Electronic Supplementary Material

Fluorescence of various buried fresh and fresh-frozen-thawed tissue types up until the point of active decay: A human taphonomy study

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A: Donor characteristics

Table S1. *Donor sex and age in years at time of death*

Donor number (arbitrary)	Sex	Age at time of death (years)
1	M	97
2	F	86
3	M	72
4	F	85
5	F	67
6	M	79
7	M	65
8	M	66
9	F	77
10	M	78
11	M	66

M: male; F: female

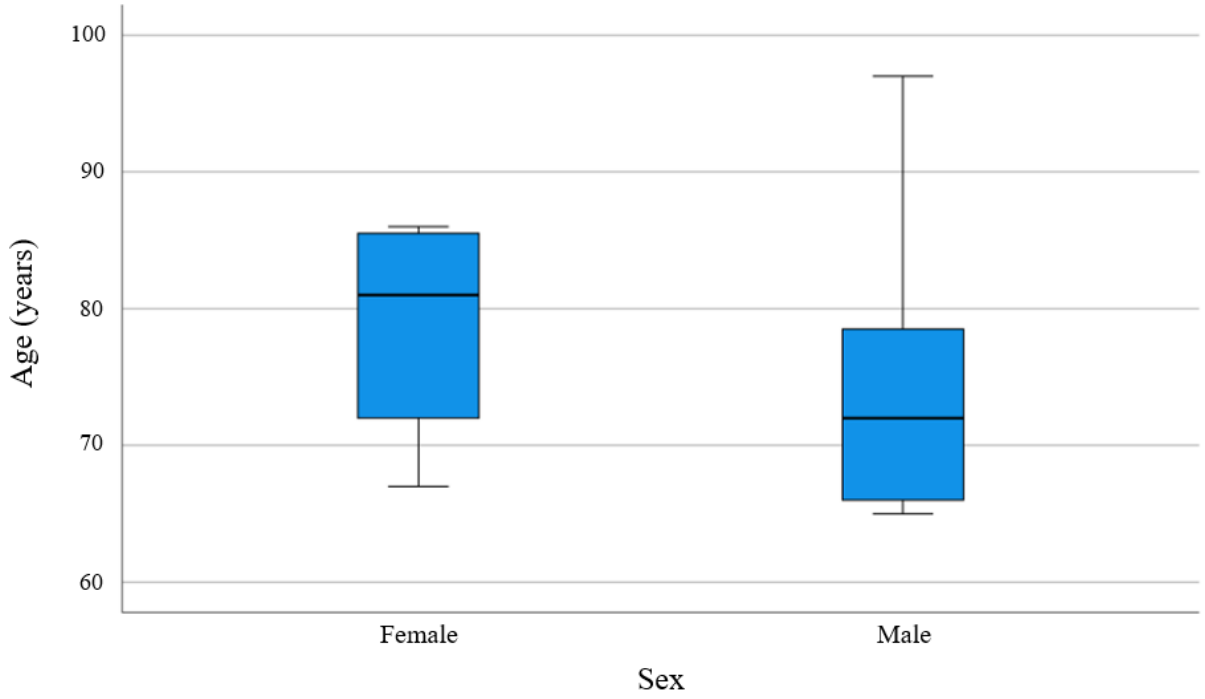


Figure S1. Boxplot of donor age (in years) grouped by sex

B: Supplementary water provided to burial pits

Table S2. *Supplementary water provided to burial pits*

Date of watering (dd/mm/yyyy)	Rainfall (mm)*	Area watered (m^2)	Amount of water (L)	Pits watered (treatment group/time point in days)
20/09/2022	2.1	0.49	5.5	FFR/all
22/09/2022	0	1	6	FFR/4, 6, 9, 14, 20, 24
04/10/2022	0	1	6	FFR/20, 24
07/10/2022	0	1	7	FFR/20, 24
12/10/2022	0	1	7	FFR/24
03/11/2022	3.4	1	6	F/24
11/11/2022	0	1	7	F/all
14/11/2022	0	1	7	F/all
05/12/2022	0.7	0.47**	1.5	F/all
09/12/2022	0.1	0.23**	0.6	F/24

* Rainfall measurements sourced from [1]

** Areas based on the dimensions of the plastic bins which contained the F hands following relocation.

C: Exhumation scheme

Table S3. *Date of interment and exhumation for each hand pair*

Date interred (dd/mm/yyyy)	Date exhumed (dd/mm/yyyy)	Time point (days)	ADD	Treatment group
20/09/2022	22/09/2022	2	31.7	FFR
20/09/2022	24/09/2022	4	58.7	FFR
20/09/2022	26/09/2022	6	82.8	FFR
20/09/2022	29/09/2022	9	115.6	FFR
20/09/2022	04/10/2022	14	179.9	FFR
20/09/2022	10/10/2022	20	257.6	FFR
20/09/2022	14/10/2022	24	300.4	FFR
03/11/2022	12/12/2022	24	340.4	F
11/11/2022	09/12/2022	14	196.9	F

D: Hand sampling diagram

Blank diagrams obtained from Lameira et al. [2].

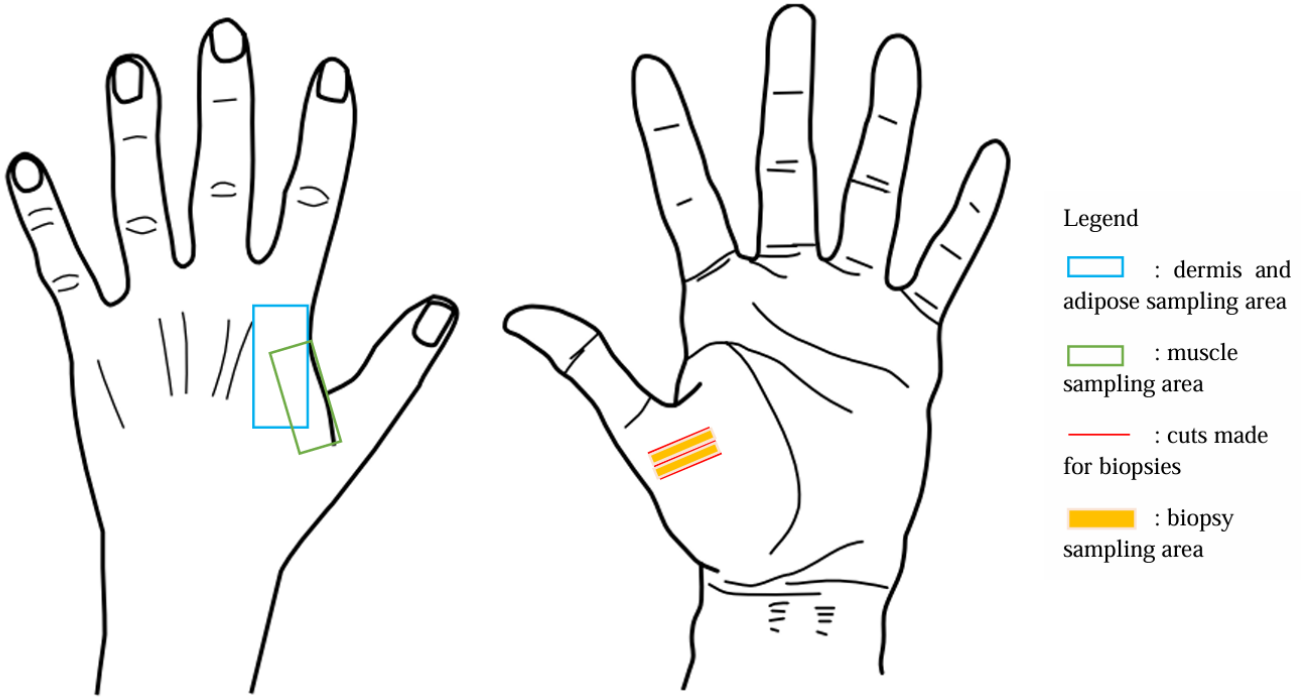


Figure S2. Diagram of tissue sampling areas on hands

E: LS55 fluorescence spectrometer measurement settings

Table S4. *LS55 settings for fluorescence measurements by tissue type*

Tissue type	Excitation slit (nm)	Emission slit (nm)	Gain
Skin	15	20	Medium
Adipose	15	12	Medium
Muscle	15	12	Medium

Excitation wavelengths were chosen based on the recommendations of [3].

Table S5. *LS55 settings for PROT and FOX fluorescence measurements*

Measurement type	Excitation wavelength (nm)	Emission range (nm)	Step size (nm)	Scanning speed (nm/min)
PROT	285	300 – 500	0.5	300
FOX	370	400 – 600	0.5	300

EEM measurements utilized the same slit sizes and gain as individual PROT-FOX measurements, with a scanning speed of 1 500 nm/min, and a range of 230 nm to 600 nm for excitation wavelengths, and 230 nm to 700 nm for emission wavelengths. Step sizes of 5 nm and 0.5 nm were used for excitation and emission wavelengths respectively.

F: Procedure for fluorescence measurements and data analysis using an ND filter

Fluorescence measurements

In case of oversaturation of peaks, a neutral density (ND) filter with optical density (OD) 0.1 was used to evenly reduce the amount of light picked up by the LS55, and provide non-saturated readings using the same settings. In these instances, a white diffuse reflectance standard tile was used to establish a spectrum of transmission for the ND filter. This was achieved by placing the white tile under the optic fiber of the LS55, and measuring its reflection at an excitation wavelength of 330 nm, with an emission range of 300 nm to 400 nm, a step size of 0.5 nm, and scanning speed of 300 nm/min. The slit sizes used were 2.5 nm for both excitation and emission slits. Care was taken to ensure that the signal was not saturated. This process was repeated after placing the ND filter in front of the detector, and taking another measurement. The ND filter was then left in place, and the white tile removed and replaced by a sample of interest, with the optical fiber 0.5 mm above the sample. Fluorescence was then measured using the settings established for PROT or FOX measurements as needed depending on which measurement was saturated. The complement PROT or FOX measurement was then taken after removal of the ND filter.

Data analysis

For a given area on a sample where an ND filter had been used, the spectra obtained with the reflectance tile plus the ND filter, and the reflectance tile alone, were entered into MATLAB® and divided by each other to produce a transmission spectrum for the ND filter. This spectrum was then used to scale fluorescence measurements prior to calculation of the various AUCs and PROT-FOX ratio.

G: Emission wavelength thresholds for calculating PROT and FOX AUCs

To set thresholds for PROT and FOX AUC calculations, measurements were plotted in Microsoft Excel, and the wavelength range which captured the emission maxima and adjacent regions for the largest number of measurements for time points 0 and 24 was determined, within a given tissue type and treatment group. These were then used to obtain the threshold which would capture the emission maxima and adjacent regions for the majority of measurements for that tissue type.

