Supporting Information

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SI Materials and Methods (for Fig. S2)

Oocyte Preparation and Two-Electrode Voltage-Clamp Recording and Analysis. Oocytes from *Xenopus laevis* were isolated and maintained as described in ref. 1. cRNA of wild-type and E157N mutant Kir4.2 was prepared using the mMessage mMachine Capped RNA transcription kit (Ambion), and 2 ng was injected into the oocytes. After 2 days, whole-cell currents were measured by conventional two-microelectrode voltage clamp with a GeneClamp 500 amplifier (Molecular Devices), as reported in ref. 2. High-potassium (96 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM Hepes, pH 7.4) and low-potassium (containing 2 mM KCl) solutions were used to superfuse oocytes, with or without the addition of Ba^{2+}. pClamp8 software (Molecular Devices) was used for data acquisition and analysis.

1. Collins A, Chuang H, Jan YN, Jan LY (1997) Scanning mutagenesis of the putative transmembrane segments of Kir2.1, an inward rectifier potassium channel. *Proc Natl Acad Sci USA* 94:5456–5460.

2. He C, *et al.* (2002) Identification of critical residues controlling G protein-gated inwardly rectifying K^+ channel activity through interactions with the beta gamma subunits of G proteins. *J Biol Chem* 277:6088–6096.

