Supporting Information

Cellular Target of a Rhodium Metalloinsertor is the DNA Base Pair Mismatch

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Table S1: Growth conditions for cell lines used.

Cell Line	Obtained from	Type of Media	% FBS	Media Supplements
CaCo2[a,b]	AMGEN	DMEM	20	100 U/mL PenStrep
Colo205[a,b]	AMGEN	RPMI 1640	10	100 U/mL PenStrep
Colo320DM[a]	AMGEN	RPMI 1640	10	100 U/mL PenStrep
Colo678[a,b]	AMGEN	DMEM	10	100 U/mL PenStrep
CW2[a,b]	AMGEN	DMEM	10	100 U/mL PenStrep
DLD-1[a,b,c,d]	AMGEN	RPMI 1640	10	100 U/mL PenStrep
HCC2998[a,b]	AMGEN	RPMI 1640	10	100 U/mL PenStrep
HCT116[a,b,c]	AMGEN	McCoy's 5A	10	100 U/mL PenStrep
HCT15[a,b,d]	AMGEN	RPMI 1640	10	100 U/mL PenStrep
HT29[a,b,d]	AMGEN	McCoy's 5A	10	100 U/mL PenStrep
KM12[a]	AMGEN	RPMI 1640	10	100 U/mL PenStrep
LoVo[a]	AMGEN	Ham's F-12K	10	100 U/mL PenStrep
Ls1034[a,b]	AMGEN	RPMI 1640	10	100 U/mL PenStrep
Ls123[a,b]	AMGEN	DMEM	10	100 U/mL PenStrep
Ls174T[a,b]	AMGEN	DMEM	10	100 U/mL PenStrep
NCI-H716[a,b]	AMGEN	RPMI 1640	10	100 U/mL PenStrep
NCI-H508[a]	AMGEN	RPMI 1640	10	100 U/mL PenStrep, 2 mM GlutaMAX
RKO[a,b]	AMGEN	DMEM	10	100 U/mL PenStrep
SW1116[a,b]	AMGEN	RPMI 1640	10	100 U/mL PenStrep
SW1463[a]	AMGEN	RPMI 1640	10	100 U/mL PenStrep
SW403[a,b]	AMGEN	RPMI 1640	10	100 U/mL PenStrep, 2 mM GlutaMAX
SW48[a,b]	AMGEN	RPMI 1640	10	100 U/mL PenStrep
SW480[a,d]	AMGEN	RPMI 1640	10	100 U/mL PenStrep
SW620[a,b,d]	AMGEN	RPMI 1640	10	100 U/mL PenStrep
SW837[a]	AMGEN	RPMI 1640	10	100 U/mL PenStrep
SW948[a,b]	ATCC	RPMI 1640	10	100 U/mL PenStrep, 2 mM GlutaMAX
WiDr[a,b,d]	AMGEN	DMEM	10	100 U/mL PenStrep
AN3-CA[c]	AMGEN	DMEM	10	100 U/mL PenStrep
DU-145[c]	ATCC	DMEM	10	100 U/mL PenStrep
HCT-116N[c]	--	RPMI 1640	10	100 U/mL PenStrep, 2 mM L-glutamine, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, 400 ug/mL Geneticin (G418)
HCT-116O[c]	--	RPMI 1640	10	100 U/mL PenStrep, 2 mM L-glutamine, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, 400 ug/mL Geneticin (G418)
HEC-1-A[c]	ATCC	McCoy's 5A	10	100 U/mL PenStrep

[a] Cell lines used in cytotoxicity assay
[b] Cell lines used in whole cell uptake assay
[c] Cell lines used in **RhCy3** fluorescence assay
[d] DLD-1/HCT15, HT29/WiDr, SW480/SW620 pairings are derived from a common patient.

Table S2: IC50 values of **RhPPO** and cisplatin in all tested cell lines.

