

SUPPLEMENTARY FIGURES

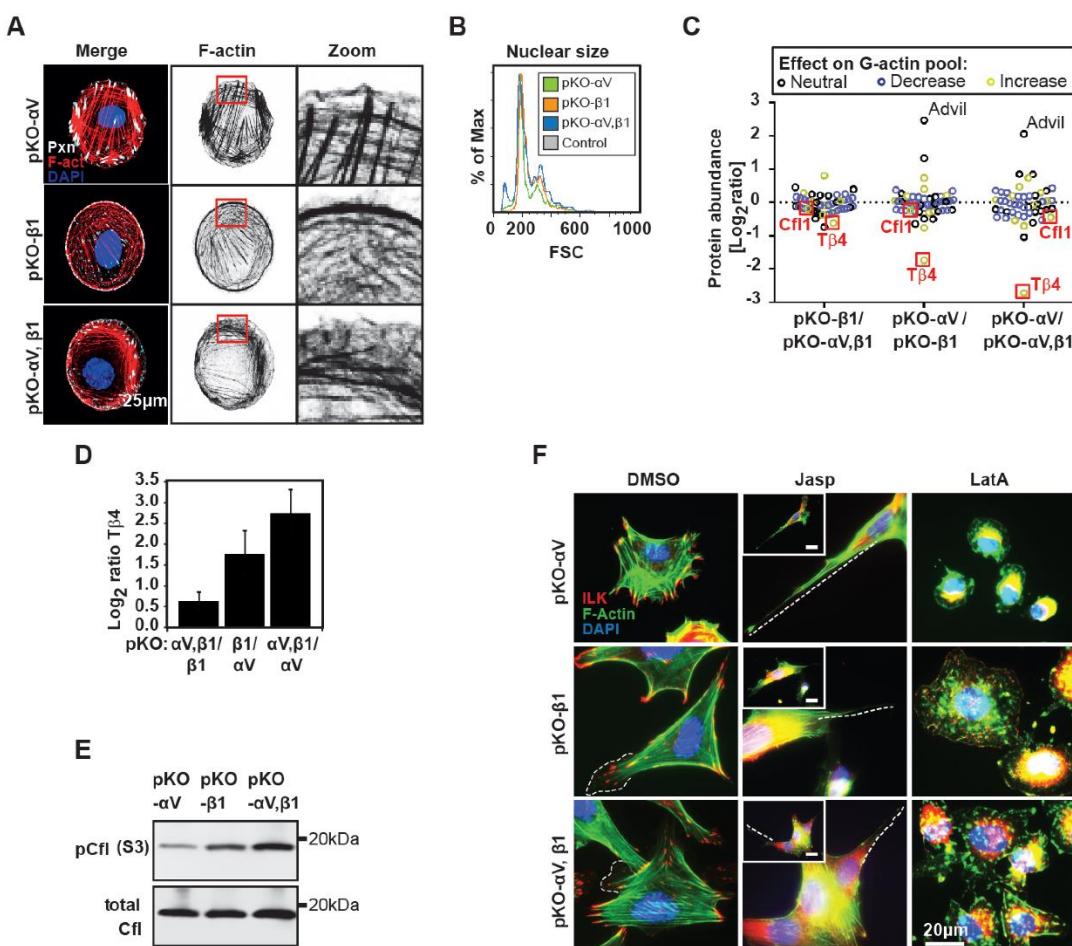


Figure S1. Characterisation of pKO- α V, pKO- β 1 and pKO- α V, β 1 cells. (A) Immunostaining of indicated cell types for Paxillin and F-actin plated for 90 minutes on circular FN-coated micropatterns. The merged images demonstrate an overlay of Paxillin, F-actin and nuclear (DAPI) staining. (B) FACS analysis of indicated cell lines upon propidium iodide staining indicates similar nuclear sizes. (C) Box plot of actin modifying proteins expressed in pKO- α V, pKO- β 1 and pKO- α V, β 1 cells (Schiller et al., 2013) classified according to their effect on G-actin pool: Neutral (black), decrease (blue), increase (yellow). Protein abundance is presented by Log₂ ratios. Thymosin β 4 (T β 4), Cofilin-1 (Cfl-1) and Advillin (Advil) are highlighted. (D) Log₂ ratio of T β 4 expression in indicated cell lines (n=3). (E) Western blot of Cofilin and phospho-Cofilin (Ser3) (representative western blot of 4 independent experiments shown). (F) Drug-induced effect on actin-cytoskeleton and cell morphology. Microscopy of indicated cells upon Latrunculin A (LatA) or Jasplakinolide (Jasp) treatment. The merged images demonstrate an overlay of ILK, F-actin and nuclear (DAPI) signal. Dashed lines highlight lamellipodia and long protrusions induced by the Jasp treatment.

