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Supplementary Materials for

Immune synapse instructs epigenomic and transcriptomic functional reprogramming in dendritic cells

Ana Alcaraz-Serna, Eugenio Bustos-Morán, Irene Fernández-Delgado, Diego Calzada-Fraile, Daniel Torralba, Ester Marina-Zárate, Erika Lorenzo-Vivas, Enrique Vázquez, Juliana Barreto de Alburquerque, Nora Ruef, Manuel José Gómez, Fátima Sánchez-Cabo, Ana Dopazo, Jens V. Stein, Almudena Ramiro, Francisco Sánchez-Madrid*

*Corresponding author. Email: fsmadrid@salud.madrid.org

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

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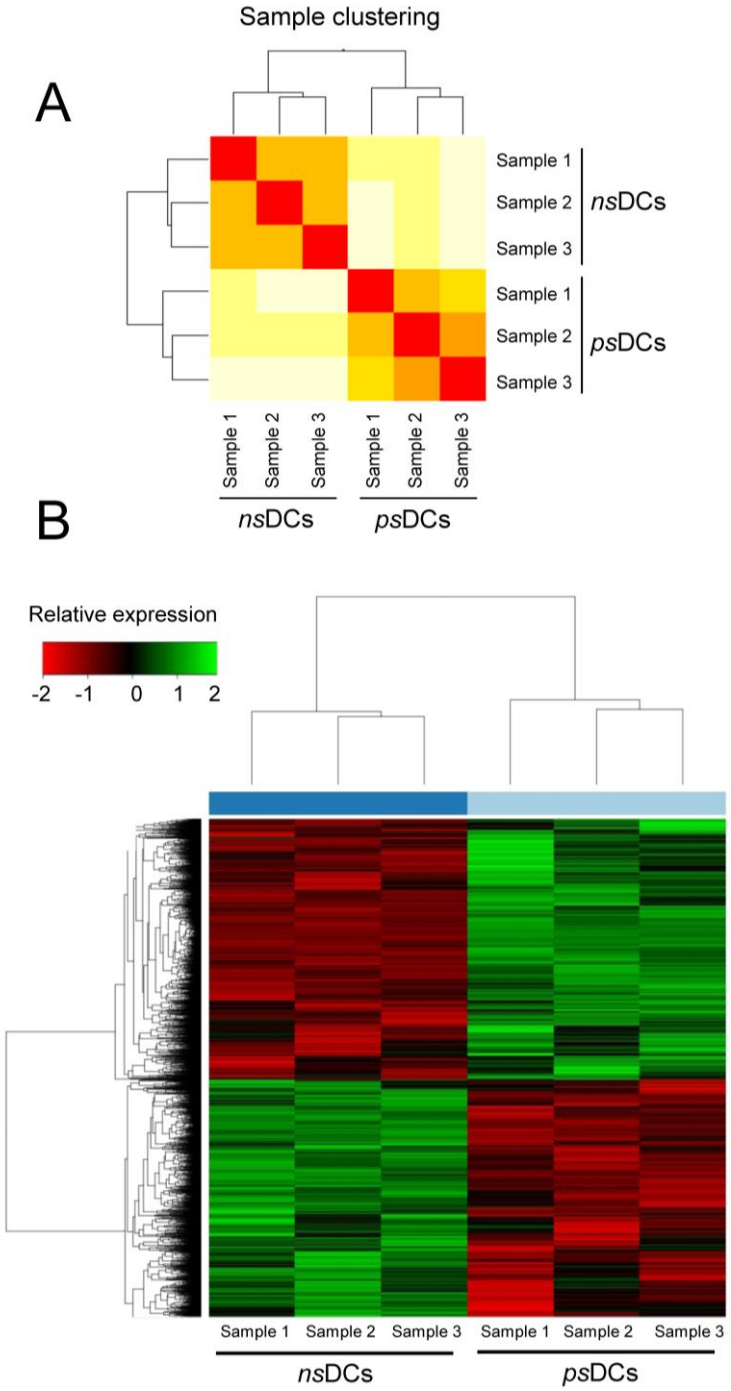
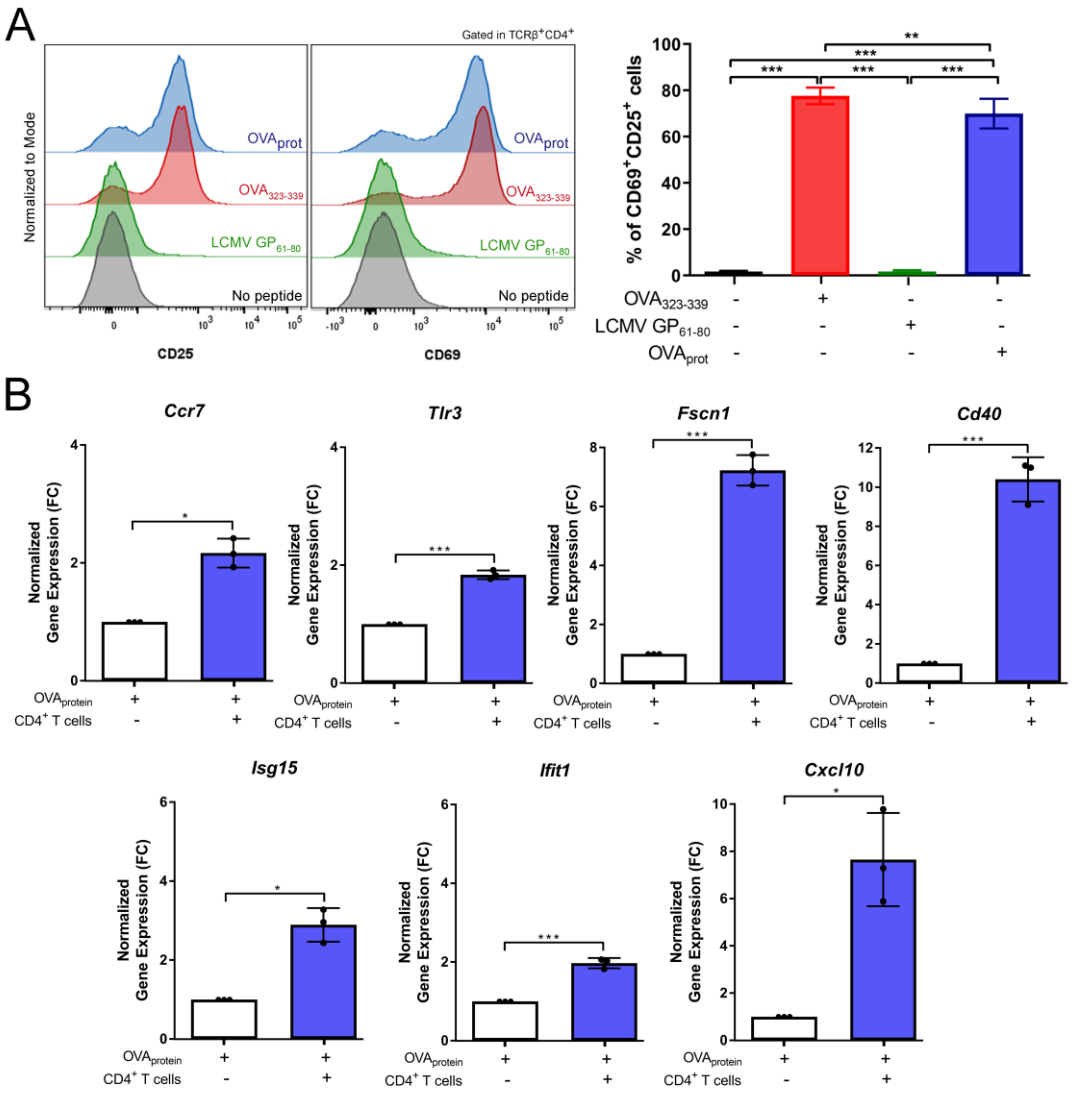
Figure S1



