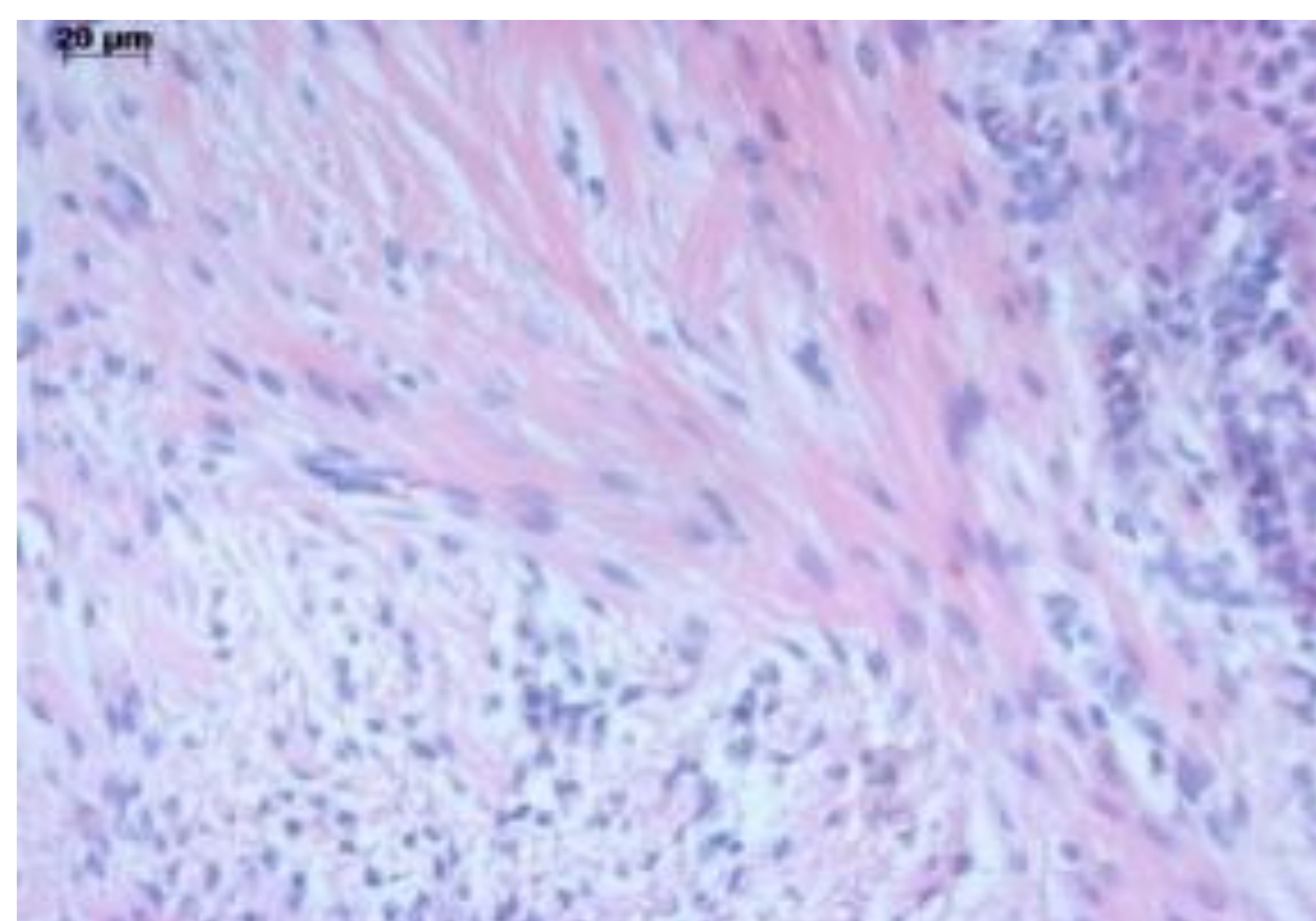
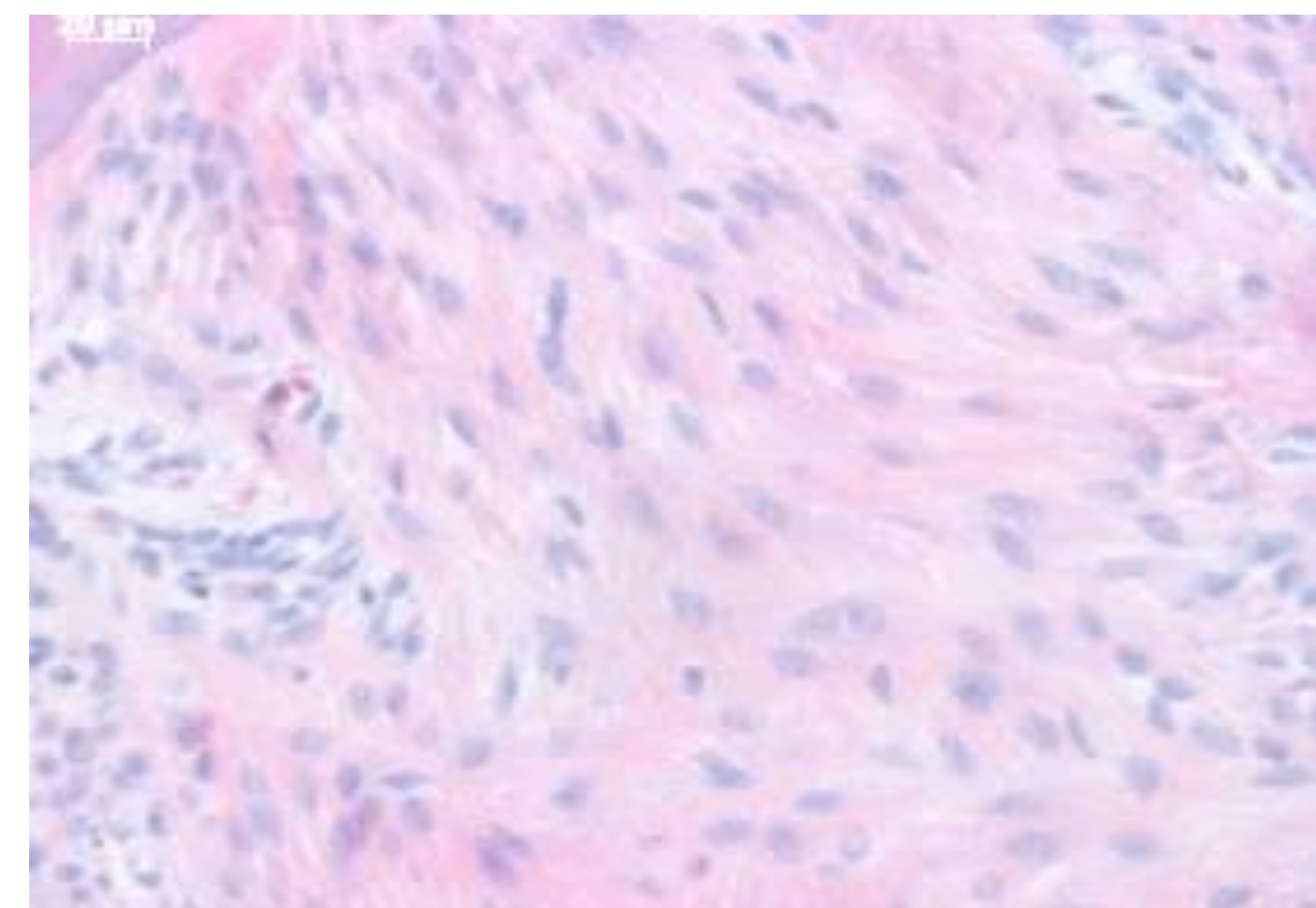


