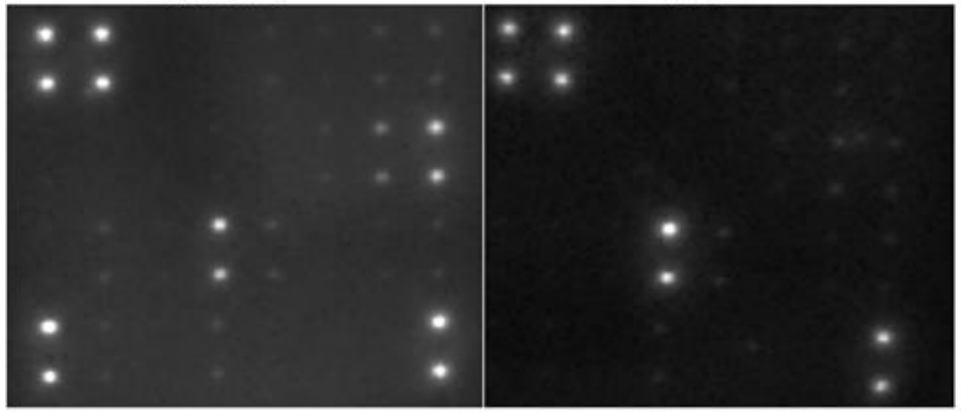
Proteomic analysis of chemokines using mouse Chemokine Array

A

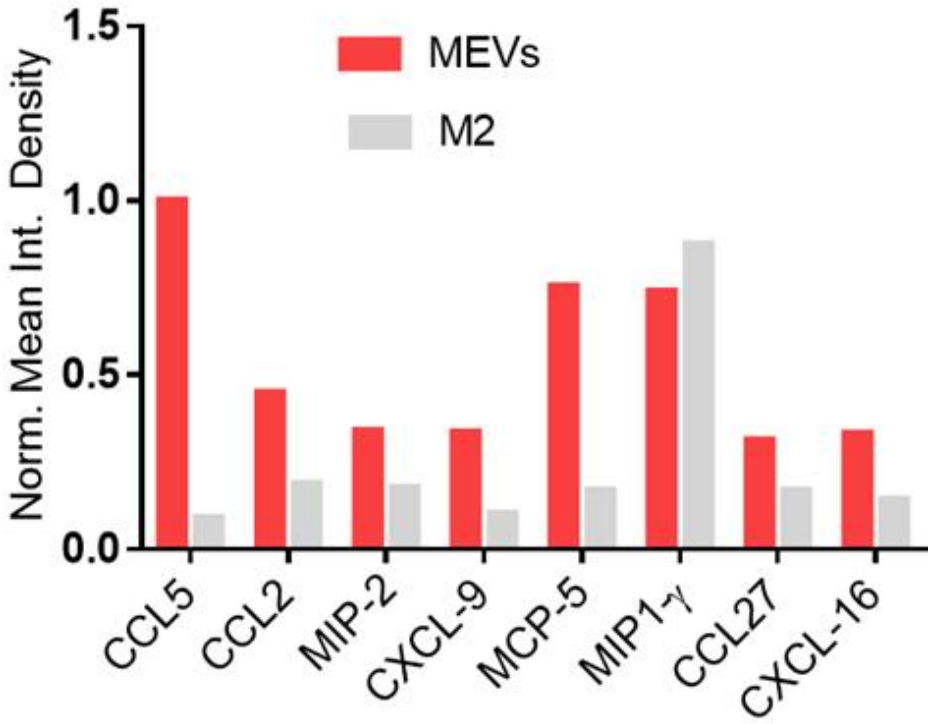
Mouse Chemokine Array

Antibody spotted in duplicate vertically								
1	POS	POS	NEG	NEG	CCL21	CXCL13	CCL27	CXCL16
2								
3	CCL11	CCL24	CX3CL1	CXCL11	CXCL1	LIX	CCL2	MCP-5
4								
5	CCL22	CXCL9	CCL3	MIP-1γ	MIP-2	CCL19	CCL20	CXCL4
6								
7	CCL5	CXCL12	CCL17	CCL1	CCL25	BLANK	BLANK	POS
8								

