Supplementary Fig. 1. Expression of HIG2 in normal human tissues. *A*, Immunohistochemistry of normal tissue sections incubated with anti-HIG2 pAb. Little or no HIG2 protein was observed in any of these tissues. *B*, Cross-inhibitory staining of HIG2 in normal kidney (i, ii) and RCC (iii, iv); (i, iii), before inhibition, (ii, iv), after inhibition with rhHIG2. Inhibition of staining by rhHIG2 protein can be seen by comparing iii and iv. Non-specific staining by anti-HIG2 pAb was almost absent. *C*, HIG2 protein in granular and papillary types of renal cell carcinoma.

Supplementary Fig. 2. *A*, FACS analysis, revealing facilitation of RCC-specific cell death by anti-HIG2 pAb. Proportions of apoptotic cells are indicated as percentages of sub-G1 populations. *B*, FACS analysis of cancer-cell lines derived from organs that have no HIG2 expression. Proportions of apoptotic cells are indicated as percentages of sub-G1 populations.

Supplementary Fig. 3. Transcriptional activation of HIG2 by the TCF4/β-catenin complex. *A*, Dual luciferase reporter assay in HEK293 cells. Error bars, SD; asterisks, $p<0.01$ by Scheffe's *F* test. *B*, ChIP assays performed on genomic fragments of *HIG2*. Nuclear extracts were obtained from RCC cell lines Caki-1 and Caki-2, which were treated with rhHIG2 or not. Samples were immunoprecipitated with an anti-β-catenin mAb prior to PCR amplification. Input chromatin represents the sonicated chromatin prior to immunoprecipitation. Immunoprecipitates with a mouse anti-FLAG monoclonal antibody served as negative controls. *C*, EMSA assay of the β-catenin/TCF4 complex using a TBM2-oligonucleotide as the probe. A supershifted band that appeared after addition of anti-β-catenin antibody (lanes 1 and 2) was not seen with anti-FLAG-antibody (lane 6). Bands corresponding to the DNA-protein complex were reduced by addition of non-labeled wild-type probe (lanes 3 and 4), but not by non-labeled mutant probe (lanes 2 and 7).

Supplementary Fig. 4. Detection of HIF2 by immunohistochemistry. RCC sections were incubated with anti-HIF1α mAb (*A*, *B*) or anti-HIG pAb (*C*, *D*) in central necrotic areas (*A*, *C*) and in non-central necrotic areas (*B*, *D*). CN, central necrosis; arrows indicate expression of HIF1α.

