Supplementary eMaterial

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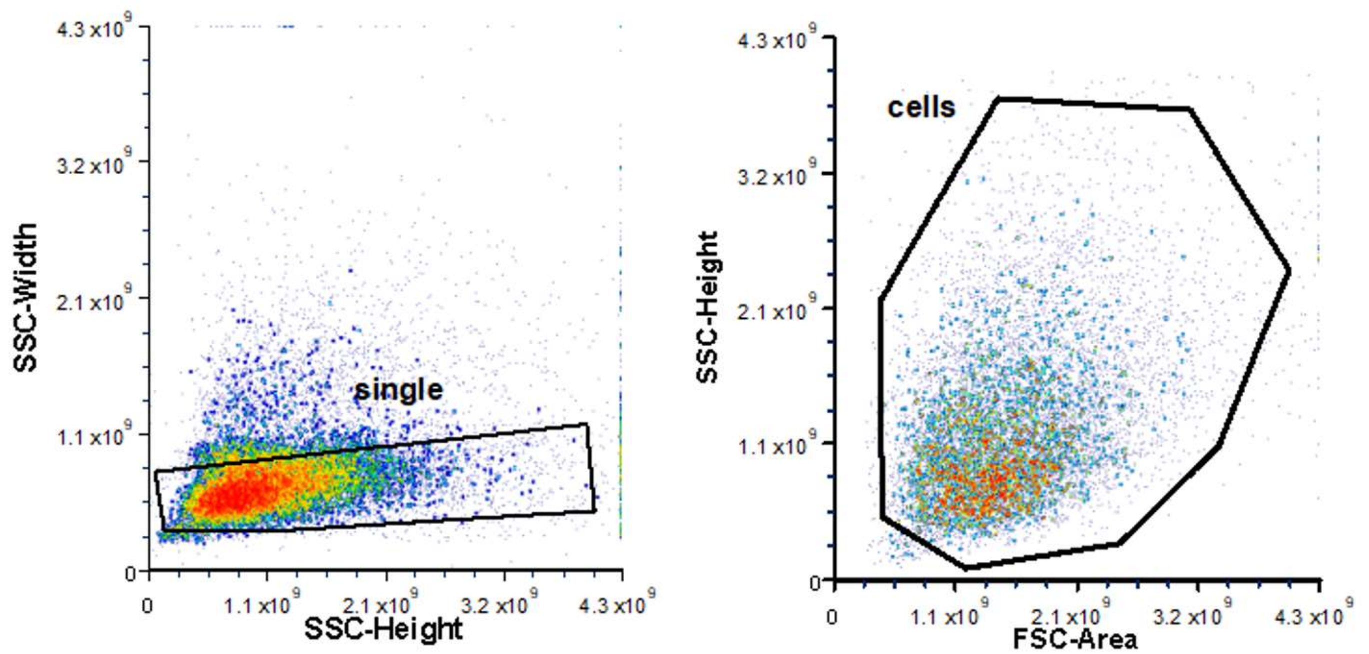
