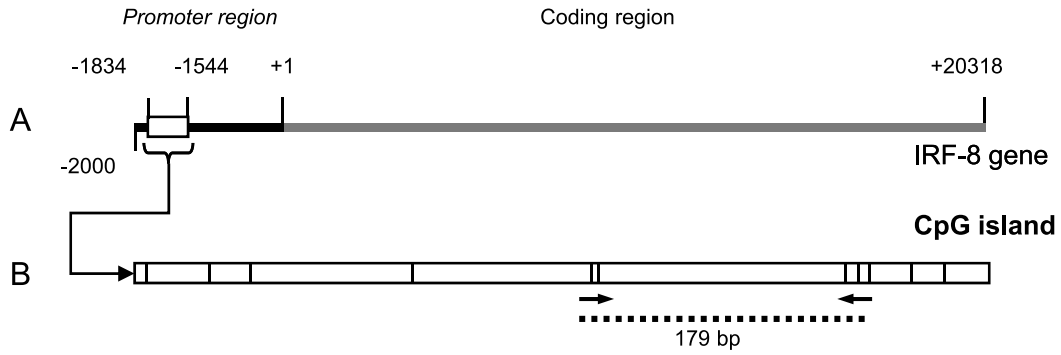

Figure W1. Analysis of a CpG island inside the promoter region of the mouse IRF-8 gene. (A) The promoter region of IRF-8 gene (MGI Identifier No. 96395) was analyzed from 2000 bp upstream the transcription initiation site (+1). The analyzed CpG island, detected by Methyl Primer Express software, is located at positions 1834 to 1544 bp before the initiation of transcription. The entire sequence, comprising the 2000-bp promoter region of IRF-8 gene and IRF-8 gene itself, is available online in the Nucleotide database of NCBI web site (http://www.ncbi.nlm.nih.gov/sites/entrez; Contig Sequence No. NT_078575.6). Black line, IRF-8 promoter sequence; gray line, IRF-8 gene sequence; white box, CpG island. (B) Detail of the analyzed CpG island. Horizontal black arrows depict primers used for detection of methylated or unmethylated status of the CpG island by DNA methylation–specific PCR. Discontinued line shows the amplicon obtained by the assay for both methylation and unmethylation primers. Vertical black thin lines show CpG sequences detected inside the analyzed CpG island.

Table W1. Murine and Human Primers Used for qRT-PCR.

Gene	Forward and Reverse Primers (5′→3′)	NCBI Accession Number	Amplicon Size (Base Pairs)
IRF-8	TGATCGAACAGATCGACAGC GCTGGTTCAGCTTTGTCTCC	NM_008320.3	187
CCL5	ATATGGCTCGGACACCACTC GTGACAAACACGACTGCAAGA	NM_013653.3	123
CCL21	GTGATGGAGGGGGTCAGGA GGGATGGGACAGCCTAAACT	NM_011124.4	109
CCL27	CTGCTGAGGAGGATTGTCCAC CACGACAGCCTGGAGGTGA	NM_011336	69
CXCL1	GCTGGGATTCACCTCAAGAA TCTCCGTTACTTGGGGACAC	NM_008176.2	180
CCR7	ACAGCGGCCTCCAGAAGAACA TGACGTCATAGGCAATGTTGAGCT	NM_007719.2	345
CCR10	GCCAGAGATGGGGACCAAGCC TGGGTTGGAAGGCCCGACTGA	NM_007721.4	143
CCL19	GGCCTGCCTCAGATTATCTGCCAT GGAAGGCTTTCACGATGTTCC	NM_011888.2	173
CX3CL1	ACGAAATGCGAAATCATGTGC CTGTGTCGTCTCCAGGACAA	NM_009142	120
CXCR3	TACCTTGAGGTTAGTGAACGTCA CGCTCTCGTTTTCCCCATAATC	NM_009910	100
CXCL12	GAGCCAACGTCAAGCATCTG CAATGCACACTTGTCTGTTG	NM_013655.3	96
CXCL10	CTCTCGCAAGGACGGTCCGC TCCGGATTCAGACATCTCTGCTCAT	NM_021274.1	166
CCR5	GCCAGAGGAGGTGAGACATCCGT GGCAGGAGCTGAGCCGCAAT	NM_009917.5	163
CCL20	GACAGATGGCCGATGAAGCTT TCACAGCCCTTTTCACCCAGT	NM_016960.1	108
IL-6	GAGGATACCACTCCCAACAGACC AAGTGCATCATCGTTGTTCATACA	NM_031168	141
IL-27 p28	CTGTTGCTGCTACCCTTGCTT CACTCCTGGCAATCGAGATTC	NM_145636.1	177
VEGF-A	AAAGGCTTCAGTGTGGTCTGAGAG GGTTGGAACCGGCATCTTTATC	NM_001025250	184
VEGF-B	TTAGAGCTCAACCCAGACACCTGTA CCTGTGAAGCAGGGCCATAA	NM_011697.3	104
VEGF-R2	GCCCTGCTGTGGTCTCACTAC CAAAGCATTGCCCATTCGAT	NM_010612	114
β-Actin	AGAGGGAAATCGTGCGT CAATAGTGATGACCTGGC	NM_007393.3	138
IRF-8 (human)	AGTAGCATGTATCCAGGACTGAT CACAGCGTAACCTCGTCTTC	NM_002163.2	196
GAPDH (human)	ATGGGGAAGGTGAAGGTCG GGGGTCATTGATGGCAACAATA	NM_002046.3	108

