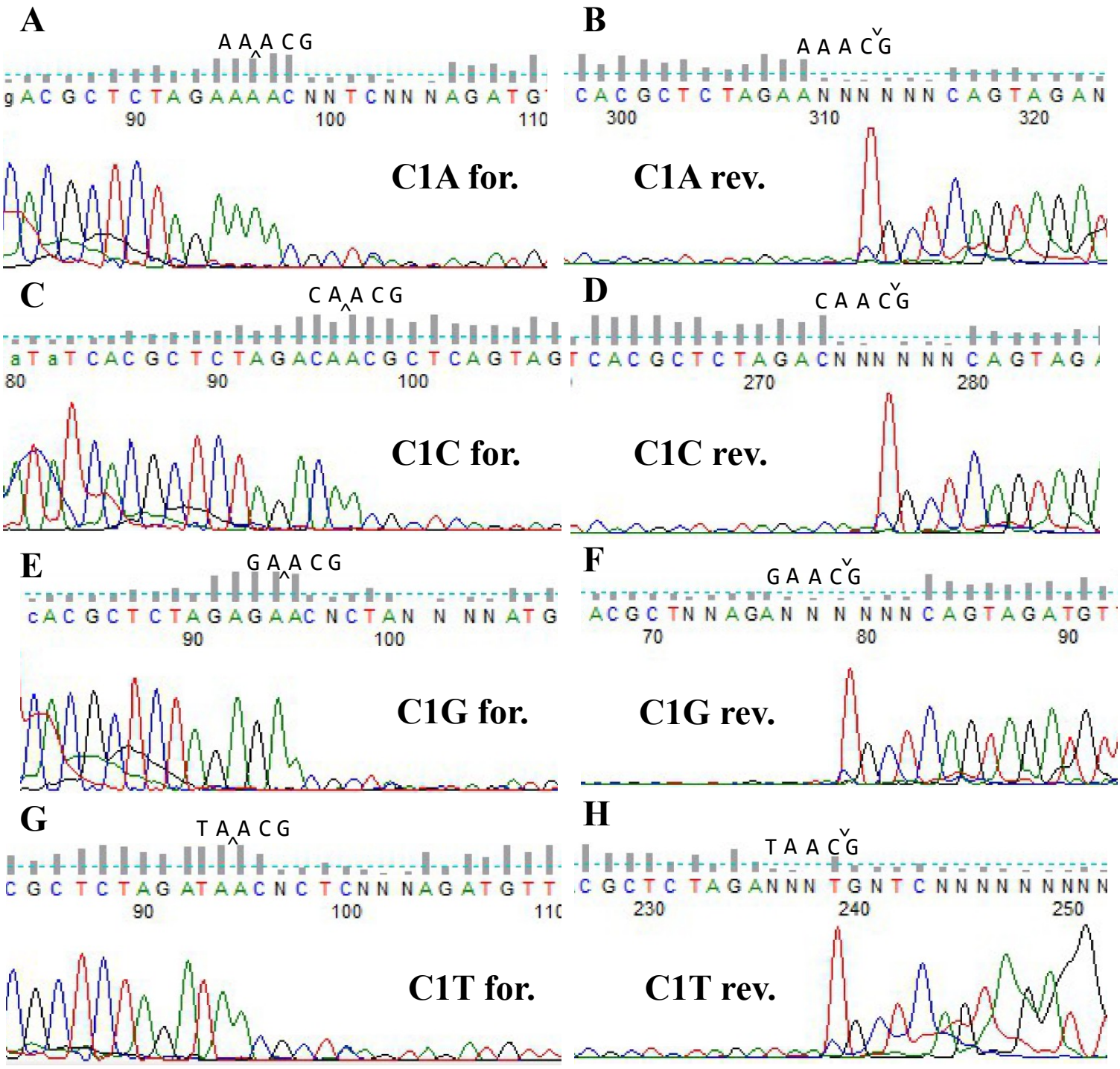
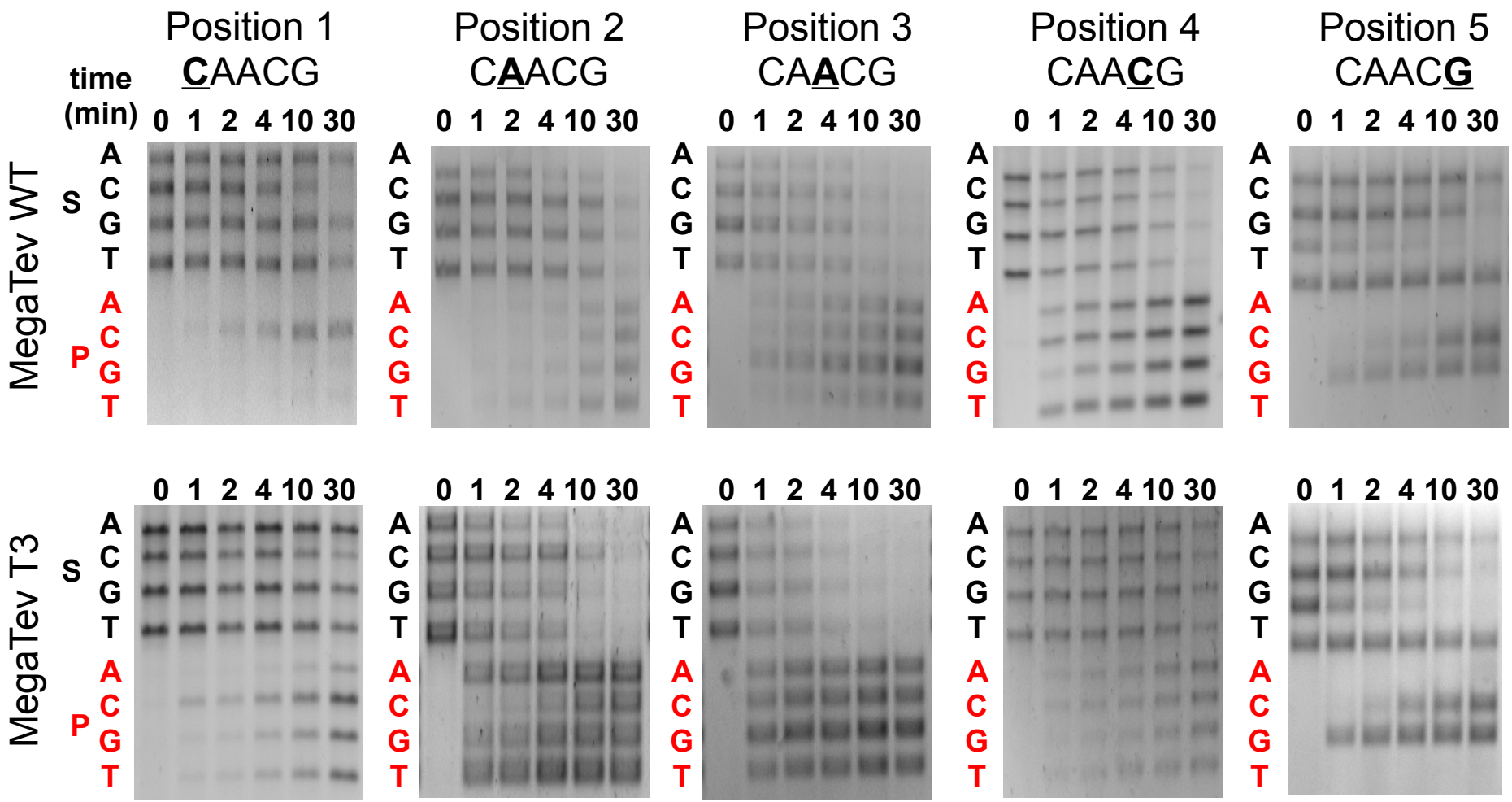
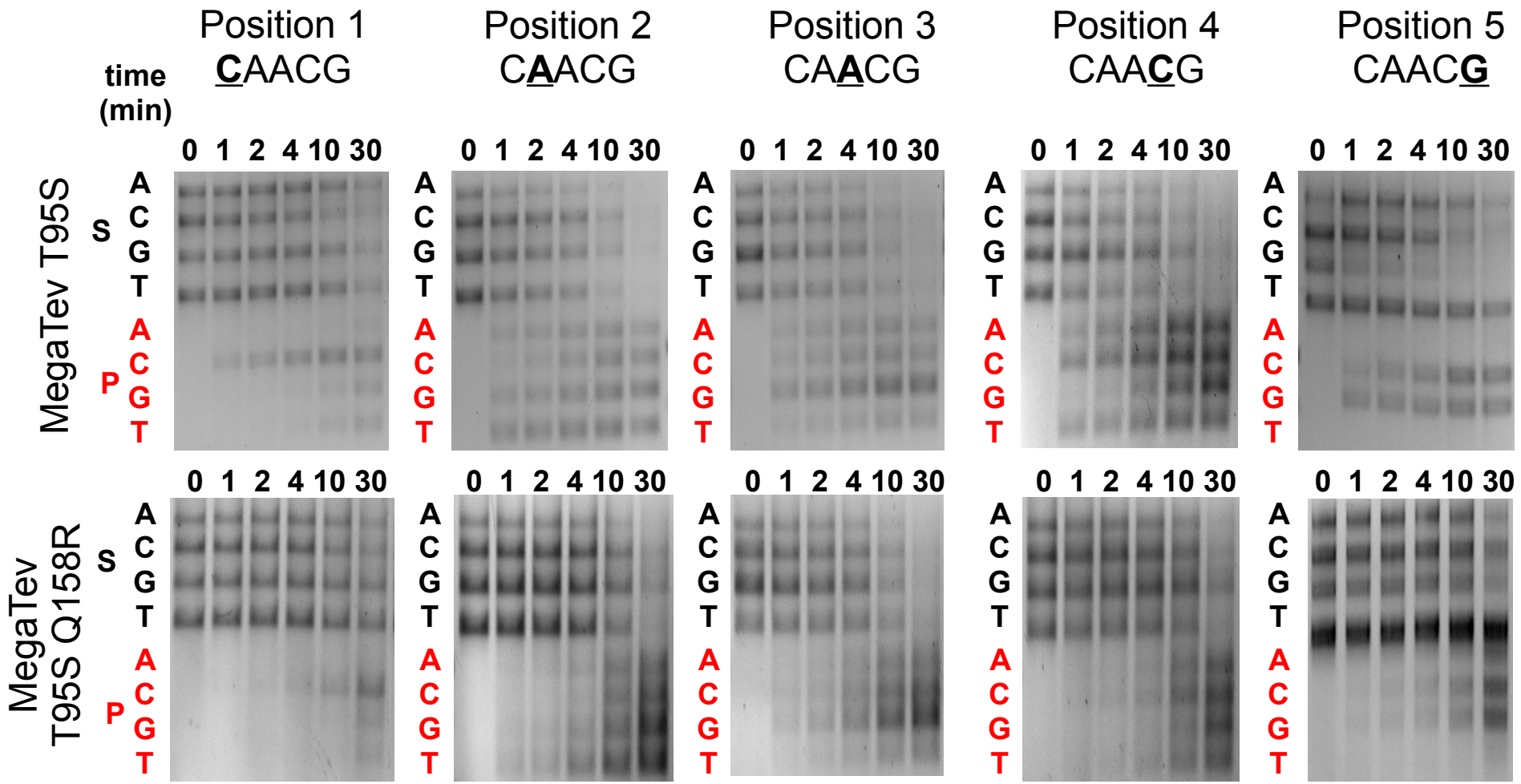
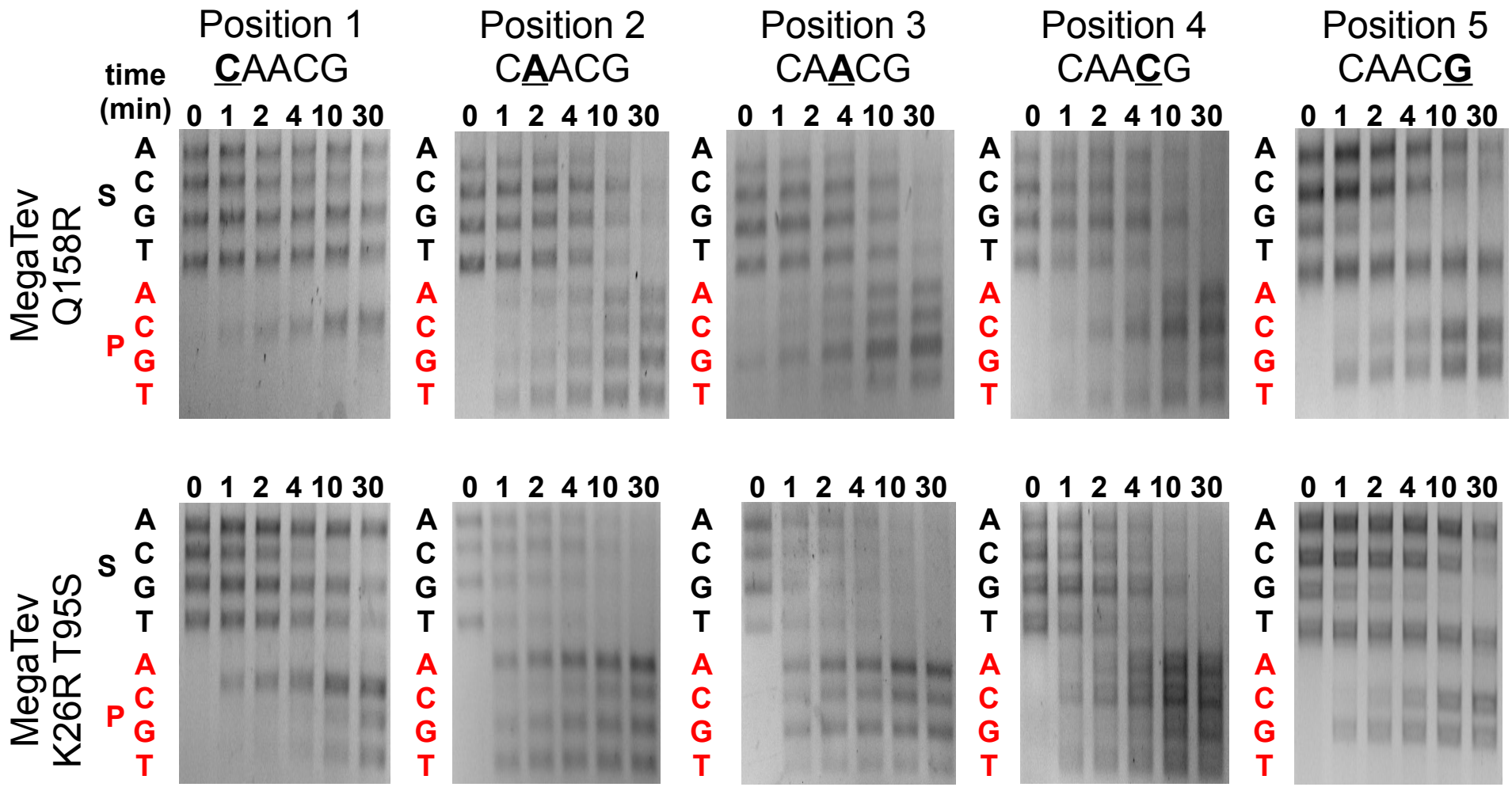
S1 Fig. The position of nicking sites of I-TevI T3 are unaffected by subsitutions at position 1 of the cleavage motif. pTox plasmids harbouring native cleavage motif (C1C [C, D]), or one of three position 1 substitutions (C1A [A, B], C1G [E, F], C1T [G, H]) were sequenced using flanking primers: one upstream of the cleavage motif (forward. [A, C, E, G]) and one downstream (reverse. [B, D, F, H], the reverse complement is shown). Sanger sequencing readouts are shown with traces for adenine (green), cytosine (blue), guanine (black), and thymine (red). The cleavage motif is given above the corresponding region of the readout, with a chevron indicating the predicted nicking position. A drop-off in fluorescence intensity is seen in each sanger readout corresponding to the predicted nicking positions in all eight cases. The 3' A or 5' T observed in each trace is an artifact of sequencing.

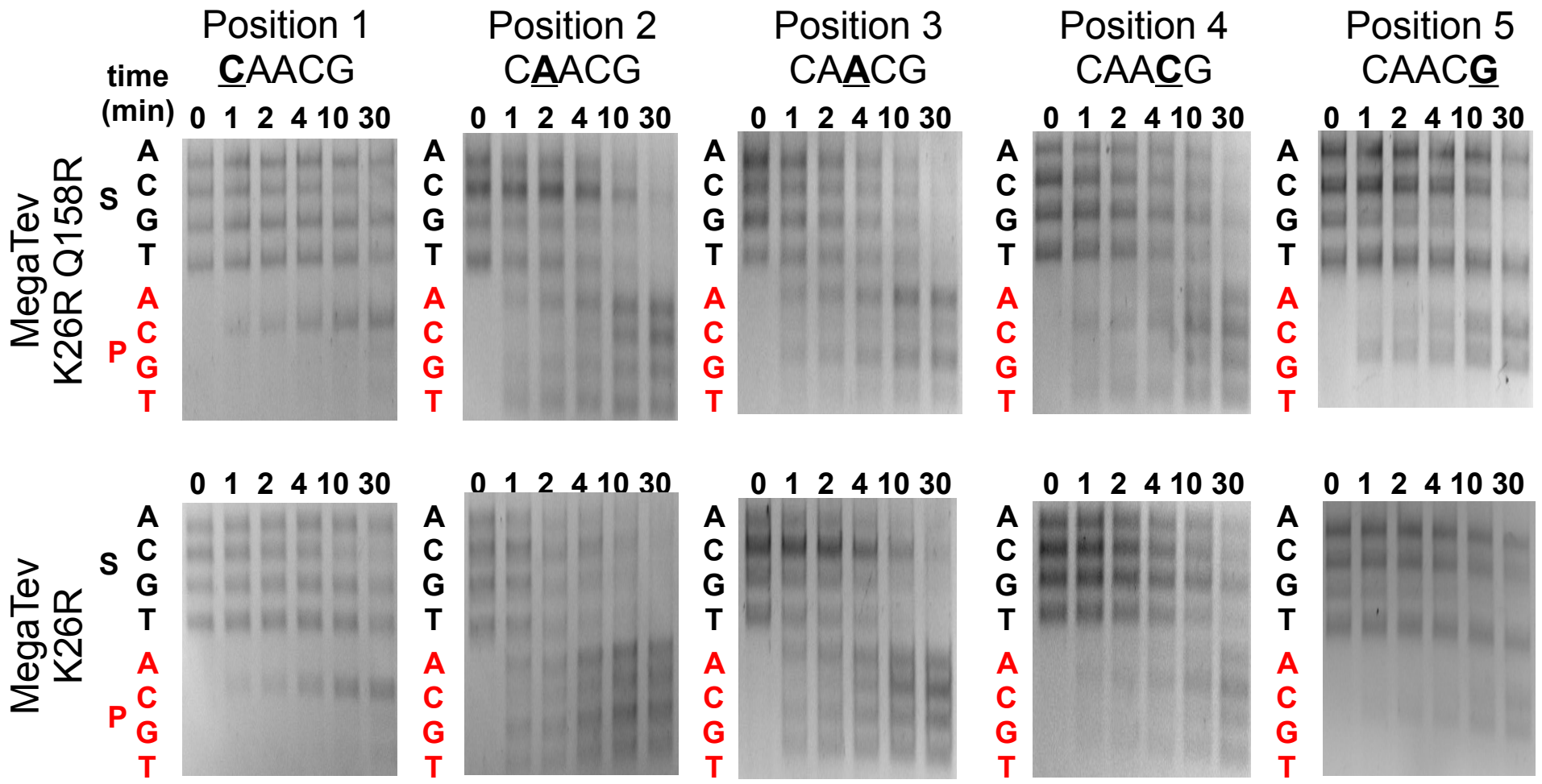

A
A A^A C G
g A C G C T C T A G A A A A C N N T C N N N A G A T G
90
100
110
C1A for.
B
A A A C^G
C A C G C T C T A G A A N N N N N N C A G T A G A N
300
310
320
C1A rev.
C
C A^A C G
a T a T C A C G C T C T A G A C A A C G C T C A G T A G
80
90
100
C1C for.
D
C A A C^G
T C A C G C T C T A G A C N N N N N N C A G T A G
270
280
C1C rev.
E
G A^A C G
c A C G C T C T A G A G A A C N C T A N N N N A T G
90
100
C1G for.
F
G A A C^G
A C G C T N N A G A N N N N N N C A G T A G A T G T
70
80
90
C1G rev.
G
T A^A C G
C G C T C T A G A T A A C N C T C N N N A G A T G T T
90
100
110
C1T for.
H
T A A C^G
C G C T C T A G A N N N T G N T C N N N N N N N N N
230
240
250
C1T rev.

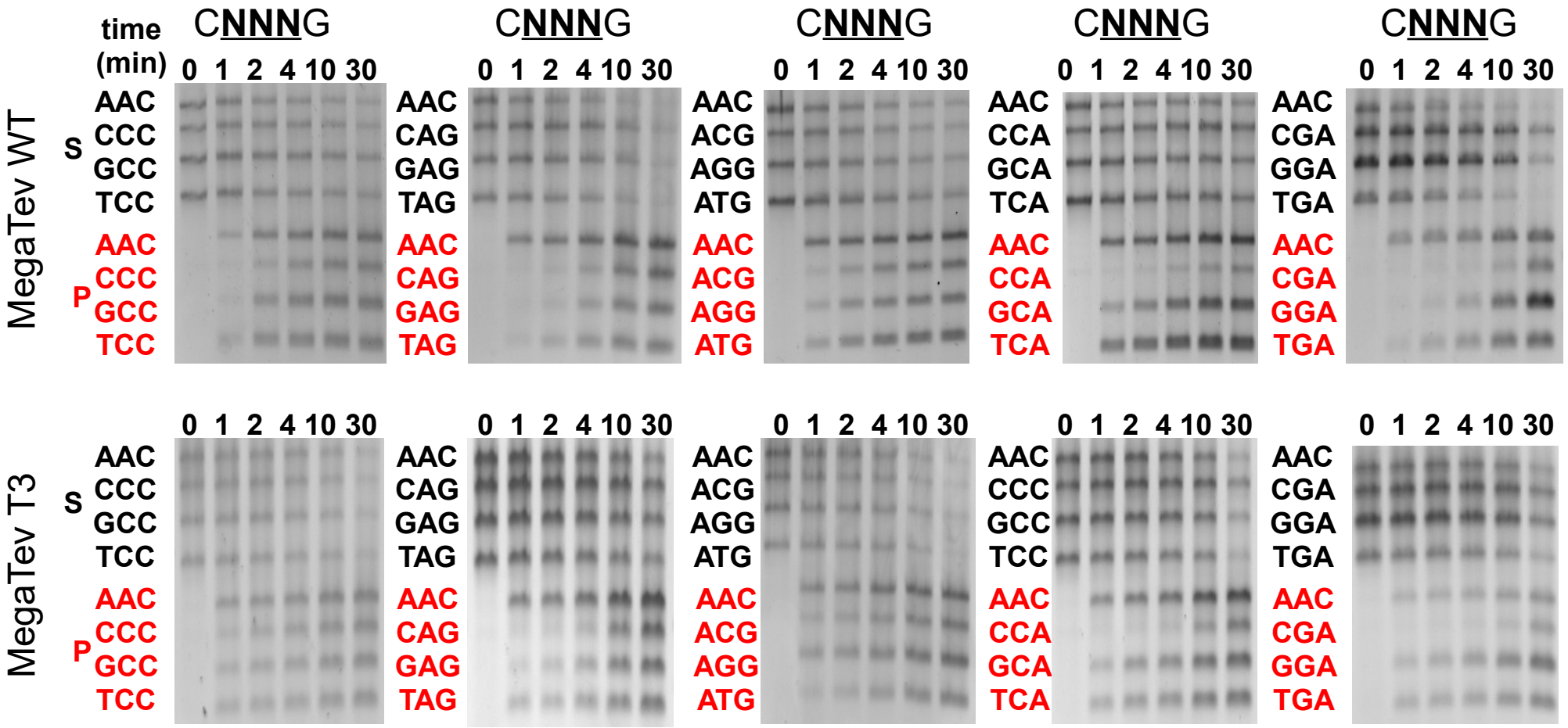
S2 Fig. Gel images of time course barcode cleavage assays for MegaTev variants. Each gel image corresponds to a position within the CAACG motif, or a related set of "triplet" substrates. The nucleotide substitution for each length substrate (S) is indicated on the left side of the gel in black font. The corresponding product (P) is indicated in red font. The images for each substrate were cropped from separate images and assembled into a composite figure.

