

Supplementary Data for:

The EGF Receptor Ligand Amphiregulin Controls Cell Division via FoxM1

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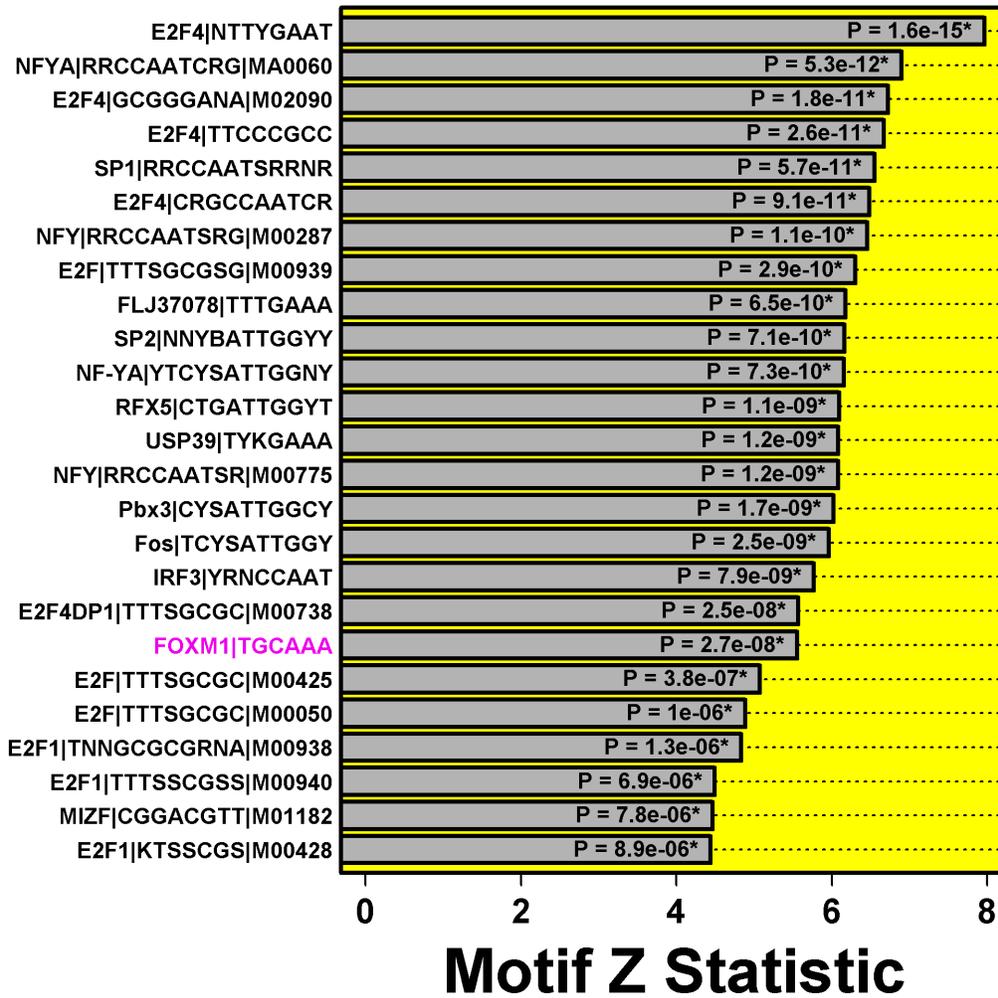


Figure S1: Transcription factor binding sites in 1 KB upstream regions of genes whose expression was decreased by AREG silencing and not normalized by EGF. The names of the transcription factor and its binding site motif are shown on the left and the p values are shown in the grey bars. Only the 25 most significant transcription factor binding sites are shown.

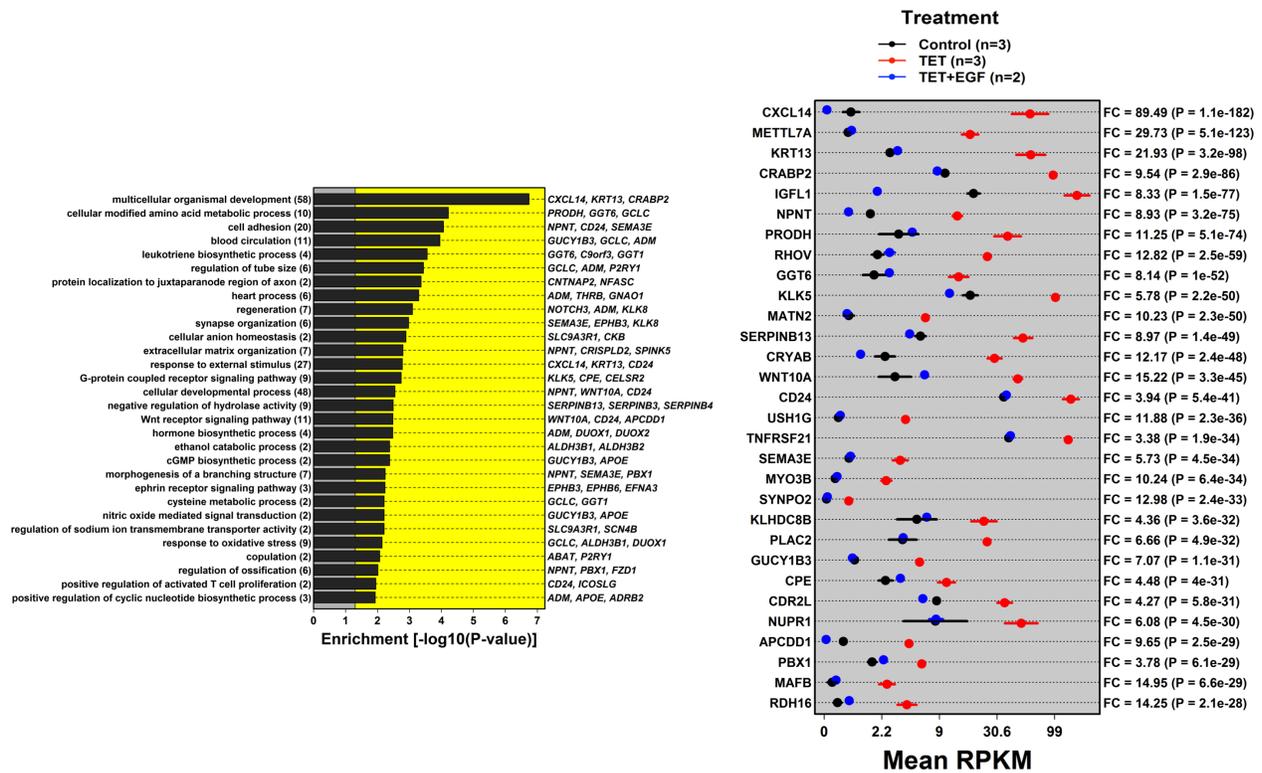


Figure S2: Genes whose expression was increased by AREG silencing and reversed by EGF are enriched for biological processes of organismal development and metabolism. N/TERT-TR-shAREG cells were incubated in the presence or absence of Tet with and without 20 ng/ml EGF for 60 h, followed by analysis of global gene expression by RNA-seq. Only the 30 most significant genes are shown. *Left panels:* bars indicate p values for the most significant GO terms with the categories on the left, (followed by the number of genes for each category in parentheses), and the most significantly altered transcripts shown on the right. *Right panels:* Gene names are indicated on the left and fold-change (FC) for Tet versus control and P-values are shown on the right. Black = control, blue = Tet, red = Tet +

EGF. Numbers underneath the panel are mean RPKM (reads per kilobase per million mapped reads). Only the 30 most significant genes are shown.

