

**Supplementary Table S1: Antigen-specific fluorochrome-labelled monoclonal antibodies used for flow cytometry assays.**

| Antigen      | Antobody            | Reporter    | Manufacture    | Clone       | Isotype     | Dilution | Cat#      |
|--------------|---------------------|-------------|----------------|-------------|-------------|----------|-----------|
| <b>LRP1</b>  | Anti-LRP1- $\alpha$ | PE          | AbD Serotec    | MCA1965     | Mouse IgG1  | 1/100    | MCA1965PE |
| <b>CD14</b>  | Anti-CD14           | FITC        | BD Biosciences | M $\phi$ P9 | Mouse IgG2b | 1/50     | 561708    |
| <b>CD16</b>  | Anti-CD16           | APC-Cy7     | BD Biosciences | 3G8         | Mouse IgG1  | 1/50     | 557758    |
| <b>CD45</b>  | Anti-CD45           | PC5         | Beckam Coulter | J.33        | Mouse IgG1  | 1/50     | IM2653    |
| <b>CD36</b>  | Anti-CD36           | PerCP-Cy5.5 | BD Biosciences | CB38        | Mouse IgM   | 1/100    | 561536    |
| <b>CD11b</b> | Anti-CD11b          | PerCP-Cy5.5 | BD Biosciences | -----       | Rat IgG2b   | 1/100    | 561114    |
| <b>CD11c</b> | Anti-CD11c          | PerCP-Cy5.5 | BD Pharmingen  | B-ly6       | Mouse IgG1  | 1/100    | 565227    |

FITC, Fluorescein isothiocyanate; APC-Cy7, Allophycocyanin dye coupled with Cyanine 7; PE, R-phycoerythrin; PC5, phycoerythrin dye coupled with Cyanine 5; PerCP-Cy5.5, Peridinin-chlorophyll-protein Complex: CY5.5 Conjugate. AbD Serotec from Bio-Rad Laboratories, Inc. (Oxford, UK), BD Biosciences/Pharmingen (San Jose, CA); Beckam Coulter (Krefeld, Germany).

**Supplementary Table S2: Specific primers for quantitative RT-PCR.**

| <b>Gen</b>                            | <b>NM/ID</b>       | <b>Sequence:<br/>sense primer (F)<br/>anti-sense primer (R)</b>  |
|---------------------------------------|--------------------|------------------------------------------------------------------|
| <b><i>LRP1</i></b>                    | 002332.2/4035      | F 5' -CTATGCACGCCCCTAAGACTT- 3'<br>R 5'-CATCGCTGGGCCTTACTCT- 3'  |
| <b><i>TNF-<math>\alpha</math></i></b> | 000594.3/7124      | F 5'-TGCACTTTGGAGTGATCGGC -3'<br>R 5'-GCTTGAGGGTTTGCTACAACA -3'  |
| <b><i>IL-1<math>\beta</math></i></b>  | 000576.2/3553      | F 5'-ATGATGGCTTATTACAGTGGCAA 3'<br>R 5'-GTCGGAGATTCGTAGCTGGA -3' |
| <b><i>CCL2</i></b>                    | 002982.3/6247      | F 5'-CTTCATTCCCCAAGGGCTC -3'<br>R 5'-GGTTTGCTTGTCCAGGTGGT -3'    |
| <b><i>CCR2</i></b>                    | 001123041.2/729230 | F 5'-GGGATGACTCACTGCTGCAT -3'<br>R 5'-GGAGTGGGGCAATCCTACAG -3'   |
| <b><i>GAPDH</i></b>                   | 001256799.2/2597   | F 5'-CTCCGGGTGATGCTTTTCCT 3'<br>R 5'-TGAAGGGGTCATTGATGGCA -3'    |

**Supplementary Table S3: Correlation analysis between LRP1 expression at cell surface of total monocytes and CVD risk parameters**

