Supplemental information

Supplemental materials and methods

Tissue dissociation

Samples from prophylactic mastectomies, TUM and JT were gently dissociated and enzymatically digested by an incubation of 30-40-minutes, at 37°C in agitation, in CO_2-indipendent medium (Gibco, Cat#18045088) supplemented by Collagenase I (2mg/mL) (Sigma, Cat#C0130), Hyaluronidase (2mg/mL) (Sigma, Cat#H3506), and DNase (25µg/mL) (Roche, Cat#5401020001). Afterwards, single cell suspensions were obtained by disrupting the fragments with a syringe plunger over a 40µM cell strainer (Fisher Scientific), washing with PBS (Eurobio, Cat#CS1PBS01-01) supplemented by 2mM EDTA (Gibco, Cat#15575020) and 1% of human serum (BioWest, Cat#S4200). Cell suspension was obtained after 10' of centrifugation. After isolation of total tissue-infiltrating cells, cells were counted in CO_2-indipendent medium plus 5% of FBS (Sigma, Cat#A7906). Peripheral blood mononuclear cells (PBMCs) were isolated using Lymphoprep solution (StemCell, Cat#07851). PBMCs were counted and collected in complete RPMI-1640 (Gibco, Cat#61870036) (supplemented by 5% FBS and Penicilin Streptomicym (Gibco, Cat#15140-122). CD14 Microbeads (Miltenyi, Cat#130-050-201) are used for the isolation of $CD14^+$ monocytes from PBMC of HD and TNBC.

Single cell-RNA-sequencing data processing

Single-cell expression was analyzed using the Cell Ranger single-cell Software Suite (v3.0.1 - 10X Genomics) to perform quality control, sample de-multiplexing, barcode processing and single-cell 3′ gene counting. Sequencing reads were aligned to the GRCh38 human reference genome. Further analysis was performed in R (v3.5.1) using the Seurat package (v3.1.1) (https://pubmed.ncbi.nlm.nih.gov/31178118/). Cells were then filtered out when expressing less than 500 genes, or when expressing more than 10% mitochondrial genes. From the 7

samples, 14134 cells (12397 cells for TUM and 1737 for JT) were kept for statistical analysis. For each sample, the gene-cell-barcode matrix of the samples was then normalized to a total of 1e4 molecules. The top 5000 variable features were identified using the "vst" method from Seurat where both lowly and highly expressed genes are transformed onto a common scale. For the 7 samples altogether, we computed the integration anchors using the Seurat v3 integration method. This method is leveraging closely-related cells (termed anchors) between datasets to compute a batch-corrected matrix. Top 30 CCA components were used to find transfer anchors between datasets and used to generated the integrated matrix for the 7 samples.

Dimension reduction and unsupervised clustering

Top 30 Principal Components were computed and Uniform Manifold Approximation and Projection (UMAP) was performed using the top 30 PCs of the integrated matrix. Clusters were identified using the *FindNeighbors* and *FindClusters* function in Seurat with a resolution parameter of 0.6 and using the first 30 principal components. Unique cluster-specific genes were identified by running the Seurat *FindAllMarkers* function using Wilcoxon test on the uncorrected matrix. Then clusters containing contaminating cells were removed from the analysis. CD3, CD4, CD8 expression was used to remove T cell clusters. CD19, MS4A1 expression was used to remove B cell clusters, CD56 expression was used to remove NK cell clusters. Signature scores were computed using the Seurat function *AddModuleScore* using the gene signature of interest and the integrated matrix. Briefly this function calculates for each individual cell the average expression of each gene signature, subtracted by the aggregated expression of control gene sets. The averaged heatmap was generated using the Average function in Seurat, which averages gene expression across clusters. Cell cycle scoring was also performed using Seurat "CellCycleScoring" function using cell cycle genes. Hallmarks gene sets from MSigDB, v.7.0 were downloaded in GMT format from https://www.gsea-

msigdb.org/gsea/msigdb/collections.jsp. These gene sets were used as modules for the "AddModuleScore" function in Seurat.

Pathway enrichment analysis

Pathways enrichment tests were performed using Reactome (25) (RRID:SCR_003485). Results were then imported in R for plotting using ggplot2 using the "Entities FDR" values.

