Supplementary methods

Generation of *40D4* mutant

A *minus* fusion-defective mutant (*40D4*) was generated by insertional mutagenesis of *Chlamydomonas* strain *B215* with plasmid pMN56 (Nelson et al., 1994) encoding the nitrate reductase gene. After screening ~ 4000 insertional mutants, clone *40D4* was identified with fusion defective phenotype. *40D4* underwent flagellar adhesion with wild type *plus* gametes and also formed pairs of gametes adhering at their apical ends, but failed to fuse, which is identical to the phenotype of the *hap2* mutant *63B10* (Liu, 2008). As expected, *40D4* was rescued for fusion by transformation with the wild-type *HAP2* gene (Fig. 3 in main text). Diagnostic PCR confirmed that the *HAP2* gene was disrupted in *40D4* (Fig. S1). The following primers were used:

P2, 5'-CTGGCTGGTGACAGGCAGCGCGAA-3';
P3, 5'-CAGCCAGGGATTCTGCTGCGAGT-3';
P15, 5'-ACTGAGTCGTACAGGCTGACTGTGC-3';
P22, 5'-CGTTGCAGCCACATGCGCTCCCACA-3';
P26, 5'-ATCGCGGACGGCCGGGTGC-3';
P61, 5'- CCCAGCTGGTCGTCAAGCCCTCCGG-3'.

As shown in Figure S1, the *HAP2* gene was disrupted near the 3' end in the coding region in *40D4* as documented with primers (P61-P22). Control PCR analysis using the parent strain *B215* is also shown. Sequencing confirmed the identity of the PCR products.

pHAP2-HAm plasmid

pHAP2-HAm plasmid sequence: lower case, cloning vector PUC19; upper case, HAP2 gene sequence; boxed, *Bgl*II or *Nru*I site; green, HAP2 coding sequence; red, the first intron; purple, 3HA tag. Note that the HAP2 cDNA encoded by this plasmid contains a Q at residue 697 (rather than L697 in cDNA GI:288563867), which corresponds to the Q at that site encoded in HAP2 gene model Cre01.g066100.

>tcgcgcgtttcggtgatgacggtgaaaacctctgacacatgcagctcccggagacggtcacagcttgtctgtaagcggatgccggg
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CTGGTTGTTCGGCCTTGAAGCGCCACTTTCGCCCGCAGGCATGGTTTTGCTAAACT
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TTATCACAGGTCTAGTCAGCGAAACCAGGACACTGATGTGTCGTGCCATCGCGGT
TGCGCTGATAGTTTACCTAGCCCAGCATTATATTCTTGCGCACGCTGAGGTCATTG
CAAGTGGGCGCTTGGAAAAATGCGTCGTCGATGGTGTTAGATCTACCGAGGTGA
GAATAGAAGTCCTGCGGTCGACGTTGGCTTGGCATTTCCTGCTCATCTGGACACC
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CACCCGGGGAGGCCTGTCGCGATACCCCTACGACGTGCCCGACTACGCCTACCCC
TACGACGTGCCCGACTACGCCGATCGATCCGGACCGTACCCCTACGACGTGCCCG
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GGCTGCTACAGCCGCCGGCAGCAGCAGTGTTTGTGCCTGAAGGCGGCGGCGGCG
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ctttcgtc>

Immunofluorescence image stacks for *hap2, HAP2-HA* and *HAP2-760* gametes
hap2, *HAP2-HA* and *HAP2-760* gametes were stained with anti-HA for immunofluorescence analysis. The serial Z-section image stacks were taken from the top to bottom of the cells using fluorescent microscopy and Micro-Manager-1.4 software. The stacks were saved using ImageJ software (NIH).

Table S1. Predicted palmitoylation sites in *Chlamydomonas* HAP2 from online server (http://csspalm.biocuckoo.org/online.php). Low threshold was used for the prediction (Veit, 2012).

