# TLRs and the germinal center antibody response

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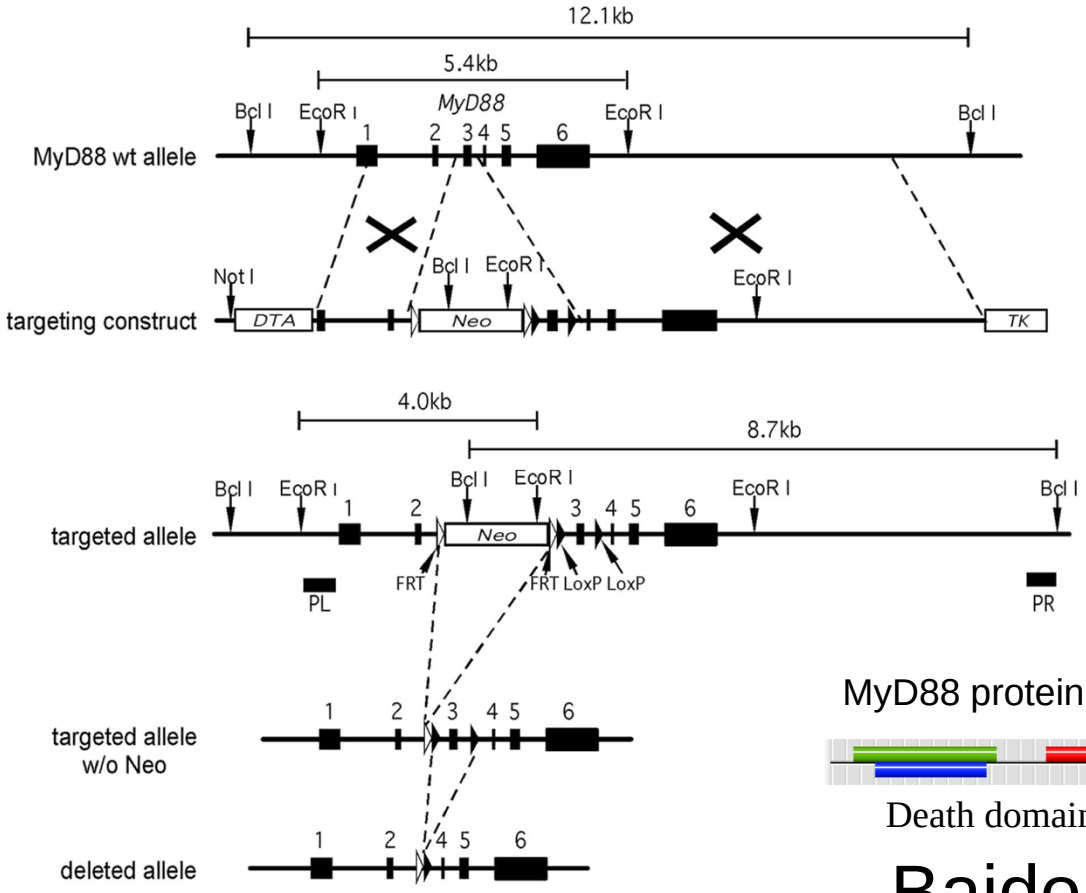
# Role of TLRs in immune responses in vivo

TLRs are expressed by macrophages, dendritic cells, B cells, epithelial cells, endothelial cells, etc.

QUESTION: What is the role of TLR signaling in particular cell types for in vivo immune responses?

