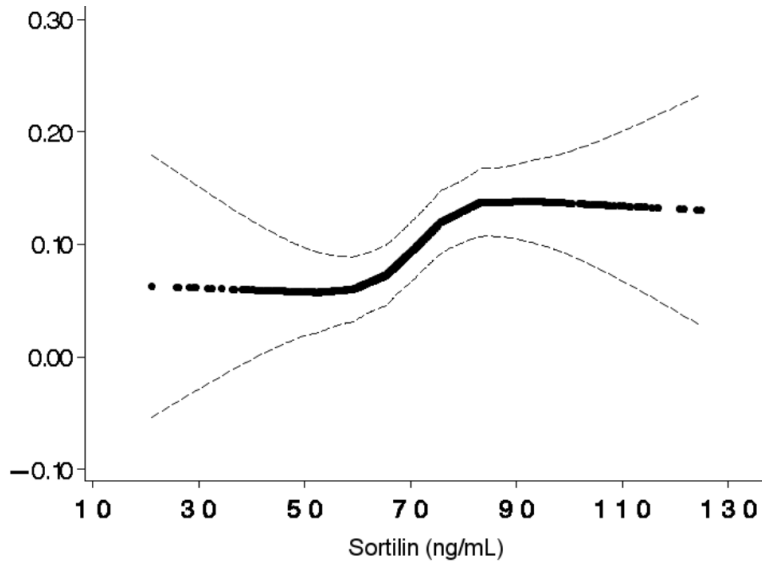


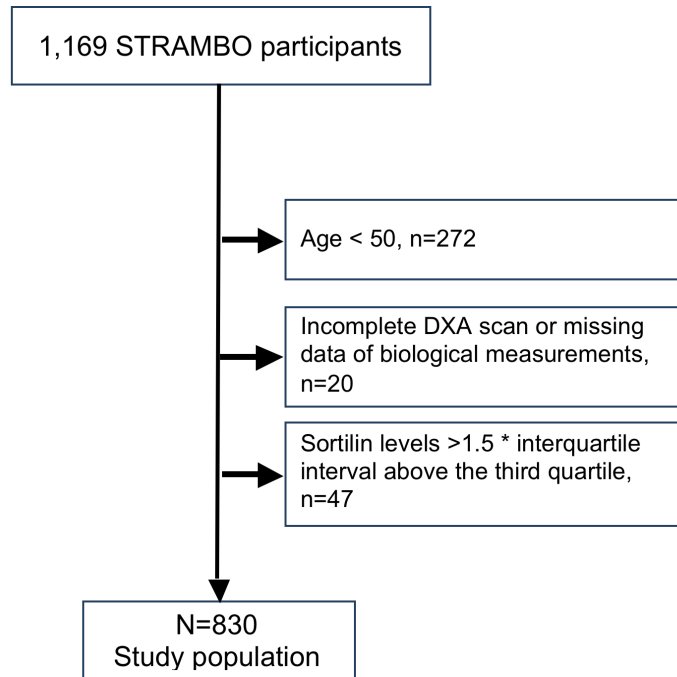
**Serum sortilin associates with aortic calcification and cardiovascular risk in men**

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Supplementary Figures and Table



**Supplementary Figure I:** Relationship between serum sortilin and MACCE by polynomial spline approach (unadjusted)



**Supplementary Figure II:** Flowchart illustrating study population. STRAMBO indicates Structure of the Aging Men’s Bones