Cell Line	**IC50** RhPPO (μM)	IC50 Cisplatin (μM)	IC50 RhPPO /IC50 Cisplatin	Cancer Type	MMR Status, (Mutated Protein)
CaCo-2	1.5 ± 0.8	9.5 ± 3.7	6.2	Colorectal	MMR+
Colo 205	0.063 ± 0.03	36.4 ± 2.8	580	Colorectal	MMR+
Colo 320DM	18.0 ± 2.9	9.5 ± 2.0	0.5	Colorectal	MMR+
Colo 678	0.81 ± 0.15	18.2 ± 0.7	22.4	Colorectal	MMR+
CW-2	9.2 ± 1.3	9.0 ± 2.1	1.0	Colorectal	MMR−, (−MLH1)
DLD-1 [c]	3.6 ± 0.3	10.7 ± 2.2	3.0	Colorectal	MMR−, (−MSH6)
HCC2998 [a]	1.6 ± 0.6	20.4 ± 1.4	12.6	Colorectal	MMR+, (−POLE)
HCT-116 [c]	0.25 ± 0.01	18.5 ± 0.9	73.3	Colorectal	MMR−, (−MLH1)
HCT-15	9.5 ± 2.5	16.1 ± 0.3	1.7	Colorectal	MMR−, (−MSH6)
HT-29	0.21 ± 0.01	22.1 ± 1.1	106	Colorectal	MMR+
KM-12	0.83 ± 0.07	13.9 ± 0.9	16.7	Colorectal	MMR−, (−MLH1)
LoVo	1.7 ± 0.2	7.0 ± 1.2	4.0	Colorectal	MMR−, (−MSH2)
Ls1034	5.6 ± 0.3	14.4 ± 1.9	2.6	Colorectal	MMR+
Ls123	0.23 ± 0.03	9.0 ± 7.0	39.7	Colorectal	MMR+
Ls174T	2.0 ± 0.2	5.4 ± 0.6	2.8	Colorectal	MMR−, (−MLH1)
NCI-H716	1.8 ± 0.6	13.9 ± 2.9	7.7	Colorectal	MMR+
NCI-H508	1.5 ± 0.4	8.8 ± 1.4	5.9	Colorectal	MMR+
RKO	0.12 ± 0.01	11.6 ± 0.7	97.5	Colorectal	MMR−, (−MLH1)
SW-1116	4.4 ± 1.2	9.1 ± 1.4	2.1	Colorectal	MMR+
SW-1463	1.6 ± 0.2	9.5 ± 1.1	6.0	Colorectal	MMR+
SW-403	0.34 ± 0.04	9.1 ± 1.5	27.1	Colorectal	MMR+
SW-48	0.34 ± 0.02	2.2 ± 0.2	6.4	Colorectal	MMR−, (−MLH1)
SW-480 [c]	0.44 ± 0.13	8.3 ± 0.6	12.0	Colorectal	MMR+
SW-620	0.33 ± 0.04	4.8 ± 0.8	14.7	Colorectal	MMR+
SW-837	1.8 ± 0.7	11.4 ± 1.3	6.2	Colorectal	MMR+
SW-948	9.7 ± 1.9	22.1 ± 1.7	2.3	Colorectal	MMR+
WiDr	0.13 ± 0.01	25.5 ± 14.1	198.7	Colorectal	MMR+
AN3-CA [c]	0.086 ± 0.003	--	--	Endometrial	MMR−, (−MLH1)
DU-145 [c]	0.67 ± 0.04	--	--	Prostate	MMR−, (−MLH1, PMS2)
HEC-1-A [c]	0.39 ± 0.02	--	--	Endometrial	MMR−, (−PMS2)
HCT-116N [b,c]	1.12 ± 0.27	--	--	Colorectal	MMR+
HCT-116O [b,c]	0.15 ± 0.06	--	--	Colorectal	MMR−, (−MLH1)

[a] HCC2998 is mutated in the POLE gene, leading to an increase in polymerase errors such as mismatches and indels. While it is not technically MMR−, it will have higher mismatch and indel occurrences than other MMR+ cell lines.

[b] IC50 values from reference [5]

[c] Cell lines used as the test set for the RhCy3 assay. Note that since the colorectal cancer cell lines examined in the cytotoxicity studies were primaily deficient in the MLH1 gene, this test set includes several cell lines of non-colorectal origin that span deficienicies in different MMR genes as well.

Table S3: Purity of genomic DNA extracted from the cancer cell lines used in this study as determined using a NanoDrop spectrophotometer.

