

Supplementary Figure and Table

YKL40/Integrin β 4 Axis Induced by the Interaction between Cancer Cells and Tumor-Associated Macrophages Is Involved in the Progression of High-Grade Serous Ovarian Carcinoma

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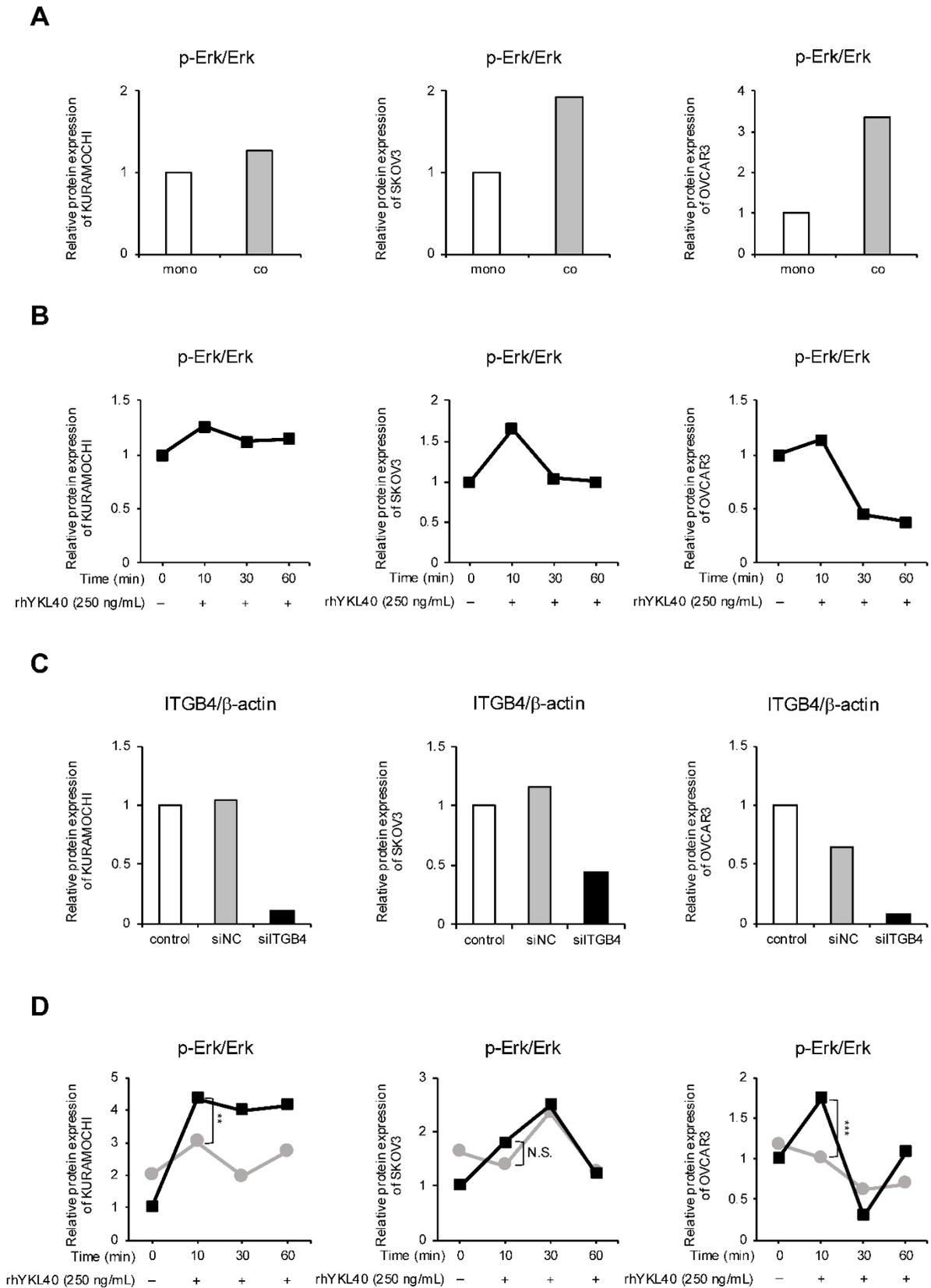
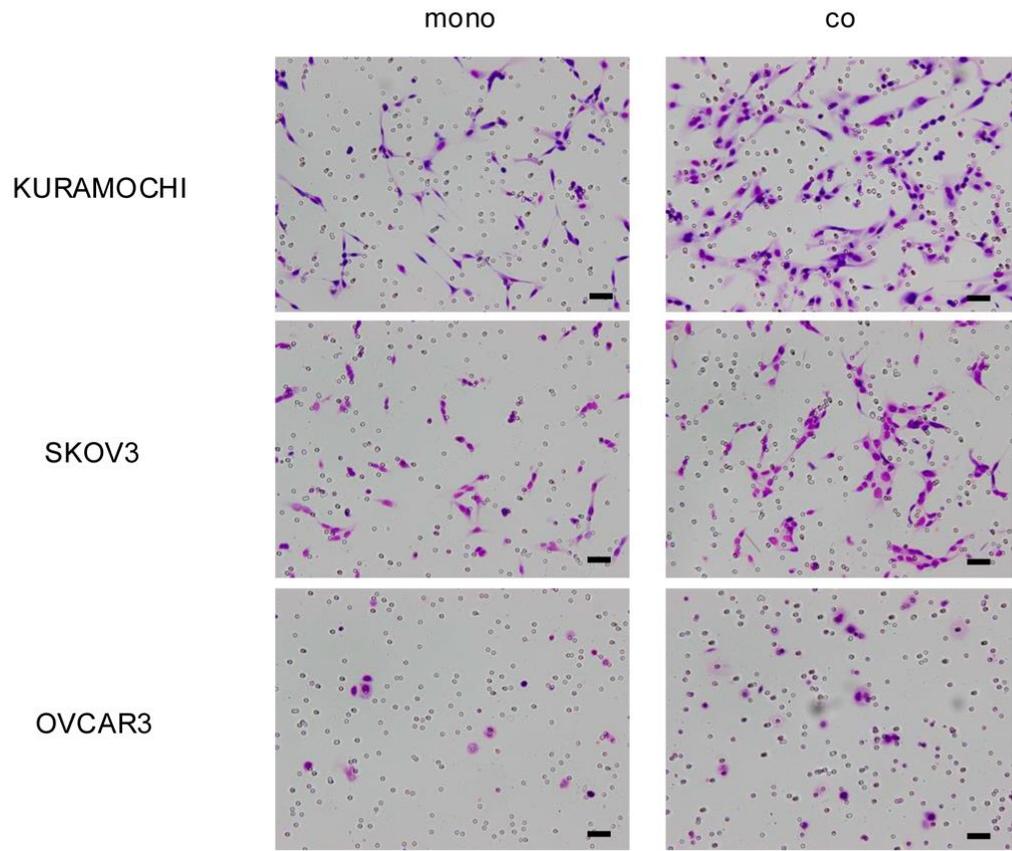