# Generation of a Conditional allele of the mouse Myd88 gene




MyD88 protein structure

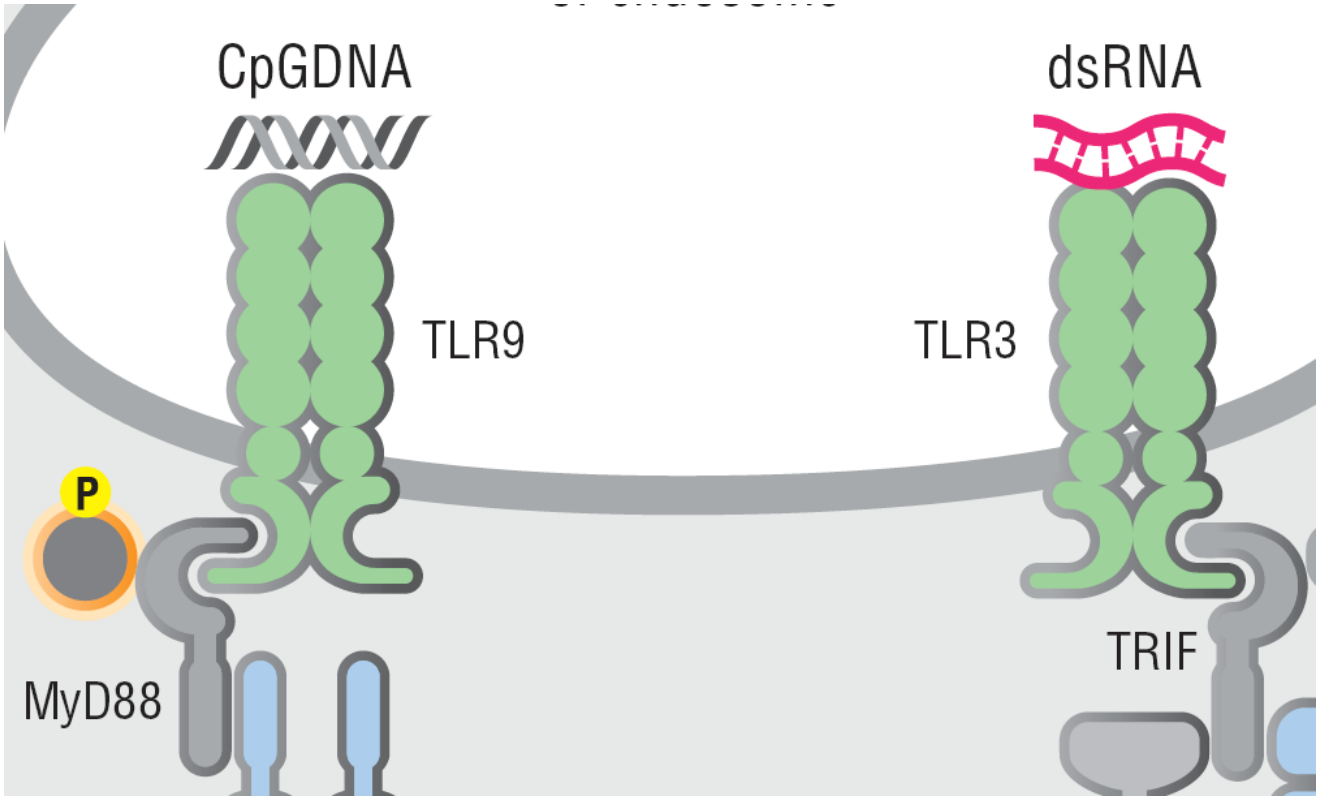


Death domain TIR domain

Baidong Hou

No residual MyD88 protein can be detected after Cre-mediated deletion

# MyD88 is a key signaling adaptor for almost all TLR signaling



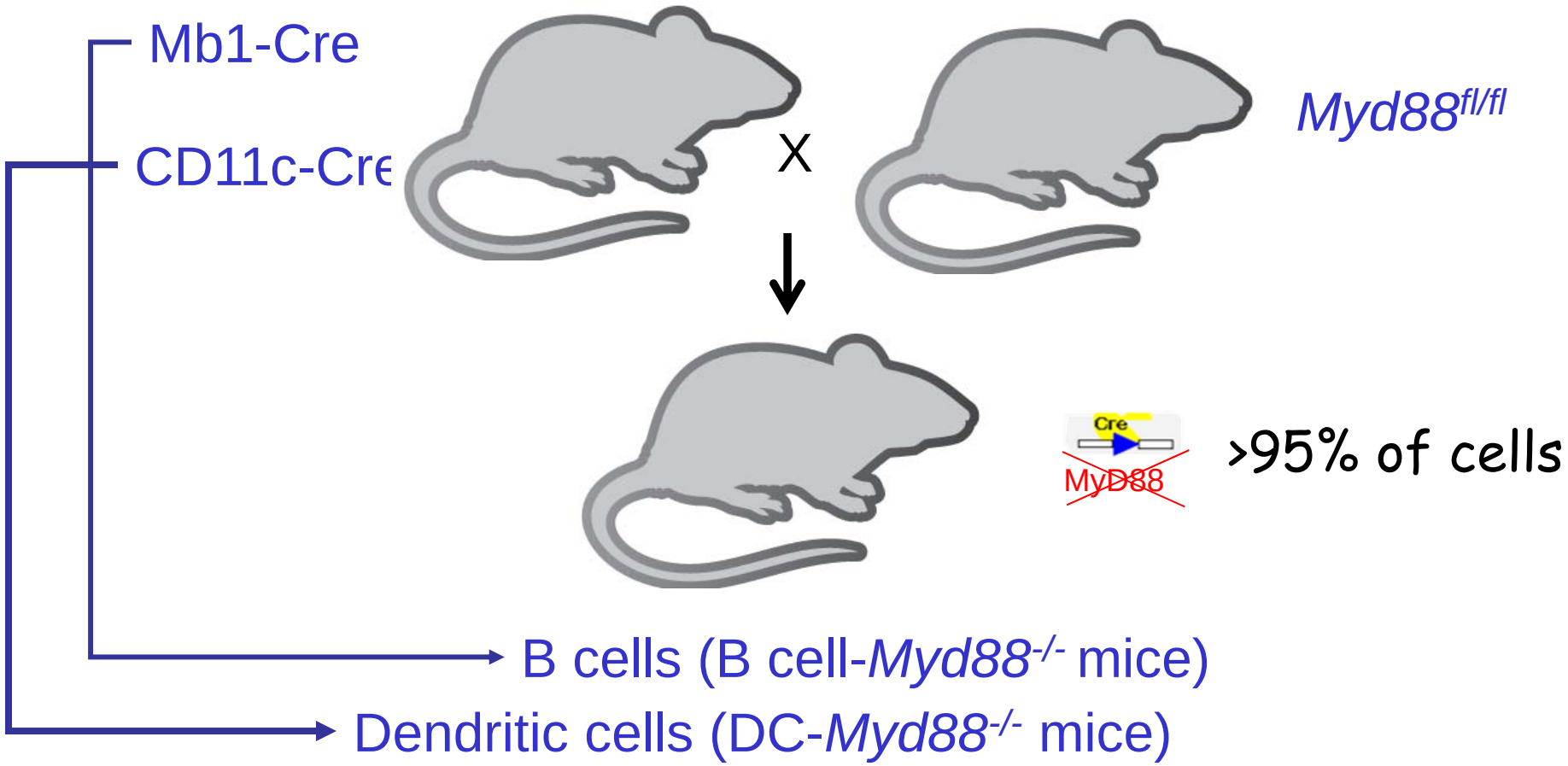

From **Immunity: The Immune Response in Infectious and Inflammatory Disease**
by DeFranco, Locksley and Robertson

All TLRs except TLR3 utilize MyD88; TLR4 utilizes MyD88 and TRIF

# TLR signaling and Antibody responses

- Antibody responses using standard adjuvants (Alum, IFA, CFA) do not depend on TLR signaling (Gavin et al, Science 12/06, etc.)
- Soluble TLR ligands (such as CpG oligonucleotides) make good adjuvants and this effect is TLR-dependent (Pasare & Medzhitov, Nature 11/05; Robert Coffman et al.)
- When TLR signaling contributes to Ab responses: how does it contribute?

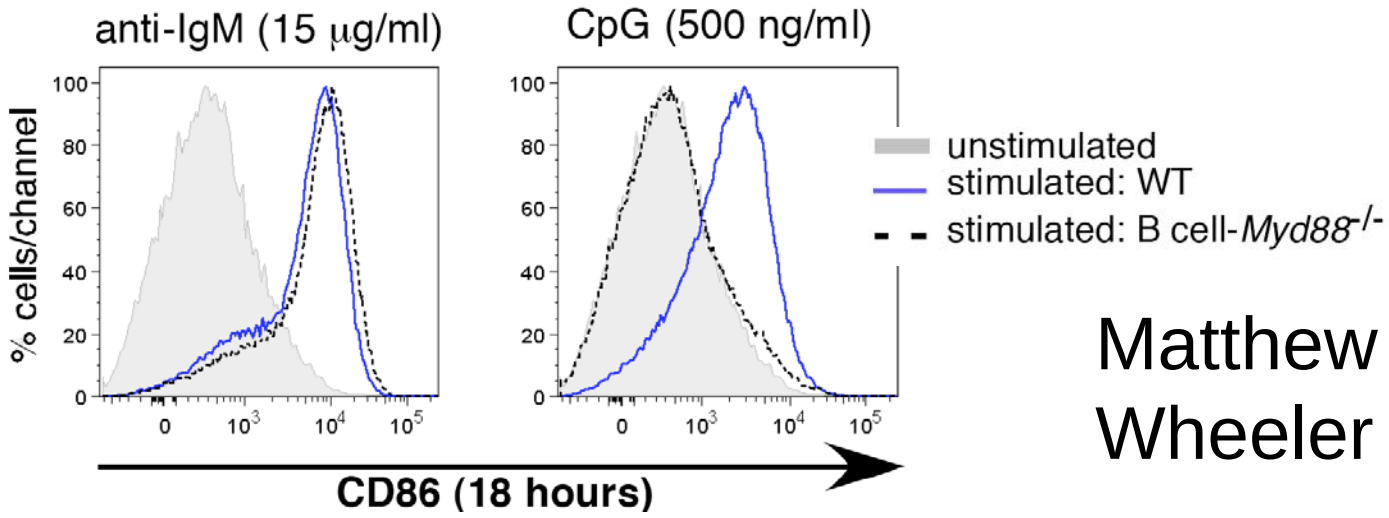
# Generation of B cell- and DC-specific MyD88 deficient mice



# Generation of B cell- and DC-specific MyD88 deficient mice

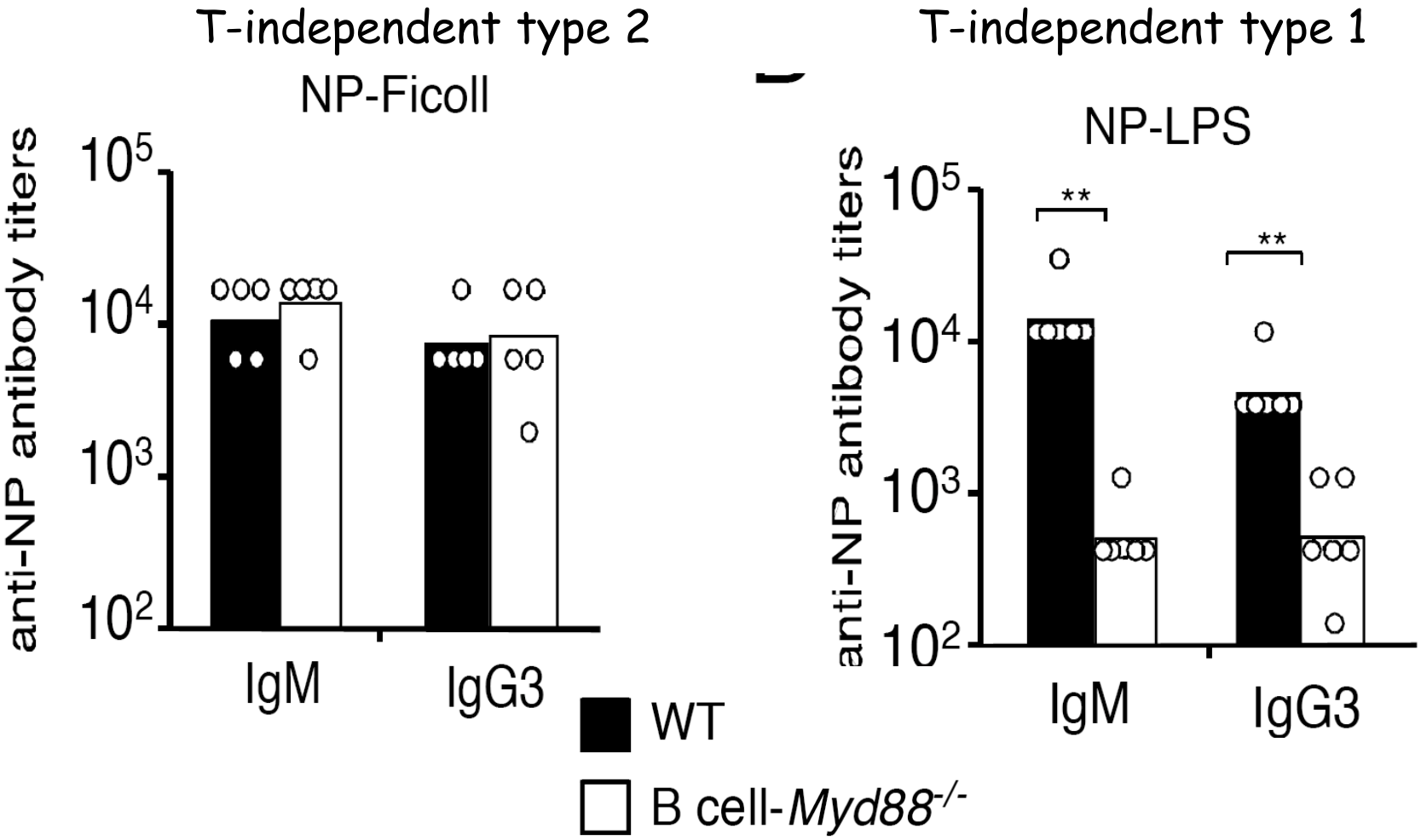
*CD11c-Cre Myd88$^{fl/fl}$ mice have normal DC subsets in spleen*

