

1 **Supplementary file**

2 **The tumor promoter cysteinyl leukotriene receptor 1 regulates PD-L1**  
3 **expression in colon cancer cells via the Wnt/ $\beta$ -catenin signalling axis**

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9 Running title: CysLT<sub>1</sub>R regulates PD-L1 expression in colon cancer.

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1 **Figure legends**

2 **Supplementary fig. S1**

3 **A**, Bar graphs showing alteration in transcript level expression of *CD274*, *CYSLTR1*, and  
4 *CTNNB1* in HT-29 colon cancer cells stimulated with IFN $\gamma$  (50 ng/mL) for 24 h.  
5 Mean  $\pm$  SEM. \*\*\*P < 0.001, two-tailed unpaired t-test.

6

7 **Supplementary fig. S2**

8 XY scatter plots showing the correlation between *CYSLTR1*, and *IFNG* transcript levels in the  
9 **A**, TCGA-COAD (n = 328), and **B**, GSE39582 (n = 585) datasets of colon cancer patients.

10 Immunofluorescence images showing PD-L1 expression following treatment of **C**, SW480  
11 cells with IFN $\gamma$  (50 ng/mL). Bars, 10  $\mu$ m.

12 **D**, Bar graph showing the mRNA expression of *CYSLTR1* in Dox-inducible conditionally  
13 knock-down of *CYSLTR1* in HCT116 cells exposed to either IFN $\gamma$  or left untreated.  
14 Mean  $\pm$  SEM. \*\*P < 0.01, \*\*\*P < 0.001, two-tailed unpaired t-test.

15

16 **Supplementary fig. S3**

17 **A**, Western blots showing alterations in the expression of the indicated proteins in IFN $\gamma$ -  
18 stimulated (50 ng/mL, 24 h) HT-29 CC cells subsequently treated with either Montelukast (Mo)  
19 or in a combination with GSK-3 $\beta$  inhibitor, CHIR-99021 (10  $\mu$ M; 24 h) (Mo + CHIR-99021).  
20 The blots are representative of three replicates, which are shown in the densitometry graph.

21 **B**, Western blots showing alterations in the expression of the indicated proteins in IFN $\gamma$ -  
22 stimulated (50 ng/mL, 24 h) HT-29 CC cells transfected with *CRISPR/Cas9-CTRL* or

1 *CRISPR/Cas9-CYSLTR1* subsequently treated with GSK-3 $\beta$  inhibitor, CHIR-99021. The blots  
2 are representative of three replicates, which are shown in the densitometry graph.

3 In all the western blot panels, GAPDH served as the loading control. Mean  $\pm$  SEM. \*P < 0.05,  
4 \*\*P < 0.01, two-tailed unpaired t test. MW, relative molecular weight expressed in kilodaltons  
5 (kDa).

6

#### 7 **Supplementary fig. S4**

8 **A**, q-RT-PCR analysis of the relative mRNA expression of *CD274* in HT-29 cell xenografts  
9 and SW480 cell xenografts in mice treated with DMSO or montelukast (Mo).

10 **B**, Immunofluorescence images showing the expression of  $\beta$ -catenin (green) and PD-L1 (red)  
11 in SW480 cell xenografts in mice treated with DMSO or Montelukast (Mo). Representative  
12 graph **B'**, showing the MFI of PD-L1 expression (n = 4 per group in each xenograft). Scale  
13 bars as indicated in the images. The white dotted line marks the outline of the xenograft  
14 section.

15 Western blots of **C**, SW480 cell xenograft. The blots show the expression of the indicated  
16 proteins of interest. Representative densitometric analysis results are shown in the graph in  
17 **C'**, for SW480 cells.

18 In all the western blot panels, GAPDH served as the loading control. The MFI in all confocal  
19 images was calculated with ImageJ software (NIH, USA). Mean  $\pm$  SEM. \*\*P < 0.01,  
20 \*\*\*P < 0.001, two-tailed unpaired t test. MW, relative molecular weight expressed in  
21 kilodaltons (kDa).

22

#### 23 **Supplementary fig. S5**

1 A, Bar graphs showing alteration in transcript level expression of *CD274*, *CYSLTRI*, and  
2 *CTNNB1* in HT-29 colon cancer cells stimulated with IFN $\gamma$  (50 ng/mL) followed by either  
3 treated with Montelukast (Mo, 10  $\mu$ M), Atezolizumab (PD-L1 neutralizing antibody) alone or  
4 in the combination of both (Mo + Atezolizumab).

