Expanded View Figures

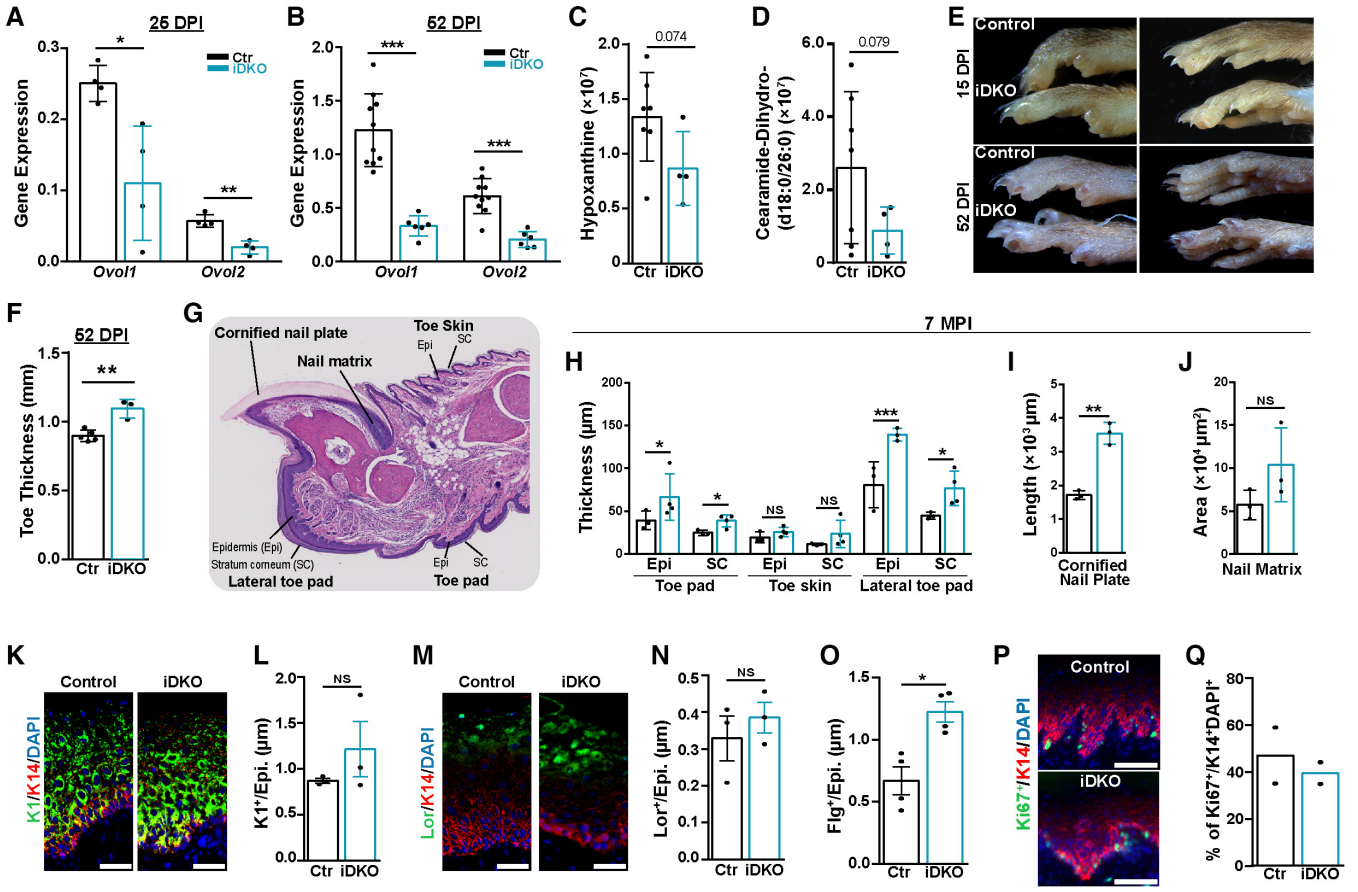


Figure EV1. Additional data on back and paw skin defects in iDKO mice. Related to Fig 1.

