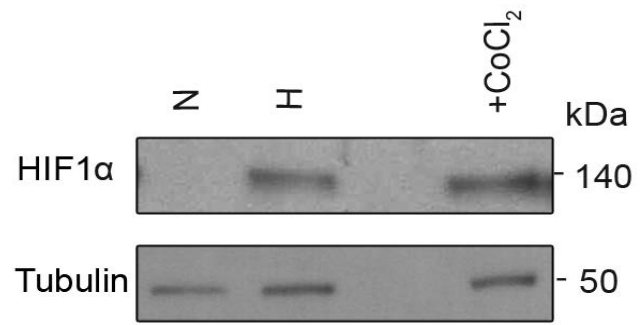
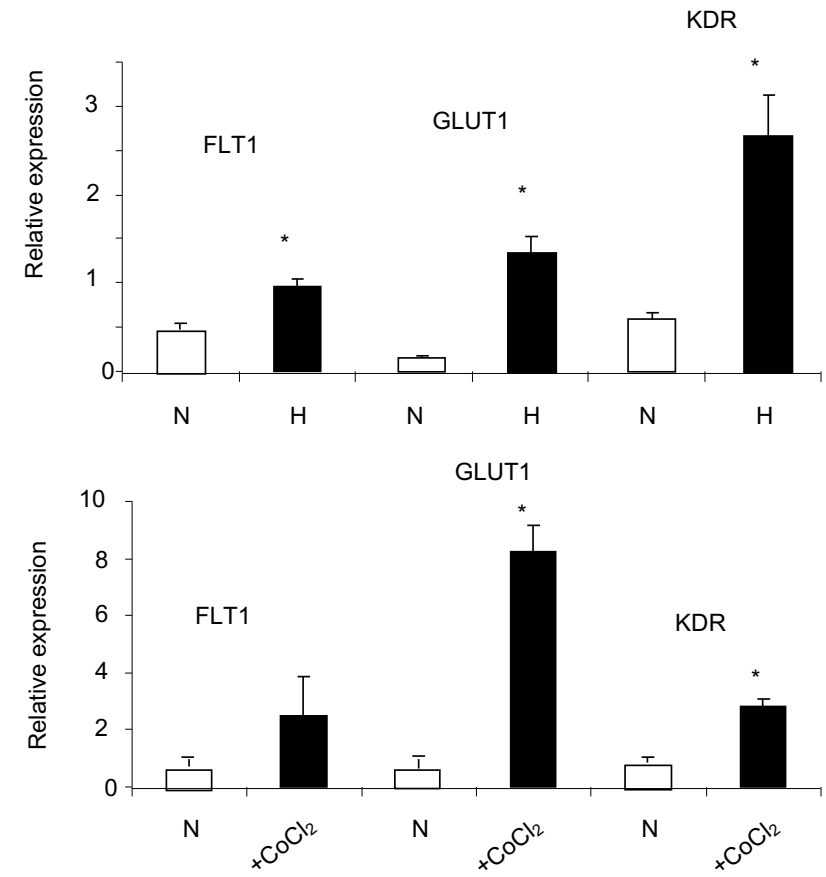
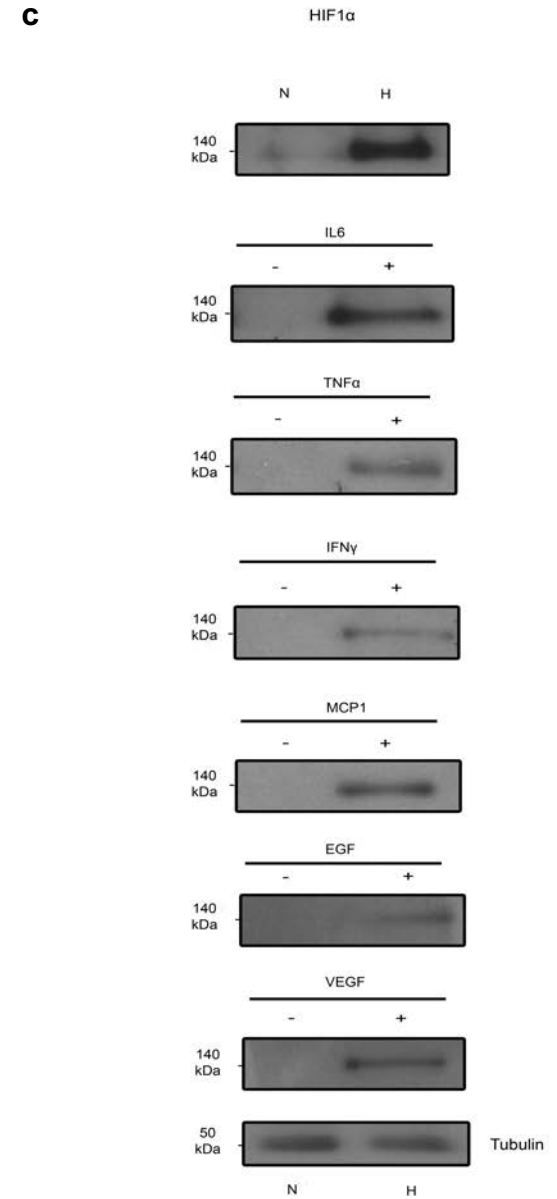
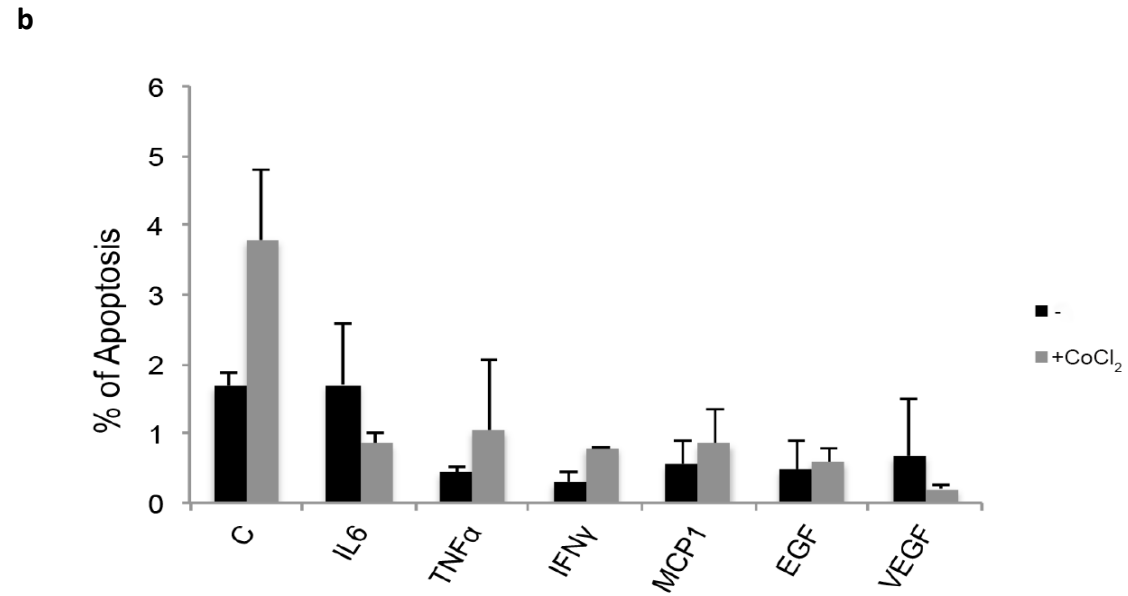
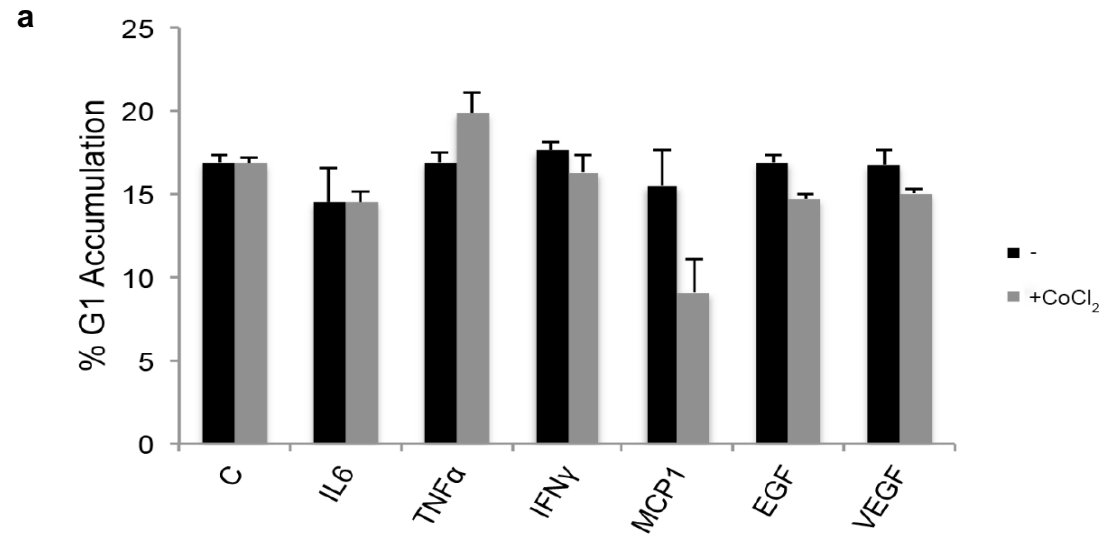


(# SREP-17-07283B)

**Pro-inflammatory cytokines activate hypoxia-inducible factor 3a via epigenetic changes in mesenchymal stromal/stem cells**

