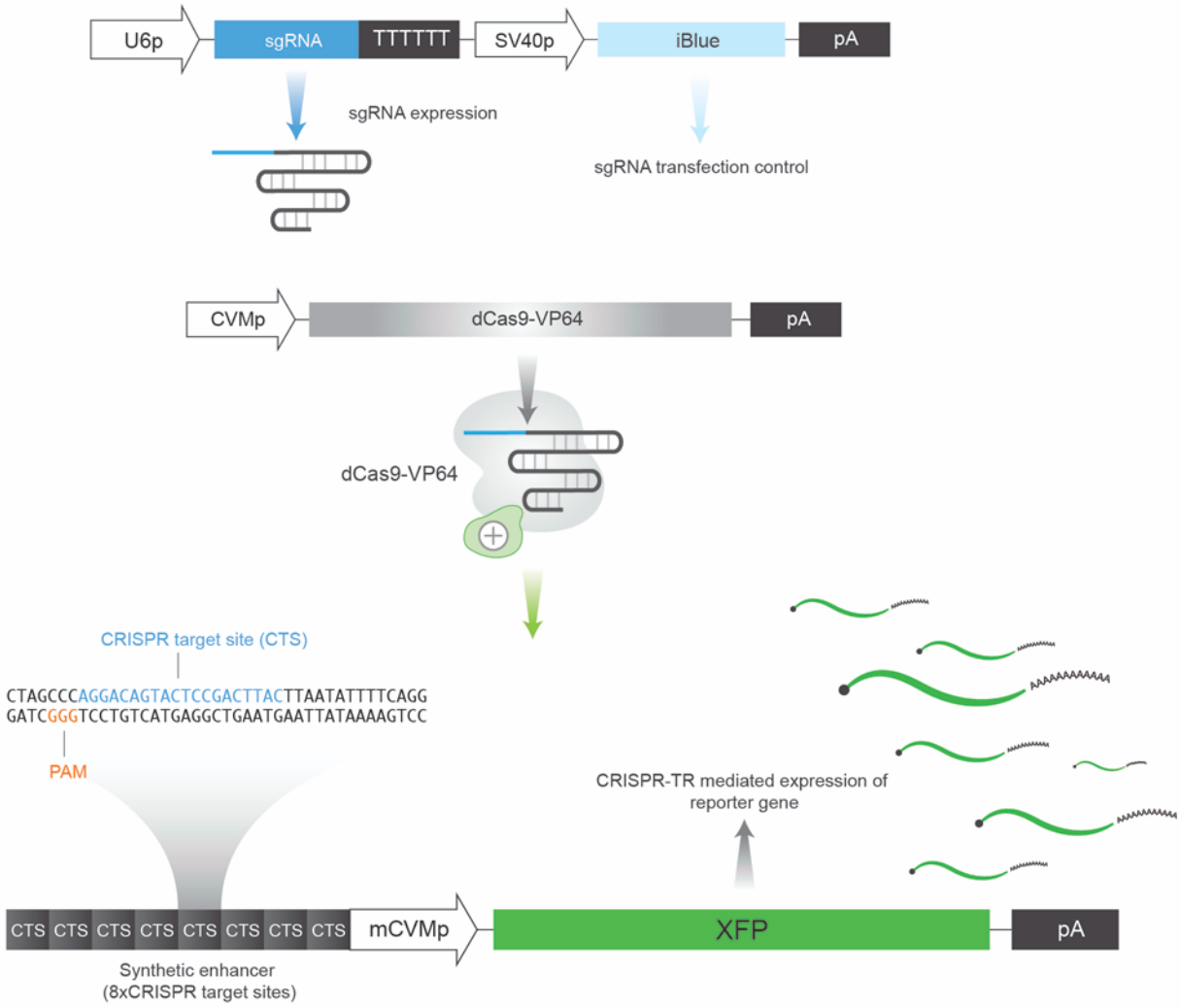
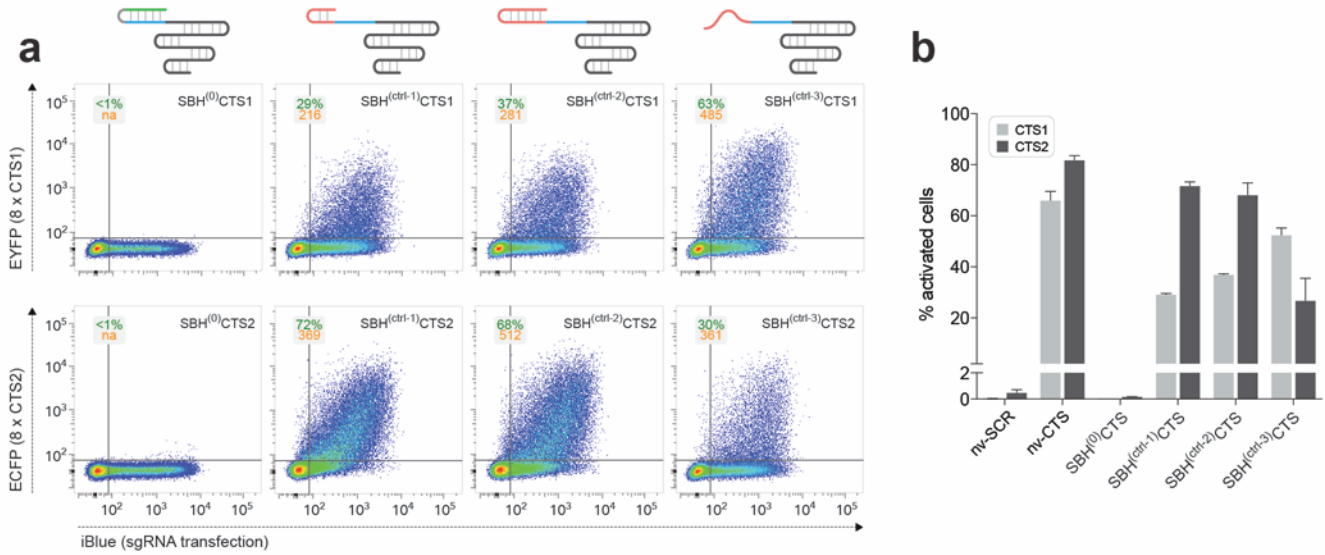
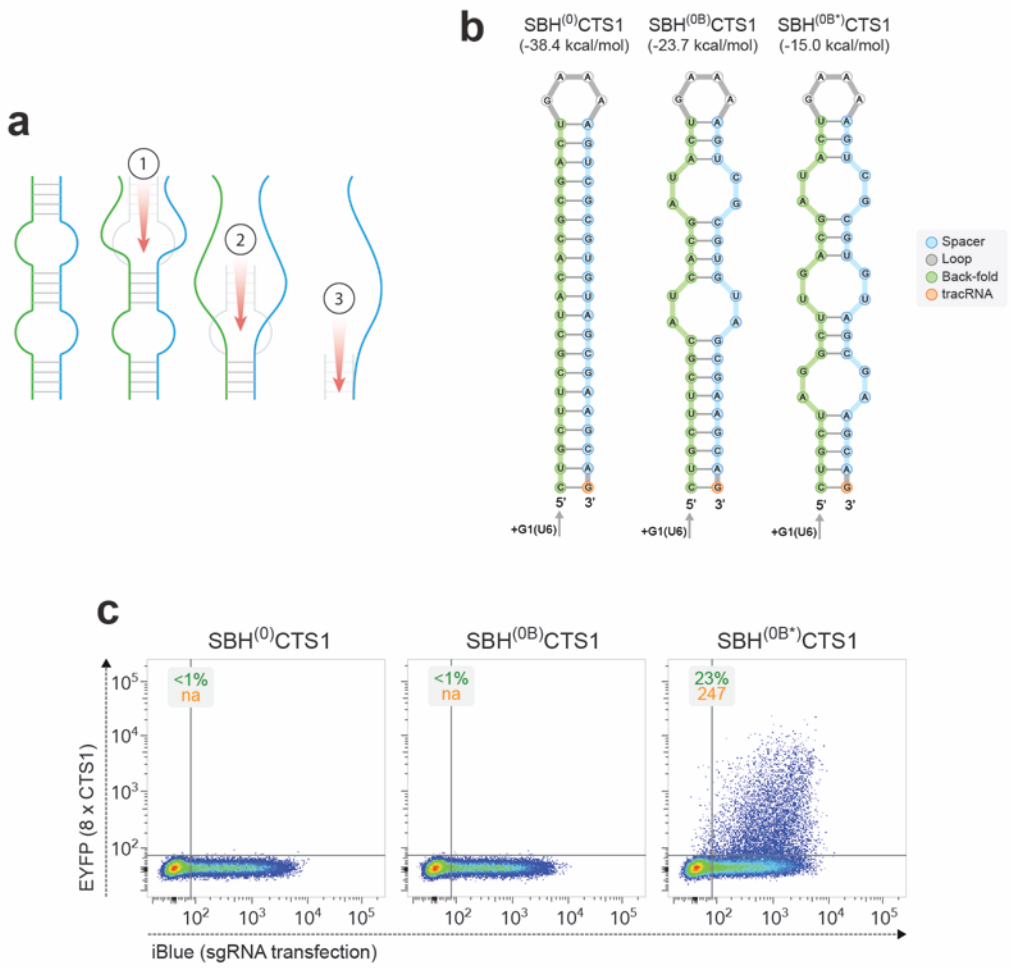