A, B RT–qPCR of the indicated genes in epidermis at 25 (n = 4 biological replicates per genotype) or 52 (Control: n = 10 biological replicates; iDKO: n = 6 biological replicates) DPI.

C, D Ion counts of the indicated lipid species Control: n = 7 biological replicates; iDKO: n = 4 biological replicates.

E Representative images of front (left) and back (right) paws of control and iDKO mice at 15 or 52 DPI.

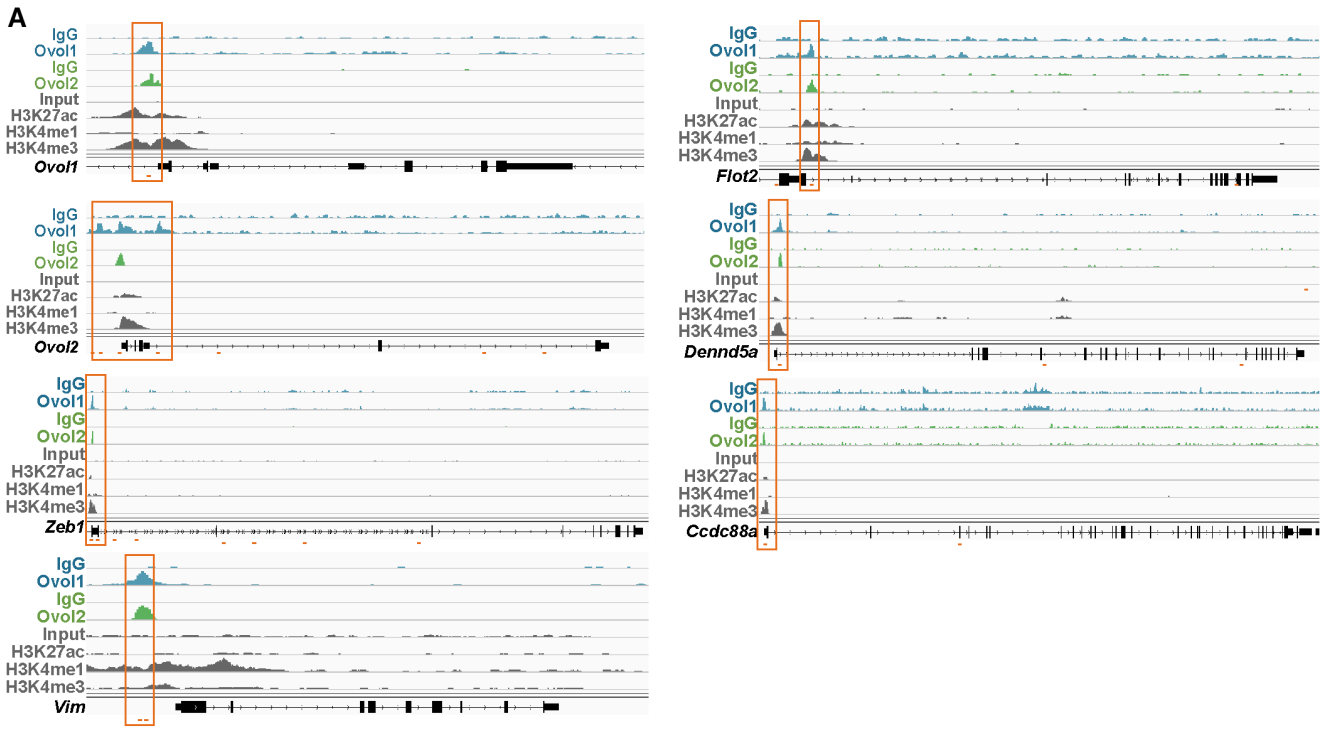
F Quantification of toe thickness in control and iDKO mice at 52 DPI. Control: n = 5 biological replicates; iDKO: n = 3 biological replicates.

