

ApoC-III is a novel inducer of calcification in human aortic valves

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Short title: Role of ApoC-III in calcific aortic valve disease

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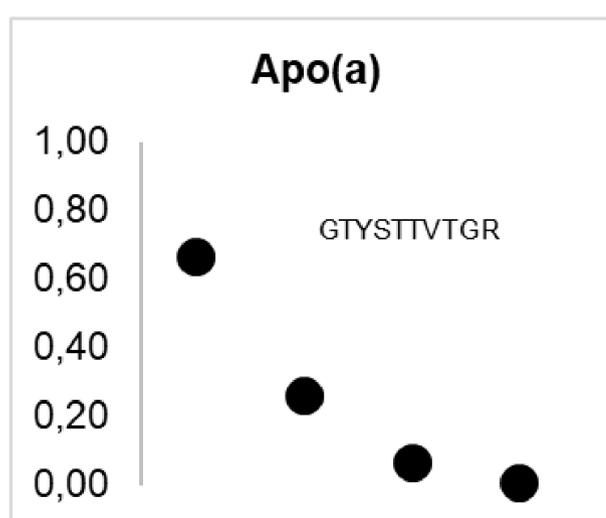
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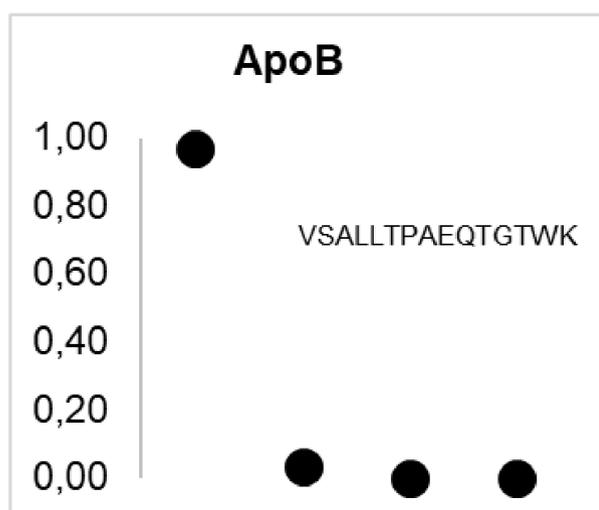
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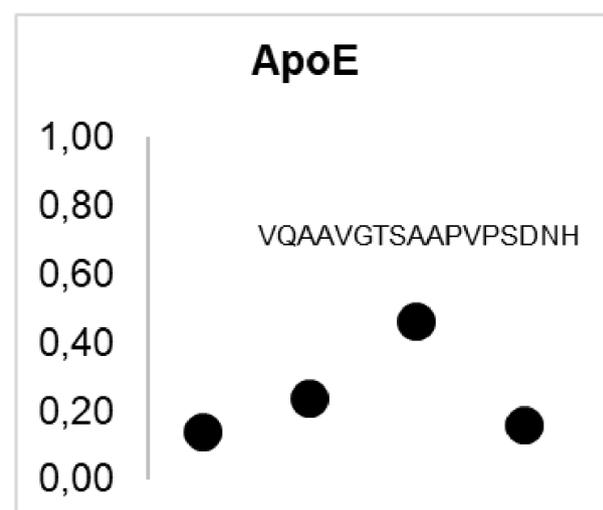
Supporting Information 1



