Supporting Information

Lapaque et al. 10.1073/pnas.0906735106

SI Materials and Methods

Transfection. Transfections were performed using Effectene (Qiagen). MARCH I construct was from P. Lehner (CIMR, Cambridge, U.K.)

RNA Extraction and cDNA Synthesis. RNA was extracted from Mel JuSo using an RNeasy Kit (Qiagen). All samples underwent 2 DNase treatments using a Turbo DNase Free kit (Ambion). cDNA synthesis was performed using SuperScript III with an Oligo$(dT)_{20}$ primer at 50 °C for 1 h, following the manufacturer's standard protocol (Invitrogen).

RT-PCR. PCR reactions were designed to detect transcripts of *MARCH I*, *MARCH VIII* and *GAPDH*. Primer pairs used in these reactions were: CCAGGAGCCAGTCAAGGT, GGTTGAGCTTGGTVTVVAT for MARCH I; CAGGAAGCCTCCACTTCG, GACGTGGAATGTCACTGAGC for MARCH VIII, GAAGGTGAAGGTCGGAGTC, CATCACGCCACAGTTTCCC for GAPDH. PCR reactions were composed of 5 μL 2× Biomix (containing Biotaq DNA polymerase) (Bioline), 0,8 ng of monocyte-derived DC or Mel JuSo cDNA, primer combinations (final reaction concentrations 2 μM for *MARCH I* and *MARCH VIII*, 0,2 μM for *GAPDH*) and brought to a total volume of 10 μL with H_2O (Sigma). DNA samples were amplified on an MJ Research PTC-200 thermal cycler. Cycling parameters were as follows: 1 min at 96 °C followed by 5 cycles of 96 °C for 20 s, 70 °C for 45 s, and 72 °C for 25 s, followed by 26 cycles of 96 °C for 25 s, 65 °C for 50 s, and 72 °C for 30 s, followed by 4 cycles of 96 °C for 30 s, 55 °C for 60 s, and 72 °C for 90 s.

Real-Time Quantitive PCR (QPCR). QPCR reactions were developed to assess the relative levels of *GAPDH* and *MARCH VII1* transcripts in infected and uninfected Mel JuSo cells. QPCR reactions consisted of 10 μL of 2× QuantiTect SYBR Green solution (Qiagen), 0.8 ng cDNA, oligonucleotides and brought to a final volume of 20 μL with H_2O (sigma). QPCR was performed on an ABI 7500 (PE Applied Biosystems). Cycling parameters were as follows: 95 °C for 15 min, followed by 40 cycles of 94 °C for 15 s, 60 °C for 30 s, and finally 72 °C for 33 s. The fluorescence levels of both SYBR Green and the internal passive dye ROX were acquired during the final step. Average cycle threshold (CT) values were produced using the ABI 7500 System SDS software. The relative expression level of each target transcript in Mel JuSo was assessed using qBase (1) by normalisation against the reference genes *GAPDH*. The oligonucleotide sequences and their final working concentration in each reaction were ACACCCACTCCTCCACCTTT, TGACAAAGTGGTCGTTGAGG (0.6 μM) for *GAPDH* and GACGTGGAATGTCACTGAG, CAGGAAGCCTCCACTTCG (0.9 μM) for *MARCH VIII*. Specificity of PCR was verified by direct sequencing.

1. Hellemans J, Mortier G, De Paepe A, Speleman F, Vandesompele J (2007) qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. *Genome Biol* 8:R19.

