

Supplemental Data

BDNF/TRKB axis provokes EMT progression to induce cell aggressiveness via crosstalk with cancer-associated fibroblasts in human parotid gland cancer

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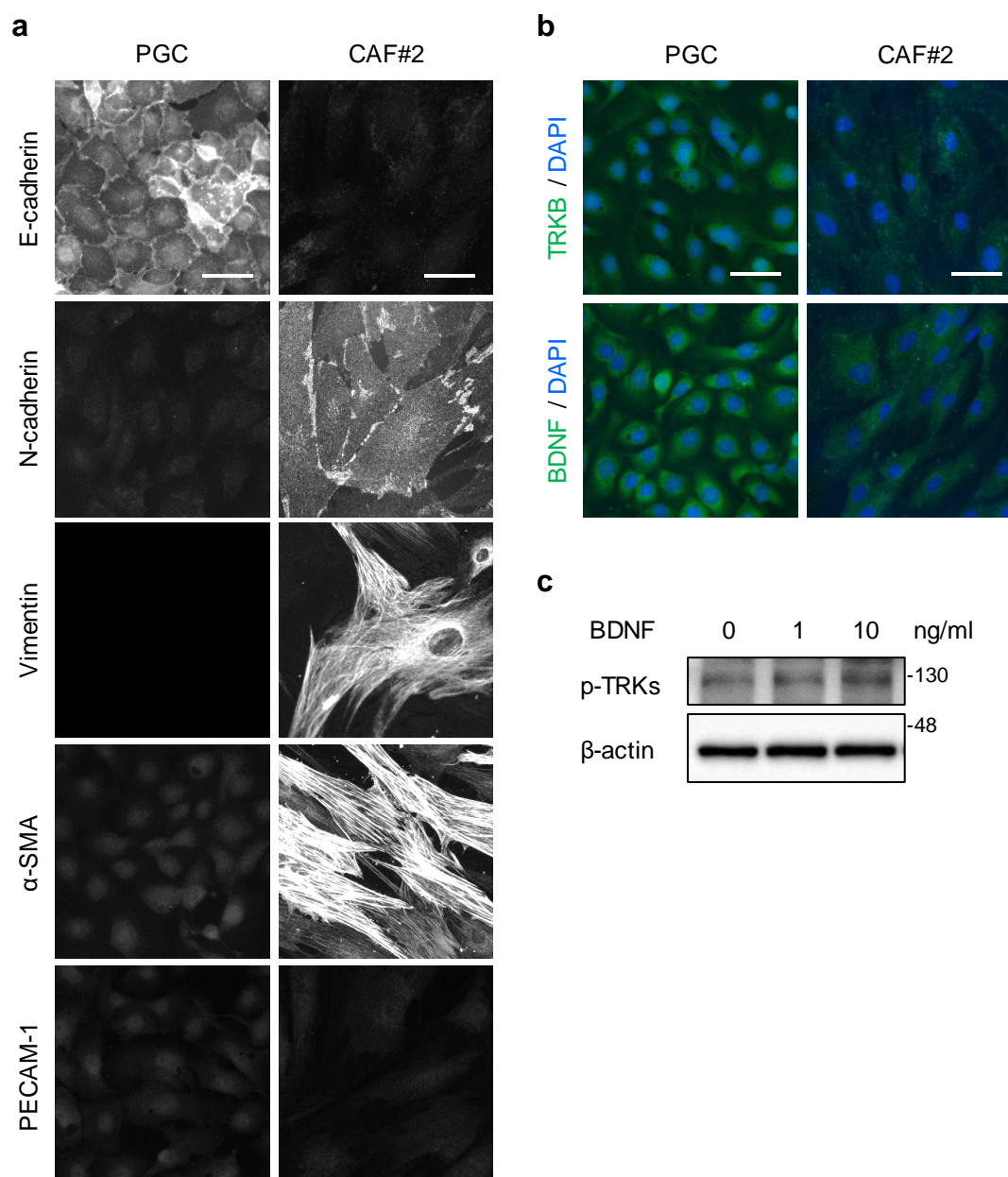
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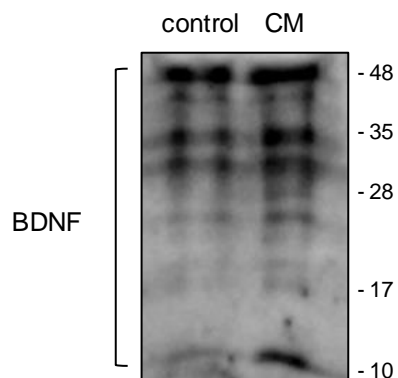
⁺These authors contributed equally to this work.

