Peli1 regulates T cell metabolism and antitumor immunity by regulating mTORC1 activation

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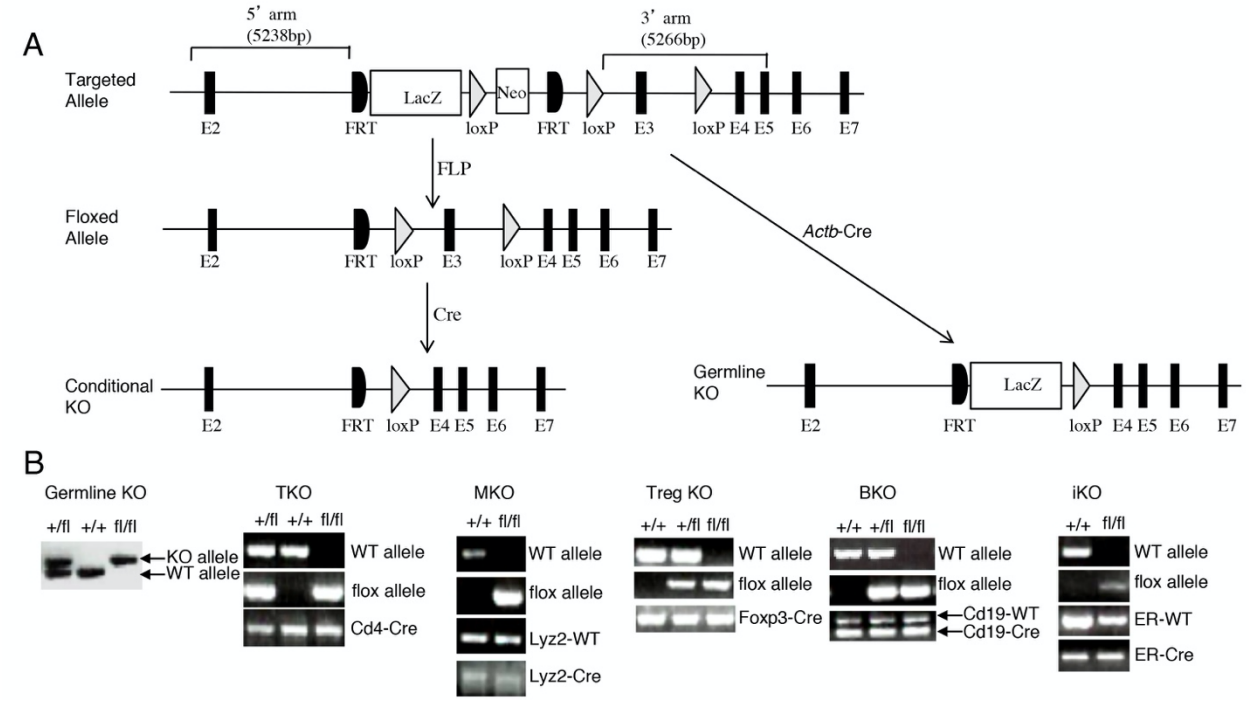
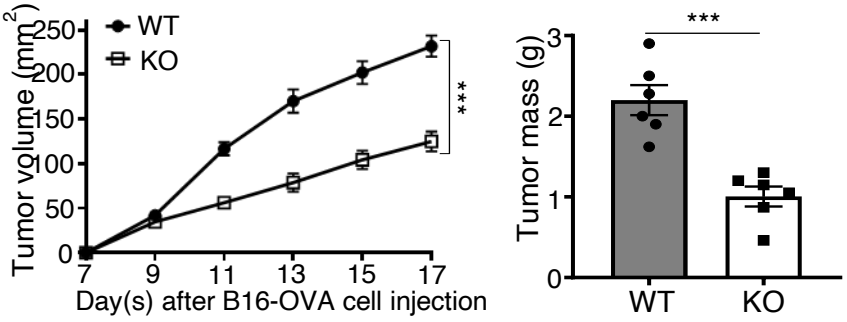
Appendix

