

# Dipeptidyl Peptidase Inhibition Enhances CD8 T Cell Recruitment and Activates Intrahepatic Inflammasome in a Murine Model of Hepatocellular Carcinoma

James M. Henderson <sup>1,2</sup>, Michelle S. W. Xiang <sup>1</sup>, Jiali Carrie Huang <sup>1</sup>, Stefanie Wetzel <sup>1</sup>, Linxuan Jiang <sup>1</sup>, Jack H. Lai <sup>3</sup>, Wengen Wu <sup>3</sup>, James G. Kench <sup>4</sup>, William W. Bachovchin <sup>3</sup>, Ben Roediger <sup>1</sup>, Geoffrey W. McCaughan <sup>1,5</sup>, Hui Emma Zhang <sup>1,\*</sup> and Mark D. Gorrell <sup>1,\*</sup>

<sup>1</sup> Centenary Institute, Faculty of Medicine and Health, The University of Sydney, Camperdown, NSW 2006, Australia; jhen7621@gmail.com (J.M.H.); m.xiang@centenary.org.au (M.S.W.X.); jhua9515@uni.sydney.edu.au (J.C.H.); christian.stefanie@yahoo.com (S.W.); ljia7302@uni.sydney.edu.au (L.J.); ben.roediger@novartis.com (B.R.); g.mccaughan@centenary.org.au (G.W.M.)

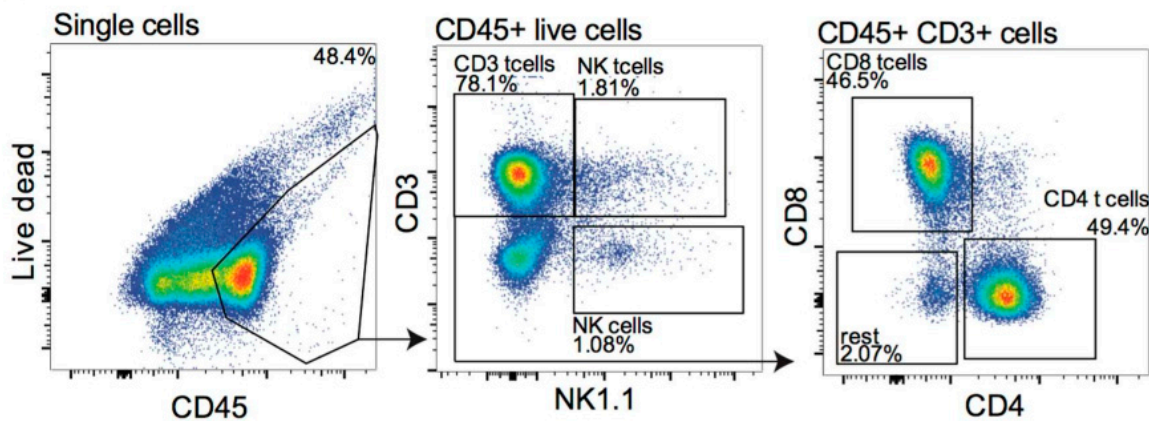
<sup>2</sup> Institute for Cardiovascular Prevention, Ludwig-Maximilians-Universität, D-80336 Munich, Germany

<sup>3</sup> Sackler School of Graduate Biomedical Science, Tufts University, Boston, MA 02111, USA; jack.lai@tufts.edu (J.H.L.); WenGen.Wu@tufts.edu (W.W.); william.bachovchin@tufts.edu (W.W.B.)

