

## Supplemental Materials

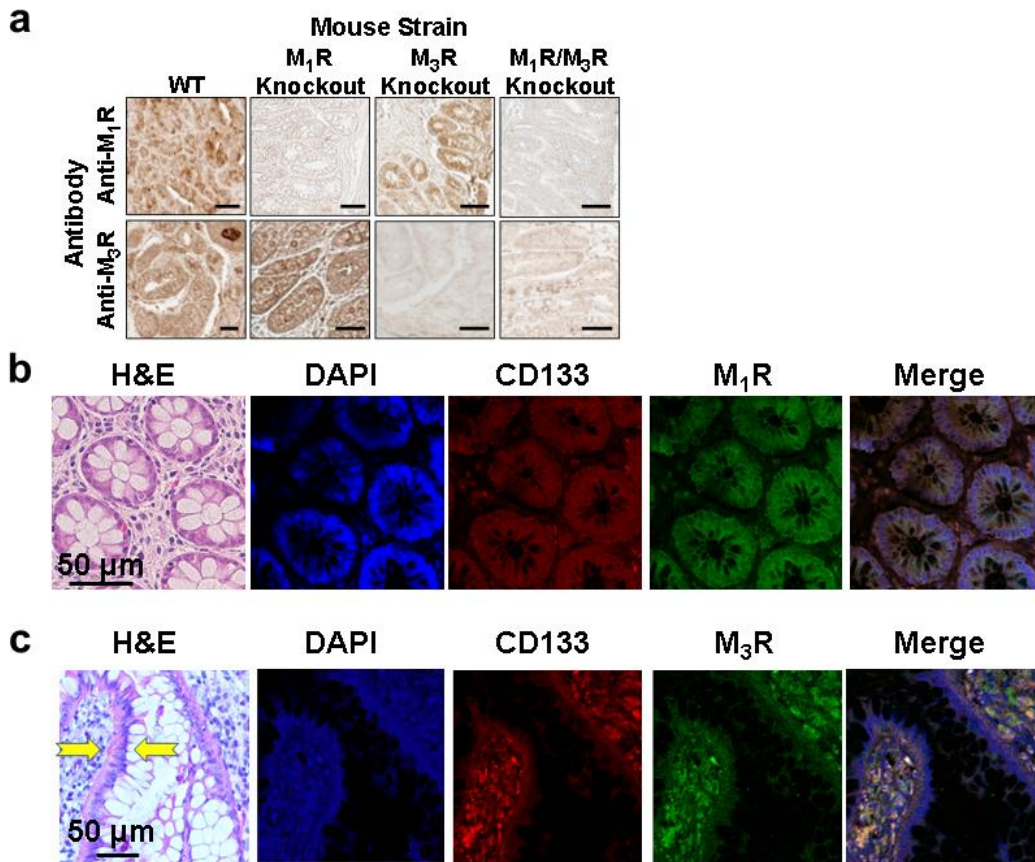
### Muscarinic receptor agonist-induced $\beta$ Pix binding to $\beta$ -catenin promotes colon neoplasia

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**Supplemental Table 1.** PCR primers used in this work.