5 Mean  $\pm$  SEM. \*P < 0.05, \*\*P < 0.01, two-tailed unpaired t-test.

6

### 7 **Supplementary fig. S6**

8 A, Western blots showing alterations in the expression of the indicated proteins in the nuclear  
9 or cytoplasmic fraction of IFN $\gamma$ -stimulated (50 ng/mL, 24 h) SW480 CC cells subsequently  
10 treated with either Montelukast (Mo) or Atezolizumab alone or in a combination of  
11 Montelukast and Atezolizumab. The blots are representative of three replicates, which are  
12 shown in densitometry graphs.

13 In the western blot panels, GAPDH or LAMIN B1 served as the loading control. Mean  $\pm$  SEM.  
14 \*P < 0.05, \*\*P < 0.01, two-tailed unpaired t test. MW, relative molecular weight expressed in  
15 kilodaltons (kDa).

16

### 17 **Supplementary fig. S7**

18 A, Box plot showing *CD274* transcript level expression in normal (n = 41) and cancer (n =  
19 286) tissue in the TCGA-COAD dataset (n = 327).

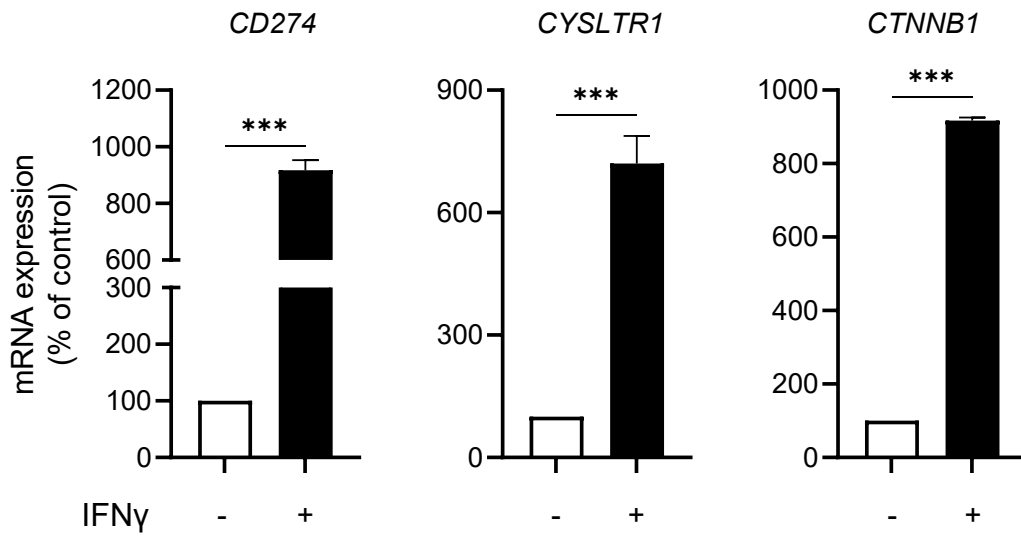
20 Mean  $\pm$  SEM. \*P < 0.05, \*\*\*P < 0.001, two-tailed unpaired t-test.

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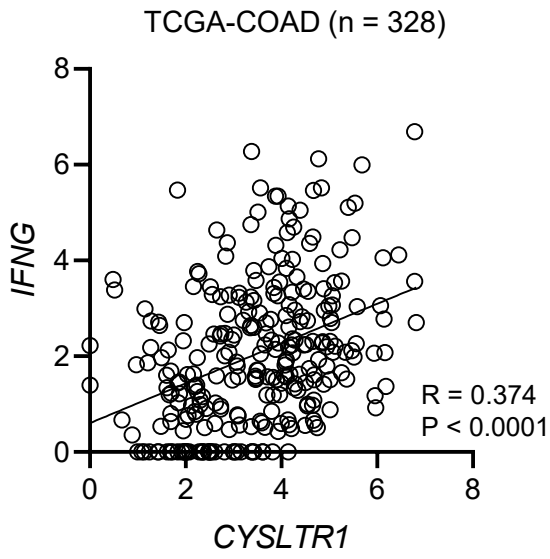
A

