# Supporting Information

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## SI Materials and Methods

**Cells, Antibodies, and Chemicals.** The malignant melanoma cell line, HTB63, and the breast cancer cell line, MCF7, were obtained from the American Type Tissue Culture Collection (ATCC), and were grown according to ATCC recommendations. The A2058 malignant melanoma cell line was a generous gift of László Kopper from the Department of Pathology and Experimental Cancer Research, Semmelweis University, Hungary, and grown in RPMI medium 1640. All growth medium contained 5U/mL penicillin, 0.5 U/mL streptomycin, 10% fetal bovine serum, and 2 mM glutamine. All cell culture reagents were obtained from Sigma-Aldrich, and cells incubated at 37 °C in a humidified atmosphere of 5% $CO_2$. Serum-free conditioned medium from the HTB63 cells was prepared from 70% confluent cells growing for 48 h. The primary antibodies, anti-$\beta$-actin, clone AC-15 (Sigma-Aldrich), and anti-phospho-MARCKS (P-MARCKS; Cell Signaling Technology) were used at 1 in 25,000 and 1 in 1,000 for Western analysis, respectively. The Wnt5a antibody was developed by our laboratory against amino acids 275–290, as described previously (1), and used at a dilution of 1 in 1,000 for Western blotting. All peroxide-conjugated IgG secondary antibodies were obtained from Dako Chemicals and used at a concentration of 1 in 10,000 for Western analysis. Unless otherwise stated, stimulation of cells with recombinant Wnt5a (rWnt5a; R&D Systems) was carried out at a concentration of 0.2 $\mu$g/mL. Recombinant Wnt3a (rWnt3a) and TGF$\beta$1 (also R&D Systems) were used at concentrations of 0.05 $\mu$g/mL and 5 ng/mL, respectively unless otherwise stated. The TGF$\beta$1-typeI receptor inhibitor, SB431542 (Tocris Cookson), was used at a concentration of 10 $\mu$M. ET-1, carbachol, Fura-2/AM, and MAPT/AM were all purchased from Sigma-Aldrich, as too were all other analytical grade chemicals (if not otherwise stated).

**Reverse Transcriptase PCR (RT-PCR).** RNA extraction, RT reaction and PCR methods were carried out as described previously (2). The specific PCR primers were as follows: Wnt-5a forward: 5′-GGATTGTTAAACTCAACTCTC-3′; Wnt-5a reverse: 5′-ACACCTCTTTCCAAACAGGCC-3′;$\beta$ -actin forward: 5′-TTCAACACCCCAGCCATGTA-3′; $\beta$-actin reverse: 5′-TTGCCAATGGTGATGACCTG-3′; FZD-2 forward: 5′-ACATCGCCTACAACCAGACC-3′, FZD-2 reverse: 5′-CTCGCCCAGAAACTTGTAGC-3′; Frizzled-5 forward: 5′-ACACCCGCTCTACAACAAGG-3′ and FZD-5 reverse: 5′-CGTAGTGGATGTGGTTGTGC-3′. For FZD2 and FZD5, the reactions were performed on 3.5 times the amount of cDNA used for the $\beta$-actin control.

1. Jönsson M, Dejmek J, Bendahl PO, Andersson T (2002) Loss of Wnt-5a protein is associated with early relapse in invasive ductal breast carcinomas. *Cancer Res* 62:409–416.

2. Ford CE, Ekström EJ, Andersson T (2009) Wnt-5a signaling restores tamoxifen sensitivity in estrogen receptor-negative breast cancer cells. *Proc Natl Acad Sci USA* 106:3919–3913.