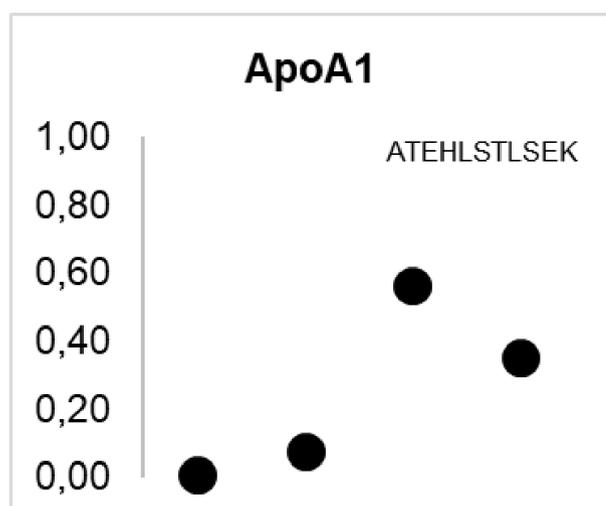
α_0 α_1 α_2 α_3



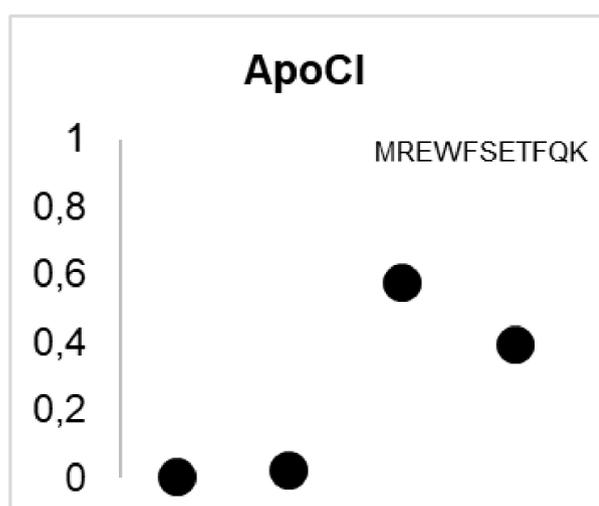
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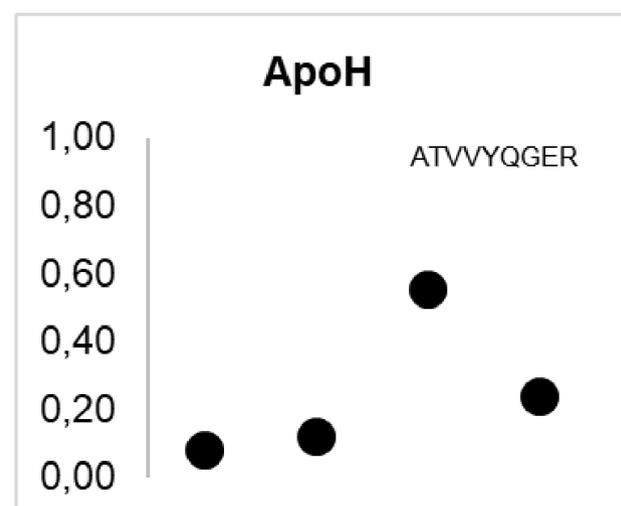
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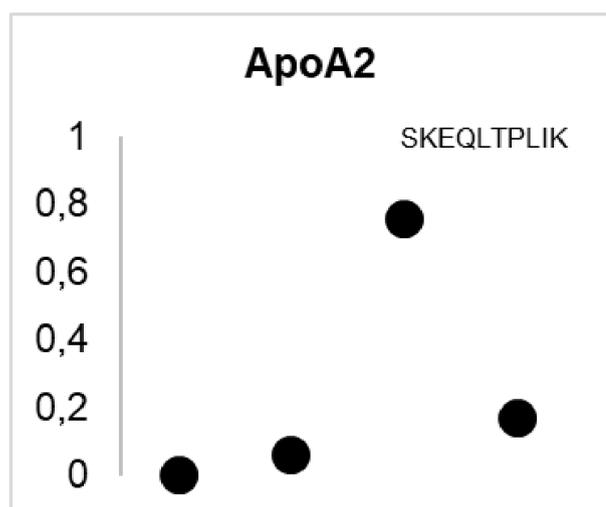
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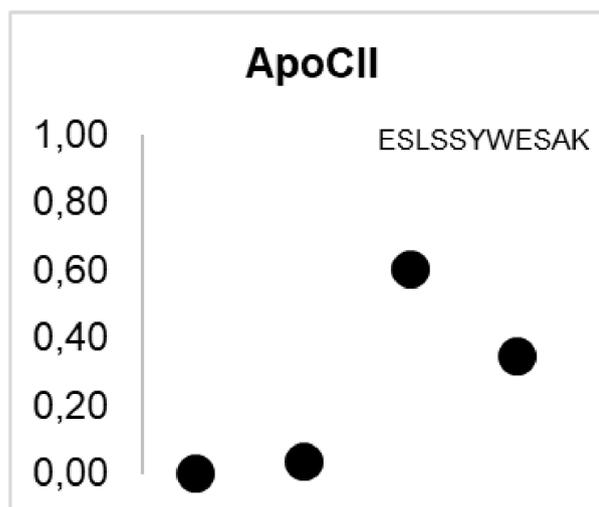
