Supplemental Material

- eTable1
- eTable2
- eFigure1
- eFigure2
- eFigure3
- eFigure4

eTable 1. Hematological parameters of fresh blood. Blood collected in the study groups of Table 1 according to the frail, pre-frail, non-frail status and in a group of 46 young.

	Frail n=40	Pre-frail n=13	Non-frail n=32	Young n=46	p ^a	p ^b	p ^c
Hemoglobin ^{d e} g/dL	11.1 ± 1.6	12.9 ± 1.3	14.1 ± 1.0	14.7±1.3	<.004	<.001	<.005
White blood cells d $n/\mu L$	6919±2002	6440±2309	6350±1213	5901±1282	NS	NS	NS
Lymphocytes f n/µL (%)	1654 ± 658 (24.0 ± 12.0)	1898 ± 696 (29.5±12.4)	2314 ± 508 (35.7±5.3)	1908±536 (32.5±6.6)	<.002	NS	NS
Granulocytes % Neutrophils Eosinophils Basophils	58.6±15.5 3.2±2.7 0.6±0.5	58.4±12.6 3.3±1.1 0.8±0.7	52.6±7.1 3.1±1.2 0.5±0.4	55.0±6.4 2.9±1.6 0.8±0.3	NS NS NS	NS NS NS	NS NS NS
Monocytes %	10.2±6.5	8.3±2.1	7.8±1.6	8.8±1.9	NS	NS	NS
cHPSC Lin1-/CD45 on PBMC of fresh bl %		N.A.	0.071±0.06	0.062±0.03		NS	

Notes. Values are expressed as mean + SD. Significant differences in the comparison with young subjects are reported below when positive.

^a comparison between pre-frail vs. frail.

 $^{^{\}rm b}$ comparison between non-frail vs. frail.

^c comparison between non-frail vs. pre-frail

^d comparison between frail vs. young. *p < 0.05

^e comparison between pre-frail vs. young. *p < 0.05

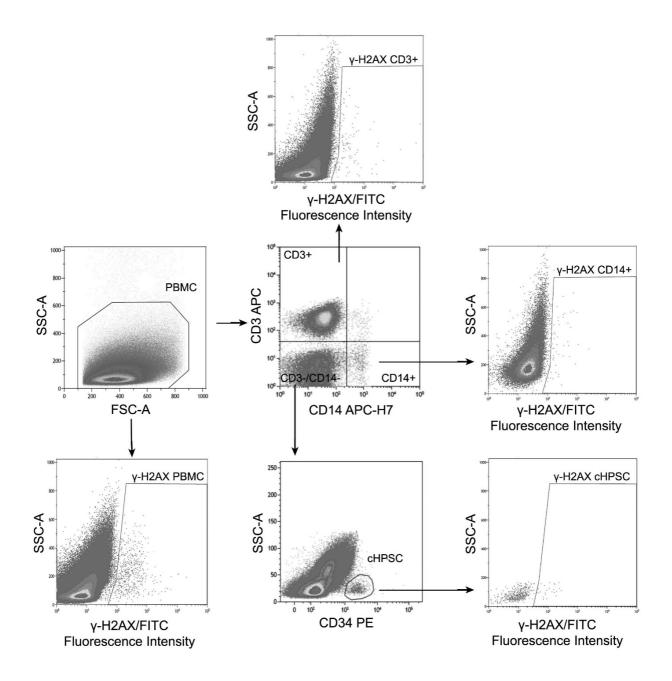
f comparison between non-frail vs. young. *p < 0.05

[§]Data obtained in randomly chosen frail (5 samples), non-frail (4 samples), young (5 samples). N.A., not available