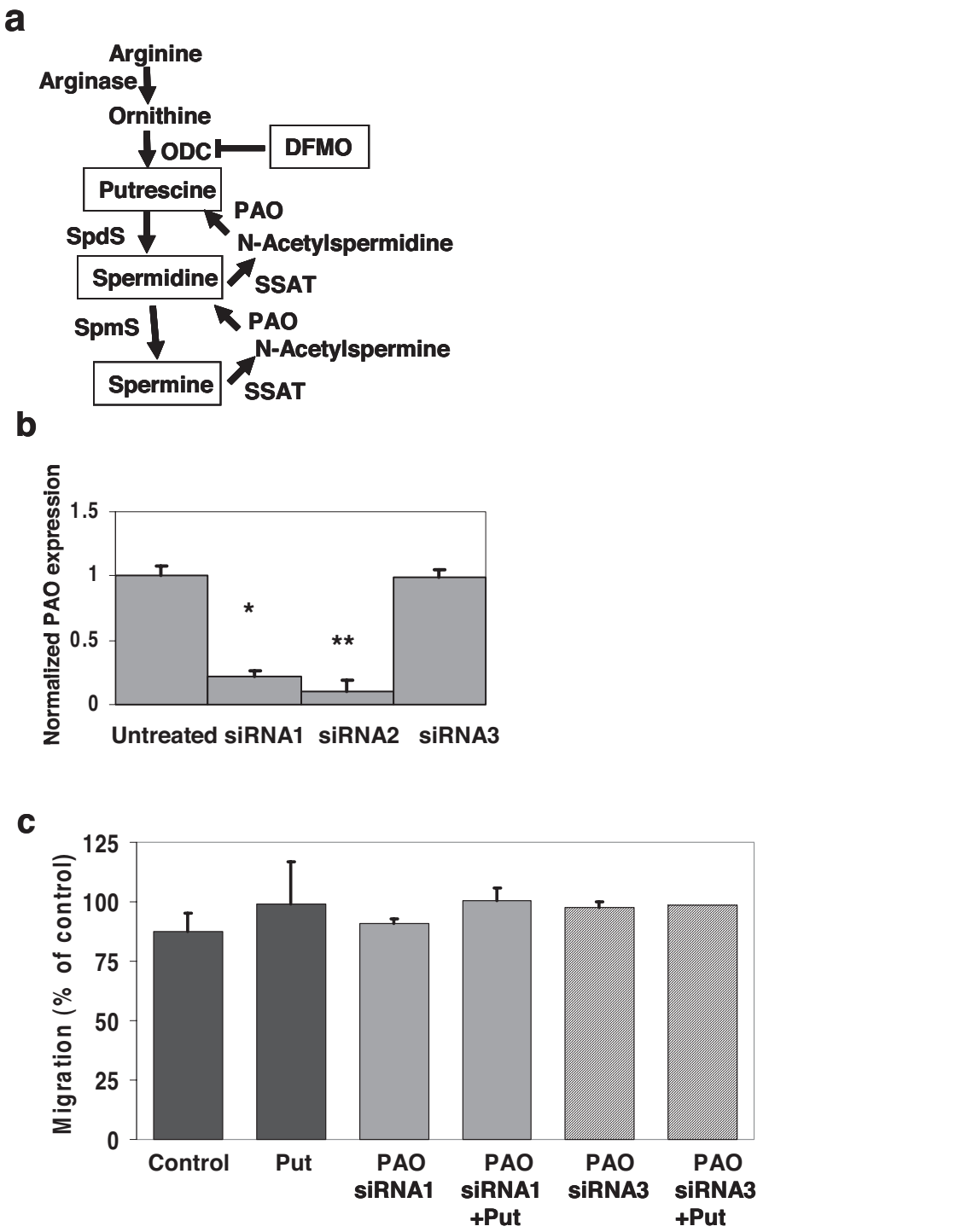


Fig. S1. Effects of suppression of polyamine oxidase message levels in MEFs. (*a*) Diagram of polyamine metabolism. (*b*) Real-time RT-PCR analysis of α9-expressing MEF cells treated with siRNA to polyamine oxidase. Cells were treated for 2 d with three unique siRNA to PAO. Levels of PAO mRNA were then measured and normalized to cellular GAPDH levels. *, $P = 0.009$; **, $P = 0.005$. (*c*) Migration of α9α4-transfected MEF cells treated with siRNA to PAO as in Fig. 2 *c* and *d*, with or without additional treatment with 100 μM putrescine.

