

Supplementary for

Radical Footprinting in Mammalian Whole Blood

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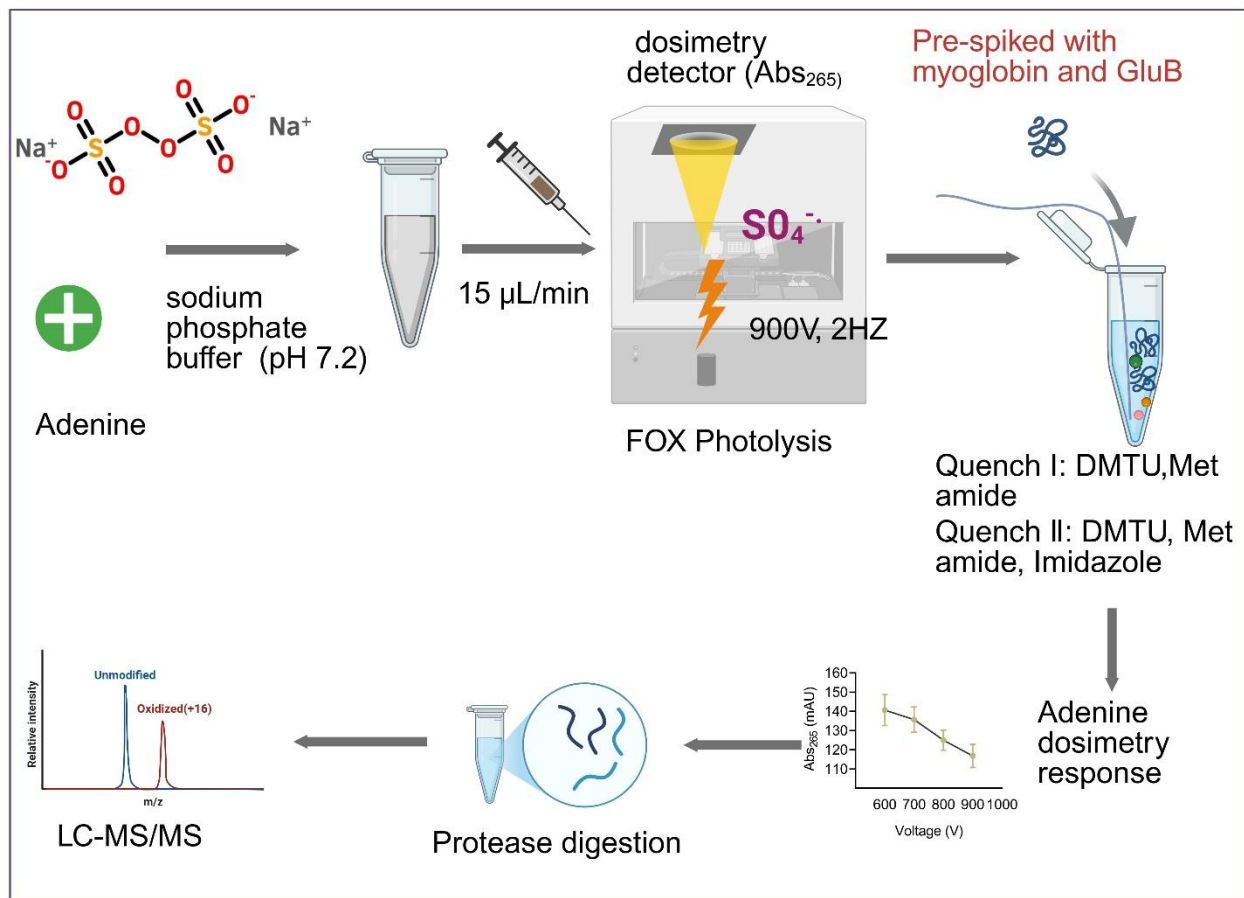
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