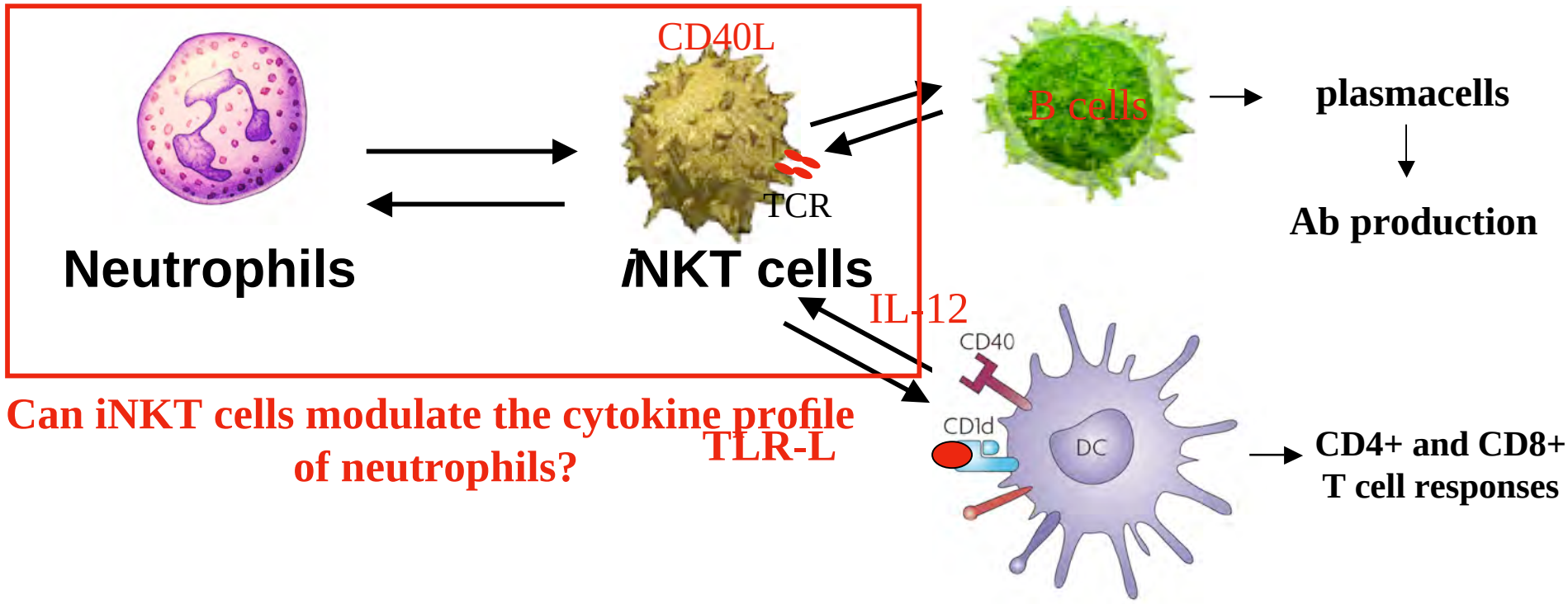
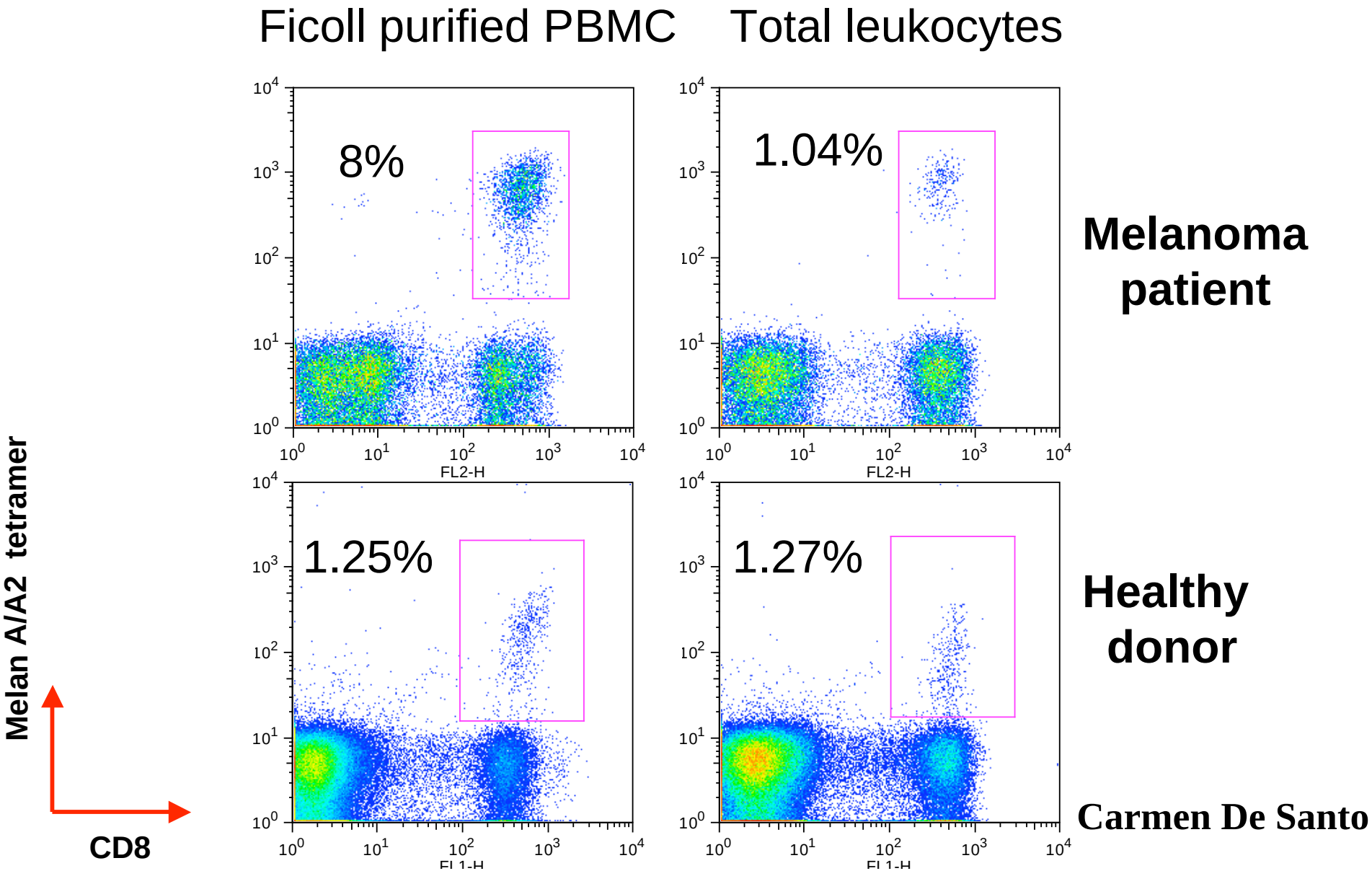


1. **The invariant TCR expressed by iNKT cells recognizes a limited repertoire of glycolipids in the context of CD1d molecules expressed by DC and B cells**
2. **iNKT cells are CD40L positive and the cross talk between iNKT cells with DC and B cells results in CD40 dependent DC maturation and B cell activation**
3. **TLR signaling events up-regulate endogenous CD1d ligand(s), which in combination with IL-12 activate iNKT cells.**

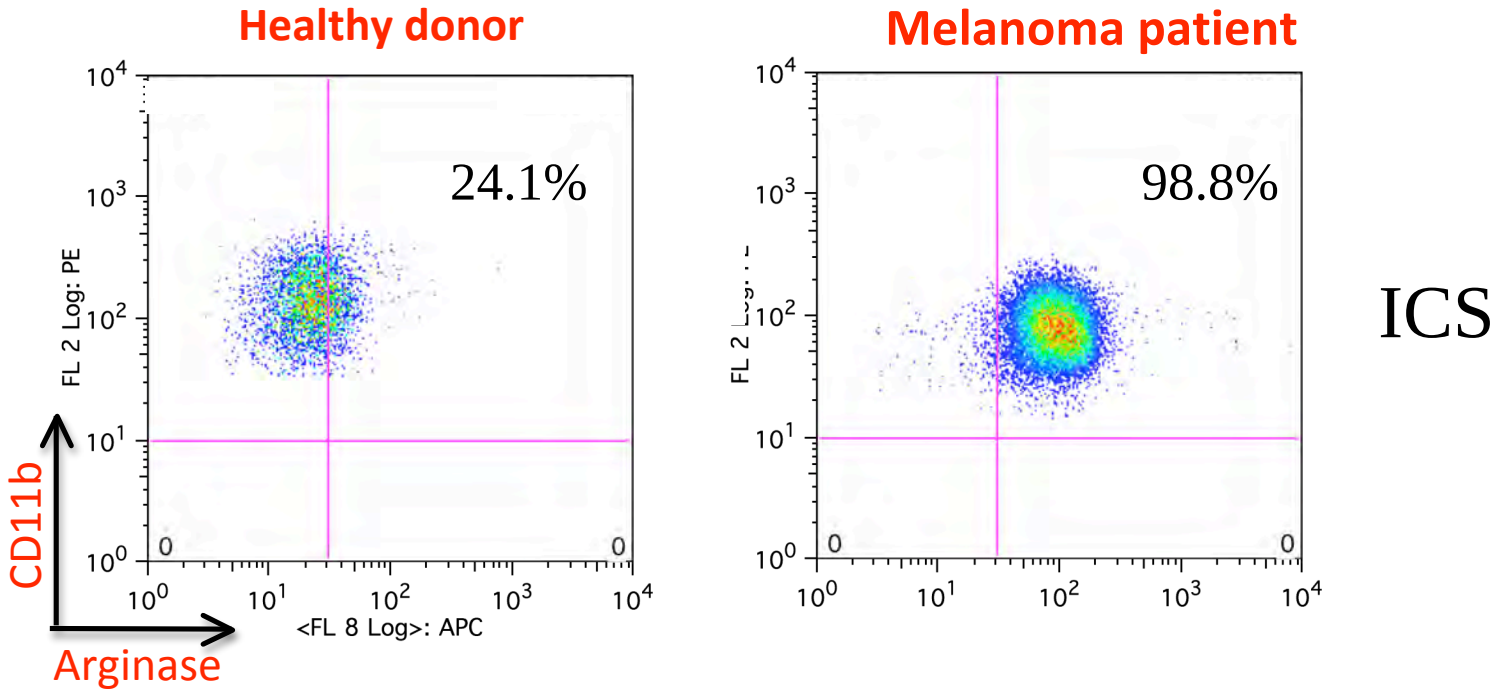
Melanoma

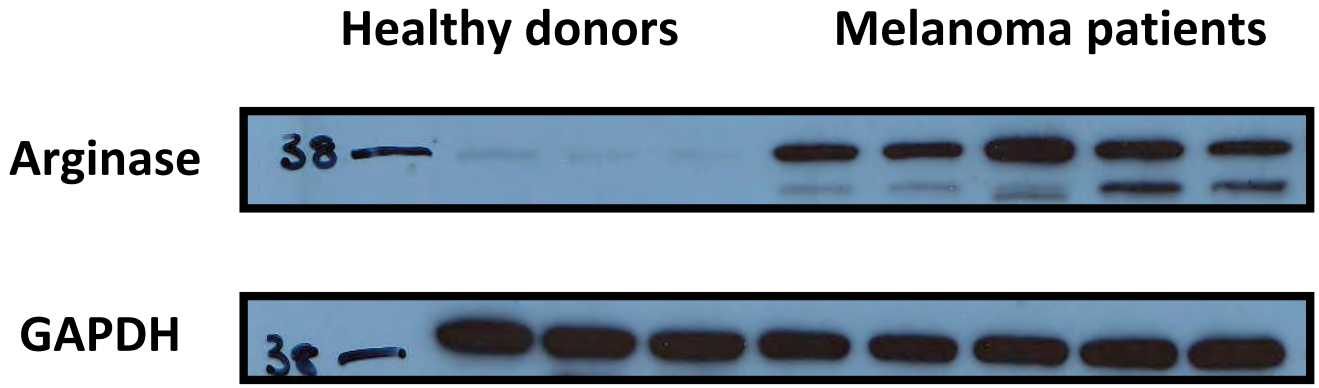
Reduced expansion of Melan-A_{26-35} specific T cells in total leukocytes



