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Supplemental Information

DeSUMOylase SENP7-Mediated Epithelial Signaling Triggers Intestinal Inflammation via Expansion of Gamma-Delta T Cells

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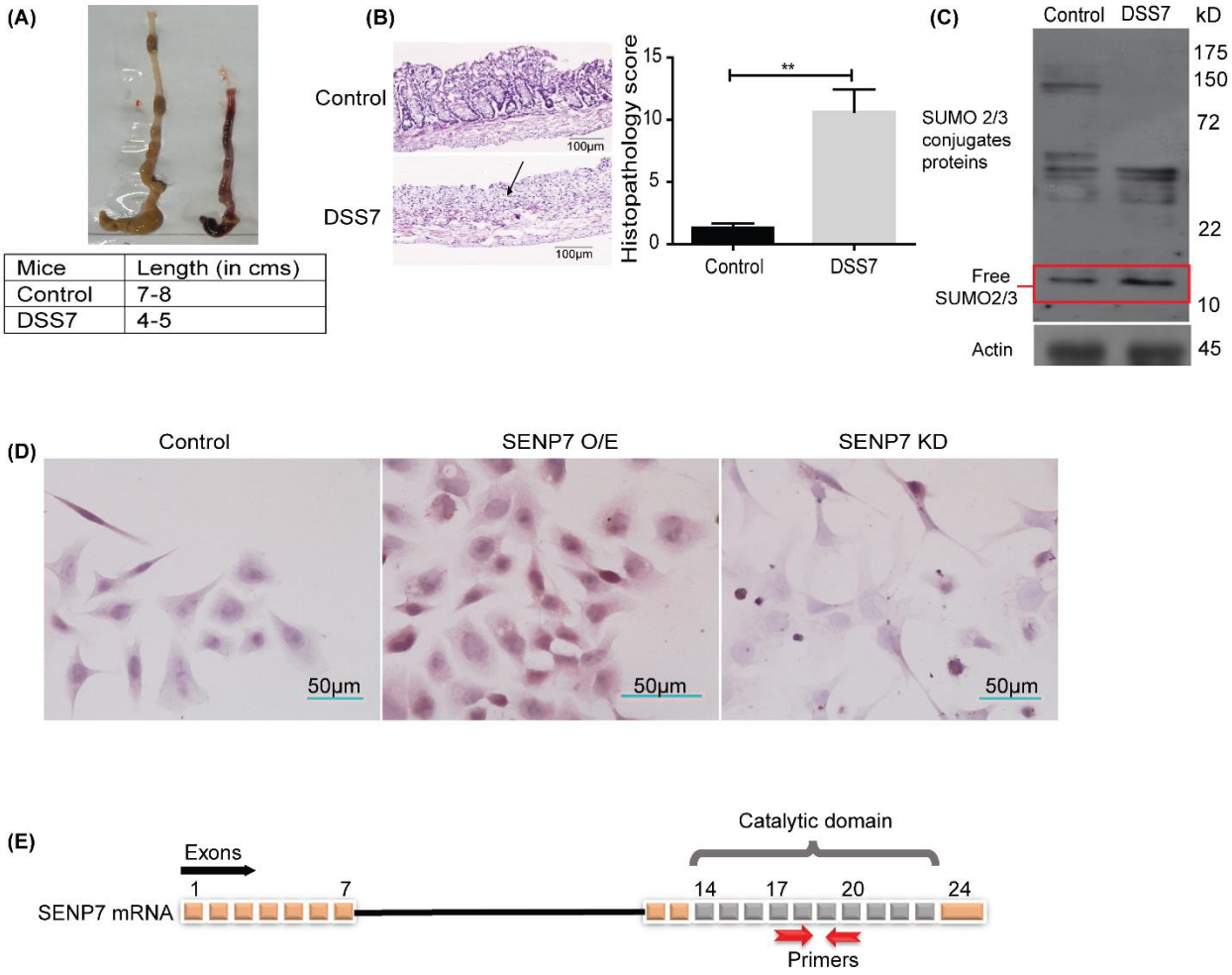
Figure S1



Mice	Length (in cms)
Control	7-8
DSS7	4-5

Figure S1: Related to Figure1*: (A) Gross morphology of mice colon with colon length in the bottom panel (n=3 mice in each category) (B) Left: Histopathology of mice colon of untreated control and 7 days DSS treated mice. Right: Graph showing histopathology score calculated by a blinded pathologist based on parameters- PMN migration, tissue ulceration, epithelial erosion, goblet cell loss, crypt abscesses (DSS7) (C) Immunoblot showing SUMO2/3 conjugated proteins in untreated control and DSS7. (D) Immunohistochemistry of fixed HCT8 cells showing SENP7 overexpression and SENP7 knockdown in comparison to control cells. (E)Graphical representation of SENP7 mRNA transcript showing the qPCR primers location in the catalytic domain which will amply all variants of SENP7 used. ** indicates p <0.01*

