INTRODUCTION

- Yes-associated protein 1 (YAP1) and the WW domain containing transcription regulator 1 (TAZ/WWTR1) are developmentally regulated Hippo-pathway effectors. Their activity is essential for the growth of whole organs, amplification of tissue-specific progenitor cells during tissue renewal and regeneration and cell proliferation.
- In cancer, YAP1/TAZ are frequently aberrantly activated through Hippo pathway mutations, focal amplification or perturbations in other upstream regulators. In the nucleus, YAP1/TAZ interact with transcription factors including TEADs, activating the expression of genes involved in oncogenic signaling.
- To identify novel regulators of YAP1/TAZ, we established a novel screening system monitoring YAP1/TAZ activity in Hippo pathway mutant MDA-MB-231 breast cancer cells modified to express luciferase (luc) under control of TEAD promoter binding-sites.
- We performed a pooled genome-wide CRISPR/Cas9 knockout screen to identify novel YAP1/TAZ regulating genes. Functional characterization of the novel candidate YAP1/TAZ modulators will aid to the further understanding of YAP1/TAZ biology in health and disease

### METHODS

- MDA-MB-231 breast cancer cells were modified to express a YAP1/TAZ dependent TEAD-luciferase reporter that can be detected using a newly developed flow cytometry-based assay (Fig. 1A, B). Fixed and permeabilized cells were stained for firefly luciferase expression using an anti-firefly luciferase antibody (EPR17790, Abcam) followed by staining with goat anti-rabbit Alexa Fluor 488 secondary antibody (Thermo). Luciferase activity was measured using the Dual-Glo Luciferase Assay System (Promega).
- Treatment of cells with Cytochalasin D caused dose-dependent inactivation of the luciferase reporter (Fig. 1C). This was detected at comparable sensitivity by both luciferase activity assay and flow cytometry, indicating that flow cytometry-based detection of luciferase protein expression is a viable method to quantify luciferase activity (Fig. 1C).



Fig. 1: Establishment of flow cytometry-based detection of luciferase protein to enable a pooled CRISPR screening approach

To detect YAP1/TAZ translocation, cells were fixed, permeabilized and stained with anti-YAP1/TAZ antibody (Santa Cruz, sc-101199) and Alexa Fluor® 488 Goat Anti-Mouse IgG (Jackson ImmunoResearch, 115-546-062).

Mechanica stress

# **Genome-wide CRISPR/Cas9 screen** for the identification of novel YAP1/TAZ modulators

Jan Naujoks<sup>1,5</sup>, Lisette Potze<sup>2</sup>, Julia Kühnlenz<sup>1</sup>, Atanas Kamburov<sup>3</sup>, Ekaterina Nevedomskaya<sup>3</sup>, Andreas Steffen<sup>3</sup>, Claudia Luther<sup>1</sup>, Anne Buttgereit<sup>1</sup>, Stefan Prechtl<sup>4</sup>, Benjamin Bader<sup>4,6</sup>, Ralf Lesche<sup>1,5</sup>, Peter Staller<sup>1,5</sup>, Martin Lange<sup>2,5</sup>, Barbara Nicke<sup>1,5\*</sup> Bayer AG, Pharmaceuticals Division, Research & Development, <sup>1</sup>Target Discovery Technologies, <sup>2</sup>Preclinical Research Oncology I, <sup>3</sup>Bioinformatics, <sup>4</sup>Lead Discovery, Berlin, Germany; Nuvisan ICB GmbH, <sup>5</sup> Therapeutic Research, <sup>6</sup>Lead Discovery, Berlin, Germany \*<u>barbara.nicke@nuvisan.com</u>

#### **Pooled whole genome CRISPR/Cas9 screening strategy**

MDA-MBA-231 cells were transduced with a three-module whole genome 150k single guide RNA (sgRNA) CRISPR knockout lentiviral library (Cellecta) targeting the entire human genome at a multiplicity of infection of 0.3 (Fig. 2). Cells were selected for viral integration using puromycin.

After 14 days of knockout generation, cells were harvested, stained for luciferase expression and cells with the highest and lowest (10%) luciferase expression were collected by flow cytometry sorting. Genomic DNA was isolated and subjected to next-generation sequencing to identify integrated sgRNAs.



Fig.2: Strategy for pooled whole genome CRISPR/Cas9 YAP1/TAZ pathway screen

#### Whole genome CRISPR/Cas9 screen identifies known and **novel YAP1/TAZ regulators**

The pooled whole genome CRISPR screening identified positive and negative regulator genes for YAP1/TAZ activity, including previously known ones such as TAOK1, AJUBA, PTPN14, LATS2, RHOA, RAC1 and TEAD1 (Fig. 3A, B).



