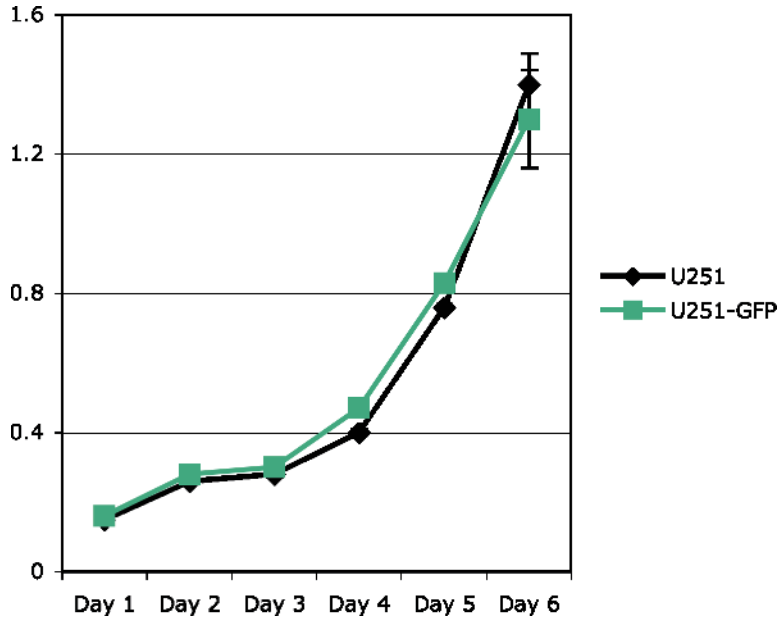


Figure W1. MTT proliferation assay of U251-MG cells either noninfected (U251) or infected with retroviruses carrying pCX_p-GFP vector for stable GFP expression (U251-GFP). Note no difference in proliferation induced by GFP expression.

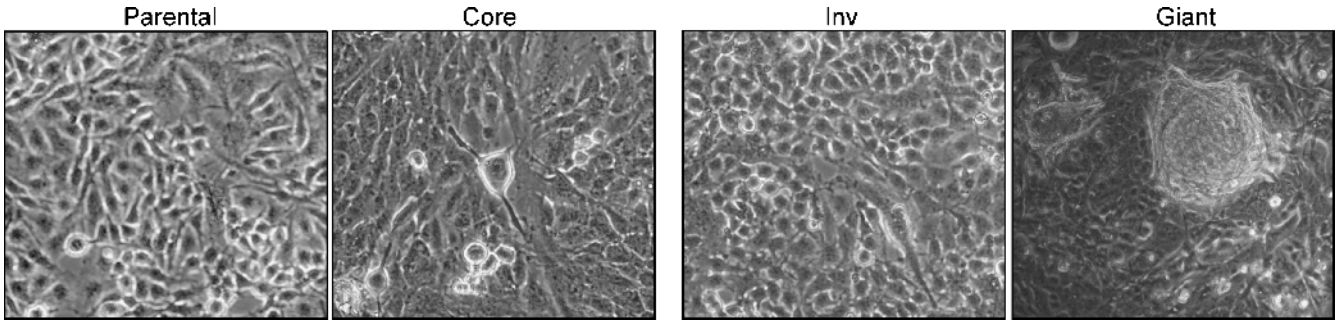
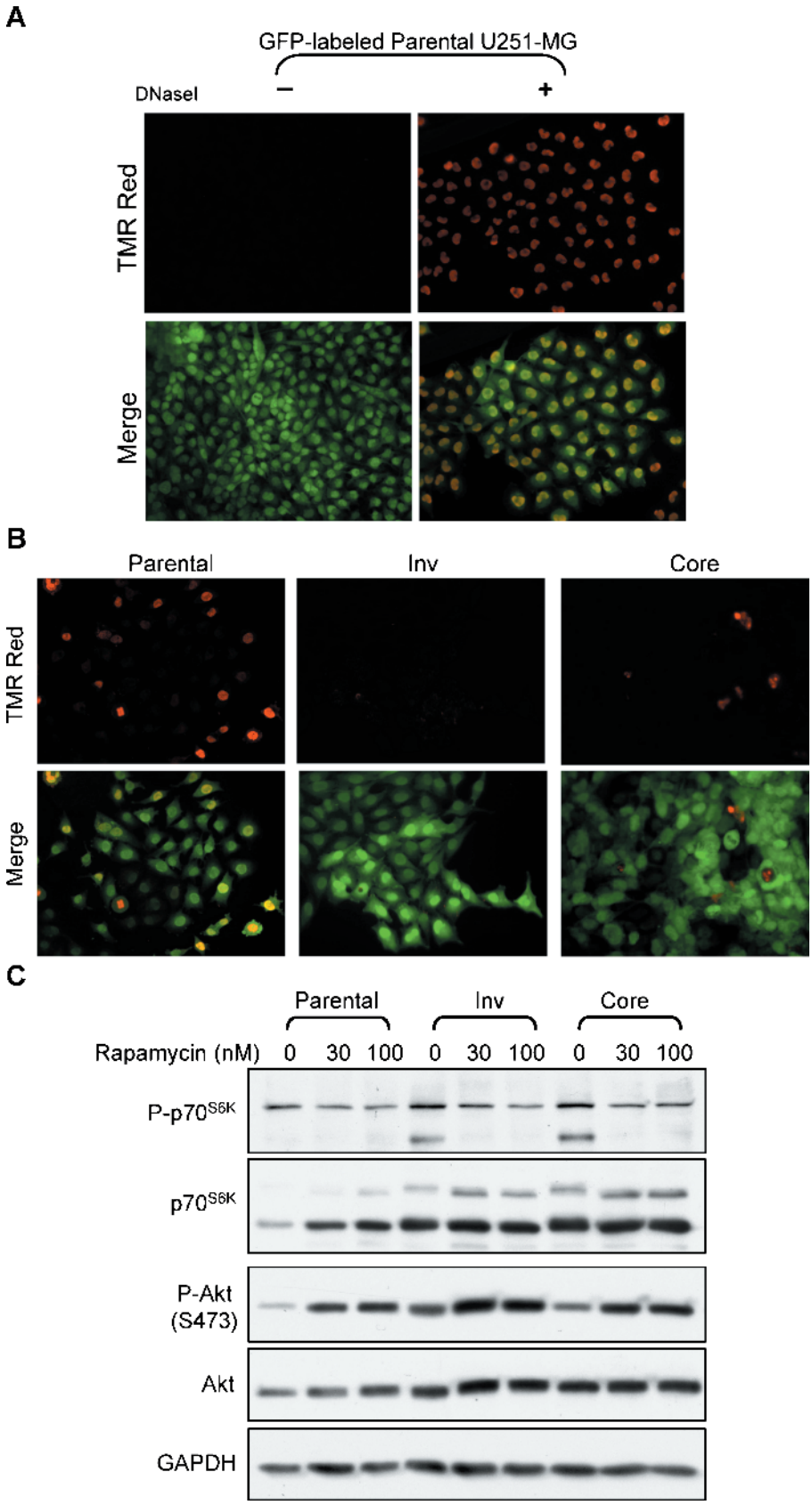


