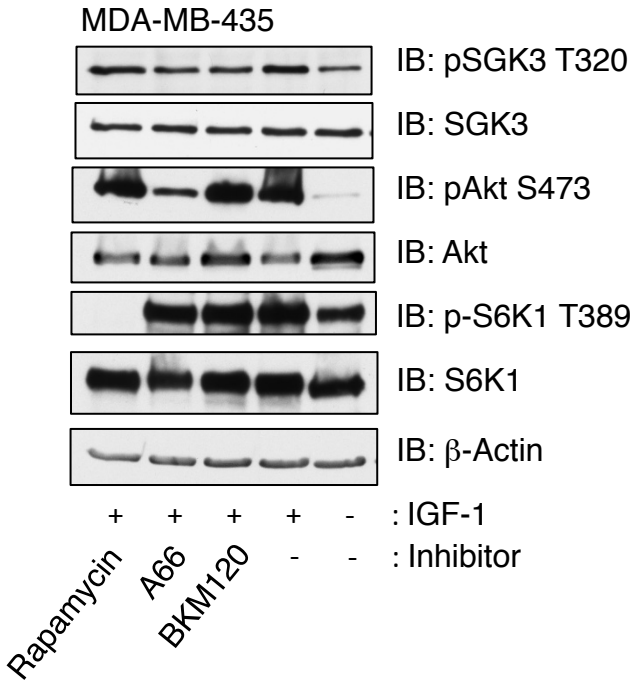

Fig. S1 related to Fig. 1D:

MDA-MB-435 cells were serum-starved for 24 hr, then pretreated with the indicated inhibitors, rapamycin (100nM), A66 (1μM) or BKM120 (1μM) for 20 min, then stimulated with IGF-1 for a further 20 min. Whole cell lysates (WCL) were immunoblotted with the indicated antibodies.