In the example below, the threshold for PROT measurements in adipose was set by identifying the range that captured the emission maxima and adjacent regions for time points 0 and 24, for F and FFR adipose, individually. The overall lowest and highest limits, 309.5 nm in FFR time point 24, and 349 nm in F time point 0 respectively, were then used as the thresholds for PROT adipose measurements, as this range ensured that the emission maxima and immediate regions of most measurements would be accounted for.

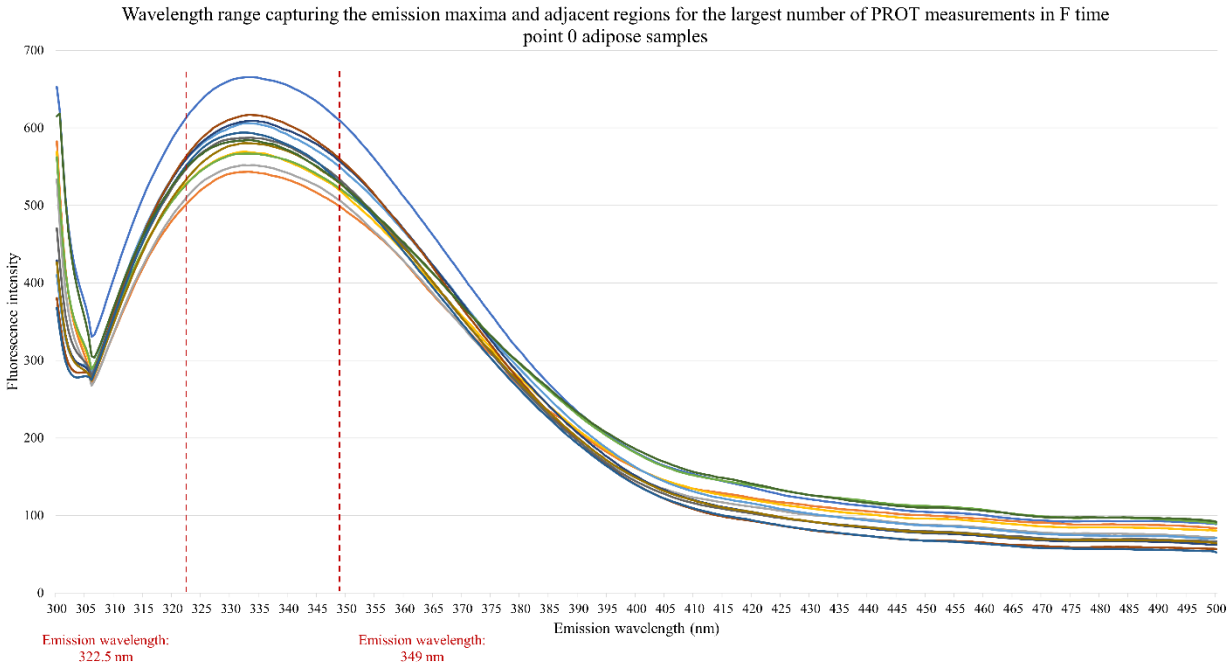


Figure S3. Line graph displaying the wavelength range capturing the emission maxima and adjacent regions for the largest number of PROT measurements in F time point 0 adipose samples

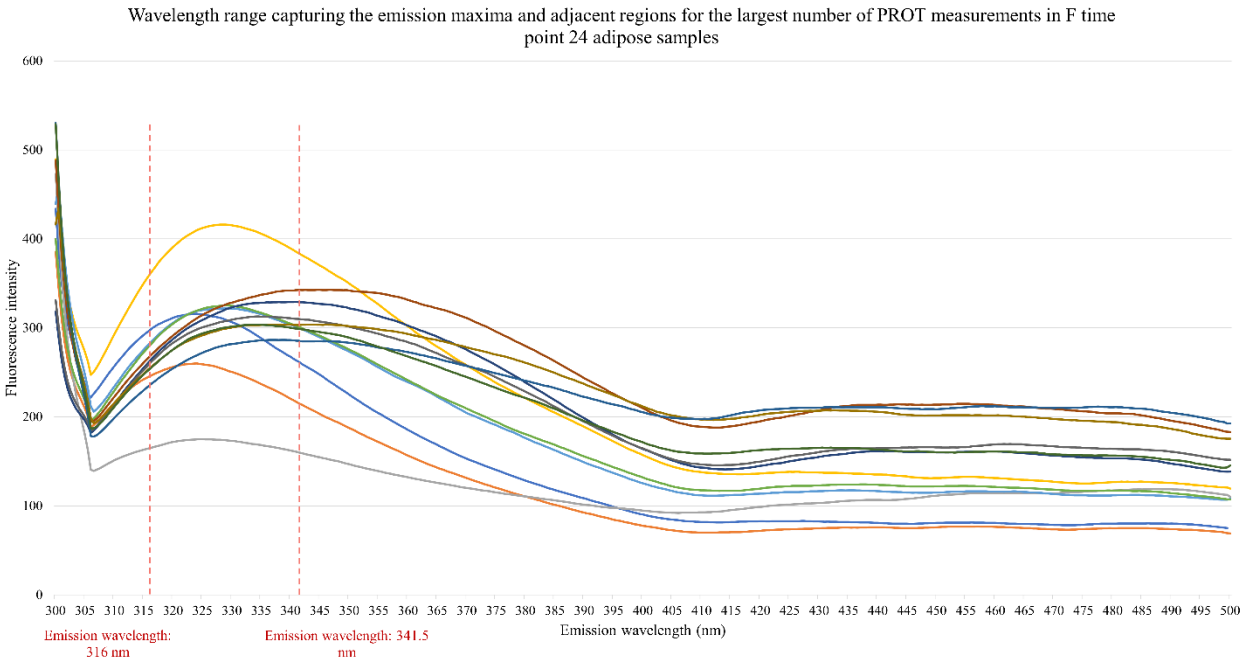


Figure S4. Line graph displaying the wavelength range capturing the emission maxima and adjacent regions for the largest number of PROT measurements in F time point 24 adipose samples

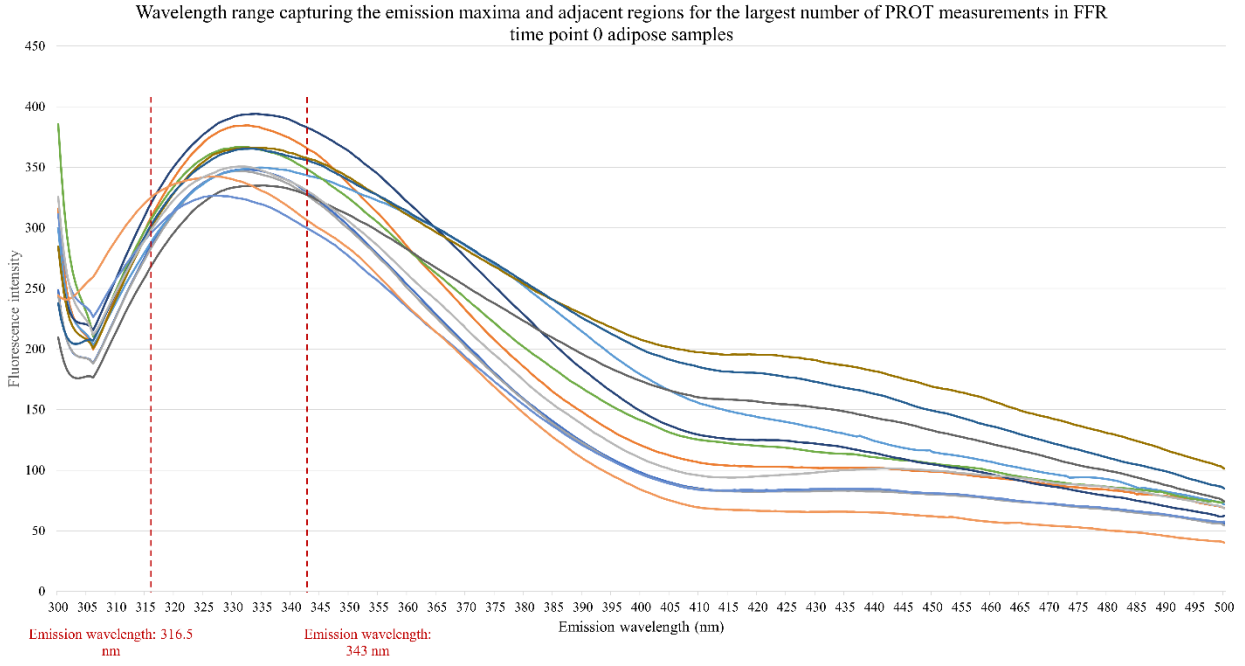


Figure S5. Line graph displaying the wavelength range capturing the emission maxima and adjacent regions for the largest number of PROT measurements in FFR time point 0 adipose samples

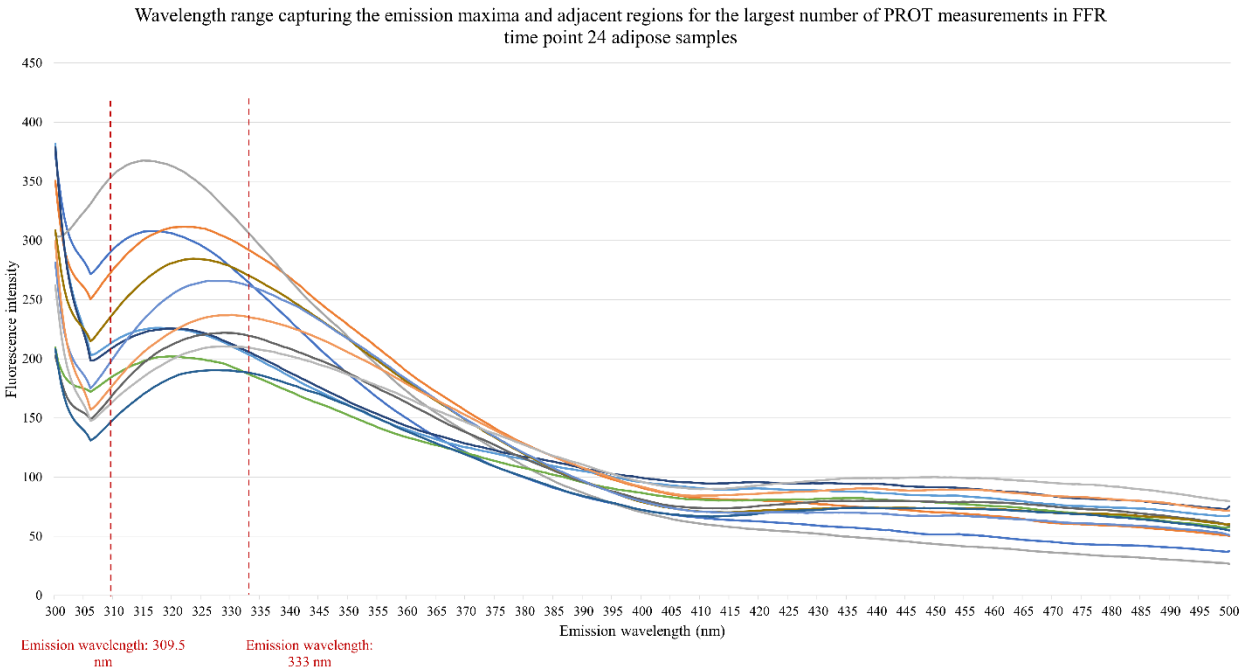


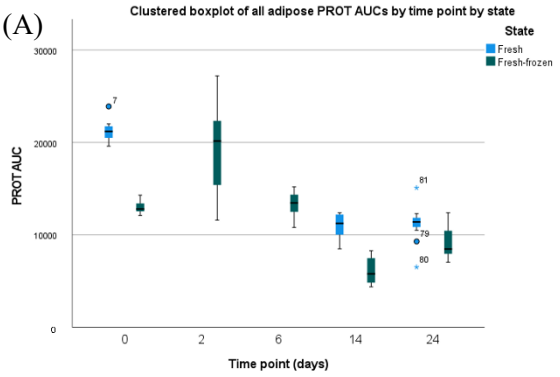
Figure S6. Line graph displaying the wavelength range capturing the emission maxima and adjacent regions for the largest number of PROT measurements in FFR time point 24 adipose samples

