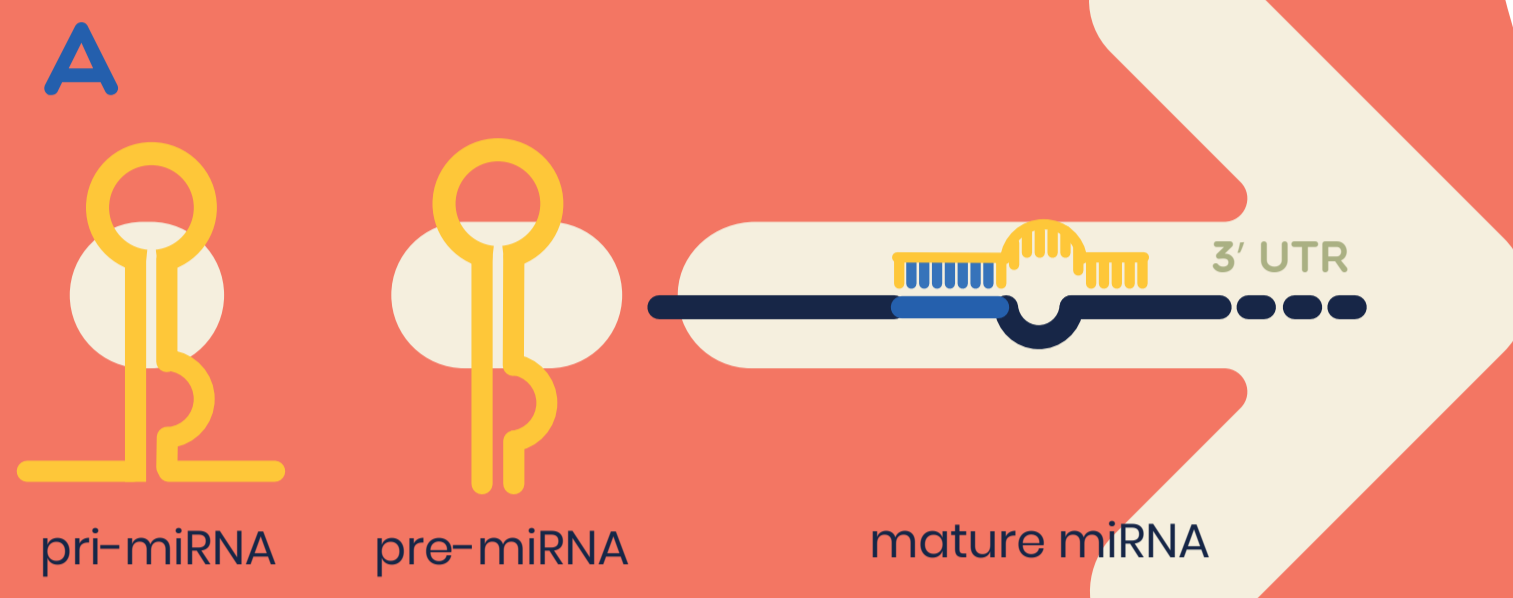


# MICRORNAS COOPERATIVELY CONTRIBUTE TO HUMAN MACROPHAGE