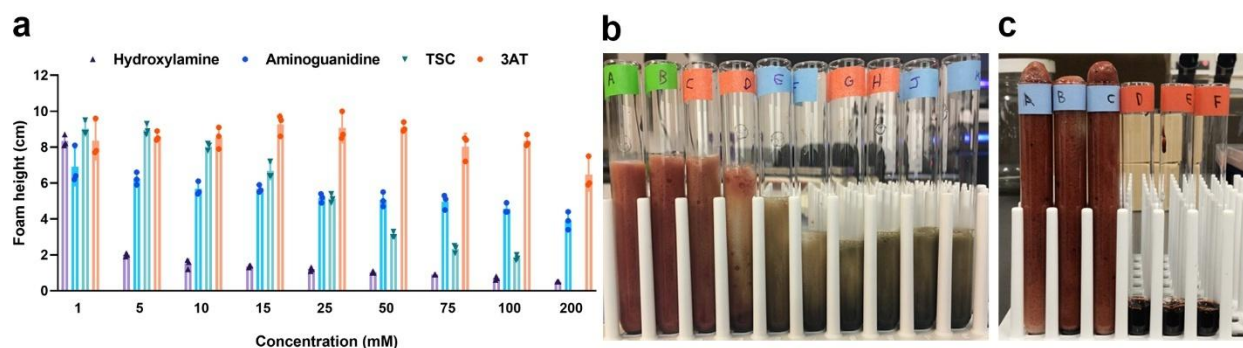
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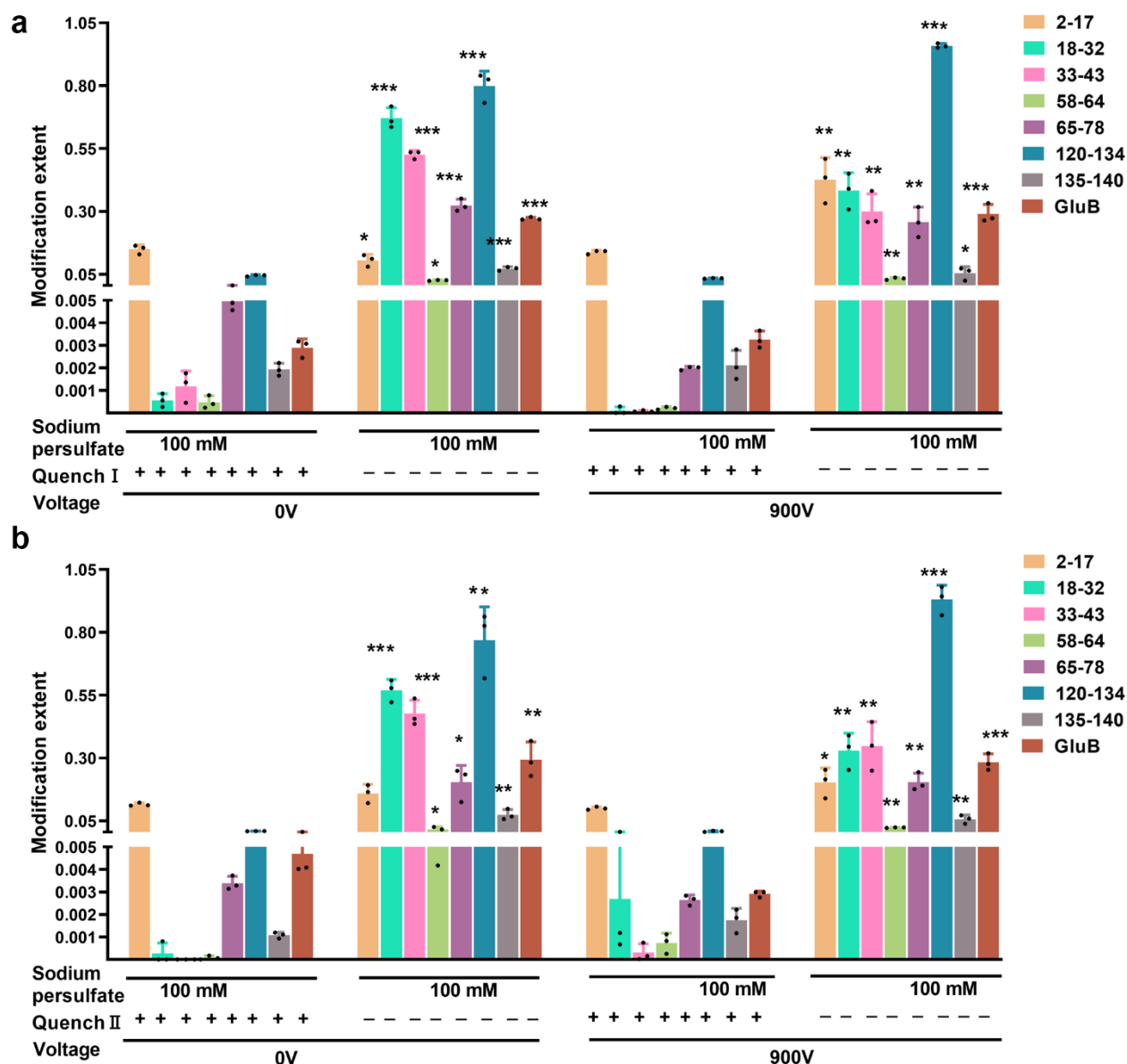
†These authors contributed equally to this work