A

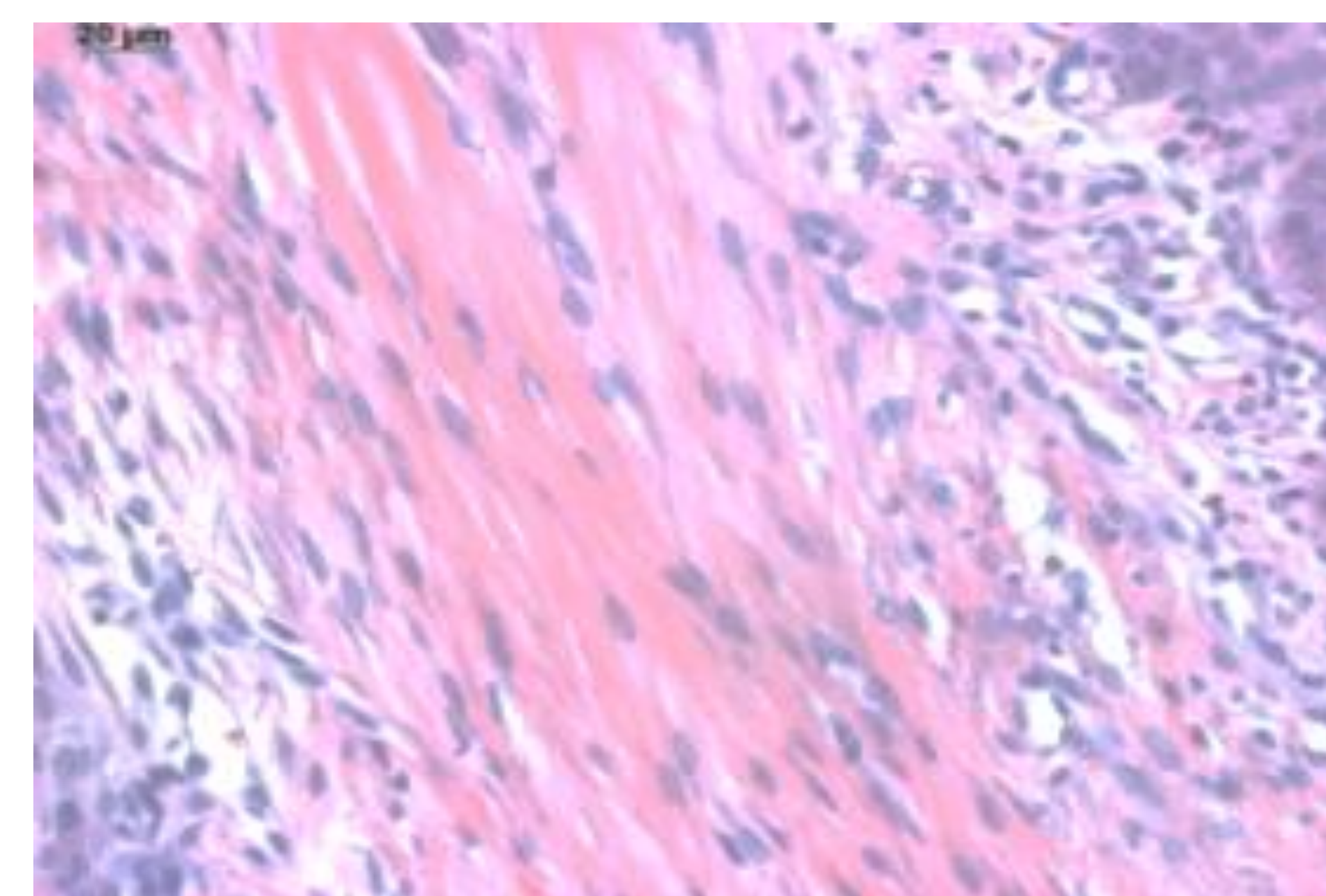


Ligature+Vehicle

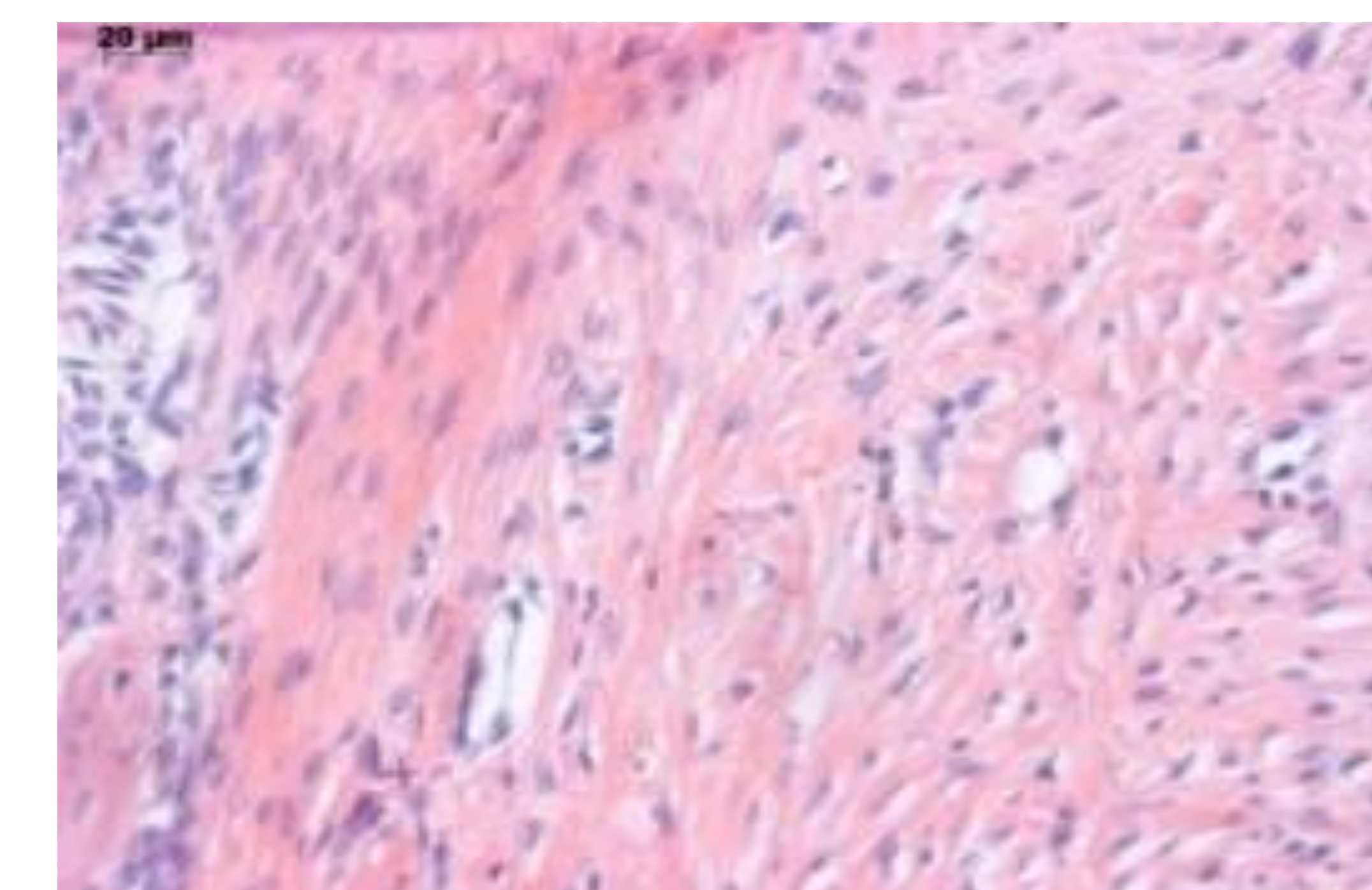


Ligature+RvE1 (0.1 µg/µl)