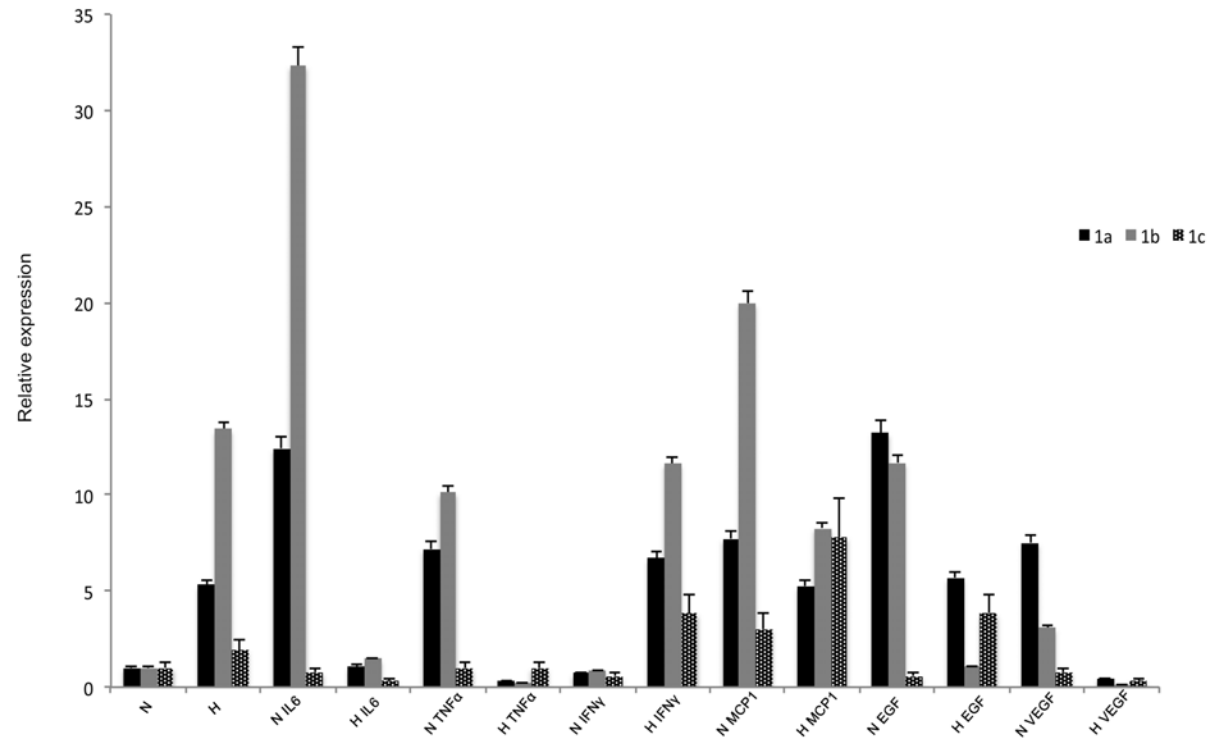
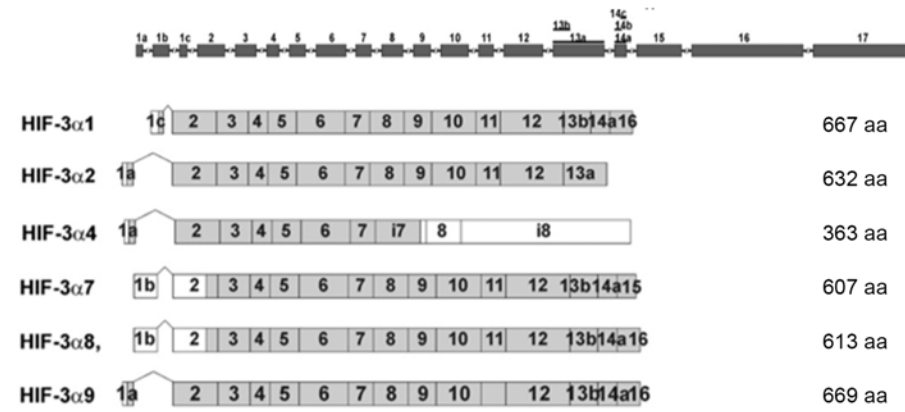
Francesca Cuomo<sup>a</sup>, Antonietta Coppola<sup>a</sup>, Chiara Botti<sup>a,§</sup>, Ciro Maione<sup>a</sup>, Amalia Forte<sup>b</sup>, Lucia Scisciola<sup>a</sup>, Giuseppina Liguori<sup>c</sup>, Ilaria Caiafa<sup>a</sup>, Matilde Valeria Ursini<sup>d</sup>, Umberto Galderisi<sup>b</sup>, Marilena Cipollaro<sup>b</sup>, Lucia Altucci<sup>a</sup> and Gilda Cobellis<sup>a</sup>

**a****b**

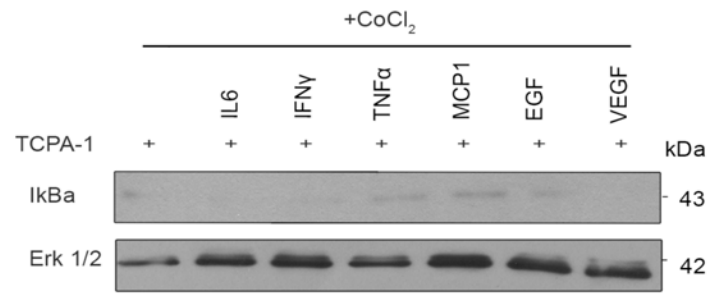


Cuomo et al Suppl. Figure 2

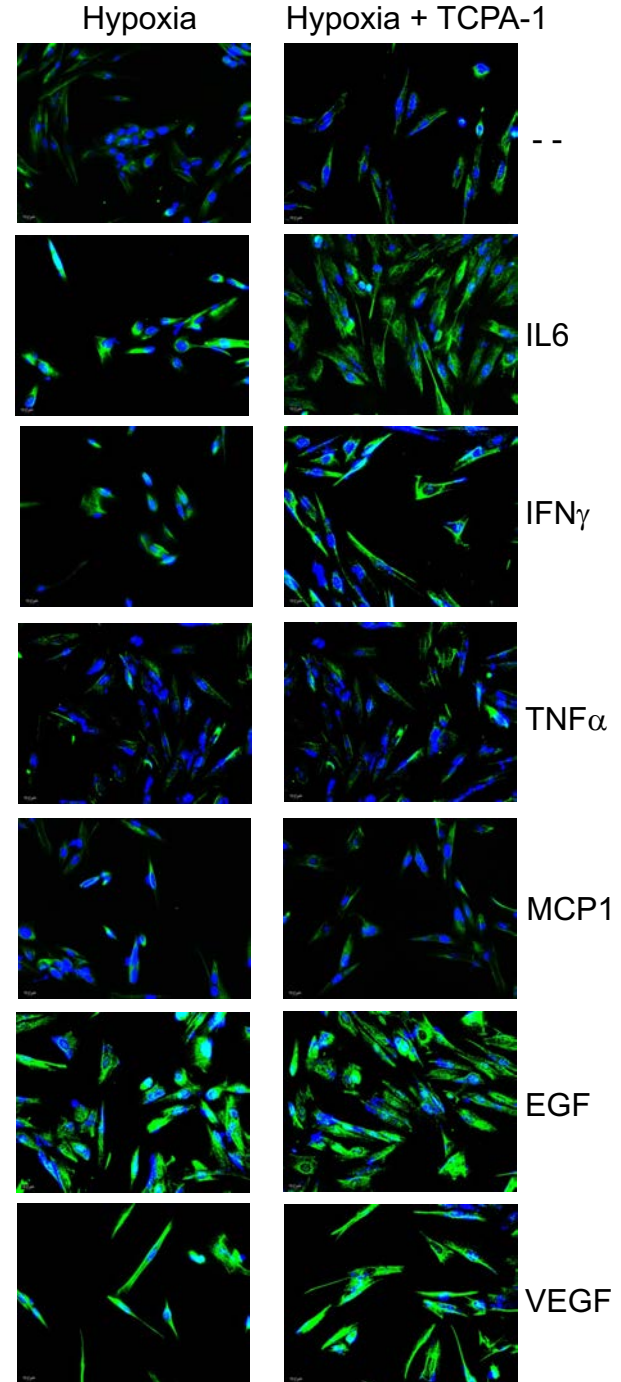
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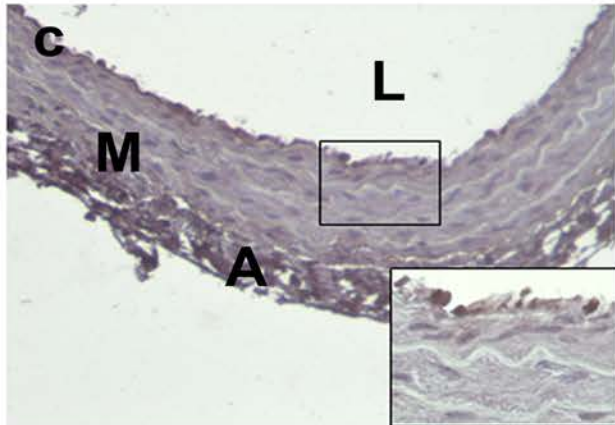
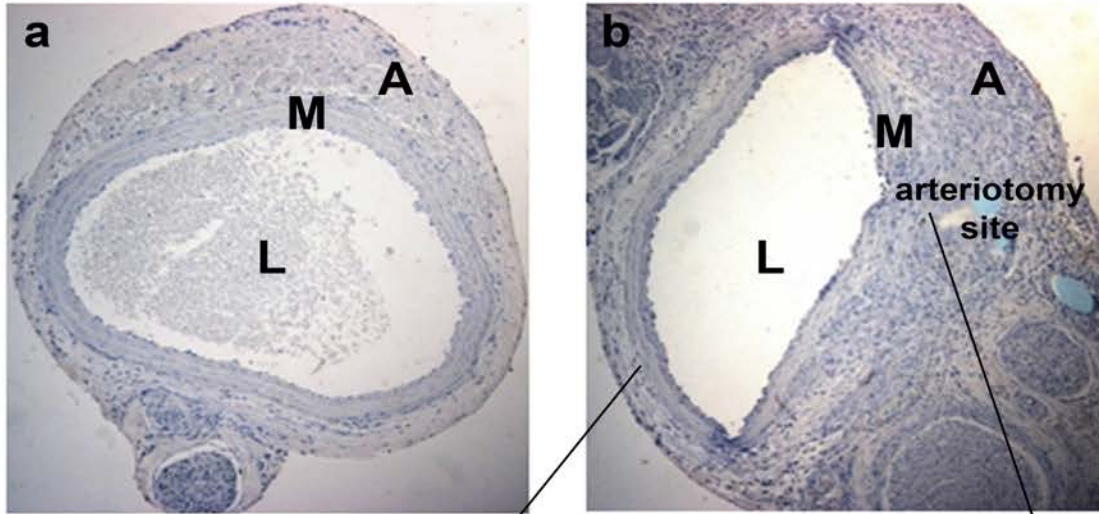


a

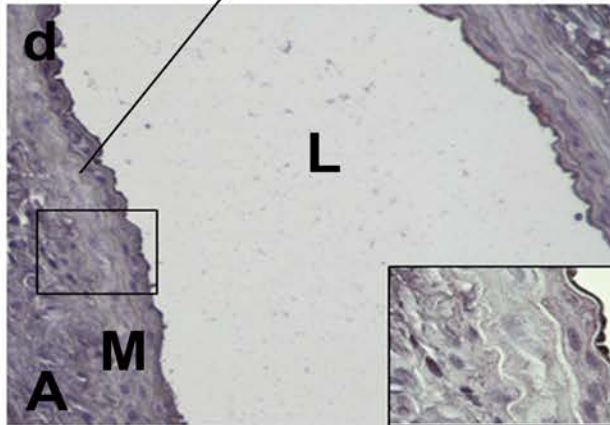


b

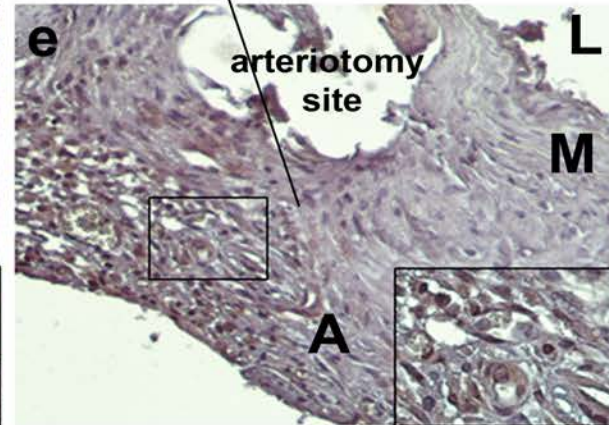




Uninjured carotid



Injured carotid  
Region distal to  
arteriotomy site



Injured carotid  
Region proximal to  
arteriotomy site

Hif1 $\alpha$

## Supplementary Figure Legends

Supplementary Figure 1: a) Immunoblotting analysis of HIF1a protein in hMSC cells cultured either in 1%O<sub>2</sub> conditions generated by GasPack method (H) or in presence of CoCl<sub>2</sub> for 24 h. Total proteins were extracted and WB probed with antibodies against HIF1 $\alpha$ . Tubulin was used as loading control. Protein molecular weights are shown. b) Expression analysis of FLT1, GLUT1 and KDR mRNAs in hMSC cells cultured either in 1%O<sub>2</sub> conditions generated by GasPack method (H) or in presence of CoCl<sub>2</sub> for 24 h. Gene expression data are reported as 2-DDCt method, normalized to housekeeping gene (beta-actin mRNA) and ALU sequences. Data are expressed as means  $\pm$  SEM. Statistical significance is reported: \*: p <0.05

