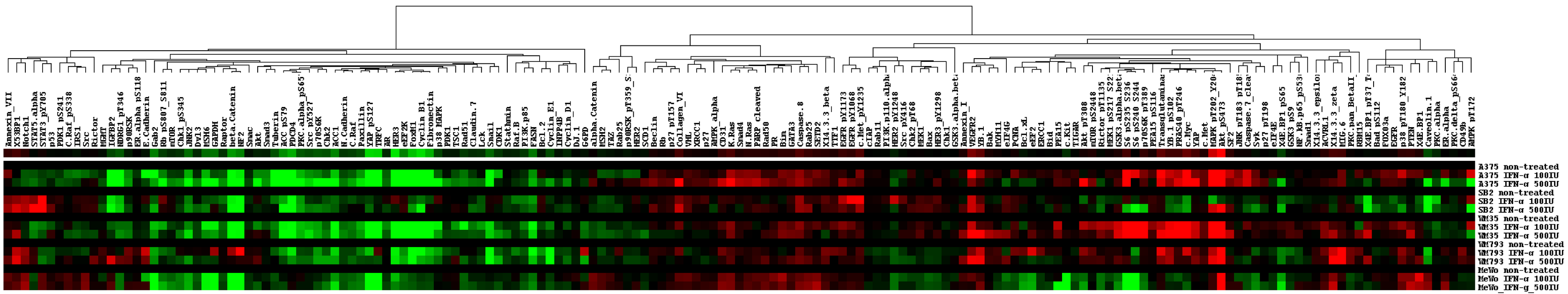
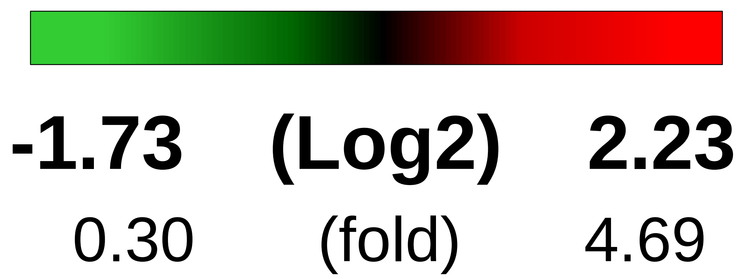
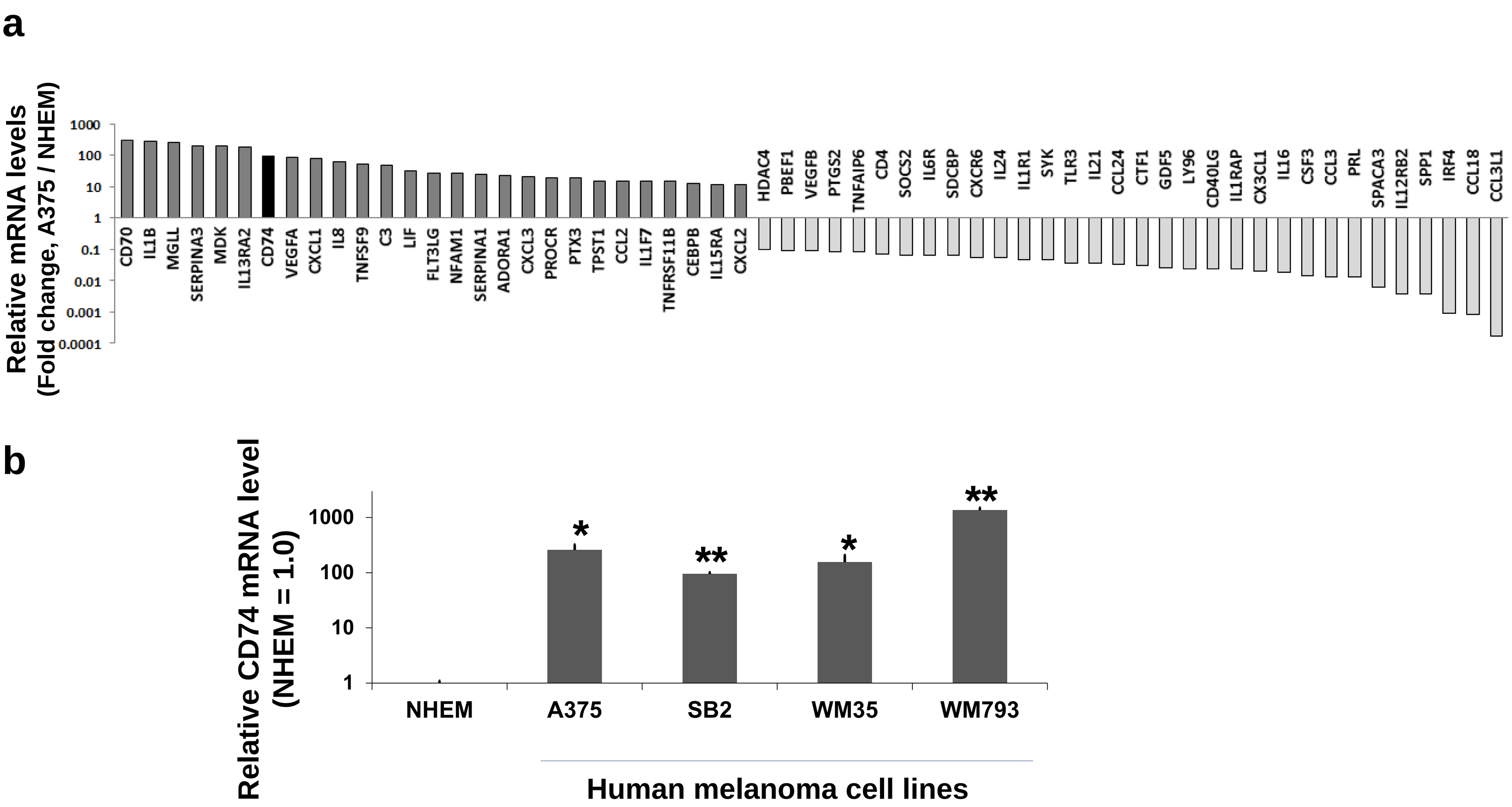
Median centered hierarchical cluster