**Supplementary Table I: Comparison of baseline characteristics of the included and excluded participants**

|  | Sortilin, ≤125 ng/mL | Sortilin, >125 ng/mL | p    |
|--|----------------------|----------------------|------|
| n  | 830                  | 47                   |      |
| Age (yrs)  | 70.3 ± 8.6           | 70.7 ± 9.1           | 0.74 |
| Weight (kg)  | 78.7 ± 11.4          | 79.7 ± 10.8          | 0.59 |
| Height (cm)  | 169.8 ± 6.6          | 169.3 ± 5.9          | 0.62 |
| BMI (kg/m <sup>2</sup> )                           | 27.6 ± 3.6           | 27.8 ± 3.4           | 0.76 |
| Fat mass, %  | 24.7 ± 5.3           | 24.8 ± 5.2           | 0.88 |
| Smoking, n (%)                                     |                      |                      |      |
| Current  | 56 (7%)              | 3 (6%)               |      |
| Former   | 503 (61%)            | 25 (53%)             | 0.93 |
| Never  | 271 (32%)            | 19 (41%)             |      |
| Alcohol intake (g/wk)                              | 109 [16; 235]        | 63 [16; 250]         | 0.93 |
| Total calcium intake (mg/d)                        | 798 ± 287            | 782 ± 294            | 0.72 |
| Occupational physical activity, n (%) <sup>#</sup> | 395 (47%)            | 25 (53%)             | 0.44 |
| Time spent outdoors (h/wk)                         | 7 [4; 10]            | 6 [3; 10]            | 0.32 |
| AAC, score   | 1 [0 ; 3]            | 0 [0 ; 2]            | 0.12 |
| AAC >5, n (%)                                      | 128 (15%)            | 3 (7%)               | 0.11 |
| Self-reported disease, n (%)                       |                      |                      |      |
| Ischemic heart disease                             | 116 (14%)            | 8 (17%)              | 0.51 |
| Diabetes mellitus - insulin                        | 15 ( 2%)             | 2 ( 4%)              | 0.36 |
| - oral agents                                      | 83 (10%)             | 3 ( 6%)              |      |
| History of stroke                                  | 30 ( 4%)             | 0 ( 0%)              | 0.18 |
| Systolic blood pressure                            | 13.4 ± 1.6           | 13.3 ± 1.9           | 0.87 |
| Diastolic blood pressure                           | 7.7 ± 1.0            | 7.7 ± 1.1            | 0.79 |
| Poor physical function                             | 142 (17%)            | 9 (19%)              | 0.66 |
| Self-reported medication, n (%)                    |                      |                      |      |
| Statins  | 212 (25%)            | 9 (19%)              | 0.37 |
| Vitamin K antagonists                              | 42 ( 5%)             | 3 ( 6%)              | 0.66 |
| ACE inhibitors                                     | 106 (13%)            | 10 (21%)             | 0.11 |
| AT receptor inhibitors                             | 131 (16%)            | 8 (17%)              | 0.92 |
| Beta-blockers                                      | 115 (14%)            | 5 (11%)              | 0.56 |
| Ca channel blockers                                | 118 (14%)            | 7 (15%)              | 0.85 |
| Diuretics  | 192 (23%)            | 10 (21%)             | 0.79 |
| Vitamin D supplements                              | 31 ( 4%)             | 2 ( 4%)              | 0.83 |
| Calcium (mmol/L)                                   | 2.36 ± 0.17          | 2.35 ± 0.18          | 0.71 |
| Phosphate (mmol/L)                                 | 1.05 ± 0.16          | 1.02 ± 0.15          | 0.25 |
| Glomerular filtration rate (mL/min)                | 72.0 ± 15.8          | 73.5 ± 13.8          | 0.52 |
| Total Cholesterol (mg/dL)                          | 206 ± 37             | 211 ± 37             | 0.33 |
| LDL-Cholesterol (mg/dL)                            | 118 ± 33             | 117 ± 34             | 0.59 |
| Testosterone (nmol/L)                              | 11.8 ± 5.2           | 11.7 ± 4.7           | 0.90 |
| 25-Hydroxycholecalciferol (ng/mL)                  | 22 ± 10              | 22 ± 8               | 0.96 |
| Parathyroid hormone (pg/mL)                        | 48 ± 24              | 48 ± 27              | 0.96 |
| Osteoprotegerin (pmol/L)                           | 3.8 [3.1; 4.8]       | 4.0 [3.2; 4.8]       | 0.70 |
| hsCRP (mg/L)                                       | 1.6 [0.8; 3.2]       | 1.4 [0.8; 2.8]       | 0.48 |

Continuous variables are presented as mean±SD. Variables with non-Gaussian distribution are presented as median [first quartile; third quartile] and categorical variables as percentage.

