Cell Reports, Volume *23*

Supplemental Information

IMP3 Stabilization of WNT5B mRNA Facilitates TAZ Activation in Breast Cancer

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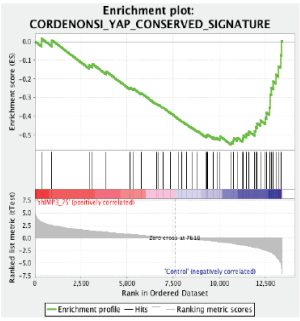
Figure S1.

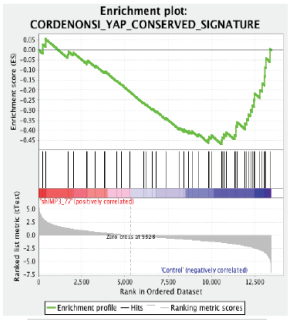
A.

shControl vs shIMP3-1



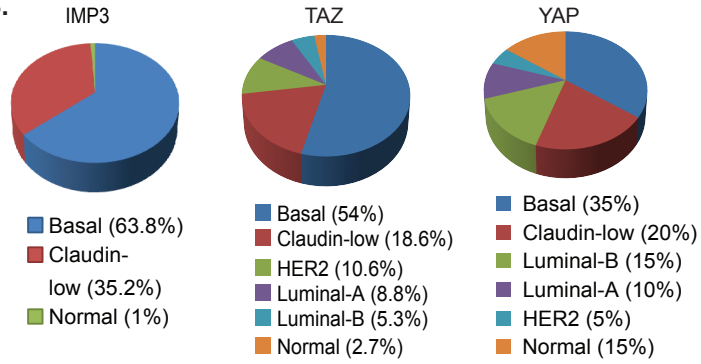
Gene	ES
GLS	-0.5381404
CRIM1	-0.5290095
PHGDH	-0.5159068
LHFP	-0.513471
FSCN1	-0.5169609
DAB2	-0.5008784
MDFIC	-0.4921103
BIRC5	-0.479332
DUSP1	-0.4776948
MARCKS	-0.4564172
ANKRD1	-0.4363506
FLNA	-0.4218539
SGK1	-0.4082211
SERPINE1	-0.3790144
TGM2	-0.3539487
AMOTL2	-0.3191373
SLIT2	-0.2828743
FGF2	-0.2476175
TGFB2	-0.2135795
DLC1	-0.1772951
THBS1	-0.1311429
CYR61	-0.0729081
CTGF	9.73E-04

shControl vs shIMP3-2

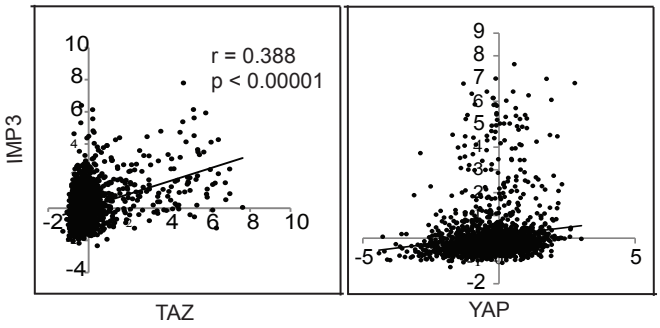


Gene	ES
TGM2	-0.4510482
MARCKS	-0.4334959
SLIT2	-0.4196395
AMOTL2	-0.4092609
GAS6	-0.4217649
HEXB	-0.4038526
SERPINE1	-0.3798229
TSPAN3	-0.3595386
FSCN1	-0.3657755
PDLIM2	-0.3440151
BICC1	-0.3216218
CRIM1	-0.2997191
TGFB2	-0.2763375
CYR61	-0.2491276
PHGDH	-0.2176879
DLC1	-0.2000943
TK1	-0.1670781
FGF2	-0.1311337
CTGF	-0.0872462
BIRC5	-0.0419462
ETV5	0.0050676

B.