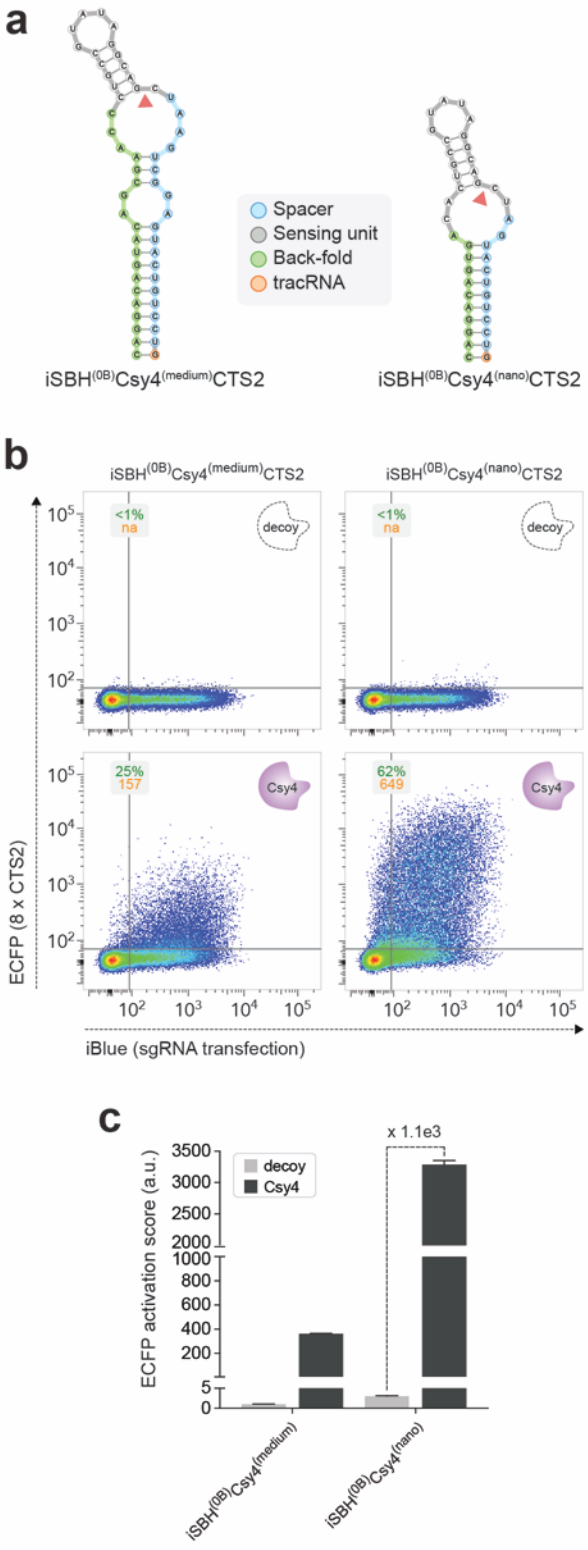
Supplementary Figure 1. Schematic of CRISPR-TR reporter assay in HEK293-T cells. A bicistronic vector is used to couple the expression of U6-driven sgRNAs with a fluorescent reporter (iBlue). The dCas9-VP64 gene is expressed under a CMV promoter from a separate plasmid. The assembled sgRNA-dCas9-VP64 complex is targeted to the synthetic enhancer (8 x CRISPR target sites (CTS)) driving the expression of a target gene (XFP), by programing the sgRNA spacer sequence. In this study two sgRNA spacers were programmed (CTS1, CTS2), to drive specific expression (with no detectable cross-talk) of two corresponding reporter target genes (8xCTS1-mCMVp-EYFP-pA and 8xCTS2-mCMVp-ECFP-pA respectively).



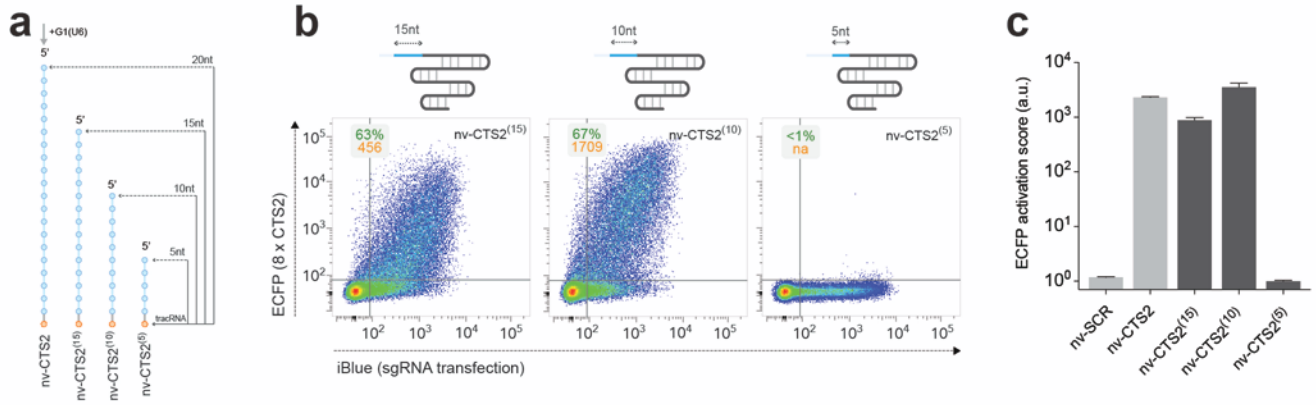
Supplementary Figure 2. Control SBH-sgRNA mimics. (a) HEK-293T cells were co-transfected with plasmids containing dCas9-VP64, 8xCTS1-mCMVp-EYFP or 8xCTS2-mCMVp-ECFP reporter, and the following SBH-sgRNAs: i) SBH$^{(0)}$CTS1 and SBH$^{(0)}$CTS2 (silent prototype SBH-sgRNAs with full spacer coverage); ii) SBH$^{(ctrl-1)}$CTS1 and SBH$^{(ctrl-1)}$CTS2 (control SBH-sgRNAs with accessible spacer and offset 5' end 10 bp hairpin structure); iii) SBH$^{(ctrl-2)}$CTS1 and SBH$^{(ctrl-2)}$CTS2 (control SBH-sgRNAs with accessible spacer and offset 5' end 20 bp hairpin structures); SBH$^{(ctrl-3)}$CTS1 and SBH$^{(ctrl-3)}$CTS2 (control SBH-sgRNAs with a scramble back-fold segment). Representative flow cytometry scatter plots (48h post-transfection) show reporter activation (EYFP, ECFP channels) plotted against sgRNA transfection (iBlue channel) for CTS1 and CTS2 spacers. Plots for SBH$^{(0)}$CTS1 and SBH$^{(ctrl-1)}$CTS1 are shared with Fig. 1d. Insets show % of activated cells (double iBlue^{+ve} and EXFP^{+ve}, green) and median reporter fluorescence intensity for this population (orange). **(b)** Graph represents percentage of activated (EXFP^{+ve}) sgRNA expressing (iBlue^{+ve}) cells for all conditions in (a) and Fig. 1d (n=3, mean +/- s.d.).