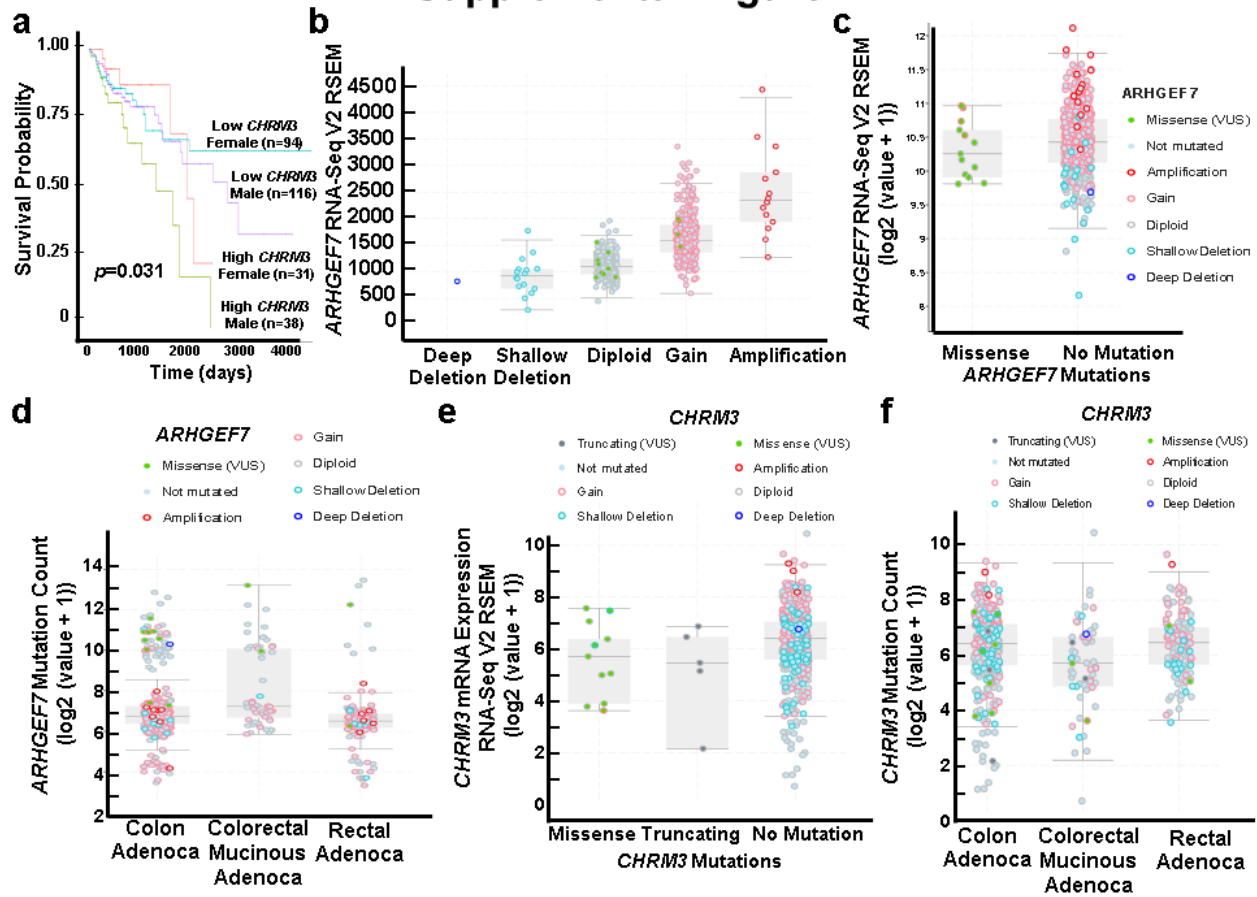
<b>Genes</b>	<b>Forward primer sequence 5'-3'</b>	<b>Reverse primer sequence 5'-3'</b>
<b>Human</b>		
<i>ARHGEF7</i>	TTCATGCGCCTGGATAAATA	CCTCTTCCGGACTTCTTGAC
<i>CHRM3</i>	TGGTTTGATGCTCCTACCTG	AGGACAGAGGAGTGGACCAG
<i>c-MYC</i>	CATACATCCTGTCCGTCCAAG	GAGTTCCGTAGCTGTTCAAGT
<i>CCND1</i>	AAGCTGTGCATCTACACCGA	CTTGAGCTTGTTCCACCAGGA
<i>PTGS2</i>	AGCACTTCACGCATCAGTTT	CGCAGTTTACGCTGTCTAGC
<i>B2M</i>	GAGGCTATCCAGCGTACTCC	ATGGATGAAACCCAGACACA
<b>Murine</b>		
<i>Axin2</i>	CAGAGGGACAGGAACCACTC	TGCCAGTTTCTTTGGCTCTT
<i>c-Myc</i>	CAGCAGCGACTCTGAAGAAG	GACTCCGACCTCTTGGCA
<i>CcnD1</i>	CAACGCACTTTCTTTCCAGA	GACTCCAGAAGGGCTTCAAT
<i>Ptgs2</i>	AGCCTTCTCCAACCTCTCCT	CAGGGATGAACTCTCTCCGT
<i>B2m</i>	CCAAGACCGTCTACTGGGAT	TCTTTCTGCGTGCATAAATTG

## Supplemental Figure 1



**Supplemental Fig. 1.** Muscarinic receptor antibody specificity. **(a)** Normal colon tissue sections obtained from WT, *Chrm1*, *Chrm3*, and dual *Chrm1/Chrm3* knockout mice were immunostained with anti-M<sub>1</sub>R and anti-M<sub>3</sub>R antibodies. Representative images confirm that M<sub>1</sub>R and M<sub>3</sub>R immunostaining, respectively, was absent in tissues from M<sub>1</sub>R- and M<sub>3</sub>R-deficient mice. Size bars, 50  $\mu$ m. **(b)** Basal CD133 and M<sub>1</sub>R staining in normal colon epithelial cells. Images show H&E (left panels) and immunofluorescent staining with DAPI (nuclear stain, blue), CD133 (red-Alexa Fluor 594), M<sub>1</sub>R, and merged images. Size bars in H&E image, 50  $\mu$ m. **(c)** Basal CD133 and M<sub>3</sub>R staining in normal colon epithelial cells. Images show H&E (left panels) and immunofluorescent staining with DAPI (nuclear stain, blue), CD133 (red-Alexa Fluor 594), M<sub>3</sub>R, and merged images. Arrows indicate epithelial lining. Size bars in H&E image, 50  $\mu$ m.