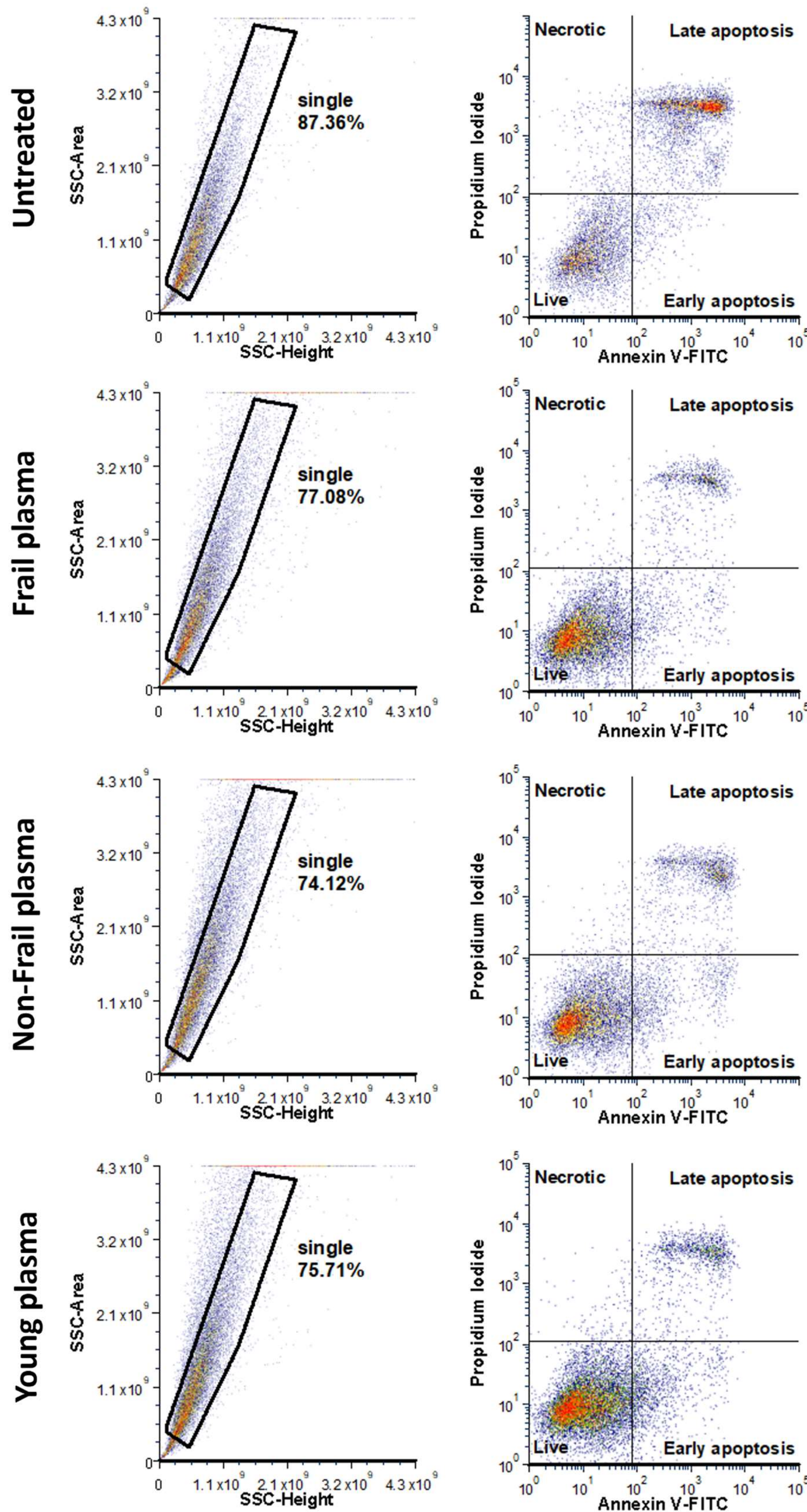
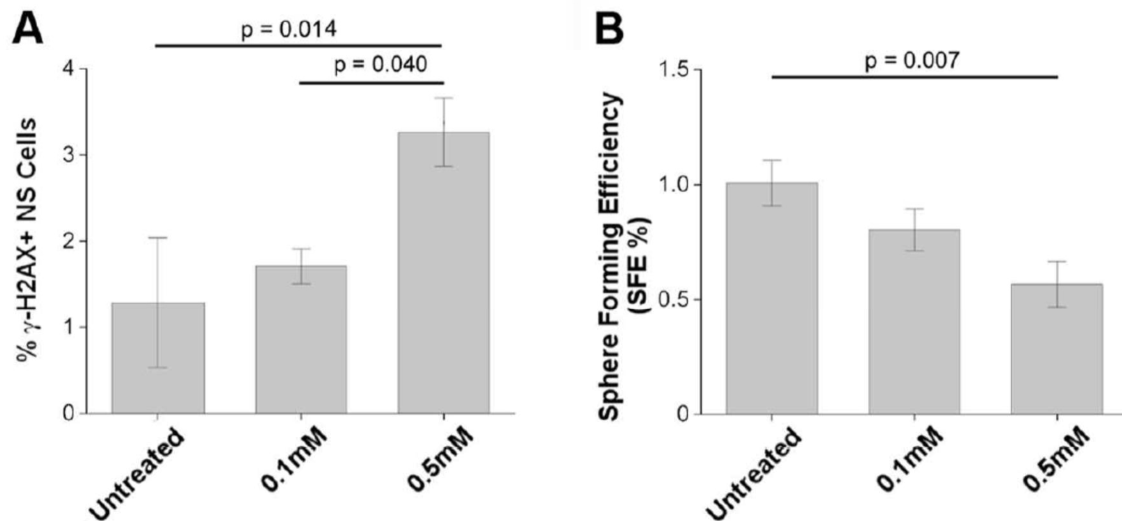


