# Supplementary Material

corresponding to:

## The stem cell transcription factor ZFP296 transforms NIH3T3 cells and promotes anchorage-independent growth of cancer cells

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# Supplemental Information

"The stem cell transcription factor ZFP296 transforms NIH3T3 cells and promotes anchorage-independent growth of cancer cells." by Mizoue, Y. *et al*.

**Materials and methods**

*Cell culture*

TIG-114 cells were obtained from JCRB. HeLa, HepG2 and TIG-114 cells were cultured in DMEM containing 10% FBS. hTERT-HME1 were from ATCC and cultured in mammary epithelial cell basal medium MEBM (Lonza). AGS cells were from European Collection of Cell Cultures and cultured in F-12 medium (Life Technologies) containing 10% FBS and 2 mM glutamine. Human umbilical vein endothelial cells (HUVECs) and human dermal lymphatic endothelial cells (HDLECs) were purchased from PromoCell (Heidelberg, Germany) and Takara, respectively, and cultured in endothelial cell growth medium 2 (PromoCell) and endothelial cell growth medium MV2 (PromoCell), respectively. BMEC1, hFOB and U266 cells were cultured in M199 medium (Life Technologies) containing 20% FBS, DMEM/Ham's F12 (FUJIFILM Wako Chemicals) containing 10% FBS, and RPMI1640 (Nacalai Tesque) containing 15% FBS, respectively. All cells were cultured in a humidified atmosphere with 5% $CO_2$ at 37$^{\circ}$C.

