Engineering of effector domains for targeted DNA methylation with reduced off-target effects

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Supplemental materials

Supplementary figures

Supplementary figure 1: Compilation of bisulfite conversion rates.

Supplemental figure 2: Compilation of the bis-seq DNA methylation data shown in aggregated form in Figure 2-4.

Supplementary figure 3: New DNA methylation peaks identified after treatment of cells with dCS, Ab-3A3L and the ISG15 sgRNA.

Supplementary figure 4: Signals of MBD-seq data in the CGIs of the *ISG15*, *VEGFA*, *ACTB* and *GAPDH* genes extracted using the multiBigwigSummary tool.

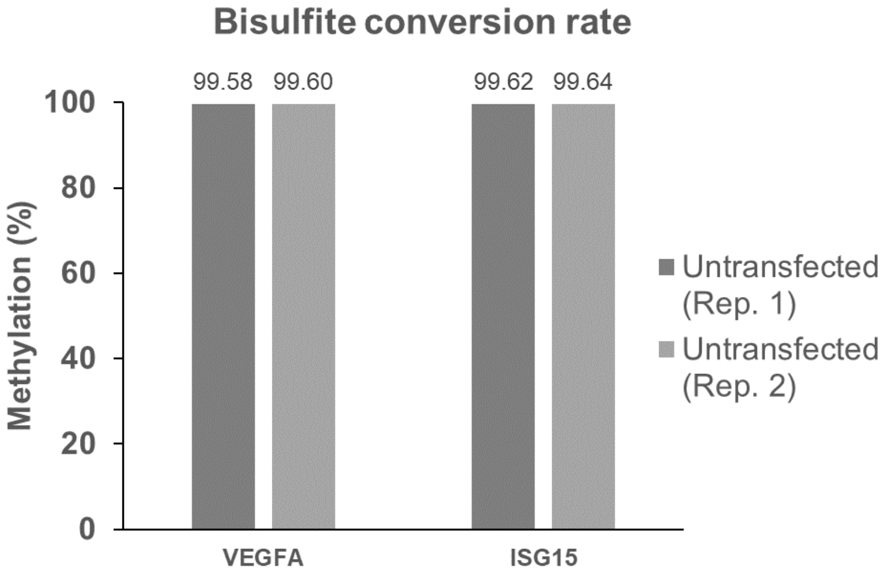
Supplementary figure 5: IGV views of MBD-seq data displayed for the two additional off-target regions *ACTB* and *GAPDH*.

Supplementary tables

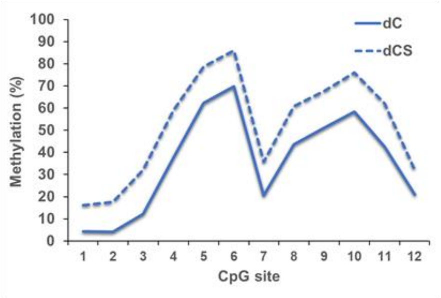
Supplementary table 1: Potential off-target binding sites of the *ISG15* sgRNA used in this study as identified by http://www.rgenome.net/cas-offinder/.

Supplementary table 2: Primers used in this work.

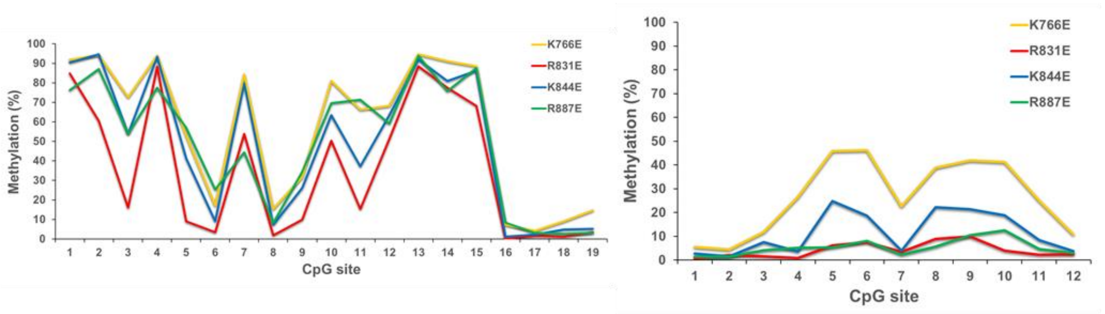
Supplementary figure 1: Compilation of bisulfite conversion rates. Bisulfite conversion was determined based on the read count of cytosine residues at non-CpG sites after bisulfite conversion of genomic DNA isolated from untransfected cells in two experimental repeats (Rep. 1 and Rep. 2).



