

Epigenetics meets proteomics in an epigenome-wide association study with circulating blood plasma protein traits

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Supplementary Information

Epigenetics meets proteomics in an epigenome-wide association study with circulating blood plasma protein traits

Zaghlool et al.

Contents:

Supplementary Figure 1: Replication in the QMDiab study.

Supplementary Figure 2: Manhattan plots for step-wise pEWAS (Figures 2a-2h). **Supplementary**

Figure 3: Step-wise inflation per protein (histograms).

Supplementary Figure 4: Enrichment/depletion barplots for various CpG designations.

Supplementary Figure 5: Association between *NLR5* methylation (cg07839457) and the urinary 1,3,7,-trimethylurate and urinary neopterin (QMDiab).

Supplementary Figure 6: Correlation plot of 72 CpG sites associated with pappalysin-1 (PAPPA).

Supplementary Figure 7: Correlation plot of CpG sites and proteins (pQTM) in the *NLR5* network.

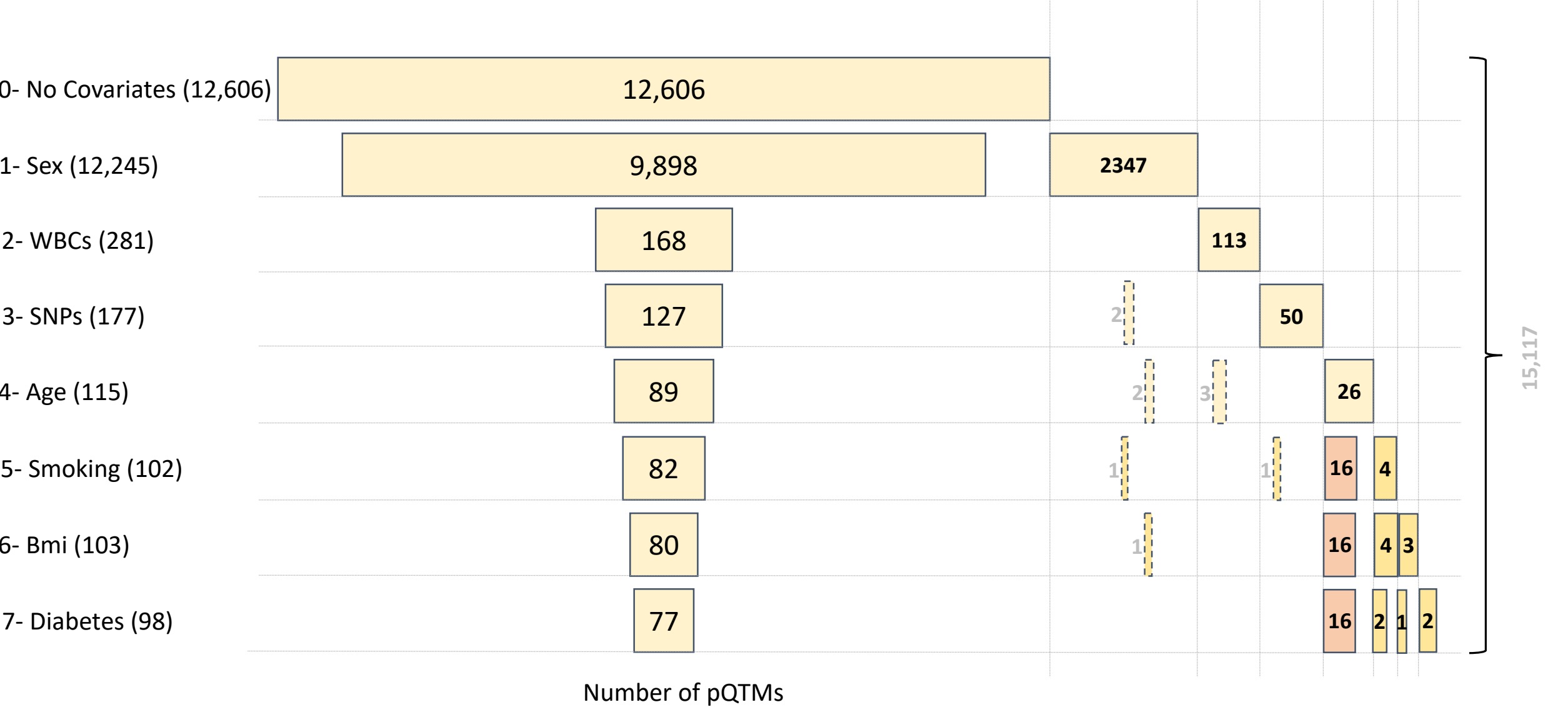
Supplementary Note 1: Replication of previous pEWAS.

Supplementary Note 2: Details of pQTM eliminated at each step of the pEWAS. **Supplementary**

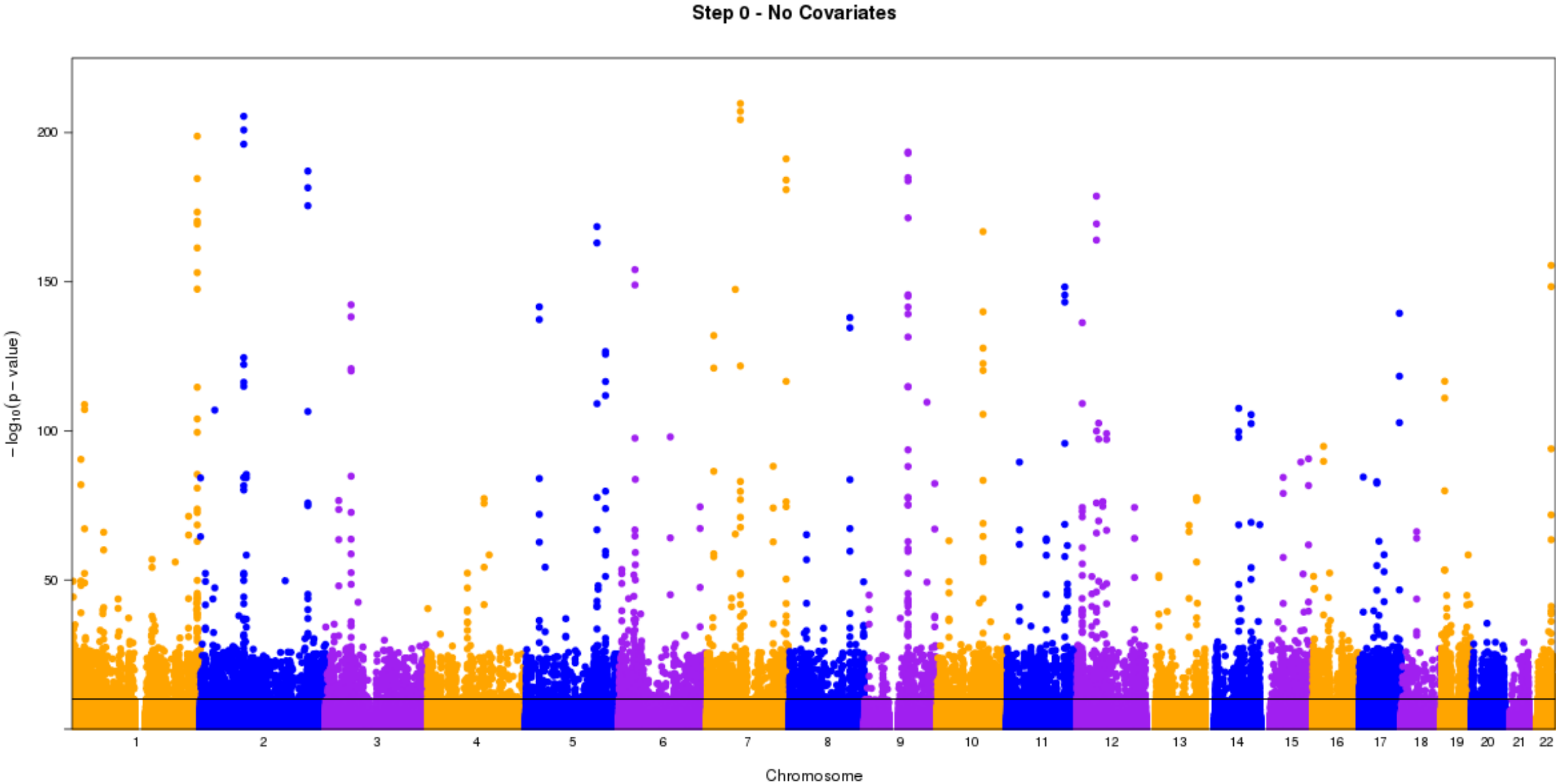
Note 3: Details for all connections in the PAPPA network.

Supplementary Note 4: Details for all connections in the *NLR5* network.

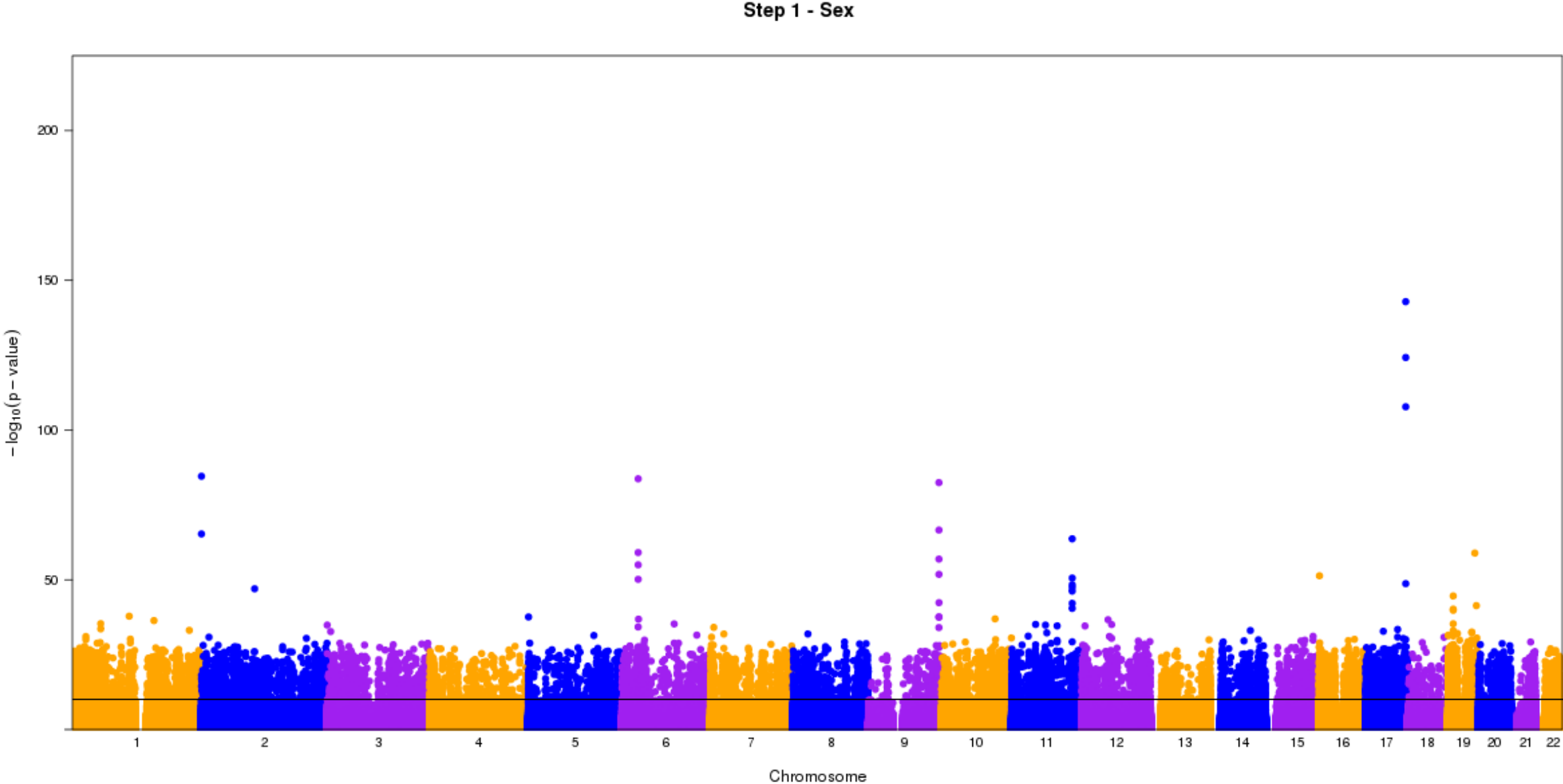
Supplementary Figure 1: Replication in the QMDiab study. We attempted replication of all pQTM from the discovery study in QMDiab. Before regressing out any covariates, 12,606 of the 38,492 initially identified pQTMs replicated at a Bonferroni significance level ($P < 0.05 / 38,492$). We show how the number of replicated pQTMs varies at each subsequent regression step. The numbers next to the covariates represents the total number of Bonferroni significant pQTMs for that step. The list of replicated pQTMs identified at the different steps of the EWAS are provided in Supplementary Data 2. Vertical alignment represents pQTMs that were already significant in a previous step.