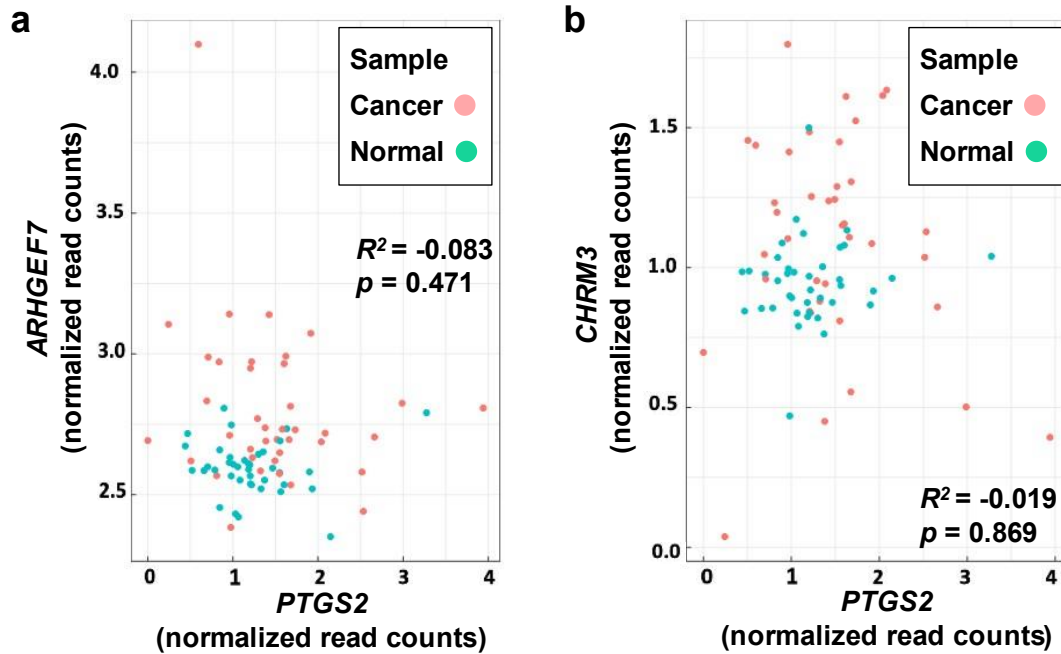
## Supplemental Figure 2



**Supplemental Fig. 2.** Expression levels of *CHRM3* and *ARHGEF7* in colorectal adenocarcinoma. (a) Effect of *CHRM3* expression levels on patient survival. Lower levels of *CHRM3* expression were associated with prolonged survival ( $p=0.031$ ). Image downloaded from UALCAN server. Survival probability was examined by Kaplan-Meier analysis and statistical significance determined using the log rank test. (b) Relationship between gene amplification and transcriptional upregulation of *ARHGEF7* in CRC. (cBioPortal analysis of the TCGA PanCancer Atlas dataset, [www.cBioportal.org](http://www.cBioportal.org)). (c) *ARHGEF7* copy number alterations in colon tumor samples obtained from 526 patients (333 colon adenocarcinomas; 137 rectal adenocarcinomas; 56 mucinous colorectal adenocarcinomas) reveal 15 missense mutations in 13 patients with an overall frequency of *ARHGEF7* mutation of 2.4%. (d) Although there is no apparent difference in *ARHGEF7* mRNA expression between colon adenocarcinoma, colorectal mucinous adenocarcinoma, and rectal adenocarcinoma, *ARHGEF7* missense mutations were more common in colon compared to rectal adenocarcinoma. (e) *CHRM3* copy number

alterations in colon tumor samples obtained from 526 patients (333 colon adenocarcinomas; 137 rectal adenocarcinomas; 56 mucinous colorectal adenocarcinomas) reveal 11 missense and 5 truncating mutations in 16 patients with an overall frequency of *CHRM3* mutation of 3.0%. (f) There is no apparent difference in *CHRM3* mRNA expression and mutations between colon, colorectal mucinous, and rectal adenocarcinomas.

### Supplemental Figure 3



**Supplemental Fig. 3.** Lack of correlation between *ARHGEF7* and *CHRM3* mRNA expression levels with *PTGS2* mRNA expression levels in human colon adenocarcinomas. Univariate mixed models incorporating participant ID as a random effect were employed to compare (a) *ARHGEF7* and (b) *CHRM3* expression levels as a function of *PTGS2* levels in colon adenocarcinomas and paired adjacent normal tissue samples (n=78 for each). Values are expressed as ‘normalized read counts’ as described in Methods. No correlation was found between *PTGS2* and *ARHGEF7* ( $p=0.471$ ) or *CHRM3* ( $p=0.869$ ) mRNA expression levels.

**Original uncut gels for immunoblots** (the following images show the respective regions of the original blots, labelled using red boxes delineating the cropped versions shown in the manuscript figures). In red, we also indicated the proteins that was targeted for analysis. To ascertain that equal amounts of cytoplasmic and nuclear proteins were included in the extracted protein *input* for immunoprecipitation, in preliminary gels we used  $\beta$ -actin and H2A loading controls, respectively. Then, we immunoblotted immunoprecipitates for expression of the *bait* protein as a second loading control (e.g., immunoblotting for  $\beta$ Pix following immunoprecipitation with anti- $\beta$ Pix antibody).

