**OMTM, Volume *10***

## Supplemental Information

# Integrated Human Evaluation of the Lysophosphatidic Acid Pathway as a Novel Therapeutic Target in Atherosclerosis

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# Supplemental Figures and Legends

## Figure S1

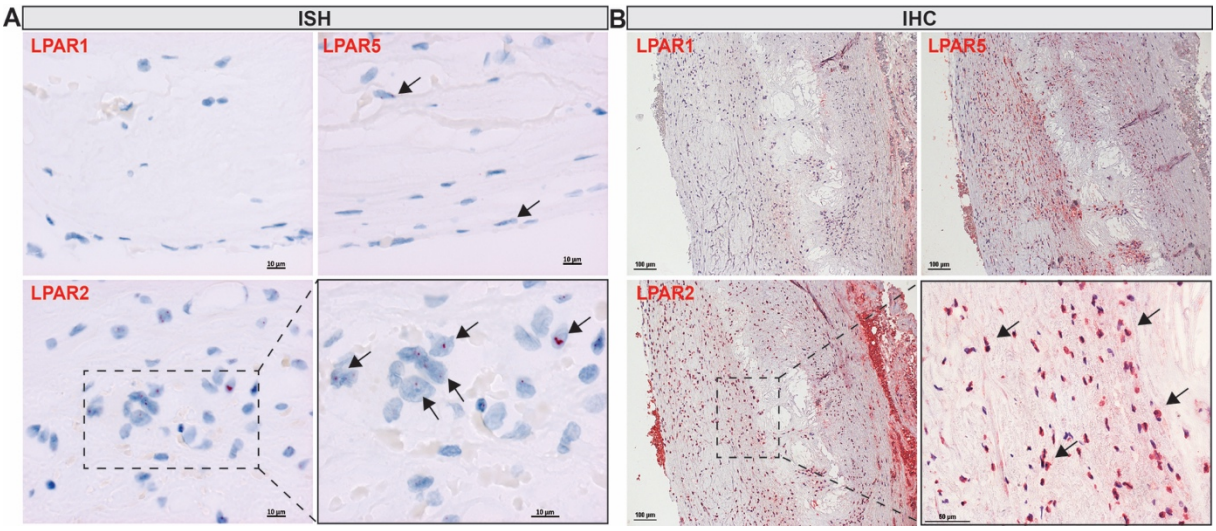


**Figure S1. Localisation of LPARs in plaques. A)** *In situ* hybridization detection of LPARs 1, 5 and 2 mRNA transcripts in human lesions. Arrows indicate the RNA probe signals (red). **B)** Immunohistochemistry staining of LPARs 1, 5 and 2 in plaques (red signal). Arrows in the enlarged LPAR2 image indicate positive signal in the cells within the necrotic core. Nuclei (purple) are stained with hematoxylin. Images taken with 20x, 40x and 63x objectives.

## Figure S2

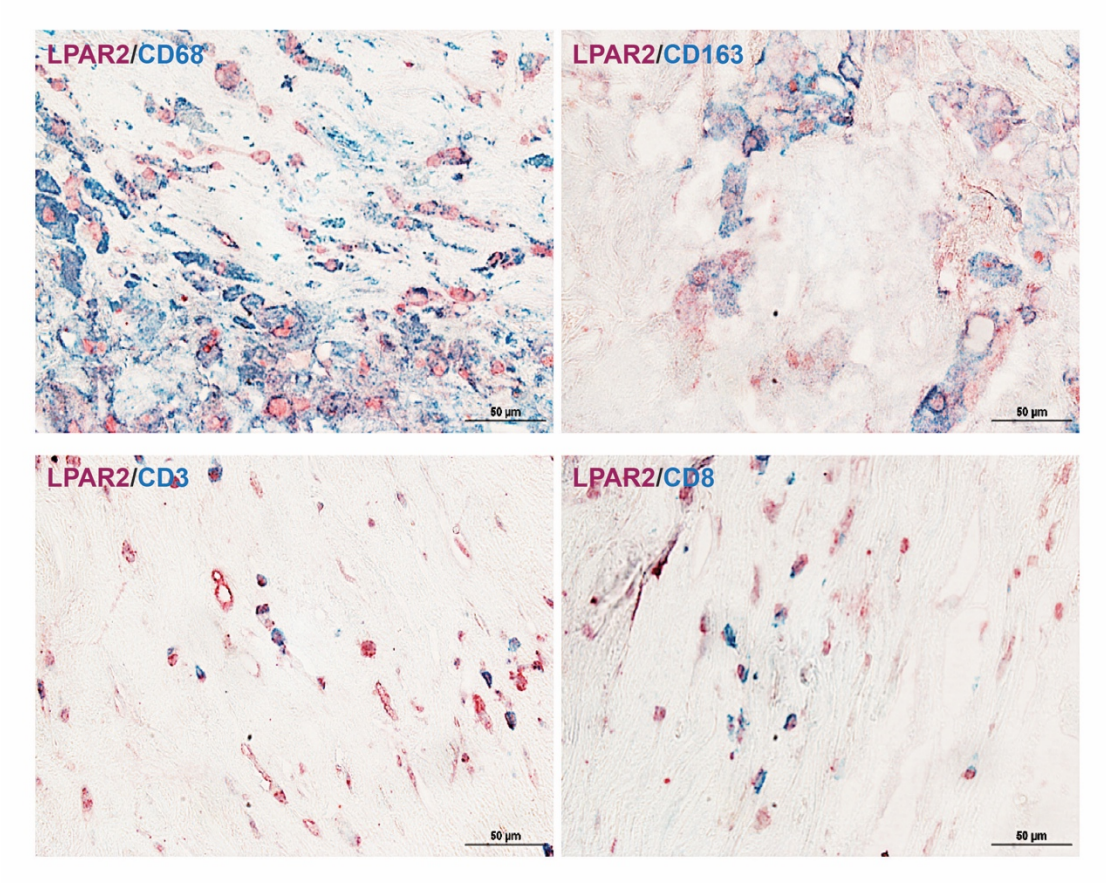


**Figure S2. Immunolocalisation of LPAR2 in plaque inflammatory cells.** Double immunohistochemistry stainings showing the colocalisation of LPAR2 (red signal) with macrophage cell markers (CD68 and CD163, green signal) and lymphocyte markers (CD3 and CD8, green signal). Images taken with the 40x objective.