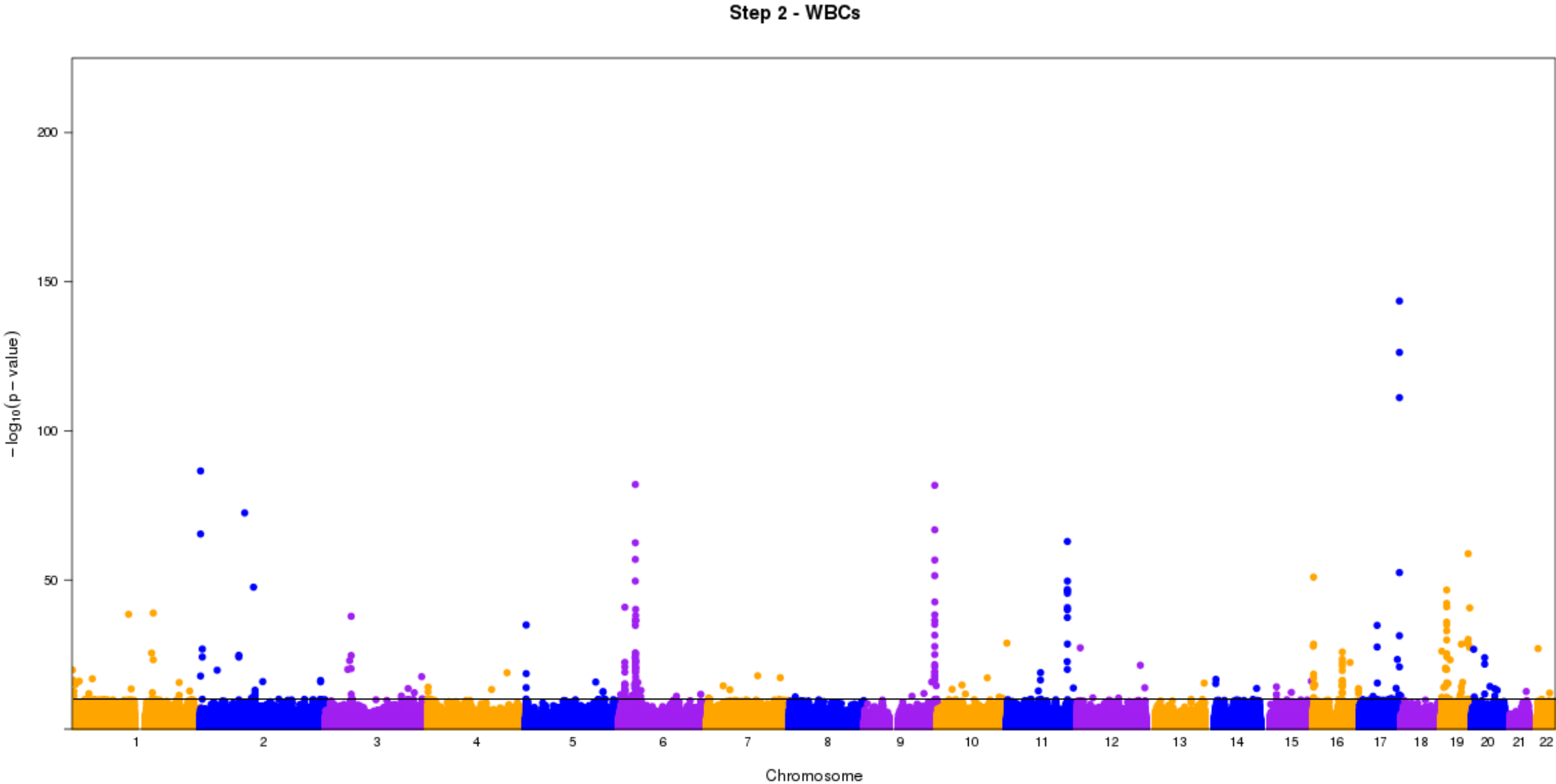
Supplementary Figure 2a: Manhattan plots for step-wise pEWAS (Step 0)



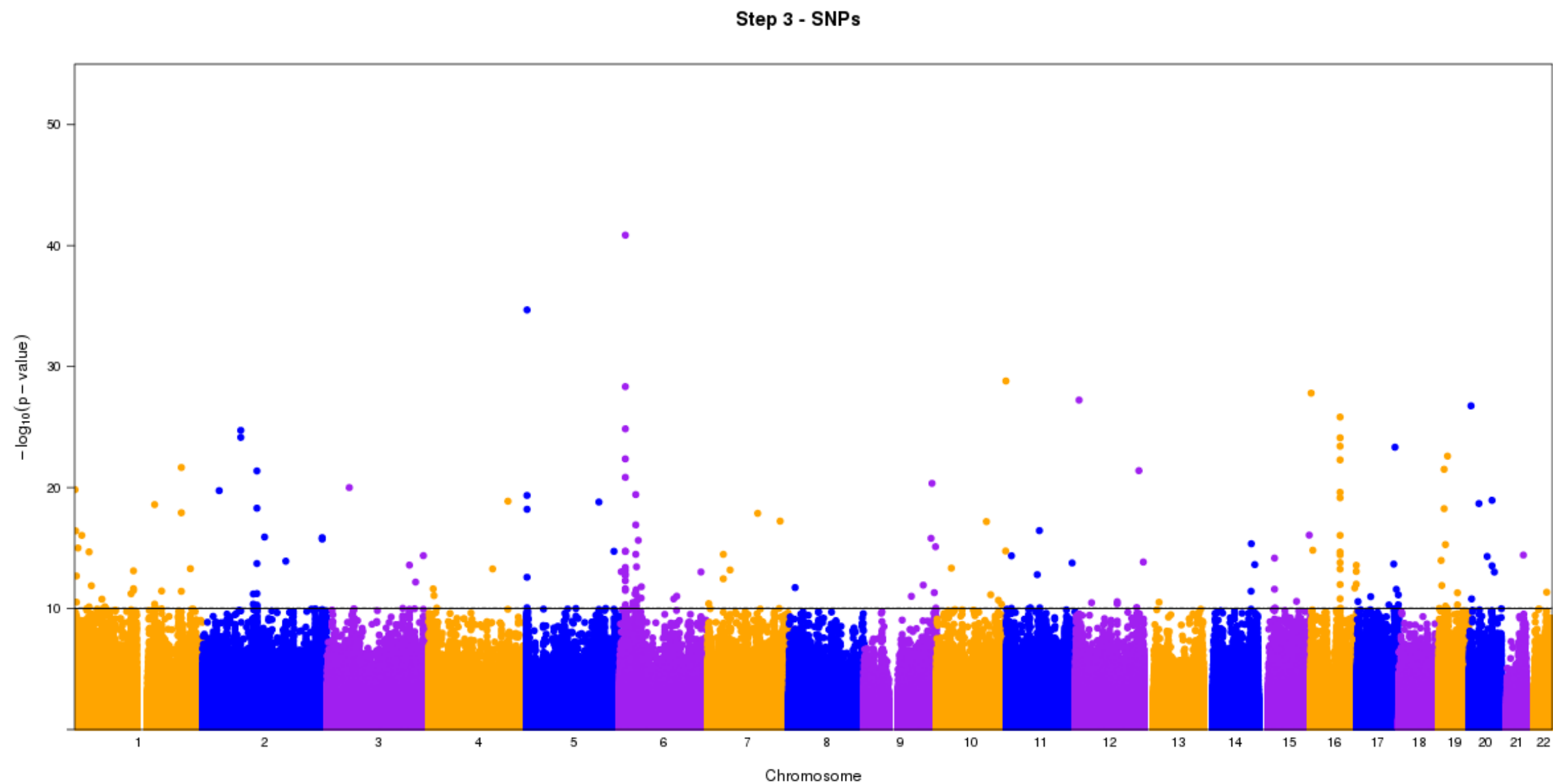
Supplementary Figure 2b: Manhattan plots for step-wise pEWAS (Step 1)



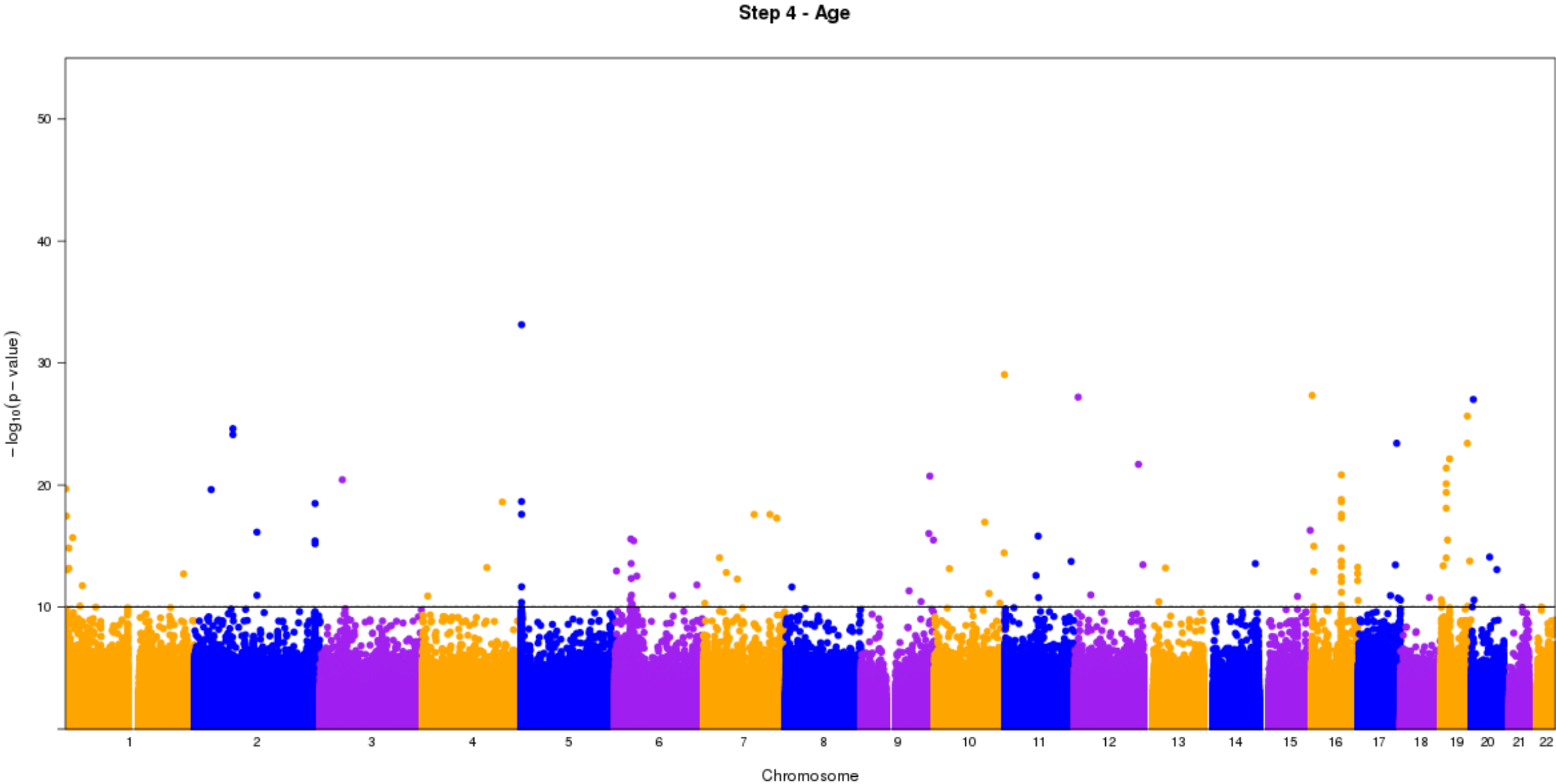
Supplementary Figure 2c: Manhattan plots for step-wise pEWAS (Step 2)



