Díaz et al., http://www.jcb.org/cgi/content/full/jcb.201209151/DC1

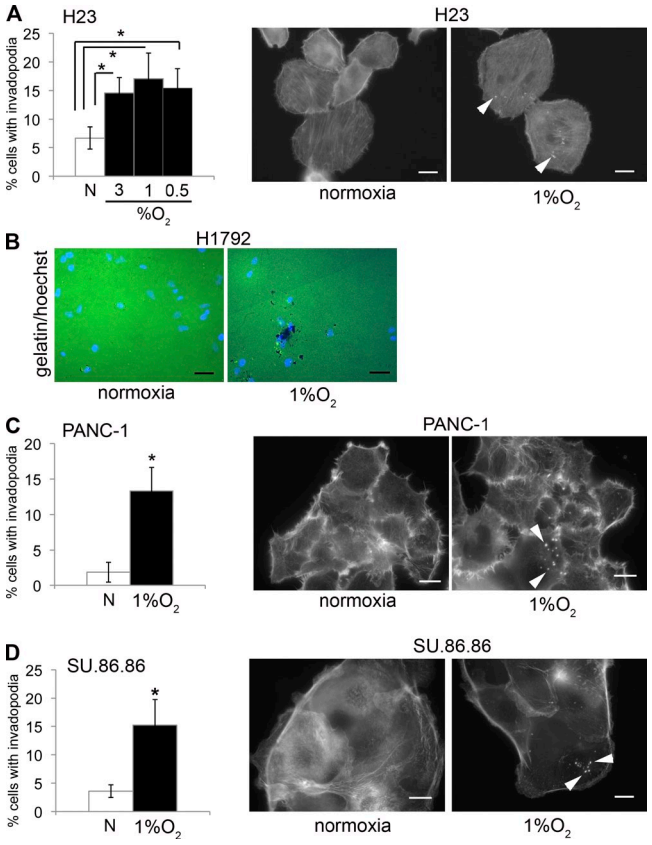

Figure S1. **Hypoxia induces invadopodia formation in different epithelial cancer cells.** (A, left) Percentage of H23 cells (lung cancer) with invadopodia after 16 h in normoxia (N) or at the indicated hypoxic conditions, $n = 2$. *, $P < 0.05$. (right) Representative images of cells stained for F-actin to detect invadopodia. (B) Representative images of H1792 cells (lung cancer) from experiment quantified in Fig. 1 D. Cells were grown on labeled gelatin (green) for 16 h in normoxia or hypoxia. Nuclei are shown in blue. (C, left) Percentage of PANC-1 cells (pancreatic cancer) forming invadopodia after 16 h in normoxia or hypoxia, $n = 3$. *, $P < 0.005$. (right) Representative images of invadopodia-associated F-actin in the same experiment. (D, left) Percentage of SU.86.86 (pancreatic cancer) cells forming invadopodia after 16 h in normoxia or hypoxia, $n = 2$. *, $P < 0.05$. (right) Representative images of invadopodia-associated F-actin in the same experiment. Arrowheads point to invadopodia. Histograms represent means ± SEM. Bars, 10 µm.