Fig.3: Pooled whole genome CRISPR/Cas9 screen identifies known and novel YAP1/TAZ regulators A) Mean log2 fold changes of sgRNAs targeting genes found in sorted cells with "low" vs "high" expression of luciferase. Annotated are known regulators of YAP1/TAZ signaling from the hit list. B) Signaling pathways regulating YAP1/TAZ activity. C) workflow for the selection and validation of candidates from the screen.

### RESULTS

### Validation of whole genome CRISPR/Cas9 screen hit results

> YAP1/TAZ cellular localization, TEAD-Luciferase activity and endogenous YAP1/TAZ target gene measurements of individual CRISPR/Cas9 knock-outs or siRNAs verified YAP1/TAZ modulation of known YAP1/TAZ regulators such as activators RHOA, TAZ and GNA12 as well as inhibitors such as AJUBA, PTPN14 and LATS2.



$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	Gene name	TEAD-Luc activity	Nuclear YAP1/TAZ	Gene name	TEAD-Luc activity	Nuclear YAP1/TAZ	
TAZ       0.17       0.80         SLC7A6OS       0.17       0.83         SLC7A6OS       0.17       0.83         BHOA       0.25       0.45         CSDE1       0.33       0.95         LATS2       1.17       1.14         DPM1       1.15       1.04         MARK2       0.33       0.70         CAND1       1.18       1.06         RBM4       0.45       0.79         RBM4       0.45       0.79         RBM4       0.45       0.79         RBM4       0.46       0.50         PTPN12       1.23       1.18         GNA12       0.48       0.66         GNA12       0.48       0.66         GNA12       0.48       0.66         SEC61B       0.53       0.59         PPP4C       0.54       0.91         MAPK14       0.55       1.11         CNBD2       0.66       0.66         NCKAP1       0.66       0.66         MED12       0.65       0.66         MED11       0.66       0.68         MLT1       0.67       0.69         SEC61B		(fold of control)	(fold of control)		(fold of control)	(fold of control)	In addition
SLC7ACOS       0.17       0.83       DPM1       1.15       1.04       signaling, I         RHOA       0.25       0.45       LTS2       1.17       1.14       protein transport         CSDE1       0.33       0.70       CAND1       1.18       1.17       1.14         MARK2       0.33       0.76       CAND1       1.18       1.06       important fr         REM4       0.45       0.79       PTPN12       1.23       1.18       1.06       important fr         REM4       0.46       0.50       PERTY12       1.23       1.18       1.04       important fr         SEC61A1       0.46       0.50       PERTY12       1.23       1.18       1.04       EMO2       0.51       1.01       AUBA       1.33       1.36       EMO2       0.60       0.83       1.53       1.23       1.84       1.05       PTPN12       1.23       1.84       1.05       PTPN12       1.33       1.36       EMO2       0.60       0.83       1.53       1.23       1.83       1.64       1.05       PTPN12       1.24       1.04       EMO2       0.61       1.11       EMO2       0.61       1.11       EMO2       0.66       0.84       0.85	TAZ	0,17	0,90	PTPN14	1.15	1.18	genes wi
RHOA       0.25       0.45       GTF3C4       1.17       1.14       Jost Stress         CSDE1       0.33       0.70       CAND1       1.18       1.01       protein trar         MARK2       0.33       0.70       CAND1       1.18       1.01       protein trar         RBM14       0.45       0.70       CAND1       1.18       1.06       Provide transmitter         SEC61A1       0.46       0.50       YEATS4       1.24       1.04       File transmitter	SLC7A6OS	0,17	0,83	DPM1	1,15	1,04	signaling I
CSDE1       0.33       0.85       LATS2       1.17       1.14       protein framprotein f	RHOA	0,25	0,45	GTF3C4	1,17	1,14	signaling, i
MARK2       0.33       0.70       CAND1       1.18       1.17         NR2F2       0.38       0.78       RBM14       0.43       0.67       UBE21       1.20       0.93       Important frequencies         RBM14       0.45       0.79       PTPN12       1.23       1.18       1.06       Important frequencies         SEC61A1       0.46       0.50       PTPN12       1.23       1.18       1.06         PTRV2       0.51       1.01       1.01       1.03       1.03       1.03         ARIGEF12       0.53       0.59       PTPN12       1.23       1.18       1.06         VEC61B       0.53       0.59       PTPN12       1.53       1.23       1.05         PPPAC       0.54       0.91       Difference       1.54       0.92       PTPN12       1.64       1.05         NPF31       0.60       0.83       Difference       Difference       PTPN12       Difference       Difference       Difference       Difference       Table 1. Summary of hits validated by additional individual TEAD-luciferase reporter assays and YAP1/TAZ cellular localization imaging       The data         NCP1       0.65       0.95       Difference       Difference       Difference       Diffe	CSDE1	0,33	0,95	LATS2	1,17	1,14	protein trar