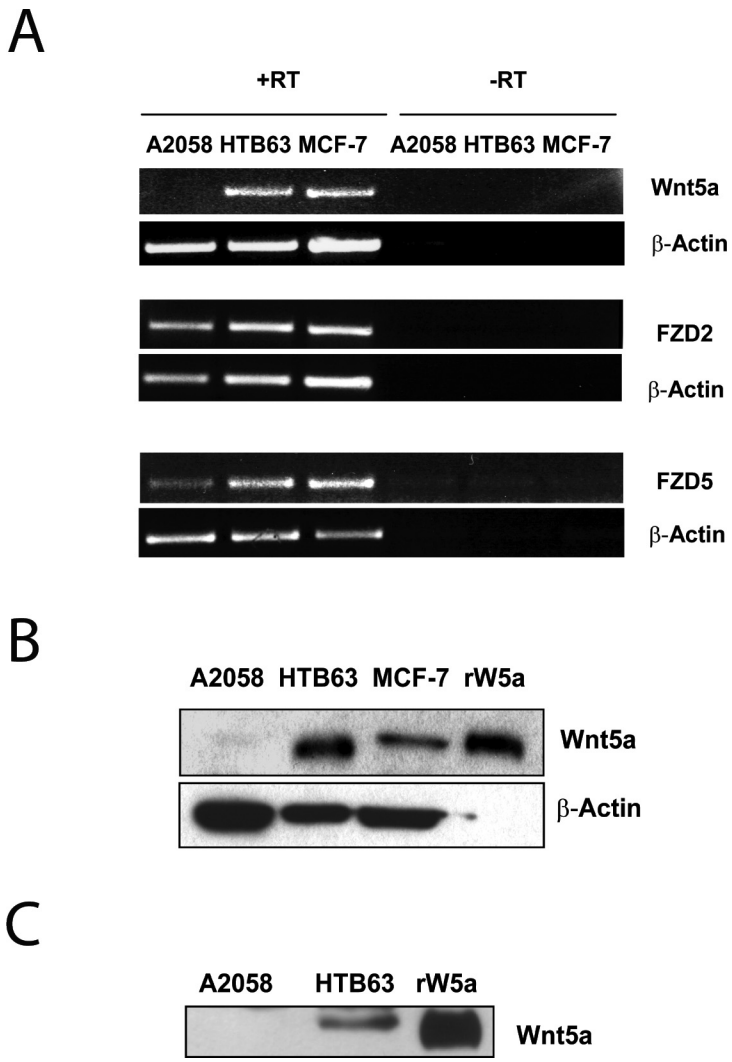


**Fig. S1.** Characterization of the A2058 and HTB63 cell lines. (*A*) A2058 and HTB63 melanoma cells have low, and have expression of Wnt5a mRNA, respectively as analyzed by RT-PCR. MCF-7 breast cancer cells were used as a positive control for the Wnt5a transcript. All cell lines express FZD5 and FZD2 at the mRNA level. Reactions performed with (+RT) and without (-RT) reverse transcriptase. (*B*) Corresponding Wnt5a protein levels, using recombinant Wnt5a (rWnt5a) as a positive control, as shown by Western analysis. (*C*) Western analysis of serum-free culture media collected from A2058 and HTB63 cells showing the absence and presence of secreted Wnt5a from these cell lines, respectively.

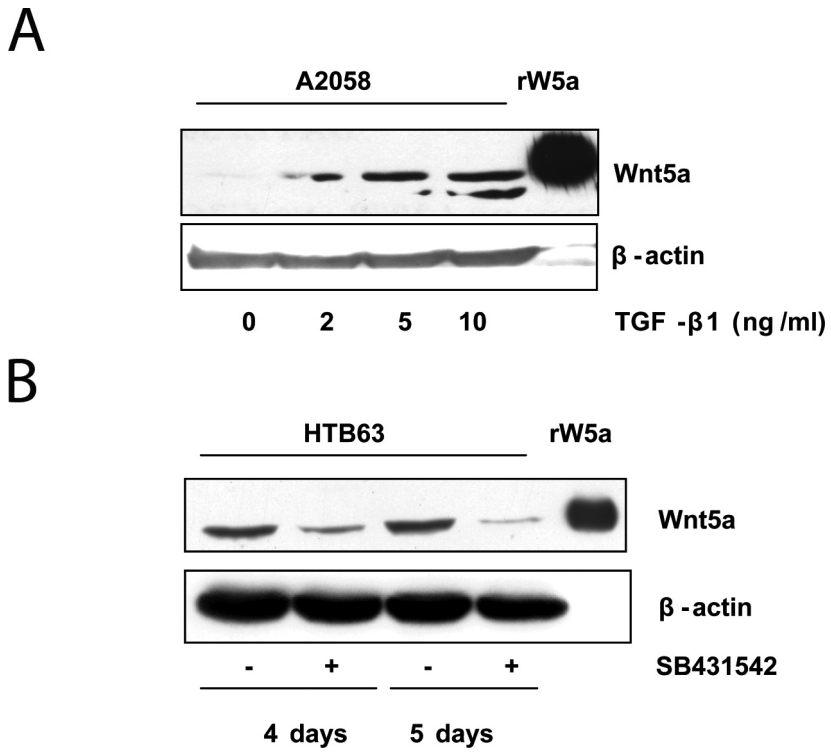


**Fig. S2.** TGF$\beta$1 up-regulates Wnt5a in human melanoma cell lines. (*A*) Western analysis of Wnt5a showing the effects of 24-h stimulation of A2058 cells (lacking endogenous expression of Wnt5a) with increasing concentrations of TGF$\beta$1. (*B*) Western analysis of Wnt5a showing the effects of 4- and 5-day treatments of the selective TGF$\beta$1 antagonist, SB431542 (10 $\mu$M), on HTB63 cells (these cells have endogenous expression of Wnt5a).

