Fig. S1

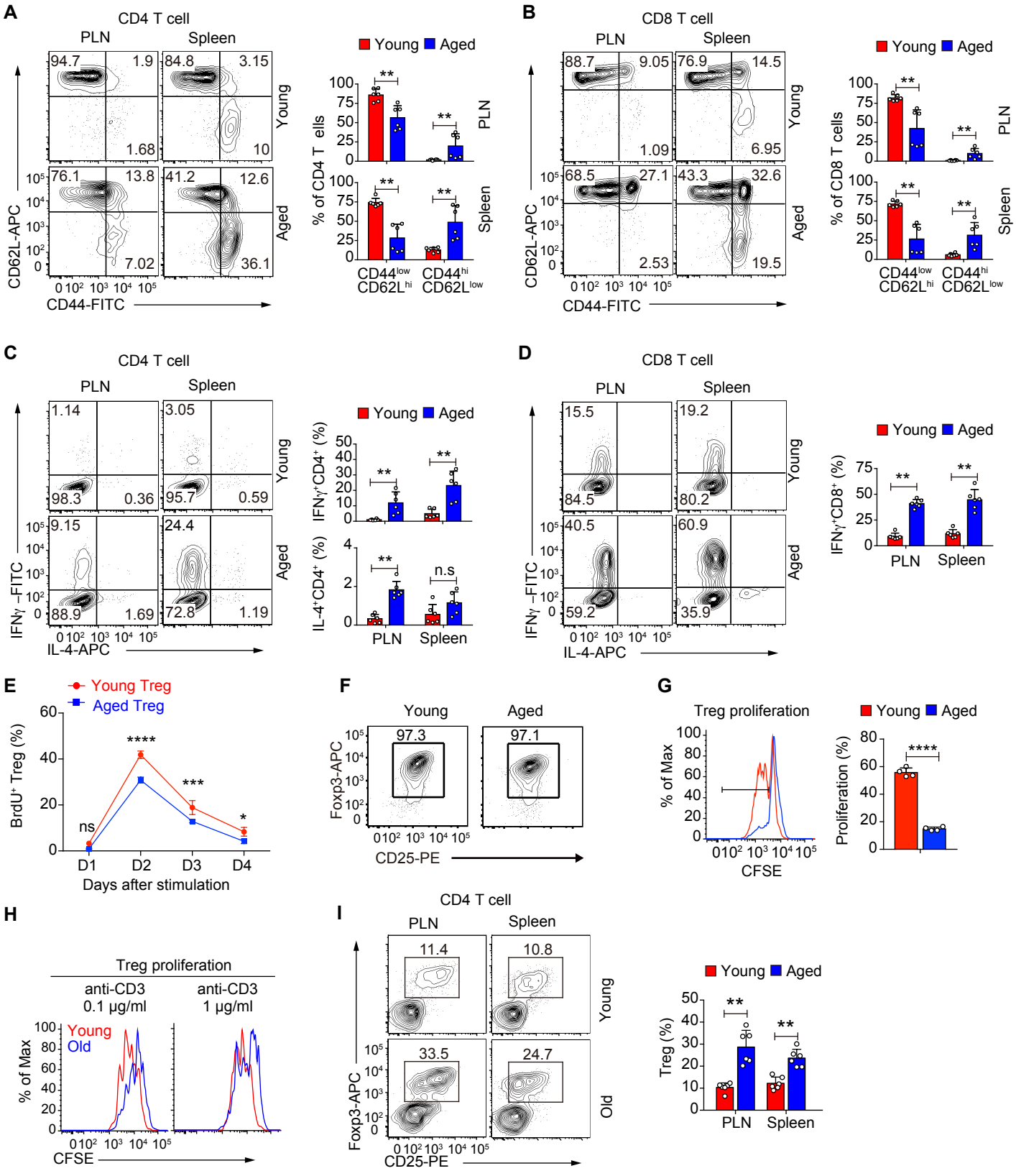

A
CD4 T cell
PLN
Spleen
Young
Aged
CD62L-APC
CD44-FITC
% of CD4 T cells
CD44low CD62Lhi
CD44hi CD62Llow
B
CD8 T cell
% of CD8 T cells
C
CD4 T cell
IFNγ –FITC
IL-4-APC
IFNγ+CD4+ (%)
IL-4+CD4+ (%)
n.s
D
CD8 T cell
IFNγ+CD8+ (%)
E
Young Treg
Aged Treg
BrdU+ Treg (%)
ns
Days after stimulation
F
Foxp3-APC
CD25-PE
G
Treg proliferation
% of Max
CFSE
Proliferation (%)
H
Treg proliferation
anti-CD3 0.1 μg/ml
anti-CD3 1 μg/ml
Young
Old
I
CD4 T cell
Treg (%)