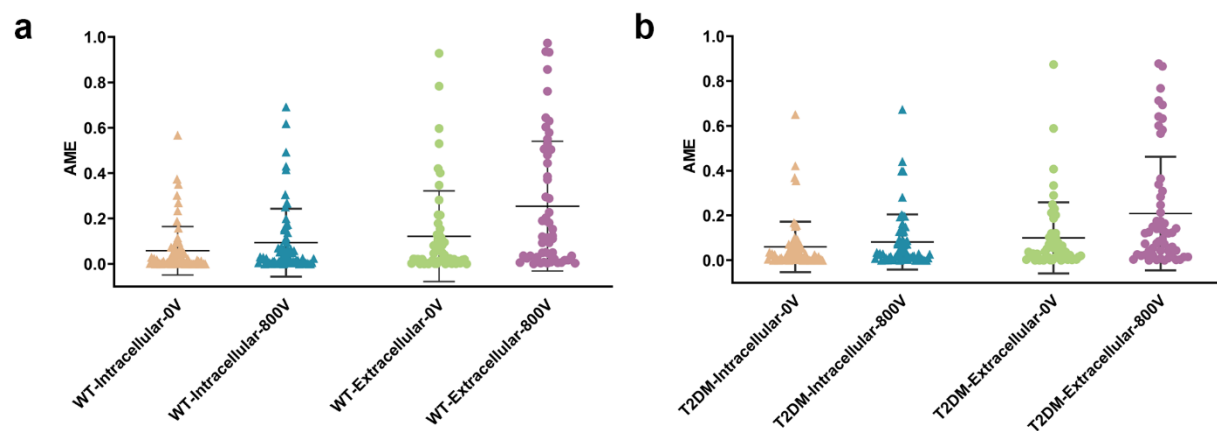
Supplementary Fig. 1 Method development of *in vitro* SO₄•⁻ RPF. FOX : Flash Oxidation ; DMTU: N'-Dimethylthiourea; GluB: [Glu]1-fibrinopeptide B ; LC-MS/MS: liquid chromatography coupled to tandem mass spectrometry. The figure was created in BioRender. Zhao, M. (2026) <https://BioRender.com/ewd9x1k>



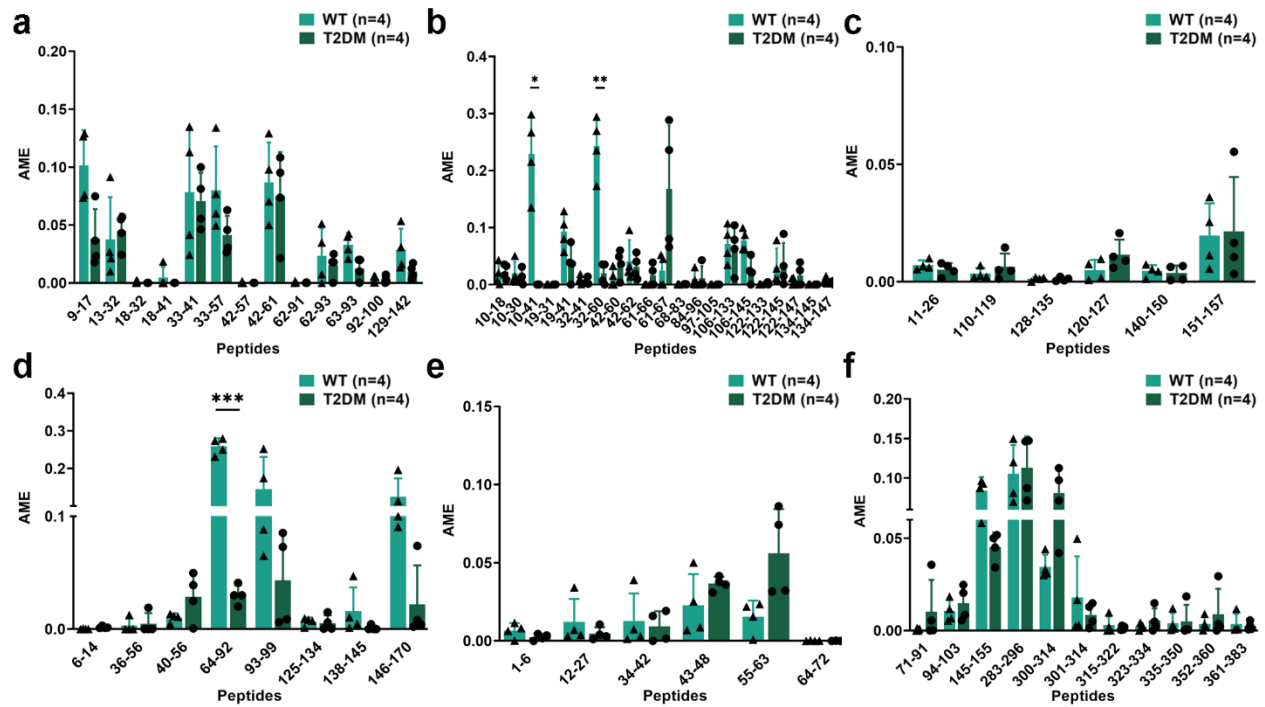
Supplementary Fig.2 Test of inhibitors of catalase. **a** Foam height after 30 minute incubation (5:1 v/v blood to inhibitor) at room temperature. TSC is insoluble at 200 mM. Error bars represent one standard deviation of triplicate measurement. **b** Representative samples of blood spiked with varying final concentrations of hydroxylamine: 0 mM (**green A-B**), 5 mM (**orange C-D**), 25 mM (**blue E-F**), 100 mM (**orange G-H**), and 142 mM (**blue J-K**). **c** Blood spiked with equal volumes of 30% H_2O_2 (**blue**) or 2.5 M $\text{Na}_2\text{S}_2\text{O}_8$ (**red**). TSC: thiosemicarbazide; 3AT: 3-amino-1:2:4-triazole. Source data are provided as a Source Data file.