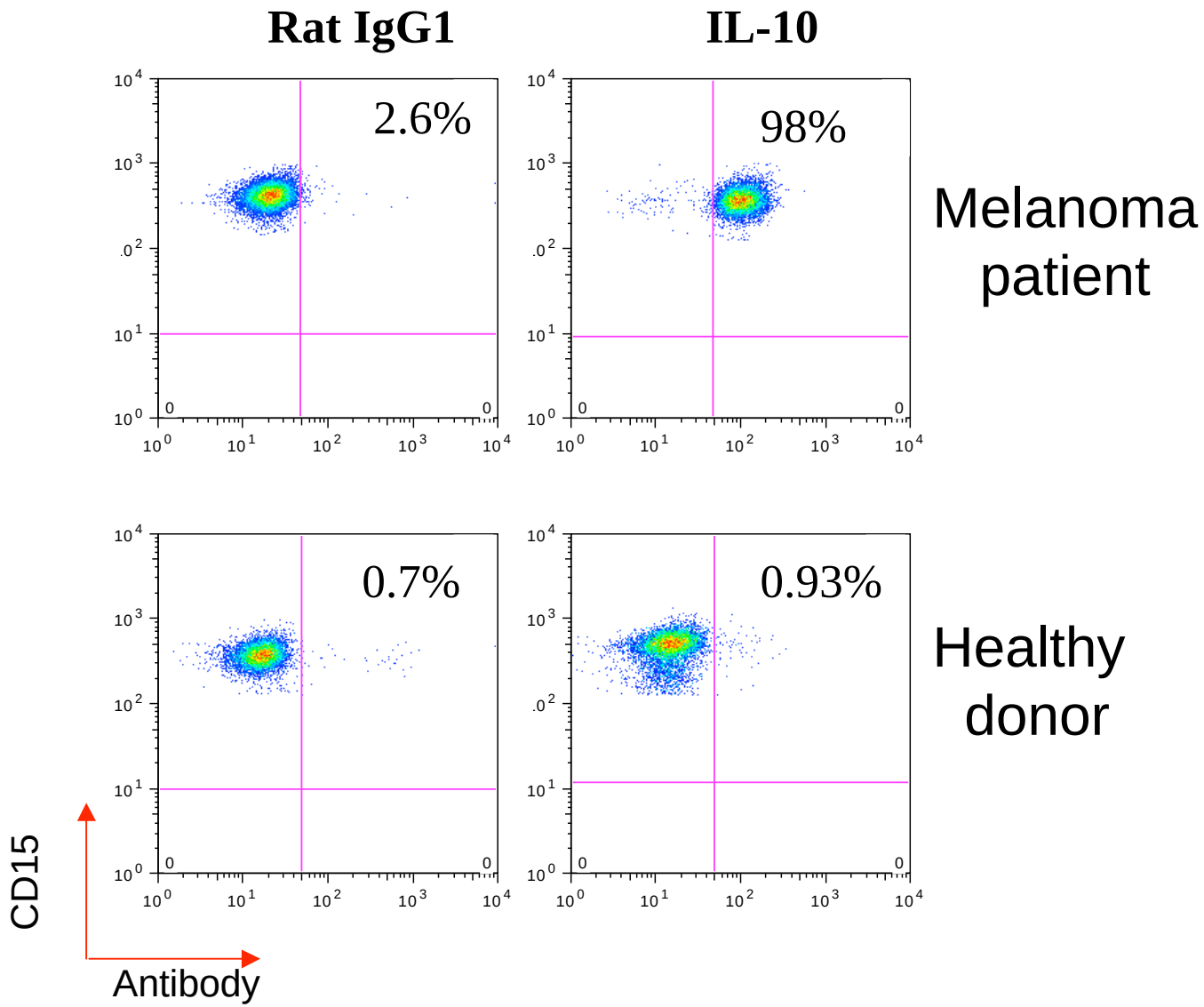
Carmen De Santo

Arginase 1 expression by neutrophils from patients with melanoma

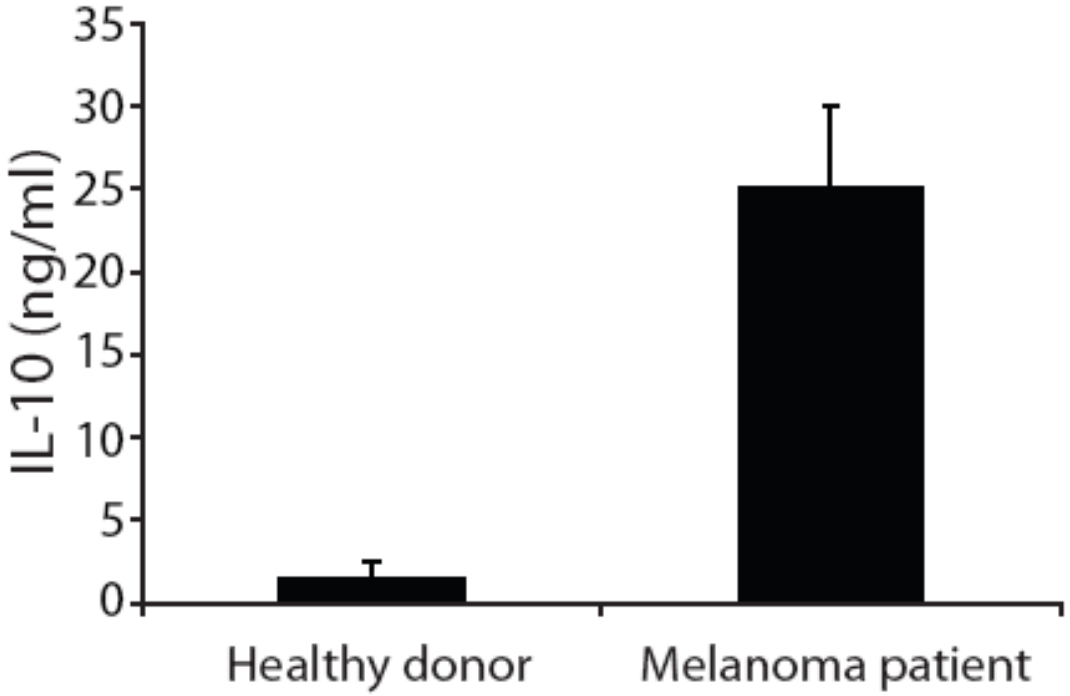




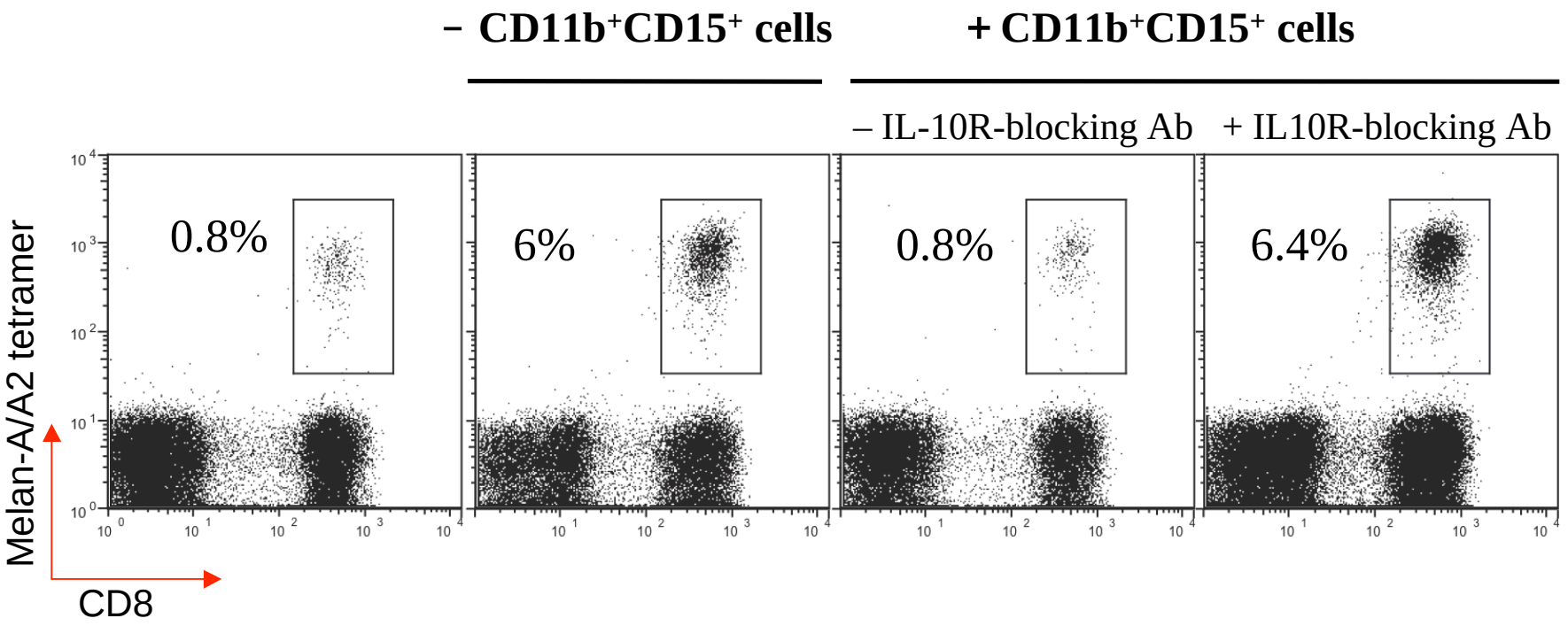
Intracellular staining with anti IL-10 Ab of neutrophils from patients with melanoma



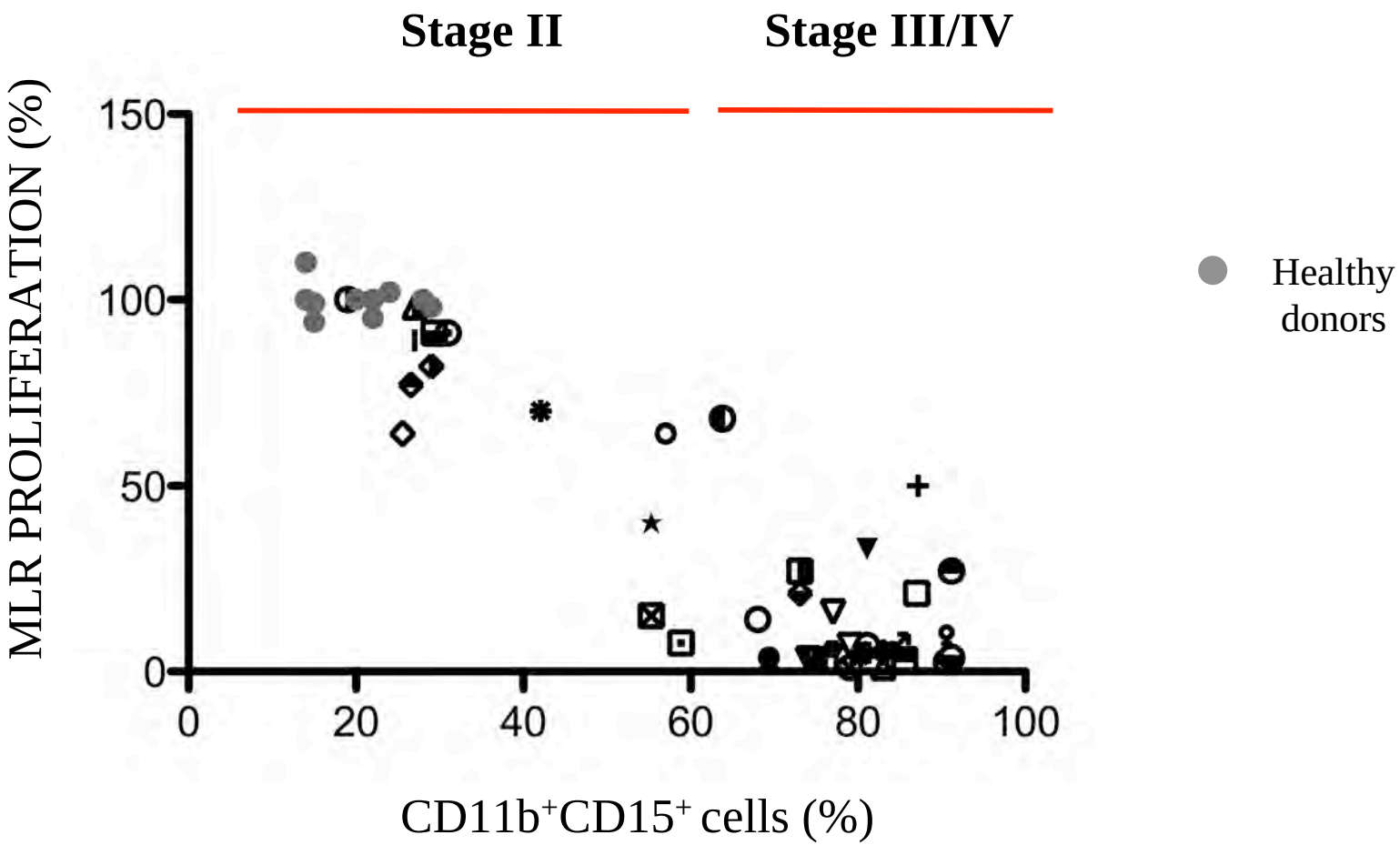
IL-10 secretion by neutrophils from patients with melanoma