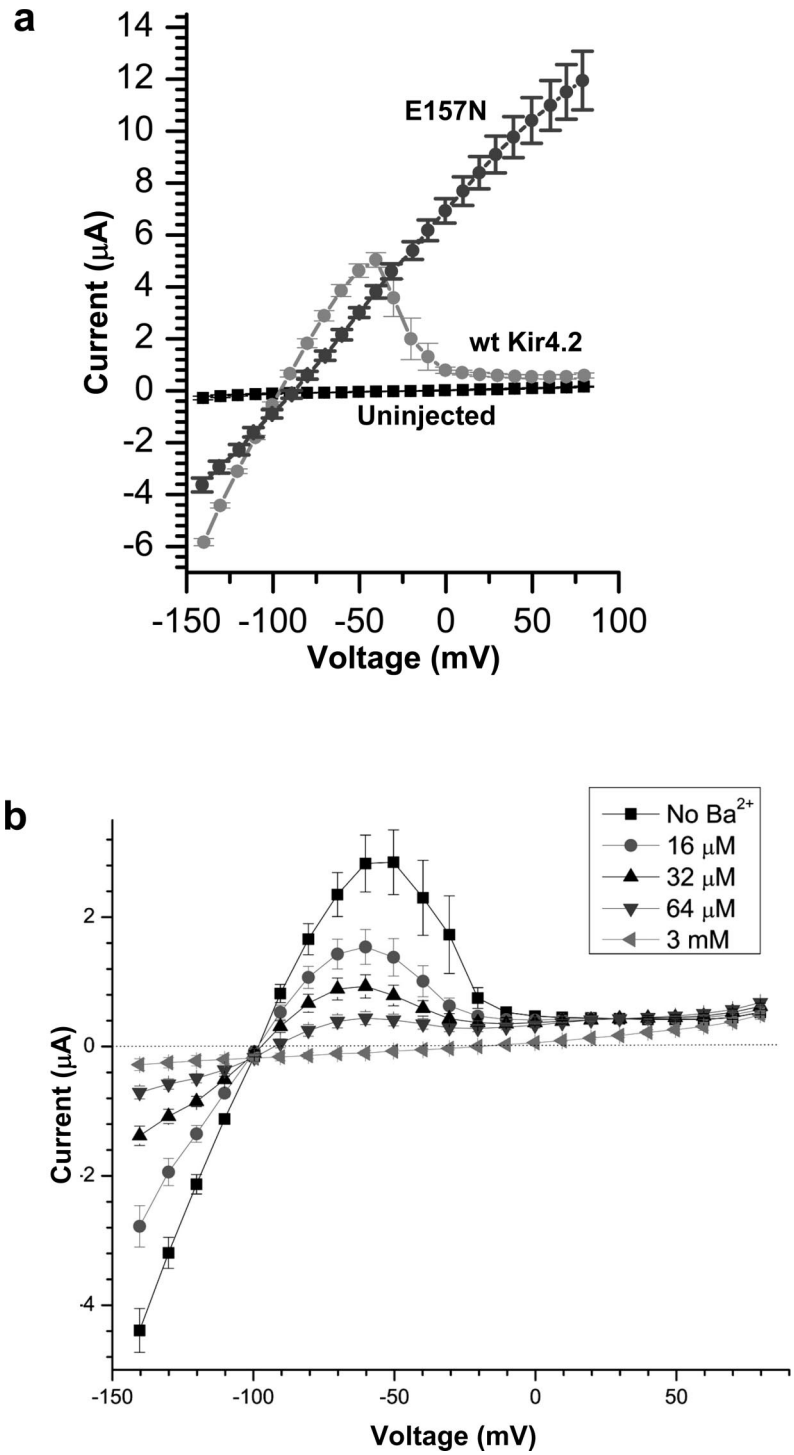


Fig. S2. Electrophysiological analysis of Kir4.2-expressing *Xenopus* oocytes. (*a*) Whole-cell current–voltage relationship of wild-type Kir4.2 and E157N in low-potassium solution (2 mM). *Xenopus* oocytes expressing wild-type Kir4.2 and Kir4.2 E157N were held at 0 V and stepped to recording membrane potentials between −140 to 80 mV at increments of 10 mV. Each membrane potential was applied every 500 ms and held for 200 ms. Currents from control uninjected oocytes are also indicated. The current values at each membrane potential were the average of the last 15 ms of the 200 ms pulse. Each point represents the mean and the SEM of recordings from six oocytes for each of the three conditions. Each oocyte was injected with 0.5 ng of cRNA, and the recordings were performed the following day. (*b*) Current–voltage relationship of wild-type Kir4.2 in low (2 mM)-potassium solution in the absence and presence of increasing concentrations of Ba^{2+}, as indicated. Whole-cell currents were recorded using the same protocol as in *a*. Depicted are the means ± SEM from 12 oocytes in two batches.

