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CRISPR-Mediated Approaches to Regulate YAP/TAZ Levels

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Abstract

The advent of CRISPR has revolutionized genomic engineering, and harnessing its power to regulate levels of the transcriptional co-activators YAP and TAZ represents an exciting new opportunity in the field of Hippo signaling. Initially repurposed from the microbial immune system to perform highly specific gene knockouts, CRISPR technology has now been expanded to modulate the transcriptional activity of any gene of interest in mammalian systems. Here, we describe strategies to employ CRISPR to genetically knock out the genes encoding for YAP (*YAP1*) or TAZ (*WWTR1*) in mammalian cell lines, as well as briefly outline an approach for utilizing CRISPR to transcriptionally modulate YAP/TAZ levels.

Keywords

Cas9; sgRNA; Hippo; LATS; CRISPRa; CRISPRi; KRAB; VP64

1 Introduction

The mammalian Hippo tumor suppressor pathway comprises a cascade of kinases that negatively regulate the transcriptional co-activator paralogs, Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ) [1, 2]. Upon Hippo pathway activation, large tumor suppressors 1 and 2 (LATS1/2) phosphorylate YAP/TAZ to induce their cytoplasmic sequestration and degradation. By contrast, when the Hippo pathway is inactive, YAP and TAZ remain unphosphorylated, allowing them to translocate to the nucleus, bind to the TEAD family of transcription factors, and induce the expression of an array of genes that promote cellular proliferation, migration, and survival [3]. The Hippo pathway was first identified as a highly conserved canonical regulator of organ size; however, recent discoveries situate this pathway as a highly integrated signaling hub, responding to complex inputs that monitor cell-cell adhesion, cell-matrix adhesion, GPCR signaling, contractile tension from the actin cytoskeleton, cell polarity, and changes in the cell cycle [3–11]. These cues converge on LATS/YAP/TAZ signaling to regulate embryological and organ development as well as adult tissue maintenance [3, 12].

With their capacity to drive proliferation, invasion, dedifferentiation, and survival, YAP/TAZ can be exploited to favor neoplastic development [13]. It is therefore unsurprising that functional impairment of the Hippo tumor suppressor pathway, with subsequent hyperactivation and/or amplification of YAP/TAZ, has been observed in many cancers [14–16]. Indeed, aberrant YAP/TAZ activity has been observed in glioblastoma, lung, colorectal, ovarian, breast, liver, and prostate cancers and is often correlated with poor prognosis [17–19].

The importance of Hippo signaling in development and tumorigenesis places it at the center of investigative efforts across many fields. Within this context, modulating YAP/TAZ activity is vital to elucidating their many roles and regulations. A recent innovation in gene editing, termed clustered regularly interspaced short palindromic repeats (CRISPR), has enabled novel methods of modulating gene activity with radically improved efficacy and efficiency, thus opening many new and exciting avenues of investigation.

CRISPR was discovered as a mechanism of microbial adaptive immunity wherein Cas9, a RNA-guided bacterial endonuclease, cleaves and destroys foreign DNA from invading viruses based on 20-nucleotide targeting sequences [20–26]. This biological tool has been adapted to allow for the specific targeting and cleavage of any region of the genome in eukaryotic cells, enabling the introduction of double strand breaks (DSBs) at a given locus of interest. These DSBs are most commonly repaired using non-homologous end joining (NHEJ), which is error-prone and frequently results in insertion/deletion (indel) mutations that can introduce frameshifts or premature stop codons, ultimately rendering a gene of interest inactive (Fig. 1a) [27]. More recently, CRISPR technology been developed to go beyond gene knockouts to enable the recruitment of transcriptional activators or repressors to the promoter region of any gene (Fig. 1b, c) [27–34]. Herein, we outline the methods for using CRISPR to knock out the genes encoding for YAP (*YAP1*) and TAZ (*WWTR1*) in mammalian cell lines as well as briefly describe the use of CRISPR activator and inhibitor systems to tightly regulate endogenous YAP/TAZ expression.

