

**Table S1. Primers used to quantitatively amplify the human miRNAs precursors and indicated genes**

	Forward primer (5'→3')	Reverse primer (5'→3')
U6	CTCGCTTCGGCAGCAC	AACGCTTCACGAATTTGCGT
5S	TACGGCCATACCACCCTGAA	TAACCAGGCCCGACCCTGCT
let-7d	AGAGGTAGTAGGTTGCATAGTT	
miR-101#	<b>AAAAA</b> TACAGTACTGTGATAACTGAA	
miR-106a	AAAAGTGCTTACAGTGCAGGTAG	
miR-106b#	<b>AAATAA</b> AGTGCTGACAGTGCAGAT	
miR-10a	TACCCTGTAGATCCGAATTTGTG	
miR-130b	CAGTGCAATGATGAAAGGGCAT	
miR-142-3p#	<b>AAATG</b> TAGTGTTCCTACTTTATGGA	
miR-142-5p#	<b>AAAAC</b> ATAAAGTAGAAAGCACTACT	
miR-146a#	<b>AAATG</b> AGAACTGAATTCATGGGTT	
miR-146b	TGAGAACTGAATTCATAGGCT	
miR-155	TTAATGCTAATCGTGATAGGGGT	
miR-15a	TAGCAGCACATAATGGTTTGTG	
miR-16	TAGCAGCACGTAATATTGGCG	
miR-17	CAAAGTGCTTACAGTGCAGGTAG	
miR-184	TGGACGGAGAACTGATAAGGGT	
miR-18a	TAAGGTGCATCTAGTGCAGATAG	
miR-193a	TGGGTCTTTGCGGGCGAGATGA	
miR-195	CCAATATTGGCTGTGCTGCTCC	
miR-198	GGTCCAGAGGGGAGATAGGTTT	
miR-199a*	ACAGTAGTCTGCACATTGGTTA	
miR-19a#	<b>AAATG</b> TGCAAATCTATGCAAAACTGA	
miR-19b#	<b>ATGTG</b> CAAATCCATGCAAAACTGA	
miR-206	TGGAATGTAAGGAAGTGTGTGG	
miR-20b	CAAAGTGCTCATAGTGCAGGTAG	
miR-21	TAGCTTATCAGACTGATGTTGA	
miR-214#	ACAGCAGGCACAGACAGGCAG	
miR-22	AAGCTGCCAGTTGAAGAAGTGT	
miR-221	AGCTACATTGTCTGCTGGGTTTC	
miR-222	AGCTACATCTGGCTACTGGGTC	
miR-23a	ATCACATTGCCAGGGATTTC	
miR-25	CATTGCACTTGTCTCGGTCTGA	
miR-26a	TTCAAGTAATCCAGGATAGGCT	
miR-29a	TAGCACCATCTGAAATCGGTTA	
miR-29C#	<b>ATAGC</b> ACCATTTGAAATCGGTTA	
miR-30a	TGTAAACATCCTCGACTGGAAG	
miR-30b	TGTAAACATCCTACACTCAGCT	
miR-30e-3p	CTTTCAGTCGGATGTTTACAGC	
miR-30e-5p#	<b>AAATG</b> TAAACATCCTTGACTGGA	
miR-31	AGGCAAGATGCTGGCATAGCT	
miR-338	AACAATATCCTGGTGCTGAGTG	
miR-342	AGG GGT GCT ATC TGT GAT TGA	
miR-34b	CAATCACTAACTCCACTGCCAT	
miR-363*	CGG GTG GAT CAC GAT GCA ATT T	
miR-373*	ACTCAAAATGGGGGCGCTTTCC	

