Autologous apoptotic cells preceding transplantation enhance survival in lethal murine graft-versus-host models

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Graft-versus-host disease (GVHD)

Medical cause

- Major cause of morbidity and mortality
  - After allogeneic transplantation
  - Not in autologous type

Bone marrow transplantation (BMT)

- Autologous bone marrow transplant
  - = self
  - Remove before treatment

- Allogeneic bone marrow transplant
  - = other
  - Good match between donor and recipient

- Umbilical cord blood transplant
  - Type of allogeneic transplant
  - Less need for perfect matching

Medical cause of GVHD

- Major cause of morbidity and mortality
  - After allogeneic transplantation
  - Not in autologous type
- Recipient has tissue antigens that are not present in the transplant donor
- Donor T cells recognize and respond to proteins on host cell
- Less occurrence when match is close
  - 30-40% in relatives
  - 60-80% in non relatives

Subtype of GVHD

- Acute GVHD (aGVHD)
  - Before day 100 post-transplant
- Chronic GVHD (cGVHD)
  - After day 100 post-transplant

Acute GVHD

- Symptoms include three main organs
  - Skin
  - Liver
  - Gastrointestinal tract

- Overall grades
  - I – mild
  - II – moderate
  - III – severe
  - IV – very severe

Chronic GVHD

- Major cause death after BMT
- Development can be progressive, quiescent or de novo
- Risk factors: age and history of aGVHD

- Symptoms
  - Dry mouth
  - Dry eyes, vision changes
  - Fatigue, muscle weakness
  - Skin rash with raised, discolored areas
  - Lung damage
  - Weight loss
  - Resemble autoimmune syndromes

Pathophysiology of GVHD

Three sequential steps

(1) Activation of APCs

(2) Donor T-cell actvation, proliferation, differentiation and migration

(3) Target tissue destruction

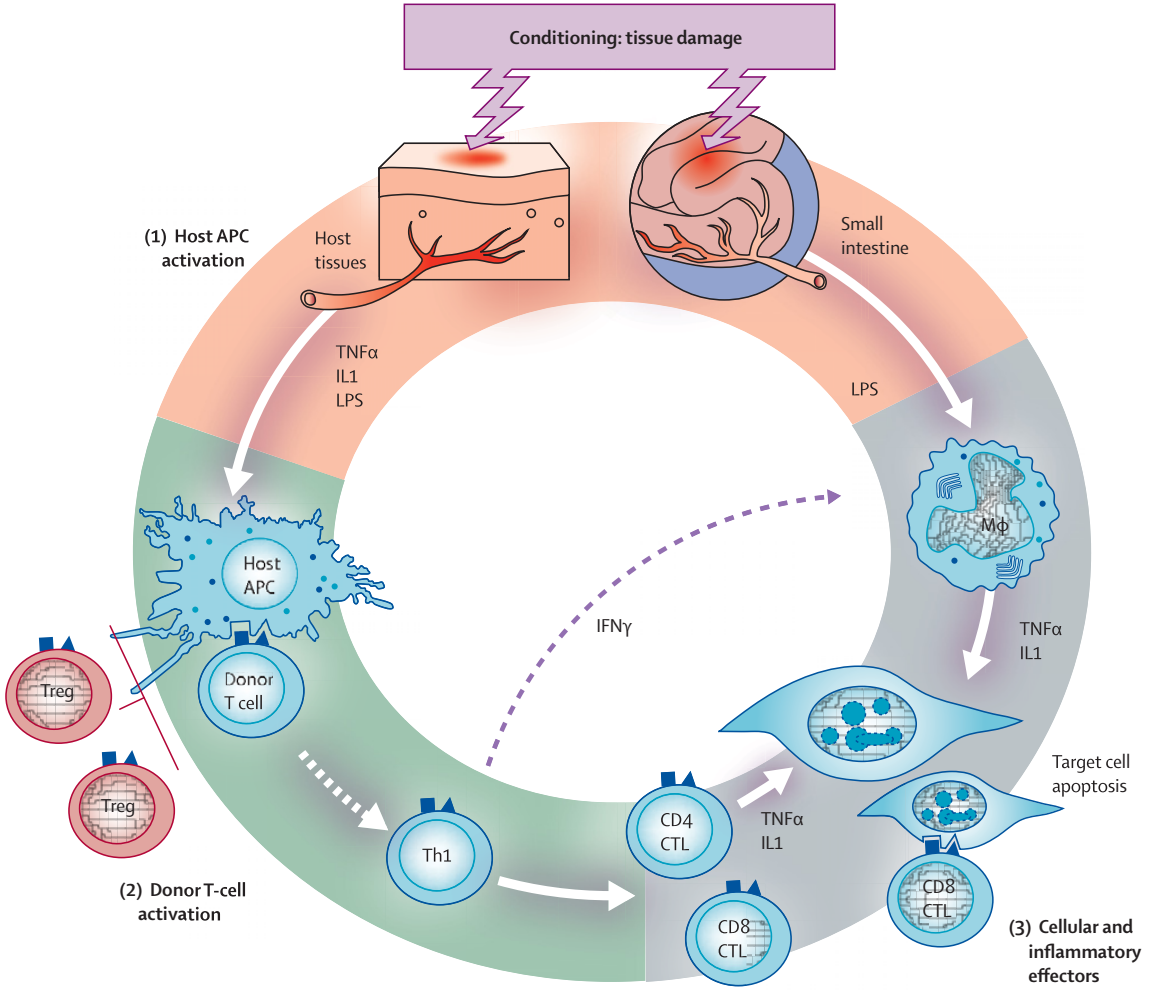


Figure 3: Pathophysiology of acute GVHD, Lancet 2009; 373: 1550–61
IL 1=interleukin 1. IFN γ=interferon γ. LPS=lipopolysaccharide. Treg=regulatory T cell. Th1=T-helper 1 cell. CTL=cytotoxic T lymphocyte.

