

Placental Malaria Infection is Associated with Downregulation of STAT-6 and ANG-1 in Decidual Macrophages

Fred Owino^{1, 2,3}, Caroline Kijogi^{3,4,5#*}, Omu Anzala^{6,7}, Edwin Walong⁸, Obiero Jael⁹, Steven G. Nyanjom¹, Agola Lelo Eric², Bernard N. Kanoi^{3,4}, Jesse Gitaka^{3,4}

¹Biochemistry Department, Jomo Kenyatta University of Agriculture and Technology, Kenya.

²Kenya Medical Research Institute, Nairobi, Kenya.

³Centre for Malaria Elimination, Institute of Tropical Medicine, Mount Kenya University, Thika, Kenya.

⁴Centre for Research in Infectious Diseases, Mount Kenya University, Thika, Kenya

⁵Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai, Japan

⁶KAVI Institute of Clinical Research, Nairobi, Kenya.

⁷University of Nairobi, Nairobi, Kenya,

⁸School of Medicine, Maseno University, Kisumu, Kenya

⁹Kenya Institute of Primate Research, Nairobi, Kenya

Shared first authorship, Caroline Kijogi

* Corresponding author, Caroline Kijogi

Supplementary Figures and Tables

Supplementary Table 1: qPCR Primer list

Oligo Name	Oligo Seq	Modification
<i>ANG-1_For</i>	AAA TGG AAG GAA AAC ACA AGG AA	AAA TGG AAG GAA AAC ACA AGG AA
<i>ANG-1_Rev</i>	ATC TGC ACA GTC TCT AAA TGG T	ATC TGC ACA GTC TCT AAA TGG T
<i>ANG-2_For</i>	GAC GGC TGT GAT GAT AGA AAT AGG	GAC GGC TGT GAT GAT AGA AAT AGG
<i>ANG-2_Rev</i>	GAC TGT AGT TGG ATG ATG TGC TTG	GAC TGT AGT TGG ATG ATG TGC TTG
<i>c-Maf_For</i>	TGC ACT TCG ACG ACC GCT TCT C	TGC ACT TCG ACG ACC GCT TCT C
<i>c-Maf_Rev</i>	TGT ACA GCT CTC ACA CAA ATT TCA TTT TGT	TGT ACA GCT CTC ACA CAA ATT TCA TTT TGT
<i>GAPDH_For</i>	ACC ACA GTC CAT GCC ATC AC	ACC ACA GTC CAT GCC ATC AC
<i>GAPDH_Rev</i>	TCC ACC ACC CTG TTG CTG TA	TCC ACC ACC CTG TTG CTG TA
<i>IRF5_For</i>	GCC TGG GCC AAG GAG AC	GCC TGG GCC AAG GAG AC
<i>IRF5_Rev</i>	CCA CTT GGC CGG ATC G	CCA CTT GGC CGG ATC G
<i>STAT-1_For</i>	CCA TCC TTT GGT ACA ACA TGC	CCA TCC TTT GGT ACA ACA TGC
<i>STAT-1_Rev</i>	TGC ACA TGG TGG AGT CAG G	TGC ACA TGG TGG AGT CAG G
<i>STAT-6_For</i>	AGA GGG GTT GCC GAG GTG A	AGA GGG GTT GCC GAG GTG A
<i>STAT-6_Rev</i>	TGT CCA CCA GGC TTT CAC AC	TGT CCA CCA GGC TTT CAC AC
<i>VEGF_For</i>	CGA AGT GGT GAA GTT CAT G	CGA AGT GGT GAA GTT CAT G
<i>VEGF_Rev</i>	TTC TGT ATC AGT CTT TCC TGG TGA G	TTC TGT ATC AGT CTT TCC TGG TGA G

Supplementary Table 1; The qPCR primer sequences used for the detection of different genes including STAT-1, IRF-5, STA-6, and cMAF for transcription factors while ANG-1, ANG-2, and VEGF for angiogenic factors