Table S6. *Thresholds for PROT and FOX measurements by tissue type*

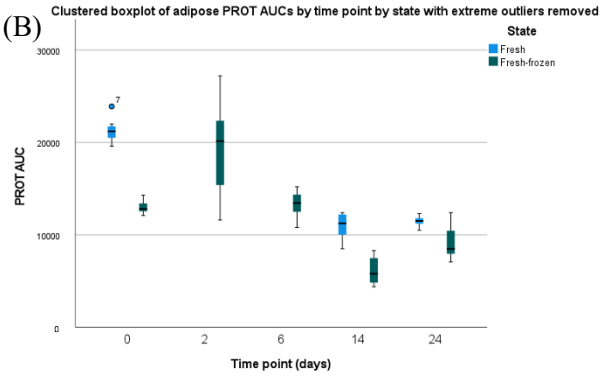
Tissue type	**Measurement type**	**Emission wavelength thresholds (nm)**
Skin	PROT	313.5 – 353.5
Skin	FOX	427.5 – 498.5
Adipose	PROT	309.5 – 349.0
Adipose	FOX	430.0 – 472.5
Muscle	PROT	313.5 – 348.0
Muscle	FOX	430.5 – 484.5

H: Example of statistical analysis carried out in SPSS®

(A)



(B)



(C)

Shapiro-Wilk test of normality for FFR adipose PROT AUCs over time

	ADD	Statistic	SW df	Sig.
AUCPROT	0.00	.942	12	.529
	31.7	.976	12	.964
	58.7	.955	12	.710
	179.9	.874	11	.088
	300.4	.926	12	.340

(D)

Levene test of homogeneity of variance for FFR adipose PROT AUCs over time

		Levene statistic	df1	df2	Sig.
AUCPROT	Based on Mean	9.456	4	54	<.001
	Based on Median	7.422	4	54	<.001
	Based on Median and with adjusted df	7.422	4	18.545	<.001
	Based on trimmed mean	9.399	4	54	<.001

(E

Welch's robust test of equality of means for FFR adipose PROT AUCs over time

AUCPROT

	Statistic[a]	df1	df2	Sig.
Welch	63.797	4	25.090	<.001

a. Asymptotically F distributed.

(

Post hoc Games-Howell test for FFR adipose PROT AUCs over time

Dependent Variable: AUCPROT

	(I) Time point (days)	(J) Time point (days)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Games-Howell	0	2	-6491.66667	1348.02278	.004	-10820.2358	-2163.0976
		6	-358.33333	439.51735	.922	-1704.1288	987.4621
		14	6835.30303	485.78484	<.001	5317.7465	8352.8595
		24	3848.33333	526.51681	<.001	2213.9577	5482.7090
	2	0	6491.66667	1348.02278	.004	2163.0976	10820.2358
		6	6133.33333	1390.84307	.005	1749.2803	10517.3864
		14	13326.96970	1406.14930	<.001	8918.8790	17735.0604
		24	10340.00000	1420.73533	<.001	5909.0083	14770.9917
	6	0	358.33333	439.51735	.922	-987.4621	1704.1288
		2	-6133.33333	1390.84307	.005	-10517.3864	-1749.2803
		14	7193.63636	594.36180	<.001	5418.4879	8968.7848
		24	4206.66667	628.09155	<.001	2335.9144	6077.4189
	14	0	-6835.30303	485.78484	<.001	-8352.8595	-5317.7465
		2	-13326.96970	1406.14930	<.001	-17735.0604	-8918.8790
		6	-7193.63636	594.36180	<.001	-8968.7848	-5418.4879
		24	-2986.96970	661.29448	.002	-4957.3747	-1016.5647
	24	0	-3848.33333	526.51681	<.001	-5482.7090	-2213.9577
		2	-10340.00000	1420.73533	<.001	-14770.9917	-5909.0083
		6	-4206.66667	628.09155	<.001	-6077.4189	-2335.9144
		14	2986.96970	661.29448	.002	1016.5647	4957.3747

Figure S7. Procedure for testing relationship significance of PROT AUCs over time in FFR adipose in SPSS®: (A) Clustered boxplot of FFR adipose PROT AUCs by time point and treatment (extreme outliers depicted as stars, outliers shown as circles). (B) Clustered boxplot of FFR adipose PROT AUCs by time point and treatment with extreme outliers removed. (C) Shapiro-Wilk test of normality for FFR adipose PROT AUCs over time. P-values (column circled in red) are used to determine normality ($p > 0.05$ data is normal, $p < 0.05$ data deviates from a normal distribution). (D) Levene test of homogeneity of variance for FFR adipose PROT AUCs over time. P-value (circled in red) is used to determine homogeneity of variance ($p > 0.05$ assumption for homogeneity of variance is met, $p < 0.05$ assumption for homogeneity of variance is not met). (E) Welch's ANOVA statistic for FFR adipose PROT AUCs over time. P-value (circled in red) is used to determine statistical significance ($p < 0.05$ a significant difference between group means exists, $p > 0.05$ a significant difference between group means does not exist). (F) *Post hoc* Games-Howell test for FFR adipose PROT AUCs over time. P-values (column circled in red) are used to evaluate the significance of relationships between multiple groups tested using the Welch's ANOVA ($p < 0.05$ a significant difference exists between group means, $p > 0.05$ there is no significant difference between group means).

I: Method for identifying fluorophores in EEMs

To identify fluorophore peaks in EEMs, ranges had to be defined to assess which peaks were present or absent. Fluorescence ranges were derived from the excitation/emission wavelengths observed for the central coordinates of each independent contour of highest fluorescence for a given region at time point 0 of EEMs, for each tissue type and state (see Figs. S8-S9 below for an illustration of this method). The only exception is the FOX peak, where time point 24 EEMs were used to define its range. The median value for the excitation and emission wavelengths respectively for each peak were used to define the ranges. For excitation wavelengths, the range extended ± 10 nm around the median, while for emission wavelengths, it extended ± 50 nm from the median. When needed, the excitation wavelength's median was rounded to the nearest multiple of 5, reflecting the measurement's 5 nm step size, or rounded up to the nearest 0.5 for the emission wavelength, corresponding to the measurement's 0.5 nm step size.

Endogenous fluorophores with optimal wavelengths within these ranges were assigned as potential contributors to each observed peak, and arbitrary names were assigned to these peaks as multiple fluorophores could be contributing to the fluorescence of one peak.

Peak presence/absence in each EEM was determined based on whether coordinates within the independent contour of a peak fell within one of the ranges defined.