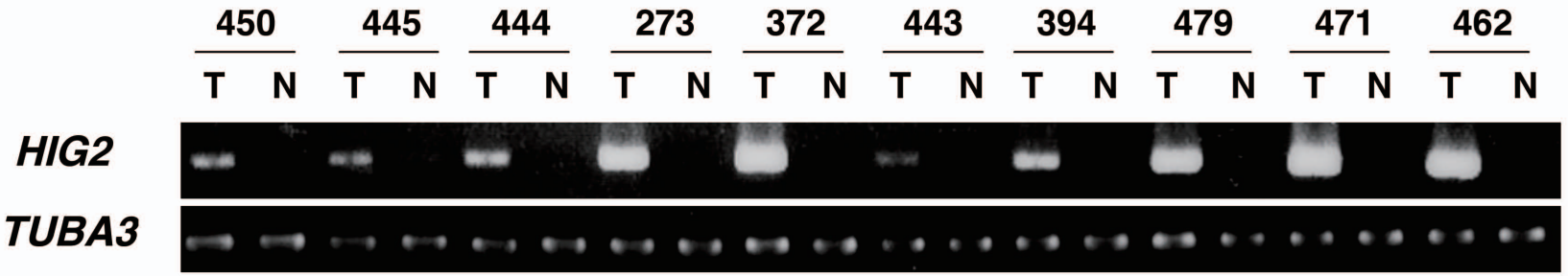

450
445
444
273
372
443
394
479
471
462
T N T N T N T N T N T N T N T N T N T N
HIG2
TUBA3

Fig. 1

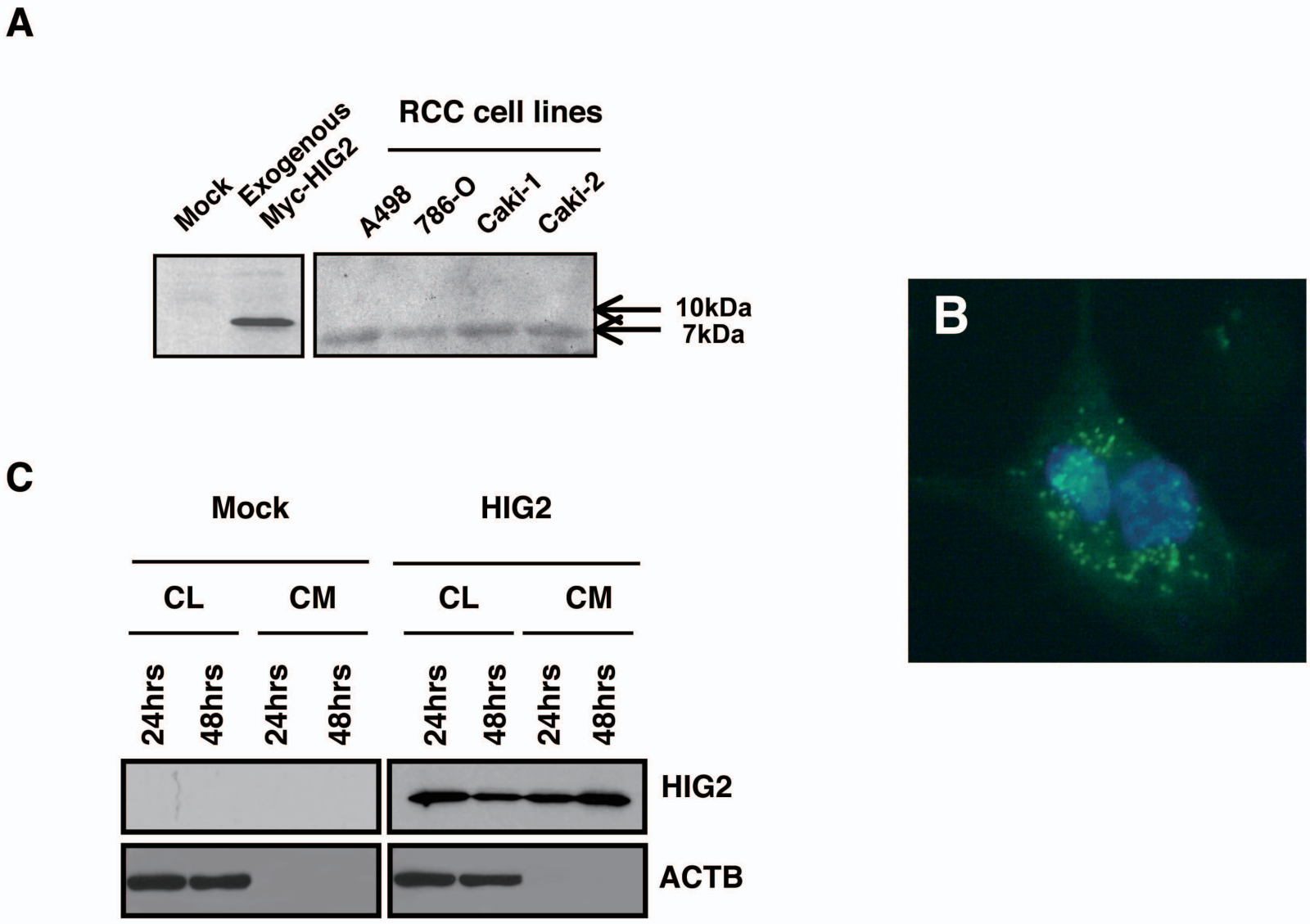

A
Mock
Exogenous Myc-HIG2
RCC cell lines
A498
786-O
Caki-1
Caki-2
10kDa
7kDa
B
C
Mock
HIG2
CL
CM
CL
CM
24hrs
48hrs
24hrs
48hrs
24hrs
48hrs
24hrs
48hrs
HIG2
ACTB

