

ERAP1 deficient mice have reduced Type 1 regulatory T cells and develop skeletal and intestinal features of Ankylosing Spondylitis.

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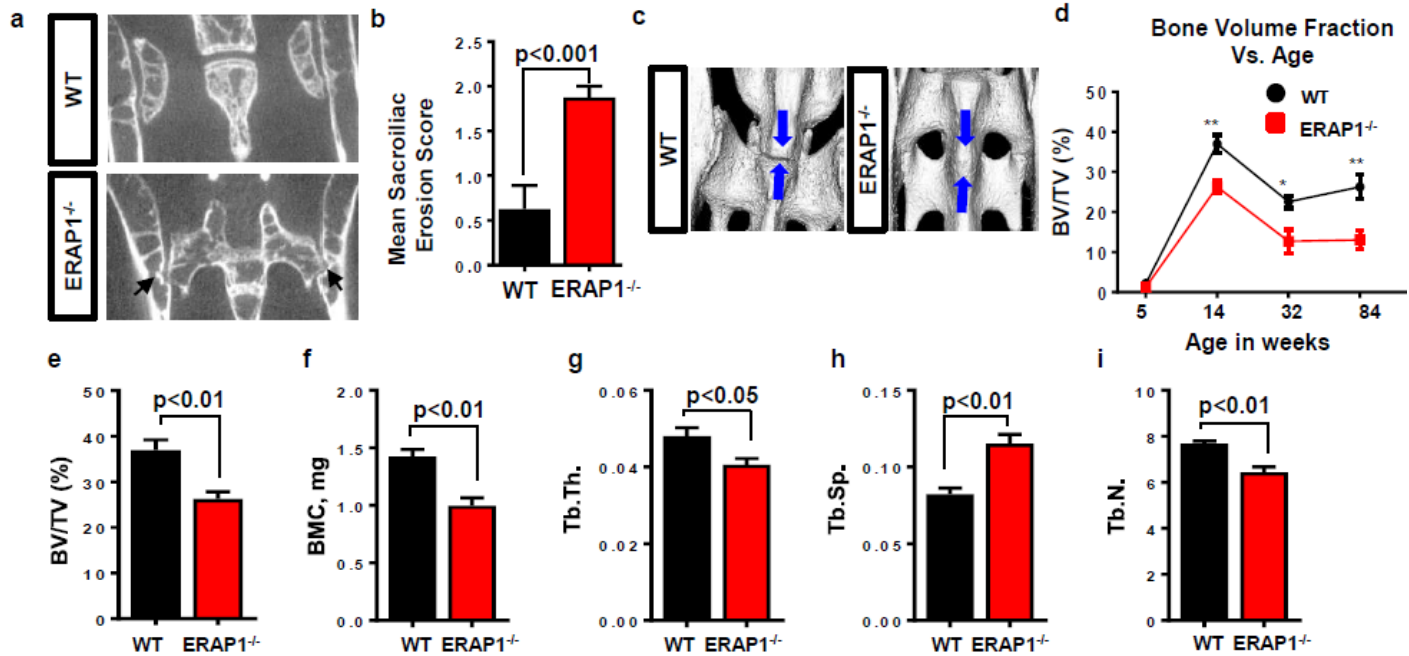
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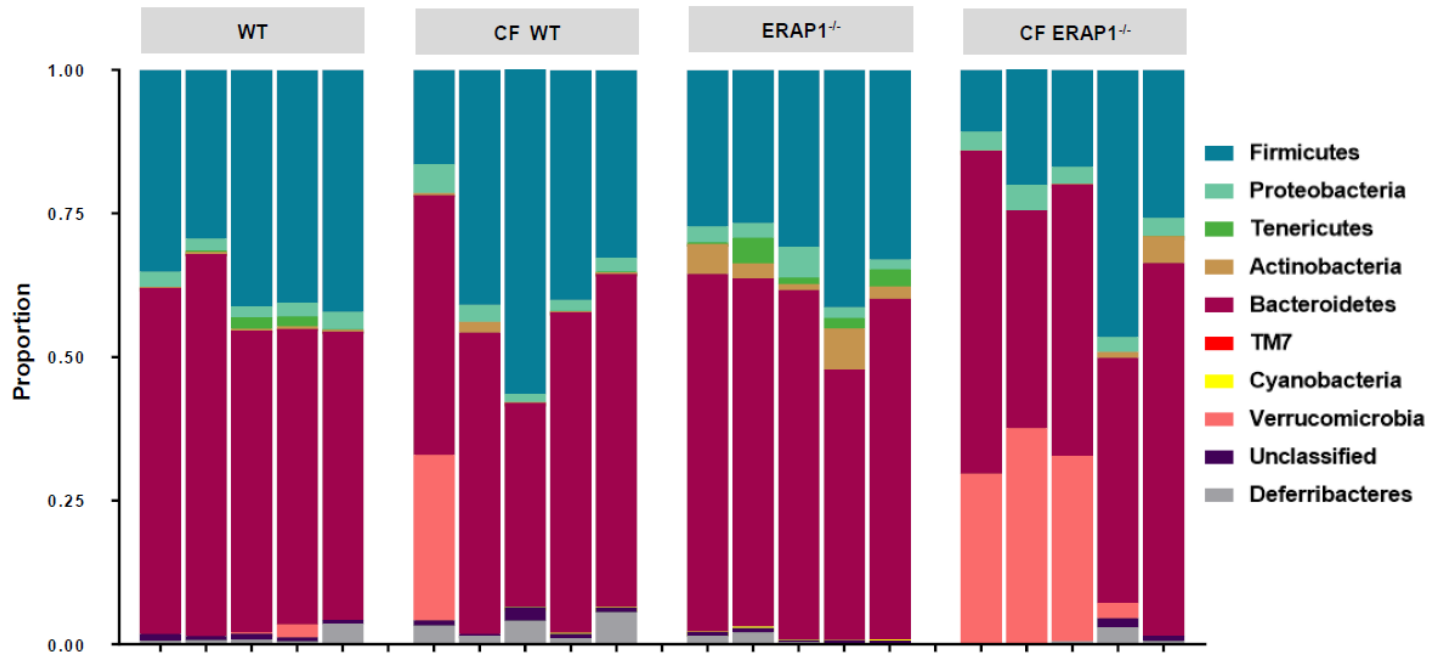
Supplemental Figures