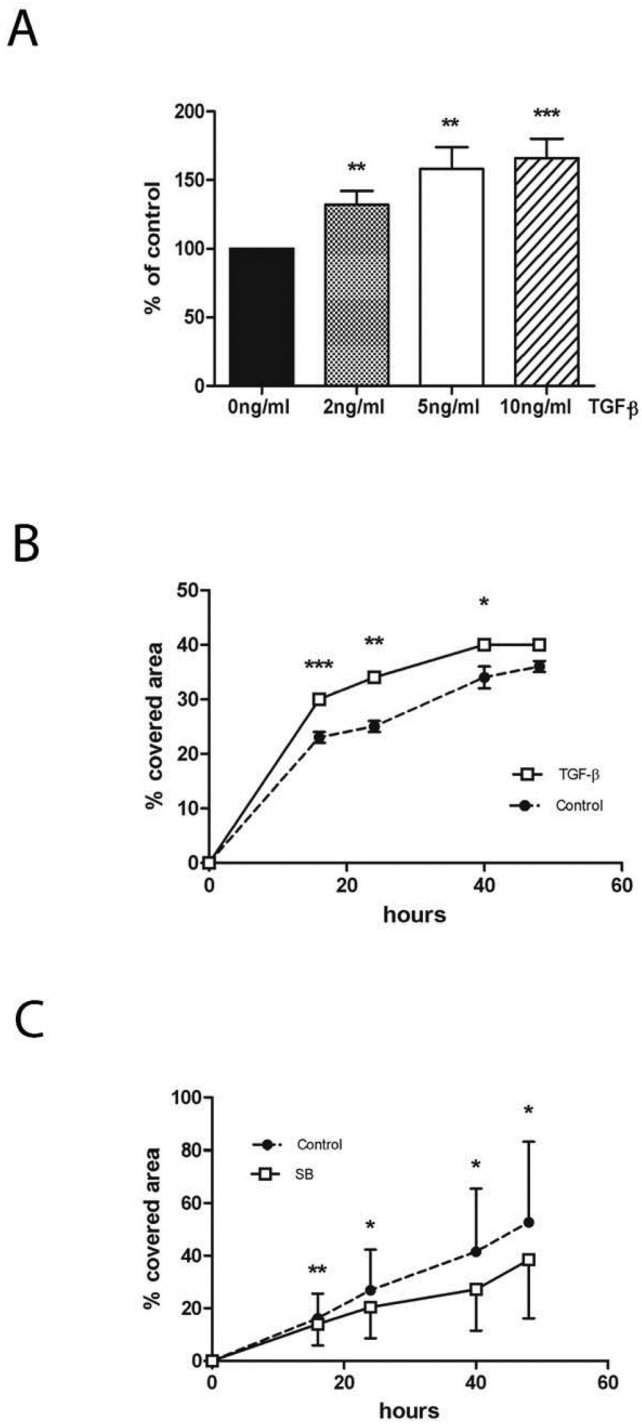


**Fig. S3.** TGF$\beta$1 promotes melanoma cell adhesion and migration. (*A*) Adhesion assay of A2058 cells stimulated with the indicated concentrations of TGF$\beta$1. (*B*) Wound-healing assay of A2058 cells stimulated with (open squares) and without (filled circles) TGF$\beta$1 at a concentration of 5 ng/mL. (*C*) Wound-healing assay of HTB63 cells in the absence (filled circles) or presence (open squares) of 10 $\mu$M SB431542.

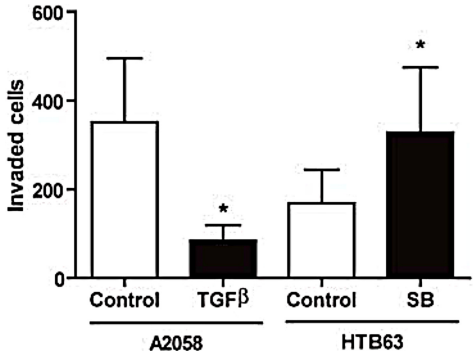


**Fig. S4.** TGF$\beta$1 inhibits melanoma cell invasion. Melanoma cell invasion was assessed using BD Matrigel invasion chambers. A2058 cells were incubated with or without 5 ng/mL of TGF$\beta$1, and HTB63 cells with or without 10 $\mu$M SB431542, during the 24 h invasion assay.