Fig. 2

Togashi A

D

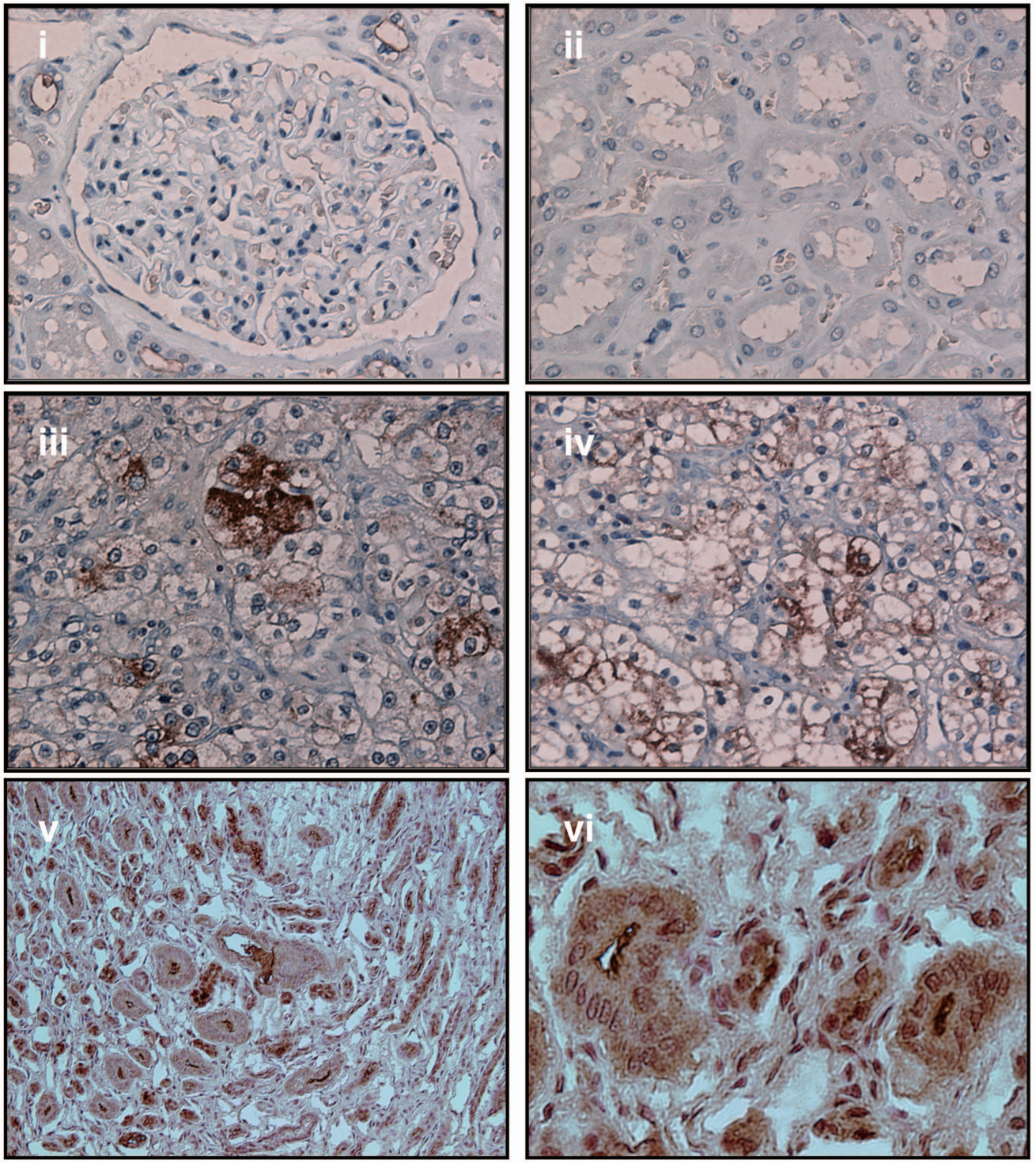


Fig. 2

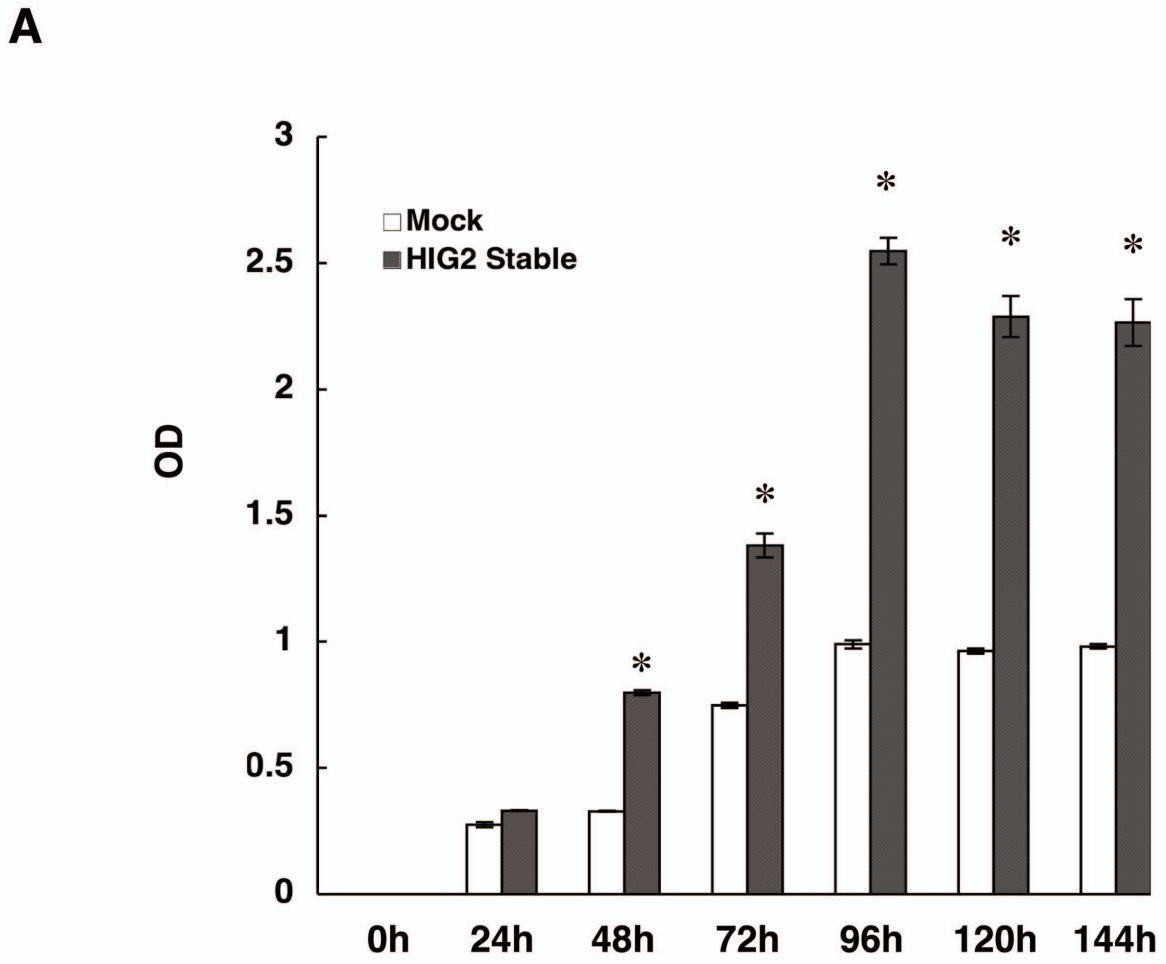


Fig. 3

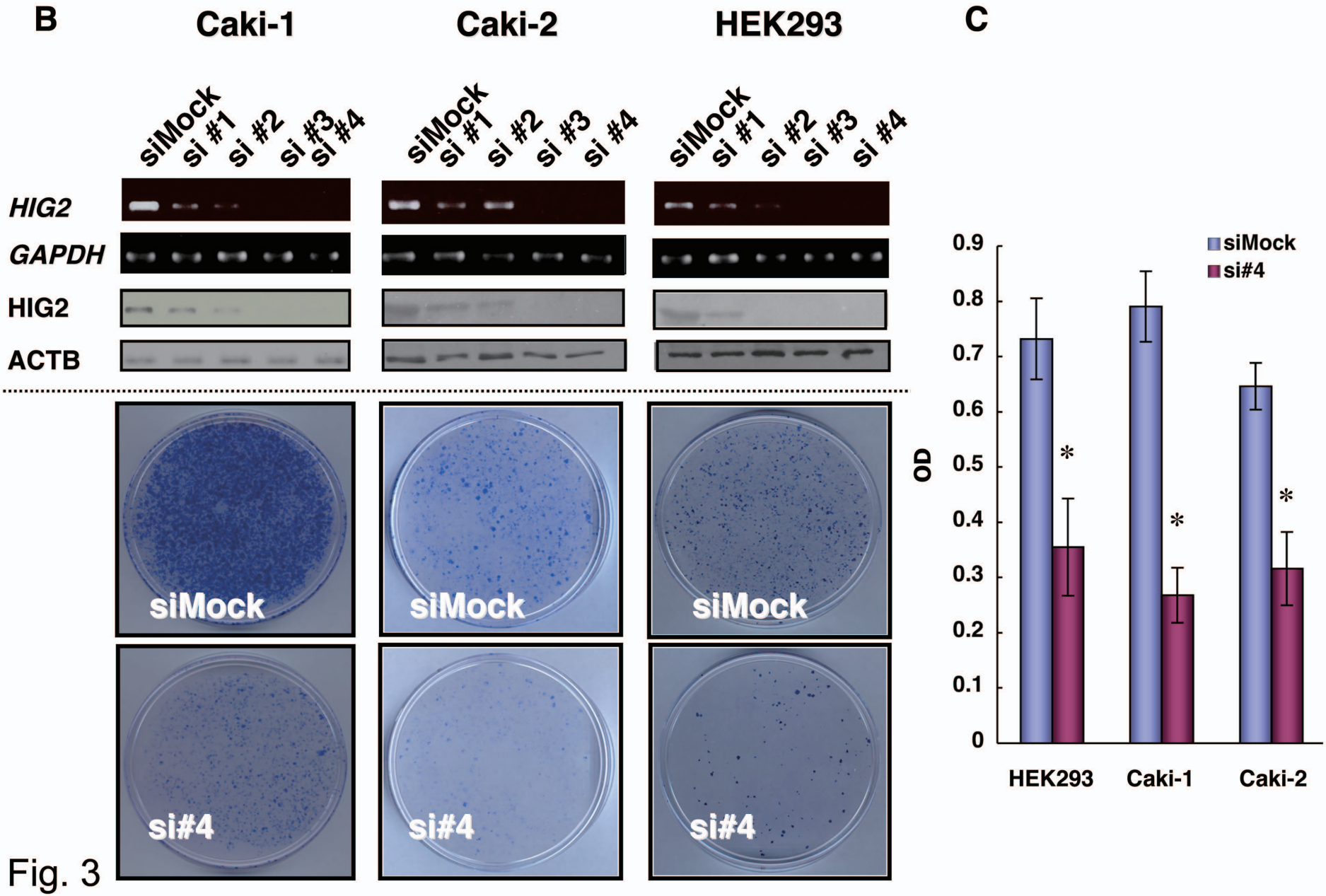
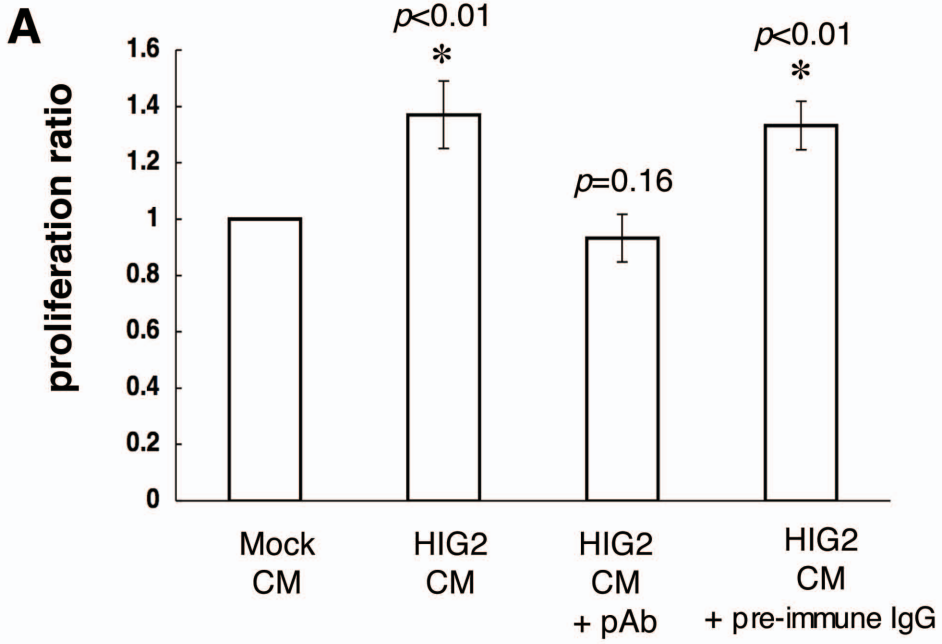
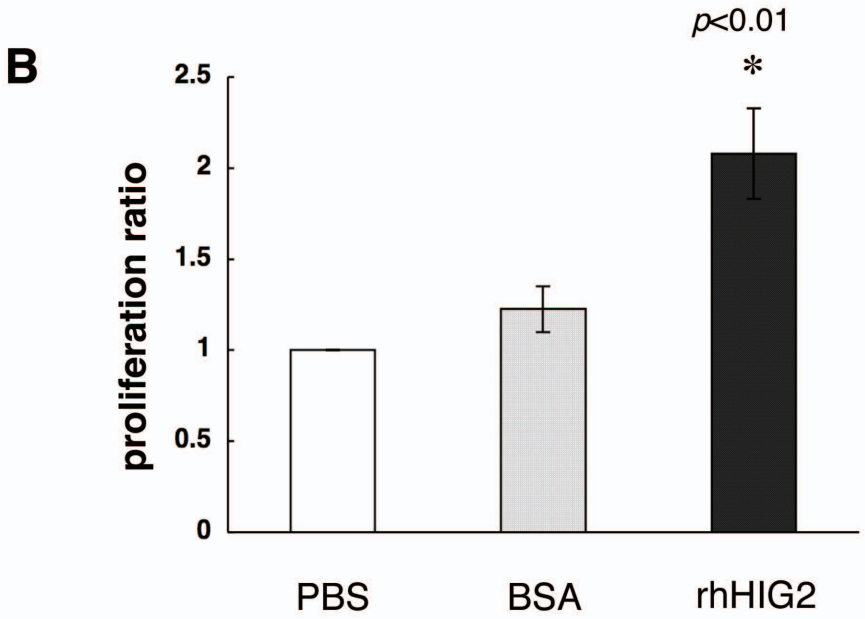