## Figure S3

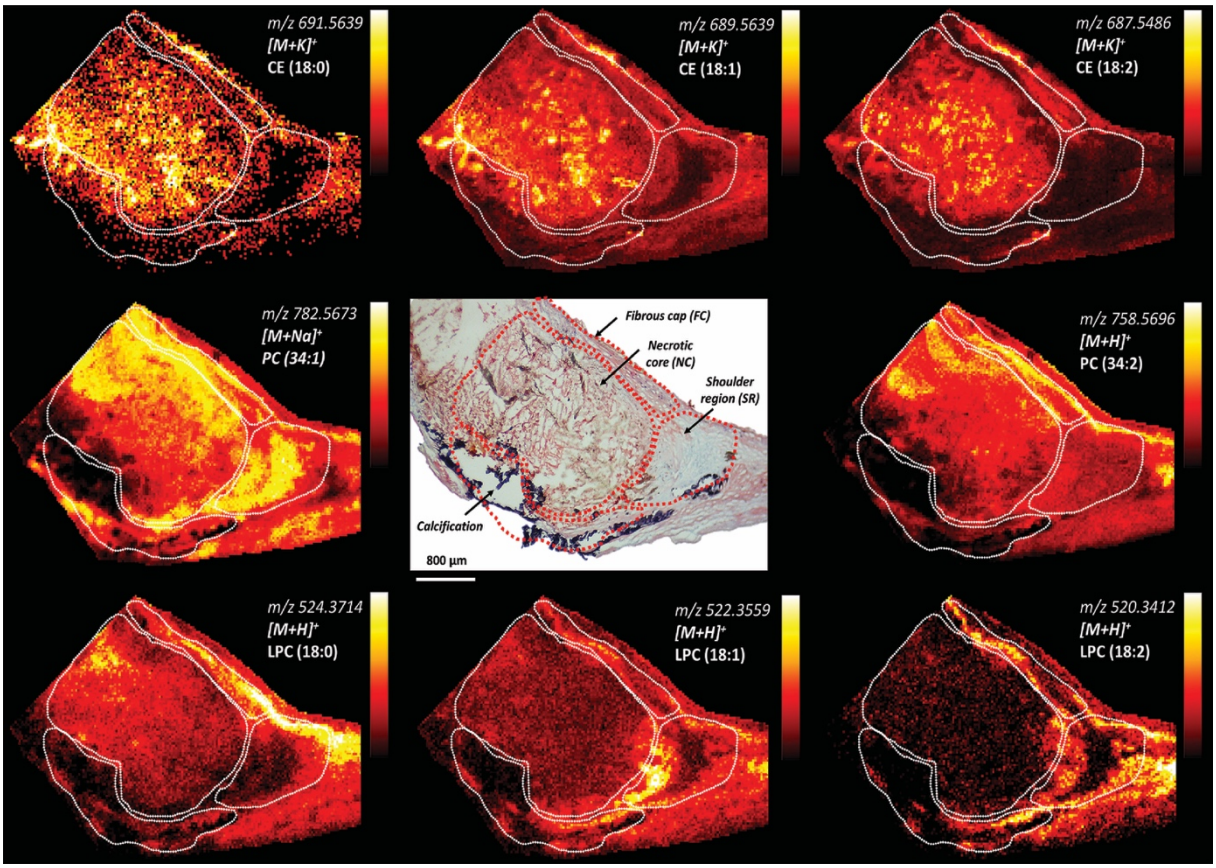


**Figure S3. Molecular histology of the human atherosclerotic plaque based on its lipid fingerprint.** Molecular distribution of the main lipid species from different classes (CE: Cholesteryl ester, PC: Phophatydilcholine and LPC: lysophosphatidylcholine) detected in human plaque tissue by mass spectrometry (MS) imaging using MALDI-FTICR in positive detection mode at 30 µm of spatial resolution. Identification of molecular species was performed by accurate MS match with database (<1 ppm) and MS/MS measurement. The different lipid classes are reported on the figure. Relative intensity scale (volcano intensity scale, 0-100%) is indicated on the side of each image. Histological regions of interest were identified by Oil-red-O staining (central picture, arrows).

**Figure S4**

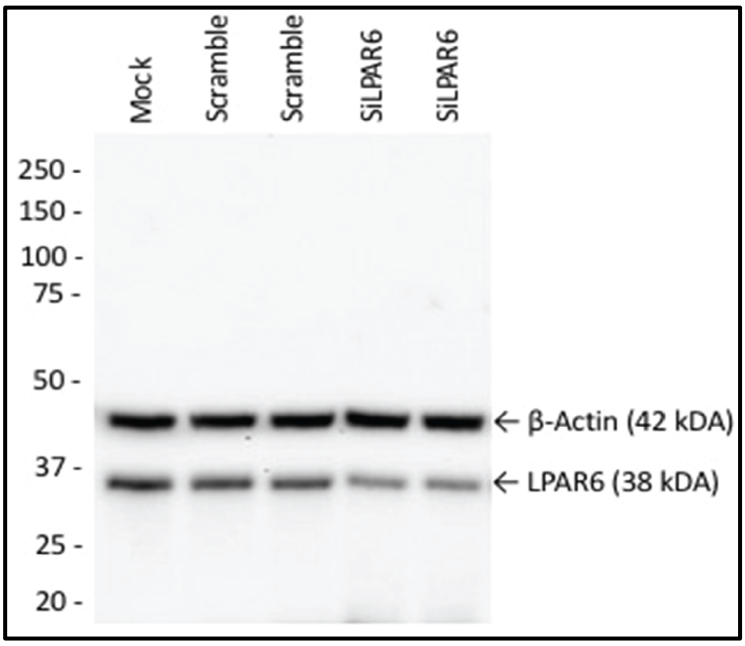


**Figure S4. Western blot analysis of LPAR6 protein levels in HUVEC lysates.** LPAR6 mRNA silencing resulted in repression of the protein levels compared to non-targeting scramble oligos and mock control. Protein levels of beta-actin were used as loading control. Ladder marks indicated on the left.

