1 (25 pts).

Describe briefly the **phenotype compared to wild-type** and **the reason behind it (5 pts each)**
Phenotype: C57BL/ScCr mice will live vs wt mice, which die due to cytokine storm. Reason: This is because C57BL/ScCr mice contain a mutation at the TLR4 gene. Without a functional TLR4 receptor, LPS can't induce cytokine storm that kill wild-type mice.
Phenotype: No activated NK cell activity in mice without DCs. Reason: During injections, DCs are needed to secrete IL-12, which usually activates NK cells in wild-type mice.
Phenotype : Susceptible to infection (or they only have IgM/IgD and not other isotypes). Reason: CD40L-CD40 signaling is required for the B cells' 2nd signals. Without it, B cells can't proliferate and can't undergo class switching.
Phenotype: No (or less) production of type I interferons (or cytokines). Reason: TLR9 is required for PDC to recognize the viral DNA (non-methylated CpG DNA)
Phenotype: No mature B cells in the peripheral organs and bone marrow (ok just say no mature B cells). Reason: Without the membrane exons, Ig μ can't be expressed on the cell surface, resulting in defective pre-BCR complex. B cell development is arrested as a result.

2 (20 pts).

a) (4 pts)

GGCATTACACTGTG/CACAGTGATGCTAA

b) (4 pts, 2 pts each).

V_K3: GTGCGGCAGGGTG

J_K2: ACTGGGCCATAA..

c) (12 pts total)

GTGCGGCA [TGCCG] [GT] ACTGGGCCATAA

N **P** (8 pts)

5 pts total if [TGCCGGT] as N nucleotides

5 pts total if [TG] as P and [CCG] as N and [GT] as P nucleotides

3 pts total if [TG] as P and [CCGGT] as N

Note: [TG] can't be P nucleotides because P nucleotides are the results of the hairpin formed by RAG-1/2, which occurs at the heptamer junction. After exonuclease deletion, P nucleotides can't be formed.

GTGCGGCAG [CC] GGGCCATAA

N (4 pts, No partial points)

3) (15 pts, 5 pts each)

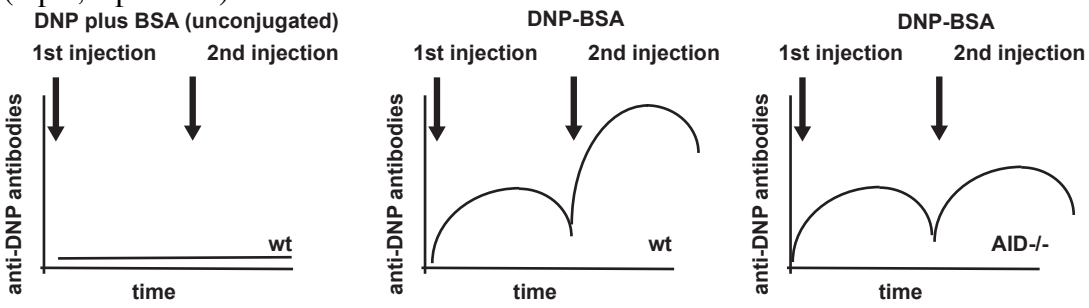
Without the Fc fragment, humira lacks 3 biological functions associated with a whole antibody:

1) Opsonization, engulfment by macrophages
2) Complement mediated killing initiated by antibodies.
3) ADCC, cellular killing by the NK cells.

(alternative answers: no cross-linking function to aggregate cell surface receptors, no neutralization that results in macrophage phagocytosis).

4 (21pts total) a)

(6 pts, 2 pts each)



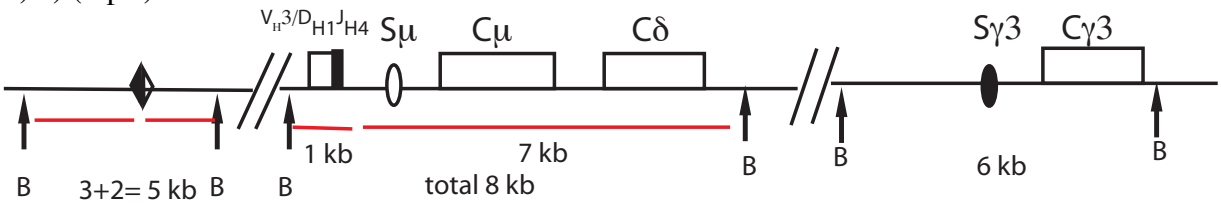
b) (15 pts, 5 pts each). Please explain briefly your answers

Unconjugated: DNP is not a protein and thus can't recruit T cells for help. Adding BSA separately can't help DNP-specific B cells.

