EV purification

B-cell lines, purified primary FL B cells, and purified tonsil B cells were seeded at 10⁶ cells/mL in RPMI1640-7% EV-depleted FCS for 2 days. EVs were isolated from cell culture media or BM plasma by differential ultracentrifugation adapted from previously described methods¹ on an Avanti J-30i centrifuge (Beckman Coulter). For culture supernatants, we first performed two regular centrifugations of 5 min at 800 g and 15 min at 2,735 g to discard cells and cells debris. The supernatant was then ultracentrifuged 90 min at 100,000 g and the resulting pellet was suspended in PBS before a second ultracentrifugation of 90 min at 100,000 g. The pellet was finally suspended in fresh PBS and centrifuged 5 min at 6,000 g to remove the remaining cell debris and EV aggregates. EVs obtained from malignant B cell cultures were quantified by flow cytometry (Cytoflex, Beckman Coulter) as the concentration of Annexin V (Tau Technologies BV) positive events in a 100 to 900 nm size region determined by a mix of Megamix-Plus FSC and Megamix-Plus SSC (Biocytex). For FL and HD BM plasma, 150 µL of plasma was diluted in 1 mL PBS and then ultracentrifuged twice 90 min at 100,000 g. The pellet was suspended in 20 µL fresh PBS and immediately used for BM-MSC stimulation.

EV characterization

The transmission electron microscopy (TEM) of EVs was performed as previously published¹. Briefly, freshly isolated EVs were fixed in 2% PFA and adsorbed on Formvar-carbon coated grids and then rinsed with TBS. The adsorbed grids were fixed for 5 min on 1% glutaraldehyde solution, washed several times in distilled water, transferred to an uranyloxalate solution for 5 min and then to a mixture of 4 % uranyl-acetate and 2 % methyl cellulose for 10 min. After blotting and air-drying, the grids were visualized on JEOL MET1400 operating at 120kV (JEOL). The size distribution and concentration of EVs were studied by Tunable Resistive Pulse Sensing (TRPS) on a qNano analyzer (Izon Science) on both NP300 and NP150 nanopores after filtration at 800 nm and 200 nm respectively. Analysis of EV protein content was performed by Western blot. EVs and EV-producing cells were lysed in RIPA buffer (Thermo Scientific) before centrifugation at 14,000g 20 min and quantification of the proteins by Bradford assay (Thermo Scientific). 10 µg proteins

separated by NuPAGE (4-12 % Bis-Tris gel, Invitrogen) were then transferred onto nitrocellulose membranes (Invitrogen) that were probed with specific antibodies against Golgin 97, Calnexin (Invitrogen), TSG101, CD81 (Life Technologies), and β -actin (Sigma Aldrich). Proteins of interest were detected and quantified on a G:BOX device (Syngene) with appropriate HRP-conjugated secondary antibodies (Invitrogen) and visualized with the Pierce ECL Plus Western blotting substrate (Thermo Scientific).

EV uptake by BM-MSC

To validate EV uptake by BM-MSC, EVs were dyed using 1.5μ M CellTrace Far Red, 5μ M CellTrace Violet (Life Technologies), or shame dyed with PBS during 30 min at 37°C. Dyed EVs were then washed by Size Exclusion Chromatography (SEC) on a Sepharose CL2-B column (GE Healthcare Life Sciences) followed by an ultracentrifugation of 90 min at 100,000 g. For confocal microscopy, CellTrace Violet-dyed EVs were added to BM-MSC for 3 days before imaging with CellMask Deep Red (Thermo Scientific) to dye cell membranes, and SytoxGreen (Life Technologies) as nuclear counterstaining. Finally, slides were analyzed by confocal microscopy on a SP8 (Leica Microsystems). ImageJ software was used for image analysis. For flow cytometry, CellTrace Far Red-dyed EVs were added to BM-MSC for 6 hours before analyzing the mean fluorescence intensity (MFI). When indicated, inhibitors of endocytosis, including 450nm sucrose, 2mM EDTA, 20 μ M Cytochalasin D, and 50 μ M Dynasore (Sigma Aldrich) or DMSO as a control vehicle, were used to treat BM-MSC 30 minutes before adding dyed-EVs.