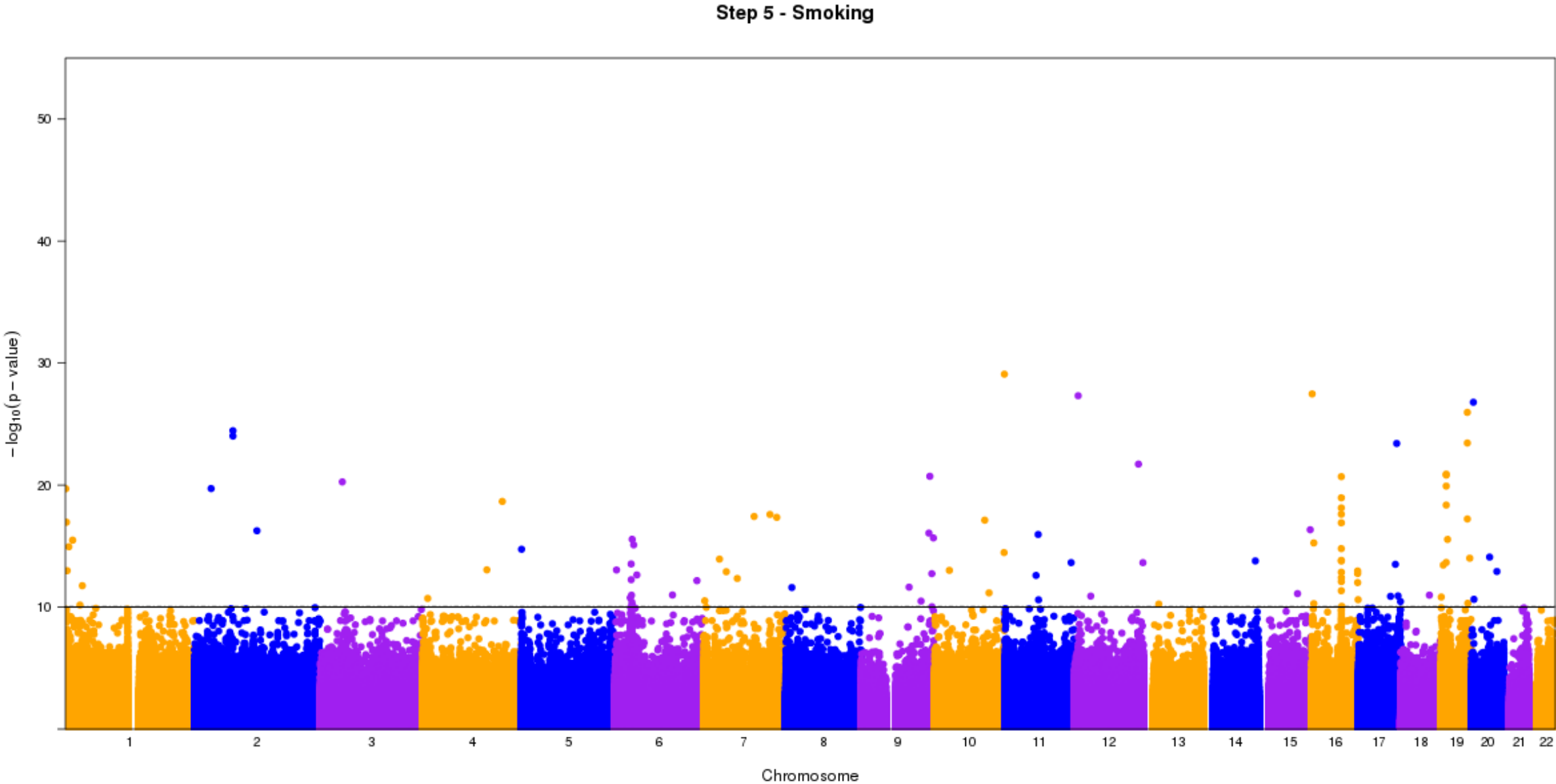
Supplementary Figure 2d: Manhattan plots for step-wise pEWAS (Step 3)

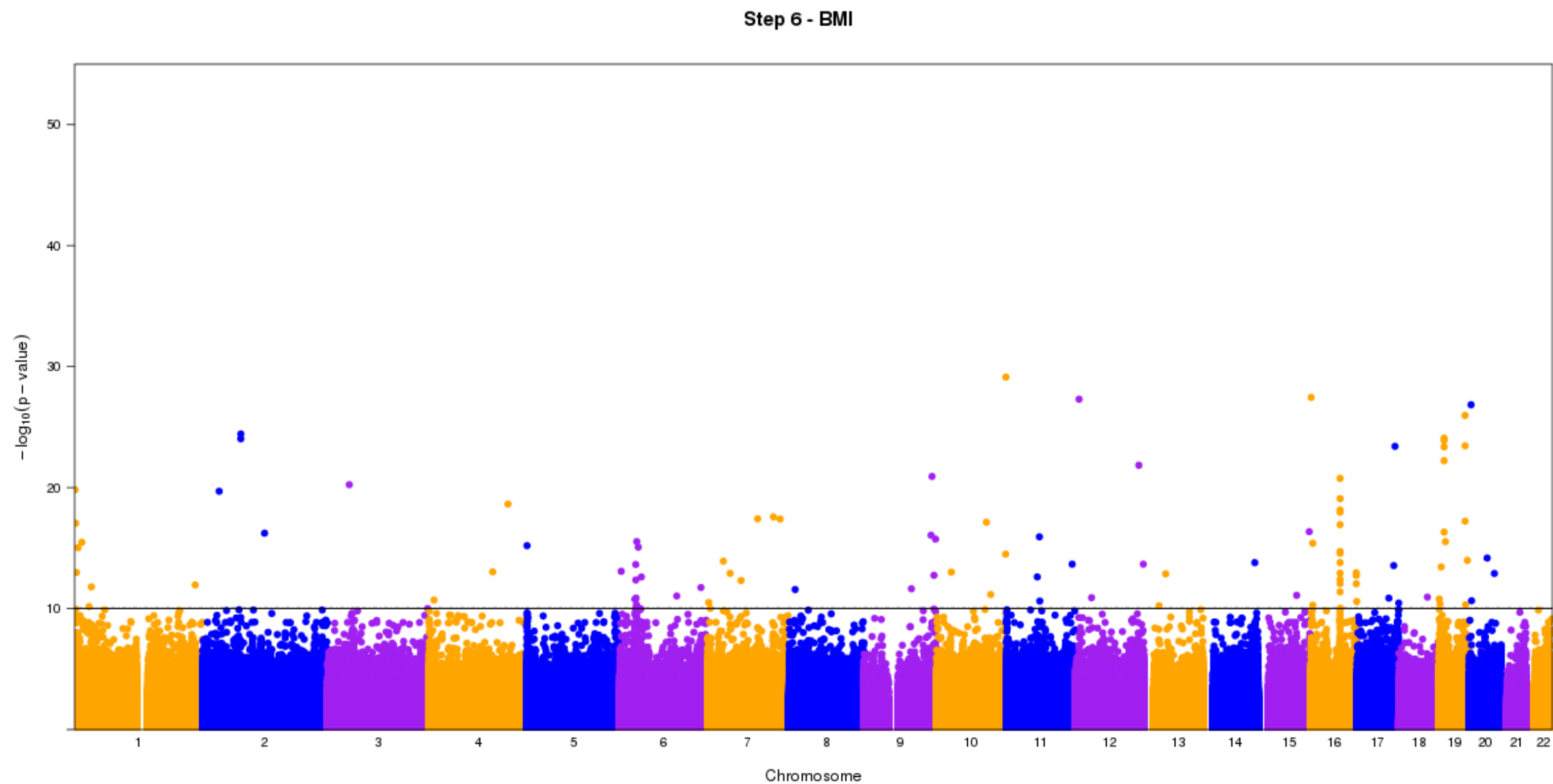


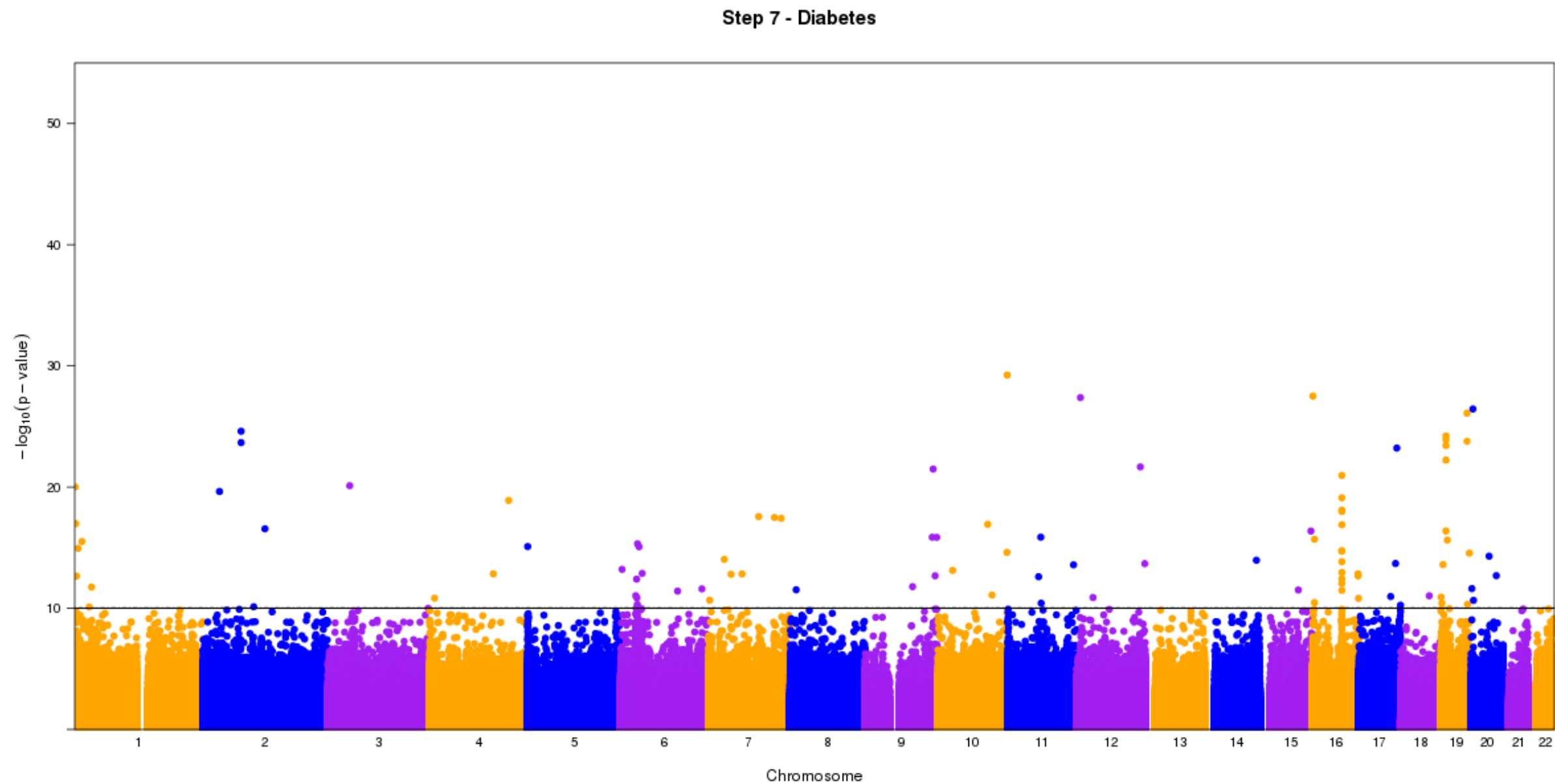
Supplementary Figure 2e: Manhattan plots for step-wise pEWAS (Step 4)



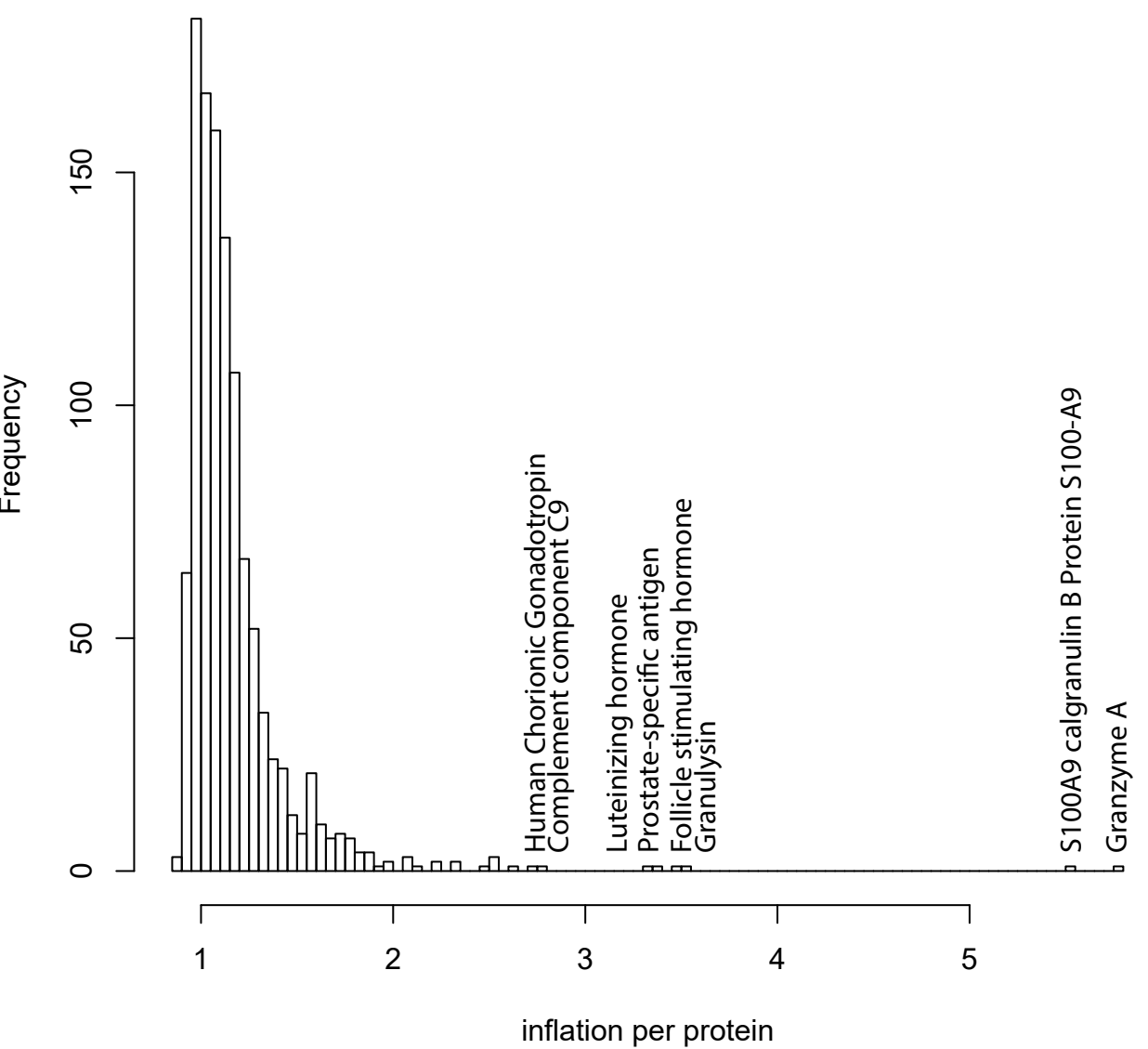
Supplementary Figure 2f: Manhattan plots for step-wise pEWAS (Step 5)



