Supplementary Information

An absorbance method for critical analysis of enzymatic degradation kinetics of poly(ethylene terephthalate) films

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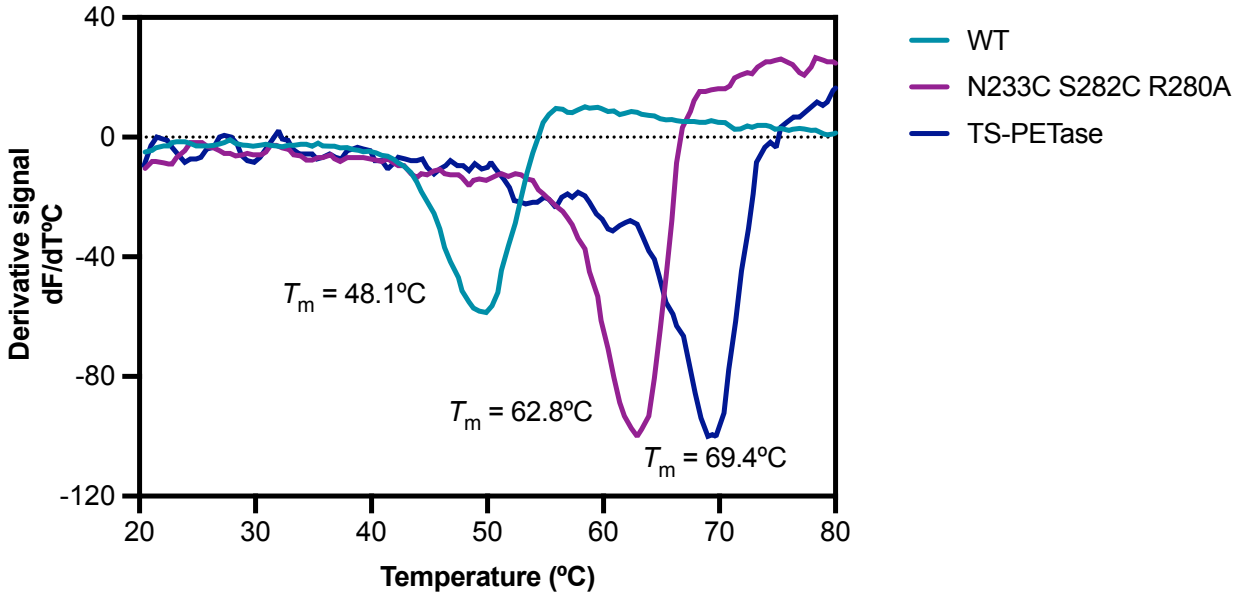
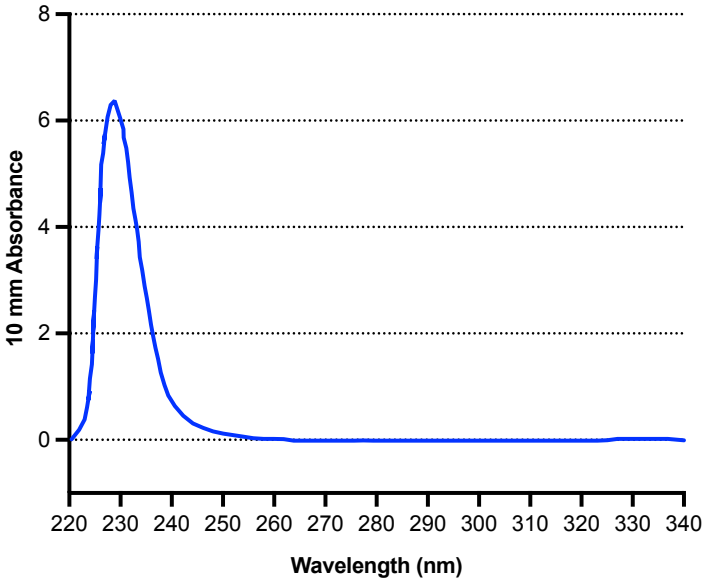
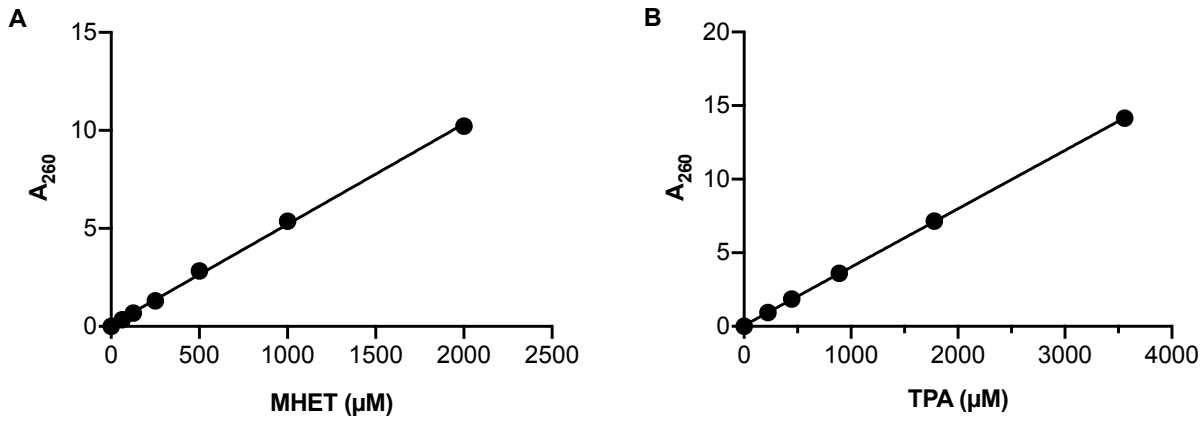


