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Dear Editor Stephanie Weldon,

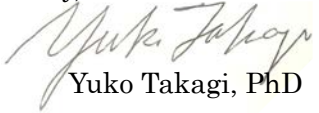
Thank you for giving us the opportunity to submit our manuscript entitled “**Introducing a gene knockout directly into amastigote stage of *Trypanosoma cruzi* by CRISPR/Cas9 system**” to JoVE.

The replication stage of *T. cruzi* in host cells, amastigote, is the least studied developmental stage in the parasite’s life cycle, although it is the most relevant stage in terms of drug development research. This is because amastigote had been considered as an obligate intracellular parasite and could not be isolated for experimental use.

Our new methodology to utilize temporal axenic culture of *T. cruzi* amastigote enables investigation of drug target essentiality specifically in amastigote stage, without going through other developmental stages. Conventional method takes 1 - 2 months to generate a knockout amastigote, but our protocol only takes 2 - 3 days. We believe our method can be applied to variety of stage-specific studies and contribute to advance our understanding of this clinically important parasite.

Please address all the correspondent regarding this manuscript to me at [yuko-takagi@aist.go.jp]. I will be happy to respond to any further questions and comments.

Sincerely,



Yuko Takagi, PhD