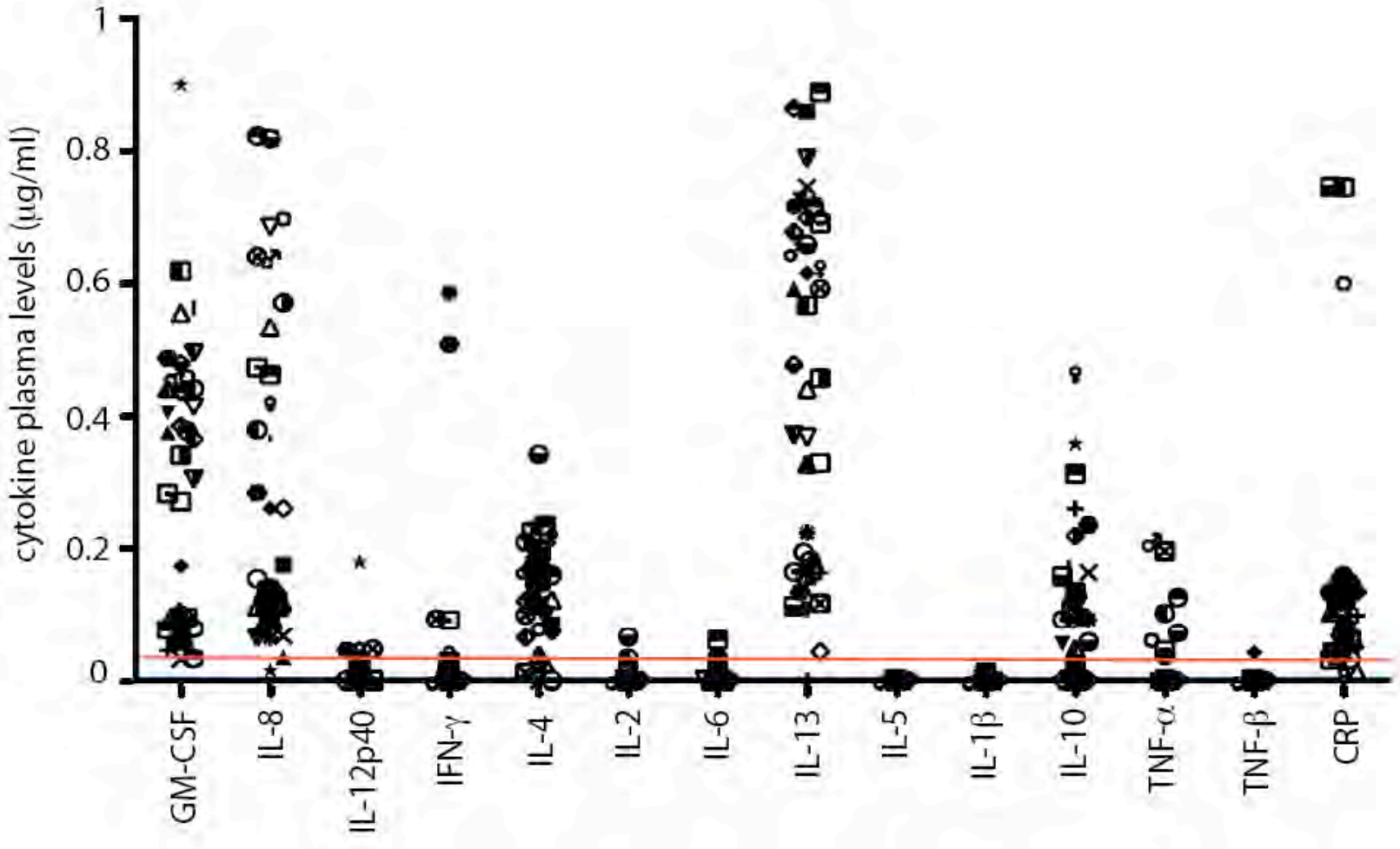
IL-10 production by neutrophils from patients with melanoma inhibits Melan-A $_{26-35}$ specific T cell response



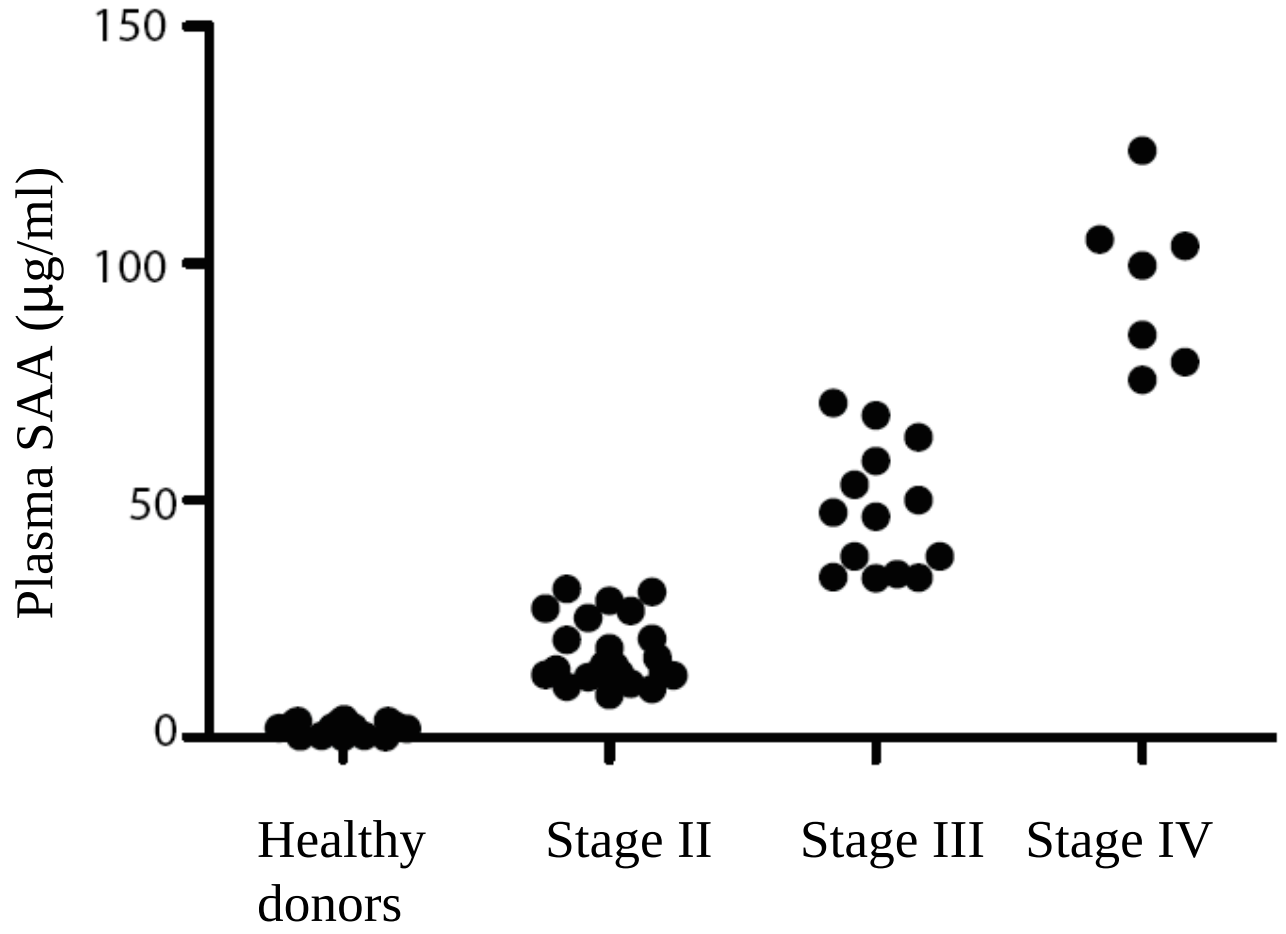
Frequency of neutrophils in patients with melanoma correlates with staging of disease and their suppressive activity



Cytokine concentration in the plasma of patients with melanoma

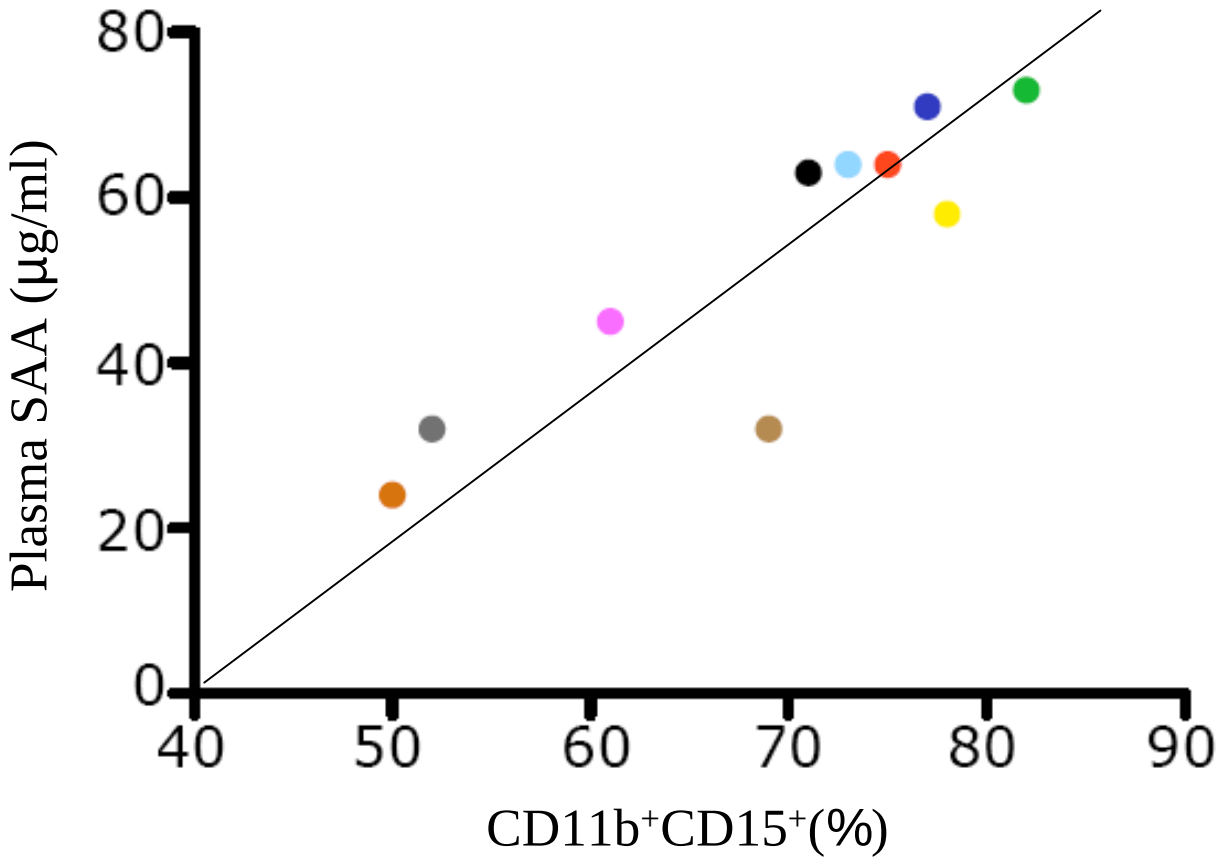


Plasma levels of **Serum Amyloid A (SAA)** in patients with melanoma correlates with disease staging