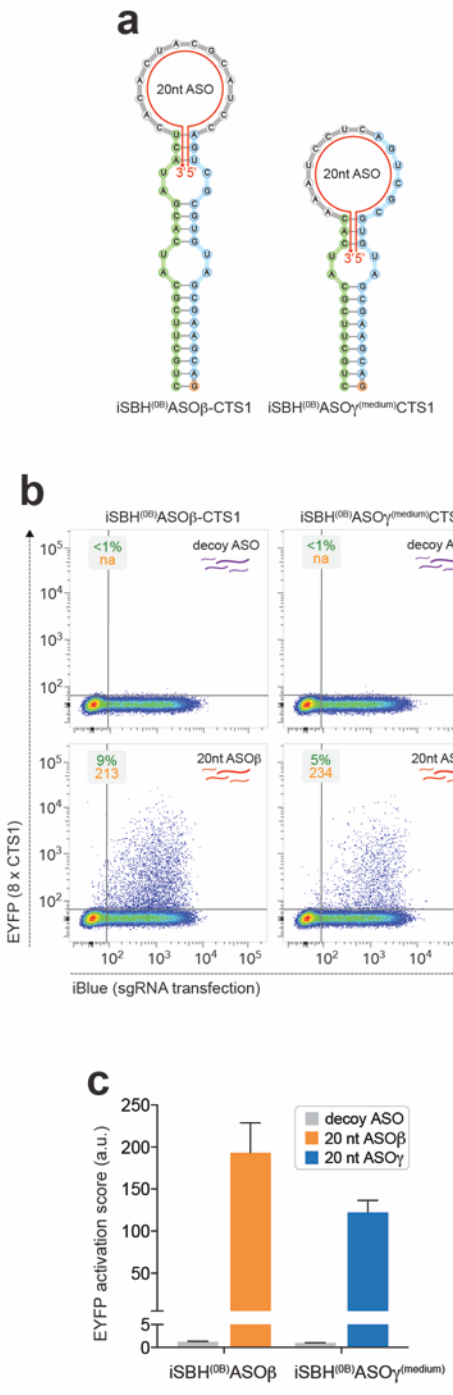
Supplementary Figure 3. Design of SBHs with reduced thermodynamic stability. (a) Hypothetical framework showing the low energy stepwise strand separation expected post-cleavage as a consequence of bulge incorporation into the SBH stem architecture. **(b)** Sequence and RNA secondary structure corresponding to the prototype SBH$^{(0)}$CTS1 design, and alternative architectures containing two (SBH$^{(0B)}$CTS1) and three (SBH$^{(0B*)}$CTS1) stem bulges. The calculated minimal free energy of each structure is shown in brackets. **(c)** Representative flow cytometry scatter plots (EYFP reporter fluorescence against iBlue sgRNA transfection) reveals complete CRISPR-TR silencing in both SBH$^{(0)}$CTS1 and SBH$^{(0B)}$CTS1 designs. In contrast, addition of a third bulge renders the sgRNA active, presumably due to excessive destabilisation of the stem structure. Insets show % of activated cells (double iBlue^{+ve} and EYFP^{+ve}, green) and median reporter fluorescence intensity for this population (orange).



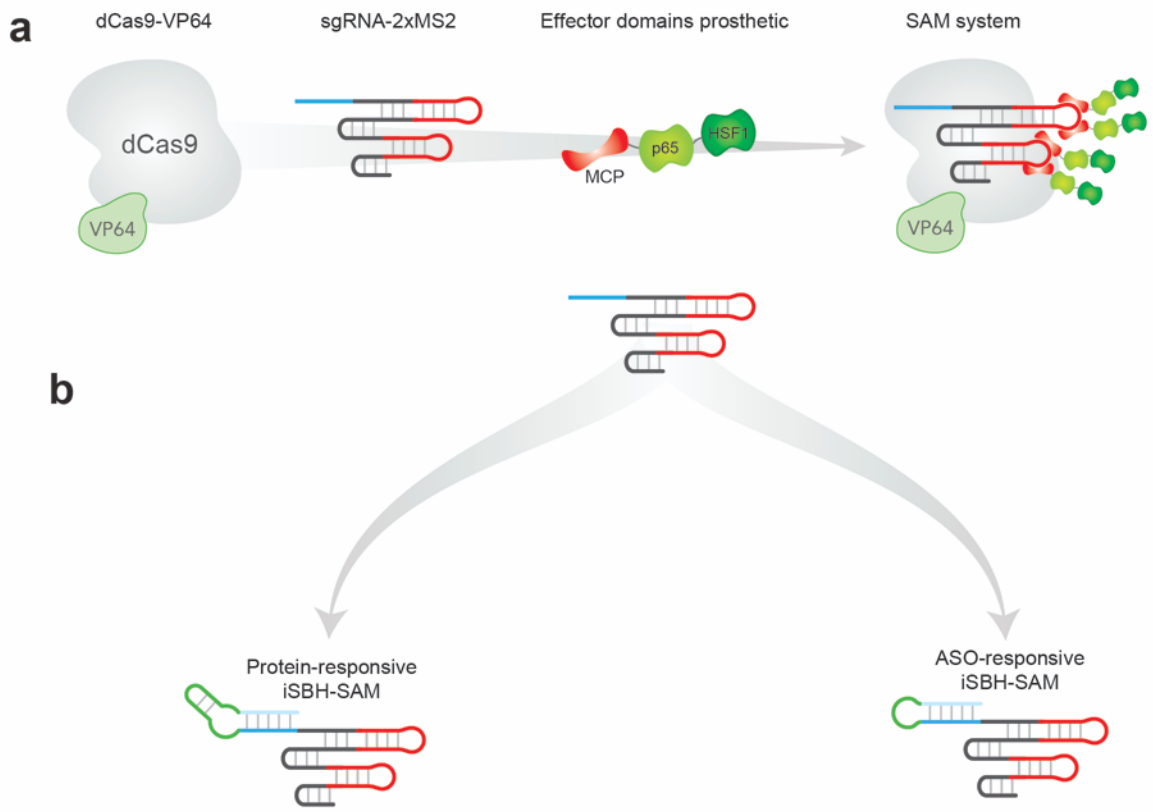
Supplementary Figure 4. Optimisation of Csy4-responsive iSBH designs for CTS2. (a) Sequence and RNA secondary structures of SBH$^{(0B)}$Csy4 medium, and nano stem design (red arrow = Csy4 cleavage site) for the CTS2 spacer. **(b)** Representative CRISPR-TR assay flow cytometry scatter plots for iSBH$^{(0B)}$Csy4$^{(medium)}$CTS2 and iSBH$^{(0B)}$Csy4$^{(nano)}$CTS2 sgRNAs in the absence (OFF-state, top) and presence (ON-state, bottom) of Csy4. Insets show % of activated cells (double iBlue^{+ve} and ECFP^{+ve}, green) and median reporter fluorescence intensity for this population (orange).**(c)** Quantification of reporter activation using the two iSBH variants in the presence of a decoy plasmid or Csy4 inducer from three biological replicates (n = 3, mean +/- SD, a.u. arbitrary units).



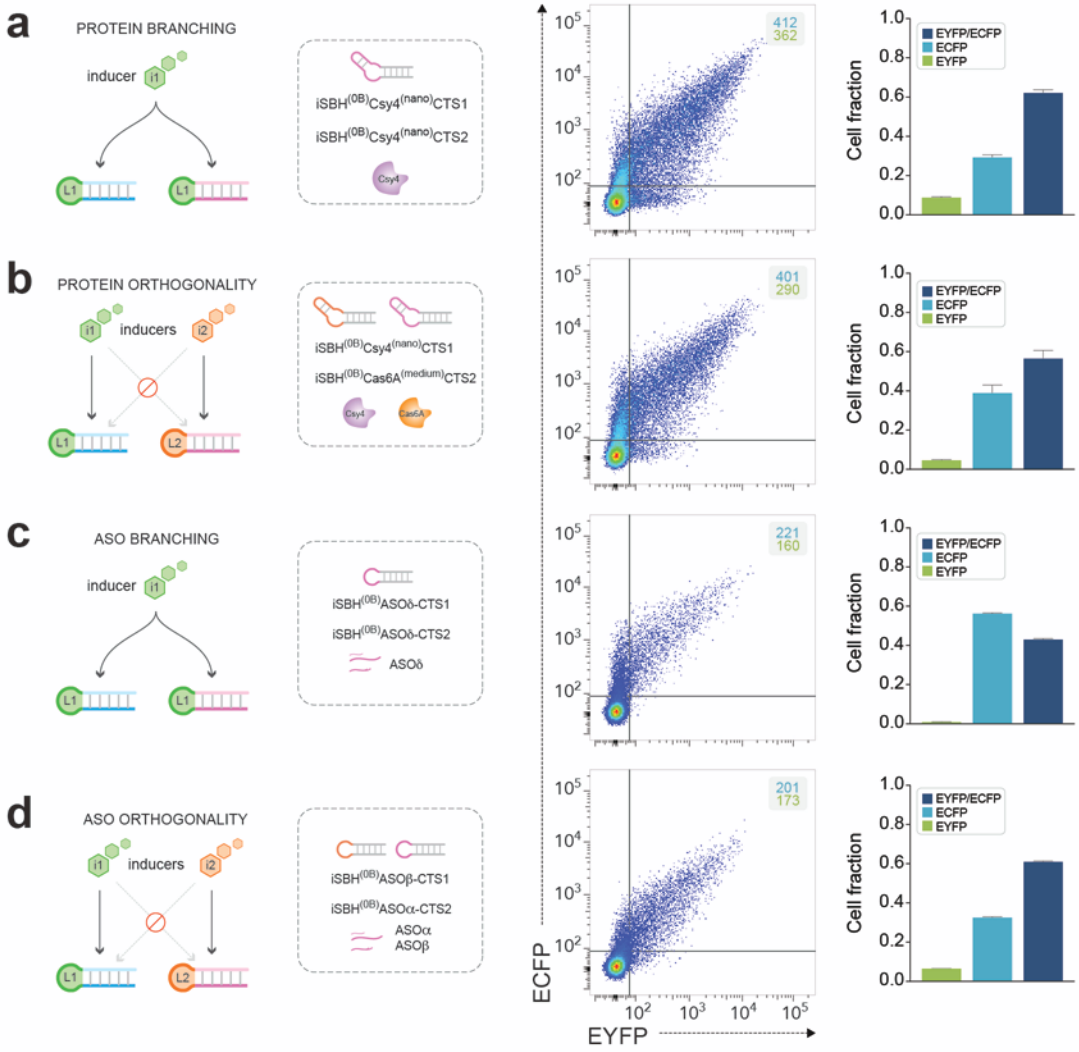
Supplementary Figure 5. Effect of sgRNA spacer shortening on CRISPR-TR activity. (a) Progressive shortening (5' to 3') of a native sgRNA spacer length (nv-CTS2 = native 20nt long spacer; nv-CTS2$^{(15,\ 10,\ 5)}$ = truncated spacers). **(b)** Representative flow cytometry scatter plots showing the impact of incremental spacer shortening (nv-CTS2$^{(15,\ 10,\ 5)}$) on reporter gene activation (axes represent ECFP reporter expression against sgRNA plasmid transfection for the three truncated sgRNA spacers). Insets show % of activated cells (double iBlue^{+ve} and ECFP^{+ve}, green) and median reporter fluorescence intensity for this population (orange). **(c)** Quantification of ECFP reporter activation from matched experiments using the following sgRNAs: nv-SCR (control 20nt scramble spacer), nv-CTS2 (matching 20nt spacer), nv-CTS2$^{(15)}$(matching 15nt spacer), nv-CTS2$^{(10)}$(matching 10nt spacer), nv-CTS2$^{(5)}$(matching 5nt spacer) (n = 3, mean +/- s.d., a.u. arbitrary units).