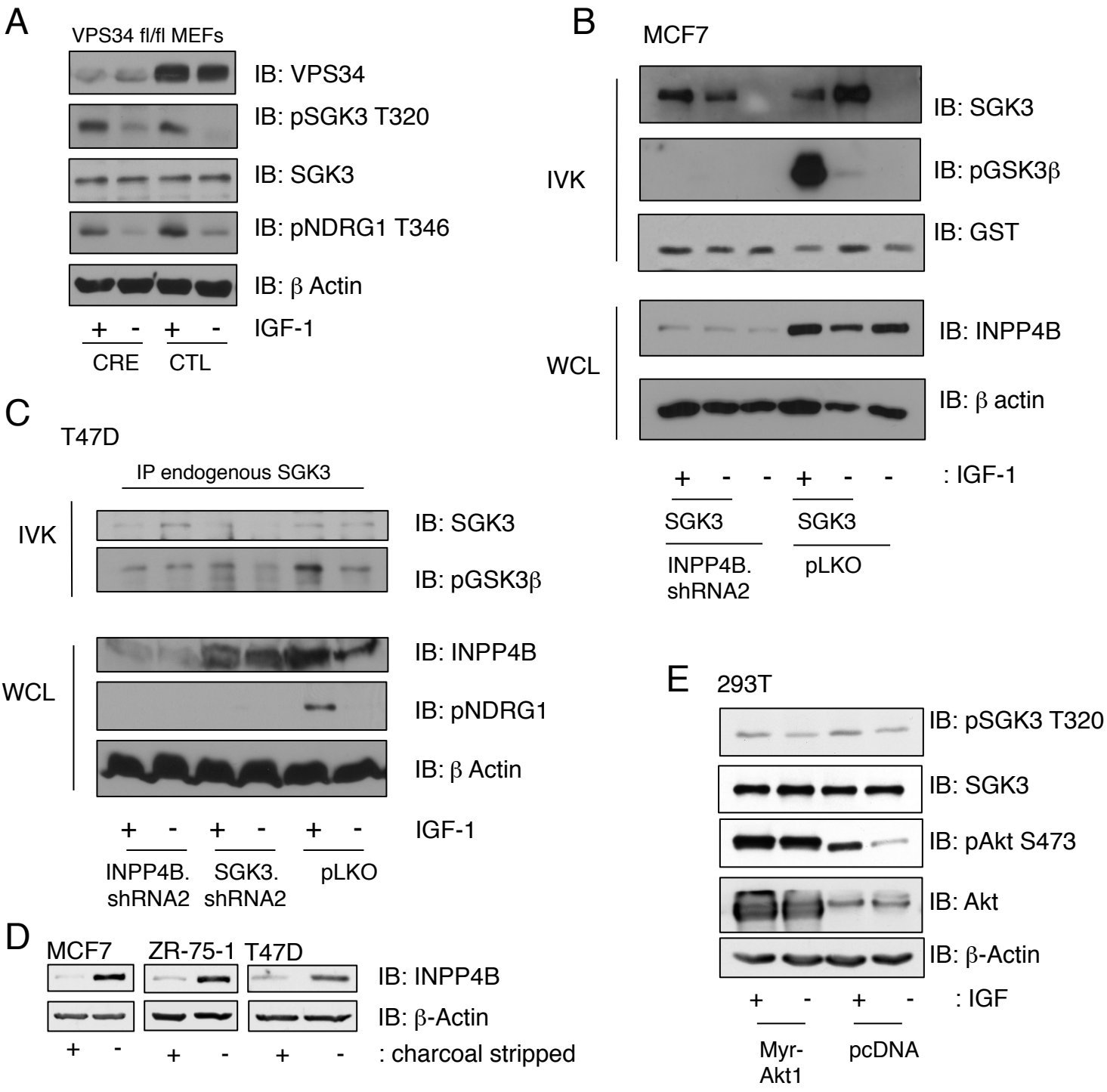

Fig. S2 related to Fig. 2:

(A) VPS34 fl/fl MEFs were infected with control (CTL) or Cre adenovirus. Control and VPS34-null MEFs were serum-starved for 18 hr and stimulated for 20 min with IGF-1. Whole cell lysates were immunoblotted with the indicated antibodies.

(B) MCF7 cells were infected with INPP4B.shRNA2 prior to transfection with SGK3-GST. Cells were serum-starved for 18 hr before stimulation with IGF-1 for 20 min. Glutathione agarose beads were used to isolate SGK3 from cell lysates, and incubated with GST-GSK3β in an *in vitro* kinase (IVK) assay.

(C) T47D cells were infected with SGK3.shRNA2 and INPP4B.shRNA2 prior to serum-starvation for 18 hr prior to IGF-1 stimulation for 20 min. SGK3 antibody was used to immunoprecipitate SGK3 for 2 hr. An in vitro kinase (IVK) assay was then performed with isolated SGK3 and GST-GSK3β peptide. Whole cell lysates (WCL) were immunoblotted with the indicated antibodies.

(D) MCF7, ZR-75-1, and T47D cells were grown in normal or charcoal stripped media for 5 days and immunoblotted as indicated.

(E) 293T cells were transfected with myristolated-Akt1 or control pcDNA prior to 24 hr serum-starvation and stimulation with IGF-1 for 20 min. Cell lysates were immunoblotted as indicated.

