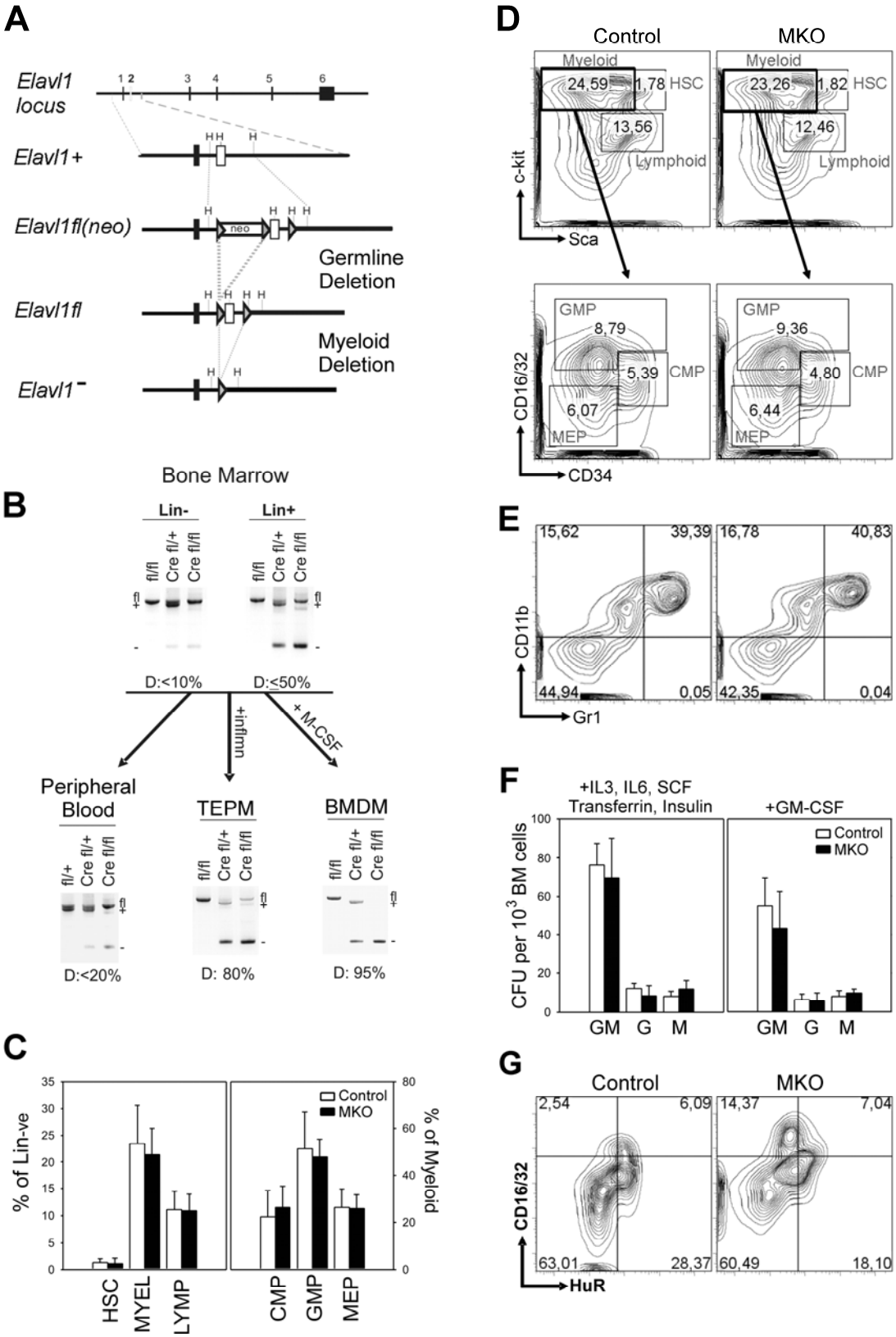
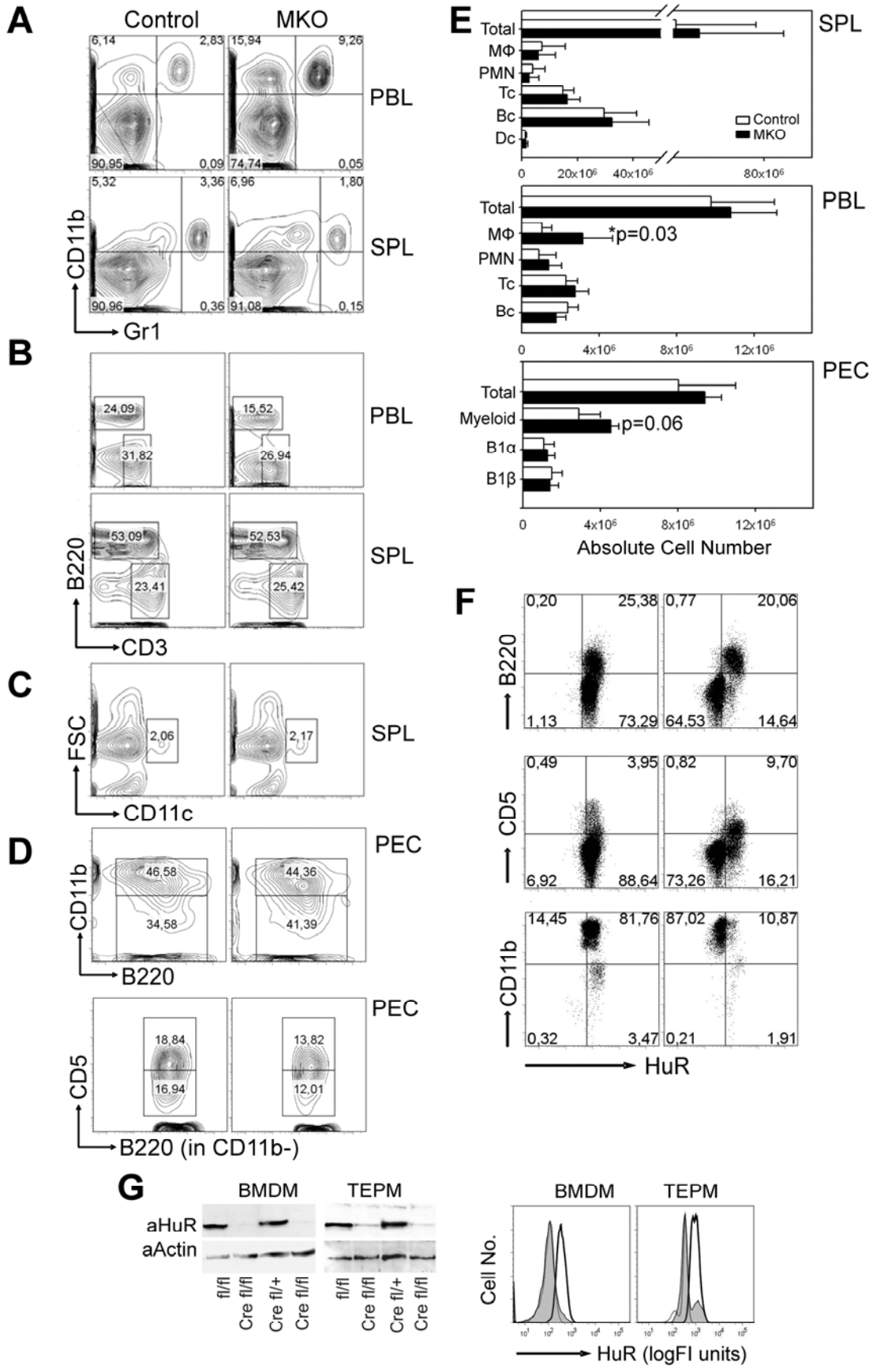
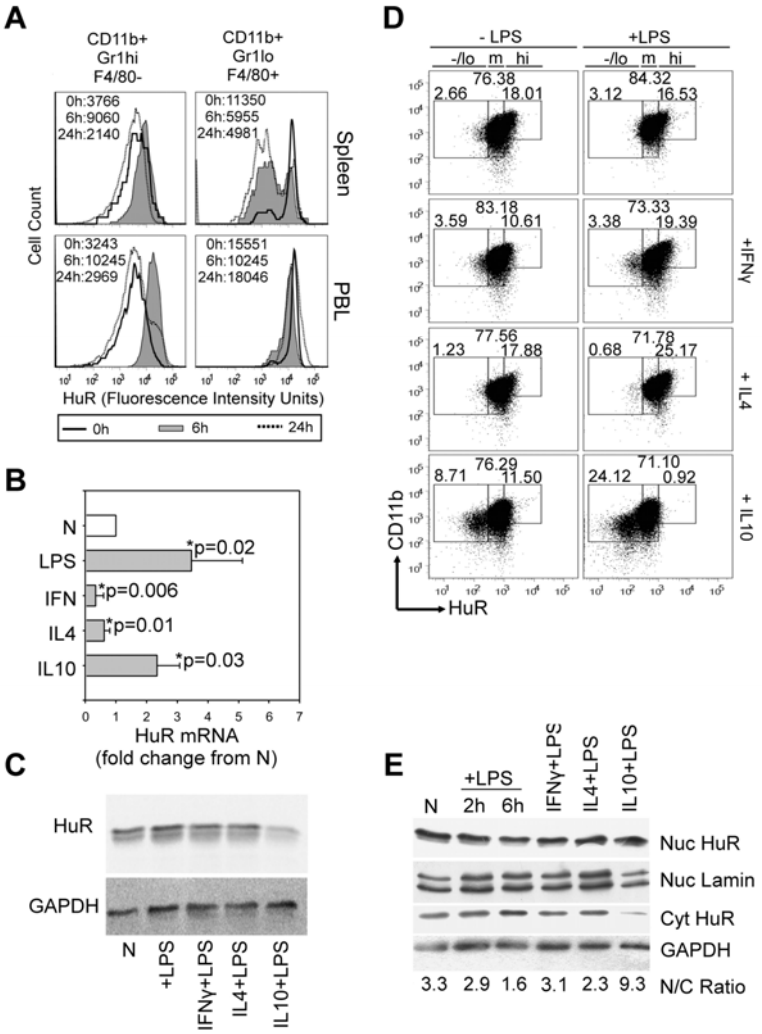
Index for Supplementary Data and Methods


