Supplementary Material

Biochemical analysis of human *POLG2* variants associated with mitochondrial disease

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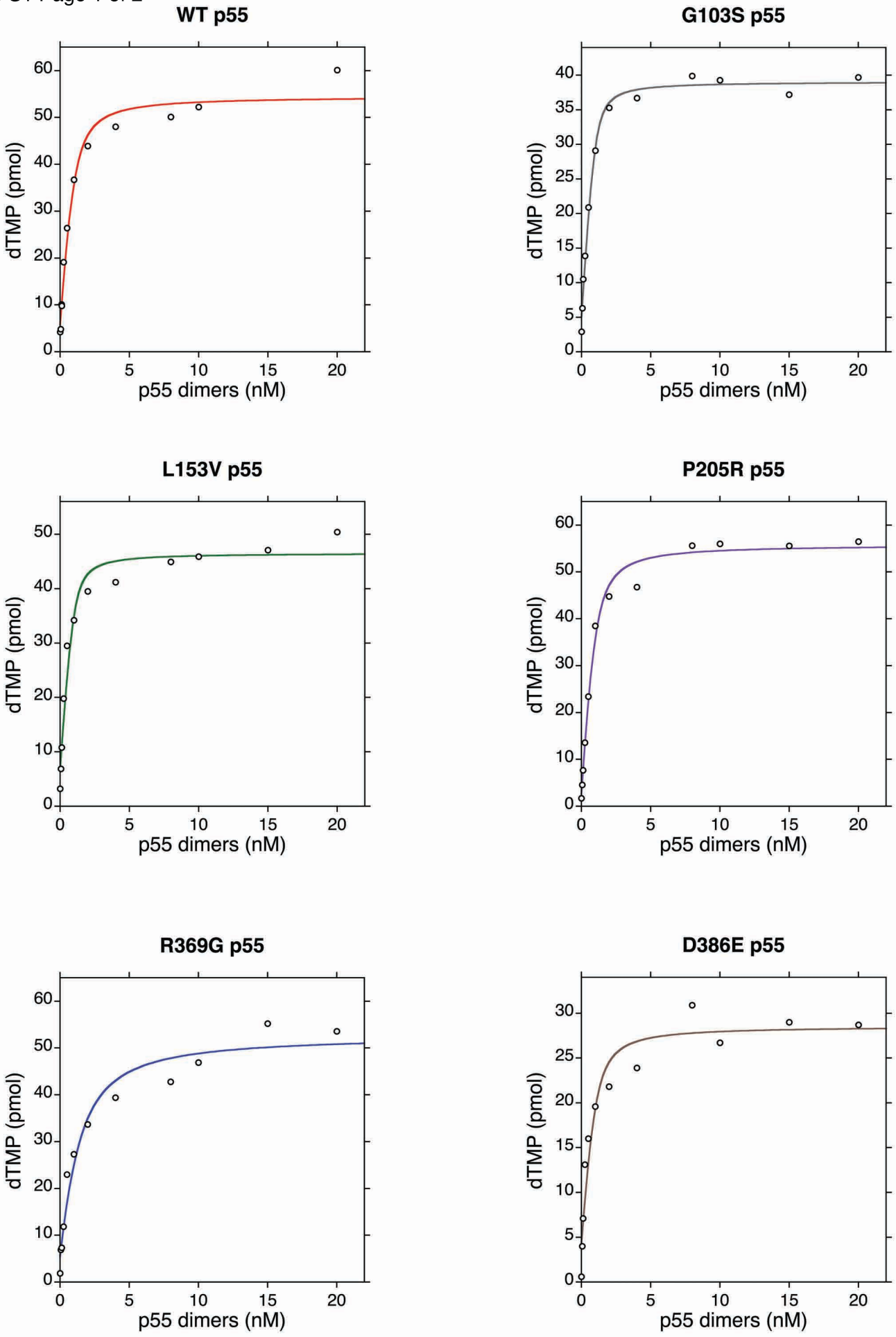
Supplementary Table: *POLG2* sequencing primers. Primer sequences were designed using genomic sequence from the Genebank database. All primers are M13 linked.

