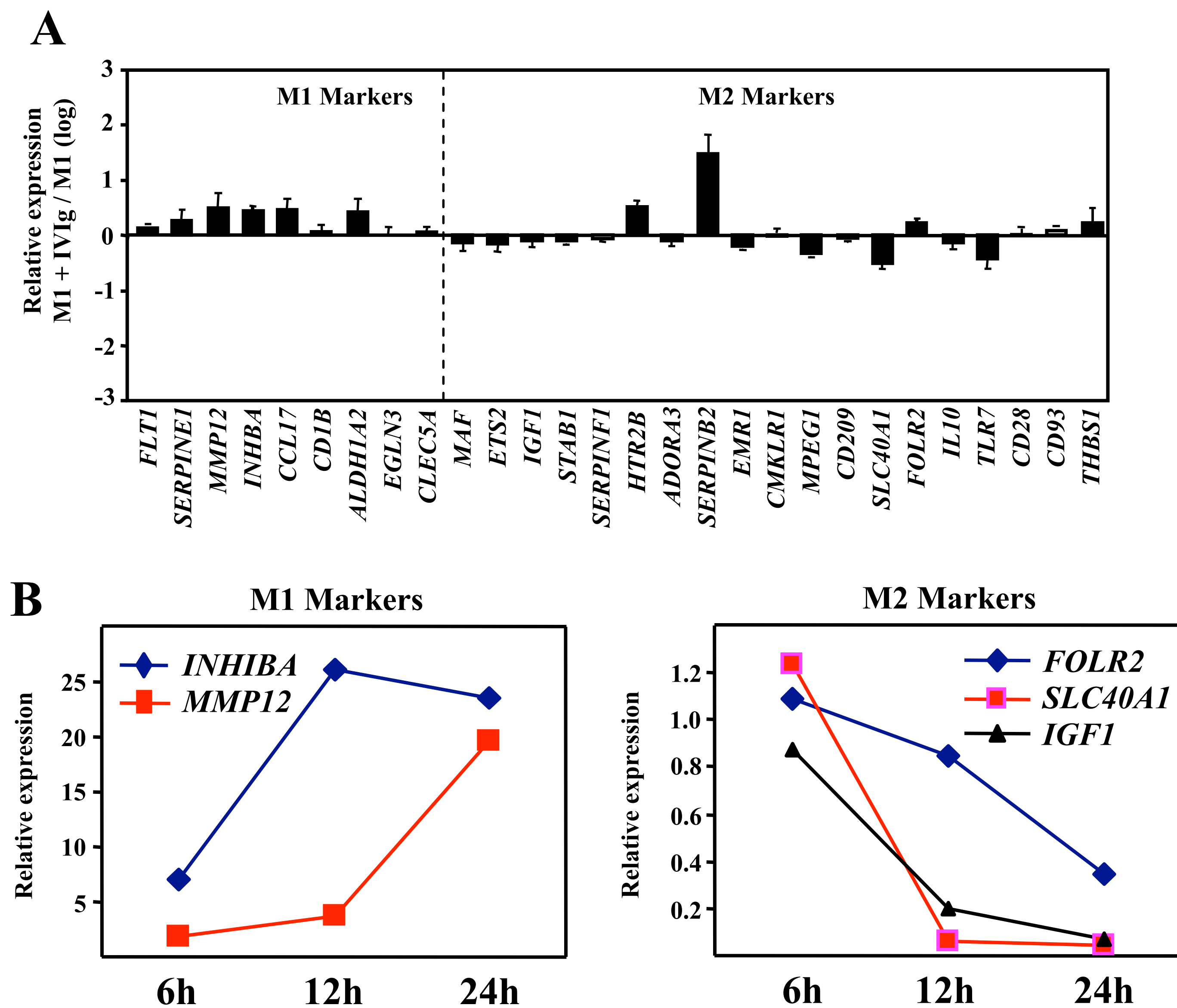