Fig. 2d

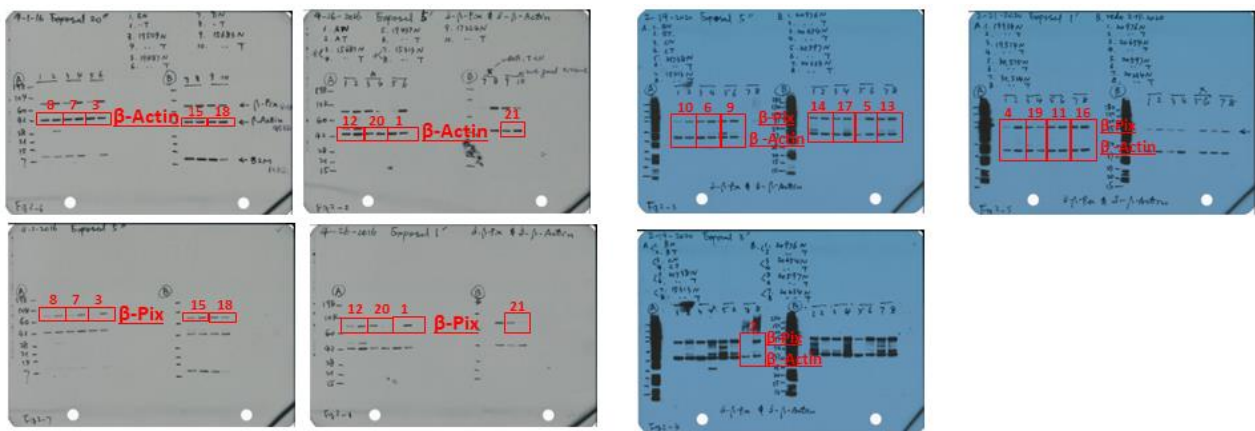


Fig. 3a

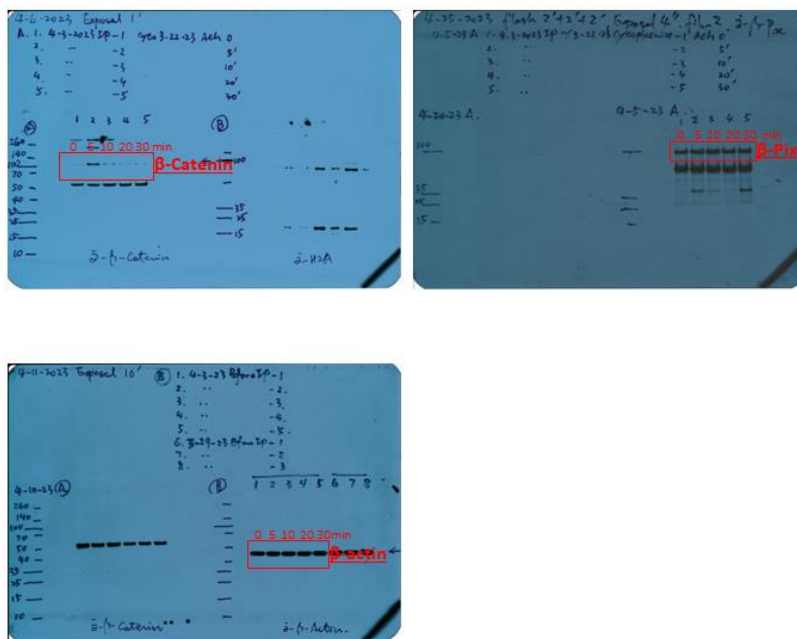


Fig. 3b

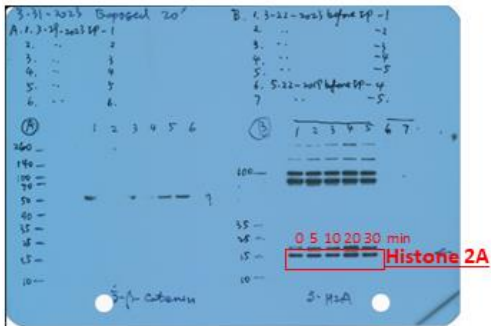
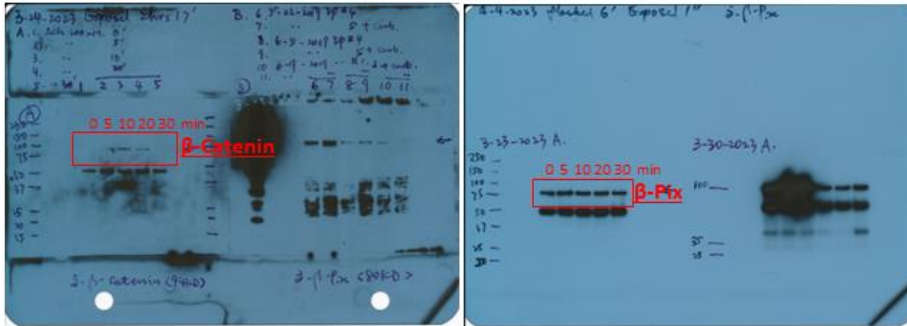


Fig. 3c

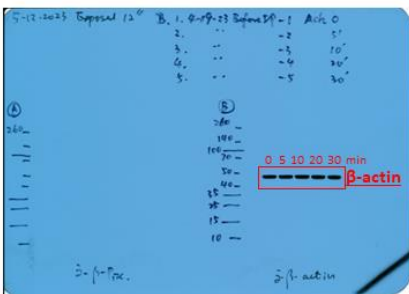
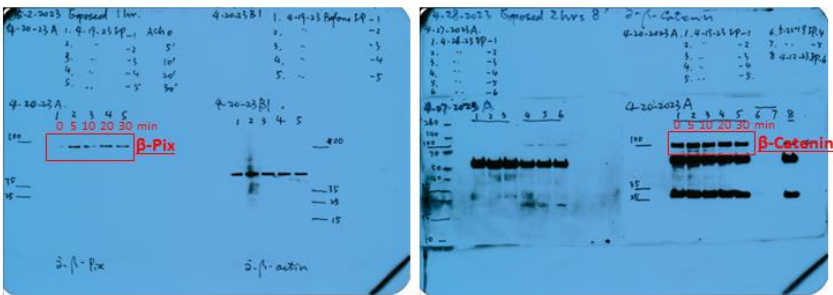