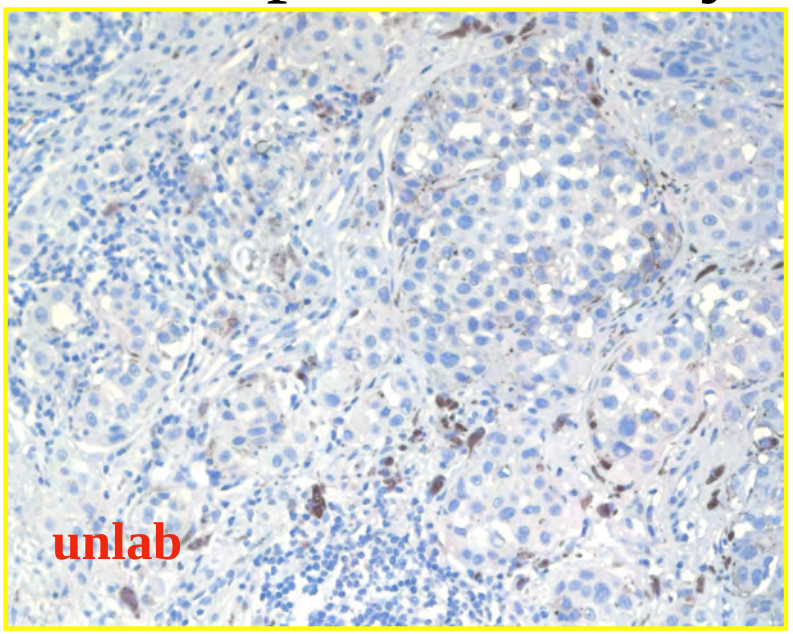


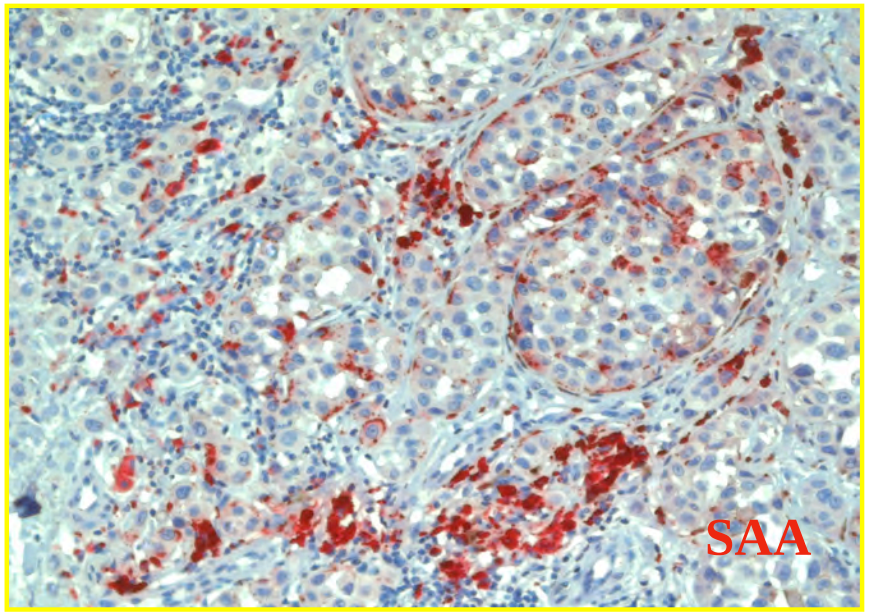
De Santo et al., *Nature Immunology* (in press)

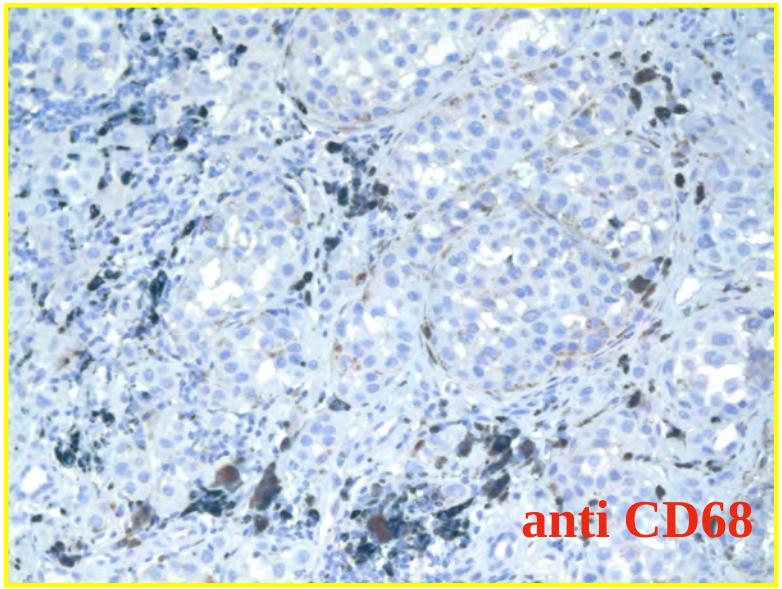
Correlation between frequency of $CD11b^{+}CD15^{+}$ cells and SAA plasma levels

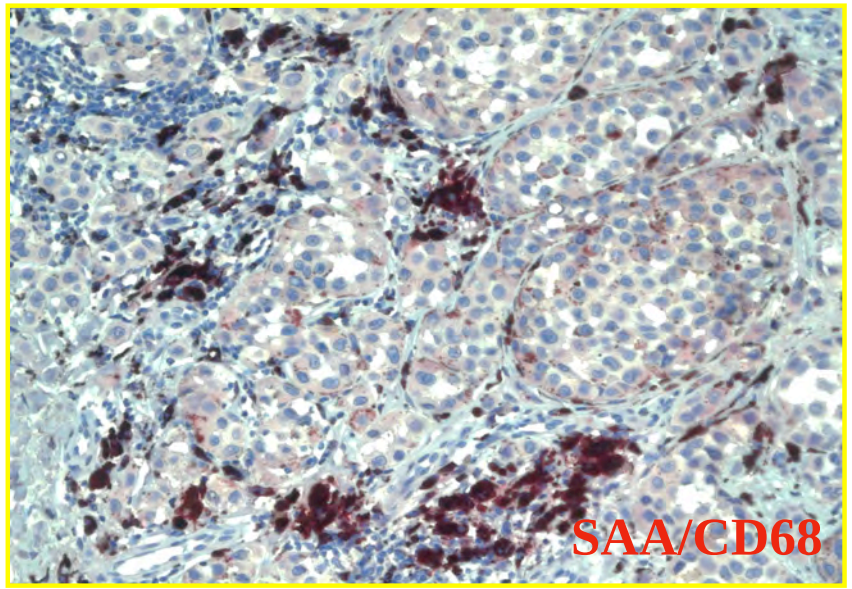


SAA production by TAM and melanoma cells









Serum Amyloid A

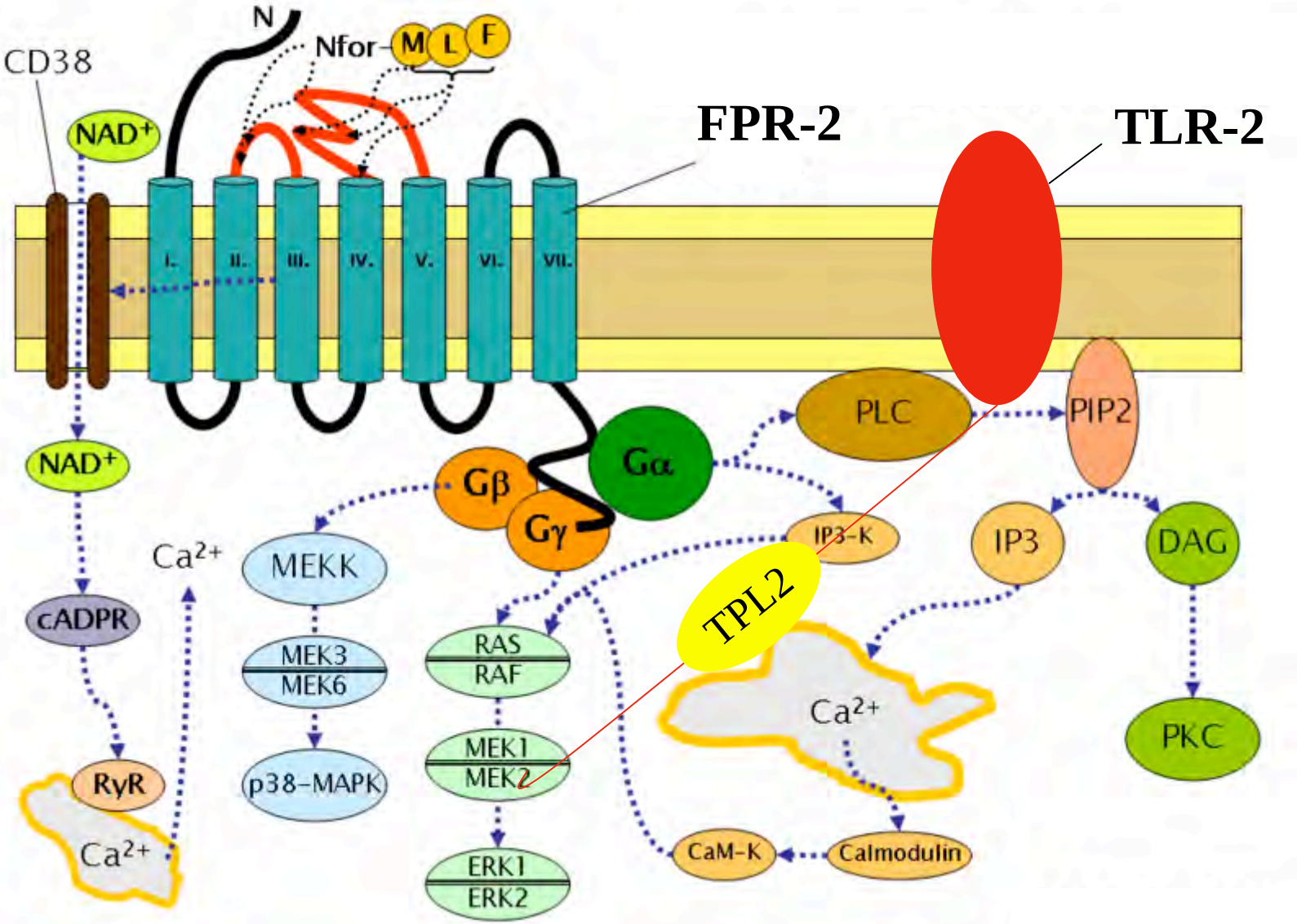
- SAA: 12Kd glycoprotein mainly secreted by hepatocytes during acute and chronic inflammation. It has also been shown to be secreted by pneumocytes ad macrophages

- SAA secretion is induced by IL-6, IL-1β, TNF α, LPS

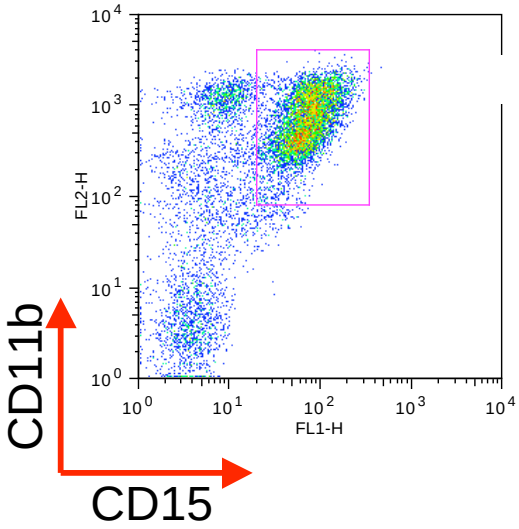
- Described activities: 1) chemoattractant, 2) induces G-CSF secretion and neutrophilia, 3) enhances ROS production, 4) opsonization of bacteria by binding to OmpA family members on G- bacteria

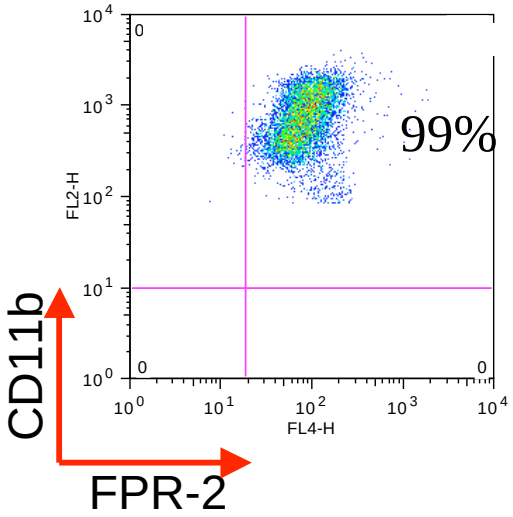
Does incubation of neutrophils with SAA induce IL-10 secretion?