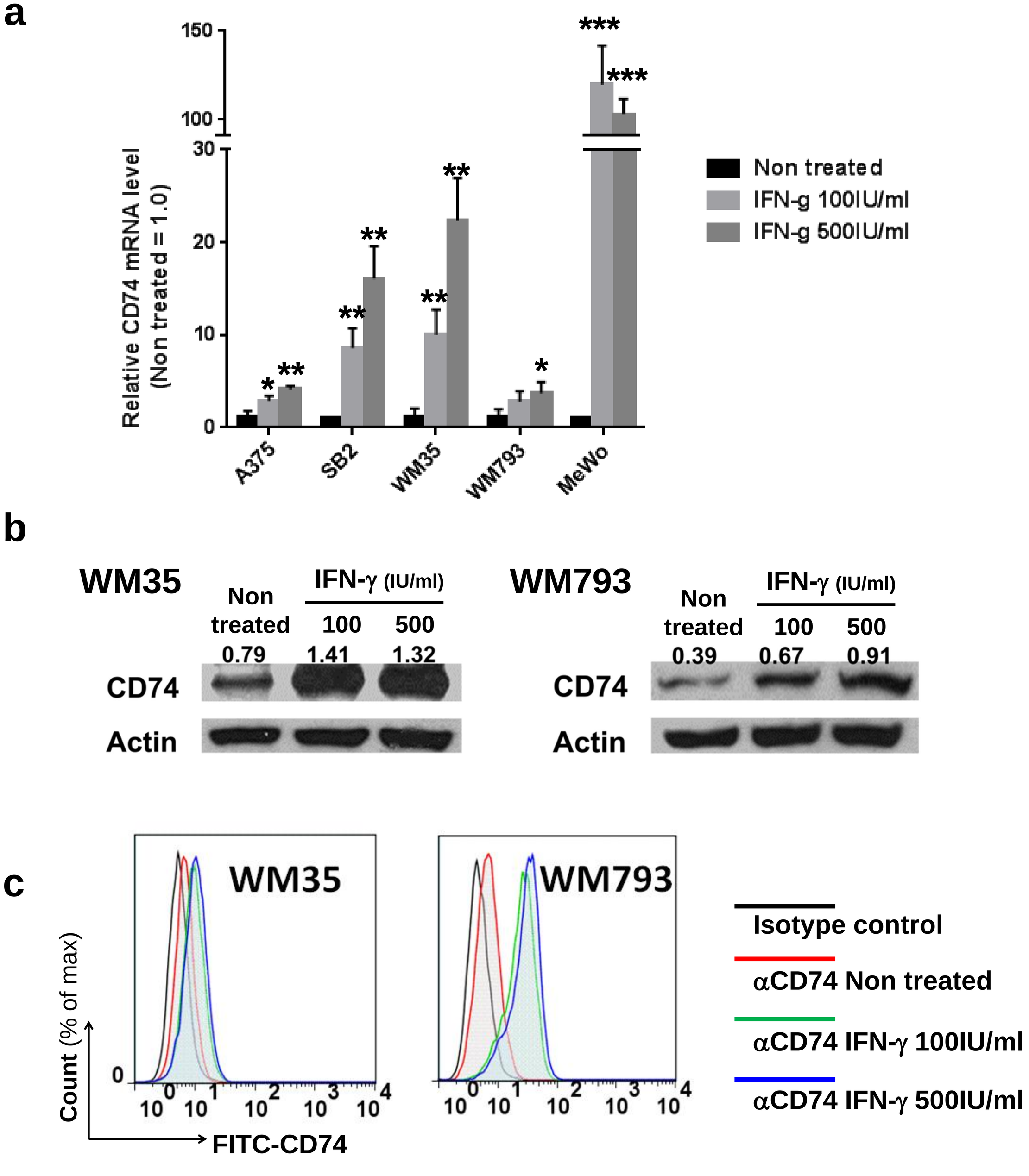




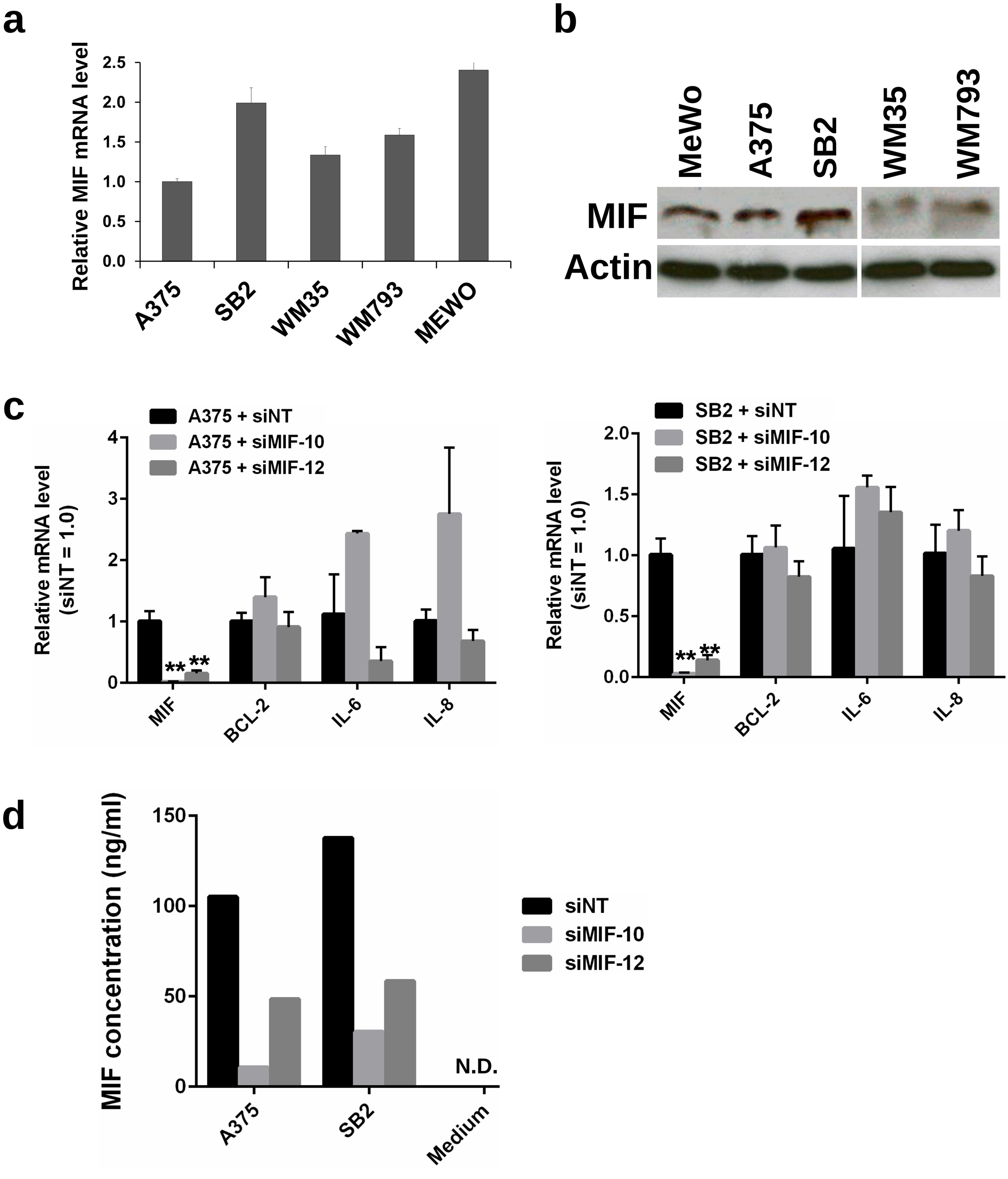
Supplementary Figure S1. Effect of IFN-γ on signaling pathways. (a) Supervised clustering heat map of RPPA analysis. Five melanoma cell lines were treated with 0, 100 or 500 IU/ml IFN-γ, and then extracted proteins were analyzed for the expression of 171 proteins and phosphorylation of signaling