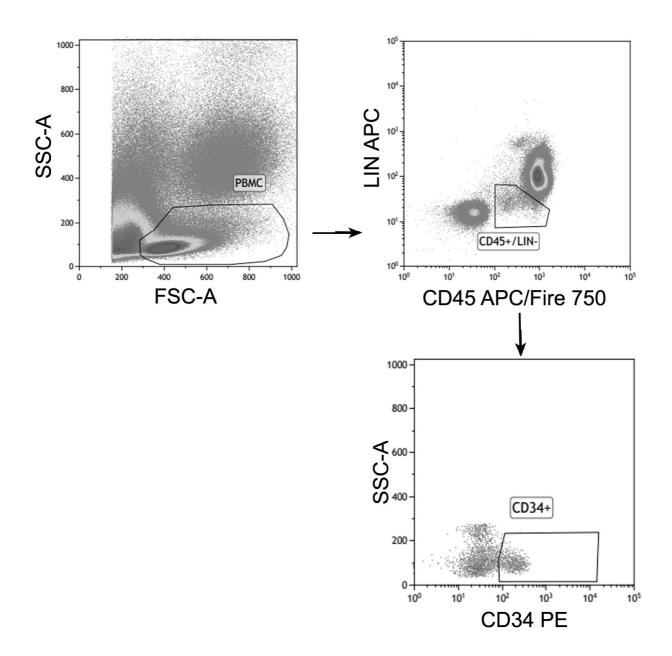
eTable 2. Description of chronic diseases and classes of drugs. Study groups of Table 1 according to the frail, pre-frail, non-frail status.