Figure S2

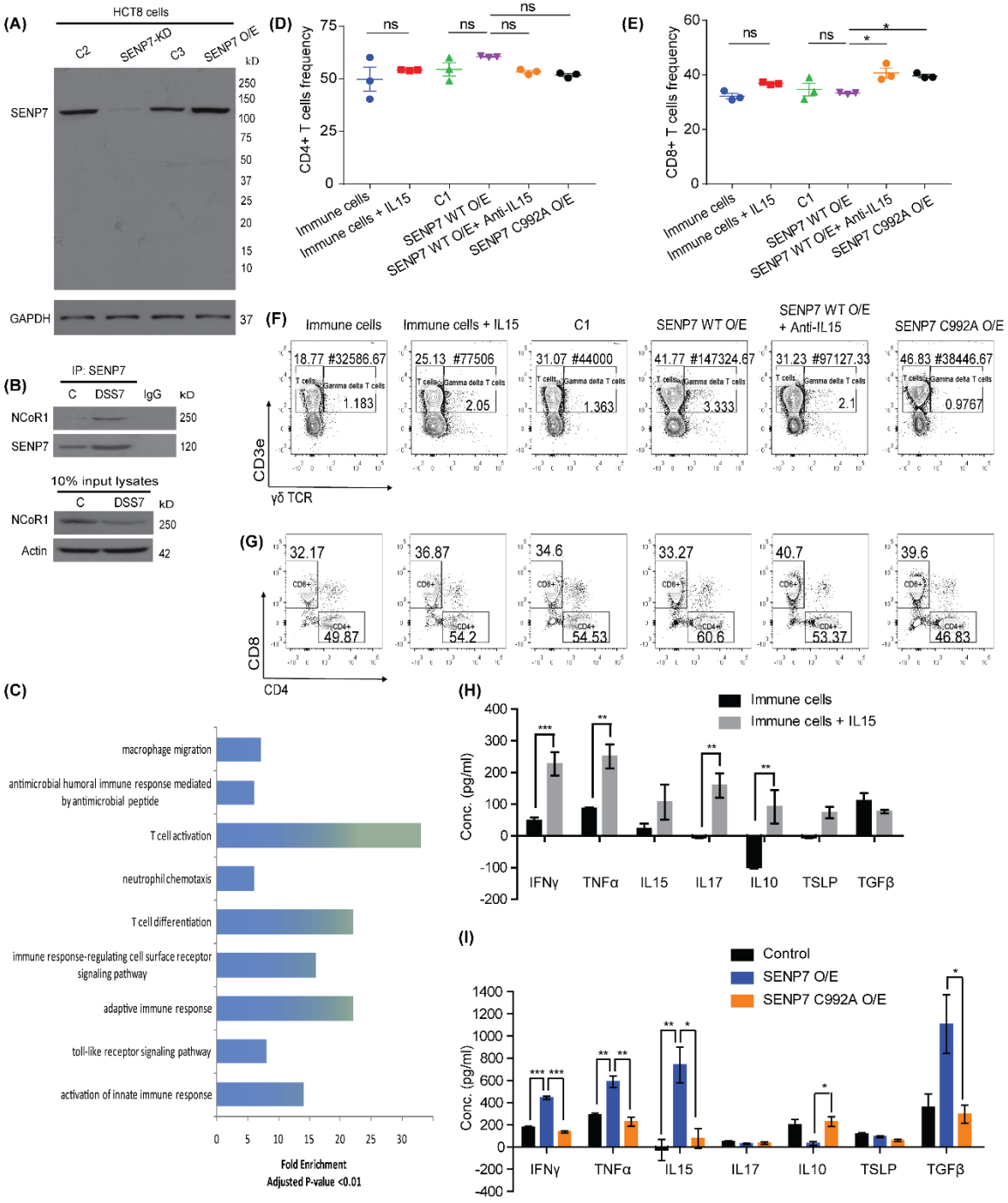

(A)
HCT8 cells
C2
SENP7-KD
C3
SENP7 O/E
kD
SENP7
GAPDH
250
150
100
75
50
37
25
20
15
10
37
(B)
IP: SENP7
C
DSS7
IgG
kD
NCoR1
SENP7
250
120
10% input lysates
C
DSS7
kD
NCoR1
Actin
250
42
(C)
macrophage migration
antimicrobial humoral immune response mediated by antimicrobial peptide
T cell activation
neutrophil chemotaxis
T cell differentiation
immune response-regulating cell surface receptor signaling pathway
adaptive immune response
toll-like receptor signaling pathway
activation of innate immune response
0
5
10
15
20
25
30
Fold Enrichment
Adjusted P-value <0.01
(D)
CD4+ T cells frequency
75
50
25
0
ns
ns
ns
ns
Immune cells
Immune cells + IL15
C1
SENP7 WT O/E
SENP7 WT O/E+ Anti-IL15
SENP7 C992A O/E
(E)
CD8+ T cells frequency
60
40
20
0
ns
ns
*
*
Immune cells
Immune cells + IL15
C1
SENP7 WT O/E
SENP7 WT O/E+ Anti-IL15
SENP7 C992A O/E
(F)
Immune cells
Immune cells + IL15
C1
SENP7 WT O/E
SENP7 WT O/E + Anti-IL15
SENP7 C992A O/E
18.77 #32586.67
1.183
25.13 #77506
2.05
31.07 #44000
1.363
41.77 #147324.67
3.333
31.23 #97127.33
2.1
46.83 #38446.67
0.9767
T cells
Gamma delta T cells
CD3e
γδ TCR
(G)
32.17
49.87
36.87
54.2
34.6
54.53
33.27
60.6
40.7
53.37
39.6
46.83
CD8+
CD4+
CD8
CD4
(H)
Immune cells
Immune cells + IL15
Conc. (pg/ml)
400
300
200
100
0
-100
-200
***
**
**
**
IFNγ
TNFα
IL15
IL17
IL10
TSLP
TGFβ
(I)
Control
SENP7 O/E
SENP7 C992A O/E
Conc. (pg/ml)
1400
1200
1000
800
600
400
200
0
-200
-400
*** ***
** **
** *
*
*
IFNγ
TNFα
IL15
IL17
IL10
TSLP
TGFβ

