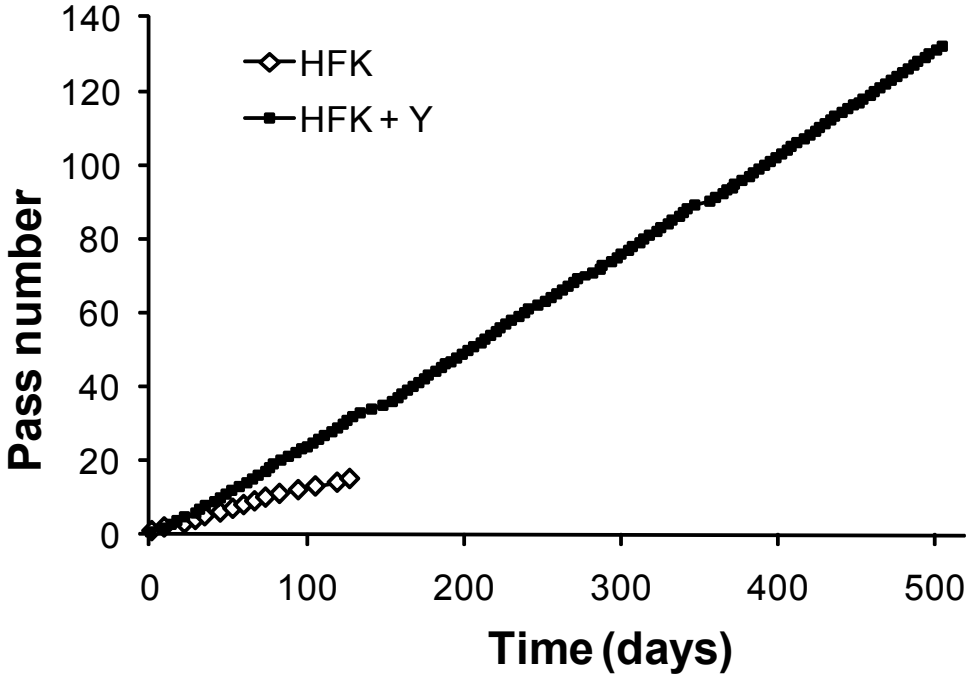
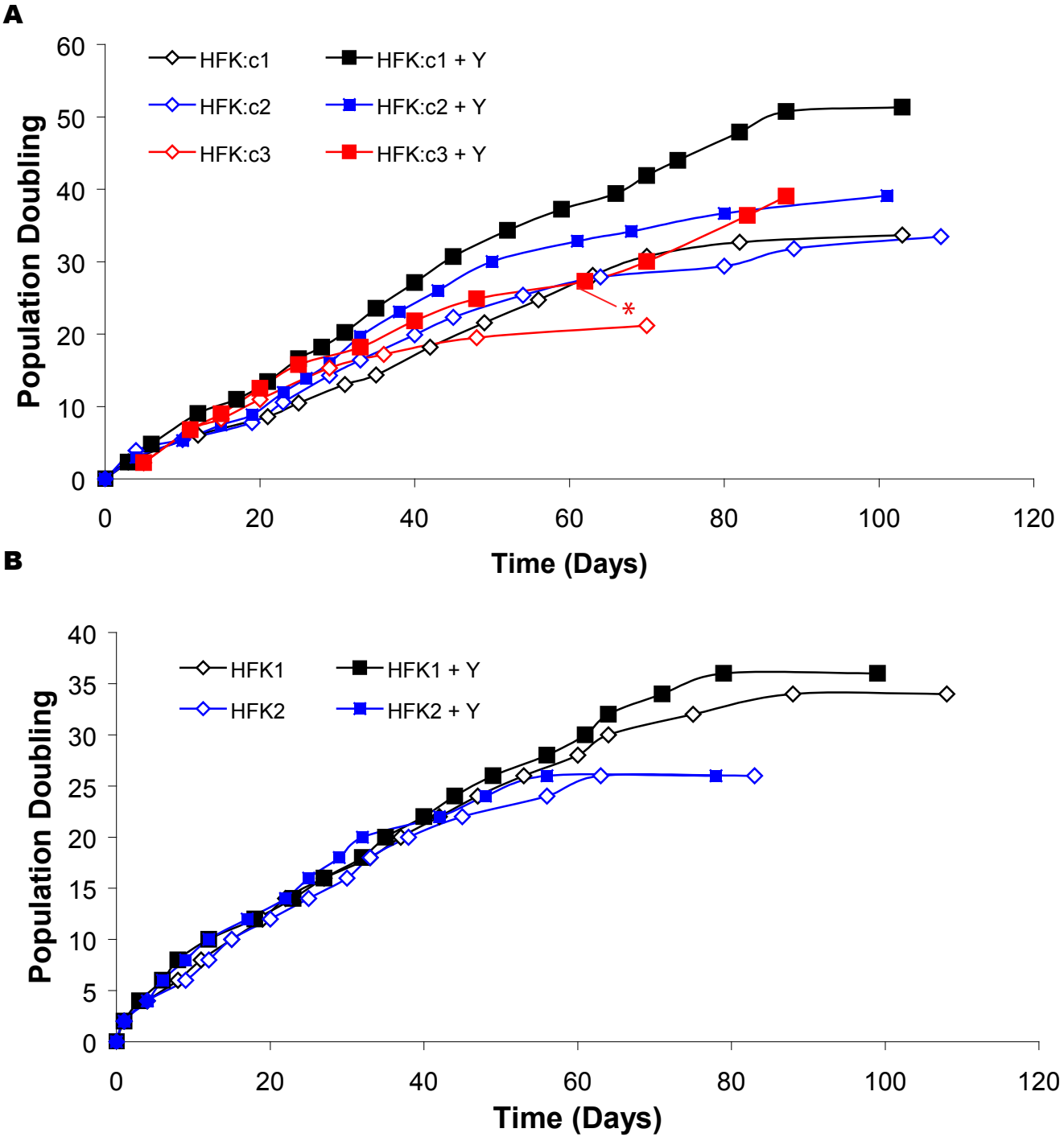
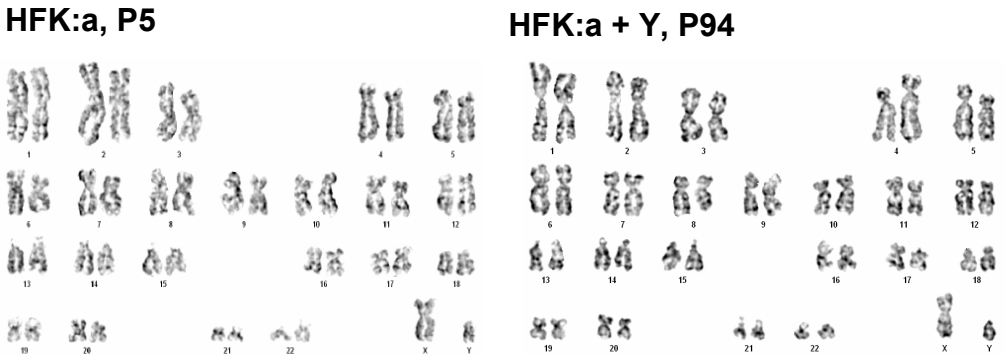


