

Supplementary material

Interferon- γ enhances the therapeutic effect of mesenchymal stem cells on experimental renal fibrosis

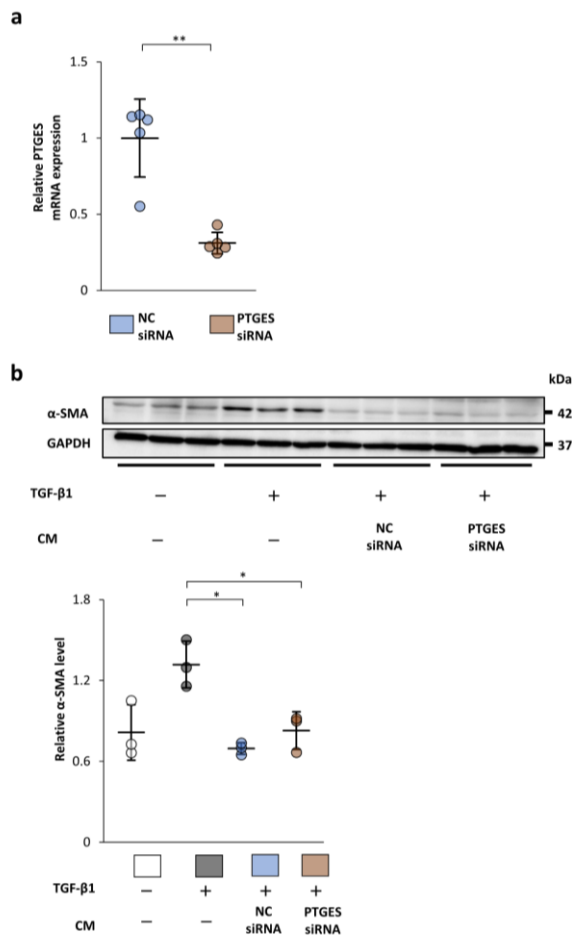
Ryo Kanai¹, Ayumu Nakashima^{1,2}, Shigehiro Doi¹, Tomoe Kimura¹, Ken Yoshida¹,
Satoshi Maeda^{2,3}, Naoki Ishiuchi¹, Yumi Yamada¹, Takeshi Ike¹, Toshiki Doi¹, Yukio
Kato^{2,3} & Takao Masaki¹

¹Department of Nephrology, Hiroshima University Hospital, 1-2-3 Kasumi, Minami-ku,
Hiroshima, Hiroshima 734-8551, Japan

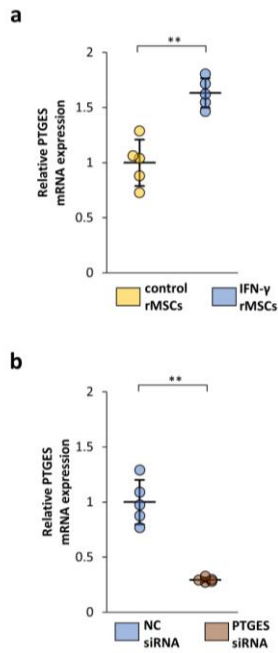
²Department of Stem Cell Biology and Medicine, Graduate School of Biomedical &
Health Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima,
Hiroshima 734-8553, Japan

³TWOCELLS Company, Limited, 16-35 Hijiyama-honmachi, Minami-ku, Hiroshima,
732-0816, Japan

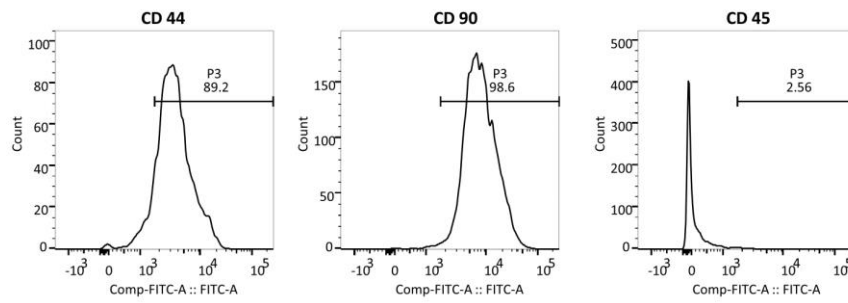
This file includes supplementary figure S1, S2, S3 and S4 and western blotting
uncropped gel images.



