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Supporting Information

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Interleukin-10-induced MARCH1 mediates intracellular sequestration of MHC class II in monocytes

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Supplemental Table I. Oligonucleotides used for quantitative PCR analysis

Gene	Oligonucleotides	Sequence
HPRT	hsHPRT F2	GACTTTGCTTTCCTTGGTCA
	hsHPRT R2	GGCTTTGTATTTTGCTTTTCC
MARCH1	hsMARCH1 E1-258 F1	TCCCAGGAGCCAGTCAAGGTT
	hsMARCH1 E2-385 R1	CAAAGCGCAGTGTCCCAGTG
MARCH2	hsMARCH2-S3	GTCTCCTTCCGCTACCACTG
	hsMARCH2-AS3	TGTCTCCTCTGCCACCTTCT
MARCH8	hsMARCH8 ORF298 F1	ACAGGAAGCCTCCACTTCG
	hsMARCH8 ORF489 R1	GACGTGGAATGTCACTGAG

Supplemental Figure legends.

Figure S1. Intracellular retention of MHC-II molecules in monocytes treated with IFN-γ and IL-10. Primary human monocytes treated with IFN-γ alone (A, upper panels) or with IFN-γ and IL-10 (B, lower panels) for 16 h were fixed, permeabilized and stained simultaneously for HLA-DM (left panels) and HLA-DR (middle panels), and analyzed by confocal microscopy. The panels on the right show the merge of fluorescent signals. The bar corresponds to 20 μm. HLA-DM molecules are localized in endosomal intracellular MHC-II loading compartments (MIICs).

Figure S2. MARCH1 and MARCH8 down-regulate HLA-DR, -DP and -DQ. 293EBNA/CIITA cells were transiently transfected either with empty expression vector (open profiles), or with MARCH1 (filled profiles in left panels), or MARCH8 (right panels). Cells were stained after 48 h for surface expression of HLA-DR (mAb L243; panels 1, 2), HLA-DQ (mAb SPV-L3, panels 3, 4), or HLA-DP (mAb B7/21; panels 5, 6) and analyzed by flow cytometry.

Figure S3. Down-regulation of wild-type but not lysine-225 mutated HLA-DRα/β by EYFP-MARCH1. 293EBNA cells were transiently co-transfected with wild-type HLA-DRα and HLA-DRβ constructs (lanes 1, 2), or with HLA-DRβ constructs carrying a lysine-to-alanine mutation at position 225 (K225A, lanes 3, 4), together with either an EYFP expression vector (lanes 1, 3), or with an expression vector expressing an EYFP-MARCH1 fusion protein (lanes 2, 4) and analyzed 48 h after transfection by flow cytometry for HLA-DR cell surface expression with mAb L243. When co-transfected with MARCH1, MHC-II molecules with a wild-type lysine at position HLA-DRβ225 were efficiently down-regulated from the plasma membrane (lane 2), while molecules with the HLA-DRβ(K225A) mutation showed strongly reduced down-regulation in the presence of MARCH1 protein (lane 4). Mean fluorescence values and standard errors are derived from duplicates of independent transfections.

Figure S1.

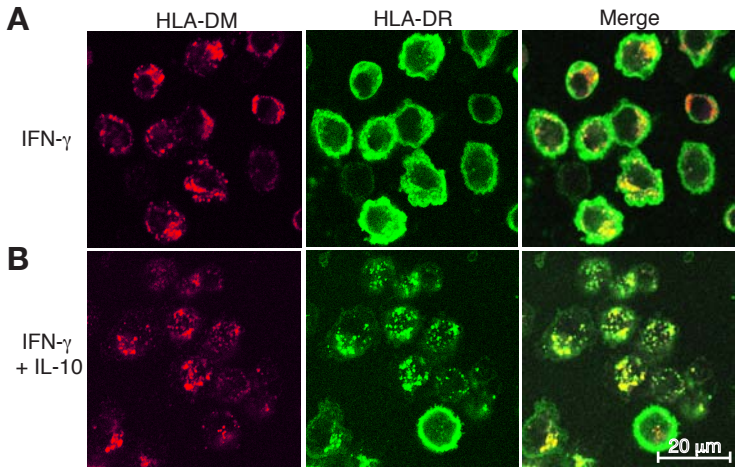

Figure S2.

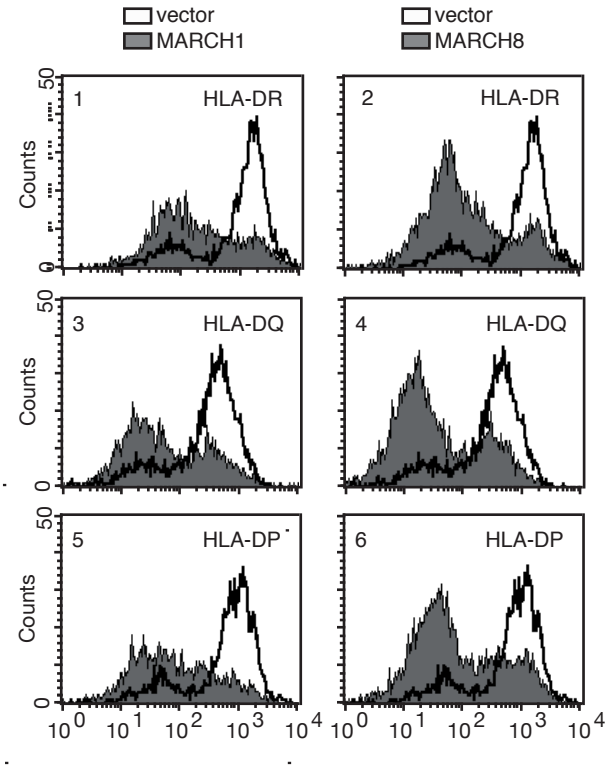

Figure S3.