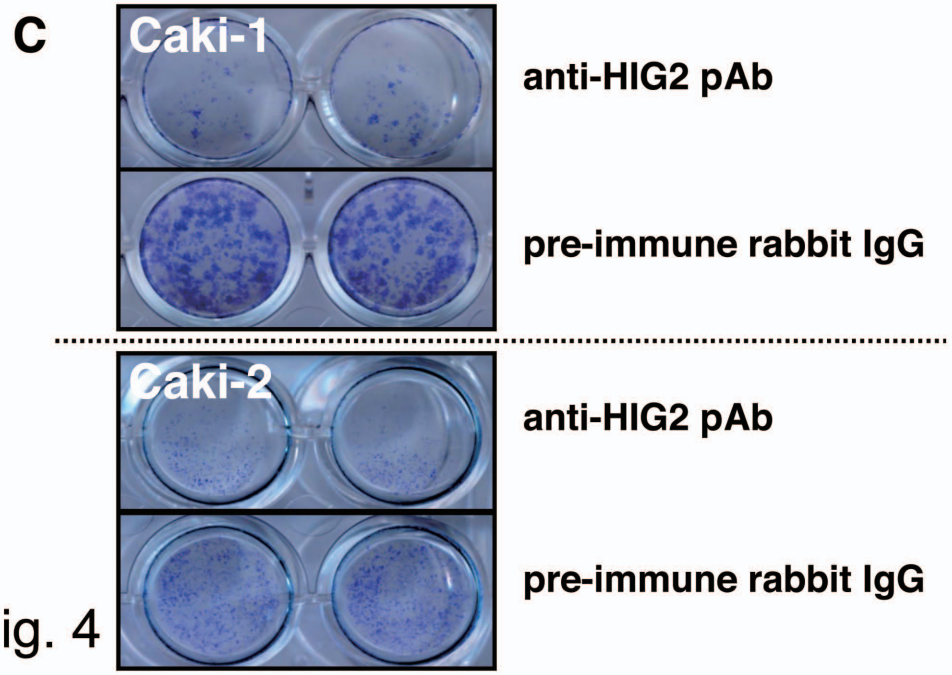

B
Caki-1
Caki-2
HEK293
siMock
si #1
si #2
si #3
si #4
HIG2
GAPDH
HIG2
ACTB
siMock
si#4
C
OD
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
siMock
si#4
*
HEK293
Caki-1
Caki-2