B



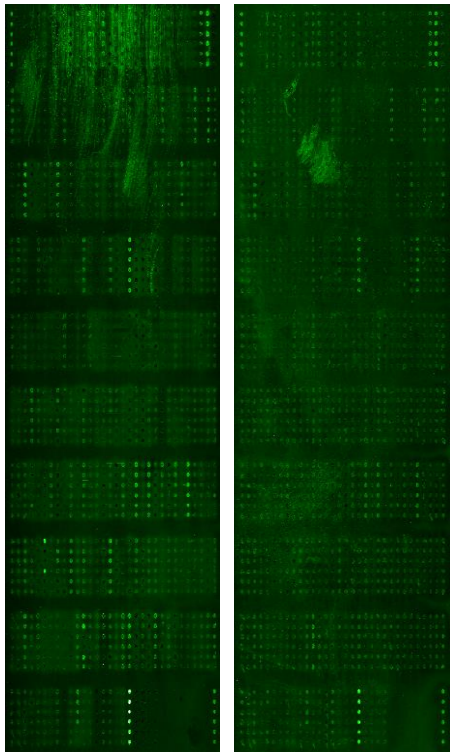
C



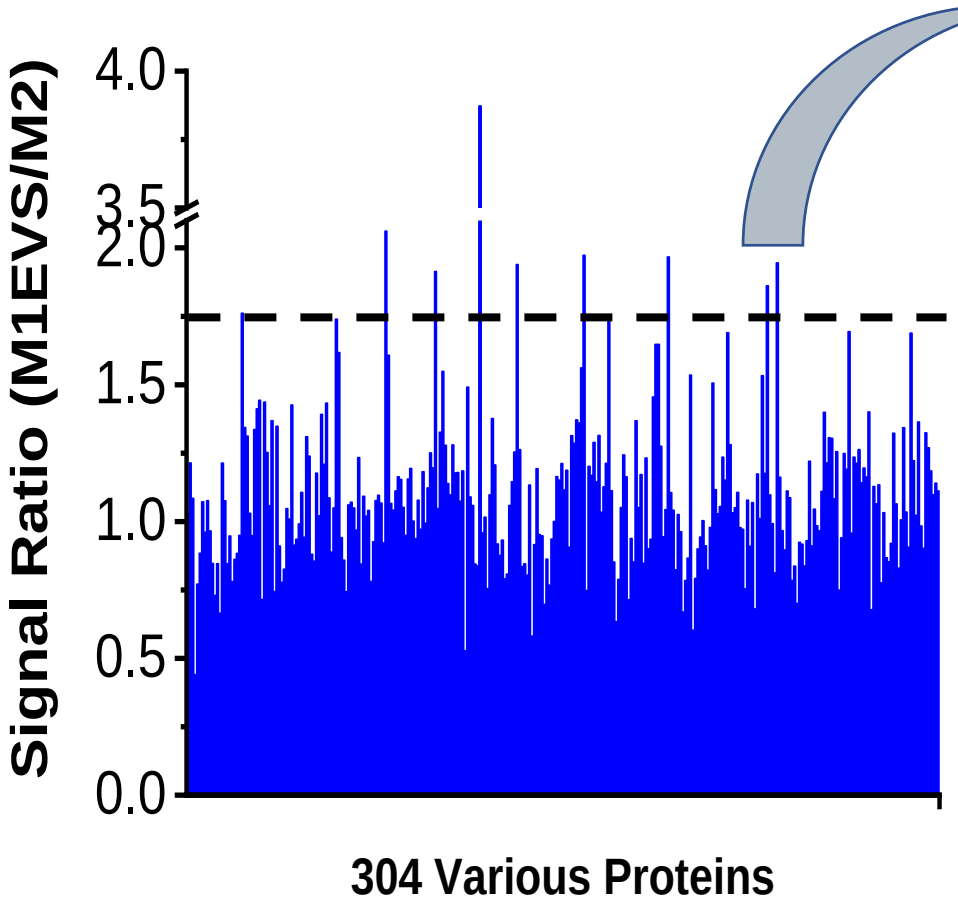
Protein (Chemokine) Analysis in M1EVs and M2 using mouse chemokines antibody array

A) List of chemokines and their position on the chemokine antibody array. **B)** Comparative study of the chemokines expressed in MEVs and M2 macrophages. Spot signal intensity is indicative of chemokine expression level. **C)** Comparison of normalized mean integrated density measurements between chemokines of MEVs and M2 macrophages