Table S1 Primer sets for qPCR

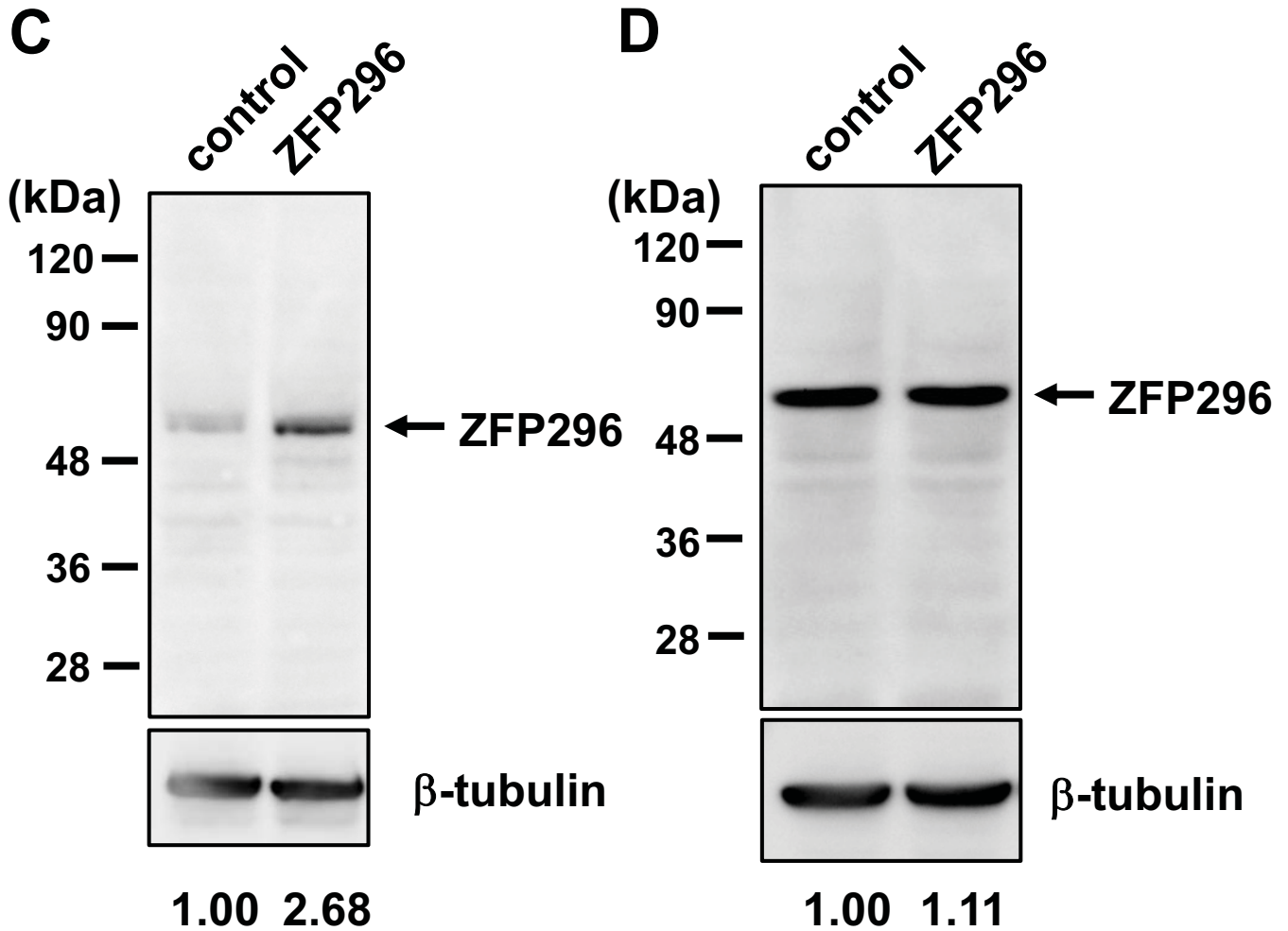| Gene | Forward primer (5′ to 3′) | Reverse primer (5′ to 3′) |
|---|---|---|
| mouse β-actin | TCCTTCTTGGGTATGGAATCCTG | GAGGTCTTTACGGATGTCAACG |