Evaluation of FL-supportive activity of BM-MSC

BM-MSC (5 x 10⁵/cm²) were stimulated by a combination of 10 ng/mL TNF and 100 ng/mL LT (R&D Systems), or by B-cell line derived EVs (ratio of 10,000 EVs/1 BM-MSC) for 3 days. Purified primary FL B cells were cultured alone or in the presence of untreated, EV-primed, or TNF/LT-primed BM-MSC at a ratio of 4:1 in RPMI-7% EV-depleted FCS. At the end of the culture, CD19/CD20^{pos}CD105^{neg} FL B cells were analyzed by flow cytometry using PECy7 anti-CD20, PECy7 anti-CD19, and APC anti-CD105 monoclonal antibodies (mAbs, Beckman Coulter). Apoptosis was measured using PE anti-active caspase-3 mAb and cytofix/cytoperm kit (Becton Dickinson). FL B-cell activation was studied by staining with PE-CF594 anti-CD86

mAb (Becton Dickison) before permeabilization (Fix and Perm kit, Invitrogen) and staining by FITC anti-Ki67 mAb (Dako).

RNAseq study of BM-MSC priming by EVs

RNA was extracted from 5 untreated, EV-primed, and TNF/LT primed BM-MSC (5 from RL, 5 from DOHH2, and 5 from SUDHL4 cell lines) using NucleoSpin RNA Kit (Macherey-Nagel). The purity and integrity of RNA were checked with the Bioanalyzer 2100 (Agilent). All samples used for RNAseq experiments displayed an RNA integrity number >9. The RNA-seq libraries were sequenced with 2 x 100 bp paired reads on the Illumina Hiseq2500 sequencer generating more of 50 million reads per sample. Raw sequencing read data was checked and visualized for quality using FastQC (v0.11.5)². Reads were aligned to the reference genome GRCh38 release 90 using STAR (v2.5.2)³. Read summarization, gene and transcripts counts were performed using the featureCounts (v1.5.0)⁴. Differential gene expression was analyzed using the DESeq2 algorithm⁵ and significant genes were defined using a Wald test (P<.01) after adjustment for multiple testing by False Discovery Rate correction (FDR).

Osteoblastic differentiation

HD BM-MSC (n=3) were plated at 3 x 10^3 cells per cm² in a 96-well flat-bottom plate in α MEM (Invitrogen) supplemented with 10% FCS, 100 IU/mL penicillin and 100 µg/mL streptomycin (Invitrogen). Osteogenesis was induced the day after by adding FL-derived EVs (ratio of 10,000 EVs/ 1 BM-MSC) or using the hMSC differenciation bulletkit – osteogenic (Lonza) as a control. The culture medium alone, the culture medium supplemented with EVs or the osteogenesis induction medium was replaced twice a week for 17 days. The mineralization was assessed using the OsteoImage Mineralization Assay (Lonza) by measuring fluorescent with the plate reader Varioskan Flash (Thermo Scientific).

EV quantitative proteomic analysis

According to Bradford Assay (Thermo Scientific), $15\mu g$ of each EV sample (4 EV production from RL cell line, 3 EV production from SUDHL4 cell line and 5 EV production from purified tonsil B cells) was dried and solubilized in 50 μ L 8M urea, 200 mM ammonium bicarbonate and then reduced in 5 mM dithiothreitol, pH 8 with

