#136 Genomic profiles of CD47 in breast tumors predict outcome and are associated with immune activation

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BACKGROUND

Targeting the innate immune system has attracted attention with the development of antibodies against CD47, an immune checkpoint for macrophage-mediated phagocytosis. Anti-CD47 antibodies block the inhibition of the phagocytic activity of macrophages caused by the up-regulation of CD47 expression on tumor cells.

AIM

In this study we aimed to identifying genomic correlates associated with the expression of CD47 in breast cancer to get insights into the immunologic characteristics of those tumors.
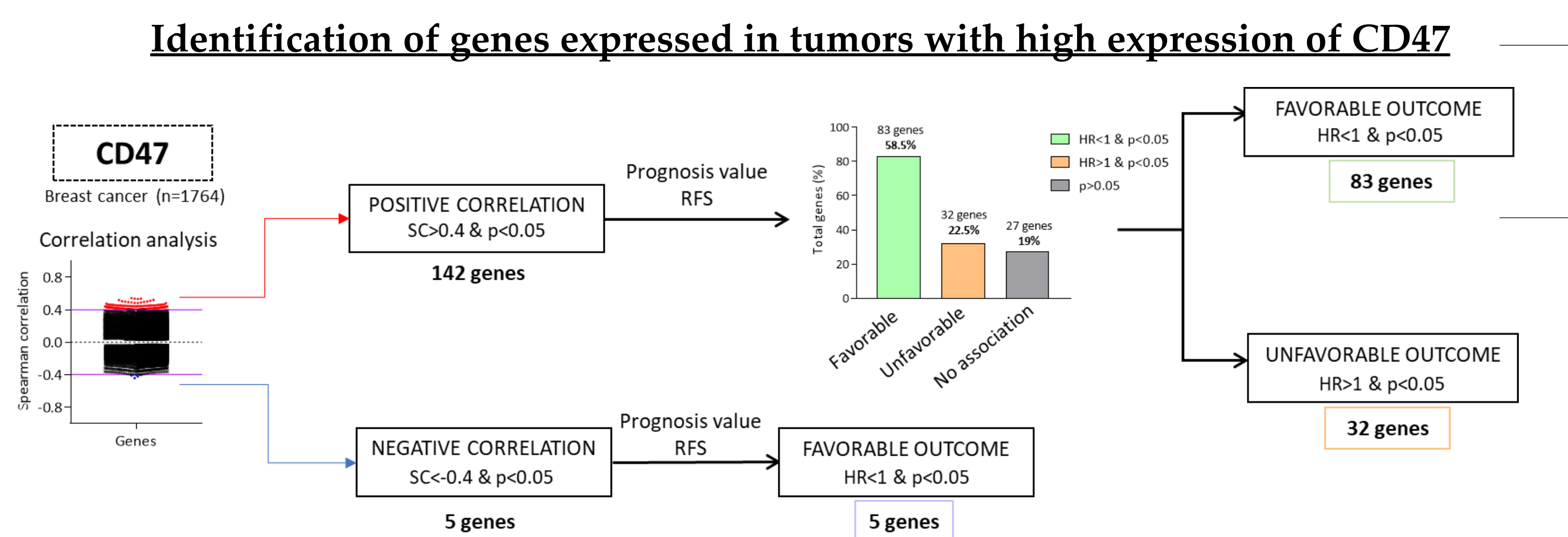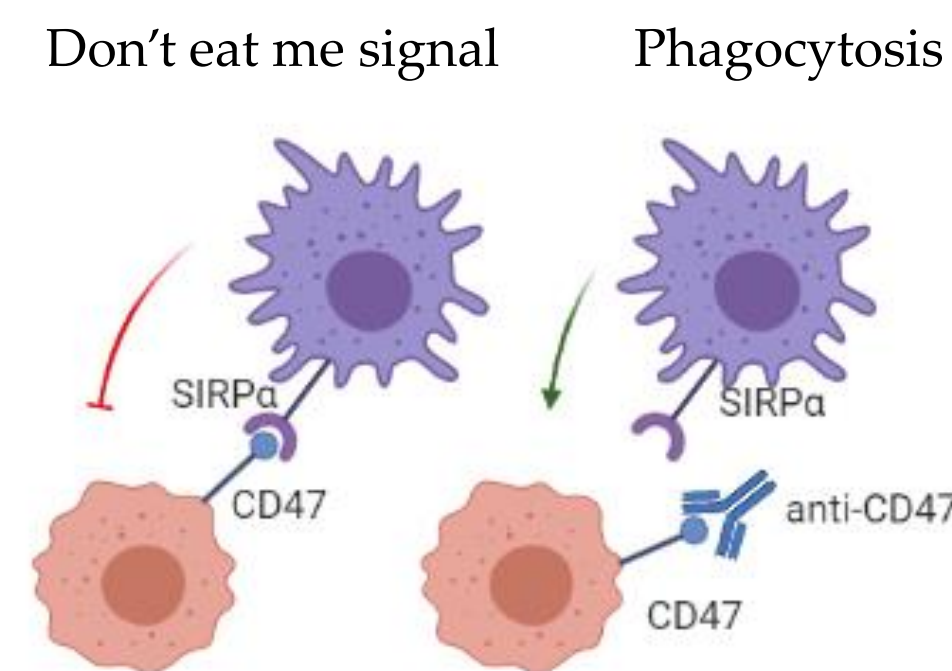
MATERIAL AND METHODS