2 Materials

We use non-transformed telomerase-immortalized retinal pigmented epithelial cells (hRPE-1) for the method described below. However, this protocol can be adapted to any adherent cell line so long as cell culture conditions are adjusted as necessary.

2.1 Cell Culture

1. 10 cm polystyrene tissue culture plates.
2. 15 cm polystyrene tissue culture plates.
3. 6-well polystyrene tissue culture plates.
4. Sterile 1.5 mL microcentrifuge tubes.
5. Complete DMEM/F12: DME/F12 media supplemented with 10% fetal bovine serum (FBS), 100 IU/mL penicillin, and 100 µg/mL streptomycin. Store at 4 °C in the dark.

6. 0.25% Trypsin/EDTA (0.53 mM). Store at 4°C in the dark.
7. Sterile phosphate-buffered saline (PBS: 1.06 mM KH₂PO₄, 155.17 mM NaCl, 2.97 mM Na₂PO₄·7H₂O). Store at 4°C.
8. Transfection reagent: Polyethylenimine (PEI) in water at a working concentration of 1 µg/mL. Store at 4°C.
9. Viral envelope/packaging plasmids (for example: psPAX2 (Addgene, #12260) and pMD2.G (Addgene, #12259) plasmids).
10. CRISPR plasmid: lentiCRISPR v2 (Addgene, #52961) (or any other applicable CRISPR vector).
11. Transduction reagent: Polybrene (10 mg/mL stock concentration). Store at −20 °C.
12. RPE-1 (ATCC, #CRL-4000) and HEK293FT cell lines (thermo fisher, #R7007).

2.2 Equipment

1. Tissue culture incubator set at 37°C with 5% humidified CO₂.
2. Tissue culture hood.
3. Hemocytometer.
4. High-speed centrifuge.
5. Phase contrast microscope equipped with a × 10 objective for cell counting.
6. Sterile cloning discs.

3 Methods

3.1 Design sgRNA Sequences Optimized to Target YAP or TAZ for Knockout

1. In order to use CRISPR to successfully knock out a gene, cells must express both Cas9 endonuclease and a single guide RNA (sgRNA) that targets Cas9 to the gene of interest. The design of a specific, functional sgRNA sequence is critical to the success of this approach and must adhere to certain parameters (*see* Note 1).
2. Custom synthesized sgRNAs should be purified using standard desalting procedures. It is also important to include additional nucleotides flanking the sgRNA sequence to facilitate proper ligation and cloning into the expression vector of choice (*see* Note 2).
3. We designed and/or purchased several sgRNAs to target *YAP1* or *WWTR1* and found these sequences to be the most efficient for gene knockout in RPE-1 cells:
YAP1: 5'-TGCCCCAGACCGTGCCCAT-3'
WWTR1: 5'-ATCCGAAGCCTAGCTCGTGG-3'

4. Numerous lentiviral expression plasmids are available to integrate both sgRNA and Cas9 expression cassettes into the genomes of infected hosts. Some all-in-one plasmids express both the sgRNA and Cas9 from the same vector [35]. Alternatively, Cas9 and sgRNAs can be expressed from different vectors [35]. These CRISPR elements can also be transiently expressed using standard expression plasmids. Several factors impact which expression system is optimal (*see Note 3*). Here, we describe methods to express Cas9 and a sgRNA from an all-in-one lentiviral vector (*see Subheading 3.2 below*).

3.2 Production of Virus Containing Cas9 and sgRNA Expression Vectors

Ensure that all institutional biosafety rules and regulations related to working with virus are strictly followed.