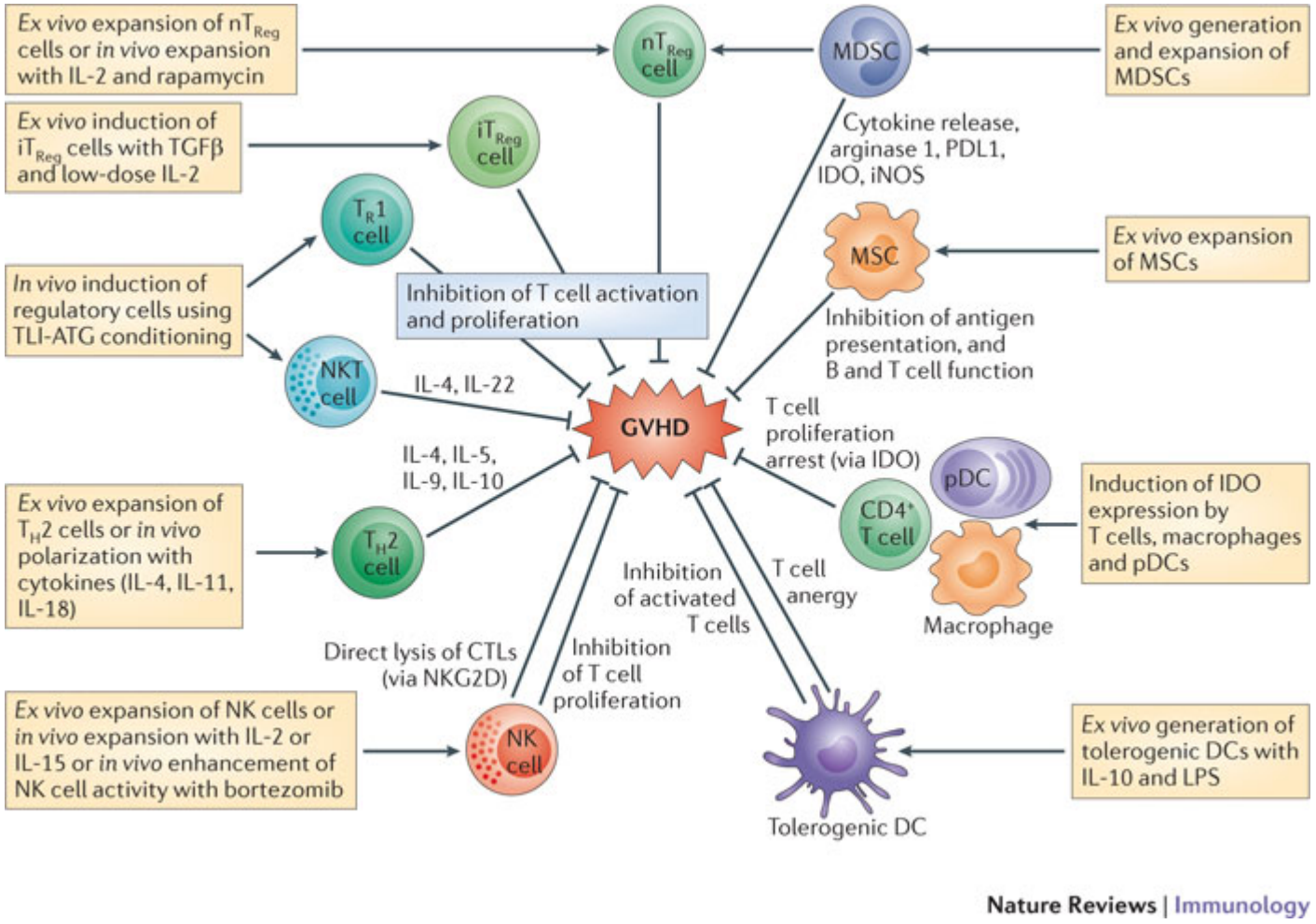
Involved cells in GVHD

- T-cell subsets
  - CD4+ Tcells & CD8+ Tcells
- T-regulatory cells (CD4+/CD25+ Treg)
  - Essential role in immune suppression following ECP
- Antigen presenting cells (APCs)
  - Main types: dendritic cells (DCs), macrophages, B cells
  - Initiation phase of aGVHD
- Natural killer cells
  - Mediated cell death by Fas-Fas-ligand-mediated apoptosis and perforin-granzime-B-mediated cytolyses
- NF-kB
  - Important for immune and inflammatory responses
  - Produce by activation of IL-1 and TNF-α

Prevention of GVHD

- Focus primarily on the effector phase
- Immunosupressive drugs
  - Significant toxicity
  - Risk for opportunistic infections
- Donor T-cell depletion
- Increase of regulatory T cells (Tregs)
- Targeting antigen-presenting cells (APCs), especially Dendritic cells (DCs)
- Extracorporeal photopheresis (ECP)

Potential targets for cellular immunotherapies in GVHD



Extracorporeal photopheresis in GVHD

- Induction of immunotolerance without general immunosuppression
- No increase of opportunistic infections
- Low risk of side effects
- Procedure: Cells exposure with 8-Methoxypsoralen (8-MOP) and UV-A light
- Process causes cellular apoptosis
- Maintain of T- and B-cell responses to novel and recall antigens

Materials and methods

- BALB/c mice
- BM transplantation
- ECP treatment
- Cell isolation and flow cytometry reagents
- Phospho flow
- In vitro mixed lymphocyte reaction (MLR) culture
- In vivo Bioluminescent imaging (BLI)
- CFSE labeling and pulsed in vivo BrdU labeling
- Cytokine analysis
- Cytotoxicity assay and in vivo tumor model
- In vivo Bcl1 tumor model

Results

Fig. 1 Host-type apoptotic cells infused prior to transplantation prolong survival