Figure S2: Related to Figure 3: *(A) Immunoblot showing SENP7 expression in HCT8 cells with SENP7 overexpressed and SENP7 knockdown condition. C2 represents control with Empty flag vector transfection and C3 represents control with scrambled siRNA transfection. (B) Colonic epithelium lysates immunoprecipitated with anti-SENP7 antibodies and probed with anti-NCoR1 and anti-SENP7 antibodies in control and DSS7 colitis mice. Ten percent input lysates showing NCoR1 expression in control and DSS7 (n=3) (C)* Gene enrichment analysis of SENP7 interacting proteins using the immunological database in the Cytoscape platform. *Probability is determined using a binomial statistic for FDR and p-values cutoff of ≤0.05 significance level. (D and E) Graph representing CD4+ T cells and CD8+ T cells frequency in different categories. Labelling: Immune cells: cells from mice MLN, Immune cells+IL15: IL15 treatment to cultured immune cells for 3 days, C1: immune cells co-cultured with CT26 cells transfected with empty Flag vector, SENP7 WT O/E: SENP7 wild type overexpressed CT26 in co-culture setup, SENP7 C992A O/E: catalytic mutant of SENP7. SENP7 WT O/E+Anti-IL15: SENP7 overexpressed epithelial cells with IL15 neutralization. Each plot represents 3 different experiments each performed independently. Results are represented as mean + s.e.m. (F and G) Representative contour plot showing T cells frequency and mean average count of γδ T cells, CD4+ and CD8+ T in different categories.* # denotes mean value count. *(H and I) ELISA for IFNγ, TNFα, IL15, IL17, IL10, TSLP, TGFβ cytokines from the supernatant of control, and SENP7 WT O/E, SENP7 WT O/E + Anti-IL15 and SENP C992A O/E cells described above Results are represented as mean + s.e.m from three independent experiments. Statistics: p-value determined by student t-test and one way ANOVA with Tukey's posttest. *p<0.05, **p< 0.01 and *** p<0.001, ns non-significant.*

Figure S3

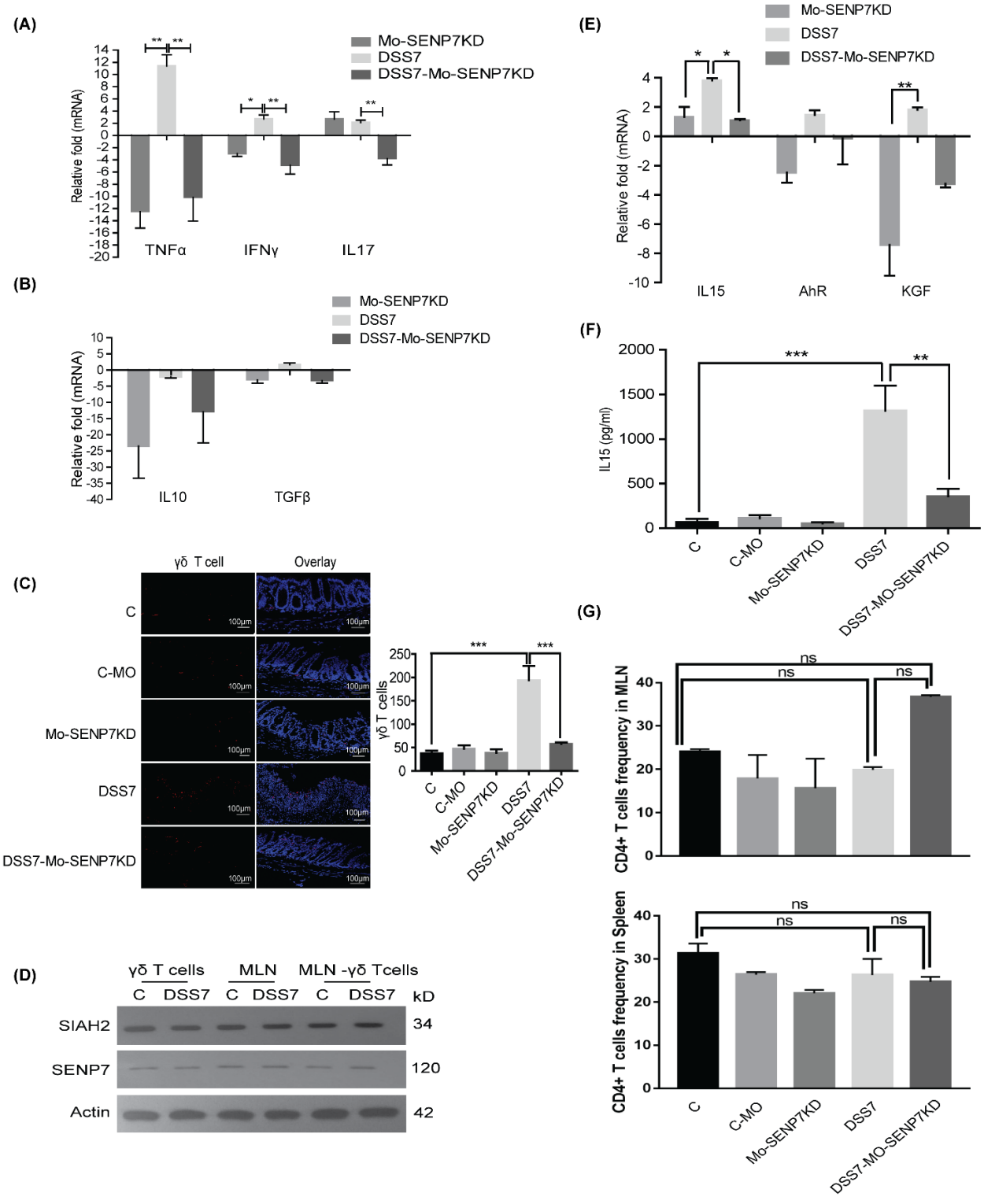

(A)
Mo-SENP7KD
DSS7
DSS7-Mo-SENP7KD
Relative fold (mRNA)
TNFα
IFNγ
IL17
(B)
Mo-SENP7KD
DSS7
DSS7-Mo-SENP7KD
Relative fold (mRNA)
IL10
TGFβ
(C)
γδ T cell
Overlay
C
C-MO
Mo-SENP7KD
DSS7
DSS7-Mo-SENP7KD
100μm
γδ T cells
C
C-MO
Mo-SENP7KD
DSS7
DSS7-Mo-SENP7KD
(D)
γδ T cells
MLN
MLN -γδ Tcells
C DSS7 C DSS7 C DSS7
kD
SIAH2
34
SENP7
120
Actin
42
(E)
Mo-SENP7KD
DSS7
DSS7-Mo-SENP7KD
Relative fold (mRNA)
IL15
AhR
KGF
(F)
IL15 (pg/ml)
C
C-MO
Mo-SENP7KD
DSS7
DSS7-MO-SENP7KD
(G)
CD4+ T cells frequency in MLN
CD4+ T cells frequency in Spleen
ns
C
C-MO
Mo-SENP7KD
DSS7
DSS7-MO-SENP7KD