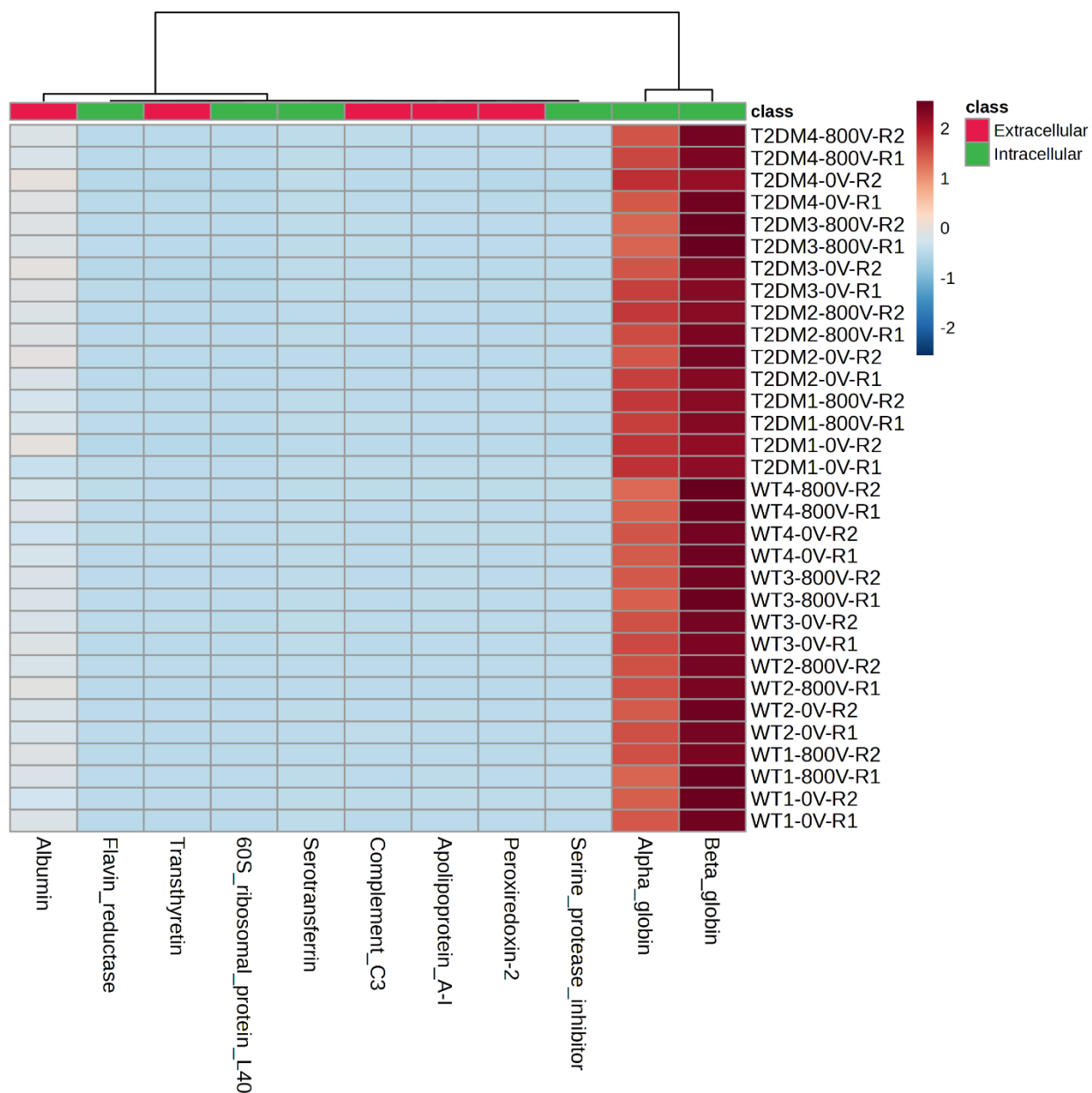
Supplementary Fig.3 Effectiveness of quench solutions. **a** Evaluation of Quench I (without imidazole). **b** Evaluation of Quench II (with imidazole). Both quenches were found to significantly reduce oxidation of most peptides with or without radical photoproduction. Statistical significance was calculated by a two-tailed unpaired t-test ($\alpha = 0.05$) with Šidák correction versus the quenched control under the same conditions. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Myoglobin peptides are denoted by their position. The myoglobin peptide corresponding to the 2–17 residues displays significant in-source oxidation. For each condition, $n = 3$ independently prepared samples. Error bars represent mean $\pm$ standard deviation (SD) across replicates. APO: Average Peptide Oxidation. Exact P values and Source data are provided as a Source Data file.