| Parameters               | Total Individuals<br>(n=227)                          | Low risk group<br>(LR)<br>(n=21)        | Intermediate group<br>(IR)<br>(n=124)   | Subclinical atherosclerosis group<br>(SCA)<br>(n=82) |
|--------------------------|-------------------------------------------------------|-----------------------------------------|-----------------------------------------|------------------------------------------------------|
| BMI, kg/m <sup>2</sup>   | <i>r</i> = -0,0835<br><i>p</i> = 0.2102               | <i>r</i> = -0,0374<br><i>p</i> = 0.8721 | <i>r</i> = 0,0713<br><i>p</i> = 0.4314  | <i>r</i> = -0,1341<br><i>p</i> = 0.2296              |
| Total Cholesterol, mg/dl | <i>r</i> = <b>-0.1718</b><br><i>p</i> = <b>0.0095</b> | <i>r</i> = 0,1678<br><i>p</i> = 0.4671  | <i>r</i> = -0,1457<br><i>p</i> = 0.1064 | <i>r</i> = -0,0685<br><i>p</i> = 0.5411              |
| Triglycerides, mg/dl     | <i>r</i> = -0,1290<br><i>p</i> = 0.0522               | <i>r</i> = 0,0327<br><i>p</i> = 0.8880  | <i>r</i> = -0,0717<br><i>p</i> = 0.4288 | <i>r</i> = -0,1087<br><i>p</i> = 0.3312              |
| LDLc, mg/dl              | <i>r</i> = -0,1235<br><i>p</i> = 0.0632               | <i>r</i> = 0,3426<br><i>p</i> = 0.1284  | <i>r</i> = -0,0302<br><i>p</i> = 0.7395 | <i>r</i> = -0,0905<br><i>p</i> = 0.4190              |
| Non-HDLc, mg/dl          | <i>r</i> = <b>-0,1611</b><br><i>p</i> = <b>0.0151</b> | <i>r</i> = 0,2796<br><i>p</i> = 0.2197  | <i>r</i> = -0,0630<br><i>p</i> = 0.4871 | <i>r</i> = -0,1043<br><i>p</i> = 0.3511              |

LRP1 levels were log-transformed to achieve normal distribution and to apply the Pearson correlation analysis. Other parameters showed normal distribution. **BMI**, body mass index; **LDLc**, low density lipoprotein-cholesterol; **non-HDLc**, non-high density lipoprotein-cholesterol. Significant value, *p*<0.05. **Bold font** indicates parameter with statistical significance.

**Supplementary Table S4: CD36, CD11b and CD11c expression at cell surface in total monocytes and monocyte subsets in LR, IR and SCA groups (Study I)**

| Monocyte marker<br>MFI, arbitrary units                 | Without Subclinical Atherosclerosis |                                               | Subclinical<br>Atherosclerosis<br>(SCA)<br>(n=82) | <i>p</i> -value*<br><i>Binary<br/>comparison</i> **    |
|---------------------------------------------------------|-------------------------------------|-----------------------------------------------|---------------------------------------------------|--------------------------------------------------------|
|                                                         | Low Risk<br>Group<br>(LR)<br>(n=21) | Intermediate Risk<br>Group<br>(IR)<br>(n=124) |                                                   |                                                        |
| CD36-Total<br>Monocytes<br>Median<br>[IQR]              | 100.0<br>[76.2-124.0]               | 91.6<br>[68.6-117.0]                          | 86.6<br>[60.2-117.0]                              | 0.4077<br><i>a, ns</i><br><i>b, ns</i><br><i>c, ns</i> |
| CD36-Classical<br>Monocytes<br>Median<br>[IQR]          | 136.0<br>[110.5-177.5]              | 124.0<br>[91.8-169.8]                         | 124.0<br>[87.4-162.0]                             | 0.6453<br><i>a, ns</i><br><i>b, ns</i><br><i>c, ns</i> |
| CD36-Intermediate<br>Monocytes<br>Median<br>[IQR]       | 57.6<br>[41.4-75.6]                 | 52.6<br>[37.7-71.6]                           | 51.6<br>[38.4-75.8]                               | 0.7196<br><i>a, ns</i><br><i>b, ns</i><br><i>c, ns</i> |
| CD36-Non-classical<br>Monocytes<br>Median<br>[IQR]      | 6.8<br>[5.9-8.8]                    | 8.4<br>[6.7-10.4]                             | 8.0<br>[7.0-9.9]                                  | 0.0934<br><i>a, ns</i><br><i>b, ns</i><br><i>c, ns</i> |
| CD11b-Total<br>Monocytes<br>Median<br>[IQR]             | 30.0<br>[19.2-40.5]                 | 23.7<br>[15.6-31.9]                           | 25.3<br>[17.7-33.5]                               | 0.1380<br><i>a, ns</i><br><i>b, ns</i><br><i>c, ns</i> |
| CD11b-Classical<br>Monocytes<br>Median<br>[IQR]         | 38.2<br>[22.2-51.3]                 | 28.5<br>[20.2-39.7]                           | 30.3<br>[23.0-43.0]                               | 0.2010<br><i>a, ns</i><br><i>b, ns</i><br><i>c, ns</i> |
| CD11b-Intermediate<br>Monocytes<br>Median<br>[IQR]      | 23.5<br>[14.1-36.3]                 | 18.2<br>[11.0-25.8]                           | 21.8<br>[13.3-32.2]                               | 0.0576<br><i>a, ns</i><br><i>b, ns</i><br><i>c, ns</i> |
| CD11b-Non-<br>classical<br>Monocytes<br>Median<br>[IQR] | 8.0<br>[5.6-8.4]                    | 6.6<br>[5.6-8.8]                              | 7.4<br>[5.6-9.1]                                  | 0.3314<br><i>a, ns</i><br><i>b, ns</i><br><i>c, ns</i> |
| CD11c-Total<br>Monocytes<br>Median<br>[IQR]             | 711<br>[639-766]                    | 597<br>[419-705]                              | 535<br>[450-656]                                  | 0.2065<br><i>a, ns</i><br><i>b, ns</i><br><i>c, ns</i> |
| CD11c-Classical<br>Monocytes<br>Median<br>[IQR]         | 643<br>[563-795]                    | 561<br>[410-700]                              | 533<br>[456-664]                                  | 0.1558<br><i>a, ns</i><br><i>b, ns</i><br><i>c, ns</i> |
| CD11c-Intermediate<br>Monocytes<br>Median<br>[IQR]      | 1606<br>[1307-1937]                 | 1460<br>[1263-1665]                           | 1468<br>[1288-1670]                               | 0.5964<br><i>a, ns</i><br><i>b, ns</i><br><i>c, ns</i> |
| CD11c-Non-<br>classical<br>Monocytes<br>Median<br>[IQR] | 1246<br>[1130-1425]                 | 1147<br>[772-1365]                            | 1020<br>[849-1224]                                | 0.0920<br><i>a, ns</i><br><i>b, ns</i><br><i>c, ns</i> |

