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DLL4 conveys Notch-dependent signals achieving selective macrophage differentiation or death

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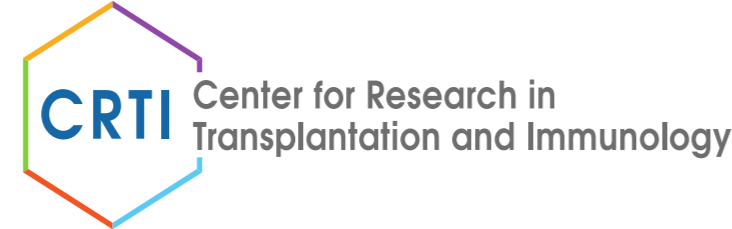
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DLL4 conveys Notch-dependent signals achieving selective macrophage differentiation or death

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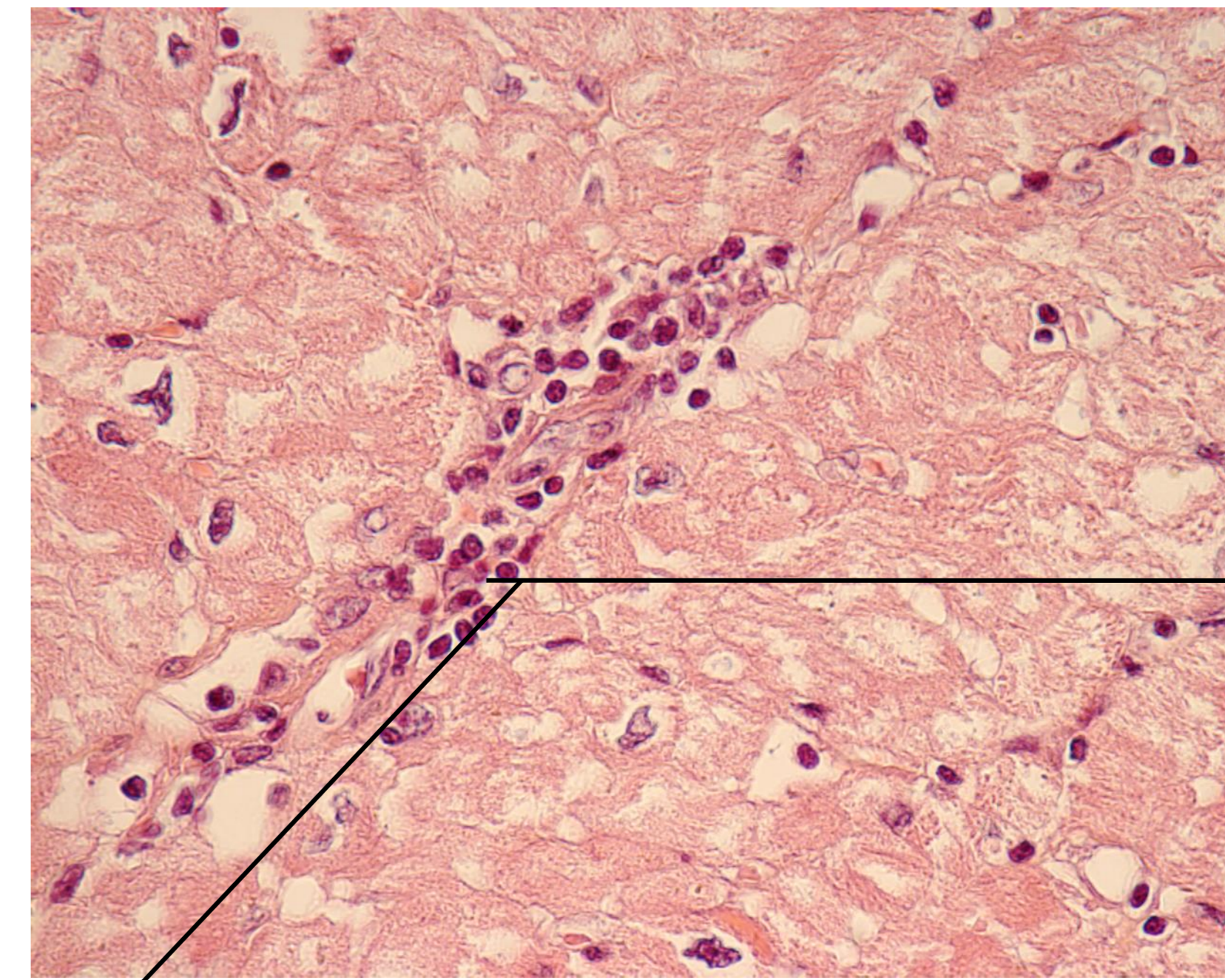