C

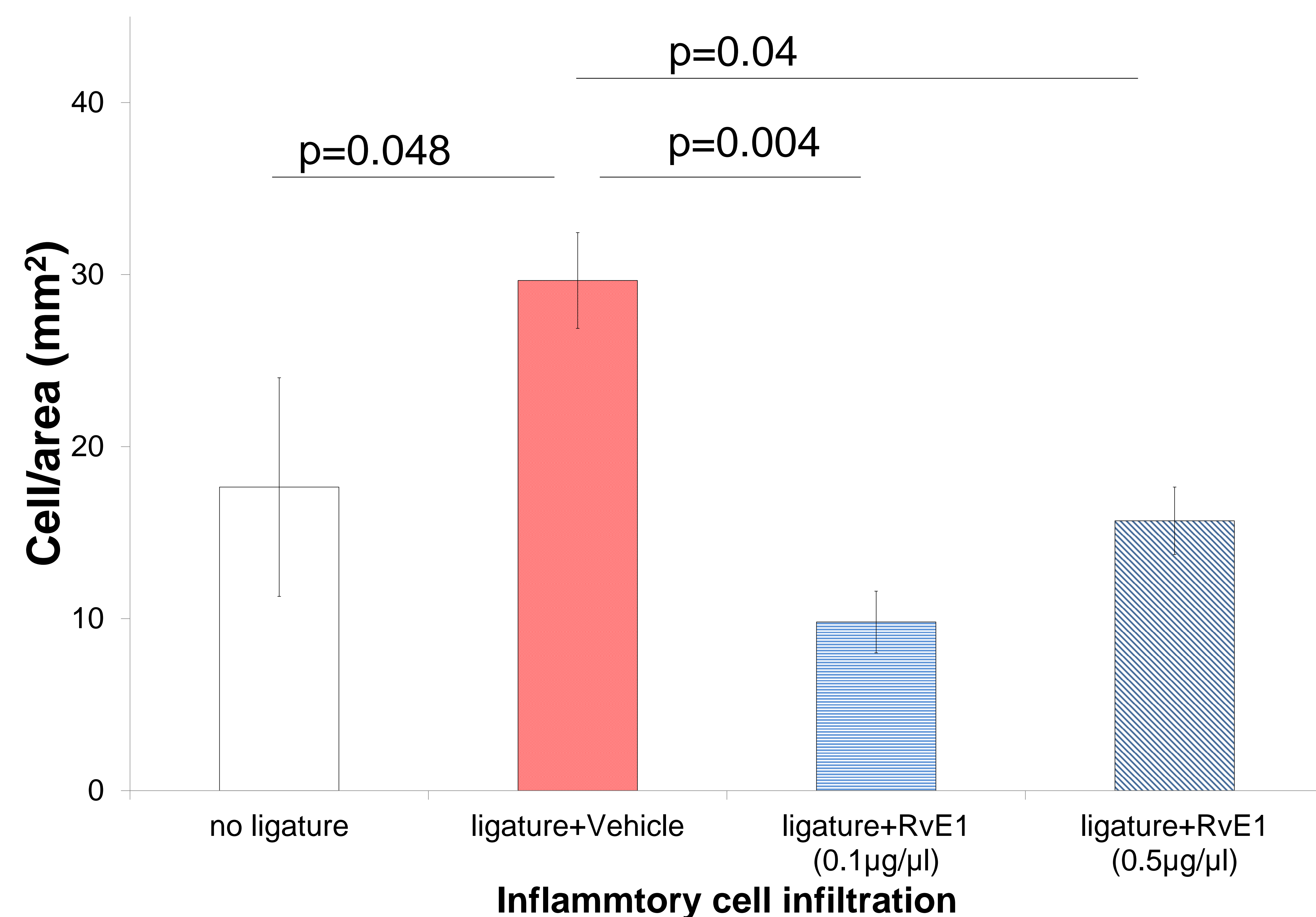


Ligature+Vehicle