vortexing at 37°C for 1 h. After cooling to room temperature, cysteines were alkylated by adding 10 mM iodoacetamide for 30 min in the dark. After diluting to 1 M urea with 200 mM ammonium bicarbonate pH 8.0, samples were digested with 0.2µg trypsine/LysC (Promega) during 2 h and then with 0.4 µg overnight by vortexing at 37°C. Samples were then loaded onto homemade C18 StageTips for desalting. Peptides were eluted using 40/60 MeCN/H2O + 0.1% formic acid, vacuum concentrated to dryness and reconstituted in 10µl injection buffer (0.3% TFA). Then Liquid chromatography-mass spectrometry (LC-MS/MS) was performed with an RSLCnano system (Ultimate 3000, Thermo Scientific) coupled online to an Orbitrap Exploris 480 mass spectrometer (Thermo Scientific). Peptides were trapped on a C18 column (75 µm inner diameter × 2 cm; nanoViper Acclaim PepMapTM 100, Thermo Scientific) with buffer A (2/98 MeCN/H2O in 0.1% formic acid) at a flow rate of 3.0 µL/min over 4 min. Separation was performed on a 50 cm x 75 µm C18 column (nanoViper Acclaim PepMapTM RSLC, 2 µm, 100Å, Thermo Scientific) regulated to a temperature of 40°C with a linear gradient of 3% to 29% buffer B (100% MeCN in 0.1% formic acid) at a flow rate of 300 nL/min over 91 min. MS full scans were performed in the ultrahigh-field Orbitrap mass analyzer in ranges m/z 375–1500 with a resolution of 120 000 at m/z 200. The top 20 most intense ions were subjected to Orbitrap for further fragmentation via high-energy collision dissociation (HCD) activation and a resolution of 15000 with the auto gain control (AGC) target set to 100%. We selected ions with charge state from 2+ to 6+ for screening. Normalized collision energy (NCE) was set at 30 and the dynamic exclusion of 40s. For identification, the data were searched against the Homo Sapiens (UP000005640) UniProt database using SequestHT through proteome discoverer (version 2.4). Enzyme specificity was set to trypsin and a maximum of two miss cleavages sites were allowed. Oxidized methionine, Met-loss, Met-loss-Acetyl and N-terminal acetylation were set as variable modifications. Carbamidomethylation of cysteins were set as fixed modification. Maximum allowed mass deviation was set to 10 ppm for monoisotopic precursor ions and 0.02 Da for MS/MS peaks. The resulting files were further processed using myProMS⁶ (v3.9.2). FDR calculation used Percolator and was set to 1% at the peptide level for the whole study. The label free quantification was performed by peptide Extracted Ion Chromatograms (XICs) computed with MassChroQ⁷ (v2.2.21). For protein guantification, XICs from proteotypic peptides shared between compared conditions (TopN matching) and missed cleavages were not allowed. Median and scale normalization was applied on the total signal to correct the XICs for each biological replicate (n=7 for FL and n=5 for tonsils). To estimate the significance of the change in protein abundance, a linear model (adjusted on peptides and biological replicates) was performed, and p-values were adjusted with a Benjamini–Hochberg FDR. Protein with at least two total peptides across three biological replicates, a 1.4-fold enrichment and an adjusted pvalue ≤ 0.05 were considered significantly enriched in sample comparison.

Analysis of EV-induced signalization pathways

NFκB p65 subunit translocation in BM-MSC was evaluated in immunofluorescence by confocal microscopy on a SP5 (Leica Microsystems) after 1 to 24 hours of stimulation by TNF/LT or FL-derived EVs. After 15 min of methanol fixation and permeabilization at -20°C cells were incubated with PBS 2 % Bovine Serum Albumine (BSA; Sigma Aldrich) and 2 % Donkey Serum (DS; SigmaAldrich) for at least 1 hour at Room Temperature (RT). Then cells were stained by anti-NFκB p65 subunit (1/400, Cell Signaling) for 3 hours at RT and, after wash, with secondary donkey anti-rabbit IgG (1/500, Jackson ImmunoResearch) for 1 hour at RT. Final nuclei were conterstained with Sytox blue (1/1000, Invitrogen).