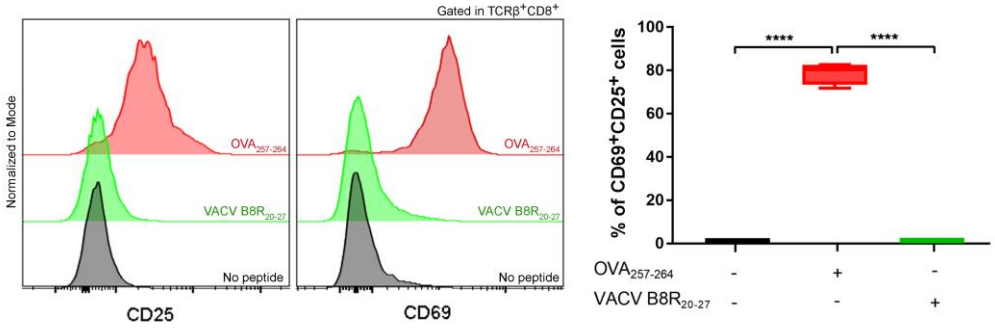
Supplementary Figure 1. Gene expression clustering reveals robustness of *psDC* RNA-Seq data. (A) Hierarchical clustering and heatmap of RNA-Seq samples, based on normalized counts for 12,005 expressed genes. n = 3. Red to white colours represent increasing euclidean distance. **(B)** Two-way hierarchical clustering and gene heatmap for differentially expressed genes in *ps*DCs versus *ns*Dcs (B-H adjusted p-value < 0.05, n =3). The colour scale represents normalized expression values.

Figure S2



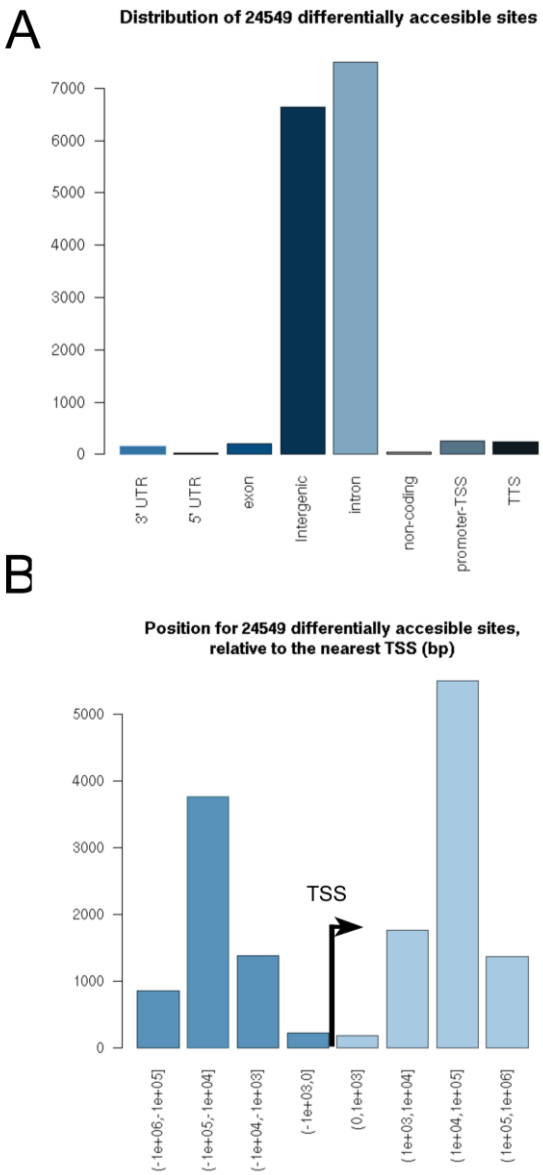
Supplementary Figure 2. CD4+ T cell activation and gene expression of *psDC* upon immune synapse in response to whole OVA protein. (A) (*Left panel*) Representative histograms for CD25 and CD69 expression from CD4+ T cells after 24h of coculture with DCs under the presence or absence of $OVA_{323-339}$, LCMV GP_{61-80} peptides or OVA protein. Gated in $TCR\beta^{+}CD4^{+}$ cells. **(*Right panel*)** Percentage of $CD25^{+}CD69^{+}$ cells gated in $TCR\beta^{+}CD4^{+}$ cells ($n \geq 3$; One-way Anova test and Tukey's post-test; ** < 0.01; *** < 0.001) **(B)** qPCR analysis of the expression levels of the genes indicated in DCs pulsed with OVA protein and in the absence or presence of CD4+ T cells (n=3; one-sample t-test; * < 0.05; *** < 0.001).

