Chioni and Grose, http://www.jcb.org/cgi/content/full/jcb.201108077/DC1

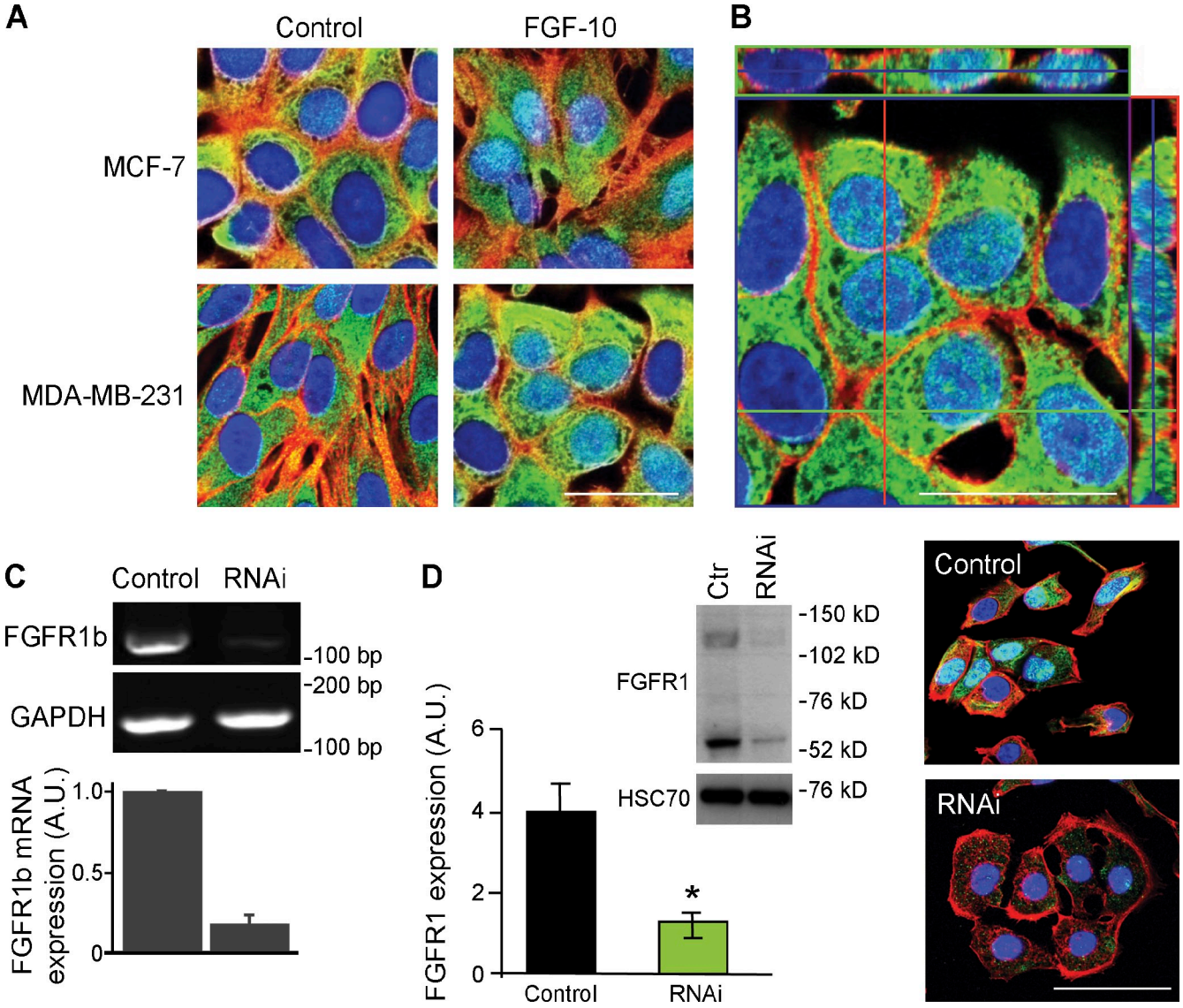


Figure S1. **Validation of FGFR1 antibody specificity.** (A and B) An anti-FGFR1 antibody (Santa Cruz Biotechnology, Inc.) specific for the C terminus of human FGFR1 (same region but different epitope to the anti-FGFR1 obtained from Abcam) showed similar results to the Abcam antibody (Fig. 3 H). 1-h stimulation with FGF-10 gave increased nuclear FGFR1 (green) staining in both MCF-7 and MDA-MB-231 cells. The green, purple, and red boxes represent the x-z and y-z scan perspectives from the confocal z stack. (C) MCF-7 and MDA-MB-231 cells, treated with a scrambled RNAi control (Ctr) or FGFR1-specific RNAi (RNAi), showed clear reduction in FGFR1 mRNA expression, as shown in a semiquantitative gel and by real-time RT-PCR (bars). (D) This knockdown of expression was clear also at the protein level, with MCF-7 cells showing clear staining for FGFR1 (green; Abcam antibody), in contrast to the absence of staining in cells treated with FGFR1 RNAi. Cells were costained for tubulin (red). Statistical significance was analyzed using Student's *t* test; *, $P < 0.05$. Bars, 50 µm. Error bars show means ± SEM. A.U., arbitrary unit.