AAC- abdominal aortic calcification, hsCRP – high-sensitivity C-reactive protein, GFR – glomerular filtration rate.

**Supplementary Table II: Baseline characteristics by sortilin quartiles**

|  | Quartile 1<br><60 ng/mL | Quartile 2<br>60 – <71 ng/mL | Quartile 3<br>71 - <84 ng/mL | Quartile 4<br>84 – 125 ng/mL | Model 1 | Model 2 |
|--|-------------------------|------------------------------|------------------------------|------------------------------|---------|---------|
| n  | 212                     | 205                          | 210                          | 203                          |         |         |
| Age (yrs)  | 71.8 ± 8.4              | 70.8 ± 8.6                   | 69.3 ± 8.7                   | 68.9 ± 8.6                   | <0.005  |         |
| Weight (kg)  | 78.6 ± 11.1             | 77.4 ± 10.6                  | 79.7 ± 12.1                  | 79.4 ± 11.3                  | 0.16    | 0.23    |
| Height (cm)  | 168.4 ± 6.8             | 168.7 ± 6.9                  | 169.2 ± 6.7                  | 169.2 ± 6.3                  | 0.49    | 0.99    |
| BMI (kg/m <sup>2</sup> )                           | 27.7 ± 3.5              | 27.2 ± 3.9                   | 27.8 ± 3.6                   | 27.7 ± 3.4                   | 0.38    | 0.28    |
| Fat mass fraction, %                               | 25.0 ± 5.2              | 24.2 ± 5.4                   | 24.6 ± 4.9                   | 25.0 ± 4.9                   | 0.46    | 0.41    |
| Smoking, n (%)                                     |                         |                              |                              |                              |         |         |
| Current  | 9 (4%)                  | 12 (6%)                      | 18 (9%)                      | 17 (8%)                      |         |         |
| Former   | 126 (60%)               | 121 (59%)                    | 132 (63%)                    | 124 (61%)                    | 0.23    | 0.37    |
| Never  | 77 (36%)                | 72 (35%)                     | 60 (28%)                     | 62 (31%)                     |         |         |
| Alcohol intake (g/wk)                              | 114 [16; 227]           | 114 [16; 244]                | 114 [16; 244]                | 114 [32; 244]                | 0.59    | 0.61    |
| Total calcium intake (mg/d)                        | 814 ± 268               | 811 ± 314                    | 781 ± 265                    | 793 ± 296                    | 0.60    | 0.61    |
| Occupational physical activity, n (%) <sup>#</sup> | 97 (46%)                | 95 (46%)                     | 106 (50%)                    | 97 (48%)                     | 0.77    | 0.40    |
| Time spent outdoors (h/wk)                         | 7.0 [4.0; 10.0]         | 7.0 [4.2; 10.0]              | 7.0 [4.0; 10.0]              | 6.8 [4.0; 10.0]              | 0.64    | 0.33    |
| AAC >5, n (%)                                      | 39 (18%)                | 28 (14%)                     | 28 (13%)                     | 33 (16%)                     | 0.43    | 0.54    |
| Self-reported disease, n (%)                       |                         |                              |                              |                              |         |         |
| Ischemic heart disease                             | 37 (17%)                | 32 (16%)                     | 29 (14%)                     | 18 (9%)                      | 0.07    | 0.18    |
| Diabetes mellitus - insulin                        | 5 (2%)                  | 5 (2%)                       | 2 (1%)                       | 3 (1%)                       |         |         |
| - oral agents                                      | 27 (13%)                | 15 (7%)                      | 23 (11%)                     | 18 (9%)                      | 0.46    | 0.55    |
| History of stroke                                  | 11 ( 5%)                | 8 ( 4%)                      | 8 ( 4%)                      | 4 ( 2%)                      | 0.39    | 0.10    |
| Systolic blood pressure (mm Hg)                    | 13.4 ± 1.7              | 13.3 ± 1.6                   | 13.3 ± 1.6                   | 13.5 ± 1.6                   | 0.43    | 0.24    |
| Diastolic blood pressure                           | 7.6 ± 1.0               | 7.6 ± 1.0                    | 7.7 ± 1.0                    | 7.8 ± 1.0                    | 0.21    | 0.43    |
| Poor physical function                             | 44 (21%)                | 32 (16%)                     | 33 (16%)                     | 33 (16%)                     | 0.44    | 0.68    |
| Self-reported medication, n (%)                    |                         |                              |                              |                              |         |         |
| Statins  | 70 (33%)                | 49 (24%)                     | 51 (24%)                     | 42 (21%)                     | <0.05   | 0.06    |
| Vitamin K antagonists                              | 11 ( 5%)                | 10 ( 5%)                     | 10 ( 5%)                     | 11 ( 5%)                     | 0.99    | 0.97    |
| ACE inhibitors                                     | 32 (15%)                | 29 (14%)                     | 31 (15%)                     | 14 ( 7%)                     | <0.05   | 0.06    |
| AT receptor antagonist                             | 40 (19%)                | 35 (17%)                     | 31 (15%)                     | 25 (12%)                     | 0.66    | 0.47    |
| Beta-blockers                                      | 29 (14%)                | 28 (14%)                     | 33 (16%)                     | 25 (12%)                     | 0.80    | 0.76    |
| Ca channel blockers                                | 33 (16%)                | 31 (15%)                     | 25 (12%)                     | 29 (14%)                     | 0.71    | 0.79    |
| Calcium (mmol/L)                                   | 2.37 ± 0.12             | 2.37 ± 0.13                  | 2.36 ± 0.17                  | 2.34 ± 0.23                  | 0.16    | 0.17    |
| Phosphate (mmol/L)                                 | 1.05 ± 0.15             | 1.07 ± 0.16                  | 1.05 ± 0.16                  | 1.01 ± 0.17                  | <0.005  | <0.001  |