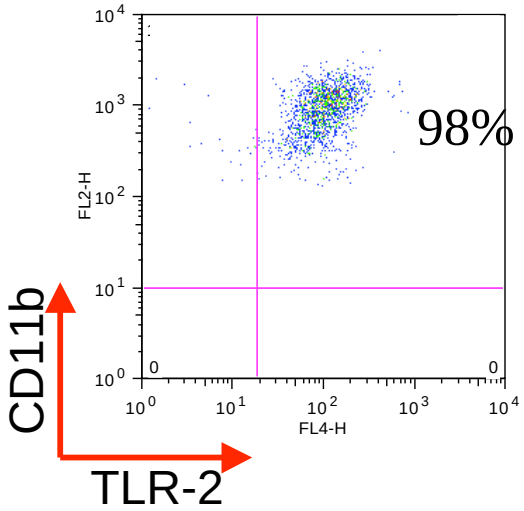
SAA binding receptors



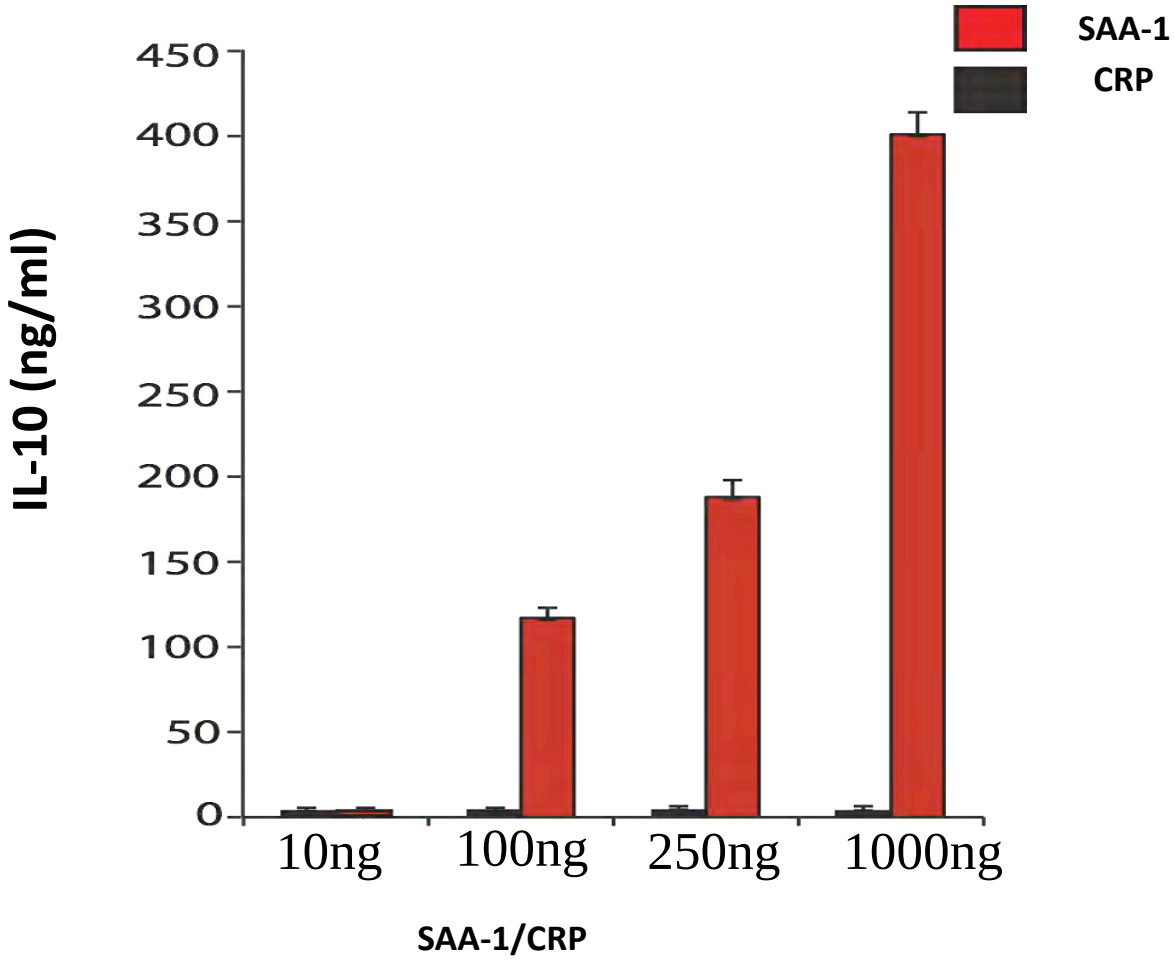
Neutrophils express both FPR2 and TLR2



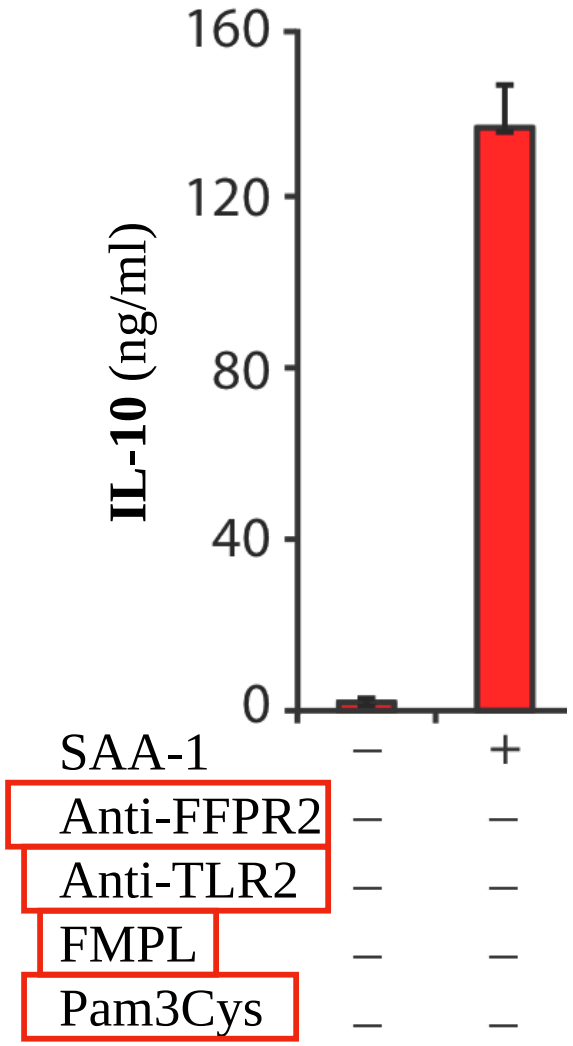




Incubation of neutrophils from healthy donors with SAA induces IL-10 secretion



SAA binding to FPR-2 controls IL-10 secretion



Conclusions (I)

A large proportion of primary melanoma and TAM secrete SAA

SAA controls the differentiation of immunosuppressive IL-10 secreting neutrophils

IL-10 secretion from SAA treated neutrophils is FPR-2 dependent

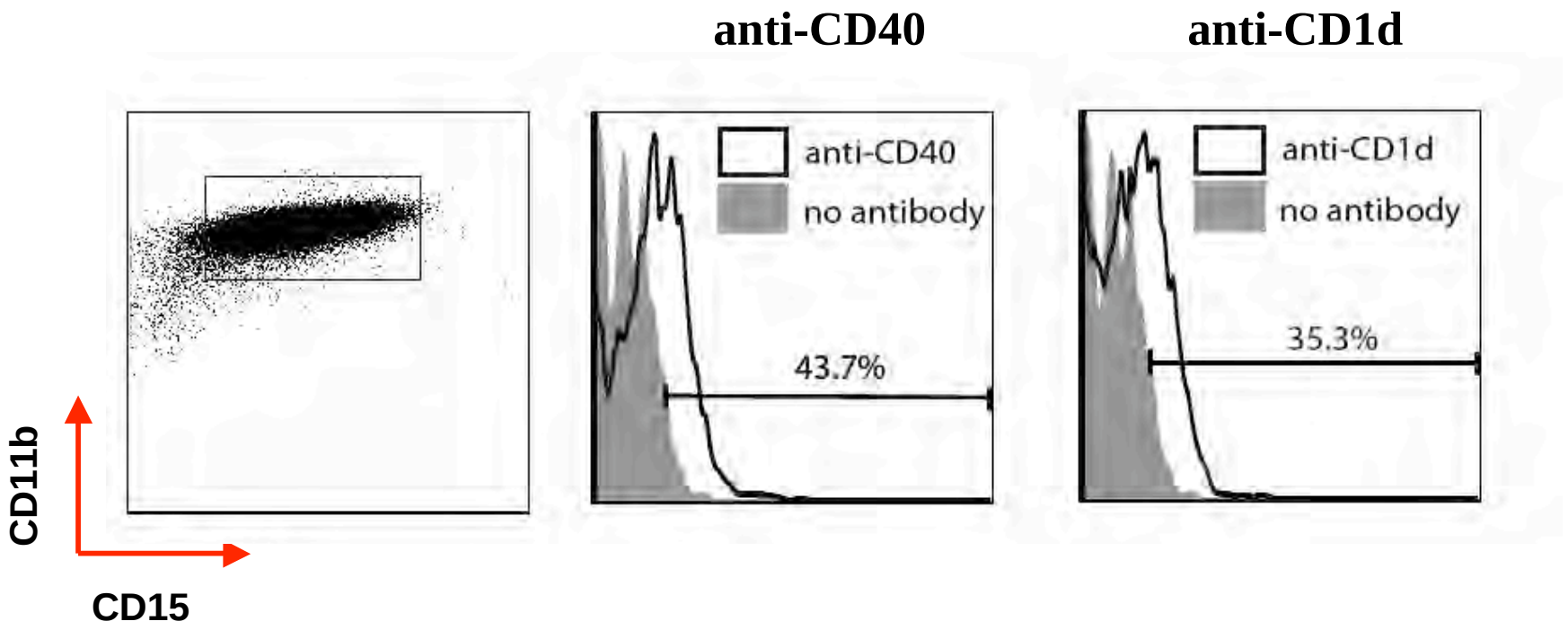
SAA levels in plasma of patients with melanoma correlates with staging of disease and frequency of IL-10 secreting neutrophils

Melanomas exploit of a physiological role of SAA

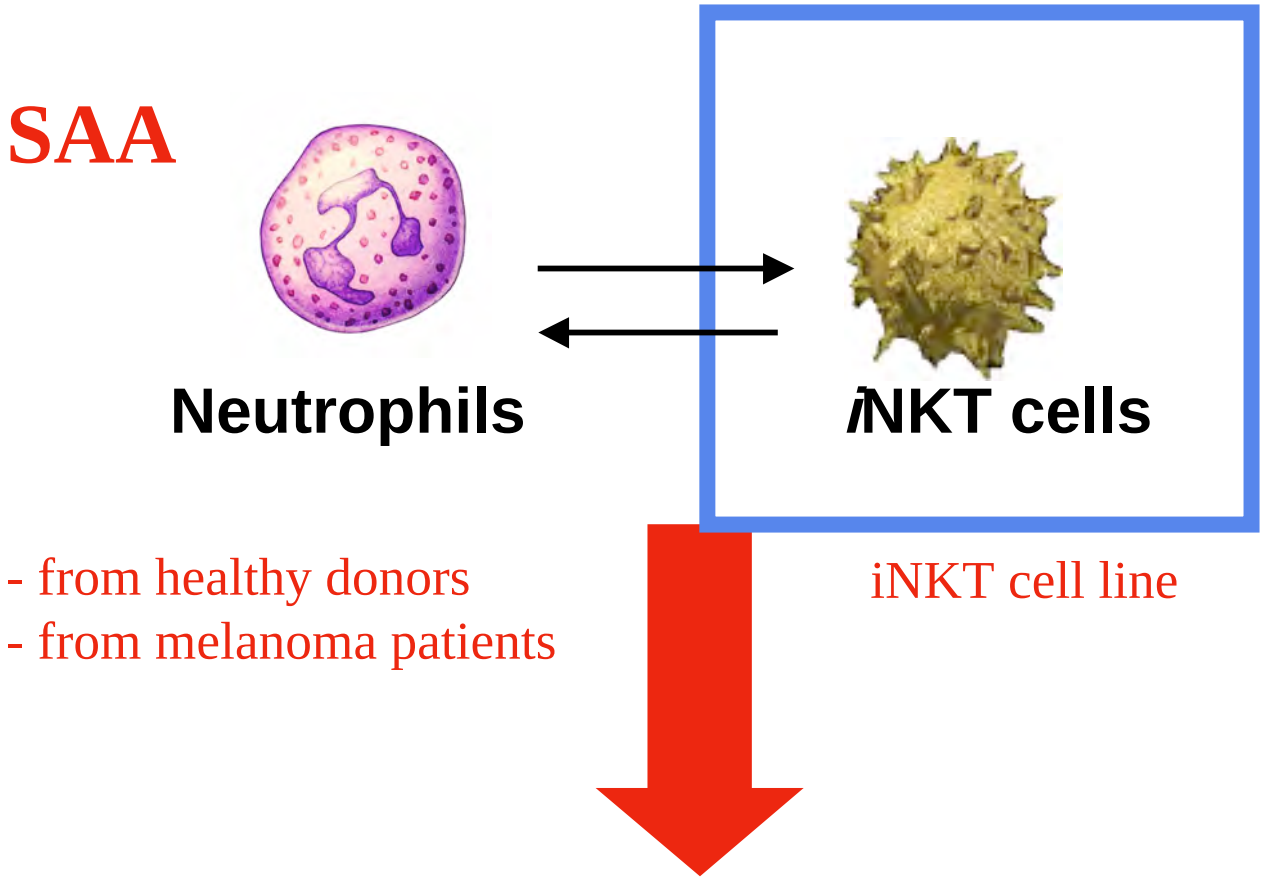
High SAA levels in melanoma patients should be considered a novel tumor evasion mechanism to differentiate IL-10 producing neutrophils

Future vaccine clinical trials in patients with melanoma should combine strategies to reduce IL-10 immunosuppressive activity