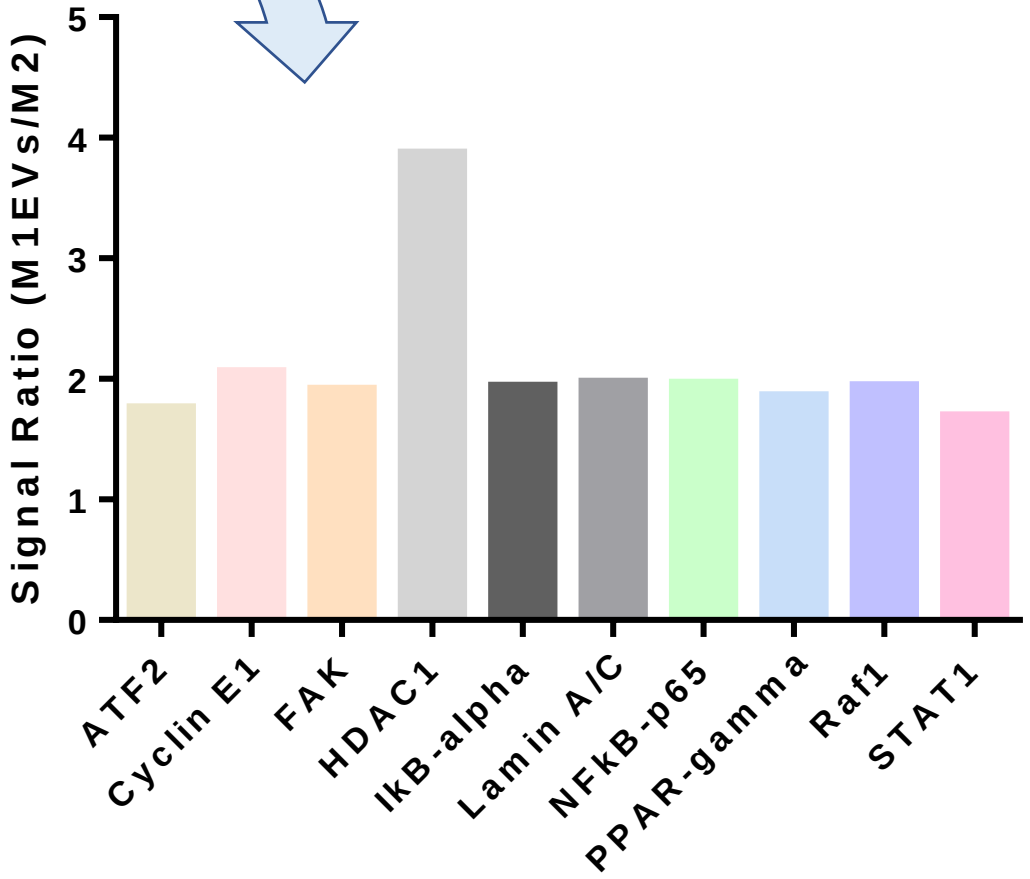
Proteomic analysis of various macrophage associated proteins using an antibody array