Identification of genes expressed in tumors with high expression of CD47

Figure 1. Flow chart of the correlation and outcome study for genes selection, describing selection criteria used.

Bioinformatic analysis

A Functional analysis	B Outcome analysis	C Infiltration association
EnrichR	KMplotter	TIMER

D Antigen and T cell markers association	E Immune signatures association	F Macrophages association
CANCERTOOL	Reference section [1-4]	GSEA

RESULTS

A — CD47-immune signature identification

SC>0.4 & (HR<1; p<0.05)

→ PTGER4, ETS1, OPTN
→ PTPRC, HLA-E, TGFBR2
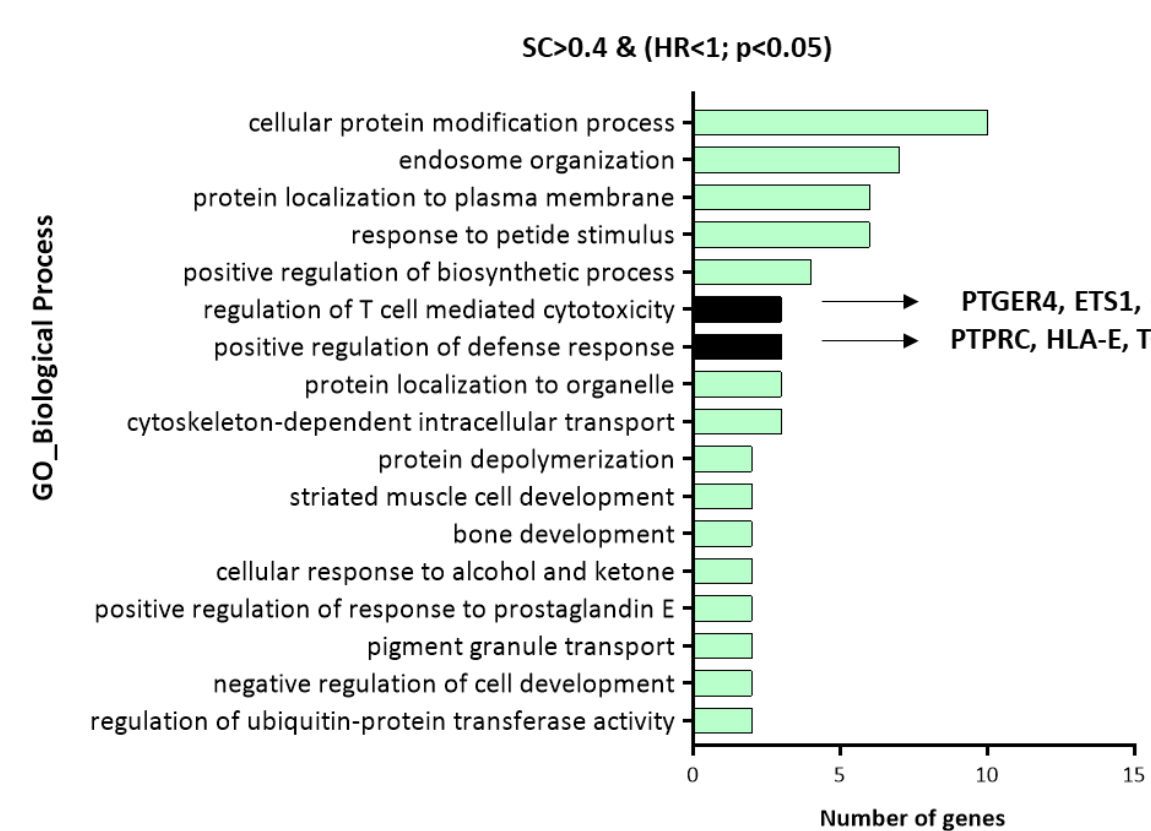
Gene	Protein	Principal function
PTGER4	Prostaglandin E2 receptor EP4 subtype	Regulates renal hemodynamics, intestinal epithelial transport, adrenal aldosterone secretion, and uterine function
ETS1	Protein C-ets-1	Controls the expression of cytokine and chemokine genes in a wide variety of different cellular contexts
PTPRC	Receptor-type tyrosine-protein phosphatase C (CD45)	Acts as a positive regulator of T-cell coactivation upon binding to DPP4
HLA-E	HLA class I histocompatibility antigen, alpha chain E	Binds nonamer self-peptides derived from the signal sequence of classical MHC class Ia molecules (VL9 peptides)
TGFBR2	TGF-beta receptor type-2	Transduces the TGFB1, TGFB2 and TGFB3 signal from the cell surface to the cytoplasm and is thus regulating a plethora of physiological and pathological processes
OPTN	Optineurin	Plays a role in the activation of innate immune response during viral infection

