Intestinal inflammation causes systemic genotoxicity

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Intestinal inflammation

- Inflammatory bowel diseases (Crohn's disease, ulcerative colitis) and celiac disease

  Chronic relapsing diseases involving many genetic and environmental factors resulting in increased risk for colorectal and small intestinal cancers among various extraintestinal manifestations

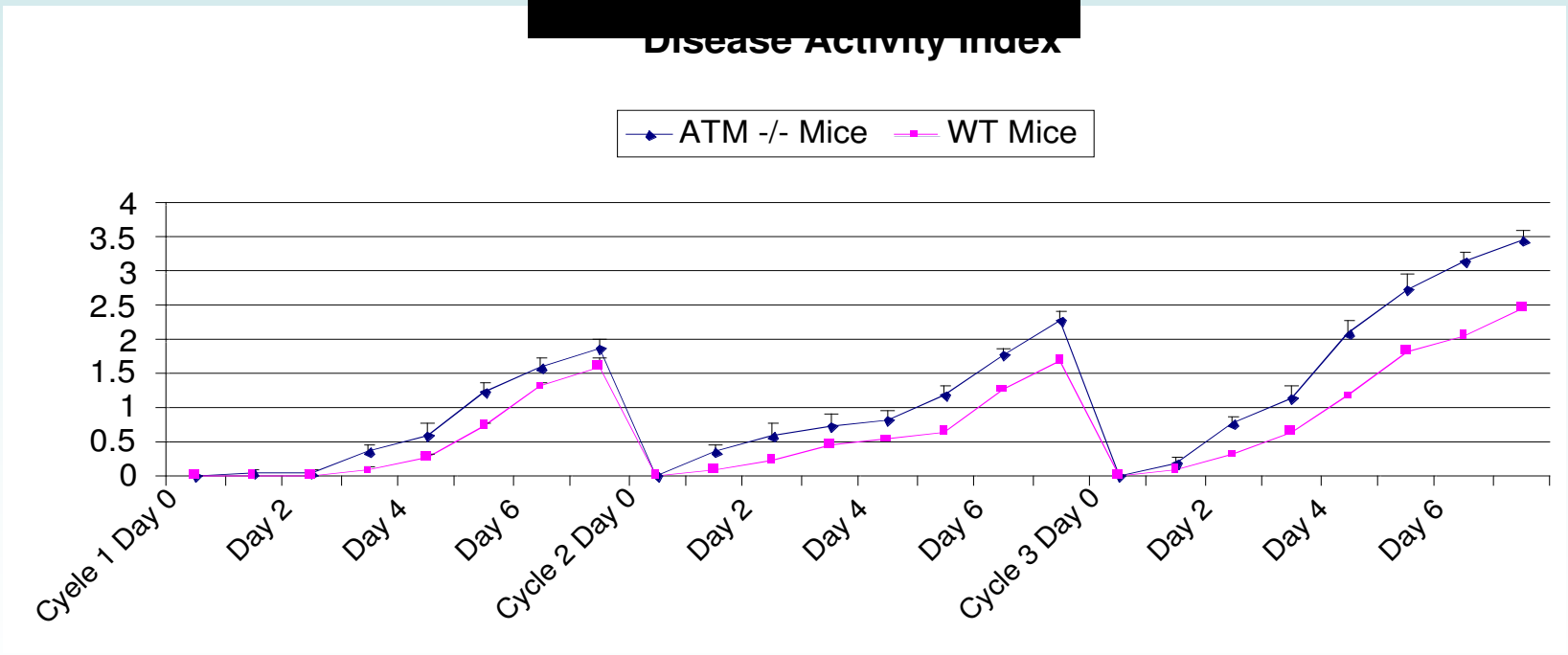
- DNA damage is beginning to be recognized as a feature of chronic inflammation
  - IBDs, rheumatoid arthritis, type 1 and 2 diabetes, chronic respiratory diseases, liver hepatitis/cirrhosis, dermatitis, and in cancer

Severity of symptoms

TABLE 1. CRITERIA FOR SCORING DISEASE ACTIVITY INDEX*

Score	Weight loss (%)	Stool consistency†	Occult blood or gross bleeding
0	None	Normal	Negative
1	1–5	Loose stool	Negative
2	5–10	Loose stool	Hemoccult positive
3	10–15	Diarrhea	Hemoccult positive
4	>15	Diarrhea	Gross bleeding

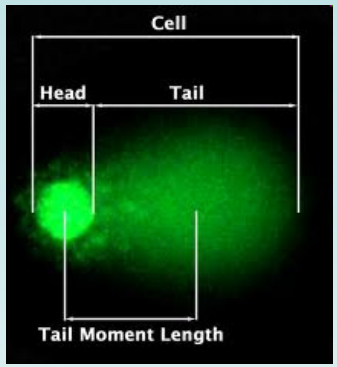
*Disease activity index = (combined score of weight loss, stool constency, and bleeding)/3.
†Normal stools = well formed pellets; loose stools = pasty stool that does not stick to the anus; and diarrhea = liquid stools that sticks to the anus.



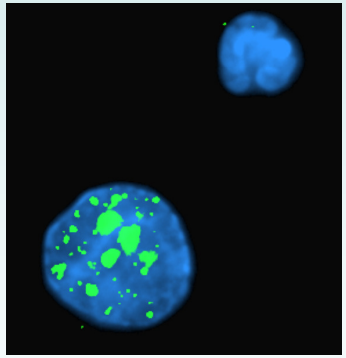
*Two Atm-/- mice died due to treatment (one after cycle 2, one end of cycle 3)

Assays of genotoxicity

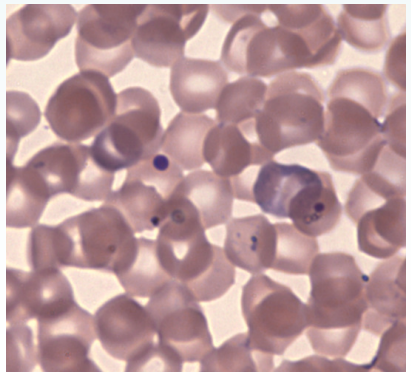
- Alkaline comet assay
  - Quantifies single and double strand breaks via the 'Tail Moment'
  - Additional hOgg1 enzyme incubation will also detect oxidative base damage



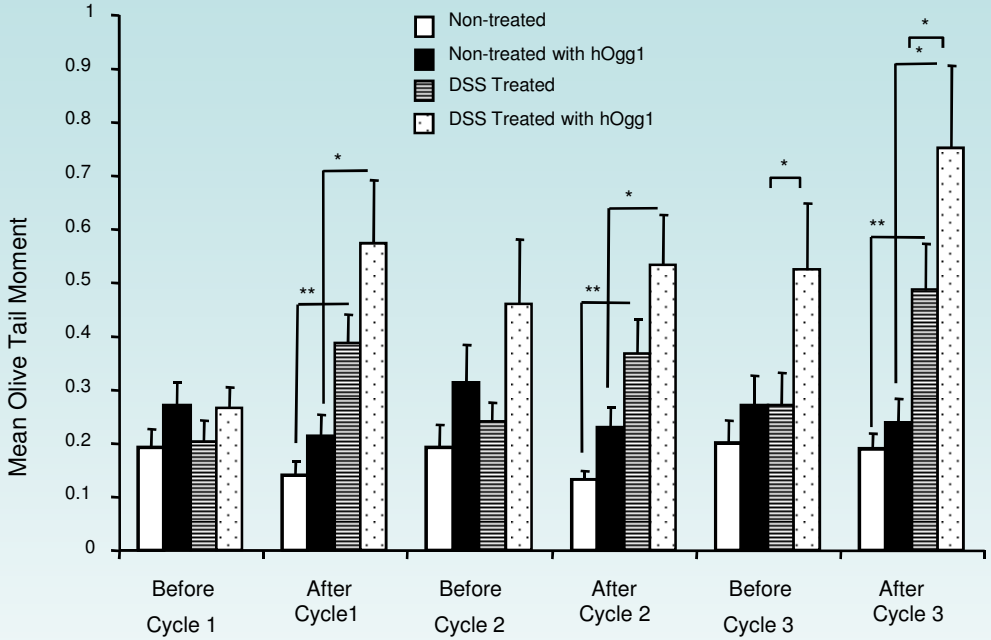
- γH2AX immunostaining
  - Identifies DNA double strand breaks--Histone 2AX becomes phosphorylated in response to double strand breaks by ATM or ATM-like kinases

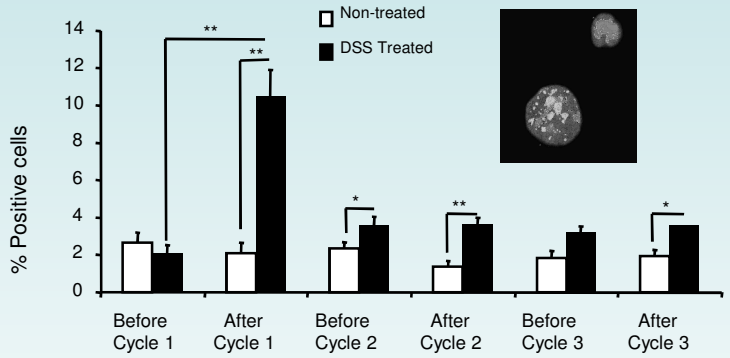


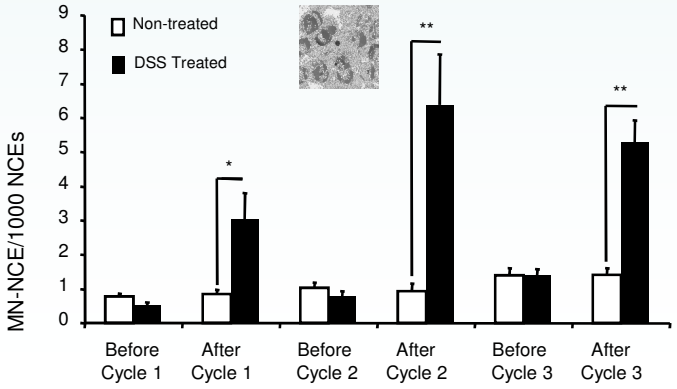
- *In vivo* micronucleus assay
  - Cytogenetic test to detect damage to chromosomes or mitotic apparatus of mammalian cells



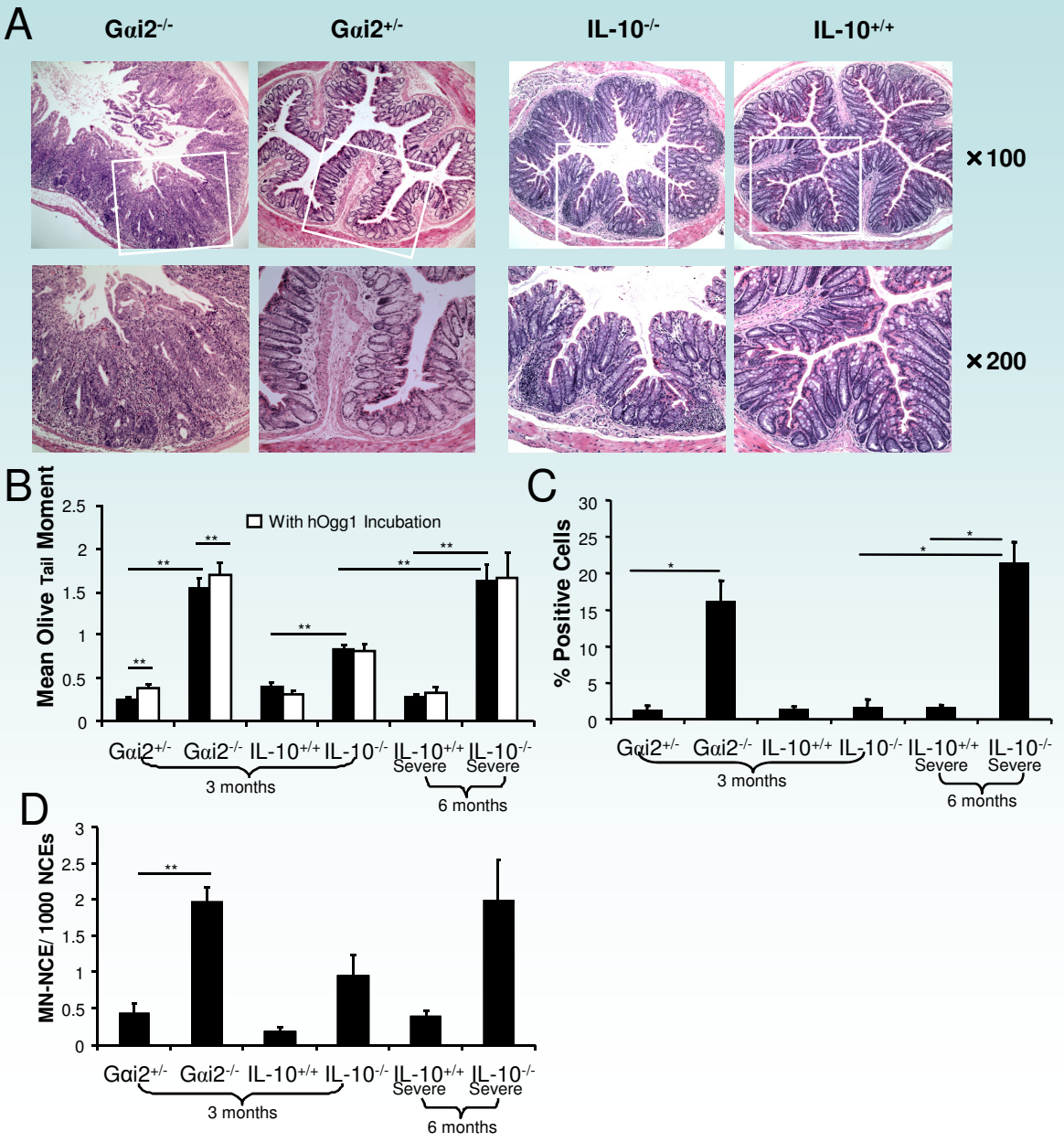
Intestinal mucosal inflammation leads to genotoxicity to peripheral leukocytes