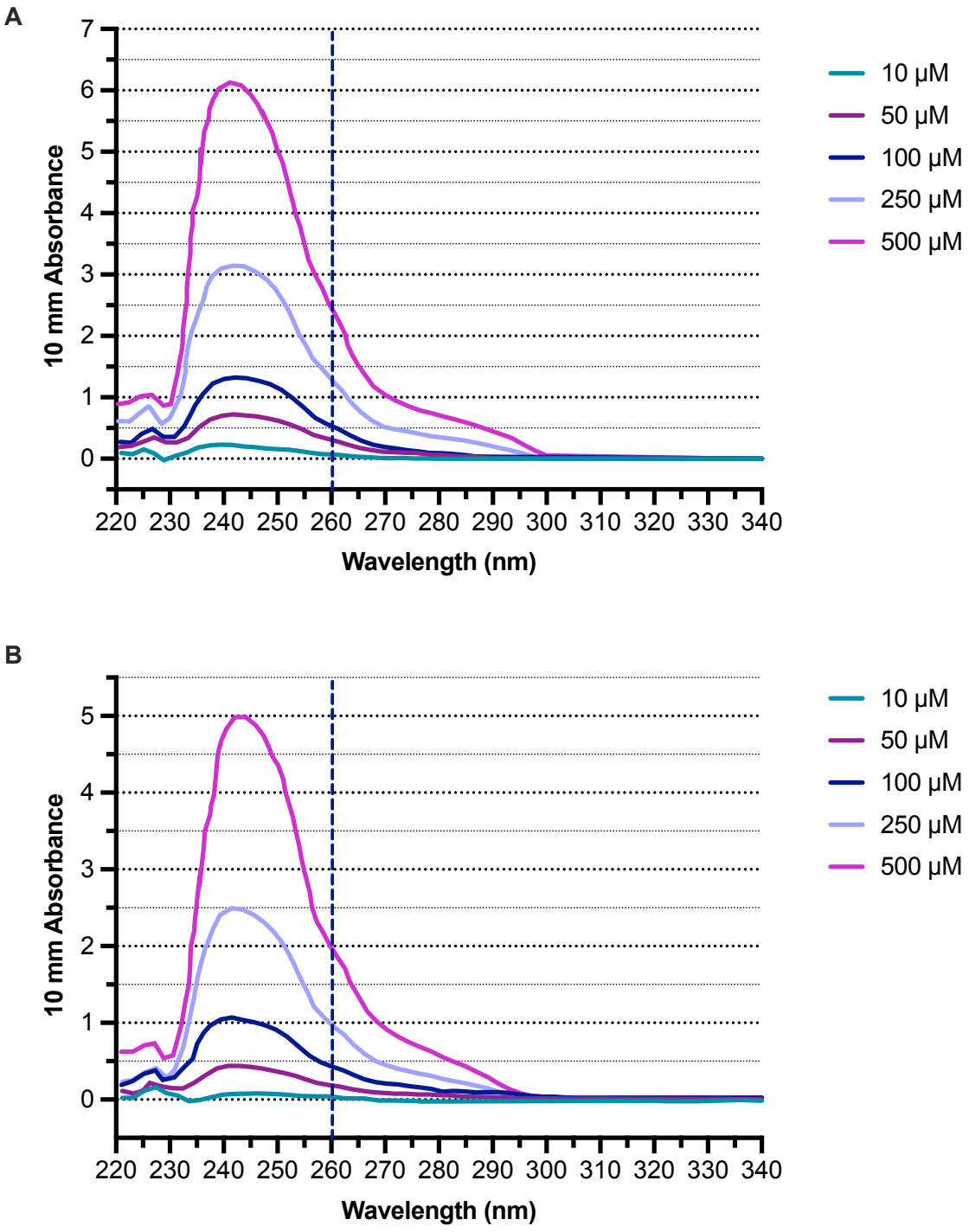
Supplementary Figure 1. First derivatives of denaturation curves of *Is*PETase variants as measured by differential scanning fluorimetry. Representative first derivatives of denaturation curves are shown. Average T_m is shown next to the curves for the various variants.