Centre de Recherche en Transplantation et Immunologie, INSERM UMR1064, Nantes

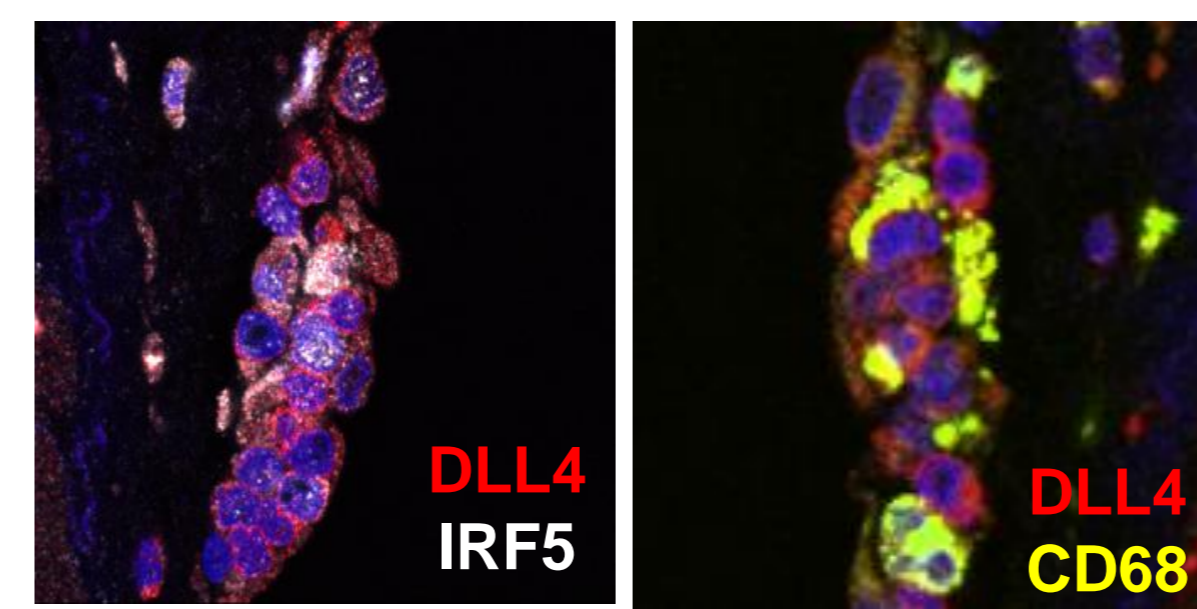
Summary

Molecular mechanisms underlying vascular and inflammatory cell network at endothelial and macrophage levels are still unclear. Here we found that microvascular inflammation associates with changes in Notch signaling at endothelium/monocyte interface including loss of endothelial Notch4 and the acquisition of the Notch ligand DLL4 in both cell types. We showed that endothelial DLL4 induces circulating monocytes to polarize into a M1-type pro-inflammatory macrophages (CD40^{high}CD64^{high}CD200R^{low} HLA-DR^{low}CD11b^{low}) eliciting the production of IL-6. DLL4 and IL-6 are both Notch-dependent and are required for macrophage polarization through selective down and upregulation of M2- and M1-type markers, respectively. Subsequently, we investigated the ability of DLL4 to interfere with M2 polarization. We found that DLL4 triggers a specific alteration of the IL-4 induced M2 phenotype through a significant inhibition of M2 markers (CD11b, CD206, CD200R). DLL4 also induces caspase3/7-dependent apoptosis specifically in M2 differentiating macrophages while DLL1 had no effect. DLL4 signals via Notch1 and DLL4-mediated apoptosis is Notch-dependent. Fully differentiated M2 macrophages became resistant to DLL4 action. DLL4 upregulates gene expression, upon M2 upon differentiation, affecting the Notch pattern (Notch1, 3, Jag1) and activity (Hes1), transcription (IRF5, STAT1) that associates with decrease in Akt but not STAT6 phosphorylation. In conclusion, our findings reveal an interplay between DLL4/Notch and IL-6/IL-6R or IL-4/IL-4R signaling pathways supporting M1 differentiation and impairing M2 differentiation via apoptosis.

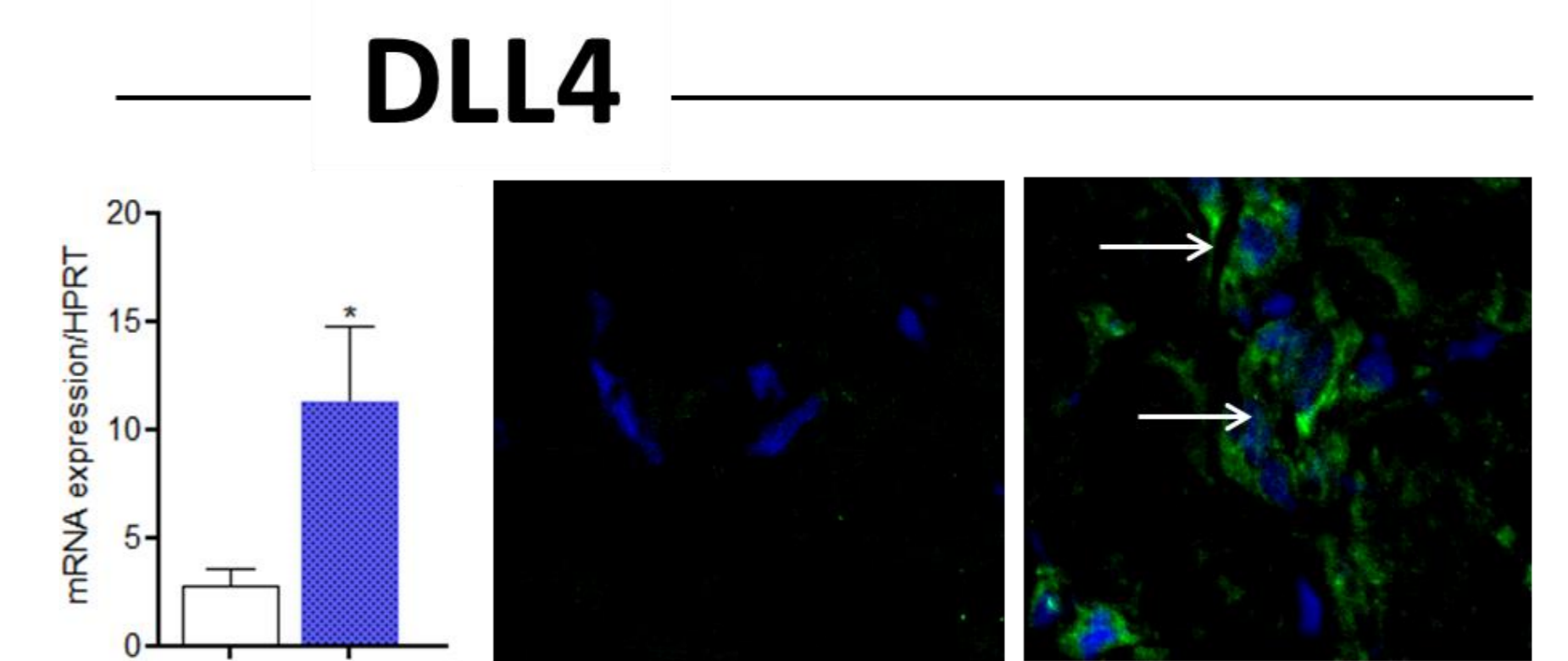
Microvascular inflammation in human cardiac allograft: a crosstalk between macrophages and graf's endothelial cells (ECs).