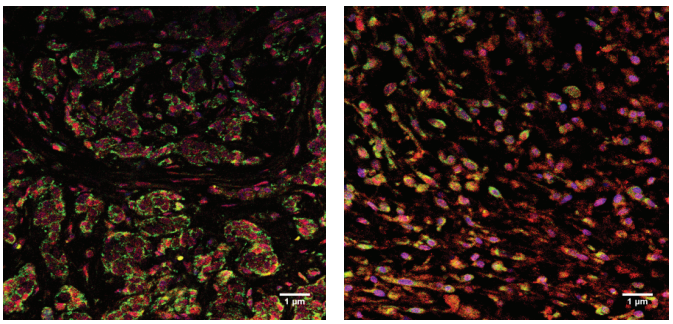


C.



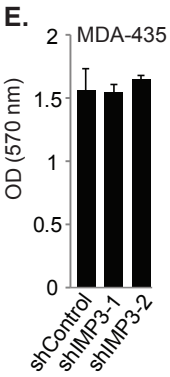
		p-value	Log odds ratio	Association
IMP3	TAZ	<0.001	2.760	Tendency towards co-occurrence
IMP3	YAP	0.335	0.239	Tendency towards co-occurrence
TAZ	YAP	0.557	-0.053	Tendency towards mutual exclisivity

D.

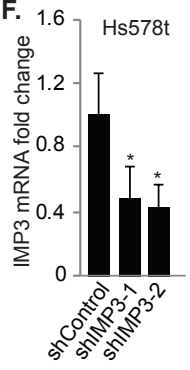


TNBC # 1 TNBC # 2

E.



F.



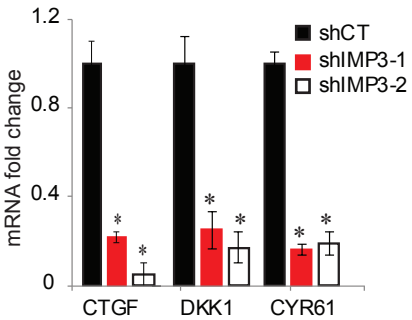
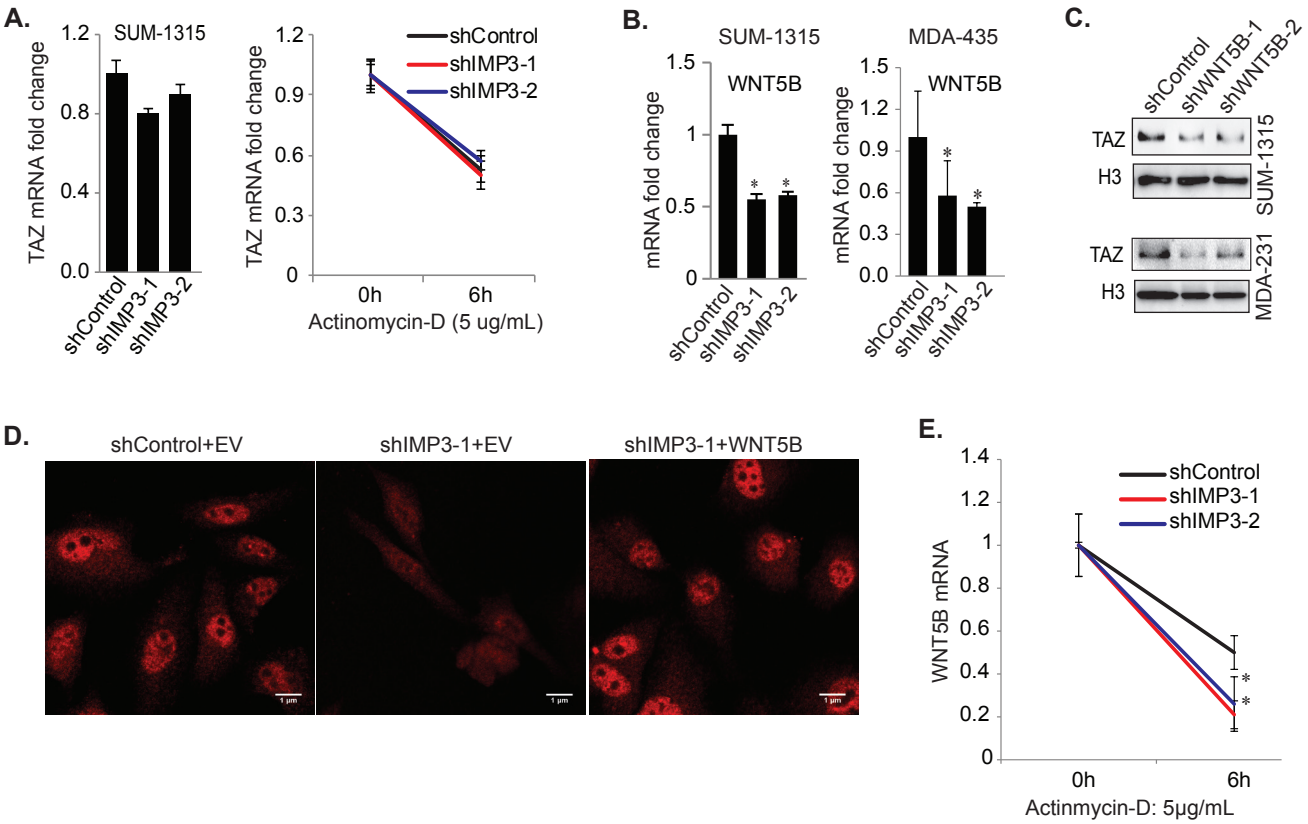
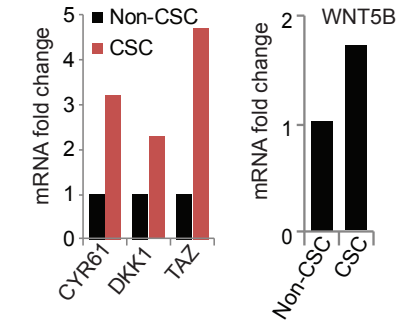