molecules (http://www.mdanderson.org/education-and-research/resources-for-professionals/scientific-resources/core-facilities-and-services/functional-proteomics-rppa-core/index.html).



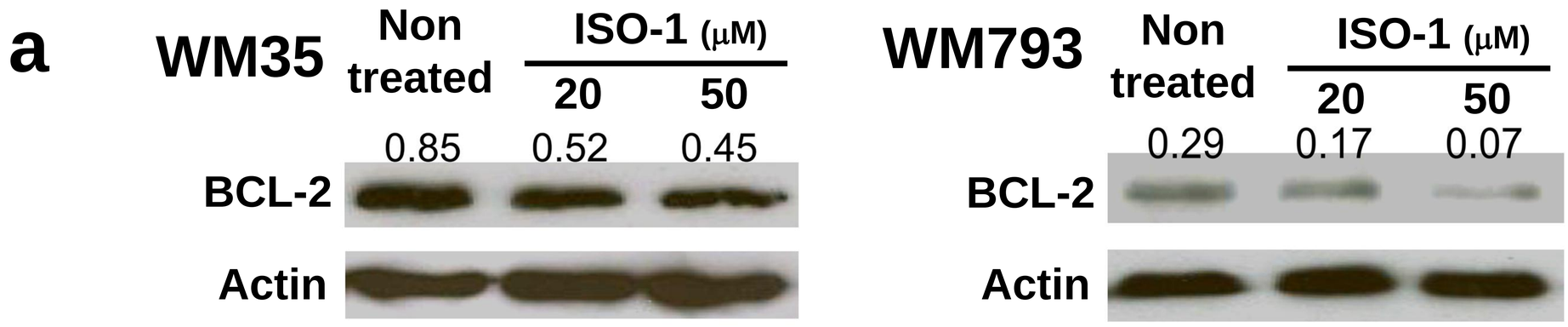
Supplementary Figure S2. Human melanoma cells express CD74. (a) Fold change of the expression of selected genes. Using the inflammatory response RT^2-Profiler PCR Array (SABioscience), 370 inflammatory-related genes were analyzed. Fold change was calculated by dividing the gene expression in A375 melanoma cells by that in normal human melanocytes (NHEMs); 10-fold or more increased and decreased genes were selected. (b) The expression of CD74 mRNA in NHEM and melanoma cell lines was determined by qRT-PCR. *$P < 0.05$, **$P < 0.01$.