Supplementary Figure S1

**Supplementary Figure S1. Establishment of PGC cells and CAFs from patients with PGC.**

a. Characterization of established PGC cells and CAFs by immunofluorescence using the indicated antibodies. Scale bars, 50 μ m. **b.** Expression of TRKB and BDNF in PGC cells and CAFs was detected by immunofluorescence analysis. Scale bars, 50 μ m. **c.** BDNF-dependent phosphorylation of TRKB in PGC cells was determined by WB analysis using anti-phospho-TRK antibody (1:1000; #4619, Cell Signaling Technology). After 24 h of serum-starvation (0.5% FBS), PGC cells were stimulated with BDNF (0, 1, and 10 ng/ml) for 30 min.

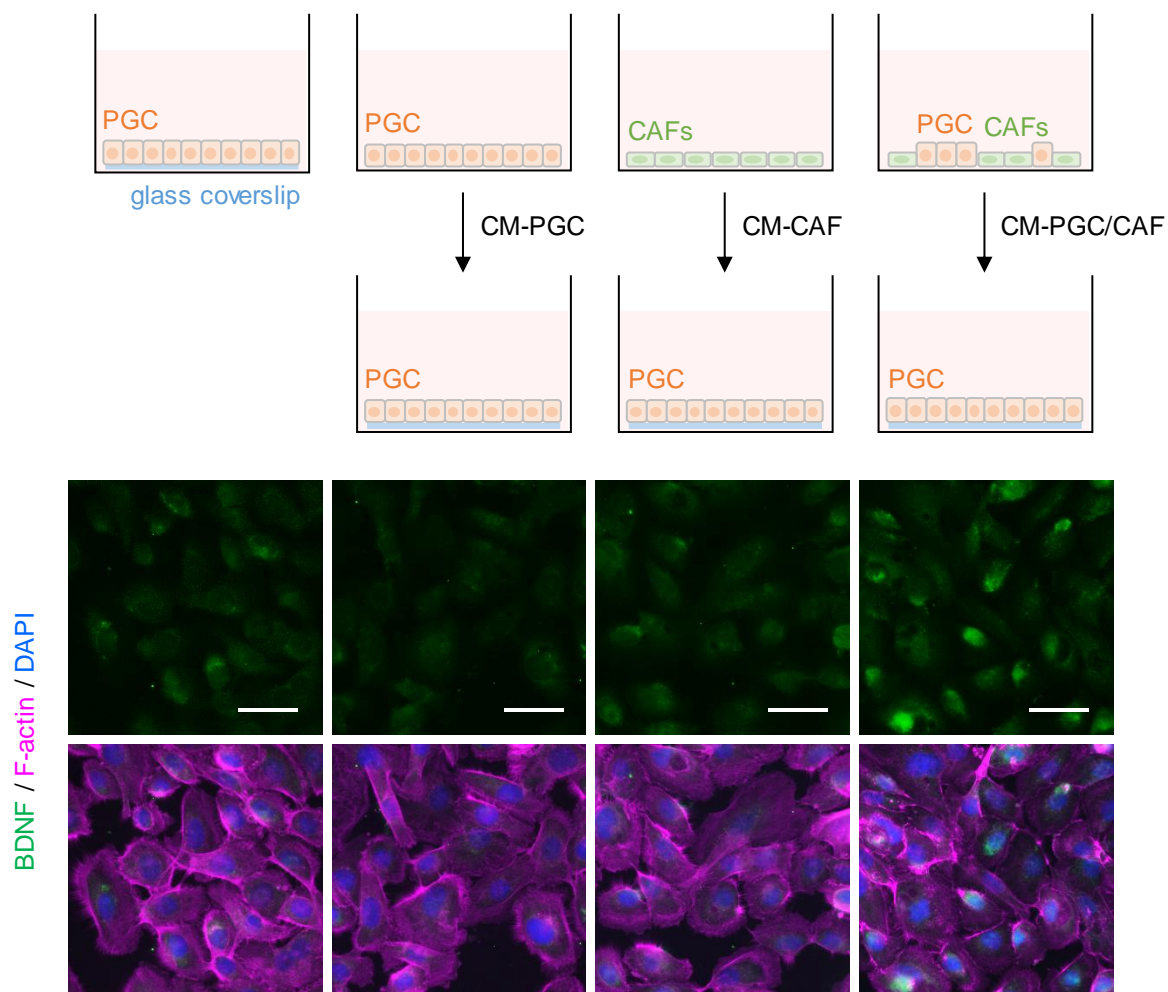
Supplementary Figure S2



Supplementary Figure S2. BDNF production in conditioned medium.