ECs and intravascular macrophages acquire DLL4 upon microvascular inflammation



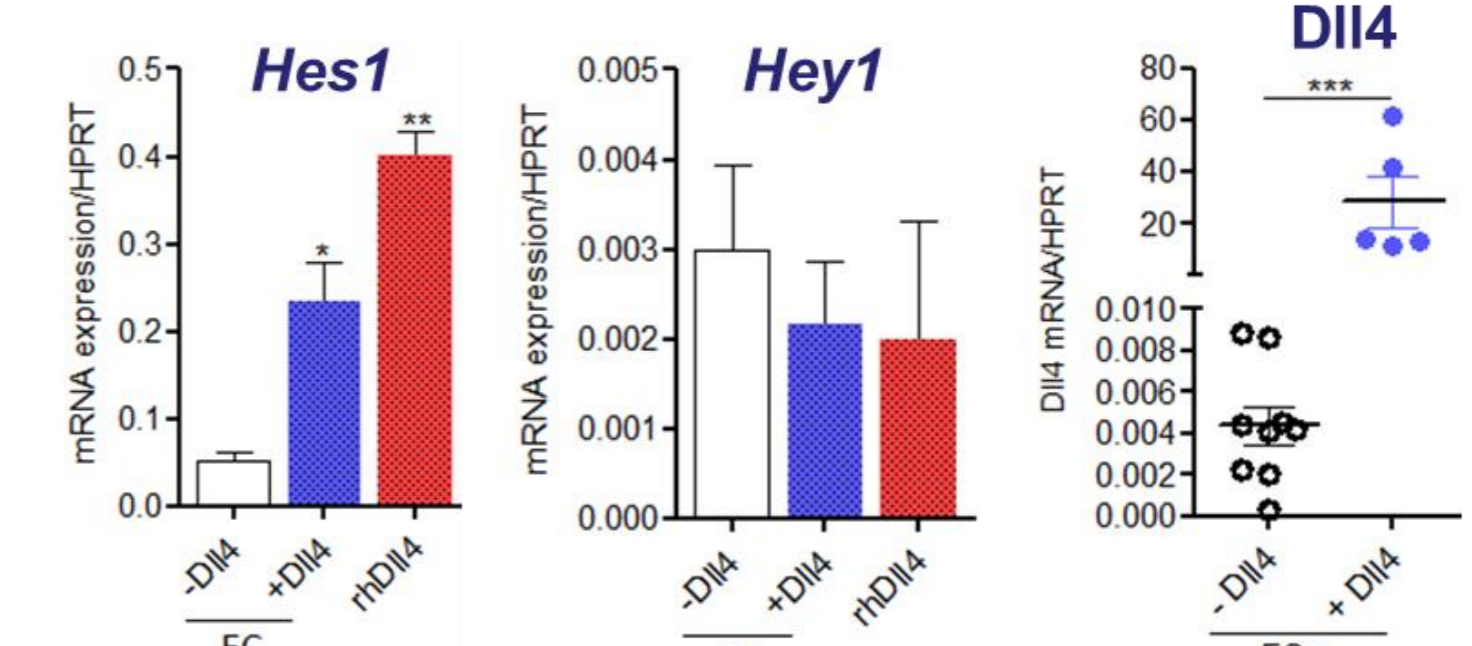
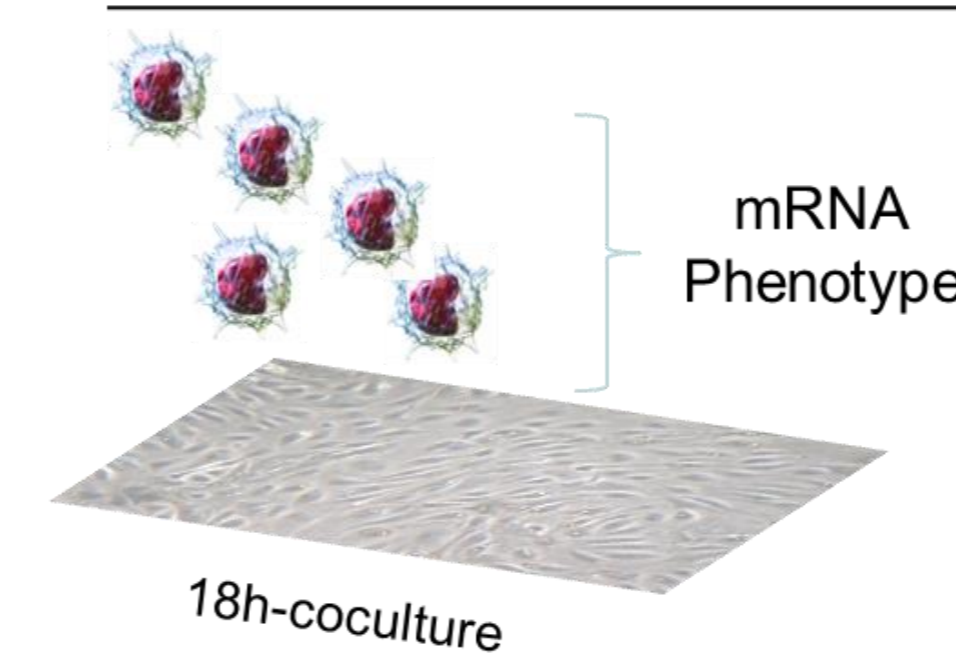
Infiltrating macrophages are M1 macrophages