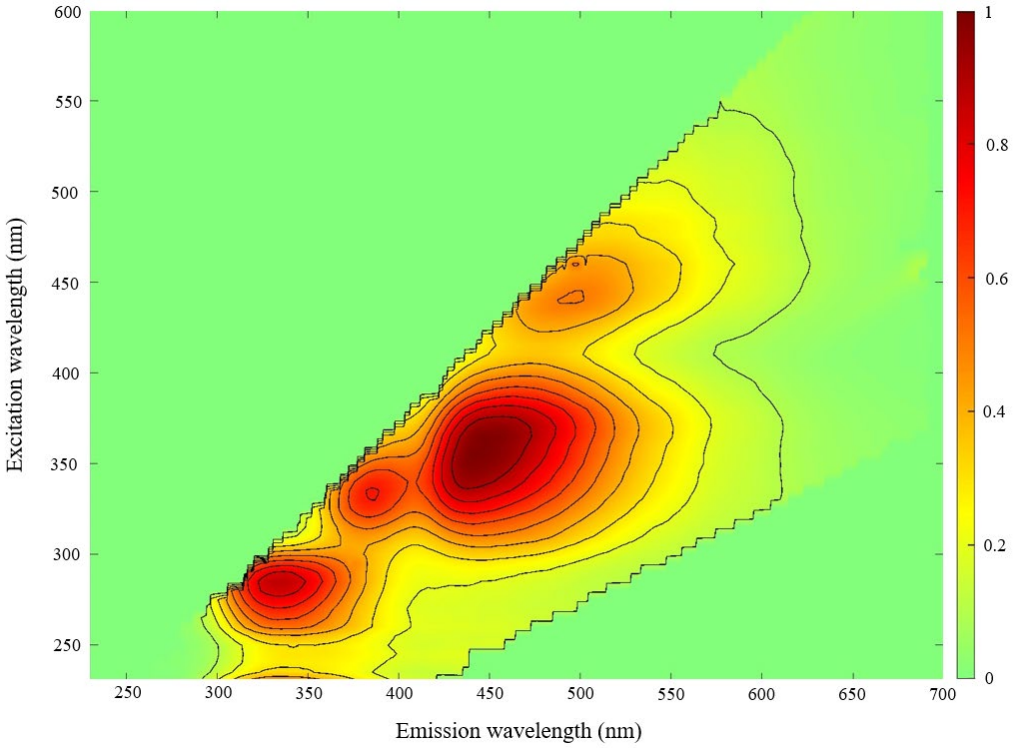


Figure S8. Unlabeled normalized EEM for FFR adipose at time point 0

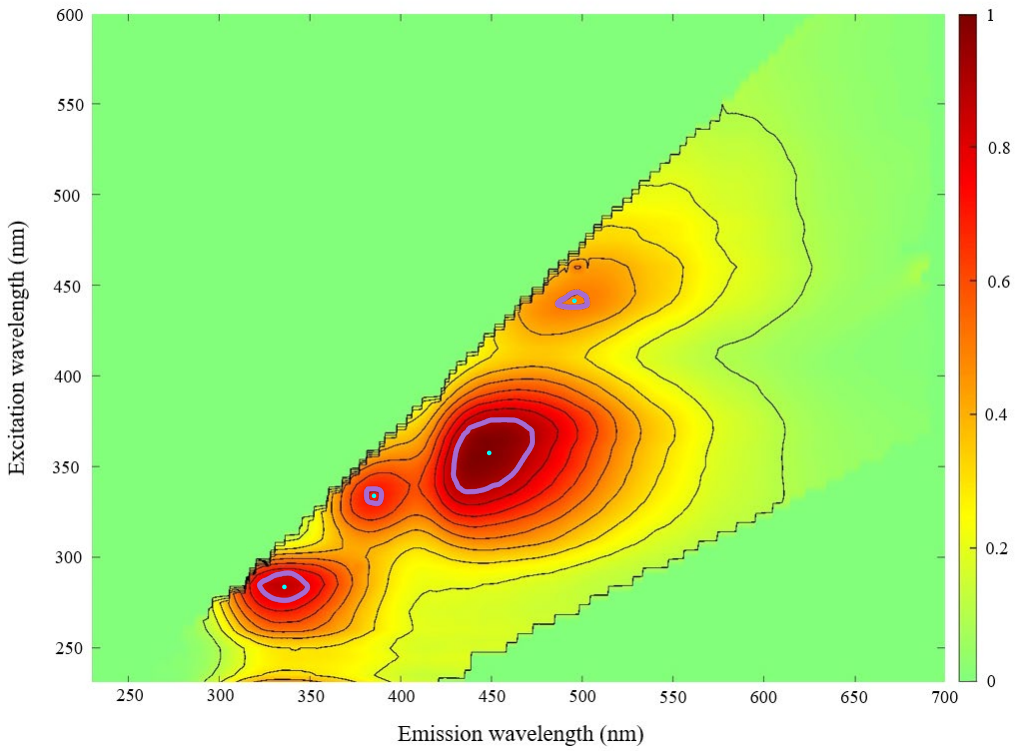


Figure S9. Central coordinates (blue dot) recorded for each independent contour of highest fluorescence (purple outline) for a given region in FFR adipose time point 0 normalized EEM

J: Precipitation

Table S7. *Total and average daily precipitation (mm) including watering for each FFR and F hand pair*

	FFR hand pairs							**F hand pairs**	
Time point (days)	2	4	6	9	14	20	24	14	24
ADD	31.7	58.7	82.8	115.6	179.9	257.6	300.4	196.9	340.4
Total precipitation (mm)	5.9	43.4	47.3	124.5	169.5	195.8	236.0	142.8	242.5
Average daily precipitation (mm)	2.9	10.8	7.9	13.8	12.1	9.8	9.8	4.9	6.2

K: Visual observations of decomposition

Table S8. *Observations of decomposition for each hand pair by time point and treatment group*

Time point (days)	ADD	Treatment group	Observations
0	0	F	Fresh, no signs of decomposition.
0	0	FFR	Fresh, no signs of decomposition.
2	31.7	FFR	Pale color, no skin slippage, *livor mortis* under fingernails, fungal growth mainly on dorsal side of the left hand with some on the fingers of both hands, bloating of left thumb.
4	58.7	FFR	Grey color, no skin slippage, some fungal growth near the wrists.
6	82.8	FFR	White/grey color, some *livor mortis* under the fingers of the left hand, skin is a bit loose but no slippage.
9	115.6	FFR	Pink/white color, skin slippage on dorsal sides and some on the palmar sides of the wrists, patches of dry skin on dorsal sides, brown discoloration at the points of severance which is more extensive on the left hand.
14	179.9	FFR	Green and grey color, extensive skin slippage on dorsal side, some brown discoloration at the points of severance, bloating in both hands.
14	196.9	F	Grey and pink color, no discoloration of extremities, extensive skin slippage.
20	257.6	FFR	White/green color, extensive skin slippage, some drying/browning at the points of severance.
24	300.4	FFR	Extensive skin slippage, some brown discoloration of fingertips and at points severance.
24	340.4	F	Brown discoloration at the points of severance, some dry patches of tissue mainly on the dorsal sides, one loosely attached fingernail remaining on the right hand, all fingernails loosely attached to left hand, thumbs slightly loose due to tissue mass loss, some blue tissue visible on the dorsal side of the left hand (likely a fungus).

L: Extreme outliers removed prior to statistical analysis

Table S9. *Extreme outliers (rounded to three significant figures) removed from datasets prior to statistical analysis*

Tissue type	**Sample name**	**Treatment group**	**ADD**	**Time point (days)**	**PROT AUC**	**FOX AUC**	**PROT-FOX ratio**
Skin	RD1	FFR	31.7	2	33 700	12 900	2.60
Skin	RD2	FFR	31.7	2	19 600	17 300	1.14
Adipose	LA1	F	340.4	24	6 520	8 490	0.768
Adipose	LA1	F	340.4	24	9 290	8 920	1.04
Adipose	LA2	F	340.4	24	15 100	16 600	0.912
Muscle	LM1	FFR	300.4	24	19 600	6 000	3.26

M: Decomposition over time by tissue type by state

Skin

Table S10. *Relationship significance between time points for FFR skin PROT AUCs*

			ADD				
			0	31.7	82.8	179.9	300.4
		Time point (days)	0	2	6	14	24
ADD	0	0					
	31.7	2	✓				
	82.8	6	✓	×			
	179.9	14	×	✓	✓		
	300.4	24	✓	✓	×	✓	

✓: significant ($p < 0.05$) relationship present; ×: significant ($p < 0.05$) relationship absent

Table S11. *Relationship significance between time points for F skin PROT AUCs*

			ADD		
			0	196.9	340.4
		Time point (days)	0	14	24
ADD	0	0			
	196.9	14	✓		
	340.4	24	✓	✓	

✓: significant ($p < 0.05$) relationship present; ×: significant ($p < 0.05$) relationship absent

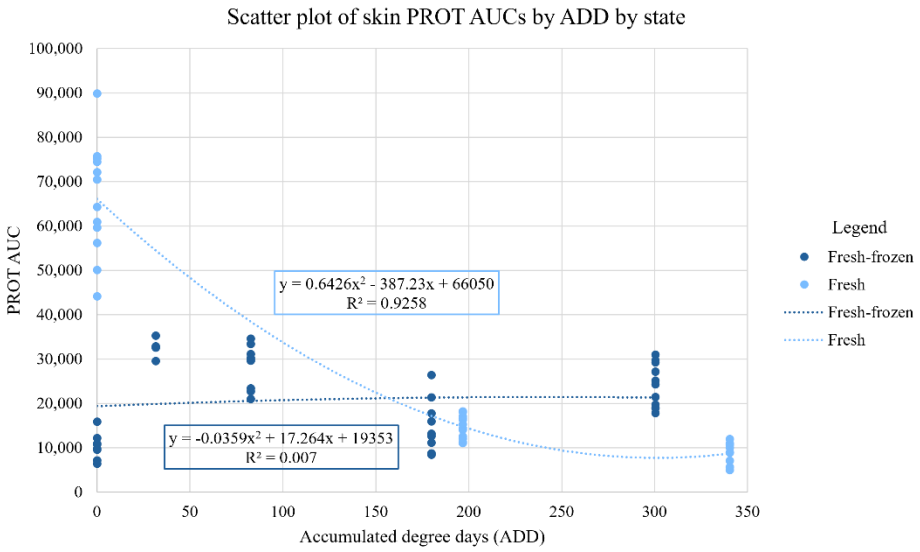


Figure S10. Scatter plot of skin PROT AUCs by ADD by state

Table S12. *Relationship significance between time points for FFR skin FOX AUCs*

			ADD				
			0	31.7	82.8	179.9	300.4
		Time point (days)	0	2	6	14	24
ADD	0	0					
	31.7	2	×				
	82.8	6	×	✓			
	179.9	14	×	×	×		
	300.4	24	×	✓	✓	✓	

✓: significant ($p < 0.05$) relationship present; ×: significant ($p < 0.05$) relationship absent

Table S13. *Relationship significance between time points for F skin FOX AUCs*

			ADD		
			0	196.9	340.4
		Time point (days)	0	14	24
ADD	0	0			
	196.9	14	✓		
	340.4	24	✓	×	

✓: significant ($p < 0.05$) relationship present; ×: significant ($p < 0.05$) relationship absent

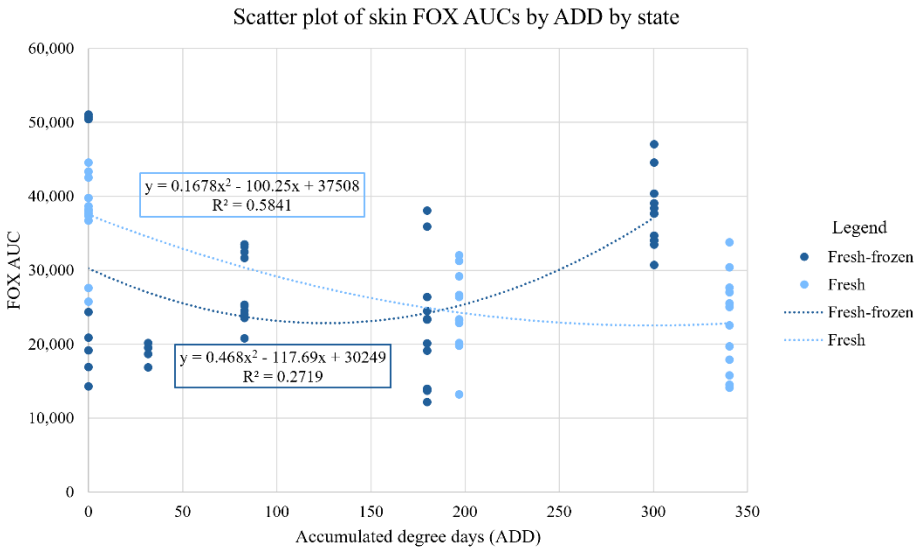


Figure S11. Scatter plot of skin FOX AUCs by ADD by state

Table S14. *Relationship significance between time points for FFR skin PROT-FOX ratios*

			ADD				
			0	31.7	82.8	179.9	300.4
		Time point (days)	0	2	6	14	24
ADD	0	0					
	31.7	2	✓				
	82.8	6	✓	✓			
	179.9	14	×	✓	✓		
	300.4	24	×	✓	✓	×	

✓: significant ($p < 0.05$) relationship present; ×: significant ($p < 0.05$) relationship absent

Table S15. *Relationship significance between time points for F skin PROT-FOX ratios*

			ADD		
			0	196.9	340.4
		Time point (days)	0	14	24
ADD	0	0			
	196.9	14	✓		
	340.4	24	✓	✓	

✓: significant ($p < 0.05$) relationship present; ×: significant ($p < 0.05$) relationship absent