Figure 2. CD47-immune signature identification. Functional analyses of the selected genes (SC>0.4, HR>1 and p<0.05). Table depicting the genes associated with the enriched biological processes "regulation of T cell mediated cytotoxicity" and "positive regulation of defense response".

B — CD47-immune signature is associated with favourable prognosis in breast cancer, especially in Basal-like and HER2+ subtypes
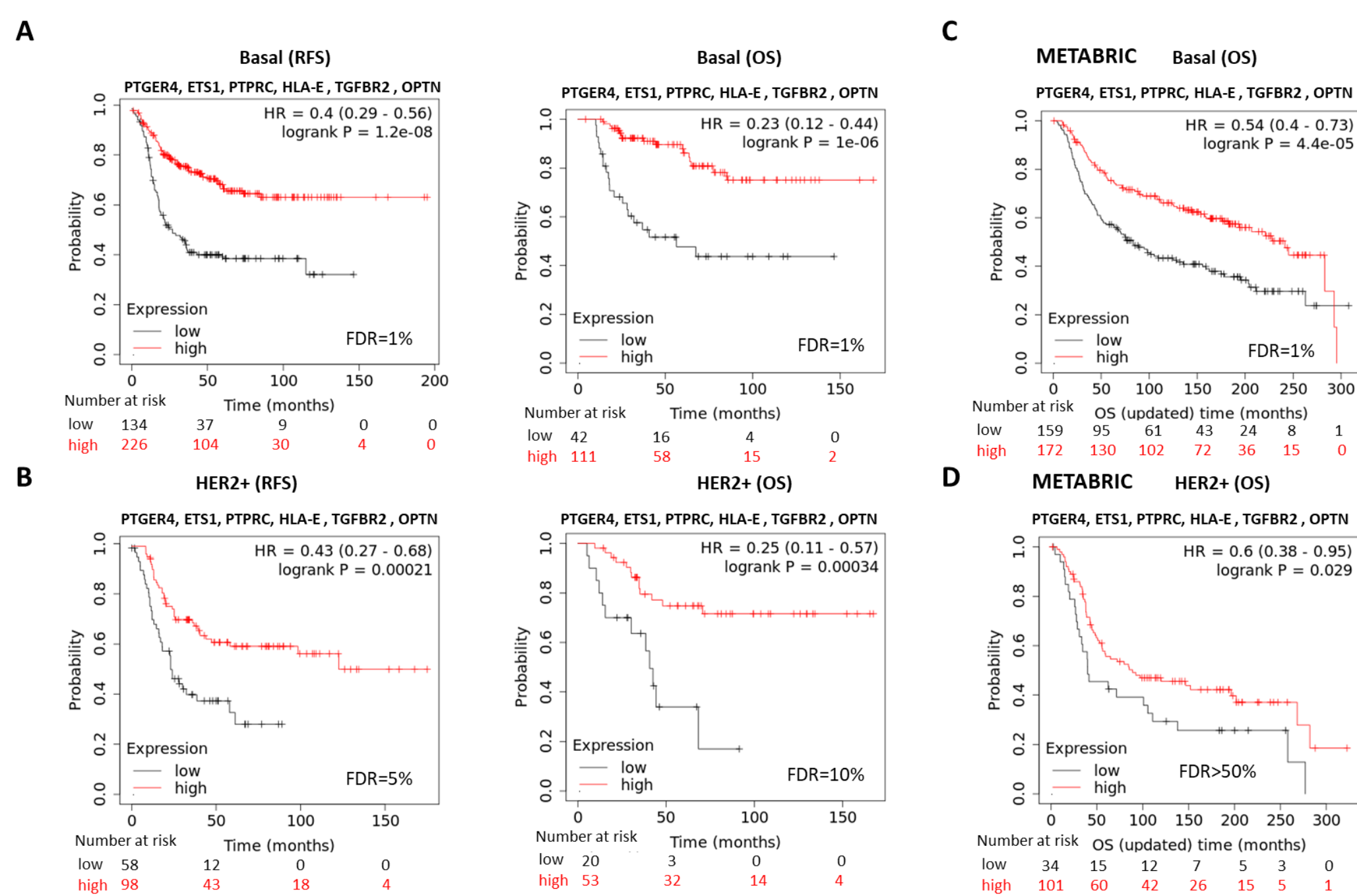