Supplementary eFigure 1. Representative flow cytometry dot plots of gating strategy. Applied to NS cells treated with plasma samples and analyzed for proliferation (Figure 2C), DNA damage (Figure 2E) and intracellular ROS (Figure 3B). Doublets were first excluded (left) and then the cell population (FSC vs SSC) was identified (right).



Supplementary eFigure 2. Representative flow cytometry dot plots related to Fig. 2D.
Dissociated NS cells derived from 12 NS cultures grown in presence of 17 frail, 16 non-frail, 16 young individual plasma sample. Each plasma sample was tested on 2-3 different independent NS cell cultures. The dissociated NS cells were stained with FITC Annexin V Apoptosis Detection Kit with Propidium Iodide (Biolegend, San Diego, CA, USA) following the manufacturer's instructions. Live, Early apoptotic, Late apoptotic, Necrotic cells were analyzed after doublets exclusion (dot plots on the left).



A) Percentage of γ-H2AX+ NS cells after treatment with different concentration of H2O2			
Cultures	Untreated	0.1 mM H2O2	0.5mM H2O2
C266	0.70	1.87	2.96
C292	2.29	1.82	3.04
C293	0.87	1.44	3.79

B) Percentage of SFE of renal cells growing as NS treated with different concentration of H2O2			
Cultures	Untreated	0.1 mM H2O2	0.5mM H2O2
C266	1.04	0.77	0.66
C292	0.88	0.72	0.44
C293	1.10	0.92	0.60

Supplementary eFigure 3. Human renal cells growing as nephrospheres (NS) for 10 days in presence of different concentrations of H2O2. (A) Percentage of DNA-damaged γ-H2AX+ NS cells after the indicated H2O2 treatments, flow cytometry data on dissociated NS cells. (B) Sphere Forming Efficiency (SFE %) of renal cells grown as NS, with the indicated concentration of H2O2, confocal microscopy data. In the graphs, the data shown in the table are expressed as mean±S.E.M. p value < 0.05 obtained with one-way ANOVA with Tukey's test for pairwise multiple comparison was considered significant (ORIGINPRO 2016 Software). Cultures from 3 different renal tissues (1 male, 2 females; median age 68 years, range 64-76 years. See Supplementary eTable 2).

Supplementary eTable 2. Nephrosphere cultures performed for treating allogeneic renal stem/progenitor cells

				Treatment: 17 frail, 16 nonfrail, 16 young plasma	Treatment: 7 frail, 4 nonfrail 4 young plasma	Treatment: 5 frail, 5 nonfrail, 5 young plasma	Treatment: H2O2 0.1mM H2O2 0.5 mM
Nephrosphere cultures established from healthy renal tissues	Sex of renal tissue	Age of patients (years)	Diagnosis for nephrectomy	Evaluated variables: SFE, Ki-67, proliferation, apoptosis, necrosis, DNA damage, flow cytometry data	Evaluated variables: DNA damage on cytospinned NS cells	Evaluated variables: intracellular ROS by DCF, flow cytometry data	Evaluated variables: DNA damage, SFE, flow cytometry data
C258	Male	66	Renal Cell Carcinoma clear cell G2		performed		
C263	Male	57	Chronic Pyelonephritis			performed	
C265	Male	75	Renal Cell Carcinoma clear cell G3			performed	
C266	Female	68	Renal Cell Carcinoma clear cell G2				performed
C284	Female	57	Renal Cell Carcinoma clear cell G1		performed		
C288	Female	61	Chronic Pyelonephritis	performed			
C292	Male	64	Renal Cell Carcinoma clear cell G4				performed
C293	Female	76	Urothelial Carcinoma High Grade				performed
C298	Male	71	Renal Cell Carcinoma clear cell G1	performed			
C299a-C299b	Female	64	Renal Cell Carcinoma clear cell G1	performed a-b			
C300	Female	48	Renal Cell Carcinoma clear cell G3-4	performed			
C302	Male	69	Renal Cell Carcinoma clear cell G4	performed			
C305	Female	76	Chronic Xanthogranulomatous pyelonephritis	performed			
C306	Female	67	Renal Cell Carcinoma clear cell G2	performed			
C308	Male	75	Renal Cell Carcinoma papillary type2 G2	performed			
C311	Male	48	Renal Cell Carcinoma clear cell G3	performed			
C312	Male	73	Renal Cell Carcinoma clear cell G2	performed			
C316	Male	77	Renal Cell Carcinoma clear cell G2			performed	
C318	Female	84	Renal Cell Carcinoma chromophobe			performed	
C319	Male	64	Oncocitoma Low Grade			performed	
C321	Male	81	Urothelial Carcinoma		performed		
	12 Males 9 Females	Median=68, Range 48-84		5 Males, 5 Females; Median=68 years, Range 48-76 years	2 Males, 1 Female; Median=66 years, Range 57-81 years	4 Males 1 Female; Median=75 years, Range 57-84 years	1 Male 2 Females; Median=68 years, Range 64-76 years

Supplementary eTable 4. Percentage of autologous DNA-damaged γ-H2AX+ cHPSC (γH2AXcHPSC)