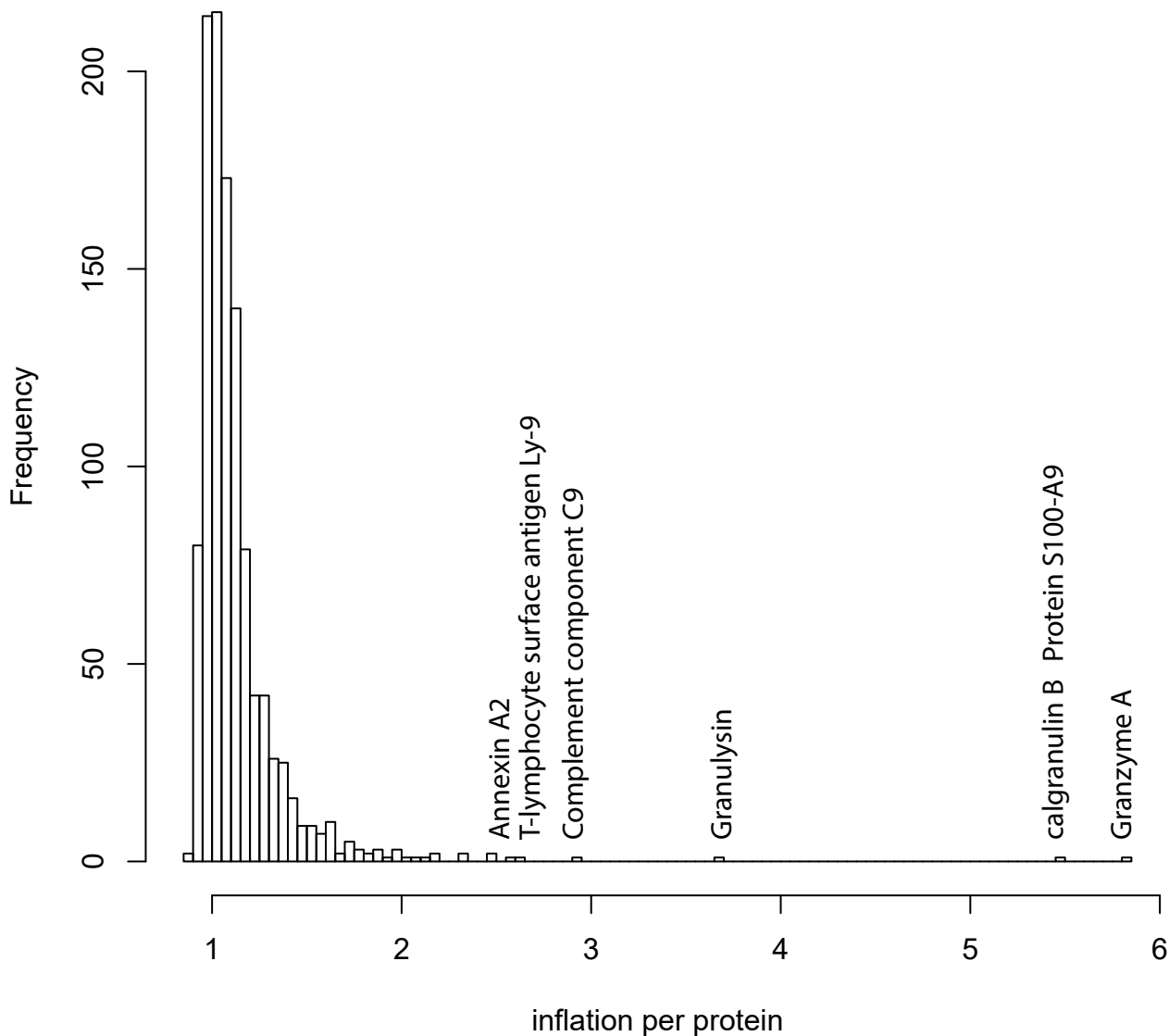




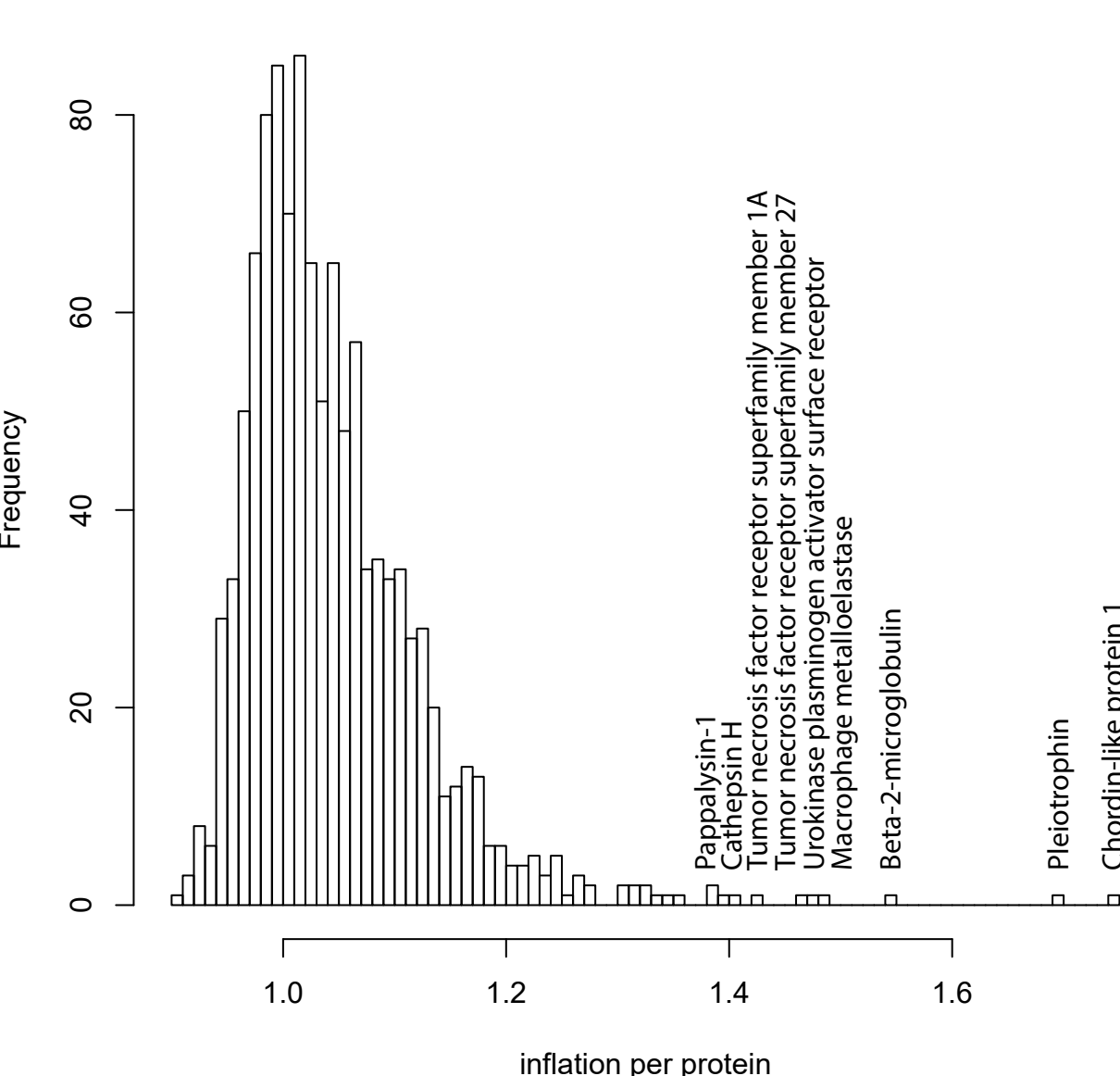
Histogram of inflation per protein (Step 0 - No Covariates)



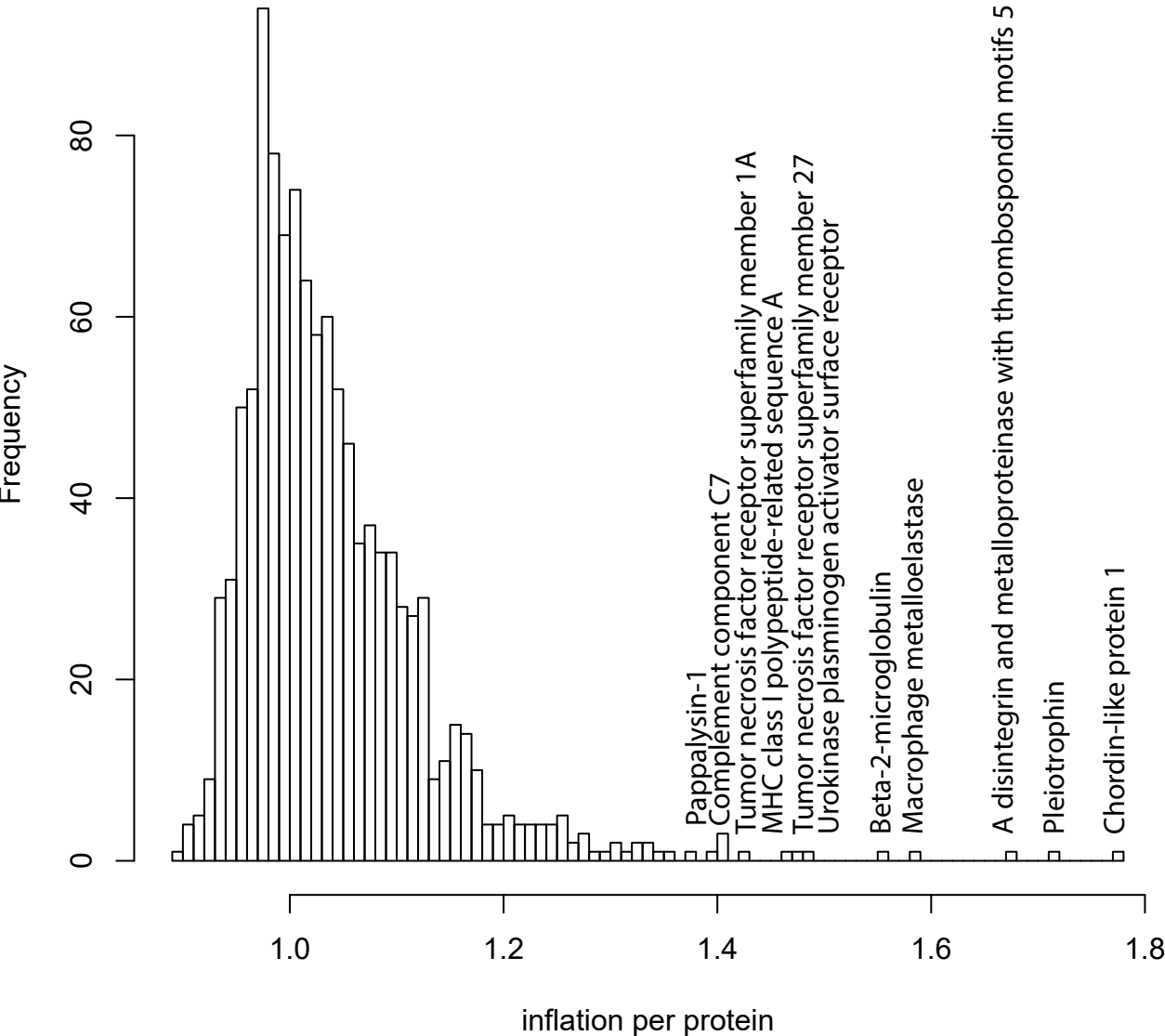
Histogram of inflation per protein (Step 1 - Sex)