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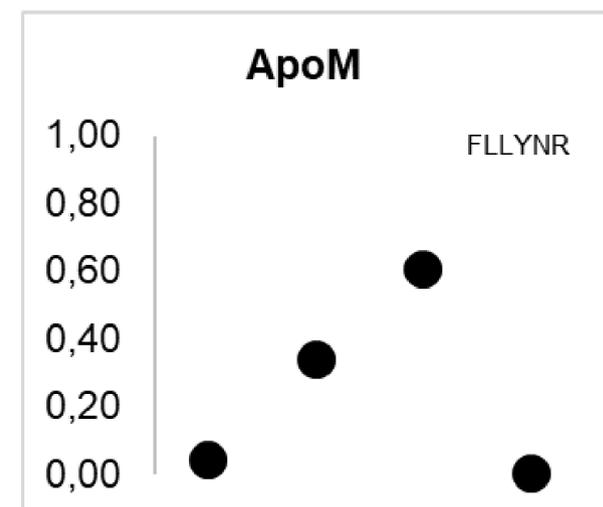
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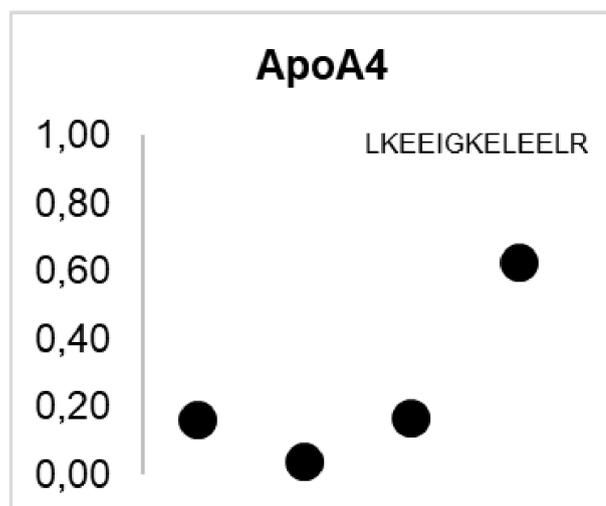
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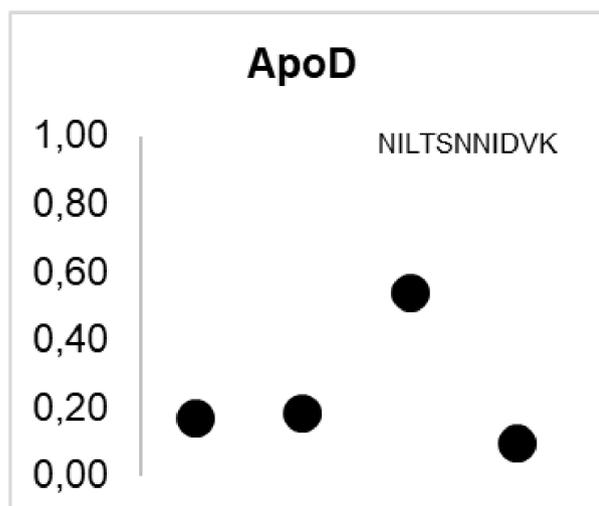
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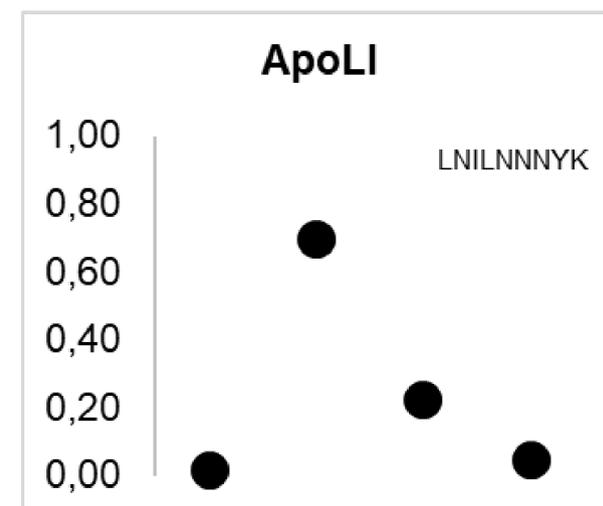
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α_0 α_1 α_2 α_3



