

**Supplementary Material**

***In vivo* reduction of Treg expansion in rodent helminth-malaria coinfection**

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## Supplementary tables and figures

Table S1. Spectral flow cytometry panels.

A) *In vivo* experiment: staining of mouse splenocytes.

| Emission (nm)                | Target       | Fluorophore    | Clone   | Supplier      | Cat. ID    |
|------------------------------|--------------|----------------|---------|---------------|------------|
| <i>Viability</i>             |              |                |         |               |            |
| 473                          | /            | LIVE/DEAD Blue | /       | Invitrogen    | L23105     |
| <i>Surface markers</i>       |              |                |         |               |            |
| 388                          | CD45         | BUV395         | 30-F11  | BD Bioscience | 564279     |
| 615                          | CD19         | BUV615         | 1D3     | BD Bioscience | 751213     |
| 661                          | CD8 $\alpha$ | BUV661         | 53-6.7  | BD Bioscience | 750023     |
| 605                          | CD3          | BV605          | 17A2    | BD Bioscience | 564009     |
| 700                          | CD4          | AF700          | RM4-5   | BD Bioscience | 557956     |
| <i>Intracellular markers</i> |              |                |         |               |            |
| 774                          | FOXP3        | PE-Cy7         | FJK-16S | Invitrogen    | 25-5773-82 |

B) *In vitro* experiment: staining of CD4<sup>+</sup> T cells.

| Emission (nm)                | Target        | Fluorophore    | Clone   | Supplier       | Cat. ID    |
|------------------------------|---------------|----------------|---------|----------------|------------|
| <i>Viability</i>             |               |                |         |                |            |
| 473                          | /             | LIVE/DEAD Blue | /       | Invitrogen     | L23105     |
| <i>Surface markers</i>       |               |                |         |                |            |
| 421                          | CD25          | BV421          | 3C7     | BioLegend      | 101923     |
| 700                          | CD4           | AF700          | RM4-5   | BD Biosciences | 557956     |
| <i>Intracellular markers</i> |               |                |         |                |            |
| 693                          | GATA-3        | BB700          | L50-823 | BD Biosciences | 566642     |
| 711                          | IFN- $\gamma$ | BV711          | XMG1.2  | BD Biosciences | 564336     |
| 774                          | FOXP3         | PE-Cy7         | FJK-16S | Invitrogen     | 25-5773-82 |

29 Table S2. Primer sequences used for the RT-qPCR of *Hp-TGM-1* gene.

| Target   | Forward sequence (5' to 3') | Reverse sequence (5' to 3') |
|----------|-----------------------------|-----------------------------|
| β-Actin  | ATGGAGGGGAATACAGCCC         | TTCTTTGCAGTCCTTCGTT         |
| Hp-TGM-1 | CTACGCAACCGGCTCAAAAGT       | TAGTGTGCGAATCCGTCCTT        |

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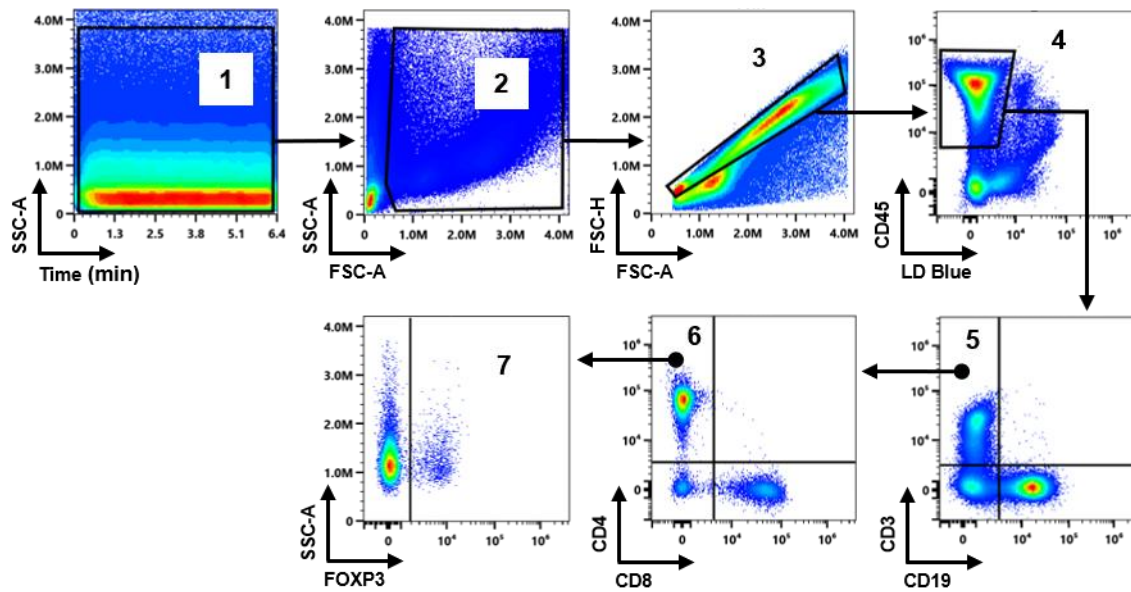