	Forward primer (5'→3')	Reverse primer (5'→3')
miR-374a#	<b>AAA</b> ATTATAATACAACCTGATAAGTG	
miR-422b	CTGGACTTGGAGTCAGAAGG	
miR-424#	<b>AAAC</b> CAGCAGCAATTCATGTTTTGAA	
miR-451#	AAAAAAACCGTTACCATTACTGAGTT	
miR-523	GAACGCGCTTCCCTATAGAGGGT	
miR-549#	<b>AAAA</b> ATGACAACCTATGGATGAGCTCT	
miR-552#	<b>AAAA</b> ACAGGTGACTGGTTAGACAA	
miR-569#	<b>AAAA</b> AGTTAATGAATCCTGGAAAGT	
mR-656#	<b>AAAA</b> AAAAATATTATACAGTCAACCTCT	
miR-765	TGGAGGAGAAGGAAGGTGATG	
miR-768#	GTTGGAGGATGAAAGTACGGAGTG	
miR-92	TATTGCACTTGTCCCGCCTG	
miR-93	CAAAGTGCTGTTCTGTCAGGTAG	
miR-95#	<b>A</b> ATTCAACGGGTATTTATTGAGCA	
miR-98#	<b>AA</b> ATGAGGTAGTAAGTTGTATTGTT	
LV-miR-155WT	<u>GAGGATCC</u> TAAAGGTAACAATGTCATCT	GAGGTCGACGAATATATTTTTCTGTTAATG
LV-miR - 155MUT@	<b><i>AATTACG</i></b> AAATCGTGATAGGGGTTTTTGCC	ACCCCTATCACGATT <b><i>TCGTA</i></b> ATTCAGCATA
pGL-KPC1-WT	AAC <u>GCTAGC</u> CTGGAACCTCCACCTTTGAA	AAG <u>CTCGAG</u> TGTATGGAATAATAATTTAATGCTCAC
pGL-KPC1-MUT	AAC <u>GCTAGC</u> CTGGAACCTCCACCTTTGAA	TAG <u>CTCGAG</u> TGTATGGAATAATAATAATTTGCTCAC
pGL-CD115-WT	GAT <u>CTCGAG</u> CAAACCTCTGCCTTCGGTCAT	GAT <u>GTCGACT</u> TAATGCTGTTAGTTTAATGTGGACAGA
pGL-CD115-MUT	GAT <u>CTCGAG</u> CAAACCTCTGCCTTCGGTCAT	GAT <u>GTCGAC</u> AATTACCTGTTAGTTTAATGTGGACAGA
pGL-SOCS-1-WT	AAT <u>GCTAGC</u> CCCGGCAGCGCCCGCCGTGC	CAT <u>CTCGAG</u> CTGCACAGCAGAAAAATAAAGC
pGL-SOCS-1- MUT	AAT <u>GCTAGC</u> CCCGGCAGCGCCCGCCGTGC	
	ACGCAGCAAATTCT	
GAPDH	GTCATCCCTGAGCTAGACGG	GGGTCTTACTCCTTGGAGGC
GM-CSFR	GCA TTC CTC CTG ATC CCA GA	CCT GGA GTC AAA CCT CAC ATT G
CD115	CTAGGGACATCATGAATGACTC	GTTTATAGAACTTGCTGTTACC

Note: # primers were designed by adding A at the 5' end (in bold) or truncating several bases at the 3' end to obtain a melting temperature (T<sub>m</sub>) of 55–68°C according to the manual for Ncode™ miRNA First-Strand cDNA Synthesis kit. @ The seeding region sequence of miR-155 was mutated in the forward and reverse primers (in italic bold). These two primers together with primers for LV-miR-155WT were used to generate LV-miR-155MUT. Restriction enzyme sites in primers were underlined.

### **Figure S1. miRNA expression analysis in human monocytes, imDCs and mDCs**

Shown are scatter plots of normalized miRNA microarray data for monocyte versus imDC (A), monocyte versus mDC (B), and imDC versus mDC (C). Dots represent individual miRNAs, and those for miR-221 and miR-155 are indicated by arrows.

### **Figure S2. Reduced secretion of IL-12p70 by mDCs from miR-155<sup>-/-</sup> mice**

BM derived imDCs ( $2.5 \times 10^5$  per well) at day 6 from WT or miR-155<sup>-/-</sup> (KO) mice were cultured in 24-well plates in 1 mL RPMI1640 culture medium supplemented with mGM-CSF (20 ng/mL). Cells were treated with LPS (0.25  $\mu$ g/ml) for 24 h. Supernatants were harvested and IL-12p70 was measured by ELISA. The data are means with 95% confidence intervals from three independent experiments. \*\* P<0.01 by two sample t test.

### **Figure S3. SOCS-1 is a target of miR-155**

(A) Multiple species sequence alignment of the SOCS-1 3' UTR including the predicted miR-155 target site sequence (in bold). Mutation of the miR155 target site sequence is shown below. (B) Luciferase reporter assays to confirm SOCS-1 3' UTR targeting by miR-155. The columns represent normalized relative luciferase activity (RLU) by means with 95% confidence intervals from two independent experiments. \*\* P<0.01 by two sample t test.