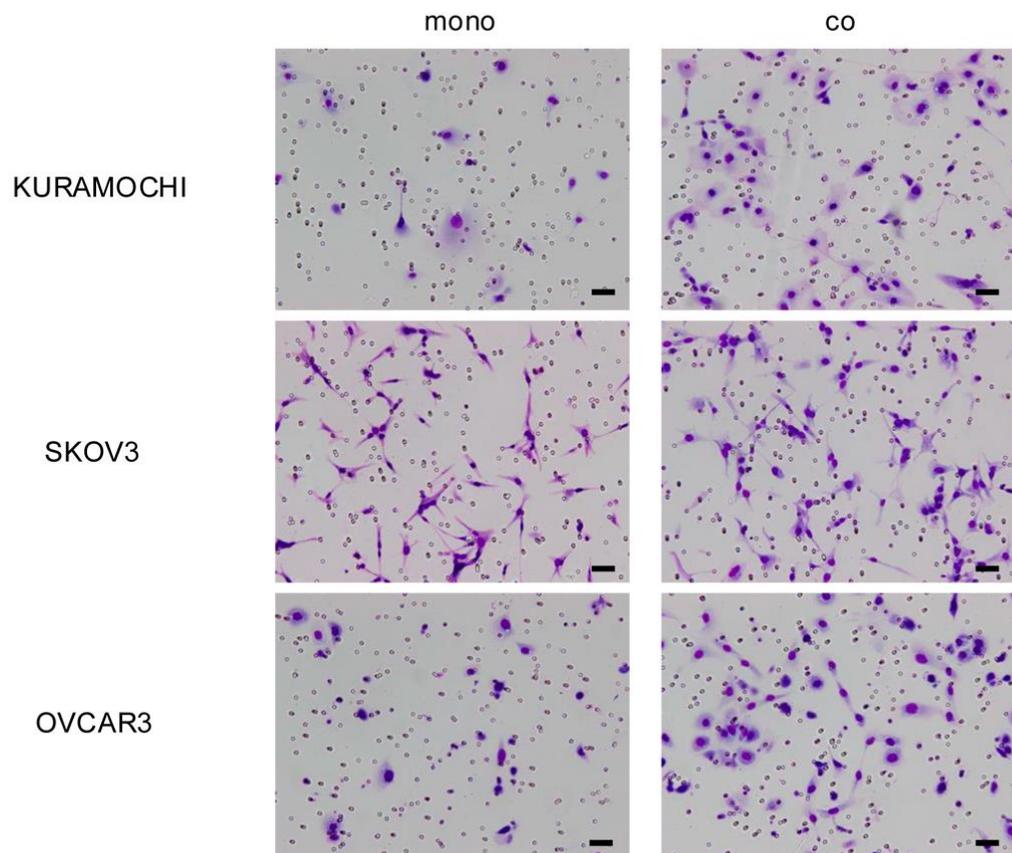
Figure S1 Quantification of bands from Western blotting shown in Figures 2C, 4A, 5B, and 5F. **A:** Expression levels of phosphorylated extracellular signal-regulated kinase (p-Erk) in epithelial ovarian cancer (EOC) cells monocultured and co-cultured with macrophages were normalized to total extracellular signal-regulated kinase (Erk) protein levels. **B:** Time-dependent expression

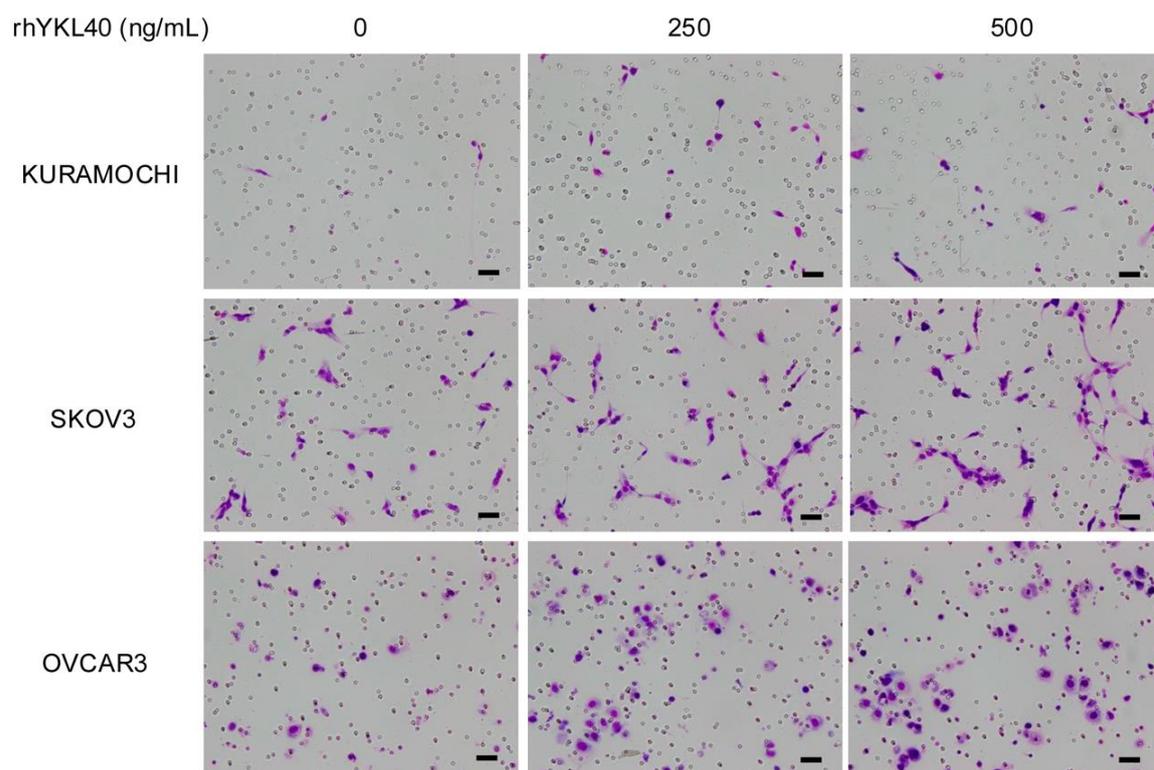
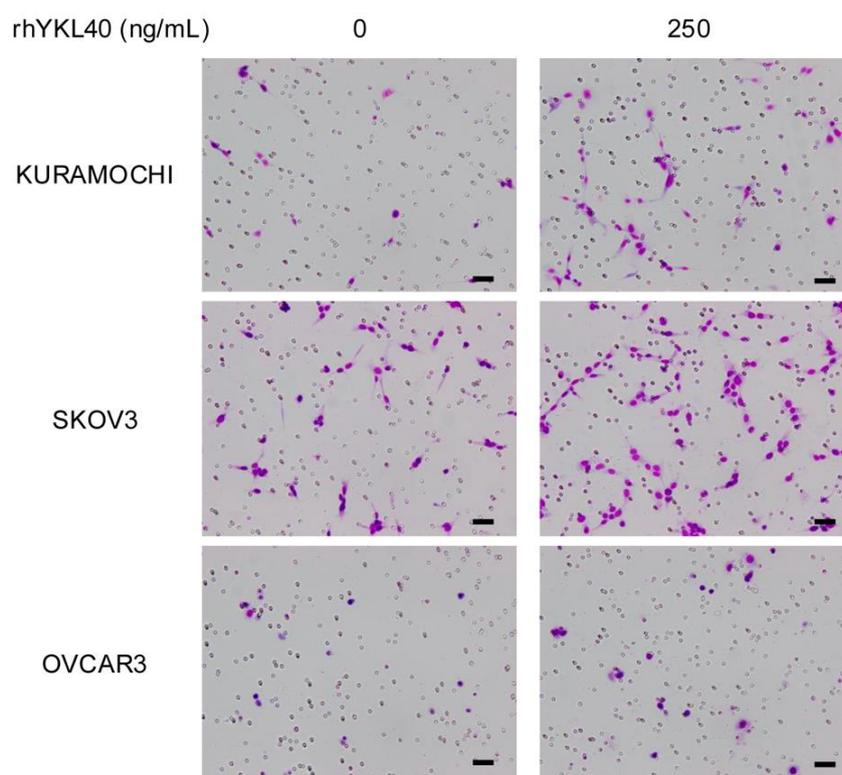
levels of p-Erk in EOC cells treated with recombinant human YKL40 (rhYKL40) were normalized to total Erk protein levels. **C:** Expression levels of integrin β 4 (ITGB4) in EOC cells, whether non-transfected (control) or transfected with negative control siRNA (siNC) or ITGB4-targeted siRNA (siITGB4), were normalized to β -actin expression levels. **D:** Expression levels of p-Erk in EOC cells were normalized to total Erk protein levels. Plots represented by squares indicate time-dependent expression levels in EOC cells transfected with siNC, whereas plots represented by circles indicate time-dependent expression levels in EOC cells transfected with siITGB4. $**P < 0.01$, $***P < 0.001$. N.S., not significant.