Activation of the canonical TGF β SMAD2/3-SMAD4 pathway was evaluated using a sensor cell line. Briefly, the liver cancer cell line Hep3B (ATCC[®] HB-8064TM) was transfected by using a SMAD2/3-SMAD4 GFP reporter lentivirus expressing GFP under the control of a minimal (m)CMV promoter and tandem repeats of the SMAD transcriptional response element (TRE) (Cellomics Technology) according to the manufacturer's instructions. Cells were transduced with lentiviral particles at Multiplicity Of Infection egal 5 in 8 µg/mL of SureENTRY transduction reagent (QIAGEN) and selected with 6µg/mL puromycin for 5 days. Stable TGF- β sensor cells were thereafter maintained in the presence of 0.5 µg/mL puromycin. Overnight stimulation of these cells was performed using either 10 ng/mL of TGF- β 1 (R&D) or EVs from RL cell line or pooled EVs from tonsil B cells. TGFBR1 inhibitor Galunisertib (R&D) was used à 10 nM for inhibition of TGF- β signaling.

Phosphorylation of SMADs, p38, and STATs was evaluated by flow cytometry using A647 anti-SMAD2(pS465/pS467)/SMAD3(pS423/pS425), PE anti-p38-MAPKr(pT180/pY182), PB anti-pSTAT1(pY701), A647 anti-pSTAT3(pY705), PE anti-pSTAT5(pY694), and A488 anti-pSTAT6(pY641) (BD Biosciences). Staining were

performed after fixation by the Cytofix/CytoPerm kit (BD Biosciences) and an additional permeabilization by -20°C pre-cooled 100% methanol.

Analysis of Affymetrix microarrays

The probe level intensities were background adjusted and quantile normalized using the Robust Multiarray Analysis (RMA) method^{8,9}. Probeset annotations were obtained from R packages AnnotationDbi¹⁰ (v1.44.0) and hgu133plus2¹¹ (v3.2.3). When multiple probesets mapped to the same Entrez Gene ID, the probeset with the highest score form R package Jetset¹² (v3.4.0) was selected. Filtering was performed by exclusion of probesets with intensity below the background threshold and/or without a gene symbol assignment. We evaluated differences in RNA expression by the empirical Bayes method¹³ from the limma R/Bioconductor package¹⁴ (v3.38.3). For the comparison of MSC subsets, significant genes were defined as P<.01 after adjustment for false discovery correction. In the same way, for the comparison of HD BM untreated or cocultured with primary FL B cells significant genes were defined using an adjusted P<.01. Conversely BM FL B cells and LN FL B cells, significant genes were defined using a non-adjusted P<.01.

Transcription factor enrichment

Enrichment for transcription factor motifs in the promoter of EV- versus TNF/LTupregulated genes was evaluated with HOMER¹⁵ by using the command findMotifs.pl with default parameter and the addition of the -bg parameter for background correction with the list of all express genes. Enrichment for transcription factor targets within EV- and TNF/LT-upregulated genes was performed with Enrichr (<u>https://maayanlab.cloud/Enrichr/</u>) by interrogating the TRRUST Transfection Factor 2019 database and results were represented as a dot blot.

Weighted Gene Correlation network analysis (WGCNA)

We used the Weighted Gene Correlation network analysis (WGCNA) R package^{16,17} (v1.69) to identify modules of co-expressed genes among the 15 samples of FL B cells studied using Affymetrix microarrays. For this analysis, we retained the top 75% most variable genes. The network was constructed by Pearson correlation considering a soft threshold power(β) of 10 identified with the function Pick Soft Threshold to avoid the selection of an arbitrary cut-off. Then, a topological overlap

Imetric (TOM) matrix was derived from the gene expression matrix and the dissimilarity of TOM was calculated. Hierarchical clustering tree was created, and modules were generated by the dynamic tree cut method, with a minimum module size of 100 and a deepSplit of 2. Each module was marked by a color. To reduce the module numbers, module with a similarity of more than 75% were merged with the MergedModule function and only 17 modules were then used for subsequent analysis, ranging from 165 genes to 4,052 genes. The module eigengenes (ME), defined as the principal component of each module and summarizing the GEP of these modules, were then assigned to each cell type and visualized as a boxplot. To determine if coexpression modules were associated with LN or BM FL B cells, we performed Mann-Whitney test or t-test depending on the results of the Shapiro-Wilk normality test.