Array Images

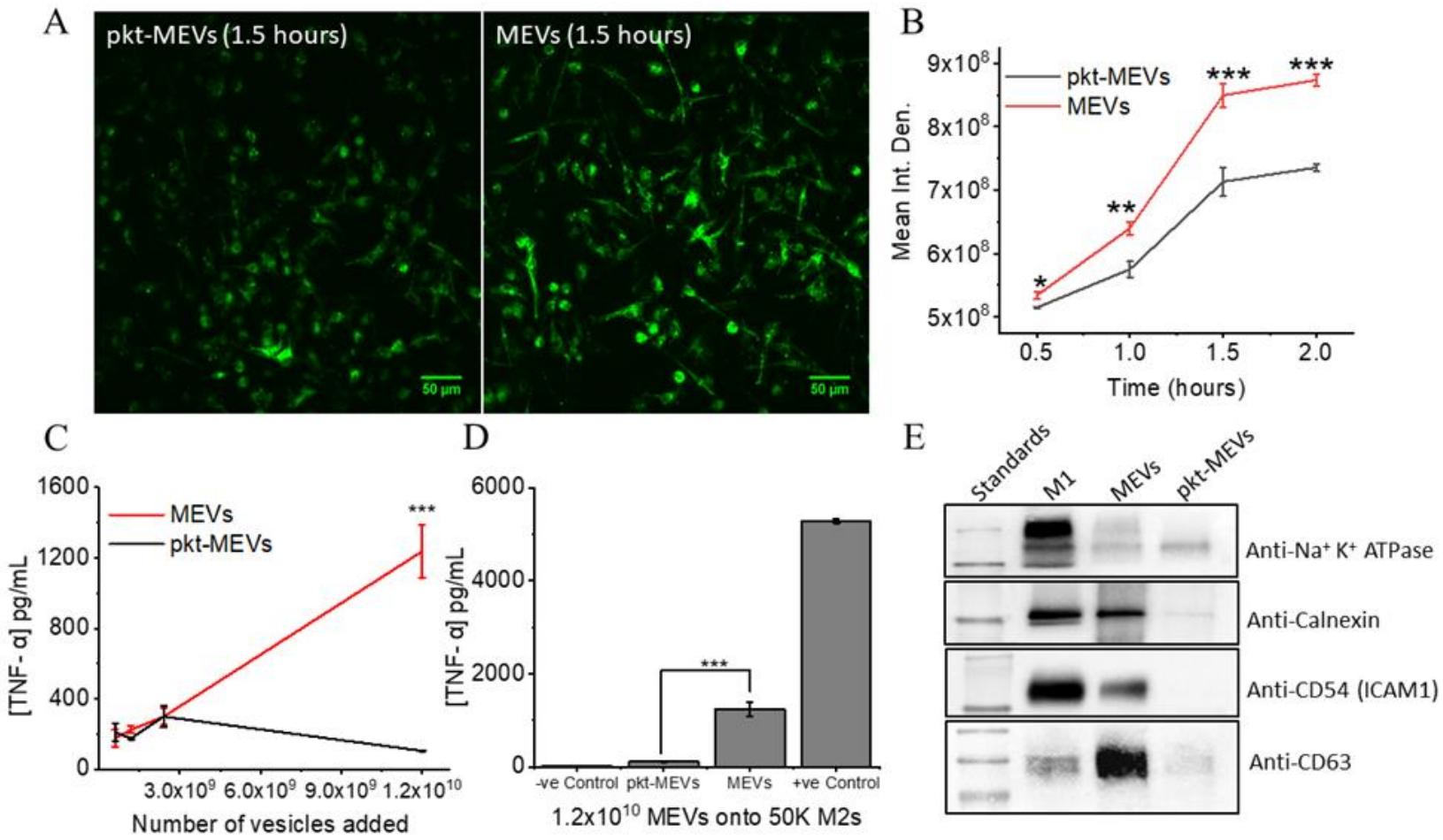


Ratio of protein expression signal intensities in MEVs compared to M2 macrophages