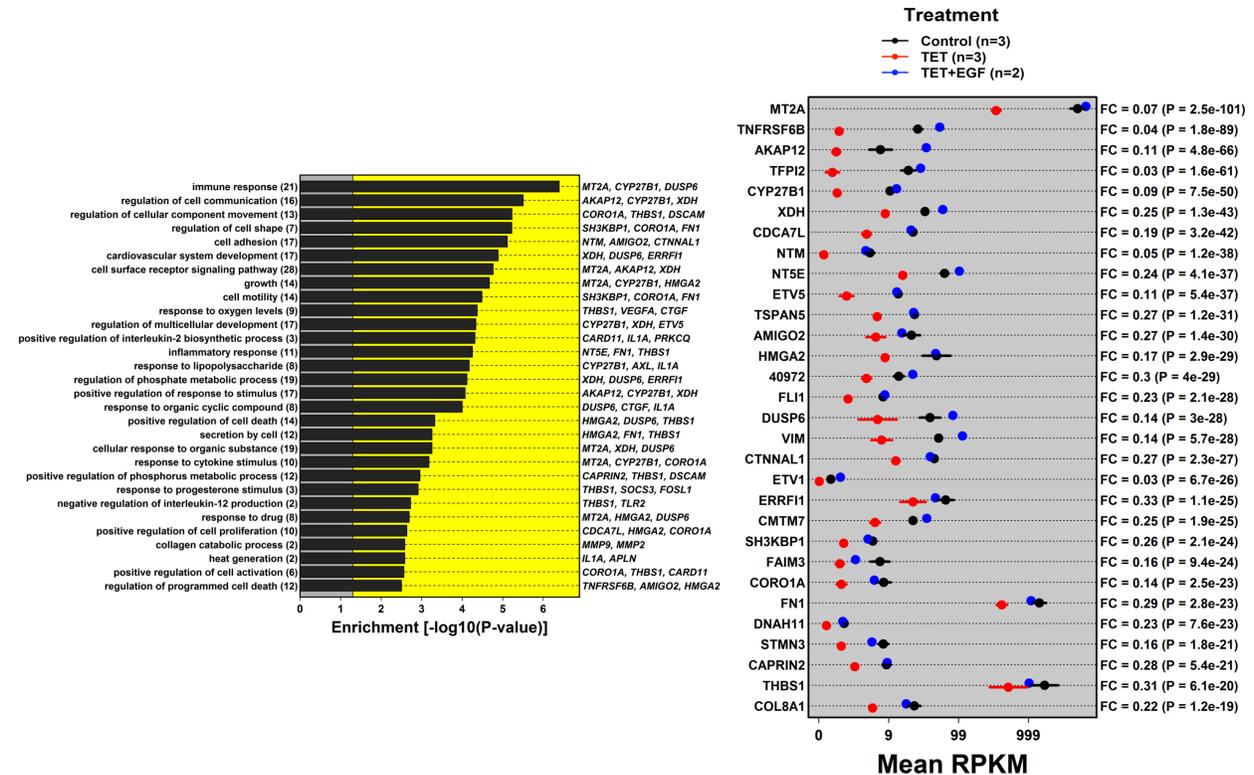


Figure S3. Genes whose expression was decreased by AREG silencing and restored by EGF are enriched for biological processes of immune responses and regulation of cell communication. Experimental description is the same as for Figure S2.