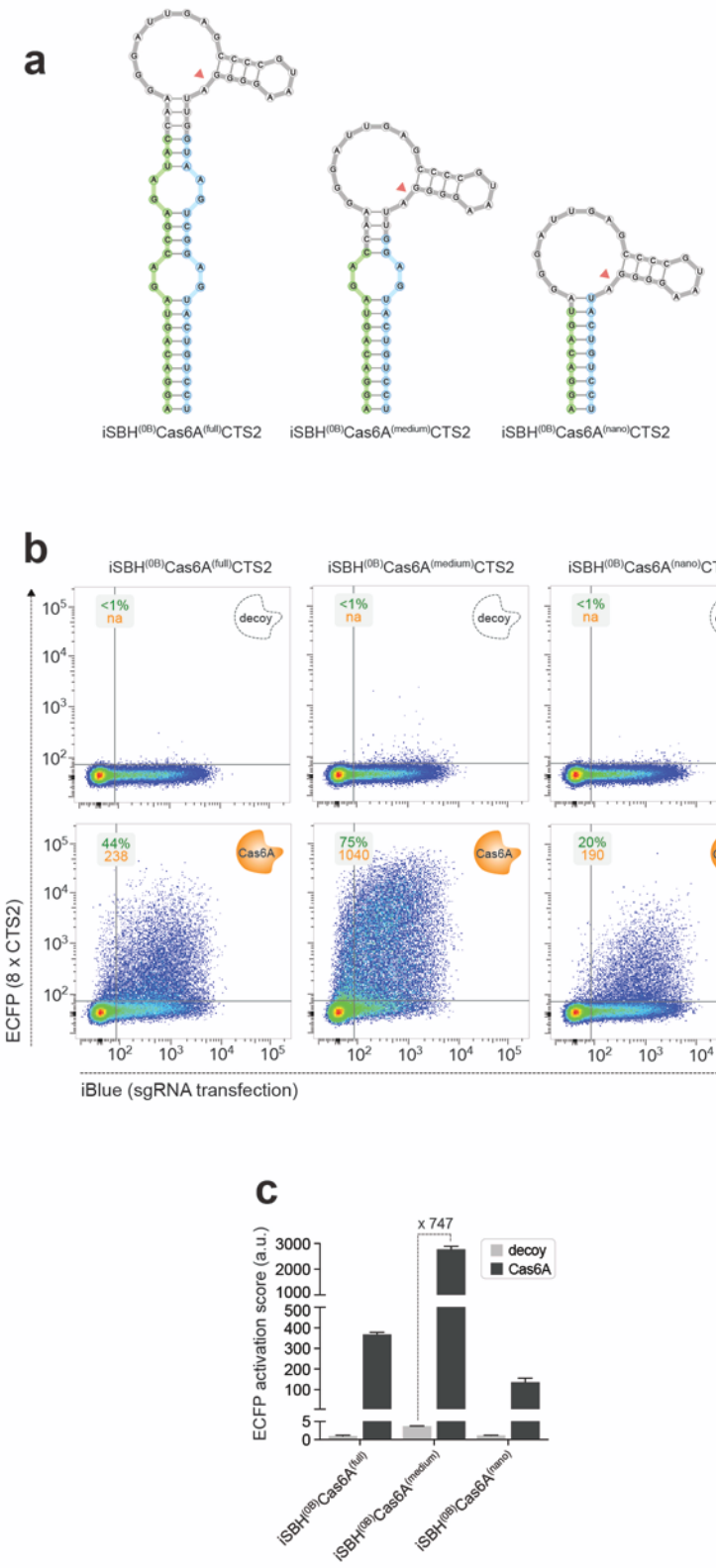
Supplementary Figure 6. Evaluation of two ASO responsive iSBH designs. (a) Sequence and RNA secondary structure of ASO-iSBH constructs with different stem length architectures. The iSBH$^{(0B)}$ASO β-CTS1 design was generated by replacing the 4nt loop in SBH$^{(0B)}$CTS1 with a 14nt sensing loop complementary to ASOβ. Extrapolating from the observation that shorter protein-responsive iSBH stems tend to display superior behavior, we also engineered iSBH$^{(0B)}$ASOγ$^{(medium)}$CTS1 by converting the distal bulge into a 14nt ASO sensing-loop, which incorporates 6 spacer nucleotides and was evolved to enforce an open ssRNA secondary structure. **(b)** Representative flow cytometry scatter plots (EYFP reporter fluorescence against iBlue sgRNA transfection) using the two ASO-responsive iSBH sgRNAs designs, in the presence of a decoy ASO (OFF-state) or active cognate ASO (ON-state). Insets show % of activated cells (double iBlue^{+ve} and EYFP^{+ve}, green) and median reporter fluorescence intensity for this population (orange). **(c)** Quantification of reporter activation scores for all conditions shown in (b) (n = 3 biological replicates, mean +/- s.d., a.u. arbitrary unit).