Figure S2

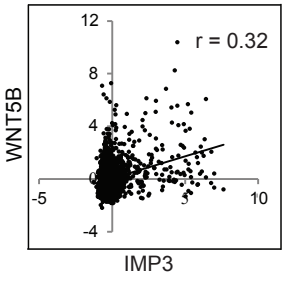




G.

cBioPortal for Cancer Genomics

	WNT5A	WNT5B	WNT3A
Basal	0	42.9	12.7
Claudin-low	7.5	21	9.5
Luminal-A	71.7	13.3	12.7
Luminal-B	17	9.5	27
HER2	3.8	5.7	30.2
Normal	0	7.6	7.9



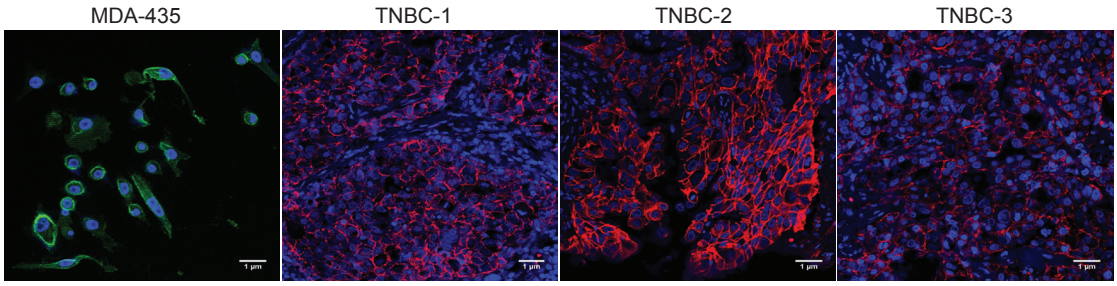


Figure S3

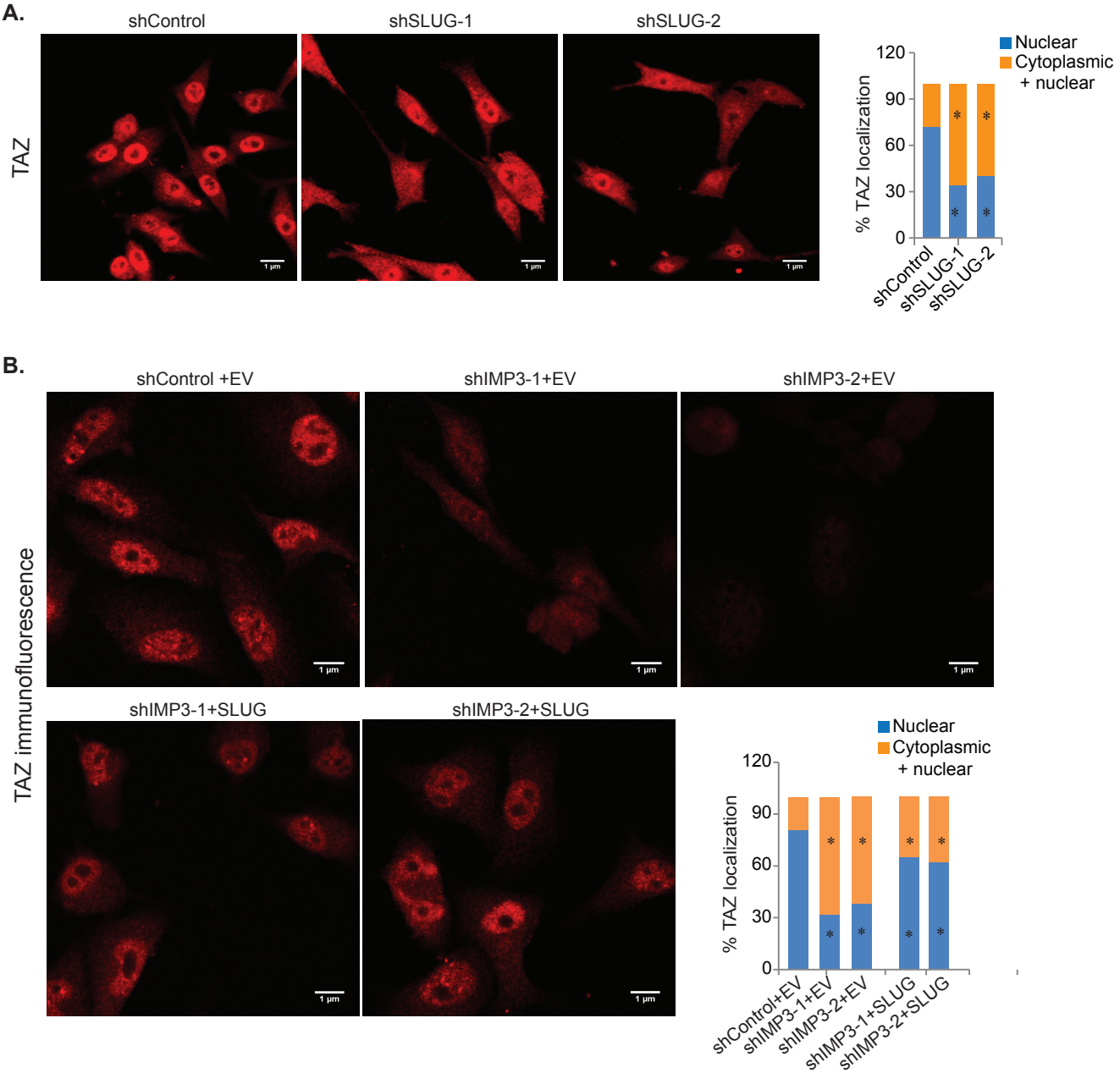
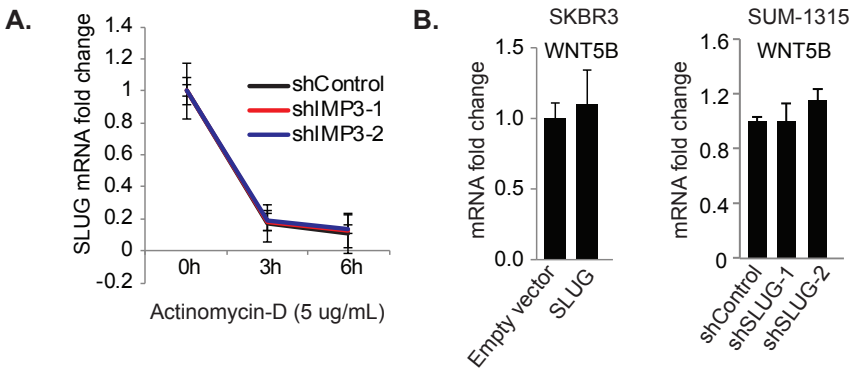

