A well-controlled BioID design for endogenous bait proteins

Supplementary Information

Giel Vandemoortele[1,2], Delphine De Sutter[1,2], Aline Moliere[1,2], Jarne Pauwels[1,2], Kris Gevaert[1,2] and Sven Eyckerman[1,2,*]

[1]VIB Center for Medical Biotechnology, VIB, B-9000 Ghent, Belgium

[2]Department of Biochemistry, Ghent University, B-9000 Ghent, Belgium

*Correspondence to Prof. Dr. Sven Eyckerman

VIB Center for Medical Biotechnology

A. Baertsoenkaai 3, B-9000 Ghent, Belgium

Tel: +32-9-264.92.18

Fax: +32-9-264.94.90

Email: sven.eyckerman@vib-ugent.be

TABLE OF CONTENTS

PCR	Construct/Purpose	Forward primer (5'-3')	Reverse primer (5'-3')
TP53 5'HR	pAav-p53-T2A-BirA*	CACTAGGGGTTCCTGCGGCCGCAAACGCG TCAGCCCTGCACAGACATTTT	CGACGAAGCTTTGGGCCAGGATTCTCCTCG ACGTCACCGCATGTTAGCAGACTTCCTCTG CCCTCTCCACTGCCCATATGGTCTGAGTCA GGCCCTTCTG
TP53 3'HR	pAav-p53-T2A-BirA*	GAAGTTATGGTACCCATTCTCCACTTCTTGT TCC	GGGTTCCTGCGGCCGCTTTTGCTAGCAGAT CACGCCACTCCACTCC
BirA*	pAav-p53-T2A-BirA*	CATCCACGCGTCATATGGGCAGTGGAGAG GGCAGAGGAAGTCTGCTAACCTGCGGGGA CGTCGAGGAGAATCCTGGCCCAAAGGACA ACACGGTGCCCCTGAAGCTGATCGC	GCTAGCAAGCTTTCATTACTTCTCTGCGCTT CTCAGGG
Lox-Neo-Lox	pAav-p53-T2A-BirA*	CAGAGAAGTAATGAAAGCTTGCTAGCATA ACTTCGTATAGCATACATTATACGAAGTTA TC	GGAACAAGAAGTGGAGAATGGGTACCATA ACTTCGTATAATGTATGCTATACGAAGTTA TAGCTGGTTCT
TP53 junction 5'HR	Screening	ATCAGCCAAGATTGCACCAT	TCAGCTCTCCGATTCTGTCC
TP53 junction 3'HR	Screening	GGGAGGATTGGGAAGACAAT	CCAGTCTCCAGCCTTTGTTC
Cassette deletion	Cre-Lox recombination	ACTCATGTTCAAGACAGAAGGGCCTGAC	TGACGCACACCTATTGCAAGCAAGG
WT allele	Sanger sequencing	ACATATTTGCATGGGGTGTG	CCTAGAATGTGGCTGATTGTAAAC
Knock-in allele	Sanger sequencing	CACTAGGGGTTCCTGCGGCCGCAAACGCG TCAGCCCTGCACAGACATTTT	ATTACTTCTCTGCGCTTCTCAG
T2A region	Illumina sequencing	TCGTCGGCAGCGTCAGATGTGTATAAGAG ACAGGGGTCAGTCTACCTCCCGCC	GTCTCGTGGGCTCGGAGATGTGTATAAGA GACAGCTCGCCGTTGGCCAGCAGGG
Neomycin	SB probe	TGCTCCTGCCGAGAAAGTAT	GCGATGCAATTTCCTCATTT
T2A	pMet7 mutagenesis	CCTTTGGGCCAAAATTCTCCTCGACGTCCC CGCA	TGCGGGGACGTCGAGGAGAATTTTGGCCC AAAGG

Table S1. Primers used for cloning or screening. HR: homology region; SB: Southern blot. The T2A sequence was encoded in the reverse p53 5'HR and forward BirA* primers.

	Sequence
gRNA1 cassette	TGTACAAAAAAGCAGGCTTTAAAGGAACCAATTCAGTCGACTGGATCCGGTACCAAGGTCGGGCAGGAA*GAGGGCCTATTTCCCAT GATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAA AATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAAC TTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCG***ATTCTCCTCGACGTCCCCGC**GTTTTAGAGCTAG AAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTCTAGACCCAGCTTTCTTG TACAAAGTTGGCATTA
gRNA2 cassette	TGTACAAAAAAGCAGGCTTTAAAGGAACCAATTCAGTCGACTGGATCCGGTACCAAGGTCGGGCAGGAA*GAGGGCCTATTTCCCAT GATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAA AATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAAC TTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCG***TCCTGGCCCAAAGGACAACA**GTTTTAGAGCTA GAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTCTAGACCCAGCTTTCTT GTACAAAGTTGGCATTA

Table S2. gRNA gBlock sequences cloned in pCR-Blunt® vector for transfection. Italics: U6 promoter sequence. Bold: gRNA sequence. Underlined: universal gRNA scaffold. Sequences were ordered as IDT gBlocks and blunt-end ligated in pCR-Blunt® vectors prior to transfection in target cells together with BE3. After parallel testing it was decided to use gRNA2 for endogenous mutagenesis.

[Excel Table S3]

Table S3. CRISPRESSO allele frequency output (column legend). Aligned sequence: sequencing read; Reference sequence: template sequence; NHEJ: indication of Non-homologous end-joining event (NHEJ); UNMODIFIED: indication of perfect match with reference sequence (no engineering event); HDR: Homology-directed repair event (if applicable); n deleted and n inserted: nucleotides deleted or inserted compared to reference sequence; n mutated: number of nucleotides subsituted compared to reference sequence; #Reads and %Reads: absolute and relative number of reads for each sequence.