Phenotype[a]	*A260/A280*[b]	*A260/A230*[c]
SW480-i	1.94	2.05
SW480-ii	1.93	2.06
SW480-iii	1.94	2.06
HCT116N-i	2.02	2.02
HCT116N-ii	2.00	2.04
HCT116N-iii	2.01	2.02
HCT116O-i	1.95	1.97
HCT116O-ii	1.97	2.01
HCT116O-iii	1.99	2.00
HCT116-i	1.97	2.06
HCT116-ii	1.98	2.05
HCT116-iii	1.94	2.03
AN3-CA-i	1.91	1.99
AN3-CA-ii	1.92	1.99
AN3-CA-iii	1.91	1.98
DU-145-i	2.00	1.97
DU-145-ii	1.95	1.90
DU-145-iii	1.96	1.91
HEC-1A-i	1.95	2.03
HEC-1A-ii	1.97	2.06
HEC-1A-iii	1.93	2.04
DLD-1-i	2.00	2.04
DLD-1-ii	1.94	1.98
DLD-1-iii	1.99	2.06

[a] For each phenotype there are three biological replicates (i, ii, iii).

[b] The ratio of absorbance at 260 nm and 280 nm is used to assess the purity of DNA. A ratio of ~1.8 is considered as "pure" for DNA; If the ratio is appreciably lower it may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm.

[c] This ratio is used as a secondary measure of nucleic acid purity. The 260/230 values for "pure" nucleic acid are commonly in the range of 2.0 - 2.2. If the ratio is appreciably lower than expected, it may indicate the presence of contaminants which absorb at 230 nm.

