

# Simple and fast method for isolation of mouse cross-presenting dendritic cells

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### Introduction

Cross-presenting CD8a<sup>+</sup> conventional dendritic cells (cDCs) play a pivotal role in the induction of protective cytotoxic T lymphocyte (CTL) responses that are vital for the eradication of cancers and viral infections. In the past, studies of CD8a<sup>+</sup> cDCs have been hampered by their scarcity and the lack of specific cell surface markers. Therefore, methods for the detection and isolation of these cells were commonly based on a multitude of immunophenotypic criteria, including the expression of CD11c and CD8a and the absence of CD3, CD4, SIRP-a and CD11b. Recently, it was demonstrated that crosspresenting cDCs in lymphoid and non-lymphoid tissues specifically express the two receptors Clec9A and XCR1. The expression of the latter has been correlated with the ability to take up and cross-present exogenous antigens<sup>1</sup>. Combining our recombinant REAfinity<sup>™</sup> Anti-XCR1 mouse mAb with MACS<sup>®</sup> Technology, we developed a new method for the fast and easy isolation of cross-presenting DCs. Using this method XCR1<sup>+</sup> DCs can be routinely enriched with remarkably high recovery and purity without the need for time-consuming

#### Using Anti-XCR1 MicroBeads cross-presenting DCs can be directly isolated from splenocytes to high purity

Mouse spleens were dissociated as described in figure 1. Crosspresenting CD8<sup>+</sup>XCR1<sup>+</sup> DCs were then isolated by positive selection with Anti-XCR1 MicroBeads, mouse.

Figure 3A shows the flow cytometric gating strategy. As above, conventional DCs were defined as CD11c<sup>+</sup>MHCII<sup>hi</sup> cells. From these, the cross-presenting DCs were further characterized by CD8α and XCR1 expression as indicated. Cellular debris and

and propidium iodide exclusion, respectively. (B) The dot plots show gated conventional DCs in the starting material as well as the depleted and the target fractions. The enrichment results in a very high purity of >90% on average (bar chart). (C) The target cells displayed the expected phenotype for crosspresenting DCs, which is CD11c<sup>+</sup>, MHCII<sup>hi</sup>, CD11b<sup>-</sup>, SIRPa<sup>-</sup>, CD8a<sup>+</sup>, and XCR1<sup>+</sup>, as examined by flow cytometry.

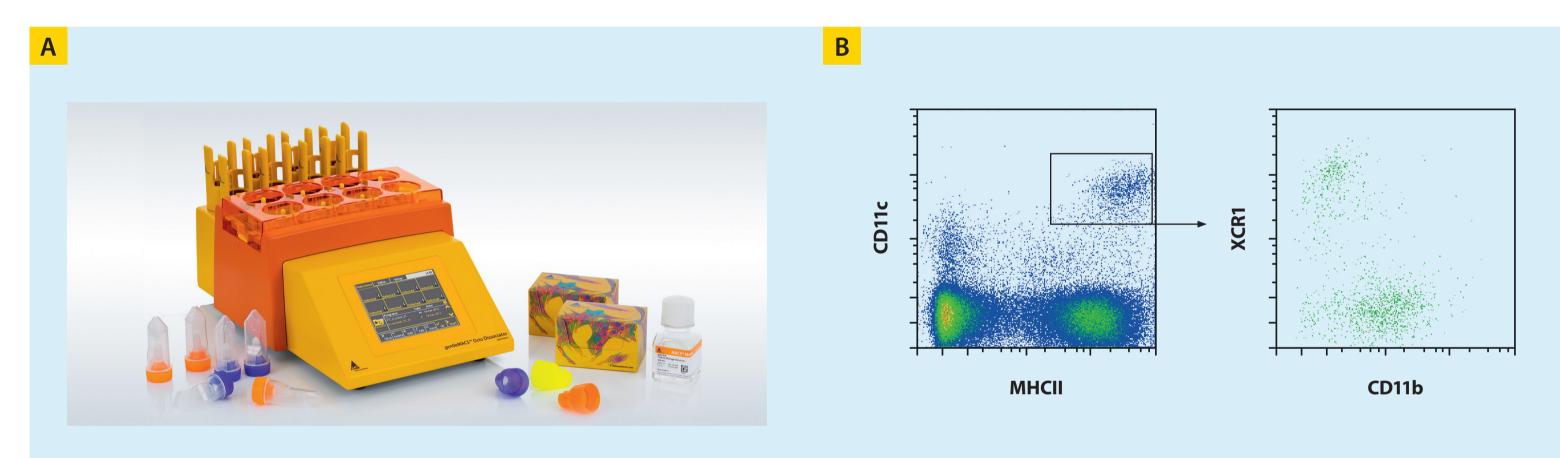
pre-depletion of non-target cells and/or laborious flow sorting. This will facilitate the study of the complexities of DC subset biology, which ultimately will help to develop new therapeutic strategies employing DCs in the future.