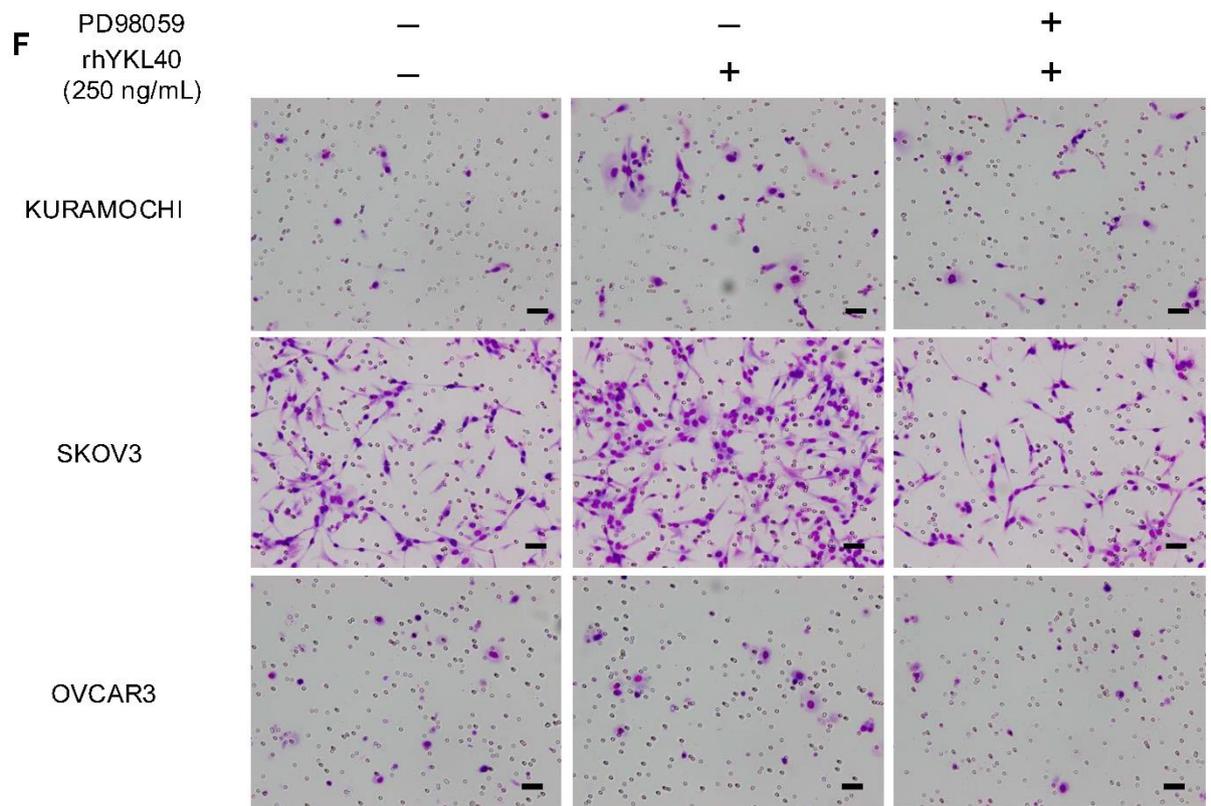
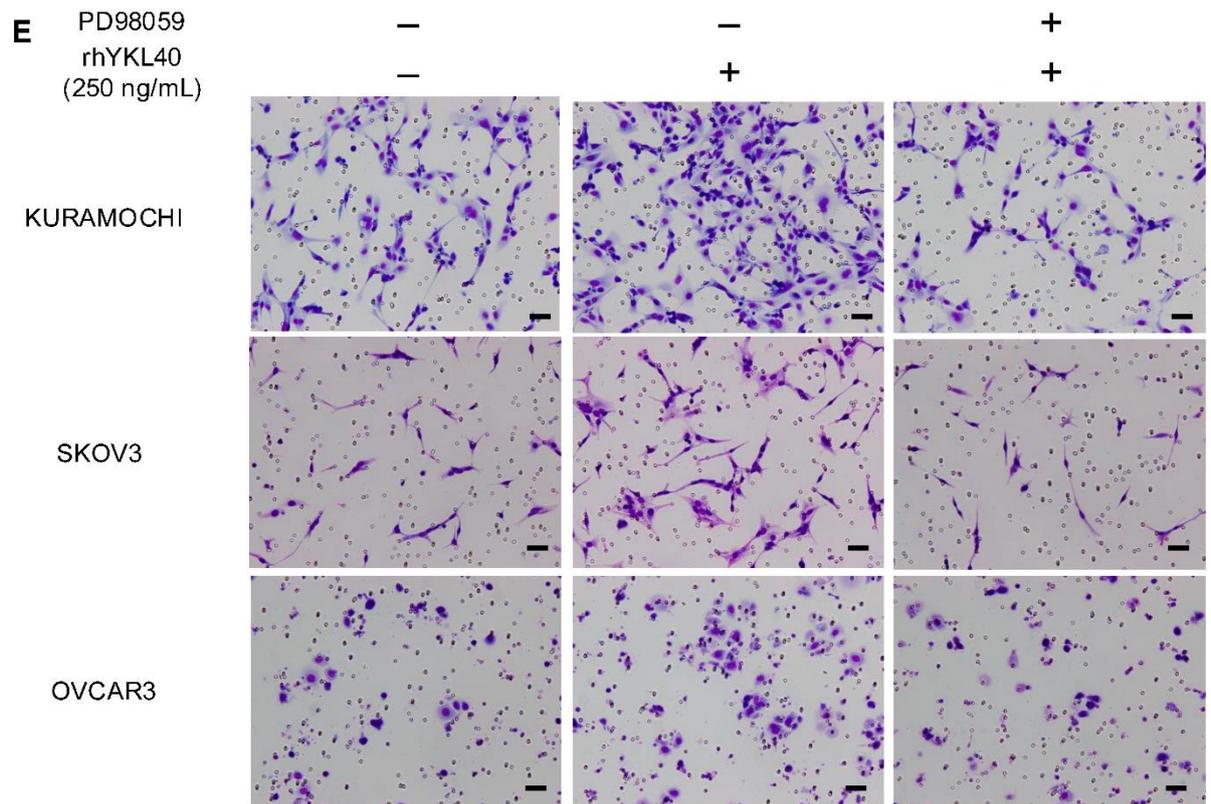
A