Identification patients and plasma (Sex(Age)	PHENOTYPE	% autologous γH2AXcHPSC	Identification patients and plasma (Sex/Age)	PHENOTYPE	% autologous γH2AXcHPSC	Identification patients and plasma (Sex/Age)	PHENOTYPE	% autologous γH2AXcHPSC
16 (F/91)	FRAIL	0.3436 a	57 (M/69)	NON-FRAIL	1.4286 d	121 (M/28)	YOUNG	0.1684 a
17 (M/70)	FRAIL	0.6468 a	58 (F/67))	NON-FRAIL	0 a,d,e	122 (M/30)	YOUNG	0.4706 a
19 (F/84)	FRAIL	0.5338 a,d,e	59 (M/85)	NON-FRAIL	0.303 a,d,e	123 (F/29)	YOUNG	3.44 a,d,e
20 (M/84)	FRAIL	2.7439 a	60 (M/87)	NON-FRAIL	0.5427 a,d,e	124 (F/30)	YOUNG	0.3891 a
21 (F/88)	FRAIL	10.48 a	61 (F/66)	NON-FRAIL	0.5357 a,d,e	125 (F/27)	YOUNG	0.2444 a
22 (M/90)	FRAIL	2.4691 b,c,d	62 (M/66)	NON-FRAIL	2.5974 a,d,e	126 (M/34)	YOUNG	0.1074 a,d,e
23 (F/85)	FRAIL	7.5145 a,b,d,e	63 (M/87)	NON-FRAIL	1.4085 a,d,e	127 (M/32)	YOUNG	1.107 a
24 (F/87)	FRAIL	3.2258 a,d,e	64 (M/70)	NON-FRAIL	2.1898 a	128 (F/31)	YOUNG	0,3953 a
25 (F/88)	FRAIL	3.3298 a,b,d,e	65 (M/71)	NON-FRAIL	0.625 a	129 (M/27)	YOUNG	0 a,d,e
26 (F/82)	FRAIL	0 a,d,e	66 (F/73)	NON-FRAIL	0.6173 a,d,e	130 (M/28)	YOUNG	0 a
27 (F/78)	FRAIL	3.7607 a,d,e	67 (F/74)	NON-FRAIL	1.2987 a,b,d,e	131 (F/29)	YOUNG	0 a,d,e
29 (M/90)	FRAIL	3.3835 a,b,d,e	68 (F/67)	NON-FRAIL	0.2257 a,d,e	132 (F/27)	YOUNG	0 a,b
30 (M/75)	FRAIL	5.0209 a	70 (M/74)	NON-FRAIL	0 a	134 (M/27)	YOUNG	0 a
31 (F/78)	FRAIL	4.7452 a	71 (F/85)	NON-FRAIL	0 a	135 (M/28)	YOUNG	0.4888 a
32 (M/88)	FRAIL	14.1791 a,d,e	72 (M/75)	NON-FRAIL	1.6051 b,d	136 (F/26)	YOUNG	0.2153 a,b
33 (F/80)	FRAIL	2.6316 a,d,e	73 (M/70)	NON-FRAIL	0.3231 b,d	137 (F/34)	YOUNG	0.2874 c,d
34 (F/85)	FRAIL	1.6514 a	74 (M/70)	NON-FRAIL	0.6494 a	138 (F/29)	YOUNG	0.363 b,c,d
37 (F/72)	FRAIL	3.4483 c,d	75 (M/65)	NON-FRAIL	0.2681 d	139 (M/27)	YOUNG	0.1127 c,d
38 (M(86)	FRAIL	1.1494 b,c,d	76 (M/85)	NON-FRAIL	0.7426 a,b	140 (F/28)	YOUNG	0 c
40 (F/92)	FRAIL	4.3478 b,c	77 (F/79)	NON-FRAIL	0.4348 a	141 (F/26)	YOUNG	0 b,c,d
41 (M/79)	FRAIL	0.8287 b,c,d	78 (M/82)	NON-FRAIL	1.6393 c,d	146 (F/30)	YOUNG	0 a,d,e
48 (F/85)	FRAIL	3.3493 a	79 (F/73)	NON-FRAIL	0.209 c,d			
			82 (F/74)	NON-FRAIL	1.4706 c,d			
			83 (M/82)	NON-FRAIL	0.2865 c,d			
			84 (F/84)	NON-FRAIL	0 c,d			

Note. These percentages were previously analyzed (*J Gerontol A Biol Sci Med Sci*. 2022;27:1279-1286) in the same plasma used for this research.
a: percentage values of γH2AXcHPSC present in the same plasma of subjects shown in Figure 2G. b: percentage of γH2AXcHPSC present in the same plasma used for the treatments shown in Figure 2 I,J,K. c: percentage of γH2AXcHPSC present in the same plasma used for the treatments shown in Figure 3B. d: percentage values of γH2AXcHPSC of the individuals shown in the PCA of Figure 4A. e: percentage values of γH2AXcHPSC of the individuals shown in the PCA of Figure 4B.
Sex: F=Female; M=Male. Age: years

Supplementary eTable 5.

