

TAZ contributes to pulmonary fibrosis by activating profibrotic functions of lung fibroblasts

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Supplementary Information

Supplementary figure legends

Supplementary Figure S1. TAZ expression in lung fibroblasts and epithelial cells.

(a) TAZ expression in lung fibroblasts and epithelial cells. Cap analysis gene expression (CAGE) data were obtained from the ZENBU database (<http://fantom.gsc.riken.jp/zenbu/>). Y-axis represents CAGE tag counts (tags per million: TPM). SAEs, Small airway epithelial cells; AECs, Alveolar epithelial cells.

(b) TAZ expression in lung fibroblasts derived from normal or idiopathic pulmonary fibrosis (IPF) lungs was analyzed using the GSE40839 microarray dataset. Probe set 202132_at. was used. * $P < 0.05$, Student's t -test.

Supplementary Figure S2. TAZ knockdown and myofibroblast phenotype in HFL-1 cells cultured on plastic tissue culture plates.

(a) TAZ and α -smooth muscle actin (α -SMA) expression was examined by immunofluorescence in HFL-1 cells seeded on plastic tissue culture plates (red and green, respectively). DAPI was used for nuclear staining (blue). Scale bar = 50 μ m. (b) HFL-1 cells were transfected with siNTC (control siRNA) or siTAZ (TAZ siRNA #1 and #2). Immunoblotting for TAZ was performed. GAPDH was used as the loading control. Molecular weight of each protein is indicated. (c) HFL-1 cells were transfected with siNTC (control siRNA) or siTAZ (TAZ siRNA #1 and #2) in the presence or absence of 5 ng/ml TGF- β stimulation. Immunoblotting for α -SMA was performed.

GAPDH was used as the loading control. Molecular weight of each protein is indicated.

Supplementary Figure S3. Scratch wound healing assay.

(a) HFL-1 cells transfected with siNTC (control siRNA) or siTAZ (TAZ siRNA #1 and #2) were grown at confluency, and wounds were generated by cell scratching. The distance of cell migration was measured after 24 h for each group. (b) Relative distance of cell migration compared to the control is shown ($n = 3$ for each group). Error bars represent standard deviations. $*P < 0.05$, Student's t -test.

Supplementary Figure S4. Venn diagram showing overlaps of TAZ-regulated genes in lung fibroblasts, those in epithelial or cancer cells, and transforming growth factor (TGF)- β -regulated genes in lung fibroblasts. The number in parentheses represents the total number of genes in each group.

Supplementary Figure S5. TAZ regulates the expression of gremlin 1.

(a) Relative expression levels of gremlin 1 (GREM1) in HFL-1 cells transfected with siNTC or siTAZ were analyzed by qRT-PCR. Error bars represent standard deviations. $*P < 0.05$, Student's t -test. (b) Correlation between TAZ and GREM1 expression was examined in lung tissues from patients with interstitial lung disease (ILD) in the Lung Genomics Research Consortium (LGRC) cohort. Spearman correlation coefficients (ρ) and the P value were calculated.

Supplementary Table S1. The commonly downregulated genes in HFL-1 cells transfected with TAZ siRNA #1 or #2.