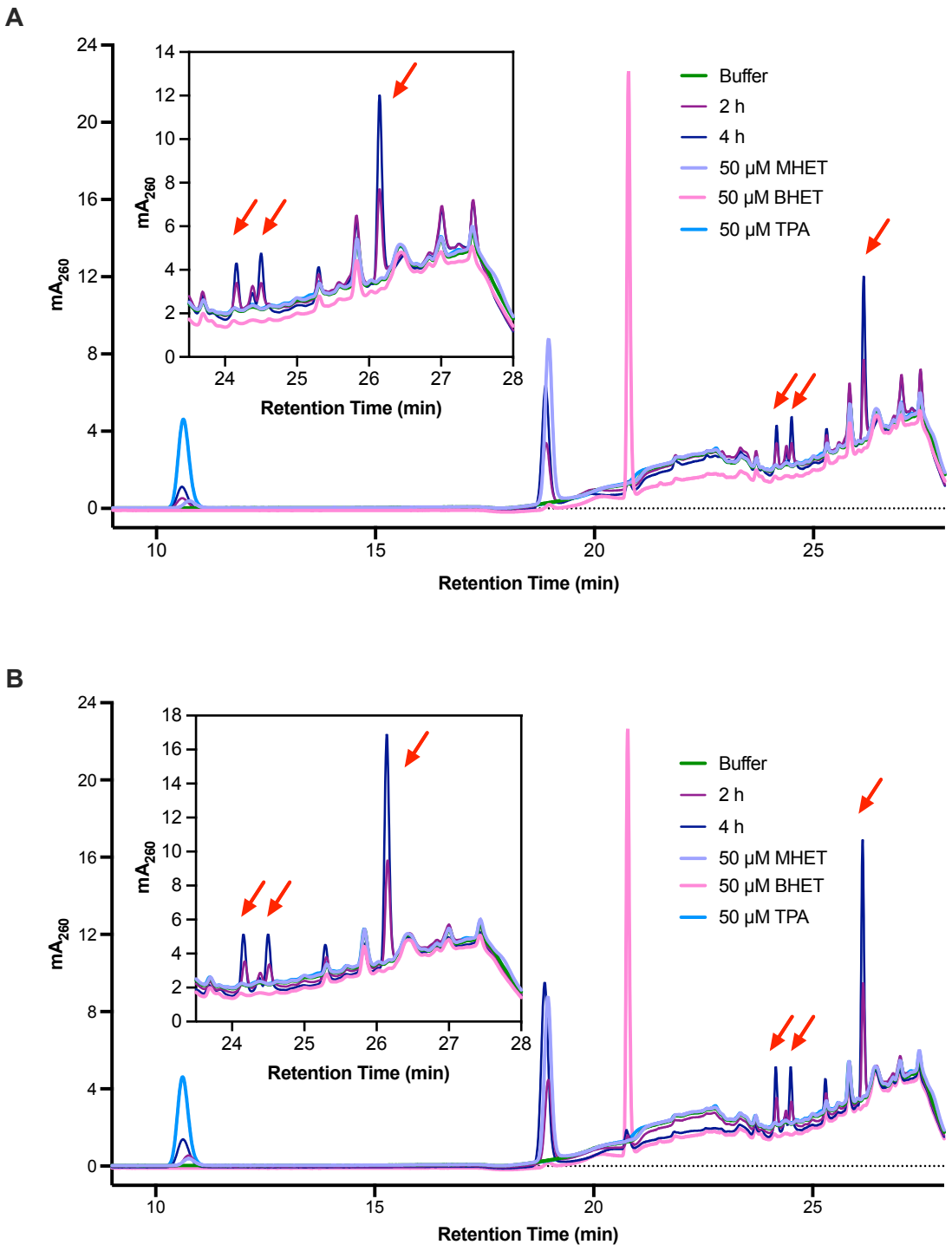
Supplementary Figure 2. Absorbance profile of DMSO in 50 mM glycine-NaOH pH 9, 50 mM NaCl, 10% DMSO (v/v). Instrument was blanked with 50 mM glycine-NaOH pH 9, 50 mM NaCl before taking the measurement.