Fig. 3d

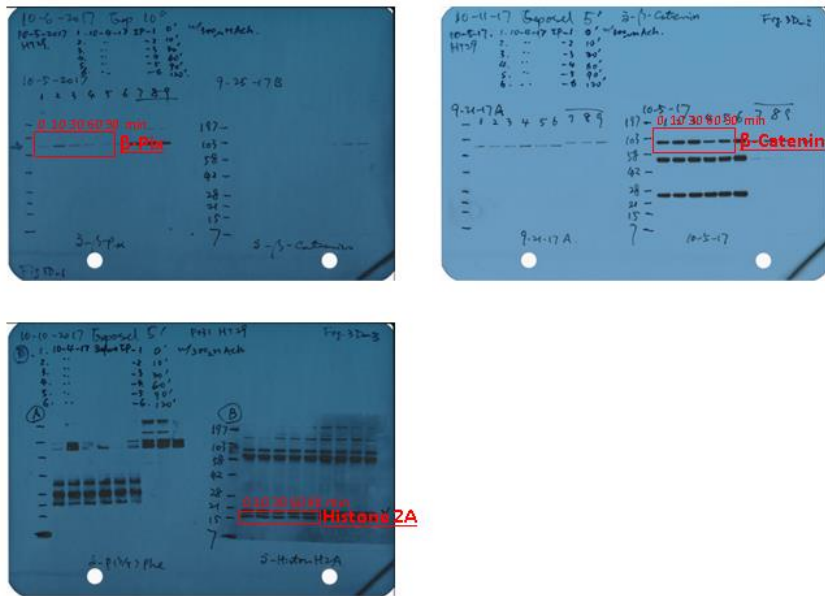


Fig. 3e

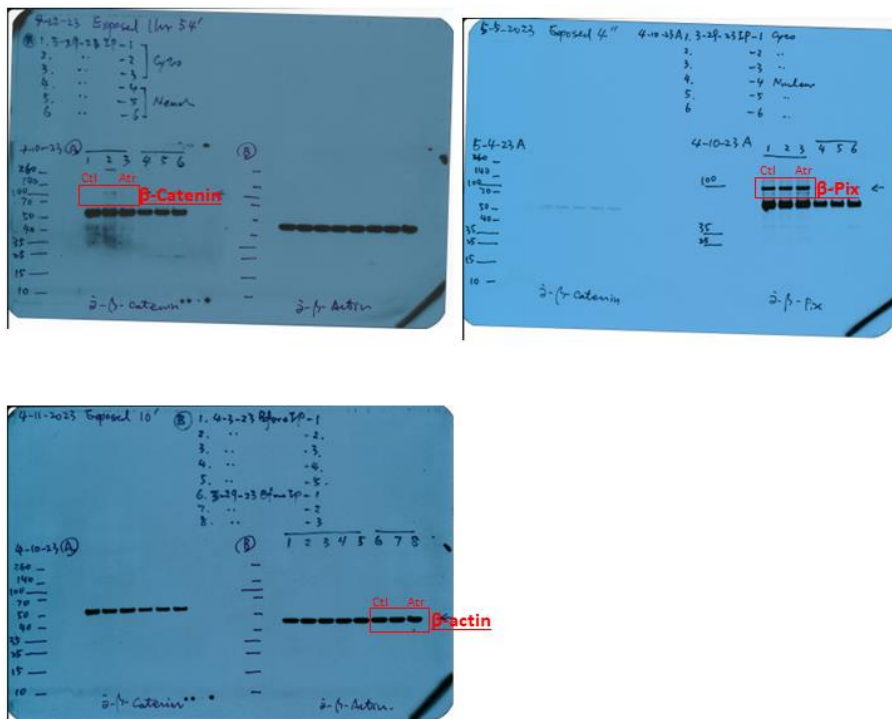




Fig. 3f

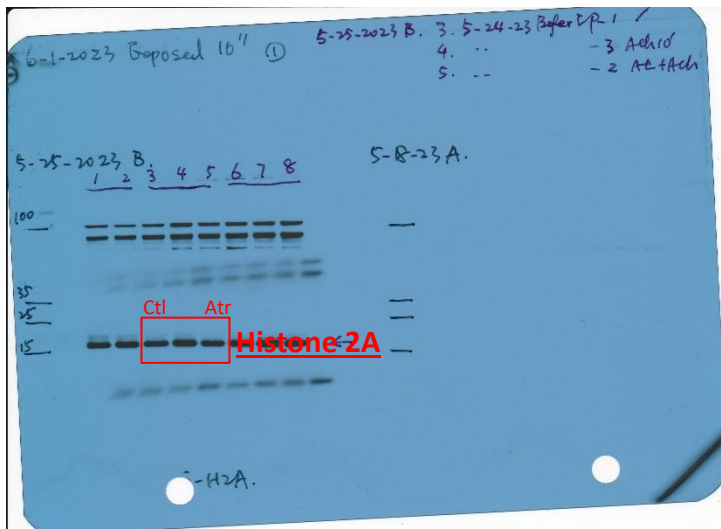
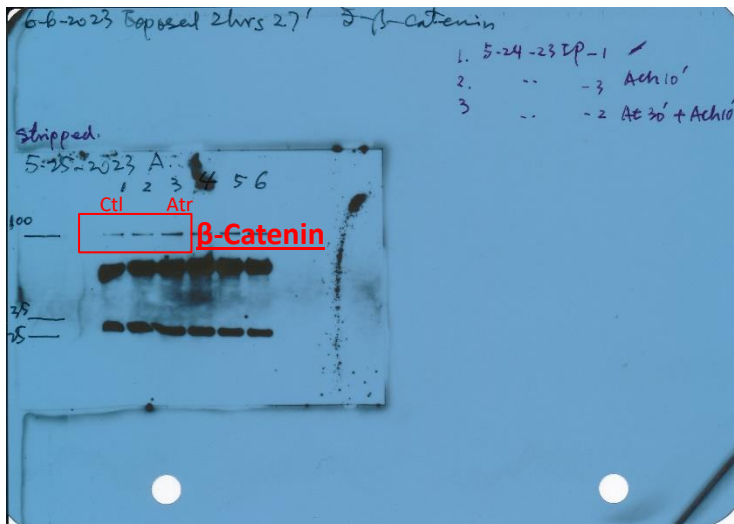
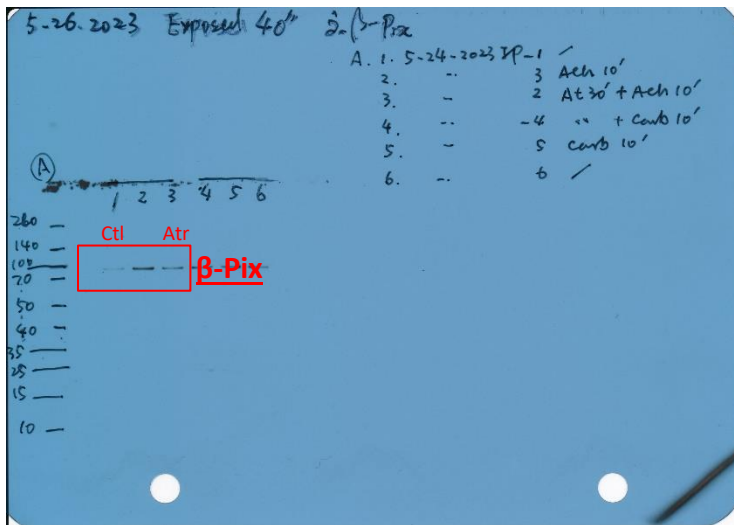