Values are median (IQR, interquartile range). Parameters were log-transformed to achieve normal distribution and to apply statistical parametric analysis, but in these table the original data were used. \* Ordinary one-way ANOVA; *ns*, non-significant. \*\**a*, *b*, and *c*: unpaired *t*-test for mean values; *a*, IR vs LR; *b*, SCA vs LR; and *c*, SCA vs IR. Significant value, *p*<0.05. MFI, mean fluorescence intensity.

**Supplementary Table S5: Clinical and biochemical parameters (Study II).**

| Parameters                                             | Without Subclinical Atherosclerosis |                                     | Subclinical Atherosclerosis Group (SCA) (n=16) | <i>p</i> -value*<br>Binary comparison** |
|--------------------------------------------------------|-------------------------------------|-------------------------------------|------------------------------------------------|-----------------------------------------|
|                                                        | Low Risk Group (LR) (n=16)          | Intermediate Risk Group (IR) (n=16) |                                                |                                         |
| Male (%)                                               | 6 (37.5)                            | 7 (43.7)                            | 10 (62.5)                                      | 0.0082 <sup>##</sup>                    |
| Ages, years#<br>Median [IQR]                           | 38.5<br>[25.2 – 42.0]               | 52.0<br>[50.0 – 54.7]               | 50.0<br>[33.2 – 55.7]                          | 0.0002<br><b>a, b</b>                   |
| BMI, kg/m2<br>Mean ± SD                                | 22.8 ± 2.5                          | 26.4 ± 3.5                          | 26.7 ± 3.0                                     | 0.0010<br><b>a, b</b>                   |
| SBP, mmHg<br>Mean ± SD                                 | 107 ± 10                            | 115 ± 13                            | 113 ± 10                                       | <i>ns</i>                               |
| DBP, mmHg<br>Mean ± SD                                 | 73 ± 7                              | 79 ± 7                              | 79 ± 5                                         | <i>ns</i>                               |
| Total Cholesterol, mg/dl<br>Mean ± SD                  | 158 ± 28                            | 203 ± 73                            | 200 ± 48                                       | 0.0353<br><b>a, b</b>                   |
| LDLc, mg/dl<br>Mean ± SD                               | 84 ± 19                             | 98 ± 39                             | 117 ± 37                                       | 0.0251<br><b>b</b>                      |
| HDLc, mg/dl<br>Mean ± SD                               | 61 ± 11                             | 58 ± 21                             | 46 ± 17                                        | 0.0442<br><b>b</b>                      |
| Triglycerides, mg/dl<br>Mean ± SD                      | 65 ± 21                             | 207 ± 408                           | 145 ± 84                                       | <i>ns</i>                               |
| Non-HDLc, mg/dl<br>Mean ± SD                           | 97 ± 23                             | 145 ± 77                            | 154 ± 47                                       | 0.0094<br><b>a, b</b>                   |
| Glucose, mg/dl<br>Mean ± SD                            | 88 ± 5                              | 97 ± 7                              | 100 ± 8                                        | <0.0001<br><b>a, b</b>                  |
| Creatinine, mg/dl<br>Mean ± SD                         | 0.8 ± 0.2                           | 0.8 ± 0.2                           | 0.8 ± 0.1                                      | <i>ns</i>                               |
| hs-CRP, mg/dl#<br>Median [IQR]                         | 0.08<br>[0.04 – 0.15]               | 0.08<br>[0.04 – 0.14]               | 0.07<br>[0.04 – 0.16]                          | <i>ns</i>                               |
| White cell count x 10 <sup>9</sup> /L#<br>Median [IQR] | 7.3<br>[5.8 – 7.8]                  | 6.5<br>[5.9 – 7.7]                  | 7.0<br>[5.5 – 8.0]                             | <i>ns</i>                               |
| Classical Monocytes, %#<br>Median [IQR]                | 76<br>[74 – 80]                     | 71<br>[65 – 75]                     | 74<br>[69 – 78]                                | <i>ns</i>                               |
| Non-Classical Monocytes, %#<br>Median [IQR]            | 8.6<br>[6.5–11.3]                   | 11.0<br>[7.5–18.7]                  | 10.6<br>[7.9–15.0]                             | <i>ns</i>                               |
| Intermediate Monocytes, %#<br>Median [IQR]             | 6.3<br>[5.0-9.6]                    | 6.5<br>[5.4-7.8]                    | 6.8<br>[5.8-8.1]                               | <i>ns</i>                               |
| CACS, Agatston Units                                   | 0                                   | 0                                   | >1.0<br>F: 1/6<br>M: 7/10                      |                                         |