**Figure S1. Gating strategy used in flow cytometry analysis to identify Treg cell subset within mouse total splenocytes.** (1) Time parameter to exclude microfluidic fluctuations. Selection of (2) splenocytes without debris and (3) single cells. Selection of (4) live LIVE/DEAD-Blue<sup>-</sup> and immune CD45<sup>+</sup> cells. Selection of (5) CD19<sup>-</sup>CD3<sup>+</sup> T cells. Selection of (6) CD8<sup>-</sup>CD4<sup>+</sup> helper T cells. Selection of (7) FOXP3<sup>+</sup> Treg cell subset within CD4<sup>+</sup> T lymphocytes.

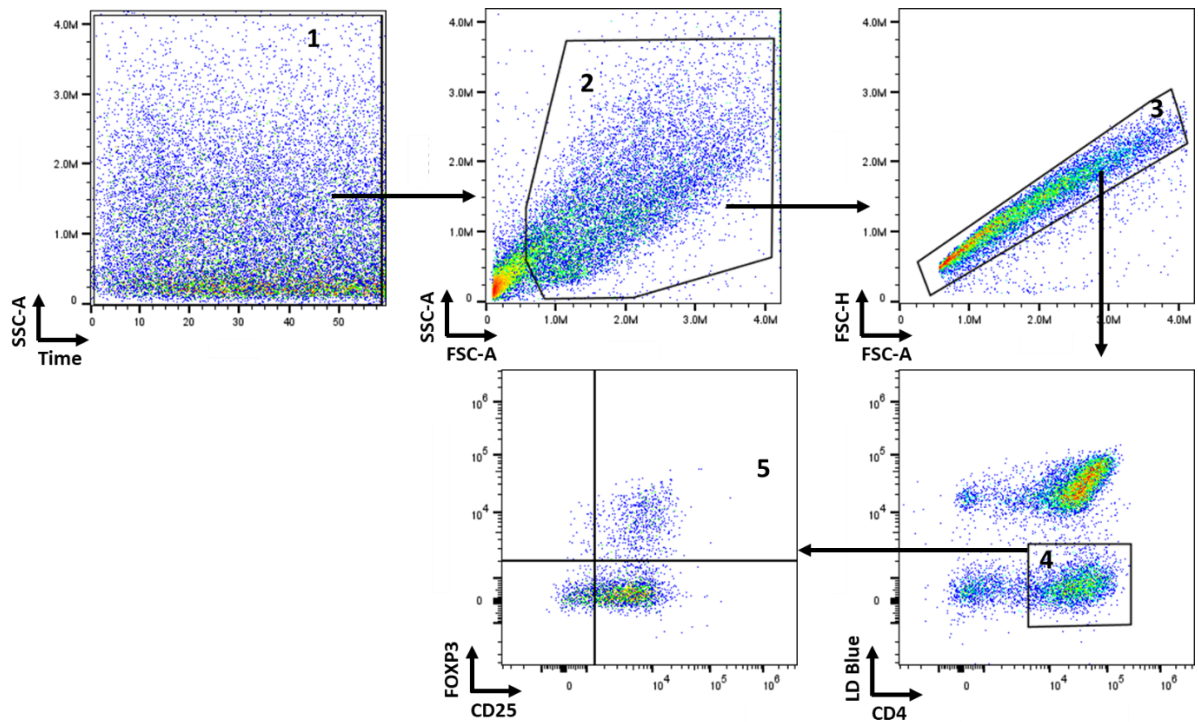


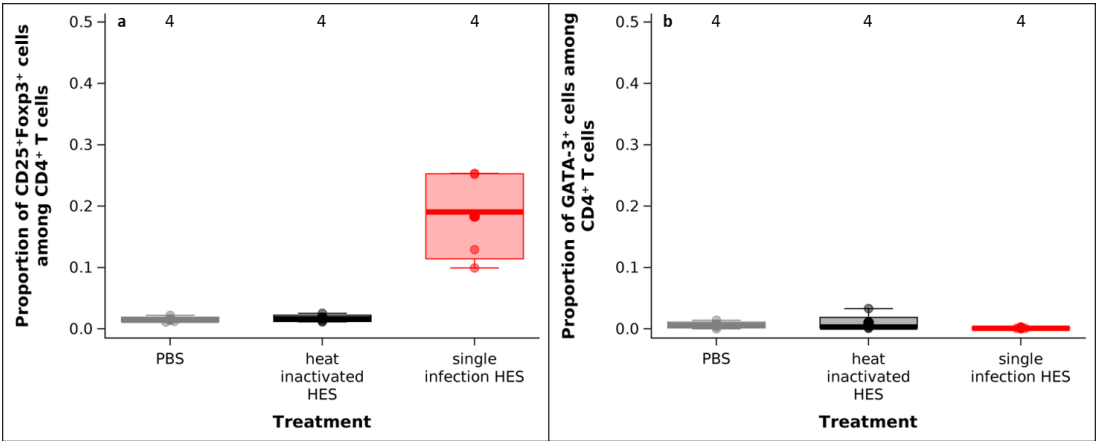
**Figure S2. Gating strategy used in flow cytometry analysis to identify Treg cells upon *in vitro* stimulation of naive CD4<sup>+</sup> T cells with HES. (1) Time parameter to exclude microfluidic fluctuations. Selection of (2) cells without debris and (3) single cells. Selection of (4) live LIVE/DEAD-Blue<sup>-</sup> and CD4<sup>+</sup> cells. Selection of (5) CD25<sup>+</sup>FOXP3<sup>+</sup> T cells.**

Table S3. Generalized linear mixed model with a beta-distribution of errors exploring the capacity of HES collected from worms in single Hp infected host to differentiate naïve CD4<sup>+</sup> T lymphocytes into CD25<sup>+</sup>Foxp3<sup>+</sup> cells *in vitro* compared to heat-inactivated HES and PBS. The proportion of CD4<sup>+</sup>IFN- $\gamma$ <sup>+</sup> cells was included in the model as a covariate. We report the parameter estimate with the 95% confidence intervals, degrees of freedom (df), F and p values. The identity of the donor mice was included in the model as a random effect and we report the parameter estimate with the standard error, the Z and p values. N = 4 donors and 12 observations. Residual variance was fixed to  $\pi^2/3$ .

| Fixed effects                                                   |                      | Estimate | 95% CI        | df   | F      | p      |