GO Cellular components

Term	Count	P-value	Fold Enrichment	Bonferroni
Nucleoplasm	380	8.32E-100	2.96	5.37E-97
Cell-cell adherens junction	112	4.24E-67	7.51	2.74E-64
Cytosol	343	2.48E-55	2.24	1.60E-52
Cytoplasm	422	2.27E-41	1.75	1.47E-38
Nucleus	417	2.88E-35	1.67	1.85E-32
Catalytic step 2 spliceosome	44	4.54E-33	10.36	2.93E-30
Membrane	220	2.53E-30	2.17	1.63E-27
Nucleolus	117	1.83E-26	2.96	1.18E-23
Spliceosomal complex	38	2.46E-25	8.76	1.59E-22
Proteasome complex	28	1.08E-20	10.11	6.94E-18
Focal adhesion	64	1.77E-18	3.55	1.14E-15
Nuclear speck	45	2.32E-18	4.85	1.49E-15
Microtubule	50	3.73E-14	3.48	2.41E-11
Proteasome accessory complex	13	4.26E-13	16.57	2.75E-10
Nuclear chromosome, telomeric region	28	3.57E-11	4.67	2.30E-08
Eukaryotic 43S preinitiation complex	11	1.00E-10	15.89	6.45E-08
MLL1 complex	14	1.32E-10	10.46	8.53E-08
U2 snRNP	12	2.15E-10	13.00	1.38E-07
Actin cytoskeleton	35	4.18E-10	3.48	2.70E-07
Proteasome core complex	12	4.32E-10	12.38	2.79E-07

GO Molecular function

Term	Count	P-value	Fold Enrichment	Bonferroni
Poly(A) RNA binding	256	5.52E-106	4.61	3.90E-103
Protein binding	679	4.44E-75	1.57	3.13E-72
Cadherin binding involved in cell-cell adhesion	111	5.31E-69	7.78	3.75E-66
RNA binding	97	1.30E-28	3.61	9.16E-26
Unfolded protein binding	31	6.35E-15	5.73	4.47E-12
Transcription coactivator activity	46	2.00E-14	3.77	1.41E-11
Translation initiation factor activity	21	4.91E-12	7.00	3.46E-09
Nucleotide binding	51	7.61E-12	2.98	5.37E-09
Threonine-type endopeptidase activity	12	8.42E-10	11.62	5.95E-07
ATP binding	126	1.56E-09	1.71	1.10E-06
Chromatin binding	48	1.29E-08	2.50	9.11E-06
Histone acetyltransferase activity	14	3.43E-07	5.93	2.42E-04
Actin filament binding	22	1.79E-06	3.39	0.001
ATP-dependent RNA helicase activity	15	2.50E-06	4.69	0.002
RNA polymerase II distal enhancer sequence-specific DNA binding	15	2.50E-06	4.69	0.002
mRNA binding	21	2.87E-06	3.42	0.002
RNA polymerase II core binding	9	3.76E-06	8.72	0.003
Protein domain specific binding	28	3.83E-06	2.74	0.003
Ribonucleoprotein complex binding	10	8.95E-06	6.78	0.006
ATPase activity	25	1.10E-05	2.78	0.008

Table S4: GO analysis of the results from an analysis of a transient transfection p53 BioID experiment using a mock vector as control. This table shows the top 20 enriched 'cellular component' (top panel) or 'molecular function' terms (bottom panel). Count: number of proteins identified related to a certain GO term. Bonferroni: Bonferroni-corrected p-values.

GO Cellular components

Term	Count	P-value	Fold Enrichment	Bonferroni
Cytosol	244	2.65E-54	2.64	1.24E-51
Extracellular exosome	211	2.29E-46	2.69	1.07E-43
Cell-cell adherens junction	60	2.20E-31	6.65	1.03E-28
Focal adhesion	63	2.37E-29	5.77	1.11E-26
Cytoplasm	264	6.74E-29	1.81	3.16E-26
Myelin sheath	40	8.02E-27	9.42	3.76E-24
Membrane	148	2.10E-25	2.41	9.82E-23
Nucleoplasm	160	1.60E-20	2.06	7.49E-18
Extracellular matrix	42	1.54E-17	5.08	7.21E-15
Proteasome complex	20	1.49E-15	11.93	6.75E-13
Mitochondrion	89	1.80E-14	2.39	8.42E-12
Nucleus	229	1.72E-13	1.51	8.05E-11
Melanosome	22	4.84E-13	7.80	2.27E-10
Proteasome core complex	12	1.94E-12	20.46	9.10E-10
Microtubule	34	4.92E-11	3.91	2.30E-08
Cytosolic small ribosomal subunit	14	4.17E-10	10.44	1.95E-07
Mitochondrial matrix	32	3.06E-09	3.50	1.43E-06
Endoplasmic reticulum chaperone complex	8	3.76E-09	26.04	1.76E-06
Ribosome	22	7.67E-09	4.75	3.59E-06
Chaperonin-containing T-complex	7	3.56E-08	27.85	1.67E-05

GO Molecular function

Term	Count	P-value	Fold Enrichment	Bonferroni
Protein binding	399	1.95E-39	1.54	1.27E-36
Poly(A) RNA binding	116	2.94E-33	3.48	1.91E-30
Cadherin binding involved in cell-cell adhesion	58	7.14E-31	6.77	4.65E-28
Unfolded protein binding	39	1.18E-30	11.99	7.67E-28
ATP binding	100	8.67E-15	2.26	5.65E-12
Threonine-type endopeptidase activity	12	3.57E-12	19.33	2.33E-09
Chaperone binding	19	1.73E-11	7.94	1.13E-08
Structural constituent of ribosome	30	1.78E-11	4.57	1.16E-08
Ubiquitin protein ligase binding	30	8.07E-09	3.54	5.26E-06
Hsp70 protein binding	11	2.29E-08	11.28	1.50E-05
GTPase activity	26	2.92E-08	3.76	1.90E-05
GTP binding	33	1.37E-07	2.91	8.91E-05
Structural constituent of cytoskeleton	17	1.41E-07	5.23	9.17E-05
Heat shock protein binding	11	2.87E-07	8.86	1.87E-04
Protein kinase binding	30	2.57E-06	2.70	0.002
Protein complex binding	21	3.19E-06	3.45	0.002
Biotin carboxylase activity	5	3.66E-06	33.83	0.002
Adenyl-nucleotide exchange factor activity	6	4.88E-06	20.30	0.003
Actin filament binding	16	8.20E-06	4.10	0.005
Transcription coactivator activity	22	1.55E-05	3.00	0.010

Table S5. GO analysis of the results from an analysis of a transient transfection p53 BioID experiment using a bi-cistronic p53-T2A-BirA* expression construct as control. This table shows the top 20 enriched 'cellular component' (top panel) or 'molecular function' terms (bottom panel). Count: number of proteins identified related to a certain cellular component. Bonferroni: Bonferroni-corrected p-values.