1. Seed 293FT cells in complete DMEM/F12 media into a 6-well plate. Cells are ready for transfection when they are attached and roughly 80% confluent.
2. When cells are ready to transfect, change the media on 293FT cells to serum supplemented media without penicillin/streptomycin added. Gently add 2 mL per well.
3. Prepare transfection mixture as follows: (Volumes provided are for 1 well within a 6-well plate; scale volumes as necessary).
 - a. Mix 200 μ L of serum free media with 16 μ L of 1 μ g/mL PEI (*see Note 4*) in a 1.5 mL microcentrifuge tube. Mix gently. Wait 5 min.
 - b. Add 2 μ g of plasmid DNA (e.g., the LentiCRISPR-v2 all-in-one plasmid containing a YAP targeting sgRNA).
 - c. Add 1.5 μ g of psPAX or equivalent lentiviral packaging plasmid.
 - d. Add 500 ng of pMD2.G or equivalent lentiviral envelope plasmid.
 - e. Mix transfection mixture gently; avoid pipetting up and down. Wait 15 min.
4. Add 200 μ L of solution gently in dropwise manner to 293FT cells in penicillin/streptomycin free media.
5. Place in 37 ° C incubator.
6. After 4 h, gently replace media with 2 mL of complete DMEM/F12 media.
7. After 48 h, collect supernatant containing virus and filter through a 0.45 μ m filter. Collect virus in a 15 mL conical vial and use for infection immediately or store at –80 ° C.

3.3 Infection

1. On the day before the infection, plate RPE-1 cells in complete DMEM/F12 media in a 6-well plate so that they will be roughly 40–50% confluent the next day.

2. Dilute viral supernatant in fresh complete DMEM/F12 media at a ratio of 1 mL of viral supernatant: 1 mL of media and add polybrene at a final concentration of 10 µg/mL. Aim for a multiplicity of infection (MOI) of ~1, which must be empirically determined (*see* Note 5).
3. Aspirate media from RPE-1 cells and replace with 2 mL of prepared viral supernatant/media mixture containing polybrene.
4. Optionally, spin the 6-well plate at $1200 \times g$ for 60 min at 32 °C to improve infection efficiency.
5. Place in 37 °C incubator.
6. Add antibiotic selection to infected cells and non-infected controls after 24 h.
7. Once resistant cells have grown sufficiently to passage, and uninfected control cells have died in antibiotic, passage cells and verify knockdown of YAP (or TAZ) via western blot. If YAP (or TAZ) levels appear significantly decreased by western blot, proceed to isolating single cell knockout clones.

3.4 Selecting Knockout Clones

1. Wash the population of cells infected with YAP (or TAZ) CRISPR reagents with sterile PBS, then trypsinize cells with 0.25% trypsin/EDTA for 5 min (or until all cells are detached from the plate).
2. Collect cells in complete medium containing 10% FBS and pellet cells at $200 \times g$ for 5 min. Resuspend cells in complete growth medium, and use a hemocytometer (or similar counting device) to count cells.
3. Plate cells sparsely on a 15 cm tissue culture plate so that they will grow up as individual colonies (*see* Note 6). Alternatively, use FACS to sort single cells into a 96-well tissue culture plate. Place plate in 37 °C incubator.
4. Change media every 3–4 days, and wait 1–2 weeks for distinct colonies of cells to become visible.
7. Once clonal populations are visible and sufficiently grown, mark where they are on the bottom of the tissue culture dish with a marker (*see* Note 7).
8. Aspirate media and wash the tissue culture plate with sterile, room temperature PBS.
9. Immerse sterile cloning discs in 0.25% trypsin/EDTA that has been pre-warmed to 37 °C.
10. Aspirate the PBS from the tissue culture plate containing the clones, and then use forceps to place trypsin saturated discs on top of each marked colony. Move quickly to avoid cells drying out (*see* Note 7).
11. Wait 5 min for cells to detach, and then use forceps to transfer individual cloning discs into separate wells of 6-well tissue culture plates containing 2 mL of pre-warmed complete media. Do not agitate the plate while waiting for cells to detach.

12. Allow clonal populations to grow in 6-well dishes, passage, and verify YAP knockout in clones via western blot (Fig. 2) (see Note 8).

