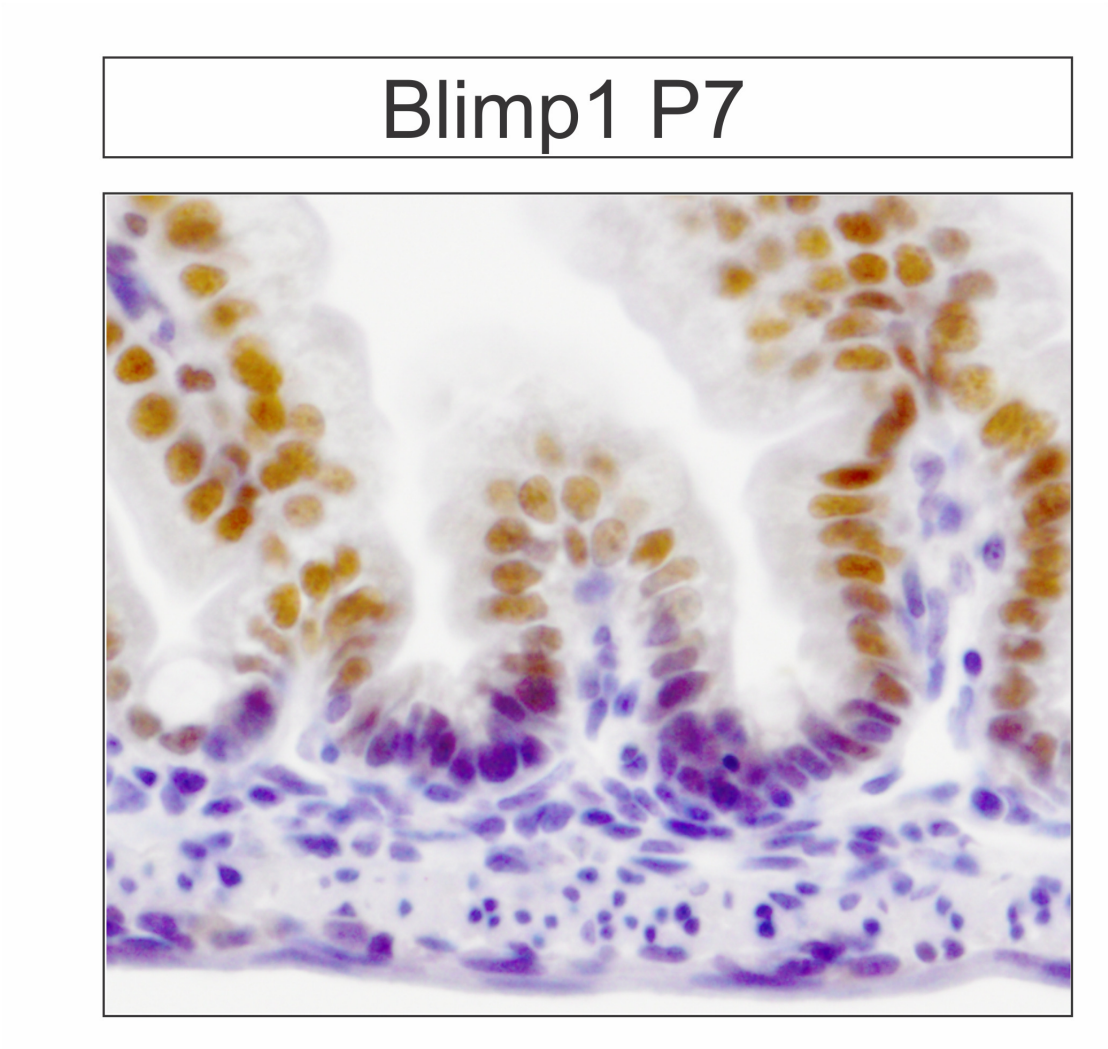
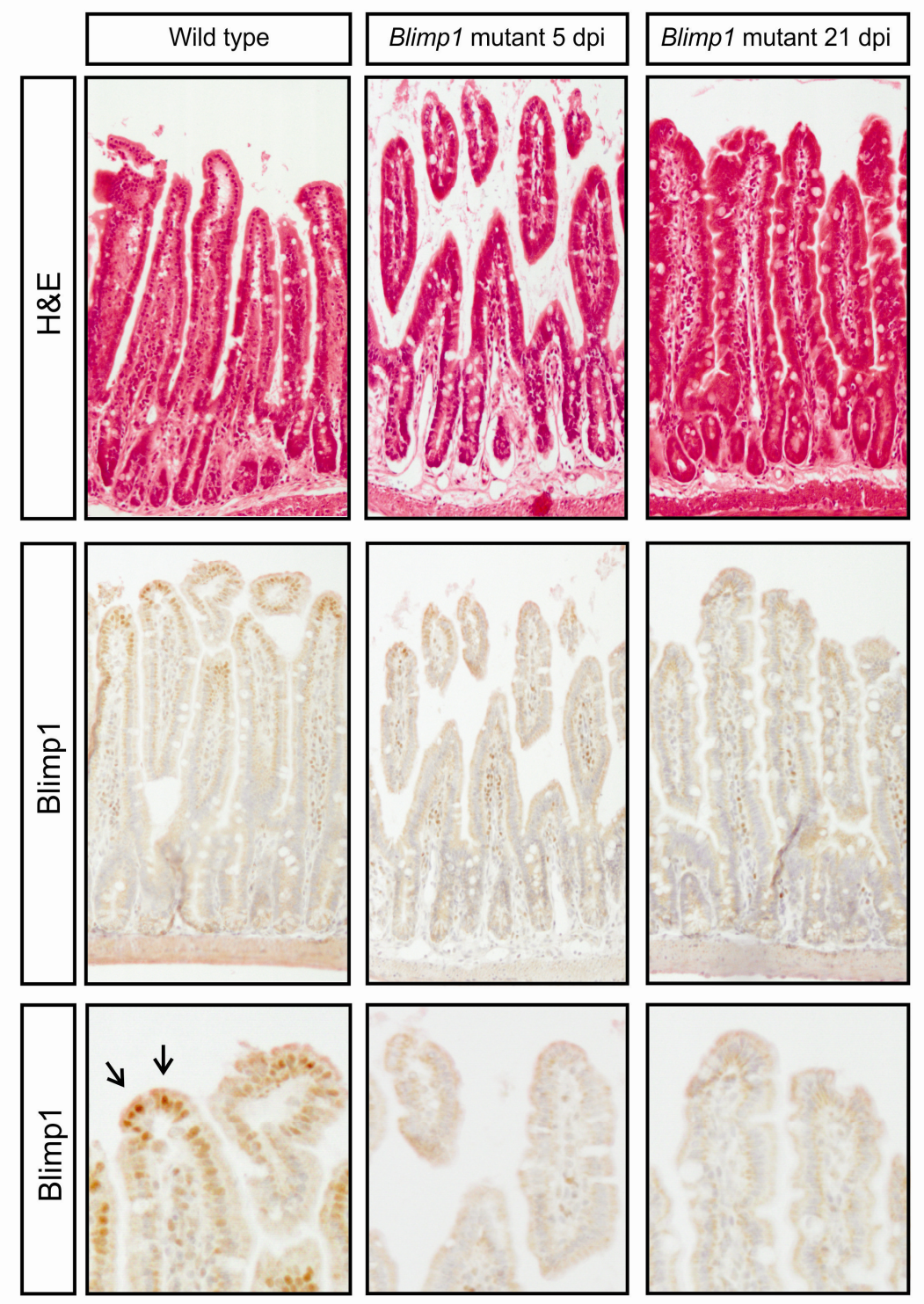
Supplementary information