*Mb-1-Cre Myd88$^{fl/fl}$ mice have normal B cell development and normal BCR signaling*

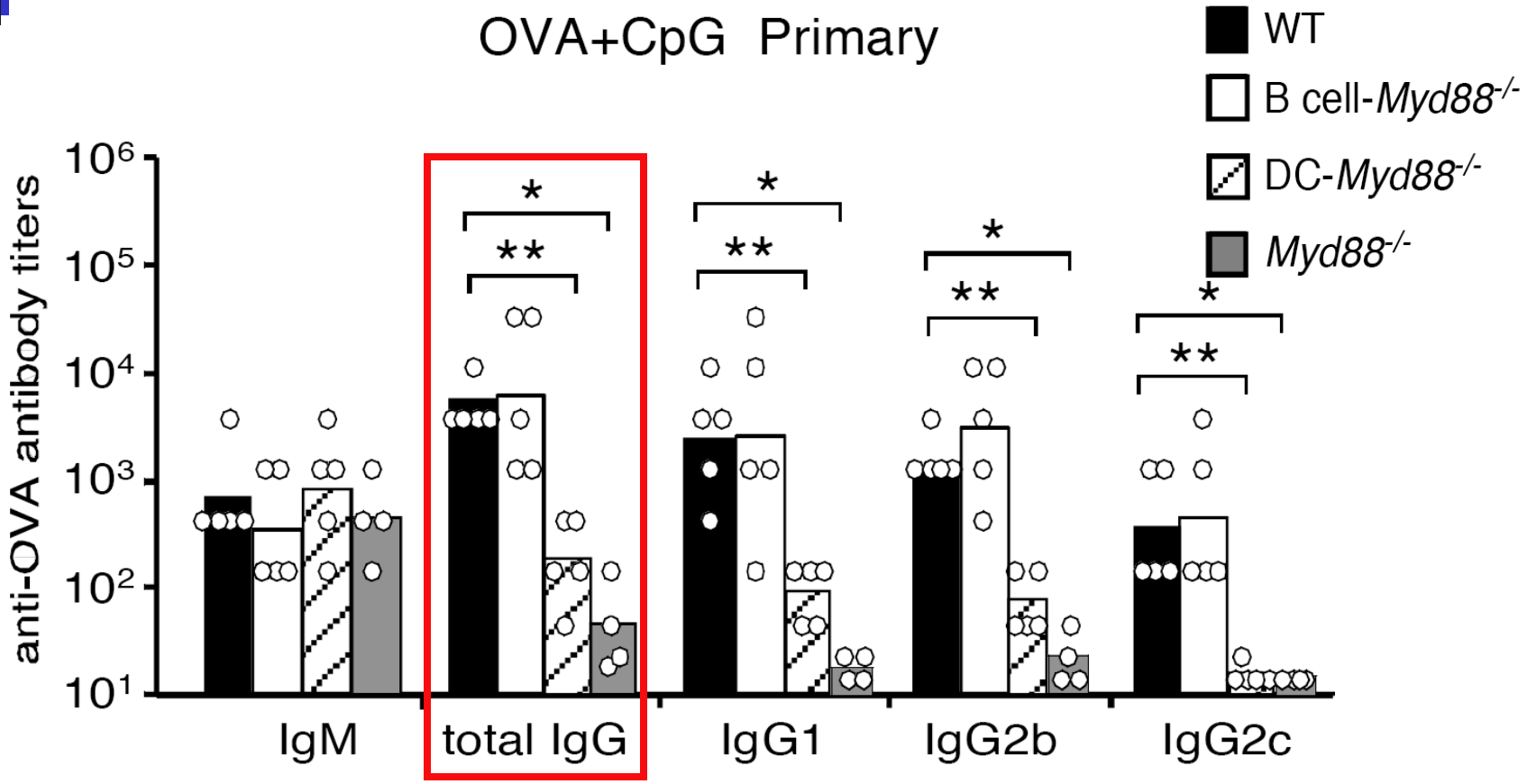



Matthew Wheeler

# TLRs in B cells are required for response to NP-LPS but not to NP-Ficoll

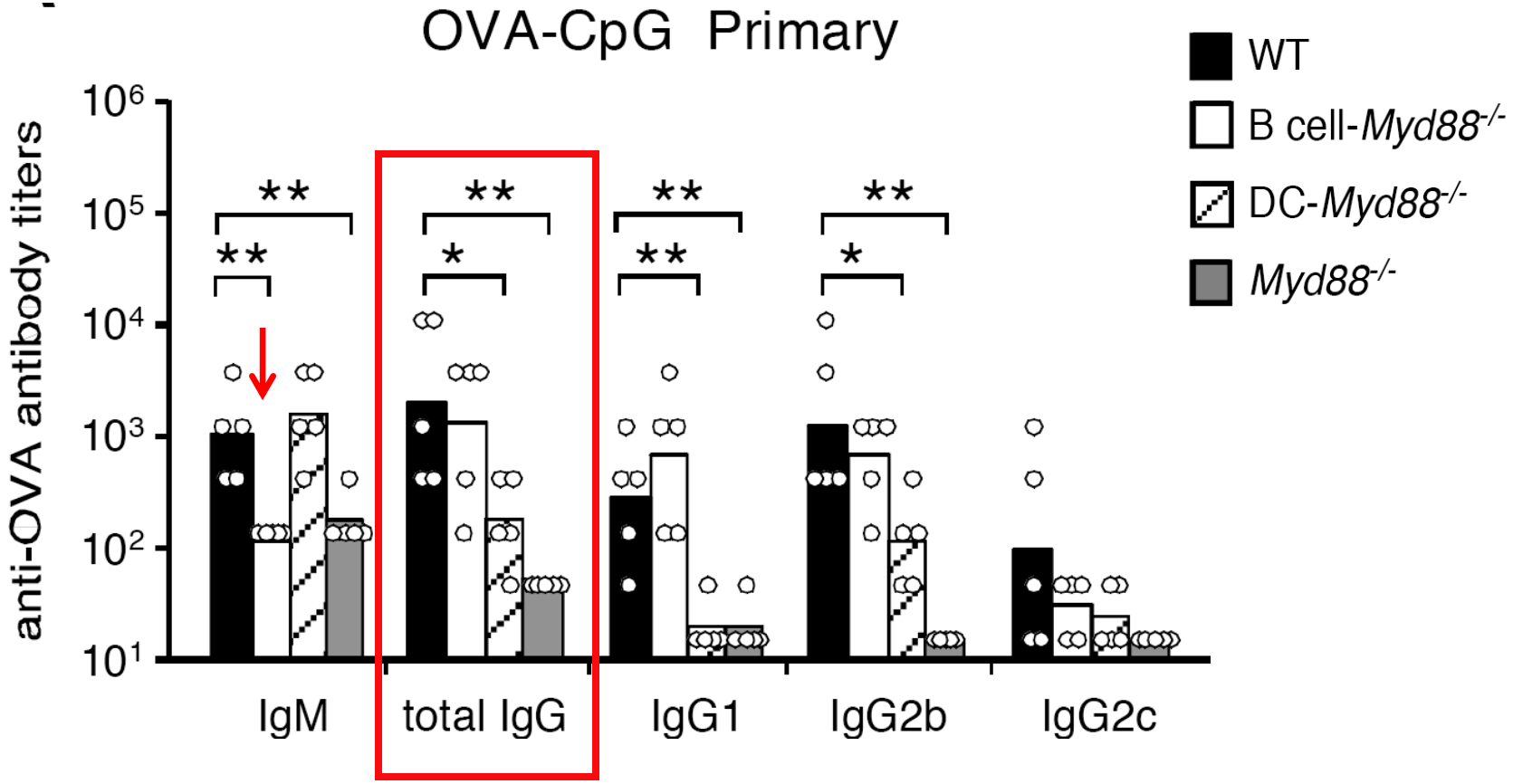


# Soluble protein antigen plus CpG oligo: TLR signaling in DCs is required for IgG response




Serum Ag-specific Ig titers at 2 weeks after i.p. immunization with Ag mixed together with CpG