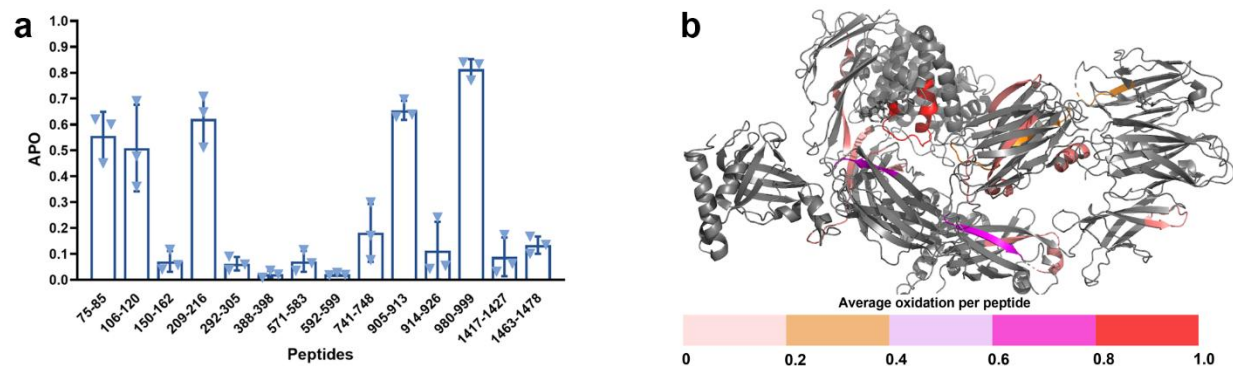
Supplementary Fig.4 Comparison of in-blood RPF of intracellular and extracellular proteins detected in WT and T2DM whole blood. **a** Comparison of modification extent of intracellular and extracellular proteins of WT. **b** Comparison of modification extent of intracellular and extracellular proteins of T2DM. Each data point represents the average AME of a peptide from 4 biological replicates. Error bars represent mean $\pm$ standard deviation (SD). AME: Average Modification Extent; T2DM: type 2 diabetes mellitus; WT: wild type. Source data are provided as a Source Data file.