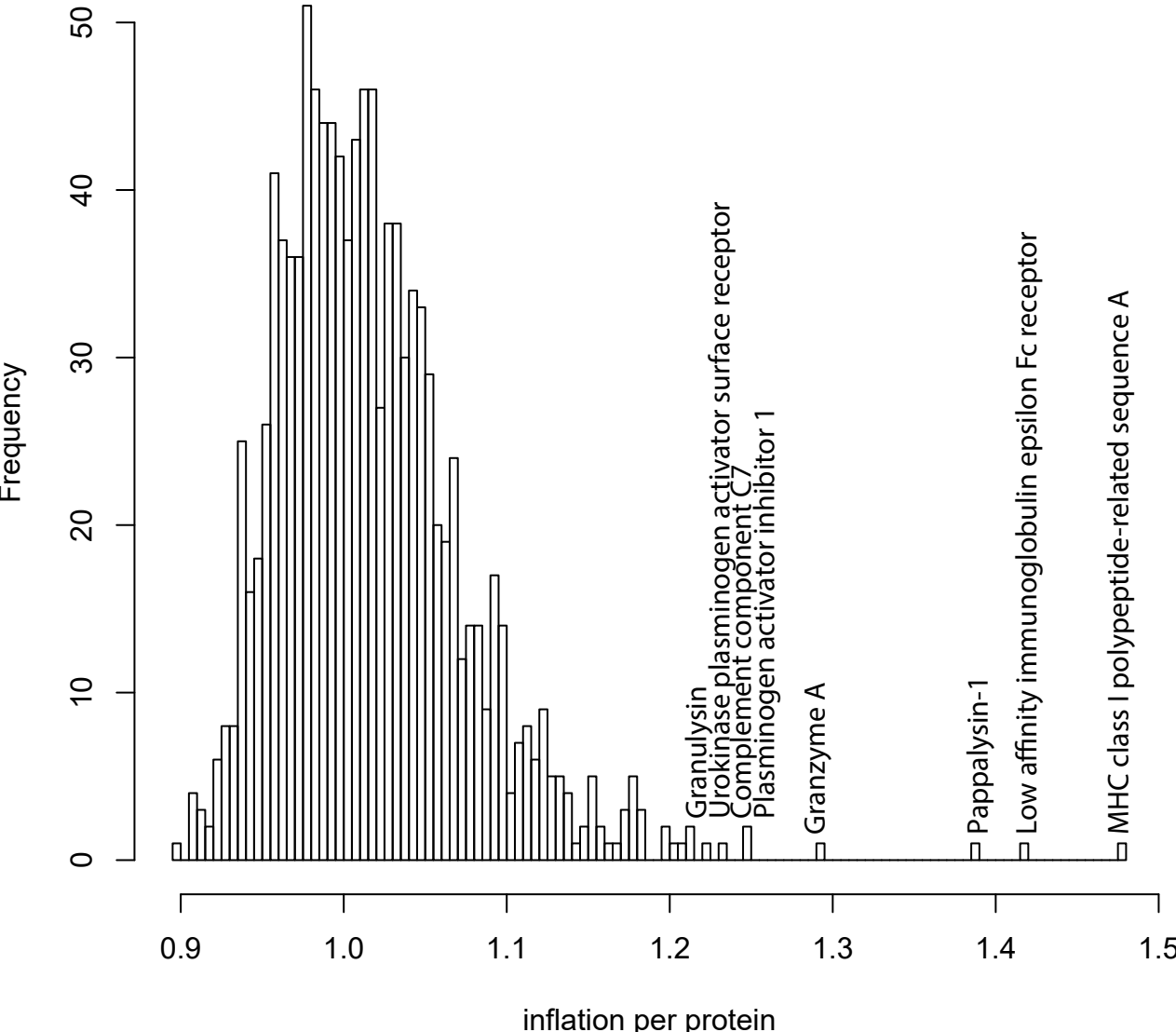
Histogram of inflation per protein (Step 2 - White Blood Counts)



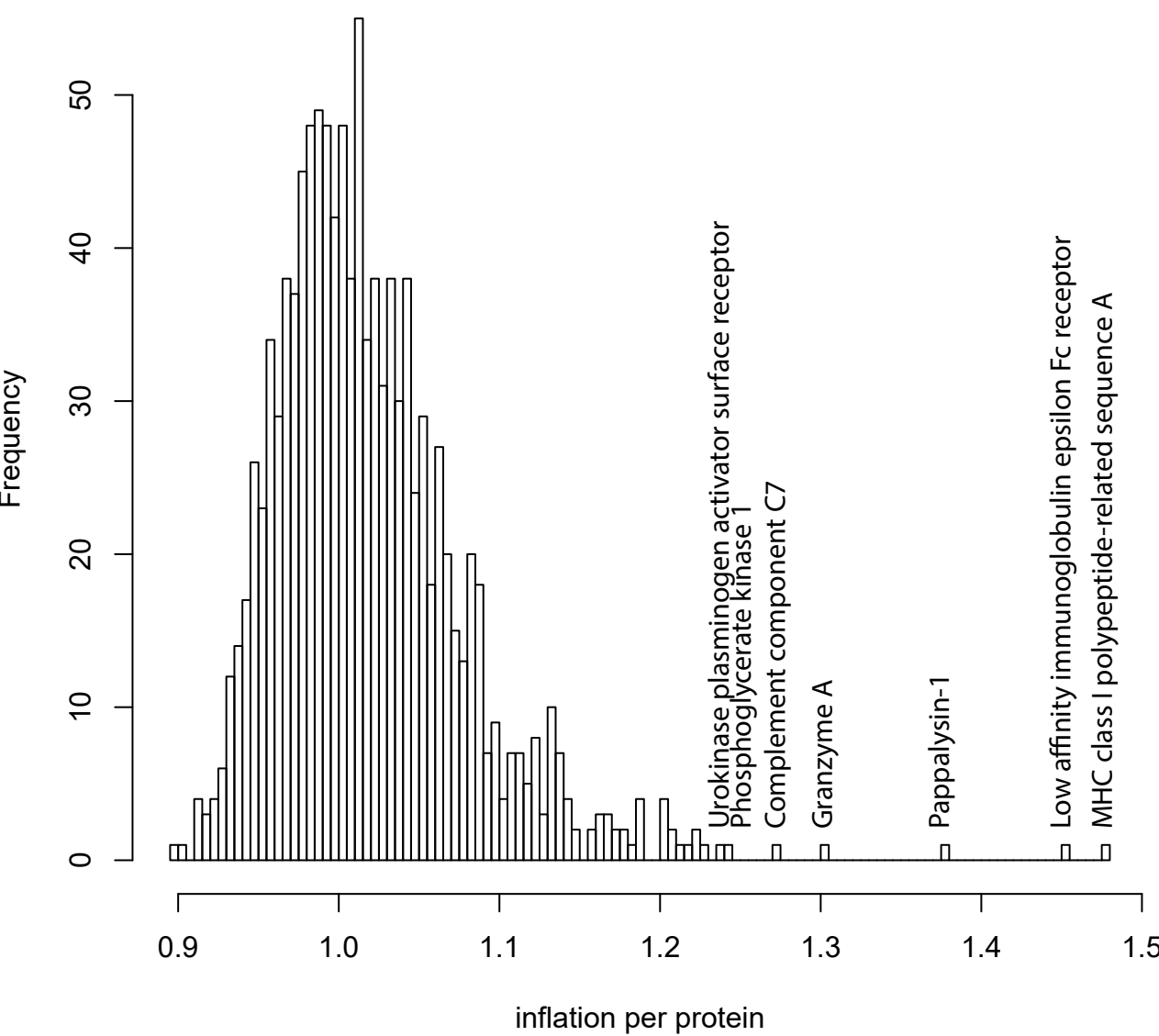
Histogram of inflation per protein (Step 3 - SNPs)



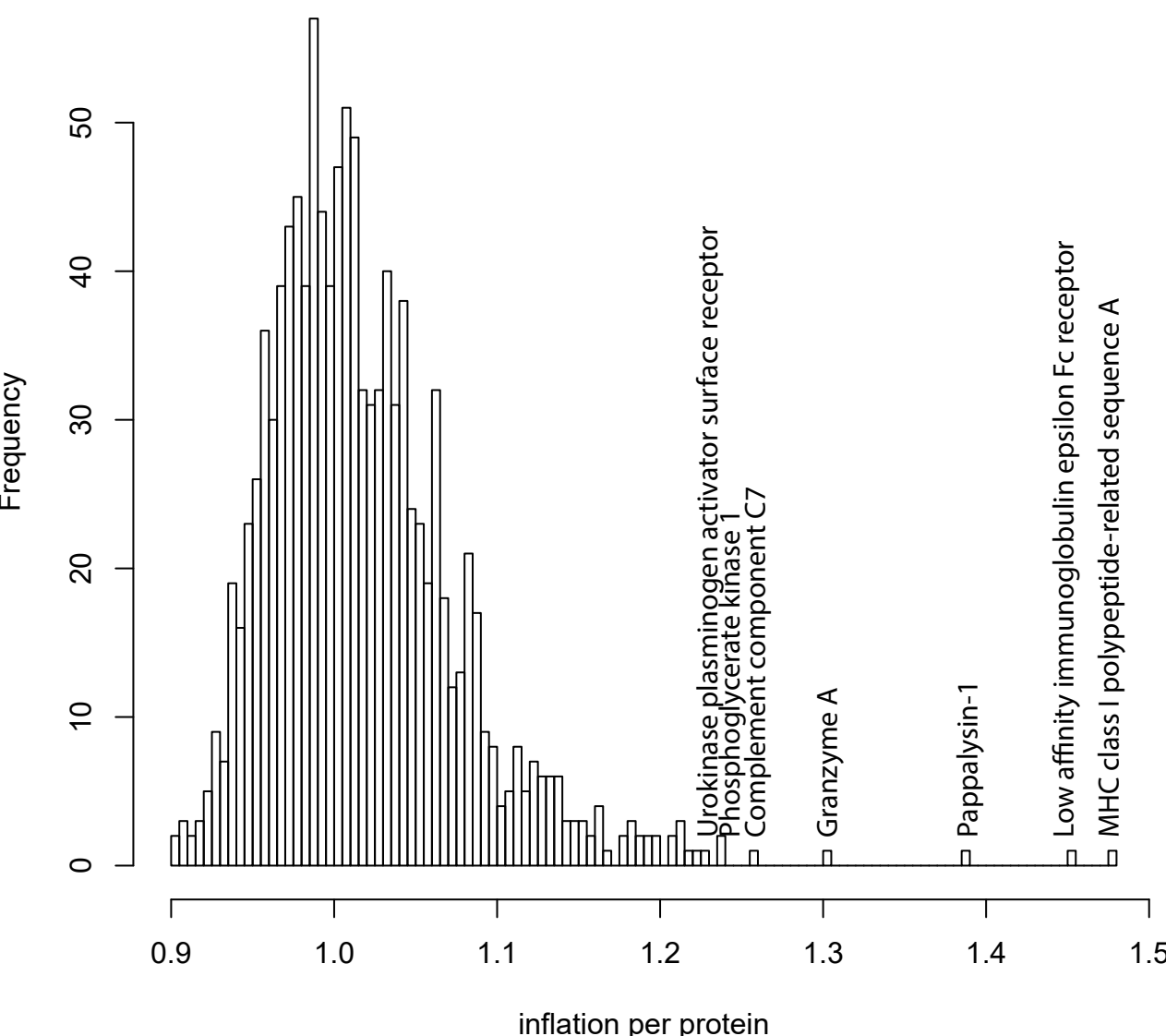
Histogram of inflation per protein (Step 4 - Age)



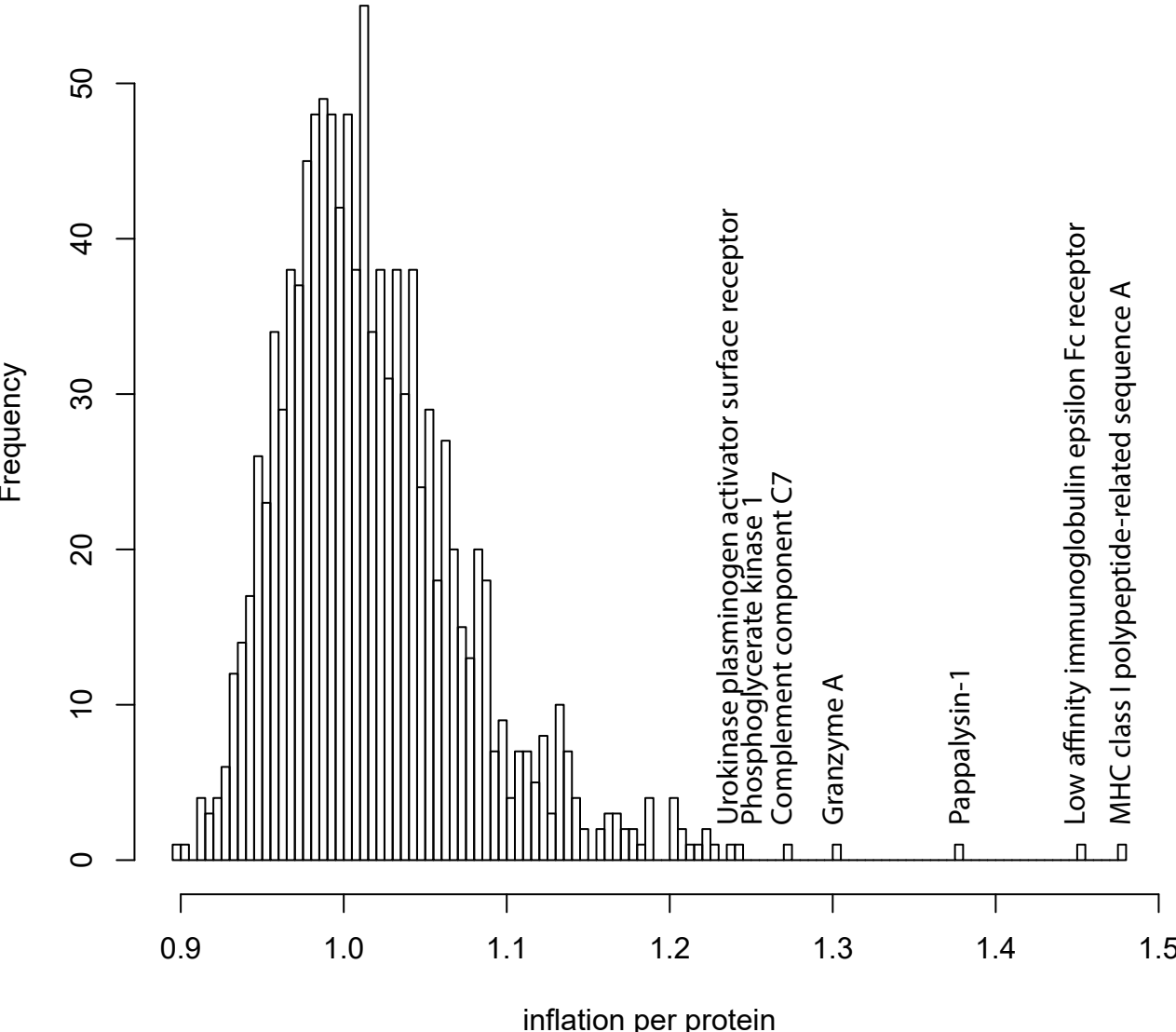
Histogram of inflation per protein (Step 5 - Smoking)