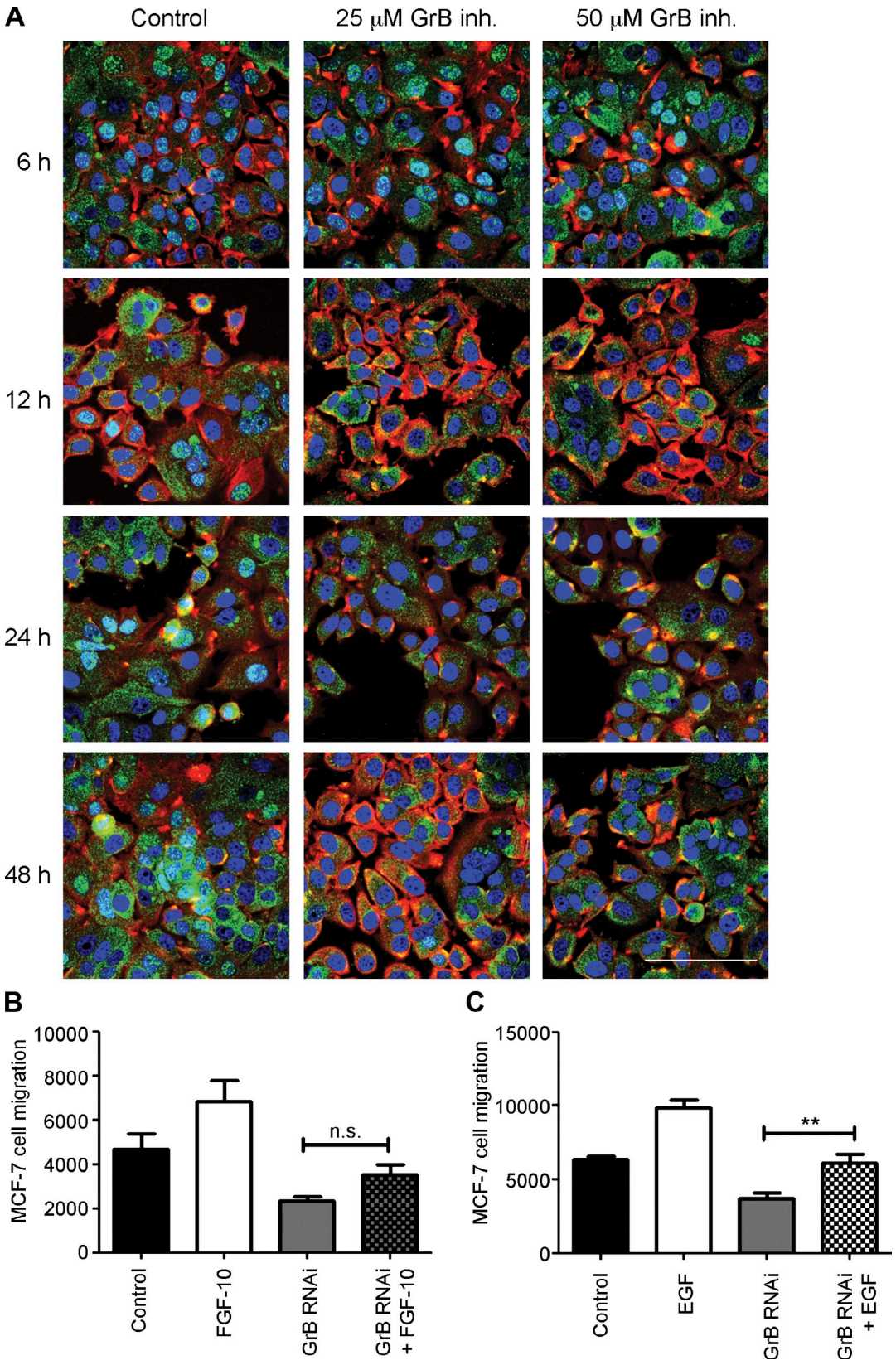


Figure S2. **GrB inhibitor treatment blocked FGFR1 nuclear localization, and RNAi-mediated knockdown of GrB reduced migration in MCF-7 cells.** (A) MCF-7 cells were treated with 25 and 50 µM GrB inhibitor (inh.) for 6, 12, 24, and 48 h. There was no change in nuclear FGFR1 localization after 6-h treatment. However, 12-h treatment with 25 µM inhibitor was sufficient to block nuclear FGFR1 localization. This effect was sustained after 24 and 48 h of treatment. Bar, 100 µm. (B) Transwell migration assays with MCF-7 cells showed that knockdown of GrB with RNAi (48 h after transfection) reduced the promigratory effect of FGF-10. (C) Furthermore, cells were still able to migrate in response to EGF stimulation. **, $P \leq 0.01$ (Student's *t* test). Error bars show means ± SEM.