### **Figure S4. Over-expression of miR-155 in DCs significantly increases IL-12 production after stimulation with potent IL-12 inducers**

(A) Enhanced IL-12 production in DCs after over-expression of miR-155 and stimulation with LPS + R848 and poly(I:C) + the cocktail.<sup>1,2</sup> ImDCs at day 5 from two donors were transfected with 40 nM of miR-155 or negative control. 6 h later, cells were treated with the LPS [0.1  $\mu$ g/mL, cat# L2654, Sigma] + imidazoquinoline compound R848 (2.5  $\mu$ g/mL, InvivoGen) or poly(I:C) (20  $\mu$ g/mL, InvivoGen) + the cocktail in AIM V serum free medium supplemented with human GM-CSF and IL-4 for 16 h. Supernatants were harvested and IL-12p70 was measured by ELISA. The data are mean  $\pm$  SEM from three replicates. \*\* P<0.01 by two sample t test. (B) Flow cytometric analysis of CD83 maturation marker in DCs after over-expression of miR-155 and stimulation with LPS + R848 and poly(I:C) + the cocktail for 16 h. Unfilled histogram indicates an isotype control.

## REFERENCES

1. Kim HJ, Kim HO, Lee K, Baek EJ, Kim HS. Two-step maturation of immature DCs with proinflammatory cytokine cocktail and poly (I: C) enhances migratory and T cell stimulatory capacity. *Vaccine* 2010; 28(16):2877–86.
2. Hemmi H, Kaisho T, Takeuchi O, Sato S, Sanjo H, Hoshino K, et al. Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. *Nat Immunol* 2002; 3(2):196–200.

