Appendix A. Supplementary data

The following are the supplementary data related to this article:

Figure S1 – The WNT5A-antagonistic peptide Box5 inhibits IL-6-induced melanoma cell invasion in HTB63 cells. Matrigel invasion of human HTB63 cells treated with either Carrier (0.1% BSA in PBS) alone or recombinant IL-6 (20 ng/ml) in the absence or presence of Box5 (100 μM). The invasion assays were performed over 24 h (n = 4) and analyzed as described in Materials and methods. The results are given as means and S.E.M; **, $p < 0.01$.

Figure S2 – siRNA silencing of WNT5A reduces Matrigel cell invasion and degradation of the reconstituted extracellular matrix component collagen (gelatin). (A) Matrigel invasion assay of human HTB63 cells transfected with either negative control siRNA (NC siRNA; 100 nM), anti-*WNT5A*-siRNA #1 (WNT5A siRNA #1; 100 nM) or anti-*WNT5A*-siRNA #2 (WNT5A siRNA #2; 100 nM), incubated for 48 h, detached and subsequently allowed to invade through a Matrigel-coated membrane. The invasion assays were performed over 24 h (n = 4) and analyzed as described in Materials and methods. (B) Gelatin-FITC degradation assay of human HTB63 cells transfected with different siRNA oligos as described in (A), detached and added to a gelatin-FITC coated surface. The gelatin-FITC degradation on this coated surface was allowed to occur over 24 h (n = 4) and then analyzed as described in Materials and methods. The results are given as means and S.E.M; **, $p < 0.01$, ***, $p < 0.001$.

Figure S3 – The basal migration of WM852 cells is significantly reduced in the presence of Box5. Transwell migration assay results of human WM852 melanoma cells treated with Carrier alone or two different concentrations of Box5 (100 or 500 μM). The migration assay was performed over 24 h (n = 6) and analyzed as described in Materials and methods. The results are given as means and S.E.M; *, $p < 0.05$

Figure S4 – 5-Aza treatment significantly increases the *WNT5A* mRNA expression in A2058 cells. qPCR analysis of *WNT5A* mRNA expression in A2058 cells treated with either DMSO alone or 5-Aza (5 μM dissolved in DMSO) for 48 h. The relative *WNT5A* mRNA expression levels were normalized against *TATA-binding protein (TBP)* mRNA expression (n = 3). The results are given as means and S.E.M; **, $p < 0.01$

Figure S5 – Short-term recombinant IL-6 stimulation triggers STAT3 but not Akt or ERK1/2 activation in HTB63 and A375 cells. Western blot analysis of tyrosine-705 phosphorylation of STAT3 (p-STAT3, Tyr705), Akt phosphorylation (p-Akt) and ERK1/2 phosphorylation (p-ERK1/2) in the human melanoma cell lines HTB63 (A) and A375 (B) treated with Carrier (0.1% BSA in PBS) alone or recombinant IL-6 (20 ng/ml) for 1, 5, 10, 30 and 60 min. Total STAT3 (t-STAT3), Akt (t-Akt) and ERK1/2 (t-ERK1/2) were used to ensure equal loading of all samples. Representative blots from three separate experiments are shown.

Figure S6 – Inhibition of STAT3 activity using S3I-201 significantly reduces the expression of the known STAT3-regulated proteins c-Myc, Bcl-2 and MMP-9. (A-C) HTB63 and A375 cells were treated with either DMSO alone or S3I-201 (100 μM dissolved in DMSO) for 24 h prior to cell lysis. These lysates were then analyzed by Western blotting for their contents of c-Myc, B-cell lymphoma-2 (Bcl-2) and Matrix metalloproteinase-9 (MMP-9) proteins. Densitometric analyses were performed, and the data were then normalized against α-tubulin. (n = 3). The results are given as means and S.E.M; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$