Figure 3. Transcriptomic expression of PTGER4, ETS1, PTPRC, HLA-E, TGFBR2, and OPTN and association with clinical outcome in Basal-like and HER2+ breast cancer patients in breast tumors in the exploratory cohort (A, B). and in the validation cohort (METABIRC project) (C, D).

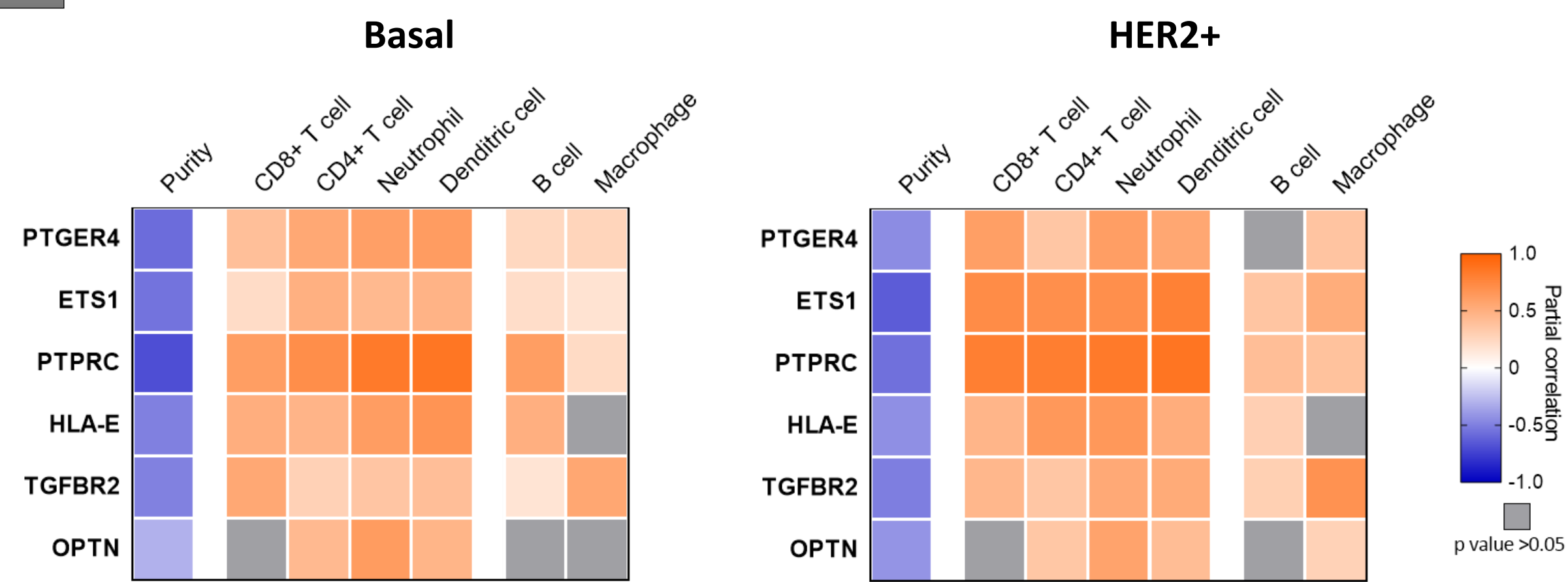### C — CD47-immune signature correlated with the presence of immune infiltrates