Supplemental figure 2: Compilation of the bis-seq DNA methylation data shown in aggregated form in Figure 2-4. In each case, the x-axis displays the individual numbered CpG sites. For the experimental conditions, refer to the legends of the individual main figures.



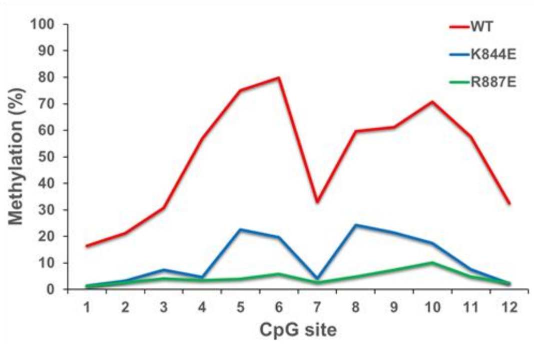
Methylation of VEGFA in Fig. 2D



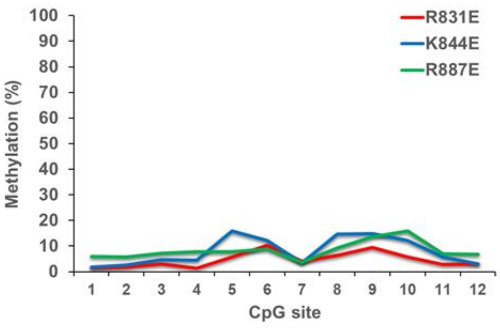
Methylation of ISG15 and VEGFA in Fig. 3B

ISG15

VEGFA

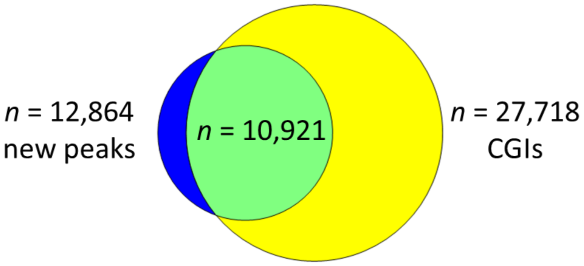


Methylation of VEGFA in Fig. 3D

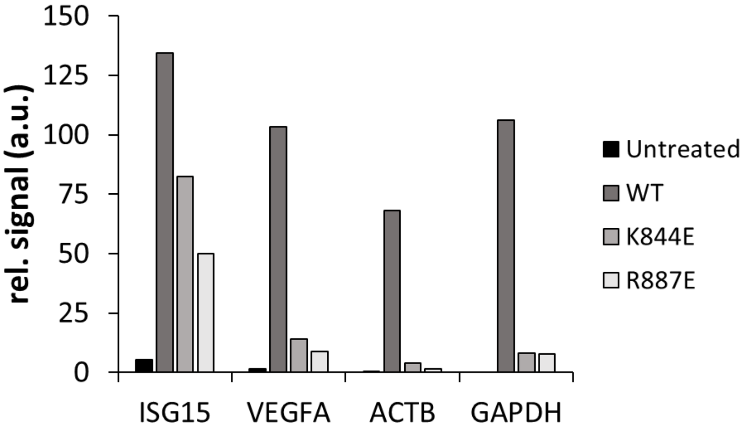


Methylation of VEGFA in Fig. 5C

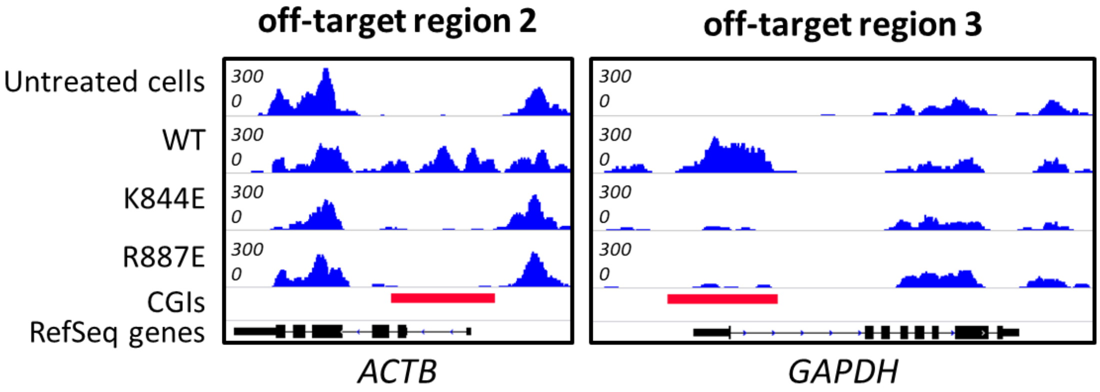
Supplementary figure 3: New DNA methylation peaks identified after treatment of cells with dCS, Ab-3A3L and the ISG15 sgRNA. Newly appearing MBD-seq peaks were intersected with all CGIs. 1943 out of 12,864 newly appearing MBD-seq peaks (15%) were not within an annotated CGI. Among them, only 263 peaks were more than 1 kb apart from the next CGI (2% of all newly appearing peaks).