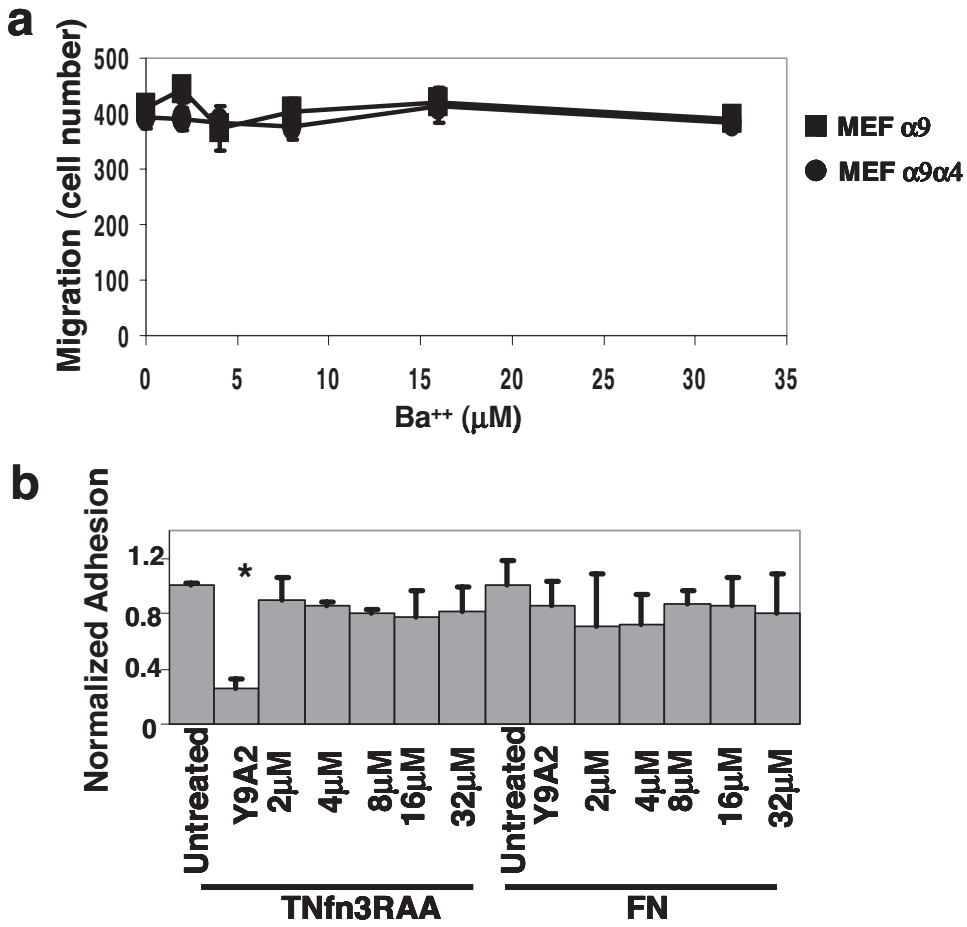


Fig. S3. Effects of barium treatment on migration and adhesion of MEFs. (*a*) Cell migration of barium-treated MEFs toward fibronectin. Cells were treated or not with Ba^{2+} as in Fig. 3*b*, and then plated onto filters coated with 5 μg/ml plasma fibronectin. (*b*) Adhesion of α9-expressing MEFs treated with Ba^{2+}. BE-3-3-3-treated cells were treated with different Ba^{2+} concentrations as above, and then plated into wells coated with 5 μg/ml TNfn3RAA or FN. Adhesion after 1 h is indicated here. All data are expressed as mean $\pm$ SD of three experiments. *, $P = 0.004$.

