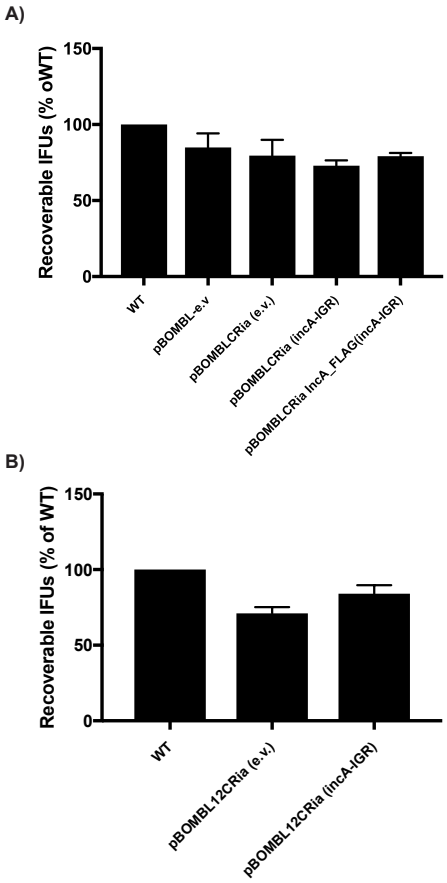
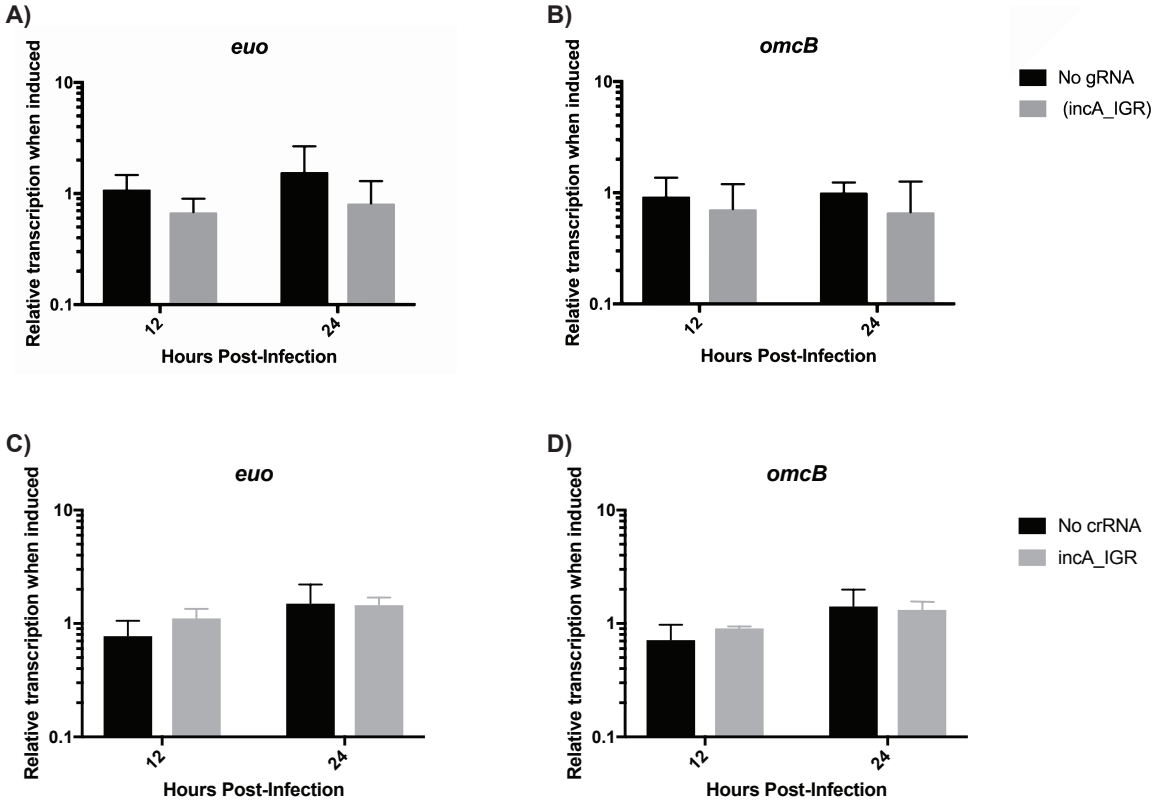
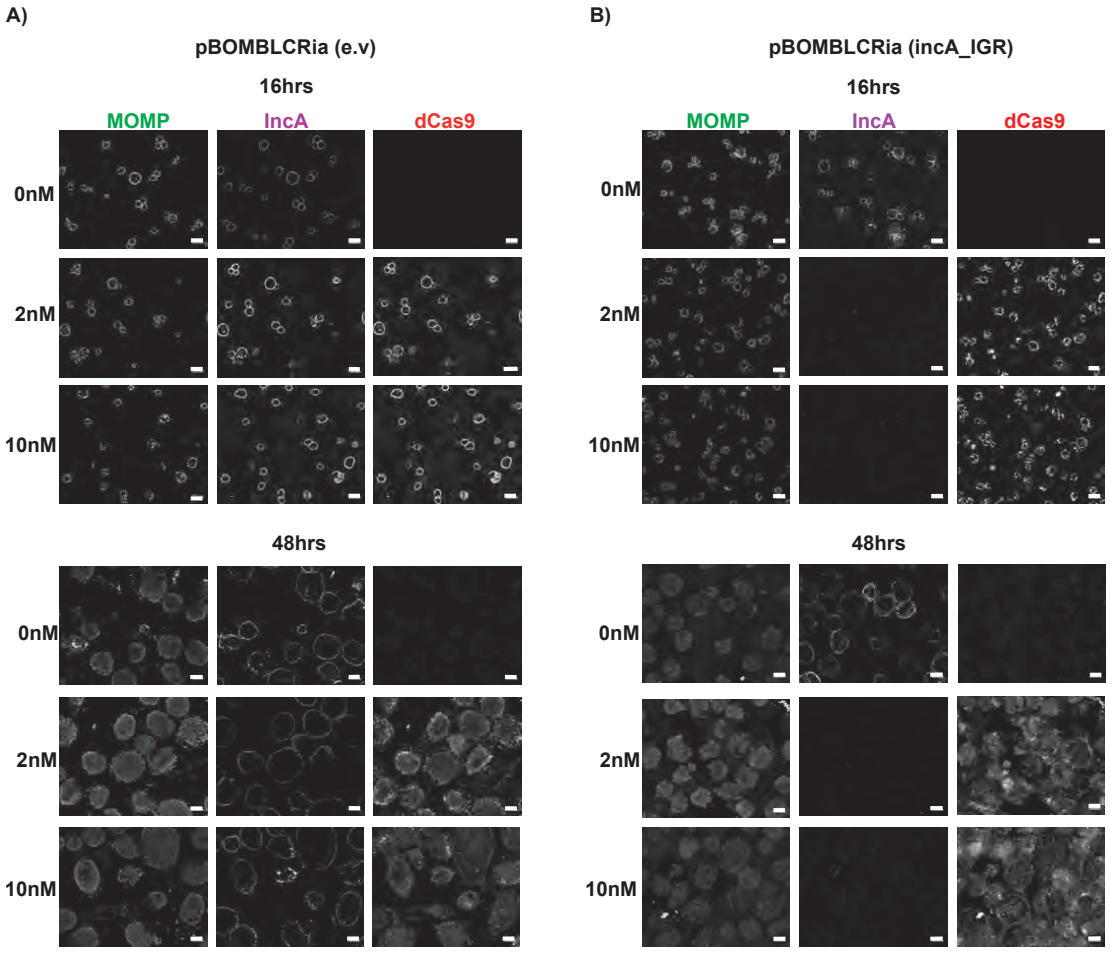


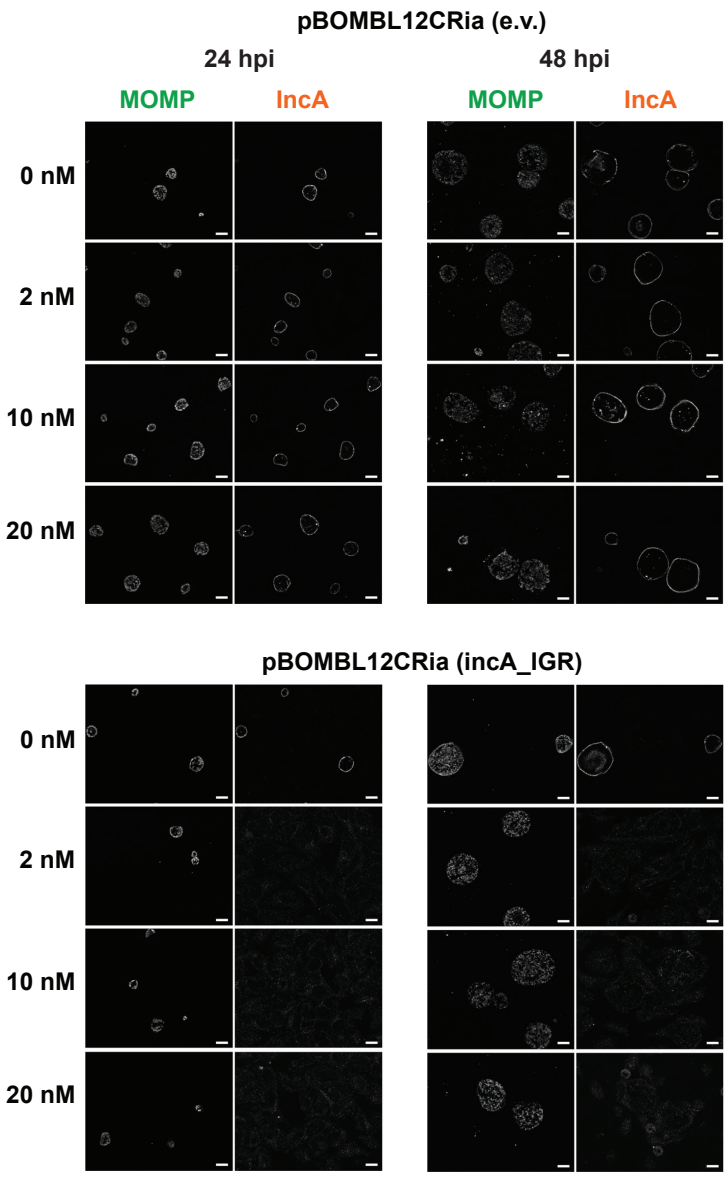
Supplemental Figure S1. Effect of vectors and plasmids used on chlamydial growth and development as assessed by IFU production. McCoy cells were infected with (A) *C. trachomatis* wild-type L2 strain, *C. trachomatis* L2 transformants carrying the pBOMBL::L2 empty vector, pBOMBLCRia::L2 empty vector, pBOMBLCRia::L2 (incA_IGR) plasmid, and pBOMBLCRia-incA_FLAG (incA_IGR) and (B) *C. trachomatis* wild-type L2 strain, pBOMBL12CRia::L2 empty vector, and pBOMBL12CRia::L2 (incA_IGR) plasmid. Infections were allowed to proceed for 24 hpi at which time the cells were harvested. The EBs were infected onto a fresh monolayer of cells, and this secondary infection was allowed to proceed for 24 hours at which time the IFUs were counted. There was no significant statistical difference between the wild-type L2 strain and the transformants as assessed by Student's two-tailed *t*-test.