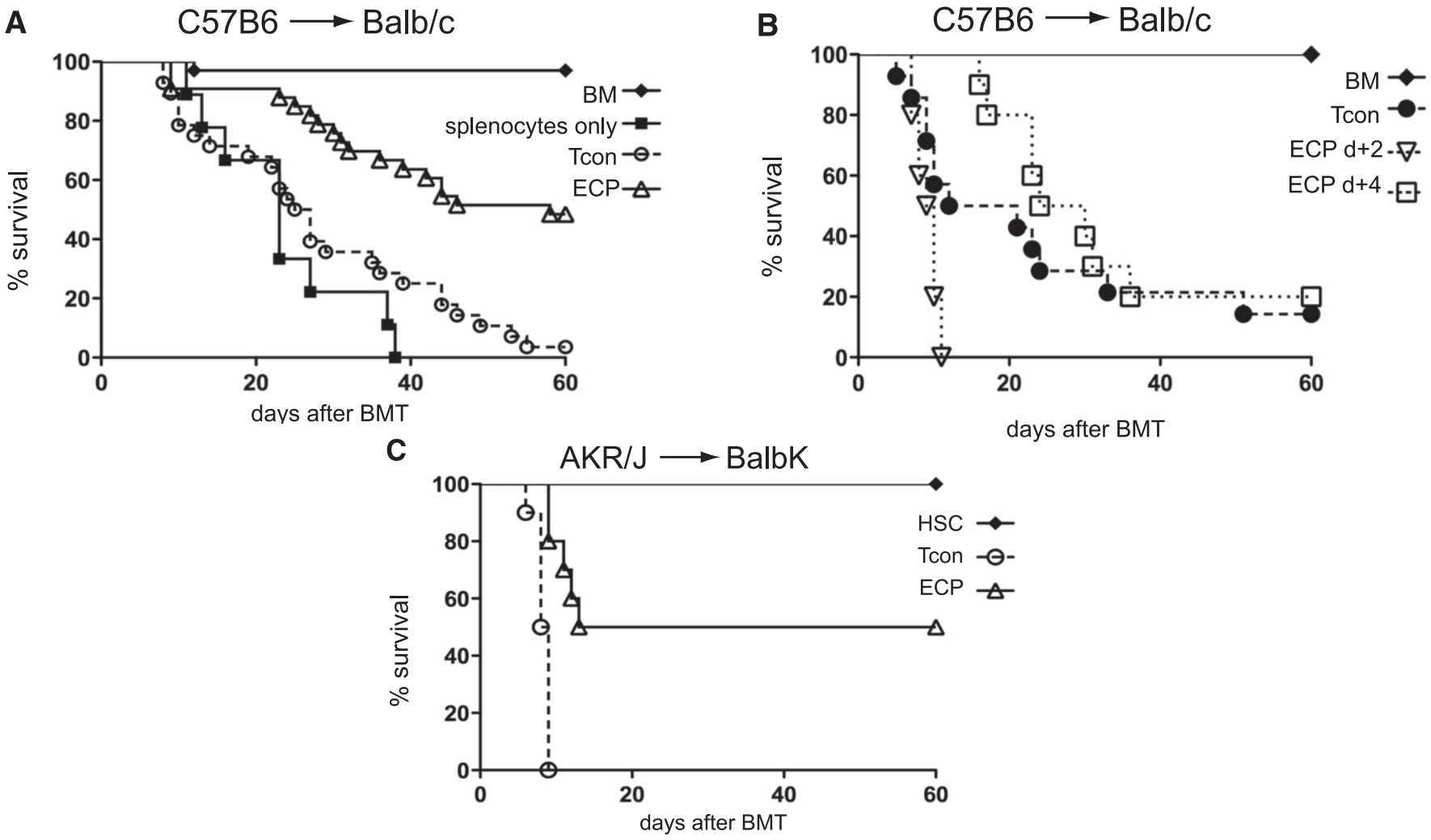
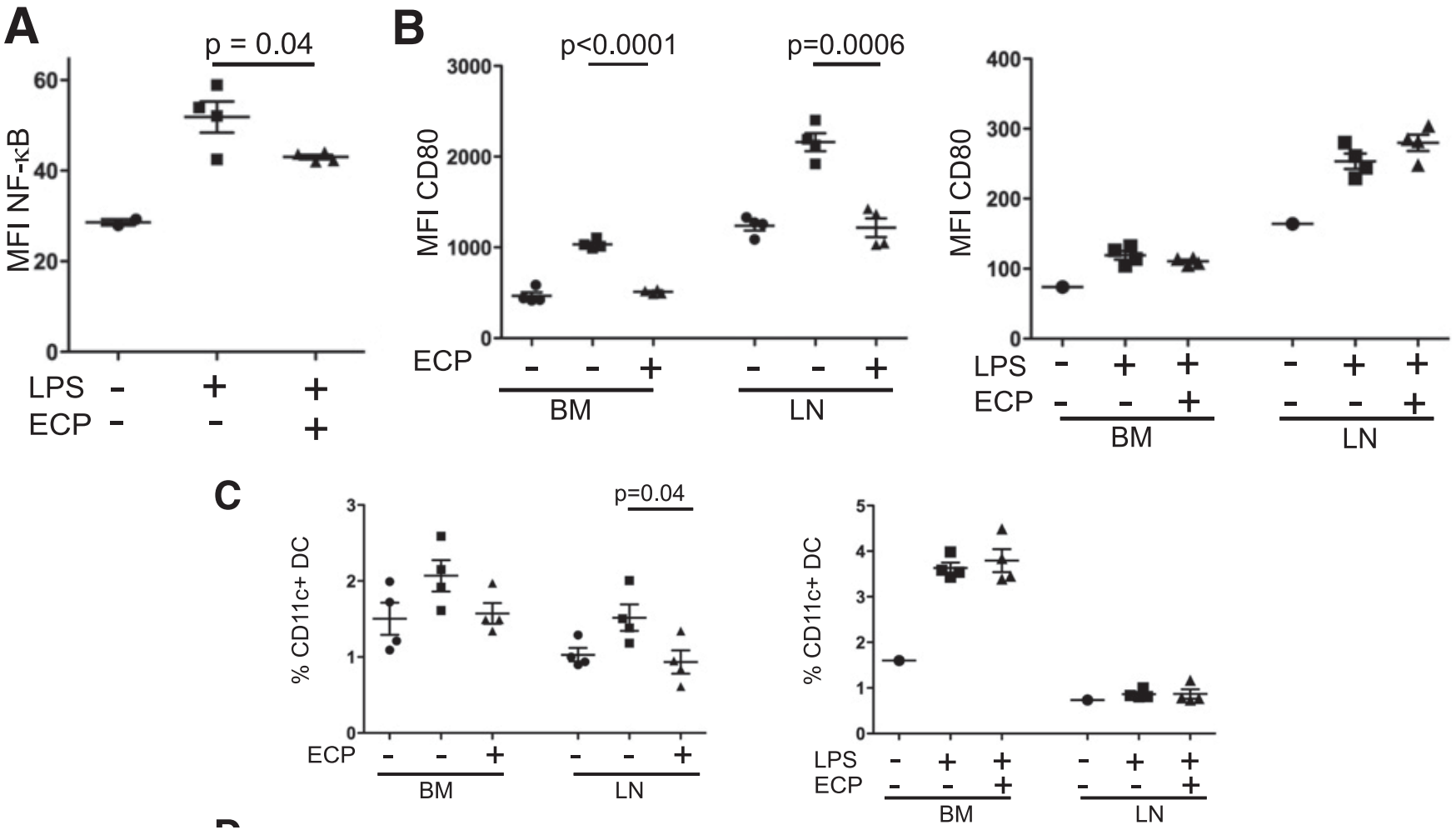
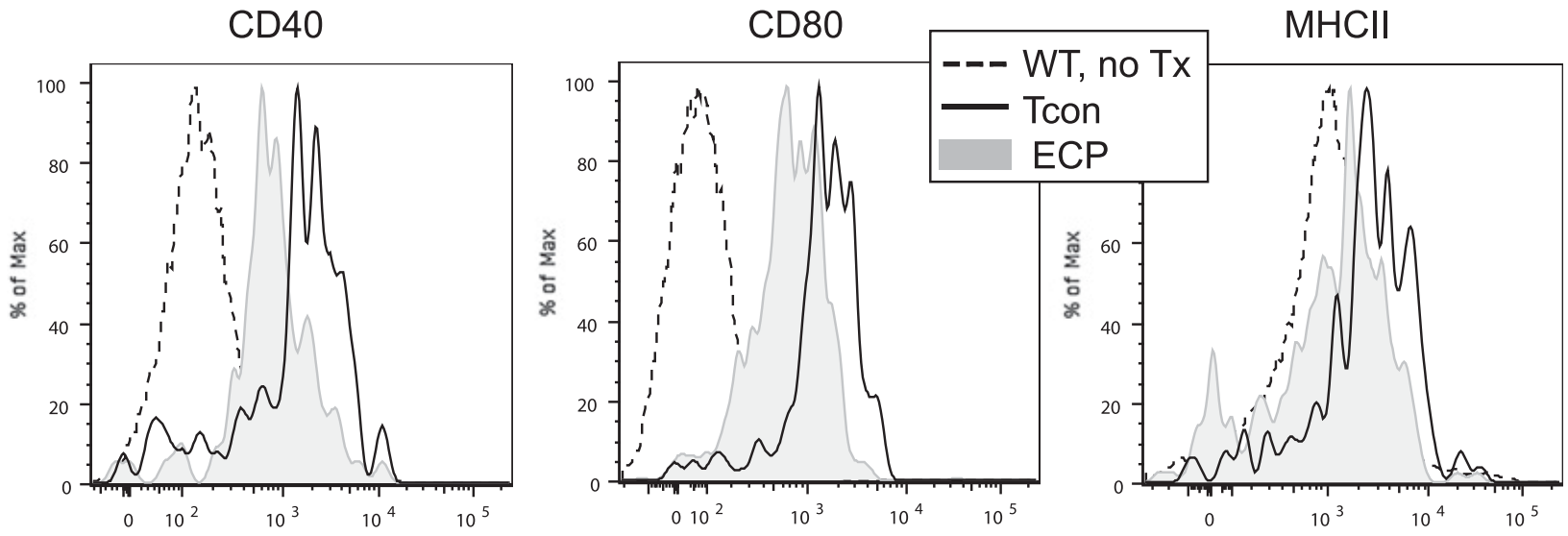


Figure 2. Uptake of apoptotic cells reduces NF-kB activation and costimulatory molecule expression in host DCs and diminishes MHCII uptake in donor T cells.