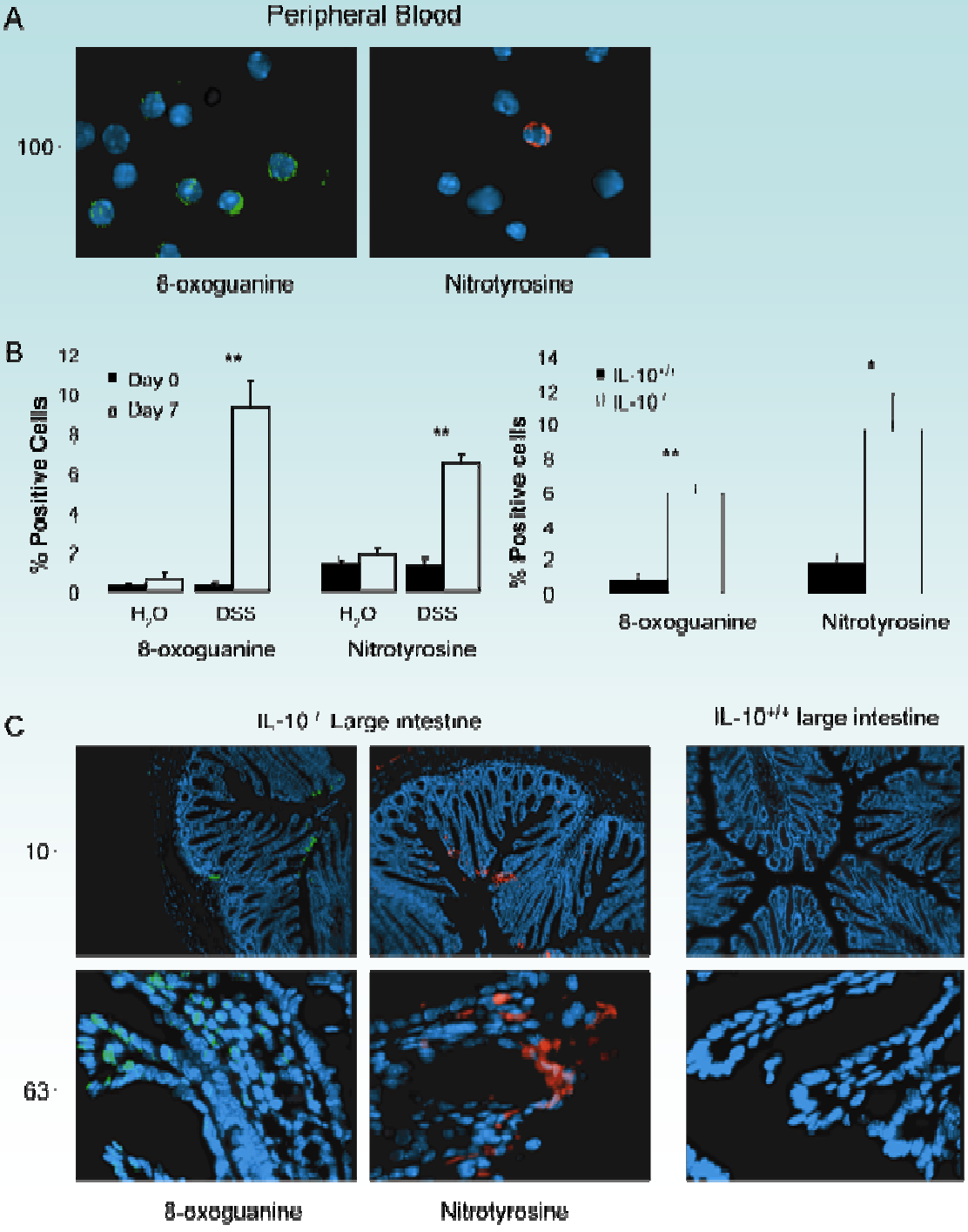






Genotoxicity in genetic models of spontaneous immune colitis




A
Peripheral Blood
100
8-oxoguanine
Nitrotyrosine
B
% Positive Cells
Day 0
Day 7
H2O
DSS
8-oxoguanine
Nitrotyrosine
% Positive cells
IL-10+/+
IL-10-/-
C
IL-10-/- Large intestine
IL-10+/+ large intestine
10
63
8-oxoguanine
Nitrotyrosine

Intestinal inflammations causes systemic genotoxicity

Publication in Cancer Research. 2009 Jun 1;69(11):4827-34
Displayed on the cover of that issue

Press release by the Jonsson Comprehensive Cancer Center
http://www.cancer.ucla.edu/index.aspx?recordid=255&page=644:

Invited for an editorial in Cell Cycle: More damaging than we think:systemic effects of intestinal inflammation. Cell Cycle 2009 Aug15;8(16):2482-3.

Hundreds of webpages on the internet entitled like:
First ever link between intestinal inflammation and systemic genotoxicity

ATAXIA TELANGIECTASIA (AT)

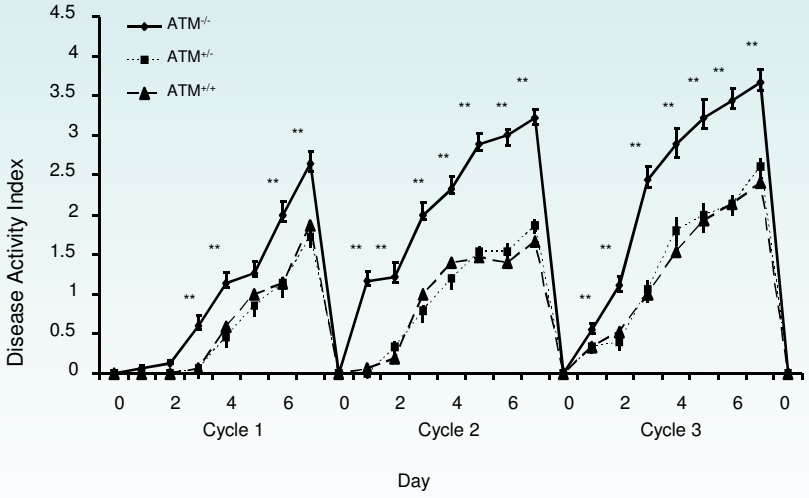
Clinical manifestation:

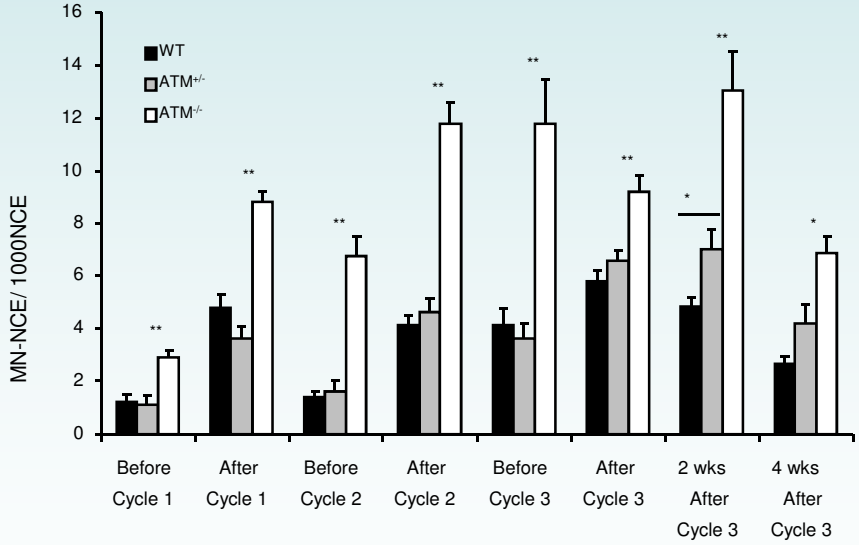
- Autosomal recessive disease (1 in 40.000-100.000 people affected)
- Early-onset progressive cerebellar ataxia
- High incidence of tumors (1000 fold increase)
- Growth retardation
- Immunodeficiency

Biological markers:

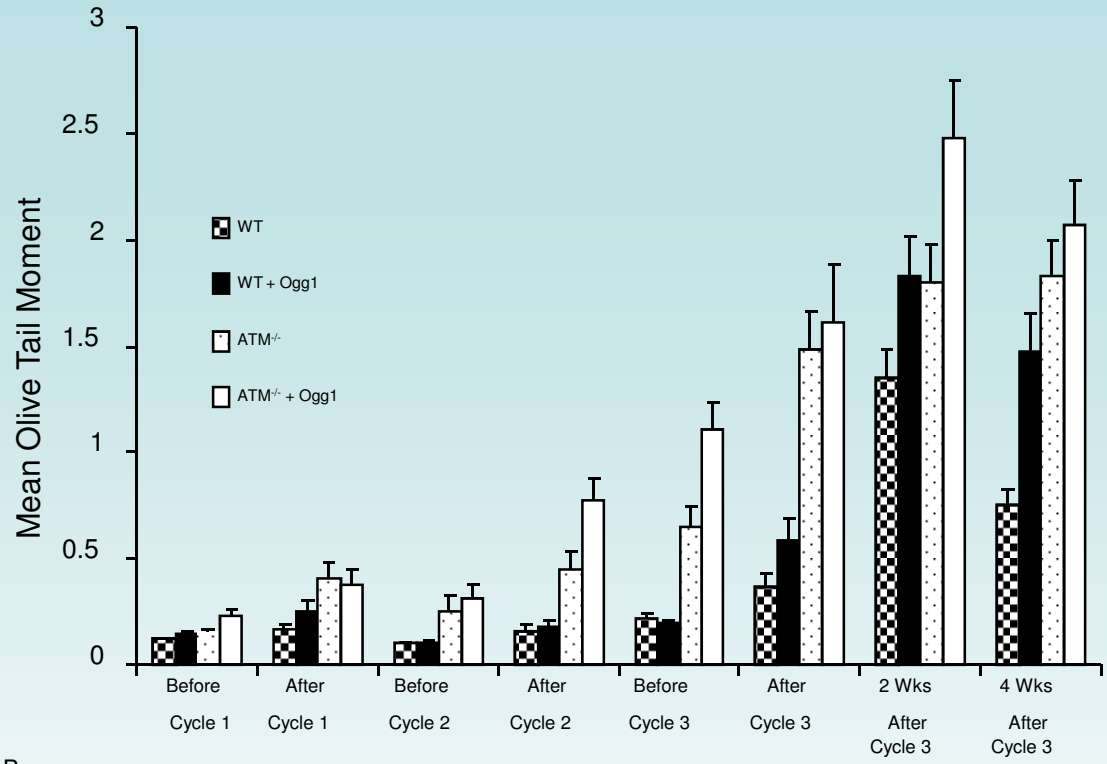
- Chromosomal instability
- Hypersensitivity to radiation
- Imbalance in antioxidant levels and antioxidative enzymes