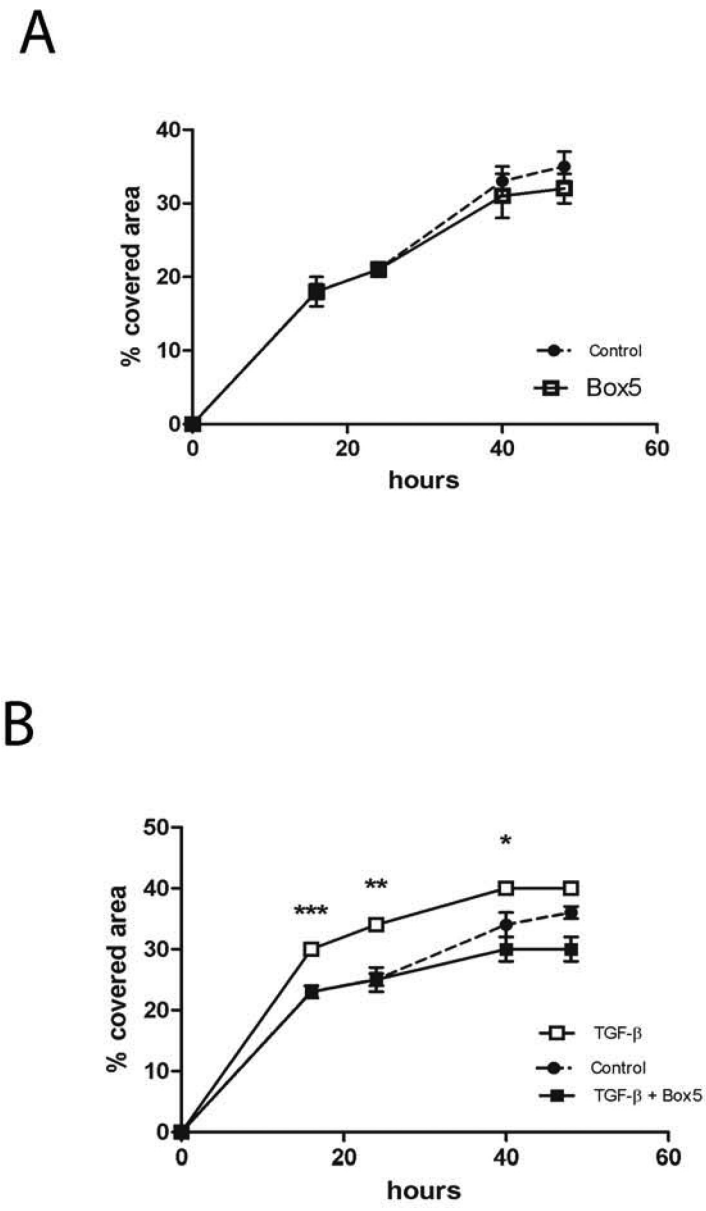


**Fig. S5.** Box5 has no effect on the basal migration of A2058 melanoma cells, but can inhibit TGF$\beta$1-induced migration. (*A*) Wound-healing analysis of A2058 cells stimulated with (open squares) or without (filled circles) 100 $\mu$M Box5. (*B*) Wound-healing assay of A2058 cells preincubated with or without 100 $\mu$M Box5 for 40 min, and then further stimulated with or without 5 ng/mL TGF$\beta$1, as indicated. All wound-healing data are expressed as percentage of the wound area closed after 0, 16, 24, 40, and 48 h.

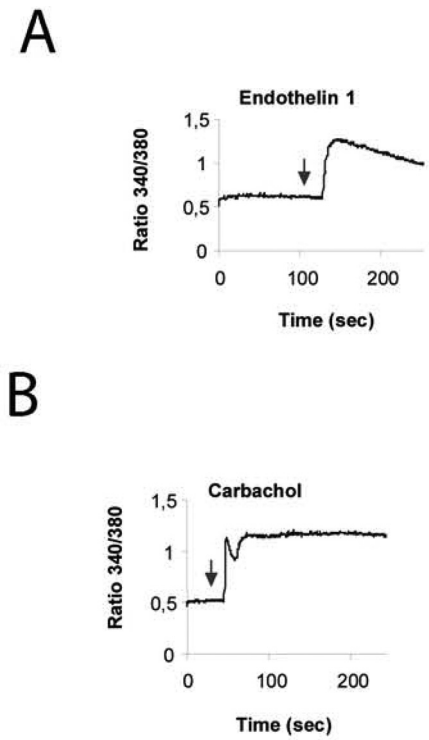


**Fig. S6.** Endothelin-1 and carbachol increase cytosolic $Ca^{2+}$ in A2058 melanoma cells. (*A*) Representative $Ca^{2+}$ trace from A2058 cells stimulated with 10 nM ET-1 (addition as indicated by arrow), using a Photon Technology International (PTI) imaging system. (*B*) Representative $Ca^{2+}$ trace from A2058 cells stimulated with 5 $\mu$M carbachol (addition as indicated by arrow), also analyzed using a PTI.