A.
shControl
shSLUG-1
shSLUG-2
TAZ
1 μm
Nuclear
Cytoplasmic
+ nuclear
% TAZ localization
120
90
60
30
0
shControl
shSLUG-1
shSLUG-2
B.
shControl +EV
shIMP3-1+EV
shIMP3-2+EV
shIMP3-1+SLUG
shIMP3-2+SLUG
TAZ immunofluorescence
Nuclear
Cytoplasmic
+ nuclear
% TAZ localization
shControl+EV
shIMP3-1+EV
shIMP3-2+EV
shIMP3-1+SLUG
shIMP3-2+SLUG
C.
mRNA fold change
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
CTGF
CYR61
DKK1
shControl+PBS
shControl+LPA
shSLUG-1+PBS
shSLUG-1+LPA
shSLUG-2+PBS
shSLUG-2+LPA

Figure S4


A.
SLUG mRNA fold change
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
shControl
shIMP3-1
shIMP3-2
0h
3h
6h
Actinomycin-D (5 ug/mL)
B.
SKBR3
WNT5B
mRNA fold change
1.5
1.0
0.5
0.0
Empty vector
SLUG
SUM-1315
WNT5B
mRNA fold change
1.6
1.2
0.8
0.4
0
shControl
shSLUG-1
shSLUG-2

Supplementary Figure Legends

Figure S1. Related to Figure 1. (A) Gene Set Enrichment Analysis (GSEA) of RNA-sequencing data showing enrichment of conserved YAP/TAZ target genes in control (shControl) cells compared to IMP3-depleted cells. (B) Expression of IMP3, TAZ and YAP mRNA in breast cancer subtypes (PAM50 classification). Charts were generated by analyzing gene expression profiles of 2509 breast cancer patients in cBioPortal for Cancer Genomics. (C) Expression of IMP3, TAZ and YAP was correlated in a cohort of 2509 human breast tumors using cBioportal. The correlation coefficient (r) was estimated using Pearson's correlation. (D) Immunofluorescence staining on human TNBC specimens showing co-localization of IMP3 (green) and TAZ (red). Scale bar = 1µm. (E) Proliferation of control and IMP3-depleted MDA-435 cells was measured using the MTT assay. (F) Total RNA extracted from control and IMP3-depleted Hs578t cells (a TNBC cell line) were used to quantify IMP3 and TAZ targets mRNA by qPCR. $p \leq 0.05$.

Figure S2. **Related to Figure 2.** (A) Total RNA was extracted from control and IMP3-depleted SUM-1315 cells and used to quantify TAZ mRNA by qPCR (bar graph, left). The same cells were treated with DMSO or actinomycin-D (5 µg/mL) for 6 hours. Total RNA was isolated and used to quantify TAZ mRNA by qPCR (line graph, right) (B) Total RNA extracted from control and IMP3-depleted SUM-1315 and MDA-435 cells were used to quantify WNT5B mRNA by qPCR. (C) Nuclear extracts from control and WNT5B-depleted SUM-1315 and MDA-231 cells were blotted for TAZ. (D) Immunofluorescence staining of TAZ (red) in control (shControl) and IMP3-depleted SUM-1315 cells (shIMP3-1) that had been transiently transfected with an empty vector (EV) or a vector expressing WNT5B for 48 hours. (E) WNT5B mRNA was quantified using total RNA extracted from control and IMP3-depleted SUM-1315 cells that had been treated with DMSO or actinomycin-D (5 µg/mL) for 6 hours. (F) Expression of TAZ target genes and WNT5B mRNA