Supplementary figure 4: Signals of MBD-seq data in the CGIs of the *ISG15*, *VEGFA*, *ACTB* and *GAPDH* genes extracted using the multiBigwigSummary tool. Note the reduced relative activity of the K844E and R887E variants at the off-target sites. Cells were treated with dCS, Ab-3A3L with indicated mutations in the DNMT3A part and the ISG15 sgRNA.



Supplementary figure 5: IGV views of MBD-seq data displayed for the two additional off-target regions *ACTB* and *GAPDH*. Cells were treated with dCS, Ab-3A3L with indicated mutations in the DNMT3A part and the ISG15 sgRNA. The wild type DNMT3A (WT) demonstrated higher off-target methylation levels than the K844E or R887E variants.



Supplementary table 1: Potential off-target binding sites of the *ISG15* sgRNA used in this study as identified by http://www.rgenome.net/cas-offinder/.

#Bulge type	crRNA	DNA	Chromosome	Position	Direction	Mismatches	Bulge Size
X	GTTCGCTGCCTCTCAGCCGCNGG	GgTCGCTGCCTCTCcaCCGgAGG	chr8	17104884	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTaGCTGCCTCTCgGtCGCCGG	chr8	43406533	-	3	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GaTCGaTtCCTCTCAGCCaCAGG	chr8	62815042	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	aTTCaCaGCaTCTCAGCCGCAGG	chr8	81805938	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	tTTCcCTtCCTCTCAGCCcCTGG	chr8	124933078	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	tTTtGCTGCCTgTCAGCtGCAGG	chr12	73776344	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTCGtTaaCTCTCAGCtGCAGG	chr12	86975240	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GgaaGCTGCCaCTCAGCCGCAGG	chr12	95397507	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	tTTCtCTGCCTCTCAGCtGgAGG	chr12	105098325	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTCcCTcCCTCTCAcCtGCTGG	chr12	112605777	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	tTTCtCTGCCaCTCAGaCGCAGG	chr12	124094533	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	aTTCcCTGCCTtTCAGCCGgTGG	chr3	51727405	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTCtCTaCCTCcCAGCCtCTGG	chr3	59181111	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTaGCTGgCTgTCAGCgGCAGG	chr3	132824091	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTaGCTGtCTCTtgGCCGCCGG	chr3	186728772	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTCtCctCCTCTCAGCCcCTGG	chr7	2508465	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GgTtGCTGCCTCTCAGCCctAGG	chr7	50567760	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTaCGCTGCCTCcCcGCCGCCGG	chr7	101460483	+	3	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTCcCTGgCcCTgAGCCGCGGG	chr7	139344168	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTaGCTGtCTCTtgGCCGCTGG	chr4	8430973	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTaGCTGCCTCTCgGtCGCCGG	chr4	25522958	+	3	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTCcCgGCtTCTCAGCCaCTGG	chr4	40337256	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTCtCTGCCTCTCtGCCtgAGG	chr4	75658461	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	tTcCGCTGaCTCTCAGCCcCGGG	chr4	109572018	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTaGCTGCacCTCAGCtGCAGG	chr4	141746720	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GgcCGCaGCCTCTCAGCCGCCGG	chr5	14664836	-	3	0