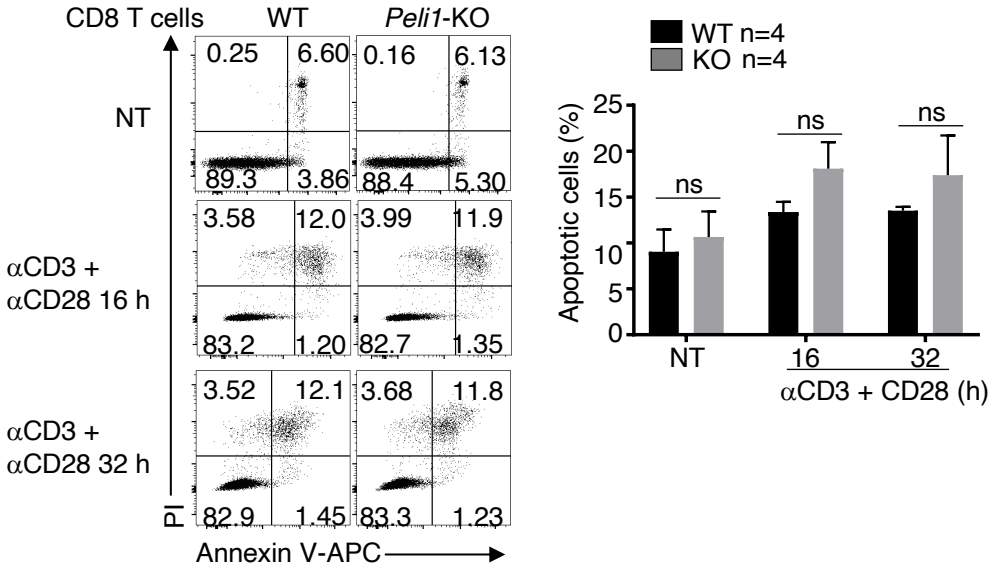
Appendix Figures



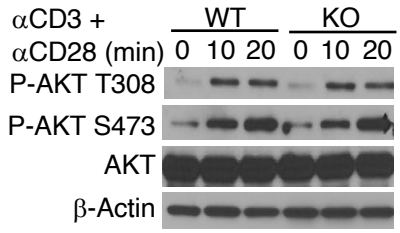
Appendix Figure S1. Generation of Peli1 germline KO and conditional KO mice.
Schematic picture of *Peli1* gene targeting (A) and genotyping PCR analyses (B) of the generated mice. Mice carrying the *Peli1* targeted allele were crossed with FLP deleter (Rosa26-FLPe) mice to generate *Peli1-flox* mice, which were further crossed with Cd4-Cre, Lyz2-Cre, Foxp3-Cre, Cd19-Cre, and CreER mice to generate T cell-conditional KO (TKO), myeloid cell-conditional KO (MKO), Treg cell-conditional (Treg KO), B cell-conditional (BKO), and tomaxifen-inducible KO (iKO) mice, respectively. Peli1 germline KO mice (obtained from KOMP) were created by crossing Peli1-targeted mice with *Actb*-Cre mice.



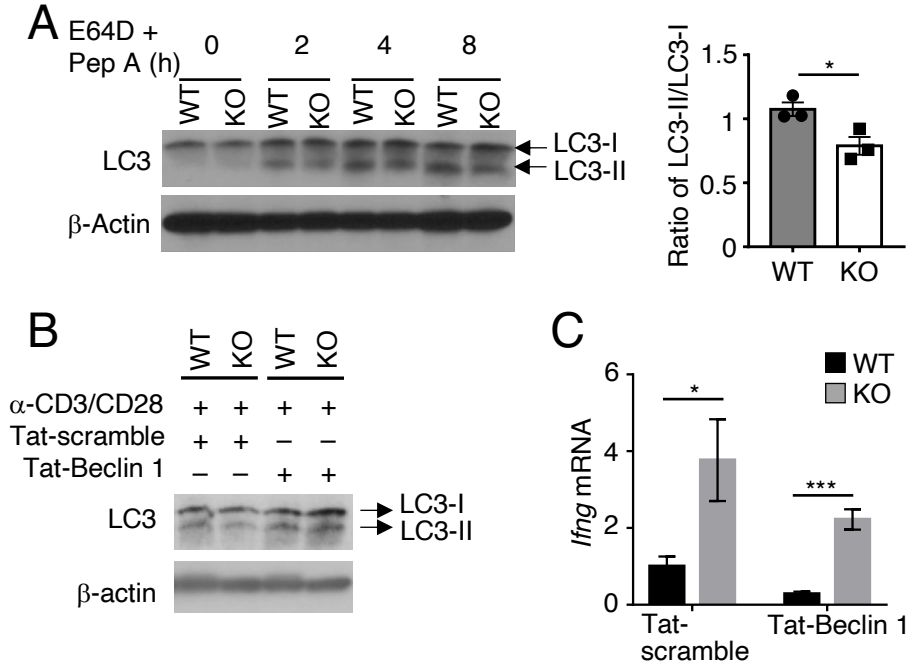
Appendix Figure S2. Peli1 deficiency suppresses tumor growth.
Tumor growth curve (left) and summary of end-point tumor masses (right) of 6-8 week-old wildtype (WT) and *Peli1*-KO (KO) mice inoculated s.c. with B16-OVA tumor cells. Data are presented as mean±SEM with P values being determined by a two-way ANOVA analysis with Bonferroni correction (left panel) and two-tailed unpaired Student's t-test (right panel). ***P<0.001.