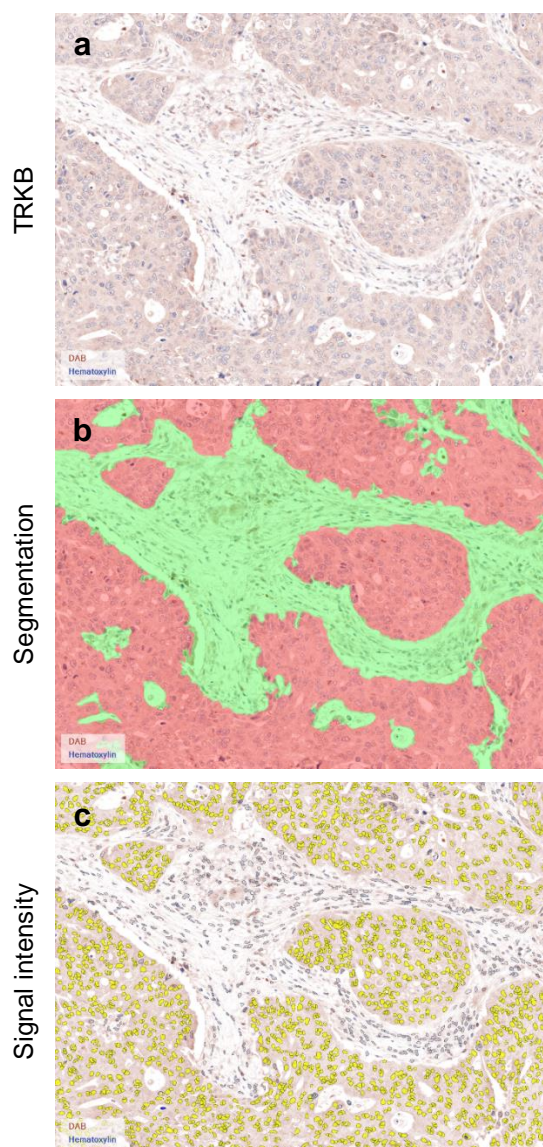
BDNF in CM from PGC/CAF co-culture was determined by WB analysis using anti-BDNF antibody. Basal medium (RPMI1640 with 10% FBS) cultured for 4 days in a cell culture incubator was used as control.

Supplementary Figure S3



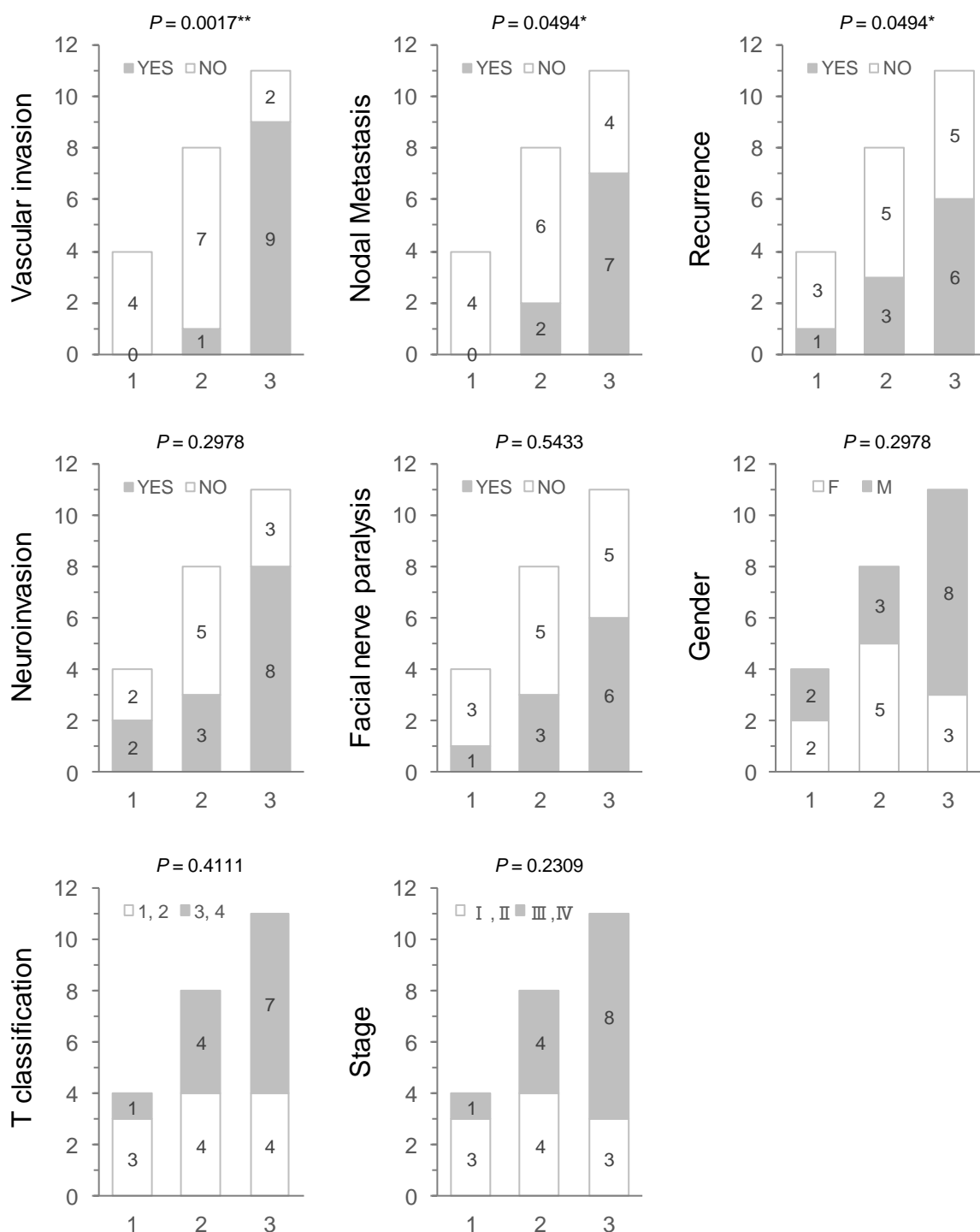
Supplementary Figure S3. Upregulation of BDNF in PGC cells directly or indirectly co-cultured with CAFs. BDNF expression in PGC cells was determined by immunofluorescence analysis. PGC cells were cultured for 4 days under the conditions described in the scheme. Scale bars, 50 μm. CM-PGC, cell-free conditioned medium from PGC cultures; CM-CAF, cell-free conditioned medium from CAF cultures; CM-PGC/CAF, cell-free conditioned medium from PGC/CAF co-cultures.