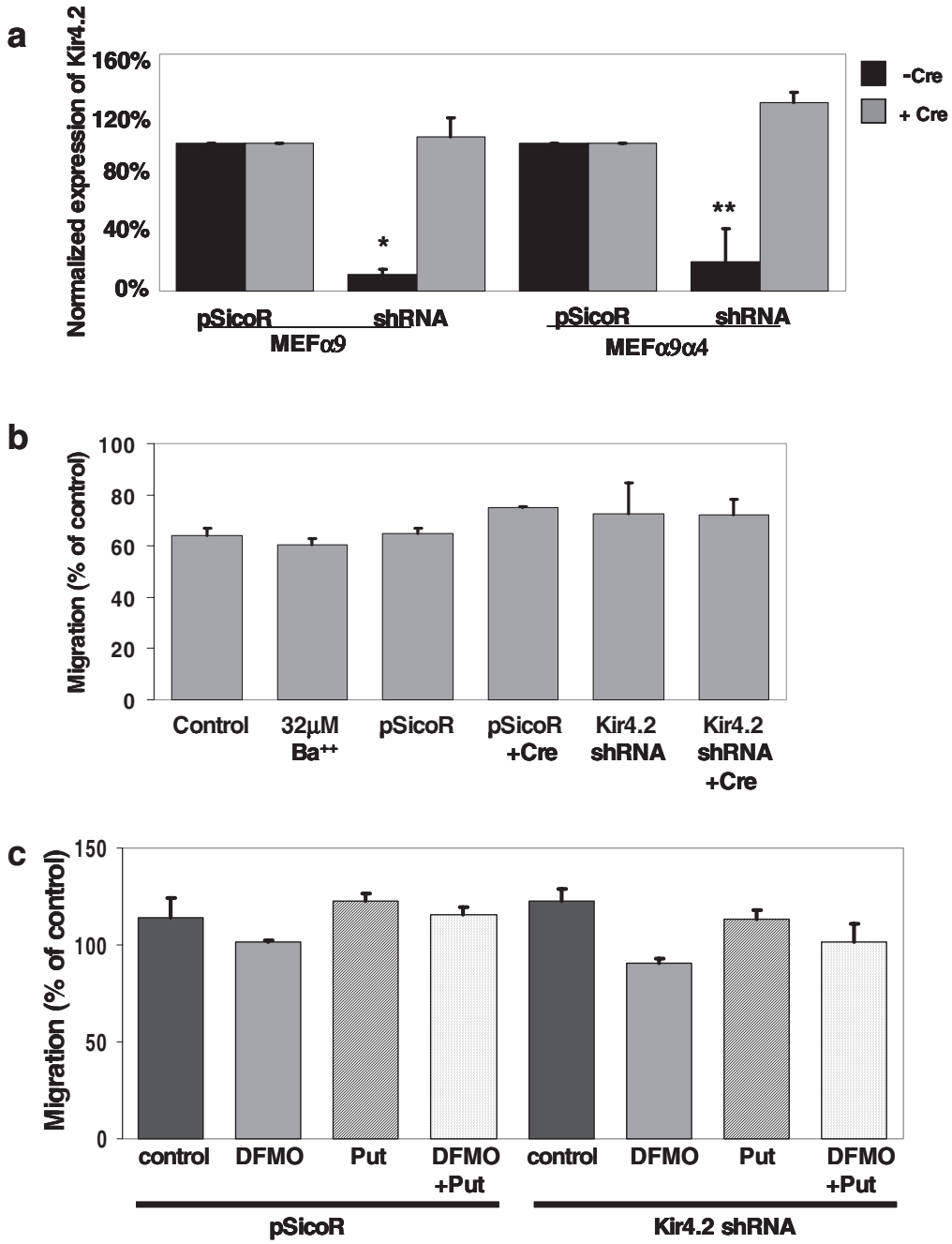
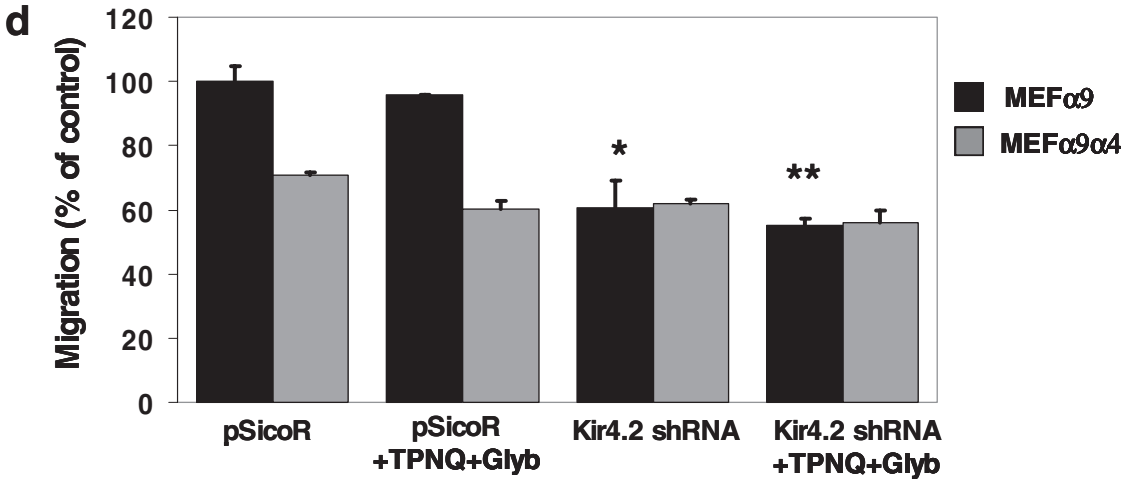
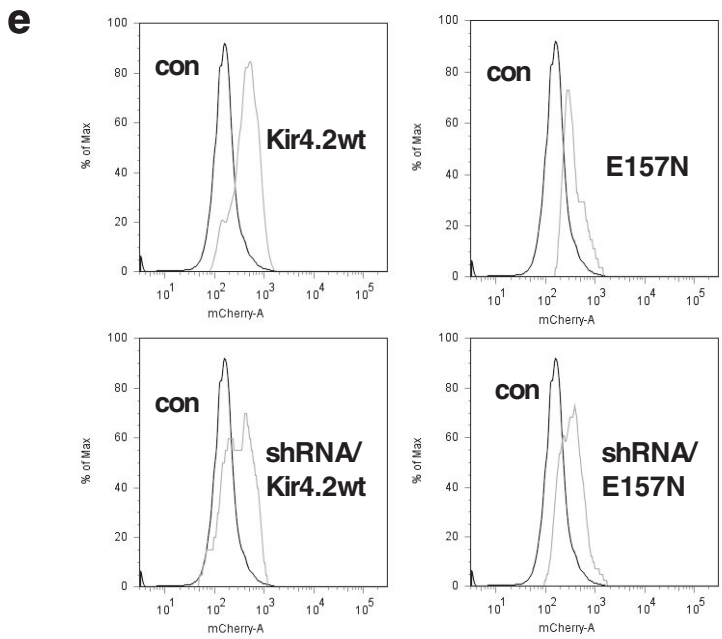