Figure 4. Association of the expression of the selected genes with immune infiltrates in breast cancer. Correlation analysis between gene expression and level of tumor immune infiltrates, and tumor purity in Basal-like, and HER2+ breast cancer tumors..

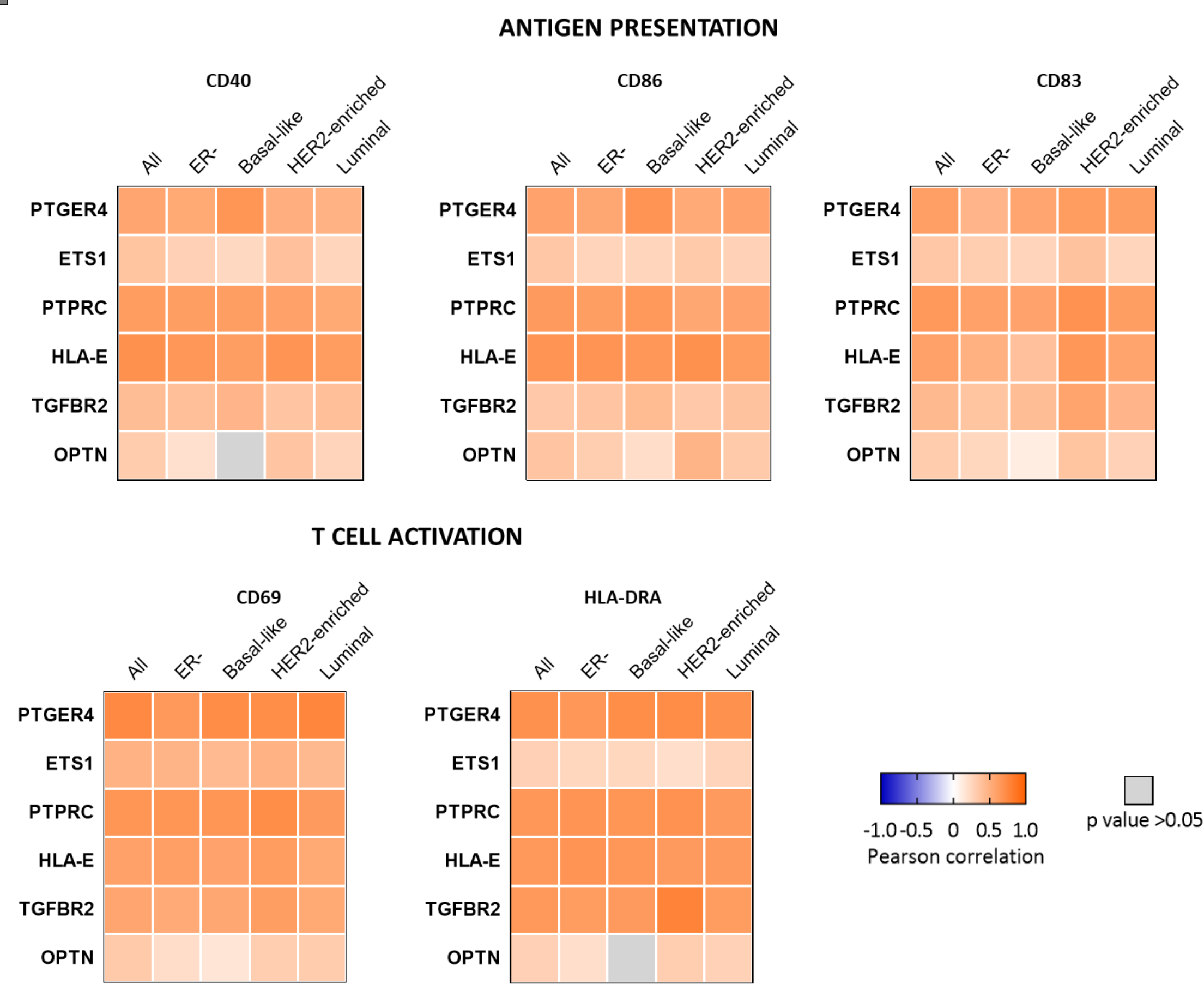### D — Association with markers of antigen presentation and T cell activation

