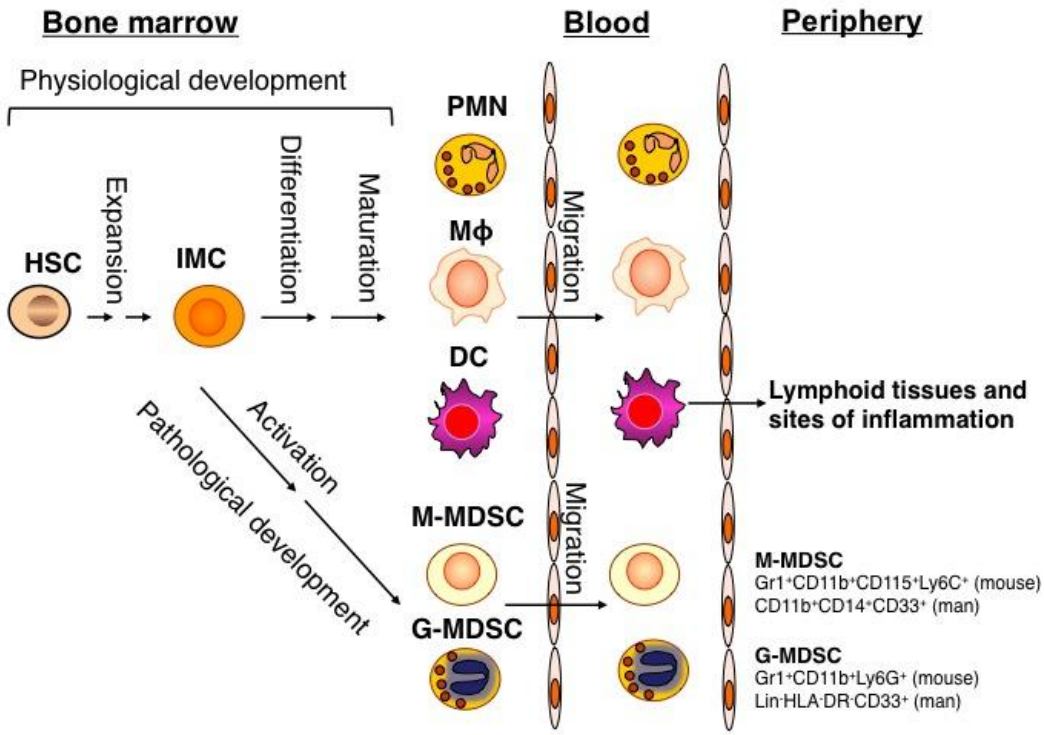
Supplemental Table S1. PIR and their ligands in humans and mice

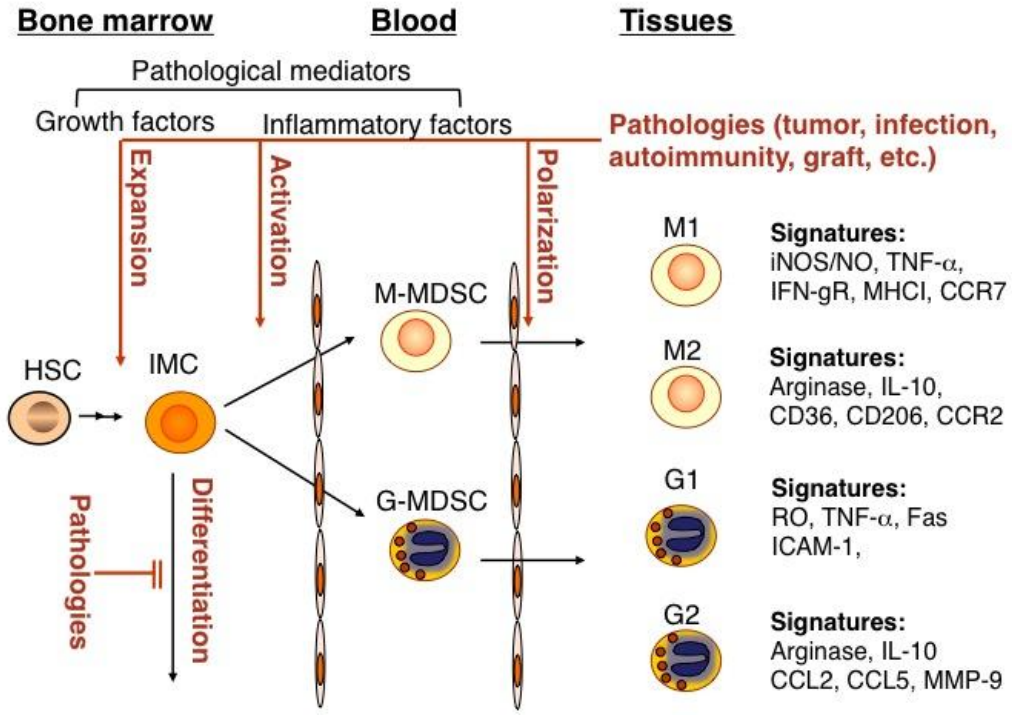
Species	Inhibitory receptor		Activating receptor	
	ITIM	Ligand	ITAM	Ligand
Mouse	PIR-B	MHC I Angptl MDI	PIR-A	MHC I
Human	LILR-B1 (ILT2, CD85j)	HLA-A, -B, -C, -E, -F and -G Angptl (?) MDI (?)	LILR-A1	HLA-A, -B, -C, -E, -F and -G (?)
	LILR-B2 (ILT4, CD85d)	HLA-A, -B, -C, -E, -F and -G Angptl	LILR-A2	HLA-A, -B, -C, -E, -F and -G (?)
	LILR-B3 (ILT5, CD85a)	HLA-A, -B, -C, -E, -F and -G (?) Angptl MDI (?)	LILR-A4	HLA-A, -B, -C, -E, -F and -G (?)
	LILR-B4 (ILT3, CD85k)	HLA-A, -B, -C, -E, -F and -G (?) Angptl (?) MDI (?)	ILT8	HLA-A, -B, -C, -E, -F and -G (?)
	LILR-B5 (CD85c)	HLA-A, -B, -C, -E, -F and -G (?) Angptl (?) MDI (?)	ILT11	HLA-A, -B, -C, -E, -F and -G (?)