Appendix Figure S3. Effect of Peli1 deficiency on apoptosis induction.
Flow cytometric analysis of apoptotic cells in wildtype (WT) or Peli1-KO (KO) CD8 T cells stimulated with anti-CD3 plus anti-CD28 for the indicated time points. Data are presented as representative FACS plot or summary graph based on 4 different pairs of mice. P values were determined by two-tailed unpaired Student's t-test. ns, not significant.



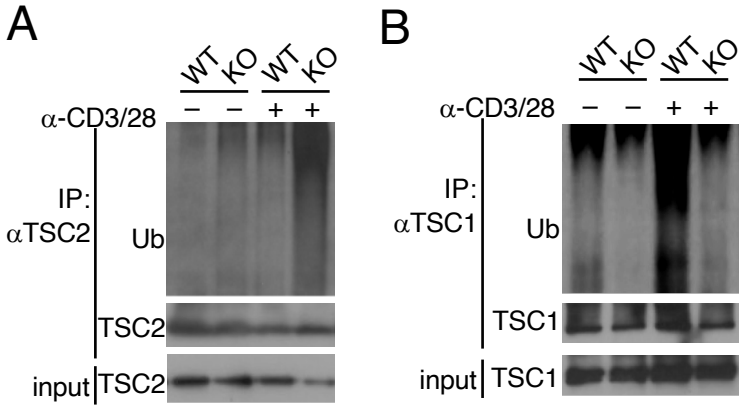
Appendix Figure S4. Dispensable role of Peli1 in AKT regulation.
Immunoblot analysis of AKT phosphorylation at serine 473 (S473) and threonine 308 (T308), total AKT expression level, and loading control β-Actin expression level in whole-cell lysates of wildtype (WT) and Peli1-KO (KO) OT-I CD8 T cells, stimulated with anti-CD3 plus anti-CD28 for the indicated time points.



Appendix Figure S5. Autophagy is partially involved in the hyper-induction of IFNγ in Peli1-deficient CD8 T cells.

A. Immunoblot analysis of LC3 in wildtype (WT) and Peli1-KO (KO) CD8 T cells that were activated for 2 h with plate-bound anti-CD3 and anti-CD28 and then treated for the indicated time points with lysosomal protease inhibitors, E64D (10 μg/ml) and pepstatin (10 μg/ml). Data are presented as a representative plot (left panel) and a summary graph of LC3-II/LC3-I ratio based on the 8 h time point of E64D/PepA treatment.

B,C. Immunoblot analysis of LC3 (**b**) and qRT-PCR analysis of *Ifng* induction (**c**) in wildtype (WT) and Peli1-KO KO) CD8 T cells that were activated for 2 h with anti-CD3 and anti-CD28 and then treated for additional 6 h with the autophagy-stimulating peptide Tat-Beclin 1 or the Tat-scrambled control peptide (Tat-scr). P values were determined by two-tailed unpaired Student's t-test. *P<0.05; ***P<0.001.



Appendix Figure S6. Ubiquitination of TSC2 and TSC1 in CD8 T cells.
TSC2 (**A**) and TSC1 (**B**) were immunoprecipitated from wildtype (WT) or Peli1-KO (KO) naïve CD8 T cells that were either untreated (–) or stimulated for 2h with anti-CD3 plus anti-CD28 (+) and subjected to immunobloting using anti-ubiquitin (Ub) and anti-TSC2 or anti-TSC1. Cell lysates (input) were also subjected to immunoblotting using anti-TSC2 or anti-TSC1.