Fig. 3

Togashi A







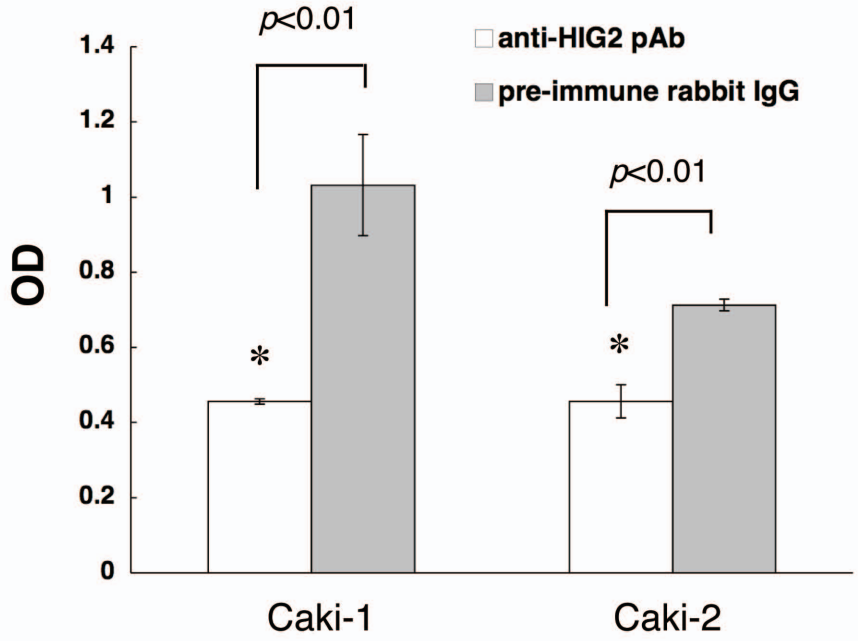


Fig. 4

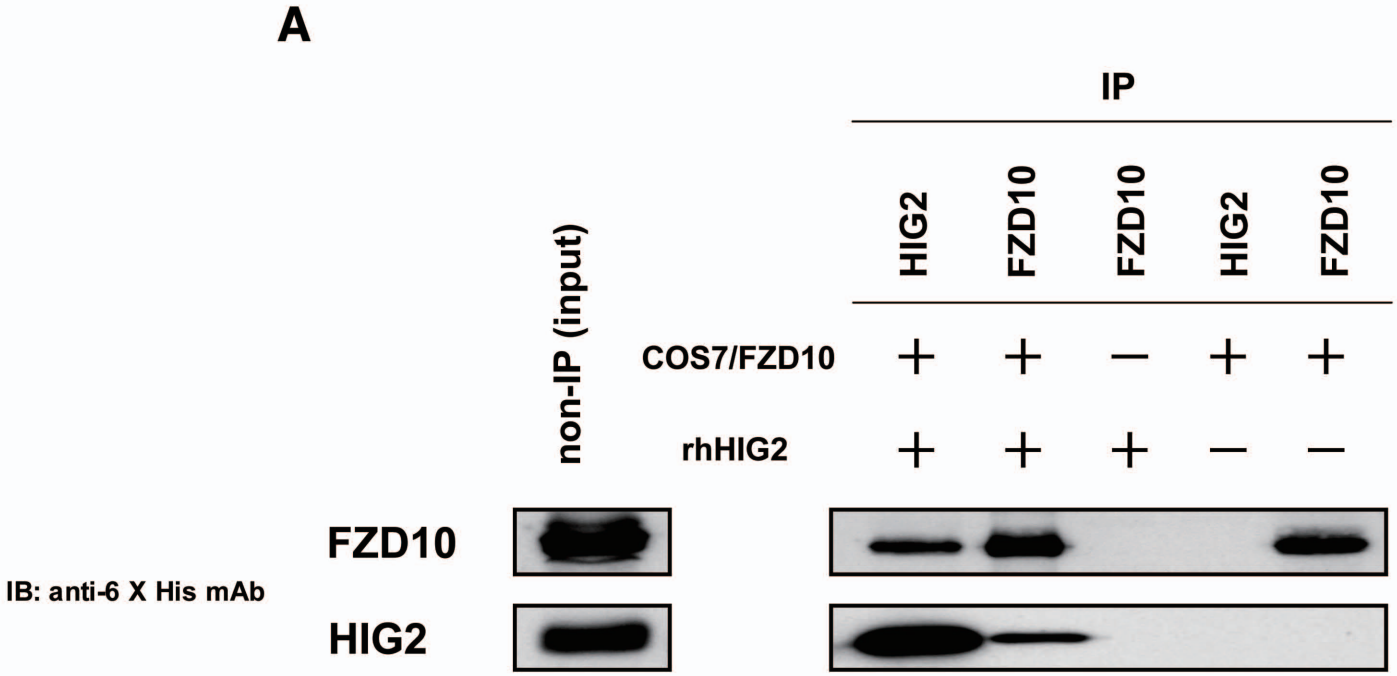

A
IP
HIG2
FZD10
FZD10
HIG2
FZD10
non-IP (input)
COS7/FZD10
+
+
−
+
+
rhHIG2
+
+
+
−
−
FZD10
HIG2
IB: anti-6 X His mAb

Fig. 5

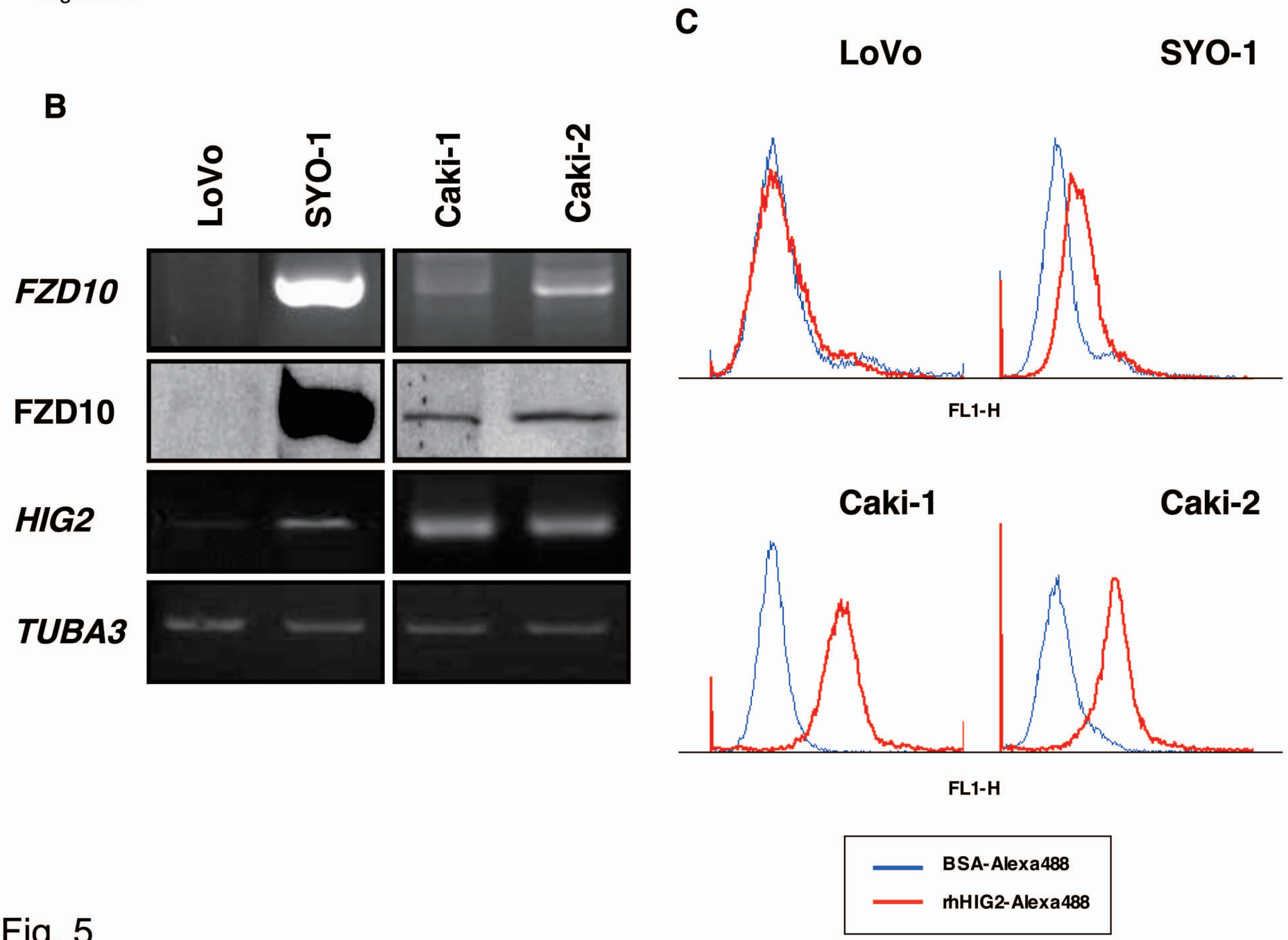

B
LoVo
SYO-1
Caki-1
Caki-2
FZD10
FZD10
HIG2
TUBA3
C
LoVo
SYO-1
FL1-H
Caki-1
Caki-2
FL1-H
BSA-Alexa488
rhHIG2-Alexa488

Fig. 5

D

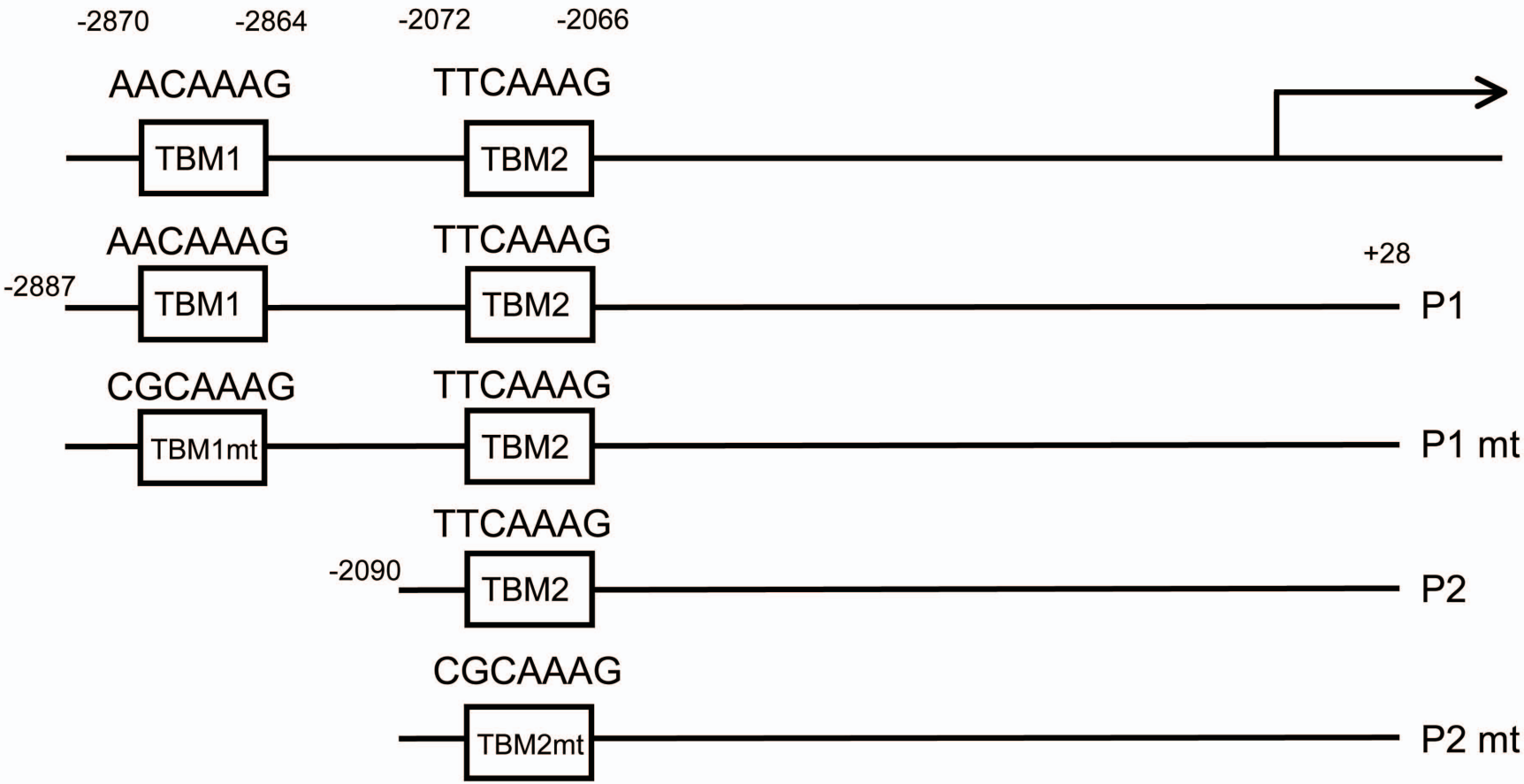

-2870
-2864
-2072
-2066
AACAAAG
TTCAAAG
TBM1
TBM2
AACAAAG
TTCAAAG
+28
-2887
TBM1
TBM2
P1
CGCAAAG
TTCAAAG
TBM1mt
TBM2
P1 mt
TTCAAAG
-2090
TBM2
P2
CGCAAAG
TBM2mt
P2 mt

