



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

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► To cite this version:

S. Pagie, Angélique Pabois, N. Gérard, Christian Labois, Sabine Pattier, et al.. DLL4 conveys Notch-dependent signals achieving selective macrophage differentiation or death. The Notch Meeting X, Oct 2017, Athènes, Greece. inserm-02159948

HAL Id: inserm-02159948

<https://inserm.hal.science/inserm-02159948>

Submitted on 19 Jun 2019

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

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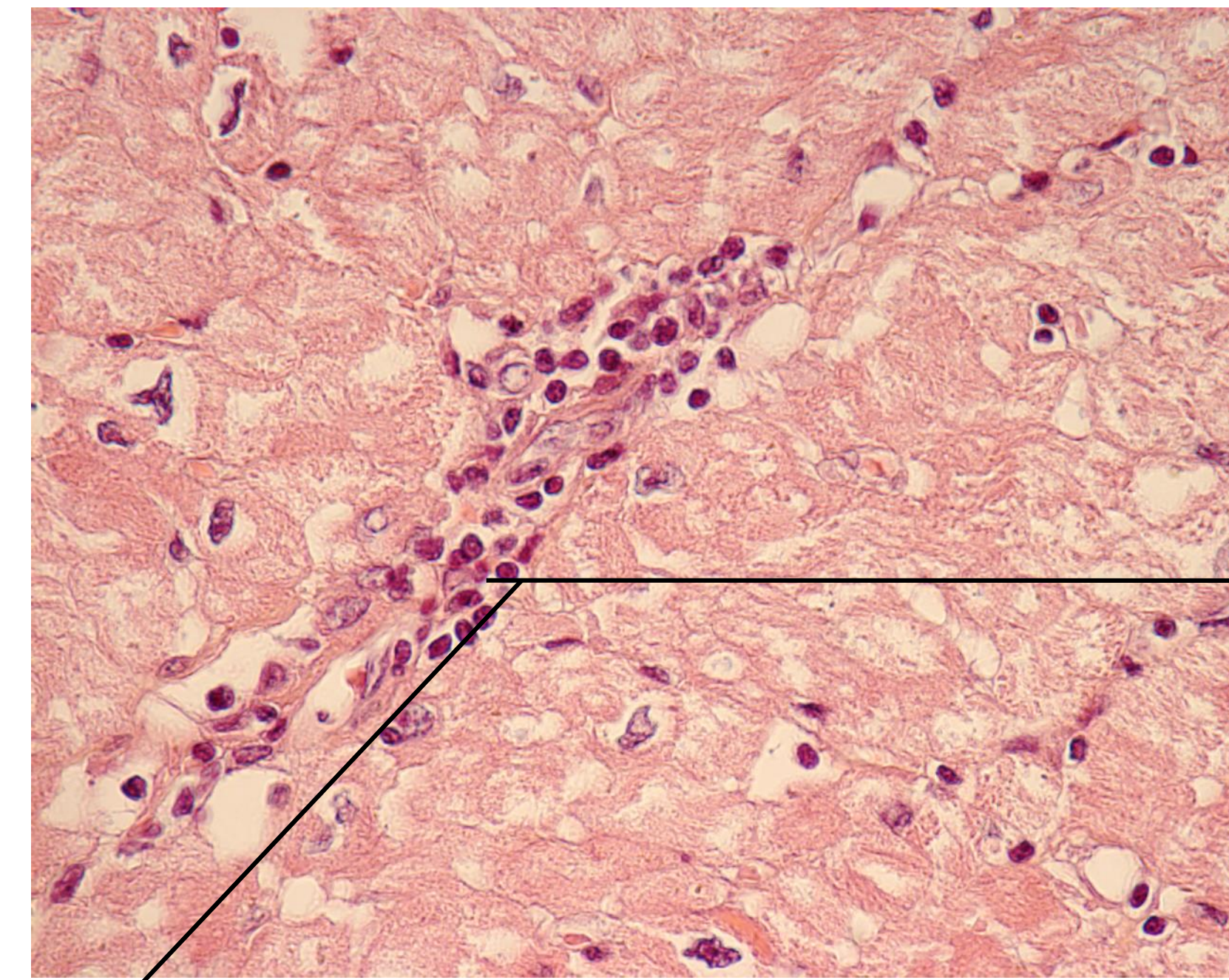