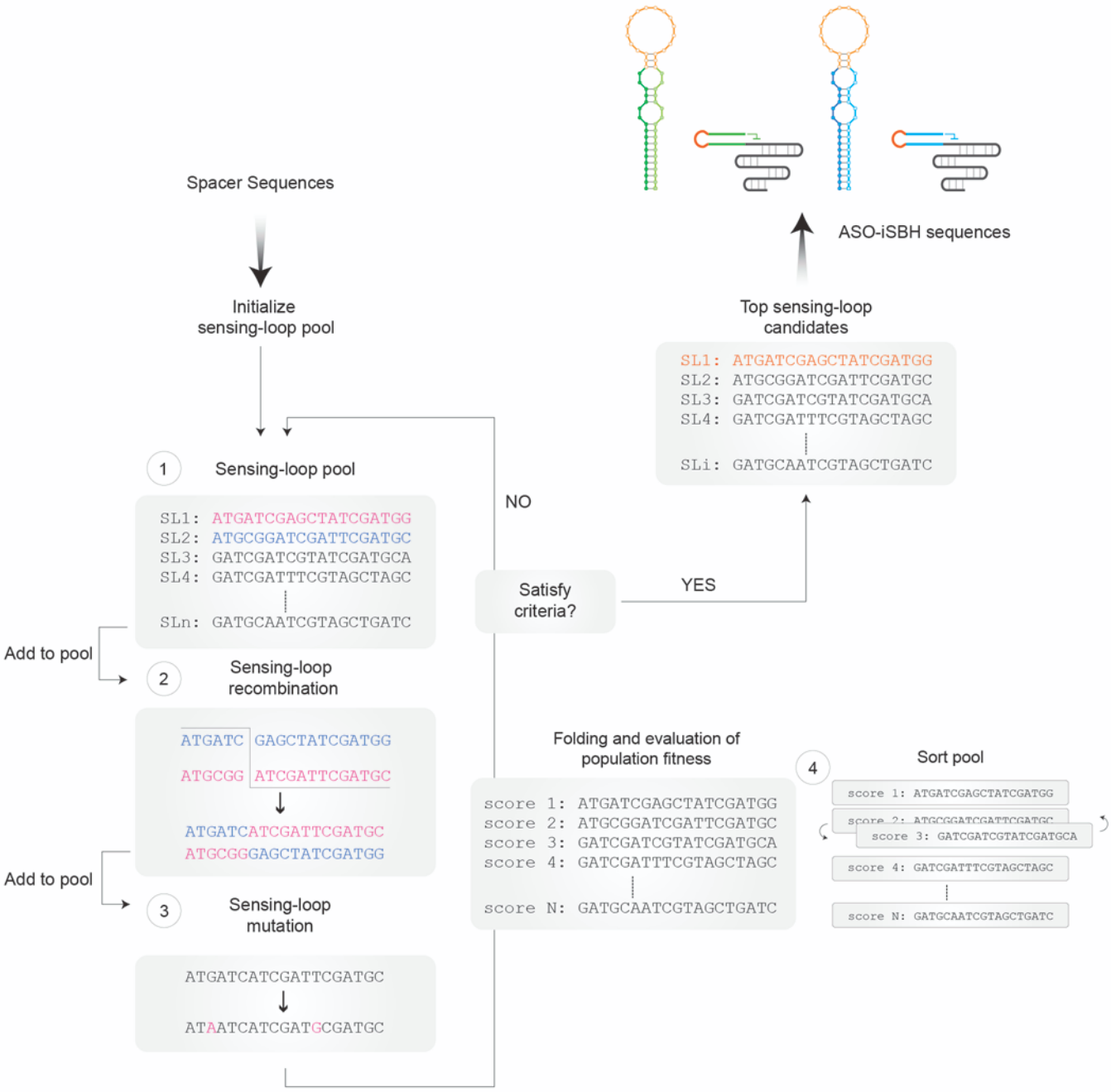
Supplementary Figure 7. iSBH for SAM-based CRISPR-TR. (a) Schematic diagram detailing the SAM system for single sgRNA CRISPR-mediated transcriptional activation of endogenous target genes[11]. dCas9-VP4 is complexed with a modified sgRNA-2xMS2 (red hairpins) capable of recruiting additional 4x p65-HSF1 effectors through MS2-MCP interactions. **(b)** Modification of sgRNA-2xMS2 with protein or ASO-responsive iSBH.



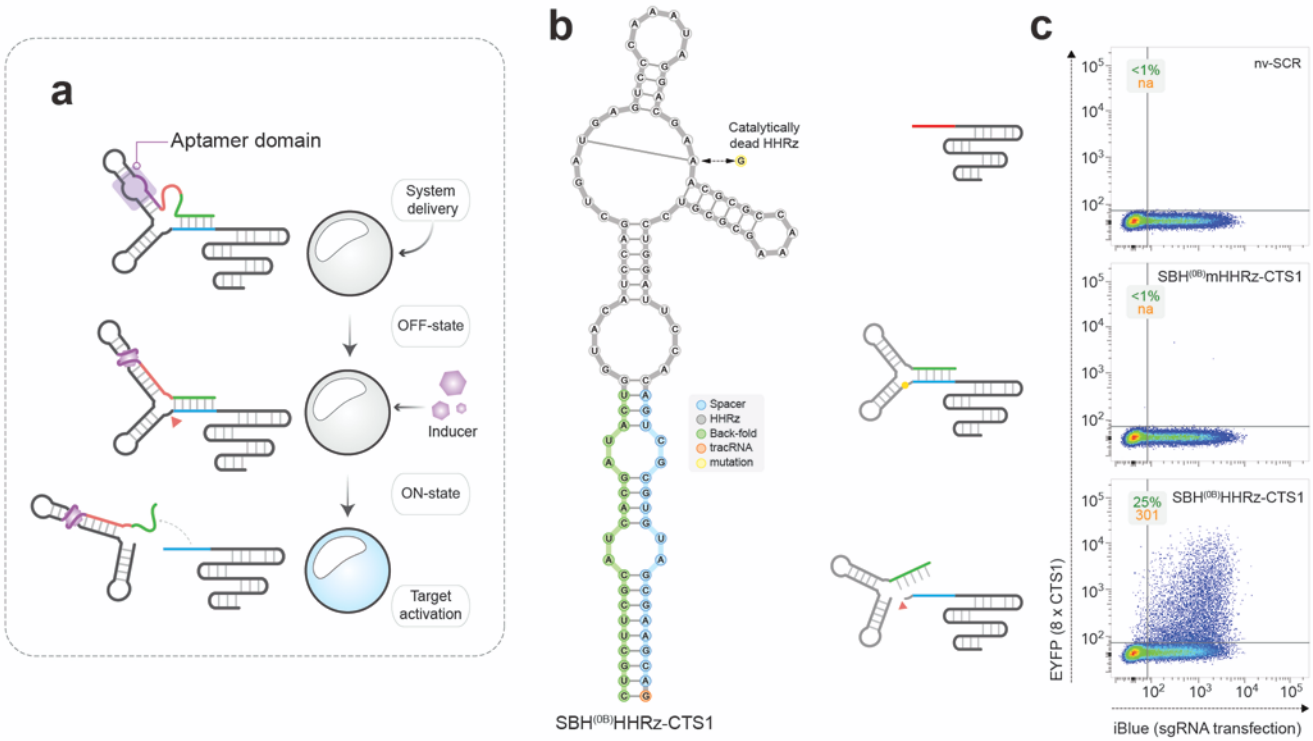
Supplementary Figure 8. Evaluation of reporter co-activation in single cells. (a-d) Flow cytometry analysis (48h post-transfection) of cell fractions expressing EYFP and ECFP reporter targets in four gene network module implementations: **(a)** branched activation with single protein inducer (related to Fig. 4b); **(b)** simultaneous orthogonal activation with two protein inducers (related to Fig. 4d); **(c)** branched activation with single ASO inducer (related to Fig. 5b); **(d)** ASO-mediated orthogonal activation of two reporter genes (related to Fig. 5d). Panels show the schematic of each gene module, system components, raw flow cytometry scatter plots and quantification of $EYFP^{+ve}$, $ECFP^{+ve}$, and double positive subpopulation frequencies within activated cells (n = 3 biological replicates, mean +/- s.d.). In all conditions between 40-60% of activated cells co-express both reporter genes. Insets show median reporter fluorescence intensity on the ECFP (light blue) and EYFP (green) channels in the double positive cell population ($ECFP^{+ve}$ and $EYFP^{+ve}$).