Neutrophils are CD1d and CD40 positive

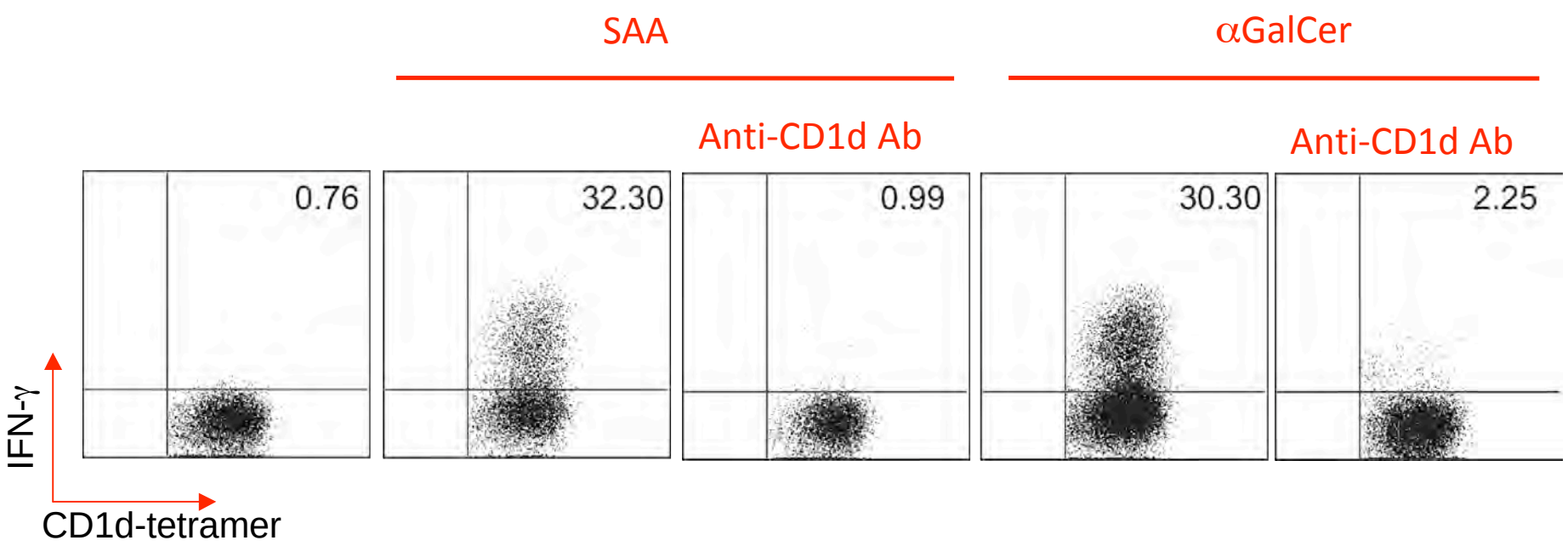


Cross-talk between neutrophils and iNKT cells

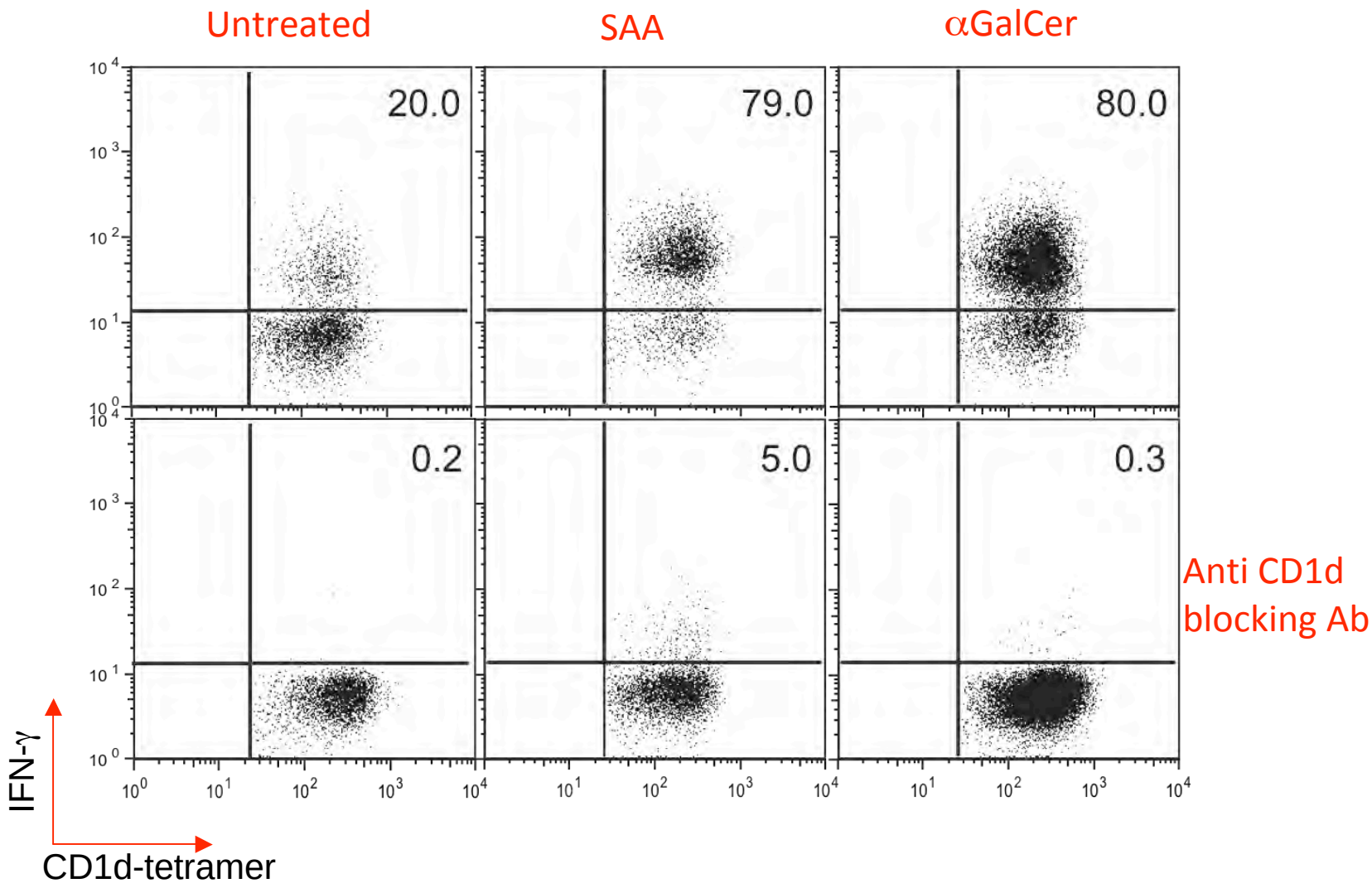


Adaptive Immune Response

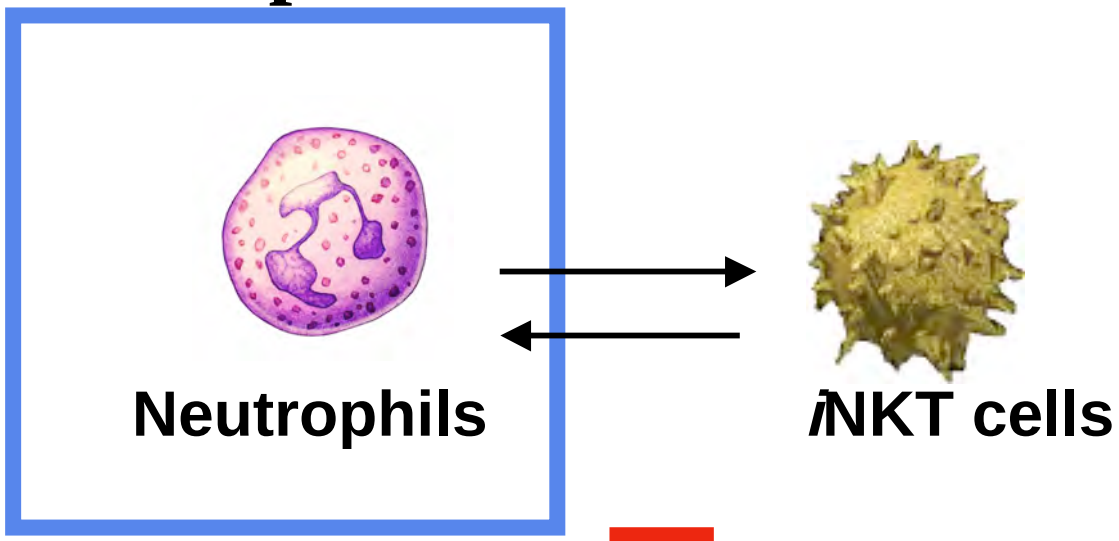
Neutrophils from healthy donors pre-treated with SAA activate iNKT cells



Neutrophils purified from melanoma patients directly activate iNKT cells



Cross-talk between innate and adaptive immune system to optimize vaccination strategies