Diabetes mellitus 16 (40) 2 (13) Dyslipidemia 12 (30) 3 (23) Ischemic heart disease 11 (27.5) 2 (13) Cerebrovascular disease 10 (25) 1 (7) Renal insufficiency 10 (25) 2 (13) Heart failure 8 (20) 1 (7) Obstructive respiratory disease 8 (20) 0 (0) Peripheral vascular disease 8 (20) 0 (0) Epilepsy 3 (7.5) 0 (0) Solid tumor 3 (7.5) 1 (7) Rheumatic disease 2 (5) 2 (13) Asthma 1 (2.5) 0 (0) Leukemia 1 (2.5) 0 (0) Classes of drugs. n (%) 1 (2.5) 0 (0) Antihypertensives 33 (82.5) 11 (3) Proton-pump inhibitors 24 (60.0) 6 (4) Oral antidiabetic agents 12 (30) 2 (1) Statins 12 (30) 6 (4) Folic acid supplements 10 (25) 1 (7) Antiepileptic drugs 3 (7.5) 0 (0)	3.1) 12 (37.5) 5.4) 3 (9.4) .7) 0 (0.0) 5.4) 1 (3.1)
Diabetes mellitus 16 (40) 2 (13) Dyslipidemia 12 (30) 3 (23) Ischemic heart disease 11 (27.5) 2 (13) Cerebrovascular disease 10 (25) 1 (7) Renal insufficiency 10 (25) 2 (13) Heart failure 8 (20) 1 (7) Obstructive respiratory disease 8 (20) 0 (0) Peripheral vascular disease 8 (20) 0 (0) Epilepsy 3 (7.5) 0 (0) Solid tumor 3 (7.5) 1 (7) Rheumatic disease 2 (5) 2 (13) Asthma 1 (2.5) 0 (0) Leukemia 1 (2.5) 0 (0) Classes of drugs. n (%) 1 (2.5) 0 (0) Antihypertensives 33 (82.5) 11 (3) Proton-pump inhibitors 24 (60.0) 6 (4) Oral antidiabetic agents 12 (30) 2 (1) Statins 12 (30) 6 (4) Folic acid supplements 10 (25) 1 (7) Antiepileptic drugs 3 (7.5) 0 (0)	5.4) 1 (3.1) 3.1) 12 (37.5) 5.4) 3 (9.4) .7) 0 (0.0) 5.4) 1 (3.1)
Dyslipidemia 12 (30) 3 (2) Ischemic heart disease 11 (27.5) 2 (1) Cerebrovascular disease 10 (25) 1 (7) Renal insufficiency 10 (25) 2 (1) Heart failure 8 (20) 1 (7) Obstructive respiratory disease 8 (20) 0 (0) Peripheral vascular disease 8 (20) 0 (0) Epilepsy 3 (7.5) 0 (0) Solid tumor 3 (7.5) 2 (1) Rheumatic disease 2 (5) 2 (1) Gastrointestinal disease 2 (5) 2 (1) Asthma 1 (2.5) 0 (0) Leukemia 1 (2.5) 0 (0) Classes of drugs. n (%)	3.1) 12 (37.5) 5.4) 3 (9.4) .7) 0 (0.0) 5.4) 1 (3.1)
Ischemic heart disease	5.4) 3 (9.4) .7) 0 (0.0) 5.4) 1 (3.1)
Cerebrovascular disease 10 (25) 1 (7.5) Renal insufficiency 10 (25) 2 (15) Heart failure 8 (20) 1 (7.5) Obstructive respiratory disease 8 (20) 1 (7.5) Peripheral vascular disease 8 (20) 0 (0.5) Epilepsy 3 (7.5) 0 (0.5) Solid tumor 3 (7.5) 1 (7.5) Rheumatic disease 2 (5) 2 (15) Gastrointestinal disease 2 (5) 2 (15) Asthma 1 (2.5) 0 (0.5) Leukemia 1 (2.5) 0 (0.5) Classes of drugs. n (%) (%) Antihypertensives 33 (82.5) 11 (3.5) Proton-pump inhibitors 24 (60.0) 6 (4.6) Oral antidiabetic agents 12 (30) 2 (1.5) Statins 12 (30) 6 (4.6) Folic acid supplements 10 (25) 1 (7.5) Antiepileptic drugs 3 (7.5) 0 (0.5) Vitamin D supplements 5 (12.5) 1 (7.5) Bisphosphonates 1 (2.5) 0 (.7) 0 (0.0) 5.4) 1 (3.1)