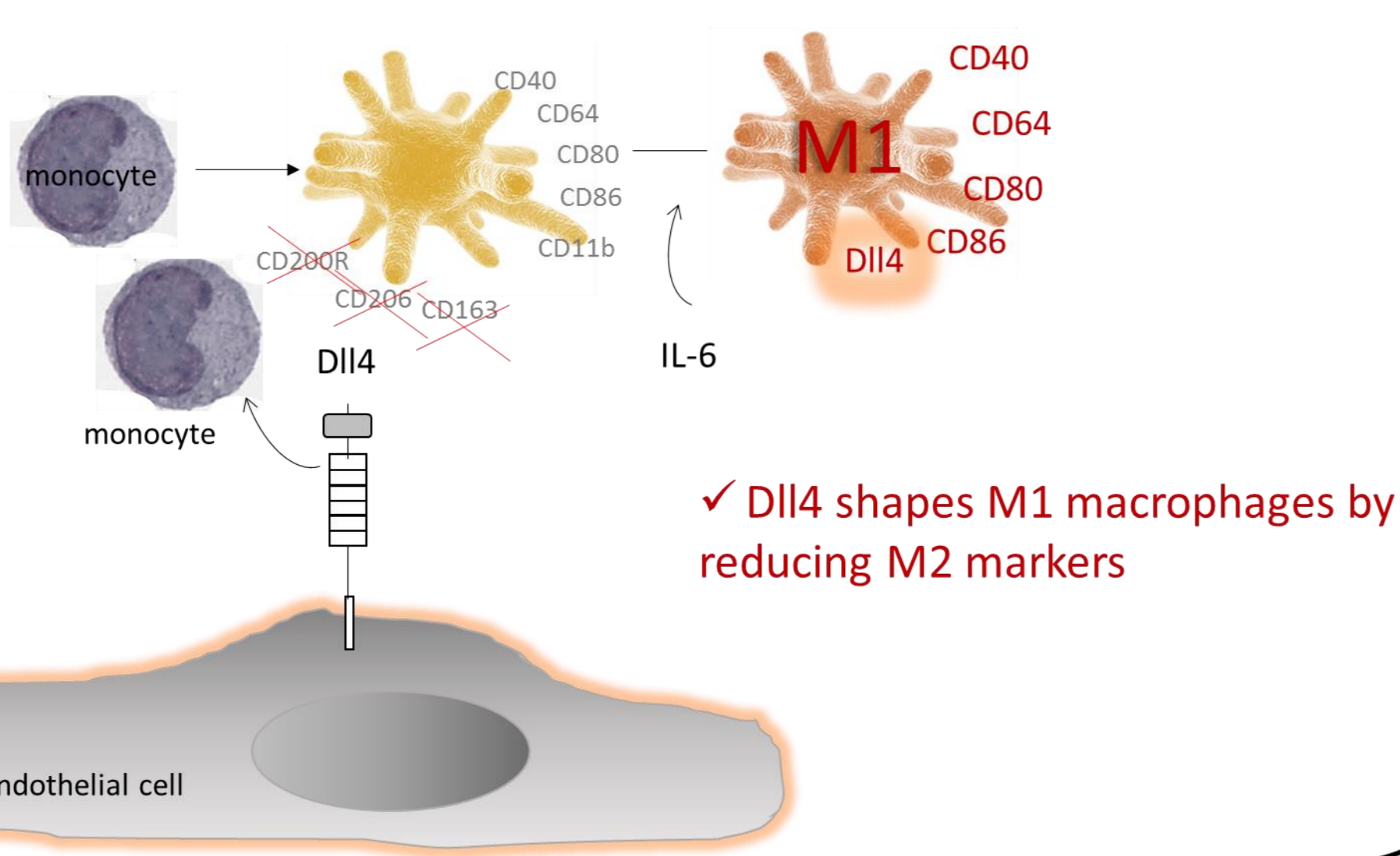
Endomyocardial biopsies without (-) or with (+) microvascular inflammation (Immunostaining for DLL4 in green)

Endothelial DLL4 induces DLL4 on monocytes *in vitro*:

Purified CD14⁺ monocytes + primary EC cocultures expressing DLL4



Upon inflammation, DLL4 is expressed by activated endothelial cells and by M1 macrophages