Supplementary Figure 2: a) G1-phase accumulation: hMSCs were cultured in normoxic (N) and hypoxic (H) conditions and treated with IL6, TNF $\alpha$ , IFN $\gamma$  MCP1, EGF, and VEGF for 24 h and analysed by flow cytometry using CellQuest software. b) Quantification of apoptotic cells in pre-G1 phase was performed by ModFit software. c) Immunoblotting analysis of HIF1a protein in hMSC cells cultured either in normoxic conditions or in presence of CoCl<sub>2</sub> for 24 h in absence or in presence of indicated cytokines. Total proteins were extracted and WB probed with antibodies against HIF1 $\alpha$ . Tubulin was used as loading control. Protein molecular weights are shown. Images derived from different part of the same gel and cropped for layout reasons and included in Supplementary Information.

Supplementary Figure 3: a) Schematic representation of the HIF3A locus and relative isoforms, modified from<sup>15</sup>.

Supplementary Figure 4: a) Immunoblotting analysis of I $\kappa$ B $\alpha$  in hMSCs cultured in CoCl<sub>2</sub>- induced hypoxia conditions and treated with IL6, IFN $\gamma$ , TNF $\alpha$ , MCP1, EGF and VEGF for 24h, after an incubation with TCPA-1 for 1hr. b) Immunofluorescence analysis of HIF3a protein in hMSCs cultured in hypoxia in absence and in presence of indicated cytokines and probed with antibodies against HIF3a. Cells were pre-treated with TCPA-1 for 1h before cytokines supplementation. Scale bars: 10 $\mu$ m)

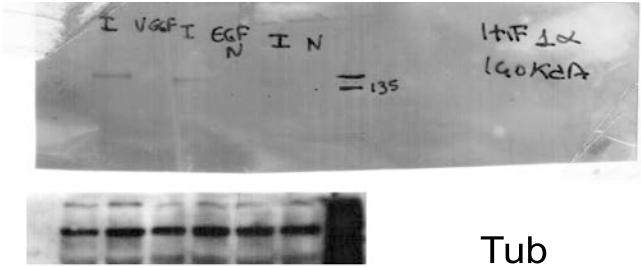
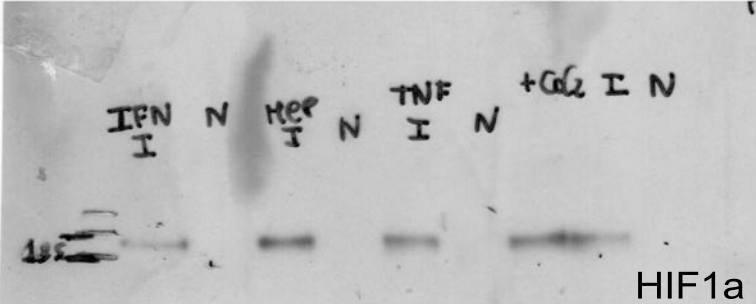
Supplementary Figure 5: Immunohistochemical analysis of Hif1 $\alpha$  expression in uninjured rat carotids and in carotids from Wistar male rats harvested 7 days after arteriotomy. a: uninjured rat carotid; b: arteriotomy-injured rat carotid harvested 7 days after injury, haematoxylin staining. The injury site is indicated in b, where arteriotomy is followed by the application of an 8.0 polypropylene stitch (light blue); c-e: representative immunohistochemical staining of Hif1 $\alpha$  in uninjured rat carotid (c) and in injured carotids harvested 7 days after arteriotomy, showing a carotid region distal (d) and proximal (e) to the arteriotomy site. a,b: 10x magnification; c-e: 20x magnification; small insets: 40x magnification of selected areas enclosed in black rectangles, representative of nuclei positive to Hif1 $\alpha$  in intima, adventitia, *vasa vasorum* and perivascular tissue. Brown staining corresponds to target protein expression. Nuclei were counterstained with haematoxylin. L: lumen; M: media; A: adventitia.



Supplementary Figure 7: Raw data (8 panels) from MS-PCR experiments used for figure 3B

Supplementary Figure 8: Raw data (8 panels) from replicate MS-PCR experiment.

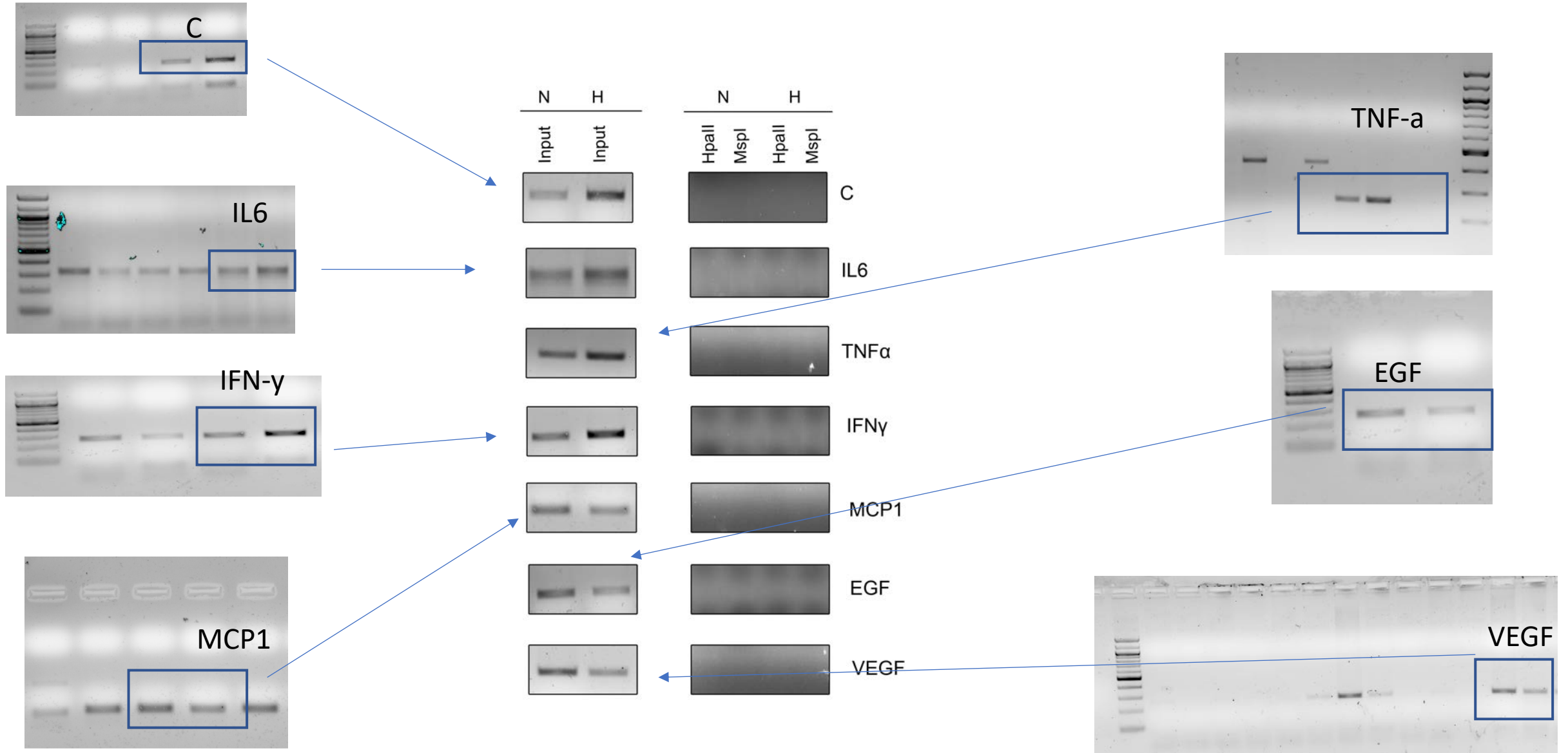
Raw data of HIF1a expression in normoxic, hypoxic and CoCl<sub>2</sub>-induced hypoxia in Suppl. Figure 2



