

## Supplementary Figure legends

**Suppl. Fig. 1. Comparison of the beginning 850nt in the first intron between human and mouse TMEPAI genes.** Identical nucleotides between human and mouse show (-).

**Suppl. Fig. 2. Effect of Smad2-specific siRNA on TGF- $\beta$ -induced pGL3ti-850 activity.** (left) HepG2 cells transfected with Smad2-specific siRNA (Nippon EGT) (1) together with pGL3ti-850 were stimulated with TGF- $\beta$ . Control siRNA (Nippon EGT) means a non-targeting siRNA. Transfection was carried out using lipofectamine 2000. (right) The expression of endogenous Smad2 was detected with an anti-Smad2 antibody (2) by western blot analysis.

**Suppl. Fig. 3. Expression of TMEPAI in tumors.** Paraffin-embedded sections of intestinal adenoma from *Apc<sup>Min/+</sup>* mice. The paraffin-embedded mouse tissues were sectioned to a 3  $\mu$ m thickness, deparaffinized in xylene, rehydrated in graded ethanol solution, and immersed in citrate-NaOH buffer (10 mM sodium citrate, pH 7.2) for 20 min at 115°C to restore antigenicity. Then, the rehydrated sections were incubated

overnight at 4°C with mouse anti-TMEPAI monoclonal antibody (Abnova, 1:100), rabbit anti-phosphorylated Smad2 antibody (3), or mouse anti- $\beta$ -catenin antibody (BD Transduction laboratories, 1:100). The sections incubated with the first antibodies were washed with PBS and subsequently colored using the Dako EnVision+system HRP (DAB) (DakoCytomation). Adenoma was stained for (i)  $\beta$ -catenin, (ii) TMEPAI and (iii) phosphorylated Smad2 antibodies using 3 consecutive sections. All sections were counterstained with hematoxylin. Lower panels show enlarged photos of the regions surrounded with squares (upper panels). The broken red and blue lines indicate the regions expressing  $\beta$ -catenin at low and high levels, respectively.

**Suppl. Fig. 4. Expression of TMEPAI mRNA in cells transfected with**

**Smad4-specific siRNA, TCF7L2-specific siRNA or both combinations.** After indicated siRNAs were transfected in MCF10A1 cells, cells were stimulated with TGF- $\beta$  for 2 h.

The expressions of TMEPAI, TCF7L2, Smad4 and  $\beta$ -actin were measured by

semi-quantitative RT-PCR and normalized using the intensity of the band

corresponding to  $\beta$ -actin. The level of induction was calculated relative to the value for

control-siRNA-transfected cells in the absence of TGF- $\beta$ . A primer set for human

Smad4 is 5'-CAAGGTGGAGAGAGTGAAAC-3' and

5'-GACGGGCATAGATCACATGA-3'. The other primer sets are described in the text.

**Suppl. Fig. 5. Induction of TMEPAI mRNA upon Wnt-3a and/or TGF- $\beta$**

**stimulation.** MDA-MB231 cells were stimulated with 200 ng/ml Wnt-3a (R&D systems) and/or TGF- $\beta$  for 2 h. The expressions of TMEPAI and  $\beta$ -actin were measured by semi-quantitative RT-PCR and normalized using the intensity of the band corresponding to  $\beta$ -actin. The level of induction was calculated relative to the value for control-siRNA-transfected cells in the absence of TGF- $\beta$ . The primer sets used here are described in the text.

**Suppl. Fig. 6. Effect of TCF7L2 $\Delta$ (1-30) on TGF- $\beta$ -induced (CAGA)<sub>12</sub>-luc activity.**

HepG2 cells transfected with TCF7L2 $\Delta$ (1-30) were stimulated with TGF- $\beta$ .

**Suppl. Fig. 7. Effect of TCF7L2 on TGF- $\beta$ -mediated pGL3ti-(mTTE)<sub>3</sub> activity.**

HepG2 cells transfected with plasmids described were stimulated with TGF- $\beta$ .

**Suppl. Fig. 8. Interaction of Smad3 with TCF7L2 in COS7 cells.** COS7 cells were transfected with indicated plasmids. Forty hours after transfection, cell lysates were immunoprecipitated with anti-Flag antibody, followed by immunoblot using anti-HA antibody (upper panel). The expressions of Flag-Smad3, HA-TCF7L2 and ALK5ca/V5 were evaluated using anti-Flag (2nd panel), anti-HA (3<sup>rd</sup> panel) and anti-V5 antibodies (lower panel), respectively.