Fig. 3g

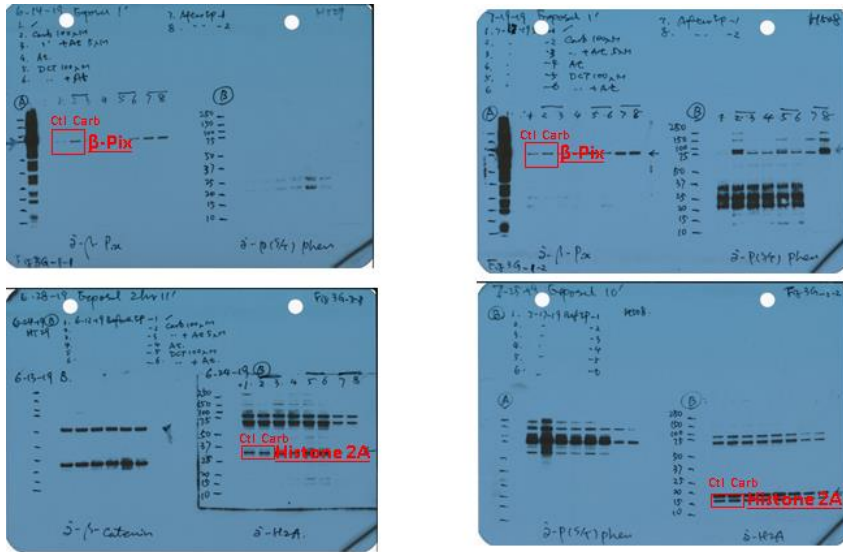


Fig. 3i

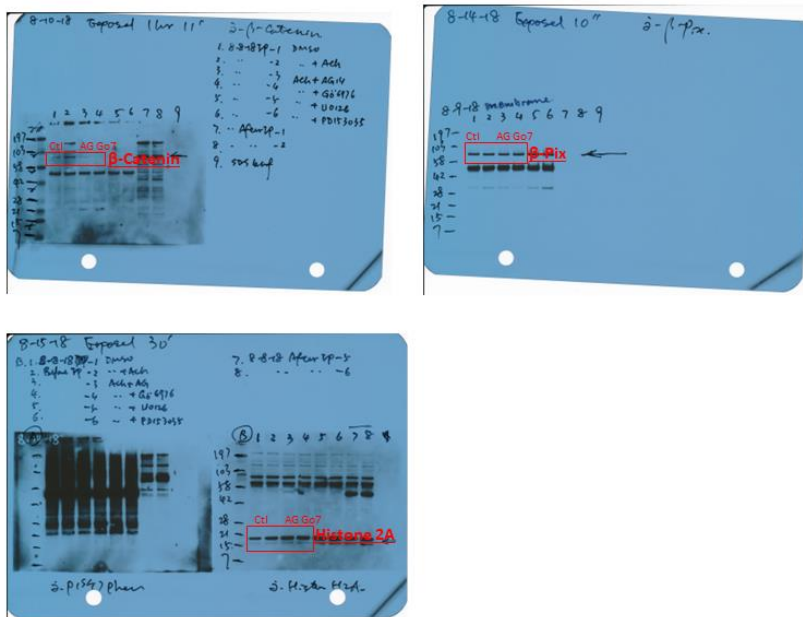


Fig. 3j

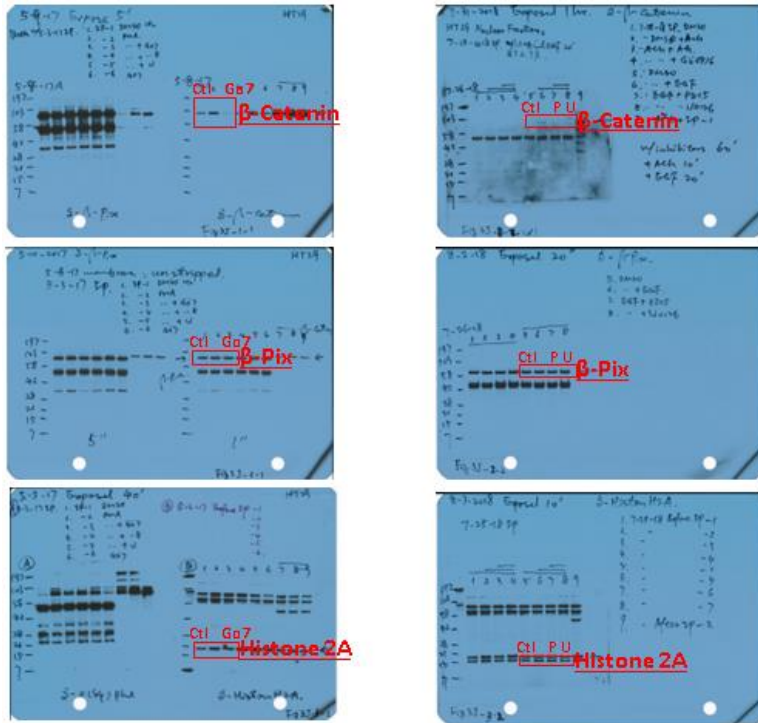


Fig. 4a

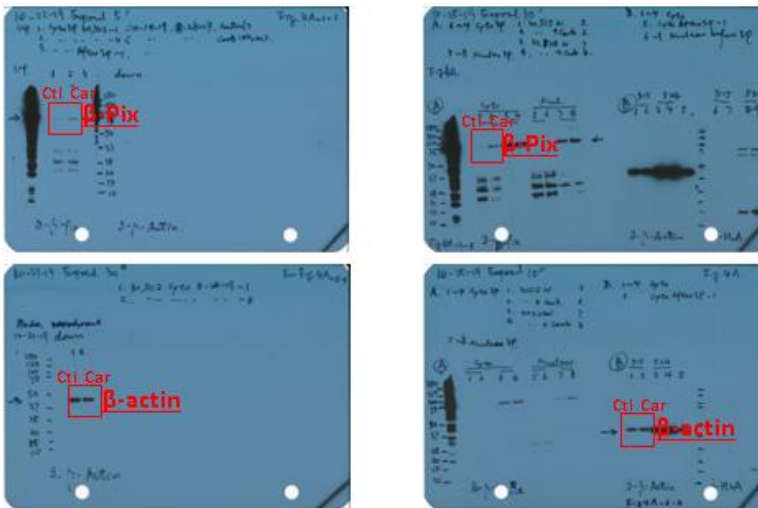