$ATM^{-/-}$ show elevated sensitivity to DSS

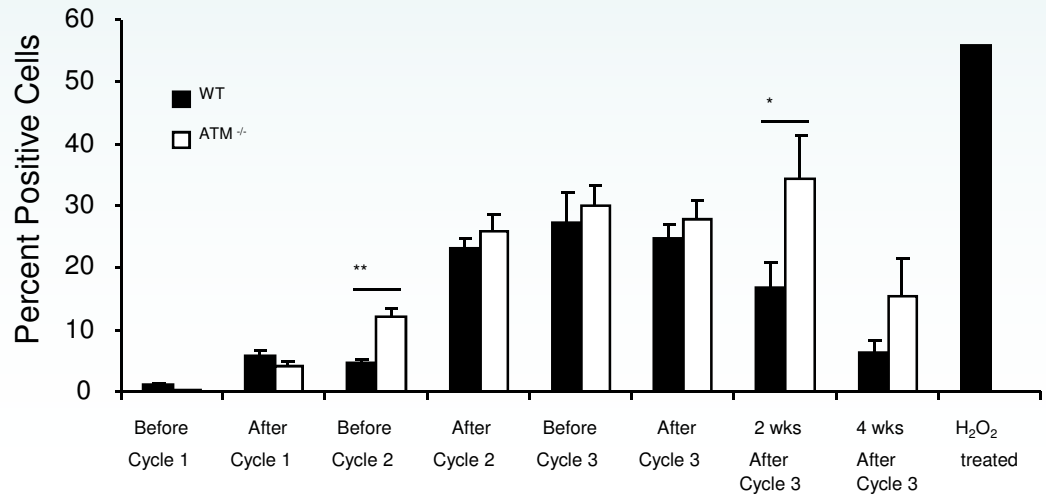




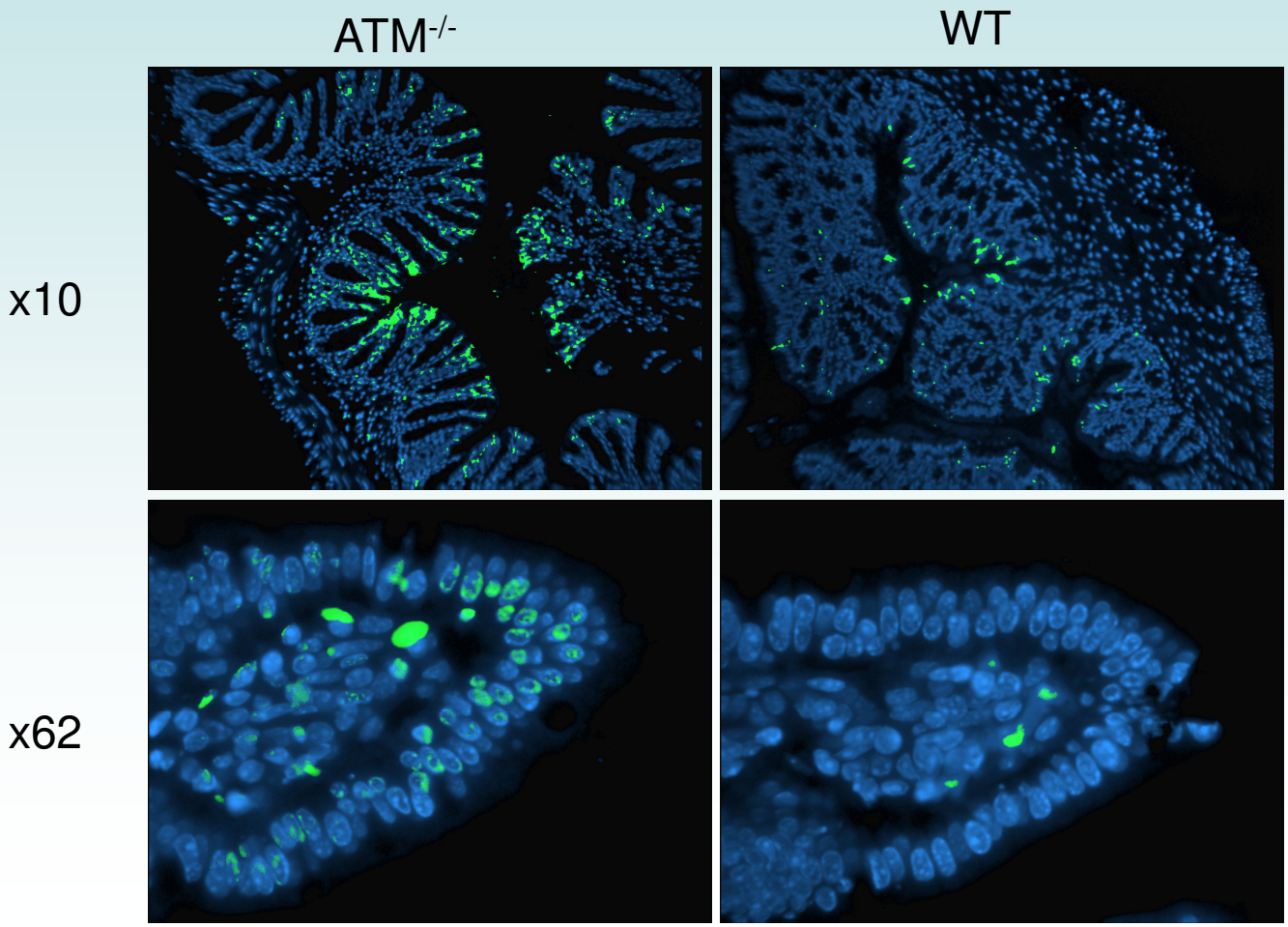
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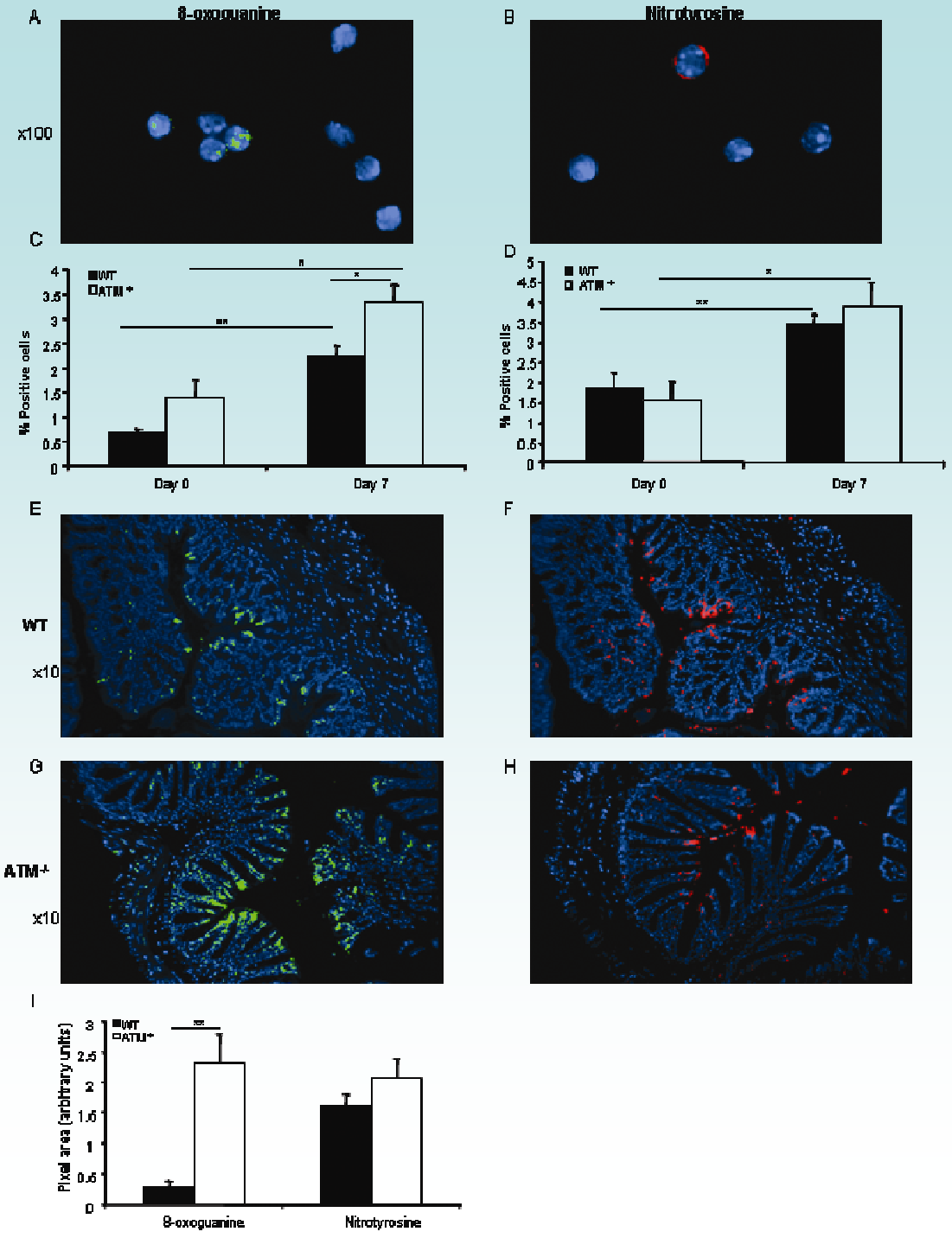

Mean Olive Tail Moment
3
2.5
2
1.5
1
0.5
0
WT
WT + Ogg1
ATM-/-
ATM-/- + Ogg1
Before Cycle 1
After Cycle 1
Before Cycle 2
After Cycle 2
Before Cycle 3
After Cycle 3
2 Wks After Cycle 3
4 Wks After Cycle 3

