Response to Reviewer 2 Comments

Point 1: A major issue raise in my previous review was not addressed in the revised manuscript.

"Point 3: It is not clear whether the increase in genome editing efficiency by using dgRNP and chemical modifications was due to chemical modifications of nucleotides or simply due to the assembly of crRNA:tracrRNA duplex".

This needs to be done by comparing the genome editing efficiency of dgRNP without and with chemical modifications.

Response 1: Done. We have compared editing efficiency of dgRNP with or without chemical modifications and add the result in the revised manuscript (**line 199-203** and **figure 3B**). According to the result, both assembly of crRNA:tracrRNA duplex and chemical modifications of nucleotides can increase genome editing efficiency in ScCas9 system.

Line 199-203 “Our results showed that the ScCas9 activities on *tyr*-NAG, *ddx21*-NAG, and *rpl9*-NCG were 12.5%, 47.6%, 14.3%, respectively, and increased to 57.5%, 73.1%, 15.0% with dgRNP, respectively. Interestingly, with chemical modified dgRNP (mdgRNP), the ScCas9 editing efficiency were dramatically improved to 88.0%, 88.9%, and 33.3% on the three loci, respectively (Figure 3B).”

Figure 3