Label transfer using a reference

Data from Azizi et al. 2018 (4) and Wu et al. 2020 (37) were downloaded from GSE114727 and https://singlecell.broadinstitute.org/single_cell/study/SCP1106, respectively. The 3 datasets, composed of 17 samples were integrated using the same method as previously described using Seurat pipeline (RRID:SCR_016341). Labels from our original dataset were transferred onto the other using *FindTransferAnchors* and TransferData functions in Seurat using top 30 PCs.

Reprocessing of other datasets

Sharma et al. Seurat object was downloaded from GSE156625 (15). Molgora et al. was downloaded from GSE151710 (18). Bassez et al. data were downloaded from http://biokey.lambrechtslab.or (29). We computed Module Score using our clusters DEG as markers.

Trajectory inference analysis

To compute pseudo-time alignment of our transcriptomes, we first used Monocle3 (v3.0.2.3.0) (RRID:SCR_018685) using the first 30 PCs of the integrated matrix to perform preprocessing and UMAP reduction. The graph was generated using default parameters and setting "minimal_branch_len = 13" parameter to simplify our graph by pruning long branches.

PHATE algorithm

To confirm our UMAP representation, we additionally used the PHATE v0.3 algorithm that was run on our integrated matrix using Phate R implementation RunPHATE available at https://github.com/scottgigante/seurat with a gamma parameter set at 0.

CytoTRACE

We then computed each cell cluster's developmental potential of using CytoTRACE computational framework, which assumes that the number of genes expressed in a cell decreases during differentiation. CytoTRACE values were computed using CytoTRACE R package (v0.3.3) using the uncorrected matrix as input data.

ROGUE

To determine the degree of homogeneity of our cell clusters, we used the ROGUE method, an entropy-based statistic used to quantify cell clusters' purity. ROGUE values were computed using ROGUE R package (v1.0) by the uncorrected matrix as input data, using platform = "UMI", filter = T parameters. The output values range from 0 (lowest purity) to 1 (highest purity). We used METABRIC to compute expression and correlations on bulk cohorts. For all the analyses we selected 1989 total patients: TNBC (N=332), Luminal (N=1409) and HER2+ (N=248).

Cell-cell communication analysis

For the analysis of CAF-myeloid interactions we merged 2 BC SC-datasets. scRNA-seq data from CAF dataset were downloaded from (https://ega-sarchive.org) under accession number EGAS00001004030. Since we did not rely on previously defined clusters, we merged the CAF dataset with our myeloid Seurat objects without integration. For the analysis of cell-cell interactions, ligand-receptor information was recovered from CellPhoneDB (https://www.cellphonedb.org) (RRID:SCR_017054) repository. This package examines the ligand-receptor interaction based on curated databases such as UniProt (RRID:SCR_018666), IUPHAR, and Ensembl (RRID:SCR_002344). Log2 mean expression and log10 of *p* values

were calculated and expressed on dot-plots. Using our combined CAF and myeloid Seurat objects, we specified CAFs as sender-cells, and myeloid as receiver-cells.

Conditioned media and Luminex assays

Cytokines and chemokines were assessed in the JT and TUM- CM from treatment-naïve TNBC (N=78) and LBC (N=103). CM were obtained after 24h, by incubating a small fragment of tissue (cut into 15-20 mg weight fragments) in complete RPMI-1640 medium supplemented with 10% FBS, 1mM Sodium Pyruvate, 10 ml Non-essential amino acids 100X and 1% of Penicillin/Streptomycin (Penicillin 10.000U/ml; Streptomycin 10.000 mg/ml). After incubation, the supernatants were diluted 1:2 (v/v) in complete RPMI medium, filtered (Millex-GP 0,22µM, Millipore, Cat#SLGP033RB) and frozen in aliquots at -80° C. Human cytokine/chemokine Magnetic Bead panels I, II, III and Human Adipocyte magnetic bead panel (Millipore, #HCYTOMAG, #HCYP2MAG, #HCYP3MAG, #HADCYMAG) were purchased from Millipore and used according to manufacturer's recommendations to measure the following cytokines in the supernatants: CCL2, CCL3, CCL4, CCL8, GM-CSF, M-CSF and CXCL12.