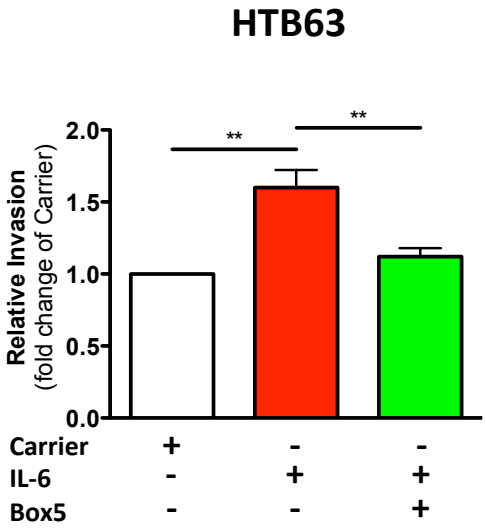
Figure S7 – siRNA interference of STAT3 does not affect WNT5A protein expression. (A) HTB63 and A375 cells were transfected with either negative control siRNA (NC siRNA; 50 nM) or anti-*STAT3*-siRNA (STAT3 siRNA; 50 nM) and incubated for 48 h prior to lysis. Western blot analysis showing STAT3 protein expression in these siRNA transfected cells. Densitometric analyses were performed, and the data were then normalized against α-tubulin (n = 3). (B) Western blot analysis of WNT5A expression in HTB63 and A375 cells treated as described in (A). Densitometric analyses were performed, and the data were then normalized against α-tubulin (n = 3). The results are given as means and S.E.M; **, $p < 0.01$; ***, $p < 0.001$.

Figure S8 – SB203580 treatment significantly reduces the basal expression of WNT5A in WM852 cells. Western blot analysis of WNT5A expression in WM852 cells treated with either DMSO alone or SB203580 (10 μM dissolved in DMSO) for 24 h. Densitometric analyses were performed, and the data were then normalized against α-tubulin (n = 3). The results are given as means and S.E.M; *, $p < 0.05$

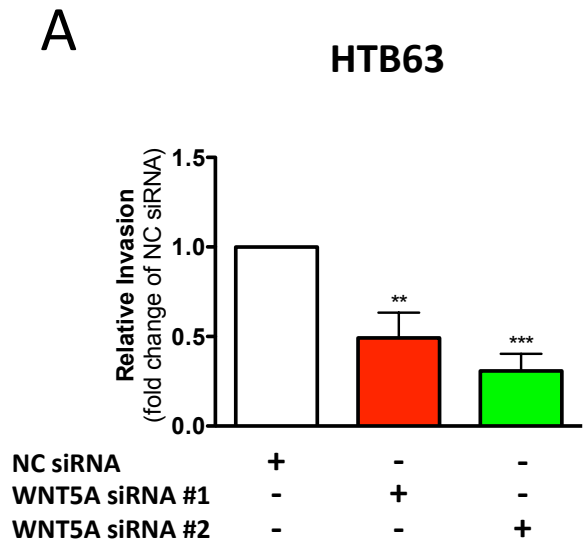
Figure S9 –TGFβ-induced WNT5A expression in HTB63 cells requires p38-MAPK activation. (A) Western blot analysis of p38-MAPK phosphorylation (p-p38) in the human melanoma cell line HTB63 incubated in the absence or presence of recombinant TGFβ1 (5 ng/ml) for 30 min, 1 h, 2 h, 3 h or 4 h. Densitometric analyses were performed, and the data were then normalized against total p38-MAPK (p38; n = 4). (B) Western blot analysis of