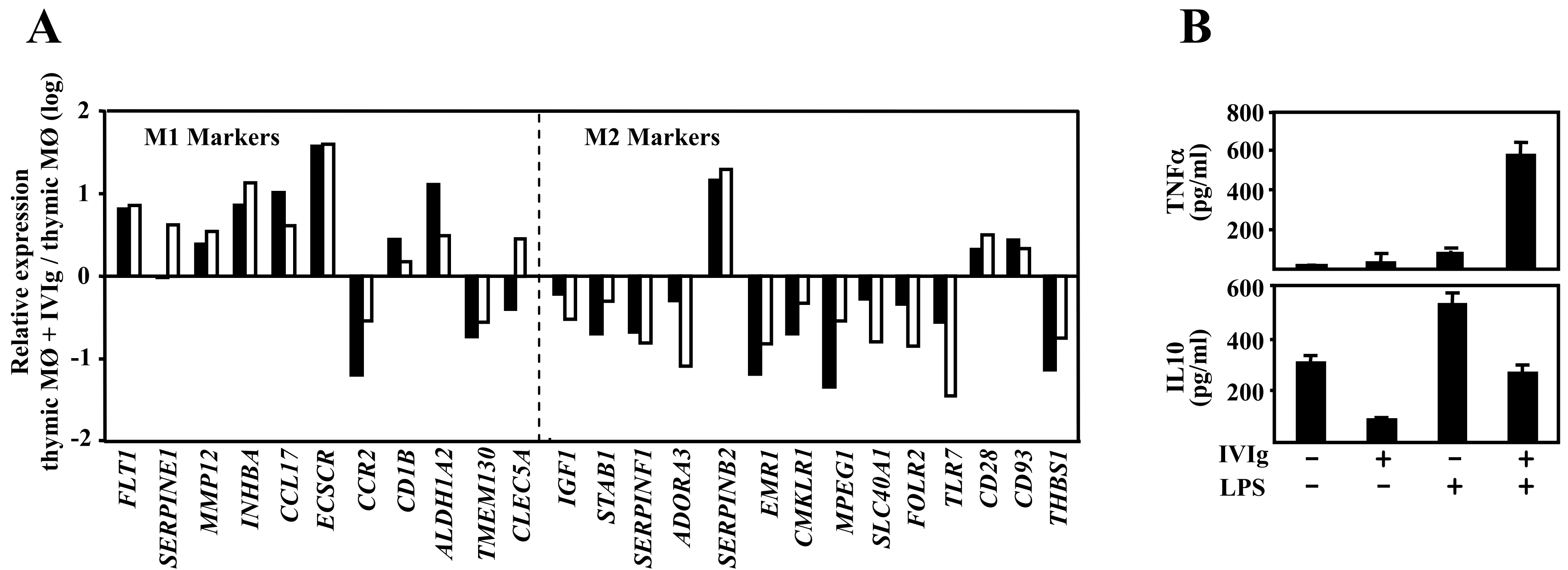
Supplementary Figure 1