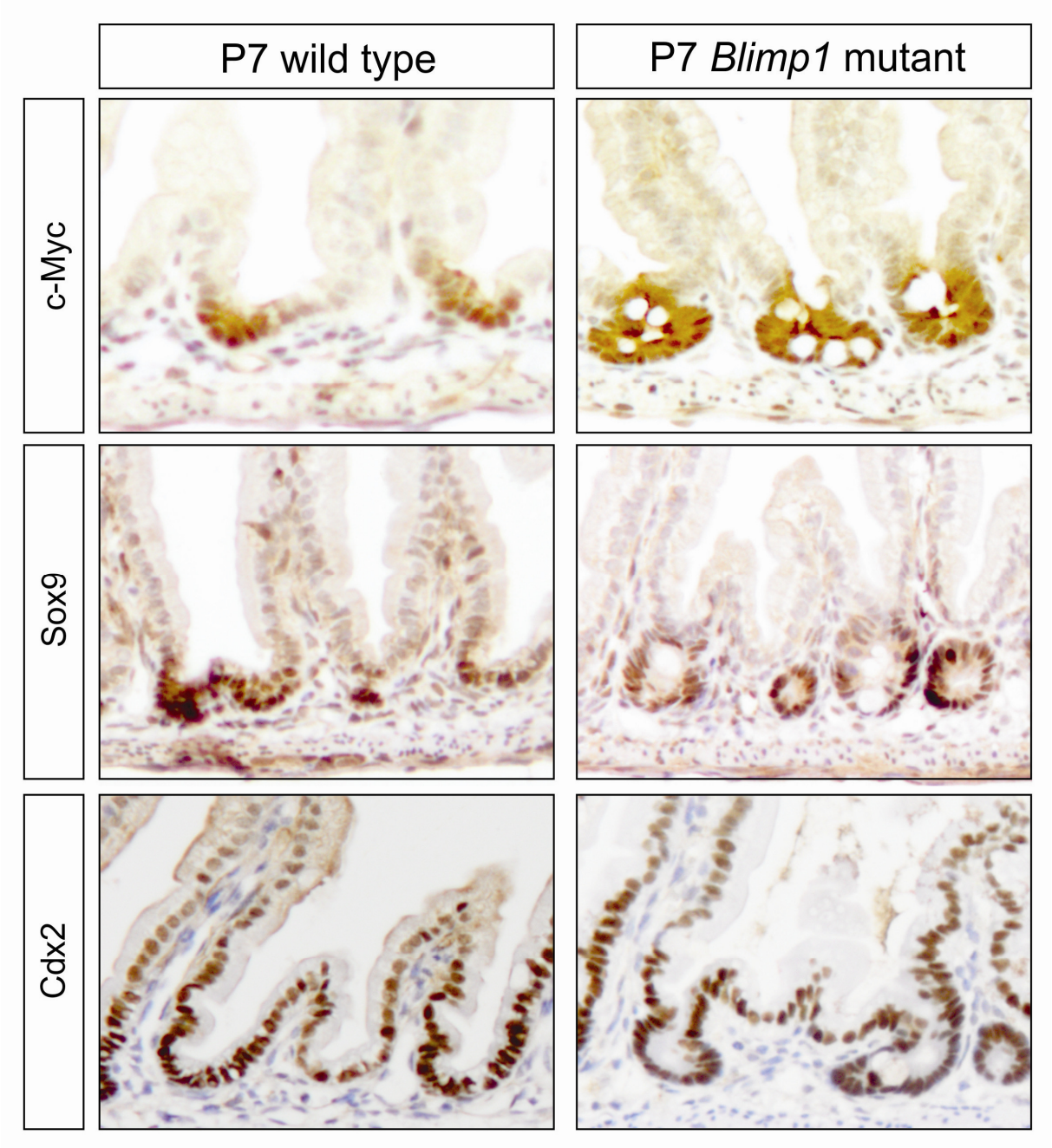
Muncan *et al*. Blimp1 regulates the transition of neonatal to adult intestinal epithelium