3.5 CRISPR-Mediated Transcriptional Regulation

Recent developments have allowed for the use of CRISPR technology in modulating the transcriptional activity of a gene of interest. CRISPR-mediated transcriptional regulation is typically accomplished by fusing a catalytically inactive Cas9 (dCas9) that is devoid of endonuclease activity to transcriptional activator or repressor proteins, such as VP64 or KRAB, respectively (Fig. 1). This allows for sgRNA-mediated targeting of transcriptional activators/repressors to the promoter region of any gene of interest and its resultant activation or repression [29–34]. Excitingly, this technology confers significant investigative advantage over current methods in performing genome wide gene activation/repression screens, allowing for their simple and cost effective execution [33, 37, 38].

While many of the considerations when using CRISPR as a means of transcriptional regulation are similar to performing CRISPR mediated gene knockout, the sgRNA targeting region shifts from the coding region of a gene to the upstream native promoter region. As such, alternate parameters regarding off-target effects and optimal sgRNA design must be accounted for. Publically available tools, such as this website furnished by the Broad institute, <https://portals.broadinstitute.org/gpp/public/analysis-tools/sgrna-design-crisprai>, incorporate the necessary considerations regarding transcriptional start sites and DNase 1 hypersensitive sites into their sgRNA design algorithms.

CRISPR activator/inhibitor systems can also be utilized to induce YAP/TAZ expression/inhibition in a way that allows for temporal control. This is accomplished by placing dCas9, fused to a transcriptional activator or inhibitor, under the control of an inducible promoter, allowing for the tunable activation of CRISPR transcriptional modulation over time. Several plasmids encoding for dCas9 fused to transcriptional activators or repressors are publically available, as are plasmids containing CRISPR transcriptional modulation elements under the inducible control of both light and chemical activation [39, 40].

4 Notes

1. When using *Streptococcus pyogenes* Cas9, the most commonly used Cas9 ortholog, the 20-nt DNA sequence to be targeted by the sgRNA must be followed at the 3' end by the protospacer adjacent motif (PAM) sequence NGG in order for the Cas9 nuclease to perform its cleavage function [41]. In addition, it is advised to select sgRNA sequences that target early exons that are common to all transcript variants and that occur upstream of functional protein domains [42, 43]. There are multiple online tools to assist with the design of sgRNAs targeting specific genes of interest, such as <http://tools.genome-engineering.org>. It is recommended to test 1–3 different sgRNAs for each gene of interest.
2. Custom sgRNA sequences can be cloned into numerous available vectors following cloning strategies described for each vector. A protocol for cloning sgRNA sequences into commonly used lentiviral CRISPR constructs is available

here: https://media.addgene.org/data/plasmids/52/52961/52961-attachment_B3xTwla0bkYD.pdf. Alternatively, pre-designed sgRNAs cloned into commercially available vectors are available for purchase from several companies.

3. In general, stable integration of Cas9 and sgRNA using lentiviral vectors, leading to prolonged expression of CRISPR elements, may increase the likelihood of successful on-target modifications [38]. Alternatively, prolonged expression of sgRNA may also lead to increased off-target effects, and since the location of genome integration is random, there is also the potential for phenotypic artifacts to arise [44]. The delivery of recombinant Cas9 protein, sgRNA delivery using non-integrating adeno-associated viral (AAV)-based vectors, delivery via transient transfection of sgRNA plasmids, or the delivery of sgRNAs as synthetic oligonucleotides are viable alternatives to that may limit off-target effects while still maintaining a high degree of efficacy [44].
4. It is important to optimize the ratio of DNA:PEI to be used for each batch of PEI. Commercially available DNA transfection reagents can also be used in place of PEI.
5. Polybrene (hexadimethrine bromide) can be used to enhance transduction efficiency, however, this chemical is not required for infection and has been shown to be toxic for some cell types. It is ideal to aim for a multiplicity of infection (MOI) of ~1. This can be achieved by testing multiple dilutions of viral supernatant: media added to cells. Typically, dilutions from 1:1 to 1:8 are performed.
6. The number of cells plated can vary based on how well they adhere after suspension and how fast they proliferate. For RPE-1 cells, plate roughly 500 cells on a 15 cm tissue culture plate. This generates dozens of clonal populations after 1–2 weeks. This method is not ideal for highly motile cells, and sorting cells via FACS into individual wells of a 96-well plate may be preferred.
7. Clonal outgrowths are typically ready for transfer when they have reached numbers in the hundreds. As a general rule for RPE cells, trypsinize colonies when the outer boundaries of the clonal population nearly fill the field of view of a $\times 10$ objective. Keep in mind that knockdown of YAP or TAZ impairs cell proliferation rates; therefore, it is important to also collect slow-growing clones that may take longer to develop (e.g. 2–4 weeks). It is recommended to collect at least 12 clones.
8. When imaging a blot to verify gene knockout, overexpose the blot to ensure knockout is complete. There are additional methods to verify gene knockout, such as sequencing, mismatch cleavage assays, and others [36].