|-----------------------------------------------------------------|----------------------|----------|---------------|------|--------|--------|
| Treatment                                                       |                      |          |               | 2,5  | 18.87  | 0.0047 |
|                                                                 | single infection HES | 0        |               |      |        |        |
|                                                                 | Heat-inactivated HES | -1.951   | -3.113/-0.790 |      |        |        |
|                                                                 | PBS                  | -2.321   | -3.467/-1.175 |      |        |        |
| Proportion of CD4 <sup>+</sup> IFN- $\gamma$ <sup>+</sup> cells |                      | -8.286   | -17.116/0.544 | 1,5  | 5.82   | 0.0607 |
| Random effect                                                   |                      | Estimate | SE            | Z    | p      |        |
| Donor mouse id                                                  |                      | 0.007    | 0.087         | 0.08 | 0.4684 |        |

Table S4. Generalized linear mixed model with a beta-distribution of errors exploring the capacity of HES collected from worms in single Hp infected host to differentiate naïve CD4<sup>+</sup> T lymphocytes into GATA-3<sup>+</sup> cells *in vitro* compared to heat-inactivated HES and PBS. The proportion of CD4<sup>+</sup>IFN- $\gamma$ <sup>+</sup> cells was included in the model as a covariate. We report the parameter estimate with the 95% confidence intervals, degrees of freedom (df), F and p values. The identity of the donor mice was included in the model as a random effect and we report the parameter estimate with the standard error, the Z and p values. N = 4 donors and 12 observations. Residual variance was fixed to  $\pi^2/3$ .

