**Supplementary Figure S1A:** Schematic diagram indicating assay workflow including assay optimization.

**Supplementary Figure S1B: Droplet digital PCR workflow**

**A:** Samples and droplet generation oil was loaded into an eight-channel droplet generator cartridge. Vacuum was applied to the droplet well, that drew sample and oil through a flow-focusing nozzle where mono-dispersed 1 nL droplets were formed. In < 2 mins, eight samples were converted into eight sets of 20,000 droplets. The surfactant stabilized droplets were pipetted into 96 well PCR plate.

**B:** ddPCR amplification to end point was performed in a Bio-Rad thermocycler.

**C:** The plate was finally transferred to the reader which sips droplets from each well and streams them single-file past a two colour detector at the rate of 1000/seconds. Droplets were assigned as positive or negative based on channel fluorescent amplitude. The number of positive and negative droplets in each channel was used to calculate the concentration of the target sequence and their Poisson based 95% confidence intervals.

**Supplementary Figure S2: Preliminary standardization for optimal expression of *A2* in *L. donovani* infected Syrian golden hamsters.**

**A-C:** One-dimensional plots of droplets measured for fluorescence signals (amplitude indicated on y-axis) emitted from *A2* with primer concentrations of 100 **(i)**, 250 **(iii)**, 500 nM/mL **(v)** in infected tissues of hamsters using a template gradient of 25-100 ng/µL (**Lanes 3 - 5**) and temperature 58˚C **(A)**, 60˚C **(B),** 62˚C **(C)**. Concentration graphs showing corresponding mRNA expression of *A2* **(ii, iv, vi)** with Poisson distribution model. Lane 1: negative controls, lane 2: non-template controls, lanes 3 - 5: template cDNA 25, 50 and 100 ng/µL respectively. EvaGreen-bound positive droplets are shown in blue while negative droplets are shown in black, with expression of the genes quantified as copies/μL.

**Supplementary Figure S3: Preliminary standardization for optimal expression of *amastin* in *L. donovani* infected Syrian golden hamsters.**

**A-C:** One-dimensional plots of droplets measured for fluorescence signals (amplitude indicated on y-axis) emitted from *amastin* with primer concentrations of 100 **(i)**, 250 **(iii)**, 500 nM/mL **(v)** in infected tissues of hamsters using a template gradient of 25-100 ng/µL (**Lanes 3 - 5**) and temperature 58˚C **(A)**, 60˚C **(B),** 62˚C **(C)**. Concentration graphs showing corresponding mRNA expression of *amastin* **(ii, iv, vi)** with Poisson distribution model. Lane 1: negative controls, lane 2: non-template controls, lanes 3 - 5: template cDNA 25, 50 and 100 ng/µL respectively. EvaGreen-bound positive droplets are shown in blue while negative droplets are shown in black, with expression of the genes quantified as copies/μL.

**Supplementary Figure S4: Optimization for expression of *A2* and *amastin* in *L. donovani* infected Syrian golden hamsters.**

**A:** Representative one-dimensional plots of droplets measured for fluorescence signals (amplitude indicated on y-axis) emitted from *A2* in infected hepatic and splenic tissues of hamsters using a template gradient of 12.5-100 ng/µL and temperature 58-62˚C **(i-iii)**. Bar graphs showing mRNA expression of *A2* **(iv-vi).**

**B:** Representative one-dimensional plots of droplets measured for fluorescence signals (amplitude indicated on y-axis) emitted from *amastin* in infected hepatic and splenic tissues of hamsters using a template gradient of 12.5-100 ng/µL and temperature 58-62˚C **(i-iii)**. Bar graphs showing mRNA expression of *amastin* **(iv-vi).**

Lane 1: negative controls, lane 2: non-template controls, lanes 3 - 6: template cDNA 12.5, 25, 50 and 100 ng/µL respectively. EvaGreen-bound positive droplets are shown in blue while negative droplets are shown in black, with expression of the genes as copies/μL. Each horizontal bar represents the mean ± SEM of at least three experiments in duplicates; \*p<0.05 and \*\*p<0.01 as compared to NTC.