Fig. S4. Migration effects of knockdown of Kir4.2 message level by shRNA. (*a*) Real-time PCR analysis of Kir4.2 cDNA. MEFs expressing wild-type α9 or α9α4 chimeric subunits were infected with lentiviruses to express the pSicoR vector alone (pSicoR) or shRNA to mouse Kir4.2 (shRNA). Cells were also infected with adenoviruses to express Cre recombinase (+Cre). RNA was isolated from all treated cells, and cDNA was generated by reverse transcription. Real-time PCR reactions were then performed by using primers to Kir4.2 and the control GAPDH gene. Data are displayed as the relative percentage of expression compared to that of GAPDH in these cells. *, $P = 0.0007$; **, $P = 0.03$. (*b*) Migration of MEFα9α4 cells expressing shRNA to Kir4.2. Cells expressing pSicoR or shRNA to Kir4.2 were treated overnight or not with cre recombinase for shRNA inactivation before addition to TNfn3RAA-coated Transwell filters. Control cells were treated or not with 32 μM Ba^{2+} before adding to coated filters. Migration after 3 h is shown here. (*c*) Migration of MEFα9α4 cells treated or not with Kir4.2 shRNA and DFMO or putrescine. Cells expressing either pSicoR or Kir4.2 shRNA were treated for 2 d with 5 mM DFMO, 100 μM putrescine, or both. Untreated control or treated cells were then plated onto Transwell filters coated with Tnfn3RAA and migration was measured after 3 h. (*d*) Migration of Kir4.2 shRNA-treated cells with or without TPNQ and Glyb. α9- or α9α4-expressing MEF cells treated with control virus (pSicoR) or Kir4.2 shRNA were incubated or not with 100 nM TPNQ and 1 mM Glyb before addition to wells. Migration after 3 h is shown. (*e*) Flow cytometric analysis of mCherry-tagged wild-type (wt) or mutant (E157N) Kir4.2 in MEFα9 cells also expressing shRNA to Kir4.2. Control nonexpressing MEFα9 cells (con) are also indicated. (*f*) Migration on Tnfn3RAA of wild-type or E157N mutant Kir4.2-expressing MEFα9α4 cells treated or not with Kir4.2 shRNA.