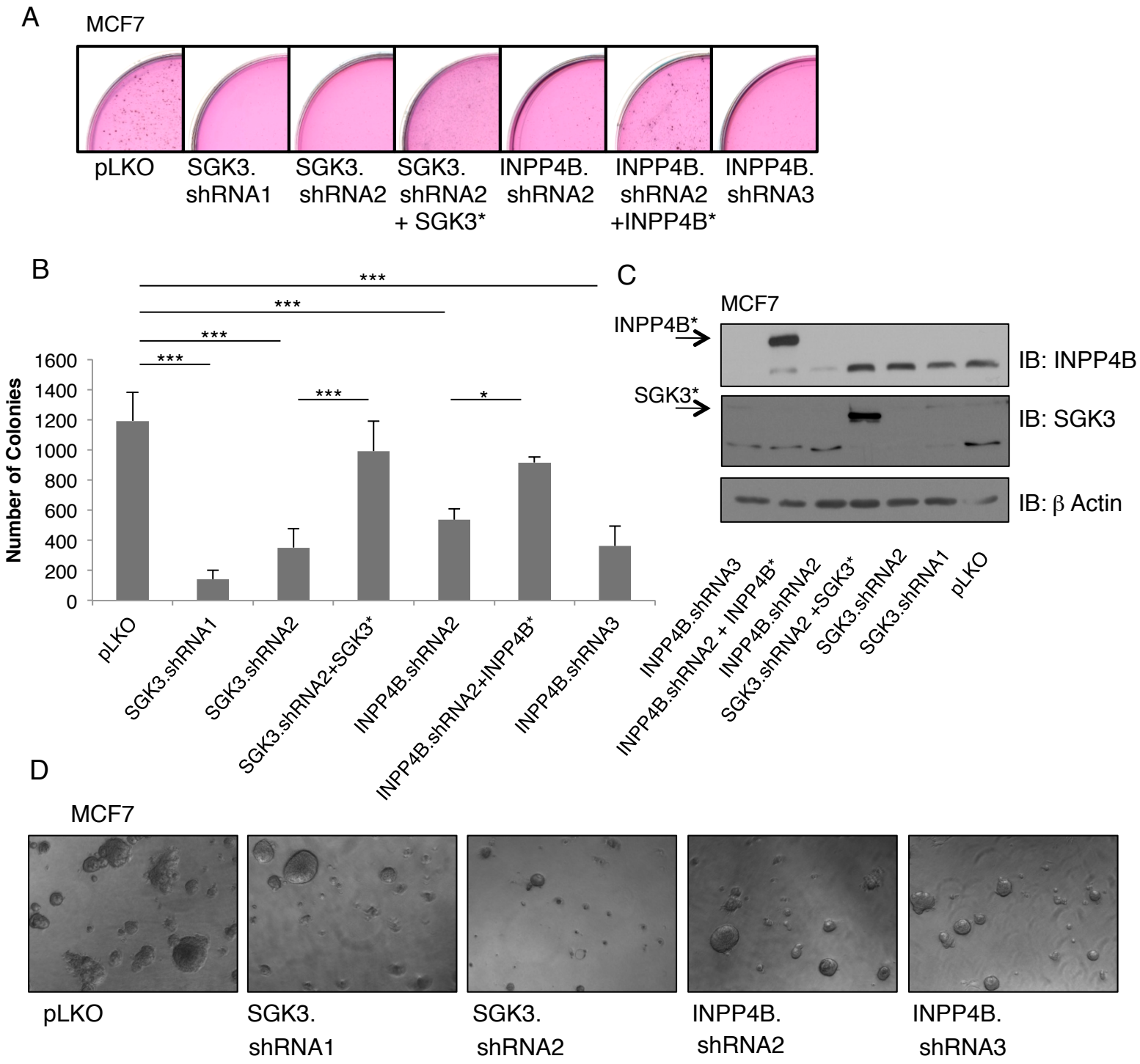

Fig. S3 related to Fig. 3 and Fig. 4:

(A) MCF7 cells infected with pLKO vector control, or two distinct INPP4B and SGK3 shRNA lentiviral vectors and grown in agar/growth media for 28 days. Additional conditions include infection of INPP4B (INPP4B*) and SGK3 (SGK*) cDNA rescue virus. Representative images of culture dishes used for the analysis in (B) and (C) are shown. Results are representative of at least 3 independent experiments.

(B) Relative number of colonies in soft agar obtained in (A) was quantitated using MatLab. Error bars represent standard deviation.

(C) Immunoblot analysis of cell lysates from MCF7 conditions in (A) prior to plating.

(D) MCF7 cells were infected with control pLKO or one of two distinct SGK3 or INPP4B shRNA lentiviruses and grown for 8 days in 3D Matrigel/culture media. Representative spheroid images for each condition are shown at 4X magnification.