NR2F2       0.38       0.78       KCTD10       1.18       1.06       EXact       Knock-out         RBM14       0.45       0.79       PTPN12       1.23       1.18       related MA         SEC61A1       0.46       0.50       EPC2       1.31       1.04       related MA         SEC61A1       0.46       0.50       EPC2       1.31       1.04       File       Knock-out         PTK2       0.51       1.01       AUBA       1.33       1.36       Cull       Knock-out         ARHGEF12       0.53       0.97       ZC3HAV1       1.54       0.92       FNTE       1.64       1.05       PTPN12       PTPN12 <td>MARK2</td> <td>0,33</td> <td>0,70</td> <td>CAND1</td> <td>1,18</td> <td>1,17</td> <td>_</td>	MARK2	0,33	0,70	CAND1	1,18	1,17	_
RAC1       0.43       0.67       UBE2/       1.20       0.93       important free         RBM14       0.45       0.79       PTPN12       1.23       1.18       important free         SEC61A1       0.46       0.50       PTPN12       1.23       1.18       important free         GNA12       0.48       0.66       1.01       AUBA       1.33       1.36       important free         GNA12       0.48       0.66       1.01       AUBA       1.33       1.36       important free         GNA12       0.48       0.66       0.53       0.59       PTPN12       1.23       1.31       1.06         PPPAC       0.54       0.91       PTPN12       1.53       1.23       Knock-out         MAPK14       0.55       1.11       Table 1. Summary of hits validated by additional individual TEAD-luciferase reporter assays and YAP1/TAZ cellular localization imaging       • CUL3 is degrada         UBE20       0.60       0.88       0.95       • The deta       • The deta         NCP9       0.65       1.06       0.98       • The deta       • The deta         NCP1       0.66       0.84       • Sece       0.70       0.65       0.87         SEC62	NR2F2	0,38	0,78	KCTD10	1,18	1,06	Knock-out
RBM14       0.45       0.79       PTPN12       1.23       1.18       Instruction of the novel potential YA         SEC61A1       0.46       0.50       PTPN12       1.23       1.18       related MA         GNA12       0.48       0.66       PTPN2       1.31       1.00       FK         PTK2       0.51       1.01       AUBA       1.33       1.36       Knock-out         ARHGEF12       0.53       0.97       2C3HAV1       1.54       0.92       PTPN12       1.23       1.18       related MA         ARHGEF12       0.53       0.97       2C3HAV1       1.54       0.92       PTPN12       1.23       1.18       Receptor         MARK14       0.55       1.11       CUL3       1.54       1.05       PTPN12       1.44       0.92       PTPN12       0.60       0.83       degrada       CUL3 is degrada       CUL3 is degrada       The detated MA         MED12       0.61       1.11       It       NCKAP1       0.66       0.84       Thm28       0.65       0.95       PTPN12       0.65       0.95       PTPN12       0.65       0.95       PTPN12       0.65       0.95       PTPN12       0.66       0.84       PTPN177       0.66 <td>RAC1</td> <td>0,43</td> <td>0,67</td> <td>UBE2I</td> <td>1,20</td> <td>0,93</td> <td>important f</td>	RAC1	0,43	0,67	UBE2I	1,20	0,93	important f
SEC61A1       0.46       0.50       YEATS4       1.24       1.04       related MA         GNA12       0.48       0.66       EPC2       1.31       1.00       Standard MA         PTK2       0.51       1.01       AUBA       1.33       1.36       Standard MA         RH0EF12       0.53       0.97       SC661B       0.53       0.97       SC661B       0.53       0.97         SEC61B       0.53       0.59       FNTB       1.64       1.05       Receptor T       •       PTPN12         MAPK14       0.55       1.11       Table 1. Summary of hits validated by additional individual TEAD-luciferase reporter assays and YAP1/TAZ cellular       •       CUL3 is degradar         UBE2D3       0.60       0.88       0.98       The deta       •       •       CUL3 is degradar         WED12       0.65       0.84       0.98       1.06       • <td>RBM14</td> <td>0,45</td> <td>0,79</td> <td>PTPN12</td> <td>1,23</td> <td>1,18</td> <td></td>	RBM14	0,45	0,79	PTPN12	1,23	1,18	