B



C**D**



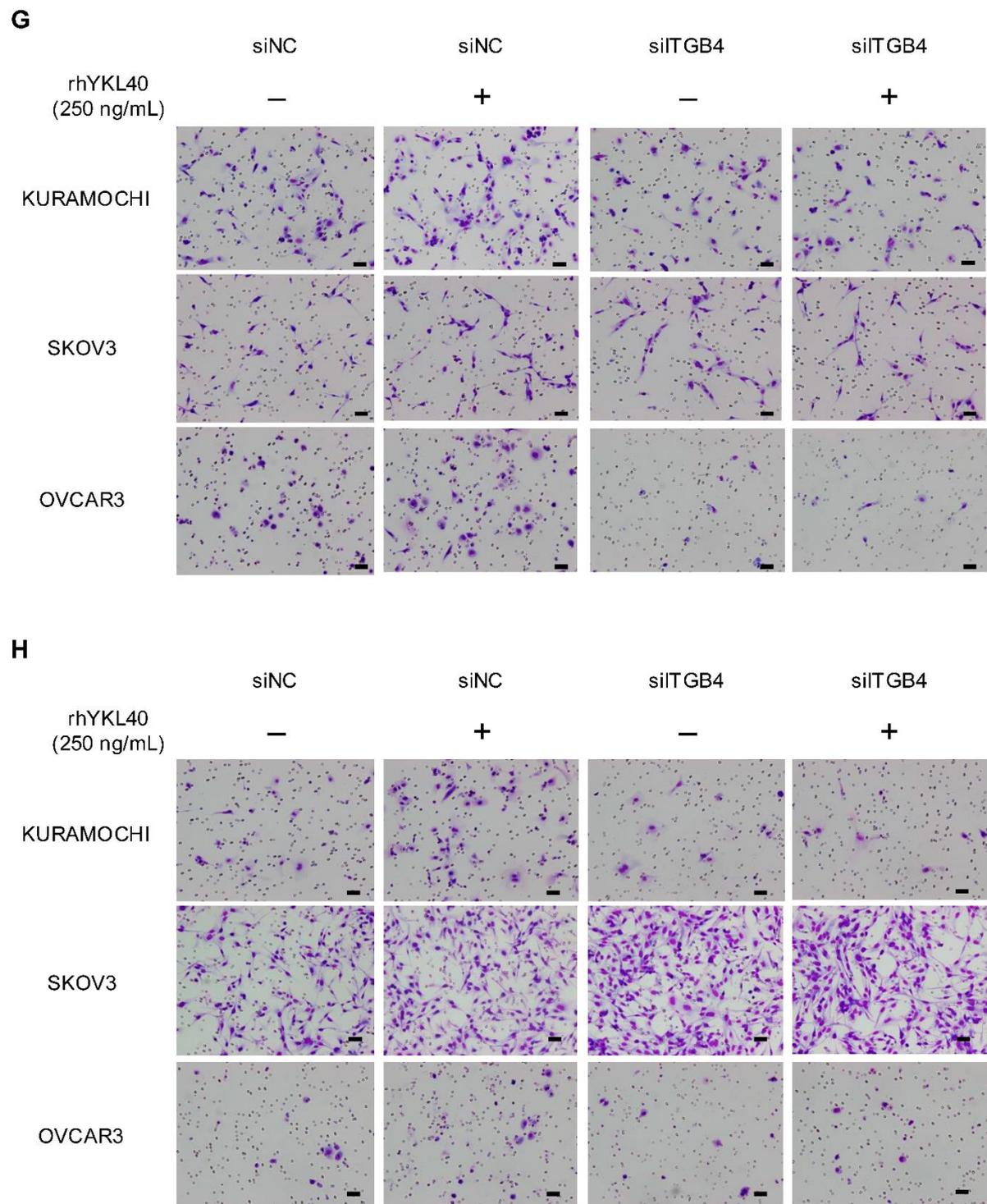


Figure S2 Representative images of transwell migration and invasion assays shown in Figures 2E (A), 2F (B), 3E (C), 3F (D), 4C (E), 4D (F), 5D (G), and 5E (H). Scale bar: 20 μ m.

| | 1,2 | 3,4 | 5,6 | 7,8 | 9,10 | 11,12 | 13,14 | 15,16 | 17,18 | 19,20 | 21,22 | 23,24 |
|---|-----------------|-------------|----------------|------------|----------------|----------------|---------------|---------------|---------------|-----------|----------|-------------------|
| A | Reference Spots | Adiponectin | Apolipoprotein | Angiogenin | Angiopoietin-1 | Angiopoietin-2 | BAFF | BDNF | C5/C5a | CD14 | CD30 | Reference Spots |
| B | | CD40 ligand | YKL-40 | Adipsin | CRP | Cripto-1 | Cystatin C | Dkk-1 | DPP4 | EGF | EMMPRIN | |
| C | | CXCL5 | Endoglin | Fas Ligand | FGF-2 | FGF-7 | FGF-19 | FLT3LG | G-CSF | GDF-15 | GM-CSF | |
| D | CXCL1 | GH | HGF | ICAM-1 | IFN- γ | IGFBP-2 | IGFBP-3 | IL-1 α | IL-1 β | IL-1ra | IL-2 | IL-3 |
| E | IL-4 | IL-5 | IL-6 | IL-8 | IL-10 | IL-11 | IL-12p70 | IL-13 | IL-15 | IL-16 | IL-17A | IL-18 Bpa |
| F | IL-19 | IL-22 | IL-23 | IL-24 | IL-27 | IL-31 | IL-32 | IL-33 | IL-34 | CXCL10 | CXCL11 | PSA |
| G | Leptin | LIF | Lipocalin-2 | CCL2 | CCL7 | M-CSF | MF | CXCL9 | CCL3/CCL4 | CCL20 | CCL19 | MMP9 |
| H | MPO | OPN | PDGF-AA | PDGF-AB | PTX3 | CXCL4 | RAGE | CCL5 | RBP4 | Relaxin-2 | Resistin | SDF-1 α |
| I | Serpin E1 | SHBG | ST2 | TARC | TFF3 | TIR | TGF- α | THBS1 | TNF- α | uPAR | VEGF | |
| J | Reference Spots | | VDB | CD31 | TIM-3 | VCAM-1 | | | | | | Negative controls |

Figure S3 Cytokine array used in this study. Coordinate of capture antibodies in Proteome Profiler Human XL Cytokine Array Kit. Green boxes show increased expression in supernatants of KURAMOCHI/macrophage co-culture compared with KURAMOCHI monoculture and macrophage monoculture.