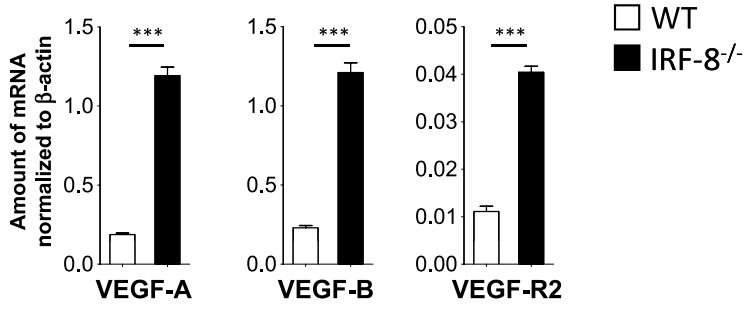

Figure W2. Expression of angiogenic factors in tumor bulks. Melanoma was excised from WT and IRF-$8^{-/-}$ mice ($n = 3$) at medium stage (20-mm mean diameter), and mRNA was extracted. qRT-PCR for the indicated angiogenic markers was performed. Histograms represent the amount of mRNA expression normalized to β-actin for each experimental condition run in triplicate (mean values ± SD). One representative experiment of two is shown.

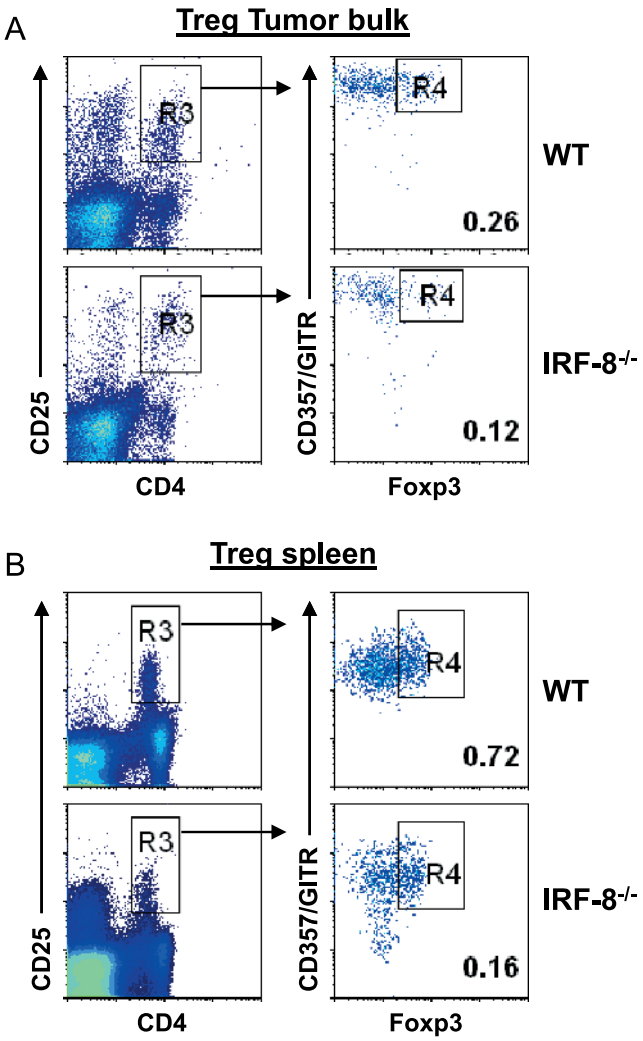

Figure W3. Frequency of Tregs in tumor and spleen of melanoma-bearing IRF-8$^{-/-}$ mice. Melanoma-bearing IRF-8$^{-/-}$ and WT mice were sacrificed at early tumor stage (12-mm mean diameter). FACS analysis of Tregs in tumor bulks (A) and spleens (B). Left-side dot plots show total live cell population; right-side plots show the population gated as indicated by the arrow. Values depicted refer to percent of positive cells over total live cells. One representative experiment of three is shown.