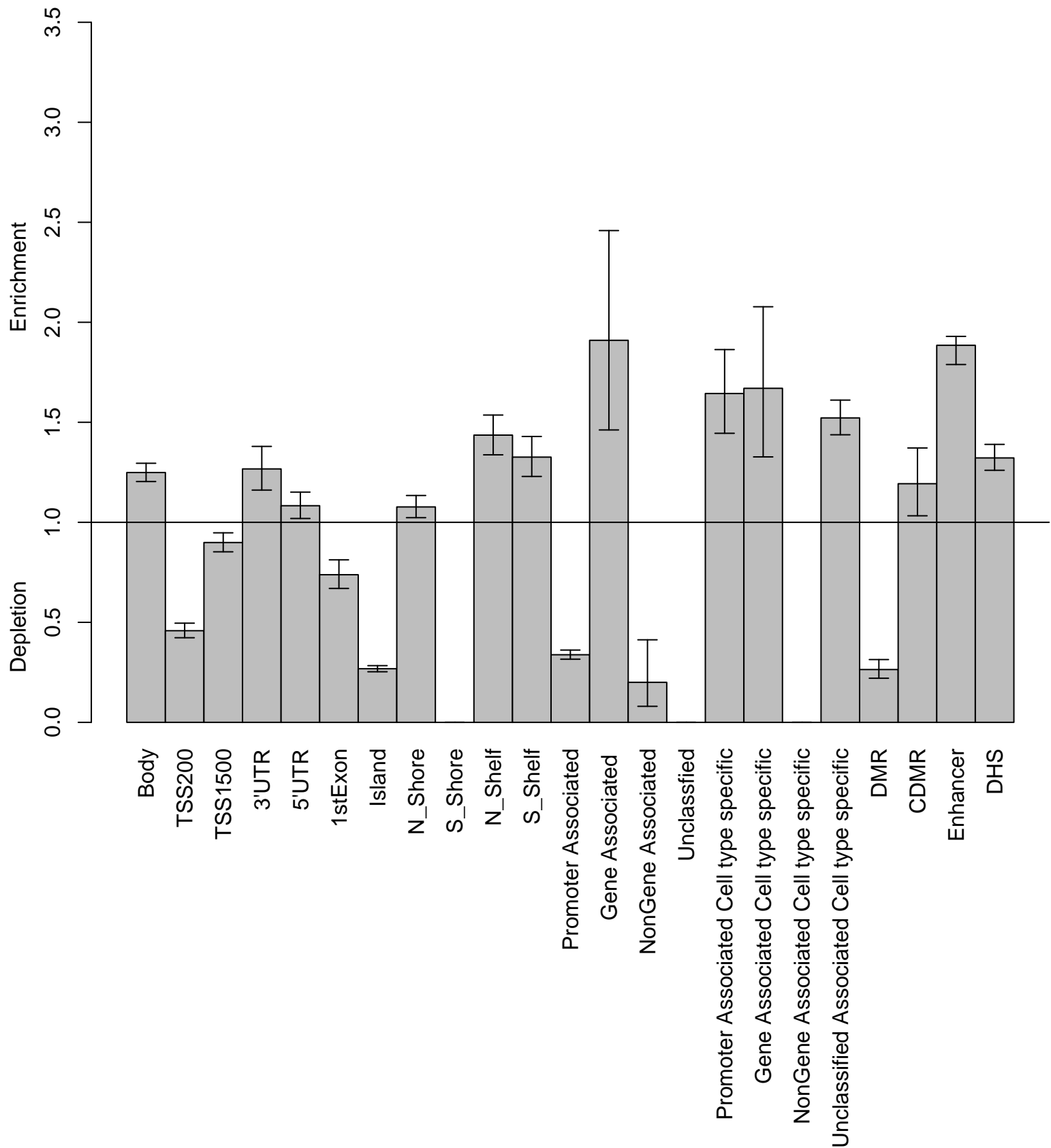
Histogram of inflation per protein (Step 6 - Body Mass Index)



Histogram of inflation per protein (Step 7 - Diabetes)

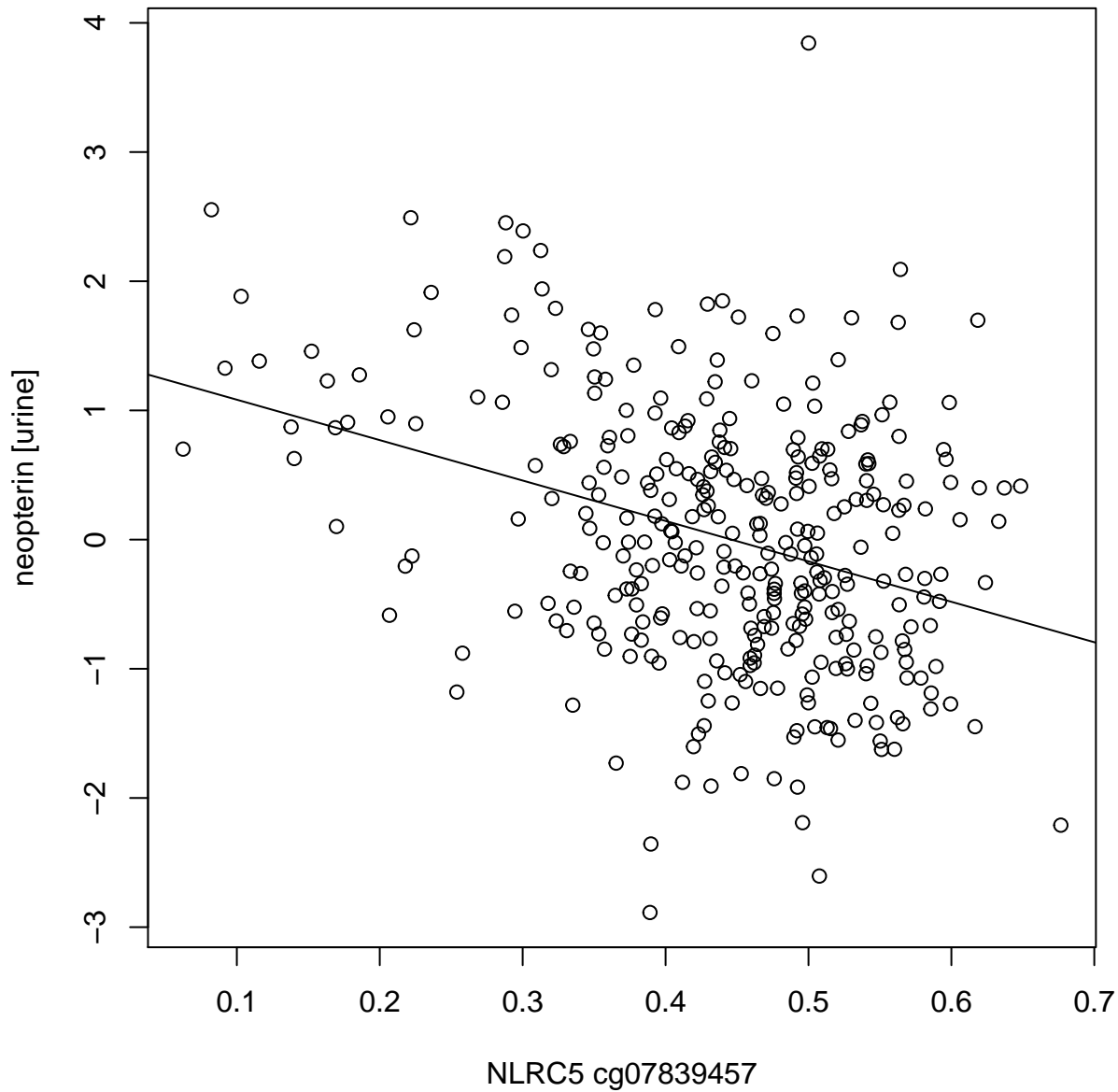
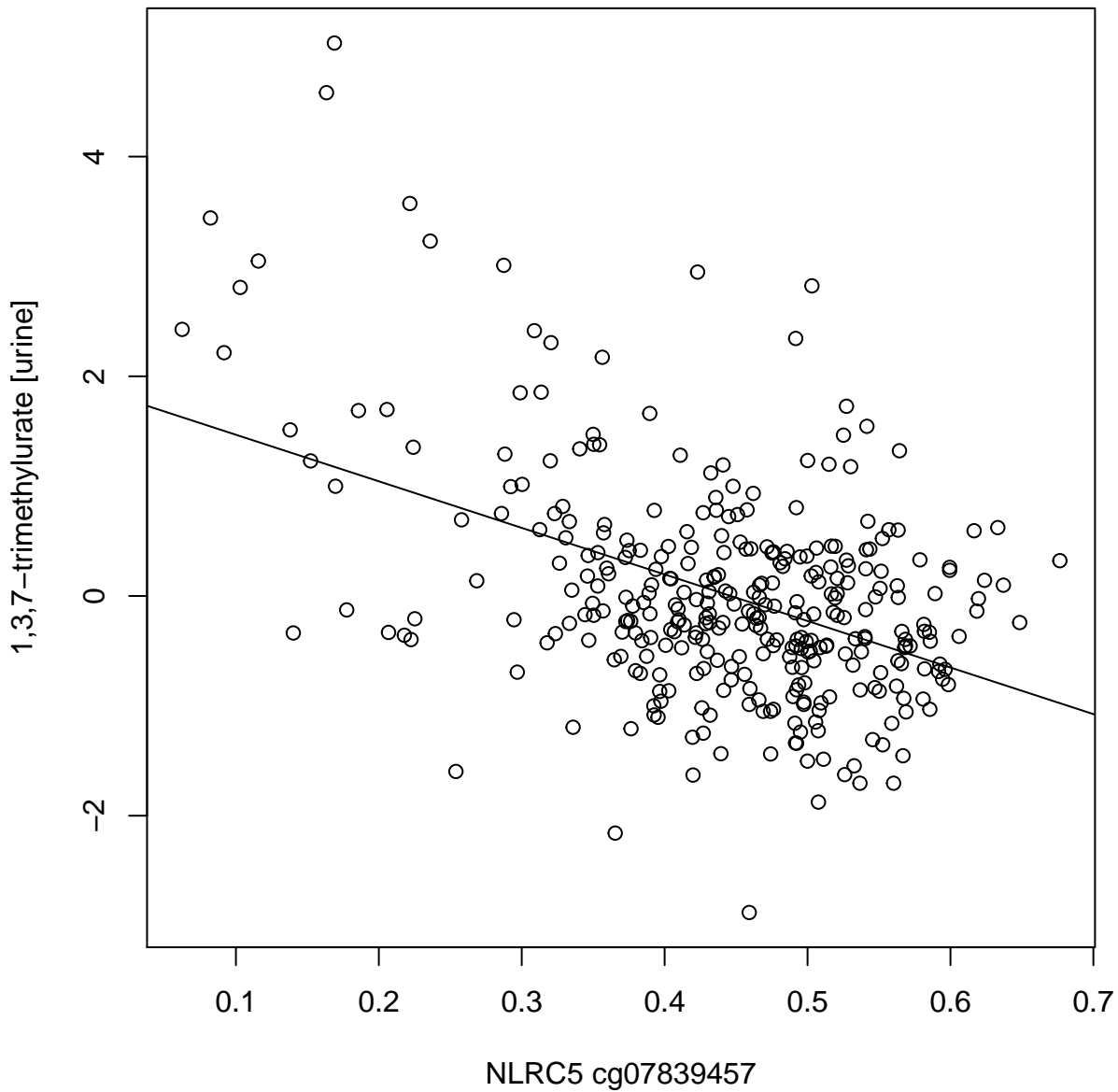


Significant CpG properties Enrichment/Depletion (Step 0)