| mouse Zfp296 | AGCTTCTCCAAGTCTCCGACC | GTGGCACAGCAACTTCCAAGG |
| human β-actin | AGCACAGAGCCTCGCCTTT | CGCGGCGATATCATCATCCA |
| human ZFP296 | CTGGACCGACAAACACCCAG | GTGAACTGTTTGCCACAGCG |
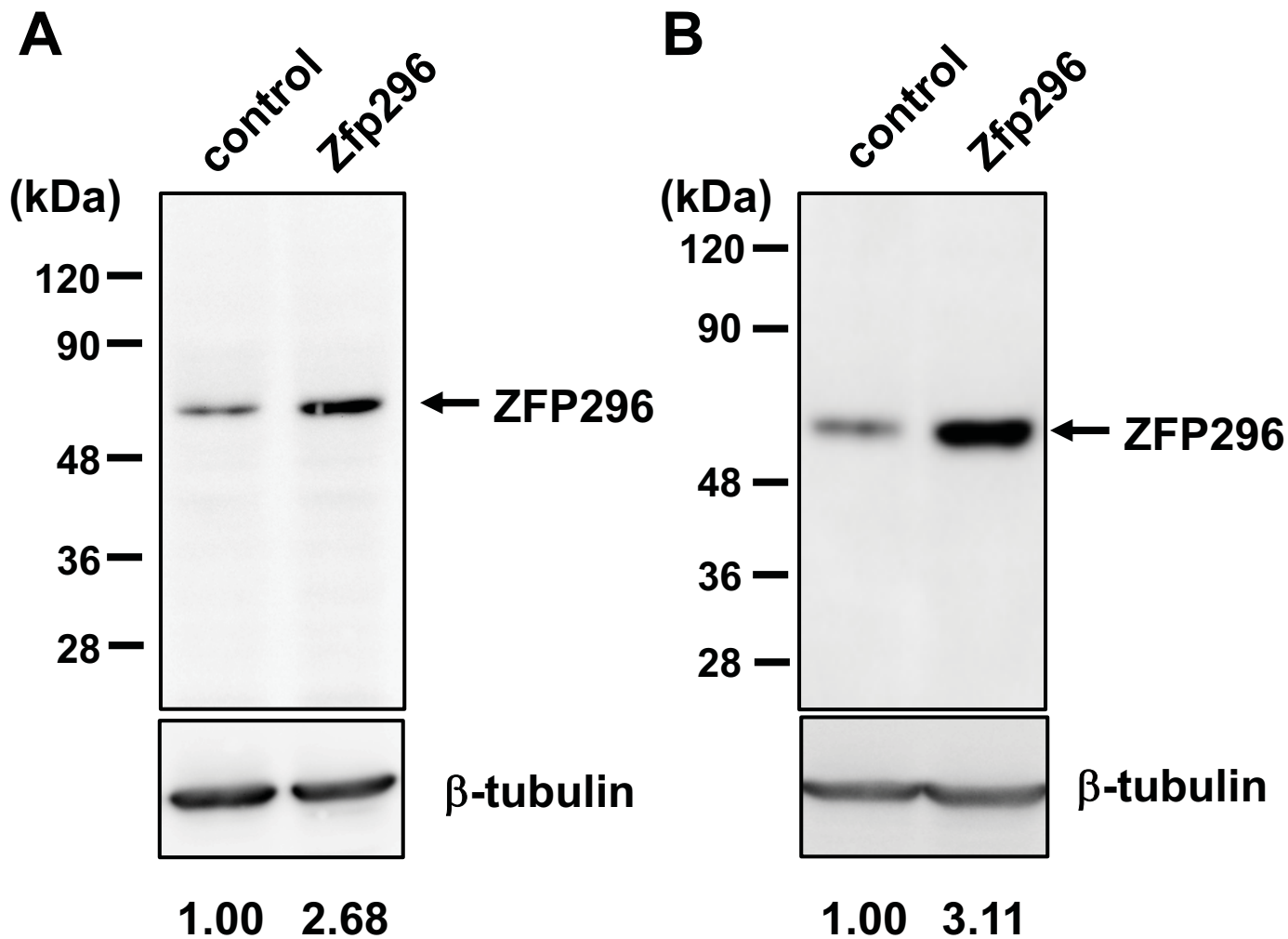
**Fig. S1   Western blot analysis**