G Representative H/E image of a control toe labeled for the indicated anatomical regions.

H–J Quantification of thickness of different anatomical regions labeled in (G). Control: n = 3 biological replicates; iDKO: n = 4 biological replicates for (H) and n = 3 biological replicates per genotype for (I, J).

K–Q Representative immunofluorescent images (K, M, P) and quantification (L, N, O, Q) of the indicated proteins in toe pads at 52 DPI. n = 3, 4, 2 biological replicates per genotype for K1/Lor, Flg, and Ki67, respectively.

Data information: Scale bar: 25 µm for (K, M, P). For (A–D, F, H–J, L, N, O, Q), data are represented as mean ± SD and statistical analysis was done with the Holm–Sidak method. NS, nonsignificant; *$P \leq 0.05$; **$P \leq 0.005$; ***$P \leq 0.0005$.
Source data are available online for this figure.



B 25 DPI

Gene	CTR25-1	DKO25-1	CTR25-2	DKO25-2	Fold Change	p-value	padj-value
Il12b	0.000	25.701	0.827	30.254	55.378	0.002	0.355
Il12rb1	29.589	18.692	50.037	25.089	1.789	0.436	1.000
Il12rb2	196.363	1474.295	1817.454	4376.560	4.203	0.000	0.005
Il6	47.073	165.887	30.188	126.921	3.858	0.004	0.496
Nfkb2	780.072	1411.211	2912.062	6030.960	1.937	0.008	0.629
Cx3cl1	525.876	754.671	658.336	1243.383	1.650	0.071	1.000
Ccl27a	78122.830	49920.407	4975.566	4987.551	1.250	0.368	1.000
Ccl17	69.937	0.000	3.722	2.952	8.782	0.067	1.000

C 52 DPI

Gene	CTR52-1	DKO52-1	CTR52-2	DKO52-2	Fold Change	pvalue	padj
Il12b	0.000	10.559	7.439	16.866	6.984	0.172	NA
Il12rb1	22.418	215.132	111.590	171.160	3.596	0.005	0.165
Il12rb2	1449.186	1120.534	1860.577	2066.410	1.077	0.696	1.000
Il6	59.248	34.316	43.148	121.186	1.311	0.615	1.000
Nfkb2	3724.648	2801.994	2524.166	2140.121	1.252	0.188	1.000
Cx3cl1	853.498	884.285	1278.821	1509.204	1.107	0.594	1.000
Ccl27a	4842.363	4599.599	12941.465	11799.403	1.075	0.653	1.000
Ccl17	1.601	3.959	0.000	4.373	9.568	0.349	NA

Figure EV2. Additional Ovol1/2 direct targets and altered expression of immune-associated genes in iDKO epidermis. Related to Fig 3.

A ChIP-seq tracks of Ovol1, Ovol2, and histone marks for the select loci.

B, C Normalized raw RNA-seq counts and *P*-values calculated by DEseq2 at 25 or 52 DPI.

Figure EV3. Gating strategies for flow cytometry and additional data on immune-associated changes in iDKO mice. Related to Fig 4.

A, D, E, M, O Gating strategies for the indicated cells shown in Fig 4.

B, C, F–L, N, P, Q Shown are matching or additional data for Fig 4 now represented as cell number per gram of tissue or frequency of live. $n = 5$ biological replicates per genotype for (B, C); $n = 3$ biological replicates per genotype for (F); Control: $n = 9$ biological replicates; iDKO: $n = 5$ biological replicates for (G); Control: $n = 13$ biological replicates; iDKO: $n = 9$ biological replicates for (H, I); Control: $n = 10$ biological replicates; iDKO: $n = 6$ biological replicates for (J); $n = 5$ biological replicates per genotype for (K, L); Control: $n = 8$ biological replicates; iDKO: $n = 4$ biological replicates for (N); Control: $n = 10$ biological replicates; iDKO: $n = 5$ biological replicates for (P); and $n = 4$ biological replicates per genotype for (Q).

