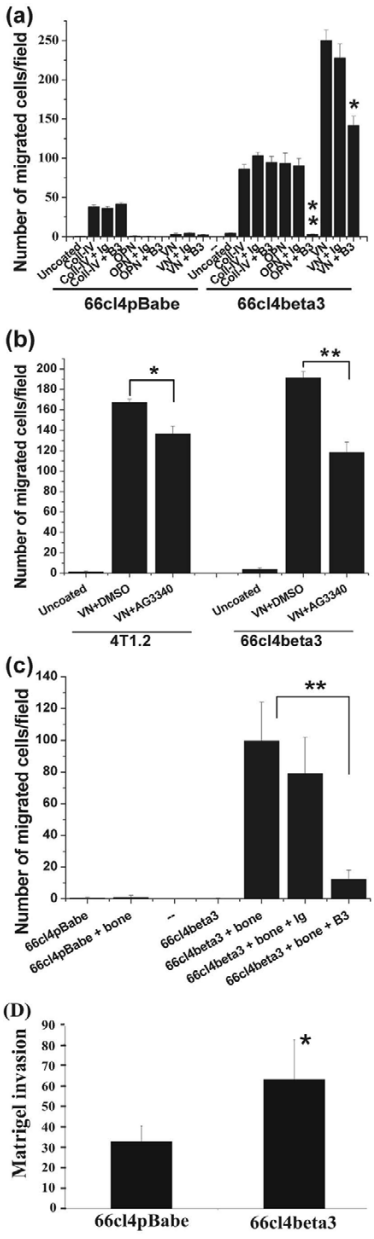
Figure 5



Expression of αvβ3 integrin promotes tumor cell migration and invasion. Migration and Matrigel invasion assays were performed in Transwell migration chambers (8 μm pore size). **(a)** Haptotactic migration in response to collagen IV, osteopontin and vitronectin and the effect of neutralizing β3 antibodies. **(b)** Effect of the matrix metalloproteinase inhibitor AG3340 (10 μM) on the haptotactic migration of 4T1.2 and 66cl4beta3 cells. **(c)** Chemotactic migration of 66cl4pBabe and 66cl4beta3 cells towards a monolayer of bone stromal cells seeded in the lower chamber. **(d)** Invasion of cells through Matrigel in response to a serum chemotactic gradient. All assays were performed in duplicate wells and the cells counted from three fields of view/membrane at 40× magnification (a, b and d) or 20× magnification (c). The experiments were repeated at least twice and the data represent the mean number of migrated cells ± standard deviation of six fields of view/condition from a representative experiment performed in duplicate wells. *$P <$ 0.01, **$P <$ 0.001.

4a). None of the substrates tested promoted rapid adhesion of the weakly metastatic line 66cl4pBabe. Exogenous expression of αvβ3 integrin in 66cl4 cells, however, resulted in markedly increased adhesion to vitronectin, but not to other matrices (Figure 4a). Bone metastatic 4T1.2 cells similarly adhered most avidly to vitronectin but poorly to other substrates, whereas 4T1.13 cells showed moderate binding to collagen IV and vitronectin (Figure 4a). In adhesion assays extended over several hours, the cells adhered to all these matrices (data not shown). Treatment of 66cl4beta3 and 4T1.2 cells with a function-blocking anti-β3 integrin antibody, but not with an isotype-matched control antibody, substantially inhibited their adhesion to vitronectin, indicating that adhesion to vitronectin was mediated specifically via αvβ3 integrin (Figure 4b). A similar inhibition of binding to vitronectin was observed in the 4T1.13 line (data not shown).

Additional experiments were conducted to assess whether tumor αvβ3 may contribute to attachment to endothelial cells, a process required for intravasation/extravasation of metastatic tumor cells *in vivo*. We compared 66cl4pBabe and 66cl4beta3 cells for their ability to attach to a confluent monolayer of microvascular endothelial bEnd.3 cells in the absence of serum. As shown in Figure 4c, both 66cl4pBabe and 66cl4beta3 cells adhered to bEnd.3 cells to the same extent, indicating that αvβ3 is not required for their adhesion to endothelial cells.

αvβ3 integrin promotes migration and invasion of 66cl4 cells

To metastasize successfully, cells need to acquire a motile phenotype and to invade through the basement membrane and surrounding stroma. Site-specific metastasis has been proposed to be regulated, in part, by adhesion to and migration towards the ECM expressed at metastatic sites. To explore the role of αvβ3 integrin in these processes, we first compared the haptotactic migration of 66cl4pBabe and 66cl4beta3 cells towards specific extracellular matrix substrates coated on the underside of the porous membranes of the transwell inserts (Figure 5a). The inserts were placed in wells containing serum-free medium. Collagen IV stimulated the haptotactic migration of 66cl4pBabe cells with a further enhancement in 66cl4beta3 cells. This migration, however, was not blocked by antibodies targeting β3 integrin (Figure 5a). Control 66cl4pBabe cells showed no haptotactic migration towards osteopontin, an abundant αvβ3 integrin ligand in bone, presumably due to their lack of αvβ3 expression. Consistent with this, 66cl4beta3 cells were strongly migratory towards osteopontin in a process blocked completely by neutralizing antibodies against integrin β3. Migration towards vitronectin was also strongly stimulated by β3 integrin expression in 66cl4beta3 cells and was partially inhibited by neutralization of αvβ3 (Figure 5a).