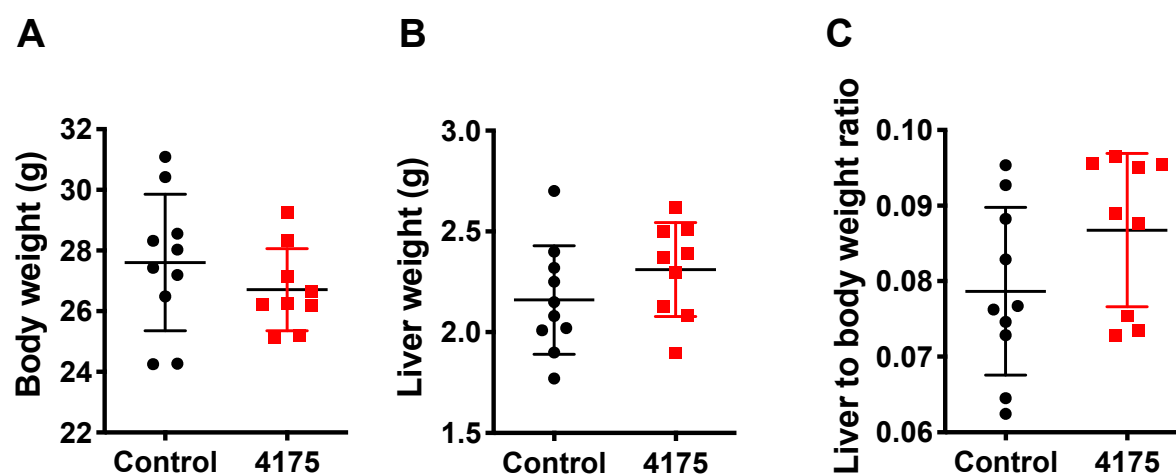
<sup>4</sup> Tissue Pathology & Diagnostic Oncology, NSW Health Pathology, Royal Prince Alfred Hospital, Camperdown, NSW 2050, Australia; James.Kench@sydney.edu.au

<sup>5</sup> A.W. Morrow Gastroenterology and Liver Centre, Royal Prince Alfred Hospital, Camperdown, NSW 2050, Australia

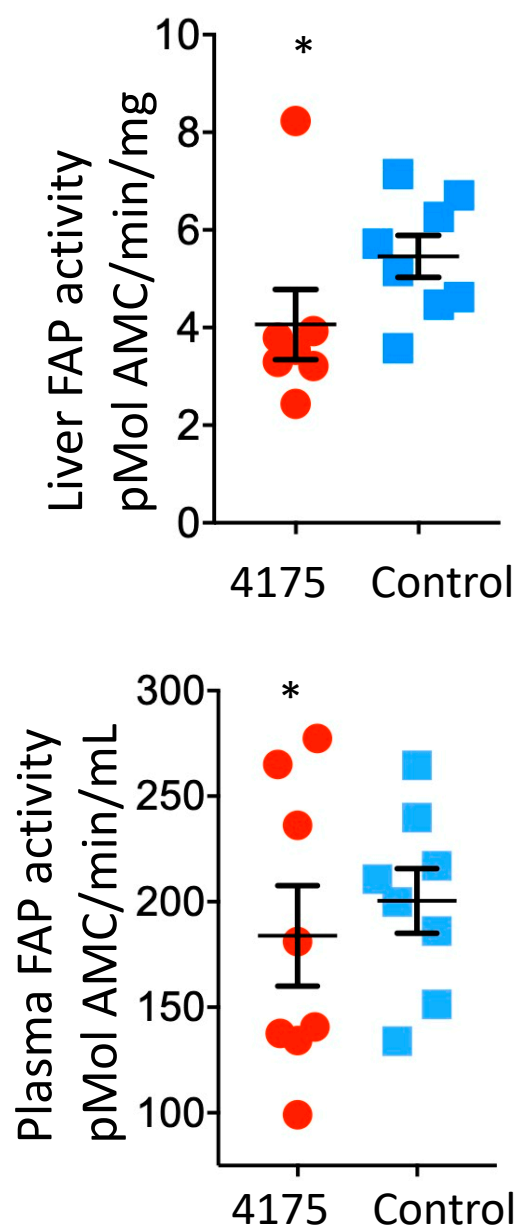
\* Correspondence: e.zhang@centenary.org.au (H.E.Z.); m.gorrell@centenary.org.au (M.D.G.); Tel.: +61-2-95656156 (M.D.G.)