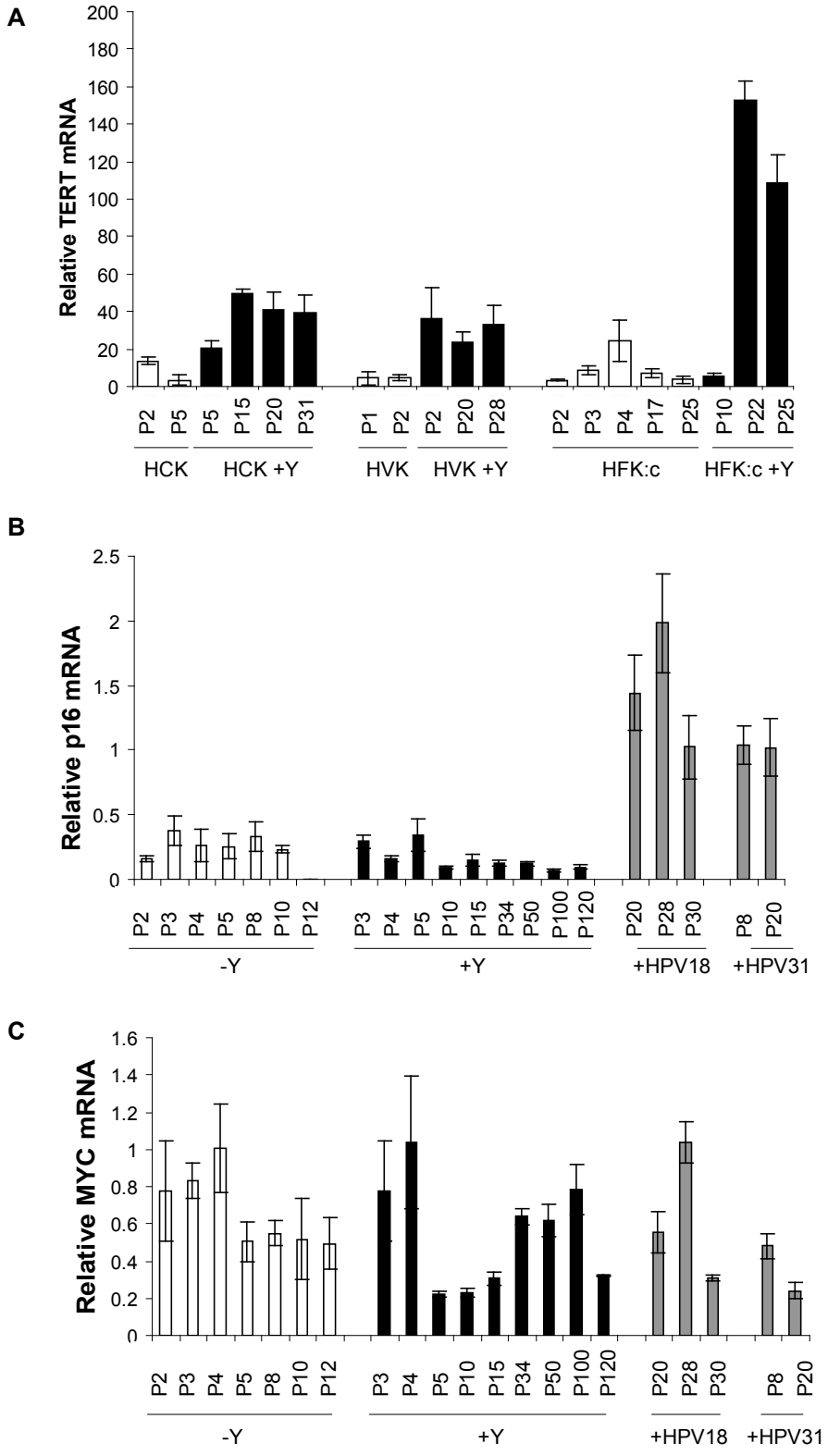

**Supplementary Figure 1. Treatment with Y-27632 immortalizes primary keratinocytes.** Human foreskin keratinocytes (HFK:a strain) were cultured in the presence (solid squares) or absence (open diamonds) of 10μM Y-27632. Cells were passed at a ratio 1:10 at the times shown, with a few exceptions when cells were split 1:20.



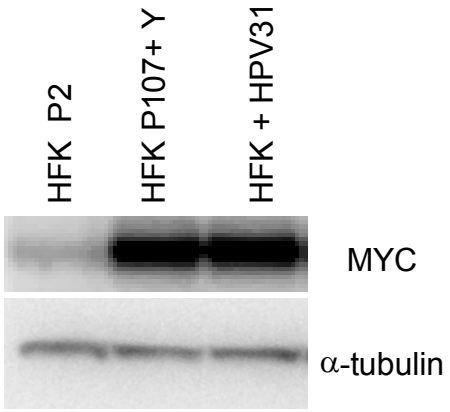

**Supplementary Figure 2: Y27632 does not immortalize keratinocytes in the absence of feeder fibroblasts.** HFKs were passed on five separate occasions on plastic in (A) serum-free 154 medium or in (B) KGM media in the presence or absence of (B) 5μM or (A)10μM Y-27632. All cells became senescent and ceased growing at the times indicated, except f or HFK:c3 +Y, where cells grew out after crisis (*).