Supplementary Figure 1. **Effect of IVIg on human M1 macrophage polarization (A), and kinetic analysis of representative M1- and M2-specific markers on IVIg-treated M2 macrophages (B).- (A)** Polarization marker expression in M1 macrophages exposed to IVIg (24 hours), as determined by qRT-PCR (n=2). Relative Expression (log scale) indicates the expression of each marker in the presence of IVIg relative to its expression in the absence of IVIg**. (B)** M2 macrophages were exposed to IVIg, and the expression level of the indicated M1 and M2 polarization markers was determined by qRT-PCR after 6, 12 or 24 hours. Results are expressed as Relative Expression, which indicates the expression of each marker in the presence of IVIg and relative to its expression in the absence of IVIg.

Downloaded from http://journals.aai.org/jimmunol/article-supplement/96830/pdf/ji_1303375_supplemental_figures_1/ by guest on 20 May 2025

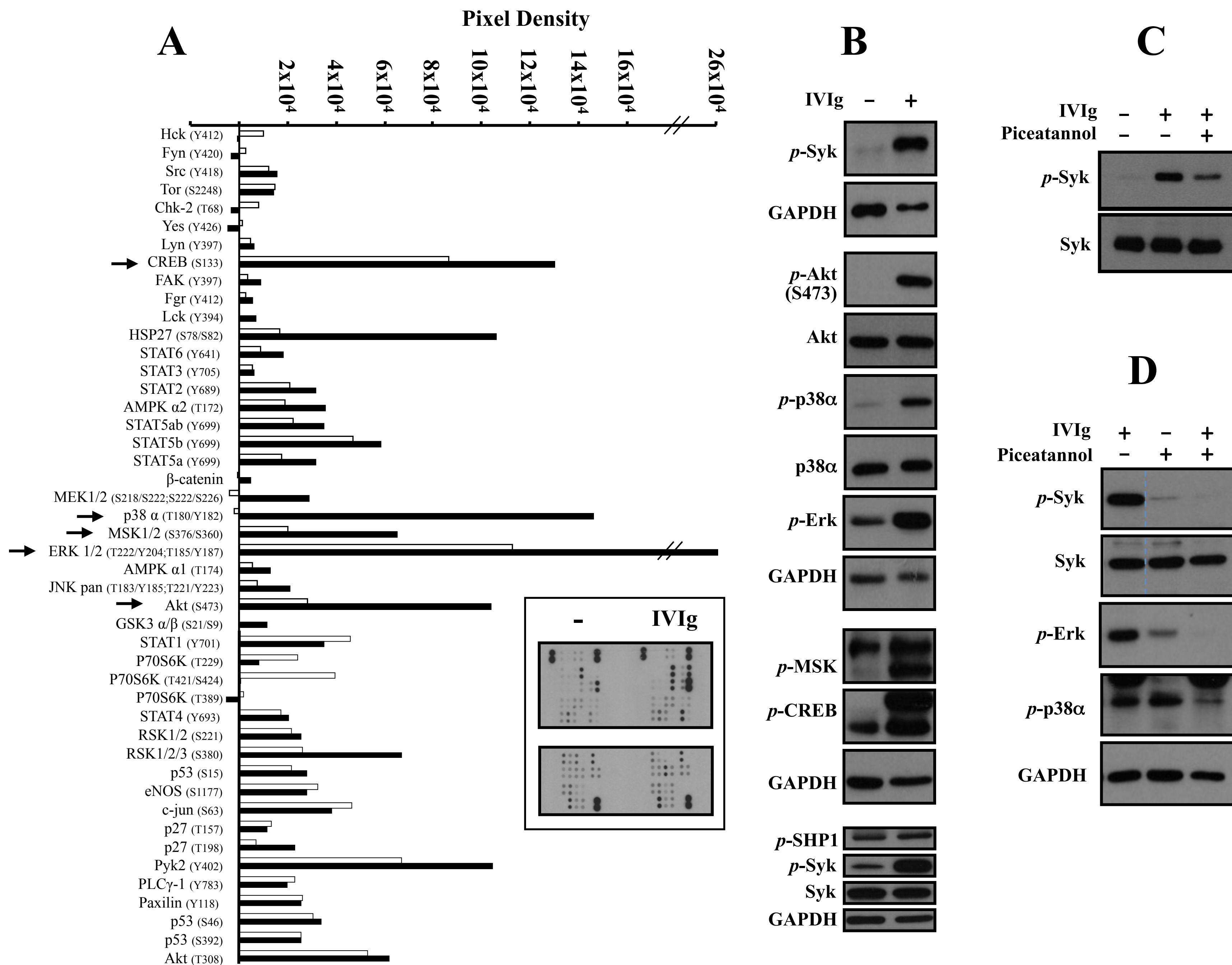
Supplementary Figure 2



Supplementary Figure 2. Effect of IVIg on *ex vivo* isolated macrophages.- (A) Thymic macrophages from two independent donors (donor#1, black bars; donor#2, empty bars) were exposed to IVIg for 24 hours, and the expression level of the indicated polarization markers was determined by qRT-PCR. Results are expressed as Relative Expression, which indicates the expression of each marker in the presence of IVIg and relative to its expression in the absence of IVIg. **(B)** CD14+ TAM from the pleural fluid of a metastatic breast adenocarcinoma were stimulated for 24 hours with 10 ng/ml LPS in the absence (-) or in the presence (+) of IVIg, and supernatants assayed for TNFα and IL-10. Each determination was done in triplicate and mean ± SD is indicated.

Downloaded from http://journals.aai.org/jimmunol/article-supplement/96830/pdf/ji_1303375_supplemental_figures_1/ by guest on 20 May 2025