[Excel table S6]

Table S6: Perseus data matrices generated for all analyses (column legend). Each sheet corresponds to a separate analysis. Significance: significant after t-test on FDR 0.05 and optimized s0 value; -log(p-value): significance; Difference: log difference; Protein IDs, Majority protein IDs, Protein names, Gene names: protein identifiers; Peptides, Razor + unique peptides, Unique peptides: number of identified peptides for every protein; Sequence coverage, Unique + razor sequence coverage, Unique sequence coverage: % sequence coverage for every protein; Mol. Weight [kDa]: molecular weight; Q-value: adjusted p-value; Score: test score; Intensity: Observed intensities; MS/MS count: number of identifications; LFQ intensity columns: Label-Free Quantitation (LFQ) intensities for every protein in each sample.

GO Cellular components

Term	Count	P-value	Fold Enrichment	Bonferroni
Nucleoplasm	156	9.41E-87	5.08	2.25E-84
Nucleus	143	9.62E-34	2.39	2.30E-31
Mediator complex	21	6.80E-31	54.40	1.62E-28
Nuclear chromatin	26	7.44E-20	12.21	1.78E-17
Transcription factor TFTC complex	12	7.44E-20	77.71	1.78E-17
STAGA complex	11	1.94E-17	71.24	4.64E-15
Nucleolus	44	2.38E-17	4.65	5.68E-15
Transcription factor TFIID complex	14	4.12E-17	35.26	9.84E-15
SWI/SNF complex	9	1.10E-12	54.40	2.63E-10
NuA4 histone acetyltransferase complex	9	7.28E-12	45.33	1.74E-09
NuRD complex	9	7.28E-12	45.33	1.74E-09
Core mediator complex	6	9.01E-10	90.67	2.15E-07
npBAF complex	7	1.42E-09	52.89	3.39E-07
nBAF complex	7	4.52E-09	45.33	1.08E-06
MLL1 complex	8	2.20E-08	25.01	5.25E-06
Protein complex	21	2.97E-08	4.62	7.09E-06
Sin3 complex	6	1.81E-07	41.85	4.34E-05
Transcription factor TFIIIC complex	5	2.08E-07	75.56	4.96E-05
Chromatin	10	5.75E-07	10.19	1.37E-04
SAGA complex	6	6.00E-07	34.00	1.43E-04

GO Molecular function

Term	Count	P-value	Fold Enrichment	Bonferroni
Transcription coactivator activity	40	1.48E-32	13.41	3.88E-30
Protein binding	179	3.12E-28	1.69	8.19E-26
RNA polymerase II transcription cofactor activity	18	7.44E-24	41.58	1.96E-21
Histone acetyltransferase activity	19	6.15E-23	32.92	1.62E-20
Chromatin binding	34	6.31E-19	7.23	1.66E-16
Nucleosomal DNA binding	15	1.31E-16	27.12	2.92E-14
RNA polymerase II distal enhancer sequence-specific DNA binding	16	1.04E-15	20.47	2.63E-13
DNA binding	61	2.41E-15	3.03	6.42E-13
Histone deacetylase activity	12	6.86E-13	25.59	1.80E-10
Vitamin D receptor binding	9	2.19E-12	49.89	5.76E-10
Poly(A) RNA binding	43	3.18E-11	3.17	8.37E-09
p53 binding	12	3.93E-10	14.89	1.03E-07
Thyroid hormone receptor binding	9	6.68E-10	27.72	1.76E-07
Transcription cofactor activity	12	7.50E-10	14.05	1.97E-07
RNA polymerase II core promoter proximal region sequence-specific DNA binding	20	5.53E-08	4.68	1.45E-05
Transcription factor binding	18	5.76E-08	5.27	1.51E-05
RNA polymerase II repressing transcription factor binding	7	6.54E-07	21.56	1.72E-04
Lysine-acetylated histone binding	6	1.76E-06	27.72	4.63E-04
Ligand-dependent nuclear receptor transcription coactivator activity	8	2.35E-06	13.04	6.17E-04
Ligand-dependent nuclear receptor binding	6	4.07E-06	23.76	0.001

Table S7: GO analysis of significantly enriched proteins from converted p53-MUTT2A-BirA* HCT116 cells when compared to isogenic non-converted p53-T2A-BirA* cells. This table shows the top 20 enriched 'cellular component '(top panel) or 'molecular function' terms (bottom panel). Count: number of proteins identified related to a certain GO term. Bonferroni: Bonferroni-corrected p-values.

GO Cellular components

Term	Count	P-value	Fold Enrichment	Bonferroni
Nucleoplasm	252	1.48E-103	4.03	5.75E-101
Nucleus	264	8.52E-49	2.17	3.32E-46
Nucleolus	89	1.73E-34	4.63	6.75E-32
Catalytic step 2 spliceosome	33	7.58E-30	15.98	2.95E-27
Mediator complex	23	3.19E-28	29.28	1.24E-25
Cytosolic large ribosomal subunit	25	7.53E-23	16.38	2.93E-20
Nuclear speck	37	2.15E-22	8.20	8.36E-20
Nuclear chromatin	31	4.50E-17	7.16	1.75E-14
Ribosome	29	5.73E-17	7.78	4.32E-14
Transcription factor TFTC complex	12	2.11E-16	38.19	8.64E-14
Cell-cell adherens junction	38	2.66E-16	5.24	8.64E-14
Spliceosomal complex	21	1.87E-14	9.95	7.26E-12
STAGA complex	11	2.62E-14	35.01	1.02E-11
Cytoplasm	188	7.43E-14	1.60	2.89E-11
Membrane	103	2.81E-13	2.09	1.09E-10
Transcription factor TFIID complex	14	4.25E-13	17.33	1.65E-10
NuA4 histone acetyltransferase complex	11	1.06E-12	27.23	4.11E-10
Focal adhesion	35	1.49E-11	3.99	5.80E-09
MLL1 complex	12	1.49E-11	18.44	5.80E-09
U2 snRNP	10	1.75E-10	22.28	6.80E-08