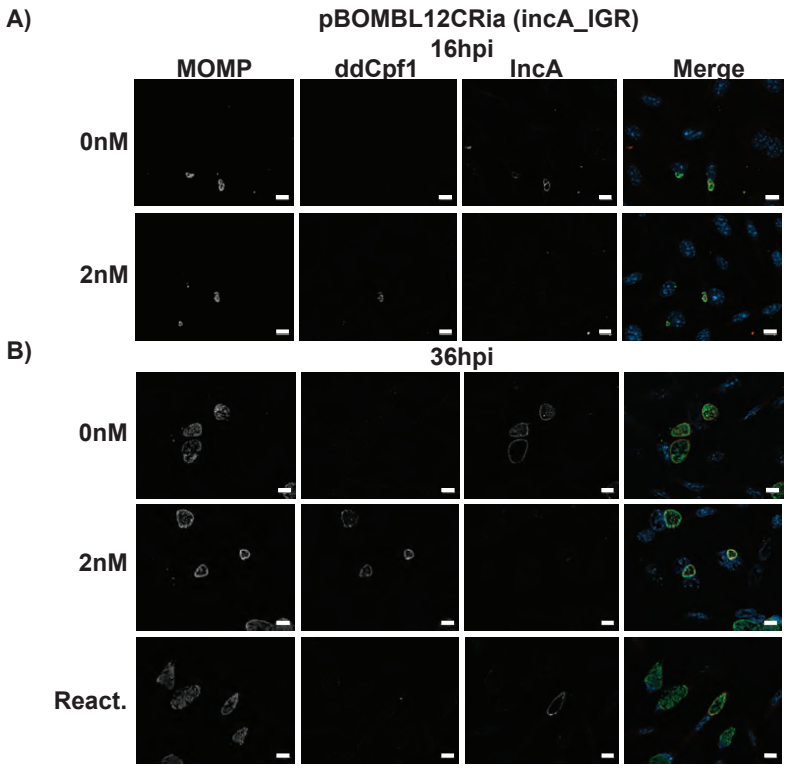
Supplemental Figure S2. Effect of dCas9 or dCas12 expression in the presence or absence of a gRNA or crRNA on developmental stage-specific gene expression in *C. trachomatis* as assessed by *euo* (A&C) and *omcB* (B&D) transcripts. (A&B) Transformants carrying dCas9 vectors were induced at 8 hpi using 10 nM aTc. RNA and DNA were isolated at 12 and 24 hpi for qPCR. (C&D) Transformants carrying dCas12 vectors were induced at 8 hpi using 2 nM aTc. RNA and DNA were isolated at 12 and 24 hpi for qPCR. All quantified cDNA was normalized to gDNA and is expressed as induced relative to uninduced samples. No statistically significant differences were detected as calculated by Student's t test.



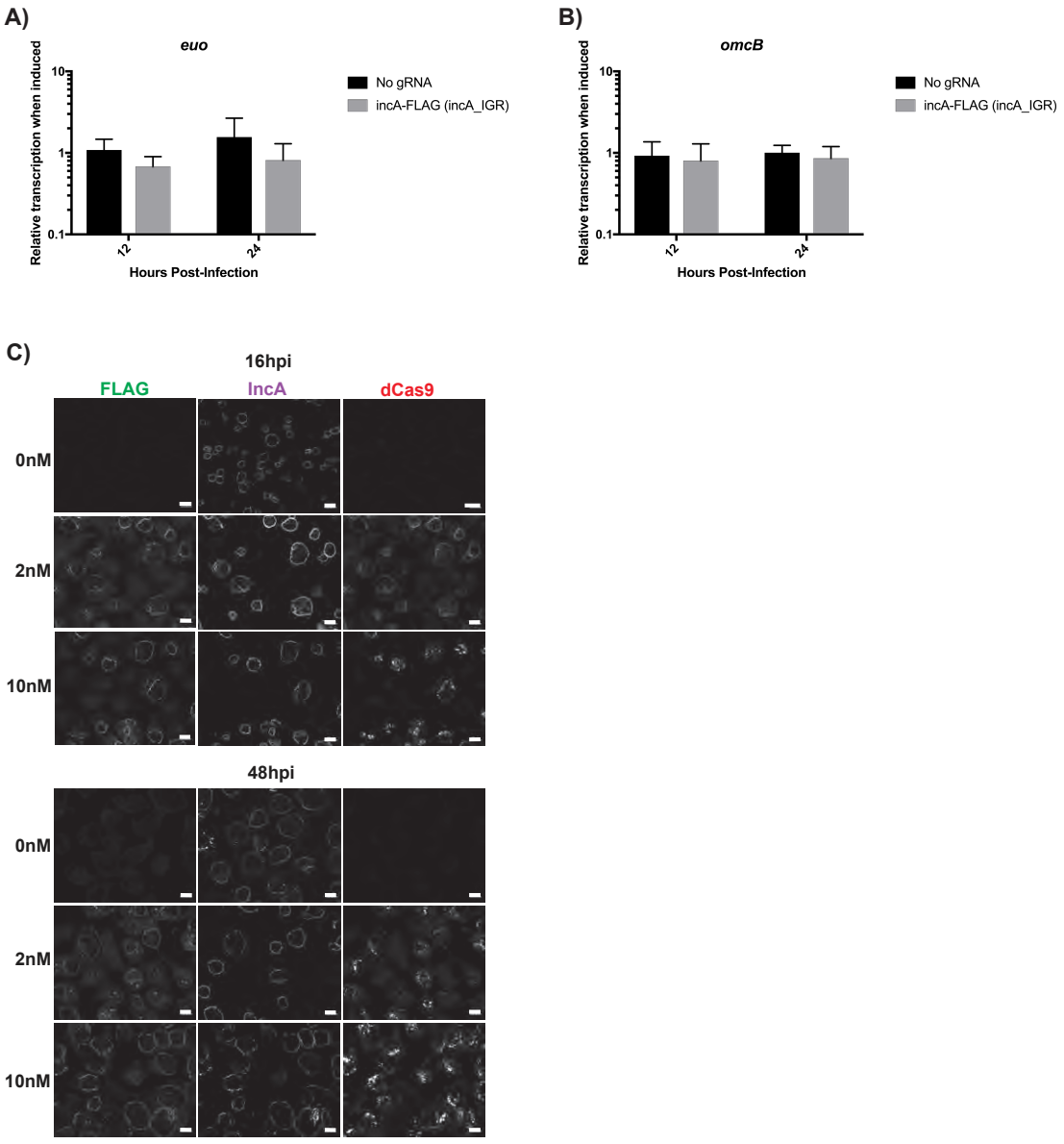
Supplemental Figure S3. Immunofluorescence time course assessment of *incA* knockdown mediated by Sa_dCas9 in *C. trachomatis* L2 transformants carrying the (A) pBOMBLCRia (e.v.) or (B) pBOMBLCRia::L2 (incA_IGR) plasmid. Samples were processed at the indicated time points for the indicated markers as described in the Materials and Methods and in the figure legend of Figure 2. All images were acquired on an AXIO Imager.Z2 with ApoTome.2 at 100x magnification. Scale bars represent 10 µm.



Supplemental Figure S4. Immunofluorescence time course assessment of *incA* knockdown mediated by As_dCas12 in *C. trachomatis* L2 transformants carrying the pBOMBL12CRia::L2 (incA_IGR) plasmid. Samples were processed at the indicated time points for the indicated markers as described in the Materials and Methods and in the figure legend of Figure 3. All images were acquired on an AXIO Imager.Z2 with ApoTome.2 at 100x magnification. Scale bars represent 10 µm.