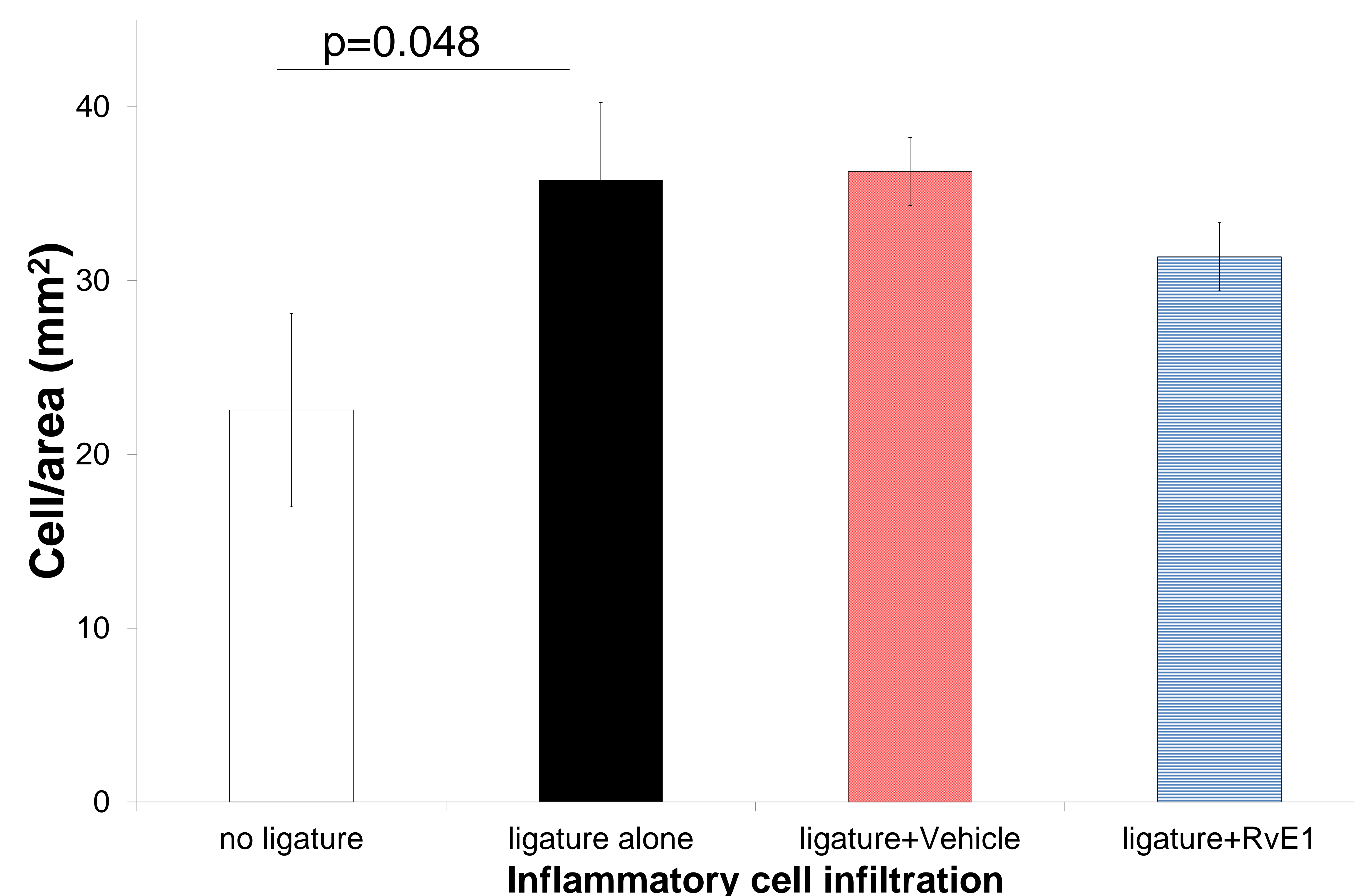


Ligature+RvE1(0.1 µg/µl)