Fig. S2

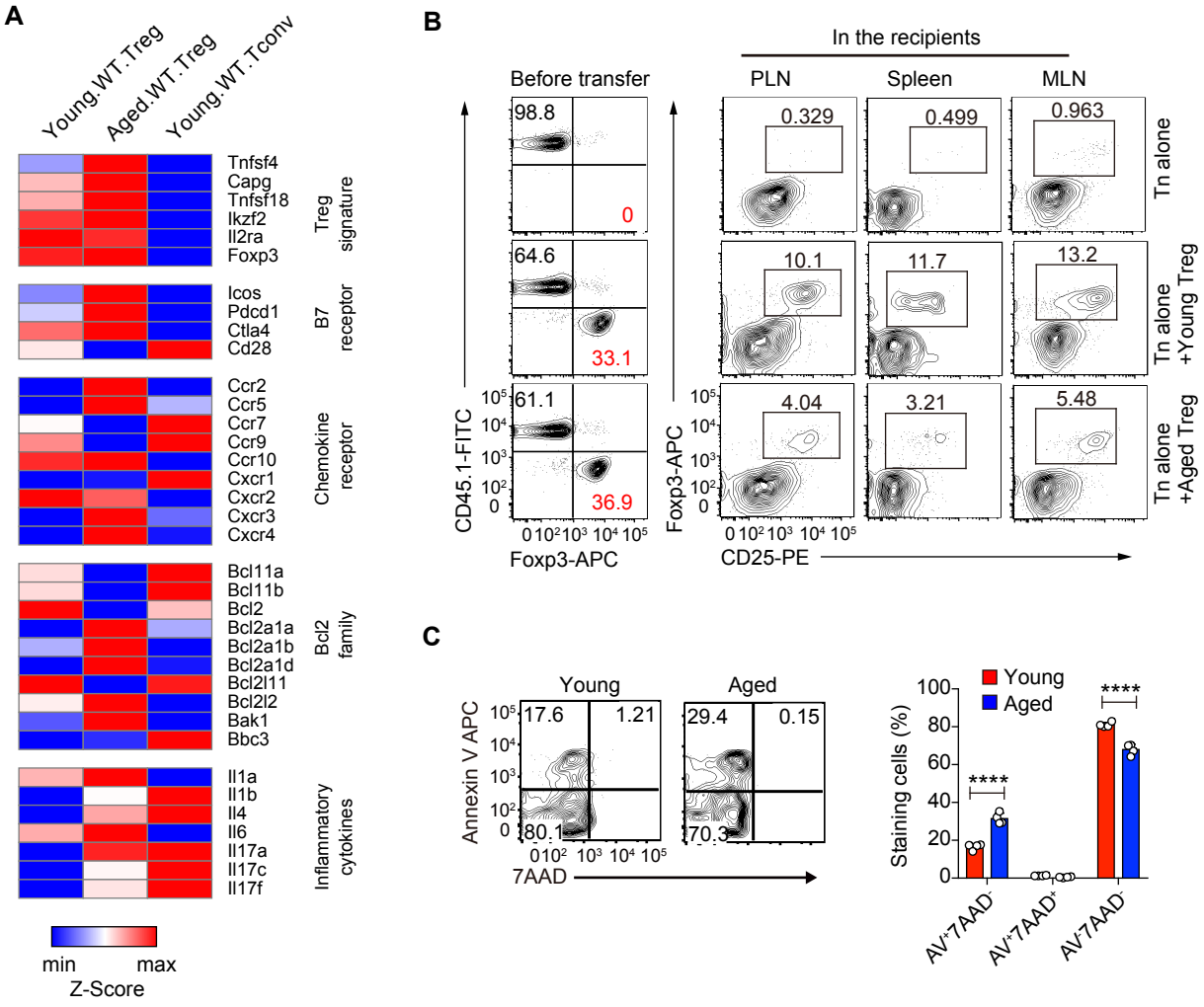

A
Young.WT.Treg
Aged.WT.Treg
Young.WT.Tconv
Tnfsf4
Capg
Tnfsf18
Ikzf2
Il2ra
Foxp3
Treg signature
Icos
Pdcd1
Ctla4
Cd28
B7 receptor
Ccr2
Ccr5
Ccr7
Ccr9
Ccr10
Cxcr1
Cxcr2
Cxcr3
Cxcr4
Chemokine receptor
Bcl11a
Bcl11b
Bcl2
Bcl2a1a
Bcl2a1b
Bcl2a1d
Bcl2l11
Bcl2l2
Bak1
Bbc3
Bcl2 family
Il1a
Il1b
Il4
Il6
Il17a
Il17c
Il17f
Inflammatory cytokines
min
max
Z-Score
B
In the recipients
Before transfer
PLN
Spleen
MLN
98.8
0
64.6
33.1
61.1
36.9
0.329
0.499
0.963
10.1
11.7
13.2
4.04
3.21
5.48
Tn alone
Tn alone +Young Treg
Tn alone +Aged Treg
CD45.1-FITC
Foxp3-APC
Foxp3-APC
CD25-PE
C
Young
Aged
17.6
1.21
80.1
29.4
0.15
70.3
Annexin V APC
7AAD
Young
Aged
Staining cells (%)
****
****
AV+7AAD-
AV+7AAD+
AV-7AAD-