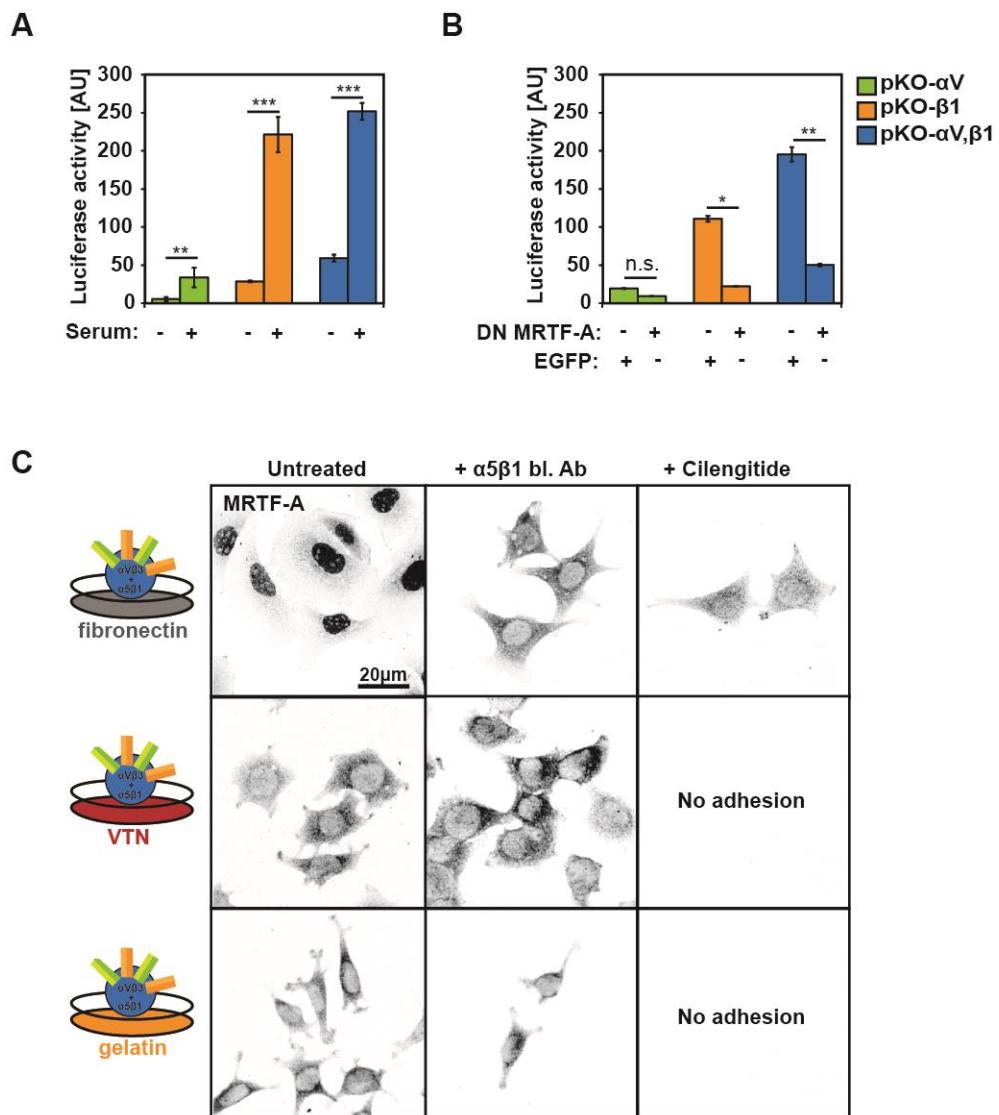


Figure S2. FN-binding integrins cooperate to induce MRTF/SRF activity. (A-B) MRTF/SRF-driven luciferase reporter activity in indicated cells plated on FN-coated cell culture dishes. Cells were either starved or treated with 40% FCS (A) or transfected with a dominant negative MRTF-A (DN MRTF-A) construct or an EGFP control vector (B). (C) pKO- α V, β 1 cells plated on fibronectin, VTN or gelatin and left untreated or treated with integrin- α 5 β 1 blocking antibodies or Cilengitide followed by immunostaining for MRTF-A. All error bars represent mean +/- s.e.m. All P-values were calculated using an unpaired Students-t-test; n.s., not significant; *P<0.05; **P<0.01; ***P<0.001.