Murine PIR-A and PIR-B (Takai, 2005), human PIR homologues, also known as leukocyte immunoglobulin-like receptors (LILR) or Ig-like transcripts (ILT) (Andre et al., 2001) and their cognate ligands are tabulated. LILRA3, ILT-9 and ILT-10 are omitted as LILRA3 is a soluble protein and ILT-9 and ILT-10 are thought to be pseudogenes (Andre, et al., 2001; Brown et al., 2004; Takai, 2005; Zheng et al., 2012). Question mark denotes that the finding has not been confirmed.

Sup. Fig 1A



Sup. Fig 1B

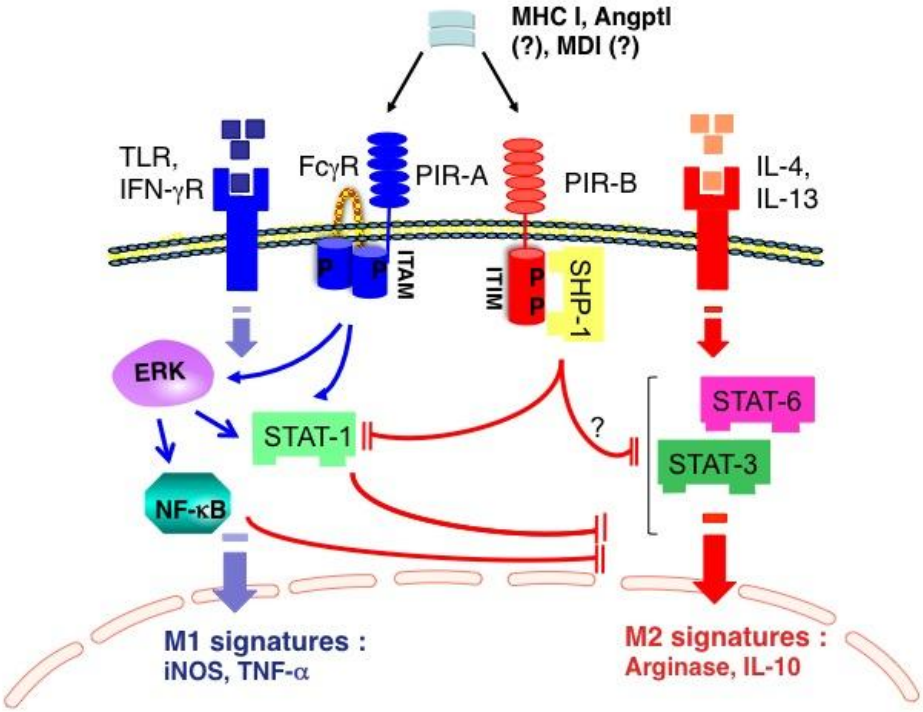


Supplementary Figure S1. Multiple stages in the development of myeloid cells and MDSC.