|                                   |                |                |                |                |        |        |
|-----------------------------------|----------------|----------------|----------------|----------------|--------|--------|
| GFR (mL/min/1.73m <sup>2</sup> )  | 69.9 ± 16.5    | 72.1 ± 15.0    | 72.0 ± 15.3    | 74.3 ± 16.0    | <0.05  | 0.38   |
| Total Cholesterol (mg/dL)         | 195 ± 38       | 205 ± 33       | 208 ± 38       | 216 ± 37       | <0.001 | <0.001 |
| LDL-Cholesterol (mg/dL)           | 111 ± 35       | 120 ± 29       | 119 ± 32       | 124 ± 33       | <0.001 | <0.005 |
| Testosterone (nmol/L)             | 11.5 ± 5.3     | 11.4 ± 5.2     | 11.8 ± 5.5     | 12.2 ± 5.0     | 0.36   | 0.44   |
| 25-Hydroxycholecalciferol (ng/mL) | 22 ± 10        | 22 ± 10        | 22 ± 10        | 23 ± 10        | 0.72   | 0.96   |
| Parathyroid hormone (pg/mL)       | 51 ± 27        | 48 ± 24        | 46 ± 22        | 47 ± 21        | 0.17   | 0.67   |
| Osteoprotegerin (pmol/L)          | 4.1 [3.3; 5.1] | 3.7 [2.9; 4.7] | 3.8 [3.0; 4.8] | 3.7 [3.1; 4.5] | <0.001 | <0.01  |
| hsCRP (mg/L)                      | 1.3 [0.7; 2.7] | 1.7 [0.8; 3.3] | 1.8 [0.9; 3.7] | 1.7 [0.9; 3.2] | <0.05  | <0.01  |

Continuous variables are presented as mean±SD. Variables with non-Gaussian distribution are presented as median [first quartile; third quartile] and categorical variables as percentage. Data are unadjusted. #weak or moderate vs. relatively high or high, AAC-abdominal aortic calcification, hsCRP – high-sensitivity C-reactive protein, GFR – glomerular filtration rate. Model 1 is unadjusted comparison; Model 2 is adjusted for age.