A. Plasma oxysterol and cholesterol precursor concentrations shown in Fig 3A

Identification patient and plasma (Sex/Age)	PLASMA PHENOTYPE	27-hydroxy cholesterol (27-OHC) µg/L	LATHOSTEROL µg/L	LANOSTEROL µg/L	24-hydroxy cholesterol (24-OHC) µg/L
19 (F/84)	frail	109.31	892.88	70.29	44.21
22 (M/90)	frail	149.06	1425,08	127.53	46.94
23 (F/84)	frail	43.38	522.56	44.09	25.96
24 (F/87)	frail	85.88	554.48	60.07	42.55
25 (F/88)	frail	149.87	1726.16	65.39	67.90
26 (F/82)	frail	105.50	1007.76	70.13	56.70
27 (F/78)	frail	118.35	1113.92	31.40	52.78
29 (M/90)	frail	51.03	671.32	47.94	33.73
30 (M/75)	frail	64.91	1086.52	56.06	24.23
32 (M/88)	frail	161.30	1945.92	104.38	43.20
33 (F/80)	frail	82.76	432.04	16.28	29.56
35 (M/85)	frail	59.57	996.04	97.73	24.59
37 (F/72)	frail	129.37	1536.56	181.83	50.04
38 (M/86)	frail	160.72	1732,08	83.76	47.70
40 (F/92)	frail	137.60	1131.04	109.92	65.20
41 (M/79)	frail	111.28	1317.04	91.69	35.53
42 (F/84)	frail	85.14	1327.48	152.55	36.76
57 (M/69)	non-frail	132.87	1521.04	59.23	25.52
58 (F/67)	non-frail	134.40	1873.20	100.38	56.41
59 (M/85)	non-frail	118.52	1330.84	50.91	57.71
60 (M/87)	non-frail	105.74	1493.16	87.79	30.24
61 (F/66)	non-frail	178.60	2374.24	190.18	50.47
62 (M/66)	non-frail	186.35	2335.36	160.95	66.13
63 (M/87)	non-frail	177.11	2140.56	96.78	43.74
66 (F/73)	non-frail	173.06	1478.48	62.08	76.10
67 (F/74)	non-frail	140.05	1638.96	68.15	65.56
68 (F/67)	non-frail	279.92	2753.96	166.02	88.67
72 (M/75)	non-frail	227.73	2733.24	155.76	73.94
73 (M/70)	non-frail	216.55	2270.04	156.72	91.26
75 (M/65)	non-frail	258.23	2779.88	156.95	70.02
78 (M/82)	non-frail	228.45	2856.28	185.98	55.48
79 (F/73)	non-frail	146.81	2056.40	111.10	58.61
82 (F/74)	non-frail	126.89	1430.96	55.36	49.82
83 (M/82)	non-frail	193.87	1908.96	106.15	63.22
84 (F/84)	non-frail	155.55	1634.24	119.28	41.33
123 (F/29)	young	137.36	1721.52	60.35	67.79
126 (M/34)	young	131.21	1601.60	50.29	53.06
129 (M/27)	young	153.92	1868.04	81.17	69.88
131 (F/29)	young	127.19	1493.92	64.69	58.82
132 (F/27)	young	114.82	1471.68	41.93	59.87
137 (F/34)	young	171.26	1898.76	77.34	79.20
138 (F/29)	young	148.34	2129.28	82.88	76.39
139 (M/27)	young	161.84	1941.00	67.34	76.61
141 (F/26)	young	113.19	1344.60	56.06	49.97
146 (F/30)	young	134.27	1504.36	50.73	65.70

(Continued)

Supplementary eTable 5 (continued)