Figure S1

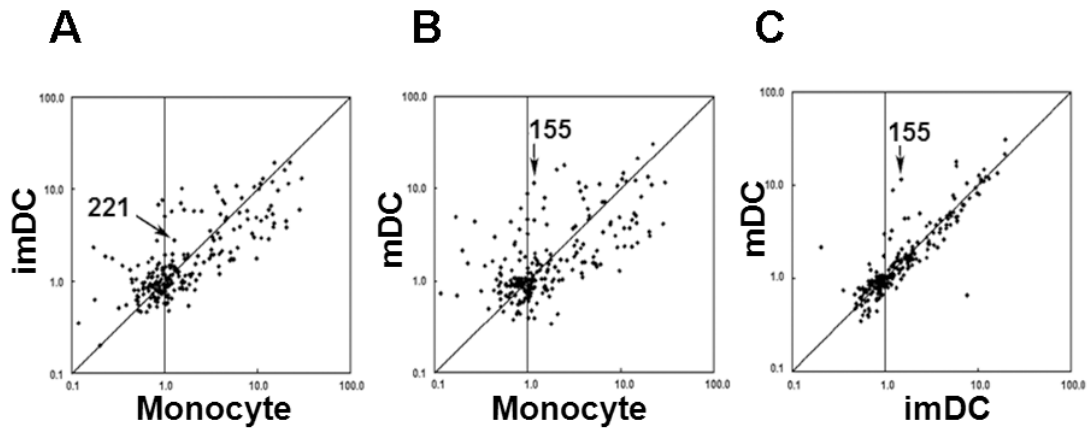


Figure S2

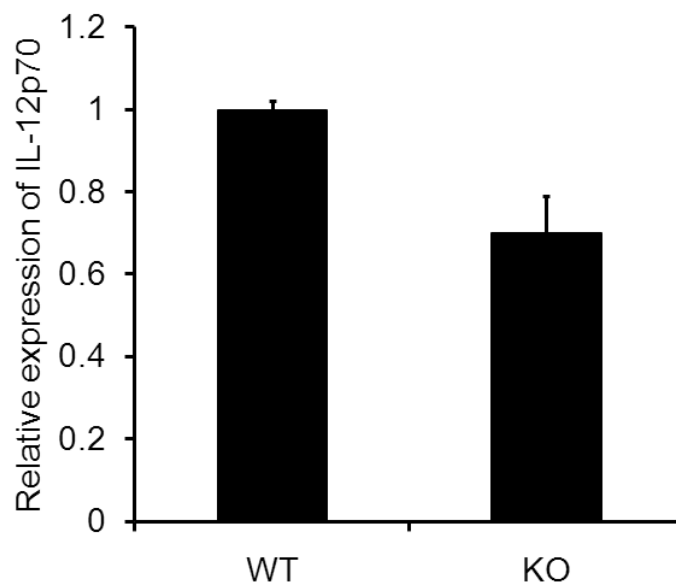
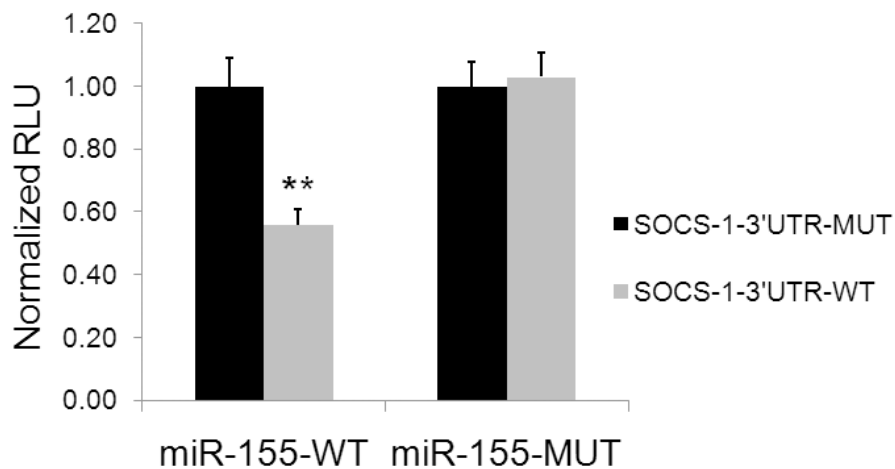


Figure S3

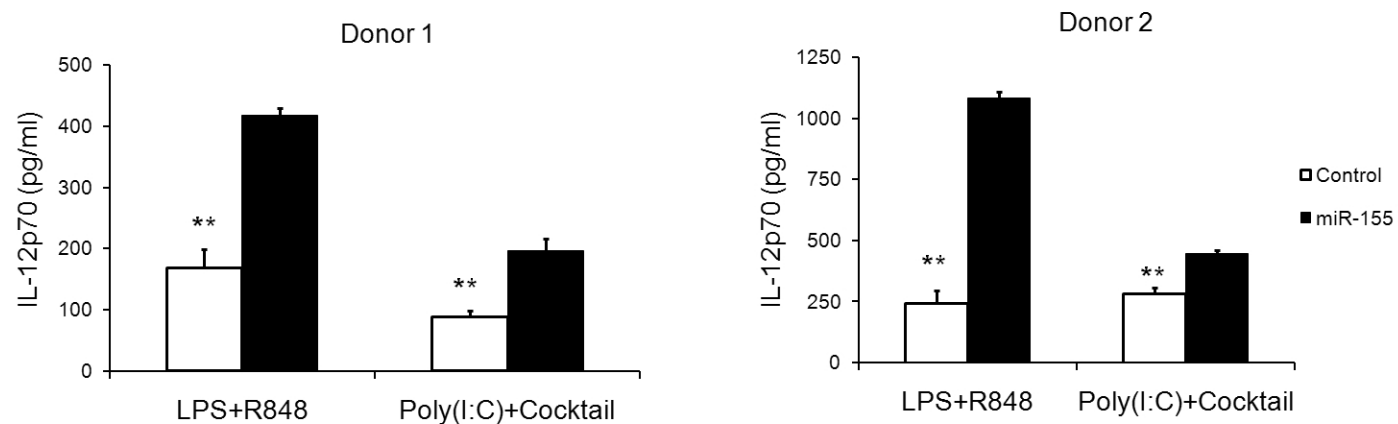
**A**

Hsa-miR-155MUT	3' tggggauagugcuaa <b>AGCAUUA</b> A 5'
Hsa-miR-155WT	3' tggggauagugcuaa <b>UCGUAAU</b> U 5'
Human	- gccgcgctgacgc <b>AGCATTAA</b> ctg-
Mouse	- --ccgctgtgcc-gc <b>AGCATTAA</b> gtg-
Rat	- --ccgccgtgccgc <b>AGCATTAA</b> gtg-
Dog	- -----cgcgcacgc <b>AGCATTAA</b> ctc-
Chicken	- -----gtac <b>AGCATTAA</b> ctg-
	<b>AATT</b>
	<b>Mutant 3'UTR</b>

**B**



**A**



**B**