# Soluble protein antigen-CpG conjugate:TLR signaling in DCs (not B cells) is required for IgG response



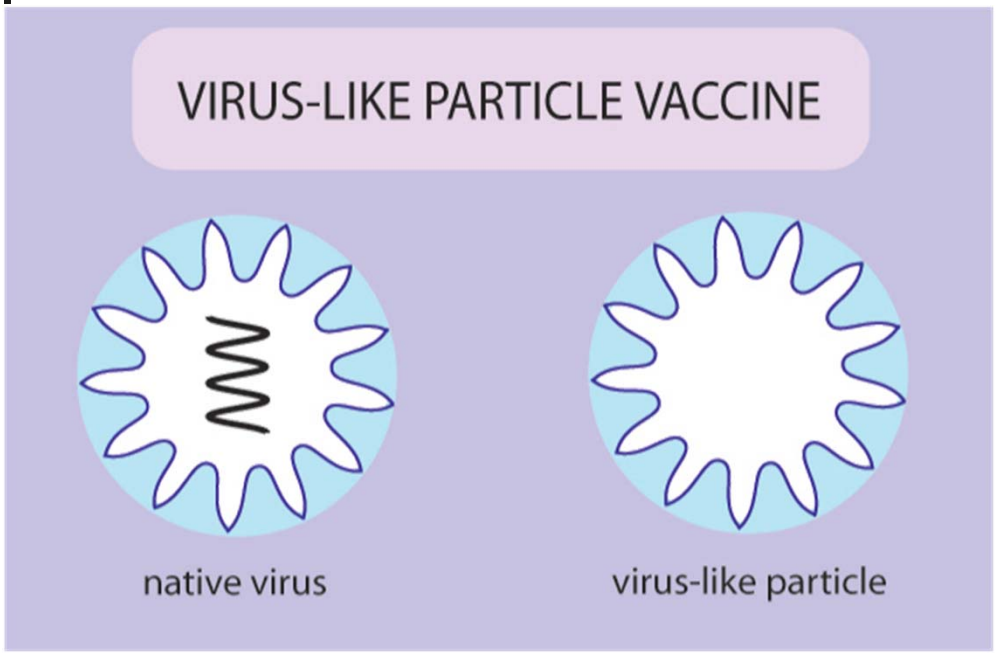

Serum Ag-specific Ig titers at 2 weeks after i.p. immunization with Ag conjugated with CpG

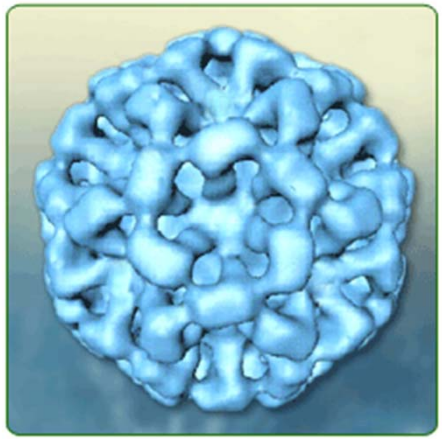
# Soluble protein antigen-CpG conjugate: TLR signaling in DCs (not B cells) is required for IgG response

Same answer seen with different soluble protein antigens including flagellin (TLR5 agonist) and Amb A1-CpG conjugate (ragweed pollen allergen)
(same answer for “strong” antigen or “weak” antigen)

Collaboration with Robert Coffman and Gary Ott, Dynavax, Inc.

# Virus-like particles: a vaccine platform



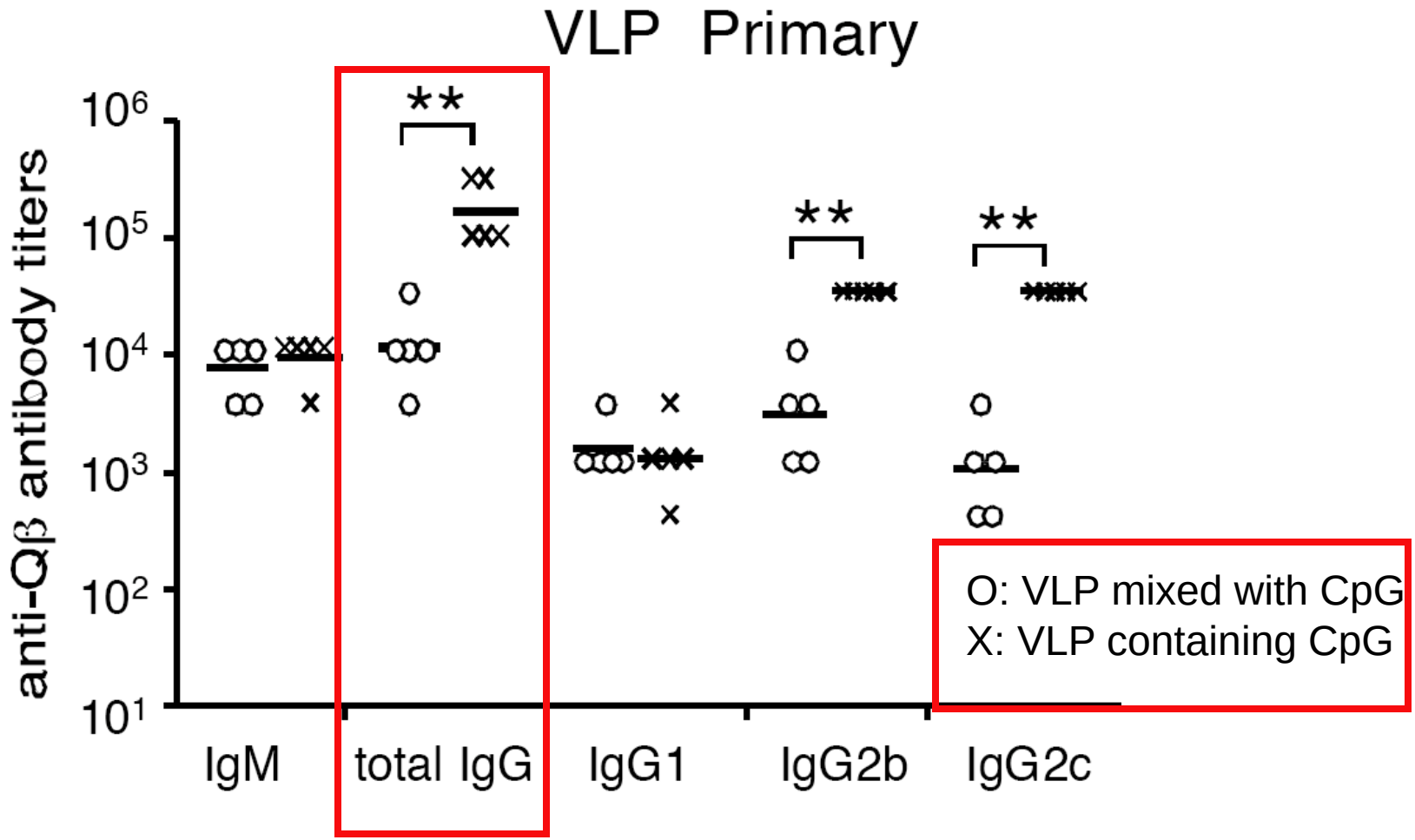



Virus-Like-Particles: a 3-D illustration of a VLP structure.

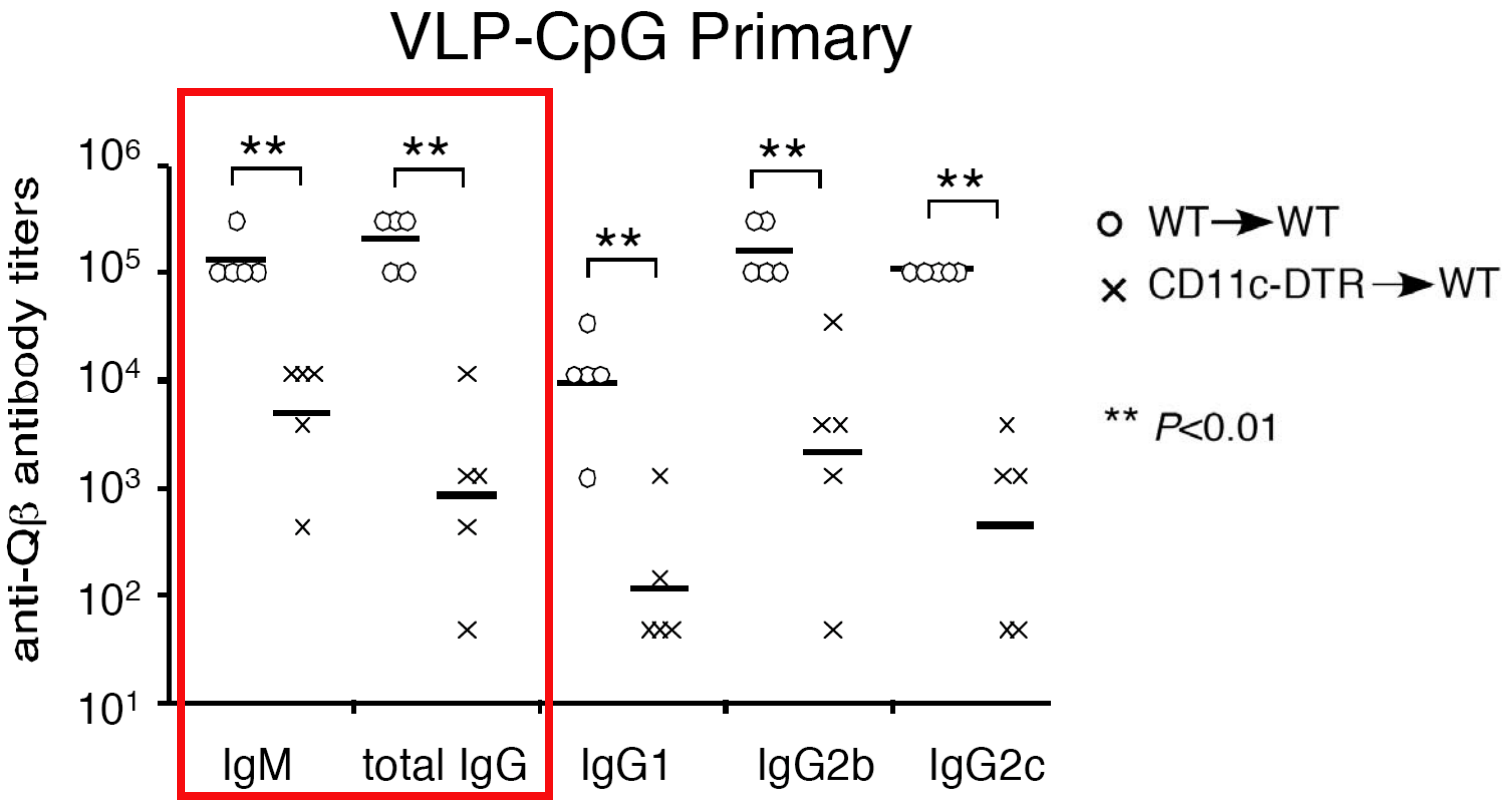
Bacteriophage Qβ-based VLPs: protein shell with TLR7 or TLR9 ligands inside (or not)