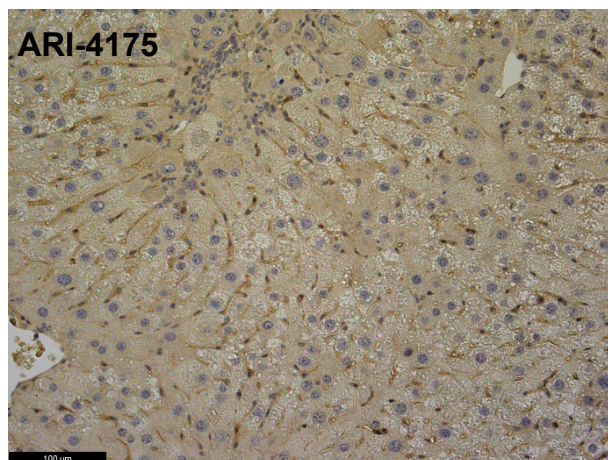
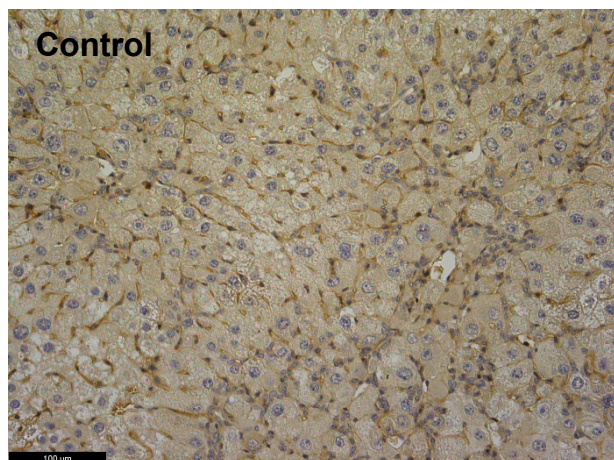
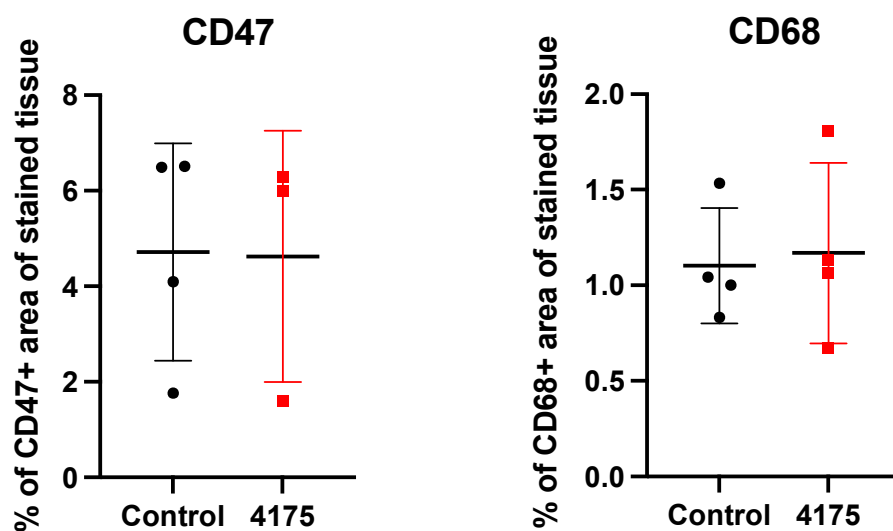
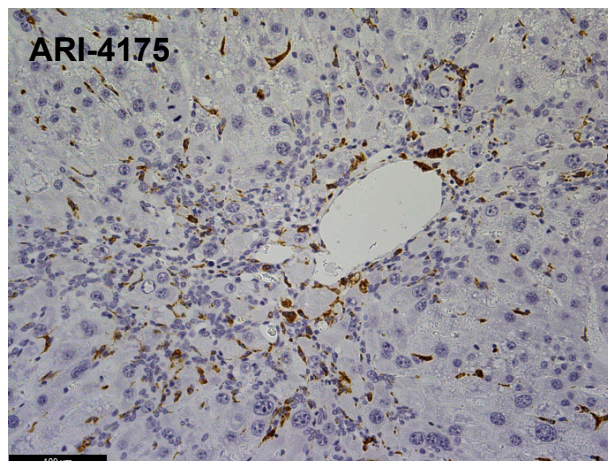
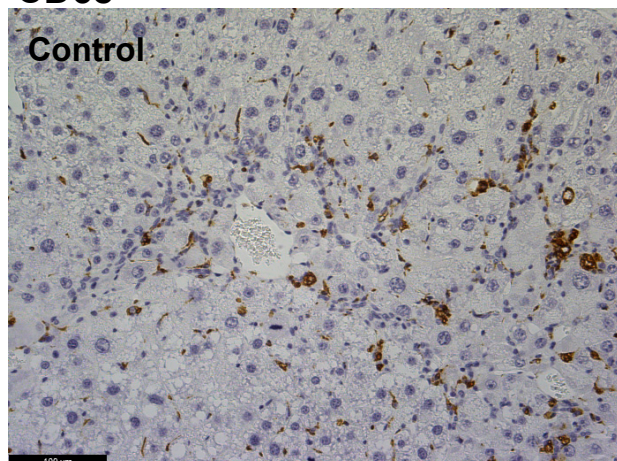
**Supplementary Figure S1.** Gating strategy for putative T cell and natural killer (NK) cell populations. Number adjacent to each outlined area is positive cells as percentage of each parent population.