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References

1. Huang J, Wu S, Barrera J, Matthews K, Pan D (2005) The Hippo signaling pathway coordinately regulates cell proliferation and apoptosis by inactivating Yorkie, the Drosophila Homolog of YAP. *Cell* 122(3):421–434. 10.1016/j.cell.2005.06.007 [PubMed: 16096061]
2. Lei QY, Zhang H, Zhao B, Zha ZY, Bai F, PeiXH, Zhao S, Xiong Y, Guan KL (2008) TAZ promotes cell proliferation and epithelialmesenchymal transition and is inhibited by the hippo pathway. *Mol Cell Biol* 28(7):2426–2436. 10.1128/MCB.01874-07 [PubMed: 18227151]
3. Meng Z, Moroishi T, Guan K-L (2016) Mechanisms of Hippo pathway regulation. *Genes Dev* 30(1):1–17. 10.1101/gad.274027.115 [PubMed: 26728553]
4. Yu F-X, Guan K-L (2013) The Hippo pathway: regulators and regulations. *Genes Dev* 27 (4):355–371. 10.1101/gad.210773.112 [PubMed: 23431053]
5. Ganem Neil J, Cornils H, Chiu S-Y, O'Rourke Kevin P, Arnaud J, Yimlamai D, Théry M, Camargo Fernando D, Pellman D (2014) Cytokinesis failure triggers hippo suppressor pathway activation. *Cell* 158 (4):833–848. 10.1016/j.cell.2014.06.029 [PubMed: 25126788]
6. Aragona M, Panciera T, Manfrin A, Giullitti S, Michielin F, Elvassore N, Dupont S, Piccolo S (2013) A mechanical checkpoint controls multicellular growth through YAP/TAZ regulation by actin-processing factors. *Cell* 154(5):1047–1059. 10.1016/j.cell.2013.07.042 [PubMed: 23954413]
7. Halder G, Dupont S, Piccolo S (2012) Transduction of mechanical and cytoskeletal cues by YAP and TAZ. *Nat Rev Mol Cell Biol* 13:591 10.1038/nrm3416 [PubMed: 22895435]
8. Paramasivam M, Sarkeshik A, Yates JR, Fernandes MJG, McCollum D (2011) Angiomotin family proteins are novel activators of the LATS2 kinase tumor suppressor. *Mol Biol Cell* 22(19):3725–3733. 10.1091/mbc.E11-04-0300 [PubMed: 21832154]
9. Wada K-I, Itoga K, Okano T, Yonemura S, Sasaki H (2011) Hippo pathway regulation by cell morphology and stress fibers. *Development* 138(18):3907–3914. 10.1242/dev.070987 [PubMed: 21831922]
10. Zhao B, Li L, Wang L, Wang C-Y, Yu J, Guan K-L (2012) Cell detachment activates the Hippo pathway via cytoskeleton reorganization to induce anoikis. *Genes Dev* 26(1):54–68. 10.1101/gad.173435.111 [PubMed: 22215811]
11. Zhao B, Wei X, Li W, Udan RS, Yang Q, Kim J, Xie J, Ikenoue T, Yu J, Li L, Zheng P, Ye K, Chinnaiyan A, Halder G, Lai Z-C, Guan K-L (2007) Inactivation of YAP oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control. *Genes Dev* 21(21):2747–2761. 10.1101/gad.1602907 [PubMed: 17974916]
12. Nishioka N, Inoue K, Adachi K, Kiyonari H, Ota M, Ralston A, Yabuta N, Hirahara S, Stephenson RO, Ogonuki N, Makita R, Kurihara H, Morin-Kensicki EM, Nojima H, Rossant J, Nakao K, Niwa H, Sasaki H (2009) The Hippo signaling pathway components Lats and Yap pattern Tead4 activity to distinguish mouse trophectoderm from inner cell mass. *Dev Cell* 16(3):398–410. 10.1016/j.devcel.2009.02.003 [PubMed: 19289085]
13. Moroishi T, Hansen CG, Guan KL (2015) The emerging roles of YAP and TAZ in cancer. *Nat Rev Cancer* 15(2):73–79. 10.1038/nrc3876 [PubMed: 25592648]
14. Johnson R, Halder G (2014) The two faces of Hippo: targeting the Hippo pathway for regenerative medicine and cancer treatment. *Nat Rev Drug Discov* 13(1):63–79. 10.1038/nrd4161 [PubMed: 24336504]
15. Harvey KF, Zhang X, Thomas DM (2013) The Hippo pathway and human cancer. *Nat Rev Cancer* 13(4):246–257. 10.1038/nrc3458 [PubMed: 23467301]
16. Zender L, Spector MS, Xue W, Flemming P, Cordon-Cardo C, Silke J, Fan S-T, Luk JM, Wigler M, Hannon GJ, Mu D, Lucito R, Powers S, Lowe SW (2006) Identification and validation of oncogenes in liver cancer using an integrative oncogenomic approach. *Cell* 125(7):1253–1267. 10.1016/j.cell.2006.05.030 [PubMed: 16814713]