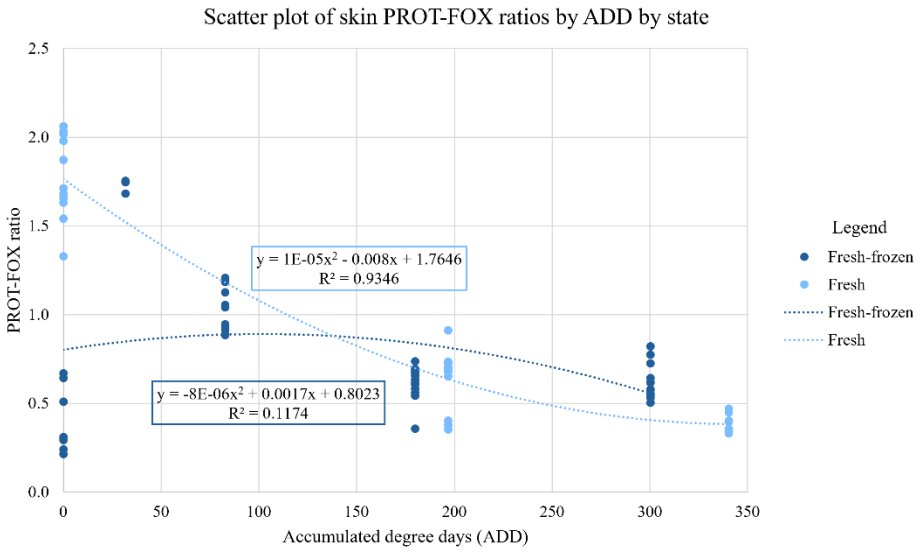


Figure S12. Scatter plot of skin PROT-FOX ratios by ADD by state

Adipose

Table S16. *Relationship significance between time points for FFR adipose PROT AUCs*

			ADD				
			0	31.7	82.8	179.9	300.4
		Time point (days)	0	2	6	14	24
ADD	0	0					
	31.7	2	✓				
	82.8	6	×	✓			
	179.9	14	✓	✓	✓		
	300.4	24	✓	✓	✓	✓	

✓: significant ($p < 0.05$) relationship present; ×: significant ($p < 0.05$) relationship absent

Table S17. *Relationship significance between time points for F adipose PROT AUCs*

			ADD		
			0	196.9	340.4
		Time point (days)	0	14	24
ADD	0	0			
	196.9	14	✓		
	340.4	24	✓	×	

✓: significant ($p < 0.05$) relationship present; ×: significant ($p < 0.05$) relationship absent

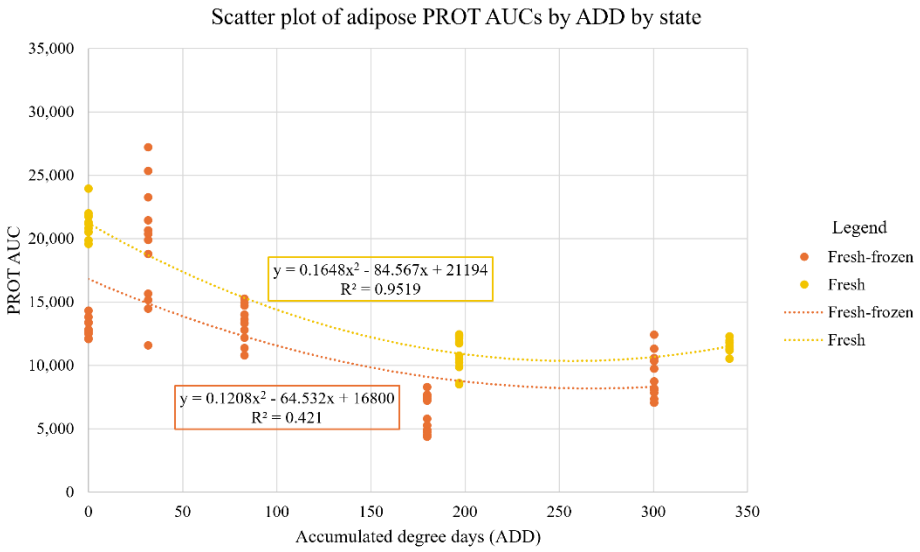


Figure S13. Scatter plot of adipose PROT AUCs by ADD by state

Table S18. *Relationship significance between time points for FFR adipose FOX AUCs*

			ADD				
			0	31.7	82.8	179.9	300.4
ADD		Time point (days)	0	2	6	14	24
	0	0					
	31.7	2	×				
	82.8	6	×	×			
	179.9	14	✓	✓	✓		
	300.4	24	✓	✓	✓	✓	

✓: significant ($p < 0.05$) relationship present; ×: significant ($p < 0.05$) relationship absent

Table S19. *Relationship significance between time points for F adipose FOX AUCs*

			ADD		
			0	196.9	340.4
ADD		Time point (days)	0	14	24
	0	0			
	196.9	14	×		
	340.4	24	✓	✓	

✓: significant ($p < 0.05$) relationship present; ×: significant ($p < 0.05$) relationship absent

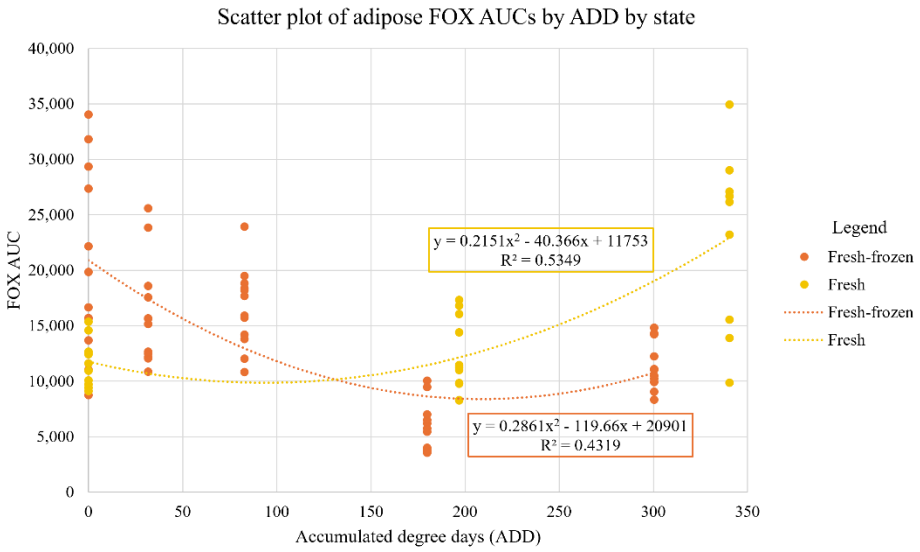


Figure S14. Scatter plot of adipose FOX AUCs by ADD by state

Table S20. *Relationship significance between time points for FFR adipose PROT-FOX ratios*

			ADD				
			0	31.7	82.8	179.9	300.4
		Time point (days)	0	2	6	14	24
ADD	0	0					
	31.7	2	✓				
	82.8	6	×	✓			
	179.9	14	✓	×	×		
	300.4	24	×	✓	×	×	

✓: significant ($p < 0.05$) relationship present; ×: significant ($p < 0.05$) relationship absent

Table S21. *Relationship significance between time points for F adipose PROT-FOX ratios*

			ADD		
			0	196.9	340.4
		Time point (days)	0	14	24
ADD	0	0			
	196.9	14	✓		
	340.4	24	✓	✓	

✓: significant ($p < 0.05$) relationship present; ×: significant ($p < 0.05$) relationship absent

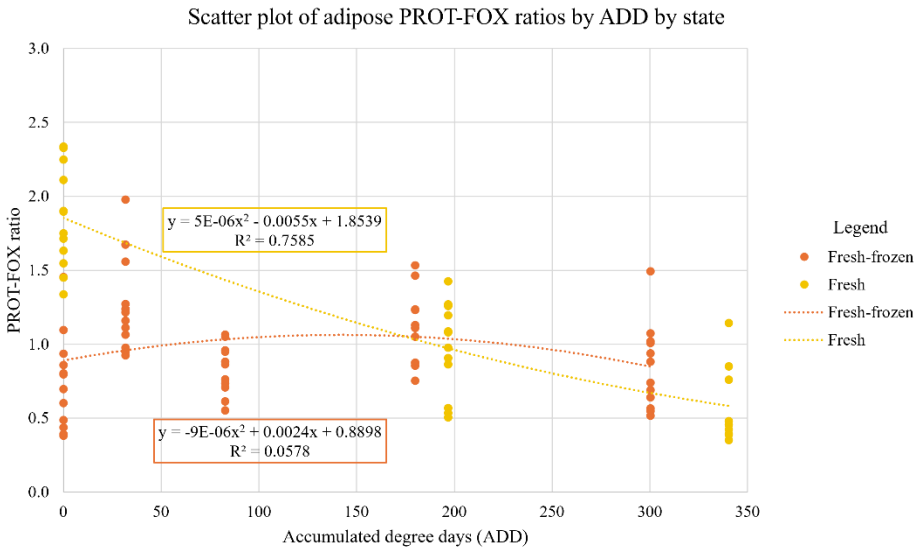


Figure S15. Scatter plot of adipose PROT-FOX ratios by ADD by state

Muscle

Table S22. *Relationship significance between time points for FFR muscle PROT AUCs*

			ADD				
			0	31.7	82.8	179.9	300.4
		Time point (days)	0	2	6	14	24
ADD	0	0					
	31.7	2	×				
	82.8	6	×	×			
	179.9	14	×	×	×		
	300.4	24	✓	✓	✓	✓	

✓: significant ($p < 0.05$) relationship present; ×: significant ($p < 0.05$) relationship absent

Table S23. *Relationship significance between time points for F muscle PROT AUCs*

			ADD		
			0	196.9	340.4
		Time point (days)	0	14	24
ADD	0	0			
	196.9	14	✓		
	340.4	24	✓	✓	

✓: significant ($p < 0.05$) relationship present; ×: significant ($p < 0.05$) relationship absent

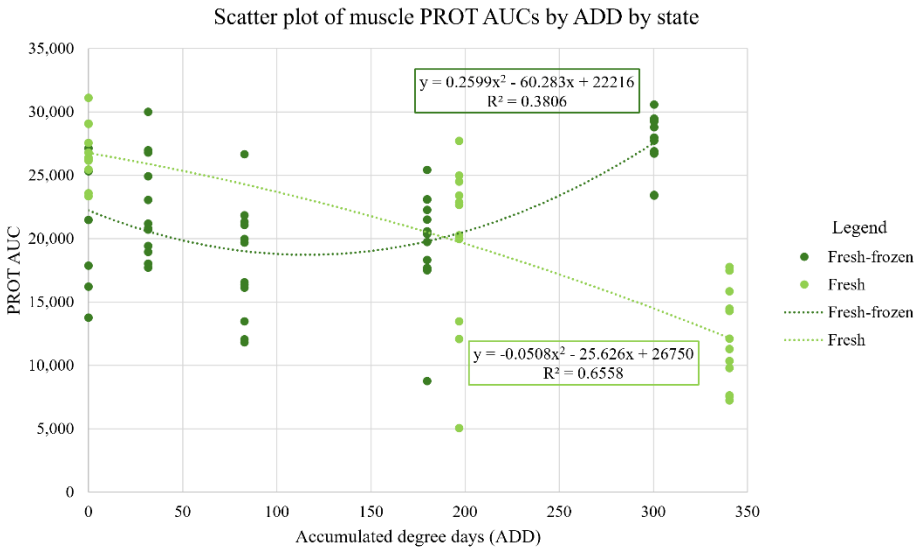


Figure S16. Scatter plot of muscle PROT AUCs by ADD by state

Table S24. *Relationship significance between time points for FFR muscle FOX AUCs*