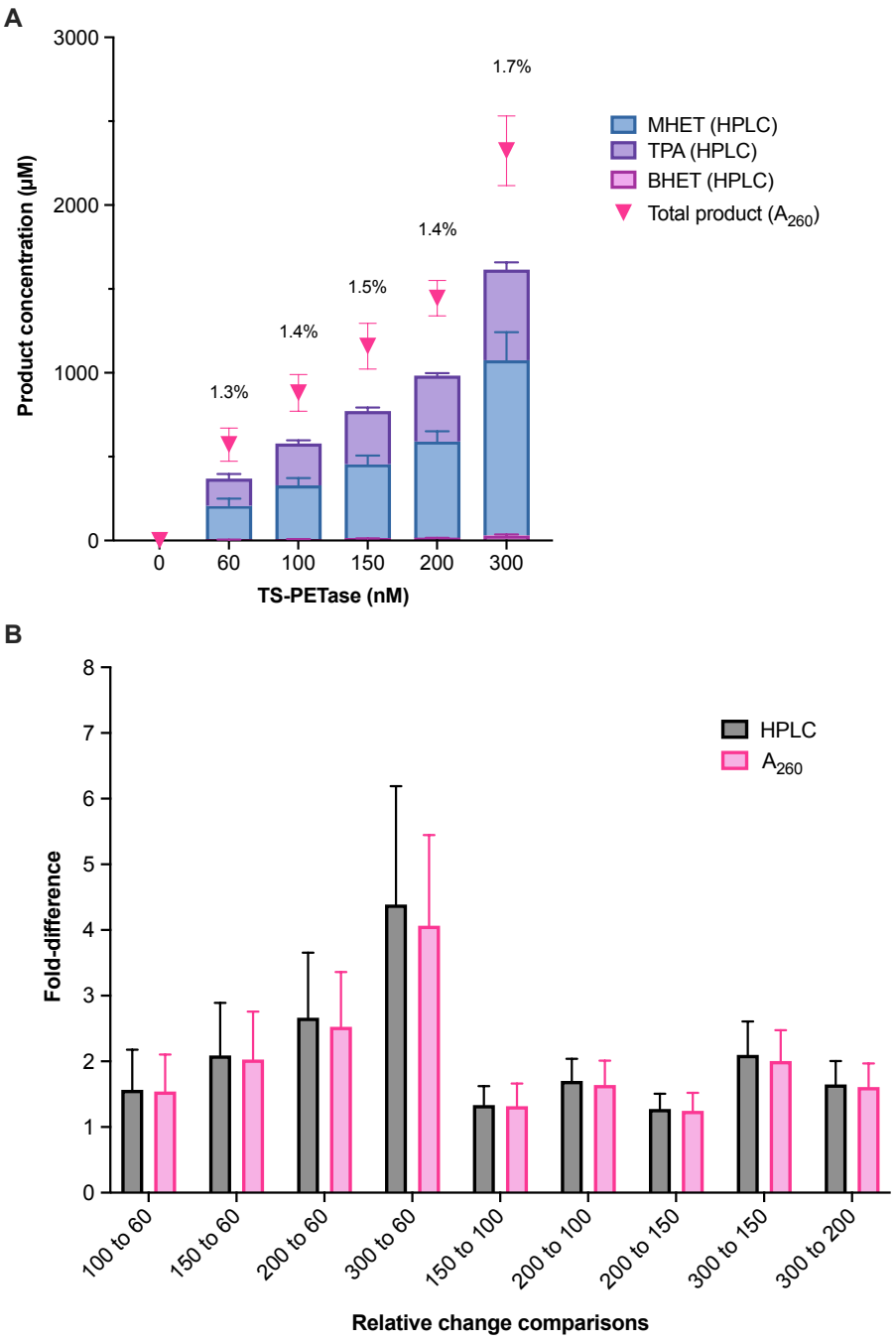
Supplementary Figure 3. Extended linear response profile of MHET and TPA as measured on the NanoDrop 1000 (ThermoFisher Scientific). Each point represents mean A_{260} at each concentration (n = 3) and error bars represent SEM. The absorbance values are based on a path length of 1 cm.



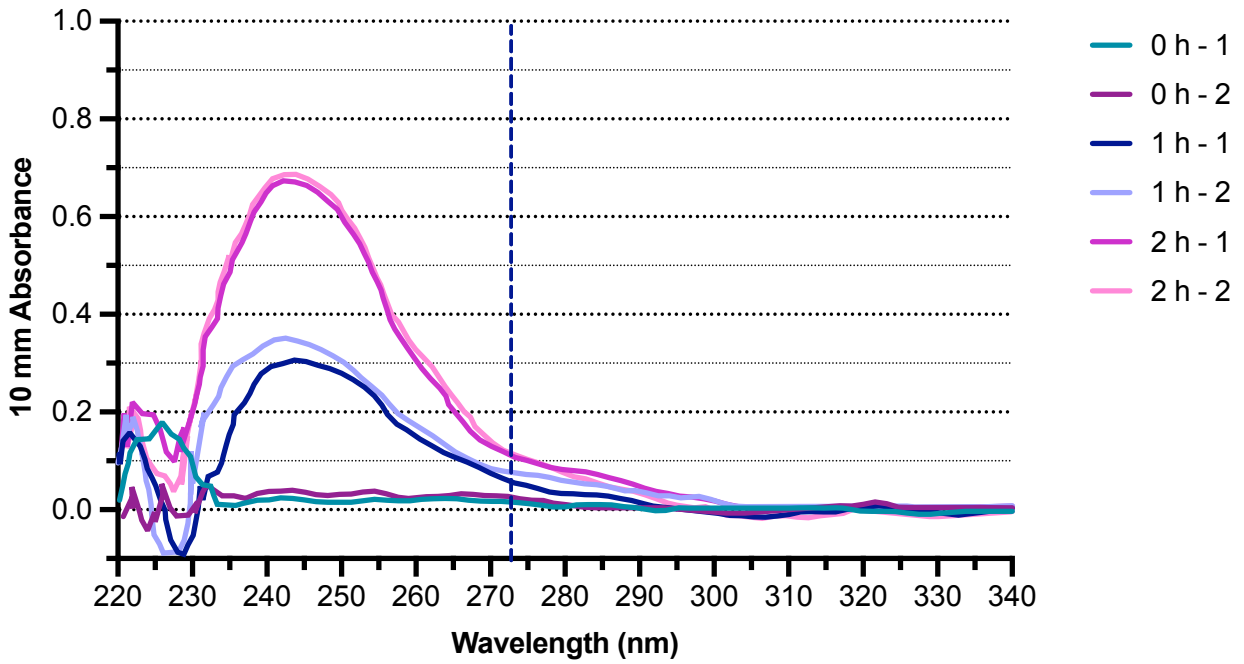
Supplementary Figure 4. UV-Absorbance profiles for MHET and TPA on NanoDrop 1000. Absorbance profiles of various concentrations of A) MHET and B) TPA used to generate standard curves. Absorbance is shown from 220 nm to 340 nm.