Appendix Tables

Appendix Table S1. Primers for mouse genotyping

Mice	Primer Sequence (5' to 3')	Amplicon
Peli1 KO		
WT allele	Forward GCTTCCTGGGTGTGTGATACATGC	425 bp
	Reverse GGATCTGTCTGGCTATGTTTTGAACC	
KO allele	Forward GCTACCATTACCAGTTGGTCTGGTGTC	560 bp
	Reverse AGAGAAATTCCAAGGCAAAATGAGG	
Peli1 flox		
WT allele	Forward TGAGTGTAGGGTTAATTGACGTAG	249 bp
	Reverse AGTCCTAACTACCTGAATAGAGCAC	
Flox	Forward TGAGTGTAGGGTTAATTGACGTAG	500 bp
	Reverse TGCGACTATAGAGATATCAACCAC	
Cd4-Cre	Forward CCCAACCAACAAGAGCTC	600 bp
	Reverse CCCAGAAATGCCAGATTACG	
Lyz2-Cre		
WT allele	Forward CTTGGGCTGCCAGAATTTCTC	350 bp
	Reverse TTACAGTCGGCCAGGCTGAC	
Cre allele	Forward CTTGGGCTGCCAGAATTTCTC	700 bp
	Reverse CCCAGAAATGCCAGATTACG	
Cd19-Cre		
WT allele	Forward AGAGGGAGGCAATGTTGTGC	588 bp
	Reverse GTCCAGGTCCCTGACGTCTG	
Cre allele	Forward AGAGGGAGGCAATGTTGTGC	420 bp
	Reverse GACGATGAAGCATGTTTAGCTGG	
Foxp3-Cre	Forward ACGTAAACGGCCACAAGTTCAGC	509 bp
	Reverse GTCGCCGATGGGGGTGTTCT	
Rosa CreER		
WT allele	Forward AAAGTCGCTCTGAGTTGTTAT	650 bp
	Reverse GGAGCGGGAGAAATGGATATG	
CreER allele	Forward AAAGTCGCTCTGAGTTGTTAT	825 bp
	Reverse CCTGATCCTGGCAATTTCG	

Appendix Table S2. Primers for human TSC1 site-directed mutagenesis

Mutation	Primers (5' to 3')
K30A	Forward ACAGCTGTCTTTGCAGAGAACCTCAAT Reverse ATTGAGGTTCTCTGCAAAGACAGCTGT
K632A	Forward TTAAAGAAAGCAGCAGGAAACACAGAG Reverse CTCTGTGTTTCCTGCTGCTTTCTTTAA

Appendix Table S3. Gene-specific primers for qRT-PCR analysis of mouse gene expression

Gene	Primers (5' to 3')	Amplicon
Tsc1	Forward ACTCTCCCTTCTACCGAGACA Reverse GAGGCTGCCGAATGAGTCTTC	61 bp
Tsc2	Forward TGCCGCAGCATCAGTGTATC Reverse TGCCAGGAGGAACTCTCCC	231 bp
Hk2	Forward GATCGCCGGATTGGAACAGA Reverse GGTCTAGCTGCTTAGCGTCC	97 bp
Glut1	Forward GCTGTGCTTATGGGCTTCTC Reverse CACATACATGGGCACAAAGC	114 bp
Hif1a	Forward AAGTGGCAACTGATGAGCAA Reverse GGCGAGAACGAGAAGAAAAA	123 bp
Myc	Forward AAACGACAAGAGGCGGACAC Reverse TGGTCACGCAGGGCAAAA	84 bp
Pgk1	Forward TGTCGCTTTCCAACAAGCTG Reverse GCTCCATTGTCCAAGCAGAAT	163 bp
Eno1	Forward TGCGTCCACTGGCATCTAC Reverse CAGAGCAGGCGCAATAGTTTTA	118 bp
Pfkp	Forward GAAACATGAGGCGTTCTGTGT Reverse CCCGGCACATTGTTGGAGA	66 bp
Pkm	Forward GCCGCCTGGACATTGACTC Reverse CCATGAGAGAAATTCAGCCGAG	145 bp
Aldoa	Forward GCGCCTTTAAATGTCCGGG Reverse TAGGGTCACCAGAACCTCGT	103 bp
Peli1	Forward CGTGAAACCAGATCAGCTCAGC Reverse GAGCTGCATTGATCTCCTGTCTT	181 bp
Ifng	Forward CAGCAACAGCAAGGCGAAA Reverse CTGGACCTGTGGGTTGTTGAC	73 bp
Actb	Forward CGTGAAAAGATGACCCAGATCA Reverse CACAGCCTGGATGGCTACGT	72 bp