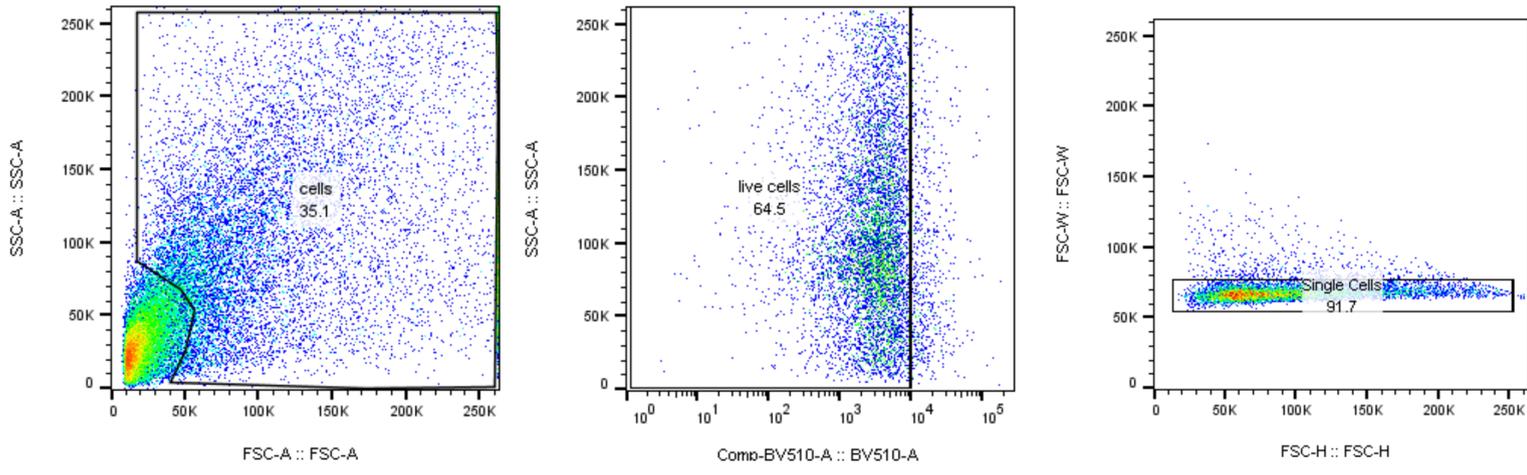
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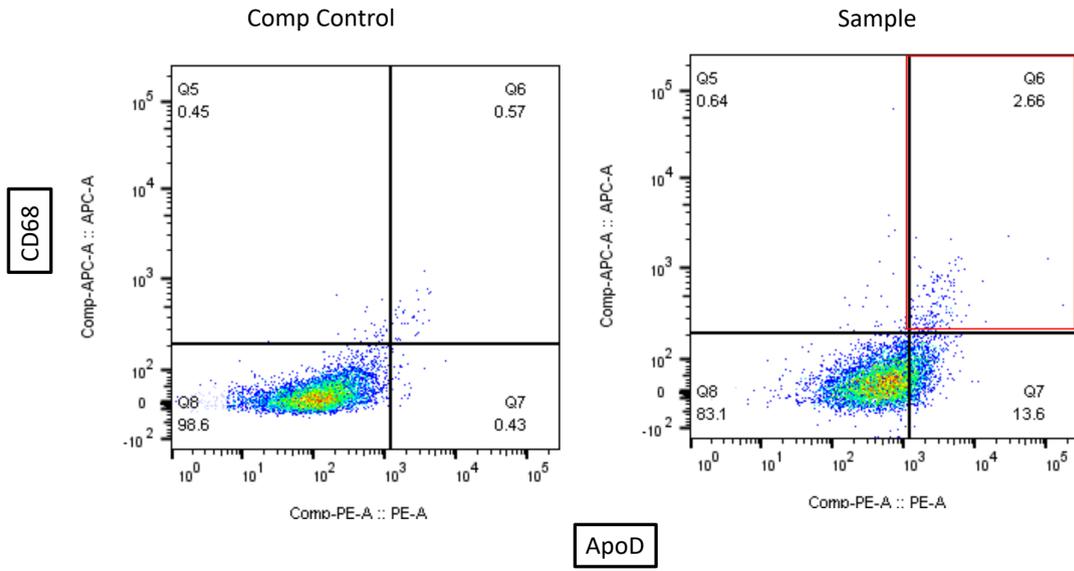
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Supporting Information 2