**Supplementary Table III: Correlation of sortilin with continuous variables**

|                                | Simple correlation |        | Partial (corrected for age) |         |
|--------------------------------|--------------------|--------|-----------------------------|---------|
|                                | r                  | p      | r                           | p-value |
| Age                            | <b>-0.14</b>       | <0.001 |                             |         |
| Weight                         | 0.05               | 0.13   | 0.04                        | 0.26    |
| Height                         | 0.05               | 0.12   | 0.01                        | 0.96    |
| Total calcium intake           | -0.04              | 0.26   | -0.04                       | 0.26    |
| Systolic blood pressure        | 0.05               | 0.13   | 0.08                        | <0.05   |
| <b>Biological measurements</b> |                    |        |                             |         |
| Total cholesterol              | <b>0.23</b>        | <0.001 | <b>0.23</b>                 | <0.001  |
| LDL-cholesterol                | <b>0.16</b>        | <0.001 | <b>0.16</b>                 | <0.001  |
| Calcium                        | -0.08              | <0.05  | -0.08                       | <0.05   |
| Phosphorus                     | -0.07              | <0.05  | -0.08                       | <0.05   |
| Glomerular filtration rate     | <b>0.11</b>        | <0.005 | 0.05                        | 0.25    |
| Testosterone                   | 0.05               | 0.14   | 0.04                        | 0.19    |
| 25-Hydroxycholecalciferol      | 0.05               | 0.13   | 0.02                        | 0.37    |
| Parathyroid hormone            | -0.07              | <0.05  | -0.03                       | 0.38    |
| C-reactive protein             | 0.06               | 0.09   | <b>0.10</b>                 | <0.01   |
| Osteoprotegerin                | -0.09              | <0.01  | -0.03                       | 0.38    |

Pearson's correlation. Given multiple tests, only coefficients with  $p < 0.01$  (marked in bold) were considered significant

**Supplementary Table IV: Multivariate Cox proportional hazard regression analysis for MACCE according to the serum sortilin levels – the thresholds were selected on the basis of the visual analysis of the LOESS curve presented in the Supplementary Figure 2**

|               | Incidence* | Model 1                         | Model 2                         | Model 3                         |
|---------------|------------|---------------------------------|---------------------------------|---------------------------------|
| <60 ng/mL     | 8.0        | 1.00                            | 1.00                            | 1.00                            |
| 60 – 80 ng/mL | 11.4       | 1.57 (0.75 – 3.29)              | 1.81 (0.86 – 3.83)              | 1.87 (0.88 – 4.00)              |
| >80 ng/mL     | 22.5       | 4.00 (1.97 – 8.14) <sup>a</sup> | 4.31 (2.09 – 8.89) <sup>a</sup> | 4.57 (2.18 – 9.61) <sup>a</sup> |

<sup>a</sup>p<0.001; \* - number of events per 1000 person-years

MACCE is defined as composite of cardiovascular death, non-fatal ST-elevation or non-ST-elevation myocardial infarction, unstable angina, and non-fatal stroke.

**Model 1** is adjusted for age, fat mass, smoking (current, former, never), and professional physical activity;

**Model 2** is adjusted as for model 1 plus health status (self-reported pharmacologically treated diabetes mellitus, systolic blood pressure, AAC) and treatments (vitamin K antagonists, angiotensin-converting enzyme inhibitors, diuretics, fibrates);

**Model 3** is adjusted as for model 2 plus biological measurements (testosterone, osteoprotegerin, LDL-cholesterol, C-reactive protein)