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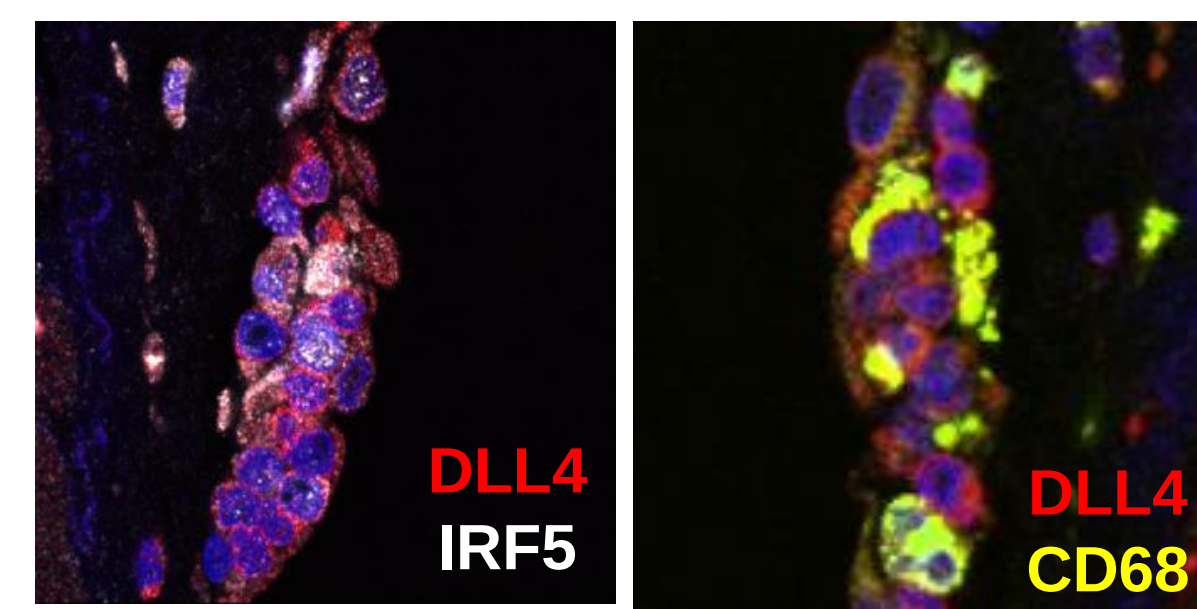
Summary

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.

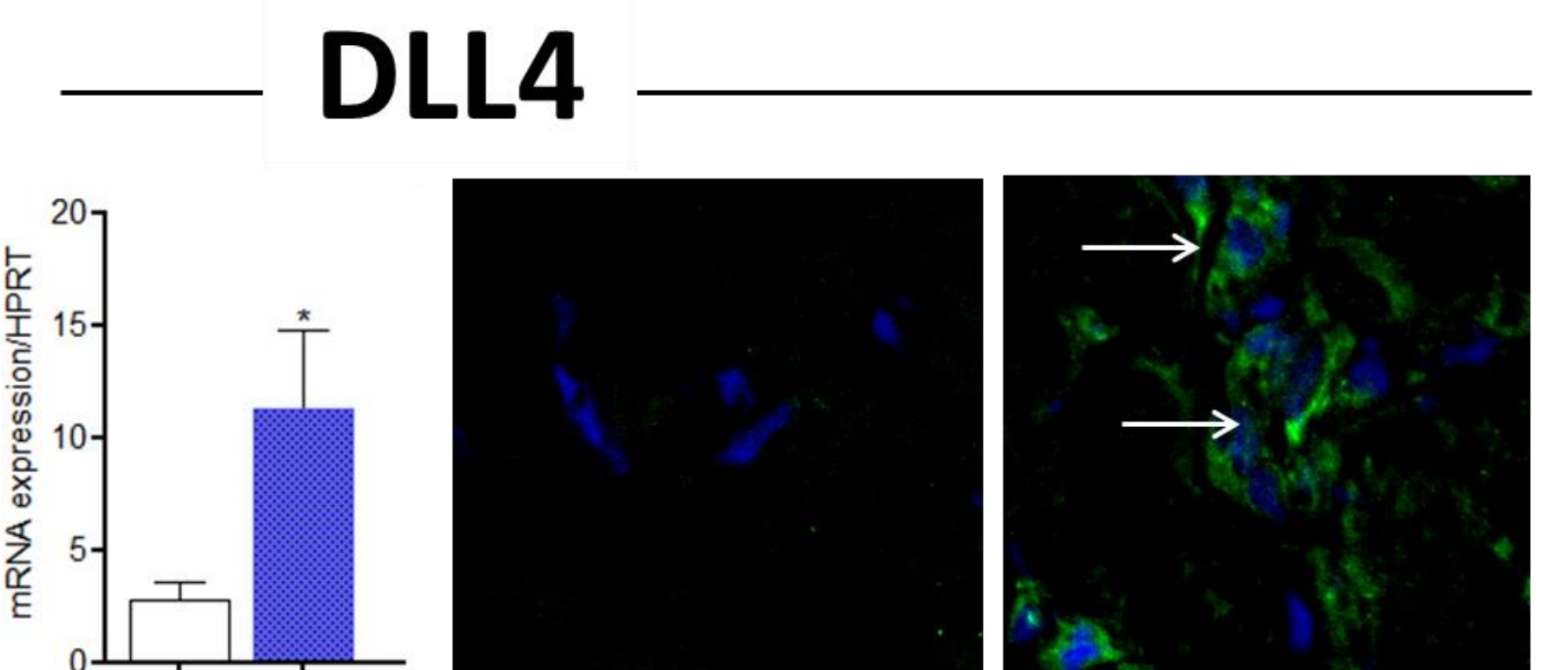
Microvascular inflammation in human cardiac allograft: a crosstalk between macrophages and graf's endothelial cells (ECs).