			ADD				
			0	31.7	82.8	179.9	300.4
		Time point (days)	0	2	6	14	24
ADD	0	0					
	31.7	2	✓				
	82.8	6	×	✓			
	179.9	14	×	×	×		
	300.4	24	×	×	✓	×	

✓: significant ($p < 0.05$) relationship present; ×: significant ($p < 0.05$) relationship absent

Table S25. *Relationship significance between time points for F muscle FOX AUCs*

			ADD		
			0	196.9	340.4
		Time point (days)	0	14	24
ADD	0	0			
	196.9	14	×		
	340.4	24	✓	✓	

✓: significant ($p < 0.05$) relationship present; ×: significant ($p < 0.05$) relationship absent

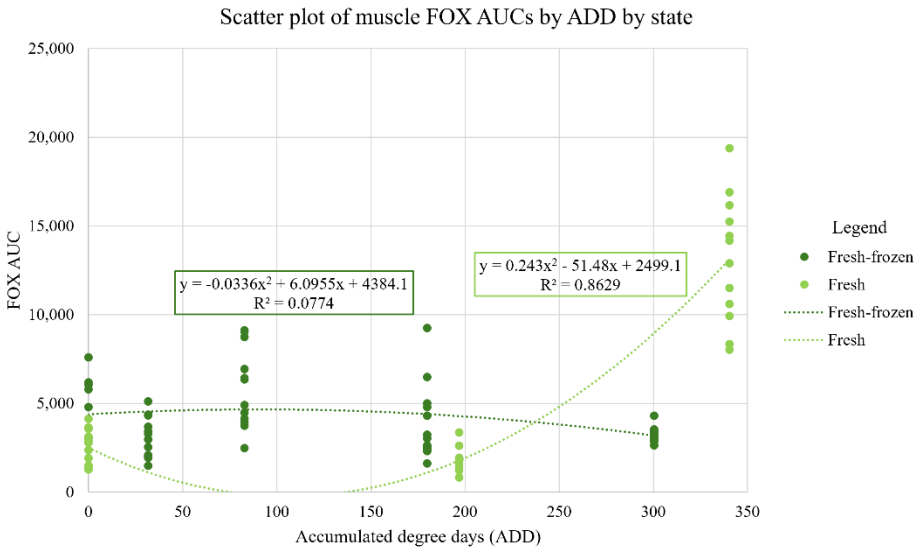


Figure S17. Scatter plot of muscle FOX AUCs by ADD by state

Table S26. *Relationship significance between time points for FFR muscle PROT-FOX ratios*

			ADD				
			0	31.7	82.8	179.9	300.4
		Time point (days)	0	2	6	14	24
ADD	0	0					
	31.7	2	✓				
	82.8	6	×	✓			
	179.9	14	×	×	×		
	300.4	24	✓	×	✓	×	

✓: significant ($p < 0.05$) relationship present; ×: significant ($p < 0.05$) relationship absent

Table S27. *Relationship significance between time points for F muscle PROT-FOX ratios*

			ADD		
			0	196.9	340.4
		Time point (days)	0	14	24
ADD	0	0			
	196.9	14	×		
	340.4	24	✓	✓	

✓: significant ($p < 0.05$) relationship present; ×: significant ($p < 0.05$) relationship absent

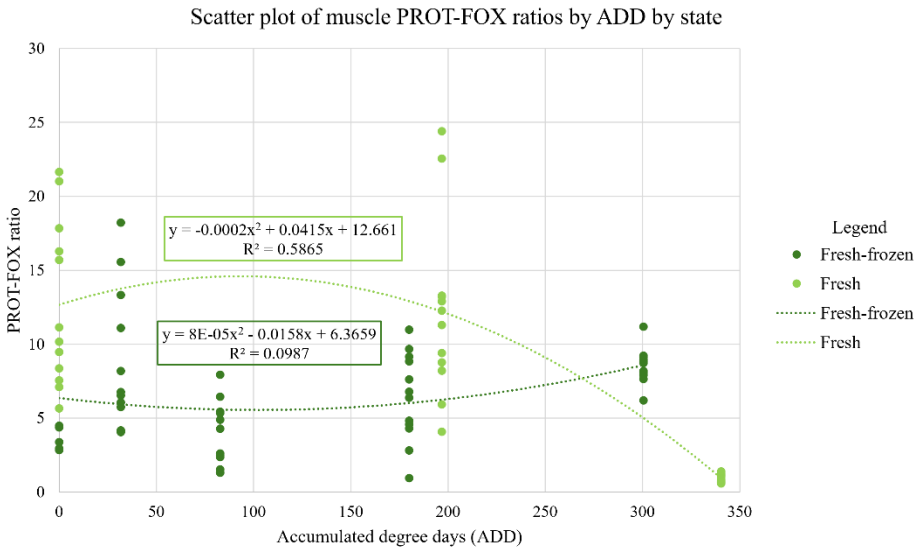


Figure S18. Scatter plot of muscle PROT-FOX ratios by ADD by state

N: Occurrence of significant ($p < 0.05$) relationships between fresh and fresh-frozen samples at corresponding time points by tissue type

Table S28. *Occurrence of significant ($p < 0.05$) relationships between F and FFR samples at corresponding time points per tissue type*

		Occurrence of significant ($p < 0.05$) relationship between F and FFR parameters		
Tissue type	**Time point (days)**	**PROT AUCs**	**FOX AUCs**	**PROT-FOX ratios**
Skin	0	✓	×	✓
	14	×	×	×
	24	✓	✓	✓
Adipose	0	✓	✓	✓
	14	✓	✓	×
	24	✓	✓	✓
Muscle	0	✓	✓	✓
	14	×	✓	✓
	24	✓	✓	✓

✓: significant ($p < 0.05$) relationship present; ×: significant ($p < 0.05$) relationship absent

O: EEM peaks excluded from fluorophore peak analysis

Table S29. *Overview of fluorophore peaks excluded from fluorophore peak analysis*

		Occurrence of peak		
Name assigned to peak	**Central peak coordinates (excitation/emission wavelengths in nm)**	**Tissue type**	**Treatment group**	**Time point (days)**
Unknown A	425/592	Adipose	FFR	24
Unknown A	430/595.5	Muscle	FFR	6
Unknown B	355/544	Adipose	FFR	24
Unknown C	545/585.5	Adipose	F	0
Unknown C	555/595	Muscle	FFR	6
Unknown D	260/375.5	Adipose	F	14
Unknown E	295/501.5	Skin	F	0
Unknown F	240/333.5	Muscle	FFR	14

P: Occurrence of fluorophore peaks

Table S30. *Overview fluorophore peak occurrences in skin EEMs*

				Occurrence of peak				
Tissue type	**Treatment group**	**ADD**	**Time point (days)**	**Peak A**	**Peak B**	**Peak C**	**Peak D**	**Peak E**
Skin	FFR	0	0	✓	×	✓	×	×
	FFR	31.7	2	✓	×	✓	×	×
	FFR	82.8	6	✓	×	✓	✓	✓
	FFR	179.9	14	✓	✓	×	✓	×
	FFR	300.4	24	✓	×	✓	×	✓
	F	0	0	✓	×	✓	×	✓
	F	196.9	14	✓	×	✓	×	✓
	F	340.4	24	✓	✓	✓	×	×

✓: peak present; ×: peak absent

Table S31. *Overview fluorophore peak occurrences in adipose EEMs*

				Occurrence of peak				
Tissue type	**Treatment group**	**ADD**	**Time point (days)**	**Peak A**	**Peak B**	**Peak C**	**Peak D**	**Peak E**
Adipose	FFR	0	0	✓	✓	✓	✓	×
	FFR	31.7	2	✓	✓	×	✓	×
	FFR	82.8	6	✓	✓	✓	×	×
	FFR	179.9	14	✓	✓	✓	✓	✓
	FFR	300.4	24	✓	✓	✓	×	✓
	F	0	0	✓	✓	×	×	✓
	F	196.9	14	×	✓	×	✓	✓
	F	340.4	24	✓	✓	×	×	✓

✓: peak present; ×: peak absent

Table S32. *Overview fluorophore peak occurrences in muscle EEMs*

				Occurrence of peak				
Tissue type	**Treatment group**	**ADD**	**Time point (days)**	**Peak A**	**Peak B**	**Peak C**	**Peak D**	**Peak E**
Muscle	FFR	0	0	✓	×	×	×	✓
	FFR	31.7	2	✓	×	×	×	×
	FFR	82.8	6	✓	✓	✓	✓	×
	FFR	179.9	14	✓	×	×	×	×
	FFR	300.4	24	✓	✓	×	×	×
	F	0	0	✓	×	×	×	×
	F	196.9	14	✓	×	×	×	×
	F	340.4	24	✓	✓	✓	✓	✓

✓: peak present; ×: peak absent

Q: Effect of multiple freeze-thaw cycles on PROT and FOX fluorescence in time point 2 samples

The ratio of one to two F-T cycles was calculated for each fluorescence parameter (PROT and FOX AUCs, and PROT-FOX ratios) by dividing the fluorescence intensity at each emission wavelength after one F-T cycle, by the values obtained after the second cycle. If no changes in fluorescence occur from one to two cycles, the ratio is expected to be 1, and if there is a change, the greater this change, the further away from 1 the ratio will be.

