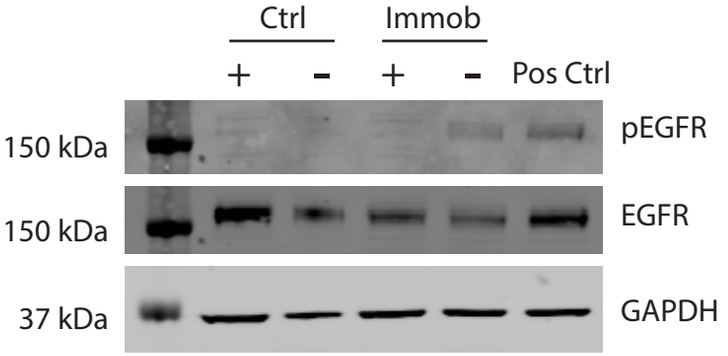
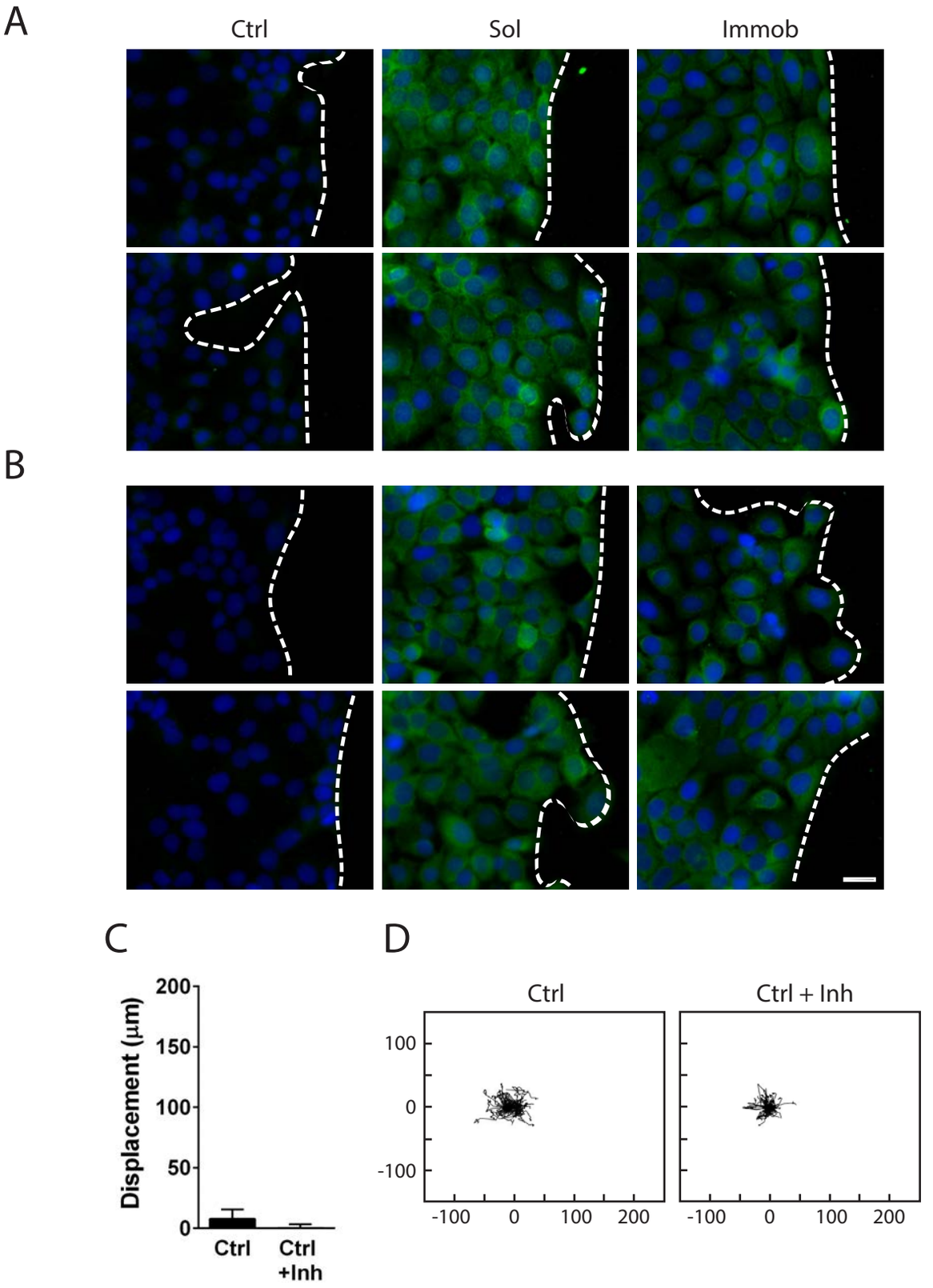
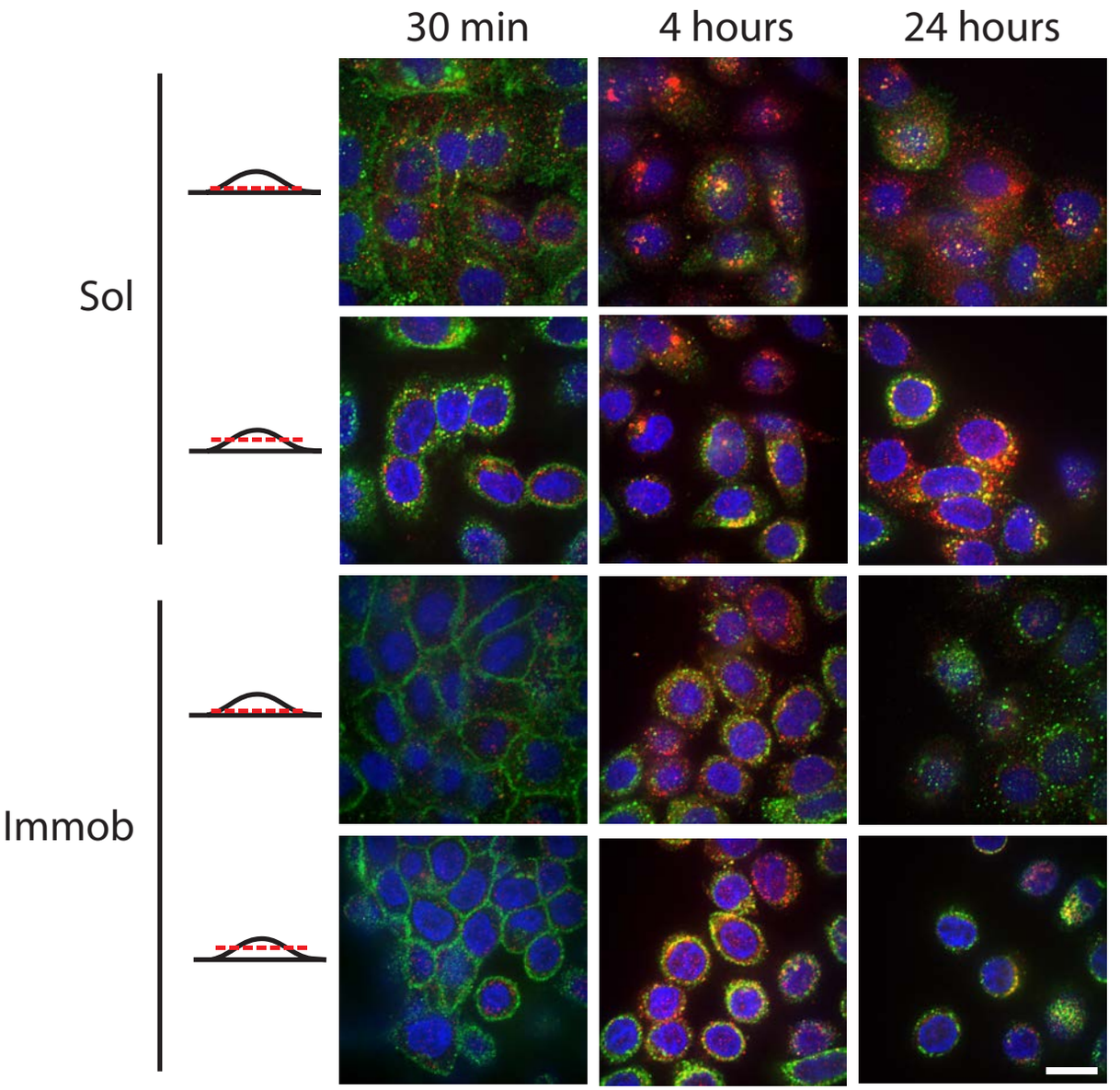


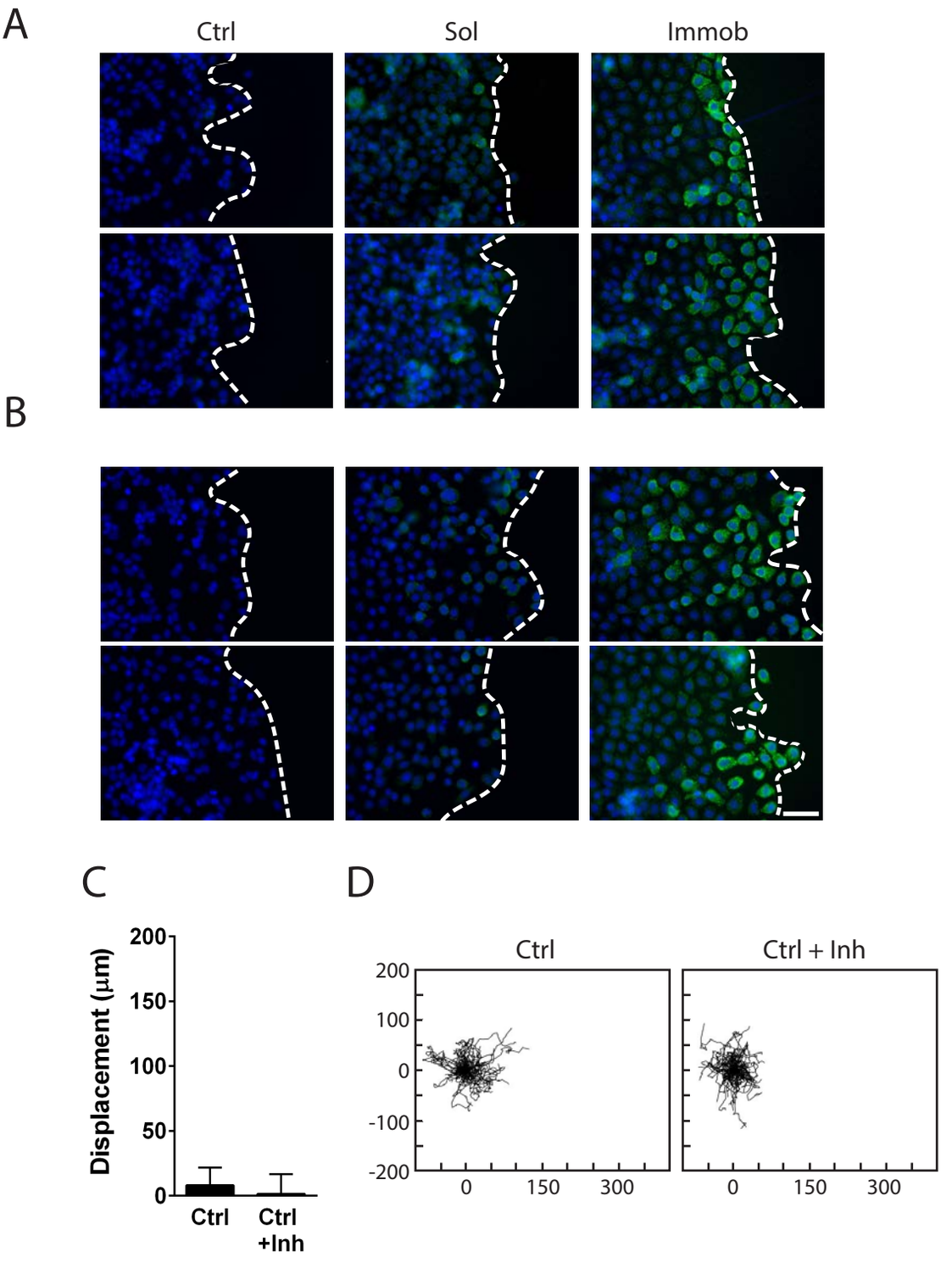
Supplemental Figure 1. Immunoblotting of pEGFR demonstrated that 2 µM AG 1478 prevents EGFR phosphorylation during cell attachment in the immobilized EGF condition without substantially decreasing EGFR levels. HaCaT cells were plated at 50,000 cells/cm^2 in 24-well plates in DMEM supplemented with 0.5% FBS and 2 µM AG 1478. After 12 hours, the control and immobilized EGF wells were refed with serum-free media, while the soluble EGF treatment condition received 20 ng soluble EGF in serum-free media. HaCaT cells were lysed after 15 min for validation of AG 1478 inhibition of pEGFR. The lysis