Fig. 5

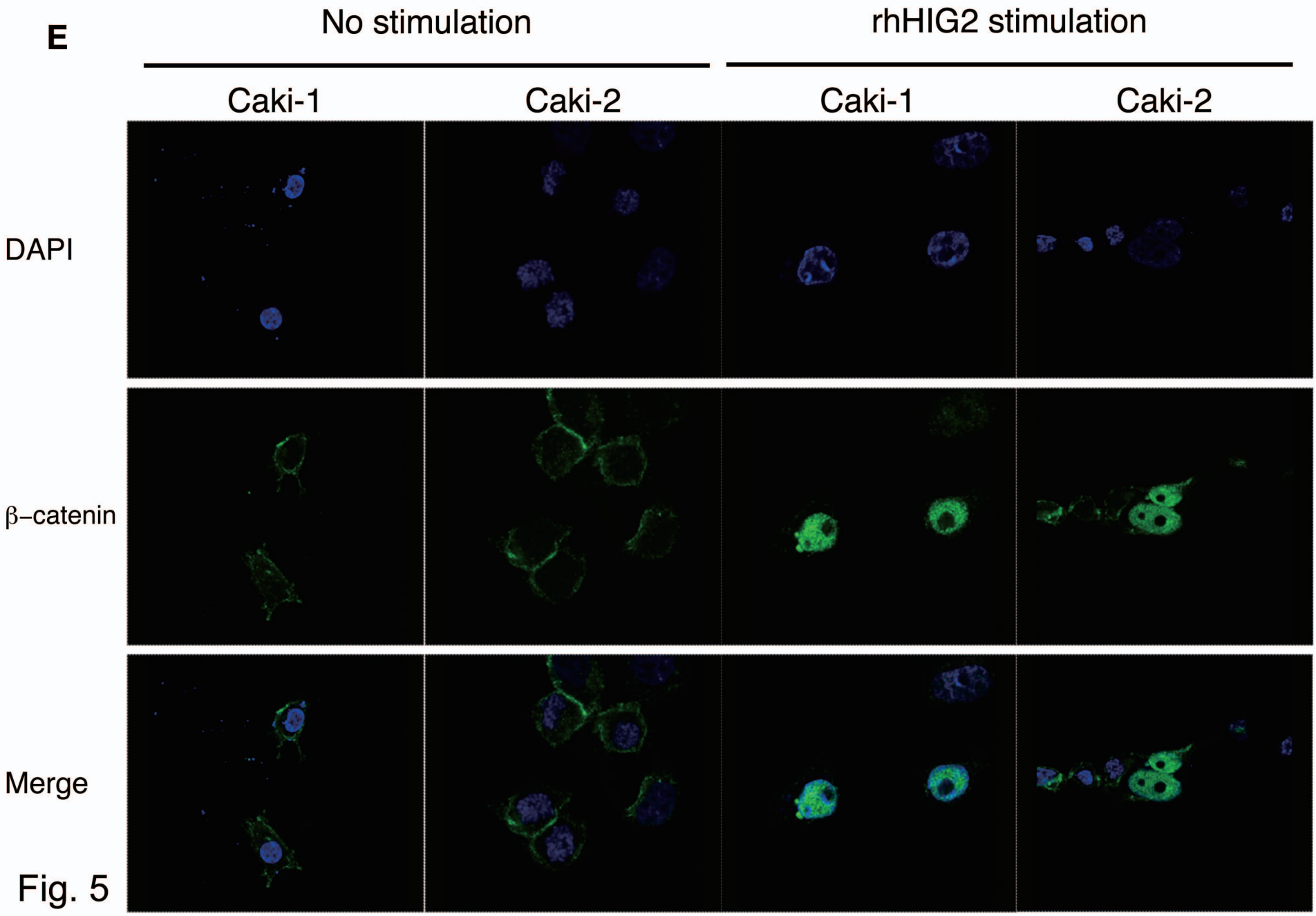

E
No stimulation
rhHIG2 stimulation
Caki-1
Caki-2
Caki-1
Caki-2
DAPI
β–catenin
Merge

