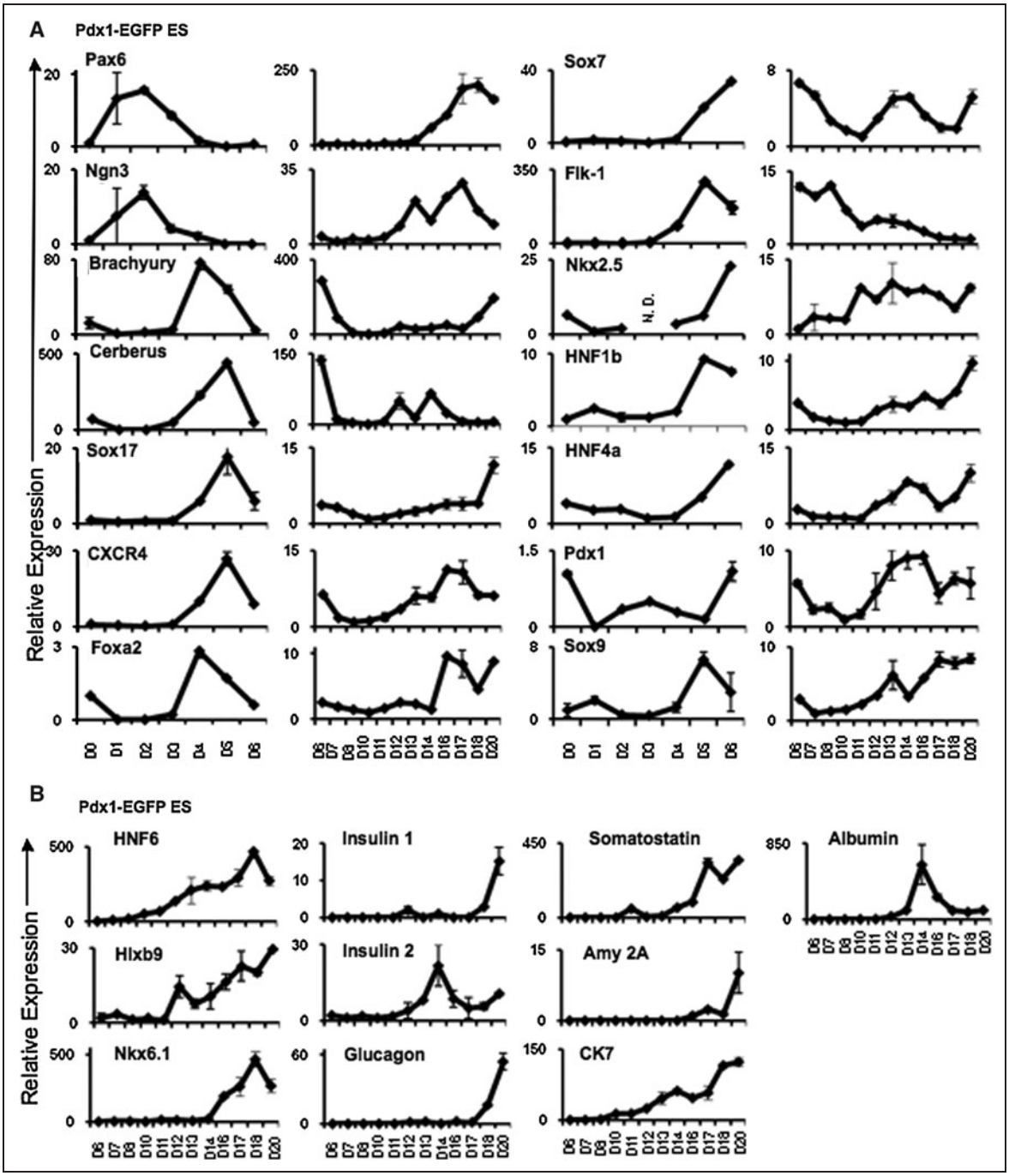
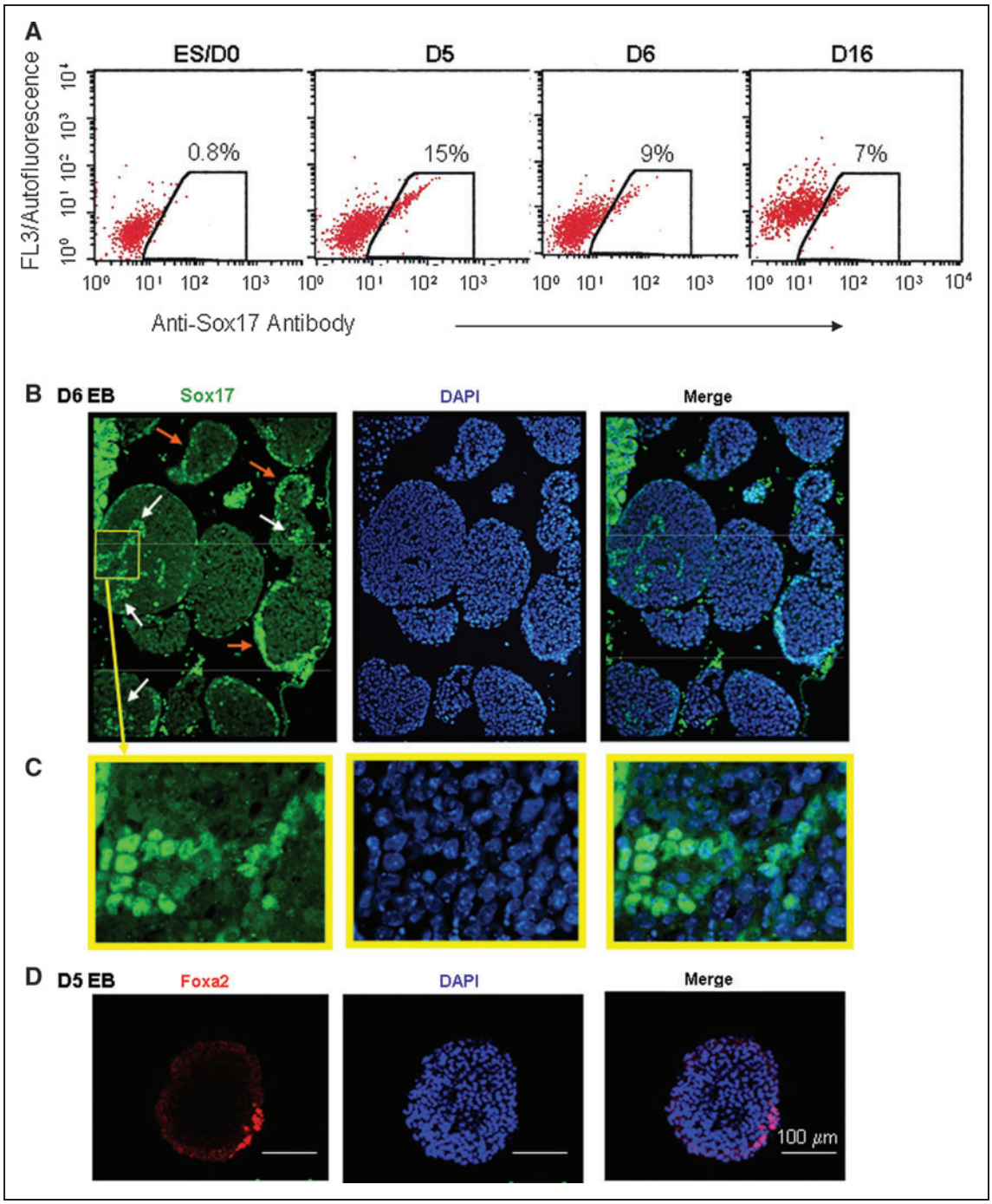
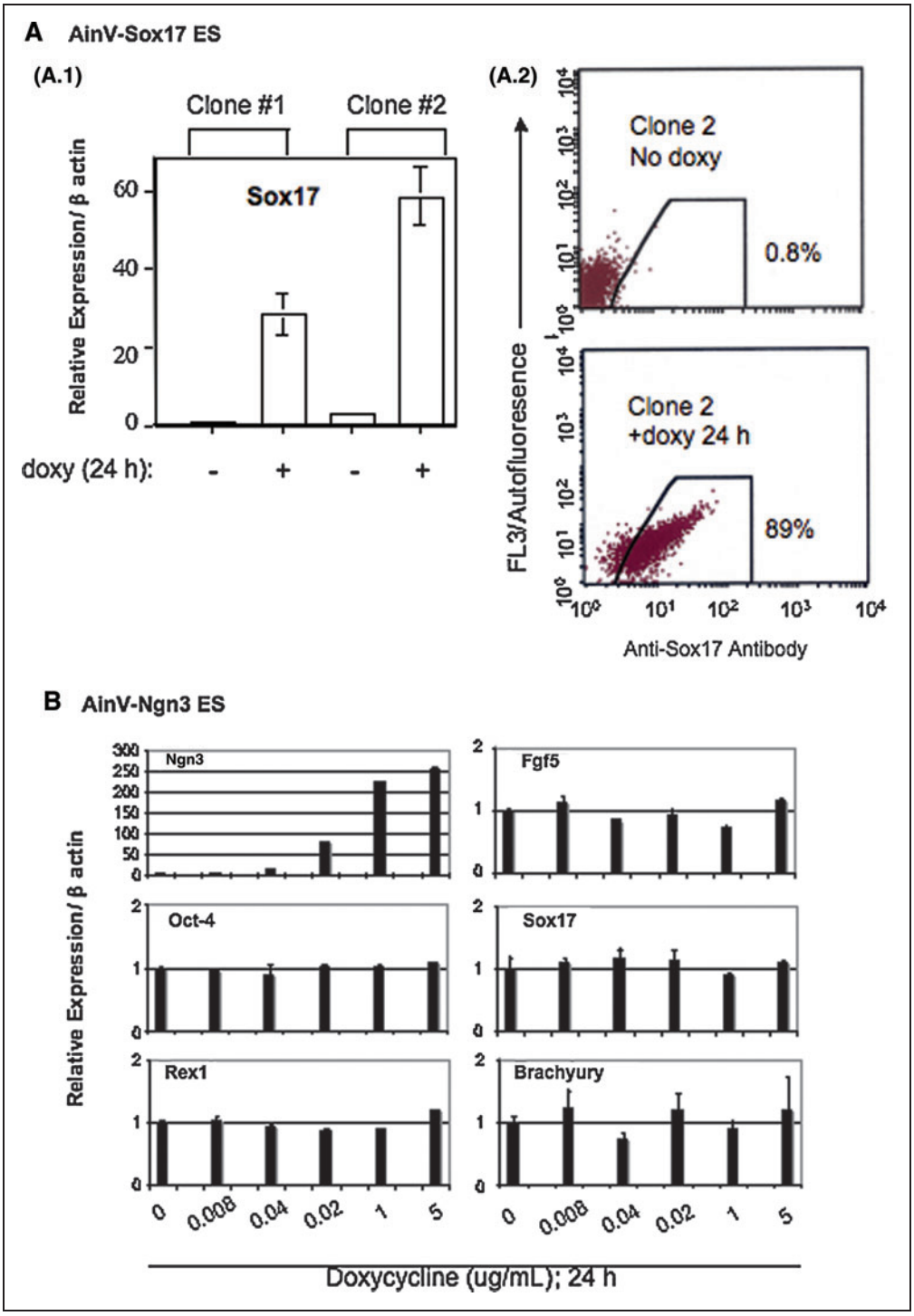
SUPPLEMENTARY DATA



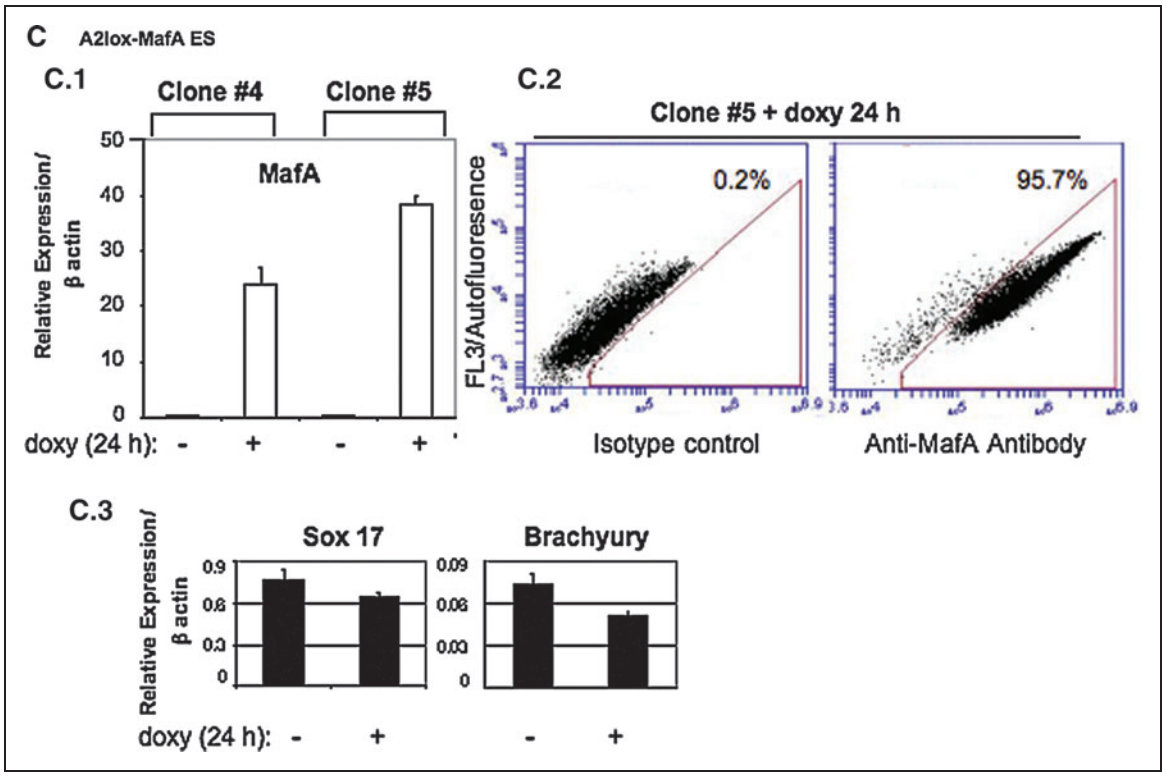
Supplementary Fig. S1. Kinetics studies of endogenous gene expression during *in vitro* differentiation of Pdx1-EGFP ES cells (Micallef *et al.*, *Stem Cell Res* 2007;1:25–36). Quantitative reverse transcription (RT)–PCR with Taqman probes was performed. β-Actin was used as internal control in these experiments. Data represent the mean and standard deviation from duplicated samples. Pdx1, pancreatic and duodenal homeobox 1; EGFP, enhanced green fluorescent protein; ES, embryonic stem; PCR, polymerase chain reaction.