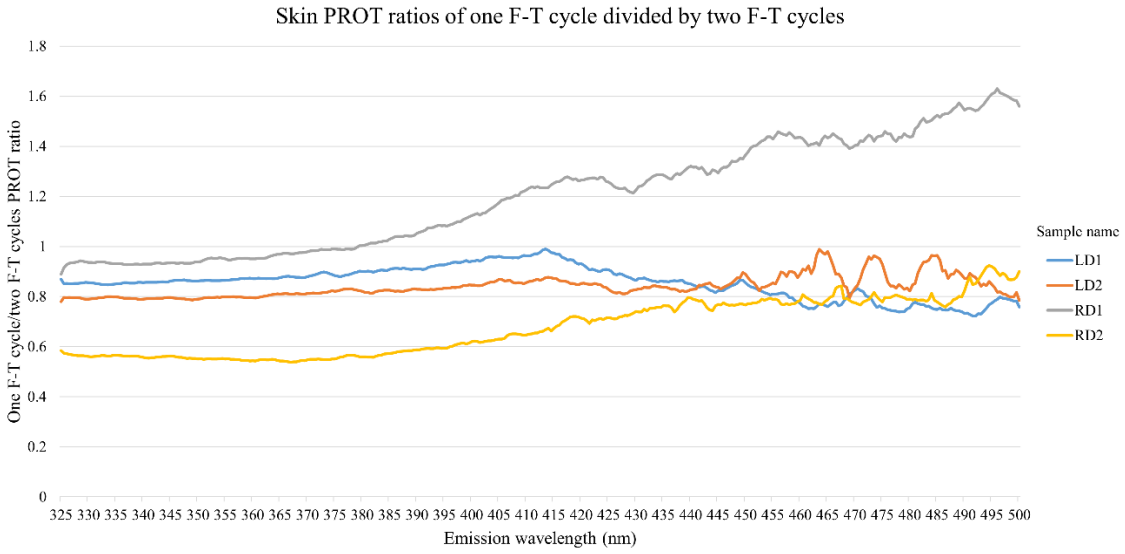


Figure S19. Skin time point 2 PROT ratios following one vs. two freeze-thaw cycles

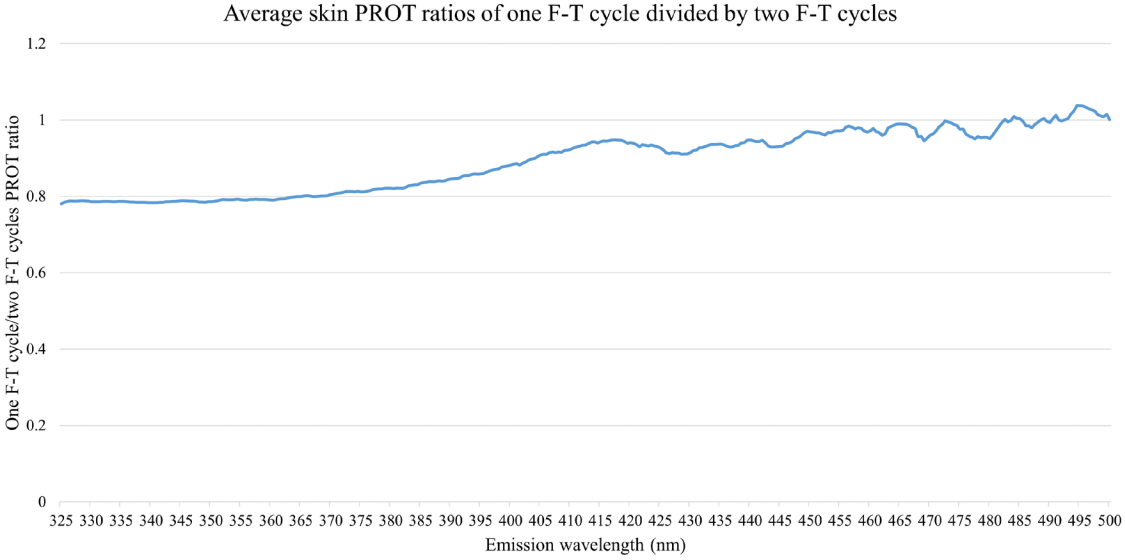


Figure S20. Average skin time point 2 PROT ratios following one vs. two freeze-thaw cycles

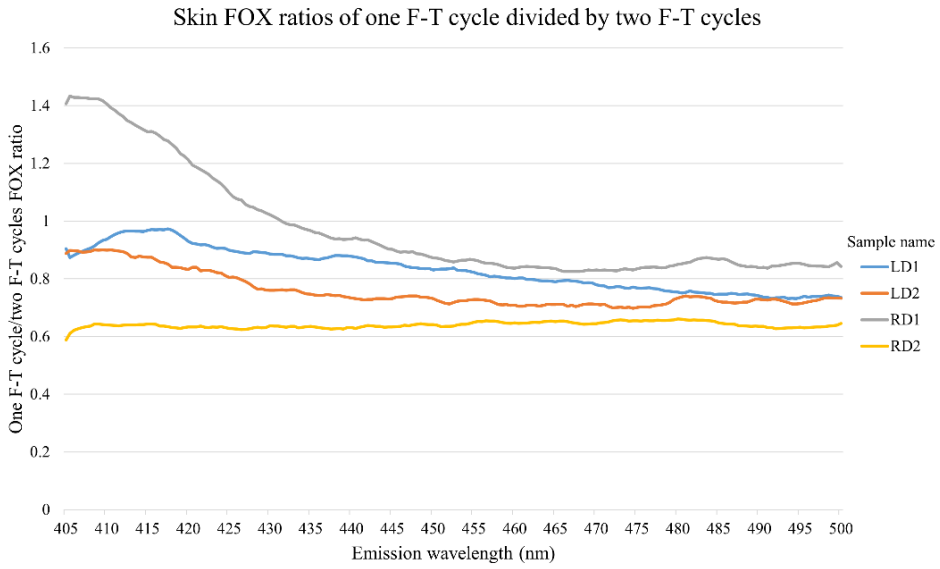


Figure S21. Skin time point 2 FOX ratios following one vs. two freeze-thaw cycles

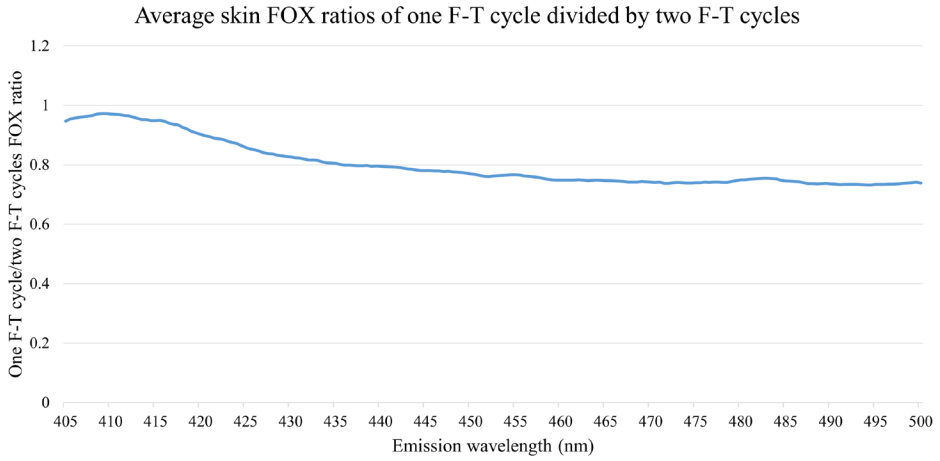


Figure S22. Average skin time point 2 FOX ratios following one vs. two freeze-thaw cycles

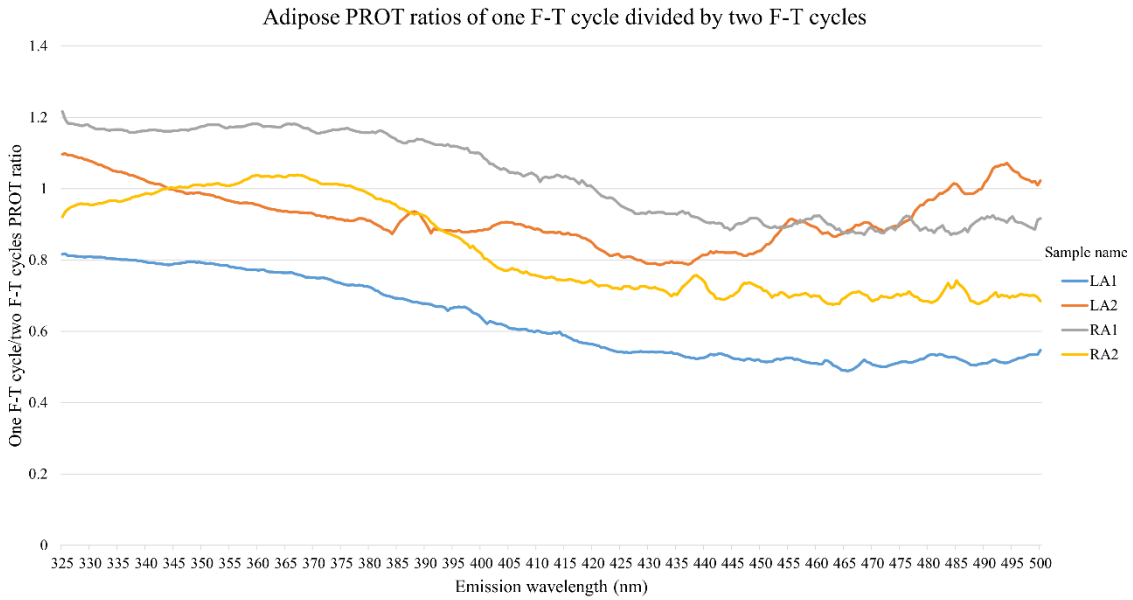


Figure S23. Adipose time point 2 PROT ratios following one vs. two freeze-thaw cycles

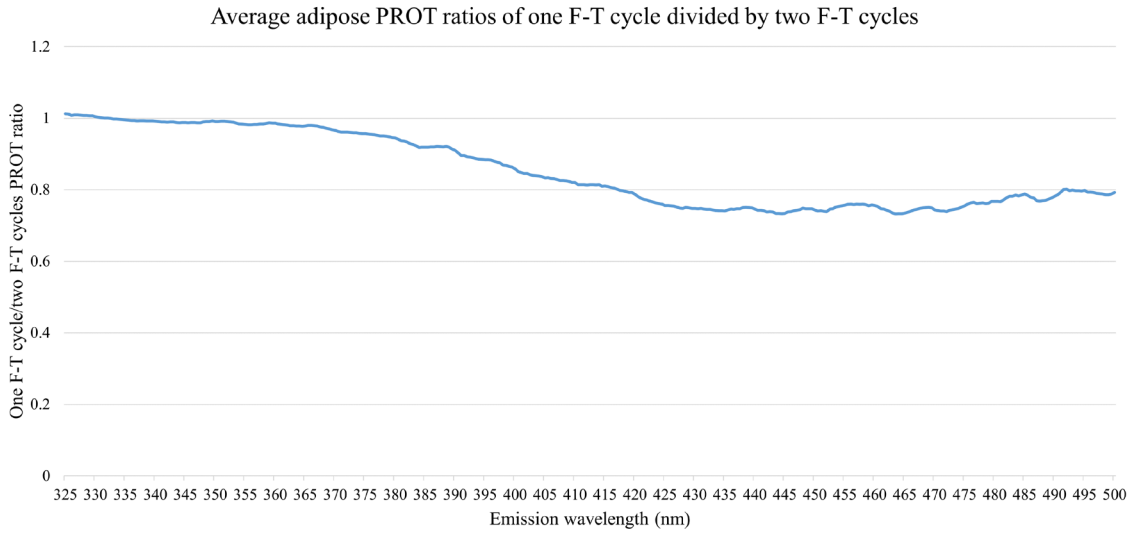


Figure S24. Average adipose time point 2 PROT ratios following one vs. two freeze-thaw cycles