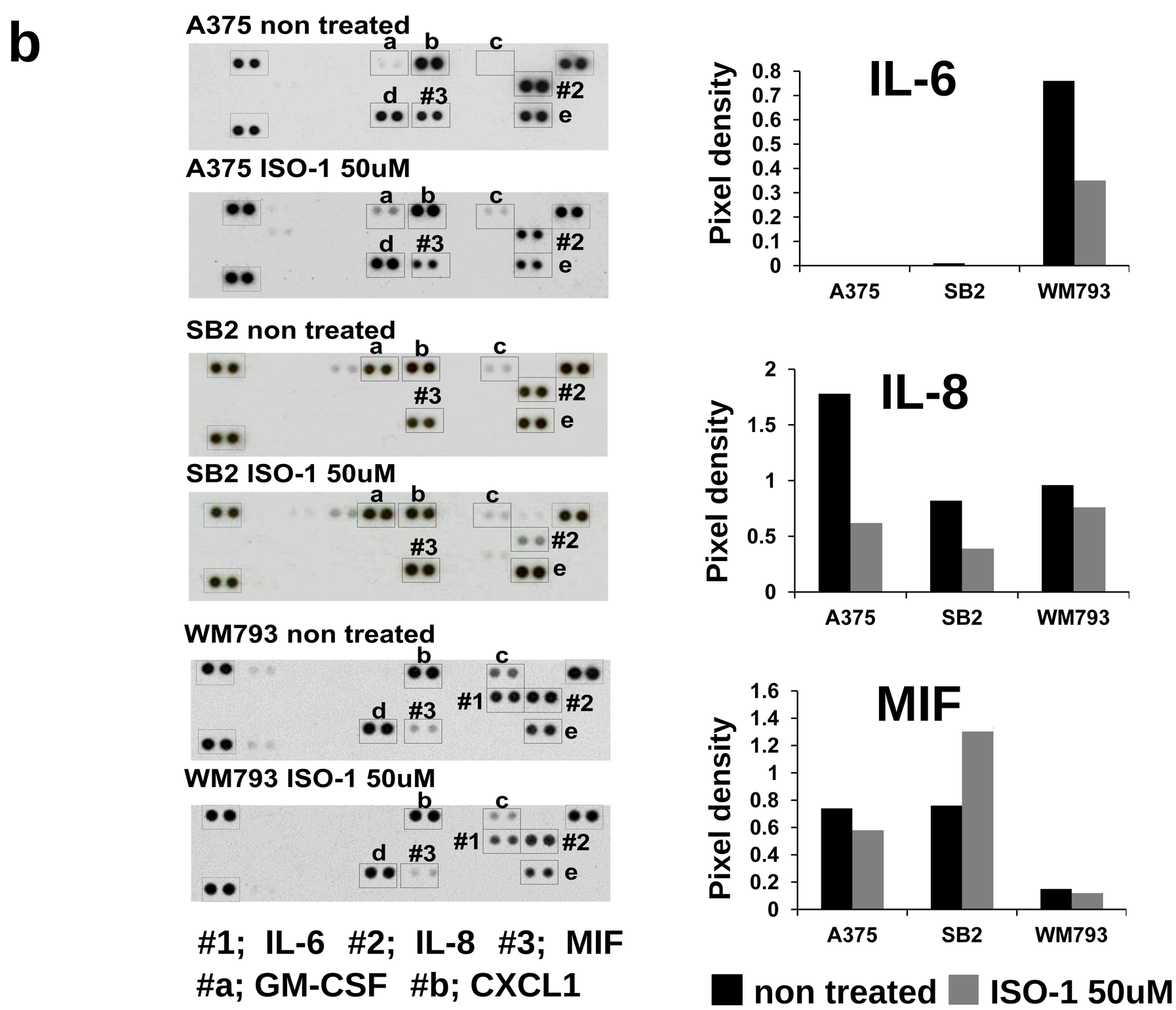


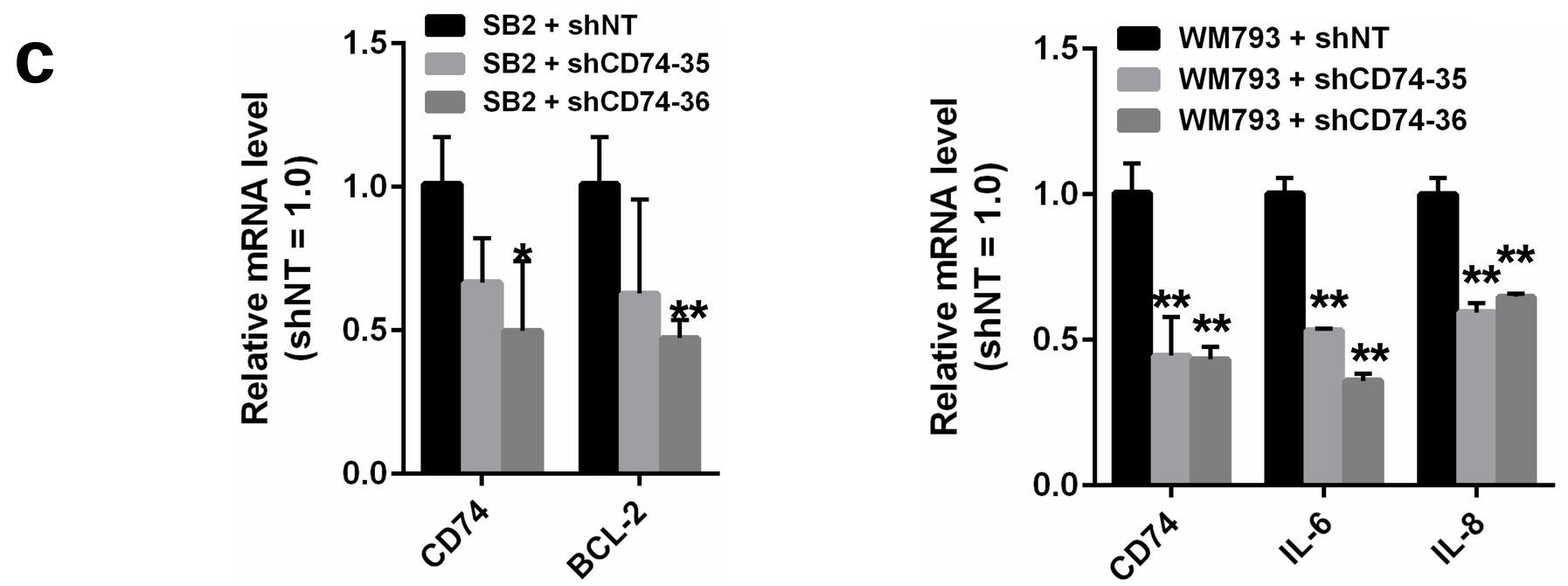
Supplementary Figure S3. IFN-γ stimulation upregulates total and cell surface CD74. Melanoma cell lines were treated with 0, 100 or 500 IU/ml IFN-γ for 48 hours and then analyzed for CD74 expression. (a) CD74 mRNA expression in IFN-g–treated cells compared with untreated cells. **P* < 0.05, ***P* < 0.01, ****P* < 0.001. (b) Western blot analysis for CD74 protein expression. Numbers above each band indicate relative expression level of CD74 to actin. (c) Flow cytometric analysis for cell surface CD74. Cells were stained with FITC-conjugated anti-CD74 antibody (M-B741) or its isotype control antibody.