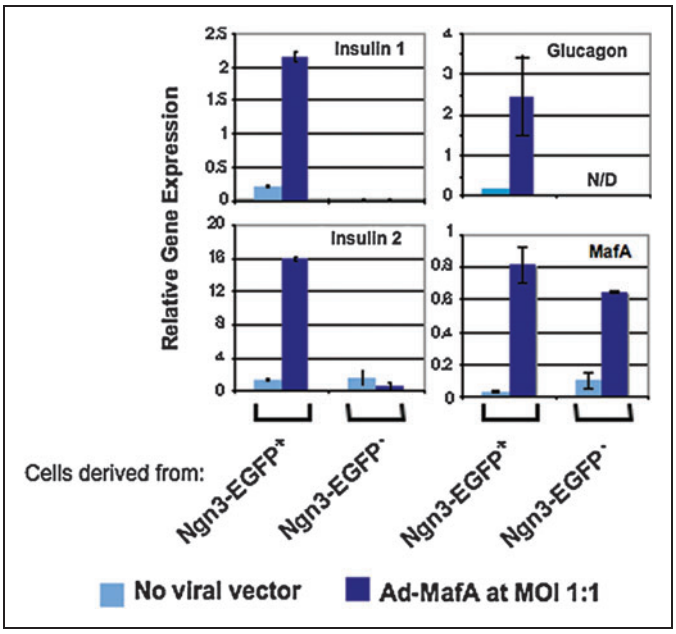
Supplementary Fig. S2. Protein expression of endogenous Sox17 or Foxa2 in embryoid body and/or attachment culture stages. R1 ES cells were differentiated. **(A)** Representative fluorescent flow cytometric analysis of Sox17 expression. **(B)** Representative immunohistological staining of Sox17. White arrows indicate intra-embryoid body staining of Sox17. Orange arrows indicate areas of outer epithelial layer that are positive for Sox17. Bright green-stained areas that are nonoverlapping with the DAPI staining are nonspecific. Magnification: 200×. **(C)** The area indicated by the yellow box in **B**. Magnification: 1000×. **(D)** Representative immunohistological staining of Foxa2. DAPI, 4′,6-diamidino-2-phenylindole.



Supplementary Fig. S3. Characterization of inducible AinV-Sox17 **(A)**, AinV-Ngn3 **(B)**, or A2lox-MafA **(C)** ES cell clones. **(A.1)** Quantitative RT-PCR analysis of Sox17 transcripts after addition of 1 μg/mL doxycycline to two undifferentiated AinV-Sox17 ES clones for 24 h. **(A.2)** Representative fluorescent flow cytometric analysis of Sox17 expression, with or without 24 h of doxycycline treatment, on undifferentiated AinV-Sox17 ES Clone #2. **(B)** A clone of AinV-Ngn3 ES cells was treated with designated graded doses of doxycycline for 24 h and analyzed for gene expression of the markers by quantitative RT-PCR. **(C.1)** Quantitative RT-PCR analysis of MafA transcripts after addition of 1 μg/mL doxycycline to two undifferentiated A2lox-MafA ES clones for 24 h. **(C.2)** Representative fluorescent flow cytometric analysis of MafA protein expression, with 24 h of doxycycline treatment, on undifferentiated Clone #5. **(C.3)** Clone #5, after treatment with or without doxycycline for 24 h, was analyzed by quantitative RT-PCR. Data represent the mean and standard deviation from duplicated samples. Ngn3, neurogenin3.



Supplementary Fig. S3. (Continued).



Supplementary Fig. S4. Effects of overexpression of MafA in sorted Ngn3-EGFP–expressing cells derived from day 16 culture. Ngn3-EGFP ES cells (Lee *et al.*, *Genes Dev* 2002;16:1488–1497), which contain an EGFP reporter gene knock-in to one allele of Ngn3, were differentiated. On day 16, cells were sorted into Ngn3-EGFP^{+} or Ngn3-EGFP^{-} populations and were plated onto Matrigel and methylcellulose-containing semisolid media, as established and described in our earlier work (Ku *et al.*, *Diabetes* 2007;56:921–929), for 8 days in the presence or absence of an adenoviral vector carrying MafA at an MOI of 1:1. At the end of the culture, cells were procured and analyzed by quantitative RT-PCR. Data represent the mean and standard deviation from duplicated samples. MOI, multiplicity of infection.