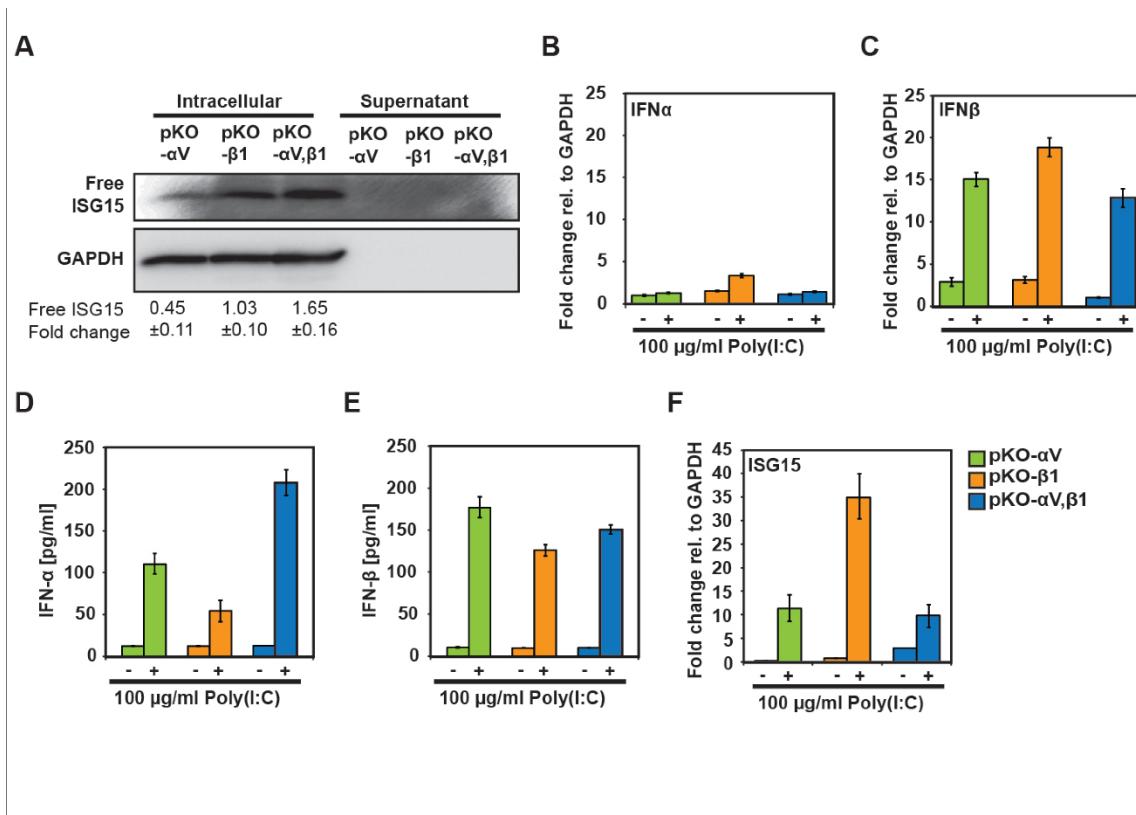


Figure S3. ISG15 expression in pKO-αV, pKO-β1 and pKO-αV,β1 cells. (A) Western blot analysis of ISG15 in total cell lysate or cell supernatants from indicated cells cultured for 72h on FN. GAPDH was used as a loading control. Densitometric quantification of western blots ($n>3$) is depicted as fold changes below the corresponding blot with +/- s.e.m. values. (B,C) qPCR analysis of Interferon α (IFN α ; B), Interferon β (IFN β ; C) levels upon 100µg/ml PolyI:C stimulation ($n=3$). (D,E) ELISA-based quantification of IFN α (D) or IFN β (E) protein from supernatants of indicated control cell cultures or cultures treated with PolyI:C. (F) qPCR analysis of *lsg15* mRNA levels upon 100µg/ml poly I:C stimulation ($n=3$). Error bars represent mean +/- s.e.m.