Supplementary Figure S4

**Supplementary Figure S4. Imaging analysis of immunohistochemistry by inForm software.**

a. Representative image of TRKB-staining tissue acquired using Vectra Polaris automated quantitative pathology imaging system. **b-c.** Tissue segmentation into carcinoma (red) and stromal areas (green) and the staining positivity (blue, negative; yellow, positive) were shown in (b) and (c), respectively.

Supplementary Figure S5



Supplementary Figure S5. Correlation between TRKB expression and clinical significance of patients with PGC (n = 23).

Histograms show the correlation between the TRKB-staining intensity which was classified into three levels (level 1 < 2 < 3, see Fig. 5b) and the clinical variables. Note, the number of patients with the poorest prognostic markers increased in a stepwise manner depending on TRKB expression levels. F, female; M, male. Statistical analyses were performed using Pearson's χ^2 test or Fisher's exact test. * $P < 0.05$; ** $P < 0.01$.

Supplementary Figure S6. Original images of Western blot.

Original images of Western blot or PVDF membrane in all experiments are shown below.

Fig. 2b. E-cadherin
Vimentin

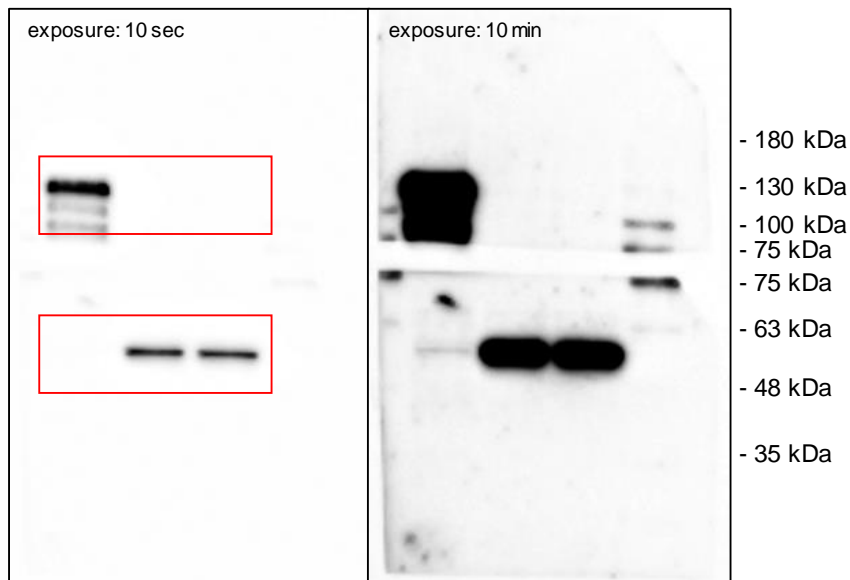


Fig. 2b. α -SMA

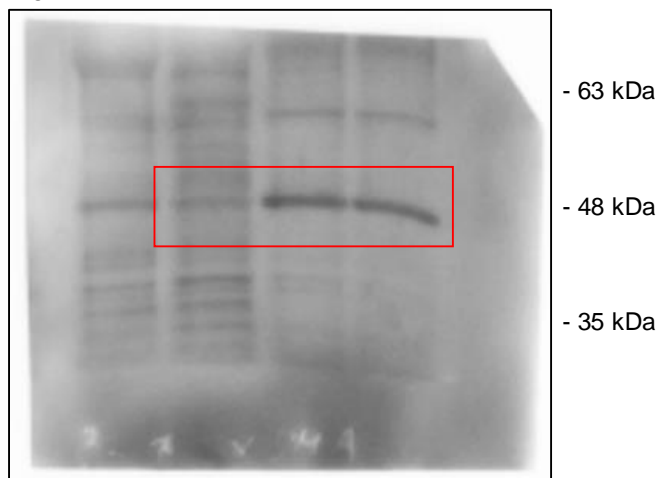


Fig. 2b. VE-cad



Fig. 2b. TRKB

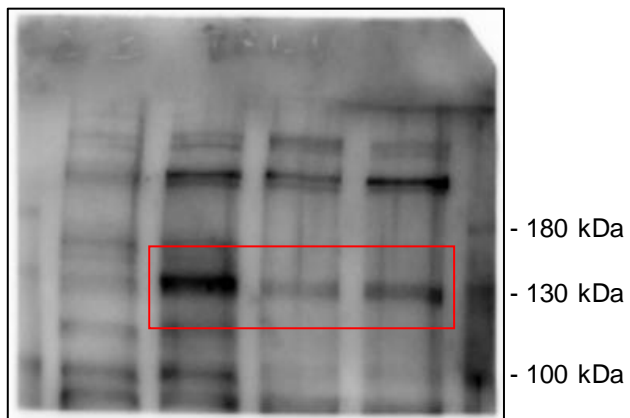


Fig. 2b. BDNF

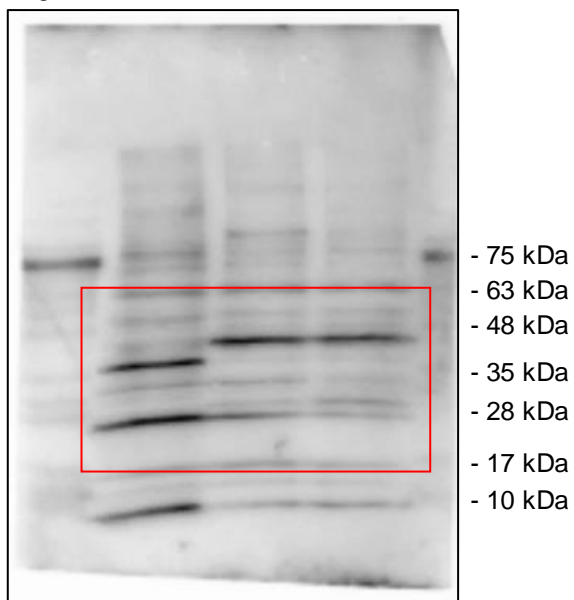


