SUPPLEMENTARY METHODS

Construction of the C. rodentium luxS mutant (TJE2)
1362 bp of luxS and flanking sequence from C. rodentium DBS100 were PCR-amplified using primers TE1 (TATAGGATCCGAACCTTACTCACAAGGCTTG) and TE2 (TAATTCTAGAAGCTGGACAGCATTACACG) and cloned into pBluescript-II SK+ on a BamHI, XbaI fragment. The kanamycin-resistance (KnR) cassette from pACYC177 (NEB) was cloned into the AgeI site in the middle of luxS. The luxS::KnR fragment was then cloned into the suicide vector pKNG101 (Kaniga et al., 1999), to generating the marker-exchange plasmid. Marker-exchange was carried out using a similar protocol as Kaniga et al., (1999). Transconjugants were selected on minimal medium containing 0.2% glucose + streptomycin. Resolvants, in which resolution of the plasmid from the chromosome had occurred, leaving only the disrupted allele, were selected on minimal medium containing 10% sucrose + Kn. The correct disruption of the locus was confirmed by PCR analysis and sequencing.

Construction of the C. rodentium croR and croI, croR mutants
1162 bp of croR and flanking sequence from C. rodentium DBS100 were PCR-amplified using primers KR1 (TATAGGCGCCCTGAGTCTATCCTCAACAAGGCTTG) and KR2 (CGTATCTAGAAGCTGGACAGCATTACACG) and cloned into pBluescript-II SK+ on an ApgI, XbaI fragment. The ’uidA, KnR cassette from pUIDK1 (Bardonnet & Blanco, 1992) was cloned into the EcoRV site in the middle of croR. The croR::’uidA, KnR fragment was then cloned into the suicide vector pKNG101 (Kaniga et al., 1999), to generating the marker-exchange plasmid. Marker exchange was carried out as above, into a DBS100 background to generate KJR025 (croR), and into an IF232 background (croI) to generate KJR026 (croI, croR).

References:
**Supplementary Fig 1** | Production of AI-2 activity by *C. rodentium* wild type (DBS100, WT) and *luxS* mutant (TJE2). Supernatant samples were harvested at intervals throughout growth in LB and their AI-2 activity was measured using the *V. harveyi* BB170 bioassay. AI-2 activity induces bioluminescence in the sensor strain, reported as light units (lu). The results shown are representative of three independent experiments.
Supplementary Fig 2 | Inactivation of *croI* does not have a substantial effect on Type III secretion by *C. rodentium*. Secreted proteins produced by DBS100 (wild type) and IF232 (*croI*) were precipitated from the supernatants of cultures grown in DMEM and analysed by 12% SDS-PAGE and staining with Coomassie blue. Three independent cultures are shown for each strain. EspA, EspB and EspD (labelled), the most abundant secreted proteins, were identified by comparison with previous work (Deng *et al*, 2004) and mass spectrometry (data not shown).
**Supplementary Fig 3**] Complementation of the surface-attachment phenotype of the *crol* mutant by the expression of *crol* in *trans*. The ability of *C. rodentium* strains DBS100 (pBluescript) [wild type, vector control], DBS100 (pSJJC45) [*crol in trans*], IF232 (pBluescript) [*crol* mutant] and IF232 (pSJJC45) to adhere to the wells of a 96-well microtitre plate was determined after incubation for 20 h at 37 degrees C. Attachment was quantified using crystal violet staining and is expressed as A595; bars show mean +/- sd (n=3).