| Fixed effects                                                   |                      | Estimate | 95% CI         | df   | F      | p      |
|-----------------------------------------------------------------|----------------------|----------|----------------|------|--------|--------|
| Treatment                                                       |                      |          |                | 2,5  | 1.41   | 0.3276 |
|                                                                 | single infection HES | 0        |                |      |        |        |
|                                                                 | heat-inactivated HES | 2.198    | -1.185/5.582   |      |        |        |
|                                                                 | PBS                  | 1.657    | -1.317/4.632   |      |        |        |
| Proportion of CD4 <sup>+</sup> IFN- $\gamma$ <sup>+</sup> cells |                      | -2.284   | -28.393/23.825 | 1,5  | 0.05   | 0.8310 |
| Random effect                                                   |                      | Estimate | SE             | Z    | p      |        |
| Donor mouse id                                                  |                      | 1.385    | 1.593          | 0.87 | 0.1924 |        |



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69 **Figure S3. *In vitro* differentiation of naïve CD4<sup>+</sup> T lymphocytes into CD25<sup>+</sup>Foxp3<sup>+</sup> and GATA-3<sup>+</sup> cells.**

70 a) Proportion of CD25<sup>+</sup>Foxp3<sup>+</sup> cells after *in vitro* exposure of naïve CD4<sup>+</sup> T lymphocytes to PBS, heat-  
71 inactivated HES collected from worms in single infected hosts, or HES collected from worms in single  
72 infected mice. The proportion of Treg cells was significantly different between the HES collected from  
73 worms in single infected hosts and the PBS and heat-inactivated groups (Bonferroni adjusted  $p = 0.008$   
74 and  $p = 0.0173$ , respectively). b) Proportion of GATA-3<sup>+</sup> cells after *in vitro* exposure of naïve CD4<sup>+</sup> T  
75 lymphocytes to PBS, heat-inactivated HES collected from worms in single infected hosts, or HES  
76 collected from worms in single infected mice. The proportion of GATA-3<sup>+</sup> cells did not differ between  
77 groups (all Bonferroni adjusted  $p$ 's  $> 0.3$ ). Dots represent individual observations; boxes represent the  
78 interquartile range (IQR); the horizontal line within each box indicates the median; the larger dot  
79 indicates the mean; whiskers extend to the most extreme values within  $1.5 \times$  IQR from the quartiles;  
80 and points beyond the whiskers indicate outliers. Numbers above the boxes indicate the sample size.  
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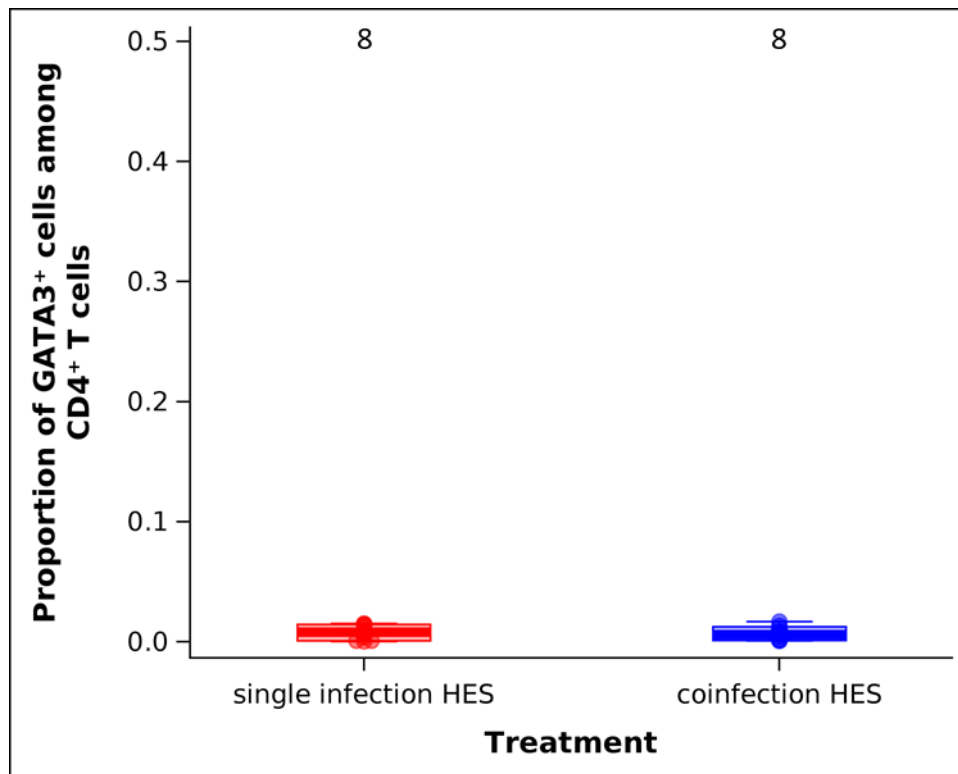