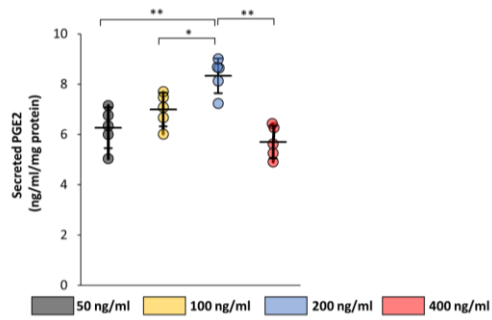
Supplementary figure S1. Effects of PTGES siRNA on the anti-fibrotic effects of IFN- γ -treated hMSCs *in vitro*. **(a)** After hMSCs were transfected with NC siRNA or PTGES siRNA, the cells were treated with IFN- γ , and then PTGES mRNA levels were evaluated by real-time PCR. PTGES mRNA levels were normalized to the level of 18S rRNA (n = 5 in each group). **(b)** After HK-2 cells were incubated with conditioned medium (CM) from IFN- γ -treated hMSCs transfected with NC siRNA or PTGES siRNA for 24 hours, the cells were treated with TGF- β 1. The figure and graph show western blot analysis of α -SMA in HK-2 cells. α -SMA protein levels were normalized to GAPDH levels (n = 3 in each group). Data are presented as the mean \pm SD. * p < 0.05, ** p < 0.01.



Supplementary figure S2. Expression of PTGES mRNA in rMSCs. **(a)** PTGES mRNA levels in rMSCs treated with or without IFN- γ were evaluated by real-time PCR. PTGES mRNA levels were normalized to 18S rRNA levels (n = 5 in each group). **(b)** After rMSCs were transfected with NC or PTGES siRNAs, rMSCs were treated with IFN- γ , and then PTGES mRNA levels were evaluated by real-time PCR. PTGES mRNA levels were normalized to the level of 18S rRNA (n = 5 in each group). Data are presented as the mean \pm SD. ** $p < 0.01$.



Supplementary figure S3. Cell surface markers on rMSCs. Flow cytometry showing the expression of surface markers on bone marrow-derived rMSCs.



Supplementary figure S4. The amount of PGE2 from hMSCs over different concentrations of IFN- γ . The amount of PGE2 in conditioned medium (CM) from hMSCs with 50, 100, 200, and 400 ng/ml IFN- γ were analyzed by ELISA (n = 5 in each group). PGE2 secretion was significantly elevated in CM from 200 ng/ml IFN- γ -treated human MSCs. Data are presented as the mean \pm SD. *p < 0.05, **p < 0.01.

