Leyme et al., http://www.jcb.org/cgi/content/full/jcb.201506041/DC1

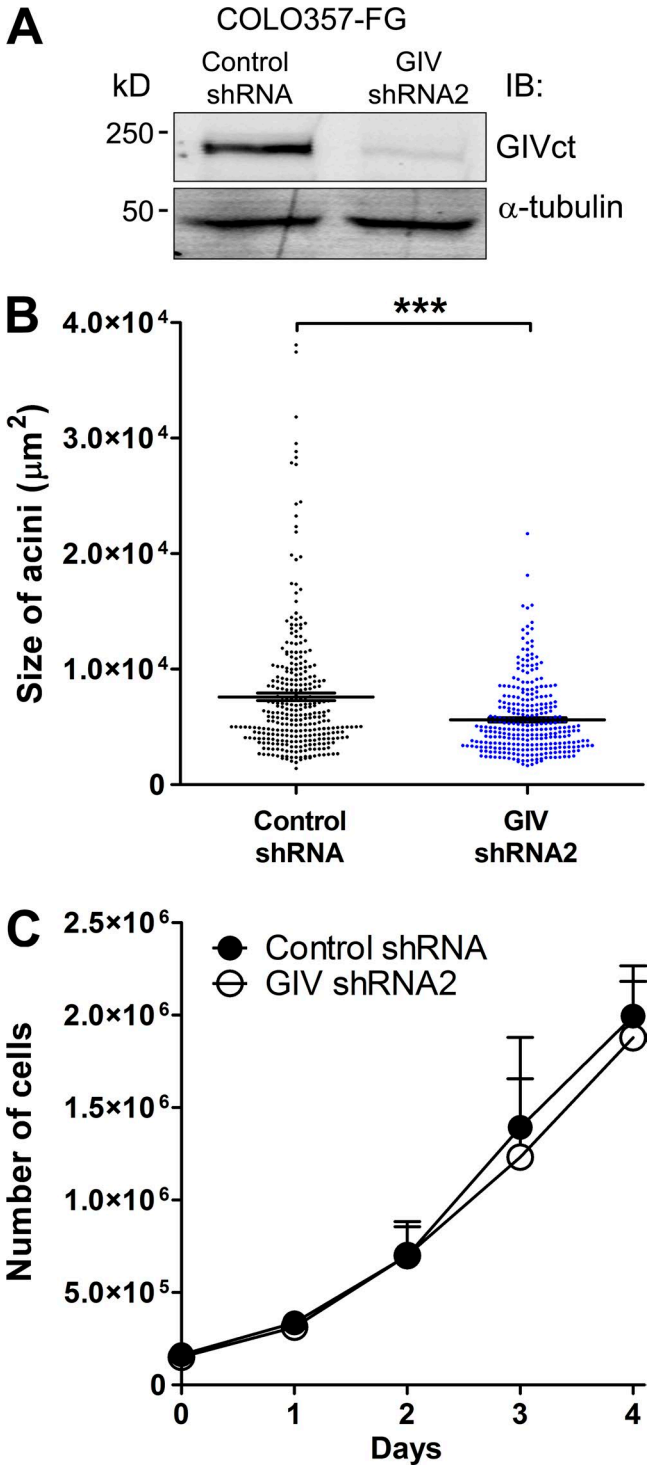

Figure S1. **GIV depletion reduces the size of COLO357-FG acini in Matrigel 3D cultures but does not impair the rate of growth on plastic dishes.** (A) COLO357-FG cells stably depleted of GIV were generated by lentiviral transduction as described in Materials and methods, and GIV expression was determined by immunoblotting (IB) with the indicated antibodies. GIV expression is reduced >90% in COLO357-FG cells expressing GIV shRNA2 compared with controls (COLO357-FG control shRNA). (B) COLO357-FG control shRNA and GIV shRNA2 cells were seeded on Matrigel, and the size of the acini was quantified after 7 d as described in Materials and methods. Each dot is the size of one acini (n = 3, 200 acini per experiment), and the horizontal line is the mean ± SEM (***, $P < 0.001$). GIV-depleted cells (GIV shRNA2) form smaller acini compared with controls (control shRNA). (C) COLO357-FG control shRNA and GIV shRNA2 cells were seeded on plastic dishes and grown in complete media for 4 d. Cells were counted every day using a hemocytometer. Results are depicted as mean ± SEM (error bars; n = 3). No significant difference in the growth rate is observed in GIV-depleted cells compared with controls.