SUSCEPTIBILITY TO *L. AMAZONENSIS* INFECTION

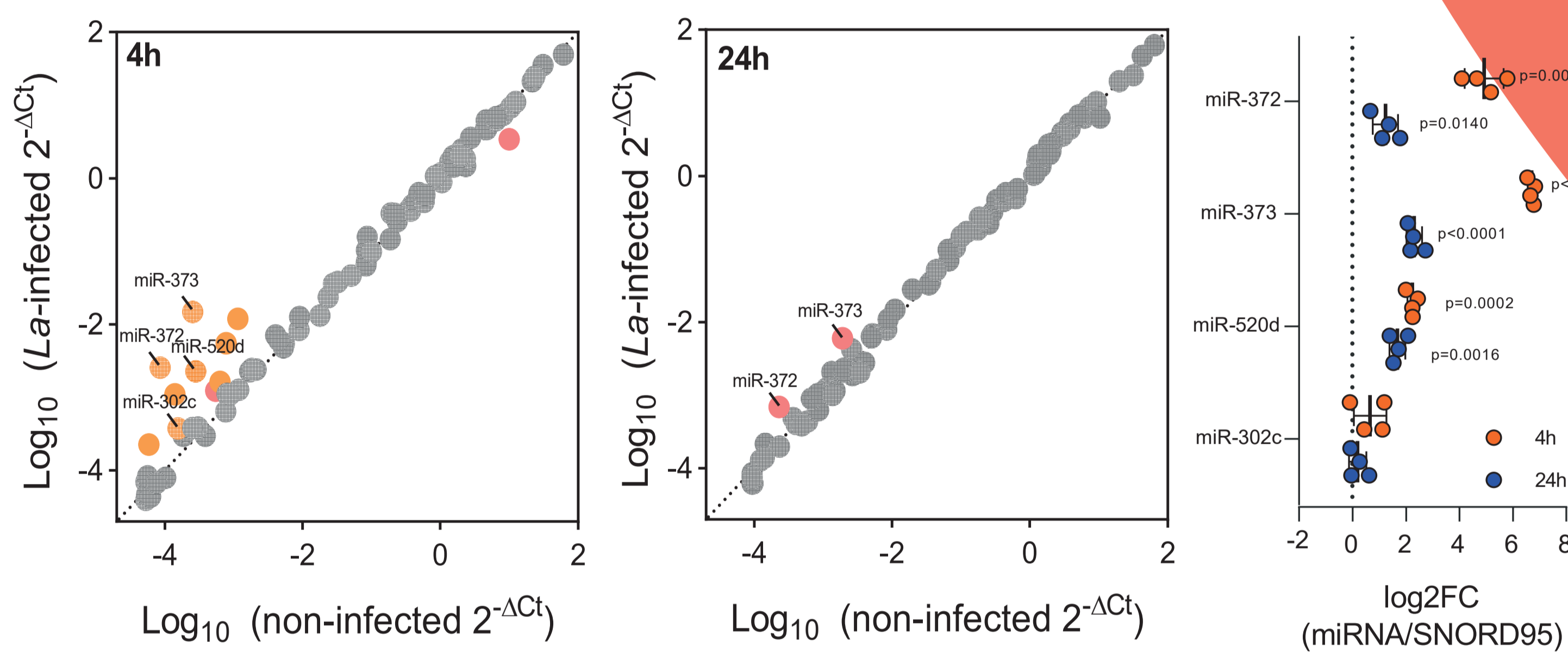
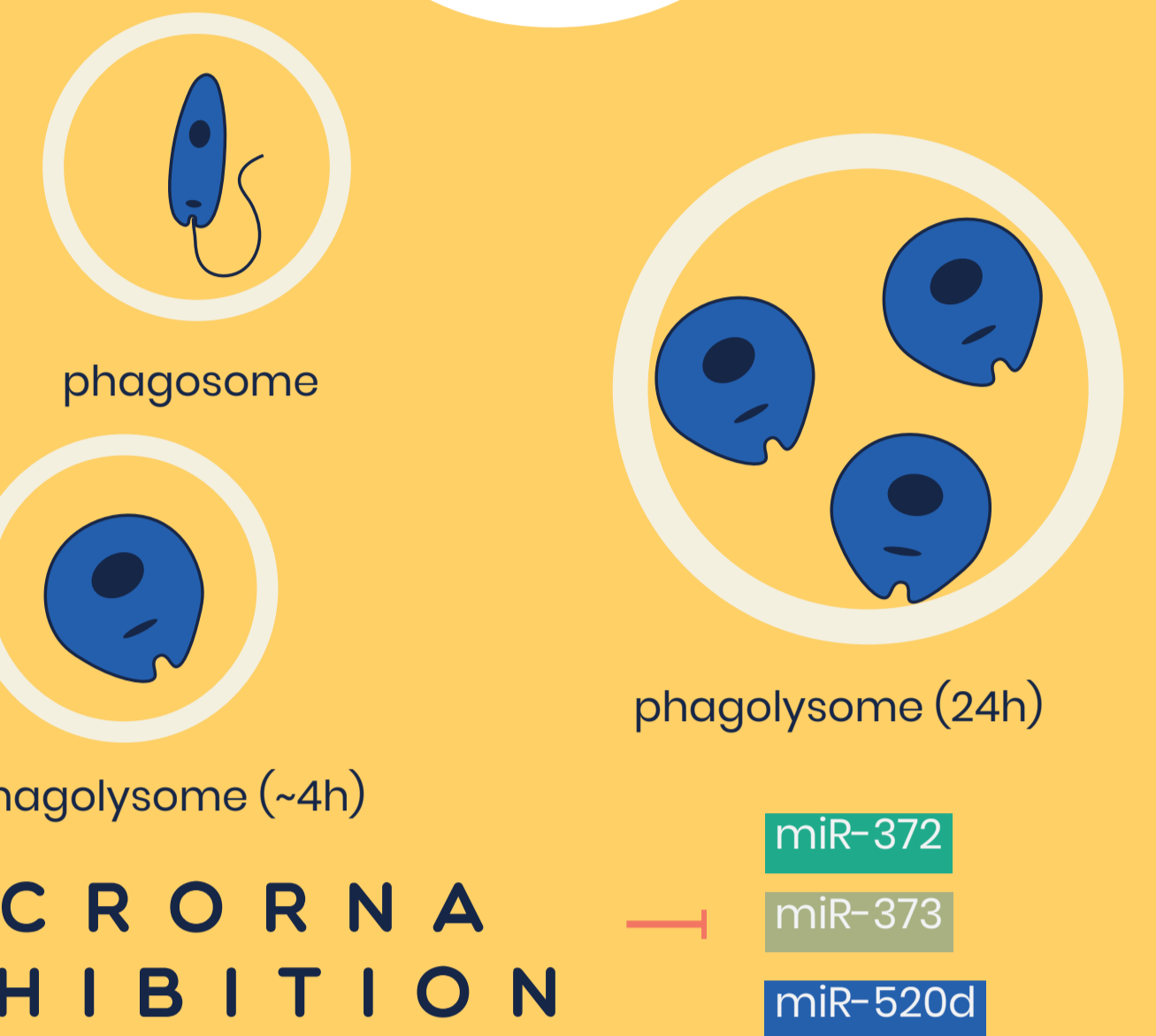
FERNANDES, JCR<sup>1,2</sup>; MUXEL, SM<sup>1</sup>; FLOETER-WINTER, LM<sup>1</sup>

