Supplementary Methods

Study protocol

The study was conducted over the course of the COVID-19 outbreak. Whole blood samples and clinical data from 407 adult patients (>18y) with confirmed COVID-19 were retrospectively collected between March 7th 2020 and September 14th 2020 in fourteen Hospitals in Spain, organized into five collaborative groups, after approval by the corresponding Ethical Committees (PR127/20, Hospital Universitari de Bellvitge; CEIm_IISPV 079/2020, Hospital Universitari Joan XXIII de Tarragona / Institut d'Investigació Sanitària Pere Virgili; PR(AMI)388/2016, Hospital Universitari Vall d'Hebron; PI-4165, Hospital Universitario La Paz; and PI/2020-145, Hospital Mutua de Terrassa). Exclusion criteria for the study were the presence of comorbidities [obesity (BMI $\geq$ 30), diabetes, hypertension, autoimmune disorders, and chronic cardiovascular or lung diseases], smoking habit and advanced age (> 61 years). According to the Biomedical Research Law 14/2007, patients signed informed consents to donate biological material for research purposes at the reference center. Clinical information has been collected, processed and stored under confidentiality policies, in accordance with the National Organic Law 3/2018, on the protection of personal data and guarantee of digital rights. Clinical data and biological samples arrived at our institution pseudonymized (de-identified) by the clinician or personnel authorized at the healthcare institution. Sensitive patient information showing the identity of the patient was only recorded at the healthcare institution. Biological samples from positive COVID-19 patients were systematically collected and appropriately preserved for research studies. To this end, peripheral blood samples were drawn in EDTA Vacutainer® blood collection tubes and stored at - 80ºC until DNA extraction.

Statistical analysis plan

To have the statistical power to demonstrate the hypotheses, we calculated a priori the minimum sample size necessary according to an epigenome-wide association study (EWAS) power calculation technique previously described (Mansell et al., 2019). We determined that, in order to obtain a statistical power of >90% including >99% of the CpGs present in the EPIC DNA methylation microarray used, taking into account 10% effect size and a significance threshold of P<0.0001, the minimum number of samples in the discovery cohort had to be 206. We were finally able to include 207 cases. We also checked the data to ensure that the assumptions for the methods used (such as linearity) were met. Briefly, the comparison between COVID-19 severity groups was applied to B-values employing an empirical Bayesian framework linear model from the limma package, a model suitable for DNA methylation data (Mansell et al., 2019). The filtered B-values were tested for linearity

by applying a quantile-quantile analysis and calculating their corresponding lambda score (lambda=4.9). Furthermore, variance analysis for each probe was calculated, obtaining that more than 89% of the CpGs presented an equal variance between COVID-19 severity groups (adjusted P > 0.05). Finally, the gvlma (Global Validation of Linear Model Assumptions) R package was used to test each CpG site's model for violations of the assumptions of linear models related to linearity, homoscedasticity, uncorrelatedness and normality of the residuals. Specifically, the data was tested for significance in skewness, kurtosis, link function and heteroscedasticity tests summarizing the results in a global omnibus test.

Reference

Mansell G, Gorrie-Stone TJ, Bao Y, et al. Guidance for DNA methylation studies: statistical insights from the Illumina EPIC array. *BMC Genomics* 2019;**20**:366.

Primers used for DNA methylation analyses in the pyrosequencing assays

Oligo ID	Sequence
CpG_cg02872426_Fw	TTT TAG TGT TTG GTT GGT TTT ATG T
CpG_cg02872426_Rv	/5Biosg/ACC AAA AAA AAT ATC CTA AAT CAA ATC AC
CpG_cg02872426_Seq	GGT TGG TTT TAT GTT TTG AG
CpG_cg04736673_Fw	GAG TGG TTA GGA ATT ATT TTT TTT GAT A
CpG_cg04736673_Rv	/5Biosg/ACC ATT ATA CAT TTA ATT TAT TTT CAA TCT
CpG_cg04736673_Seq	AAT TAT TTT TTT TGA TAT ATT TAT T
CpG_cg05030953_Fw	AAG GAG TTT ATT AGT TTT AAG GTA GT
CpG_cg05030953_Rv	/5Biosg/AAA ATT CTA AAA CAA TAA AAA AAC CTA ACA
CpG_cg05030953_Seq	AGT TTA TTA GTT TTA AGG TAG TT
CpG_cg07796016_Fw	/5Biosg/GGT TTT GGG TAA TTT GGT TTA ATT TAA GT
CpG_cg07796016_Rv	TCT AAC TCT TTA ACC AAT TAC CTA ATA ACT
CpG_cg07796016_Seq	CTT TAA CCA ATT ACC TAA TAA CTT T
CpG_cg08309069_Fw	TGG TGA AGT TTT GTT TGA AAG TAT TAG G
CpG_cg08309069_Rv	/5Biosg/TTA AAT ACA ACA ACC CAA AAT CAC AA
CpG_cg08309069_Seq	TGT ATT ATA ATT AAA TTT TTA AAG T
CpG_cg13452062_Fw	TTG TTA ATA ATT ATA GGA GTT TGG AAG TA
CpG_cg13452062_Rv	/5Biosg/AAC CAC AAC TAC AAA CTC TTC T
CpG_cg13452062_Seq	ATA GGA GTT TGG AAG TAT
CpG_cg13571460_Fw	ATA TGG GGA GGT TTG ATT TT
CpG_cg13571460_Rv	/5Biosg/CCA AAA CAA CCC CAA CTC
CpG_cg13571460_Seq	GGG GAG AGA AGG AGA
CpG_cg14859874_Fw	AGT TGG GGT ATG AGA GTT TTT A
CpG_cg14859874_Rv	/5Biosg/CAA AAA TAA ACC ATC TCC TAT AAC CTA AAT
CpG_cg14859874_Seq	GGA GAA GTT GAA GTT GTA TA

