

TNF- α regulates the proteolytic degradation of ST6Gal-1 and endothelial cell-cell junctions through upregulating expression of BACE1

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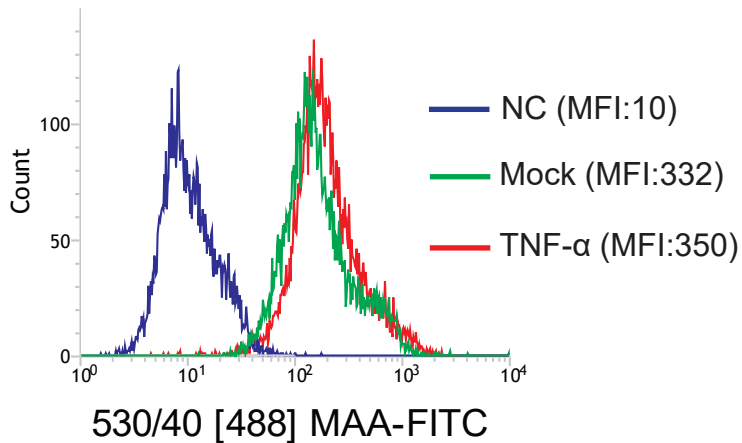
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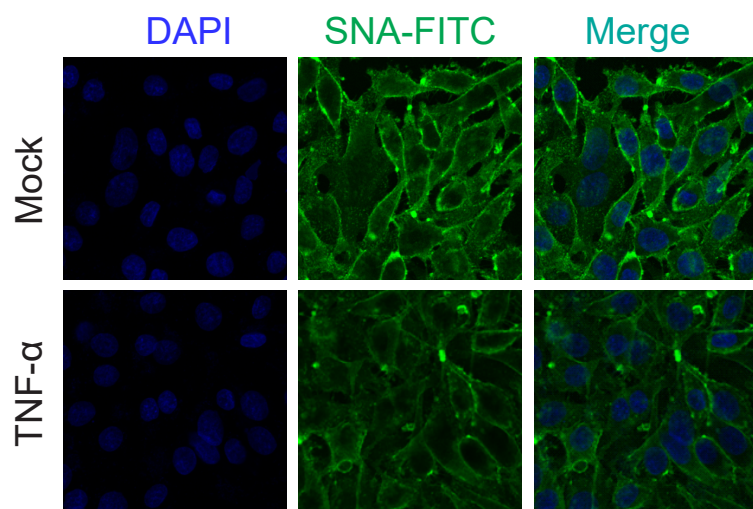
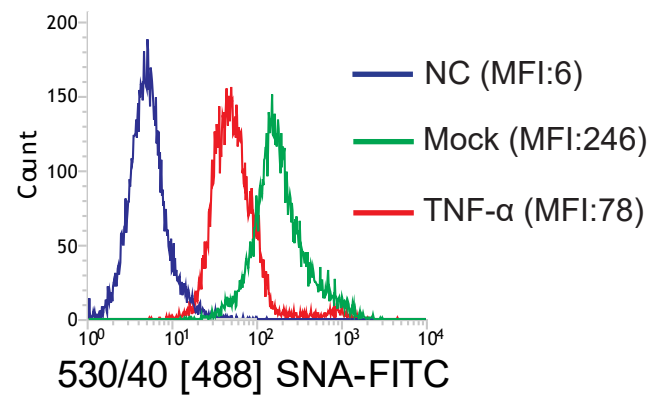
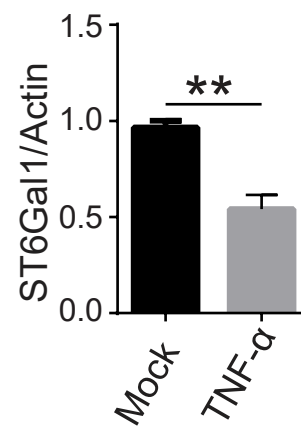
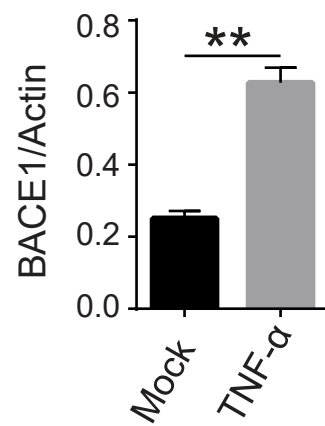
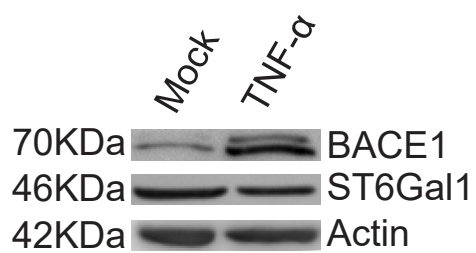
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Supplementary Figure 1



Supplementary Fig. 1 Flow cytometry data for total protein α -2, 3 sialylation levels in EA.hy926 cells treated with TNF- α . EA.hy926 cells were treated with TNF- α (50 ng/ml) for 24 h and then incubated with MAA-FITC (2 μ g/ml) for 1 h at room temperature. After being washed three times in PBS, the cells were analyzed using a BD Influx Cell Sorter (BD Biosciences, USA). The graphs represent the relative results of three independent assays. MFI, mean fluorescence intensity. NC, EA.hy926 cells unstained with FITC-labeled SNA. Mock, EA.hy926 without treatment.