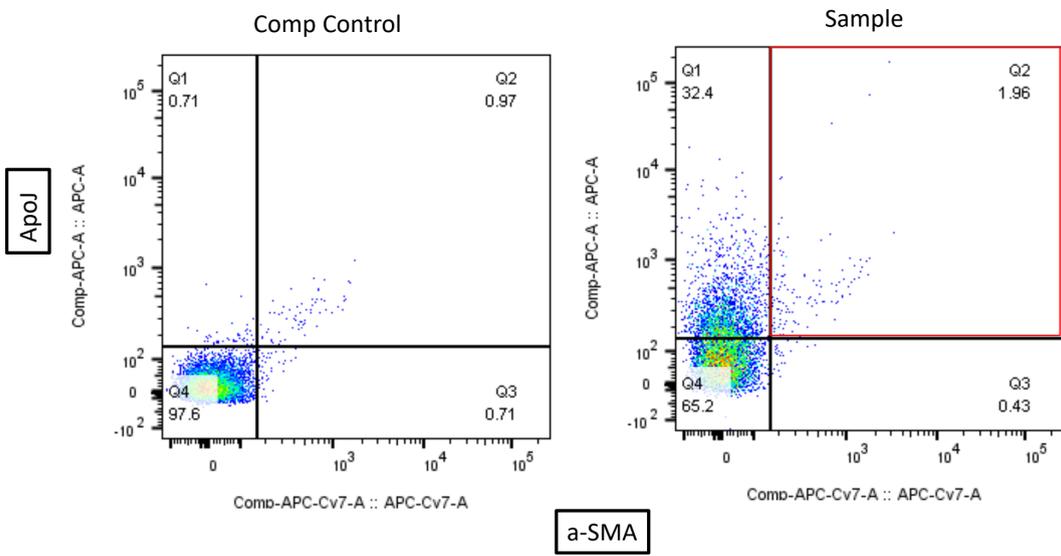
Gating Strategy



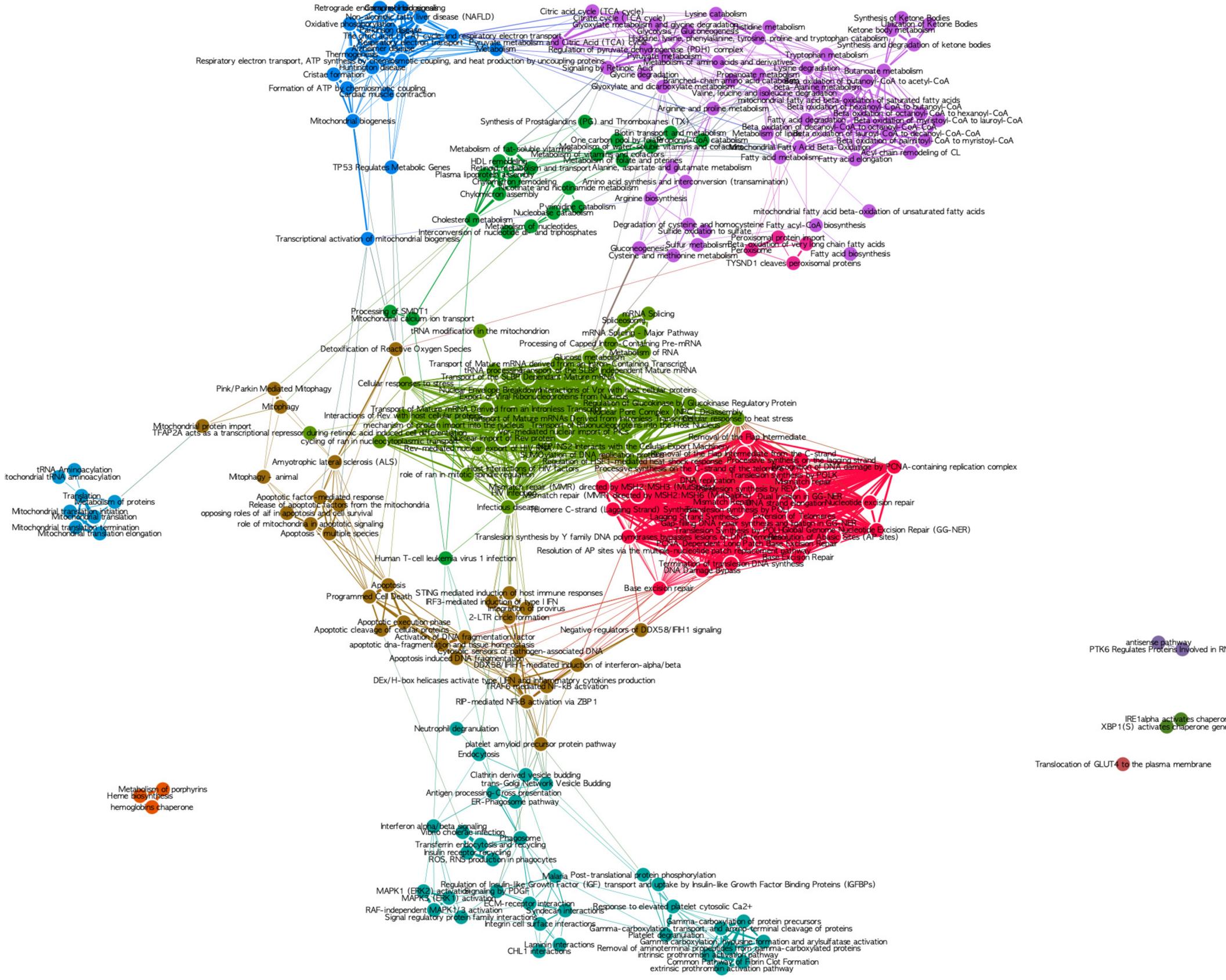
CD68 (APC) + ApoD (PE)



ApoJ (APC) + aSMA (APC-cy7)