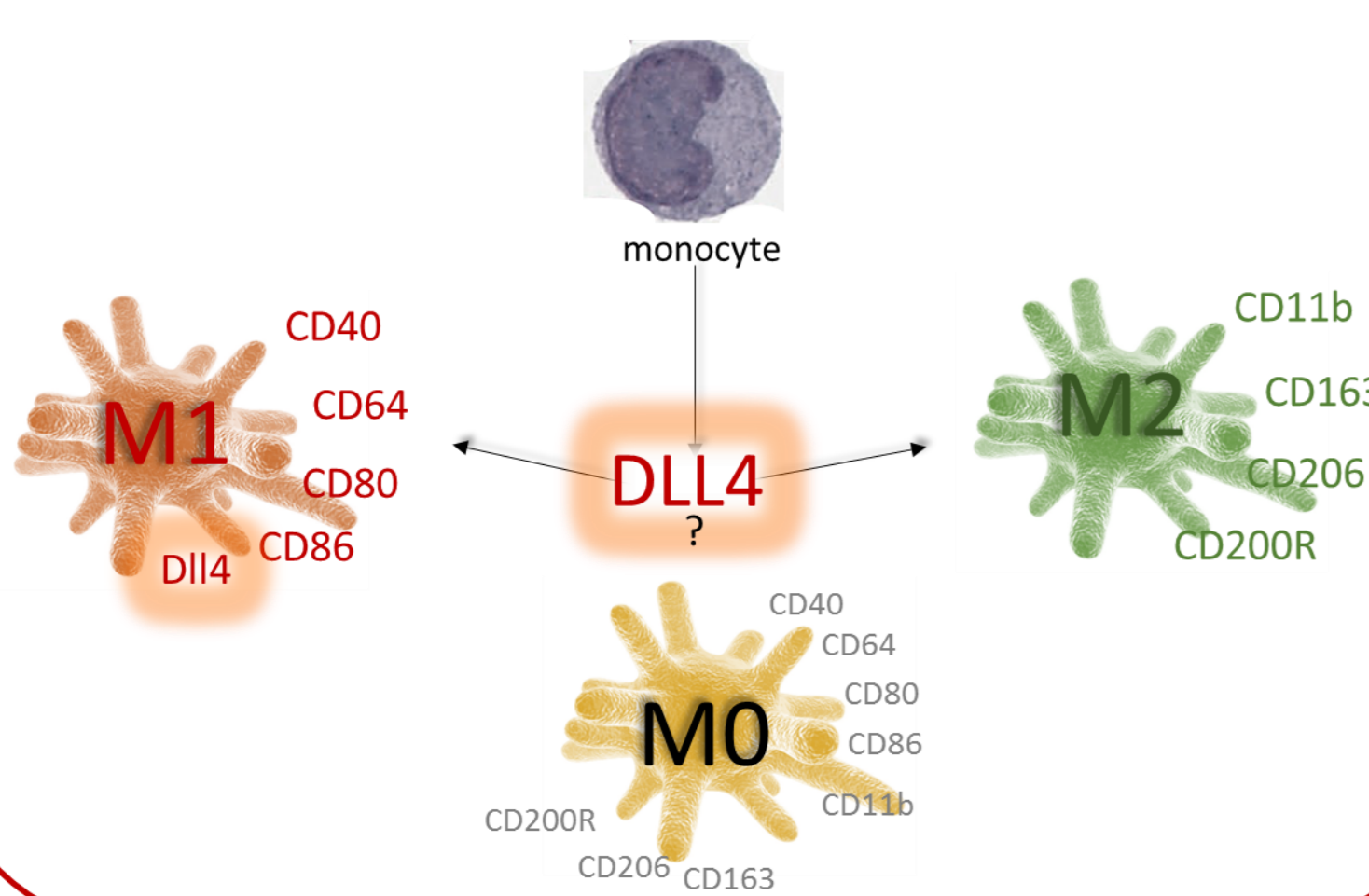
✓ DLL4 shapes M1 macrophages by reducing M2 markers

DLL4 engages a functional crosstalk between ECs and monocytes promoting M1 macrophage polarization and intravascular proinflammatory mediators

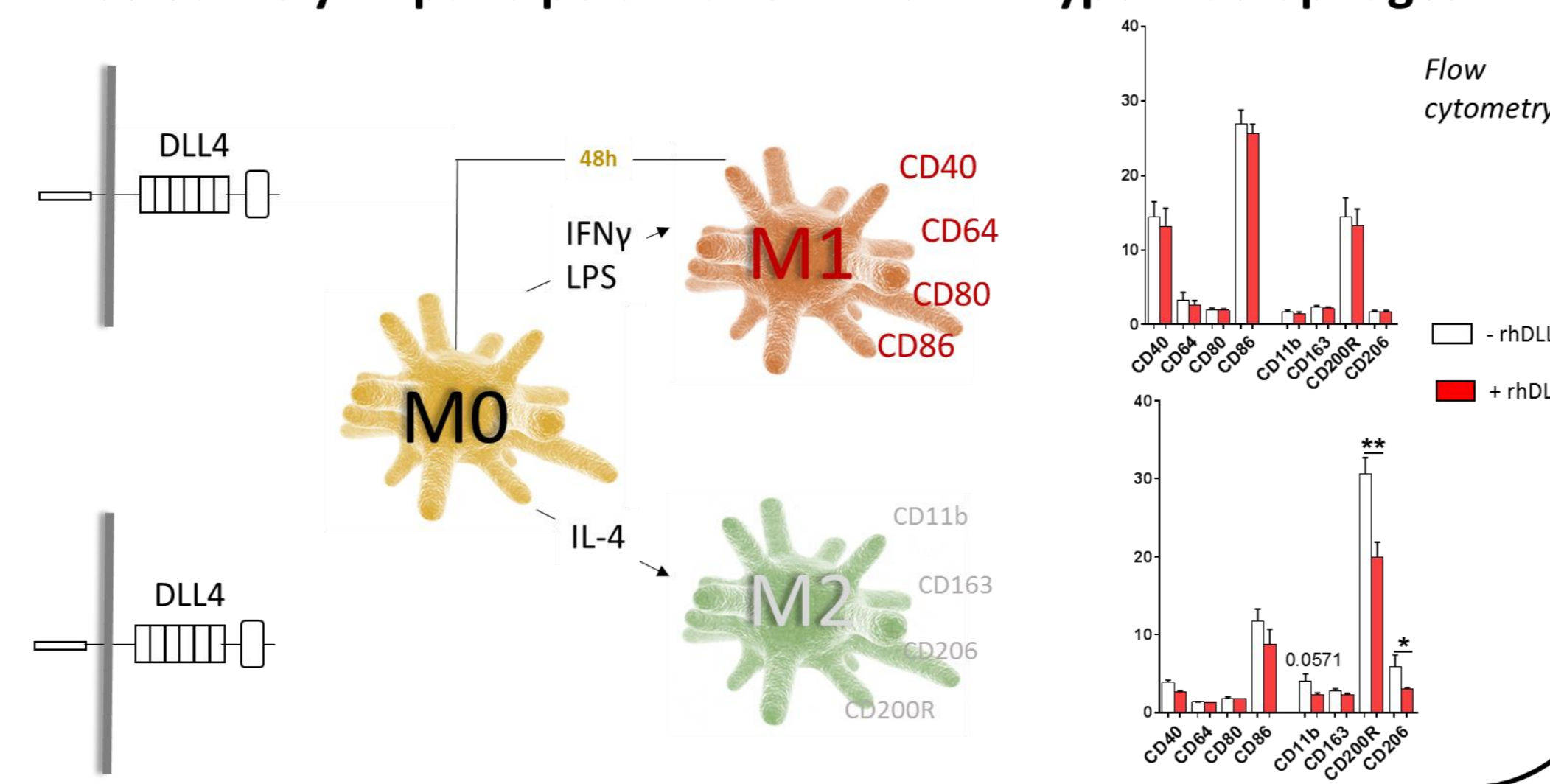
Pabois A., Pagie S. et al., *Biochem. Pharmacol.*, 2016.