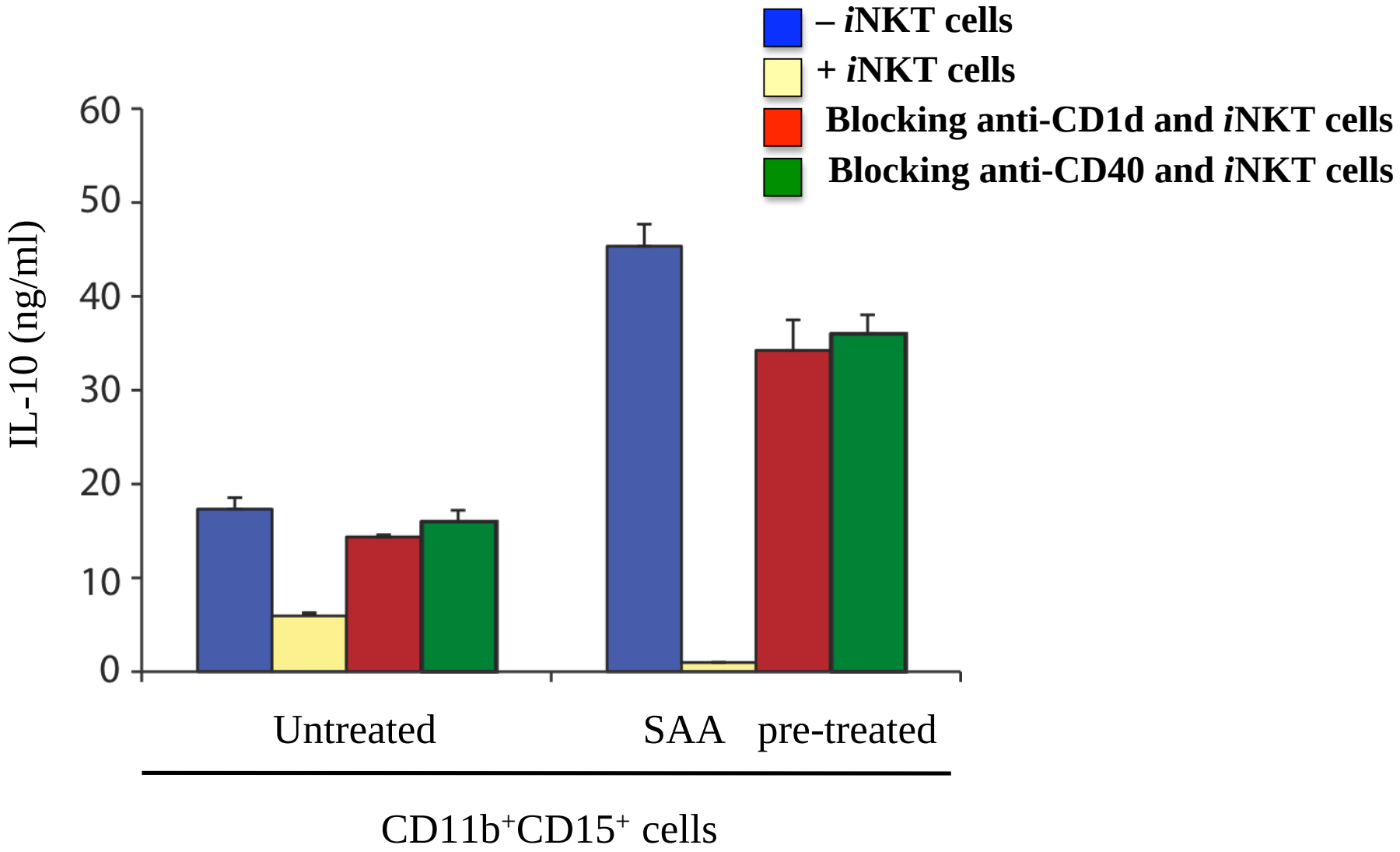


- from melanoma patients

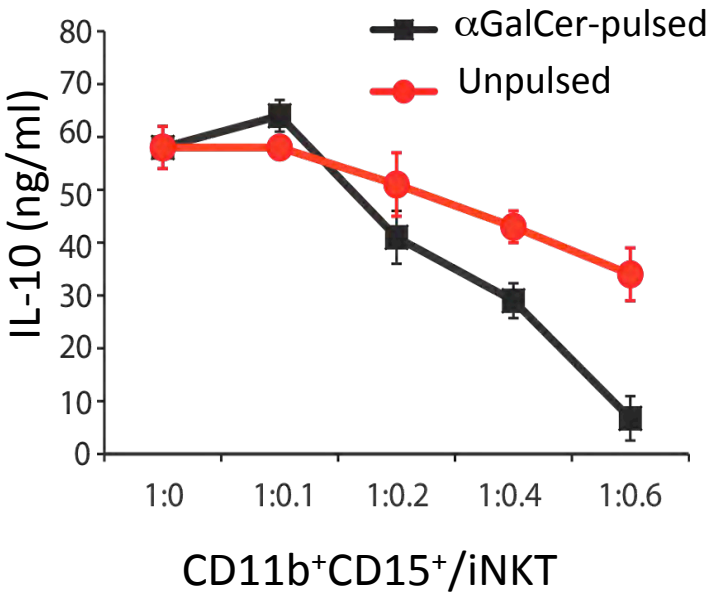
iNKT cell line

Adaptive Immune Response

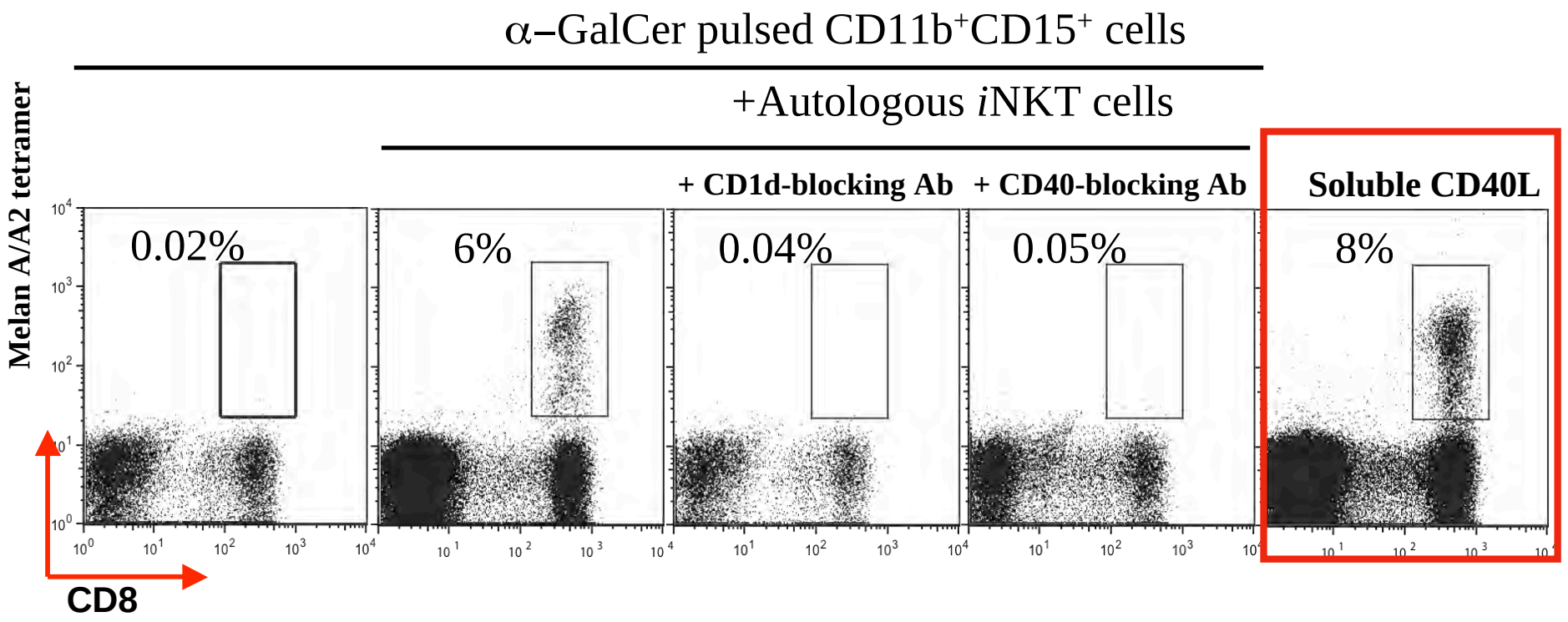
Reduction of IL-10 secretion after incubating neutrophils from melanoma patients with iNKT cells



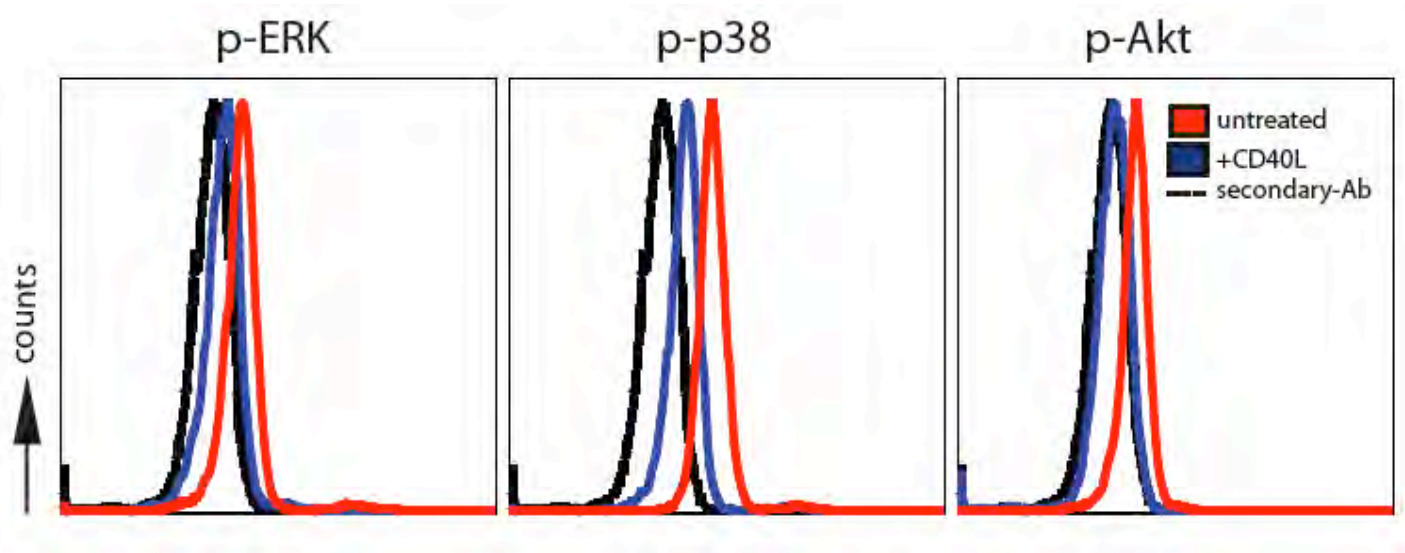
Reduction of IL-10 secretion from SAA treated neutrophils is dependent on the numbers of iNKT cells

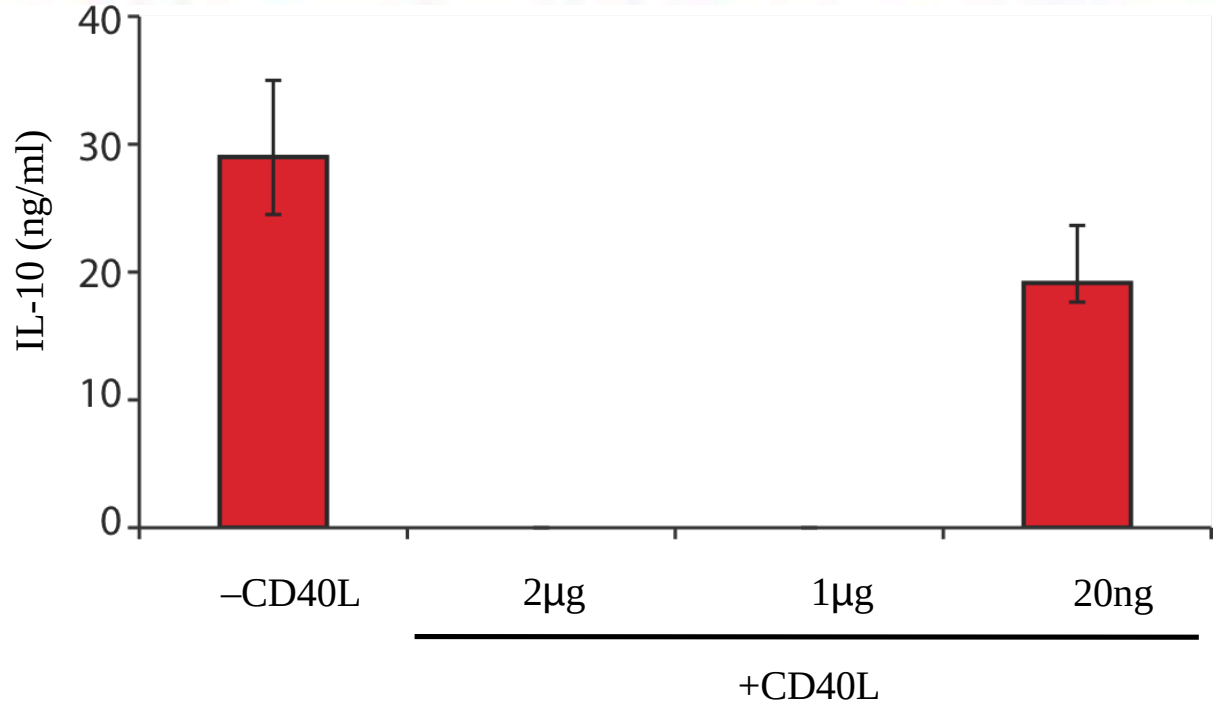


Harnessing iNKT cells abolishes neutrophils' suppressive activity and restores Melan-A_{26-35} specific CD8+ T cell response