R Quantification of spleen weight at 4–6 MPI. $n = 7$ biological replicates per genotype.

S, T Luminex measurements of the indicated cytokines in serum of control and iDKO mice at 4–6 MPI. $n = 8$ biological replicates per genotype.

Data information: For (F–L, N, P–T), data are represented as mean ± SD and statistical analysis was done with the Holm–Sidak method. NS, nonsignificant; $*P \leq 0.05$; $**P \leq 0.005$.

Source data are available online for this figure.

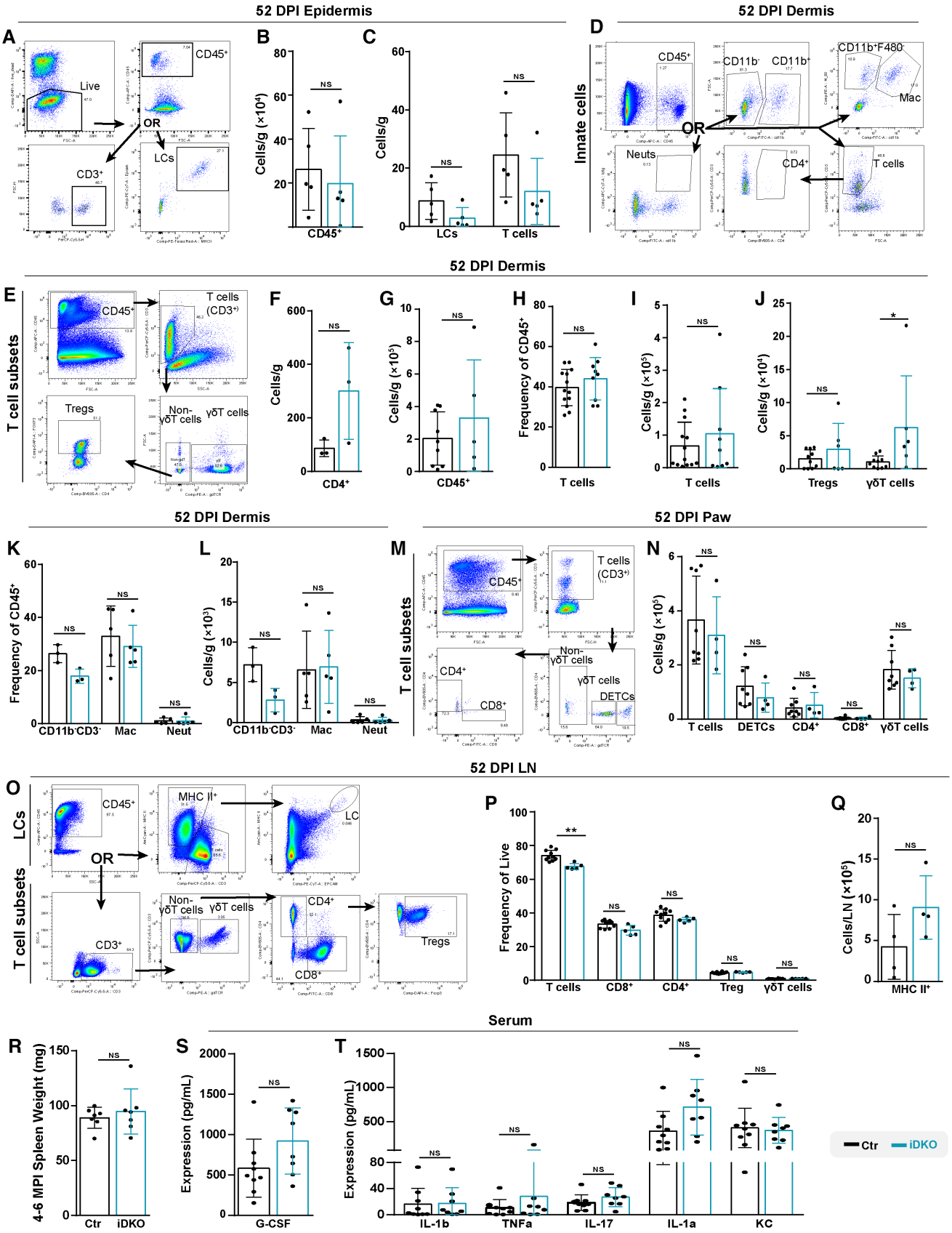

52 DPI Epidermis
52 DPI Dermis
52 DPI Dermis
52 DPI Dermis
52 DPI Paw
52 DPI LN
Serum
Live
CD45+
OR
LCs
CD3+
Cells/g (×10⁴)
Cells/g
CD45+
LCs
T cells
Innate cells
CD11b-
CD11b+
CD11b+F480-
Mac
Neuts
CD4+
T cells
T cell subsets
T cells (CD3+)
Tregs
Non-γδT cells
γδT cells
Cells/g (×10⁵)
Frequency of CD45+
Cells/g (×10³)
CD11b-CD3-
Neut
DETCs
CD8+
MHC II+
LC
Frequency of Live
Treg
Cells/LN (×10⁵)
4-6 MPI Spleen Weight (mg)
Ctr
iDKO
Expression (pg/mL)
G-CSF
IL-1b
TNFa
IL-17
IL-1a
KC
NS

Figure EV3.

