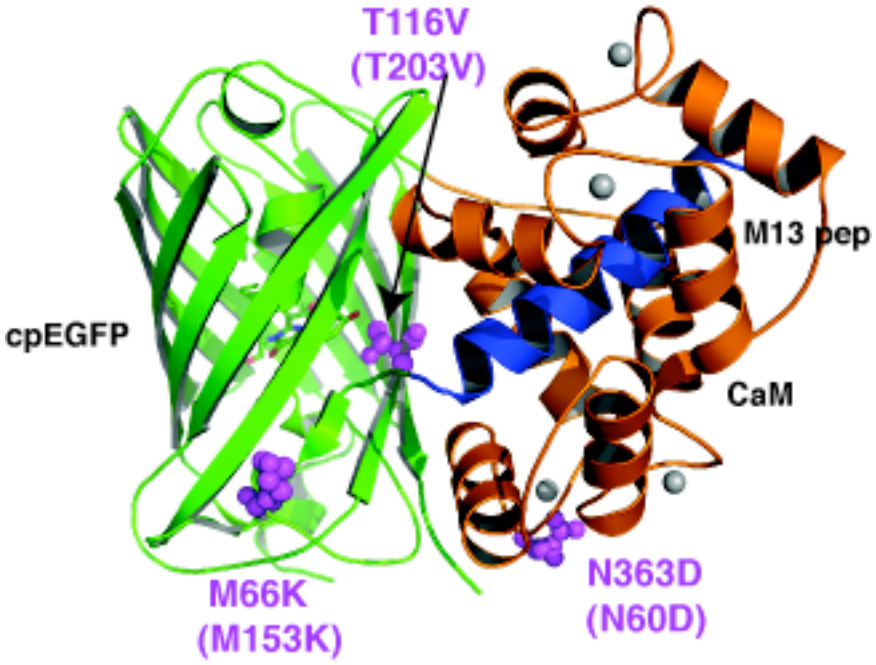
a

```
GCaMP2 MRGSHHHHHHGMASMTGGQQMGRDLYDDDDKDLATMVDSSRRKWNKTGHA      50
       VRAIGRLSSLENVYIMADKQKNGIKANFKIRHNIEDGGVQLAYHYQQNTP     100
       IGDGPVLLPDNHYLSTQSKLSKDPNEKRDHMVLLEFVTAAGITLGMDELY     150
       KGGTGGSMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKL     200
       TLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGY     250
       IQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLE     300
       YNTRDQLTEEQIAEFKEAFSLFDKDGDGTITTKELGTVMRSLGQNPTEAE     350
       LQDMINEVDADGNGTIDFPEFLTMMARKMKDTDSEEEIREAFRVFDKDGN     400
       GYISAAELRHVMTNLGEKLTDEEVDEMIREADIDGDGQVNYEEFVQMMTAK*   450
```

b



Supplementary Figure 2. Summary of positions where mutants were generated.

(a) Protein sequence of GCaMP2. The sites of side-directed mutagenesis are highlighted. Arginine at position 2 was deleted. Residues in red surround the

chromophore (highlighted in green). Residues in blue are known to contribute to GFP thermodynamic stability. Residues in pink are at the interface of M13 peptides and calmodulin (CaM). Residues in purple are responsible for the calcium-binding in EF-hands of CaM. **(b)** Structural model of GCaMP3, based on the structure of calcium-bound monomeric GCaMP2. The mutated amino acids are highlighted in purple.