CpG_cg17178900_Fw	GGG TTT TAG TTG TAG TAG TAG ATG TT
CpG_cg17178900_Rv	/5Biosg/CCT TCA CCC ACC TAT ACT CAT AT
CpG_cg17178900_Seq	AGT TGT AGT AGT AGA TGT TTT
CpG_cg24795173_Fw	/5Biosg/TAG GTT GTT GGA TGT TTG TTT GTA TTT AT
CpG_cg24795173_Rv	AAC TCC TTT ATC CAC CAT ACC TAT ACT TTT
CpG_cg24795173_Seq	CCA CCA TAC CTA TAC TTT TA
CpG_cg01097406_Fw	ATT GGT TTT TTG TTA GGA AAA TGT TG
CpG_cg01097406_Rv	/5Biosg/ATT CTA AAA TAA CAA AAA CAA ACC TTC TAC
CpG_cg01097406_Seq	TTA GAG GTG GAT TTA TT
CpG_cg01808126_Fw	AGG GTT TAA AAT TTA GAT TAT AAA AT
CpG_cg01808126_Rv	/5Biosg/TAC CAC ACA CAC CAT AAT CAA TAT ATC
CpG_cg01808126_Seq	ATA AAT TAT TTA TTT TTA ATA AG
CpG_cg02159489_Fw	GGT TTT AGT GGG ATT TGA GTT TAT
CpG_cg02159489_Rv	/5Biosg/TCC AAA TAA TCC TCC TAC ATC AAA C
CpG_cg02159489_Seq	GTT ATA GAG GGT TGA G
CpG_cg11671940_Fw	TTG AGG GTA TTG TAA AAT AAT AAG TAG ATG
CpG_cg11671940_Rv	/5Biosg/CAC CCT AAA CCT CTC AAT CAC AAA AT
CpG_cg11671940_Seq	GTA TTG TAA AAT AAT AAG TAG ATG T
CpG_cg11822515_Fw	ATG TAT TGA GTG TTA TGA TAG GGA AGT A
CpG_cg11822515_Rv	/5Biosg/ATT CCA TAC ATA CTA TCT CTC ATA TCA TT
CpG_cg11822515_Seq	AGA AGT ATT ATA GGT TTT TTT AAA
CpG_cg15532640_Fw	TTT AGG GAG GGA ATT GAG AAG
CpG_cg15532640_Rv	/5Biosg/AAC CCC TAA AAA CAC TAA ATT TCC
CpG_cg15532640_Seq	GAG ATG TTG ATG AGG T
CpG_cg16814680_Fw	/5Biosg/AAA TTA ATA TTT TTG GTT TTA TTT TTA AA
CpG_cg16814680_Rv	AAA ATT CCC CTT CTC AAC TAA CT
CpG_cg16814680_Seq	CCT TCT CAA CTA ACT ACT C
CpG_cg18523915_Fw	TTT TTT ATT GGT TGA ATT AAT TTG GAA GTT
CpG_cg18523915_Rv	/5Biosg/CTA CCT TTC TAA ACC TTT ATA CTA TAT CAC
CpG_cg18523915_Seq	AAT TTG GAA GTT AGA GAG TA
CpG_cg21139150_Fw	GAA GAG GGT GGT TGT AGT TAT T
CpG_cg21139150_Rv	/5Biosg/CCA AAA CTT AAA CTT ACA ATC TTA AAT CC
CpG_cg21139150_Seq	GAG ATT TGA GAT TTT TAA GTT G
CpG_cg25134647_Fw	GGG TTA GGA ATT TGA AGT GAT TTA GTT G
CpG_cg25134647_Rv	/5Biosg/CAC CAT ACC TAA CCA ATC CTA ACT A
CpG_cg25134647_Seq	ATG GTA TGG TTT AGG G
CpG_cg26035071_Fw	GGG GTT TTA TTT ATT TGG ATT TTT GTT
CpG_cg26035071_Rv	/5Biosg/TCT CCA ACT CCT AAC CTC AAA TAA TCT A
CpG_cg26035071_Seq	ATT TGT TTT GTA TTG GTT GT