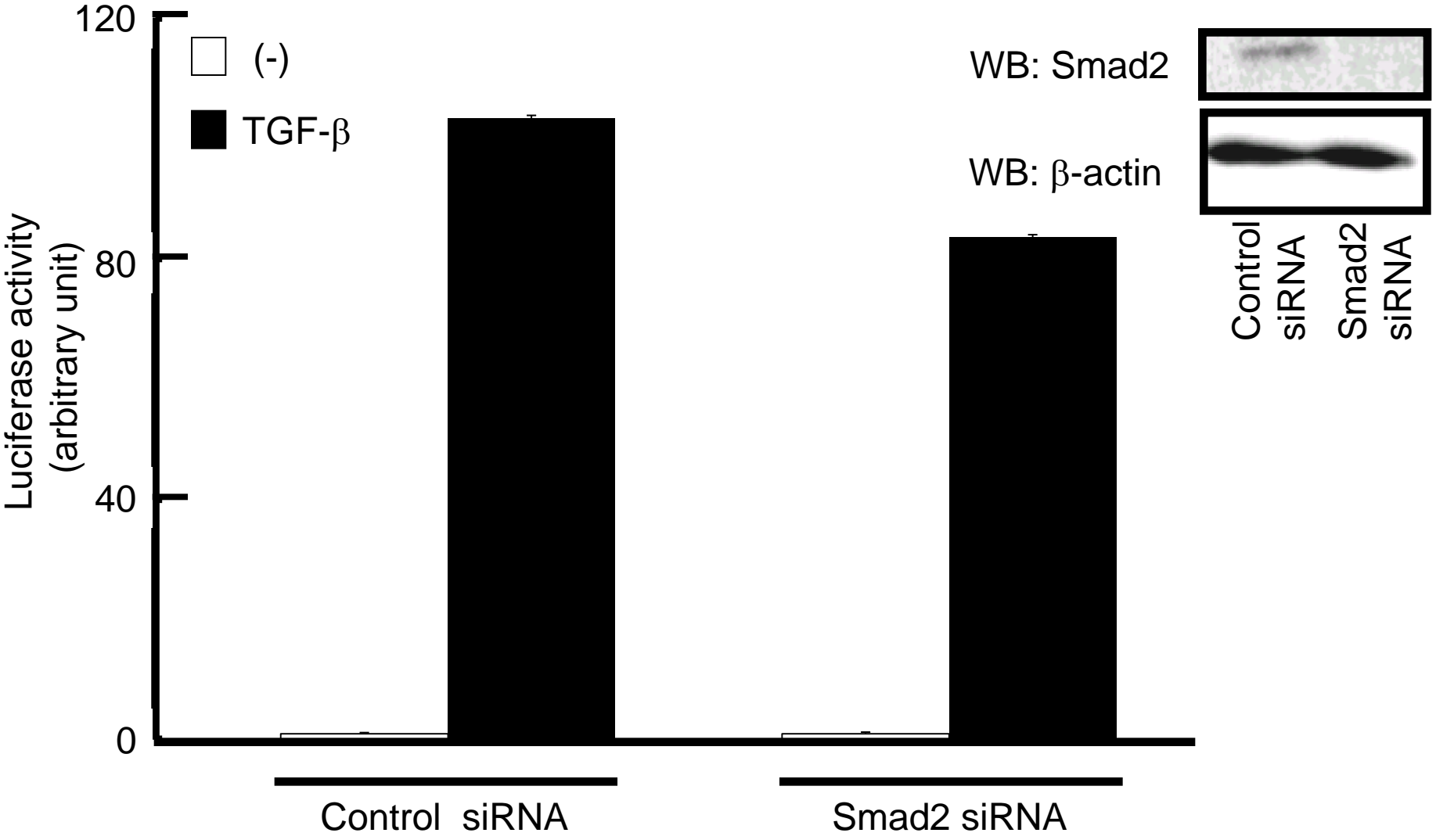
#### **Supplementary references**

1. Horiguchi K., Shirakihara T., Nakano A., Imamura T., Miyazono K., and Saitoh M. (2009) *J. Biol. Chem.* **284**, 245-253.
2. Nakao A., Imamura T., Souchelnytskyi S., Kawabata M., Ishisaki A., Oeda E., Tamaki K., Hanai J., Heldin C.-H., Miyazono K., and ten Dijke P. (1997) *EMBO J.* **16**, 5353-5362.