Figure S3: Related to Figure 4: Mucosal γδ T cells dependent lowering of inflammation in DSS treated SENP7 knockdown mice. *(A and B) qPCR analysis showing expression of IFNγ, TNFα, IL17, TGFβ, IL10 in different categories. Actin was used for normalization (n=3). (C) Representative immunostaining images showing γδ T cells (red color) in intraepithelial region mice colonic sections. Quantification of γδ T cells in all categories is represented as mean+s.e.m (representative of at least 5 different fields) (as described in Figure 4, labeled as C: untreated control, C-MO: Negative control, Mo-SENP7KD: SENP7 knockdown, DSS7: seven days of DSS treatment, DSS7-Mo-SENP7KD: SENP7 knockdown with 7 days DSS treatment), (n=3 in each category). (D)) Immunoblot showing SIAH2 and SENP7 expression in γδ T cells, total immune cells from MLN and remaining immune cells after γδ T cells sorting from untreated control mice and DSS7 mice. Actin was used as a loading control. Immunoblot represents pooled γδ T cells lysates from 5 mice in each group. (E) qPCR gene expression analysis of γδ T cells relevant genes: IL15, Aryl hydrocarbon receptor (AhR) and keratinocytes growth factor (KGF) in the indicated group. The data was calculated as averaged fold change compared to a baseline obtained from values of the control group (untreated mice). β-actin was used for normalization (n=3). (F) ELISA of IL15 from the mucosal collections of mice intestine in the mentioned groups. Results are represented as mean + s.e.m from three independent experiments. (G) Immunophenotyping shows CD4+ T cell and CD8+ T cells distribution in MLN and Spleen in different categories of mice (For flow cytometry, cells were acquired on BD FACS Canto and analyzed on FlowJo Vx0.7and represented in the form of a graph (mean + s.e.m) prepared on Prism v6.01. Each plot represents 3 different experiments each performed independently. Statistical differences were calculated on Prism by performing 2-way ANNOVA with Tukey's test. *p<0.05, **p< 0.01 and ***p <0.001, ns non-significant.*