Geneset and biological pathway enrichment analysis

Genes were ranked according to the Wald or eBayes tests t statistic and Gene Set Enrichment Analysis (GSEA) were performed with the R package fgsea¹⁸ (v1.8.0). GSEA preRanked analysis was used to identify whether the genes upregulated in EV or TNF/LT signatures were associated with gene expression differences between FL BM-MSC and FL LSC or between invaded FL BM-MSC and non-invaded FL BM-MSC. Moreover, GSEA preRanked was used to explore MySigdb HALLMARK pathways and REACTOME pathway databases.

GO terms enrichment was computed with the topGO¹⁹ R package (v2.34.0), following the default algorithm and using the Fisher's exact test p.value to sort the enriched pathways. This analysis was performed on the 1,515 significant upregulated genes in EV-primed BM-MSC and on the genes belonging to the significant WGCNA modules.

Analysis of the similarities between BM-MSC GEP and mouse scRNAseq data

Processed single cell RNA-seq data from mouse bone marrow stromal cells were downloaded from the Institute cell Broad single portal (dataset 1. http://singlecell.broadinstitute.org)²⁰ and the BioTuring Single-cell Browser (dataset 2, http://bioturing.com)²¹. Endothelial cells (EC-sinusoidal, EC-arteriolar, and EC-arterial clusters) were discarded from dataset 1 in order to retain only mesenchymal cells. Cells from mice exposed to fluorouracil were discarded from dataset2 to retain only cells from mice at a steady state. Genes upregulated in human EV-primed or TNF/LT-primed BM-MSC RNAseq data were used as signatures for gene set scoring. Gene names were converted to their mouse equivalent with a home-made R script and the homologene package²² (v1.4.68). Signature scores were computed with the AddModuleScore function of the Seurat package²³ (v3.1.5). This function calculates for each individual cell the average expression of each gene signature, subtracted by the aggregated expression of control gene sets. All the analyzed genes are binned into 25 bins based on their averaged expression, and for each gene of the gene signature, 100 control genes are randomly selected from the same bin as the gene. For each gene signature, cell scores were visualized on t-SNE plots using the FeaturePlot function of the Seurat package.

Analysis of the interactome between FL B cells and stromal cells

Nichnet R package (v0.1.0) allows the prediction of ligand-target links between interacting cells by combining their expression data with prior knowledge on signaling and gene regulatory networks. We first restricted our lists of BM-MSC-specific ligands to those identified in the EV signature and the TNF/LT signature according to our DESeq2 analysis (FDR<.01). To infer receptor activity, we used as a target geneset the list of genes identified as upregulated in LN FL B cells or BM FL B cells compared to normal centrocytes by using the eBayes test followed by adjustment for false discovery by Bonferroni correction (FDR<.01). As a background, we considered genes that were not differentially expressed but had a Log2 expression>5.5. The best-upstream ligands were then filtered by the genes that fall within the genes upregulated in sender cells (EV or TNF/LT primed BM-MSC). Ligand-target interactions were then represented through a circos visualization (circlize²⁴ R package, v0.4.9) including all ligands associated with upregulated receptors in FL B cells with at least one interaction with regulatory potential score upper than the 90th percentile of all the interactions. Ligands and receptors were then color-tagged according to their cell of origin. Links between ligands and targets were drawn with a transparency scale proportional to ligand-receptors interaction weights. Width of sectors was determined as the sum of the regulatory potential scores.

