Supplementary Figure 4.

3) Insertion of new Multiple Cloning Site (MCS)

Supplementary Fig. 4. Insertion of new multiple cloning site into mCherry in pEGFP-C3 background. See Materials and Methods for explanation.

C terminal mCherry sequence (stop codon removed and bp's inserted):

```
                                  EcoRI        HindIII
5'-GGACGAGCTGTACAAGTACGAATTCTCCCTGGAAGCTTG-3'
3'-CCTGCTCGACATGTTCATGCTTAAGAGGGACCTTCGAAC-5'
```

New MCS

```
          E  H  M  A  S  M  T  G  G  Q  Q  M  G  R  D  P  L  E  X  X
5'-GAATTACATATGGCTAGCATGACTGGTGGACAGCAAATGGGTCGGGATCCTCTCGAGGGCGGCAAGCTT-3'
          NdeI                                  BamHI  XhoI        HindIII
```

- Underline – new restriction enzyme sites in the new MCS
- **Boldface** - Φ10 recognition sequence
- *Italics* – EcoRI site that was destroyed upon ligation of the new MCS into mCherry-pRSETB (stop codon removed)
- Oligos for New MSC: 1859 (forward sequence)
  1860 (reverse sequence)