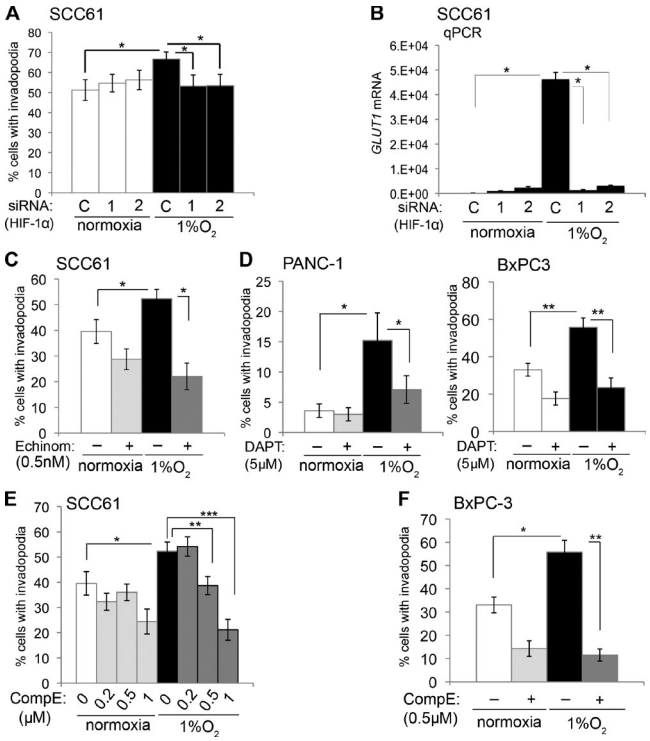

Figure S2. **HIF-1α mediates hypoxia-induced invadopodia formation.** (A) Percentage of SCC61 cells forming invadopodia after transfection with control (C) or HIF-1α siRNAs 1 and 2 and growth under or normoxia or hypoxia for 16 h, $n = 3$. *, $P < 0.005$. (B) qPCR analysis of GLUT-1 mRNA in SCC61 cells transfected with control or HIF-1α siRNAs 1 and 2, measured after 16 h of normoxia or hypoxia. Means ± SD for GLUT1 mRNA levels normalized to Actin mRNA are represented. $n = 2$. *, $P < 0.01$. (C) Percentage of SCC61 cells forming invadopodia after treatment with the HIF-1α inhibitor Echinomycin for 16 h in normoxia or hypoxia, $n = 2$. *, $P < 0.01$. (D) Percentage of PANC-1 or BxPC3 cells forming invadopodia after treatment with DAPT or same volume of vehicle for 16 h in normoxia or hypoxia, $n = 3$ for each cell line. *, $P < 0.05$; **, $P < 0.005$. (E) Percentage of SCC61 cells forming invadopodia after treatment with the indicated micromolar concentrations of compound E (CompE) in normoxia or hypoxia for 16 h, $n = 2$. *, $P < 0.01$; **, $P < 0.005$; ***, $P < 1 \times 10^{-7}$. (F) Percentage of BxPC3 cells forming invadopodia after treatment the indicated concentration of compound E or corresponding volume of vehicle in normoxia or hypoxia for 16 h, $n = 3$. *, $P < 5 \times 10^{-5}$; **, $P < 3 \times 10^{-10}$. Histograms represent means ± SEM.