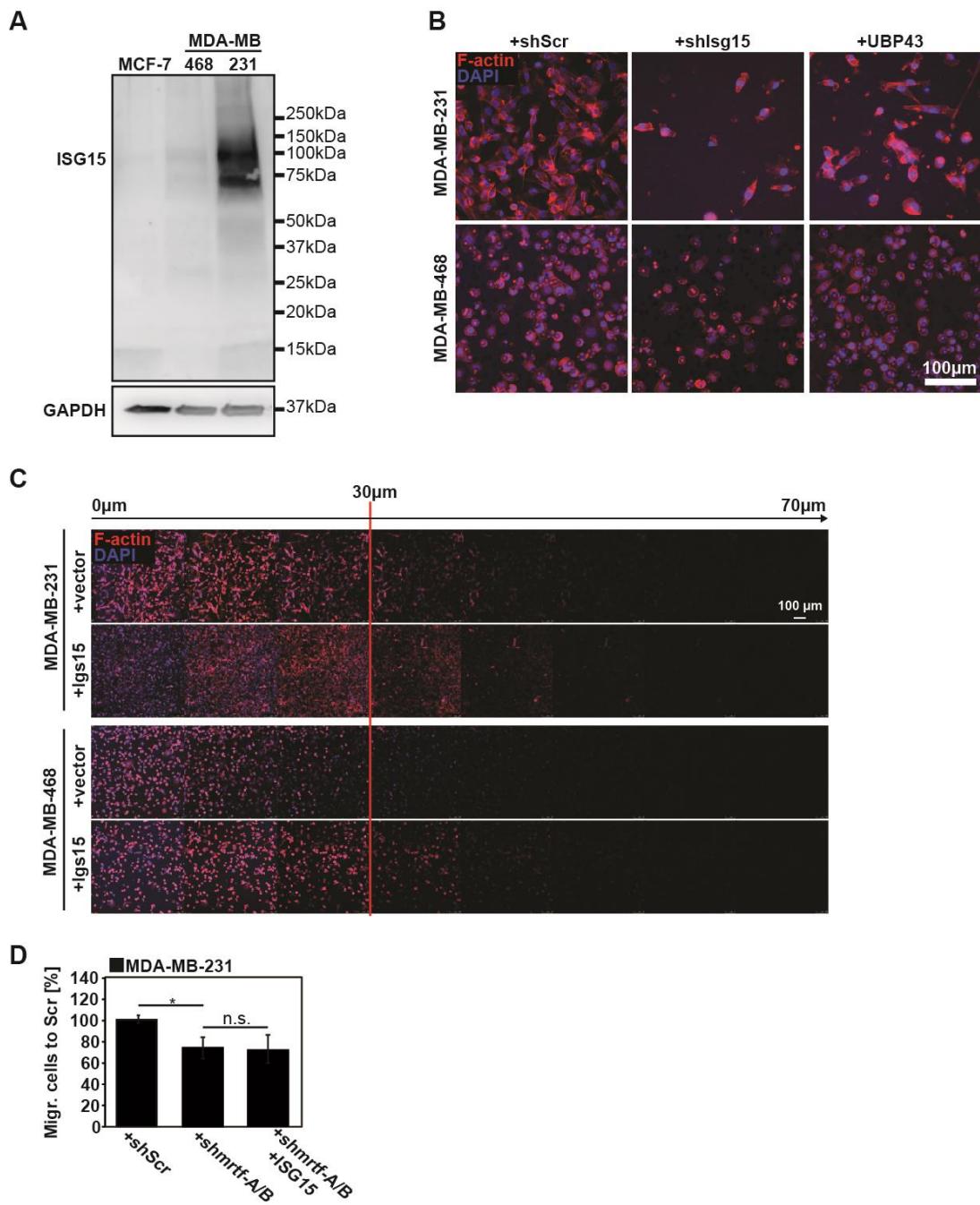


Figure S4. ISG15 in breast cancer cell migration and invasion. (A) Western blot analysis of MCF-7, MDA-MB-468 and MDA-MB-231 cell lysates with anti-ISG15 specific antibody. GAPDH was used to control loading. (B) Fibronectin-coated transwell cell migration assay with MDA-MB-231 and MDA-MB-468 cells expressing scrambled (shScr), specific Isg15 shRNAs, or overexpressing ISG15-specific UBP43 peptidase (n=4). Transmigrated cells were stained with Phalloidin-TRITC and DAPI. (C) Confocal microscopy analysis

of Matrigel invasion of MDA-MB-231 and MDA-MB-468 cells transduced with either lsg15 expression construct or an empty vector. Cells were stained for F-actin with Phalloidin-TRITC and nuclei were labelled with DAPI. Serial optical sections were captured at 10 μm interval and are presented as a sequence in which the individual optical sections are placed alongside one another, with increasing depth from left to right. (D) Quantification of MRTF-A/B depletion with and without ISG15 overexpression on fibronectin-coated transwell migration of MDA-MB-231 cells (n=4). All error bars represent mean +/- s.e.m. All P-values were calculated using an unpaired Students-t-test; n.s., not significant; *P<0.05.