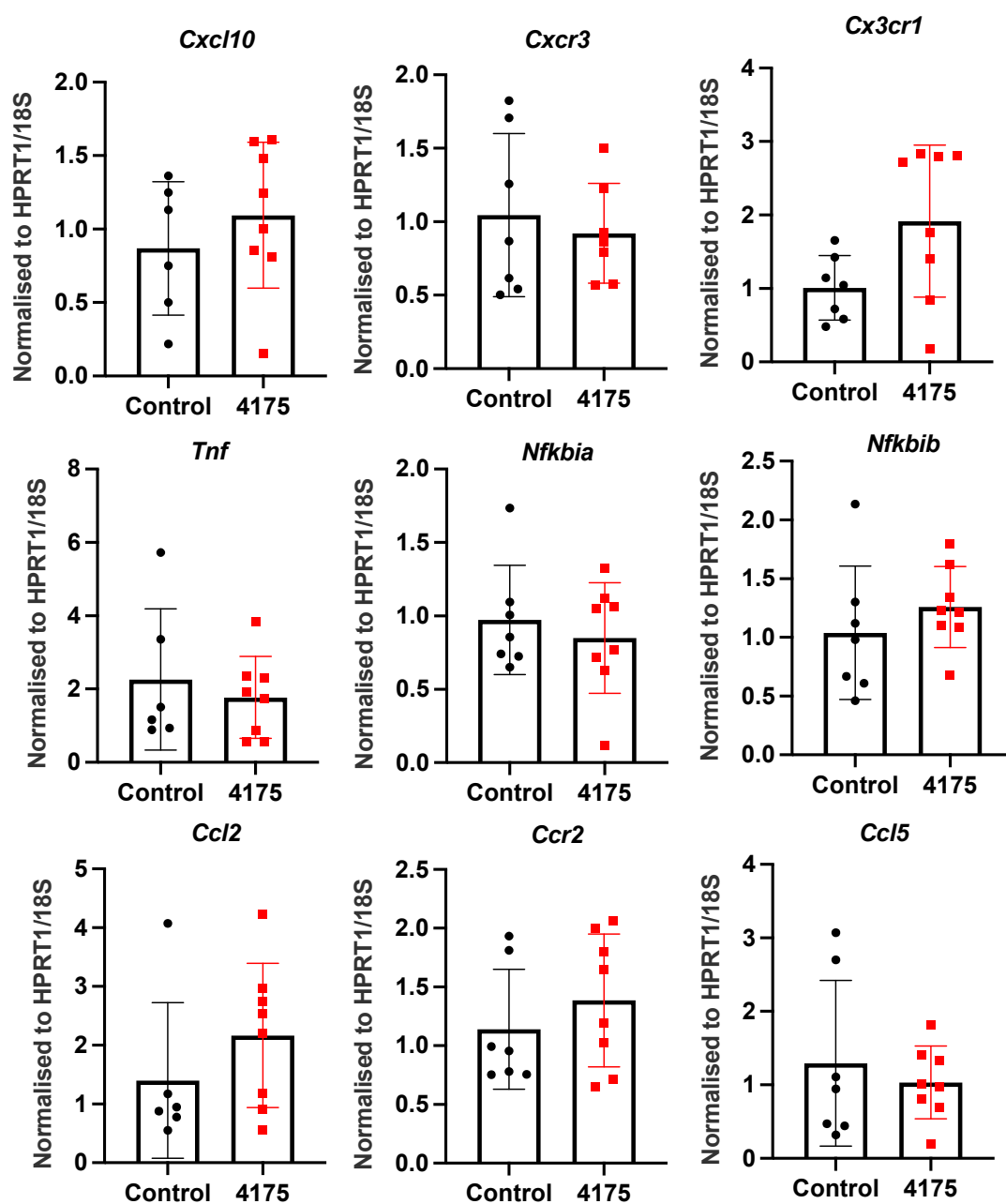
**Supplementary Figure S2.** Bodyweight and liver weight at 22 weeks of age. Mouse body weight (**A**), mouse liver weight (**B**), and liver to body weight ratio (**C**). Mean  $\pm$  SD, n = 9-10 per group. Mann-Whitney U test.