Cytometric Bead Array (CBA)

Supernatants from mono-derived LAM -T cell co-cultures were collected at day 3 to measure hIL-10 and GrzmB by the Flex Set (BD, Cat# BDB558274, Cat# BDB560304) kit. CBA was performed according to the manufacturer's protocol. The data were analyzed by BD FACS-Verse in the PE channel. hIL-10 and GrzmB data were generated by FCAP Array Software v3.0 (BD Bioscience) and reported as pg/mL.

Human-Procarta Plex assay in FAP^+CD29^+ supernatants

FAP^+CD29^+ supernatants generated as described above were analyzed by Human-Procarta 17-plex (Thermo Fisher Scientific, Cat#MX323EP) following the manufacturer's protocol. Data

were acquired using a BIO-PLEX 200 plate reader and analyzed with the Bio-Plex Manager 6.1 software. IL-6, CCL2 and VEGF-α data were reported as pg/mL.

Bulk RNA-sequencing *in vitro*

Magnetically-enriched circulating CD14+ monocytes (CD14 Microbeads-Miltenyi) from HD (N=3) and TNBC (N=3) patients were co-culture with $FAP^{+}CD29^{+}$. After 3 hours of culture FACS-sorted monocytes were isolated by FACS-ARIA III (BD) cell sorter directly collected on lysing TCL buffer (Qiagen, Cat#1070498) containing 1%β-mercatpoethanol (LifeTechnologies, Cat#31350-010) before storage at -80°. RNA extraction was performed from *ex vivo* circulating isolated $CD14^{+}$ and $FAP^{+}CD29^{+}$ co-cultured monocytes by Single Cell RNA purification Kit (Norgen, Cat#51800) according to the manufacturer's protocol. A step of DNase I digestion (RNase-Free Dnase Set-Qiagen, Cat#79254) was added. Total RNA was analyzed using Agilent RNA 6000 Pico Kit on the Bioanalyzer 2100 Bioanalyzer system. RNA quality was checked by RNA Integrity Number (RIN). RNA sequencing libraries were prepared using the SMARTer Stranded Total RNA-Seq Kit v2 - Pico Input Mammalian (Clontech/Takara). The input quantity of total RNA was 10 ng/each condition. A first step of RNA fragmentation, using a proprietary fragmentation mix at 94° C. After fragmentation, indexed cDNA synthesis was performed. Then the ribodepletion step was performed, using probes specific to mammalian rRNA. PCR amplification was done to amplify the cDNA libraries. Library quantification and quality assessment was performed by Qubit fluorometric assay (Invitrogen) with dsDNA HS (High Sensitivity) Assay Kit and LabChip GX Touch using a High Sensitivity DNA chip (Perkin Elmer). Libraries were then equimolarly pooled and quantified by qPCR using the KAPA library quantification kit (Roche). Sequencing was performed on the NovaSeq 6000 (Illumina), targeting between 10 and 15 M reads per sample and using paired-end 2 x 100 bp.

RNA-sequencing, data processing and analysis

Aligned files were processed using Curie pipeline v3.1.8 https://github.com/bioinfo-pf-curie/RNA-seq. The pipeline is built using Nextflow and processes RNA-seq from raw sequencing reads to count table for downstream analysis. Briefly, the overall quality of raw sequencing data is first checked using FastQC. Reads were then aligned on a ribosomal RNAs database and on the genome reference using STAR 2.6.1a to the hg38 human reference genome. Additional controls on aligned data are performed to infer strandness, complexity, gene-based saturation, read distribution or duplication level. The aligned data are then used to generate a final count matrix with all genes and all samples. PCA was done with the "prcomp" function in R using the top twenty percent most variable genes. PC1 and PC2 are shown in Figures XX. Differentially expressed genes (DEGs) were identified with DESeq2 (DESeq function) using adjusted p-value < 0.05; genes were first filtered on minimum expression (median reads per group ≤ 5). For pairwise comparisons of HD and TNBC donors following $FAP^{+}CD29^{+}$ co-culture, sample information was used as a term in the design formula to adjust for statistical confounding effects. "Lfcshrink" function from DESeq2 was then performed on the differential genes to give more realistic fold-change estimates, using the apeglm method (https://academic.oup.com/bioinformatics/article/35/12/2084/5159452). Volcano plots were plotted using the Enhanced Volcano package v1.12 (Blighe K, Rana S, Lewis M (2021). EnhancedVolcano: Publication-ready volcano plots with enhanced colouring and labeling.) Heatmaps were produced using pheatmap R package (v 1.0.12), using TPM values given following STAR alignment.