Western blotting uncropped gel images

Figure 1

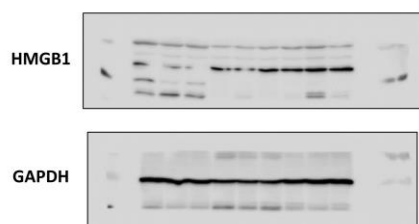


Figure 2

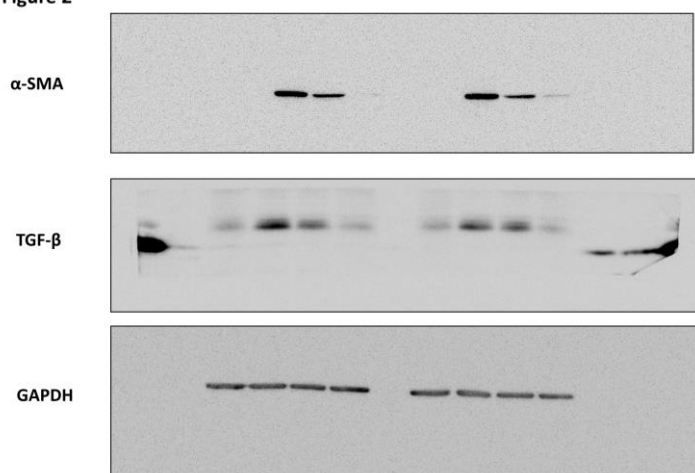


Figure 4

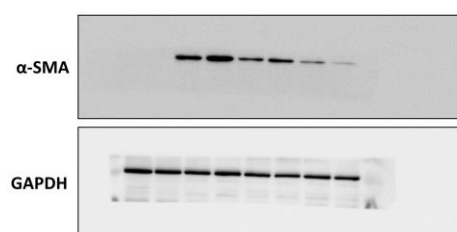


Figure 5

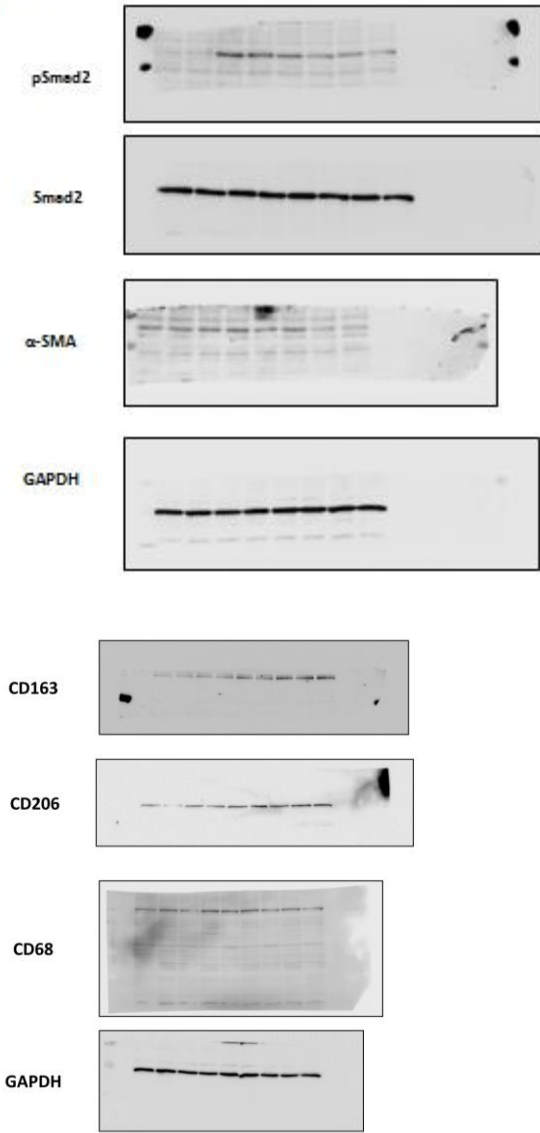


Figure 6

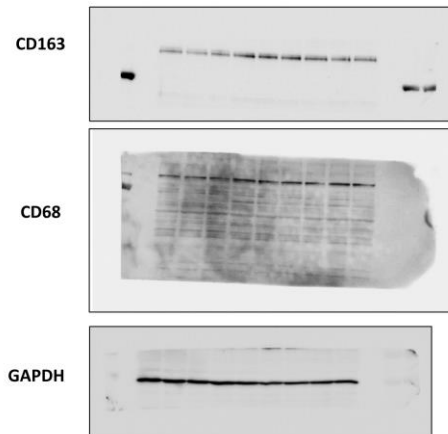
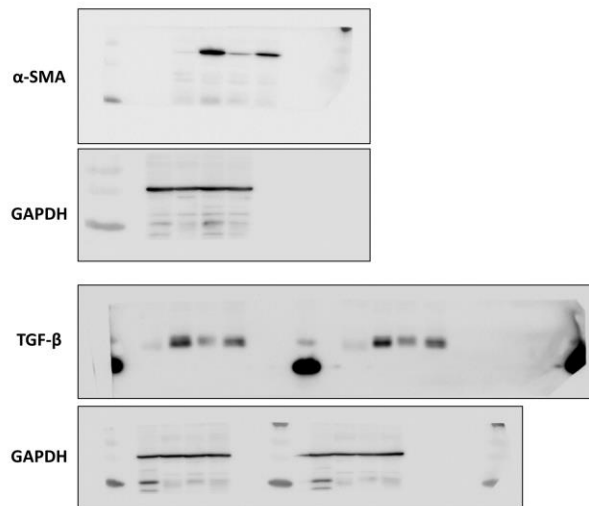


Figure 7



Supplementary Figure S1