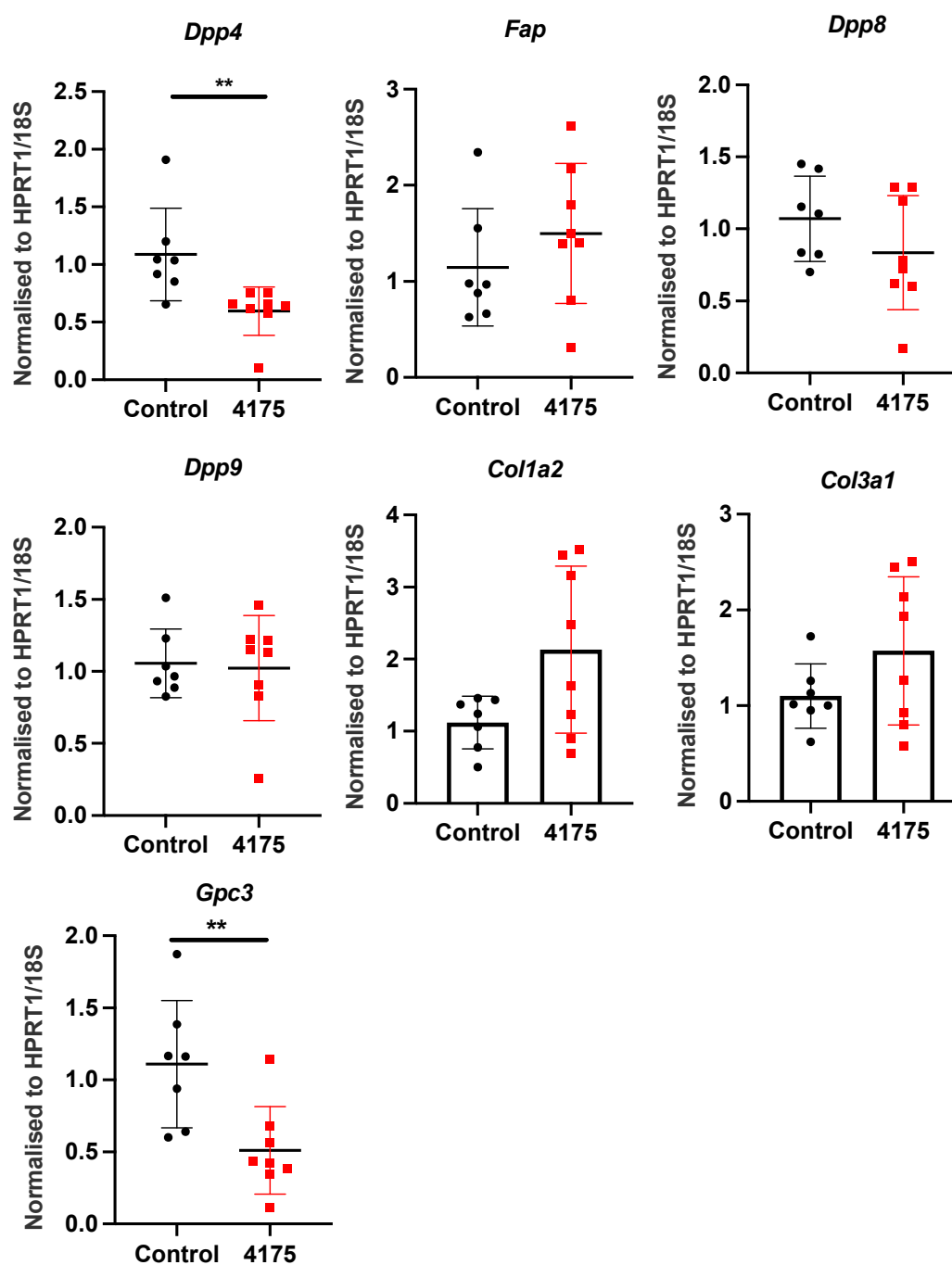


**Supplementary Figure S3.** FAP enzymatic activities in liver and blood plasma at 22 weeks of age. Mean  $\pm$  SD,  $n = 9$ -10 per group. Mann-Whitney U test, \*  $p < 0.05$ .