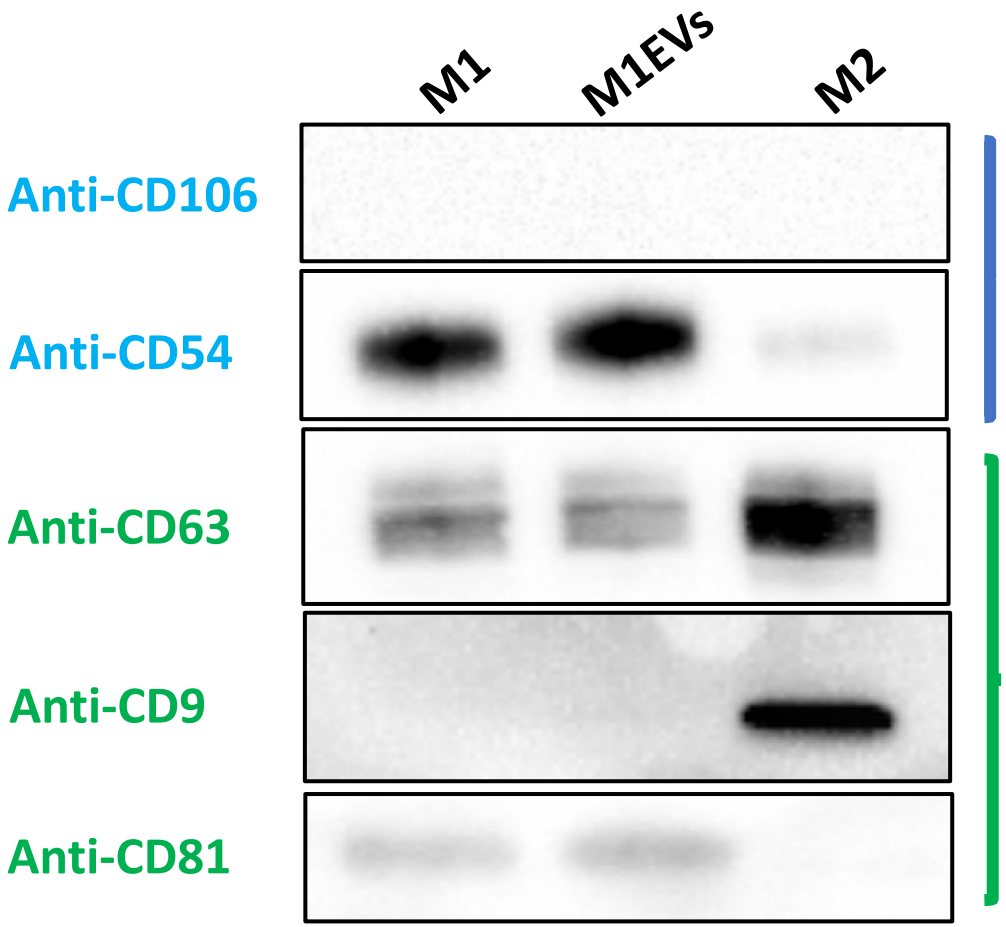
Proteins with signal ratio above 1.75

Proteolytic digestion of MEV membrane proteins limits uptake and inhibits repolarization capacity



A) Widefield images of M2 macrophages following incubation with proteinase-K treated fluorescently-labeled MEVs(pkt-MEVs) or untreated fluorescently-labeled MEVs. B) Quantitation of fluorescence intensity in M2 macrophages treated with pkt-MEVs or untreated MEVs. C) Dose response of TNF-α release by M2 macrophages after incubation with different numbers of MEVs or pkt-MEVs. D) Quantitation of TNF-α released by M2 macrophages after incubation with equal number of pkt-MEVs, MEVs and positive-control [LPS (20 ng/mL) + IFN-γ (20 ng/mL)] for 24 hours. E) Western blot analysis of MEV membrane anchored proteins before and after proteinase-K treatment. Equal amounts of total proteins extracted from M1 macrophages, MEVs and pkt-MEVs were immunoblotted for $Na^+ K^+$ ATPase , calnexin, CD54, CD63.

Validation of selected exosomal-marker proteins on M1EVs

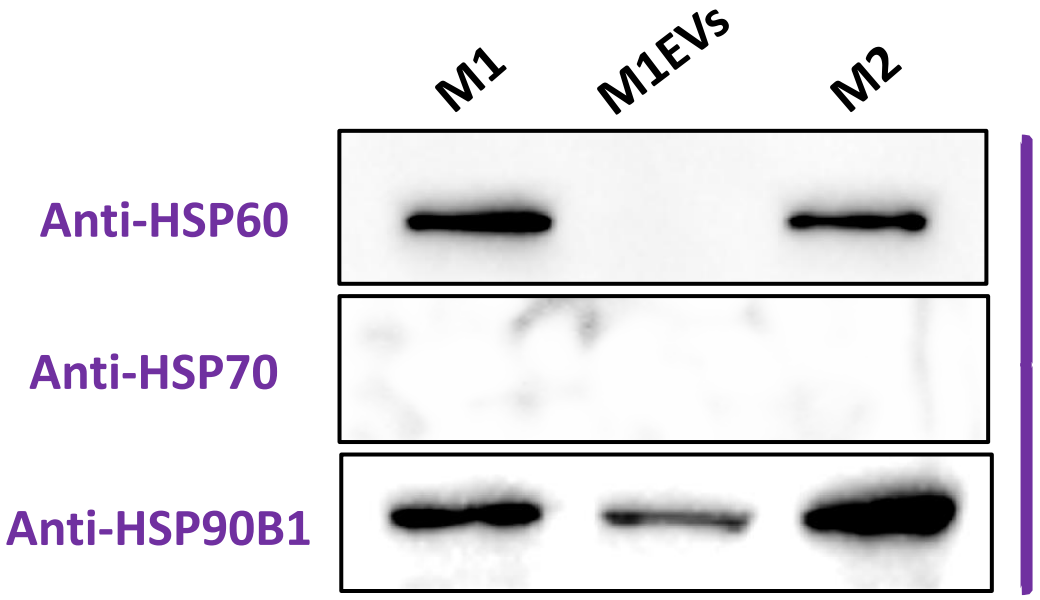


Cell adhesion molecules like ICAM1/CD54 and VCAM1(CD106) are involved in adhesion as well as activation

