Supplementary Tables

Construct	Gene size (bp)	Gene size (kDa)	Mechanism of activation	Activator	Editing Kinetics Reported	Earliest Timepoint at which indels detected	Single-component	Dose-dependent editing demonstrated	Specificity relative to WT Cas9	Reference
ciCas9	4521	173	Disruption of autoinhibitory BCL-xL/BH3 Protein-peptide interaction	A-385358, WEHI-539, ABT-737, et al.	Indels + DSBs	30 min (AAVS1, VEGFAg2, EMX1*) 2 h (MYC sgRNA4 and sgRNA5, VEGFAg2)	Yes	Yes	Improved	This report
iCas9	8058	305	ERT2-mediated nuclear localization	4-HT	Indels (no statistics, errors, or clear indication of replicates reported for time points before 12 h)	2h (EMX1, no statistics or replicates reported)	Yes	No*	Improved	8
intein-Cas9	5451	208	Excision of intein	4-HT	Indels (12 - 96h), reported in [8]	Not tested prior to 12 h	Yes	No	Improved	10
split-Cas9	4974	189	Reconstitution of split-Cas9 via induced dimerization	Rapamycin	Indels (12 - 96h), reported in [8]	Not tested prior to 12 h	No	No	Not reported	11
arC9	4905	189	Allosteric effect of 4HT binding	4-HT	No	NA	Yes	Yes	Not reported	12
paCas9	5469*	207	Reconstitution of split-Cas9 via induced dimerization	Blue light	Indels	9h (EMX1)	No	No	Not reported	9
split-Cas9-ERT	6924*	263	Reconstitution of split-Cas9 via Rapamyxin induced dimerization + 4-HT induced ERT2-mediated nuclear localization	Rapamycin and/or 4HT	No	NA	No	Demonstrated for transcriptional control, but not editing	Not reported	13
DIG-Cas9, PRO-Cas9	4566	176	Small-molecule mediated stabilization of destabilized ligand binding domain fused to Cas9	Digoxin, Progesterone	No	NA	Yes	No	Not reported	14
DD-Cas9	Not reported	Not reported	Small-molecule mediated stabilization of destabilized ligand binding domain fused to Cas9	Shield-1 (rapalog)	No	NA	Yes	No	Not reported	15
DD regulated Cas9 (DD.SpCas9.DD)	5175 (DHFR), 5697 (ER50)	198 (DHFR), 218 (ER50)	Small-molecule mediated stabilization of destabilized ligand binding domains (DHFR or ER50) fused to Cas9	Trimethoprim (DHFR), 4HT (ER50), CMP8 (ER50)	No	NA	Yes	Yes	Improved	16

Supplementary Table 1 | Comparison of inducible Cas9 variants. NA – not applicable. *Size (bp) includes 2 stop codons. Indicated split-Cas9 constructs delivered via 2 plasmids.

Method	Site specific	Multiplex	Absolute quantitation demonstrated	References
Immunodetection	No	Yes	No	17
Comet	No	No	No	18
Pulse-field gel electrophoresis	No	No	No	19
BLESS/DSB-capture	No	Yes	No	24,31
GUIDE-Seq	No	Yes	No	20
qPCR	Yes	No	No	21,22
Enzymatic methods	Yes	Yes	No	23
DSB-ddPCR	Yes	No	Yes	This work

Supplementary Table 2 l Comparison of methods for detecting DSBs.

Time (h)	0	0.167	0.5	2	4	8	24
AAVS1	0.15±0.09	0.38±0.03	0.47±0.02	2.59±0.14	5.64±0.52	10.41±1.52	17.04±0.84
MYC sgRNA5	0.37±0.07	0.47±0.11	0.44±0.12	1.42±0.04	2.31±0.31	6.05±0.61	12.07±0.75
VEGFA sgRNA2	0.0012±0.0006	0.0069±0.0031	0.009±0.008	0.44±0.12	0.60±0.05	1.43±0.13	5.08±1.62
VEGFA sgRNA3	1.59±0.11	2.05±0.09	2.49±0.39	6.92±0.47	10.52±0.58	15.76±0.33	26.72±0.58

Supplementary Table 3 | Indel kinetics for multiple sgRNAs. Indel frequencies (% ± s.e.m., n = 3 cell culture replicates) plotted in **Fig. 1d**.

Time (h)	0.167	0.5	2	4	8	24
AAVS1	0.0348	0.0121	5.71E-05	0.000249	0.00126	1.81E-05
MYC sgRNA5	0.254	0.317	8.56E-05	0.00191	0.000373	5.091E-05
VEGFA sgRNA2	0.0689	0.196	0.0103	0.000148	0.000199	0.0174
VEGFA sgRNA3	0.0154	0.0464	0.000195	5.73E-05	1.13E-06	9.02E-07

Supplementary Table 4 | *P*-values for comparisons to respective zero hour time point. *P*-values for comparisons of each time point to the zero hour time point for each sgRNA as plotted in **Fig. 1d**. All sgRNAs exhibit statistically significant increase in indels by 2 hours. *P*-values were calculated using a one-sided two-sample Student's t-test (n = 3 cell culture replicates).