Exons	Primer Names	Primer Sequences (5'-3')	Length
E1	POLG2-E1F2	CTC GT TGT AAA ACG ACG GCC AGT AACGGTAGTGGTGGCTTGTT	43
	POLG2-E1R2	CTG CT CAG GAA ACA GCT ATG ACC CCAGTTGCAATCCCCAAG	41
E2-3	POLG2-E2-3F	CTC GT TGT AAA ACG ACG GCC AGT CCACCAAGCTTAGCCAACAT	43
	POLG2-E2-3F3	CTC GT TGT AAA ACG ACG GCC AGT TAGGCGTGAGCCACCAAG	41
	POLG2-E2-3R	CTG CT CAG GAA ACA GCT ATG ACC ACACATCTGAGCCCAACAAA	43
E4	POLG2-E4F	CTC GT TGT AAA ACG ACG GCC AGT TGCTGAATAAAAAGTATCGGACAA	47
	POLG2-E4R	CTG CT CAG GAA ACA GCT ATG ACC GGCTCTGTGGCAATAGTGGA	43
E5	POLG2-E5F	CTC GT TGT AAA ACG ACG GCC AGT CCGTCTCAAAAACAAACAAACA	45
	POLG2-E5R2	CTG CT CAG GAA ACA GCT ATG ACC AGAAACCGAGCACACTAACATT	45
E6	POLG2-E6F	CTC GT TGT AAA ACG ACG GCC AGT CTGCCAAACATCTAAGTGGAGA	45
	POLG2-E6R	CTG CT CAG GAA ACA GCT ATG ACC TGAGTTCAAAGGAAATGCTCA	44
	POLG2-E6R2	AACTCACCTGTCTTAGTTCC	20
E7	POLG2-E7F	CTC GT TGT AAA ACG ACG GCC AGT TTGAAGAGGGTGATTTGTGG	43
	POLG2-E7R	CTG CT CAG GAA ACA GCT ATG ACC TTCTGTAAAAGGGAAACTGAGGA	46
E8	POLG2-E8F	CTC GT TGT AAA ACG ACG GCC AGT CCCTGAGTCTGTGACTCTTGA	44
	POLG2-E8R	CTG CT CAG GAA ACA GCT ATG ACC AATGAAAGCAAGCACCACAA	43

Figure Legends

Figure S1. Determination of the affinities of p55 variants for p140 ($K_{d(p140)}$). Representative p140-binding isotherms for wild-type and each of the p55 variants. Reactions (50 μl) contained 1 nM of p140 and varying amounts (0 – 20 nM) of dimeric p55 or variant at the fixed concentration of 220 mM NaCl. Reaction times were 10 minutes at 37^{o}C. On the Y-axis, pmol dTMP represents the amount of dTMP incorporated into the poly(rA)•oligo$(dT)_{12\text{-}18}$ (polymerase activity) as measured by counting TCA-insoluble radioactivity. Using the KaleidaGraph program each $K_{d(p140)}$ value was estimated from the quadratic curve fits by nonlinear regression analysis as described in Materials and Methods. Each version of p55 was measured at least twice and the average $K_{d(p140)}$ value ± the standard deviation is reported in Table 2.

Figure S2. Determination of the dissociation constants for DNA binding ($K_{d(DNA)}$) of p55 and p55 variants. Representative DNA-binding isotherms for wild-type and each of the p55 variants. Electrophoretic mobility shift assays were performed to estimate the $K_{d(DNA)}$ values using various concentrations of p55 or p55 variant (0 – 160 nM dimers) incubated separately with 15 nM of double stranded oligonucleotide in 20 μl binding reactions (Materials and Methods). The fraction of DNA bound is plotted against the concentration of p55 or variant dimers and binding isotherms were fit to a quadratic equation by nonlinear regression analysis using the KaleidaGraph program to estimate $K_{d(DNA)}$ values. Each version of p55 was measured at least twice and the average $K_{d(DNA)}$ value ± the standard deviation is reported in Table 2.