B. Plasma oxysterol concentrations previously analyzed (*J Gerontol A Biol Sci Med Sci*. 2022;27:1279-1286)

Identification patient and plasma (Sex/Age)	PLASMA PHENOTYPE	7β-hydroxy-cholesterol (7βOHC) µg/L	7-keto-cholestero (7KC) µg/L	5β,6β-epoxy-cholsterol (5β6β-EC) µg/L	5α,6α-epoxy-cholesterol (5α6α-EC) µg/L	3β,5α,6β-3hydroxy-cholesterol (3β,5α,6β-3OHC) µg/L
19 (F/84)	frail	23	42.14	54.74	50.23	31.98
22 (M/90)	frail	16	33.82	44.90	41.57	29.34
23 (F/84)	frail	15	25	21.51	18.51	7.96
24 (F/87)	frail	17.92	37.86	38.68	35.66	15.90
25 (F/88)	frail	18.52	39.38	46.26	29.13	25.18
26 (F/82)	frail	19.12	36.42	48.14	38.44	29.02
27 (F/78)	frail	14.08	30.3	39.76	35.16	21.26
29 (M/90)	frail	24.6	47.64	61.90	49.86	30.50
30 (M/75)	frail	14.36	24.54	29.71	19.31	8.02
32 (M/88)	frail	16.08	28.76	28.55	24.07	11.20
33 (F/80)	frail	25.64	52.54	58.32	48.88	38.30
35 (M/85)	frail	12.88	25.16	22.51	24.92	9.92
37 (F/72)	frail	21.76	43	57.59	40.19	33.56
38 (M/86)	frail	18.24	31.7	55.43	43.89	26.36
40 (F/92)	frail	24.6	43.58	50.84	43.55	19.04
41 (M/79)	frail	23.36	44.16	46.33	46.03	26.70
42 (F/84)	frail	25.48	47.06	50.01	52.59	27.02
57 (M/69)	non-frail	8.12	21.98	4.86	17.09	6.24
58 (F/67)	non-frail	9	34.98	9.87	23.33	13.46
59 (M/85)	non-frail	9.8	24.28	14.72	22.32	16.84
60 (M/87)	non-frail	8.44	30.24	6.75	25.26	11.12
61 (F/66)	non-frail	11.36	37.5	14.30	37.62	19.10
62 (M/66)	non-frail	8.96	26.14	16.03	30.05	17.10
63 (M/87)	non-frail	6.96	17.02	10.01	21.08	9.30
66 (F/73)	non-frail	11.12	35.74	19.19	41.08	18.02
67 (F/74)	non-frail	8	29.14	8.99	17.93	8.20
68 (F/67)	non-frail	10.44	29.32	15.89	33.08	18.70
72 (M/75)	non-frail	7.04	22.76	5.21	16.81	6.66
73 (M/70)	non-frail	8.56	24.96	10.69	26.03	9.98
75 (M/65)	non-frail	8.72	29.2	10.82	29.32	13.06
78 (M/82)	non-frail	8.28	23.14	15.05	25.01	14.84
79 (F/73)	non-frail	6.6	19.44	3.33	12.94	4.76
82 (F/74)	non-frail	7.16	26.48	5.82	19.07	9.66
83 (M/82)	non-frail	8.56	22.72	6.96	23.05	9.74
84 (F/84)	non-frail	6.48	15.48	7.51	11.98	6.34
123 (F/29)	young	5.4	20.18	6.99	13.09	8.10
126 (M/34)	young	6.56	29.04	10.19	19.25	10.70
129 (M/27)	young	6.36	29.16	9.51	25.81	8.90
131 (F/29)	young	5.24	18.28	5.95	8.77	6.50
132 (F/27)	young	6.2	27.08	10.88	29.23	10.92
137 (F/34)	young	6.88	29.74	12.35	26.75	12.12
138 (F/29)	young	5.76	21.38	9.54	15.45	10.54
139 (M/27)	young	5.68	25.9	8.37	18.11	8.58
141 (F/26)	young	5.8	24.52	6.48	13.75	5.28
146 (F/30)	young	6.32	30.18	7.95	10.37	7.08

Note. Each analyte of this Supplementary eTable was evaluated in the same batch of plasma. All the concentrations of this analytes were all used as variables of the oxidative status domain for the PCA shown in Figure 4, as summarized in Supplementary Table 1B.
Sex: F=Female; M=Male. Age: years

Supplementary eTable 6.

Intracellular ROS in NS cells

ID Culture (SEX/AGE)	ID plasma donors	PLASMA PHENOTYPE	SEX plasma donors	AGE plasma donors	MEDIAN of intracellular ROS fluorescence
C263 (M/57)		untreated			303.24
C263	137	young	F	34	243.04
C263	82	non-frail	F	74	284.08
C263	38	frail	M	86	634.39
C265 (M/75)		untreated			258.5
C265	141	young	F	26	357.1
C265	83	non-frail	M	82	311.94
C265	40	frail	F	92	316.58
C316 (M/77)		untreated			504.48
C316	138	young	F	29	493.03
C316	84	non-frail	F	84	605.15
C316	22	frail	M	90	686.15
C318 (F/84)		untreated			219.85
C318	139	young	M	27	256.02
C318	78	non-frail	M	82	260.00
C318	37	frail	F	72	537.59
C319 (M/64)		untreated			131.29
C319	140	young	F	28	118.94
C319	79	non-frail	F	73	100.06
C319	41	frail	M	79	162.24

Ratio of medians treated/untreated

ID Culture (SEX/AGE)	C263 (M/57)	C265 (M/75)	C316 (M/77)	C318 (F/84)	C319 (M/64)	Mean of ratios treated/ untreated	SD
Untreated	1.00	1.00	1.00	1.00	1.00	1.00	0.00
Young	0.80	1.38	0.98	1.16	0.91	1.05	0.23
Non-frail	0.94	1.21	1.20	1.18	0.76	1.06	0.20
Frail	2.09	1.23	1.36	2.45	1.24	1.67	0.56