Fig. S3

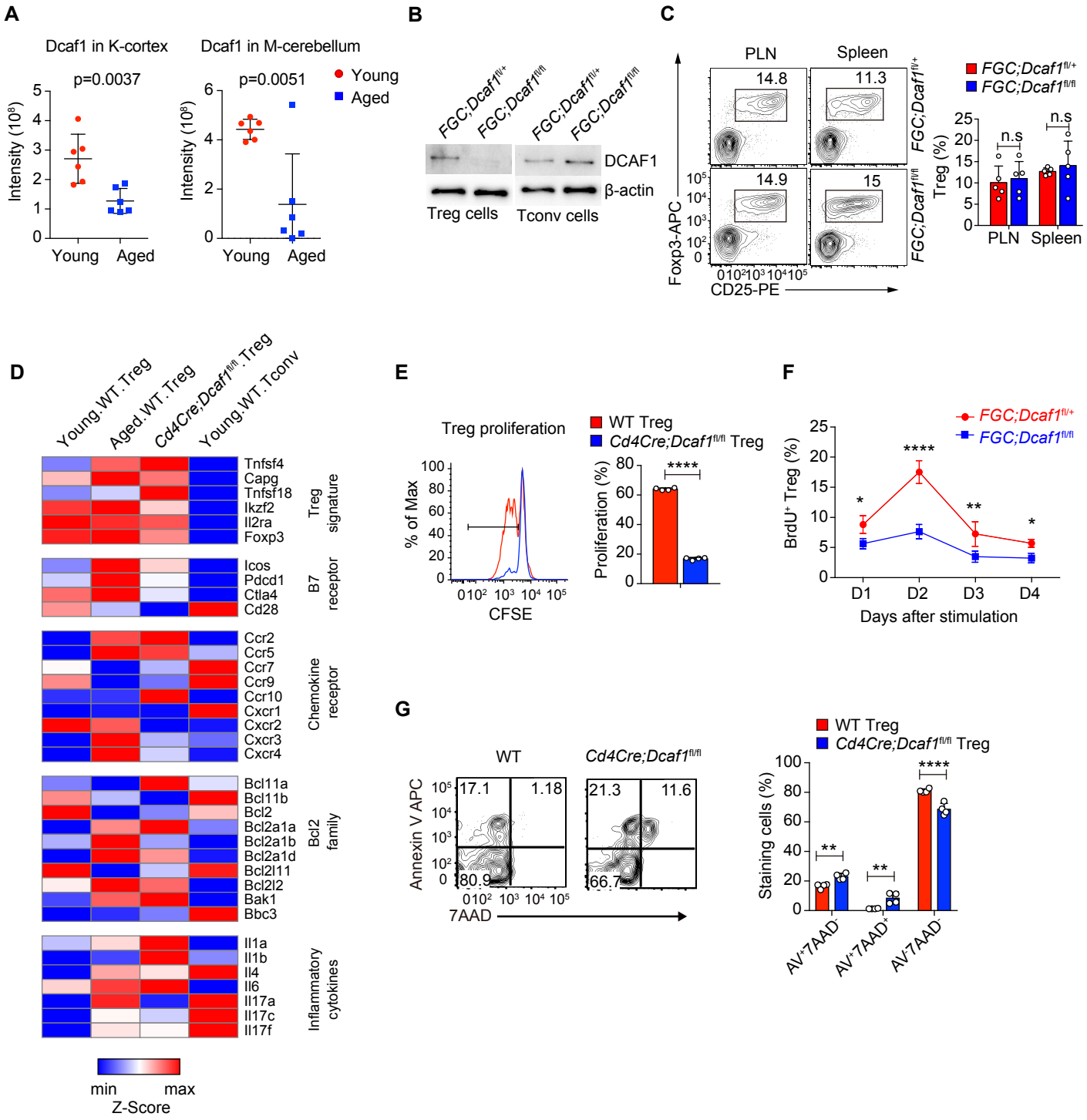

A
Dcaf1 in K-cortex
p=0.0037
Intensity (10^8)
Young
Aged
Dcaf1 in M-cerebellum
p=0.0051
Young
Aged
B
FGC;Dcaf1fl/+
FGC;Dcaf1fl/fl
FGC;Dcaf1fl/+
FGC;Dcaf1fl/fl
DCAF1
β-actin
Treg cells
Tconv cells
C
PLN
Spleen
14.8
11.3
14.9
15
FGC;Dcaf1fl/+
FGC;Dcaf1fl/fl
Foxp3-APC
CD25-PE
Treg (%)
n.s
n.s
PLN
Spleen
D
Young.WT.Treg
Aged.WT.Treg
Cd4Cre;Dcaf1fl/fl.Treg
Young.WT.Tconv
Tnfsf4
Capg
Tnfsf18
Ikzf2
Il2ra
Foxp3
Treg signature
Icos
Pdcd1
Ctla4
Cd28
B7 receptor
Ccr2
Ccr5
Ccr7
Ccr9
Ccr10
Cxcr1
Cxcr2
Cxcr3
Cxcr4
Chemokine receptor
Bcl11a
Bcl11b
Bcl2
Bcl2a1a
Bcl2a1b
Bcl2a1d
Bcl2l11
Bcl2l2
Bak1
Bbc3
Bcl2 family
Il1a
Il1b
Il4
Il6
Il17a
Il17c
Il17f
Inflammatory cytokines
min
max
Z-Score
E
Treg proliferation
% of Max
CFSE
WT Treg
Cd4Cre;Dcaf1fl/fl Treg
Proliferation (%)
****
F
FGC;Dcaf1fl/+
FGC;Dcaf1fl/fl
BrdU+ Treg (%)
****
*
**
*
D1
D2
D3
D4
Days after stimulation
G
WT
Cd4Cre;Dcaf1fl/fl
17.1
1.18
21.3
11.6
80.9
66.7
Annexin V APC
7AAD
WT Treg
Cd4Cre;Dcaf1fl/fl Treg
Staining cells (%)
****
**
**
AV+7AAD-
AV+7AAD+
AV-7AAD-