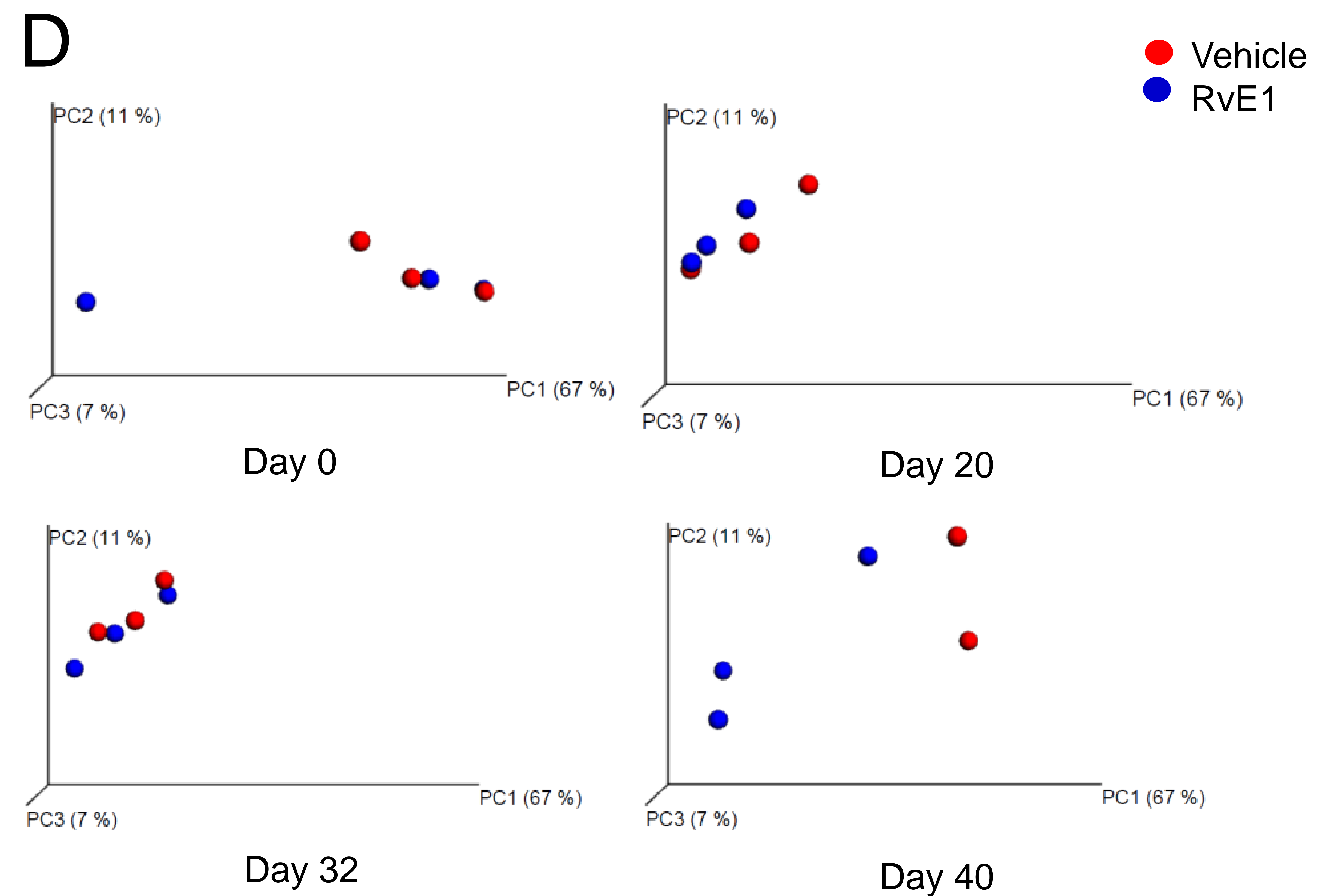