Figure S4

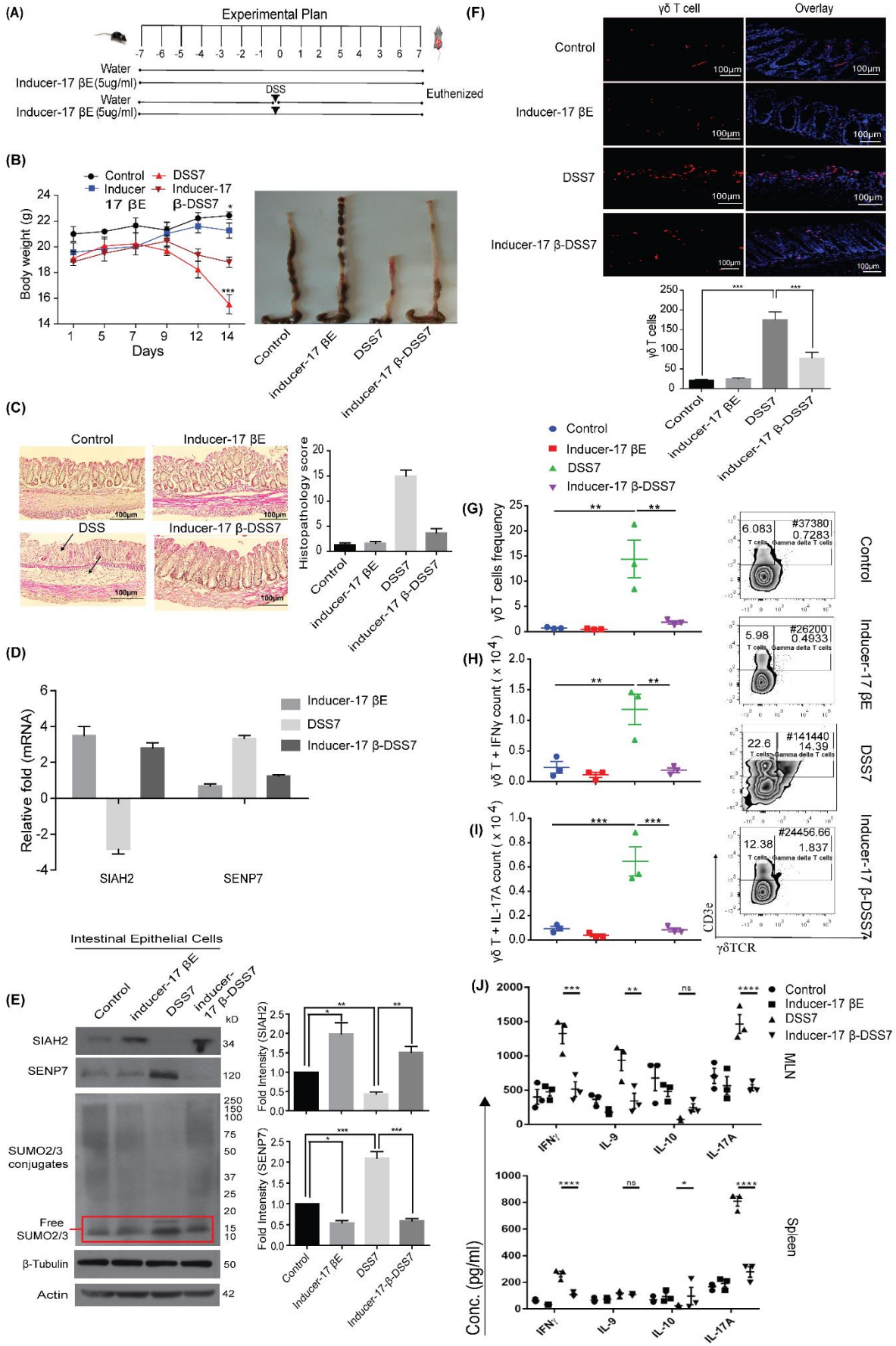

(A)
Experimental Plan
-7 -6 -5 -4 -3 -2 -1 0 1 2 5 4 3 6 7
Water
Inducer-17 βE(5ug/ml)
DSS
Euthenized
Water
Inducer-17 βE (5ug/ml)
(B)
Control
Inducer 17 βE
DSS7
Inducer-17 β-DSS7
Body weight (g)
Days
Control
Inducer-17 βE
DSS7
Inducer-17 β-DSS7
(C)
Control
Inducer-17 βE
DSS
Inducer-17 β-DSS7
100μm
Histopathology score
(D)
Relative fold (mRNA)
Inducer-17 βE
DSS7
Inducer-17 β-DSS7
SIAH2
SENP7
(E)
Intestinal Epithelial Cells
Control
inducer-17 βE
DSS7
Inducer-17 β-DSS7
kD
SIAH2
SENP7
SUMO2/3 conjugates
Free SUMO2/3
β-Tubulin
Actin
Fold Intensity (SIAH2)
Fold Intensity (SENP7)
(F)
γδ T cell
Overlay
Control
Inducer-17 βE
DSS7
Inducer-17 β-DSS7
100μm
γδ T cells
(G)
γδ T cells frequency
(H)
γδ T + IFNγ count (x 10 4)
(I)
γδ T + IL-17A count (x 10 4)
6.083
#37380
0.7283
5.98
#26200
0.4933
22.6
#141440
14.39
12.38
#24456.66
1.837
CD3e
γδTCR
(J)
Control
Inducer-17 βE
DSS7
Inducer-17 β-DSS7
MLN
Spleen
Conc. (pg/ml)
IFNγ
IL-9
IL-10
IL-17A
ns

Figure S4: Related to Figure 4 *(A) Schematic representation of SIAH2 overexpressed mice using 17 Beta Estradiol (SIAH2 Inducer). (n=3) (B) Daily curve of mice weight change and gross morphology of mice colon in different categories. C-untreated control, Inducer 17-βE: Mice given 17 β-Estradiol in drinking water. Inducer 17-β-DSS7: Inducer 17-β-DSS7 treatment followed by 7 days DSS treatment (n=3) (C) Left: Histopathology showing inflammation in DSS7 treated mice as marked by the infiltration of immune cells but in inducer 17-β-DSS7 group mice there no such sign of inflammation. Right: Histopathology score calculated on the basis of parameter PMN migration, Ulceration, Epithelial erosion, goblet cell loss, and crypt abscesses (D) qPCR analysis of SIAH2 and SENP7 in mice colonic epithelium. β-actin was used for normalization. Data represented as mean+s.e.m. (E) Immunoblot showing SIAH2, SENP7 and SUMO2/3 expression in control and Inducer 17-β-estradiol treated mice. Actin and β-tubulin used as a loading control. (F) Immunostaining images showing γδ T cells (red color) in intraepithelial region mice colon in indicated categories. (n=3) (G-I) Representative graph and contour plot showing immunophenotyping and intracellular cytokines staining of γδ T cells, IFNγ and IL17A in intraepithelial lymphocytes of mice in all categories (n=3). For flow cytometry cells were acquired on BD FACS Canto and analyzed on FlowJo and represented in the form of a graph (mean+s.e.m values) prepared Statistical significance is calculated using one way ANOVA with Tukey's posttest. **< 0.01 and **** <0.001, ns non-significant. (J) ELISA showing different secreted cytokines IFNγ, IL17, IL9 and IL10 in cultured immune cells from MLN and spleen (n=3). Results represented as mean+s.e.m. *p<0.05, **p< 0.01 and ***p <0.001, ns non-significant.*