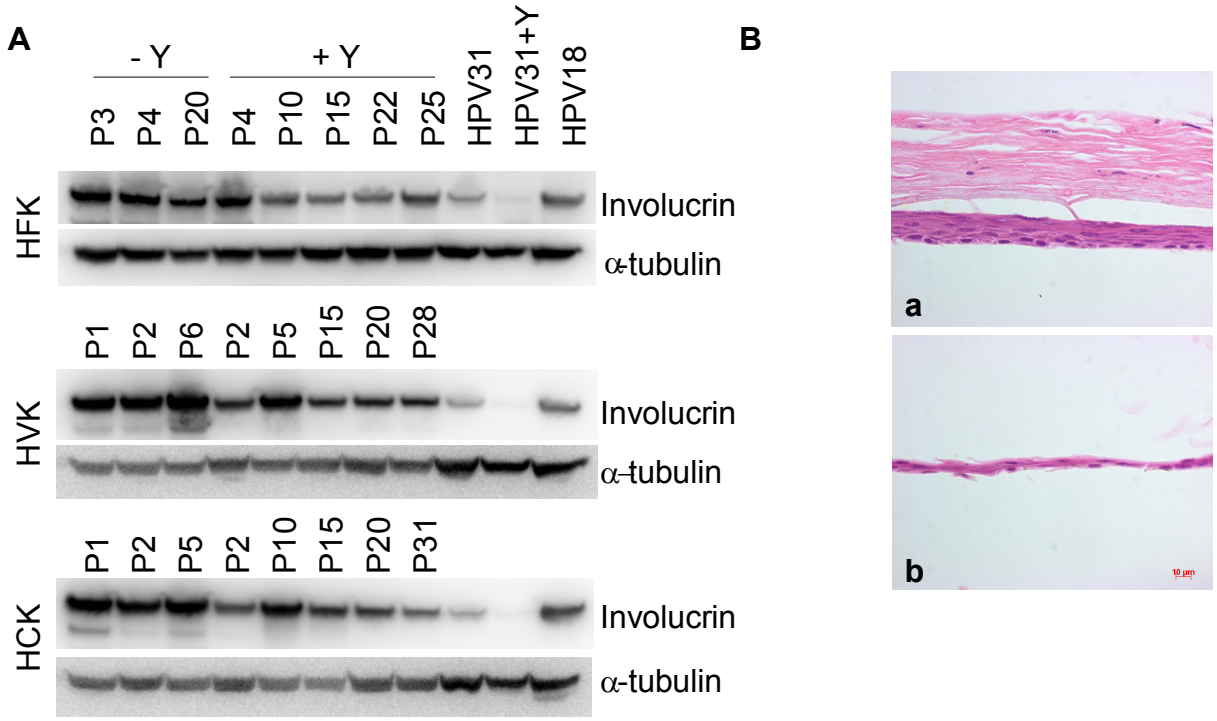

**Supplementary Figure 3 Y-27632 immortalized keratinocytes show no major chromosomal abnormalities**
Karyotype of the HFK:a strain at passage 5 and after 95 passages in Y-27632.




**Supplementary Figure 4.** Relative mRNA levels of hTERT, p16INK4 and MYC in Y-27632-treated cells. A. Levels of hTERT mRNA were determined in HFK:c strain , HCK and HVK cells cultured in the absence or presence of Y-27632 by QRT-PCR. B. Levels of myc mRNA were determined in HFK:a strain cultured in the absence or presence of Y-27632, HPV18 immortalized keratinocytes and HPV31 containing CIN612 cells QRT-PCR. C. Levels of p16INK4 mRNA were determined in HFK:a strain cultured in the absence or presence of Y-27632 , HPV18 immortalized keratinocytes and HPV31 containing CIN612 cells QRT-PCR. In each case, the levels of mRNA were standardized relative to GAPDH transcript levels. Each value represents the mean of replicate values +/- SD.