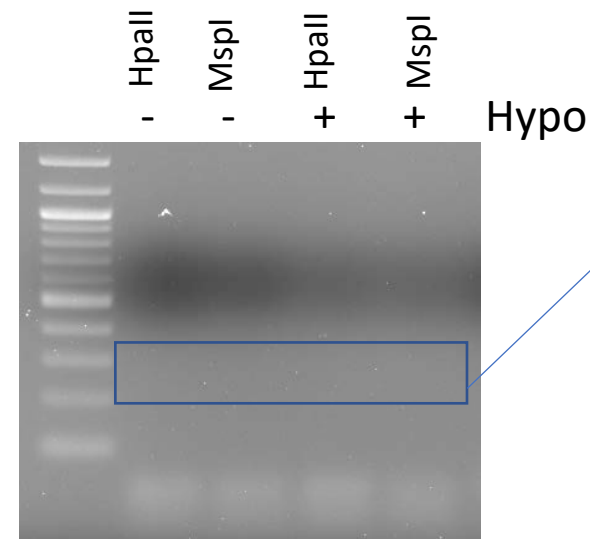
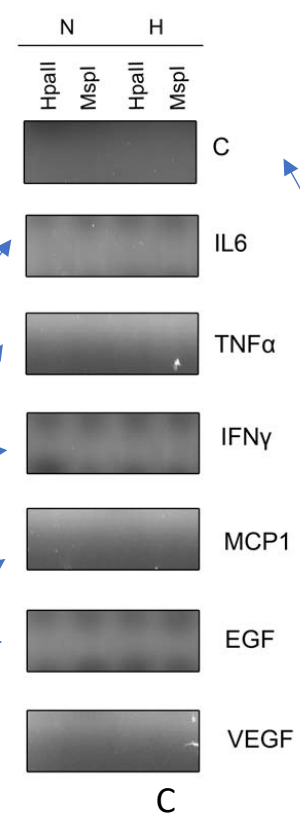
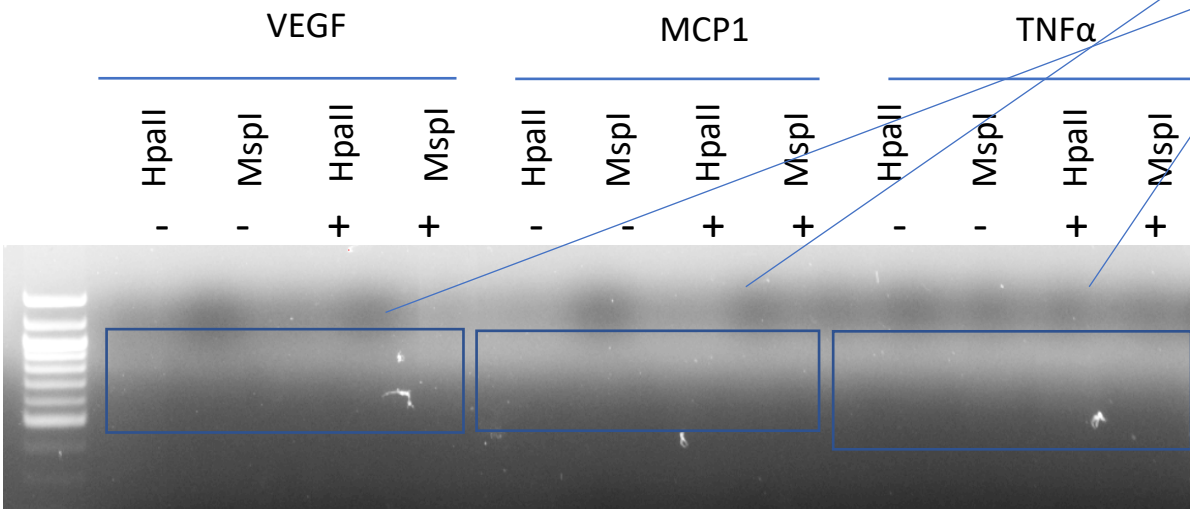
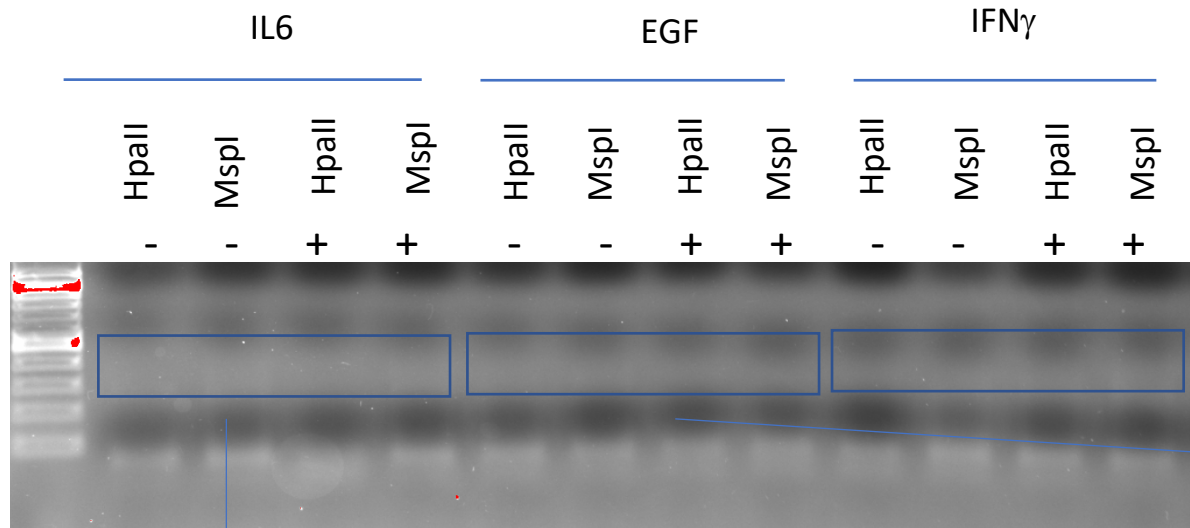
Tub

Raw data from MS-PCR experiments used for figure 3B

# INPUT 1



# MS-PCR Amplicon #1

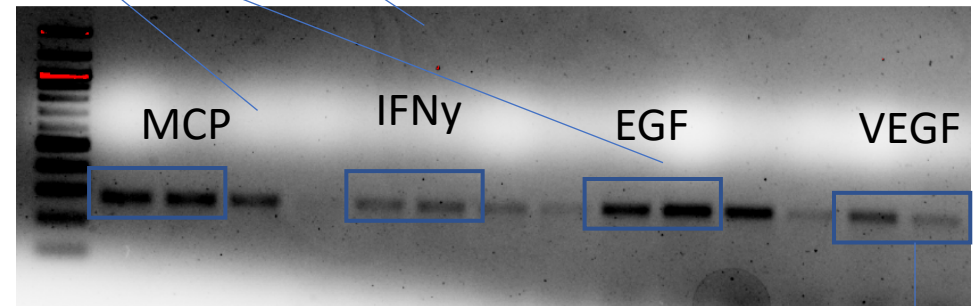
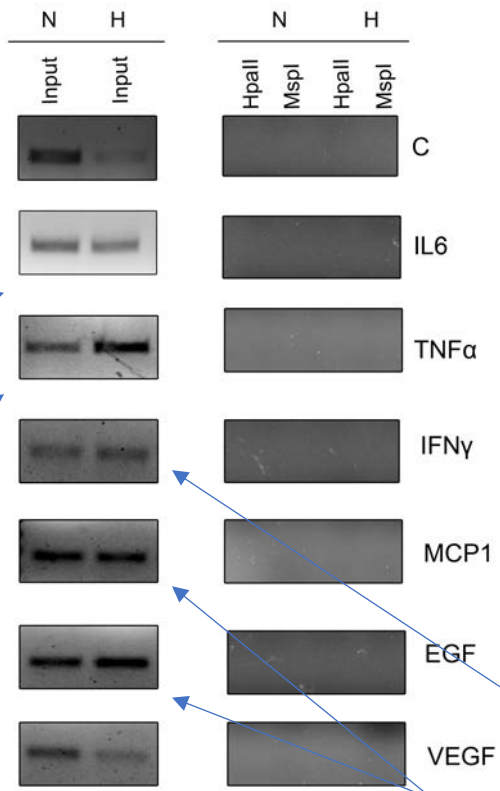
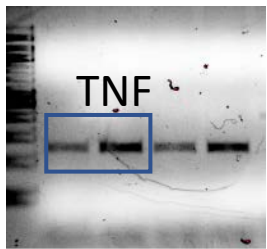
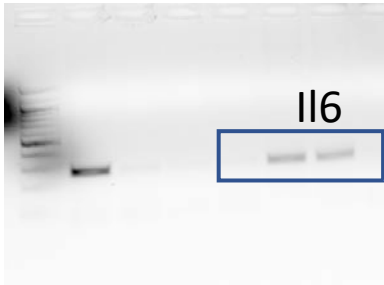
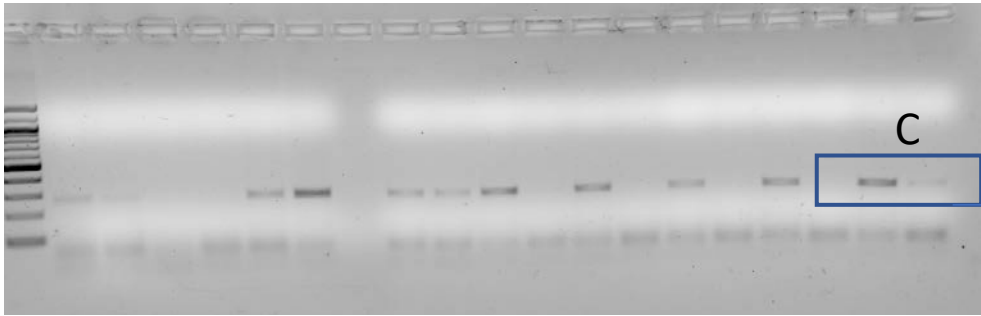