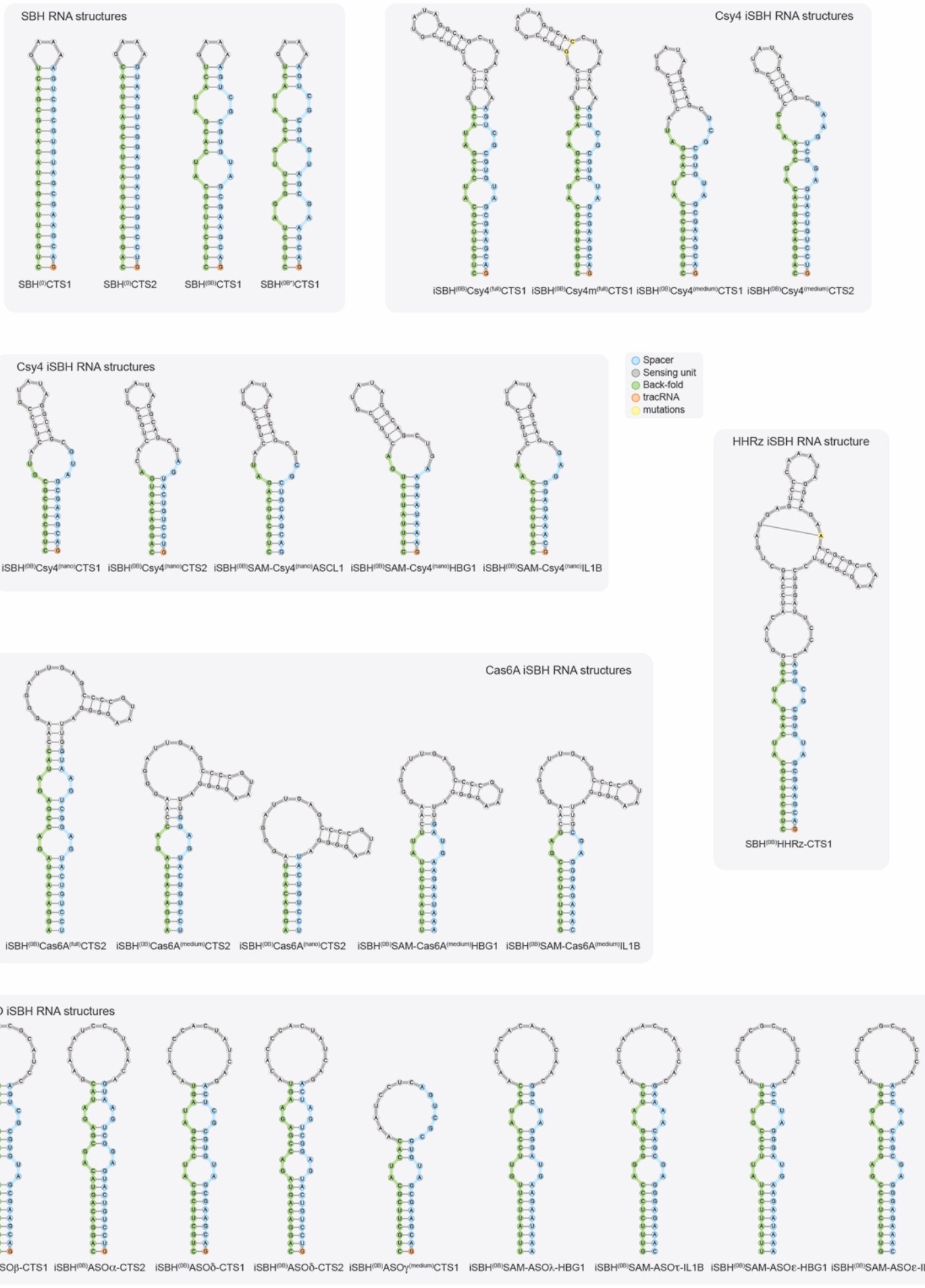
Supplementary Figure 9. Optimisation of Cas6A-responsive iSBH designs for CTS2 spacer. (a) Sequence and RNA secondary structures of SBH$^{(0B)}$Cas6A$^{(full)}$ sgRNA compared to the *medium*, and *nano* stems (CTS2 spacer; red arrow = Cas6A cleavage site) obtained by grafting the Cas6A RNA box onto the distal or proximal SBH$^{(0B)}$CTS2 bulge. **(b)** Representative CRISPR-TR assay flow cytometry scatter plots for iSBH$^{(0B)}$Cas6A$^{(full)}$CTS2, iSBH$^{(0B)}$Cas6A$^{(medium)}$CTS2 and iSBH$^{(0B)}$Cas6A$^{(nano)}$CTS2 sgRNAs in the absence (OFF-state, top) and presence (ON-state, bottom) of Cas6A. Insets show % of activated cells (double iBlue^{+ve} and ECFP^{+ve}, green) and median reporter fluorescence intensity for this population (orange). **(c)** Quantification of ECFP reporter activation score using the three iSBH variants in the presence of a decoy plasmid or Cas6A inducer from three biological replicates (n = 3, mean +/- s.d., a.u. arbitrary units).



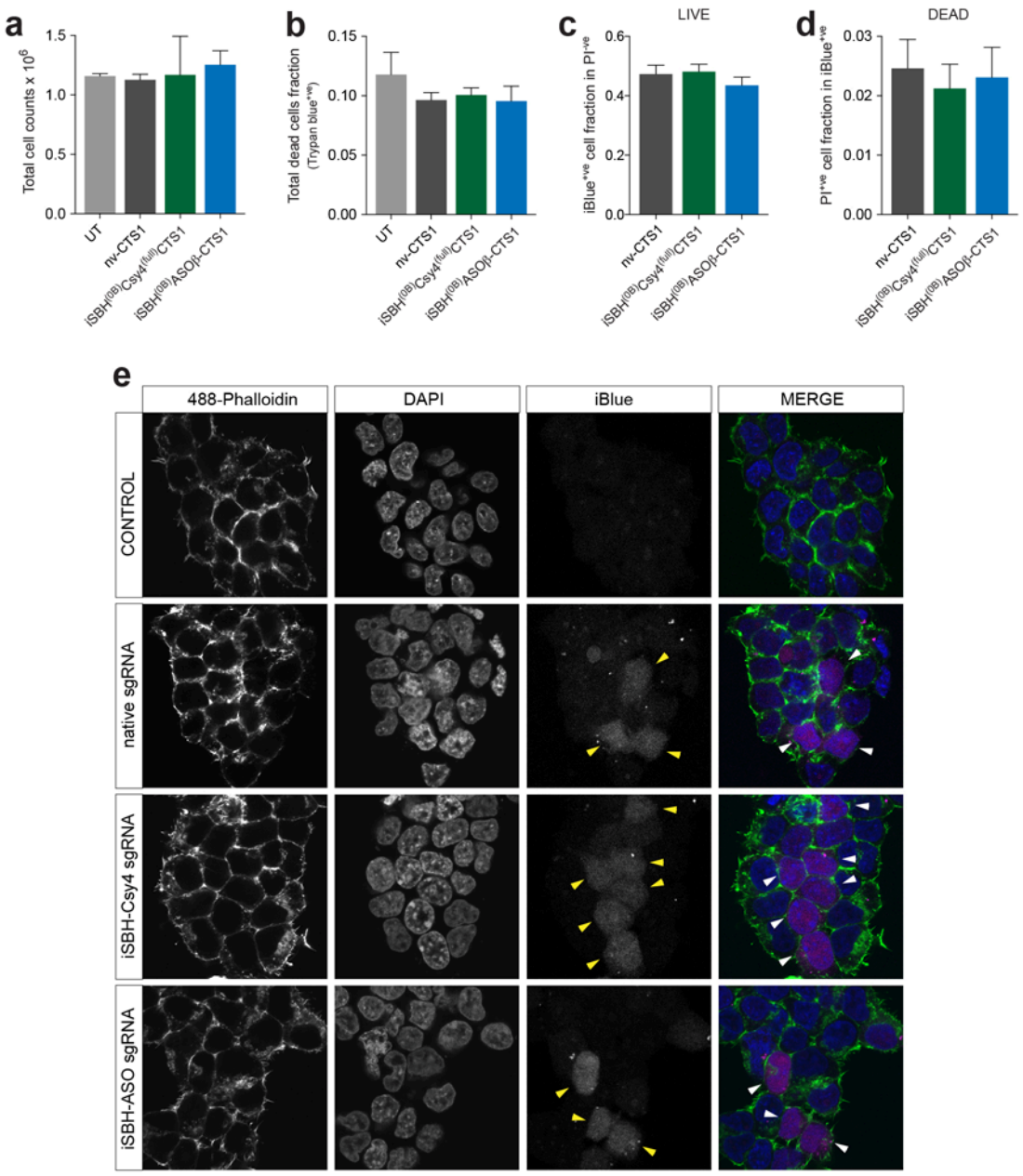
Supplementary Figure 10. *In silico* evolution of a common ASO sensing-loop satisfying ASO-iSBH folding conditions across multiple spacers using *iSBHfold*. For an input set of spacers (sp1, sp2, etc.) the algorithm aims to evolve a shared sensing-loop which can be grafted onto all SBH$^{(0B)}$spX while ensuring accessibility to ASO binding by enforcing structural constraints (open ssRNA loop conformation). Loops satisfying these conditions are evolved from an initial pool of 20nt RNA sequences [1]. A custom-made genetic algorithm is then run iteratively on this pool over several generations to optimise the sensing-loop sequences. For each iteration the following steps are performed: i) initial sequences are recombined to generate offspring sequences which are added to the original parent population [2]; ii) the pool is further enriched with sequences obtained by randomly mutating the existing segments as well as new fully randomised ones [3]. iii) finally, each sequence is folded within the corresponding iSBH using NUPACK[21] and attributed a folding score (FSi) measuring the similarity between the predicted RNA secondary structure and that of the target ASO-iSBH [4]. The ensuing pool is then sorted and only the fittest sequences are considered for the next iteration (repeat steps [1] to [4]). Once a user-defined criteria based on score or number of iterations is reached, the system outputs a list of top sensing-loop candidates used to create the ASO-responsive iSBHs.