Validation of differentially expressed genes by qPCR

To confirm the differential gene expression in BM-MSC, cDNA were obtained using the Fluidigm reverse transcription Master Mix and were then preamplified for 14 cycles in the presence of Pre-Amp Master Mix (Fluidigm) and a pool of Taqman assays targeting the genes of interest (Applied Biosystems). Gene expression was then measured with the TaqMan Gene Expression Master Mix (Applied Biosystems) on a 48.48 Dynamic Array IFC (Fluidigm). The relative quantification in gene expression was determined using the $2-\Delta\Delta$ CT method²⁵ with *EIF2B1* as housekeeping gene and untreated BM-MSC as a calibrator. In order to confirm the gene expression level in FL B cells, cDNA were generated using Superscript II reverse transcriptase (Invitrogen) and gene expression was measured using the TaqMan universal master mix and Taqman assays on a StepOnePlus instrument. *ABL1* and *HPRT1* were used as housekeeping genes and a mix of peripheral blood mononuclear cells (PBMC) as a calibrator.

Data availability

All the GEP data, obtained or used in this study, are available under the GEO accession numbers reported in the Supplemental Table S1. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) *via* the PRIDE²⁶ partner repository with the dataset reported in the Supplemental Table S1.

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SUPPLEMENTAL TABLES

Table S1_Summary of the GEO and PRIDE deposits for the expression and

proteomic data used in this study

View Excel file: Table S1_Online data deposits.xlsx

Table S2_Signature of the primed BM-MSC

Table S2A_Signature of EVs-primed BM-MSC including the 1218 genes specifically upregulated (red line) and the 1291 specifically downregulated (green line) genes compared with untreated BM-MSC (FDR<1%)

Table S2B_Signature of the TNF/LT-primed BM-MSC including the 1312 specifically upregulated (red line) and the 1083 specifically downregulated (green line) genes compared with untreated BM-MSC (FDR<1%)

View Excel file: Table S2_Signature of the primed BM-MSC.xlsx

Table S3_Genes differentially expressed in FL BM-MSC versus FL LN-LSC (FDR<0.01)

Red line indicates genes upregulated in FL BM-MSC (1050) and green line genes upregulated in FL-LSC (874)

View Excel file: Table S3_FL_BM-MSC vs FL_LN-LSC.xlsx

Table S4_1716 upregulated genes in FL B-cell primed BM-MSC compared with untreated BM-MSC (FDR<1% and Log2FC >0.80)

View Excel file: Table S4_ FL B cell primed BM_MSC upregulated genes.xlsx

Table S5_GSEA on primed BM-MSC

Table S5A_GSEA of the MSigDB Hallmark pathways on the t-score ranked comparison FL derived EVs primed BM-MSC versus untreated BM-MSC Red line indicates significant enriched pathways (FDR<5%) Table S5B_GSEA of the MSigDB Hallmark pathways on the t-score ranked comparison TNF/LT primed BM-MSC versus untreated BM-MSC Red line indicates significant enriched pathways (FDR<5%)

View Excel file: Table S5 GSEA on primed BM-MSC.xlsx

Table S6_381 overexpressed proteins in EVs from FL B cell lines compared with EVs from tonsils B cells (FDR<5% and ratio > 1.4) Table S6_EV proteomic data.xlsx

Table S7_Genes differentially expressed in BM FL B cells versus LN FL B cells (FDR<1%)

Red line indicates genes upregulated in BM FL B cells (250) and green line genes upregulated in LN FL B cells (345)

Table S7_BM_FL B vs LN_FL B.xlsx

Table S8_ Genes differentially expressed in FL B cell versus HD CC

Table S8A_Genes differentially expressed in BM_FL_B cell versus HD _CC (FDR<0.01)

Red line indicates genes upregulated in BM FL B cells (879) and green line genes downregulated in BM FL B cells (1622)

Table S8B_Genes differentially expressed in LN_FL_B cell versus HD _CC (FDR<0.01)

Red line indicates genes upregulated in LN_FL B cells (1194) and green line genes downregulated in LN_FL B cells (2250)

Table S8_FL vs CC.xlsx

SUPPLEMENTAL FIGURES



Supplemental Figure 1: Analysis of EV protein content by Western Blot

Purified FL-derived vesicles contained conventional EV markers, including the endosome-associated protein TSG101 and the tetraspanin CD81 in the absence of the contamination markers Golgin and Calnexin.