A**B****C**

Supplementary Fig. 2 TNF- α treatment decreases ST6Gal-I levels but upregulates BACE1 in primary HUVECs. (A) Primary HUVECs were treated with TNF- α (50 ng/ml) for 24 h. After FITC-labeled SNA staining, α 2, 6 sialylation expression was examined by confocal fluorescence microscopy. Mock, HUVECs without treatment. (B) Flow cytometry was then used to determine α 2, 6 sialylation expression. The data are the mean \pm SD of three independent assays. The MFI was quantified using BD FACS Software. NC, HUVECs unstained with FITC-labeled SNA. Mock, HUVECs without TNF- α treatment. (C) Western blotting was conducted to characterize ST6Gal-I and BACE1 levels in HUVECs after TNF- α treatment. The data are the mean \pm SD of three independent assays (** p <0.01). ns, not significant.

Fig.2A

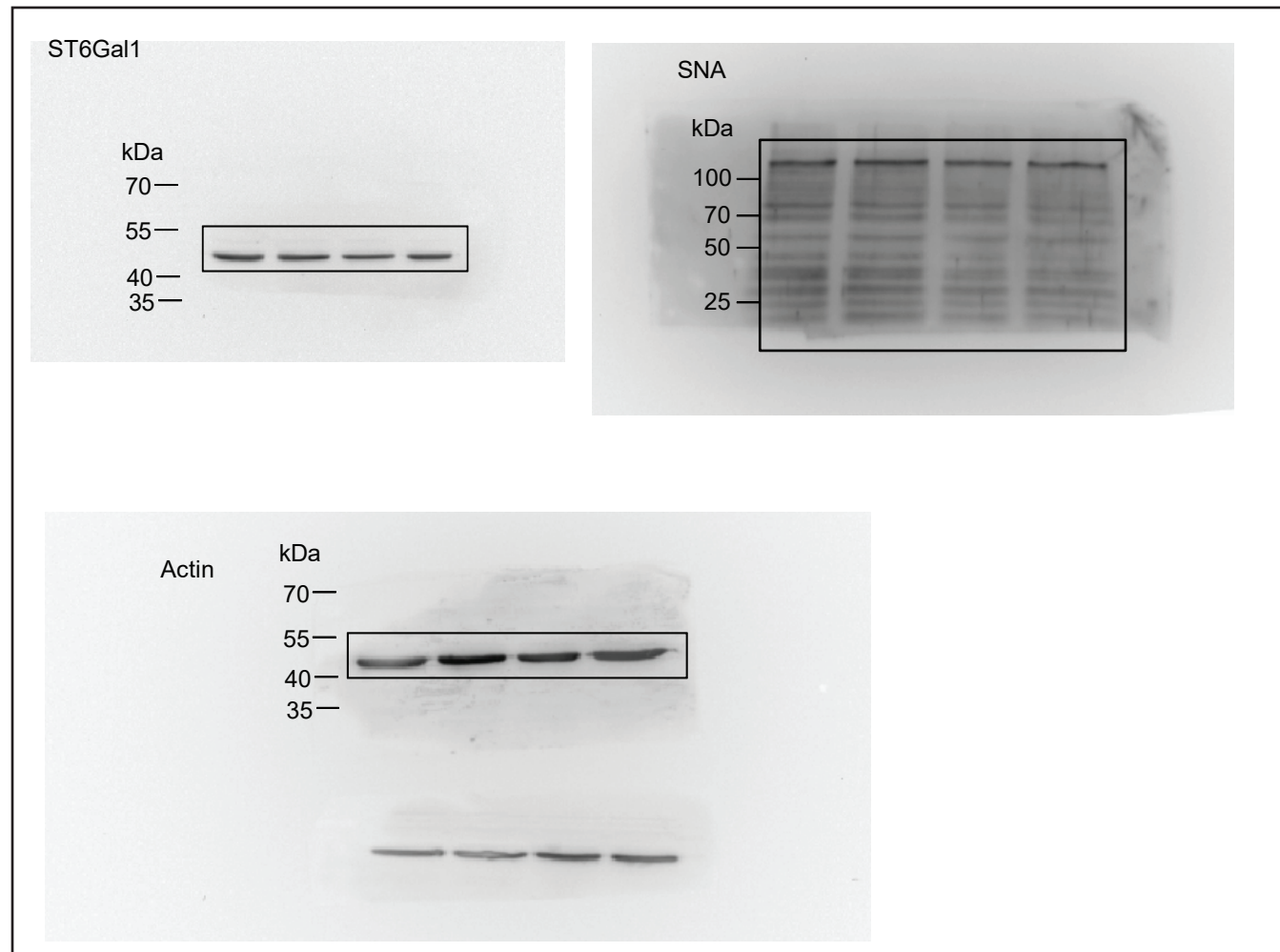


Fig.3A

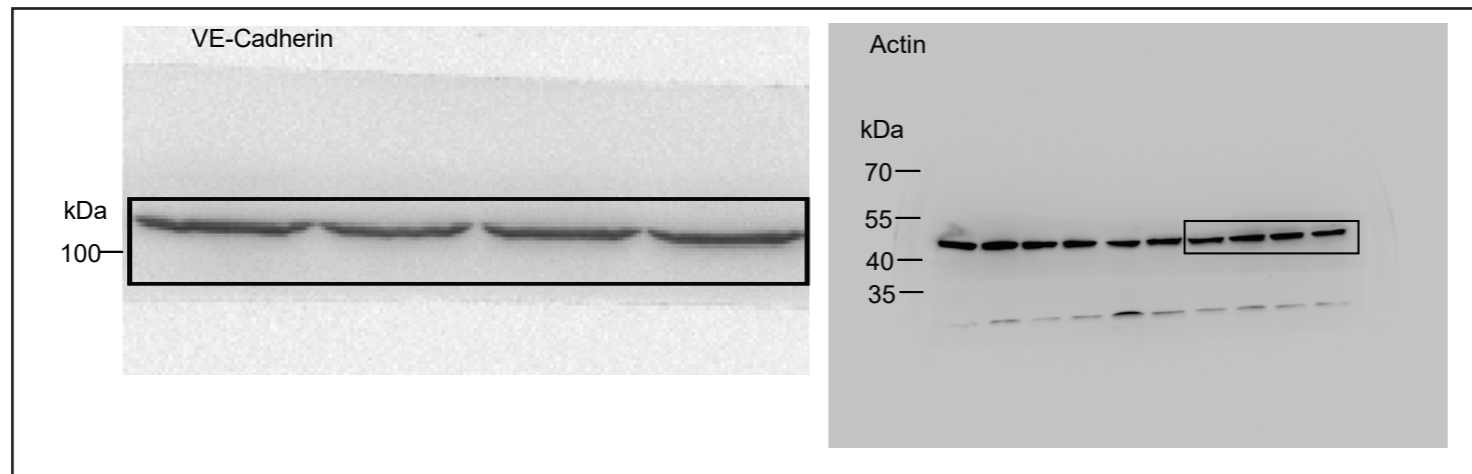


Fig.3B

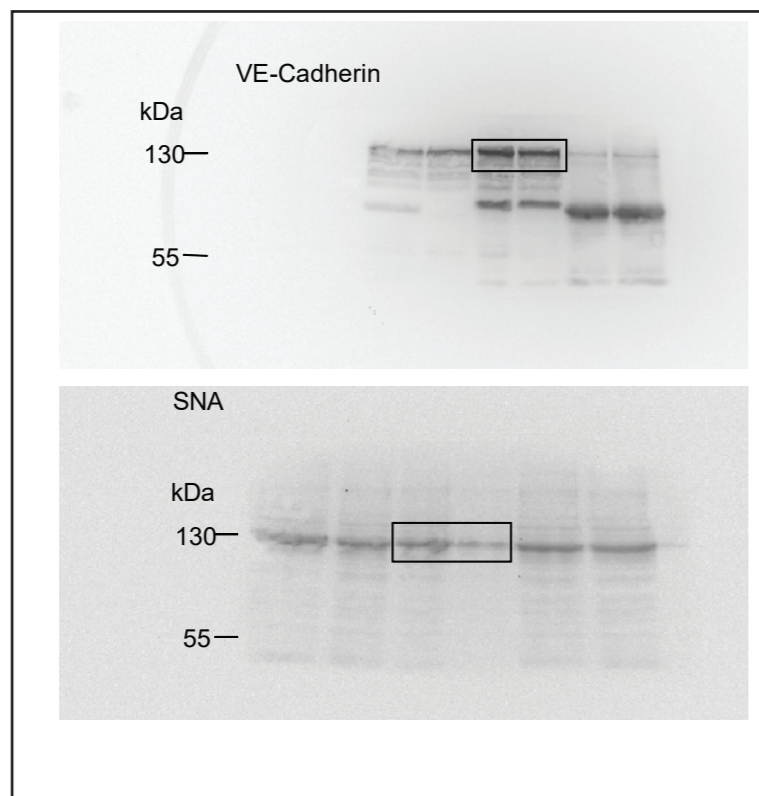


Fig.3C