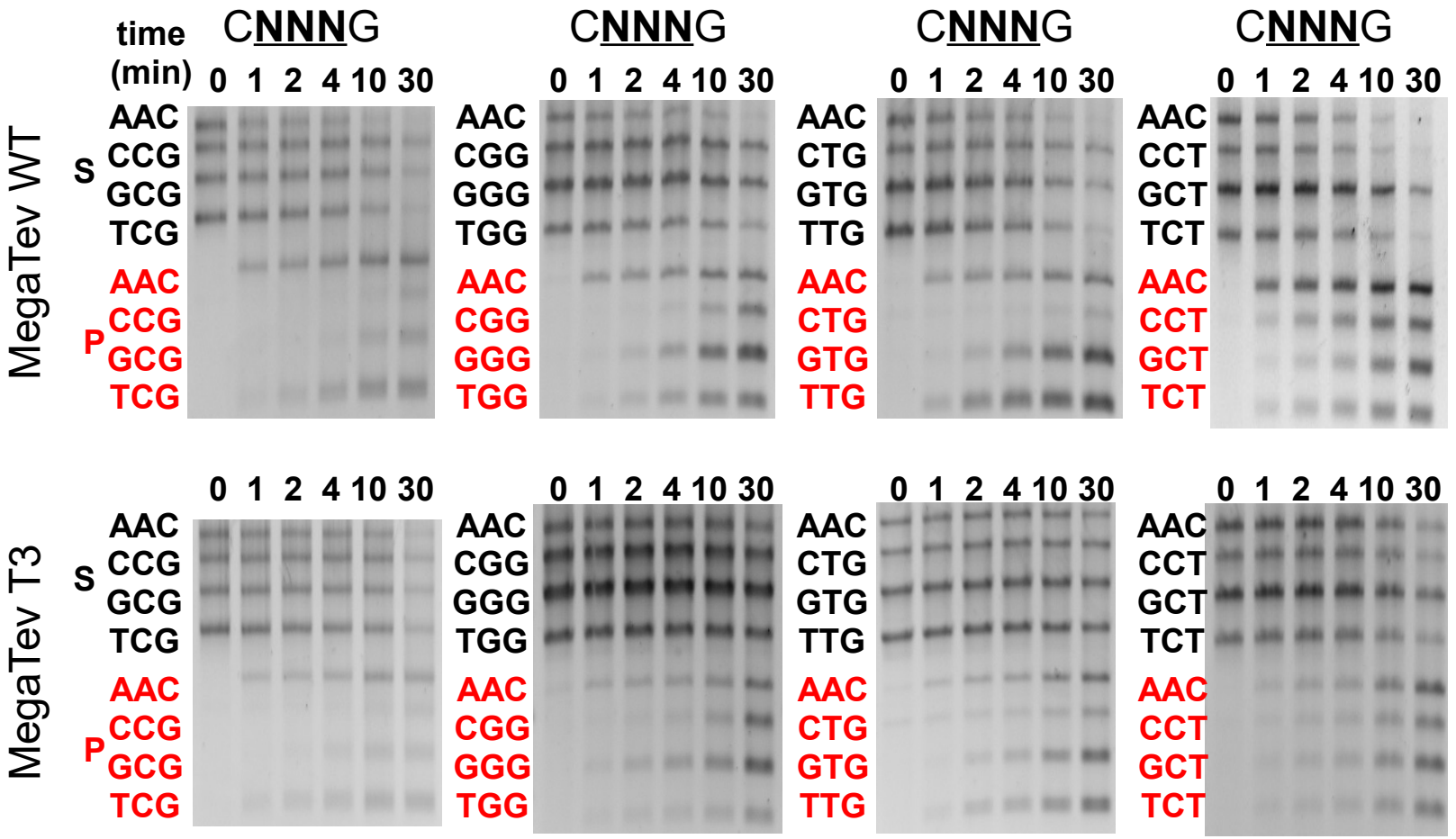

Position 1
CAACG
Position 2
CAACG
Position 3
CAACG
Position 4
CAACG
Position 5
CAACG
time (min)
0 1 2 4 10 30
MegaTev WT
MegaTev T3
S
P
A
C
G
T


Position 1
CAACG
Position 2
CAACG
Position 3
CAACG
Position 4
CAACG
Position 5
CAACG
time (min)
0 1 2 4 10 30
MegaTev T95S
MegaTev T95S Q158R
S
P
A
C
G
T


Position 1
CAACG
Position 2
CAACG
Position 3
CAACG
Position 4
CAACG
Position 5
CAACG
time (min)
0 1 2 4 10 30
S
P
A
C
G
T
MegaTev Q158R
MegaTev K26R T95S

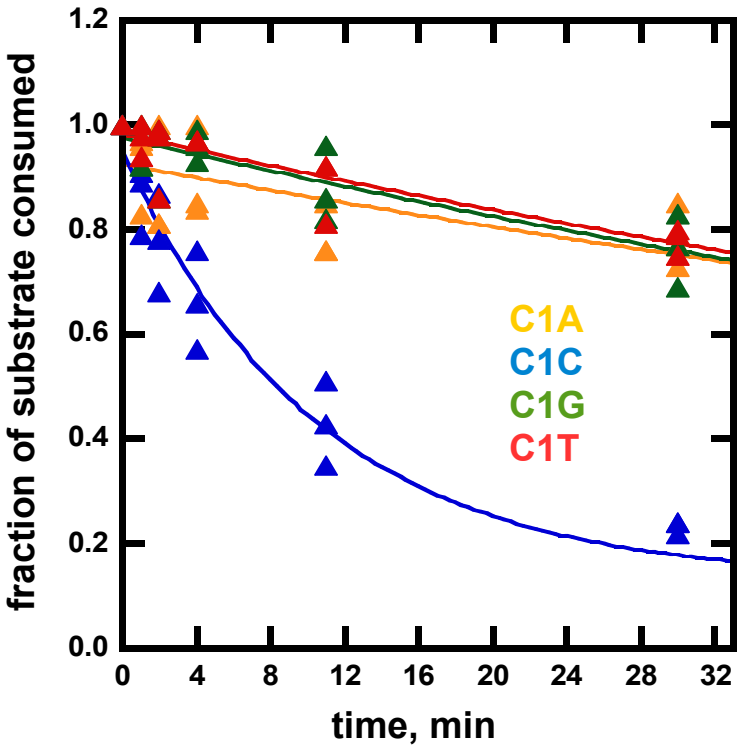

Position 1
CAACG
Position 2
CAACG
Position 3
CAACG
Position 4
CAACG
Position 5
CAACG
time (min)
0 1 2 4 10 30
S
P
A C G T
MegaTev K26R Q158R
MegaTev K26R


MegaTev WT
MegaTev T3
time (min)
0 1 2 4 10 30
CNNNG
S
P
AAC
CCC
GCC
TCC
CAG
GAG
TAG
ACG
AGG
ATG
CCA
GCA
TCA
CGA
GGA
TGA


MegaTev WT
MegaTev T3
time (min)
0 1 2 4 10 30
CNNNG
S
P
AAC
CCG
GCG
TCG
CGG
GGG
TGG
CTG
GTG
TTG
CCT
GCT
TCT

S3 Fig. Barcode assay kinetic data. MegaTev variants were assayed against four substrates of varying lengths (2200 [yellow ▲], 1900 [blue ▲], 1600 [green ▲], and 1320 bp [red ▲]). Each plot represents reaction progress over time for a combination of substrates and MegaTev enzyme. Substrates harboured I-TevI cleavage motifs, one of which was the native cleavage motif (5' – CAACG – 3'), and the others were comprised of NNN triplet substitutions (5' – CNNNG – 3'), position 1 substitutions (5' – NAACG – 3'), or control substrates (as described in the text). The controls included all native cleavage motifs, one of each of four lengths, or a single native cleavage motif among cleavage resistant motifs (5' – AAACA – 3'). The equation of fit is explained in detail in the text (see Equation 1). Note that the equation given below is superficially different; using the identities $y = f_S$, and $x = t$, the equations below become Equation 1.

MegaTev-wt pos1



y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.00027691	0.081058
m2	0.92428	0.084159
m3	0.0068968	0.0024033
Chisq	0.10323	NA
R^2	0.88471	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.13191	0.052163
m2	0.81883	0.054531
m3	0.095529	0.018596
Chisq	0.09732	NA
R^2	0.94235	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-5.4426e-5	0.051252
m2	0.97499	0.053392
m3	0.0083109	0.0015143
Chisq	0.041817	NA
R^2	0.95536	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-5.2976e-5	0.040737
m2	0.98257	0.04242
m3	0.0079598	0.0011796
Chisq	0.026368	NA
R^2	0.9717	NA

MegaTev-wt pos2

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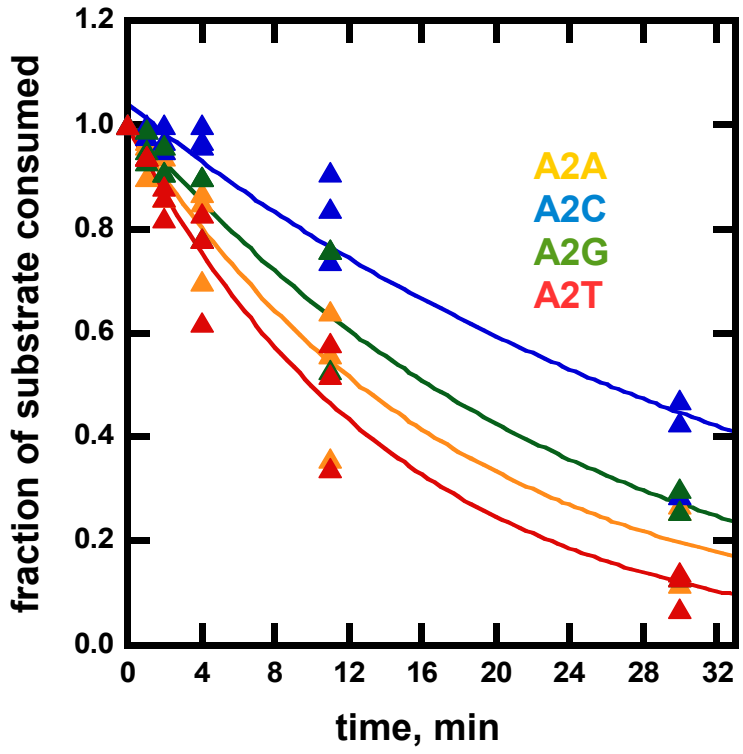


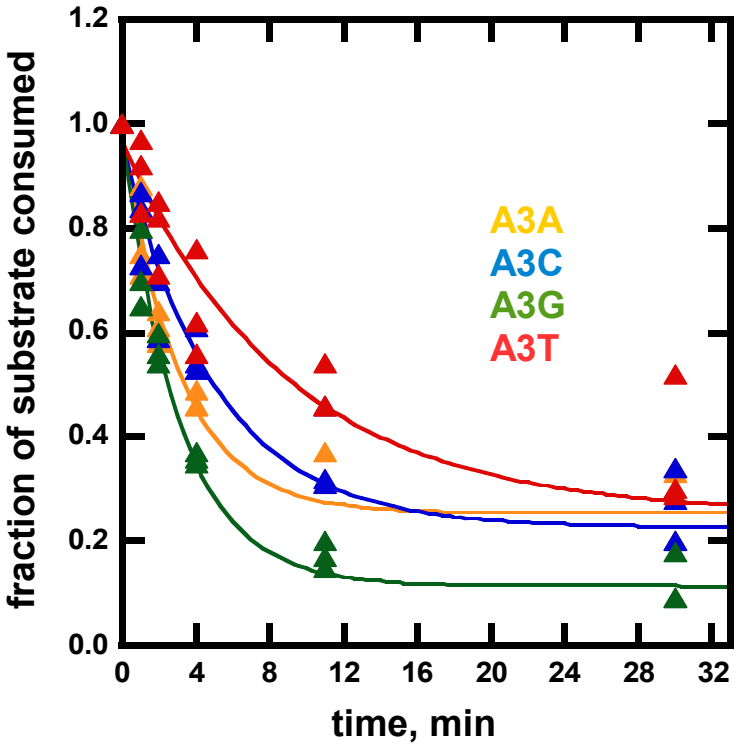
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.020717	0.064804
m2	0.98356	0.06443
m3	0.057261	0.0095278
Chisq	0.085323	NA
R^2	0.95714	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.021742	0.064941
m2	1.061	0.066745
m3	0.027244	0.0035897
Chisq	0.068489	NA
R^2	0.95646	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.032002	0.067897
m2	1.0436	0.068519
m3	0.041123	0.0060596
Chisq	0.079497	NA
R^2	0.958	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.0074564	0.051061
m2	1.0029	0.050855
m3	0.06849	0.0094489
Chisq	0.062342	NA
R^2	0.9717	NA

MegaTev-wt pos3



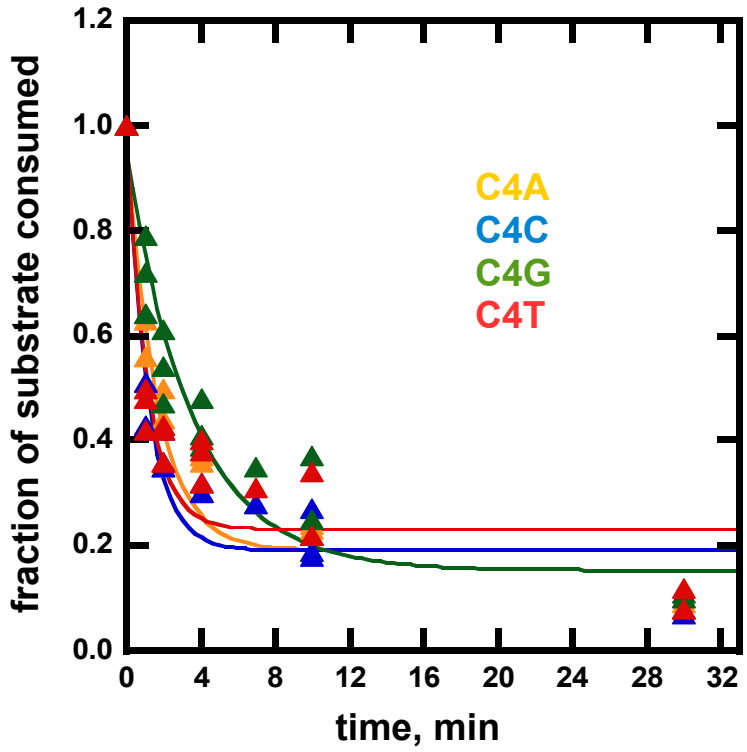
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.25416	0.034701
m2	0.73301	0.053155
m3	0.32299	0.057994
Chisq	0.11387	NA
R^2	0.92303	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.22681	0.042542
m2	0.73696	0.055635
m3	0.19882	0.041447
Chisq	0.12825	NA
R^2	0.91645	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.11418	0.020585
m2	0.86968	0.031591
m3	0.32499	0.029186
Chisq	0.040198	NA
R^2	0.97951	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.25285	0.065892
m2	0.71295	0.07214
m3	0.11299	0.033475
Chisq	0.18991	NA
R^2	0.86821	NA

MegaTev-wt pos4



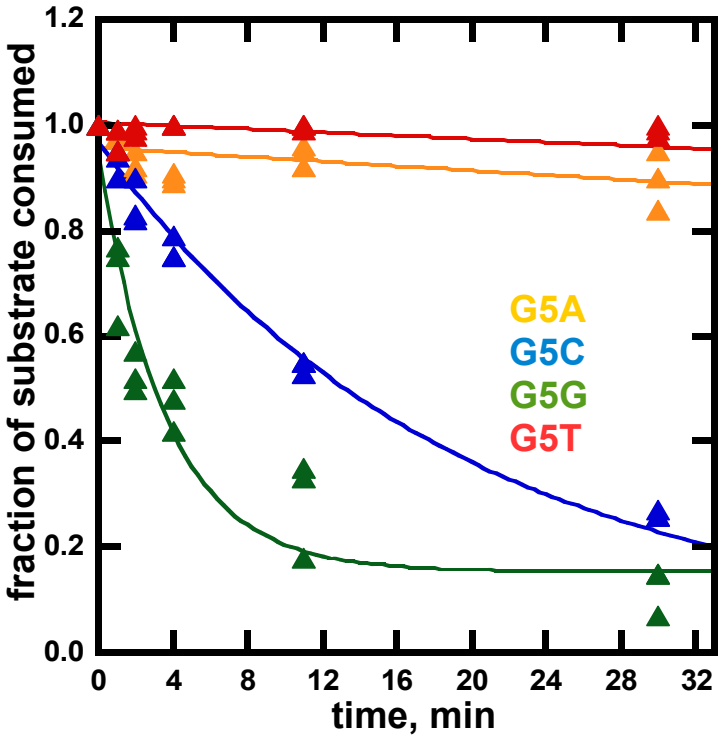
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.19034	0.034929
m2	0.78129	0.061262
m3	0.61458	0.11232
Chisq	0.14368	NA
R^2	0.91069	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.19078	0.030804
m2	0.79212	0.059087
m3	0.87436	0.1546
Chisq	0.13225	NA
R^2	0.9183	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.15292	0.041832
m2	0.79656	0.055731
m3	0.28242	0.050818
Chisq	0.1204	NA
R^2	0.92764	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.23094	0.03561
m2	0.75136	0.068694
m3	0.89702	0.19515
Chisq	0.17869	NA
R^2	0.88208	NA

MegaTev-wt pos5



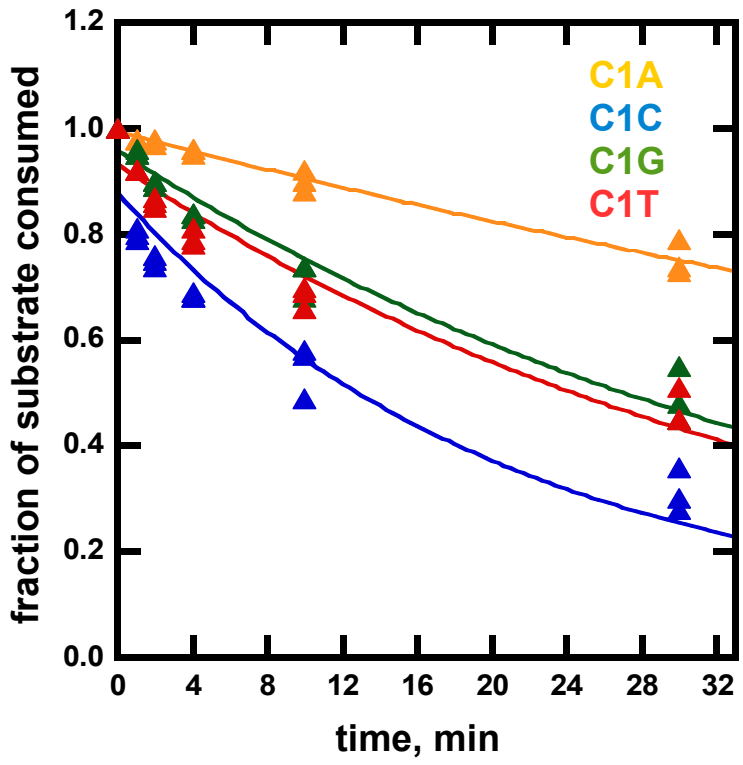
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.14508	0.21467
m2	1.1026	0.20828
m3	0.0020278	0.0012351
Chisq	0.024798	NA
R^2	0.97153	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.040771	0.032027
m2	0.92592	0.031917
m3	0.052988	0.0044958
Chisq	0.019759	NA
R^2	0.988	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.15434	0.040028
m2	0.79079	0.058246
m3	0.27512	0.052268
Chisq	0.13873	NA
R^2	0.92051	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.31227	0.62245
m2	1.3174	0.61725
m3	0.0012055	0.0011009
Chisq	0.014235	NA
R^2	0.98478	NA

MegaTev-T3 pos1



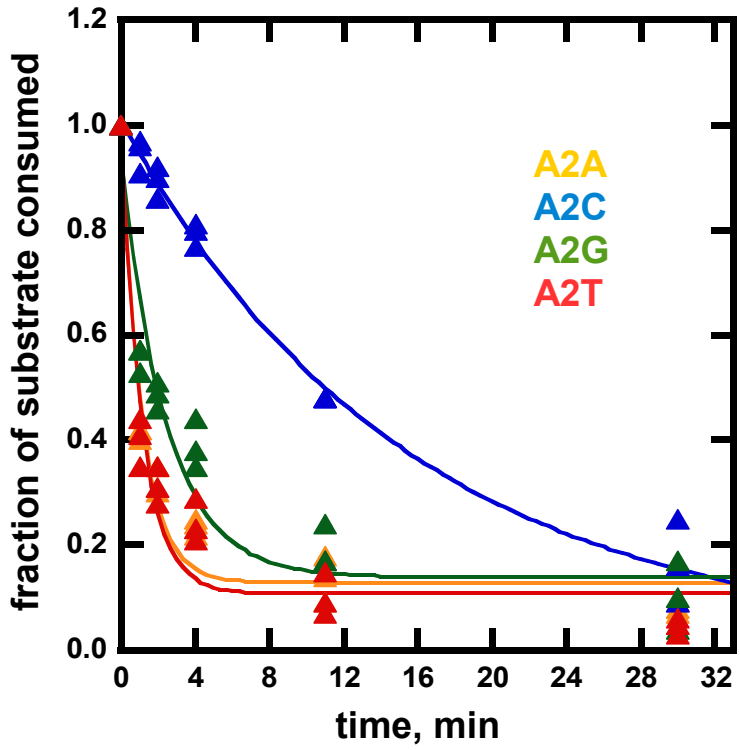
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	6.4186e-5	0.014334
m2	0.99352	0.014932
m3	0.0093258	0.00043412
Chisq	0.003281	NA
R^2	0.99653	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.067904	0.069893
m2	0.80943	0.069723
m3	0.048876	0.0102
Chisq	0.089982	NA
R^2	0.92923	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.010732	0.038395
m2	0.94772	0.039551
m3	0.02444	0.0021671
Chisq	0.023805	NA
R^2	0.97945	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.016272	0.046755
m2	0.91579	0.048062
m3	0.0262	0.0029144
Chisq	0.035407	NA
R^2	0.96869	NA

