Figure S2

A) Young WT Treg vs Aged WT Treg vs Young WT Tconv

B) Before transfer

In the recipients

Treg signature

Foxp3

B7 receptor

Chemokine receptor

Bcl2 family

Inflammatory cytokines

CD45+FITC

Foxp3-APC

CD25-PE

C) Young vs Aged

Annexin V-APC

7AAD

Staining cells (%)

Young

Aged
Fig. S4

A-B: Western blot analysis of pERK, ERK, and β-actin in Young Treg, Aged Treg, WT Treg, and Cd4Cre; Dcaf1fl/fl Treg at 0 min and 30 min after IL-2 (50U) stimulation.

C-D: Western blot analysis of pSTAT5, STAT5, and β-actin in Young Treg, Aged Treg, WT Treg, and Cd4Cre; Dcaf1fl/fl Treg at 0 min and 30 min after IL-2 (50U) stimulation.

E: Western blot analysis of pERK, pan ERK, and β-actin in Naive, WT, ERCre; Dcaf1fl/fl, WT, ERCre; Dcaf1fl/fl, WT, ERCre; Dcaf1fl/fl, and WT, ERCre; Dcaf1fl/fl Treg at Day 1, Day 2, Day 3, Day 4, Day 5, Day 6, and Day 7.

F: Relative mRNA amounts of p16Ink4a in WT and ERCre; Dcaf1fl/fl Treg at Days 0, 2, 3, and 4 after stimulation.

G: Flow cytometry analysis of pERK-PE in WT and ERCre; Dcaf1fl/fl Treg treated with DMSO or MEKI at Days 1 and 2 after stimulation.

H: Relative mRNA amounts of p16Ink4a in DMSO and MEKI-treated WT and ERCre; Dcaf1fl/fl Treg at Day 7 after stimulation.

**: p < 0.01, ****: p < 0.0001 compared to WT by two-way ANOVA with Holm-Sidak's multiple comparisons test.
Identification of Dcaf1 interacting proteins shared by T and non-T cells

Non-T cells (Nakagawa, T. et al. 2015)

T cells (Guo, Z. et al. 2016)

NER Pathway
Protein Ubiquitination Pathway
Hypusine Biosynthesis
Oxidized GTP and dGTP Detoxification
Remodeling of Epithelial Adherens Junctions
Cyclins and Cell Cycle Regulation
Telomerase Signaling
Dolichyl-diphosphooligosaccharide Biosynthesis
Cleavage and Polyadenylation of Pre-mRNA
Epithelial Adherens Junction Signaling
Wnt signaling β-catenin Signaling
Glutathione Redox Reactions I
NF-κB signaling
Clathrin-mediated Endocytosis Signaling
Breast Cancer Regulation by Slathmin1
AMPK Signaling
Glutathione-mediated Detoxification
DNA Methylation and Transcriptional Repression Signaling
Cell Cycle Regulation by BTG Family Proteins
Huntington's Disease Signaling
Amyloid Processing
Xenobiotic Metabolism Signaling
Unfolded protein response
Role of CHK Proteins in Cell Cycle Checkpoint Control

(p<0.05)

** GST activity in aged Treg cells

GST activity in Dcaf1-deficient Treg cells
Fig. S6

A

WT

pERK-PE

% of Max

0 10^2 10^3 10^4 10^5

ERec;Dcaf1^+/+*^1

MIT

MIT-Gstp1

***

****

pERK MFI

0 100 200 300 400 500

WT ERec;Dcaf1^+/+*^1

MIT

MIT-Gstp1

***
Young Treg
DCAF1 HIGH
GSTP1
ROS
p16^{ink4a}
p21^{Cip1}
p19^{Arf} SA-β-gal
Normal proliferation
Controlled inflammation
Healthy state

Old Treg
DCAF1 LOW
GSTP1
ROS
p16^{ink4a}
p21^{Cip1}
p19^{Arf} SA-β-gal
Impaired proliferation
Aberrant inflammation
Inflamm-ageing
Age-related morbidity

Treg Naive Tconv Effector/Memory Tconv
1. Full unedited gel for Figure 3A

- Anti-DCAF1 antibody 1:1000 dilution
- Anti-Actin antibody 1:2000 dilution

2. Full unedited gel for Figure 6A

- Anti-DCAF1 antibody 1:1000 dilution
- Anti-Actin antibody 1:2000 dilution

3. Full unedited gel for Figure 7F

For IP

- Anti-MYC HRP antibody 1:5000 dilution

For Input, in separate membrane

- Anti-MYC HRP antibody 1:5000 dilution
- Anti-FLAG HRP antibody 1:5000 dilution
- Anti-Actin antibody 1:2000 dilution

4. Full unedited gel for Figure 7G

For IP

- Anti-DCAF1 antibody 1:1000 dilution

For Input

- Anti-DCAF1 antibody 1:1000 dilution
- Anti-GSTP1 antibody 1:1000 dilution
- Anti-pERK antibody 1:1000 dilution
- Anti-panERK antibody 1:1000 dilution

5. Full unedited gel for Figure S3B

- Anti-DCAF1 antibody 1:1000 dilution
- Anti-Actin antibody 1:2000 dilution

6. Full unedited gel for Figure S4A

- Anti-pERK antibody 1:1000 dilution
- Anti-panERK antibody 1:1000 dilution
- Anti-Actin antibody 1:2000 dilution