CAF-monocyte migration assays

To test the migration of monocytes we used the xCELLigence Real-Time Cell Analyzer Dual Purpose (RTCA DP) (ACEA Bioscience). The Electronic Agilent Technologies CIM-plate 16 (Thermo Fisher Scientific, Cat# NC0552303) was used. CIM-plate 16-well is composed by upper chambers coated with gold microelectrode sensors and the lower chambers in which the

chemoattractant is placed. Monocyte migration index, generated as impedance signal, was calculated for each condition. Migration assays used CAF supernatants (generated in DMEM 1% FBS), DMEM 1%FBS as negative control, and recombinant CXCL12 (150 ng/mL) (Peprotech, Cat#300-28B). Control media and CAF supernatants were placed in the lower chamber. After equilibration at 37°C for 1 hour and baseline background measurements $CD14^+$ monocytes ($4x10^5$) enriched from HDs, pre-treated or not with 50 µg of AMD3100 (CXCR4 antagonist I, Sigma-Aldrich, Cat#239820) for 3 hours at 37°C, were added in the upper chambers. As the peak of monocyte migration mediated by CXCL12 was observed at 1½ - 2 hours, the mean cell index of duplicates was evaluated at 2 hours for each condition.

Immunohistochemistry

A naïve, primary untreated patients (N=6) have been studied for multicolor immunohistochemistry (IHC) analyses. IHC were performed on residual surgery samples prior any treatments. Multiplex immunohistochemistry (IHC) was performed using a Leica Bond RX 3µm, dewaxed (Leica Bond Dewax solution) Formalin-Fixed Paraffin-Embedded (FFPE) of above described TNBC samples. Heat-induced epitope retrieval was obtained with Leica HIER1 (pH 6) solution. Endogenous peroxidases were blocked with Dako REAL Peroxidase-Blocking solution for 10 min. Prior to antibody staining, slides were incubated with Dako Protein Block Serum-Free solution for 10 min. Multiplex staining was performed using AKOYA Biosciences Opal 7-Color Automation IHC Kit (Cat#NEL821001KT).

For each multiplex IHC step, primary antibodies listed [Human Stabilin-1 (Clone 840449) (Biotechne, Cat# MAB3825), CD11c/Integrin Alpha X Monoclonal (Clone 2F1C10) (Protein tech, Cat#60258; RRID:AB_2883128), Recombinant Anti-Apolipoprotein E antibody EP1374Y (Ab-cam, Cat#ab52607; RRID:AB_867704), Cytokeratin (Clone AE1/AE3) (Dako, Cat#M3515), Spectral DAPI (Akoyabio, Cat# SKU FP1490)] were incubated for 60 min, followed by Opal-TM Polymer Horseradish peroxidase (HRP) and Opal-TM corresponding

fluorophores (APOE-Opal 540, STAB1-Opal 570, CD11c-Opal 520, Cytokeratin-Opal 690, DAPI) according to manufacturer instructions. After each step, Leica HIER1 (pH 6) solution was used to remove antibodies construction for 15min at 95°C. Images were acquired on a Vectra3 from PerkinElmer and analysed by Halo Software (RRID:SCR_018350) from Indica Labs. Triple positive $APOE^{+}CD11c^{+}STAB1^{+}$ were counted in the selected areas (10/20 areas for each tissue/pt).