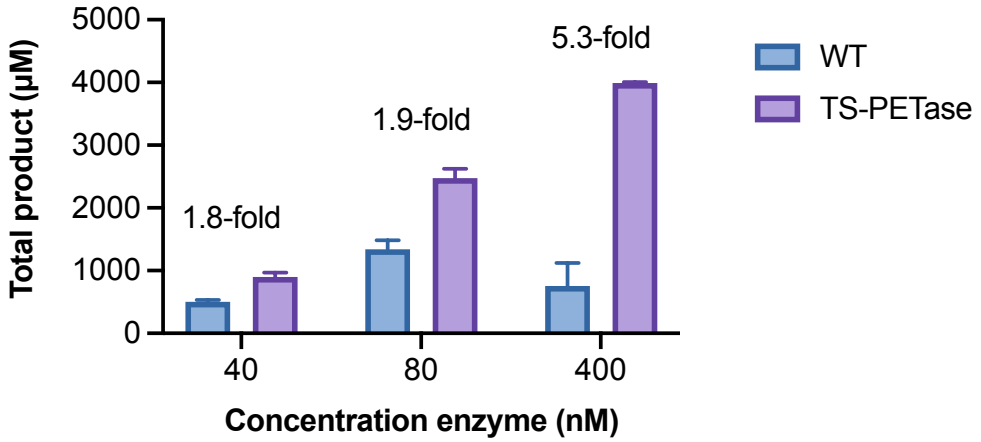
Supplementary Figure 5. HPLC curves of reaction supernatants from PET films incubated with 80 nM of *Is*PETase variants at 30ºC. HPLC curves of supernatants of A) wild-type *Is*PETase and B) TS-PETase incubated with PET film at various time points. The red arrows indicate the peaks that appear exclusively in enzyme reactions.