Supplementary Table 2

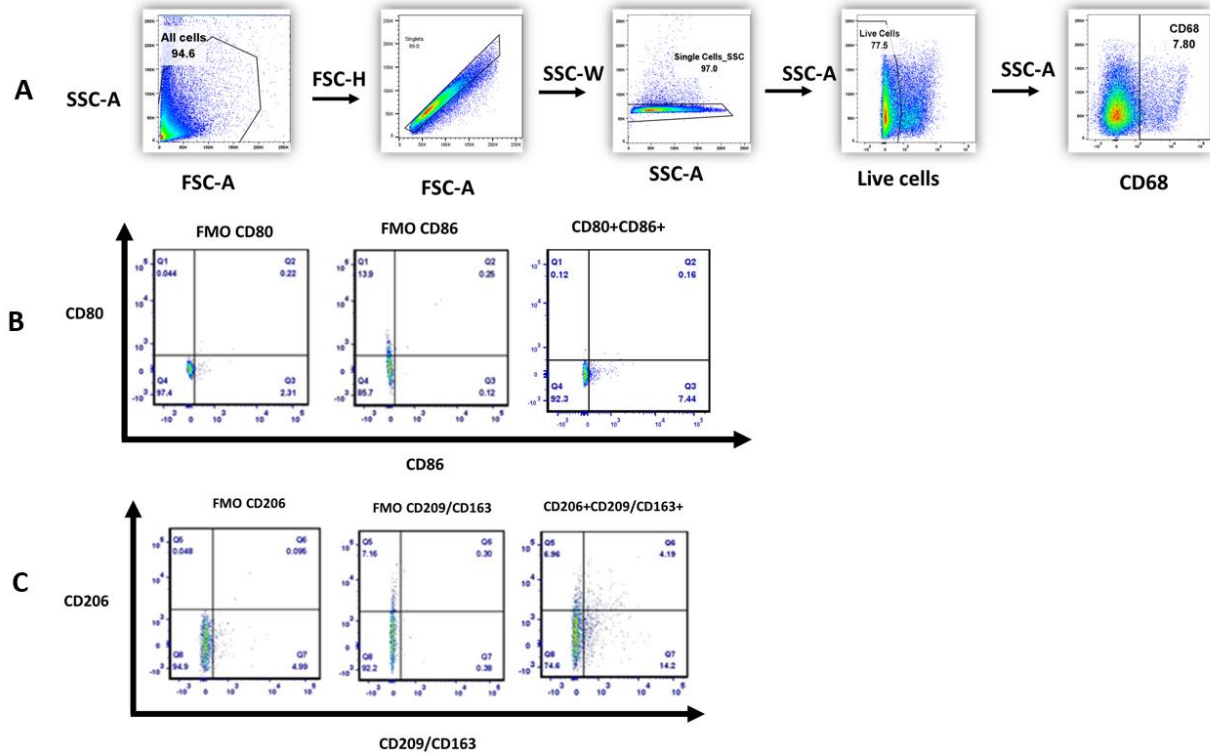
		Histology		
		+	-	TOTAL
PCR	+	12	11	23
	-	11	17	28
	TOTAL	23	28	<u>51</u>

Key

1. Sensitivity = 52.17%
2. Specificity = 60.71%

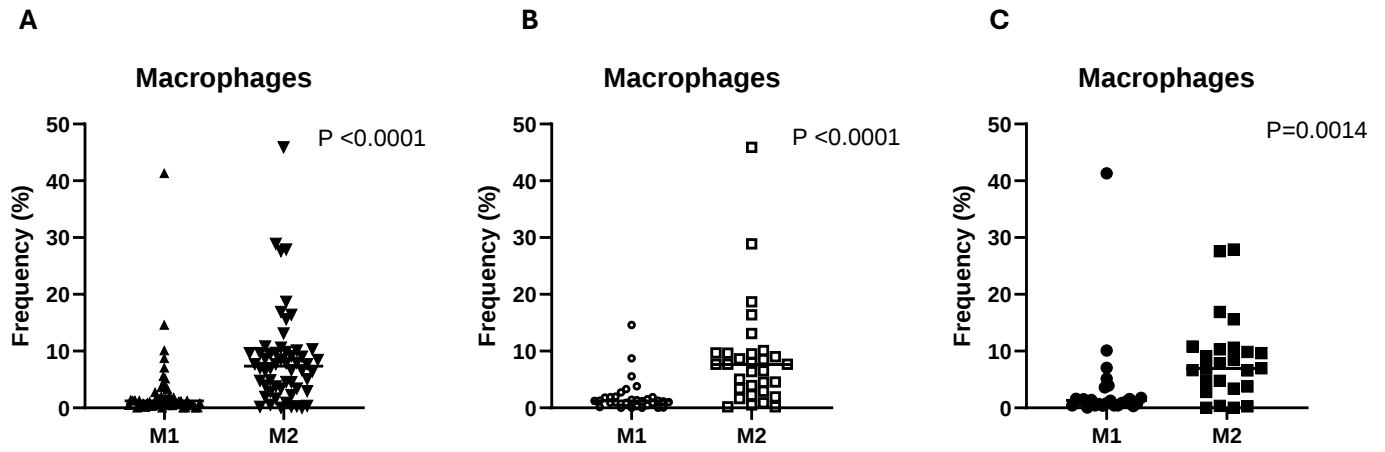
Supplementary Table 2; PCR as an alternative placental malaria diagnostic method against the gold standard, histology, showed 52.17% sensitivity and as specificity of 60.71%.