**Supplementary Table V: Multivariate Cox proportional hazard regression analysis for MACCE according to sortilin quartiles adjusted for LDL-C and TC separately and combined.**

|                                 | n/N (%)     | Incidence* | Adjusted for<br>LDL-C <sup>#</sup> | Adjusted for<br>TC              | Adjusted for<br>LDL-C and TC    |
|---------------------------------|-------------|------------|------------------------------------|---------------------------------|---------------------------------|
| Per SD (17.6 ng/ml)             |             |            | 1.70 (1.30 – 2.20) <sup>b</sup>    | 1.67 (1.31 – 2.10) <sup>b</sup> | 1.66 (1.24 – 2.08) <sup>b</sup> |
| <b>Sortilin quartile</b>        |             |            |                                    |                                 |                                 |
| Quartile 1                      | 12/178 (6)  | 8.0        | 1.00                               | 1.00                            | 1.00                            |
| Quartile 2                      | 12/180 (6)  | 7.4        | 1.44 (0.60 – 3.46)                 | 1.40 (0.59 – 3.33)              | 1.40 (0.60 – 3.35)              |
| Quartile 3                      | 26/187 (13) | 19.8       | 3.42 (1.61 – 7.25) <sup>a</sup>    | 3.21 (1.51 – 6.79) <sup>a</sup> | 3.37 (1.50 – 6.71) <sup>a</sup> |
| Quartile 4                      | 24/185 (14) | 20.3       | 3.82 (1.77 – 8.26) <sup>b</sup>    | 3.69 (1.67 – 7.71) <sup>b</sup> | 3.68 (1.66 – 7.77) <sup>b</sup> |
| <b>Sortilin ≥ vs &lt;median</b> |             |            |                                    |                                 |                                 |
| ≤71 ng/ml                       | 24/358 (6)  | 7.8        | 1.00                               | 1.00                            | 1.00                            |
| >71 ng/ml                       | 50/372 (13) | 20.0       | 3.00 (1.77 – 5.08) <sup>b</sup>    | 2.93 (1.70 – 4.90) <sup>b</sup> | 2.92 (1.70 – 4.91) <sup>b</sup> |
| <b>Sortilin –ROC curve</b>      |             |            |                                    |                                 |                                 |
| ≤76 ng/ml                       | 33/466 (6)  | 7.9        | 1.00                               | 1.00                            | 1.00                            |
| >76 ng/ml                       | 41/264 (15) | 21.4       | 3.08 (1.89 – 5.03) <sup>b</sup>    | 2.98 (1.79 – 4.91) <sup>b</sup> | 2.98 (1.70 – 4.82) <sup>b</sup> |

<sup>a</sup>p<0.005, <sup>b</sup>p<0.001; \* - number of events per 1000 person-years; <sup>#</sup>same data as Table 1 – model 4

Quartile 1, <60 ng/mL; Quartile 2, 60- 71 ng/mL; Quartile 3, >71-84 ng/mL; Quartile 4, >84-125 ng/mL

MACCE is defined as composite of cardiovascular death, non-fatal ST-elevation or non-ST-elevation myocardial infarction, unstable angina, and non-fatal stroke.

Model 4 from Table 1; adjusted for: age, fat mass, smoking (current, former, never), alcohol intake and physical activity, health status (self-reported pharmacologically treated diabetes mellitus, systolic blood pressure, AAC) and medications (vitamin K antagonist, angiotensin-converting enzyme inhibitors, diuretics, fibrates); biological measurements (testosterone, osteoprotegerin, C-reactive protein).



**Supplementary Table VI: Multivariate Cox proportional hazard regression analysis adjusted for traditional Framingham risk factors for MACCE, and separated by MACE and stroke according to sortilin quartiles and above/below median**