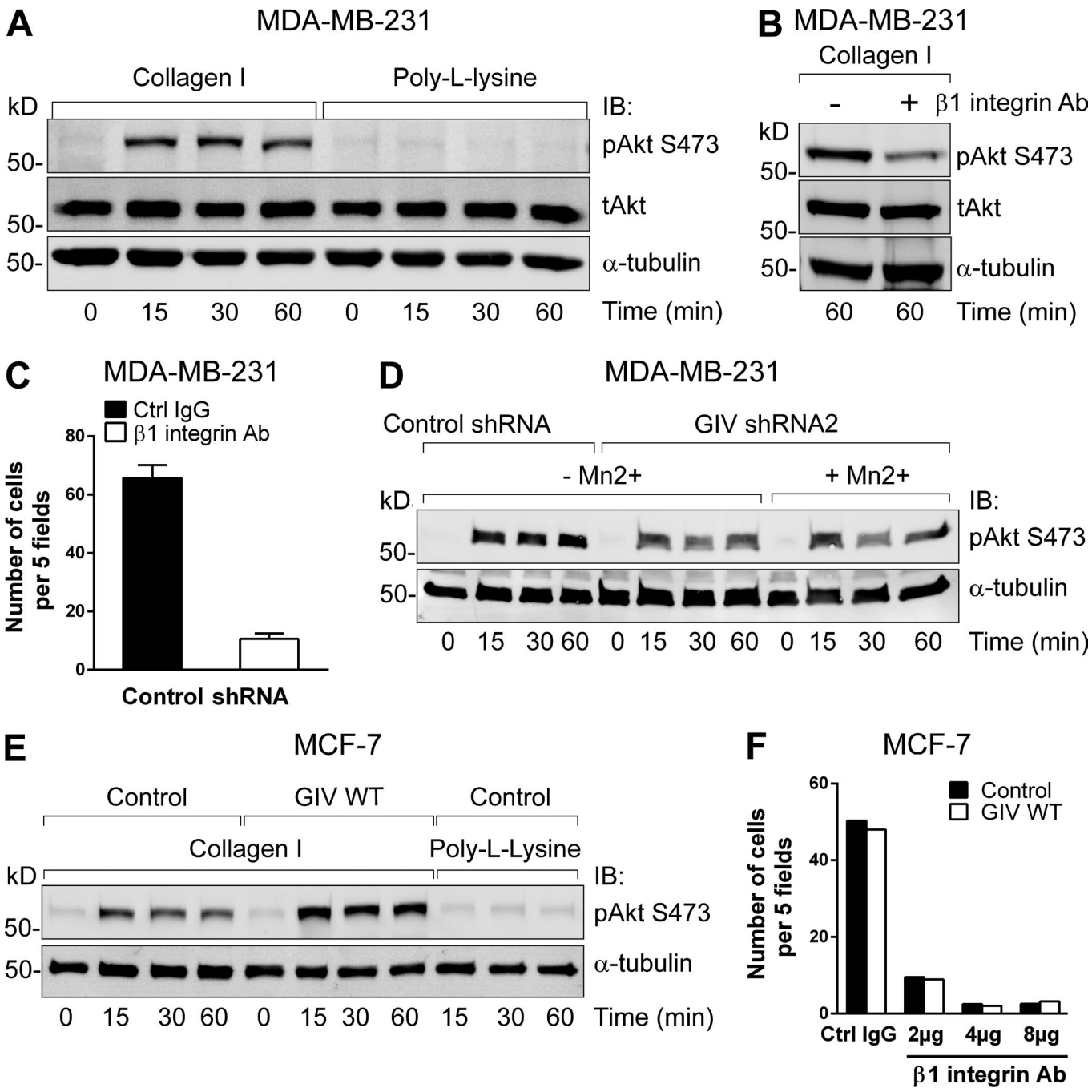

Figure S2. **MDA-MB-231 or MCF-7 cell responses to collagen I stimulation specifically depend on β1 integrin.** (A) Control MDA-MB-231 cells were seeded on collagen I or the nonintegrin substrate poly-L-lysine following the protocol described in Fig. 2 B. Collagen I promotes Akt phosphorylation, whereas poly-L-lysine has no effect. (B) Control MDA-MB-231 cells were stimulated with collagen I as described in Fig. 2 B but in the presence of the β1-integrin blocking antibody P5D2 (or a control IgG). Akt activation at 60 min is reduced ~60% upon β1-integrin blockade. (C) Haptotaxis was measured as in Fig. 4 A but in the presence of the β1-integrin blocking antibody AIIB2 (or a control IgG). Cell migration is reduced ~90% upon β1-integrin blockade. (D) MDA-MB-231 control shRNA or GIV shRNA2 cells were seeded on collagen I following the protocol described in Fig. 2 B, except that some cells were incubated in the presence of 1 mM $MnCl_2$ during the 1 h in suspension and during the time course of attachment. $MnCl_2$ does not rescue the Akt activation defect of GIV-depleted cells. (E) Control MCF-7 cells were seeded on collagen I or the nonintegrin substrate poly-L-lysine following the protocol described in Fig. 2 B. Collagen I promotes Akt phosphorylation, whereas poly-L-lysine has no effect. MCF-7 GIV WT cells seeded on collagen I are shown just for comparison with control cells. (F) Cell adhesion assays for control MCF-7 cells or cells overexpressing GIV were performed as in Fig. 2 A but in the presence of 2–8 µg of the β1-integrin blocking antibody AIIB2 (or 8 µg of a control IgG). MCF-7 cell adhesion is almost completely abolished upon β1-integrin blockade. Results are depicted as mean ± SEM (error bars).