Supplementary Figure 11. HHRz-based iSBH spacer release mechanism. (a) Theoretical framework underlying the use of allosteric hammerhead ribozymes (aHHRz) for iSBH-sgRNA-based CRISPR-TR conditional control of gene expression. An iSBH equipped with an engineered aHHRz containing a ligand-sensing aptamer domain is delivered to cells. In the absence of a cognate ligand (OFF-state) the aHHRz assumes an inactive conformation, sequestering the spacer and inhibiting CRISPR-TR. Delivery of the cognate ligand promotes a change in conformation (strand displacement), which allows the aHHRZ to resume its catalytic activity. aHHRz cleavage facilitates back-fold dissociation from the spacer, rendering the sgRNA competent for CRISPR-TR-mediated activation of its programmed target gene (ON-state). **(b)** Sequence and RNA secondary structure of a prototype HHRz-SBH. The active HHRz is directly fused to the SBH$^{(0B)}$CTS1. A to G point mutation (yellow) renders the HHRz inactive (mHHRz). **(c)** Representative CRISPR-TR assay flow cytometry scatter plots with nv-SCR, SBH$^{(0B)}$HHRz-CTS1, and SBH$^{(0B)}$mHHRz-CTS1 sgRNAs (EYFP reporter fluorescence against iBlue sgRNA transfection). Insets show % of activated cells (double iBlue^{+ve} and EYFP^{+ve}, green) and median reporter fluorescence intensity for this population (orange).



Supplementary Figure 12. Catalogue of silent (SBH) and and inducible (iSBH) designs used in the study organised by inducer and target type.



Supplementary Figure 13. Analysis of cellular toxicity and stress following delivery of sgRNA variants. HEK293-T cells were transfected with either a native sgRNA (nv-CTS1; NV), Csy4-responsive iSBH-sgRNA (iSBH$^{(0B)}$Csy4$^{(nano)}$CTS1; Csy4-SBH), or ASO-responsive iSBH-sgRNA (iSBH$^{(0B)}$ASO β-CTS1; ASO-SBH). Untransfected control cells were treated with PEI in the absence of plasmid DNA (UT). Cells were harvested 48h post-transfection, counted (hemocytometer) and stained with Propidium iodide (PI) before flow cytometry analysis. **(a)** Total cell counts for UT, NV, Csy4-SBH and ASO-SBH conditions. **(b)** Fraction of total dead cells measured by Trypan Blue staining. **(c)** Flow cytometry quantification of sgRNA expressing cell fraction (iBlue+ve) amongst live cells (PI-ve).**(d)** Flow cytometry quantification of dead cell fraction (PI+ve) amongst cells expressing sgRNAs (iBlue+ve). **(e)** Confocal microscopy analysis of cellular morphology (488-Phalloidin, green) and nuclear integrity (DAPI, blue) in UT cells compared to cells transfected with nv-CTS1, iSBH$^{(0B)}$Csy4$^{(nano)}$CTS1 and iSBH$^{(0B)}$ASOβ-CTS1 sgRNA variants (iBlue, magenta).

U6-sgRNA-6xT cassette (U6 promoter, scramble spacer segment with 2x BbsI sites, sgRNA scaffold, terminator)

GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTGGAATT
AATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTT
GCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTG
GCTTTATATATCTTGTGGAAAGGACGAAACACCGGGTCTTCGAGAAGACCTGTTTTAGAGCTAGAAATAGCA
AGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTT

Human codon optimized Cas6A (Kozak sequence, 5' NLS, TTCas6A)

GCCACCATGGCCCCAAAGAAGAAGCGGAAGGTCGGTATCCACGGAGTCCCAGCAGCCGAAAACCTG
TACTTCCAATCCAATGCAGCTAGCGTGCTTGCCGCGCTTGTGCTCGTGTTGGAAGGGGAAGGCCTC
CCGGAGCCGTTGGGTCTTAGGGGTTTCTTTTACGGCCTTTTGCGCGAGGTAGCGCCAGAGGTACAC
GATCAAGGAGAAAATCCTTTTGCTCTGGGATTCGGCGGGAGGGAAGGCGCAGCTTGGGCTAGGGTT
AGTCTTCTCGTAGAAGGGTTGTATGCGCGACTCGCACCTAGACTCTATGCGCTTGAAGGTGAGGAA
GTTCGCCTTGGACCTCCGTTCCGGGTACGCGCAGTCTTGCAAGAGGGCCATCCTTGGGCTGGAGTT
TCAACGTATCCACGCTTGTTCCAGGGACCTCCCTCCAGAGATCTGGCCCTCCGATTTGCAAGTCCA
ACCTTCTTCCGCCGGAAAGGCGTTCACTACCCCGTGCCGGAACCTCGCCTGGTTCTGGAGTCCCTC
CTGCGGCGGTTGGAAGCATTCGGTCCACTTAAAGCCCCGGAAGGAGTGAGAGAAGCTCTTCTGGAG
AGAACCACGGTGCGATCATTGGAGGGACGCACACTTCCGGCGAGGACGGAAGTTGACACAGCTGGA
TTTGTAGGCAGGGTCGTGTACCACCTGCCTAGAGCGACAGAGGAAGAAGCACTCTGGTTGTCTGCG
TTGGGCCGATTCGCGTTCTATTCAGGAGTAGGTGCAAAGACCAGCTTGGGATATGGTAGAGCACGA
GCAGAATCTGCGTAG

Supplementary Figure 14. DNA sequences of U6-sgRNA-6xT cassette and the human codon optimized Cas6A.

Oligo/Primer name	Sequence (5'-3')
Fwd_sgRNA-cassette_SpeI	TAGACTAGTGAGGGCCTATTTCCCATG
Rev_sgRNA-cassette_BcoDI	CTAGTCTCTGCTAGCAAAAAAGCACCGACTCGG
Fwd_SV40_NcoI	CTAGCCTAACTCCGCCCAGTTCC
Rev_SV40	GATCAGATCCGAAAATGGATATAC
Fwd_iBlue	GTATATCCATTTTCGGATCTGATCGCCACCATGTCGGTACC
Rev_iBlue_BsrGI	CTACTATGTACATCATTTGGACTGAGACTGTGC
HHRz_oligo_1	TAGGAAGACTACACCGCTGCTTCGCTACACGCGACTGGTACA
HHRz_oligo_2	CGCGTTTCGTCCTATTTGGGACTCATCAGCTGGATGTACCAGTCGCGTGTAG
HHRz_oligo_2m	CGCGTCTCGTCCTATTTGGGACTCATCAGCTGGATGTACCAGTCGCGTGTAG
HHRz_oligo_3	CCAAATAGGACGAAACGCGCCAAAGCGCGTCCTGGATTCCACAGTCGCGTG
HHRz_oligo_3m	CCAAATAGGACGAGACGCGCCAAAGCGCGTCCTGGATTCCACAGTCGCGTG
HHRz_oligo_4	CTAGAAGACTAAAACTGCTTCGCTACACGCGACTGTGGAATCC
HHRz_oligo_flanking _fwd	TAGGAAGACTACACCGCTGC
HHRz_oligo_flanking _rev	CTAGAAGACTAAAACTGCTTCGCT
Fwd_pX330_NLS	GACCTGCATCCCAAGCTTACGCGTGCCACCATGGCCCCAAAGAAGAAGCG
Rev_pX330_NLS	GGTCGCTAGCTGCATTGGATTGGAAGTACAGGTTTTCGGCTGCTGGGACTCCGTG

Supplementary Table 1. Cloning oligonucleotides and PCR primers used in the study.