Supplemental Figure S5. Immunofluorescence analysis of As_dCas12 (ddCpf1) in *C. trachomatis* L2 transformants carrying the pBOMBL12CRia::L2 (incA_IGR) plasmid before and after the removal of aTc. Transformants were induced at 4 hpi with 2 nM aTc. Samples were collected and stained as described in the Materials and Methods at 16 hpi (A) or 36 hpi (B). At 16 hpi, "React." samples were rinsed 2x with dPBS and replenished with DMEM and allowed to proceed until 36 hpi. All images were acquired on an AXIO Imager.Z2 with ApoTome.2 at 100x magnification. Scale bars represent 10 µm.



Supplemental Figure S6. Effect of dCas9 and IncA_FLAG co-expression in the presence of a gRNA targeting *incA* on developmental stage-specific gene expression in *C. trachomatis* as assessed by (A) *euo* and (B) *omcB* transcripts. The transformant carrying the pBOMBLCRia-incA_FLAG (incA_IGR) plasmid was induced at 8 hpi using 10 nM aTc. RNA and DNA were isolated at 12 and 24 hpi for qPCR. All quantified cDNA was normalized to gDNA and is expressed as induced relative to uninduced samples. No statistically significant differences were detected as calculated by Student's t test. (C) Immunofluorescence time course assessment of

62 *incA* in *C. trachomatis* L2 transformants carrying pBOMBLCRia-incA_FLAG (incA_IGR)
63 plasmid. Samples were processed at the indicated time points for the indicated markers as
64 described in the Materials and Methods and in the figure legend of Figure 5. All images were
65 acquired on an AXIO Imager.Z2 with ApoTome.2 at 100x magnification. Scale bars represent 10
66 μm.