17. Steinhardt AA, Gayyed MF, Klein AP, Dong J, Maitra A, Pan D, Montgomery EA, Anders RA (2008) Expression of Yes-associated protein in common solid tumors. *Hum Pathol* 39 (11):1582–1589. 10.1016/j.humpath.2008.04.012 [PubMed: 18703216]
18. Bhat KP, Salazar KL, Balasubramanian V, Wani K, Heathcock L, Hollingsworth F, James JD, Gumin J, Diefes KL, Kim SH, Turski A, Azodi Y, Yang Y, Doucette T, Colman H, Sulman EP, Lang FF, Rao G, Copray S, Vaillant BD, Aldape KD (2011) The transcriptional coactivator TAZ regulates mesenchymal differentiation in malignant glioma. *Genes Dev* 25(24):2594–2609. 10.1101/gad.176800.111 [PubMed: 22190458]
19. Cordenonsi M, Zanconato F, Azzolin L, Forcato M, Rosato A, Frasson C, Inui M, Montagner M, Parenti AR, Poletti A, Daidone MG, Dupont S, Basso G, Bicciato S, Piccolo S (2011) The Hippo transducer TAZ confers cancer stem cell-related traits on breast cancer cells. *Cell* 147(4):759–772. 10.1016/j.cell.2011.09.048 [PubMed: 22078877]
20. Horvath P, Barrangou R (2010) CRISPR/Cas, the immune system of bacteria and archaea. *Science* 327(5962):167–170. 10.1126/science.1179555 [PubMed: 20056882]
21. Makarova KS, Haft DH, Barrangou R, Brouns SJ, Charpentier E, Horvath P, Moineau S, Mojica FJ, Wolf YI, Yakunin AF, van der Oost J, Koonin EV (2011) Evolution and classification of the CRISPR-Cas systems. *Nat Rev Microbiol* 9(6):467–477. 10.1038/nrmicro2577 [PubMed: 21552286]
22. Bhaya D, Davison M, Barrangou R (2011) CRISPR-Cas systems in bacteria and archaea: versatile small RNAs for adaptive defense and regulation. *Annu Rev Genet* 45:273–297. 10.1146/annurev-genet-110410-132430 [PubMed: 22060043]
23. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, Zhang F (2013) Multiplex genome engineering using CRISPR/Cas systems. *Science* 339(6121):819–823. 10.1126/science.1231143 [PubMed: 23287718]
24. Mali P, Yang L, Esvelt KM, Aach J, Guell M, DiCarlo JE, Norville JE, Church GM (2013) RNA-guided human genome engineering via Cas9. *Science* 339(6121):823–826. 10.1126/science.1232033 [PubMed: 23287722]