Figure S5

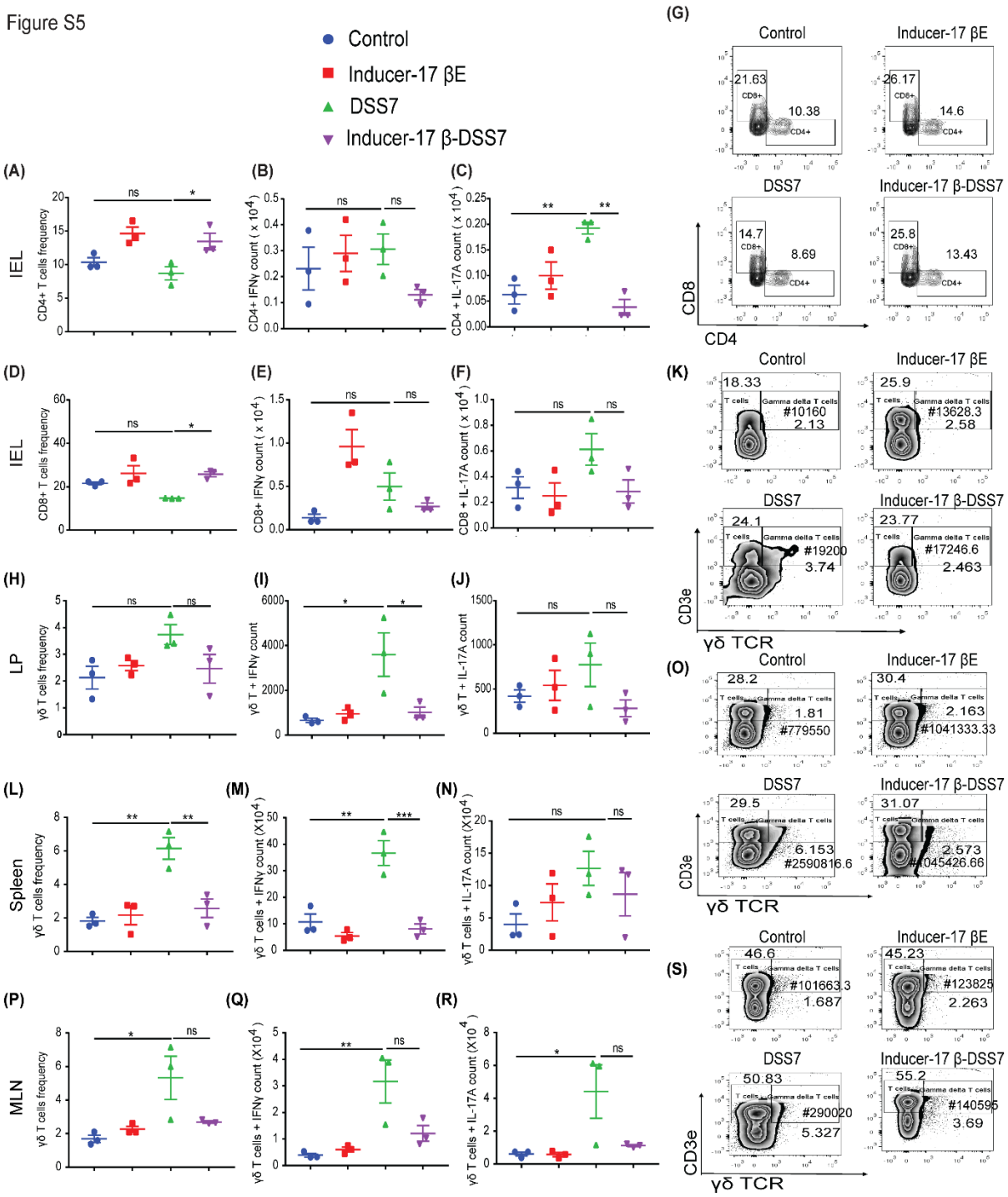

Control
Inducer-17 βE
DSS7
Inducer-17 β-DSS7
(A)
IEL
CD4+ T cells frequency
(B)
CD4+ IFNγ count (x 10^4)
(C)
CD4 + IL-17A count (x 10^4)
(D)
IEL
CD8+ T cells frequency
(E)
CD8+ IFNγ count (x 10^4)
(F)
CD8 + IL-17A count (x 10^4)
(H)
LP
γδ T cells frequency
(I)
γδ T + IFNγ count
(J)
γδ T + IL-17A count
(L)
Spleen
γδ T cells frequency
(M)
γδ T cells + IFNγ count (X10^4)
(N)
γδ T cells + IL-17A count (X10^4)
(P)
MLN
γδ T cells frequency
(Q)
γδ T cells + IFNγ count (X10^4)
(R)
γδ T cells + IL-17A count (X10^4)
ns
*
**
***
(G)
Control
Inducer-17 βE
21.63
CD8+
10.38
CD4+
26.17
14.6
DSS7
Inducer-17 β-DSS7
14.7
8.69
25.8
13.43
CD8
CD4
(K)
Control
Inducer-17 βE
18.33
T cells
Gamma delta T cells
#10160
2.13
25.9
#13628.3
2.58
DSS7
Inducer-17 β-DSS7
24.1
#19200
3.74
23.77
#17246.6
2.463
CD3e
γδ TCR
(O)
Control
Inducer-17 βE
28.2
1.81
#779550
30.4
2.163
#1041333.33
DSS7
Inducer-17 β-DSS7
29.5
6.153
#2590816.6
31.07
2.573
#1045426.66
CD3e
γδ TCR
(S)
Control
Inducer-17 βE
46.6
#101663.3
1.687
45.23
#123825
2.263
DSS7
Inducer-17 β-DSS7
50.83
#290020
5.327
55.2
#140595
3.69
CD3e
γδ TCR

***Figure S5: Related to Figure 4:* Frequency of various T cell populations in different group mice.** *(A-G)Population frequencies of different T cells in intraepithelial lymphocytes (IEL) of different categories. C-untreated control, Inducer 17-β-E: Mice given 17 β-Estradiol in drinking water. Inducer 17-β-DSS7: Inducer 17-β-DSS7 treatment followed by 7 days DSS treatment (A) CD4+T cells (B) CD4 + IFNγ T cells (C) CD4 + IL17A T cells (D) CD8+ T cell (E) CD8 + IFNγ T cells (F) CD8+ IL17A T cells (G) Contour plot showing CD4+ and CD8+ T cells in IELs. (H-J) Graph representing γδ T cells, γδ T cell+ IFNγ and γδ T cells+ IL17A population in different categories in Lamina propria (LP). (K) Contour plot representing γδ T cells in Lamina propria (L-N) Graph representing γδ T cells population along with intracellular staining of IFNγ and IL17A in the spleen (O) Contour plot representing γδ T cells population in the spleen. (P-R) Graph representing γδ T cells population along with intracellular staining of IFNγ and IL17A in mesenteric lymph nodes (MLN). (S) Contour plot representing γδ T cells population in MLN. For flow cytometry, cells were acquired on BD FACS Canto and analyzed on FlowJo Vx0.7and represented in the form of graph (mean + s.e.m). # denotes mean value count. Each plot represents 3 different experiments each performed independently. Statistical differences were calculated on Prism by performing 2-way ANNOVA with Tukey's test. *p<0.05, **p< 0.01 and ***p <0.001, ns non-significant*