Site	Peptide	Score
2	******MCRAIAVAL	42.224
71	ALEFSLSCLNSPDGR	10.84
136	LRPSNKVCKDGDWED	4.397
164	VADSQGFCCECSSSQ	4.397
661	LASLAASCCGGGGGA	9.477

Figure

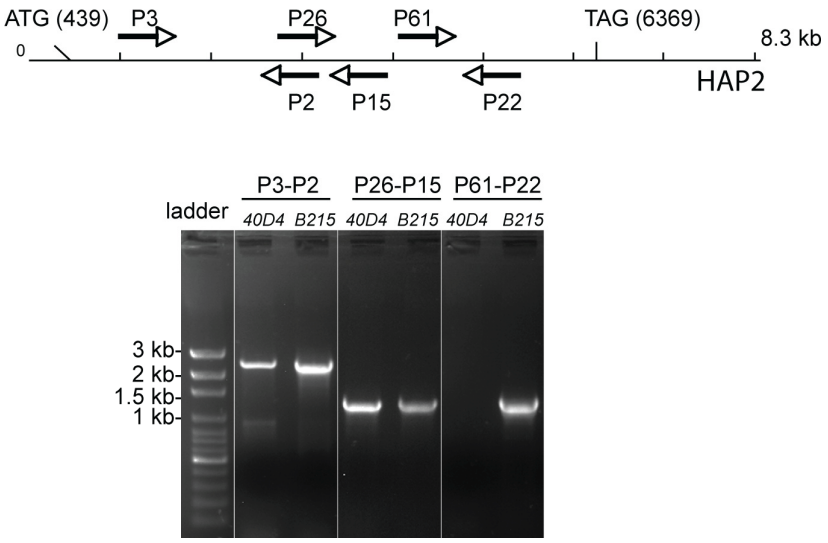
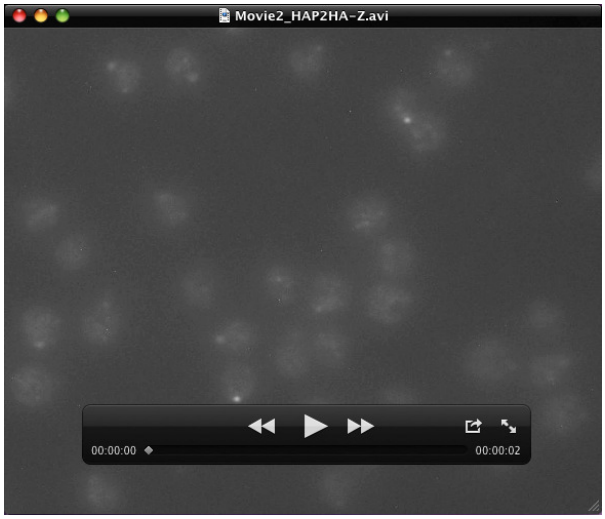
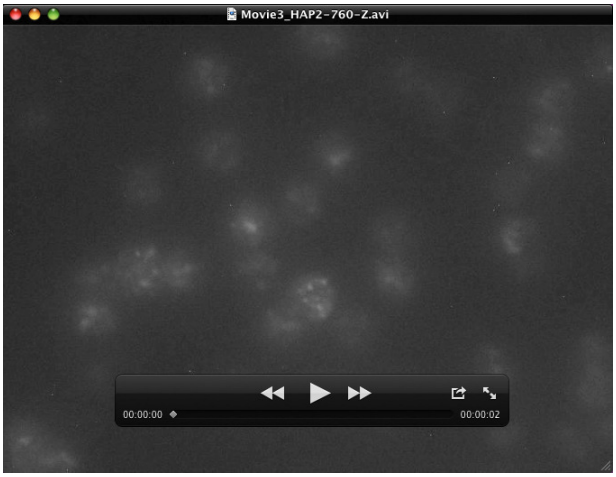


Fig. S1. Diagram and DNA gel image show diagnostic PCR results using *B215* and *40D4* genomic DNA as template. The location of primers is shown in the diagram of the *HAP2* gene. The PCR amplifications from primer sets P3-P2 and P26-P15 are positive in both *B215* and *40D4*. There is no PCR product when using primer P61-P22 in *40D4* indicating that the *HAP2* gene is disrupted at the 3' end.





Movies 1-3. Movie 1 (hap2-Z.avi), Movie 2 (HAP2HA-Z.avi) and Movie 3 (HAP2-760-Z.avi) are anti-HA immunofluorescence image stacks of *hap2*, *HAP2-HA* and *HAP2-760* gametes, respectively.

Supplementary Reference:

Nelson JA, Savereide PB, Lefebvre PA. (1994). The CRY1 gene in *Chlamydomonas reinhardtii*: structure and use as a dominant selectable marker for nuclear transformation. *Mol Cell Biol.* 14(6):4011-9.