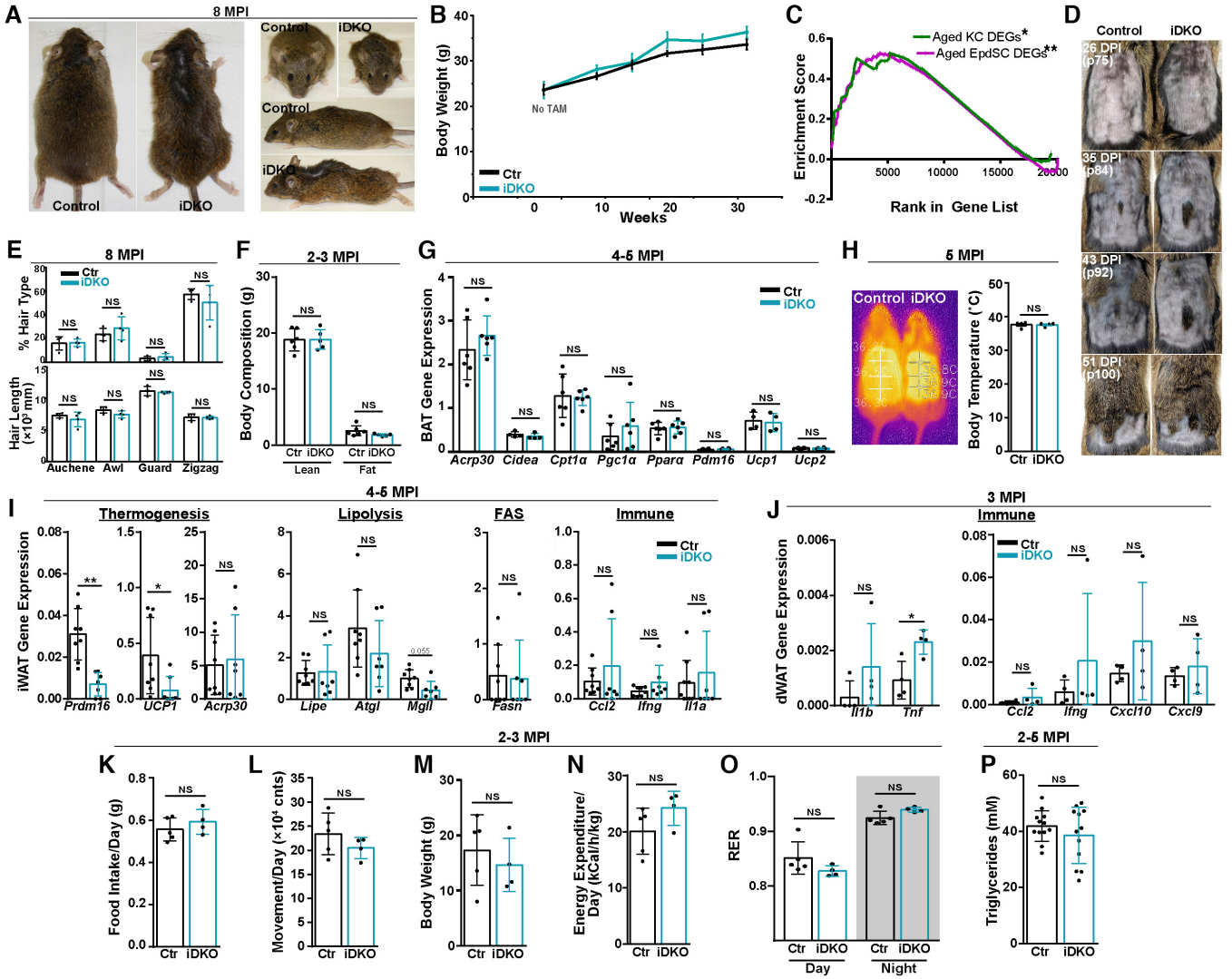


Figure EV4. Characterization of the aging, hair, and whole-body metabolic phenotypes in iDKO mice. Related to Fig 5.

A Representative images of control and iDKO mice at 8 MPI.

B Body weight measurements of non-TAM-injected control and iDKO mice over time. $n = 9$ biological replicates per genotype.

C GSEA using genes known to be upregulated in aged skin over young.

D Representative images of shaved back skin from control and iDKO males at the indicated times. "p" = postnatal day.

E Quantitative analysis of the type (top) and length (bottom) of hairs plucked from control and iDKO mice at 8 MPI. Control: $n = 4$ Biological replicates per genotype.

F EchoMRI measurements of body composition at 2–3 MPI. Control: $n = 6$ biological replicates; iDKO: $n = 5$ biological replicates.

G RT–qPCR of the indicated genes in BAT. $n = 6$ biological replicates per genotype.

H Representative FLIR thermogun image and quantification of body temperature at 5 MPI. $n = 4$ biological replicates per genotype.

I, J RT–qPCR of the indicated genes in iWAT (I; Control: $n = 8$ biological replicates; iDKO: $n = 7$ biological replicates) and dWAT (J; $n = 4$ biological replicates per genotype) of control and iDKO mice at 4–5 MPI. FAS, fatty acid synthesis.

K–O Results from metabolic cage analysis of control and iDKO mice at 2–3 MPI. Shown are data on food intake (K), and movement (L), body weight (M), energy expenditure (N), and RER (O). Control: $n = 5$ biological replicates; iDKO: $n = 4$ biological replicates.

P Quantification of serum triglyceride levels. $n = 12$ biological replicates per genotype.

Data information: For (B), data are represented as mean ± SEM. Two-way ANOVA and Sidak's multiple comparisons test for individual time points were performed, and none was statistically significant. For (E–P), data are represented as mean ± SD and statistical analysis was done with the Holm–Sidak method. NS, nonsignificant; $^*P \leq 0.05$; $^{**}P \leq 0.005$.

Source data are available online for this figure.