Fig. 4g

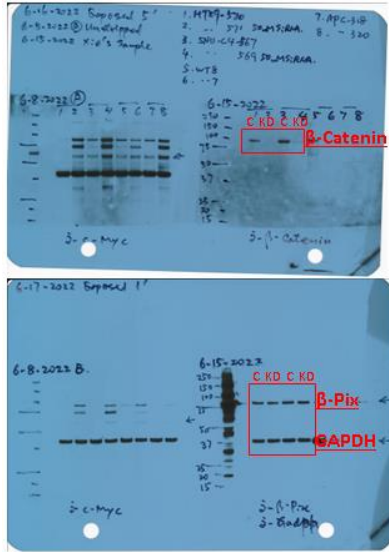


Fig. 4h

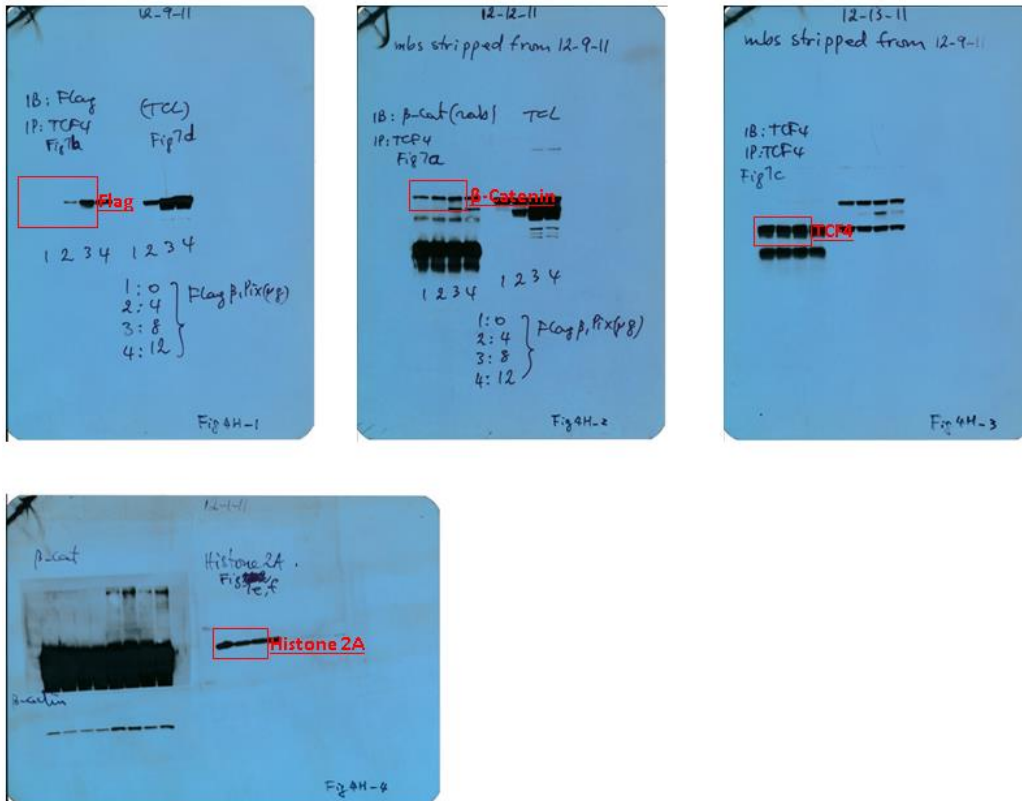


Fig. 5a

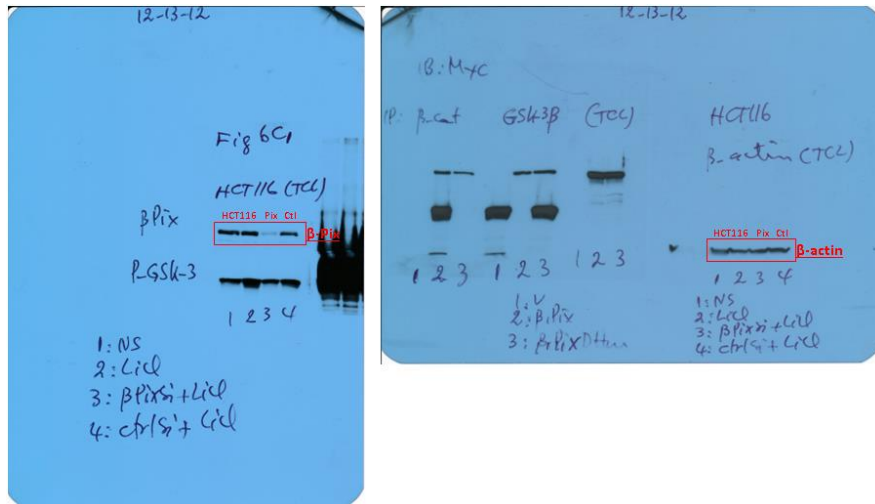


Fig. 5c