# Supplementary Fig. 1 (Nakano et al)



Supplementary Fig. 2 (Nakano et al)

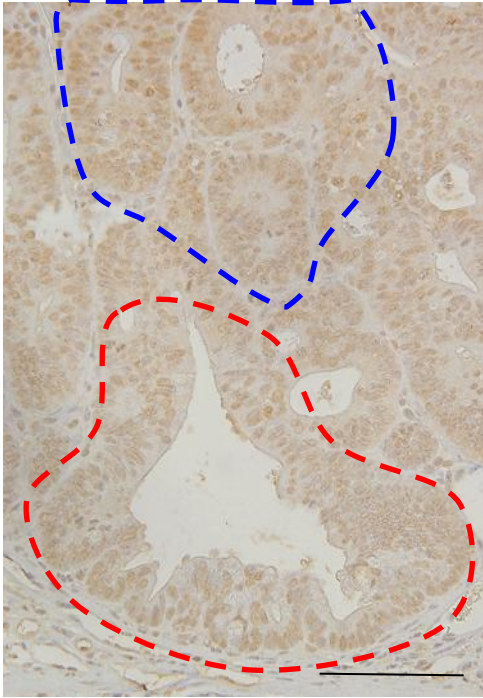
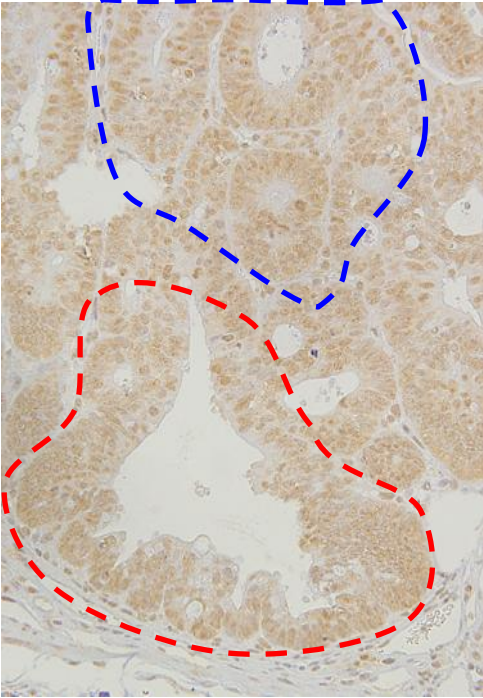
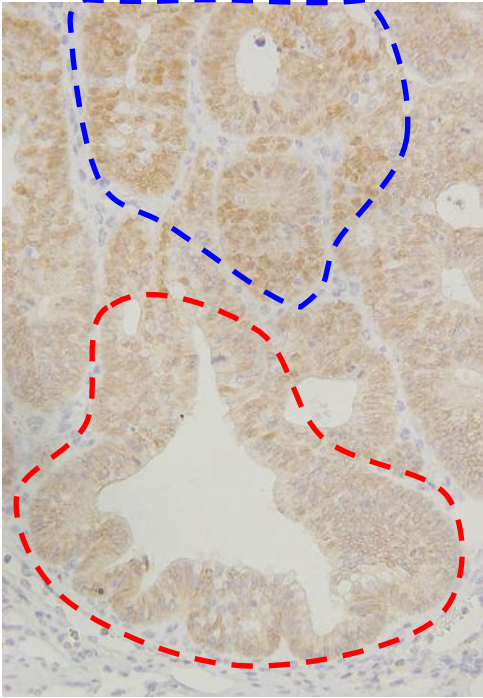
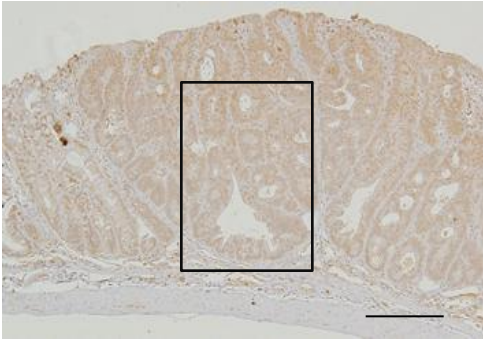
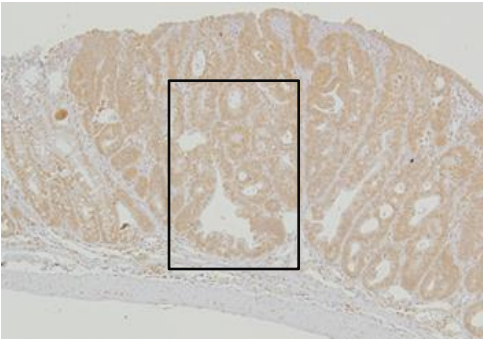
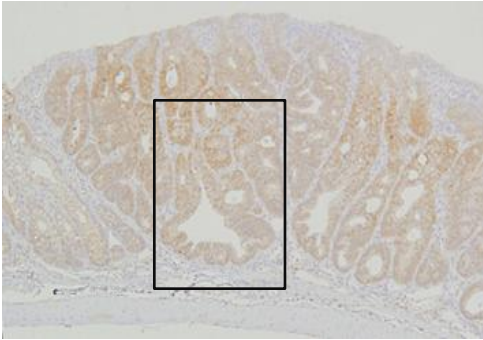