Figure 5. Relationship between selected genes expression and immune activation markers. Relationship between markers of antigen presentation (CD40, CD83 and CD86) expression and T cell activation (CD69 and HLA-DRA) and the expression of the selected genes using CANCERTOOL in METABIC cohort.

E — CD47-immune signature correlated with gene signatures of T cell activation
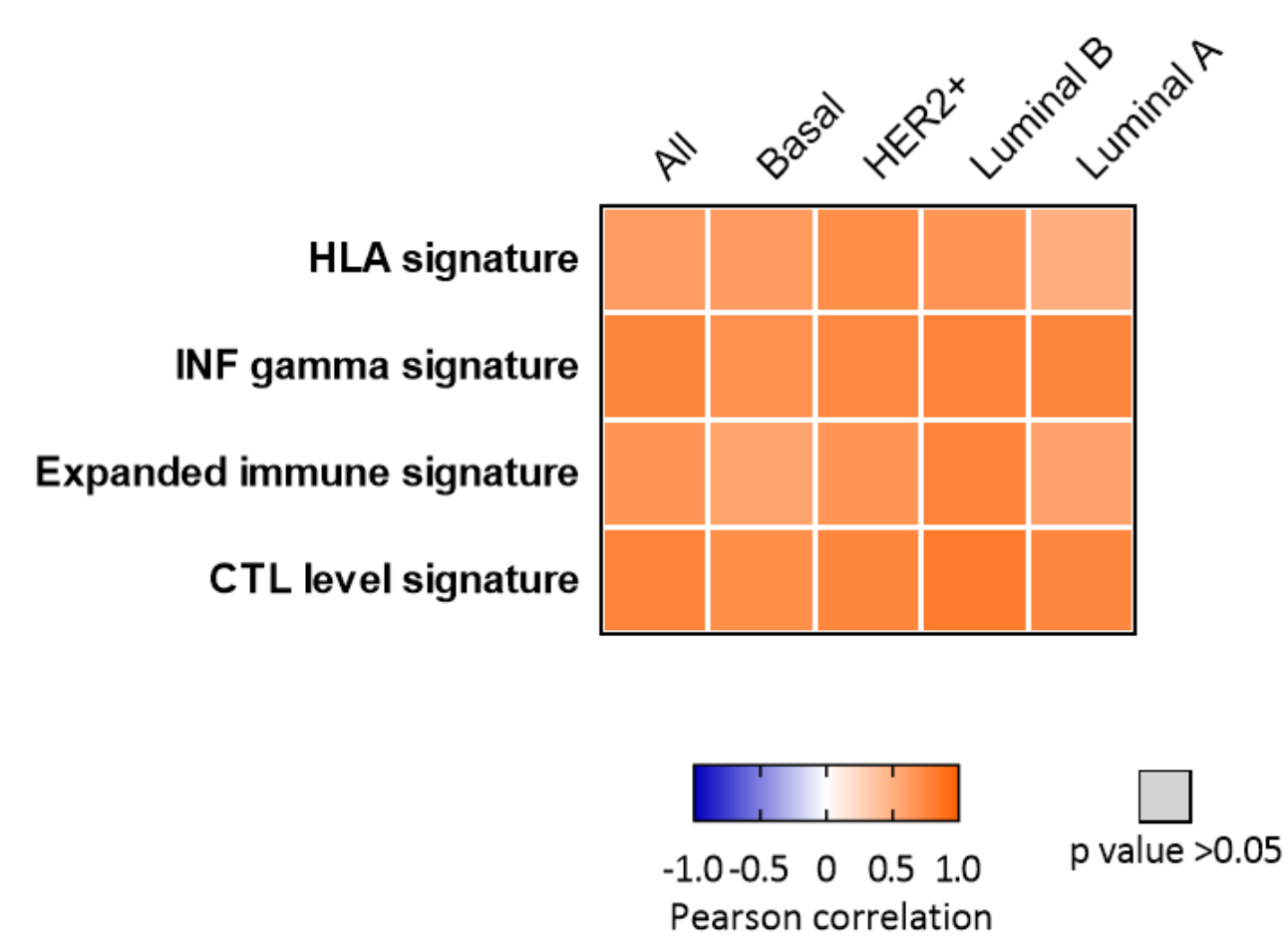