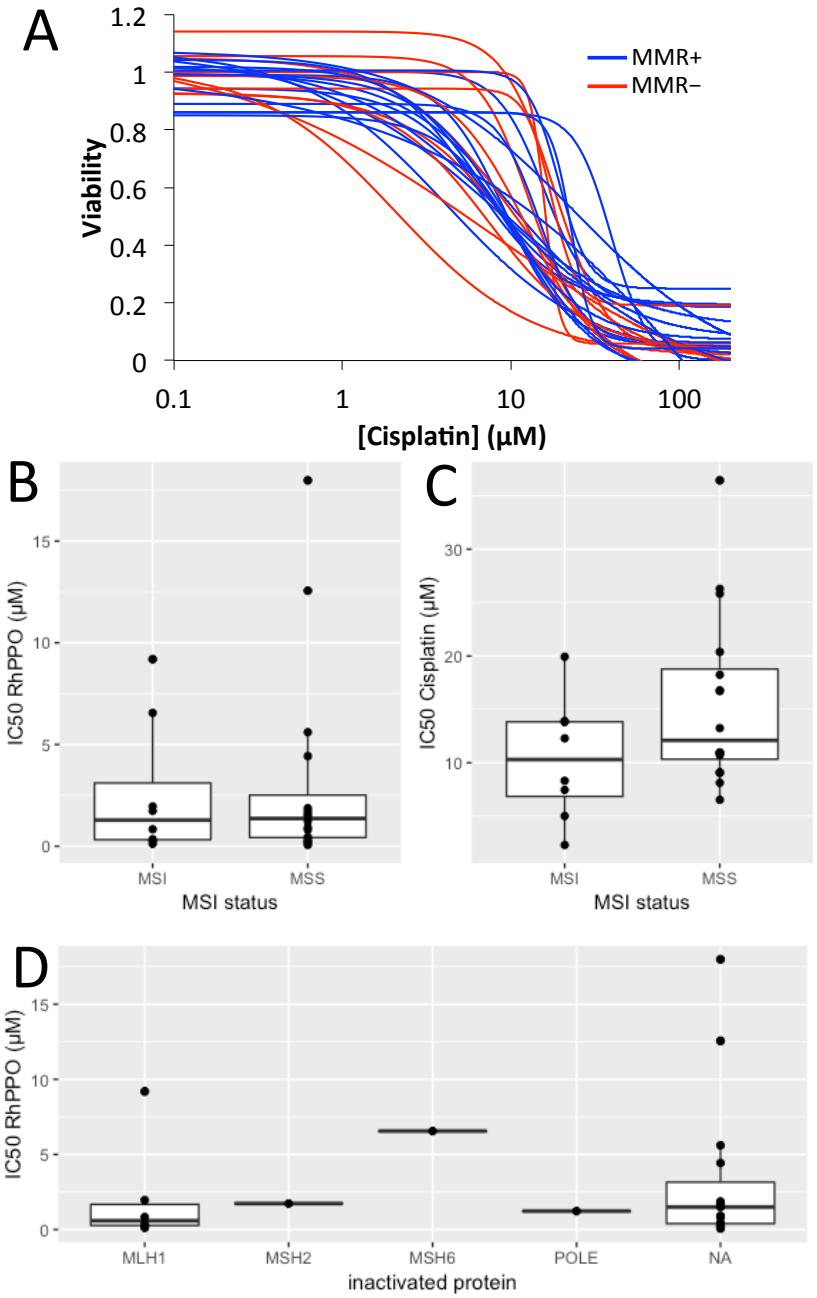

Figure S1. Cytotoxicity of a therapeutic in 27 colorectal cancer cell lines. A) Dose response curves of cisplatin in MMR− and MMR+ CRC cell lines. B) Comparison of the IC50 values of **RhPPO** in MMR+ (MSS) and MMR− (MSI) cell lines shown as boxplots. The average IC50 of **RhPPO** in MMR+ cells is 3.22 µM and the median is 1.37 µM. The average IC50 of **RhPPO** in MMR− cells is 2.62 µM and the median is 1.28 µM. C) Comparison of the IC50 values of cisplatin in MMR+ and MMR− cell lines shown as boxplots. The average IC50 of cisplatin in MMR+ cells is 15.65 µM and the median is 12.09 µM. The average IC50 of cisplatin in MMR− cells is 10.38 µM and the median is 10.30 µM. D) Comparison of the IC50 values of **RhPPO** broken down based on mutated MMR (or POLE) protein (MMR+ = NA). For MLH1 deficient cells, the IC50 of **RhPPO** is 2.06 µM and the median is 0.59 µM.