Supplementary Figure S4. Melanoma cells express and secrete MIF. (a) The MIF mRNA expression in melanoma cells were measured by qRT-PCR. (b) Melanoma cell lines were subjected to Western blot analysis to determine MIF protein levels. (c and d) A375 and SB2 cells were transfected with 30 nM siRNA targeting MIF (siMIF) or non-target siRNA control (siNT). Three days after transfection, cells were harvested and culture supernates were collected. (c) qRT-PCR was performed to determine mRNA levels of indicated genes. $^{**}P < 0.01$. (d) MIF concentration in cell culture supernates was measured using a human MIF ELISA kit (R&D systems). N.D., not detected.

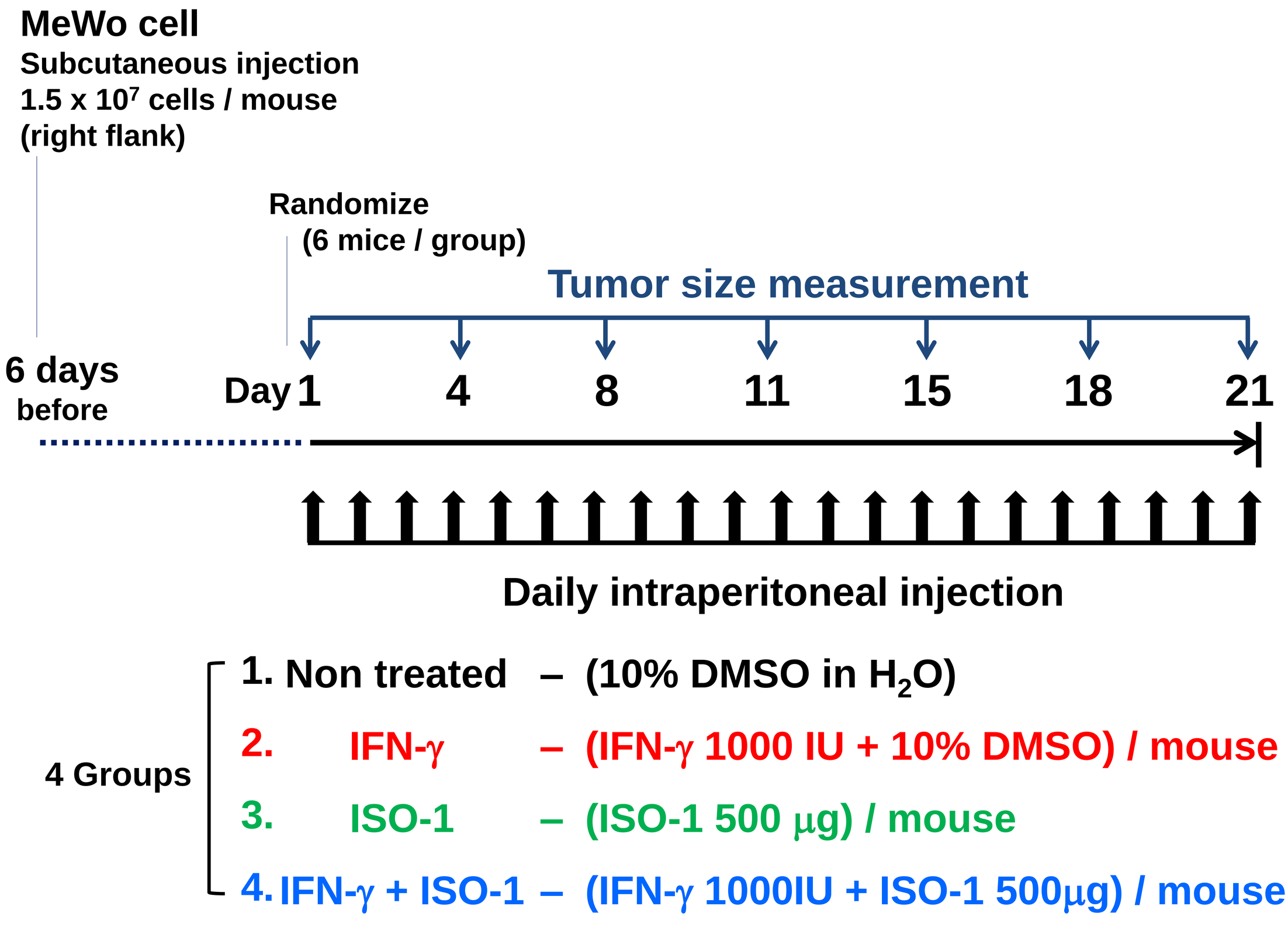






Supplementary Figure S5. MIF-CD74 autocrine interaction regulates expressions of BCL-2, IL-6 and IL-8. (a) Cells were treated with ISO-1 for 48 hours and then analyzed for BCL-2 protein expression by Western blot analysis. (b) Cells were treated with 50 μM ISO-1 for 48 hours and then cell culture supernates were subjected to the focused cytokine array. Right graphs indicate relative pixel densities of IL-6, IL-8 and MIF. (c) SB2 and WM793 cells were knocked down CD74 by shRNA. The mRNA expressions of indicated genes were determined by qRT-PCR. shNT, non-target shRNA. **P* < 0.05, ***P* < 0.01.

Mouse strain: SCID-beige mice