NAME	Plasmid backbone	Insert (5' overhang CACC, 3' overhang GTTT)
nv-SCR	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GGGTCTTCGAGAAGACCT
nv-CTS1	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GAGTCGCGTGTAGCGAAGCA
nv-CTS2	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GTAAGTCGGAGTACTGTCCT
SBH(0)CTS1	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GCTGCTTCGCTACACGCGACTGAAAAGTCGCGTGTAGCGAAGCA
SBH(0)CTS2	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GCAGGACAGTACTCCGACTTACGAAAGTAAGTCGGAGTACTGTCCT
SBH(ctrl-1)CTS1	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GAAAGCAGCCGAAAGGCTGCTTTCAGTCGCGTGTAGCGAAGCA
SBH(ctrl-1)CTS2	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GACAAGCAGCCGAAAGGCTGCTTGTCGTAAGTCGGAGTACTGTCCT
SBH(ctrl-2)CTS1	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GCAGGACAGTACTCCGACTTACGAAAGTAAGTCGGAGTACTGTCCTGAGTCGCGTGTAGCGAAGCA
SBH(ctrl-2)CTS2	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GCTGCTTCGCTACACGCGACTGAAAAGTCGCGTGTAGCGAAGCAGGTAAGTCGGAGTACTGTCCT
SBH(ctrl-3)CTS1	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GTAGCGATCAGTAGTCAGAATGAAAAGTCGCGTGTAGCGAAGCA
SBH(ctrl-3)CTS2	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GACGTAGAGTTAGTTAGCTAGAGAAAGTAAGTCGGAGTACTGTCCT
SBH(5)CTS1	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GCGCTACACGCGACTGAAAAGTCGCGTGTAGCGAAGCA
SBH(5)CTS2	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GAGTACTCCGACTTACGAAAGTAAGTCGGAGTACTGTCCT
SBH(10)CTS1	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GCACGCGACTGAAAAGTCGCGTGTAGCGAAGCA
SBH(10)CTS2	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GTCCGACTTACGAAAGTAAGTCGGAGTACTGTCCT
SBH(15)CTS1	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GGACTGAAAAGTCGCGTGTAGCGAAGCA
SBH(15)CTS2	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GCTTACGAAAGTAAGTCGGAGTACTGTCCT
wt-CTS1(5)	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GAAGCA
wt-CTS2(5)	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GTCCT
wt-CTS1(10)	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GTAGCGAAGCA
wt-CTS2(10)	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GTACTGTCCT
wt-CTS1(15)	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GCGTGTAGCGAAGCA
wt-CTS2(15)	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GTCGGAGTACTGTCCT
SBH(0B)CTS1	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GCTGCTTCGCATCACGATACTGAAAAGTCGCGTGTAGCGAAGCA
SBH(03xB)CTS1	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GCTGCTAGGCTTGACGATACTGAAAAGTCGCGTGTAGCGAAGCA
iSBH(0B)Csy4(full)CTS1	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GCTGCTTCGCATCACGATACTGTTCACTGCCGTATAGGCAGCTAAGAAAAGTCGCGTGTAGCGAAGCA
iSBH(0B)Csy4m(full)CTS1	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GCTGCTTCGCATCACGATACTGTTCAGTGCCGTATAGGCACCTAAGAAAAGTCGCGTGTAGCGAAGCA
iSBH(0B)Csy4(medium)CTS	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GCTGCTTCGCATCACGATACTGCCGTATAGGCAGCTCGCGTGTAGCGAAGCA
iSBH(0B)Csy4(medium)CTS	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GCAGGACAGTACAGCGAACCCTGCCGTATAGGCAGCTAAGTCGGAGTACTGTCCT
iSBH(0B)Csy4(nano)CTS1	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GCTGCTTCGCGTACTGCCGTATAGGCAGCGTAGCGAAGCA
iSBH(0B)Csy4(nano)CTS2	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GCAGGACAGTGACACTGCCGTATAGGCAGCTAGTACTGTCCT
SAM_nv-SCR	SAM_U6-sgRNA-2xMS2	GGGTCTTCGAGAAGACCT
SAM_nv-HBG1	SAM_U6-sgRNA-2xMS2	GGCTAGGGATGAAGAATAAA
SAM_nv-IL1B	SAM_U6-sgRNA-2xMS2	GAAAACAGCGAGGGAGAAAC
iSBH(0B)Csy4(nano)ASCL1	SAM_U6-sgRNA-2xMS2	GCTGCTGCAGATACTGCCGTATAGGCAGCTCGCTGCAGCAG
iSBH(0B)Csy4(nano)HBG1	SAM_U6-sgRNA-2xMS2	GCTTTATTCTGACTGCCGTATAGGCAGCTGAAGAATAAA
iSBH(0B)Csy4(nano)IL1B	SAM_U6-sgRNA-2xMS2	GCGTTTCTCCAAACTGCCGTATAGGCAGCGAGGGAGAAAC
iSBH(0B)Cas6A(full)CTS2	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GAGGACAGTAGACCGAGATACCAAGGGATTGAGCCCCGTAAGGGGATTGGTAAGTCGGAGTACTGTCCT
iSBH(0B)Cas6A(medium)CT	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GAGGACAGTAGACCAAGGGATTGAGCCCCGTAAGGGGATTGGAGTACTGTCCT
iSBH(0B)Cas6A(nano)CTS2	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GAGGACAGTAGGGATTGAGCCCCGTAAGGGGATACTGTCCT
iSBH(0B)Cas6A(medium)HB	SAM_U6-sgRNA-2xMS2	GTTTATTCTTATTCAAGGGATTGAGCCCCGTAAGGGGATTGATGAAGAATAAA
iSBH(0B)Cas6A(medium)IL1	SAM_U6-sgRNA-2xMS2	GTTTCTCCCGAGCAAGGGATTGAGCCCCGTAAGGGGATTGCGAGGGAGAAAC
iSBH(0B)ASO1-CTS1	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GCTGCTTCGCATCACGATACTCACACTACGCATCCAGTCGCGTGTAGCGAAGCA
iSBH(0B)ASO2-CTS2	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GCAGGACAGTACAGCGAGATACGAACATCCCTAACAGTAAGTCGGAGTACTGTCCT
iSBH(0B)ASO-CTS1	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GCTGCTTCGCATCACGATAGTACACCCACTATCAGACTCGCGTGTAGCGAAGCA
iSBH(0B)ASO-CTS2	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GCAGGACAGTAGACCGAGAAGTACACCCACTATCAGACTAGTCGGAGTACTGTCCT
iSBH(0B)ASO1(medium)CTS	pcDNA3.1_U6-sgRNA-6T_SV40-iBlue-pA	GCTGCTTCGCATCACAAATCCTCAGTCGCGTGTAGCGAAGCA
iSBH(0B)ASO1-HBG1	SAM_U6-sgRNA-2xMS2	GTTTATTCTTGTTCCCATGCCAACACACACAACGGCTAGGGATGAAGAATAAA
iSBH(0B)ASO2-IL1B	SAM_U6-sgRNA-2xMS2	GGTTTCTCCCAGGCTGAATTCAACCAAACCAACACGAAAACAGCGAGGGAGAAAC
iSBH(0B)ASO-HBG1	SAM_U6-sgRNA-2xMS2	GTTTATTCTTATTCCCGTGGTTACCGCGCCTCCACACCTAGGGATGAAGAATAAA
iSBH(0B)ASO-IL1B	SAM_U6-sgRNA-2xMS2	GGTTTCTCCCGAGCTGAGGGTTACCGCGCCTCCACACCAACAGCGAGGGAGAAAC
SBH(0B)HHRz-CTS1	SAM_U6-sgRNA-2xMS2	GCTGCTTCGCATCACGATACTGGTACATCCAGCTGATGAGTCCCAAATAGGACGAAACGCGCCAAAGCGCGTCCTGGATTCCACAGTCGCGTGTAGCGAAGCA
SBH(0B)mHHRz-CTS1	SAM_U6-sgRNA-2xMS2	GCTGCTTCGCATCACGATACTGGTACATCCAGCTGATGAGTCCCAAATAGGACGAGACGCGCCAAAGCGCGTCCTGGATTCCACAGTCGCGTGTAGCGAAGCA

Supplementary Table 2. Sequence of native sgRNA spacer and SBH / iSBH hairpins used in the study together with the corresponding cloning vectors.

NAME	Sequence	Corresponding ASO-iSBH
ASOα-14	T*G*T*TAGGGATGT*T*C*G	iSBH$^{(0B)}$ASOα-CTS2
ASOα-255	T*A*C*T*GTTAGGGATGTTCGTAT*C*T*C*G	iSBH$^{(0B)}$ASOα-CTS2
ASOβ	A*C*T*G*GATGCGTAGTGT*G*A*G*T	iSBH$^{(0B)}$ASOβ-CTS1
ASOγ	C*A*C*G*CGACTGAGGATT*T*G*T*G	iSBH$^{(0B)}$ASOγ$^{(medium)}$CTS1
ASOα	T*A*C*T*GTTAGGGATGTT*C*G*T*A	iSBH$^{(0B)}$ASOα-CTS2
ASOδ	A*G*T*C*TGATAGTGGGTG*T*A*C*T	iSBH$^{(0B)}$ASOδ-CTS1
		iSBH$^{(0B)}$ASOδ-CTS2
ASOλ	G*C*C*G*TTGTGTGTGTGT*T*G*G*C	iSBH$^{(0B)}$ASOλ-HBG1
ASOτ	T*T*C*G*TGTTGGTTTGGT*T*G*A*A	iSBH$^{(0B)}$ASOτ-IL1B
ASOε	G*G*T*G*TGGAGGCGCGGT*A*A*C*C	iSBH$^{(0B)}$ASOε-HBG1
		iSBH$^{(0B)}$ASOε-IL1B

Supplementary Table 3. ASO sequences used in the study.

Target mRNA	Forward Primer Sequence (5'-3')	Reverse Primer Sequence (5'-3')
GAPDH	AACAGCGACACCCACTCCTC	CATACCAGGAAATGAGCTTGACAA
dCas9-VP64 (SAM)	AACCTATGCCCACCTGTTCG	ATCCAGGATTGTCTTGCCGG
HBG1	GTTGTCTACCCATGGACCCA	TCTCCCAAGGAAGTCAGCAC
IL1B	CGAATCTCCGACCACCACTA	AGGGAAAGAAGGTGCTCAGG

Supplementary Table 4. RT-qPCR primers used in the study.