Fig. S4

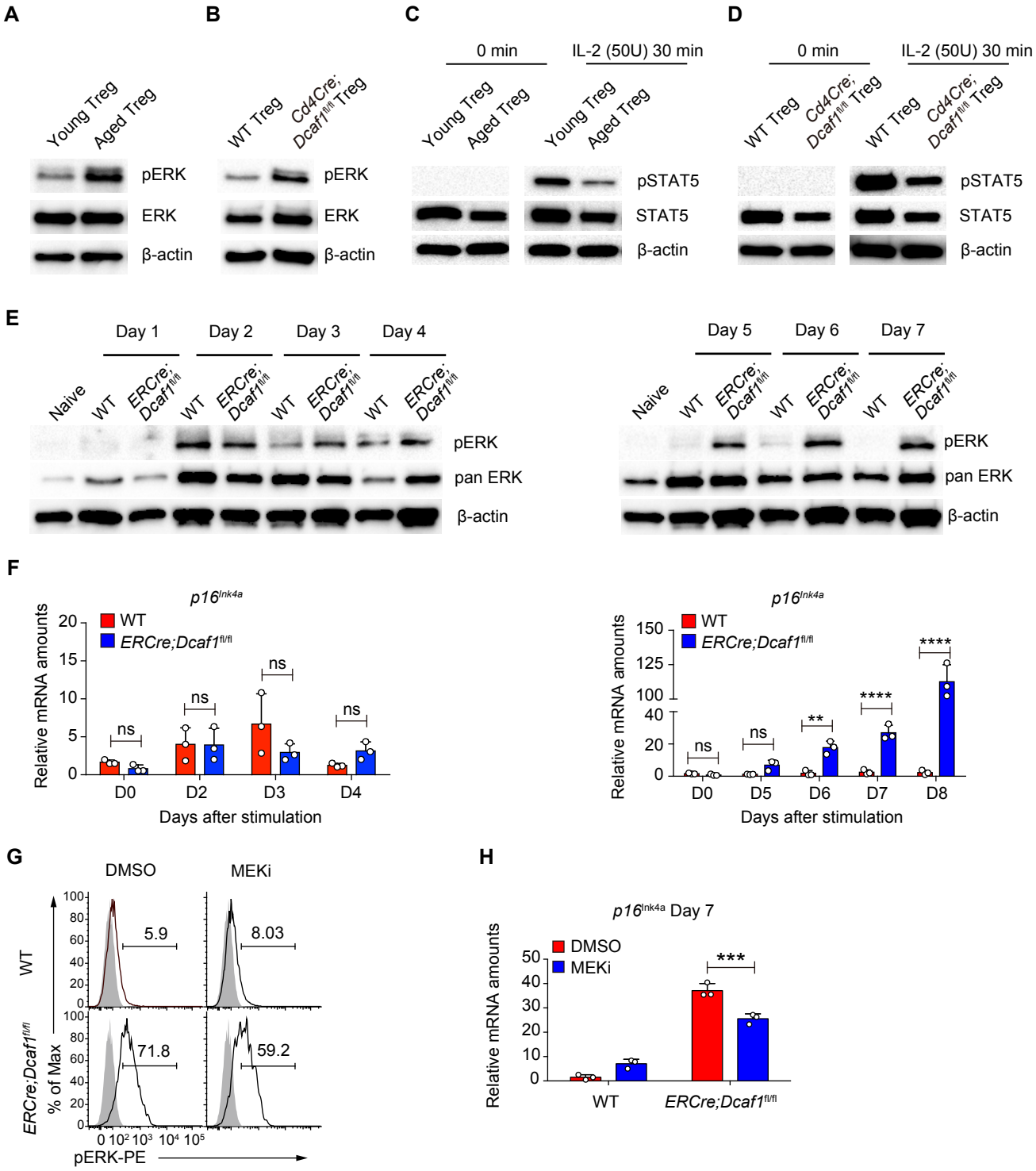

A
Young Treg
Aged Treg
pERK
ERK
β-actin
B
WT Treg
Cd4Cre; Dcaf1fl/fl Treg
pERK
ERK
β-actin
C
0 min
IL-2 (50U) 30 min
Young Treg
Aged Treg
pSTAT5
STAT5
β-actin
D
0 min
IL-2 (50U) 30 min
WT Treg
Cd4Cre; Dcaf1fl/fl Treg
pSTAT5
STAT5
β-actin
E
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Naive
WT
ERCre; Dcaf1fl/fl
pERK
pan ERK
β-actin
F
p16Ink4a
WT
ERCre;Dcaf1fl/fl
Relative mRNA amounts
ns
**
****
D0
D2
D3
D4
D5
D6
D7
D8
Days after stimulation
G
DMSO
MEKi
WT
ERCre;Dcaf1fl/fl
% of Max
5.9
8.03
71.8
59.2
pERK-PE
H
p16Ink4a Day 7
DMSO
MEKi
***
Relative mRNA amounts
WT
ERCre;Dcaf1fl/fl