**CD47****CD68**

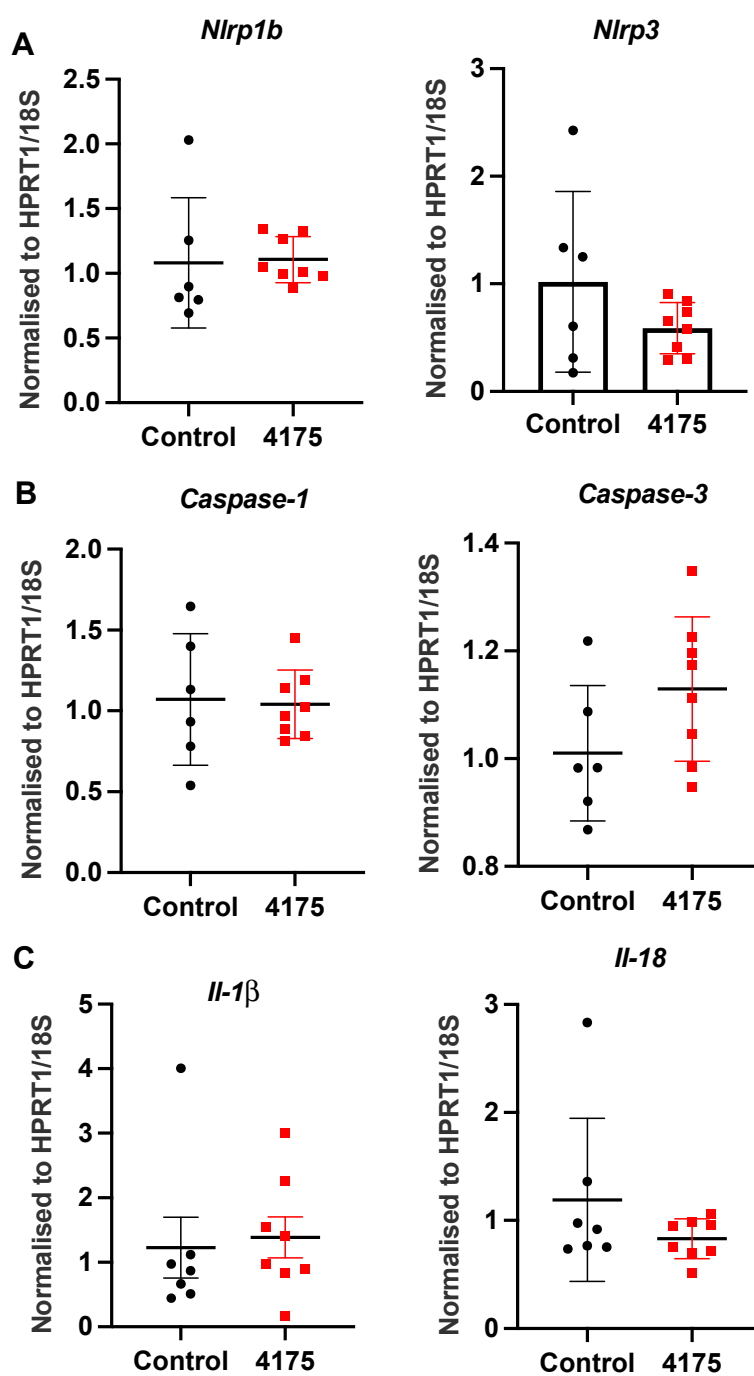
**Supplementary Figure S4.** Assessment of CD47 and CD68 immunostains following ARI-4175 treatment. Most cells were CD47<sup>+</sup> but a sinusoidal pattern was evident. CD68 immunopositivity appeared restricted to cells of macrophage/ dendritic cell morphology. Representative immunohistochemistry images are shown. The stained area was measured and divided by total tissue area to calculate proportion of positive staining. Mean ± SD, n = 3-4 per group.





