# On the way to a CRISPR/Cas9 *in vivo* screen for new regulators of antibody-secreting cells

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## Abstract

Antibody-secreting cells (ASCs) are generated once B lymphocytes encounter their cognate antigen in secondary lymphoid organs. While they are a very rare population of cells, ASCs secrete huge amounts of antigen-specific antibodies and are essential for the humoral immunity against pathogens. Despite their discovery since 1875, the intricacies of their development and function in the microenvironment of lymphoid organs are not fully comprehended. To identify new regulators for the development of ACSs, we have established an *in vivo* model for CRISPR/Cas9 negative selection screenings, that could specifically interrogate, in parallel, the function of many genes expressed on ASCs.

The model requires the transduction of Cas9-exppressing *Igh*$^{B1\text{-}8hi/+}$ naïve B cells *in vitro* with lentiviral (LV) particles carrying gene-specific sgRNAs, the subsequent transfer of transduced naïve B cells to recipient mice, the immunization of these mice with the hapten NP to induce a T-dependent or a T-independent immune response and, finally, the evaluation of sgRNAs abundance on the generated ASCs. The main challenge was the LV transduction of naïve B cells. Conditional overexpression from the *Rosa26* locus, of the solute carrier Slc7a1, the receptor for the ecotropic LV, improved the transduction of B cells without activation.

We used single sgRNAs to validate our *in vivo* model for CRISPR/Cas9 screenings. Naïve B cells, transduced *in vitro* with LV particles carrying a non-targeting control sgRNA, differentiated to NP-specific ASCs *in vivo*, after being transferred to recipient mice that were later immunized with NP-KLH/Alum or NP-Ficoll. In contrast, *in vivo* ASCs differentiation was completely blocked when the transferred B cells were previously transduced with LV particles carrying a *Prdm1*-specific sgRNA. We would like to study at once, around 400 genes highly expressed on ASCs and involved on signal transduction, adhesion, endoplasmic reticulum, transcriptional regulation, or metabolism. Our study might uncover novel molecular mechanisms regulating antibody-mediated immune responses.