Flow cytometry human

All cells were stained with LIVE/DEAD (Thermo Fisher Scientific, Cat#L34957) in PBS plus 2% FBS, according to the manufacturer instructions. FcR-block (Miltenyi, Cat#130-059-901) was consistently added in all surface staining to avoid unspecific binding. BODIPY FL C16 (Thermo Fisher) (Cat#D3821) was diluted (1:1000) in PBS plus 2% FBS in combination of surface staining. All cells from normal breast, tumor and JT tissues were stained with surface monoclonal Abs listed: Mouse Anti-Human MSR1 CD204- BV605 (Clone U23-56) (BD Biosciences Cat# 742440, RRID:AB_2740783), Mouse Anti-Human CD14- Alexa Fluor-488 (Clone M5E2) (BD Biosciences Cat# 557700, RRID:AB_396809), Anti-human CD3-Alexa Fluor-700 (Clone UCHT1) (BioLegend Cat# 300424, RRID:AB_493741), anti-human CD163- PE/Cy7 (Clone GHI/61) (BioLegend Cat# 333614, RRID:AB_2562641), Mouse Anti-Human CD206-PE-CF594 (Clone 19.2) (BD Biosciences Cat# 564063, RRID:AB_2732052), Mouse Anti-Human CD16-BUV737 (Clone 3G8) (BD Biosciences Cat# 564434, RRID:AB_2869578), Mouse Anti-Human CD11c- BUV395 (Clone B-ly6) (BD Biosciences Cat# 563787, RRID:AB_2744274), Mouse Anti-Human CD45-APC-Cy7 (Clone 2D1) (BD Biosciences Cat# 557833, RRID:AB_396891), Mouse Anti-Human CD19-Alexa Fluor700 (Clone HIB19), anti-human HLA-DR- Brilliant Violet 711 (Clone L243) (BioLegend Cat# 307644, RRID:AB_2562913), Mouse Anti-Human CD56-Alexa Fluor700 (Clone B159), Mouse Anti-Human CD14-PerCP-Cp5.5 (Clone M5E2) (BD Biosciences Cat# 550787,

RRID:AB_393884), anti-human CD45-Brilliant Violet 421 (Clone 2D1) (BioLegend Cat# 368521, RRID:AB_2687374), anti-human CD184 (CXCR4)-Brilliant Violet 605 (Clone 12G5) (BioLegend Cat# 306522, RRID:AB_2563631), anti-human CD9- PerCP/Cyanine5.5 (Clone HI9a) (BioLegend Cat# 312110, RRID:AB_2728251), anti-human CD63- PE/Cy7 (Clone H5C6) (BioLegend Cat# 353010, RRID:AB_10915475), anti-human CD14- Brilliant Violet 650 (Clone M5E2) (BioLegend Cat# 301836, RRID:AB_2563799), anti-human CD326 (EpCAM)- Brilliant Violet 786 (Clone 9C4) (BioLegend Cat# 324238, RRID:AB_2632937), anti-human HLA-DR- Brilliant Violet 650 (Clone L243) (BioLegend Cat# 307650, RRID:AB_2563828), anti-human CD29- PE/Cy7 (Clone TS2/16) (BioLegend Cat# 303025, RRID:AB_2716097), Human Fibroblast Activation Protein alpha/FAP-PE (Clone 427819) (R&D Systems, Cat#FAB3715P-100), TEM8/ANTXR1-Alexa Fluor405 (Clone 200C1339(SB20)) (Novusbio, Cat#NB100-56585AF750), anti-human CD31- Brilliant Violet 605 (Clone WM59) (BioLegend Cat# 303122, RRID:AB_2562149), anti-human CD235a (Glycophorin A)- PE/Dazzle594 (Clone HI264) (BioLegend Cat# 349120, RRID:AB_2814278), STAB1-AlexaFluor488 (AK1013/03 or 9-11). For intracellular staining (APOE, S100A8-9 and CXCL12), a fixation-permeabilization buffer (FOXP3 fixation-permeabilization buffer) (Thermo Fischer, Cat#00-5521-00) was used. Abs listed: Anti-Apolipoprotein E- Alexa Fluor647 (Clone EP1374Y) (Abcam, Cat# ab196194), Anti-Apolipoprotein E-Alexa Fluor488 (Clone EP1374Y) (Abcam, Cat# ab196463), Mouse Anti-Human S100A8/A9 (MRP-8/14)- Alexa Fluor647 (Clone 5.5) (BD Biosciences Cat# 566010, RRID:AB_2732837), Human/Mouse CXCL12/SDF-1-APC (Clone 79018) (R&D Systems Cat# IC350A, RRID:AB_1964550).