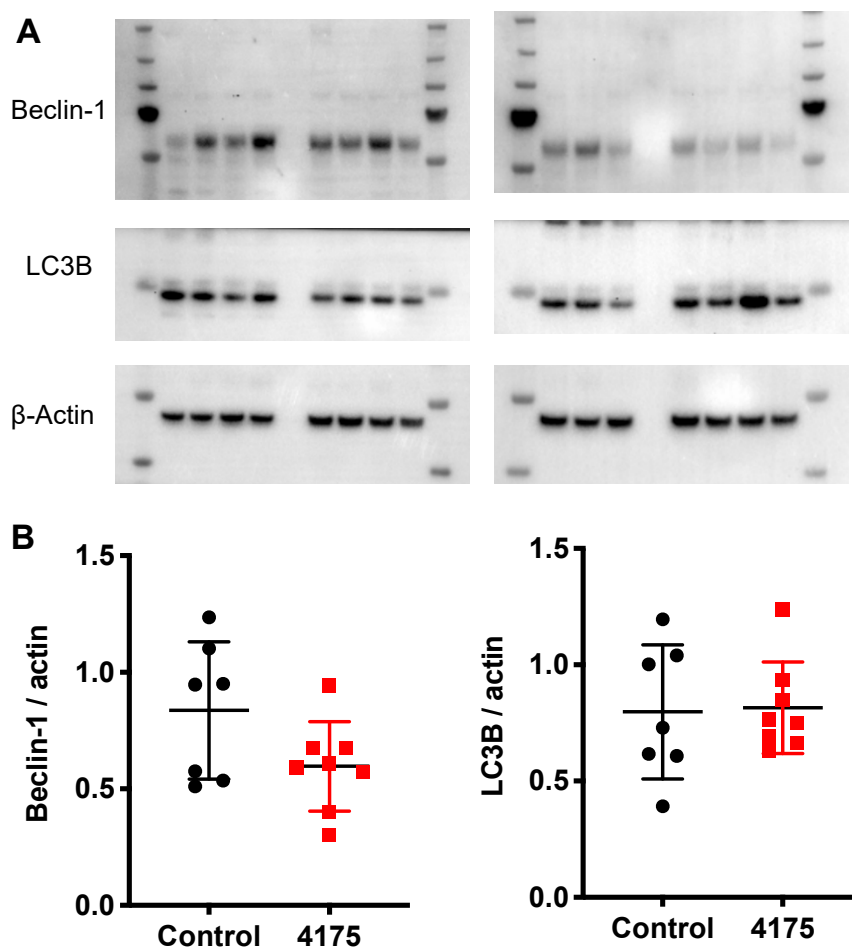
**Supplementary Figure S5.** Intrahepatic gene expression levels of *Cxcl10*, *Cxcr3*, *Cxcr3r1*, *Tnf- $\alpha$* , *Nfkb1a*, *Nfkb1b*, *Ccl2*, *Ccr2*, *Ccl5*, *Dpp4*, *Fap*, *Dpp8*, *Dpp9*, *Col1a2*, *Col3a1* and *Gpc3* in control and ARI-4175 treated mice. Gene expression was normalised to *Hprt1/18S*. Mean  $\pm$  SD, n = 7-8 per group. Mann-Whitney U test, p values \* < 0.05, \*\* < 0.01.