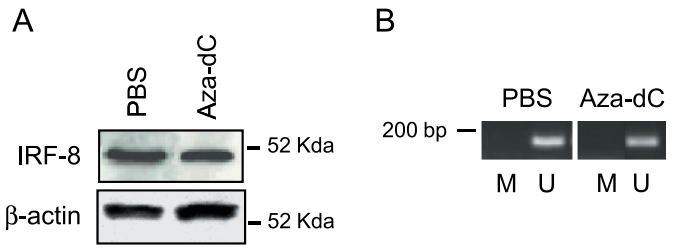

Figure W4. WT mice ($n = 8$) were injected s.c. with B16-F10 melanoma cells. At day 17 post-injection, mice were injected i.p. with PBS or 5-Aza-dC. Twenty-four hours later, mice were sacrificed and melanoma was excised. (A) Western blot analysis of intratumoral IRF-8 expression in WT-melanoma lesions. Numbers represent the size of protein weight markers. One representative experiment of two is shown. (B) DNA methylation assay specific for IRF-8 promoter region in WT-melanoma lesions at the indicated experimental conditions. M, methylation primers; U, unmethylation primers. Numbers represent length of DNA molecular weight markers.

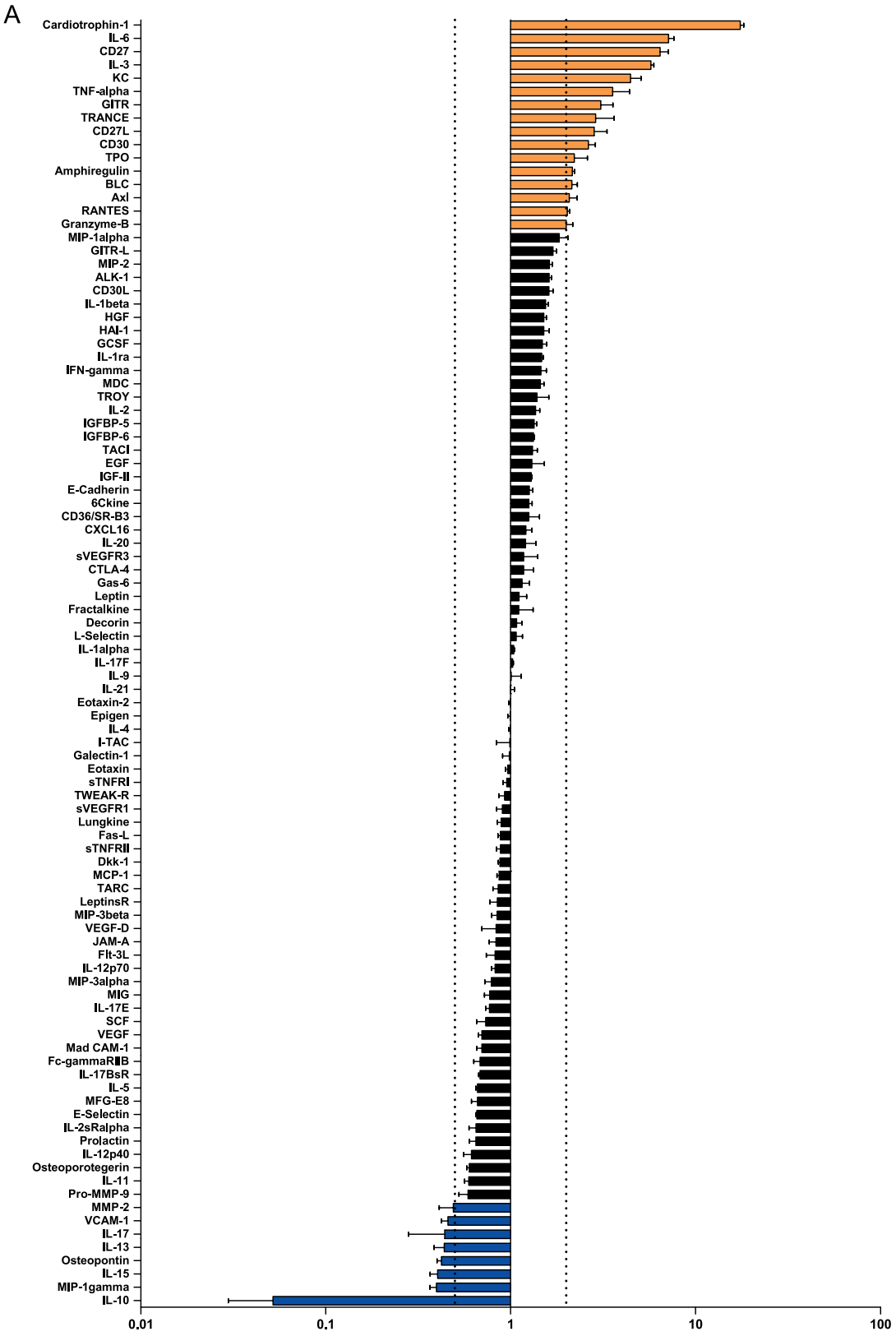