A
Elavl1 locus
Elavl1+
Elavl1fl(neo)
neo
Germline Deletion
Elavl1fl
Myeloid Deletion
Elavl1⁻
B
Bone Marrow
Lin-
Lin+
fl/fl
Cre fl/+
Cre fl/fl
D:<10%
D:≤50%
+Infl/nn
+M-CSF
Peripheral Blood
TEPM
BMDM
D:<20%
D: 80%
D: 95%
C
% of Lin-ve
% of Myeloid
Control
MKO
HSC
MYEL
LYMP
CMP
GMP
MEP
D
Control
MKO
Myeloid
24,59
1,78 HSC
13,56
Lymphoid
23,26
1,82 HSC
12,46
c-kit
Sca
GMP
8,79
5,39 CMP
6,07
MEP
9,36
4,80 CMP
6,44
CD16/32
CD34
E
15,62
39,39
44,94
0,05
16,78
40,83
42,35
0,04
CD11b
Gr1
F
+IL3, IL6, SCF
Transferrin, Insulin
+GM-CSF
Control
MKO
CFU per 10³ BM cells
GM
G
M
G
Control
MKO
2,54
6,09
63,01
28,37
14,37
7,04
60,49
18,10
CD16/32
HuR

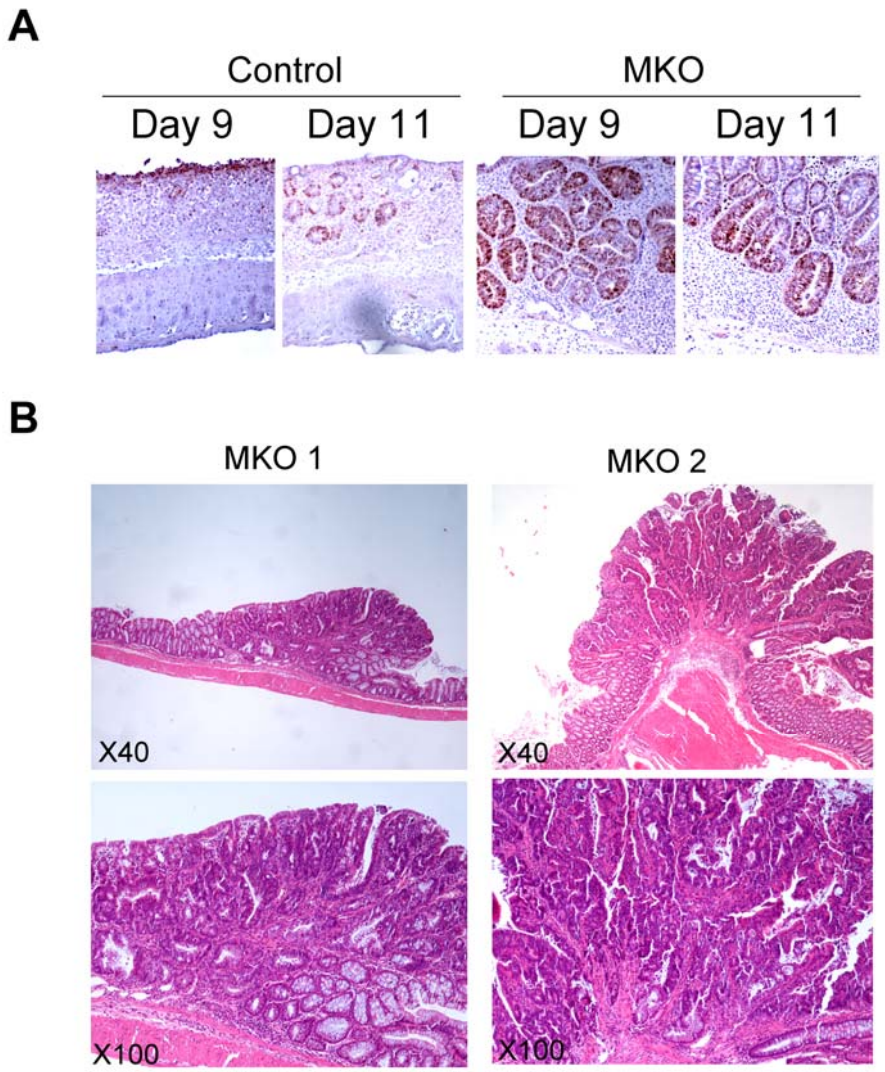
Supplementary Figure 1. The myeloid-restricted inactivation of the *Elavl1* locus does not block myelopoiesis. (A) Wild-type (*Elavl1*$^{+}$), the neo-containing (*Elavl1*$^{fl neo}$), the neo-less floxed (*Elavl1*^{fl}) alleles and the Cre-recombined (*Elavl1*$^{-}$) loci on mouse chromosome 8; noted are the loxP sequences (triangles), marker (neo) and the ATG containing exon 2 (white box); (H) denotes HindIII restriction sites for loci mapping. **(B)** Detection of native (+), targeted (fl) and Cre-recombinant (-) loci in DNA extracts from sorted bone marrow progenitor (Lineage negative; Lin-) and differentiated (Lineage positive; Lin+) cells; peripheral blood cells; exudate peritoneal macrophages (TEPM); and bone-marrow derived (BMDM) macrophage populations from control and test mice. D=estimated recombination efficiency. Data derived from semiquantitative PCR on genomic DNA. **(C & D)** Flow cytometric detection (D) and enumeration (C) of hemopoietic stem (HSC) and progenitor subsets in Lin- bone marrow-derived fractions from control and MKO mice by means of surface ckit and Sca-1 expression. Gated myeloid progenitors were analysed further for the presence of granulocyte/ monocyte (GMP), common myeloid (CMP) and megakaryocyte/erythroid (MEP) progenitors by means of their surface CD16/32 and CD34 expresssion. Data presented as mean numbers ($\pm$SEM) derived from n=6 mice/genotype at the age of 10-12 weeks. **(E)** Flow cytometric detection of differentiated monocytic ($CD11b^{+}Gr1^{int}$) and granulocytic/polymorphonuclear ($CD11b^{+}Gr1^{hi}$) cells Lin+ bone marrow-derived fractions from control and MKO mice. **(F)** Colony forming assays in methycellulose media supplemented with myelotrofic factors using Lin^{-} bone marrow progenitors from control and MKO mice. The bar graph depicts the mean number ($\pm$SEM) of granulomonocytic (GM), granulocytic (G) or monocytic (M) colonies derived by seeding 10^{3} cells and measured by light microscopy **(G)** Flow cytometric detection of HuR positive and negative myeloid subsets in lineage- enriched bone marrow cells from control and MKO mice, cultured in the presence of GM-CSF; The quadrants were defined based on isotype matched control which gives high background values; still note that the deleted subsets are present primarily in the committed $CD16/32^{int/hi}$ subsets.


A
Control
MKO
6,14
2,83
15,94
9,26
PBL
90,95
0,09
74,74
0,05
5,32
3,36
6,96
1,80
SPL
90,96
0,36
91,08
0,15
CD11b
Gr1
B
24,09
15,52
PBL
31,82
26,94
53,09
52,53
SPL
23,41
25,42
B220
CD3
C
2,06
2,17
SPL
FSC
CD11c
D
46,58
44,36
PEC
34,58
41,39
CD11b
B220
18,84
13,82
PEC
16,94
12,01
CD5
B220 (in CD11b-)
E
Total
MΦ
PMN
Tc
Bc
Dc
Control
MKO
SPL
0
20x10⁶
40x10⁶
80x10⁶
Total
MΦ
*p=0.03
PMN
Tc
Bc
PBL
0
4x10⁶
8x10⁶
12x10⁶
Total
Myeloid
p=0.06
B1α
B1β
PEC
0
4x10⁶
8x10⁶
12x10⁶
Absolute Cell Number
F
0,20
25,38
0,77
20,06
B220
1,13
73,29
64,53
14,64
0,49
3,95
0,82
9,70
CD5
6,92
88,64
73,26
16,21
14,45
81,76
87,02
10,87
CD11b
0,32
3,47
0,21
1,91
HuR
G
BMDM
TEPM
aHuR
aActin
fl/fl
Cre fl/fl
Cre fl/+
Cre fl/fl
fl/fl
Cre fl/fl
Cre fl/+
Cre fl/fl
BMDM
TEPM
Cell No.
HuR (logFI units)

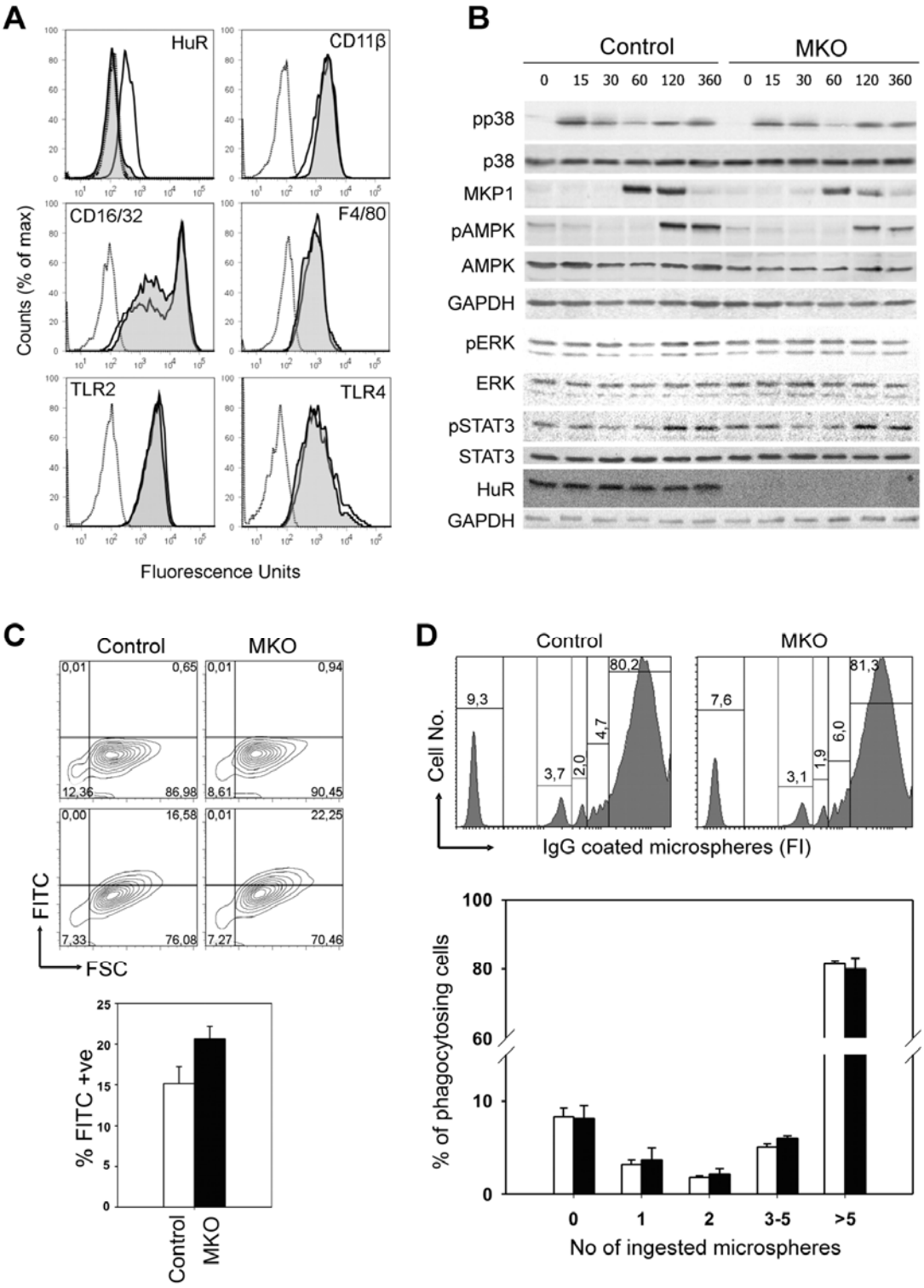
Supplementary Figure 2. Metrics of immune subsets in MKO mice. Flow cytomeric detection of **(A)** Macrophages ($CD11b^{hi}Gr1^{int}$) & neutrophils ($CD11b^{hi}Gr1^{hi}$), **(B)** $CD3^+$ T & $B220^+$ B lymphocytes, **(C)** $CD11c^+$ dendritic cells & **(D)** $CD11b^+B220^+B1\alpha$ or $CD5^+B220^+B1\beta$ cells in the indicated peripheral compartments of control and MKO mice. PBL:Peripheral blood; SPL:Spleen; PEC: Peritoneal cavity. **(E)** Bar graphs depicting total and subset numbers ($\pm$SD) for macrophages (MΦ), polymorphonuclear cells(PMN) or total myeloids; T & B lymphocytes (Tc, Bc); dendritic cells (Dc); and peritoneal B1α/B1β subsets in selected peripheral compartments. Data derived from 10-14 mice/genotype at the age of 12 weeks. **(F)** Flow cytometric detection of HuR^+ or HuR^- cells in peritoneal cavity populations indicating the loss of HuR in myeloid but not lymphoid populations. Quadrants were set by isotype staining. **(G)** ***Left:*** Western blot of total macrophage extracts probed with αHuR Ab showing the loss of HuR protein in $LysMCre^+Elavl1^{fl/fl}$ macrophages. α-actin is shown for quantitation. ***Right:*** Flow cytometric detection of intracellular mHuR protein in $LysMCre^+Elavl1^{fl/+}$ macrophages (open histogram), and its respective loss in $LysMCre^+Elavl1^{fl/fl}$ macrophages (shaded histogram). The dotted histogram depicts the isotype-matched background staining.



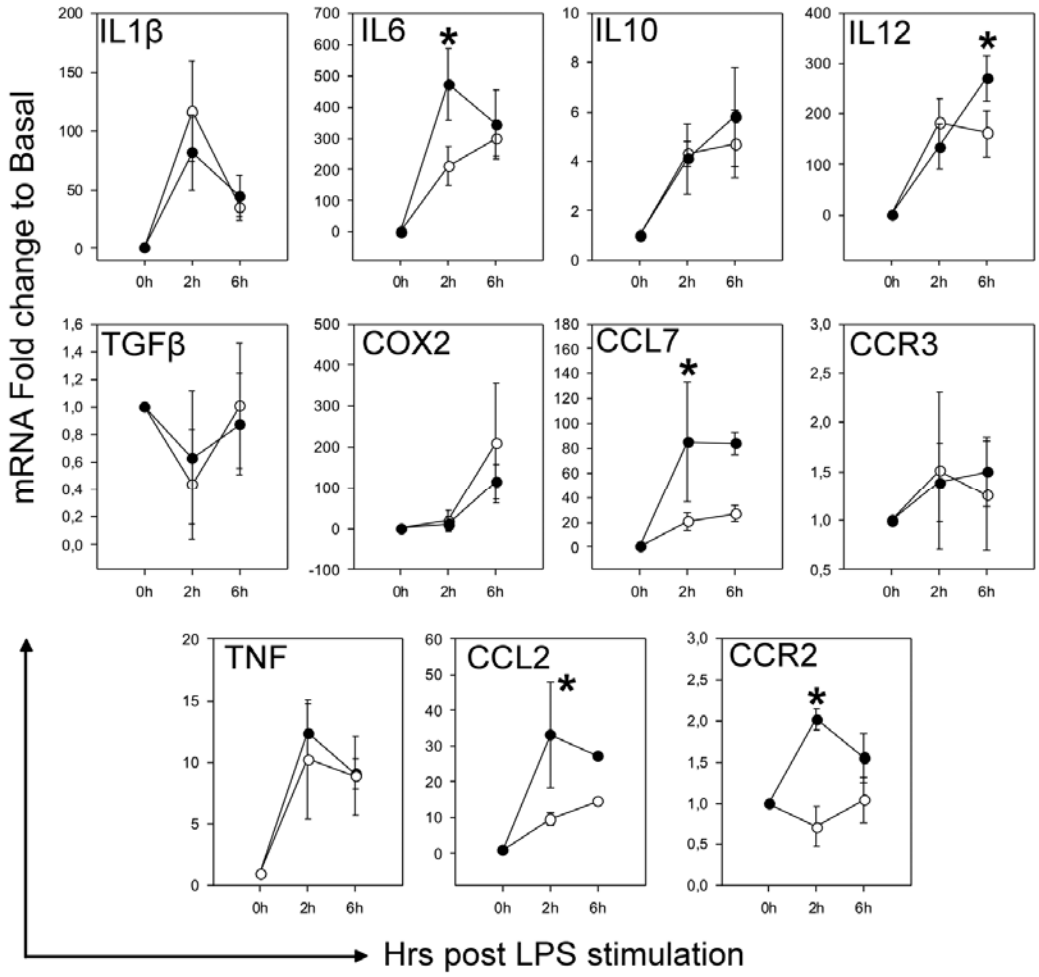
Supplementary Figure 3. HuR responds to macrophage activating and polarizing signals but at different levels. **(A)** Flow cytometric HuR detection in macrophages ($CD11\beta^{+}Gr1^{lo}F4/80^{hi}$) and PMN ($CD11\beta^{+}Gr1^{h}iF4/80^{-}$) from the blood of control mice challenged with LPS for the indicated time points. Representative fluorescence intensity values are indicated. **(B)** qRT-PCR detection of *Elavl1* mRNA using oligos for exon 2 in mouse macrophages cultured with LPS (for 2hrs) or IFNγ, IL4 or IL10 (for 4hrs). Bar graphs present data as fold changes to untreated (N) cultures. Similar results were obtained via the detection of exon 4 containing transcripts. **(C)** Detection of macrophage HuR in the presence of the indicated stimuli via immunoblots. GAPDH is shown as a loading control. Note that a difference is observed only in the case of LPS+IL10. **(D)** Flow cytometric detection and electronic separation of macrophage cultures with respect to their HuR content and their response to activating and polarizing signals for 24hrs. Note the changes between HuR low, medium and high expressors in response to the different signals. **(E)** Immunodetection of HuR in nuclear and cytoplasmic extracts in the presence or absence of activating or polarizing stimuli. LAMIN and GAPDH are shown as nuclear and total loading controls respectively. Numbers indicate representative changes in nucleocytoplasmic distribution of HuR.