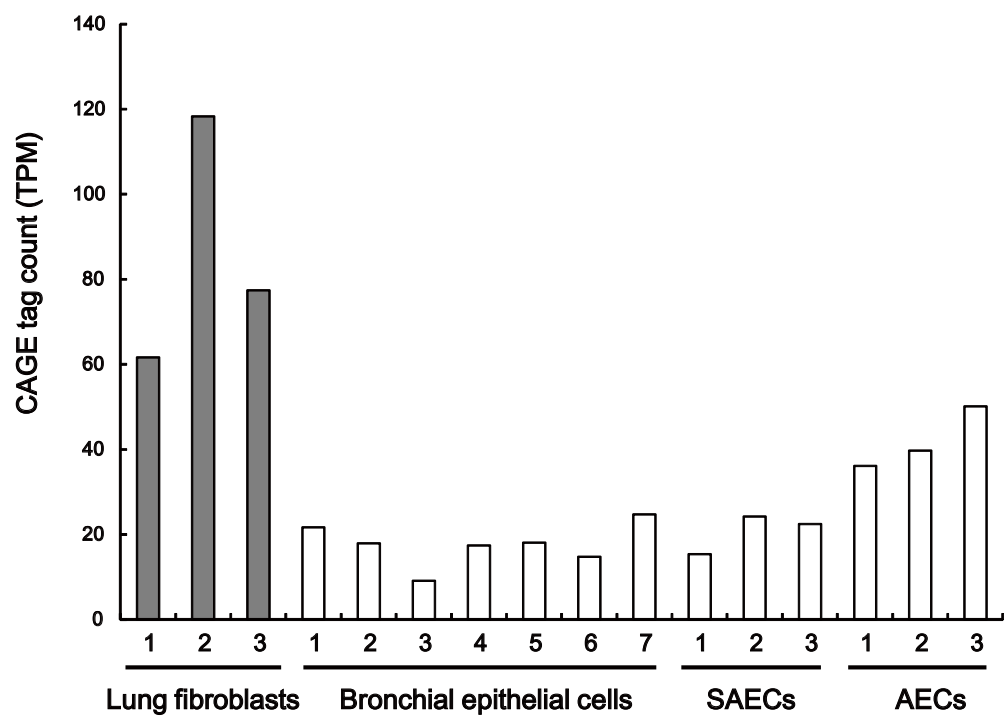
Gene symbol	Gene name
<i>ABCF1</i>	ATP-binding cassette, sub-family F (GCN20), member 1
<i>ABHD2</i>	abhydrolase domain containing 2
<i>ACSS1</i>	acyl-CoA synthetase short-chain family member 1
<i>ADAMTS15</i>	ADAM metalloproteinase with thrombospondin type 1 motif, 15
<i>ADH1B</i>	alcohol dehydrogenase 1B (class I)
<i>ADM2</i>	adrenomedullin 2
<i>AHNAK</i>	AHNAK nucleoprotein
<i>ALDH1L2</i>	aldehyde dehydrogenase 1 family, member L2
<i>ALDH6A1</i>	aldehyde dehydrogenase 6 family, member A1
<i>ANKRD13A</i>	ankyrin repeat domain 13A
<i>AOX1</i>	aldehyde oxidase 1
<i>ARID5B</i>	AT rich interactive domain 5B (MRF1-like)
<i>ARL6IP1</i>	ADP-ribosylation factor-like 6 interacting protein 1
<i>ASNS</i>	asparagine synthetase
<i>ATP2B4</i>	ATPase, Ca ⁺⁺ transporting, plasma membrane 4
<i>AURKAPS1</i>	aurora kinase A pseudogene 1
<i>B3GALT2</i>	beta 1,3-galactosyltransferase, polypeptide 2
<i>BCYRN1</i>	brain cytoplasmic RNA 1 (non-protein coding)
<i>C20orf194</i>	chromosome 20 open reading frame 194
<i>CAV1</i>	caveolin 1, caveolae protein, 22kDa
<i>CBX5</i>	chromobox homolog 5 (HP1 alpha homolog, Drosophila)
<i>CCBE1</i>	collagen and calcium binding EGF domains 1
<i>CDC25B</i>	cell division cycle 25 homolog B (S. pombe)
<i>CELF2</i>	CUG triplet repeat, RNA binding protein 2
<i>CHSY1</i>	chondroitin sulfate synthase 1
<i>COL1A2</i>	collagen, type I, alpha 2
<i>CORO1C</i>	coronin, actin binding protein, 1C
<i>COX15</i>	COX15 homolog, cytochrome c oxidase assembly protein (yeast)
<i>CRIM1</i>	cysteine rich transmembrane BMP regulator 1 (chordin-like)
<i>CTGF</i>	connective tissue growth factor