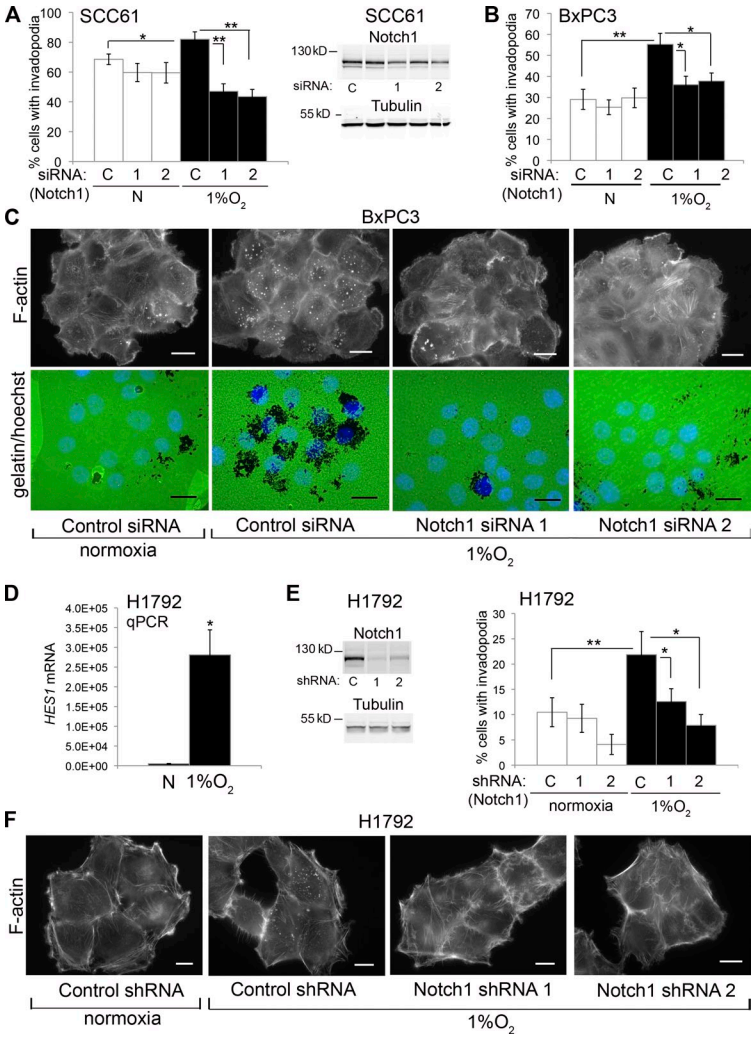

Figure S3. **Hypoxia-induced invadopodia formation is mediated by NOTCH signaling.** (A, left) Percentage of SCC61 cells forming invadopodia after transfection with control (C) or Notch1 siRNAs 1 and 2 and growth under or normoxia (N) or hypoxia for 16 h, $n = 3$. *, $P < 0.05$; **, $P < 0.0005$. (right) Total Notch1 protein levels analyzed by immunoblotting on extracts from SCC61 cells transfected with four individual siRNA oligos for Notch1. Both 1 and 2 decreased Notch1 levels by 75% with respect to control siRNA. (B) Percentage of BxPC3 cells forming invadopodia after transfection with control or Notch1 siRNAs 1 and 2 and growth under or normoxia or hypoxia for 16 h, $n = 3$. *, $P < 0.05$; **, $P < 0.001$. (C) Representative images from the experiment shown in B. Cells were stained for F-actin to visualize invadopodia and grown on green-labeled gelatin to assess gelatin degradation. Blue, Hoechst. (D) qPCR analysis of HES1 mRNA in H1792 cells grown in normoxia or hypoxia for 16 h, $n = 2$. Means ± SD for HES1 mRNA levels normalized to Actin mRNA are represented. *, $P < 0.05$. (E, left) Total Notch1 protein levels analyzed by immunoblotting on extracts from H1792 cells stably expressing shRNA control or shRNAs targeting NOTCH1 (1 and 2). (right) Percentage of H1792 cells stably expressing shRNA control or shRNAs targeting NOTCH1 (1 and 2) that form invadopodia after 16 h in normoxia or hypoxia, $n = 3$. *, $P < 0.05$; **, $P < 0.005$. (F) Representative images of invadopodia-associated F-actin in H1792 cells from E. Histograms in A, B, and E represent means ± SEM. Bars, 10 μm.