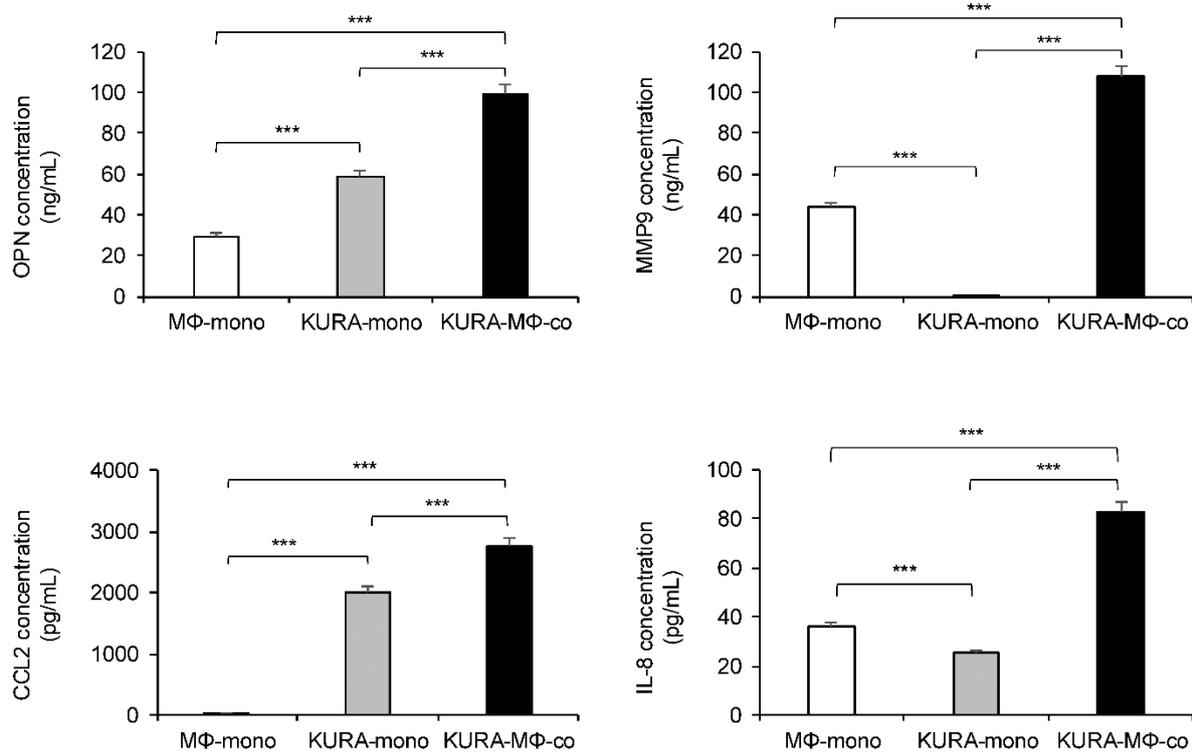
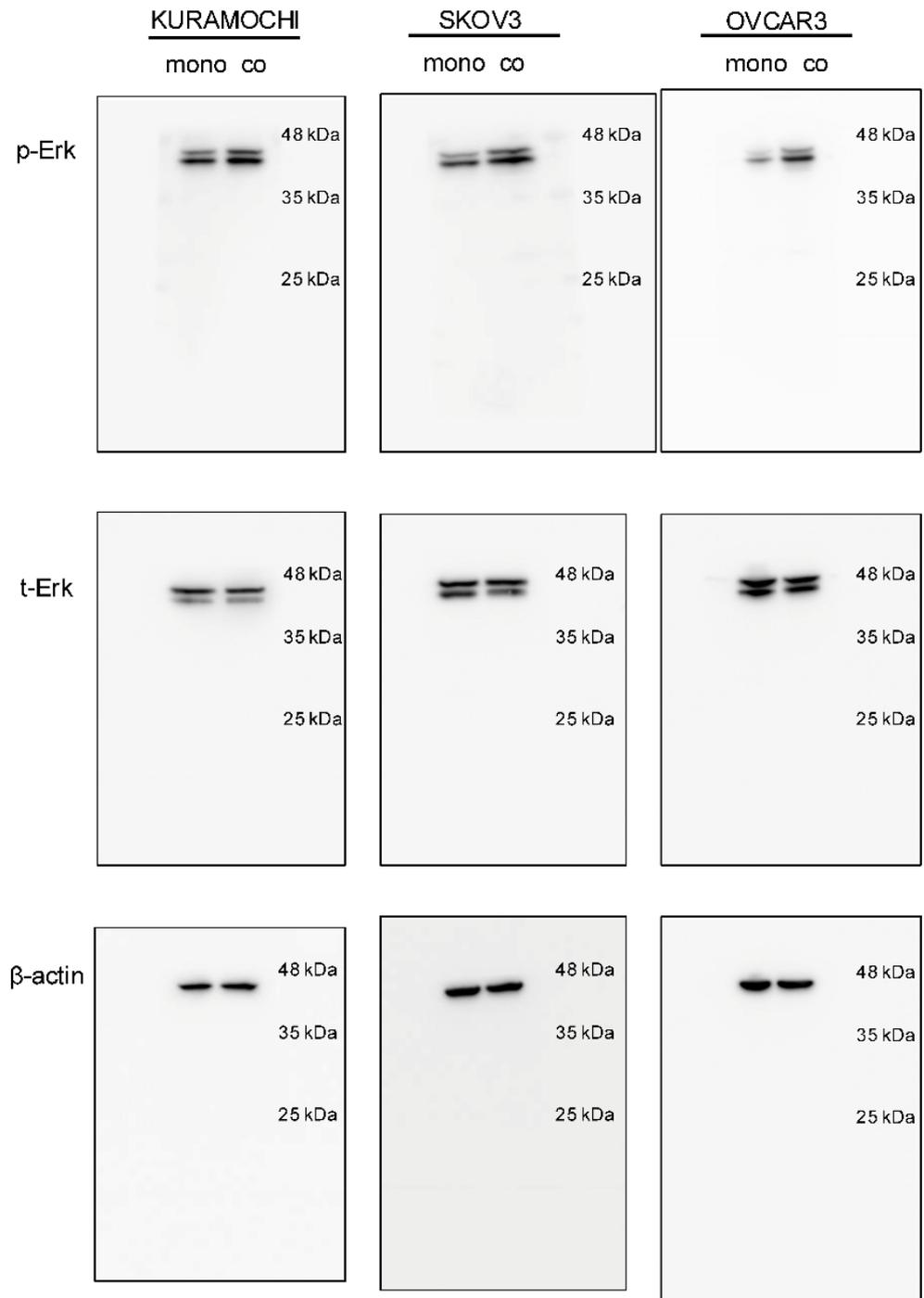
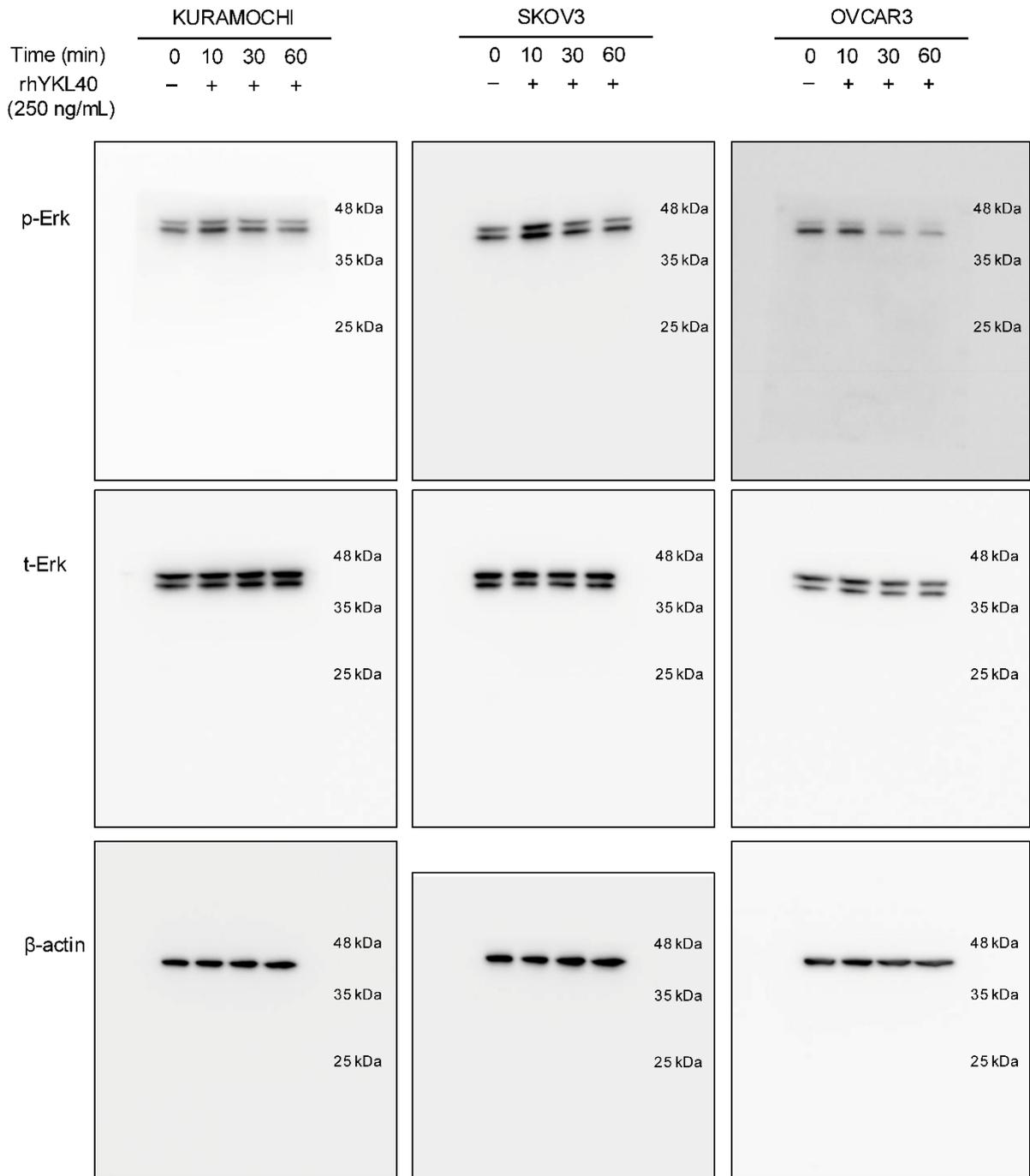