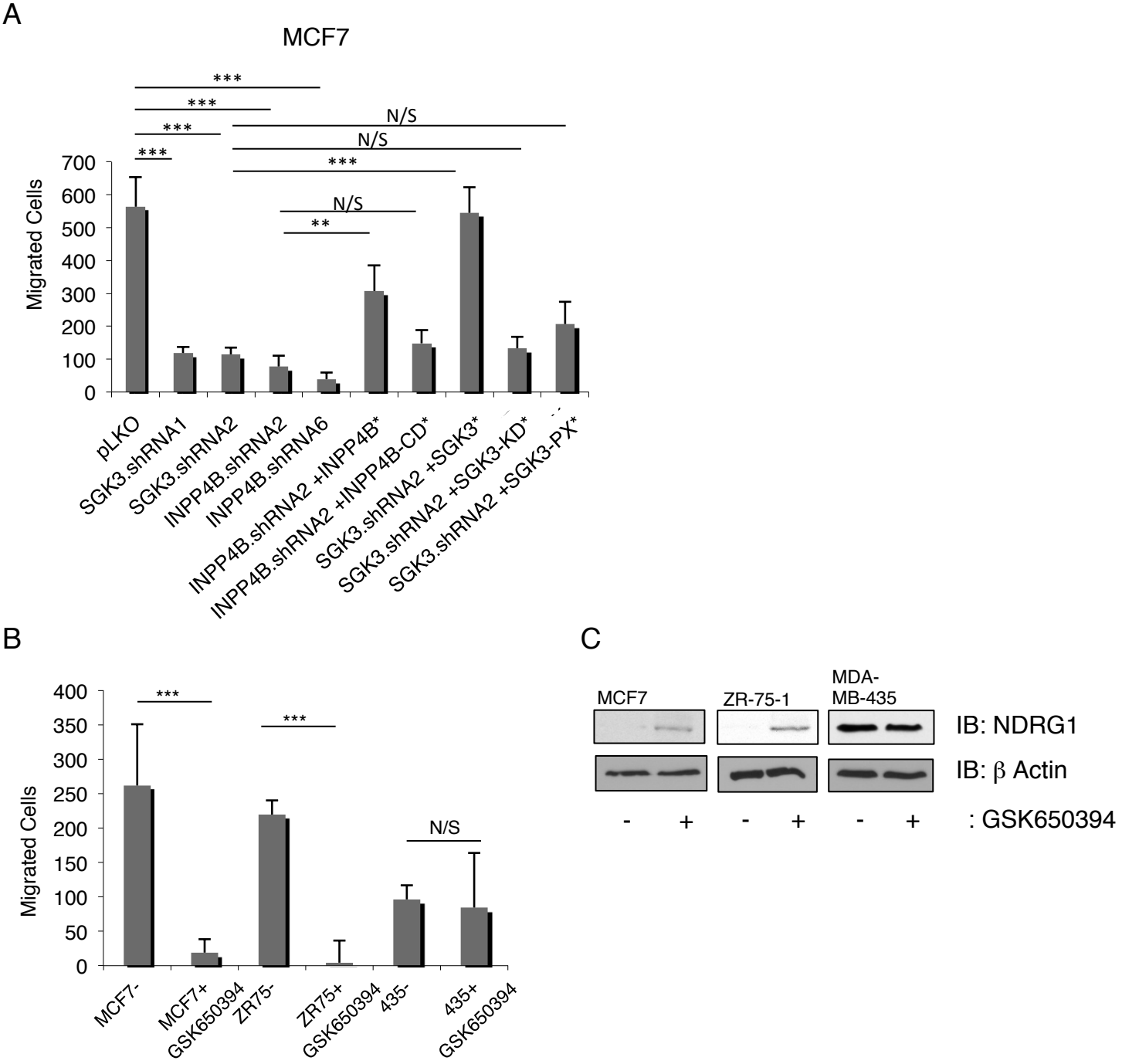

Fig. S4 related to Fig. 5:

(A) MCF7 cells infected with pLKO vector control, or two distinct INPP4B and SGK3 shRNA lentiviral vectors with additional infection of INPP4B (INPP4B*) and SGK3 (SGK*) cDNA rescue viruses and their respective catalytically inactivating mutants. Cells were migrated for 24 hr. Error bars represent standard deviation.

(B) MCF7, ZR-75-1, and MDA-MB-435 (435) cells were pre-treated 1 hr with with GSK650394 prior to assay. Cells were migrated for 24, 18, and 4 hrours respectively, with SGK inhibitor treatment at 10 hr intervals. Error bars represent standard deviation.

(C) Immunoblot analysis of cell lysates collected in parallel with (B).