Fig. S5

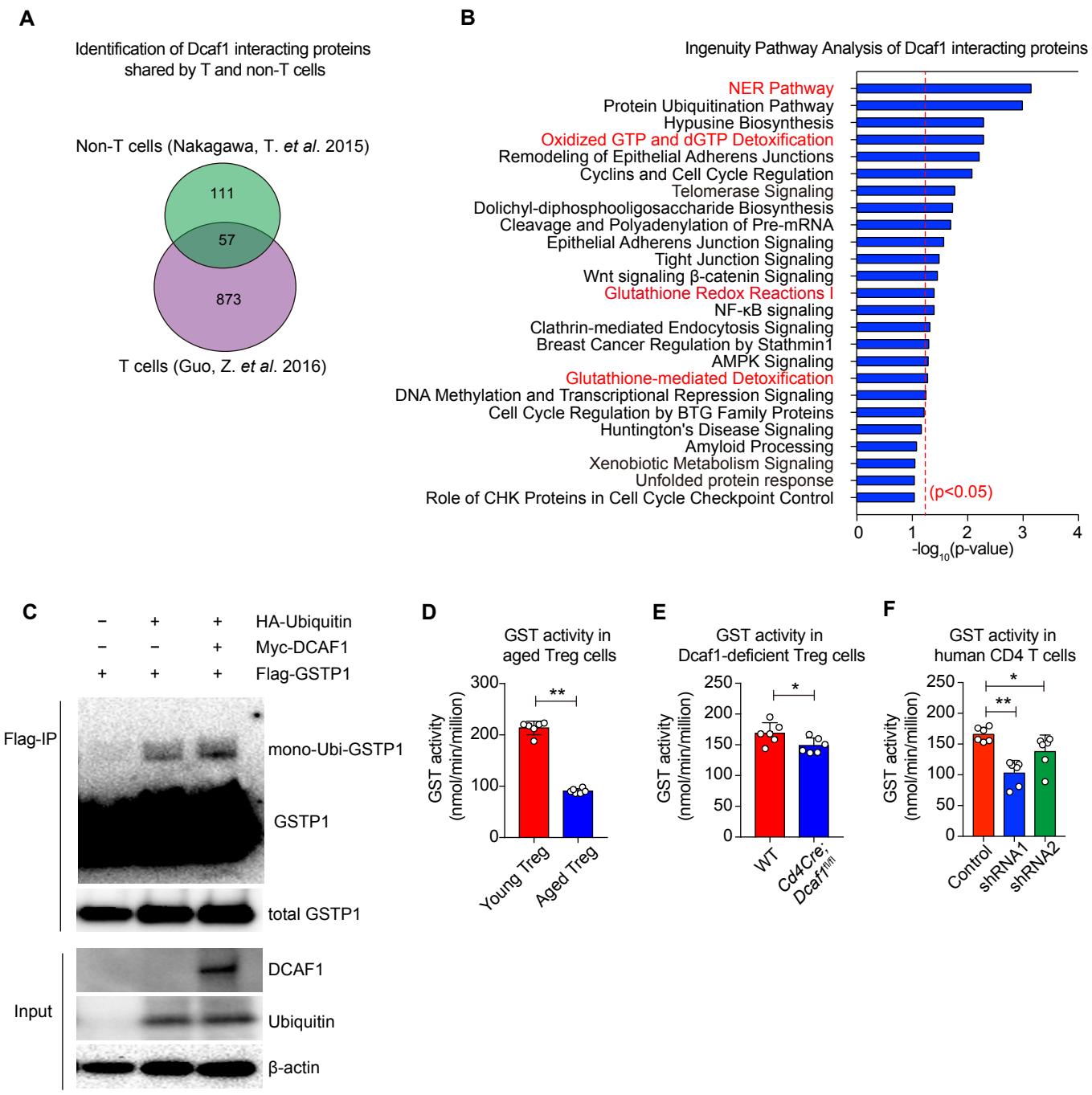

A
Identification of Dcaf1 interacting proteins shared by T and non-T cells
Non-T cells (Nakagawa, T. et al. 2015)
111
57
873
T cells (Guo, Z. et al. 2016)
B
Ingenuity Pathway Analysis of Dcaf1 interacting proteins
NER Pathway
Protein Ubiquitination Pathway
Hypusine Biosynthesis
Oxidized GTP and dGTP 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
Tight Junction Signaling
Wnt signaling β-catenin Signaling
Glutathione Redox Reactions I
NF-κB signaling
Clathrin-mediated Endocytosis Signaling
Breast Cancer Regulation by Stathmin1