Variant	BH3 peptide sequence	K_i (nM)
G22 (ciCas9)	APPNLWAAQRYGRELRRMADE**G**EGSFK	48 ± 5
A22	APPNLWAAQRYGRELRRMADE**A**EGSFK	36 ± 2
V22	APPNLWAAQRYGRELRRMADE**V**EGSFK	20 ± 3
L22	APPNLWAAQRYGRELRRMADE**L**EGSFK	1.7 ± 0.2
F22	APPNLWAAQRYGRELRRMADE**F**EGSFK	< 0.5

Supplementary Table 5 | BH3 variant peptide sequences and BCL-xL K_i's.

Compound	BCL-xL K_i (nM)	Selectivity	Dose (mg/kg)	C_{max} (μM)	Exposure	Dosing	Species	Reference
ABT-263	0.40	dual BCL-2 / BCL-xL	100	7.66†	> 3.8 μM for 5.7 h†	p.o.	Mouse	[43]
ABT-263	0.40	dual BCL-2 / BCL-xL	315§	4.46§	>1.7 μM for 24 hours§	p.o.	Human	[44]
ABT-737	0.080	dual BCL-2 / BCL-xL	100	5.94	Not reported	i.p.	Mouse	[43]
A-1155463	< 0.010	Selective BCL-xL	5*	1.8*	> 0.18 μM for 12 hours*	i.p.	Mouse	[39]

Supplementary Table 6 | Pharmacokinetic properties of BH3/BCL-xL disruptors in mice and humans. All three compounds have been shown to be well tolerated in mice in multi-day dosing regimes, lasting between 14 to 21 days. †C_{max} and exposure were determined on day three of a three day consecutive daily dosing regime, steady-state pharmacokinetic study. §Dose given as (mg/day). C_{max} and exposure were determined on day 1 of dosing regime of consecutive daily doses for 14 days. 315 mg/day represents the max tolerated dose in humans. *C_{max} and exposure were determined following a single i.p. dose in mice.

Supplementary Note 1: Peptide Sequences for constructs and domains

Cas9:
MDYKDDDDKDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKN
LIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRL
EESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLA
LAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILS
ARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSK
DTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKR
YDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPI
LEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASA
QSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSG
EQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDL
LKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKR
RRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDI
QKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIE
MARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYL
QNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDN
VPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVE
TRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREIN
NYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKAT
AKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLS
MPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYS
VLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLP
KYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDN
EQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAE
NIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQL
GGDSRADPKKKRKV

ciCas9:
MDYKDDDDKDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKN
LIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRL
EESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLA
LAHMIKFRGHFLIEGDLNPDNSSNRELVVDFLSYKLSQKGYSWSQFSDVEENR
TEAPEGTESEMETPSAINGNPSWHLADSPAVNGATGHSSSLDAREVIPMAAVK
QALREAGDEFELRYRRAFSDLTSQLHITPGTAYQSFEQVVNELFRDGVNWGRI
VAFFSFGGALCVESVDKEMQVLVSRIAAWMATYLNDHLEPWIQENGGWDTFV
ELYGNNGSGTASGTGSGTGSATGSGTVNTEITKAPLSASMIKRYDEHHQDLTLL
KALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELL
VKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTF
RIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFD
KNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFK
TNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNE

ENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSR
KLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDS
LHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ
KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQEL
DINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNY
WRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDS
RMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNA
VVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFF
KTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQ
TGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSK
KLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKR
MLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHY
LDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPA
AFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDSRADPKKKRK
VTGSGTAPPNLWAAQRYGRELRRMADEGEGSFK

BCL-xL (res 2-197):
SNRELVVDFLSYKLSQKGYSWSQFSDVEENRTEAPEGTESEMETPSAINGNPS
WHLADSPAVNGATGHSSSLDAREVIPMAAVKQALREAGDEFELRYRRAFSDLT
SQLHITPGTAYQSFEQVVNELFRDGVNWGRIVAFFSFGGALCVESVDKEMQVL
VSRIAAWMATYLNDHLEPWIQENGGWDTFVELYGNN

FLAG:
DYKDDDDK

NLS:
SRADPKKKRKV

enhanced specificity-ciCas9 (e-ciCas9):
MDYKDDDDKDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKN
LIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRL
EESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLA
LAHMIKFRGHFLIEGDLNPDNSSNRELVVDFLSYKLSQKGYSWSQFSDVEENR
TEAPEGTESEMETPSAINGNPSWHLADSPAVNGATGHSSSLDAREVIPMAAVK
QALREAGDEFELRYRRAFSDLTSQLHITPGTAYQSFEQVVNELFRDGVNWGRI
VAFFSFGGALCVESVDKEMQVLVSRIAAWMATYLNDHLEPWIQENGGWDTFV
ELYGNNGSGTASGTGSGTGSATGSGTVNTEITKAPLSASMIKRYDEHHQDLTLL
KALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELL
VKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTF
RIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFD
KNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFK
TNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNE
ENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSR
KLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDS

LHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ
KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQEL
DINRLSDYDVDHIVPQSFLADDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNY
WRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDS
RMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNA
VVGTALIKKYPALESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFF
KTEITLANGEIRKAPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQ
TGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSK
KLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKR
MLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHY
LDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPA
AFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDSRADPKKKRK
VTGSGTAPPNLWAAQRYGRELRRMADEGEGSFK

Supplementary References

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