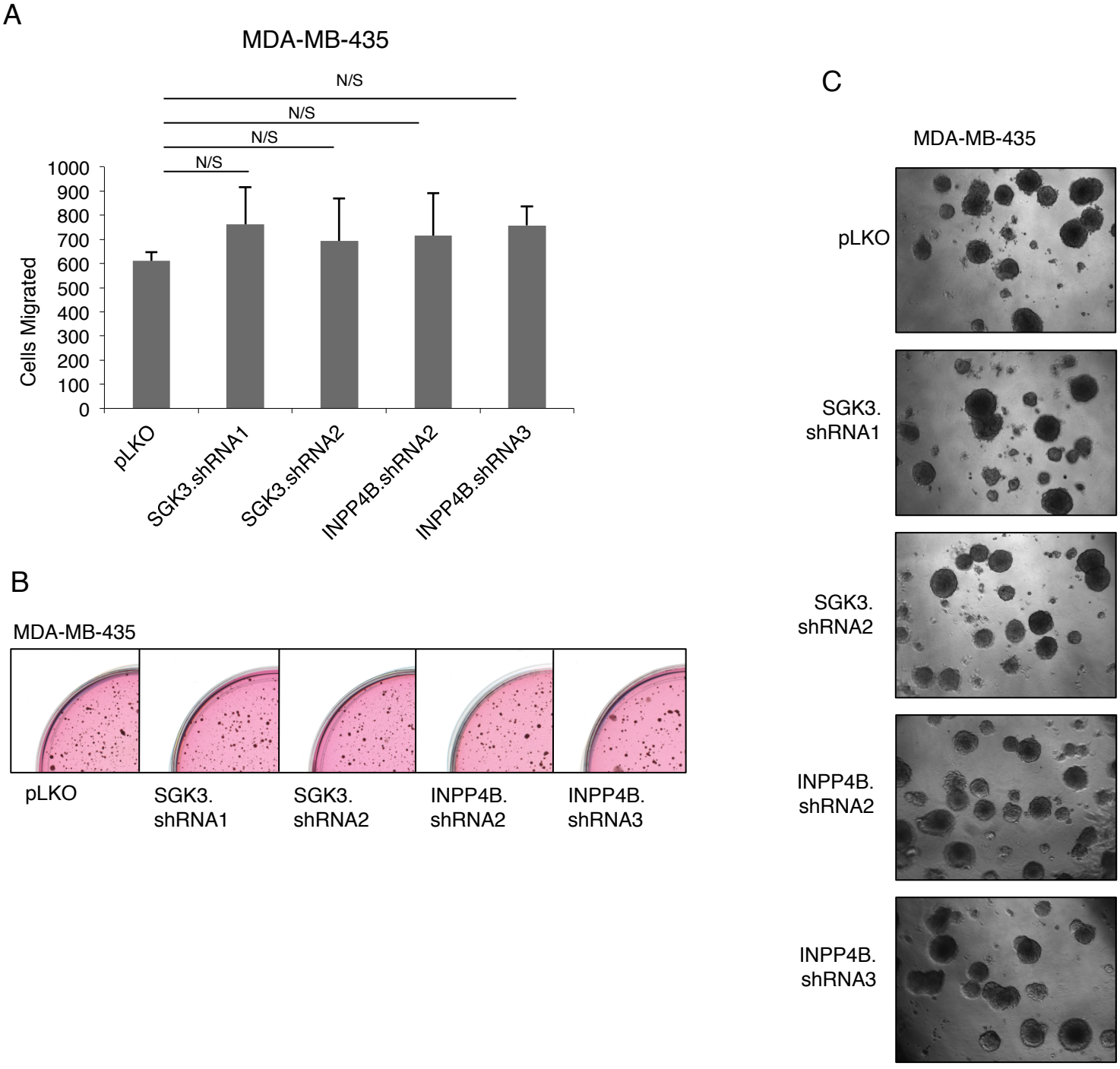

Fig. S5 related to Fig.1, Fig. 3, Fig. 4 and Fig. 5:

(A) MDA-MB-435 cells were infected with control pLKO or one of two distinct SGK3 or INPP4B shRNA lentiviruses. Cells were migrated for 4 hours. Error bars represent standard deviation.

(B) Representative images of culture dishes of MDA-MB-435 cells infected with pLKO vector control, or two distinct INPP4B and SGK3 shRNA lentiviral vectors grown in agar/growth media for 28 days to assay anchorage-independent growth.