Supplementary Figure 1



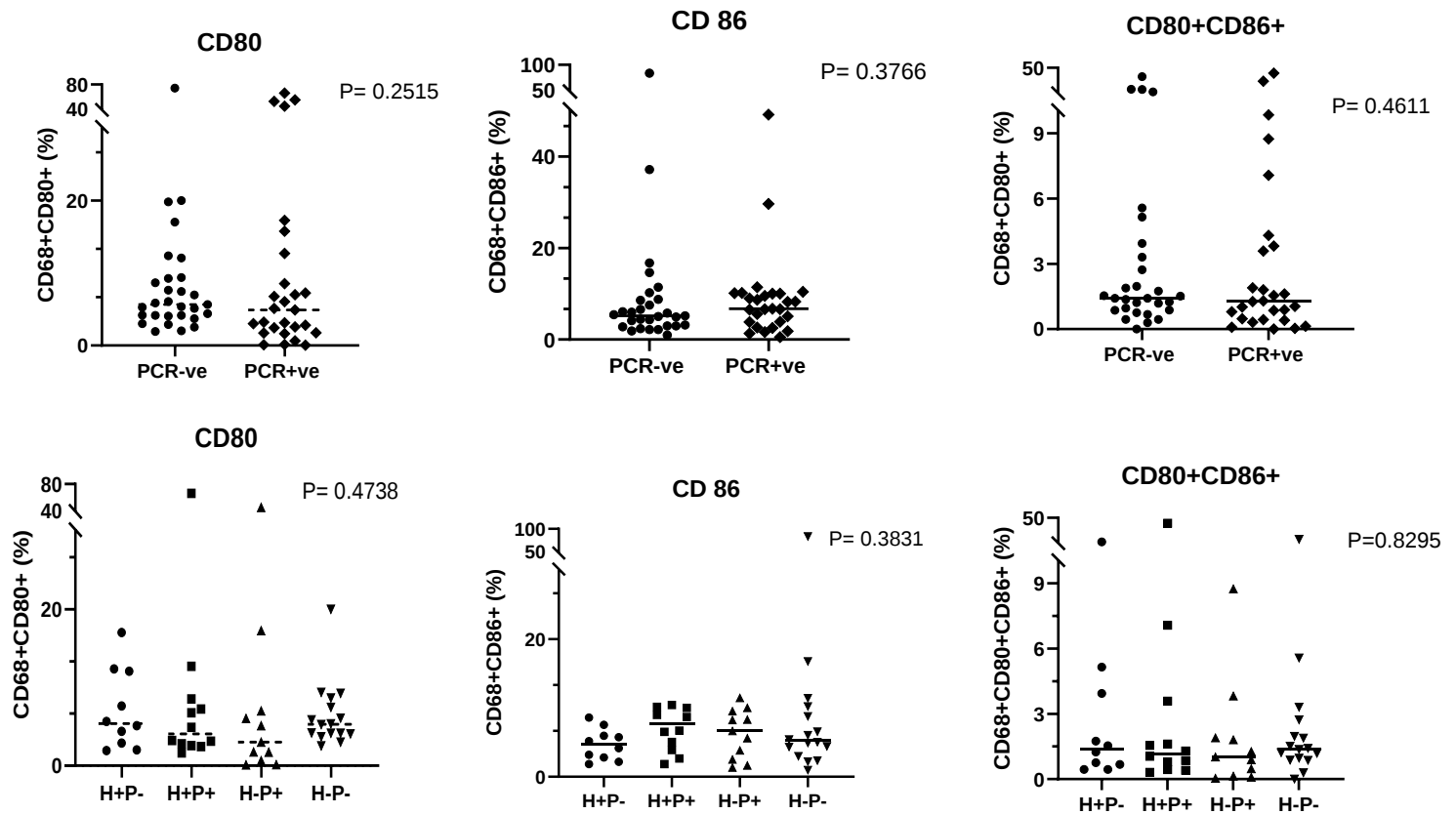
Supplementary Figure 1; Representative dot plots depicting the gating strategy of the different markers used to define M1 and M2 macrophage subsets and their corresponding FMOS in flow cytometry analysis. Gating for the macrophage pan marker CD68, is illustrated in **A** while in **B** the FMOS for CD80, CD86, and full staining M1(CD80+CD86+) is displayed. **C** shows the FMOS for CD206/CD163, and CD209 representing the M2 subset.