Supplemental Figure 2: Uptake of FL-derived EVs by BM-MSC

A) Evaluation by confocal microscopy of the uptake by BM-MSC of 5µM CellTrace Violet-dyed EVs produced by malignant B cells (Violet). Nuclei were stained with SytoxGreen (Green) and plasma membrane with CellMask Red (red).

B) Flow cytometry analysis of the 6-hour uptake of CellTrace Far Red-dyed FLderived EVs versus shame-dyed FL-derived EVs by BM-MSC, as evaluated by the mean fluorescence intensity (MFI). Data are expressed as the mean of 3 independent experiments +/- SD. * P<.05.



Supplemental Figure 3: TNF/LT-primed and FL B-cell-primed BM-MSC signatures were related to FL invaded BM-MSC phenotype

A) Principal component analysis based on the 2389 differentially expressed genes between HD_BM-MSC and FL_BM-MSC (FDR <1%) highlighting invaded FL BM-MSC (FL_I BM-MSC) and non-invaded FL BM-MSC (FL_NI BM-MSC). **B**) GSEA enrichment score for the TNF/LT signature (1312 upregulated genes) between FL_I BM-MSC and FL_NI BM-MSC. **C**) GSEA enrichment score for the 1716 genes upregulated in BM-MSC primed by coculture with primary FL B cells between FL BM-MSC and HD BM-MSC. **D**) Venn diagram representing the number of genes upregulated in BM-MSC by FL-derived EVs *versus* primary FL B cells. **E-F**) GSEA enrichment score for the specific FL B-cell primed signature (1,551 upregulated

genes) between FL BM-MSC and FL LN -MSC (E) and between FL_I BM-MSC and FL_NI BM-MSC (F).



Supplemental Figure 4. Study of the TNF/LT signature using GSEA on REACTOME pathways



Supplemental Figure 5: Osteoblastic differentiation of EV primed BM-MSC Functional osteoblastic differentiation of 3 HD BM-MSC evaluated by fluorescent mineralization assay after 17 days of induction with osteogenic induction medium or EVs form FL B-cell lines. Results are expressed as fluorescence arbitrary unit (AU).



Supplemental Figure 6: Priming of BM-MSC by FL-derived EVs did not trigger NF-κB activation but induced STAT6 phosphorylation

A) BM-MSC were treated by TNF/LT or FL-derived EVs for 24 hours before fixation and permeabilization by methanol and staining for NF- κ B p65 subunit (green). Nuclei were conterstained with Sytox blue. Scale bar, 100 μ m; **B**) Evaluation of STAT6, STAT3, STAT5, and STAT1 phosphorylation after 24 hours stimulation by EVs from FL B-cell lines. Shown is on experiment representative of 3.



Supplemental Figure 7: WGCNA of FL B cells purified from LN and BM

A) Gene clustering dendrogram and module delimitation based on topological overlap, with different colors representing different coexpression gene modules. **B**) The relationship between the module eigengenes and the traits (BM_FL *vs* LN_FL) was then represented as boxplots. * P<.05



Supplemental Figure 8: Induction of CD40 and IL-4 pathways in BM-MSC by FLderived EVs

Expression of *STAT6, CD40*, and *IL4R* was evaluated by qPCR in BM-MSC treated by EVs purified from BM plasma obtained from HD (n=6) versus FL patients (n=13). Results expressed as fold change of untreated BM-MSC. * P<.05, ** P<.01