ECs and intravascular macrophages acquire DLL4 upon microvascular inflammation

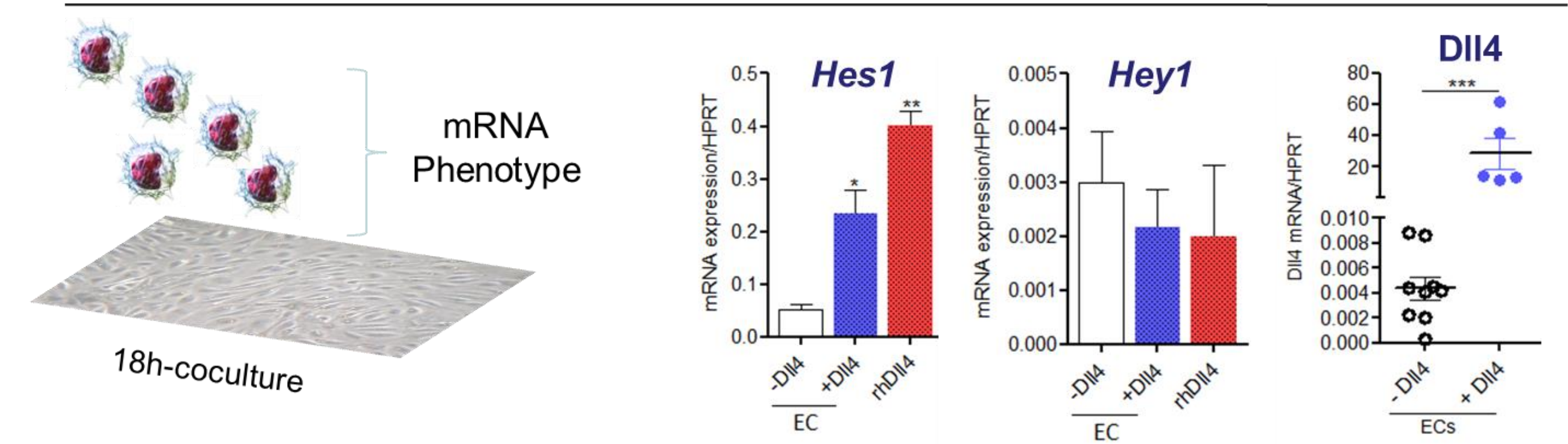


Infiltrating macrophages are M1 macrophages



Endothelial DLL4 induces DII4 on monocytes *in vitro*:

Purified CD14⁺ monocytes + primary EC cocultures expressing DII4

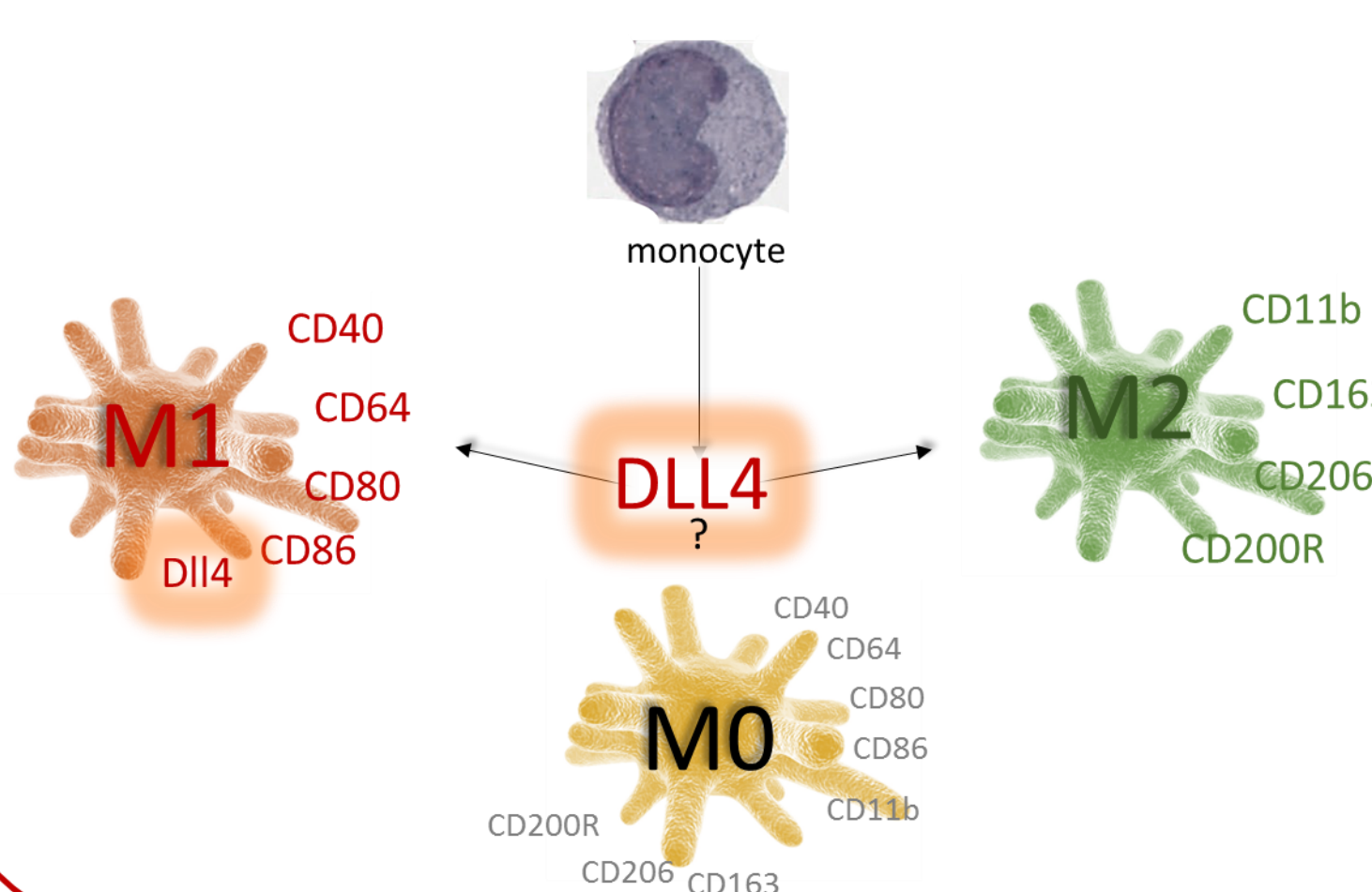


DLL4 engages a functional crosstalk between ECs and monocytes promoting M1 macrophage polarization and intravascular proinflammatory mediators