Table S1. List of PCR oligos used in the study

Primer (Target) Name	Sequence
hISG15 fw	CGCAGATCACCCAGAAGATCG
hISG15 rev	TTCGTCGCATTGTCCACCA
hITGaV fw	GCTGTCGGAGATTCAATGGT
hITGaV rev	TCTGCTGCCAGTAAAATTGT
hITGb1 fw	CCTACTTCTGCACGATGTGATG
hITGb1 rev	CCTTGCTACGGTGGTTACATT
hMRTF-A1 fw	CAAACGGAAGATCGTCCCG
hMRTF-A1 rev	TTGAGGTCATCGGCTAGTCTG
hMRTF-A2 fw	ACTAGCCGATGACCTCAATGA
hMRTF-A2 rev	TTCACCTGGCCCACAATGATG
hSRF fw	CGAGATGGAGATCGGTATGGT
hSRF rev	GGGTCTTCTTACCCGGCTTG
hSRFb fw	CCGGCAAGGCAGTGATTCA
hSRFb rev	CTCATTCTCTGGTCTGTTGTGG
mGAPDH CHIP fw	CCCTGCTTATCCAGTCCTAGCTCAAGG
mGAPDH CHIP fw	CTCGGGAAAGCAGCATTCAAGGTCTCTGG
mGapdh fw	TCCTGCACCACCAACTGCTTAGC
mGapdh rev	TGGATGCAGGGATGATGTTCTGG
mIFNalpha fw	CTTCGTGTTGGTAGTGTGATGGT
mIFNalpha rev	GGGGATGATTCCAGCCGA
mIFNbta fw	CAGCTCCAAGAAAGGACGAAC
mIFNbta rev	GGCAGTGTAACTCTTCTGCAT
mISG15 CHIP fw	GAAGAC CCTATCAAGGAATGGA
mISG15 CHIP rev	TCCTTAATTCCAGGGGACCT
mISG15 fw A	GTGAGAGCAAGCAGCCAGAAG
mISG15 fw B	GACCTAAAGGTGAAGATGCTGG
mISG15 rev	ACCAACACTGGCTCTGGATG
mISG15 rev	ACCAACACTGGCTCTGGATG
mItgaV fw	CCGTGGACTTCTCGAGCC
mItgaV rev	CTGTTGAATCAA ACTCAATGGC
mItgb1 fw	CCTTGCTGCTGATTGGAAAC
mItgb1 rev	TGCAAAATCCGCCTGAGTAG
mItgb3 fw	CCACACGAGGCGTGAAC
mItgb3 rev	CTTCAGGTTACATCGGGGTGA
mMRTF-A fw	CCAGGACCGAGGACTATTG
mMRTF-A rev	CGAAGGAGGA ACTGTCTGCTA
mSRF fw	GGCCCGCTGAAGATCAAGAT
mSRF rev	CACATGGCCTGTCTCACTGG
mTln1 CHIP fw	TACTCCTTGAGCCCTGTC
mTln1 CHIP rev	CTACCAAGCTAACAGAGGCAGAG
mVCL CHIP fw	CTGGCGCTGCCTGAGGTGAGGATAT