Fig. 5

Togashi A

F

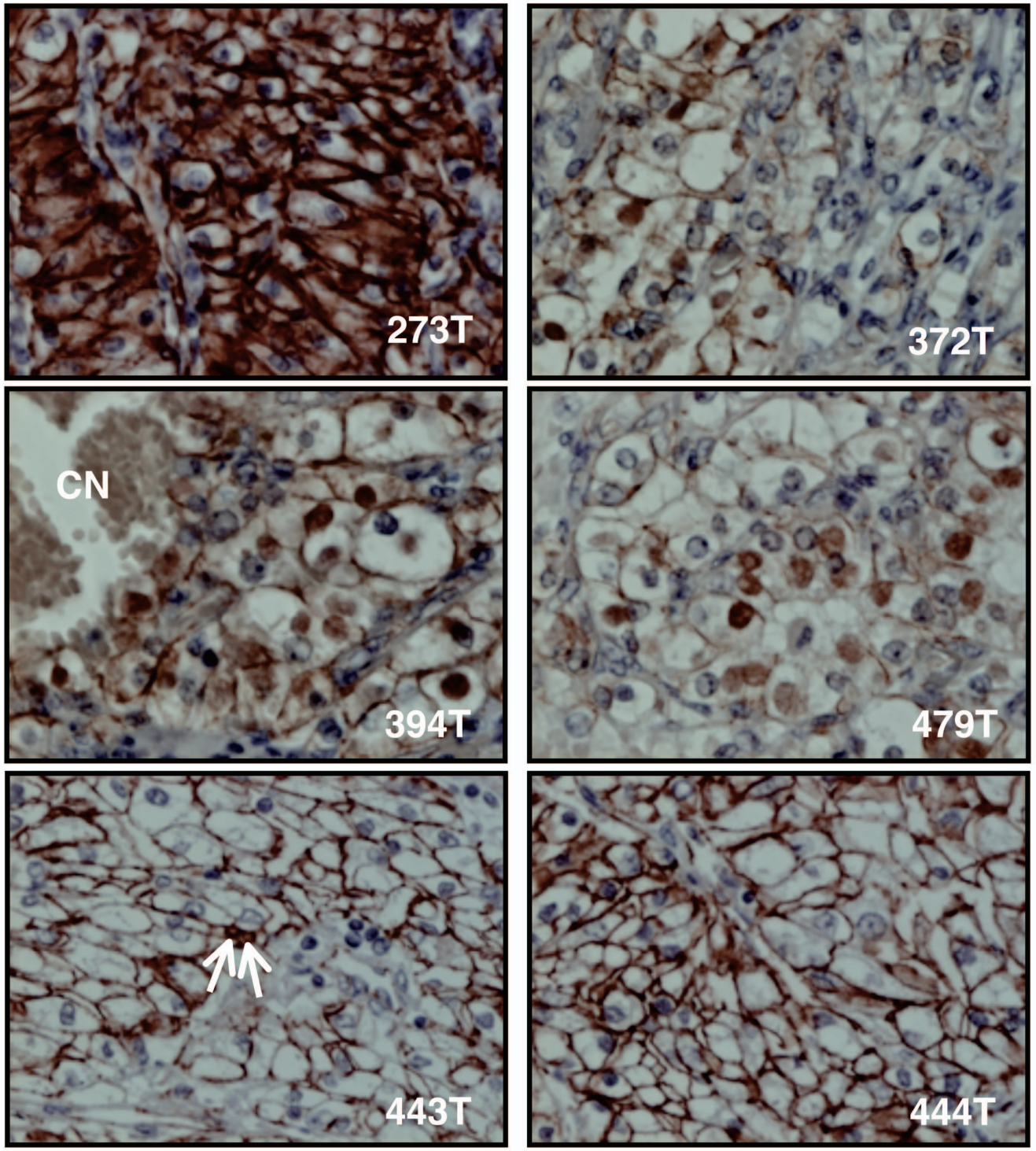


Fig. 5

Togashi A

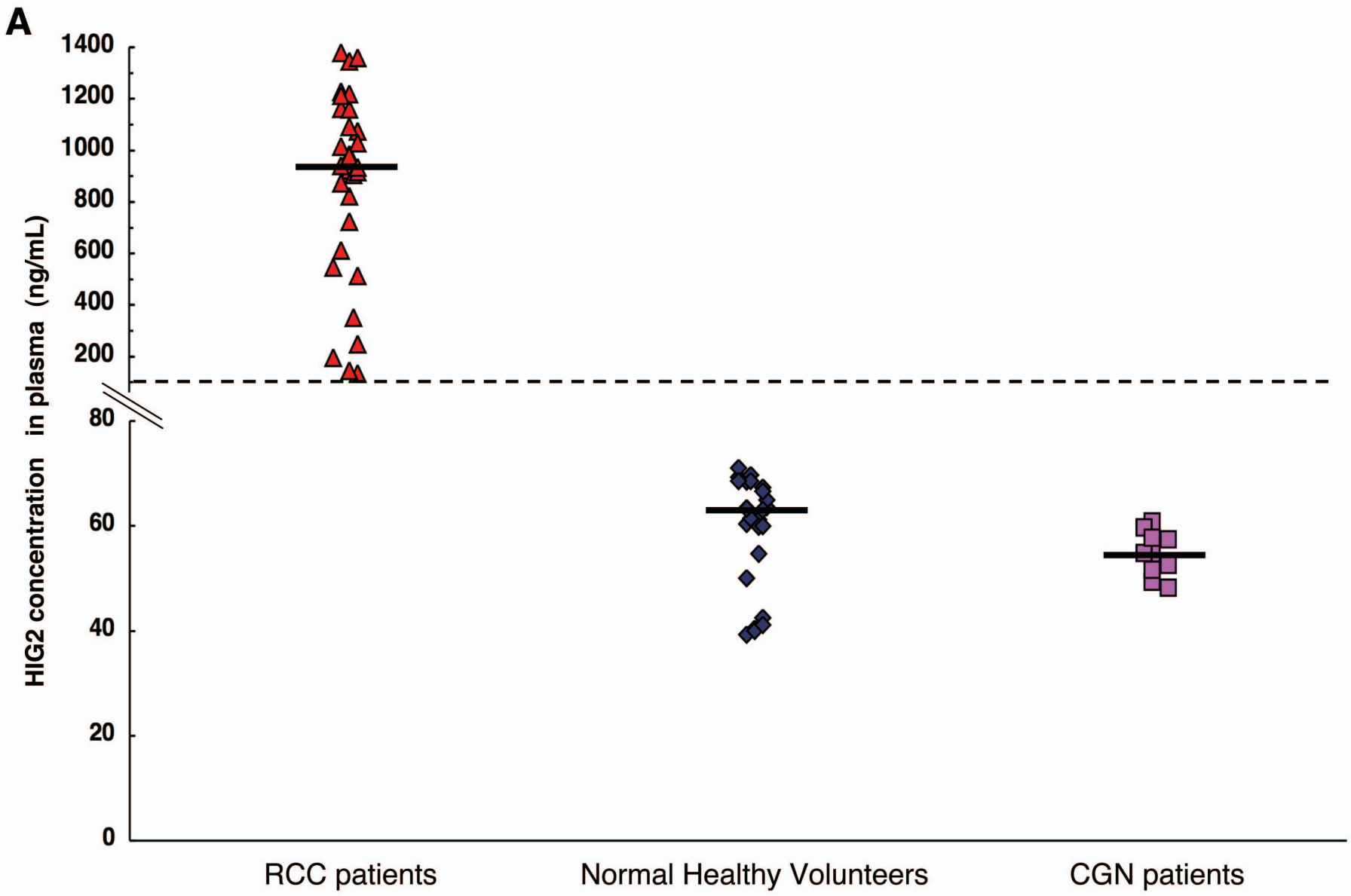


Fig. 6

B

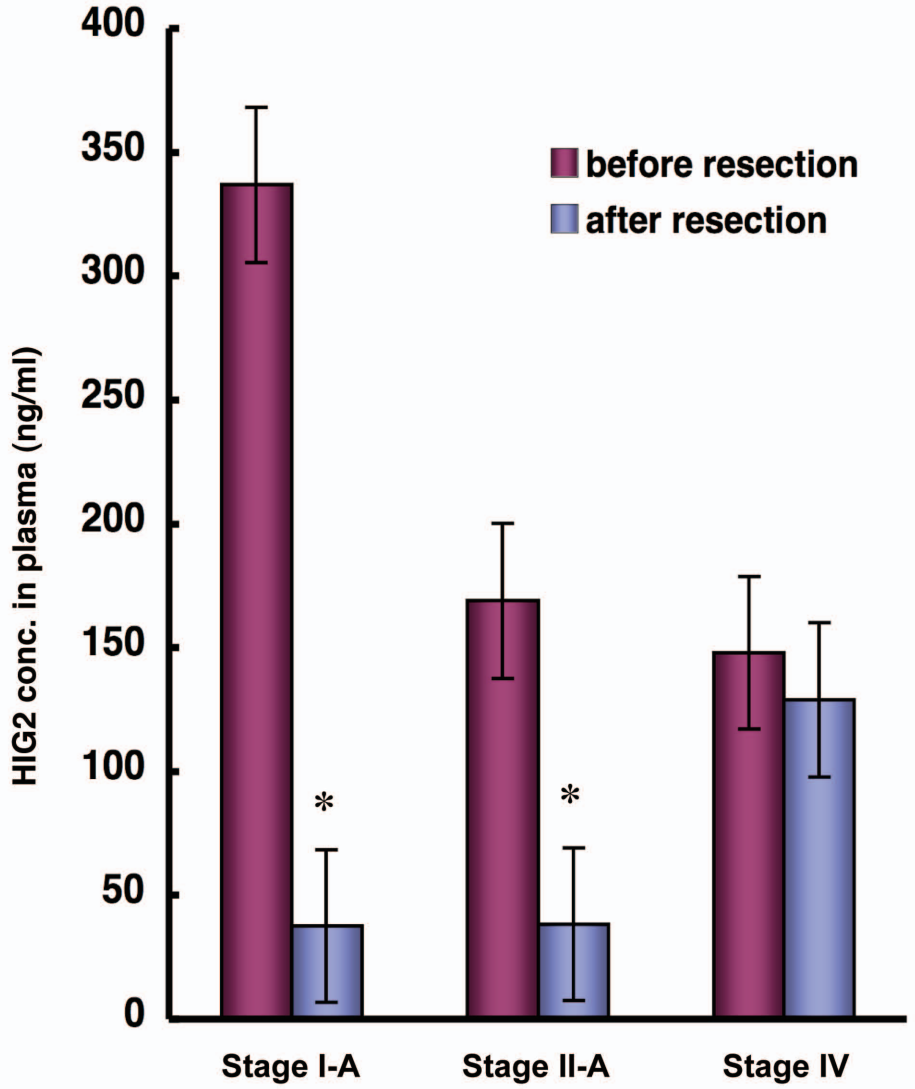

400
350
300
250
200
150
100
50
0
HIG2 conc. in plasma (ng/ml)
before resection
after resection
*
*
Stage I-A
Stage II-A
Stage IV

Fig. 6