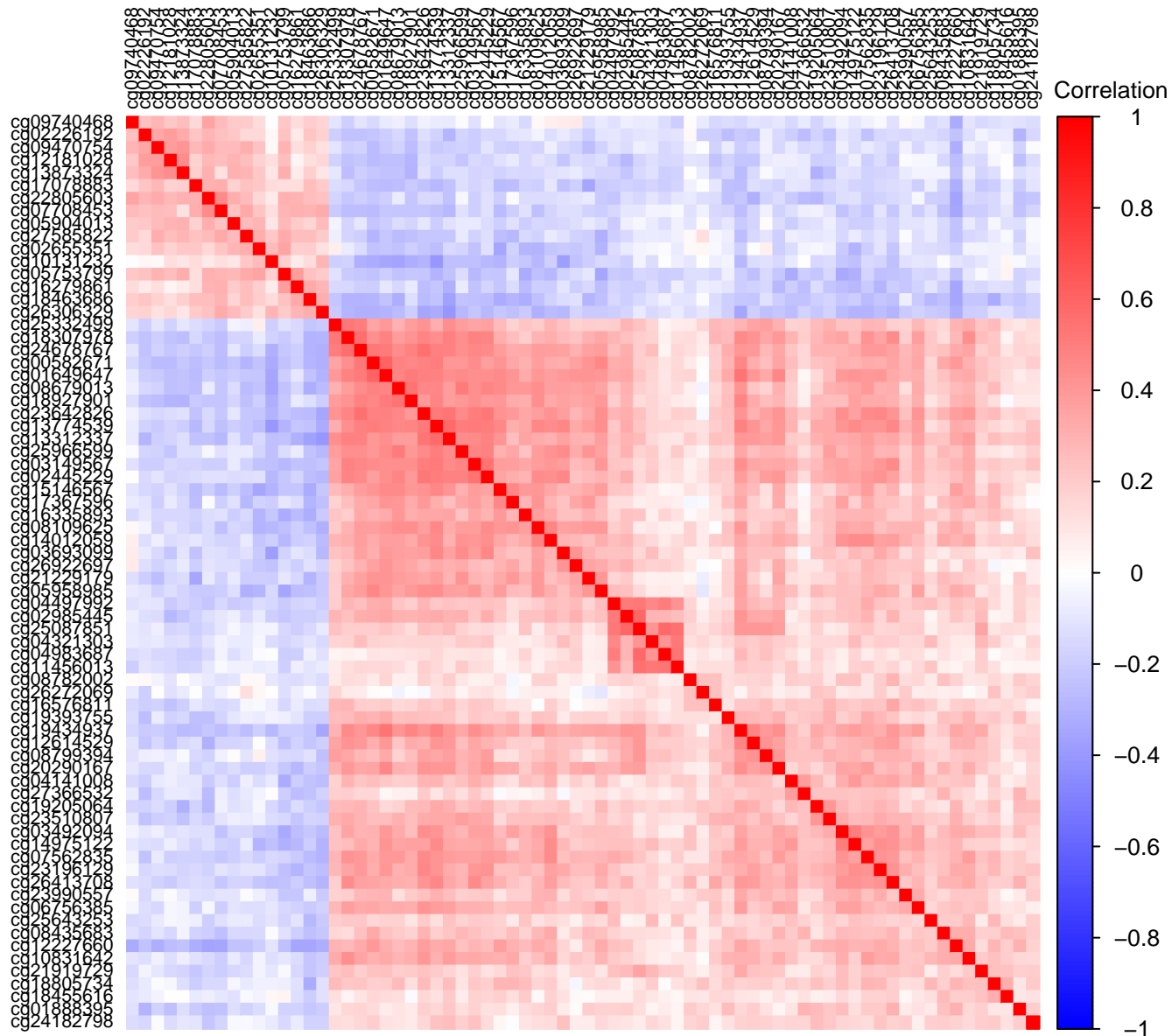


Supplementary Figure 4: Enrichment/depletion barplots for various CpG designations. Enrichment/depletion is computed using the fisher's exact test and the error bars represent the 95% confidence interval for the odds ratio.

Supplementary Figure 5: Association between NLRC5 methylation (cg07839457) and the urinary 1,3,7,-trimethylurate and urinary neopterin (QMDiab).

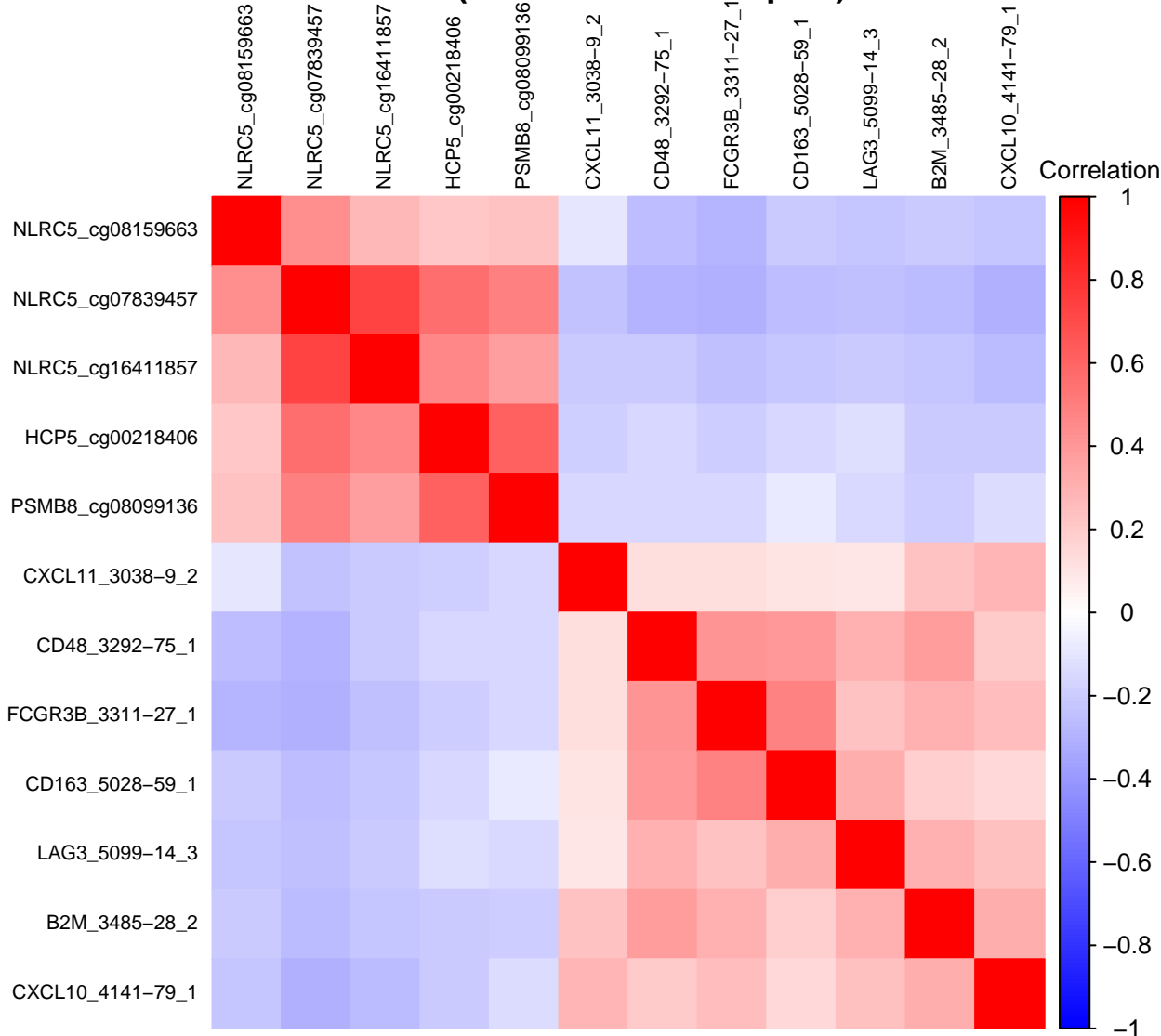


72 CpGs associated with Pappalysin-1 (based on 944 samples)



Supplementary Figure 6: Correlation plot of 72 CpG sites associated with pappalysin-1 (PAPPA).

NLRC5 Network CpGs and Proteins Correlations (based on 944 samples)



Supplementary Figure 7: Correlation plot of CpG sites and proteins (pQTM) in the NLRC5 network.

Supplementary Note 1: Replication of previous pEWAS

In a previous EWAS with three Olink panels (cardiovascular, inflammation, and oncology) [1], *NLRC5* methylation (cg07839457) was associated with *CXCL9*, *CXCL11*, *IL-12* and *IL-18*. 121 protein biomarkers were measured in that study, of which we cover 85 (70%) in our panel. Ahsan et al. reported 188 pQTM (44 proteins and 169 CpG sites) of which we shared 27 proteins (SOMAscan) and are able to attempt replication of 114 pQTMs. We attempted to replicate these 114 pQTMs and only replicated 7 of them (6%) at Bonferroni significance ($p < 0.05/114 = 4.39 \times 10^{-4}$), 18 (15.8%) at nominal significance ($p < 0.05$) (**Supplementary Data 10**). However, 64 pQTMs (56%) displayed the same trend of association between the two studies. Most importantly, we did replicate the association between C-X-C motif chemokine 11 (*CXCL11*) and 2 *NLRC5* methylation loci (cg07839457 and cg16411857). Our other main associations could not be checked as they were not included in their panel. Although Pappalysin-1 was measured in Ahsan et al., it did not pass QC and was excluded from their study.

Supplementary Note 2: Details of pQTM eliminated at each step of the pEWAS

Identification of pQTMs driven by gender

After regressing out gender, 2,708 pQTMs (71 proteins and 1,192 CpG sites) were no longer significant. However, in some cases pQTMs became significant due to the correction for a potential confounder. This was in particular the case for gender, where 2,347 new pQTMs reached Bonferroni significance and were replicated. As expected, many of the pQTMs driven by gender implicated sex-related proteins, such as follicle stimulating hormone (*CGA FSHB*; 332 eliminated pQTMs), leptin (*LEP*; 313 pQTMs), human chorionic gonadotropin (*CGA CGB*; 207 pQTMs), and prostate-specific antigen (*KLK3*; 191 pQTMs). The CpG sites of gender-driven pQTMs included previously identified autosomal gender-related sites, such as cg11955727 (chr2:84,105,546; 36 pQTMs), cg20926353 (*TLE1*; 35 pQTMs), and cg00399683 (chr7:153,109,375; 34 pQTMs) [2] [3].

Identification of pQTMs driven by white blood cell composition

After regressing out white blood composition, 12,077 pQTMs (21 proteins and 10,751 CpG sites) were no longer significant. Those pQTMs were dominated by calgranulin B (*S100A9*), a calcium binding protein that is expressed in neutrophils, with 8,495 pQTMs, granzyme A (*GZMA*), a cytotoxic serine protease present in T-lymphocytes, with 2,499 pQTMs, myeloblastin (*PRTN3*) found in polymorphonuclear leukocyte granules, with 528 pQTMs, granulysin (*GNLY*; 315 pQTMs), and matrix metalloproteinase-9 (*MMP9*; 193 pQTMs), among others.

Identification of pQTMs driven by genetic variation

After regressing out genetic variation, 154 pQTMs (62 proteins and 134 CpG sites) were no longer significant. For 90% of these 62 proteins, a genome-wide significant pQTL was already identified in our previous GWAS [4]. In addition, for 43% of these 134 CpG sites, a meQTL was already identified in BIOS [5].

Identification of pQTM driven by age

Regressing out age eliminated 88 pQTMs (61 CpG sites and 18 proteins). Of these 61 CpG sites, 39 (64%) were previously linked to age in at least one other EWAS [3, 6-9]. Proteins involved in the age-driven pQTMs included A disintegrin and metalloproteinase with thrombospondin motifs 5 (*ADAMTS5*; 37 pQTMs), pleiotrophin (*PTN*; 6 pQTMs), macrophage metalloelastase (*MMP12*; 4 pQTMs), follistatin-related protein 3 (*FSTL3*; 2 pQTMs), and chordin-like protein 1 (*CHRD1*; 1 pQTM). These proteins were previously associated with chronological age [10]. In KORA, *CHRD1* and *PTN* are also associated with 12 and 7 of the clock CpG sites identified in Horvath et al. [6], respectively. Interestingly, these two proteins were also previously identified as the strongest associations with chronological aging in the TwinsUK study using an earlier version of the SOMAscan assay [10]. *CHRD1* was also significantly associated with birthweight and the individual Framingham 10-year cardiovascular risk score. *PTN* is a secreted growth factor with many functions in multiple tissues and known to be a marker for cardiovascular risk and osteoporosis which are both age related diseases. The age-driven pQTMs also included 11 associations with dermatopontin (*DPT*) and seven with beta-2-microglobulin (*B2M*).

Identification of pQTMs driven by smoking, BMI and diabetes

Regressing out smoking identified 17 pQTMs, (17 CpG sites and 4 proteins). The leading CpG sites were close to the *AHRR* and *F2RL3* genes. Both are known smoking associated methylation sites [11, 12]. Polymeric immunoglobulin receptor (*PIGR*), which we previously identified in association with smoking and CpG methylation of *AHRR* [13], was in eight of the pQTMs driven by smoking. Furthermore, pappalysin-1 (*PAPPA*) was in seven of the pQTMs driven by smoking. Regressing out body mass index excluded two additional pQTMs, one between C-reactive protein (*CRP*) and cg18181703 at *SOC3*, and the second between platelet glycoprotein Ib alpha chain (*GP1BA*) and cg0008629 at *ROD1*. Both *CRP* and *SOC3* were previously associated body mass index [14, 15]. *GP1BA* is associated with body mass index in KORA ($p=6.66 \times 10^{-6}$) and was also previously linked to atherosclerosis and inflammation in mice [16]. Finally, seven pQTMs were eliminated after regressing out diabetes. These seven pQTMs covered three proteins:

pappalysin-1 (*PAPPA*; 5 pQTM), beta-2-microglobulin (*B2M*, 1 pQTM) and stem cell growth factor-alpha (*CLEC11A*; 1 pQTM). Two of the CpG sites in the pQTMs that were driven by T2D (cg00851028 and cg16463452) were weakly associated with T2D and HOMA-IR ($p=1.38 \times 10^{-4}$ and 0.0435, respectively) in a previous diabetes EWAS [17].