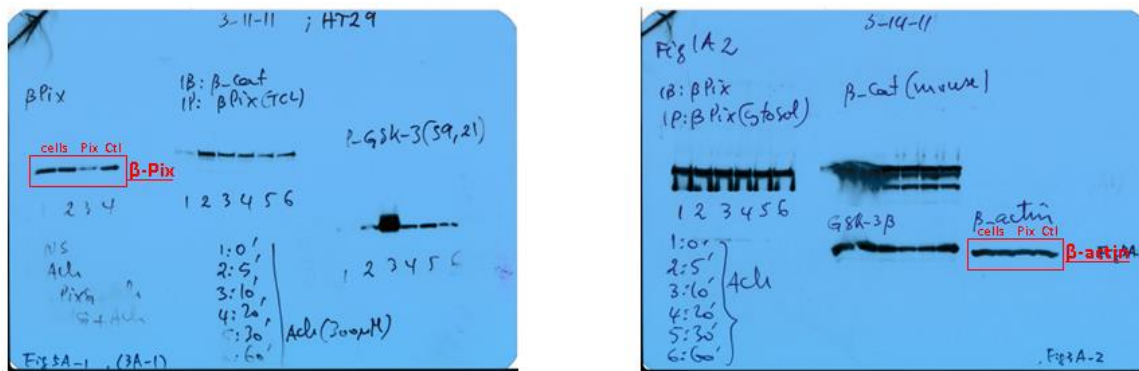


Fig. 5e

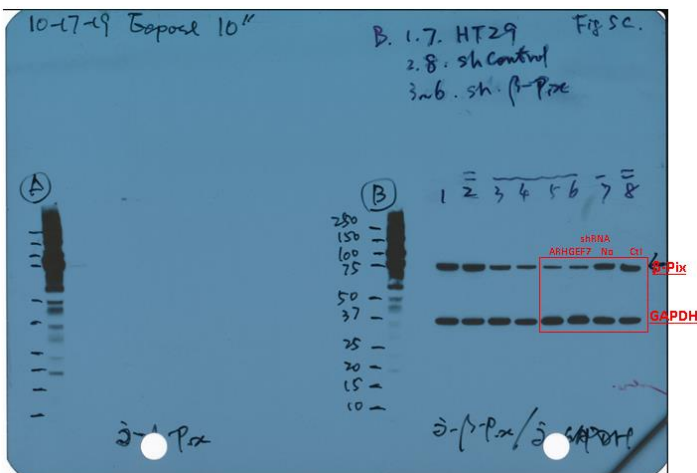


Fig. 6g

Fig. 6g\_Ctl & CKO1,2

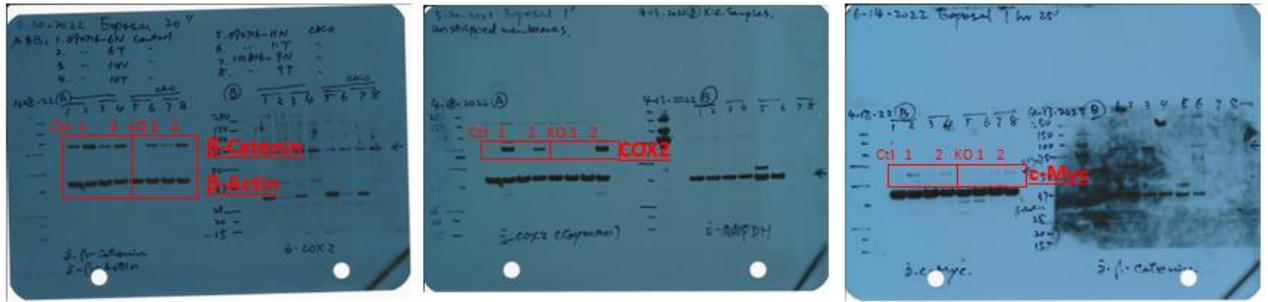


Fig. 6g\_Ctl & CKO 3,4,5,6