(C) MDA-MB-435 cells generated in (A) were grown for 8 days in 3D Matrigel/culture media. Representative spheroid images for each condition are shown at 4X magnification.

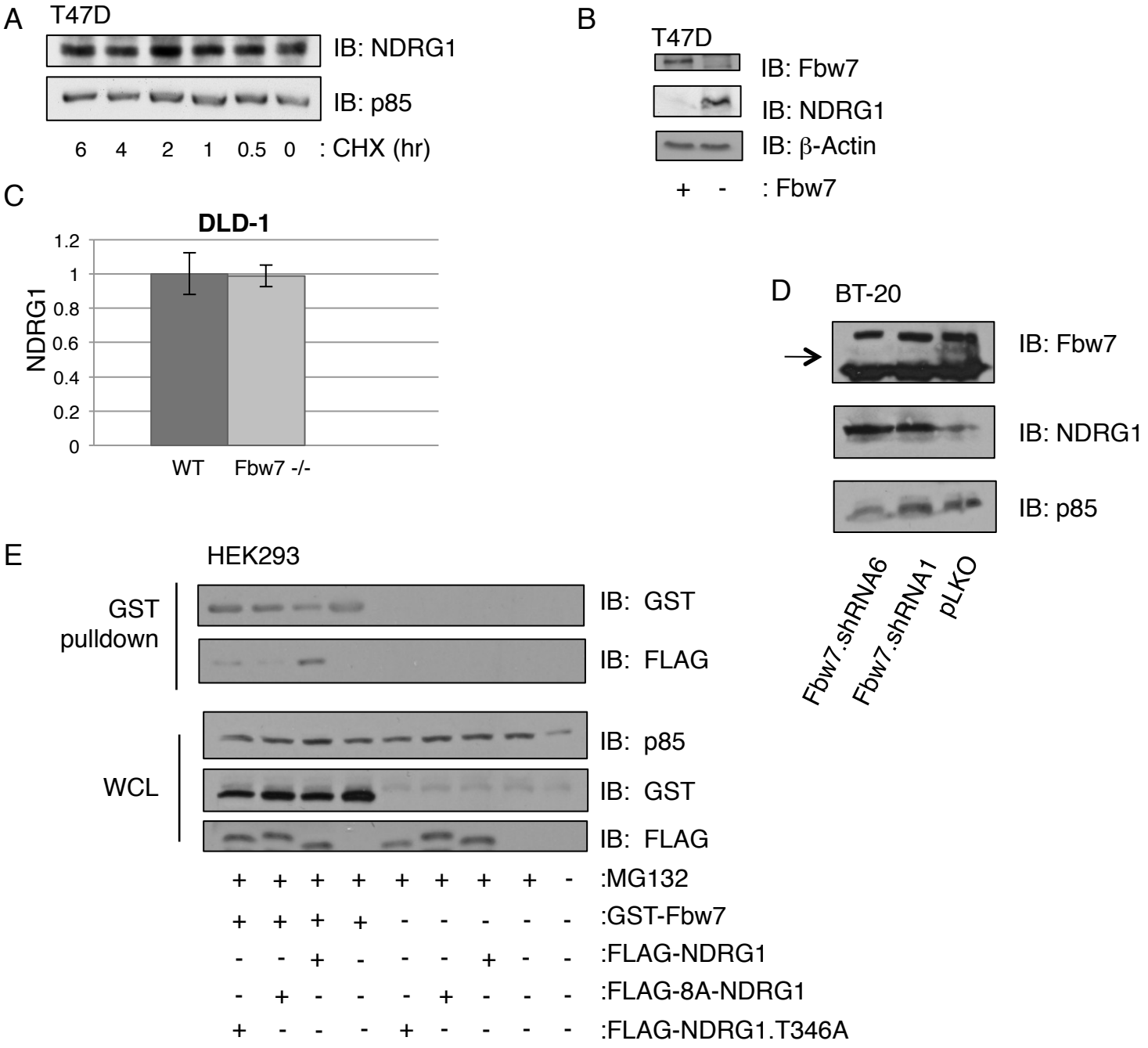

Fig. S6 related to Fig. 6:

(A) T47D cells were treated with cycloheximide or control DMSO for the indicated times and cell lysates immunoblotted with anti-NDRG1 and anti-p85.