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)
0
1
2
3
4
-log10(p-value)
C
HA-Ubiquitin
Myc-DCAF1
Flag-GSTP1
Flag-IP
mono-Ubi-GSTP1
GSTP1
total GSTP1
Input
DCAF1
Ubiquitin
β-actin
D
GST activity in aged Treg cells
GST activity (nmol/min/million)
Young Treg
Aged Treg
E
GST activity in Dcaf1-deficient Treg cells
GST activity (nmol/min/million)
WT
Cd4Cre; Dcaf1fl/fl
F
GST activity in human CD4 T cells
GST activity (nmol/min/million)
Control
shRNA1
shRNA2

Fig. S6

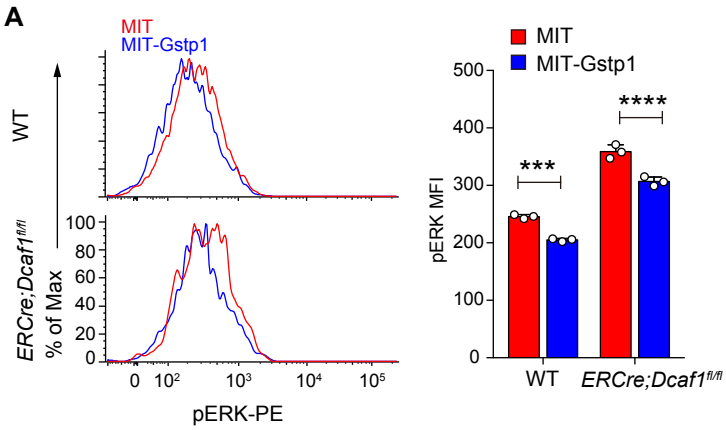
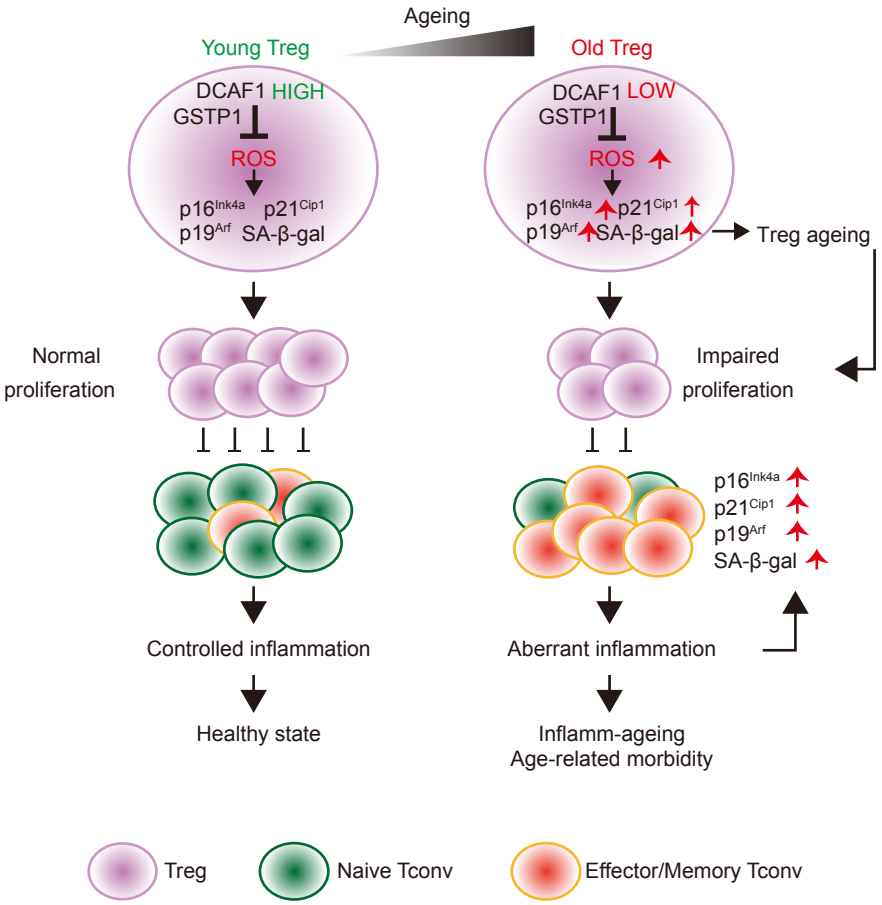
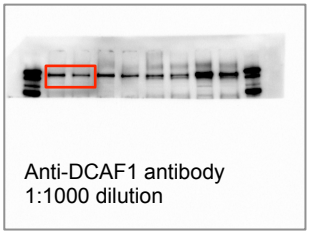
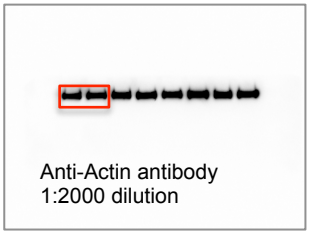