Supplemental Figure 1



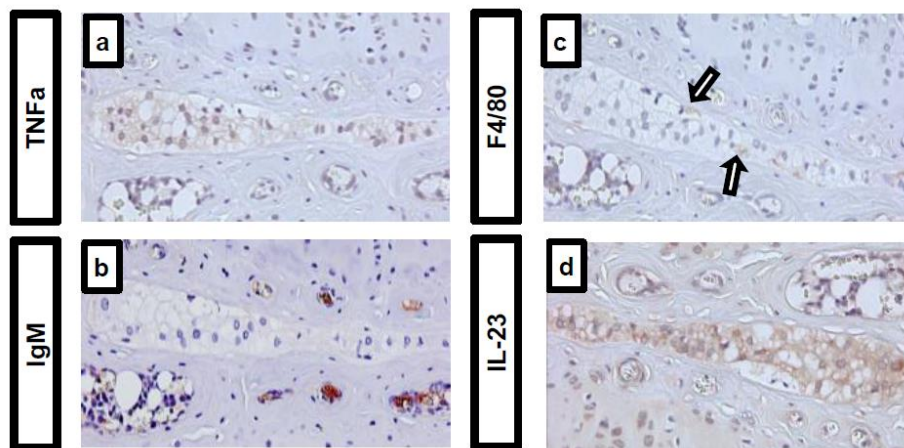
Supplemental Figure 1. μ CT analysis of sacral region. The spines from 84-week-old female ERAP1^{-/-} and WT female mice (n=8) were harvested and analyzed with μ CT. **(a)** Representative 2D radiograph demonstrating erosions (**black arrows**) at the SI joint. **(b)** SI joints were assessed for erosions bilaterally, averaged per side and graphed. **(c)** Representative images demonstrating calcification of the anterior longitudinal ligament between L6 and S1 in ERAP1^{-/-} animals, depicted with **blue arrows**. The spines from 14-week-old ERAP1^{-/-} and WT female mice (n=5) were harvested and analyzed with μ CT. **(d)** Graph comparing BV/TV% of the trabecular bone of S1 vertebra between WT and ERAP1^{-/-} mice at various ages. Bar graphs demonstrating BV/TV **(e)**, BMC **(f)**, Tb.Th. **(g)**, Tb.Sp. **(h)**, and Tb.N. **(i)** of the trabecular bone of S1 vertebra. Values on graphs are the mean \pm SEM. p values calculated by student's t-test, where * - p<0.05 and ** - p<0.01 compared to age-matched WT mice.