Renal insufficiency 10 (25) 2 (15) Heart failure 8 (20) 1 (7. Obstructive respiratory disease 8 (20) 1 (7. Peripheral vascular disease 8 (20) 0 (0. Epilepsy 3 (7.5) 0 (0. Solid tumor 3 (7.5) 1 (7. Rheumatic disease 2 (5) 2 (15) Gastrointestinal disease 2 (5) 2 (15) Asthma 1 (2.5) 0 (0. Leukemia 1 (2.5) 0 (0. Classes of drugs. n (%) 2 Antihypertensives 33 (82.5) 11 (8. Proton-pump inhibitors 24 (60.0) 6 (4. Oral antidiabetic agents 12 (30) 2 (15) Statins 12 (30) 6 (4. Folic acid supplements 10 (25) 1 (7. Antiepileptic drugs 3 (7.5) 0 (0. Vitamin D supplements 7 (17.5) 0 (0. Calcium supplements 5 (12.5) 1 (7. Bisphosphonates 1 (2.5) 4 (30. Antiarrhythmics 11 (27.5) 4 (30. Oral an	5.4) 1 (3.1)
Heart failure	
Obstructive respiratory disease 8 (20) 1 (7.2) Peripheral vascular disease 8 (20) 0 (0.2) Epilepsy 3 (7.5) 0 (0.2) Solid tumor 3 (7.5) 1 (7.2) Rheumatic disease 3 (7.5) 2 (1.2) Gastrointestinal disease 2 (5) 2 (1.2) Asthma 1 (2.5) 0 (0.2) Leukemia 1 (2.5) 0 (0.2) Classes of drugs. n (%) 33 (82.5) 11 (3.2) Antihypertensives 33 (82.5) 11 (3.2) Proton-pump inhibitors 24 (60.0) 6 (4.2) Oral antidiabetic agents 12 (30) 2 (1.2) Statins 12 (30) 6 (4.2) Folic acid supplements 10 (25) 1 (7.2) Antiepileptic drugs 3 (7.5) 0 (0.2) Vitamin D supplements 7 (17.5) 0 (0.2) Calcium supplements 5 (12.5) 1 (7.2) Bisphosphonates 1 (2.5) 0 (0.2) Antiarrhythmics 11 (27.5) 4 (30.2) Oral anticoagulants	.7) 0 (0.0)
Peripheral vascular disease 8 (20) 0 (0.00) Epilepsy 3 (7.5) 0 (0.00) Solid tumor 3 (7.5) 1 (7.00) Rheumatic disease 3 (7.5) 2 (1.00) Gastrointestinal disease 2 (5) 2 (1.00) Asthma 1 (2.5) 0 (0.00) Leukemia 1 (2.5) 0 (0.00) Classes of drugs. n (%) 33 (82.5) 11 (8.00) Antihypertensives 33 (82.5) 11 (8.00) 6 (4.00) Oral antidiabetic agents 12 (30) 2 (1.00) 6 (4.0	
Epilepsy 3 (7.5) 0 (0.00) Solid tumor 3 (7.5) 1 (7.00) Rheumatic disease 3 (7.5) 2 (1.00) Gastrointestinal disease 2 (5) 2 (1.00) Asthma 1 (2.5) 0 (0.00) Leukemia 1 (2.5) 0 (0.00) Classes of drugs. n (%) 33 (82.5) 11 (3.00) Antihypertensives 33 (82.5) 11 (3.00) 6 (4.00) Oral antidiabetic agents 12 (30) 2 (1.00) 6 (4.00) Statins 12 (30) 6 (4.00) 6 (4.00) 6 (4.00) Folic acid supplements 10 (25) 1 (7.00) 1 (7.00) Antiepileptic drugs 3 (7.5) 0 (0.00) 0.00 Vitamin D supplements 5 (12.5) 1 (7.00) 0.00 Calcium supplements 5 (12.5) 1 (7.00) 0.00 Antiarrhythmics 11 (27.5) 4 (3.00) 0.00 Antiplatelet agents 19 (47.5) 4 (3.00) 0.00	.7) 0 (0.0)
Solid tumor 3 (7.5) 1 (7.5) Rheumatic disease 3 (7.5) 2 (12.5) Gastrointestinal disease 2 (5) 2 (12.5) Asthma 1 (2.5) 0 (0.5) Leukemia 1 (2.5) 0 (0.5) Classes of drugs. n (%) 33 (82.5) 11 (3.5) Antihypertensives 33 (82.5) 11 (3.5) Proton-pump inhibitors 24 (60.0) 6 (4.6) Oral antidiabetic agents 12 (30) 6 (4.6) Folic acid supplements 10 (25) 1 (7.5) Antiepileptic drugs 3 (7.5) 0 (0.5) Vitamin D supplements 7 (17.5) 0 (0.5) Calcium supplements 5 (12.5) 1 (7.5) Bisphosphonates 1 (2.5) 0 (0.5) Antiarrhythmics 11 (27.5) 4 (30.6) Oral anticoagulants 15 (37.5) 1 (7.5) Antiplatelet agents 19 (47.5) 4 (30.6)	.0) 3 (9.4)