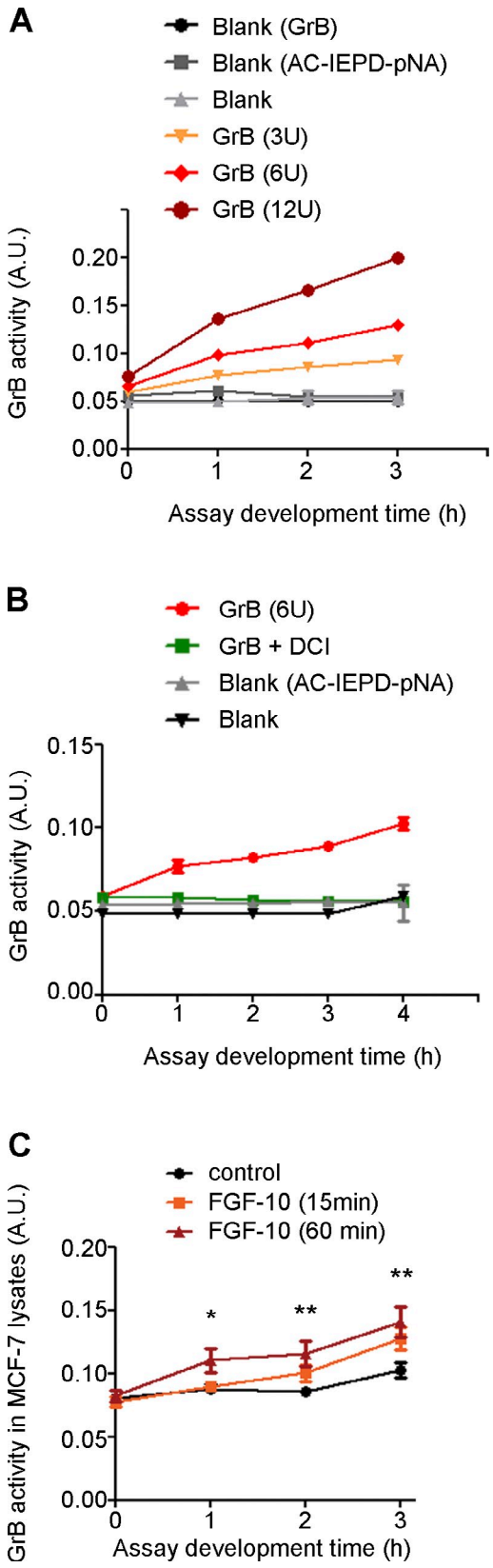


Figure S3. **Recombinant GrB activity assay.** Before assaying GrB activity in cell lysates, recombinant GrB was used to confirm that cleavage of the Ac-IEPD-pNA substrate could be detected. (A) Increasing the concentration of recombinant GrB in the assay reaction mix resulted in markedly increased signal (read at 405 nm; A.U., arbitrary units), which increased over incubation time. Signal from all the negative controls (buffer alone [blank]; substrate plus buffer [AC-IEPD-pNA]; and GrB plus buffer in the absence of substrate [GrB]) remained the same as at baseline throughout the course of the assay. (B) Recombinant GrB activity was inhibited by the addition of 50 μM DCI inhibitor to the reaction mix. (C) GrB activity assay with MCF-7 cell lysates with or without FGF-10/heparin treatment (100 ng/ml and 7.5 μg/ml, respectively). Activity was measured at 405-nm absorbance at hourly intervals after the addition of 200 μM Ac-IEPD-pNA substrate. FGF-10 treatment significantly increased GrB activity in MCF-7 cells. *, $P \leq 0.05$; **, $P \leq 0.01$ (Student's *t* test). Error bars show means ± SEM.