Supplementary Figure 2



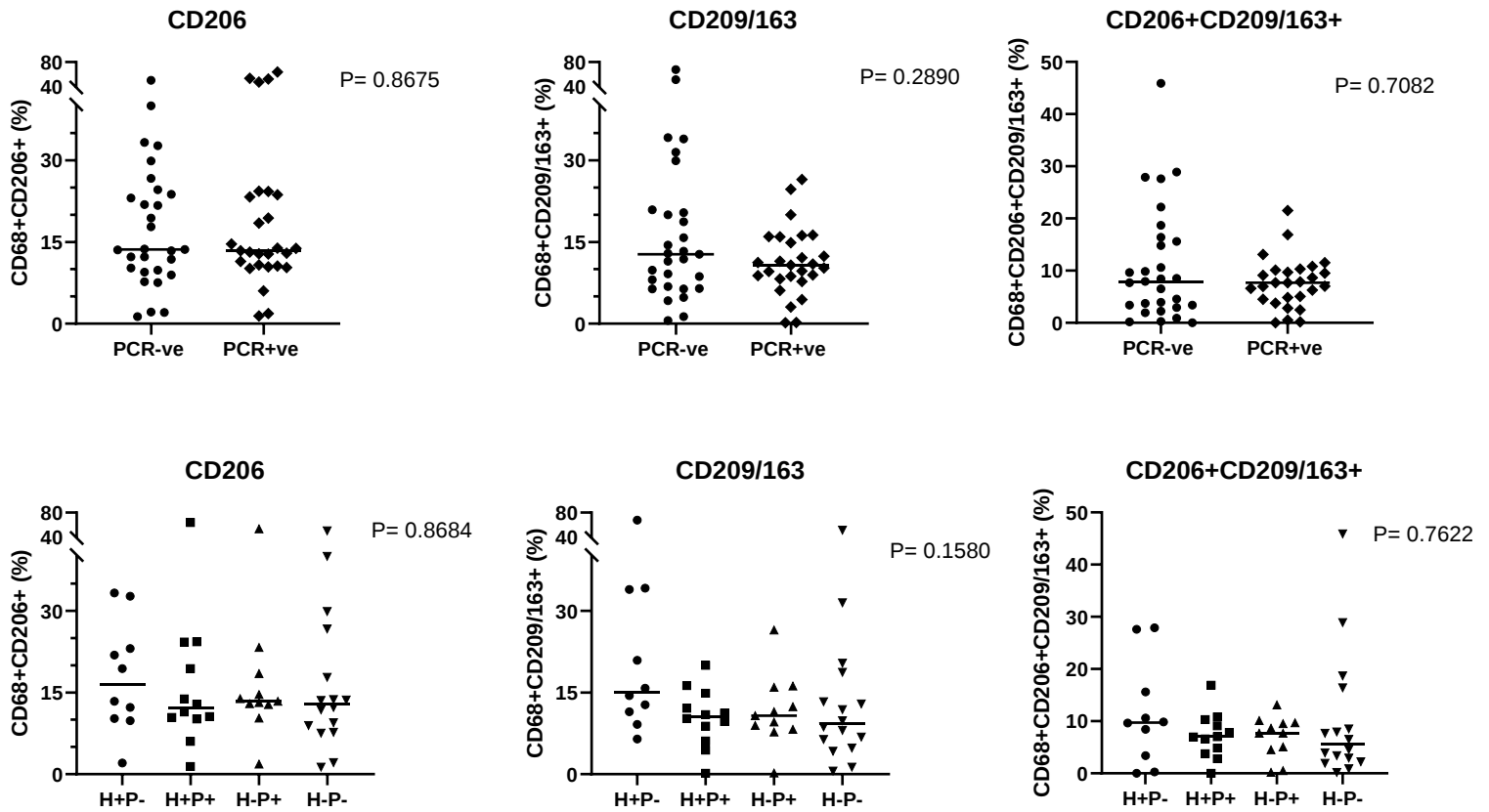
Supplementary Figure 2; Comparison of the distribution of M1 and M2 like macrophages from the placentas of all women (**A**) ($P=0.0001$), uninfected women only (**B**) ($P=0.0001$) and the infected women only (**C**) ($P=0.0014$) by Mann-Whitney U test.