Figure W2. Morphology of parental U251-MG cells and of the cells isolated from the animals 1 (Core and Inv) and 9 (giant) from Figure 1*A*. Bright field images at an original magnification of ×400.

Figure W3. Resistance to apoptosis induced by rapamycin treatment of *in vivo*–selected Inv and Core cells. (A) Control TMR Red apoptosis assay showing 100% apoptosis by treatment with DNase I of parental U251-MG cells labeled with GFP. Apoptosis was recognized by treating the fixed permeabilized cells with terminal deoxynucleotidyl transferase followed by treatment with TMR Red to label the DNA strand breaks. Double-labeled nuclei appear yellow in the merged image. (B) Parental, Inv, and Core cells were treated with 100 nM rapamycin for 72 hours and assayed for apoptosis. Four images were recorded per chamber in both green and red channels with a Zeiss Axiovert 200M inverted microscope, and the number of red cells (apoptotic) was calculated from the total number of cells (green cells; graph in Figure 4*D*). (C) Parental, Inv, and Core cells were treated with the indicated doses of rapamycin for 72 hours. Cells were lysed and analyzed by immunoblot analysis with antibodies for P-$p70^{S6K}$, a downstream target of mTORC1, and P-Akt (Ser473), target of mTORC2. Note efficient inhibition of $p70^{S6K}$ phosphorylation by rapamycin treatment. In contrast, Akt phosphorylation was increased in the same conditions. Note also higher basal Akt phosphorylation of Inv cells compared with Core cells.


A
GFP-labeled Parental U251-MG
DNaseI
–
+
TMR Red
Merge
B
Parental
Inv
Core
TMR Red
Merge
C
Parental
Inv
Core
Rapamycin (nM) 0 30 100 0 30 100 0 30 100
P-p70S6K
p70S6K
P-Akt (S473)
Akt
GAPDH

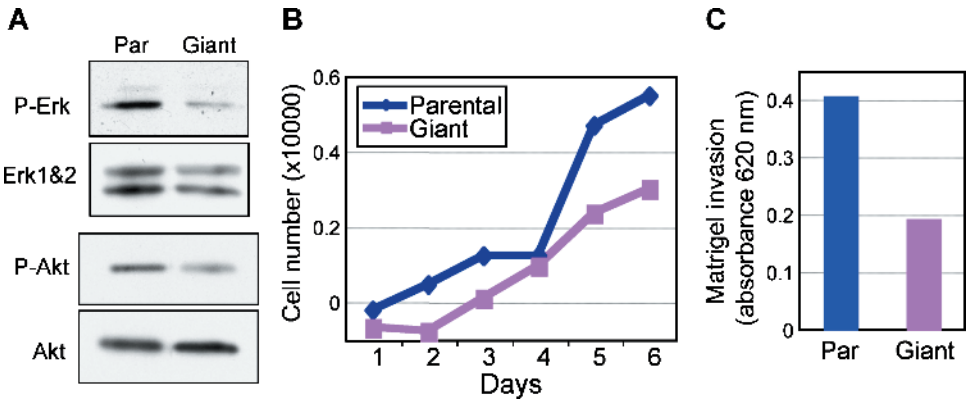


Figure W4. Reduced signaling, proliferation, and invasion of giant cells in comparison to parental U251 cells. (A) Western blot analysis of protein extracts from parental (Par) and giant cells with antibodies against phosphorylated Erk and Akt (S473) and control total Erk1 and 2 and Akt. (B) MTT assay of parental and giant cells showing proliferation during a 6-day period. (C) Matrigel invasion assay showing very reduced invasion of giant cells compared with parental (Par) cells.