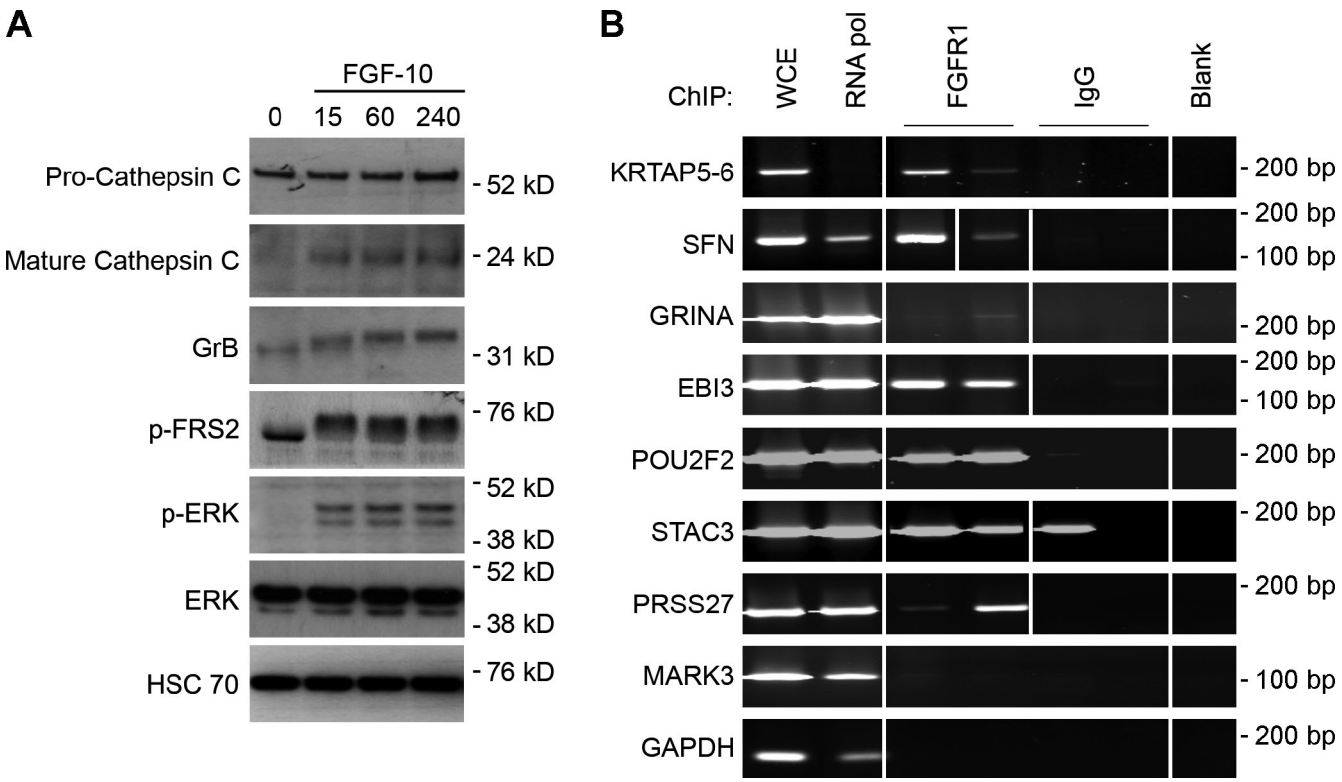


Figure S4. **Effect of FGF-10 stimulation on Cathepsin C and GrB protein levels and validation of ChIP on chip results by independent ChIP.** (A) MCF-7 cells were stimulated with 100 ng/ml FGF-10 in the presence of 7.5 µg/ml heparin in serum-free media for 15, 30, and 60 min. Cell lysates were prepared and Western blotting. Stimulation with FGF-10 increased levels of pro– and mature Cathepsin C as well as GrB. As expected, FGF-10 stimulation also activated the FRS2–ERK pathway. (B) Eight potential FGFR1 target genes were identified by ChIP on chip screening using an FGFR1 antibody (Santa Cruz Biotechnology, Inc.) on chromatin prepared from MCF-7 cells. PCR primers were designed using sequences