Supplementary Figure 3



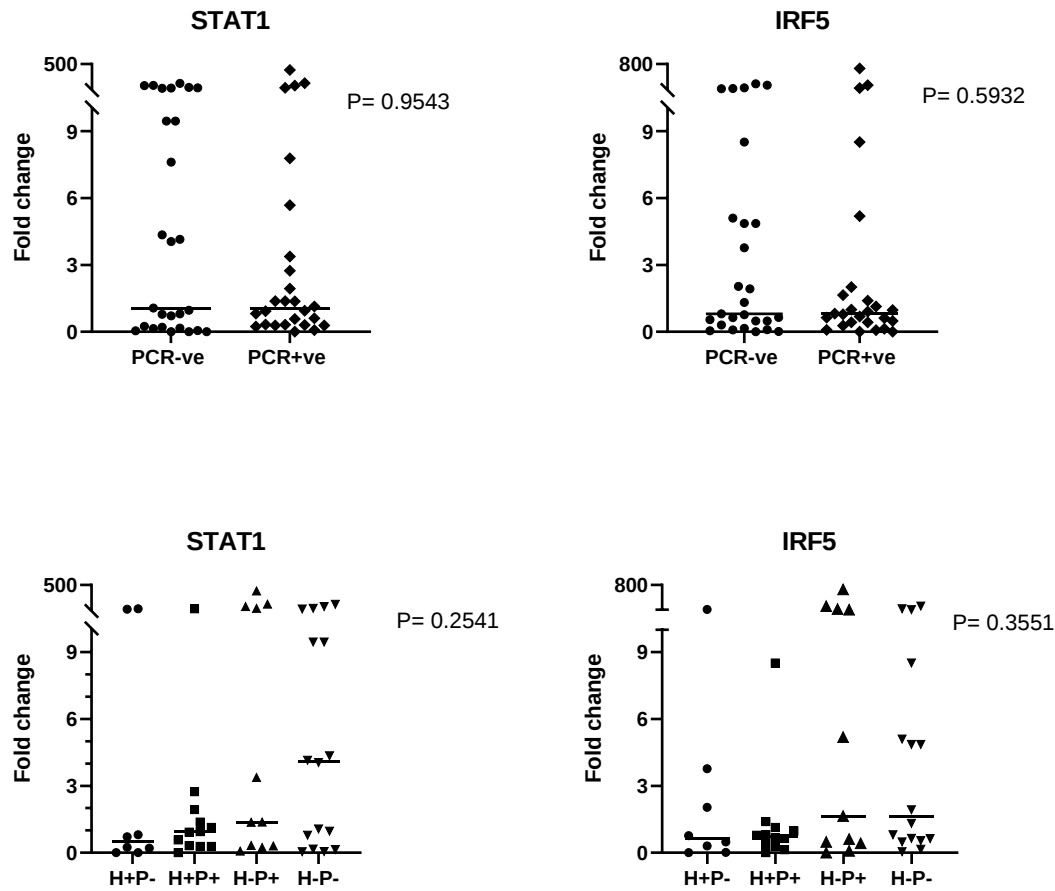
Supplementary Figure 3; Upper panel indicates analysis of M1 surface marker expression when samples were classified using PCR stratification. Lower panel: when stratification was by both histology and PCR. Mann-Whitney U test was used for pairwise comparisons ANOVA and Kruskal- Walis were used for multiple comparisons.