HT-29

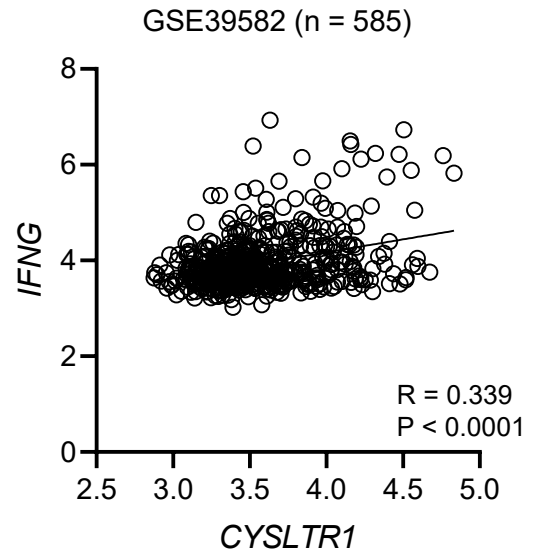


Supplementary fig. S2

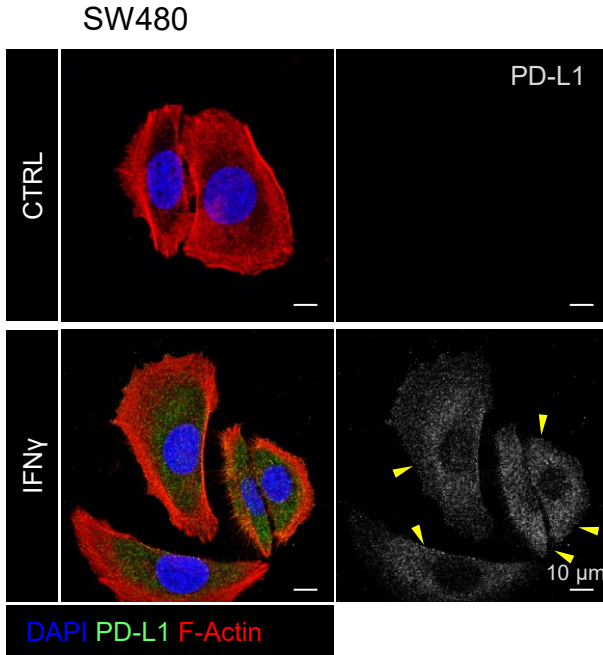
**A**



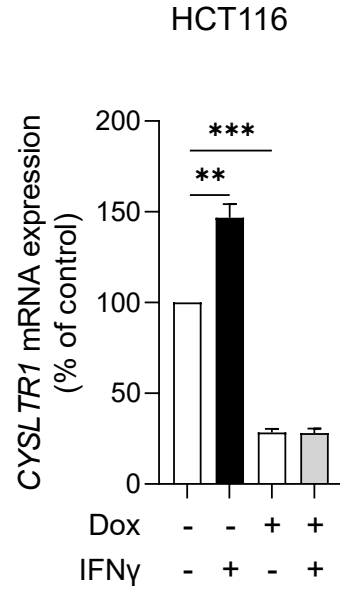
**B**



**C**

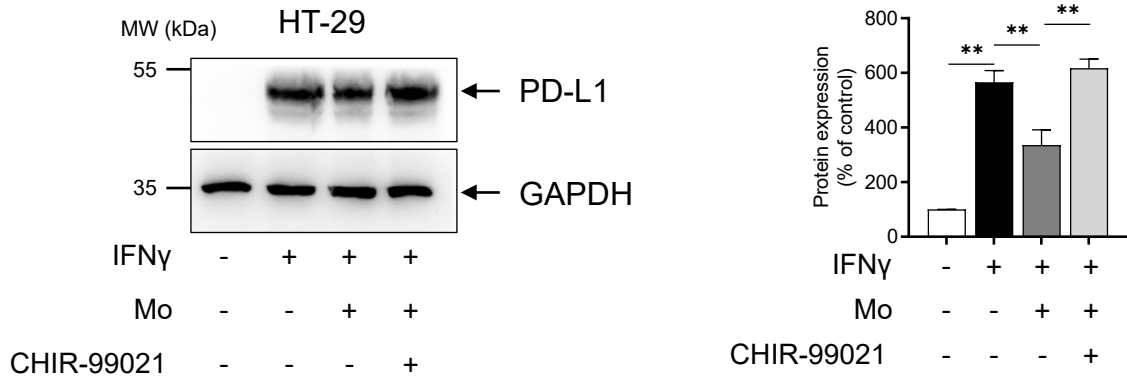