B

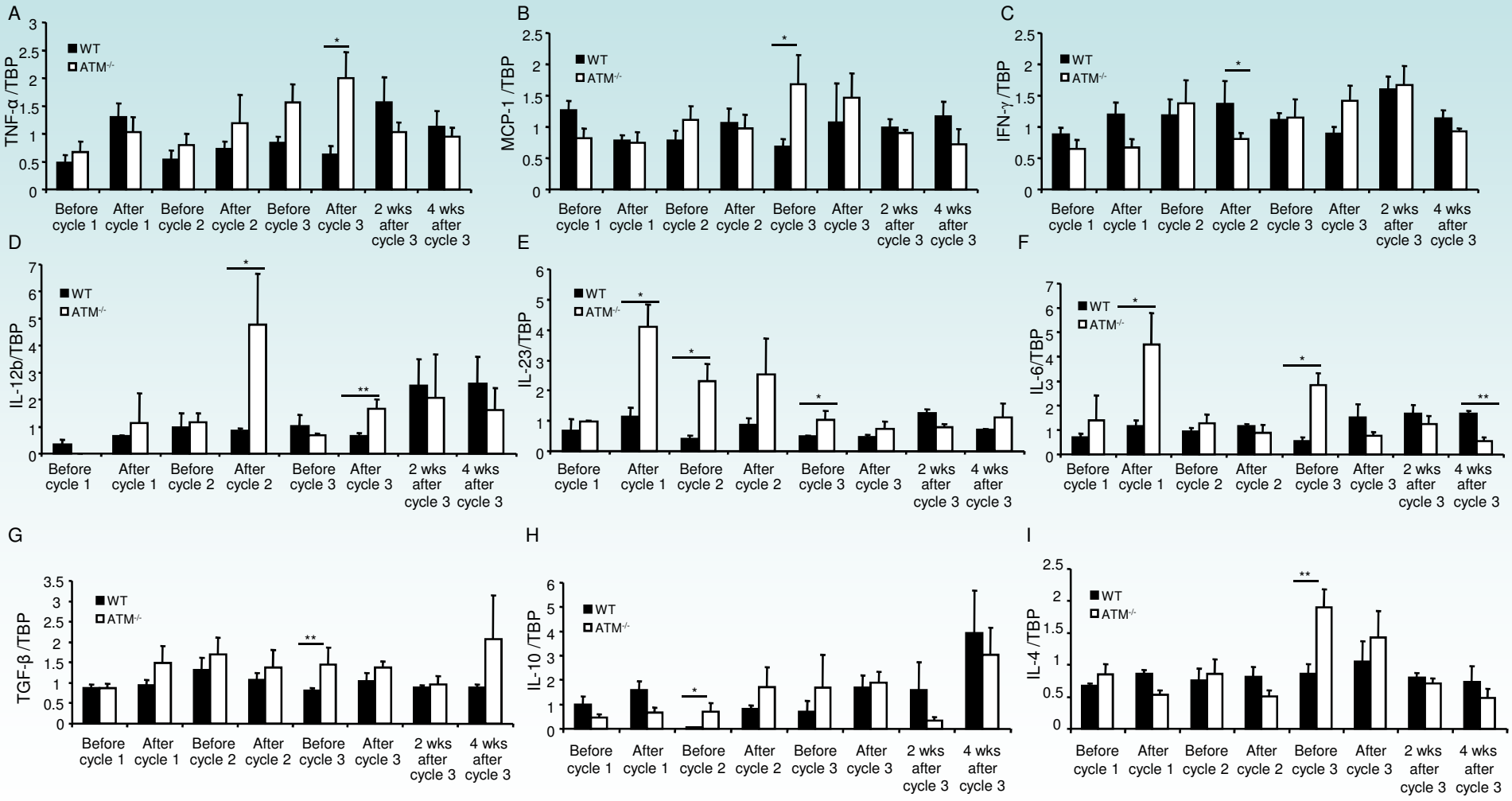

Percent Positive Cells
60
50
40
30
20
10
0
WT
ATM -/-
**
*
Before Cycle 1
After Cycle 1
Before Cycle 2
After Cycle 2
Before Cycle 3
After Cycle 3
2 wks After Cycle 3
4 wks After Cycle 3
H2O2 treated

8-oxoguanine in distal colon of DSS-treated ATM$^{-/-}$ mice

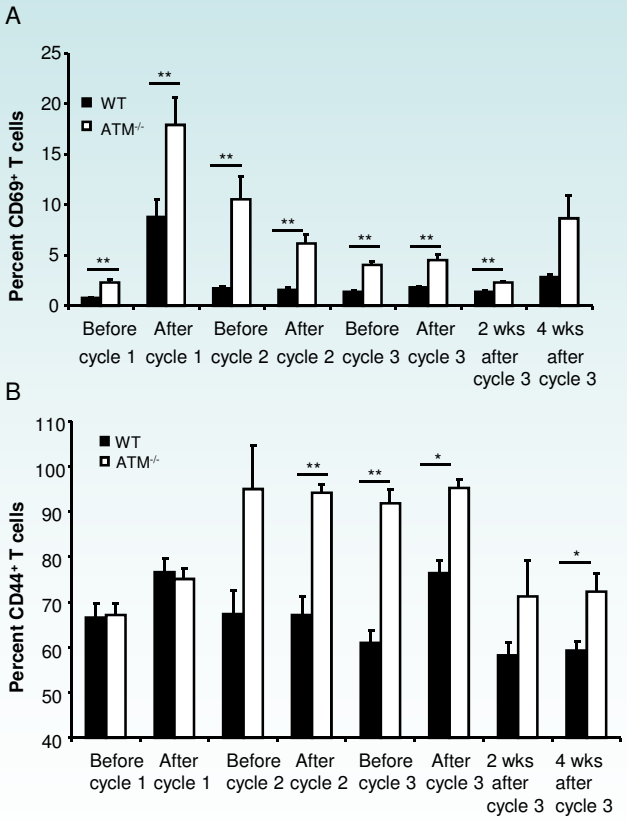



A
8-oxoguanine
x100
B
Nitrotyrosine
C
WT
ATM+
% Positive cells
Day 0
Day 7
D
WT
ATM+
% Positive cells
Day 0
Day 7
E
WT
x10
F
G
ATM+
x10
H
I
WT
ATM+
Pixel area (arbitrary units)
8-oxoguanine
Nitrotyrosine

Cytokine panel in peripheral blood



Surface activation markers in peripheral blood



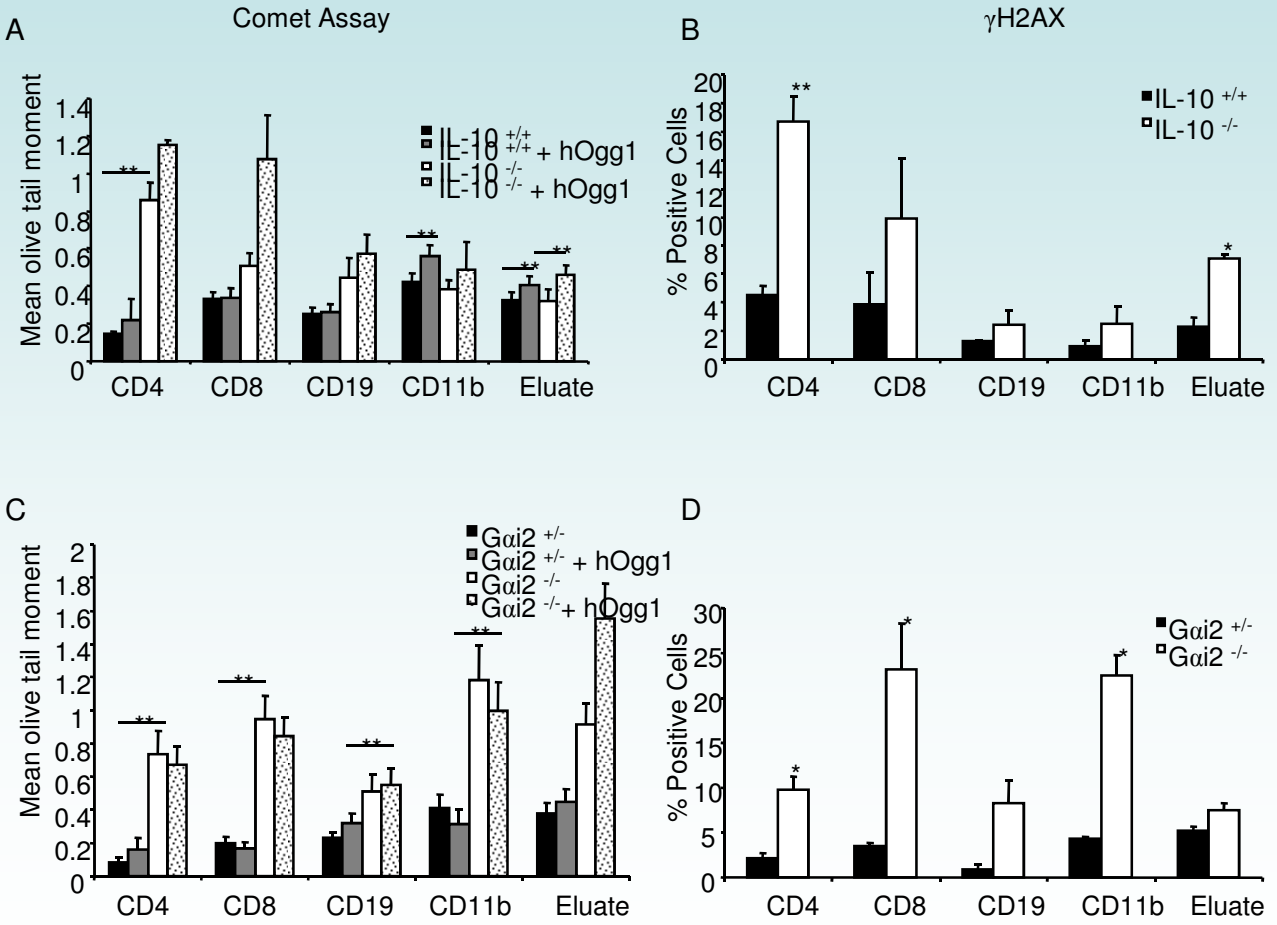
Conclusions

- Damage is mediated by inflammation derived RONS including nitrotyrosine and 8-oxoguanine
- Observed genotoxicity is proportional to clinical symptoms (treatment with DSS or age)
- Systemic distribution of cytokines
- *Atm* plays a critical role in maintaining genetic stability during intestinal inflammation

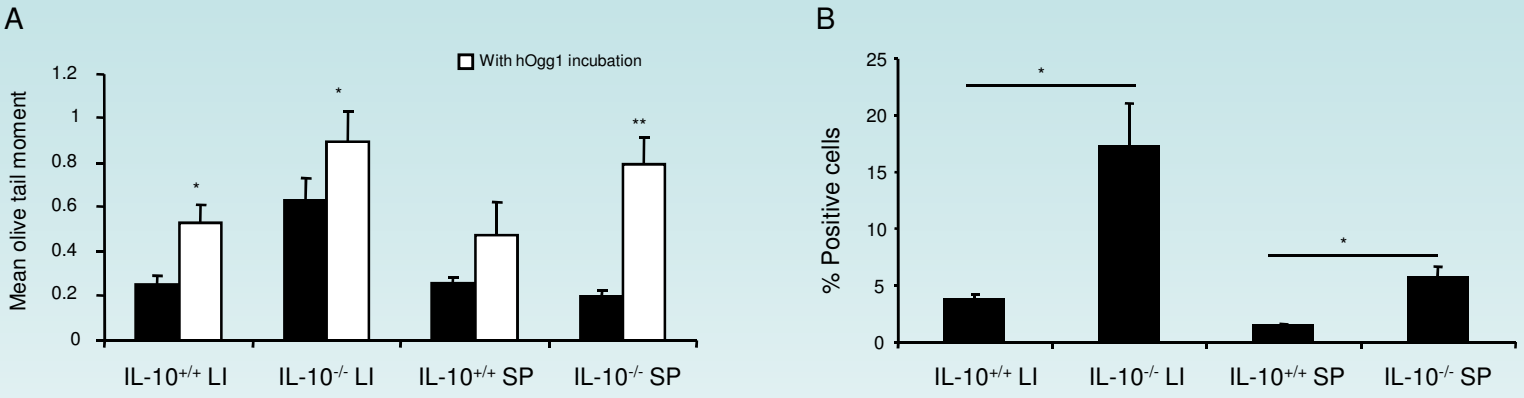
Atm deficient mice exhibit increased sensitivity to DSS-induced colitis characterized by elevated DNA damage and persistent immune activation

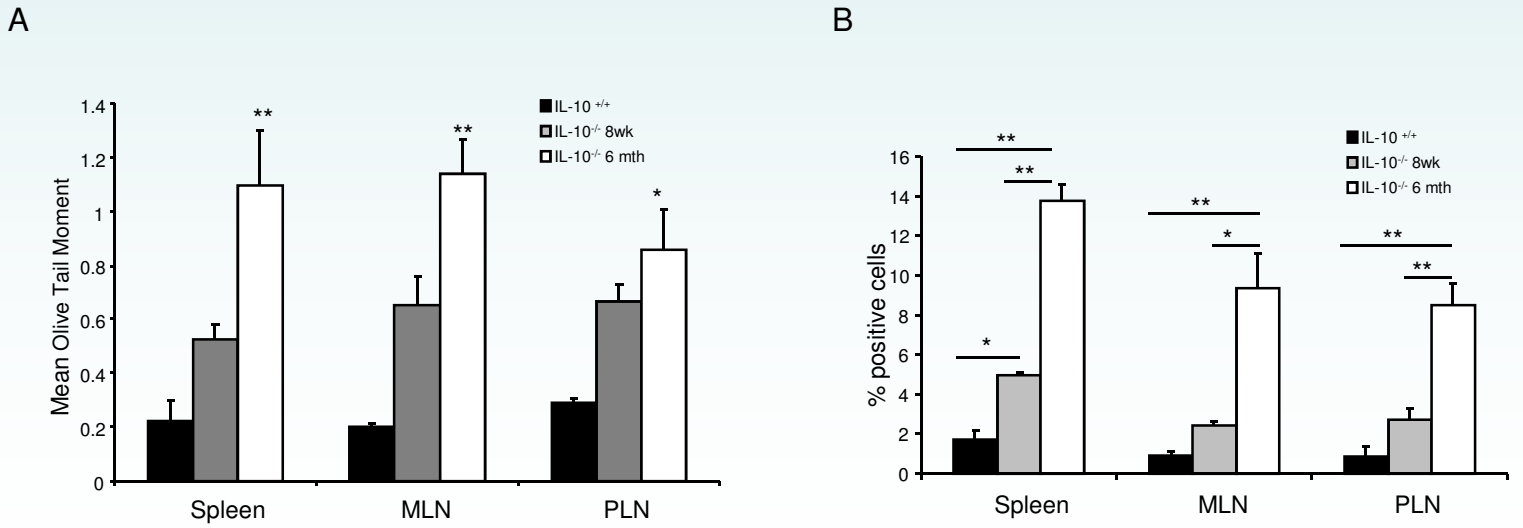
- The lack of ATM causes further DNA damage and genetic instability (induced by byproducts of inflammation), along with a more potent immune response, possibly due to other pathways alerting and further activating the immune response.
- ATM therefore can be inferred to play a role in immunoregulation and maintenance of genetic stability during inflammation

Which cell types are sensitive to DNA damage?