X	GTTCGCTGCCTCTCAGCCGCNGG	GTTCGCTGCCTgaCAtCtGCAGG	chr16	4884173	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTgCGCTGCCTCcCAGgaGCCGG	chr16	12213529	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GaTCGCTGCtTCcCAGCCtCAGG	chr16	27250582	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTCGCTGCCTCTCAGCCGCCGG	chr1	948874	-	0	0
X	GTTCGCTGCCTCTCAGCCGCNGG	tTTCcCTGtCTCTCAGCtGCAGG	chr1	17986443	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTgCcCTcCtTCTCAGCCGCTGG	chr1	26508857	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GacCcCTGCCTCTCAGCCcCTGG	chr1	32716729	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTCcCTGaCTCTCAcCCtCGGG	chr1	41760952	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTaGCcatCTCTCAGCCGCTGG	chr1	146710912	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTCaCTGCCTCTCAGCtGtGGG	chr1	215131205	+	3	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GgTCctTGCCTCTCAGCtGCTGG	chr1	220325057	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	tTcCtCTGCCTCTCAGCCtCCGG	chr1	225931264	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTgCGCTtCCTCTgAGCCaCTGG	chr13	26423100	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GcTgGCcGCCTCTCcGCCGCTGG	chr2	90284246	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GcTgGCcGCCTCTCcGCCGCTGG	chr2	90284228	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GcTgGCcGCCTCTCcGCCGCTGG	chr2	90527824	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GcTgGCcGCCTCTCcGCCGCTGG	chr2	90527806	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GgTgGCcGCCTCTCcGCCGCTGG	chr2	91750692	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTCcCTGCCTCcCAGCCcaGGG	chr2	96763987	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTacCTGCCTCTCAGCCctGGG	chr2	161960608	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTgCGCTGCCTCTgccCCGCGGG	chr2	175205238	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTCcCTGCaTCTCAtCCcCAGG	chr2	199599312	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTaGCTGtCTCTtgGCCGCCGG	chr2	201990826	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GcTgGCTGCtTCTCAGCaGCTGG	chr22	27533888	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	aTTaGCTGCCTCTCgGtCGCCGG	chr22	39507745	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTtcCcGCCTCTCAGgCGCGGG	chr22	41864477	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	tcTCGCTGCgcCTCAGCCGCAGG	chr19	2207686	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTCctTcCCTCTCAGCCtCTGG	chr19	8828214	-	4	0

X	GTTCGCTGCCTCTCAGCCGCNGG	GTTaGCTGtCTCTtgGCCGCTGG	chr19	23369142	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GgTCGCTGaCTCaCAGCCGCAGG	chr19	46010136	-	3	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GcTaGCTGtCTCTCAGtCGCTGG	chr19	51253303	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	tgTCtCTGCCTCTCAGCCcCCGG	chr19	51585940	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	aTggGCTGCCaCTCAGCCGCGGG	chr21	44775160	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTaGCTGgCTCTCAGCCtCAGG	chr15	28106352	+	3	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTaGCTtCCTtTCAGCCtCTGG	chr15	94806594	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTaGCTGCCTCTCcGtCGCTGG	chrY	9938947	+	3	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTtGgTGCCTgTCAGCCGtGGG	chr17	4982094	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GcTtGCTGCCTtTCAGCaGCTGG	chr17	17143558	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTcCtCTGCCTaTCAGaCGCCGG	chr17	21187912	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GcTaGCTGtCTCTCAGtCGCCGG	chr17	66065887	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GgTCctTGCCTCgCAGCCGCCGG	chr6	5260751	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTgCGCctCCTCTCAGCaGCCGG	chr6	40638930	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTgCGCTGggTCTCAGCCcCGGG	chr6	111303336	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTCtCTGCCTgTgAGCCcCTGG	chr6	145779528	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTaGCcGtCTCTCAGtCGCCGG	chr6	153003705	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTCcCTGCCTCTtAGCtGgAGG	chr14	24576358	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTCaCTGCCTCTgAGCtGgTGG	chr14	47936202	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTgGgTGCCTCTCAGCCGtGGG	chr14	56824336	+	3	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTCGCTGCgcCgCgGCCGCCGG	chr14	75745570	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	cTTCcCTaCCTgTCAGCCGCTGG	chr14	78346500	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTaGCTGtCTCTtgGCCGCCGG	chr14	101561691	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTCaCTGCCTCTCtcCCcCAGG	chr20	15601656	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	cTTgGCTcCCTCTCAGCtGCTGG	chr9	87015605	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GcTgGCTGCCTCTCAGCaGtGGG	chr9	95402183	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	tcTCtCTGaCTCTCAGCCGCTGG	chr9	128801012	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	ccTCcCTGCCTCTCAGCCcCTGG	chr9	132005010	-	4	0