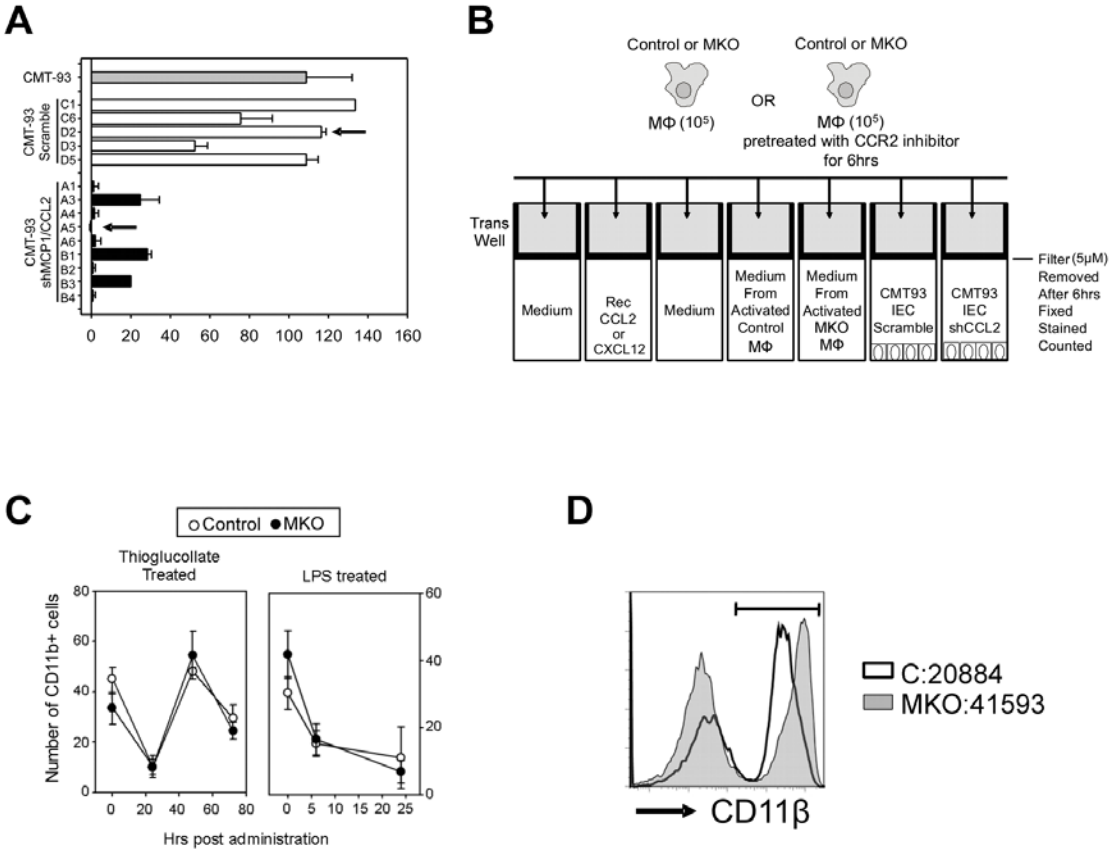
Supplementary Figure 4. Myeloid deletion of HuR accelerates epithelial restitution and may support spontaneous tumorigenesis. (A) Detection of proliferating epithelia by Ki67 staining in colonic tissue from control and MKO mice between days 3-12 of the acute DSS protocol; note the rapid regeneration of the epithelium in the mutant mice. **(B)** Histology from the two different, DSS-treated, MKO mice that developed spontaneous neoplastic lesions (1 each) in the form of early and late stage adenomas. For both sets, photomicropgraphs derived from colonic paraffin sections stained with DAB/Haematoxylin or Haematoxylin /Eosin. Magnification: (x40 & x100).


A
HuR
CD11β
CD16/32
F4/80
TLR2
TLR4
Counts (% of max)
Fluorescence Units
B
Control
MKO
0 15 30 60 120 360 0 15 30 60 120 360
pp38
p38
MKP1
pAMPK
AMPK
GAPDH
pERK
ERK
pSTAT3
STAT3
HuR
GAPDH
C
Control
MKO
0,01 0,65 12,36 86,98
0,01 0,94 8,61 90,45
0,00 16,58 7,33 76,08
0,01 22,25 7,27 70,46
FITC
FSC
% FITC +ve
Control
MKO
D
Control
MKO
Cell No.
9,3 3,7 2,0 4,7 80,2
7,6 3,1 1,9 6,0 81,3
IgG coated microspheres (FI)
% of phagocytosing cells
No of ingested microspheres
0 1 2 3-5 >5

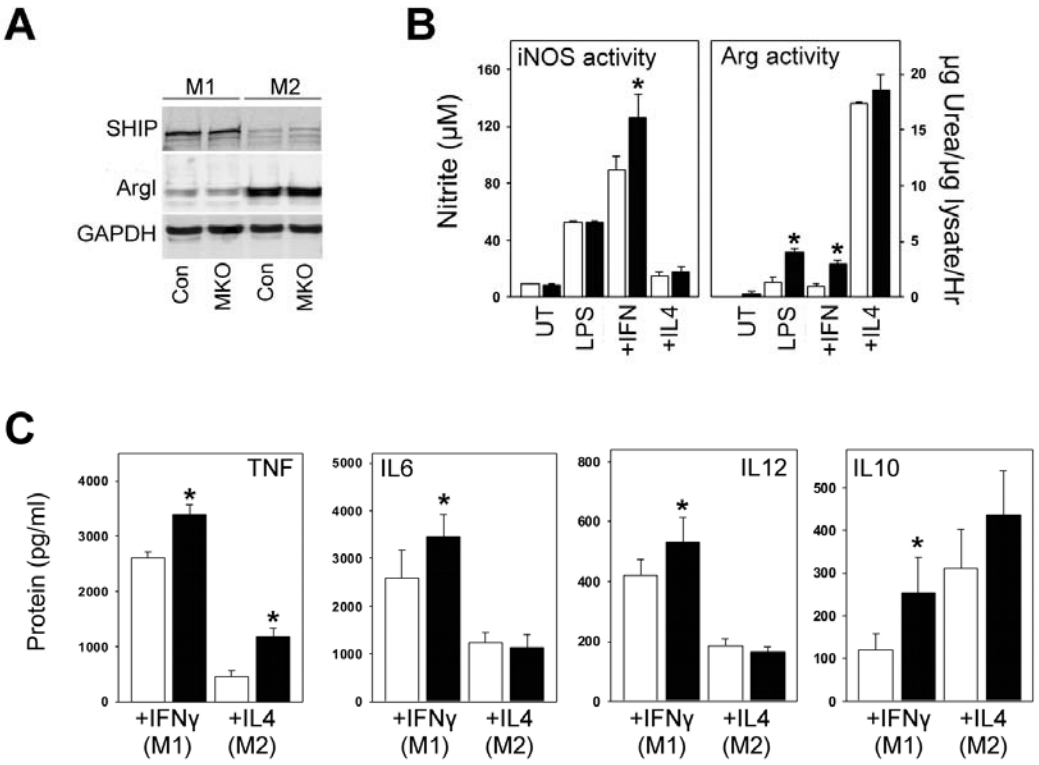
Supplementary Figure 5. The loss of HuR does not impair macrophage maturation, phagocytosis/pinocytosis nor TLR signals controlling ARE-containing mRNAs. (A) Flow cytometric detection of HuR, macrophage maturation markers (CD11β, F4/80, CD16/32) and central TLRs on the surface of differentiated BMDMs from control (open histograms) and MKO (shaded histograms) mice. Dotted lines represent isotype staining for each antibody. **(B)** Immunodetection of total and phosphorylated forms of p38, ERK, AMPK STAT3 and of total phosphatase MKP1 in whole extracts from control and MKO BMDM, stimulated with 100ng/ml LPS for the indicated time points. HuR protein content is also indicated, whereas the GAPDH protein is shown as a loading control. **(C)** Flow cytometric detection and percentile enumeration of $FITC^{+ve}$ BMDM from control and MKO mice following incubation with soluble FITC for pinocytic uptake. **(D)** Flow cytometric detection of ingested FITC-labeled microspheres by BMDM from control and MKO mice. Histograms depict the number of cells ingesting increasing number of microspheres in 1hr of incubation. Data obtained from 2 independent experiments are depicted in the bar graph as a distribution of phagocytosing cells with respect to their ingested microspheres.



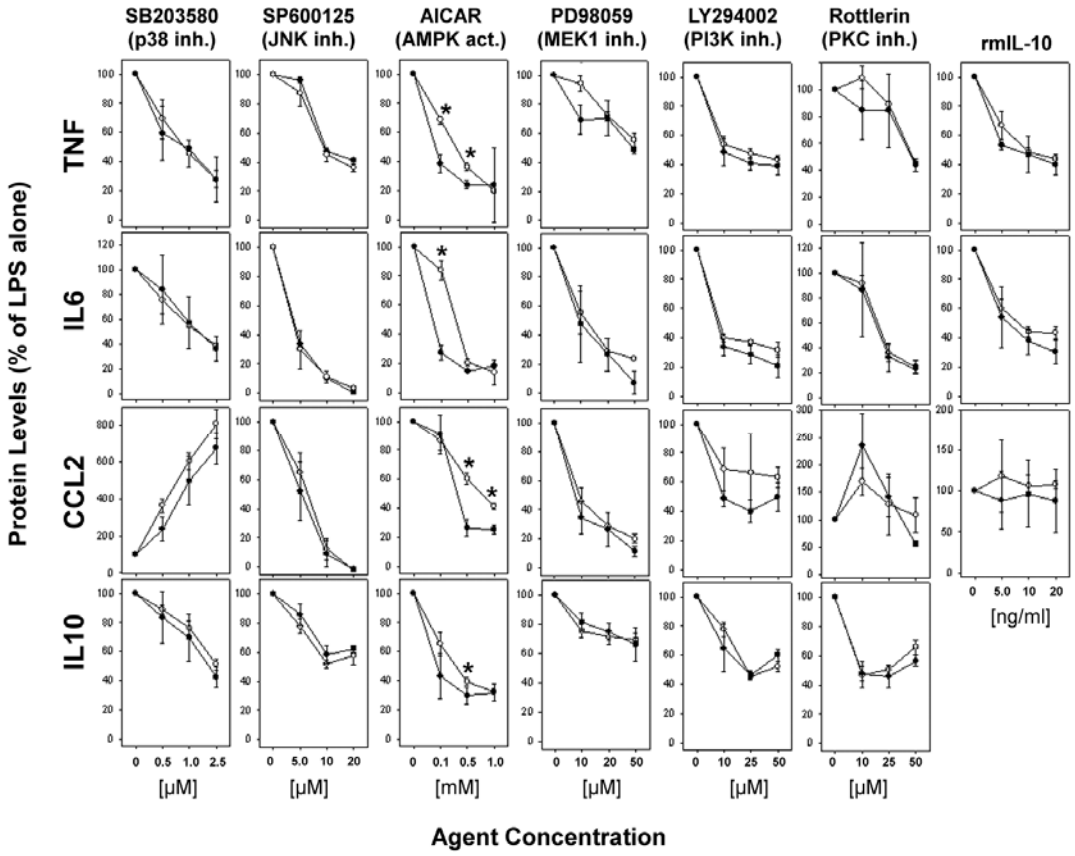
Supplementary Figure 6. Changes in the kinetics of cytokine mRNAs in HuR-null macrophages. Line graphs depicting differences in the temporal accumulation cytokine/chemokine mRNAs following stimulation with LPS. Data derived from the qRT-PCR detection of control (○) and MKO (●) macrophage mRNAs as in **Figures 4A & D** but here are presented as fold changes (±SD) to the corresponding untreated macrophages.



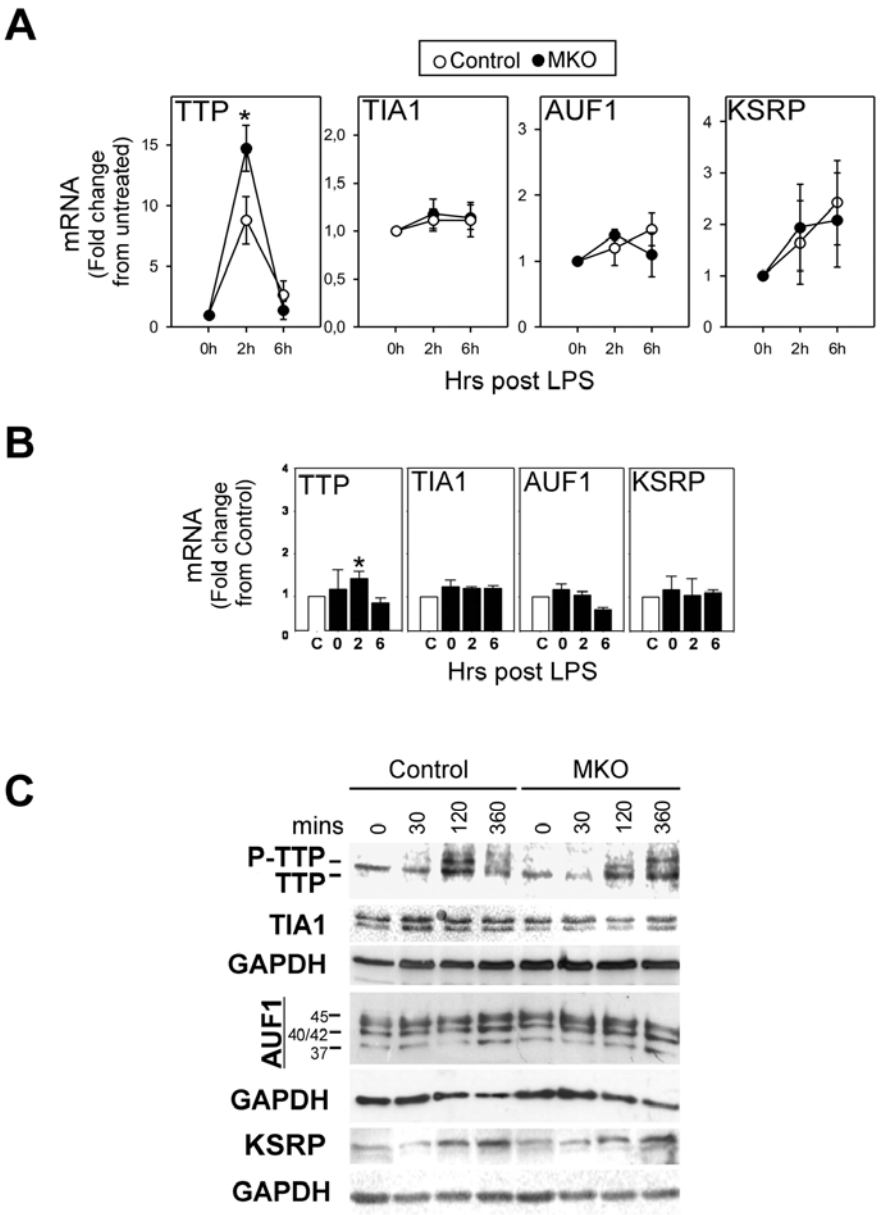
Supplementary Figure 7. Supportive data for migration assays presented in Figure 5. (A) Estimation of secreted CCL2 protein (mean values± SEM) in supernatants from cultured CMT93 intestinal epithelial cells transduced with lentiviral shRNA against *Ccl2* mRNA or a scramble control. Data derived from isolated clones; arrows indicate the clones that have been used in the study. **(B)** Diagrammatic representation of the transwell assays used for the data in **Figure 5A**. **(C)** Flow cytometric enumeration of CD11b expressing myeloid cells in the bone marrow of control and MKO mice during thioglycollate induced aseptic peritonitis or LPS challenge. Values derived as in **Figure 5B**. **(D)** Flow cytometric comparison of the expression of CD11β on the surface of gated blood macrophages (i.e. F4/80+Gr1^{lo}) challenged with LPS for 12 hrs. Indicated are representative fluorescent intensity values for control and MKO macrophages**.**



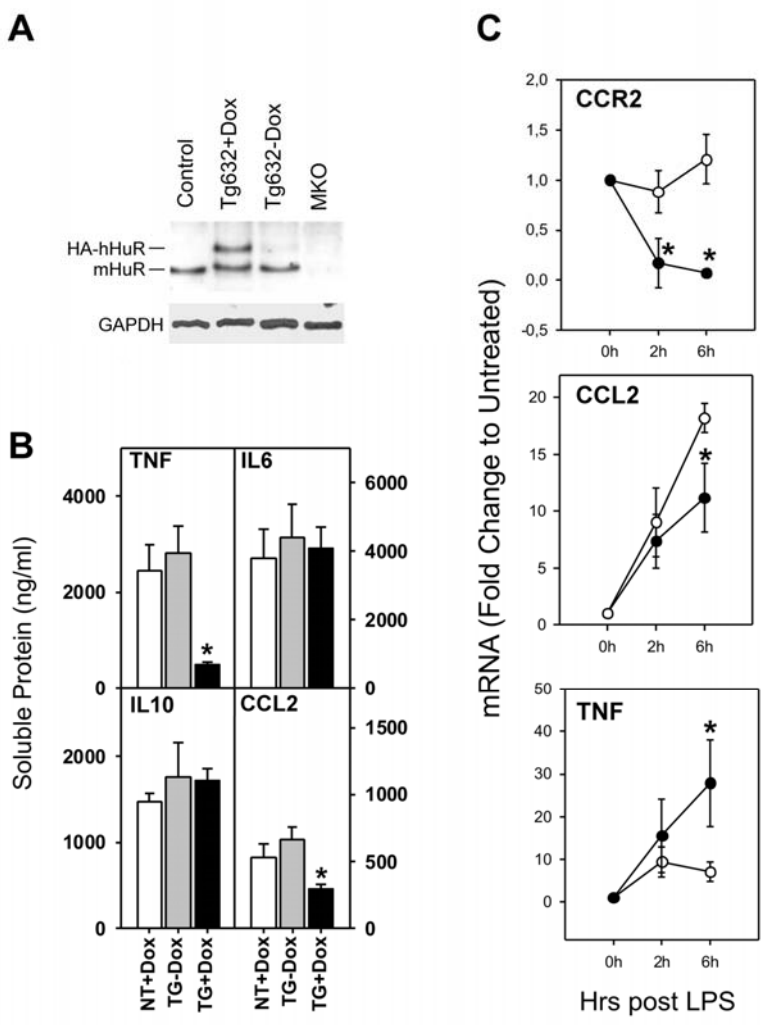
Supplementary Figure 8. HuR-null macrophages can be polarized to M1 and M2 but do maintain changes in cytokine expression profiles. (A) Expression of SHIP and arginase I (Arg I) in cell lysates as markers of M1 vs M2 polarization following the exposure of macrophages to IFNγ or IL4. GAPDH is shown as a loading control. **(B)** iNOS activity (nitrite in supernatants) and Arginase activity (urea in lysates) of macrophage cultures following exposure to LPS after polarization by IFNγ or IL4. **(C)** Detection of TNF, IL6, IL12 and IL10 proteins in supernatants from macrophage cultures following exposure to LPS after polarization by IFNγ or IL4. Bar graphs depict mean values (±SD) from two experiments with three individual macrophage cultures each. For all protein measurements: open bar = control; closed bar = MKO. * denotes significant increases with $p \leq 0.01$



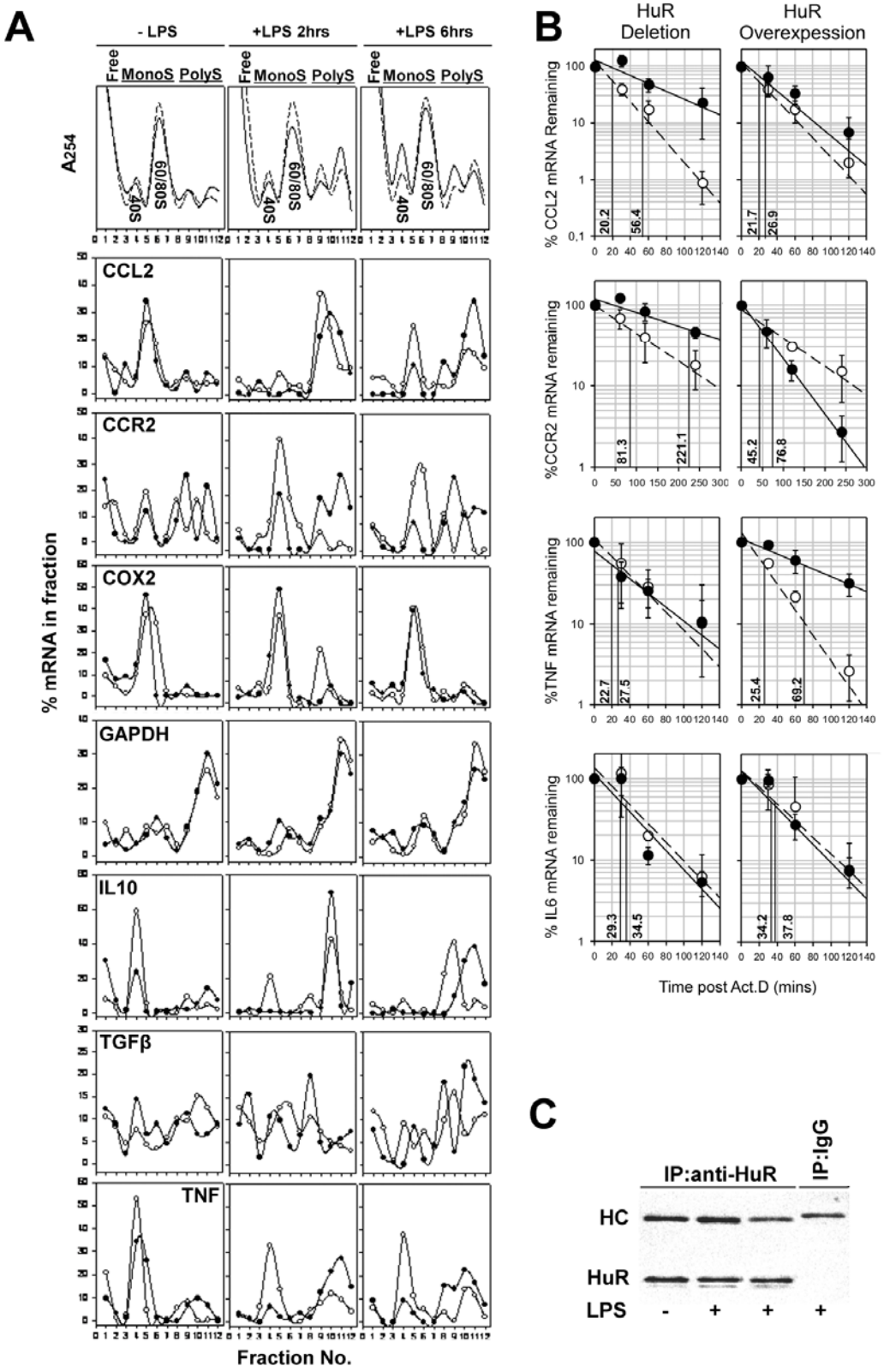
Supplementary Figure 9. Search for signalling aberrations in HuR-null macrophages by means of pharmacological inhibition. Cultured macrophages ($5x10^5$)were pre-treated with increasing concentrations of the indicated inhibitors for a period of 1hr (SB203580, SP600125, AICAR, LY294002, Rottlerin) or 12 hrs (PD98059) and then stimulated with LPS (100ng/ml) in medium containing agent solvent (ethanol, saline or DMSO) for an additional 12hrs. Cytokine concentrations in supernatants were determined via specific ELISAs. Values ($\pm$SEM) were normalized and compared to LPS alone. Line graphs depicting data from at least two experiments with macrophages derived from individual control (○) or MKO (●) mice (n=4/group/experiment). * denotes statistical differences with $p<0.05$.



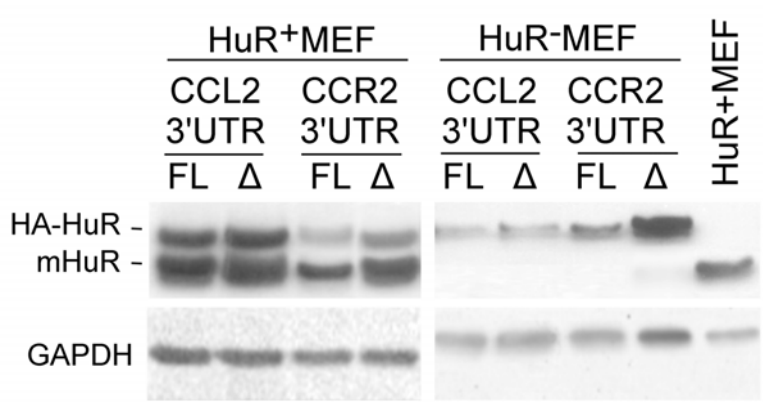
Supplementary Figure 10. Detection of mRNA normalized to untreated **(A)** or control values **(**white bar; **B)** and protein **(C)** for known ARE-binding factors TTP, TIA1,AUF1 and KSRP in extracts from LPS-stimulated macrophages from control and MKO mice. Protein data are shown as representative immunoblots for TTP, phospho-TTP, TIA1, TIAR, the 4 isoforms of AUF1 and KSRP. GAPDH is shown as loading control. * denotes significant increases with $p<0.05$



Supplementary Figure 11. Suppressive effects of HuR overexpression on cytokine production. (A) Immunoblots for HuR demonstrating the induction of HA-HuR in cultured bone marrow derived macrophages from *Tg632*$^{+}$ transgenic mice and in the presence of 5 μg/ml Doxycycline. Shown are control, untreated *Tg632*$^{+}$ and MKO macrophages for comparisons. GAPDH is also shown as a loading control. **(B)** TNF, IL6, IL10 and CCL2 protein levels in supernatants from cultured macrophages derived from *Tg632*-with or without Dox- and control mice (NT). Cytokines were detected via ELISA. Bar graphs depict mean values±SEM from n>3 independent cultures per group. **(C)** Line graphs depicting differences in the temporal accumulation cytokine/chemokine *Ccr2*, *Ccl2* and *Tnf* mRNAs following stimulation with LPS. Values derived from the qRT-PCR detection of unstimulated *Tg632*$^{+}$ (○) and Dox-stimulated *Tg632*$^{+}$ (●) macrophage mRNAs, normalized to *Gapdh* and presented as fold changes (±SD) to the corresponding untreated macrophages.


A
- LPS
+LPS 2hrs
+LPS 6hrs
Free
MonoS
PolyS
A254
S40
S08/09
CCL2
CCR2
COX2
GAPDH
IL10
TGFβ
TNF
% mRNA in fraction
Fraction No.
B
HuR Deletion
HuR Overexpression
% CCL2 mRNA Remaining
%CCR2 mRNA remaining
%TNF mRNA remaining
% IL6 mRNA remaining
20.2
56.4
21.7
26.9
81.3
221.1
45.2
76.8
22.7
27.5
25.4
69.2
29.3
34.5
34.2
37.8
Time post Act.D (mins)
C
IP:anti-HuR
IP:IgG
HC
HuR
LPS
- + + +

Supplementary Figure 12. Differential effects of HuR deficiency on inflammatory mRNA tranlanslation and stability. (A) Polysome analysis in untreated and LPS-treated (2 and 6hrs) macrophages and subsequent fractionation of cytoplasmic extracts in sucrose gradients. The first panel shows representative UV absorption profiles (OD 254) of eluted fractions indicating the peaks corresponding to free RNA (fractions 1-3), monosomal (MonoS; 40S, 60-80S-fractions 4-7) and polysomal (PolyS; fractions 8-12) fractions from HuR^+ or HuR^- macrophages. The RNA in each fraction was extracted and analysed via qRT-PCR. The quantity of mRNAs in each fraction is presented as the percentage of the total quantity measured. Data presented from 3 independent control (○) and MKO (●) cultures. For statistical measurements, clustered monosomal and polysomal values derived from each experiment are compared in **Figure 6B**. **(B)** Decay of inflammatory mRNAs in HuR^+ and HuR^- and HuR overexpressing macrophages in the presence or absence of LPS. Shown are semilogarithmic plots with data (mean values $\pm$ SEM and regression lines) from 3 independent experiments. Numbers indicate estimated half lives from the mean values. In each case left panels indicate difference between control (HuR^+ - ○) and MKO (HuR deletion -●) states whereas the right panels indicate differences between non Dox-treated *Tg632*$^+$ (control -○) and Dox treated (HuR overexpressing -●) states. The complete set of estimated half lives from each individual experiment is presented in **Figure 6C. (C)** Representative anti-HuR immunoblot of the anti-HuR-IP or mIgG1-IP material derived from HuR^+ and HuR^- macrophages. The heavy chain (HC) of the antibodies is indicated.



Supplementary Figure 13. Detection of transfected HuR in mouse embryonic fibroblasts bearing UTR sensors. Immunodetection of HuR in extracts from clonal MEF populations bearing full length (FL) of truncated (Δ) versions of *Ccl2* and *Ccr2* 3'UTR fragments fused to GFP and following transfection with the pEBB-HA-HuR construct (see Figure 7). GAPDH is shown as a loading control. The estimated transfection efficiencies ranged from 30 to 60 %.

Supplementary Table 1. Chemokines, cytokines & other innate mRNAs with differential expression in HuR deficient macrophages

Gene Name	Gene Description	ENSEMBL ID	Fold Change*			p value*			ARE**
			0	2	6	0	2	6	
Ccl2	chemokine (C-C motif) ligand 2	ENSMUSG00000035385	1,5	1,6		0,002	0,001		NR
Ccl7	chemokine (C-C motif) ligand 7	ENSMUSG00000035373		2			0,03		332-344
Ccl12	chemokine (C-C motif) ligand 12	ENSMUSG00000035352		1,9			0,001		52-64
Ccl22	chemokine (C-C motif) ligand 22	ENSMUSG00000031779			1,8			0,006	941-953
Ccr2	chemokine (C-C motif) receptor 2	ENSMUSG00000049103	1,7	1,6	2	0,001	0,01	0,007	1560-1572
Ccr3	chemokine (C-C motif) receptor 3	ENSMUSG00000035448		2	2		0,002	0,003	NR
Cxcl7/Ppbp	chemokine (C-X-C motif) ligand 7	ENSMUSG00000029372		1,8			1E-04		NR
Cxcl9	chemokine (C-X-C motif) ligand 9	ENSMUSG00000029417		2			0,01		NR
Cxcl11	chemokine (C-X-C motif) ligand 11	ENSMUSG00000060183		1,8			0,001		N/A
Cxcr6	chemokine (C-X-C motif) receptor 6	ENSMUSG00000048521			2			5E-06	N/A
Cx3cl1	chemokine (C-X3-C motif) ligand 1	ENSMUSG00000031778			1,5			0,02	N/A
Ifnb1	interferon beta 1, fibroblast	ENSMUSG00000048806		1,5			0,003		NR
Il6	interleukin 6	ENSMUSG00000025746		2			0,004		188-200
Tgfb3	transforming growth factor, beta 3	ENSMUSG00000021253	-1,8			0,01			N/A
Tnfsf8	tumor necrosis factor (ligand) superfamily, member 8	ENSMUSG00000028362			1,5			1E-04	NR
Tnfsf4	tumor necrosis factor (ligand) superfamily, member 4	ENSMUSG00000026700			1,7			0,003	NR
Tnfsf10	tumor necrosis factor (ligand) superfamily, member 10	ENSMUSG00000039304		2			9E-05		NA
Tnfrsf26	tumor necrosis factor receptor superfamily, member 26	ENSMUSG00000045362	-1,5			1E-04			NR
Cfh	complement component factor h	ENSMUSG00000026365	-1,7	-1,5		0,002	0,004		NR
F13a1	coagulation factor XIII, A1 subunit	ENSMUSG00000039109	-1,6	-1,7		0,001	0,006		N/A
F3	coagulation factor III	ENSMUSG00000028128		1,6			0,02		606-618
Fap	fibroblast activation protein	ENSMUSG00000000392			1,6			2E-06	N/A
Mmp8	matrix metallopeptidase 8	ENSMUSG00000005800		1,7			2E-04		N/A
Mmp9	matrix metallopeptidase 9	ENSMUSG00000017737	2,1			0,02			396-408
Plau	plasminogen activator, urokinase	ENSMUSG00000021822				0,001			875-887

* Fold Change and corresponding p values following the bioinformatic comparison of control and test RNAs at individual timepoints following exposure to LPS for 0,2 & 6 Hrs

** Obtained from ARED Organism where NR=Not Reported; NA=Not Any

Supplementary Table 2. Putative HuR binding sites in CCL2 and CCR2 3'UTRs

Gene	Position*	Sequence
Ccl2	**636**	5'-AAGGTGTGGATCCATTTTTC-3'
Ccr2	**1273**	5'-CTTTTTTTAAGCAGGGAAGG-3'
	1332	5'-TGGTTCTTACTTTGGGTCAT-3'
	1472	5'-AATAATTTTATTGTTGTAGA-3'
	2627	5'-TTATGTTTTTGTGGGTAAAA-3'
	2630	5'-TGTTTTTGTGGGTAAAACAA-3'

According to Lopez de Silanes et al. *Nucleotide positions refer to the respective ENSEMBL accession number

SUPPLEMENTARY METHODS

Flow cytometric analysis and sorting of bone marrow progenitors and colony forming assays

Single cell suspensions were prepared from the bone marrow of control or MKO mice, and stained with a Lineage (Lin) antibody cocktail (CD3, CD45R/B220, Ly6G and Ly-6C, and TER-119; Biolegend). For the detection of recombination, Lin+ and Lin- cells were separated via FACS sorting using a FACSVantage^{TM} instrument (BD Biosciences) and used for DNA extraction. For the assessment of stem and progenitor pools, Lin^{-} progenitor cells were enriched by depletion of Lineage positive cells using magnetic sorting (BD IMag^{TM}) according to manufacturer protocol; cells were then analysed further using antibodies against: cKit and Sca for progenitors; CD16/32 and CD34 for myeloid progenitors; and IL7R for lymphoid progenitors. For colony forming assays, sorted Lin- cells were cultured in Methylcellulose media (Methocult^{TM}, StemCell Technologies) enriched in recombinant SCF, IL3 and IL6 (Methocult #03534) or GMCSF (Methocult #3001) according to manufacturers' instructions. Colonies were detected between days 7-10 and analysed via light and phase contrast microscopy.

Flow cytometric analysis of peripheral subsets and cultured macrophages

Single cell suspensions were prepared from mouse bone marrow, peritoneal cavity, peripheral blood, spleens or cultured macrophages and surface-stained via standard procedures. Antibodies used were against: CD11b, Gr1, F4/80, CD16/32, B220, CD5 (from BD Biosciences or Biolegend), TLR2, TLR4 (from eBioscience) and the corresponding IgG isotype. For the intracellular detection of HuR, cells were first surface stained, treated with FcBlock and then fixed/permeabilized with Cytofix and CytoPerm (BD Biosciences). After extensive washing, cells were stained for the intracellular detection of HuR using a specific biotin-conjugated anti-HuR antibody (3A2; Santa Cruz) or the corresponding mouse IgG1 isotype control (R&D). Detection was performed using fluorescent streptavidin conjugates (BD Biosciences) using a FACSCantoII^{TM} (BD) instrument. Data were acquired with FACSDIVA^{TM} software (V.6-BD Biosciences) and analyzed using FlowJo^{TM} (V 7.2.5; TreeStar).

Detection of proliferating epithelia

For the assessment of proliferating cells, deparaffinised colon sections were treated for epitope unmasking, blocked and incubated with Ki67 antibody (Abcam). Detection was performed using streptavidin-conjugated secondary antibody (Southern Biotech) with DAB substrate and haematoxylin counterstaining (Sigma).

Assays for Macrophage Endocytic Functions. BMDMs were either incubated with 100 μg/ml FITC (Sigma) or fluorescently labelled IgG coated microspheres (Polysciences) and analysed as described in *Macrophages: A Practical Approach.* Ed. Donna M .Paulnock 2000. Oxford University Press.