(A)   Confirmation of mouse ZFP296 overexpression in NIH3T3 cells.

(B)   Confirmation of mouse ZFP296 overexpression in C2C12 cells.

  Representative data from  multiple experiments are shown. The numbers below the

gel indicate the expression level of ZFP296, relative to control cells, which was

calculated by dividing the density of ZFP296 bands by that of β-tubulin bands,

followed by setting the value of control cells as 1.

**C**

| (kDa) | control | ZFP296 |
|-------|---------|--------|

120 —
90 —
← ZFP296
48 —
36 —
28 —

β-tubulin

**1.00  2.68**

**D**

| (kDa) | control | ZFP296 |
|-------|---------|--------|

120 —
90 —
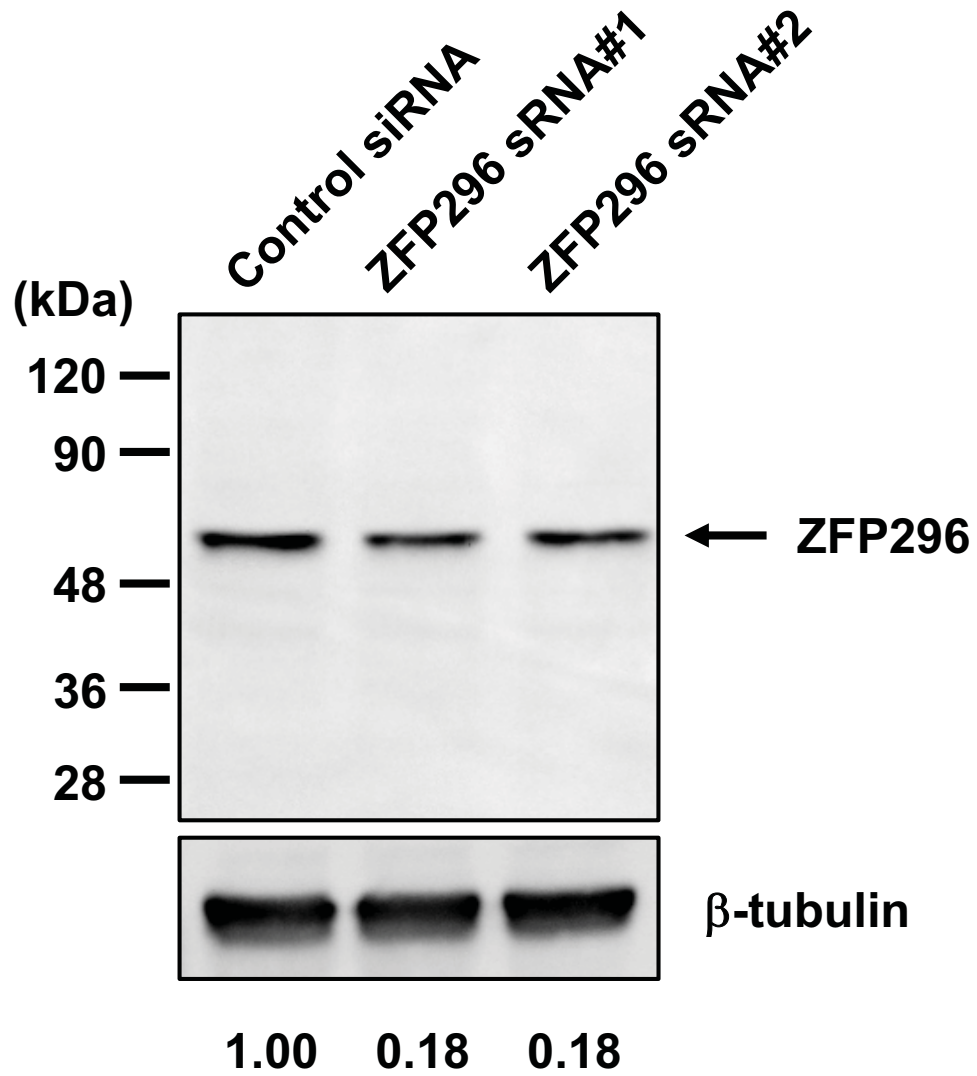← ZFP296
48 —
36 —
28 —

β-tubulin

**1.00  1.11**

**Fig. S1    Western blot analysis**

(C) Confirmation of human ZFP296 overexpression in HT1080 cells.

(D) Confirmation of human ZFP296 overexpression in HCT116 cells.

 Representative data from  multiple experiments are shown. The numbers below the gel