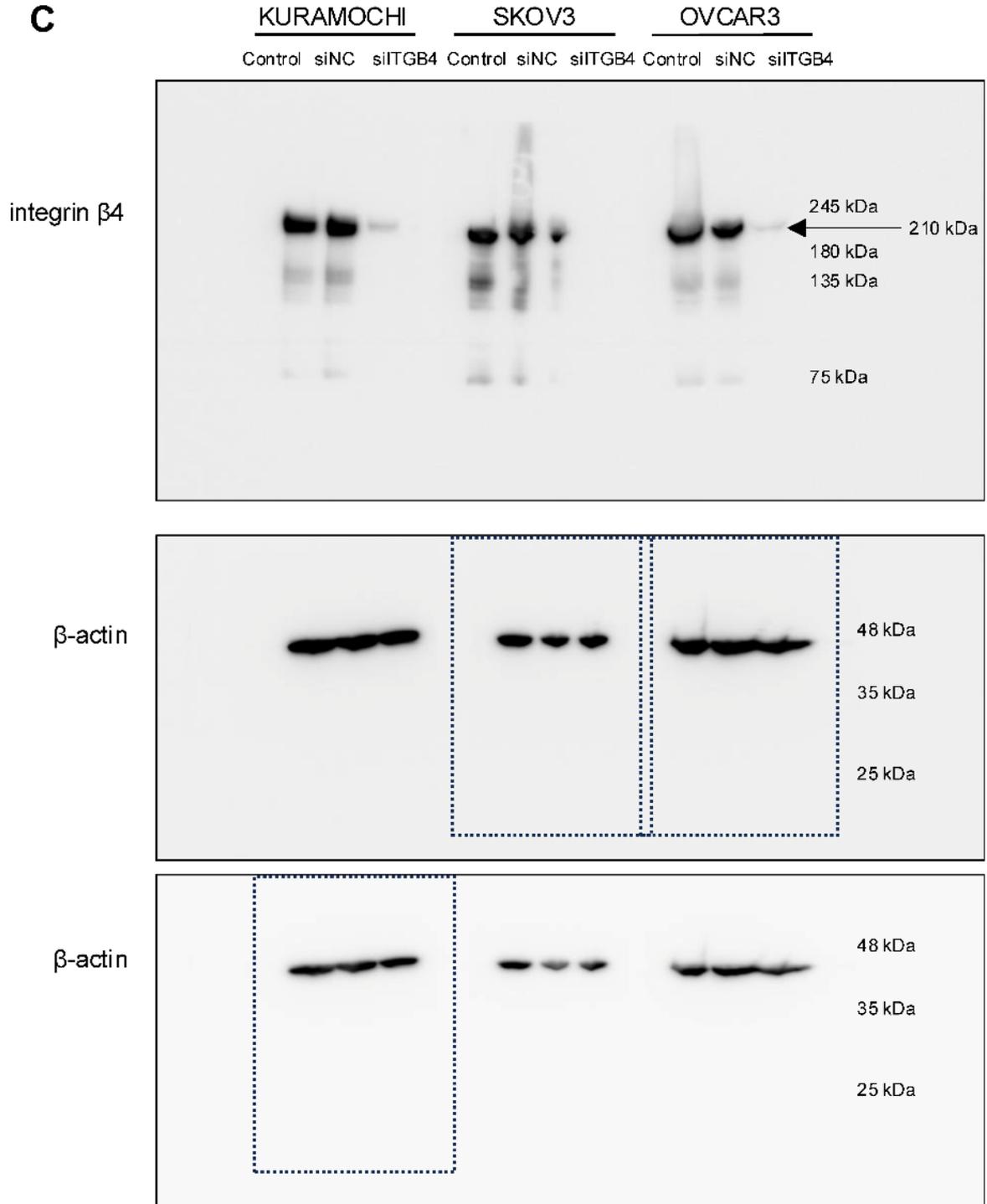
Figure S4 Increased secretion of osteopontin (OPN), interleukin (IL)-8, CCL2, and matrix metalloproteinase (MMP) 9 in KURAMOCHI/macrophage co-culture. ELISA was performed to investigate the secretion of OPN, IL-8, CCL2, and MMP9 in the supernatants of three different cultures: KURA-Mφ-co, KURAMOCHI/macrophage co-culture; KURA-mono, KURAMOCHI cell monoculture; Mφ, macrophage monoculture. *** $P < 0.001$.

