Figure S1. Highly purified preparations of pol III contain POLR3GL. A. Protocol for simple- (Flag tag) or double (Flag and His tags) affinity chromatography purification of tagged pol III. B. Silver-stained protein gel showing the material obtained after Flag, or Flag and His tag, purification. The identities of the bands labeled POLR3A, POLR3B, POLR3E, POLR3C, POLR3D, POLR1C, and POLR3F were confirmed by western blotting (not shown). C. Human POLR3G and POLR3GL amino acid sequences. The regions highlighted in blue (POLR3G) or green (POLR3GL) correspond to one or several peptides detected by mass spectrometry. D. The genome was searched for regions with homology to the various pol III subunits coding sequences. The first column shows the name of the sequence used in the search (and the chromosome on which it is located). The second and third columns show the genomic location and the name of homologous regions. The percentage identity and the presence or absence of the homologous sequence in the EST database are also indicated.

FigureS2. POLR3G/POLR3GL protein family. The phylogenetic tree was constructed with the Nearest neighbor interchange (NNI) and the LG substitution model using PhyML. In red, blue, and green, species with one, two, and three POLR3G/POLR3GL homologues, respectively. Three distinct groups are highlighted in grey boxes. The sequences in the first major cluster (second grey box) are POLR3G-like, those in the bottom cluster (third grey box) POLR3G-like. Scale bar indicates 0.1 change per amino acids. The analysis was performed with 1000 bootstrap trials to provide confident estimates for phylogenetic tree topologies. Bootstrap probabilities are shown in percentages. Values below 50% are not shown.

Figure S3. *C. intestinalis* and *P. marinus* have a gene closer in sequence to human *POLR3GL* than human *POLR3G*. A. Alignment of the *C. intestinalis* protein sequence with human POLR3G. B. As in B but with human POLR3GL. C. Alignment of the *P. marinus* protein sequence with human POLR3G. D. As in B but with human POLR3GL. The sequences were aligned with ClustalX with the default parameters.

Figure S4. POLR3G- and POLR3GL-containing pol III. A. POLR3G and POLR3GL lie in separate complexes. Immunoprecipitation was performed with antibodies directed against POLR3G or POLR3GL, as indicated above the lanes, and the precipitated proteins were then analyzed by immunoblots with antibodies directed against POLR3C, POLR3G, or POLR3GL, as indicated on the left. B. HeLa whole cell extract elution profile on a Superose 6 gel filtration column. The x-axis shows fraction number as well as elution volume, and the y-axis the absorbance at 280 nm (in "milli arbitrary units" mAU). The elution of size markers is indicated. C. Western blotting with antibodies directed against POLR3A and POLR3C in fractions 1 to 26. D. The fractions indicated above the lanes were used as starting material for immunoprecipitations with pre-immune (pre) or anti-POLR3C antibodies. The immunoprecipitated material was then analyzed by western blot with antibodies directed against the subunits indicated on the left. E. POLR3G, POLR3GL, or GFP as a negative control were fused to GST and used in GST pull-down experiments with *in vitro* translated, radiolabeled POLR3C, POLR3F, BRF1, and BRF2.

Figure S5. *POLR3GL* is relatively more expressed than *POLR3G* in non-dividing cells compared to dividing cells. A. *POLR3GL* and *POLR3G* mRNA levels were measured by RT-PCR in human IMR90 cells placed either in medium containing 10% fetal calf serum or no serum, as described in Methods. The data were normalized relative to levels of *POLR3C* mRNA, and the level of *POLR3G* mRNA in non-treated cells was set to 1. B. As in A but in IMR90Tert cells plated at the required concentrations to obtain cells at 25, 50, and 100% confluency 18 h later; the level of *POLR3G* was set to 1 in cells at 25% confluency. C. *POLR3GL* and *POLR3G* mRNA levels were measured by RT-PCR in human Feo and 4A cells at similar densities, and normalized to *POLR3C* mRNA levels. The level of *POLR3G* mRNA was set at 1. D. *Polr3g* and *Polr3gl* mRNA levels were measured by RT-PCR in mouse liver: for comparison, expression from two genes that are silent in liver, *Ucp1* and *Pdk4*, was also measured. The *Polr3g* level was set at 1. E. *Polr3g*, *Polr3gl*, and *Polr3c* mRNA levels were measured in Hepa 1-6 cells. The *Polr3g* level was set at 1. All experiments were repeated three times with different preparations of cDNA. In all panels, two stars indicate a p-value >0.1 and one star indicates a p-value >0.5.

Figure S6. Several pol III-occupied loci are likely to be deleted or rearranged in Hepa 1-6 cells. The three upper panels and three lower panels show examples of tRNA genes that appear completely or partially deleted, respectively, in Hepa 1-6 cells. Note the differences in the tag accumulation scales (y-axis) for the input and the POLR3D tracks in the UCSC browser views to allow visualization of the input signal.

Figure S7. MYC binds to the TSSs of all pol III subunit encoding genes except that of *POLR3GL*. A. UCSC browser views of the *POLR3A* promoter region showing MYC, MAX, and pol II (antibody directed against the N-terminus of POLR2A, Santa Cruz sc-899) tag accumulation, as indicated on the right, in P493-6 cells at time 0, 1 h, and 24 h after induction of MYC, or in SCLC H128_1, SCLC H2171_1, U87, and MM.1S cells, as indicated on the left. The y-axis shows tag accumulation and the scales in all panels go from 0 to 10. B-K. As in A, but for the gene promoter regions indicated at the bottom. Based on the data of (Lin et al. 2012).

Figure S8. Spearman's rank correlation of scores obtained considering only tags sequenced once (non-redundant tags) and non-redundant tags as well as redundant tags with a cutoff at 50 (i.e. tags sequenced more than 50 times were counted as 50) for the indicated samples. Panels *c*: x-axis, scores obtained with non-redundant tags only; y-axis, scores obtained with the sum of non-redundant tags and redundant tags counted up to a maximum of 50; in blue the x=y line; in red, the regression line. Panels *b*: correlation coefficients. Panels *a*: distribution histograms representing, for each non-redundant tag score interval of 0.5 (IMR90), 0.5 (Liver-rep1), 0.5 (liver-rep2), 0.5 Hepa 1-6-rep1), and 0.5 (Hepa 1-6-rep2) (see in each case x-axis at the bottom of corresponding Panel *c*), the number of genes in that interval (see in each case the y-axis at the right of the panel: the numbers in green correspond to the lowest, middle, and highest number of genes). Panels *d*: as in panels *a* but for each

non-redundant + redundant tag score interval of 0.5 (IMR90), 1 (Liver-rep1), 1 (liver-rep2), 1 (Hepa 1-6-rep1), and 1 (Hepa 1-6-rep2).