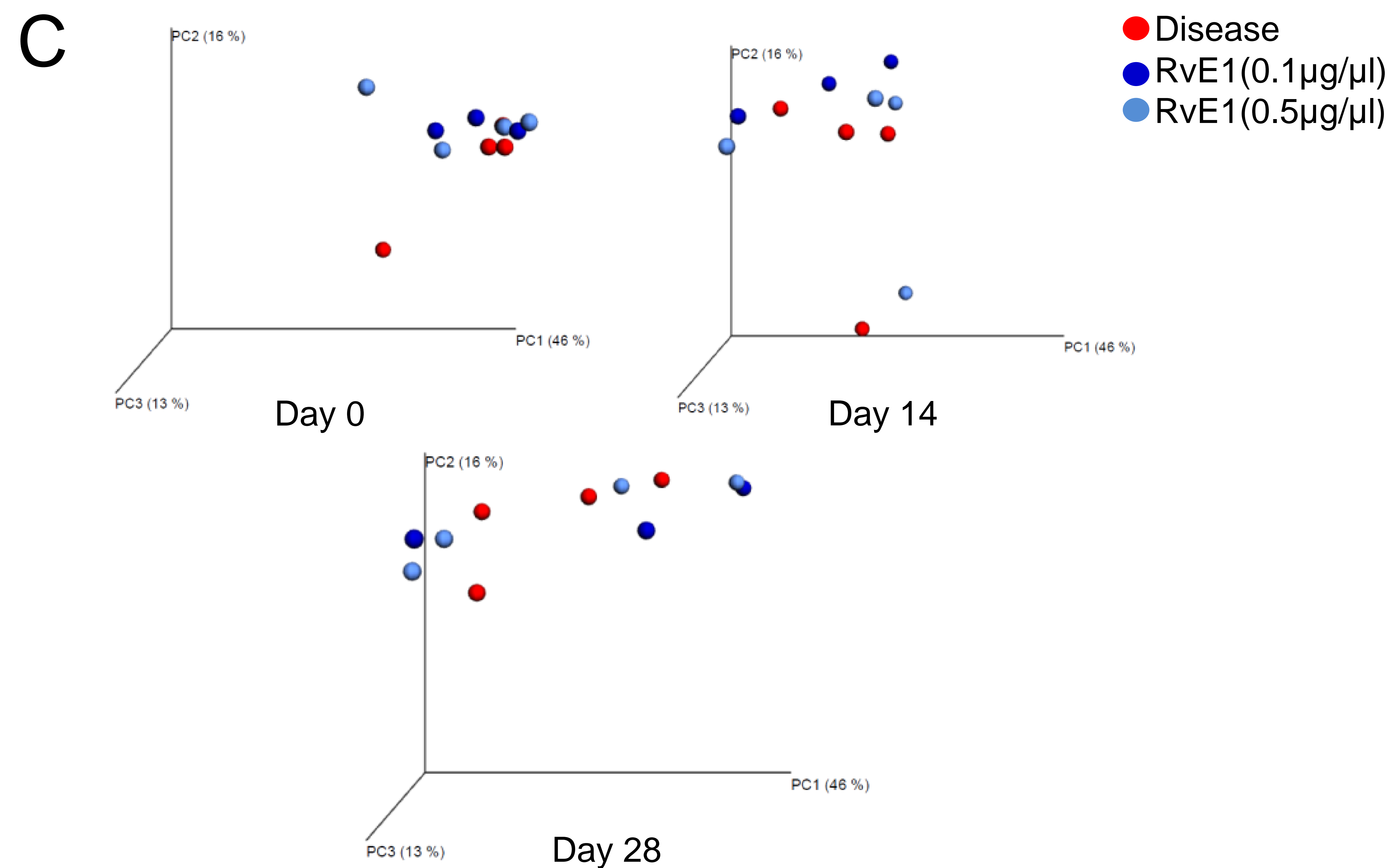
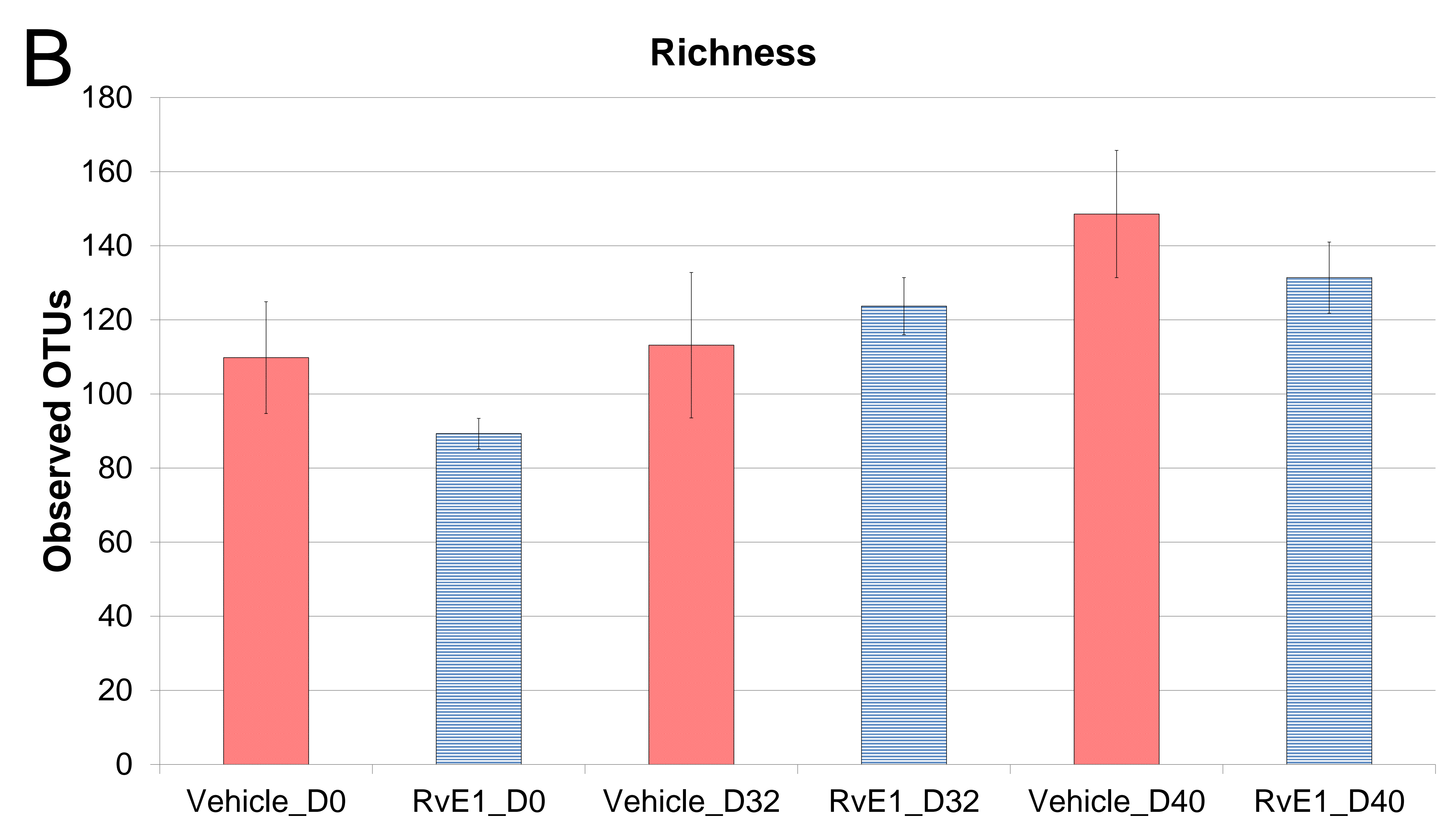
