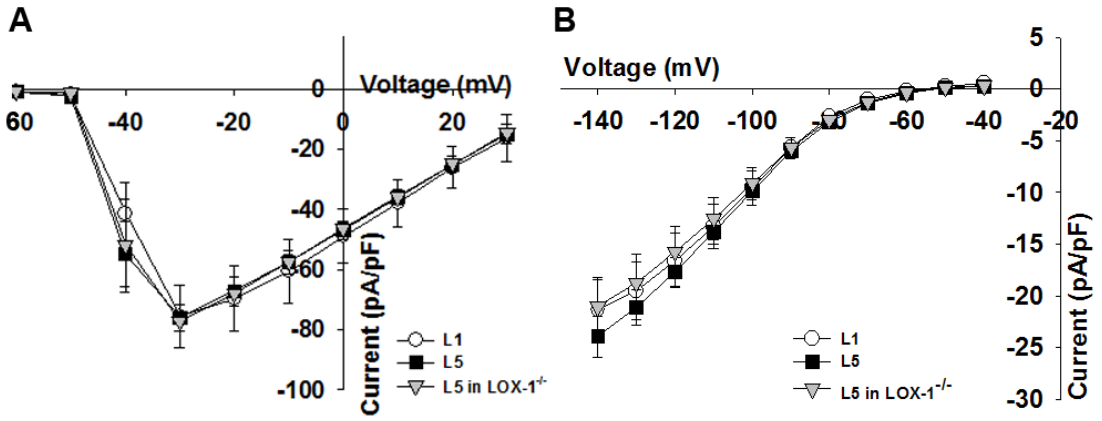

Figure S4. Effect of L5 on sodium (I_{Na}) and inward rectifier potassium current (I_{K1}) in mice cardiomyocytes. A, Comparison of the I-V relationships of I_{Na} between 3 groups of cardiomyocytes. n=3 per group. **B,** Comparison of the I-V relationships of I_{K1} between 3 groups of cardiomyocytes. n=6 per group.

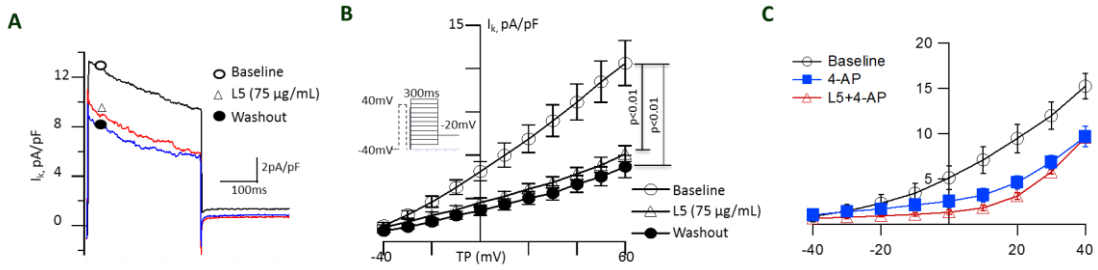

Figure S5. Decreased 4-AP sensitive potassium current (I_K) by L5 in H9c2 cells.

A, Representative outward potassium currents were elicited with voltages ranging from –40 mV to 60 mV (at 10-mV intervals) from a holding potential of –40 mV. **B,** Current-voltage relationship of I_K. **C,** 4-Aminopyridine was used to confirm the potassium current which L5 inhibits is 4-AP sensitive potassium currents. n=7 per group.

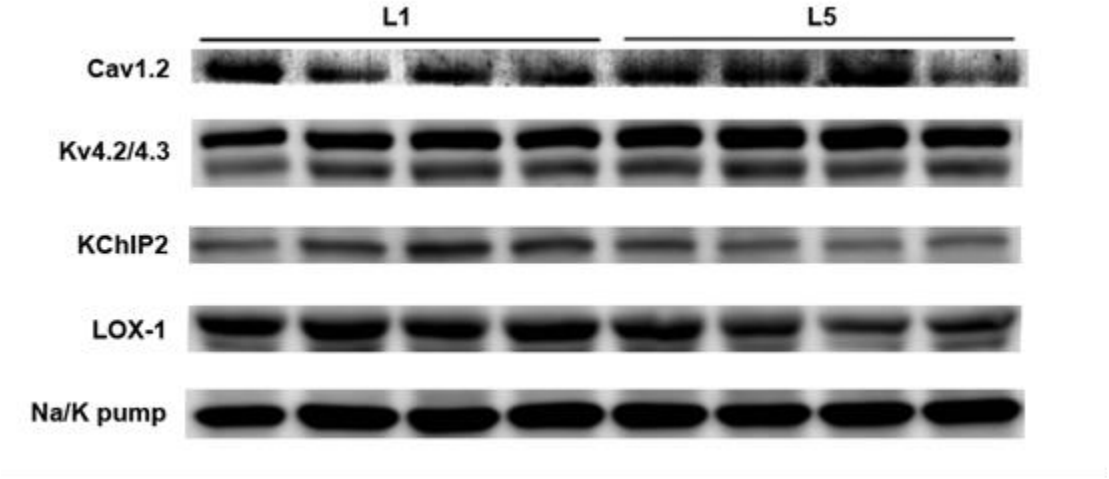

Figure S6. Comparison of membrane fraction proteins between L1- and L5-treated rat ventricular myocytes. It showed that there is no significant difference of Cav1.2, Kv4.2/4.3, KChIP2, and LOX-1 protein expression between 30 min L1- and L5-treated rat ventricular myocytes. Sodium-potassium ATPase (Na/K) was used as membrane fraction internal control.

Supplementary Table

Table S1. Effects of L5 on Electrocardiographic Parameters in Mice

	L1	**L5**	**L5 in LOX-1$^{-/-}$**
Heart rate (beats/min)	402.40±52.72	447.44±32.64	409.67±21.24
RR interval (ms)	155.88±17.32	137.15±10.41	147.13±14.60
PR interval (ms)	41.22±3.49	41.34±0.77	40.98±3.33
QRS interval (ms)	10.41±0.84	11.40±0.33	11.22±0.65
QT interval (ms)	17.53±0.54	18.81±1.96	16.61±1.82
QTc (ms)	14.30±1.12	17.77±0.94*	13.41±0.91$^{\#}$

Values are means±S.E. QTc is calculated by the method according to Mitchell GF et al. *P<0.05 vs. L1; $^{\#}P$<0.05 vs. L5.