Fig. 2b. β -actin

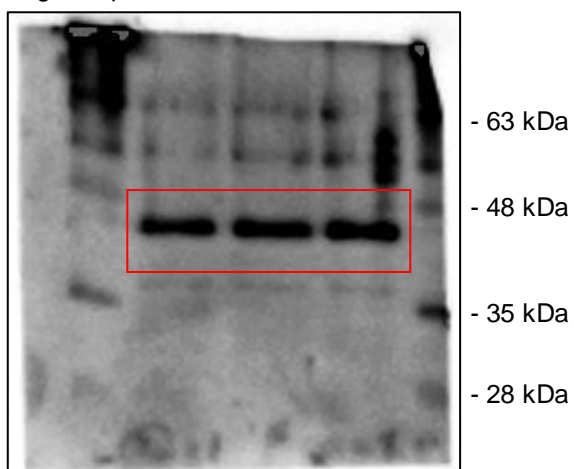


Fig. 2f. Snail

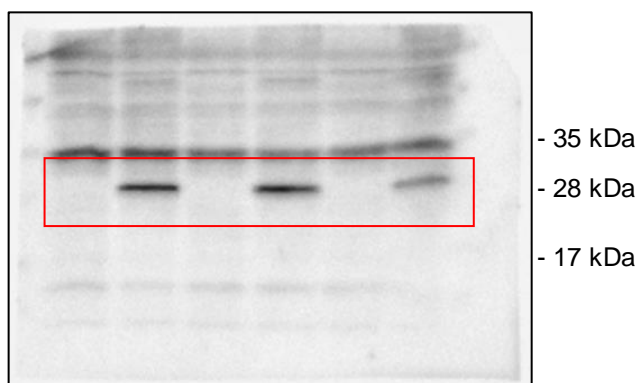


Fig. 2f. E-cadherin

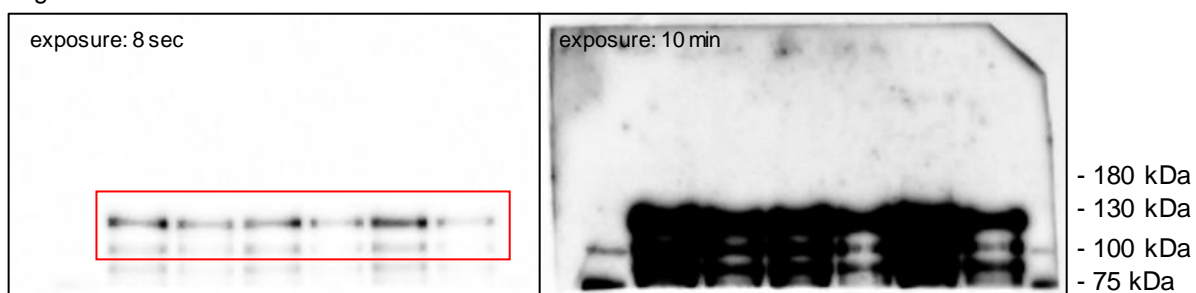


Fig. 2f. BDNF

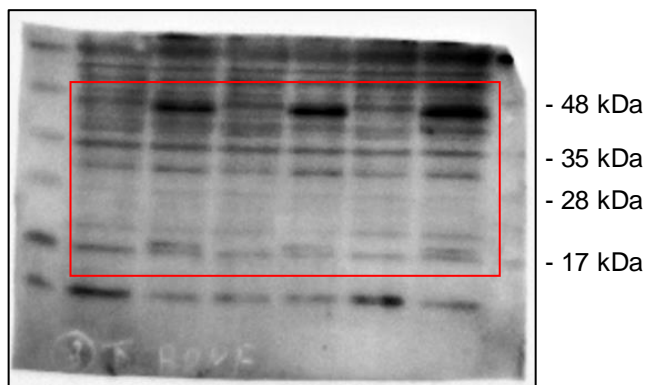


Fig. 2f. β -actin

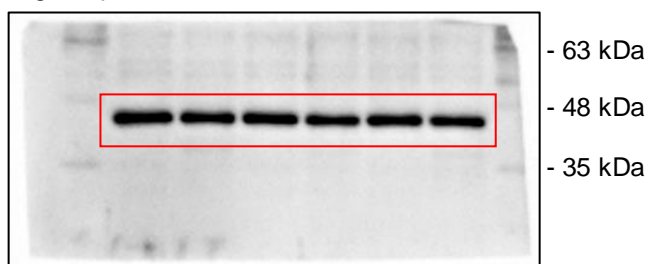


Fig. 2g. p-TRK

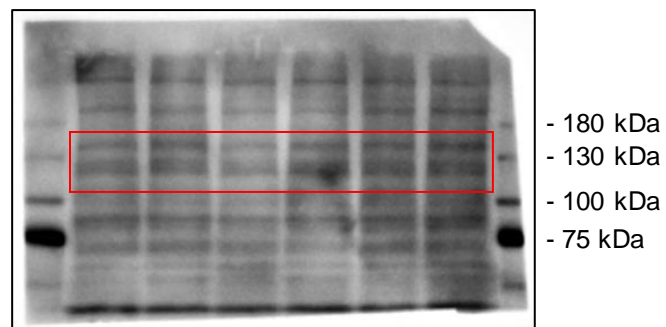


Fig. 2g. p-Akt

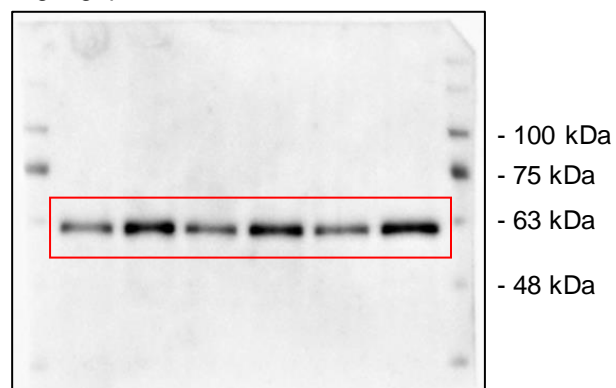


Fig. 2g. p-Erk1/2

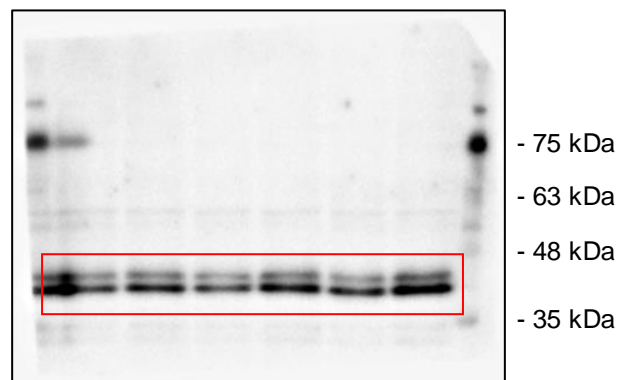