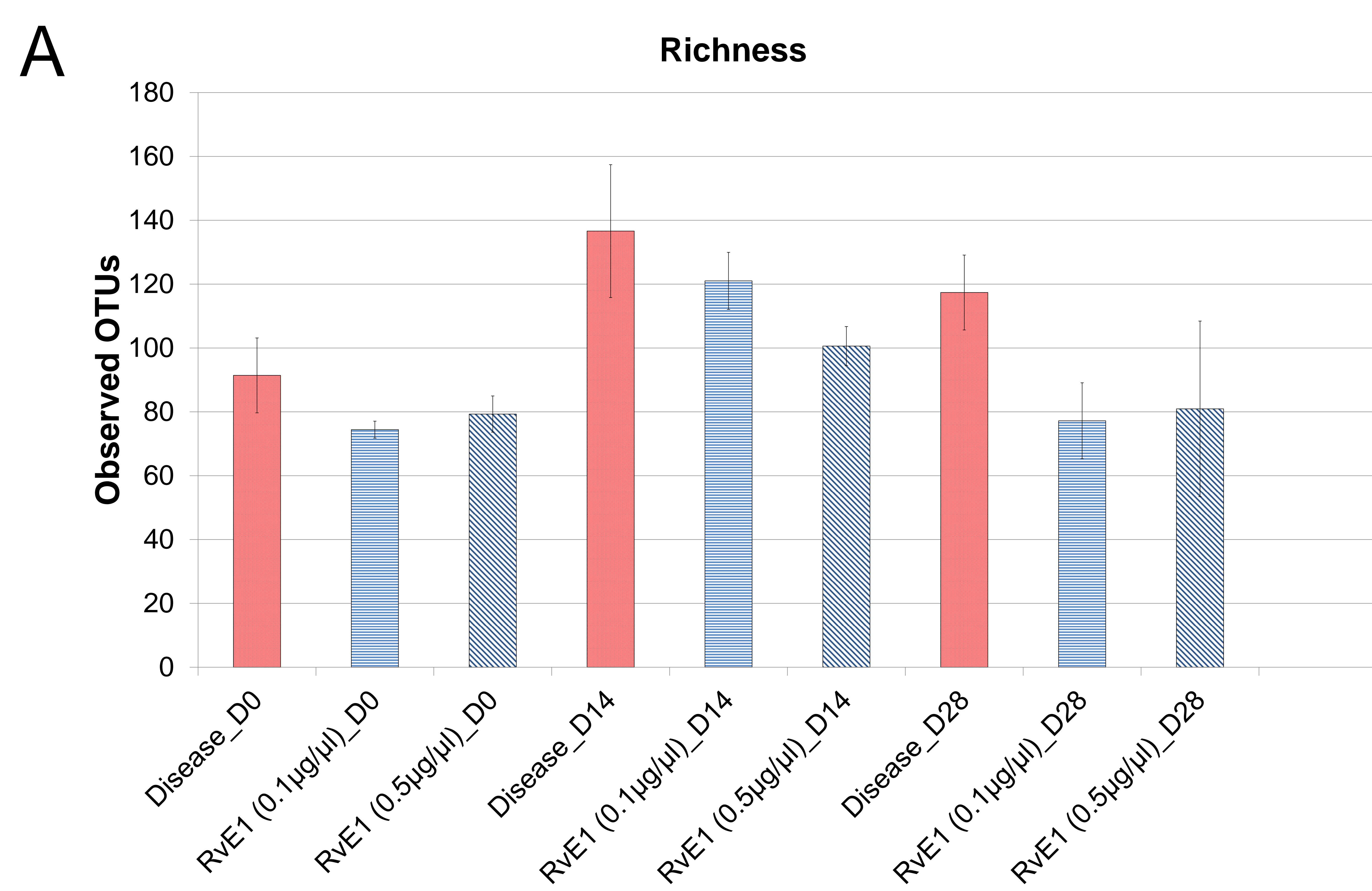
B