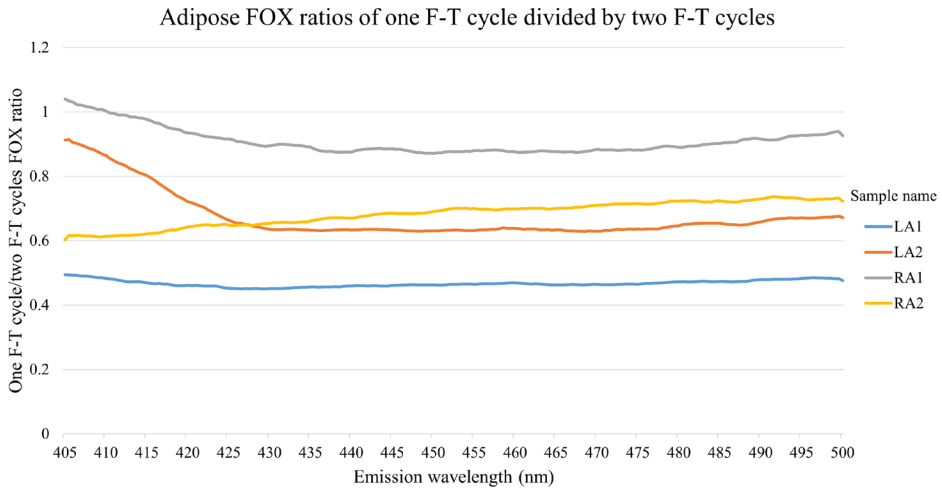


Figure S25. Adipose time point 2 FOX ratios following one vs. two freeze-thaw cycles

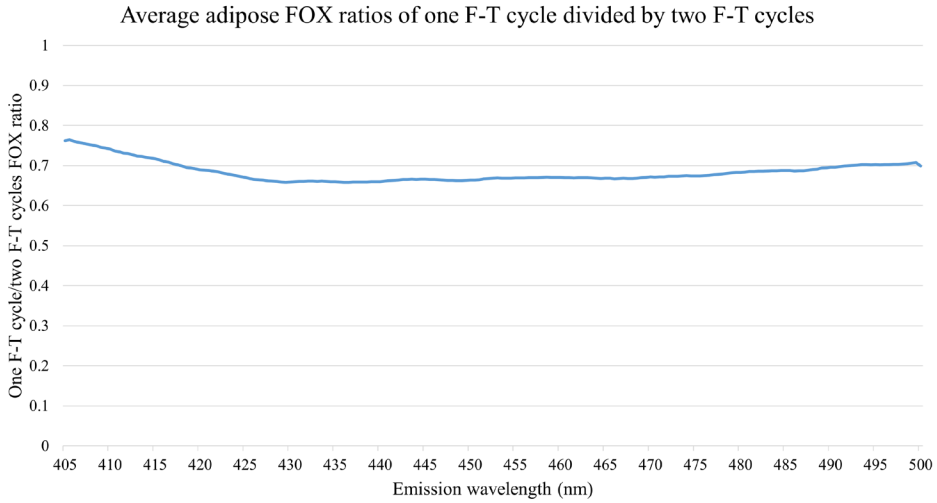


Figure S26. Average adipose time point 2 FOX ratios following one vs. two freeze-thaw cycles

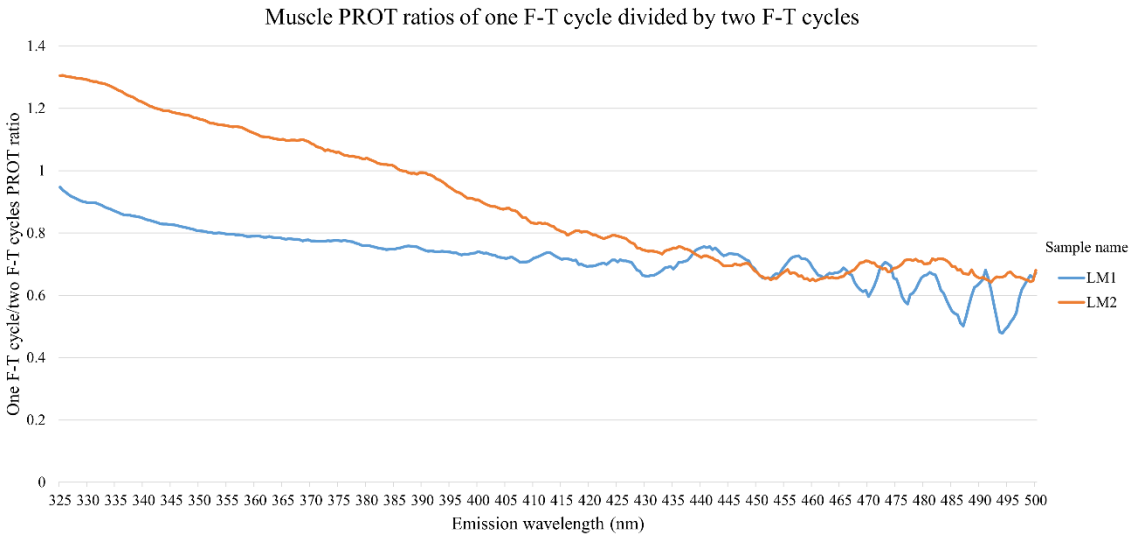


Figure S27. Muscle time point 2 PROT ratios following one vs. two freeze-thaw cycles

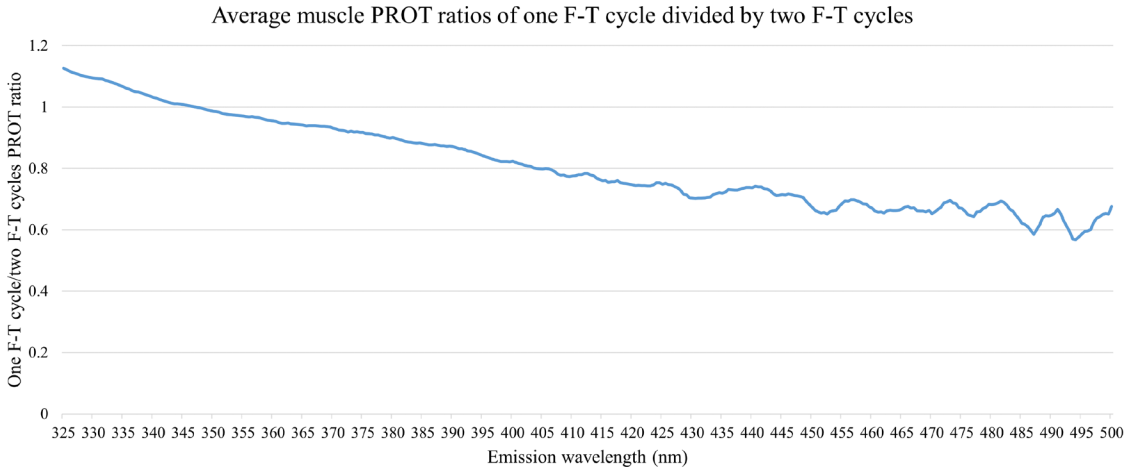


Figure S28. Average muscle time point 2 PROT ratios following one vs. two freeze-thaw cycles

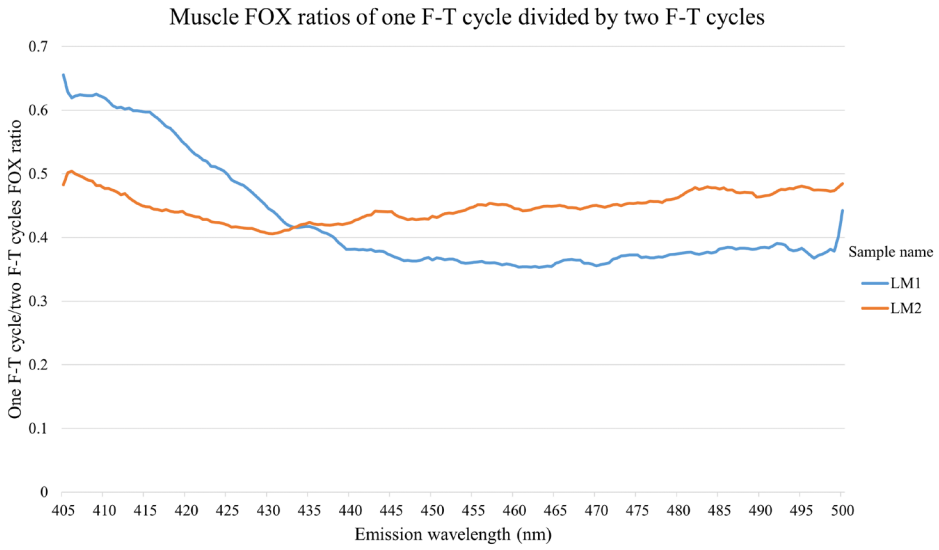


Figure S29. Muscle FOX ratios following one vs. two freeze-thaw cycles

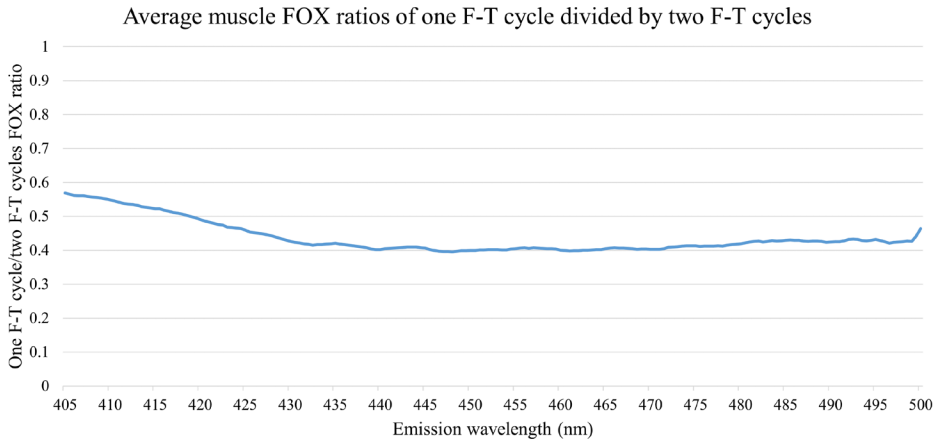


Figure S30. Average muscle time point 2 FOX ratios following one vs. two freeze-thaw cycles

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3. van Dam, A. (2022, September 21). [Personal communication].