GNA12       0.48       0.66       FC2       1.31       1.00       ➡       Knock-out of Receptor T         PTK2       0.51       1.01       AJUBA       1.33       1.36       Receptor T         ARHGEF12       0.53       0.97       ZGHAV1       1.54       0.92       PTPN12       PTPN12         SEC61B       0.53       0.59       FNTB       1.64       1.05       PTPN12         CNB02       0.60       0.65       1.11       Table 1. Summary of hits validated by additional individual TEAD-luciferase reporter assays and YAP1/TAZ cellular localization imaging       • CUL3 is degrada         MED12       0.61       1.11       Table 1. Summary of hits validated by additional individual TEAD-luciferase reporter assays and YAP1/TAZ cellular localization imaging       • The deta         NCKAP1       0.66       0.88       Incessory       • CUL3 is degrada       • The deta         NCKAP1       0.66       0.84       • • • • • • • • • • • • • • • • • • •	SEC61A1	0,46	0,50	YEATS4	1,24	1,04	related MA
PTK2       0.51       1.01       AJUBA       1.33       1.36       PTK0CK04T         ELMO2       0.52       0.81       CUL3       1.53       1.23       Receptor T         ARHGEF12       0.53       0.97       ZC3HAV1       1.54       0.92       PTPN12         SEC61B       0.53       0.55       1.11       CUL3       1.54       0.92       PTPN12         MAPK14       0.55       1.11       Table 1. Summary of hits validated by additional individual TEAD-luciferase reporter assays and YAP1/TAZ cellular localization imaging       CUL3 is degrada         UBE2D3       0.60       0.83       The deta         MED12       0.61       1.11       Table 1. Summary of hits validated by additional individual TEAD-luciferase reporter assays and YAP1/TAZ cellular localization imaging       The deta         NOP9       0.65       1.06       VEZF1       0.65       0.95         VEZF1       0.65       0.95       0.06       0.73       0.95         SRP14       0.69       1.00       TGB5       0.69       0.71       0.87         SRP14       0.71       0.87       9       1.02       1.64       1.05       1.02         MEM26       0.71       0.88       0.71<	GNA12	0,48	0,66	EPC2	1,31	1,00	Knock-out
ELMO2 $0.52$ $0.81$ CU3 $1.53$ $1.23$ Receptor T         ARHGEF12 $0.53$ $0.97$ $2C3HAV1$ $1.54$ $0.92$ $PTPN12$ PPP4C $0.54$ $0.91$ $1.64$ $1.05$ $PTPN12$ MAPK14 $0.55$ $1.11$ $CUL3$ $1.64$ $1.05$ $PTPN12$ CNBD2 $0.60$ $0.65$ $0.83$ $Table 1.$ Summary of hits validated by additional individual TEAD-luciferase reporter assays and YAP1/TAZ cellular localization imaging       Table 1. Summary of hits validated by additional individual TEAD-luciferase reporter assays and YAP1/TAZ cellular localization imaging       The deta         MED12 $0.61$ $1.11$ $0.63$ $0.95$ The deta         NOP9 $0.65$ $0.98$ $0.95$ $0.96$ $0.96$ $0.65$ $0.98$ NLLT1 $0.67$ $1.03$ $0.95$ $0.96$ $0.73$ $0.94$ SEC62 $0.70$ $0.65$ $0.94$ $0.94$ $0.94$ $0.94$ MED26 $0.71$ $0.87$ $0.94$ $0.72$ $0.72$ $0.72$ $0.72$ $0.72$ $0.83$	PTK2	0,51	1,01	AJUBA	1,33	1,36	
ARHGEF12       0.63       0.97       SEC618       0.53       0.97         SEC618       0.53       0.59       PPP40       0.54       0.91         MAPK14       0.55       1.11       1.64       1.05       • PTPN12         CNBD2       0.60       0.65       1.11       • Table 1. Summary of hits validated by additional individual TEAD-luciferase reporter assays and YAP1/TAZ cellular localization imaging       • CUL3 is degrada         ILK       0.60       0.88       • • • • • • • • • • • • • • • • • • •	ELMO2	0,52	0,81	CUL3	1,53	1,23	Receptor T
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PPP4C $0.54$ $0.91$ that seqMAPK14 $0.55$ $1.11$ that seqCNBD2 $0.60$ $0.65$ $1.11$ that seqNFS1 $0.60$ $0.68$ additional individual TEAD-luciferase reporter assays and YAP1/TAZ cellular localization imagingCUL3 is degradaMED12 $0.61$ $1.11$ Table 1. Summary of hits validated by additional individual TEAD-luciferase reporter assays and YAP1/TAZ cellular localization imagingThe detaMED12 $0.61$ $1.11$ Table 2. Summary of hits validated by additional individual TEAD-luciferase reporter assays and YAP1/TAZ cellular localization imagingThe detaMED12 $0.61$ $1.11$ $MARK2$ PTPN12CECF1 $0.65$ $0.95$ $0.65$ $0.84$ MLT1 $0.66$ $0.84$ $MARK2$ PTPN12MCKAP1 $0.66$ $0.84$ $MARK2$ PTPN12MED26 $0.71$ $0.87$ $CDC27$ $0.71$ $0.87$ GPN2 $0.71$ $0.87$ $CIL3 Isadditional individual TEAD-luciferasereporter assays and YAP1/TAZ target geelectrophoresis) measurement of endogenous YAP1/TAZ target geelectrophoresis) measurement of the novel potential YAMED260.710.730.73<$	SEC61B	0,53	0,59	FNTB	1,64	1,05	• PIPN12