MegaTev-T3 pos2



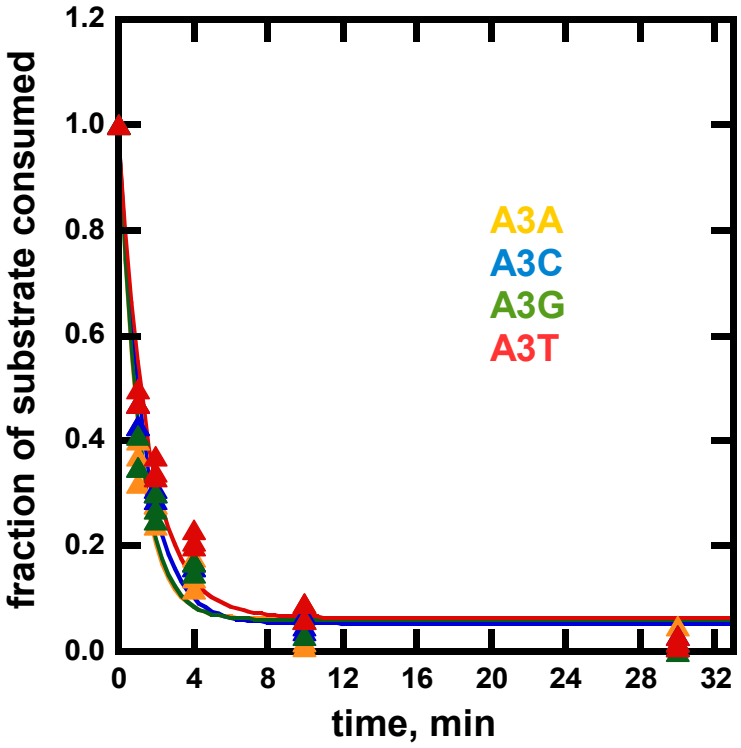
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.12934	0.023292
m2	0.85358	0.044921
m3	0.87859	0.10924
Chisq	0.076452	NA
R^2	0.9576	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.013363	0.029881
m2	0.99525	0.029707
m3	0.065148	0.0051962
Chisq	0.020289	NA
R^2	0.99027	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.13886	0.034328
m2	0.8025	0.056289
m3	0.41126	0.067869
Chisq	0.12486	NA
R^2	0.92777	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.10847	0.026417
m2	0.87251	0.050826
m3	0.86804	0.11923
Chisq	0.097894	NA
R^2	0.94855	NA

MegaTev-T3 pos3



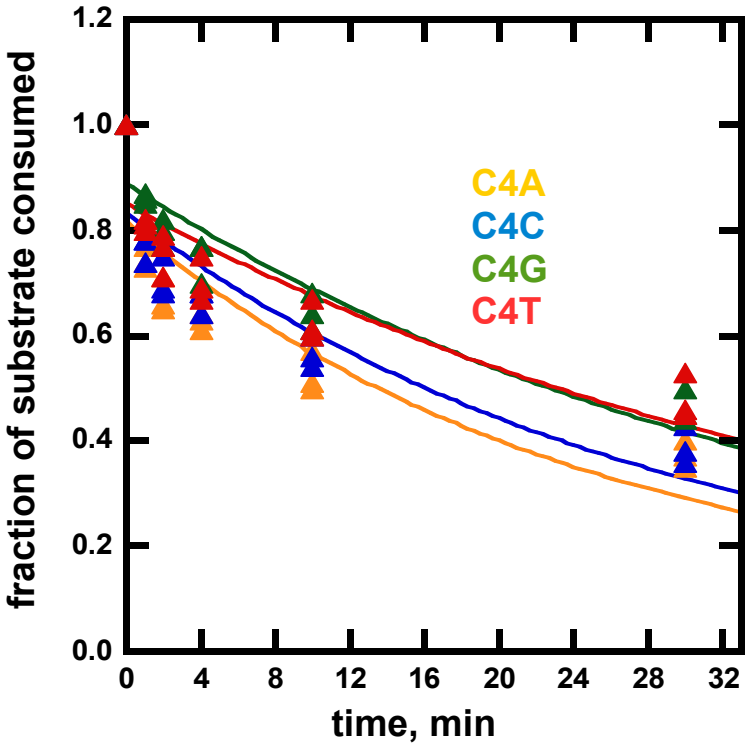
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.062893	0.01928
m2	0.92328	0.037609
m3	0.93281	0.090812
Chisq	0.05355	NA
R^2	0.97415	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.053289	0.01581
m2	0.92973	0.029327
m3	0.73181	0.053423
Chisq	0.032728	NA
R^2	0.98437	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.060967	0.020422
m2	0.92293	0.039502
m3	0.8943	0.090758
Chisq	0.059104	NA
R^2	0.97154	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.064563	0.01986
m2	0.91048	0.035623
m3	0.62808	0.056516
Chisq	0.048584	NA
R^2	0.97621	NA

MegaTev-T3 pos4



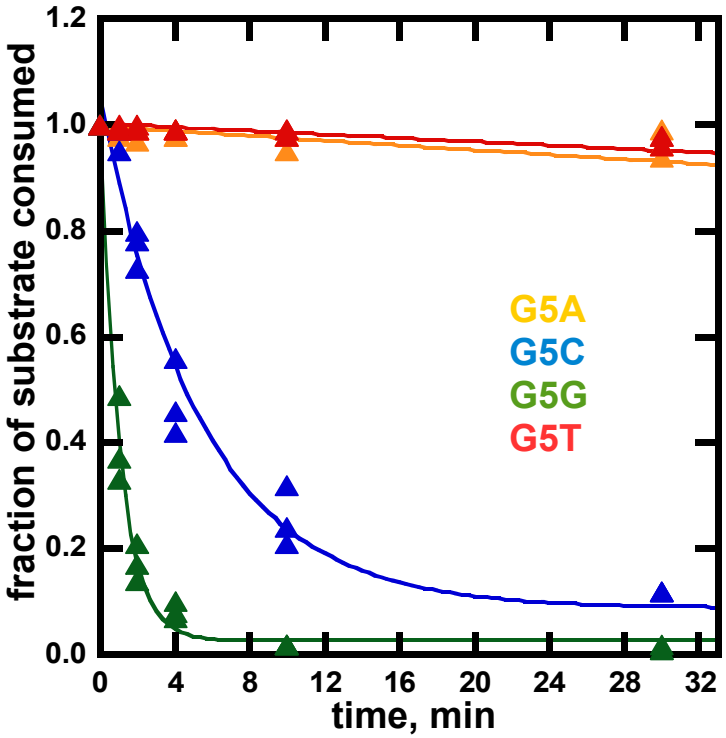
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.07577	0.10316
m2	0.74065	0.10387
m3	0.041234	0.013189
Chisq	0.18353	NA
R^2	0.83169	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.043316	0.09368
m2	0.78877	0.095292
m3	0.034003	0.008919
Chisq	0.14544	NA
R^2	0.86461	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.021023	0.068464
m2	0.86658	0.070383
m3	0.02613	0.0044984
Chisq	0.075911	NA
R^2	0.92808	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.020292	0.087175
m2	0.82963	0.089878
m3	0.02368	0.0054637
Chisq	0.12258	NA
R^2	0.87487	NA

MegaTev-T3 pos5



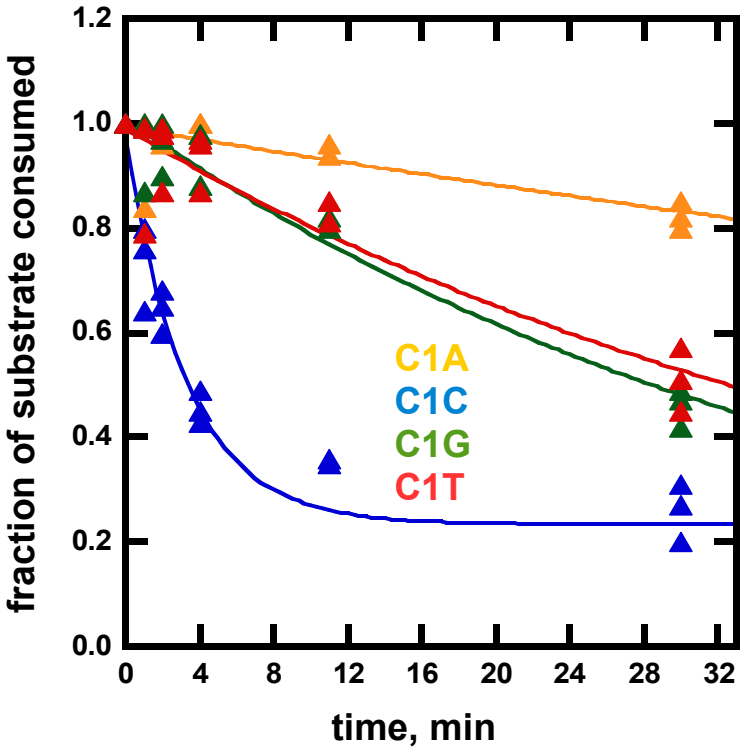
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.04104	0.11348
m2	1.0388	0.10939
m3	0.0022215	0.00084633
Chisq	0.011265	NA
R^2	0.98771	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.087667	0.034273
m2	0.96671	0.04373
m3	0.18621	0.022678
Chisq	0.077213	NA
R^2	0.96839	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.026527	0.01233
m2	0.97115	0.024107
m3	0.94357	0.05611
Chisq	0.021999	NA
R^2	0.99024	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.25872	0.26558
m2	1.262	0.26249
m3	0.0013947	0.00063384
Chisq	0.0052941	NA
R^2	0.9943	NA

MegaTev T95S pos1



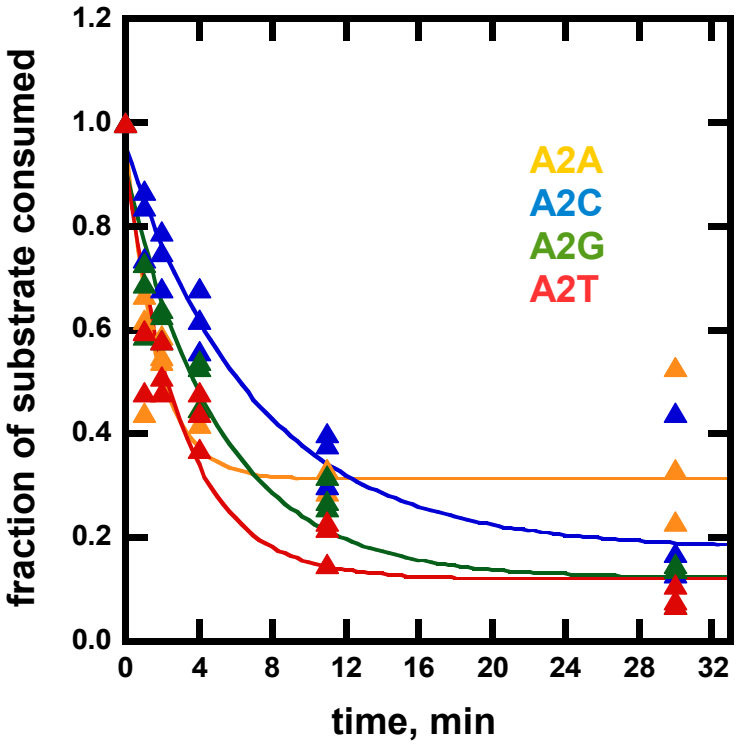
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.003106	0.044054
m2	0.99526	0.045408
m3	0.0058548	0.0011729
Chisq	0.027741	NA
R^2	0.97034	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.23449	0.037392
m2	0.73761	0.055064
m3	0.30253	0.055294
Chisq	0.10873	NA
R^2	0.92322	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.0079427	0.048921
m2	1.0159	0.050505
m3	0.024333	0.0025807
Chisq	0.036173	NA
R^2	0.97299	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.00495	0.064505
m2	0.99393	0.066838
m3	0.020755	0.0030328
Chisq	0.06266	NA
R^2	0.94885	NA

MegaTev T95S pos2



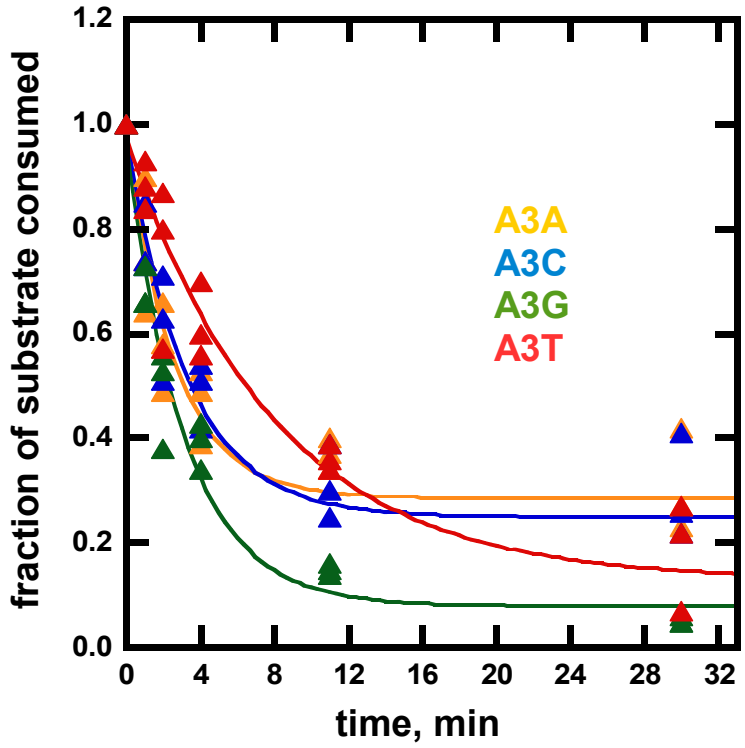
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.31188	0.043897
m2	0.6555	0.078212
m3	0.59496	0.16248
Chisq	0.23485	NA
R^2	0.81546	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.17904	0.050323
m2	0.77663	0.059173
m3	0.14193	0.031114
Chisq	0.13931	NA
R^2	0.91674	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.12238	0.040114
m2	0.78879	0.052266
m3	0.19654	0.036025
Chisq	0.11316	NA
R^2	0.93438	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.1207	0.040596
m2	0.79733	0.062159
m3	0.32255	0.062283
Chisq	0.15573	NA
R^2	0.9121	NA

MegaTev T95S pos3



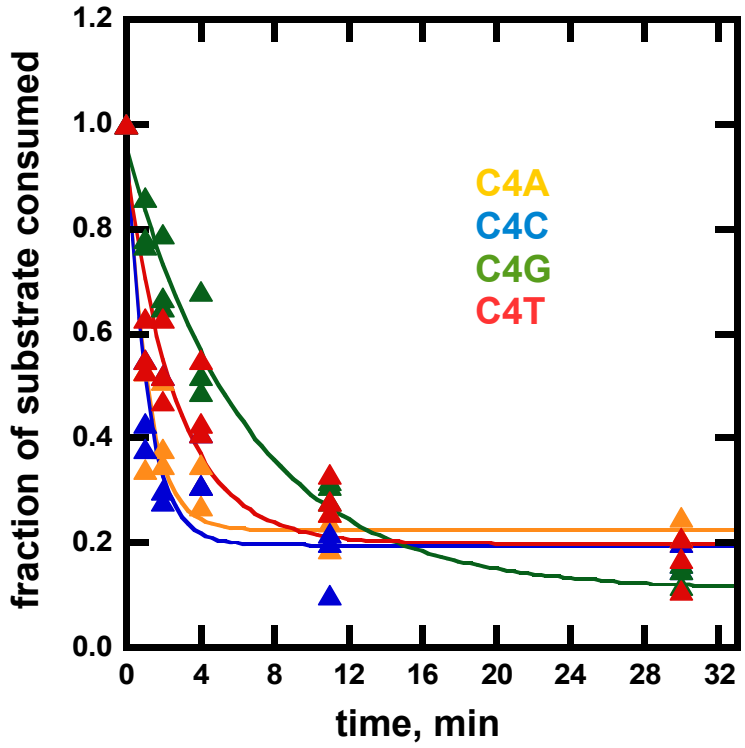
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.28559	0.042886
m2	0.70071	0.068791
m3	0.37838	0.088729
Chisq	0.18788	NA
R^2	0.86765	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.24982	0.039975
m2	0.73126	0.060179
m3	0.30552	0.063136
Chisq	0.14672	NA
R^2	0.90292	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.079482	0.027445
m2	0.88296	0.041892
m3	0.31934	0.037621
Chisq	0.070804	NA
R^2	0.96553	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.12834	0.048705
m2	0.84633	0.055257
m3	0.12676	0.02406
Chisq	0.11731	NA
R^2	0.9387	NA

MegaTev T95S pos4



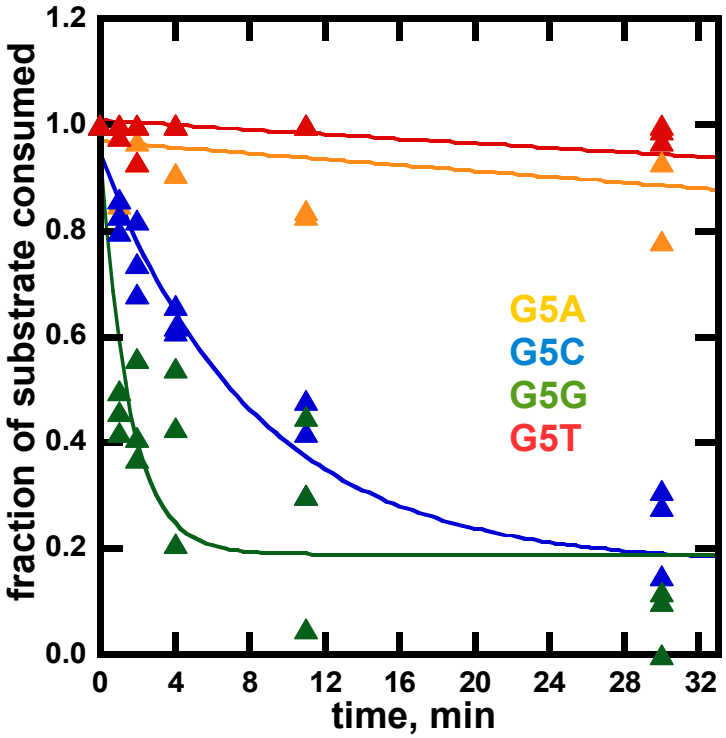
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.22453	0.030833
m2	0.75978	0.059689
m3	0.89549	0.16676
Chisq	0.13495	NA
R^2	0.91018	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.19613	0.035054
m2	0.78689	0.067793
m3	0.89096	0.18179
Chisq	0.17409	NA
R^2	0.89391	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.1125	0.034081
m2	0.84332	0.04113
m3	0.15412	0.021443
Chisq	0.068494	NA
R^2	0.96377	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.19876	0.044415
m2	0.726	0.070366
m3	0.36192	0.084569
Chisq	0.1974	NA
R^2	0.87054	NA

MegaTev T95S pos5



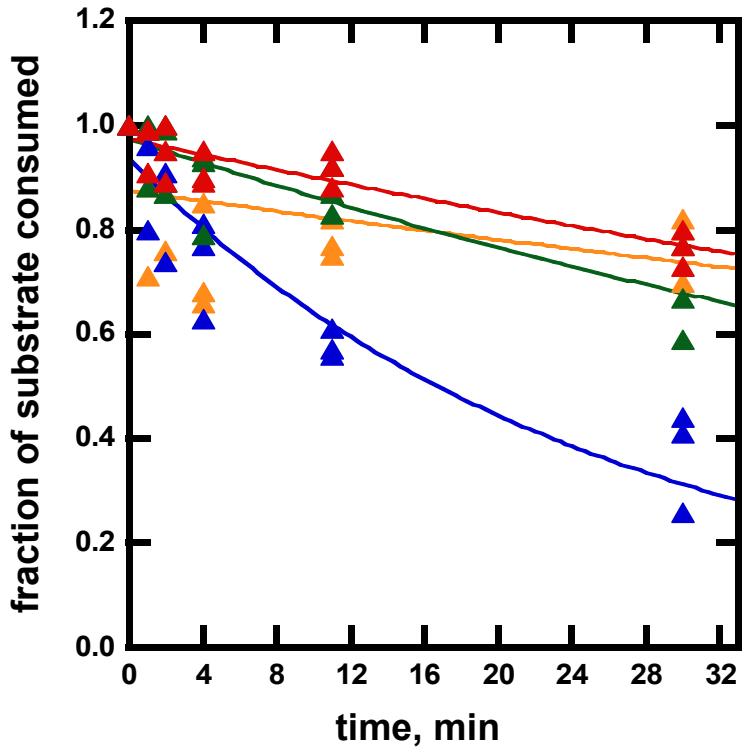
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.063471	0.16335
m2	1.0325	0.15427
m3	0.0027869	0.0020205
Chisq	0.078241	NA
R^2	0.91694	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.17062	0.045821
m2	0.77657	0.051349
m3	0.12189	0.023499
Chisq	0.099737	NA
R^2	0.9378	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.18952	0.055941
m2	0.76419	0.10117
m3	0.63969	0.1941
Chisq	0.39154	NA
R^2	0.7819	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.11144	0.17927
m2	1.1202	0.17425
m3	0.0019767	0.00096094
Chisq	0.015064	NA
R^2	0.98396	NA

MegaTev T95S/Q158R pos1



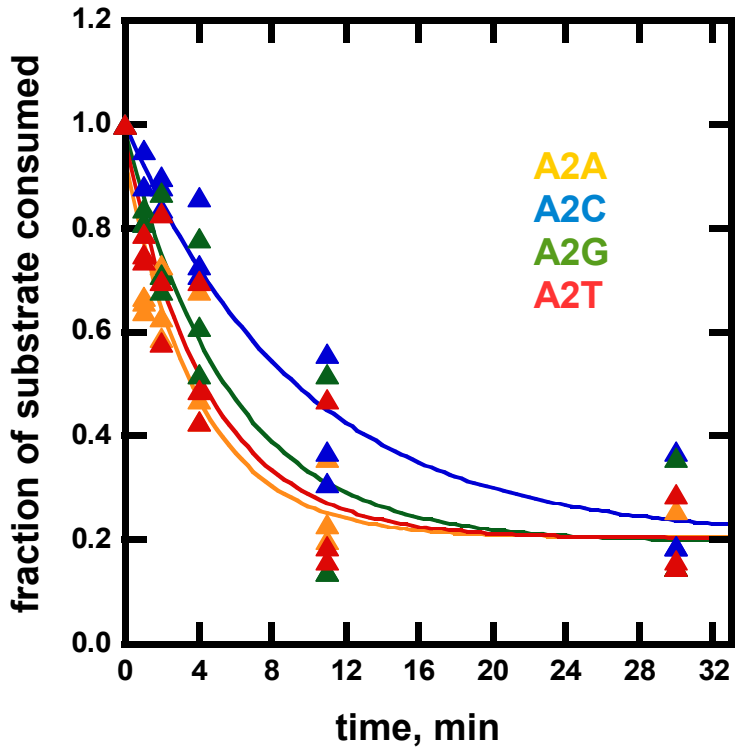
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.0020138	0.11603
m2	0.87648	0.11912
m3	0.0056497	0.0034601
Chisq	0.2031	NA
R^2	0.77588	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.047326	0.083737
m2	0.88739	0.084607
m3	0.040194	0.0085418
Chisq	0.12017	NA
R^2	0.91508	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	7.1902e-5	0.06238
m2	0.9739	0.064974
m3	0.012054	0.0021164
Chisq	0.062284	NA
R^2	0.93803	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.00046328	0.03911
m2	0.9743	0.040716
m3	0.0078091	0.001136
Chisq	0.024279	NA
R^2	0.9734	NA