UNIVERSIDADE DE SÃO PAULO



**Graphical abstract.** (A) miRNAs are first transcribed as pri-miRNA and processed as pre-miRNA, the mature miRNA recognizes the intended target on its 3' UTR region (B) human miRNA family miR-372/372/373/302b/520d are upregulated upon *L. amazonensis* infection. Inhibition of miRNAs impairs infectivity.

mRNA degradation or storage in P bodies  
Translational repression  
Peptide blockage or degradation

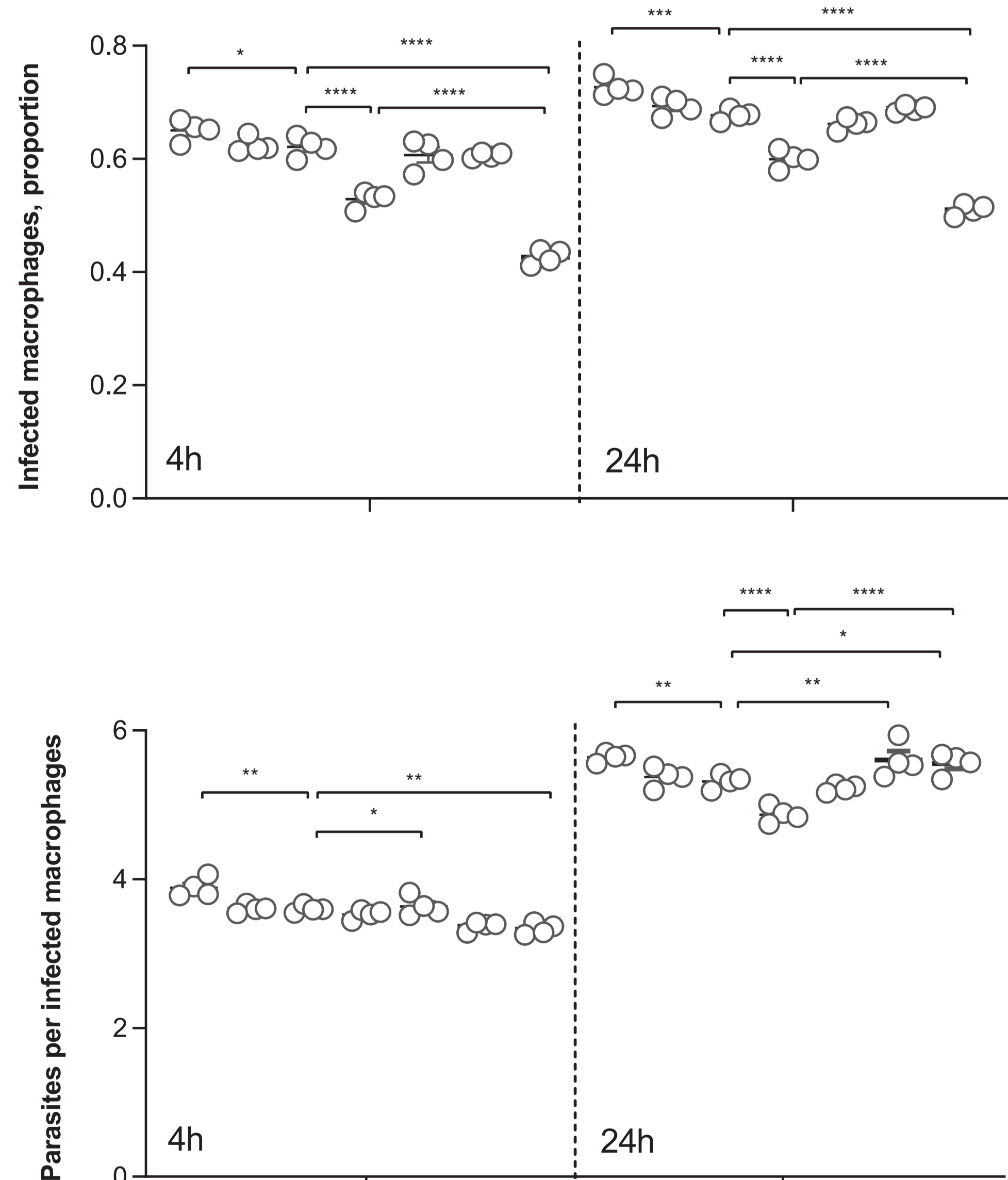


**Figure 1. miR-302/372/373/520 family is upregulated in macrophages infected with *Leishmania amazonensis*.**

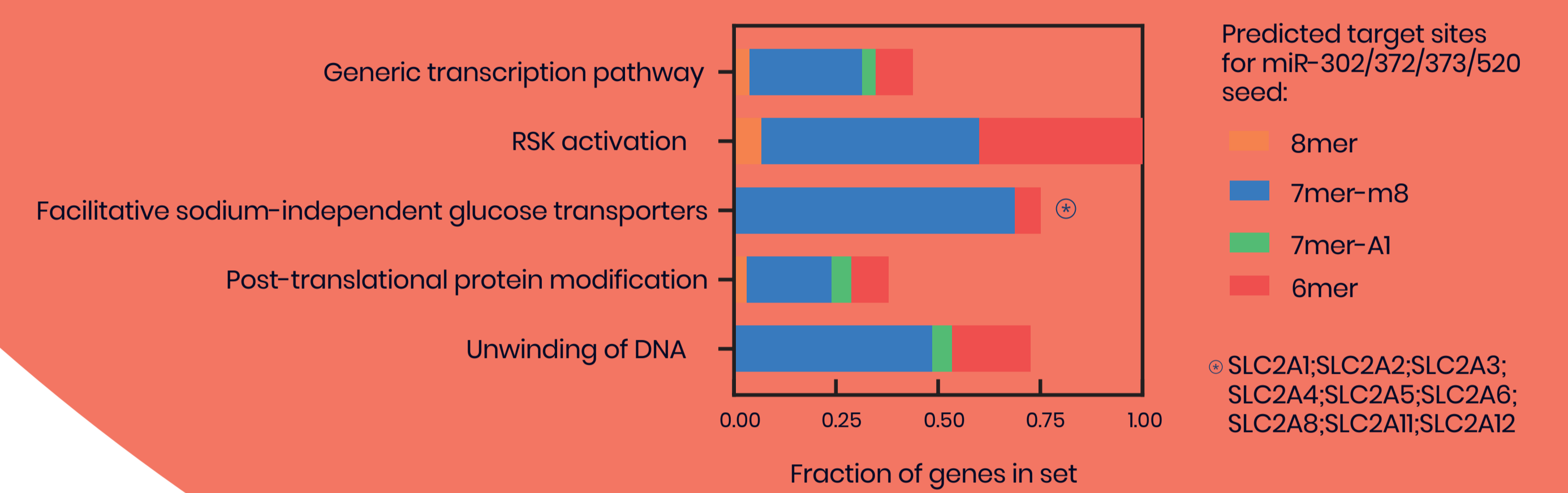
Each dot represents the expression of immune response-related microRNAs in non-infected and *L. amazonensis*-infected THP-1 macrophages after 4h or 24h. miRNAs sharing the seed sequence are named and were validated by RT-qPCR (bars graphs). Orange dots in the scatter plots are statistically significant (P adjusted for multiple comparisons < 0,05).

**Figure 2. Inhibition of the miR-302/372/373/520 family impairs *Leishmania amazonensis* infection.**

Graphs representative of *L. amazonensis* infection of THP-1 macrophages after 4h or 24h. Proportion of infected macrophages and number of internalized parasites per infected macrophage. miR-372, miR-373 or miR-520d were inhibited either alone or in combination. \*p<0,05, \*\* p<0,01, \*\*\* p<0,001, \*\*\*\* p<0,0001. P values were determined with ANOVA Dunnet's post-hoc test.