# Supplemental Tables and Legends

## Table S1

| Cell type markers | Gene Symbol | Pearson r | p-value | Significance level |
|---|---|---|---|---|
| ***Smooth muscle cells*** | | | | |
| Myosin heavy chain 11 | *MYH11* | 0.2759 | 0.0018 | ** |
| Smoothelin | *SMTN* | 0.2057 | 0.0209 | * |
| Alpha smooth muscle actin | *ACTA2* | 0.3752 | < 0.0001 | **** |
| Myocardin | *MYOCD* | 0.3399 | < 0.0001 | **** |
| Transgelin | *TAGLN* | 0.4809 | < 0.0001 | **** |
| ***Endothelial cells*** | | | | |
| von Willebrand factor | *VWF* | 0.01506 | 0.8671 | ns |
| PECAM-1 (CD31) | *PECAM1* | 0.5245 | < 0.0001 | **** |
| ***Dendritic cells*** | | | | |
| ITGAX (CD11c) | *ITGAX* | -0.1212 | 0.1746 | ns |
| LY75 (CD205) | *LY75* | 0.04661 | 0.6028 | ns |
| CD80 | *CD80* | -0.1459 | 0.1018 | ns |
| ***T Lymphocytes*** | | | | |
| CD11b | *ITGAM* | -0.1982 | 0.0261 | * |
| ITGAL | *ITGAL* | -0.1949 | 0.0287 | * |
| CD27 | *CD27* | -0.07268 | 0.4187 | ns |
| CD28 | *CD28* | 0.2105 | 0.018 | * |
| CD3 delta | *CD3D* | -0.02751 | 0.7598 | ns |
| CD4 | *CD4* | 0.1938 | 0.0297 | * |
| CD8A | *CD8A* | -0.07039 | 0.4335 | ns |
| PTPRC (CD45RA) | *PTPRC* | 0.3796 | < 0.0001 | **** |
| CD69 | *CD69* | -0.06686 | 0.4552 | ns |
| ITGAE | *ITGAE* | 0.08025 | 0.3698 | ns |
| FABP4 | *FABP4* | 0.01908 | 0.8314 | ns |
| ***Macrophages*** | | | | |
| CD83 | *CD83* | -0.02925 | 0.7451 | ns |
| CD86 | *CD86* | 0.3196 | 0.0003 | *** |
| CD163 | *CD163* | 0.2817 | 0.0014 | ** |
| TNFRSF9 | *TNFRSF9* | -0.1674 | 0.0611 | ns |
| CD40 | *CD40* | -0.1155 | 0.1977 | ns |
| CD36 | *CD36* | 0.1858 | 0.0365 | * |
| **Inlfammation/Apoptosis Calcification markers** | | | | |
| IL-1beta | *IL1B* | 0.05357 | 0.5513 | ns |
| NFkB | *NFKB1* | 0.3644 | < 0.0001 | **** |
| TNF-alpha | *TNFA* | -0.2183 | 0.0141 | * |
| MCP-1 | *CCL2* | 0.3241 | 0.0002 | *** |
| Caspase-3 | *CASP3* | 0.1682 | 0.0598 | ns |

| | | | | |
|---|---|---|---|---|
| Caspase-7 | *CASP7* | 0.08284 | 0.3564 | ns |
| Caspase-9 | *CASP9* | 0.1407 | 0.116 | ns |
| BCL2 | *BCL2* | 0.1983 | 0.026 | * |
| RANTES | *CCL5* | -0.2318 | 0.009 | ** |
| BMP4 | *BMP4* | | < 0.0001 | **** |
| **Extracellular matrix/degradation** | | | | |
| MMP9 | *MMP9* | 0.03487 | 0.6982 | ns |
| TIMP1 | *TIMP1* | 0.3178 | 0.0003 | *** |
| Sulfatase 1 | *SULF1* | 0.6295 | < 0.0001 | **** |
| Sulfatase 2 | *SULF2* | 0.1564 | 0.0803 | ns |
| **Growth factors** | | | | |
| TGFB1 | *TGFB1* | 0.3565 | < 0.0001 | **** |
| TGFA | *TGFA* | -0.1275 | 0.155 | ns |
| IGF1 | *IGF1* | 0.4746 | < 0.0001 | **** |
| PDGFA | *PDGFA* | -0.3214 | 0.0002 | *** |
| PDGFB | *PDGFB* | -0.2063 | 0.0205 | * |
| PDGFC | *PDGFC* | 0.3403 | < 0.0001 | **** |
| PDGFD | *PDGFD* | 0.4509 | < 0.0001 | **** |
| **Chemokines and receptors** | | | | |
| CCR2 | *CCR2* | 0.425 | < 0.0001 | **** |
| CCR5 | *CCR5* | 0.0959 | 0.2835 | ns |
| Interleukin 10 | *IL10* | -0.2681 | 0.0024 | ** |
| Interferon gamma | *INFG* | -0.2756 | 0.0018 | ** |
| IL2 | *IL2* | -0.1016 | 0.2578 | ns |
| IL6 | *IL6* | 0.01629 | 0.8557 | ns |
| IL4 | *IL4* | -0.2411 | 0.0065 | ** |
| IL5 | *IL5* | -0.01216 | 0.8925 | ns |
| IL9 | *IL9* | -0.172 | 0.0542 | ns |

**Table S1. Expression correlation analyses between PPAP2B and genes of interest in plaques.** Pearson correlation analyses were calculated from n=127 human plaque microarrays, p-values are corrected for multiple comparisons according to the Bonferroni method. Correlation considered weak if $r < 0.3$ moderate if $0.3 < r < 0.5$ and strong if $r > 0.5$.