Supplementary Figure S6. The experimental schema of the xenograft mouse model study. MeWo cells (1.5×10^7) were subcutaneously inoculated into the flanks of 24 SCID Beige mice. Six days after cell inoculation, mice were randomly grouped into the following four treatment groups (6 mice per group): #1, untreated control; #2, IFN-γ (1000 IU/day); #3, ISO-1 (500 μg/day); #4, IFN-γ (1000 IU/day) and ISO-1 (500 μg/day). Each mouse received daily intraperitoneal injections for 21 successive days. Tumor size was measured every 3 or 4 days during treatment.

Supplementary Table 1

(a)Primer sequences used for quantitative real-time PCR studies

CD74	sense	5'-GAGCTGTCGGGAAGATCAGA-3'
	antisense	5'-AGGAAGTAGGCGGTGGTG-3'
MIF	sense	5'-CGGACAGGGTCTACATCAA-3'
	antisense	5'-CTTAGGCGAA-GGTGGAGTT-3'
IL-8	sense	5'-TGGCAGCCTTCCTGATTTCT-3'
	antisense	5'-TTAGCACTCCTTGGCAAAACTG-3'
IL-6	sense	5'- CACAGACAGCCACTCACCTC-3'
	antisense	5'-TTTTCTGCCAGTGCCTCTTT-3'
BCL-2	sense	5'-CATGTGTGTGGAGAGCGTCAA-3'
	antisense	5'-GCCGGTTCAGGTACTCAGTCA-3'
GAPDH	sense	5'-TGGGTGTGAACCATGAGAAG-3'
	antisense	5'-GCTAAGCAGTTGGTGGTGC-3'

(b) Primer sequences used for constructing sh-RNA

Forward Primer #35 (CD74 3'UTR)

5'-GATCCGCCACACAGCTACAGCTTTCTTCaagcttCAAGAAAGCTGTAGCTGTGTGGTTTTTTg-3'

Reverse Primer #35 (CD74 3'UTR)

5'-AATTCAAAAAACCACACAGCTACAGCTTTCTTCaagcttCAAGAAAGCTGTAGCTGTGTGGcg-3'

Forward Primer #36 (CD74 CDS)

5'-GATCCGCGCGACCTTATCTCCAACAATCaagcttCATTGTTGGAGATAAGGTCGCGTTTTTTg-3'

Reverse Primer #36 (CD74 CDS)

5'-AATTCAAAAAACGCGACCTTATCTCCAACAATCaagcttCATTGTTGGAGATAAGGTCGCGcg-3'

Forward Primer NT (control)

5'-GATCCGCAACAAGATGAAGAGCACCAACaagcttCTTGGTGCTCTTCATCTTGTTGTTTTTTg-3'

Reverse Primer NT (control)

5'-AATTCAAAAAACAACAAGATGAAGAGCACCAACaagcttCTTGGTGCTCTTCATCTTGTTGcg-3'

Note: loop AAGCTT = Hind III site