A
MIT
MIT-Gstp1
WT
ERCre;Dcaf1fl/fl
% of Max
100
80
60
40
20
0
0
10²
10³
10⁴
10⁵
pERK-PE
MIT
MIT-Gstp1
500
400
300
200
100
0
pERK MFI
***
****
WT
ERCre;Dcaf1fl/fl

Fig. S7

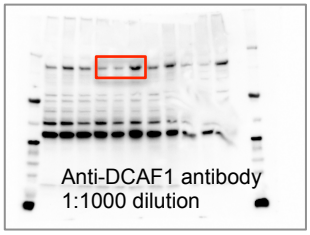

Ageing
Young Treg
Old Treg
DCAF1 HIGH
GSTP1
ROS
p16Ink4a p21Cip1
p19Arf SA-β-gal
DCAF1 LOW
GSTP1
ROS
p16Ink4a p21Cip1
p19Arf SA-β-gal
Treg ageing
Normal proliferation
Impaired proliferation
p16Ink4a
p21Cip1
p19Arf
SA-β-gal
Controlled inflammation
Aberrant inflammation
Healthy state
Inflamm-ageing
Age-related morbidity
Treg
Naive Tconv
Effector/Memory Tconv

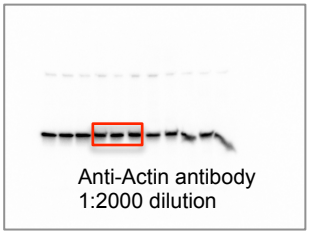
1. Full unedited gel for Figure 3A



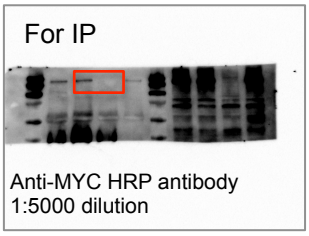


2. Full unedited gel for Figure 6A

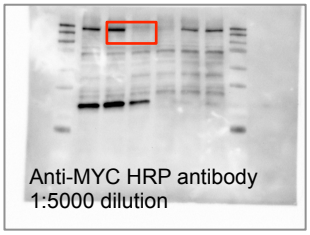


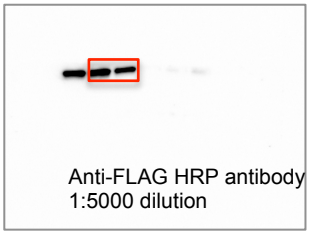