**D**

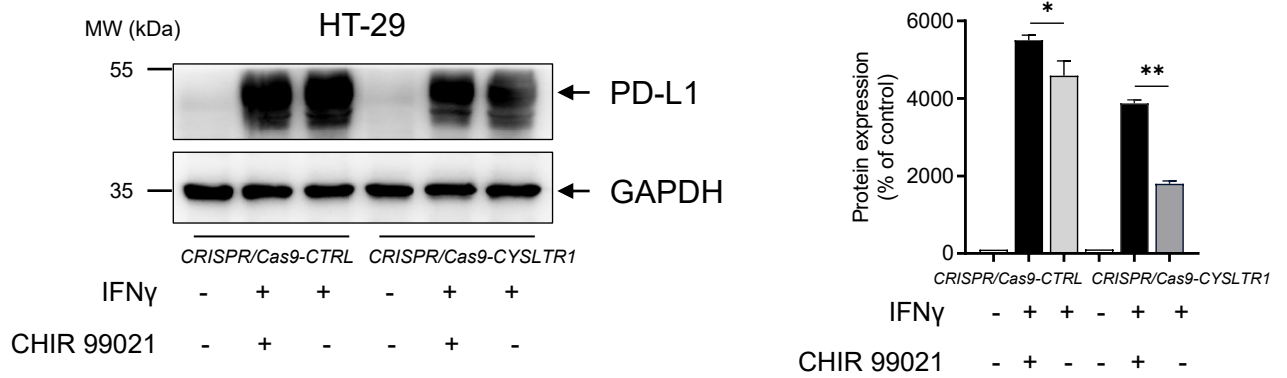


# Supplementary fig. S3

**A**

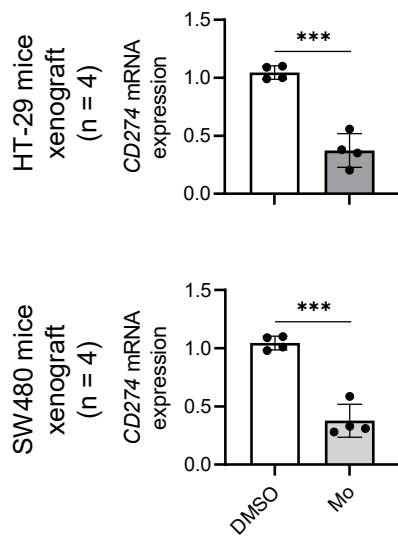


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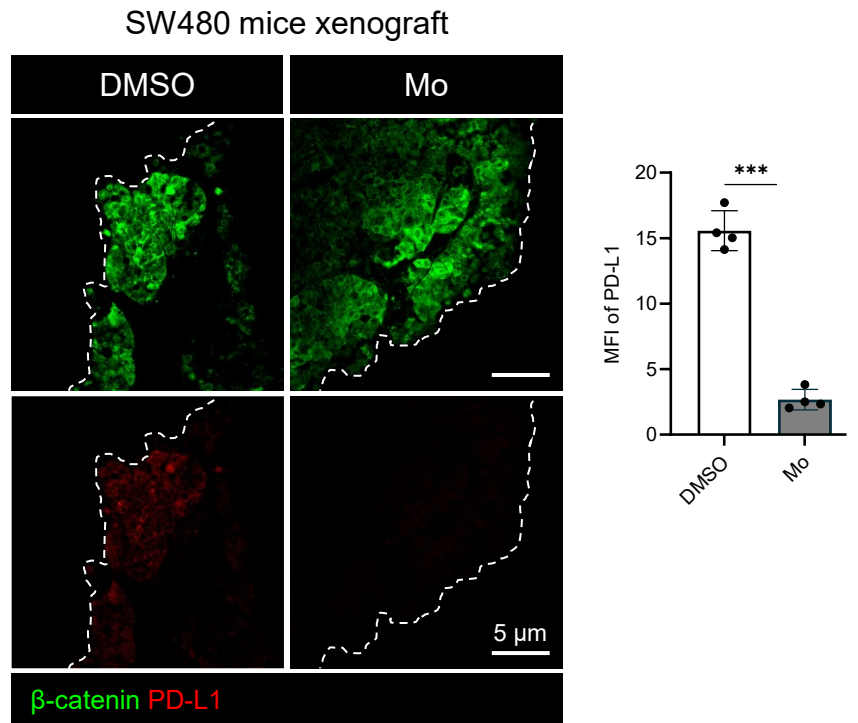