<i>CXCL12</i>	chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)
<i>DAB2</i>	disabled homolog 2, mitogen-responsive phosphoprotein (Drosophila)
<i>DDIT4</i>	DNA-damage-inducible transcript 4
<i>DOCK5</i>	dedicator of cytokinesis 5
<i>EIF1AX</i>	eukaryotic translation initiation factor 1A, X-linked
<i>ELL2</i>	elongation factor, RNA polymerase II, 2
<i>ELOVL2</i>	elongation of very long chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 2
<i>EMG1</i>	EMG1 nucleolar protein homolog (S. cerevisiae)
<i>FAM107B</i>	family with sequence similarity 107, member B
<i>FOXM1</i>	forkhead box M1
<i>FST</i>	follostatin
<i>FSTL1</i>	follostatin-like 1
<i>GARS</i>	glycyl-tRNA synthetase
<i>GAS5</i>	growth arrest-specific 5 (non-protein coding)
<i>GDAP1</i>	ganglioside-induced differentiation-associated protein 1
<i>GFRA1</i>	GDNF family receptor alpha 1
<i>GREM1</i>	gremlin 1, cysteine knot superfamily, homolog (Xenopus laevis)
<i>GRPR</i>	gastrin-releasing peptide receptor
<i>GUCY1A2</i>	guanylate cyclase 1, soluble, alpha 2
<i>GUCY1B3</i>	guanylate cyclase 1, soluble, beta 3
<i>HADHA</i>	hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), alpha subunit
<i>HDDC3</i>	HD domain containing 3
<i>HEBP1</i>	heme binding protein 1
<i>HIST1H2BK</i>	histone cluster 1, H2bk
<i>HMGN2</i>	high-mobility group nucleosomal binding domain 2
<i>HSPB6</i>	heat shock protein, alpha-crystallin-related, B6
<i>IGFBP3</i>	insulin-like growth factor binding protein 3
<i>IL6R</i>	interleukin 6 receptor
<i>IMPDH2</i>	IMP (inosine monophosphate) dehydrogenase 2
<i>INHBE</i>	inhibin, beta E
<i>KCNE4</i>	potassium voltage-gated channel, Isk-related family, member 4
<i>KCNJ2</i>	potassium inwardly-rectifying channel, subfamily J, member 2
<i>KCNN2</i>	potassium intermediate/small conductance calcium-activated channel, subfamily N, member 2

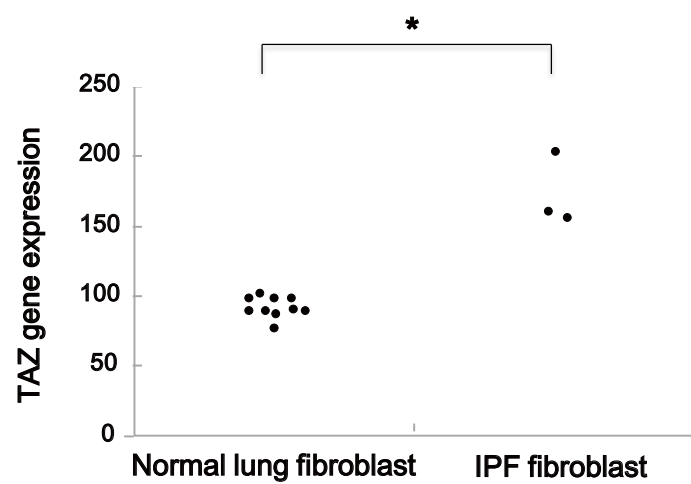
<i>KIAA1644</i>	KIAA1644
<i>MASP1</i>	mannan-binding lectin serine peptidase 1 (C4/C2 activating component of Ra-reactive factor)
<i>MATN2</i>	matrilin 2
<i>MICALL1</i>	MICAL-like 1
<i>MOV10</i>	Mov10, Moloney leukemia virus 10, homolog (mouse)
<i>NAV2</i>	neuron navigator 2
<i>PCK2</i>	phosphoenolpyruvate carboxykinase 2 (mitochondrial)
<i>PHACTR4</i>	phosphatase and actin regulator 4
<i>PMP22</i>	peripheral myelin protein 22
<i>PNPO</i>	pyridoxamine 5'-phosphate oxidase
<i>PPAP2B</i>	phosphatidic acid phosphatase type 2B
<i>PPP1R3C</i>	protein phosphatase 1, regulatory (inhibitor) subunit 3C
<i>PRSS23</i>	protease, serine, 23
<i>RBMS2</i>	RNA binding motif, single stranded interacting protein 2
<i>RELN</i>	reelin
<i>RGMB</i>	RGM domain family, member B
<i>RLIM</i>	ring finger protein, LIM domain interacting
<i>RPL23P8</i>	ribosomal protein L23 pseudogene 8
<i>S100A10</i>	S100 calcium binding protein A10
<i>SH3D19</i>	SH3 domain containing 19
<i>SLC7A14</i>	solute carrier family 7 (cationic amino acid transporter, y ⁺ system), member 14
<i>SLC7A5</i>	solute carrier family 7 (cationic amino acid transporter, y ⁺ system), member 5
<i>SULF1</i>	sulfatase 1
<i>TGFBR3</i>	transforming growth factor, beta receptor III
<i>TMBIM6</i>	transmembrane BAX inhibitor motif containing 6
<i>TRIM25</i>	tripartite motif-containing 25
<i>TXNIP</i>	thioredoxin interacting protein
<i>WASF2</i>	WAS protein family, member 2
<i>WNT2</i>	wingless-type MMTV integration site family member 2
<i>WWTR1</i>	WW domain containing transcription regulator 1
<i>XPB1</i>	X-box binding protein 1