Protein Analysis via Immunoblots and Enzymatic assays

Tissue or whole cell lysates were prepared in RIPA Buffer. Nuclear and Cytoplasmic extracts were prepared using NE-PER (Pierce) according to manufacturer's instructions. Equimolar amounts of protein were analyzed on SDS-polyacrylamide gels (10-12%) and blotted onto nitrocellulose membranes (Schleicher & Schuell). Probing antibodies included: HuR (3A2), p38 (H-147), MKP1 (C19), pERK (E-4), ERK (K-23), AMPKα (H-300), pAMPKα (Thr172), FBP2/KSRP(S-18), TIA1 (C-20), Arginase I (N-20) and SHIP-1(P1C1) from Santa Cruz; pP38 (3D7), pSTAT3 (D3A7), and STAT3 (79D7) from Cell Signalling; GAPDH (6C5) from Ambion; hnRNPD/AUF1 from Upstate; and TTP from Aviva Systems Biology. Detection was performed using horseradish peroxidase-conjugated secondary antibodies and visualized by enhanced chemiluminescence (ECL+; Amersham). Arginase and NO activities were measured as in Ho,V.W., and Sly,L.M. 2009. "Derivation and characterization of murine alternatively activated (M2) macrophages". *Methods Mol Biol.* **531:173-85.**:173-185.

Macrophage Polarization Assays

The generation of polarized M1/M2 macrophages was performed as described in (32). Briefly, BMDMs were cultured for 3 days with IFNγ (5 μγ/ml; Peprotech) or IL-4 (10 μg/ml; R&D), then stimulated with 100 ng/ml LPS.

Assays of Pharmacological Interference.

BMDMs ($5x10^5$) from individual animals were seeded onto 24-well plates in duplicates and left to adhere overnight in a 5%CO_2, 37^0C incubator. Cells were then pre-treated with SB203580 (p38/SAPK inhibitor; Santa Cruz Chemicals), SP600125 (JNK inhibitor; Sigma Aldrich), PD98059 (MEK1/ ERK inhibitor; Sigma Aldrich), LY294002 (PI3 kinase inhibitor), AICAR (5-Aminoimidazole-4-carboxyamide ribonucleoside; AMP kinase agonist; Cell Signalling), Rottlerin (PKC inhibitor; ACROS organics); and recombinant mouse interleukin 10 (IL10; R&D) for the indicated times and with the indicated concentrations. Subsequently, cells were treated with LPS (100ng/ml) for an additional 12 hrs and supernatants used for mouse TNF, IL6, CCL2 and IL10 ELISAs.

Knock-down of CCL2/MCP1 in CMT-93 intestinal epithelial cells

The C57Bl/6 mouse-derived colonic epithelial carcinoma cell line CMT-93 was purchased from ATCC/LGC and maintained DMEM+10% FBS. For the knockdown of CCL2/MCP1, cells were transduced with MCP1 shRNA (sc43914-V) or scramble shRNA (sc-108080) containing lentiviral particles obtained from Santa Cruz and in the presence of Polybrene, under standard Biosafety Level 2 conditions. On the 6^{th} day post infection, cells were selected via the incorporation of 5μg/ml Puromycin dihydrochloride (Sigma). Colonies were lifted on the 14^{th} day and analysis of CCL2 knock-down was performed by its estimation in supernatants via ELISA.

RNA Labeling and Affymetrix Expression Array processing

300 ng of total RNA was used to generate biotinylated complementary RNA (cRNA) for each treatment group using the Total RNA Target Labeling protocol (Affymetrix, Santa Clara, CA) as described at the GeneChip® Whole Transcript (WT) Sense Target Labeling Assay Manual v4 (701880 Rev. 4). In short, isolated total RNA was checked for integrity using the RNA 6000 Nano LabChip kit on the Agilent Bioanalyzer 2100 (Agilent Technologies, Inc., Palo Alto, CA) and concentration using the ND-1000Nanodrop (Thermo Fisher Scientific, Wilmington, Delaware USA). Poly-A RNA control kit and RNA of interest were reverse transcribed using a T7-$(N)_6$ primer and Superscript II reverse transcriptase GeneChip® WT cDNA Synthesis Kit (Affymetrix, Santa Clara, CA). Polymerase I from the same kit was used for second strand cDNA synthesis. Biotinylated cRNA was synthesized from the double stranded cDNA using

T7 RNA polymerase and a biotin-conjugated pseudouridine containing nucleotide mixture provided in the IVT Labeling Kit (Affymetrix, Santa Clara, CA). The cRNA was purified with GeneChip® Sample Cleanup Modules (Affymetrix, Santa Clara, CA). 10 μg of purified cRNA were used for second cycle cDNA synthesis with Random primers and Superscript II reverse transcriptase. GeneChip® Sample Cleanup Module (Affymetrix, Santa Clara, CA) was used to purify the resulted single stranded DNA (ssDNA). Fragmentation of 5.5μg ssDNA was performed and the resulted product was labeled with DNA Labeling Reagent, GeneChip® WT Terminal Labeling Kit (Affymetrix, Santa Clara, CA). The product was hybridized for 16 hours to MOGene 1.0 ST arrays in an Affymetrix GeneChip® Hybridization Oven 640. Immediately following hybridization, the GeneChip® arrays are washed and stained with streptavidin-phycoerythrin conjugate, GeneChip® Hybridization wash and stain kit (Affymetrix, Santa Clara, CA), using automated protocol on a GeneChip® Fluidics Station 450, followed by scanning on an Affymetrix GeneChip® Scanner 3000 at 570nm. The Affymetrix eukaryotic hybridization control kit and Poly-A RNA control kit were used to ensure efficiency of hybridization and cRNA amplification. All cRNA were synthesized and processed simultaneously. Images and data were acquired using the Affymetrix® GeneChip® Command Console® Software (AGCC) where initial quality check of the experiment was performed. The Partek Software Genomics suite (Partek Incorporated Missouri 63141, USA) was used for the analysis of the data. Background correction applied to the data using RMA background correction adjusted for GC content and quantile normalization was performed with median polish probeset summarization. A principal Component analysis produced the initial list for statistical selection.

Primer Sets used in this study

Ccl2 sense	5' AGCACCAGCACCAGCCAACT 3'
Ccl2 antisense	5' TTCCTTCTTGGGGTCAGCAC 3'
*Ccl7*sense	5' AGCTACAGAAGGATCACCAG 3'
Ccl7 antisense	5' CACATTCCTACAGACAGCTC 3'
Ccr2 sense	5' GGTCATGATCCCTATGTGGG 3'
Ccr2 antisense	5' CTGGGCACCTGATTTAAAGG 3'
Ccr3 sense	5' ATGGCATTCAACACAGATGAAATCAAG 3'
Ccr3 antisense	5' GGATAGCGAGGACTGCAGGAAAAC 3'
Tnfa sense	5' CACGCTCTTCTGTCTACTGA 3'
Tnfa antisense	5' ATCTGAGTGTGAGGGTCTGG 3'
Il1b sense	5' TTGTTGATGTGCTGCTGTGA 3'
Il1b antisense	5' TGTGAAATGCCACCTTTTGA 3'
Il6 sense	5' CTTCTTGGGACTGATGCTGGTGAC 3'
Il6 antisense	5' TCCAGGTAGCTATGGTACTCCAGA 3'
Il10 sense	5' TGGCCTTGTAGACACCTTGG 3'
Il10 antisense	5' AGCTGAAGACCCTCAGGATG 3'
Il12b sense	5' TGTCCTCAGAAGCTAACCAT 3'
Il12b antisense	5' CCAGTCCACCTCTACAACAT 3'
Tgfb1 sense	5' TGACGTCACTGGAGTTGTACGG 3'
Tgfb1 antisense	5' GGTTCATGTCATGGATGGTGC 3'
Cox2 sense	5' TCAGTTTTTCAAGACAGATC 3'
Cox2 antisense	5' TCTCTACCTGAGTGTCTTTG 3'
B2M sense	5' TTCTGGTGCTTGTCTCACTGA 3'
B2M antisense	5' CAGTATGTTCGGCTTCCCATTC 3'
Gapdh sense	5' TGCACCACCACCTGCTTAGC 3'
Gapdh antisense	5' GGCATGGACTGTGGTCATGAG 3'
Auf1 sense	5' AGAGAGTACTTTGGTGGTTTTGG 3'
Auf1 antisense	5' TGATACTGTTCCTTTGACATGGC 3'
Ttp sense	5' CTCTTCACCAAGGCCATTC 3'
Ttp antisense	5' CCAGTCAGGCGAGAGGTGAC 3'
Tia1 sense	5' TGGAGGACGAGATGCCCAAG 3'
Tia1 antisense	5' TTACCCATTATCTTCCGCCC 3'
Ksrp sense	5’ ACTGGAGCACCTGAGTCTGT 3’
Ksrp antisense	5’ CGTTGTCGTGAAACTGTCCT 3’
Elavl1exon2 sense	5’ AGGACACAGCTTGGGCTACG 3’
Elavl1exon2 antisense	5’ CGTTCAGTGTGCTGATTGCT 3’
Elavl1exon4 sense	5’ GTTTGTCCAGAGGGGTTGCC 3’
Elavl1exon4 antisense	5’ TGGTACAGCTGCGAGAGGAG 3’
iNos sense	5’ ATGGCTTGCCCCTGGAAGTT 3’
iNos antisense	5’ TGATGGACCCCAAGCAAGAC 3’
Ifnγ sense	5’ GCTGTTACTGCCACGGCACA 3’
Ifnγ antisense	5’ TGCTGATGGCCTGATTGTCT 3’