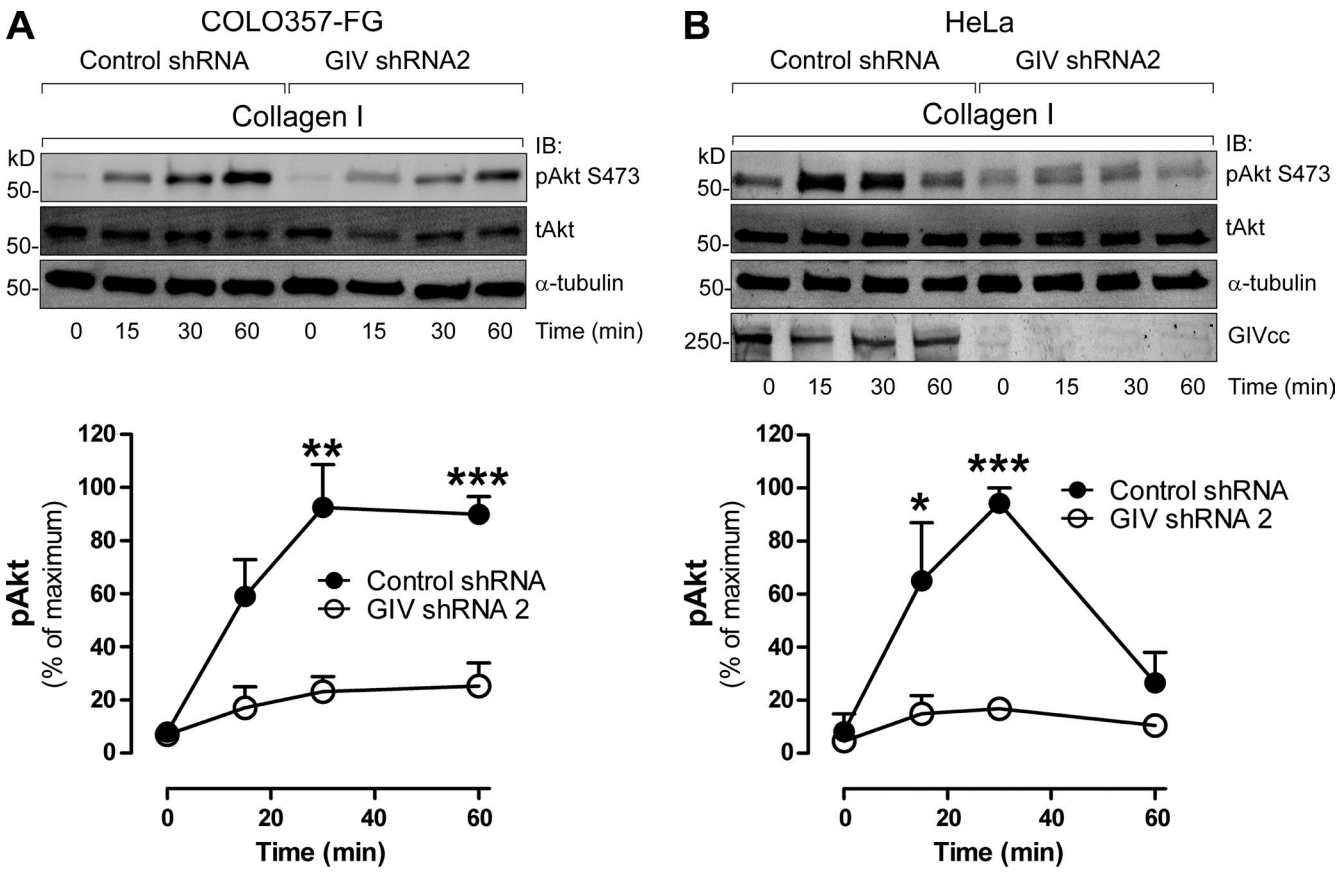

Figure S3. **GIV depletion impairs Akt activation in COLO357-FG and HeLa cells upon collagen I stimulation.** (A) COLO357-FG and (B) HeLa control shRNA and GIV shRNA2 cells were stimulated with collagen I as described in Fig. 2 B. Representative immunoblots for the time course of Akt activation (as measured by levels of pAkt) upon collagen I stimulation are shown on the top part of the panels, and quantification of Akt activation (as described in Materials and methods) expressed as mean ± SEM (error bars; $n = 3$; *, $P < 0.05$; **, $P < 0.01$) is shown below. Akt activation in response to collagen I stimulation is reduced ~75–80% in COLO357-FG GIV shRNA2 cells compared with controls and ~60–80% in HeLa cells.

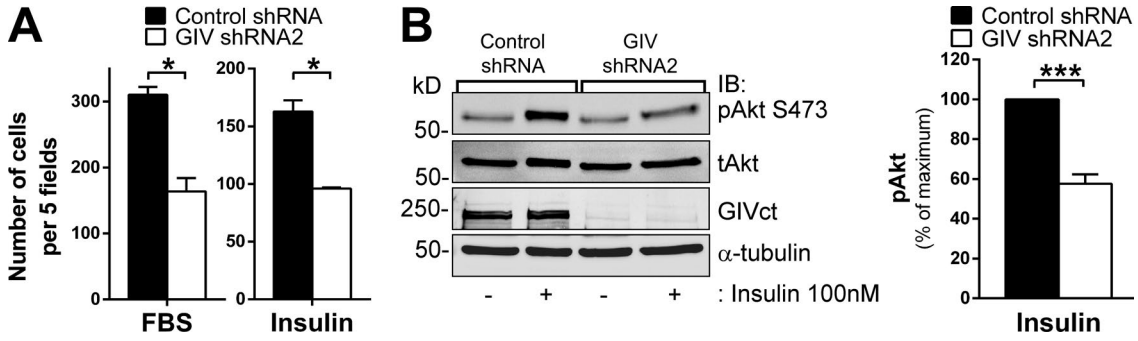

Figure S4. **GIV is required for MDA-MB-231 chemotactic cell migration and efficient Akt signaling in response to soluble factors.** (A) Serum-starved MDA-MB-231 control shRNA and GIV shRNA2 cells were added to the upper compartment of a Boyden chamber, and the lower compartment was filled with media containing 10% FBS (left) or 100 nM insulin (right). The number of migrated cells on the underside of the filter was counted at 5 h, as described in Materials and methods and expressed as mean ± SEM ($n = 3$; *, $P < 0.05$). (B) Serum-starved MDA-MB-231 control shRNA and GIV shRNA2 cells were stimulated or not with 100 nM insulin for 10 min. (Left) Representative immunoblots for Akt activation by insulin in MDA-MB-231 cells. (Right) Quantification of insulin-mediated Akt activation (pAkt) expressed as mean ± SEM (error bars; $n = 3$; ***, $P < 0.001$).