Figure W5. Differential cytokine release by immune cells from melanoma-bearing or naïve WT *versus* IRF-8$^{-/-}$ mice. Cytokines from supernatants of spleen cell–B16 melanoma co-cultures (24 hours) were measured using a protein array kit and quantified using ImageJ software. (A) Full array of differentially expressed cytokines in spleen cells of melanoma-bearing WT *versus* IRF-8$^{-/-}$ mice. Orange, cytokines upregulated in WT cells; blue, cytokines upregulated in IRF-8$^{-/-}$ cells; black, cytokines not differentially expressed. Analysis of protein expression of WT-upregulated cytokines (B) and of IRF-8–upregulated cytokines (C) in tumor-bearing and naïve mice. Values are expressed in arbitrary units. One experiment of two is shown.

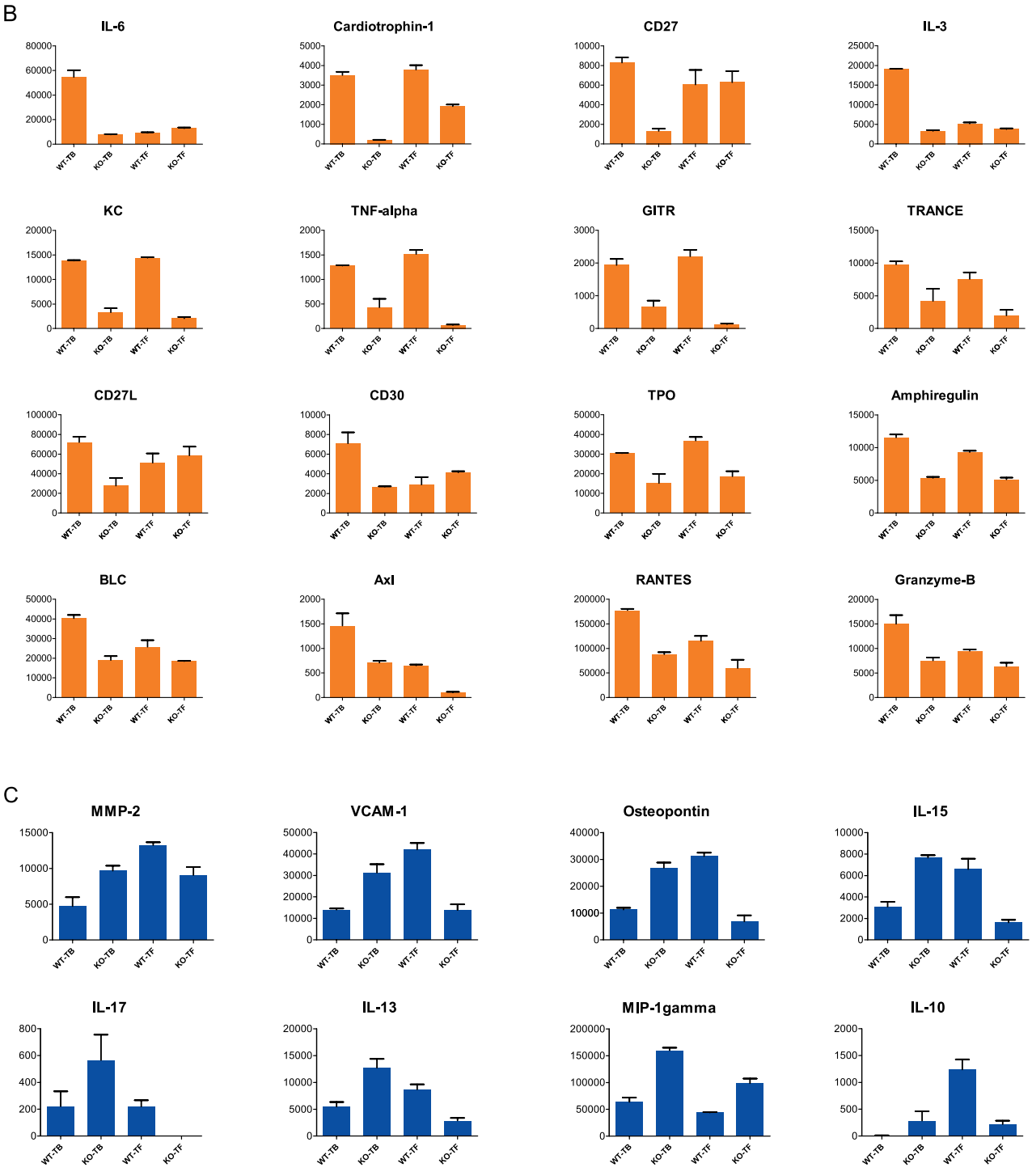

Figure W5. (*continued*).