Supplementary Fig. 3 (Nakano et al)

$\beta$ -catenin

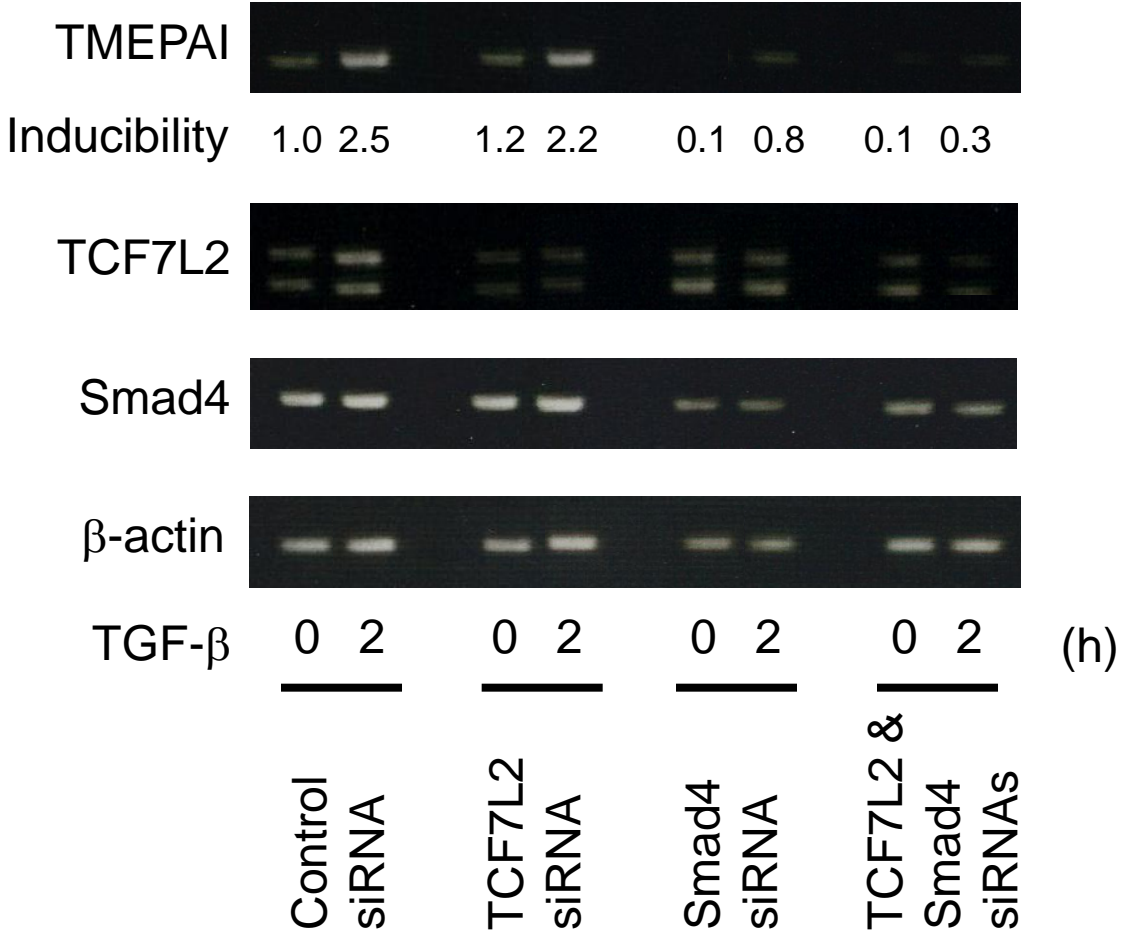
TMEPAI

Phosphorylated  
Smad2



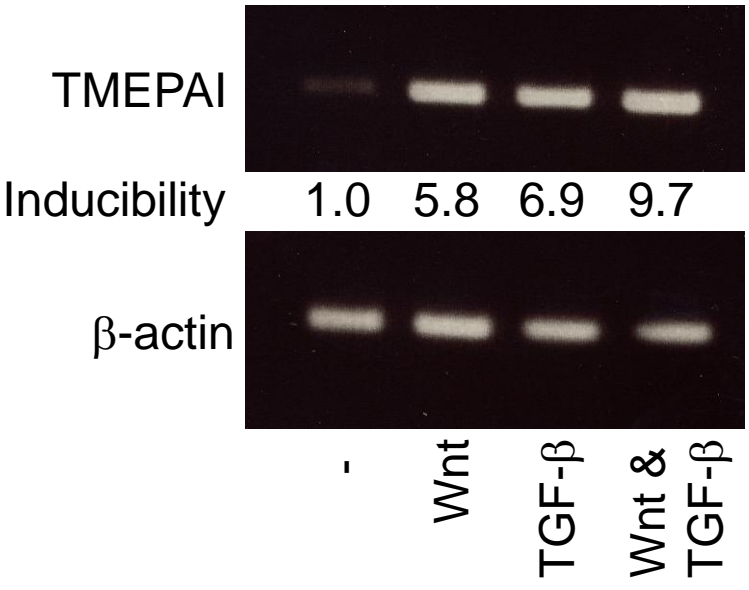
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Supplementary Fig. 4 (Nakano et al)