**Table S2**

| | LPAR1 | | | | LPAR2 | | |
|---|---|---|---|---|---|---|---|
| **Cell type markers** | Gene Symbol | Pearson r | p-value | Significance level | Pearson r | p-value | Significance level |
| ***Smooth muscle cells*** | | | | | | | |
| Myosin heavy chain 11 | *MYH11* | 0.6706 | < 0.0001 | **** | -0.4431 | < 0.0001 | **** |
| Smoothelin | *SMTN* | 0.4657 | < 0.0001 | **** | -0.4536 | < 0.0001 | **** |
| Alpha smooth muscle actin | *ACTA2* | 0.6343 | < 0.0001 | **** | -0.4332 | < 0.0001 | **** |
| Myocardin | *MYOCD* | 0.6585 | < 0.0001 | **** | -0.5676 | < 0.0001 | **** |
| Transgelin | *TAGLN* | 0.5662 | < 0.0001 | **** | -0.5853 | < 0.0001 | **** |
| ***Endothelial cells*** | | | | | | | |
| von Willebrand factor | *VWF* | -0.3296 | 0.0002 | *** | 0.4895 | < 0.0001 | **** |
| PECAM-1 (CD31) | *PECAM1* | -0.1619 | 0.0701 | ns | 0.2939 | 0.0008 | *** |
| ***Dendritic cells*** | | | | | | | |
| ITGAX (CD11c) | *ITGAX* | -0.5713 | < 0.0001 | **** | 0.5664 | < 0.0001 | **** |
| LY75 (CD205) | *LY75* | -0.002261 | 0.9799 | ns | 0.4875 | < 0.0001 | **** |
| CD80 | *CD80* | -0.3983 | < 0.0001 | **** | 0.4718 | < 0.0001 | **** |
| ***T Lymphocytes*** | | | | | | | |
| CD11b | *ITGAM* | -0.5282 | < 0.0001 | **** | 0.4891 | < 0.0001 | **** |
| ITGAL | *ITGAL* | -0.4416 | < 0.0001 | **** | 0.6066 | < 0.0001 | **** |
| CD27 | *CD27* | -0.419 | < 0.0001 | **** | 0.3069 | 0.0005 | *** |
| CD28 | *CD28* | -0.2708 | 0.0022 | ** | 0.4252 | < 0.0001 | **** |
| CD3 delta | *CD3D* | -0.3652 | < 0.0001 | **** | 0.1419 | 0.113 | ns |
| CD4 | *CD4* | -0.5254 | < 0.0001 | **** | 0.08309 | 0.355 | ns |
| CD8A | *CD8A* | -0.3322 | 0.0001 | *** | 0.2653 | 0.0027 | ** |
| PTPRC (CD45RA) | *PTPRC* | -0.2482 | 0.0049 | ** | 0.3816 | < 0.0001 | **** |
| CD69 | *CD69* | -0.07406 | 0.408 | ns | 0.4384 | < 0.0001 | **** |
| ITGAE | *ITGAE* | -0.1859 | 0.0364 | * | 0.5556 | < 0.0001 | **** |
| FABP4 | *FABP4* | -0.3392 | < 0.0001 | **** | 0.4484 | < 0.0001 | **** |
| ***Macrophages*** | | | | | | | |
| CD83 | *CD83* | -0.4417 | < 0.0001 | **** | 0.465 | < 0.0001 | **** |
| CD86 | *CD86* | -0.4989 | < 0.0001 | **** | 0.5162 | < 0.0001 | **** |
| RANK | *TNFRSF11A* | -0.4012 | < 0.0001 | **** | 0.6283 | < 0.0001 | **** |
| CD163 | *CD163* | -0.4111 | < 0.0001 | **** | 0.5445 | < 0.0001 | **** |
| TNFRSF9 | *TNFRSF9* | -0.621 | < 0.0001 | **** | 0.3327 | 0.0001 | *** |
| CD40 | *CD40* | -0.5126 | < 0.0001 | **** | 0.4241 | < 0.0001 | **** |
| CD36 | *CD36* | -0.3921 | < 0.0001 | **** | 0.5142 | < 0.0001 | **** |
| **Inlfammation/Apoptosis Calcification markers** | | | | | | | |
| IL-1beta | *IL1B* | -0.2719 | 0.0021 | ** | 0.242 | 0.0063 | ** |
| NFkB | *NFKB1* | -0.1226 | 0.1714 | ns | 0.1519 | 0.0896 | ns |
| TNF-alpha | *TNFA* | -0.484 | < 0.0001 | **** | 0.3666 | < 0.0001 | **** |
| MCP-1 | *CCL2* | -0.1699 | 0.0571 | ns | 0.01676 | 0.8522 | ns |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Caspase-3 | *CASP3* | -0.05183 | 0.5644 | ns | 0.5511 | < 0.0001 | **** |
| Caspase-7 | *CASP7* | -0.005042 | 0.9553 | ns | 0.3474 | < 0.0001 | **** |
| Caspase-9 | *CASP9* | -0.02516 | 0.7797 | ns | 0.2577 | 0.0036 | ** |
| BCL2 | *BCL2* | 0.4797 | < 0.0001 | **** | -0.4165 | < 0.0001 | **** |
| RANTES | *CCL5* | -0.4499 | < 0.0001 | **** | 0.44 | < 0.0001 | **** |
| BMP4 | *BMP4* | 0.04733 | 0.5987 | ns | 0.4274 | < 0.0001 | **** |
| **Extracellular matrix/degradation** | | | | | | | |
| MMP9 | *MMP9* | -0.4874 | < 0.0001 | **** | 0.4547 | < 0.0001 | **** |
| TIMP1 | *TIMP1* | -0.3947 | < 0.0001 | **** | 0.334 | 0.0001 | *** |
| Sulfatase 1 | *SULF1* | -0.325 | 0.0002 | *** | 0.5368 | < 0.0001 | **** |
| Sulfatase 2 | *SULF2* | 0.3379 | 0.0001 | *** | -0.3382 | 0.0001 | *** |
| **Growth factors** | | | | | | | |
| TGFB1 | *TGFB1* | -0.2904 | 0.001 | *** | -0.4719 | < 0.0001 | **** |
| TGFA | *TGFA* | -0.3053 | 0.0005 | *** | 0.5867 | < 0.0001 | **** |
| IGF1 | *IGF1* | -0.1903 | 0.0328 | * | 0.4513 | < 0.0001 | **** |
| PDGFA | *PDGFA* | 0.5479 | < 0.0001 | **** | 0.02336 | 0.7951 | ns |
| PDGFB | *PDGFB* | -0.408 | < 0.0001 | **** | 0.1198 | 0.1813 | ns |
| PDGFC | *PDGFC* | 0.5948 | < 0.0001 | **** | -0.1977 | 0.0265 | * |
| PDGFD | *PDGFD* | 0.6034 | < 0.0001 | **** | -0.4602 | < 0.0001 | **** |
| **Chemokines and receptors** | | | | | | | |
| CCR2 | *CCR2* | -0.07643 | 0.3931 | ns | 0.3701 | < 0.0001 | **** |
| CCR5 | *CCR5* | -0.4306 | < 0.0001 | **** | 0.4636 | < 0.0001 | **** |
| Interleukin 10 | *IL10* | -0.3689 | < 0.0001 | **** | 0.5818 | < 0.0001 | **** |
| Interferon gamma | *INFG* | -0.3075 | 0.0005 | *** | -0.03486 | 0.6984 | ns |
| IL2 | *IL2* | -0.1837 | 0.0395 | * | -0.2099 | 0.0183 | * |
| IL6 | *IL6* | -0.03277 | 0.7145 | ns | 0.2466 | 0.0052 | ** |
| IL4 | *IL4* | -0.1667 | 0.0621 | ns | -0.2958 | 0.0008 | *** |
| IL5 | *IL5* | -0.007118 | 0.9369 | ns | -0.4802 | < 0.0001 | **** |
| IL9 | *IL9* | -0.1642 | 0.0662 | ns | -0.206 | 0.0206 | * |