## Results

## Dissociation of mouse spleen with the gentleMACS<sup>™</sup> Octo Dissociator with Heaters

Gentle, rapid, and efficient generation of single-cell splenocyte suspensions from mice was performed using Miltenyi Biotec's Spleen Dissociation Kit together with the gentleMACS<sup>™</sup> Octo Dissociator with Heaters, which combines mechanical dissociation with enzymatic degradation of the extracellular matrix. The process is optimized for a high yield of leukocytes with high viability, especially DCs (3×10<sup>7</sup> CD11c<sup>+</sup> cells per gram

of spleen), while preserving all cell surface epitopes. Figure 1A shows the instrument and all required materials. Phenotypic characterization of the DCs among dissociated splenocytes was performed by flow cytometry as indicated (B). The cell suspension was then ready for subsequent cell isolation.



dead cells were excluded by FSC/SSC scatter characteristics

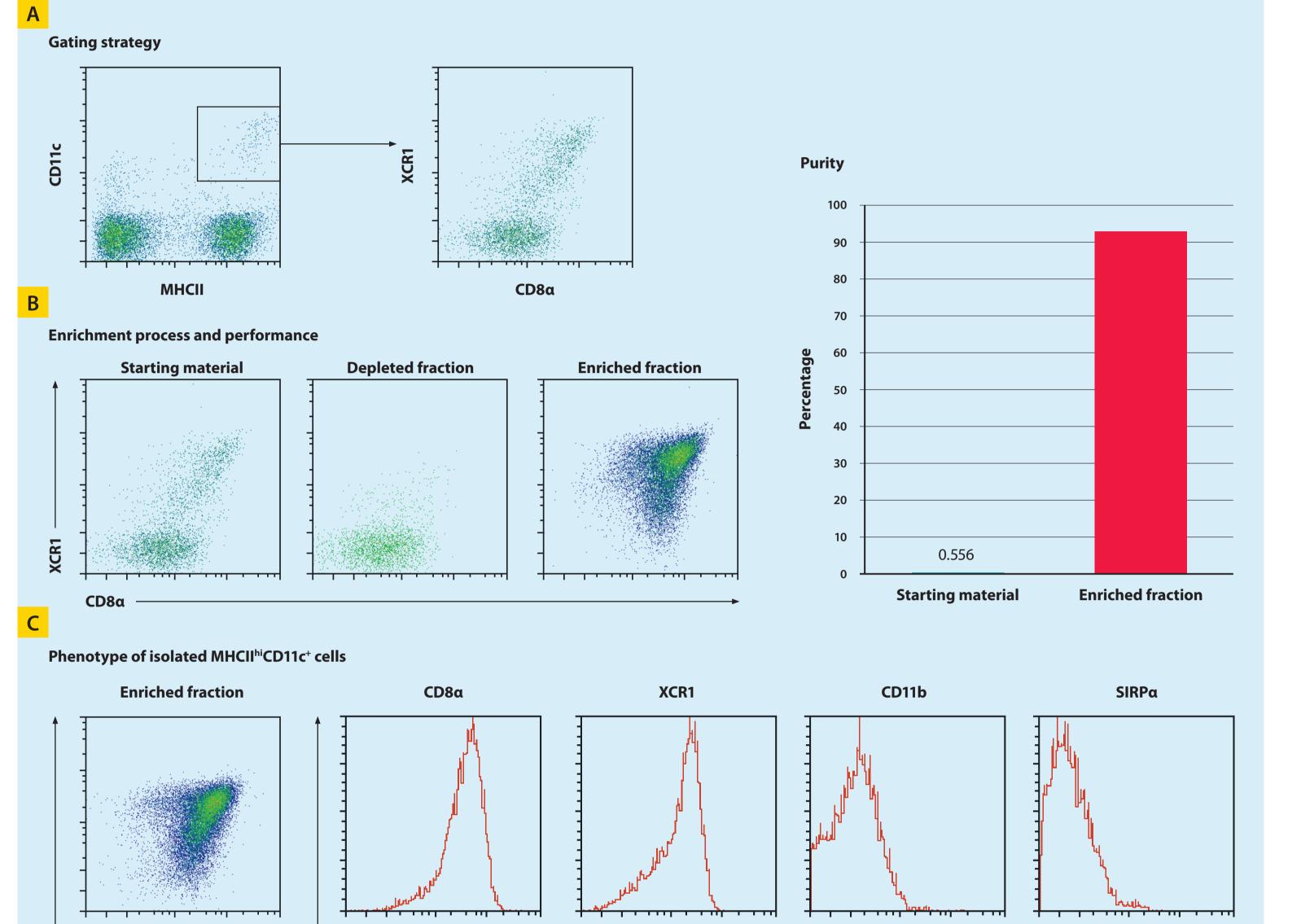
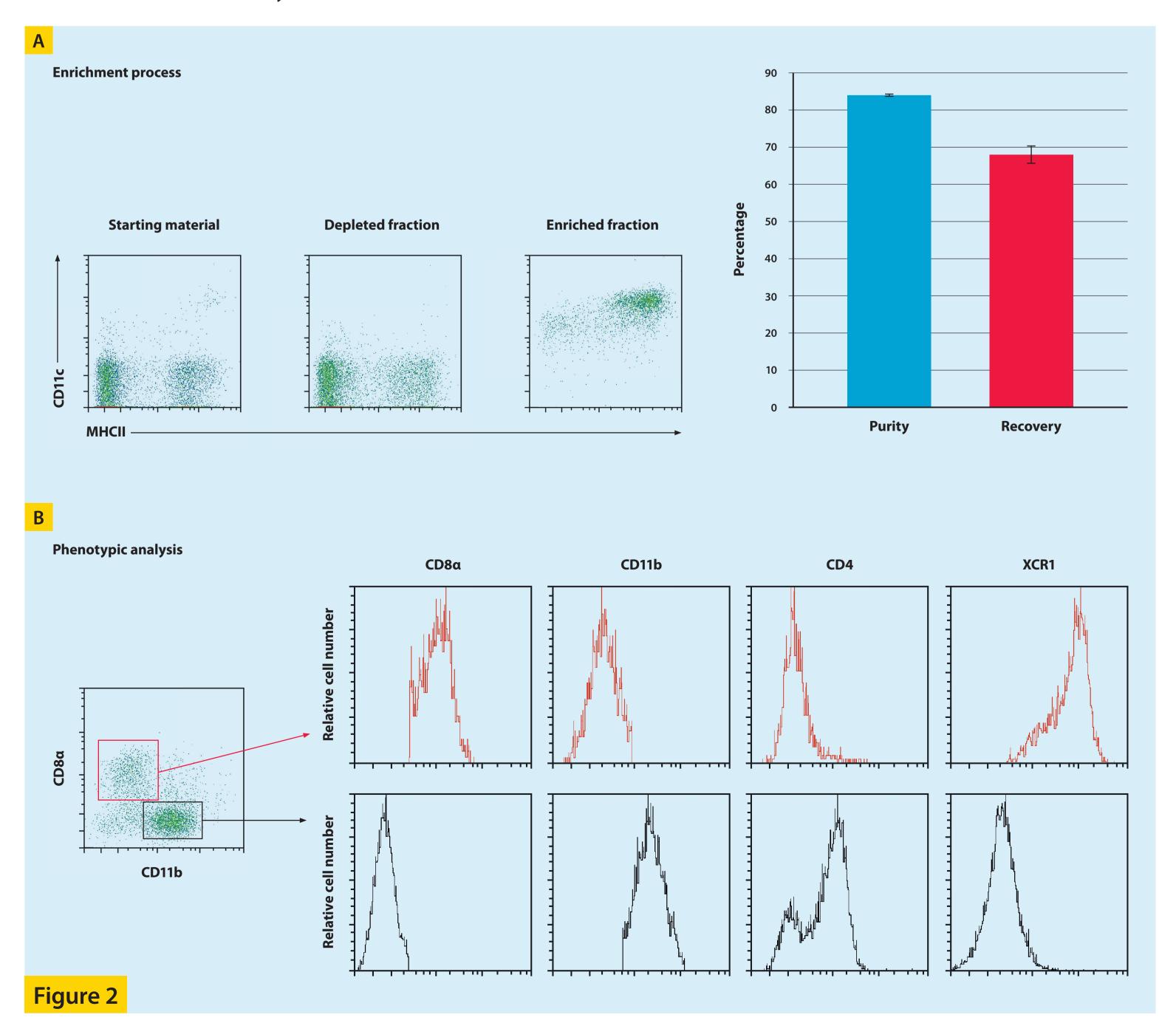