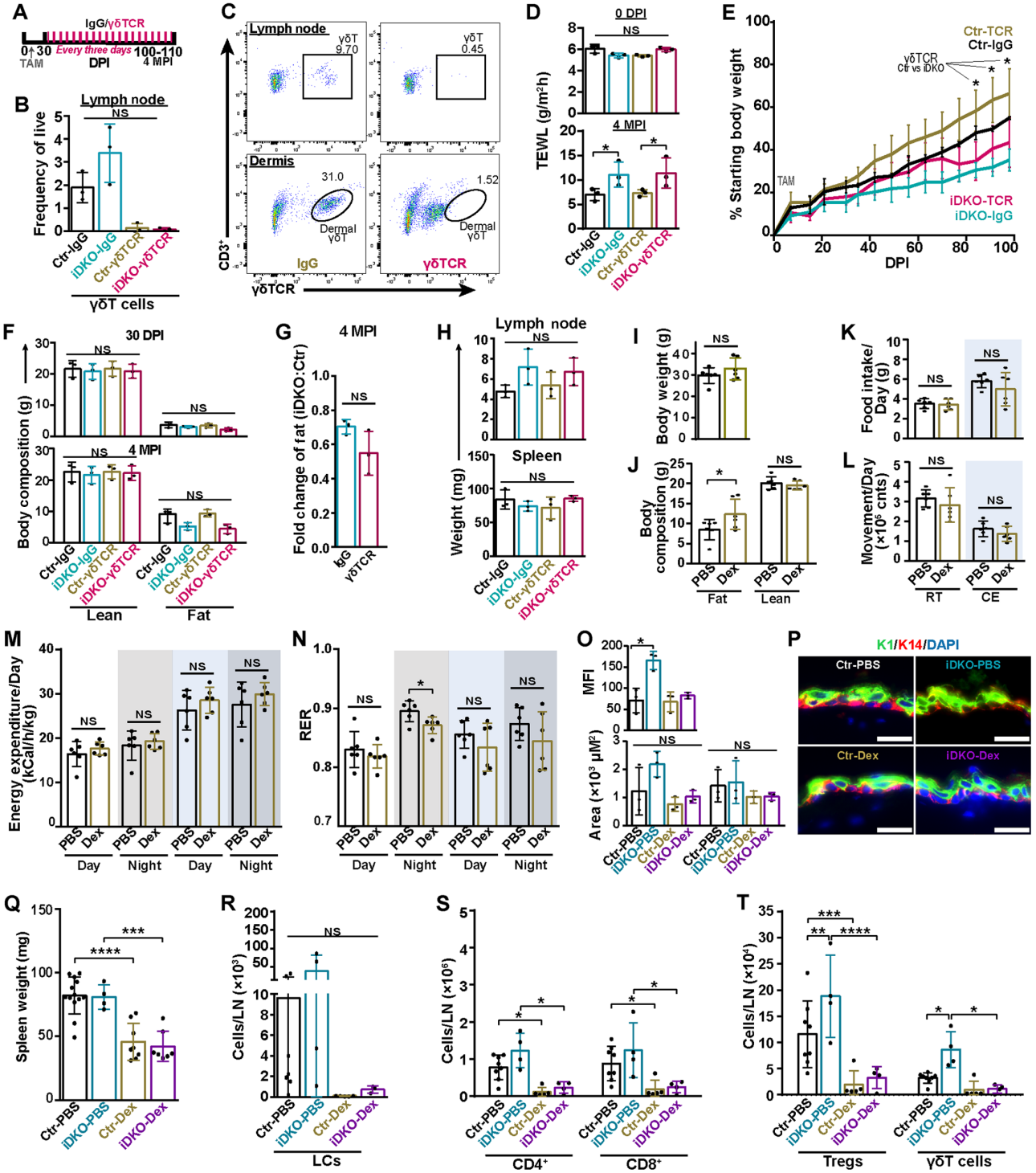

A
IgG/γδTCR
0 30
TAM
Every three days
DPI
100-110
4 MPI
B
Lymph node
NS
Frequency of live
Ctr-IgG
iDKO-IgG
Ctr-γδTCR
iDKO-γδTCR
γδT cells
C
Lymph node
γδT 9.70
γδT 0.45
Dermis
31.0
1.52
Dermal γδT
IgG
γδTCR
CD3+
γδTCR
D
0 DPI
NS
TEWL (g/m²h)
4 MPI
E
Ctr-TCR
Ctr-IgG
γδTCR Ctr vs iDKO
iDKO-TCR
iDKO-IgG
% Starting body weight
TAM
DPI
F
30 DPI
Body composition (g)
4 MPI
Lean
Fat
G
4 MPI
Fold change of fat (iDKO:Ctr)
IgG
γδTCR
H
Lymph node
Spleen
Weight (mg)
I
Body weight (g)
J
Body composition (g)
PBS
Dex
Fat
Lean
K
Food intake/ Day (g)
L
Movement/Day (×10⁶ cnts)
RT
CE
M
Energy expenditure/Day (kCal/h/kg)
Day
Night
N
RER
O
MFI
Area (×10³ μM²)
P
K1/K14/DAPI
Ctr-PBS
iDKO-PBS
Ctr-Dex
iDKO-Dex
Q
Spleen weight (mg)
R
Cells/LN (×10³)
LCs
S
Cells/LN (×10⁶)
CD4⁺
CD8⁺
T
Cells/LN (×10⁴)
Tregs
γδT cells

Figure EV5.

Figure EV5. Characterization of effects of γδTCR blocking antibody and Dex on iDKO phenotypes. Related to Fig 6.

A Schematic depicting treatment strategy for IgG/γδTCR blocking antibody experiments in (B–H; $n = 3$ biological replicates per genotype). Tissues were harvested at 4 MPI.
B Quantification of γδTCR+ cells in skin-draining LNs.
C Representative flow cytometry plots of γδTCR staining from the indicated tissues.
D TEWL measurements after the indicated treatments.
E Body weight over time represented as a percentage of the starting weight.
F EchoMRI measurements of body composition at the indicated times.
G iDKO/Ctr fold change in fat content at 4 MPI, calculated from (F).
H Quantification of LN or spleen weight form the indicated mice.