indicate the expression level of ZFP296, relative to control cells.
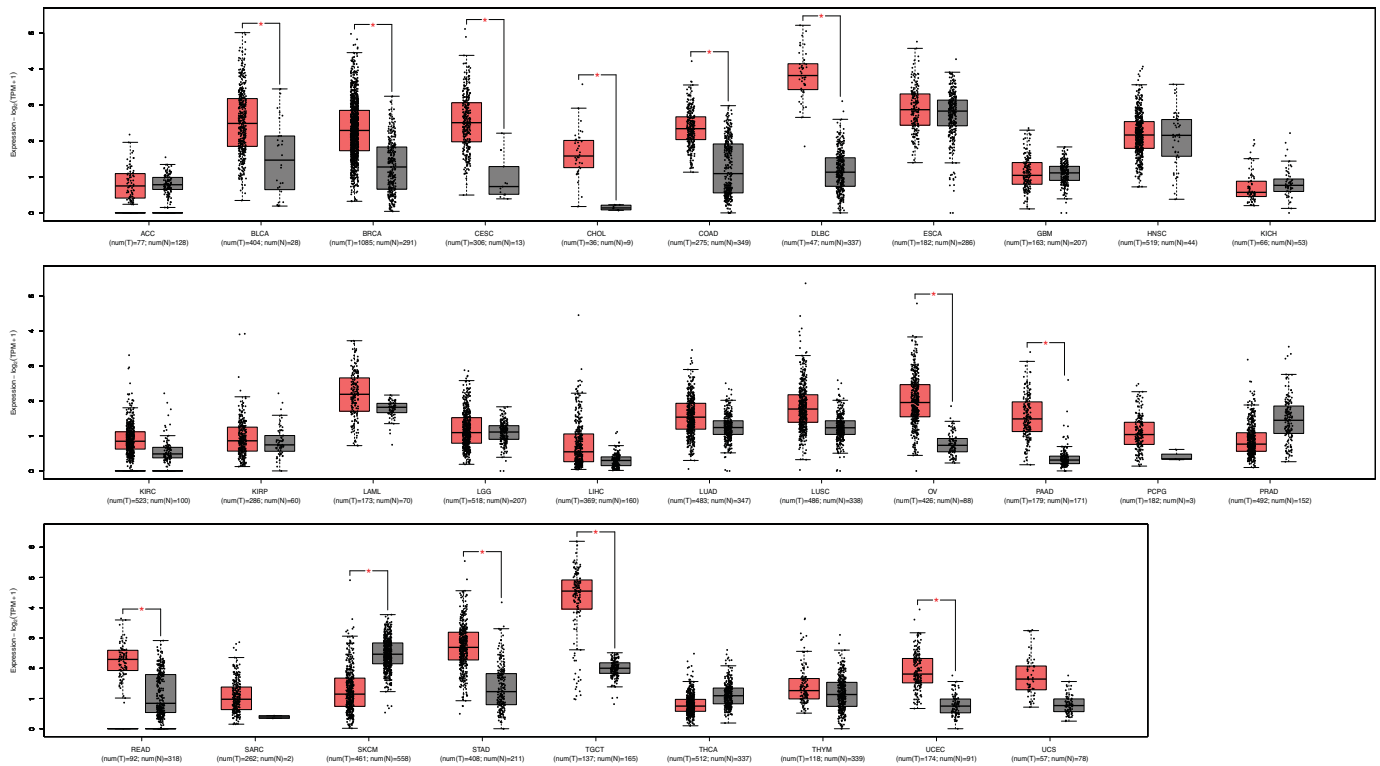
**E**



**Fig. S1   Western blot analysis**

(E) Confirmation of ZFP296 knockdown in MCF7 cells.

   Representative data from  multiple experiments are shown. The numbers below the gel indicate the expression level of ZFP296, relative to control cells.

**Fig. S2    Expression of ZFP296 in several human tumors (red box) and normal tissues (grey box).**

Data from the Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) were analyzed by using GEPIA2. The abbreviations for each cancer in the figure are as follows.

ACC    Adrenocortical carcinoma
BLCA  Bladder urothelial carcinoma
BRCA  Breast invasive carcinoma
CESC  Cervical squamous cell carcinoma and
            endocervical adenocarcinoma
CHOL  Cholangiocarcinoma
COAD Colon adenocarcinoma
DLBC  Lymphoid neoplasm diffuse large B-cell
            lymphoma
ESCA  Esophageal carcinoma
GBM    Glioblastoma multiforme
HNSC  Head and Neck squamous cell carcinoma
KICH   Kidney chromophobe
KIRC   Kidney renal clear cell carcinoma
KIRP   Kidney renal papillary cell carcinoma
LAML  Acute myeloid leukemia
LGG    Brain lower grade glioma
LIHC    Liver hepatocellular carcinoma

LUAD Lung adenocarcinoma
LUSC  Lung squamous cell carcinoma
MESO Mesothelioma
OV       Ovarian serous cystadenocarcinoma
PAAD Pancreatic adenocarcinoma
PCPG  Pheochromocytoma and paraganglioma
PRAD Prostate adenocarcinoma
READ Rectum adenocarcinoma
SARC  Sarcoma
SKCM Skin cutaneous melanoma
STAD  Stomach adenocarcinoma
TGCT  Testicular germ cell tumors
THCA  Thyroid carcinoma
THYM Thymoma
UCEC Uterine corpus endometrial carcinoma
UCS     Uterine carcinosarcoma
UVM    Uveal melanoma