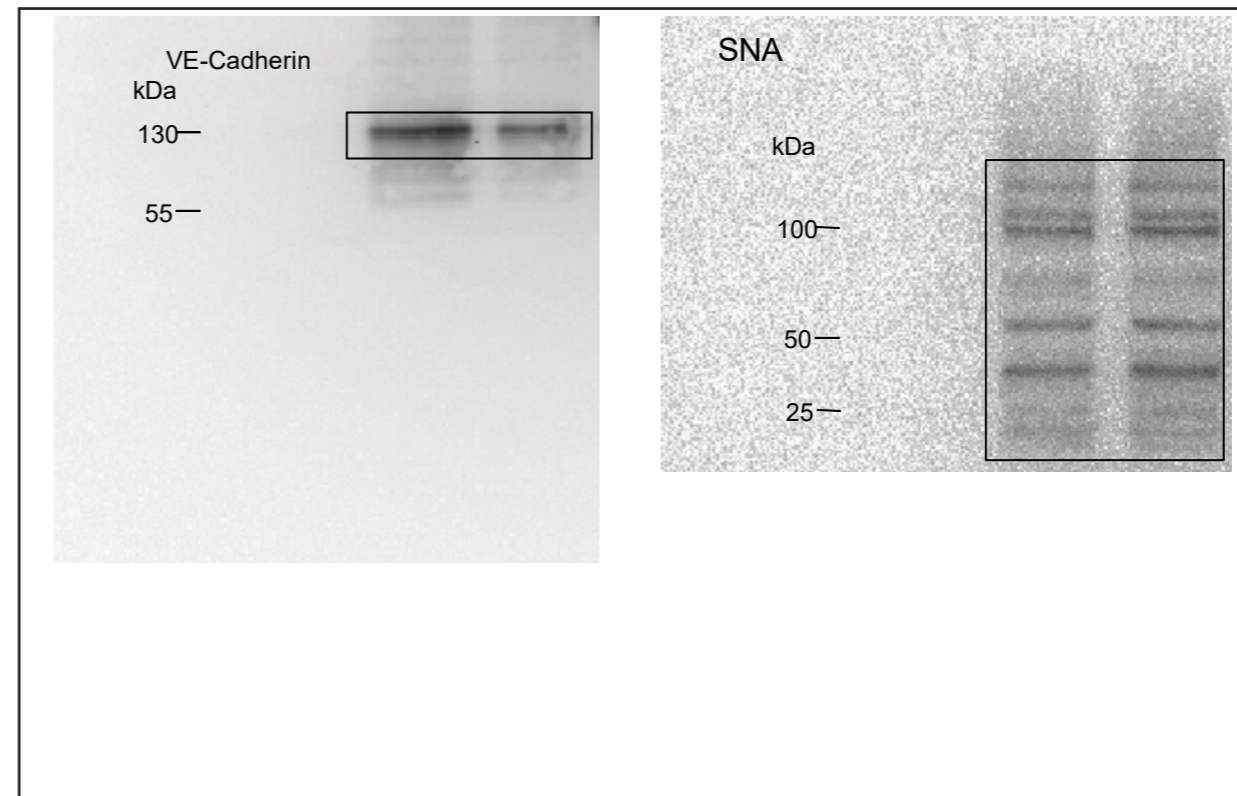


Fig.4A

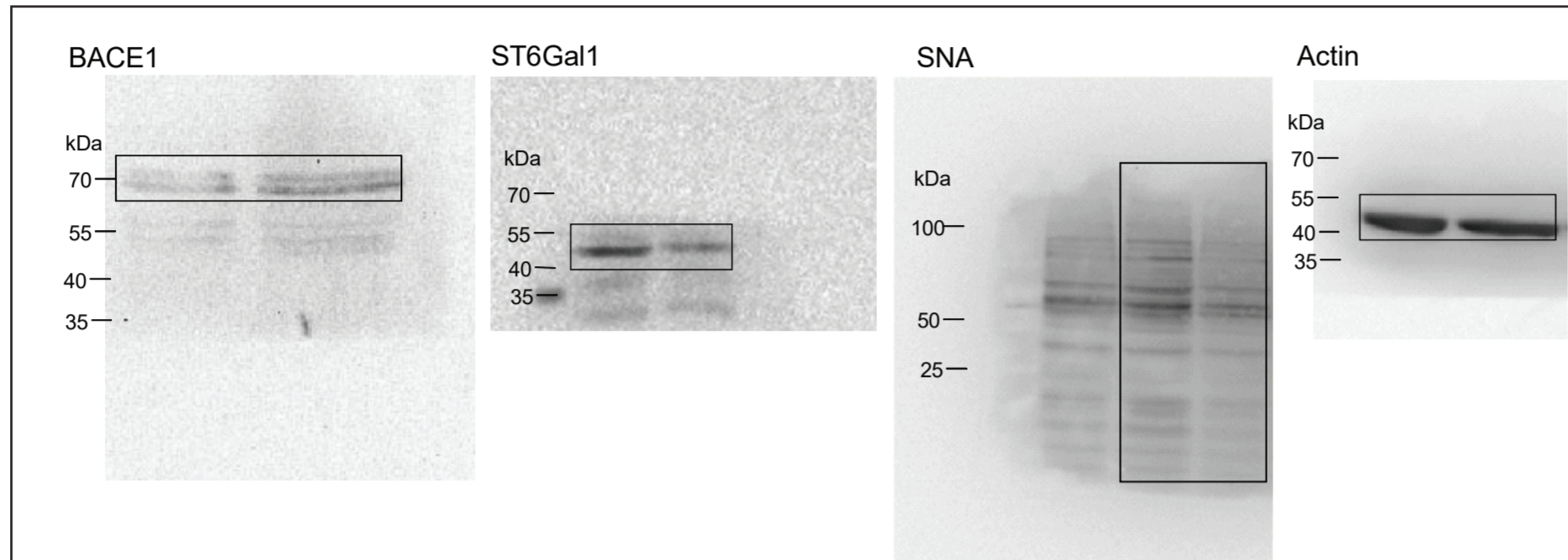


Fig.4B

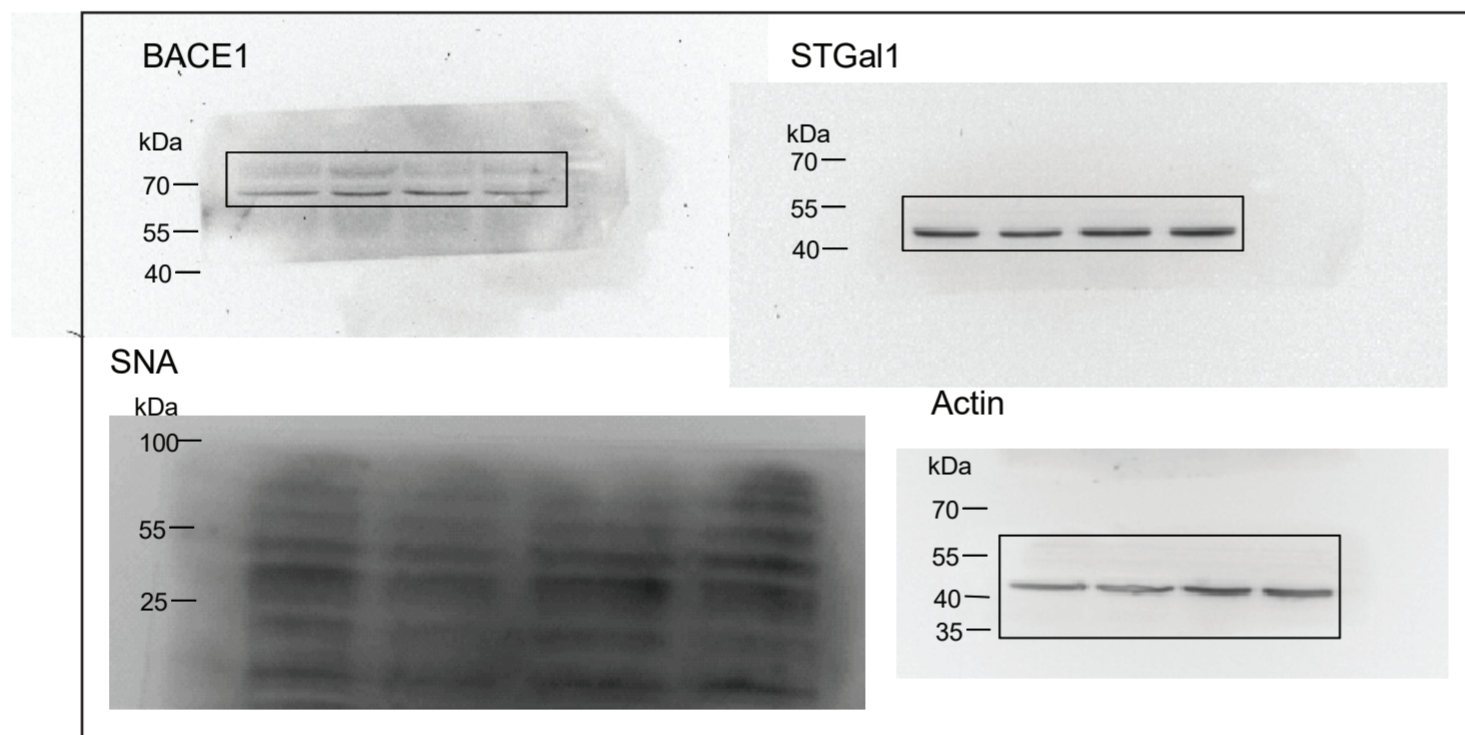


Fig.4D

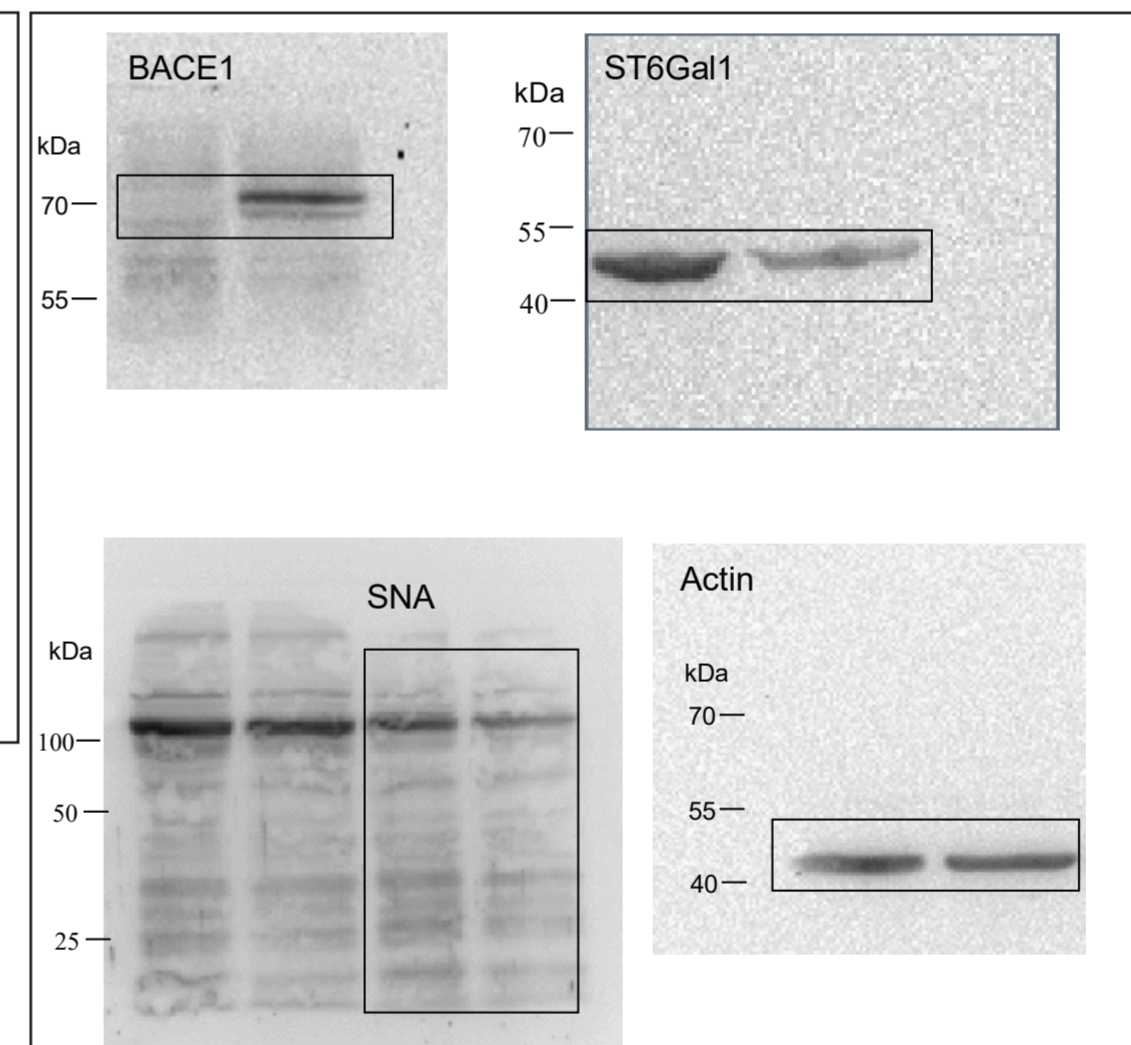


Fig.4F