(B) T47D cells were infected with pLENTI-Fbw7 or control virus and immunoblotted with the indicated antibodies.

(C) Quantitative Q-PCR analysis to determine the relative mRNA expression of *NDRG1* in wild-type or *Fbw7*-/- DLD-1 cell lines. Error bars represent standard deviation.

(D) BT-20 cells were infected with two distinct Fbw7 shRNA or control pLKO, and cell lysates immunoblotted with the indicated antibodies.

(E) HEK293 transfected with GST-Fbw7 and NDRG1, 8A-NDRG1, or NDRG1.T346A and treated with MG132 for 18 hr. Glutathione agarose was used to isolate Fbw7 and associated proteins. Pulldown and whole cell lysates (WCL) were immunoblotted with the indicated antibodies.

Supplemental Methods

Plasmids

SGK3-GST was provided by Sandra Marmiroli (University of Modena and Reggio Emilia). FLAG- p110α/ pCMV2 (#16643) FLAG-p110α H1047R/ pCMV2 (#16639) and FLAG-p110α E545K/ pCMV2 (#16642) plasmids were obtained from Addgene. HA-Fbw7 and HA-GSK3 were described previously (Wei et al., 2005). NDRG1-FLAG was provided by Sushant Kachhap (Johns Hopkins Medical Institute) (Kachhap et al., 2007). pEAK-FLAG/INPP4B was provided by Lewis Cantley (Weill Cornell Medical College). FLAG-VPS34 was provided by Junying Yuan (Harvard Medical School). pHAGE-N-eGFP was provided by Wade Harper (Harvard Medical School). His-Ub was previously published (Yang et al., 2009). Additional information on plasmids and shRNA vectors, including oligonucleotide sequences, can be found in the Supplemental Information online.

Vector Construction

FLAG-INPP4B.C842A was generated by site- directed mutagenesis with the following primers: sense, 5'-AATGGTATTCGTTTCACCTGTGCTAAAAGTGCCAAAGACAGGAC -3'; anti-sense: 5'- GTCCTGTCTTTGGCACTTTTAGCACAGGTGAAACGAATACCATT-3'. FLAG-1A-NDRG1 was constructed by site- directed mutagenesis with the

following primers: 346A-sense: 5'-ACCCGCAGCCGCTCCCACGCCAGCGAGGGCA-3'; 346A-anti-sense, 5'-TGCCCTCGCTGGCGTGGGAGCGGCTGCGGGT-3'. FLAG-8A-NDRG1 was generated using site- directed mutagenesis and the following primers: 328A/ 330A-sense, 5' TGCGGTCCCGCGCAGCCGCTGGTTCCAGC 3'; 328A/ 330A-anti-sense, 5' GCTGGAACCAGCGGCTGCGCGGGACCGCA 3'; 342A –sense, 5' GATGGCACCCGCGCCCGCTCCCACGC 3'; 342A-anti-sense, 5' GCGTGGGAGCGGGCGCGGGTGCCATC 3'; 352A/362A-sense, 5' CGAAGCCGCTCCCACGCCAGCGAGGGCACCCGCAGCCGCTC 3'; 352A/362A-anti-sense, 5' CTCGCTGGCGTGCGAGCGGCTGCGGGTGCCCTCGCTGGCGTGGGAGC 3'; 346A-sense: 5' ACCCGCAGCCGCTCCCACGCCAGCGAGGGCA 3'; 346A-anti-sense, 5' TGCCCTCGCTGGCGTGGGAGCGGCTGCGGGT 3'; 356A/366A-sense, 5'AAGCCGCTCCCACGCCAGCGAGGGCACCCGCAGCCGCTCGCAC 3'; 356A/ 366A-antisense, 5' CTCGCTGGCGTGCGAGCGGCTGCGGGTGCCCTCGCTGGCGTGGGAGCGG CTT 3'. shRNA-resistant SGK3 and INPP4B were made by Gateway Cloning (Life Technologies) into lentiviral pHAGE-N-eGFP vector followed by site-directed mutagenesis. SGK3shRNA2-resistant SGK3-GFP (SGK3*) was generated by site-directed mutagenesis with the following primers: sense, 5' AGATTTTATTAAACAAAGACGAGCAGGACTGAATGAGTTTATTCAGAACCTA GTTAGGTATCCAGAACTT 3': anti-sense, 5'