Conjugated: DNP is not a protein and thus can't recruit T cell help. Using conjugated DNP to BSA would allow the DNP B cells to express BSA peptides to be presented by their MHCs on the cell surface. This in turn allows recruiting of BSA-specific T cell to deliver the 2nd signals, resulting in normal primary response. There is greater secondary response due to somatic hypermutation and class switching or the presence of memory B cells.

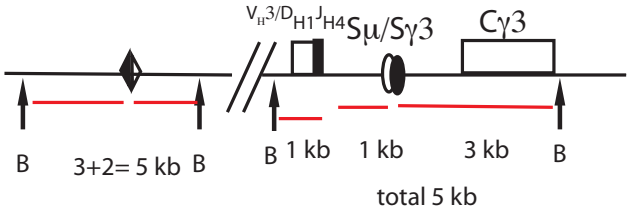
Conjugated DNP-BSA in AID$^{-/-}$ mice: Without AID, there is no class switching and no somatic hypermutation, resulting in defective secondary antibody response, which is usually composed of mostly IgG.

5) a) (6 pts)



Ok not to draw the Sγ3 map

b) (6 pts)



Ok not to draw the signal joint map

c) (12 pts, 2 pts each, ok not to indicate 1x)

liver	Myeloma 1	Myeloma 1	Myeloma 2	Myeloma 1	Myeloma 2
2X 4kb	1X 5 kb 1X 4 kb	1X 9 kb 1X 8 kb	1X 9 kb	2X 6 kb	1X 6 kb 1X 5 kb

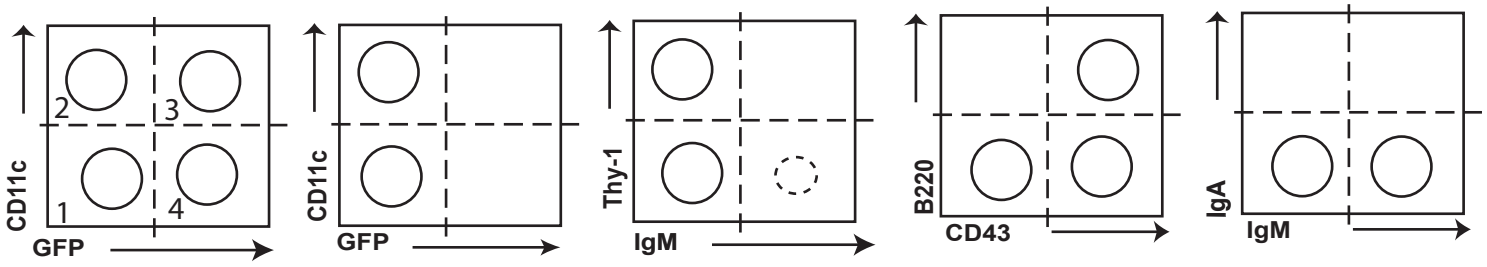
d) (12 pts) (3 pts each)

WT Liver	Tg liver	WT spleen	Tg Spleen
2X 11 kb	5X 12 kb		5X 12 kb

total: 36 pts

e) (3 pts) Due to allelic exclusion that suppresses V to DJ rearrangements, there are less signals (bands) in the Tg spleen. There are still a few DJ rearrangements, however, as well as the 12 kb transgenic bands.

6 (30 pts)
a)



Ok to leave quadrant 2 blank (because it is hard to see pDCs in spleen, which are only ~1%)
Ok to draw a normal circle in IgM+ for Jkappa mutant mice.

b) (8 pts)
Quadrant (1.5 pts each):
1: T cells (or B cells, stromal cells but can't say monocytes or macrophages)
2: pDCs (or none)
3: cDCs
4: monocytes (or macrophages)

2 pts: GFP+ cells also express the Diphteria toxin receptor and thus are eliminated when diphtheria toxin is injected.

c) (12 pts, 4 pts each).
Without Jκ, B cells will rearrange the λ gene segments. The Ig λ proteins will pair with μ to form IgM. There might be less IgM due to the loss of the κ light chains. Loss of Jκ has no effect on development of Thy-1+ T cells.

Without the heavy chain J gene segments, B cell development stops at pre-B cell stage because the pre-BCR can't form and thus immature and mature B cells B220+ CD43- cells are absent in these knockout mice.

Without Sμ, no class switching can occur and thus there are no IgA+ cells that are present in wt mice but IgM B cells are not affected.