| | | **LPAR3** | | | **LPAR4** | | |
|---|---|---|---|---|---|---|---|
| **Cell type markers** | Gene Symbol | Pearson r | p-value | Significance level | Pearson r | p-value | Significance level |
| ***Smooth muscle cells*** | | | | | | | |
| Myosin heavy chain 11 | *MYH11* | -0.2946 | 0.0008 | *** | 0.1502 | 0.0933 | ns |
| Smoothelin | *SMTN* | 0.4251 | < 0.0001 | **** | -0.3744 | < 0.0001 | **** |
| Alpha smooth muscle actin | *ACTA2* | -0.2514 | 0.0045 | ** | 0.1165 | 0.1939 | ns |
| Myocardin | *MYOCD* | -0.09252 | 0.3028 | ns | 0.03997 | 0.6568 | ns |
| Transgelin | *TAGLN* | 0.03132 | 0.7278 | ns | -0.1038 | 0.2472 | ns |
| ***Endothelial cells*** | | | | | | | |
| von Willebrand factor | *VWF* | -0.3106 | 0.0004 | *** | 0.1736 | 0.0519 | ns |
| PECAM-1 (CD31) | *PECAM1* | -0.1621 | 0.0698 | ns | 0.1127 | 0.2089 | ns |
| ***Dendritic cells*** | | | | | | | |
| ITGAX (CD11c) | *ITGAX* | 0.3511 | < 0.0001 | **** | -0.07009 | 0.4336 | ns |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| LY75 (CD205) | *LY75* | -0.4494 | < 0.0001 | **** | 0.06596 | 0.4613 | ns |
| CD80 | *CD80* | -0.2953 | 0.0007 | *** | 0.1664 | 0.0614 | ns |
| ***T Lymphocytes*** | | | | | | | |
| CD11b | *ITGAM* | 0.4518 | < 0.0001 | **** | -0.4083 | < 0.0001 | **** |
| ITGAL | *ITGAL* | -0.1041 | 0.2458 | ns | -0.01843 | 0.8377 | ns |
| CD27 | *CD27* | 0.03483 | 0.6987 | ns | -0.2123 | 0.017 | * |
| CD28 | *CD28* | -0.254 | 0.0041 | ** | -0.2748 | 0.0018 | ** |
| CD3 delta | *CD3D* | 0.1412 | 0.1148 | ns | -0.1471 | 0.1001 | ns |
| CD4 | *CD4* | 0.2873 | 0.0011 | ** | -0.4718 | < 0.0001 | **** |
| CD8A | *CD8A* | 0.05853 | 0.515 | ns | -0.09318 | 0.2994 | ns |
| PTPRC (CD45RA) | *PTPRC* | -0.2953 | 0.0007 | *** | 0.3897 | < 0.0001 | **** |
| CD69 | *CD69* | -0.4398 | < 0.0001 | **** | 0.02757 | 0.7583 | ns |
| ITGAE | *ITGAE* | -0.2002 | 0.024 | * | 0.03967 | 0.6579 | ns |
| FABP4 | *FABP4* | -0.02759 | 0.7582 | ns | -0.07478 | 0.4034 | ns |
| ***Macrophages*** | | | | | | | |
| CD83 | *CD83* | 0.06016 | 0.5034 | ns | -0.09816 | 0.2742 | ns |
| CD86 | *CD86* | -0.005174 | 0.9541 | ns | -0.09719 | 0.279 | ns |
| RANK | *TNFRSF11A* | 0.02527 | 0.7788 | ns | -0.1704 | 0.0564 | ns |
| CD163 | *CD163* | -0.268 | 0.0024 | ** | 0.02635 | 0.7696 | ns |
| TNFRSF9 | *TNFRSF9* | 0.3201 | 0.0003 | *** | -0.2192 | 0.0137 | * |
| CD40 | *CD40* | 0.3923 | < 0.0001 | **** | 0.4597 | < 0.0001 | **** |
| CD36 | *CD36* | -0.1493 | 0.0939 | ns | -0.1578 | 0.0765 | ns |
| **Inlfammation/Apoptosis Calcification markers** | | | | | | | |
| IL-1beta | *IL1B* | -0.01481 | 0.8692 | ns | -0.1345 | 0.1333 | ns |
| NFkB | *NFKB1* | 0.007307 | 0.9353 | ns | -0.1105 | 0.2179 | ns |
| TNF-alpha | *TNFA* | 0.1344 | 0.1334 | ns | -0.1546 | 0.0839 | ns |
| MCP-1 | *CCL2* | -0.001336 | 0.9882 | ns | -0.1377 | 0.1243 | ns |
| Caspase-3 | *CASP3* | -0.4667 | < 0.0001 | **** | 0.2803 | 0.0015 | ** |
| Caspase-7 | *CASP7* | -0.446 | < 0.0001 | **** | 0.106 | 0.2374 | ns |
| Caspase-9 | *CASP9* | -0.3026 | 0.0006 | *** | 0.3252 | 0.0002 | *** |
| BCL2 | *BCL2* | 0.487 | < 0.0001 | **** | -0.1836 | 0.0396 | * |
| RANTES | *CCL5* | 0.07816 | 0.3843 | ns | -0.2627 | 0.003 | ** |
| BMP4 | *BMP4* | -0.3192 | 0.0003 | *** | 0.162 | 0.07 | ns |
| **Extracellular matrix/degradation** | | | | | | | |
| MMP9 | *MMP9* | -0.004226 | 0.9625 | ns | -0.1193 | 0.1834 | ns |
| TIMP1 | *TIMP1* | -0.009881 | 0.9126 | ns | -0.2127 | 0.0168 | * |
| Sulfatase 1 | *SULF1* | -0.2694 | 0.0023 | ** | -0.08024 | 0.3718 | ns |
| Sulfatase 2 | *SULF2* | -0.5118 | < 0.0001 | **** | 0.1538 | 0.0856 | ns |
| **Growth factors** | | | | | | | |
| TGFB1 | *TGFB1* | 0.4135 | < 0.0001 | **** | -0.3427 | < 0.0001 | **** |
| TGFA | *TGFA* | 0.4058 | < 0.0001 | **** | 0.123 | 0.17 | ns |
| IGF1 | *IGF1* | -0.2969 | 0.0007 | *** | 0.1296 | 0.148 | ns |
| PDGFA | *PDGFA* | -0.5459 | < 0.0001 | **** | 0.3771 | < 0.0001 | **** |
| PDGFB | *PDGFB* | 0.2634 | 0.0029 | ** | -0.1694 | 0.058 | ns |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| PDGFC | *PDGFC* | -0.4801 | < 0.0001 | **** | 0.269 | 0.0023 | ** |
| PDGFD | *PDGFD* | -0.2303 | 0.0095 | ** | 0.2796 | 0.0015 | ** |
| **Chemokines and receptors** | | | | | | | |
| CCR2 | *CCR2* | -0.3749 | < 0.0001 | **** | 0.05098 | 0.5692 | ns |
| CCR5 | *CCR5* | -0.06846 | 0.4444 | ns | -0.2076 | 0.0192 | * |
| Interleukin 10 | *IL10* | -0.1088 | 0.2254 | ns | -0.1253 | 0.1622 | ns |
| Interferon gamma | *INFG* | 0.1622 | 0.0696 | ns | -0.1909 | 0.0323 | * |
| IL2 | *IL2* | 0.3133 | 0.0004 | *** | -0.2224 | 0.0123 | * |
| IL6 | *IL6* | -0.2154 | 0.015 | * | -0.05445 | 0.5432 | ns |
| IL4 | *IL4* | 0.4896 | < 0.0001 | **** | -0.3051 | 0.0005 | *** |
| IL5 | *IL5* | 0.404 | < 0.0001 | **** | 0.02027 | 0.8218 | ns |
| IL9 | *IL9* | 0.4855 | < 0.0001 | **** | -0.2934 | 0.0009 | *** |