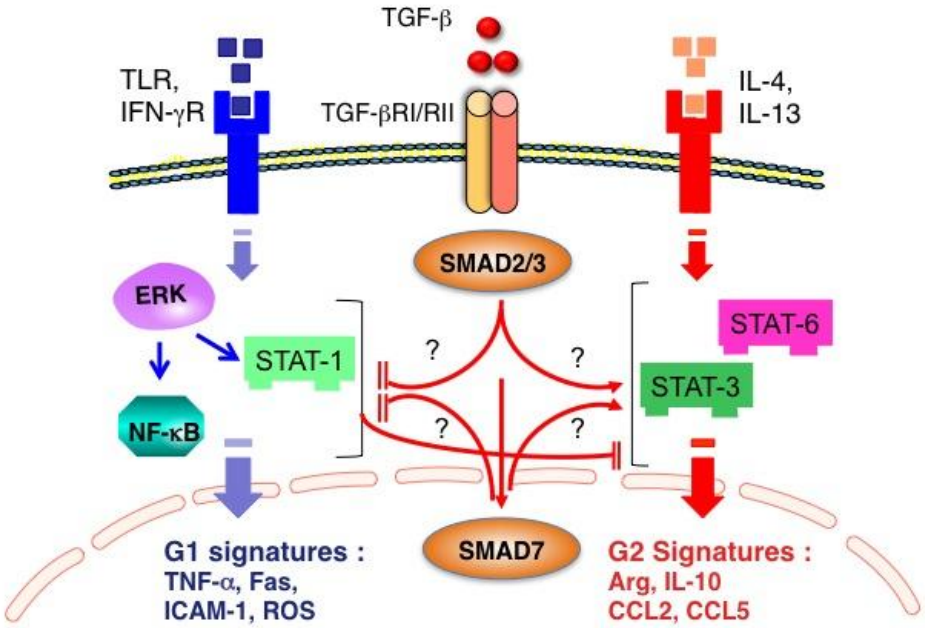
A. MDSC development from expansion to activation and functional polarization

is a multiple-step process, a “multiple signal model” in which multiple factors/signals are necessary for this process is proposed in the article. Under normal physiological conditions, hematopoietic stem cells (HSC) undergo a series of expansion, differentiation, and maturation in bone marrow. Mature myeloid cells migrate to the periphery via blood vessels and replenish the peripheral pool of myeloid cells. Under pathological conditions, mediators of pathology deter and divert normal HSC development to pathological development, distinguished by an increase in immature myeloid cells (IMC) expansion and activation. These immature myeloid cells, i.e., MDSC, migrate to the peripheral lymphoid tissues and sites of inflammation. Based on lineage markers, MDSC can be classified into $Gr1^{+}CD11b^{+}CD115^{+}Ly6C^{+}$ monocytic (M)-MDSC and $Gr1^{+}CD11b^{+}Ly6G^{+}$ granulocytic (G)-MDSC in mice (Huang et al., 2006; Movahedi et al., 2008). A consensus regarding the markers for human MDSC has not been reached. Depending on cancer type, human MDSC are characterized as $CD11b^{+}CD14^{+}CD33^{+}$ or $Lin^{-}HLA^{-}DR^{-}CD33^{+}$ myeloid cells (Ostrand-Rosenberg and Sinha, 2009; Raychaudhuri et al., 2011). B. The inflammatory mediators of pathology can regulate three developmental stages of MDSC from expansion to activation and polarization. In terms of polarization, these mediators drive MDSC subsets to skew into M2 M-MDSC and G2 G-MDSC. Polarized MDSC subsets can be distinguished by a distinct set of signature genes related to their function. M2/G2 cells produce arginase, anti-inflammatory cytokines and chemokines, eventually converging to facilitate tumorigenesis and angiogenesis as well as probably metastasis and tissue remodeling. In marked contrast, M1 and G1 cells produce iNOS, NO, inflammatory cytokines and chemokines, leading to their anti-tumor effects. Definitive evidence as to whether MDSC polarization is an irreversible process or a reversible hyperactivation state remains elusive.

Sup. Fig 2A



Sup. Fig 2B



Supplementary Figure S2. Signaling pathways regulating the polarization of MDSC subsets.

A. TLR4 ligand, e.g. LPS, IFN-γ, IL-4, and IL-13 are present in the tumor microenvironment and other pathologies. Stimulation with TLR4 ligand and IFN-γ activates ERK, NK-κB and STAT1 and, as a consequence, polarizes