Collaboration with Martin Bachmann, Philippe Saudan, Cytos Inc.

# Incorporation of CpG into VLPs strongly enhances the IgG antibody response

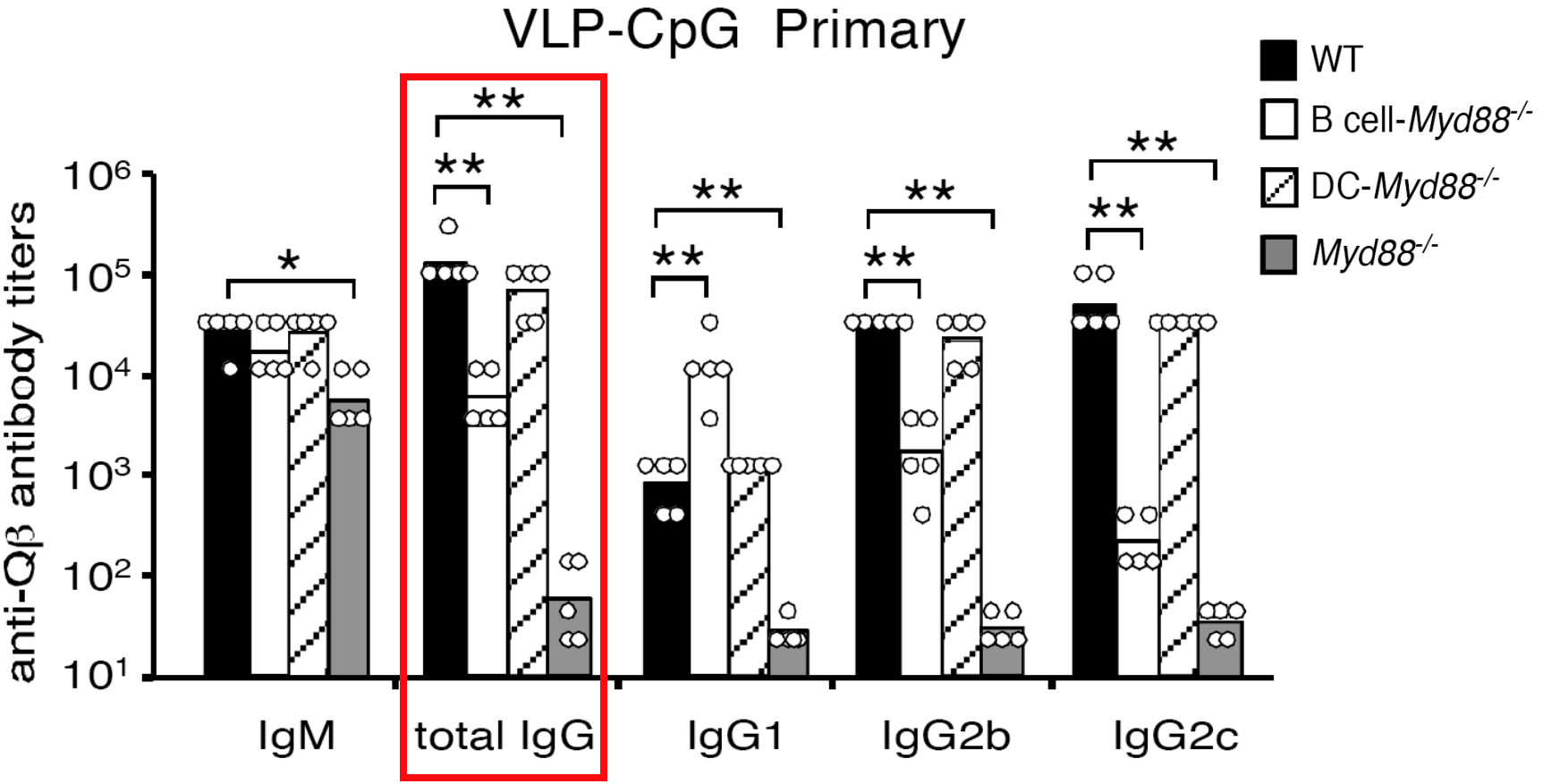


# IgG response to VLPs requires conventional dendritic cells (and T cells)



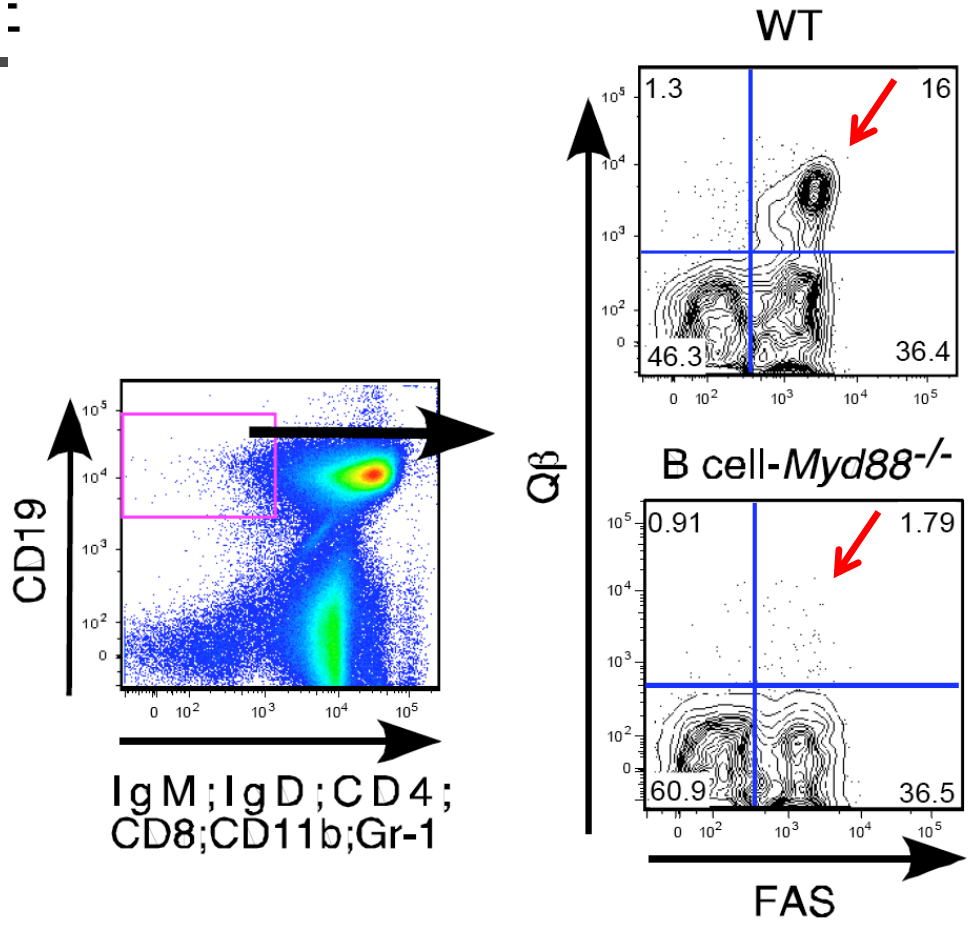

IgG response is highly dependent on T cells as well (weak response in T cell-deficient mice)