# Supplementary fig. S4

**A**

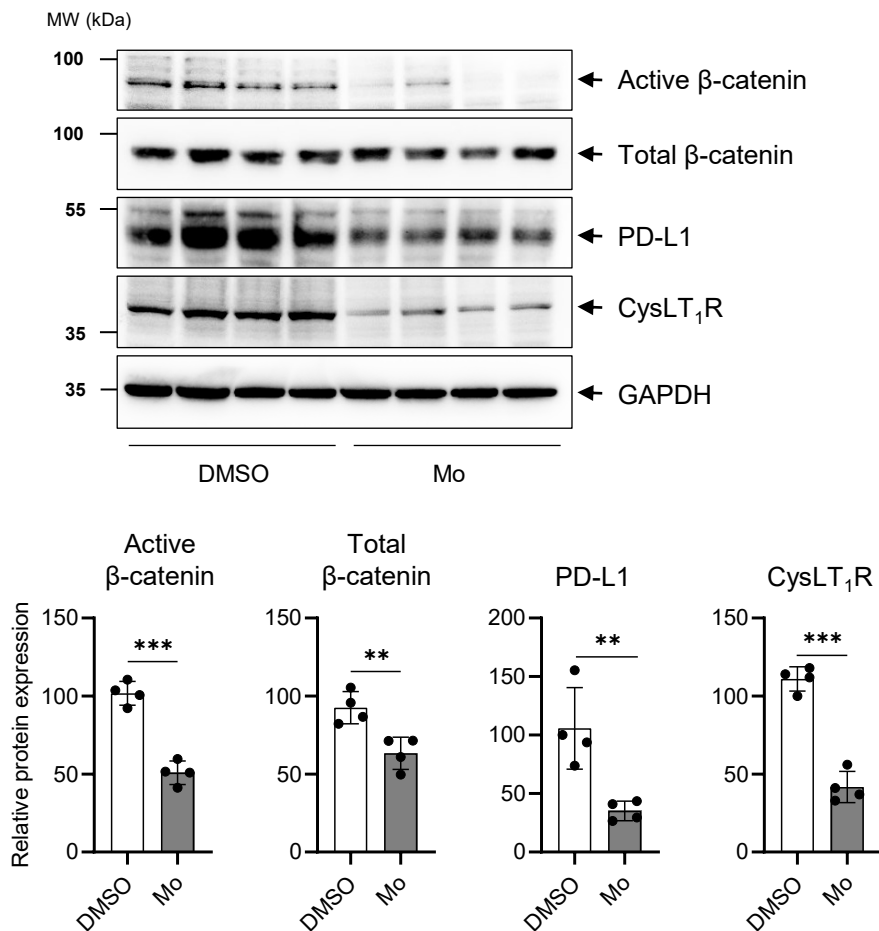


**B**



**C**

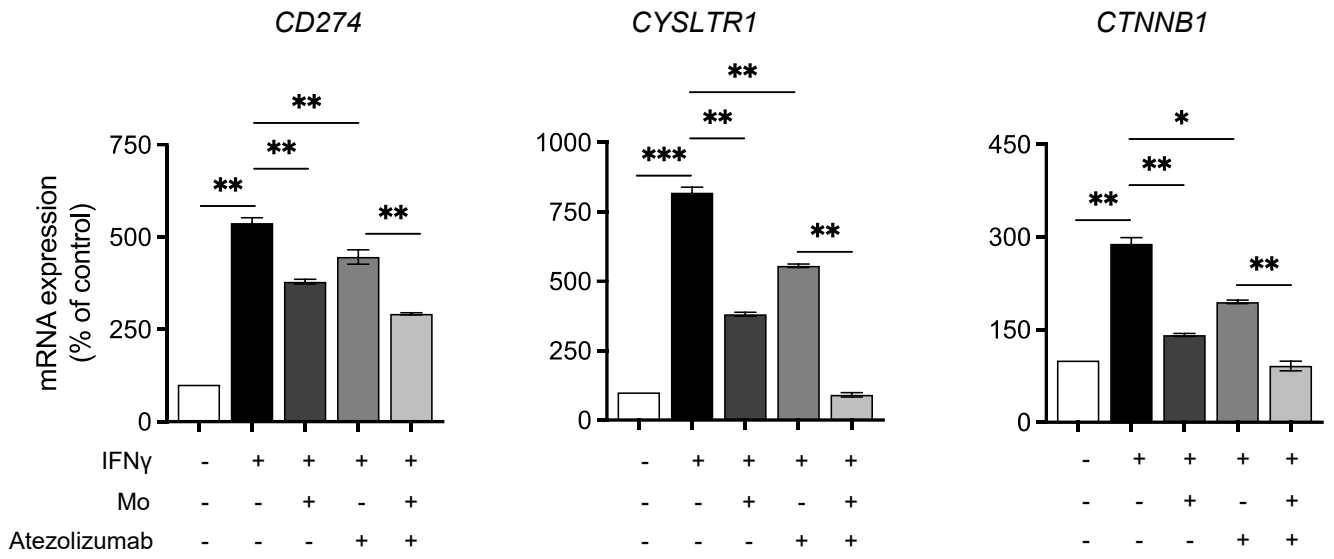
SW480 mice xenograft (n = 4)