Hypo

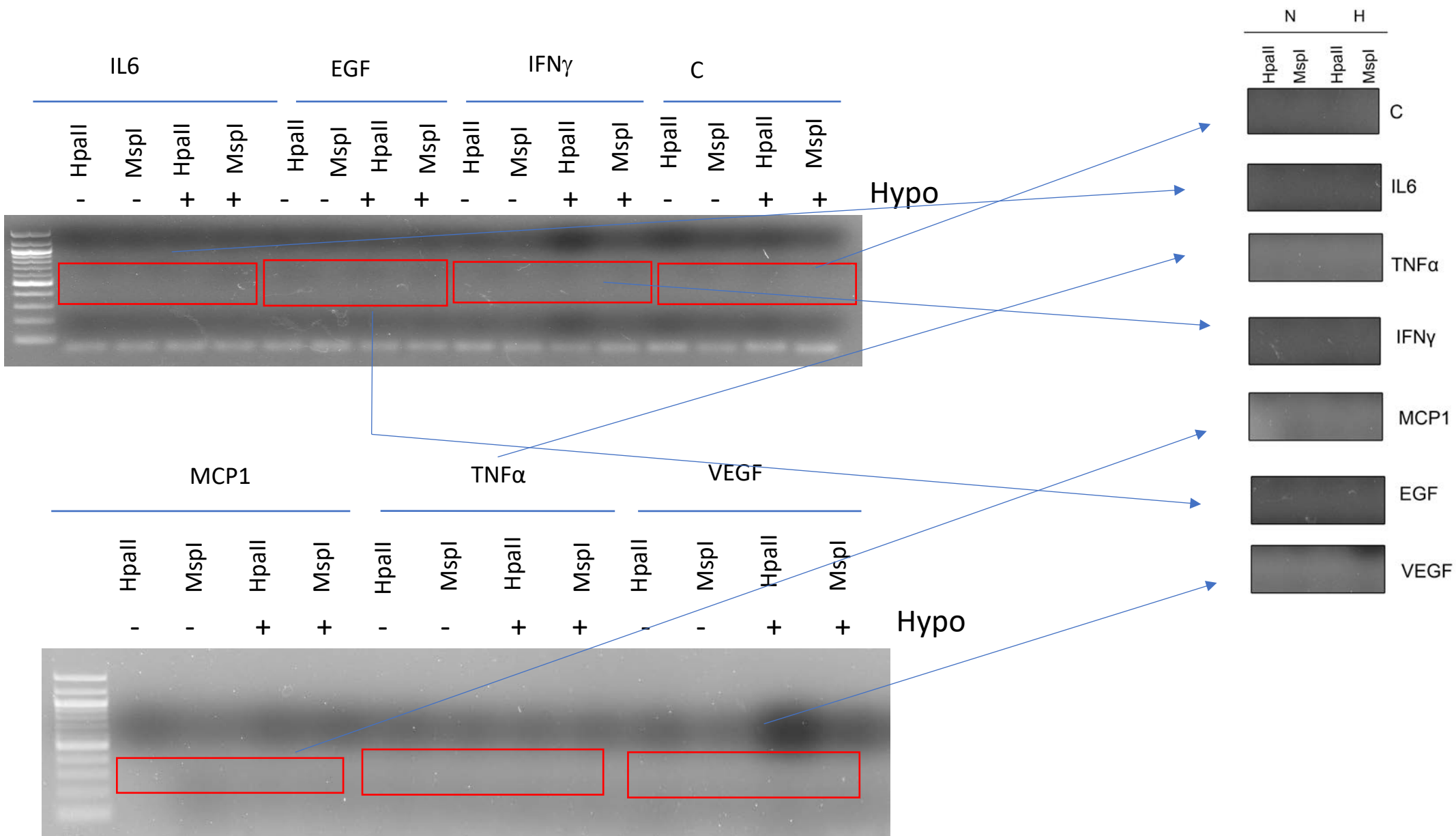
Hypo

Hypo

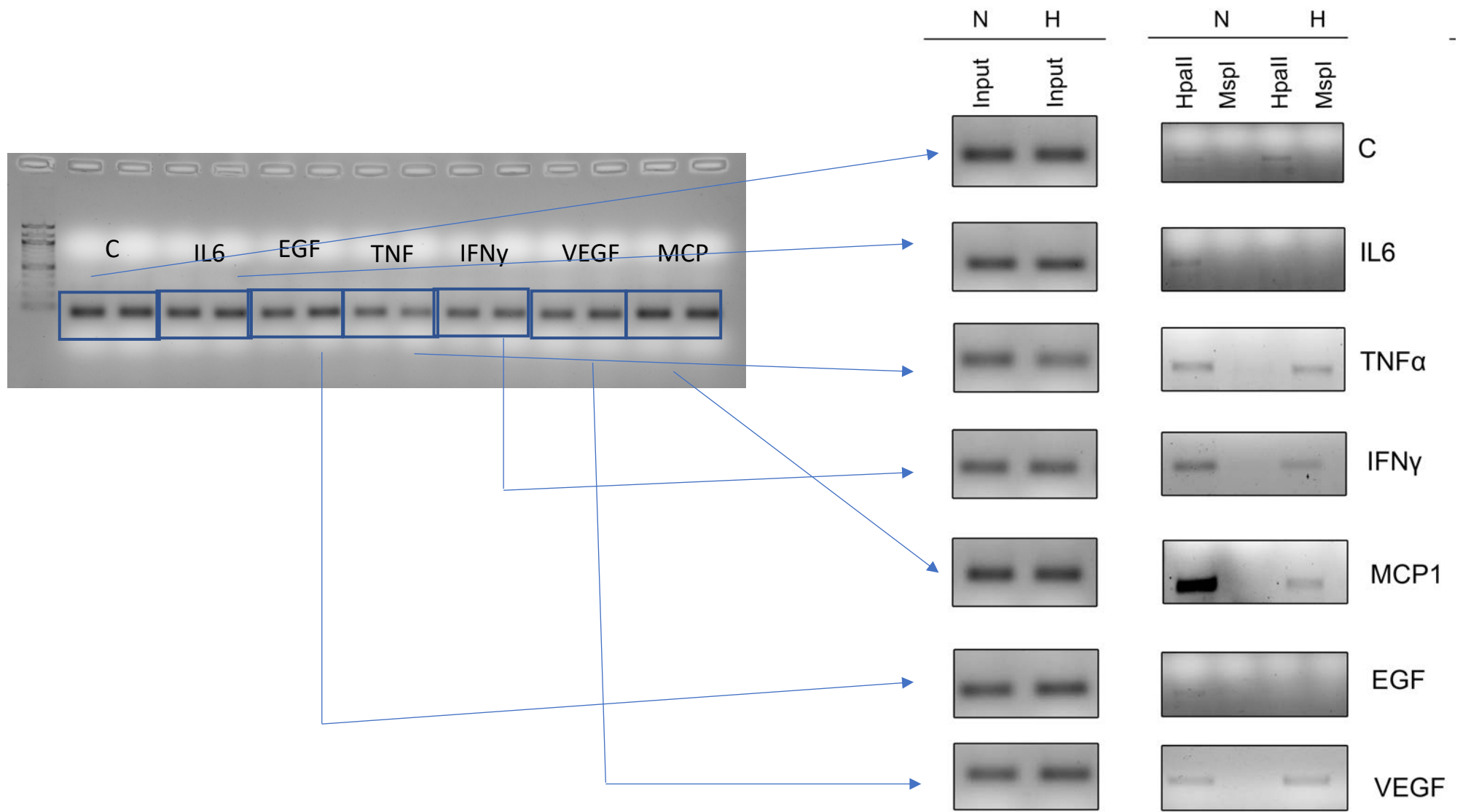
# INPUT 2



# MS-PCR Amplicon #2



# INPUT 3





# MS-PCR Amplicon #3

IL6

EGF

C

	N	H
	HpaII	MspI
	HpaII	MspI

C

IL6

TNF $\alpha$

IFN $\gamma$

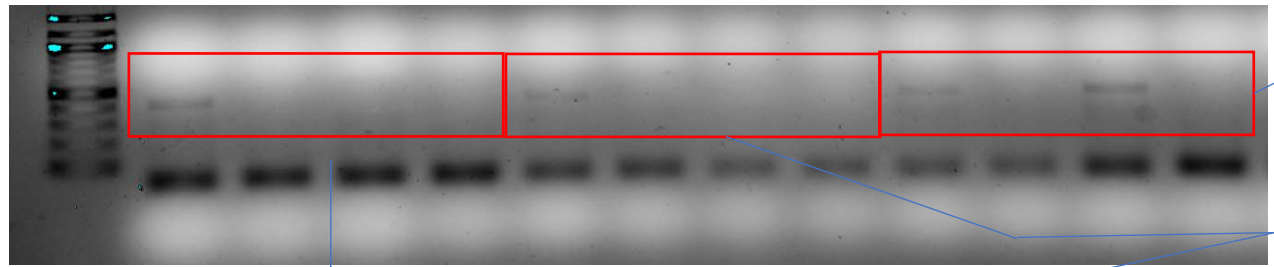
MCP1

EGF

VEGF

IFN $\gamma$

Hypo

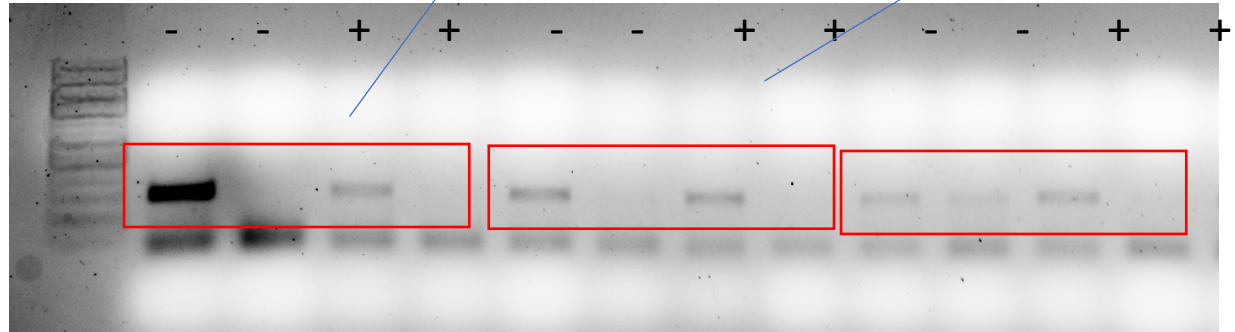


Hypo

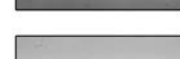
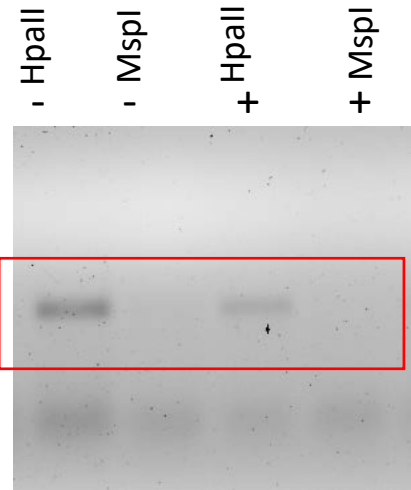
MCP1

TNF $\alpha$

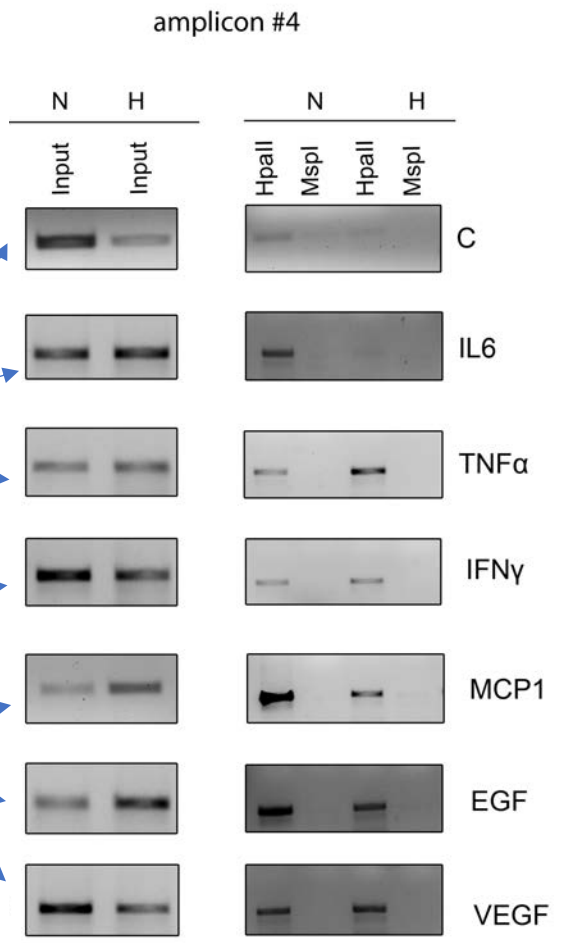
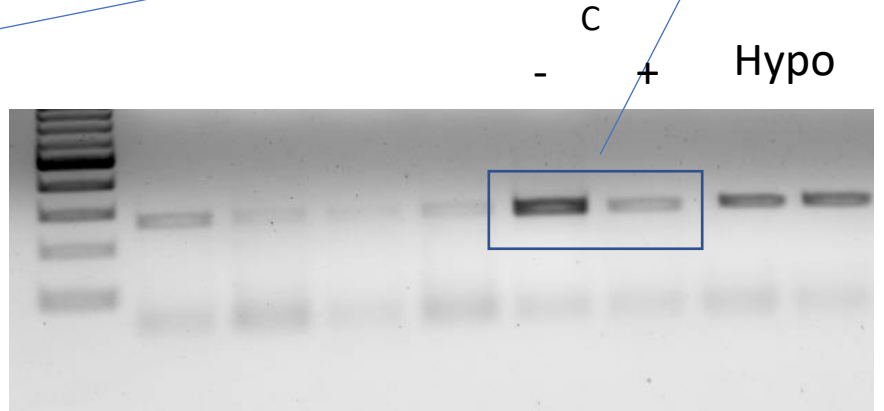
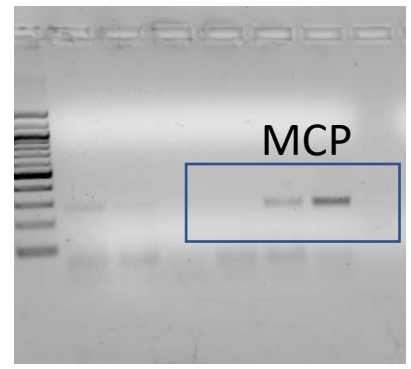
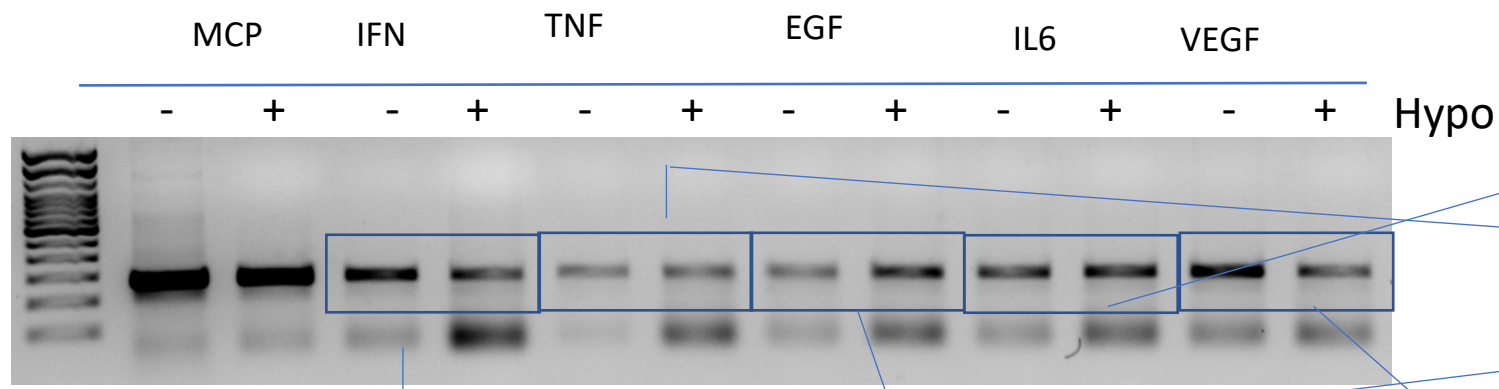
VEGF