Supplementary Figure 4



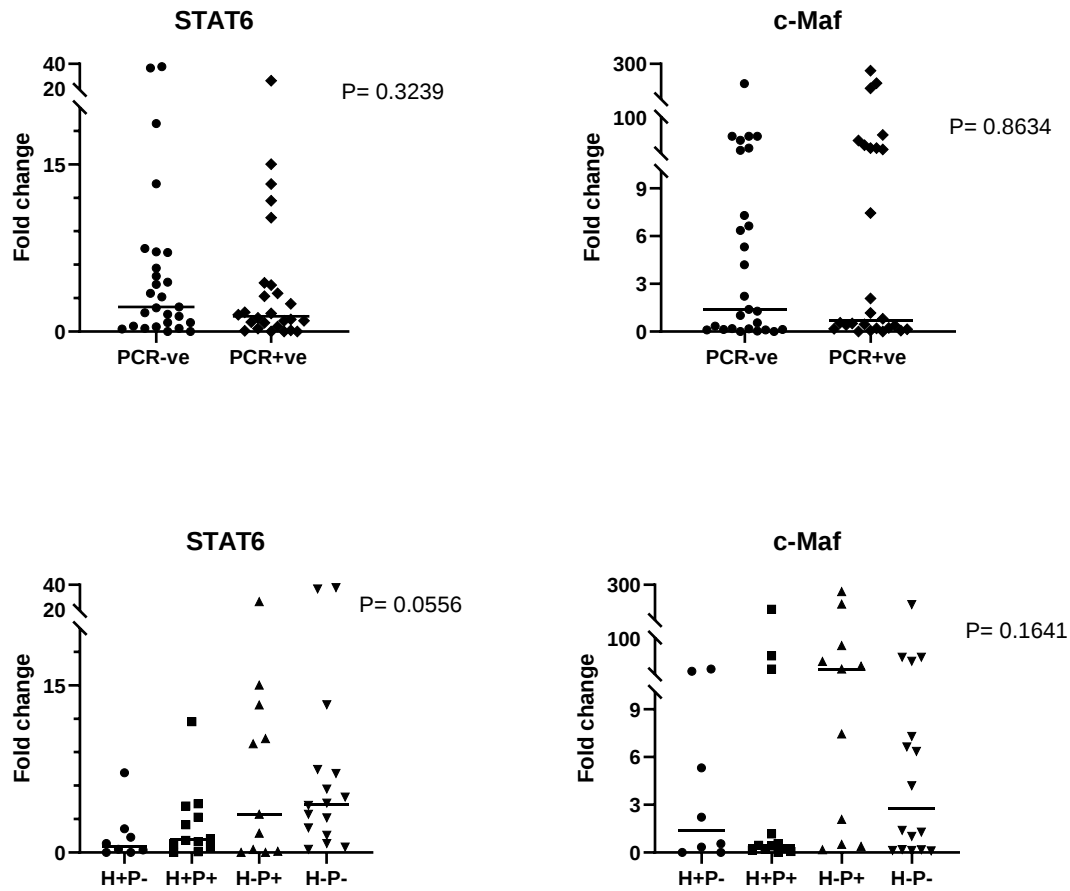
Supplementary Figure 4: Upper panel indicates analysis of M2 surface marker expression when samples were classified using PCR stratification. Lower panel: when stratification was by both histology and PCR. Mann-Whitney U test was used for pairwise comparisons ANOVA and Kruskal- Wallis were used for multiple comparisons.