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UBE2D30.600.80ILK0.600.88MED120.611.11KT1120.630.95EHMT10.640.98NOP90.651.06VEZF10.650.95PLAGL20.650.84TRIM280.651.05NCKAP10.660.84MLLT10.671.03SRP140.691.00ITGB50.690.73SEC620.700.65GPN20.710.87CDC270.710.87ARF40.710.94DNMT3B0.711.02MAPK10.721.14UVRAG0.720.83TP53INP20.720.83TP53INP20.730.93WDR920.730.91FDX10.730.99HM130.730.99HM130.730.99HM130.730.99HM130.730.99HM130.730.99HM130.730.99HM130.730.99HM130.730.99HM130.730.99HM130.730.99HM130.730.99HM130.730.99HM130.730.99HM130.730.99HM130.730.99HM130.730.99HM130.730.99HM130.730.99 </td <td>NFS1</td> <td>0,60</td> <td>0,83</td> <td>additional</td> <td>individual TE</td> <td>AD-luciferase</td> <td>doarada</td>	NFS1	0,60	0,83	additional	individual TE	AD-luciferase	doarada
ILK0,600.88MED120,611,11KTI120,630.95EHMT10,640,98NOP90,651,06VEZF10,660.95PLAGL20,650,95PLAGL20,650,84MLIT10,660.84MLIT10,660.84MLIT10,671,03SRP140,691,00ITGB50,690,73SEC620,700,65GPN20,710.87ARF40,710,94MED260,710.87MARK3PTPN12MARK4DNMT3B0,71UVRAG0,72MARK10,72MARK10,72MARK10,72MARK3PTPN1OR0,73MP260,71MARK4DNMT3BDNMT3B0,71UVRAG0,72MARK3PTPN1OR0,73MP53INP20,720,730,93WDR920,730,730,93WDR920,730,710,94MH130,73MP320,73MH130,730,95MH130,73MH130,73MP32MH130,73MP32MH130,73MH13MH13MH13MH13MH13MH14MH15 <th< td=""><td>UBE2D3</td><td>0,60</td><td>0,80</td><td>reporter as</td><td>says and YAP</td><td>1/TAZ cellular</td><td>uegraua</td></th<>	UBE2D3	0,60	0,80	reporter as	says and YAP	1/TAZ cellular	uegraua
MED12       0.61       1.11         KT112       0.63       0.95         EHMT1       0.64       0.98         NOP9       0.65       1.06         VEZF1       0.65       0.84         MLLT1       0.66       0.84         MLLT1       0.66       0.84         MLLT1       0.66       0.84         MLLT1       0.67       1.03         SRP14       0.69       1.00         ITGB5       0.69       0.73         SEC62       0.70       0.65         GPN2       0.71       0.87         CDC27       0.71       0.87         MED26       0.71       0.88         DNMT3B       0.71       1.02         MARK1       0.72       1.14         UVRAG       0.72       0.88         TP53INP2       0.72       0.88         CCNC       0.72       0.88         PTPN1       0.73       0.79         TAF13       0.73       0.93         WDR92       0.73       0.91         FDX1       0.73       0.99         MM13       0.73       0.99         MM13	ILK	0,60	0,88	localization	imaging		• The deta
KTI12       0,63       0,95         EHMT1       0,64       0,98         NOP9       0,65       1,06         VEZF1       0,65       0,95         PLAGL2       0,65       0,84         TRIM28       0.65       1,05         NCKAP1       0,66       0,84         MLIT1       0,67       1,03         SRP14       0,69       1,00         ITGB5       0,69       0,73         SEC62       0,70       0,65         GPN2       0,71       0,87         ARF4       0,71       0,94         MARK2       PTPN12         ITGB5       0,69       0,73         SEC62       0,70       0,65         GPN2       0,71       0,87         ARF4       0,71       0,87         ARF4       0,71       0,88         DNMT3B       0,71       1,02         MAPK1       0,72       1,14         UVRAG       0,72       0,88         TFS3INP2       0,73       0,93         WDR92       0,73       0,91         FDX1       0,73       0,95         HM13       0,73	MED12	0,61	1,11	looanzallon			
EHMT1       0,64       0,98         NOP9       0,65       1,06         VEZF1       0,65       0,95         PLAGL2       0,65       0,84         TRIM28       0,65       1,05         NCKAP1       0,66       0,84         MLLT1       0.67       1,03         SRP14       0,69       1,00         ITGB5       0.69       0,73         SEC62       0,70       0,65         GPN2       0,71       0,87         CDC27       0,71       0,87         CDC27       0,71       0,87         DNMT3B       0,71       1,02         MAPK1       0,72       1,14         UVRAG       0,72       0,88         TPSINP2       0,72       0,88         CCNC       0,72       0,88         PTPN1       0,73       0,93         WDR92       0,73       0,91         FDX1       0,73       0,93         WDR92       0,73       0,91         FDX1       0,73       0,95         G30rf17       0,75       0,90	KTI12	0,63	0,95	۸			
NOP90,651,06VEZF10,650,95PLAGL20,650,84MLW280,651,05NCKAP10,660,84MLLT10,671,03SRP140,691,00ITGB50,690,73SEC620,700,65GPN20,710,87CDC270,710,87CDC270,710,87CDC270,710,87MED260,710,87MED260,710,88DNMT3B0,711,02MAPK10,721,14UVRAG0,720,88CCNC0,720,88CCNC0,720,83TP53INP20,720,88CCNC0,721,02PTPN10,730,93WDR920,730,91FDX10,730,93HM130,730,95C30rf170,750,90	EHMT1	0,64	0,98	A	MARK2		PTPN12
VEZF10.650.95PLAGL20.650.84TRIM280.651.05NCKAP10.660.84MLLT10.671.03SRP140.691.00ITGB50.690.73SEC620.700.65GPN20.710.87CDC270.710.87ARF40.710.94MED260.710.88DNMT3B0.711.02MAPK10.721.14UVRAG0.720.72PTPN10.730.79TAF130.730.93WDR920.730.91FDX10.730.95G30ft170.750.90	NOP9	0,65	1,06	ω 0.5	_	<u> </u>	т