Aim of our study:

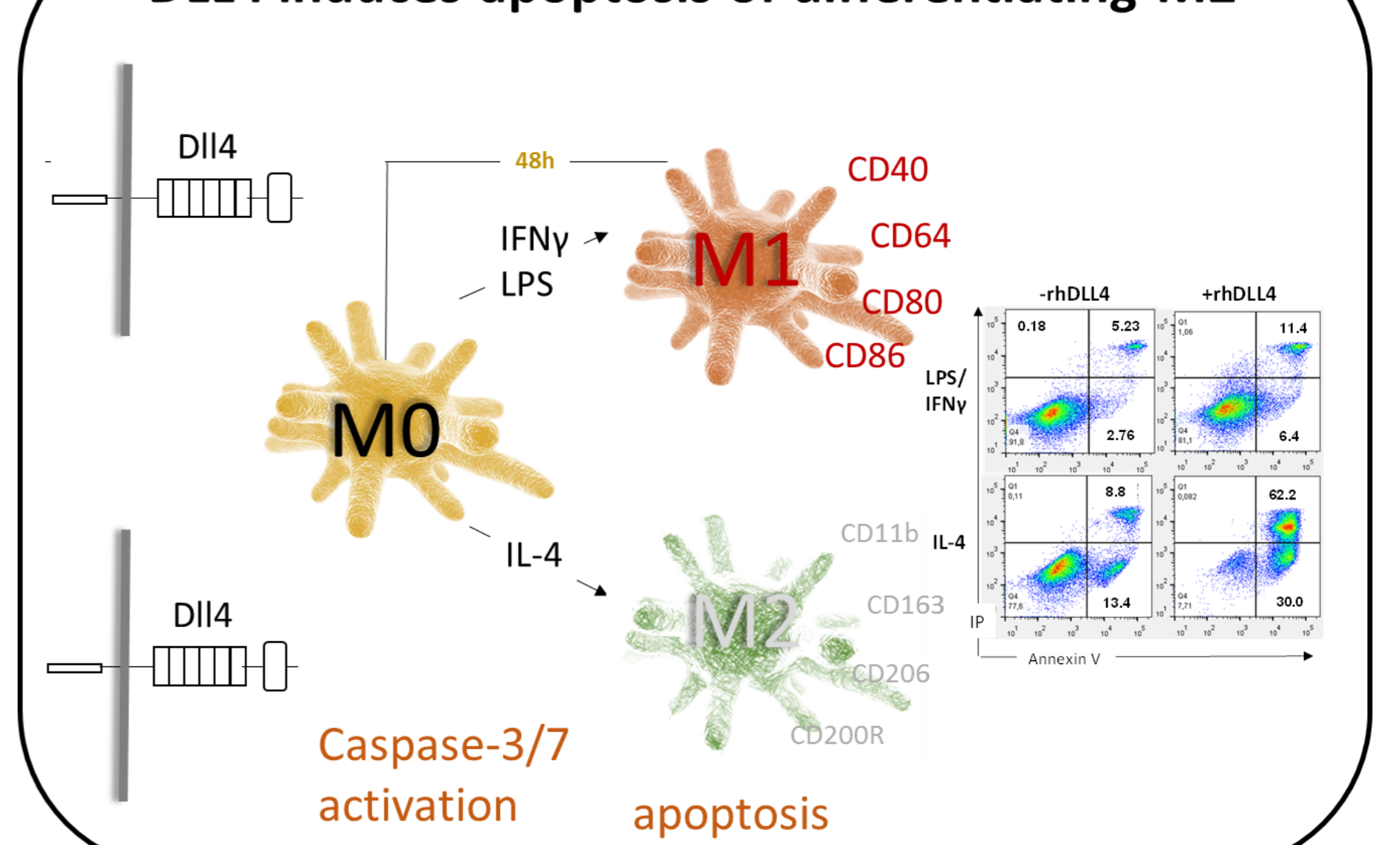
Deciphering the role of the Notch ligand DLL4 in macrophage differentiation and polarization