Supplementary Fig.5 Comparison of in-blood RPF of top 6 intracellular proteins detected in WT and T2DM whole blood. (a-f) Peptide-level oxidation of peptides from six intracellular proteins in 4 mice of each condition, after subtraction of 0V background oxidation values. For each condition, $n = 4$ biological replicates, where each biological replicate represents an independently prepared sample from an individual mouse; All statistics performed were unpaired two-tailed t-test with Šidák correction. Error bars represent mean $\pm$ standard deviation (SD). ; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. **a** alpha-globin; **b** beta-globin; **c** peroxiredoxin-2; **d** Flavin reductase; **e** 60S ribosomal protein-L40; **f** Serine protease inhibitor. AME: Average Modification Extent; T2DM: type 2 diabetes mellitus; WT: wild type. Exact P values and Source data are provided as a Source Data file.



Supplementary Fig.6 Hierarchical clustering heatmaps on the abundance of top intracellular and extracellular proteins detected from T2DM and WT whole blood. Each colored cell on the map corresponds to an intensity value. Red: high; blue: low. T2DM: type 2 diabetes mellitus; WT: wild type. Source data are provided as a Source Data file.



Supplementary Fig.7 *In vitro* SO₄• RPF of protein complement C3. **a** Modification extent of peptides; n = 3 independently prepared samples; Error bars represent mean ± standard deviation (SD). Only oxidized peptides were included. **b** Average oxidation per peptide mapped on structure of complement C3 (PDB : 2a73). Peptides with non-measurable oxidation are shown in grey. Color represents the amount of oxidation detected. APO: Average Peptide Oxidation. Source data are provided as a Source Data file.

Supplementary Table 1. Ordinary one-way ANOVA analysis of RBCs cell area measurement.

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted Exact <i>P</i> Value
Blood vs. Blood + 2 M NaCl	-0.00996	-0.03273 to 0.01282	No	ns	0.5524
Blood vs. Blood + 2 M Na ₂ S ₂ O ₈	-0.02142	-0.04420 to 0.001354	No	ns	0.0697
Blood + 2 M NaCl vs. Blood + 2 M Na ₂ S ₂ O ₈	-0.01147	-0.03424 to 0.01131	No	ns	0.4561

Supplementary Table 2. Ordinary one-way ANOVA analysis of RBCs cell length measurement.

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted Exact <i>P</i> Value
Blood vs. Blood + 2 M NaCl	-0.5877	-0.9097 to -0.2657	Yes	***	0.0001
Blood vs. Blood + 2 M Na ₂ S ₂ O ₈	-0.7411	-1.063 to -0.4191	Yes	****	<0.0001
Blood + 2 M NaCl vs. Blood + 2 M Na ₂ S ₂ O ₈	-0.1534	-0.4754 to 0.1686	No	ns	0.4947

Supplementary Table 3. Ordinary one-way ANOVA analysis of hemolysis measurement.

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted Exact <i>P</i> Value
10% Triton-X-100 vs. 200 mM NaCl	99.98	96.99 to 103.0	Yes	****	<0.0001
10% Triton-X-100 vs. 200 mM Na ₂ S ₂ O ₈	99.63	96.64 to 102.6	Yes	****	<0.0001
200 mM NaCl vs. 200 mM Na ₂ S ₂ O ₈	-0.3477	-3.342 to 2.646	No	ns	0.9332

Supplementary Table 4. Sequence coverage of top 11 proteins in T2DM and WT whole blood.

Protein Name	Average sequence coverage (%)		Class
	T2DM (n=4)	WT (n=4)	
Alpha globin	99.84 ± 0.29	99.95± 0.18	Intracellular
Beta globin	99.48 ± 0.82	99.83 ± 0.30	Intracellular
Flavin reductase	75.49 ± 5.48	82.25 ± 6.28	Intracellular
Transthyretin	76.34 ± 8.38	78.54 ± 9.09	Extracellular
Peroxiredoxin-2	75.99 ± 4.66	75.22 ± 1.21	Intracellular
Apolipoprotein A-I	73.39 ± 7.78	73.44 ± 7.89	Extracellular
Complement c3	70.59 ± 4.35	73.04 ± 4.64	Extracellular
Serotransferrin	70.35 ± 4.21	70.45 ± 4.97	Extracellular
Albumin	72.15 ± 2.89	70.29 ± 4.07	Extracellular
Serine protease inhibitor	60.69 ± 4.82	60.49 ± 5.40	Intracellular
60S ribosomal protein L40	52.06 ± 1.84	52.05 ± 2.10	Intracellular

Supplementary Table 5. Comparison on modification extent of intracellular and extracellular peptides in WT mice using Mann Whitney analysis.