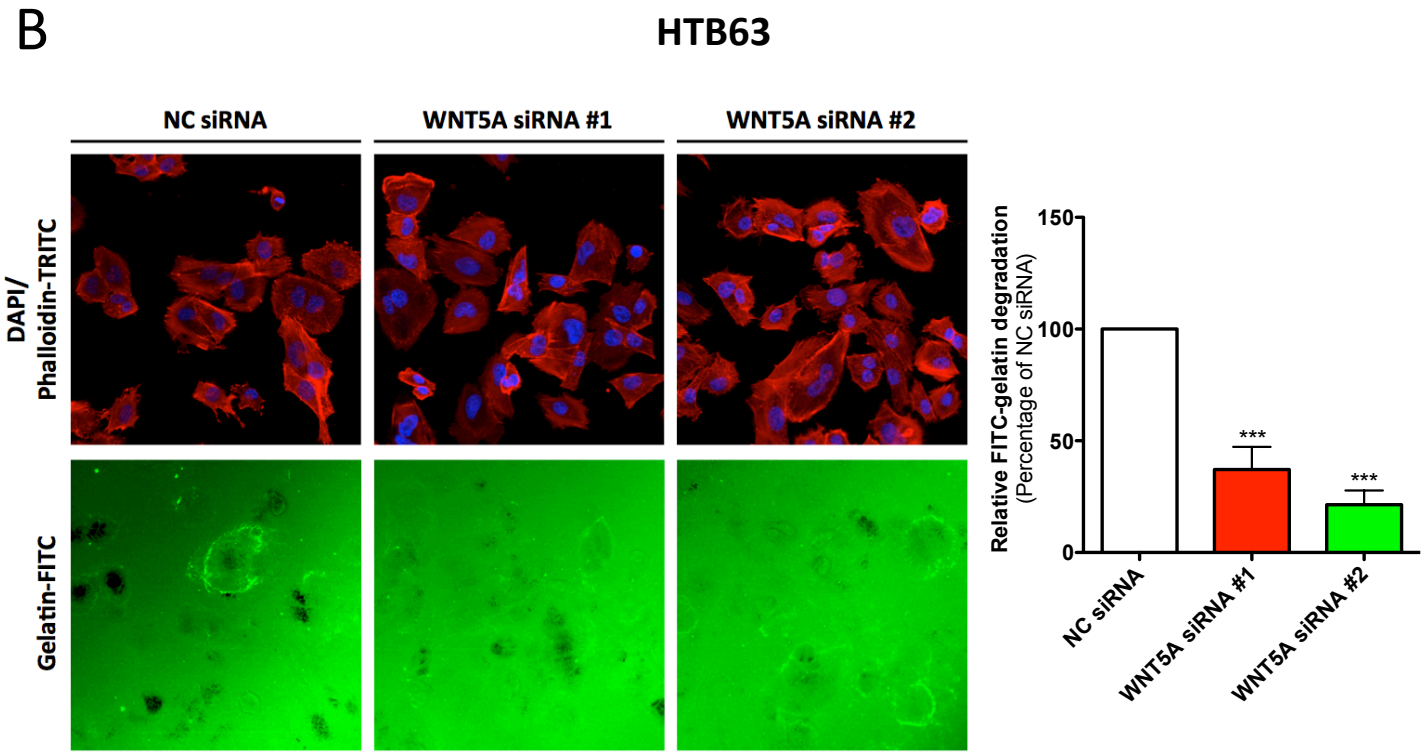
WNT5A expression in HTB63 cells treated with either Carrier (0.1% BSA in PBS) alone or recombinant TGFβ1 (5 ng/ml) for 24 h in the absence (only DMSO) or presence of SB203580 (10 μM dissolved in DMSO). Densitometric analyses were performed, and the data were then normalized against α-tubulin (n = 4). The results are given as means and S.E.M; ns, not significant; *, $p < 0.05$; ***, $p < 0.001$

SUPPLEMENTARY FIGURE 1



SUPPLEMENTARY FIGURE 2





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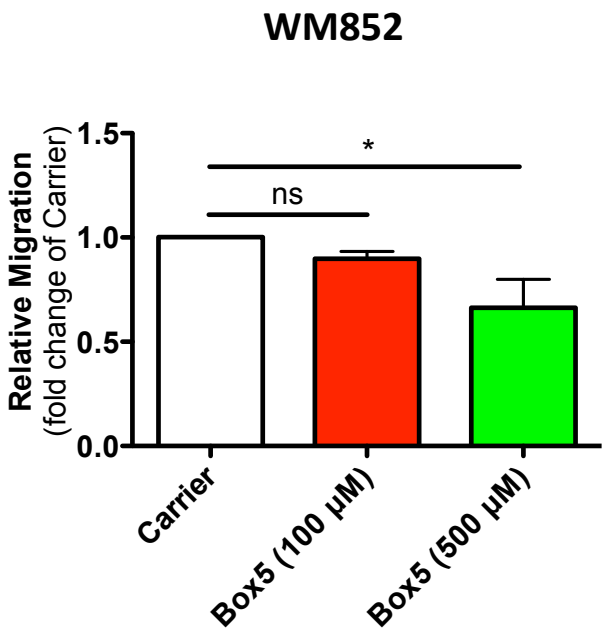

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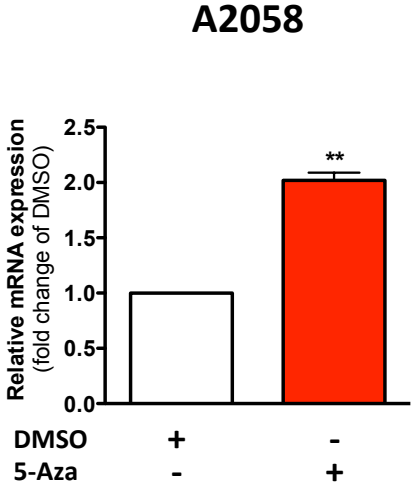