Figure S3



Supplementary Figure 3. CD8+ T cell activation upon immune synapse with cognate and control peptides. (*Left panel*) Representative histograms for CD25 and CD69 from CD8+ T co-cultured with DCs under the presence or absence of $OVA_{257-264}$ or VACV $B8R_{20-27}$. Gated in $TCR\beta^+CD8^+$ cells. **(*Right panel*)** Percentage of $CD25^+CD69^+$ cells gated in $TCR\beta^+CD8^+$ (n=4; One-way Anova test and Tukey's post-test; **** < 0.0001).

Figure S4



Supplementary Figure 4. Distribution and position of differentially DNA accessible sites in *psDC* revealed by ATAC-Seq. (A) Histogram of the distribution of the differentially accessible sites (FDR < 0.05) in relation to already known DNA sequences as obtained with HOMER´s annotatePeak function (3´or 5´UTR, exon, intergenic, intron, non-coding, transcription starting site (TSS) or promoter-TSS). n = 2. **(B)** Histograms showing the position of the differentially accessible sites relative to the nearest TSS. Distances are described in pair bases (pb) and the arrow indicates the direction of TSS-downstream positioned sequences. n = 2.

Supplementary Video 1. CCL19-dependent chemotaxis of psDCs and nsDCs in an under-agarose assay. Time-lapse of the migratory movement of nsDCs (red) and psDCs (green) towards a CCL19 gradient (right side of the video). Tracks with a time-colored scale are shown in the image as tracked by Imaris. Scale bar of 30 µm.

Peptide name	Sequence	Molecular weight	Use
OVA(323-339)	ISQAVHAAHAEINEAGR	1773.9 g/mol	5 µg/mL
LCMV GP(61-80)	GLKGPDIYKGVYQFKSVEFD	2290.6 g/mol	10 µg/mL
OVA(257-264)	SIINFEKL	963.2 g/mol	1 µg/mL
VACV B8R(20-27)	TSYKFESV	960.1 g/mol	1 µg/mL

Supplementary Table S1. List of peptides with their corresponding sequence, concentration of use and molecular weight supplied by GenScript. Used to pulse DCs.

Gene	Application	Forward Sequence	Reverse Sequence
Cd40	qPCR	GACTCAGGCGAATTCTCAGC	GTCTCAGTGGCCATCTCCAT
Ccr7	qPCR	CAGGAAAAACGTGCTGGTGGT	ACTCGTACAGGGTGTAGTCCA
Fscn1	qPCR	AACCCCTTGCCTTTCAAACT	CATGGAAAGAAGGGGACAGA
Tlr3	qPCR	AGGTTGACGCACCTGTTCTC	CCCTTTCATGATTCAGCCCAGA
Cxcl10	qPCR	CCAGTGCTGCCGTCATTTTC	GGCTCGCAGGGATGATTTC
Isg15	qPCR	CTAGAGCTAGAGCCTGCAG	AGTTAGTCACGGACACCAG
Ifit1	qPCR	CAAGGCAGGTTTCTGAGGAG	GACCTGGTCACCATCAGCAT
Ywhaz	qPCR	CGTTGTAGGAGCCCGTAGGTCAT	TCTGGTTGCGAAGCATTGGG
M2B	qPCR	TTCTGGTGCTTGTCTCACTGA	CAGTATGTTCGGCTTCCCATTC
Ccr7	ChIP-qPCR	TGGTGAGCGGAACTCTAGGA	GCTCCAGACACCCCAGTTAC
Fscn1	ChIP-qPCR	ACTGCCGCTTTCTCGTCG	CTCGGTGCCGCCAAAGTAG
Cd40	ChIP-qPCR	CTTCCAGAAGGTCGGGGTTC	GGGTCAGACAAAGCTGTCCA
Gapdh	ChIP-qPCR	TCATCCACCTCCCCACAGTA	TGTGAACGGGTGAGTTCCAG

Supplementary Table S2. List of primers, with their corresponding sequence, used for qPCR and ChIP-qPCR**.**