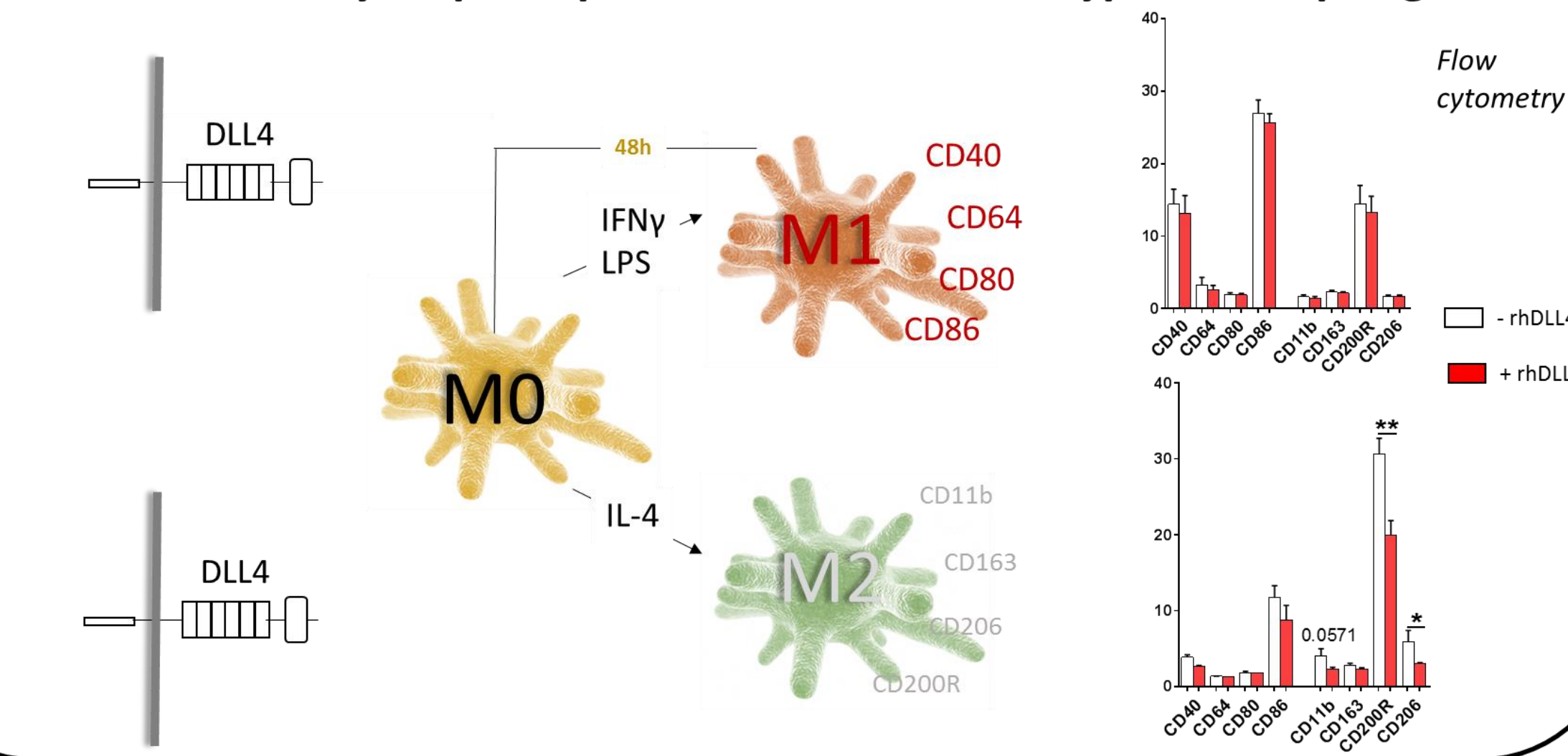
Pabois A., Pagie S. et al., *Biochem. Pharmacol.*, 2016.

Aim of our study:

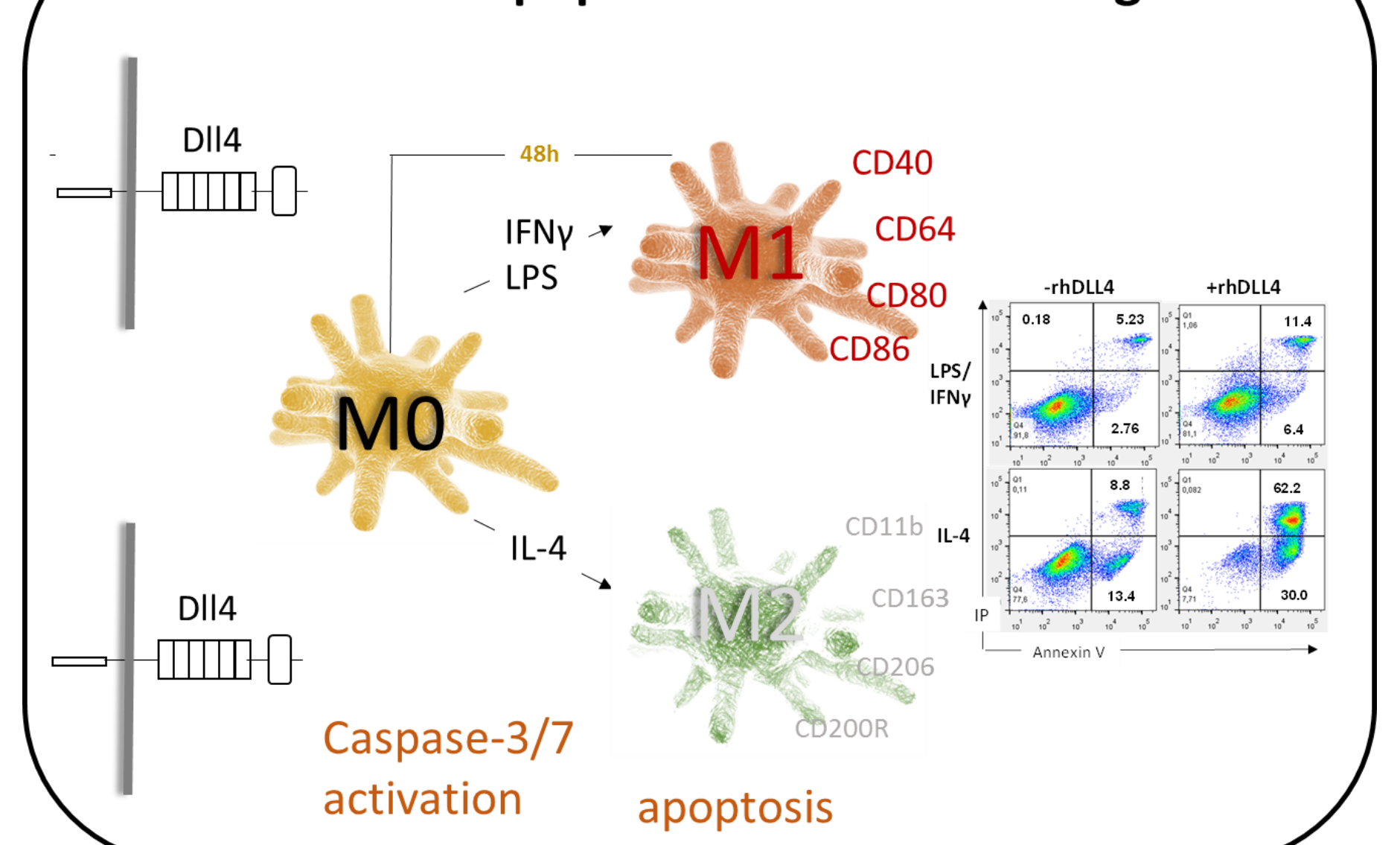
Deciphering the role of the Notch ligand DLL4 in macrophage differentiation and polarization