|                                 | MACCE<br>(n=76)                 | MACE<br>(n=43)                  | Stroke<br>(n=33)                 |
|---------------------------------|---------------------------------|---------------------------------|----------------------------------|
| Per SD (17.6 ng/mL)             | 1.57 (1.22 – 2.01) <sup>c</sup> | 1.31 (0.96 – 1.80) <sup>c</sup> | 1.91 (1.31 – 2.77) <sup>c</sup>  |
| <b>Sortilin quartiles</b>       |                                 |                                 |                                  |
| Quartile 1                      | 1.00                            | 1.00                            | 1.00                             |
| Quartile 2                      | 1.43 (0.60 – 3.39)              | 1.54 (0.50 – 4.80)              | 0.73 (0.20 – 2.70)               |
| Quartile 3                      | 3.37 (1.62 – 7.07) <sup>b</sup> | 3.67 (1.50 – 9.00) <sup>b</sup> | 1.74 (0.56 – 5.39)               |
| Quartile 4                      | 3.45 (1.63 – 7.29) <sup>b</sup> | 2.33 (0.87 – 6.30)              | 3.90 (1.48 – 10.28) <sup>a</sup> |
| <b>Sortilin ≥ vs &lt;median</b> |                                 |                                 |                                  |
| ≤71 ng/ml                       | 1.00                            | 1.00                            | 1.00                             |
| >71 ng/ml                       | 2.83 (1.71 - 4.68) <sup>c</sup> | 2.48 (1.32 – 4.64) <sup>b</sup> | 3.04 (1.44 – 6.42) <sup>b</sup>  |

<sup>a</sup>p<0.01, <sup>b</sup>p<0.005, <sup>c</sup>p<0.001; n- number of events

Quartile 1, <60 ng/mL; Quartile 2, 60- 71 ng/mL; Quartile 3, >71-84 ng/mL; Quartile 4, >84-125 ng/mL

Data are adjusted for traditional Framingham risk factors including age, LDL-C, lipid lowering treatment, diabetes, systolic blood pressure, antihypertensive treatment, cigarette use, BMI, hsCRP

MACCE is defined as composite of cardiovascular death, non-fatal ST-elevation or non-ST-elevation myocardial infarction, unstable angina, and non-fatal stroke. MACE is defined as composite of cardiovascular death, non-fatal ST-elevation or non-ST-elevation myocardial infarction, and unstable angina.

**Supplementary Table VII: The distribution of self-reported ischemic heart disease, history of myocardial infarction and AAC score in the cohort at baseline**

| Age range      | IHD      | IHD prior MI | AAC score |
|----------------|----------|--------------|-----------|
| 50 – 60 years  | 1 (1%)   | 1 (1%)       | 0 [0; 1]  |
| >60 – 70 years | 30 (9%)  | 19 (6%)      | 0 [0; 2]  |
| >70 – 80 years | 53 (16%) | 15 (5%)      | 2 [0; 4]  |
| >80 years      | 32 (28%) | 10 (9%)      | 4 [0; 8]  |
| P for trend    | <0.001   | 0.08         | <0.001    |

IHD – ischemic heart disease, MI – myocardial infarction; N (%) per group;

AAC score is presented as median and interquartile range per age group.

**Supplementary Table VIII: AAC score according to the presence of self-reported ischemic heart disease and history of myocardial infarction at baseline**

|             | No IHD reported<br>(n = 729) | IHD without MI<br>(n=71) | IHD with MI<br>(n=45) | p <sup>1</sup> | p <sup>2</sup> | p <sup>3</sup> |
|-------------|------------------------------|--------------------------|-----------------------|----------------|----------------|----------------|
| Age (years) | 69 ± 9                       | 77 ± 6                   | 73 ± 8                |                |                |                |
| AAC score   | 1 [0; 3]                     | 4 [0; 8]                 | 2 [0; 8]              | <0.001         | <0.005         | <0.001         |

IHD – ischemic heart disease, MI – myocardial infarction; AAC score is presented as median and interquartile range per age group. p<sup>1</sup> – Kruskal-Wallis test; p<sup>2</sup> – analysis of covariance using log-transformed AAC adjusted for age, weight, smoking, diabetes mellitus and hypertension; p<sup>3</sup> – trend for log-transformed AAC adjusted for age, weight, smoking, diabetes mellitus and hypertension

**Supplementary Table IX: Sortilin serum levels according to AAC categories**

| AAC group  | Sortilin (ng/mL) |
|------------|------------------|
| AAC = 0    | 71 ± 17          |
| AAC 1 to 2 | 73 ± 18          |
| AAC 3 to 5 | 71 ± 18          |
| AAC >5     | 74 ± 18          |