PLAGL20.650.84TRIM280.651.05NCKAP10.660.84MLLT10.671.03SRP140.691.00ITGB50.690.73SEC620.700.65GPN20.710.87CDC270.710.87CDC270.710.87MED260.710.88DNMT3B0.711.02MAPK10.721.14UVRAG0.720.88DNMT3B0.711.02MAPK10.721.14UVRAG0.720.88DNMT3B0.711.02MAPK10.721.14UVRAG0.720.83TP53INP20.730.93WDR920.730.93WDR920.730.91HM130.730.95G30rf170.750.90	VEZF1	0,65	0,95		<b>▖▖▖</b> ▖ ▖	- ang	- T
TRIM28       0,65       1,05         NCKAP1       0,66       0,84         MLLT1       0,67       1,03         SRP14       0,69       1,00         ITGB5       0,69       0,73         SEC62       0,70       0,65         GPN2       0,71       0,87         CDC27       0,71       0,87         ARF4       0,71       0,94         MED26       0,71       0,88         DNMT3B       0,71       1,02         MAPK1       0,72       1,14         UVRAG       0,72       0,88         CCNC       0,72       1,02         PTPN1       0,73       0,79         TAF13       0,73       0,93         WDR92       0,73       0,91         FDX1       0,73       0,95         C30rf17       0,75       0,90	PLAGL2	0,65	0,84	<sup>5</sup> -0.5 -	<b>₽</b> ₽ <sup>⊥</sup> <sup>-</sup>	5 <sup>0.5</sup>	
NCKAP1       0,66       0,84         MLLT1       0,67       1,03         SRP14       0,69       1,00         ITGBS       0,69       0,73         SEC62       0,70       0,65         GPN2       0,71       0,87         CDC27       0,71       0,87         CDC27       0,71       0,87         MED26       0,71       0,88         DNMT3B       0,71       1,02         MAPK1       0,72       1,14         UVRAG       0,72       0,83         TP53INP2       0,72       0,88         CCNC       0,72       0,83         TP53INP2       0,73       0,93         WDR92       0,73       0,91         FDX1       0,73       0,95         G30f17       0,75       0,90	TRIM28	0,65	1,05	<sup>3</sup> .1.0 −			
MLLT1       0,67       1,03         SRP14       0,69       1,00         ITGB5       0,69       0,73         SEC62       0,70       0,65         GPN2       0,71       0,87         CDC27       0,71       0,87         ARF4       0,71       0,94         MED26       0,71       0,88         DNMT3B       0,71       1,02         MAPK1       0,72       0,83         TP53INP2       0,72       0,88         CCNC       0,72       0,83         TP53INP2       0,72       0,83         TP53INP2       0,73       0,93         WDR92       0,73       0,91         FDX1       0,73       0,95         C3oft17       0,75       0,90	NCKAP1	0,66	0,84	ິ <sub>ດີ 1.5</sub>	Ŧ	0.0	<b>I</b>
SRP14       0,69       1,00         ITGB5       0,69       0,73         SEC62       0,70       0,65         GPN2       0,71       0,87         CDC27       0,71       0,87         ARF4       0,71       0,94         MED26       0,71       0,88         DNMT3B       0,71       1,02         MAPK1       0,72       0,83         TP53INP2       0,72       0,88         CCNC       0,72       0,88         CCNC       0,72       1,02         PTPN1       0,73       0,93         WDR92       0,73       0,91         FDX1       0,73       0,95         C3orf17       0,75       0,90	MLLT1	0,67	1,03			- <u> </u>	
ITGB5       0,69       0,73         SEC62       0,70       0,65         GPN2       0,71       0,87         CDC27       0,71       0,87         ARF4       0,71       0,94         MED26       0,71       0,88         DNMT3B       0,71       1,02         MAPK1       0,72       1,14         UVRAG       0,72       0,88         CCNC       0,72       0,88         CCNC       0,72       0,88         CNC       0,72       1,02         PTPN1       0,73       0,79         TAF13       0,73       0,93         WDR92       0,73       0,91         FDX1       0,73       0,95         C3orf17       0,75       0,90	SRP14	0,69	1,00	-2.0 ' ' '	( 6) + 61 6 81 1P 0H	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	+ 5 c 8 1 N 0 H
SEC62       0,70       0,65         GPN2       0,71       0,87         CDC27       0,71       0,87         ARF4       0,71       0,94         MED26       0,71       0,88         DNMT3B       0,71       1,02         MAPK1       0,72       1,14         UVRAG       0,72       0,83         TP53INP2       0,72       0,88         CCNC       0,72       1,02         PTPN1       0,73       0,79         TAF13       0,73       0,93         WDR92       0,73       0,91         FDX1       0,73       0,95         C3orf17       0,75       0,90	ITGB5	0,69	0,73	NONTRO	1 & O A. & CO A & SA & SA	NON RECT RO	4. 6. 0 4 6. 8 8 4 V