D day+3


CD40
CD80
MHCII
WT, no Tx
Tcon
ECP
% of Max
100
80
60
40
20
0
0
10^2
10^3
10^4
10^5

E

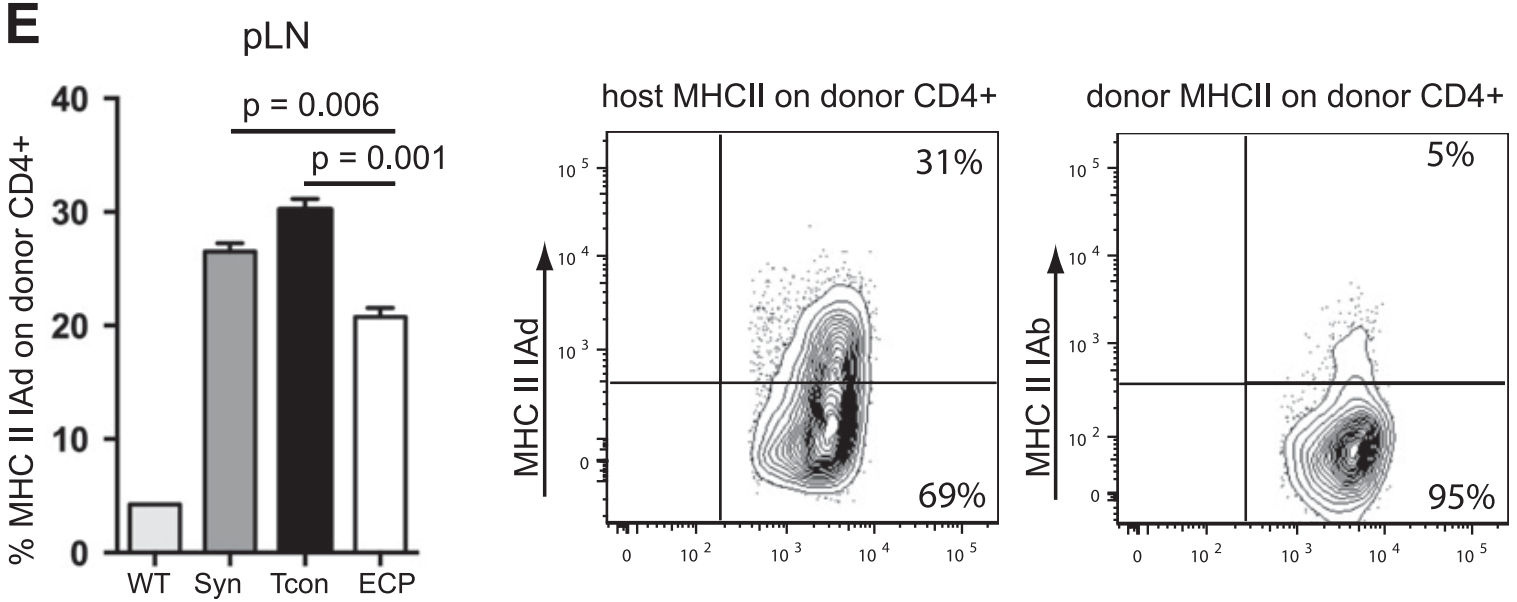

pLN
p = 0.006
p = 0.001
% MHC II IAd on donor CD4+
40
30
20
10
0
WT
Syn
Tcon
ECP
host MHCII on donor CD4+
donor MHCII on donor CD4+
MHC II IAd
MHC II IAb
31%
69%
5%
95%
10^5
10^4
10^3
10^2
0

Figure 3. Apoptotic cells reduce T-cell proliferation in vitro and in vivo

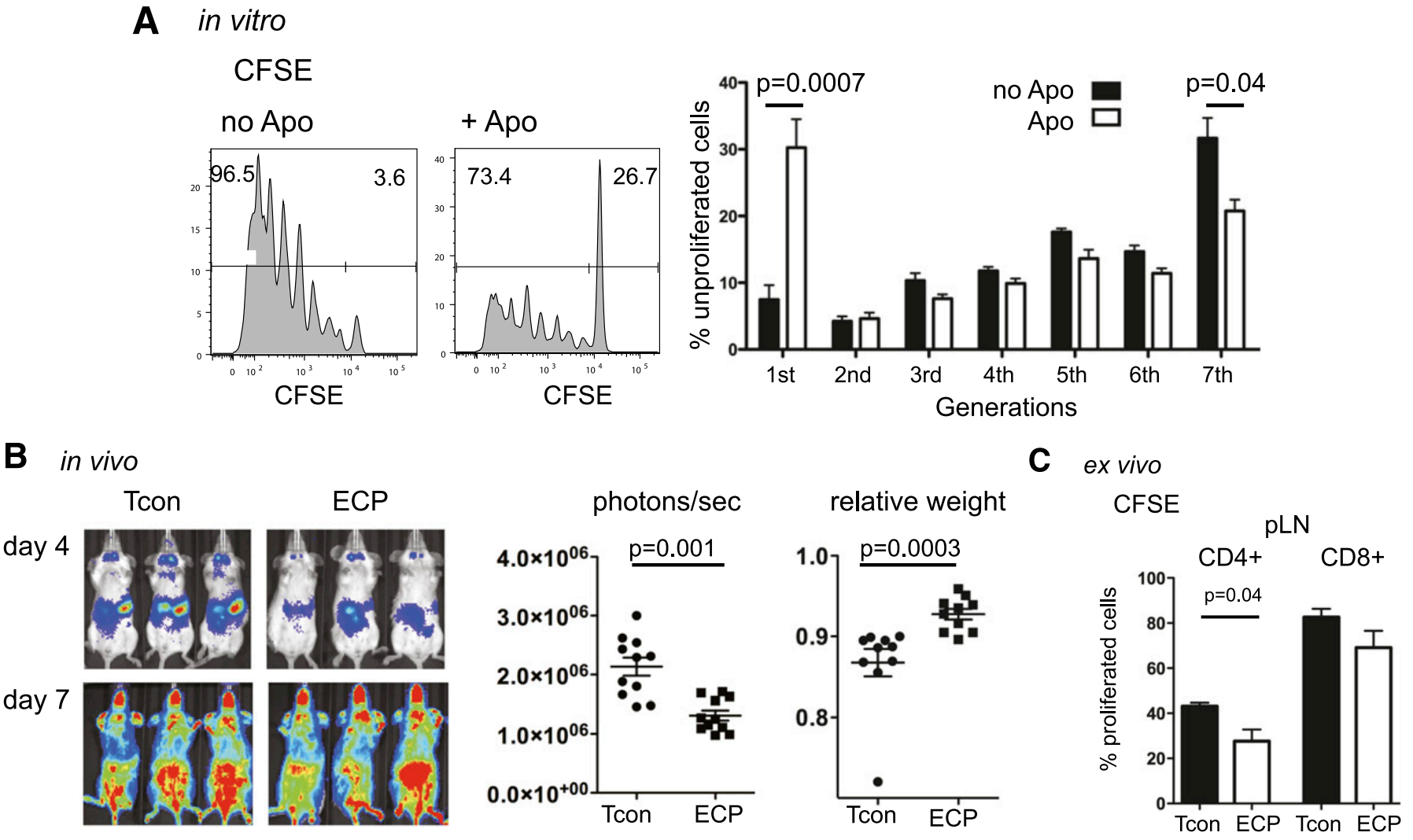


Figure 4. CD4+ T cells in ECP-treated mice show reduced expression of homing and activation markers.