buffer was composed of 50 mM β-glycerophosphate, pH 7.3 (Boston BioProducts, Ashland, MA), 10 mM NaPP (Boston BioProducts), 30 mM NaF (Boston BioProducts), 50 mM Tris, pH 7.5, 2% Triton X-100, 150 mM NaCl, 1 mM benzamidine (Millipore), 2 mM EGTA (Boston BioProducts), 100 mM sodium orthovanadate (Boston BioProducts), 1 mM DTT, 10 µg/mL aprotinin (Sigma), 10 µg/mL leupeptin (Sigma), 1 µg/mL pepstatin (Sigma), 1 µg/mL microcystin-LR (Sigma), and 1 mM PMSF (Sigma). Protein content of the lysate was measured by a bicinchoninic acid (BCA) assay, and 20 µg protein per sample was separated on NuPage® Novex 4-12% Bis-Tris Protein Gels and transferred onto nitrocellulose membranes (Bio-Rad, Hercules, CA). Membranes were blocked with Odyssey blocking buffer (Li-cor Biosciences, Lincoln, NE) for 1 hour at room temperature and incubated overnight at 4°C in primary antibody diluted in Odyssey blocking buffer. Anti-pEGFR (Tyr1068, #2236, 1:500), EGFR (#2232, 1:1000), and GAPDH (#2118, 1:10000) were purchased from Cell Signaling Technology. Membranes were incubated for 1 hour with goat anti-rabbit IRDye® 680 (Li-cor Biosciences, 1:15000) to detect EGFR and GAPDH, or goat anti-mouse IRDye® 800 (Li-cor Biosciences, 1:15000) to detect pEGFR. Membranes were washed again and analyzed using the Li-cor BioScience Odyssey 9120 Infrared Imaging System (Li-cor Biosciences).



Supplemental Figure 2. Additional images of immunofluorescent staining for pERK at (A) 30 minutes or (B) 2 hours. Green indicates pERK and blue is DAPI nuclei stain, scale bar = 25 µm. MEK inhibition does not impact baseline collective migration behavior, as evidenced by (C) cell sheet displacement at 8 hours and (D) windrose plots (µm) of individual cell tracks. n = 75.



Supplemental Figure 3. Additional images of immunofluorescent staining for EGFR (green) and EEA1 (red). Red line indicates cellular plane for panels, scale bar =100 µm.



Supplemental Figure 4. Additional images of immunofluorescent staining for pPLCg1 at (A) 30 minutes or (B) 4 hours. Green indicates pPLCγ1 and blue is DAPI nuclei stain, scale bar = 50 μm. PLCγ1 inhibition does not impact baseline collective migration behavior, as evidenced by (C) cell sheet displacement at 8 hours and (D) windrose plots (μm) of individual cell tracks. n = 75.