Supplementary Figure 5



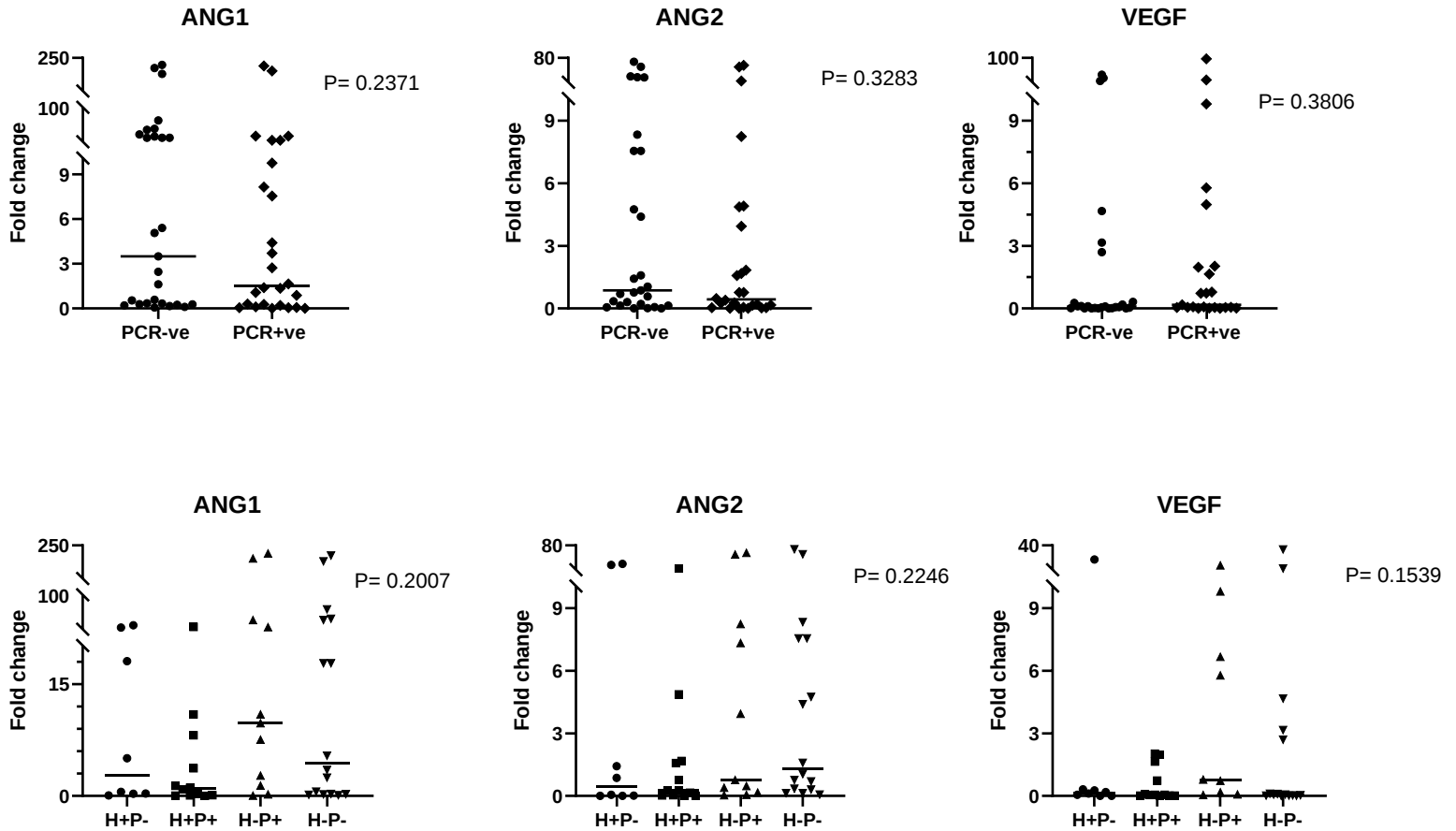
Supplementary Figure 5; Gene expression analysis of M1 transcription factors (*STAT-1* and *IRF-5*) as stratified by PCR (upper panel) and when stratification was by both histology and PCR (lower panel). Mann-Whitney U test was used for pairwise comparisons ANOVA and Kruskal- Wallis were used for multiple comparisons.

Supplementary Figure 6



Supplementary Figure 6; Gene expression analysis of M2 transcription factors (*STAT-6* and *c-Maf*) as stratified by PCR (upper panel) and when stratification was by both histology and PCR (lower panel). Mann-Whitney U test was used for pairwise comparisons ANOVA and Kruskal- Walis were used for multiple comparisons.

Supplementary Figure 7



Supplementary Figure 7; Gene expression analysis of Angiogenic factors (*ANG-1*, *ANG-2* and *VEGF*) as stratified by PCR (upper panel) and when stratification was by both histology and PCR (lower panel). Mann-Whitney U test was used for pairwise comparisons ANOVA and Kruskal- Walis were used for multiple comparisons.