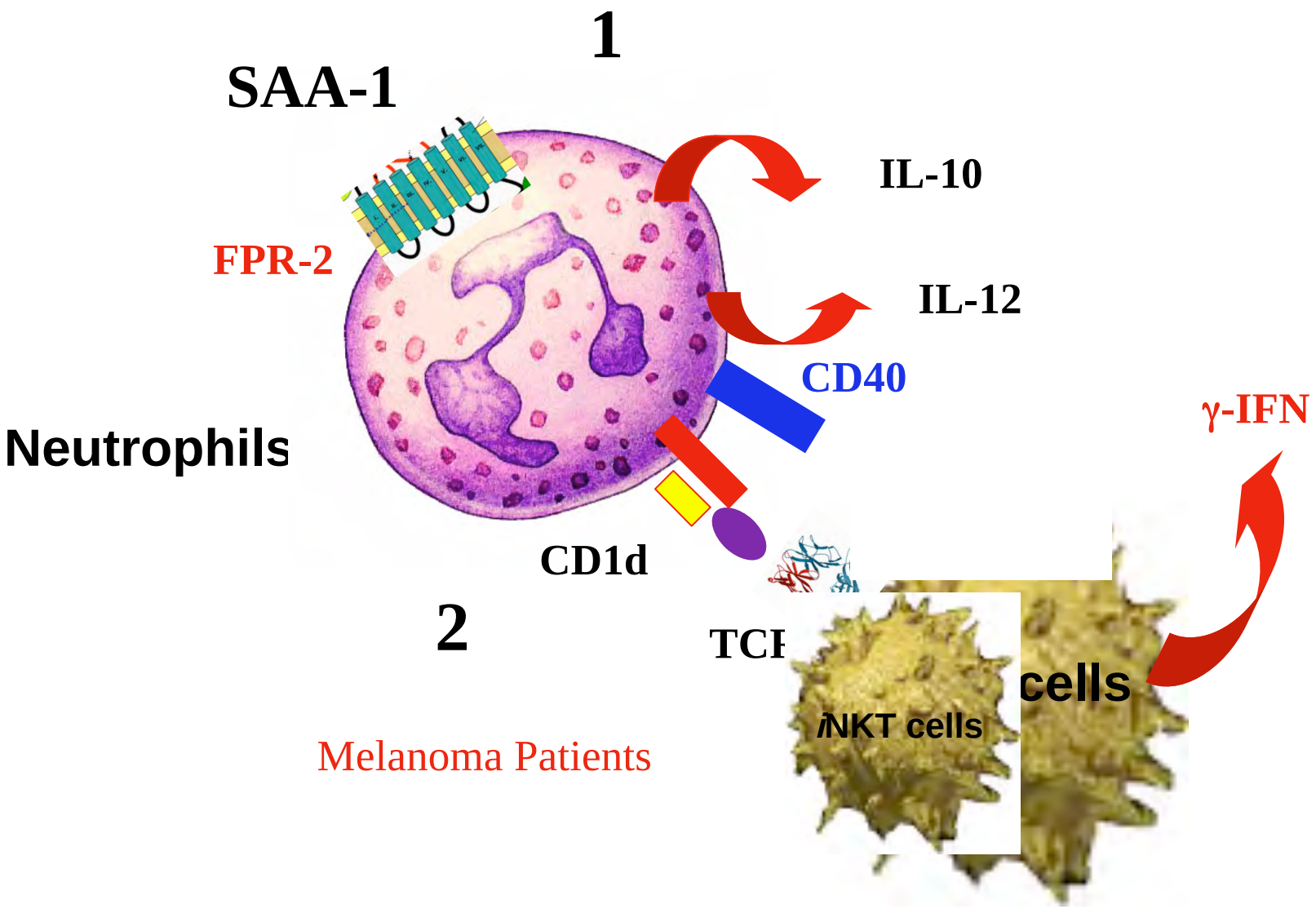


Reduction of IL-10 secretion from SAA treated neutrophils is CD40 dependent

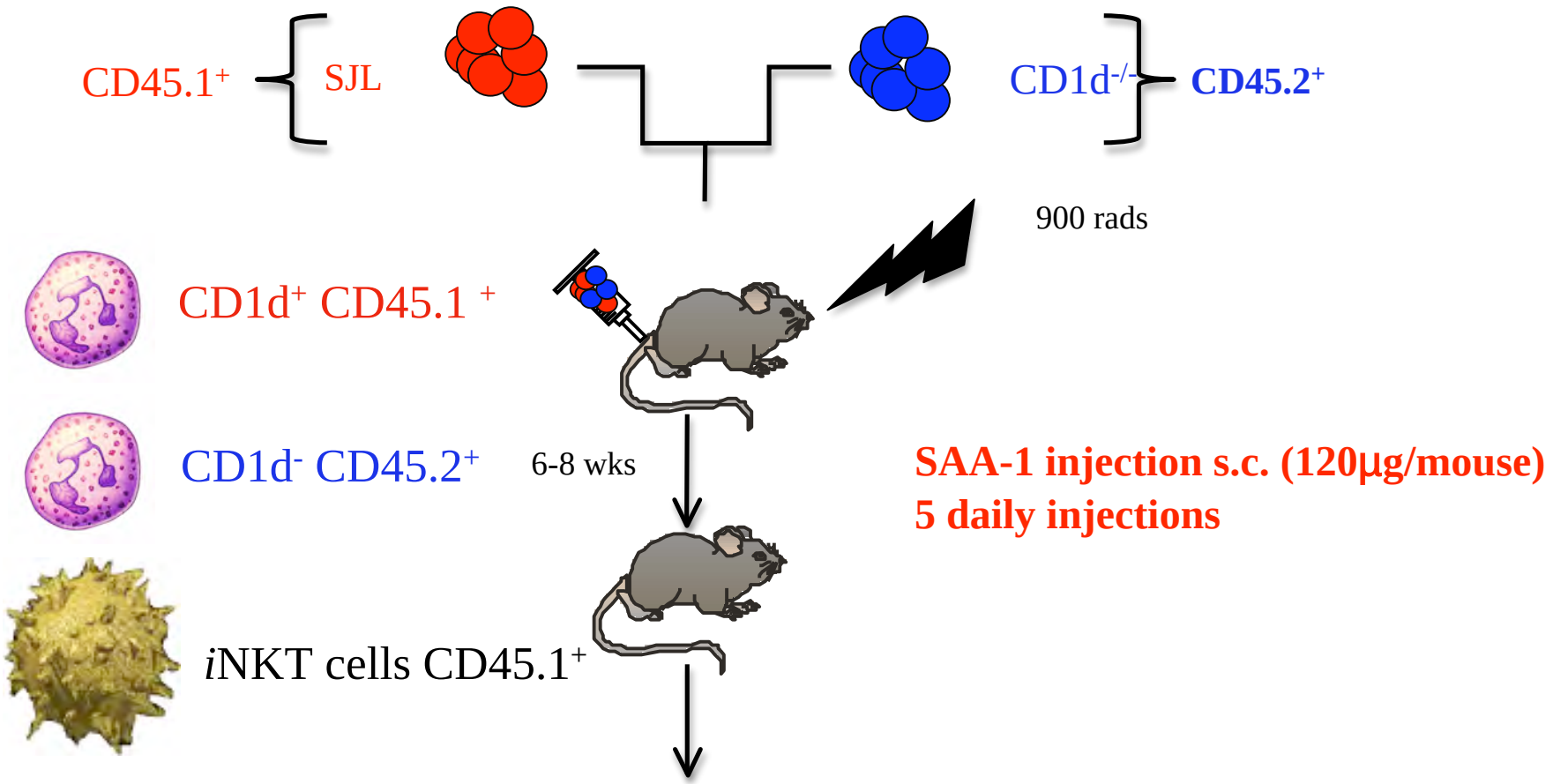




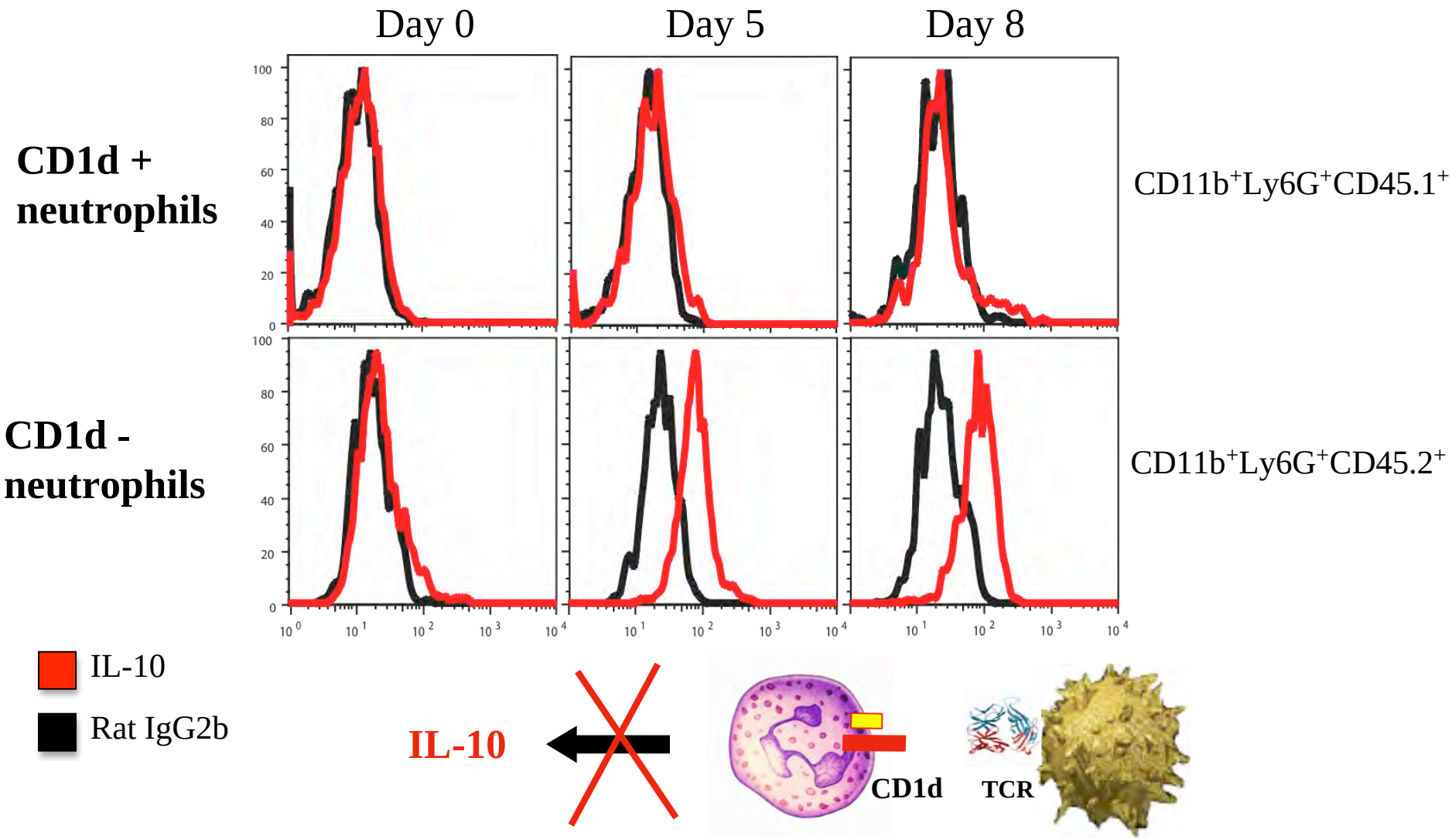
SAA treated neutrophils can interact with iNKT cells



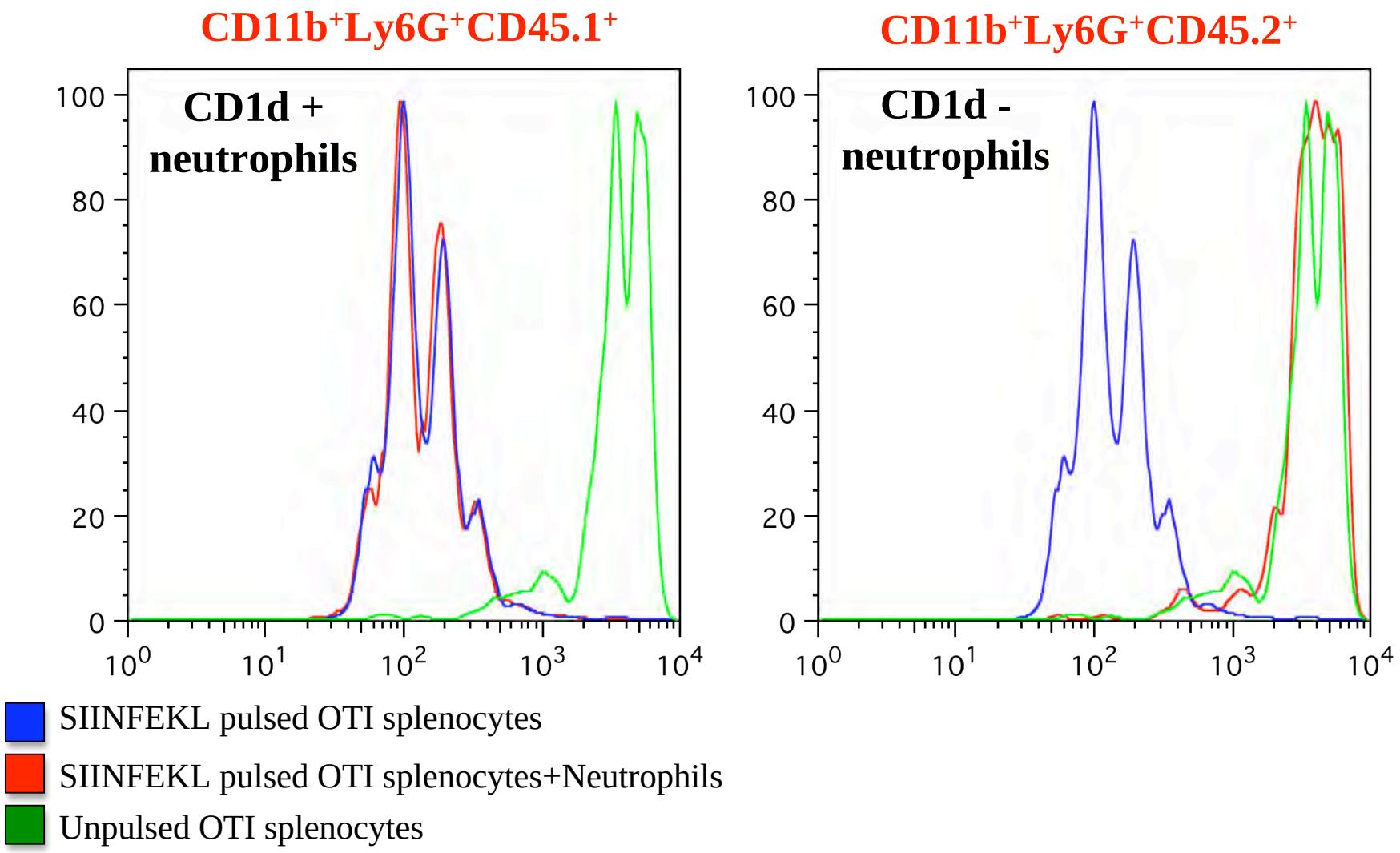
$CD1d^{+/+}/CD1d^{-/-}$ mixed bone marrow chimera mice



Injection of SAA-1 into CD1d mixed bone marrow chimeras results in the expansion of IL-10 neutrophils only from CD1d - neutrophils



CD1d- IL-10 secreting neutrophils from CD1d $^{+/+}$/CD1$^{-/-}$ chimera inhibit OT-I proliferation



Conclusions (II)

- Incubation of neutrophils with SAA facilitates their cross-talk with iNKT cells, which results in iNKT cell activation and reduced secretion of IL-10

- SAA dependent interaction between neutrophils and iNKT cells is CD1d and CD40 dependent

- Injection of SAA in $CD1d^{+/+}$ /$CD1d^{-/-}$ mixed bone marrow chimeras induces IL-10 secretion mainly from CD1d- neutrophils

Harnessing iNKT cells should be considered in melanoma patients to reduce the activity of immunosuppressive neutrophils

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