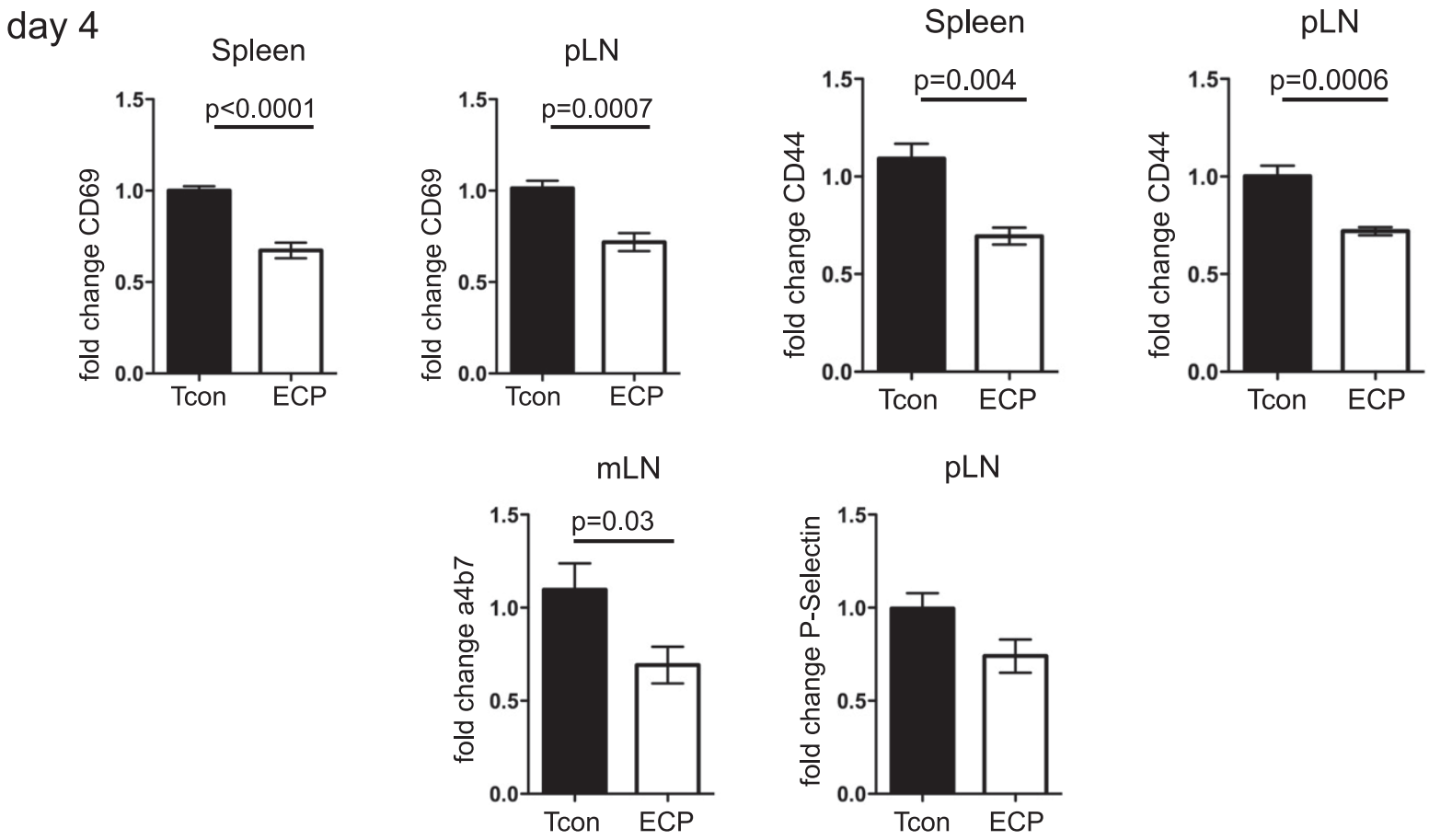
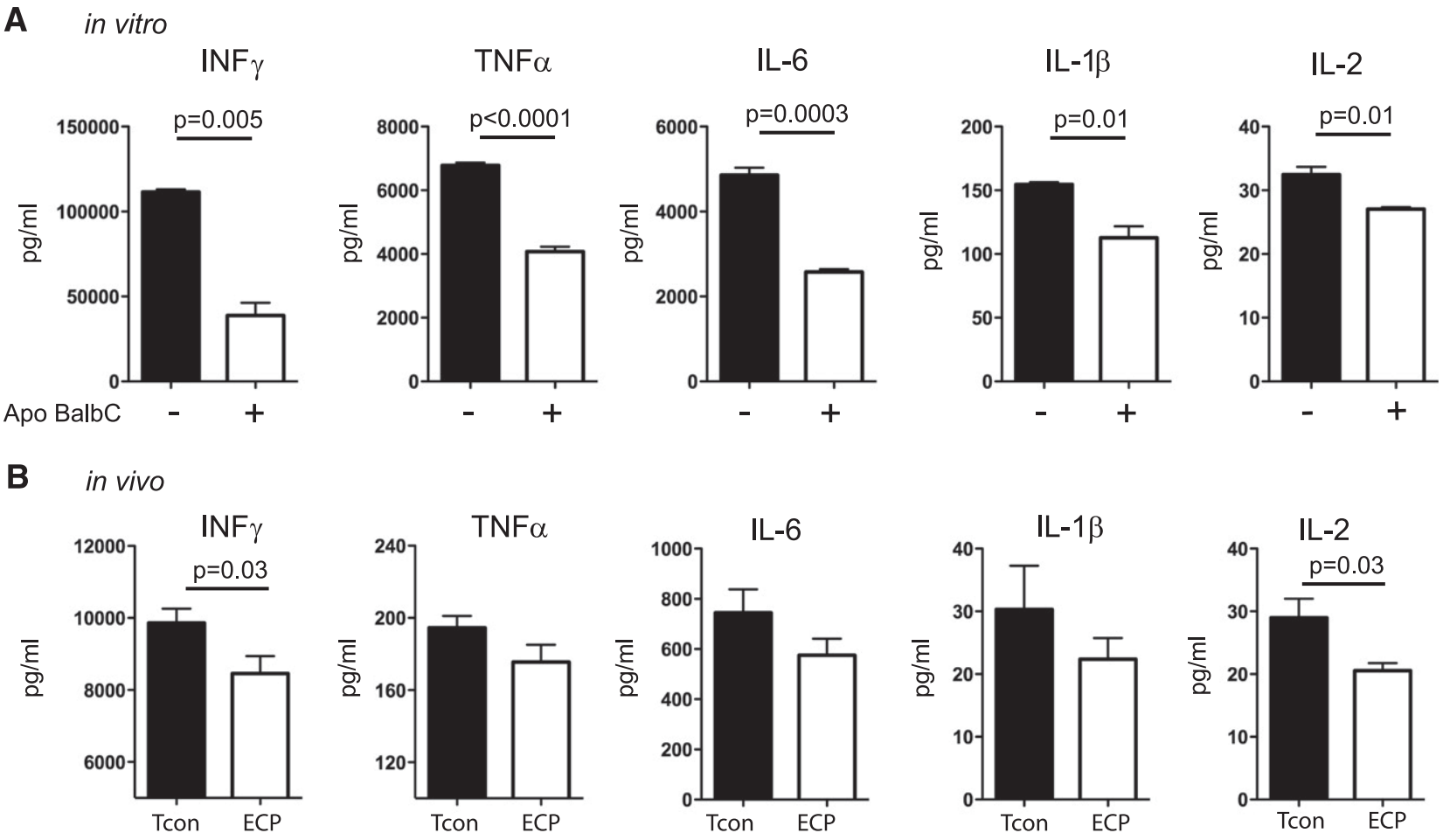


Figure 5. ECP treatment reduces proinflammatory cytokine secretion in vitro and in vivo and requires host type IL-10 for its beneficial effect.