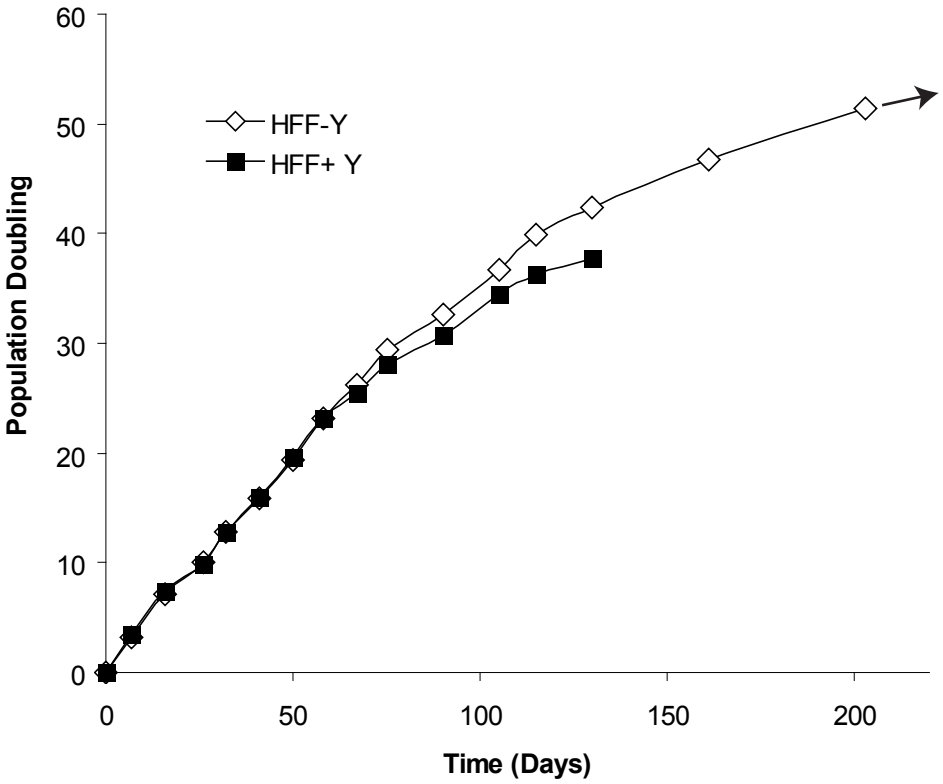

**Supplementary Figure 5 MYC protein levels are upregulated in Y-27632 immortalized cells at a late passage.** Immunoblot analysis of MYC protein in HFK:a strain at passage 2, or after 107 passages in 10μM Y-27632. CIN-612 cells, containing the oncogenic HPV31, are included as a comparison. Tubulin is included as a loading control.




**Supplementary Figure 6. Y-27632 inhibits differentiation in monolayer and organotypic raft culture**

A. Immunoblot analysis of Involucrin proteins in HFK strain c, HVK, and HCK cells in the absence or presence of 10μM Y-27632, collected at the pass indicated. Cells containing oncogenic HPV31 and HPV18 viruses are included as controls. HPV 31 +Y was grown in 10μM Y-27632 for 15 passes.

B. HFK P1 keratinocytes grown in organotypic raft culture for 14 days in raft media (a) without or (b) with 10μM Y-27632 .




**Supplementary Figure 7. Treatment with Y-27632 does not immortalize primary human foreskin fibroblasts.** Human foreskin fibroblasts were cultured in the presence (solid squares) or absence (open diamonds) of 10μM Y-276T32. Cells were passed when confluent at the times shown. Arrow indicates continued growth.

| STR locus | Allele present | |
|---|---|---|
| | HFK:a P4 | HFK:a +Y, P94 |
| D5S818 | 11 | 11 |
| D13S317 | 11,13 | 11,13 |
| D7S820 | 10 | 10 |
| D16S539 | 11,12 | 11,12 |
| vWA | 16,17 | 16,17 |
| TH01 | 6,9 | 6,9 |
| Amelogenin | X,Y | X,Y |
| TPOX | 8,11 | 8,11 |
| CSF1PO | 11,12 | 11,12 |

**Supplementary Table 1. Short Tandem Repeat Analysis of HFK:a strain**
The HFK:a strain was analyzed at P4 and after 94 passages in the presence of Y-27632