AAGTTCTGGATACCTAACTAGGTTCTGAATAAACTCATTCAGTCCTGCTCGTC TTTGTTTAATAAAATCT 3'. INPP4BshRNA2-resistant INPP4B-GFP (INPP4B*) was generated using site-directed mutagenesis with the following primers: sense, 5'-TTCATCCTTGCATGCAAGGATCTCGTGGCACCAGTGAGGGATCGTAAACTGA ATACACTGGTGC-3': anti-sense, 5'-GCACCAGTGTATTCAGTTTACGATCCCTCACTGGTGCCACGAGATCCTTGCA TGCAAGGATGAA-3'. All point mutations were verified by DNA sequencing.

shRNA Vectors

shRNA-mediated silencing of Fbw7, INPP4B, SGK3, and Akt1 each required the synthesis of a set of oligonucleotides composed of a target shRNA sequence and its complement against each respective gene. Two hairpins were generated against each gene as follows: Fbw7.shRNA1-sense, 5'-AACCTTCTCTGGAGAGAGAAA-3'; Fbw7.shRNA1-anti-sense, 5'-TTTCTCTCTCCAGAGAAGGTT-3'; Fbw7.shRNA6-sense, 5'-CCAGAGACTGAAACCTGTCTA-3'; Fbw7.shRNA6-anti-sense, 5'-TAGACAGGTTTCAGTCTCTGG-3'; INPP4B.shRNA2-sense, 5'-CCGGCCATCTGAGTATCCCATCTATCTCGAGATAGATGGGATACTCAGATGG TTTTTG-3'; INPP4B.shRNA2-anti- sense, 5'-AATTCAAAAACCATCTGAGTATCCCATCTATCTCGAGATAGATGGGATACTCA GATGG-3'; INPP4B.shRNA6-sense, 5'-

CCGGAGATACTCCAGCACCGAAATTCTCGAGAATTTCGGTGCTGGAGTATTT TTTG-3’; INPP4B.shRNA6-anti-sense, 5’-AATTCAAAAAAGATACTCCAGCACCGAAATTCTCGAGAATTTCGGTGCTGGA GTATCT-3’; SGK3.shRNA1-sense, 5'-CCGGGCGAGACCCTAGTTAAGAGAACTCGAGTTCTCTTAACTAGGGTCTCG CTTTTT-3'; SGK3.shRNA1-anti-sense, 5’-AATTCAAAAAGCGAGACCCTAGTTAAGAGAACTCGAGTTCTCTTAACTAGGG TCTCGC-3’; SGK3.shRNA2-sense, 5’-CCGGGCAGGACTAAACGAATTCATTCTCGAGAATGAATTCGTTTAGTCCTGC TTTTT-3’; SGK3.shRNA2-anti-sense, 5’-AATTCAAAAAGCAGGACTAAACGAATTCATTCTCGAGAATGAATTCGTTTAGT CCTGC-3’; Akt1.shRNA, sense, 5′-CCGGGAGTTTGAGTACCTGAAGCTGCTCGAGCAGCTTCAGGTACTCAAACT CTTTT TG-3′; Akt1.shRNA, anti-sense, 5′-AATTCAAAAAGAGTTTGAGTACCTGAAGCTGCTCGAGCAGCTTCAGGTACTC AAACTC-3′

The primer sets were each annealed and ligated into pLKO. Lentiviral supernatants were produced by transfection of HEK293T cells with VSVG, Δ8.2, and control-PLKO, Fbw7, INPP4B, or SGK3 shRNA-containing vectors for 48 hr.

RT-PCR

After mixing the resulting cDNA template with NDRG1 (sense: 5′-CCTGAGATGGTAGAGGGTCTC-3′ and antisense: 5′-CCAATTTAGAATTGCATTCCACC-3′) or glyceraldehyde-3-phosphate dehydrogenase (sense: 5′-GCAAATTCCATGGCACCGT-3′ and antisense: 5′-TCGCCCCACTTGATTTTGG-3′) primers quantitative RT-PCR was performed using SYBR Green PCR master mix in an ABI Prism 7500 sequence detector (Applied Biosystems, Foster City, CA).