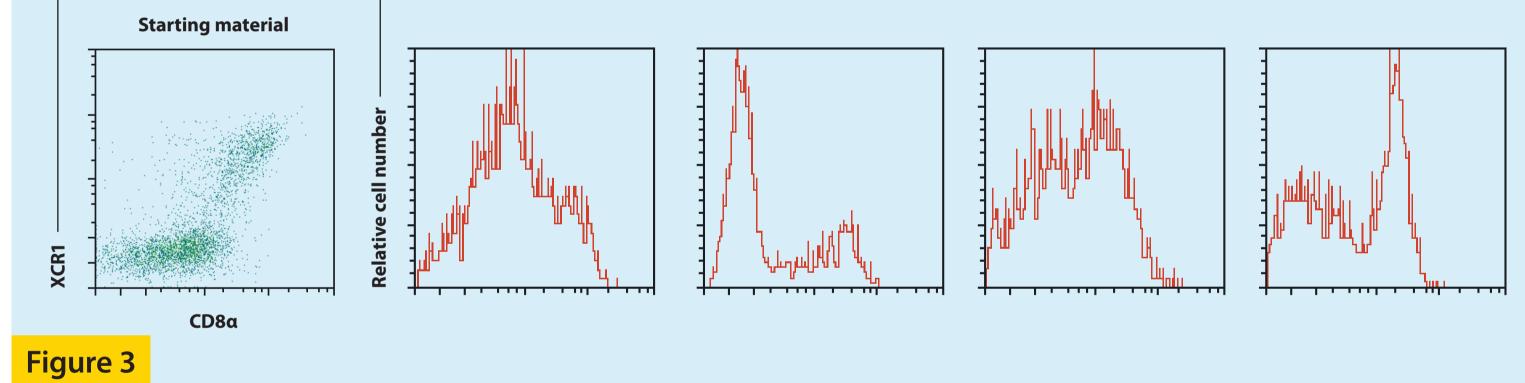
Figure 1

## Splenic DCs isolated with CD11c MicroBeads UltraPure contain the XCR1<sup>+</sup> subset of cross-presenting DCs

Splenocytes were prepared as described above and DCs were isolated with CD11c MicroBeads UltraPure according to the manufacturer's instructions. (A) The starting material as well as the enriched and depleted fractions were analyzed by flow cytometry (dot plots) and the high purity and recovery in the target cell fraction were validated (bar chart). Cellular debris and dead cells were excluded by FSC/SSC scatter characteristics