Note. Sex: F=Female; M=Male. Age: years

Supplementary eTable 8. Principal component analysis (PCA) related to Figure 4

	PCs	Explained Variance (%)	Cumulative Explained Variance (%)	Dominating Original Variables
Related to Figure 4A - Variables concerning: oxidative status, cytokines,γ-H2AXcHPSC	PC1	30	30	CD40, IL-6, TGFα, IL-4, IL-8, TNFα, LATHOSTEROL*, 7BOHC, 7KC, 5B6B-EPOXY, 5A6A-EPOXY, TRIOL, 27OHC*, 24OHC*
	PC2	12	42	CCL2*, IL-4*, FLT-3LIGAND*, CCL4*, IL-10*
Related to Figure 4B - Variables concerning: oxidative status, cytokines, γ-H2AXcHPSC, SFE, γ-H2AXNSC	PC1	32	30	CD40*, IL-6*, CXCL10*, TGFα*, IL-4*, TNFα*, LATHOSTEROL, 7BOHC*, 7KC*, 5B6B-EPOXY*, 5A6A-EPOXY*, TRIOL*, 27OHC, 24OHC, γ-H2AXRSC*, SFE
	PC2	12	44	IL-1A*, CXCL10, TGFα, CCL2, IL-4, IL-8, FLT-3LIGAND, LATHOSTEROL, LANOSTEROL, 27OHC
Related to Figure 4C - Variables concerning: oxidative status	PC1	58	58	LATHOSTEROL, 7BOHC*, 7KC*, 5B6B-EPOXY*, 5A6A-EPOXY*, TRIOL*, 27OHC, 24OHC
	PC2	27	85	LATHOSTEROL, LANOSTEROL, 5A6A-EPOXY, TRIOL, 27OHC, 24OHC
Related to Figure 4D - Variables concerning: cytokines	PC1	25	25	CD40, IL-1α, IL-6, CXCL10, IL-4, IL-8, IL-15, TNFα, IL-1β, IL-2
	PC2	12	37	CX3CL1, CD40, IL-6*, CCL11, FLT-3LIGAND, CCL4, IL-1β*, IL-2*, IL-10
Related to Figure 4E - Variables concerning: oxidative status, cytokines	PC1	31	31	CD40, IL-6, TGFα, IL-4, IL-8, TNFα, LATHOSTEROL*, 7BOHC, 7KC, 5B6B-EPOXY, 5A6A-EPOXY, TRIOL, 27OHC*, 24OHC*
	PC2	12	43	CCL2*, IL-4*, FLT-3LIGAND*, CCL4*, IL-10*

Note. First two principal components (PCs) of each considered variable combination described by the proportion of cumulative explained variance and dominating original variables (* indicates negative load).. The panels of Figure 4 to which the data were referred are reported.

Supplementary eMethods

Comorbid conditions
The comorbid conditions considered in this research are routinely recorded in the Acute Geriatrics Unit for all inpatients or outpatients. To be recorded, each disease needed to be confirmed by the participants' medical history. Here, some additional details about these conditions are provided. Heart failure was identified if there was any record of access to the emergency department or hospitalization with this diagnosis, along with evidence of active drug treatment. Ischemic heart disease encompassed all previous episodes defined as myocardial infarction, as confirmed by electrocardiogram and/or changes in the levels of cardiac enzymes, or any history of coronary artery bypass graft or coronary stenting. Peripheral vascular disease included all documented instances of *claudicatio intermittens*, a history of bypass interventions on peripheral arteries, and all abdominal or thoracic aneurysms that had not undergone surgical treatment. Cerebrovascular diseases included transient ischemic attack (TIA), minor stroke or stroke with minor or no *sequelae*, and strokes with evident *sequelae*. Epilepsy included any history of seizures controlled by a specific drug therapy. Chronic obstructive pulmonary disease (COPD) and asthma were recorded if previously certified by a specialist. Solid tumors and leukemia were recorded if a medical history emerged within the previous 5 years. However, patients needed to be free from active treatments for at least one year, and clinical remission had to be certified by a specialist for enrollment in this study. Rheumatic diseases included rheumatoid arthritis, connective tissue disease, and *polymyalgia rheumatica*. Gastrointestinal diseases were recorded in cases of a history of peptic ulcer or gastrointestinal bleeding. We excluded inflammatory bowel diseases because of their chronic inflammatory phenotype and the recurrent need for specific drug treatments. Regarding renal insufficiency, individuals with moderate or severe chronic kidney disease were not enrolled. However, individuals with a diagnosis of mild (stage 2, 60-89 mL/min/1.73 m^2) or mild–moderate (stage 3a, 45-59 mL/min/1.73 m^2) reduction of the glomerular filtration rate with a normal to mildly increased albumin:creatinine ratio (<3 mg/mmol) were included. For the purpose of overall data integration, the older adults were stratified by the comorbidity cutoff 2, generating subgroups with ≤2 and >2 pathologies (1).