Supplementary Figure S1

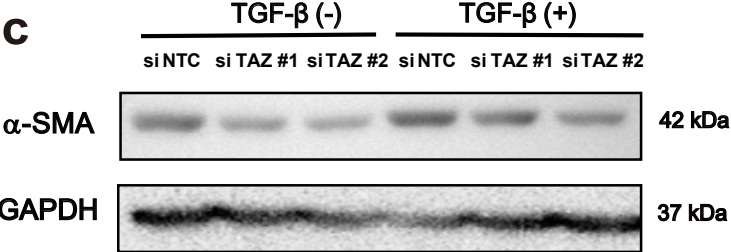
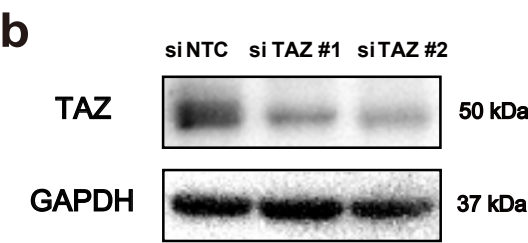
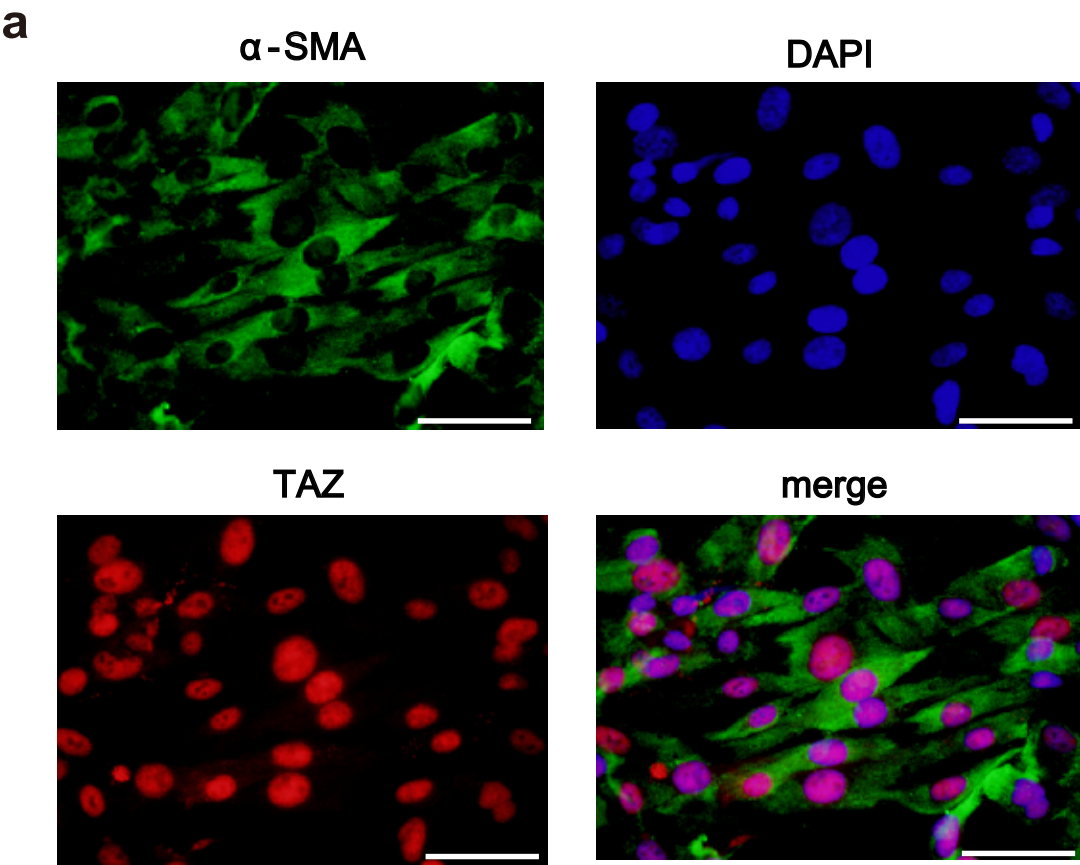
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