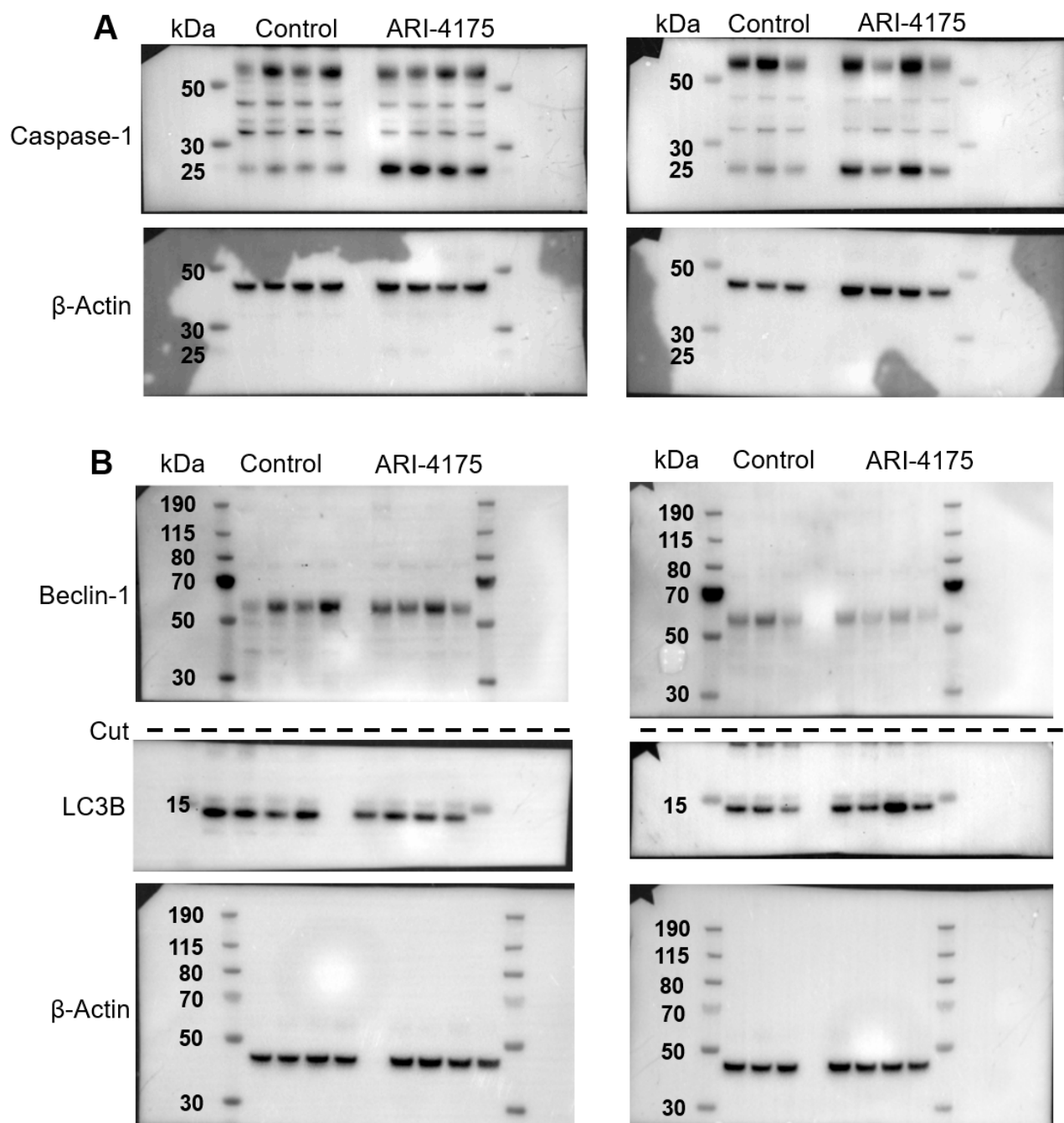


**Supplementary Figure S6.** Assessment of inflammasome pathway in control and ARI-4175 treated HCC livers at the mRNA level. The gene expression levels of *Nlrp1b*, *Nlrp3* (A), *pro-caspase-1*, *pro-caspase-3* (B), and *Il-1 $\beta$* , *Il-18* (C) were normalised to *Hprt1/18S*. Mean  $\pm$  SD, n = 7-8 per treatment group. Mann-Whitney U test.





**Supplementary Figure S7.** Assessment of autophagy pathway components in control and ARI-4175 treated HCC livers. Immunoblotting (A) and densitometry (B) for autophagy markers Beclin-1 and LC3B were quantified in liver lysates.  $\beta$ -actin was the loading control. Mean  $\pm$  SD,  $n = 7$ -8 per group. Mann-Whitney U test.



**Supplementary Figure S8.** Full-length immunoblots for (A) Caspase-1 and (B) Autophagy markers Beclin-1 and LC3B in control and ARI-4175 treated HCC liver lysates.  $\beta$ -actin was the loading control.  $n = 7$  for control and  $n = 8$  for ARI-4175 treatment.