# Myd88 signaling in B cells is required for optimal IgG response to VLP-CpG



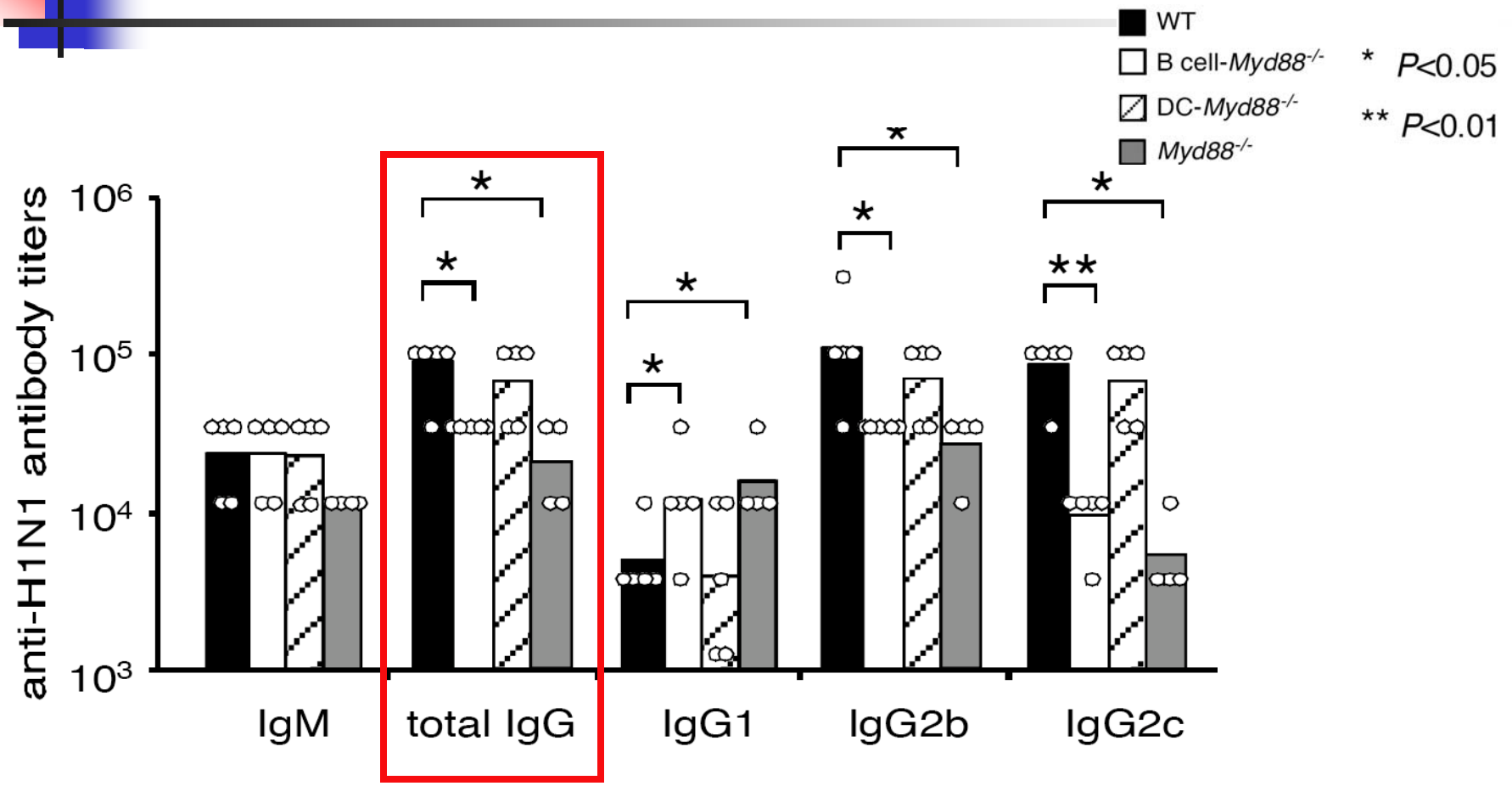

IgG response is almost totally dependent on MyD88 --> role in B cells is most important but another cell type can contribute

# VLPs engage B cell MyD88 signaling to promote the germinal center response




Numbers of antigen-specific germinal center B cells on day 7 (similar result later as well); Requires TLR ligand inside VLP

# TLR signaling in B cells also enhances the Ab response to inactivated influenza virus




Serum Ag-specific Ig titers at 2 weeks after i.p. immunization

# Summary of VLP data

•B cell MyD88 enhances the T cell-dependent Germinal Center response to virus-like particles containing TLR7 or TLR9 ligands

•The TLR ligand must be in  the VLP to have this effect

•Deletion of MyD88 from DCs does not decrease the IgG response but DCs are required (probably to activate CD4 T cells initially)

•Magnitude of B cell MyD88 enhancement of response to VLPs correlates with epitope density (greater density--> more enhancement)

•This enhancement by B cell MyD88 can also be seen with enveloped virus particles (inactivated influenza virus)

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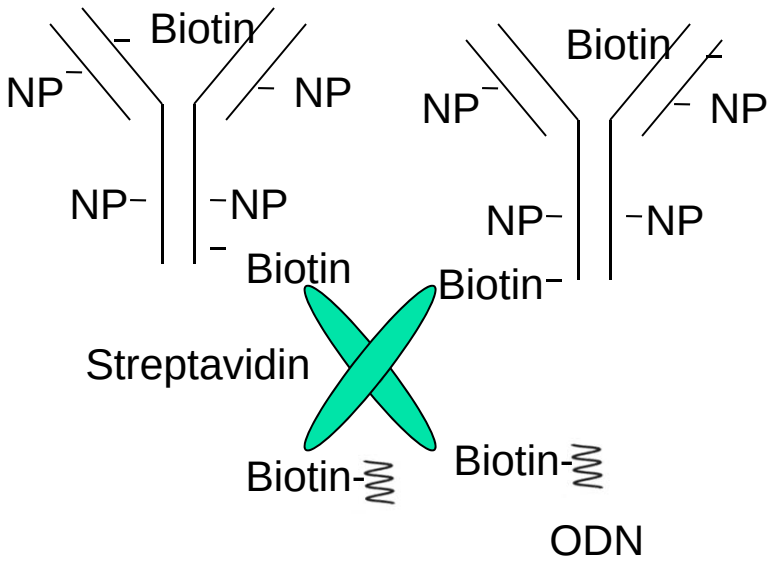
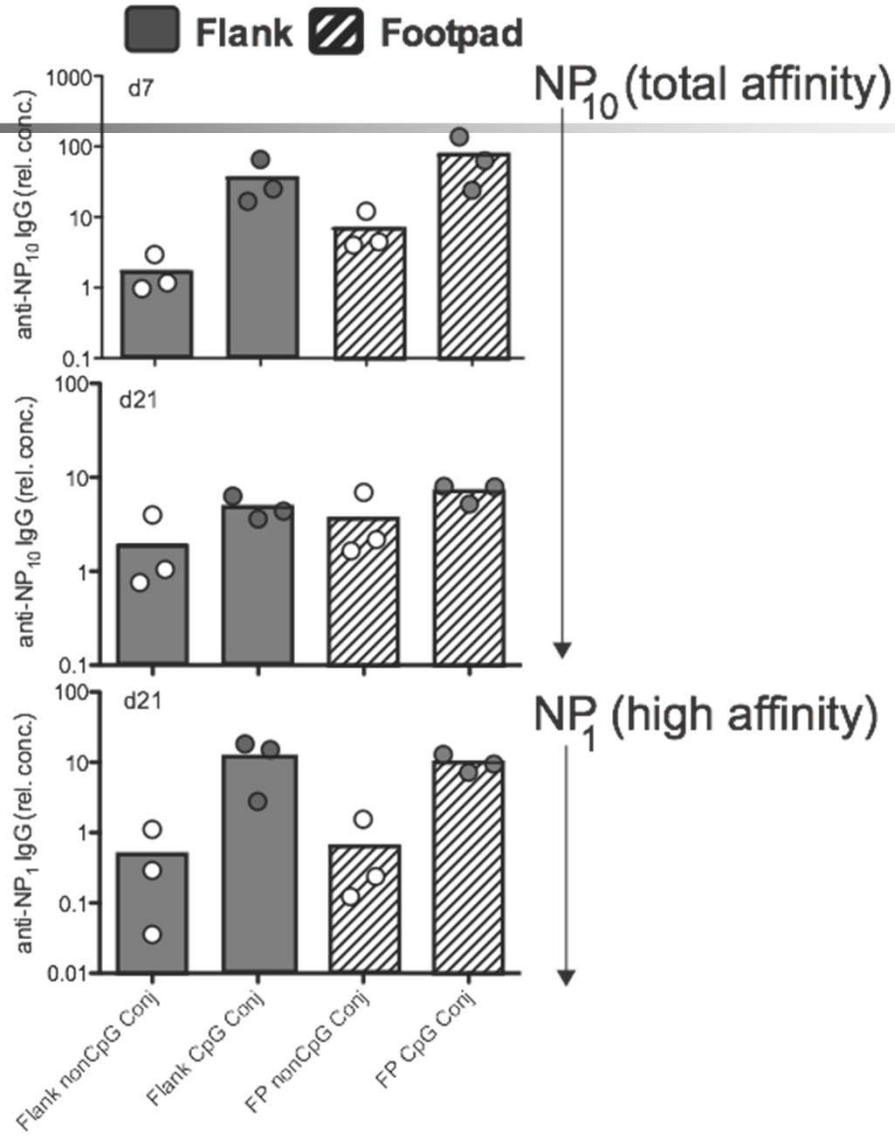
# Role of MyD88 signaling for germinal center response