X	GTTCGCTGCCTCTCAGCCGCNGG	GTTCtCTGCCTCaCAGgaGCCGG	chr9	138022430	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	cTTtGCTtCCTCTCAGCaGCCGG	chr9	137943631	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTCaCTGCCaCTCAGCCtgAGG	chrX	44601032	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTCcCTGCCaaTCAGCCtCTGG	chrX	53032189	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTaGCTGCCTCTCgGtCGCCGG	chr18	52057540	-	3	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTgGCaGCaTCTCAGCCGCAGG	chr18	56452683	-	3	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTCcCTGCaTCTCAaCCaCAGG	chr11	6072400	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTCGCTGCCTCaCAGttcCTGG	chr11	18765551	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GcTCtCTGCCTCTgAGCCtCAGG	chr11	48542507	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GcTCtCTGCCTCTgAGCCtCAGG	chr11	48643596	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GaTCtCTGCCTCTgAGCCtCAGG	chr11	48607292	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GcTCtCTGCCTCTgAGCCtCAGG	chr11	51400389	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GcTCtCTGCCTCTgAGCCtCGGG	chr11	51431909	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GcTCtCTGCCTCTgAGCCtCAGG	chr11	55081356	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GcTCtCTGCCTCTgAGCCtCAGG	chr11	55151318	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTCtCTGCCTCTgAGCCtCAGG	chr11	55203729	+	3	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GaTCtCTGCCTCTgAGCCtCAGG	chr11	55238900	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GcTCtCTGCCTCTgAGCCtCAGG	chr11	55193084	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	tTTaGCTGCCTCTCAaCtGCAGG	chr11	64007861	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GccCaCaGCCTCTCAGCCGCAGG	chr11	69354395	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTCcaTGCCTCTCAGCttCTGG	chr11	73316844	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GagCcCTGCCTCTCAGCCtCGGG	chr11	76886328	+	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GgTgGCTGaCTCTCAGCaGCCGG	chr11	117917816	-	4	0
X	GTTCGCTGCCTCTCAGCCGCNGG	GTTaGCcGCCTCTCgGgCGCTGG	chr11	125555674	+	4	0

Supplementary table 2: Primers used in this work.

Nucleotides highlighted by red color are inline barcodes, nucleotides printed in green are indices.
NNNNN – random nucleotides, which were introduced to increase the sequence diversity of the library.

	ISG15 primers for bis PCR1
FP1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNN**AGAGC**TTAGGTGTTTTTAGGGTGTTGG
RP1	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNN**AGCAT**CACAAACTCCTATACTAACAAAAATAAAT
FP2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNN**GACAT**TTAGGTGTTTTTAGGGTGTTGG
RP2	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNN**GTCGT**CACAAACTCCTATACTAACAAAAATAAAT
FP3	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNN**ATAGA**TTAGGTGTTTTTAGGGTGTTGG
RP3	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNN**AAGCG**CACAAACTCCTATACTAACAAAAATAAAT
FP4	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNN**GTGTA**TTAGGTGTTTTTAGGGTGTTGG
RP4	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNN**GTCAC**CACAAACTCCTATACTAACAAAAATAAAT

	VEGFA primers for bis PCR1
FP1	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNN**AGAGC**GTTTGTTATTTTTTATTTGAAT
RP1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNN**AGCAT**AATCACTCACTTTACCCCTATC
FP2	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNN**TGTCG**GTTTGTTATTTTTTATTTGAAT
RP2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNN**TCAGC**AATCACTCACTTTACCCCTATC
FP3	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNN**GACAT**GTTTGTTATTTTTTATTTGAAT
RP3	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNN**GTCGT**AATCACTCACTTTACCCCTATC
FP4	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNN**CAGAT**GTTTGTTATTTTTTATTTGAAT
RP4	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNN**CGTGT**AATCACTCACTTTACCCCTATC

	PCR2 primers with indices and Illumina adapters
P5 primers	AATGATACGGCGACCACCGAGATCTACAC**ATTACTCG**ACACTCTTTCCCTACACGACGCTCTTCCGATCT
	AATGATACGGCGACCACCGAGATCTACAC**TCCGGAGA**ACACTCTTTCCCTACACGACGCTCTTCCGATCT
	AATGATACGGCGACCACCGAGATCTACAC**CGCTCATT**ACACTCTTTCCCTACACGACGCTCTTCCGATCT
	AATGATACGGCGACCACCGAGATCTACAC**GAGATTCC**ACACTCTTTCCCTACACGACGCTCTTCCGATCT
	AATGATACGGCGACCACCGAGATCTACAC**ATTCAGAA**ACACTCTTTCCCTACACGACGCTCTTCCGATCT
	AATGATACGGCGACCACCGAGATCTACAC**GAATTCGT**ACACTCTTTCCCTACACGACGCTCTTCCGATCT
P7 primers	CAAGCAGAAGACGGCATACGAGAT**CGAGTAAT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
	CAAGCAGAAGACGGCATACGAGAT**TCTCCGGA**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
	CAAGCAGAAGACGGCATACGAGAT**AATGAGCG**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
	CAAGCAGAAGACGGCATACGAGAT**GGAATCTC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
	CAAGCAGAAGACGGCATACGAGAT**TTCTGAAT**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
	CAAGCAGAAGACGGCATACGAGAT**ACGAATTC**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT