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Supplemental Figures and Tables

Figure S1. CLEC14A is an ECM protein and binds to MMRN2

(A) Domain structure of the 4 members of the endosialin family, endosialin/TEM1, C1qR/CD93, thrombomodulin/CD141 and CLEC14A/EGFR5.

(B) Confocal images of HUVECs plated on Matrigel and then stained for CLEC14A and FN1. Nuclei are stained with DAPI (blue). Region of colocalization are indicated with arrowhead (overlay). In the lower right panel, the white spots indicate the overlay of CLEC14A and FN1, according to the pixel intensity (last panel, Imaris software).

(C) CLEC14A immunoprecipitation performed on lysate of HUVEC cultured in M199 complete and grown at confluence. Sheep IgG have been used as control. Lys tot = total lysate, 15 μg. Depl = depleted lysate, after IP with anti-CLEC14A antibody or IgG. Immunoblot analysis was performed for CLEC14A and MMRN2.

Figure S2. CLEC14A and MMRN2 are required in EC morphogenesis

(A) Immunoblot analysis for CLEC14A and MMRN2 of the total cell lysate (RIPA modified buffer, see above) of HUVECs used for the matrigel assay (Figure 5A) showing the efficient silencing with two independent siRNA. β-tubulin was used as loading control. The different panels are grouped images from different parts of the same gel.

(B) Representative phase contrast pictures of the matrigel assay (Figure 5A) of HUVECs transfected with control pool siRNA (siCtl) or siRNA for CLEC14A and MMRN2. Scale bar = 200 μm.

(C) Immunoblot analysis for CLEC14A and MMRN2 of the total cell lysate of HUVECs used for the 3D tubulogenesis (Figure 5B) assay, showing the efficient silencing with two independent siRNA. β-tubulin was used as loading control.

(D) Representative phase contrast pictures of the 3D assay tubulogenesis (Figure 5B) of HUVECs transfected with control pool siRNA (siCtl) or siRNA for CLEC14A and MMRN2.

(E) Immunohistochemistry for CLEC14A and the EC marker PECAM1 of consecutive sections of a panel of human tumors. The bottom right panels show positive staining for CLEC14A and MMRN2 in artery and vein of normal mammary tissue adjacent to the tumor. Ar=artery; V=vein.

Figure S3. Clec14a co-localizes with the matrixome proteins Mmrn2 and Lama4 in tumor vessels of Rip-Tag2 and HPV16/E_2 mice

(A) Confocal images of pancreatic tumor islet of RIP-Tag2 mice. Sections were stained with Clec14a and Mmrn2 or Lama4. Nuclei are stained with DAPI (blue)

(B) Confocal images of invasive cervical carcinoma of HPV16/E_2 mice. Sections were stained with Clec14a and Meca32, Clec14a and Mmrn2, or Clec14a and Lama4. Nuclei are stained with DAPI (blue)

Table S1. Legend column header

Explanation of the column content of the output file (identified proteins) obtained from MaxQuant analysis and contained in Tables S2,S7.

Table S2. Proteins identified, quantified and significantly regulated during HUVECs morphogenesis and spreading

"Identified proteins" sheet. Proteins identified in the MS analysis of HUVECs morphogenesis and spreading. L/H indicates the ratio Sample/SILAC. _1/2/3 indicates the three independent experiments. NaN = not quantified.

"Quantified proteins" sheet. List of the identified proteins that have been quantified with maximum one missing value per experimental condition in the three MS measurements. The column Sample/0h reports the median change in protein expression levels calculated as ratio between the median "Ratio L/H normalized" (as in "identified proteins" sheet) and the median "Ratio L/H normalized 0h" (as in "identified proteins" sheet). Proteins that passed the ANOVA test (Materials and Methods) are indicated in the column "ANOVA significant".

Detailed explanation of the column headers is reported in Table S1.

Table S3. Normalized ratio correlation between replicates

Expression profile correlation between independent experiments was calculated by mean of the Spearman rank correlation. For this purpose, the 1,401 SILAC ratios (Sample/SILAC) of the proteins that passed the ANOVA test (Table S2) were considered. In yellow are indicated the values used to calculate the average reproducibility between independent experiments as reported in the Results section.

Table S4. Quantified proteins significantly up and downregulated in HUVECs morphogenesis and spreading

This table reports the significantly up and downregulated proteins shown in Figure 2. These are proteins that passed ANOVA test and with a minimum effect size of 1.5 fold change, which is more than 1 standard deviation (SD) from the average of the all calculated ratios in each of the morphogenesis and spreading conditions (Sample/0h average $\text{ratio}_{1,401\text{prot}}$=0.01; SD=0.534).

Table S5. KEGG pathways regulated in EC morphogenesis and spreading

“One-dimensional analysis” sheet. Contains the list of the significantly regulated KEGG categories (FDR 5%, Supplemental Material and Methods) in the different experimental conditions of EC morphogenesis and spreading, with associated the median regulation levels and the statistical significance. Median values were used for the heat map in Figure 3A.

“Protein lists” sheet. Provide the detailed list of the proteins (among the 1,401 that passed the ANOVA test) belonging to the KEGG categories reported in the “One-dimensional analysis” sheet and that were highlighted in the Results and Discussion sections of the main manuscript.

Table S6. ECM proteome and “matrixome” of HUVECs in culture

Proteins identified by LC MS/MS in three independent replicates of ECM produced and isolated from HUVECs grown in culture. The column “LFQ Median/Total Sum (%)” reports the estimated percentage of each protein in the total ECM mass analyzed. LFQ median is the median of the three replicates, Exp 1-3. Total Sum is the sum of all LFQ median (=1.190 E11). LFQ intensity = Label Free Quantification intensity as reported in [1]. Column headers are according to Table S1.

Table S7. HUVEC matrixome proteins regulated in EC morphogenesis and spreading

List of the 69 proteins identified as matrixome proteins and significantly regulated (which passed the ANOVA test, see Material and Methods) during EC morphogenesis and spreading. For each protein, the ratios (according to Table S2), and “LFQ Median Intensity” (according to Table S6) are reported and shown in Figure 4B.

Table S8. Enriched proteins in MS-based analysis of CLEC14A immunoprecipitates

List of the 53 proteins identified and measured enriched in CLEC14A MS-based immunoprecipation of HUVECs grown over-night on matrigel, and which were quantified in the MS proteomic analysis of EC morphogenesis and spreading (significantly regulated proteins are indicated in the column “Significantly upregulated in Matr 30h”) (Figure 4D). The enrichment was calculated as ratio of the LFQ intensity

between CLEC14A and IgG immunoprecipitates and required to be a minimum of 2 fold (CLEC14A/IgG > 1, in $\log_2$ scale) in the two experiments. LFQ intensity = Label Free Quantification intensity as reported in [1].

Table S9. Annotated spectra proteins identified with single peptide (Table S2) and CLEC14A phosphorylation site

Reference

1. Luber, C.A., Cox, J., Lauterbach, H., Fancke, B., Selbach, M., Tschopp, J., Akira, S., Wiegand, M., Hochrein, H., O'Keeffe, M., et al. (2010). Quantitative proteomics reveals subset-specific viral recognition in dendritic cells. Immunity *32*, 279-289.