Values are mean ± SD (standard deviation) for normal distribution, or median (IQR, interquartile range) for non-normal distribution. **BMI**, body mass index; **SBP**, systolic blood pressure; **DBP**, diastolic blood pressure; **LDLc**, low density lipoprotein-cholesterol; **HDLc**, high density lipoprotein-cholesterol; **non-HDLc**, non-high-density lipoprotein-cholesterol; **hs-CRP**, high sensitivity - C-reactive protein; **CACS**, coronary artery calcium score. #Parameters were log-transformed to achieve normal distribution and to apply statistical parametric analysis, but in these table the original data were used. <sup>##</sup>Contingency analysis and Chi-square test. \* Ordinary one-way ANOVA; *ns*, non-significant. \*\***a, b**, and **c**: unpaired *t*-test for mean values; **a**, IR vs LR; **b**, SCA vs LR; and **c**, SCA vs IR. Letters with significant value, *p*<0.05, are shown; *ns*, non-significant *p* value.

**Supplementary Table S6: Correlation analysis between cell surface LRP1 expression and pro-inflammatory factor in total monocytes (Study II)**

|          | LRP1 expression in total monocytes (MFI values)<br>vs.                      |                                                                             |                                                           |                                                           | LRP1/GADPH mRNA levels (relative expression)<br>vs.                         |                                                                             |                                                           |                                                           |
|----------|-----------------------------------------------------------------------------|-----------------------------------------------------------------------------|-----------------------------------------------------------|-----------------------------------------------------------|-----------------------------------------------------------------------------|-----------------------------------------------------------------------------|-----------------------------------------------------------|-----------------------------------------------------------|
|          | <i>TNF<math>\alpha</math>/GA<br/>DPH<br/>(Relative<br/>Expression<br/>)</i> | <i>IL-<br/>1<math>\beta</math>/GADPH<br/>(Relative<br/>Expression<br/>)</i> | <i>CCL2/GA<br/>DPH<br/>(Relative<br/>Expression<br/>)</i> | <i>CCR2/GA<br/>DPH<br/>(Relative<br/>Expression<br/>)</i> | <i>TNF<math>\alpha</math>/GA<br/>DPH<br/>(Relative<br/>Expression<br/>)</i> | <i>IL-<br/>1<math>\beta</math>/GADPH<br/>(Relative<br/>Expression<br/>)</i> | <i>CCL2/GA<br/>DPH<br/>(Relative<br/>Expression<br/>)</i> | <i>CCR2/GA<br/>DPH<br/>(Relative<br/>Expression<br/>)</i> |
| <i>r</i> | <b>-0.376</b>                                                               | <b>-0.387</b>                                                               | -0.257                                                    | -0.173                                                    | 0.009                                                                       | -0.008                                                                      | -0.0879                                                   | -0.2561                                                   |
| <i>p</i> | <b>0.008</b>                                                                | <b>0.007</b>                                                                | 0.077                                                     | 0.241                                                     | 0.949                                                                       | 0.959                                                                       | 0.5527                                                    | 0.079                                                     |

All parameters were log-transformed to achieve normal distribution and to apply the Pearson correlation analysis. Significant value,  $p < 0.05$ . **Bold font** indicates parameter with statistical significance. **MFI**, mean fluorescence intensity; (n= 48)