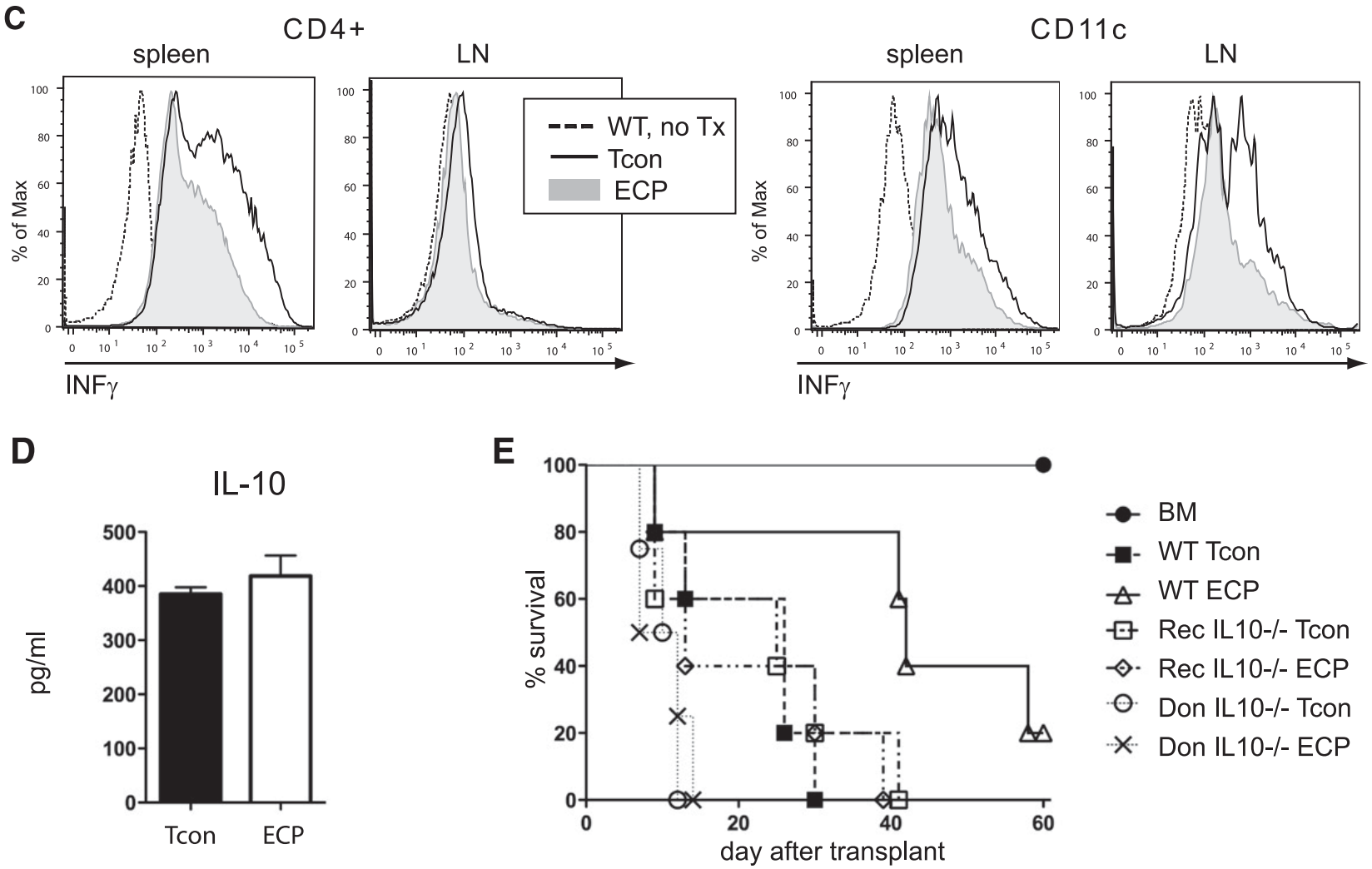
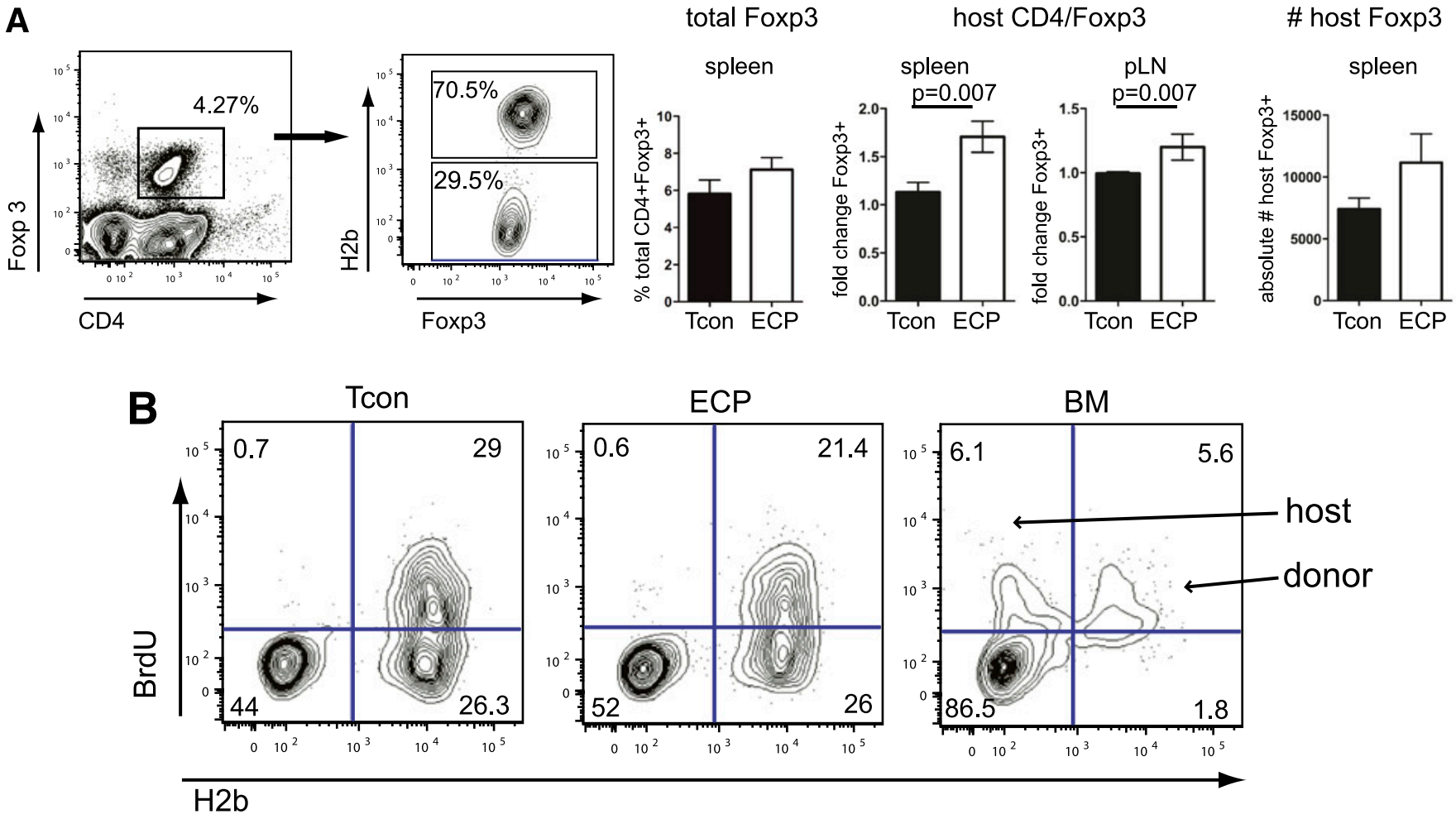