Supporting Information 4A

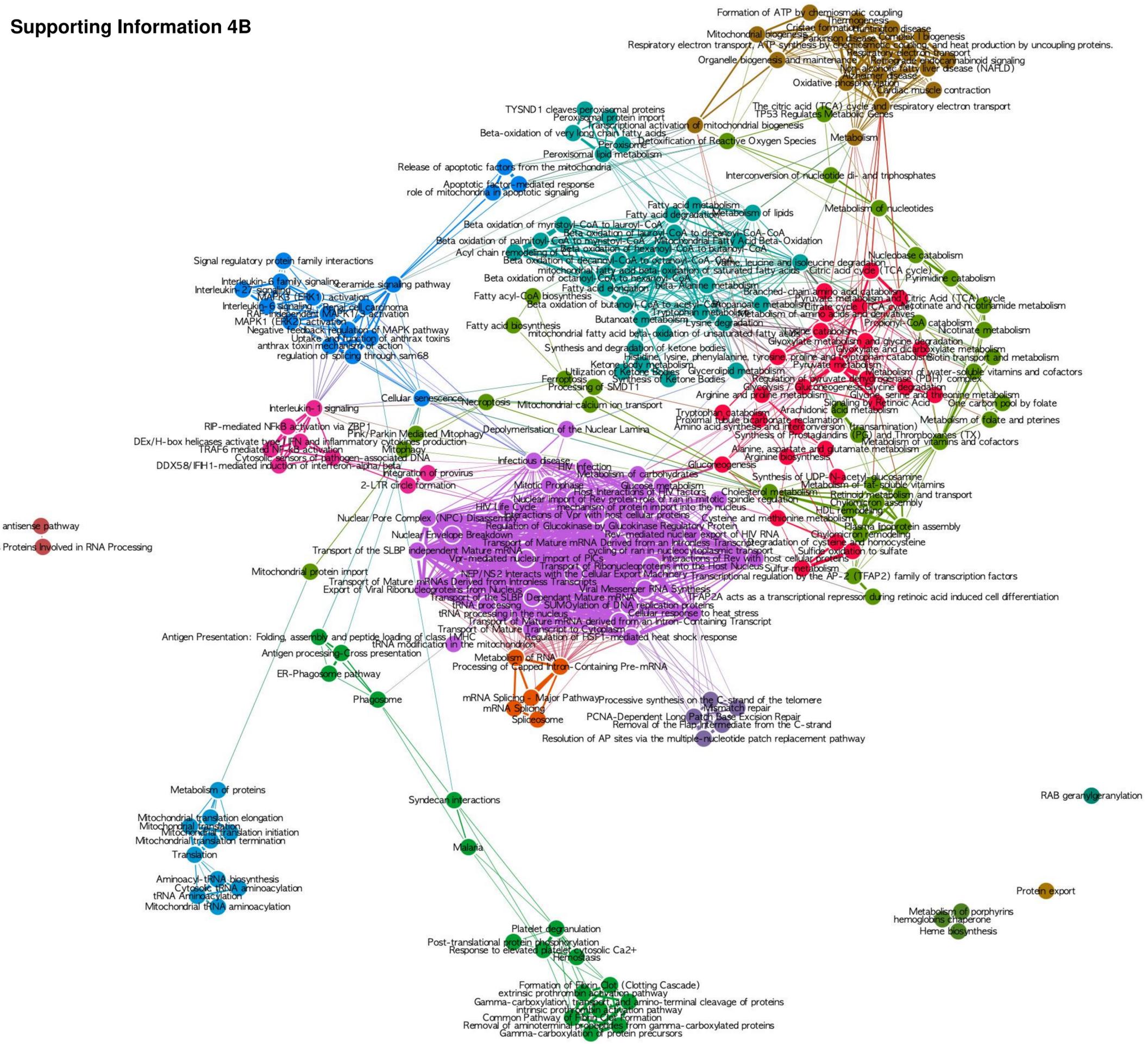


antisense pathway
PTK6 Regulates Proteins Involved in RNA

IRE1alpha activates chaperones
XBP1(S) activates chaperone genes

Translocation of GLUT4 to the plasma membrane

Supporting Information 4B



antisense pathway
Proteins Involved in RNA Processing

Metabolism of proteins
Mitochondrial translation elongation
Mitochondrial translation initiation
Mitochondrial translation termination
Translation
Aminoacyl-tRNA biosynthesis
Cytosolic tRNA aminoacylation
tRNA Aminoacylation
Mitochondrial tRNA aminoacylation

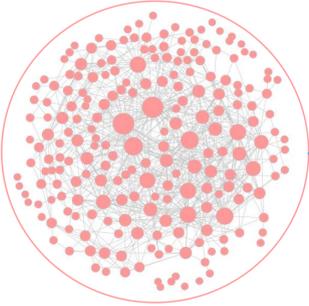
Syndecan interactions
Malana

Platelet degranulation
Post-translational protein phosphorylation
Response to elevated platelet cytosolic Ca²⁺
Hemostasis
Formation of Fibrin Clot (Clotting Cascade)
extrinsic prothrombin activation pathway
Gamma-carboxylation, transport and amino-terminal cleavage of proteins
intrinsic prothrombin activation pathway
Common Pathway of Fibrin Clot formation
Removal of aminoterminal propeptides from gamma-carboxylated proteins
Gamma-carboxylation of protein precursors

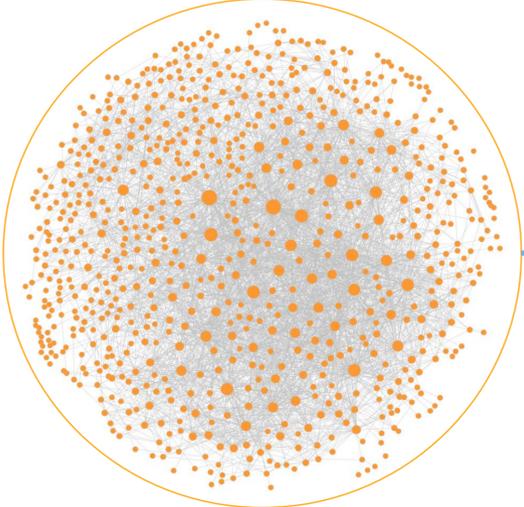
RAB geranylgeranylation
Protein export
Metabolism of porphyrins
hemoglobins chaperone
Heme biosynthesis