| | | **LPAR5** | | | **LPAR6** | | |
|---|---|---|---|---|---|---|---|
| **Cell type markers** | Gene Symbol | Pearson r | p-value | Significance level | Pearson r | p-value | Significance level |
| ***Smooth muscle cells*** | | | | | | | |
| Myosin heavy chain 11 | *MYH11* | 0.3653 | < 0.0001 | **** | -0.2408 | 0.0066 | ** |
| Smoothelin | *SMTN* | -0.3695 | < 0.0001 | **** | -0.5444 | < 0.0001 | **** |
| Alpha smooth muscle actin | *ACTA2* | -0.3167 | 0.0003 | *** | -0.2255 | 0.0111 | * |
| Myocardin | *MYOCD* | -0.407 | < 0.0001 | **** | -0.2115 | 0.0174 | * |
| Transgelin | *TAGLN* | -0.422 | < 0.0001 | **** | -0.2844 | 0.0013 | ** |
| ***Endothelial cells*** | | | | | | | |
| von Willebrand factor | *VWF* | 0.472 | < 0.0001 | **** | 0.6393 | < 0.0001 | **** |
| PECAM-1 (CD31) | *PECAM1* | 0.1995 | 0.0251 | * | 0.163 | 0.0683 | ns |
| ***Dendritic cells*** | | | | | | | |
| ITGAX (CD11c) | *ITGAX* | 0.4244 | < 0.0001 | **** | -0.3362 | 0.0001 | *** |
| LY75 (CD205) | *LY75* | 0.4917 | < 0.0001 | **** | 0.4139 | < 0.0001 | **** |
| CD80 | *CD80* | 0.5655 | < 0.0001 | **** | 0.4392 | < 0.0001 | **** |
| ***T Lymphocytes*** | | | | | | | |
| CD11b | *ITGAM* | 0.3943 | < 0.0001 | **** | 0.3279 | 0.0002 | *** |
| ITGAL | *ITGAL* | 0.5303 | < 0.0001 | **** | 0.511 | < 0.0001 | **** |
| CD27 | *CD27* | 0.2685 | 0.0024 | ** | 0.3421 | < 0.0001 | **** |
| CD28 | *CD28* | 0.6452 | < 0.0001 | **** | 0.5678 | < 0.0001 | **** |
| CD3 delta | *CD3D* | 0.2161 | 0.0151 | * | 0.2485 | 0.005 | ** |
| CD4 | *CD4* | 0.1155 | 0.1977 | ns | 0.05921 | 0.5102 | ns |
| CD8A | *CD8A* | 0.2882 | 0.0011 | ** | 0.3144 | 0.0003 | *** |
| PTPRC (CD45RA) | *PTPRC* | 0.4544 | < 0.0001 | **** | 0.6948 | < 0.0001 | **** |
| CD69 | *CD69* | 0.5144 | < 0.0001 | **** | 0.5524 | < 0.0001 | **** |
| ITGAE | *ITGAE* | 0.2536 | 0.004 | ** | 0.2311 | 0.0089 | ** |
| FABP4 | *FABP4* | 0.1492 | 0.0942 | ns | 0.1873 | 0.035 | * |
| ***Macrophages*** | | | | | | | |
| CD83 | *CD83* | 0.4243 | < 0.0001 | **** | 0.2171 | 0.0146 | * |
| CD86 | *CD86* | 0.4769 | < 0.0001 | **** | 0.5662 | < 0.0001 | **** |
| RANK | *TNFRSF11A* | 0.5756 | < 0.0001 | **** | 0.4303 | < 0.0001 | **** |
| CD163 | *CD163* | 0.4787 | < 0.0001 | **** | 0.5628 | < 0.0001 | **** |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| TNFRSF9 | *TNFRSF9* | 0.2011 | 0.0239 | * | -0.01977 | 0.8261 | ns |
| CD40 | *CD40* | 0.4126 | < 0.0001 | **** | 0.2321 | 0.0089 | ** |
| CD36 | *CD36* | 0.3239 | 0.0002 | *** | 0.3013 | 0.0006 | *** |
| **Inlfammation/Apoptosis Calcification markers** | | | | | | | |
| IL-1beta | *IL1B* | 0.4214 | < 0.0001 | **** | 0.3324 | 0.0001 | *** |
| NFkB | *NFKB1* | 0.2796 | 0.0015 | ** | 0.3435 | < 0.0001 | **** |
| TNF-alpha | *TNFA* | 0.3693 | < 0.0001 | **** | 0.3589 | < 0.0001 | **** |
| MCP-1 | *CCL2* | 0.1886 | 0.0344 | * | 0.334 | 0.0001 | *** |
| Caspase-3 | *CASP3* | 0.4408 | < 0.0001 | **** | 0.5471 | < 0.0001 | **** |
| Caspase-7 | *CASP7* | 0.4525 | < 0.0001 | **** | 0.5657 | < 0.0001 | **** |
| Caspase-9 | *CASP9* | 0.365 | < 0.0001 | **** | 0.3399 | < 0.0001 | **** |
| BCL2 | *BCL2* | -0.3679 | < 0.0001 | **** | -0.2746 | 0.0019 | ** |
| RANTES | *CCL5* | 0.4579 | < 0.0001 | **** | 0.3982 | < 0.0001 | **** |
| BMP4 | *BMP4* | 0.2555 | 0.0039 | ** | -0.0006809 | 0.994 | ns |
| **Extracellular matrix/degradation** | | | | | | | |
| MMP9 | *MMP9* | 0.218 | 0.0142 | * | 0.1144 | 0.2023 | ns |
| TIMP1 | *TIMP1* | 0.3771 | < 0.0001 | **** | 0.2514 | 0.0045 | ** |
| Sulfatase 1 | *SULF1* | 0.4413 | < 0.0001 | **** | 0.4089 | < 0.0001 | **** |
| Sulfatase 2 | *SULF2* | 0.3157 | 0.0003 | *** | 0.3571 | < 0.0001 | **** |
| **Growth factors** | | | | | | | |
| TGFB1 | *TGFB1* | -0.4978 | < 0.0001 | **** | -0.2573 | 0.0036 | ** |
| TGFA | *TGFA* | 0.5957 | < 0.0001 | **** | 0.57 | < 0.0001 | **** |
| IGF1 | *IGF1* | 0.4343 | < 0.0001 | **** | 0.6966 | < 0.0001 | **** |
| PDGFA | *PDGFA* | 0.08341 | 0.3531 | ns | 0.2096 | 0.0185 | * |
| PDGFB | *PDGFB* | 0.1527 | 0.0878 | ns | 0.04191 | 0.6412 | ns |
| PDGFC | *PDGFC* | 0.06585 | 0.4638 | ns | 0.2563 | 0.0038 | ** |
| PDGFD | *PDGFD* | -0.2219 | 0.0125 | * | 0.1722 | 0.0539 | ns |
| **Chemokines and receptors** | | | | | | | |
| CCR2 | *CCR2* | 0.5877 | < 0.0001 | **** | 0.7626 | < 0.0001 | **** |
| CCR5 | *CCR5* | 0.4241 | < 0.0001 | **** | 0.4083 | < 0.0001 | **** |
| Interleukin 10 | *IL10* | 0.4787 | < 0.0001 | **** | 0.3627 | < 0.0001 | **** |
| Interferon gamma | *INFG* | -0.01569 | 0.8616 | ns | 0.01233 | 0.891 | ns |
| IL2 | *IL2* | -0.175 | 0.0501 | ns | -0.1519 | 0.0895 | ns |
| IL6 | *IL6* | 0.3142 | 0.0003 | *** | 0.2623 | 0.0029 | ** |
| IL4 | *IL4* | -0.1973 | 0.0268 | * | -0.4218 | < 0.0001 | **** |
| IL5 | *IL5* | -0.4005 | < 0.0001 | **** | -0.2844 | 0.0012 | ** |
| IL9 | *IL9* | -0.2452 | 0.0056 | ** | -0.3565 | < 0.0001 | **** |