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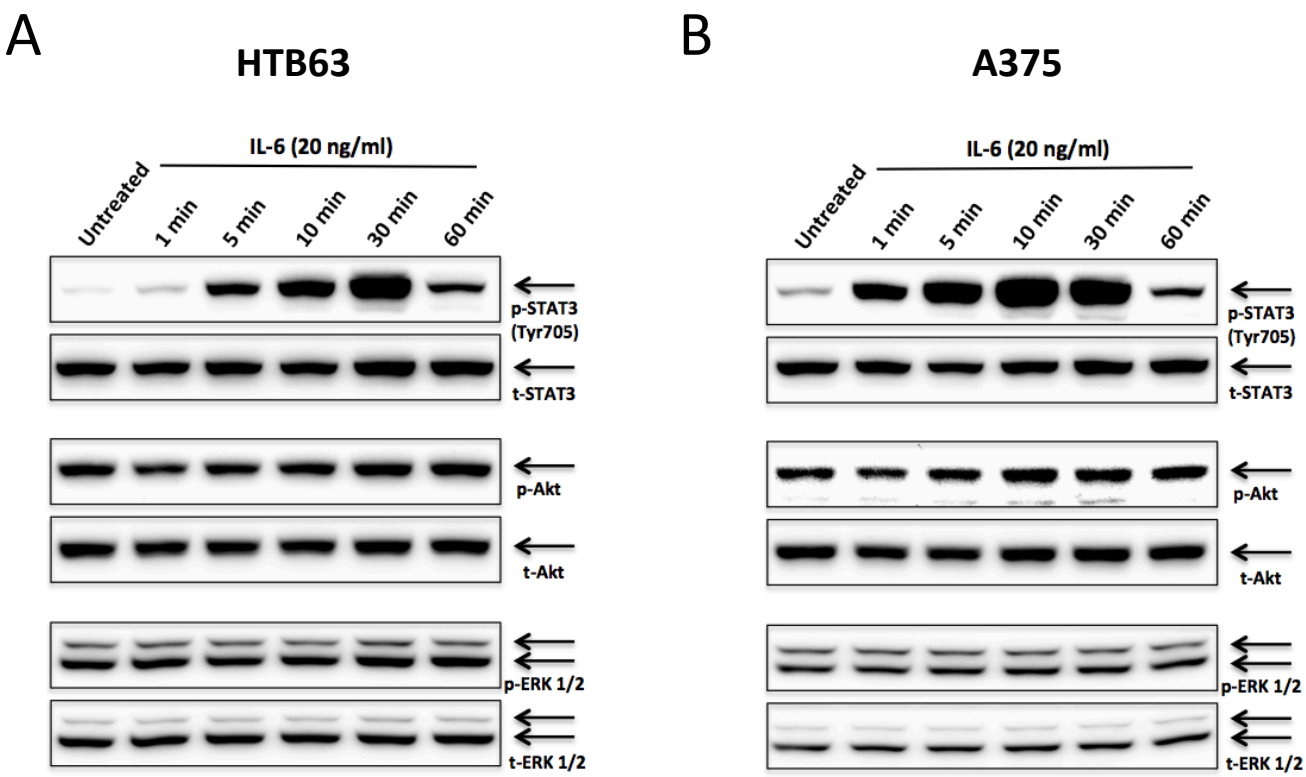

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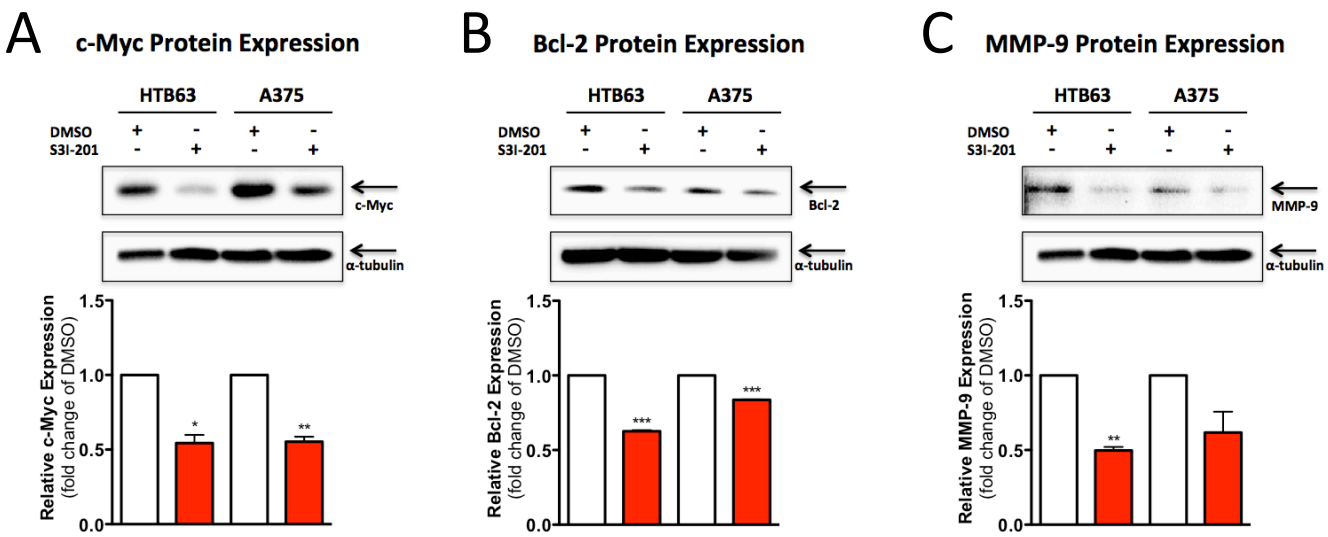