Figure 6. Relationship between selected genes expression and signatures of immune activation. Heat map of Pearson correlation coefficient (R) of the expression of the CD47-immune signature and the HLA signature, IFN gamma signature, expanded immune gene signature, and CTL level signature in all (n=1988), basal (n=334), HER2+ (n=137), Luminal B (n=680), and Luminal A (n=837) breast cancer.

F — Gene-set enrichment analysis (GSEA) confirm the association of the CD47-immune signature with pro-tumoral macrophages
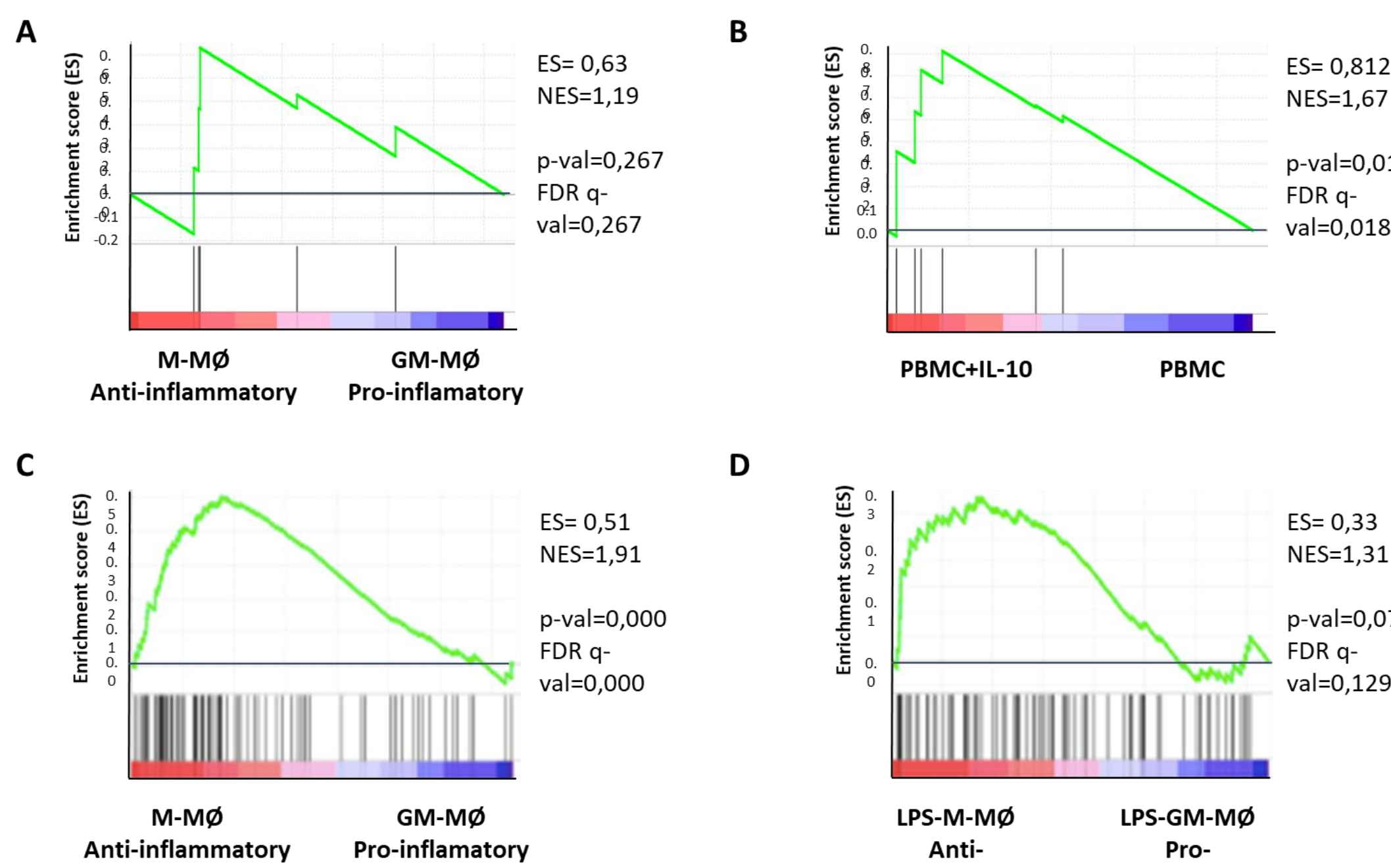