CD9,CD63 & CD81 are transmembrane proteins and play a role in activation

Heat shock proteins on M1EVs

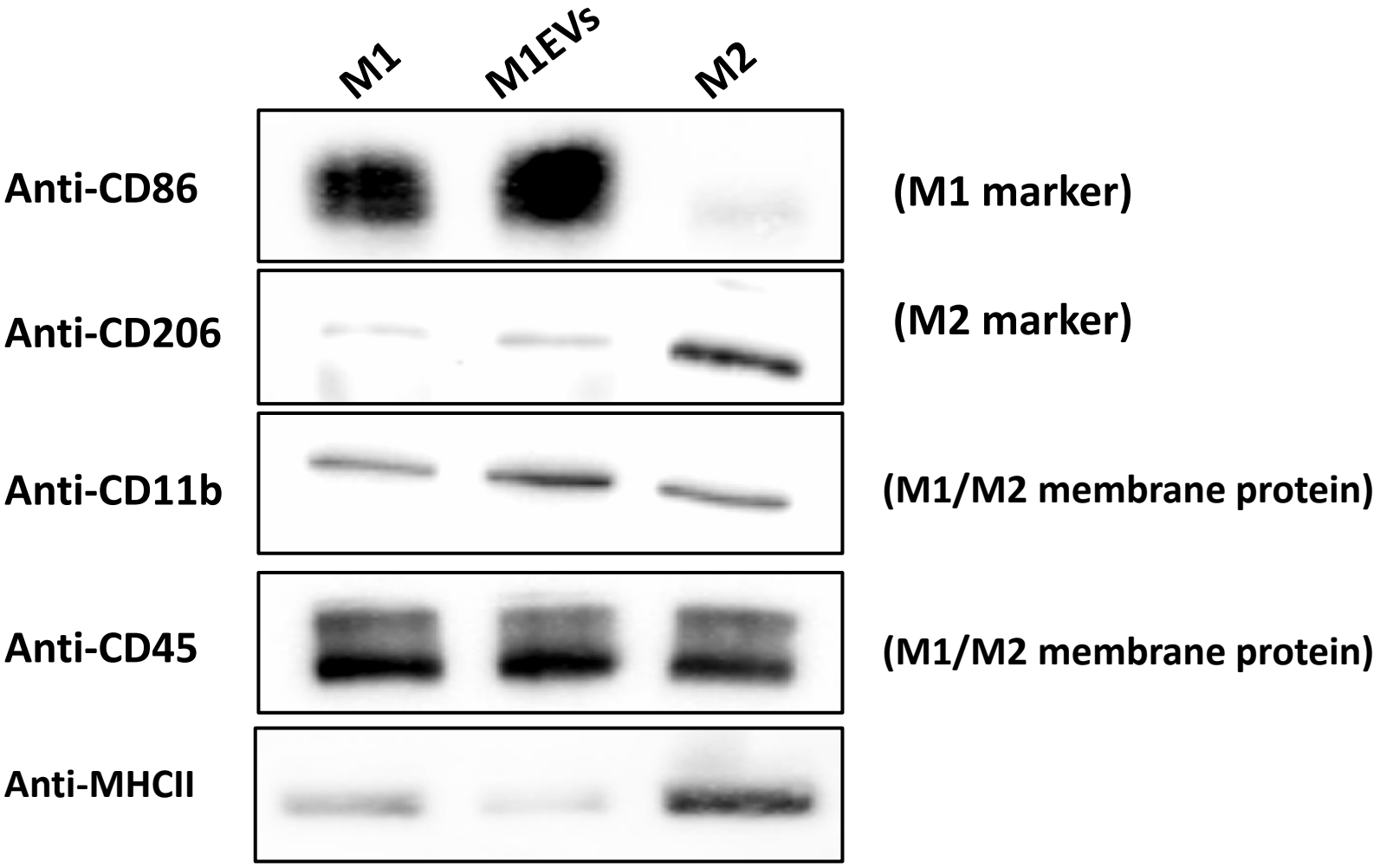
Some of the heat shock proteins like HSP60, HSP70 are cytoplasmic while others like HSP90B1(gp96) are found in endoplasmic reticulum