Rheumatic disease 3 (7.5) 2 (19) Gastrointestinal disease 2 (5) 2 (19) Asthma 1 (2.5) 0 (0.00) Leukemia 1 (2.5) 0 (0.00) Classes of drugs. n (%) 33 (82.5) 11 (80) Antihypertensives 33 (82.5) 11 (80) Proton-pump inhibitors 24 (60.0) 6 (40) Oral antidiabetic agents 12 (30) 2 (19) Statins 12 (30) 6 (40) Folic acid supplements 10 (25) 1 (7.00) Antiepileptic drugs 3 (7.5) 0 (0.00) Vitamin D supplements 7 (17.5) 0 (0.00) Calcium supplements 5 (12.5) 1 (7.00) Bisphosphonates 1 (2.5) 0 (0.00) Antiarrhythmics 11 (27.5) 4 (30) Oral anticoagulants 15 (37.5) 1 (7.00) Antiplatelet agents 19 (47.5) 4 (30)	.0) 1 (3.1)
Gastrointestinal disease 2 (5) 2 (15) Asthma 1 (2.5) 0 (0.5) Leukemia 1 (2.5) 0 (0.5) Classes of drugs. n (%) 33 (82.5) 11 (3.5) Antihypertensives 33 (82.5) 11 (3.5) Proton-pump inhibitors 24 (60.0) 6 (4.6) Oral antidiabetic agents 12 (30) 2 (1.5) Statins 12 (30) 6 (4.6) Folic acid supplements 10 (25) 1 (7.5) Antiepileptic drugs 3 (7.5) 0 (0.5) Vitamin D supplements 7 (17.5) 0 (0.5) Calcium supplements 5 (12.5) 1 (7.5) Bisphosphonates 1 (2.5) 0 (0.5) Antiarrhythmics 11 (27.5) 4 (3.6) Oral anticoagulants 15 (37.5) 1 (7.5) Antiplatelet agents 19 (47.5) 4 (3.6)	.7) 0 (0.0)
Asthma 1 (2.5) 0 (0.00) Leukemia 1 (2.5) 0 (0.00) Classes of drugs. n (%) 33 (82.5) 11 (3.00) Antihypertensives 33 (82.5) 11 (3.00) 6 (4.00) Proton-pump inhibitors 24 (60.0) 6 (4.00) 6 (4.00) Oral antidiabetic agents 12 (30) 6 (4.00) 6 (4.00) Folic acid supplements 10 (25) 1 (7.00) Antiepileptic drugs 3 (7.5) 0 (0.00) Vitamin D supplements 7 (17.5) 0 (0.00) Calcium supplements 5 (12.5) 1 (7.00) Bisphosphonates 1 (2.5) 0 (0.00) Antiarrhythmics 11 (27.5) 4 (3.00) Oral anticoagulants 15 (37.5) 1 (7.00) Antiplatelet agents 19 (47.5) 4 (3.00)	5.4) 0 (0.0)
Leukemia 1 (2.5) 0 (0.5) Classes of drugs. n (%) 33 (82.5) 11 (3.5) Antihypertensives 33 (82.5) 11 (3.5) Proton-pump inhibitors 24 (60.0) 6 (40.5) Oral antidiabetic agents 12 (30) 2 (12.5) Statins 12 (30) 6 (40.5) Folic acid supplements 10 (25) 1 (7.5) Antiepileptic drugs 3 (7.5) 0 (0.5) Vitamin D supplements 7 (17.5) 0 (0.5) Calcium supplements 5 (12.5) 1 (7.5) Bisphosphonates 1 (2.5) 0 (0.5) Antiarrhythmics 11 (27.5) 4 (30.5) Oral anticoagulants 15 (37.5) 1 (7.5) Antiplatelet agents 19 (47.5) 4 (30.5)	5.4) 2 (6.3)
Classes of drugs. n (%) 33 (82.5) 11 (8) Proton-pump inhibitors 24 (60.0) 6 (40) Oral antidiabetic agents 12 (30) 2 (12) Statins 12 (30) 6 (40) Folic acid supplements 10 (25) 1 (7) Antiepileptic drugs 3 (7.5) 0 (0) Vitamin D supplements 7 (17.5) 0 (0) Calcium supplements 5 (12.5) 1 (7) Bisphosphonates 1 (2.5) 0 (0) Antiarrhythmics 11 (27.5) 4 (30) Oral anticoagulants 15 (37.5) 1 (7) Antiplatelet agents 19 (47.5) 4 (30)	.0) 2 (6.3)
Antihypertensives 33 (82.5) 11 (8 Proton-pump inhibitors 24 (60.0) 6 (40 Oral antidiabetic agents 12 (30) 2 (13 Statins 12 (30) 6 (40 Folic acid supplements 10 (25) 1 (7 Antiepileptic drugs 3 (7.5) 0 (0 Vitamin D supplements 7 (17.5) 0 (0 Calcium supplements 5 (12.5) 1 (7 Bisphosphonates 1 (2.5) 0 (0 Antiarrhythmics 11 (27.5) 4 (30 Oral anticoagulants 15 (37.5) 1 (7 Antiplatelet agents 19 (47.5) 4 (30	.0) 0 (0.0)