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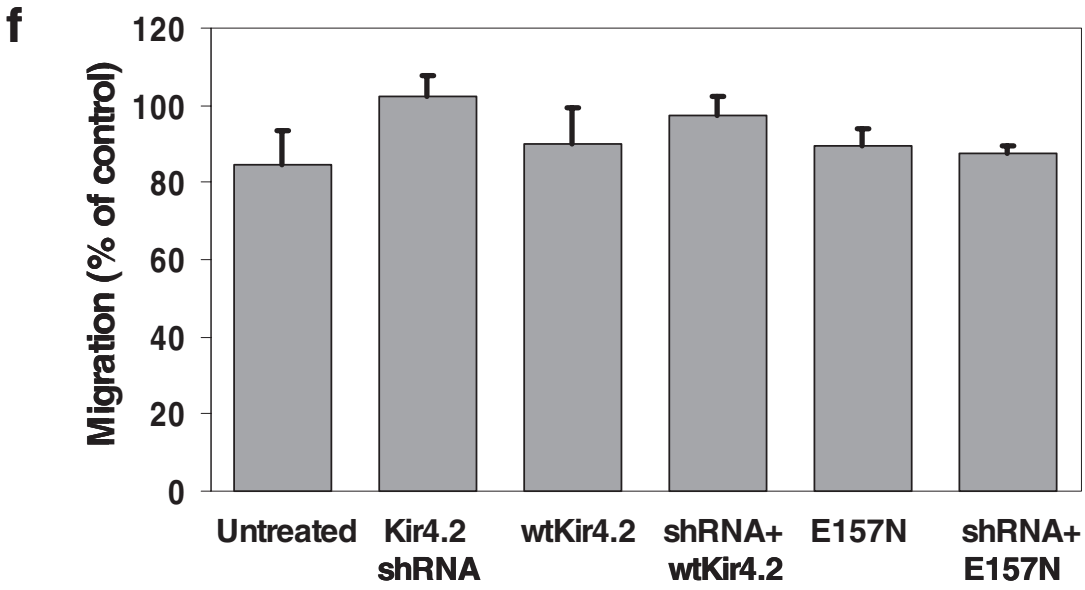

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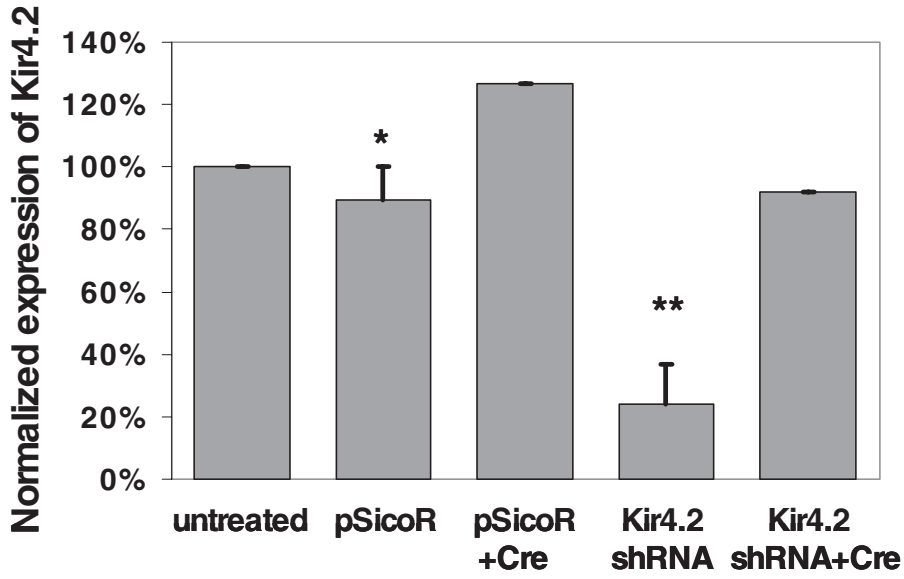

Fig. S5. Real-time PCR analysis of Kir4.2 mRNA levels in HMVECs. cDNA generated from RNA of untreated cells, cells expressing pSicoR or Kir4.2 shRNA, or cells expressing the shRNA and Cre recombinase were amplified by using primers to human Kir4.2 or GAPDH as a control. Levels are expressed as percentage of message relative to GAPDH levels in each sample. *, $P = 0.50$; **, $P = 0.001$.