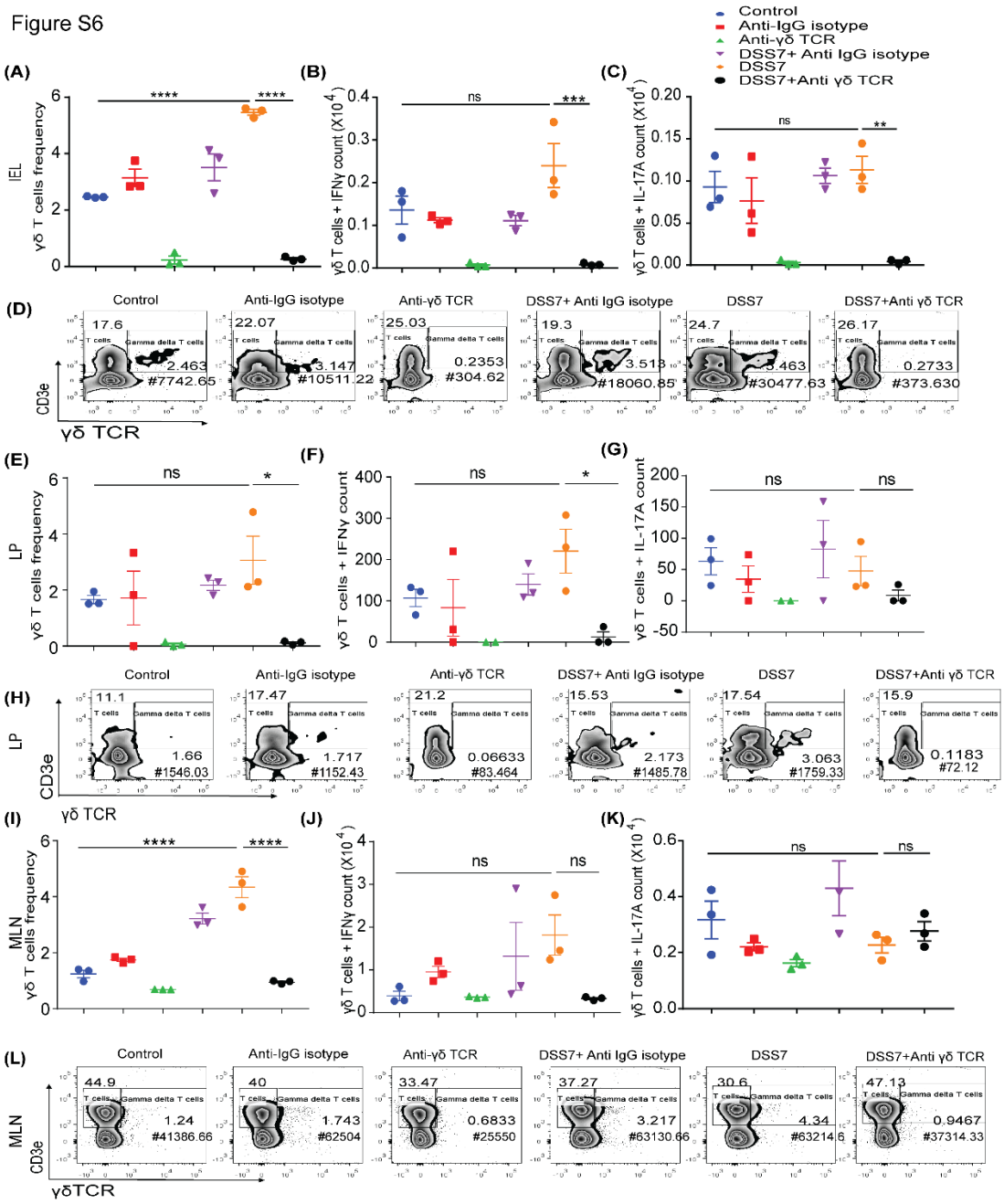


Figure S6: Related to Figure 5: Frequency of γδ T cell population in different group mice. *Categories are labeled as: C-untreated control, Inducer 17-βE: Mice given 17 β-Estradiol in drinking water. Inducer 17-β-DSS7: Inducer 17-β-DSS7 treatment followed by 7 days DSS treatment (A-C) Graph showing γδ T cells in intraepithelial lymphocytes (IELs) along with intracellular staining of IFNγ and IL17A. (D) Contour plot showing γδ T cells frequency and mean count values in all categories. (E-G) Graph representing γδ T cells, γδ T cells+ IFNγ, γδ T cells+ IL17A population in all categories in Lamina propria (LP) (H) Contour plot representing γδT cells frequency and mean count in LP. (I-K) Graph representing γδ T cells, γδ T cells+ IFNγ, γδ T cells+ IL17A population in MLN (L) Contour plot representing γδ T cells population in all categories. # denotes mean value count. For flow cytometry, cells were acquired on BD FACS Canto and analyzed on FlowJo Vx0.7and represented in the form of graph (mean + s.e.m).*

*Each plot represents 3 different experiments each performed independently. Statistical differences were calculated on Prism by performing 2-way ANNOVA with Tukey's test. *p<0.05, **p< 0.01 and ***p <0.001, ns non-significant*