Figure 7. Gene-set enrichment analysis (GSEA) between gene expression and macrophage signatures. Gene-set enrichment analysis (GSEA) of the 6-gene CD47-immune signature on (A) the ranked comparison of M-MØ or M2 and GM-MØ or M1 whole transcriptomes or (B) the transcriptomes of adherent human peripheral blood mononuclear cells either untreated (PBMC) or treated with 10ng/ml IL-10 for 24h (PBMC+IL-10). GSEA of the genes that positively correlate with CD47 and are associated with good prognosis on (C) the ranked comparison of M-MØ or M2 and GM-MØ or M1 whole transcriptomes or (D) the ranked comparison of the transcriptome of LPS-treated M-MØ or M2 and LPS-treated GM-MØ or M1 transcriptomes.

CONCLUSIONS

- We described a transcriptomic immune signature formed by six gene (PTGER4, ETS1, PTPRC, HLA-E, TGFBR2, and OPTN) that is expressed in **breast tumors with high expression of CD47** and is **associated with favorable outcome**. The combination of these genes, predicted favorable prognosis in all breast tumors, and **particularly in the HER2+ and Basal-like subtype.**

- The described signature is linked with the **presence of T cells, dendritic cells and neutrophil infiltrates.** Although the strongest effect was observed in the Basal-like and HER2+ population, such association was also identified in **all of breast cancer patients.**

- A **strong positive correlation** was observed between the whole expression of the signature and that of described **markers of T cell activation and antigen presentation and signatures of T cell activation**. No increase in the **macrophage** population was detected, but those were **pro-tumoral.**

- It is relevant to **explore if this signature** could help identify patients that would **respond to anti-CD47 agents.**

INFO, REFERENCES AND ACKNOWLEDMENT

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References:
1. Noblejas-López M del M. et al. Expression of MHC class I, HLA-A and HLA-B identifies immune-activated breast tumors with favorable outcome. Oncoimmunology. Taylor & Francis; 2019;8:e1629780.
2. Jiang P, et al. Signatures of T cell dysfunction and exclusion predict cancer immunotherapy response. Nat Med. 2018;24:1550–8.
3. Ayers M, et al. IFN-γ-related mRNA profile predicts clinical response to PD-1 blockade. J Clin Invest. 2017;127:2930–40.
4. Rooney MS, et al. Molecular and Genetic Properties of Tumors Associated with Local Immune Cytolytic Activity. Cell. Elsevier Inc.; 2015;160:48–61.

Noblejas-López MDM et al. Genomic profiles of CD47 in breast tumors predict outcome and are associated with immune activation. Submitted.