SUPPLEMENTARY METHODS

Antibodies used for Western Blotting: anti-CD43 (clone DF-T1, DakoCytomation), anti-CD45 (clones 2B11+PD7/26, DakoCytomation), anti-phosphotyrosine (clone 4G10, Upstate), anti-Syk (Cell Signaling Technology), anti-phospho-Zap-70 (Tyr319) / Syk (Tyr352) (Cell Signaling Technology), anti-rabbit IgG-HRP (Cell Signaling Technology), goat anti-mouse IgG-HRP (Pierce), streptavidin-HRP (Pierce). Protein bands were visualized using ECL Plus (Amersham) and SuperSignal West Femto (Pierce).

Antibodies used for immunofluorescence: mouse monoclonal anti-human CD43 (clone DF-T1, DakoCytomation), mouse monoclonal anti-human CD45 (clones 2B11+PD7/26, DakoCytomation), biotin-conjugated mouse monoclonal anti-human CD45 (clone H130, BD Pharmingen), mouse monoclonal anti-human DC-SIGN (clone 120612, R&D Systems), rabbit anti-human anti-phospho-Zap-70 (Tyr319)/Syk(Tyr352) (Cell Signaling Technology), rabbit polyclonal anti-human CCR5 (Abcam), Alexa-633 (or 488)-conjugated goat anti-mouse IgG (Invitrogen), Alexa-488 (or 594)-conjugated streptavidin (Invitrogen), and Alexa-594-conjugated goat anti-rabbit IgG (Invitrogen).

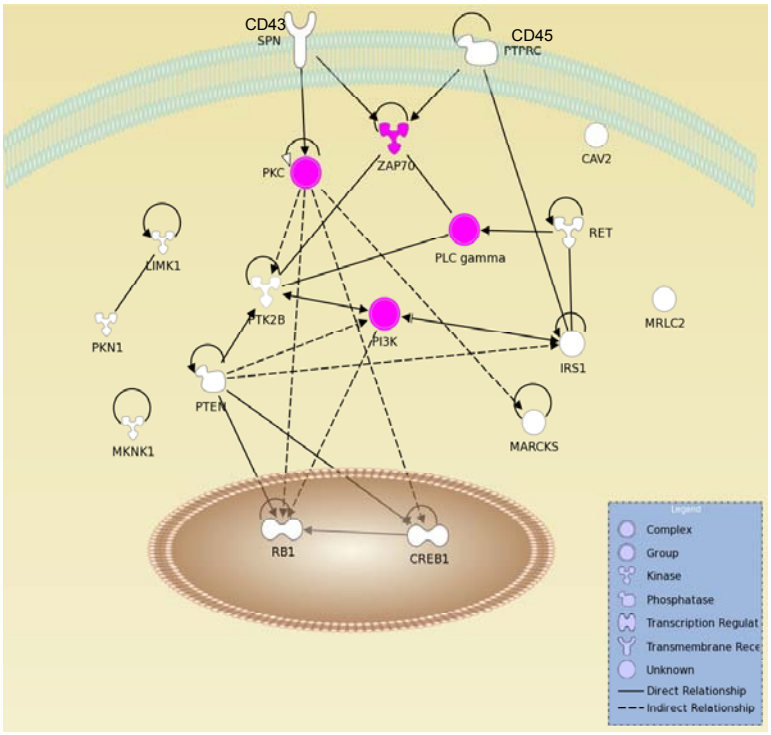
SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Ingenuity Pathway Analysis based on the kinome screen of galectin-1 stimulated MDDCs. A) Phosphorylated proteins identified by Kinex array were ranked by corrected fold change compared to buffer control and those proteins with corrected fold change >1.3 (12 proteins) were used for Ingenuity Pathway Analysis. To encompass small changes in Phosphorylation that may have large consequences in signaling cascades, a very liberal fold change of 1.3 was used to parse our list. Our candidate list was further filtered to include only those proteins with a detected change in phosphorylation rather than a change in total protein levels, resulting in 32 proteins, of which the top dozen candidates with a fold change above 1.3 are shown in this figure and Table 1. This figure is organized by cellular localization. Our candidate receptors on the cell surface, CD45 (PTPRC) and CD43 (SPN), as well as candidate signaling pathways (ZAP70/Syk, PKC, PLCgamma, and PI3K) are included for reference. Proteins were labeled according to their abbreviations from Table 1. Solid lines indicate direct interactions and dashed lines indicate indirect interactions. Proteins highlighted in pink were targeted in the chemical inhibition assay (Fig. 3). **B)** Images of Kinex array chips identifies phosphorylated proteins at 5 minutes after galectin-1 or Buffer control (80μM DTT + 10μg/ml polymyxin B) treatment. Pink dots represents targets identified in galectin-1 treated cell lysates, Blue dots indicate targets identified in Buffer control treated cells.

Supplemental Figure 2. Galectin-1 matures murine bone marrow derived DCs. (A) DC activation markers on murine bone marrow derived dendritic cells (BMDCs) cultured with LPS (1μg/ml), Buffer control (80μM DTT + 10μg/ml polymyxin B) or increasing concentrations of galectin-1 (5μM, 10μM, 20μM) for 48 hours. All galectin-1 induction experiments were performed with excess polymyxin B, which binds to and abrogates any potentially confounding effects of endotoxin from the galectin-1 stock. Data are expressed as mean fluorescence intensity (MFI) for each marker. **(B)** IL-6 cytokine secretion by BMDCs measured by ELISA of supernatant following 48 hour culture with indicated treatments.

Supplemental Figure 1

A



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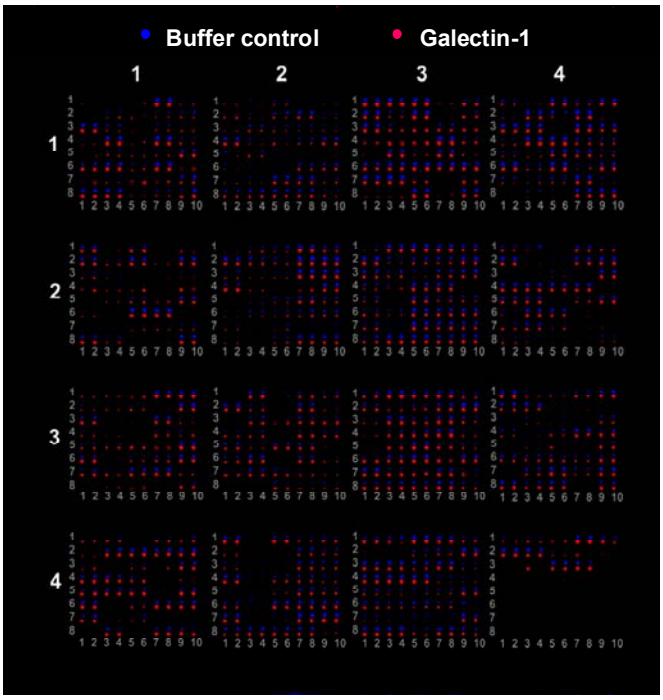

Supplemental Figure 2