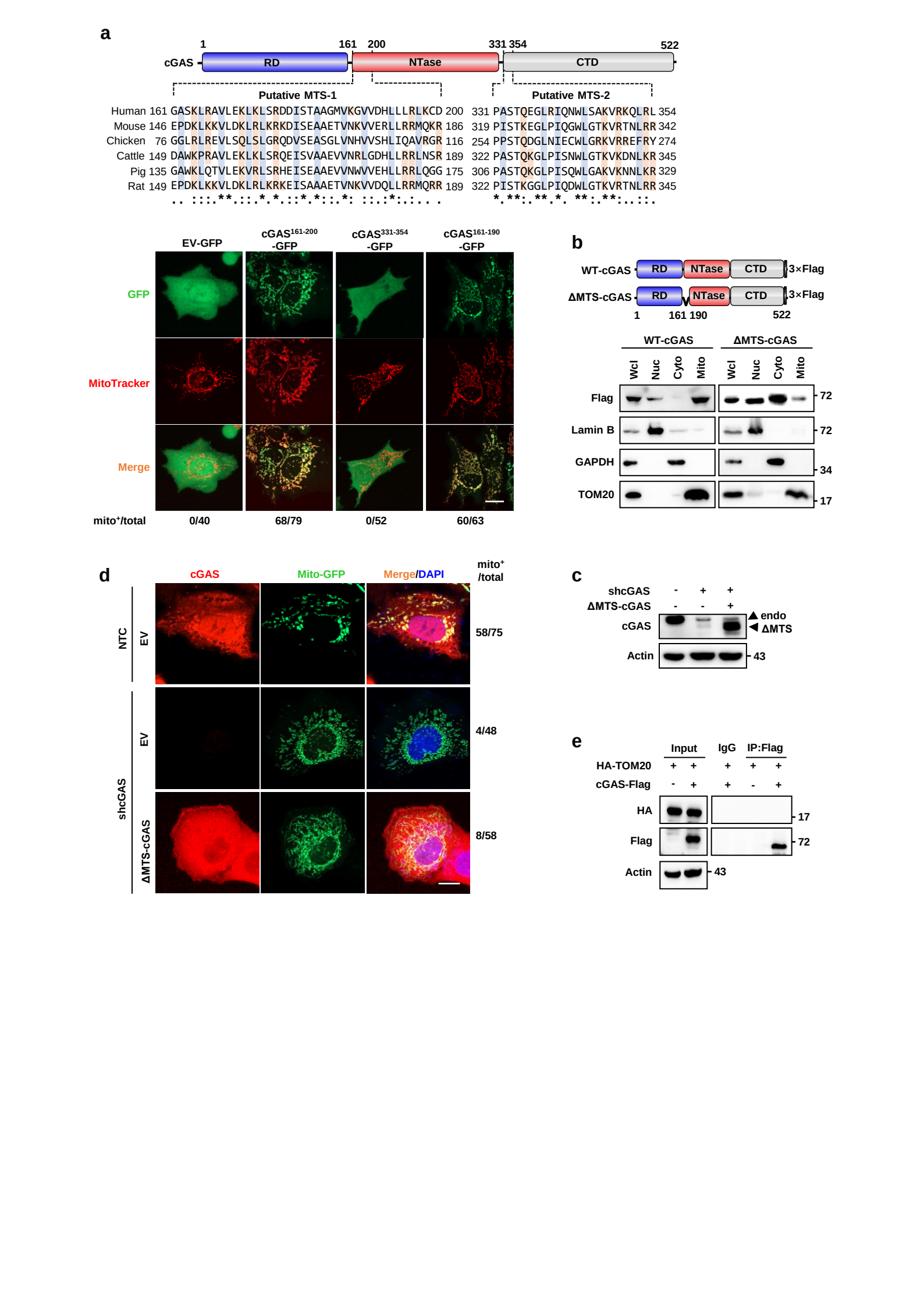
a
1
161
200
331
354
522
cGAS
RD
NTase
CTD
Putative MTS-1
Putative MTS-2
Human 161 GASKLRAVLEKLKLSRDDISTAAGMVKGVVDHLLLRLKCD 200 331 PASTQEGLRIQNWLSAKVRKQLRL 354
Mouse 146 EPDKLKKVLDKLRLKRKDISEAAETVNKVVERLLRRMQKR 186 319 PISTKEGLPIQGWLGTKVRTNLRR 342
Chicken 76 GGLRLREVLSQLSLGRQDVSEASGLVNHVVSHLIQAVRGR 116 254 PPSTQDGLNIECWLGRKVRREFRY 274
Cattle 149 DAWKPRAVLEKLKLSRQEISVAAEVVNRLGDHLLRRLNSR 189 322 PASTQKGLPISNWLGTKVKDNLKR 345
Pig 135 GAWKLQTVLEKVRLSRHEISEAAEVVNWVVEHLLRRLQGG 175 306 PASTQKGLPISQWLGAKVKNNLKR 329
Rat 149 EPDKLKKVLDKLRLKRKEISAAAETVNKVVDQLLRRMQRR 189 322 PISTKGGLPIQDWLGTKVRTNLRR 345
EV-GFP
cGAS161-200 -GFP
cGAS331-354 -GFP
cGAS161-190 -GFP
GFP
MitoTracker
Merge
mito+/total
0/40
68/79
0/52
60/63
b
WT-cGAS
ΔMTS-cGAS
RD
NTase
CTD
3×Flag
1
161 190
522
WT-cGAS
ΔMTS-cGAS
Wcl
Nuc
Cyto
Mito
Flag
Lamin B
GAPDH
TOM20
72
72
34
17
d
cGAS
Mito-GFP
Merge/DAPI
mito+ /total
NTC
EV
shcGAS
EV
ΔMTS-cGAS
58/75
4/48
8/58
c
shcGAS
ΔMTS-cGAS
cGAS
endo
ΔMTS
Actin
43
e
Input
IgG
IP:Flag
HA-TOM20
cGAS-Flag
HA
Flag
Actin
17
72
43

Supplementary information, Fig S3 cGAS is actively imported in mitochondria through mitochondrial targeting sequence

a, Alignment of the Mitochondrial Targeting Sequence (MTS) of cGAS from different species. Positively charged residue are highlighted in orange and hydrophobic residues are marked in blue. Representative fluorescence images show the localization of Human cGAS peptides, 161-200, 331-354 or 161-190 stably expressed in Hep3B cells. The mitochondria were marked by MitoTracker in the cells. Scale