CAVD pathways

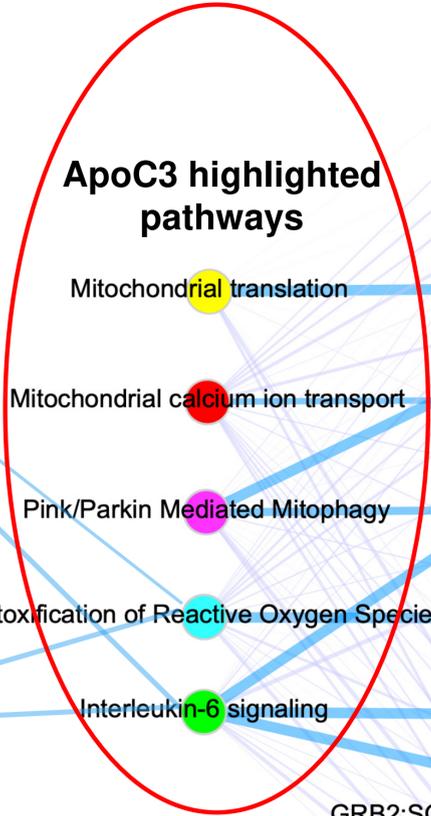
Calcificasome



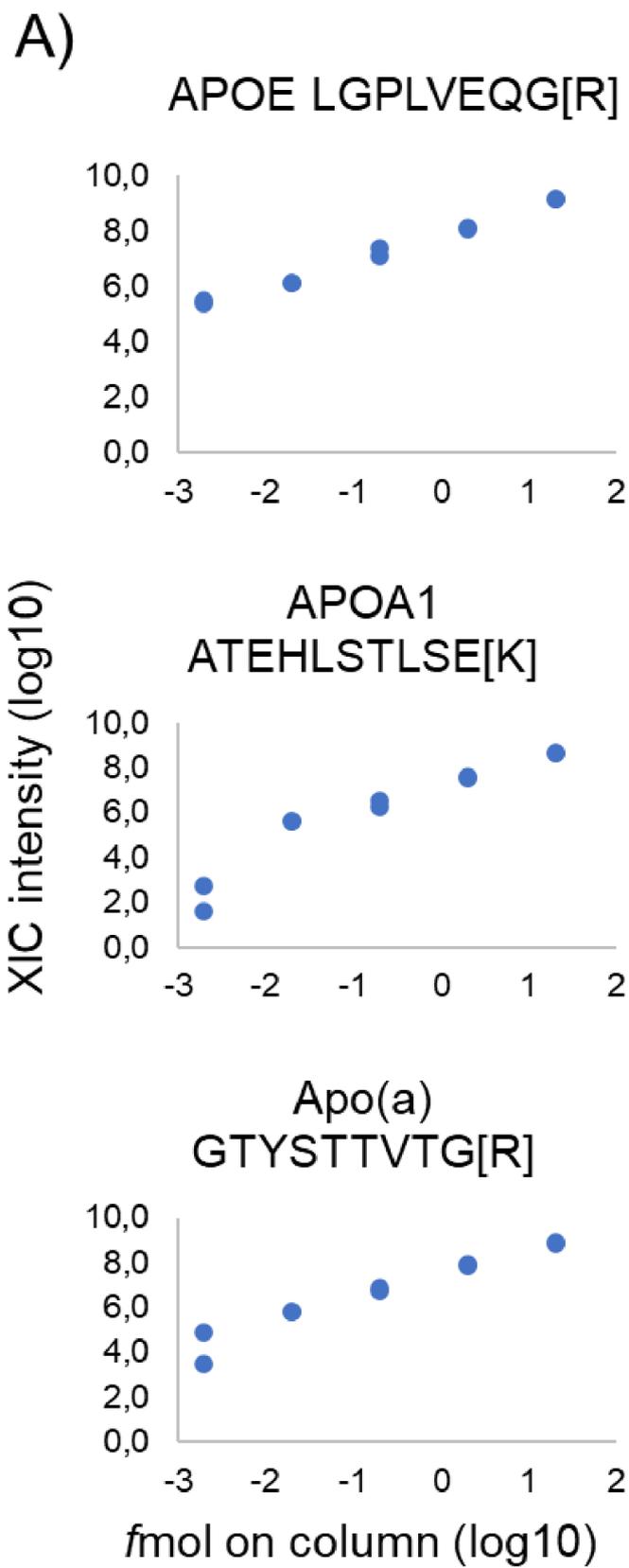
Inflammasome



ApoC3 highlighted pathways



- Retinoid metabolism and transport
- Integrin cell surface interactions
- MHC class II antigen presentation
- MAP2K and MAPK activation
- Proteasome - Homo sapiens (human)
- Toll-Like Receptors Cascades
- PD-1 signaling
- Vesicle-mediated transport
- Phagosome - Homo sapiens (human)
- IGF1R signaling cascade
- GRB2:SOS provides linkage to MAPK signaling for Integrins
- ECM proteoglycans
- Activation of Matrix Metalloproteinases
- Renin-angiotensin system - Homo sapiens (human)
- TCR signaling
- PPAR signaling pathway



B)

