The spectraplakin Dystonin antagonizes YAP activity and suppresses tumourigenesis

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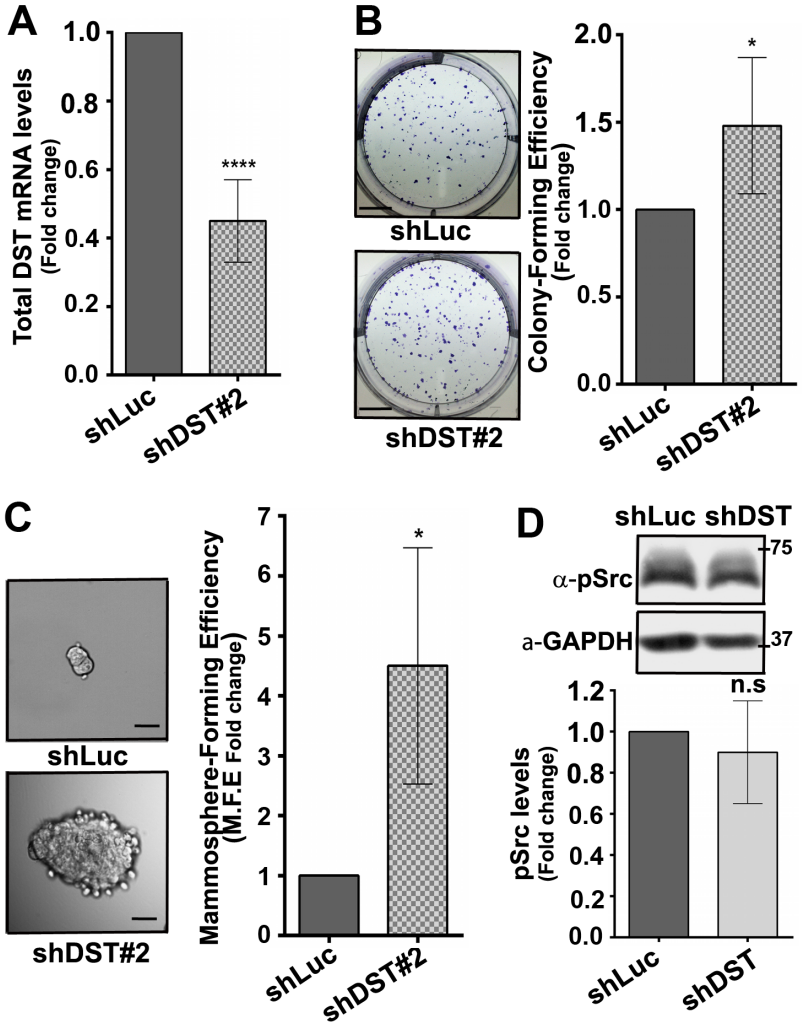
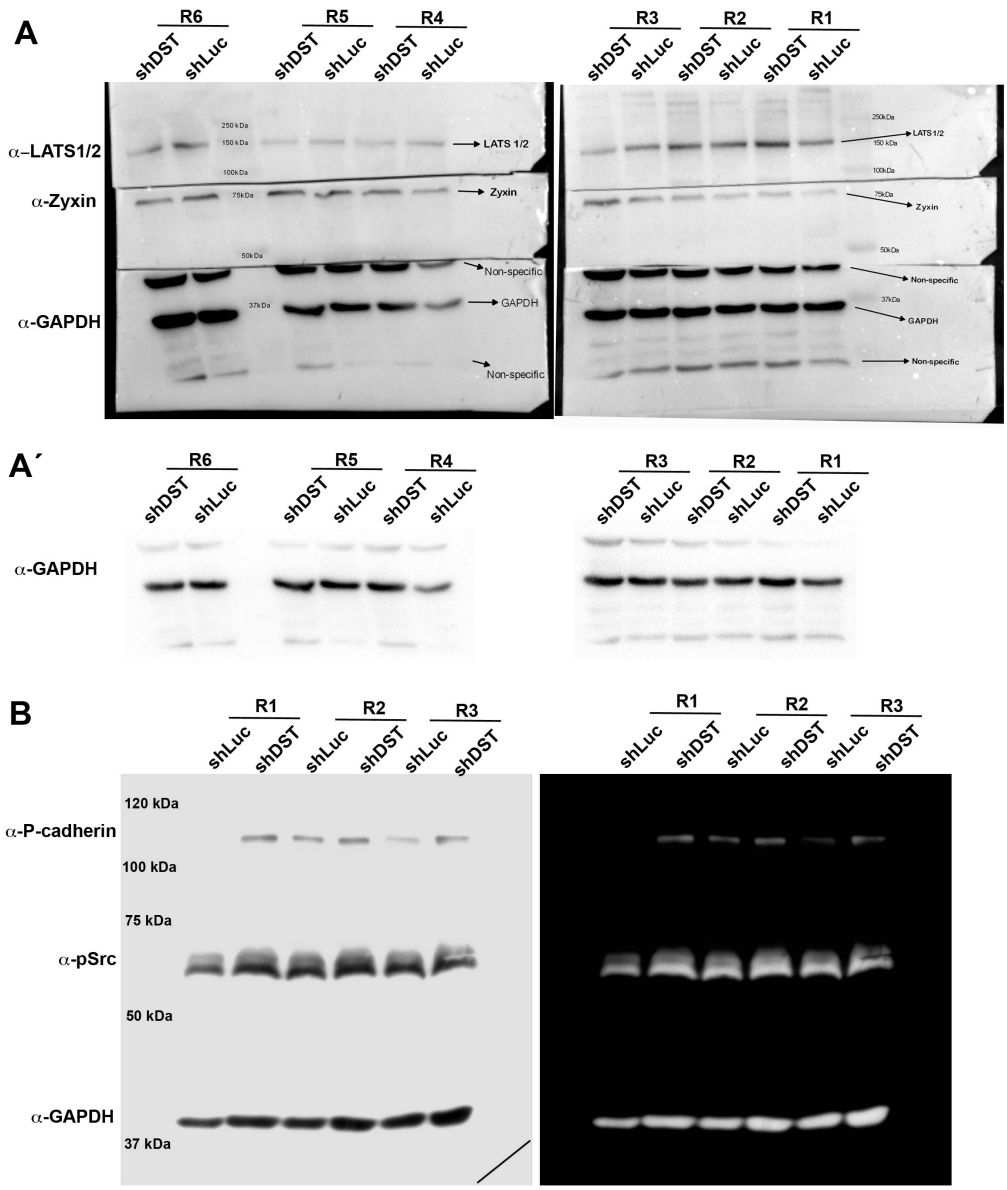
Supplementary Information