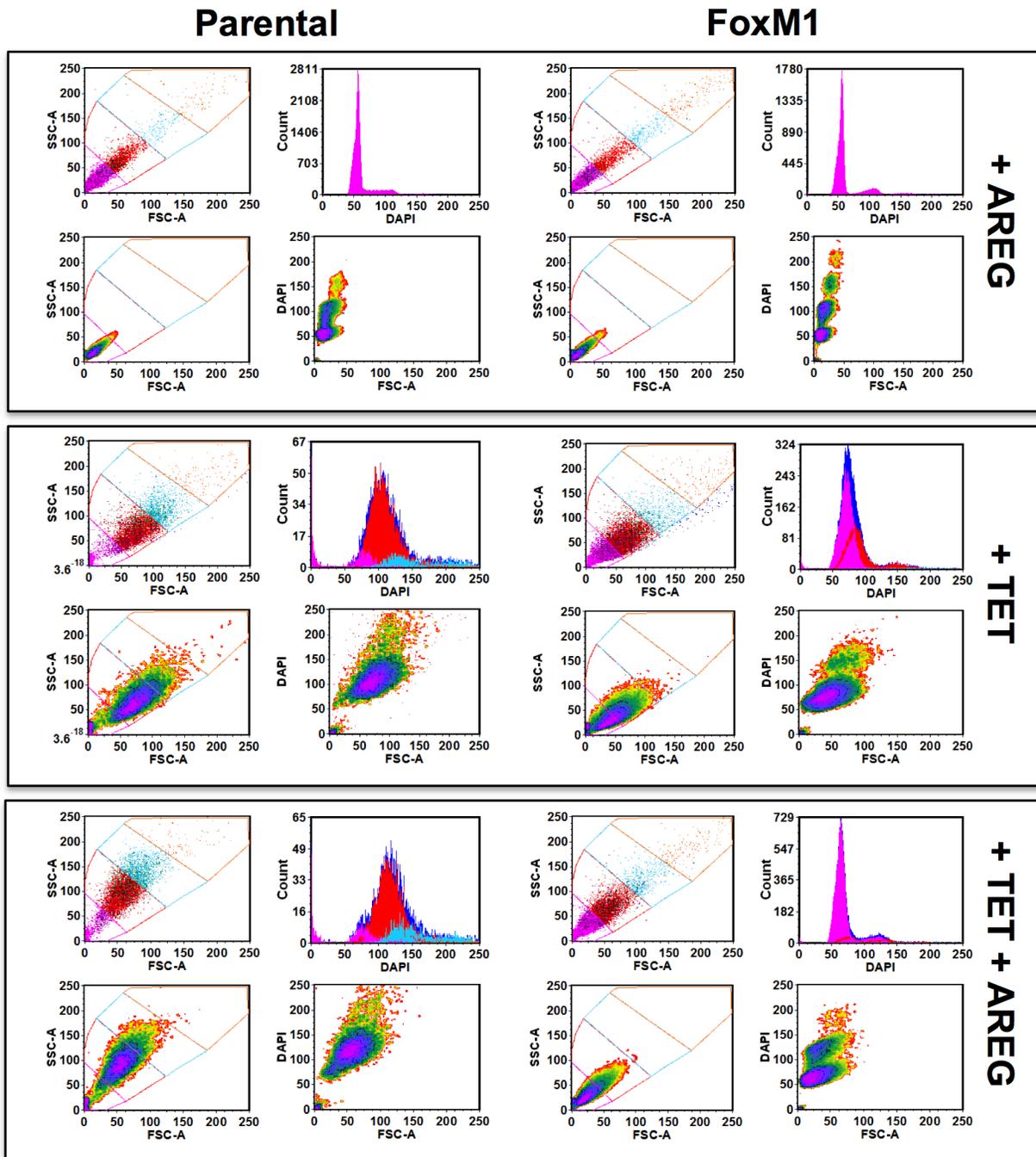


Figure S4: FoxM1 expression normalizes cell cycle distribution in AREG knockdown cells. N/TERT-TR-shAREG keratinocytes (parental) and N/TERT-TR-shAREG cells stably infected with a constitutive FoxM1 expression construct (FoxM1-

rescue cells) were subjected to six day growth assays in the presence or absence of 1 $\mu\text{g/ml}$ Tet with and without 100 ng/ml recombinant human AREG followed by assessment of cell cycle distribution of DAPI-stained keratinocytes by flow cytometry. Experimental conditions are indicated to the right. Representative flow cytometry analyses (n=4) for each of the three treatments are shown. Forward (FSC) and side scatter (SSC) reflect cell size and cell complexity. *Upper panels:* Different colours in histograms (right) represent cells of different size and complexity as indicated by the gates in the dot blots (left). *Lower panels:* Density blots represent FSC and SSC (left), and DAPI versus cell size (right).