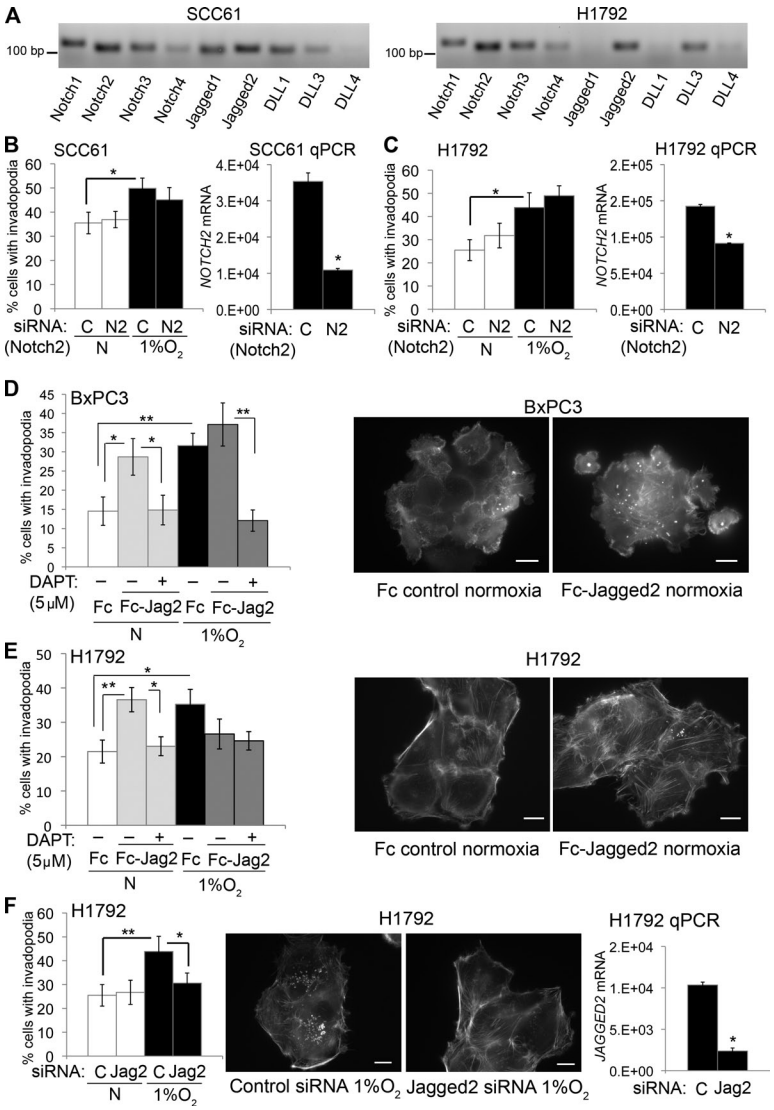

Figure S4. **Active NOTCH signaling induces invadopodia formation.** (A) RT-PCR analysis of mRNA expression for Notch signaling pathway components (receptors and ligands) in SCC61 and H1792 cells. (B, left) Percentage of SCC61 cells forming invadopodia after transfection with control (C) or NOTCH2 (N2) siRNA pools and grown under or normoxia (N) or hypoxia for 16 h, $n = 2$. *, $P < 0.005$. (right) qPCR analysis of NOTCH2 mRNA in SCC61 cells transfected with control or NOTCH2 siRNA pools. Means ± SD for NOTCH2 mRNA levels normalized to Actin mRNA are represented, $n = 2$. *, $P < 0.01$. (C, left) Percentage of H1792 cells forming invadopodia after transfection with control or NOTCH2 siRNA pools and grown under or normoxia or hypoxia for 16 h, $n = 2$. *, $P < 0.0005$. (right) qPCR analysis of NOTCH2 mRNA in H1792 cells transfected with control or NOTCH2 siRNA pools. Means ± SD for NOTCH2 mRNA levels normalized to Actin mRNA are represented, $n = 2$. *, $P < 0.05$. (D, left) Percentage of BxPC3 cells forming invadopodia plated on Fc-JAG2– or Fc control–covered coverslips and grown under the indicated conditions for 16 h, $n = 3$. *, $P < 0.01$; **, $P < 0.005$. (right) Representative pictures of cells plated on Fc control or Fc-JAG2 coverslips in normoxia and stained for F-actin. (E, left) Percentage of H1792 cells forming invadopodia after plating on Fc-JAG2– or Fc control–covered coverslips and grown under the indicated conditions for 16 h, $n = 3$. *, $P < 0.01$; **, $P < 0.005$. (right) Representative pictures of cells plated on Fc control or Fc-JAG2 coverslips in normoxia stained for F-actin are shown. (F, left) Percentage of H1792 cells forming invadopodia after transfection with control or JAG2 siRNA pools and grown under normoxia or hypoxia for 16 h, $n = 2$. *, $P < 0.005$; **, $P < 0.0005$. (middle) Representative images of invadopodia-associated F-actin in cells from the same experiment. (right) qPCR analysis of JAG2 mRNA in SCC61 cells transfected with control or JAG2 siRNA pools. Means ± SD for JAG2 mRNA levels normalized to Actin mRNA are represented, $n = 2$. *, $P < 0.005$. Histograms represent means ± SEM. Bars, 10 μm.

