a

Lower in AGN-treated / Higher in AGN-treated

P value ($-\log_{10}$) vs Fold change ($\log_2$, AGN vs Veh)

TMED9, TMED10, TMED5, IFNG

b

PD-1-PE MFI

ns, *

AGN: − + − +
αCD3: − − + +

c

CD137-APC MFI

ns, *ns*

AGN: − + − +
αCD3: − − + +

d

CD137⁺ (%)

ns, *ns*

AGN: − + − +
αCD3: − − + +

e

Rest / Act
AGN: − + − +

50kDa — PD-1
20kDa — TMED10
50kDa — Tubulin

f

sgCtrl vs sg*Tmed10*

Overlap
1477 | 956 | 268

Vehicle vs AGN

Hypergeometric
p = 1.86 x 10⁻⁵²⁴

g

IFNγ (ng · mL⁻¹)

****, ****, *ns*

Resting / Activated

■ Vehicle
■ Vehicle + αPD-1
■ AGN
■ AGN + αPD-1

h

CTLA-4-PE-Cy7 MFI

ns, ***

AGN: − + − +
αCD3: − − + +

Supplementary Figure S4

Supplementary Figure S4. AGN192403 phenocopies Tmed10 KO in murine and human CD8 T cells.

a.　　　Proteomic differences between OT-I/Cas9 CD8 T cells treated with vehicle or AGN192403 (AGN, 100μM) after activation with anti-CD3 antibody for 24h as measured by mass spectrometry. The data is based on three independent spleens for each treatment. Statistical analysis was performed by a Student's t test.

b.　　　Quantification of PD-1-PE abundance by flow cytometry of resting and activated human CD8 T cells in the presence or absence of AGN192403 (AGN; 100μM). Each datapoint indicates data obtained with CD8 T cells from independent donors. Error bars denote SD. Statistical analysis was performed with a Friedman test, followed by a Dunn's post-hoc analysis.

c.　　　As in b, but for CD137-APC abundance.

d.　　　As in b, but for percentage of CD137+ cells.

e.　　　Western blot analysis of PD-1 and TMED10 abundance in OT-I/Cas9 CD8 T cells, before or after activation with anti-CD3 antibody for 24h in the presence or absence of AGN192403 (AGN; 100 μM). The size markings indicate the size of the closest molecular weight marker.

f.　　　Overlap of significantly differentially expressed genes between activated OT-I/Cas9 CD8 T cells carrying either a non-targeting control sgRNA or an sgRNA targeting Tmed10 (green) and activated OT-I/Cas9 CD8 T cells treated with vehicle or AGN192403 (AGN; 100μM; blue). Statistical p-value was determined by hypergeometric test.

g.　　　Cytokine release of IFNγ as measured by cytometric bead array of vehicle- or AGN192403-treated OT-I/Cas9 CD8 T cells, after activation, or not, with CD3-antibody in the presence or absence of PD-1 antibody (10 μg/mL). Each datapoint indicates data obtained with CD8 T cells from an independent spleen. Error bars denote SD. Statistical analysis was performed with a one-way ANOVA, followed by a Tukey post-hoc test.

h.　　　Quantification of flow cytometry measurements for the abundance of CTLA-4-PE-Cy7 in resting and activated OT-I/-Cas9 CD8 T cells treated with, or not, AGN192403. Each datapoint indicates data obtained with CD8 T cells from an independent spleen. Error bars denote SD. Statistics were performed with a Student t test for each activation condition.

$*$ P < 0.05; $**$ P < 0.01; $***$ P < 0.001; $****$ P < 0.0001.