Proton-pump inhibitors 24 (60.0) 6 (40.0) Oral antidiabetic agents 12 (30) 2 (13.0) Statins 12 (30) 6 (40.0) Folic acid supplements 10 (25) 1 (7.0) Antiepileptic drugs 3 (7.5) 0 (0.0) Vitamin D supplements 7 (17.5) 0 (0.0) Calcium supplements 5 (12.5) 1 (7.0) Bisphosphonates 1 (2.5) 0 (0.0) Antiarrhythmics 11 (27.5) 4 (30.0) Oral anticoagulants 15 (37.5) 1 (7.0) Antiplatelet agents 19 (47.5) 4 (30.0)	
Oral antidiabetic agents 12 (30) 2 (15) Statins 12 (30) 6 (40) Folic acid supplements 10 (25) 1 (7) Antiepileptic drugs 3 (7.5) 0 (0) Vitamin D supplements 7 (17.5) 0 (0) Calcium supplements 5 (12.5) 1 (7) Bisphosphonates 1 (2.5) 0 (0) Antiarrhythmics 11 (27.5) 4 (30) Oral anticoagulants 15 (37.5) 1 (7) Antiplatelet agents 19 (47.5) 4 (30)	84.6) 21 (65.6)
Statins 12 (30) 6 (40) Folic acid supplements 10 (25) 1 (7.5) Antiepileptic drugs 3 (7.5) 0 (0.5) Vitamin D supplements 7 (17.5) 0 (0.5) Calcium supplements 5 (12.5) 1 (7.5) Bisphosphonates 1 (2.5) 0 (0.5) Antiarrhythmics 11 (27.5) 4 (30) Oral anticoagulants 15 (37.5) 1 (7.5) Antiplatelet agents 19 (47.5) 4 (30)	6.2) 7 (21.9)
Folic acid supplements 10 (25) 1 (7. Antiepileptic drugs 3 (7.5) 0 (0. Vitamin D supplements 7 (17.5) 0 (0. Calcium supplements 5 (12.5) 1 (7. Bisphosphonates 1 (2.5) 0 (0. Antiarrhythmics 11 (27.5) 4 (30. Oral anticoagulants 15 (37.5) 1 (7. Antiplatelet agents 19 (47.5) 4 (30.	5.4) 1 (3.1)
Antiepileptic drugs 3 (7.5) 0 (0.00) Vitamin D supplements 7 (17.5) 0 (0.00) Calcium supplements 5 (12.5) 1 (7.00) Bisphosphonates 1 (2.5) 0 (0.00) Antiarrhythmics 11 (27.5) 4 (30) Oral anticoagulants 15 (37.5) 1 (7.00) Antiplatelet agents 19 (47.5) 4 (30)	6.2) 11 (34.4)
Vitamin D supplements 7 (17.5) 0 (0.00) Calcium supplements 5 (12.5) 1 (7.00) Bisphosphonates 1 (2.5) 0 (0.00) Antiarrhythmics 11 (27.5) 4 (30) Oral anticoagulants 15 (37.5) 1 (7.00) Antiplatelet agents 19 (47.5) 4 (30)	.7) 1 (3.1)
Calcium supplements 5 (12.5) 1 (7.5) Bisphosphonates 1 (2.5) 0 (0.5) Antiarrhythmics 11 (27.5) 4 (30.5) Oral anticoagulants 15 (37.5) 1 (7.5) Antiplatelet agents 19 (47.5) 4 (30.5)	.0) 1 (3.1)
Bisphosphonates 1 (2.5) 0 (0.00) Antiarrhythmics 11 (27.5) 4 (30) Oral anticoagulants 15 (37.5) 1 (7.00) Antiplatelet agents 19 (47.5) 4 (30)	.0) 0 (0.0)
Antiarrhythmics 11 (27.5) 4 (30) Oral anticoagulants 15 (37.5) 1 (7.5) Antiplatelet agents 19 (47.5) 4 (30)	.7) 0 (0.0)
Oral anticoagulants 15 (37.5) 1 (7.5) Antiplatelet agents 19 (47.5) 4 (30.5)	.0) 0 (0.0)
Antiplatelet agents 19 (47.5) 4 (30	0.8) 1 (3.1)
	.7) 2 (6.3)
	0.8) 5 (156)
Benzodiazepines 7 (17.5) 0 (0.	.0) 2 (6.3)
Antipsychotics 6 (15) 1 (7.	.7) 0 (0.0)
Opioids 3 (7.5) 0 (0.	.0) 0 (0.0)
Non steroidal anti-inflammatory drugs 0 (0.0) 1 (7.	
Corticosteroids 6 (15) 1 (7.	.7) 0 (0.0)
Drugs for the genitourinary system 8 (20) 2 (1:	
Antidepressants 5 (12.5) 0 (0.	.7) 1 (3.1)
Respiratory medications 7 (17.5) 2 (1:	.7) 1 (3.1) 5.4) 3 (9.4)