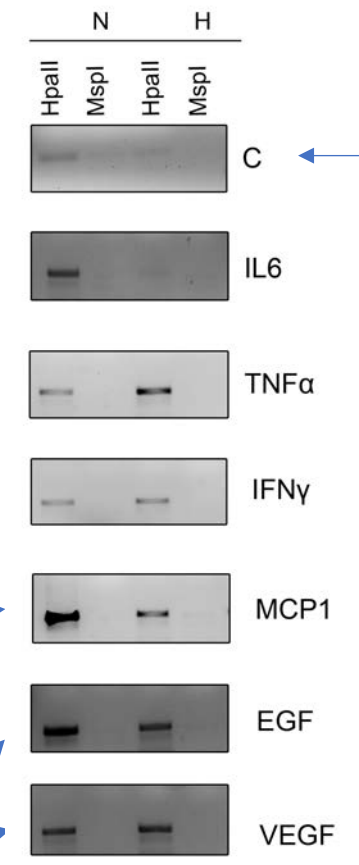
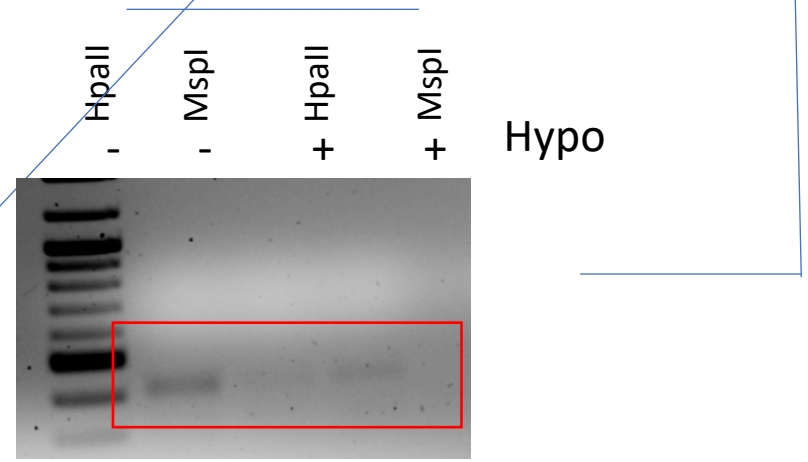
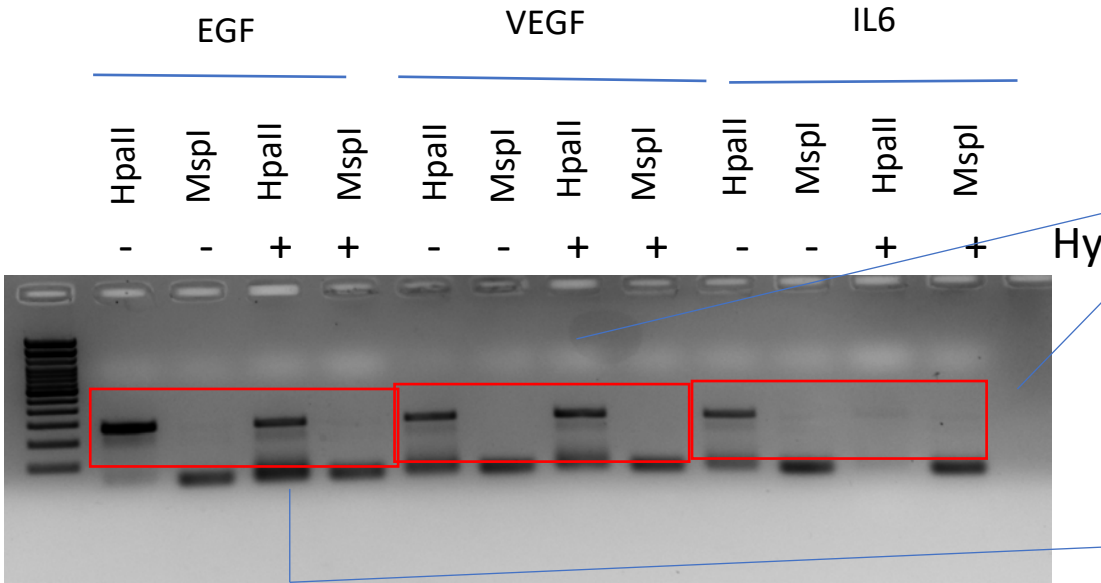
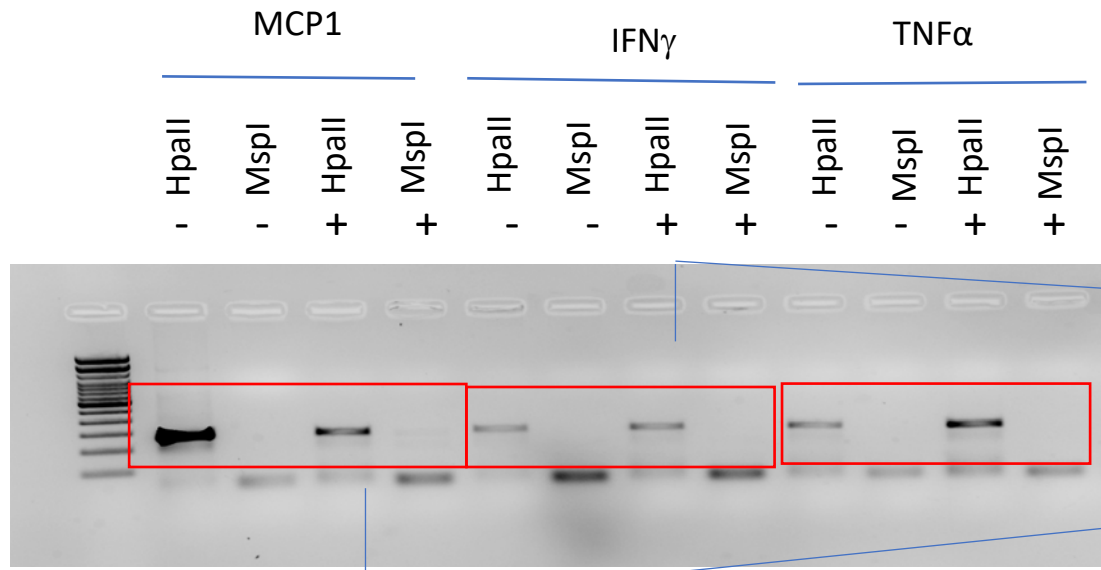
Hypo



# INPUT #4



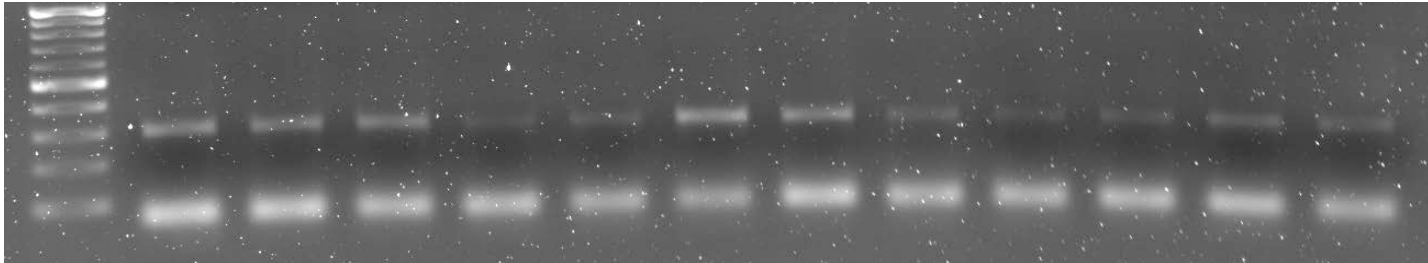
# MS-PCR Amplicon #4



Raw Replicate data from MS-PCR experiment

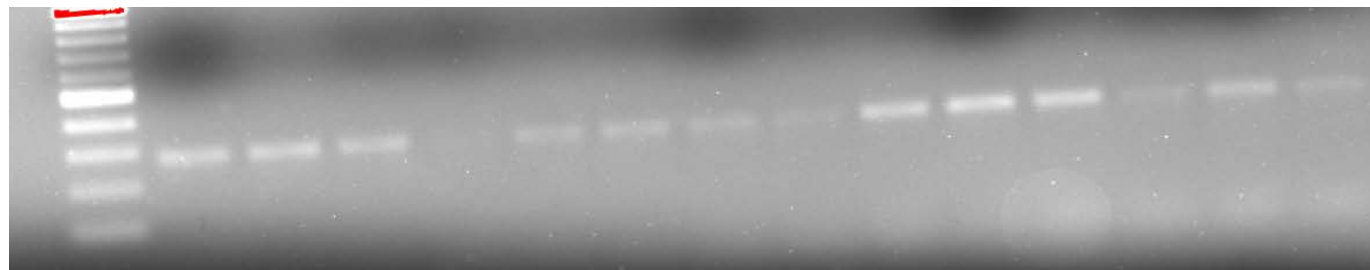
AMPLICON INPUT #1

IL6		IFN $\gamma$		TNF $\alpha$		MCP1		EGF		VEGF	
-	+	-	+	-	+	-	+	-	+	-	+



AMPLICON INPUT #2

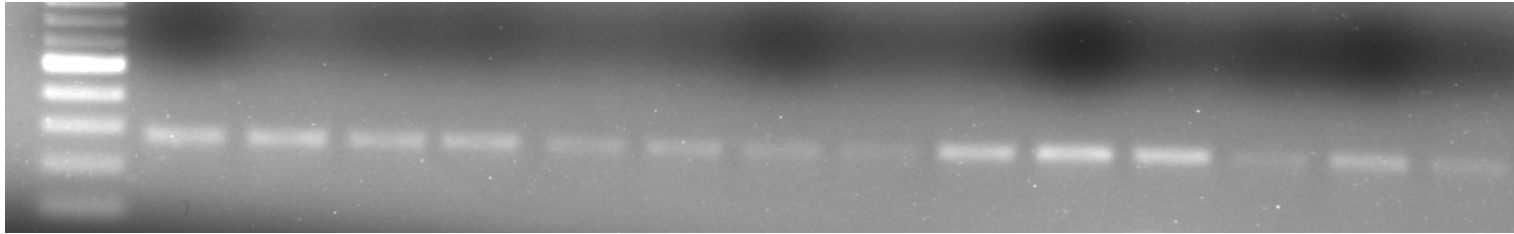
C		IL6		IFN $\gamma$		TNF $\alpha$		MCP1		EGF		VEGF	
-	+	-	+	-	+	-	+	-	+	-	+	-	+