Figure S7

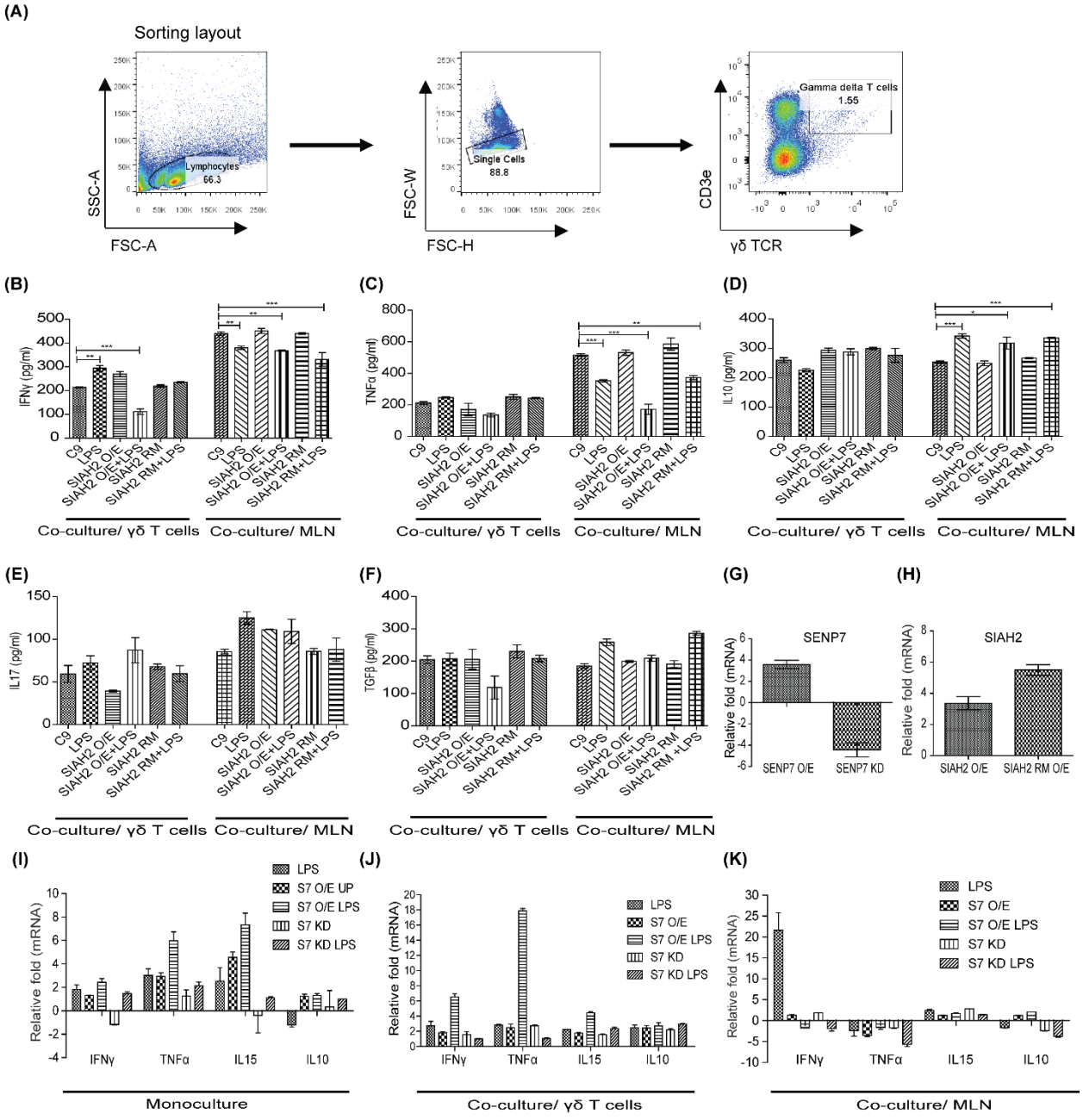


Figure S7: Related to Figure 6*: (A) Sorting layout for isolating γδ T cells from spleen and mesenteric lymph nodes of mice. Spleen and lymph nodes of mice were processed into single-cell suspension and then stained with flourescenated anti-mouse γδ TCR and anti-mouse CD3e antibody after RBC lysis. The stained cells were incubated for 30 minutes and thereafter washed twice before sorting them on BD FACS Aria III. Width (W) vs Height (H) gating was done to remove doublet populations. (B-F) ELISA showing secreted cytokines (B) IFNγ, (C) TNFα (D) IL10 (E)*

*IL17 and (F) TGFβ in SIAH2 and SIAH2 Ring Mutant (SIAH2 RM) overexpressed CT26 epithelial cells in co-culture setup. C9: control epithelial cells with empty vector GFP. (G and H) qPCR showing SENP7 and SIAH2 expression in indicated samples. GAPDH used for normalization. (I-K) qPCR showing gene expression of cytokines in (I) monoculture, (J) co-culture with γδ T cells (K) co-culture with total immune cells from MLN. GAPDH used for normalization. Each plot represents 3 different experiments each performed independently. Statistical differences were calculated on Prism by performing 2-way ANNOVA with Tukey's test. *p<0.05, **p< 0.01 and ***p <0.001, ns non-significant.*

Table S4: List of primers used in qPCR (Related to STAR methods details Quantitative real-time PCR)

Gene Name	**Forward Primer (Direction 5'-3')**	**Reverse Primer (Direction 5'-3')**
Mouse SENP7	AGCCTTGGTTTCTGTTGCTT	TGCTGACACACAGGCTTACA
Human SENP7	GTCGTCTCACTGGTATCTCGCA	TCGGTACTTTGGGSSTCCTCTGC
Mouse SIAH2	CACTGACAGCATGTAGATATCGTG	CTGTTTCCCTGTAAGTATGCTACC
Human SIAH2	AGGTTGCCCTCTGCCGATA	ACATAGGTGAGTGGCCAAATCTC
Mouse β-Actin	TCTACGAGGGCTATGCTCTCC	GGATGCCACAGGATTCCATAC
Human GAPDH	CTCACCGGATGCACCAATGTT	CGCGTTGCTCACAATGTTCAT
Mouse TNF-α	CATCTTCTCAAAATTCGAGTGACAA	TGGGAGTAGACAAGGTACAACCC
Mouse IFN-γ	TCTTGGCTTTGCAGCTCTTC	TGTTGCTGATGGCCTGATTG
Mouse IL-10	TGCACTACCAAAGCCACAAG	TCAGTAAGAGCAGGCAGCAT
Mouse TGF-β	TAATGGTGGACCGCAACAACGC	GACGGAATACAGGGCTTTCG
Mouse KGF	CGCAAATGGATACTGACACG	GGGCTGGAACAGTTCACACT
Mouse IL15	CATCCATCTCGTGCTACTTGTGTT	CATCTATCCAGTTGGCCTCTGTTT
Mouse AhR	ACCAGAACTGTGAGGGTTGG	TCTGAGGTGCCTGAACTCCT