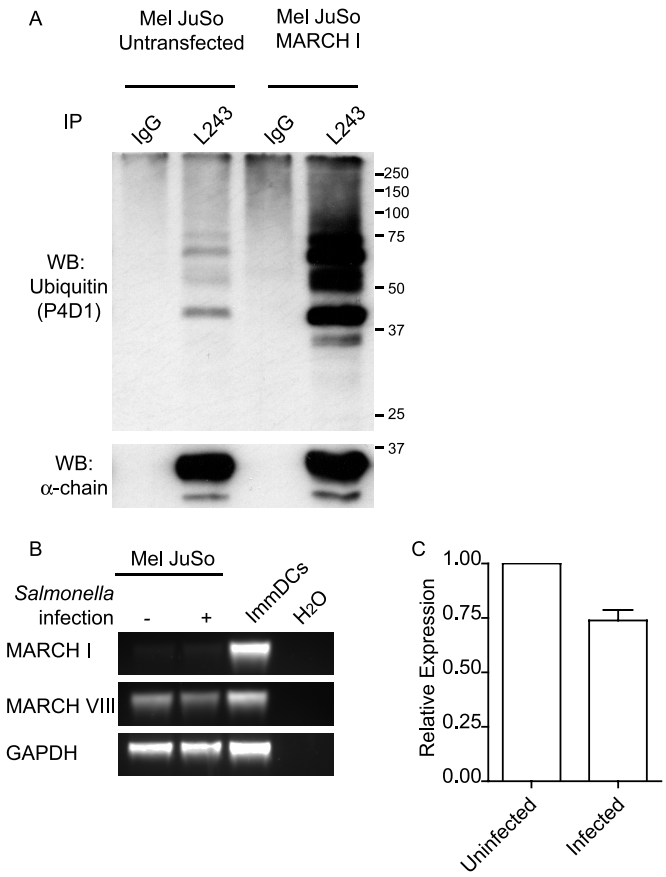

Fig. S1. HLA-DR is constitutively ubiquitinated in Mel JuSo cells but transcript levels of MARCH I and MARCH VIII are not up-regulated upon *Salmonella* infection. (*A*) HLA-DR was immunoprecipitated with L243 from 1×10^6 WT Mel JuSo or MARCH I transfected Mel JuSo cells. After standard PAGE electrophoresis and western transfer ubiquitinated DR was detected with HRP-P4D1 (top panels) and levels of DRα and assessed with HRP-Tal1B5 (bottom panel). Control immunoprecipitations were performed with isotype matched control antibody (IgG2a). (*B*) RT-PCR analysis of MARCH I, MARCH VIII and GAPDH, expression in uninfected or *Salmonella*-infected Mel JuSo cells. Mel JuSo cells were infected with *Salmonella* for 16–20 h and FACS sorted into infected and uninfected cell populations. Immature DCs (ImmDCs) were derived as described in *Materials and Methods*. RT-PCR conditions are described in *SI Materials and Methods*. (C) Mel JuSo cells were infected with WT *Salmonella* and FACS sorted into infected and uninfected cell populations. mRNA expression of MARCH VIII and GAPDH was measured by QPCR. Expression of MARCH VIII in infected and uninfected cells was normalised against GAPDH. Graph shows the fold change of MARCH VIII in 2 independent infections.

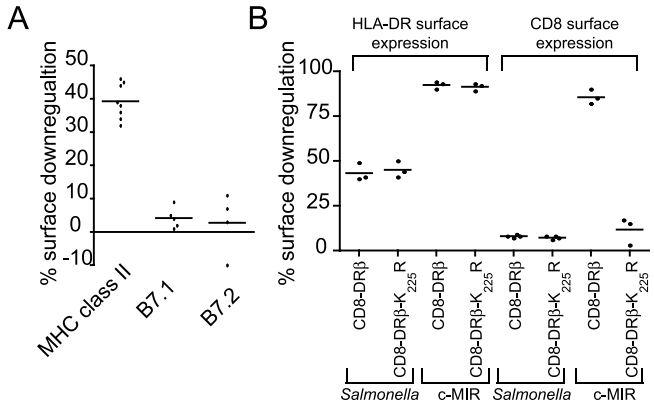

Fig. S2. Surface receptors ubiquitinated by MARCH I and VIII are not targets for *Salmonella*-induced down-regulation. (*A*) Mel JuSo cells, stably expressing B7.1 or B7.2 were infected with *Salmonella*. The levels of HLA-DR and B7 expression in infected compared to uninfected cells were measured by FACS. Dots represent the percentage down regulation for each molecule. Data points for HLA-DR are from both B7.1 and B7.2 transfectants. (*B*) Mel JuSo cells, stably expressing either CD8-DRβ or CD8-DRβ-K_{225}R, were infected by *Salmonella* or transfected with c-MIR (MARCH VIII). Surface HLA-DR and CD8 was analyzed by FACS and the percentage down-regulation plotted as dots. Down-regulation by c-MIR was calculated as mean fluorescence of c-MIR transfected cells/mean fluorescence of untransfected cells $\times$ 100.

Table S1. List of antibodies used in this study.

Specificity	Clones	Used for	Source
HLA-DR	L243	IP, FACS	Prof. J. Trowsdale, Cambridge
HLA-DRα	TAL1B5	WB	Prof. J. Trowsdale, Cambridge
HLA-DRβ	LC2.1	WB	Prof. J. Kaufman, Cambridge
HLA-DP	HI-43	FACS	Biolegend
HLA-DQ	SPV-L3	FACS	Abcam
CD8	OKT8	FACS	Prof. J. Trowsdale, Cambridge
CD1a	HI149	FACS	BD Pharmingen
CD1b	M-T101	FACS	BD Pharmingen
CD1c	AD5–9E7	FACS	Miltenyi Biotech
CD1d	CD1d42	FACS	BD Pharmingen
B7.1	2D10.4	FACS	BD Pharmingen
B7.2	IT2.2	FACS	BD Pharmingen
HA-PE	GG8-IF3.3	FACS	Miltenyi Biotech
Ubiquitin-HRP	P4D1	WB	Santa-Cruz
Clathrin	Rabbit polyclonal	WB	Prof Robinson, Cambridge
AP-2	31	WB	BD transduction laboratories
Actin	AC-74	WB	Sigma
CD74/Ii	M-B741	FACS	BD Biosciences

IP, immunoprecipitation; WB, western blot; FACS, flow cytometry. Secondary antibodies: PE conjugated anti–mouse IgG and IgG isotype controls were from Dako.