EGFP shRNA

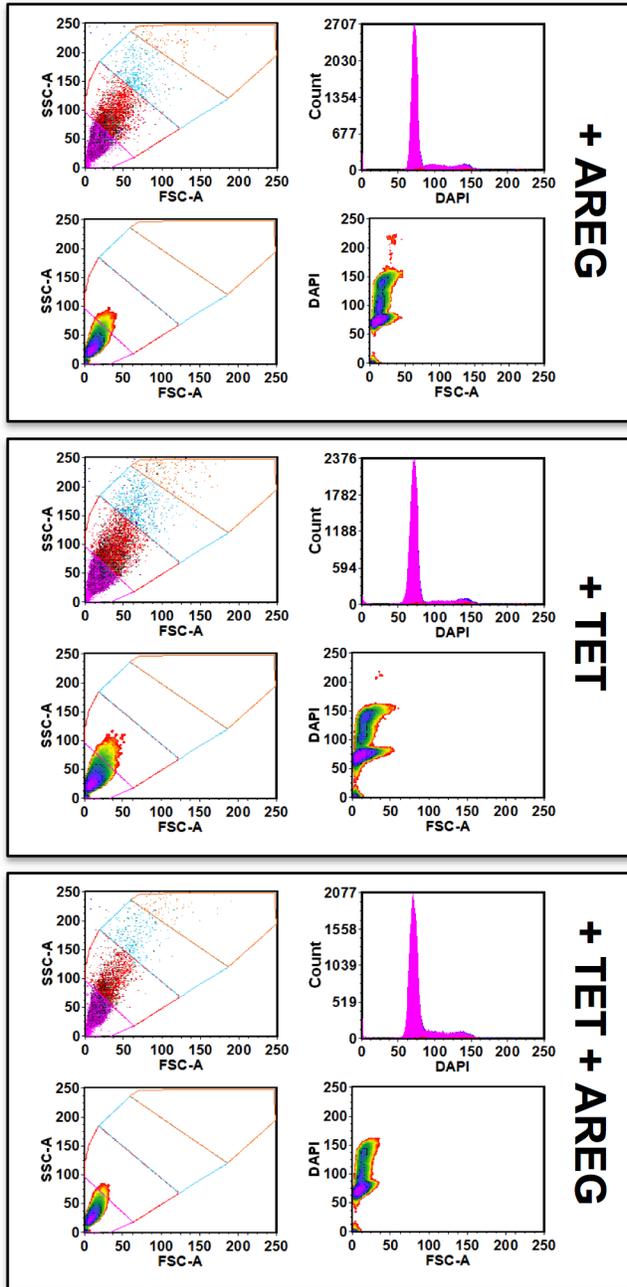


Figure S5: Lack of effect of an irrelevant shRNA on cell cycle parameters.

N/TERT-TR-shAREG keratinocytes (parental), or N/TERT-TR keratinocytes carrying a Tet-inducible shRNA targeting EGFP (N/TERT-TR-shEGFP) were subjected to six day

growth assays in the presence or absence of 1 $\mu\text{g/ml}$ Tet with and without 100 ng/ml rhAREG followed by assessment of cell cycle distribution of DAPI-stained keratinocytes by flow cytometry. Representative flow cytometry analyses (n=3) for three treatments as indicated on the right are shown. Forward (FSC) and side scatter (SSC) reflect cell size and cell complexity. *Upper panels:* Different colours in histograms (right) represent cells of different size and complexity as indicated by the gates in the dot blots (left). *Lower panels:* Density blots represent FSC and SSC (left), and DAPI versus cell size (right).

Lists of differentially regulated genes (Excel Data Files)

File S1. TET versus Control Decreased

File S2. TET versus Control Increased

File S3. TET-Decreased and not restored by EGF

File S4. TET-Decreased and restored by EGF

File S5. TET-Increased and not repressed by EGF

File S6. TET-Increased and repressed by EGF