WT p55
G103S p55
L153V p55
P205R p55
R369G p55
D386E p55
dTMP (pmol)
p55 dimers (nM)

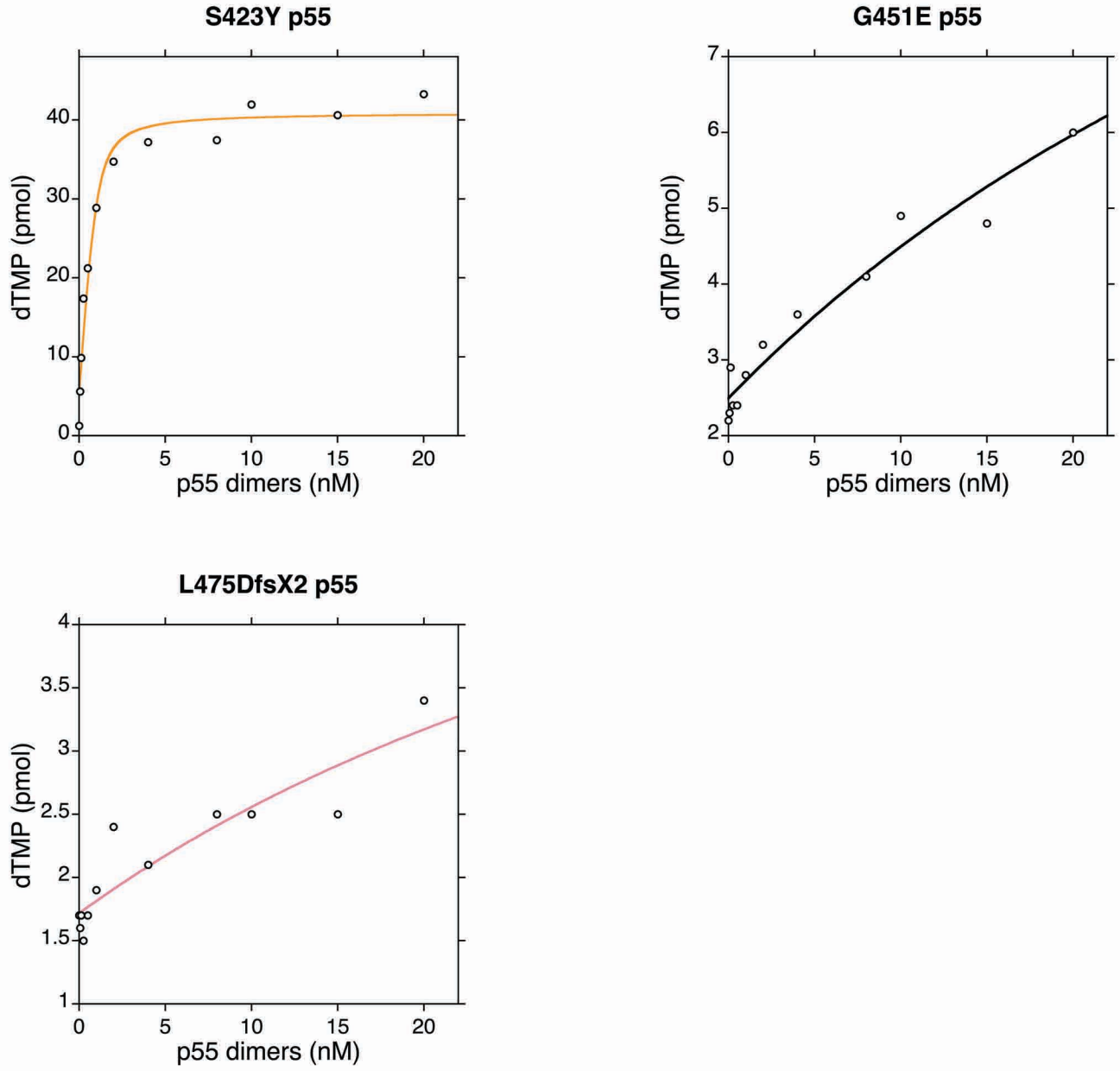
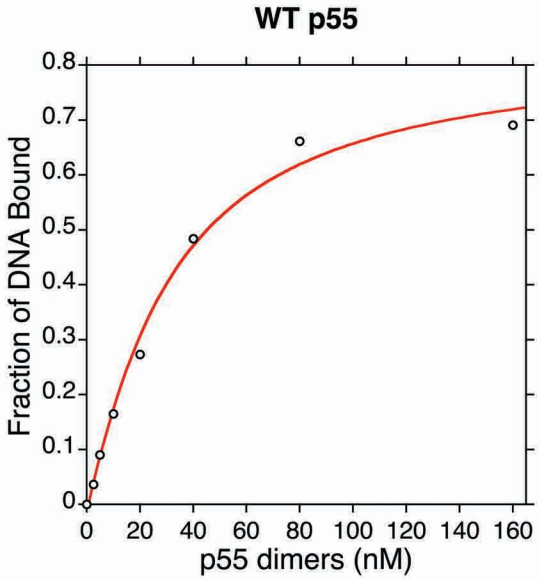
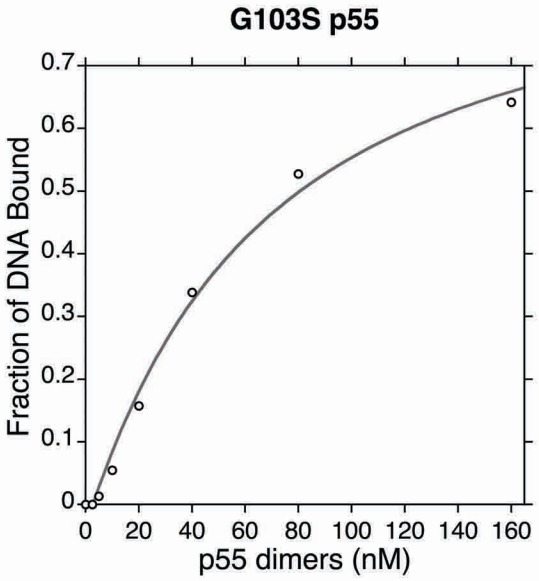