Supplementary Note 3: Details for all connections in the PAPPa network

KORA clinical phenotypes

Relationship	Details
<i>GABBR1</i> Alcohol Consumption	See Supplementary Data 8
PAPPa Alcohol Consumption	See Supplementary Data 7
PAPPa Metabolic Syndrome	See Supplementary Data 7
PAPPa Total Cholesterol	See Supplementary Data 7
PAPPa Triglycerides	See Supplementary Data 7
PAPPa LDL	See Supplementary Data 7

Disease Association (External Studies)

Relationship	Details	Reference
PAPPa Cardiovascular Disease	PAPPa is a strong predictor for adverse cardiovascular events in patients with type 2 diabetes.	[18]
PAPPa Heart Failure	High levels of PAPPa have been shown to be associated with increased risk of heart failure.	[19]
PAPPa Type 2 Diabetes	PAPPa also is a strong predictor for adverse cardiovascular events in patients with type 2 diabetes.	[18]
PAPPa Wound Healing	PAPPa also has a role in bone formation, inflammation, wound healing, and female fertility.	[20]
PAPPa Atherosclerosis	PAPPa is considered a marker of response to injury or diseases such as atherosclerosis or lesion progressio	[21]
PAPPa Cancer	PAPPa acts as an oncogene, promoting tumor cell proliferation, invasion, and metastasi	[22]

Molecular Association (External Studies)

Relationship	Details	Reference
PRG2 PAPPA	In serum from pregnant human, human pro-PRG2 protein decreases proteolysis activity of human PAPPA protein.	[23]
TNF PAPPA	In primary culture human dermal fibroblasts, actinomycin D decreases expression of human PAPPA protein that is increased (in a time-dependent and dose-dependent manner) by TNF-alpha [TNF] protein.	[24]
TNF ELAVL1	Human INTERFERON GAMMA [IFNG] protein in cell culture and human IL1B protein in cell culture and human TNF-alpha [TNF] protein in cell culture decrease expression of human HUR [ELAVL1] mRNA in Dld 1 cells.	[25]
TGFB1 PAPPA	TGF-beta1 [TGFB1] is involved in expression of PAPP-A mRNA.	[26]
PAPPA ELAVL1	Binding of human ELAVL1 protein and human PAPPA mRNA occurs.	[27]
TGFB1 GABBR1	Transgenic TGF-beta1 [TGFB1] protein is involved in expression of mouse Gabbr1 mRNA in kidney from mouse exhibiting glomerular disease.	[28]
IGF1 TGFB1	In fibroblasts from lung of fetal human, TGFB-1 protein increases expression of human IGF1 mRNA.	[29]
IGF1 TNF	Tumor necrosis factor alpha [TNF] is involved in downregulation of Insulin-like growth factor-1 [IGF1].	[30]
GABBR1 TNF	In POMC neurons from 16 week-old male mouse, homozygous mutant mouse Gabbr1 gene (knockout) increases expression of mouse Tnfa [Tnf] mRNA in hypothalamus from 16 week-old male mouse that involves high fat diet.	[31]
PRG2 TNF	MBP [PRG2] protein increases release of mouse Tnf alpha [Tnf] protein from cultured mast cells from mouse bone marrow that involves rat Scgf [Clec11a] protein.	[32]
CDC42EP4 TNF	In human keratinocytes, human TNF protein increases expression of human CDC42EP4 mRNA.	[33]
SH3PXD2A ELAVL1	Binding of human ELAVL1 protein and human SH3PXD2A mRNA occurs.	[27]
PRDM2 SOD2	Binding of human PRDM2 protein and human SOD2 protein occurs.	[34]
SOD2 TNF	TNF increases activity of SOD2.	[35]
METRNL IL10	Circulating METRNL protein increases expression of mouse IL10 mRNA in mouse subcutaneous white adipose tissue.	[36]

IL10 TNF	Uncleavable transmembrane form mutant TNF protein increases production of mouse IL10 protein in mouse tumor derived from 4T1 cells.	[37]
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Supplementary Note 4: Details for all connections in the NLRC5 network

KORA clinical phenotypes

Relationship	Details
NLRC5 LDL	See Supplementary Data 8
NLRC5 HDL	See Supplementary Data 8
NLRC5 Total Cholesterol	See Supplementary Data 8
CD48 LDL	See Supplementary Data 7
CD48 Total Cholesterol	See Supplementary Data 7
CD163 HDL	See Supplementary Data 7
CD163 Hypertension	See Supplementary Data 7
CD163 Triglycerides	See Supplementary Data 7
CD163 Metabolic Syndrome	See Supplementary Data 7
CD163 Body Mass Index	See Supplementary Data 7
CD163 Type 2 Diabetes	See Supplementary Data 7
CXCL10 HDL	See Supplementary Data 7
CXCL10 Hypertension	See Supplementary Data 7
CXCL10 Triglycerides	See Supplementary Data 7
FCGR3B LDL	See Supplementary Data 7
FCGR3B Total Cholesterol	See Supplementary Data 7
FCGR3B Hypertension	See Supplementary Data 7
B2M LDL	See Supplementary Data 7
B2M HDL	See Supplementary Data 7
B2M Total Cholesterol	See Supplementary Data 7
B2M Hypertension	See Supplementary Data 7
B2M Body Mass Index	See Supplementary Data 7

QMDiab Metabolic Associations

Relationship	Details
NLRC5 neopterin	See Supplementary Data 9
HCP neopterin	See Supplementary Data 9
PSMB8 neopterin	See Supplementary Data 9
NLRC5 1,3,7-trimethylurate	See Supplementary Data 9
HCP 1,3,7-trimethylurate	See Supplementary Data 9
PSMB8 1,3,7-trimethylurate	See Supplementary Data 9

Disease Association (External Studies)

Relationship	Details	Reference
<i>NLRC5</i> Cancer	<i>NLRC5</i> has been suggested as a promising entry in tumor immunology.	[38] [39]
<i>NLRC5</i> HIV	<i>NLRC5</i> methylation associates with HIV infection.	[40]
<i>NLRC5</i> Cardiovascular Disease	<i>NLRC5</i> methylation associates with circulating IL-18 levels which have been associated to cardiovascular disease. <i>NLRC5</i> methylation also associates with Soluble Tumor Necrosis Factor Receptor 2 (sTNFR2) a marker of cardiovascular disease risk in people with diabetes. <i>NLRC5</i> methylation is linked to gene expression and inversely associated with the risk of incident coronary heart disease.	[41] [42] [43]
<i>NLRC5</i> BMI and Obesity	<i>NLRC5</i> methylation was shown to be associated with BMI and obesity in Africans.	[44]
<i>NLRC5</i> Lupus	<i>NLRC5</i> methylation associates with lupus.	[42]
<i>NLRC5</i> Rheumatoid Arthritis	<i>NLRC5</i> methylation associates rheumatoid arthritis.	[45]

Other Molecular Association (External Studies)

Relationship	Details	Reference
NLRC5 IL6	Interference of mouse <i>Nlrc5</i> mRNA by siRNA increases expression of mouse <i>Il6</i> mRNA in RAW 264.7 cells that is increased by lipopolysaccharide.	[46]
NLRC5 IRF3	NLRC5 ablation reduces MHC class I expression, and enhances IKK and IRF3 phosphorylation in response to TLR stimulation or viral infection	[47]
NLRC5 B2M	Human NLRC5 protein increases expression of human B2M protein in OSE cells that is increased by human RFX5 protein.	[48]
NLRC5 IL10	Mouse <i>Nlrc5</i> protein is necessary for expression of mouse <i>Il10</i> mRNA in RAW 264.7 cells that is increased by lipopolysaccharide.	[46]

NLRC5 HLA	Human NLRC5 protein increases expression of human HLA-A mRNA in EBA cells that is increased by human RFXANK isoform b protein.	[48]
NLRC5 MHC Class I	The expression of MHC class I genes is regulated by NLRC5 in coordination with the RFX components through an enhanceosome-dependent manner. It has recently been discovered that another member of the NLR protein family, NLRC5, transcriptionally activates MHC class I genes, and thus acts as CITA (MHC class I transactivator), a counterpart to CIITA.	[49, 50]
PSMB8 IL6	In dendritic cells from human peripheral blood, IL-1beta [IL1B] protein and IL6 protein and TNF-alpha [TNF] protein increase expression of human LMP7 [PSMB8] protein.	[51]
PSMB8 HLA	Repression of human ERAP1 gene by human PRDM1 protein and repression of human MECL1 [PSMB10] gene by human PRDM1 protein and repression of human LMP7 [PSMB8] gene by human PRDM1 protein and repression of human TAPASIN [TAPBP] gene by human PRDM1 protein decrease expression of human Hla-abc protein(s) in cell surface from Hela cells that is increased by IFN gamma [IFNG] protein.	[52]
PSMB8 MCH Class I	Mutant mouse Psmb8 gene (allele Psmb8 ^{tm1Hjf} /Psmb8 ^{tm1Hjf}) (knockout [homozygous]) in 129P2/OlaHsd mouse decreases level of mouse Mhc class I complex in cell surface.	[53]
PSMB8 NFKB1	Human LMP7 [PSMB8] protein and human LMP2 [PSMB9] protein are involved in production of human p50 [product of NFKB1] protein that is mediated by Proteasome [26s Proteasome] complex.	[54]
NFKB1 IL6	In U937 cells, human IL6 protein increases activity of human NF-kappa-B [NFKB1] protein.	[55]
NFKB1 IRF3	Binding of immobilized human IRF3 protein and p50 [product of NFKB1] protein occurs in a cell-free system.	[56]
NFKB1 IL10	In human mononuclear cells expressing human CD14 protein, benzyl adenine and resiquimod	[57]

	increase induction of human IL10 protein that involves signaling of NF-kB [NFKB1] protein.	
NFKB1 MHC Class I	Interference of human IKKA [CHUK] mRNA by siRNA decreases binding of a DNA fragment containing a NFkB binding site from Mhc Class I gene(s) and a protein complex consisting of human p50 [product of NFKB1] and of human p65 [RELA] in a nuclear extract from Sk Br 3 cells.	[58]
NFKB1 B2M	Binding of promoter fragment containing a NF-kappa B response element from human B2M gene and a protein-protein complex consisting of human p50 [product of NFKB1] and of human C-Rel [REL] and of human p65 [RELA] and of human RELB occurs in a nuclear extracts from MSH cells.	[59]
NFKB1 CXCL11	Interference of human NFKB subunit P105/P50 [NFKB1] mRNA by antisense oligonucleotide decreases production of human CXCL11 protein in cultured keratinocytes treated with human IFNG protein from foreskin of neonatal human that is increased by human IL18 protein.	[60]
NFKB1 CXCL10	P50 [product of NFKB1] protein increases expression of human IP-10 [CXCL10] protein in HepG2 cells.	[61]
B2M MHC Class I	Binding of human B2M protein and human HLA class I [MHC CLASS I] protein(s) occurs in human platelets.	[62]
B2M HLA	Binding of human B2M protein and human HLA-A protein and human HLA-F protein occurs.	[63]
B2M IRF3	IRF3 protein increases transactivation of human B2M gene.	[59]
B2M IL6	Beta 2-microglobulin [B2M] increases induction of interleukin-6 [IL6]	[64]
IL10 FCGR3A/FCGR3B	In cell surface from isolated peripheral blood monocytes of human, IL10 protein increases expression of human CD16 [FCGR3A] protein.	[65]
IRF3 LAG3	Mouse Irf3 protein is necessary for expression of mouse Lag3 mRNA in conventional dendritic cells expressing mouse Cd11b [Itgam] protein from mouse lung that is increased by house dust mite allergens.	[66]
IL6 LAG3	IL-6 is involved in Expression of LAG-3 mRNA.	[67]
IL10 LAG3	L-10 is involved in Expression of LAG-3 mRNA.	[67]

HLA LAG3	Mutant mouse Qa-1 [H2-Q1] protein (substitution p.D227K) decreases expression of mouse Lag3 protein in cell surface from spleen effector CD8+ T cells from chronically infected Mus (mouse) infected by LCMV Cl 13.	[68]
IL10 CXCL11	IL10 protein decreases production of I-TAC [CXCL11] protein in neutrophils 2 hours after initial treatment that is increased by LPS [lipopolysaccharide] and Tnf protein(s).	[69]
IRF3 CXCL10	Activation of IRF3 protein by double-stranded DNA increases production of CXCL10 protein.	[70]
IL6 CXCL10	IL6 protein increases production of human IP-10 [CXCL10] protein in cultured Homo sapiens (human) monocyte-derived macrophages that is dependent on phosphorylation of human STAT3 protein.	[71]
IL10 CXCL10	IL10 protein decreases production of IP-10 [CXCL10] protein	[72]
CD163 IL6	The cytokine IL-6 which exerts pro- and anti-inflammatory effects depending on the signaling pathway activated strongly upregulates CD163.	[73]
CD163 IL10	CD163 is induced by IL-10 and glucocorticoids while proinflammatory cytokines like TNF reduce its expression.	[73]
IL6 CD48	In ANBL-6 cells, constitutively active mutant n-Ras protein (unspecified protein mutation) is involved in expression of MEM-102 [CD48] mRNA that involves IL-6 protein.	[74]

Supplementary References

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