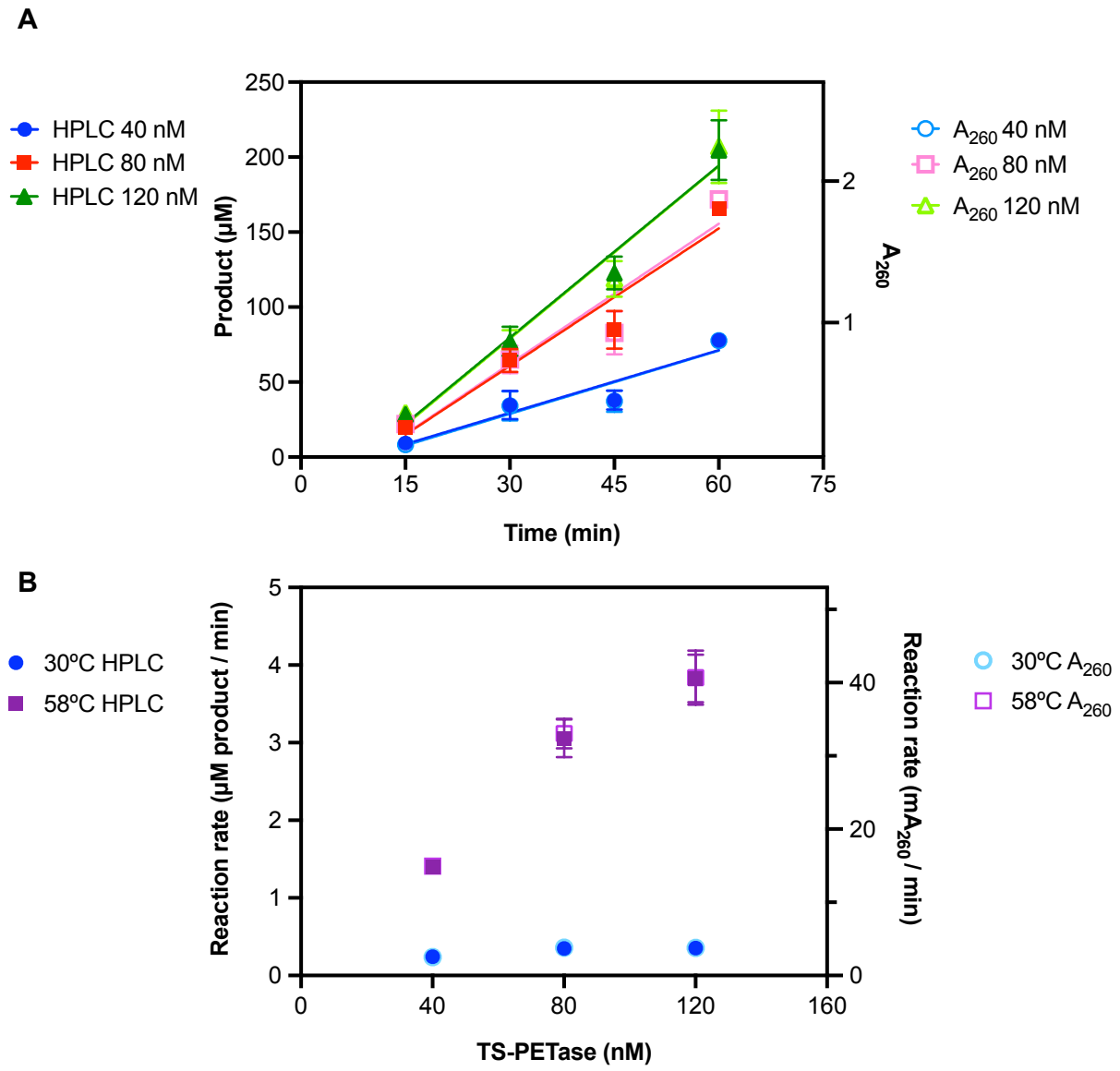
Supplementary Figure 6. Reaction supernatants of TS-PETase containing BHET. The reactions with PET film were performed at 58ºC and assayed at 24 hours. A) HPLC quantification and A_{260} estimation of total product concentrations ($n = 3$) using the MHET extinction coefficient. Error bars show SEM. Average percentage of BHET in the reaction is indicated above each bar. B) Fold-difference in total product accumulated between samples based on HPLC and A_{260} ($n = 3$ for each concentration). Fold-difference for "Concentration 1" to "Concentration 2" is calculated based on mean of "Concentration 1" divided by "Concentration 2". The error bars show standard deviation calculated using ratio distribution.



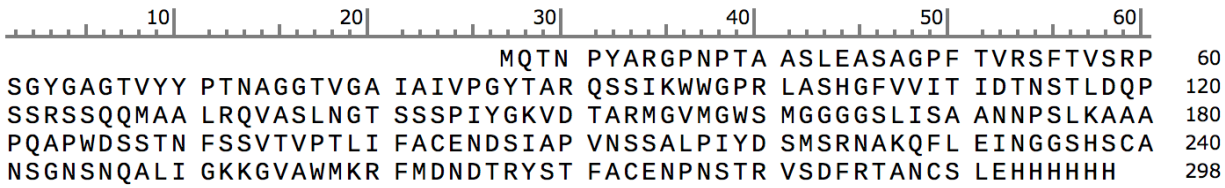
Supplementary Figure 7. Bulk absorbance profiles of reaction supernatants. PET films were incubated with 80 nM of *Is*PETase WT in duplicate (indicate as -1 and -2 on figure). Supernatant absorbance profiles were recorded at time 0, 1h, and 2h.



Supplementary Figure 8. HPLC quantification of products in day 6 enzyme reaction supernatants (30ºC). Absolute quantification of products in reaction supernatants ($n \geq 3$) for *Is*PETase WT and TS-PETase by HPLC. Fold-difference of total product concentrations between TS-PETase and WT are shown above each set of bars. Error bars represent SEM from two independent experiments.

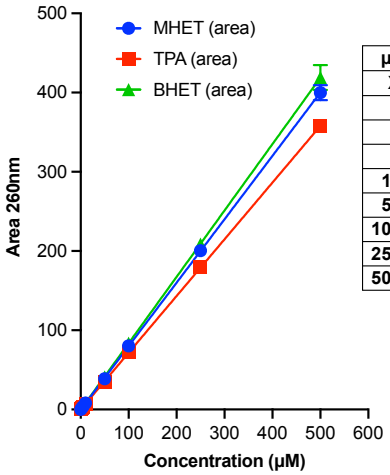


Supplementary Figure 9. Initial rates of TS-PETase as measured by bulk absorbance and HPLC. Filled symbols represent HPLC data, open symbols represent bulk absorbance data measured on the NanoDrop 1000 (path length = 1 cm). A) Initial rate curves interpolated from four time points (n = 3 per time point) for TS-PETase incubated with PET films at 58ºC. Each time point is measured in duplicate, and the supernatant of each reaction are measured by both HPLC and bulk absorbance (A_{260}). B) Rate vs. enzyme concentration profiles for three TS-PETase concentrations as measured by HPLC and bulk absorbance (A_{260}) at 30ºC and 58ºC. Error bars show SEM for both graphs.