S423Y p55
dTMP (pmol)
p55 dimers (nM)
0
10
20
30
40
0
5
10
15
20
G451E p55
dTMP (pmol)
p55 dimers (nM)
2
3
4
5
6
7
0
5
10
15
20
L475DfsX2 p55
dTMP (pmol)
p55 dimers (nM)
1
1.5
2
2.5
3
3.5
4
0
5
10
15
20

Figure S1.


WT p55
Fraction of DNA Bound
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8
0 20 40 60 80 100 120 140 160
p55 dimers (nM)


G103S p55
Fraction of DNA Bound
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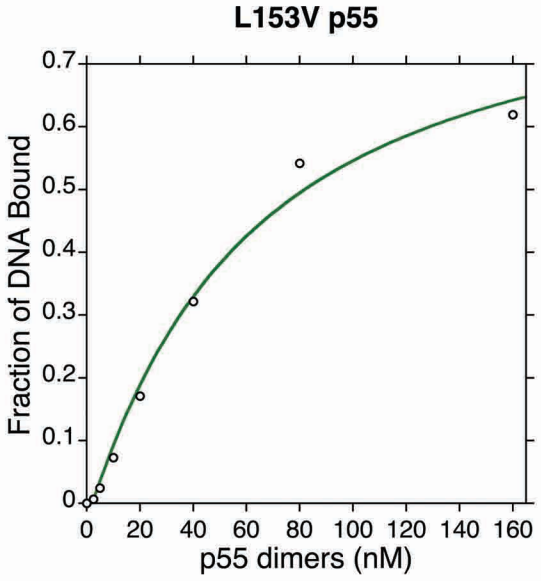
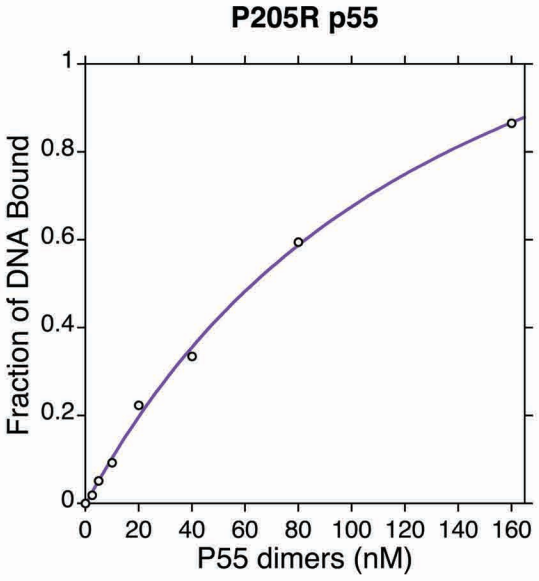
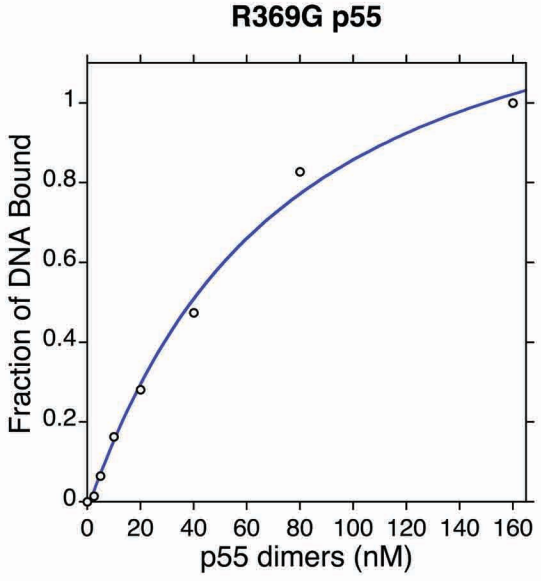
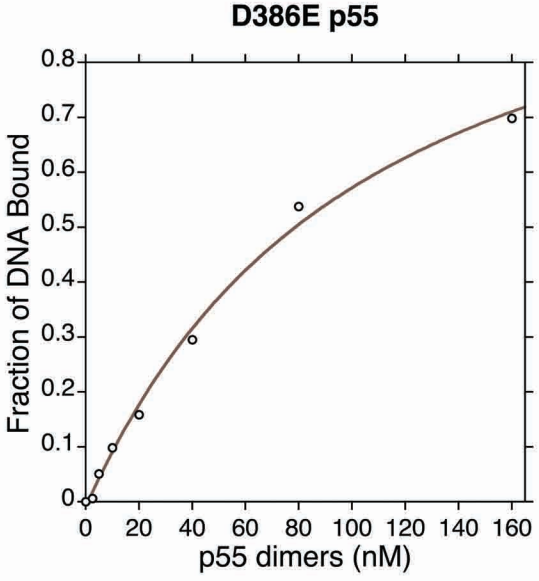
0 20 40 60 80 100 120 140 160
p55 dimers (nM)


L153V p55
Fraction of DNA Bound
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7
0 20 40 60 80 100 120 140 160
p55 dimers (nM)


P205R p55
Fraction of DNA Bound
0 0.2 0.4 0.6 0.8 1
0 20 40 60 80 100 120 140 160
P55 dimers (nM)


R369G p55
Fraction of DNA Bound
0 0.2 0.4 0.6 0.8 1
0 20 40 60 80 100 120 140 160
p55 dimers (nM)


D386E p55
Fraction of DNA Bound
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8
0 20 40 60 80 100 120 140 160
p55 dimers (nM)