GO Molecular function

Term	Count	P-value	Fold Enrichment	Bonferroni
Poly(A) RNA binding	148	6.17E-70	5.41	2.67E-67
Protein binding	346	1.23E-44	1.63	5.31E-42
Transcription coactivator activity	47	1.15E-27	7.82	4.99E-25
RNA polymerase II transcription cofactor activity	20	7.30E-22	22.93	3.15E-19
RNA binding	57	2.61E-20	4.30	1.13E-17
Histone acetyltransferase activity	19	1.98E-17	16.34	8.54E-15
Cadherin binding involved in cell-cell adhesion	38	8.49E-17	5.41	4.80E-14
Vitamin D receptor binding	12	1.79E-15	33.02	7.67E-13
Structural constituent of ribosome	31	1.89E-14	5.76	8.15E-12
Chromatin binding	40	5.77E-14	4.22	2.49E-11
Nucleosomal DNA binding	16	1.12E-13	14.36	4.85E-11
DNA binding	89	1.31E-12	2.19	5.64E-10
RNA polymerase II distal enhancer sequence-specific DNA binding	17	2.22E-12	10.79	9.61E-10
Thyroid hormone receptor binding	12	1.32E-11	18.34	5.72E-09
Transcription cofactor activity	15	1.28E-09	8.72	5.52E-07
Histone deacetylase activity	12	1.31E-09	12.70	5.67E-07
Transcription factor binding	26	2.79E-08	3.78	1.20E-05
p53 binding	13	6.11E-08	8.01	2.64E-05
Nucleotide binding	27	3.86E-07	3.20	1.67E-04
RNA polymerase II core promoter proximal region sequence-specific DNA binding	26	1.91E-06	3.02	8.25E-04

Table S8: GO analysis of significantly enriched proteins from converted p53-MUTT2A-BirA* HCT116 cells when compared to parental HCT116 cells. This table shows the top 20 enriched 'cellular component' (top panel) or 'molecular function' terms (bottom panel). Count: number of proteins identified related to a certain GO term. Bonferroni: Bonferroni-corrected p-values.

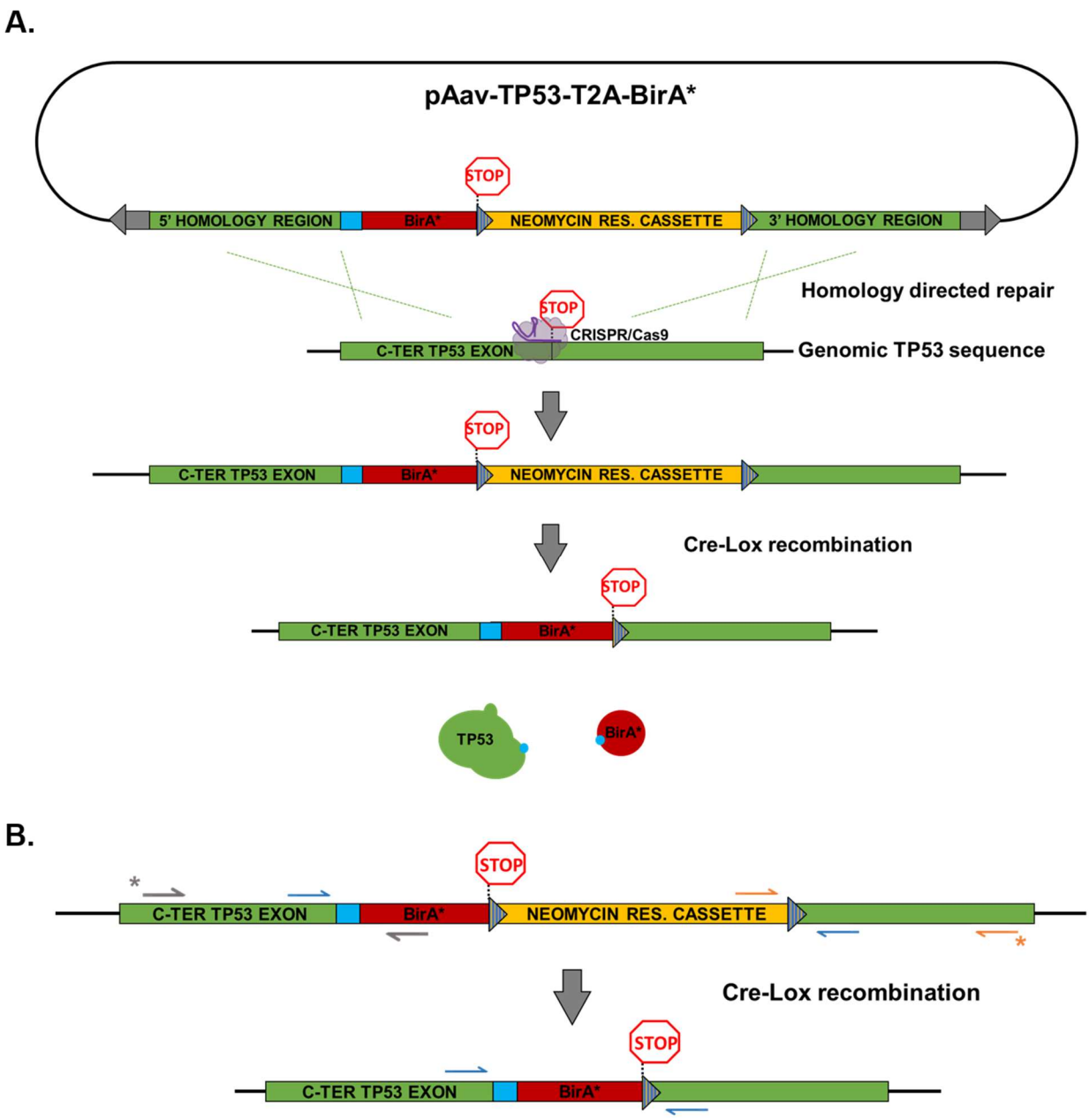

Figure S1. Targeting strategy and primer scheme used for PCR screening. (A) Knock-in of T2A-BirA* to the p53 C-terminus. The pAav-TP53-T2A-BirA* plasmid was packaged in rAAV and served as HDR repair template for the Cas9-induced DNA lesion site. After a first screening round to confirm integration, cells were treated with TAT-Cre for Cre-Lox recombination leading to the removal of the neomycin resistance cassette. The final HCT116 *TP53*$^{+/T2A\text{-}BirA*}$ cell line contains only a remnant Lox site as a scar sequence. Cyan:

T2A sequence. Purple: CRISPR/Cas9. (B) PCR screening strategy. Arrows are indicative for primer location on the altered locus for PCR screening. Grey arrows: primers used for 5’ HR PCR (1706 nt). Orange arrows: primers used for 3’ HR PCR (1467 nt). Blue arrows: primers used for cassette deletion PCR (WT amplicon: 163 nt; recombined amplicon: 1230 nt; not recombined amplicon: 2879 nt). Asterisks indicate location of the primer outside the HR to exclude false-positive PCR amplicon in case of random integration.

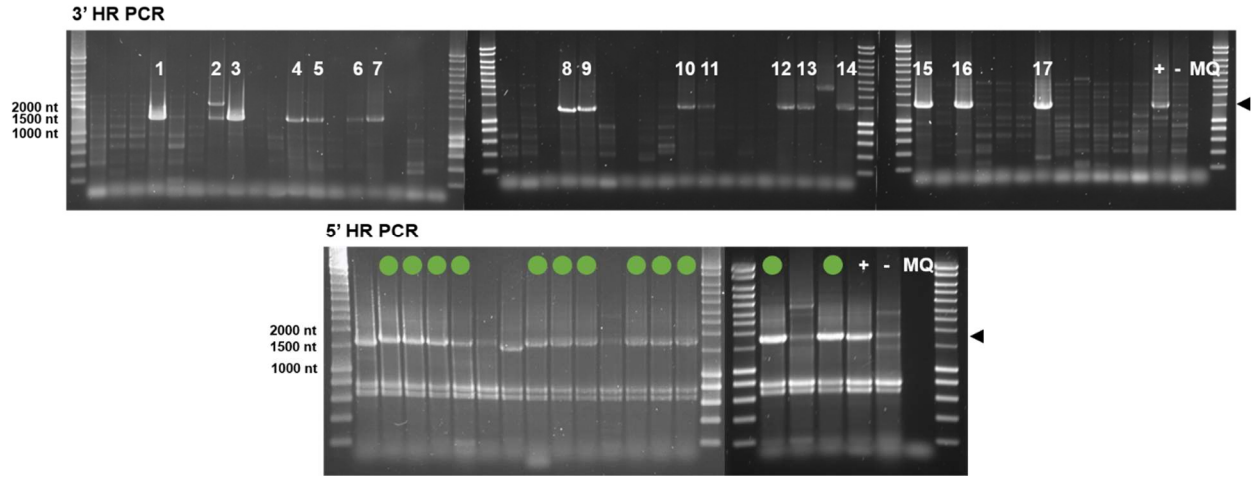

Figure S2. PCR screening of neomycin resistant clonal populations. PCR screening of clonal populations for HDR on agarose gels. Lysates were obtained by adding the cell suspension to DirectPCR lysis reagent. 17 clones showed a correct amplicon for 3' HR (1467 nt). These 17 clones were retained for the 5' HR PCR of which 12 were positive for the 5' HR PCR (1706 nt) as indicated in green. +: Positive control consisting of the neomycin resistant pool of HCT116 cells subjected to transfection and infection. -: Parental (non-engineered) HCT116 cells. MQ: No input DNA negative control.

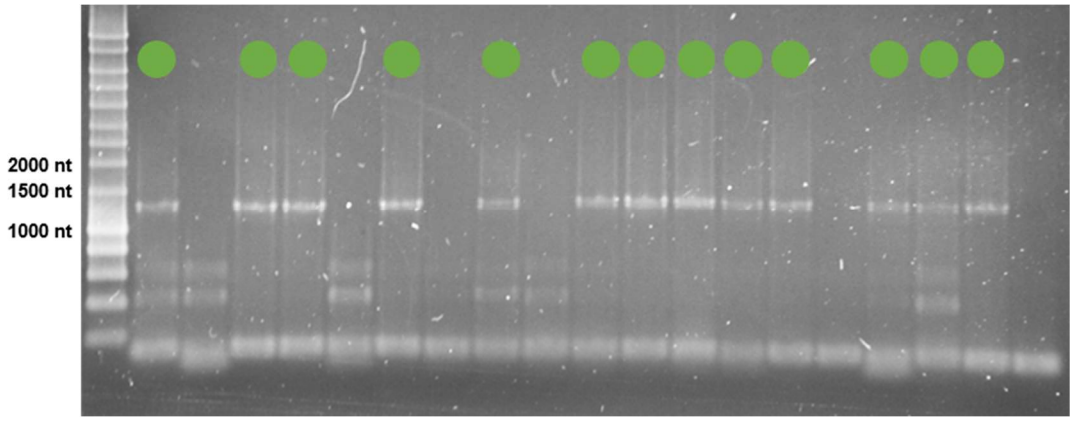

Figure S3. PCR for assessing the removal of the selection cassette upon TAT-Cre treatment. Agarose gel showing a typical post-recombination PCR screening. Lysates were obtained by adding the cell suspension to DirectPCR lysis reagent. Confirmed HDR positive clones were subjected to TAT-Cre treatment and screened by PCR for neomycin selection cassette removal by Cre-Lox recombination. WT amplicon: 163 nt; recombined amplicon: 1230 nt; not recombined amplicon: 2879 nt. 13 out of 19 clones showed recombination as indicated in green. Contrast adjusted to +40%. Empty lanes are possibly due to not-recombined amplicons that were not amplified as elongation time did not allow for PCR amplification of this size. Alternatively, this can be due to varying amounts of input DNA obtained from the lysis reagent.