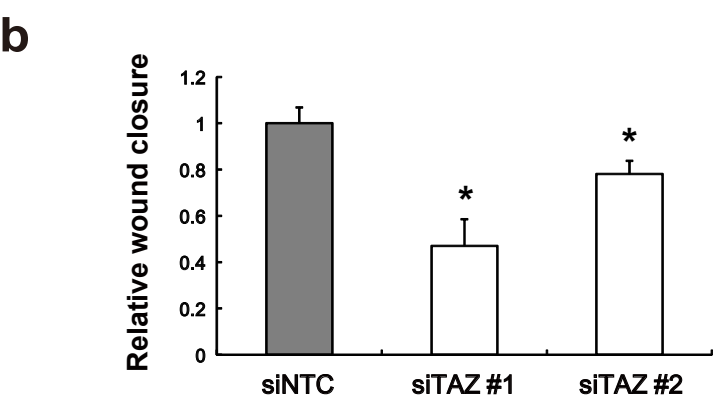
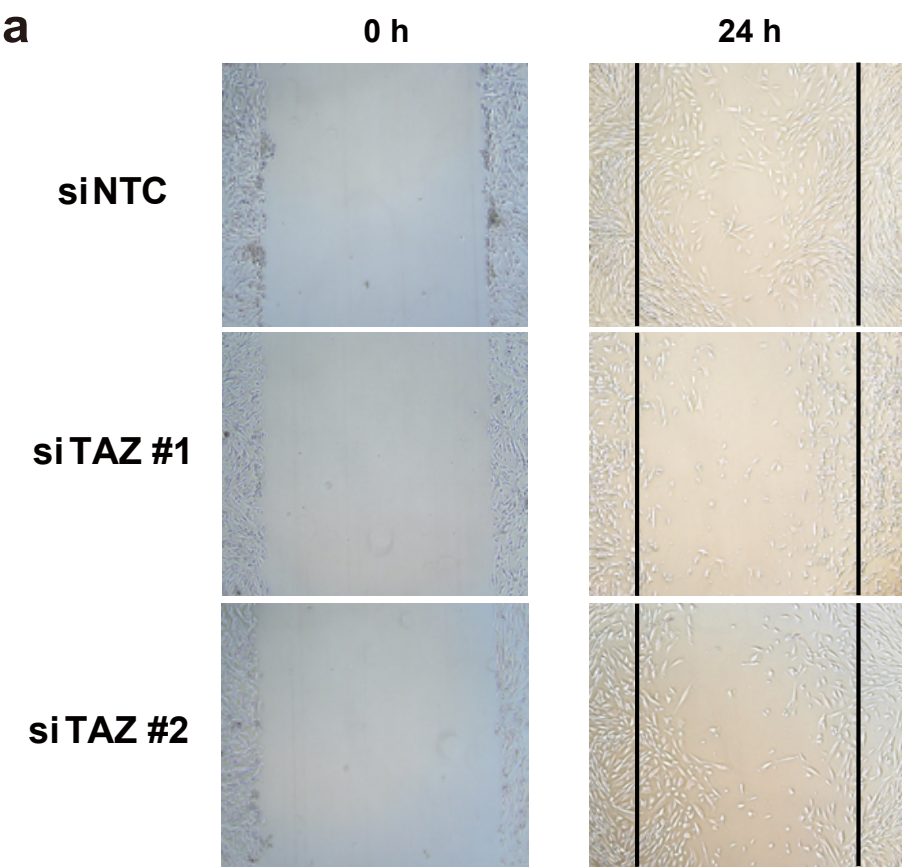
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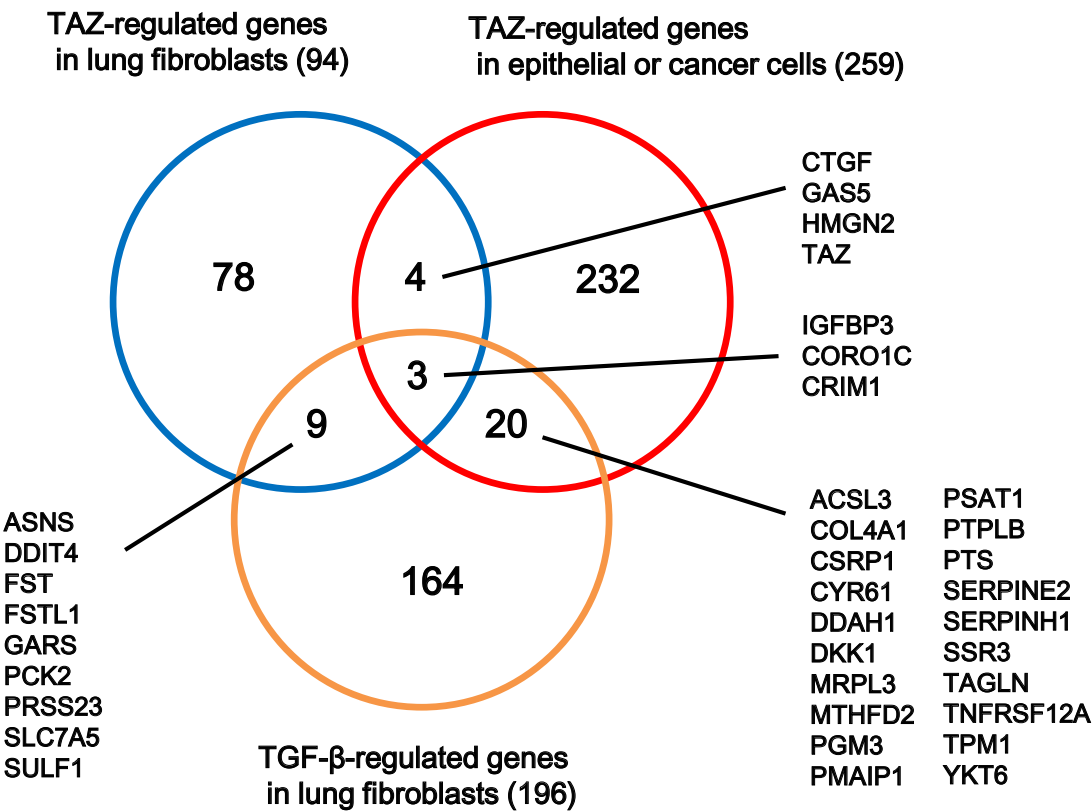
Supplementary Figure S2