Supplementary Figure 10. Protein sequence of mature *Is*PETase cloned into p21a. Sequence is numbered with respect to the start of the signal peptide.

A

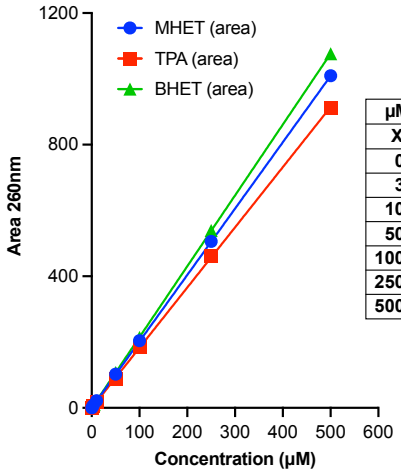


μM	MHET (area)		
X	A:Y1	A:Y2	A:Y3
0.0	0.0	0.0	0.0
3.0	2.5	2.3	2.5
5.0	4.3	4.1	3.8
10.0	8.1	7.6	8.6
50.0	38.2	39.7	38.6
100.0	80.9	79.9	79.9
250.0	207.6	200.3	193.8
500.0	417.7	396.6	385.3

μM	TPA (area)		
X	B:Y1	B:Y2	B:Y3
0.0	0.0	0.0	0.0
3.0	2.4	2.3	2.2
5.0	3.1	3.8	3.8
10.0	7.4	6.3	7.6
50.0	36.6	30.6	35.6
100.0	72.9	74.6	71.9
250.0	181.2	178.1	179.3
500.0	361.9	351.3	359.4

μM	BHET (area)		
X	C:Y1	C:Y2	C:Y3
0.0	0.0	0.0	0.0
1.0	2.1	2.0	1.9
2.5	5.2	5.4	4.9
5.0	10.9	10.1	9.8
10.0	22.4	20.5	22.3
50.0	113.2	103.3	106.3
100.0	223.8	210.5	208.0
250.0	557.0	538.2	522.5
500.0	1103.6	1058.9	1067.3

B



μM	MHET (area)		
X	A:Y1	A:Y2	A:Y3
0.0	0.0	0.0	0.0
3.0	6.3	7.2	6.8
10.0	20.9	21.6	22.3
50.0	101.4	102.8	103.8
100.0	204.3	202.2	205.9
250.0	502.3	504.6	511.4
500.0	1013.2	999.3	1015.5

μM	TPA (area)		
X	B:Y1	B:Y2	B:Y3
0.0	0.0	0.0	0.0
3.0	5.8	5.7	5.7
10.0	15.0	18.7	18.6
50.0	80.3	92.9	92.2
100.0	187.3	185.0	184.2
250.0	461.2	461.5	460.5
500.0	916.7	914.8	907.2

μM	BHET (area)		
X	C:Y1	C:Y2	C:Y3
0.0	0.0	0.0	0.0
1.0	2.1	2.0	1.9
2.5	5.2	5.4	4.9
5.0	10.9	10.1	9.8
10.0	22.4	20.5	22.3
50.0	113.2	103.3	106.3
100.0	223.8	210.5	208.0
250.0	557.0	538.2	522.5
500.0	1103.6	1058.9	1067.3

Supplementary Figure 11. HPLC standard curves for TPA, MHET, BHET (*n* = 3 for each concentration, error bars represent SEM). A) 2 μL injection standard curves. B) 5 μL injection standard curves. Tables show triplicate values for area under the A_{260} HPLC curves for all three standards.

Supplementary Table 1. HPLC quantification of TPA, MHET, and BHET in reaction supernatants in Figure 2D and Supplementary Figure 9

Variant and concentration	Temperature (ºC)	Time (minutes)	Replicate #	MHET (μM)	TPA (μM)	BHET (μM)
Wild-type 40 nM	30ºC	60	1	5.9	0.0	0.0
			2	5.7	0.0	0.0
			3	14.7	3.9	1.0
		120	1	14.4	4.0	1.0
			2	12.4	3.4	0.0
			3	24.4	6.6	1.8
		180	1	24.1	6.7	1.6
			2	23.2	6.6	1.7
			3	30.5	9.0	1.9
		240	1	27.7	8.6	1.8
			2	30.7	9.5	1.8
			3	5.9	0.0	0.0
Wild-type 60 nM	30ºC	60	1	6.3	0.0	0.0
			2	5.7	0.0	0.0
			3	4.5	0.0	0.0
		120	1	14.4	4.1	0.0
			2	15.5	4.5	0.0
			3	17.1	4.9	1.0
		180	1	25.9	7.5	1.4
			2	30.8	8.8	1.8
			3	26.6	7.8	1.5
		240	1	36.2	11.1	1.9
			2	37.7	11.6	2.1
			3	39.2	11.9	2.0
TS-PETase 40 nM	30ºC	60	1	7.6	0.0	0.0
			2	10.0	0.0	0.0
			3	6.5	0.0	0.0
		120	1	19.5	4.2	0.0
			2	22.6	4.9	0.0
			3	18.2	5.4	1.4
		180	1	36.1	8.0	2.5
			2	32.2	7.5	0.0
			3	25.4	6.0	1.9
		240	1	41.2	9.8	2.5
			2	37.6	9.7	2.4
			3	39.1	9.8	2.8
TS-PETase 80 nM	30ºC	60	1	12.7	0.0	0.0
			2	13.0	0.0	0.0
			3	6.6	0.0	0.0