MegaTev T95S/Q158R pos2



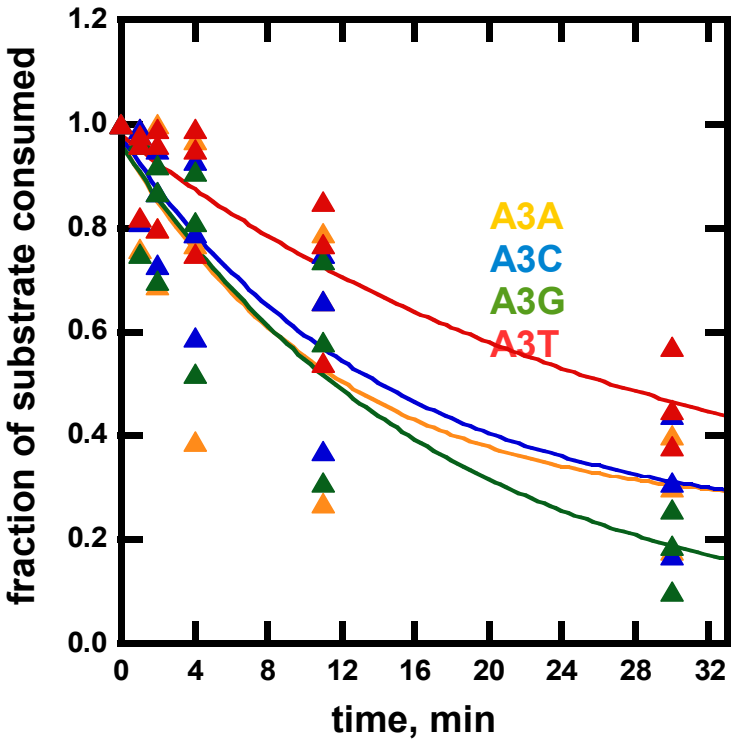
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.20594	0.046433
m2	0.72659	0.061173
m3	0.24988	0.055454
Chisq	0.13301	NA
R^2	0.90397	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.20675	0.064398
m2	0.79405	0.064054
m3	0.10702	0.024629
Chisq	0.097529	NA
R^2	0.93059	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.19726	0.066024
m2	0.78921	0.077232
m3	0.17626	0.048051
Chisq	0.20134	NA
R^2	0.8775	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.20428	0.05301
m2	0.76398	0.066907
m3	0.22109	0.052309
Chisq	0.15772	NA
R^2	0.89729	NA

MegaTev T95S/Q158R pos3



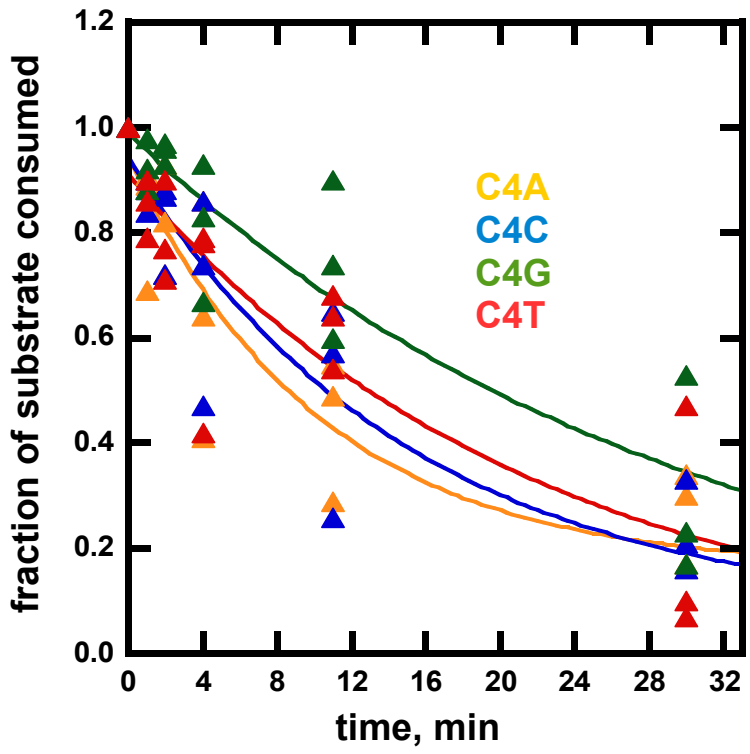
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.25221	0.17449
m2	0.71219	0.16476
m3	0.086468	0.055358
Chisq	0.44336	NA
R^2	0.68517	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.2212	0.16712
m2	0.75402	0.15439
m3	0.070408	0.036997
Chisq	0.22605	NA
R^2	0.81055	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.028476	0.24184
m2	0.9319	0.22382
m3	0.058648	0.032774
Chisq	0.25757	NA
R^2	0.83496	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.2028	0.45907
m2	0.77576	0.44045
m3	0.036001	0.035733
Chisq	0.14542	NA
R^2	0.79712	NA

MegaTev T95S/Q158R pos4



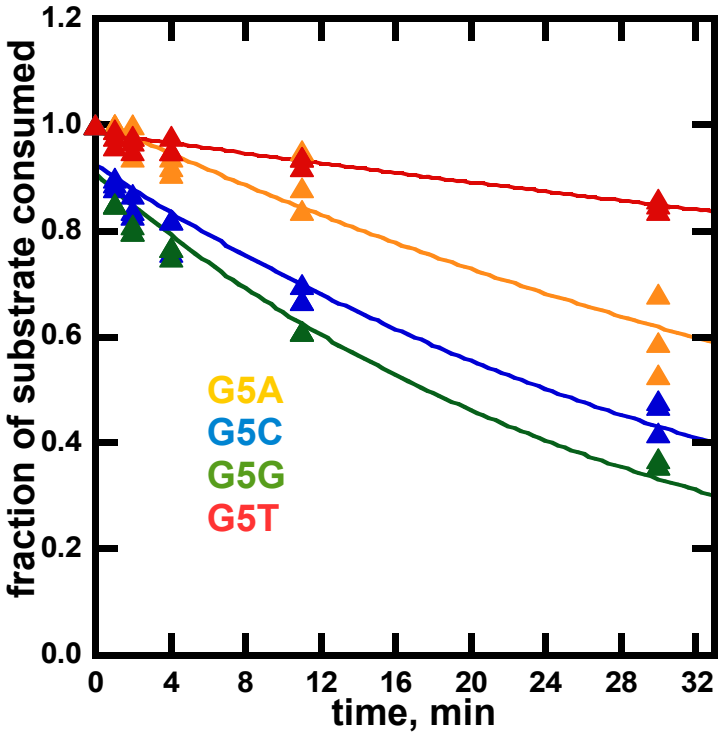
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.16141	0.085658
m2	0.78453	0.090063
m3	0.09778	0.032858
Chisq	0.27017	NA
R^2	0.84463	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.073486	0.098503
m2	0.87107	0.098012
m3	0.066959	0.020335
Chisq	0.22662	NA
R^2	0.87596	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.0184	0.1078
m2	1.0084	0.10984
m3	0.033973	0.007923
Chisq	0.19272	NA
R^2	0.88789	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.00093228	0.12801
m2	0.91041	0.12833
m3	0.046626	0.015392
Chisq	0.29533	NA
R^2	0.83317	NA

MegaTev T95S/Q158R pos5



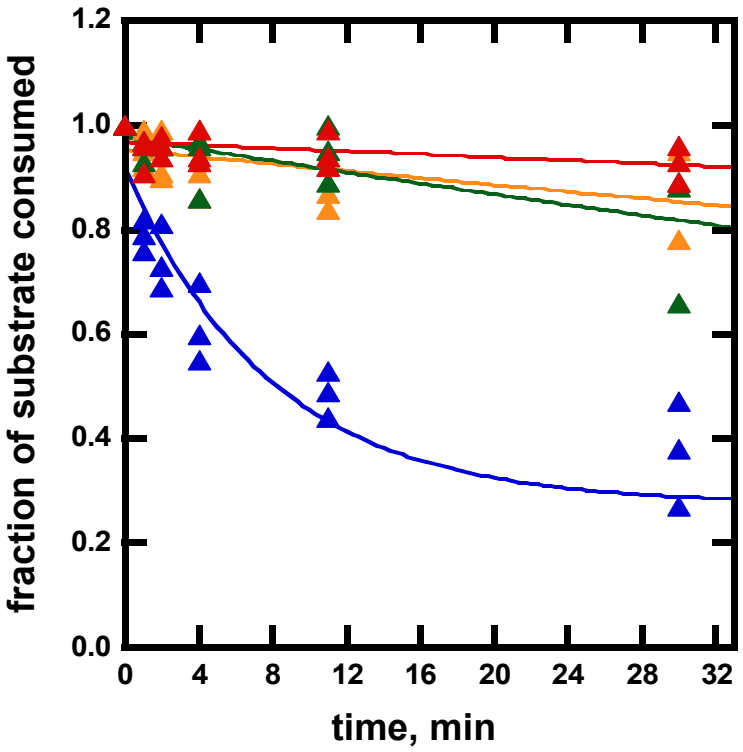
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.0029847	0.042815
m2	1.0125	0.044483
m3	0.016264	0.0016409
Chisq	0.029384	NA
R^2	0.97403	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.011407	0.045335
m2	0.91341	0.046668
m3	0.025905	0.0027726
Chisq	0.033285	NA
R^2	0.97029	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.022233	0.049418
m2	0.88538	0.050286
m3	0.034937	0.0042712
Chisq	0.040669	NA
R^2	0.96708	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.0072034	0.014673
m2	0.99203	0.014818
m3	0.0049198	0.00037166
Chisq	0.0030346	NA
R^2	0.99661	NA

MegaTev Q158R pos1



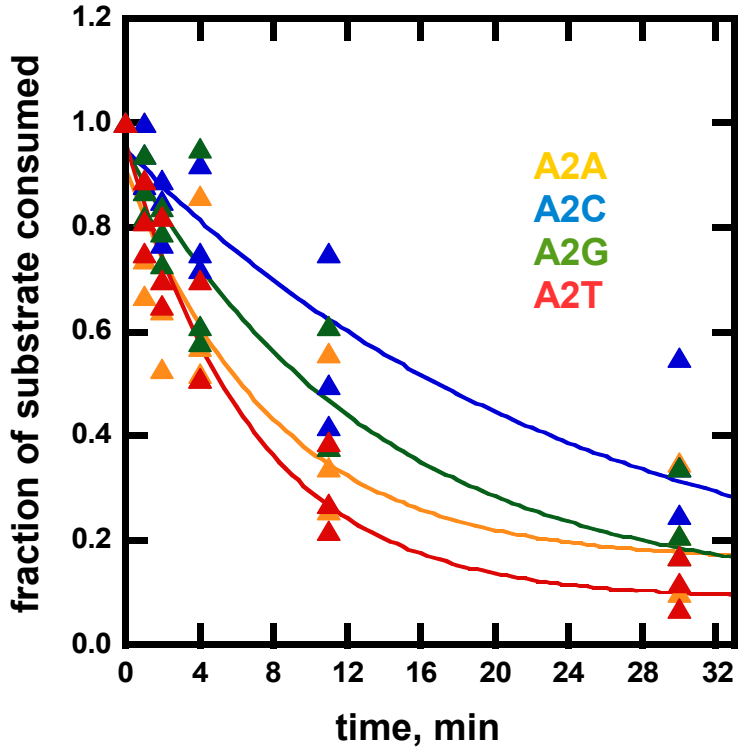
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.026509	0.075463
m2	0.98035	0.071863
m3	0.0036023	0.0015444
Chisq	0.04792	NA
R^2	0.94574	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.2751	0.061319
m2	0.64376	0.069715
m3	0.12762	0.040154
Chisq	0.1872	NA
R^2	0.84751	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.0028867	0.060361
m2	0.97958	0.062173
m3	0.0058883	0.0016267
Chisq	0.055643	NA
R^2	0.94057	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.62468	1.1541
m2	1.5926	1.1488
m3	0.00093586	0.0011221
Chisq	0.014588	NA
R^2	0.98351	NA

MegaTev Q158R pos2



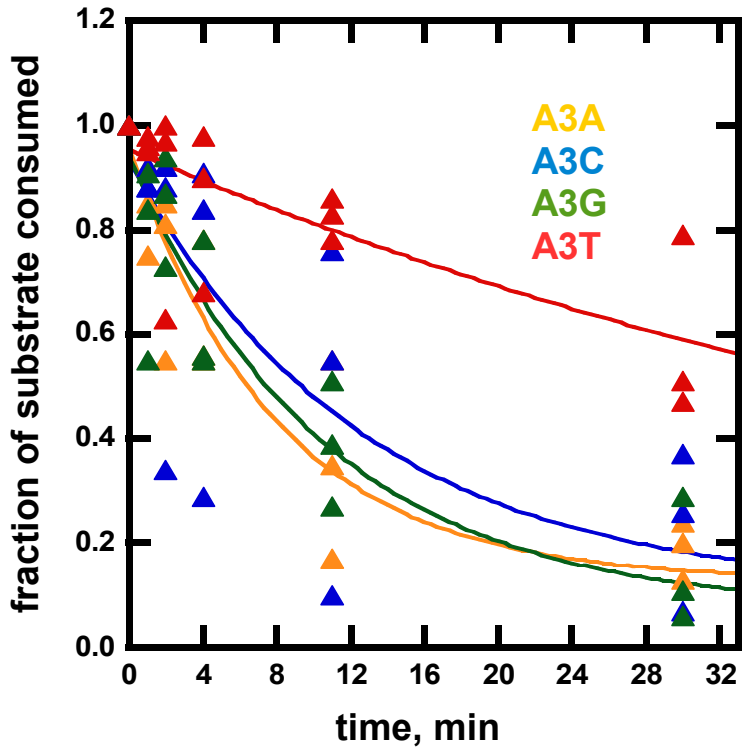
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.16104	0.082495
m2	0.75028	0.093645
m3	0.12699	0.04607
Chisq	0.33715	NA
R^2	0.80727	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.052907	0.10489
m2	0.89651	0.10585
m3	0.041104	0.01089
Chisq	0.1897	NA
R^2	0.87581	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.098367	0.082373
m2	0.84933	0.082675
m3	0.075774	0.020712
Chisq	0.18179	NA
R^2	0.89768	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.088549	0.039789
m2	0.8743	0.047057
m3	0.14455	0.022344
Chisq	0.088491	NA
R^2	0.95654	NA

MegaTev Q158R pos3



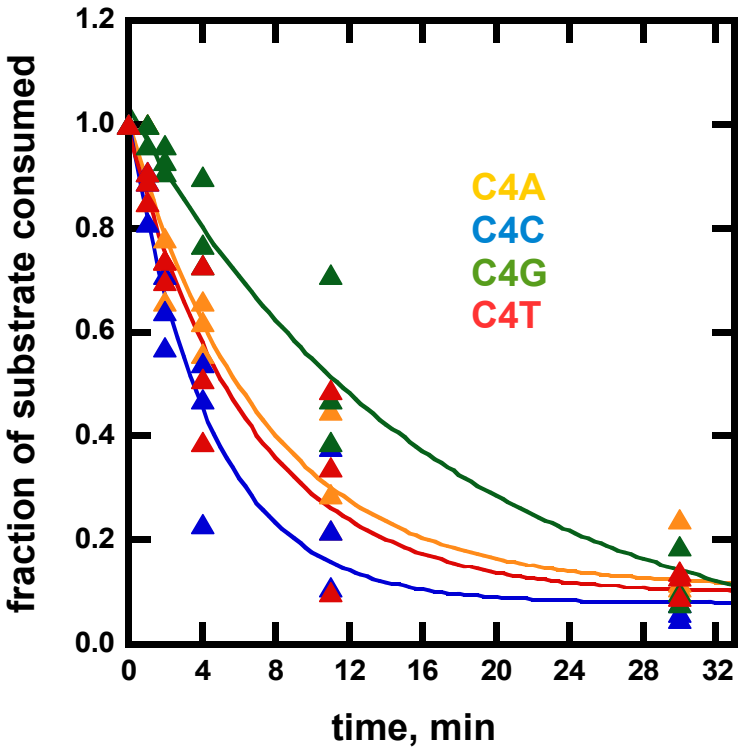
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.12894	0.066185
m2	0.82865	0.074762
m3	0.12502	0.032826
Chisq	0.21362	NA
R^2	0.88945	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.10782	0.16591
m2	0.82404	0.16748
m3	0.079503	0.045933
Chisq	0.78147	NA
R^2	0.66138	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.073303	0.083576
m2	0.86696	0.087203
m3	0.09477	0.027848
Chisq	0.24734	NA
R^2	0.87797	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.00086479	0.1177
m2	0.95224	0.12231
m3	0.016044	0.0047558
Chisq	0.22204	NA
R^2	0.81383	NA

MegaTev Q158R pos4



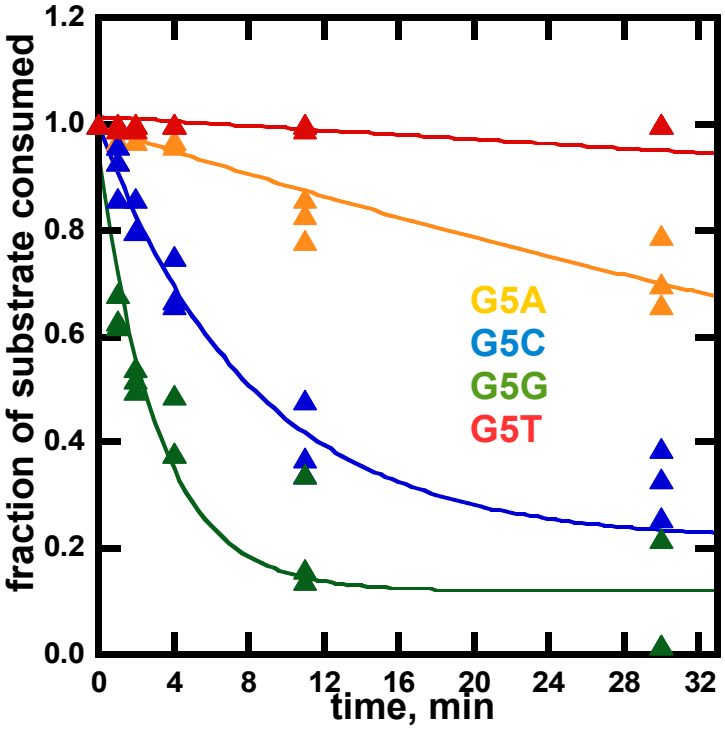
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.11006	0.043849
m2	0.89656	0.051368
m3	0.14026	0.023149
Chisq	0.10467	NA
R^2	0.95123	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.080112	0.043863
m2	0.92785	0.059729
m3	0.22519	0.039152
Chisq	0.14767	NA
R^2	0.93786	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.027379	0.064182
m2	1.0607	0.063762
m3	0.061044	0.0095634
Chisq	0.088146	NA
R^2	0.9628	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.096095	0.053281
m2	0.89161	0.064085
m3	0.15247	0.0313
Chisq	0.16596	NA
R^2	0.9246	NA

MegaTev Q158R pos5



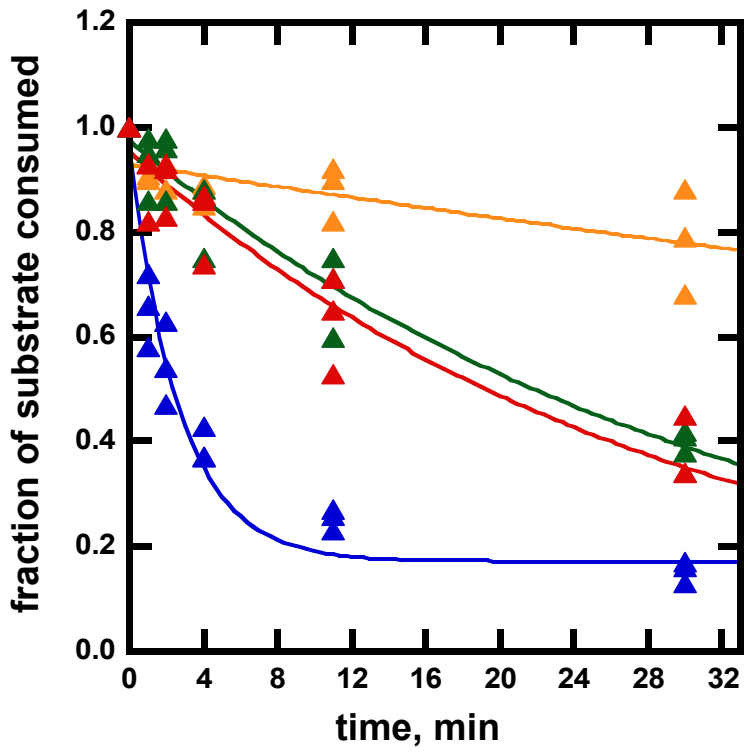
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.0015124	0.042501
m2	0.99347	0.044274
m3	0.011781	0.0013992
Chisq	0.028909	NA
R^2	0.97125	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.21553	0.046915
m2	0.78238	0.05268
m3	0.12267	0.024071
Chisq	0.10525	NA
R^2	0.93555	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.12043	0.038285
m2	0.82409	0.058334
m3	0.31749	0.055887
Chisq	0.13737	NA
R^2	0.92637	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.099781	0.16927
m2	1.1139	0.16463
m3	0.00196	0.00089618
Chisq	0.01283	NA
R^2	0.98642	NA

MegaTev K26R/T95S pos1



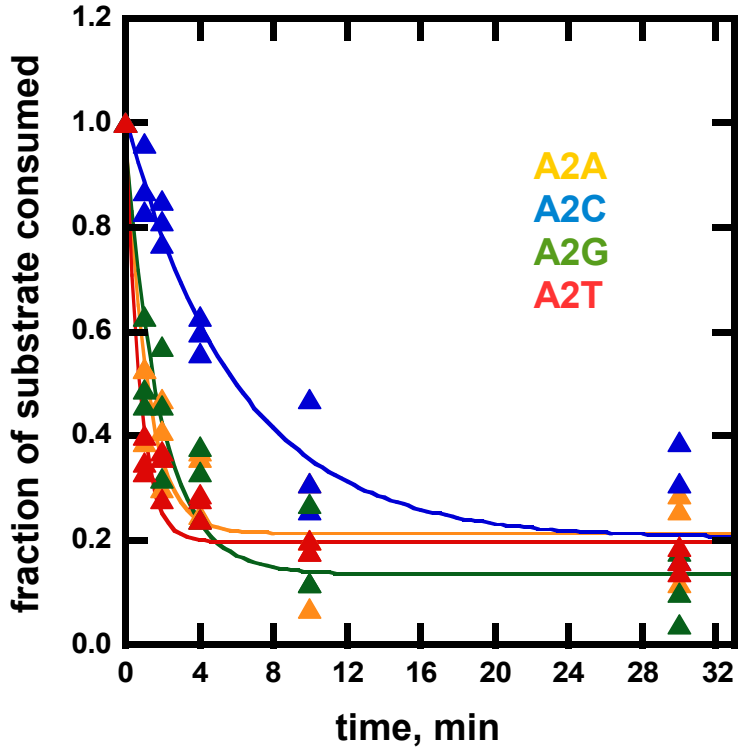
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.0023415	0.058185
m2	0.93195	0.059938
m3	0.0058955	0.0016487
Chisq	0.05172	NA
R^2	0.93907	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.17137	0.030238
m2	0.79213	0.0482
m3	0.36988	0.05401
Chisq	0.092431	NA
R^2	0.94464	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.0075774	0.053106
m2	0.96691	0.05433
m3	0.030905	0.0036665
Chisq	0.04625	NA
R^2	0.96649	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.016369	0.061794
m2	0.93768	0.062926
m3	0.034377	0.0049501
Chisq	0.063433	NA
R^2	0.95442	NA