D



Supplemental Figure 1: RvE1 treatment inhibits inflammatory infiltrate. (A)(B) In the prevention experiment, inflammatory cell count in the RvE1 groups (RvE1 (0.1 µg/µl) and RvE1 (0.5 µg/µl)) was significantly lower than that in the disease (ligature + vehicle) group. There was no significant difference of inflammatory cell count between the RvE1 (0.1 µg/µl) group and the RvE1 (0.5 µg/µl) group. (n=4 in the health (no ligature) group, the disease (ligature + vehicle) group, and ligature+ RvE1 (0.1 µg/µl) group; n=3 in the ligature+RvE1 (0.5 µg/µl) group) (C)(D) In the treatment experiment, inflammatory cell count in the RvE1 group tended to be lower than that in the ligature alone group and the vehicle group. However, the differences were not statistically significant. (n=6 in the no ligature group and the ligature alone group; n=3 in the ligature + vehicle group and the ligature + RvE1 group; the p-values are calculated by pair-wise t-test following one-way analysis of variance.)



Supplemental Figure 2: Microbial community diversity is associated with the state of inflammation. (A) In the prevention experiment, the number of observed OTUs in the three groups increased during disease progression and the number is the highest on day 14. On day 28, the number of observed OTUs in the RvE1 groups (RvE1 (0.1 µg/µl) and RvE1 (0.5 µg/µl)) tends to return to baseline number, but the disease group does not. (B) In the treatment experiment, the number of observed OTUs in the two groups gradually increases. On day 40, the number of observed OTU in the RvE1 group is lower than that in the vehicle group. The results indicate that inflammation in the local environment of the disease group or the vehicle group might be more severe than that in the RvE1 groups as more species are expected in an inflammatory environment compared to an inflammation-controlled environment. (C)(D) Principal coordinate analysis (PCoA) plots depict distances among microbial communities based on qualitative community metrics. In the prevention experiment (C), the plots reveal that there are significant shifts of microbial communities during disease progression. At the end of the experiment, microbial communities in three groups are not clearly separated. However, the communities within the disease group appear to be closer to each other than do communities of other two groups. In the treatment experiment (D), the plots show that inflammation induced by ligature placement drive the compositional change in the microbial community. The microbial community in the RvE1 group clusters apart from the microbial community in the Vehicle group at the end of treatment phase (day 40). Larger values of each component (PC) explain more of the variance in the data.

Disease vs. Health		RvE1(0.5µg/ml) vs. Disease	
Processes	False Discovery Rate (FDR)	Processes	False Discovery Rate (FDR)
Immune response	2.42E-16	†Response to lipopolysaccharide	2.07E-05
Immune system process	3.24E-16	†*Response to external stimulus	2.07E-05
Regulation of immune system process	6.19E-13	†Response to molecule of bacterial origin	2.07E-05
Defense response	1.08E-12	Response to lipid	4.62E-05
Antigen processing and presentation of peptide or polysaccharide antigen via MHC class II	4.75E-11	Response to chemical	1.09E-04
Antigen processing and presentation of peptide antigen	6.48E-10	Response to bacterium	1.09E-04
*Response to external stimulus	3.39E-09	*Collagen catabolic process	2.13E-04
Antigen processing and presentation	5.94E-09	Multicellular organismal catabolic process	2.31E-04
Antigen processing and presentation of peptide antigen via MHC class II	7.06E-09	*Response to other organism	2.35E-04
Cell activation	7.06E-09	*Response to external biotic stimulus	2.35E-04
Positive regulation of immune system process	7.06E-09	Cellular response to oxygen-containing compound	2.44E-04
Collagen metabolic process	2.41E-08	Response to biotic stimulus	2.52E-04
*Response to other organism	3.08E-08	Response to oxygen-containing compound	4.44E-04
*Response to external biotic stimulus	3.08E-08	Response to organic substance	4.44E-04
Multicellular organismal macromolecule metabolic process	4.03E-08	Response to endogenous stimulus	4.70E-04
Inflammatory response	4.32E-08	Homeostatic process	4.83E-04
Negative regulation of mononuclear cell proliferation	4.32E-08	Cellular response to chemical stimulus	5.04E-04
Negative regulation of lymphocyte proliferation	4.32E-08	Cellular response to molecule of bacterial origin	5.83E-04
Regulation of immune response	5.14E-08	Tissue remodeling	5.83E-04
*Collagen catabolic process	5.14E-08	Response to organic cyclic compound	7.98E-04

Supplemental Table 1. Gene ontology analysis of differentially expressed genes in the two comparisons (Disease vs. Health; RvE1 (0.5µg/µl) vs. Disease)

The selected significant biological processes (GO categories) are listed by the order of false discovery rate (<0.05).

*The common processes are shared in the two comparisons.

† The top three biological processes in the comparison between the RvE1 (0.5µg/ml) group and the disease group were described below:

Response to lipopolysaccharide (GO: 0032496):

Any process that results in a change in state or activity of an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a lipopolysaccharide stimulus; lipopolysaccharide is a major component of the cell wall of gram negative bacteria. In this experiment, the algorithm identified 10 differentially expressed genes (*Mmp3*, *Cxcl1*, *Ccl3*, *Cxcl2*, *Hmgcs2*, *Nfkb1a*, *Acp5*, *Il18bp*, *Trem2*, *Nos2*). These genes are all down-regulated.

†Response to external stimulus (GO:0009605):

Any process that results in a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of an external stimulus. In this experiment, the algorithm identified 19 differentially expressed genes (*Ccl9*, *Mmp13*, *Mmp3*, *Mt3*, *Pla2g7*, *Rac2*, *Pdk4*, *Cxcl1*, *Ccl3*, *Cxcl2*, *Hmgcs2*, *Nfkb1a*, *Ptprc*, *Ctss*, *Acp5*, *Slc6a19*, *Il18b*, *Trem2*, *Nos2*). These genes are generally down-regulated except one (*Slc6a19* is up-regulated).

†Response to molecule of bacterial origin (GO: 0002237):

Any process that results in a change in state or activity of an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a stimulus by molecules of bacterial origin such as peptides derived from bacterial flagellin. In this experiment, the algorithm identified 10 differentially expressed genes (*Mmp3*, *Cxcl1*, *Ccl3*, *Cxcl2*, *Hmgcs2*, *Nfkb1a*, *Acp5*, *Il18bp*, *Trem2*, *Nos2*). These genes are all down-regulated.