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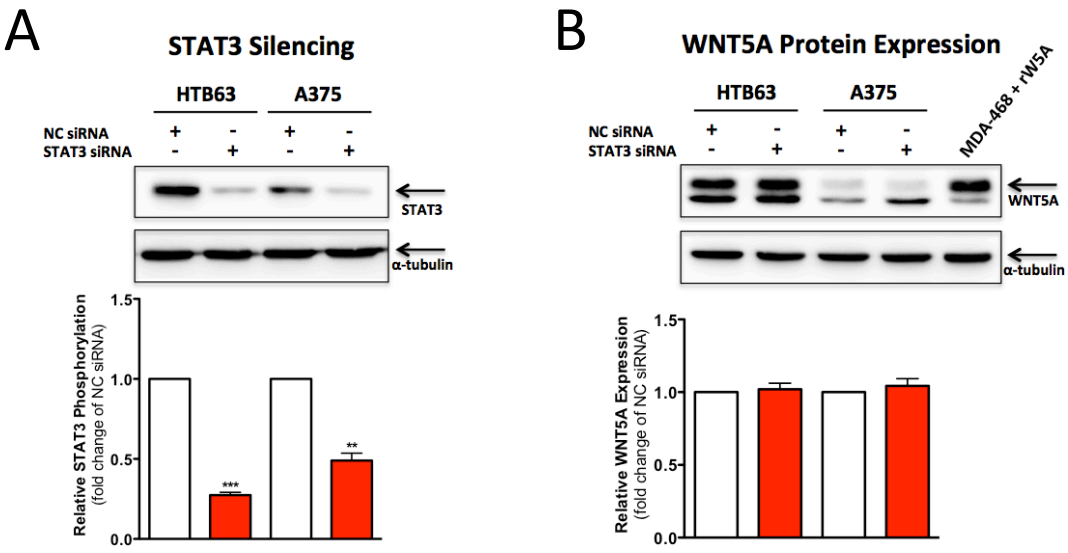

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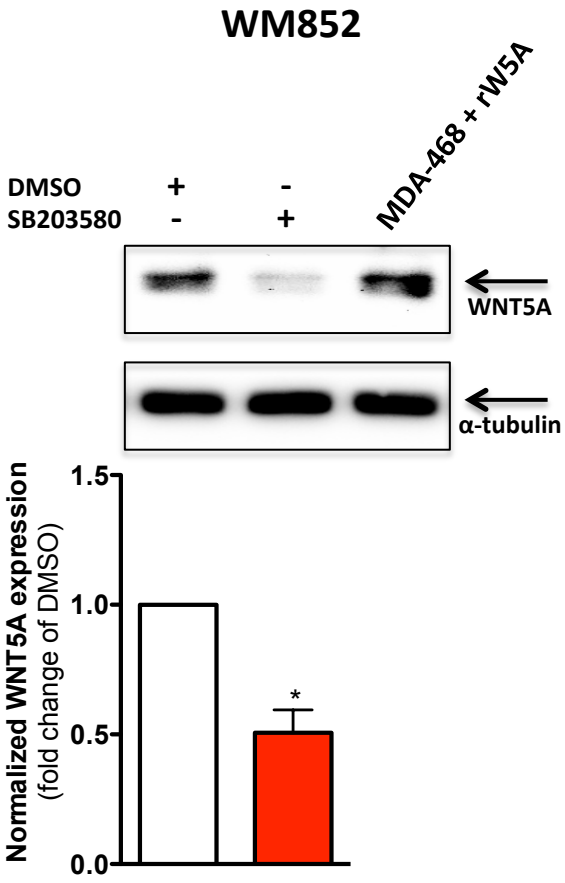

SUPPLEMENTARY FIGURE 9