GPN2       0,71       0,87         CDC27       0,71       0,87         ARF4       0,71       0,94         MED26       0,71       0,88         DNMT3B       0,71       1,02         MAPK1       0,72       1,14         UVRAG       0,72       0,83         TP53INP2       0,72       0,88         CCNC       0,72       1,02         PTPN1       0,73       0,79         TAF13       0,73       0,91         FDX1       0,73       0,95         G3orf17       0,75       0,90	SEC62	0,70	0,65	P. P.	· ·	b. b.	·
CDC27       0,71       0,87         ARF4       0,71       0,94         MED26       0,71       0,88         DNMT3B       0,71       1,02         MAPK1       0,72       1,14         UVRAG       0,72       0,83         TP53INP2       0,72       0,88         CCNC       0,72       1,02         PTPN1       0,73       0,79         TAF13       0,73       0,93         WDR92       0,73       0,91         FDX1       0,73       0,95         C3orf17       0,75       0,90	GPN2	0,71	0,87		ote of knocko	ut of hits on VA	D1/TA7 torget de
ARF4       0,71       0,94         MED26       0,71       0,88         DNMT3B       0,71       1,02         MAPK1       0,72       1,14         UVRAG       0,72       0,83         TP53INP2       0,72       0,88         CCNC       0,72       1,02         PTPN1       0,73       0,79         TAF13       0,73       0,93         WDR92       0,73       0,91         FDX1       0,73       0,95         C3orf17       0,75       0,90	CDC27	0,71	0,87	FIG. 5. Elle			AF ITAL LAIYEL YE
MED26       0,71       0,88         DNMT3B       0,71       1,02         MAPK1       0,72       1,14         UVRAG       0,72       0,83         TP53INP2       0,72       0,88         CCNC       0,72       1,02         PTPN1       0,73       0,79         TAF13       0,73       0,93         WDR92       0,73       0,91         FDX1       0,73       0,95         C3orf17       0,75       0,90	ARF4	0,71	0,94	electrophore	esis) measuren	nent of endogene	ous YAP1/TAZ tar
DNMT3B       0,71       1,02         MAPK1       0,72       1,14         UVRAG       0,72       0,83         TP53INP2       0,72       0,88         CCNC       0,72       1,02         PTPN1       0,73       0,79         TAF13       0,73       0,93         WDR92       0,73       0,91         FDX1       0,73       0,95         C3orf17       0,75       0,90	MED26	0,71	0,88				
MAPK1       0,72       1,14         UVRAG       0,72       0,83         TP53INP2       0,72       0,88         CCNC       0,72       1,02         PTPN1       0,73       0,79         TAF13       0,73       0,93         WDR92       0,73       0,91         FDX1       0,73       0,95         C3orf17       0,75       0,90	DNMT3B	0,71	1,02				
UVRAG       0,72       0,83         TP53INP2       0,72       0,88         CCNC       0,72       1,02         PTPN1       0,73       0,79         TAF13       0,73       0,93         WDR92       0,73       0,91         FDX1       0,73       0,69         HM13       0,73       0,95         C3orf17       0,75       0,90	MAPK1	0,72	1,14				
TP53INP2       0,72       0,88         CCNC       0,72       1,02         PTPN1       0,73       0,79         TAF13       0,73       0,93         WDR92       0,73       0,91         FDX1       0,73       0,69         HM13       0,73       0,95         C3orf17       0,75       0,90	UVRAG	0,72	0,83				
CCNC       0,72       1,02         PTPN1       0,73       0,79         TAF13       0,73       0,93         WDR92       0,73       0,91         FDX1       0,73       0,69         HM13       0,73       0,95         C3orf17       0,75       0,90	TP53INP2	0,72	0,88		pled whole ger	nome CRISPR/	Casy screen Ider
PTPN1       0,73       0,79         TAF13       0,73       0,93         WDR92       0,73       0,91         FDX1       0,73       0,69         HM13       0,73       0,95         C3orf17       0,75       0,90	CCNC	0,72	1,02	MB-2	31 breast can	cer cells	
TAF13       0,73       0,93         WDR92       0,73       0,91         FDX1       0,73       0,69         HM13       0,73       0,95         C3orf17       0,75       0,90	PTPN1	0,73	0,79		tional obaracte	vrization of the r	noval potential V/A
WDR92       0,73       0,91         FDX1       0,73       0,69         HM13       0,73       0,95         C3orf17       0,75       0,90	TAF13	0,73	0,93			inzation of the f	iovel potential YA
FDX1       0,73       0,69         HM13       0,73       0,95         C3orf17       0,75       0,90	WDR92	0,73	0,91				
HM130,730,95C3orf170,750,90	FDX1	0,73	0,69				
C3orf17 0,75 0,90 Mathematic Andrea Sturz Malania Barthald and Thika	HM13	0,73	0,95				
	C3orf17	0,75	0,90	We th	hank Andrea S	turz, Melanie B	erthold and Thiba