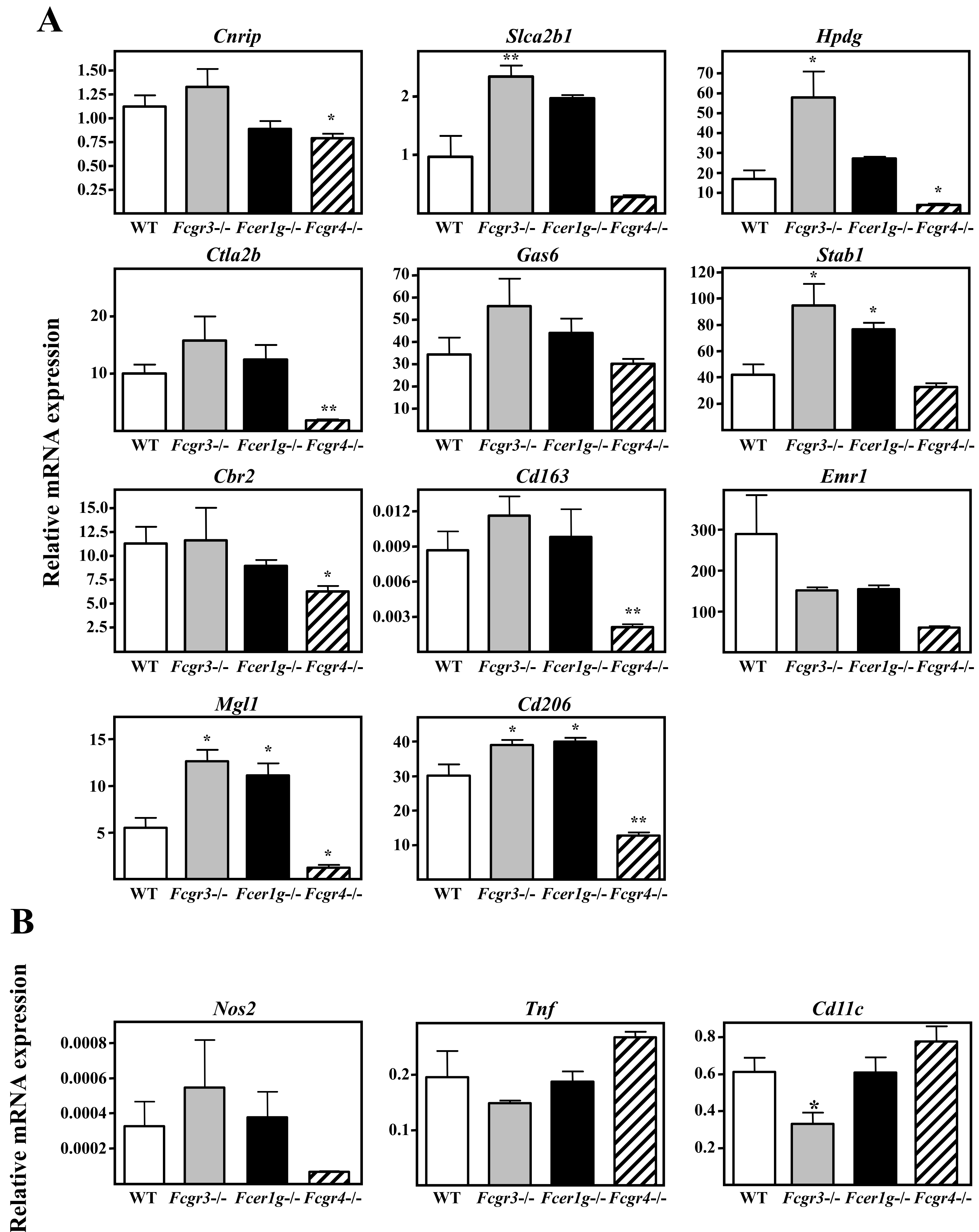
Supplementary Figure 3



Supplementary Figure 3. IVIg-triggered intracellular signaling in human macrophages (A,B) and in the presence of the Syk tyrosine kinse inhibitor piceatannol (C,D).- (A) M2 macrophages were left untreated (-) or exposed to IVIg for 15 minutes, and the phosphorylation state of the indicated signaling molecules was determined using the Proteome Profiler® protein array (R&D Systems, Inc, USA), which detects the relative phosphorylation levels of 46 intracellular serine/threonine/tyrosine kinases. Results are shown as the pixel density of each spot after densitometric analysis of the blot (insert). Arrows indicate the kinases specifically mentioned in the text. **(B)** M2 macrophages were left untreated (-) or exposed to IVIg for 15 minutes, and the phosphorylation state of the indicated molecules was determined by Western blot using specific antibodies. Where indicated, the level of GAPDH, Syk, Akt and p38α was used as a loading control. **(C)** M2 macrophages were left untreated (-) or exposed to IVIg for 15 minutes either in the absence or presence of piceatannol, and the level of Syk phosphorylation and total protein state was determined by Western blot. **(D)** M2 macrophages were left untreated (-) or exposed to IVIg for 15 minutes either in the absence or absence of piceatannol, and the phosphorylation state of Syk, ERK1/2 and p38MAPK was determined by Western blot using phosphorylation-specific antibodies. Where indicated, the level of Syk and GAPDH was used as a loading control.

Downloaded from http://journals.aai.org/jimmunol/article-supplement/96830/pdf/ji_1303375_supplemental_figures_1/ by guest on 20 May 2025

Supplementary Figure 4



Supplementary Figure 4. Basal state of transcriptomic polarization of bone marrow-derived M2 macrophages polarization.- Expression of genes associated to either M2 **(A)** or M1 **(B)** polarization in bone marrow-derived M2 macrophages BMDM from wild type (WT), *Fcgr3-/-*, *Fcer1g-/-* and *Fcgr4-/-* mice, as determined by qRT-PCR. Relative Expression indicates the expression of each marker relative to the expression of the *Tbp* gene in each strain. Shown are the mean ± SD of four independent experiments (*, $p<0.05$; **, $p<0.01$).

Downloaded from http://journals.aai.org/jimmunol/article-supplement/96830/pdf/ji_1303375_supplemental_figures_1/ by guest on 20 May 2025