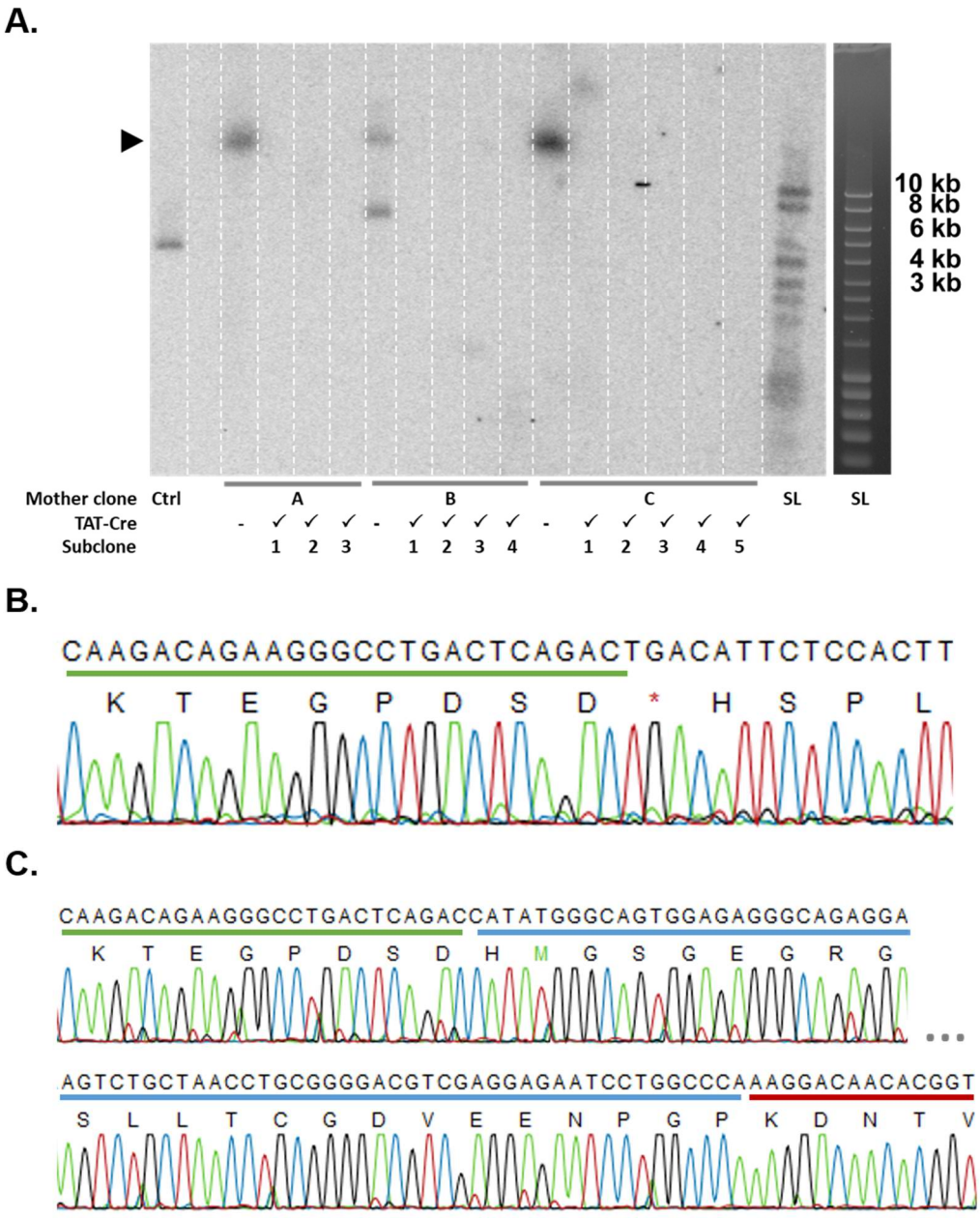

Figure S4. Validation of targeted T2A-BirA* insertion. (A) Southern blot of engineered T2A-BirA* clones prior and after TAT-Cre treatment to verify single integration and subsequent removal of the neomycin selection cassette. A total of 3, 4 and 5 TAT-Cre treated single cell subclones derived from 3 mother clones (A, B and C respectively) were assayed for cassette removal. A [α-^{32}P] Southern blot probe was generated against the neomycin resistance cassette. Expected size of the EcoRI digested HCT116 *TP53*$^{+/T2A\text{-}BioID}$ DNA

fragment containing the probed selection cassette: ±20 kb. Ctrl: positive control from an ERN1 FLAG-tagged cell line containing the cassette with expected fragment size of 3537 bp. SL: Smartladder (left: post transfer on the membrane, right: SL on 0.7% agarose gel prior to transfer). Mother clone B showed double integration of the selection cassette, leading to exclusion of all derived post TAT-Cre expanded clones for further experiments. Clones 1-4 in the T2A mutagenesis Western blot experiment (Figure 2A) correspond to clone A1, A3, C1 and C3 respectively. (B) Sanger sequencing result of unaltered WT allele in knock-in clone C3 (clone 4). Green line: C-terminus of p53 (C) Sanger sequencing result of T2A-BirA* allele of the HCT116 *TP53*$^{+/\text{T2A-BirA*}}$ clone C3 (clone 4). Blue line: Linker sequence and T2A sequence. Red line: N-terminus of BirA*