was analyzed in the breast cancer stem cells ($CD44^+CD24^-ESA^+$) and bulk populations of breast tumor cells using a published database (GEO accession no. **GSE6883**). Gene expression was analyzed in GEO2R. (G) Expression of WNT5A, WNT5B and WNT3A in breast cancer subtypes (PAM50) from cBioPortal (left chart); IMP3 and WNT5B expression was correlated in patients using cBioPortal (right plot). (H) Immunofluorescence staining of β-catenin in MDA-435 cell line (left, green) and three TNBC specimens (red). Nuclei were stained with DAPI (blue). Scale bar = 1μm.

Figure S3. **Related to Figure 3.** (A) Immunofluorescence staining of TAZ in control and SLUG-depleted SUM-1315 cells. Scale bar = 1μm. The graphs show the quantification of TAZ localization in either the nucleus only or in both cytoplasm and nucleus. (B) Immunofluorescence staining of TAZ (red) in control (shControl) and IMP3-depleted SUM-1315 cells (shIMP3-1 & shIMP3-2) that had been transiently transfected with an empty vector (EV) or a vector expressing SLUG for 48 hours. Scale bar = 1μm. The graphs show the quantification of TAZ localization. (C) Total RNA extracted from vehicle (phosphate-buffered saline) or LPA (1μm, 2h) treated control (shControl) and SLUG-depleted SUM-1315 cells was used to quantify TAZ target genes by qPCR. $p \leq 0.05$.

Figure S4. Related to Figure 4. (A) Control and IMP3-depleted SUM-1315 cells were treated with DMSO or actinomycin-D (5 μg/mL) for indicated time periods and SLUG mRNA was quantified by qPCR. (B) SKBR3 cells were transfected with an empty vector or a SLUG-expressing construct for 48h and WNT5B mRNA was quantified by qPCR (left). Right graph shows WNT5B mRNA quantification in SLUG-depleted SUM-1315 cells. $p \leq 0.05$.

Supplemental Experimental Procedures

Reagents: shRNAs specific for GFP (RHS4459), IMP3 (TRCN0000074675, TRCN0000074677), SLUG (TRCN0000015389, TRCN0000015390) were obtained from Open Biosystems (Rockford, IL, USA). shRNAs targeting WNT5B (TRCN0000437705, TRCN0000123197) were obtained from Sigma (St. Louis, MO, USA). TAZ specific shRNAs were provided by Dr. Junhao Mao (University of Massachusetts Medical School, Worcester, MA, USA). Antibodies for IMP3 (RN009P) and SLUG (L40C6) were purchased from MBL International (Woburn, MA, USA) and Cell Signaling (Danvers, MA, USA), respectively. TAZ (H-70), β-catenin (E5) and WNT5B (G-4) specific antibodies were obtained from Santa Cruz Biotechnology (Dallas, Texas, USA). Histone-H3, LATS1, pLATS1(T1079) and pYAP(S127) were purchased from Cell Signaling (Danvers, MA, USA) respectively. Beta-actin antibody was purchased from Sigma (St. Louis, MO, USA). A luciferase reporter construct for the SLUG promoter was obtained from Dr. Yibin Kang (Princeton University). Lipofectamine-2000 and Fugene-6 were procured from Invitrogen (CA, USA) and Promega Corporation (WI, USA), respectively. Dharmafect-4 transfection reagent was obtained from Dharmacon (Lafayette, CO, USA). Lysophosphatidic acid (LPA) and actinomycin-D were purchased from and Sigma (St. Louis, MO, USA) respectively. Micro-RNA inhibitor (Anti-miR145-5p) and corresponding negative control (NC1) were purchased from Integrated DNA Technologies (San Diego, CA, USA).