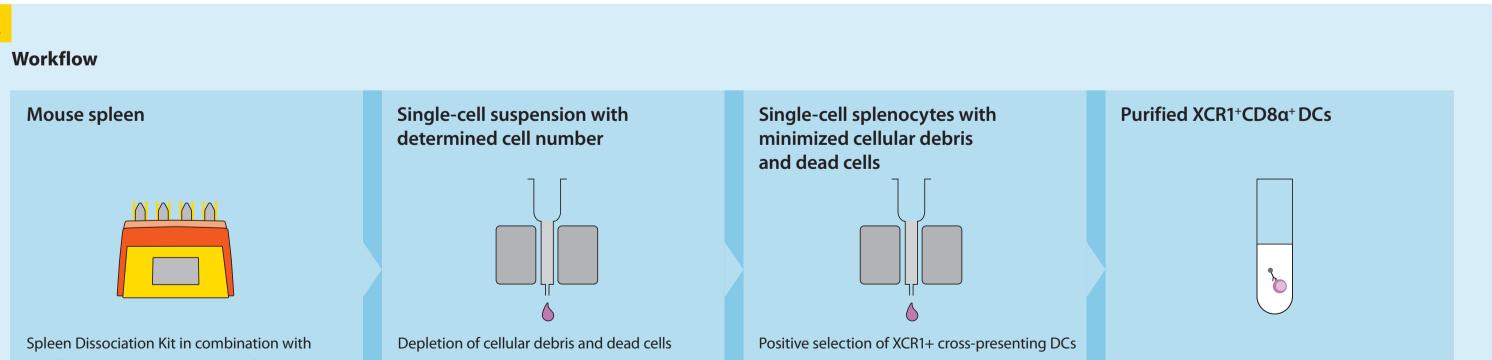
and propidium iodide exclusion, respectively. Data of a typical experiment performed in duplicates is shown. (B) The expected phenotypes for conventional CD11b<sup>+</sup> (CD11c<sup>+</sup>, MHCII<sup>hi</sup>, CD11b<sup>+</sup>, CD4<sup>-</sup>/<sup>+</sup>, CD8a<sup>-</sup>, XCR1<sup>-</sup>) and CD8a<sup>+</sup> cross-presenting (CD11c<sup>+</sup>, MHCII<sup>hi</sup>, CD11b<sup>-</sup>, CD4<sup>-</sup>, CD8a<sup>+</sup>, XCR1<sup>+</sup>) DCs were confirmed by flow cytometry.





#### From splenocytes to cross-presenting XCR1+CD8α+ DCs in just 35 minutes

In the first step, a splenocyte suspension is generated using Miltenyi Biotec's Spleen Dissociation Kit in combination with the gentleMACS Octo Dissociator with Heaters. Afterwards cellular debris as well as dead cells are depleted. The crosspresenting DCs are then specifically labeled with Anti-XCR1 MicroBeads, unbound beads washed out by centrifugation, and the XCR1<sup>+</sup> cells purified over two consecutive MACS MS Columns. The entire procedure starting from unprocessed mouse spleen to purified XCR1<sup>+</sup>CD8a<sup>+</sup> cross-presenting DCs takes only 70 minutes. The resulting cells are then ready for flow cytometric analysis and downstream applications.



	gentleMACS <sup>™</sup> Octo Dissociator with Heaters		<ol> <li>Direct magnetic labeling of XCR1<sup>+</sup> with Anti-XCR1 MicroBeads</li> <li>Magnetic separation using two MS Columns</li> </ol>	
Sample preparation: 36 minutes		XCR1 <sup>+</sup> cell isolation: 35 minutes		
Figure 4				

### Conclusions

- DCs with high viability and preserved epitopes can be obtained from mouse spleen using the gentleMACS Octo Dissociator with Heaters and the appropriate tissue dissociation kit from Miltenyi Biotec.
- Pan DCs containing the cross-presenting subset can be enriched from dissociated tissues with CD11c MicroBeads UltraPure enabling the detailed analysis of distinct DC subsets.
- Highly pure XCR1+CD8α+ cross-presenting DCs can be isolated with our novel Anti-XCR1 MicroBeads, mouse, in just 35 minutes.

References

1. Bachem, A. et al. (2012) Front. Immunol. 3: 214.

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