**Figure S4. *In vitro* differentiation of naïve CD4<sup>+</sup> T lymphocytes into GATA-3<sup>+</sup> cells.** Proportion of GATA-3<sup>+</sup> cells after *in vitro* exposure of naïve CD4<sup>+</sup> T lymphocytes to HES collected from worms in single infected mice, or from worms in coinfecting mice. The proportion of GATA-3<sup>+</sup> cells did not differ between the single infection HES and the coinfection HES ( $p = 0.5512$ ). Dots represent individual observations; boxes represent the interquartile range (IQR); the horizontal line within each box indicates the median; the larger dot indicates the mean; whiskers extend to the most extreme values within  $1.5 \times \text{IQR}$  from the quartiles; and points beyond the whiskers indicate outliers. Numbers above the boxes indicate the sample size.

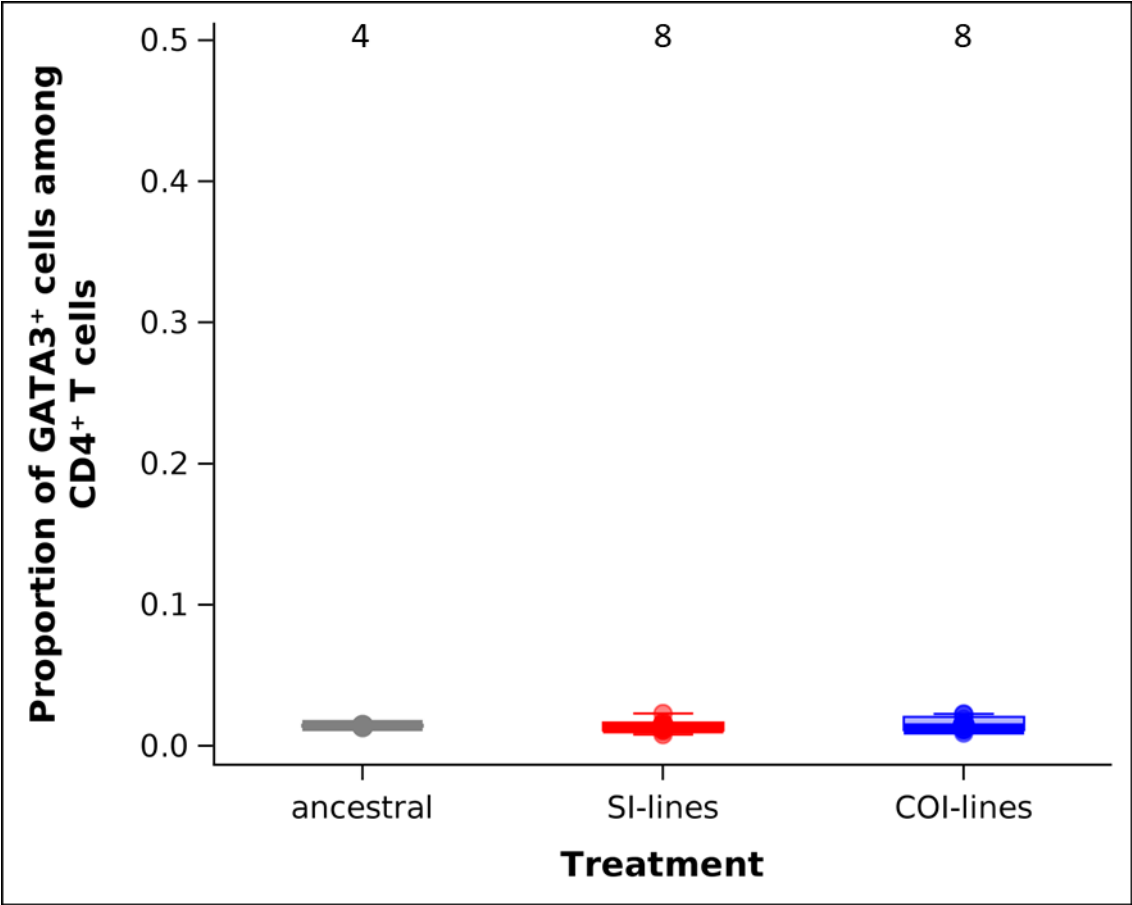
Table S5. Generalized linear mixed model with a beta-distribution of errors exploring the effect of treatment (single Hp infection, coinfection) on the capacity of HES to expand the population of GATA-3<sup>+</sup> cells *in vitro*. We report the parameter estimate with the 95% confidence intervals, degrees of freedom (df), F and p values. The identity of the donor mice used for the *in vitro* tests was included as a random effect, and we report the parameter estimate with the standard error, the Z and p values. N = 8 donor mice and 16 observations. Residual variance was fixed to  $\pi^2/3$ .