Table Analyzed	WT-EXtra vs.Intra		
Column B	WT-Intracellular		
vs.	vs.		
Column A	WT-Extracellular		
Mann Whitney test			
<i>P</i> value	<.0001		
Exact or approximate <i>P</i> value?	Exact		
P value summary	***		
Significantly different (<i>P</i> < 0.05)?	Yes		
One- or two-tailed <i>P</i> value?	Two-tailed		
Sum of ranks in column A	B	4018	3122
Mann-Whitney U	977		
Difference between medians			
Median of column A	0.05383	n=54	
Median of column B	0.01055	n=65	
Difference: Actual	-0.04328450		
Difference: Hodges-Lehmann	-0.03223750		

Supplementary Table 6. Comparison on modification extent of intracellular and extracellular peptides in T2DM mice using Mann Whitney analysis.

Table Analyzed	T2DM-EXtra vs.Intra		
Column B	T2DM-Intracellular		
vs.	vs.		
Column A	T2DM-Extracellular		
Mann Whitney test			
<i>P</i> value	<.0001		
Exact or approximate <i>P</i> value?	Exact		
<i>P</i> value summary	***		
Significantly different (<i>P</i> < 0.05)?	Yes		
One- or two-tailed <i>P</i> value?	Two-tailed		
Sum of ranks in column A	B	4037	3103
Mann-Whitney U	958		
Difference between medians			
Median of column A	0.04153	n=54	
Median of column B	0.0103	n=65	
Difference: Actual	-0.03123		
Difference: Hodges-Lehmann	-0.02703		

Supplementary Table 7. Comparison on modification extent of Tf in T2DM and WT mice using unpaired two-tailed t test with Šídák correction.

Peptide	Below threshold?	P value	Mean of WT	Mean of T2DM	Difference	SE of difference	t ratio	df	Adjusted <i>P</i> Value
47-55	No	0.143203	0.004625	0.009913	-0.00529	0.00314	1.684	6	0.75117
123-134	No	0.051143	0.02733	0.006113	0.02121	0.008728	2.43	6	0.438683
226-236	No	0.013671	0.1116	0.02902	0.08262	0.02396	3.448	6	0.152258
265-273	No	0.49503	0.003383	0.00184	0.001543	0.002124	0.7262	6	0.922017
274-287	No	0.234619	0.006925	0.03071	-0.02379	0.01801	1.321	6	0.882234
279-287	No	0.304854	0.0063	0.01808	-0.01178	0.0105	1.122	6	0.921561
288-295	No	0.84669	0.02857	0.02458	0.003993	0.01978	0.2019	6	0.976496
298-310	No	0.125529	0.1903	0.2356	-0.04531	0.02547	1.779	6	0.73851
447-455	No	0.333453	0.01525	0.006958	0.008293	0.007885	1.052	6	0.921561
456-467	No	0.471555	0.02901	0.05394	-0.02493	0.03245	0.7681	6	0.922017
548-554	Yes	0.000126	0.4031	0.003288	0.3998	0.04584	8.723	6	0.001756
555-565	Yes	0.000008	0.07421	0.005438	0.06878	0.004892	14.06	6	0.000121
555-572	No	0.914019	0.042	0.03891	0.003087	0.02742	0.1126	6	0.976496
566-572	Yes	0.001377	0.1782	0.3768	-0.1986	0.03545	5.603	6	0.017752
668-681	No	0.363026	0.01756	0.03075	-0.01318	0.01339	0.9842	6	0.921561

Supplementary Table 8. Comparison on modification extent of C3 in T2DM and WT mice using unpaired two-tailed t test with Šídák correction.

Peptide	Below threshold?	P value	Mean of WT	Mean of T2DM	Difference	SE of difference	t ratio	df	Adjusted <i>P</i> Value
75-85	Yes	0.000004	0.7415	0.000425	0.741	0.04692	15.79	6	0.000057
106-120	No	0.006587	0.3018	0.1086	0.1933	0.0475	4.069	6	0.063948
150-162	No	0.424587	0.00835	0.00355	0.0048	0.005604	0.8565	6	0.883244
209-216	No	0.011241	0.4849	0.2924	0.1925	0.05333	3.609	6	0.086468
292-305	No	0.247648	0.00935	0.002025	0.007325	0.005721	1.28	6	0.818646
388-398	No	0.415453	0.0428	0.01771	0.02509	0.02869	0.8745	6	0.883244
571-583	No	0.00478	0.008482	0.6172	-0.6087	0.1397	4.358	6	0.051338
592-599	No	0.008431	0.0472	0.4453	-0.3981	0.1033	3.853	6	0.07337
741-748	Yes	0.001684	0.05974	0.4301	-0.3704	0.06875	5.387	6	0.021677
905-913	Yes	0.001736	0.6413	0.179	0.4623	0.08633	5.355	6	0.021677
914-926	No	0.70377	0.07534	0.102	-0.02668	0.06687	0.3989	6	0.912248
980-999	No	0.270969	0.5288	0.3597	0.1692	0.1395	1.212	6	0.818646
1417-1427	No	0.039687	0.3493	0.06841	0.2809	0.1073	2.618	6	0.246836
1463-1478	No	0.983547	0.1131	0.1147	-0.00166	0.07734	0.0215	6	0.983547