25. Jinek M, East A, Cheng A, Lin S, Ma E, Doudna J (2013) RNA-programmed genome editing in human cells. *elife* 2:e00471 10.7554/eLife.00471 [PubMed: 23386978]
26. Cho SW, Kim S, Kim JM, Kim JS (2013) Targeted genome engineering in human cells with the Cas9 RNA-guided endonuclease. *Nat Biotechnol* 31(3):230–232. 10.1038/nbt.2507 [PubMed: 23360966]
27. Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, Zhang F (2013) Genome engineering using the CRISPR-Cas9 system. *Nat Protoc* 8:2281 10.1038/nprot.2013.143 [PubMed: 24157548]
28. Hsu PD, Lander ES, Zhang F (2014) Development and applications of CRISPR-Cas9 for genome engineering. *Cell* 157(6):1262–1278. 10.1016/j.cell.2014.05.010 [PubMed: 24906146]
29. Qi LS, Larson MH, Gilbert LA, Doudna JA, Weissman JS, Arkin AP, Lim WA (2013) Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. *Cell* 152(5):1173–1183. 10.1016/j.cell.2013.02.022 [PubMed: 23452860]
30. Tanenbaum ME, Gilbert LA, Qi LS, Weissman JS, Vale RD (2014) A protein-tagging system for signal amplification in gene expression and fluorescence imaging. *Cell* 159(3):635–646. 10.1016/j.cell.2014.09.039 [PubMed: 25307933]
31. Gilbert LA, Larson MH, Morsut L, Liu Z, Brar GA, Torres SE, Stern-Ginossar N, Brandman O, Whitehead EH, Doudna JA, Lim WA, Weissman JS, Qi LS (2013) CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. *Cell* 154 (2):442–451. 10.1016/j.cell.2013.06.044 [PubMed: 23849981]
32. Perez-Pinera P, Kocak DD, Vockley CM, Adler AF, Kabadi AM, Polstein LR, Thakore PI, Glass KA, Ousterout DG, Leong KW, Guilak F, Crawford GE, Reddy TE, Gersbach CA (2013) RNA-guided gene activation by CRISPR-Cas9-based transcription factors. *Nat Methods* 10:973 10.1038/nmeth.2600 [PubMed: 23892895]
33. Gilbert LA, Horlbeck MA, Adamson B, Villalta JE, Chen Y, Whitehead EH, Guimaraes C, Panning B, Ploegh HL, Bassik MC, Qi LS, Kampmann M, Weissman JS (2014) Genome-scale CRISPR-mediated control of gene repression and activation. *Cell* 159(3):647–661. 10.1016/j.cell.2014.09.029 [PubMed: 25307932]