MegaTev K26R/T95S pos2



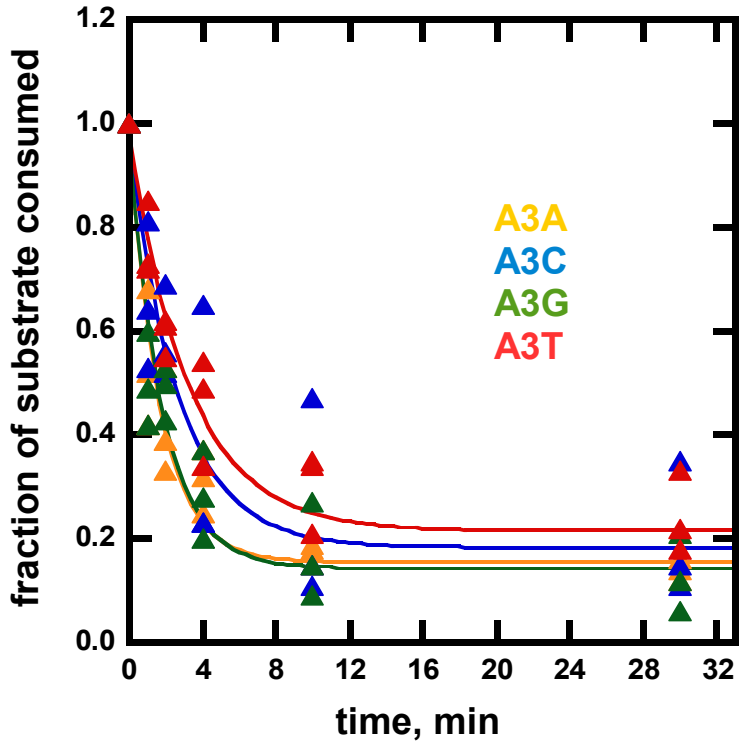
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.21336	0.03481
m2	0.77081	0.0666
m3	0.84754	0.17216
Chisq	0.16815	NA
R^2	0.8934	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.20425	0.045937
m2	0.80583	0.056968
m3	0.16736	0.032183
Chisq	0.12972	NA
R^2	0.92647	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.1362	0.037465
m2	0.82452	0.064412
m3	0.52415	0.094634
Chisq	0.16047	NA
R^2	0.91161	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.19688	0.024161
m2	0.79614	0.05014
m3	1.3198	0.22341
Chisq	0.095329	NA
R^2	0.94043	NA

MegaTev K26R/T95S pos3



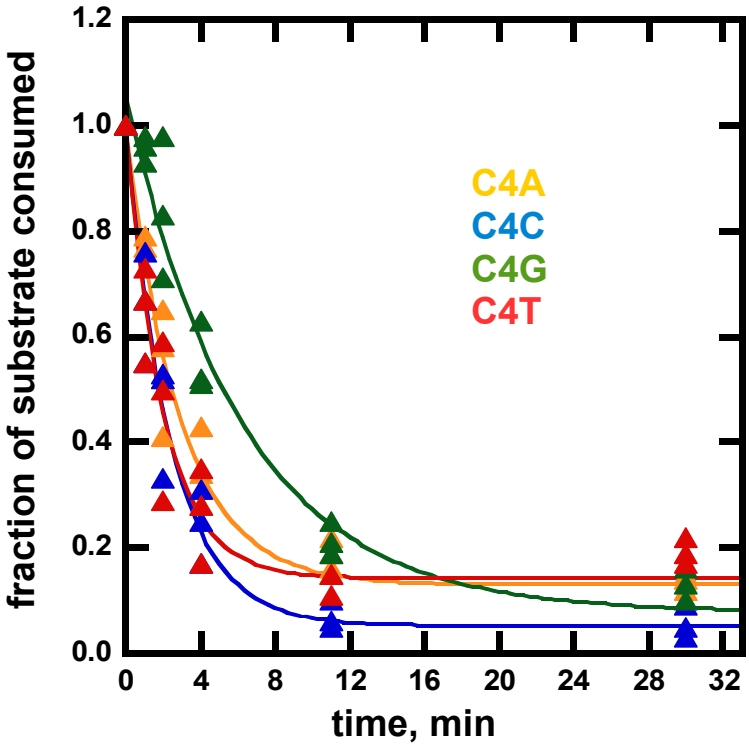
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.15515	0.032395
m2	0.81822	0.057318
m3	0.59152	0.09531
Chisq	0.12616	NA
R^2	0.92759	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.18317	0.059482
m2	0.78784	0.092965
m3	0.37008	0.10575
Chisq	0.34304	NA
R^2	0.81877	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.14259	0.036178
m2	0.81787	0.062651
m3	0.53997	0.095429
Chisq	0.15153	NA
R^2	0.9147	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.2146	0.037207
m2	0.7666	0.054966
m3	0.3088	0.055577
Chisq	0.12154	NA
R^2	0.92424	NA

MegaTev K26R/T95S pos4



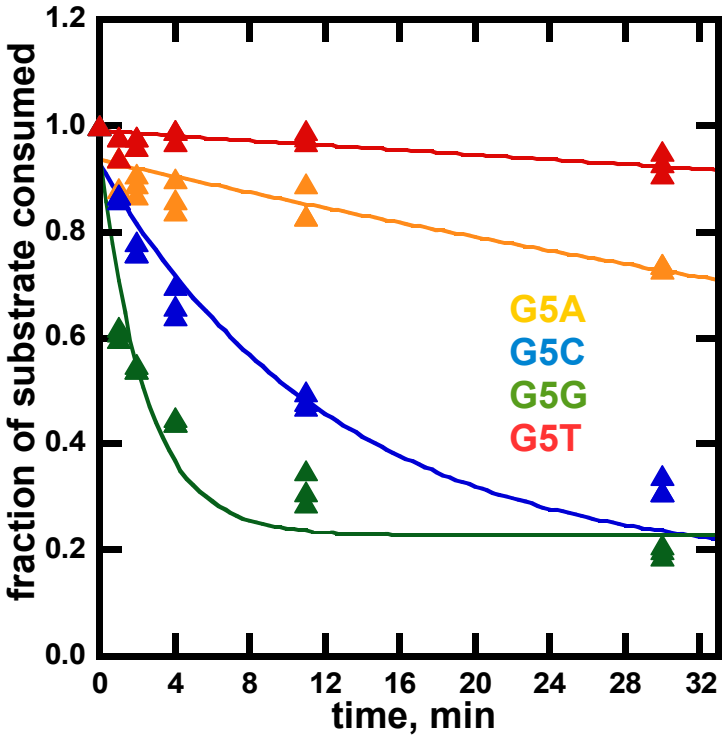
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.12972	0.027714
m2	0.86575	0.04338
m3	0.34707	0.042323
Chisq	0.075323	NA
R^2	0.96175	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.052011	0.024693
m2	0.94087	0.040589
m3	0.41523	0.04208
Chisq	0.064867	NA
R^2	0.97139	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.077682	0.039087
m2	0.9776	0.047841
m3	0.16119	0.022392
Chisq	0.093332	NA
R^2	0.9634	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.14209	0.031293
m2	0.85372	0.053703
m3	0.49871	0.072186
Chisq	0.11194	NA
R^2	0.94098	NA

MegaTev K26R/T95S pos5



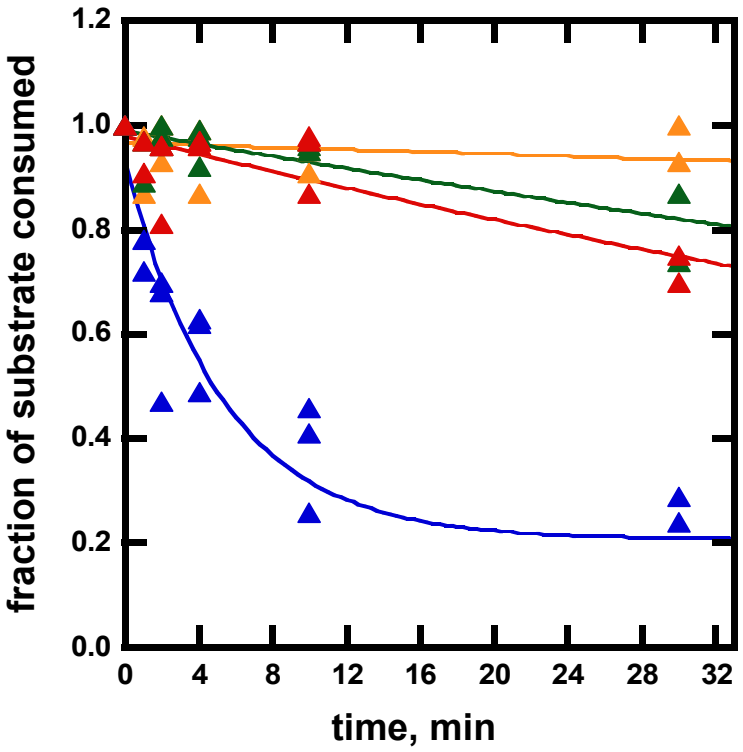
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.00047886	0.041539
m2	0.93406	0.043276
m3	0.0083843	0.0012844
Chisq	0.027478	NA
R^2	0.96766	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.16758	0.053877
m2	0.75991	0.054462
m3	0.080298	0.016397
Chisq	0.083428	NA
R^2	0.93979	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.22841	0.034958
m2	0.72074	0.057225
m3	0.40859	0.076408
Chisq	0.12912	NA
R^2	0.90929	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.13462	0.097474
m2	1.1247	0.094452
m3	0.0020631	0.00057061
Chisq	0.0056123	NA
R^2	0.99381	NA

MegaTev K26R/Q158R pos1



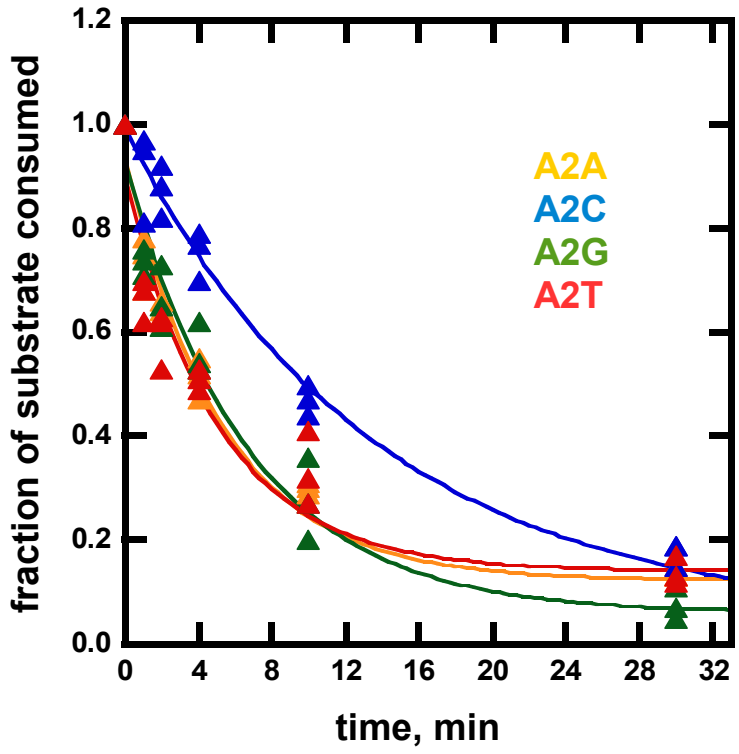
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-4.6019	67.127
m2	5.5679	67.118
m3	0.00019019	0.0025268
Chisq	0.031282	NA
R^2	0.96507	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.20691	0.062456
m2	0.7246	0.076685
m3	0.18768	0.051353
Chisq	0.17559	NA
R^2	0.86683	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.0023531	0.042864
m2	0.99083	0.044384
m3	0.0061857	0.0013599
Chisq	0.024809	NA
R^2	0.97293	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.0010714	0.056941
m2	0.98	0.059587
m3	0.0089022	0.0019976
Chisq	0.045259	NA
R^2	0.95146	NA

MegaTev K26R/Q158R pos2



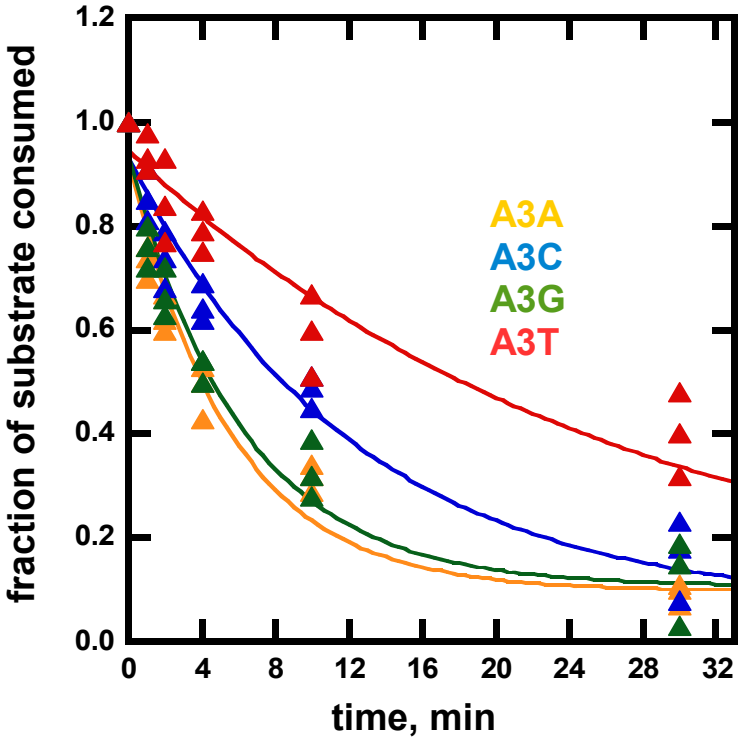
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.12239	0.030276
m2	0.81497	0.038803
m3	0.18937	0.024238
Chisq	0.060866	NA
R^2	0.96508	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.046216	0.036581
m2	0.94616	0.036684
m3	0.074632	0.0081468
Chisq	0.035526	NA
R^2	0.98196	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.059604	0.035195
m2	0.87078	0.042451
m3	0.15131	0.020287
Chisq	0.070968	NA
R^2	0.96388	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.14042	0.044623
m2	0.7603	0.05774
m3	0.19632	0.039944
Chisq	0.13505	NA
R^2	0.91564	NA

MegaTev K26R/Q158R pos3



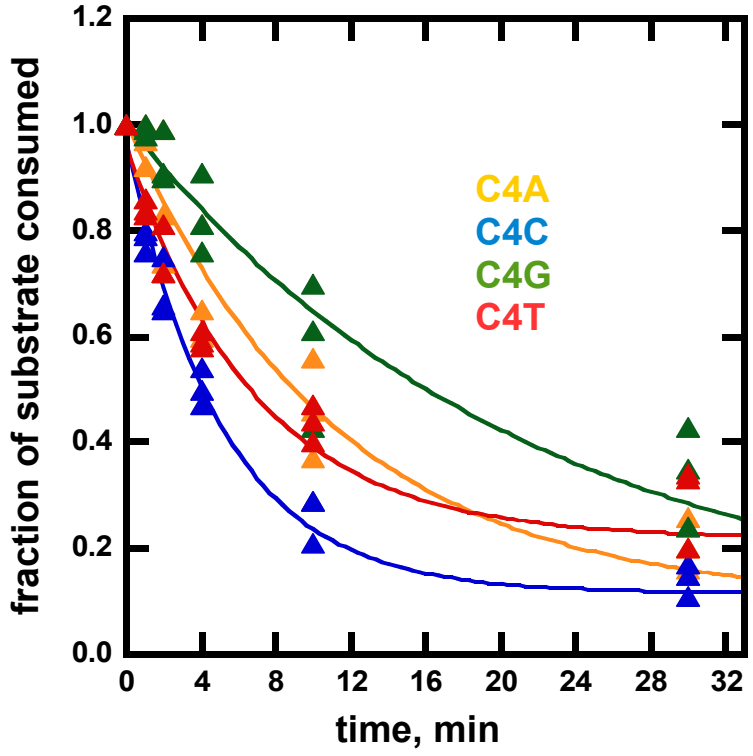
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.097534	0.033936
m2	0.83072	0.043005
m3	0.18148	0.025355
Chisq	0.074528	NA
R^2	0.95903	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.065676	0.047816
m2	0.86543	0.048638
m3	0.082159	0.013233
Chisq	0.068317	NA
R^2	0.96068	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.10647	0.035422
m2	0.82953	0.043593
m3	0.16269	0.023323
Chisq	0.075686	NA
R^2	0.95806	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.046285	0.07322
m2	0.89831	0.074097
m3	0.037612	0.0068908
Chisq	0.090396	NA
R^2	0.93359	NA

MegaTev K26R/Q158R pos4



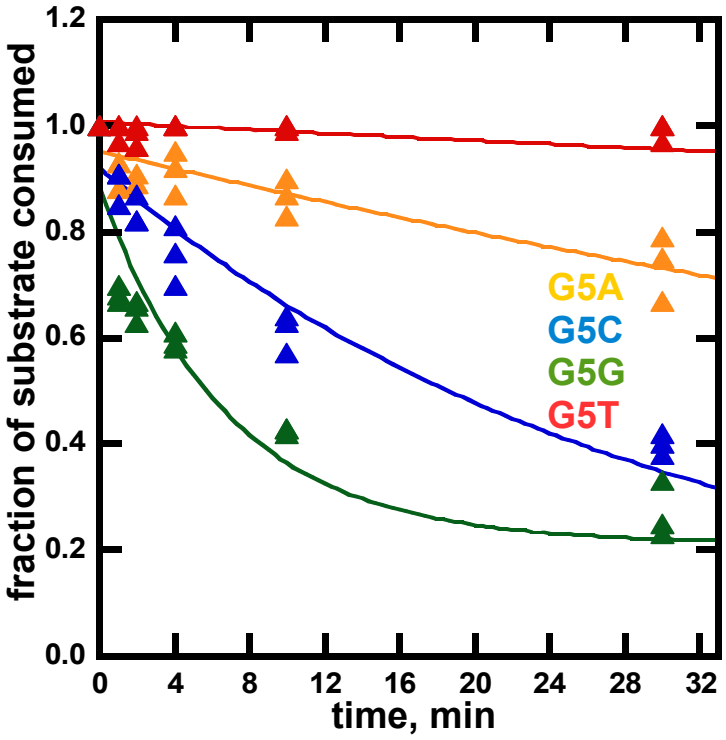
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.10024	0.056598
m2	0.90507	0.058823
m3	0.090964	0.017086
Chisq	0.10889	NA
R^2	0.94522	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.11641	0.02412
m2	0.85401	0.031223
m3	0.19663	0.019257
Chisq	0.039495	NA
R^2	0.97909	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.051778	0.075175
m2	0.95149	0.075149
m3	0.046934	0.0088426
Chisq	0.10212	NA
R^2	0.94	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.21859	0.04191
m2	0.74175	0.050092
m3	0.14654	0.027322
Chisq	0.09822	NA
R^2	0.93312	NA

MegaTev K26R/Q158R pos5



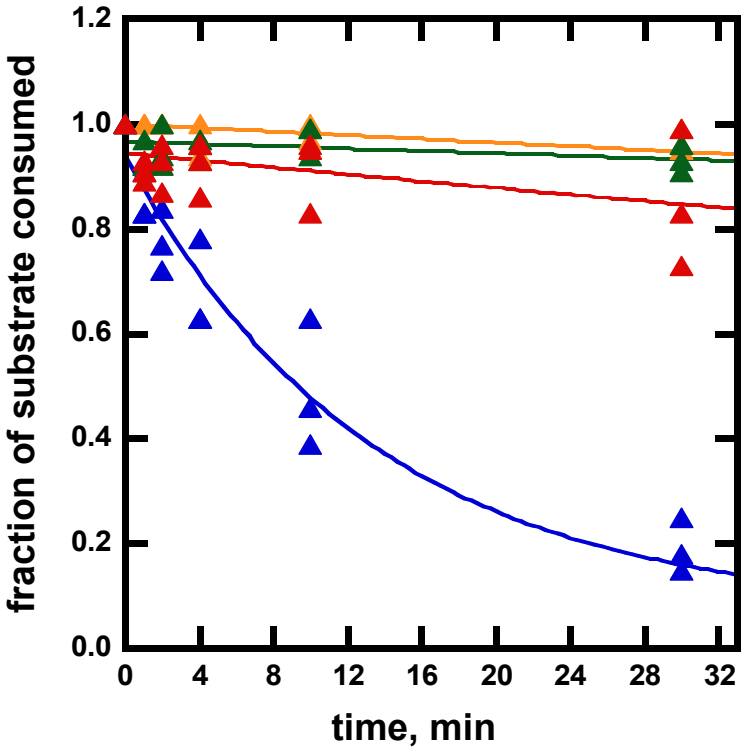
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.0002378	0.041986
m2	0.95091	0.043729
m3	0.0087586	0.0013015
Chisq	0.028111	NA
R^2	0.96824	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.035181	0.059078
m2	0.88479	0.060039
m3	0.03465	0.0051239
Chisq	0.058	NA
R^2	0.95317	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.21358	0.052693
m2	0.67069	0.0633
m3	0.14917	0.038785
Chisq	0.15739	NA
R^2	0.877	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.39189	0.43115
m2	1.3982	0.42732
m3	0.001247	0.00076141
Chisq	0.0079874	NA
R^2	0.99148	NA

MegaTev K26R pos1



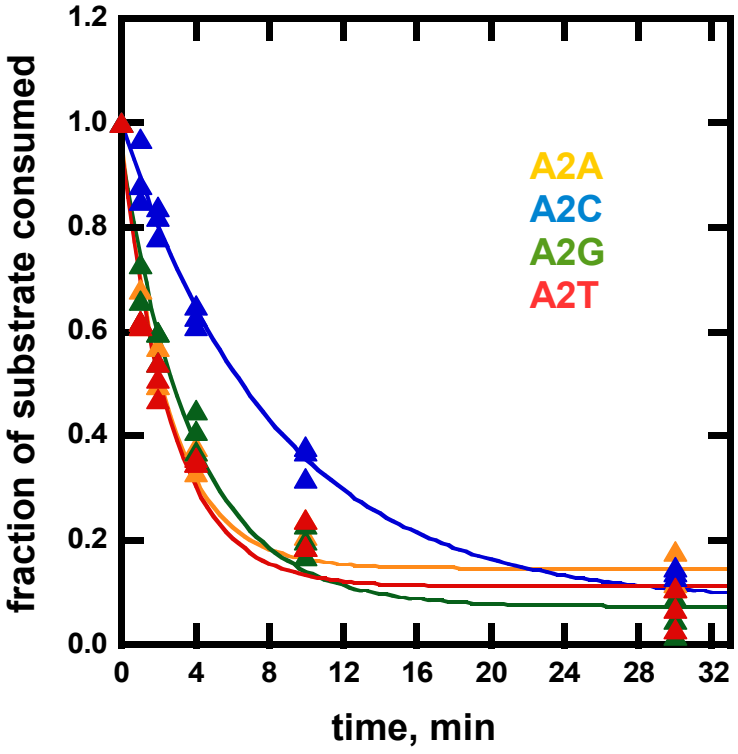
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.18643	0.33668
m2	1.1861	0.33193
m3	0.0015082	0.00098842
Chisq	0.012659	NA
R^2	0.98635	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.069828	0.0585
m2	0.86876	0.058756
m3	0.075592	0.014436
Chisq	0.092257	NA
R^2	0.94666	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-2.6177	13.947
m2	3.5842	13.941
m3	0.00031402	0.0014366
Chisq	0.017343	NA
R^2	0.98047	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.028536	0.09364
m2	0.97274	0.088973
m3	0.0035268	0.0018846
Chisq	0.06886	NA
R^2	0.92249	NA