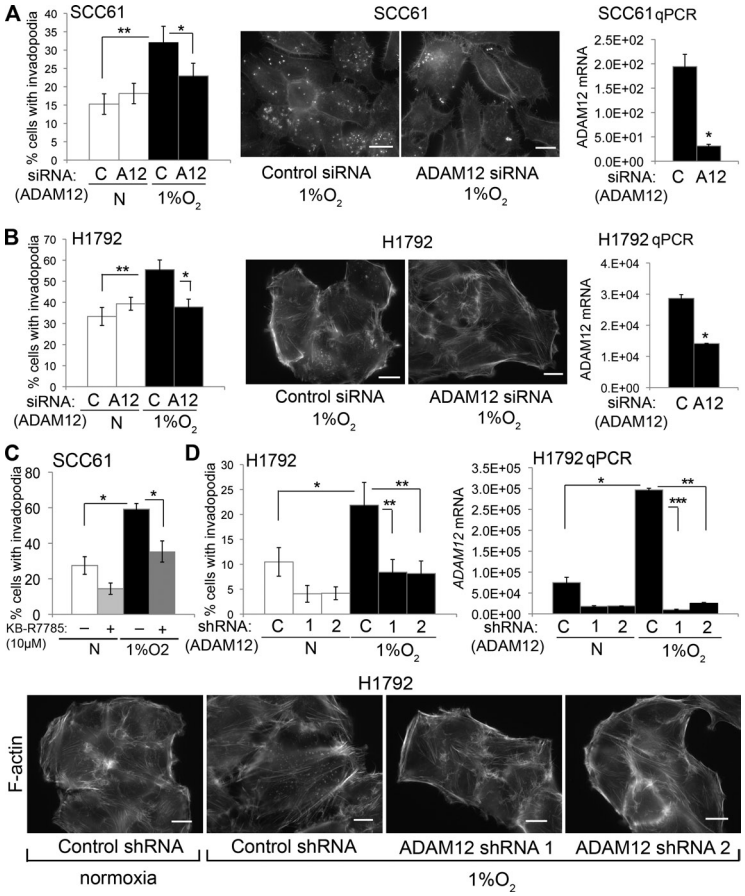

Figure S5. **ADAM12 mediates hypoxia-induced invadopodia formation.** (A, left) Percentage of SCC61 cells forming invadopodia after transfection with control (C) or ADAM12 siRNA pools grown under or normoxia (N) or hypoxia for 16 h, $n = 3$. *, $P < 0.05$; **, $P < 0.001$. (middle) Representative images of cells from the same experiment stained for F-actin. (right) qPCR analysis of ADAM12 mRNA in SCC61 cells transfected with control or ADAM12 siRNA pools. Means ± SD for ADAM12 mRNA levels normalized to Actin mRNA are represented, $n = 2$. *, $P < 0.05$. (B, left) Percentage of H1792 cells forming invadopodia after transfection with control or ADAM12 siRNA pools and grown under or normoxia or hypoxia for 16 h, $n = 3$. *, $P < 0.01$; **, $P < 0.001$. (middle) Representative images of cells from the same experiment stained for F-actin. (right) qPCR analysis of ADAM12 mRNA in H1792 cells transfected with control or ADAM12 siRNA pools. Means ± SD for ADAM12 mRNA levels normalized to Actin mRNA are represented, $n = 2$. *, $P < 0.05$. (C) Percentage of SCC61 cells forming invadopodia after treatment with the ADAM inhibitor KB-R7785 in normoxia or hypoxia for 16 h, $n = 2$. *, $P < 0.01$. (D, left) Percentage of H1792 cells stably expressing shRNA control or shRNAs targeting ADAM12 (1 and 2) that form invadopodia after 16 h in normoxia or hypoxia, $n = 3$. *, $P < 0.05$; **, $P < 0.01$. (right) qPCR analysis of ADAM12 mRNA in H1792 cells stably expressing control or ADAM12 shRNAs 1 or 2. Means ± SD for ADAM12 mRNA levels normalized to *18S* rRNA mRNA are represented, $n = 2$. *, $P < 0.01$; **, $P < 0.001$; ***, $P < 0.005$. (bottom) Representative pictures of H1792 cells from the same experiment stained for F-actin. Histograms in A–C represent means ± SEM. Bars, 10 μm.