| Fixed effect   |           | Estimate | 95% CI       | df   | F      | p      |
|----------------|-----------|----------|--------------|------|--------|--------|
| Treatment      |           |          |              | 2,14 | 0.17   | 0.8429 |
|                | ancestral | 0        |              |      |        |        |
|                | SI-lines  | -0.020   | -0.333/0.294 |      |        |        |
|                | COI-lines | 0.048    | -0.262/0.358 |      |        |        |
| Random effect  |           | Estimate | SE           | Z    | p      |        |
| Donor mouse id |           | 0.053    | 0.053        | 1.01 | 0.1574 |        |

Table S6. Generalized linear mixed model with a beta-distribution of errors investigating the effect of Hp serial passage on the capacity of HES to expand the population of GATA-3<sup>+</sup> cells *in vitro*. We compared HES collected from worms of the ancestral population, from worms passaged in single infected (SI-lines) or in coinfecting (COI-lines) hosts for eight generations. The proportion of CD4<sup>+</sup>IFN- $\gamma$ <sup>+</sup> cells was included in the model as a covariate. We report the parameter estimate with the 95% confidence interval (95% CI), degrees of freedom (df), F and p values. The identity of the donor mice used for the *in vitro* tests was included as a random effect. For the random effect, we report the parameter estimate with the standard error, the Z and p values. N = 4 donor mice and 20 observations. Residual variance was fixed to  $\pi^2/3$ .

| Fixed effects                                                   |                     | Estimate | 95% CI         | df   | F      | p      |
|-----------------------------------------------------------------|---------------------|----------|----------------|------|--------|--------|
| Treatment                                                       |                     |          |                | 1,6  | 0.40   | 0.5512 |
|                                                                 | single Hp infection | 0        |                |      |        |        |
|                                                                 | coinfection         | -0.104   | -0.508/0.300   |      |        |        |
| Proportion of CD4 <sup>+</sup> IFN- $\gamma$ <sup>+</sup> cells |                     | 4.415    | -18.749/27.579 | 1,6  | 0.22   | 0.6574 |
|                                                                 |                     |          |                |      |        |        |
| Random effect                                                   |                     | Estimate | SE             | Z    | p      |        |
| Donor mouse id                                                  |                     | 1.481    | 0.942          | 1.57 | 0.0579 |        |

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**Figure S5. Proportion of GATA-3<sup>+</sup> cells after *in vitro* exposure of naïve CD4<sup>+</sup> T lymphocytes to HES collected from worms of the ancestral population, SI-lines or COI-lines.** The proportion of GATA-3<sup>+</sup> cells did not differ between any of the groups (all Bonferroni adjusted p = 1). Dots represent individual observations; boxes represent the interquartile range (IQR); the horizontal line within each box indicates the median; the larger dot indicates the mean; whiskers extend to the most extreme values within 1.5 × IQR from the quartiles; and points beyond the whiskers indicate outliers. Numbers above the boxes indicate the sample size.