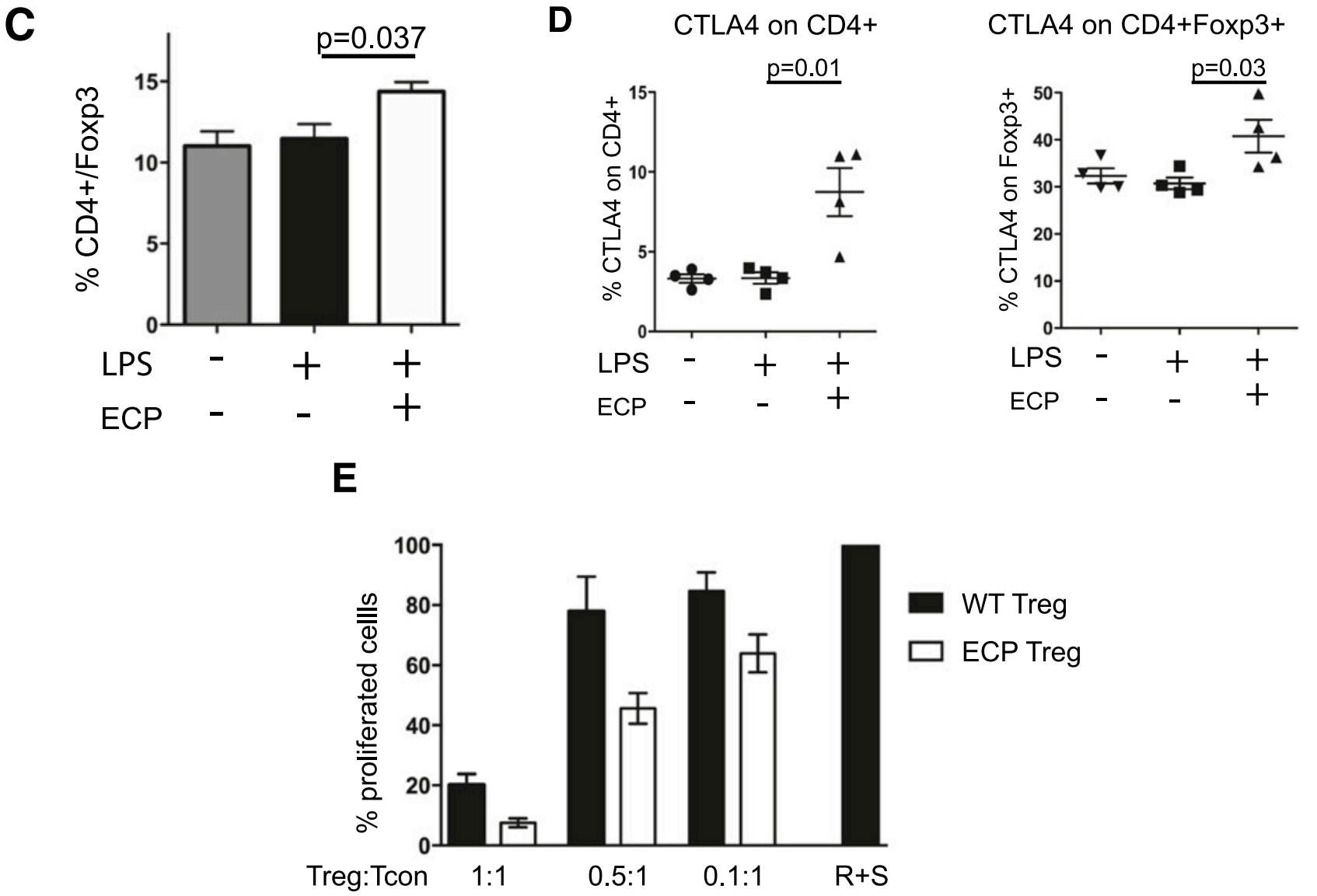
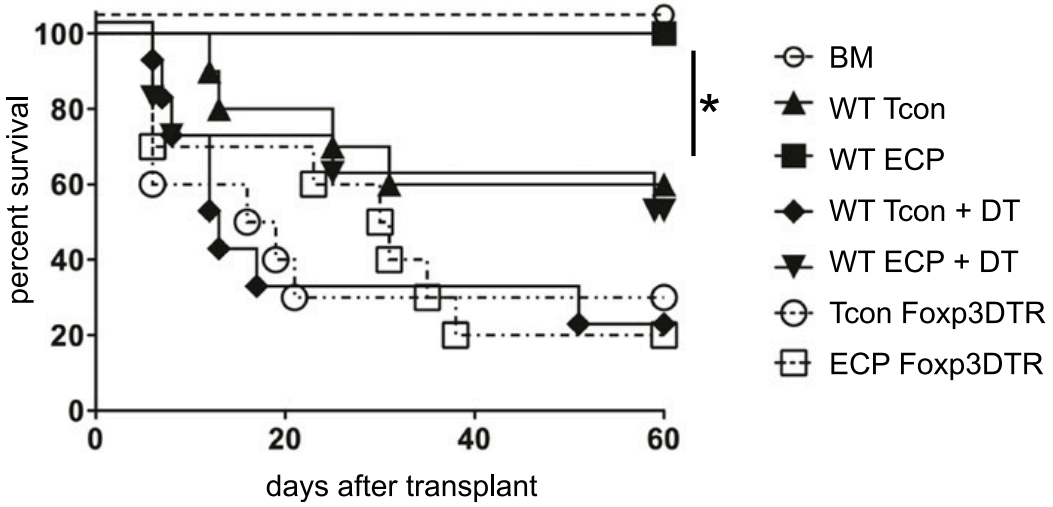