Plasma Cytokines quantification
The concentrations (pg/mL) of 38 cytokines (Supplementary eTable 7A) in the plasma of frail, non-frail, and young individuals were evaluated using the Human Magnetic Luminex Screening Assay, performed as a service by Labospace s.r.l. (Milano, Italy). At the time of the analysis, the aliquots of collected plasma, needed a 2-fold dilution, except for analyses of the RANTES and PDGF BB, which needed a 50-fold dilution. The concentrations of all cytokines, tested in duplicate in each plasma sample, were evaluated according to the manufacturer's instructions. Briefly, 50 µl of plasma sample or standard (for the standard curve preparation) followed by 50 µl of Microparticle Cocktail were added to the wells of a 96-well plate and incubated for 2 hours at RT on a shaker. Cytokine-specific antibodies were precoated onto magnetic microparticles embedded with fluorophores at set ratios for each unique microparticle region. The immobilized antibodies bound the cytokines of interest, and each well was then washed three times with wash buffer. After washing, 50 µL of diluted biotinylated antibody cocktail specific to the cytokines of interest was added to each well. This was followed by incubation for 1 hour at RT on a shaker, after which each well was washed three times with wash buffer. Following washes, 50 µL of diluted streptavidin-phycoerythrin conjugate (streptavidin-PE), which binds to the biotinylated antibody, was added to each well. The mixture was then incubated for 30 minutes at RT on a shaker, and again, each well was washed three times with wash buffer. The microparticles were resuspended in buffer, and the plate was read within 90 minutes on a Bio-Rad Bio-Plex100 (Bio-Rad). The specific characteristics of each kit used are summarized in Supplementary eTable 7A.
The Quality Control on the instrument tests have been performed using:
Bio-Plex Calibration kit code 171203060 lot 64270078 and Bio-Plex Validation kit code 171203001 lot 64239064.
All the data obtained have been checked by the technical Dept. of Labospace. The Quality Control parameters to be satisfied were: the % ratio between the values of the standard curve of each analyte with the values provided by the manufacturer of the kits used, and the similarity of the replicates evaluated by the Coefficient of Variation % (CV%) that had not to exceed 15%. For each cytokine the mean Coefficient of Variation % has been reported in the Supplementary eTable 7A

Statistical analysis, principal component analysis (PCA)
The different variables were checked to see whether there were differences among female, male, female+male (combined gender) in each single group (frail, non-frail, young). No differences were evidenced, therefore, were presented the data of combined gender to keep more robust the statistically analysis.

In PCA, the variables, first examined individually for their differences in the frail, non-frail and young groups, were then also examined collectively through principal component analysis (PCA) (2). The focus of PCA was on understanding the combined behavior of all variables rather than evaluating each individually. We previously conducted a sensitivity analysis by incorporating age and sex variables into the principal component analysis (PCA) to evaluate their impact on the explained variability and overall data structure. Our findings indicated that these two variables did not alter the overall data structure and we decided not to include age and sex in PCA. Therefore, the variables used were grouped into 5 different domains: i) plasma oxidative status (oxysterols and cholesterol

precursors), ii) plasma inflammatory cytokines, iii) DNA-damaged homologous cHPSCs (herein referred as to γ-H2AXcHPSCs), iv) SFEs, and v) DNA-damaged allogeneic NS cells (herein referred as to γ-H2AXNSCs). The intent was to uncover both domain-specific characteristics and overarching trends. In the different domains, the variables considered promising for expressing differences, due to their biological interest were i) all analyzed oxysterols and cholesterol precursors; ii) inflammatory cytokines (CX3CL1, CXCL9, CXCL10, CCL2, CD40, TGFa, TNFa, TNFb, IL-1a, IL-1b, IL-2, IL-4, IL-6, IL-8, IL-10, IL-15, IL17, CCL4, CCL11, FLT-3LIGAND, RANTES); iii) γ-H2AXcHPSC; iv) SFE; and v) γ-H2AXNSC. This multifaceted approach allowed for a thorough and nuanced understanding of the data structure. Prior to PCA, all variables were standardized to ensure equal contribution to the overall variance. Significant variables and potential outliers were identified through PCA. The highly contributing (dominating) variables were defined as those with loadings in absolute value greater than half the maximum absolute loading for a given principal component (2). The sign of the loading was also considered for interpretation. The relationship between variables was also interpreted based on the loadings on the PCs. PCA was performed through R software, version 4.1.2, 2021-11-01, using the *prcomp* function (R Core Team, 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. *http://www.R-project.org/*).

Supplementary eReferences

1. Johnston MC, Crilly M, Black C, Prescott GJ, Mercer SW (2018). Defining and measuring multimorbidity: a systematic review of systematic reviews. Eur J Public Health, 29:182–189.

2. Jolliffe IT. Principal component analysis Second Edition. New York: Springer; 2002.