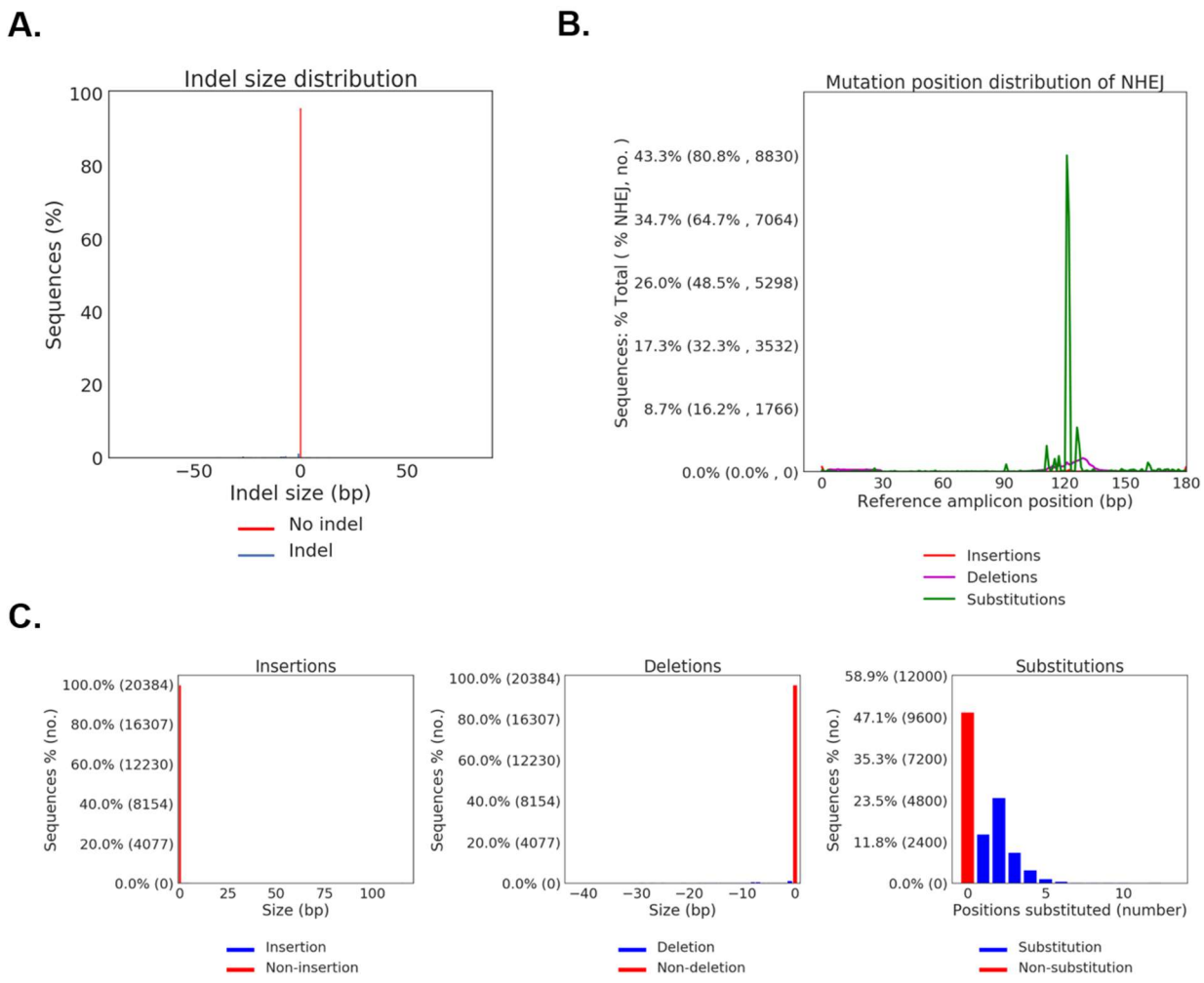

Figure S5. CRISPRESSO analysis of the T2A region after BE3 base editing. (A) Insertion and deletion (Indel) size distribution after BE3 treatment. Formation of indel mutations was negligible, with nearly all mutated amplicons bearing substitutions. (B) Position distribution of mutations in BE3-treated cells. Substitutions are exclusively prevalent in the mutation window of BE3. (C) Mutational size pattern of BE3-treated cells. Negligible insertions (left panel) and deletions (middle panel) were discerned. Substitutions (right panel) vary in size from 1 to 5 nucleotides, in accordance with the number of available residues in the T2A target sequence.