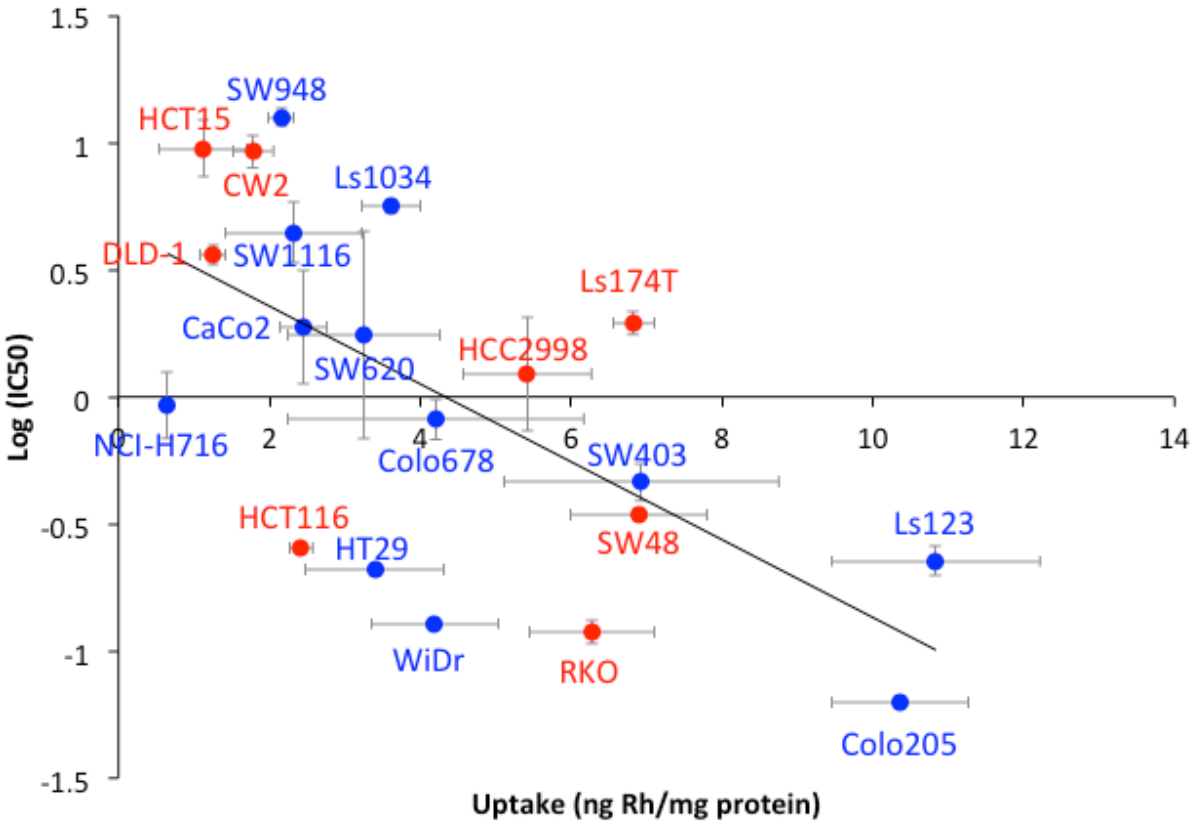

Figure S2: A correlation between whole cell uptake and IC50 for **RhPPO**. MMR– cell lines are shown in red and MMR+ are shown in blue, with all cell lines labeled.