Cells: The human breast cancer cell line SUM-1315 was obtained from Dr. Stephen Ethier (Medical University of South Carolina). MDA-435, MDA-231, Hs578t, SKBR3 and HEK293T cell lines were obtained from American Type Culture Collection (ATCC). These cell lines were authenticated using the University of Arizona Genetics Core. IMP3, SLUG, TAZ and WNT5B-

depleted cell lines were generated by infecting cells with pLKO.1 based lentiviruses expressing the corresponding shRNAs and subsequent selection in puromycin (1-2µg/mL). IMP3 and SLUG expression constructs were generated as described previously (Samanta et al., 2016). A WNT5B expression vector (pCDNA-WNT5B #35912) was purchased from Addgene (Cambridge, MA). Transient transfection of IMP3, SLUG and WNT5B was performed using Fugene-6 reagent. An shRNA resistant IMP3-expression construct was generated as described previously (Samanta et al., 2013). A constitutively active TAZ expression vector (4SA-TAZ) was obtained from Dr. Bob Varelas (Boston University). Transfection of anti-miR145-5p was performed using Dharmafect-4 transfection reagent.

IMP3-specific guide RNAs (gRNA) were designed using the CHOPCHOP (https://chopchop.rc.fas.harvard.edu/) and cloned into Lenti-CRISPR-V2 plasmid (Addgene # 52961) following the published protocol (Sanjana et al., 2014; Shalem et al., 2014). Subsequent production of lentiviral particles of the gRNAs and transduction into the cells followed by puromycin selection was performed as mentioned elsewhere. A scrambled gRNA was also designed and used as control. The gRNA sequences are listed in Table S3.

RNA-sequencing analysis: RNA-sequencing analysis was performed using total RNA extracted form control (shControl) and IMP3-depleted SUM-1315 cells. IMP3 was depleted using two different shRNAs (shIMP3-1 and shIMP3-2) and each sample was prepared in duplicate. Sequencing was performed using Illumina HiSeq-2000 instrument in the deep sequencing core facility at University of Massachusetts Medical School. The sequencing library that was prepared from total RNA by the core facility can be used to detect mRNA, small RNAs and long-non-coding RNAs. Raw reads were trimmed to remove the first 20 nucleotides using fastx_toolkit (v.0.0.14) followed by alignment to the reference human genome (hg19) using TopHat (v.2.0.9) (Kim et al.,

2013) with the following parameters: " -G [ucsc_hg19_knownGene] --b2-very-sensitive". The hg19 annotation file was downloaded from https://support.illumina.com/sequencing/sequencing_software/igenome.html, which contains both protein coding genes and miRNAs. Differential gene expression analysis was performed using Cufflinks (v2.2.1) (Trapnell et al., 2013).

Gene set enrichment analysis: Gene Set Enrichment Analysis (GSEA (Baas et al., 2006; Laudanski et al., 2006; Palacios et al., 2007; Pasieka et al., 2006; Subramanian et al., 2005) was performed using conserved YAP/TAZ target gene set available from molecular Signatures Database (version 6.1) (MolSigDB, http://software.broadinstitute.org/gsea/msigdb/index.jsp). The FPKM (Fragments Per Kilobase of transcript per Million mapped reads) values of all genes calculated by cuffdiff and were imported into GSEA. GSEA was performed separately for shIMP3-1 and shIMP3-2 compared to control cells (shGFP).

cBioPortal data processing: The mRNA expression data of human breast cancer samples was downloaded from cBioPortal for Cancer Genomics and Pearson's correlation analysis was performed to calculate correlation coefficient (*p*). The cBioportal data are expressed as Z-Scores, which is the relative expression of an individual gene in a tumor sample to the expression distribution of that gene in a reference population. The reference population is all of the samples that are diploid for the gene in question. The Z-score indicates the number of standard deviations away from the mean of expression in the reference population, which can be positive or negative. This measure is useful to determine whether a gene is up- or down-regulated relative to the normal samples or all other tumor samples.

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