C
CD4+
spleen
LN
CD11c
spleen
LN
WT, no Tx
Tcon
ECP
% of Max
INFγ
D
IL-10
pg/ml
Tcon
ECP
E
% survival
day after transplant
BM
WT Tcon
WT ECP
Rec IL10-/- Tcon
Rec IL10-/- ECP
Don IL10-/- Tcon
Don IL10-/- ECP

Figure 6. ECP treatment induces host-type Foxp31 Tregs that substantially contribute to but are not solely responsible for improved outcome.




C
p=0.037
% CD4+/Foxp3
15
10
5
0
LPS - + +
ECP - - +
D
CTLA4 on CD4+
p=0.01
% CTLA4 on CD4+
15
10
5
0
LPS - + +
ECP - - +
CTLA4 on CD4+Foxp3+
p=0.03
% CTLA4 on Foxp3+
50
40
30
20
10
0
LPS - + +
ECP - - +
E
% proliferated cellls
100
80
60
40
20
0
Treg:Tcon 1:1 0.5:1 0.1:1 R+S
WT Treg
ECP Treg

F


100
80
60
40
20
0
percent survival
0
20
40
60
days after transplant
*
BM
WT Tcon
WT ECP
WT Tcon + DT
WT ECP + DT
Tcon Foxp3DTR
ECP Foxp3DTR

G

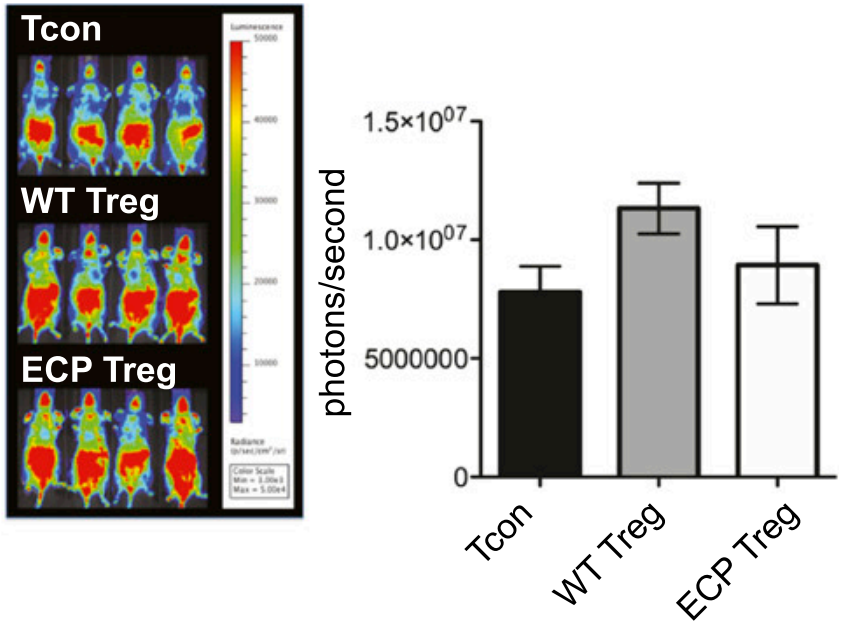
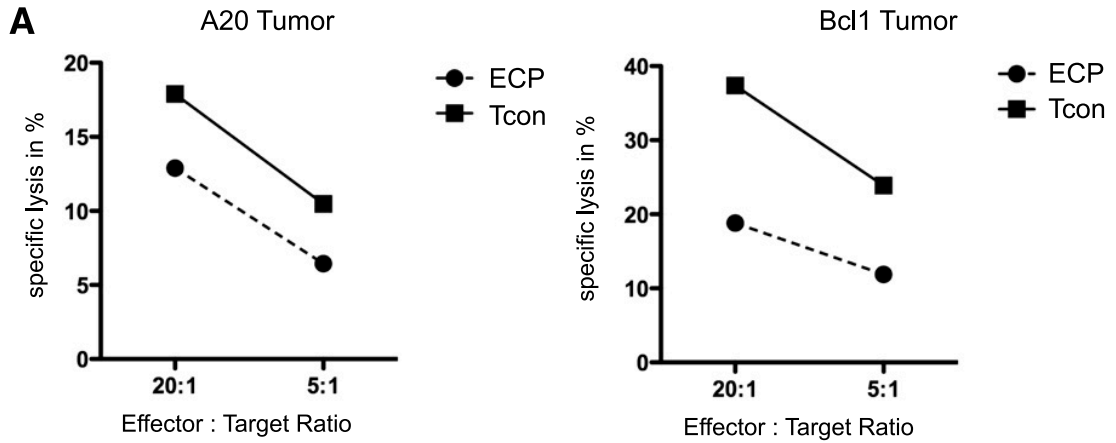
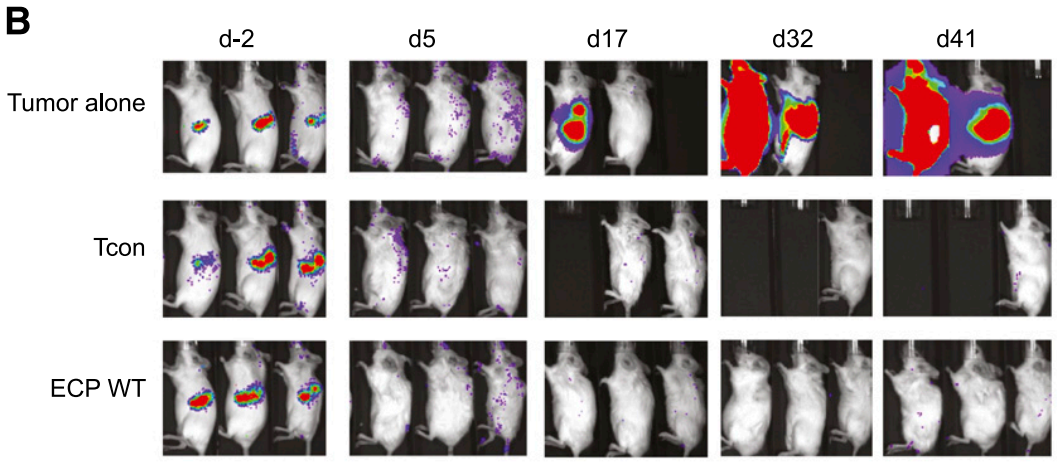
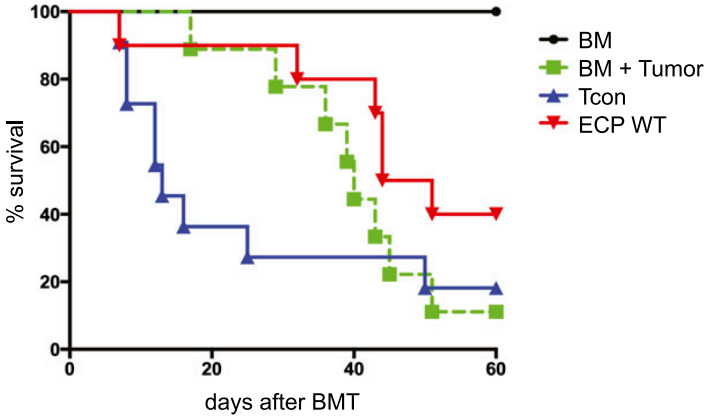

Tcon
WT Treg
ECP Treg
photons/second
1.5×10^07
1.0×10^07
5000000
0
Tcon
WT Treg
ECP Treg

Figure 7. GVT effect is maintained after ECP treatment.







Summary of results I

- host-type ECP-treated cells prior to transplantation diminishes GVHD and significantly improves survival
- ECP treatment at day -5 did not improve outcomes
- Depends to apoptotic cells that reduce NF-kB and inhibit maturation of host DCs
- Inhibition of NF-kB activation in DCs reduce T-cell activation in GVHD
- Reduce donor T-cells contribute to reduce proinflamatory signals

Summary of results II

- Tregs increased in patients with acute GVHD when successfully treated with ECP
- Tregs inhibit T-cell activation by inhibition of CD28 signaling
- Increase suppressive capacity by expression of CTLA4
- IL10 are required in the recipient at the time of ECP
- lower MHCII expression on donor T cells in ECP-treated mice
- T- and B-cell responses to novel and recall antigens remained intact
- Prophylactic ECP delayed the induction of GVHD

Conclusion

- prophylactic ECP prior to BMT reducing transplant complicantions and improved survival in a murine BMT model
- ECP treatment is safer than many other immunosuppressive approaches

Critical review

- long term impact of ECP?
- Exact immunological effect of ECP?