DLL4 selectively impairs polarization into M2-type macrophages

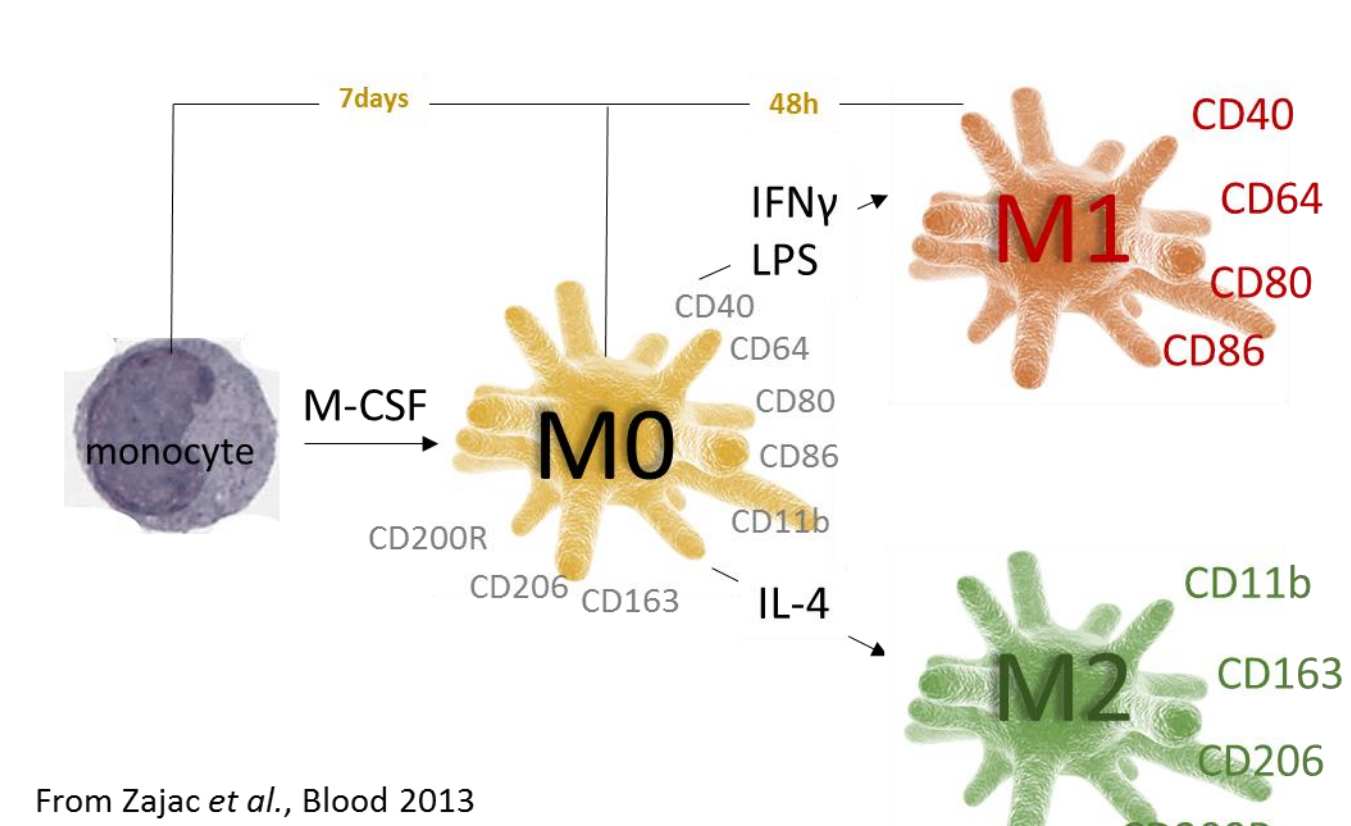


DLL4 induces apoptosis of differentiating M2

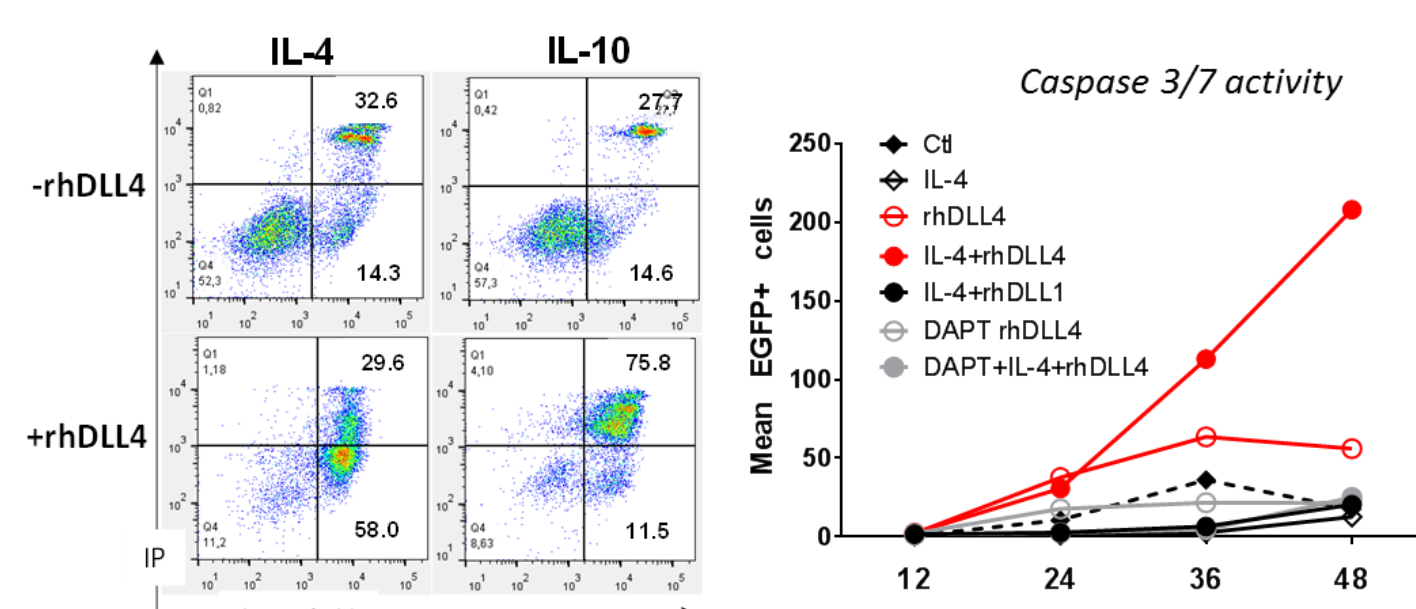


Protocol

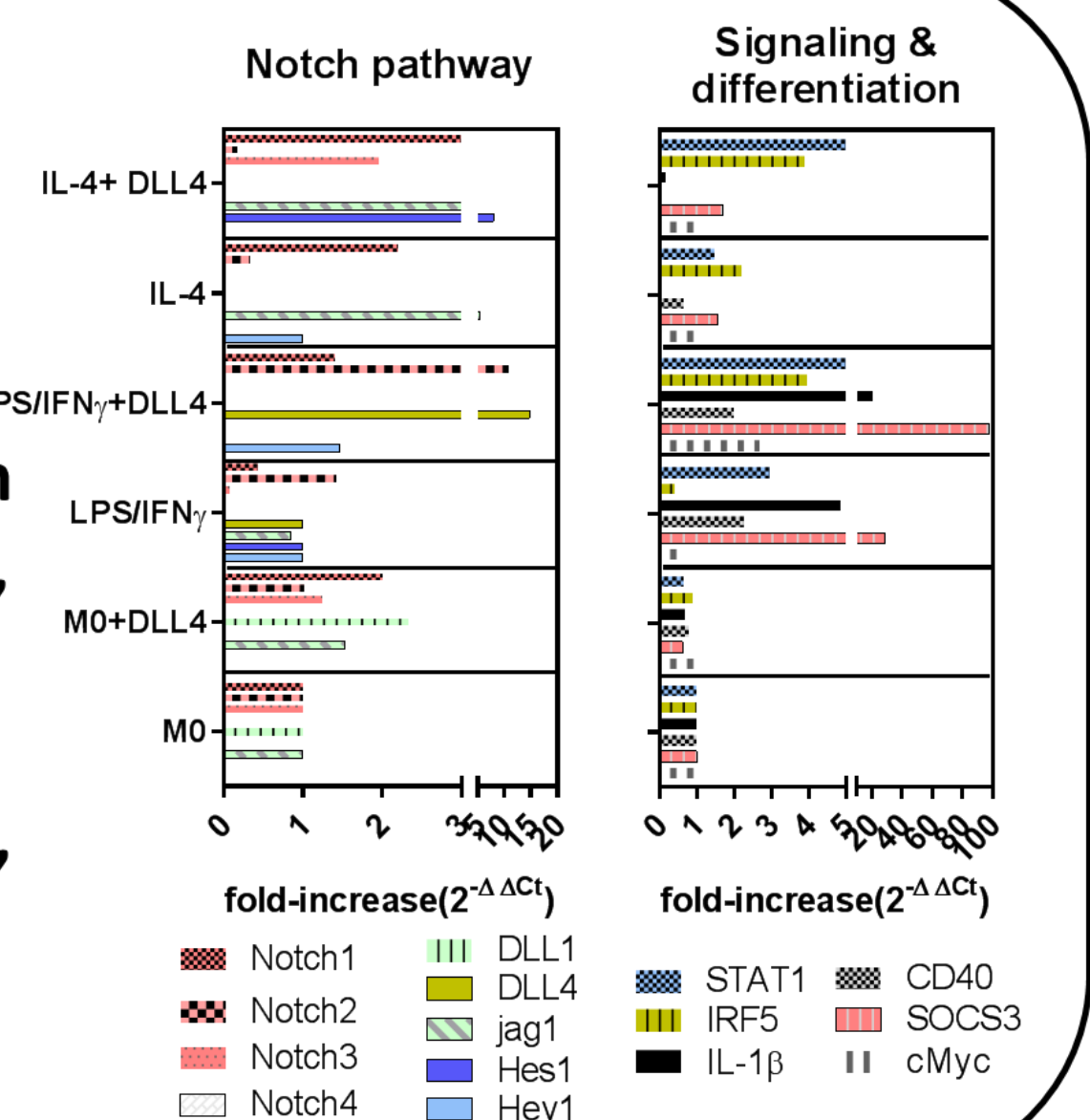
Macrophage Differentiation & Polarization *in vitro*



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*.

Quillard T. et al. ATVB 2008

Quillard T. et al. PlosOne 2009

Quillard T. et al. Biochem Pharmacol. 2010

Quillard T. et al. JM 2013

Pabois A. et al., Biochem. Pharmacol. 2014

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.