**Table S2. Expression correlation analyses between LPARs and various markers in plaques.**

Pearson correlation analyses were calculated from n=127 human plaque microarrays, p-values are corrected for multiple comparisons according to the Bonferroni method. Correlation considered weak if $r < 0.3$, moderate if $0.3 < r < 0.5$ and strong if $r > 0.5$.

**Table S3**

| Correlation | Pearson r | 95% confidence interval | P (two-tailed) | Significance level |
|---|---|---|---|---|
| PPAP2B vs. LPAR1 | 0,1586 | -0.01608 to 0.3239 | 0,0749 | ns |
| PPAP2B vs. LPAR2 | -0,4009 | -0.5376 to -0.2437 | < 0.0001 | **** |
| PPAP2B vs. LPAR3 | -0,2321 | -0.3906 to -0.06033 | 0,0086 | ** |
| PPAP2B vs. LPAR4 | -0,1145 | -0.2831 to 0.06098 | 0,1999 | ns |
| PPAP2B vs. LPAR5 | -0,09513 | -0.2650 to 0.08046 | 0,2874 | ns |
| PPAP2B vs. LPAR6 | 0,366 | 0.2048 to 0.5079 | < 0.0001 | **** |

**Table S3. Expression correlation analyses between PPAP2B and various LPARs in plaques.** Pearson correlation analyses were calculated from n=127 human plaque microarrays, p-values are corrected for multiple comparisons according to the Bonferroni method. Correlation considered weak if $r < 0.3$ moderate if $0.3 < r < 0.5$ and strong if $r > 0.5$.