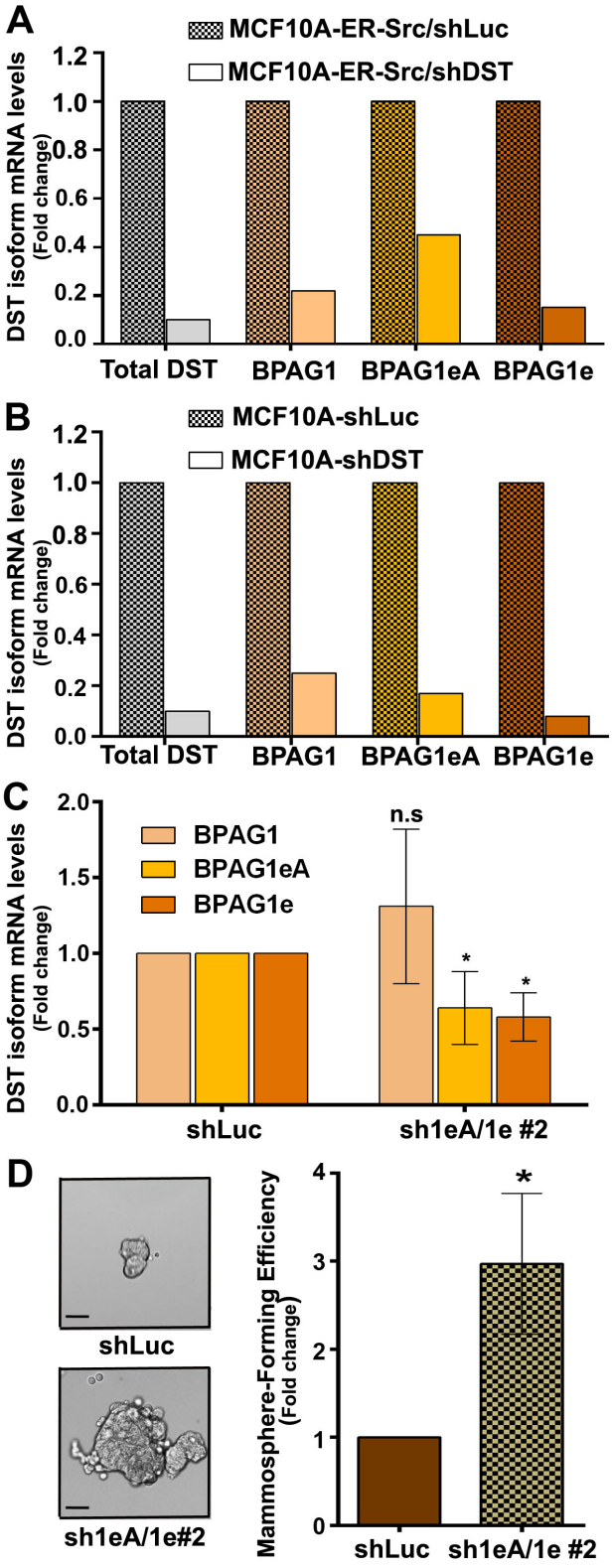
Supplementary Figures



Supplementary Figure S1: Knocking down DST promotes transformation in MCF10A cells independently of Src activation. (A) Fold change of total DST mRNA levels between shLuc- and shDST#2-expressing MCF10A cells, normalized to GAPDH. Data are from four biological replicates performed in triplicates. **(B)** (Left panels) Representative images of colonies from shLuc- or shDST#2-expressing MCF10A cells grown in clonogenic assays. Scale bars represent 5 mm. (Right panel) Fold change in colony-forming efficiency between shLuc- and shDST#2-expressing MCF10A cells. Data are from four biological replicates performed in triplicates. **(C)** (Left panels) Representative images of shLuc- or shDST#2-expressing MCF10A mammospheres. Scale bars represent 50 μm. (Right panel) Fold change in mammosphere-forming efficiency between shLuc- and shDST#2-expressing MCF10A cells. Data are from three biological replicates performed in triplicates. **(D)** (Upper panels) Western blots on protein extracts from shLuc- or shDST-expressing MCF10A cells, blotted with anti-pSrc (upper bands) or anti-GAPDH (lower bands). (Lower panels) Ratio of pSrc levels between shLuc- and shDST-expressing MCF10A cells, normalized to GAPDH. Data are from three biological replicates. For all quantifications, error bars indicate SD.; ns indicates non-significant; * indicates $P<0.05$; **** indicates $P<0.0001$.



Supplementary Figure S2: Knocking down DST promotes Zyxin accumulation, destabilizes LATS1/2, but has no major effect on pSrc levels. (A) Western blots on protein extracts from shLuc- or shDST-expressing MCF10A cells, blotted with anti-LATS1/2 (upper blots), or anti-Zyxin (middle blots) or anti-GAPDH (lower blots). R1, R2, R3, R4, R5 and R6 represent six biological replicates. Following protein transfer, membranes were cut in three pieces according to the molecular weight of the ladders and used for blotting with the anti-LATS1/2 (upper blots) or anti-Zyxin (middle blots) or anti-GAPDH (lower blots) antibodies. Blots loaded with replicates R1 to R3 (right) were exposed for 25 seconds. Blots loaded with replicates R4 to R6 (left) were exposed for 60 seconds. **(A´)** corresponds to lower exposure of membranes blotted with the anti-GAPDH antibody shown in A. The blot loaded with replicates R1 to R3 (right) was exposed for 0.82 seconds. The blot loaded with replicates R4 to R6 (left) was exposed for 21 seconds. **(B)** Western blots on protein extracts from shLuc- or shDST-expressing MCF10A cells, blotted with the anti-P-cadherin (upper bands) or anti-pSrc (middle bands) or anti-GAPDH (lower bands) antibodies. R1, R2 and R3 represent three biological replicates loaded on the same gel. The original film is shown on the right panel. The black line on the right bottom corner marks the reference for loading. Quantification of each biological replicate was performed using the blot shown on the left.