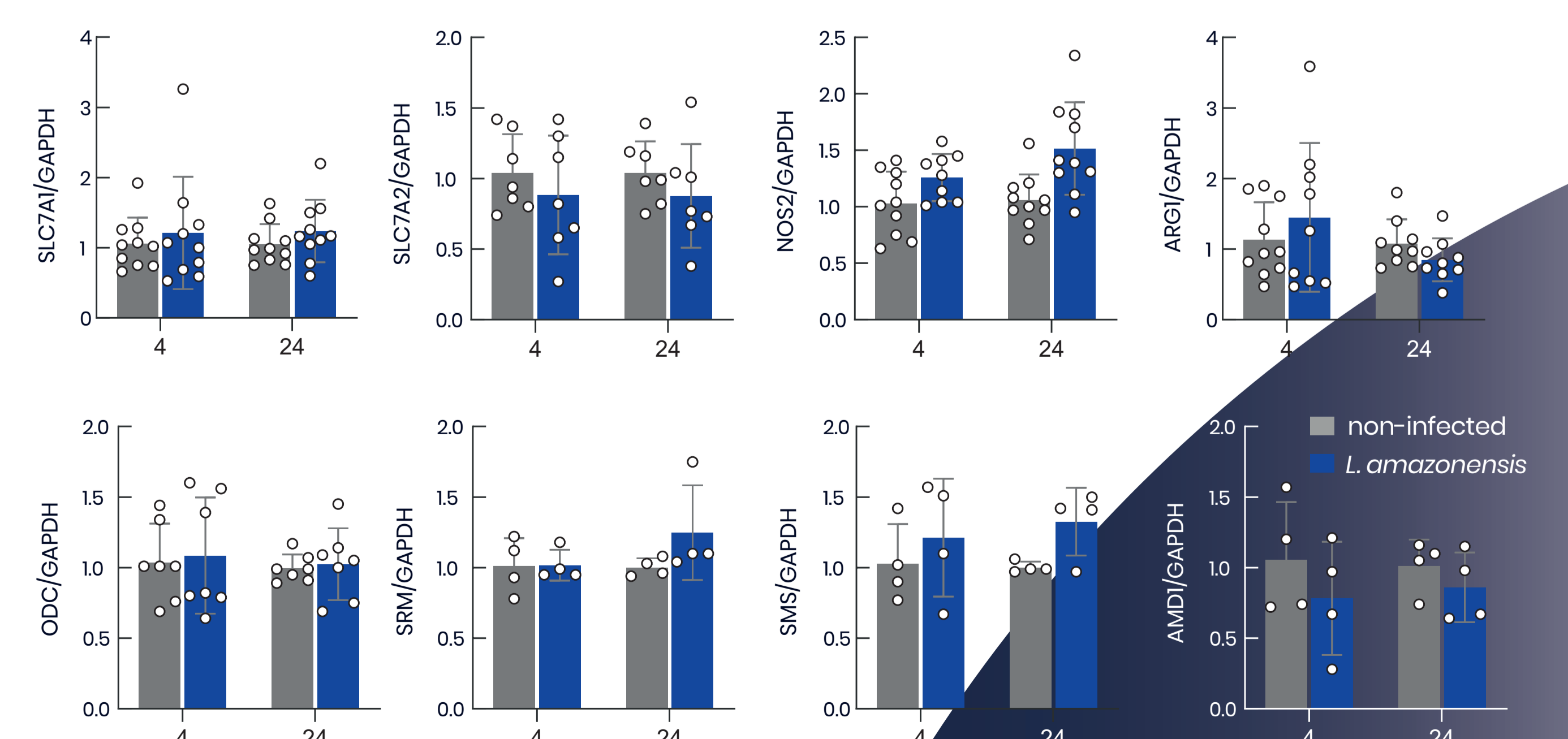
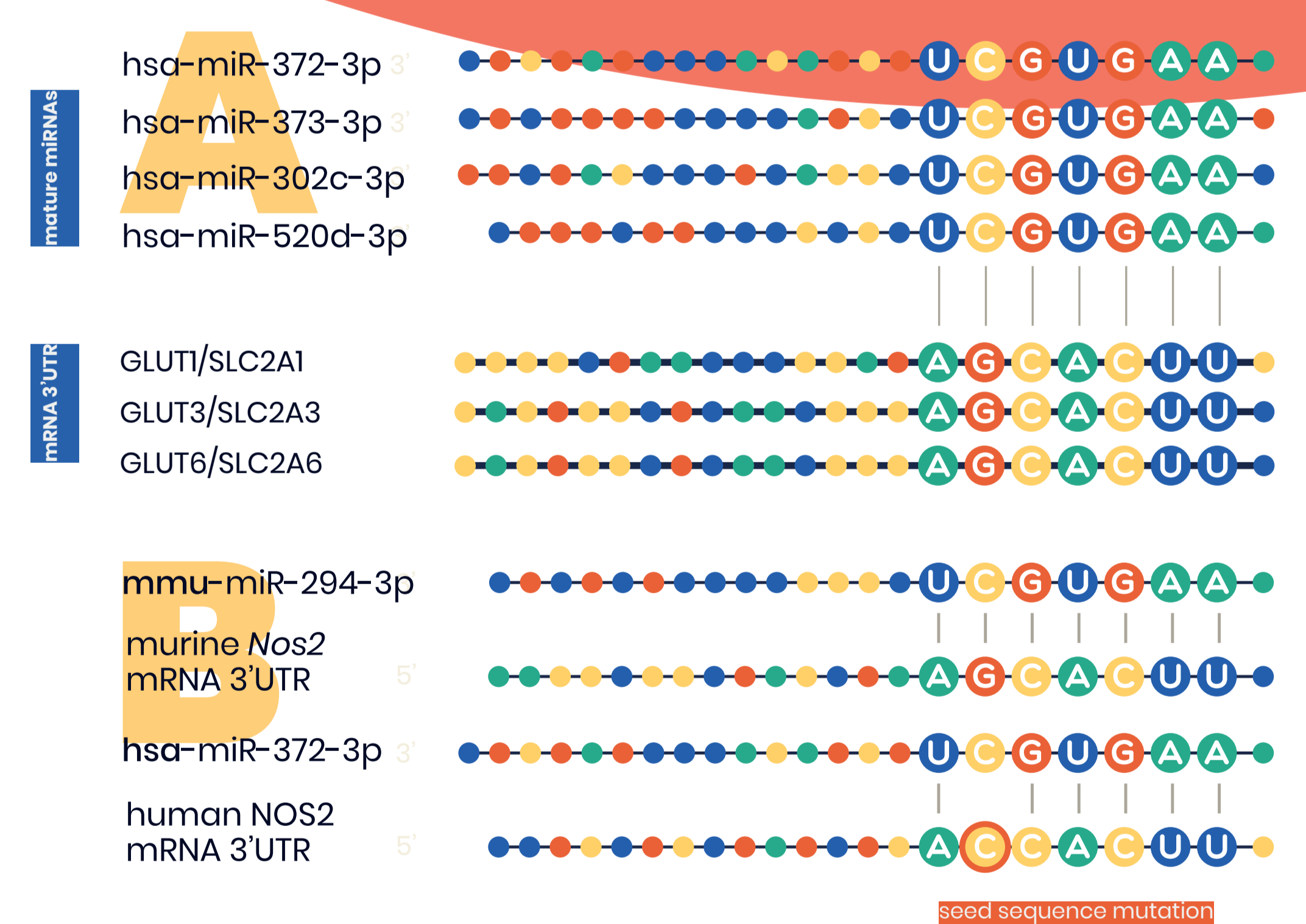
HiPerfect Transfection Reagent	-	+	+	+	+	+	-	+	+	+	+	+
NC scrambled inhibitor	-	-	-	-	-	-	-	-	-	-	-	-
miR-372 inhibitor	-	-	-	+	-	-	-	-	-	+	-	-
miR-373 inhibitor	-	-	-	-	+	-	-	-	-	-	+	-
miR-520d inhibitor	-	-	-	-	-	+	-	-	-	-	-	+



**Figure 3. Glucose transporters are enriched among miR-302/372/373/520 family predicted target genes with conserved seed-sites.** Gene list for miR-302/372/373/520 family targets retrieved from TargetScan Human database, submitted to Enrichr and the top 5 BioPlanet 2019 pathways combined scores are represented.

**Figure 4. miRNAs sharing the AAGUGCU seed sequence have glucose transporters as putative targets, but not NOS2 human mRNA.**

A) putative targets related to glucose uptake: GLUT1/SLC2A1, GLUT3/SLC2A3, and GLUT6/SLC2A6, B) the validated target site for the murine homologous miR-294 is not conserved in human NOS2 mRNA.



**Figure 5. Human THP-1-derived macrophages infected with *L. amazonensis* had no statistically significant changes in expression of mRNAs related to polyamine synthesis and nitric oxide production.**

SUPPORTED BY



GRANT: 2017/21906-9