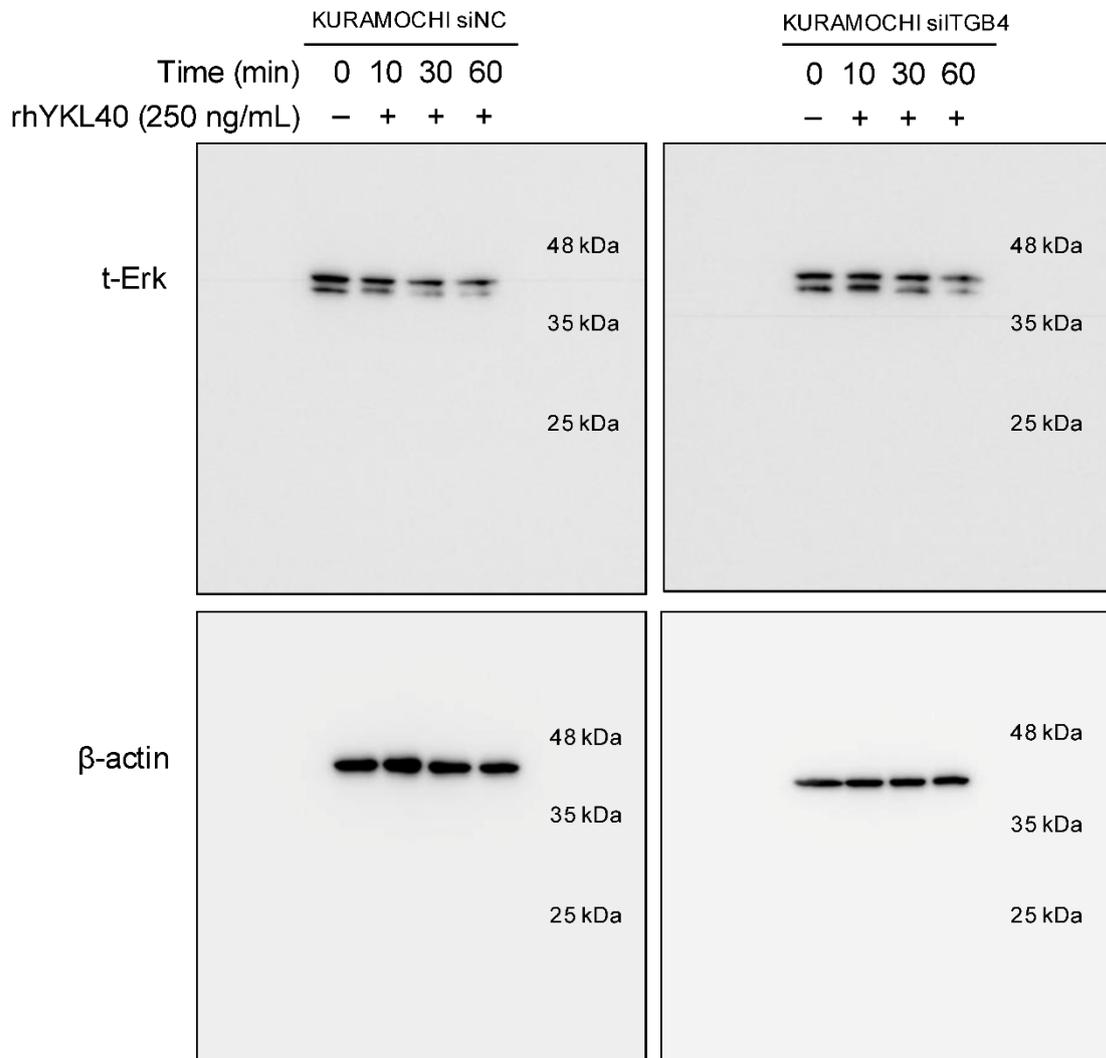
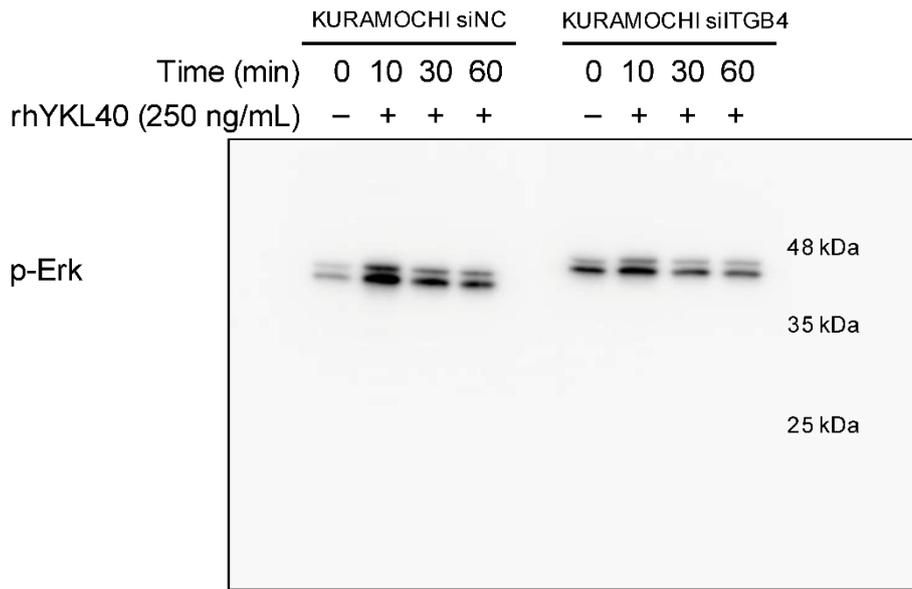
A



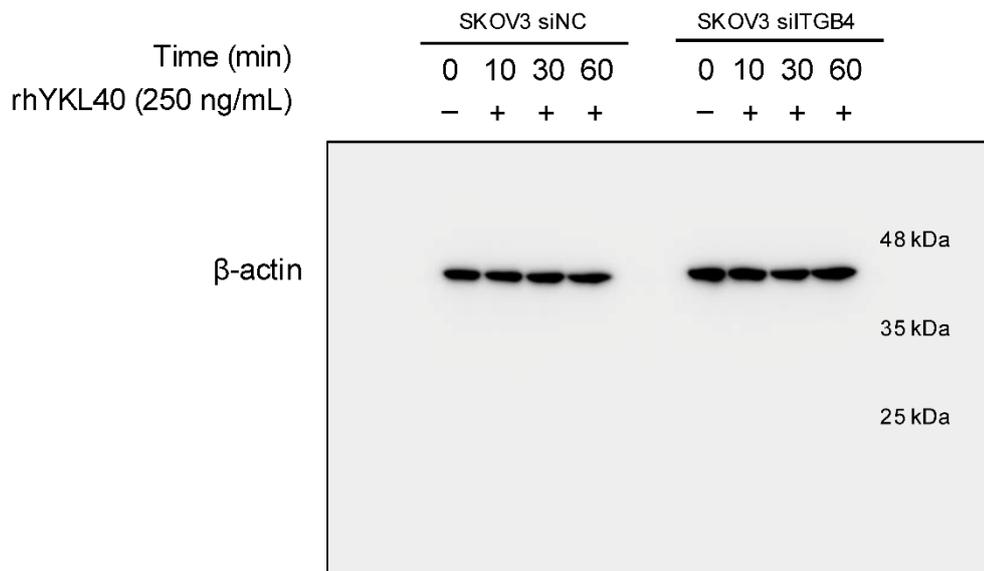
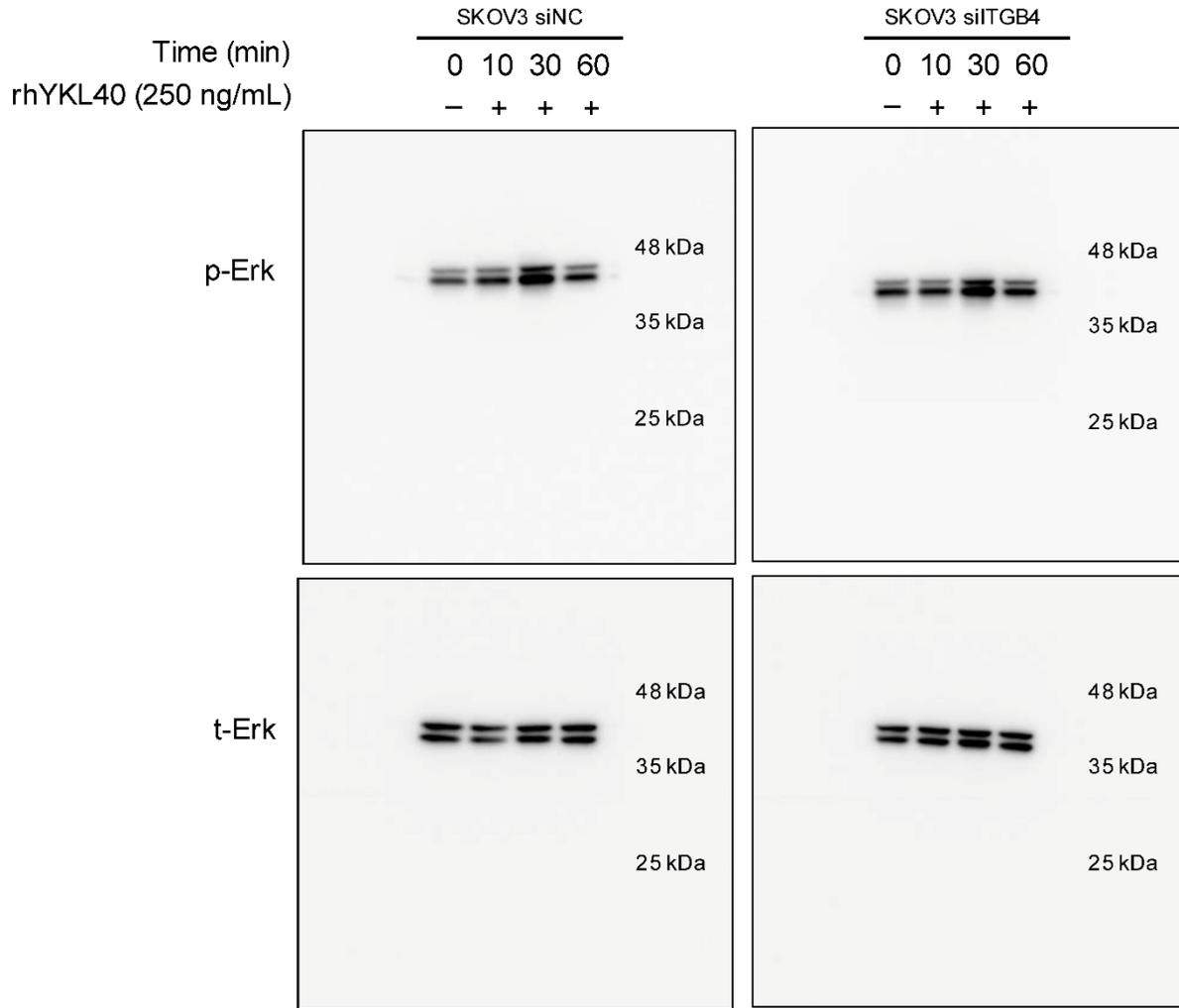
B