Fig. 2g. β -actin

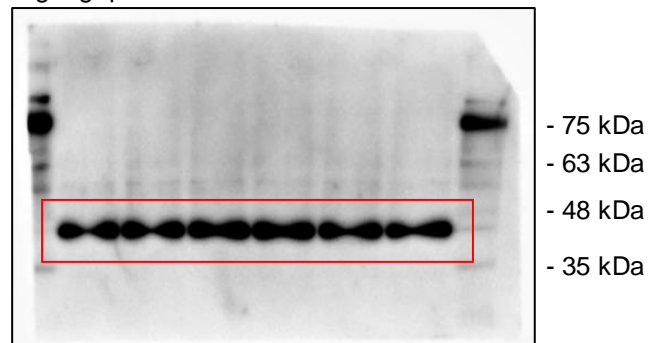


Fig. 3b. Snail

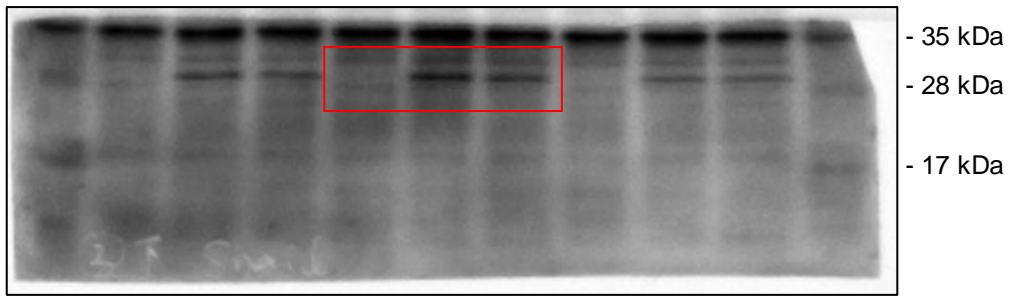


Fig. 3b. E-cadherin
 β -actin

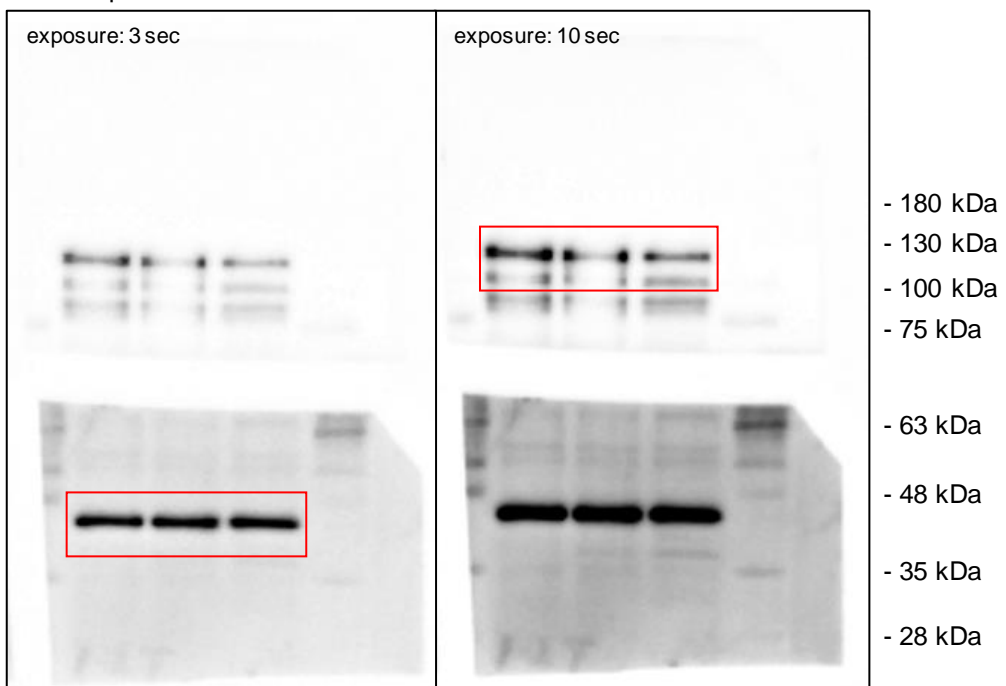


Fig. 4b. p-TRKs

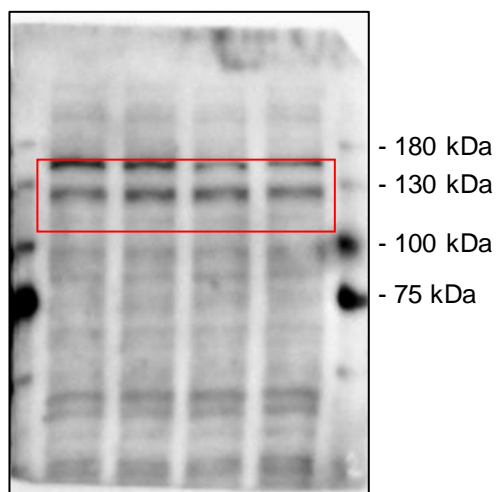


Fig. 4b. β -actin

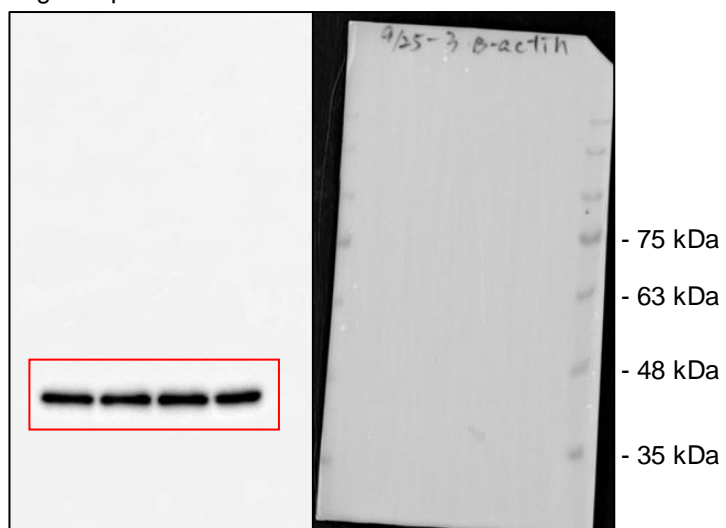


Fig. S1c. p-TRKs

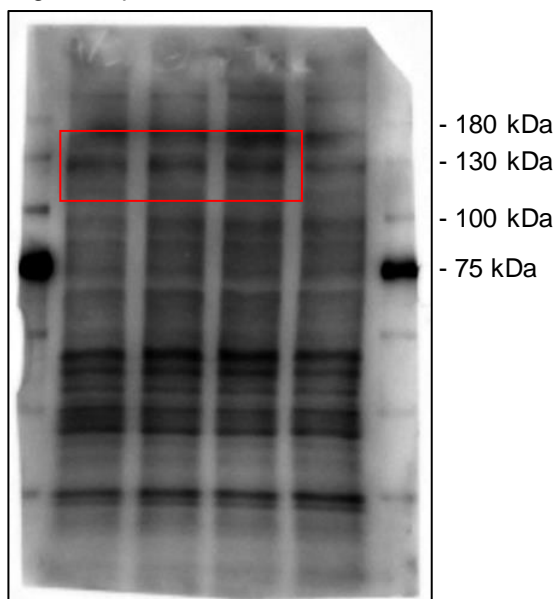


Fig. S1c. β -actin

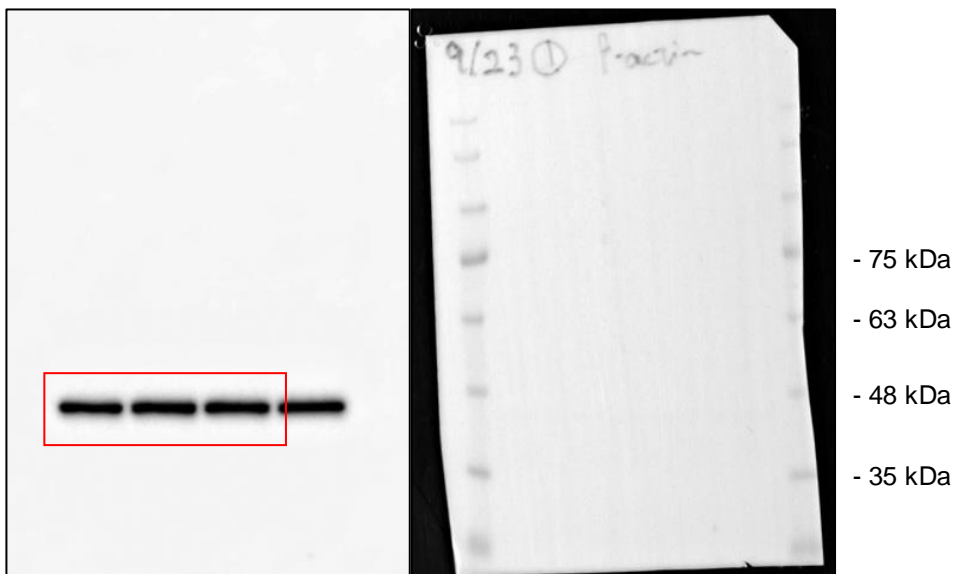


Fig. S2. BDNF