I–N Control mice were treated with either PBS or Dex using the strategy depicted in Fig 6. (I, J) Body weight (I) and composition (J) of the indicated mice at the indicated times. (K–N) Results from metabolic cage analysis of 4 MPI control and iDKO mice at RT (white band) or after CE (blue band). Gray bands designate night. Shown are data on food intake (K), movement (L), energy expenditure (M), and RER (N). $n = 6$ biological replicates per condition.
O Quantification of the mean fluorescent intensity (MFI) (top) and area (bottom) of filaggrin staining from Fig 6E. $n = 3$ biological replicates per genotype/condition.
P Representative K1/K14 immunofluorescent images of back skin from the indicated mice.
Q Spleen weight after the indicated treatments. Control-PBS: $n = 13$ biological replicates; iDKO-PBS: $n = 4$ biological replicates; Control-Dex: $n = 8$ biological replicates; iDKO-Dex: $n = 7$ biological replicates.
R–T Additional data for Fig 6 showing flow cytometry analysis of LNs at 4 MPI. Control-PBS: $n = 7$ biological replicates; iDKO-PBS: $n = 4$ biological replicates; Control-Dex: $n = 5$ biological replicates; iDKO-Dex: $n = 3$ biological replicates for (R). Control-PBS: $n = 8$ biological replicates; iDKO-PBS: n = 4 biological replicates; Control-Dex: $n = 5$ biological replicates; iDKO-Dex: $n = 4$ biological replicates for (S, T).

Data information: Scale bar: 20 μm for (P). For (B, G–I, O, Q–R), data are represented as mean ± SD and statistical analysis was done using One-way ANOVA and Tukey's multiple comparisons test. For (D–F, J–O, S, T), data are represented as mean ± SD and statistical analysis was done using two-way ANOVA and Tukey's multiple comparisons test. NS, nonsignificant; *$P \leq 0.05$; **$P \leq 0.005$; ***$P \leq 0.0005$; ****$P \leq 0.00005$.
Source data are available online for this figure.