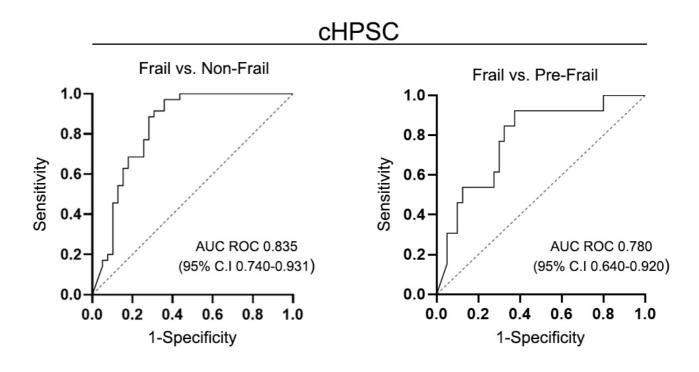
eFigure 1. Gating strategy of flow cytometry. Gating strategy of flow cytometry for the analysis of PBMC isolated by Ficoll-Paque sedimentation and the respective CD31+, CD14+ and CD34+/CD3-/CD14- cell subpopulations. γ -H2AX positive cells are also indicated. CD3 indicate the T lymphocytes, CD14 the monocytes and CD34 the cHPSCs within CD3-/CD14- cells. γ -H2AX was evaluated as a marker of DNA damage. SSC: side scatter. FSC: forward scatter.



eFigure 2. Gating strategy of flow cytometry. Gating strategy of flow cytometry for the analysis of cHPSC CD45+/Lin1-/CD34+in mononucleated cells (PBMC) of fresh whole blood. SSC: side scatter. FSC: forward scatter.



eFigure 3. Receiver-operating characteristics (ROC) curve. Evaluation of the comparison of percentage of DNA damaged cHPSC of 40 frail versus 32 non-frail subjects (left) and versus 13 pre-frail subjects (right). The area under the curve (AUC) and the 95% confidence interval (CI) are reported.



eFigure 4. Representation of oxidative stress evaluated in plasma. 5α , 6α -epoxycholesterol (5α , 6α EC), left; 3β , 5α , 6β -3OH-cholesterol (triol) (3β , 5α , 6β -3OHC), right; 5β , 6β -epoxycholsterol (5β , 6β EC), bottom. The level of the oxysterols has been evaluated in 17 frail, 9 pre-frail, 18 non-frail, 10 young samples. * p<0.01. Data expressed as means±SD.