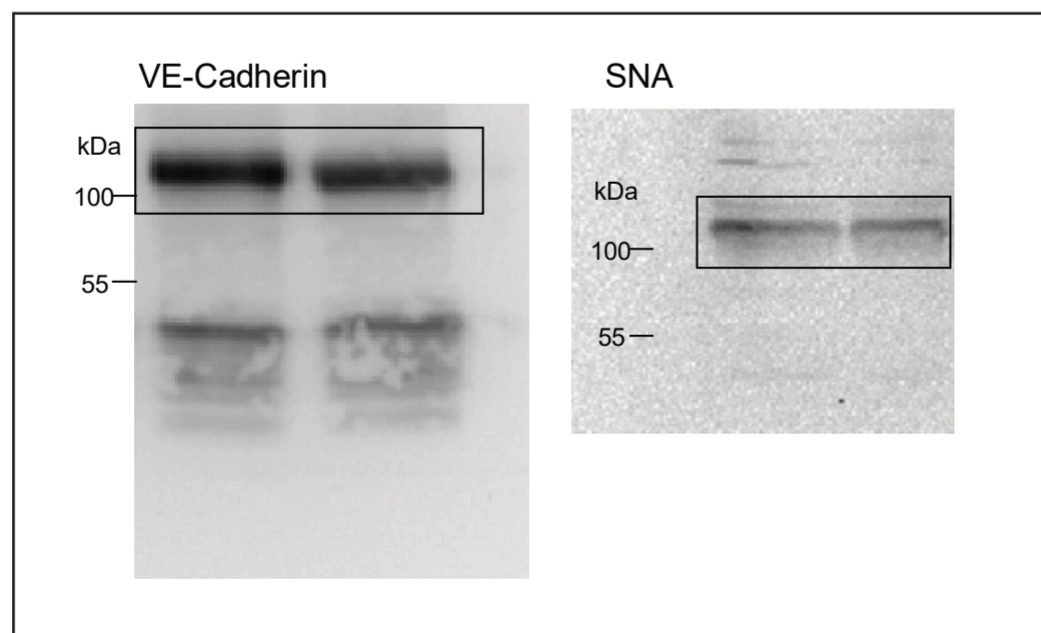


Fig.6A

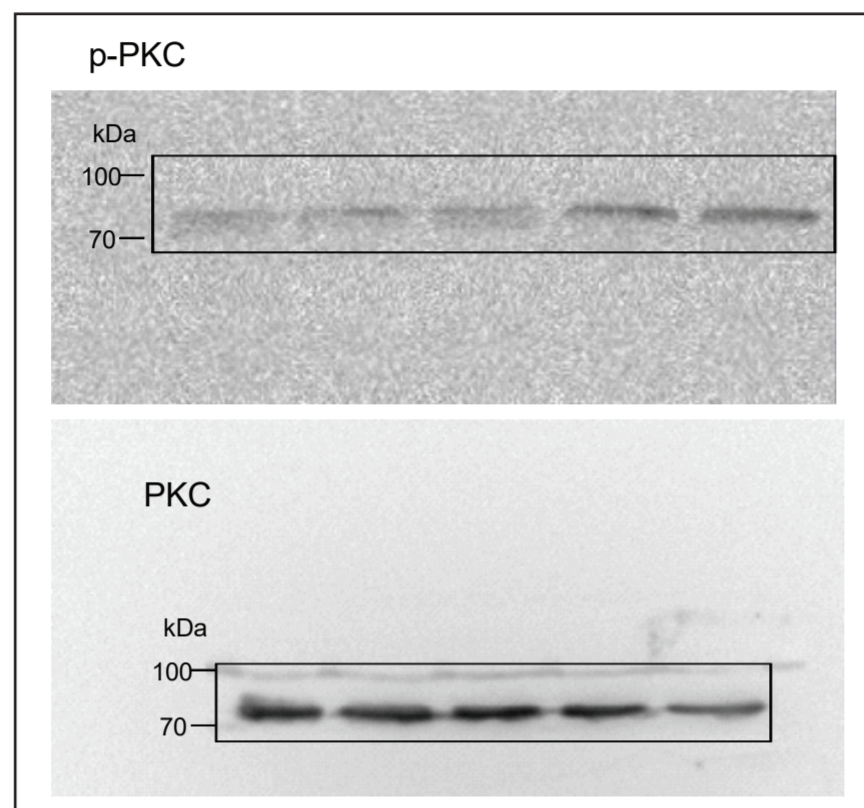


Fig.6B

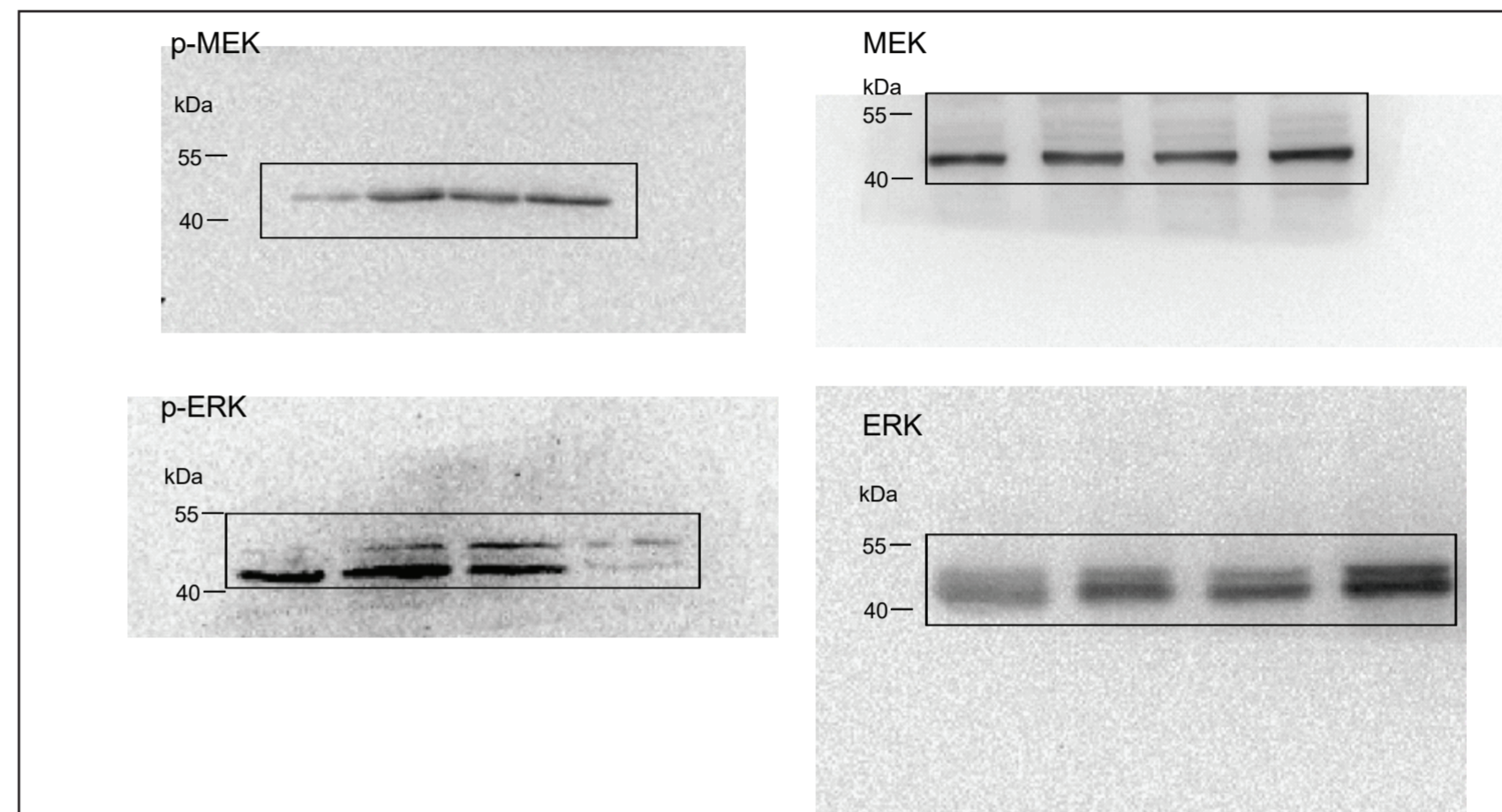


Fig.6C

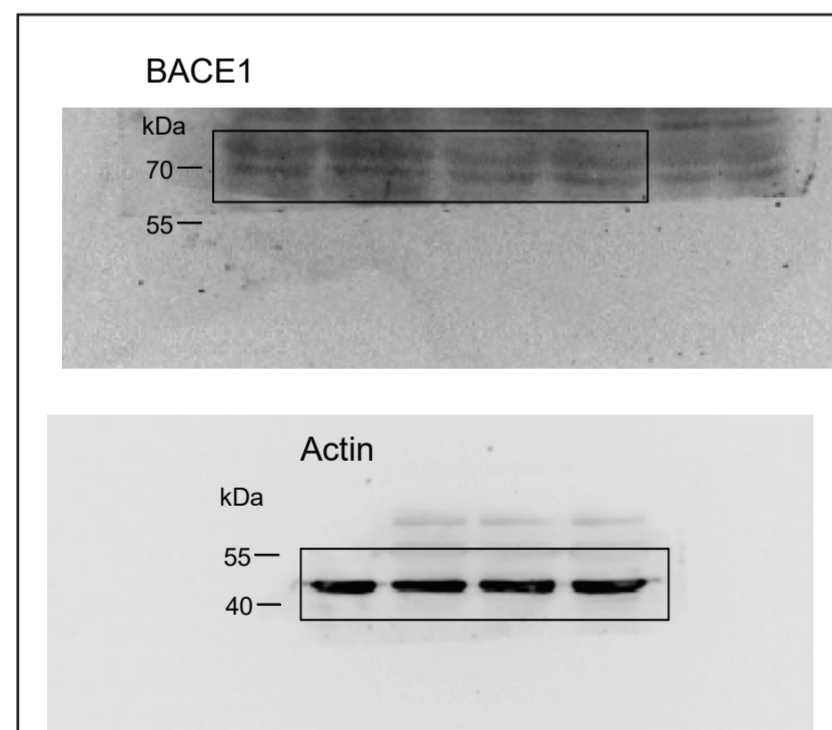


Fig.6D