A

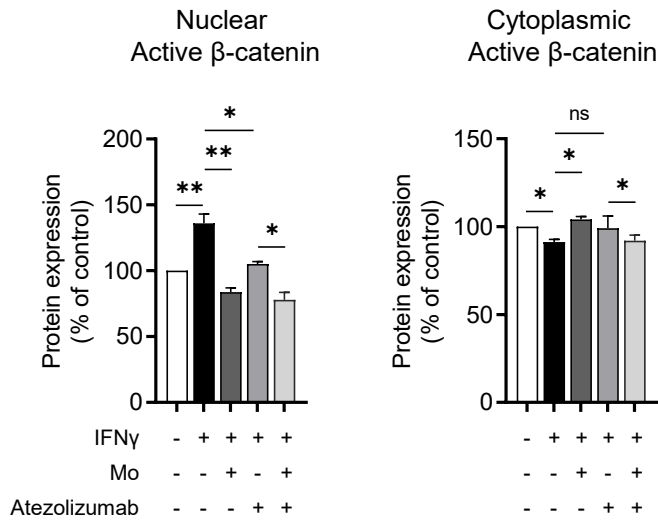
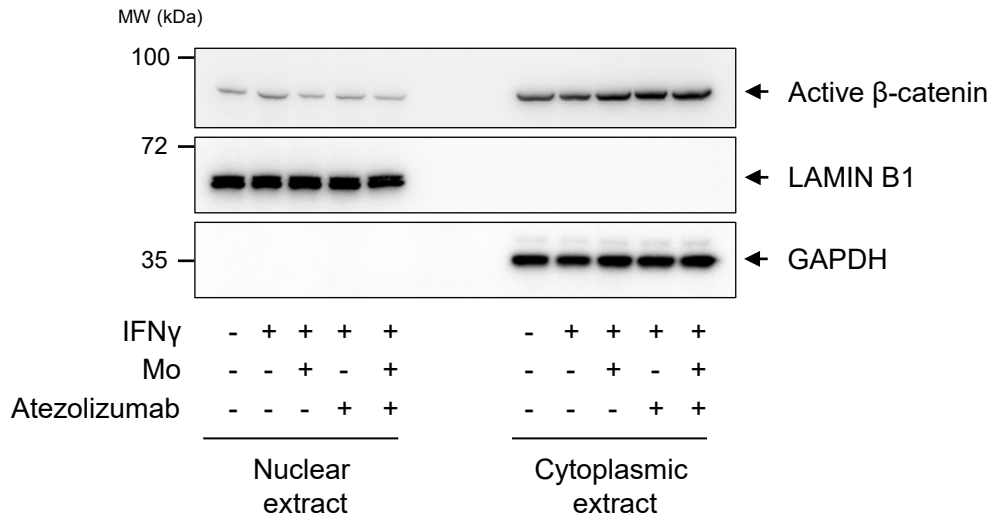
HT-29



Supplementary fig. S6

A

SW480



# Supplementary fig. S7

**A**