		120	1	21.9	5.4	0.0
			2	28.7	5.7	0.0
			3	23.6	5.7	1.6
		180	1	47.7	10.9	2.7
			2	44.9	9.5	2.5
			3	33.4	8.3	2.1
		240	1	57.2	14.4	3.3
			2	53.7	13.6	3.2
			3	55.1	14.0	3.3
TS-PETase 120 nM	30ºC	60	1	11.0	0.0	0.0
			2	10.5	0.0	0.0
			3	10.5	6.3	0.0
		120	1	25.7	6.6	0.0
			2	28.0	6.3	0.0
			3	26.4	10.4	1.8
		180	1	39.0	9.4	0.0
			2	43.9	10.3	0.0
			3	40.7	16.8	2.2
		240	1	54.0	14.2	3.0
			2	58.7	15.6	2.9
			3	62.7	16.8	2.9
TS-PETase 40 nM	58ºC	15	1	10.9	0.0	0.0
			2	10.1	0.0	0.0
			3	7.0	0.0	0.0
		30	1	35.5	4.6	3.3
			2	36.6	5.0	3.1
			3	14.5	0.0	1.3
		45	1	32.5	5.2	2.6
			2	38.6	5.9	3.2
			3	21.1	3.2	1.7
		60	1	61.1	8.7	5.7
			2	62.5	8.8	5.6
			3	65.9	10.2	5.2
TS-PETase 80 nM	58ºC	15	1	22.4	0.0	0.0
			2	18.5	0.0	0.0
			3	16.8	0.0	1.3
		30	1	58.1	7.3	5.3
			2	60.4	8.0	5.1
			3	40.1	5.7	3.4
		45	1	55.9	8.8	4.2
			2	61.2	10.0	4.7
			3	89.1	14.0	6.7
		60	1	128.7	18.7	10.7
			2	134.2	19.7	10.6
			3	141.7	20.7	11.7

TS-PETase 120 nM	58ºC	15	1	28.2	3.5	2.4
			2	25.4	3.4	0.0
			3	18.5	2.9	1.4
		30	1	63.7	10.1	6.8
			2	74.1	11.0	6.8
			3	47.9	7.3	3.9
		45	1	82.9	13.6	5.8
			2	112.8	19.0	8.1
			3	101.4	17.3	7.1
		60	1	179.8	25.0	13.1
			2	190.3	26.4	14.0
			3	132.7	23.9	8.9

Supplementary Table 2. Cloning primer sequences

Primer Name	Primer Sequence
*Is*PETase F	TTTAAGAAGGAGATATACATATGCAGACCAATCCGTATGCA
*Is*PETase R	TGGTGGTGGTGGTGCTCGAGGCTACAATTTGCGGTACGAAAA
p21a F	CTCGAGCACCACCAC
p21a R	ATGTATATCTCCTTCTTAAAGTTAAACA
R280A F	CCGAATAGCACCGCTGTTAGCGATTTTCGTACCGCAAATT
R280A R	CGCTAACAGCGGTGCTATTCGGATTTTCGCAGGCAAAG
D186H F	CTCCGTGGCATAGCAGCACCAATTTTAGCAGCGTTACCGTTCCGAC
D186H R	GTGCTGCTATGCCACGGAGCCTGCGGTGCG
N233C F	GTTTCTGGAAATTTGTGGTGGCAGCCATAGCTGTGCAAATAGC
N233C R	ATGGCTGCCACCACAAATTTCCAGAAACTGTTTTGCATTACGG
R280A S282C F	AAAATCCGAATAGCACCGCTGTTTGTGATTTTCGTACCGCAAATTG TAGCCTCGAG
R280A S282C R	CGGTACGAAAATCACAAACAGCGGTGCTATTCGGATTTTCGCAGGC AAAGGTGCT
S121E F	CACCCTGGATCAGCCGGAAAGCCGTAGCAGTCAGCAGATGGCAGC ACTGCGTCAGGTTGC
S121E R	CATCTGCTGACTGCTACGGCTTTCCGGCTGATCCAGGGTGCTGTTG GTATCAATGGTAAT