mVCL CHIP rev	AGGGATTGCGACCGGATTCCCGAAC
mVcl fw	GAGGCTGA ACTGCTTCAATCA
mVcl rev	CCAGATTGACGAGGTGCCTA

h=human; m=murine

Table S2. List of antibodies used in this study

Antigen	Monoclonal/ Polyclonal	Species	Source	Cat.No.	Dilution			
					IF	IB	functional blocking	FACS
9EG7	mono	rat	Pharmingen	550531				1:200
Cofilin	poly	rabbit	Cell Signaling	3312		1:1000		
DNaseI-488		bovine	Life Technologies	D12371	1:300			
GAPDH	mono	mouse	Merck/Millipore	CS207795		1:1000		
Histone H3	mono	mouse	Cell Signaling	3638S		1:2000		
ILK	mono	rabbit	BD Transduction Laboratories	611803 clone 3	1:300			
Integrin $\alpha 5$ -PE	mono	mouse	Bioscience	12-4900-41				1:200
Integrin αV -FITC	mono	mouse	Stemcell Technologies	60043FL.1				1:200
Integrin $\beta 1$ -PE	mono	mouse	Biolegend	303003				1:200
Integrin $\alpha 5\beta 1$	mono	rat	Merck/Millipore	MAB2575 clone BMC5		1:100		
ISG15	poly	rabbit	Cell Signaling	2743S	1:300	1:1000		
MRTF-A	poly	rabbit	Guido Posern		1:300	1:1000		
Paxillin	mono	mouse	BD Transduction Laboratories	610051 clone 349	1:300			
pCofilin (Ser3)	poly	rabbit	Cell Signaling	3311		1:1000		
Phalloidin-TRITC		probe	Sigma	P1951	1:200			
SRF	mono	rabbit	Cell Signaling	5147S		1:1000		
Talin	mono	mouse	Sigma	T3287 clone 8d4	1:300			
Vimentin	mono	mouse	Merck/Millipore	CS207806		1:1000		
Vinculin	mono	mouse	Sigma	V9131		1:1000		
β -Actin	mono	mouse	Sigma	A5441-2ML	1:300	1:5000		
β -Tubulin	mono	mouse	Sigma	T5201		1:2000		