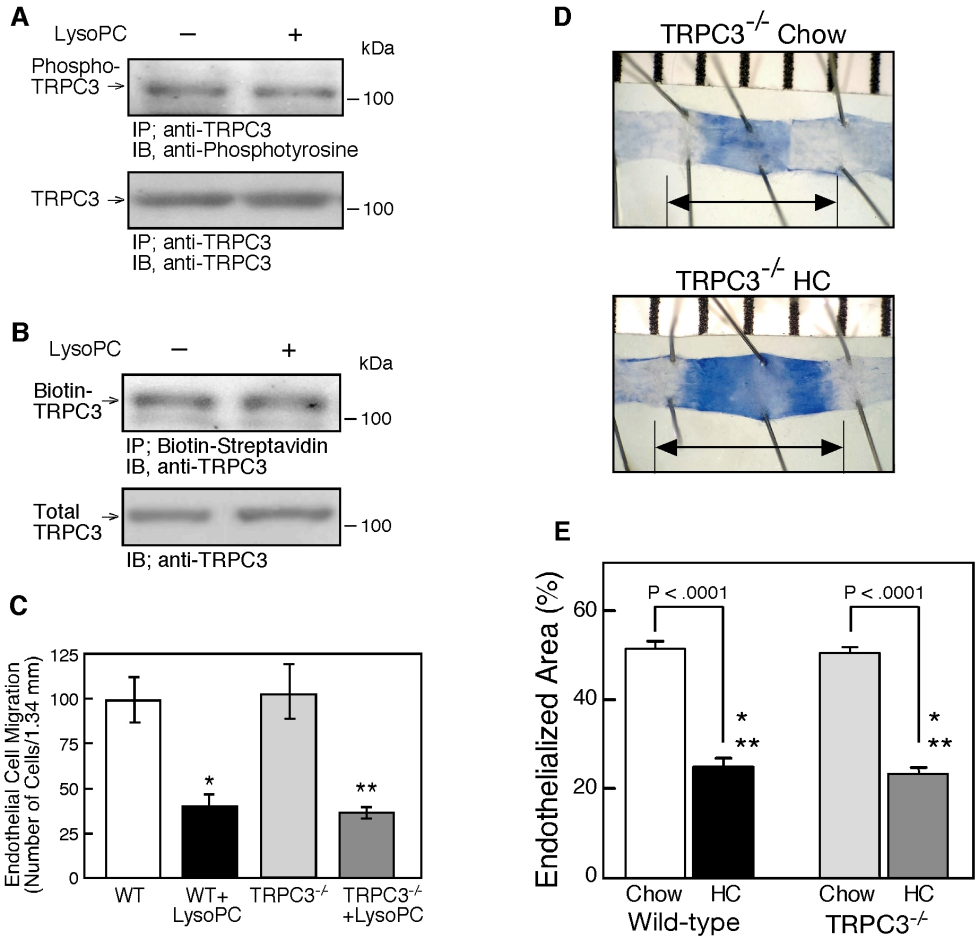
Supplemental Material

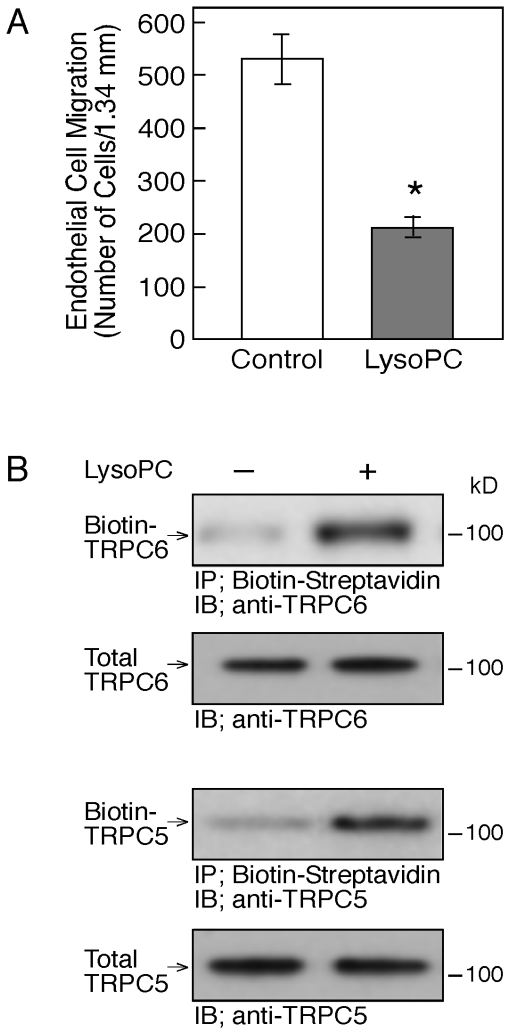
Figure Legends

Supplemental Fig 1. Lysophosphatidylcholine (lysoPC) does not induce phosphorylation or externalization of canonical transient receptor potential (TRPC) 3 protein in wild-type (WT) endothelial cells (ECs) and the anti-migratory effect of lysoPC is not attenuated in TRPC3-deficient (*TRPC3*$^{-/-}$) ECs. (A) WT ECs were incubated with or without lysoPC for 15 minutes. Tyrosine phosphorylation of TRPC3 was evaluated as an indicator of TRPC3 activation (n = 3, top panel). Prior to incubation, an aliquot of cell lysate was removed for immunoblot analysis to determine total TRPC3 protein levels (bottom panel). (B) WT ECs were incubated with or without lysoPC for 1 hour. Cell surface proteins were biotinylated and immunoblot analysis was performed for biotinylated TRPC3 (n = 3, top panel). Prior to incubation with streptavidin-agarose beads, and aliquot of cell lysate was removed for immunoblot analysis to determine total TRPC3 protein levels (bottom panel). (C) Migration assay was initiated in quiescent WT and *TRPC3*$^{-/-}$ ECs, lysoPC (10 μM) added, and migration quantitated at 24 hours. Graph represents migration results by mean ± SD (n = 3, * $P < .001$ compared with WT control ECs, ** $P < .001$ compared with *TRPC3*$^{-/-}$ control). (D) Representative images 120 hours after carotid electrocautery injury. The area without an intact endothelial monolayer stained with Evans Blue. The arrow identifies the length of the original injury. (E) Reendothelialization results shown as the percent of reendothelialized area relative to the total injured area. Results are expressed as the mean ± standard error for each group: wild-type chow diet (n = 10), wild-type HC diet (n = 10), *TRPC3*$^{-/-}$ chow diet (n = 5), and *TRPC3*$^{-/-}$ HC diet (n = 5, * P = NS compared with WT HC).

Supplemental Fig 2. Lysophosphatidylcholine (lysoPC) inhibits migration and induces externalization of canonical transient receptor potential (TRPC) 5 and 6 proteins in human endothelial cells (ECs). (A) Migration assay was initiated in quiescent human EA.hy926 ECs, lysoPC (12.5 μM) added, and migration quantitated at 24 hours. The panel represents migration results by mean ± SD (n = 3, * $P < .001$ compared with control ECs). (B) Human EA.hy926 ECs were incubated with or without lysoPC (12.5μM) for 1 hour. Cell surface proteins were biotinylated and immunoblot analysis was performed for biotinylated TRPC6 (top panel) and TRPC5 (third panel). Prior to incubation with streptavidin-agarose beads, an aliquot of cell lysate was removed for immunoblot analysis to determine total TRPC6 (second panel) and TRPC5 (bottom panel) protein levels as appropriate.



Supplemental Figure 1



Supplemental Figure 2