C



D

D



D

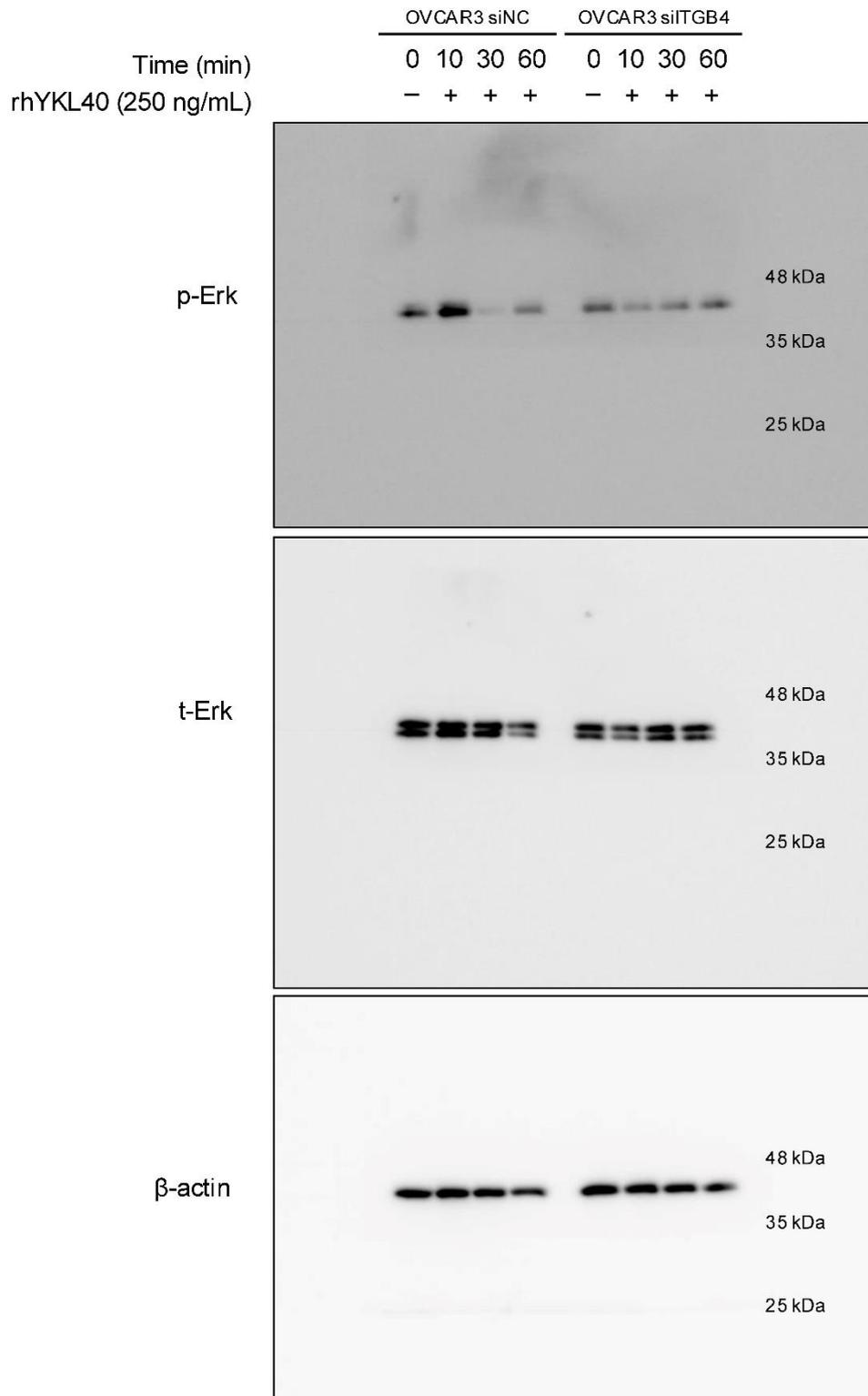


Figure S5 The original Western blotting images corresponding to Figures 2C, 4A, 5B, and 5F are provided as Figure S5A-D, respectively. The protein markers are not indicated on these original membranes.

Table S1 The sequences of the primers for qPCR.

| Primers | |
|---------|----------------------------------------|
| CD163 | Forward : 5'-CGAGTTAACGCCAGTAAG-3' |
| | Reverse : 5'-GAACATGTCACGCCAGC-3' |
| CD204 | Forward : 5'-CCAGGGACATGGGAATGCAA-3' |
| | Reverse : 5'-CCAGTGGGACCTCGATCTCC-3' |
| ITGB4 | Forward : 5'-GCTTCACACCTATTTCCCTGTC-3' |
| | Reverse : 5'-GACCCAGTCCTCGTCTTCTG-3' |
| GAPDH | Forward : 5'-GCACCGTCAAGCCTGAGAAT-3' |
| | Reverse : 5'-ATGGTGGTCAAGACGCCAGT-3' |