th functions in actin cytoskeleton

Integrin signaling, ER stress and

nsport, amongst others, were identified as potential novel YAP1/TAZ regulators.

or knock-down of MARK2, a member of the Par-1 family of serine/threonine protein kinases for regulation of cell polarity consistently inhibited YAP1/TAZ activity in all assays tested. The highly ARK4 protein has previously been shown to be an activator of YAP1/TAZ.

Luciferase activity (RLU, relative to control)

or knock-down of breast cancer tumor suppressors PTPN12 (Protein Tyrosine Phosphatase, Non-Type 12) or CUL3 (Cullin 3) consistently induced YAP1/TAZ activity in all assays tested:

interference suggests a conserved function with PTPN14, a known YAP1/TAZ inhibitory protein uesters YAP1/TAZ in the cytoplasm.

the core scaffolding protein of the CUL3–RING ubiquitin ligase complex which targets proteins for ation by the proteasome.

ailed mechanisms of how PTPN12 and CUL3 regulate YAP1/TAZ are currently being explored.



ene expression. A,B) siRNA mediated knockdown followed by qPCR and Protein (capillary rget genes.

#### CONCLUSIONS

ntified previously known as well as novel positive and negative regulators of YAP1/TAZ in MDA-

AP1/TAZ modulators will aid to the further understanding of YAP1/TAZ biology in health and disease

## **ACKNOWLEDGMENTS**

aud Jourdan for technical assistance