## Table S4

| [a]Compound Name | | Ion Cluster | [b]*m/z calcd* | [c]*m/z measd* | [d] Error (ppm) |
|---|---|---|---|---|---|
| CE (18:2) | Cholesteryl linoleate | $[M+K]^+$ | 687,5477 | 687,5486 | 1,3 |
| CE (18:1) | Cholesteryl oleate | $[M+K]^+$ | 689,5633 | 689,5639 | 0,9 |
| CE (18:0) | Cholesteryl stearate | $[M+K]^+$ | 691,5790 | 691,5798 | 1,2 |
| PC (34:1) | Phophatydilcholine | $[M+Na]^+$ | 782,5670 | 782,5673 | 0,3 |
| PC (34:2) | Phophatydilcholine | $[M+H]^+$ | 758,5694 | 758,5696 | 0,2 |
| LPC (18:2) | LysoPC | $[M+H]^+$ | 520,3409 | 520,3412 | 0,6 |
| LPC (18:1) | LysoPC | $[M+H]^+$ | 522,3554 | 522,3559 | 1,0 |
| LPC (18:0) | LysoPC | $[M+H]^+$ | 524,3710 | 524,3714 | 0,8 |
| PA (36:2) | Phosphatidic acid | $[M-H]^-$ | 699,4970 | 699,4976 | 0,9 |
| LPA (18:0) | LysoPA | $[M-H2O-H]^-$ | 419,2568 | 419,2572 | 1,0 |

**Table S4. Analysis of lipid species in human carotid plaques.**

Molecular species (a) observed in human plaque by positive and negative ionization detection mode based on m/z value. (b) m/z calculated from molecular formula and (c) m/z measured using FTICR-MS (d) mass accuracy error unit in part per million (ppm).

# Supplemental Methods

### Proteomic analysis of plaques

Atherosclerotic plaques from n=18 BiKE patients (n=9 symptomatic + 9 asymptomatic; matched for male gender, age and statin medication) were analysed using LC-MS/MS as previously described [1]. Briefly, protein samples were digested by trypsin and the resulting tryptic peptides were TMT-labeled and pooled. Pooled samples were cleaned by Strong Cation exchange columns (Phenomenex) and subjected to LC-MS/MS analysis. The sample pools were separated on a 4 hour gradient using an UPLC-system (Dionex UltiMate™ 3000) coupled to a Q-Exactive mass spectrometer (Thermo Fischer Scientific, San Jose, CA, USA). The fragment spectra from the mass spectrometer were matched to a database consisting of theoretical fragment spectra from all human proteins and filtered at a 1% False Discovery Rate on the peptide level to obtain protein identities (Uniprot). Quantitative information was acquired using the TMT reporter ion intensities.

### Antibodies

For immunohistochemical studies, the following primary antibodies with the relative concentrations were used: PPAP2B 1:50, LPAR1 1:500 (both from Novus Biologicals, Littleton, CO, USA), LPAR2 1:250, LPAR5, LPAR6 1:400 (all from LSBio, Seattle, WA, USA), smooth muscle α- actin 1:1000 (SMA, DAKO Sweden AB, Stockholm, SE), anti-CD68 1:50 (Novocastra, Bromma, SE) and anti-CD31 1:200 (Abcam, Cambridge, UK).

### Immunohistochemical (IHC) stainings

IHC reagents were from Biocare Medical (Concord, CA). Tissues were treated as previously described [2]. In brief, tissues were fixed for 48 hours in 4% Zn-formaldehyde at room temperature and paraffin-embedded. Isotype rabbit and mouse IgG were used as negative controls. 5 µm tissue sections were deparaffinized in Tissue Clear and rehydrated in graded ethanol. For antigen retrieval, slides were heated in DIVA buffer (pH 6.0) for 20 min with peak at 121°C. Blocking was performed with Background Sniper for 20 min and primary antibodies were diluted in Da Vinci Green solution, and incubated at room temperature for 1 hour. The single stainings were detected by a probe-polymer system for rabbit, followed by Warp Red chromogen. For double staining, a double-stain probe-polymer system containing alkaline phosphatase and horseradish peroxidase was applied, followed by detection with Warp Red and Vina Green. All slides were counterstained with Hematoxylin QS (Vector Laboratories, Burlingame, CA) and mounted in Pertex (Histolab, Gothenburg, Sweden). Images were scanned by an automated ScanScope slidescanner (Hamamatsu, Kista, Sweden) or acquired by a Nikon OPTIPHOT-2 microscope equipped with a digital camera and processed with NIS-Elements software. Magnifications are indicated in the figure legends.

### *In vitro* experiments

Primary human umbilical vein endothelial cells (HUVECs, Lonza, The Netherlands) were cultured in EBM2 medium supplemented with bullet kit (EGM-2, Lonza) under normoxic conditions (21% $O^2$). Passages 5 to 7 were used throughout the study. Knockdown of LPAR6 and PPAP2B was achieved by transfection with a mix of 4 specific siRNA sequences directed against the human mRNA (SMARTpool siGENOME, GE Dharmacon, Lafayette, CO) in 70% subconfluent HUVEC cultures. Cells were incubated for 1 hour in a small volume of EGM-2 medium supplemented with DharmaFECT 1 (GE Dharmacon, Lafayette, CO) according to manufacturer's instructions. After 1-2 hours cells were supplemented with extra EGM-2 medium to complement medium volumes. As controls, HUVECs were transfected with a mix of 4 scrambled, non-targeting siRNAs (siSham Smartpool; GE Dharmacon, Lafayette, CO). 48h after siRNA transfection HUVECs were treated with 10 µM Lysophosphatidic acid (Santa Cruz, Dallas, TX). 2 hours after LPA treatment cells were harvested and RNA was isolated with the NucleoSpin RNA kit according to the manufacturer's protocol (Macherey-Nage, Düren, Germany). Isolated RNA (500 ng) was reverse transcribed into cDNA (iSCript Adv cDNA kit for RT-qPCR, Bio-Rad, Hercules, CA) and analyzed by real-time fluorescence assessment of SYBR® Green signal in the iCycler iQ Detection system (Bio-Rad). Primers were designed for the human genes of interest. mRNA levels were analyzed and corrected for the housekeeping gene beta-actin. Reported values were normalized to sham that was arbitrarily assigned an average value of 1 [3].

## Supplemental References

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