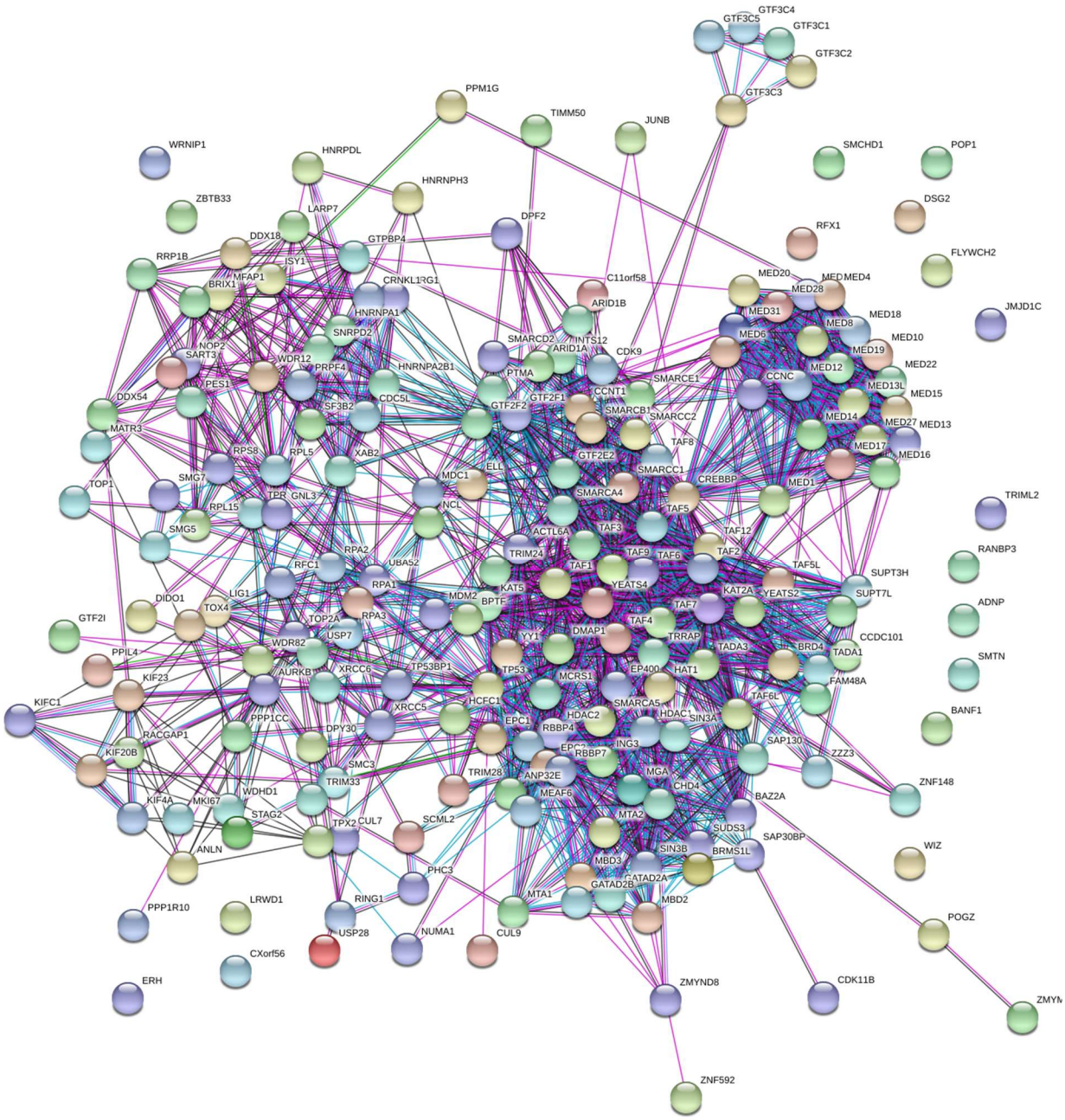

Figure S6. STRING analysis of proximal proteins for endogenous p53. All candidate proximal proteins detected in the edited p53-MUTT2A-BirA* HCT116 cells when isogenic p53-T2A-BirA* cells were used as control. Text mining associations are disabled. Minimum required interaction score was set on medium confidence (0.400).

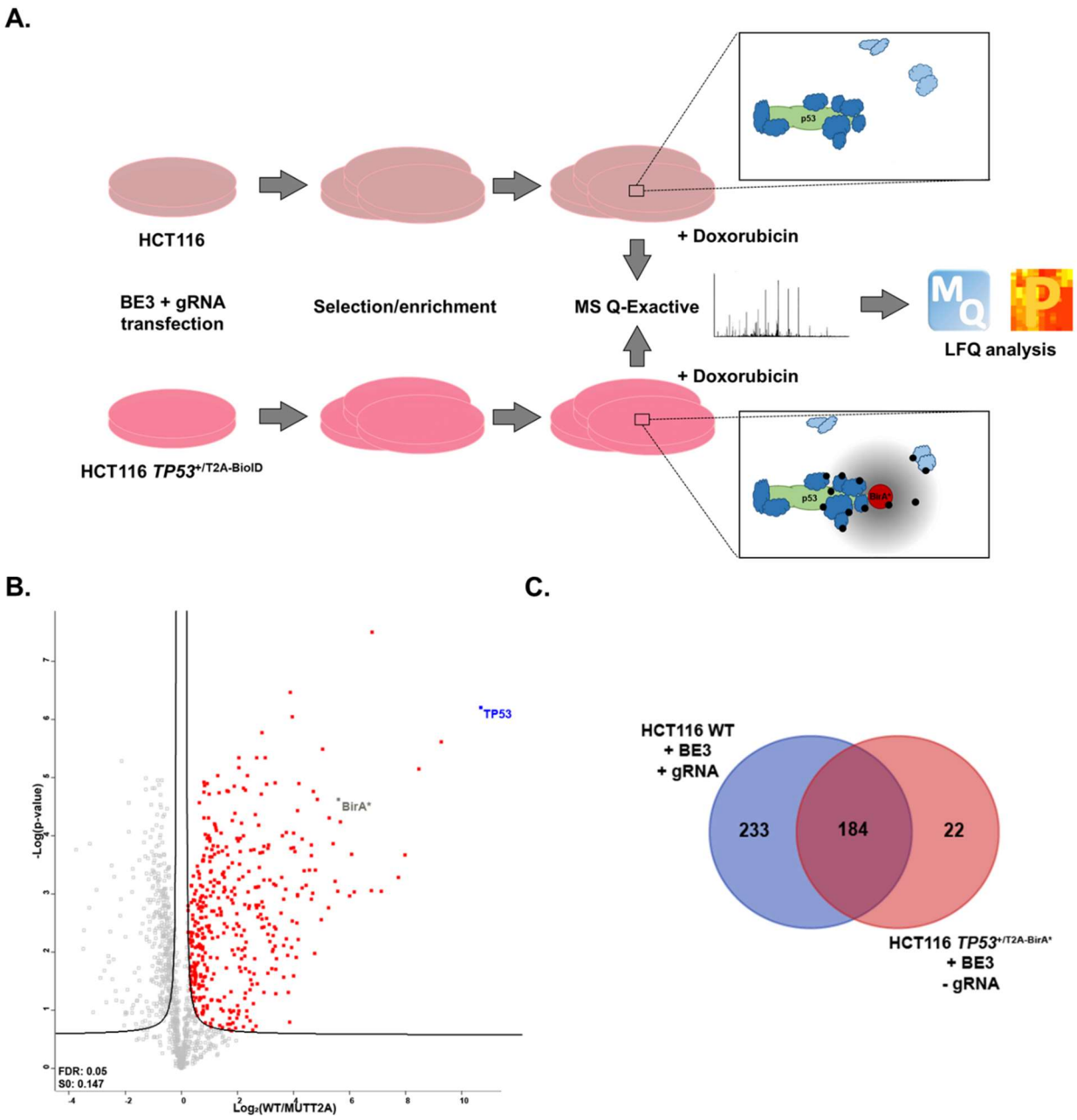

Figure S7. Evaluation of BE3 transfected non-engineered WT cells as control for p53 BioID. (A) Proteomics workflow when using transfected HCT116 WT cells as control condition. (B) Volcano plot showing the differential LFQ intensity levels (X-axis) and the p-values (Y-axis) for HCT116 *TP53*$^{+/T2A\text{-}BirA*}$ after T2A conversion in presence of 1µM doxorubicin. (C) Venn diagram comparing significant hits in analyses using the two different control conditions. Comparing the two different controls showed an

overlap of 184 significant proteins. 233 proteins were exclusively identified in a significant manner when using the HCT116 BE3-transfected control.