Supplementary Figure S3

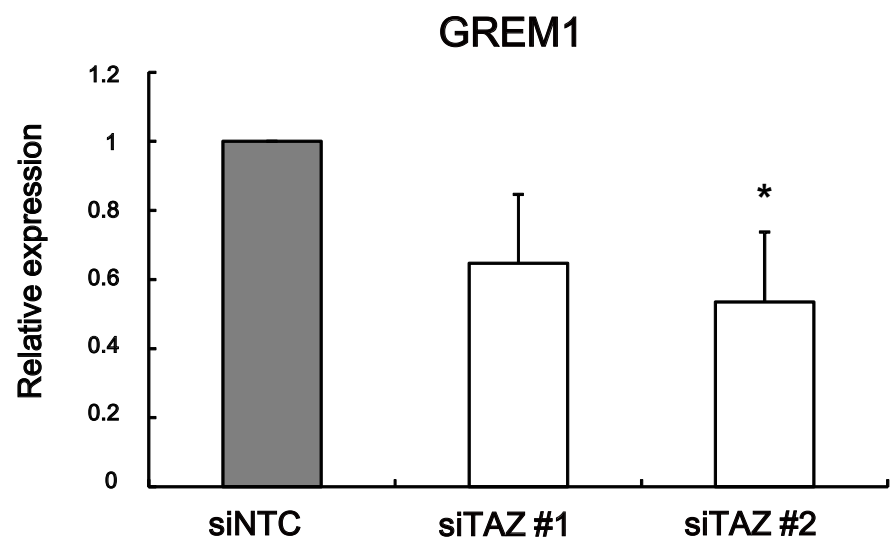


Supplementary Figure S4



Supplementary Figure S5

a



b