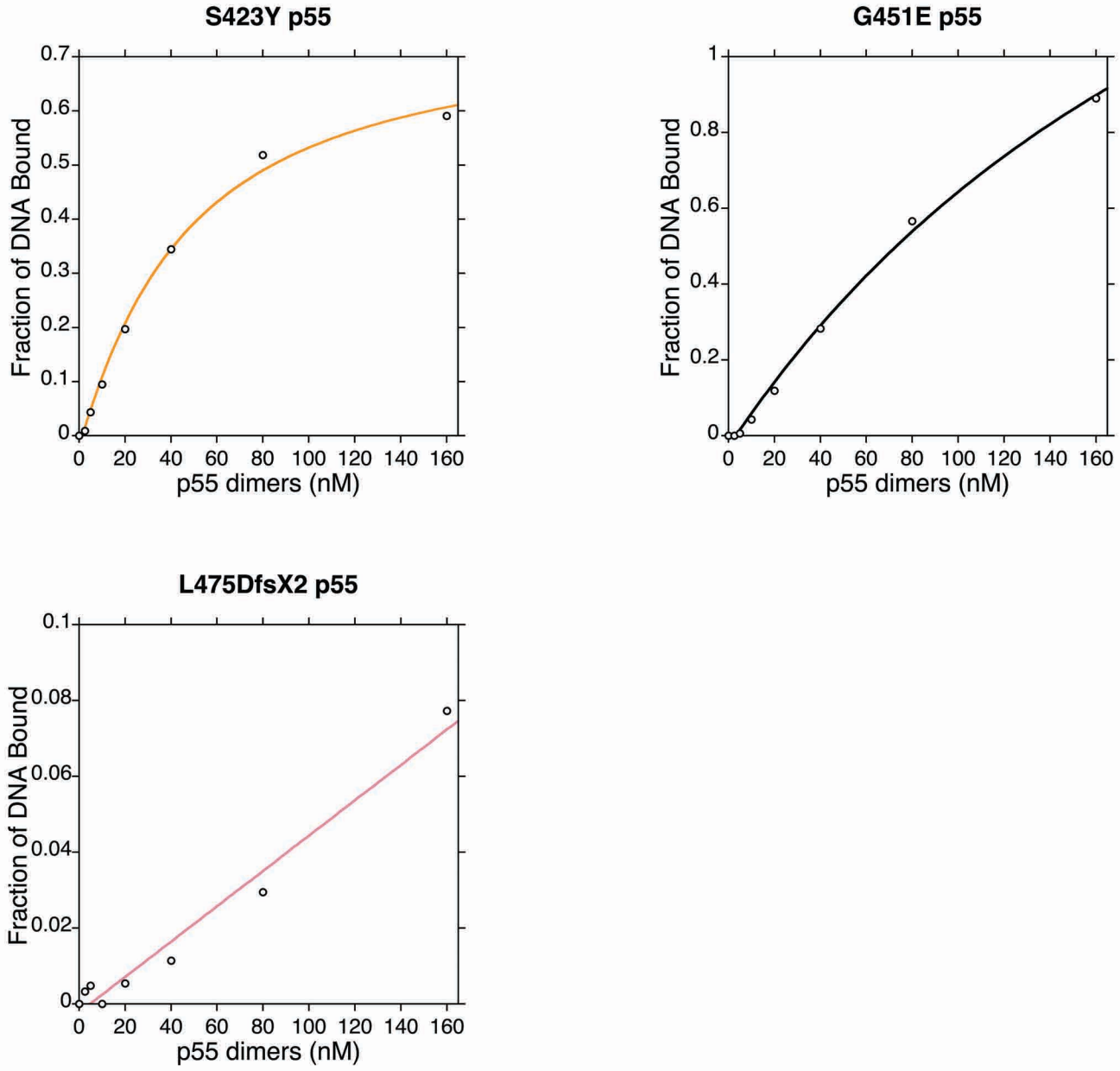

S423Y p55
Fraction of DNA Bound
p55 dimers (nM)
G451E p55
Fraction of DNA Bound
p55 dimers (nM)
L475DfsX2 p55
Fraction of DNA Bound
p55 dimers (nM)

Figure S2.