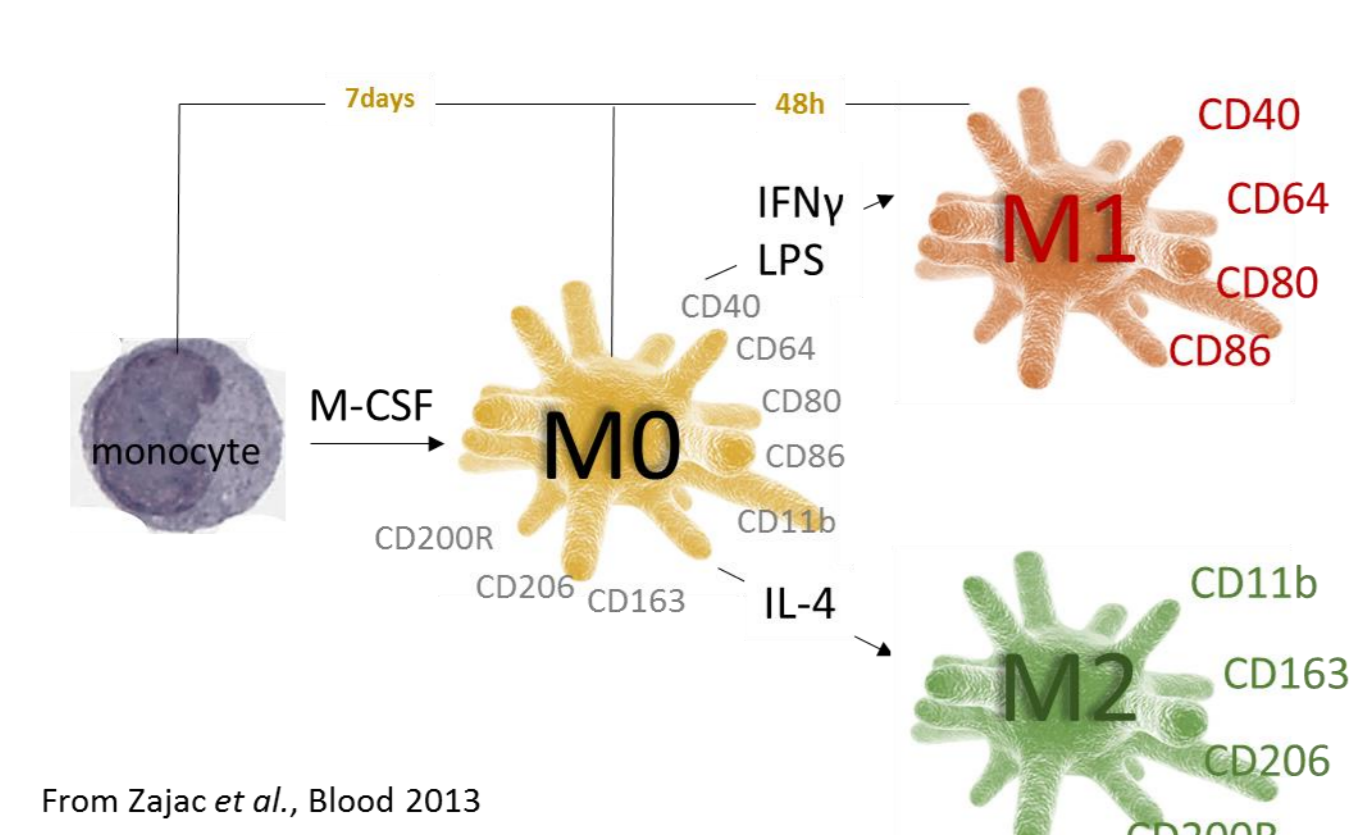
DLL4 selectively impairs polarization into M2-type macrophages