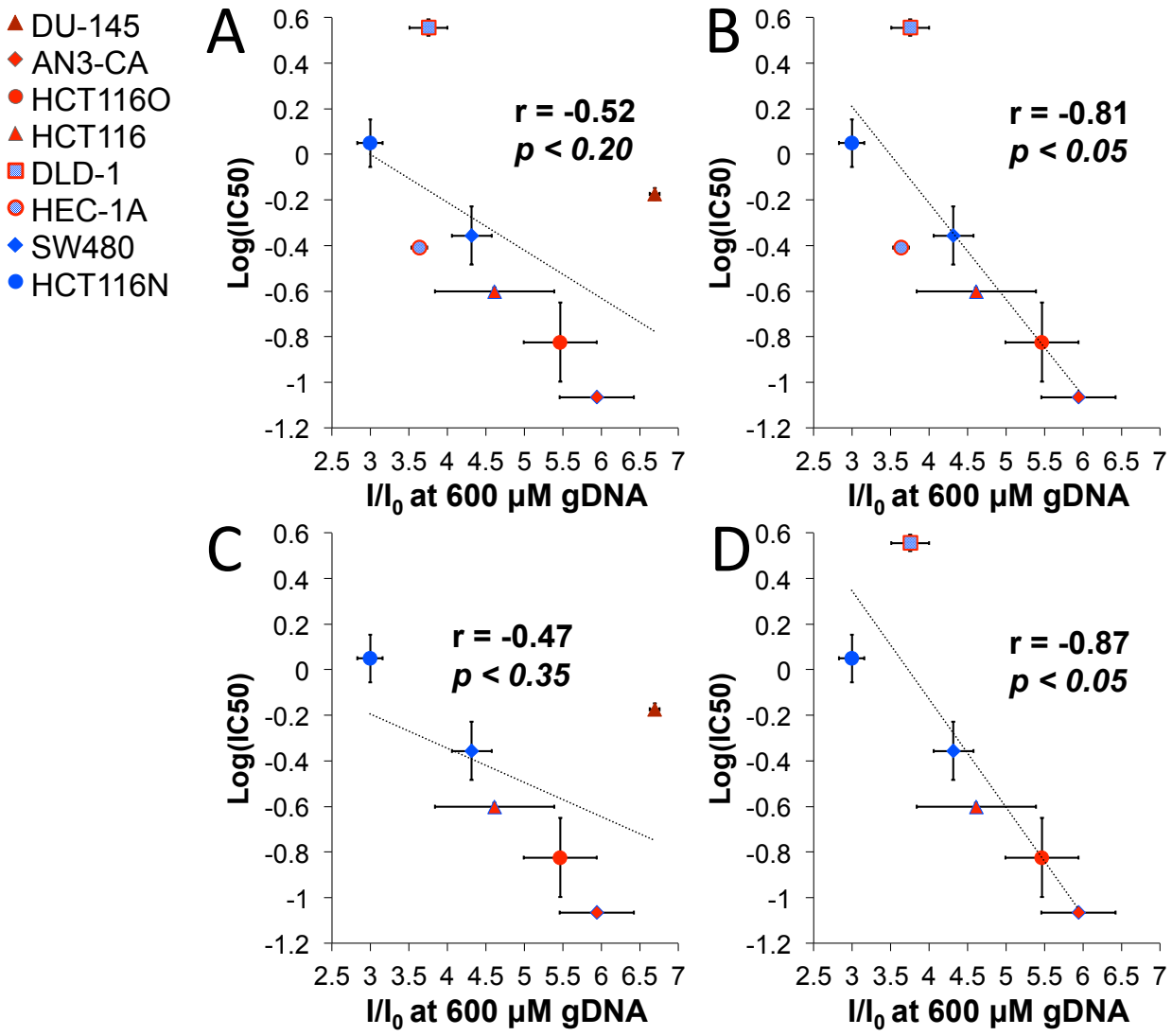

Figure S3. Scatterplots comparing fluorescence output of **RhCy3** with gDNA versus the Log(IC50) of **RhPPO** in various cell lines. A) shows all data and has a Pearson's r = -0.52, making it significant at the $p < 0.2$ level. B) By removing the possible outlier, DU145, the Pearson's r increases to -0.81, which is significant at the $p < 0.05$ level. C) When comparing only MMR+ cell lines and MMR– cell lines with mutations in the MLH1 gene, r = -0.47 with significance at the $p < 0.35$ level. D) When comparing only MMR+ cell lines and MMR– cell lines that are not mutated in PMS2, r increases to -0.87 with significance at the $p < 0.05$ level.

Supplemental Discussion on RhCy3 as a reporter on gDNA lesions

There is a clear relationship between the identity of the deficient MMR protein and **RhCy3** fluorescence output. Titrations of **RhCy3** with genomic DNA extracted from cell lines with a deficiency in the MLH1 protein (i.e. HCT116O, AN3-CA, DU-145, and HCT116) resulted in the highest fluorescence intensities, indicating there are an abundance of mismatches, indels, and/or abasic sites present in these cell lines. The final two MMR− cell lines, DLD-1 and HEC-1-A, display low fluorescence intensity that is comparable to the intensity observed for the MMR+ cell lines, HCT116N and SW480. It may be possible to explain the low intensity observed with DLD-1 and HEC-1-A by considering their specific MMR-deficiencies: MSH6 and PMS2, respectively. Functioning MMR generally involves two heterodimers, MutSα (MSH2 + MSH6) and MutLα (MLH1 + PMS2), however other homologues to these heterodimers also exist: MutSβ (MSH2 + MSH3), MutLβ (MLH1 + MLH2), and MutLγ (MLH1 + MLH3).[6] These different MutS and MutL homologues have different roles in the cell, with MutSα and MutLα correcting mismatches and some indels, and MutSβ, MutLβ, and MutLγ contributing to the correction of long and short indels but not mismatches. Therefore, a cell line deficient in MSH2 or MLH1 cannot correct any mismatches or indels, but a cell line deficient in MSH6 or PMS2 may still be able to correct some indels via MutSβ, MutLβ, and MutLγ homologues. Considering this, the fluorescence of DLD-1 and HEC-1-A may be relatively low because these cell lines have MMR machinery that can correct some indels, meaning they will have fewer total lesions than MLH1-deficient cells that correct neither mismatches nor indels. It is also of note that the baseline fluorescence in the presence of DNA from MMR+ cells and the variations between DNA from different MMR− cells may be due to **RhCy3** binding abasic sites or other thermodynamically destabilized lesions that are not associated with MMR pathways. Overall **RhCy3** shows great promise as a direct reporter on the number of thermodynamically destabilized lesions in gDNA, and it may also have the potential to be developed into an early diagnostic reporter of MMR-deficiencies in cells.

References

[1] A. C. Komor, J. K. Barton, *J. Am. Chem. Soc.* **2014**, *136*, 14160–14172.
[2] A. Nano, A. N. Boynton, J. K. Barton, *J. Am. Chem. Soc.* **2017**, *139*, 17301–17304.
[3] A. C. Komor, C. J. Schneider, A. G. Weidmann, J. K. Barton, *J. Am. Chem. Soc.* **2012**, *134*, 19223–19233.
[4] K. M. Boyle, J. K. Barton, *J. Am. Chem. Soc.* **2018**, *140*, 5612–5624.
[5] J. M. Bailis, A. G. Weidmann, N. F. Mariano, J. K. Barton, *Proc. Natl. Acad. Sci.* **2017**, *114*, 6948–6953.
[6] T. A. Kunkel, D. A. Erie, *Annu. Rev. Genet.* **2015**, *49*, 291–313.