3. Full unedited gel for Figure 7F

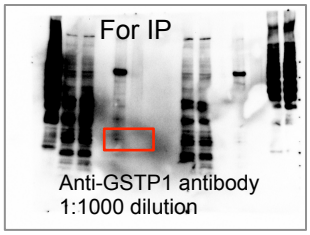


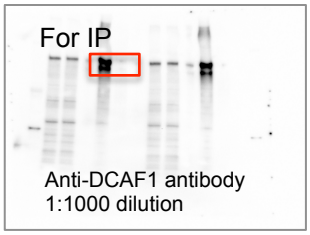
For Input, in separate membrane

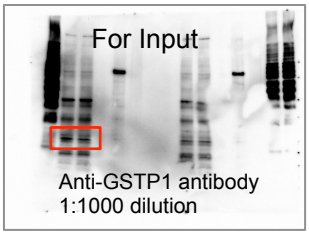


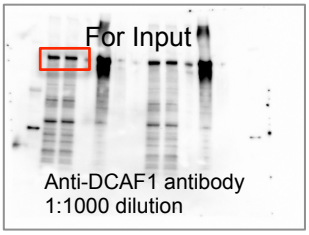


4. Full unedited gel for Figure 7G

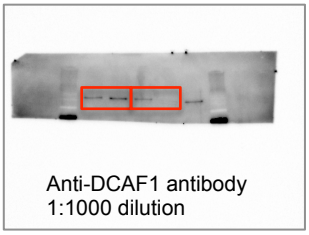


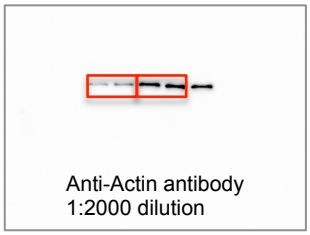




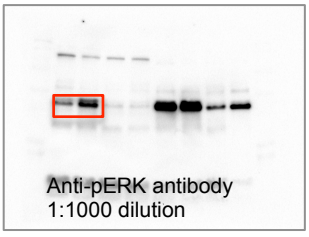


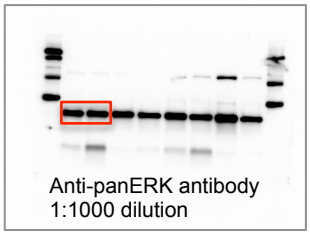
5. Full unedited gel for Figure S3B



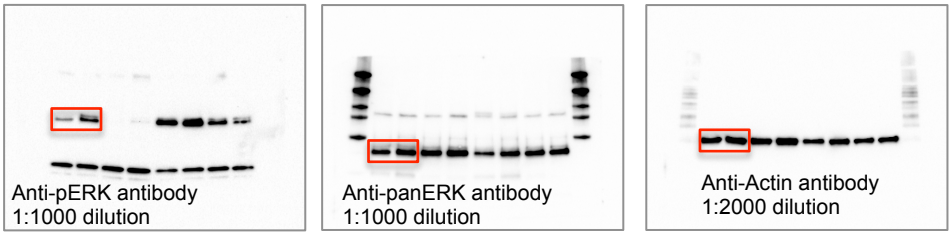


6. Full unedited gel for Figure S4A





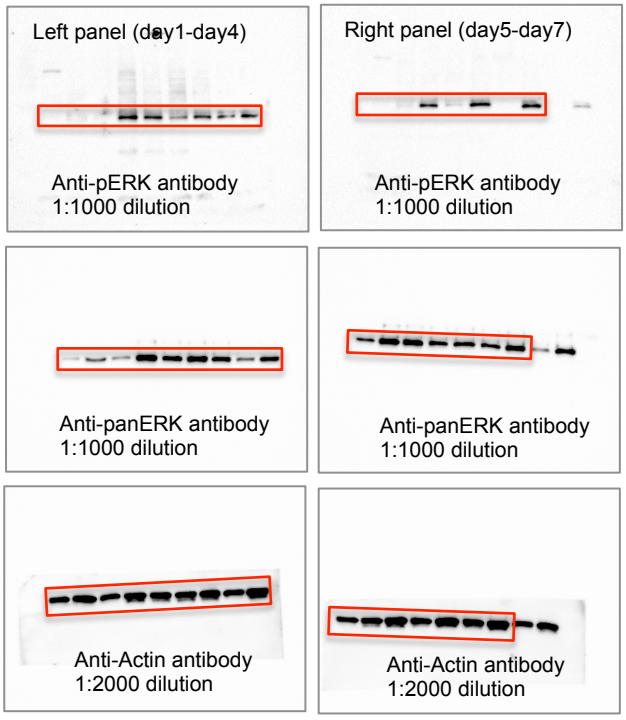
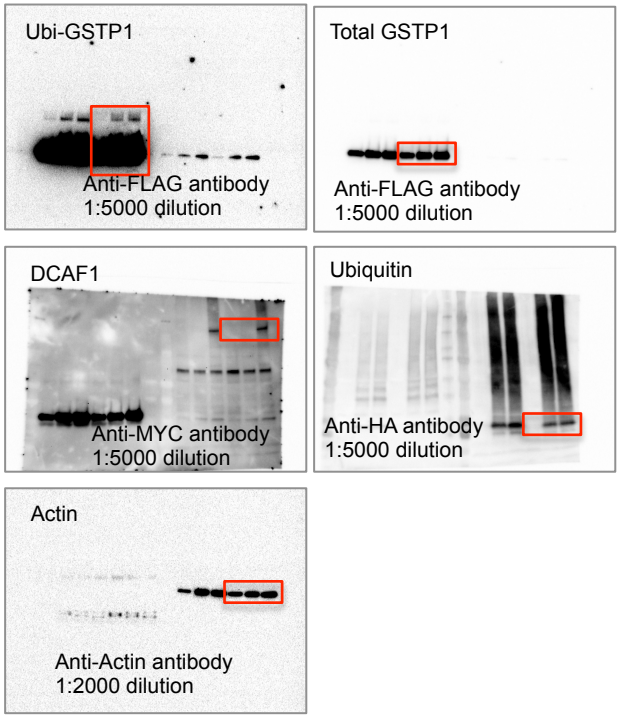
7. Full unedited gel for Figure S4B



8. Full unedited gel for Figure S4C

9. Full unedited gel for Figure S4D

10. Full unedited gel for Figure S4E



11. Full unedited gel for Figure S5C