DLL4 induces apoptosis of differentiating M2

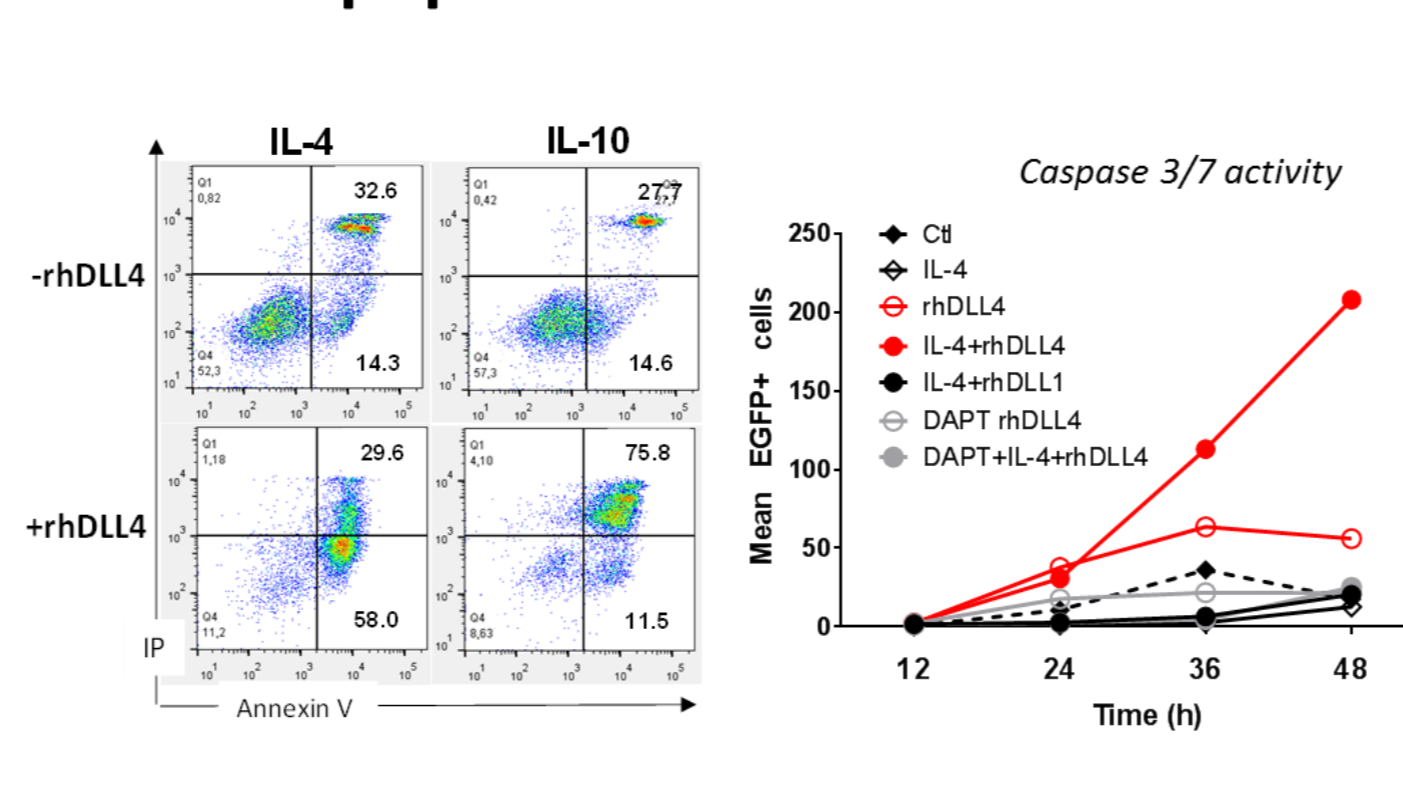


Protocol Macrophage Differentiation & Polarization *in vitro*

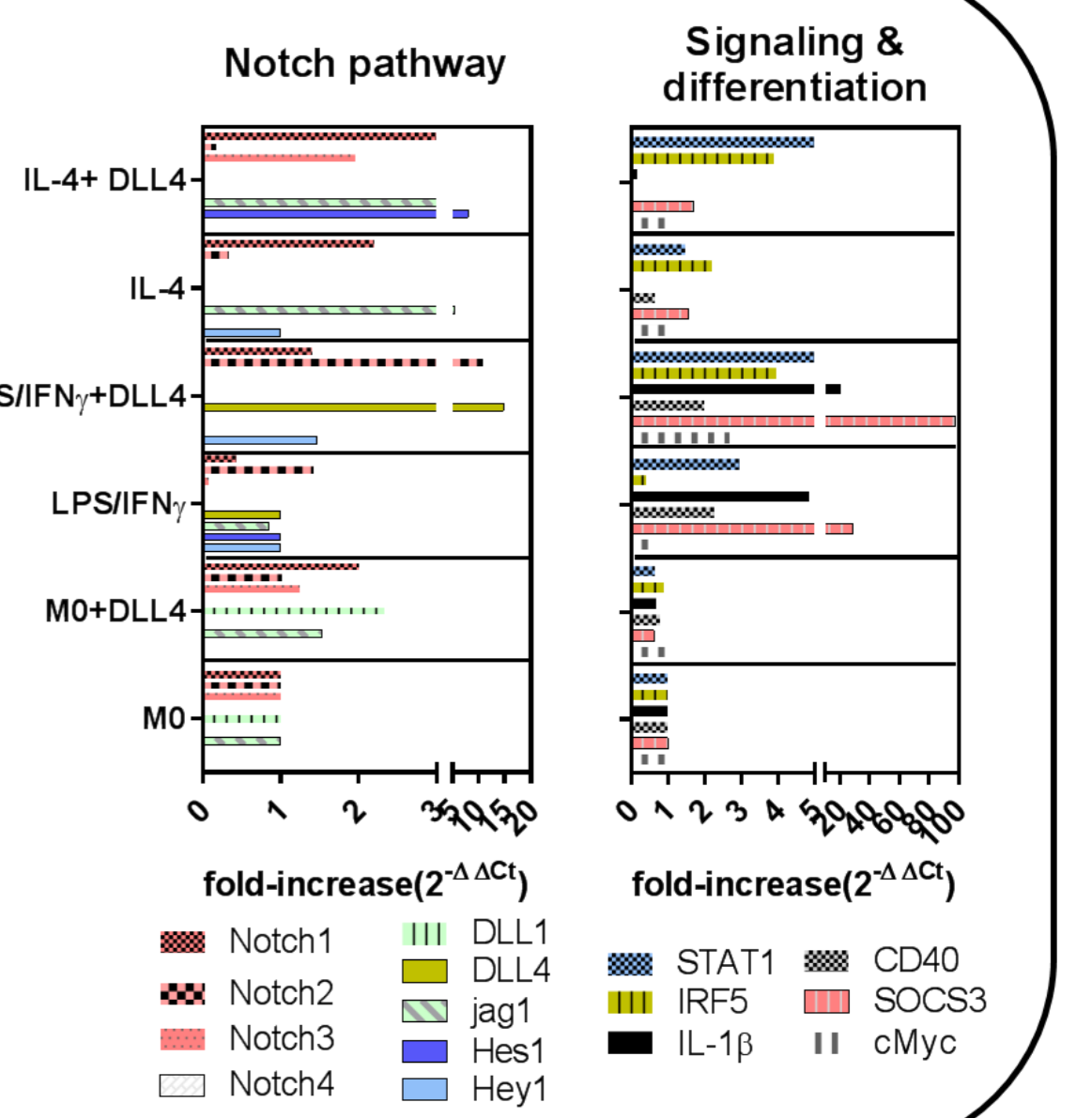


From Zajac et al., *Blood* 2013

DLL4-mediated apoptosis is Notch-dependent DLL4 induces apoptosis of M2a (IL-4) and M2c (IL-10) DLL1 has no apoptotic effect



DLL4 upregulates gene expression, upon M2 upon differentiation, affecting the Notch pattern (Notch1, 3, Jag1) and activity (Hes1), transcription (IRF5, STAT1)



Pagie S. et al., 2017, *in revision*.

In conclusion,

Our findings reveal an interplay between DLL4/Notch and IL-6/IL-6R or IL-4/IL-4R signaling pathways supporting M1 differentiation and impairing M2 differentiation via apoptosis.