For CAF-monocyte co-cultures, $CD14^+$ isolated monocytes were analysed by FC. All cells were stained with surface monoclonal Abs, listed: Human/Mouse TREM2 Alexa Fluor-350 (Clone 237920) (R&D Systems, Cat# FAB17291U-100UG), anti-human CD9-PerCP/Cyanine5.5

(Clone HI9a), anti-human CD63-PE/Cy7 (Clone H5C6), Anti-Apolipoprotein E-Alexa Fluor488 (Clone EP1374Y), Mouse Anti-Human CD14-PerCP-Cp5.5 (Clone M5E2) (BD Biosciences Cat# 550787, RRID:AB_393884), Mouse Anti-Human MSR1 CD204- BV605 (Clone U23-56), Mouse Anti-Human CD206-PE-CF594 (Clone 19.2), STAB1-AlexaFluor488 (AK1013/03 or 9-11), anti-human HLA-DR- Brilliant Violet 711 (Clone L243), Mouse Anti-Human CD16-BUV737 (Clone 3G8), Mouse Anti-Human CD14- BV650 (Clone M5E2), Mouse Anti-Human CD11c- BUV395 (Clone B-ly6) (BD Biosciences Cat# 563787, RRID:AB_2744274).

FMO staining has been performed for all markers as negative control. Cells were analyzed by BD-FORTESSA (BD-Bioscience). Data were analyzed by FlowJo software (version 10.5.2) FlowJo (RRID:SCR_008520) (Treestar).

Flow cytometry mouse

Single cell suspensions were prepared from tumors upon sacrifice. Tumors were minced and digested with Collagenase IV (Sigma, Cat #C5138-5G) for 30 min at 37°C. Cells were filtered through 70μm strainers, washed with PBS and stained for flow cytometry. The following antibodies were used: CD45–AlexaFluor700 (clone 30-F11) (BioLegend Cat# 103128, RRID:AB_493715); CD11b-PerCPCy5.5 (clone M1/70) (BioLegend Cat# 101228, RRID:AB_893232); I-A/I-E-BV650 (clone M5/114.15.2) (BioLegend Cat# 107641, RRID:AB_2565975); Ly6C-BV421 (clone HK1.4) (BioLegend, Cat #128031); FOLR2-APC (clone 10/FR2) (BioLegend Cat#153305); Ly6G –BV711 (clone 1A8) (BioLegend, Cat#127643); CD64 –BV605 (clone X54-5/7.1) (BioLegend Cat# 139323, RRID:AB_2629778), STAB1-AlexaFluor488 (AK1013/03 or 9-11); CD206 –PeCy7 (clone C068C2) (BioLegend Cat# 141719, RRID:AB_2562247); CD9-PE (clone MZ3) (BioLegend Cat# 124805, RRID:AB_1279327); CD63-PECF594 (clone NVG-2) (BioLegend Cat# 143914, RRID:AB_2565504); TCRβ-PE (clone H57-597) (BioLegend, Cat#109208); CD8-BV785

(clone 53-6.7) (BioLegend Cat# 100749, RRID:AB_11218801); CD4-PECF594 (clone RM4-5) (BioLegend Cat# 100566, RRID:AB_2563685); FOXP3-APC (clone MF-14) (BioLegend Cat# 126408, RRID:AB_1089115); CD19-PacificBlue (clone 6D5) (BioLegend Cat# 115523, RRID:AB_439718); NK1.1-BV650 (clone PK136) (BioLegend, Cat#108736). Cells were incubated with Fc block prior to staining. Cell viability was determined by Aqua LIVE/Dead-405 nm staining (Invitrogen, Cat#L34957), negative cells were considered viable. Foxp3/Transcription Factor Staining Buffer Set (eBioscience, Cat #00-5523-00) was used for intracellular staining (STAB1 and FOXP3). Lymphoid and myeloid panels were performed in $Trem2^{+/+}$ (N=4) and $Trem2^{-/-}$ (N=5 or 6) mice, the discrepancy in the number of mice is due to tumor size. Cells were analysed on BD FACSymphony (BD Bioscience). Data were analyzed with FlowJo software (Treestar) and Cytobank (RRID:SCR_014043).

Software versions

Data were collected using Cell Ranger software (10X Genomics) v.3.0.1 (Cat#CG000183), and analyzed using R v.3.5.1, and the following packages and versions in R for analysis: Seurat v3.1.1, Monocle v3.0.2.3.0 (RRID:SCR_018685), CytoTRACE v0.3.3, ROGUE v1.0, phateR v1.0.4. Figures were produced using the following packages and versions in R: RColorBrewer v1.1-2 RColorBrewer (RRID:SCR_016697); pheatmap v1.0.13 pheatmap (RRID:SCR_016418); ggplot v3.2.0 ggplot2 (RRID:SCR_014601); ggsignif v0.6.0.