guided by the chip analysis. Whole-cell extract (WCE; input before ChIP) as well as a sample immunoprecipitated with RNA polymerase II antibody was used as a positive control. Two samples chromatin immunoprecipitated with FGFR1 or IgG control antibodies are shown for each gene. *GRINA* binding was very low, and *MARK3* showed no amplification; hence, we did not investigate it further. *STAC3* showed strong binding; however, one of the five IgG samples also amplified the gene. Nevertheless, we carried on investigating *STAC3*. Primers for the promoter region of *GAPDH* were used as a negative control for the samples immunoprecipitated with FGFR1 and IgG.

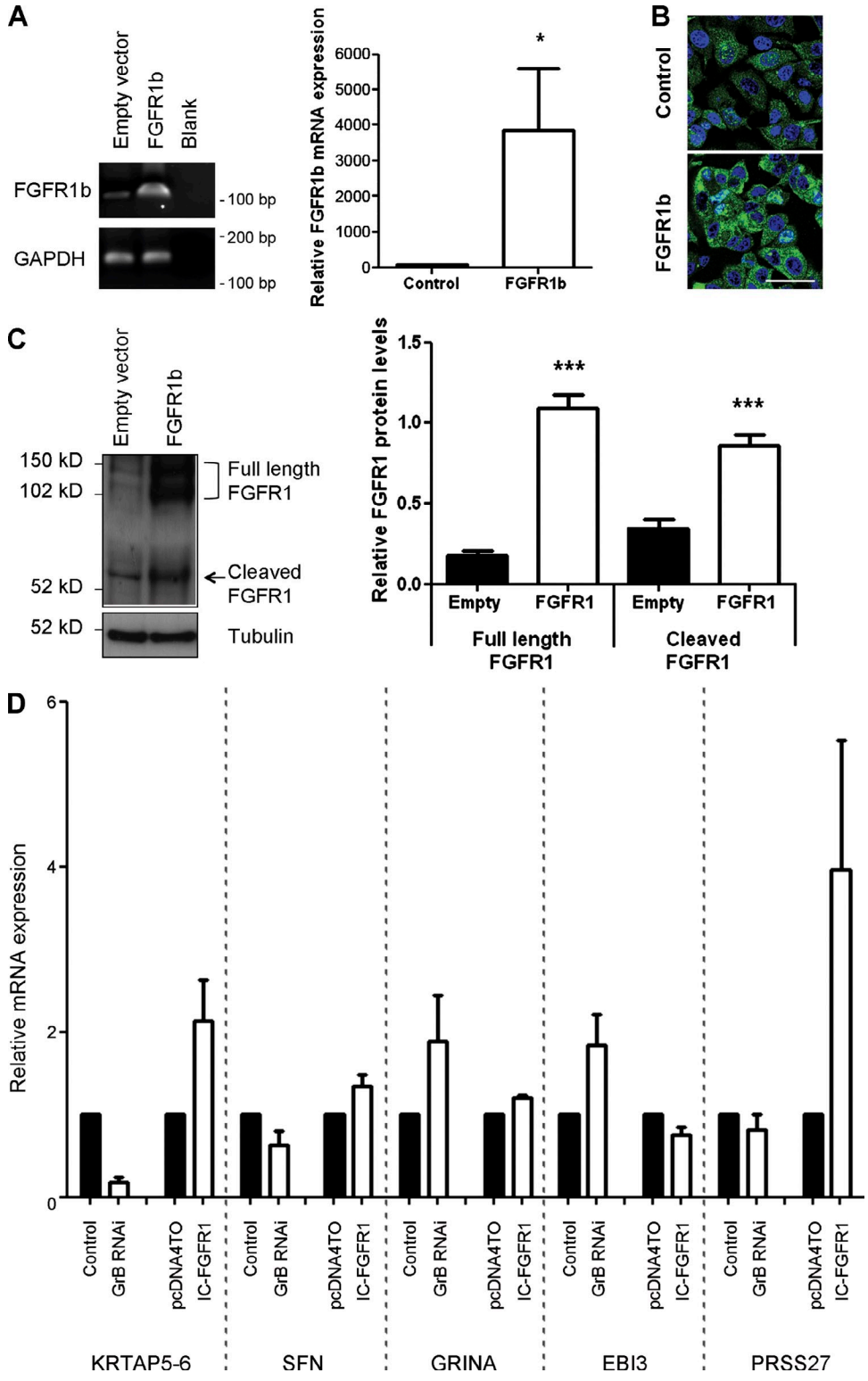
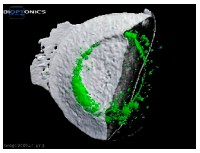