M-MDSC into an M1 phenotype, as defined by an up-regulation of the M1 hallmark genes, iNOS and TNF-α. In marked contrast, stimulation with IL-4/IL-13 activates STAT3/5 and polarizes M-MDSC into the M2 phenotype, characterized by an up-regulation of the M2 related genes, arginase and IL-10 (Greifenberg et al., 2009; Huang, et al., 2006; Movahedi, et al., 2008; Sinha et al., 2005). PIR-A and PIR-B are highly expressed in M-MDSC in a paired manner (Takai, 2005). Upon MHC I binding to PIR-A and PIR-B, the PIR-A/FcγR complex is activated, resulting in an enhanced M1 pathway. The M1 pathway is thought to antagonize the M2 pathway. Meanwhile, MHC I can also activate PIR-B and blunt the M1 and M2 pathways. Whether Angptl and MDI, another two PIR-B ligands, have similar effects on the M1/M2 pathway has not yet been ascertained. The affinity, expression level, and distribution of ligands for PIR-A and PIR-B are proposed to act as regulators of MDSC polarization (Ma et al., 2011). B. Similar to Figure 2A, signals from LPS/IFN-γ and IL-4/IL-13 stimulate G-MDSC to polarize into G1 and G2 cells, respectively, under various pathological conditions. The G1 phenotype is characterized by the G1 hallmark genes, TNF-α, Fas, ICAM-1, and ROS, and while the G2 phenotype is characterized by G2 related genes, arginase, IL-10 and CCL2/5. TGF-β is known as a negative regulator of G-MDSC polarization (Fridlender and Albelda, 2012; Fridlender et al., 2009). In most cell types, TGF-β binding can activate TGF-βRII followed by recruitment of TGF-βRI. TGF-βRII/RI engagement leads to phosphorylation/activation of SMAD2/3, followed by SMAD7 transcription and NF-κB inhibition. Current data support the concept that TGF-β inhibits the G1 pathway while promoting the G2 pathway. It is still an open question as to whether, and how, SMAD2/3 and SMAD7 participate in TGF-β-mediated G1/G2 polarization.

References:

Andre, P., Biassoni, R., Colonna, M., et al. (2001). New nomenclature for MHC receptors. Nat. Immunol*., 2*, 661.

Brown, D., Trowsdale, J., and Allen, R. (2004). The LILR family: modulators of innate and adaptive immune pathways in health and disease. Tissue antigens*, 64*, 215-225.

Fridlender, Z. G., and Albelda, S. M. (2012). Tumor Associated Neutrophils: Friend or Foe? Carcinogenesis*, 33*, 949-955.

Fridlender, Z. G., Sun, J., Kim, S., et al. (2009). Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN. Cancer Cell*, 16*, 183-194.

Galluzzi, L., Senovilla, L., Zitvogel, L., and Kroemer, G. (2012). The secret ally: immunostimulation by anticancer drugs. Nat. Rev. Drug discov., *11*, 215-233.

Greifenberg, V., Ribechini, E., Rossner, S., and Lutz, M. B. (2009). Myeloid-derived suppressor cell activation by combined LPS and IFN-gamma treatment impairs DC development. Eur. J. Immunol., *39*, 2865-2876.

Huang, B., Pan, P. Y., Li, Q., et al. (2006). Gr-1+CD115+ immature myeloid suppressor cells mediate the development of tumor-induced T regulatory cells and T-cell anergy in tumor-bearing host. Cancer Res., *66*, 1123-1131.

Ma, G., Pan, P. Y., Eisenstein, S., et al. (2011). Paired immunoglobin-like receptor-B regulates the suppressive function and fate of myeloid-derived suppressor cells. Immunity, *34*, 385-395.

Movahedi, K., Guilliams, M., Van den Bossche, J., et al. (2008). Identification of discrete tumor-induced myeloid-derived suppressor cell subpopulations with distinct T cell-suppressive activity. Blood, *111*, 4233-4244.

Nagaraj, S., Youn, J. I., Weber, H., et al. (2010). Anti-inflammatory triterpenoid blocks immune suppressive function of MDSCs and improves immune response in cancer. Clin. Cancer Res., *16*, 1812-1823.

Ostrand-Rosenberg, S., and Sinha, P. (2009). Myeloid-derived suppressor cells: linking inflammation and cancer. J. Immunol., *182*, 4499-4506.

Ozao-Choy, J., Ma, G., Kao, J., et al. (2009). The novel role of tyrosine kinase inhibitor in the reversal of immune suppression and modulation of tumor microenvironment for immune-based cancer therapies. Cancer Res., *69*, 2514-2522.

Raychaudhuri, B., Rayman, P., Ireland, J., et al. (2011). Myeloid-derived suppressor cell accumulation and function in patients with newly diagnosed glioblastoma. Neuro. Oncol., *13*, 591-599.

Sinha, P., Clements, V. K., and Ostrand-Rosenberg, S. (2005). Interleukin-13-regulated M2 macrophages in combination with myeloid suppressor cells block immune surveillance against metastasis. Cancer Res., *65*, 11743-11751.

Takai, T. (2005). Paired immunoglobulin-like receptors and their MHC class I recognition. Immunology, *115*, 433-440.

Ugel, S., Delpozzo, F., Desantis, G., et al. (2009). Therapeutic targeting of myeloid-derived suppressor cells. Curr. Opin. Pharmacol., *9*, 470-481.

Zheng, J., Umikawa, M., Cui, C., et al. (2012). Inhibitory receptors bind ANGPTLs and support blood stem cells and leukaemia development. Nature*, 485*, 656-660.