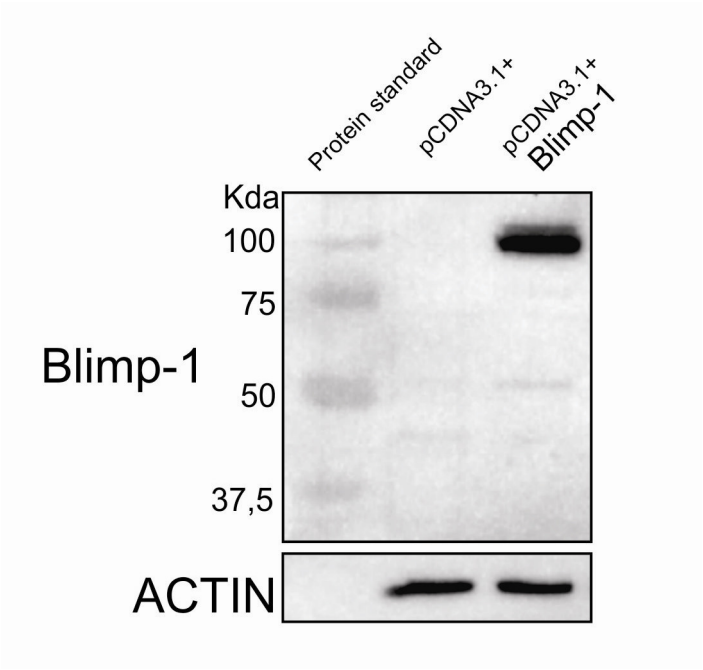
Supplementary Figure S1. Enlarged magnification of the intervillus pockets at P7. This figure shows exclusion of Blimp1 expression from the intervillus pocket. 200X