bars, 20 μm. Values shown on the bottom are the numbers of cells displaying mitochondrial cGAS relative to the total numbers of cells examined. **b**, Western blotting analysis of the protein levels of cGAS in subcellular fractions including nucleus, cytosol and mitochondria in Hep3B cells overexpressing wild type cGAS ($cGAS^{WT}$) or cGAS mutant depleting the potential MTS ($cGAS^{\Delta MTS}$). Fractionation fidelity was verified by detection of Lamin B in the nuclear fraction, GAPDH in the cytosolic fraction and TOM20 in the mitochondrial fraction. Diagrammatic representation of wild type cGAS ($cGAS^{WT}$) or cGAS mutant depleting the potential MTS ($cGAS^{\Delta MTS}$). **c**, Western blotting analysis of the protein levels of cGAS in Hep3B cells with endogenous cGAS knockdown and ΔMTS-cGAS overexpression. β-Actin served as the loading control. **d**, Representative immunofluorescence images staining for cGAS in Hep3B cells with endogenous cGAS knockdown and ΔMTS-cGAS overexpression. The mitochondria were marked by Mito-GFP expression in the cells. The nucleus was stained with DAPI. Scale bars, 20 μm. Values shown in the right are the numbers of cells displaying mitochondrial cGAS relative to the total numbers of cells examined. **e**, Co-IP assay of the protein interaction between cGAS and TOM20 in Hep3B cells. Hep3B cells were infected with lentivirus containing Flag-EV or cGAS-Flag and HA-TOM20 plasmids. Cell lysates were immunoprecipitated with an anti-Flag antibody, followed by Western blotting analysis with antibodies against Flag and HA tags.