Supplemental Figure 2



Supplemental Figure 2. Relative abundance of phyla in WT, CF WT, ERAP1^{-/-} and CF ERAP1^{-/-} fecal samples. 16s rRNA phenotyping was performed with illumina on fecal samples of 14-week-old male and female mice (n=5). Stacked bar graph showing relative abundance of phyla in fecal samples of WT, CF WT, ERAP1^{-/-}, and CF ERAP1^{-/-} mice.

Supplemental Figure 3



Supplemental Figure 3. Histopathological analysis of the ankylosed intervertebral joints from CF ERAP1^{-/-} mice. 14-week-old cross-fostered ERAP1^{-/-} female and male mice were

sacrificed. Their spines were harvested, fixed, decalcified and stained. Representative immunohistochemistry images of L6/S1 intervertebral disc at 20X magnification for TNF α (a), IgM (b), F4/80 (c), with **arrows** pointing at F4/80+ macrophages, and IL-23 (d).

Supplemental Table 1

Supplemental Table 1. Genera that were significantly different between WT and ERAP1 ^{-/-} mice.			
Increased in ERAP1 ^{-/-}			
Genus	WT vs ERAP1 ^{-/-}	ERAP1 ^{-/-} vs CF ERAP1 ^{-/-}	WT vs CF ERAP1 ^{-/-}
Bacteroides	****	NS	**
Clostridiales_unclassified	*	**	NS
Odoribacter	***	***	NS
Parabacteroides	*	NS	*
Prevotella	****	*	NS
YS2_unclassified	*	*	NS
Decreased in ERAP1 ^{-/-}			
Genus	WT vs ERAP1 ^{-/-}	ERAP1 ^{-/-} vs CF ERAP1 ^{-/-}	WT vs CF ERAP1 ^{-/-}
Christensenellaceae_unclassified	*	NS	*
Lachnospiraceae_unclassified	**	NS	NS
S24.7_unclassified	*	NS	NS

Table summarizing differences of the genus-level OTUs that were found to be significantly increased (top half of the table) or decreased (bottom half of the table) in fecal samples of ERAP1^{-/-} mice compared to WT. Differences between ERAP1^{-/-} and CF ERAP1^{-/-} and WT and CF ERAP1^{-/-} mice are summarized in columns 2 and 3, respectively, with arrows representing direction of change in CF ERAP1^{-/-} fecal samples. p values calculated by student's t-test, where * - p<0.05, ** - p<0.01, *** - p<0.001, **** - p<0.0001 and NS - not significant.

Supplemental Table 2. Details of antibodies used for Immunohistochemistry staining.

Primary Antibody	Ab Vendor:	Pretreatment:	Primary:	Staining System:
Rabbit anti – IL23 Polyclonal	Abcam #115759 Cambridge, MA	0.03% Pronase E in TBS for 10 minutes at 37°C	1:100 in NAD – 2 Hours	ProMark Rabbit on Rodent HRP Polymer™ - 30 minutes AEC – 5 minutes CATHE Hematoxylin 1:10 – 1 minute
Rat anti – F4/80 Monoclonal	Bio-Rad # <i>MCA497G</i> Hercules, CA	Overnight Retrieval overnight at 56°C	1:100 in NAD Overnight	ProMark Rat on Mouse HRP Polymer™ 15 minutes – Probe 15 minutes – Polymer AEC – 5 minutes CATHE Hematoxylin 1:10 – 1 minute
Rabbit anti – IgM Polyclonal	ThermoFisher #61-6800 <i>Rockford, IL</i>	Overnight Retrieval overnight at 56°C	1:300 in NAD – 60 minutes	ProMark Rabbit on Rodent HRP Polymer™ - 30 minutes AEC – 5 minutes CATHE Hematoxylin 1:5 – 5 minutes
Rabbit anti-TNF alpha Polyclonal	Abcam #6671 Cambridge, MA	Overnight Retrieval overnight at 56°C	1:100 in NAD – 2 Hours	ProMark Rabbit on Rodent HRP Polymer™ - 30 minutes AEC – 5 minutes CATHE Hematoxylin 1:10 – 1 minute