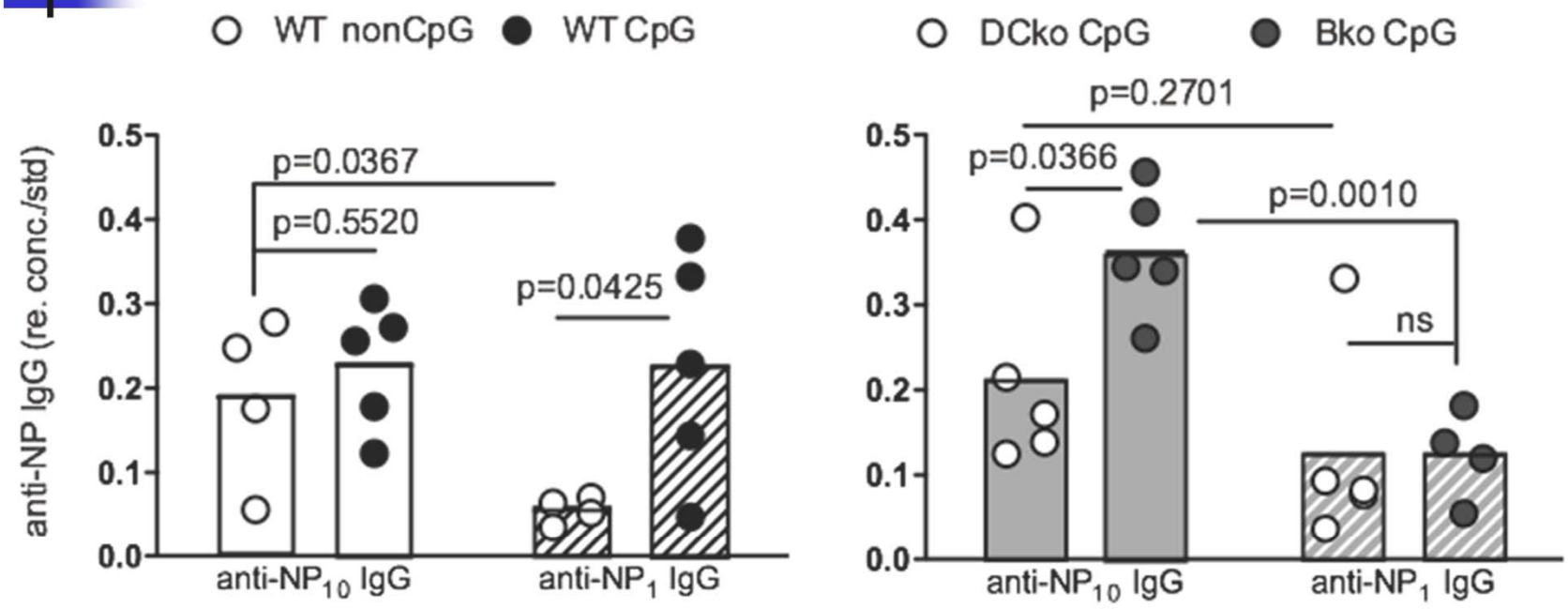



Oligomerized NP-CGG + attached CpG or non-CpG oligonucleotide (ODN)

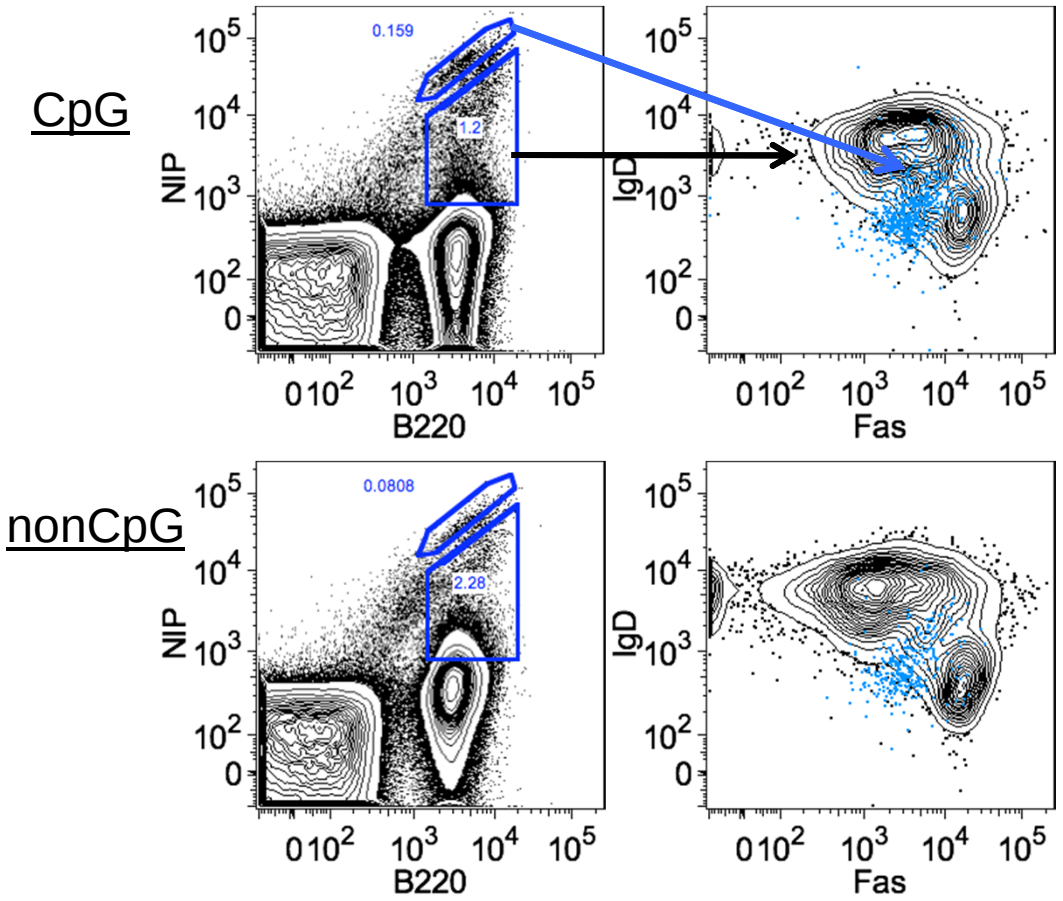
# Inclusion of TLR9 ligand boosts the total anti-NP response at early times and the high affinity anti-NP at all times