Supplementary Figure S2. No phenotype upon conditional deletion of Blimp1 from the adult intestine. Histological analysis of β-naphtoflavone injected *Cyp1a1Cre*$^{+}$*Blimp1*$^{+/+}$ (wild type) versus β-naphtoflavone injected *Cyp1a1Cre*$^{+}$*Blimp1*$^{-/-}$ (mutant) showed no phenotype at either 5 or 21 days post injection (dpi). Immunohistochemistry for Blimp1 confirmed loss of Blimp1 from the villus tips at both time points. Original magnifications 200x.



Supplementary Figure S3. No ectopic expression of c-Myc, Sox9 and Cdx2 in Blimp1 mutant animals. Immunohistochemistry for c-Myc and Sox9 showed no clear difference in the number of c-Myc and Sox9 positive cells in Blimp1 mutant animals. Expression of Cdx2 was highest in the crypt and diminished towards the villus tip at P7 in wild type mice. Expression of Cdx2 was not noticeably different in Blimp1 mutant animals. Original magnifications 200x.



Supplementary Figure S4. **Assessment of Blimp1 antibody specificity by western blotting.**A western blot of mock and Blimp1 transfected HCT116 cells shows specific detection of transfected Blimp1 with the anti-Blimp1 antibody.

Supplementary Methods

Cell culture and overexpression of Blimp-1

HCT116 colon cancer cells were cultured in DMEM, supplemented with 10% FCS, and 1% penicillin and streptomycin. For overexpression of Blimp-1, 10^6 cells were seeded in a 6-well plate and left to adhere overnight. The Blimp1 expression vector (a kind gift from Kenneth Wright) and the empty control vector were transfected using Lipofectamine 2000 (Invitrogen) according to manufacturers instructions. Cells were left for 24 hours before harvesting.

Immunoblotting

Cells were lysed in cell lysis buffer (Cell Signaling Technology, Leiden, Netherlands), and boiled in sample buffer containing 0.25M Tris-HCl pH 6.8, 8% SDS, 30% glycerol, 0.02% bromophenol blue and 1% β-ME. Separation was done on 10% SDS-PAGE, and proteins were transferred to a PVDF membrane. Specific detection was done by incubating the blot overnight in TBS with 0.1% Tween-20 with 1% BSA with anti-BLIMP-1 (1:500; Santa Cruz Biotechnology, CA, USA) and anti-ACTIN (1:2000; Santa Cruz Biotechnology, CA, USA) antibodies. Antibody binding was visualized using the Lumi-Light western blotting substrate (Roche).