- **HSP60,HSP70, and HSP90B1 have all been implicated in macrophage activation**
- **M1EVs only contain HSP90B1**

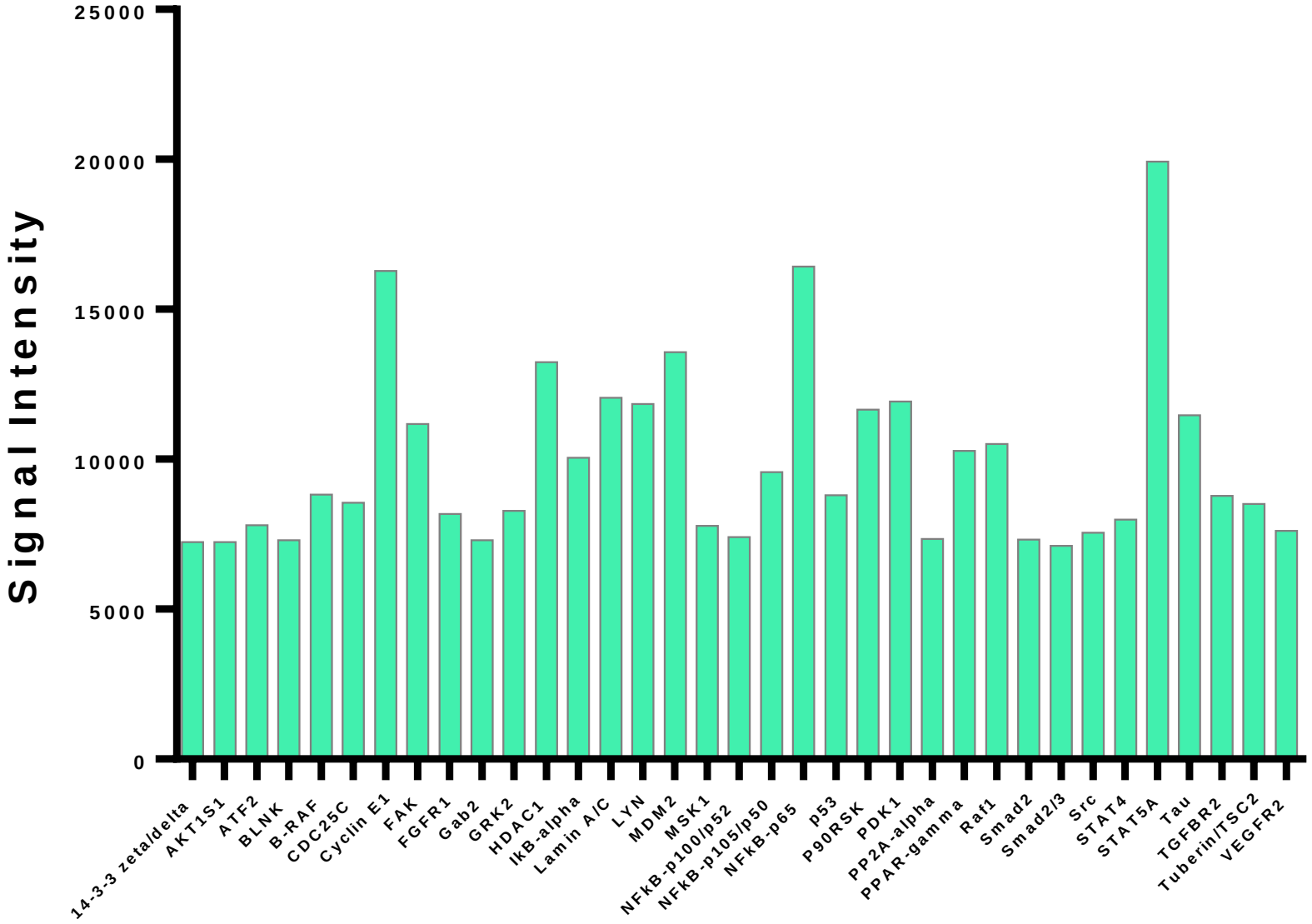
HSP90B1 is a chaperone for Toll Like Receptors

Identification of macrophage membrane-marker proteins on M1EVs



MEVs contain similar surface proteins as the parent macrophage

Additional proteins present on M1EVs



We've identified a large number of proteins present on the surface of MEVs.

The challenge now is identifying which are important for the ability of MEVs to target cells and alter macrophage polarization