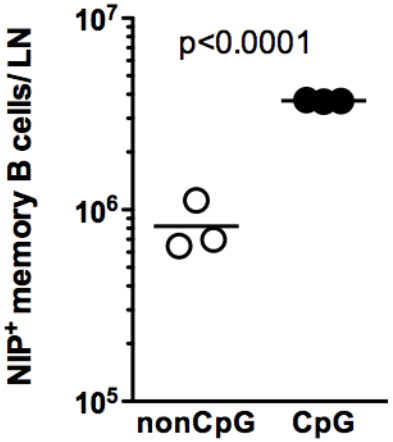


# Ablation of MyD88 in dendritic and B cells compromises affinity maturation

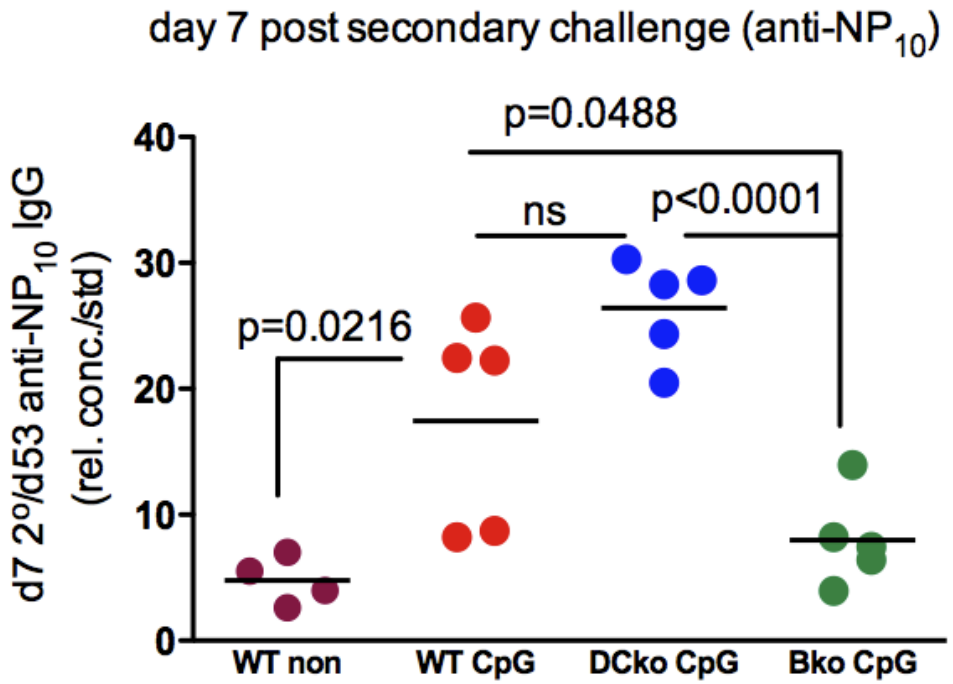


# Increased number of memory anti-NP B cells with inclusion of TLR9 ligand in the NP-CGG complexes



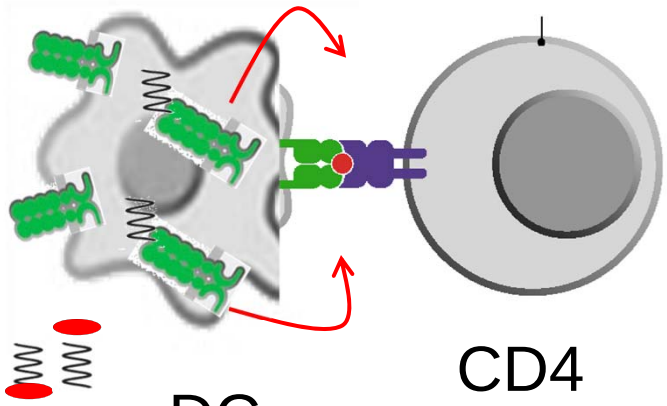



# Memory anti-NP response is decreased in absence of MyD88 in B cells



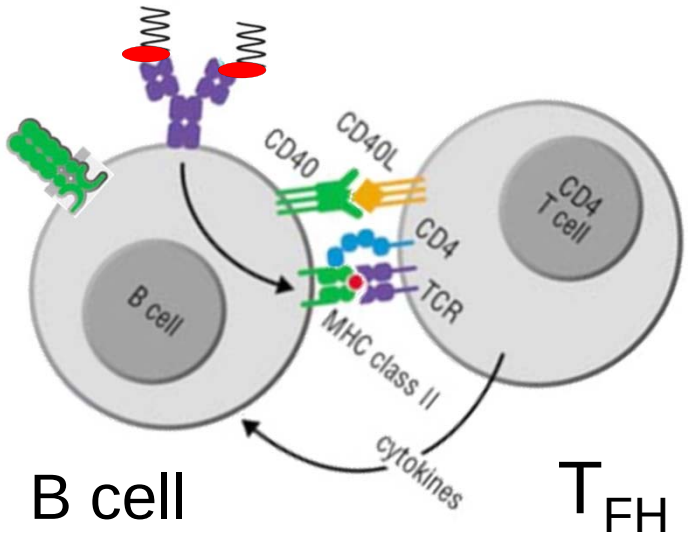
# Augmentation of IgG response to soluble protein antigen by DC cell TLR signaling

1. Protein-CpG conjugate



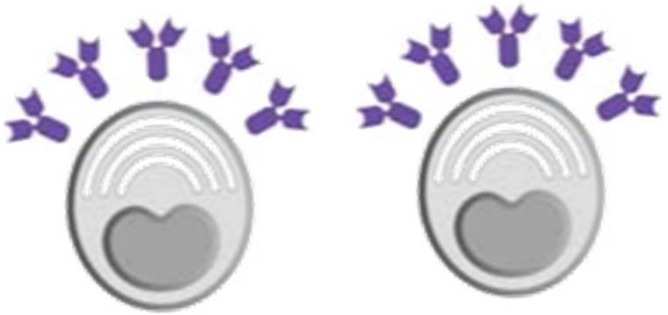
DC

CD4
T cell




B cell

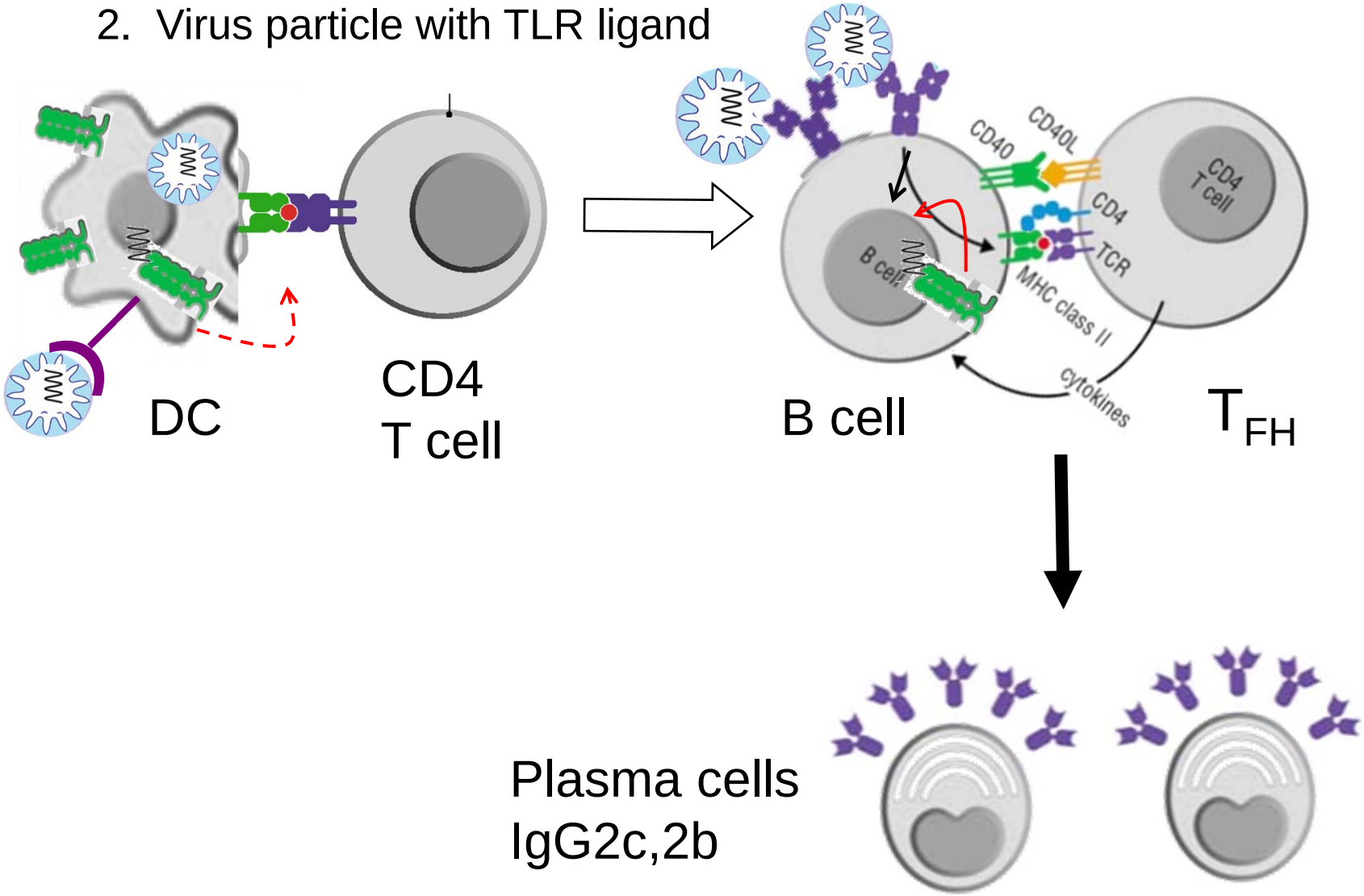
$T_{FH}$



Plasma cells
IgG2c,2b

# Augmentation of IgG response to virus by B cell TLR signaling

2. Virus particle with TLR ligand



# Summary/Implications

- VLPs, inactivated influenza virus particles, and oligovalent antigens engage BCR and B cell TLR7/TLR9/MyD88 signaling to induce a strong germinal center response
- These results are surprising:
  - 1) expectation that “second signals”are less important if BCR signaling is vigorous
  - 2) strong BCR signaling has been associated with TI-2 and extrafollicular TD antibody responses NOT GC response (other contexts)
  - 3) B cell TLR stimulation has been associated with TI and extrafollicular TD responses not GC responses (Salmonella, etc.)
- Strikingly, physical properties corresponding to many viruses --> especially engage this mechanism. Suggests this is an evolved response to promote protection from virus infection

# Acknowledgements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