MegaTev K26R pos2



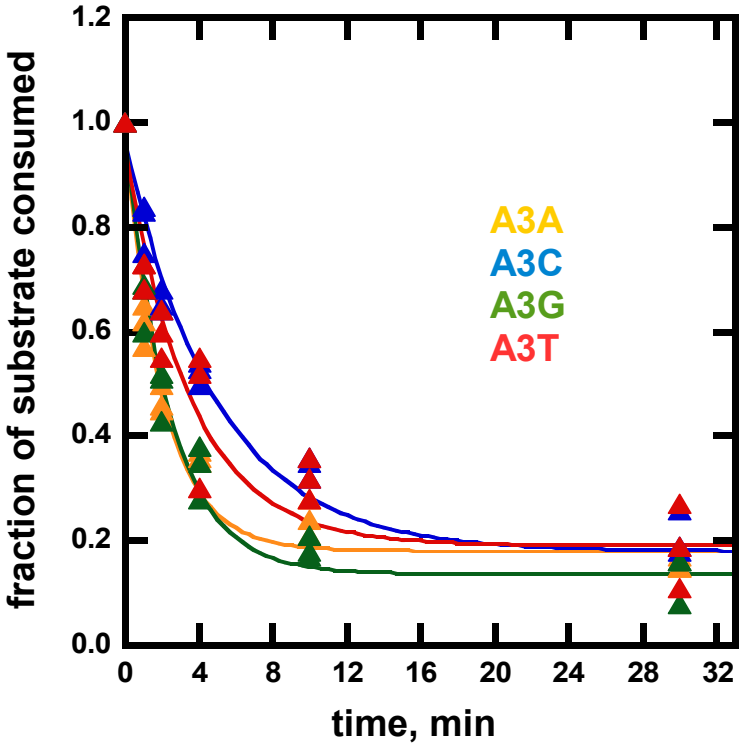
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.14694	0.024994
m2	0.81841	0.039637
m3	0.38855	0.045161
Chisq	0.062121	NA
R^2	0.96407	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.08278	0.022637
m2	0.91795	0.025546
m3	0.121	0.009538
Chisq	0.0242	NA
R^2	0.98833	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.073651	0.028282
m2	0.86988	0.039399
m3	0.2554	0.030039
Chisq	0.063026	NA
R^2	0.96824	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.11235	0.027525
m2	0.84221	0.042966
m3	0.36855	0.045565
Chisq	0.073298	NA
R^2	0.96027	NA

MegaTev K26R pos3



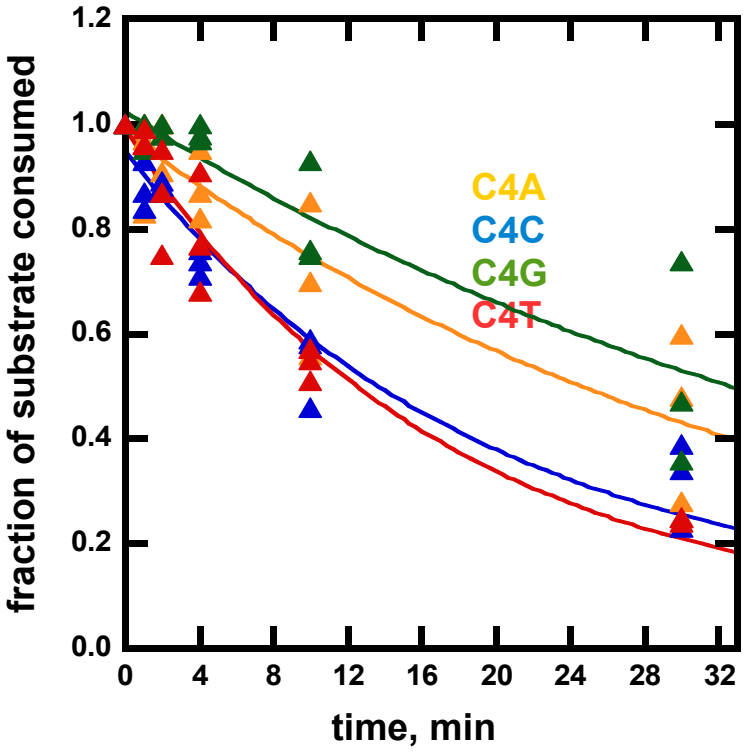
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.1806	0.026243
m2	0.79132	0.044232
m3	0.4843	0.06297
Chisq	0.076072	NA
R^2	0.95273	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.17991	0.031307
m2	0.78421	0.040864
m3	0.20281	0.028225
Chisq	0.067745	NA
R^2	0.9584	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.13665	0.022854
m2	0.8418	0.036987
m3	0.41673	0.043425
Chisq	0.053793	NA
R^2	0.97026	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.19097	0.040226
m2	0.76295	0.057712
m3	0.28127	0.054364
Chisq	0.13471	NA
R^2	0.91627	NA

MegaTev K26R pos4



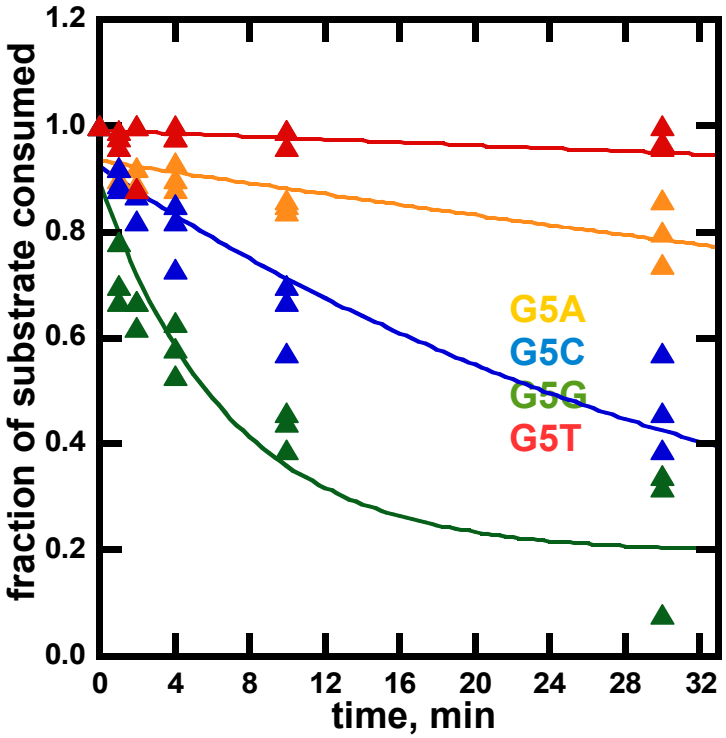
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.0097642	0.09245
m2	0.97586	0.094814
m3	0.028024	0.0057781
Chisq	0.13897	NA
R^2	0.90248	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.076018	0.061012
m2	0.87107	0.060656
m3	0.052815	0.0091856
Chisq	0.071639	NA
R^2	0.95199	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.0027385	0.082203
m2	1.0256	0.084921
m3	0.021843	0.0038887
Chisq	0.10875	NA
R^2	0.92074	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.049325	0.05566
m2	0.94194	0.055185
m3	0.059166	0.0090551
Chisq	0.064778	NA
R^2	0.9643	NA

MegaTev K26R pos5



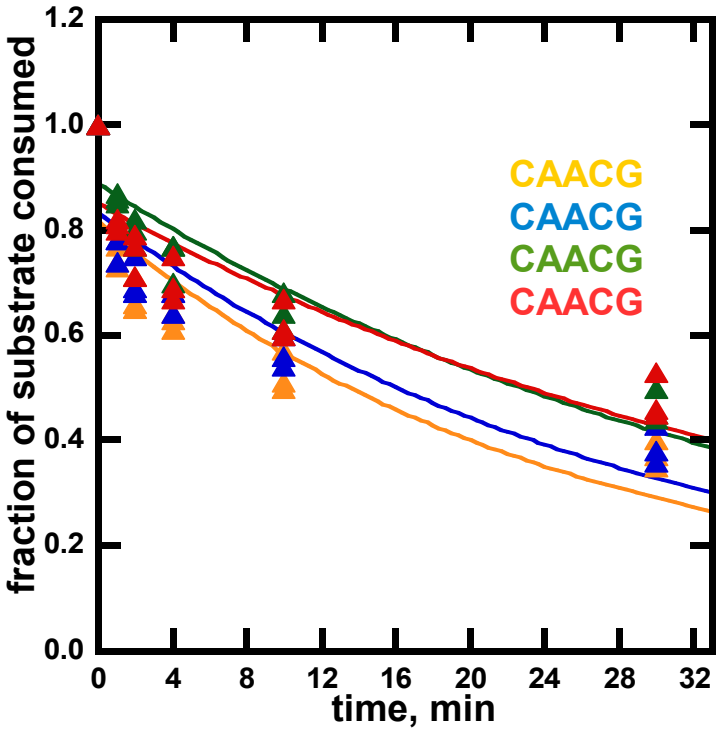
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.0023401	0.044102
m2	0.93622	0.045359
m3	0.005804	0.0012469
Chisq	0.029583	NA
R^2	0.96454	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.021355	0.069473
m2	0.90168	0.071363
m3	0.026772	0.0044911
Chisq	0.078263	NA
R^2	0.93203	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.19635	0.057373
m2	0.69776	0.068462
m3	0.14572	0.039502
Chisq	0.18326	NA
R^2	0.86864	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.83266	1.9375
m2	1.8234	1.9318
m3	0.00077719	0.0012492
Chisq	0.01729	NA
R^2	0.98129	NA

MegaTev-wt all native cleavage motif



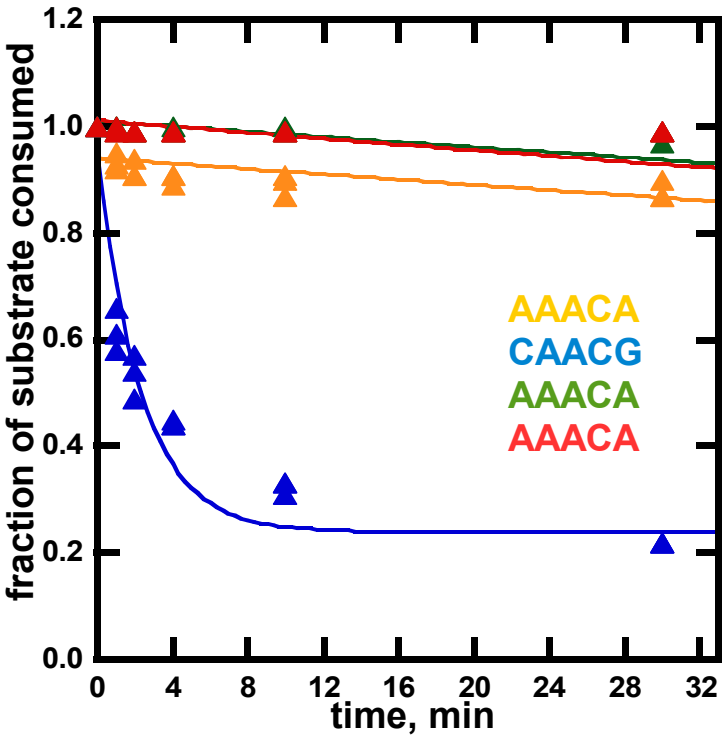
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.07577	0.10316
m2	0.74065	0.10387
m3	0.041234	0.013189
Chisq	0.18353	NA
R^2	0.83169	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.043316	0.09368
m2	0.78877	0.095292
m3	0.034003	0.008919
Chisq	0.14544	NA
R^2	0.86461	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.021023	0.068464
m2	0.86658	0.070383
m3	0.02613	0.0044984
Chisq	0.075911	NA
R^2	0.92808	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.020292	0.087175
m2	0.82963	0.089878
m3	0.02368	0.0054637
Chisq	0.12258	NA
R^2	0.87487	NA

MegaTev-wt native cleavage motif (CAACG) with cleavage motif knockouts (AAACA)



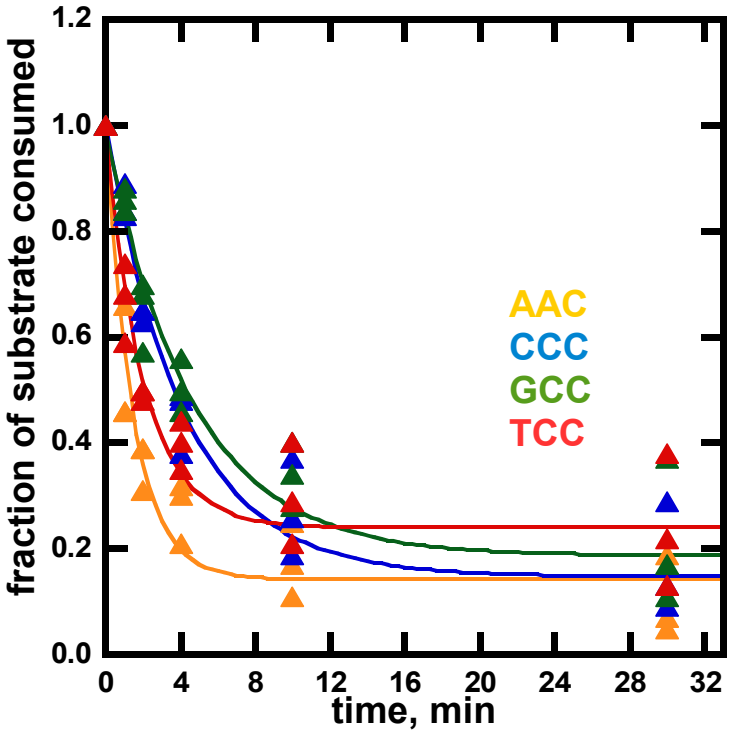
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.08168	0.10239
m2	1.023	0.097575
m3	0.0025263	0.0010328
Chisq	0.018352	NA
R^2	0.97802	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.23975	0.035082
m2	0.71427	0.057289
m3	0.43028	0.081452
Chisq	0.12873	NA
R^2	0.90732	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.074901	0.094063
m2	1.0856	0.090377
m3	0.0023075	0.00073045
Chisq	0.0095144	NA
R^2	0.98982	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.00024741	0.073951
m2	1.0118	0.069863
m3	0.0028234	0.00096084
Chisq	0.01698	NA
R^2	0.9818	NA

MegaTev WT



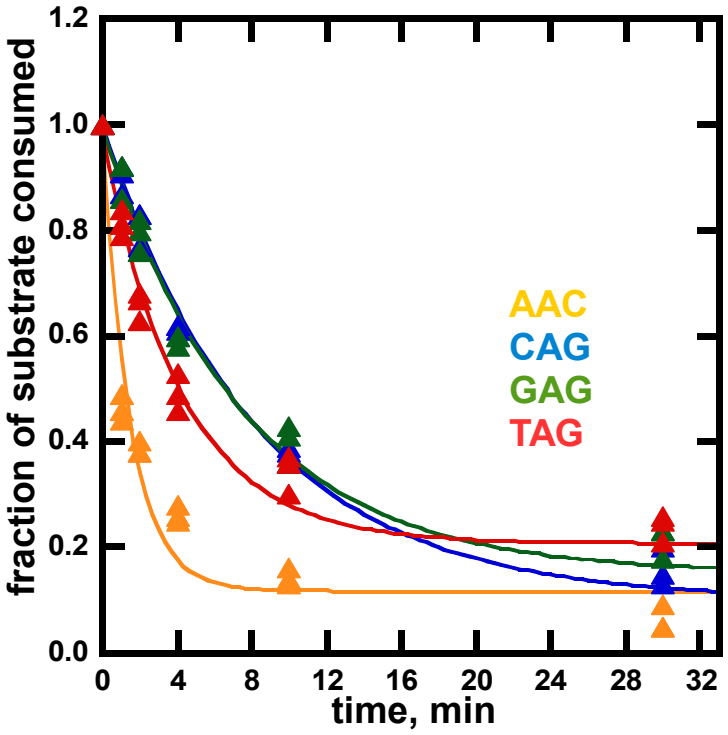
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.14201	0.02975
m2	0.84122	0.05419
m3	0.67307	0.099882
Chisq	0.11209	NA
R^2	0.93797	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.14767	0.033756
m2	0.85608	0.046325
m3	0.24277	0.034368
Chisq	0.087235	NA
R^2	0.95525	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.18691	0.041073
m2	0.80187	0.054721
m3	0.21868	0.039548
Chisq	0.12173	NA
R^2	0.93067	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.239	0.036179
m2	0.74429	0.061169
m3	0.49021	0.093605
Chisq	0.14536	NA
R^2	0.90313	NA

MegaTev WT



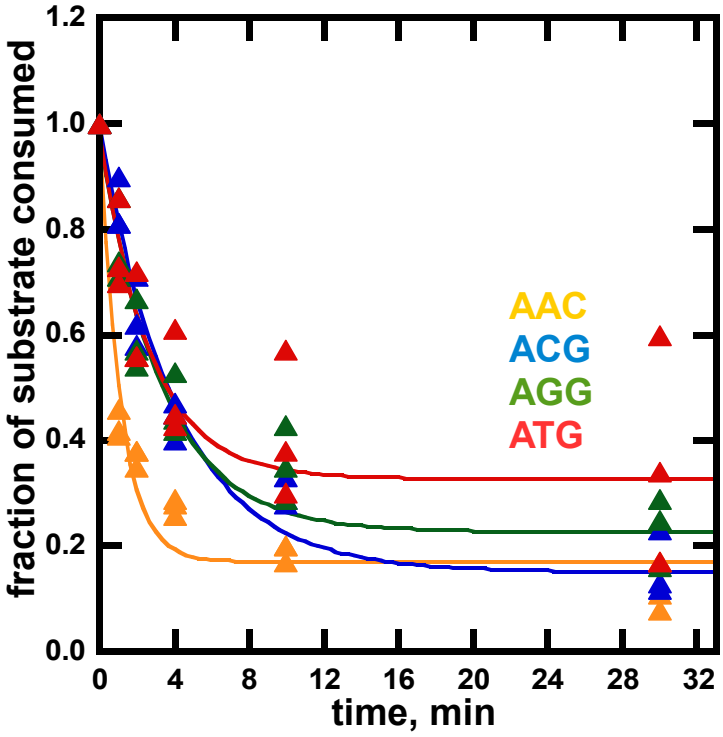
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.11701	0.025886
m2	0.85295	0.047014
m3	0.66413	0.084288
Chisq	0.084412	NA
R^2	0.95383	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.10068	0.022984
m2	0.89553	0.026015
m3	0.12217	0.01004
Chisq	0.025187	NA
R^2	0.98727	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.15049	0.03067
m2	0.84128	0.035722
m3	0.13419	0.015913
Chisq	0.04893	NA
R^2	0.97274	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.20748	0.03086
m2	0.7723	0.042036
m3	0.23658	0.033806
Chisq	0.071851	NA
R^2	0.95475	NA

MegaTev WT



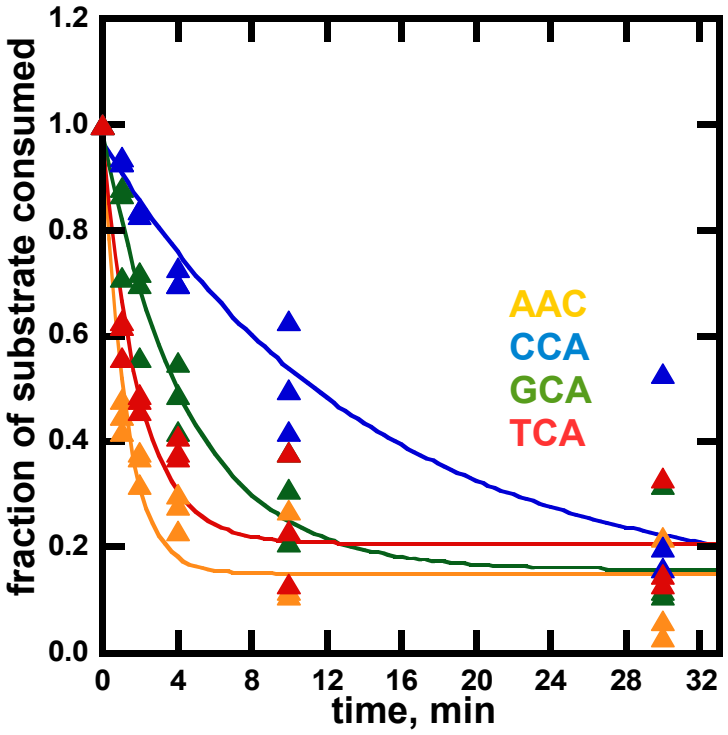
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.17076	0.025901
m2	0.81166	0.05005
m3	0.88978	0.12998
Chisq	0.094888	NA
R^2	0.94269	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.1513	0.031024
m2	0.84433	0.042612
m3	0.24347	0.032132
Chisq	0.073807	NA
R^2	0.96085	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.22778	0.039305
m2	0.74662	0.057484
m3	0.29907	0.058163
Chisq	0.1332	NA
R^2	0.9136	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.32826	0.057119
m2	0.65444	0.089381
m3	0.37157	0.12279
Chisq	0.317	NA
R^2	0.7713	NA

MegaTev WT



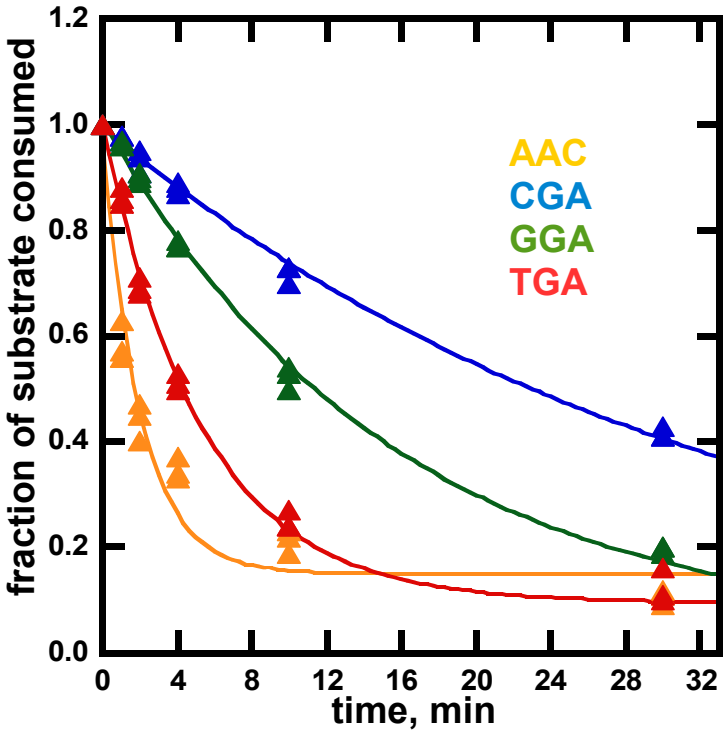
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.15014	0.029297
m2	0.83121	0.055573
m3	0.81304	0.12709
Chisq	0.11718	NA
R^2	0.93335	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.12332	0.077646
m2	0.84423	0.077491
m3	0.071139	0.01816
Chisq	0.15137	NA
R^2	0.90908	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.15762	0.041192
m2	0.82483	0.054986
m3	0.22022	0.038875
Chisq	0.12292	NA
R^2	0.93362	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.20708	0.035308
m2	0.76246	0.060171
m3	0.50581	0.092492
Chisq	0.14036	NA
R^2	0.90999	NA