Supplementary Figure S3: Expressing shBPAG1eA/1e affects BPAG1eA and BPAG1e expression specifically and promotes mammosphere formation (A) Fold change of BPAG1 or BPAG1eA or BPAG1e mRNA levels between shLuc- or shDST-expressing MCF10A-ER-Src cells, normalized to GAPDH. **(B)** Fold change of BPAG1 or BPAG1eA or BPAG1e mRNA levels between shLuc- or shDST-expressing MCF10A cells, normalized to

GAPDH. **(C)** Fold change of BPAG1 or BPAG1eA or BPAG1e mRNA levels between shLuc- and shBPAG1eA/1e#2-expressing MCF10A cells, normalized to GAPDH. Data are from three biological replicates, performed in triplicates. **(D)** (Left panels) Representative images of shLuc- or shBPAG1eA/1e#2-expressing MCF10A mammospheres. Scale bars represent 50 μm. (Right panel) Fold change in mammosphere-forming efficiency between shLuc- and shBPAG1eA/1e#2-expressing MCF10A cells. Data are from three biological replicates, performed in triplicates. For all quantifications, error bars indicate SD.; ns indicates non-significant; * indicates $P<0.05$.

Supplementary Tables

shDST	GCTTATGACTGGAGTGAGA	All DST Isoforms
shDST # 2	AGAGAAAGATTCAGGAAAA	All DST Isoforms
shBPAG1eA/1e	ACCGTTAGAAGTAGAGCTT	BPAG1eA ad BPAG1e
shBPAG1eA/1e # 2	CCTCCTACCTTTTAGTCTA	BPAG1eA ad BPAG1e
shLuciferase	AACGTACGCGGAATACTTC	Non-targeting

Supplementary Table S1: shRNA sequences used in this study

Replicate No.	**MCF10A-shLuc**	**MCF10A-shDST**
1	8.75	10.75
2	13.50	14.30
3	11.25	10.00
4	13.25	15.75
Average ± S.D.	**11.69 ± 2.20**	**12.70 ± 2.77**

[p-value > 0.05; Paired t-test - Statistically non-significant]

Supplementary Table S2: Cell number quantification 72 hours after treatment of MCF10A-shLuc and MCF10A-shDST cells with Tet for evaluating YAP sub-cellular localization and the expression of YAP target genes.

GAPDH forward	CTCTGCTCCTCCTGTTCGAC	Ensemble Release 80
GAPDH reverse	ACCAAATCCGTTGACTCCGAC	
Total DST forward	GATGCAGATCCGAAAACCCCT	Ensembl Release 85
Total DST reverse	CTCAGTGCGGTCCAGTTGTA	
Long DST Isoforms (BPAG1) forward	CCACAGGCCACACACTTCTT	Ensembl Release 80
Long DST Isoforms (BPAG1) reverse	GGGATGCGGAGCCAGATTT	
BPAG1eA forward	TGTTGAAGCATATTTAAGGAGCCAC	Ensemble Release 80
BPAG1eA reverse	AGGTAGGAGGTCTGGAAAAAGC	
BPAG1e forward	TCAGGGCAGCAATATCAGTG	Ensemble Release 87
BPAG1e reverse	ACTAACCGGCTCAGCAAAGA	
CTGF forward	CTCGCGGCTTACCGACTG	NCBI Release 105
CTGF reverse	GGCTCTGCTTCTCTAGCCTG	
CYR61 forward	CCTTGTGGACAGCCAGTGTA	NCBI Release 105
CYR61 reverse	ACTTGGGCCGGTATTTCTTC	
ITGB6 forward	AATCGGTCTGCACAGCAAGA	Ensemble Release 94
ITGB6 reverse	ACAGCCACCTTGTACGTGAT	
Cyclin D1 forward	GATCAAGTGTGACCCGGACTG	NCBI Release 105
Cyclin D1 reverse	CCTTGGGGTCCATGTTCTGC	
Zyxin forward	AGAAGAAGTTCGGCCCTGTG	Ensemble Release 94
Zyxin reverse	GGAGGCAGGGGAAAGTCTTC	

Supplementary Table S3: Primers used for qRT-PCR.

Ensembl Release 95	**NCBI Release 109**	**In this study**
DST-207 (ENST00000421834.6)	Isoform 1 (NM_183380)	Not included
DST-202 (ENST00000312431.10)	Isoform 2 (NM_001144769.2)	BPAG1
	Isoform 3 (NM_001144770.1)	
DST-201 (ENST00000244364.10)	Isoform 1eA (NM_015548)	BPAG1eA
DST-205 (ENST0000030765.10)	Isoform 1e (NM_001723)	BPAG1e

Supplementary Table S4: DST isoforms nomenclature and accession numbers in Ensembl (Release 95), NCBI (Release 109) and in this study.