Supplementary Table 9. Comparison on C3 level in T2DM and WT mice using unpaired two-tailed t test with Welch's correction.

Table Analyzed	C3-T2DM/WT		
Column B	T2DM		
vs.	vs.		
Column A	WT		
Unpaired t test with Welch's correction			
<i>P</i> value	<0.0001		
<i>P</i> value summary	****		
Significantly different (P < 0.05)?	Yes		
One- or two-tailed <i>P</i> value?	Two-tailed		
Welch-corrected <i>t</i>	df	t=7.42 0	df=9.86 6
How big is the difference?			
Mean of column A	292542		
Mean of column B	637335		
Difference between means (B - A) ± SEM	344792 ± 46470		
95% confidence interval	241060 to 448525		
R squared (eta squared)	0.848		

Supplementary Table 10. Comparison on C3b level in T2DM and WT mice using unpaired two-tailed t test with Welch's correction.

Table Analyzed	C3b-T2DM/WT		
Column B	T2DM		
vs.	vs.		
Column A	WT		
Unpaired t test with Welch's correction			
<i>P</i> value	0.0001		
<i>P</i> value summary	***		
Significantly different (P < 0.05)?	Yes		
One- or two-tailed <i>P</i> value?	Two-tailed		
Welch-corrected t	df	t=5.336	df=7.211
How big is the difference?			
Mean of column A	29888		
Mean of column B	181328		
Difference between means (B - A) ±			
SEM	151440 ± 28380		
95% confidence interval	84728 to 218153		
R squared (eta squared)	0.7979		
Data analyzed			
	column		
Sample size	A	8	
	column		
Sample size	B	8	

Supplementary Table 11. Comparison on Fold changes of C3b and C3 level in T2DM and WT mice using unpaired two-tailed t test with Welch's correction.

Table Analyzed	Fold changes-C3b/C3-T2DM/WT		
Column B	C3b		
vs.	vs.		
Column A	C3		
Unpaired t test with Welch's correction			
<i>P</i> value	0.0052		
<i>P</i> value summary	**		
Significantly different (<i>P</i> < 0.05)?	Yes		
One- or two-tailed <i>P</i> value?	Two-tailed		
Welch-corrected t	df	t=3.883	df=7.519
How big is the difference?			
Mean of column A	2.251		
Mean of column B	6.408		
Difference between means (B - A) ±			
SEM	4.157 ± 1.071		
95% confidence interval	1.661 to 6.654		
R squared (eta squared)	0.6673		

Supplementary Table 12. Comparison on serum iron, TIBC and saturation of Tf in T2DM and WT mice.

	Discovery?	P value	Mean of WT	Mean of T2DM	Difference	SE of difference	t ratio	df	q value
Serum Iron (ug/dl)	Yes	0.00036	173.3	284.8	-111.5	23.86	4.672	14	0.000483
TIBC (ug/dl)	Yes	0.000478	555.4	360.1	195.3	43.19	4.523	14	0.000483
% saturation of transferrin	Yes	<0.000001	30.7	80.36	-49.66	4.756	10.44	14	<0.000001

Significance analysis was performed using two tailed unpaired t test with False Discovery Rate correction with two-stage step-up procedure of Benjamini, Krieger & Yekutieli.

Supplementary Table 13. Body weight and blood glucose levels of mice (n =4, biological samples).

Mice	Body Weight (mg)	Blood Glucose(mg/dL)
T2DM-1	49.09	500
T2DM-2	47.04	586
T2DM-3	48.38	546
T2DM-4	45.64	586
WT-1	32.53	156
WT-2	34.90	172
WT-3	40.13	293
WT-4	39.12	185