MegaTev WT



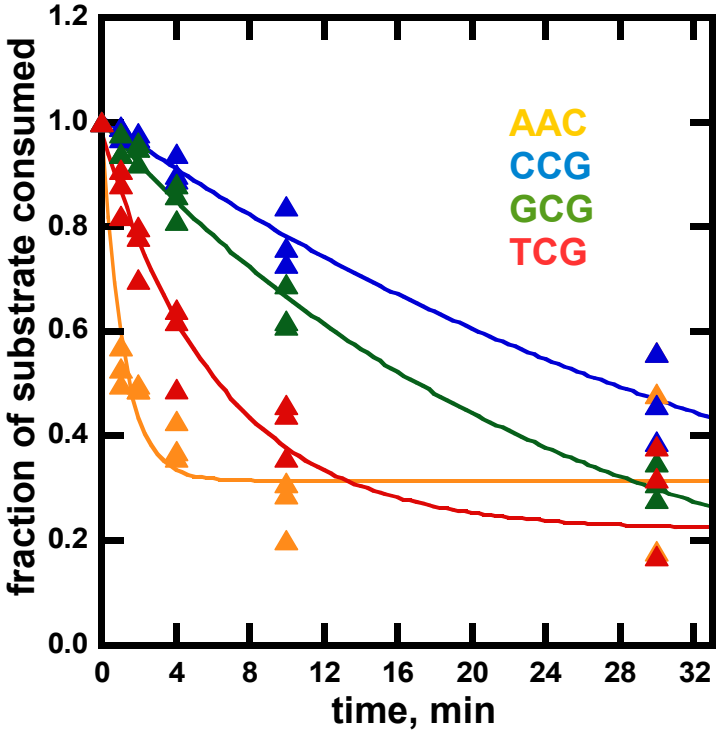
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.15012	0.027135
m2	0.81742	0.045927
m3	0.49223	0.064232
Chisq	0.081922	NA
R^2	0.95226	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.0057274	0.015214
m2	0.98982	0.015558
m3	0.030225	0.0010138
Chisq	0.0037855	NA
R^2	0.99725	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.033684	0.017569
m2	0.9748	0.01744
m3	0.065007	0.0031449
Chisq	0.0070366	NA
R^2	0.9964	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.095313	0.016786
m2	0.90434	0.021486
m3	0.18847	0.012042
Chisq	0.018656	NA
R^2	0.99107	NA

MegaTev WT



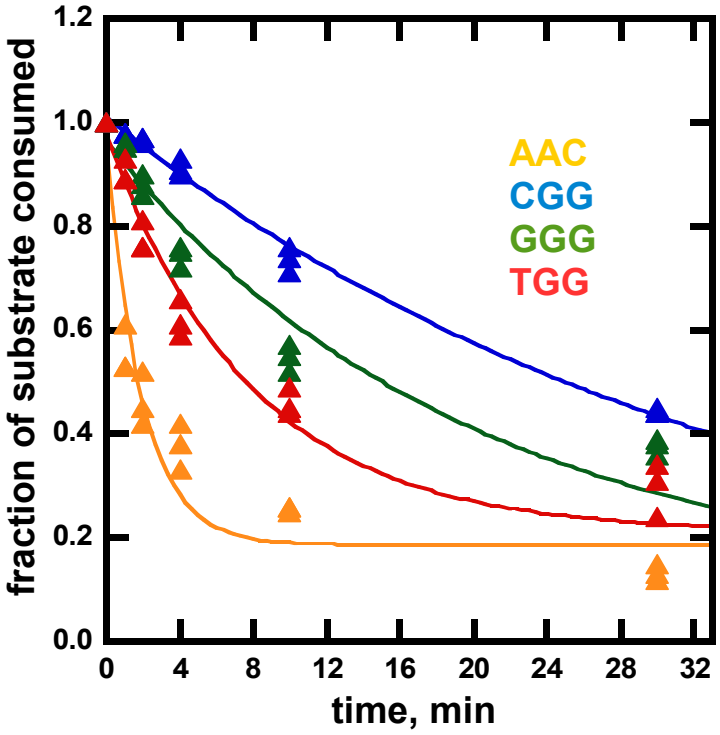
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.3142	0.042775
m2	0.67043	0.082057
m3	0.8585	0.2475
Chisq	0.2552	NA
R^2	0.80681	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.00019529	0.038219
m2	1.0081	0.039319
m3	0.02552	0.0021108
Chisq	0.023629	NA
R^2	0.98229	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.011763	0.029633
m2	0.98879	0.029831
m3	0.041364	0.0028489
Chisq	0.015156	NA
R^2	0.99073	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.22088	0.045965
m2	0.75836	0.05612
m3	0.15805	0.032006
Chisq	0.12492	NA
R^2	0.92027	NA

MegaTev WT



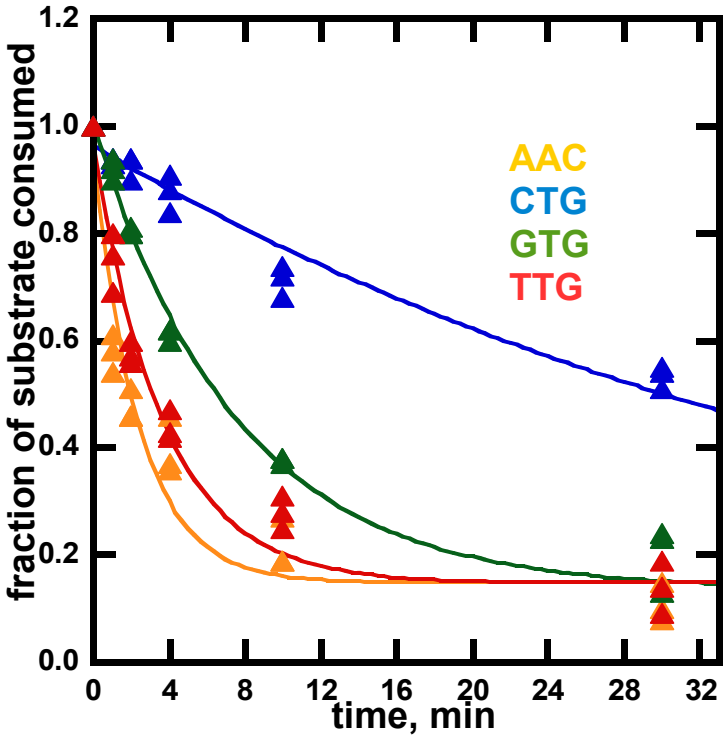
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.18593	0.033205
m2	0.7743	0.05698
m3	0.52013	0.088507
Chisq	0.12564	NA
R^2	0.92078	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.0035108	0.017768
m2	1.005	0.018217
m3	0.028239	0.0010867
Chisq	0.0051357	NA
R^2	0.99626	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.10098	0.061738
m2	0.86247	0.061448
m3	0.051319	0.0090285
Chisq	0.072088	NA
R^2	0.95014	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.21076	0.041804
m2	0.76616	0.048001
m3	0.12803	0.022546
Chisq	0.087138	NA
R^2	0.94292	NA

MegaTev WT



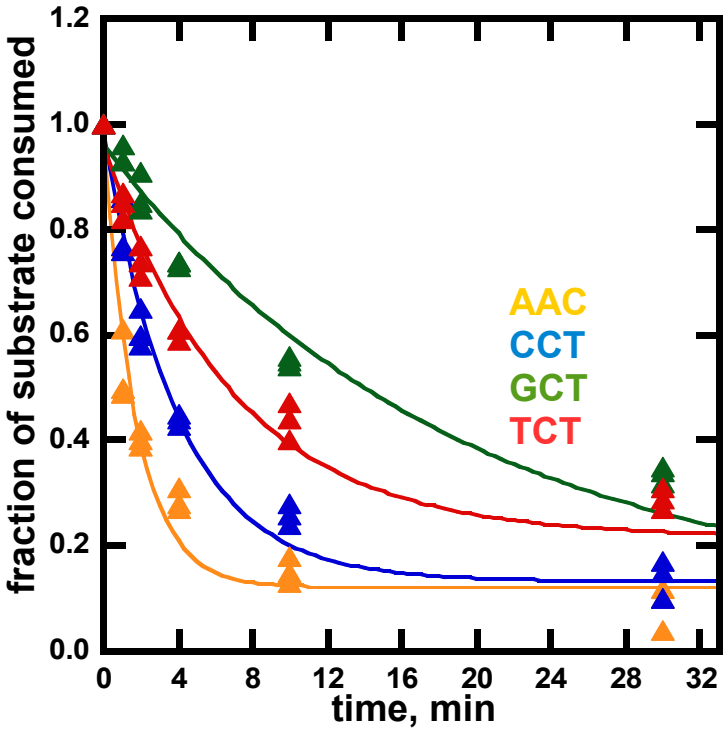
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.14855	0.034145
m2	0.79704	0.055179
m3	0.41458	0.068126
Chisq	0.11977	NA
R^2	0.92927	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.010031	0.039388
m2	0.95277	0.040679
m3	0.022104	0.0020256
Chisq	0.024974	NA
R^2	0.97772	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.1354	0.02761
m2	0.8699	0.032067
m3	0.13294	0.013704
Chisq	0.039329	NA
R^2	0.97934	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.14768	0.028834
m2	0.81814	0.040867
m3	0.27036	0.034747
Chisq	0.067673	NA
R^2	0.96166	NA

MegaTev WT



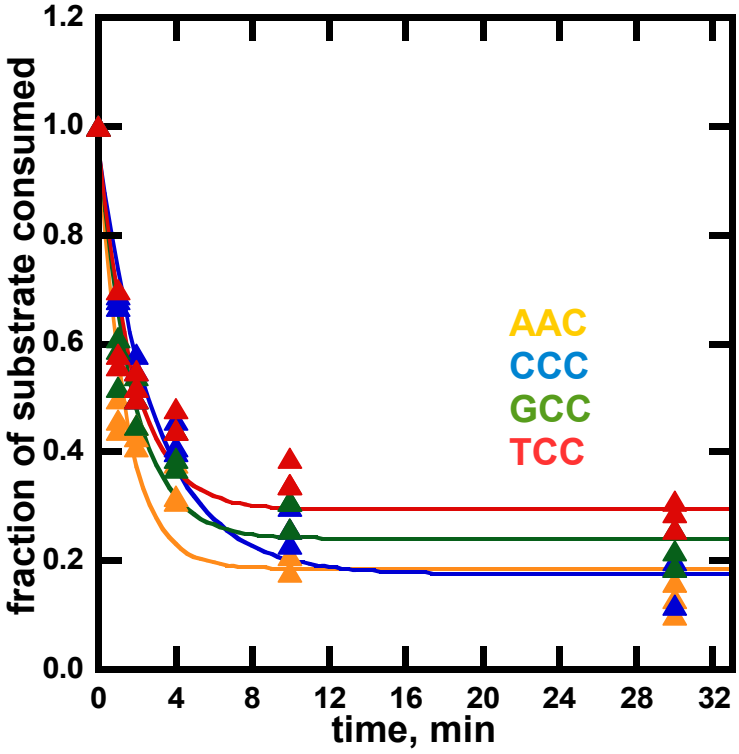
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.12159	0.024517
m2	0.85047	0.043007
m3	0.57	0.066354
Chisq	0.071175	NA
R^2	0.96093	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.133	0.024072
m2	0.85039	0.033399
m3	0.25199	0.025753
Chisq	0.045309	NA
R^2	0.97592	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.089635	0.051243
m2	0.87149	0.050904
m3	0.053977	0.0079434
Chisq	0.051253	NA
R^2	0.96555	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.21703	0.038829
m2	0.74145	0.046168
m3	0.14392	0.024798
Chisq	0.083126	NA
R^2	0.94267	NA

MegaTev T3



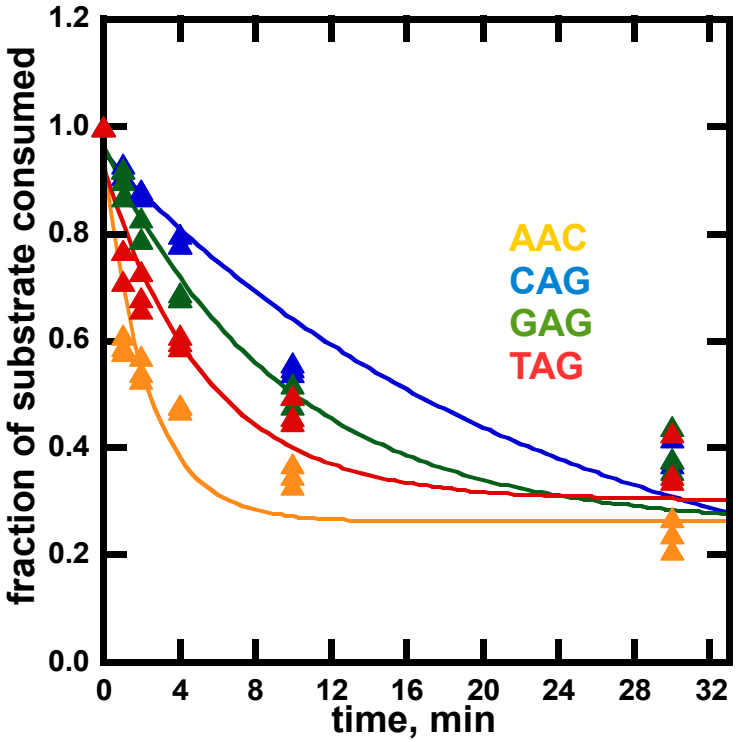
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.18568	0.032297
m2	0.78242	0.059493
m3	0.70846	0.1244
Chisq	0.13483	NA
R^2	0.91558	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.17563	0.028764
m2	0.78501	0.043822
m3	0.3406	0.046798
Chisq	0.076716	NA
R^2	0.95274	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.24054	0.032847
m2	0.72589	0.057262
m3	0.55522	0.10092
Chisq	0.12637	NA
R^2	0.90998	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.29363	0.03632
m2	0.67502	0.063011
m3	0.54408	0.11713
Chisq	0.15321	NA
R^2	0.87836	NA

MegaTev T3



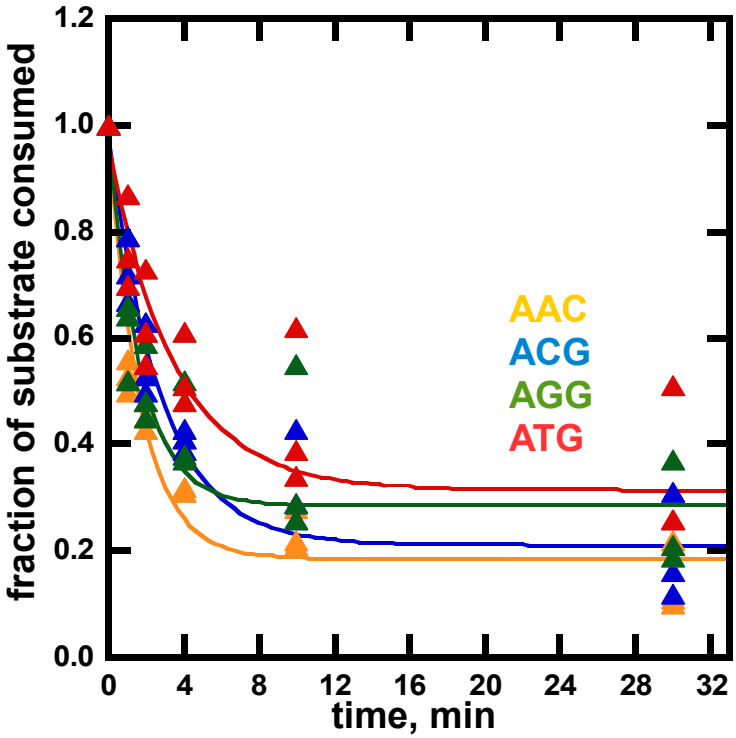
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.26354	0.039756
m2	0.68007	0.065004
m3	0.43228	0.097454
Chisq	0.16568	NA
R^2	0.87332	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.070783	0.058586
m2	0.87961	0.05881
m3	0.043503	0.0067517
Chisq	0.060197	NA
R^2	0.95623	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.25536	0.053611
m2	0.70786	0.057997
m3	0.10538	0.024841
Chisq	0.11689	NA
R^2	0.9105	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.30336	0.050709
m2	0.62114	0.064609
m3	0.18522	0.051896
Chisq	0.16848	NA
R^2	0.85283	NA

MegaTev T3



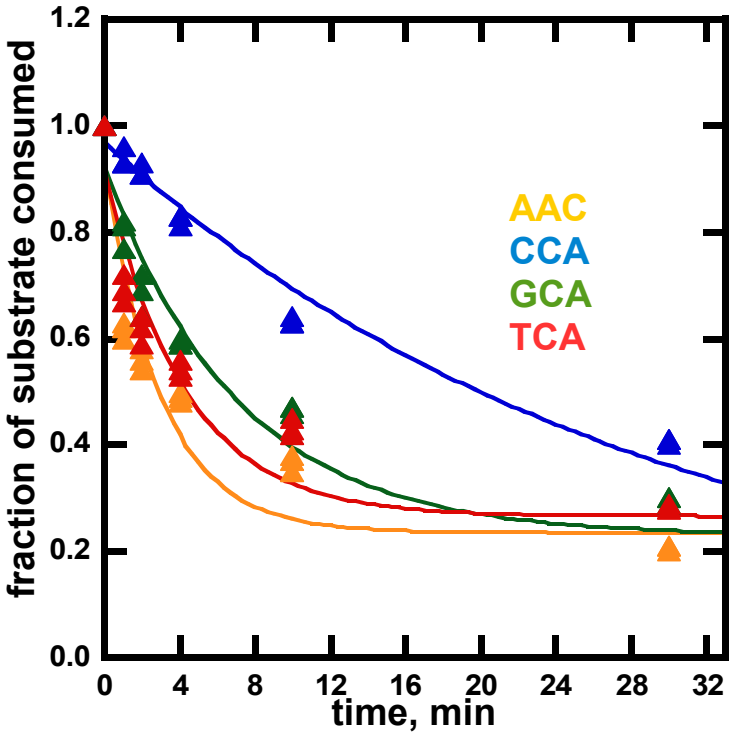
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.18553	0.031649
m2	0.78049	0.055854
m3	0.58499	0.096309
Chisq	0.11988	NA
R^2	0.92468	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.21118	0.037158
m2	0.76768	0.057677
m3	0.36179	0.066108
Chisq	0.13228	NA
R^2	0.91763	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.28452	0.044676
m2	0.69017	0.078824
m3	0.58434	0.15354
Chisq	0.23876	NA
R^2	0.82818	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.31448	0.056782
m2	0.64847	0.082154
m3	0.28898	0.09308
Chisq	0.27259	NA
R^2	0.79605	NA

MegaTev T3



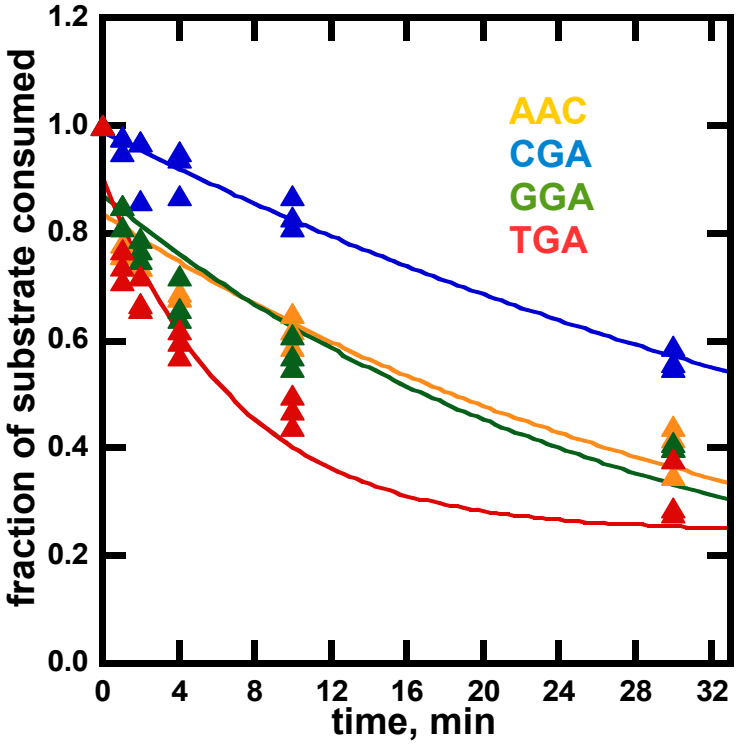
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.23545	0.042848
m2	0.68952	0.064705
m3	0.33111	0.076912
Chisq	0.1676	NA
R^2	0.87702	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.028584	0.038135
m2	0.93933	0.03876
m3	0.034577	0.0031078
Chisq	0.02416	NA
R^2	0.98214	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.23002	0.046608
m2	0.69364	0.055309
m3	0.14295	0.031569
Chisq	0.11913	NA
R^2	0.90937	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.26574	0.046533
m2	0.65075	0.063308
m3	0.23559	0.060202
Chisq	0.16297	NA
R^2	0.86849	NA

MegaTev T3



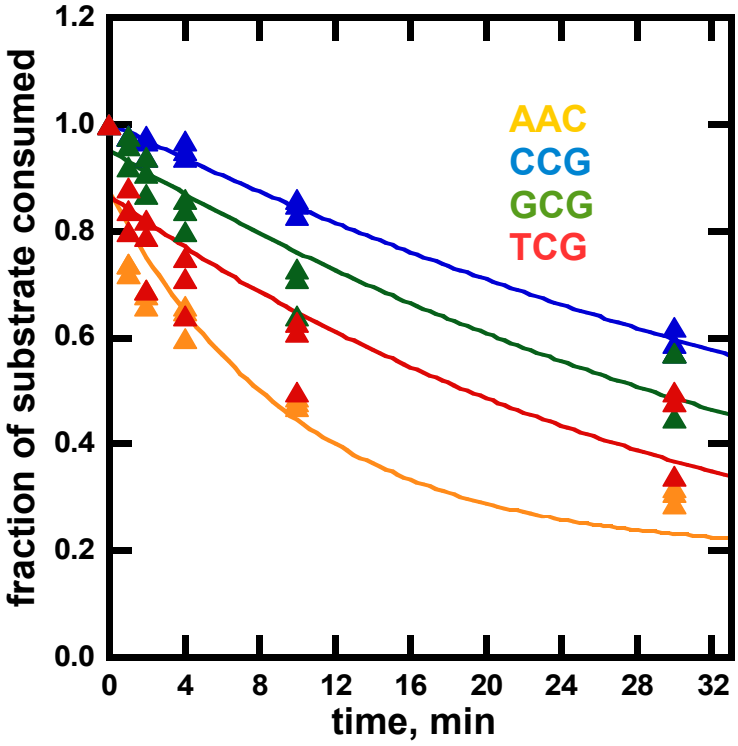
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.022858	0.090488
m2	0.8126	0.092689
m3	0.028951	0.007021
Chisq	0.13344	NA
R^2	0.87169	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.0007839	0.032888
m2	0.98976	0.034091
m3	0.018296	0.0014072
Chisq	0.017355	NA
R^2	0.98442	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.051123	0.081766
m2	0.81852	0.082989
m3	0.035549	0.0078975
Chisq	0.11155	NA
R^2	0.90176	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.24591	0.052899
m2	0.65601	0.062897
m3	0.14392	0.038184
Chisq	0.15428	NA
R^2	0.87397	NA