	Mass [m/z]	CS [z]	Start [min]	End [min]	SEQUENCE_APOLIPOPROTEIN	Observed
PRM set 1	384,212	3	6	9	KATVVYQGER_APOH	yes
	405,8787	3	8	11	ATEHLSTLSEK_APOA1	yes
	408,55	3	8	11	ATEHLSTLSEK[label]_APOA1	no
	644,3362	2	8	12	TQVNTQAEQLR_APOA4	yes
	438,5772	2	9	12	AKLEEQAQQIR_APOE	yes
	511,7669	2	9	12	ATVVYQGER_APOH	yes
	484,7798	2	12	15	LGPLVEQGR_APOE	yes
	489,78	2	12	15	LGPLVEQGR[label]_APOE	yes
	644,8226	2	15	19	ELDESLQVAER_APOJ	yes
	858,9292	2	16	19	DALSSVQESQVAQQAR_APOC3	yes
	413,2345	2	17	20	FLLYNR_APOM	yes
801,4303	2	20	23	VSALLTPAEQTGTWK_APOB	yes	
PRM set 2	496,7213	2	6	12	TDASDVKP[C]_APOH	yes
	405,8787	3	8	11	ATEHLSTLSEK_APOA1	yes
	408,55	3	8	11	ATEHLSTLSEK[label]_APOA1	no
	521,7618	2	9	12	GTYSTTVTGR_apo(a)	yes
	526,77	2	9	12	GTYSTTVTGR[label]_apo(a)	yes
	436,2534	3	9,5	12,5	VLNQELR_APOD	yes
	477,7337	2	10	15	FMETVAEK_CLUS(APOJ)	yes
	815,8996	2	12	15	VTEPISAESGEQVER_APOL1	yes
	471,2869	2	15	18	EQLTPLIK_APOA2	yes
	529,2981	3	15	18	LKEEIGKELEELR_APOA4	yes
	509,2489	3	19	23	AEPLAFTFSHDYK_APOB	yes
981,4479	2	25	35	TPEYYPNAGLIMNY[C]R_LPA	no	
PRM set 3	473,248	2	7	10	VAQELEEK_APOL1	yes
	486,7535	2	8	11	SPELQAEAK_APOA2	yes
	405,8787	3	8	11	ATEHLSTLSEK_APOA1	yes
	408,55	3	8	11	ATEHLSTLSEK[label]_APOA1	no
	519,2667	2	9	12	TAAQNLYEK_APOC2	yes
	386,2361	3	11	14	SKEQLTPLIK_APOA2	yes
	810,9025	2	11	14	VQAAVGTSAAPVPSDNH_APOE	yes
	526,7484	2	12	15	EFGNTLEDK_APOC1	yes
	415,9065	3	14	19	TLLSNLEEAKK_CLUS(APOJ)	no
	553,3037	2	15	18	LNILNNYK_APOL1	yes
	615,8381	2	16	19	NILTSNNIDVK_APOD	yes
	449,7189	2	16	19	DYWSTVK_APOC3	yes
	496,9029	3	18	23	MREWFSETFQK_APOC1	yes
	643,7986	2	20	24	ESLSSYWESAK_APOC2	yes
	806,8963	2	30	35	LLDNWDSVTSTFSK_APOA1	yes

Supporting Information Legend

Supporting Information 1: Targeted PRM mass spectrometry for the selected apolipoprotein peptides across four high-density lipoprotein (HDL) size fractions from large to small (alpha0, alpha1, alpha2, alpha3).

Supporting Information 2: Fluorescence-activated cell sorting of freshly isolated primary human valve cells: top panel: gating strategy; middle panel: CD68 and apoD expression; lower panel: alpha-smooth muscle actin (α SMA) and apoJ expression

Supporting Information 3:

A) Proteins with $q \leq 0.1$ between NM, PM and PM + apoC-III increased in PM + apoC-III vs. NM (n=4 donors, day7 and day14 data).

B) Proteins with $q \leq 0.1$ between NM, PM and PM + apoC-III increased in PM + apoC-III vs. PM (n=4 donors, day7 and day14 data).

Supporting Information 4: ApoC-III Functional Analyses

A) The network of significantly enriched pathways (two-sided hypergeometric test p-value < 0.05) in the differential proteome of apoC-III vs. NM based on unbiased community detection.

B) The network of significantly enriched pathways (two-sided hypergeometric test p-value < 0.05) in the differential proteome of apoC-III vs. PM based on unbiased community detection.

Supporting Information 5: Interactome-based association analysis

Network association of select apoC-III-associated pathways (middle column, derived from the data in Fig. 6A/B) with previously published inflammation and calcification modules (left) and calcific aortic valve disease (CAVD)-related pathways (right column; Experimental procedures for references). Edge thickness represents degree of association.

Supporting Information 6:

A) Standard curve with three stable isotope labeled peptides fully labeled (^{13}C , ^{15}N) at the Arg or Lys residues. Valve peptide samples were mixed with standards to a final concentration of 4 fmol apoA-I, 4 fmol apoE, and 0.4 fmol apo(a) on column. B) PRM inclusion list (peptides in bold represent the stable isotope labeled peptides). Start and end time represent the start and end time of the retention time window.

Supporting Table 1: CAVD tissue proteome (all data)

Supporting Table 2: Proteome data of apolipoprotein treated human VICs in cell culture

Table S3:

Protein	Supplier	Host species	Dilution
Lipoprotein (a)	abcam (MA, USA)	Goat polyclonal	1:50
Apolipoprotein A-I	abcam (MA, USA)	Rabbit monoclonal [EP1368Y]	1:100
Apolipoprotein A-II	abcam (MA, USA)	Mouse monoclonal	1:100
Apolipoprotein A-IV	abcam (MA, USA)	Mouse monoclonal [CL0468]	1:100
Apolipoprotein B	abcam (MA, USA)	Goat polyclonal	1:100
Apolipoprotein C-III	abcam (MA, USA)	Rabbit monoclonal [EP1372Y]	1:100
Apolipoprotein D	abcam (MA, USA)	Rabbit monoclonal [EPR2916]	1:100
Apolipoprotein E	abcam (MA, USA)	Mouse monoclonal [E6D7]	1:100
Apolipoprotein J	abcam (MA, USA)	Rabbit monoclonal [EPR2911]	1:400
Apolipoprotein M	abcam (MA, USA)	Goat polyclonal	1:100