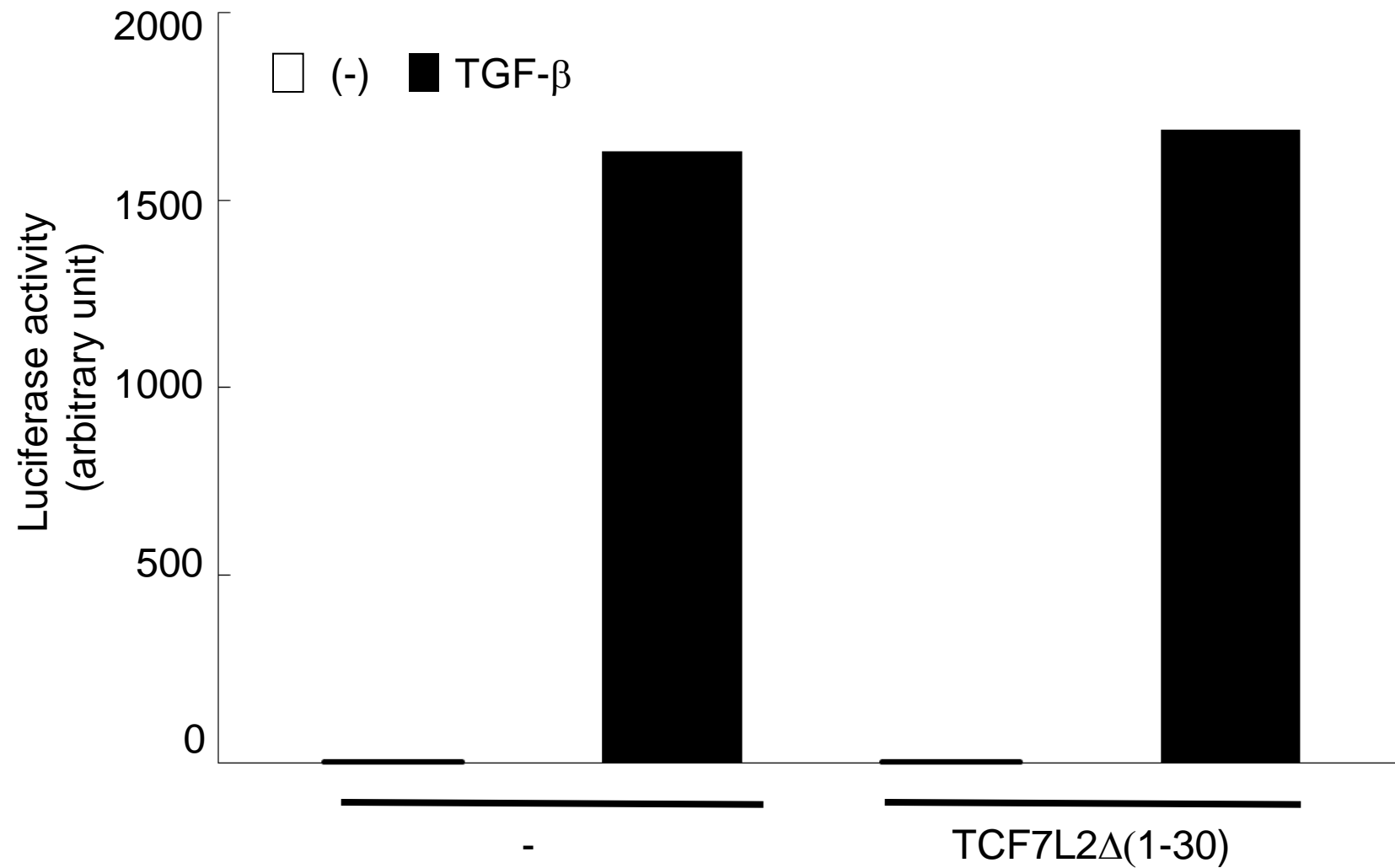




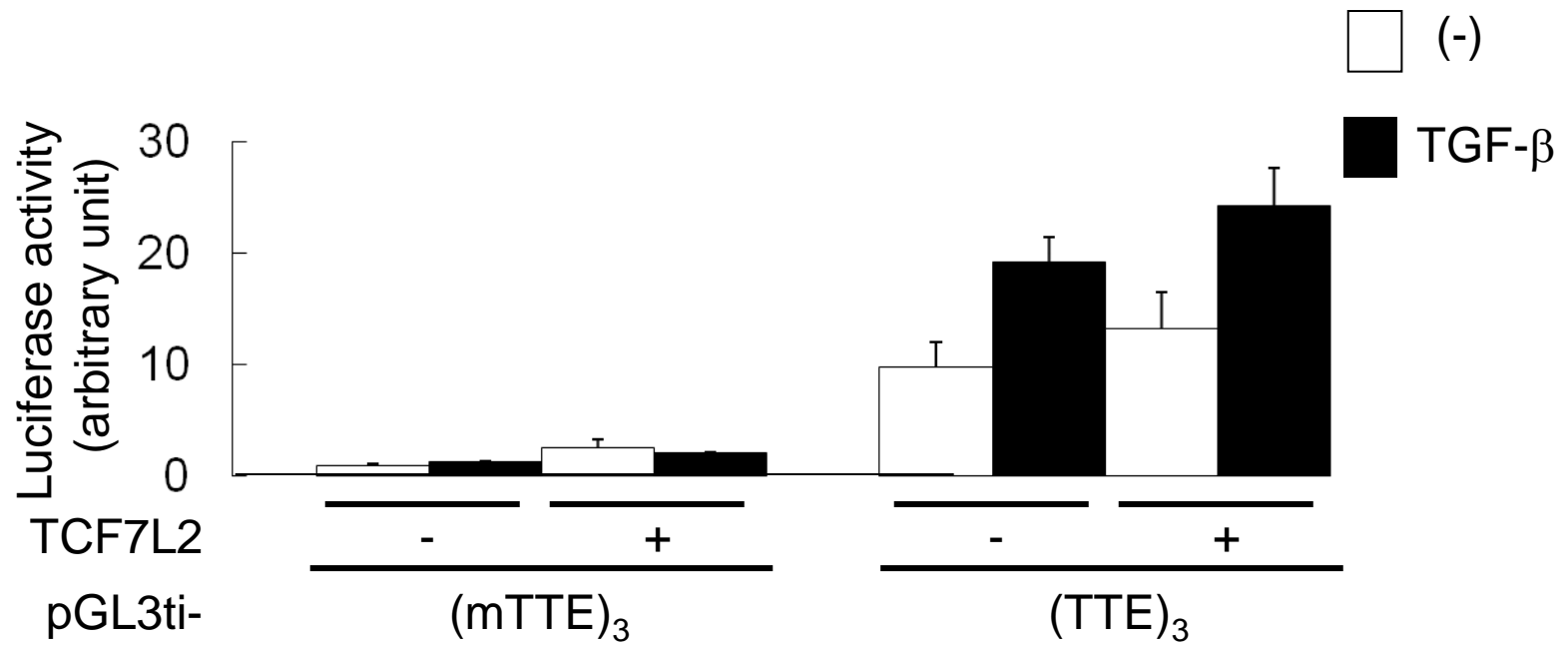
Supplementary Fig. 5 (Nakano et al)



Supplementary Fig. 6 (Nakano et al)



Supplementary Fig. 7 (Nakano et al)



Supplementary Fig. 8 (Nakano et al)