MegaTev T3



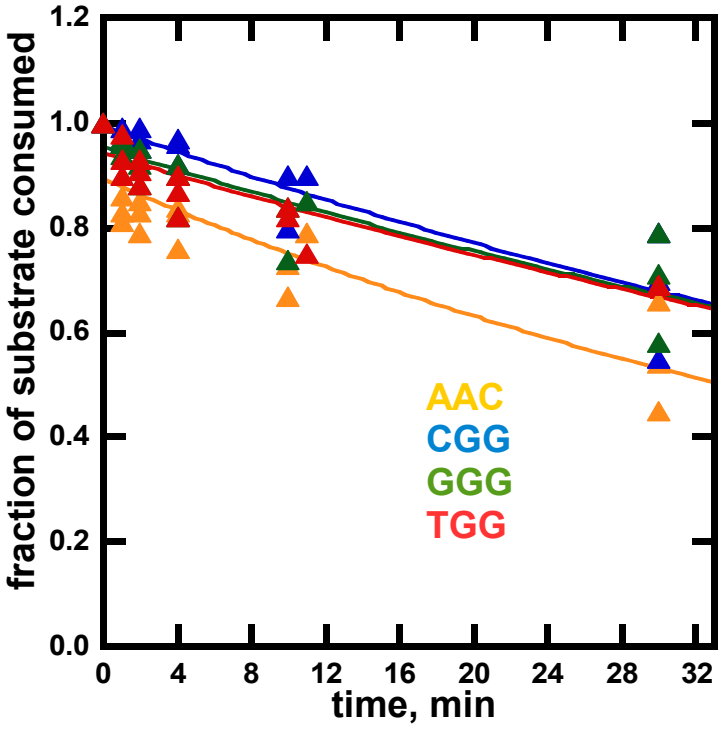
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.19969	0.061316
m2	0.6757	0.065513
m3	0.10098	0.028253
Chisq	0.1454	NA
R^2	0.88075	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	-0.00086981	0.01629
m2	1.0044	0.0169
m3	0.01729	0.00066062
Chisq	0.0042551	NA
R^2	0.99617	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.014747	0.056873
m2	0.93773	0.058687
m3	0.022897	0.0030621
Chisq	0.052119	NA
R^2	0.9539	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.04064	0.095863
m2	0.82588	0.097957
m3	0.030748	0.0077972
Chisq	0.15053	NA
R^2	0.86517	NA

MegaTev T3



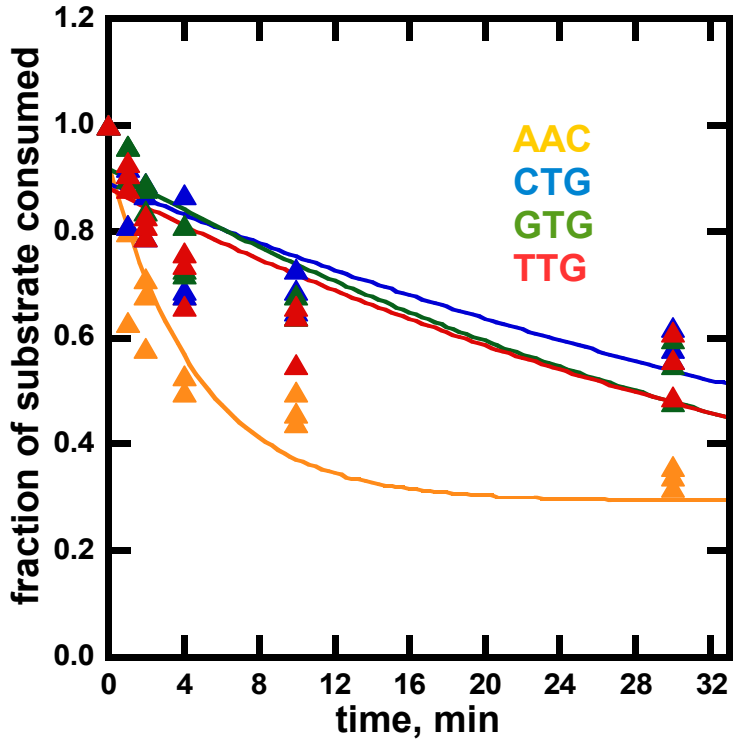
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.0047237	0.073381
m2	0.88854	0.076137
m3	0.01745	0.0033746
Chisq	0.086361	NA
R^2	0.9096	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.00013591	0.05
m2	0.99388	0.052043
m3	0.012709	0.0017131
Chisq	0.040022	NA
R^2	0.96128	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.002301	0.058519
m2	0.95134	0.060938
m3	0.011684	0.0020148
Chisq	0.054802	NA
R^2	0.94228	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.0024041	0.043925
m2	0.94005	0.045744
m3	0.011544	0.0015225
Chisq	0.030875	NA
R^2	0.96579	NA

MegaTev T3



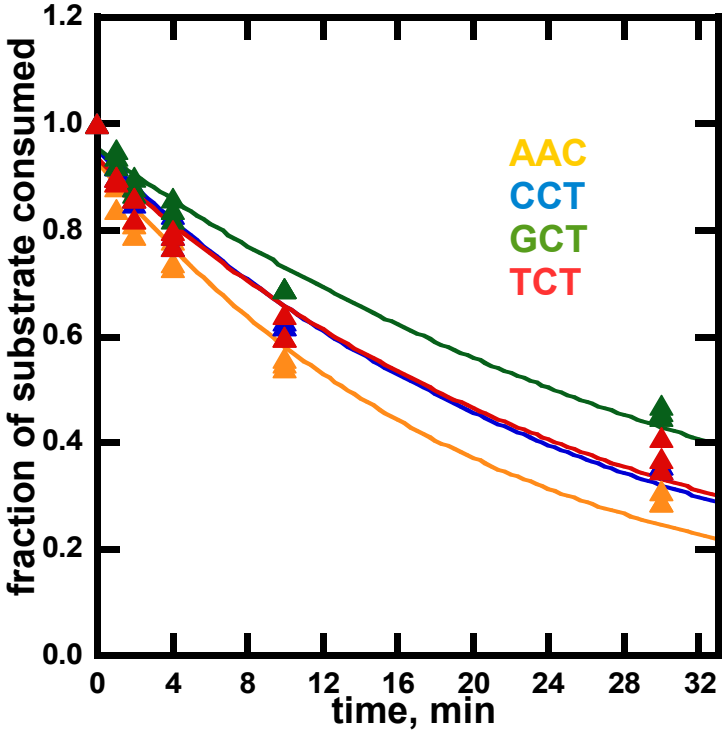
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.29406	0.054142
m2	0.63304	0.071237
m3	0.20892	0.062605
Chisq	0.20609	NA
R^2	0.8316	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.010419	0.085908
m2	0.87974	0.089146
m3	0.017002	0.0039337
Chisq	0.11833	NA
R^2	0.87706	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.021244	0.078845
m2	0.89637	0.08141
m3	0.022338	0.0043482
Chisq	0.1001	NA
R^2	0.90685	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.022697	0.095573
m2	0.85848	0.098812
m3	0.021067	0.0052439
Chisq	0.14688	NA
R^2	0.8558	NA

MegaTev T3



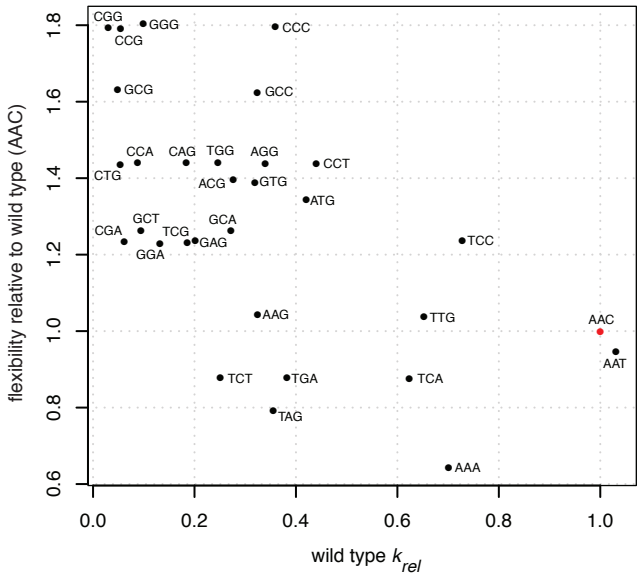
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.058155	0.045283
m2	0.87075	0.045081
m3	0.051061	0.0065148
Chisq	0.038671	NA
R^2	0.97306	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.027782	0.035134
m2	0.92215	0.035523
m3	0.038233	0.0032855
Chisq	0.020886	NA
R^2	0.98477	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.013821	0.033711
m2	0.94054	0.03461
m3	0.027205	0.0021224
Chisq	0.018444	NA
R^2	0.98457	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.03217	0.044877
m2	0.90261	0.045474
m3	0.036701	0.0040815
Chisq	0.033795	NA
R^2	0.97401	NA

S4 Fig. Correlation between relative flexibility of CNNNG sequences and relative cleavage activity. Plot of the relative rate of cleavage (*k*rel) with cumulative flexibility of each cleavage motif (CNNNG) relative to the native sequence (CAACG). Cumulative flexibility was determined from the sum of TRX values (1) from each pair of adjacent bases in the each of the CNNNG cleavage motifs. The relative cleavage activity is taken from data shown in Fig. 4 and in S3 Fig.


CGG
GGG
CCC
CCG
GCG
GCC
CCA
CAG
TGG
AGG
CCT
CTG
ACG
GTG
ATG
GCT
GCA
CGA
TCG
GAG
TCC
GGA
AAG
TTG
AAC
AAT
TCT
TGA
TCA
TAG
AAA
flexibility relative to wild type (AAC)
wild type k_{rel}
0.6
0.8
1.0
1.2
1.4
1.6
1.8
0.0
0.2
0.4
0.6
0.8
1.0

Supplemental Table S1: Bacterial strains used in this study

Strains	Description	Source
E.coli – NEB5α	F^-, φ80d*lacZ*ΔM15, Δ(*lacZYA-argF*)U169, *deoR*, *recA*1, *endA*1, *hsdR*17(rk^-, mk^+), *phoA*, *supE*44, λ^-, *thi*-1, *gyrA*96, *relA*1	N.E.B.
E.coli - ER2566	F- λ- *fhuA*2 [lon] *ompT lacZ*::T7 gene 1 *gal sulA*11 Δ(*mcrC-mrr*)114::IS10 R(*mcr*-73::miniTn10-*TetS*)2 R(*zgb*-210::Tn10)(*TetS*) *endA*1 [dcm]	N.E.B.
E.coli - BW25141(λDE3)	F^- *lacI*^{q} *rrnB*$_{T14}$ D*lacZ*$_{WJ16}$ D*phoBR580 hsdR514* D*araBAD*$_{AH33}$ D*rhaBAD*$_{LD78}$ *galU95 endA*$_{BT333}$ *uidA*(D*MluI*)::*pir*+ *recA*1, λDE3 lysogen	Ref 34

Supplemental Table S2: Plasmids used in this study

Plasmids	Description	Source
pACYCDuet-1(PciI)	*ori*$_{p15A}$, cm, pACYCDuet-1 with a PciI site substituted for the NcoI site	Novagen
p11-lacY-wtx1 (pTox)	*ori*$_{pBR322}$, amp	Ref 34
pKox	*ori*$_{pBR322}$, kan, pTox with kan^r inserted into ScaI site of amp^r	Ref 34
pSP72	*ori*$_{pBR322}$, amp	Promega
pToxTO20	p11-lacY-wtx1, that contains a 42-bp hybrid I-TevI/I-OnuI E1 homing site (*td* bases -27 to -8 fused to the I-OnuI E1 site) cloned into the XbaI and SphI sites(DE1064/1065)	Ref 34
pToxTO20 C1A/G5A	Similar to pToxTO20, with C1A and G5A substitution	Ref 34
pToxTO20 C1N/G5N	Similar to pToxTO20, with either a C1 or G5 substitution	Ref 34
pKoxTO20	Similar to pToxTO20, except derived from pKox	Ref 34
pKoxTO20 CNNNG	Similar to pKoxTO20, with one of the triplet substitutions (*e.g.* CCCGG)	Ref 34
pACYCOnuE1(E22Q) (+H)	pACYCDuet-1(PciI), containing the I-OnuI E1 gene with a E22Q mutation cloned into the BamHI and XhoI sites	Ref 34
pTevN169-OnuE1(E22Q) (+H)	pACYCOnuE1(E22Q)(+H), with residues 1-N169 of I-TevI (DE) cloned into the PciI and BamHI sites (+6xHis)	Ref 34

Supplemental Table S3: Oligonucleotides used in this study

Name	Sequence (5'-3')	Notes[1]
DE410	GGAAGAAGTGGCTGATCTCAGC	Forward primer to generate all cycle-seq products for target sites cloned into pTox
DE411	CAGACCGCTTCTGCGTTCTG	Reverse primer to generate all cycle-seq products for target sites cloned into pTox
DE840	GCCGCCATGGGTAAAAGCGGAATTTATCAGATT	Forward primer for MegaTev cloning, NcoI site underlined
DE1045	CGCGGATCCATTTCTGCATTTACTACAAG	Reverse primer for TevN169 cloning, BamHI site underlined
DE1424	CGTTTGGTGATACATGTTCTACG	Reverse primer for I-TevI linker cloning.
DE1912	CGTAGAACATGTATCACCAAACG	Reverse primer for mutagenesis of the I-TevI nuclease domain, PciI site is underlined
DE2054	CTAGAAAACGCTCAGTAGATGTTTTGGTCCACATATTT AACCTTTTGCATG	Top strand of C1A substrate for insertion into pTox XbaI/SphI
DE2055	CAAAAGGTTAAATATGTGGACCAAAACATCTACTGAG CGTTTT	Bottom strand of C1A substrate for insertion into pTox XbaI/SphI
DE2056	CTAGATAACGCTCAGTAGATGTTTTGGTCCACATATTT AACCTTTTGCATG	Top strand of C1T substrate for insertion into pTox XbaI/SphI
DE2057	CAAAAGGTTAAATATGTGGACCAAAACATCTACTGAG CGTTAT	Bottom strand of C1T substrate for insertion into pTox XbaI/SphI
DE2058	CTAGACAACACTCAGTAGATGTTTTGGTCCACATATTT AACCTTTTGCATG	Top strand of G5A substrate for insertion into pTox XbaI/SphI
DE2059	CAAAAGGTTAAATATGTGGACCAAAACATCTACTGAG TGTTGT	Bottom strand of G5A substrate for insertion into pTox XbaI/SphI
DE2060	CTAGACAACCCTCAGTAGATGTTTTGGTCCACATATTT AACCTTTTGCATG	Top strand of G5C substrate for insertion into pTox XbaI/SphI
DE2061	CAAAAGGTTAAATATGTGGACCAAAACATCTACTGAG GGTTGT	Bottom strand of G5C substrate for insertion into pTox XbaI/SphI
DE2222	CCCAAACAGGTCGCTGAAATGC	Forward primer for generating the 2200 bp barcode assay substrate from pTox or pKox templates.
DE2223	TGTCACGCTCGTCGTTTGGTATGGC	Reverse primer for generating the 2200 bp barcode assay substrate from pTox templates.
DE2224	ATGACGACCGTAGTGATGAATCTCTCC	Forward primer for generating the 1900 bp barcode assay substrate from pTox or pKox templates.
DE2225	TCATGGTTATGGCAGCACTGC	Reverse primer for generating the 1900 bp barcode assay substrate from pTox templates.
DE2226	AAAAAAATCGAGATAACCGTTGGC	Forward primer for generating the 1600 bp barcode assay substrate from pTox or pKox templates.
DE2227	CCGCGCCACATAGCAGAACTTTAAAAGTGC	Reverse primer for generating the 1600 bp barcode assay substrate from pTox or pKox templates.
DE2228	ATTGTCCATATTGCATCAGACATTGC	Forward primer for generating the 1300 bp barcode assay substrate from pTox or pKox templates.
DE2229	ACTTCACCAGCGTTTCTGG	Forward primer for generating the 1300 bp barcode assay substrate from pTox or pKox templates.
DE2230	AAATTAATAGGTTGTATTGATGTTGGACGAGTCG	Reverse primer for generating the 2200 bp native I-TevI target barcode assay substrate from pKox templates.
DE2231	AAATTGCAGTTTCATTTGATGCTCG	Reverse primer for generating the 1900 bp native I-TevI target barcode assay substrate from pKox templates.
DE2296	TGAGACACAACGTGGCTTTGTTGAATAAATCG	Reverse primer for generating the 1900 bp non-native I-TevI target barcode assay substrate from pKox templates.
DE2297	TCCATGTTGGAATTTAATCGCGGCCTCG	Reverse primer for generating the 2200 bp non-native I-TevI target barcode assay substrate from pKox templates.

[1] underlined nucleotides refer to restriction enzyme sites

Table S.4. (Part 1 of 3) Survival Rates Determned from *in vivo* Bacterial 2-Plasmid Surival Assay

Substrate	wt	K26R T95S Q158R (T3)	T95S/Q158R	Q158R	K26R/Q158R	T95S	K26R/T95S	K26R
CAACG	89.00±2.65	139.37±83.39	92.30±1.40	95.67±13.05	118.00±41.76	131.67±33.65	77.30±34.13	64.30±6.55
CTCGG	0.00±0.00	0.00±0.00	12.27±6.40	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
CGTGG	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.13±0.06	0.00±0.00	0.00±0.00	0.00±0.00
CCGGG	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
CGCGG	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
CGAAG	13.50±6.06	84.67±31.90	81.33±13.32	53.33±9.29	86.00±34.04	23.33±4.51	15.33±2.52	31.33±18.77
CGCAG	0.00±0.00	21.00±6.24	9.13±1.96	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
CTGGG	0.00±0.00	88.33±33.95	42.77±11.08	1.50±1.22	3.33±1.21	18.23±14.65	17.87±13.97	0.00±0.00
CGCCG	0.00±0.00	71.33±33.84	28.67±23.46	0.37±0.06	0.90±0.10	43.00±28.05	29.90±24.06	0.00±0.00
CGGAG	0.00±0.00	80.33±13.80	55.10±13.69	5.90±4.28	5.93±4.27	23.50±18.30	25.93±24.10	0.00±0.00
CGGGG	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
CGCTG	0.00±0.00	13.33±0.58	17.33±7.02	0.20±0.00	0.10±0.00	0.10±0.00	0.10±0.00	0.00±0.00
CCCAG	0.00±0.00	4.97±6.26	8.57±3.74	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
CCAGG	0.00±0.00	2.33±0.55	2.23±3.87	1.50±0.26	23.00±3.00	0.00±0.00	0.00±0.00	0.00±0.00
CCCCG	0.00±0.00	64.00±53.23	76.33±55.79	4.53±2.27	4.27±2.57	42.33±26.01	35.20±31.70	0.00±0.00
CAAGG	1.60±0.82	54.00±24.25	48.33±26.27	75.00±36.59	73.67±30.89	6.47±5.20	7.87±6.47	26.33±9.07
CGAGG	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.40±0.17	0.00±0.00	0.00±0.00	0.00±0.00
CACGG	0.07±0.12	24.67±21.94	34.27±9.50	0.00±0.00	2.00±0.35	0.00±0.00	0.00±0.00	0.00±0.00
AAACG	0.00±0.00	3.07±2.16	37.00±1.73	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
GAACG	0.00±0.00	4.93±2.78	21.67±2.89	0.00±0.00	0.40±0.10	0.00±0.00	0.00±0.00	0.00±0.00
TAACG	0.00±0.00	107.33±51.52	2.60±0.66	8.30±4.12	15.67±8.14	0.00±0.00	0.00±0.00	0.00±0.00
CAACA	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
CAACC	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
CAACT	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Table S.4. (Part 2 of 3) Survival Rates Determned from *in vivo* 2-Plasmid Surival Assay

Substrate	C39R	I86V	C39R/I86V	C39R/T95S	I86V/T95S	C39R/I86V/T95S
CAACG	92.33±11.02	88.60±26.38	72.50±35.57	100.17±37.52	66.17±29.10	86.43±30.51
CTCGG	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
CGTGG	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
CCGGG	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
CGCGG	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
CGAAG	27.67±11.24	20.67±10.79	17.33±10.41	42.67±6.43	15.77±6.64	17.67±5.13
CGCAG	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
CTGGG	0.00±0.00	0.00±0.00	0.00±0.00	29.33±12.70	10.53±9.04	25.40±14.25
CGCCG	0.00±0.00	0.00±0.00	0.00±0.00	58.33±28.43	33.43±22.13	32.67±20.01
CGGAG	0.00±0.00	0.00±0.00	0.00±0.00	5.73±1.10	17.37±13.56	26.97±24.11
CGGGG	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
CGCTG	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.10±0.00	0.30±0.00
CCCAG	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
CCAGG	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
CCCCG	0.00±0.00	0.00±0.00	0.00±0.00	74.33±16.56	46.47±32.10	45.00±22.91
CAAGG	14.27±7.75	4.60±2.56	14.27±13.13	4.43±3.49	4.70±3.82	7.47±6.12
CGAGG	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
CACGG	0.00±0.00	0.00±0.00	0.00±0.00	2.43±3.09	0.00±0.00	0.00±0.00
AAACG	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
GAACG	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
TAACG	0.00±0.00	0.00±0.00	0.00±0.00	2.43±2.57	0.00±0.00	0.00±0.00
CAACA	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
CAACC	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
CAACT	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Table S.4. (Part 3 of 3) Survival Rates Determned from *in vivo* 2-Plasmid Surival Assay

Substrate	K26S	K26S/T95S	K26S/C39R/T95S
CAACG	73.33±22.05	104.00±30.64	116.33±43.02
CTCGG	0.00±0.00	0.00±0.00	0.00±0.00
CGTGG	0.00±0.00	0.00±0.00	0.00±0.00
CCGGG	0.00±0.00	0.00±0.00	0.00±0.00
CGCGG	0.00±0.00	0.00±0.00	0.00±0.00
CGAAG	0.00±0.00	7.87±7.06	20.00±18.25
CGCAG	0.00±0.00	0.00±0.00	0.00±0.00
CTGGG	0.00±0.00	1.86±0.22	5.82±3.82
CGCCG	0.00±0.00	2.50±1.83	12.83±10.25
CGGAG	0.00±0.00	0.08±0.13	0.99±1.41
CGGGG	0.00±0.00	0.00±0.00	0.00±0.00
CGCTG	0.00±0.00	0.00±0.00	0.00±0.00
CCCAG	0.00±0.00	0.00±0.00	0.00±0.00
CCAGG	0.00±0.00	0.00±0.00	0.00±0.00
CCCCG	0.00±0.00	4.13±0.68	12.67±20.21
CAAGG	0.00±0.00	0.00±0.00	0.00±0.00
CGAGG	0.00±0.00	0.00±0.00	0.00±0.00
CACGG	0.00±0.00	0.00±0.00	0.00±0.00
AAACG	0.00±0.00	0.00±0.00	0.00±0.00
GAACG	0.00±0.00	0.00±0.00	0.00±0.00
TAACG	0.00±0.00	0.00±0.00	0.80±0.44
CAACA	0.00±0.00	0.00±0.00	0.00±0.00
CAACC	0.00±0.00	0.00±0.00	0.00±0.00
CAACT	0.00±0.00	0.00±0.00	0.00±0.00

Supplementary References

1. Heddi,B., Oguey,C., Lavelle,C., Foloppe,N. and Hartmann,B. (2010) Intrinsic flexibility of B-DNA: The experimental TRX scale. *Nucleic Acids Res.,* **38,** 1034-1047.