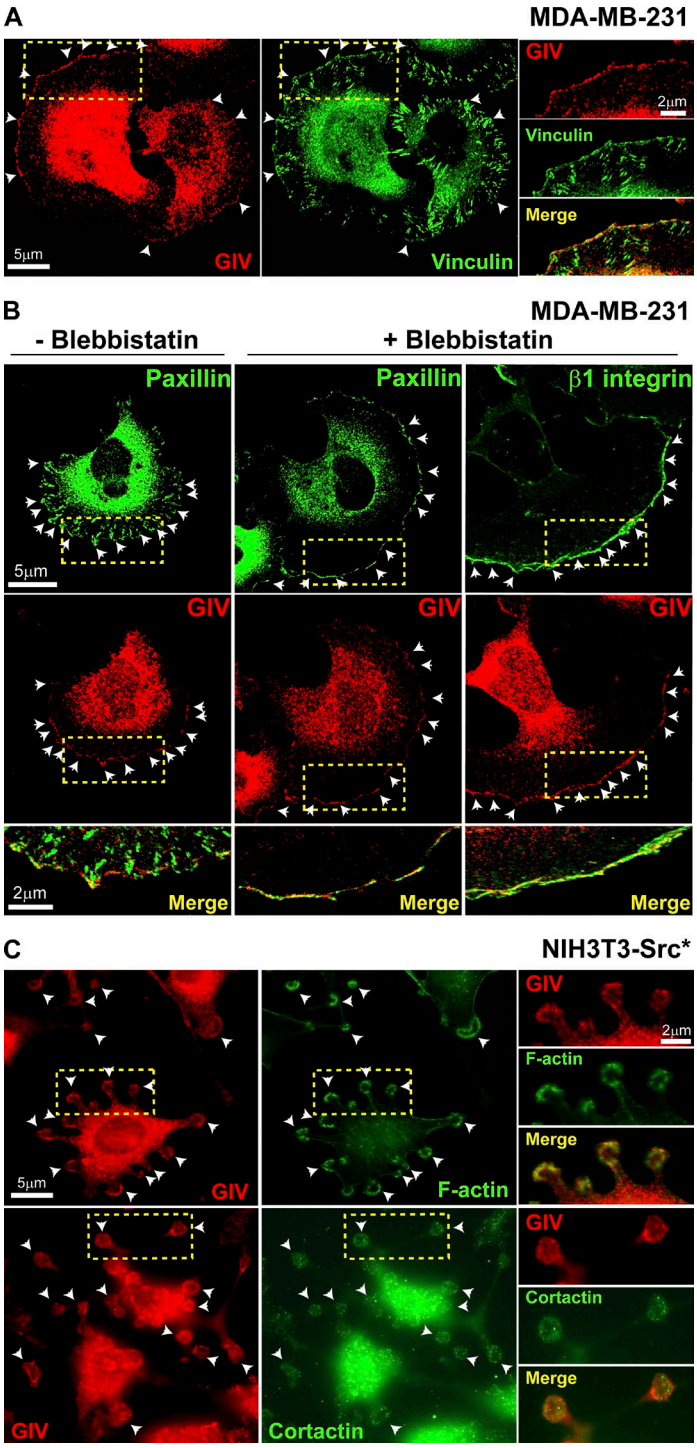

Figure S5. **GIV localization in different subcellular adhesive structures.** (A) GIV partially colocalizes with vinculin. Control MDA-MB-231 cells were seeded on collagen I for 60 min following the protocol described in Fig. 2 B, except that the coated surfaces were glass coverslips and that cells were fixed to stop the reactions. After staining for GIV (red) or vinculin (green), cells were imaged by confocal microscopy. White arrowheads indicate colocalization, and the boxed areas are shown enlarged on the right. GIV colocalizes with vinculin at the PM but not in the strip-shaped vinculin-positive structures. (B) GIV is associated with nascent focal adhesions. Control MDA-MB-231 cells were seeded on collagen I for 30 min following the protocol described in Fig. 2 B, except that they were incubated with 50 μM blebbistatin (or an equivalent volume of DMSO) during the 1 h in suspension. Fixed cells were costained for GIV (red) and paxillin (green) or GIV (red) and β1 integrin (P5D2 antibody; green). White arrowheads indicate colocalization, and the boxed areas are shown enlarged on the bottom. The blockade of focal adhesion maturation by blebbistatin was confirmed by the loss of strip-shaped structures positive for paxillin while the paxillin staining at the cell edge was preserved, presumably reflecting nascent focal adhesions. GIV colocalizes with paxillin and β1 integrins at the PM in blebbistatin-treated cells, suggesting that the colocalization occurs in nascent focal adhesions. (C) GIV localizes in invadosomes of NIH3T3-Src* cells. NIH3T3-Src* cells were seeded on noncoated glass coverslips and grown in complete media for 24 h before fixation. Cells were stained for GIV (red) and F-actin (phalloidin, green; top) or cortactin (green; bottom) and imaged by confocal microscopy. White arrowheads indicate colocalization, and the boxed areas are shown enlarged on the right. GIV colocalizes with F-actin and cortactin in the ring-shaped invadosomes characteristic of NIH3T3-Src* cells.