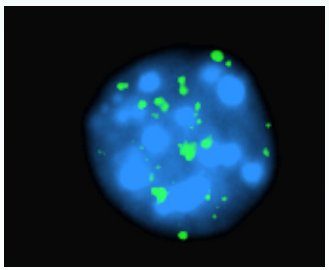


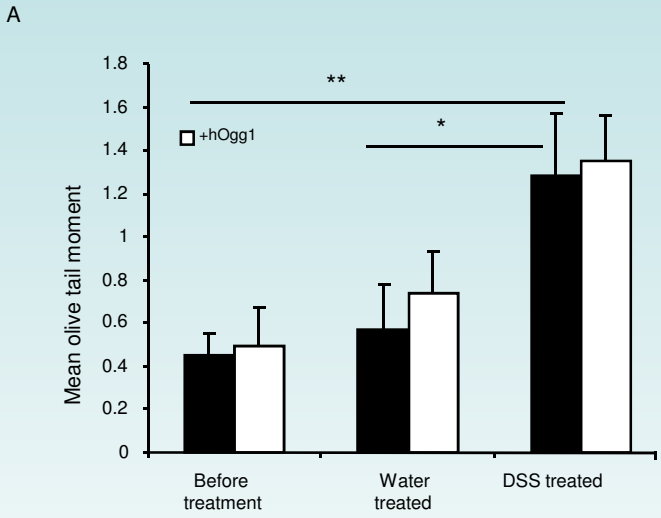
Sensitivity of various organs to genotoxicity

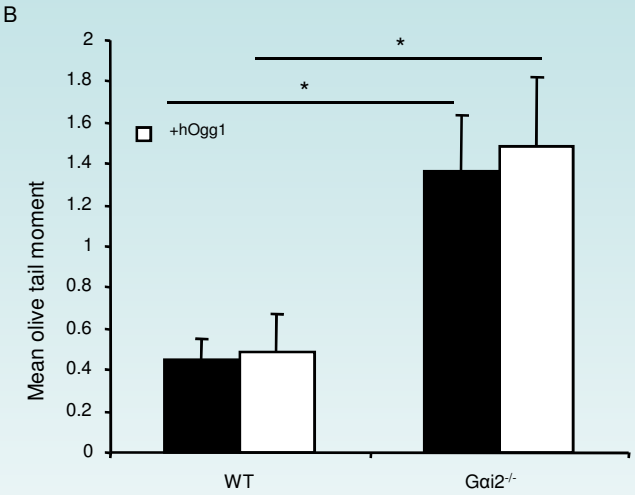


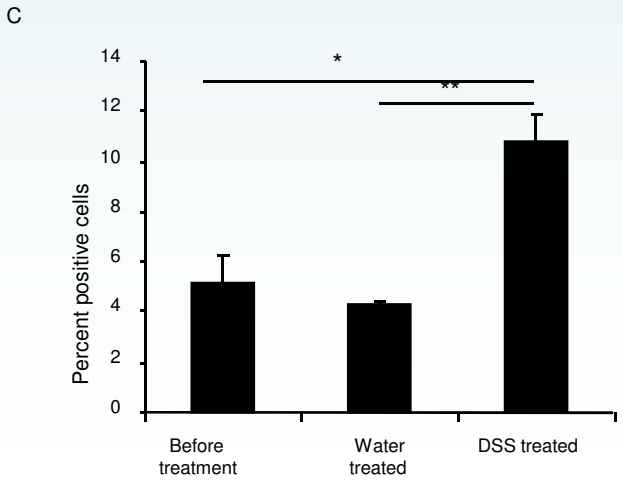


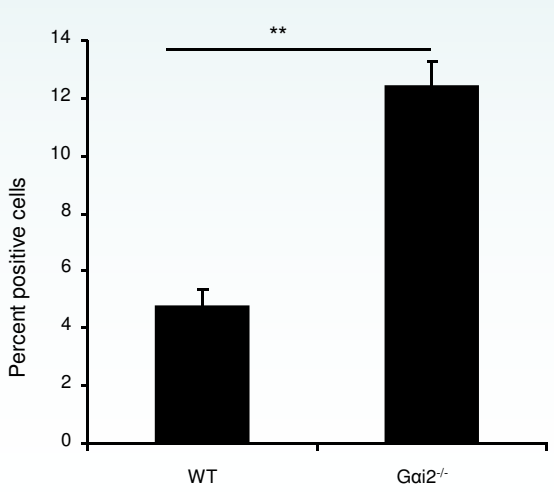
Genotoxicity to hepatocytes in mice with intestinal inflammation



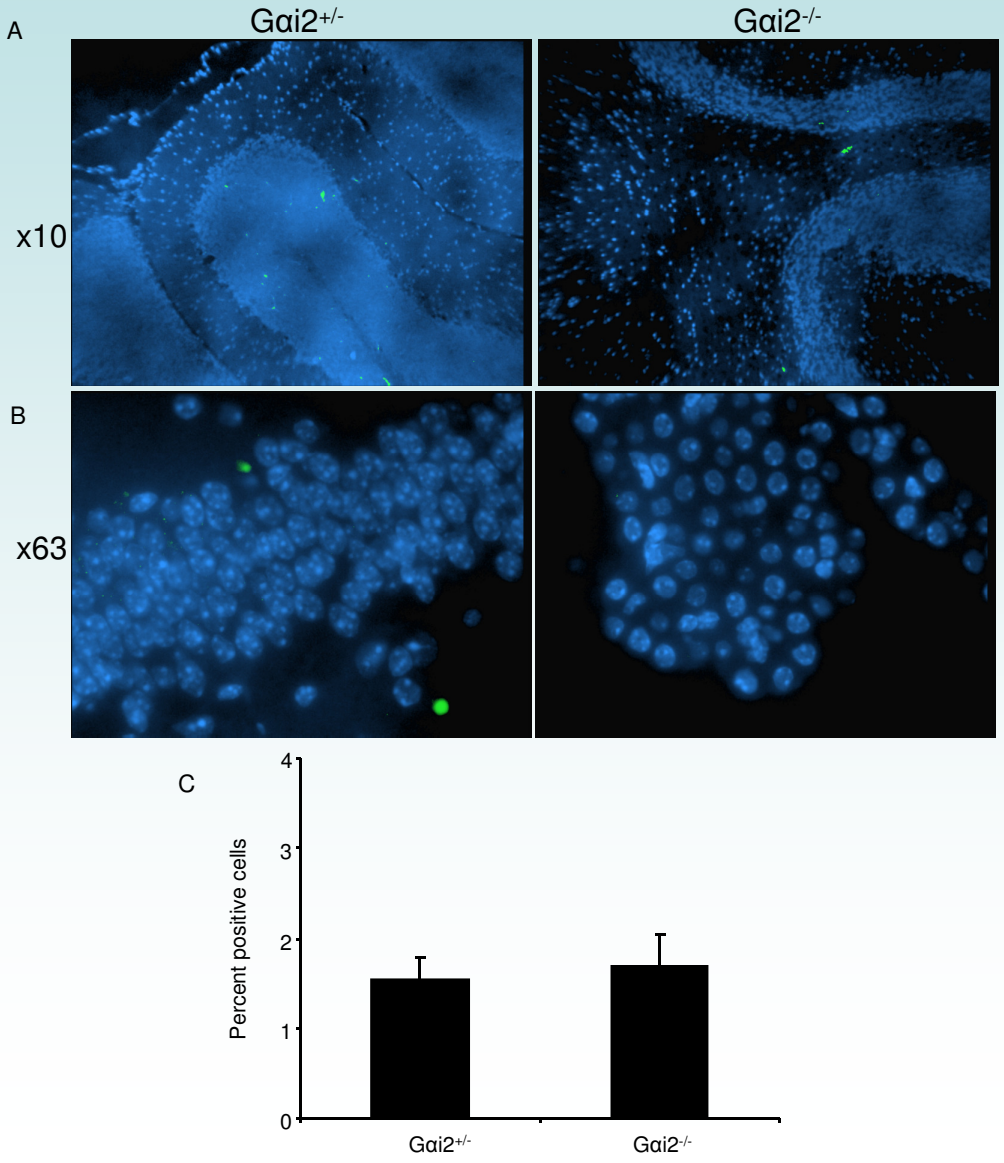








Brain does not manifest DNA damage due to intestinal inflammation



Conclusions

- Intestinal inflammation causes both local and systemic genotoxicity to multiple cell types, which demonstrate different sensitivities and damage load
- Decreased expression of ATM is not responsible for DNA damage as has been shown to be the cause in RA (not shown)
- Damage to lymphoid organs associate with severity of inflammation

Inflammation associated systemic genotoxicity may explain higher occurrences of extraintestinal manifestations due to a systemic chronic inflammatory response

Exploring mechanisms of inflammation-associated genotoxicity

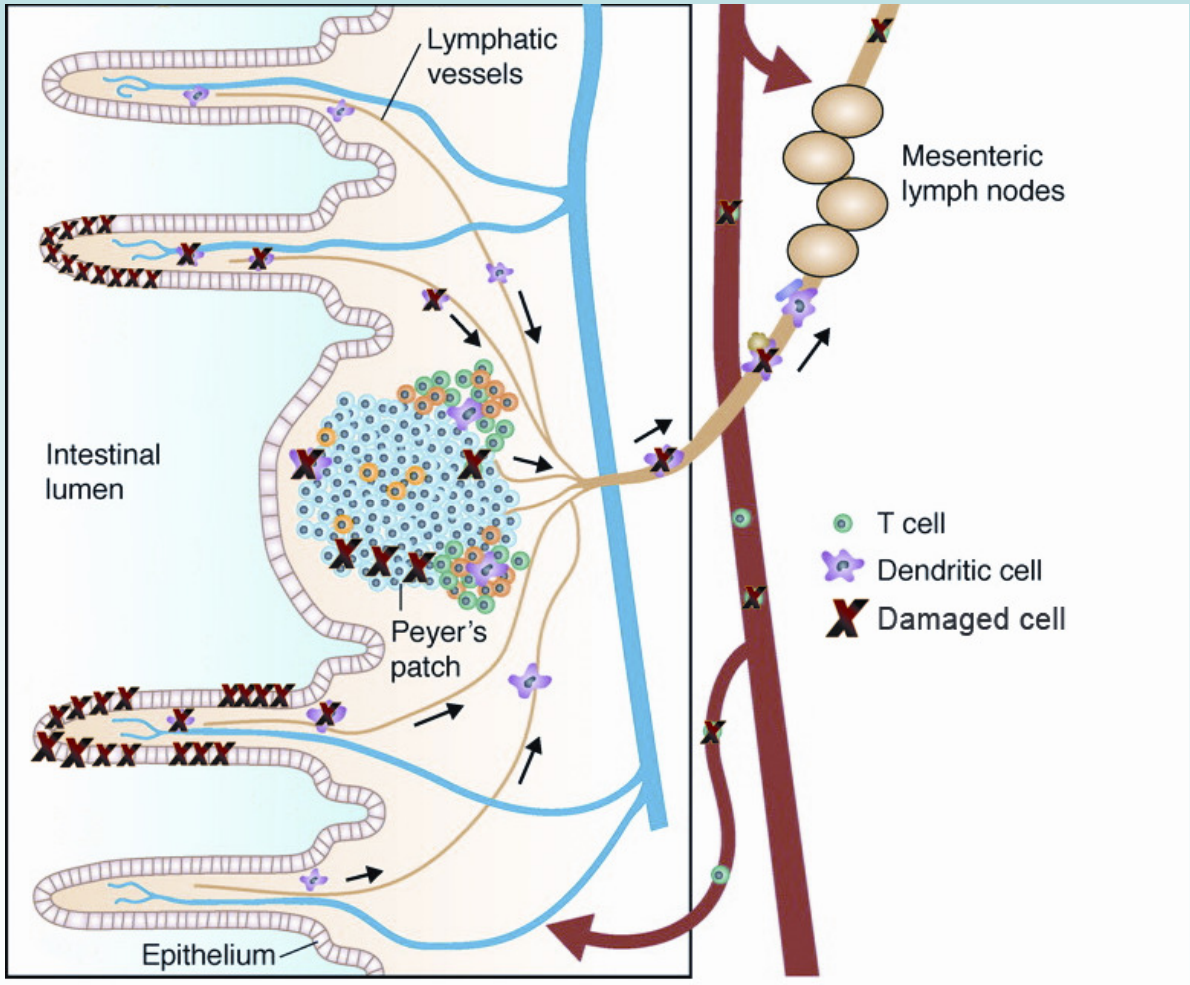
Hypotheses:

Induction of DNA damage may require:

1) Presence of the cells at the site of inflammation, which then circulate systemically (does not explain damage to hepatocytes)

2) Presence of multiple cell types (classically activated monocytes/macrophages) which have the capability of releasing RONS during oxidative burst and damaging neighboring cells/themselves

3) TNF receptor-mediated signaling and its downstream mediators directly cause damage within the cell

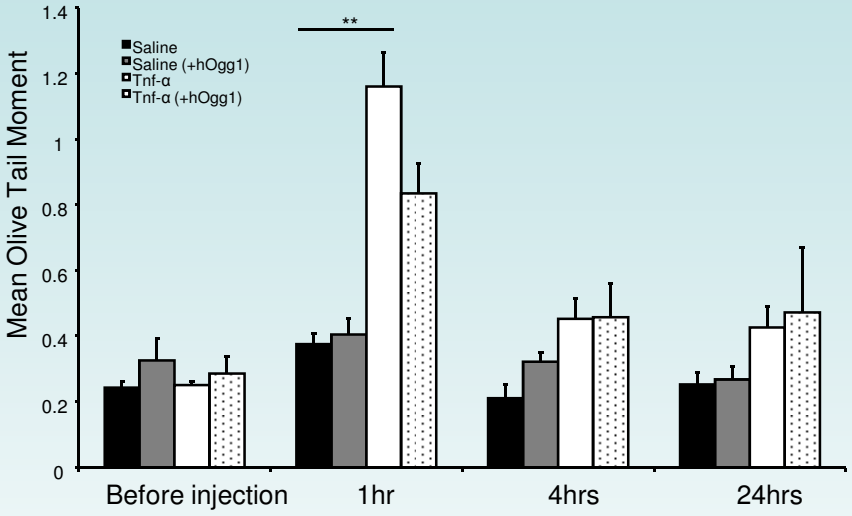

Lymphatic vessels
Mesenteric lymph nodes
Intestinal lumen
Peyer's patch
Epithelium
T cell
Dendritic cell
Damaged cell

Why TNF-α?

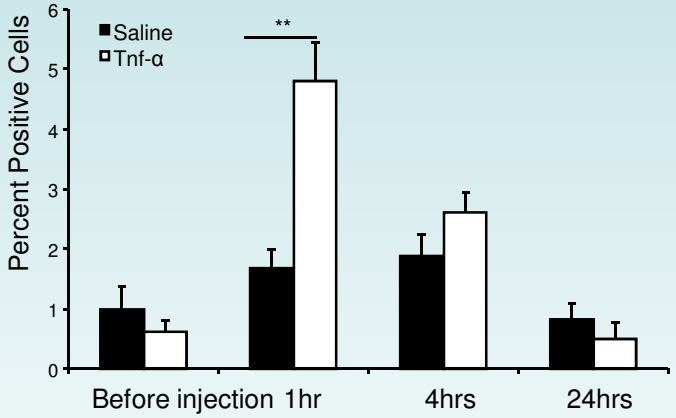
- TNF-α is highly upregulated in patients with IBDs (measured in mucosa, serum, stool) and implicated in pathogenesis
  - Chronic liver hepatitis
  - metabolic diseases
  - Rheumatoid arthritis and other autoimmune disorders
- Systemic and persistent presence may explain systemic genotoxicity

TNF-α is sufficient to induce genotoxicity *in vivo* in wildtype mice

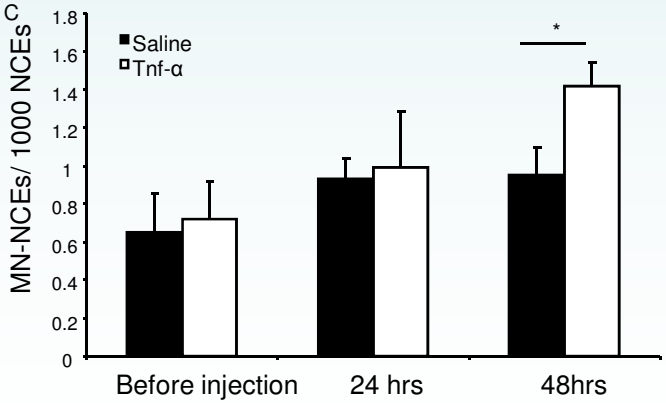
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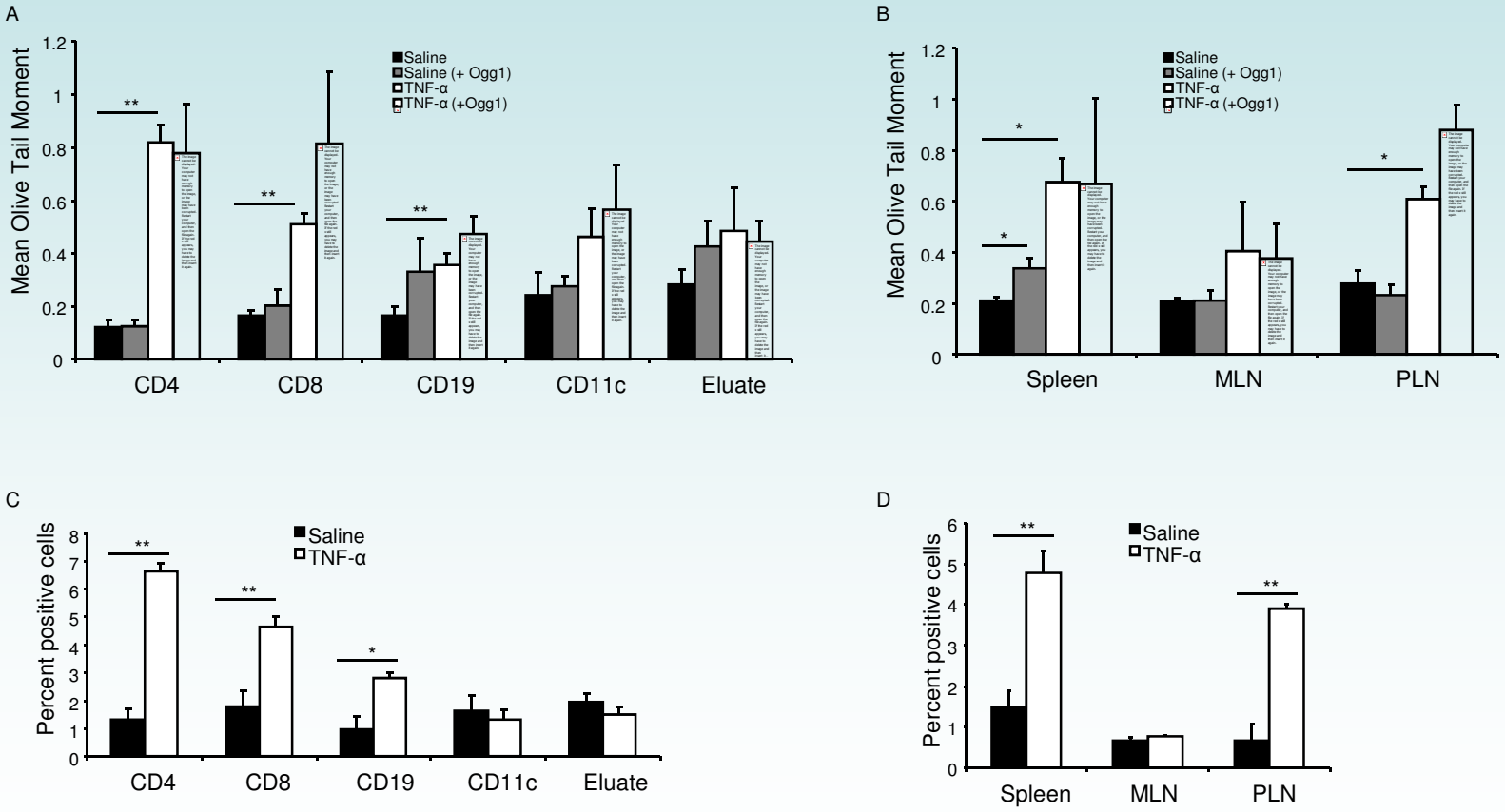
B



C

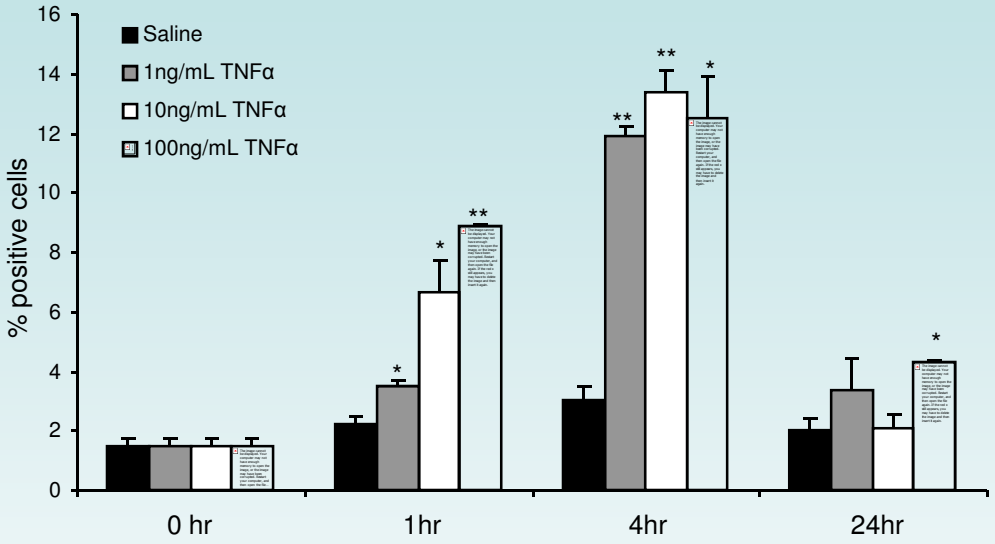


Profile of genotoxicity is similar to IBD models

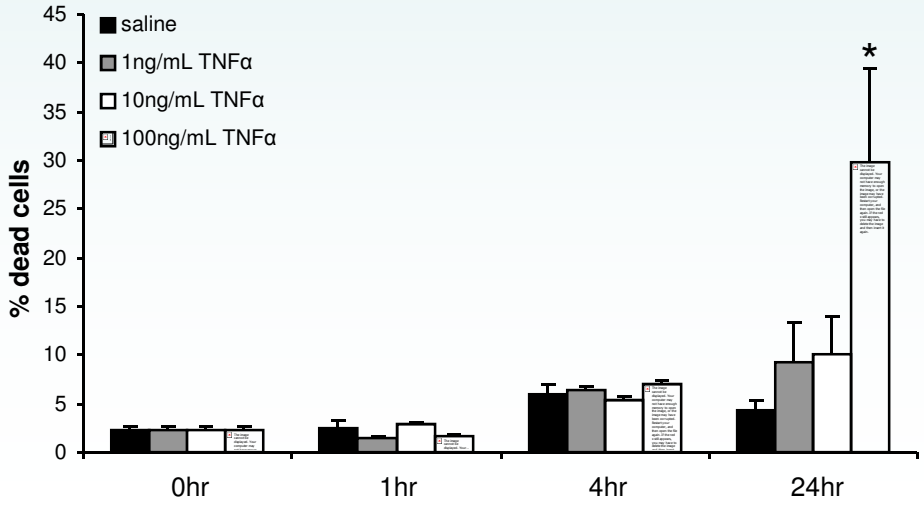


TNF-α causes DNA damage in primary splenic T-cells

γH2AX foci formation in non-apoptotic cells

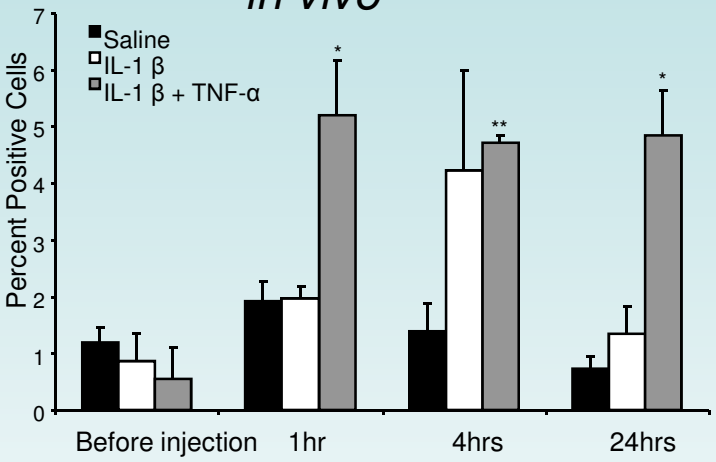


Apoptotic cells (PI+, TO+)