Raw Replicate data from MS-PCR experiment

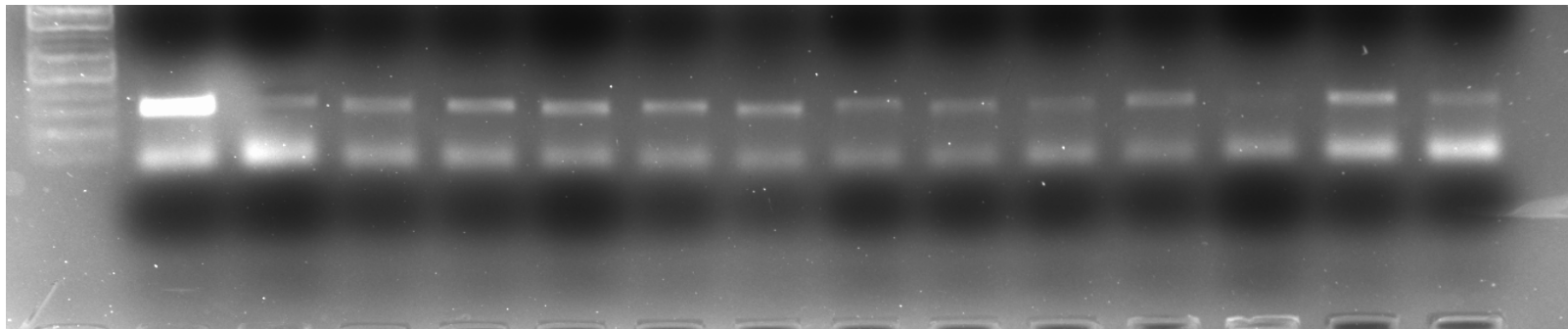
INPUT #3

C		IL6		IFN $\gamma$		TNF $\alpha$		MCP1		EGF		VEGF	
-	+	-	+	-	+	-	+	-	+	-	+	-	+

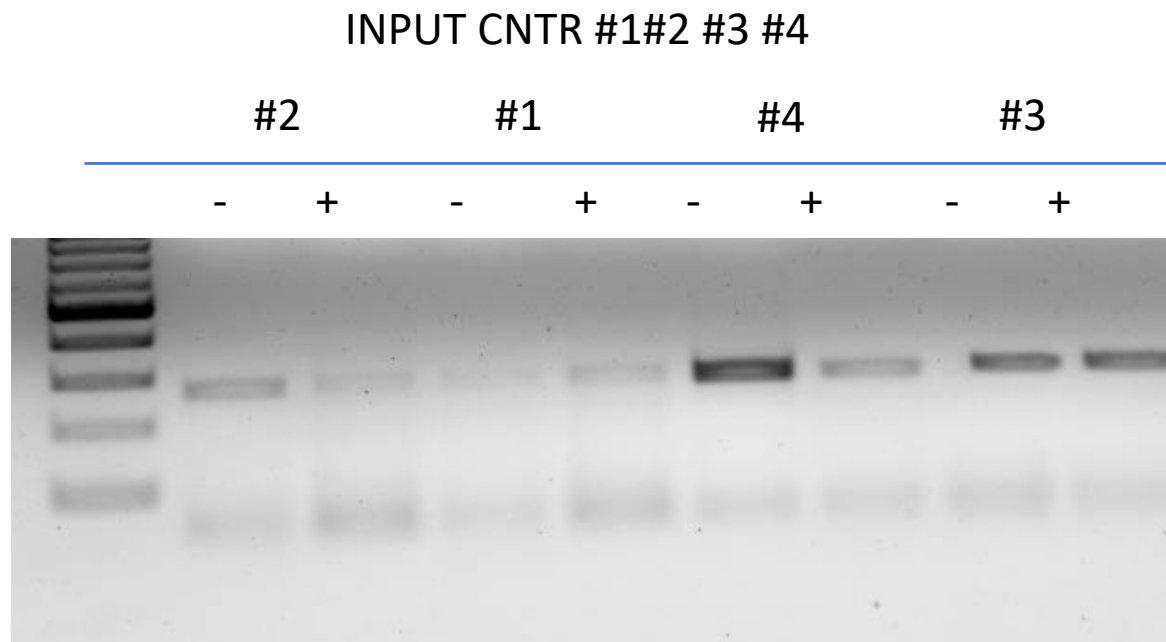


INPUT #4

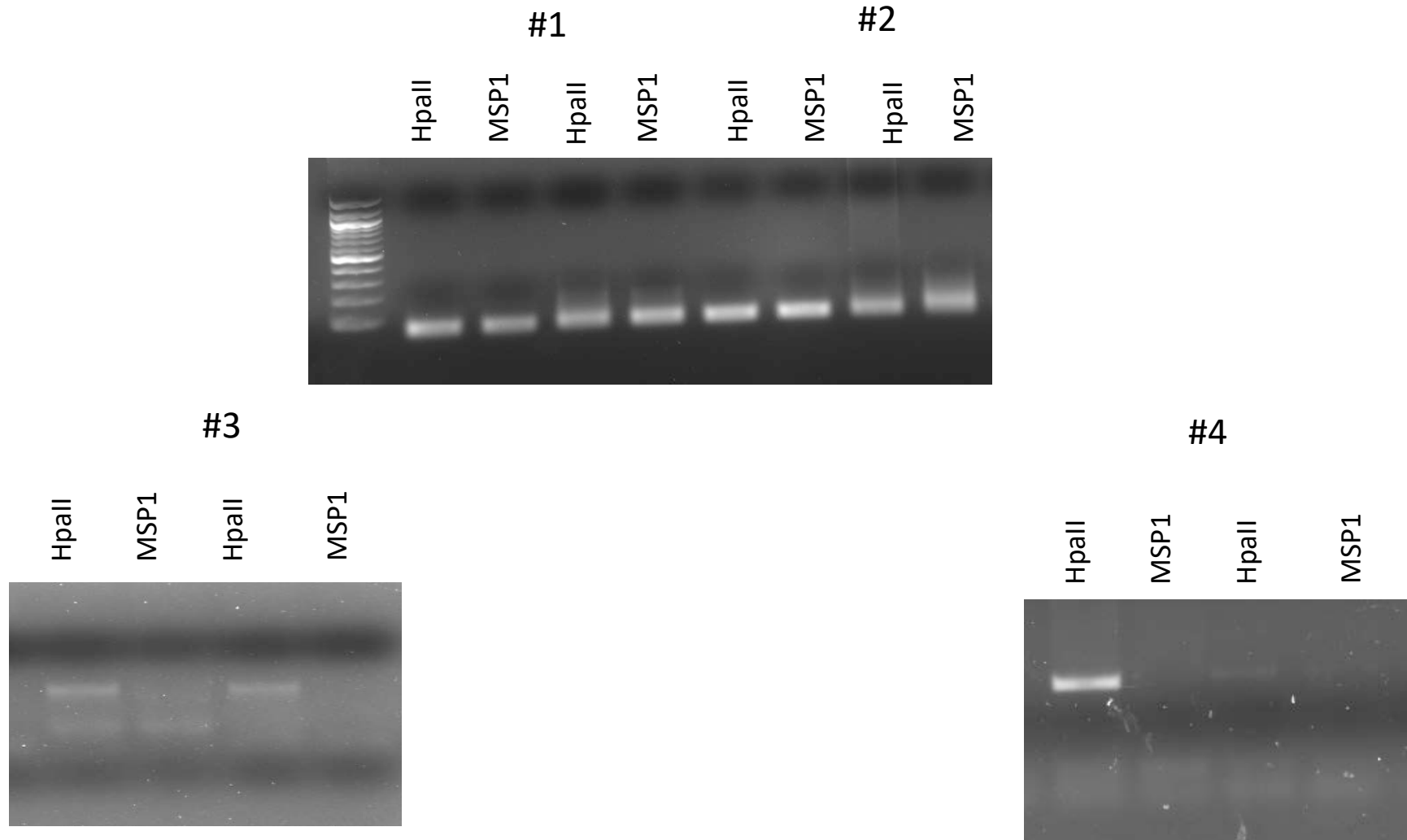
C		IL6		IFN $\gamma$		TNF $\alpha$		MCP1		EGF		VEGF	
-	+	-	+	-	+	-	+	-	+	-	+	-	+