Figure S5. **Successful overexpression of FGFR1b and mRNA expression levels of FGFR1 target genes after RNAi-mediated knockdown of GrB or transient transfection of IC-FGFR1.** (A–C) MCF-7 cells were transfected transiently with full-length human FGFR1b. (A) Real-time PCR revealed a significant increase in the FGFR1b mRNA level (FGFR1 expression was normalized with both GAPDH and HPRT). *, $P \leq 0.05$. (B and C) Immunofluorescence staining and Western blotting with an anti-FGFR1 antibody also showed that there was a significant increase in FGFR1b protein level (both full-length and cleaved FGFR1; sixfold and 2.5-fold increase, respectively) 48 h after transfection. Statistical significance was analyzed by Student's *t* test (***, $P < 0.001$). Bar, 25 µm. (D) MCF-7 cells were treated with RNAi to GrB (72 h) or transiently transfected (48 h) with an IC-FGFR1 construct. Real-time PCR revealed changes in the expression levels of KRTAP5-6, SFN, GRINA, EBI3, and PRSS27 FGFR1 target genes after treatment with GrB RNAi or IC-FGFR1. Real-time PCR was analyzed using the $2^{-\Delta\Delta Ct}$ method and using expression of HPRT and GAPDH as housekeeping genes for normalization. Similar results were observed with both housekeeping genes. Data presented in the figure are normalized with HPRT. $n \geq 3$ independent experiments in triplicate. Error bars show means ± SEM.



Video 1. **Optical projection tomography of a 3D organotypic breast cancer model.** MDA-MB-231 cells were seeded onto a collagen Matrigel stroma containing embedded fibroblasts and cultured for 10 d before fixation. They were stained with an antibody to the neonatal Nav1.5 voltage-gated sodium channel (green), a marker for metastatic cells (Chioni et al., 2005), and the images were acquired by Bioptonics (Medical Research Council).

Reference

Chioni, A.M., S.P. Fraser, F. Pani, P. Foran, G.P. Wilkin, J.K. Diss, and M.B. Djamgoz. 2005. A novel polyclonal antibody specific for the Na(v)1.5 voltage-gated Na(+) channel 'neonatal' splice form. *J. Neurosci. Methods*. 147:88–98. http://dx.doi.org/10.1016/j.jneumeth.2005.03.010