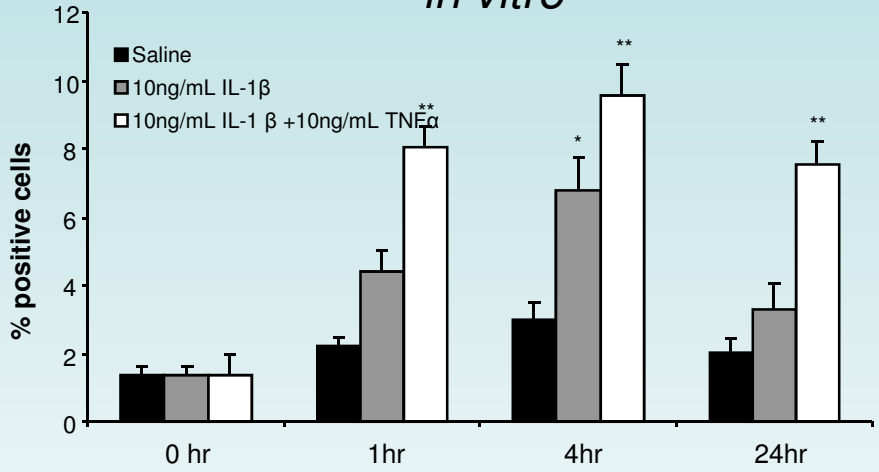


Co-administration of TNF-α and IL-1β results in sustained DNA damage *in vivo* and *in vitro*

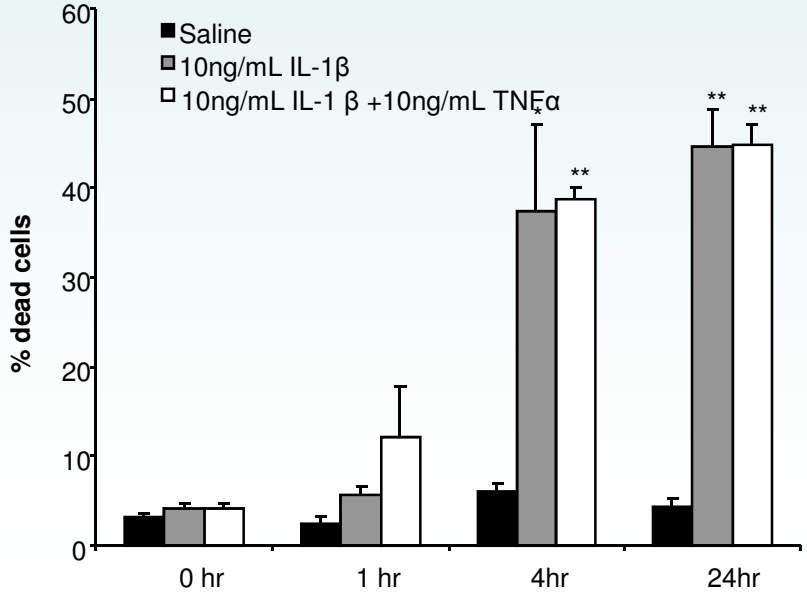
in vivo



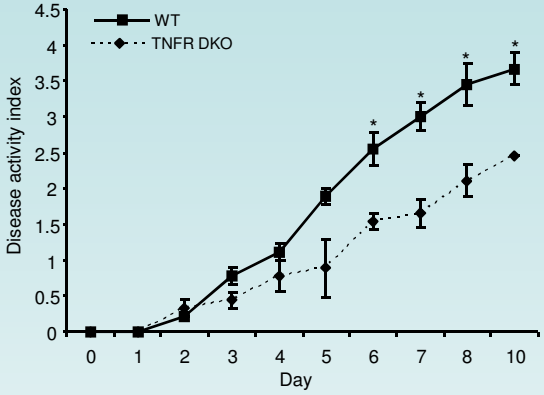
in vitro

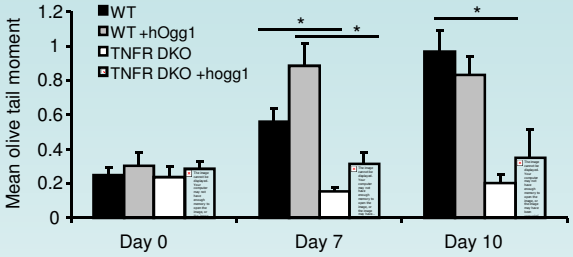


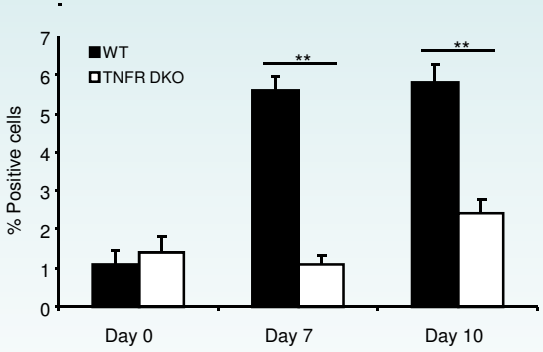
Cell viability

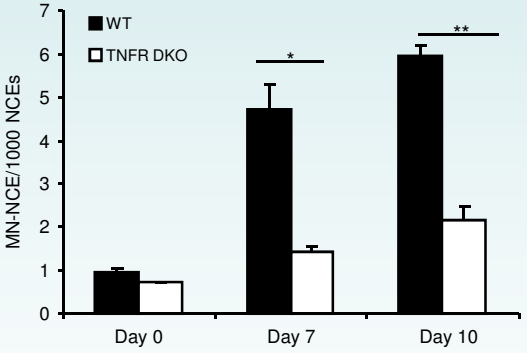


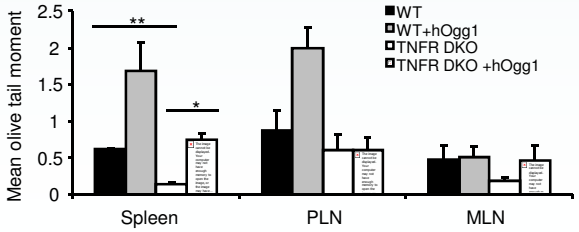
TNFR1$^{-/-}$/TNFR2$^{-/-}$ mice are less sensitive to DSS-induced colitis

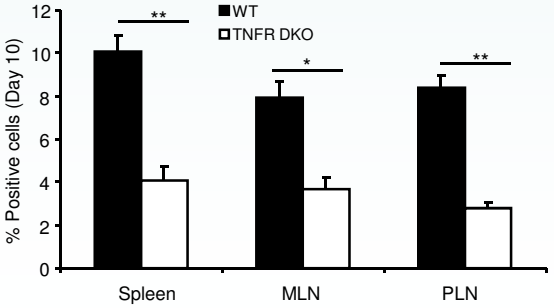




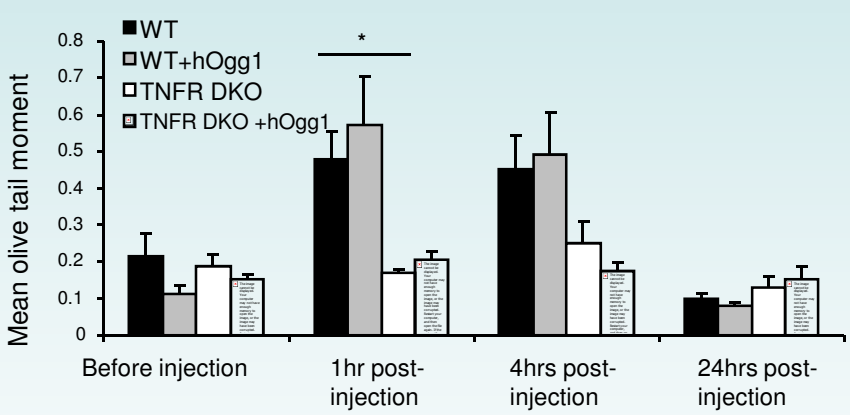


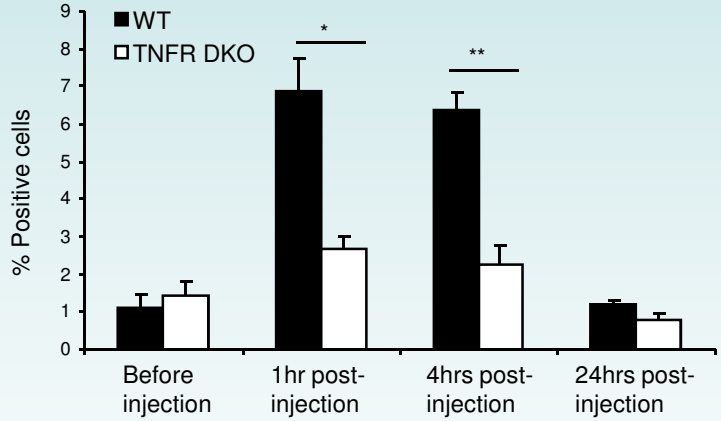




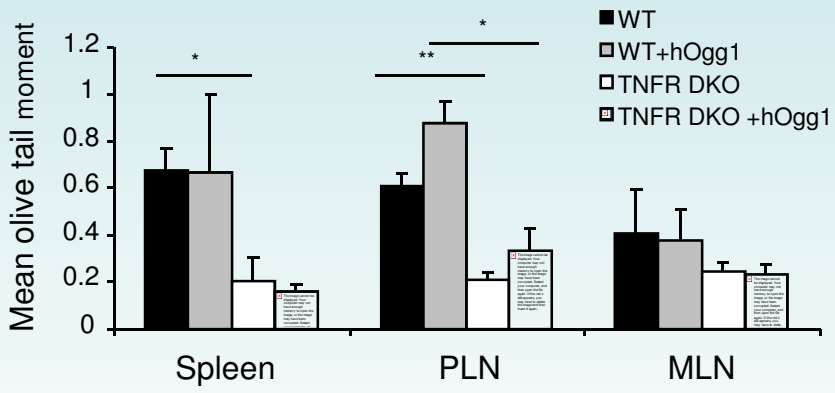


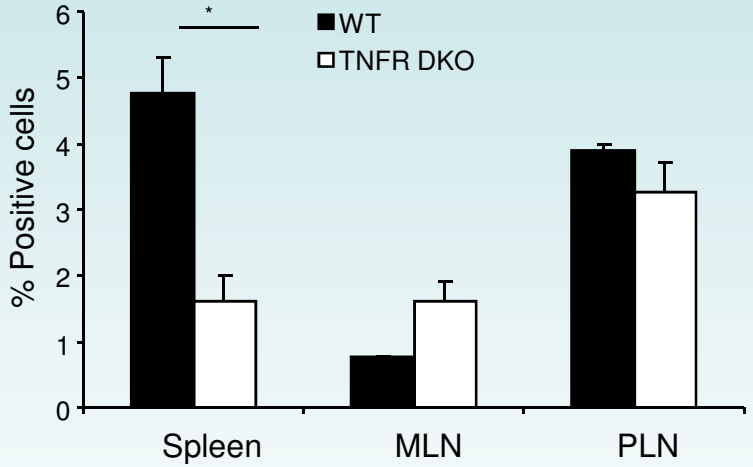
Genotoxicity in TNFR1$^{-/-}$/TNFR2$^{-/-}$ mice after injection with TNF-a