34. Maeder ML, Linder SJ, Cascio VM, Fu Y, Ho QH, Joung JK (2013) CRISPR RNA-guided activation of endogenous human genes. *Nat Methods* 10:977 10.1038/nmeth.2598 [PubMed: 23892898]
35. Sanjana NE, Shalem O, Zhang F (2014) Improved vectors and genome-wide libraries for CRISPR screening. *Nat Methods* 11:783 10.1038/nmeth.3047 [PubMed: 25075903]
36. Zischewski J, Fischer R, Bortesi L (2017) Detection of on-target and off-target mutations generated by CRISPR/Cas9 and other sequence-specific nucleases. *Biotechnol Adv* 35(1):95–104. 10.1016/j.biotechadv.2016.12.003 [PubMed: 28011075]
37. Konermann S, Brigham MD, Trevino AE, Joung J, Abudayyeh OO, Barcena C, Hsu PD, Habib N, Gootenberg JS, Nishimasu H, Nureki O, Zhang F (2014) Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex. *Nature* 517:583 10.1038/nature14136 [PubMed: 25494202]
38. Shalem O, Sanjana NE, Hartenian E, Shi X, Scott DA, Mikkelsen TS, Heckl D, Ebert BL, Root DE, Doench JG, Zhang F (2014) Genome-scale CRISPR-Cas9 knockout screening in human cells. *Science* 343(6166):84–87. 10.1126/science.1247005 [PubMed: 24336571]
39. Nihongaki Y, Yamamoto S, Kawano F, Suzuki H, Sato M (2015) CRISPR-Cas9-based photoactivatable transcription system. *Chem Biol* 22(2):169–174. 10.1016/j.chembiol.2014.12.011 [PubMed: 25619936]
40. Lu J, Zhao C, Zhao Y, Zhang J, Zhang Y, Chen L, Han Q, Ying Y, Peng S, Ai R, Wang Y (2018) Multimode drug inducible CRISPR/Cas9 devices for transcriptional activation and genome editing. *Nucleic Acids Res* 46(5):e25 10.1093/nar/gkx1222 [PubMed: 29237052]
41. Mojica FJ, Diez-Villasenor C, Garcia-Martinez J, Almendros C (2009) Short motif sequences determine the targets of the prokaryotic CRISPR defence system. *Microbiology* 155 (Pt 3):733–740. 10.1099/mic.0.023960-0 [PubMed: 19246744]
42. Doench JG, Hartenian E, Graham DB, Tothova Z, Hegde M, Smith I, Sullender M, Ebert BL, Xavier RJ, Root DE (2014) Rational design of highly active sgRNAs for CRISPR-Cas9-mediated gene inactivation. *Nat Biotechnol* 32(12):1262–1267. 10.1038/nbt.3026 [PubMed: 25184501]
43. Doench JG, Fusi N, Sullender M, Hegde M, Vaimberg EW, Donovan KF, Smith I, Tothova Z, Wilen C, Orchard R, Virgin HW, Listgarten J, Root DE (2016) Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9. *Nat Biotechnol* 34(2):184–191. 10.1038/nbt.3437 [PubMed: 26780180]
44. Agrotis A, Ketteler R (2015) A new age in functional genomics using CRISPR/Cas9 in arrayed library screening. *Front Genet* 6:300 10.3389/fgene.2015.00300 [PubMed: 26442115]

Fig. 1. CRISPR technology can be used to modulate gene expression. **(a)** Cas9 can be specifically targeted to a region of the gene encoding YAP by a 20 nucleotide sgRNA, where it subsequently introduces DSBs that are repaired by error-prone NHEJ leading to indel mutations and gene inactivation. **(b)** Catalytically inactive Cas9 (dCas9) fused to a transcriptional repressor (e.g. KRAB) can be specifically targeted to the promoter region of *YAP1* by a sgRNA, thus repressing YAP transcription. **(c)** Catalytically inactive Cas9 (dCas9) fused to a transcriptional activator (e.g. VP64) can be specifically targeted to the promoter region of *YAP1* by a sgRNA, thus promoting YAP transcription

Fig. 2. Genetic knockout of YAP/TAZ using CRISPR. **(a)** Two clones of RPE-1 cells stably expressing Cas9 and a sgRNA targeting the *YAP1* gene were isolated and total YAP and TAZ levels were assessed by western blotting. Clone 2 shows complete loss of *YAP1* protein. **(b)** Two clones of RPE-1 cells stably expressing Cas9 and a sgRNA targeting the *WWTR1* gene were isolated and total YAP and TAZ levels were assessed by western blotting. Clone 2 shows complete loss of TAZ protein