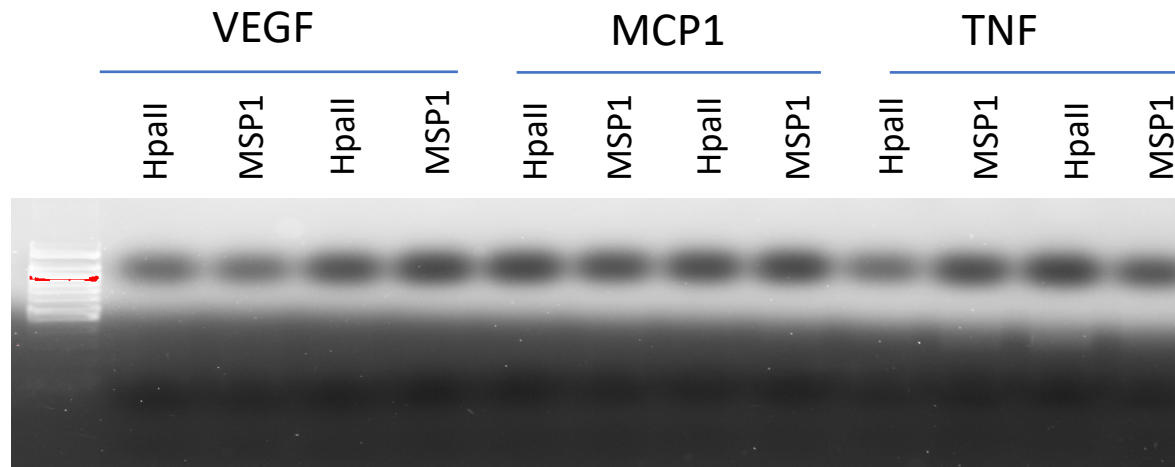
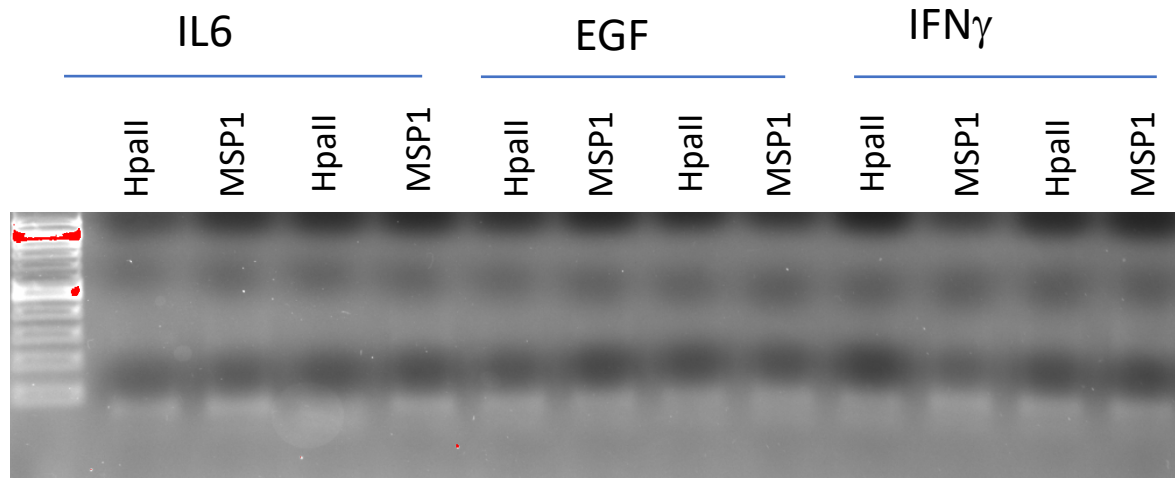
Raw Replicate data from MS-PCR experiment



Raw Replicate data from MS-PCR experiment

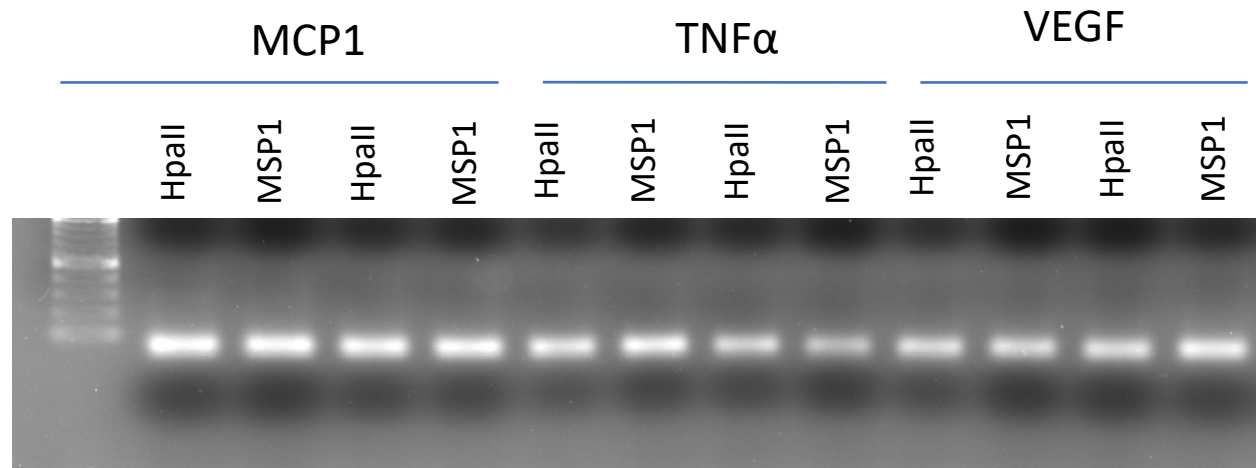
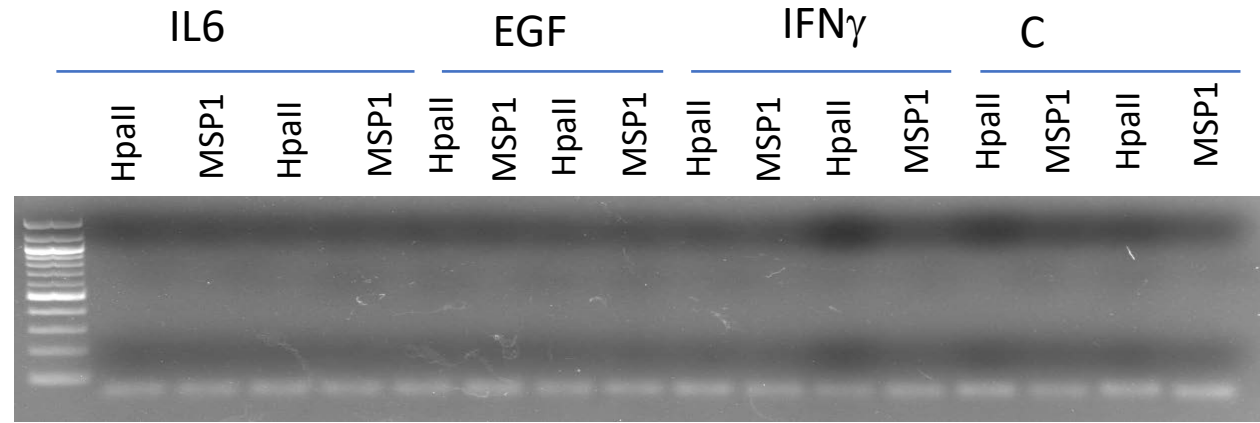


Raw Replicate data from MS-PCR experiment: amplicon #1

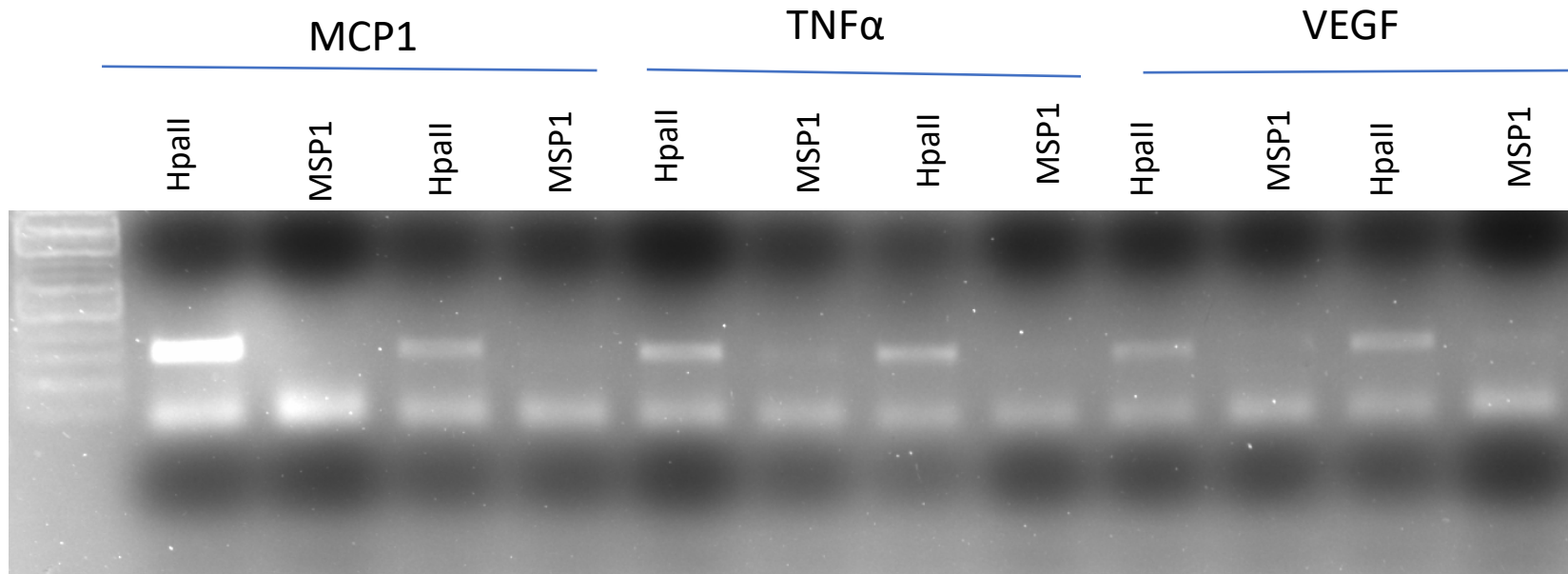
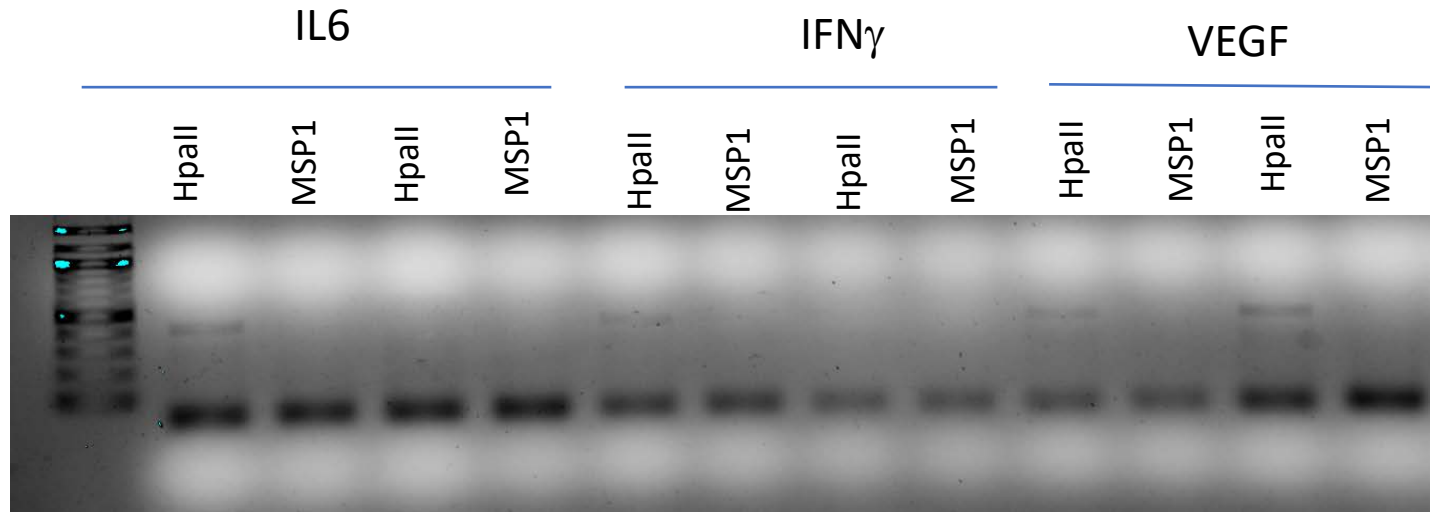




Raw Replicate data from MS-PCR experiment: amplicon #2



Raw Replicate data from MS-PCR experiment: amplicon #3



Raw Replicate data from MS-PCR experiment: amplicon #4