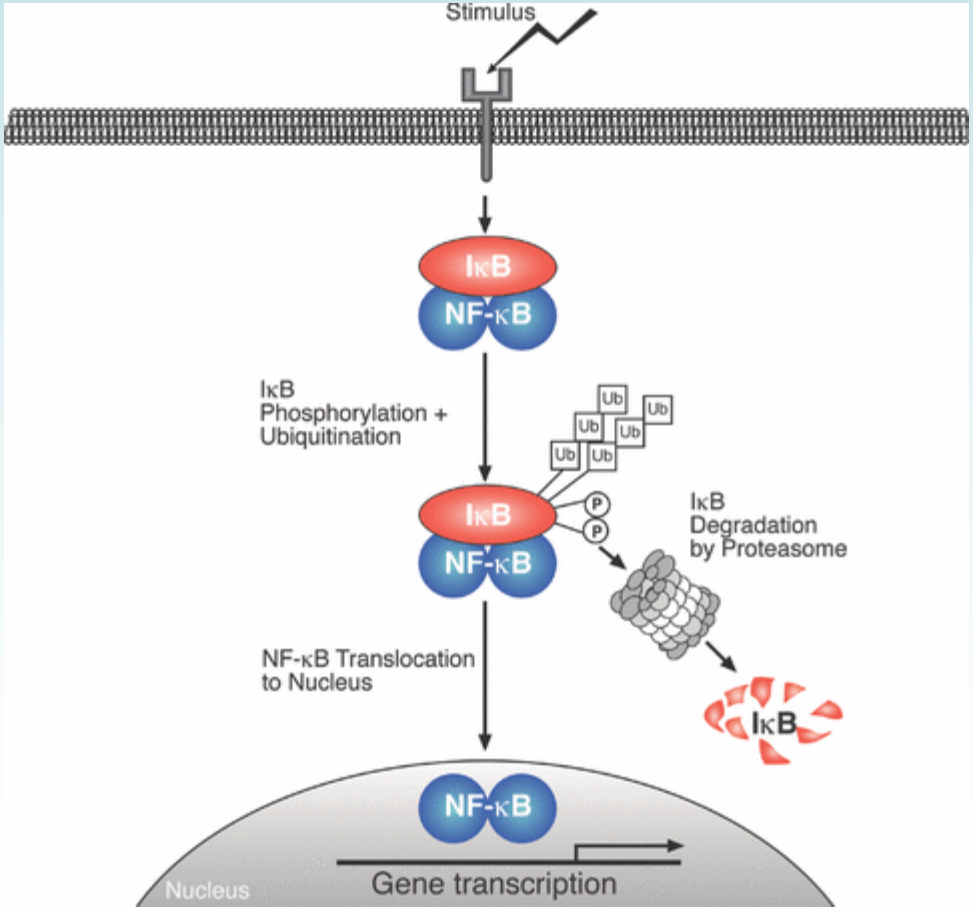


Genotoxicity in peripheral organs of TNFR1$^{-/-}$/TNFR2$^{-/-}$ mice 1hr after TNF-α injection



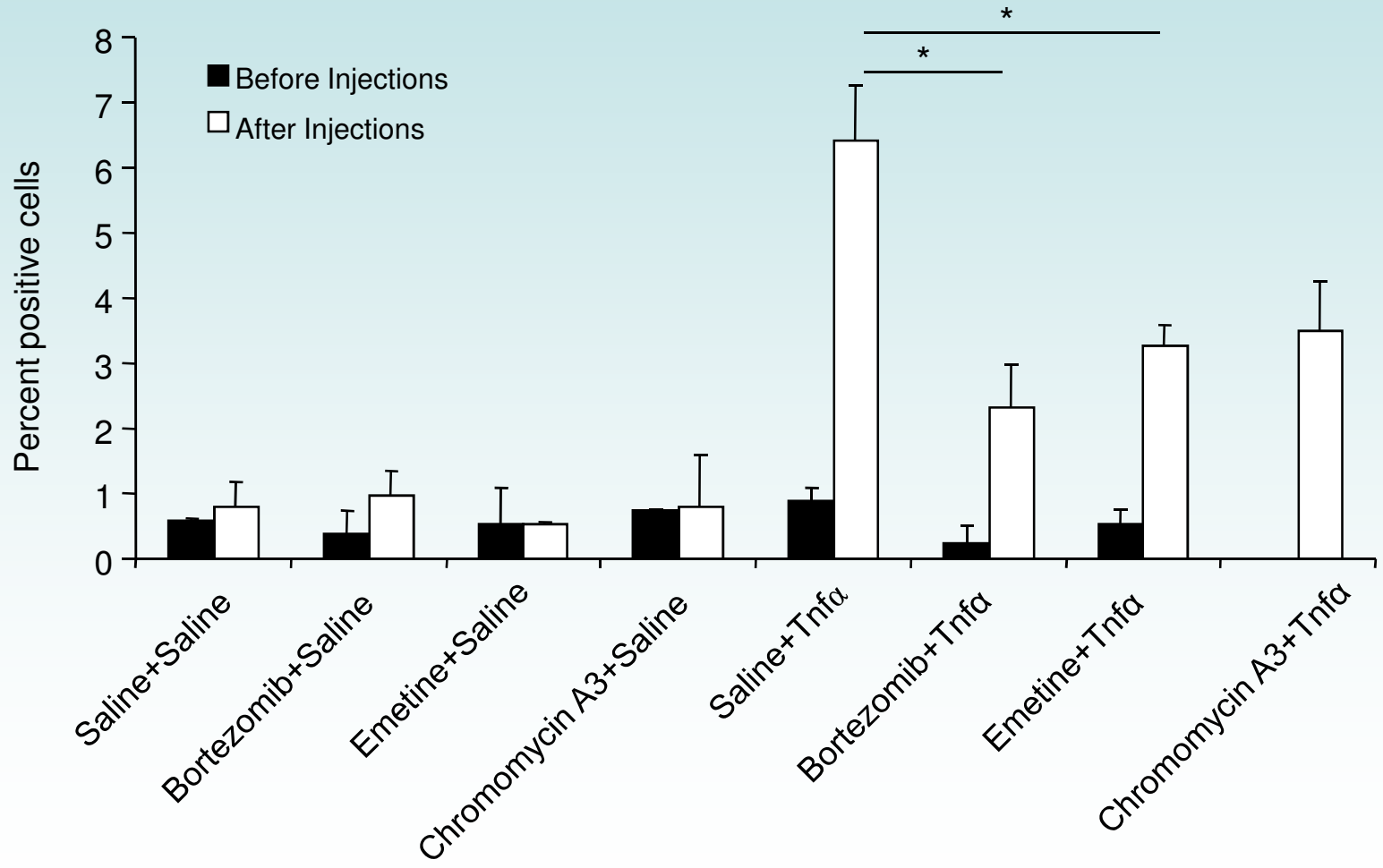


TNF-α mediated signaling via the TNFR involves activation of proinflammatory cell survival/proapoptotic genes

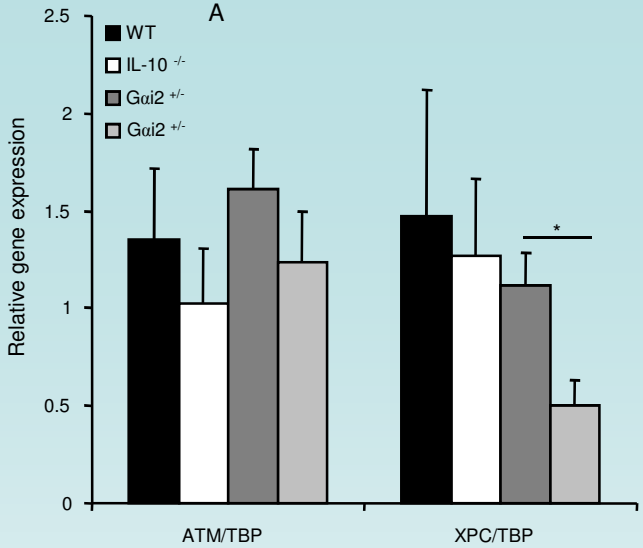


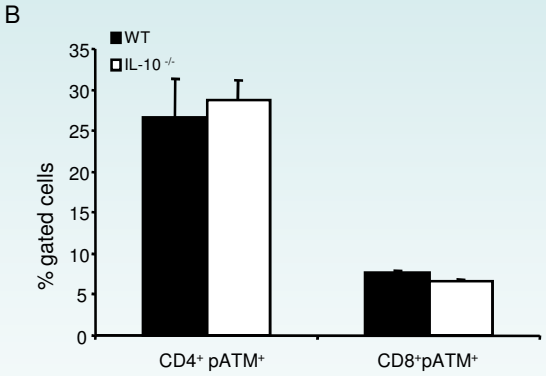
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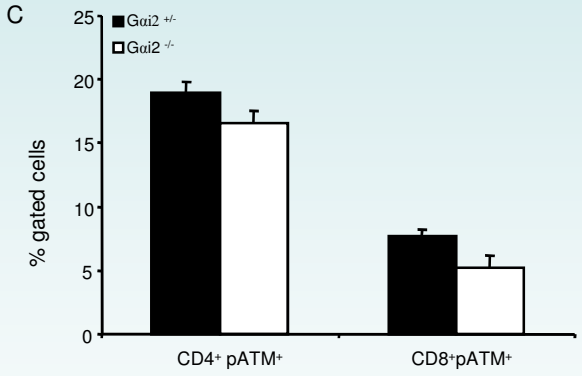
Effect of NF-κB inhibitors on TNFα-induced genotoxicity

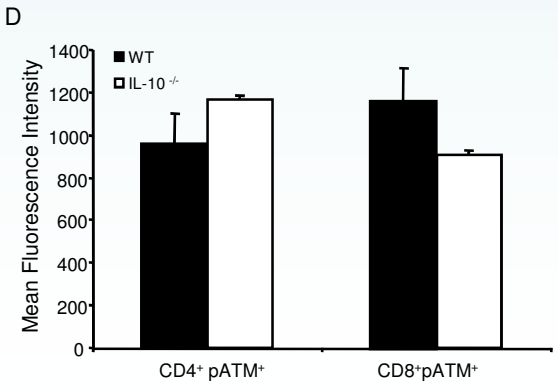


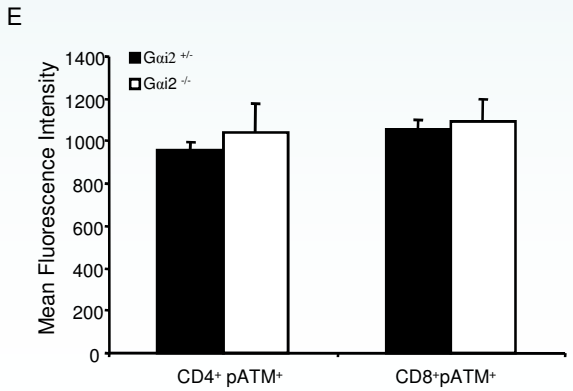
Decreased ATM expression is not responsible for increased genotoxicity in genetic models











Conclusions

- TNF-α is sufficient to cause genotoxicity in wildtype mice, which is reduced when administered NF-κB inhibitors
- TNF-α + IL-1β induces prolonged genotoxicity without successful repair even after 24 hrs

- TNF-α administration to splenic T-cells is sufficient to cause DNA damage in the absence of other cell types
- DNA damage and cytotoxicity are both increased when T-cells are co-incubated with $CD11b^{+}$ monocytes

- TNFR mediated signaling is heavily involved in DNA damage resulting from DSS-induced colitis
- TNF-a may increase intracellular ROS and cause stronger TNFR mediated signaling—DNA damage may be a side effect of this process



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