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Supplementary Materials for

ETV7 is an essential component of a rapamycin-insensitive mTOR complex in cancer

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Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/4/9/eaar3938/DC1)

Movie S1 (.mp4 format). Induction of non-targeting ETV7shRNA in human DAOY medulloblastoma cells.

Movie S2 (.mp4 format). Induction of targeting ETV7shRNA in human DAOY medulloblastoma cells.



Fig. S1. ETV7 is expressed in pediatric malignancies. Histograms of expression levels of ETV7 in (A) pediatric ALL (19) and (B) pediatric AML (18) using the Affymetrix U133Av2 array. The division of ETV7⁺ (red) and ETV7⁻ (green) tumors (relative expression value 200) was based on the hybridization level of ETV7 in non- ETV7 expressing T-ALL samples on the same array. (C) Heat map of the relative ETV7-expression levels in 50 solid tumor xenografts of the St. Jude expanded panel of pediatric preclinical tumor models using the HG-U133Plus2 GeneChip. Numbers to the right of the heat map represent the relative ETV7 RNA hybridization signals in these xenografts, as determined by the Affymetrix HG-U133Plus2 GeneChip (20). The following tumors were included: BT-36, -41, -44, -54 (ependymoma, p, p, s, s); SK-NEP-1, EW-5, -8, TC-71, CHLA258 (Ewings sarcoma, pr, p, pr, p); GBM2, BT-39, -56, D456, D212, D645 (glioblastoma, p, r, sr, p, s, p); BT-45, -46, -50, -28 (medulloblastoma, p, p, p, p); NB-SD, -1771, -1691, -EBc1, -1643, -1382, CHLA-79, SK-N-AS (neuroblastoma, p, p, p, p, p, p, p, p, s); OS-1, -2, -17, -29, -31, -33, -9 (osteosarcoma, p, p, p, s, p, p, p); Rh30R, Rh10, Rh28, Rh30, Rh41, Rh65, Rh36 (ARMS, r, p, p, p, sr); Rh18, Rh36 (ERMS, p; sr); Bt-29, KT-16, -12, -14 (rhabdoid, p, p, s, p); KT-13, -10, -11, -5 (Wilms tumor, p, p, p, s) (p=primary, s=secondary, pr=primary relapse, sr= secondary relapse, r=relapse). (D) A histogram representing the average ETV7 expression per pediatric tumor type xenograft in the HG-U133Plus2 array as compared with the average ETV7 signal in all tumor xenografts. (E) Histogram of ETV7 expression levels using the Affymetrix U133Av2 array of 46 primary human medulloblastomas (32). The division of ETV7⁺ and ETV7⁻ tumors (relative expression value 200) was based on the hybridization level of ETV7 in non- ETV7-expressing T-ALL samples on the same U133Av2 array.



В

А



Fig. S2. PI3K and PTK2 signaling and anti–p-tyr Western blot of cell lysate of vector and ETV7 mouse pre-B cells. (**A**) Cell lysates from wild type (WT) and *Arf^{-/-}*mouse pre-B cells (ARF^{-/-}) expressing GFP (Vec) or ETV7 (ETV7) immunoblotted for p-PTK2^{Tyr397} and total PTK2. (**B**) Left panel, Ponceau-S stained nitrocellulose blot of a PAGE gel of lysates of 0.5x10⁶ vector and ETV7 transduced mouse pre-B cells. Right panel, anti-p-tyrosine immunoblot of the same gel.



Fig. S3. ETV7 expression in human pediatric tumor xenografts. (**A**) IPs (ETV7 IP, IgG Ctrl) of lysates from Karpas-299 cells (K-299), and xenograft tumors (BT-28, medulloblastoma; BT-39, glioblastoma) immunoblotted for mTOR, ETV7 or p-4E-BP1^{Thr37/46}. (**B**) ETV7 immunostaining of a BT41 ependymoma xenograft section (no ETV7 expression), a BT28 medulloblastoma xenograft (intermediate ETV7 expression), a BT39 glioblastoma xenograft section (high ETV7 expression) and a BT39 section competed with the peptide against which the anti-ETV7 antibody was raised. Numbers in the lower left corner of the micrographs represent the relative ETV7 RNA hybridization signals in these xenografts, as determined by the Affymetrix HG-U133Plus2 GeneChip, shown in fig. S1C.



Fig. S4. Sequence comparison of ETV6 and ETV7 PNT domains and the position of $\Delta 9$, $\Delta 27$, $\Delta 120/\Delta 115$, $\Delta 120/\Delta 159/+60$ mutations in the ETV7 PNT domain, and the KALK mutation in the ETV7 ETS domain. (A) The upper part shows the protein sequence comparison of the ETV6 PNT domain (amino acid 44 to 124) with that of the ETV7 PNT domain (amino acid 37 to 117). The line in the middle shows the homology between the two PNT domains. The red and yellow highlights in the ETV6 PNT sequence indicate residues forming the ML and EH interaction surfaces (65), respectively. The sequences in the ETV7 PNT domain coinciding with the ETV6 ML and EH surfaces are also highlighted in red and yellow. $\Delta 9$ ($\Delta E^{81}MN^{83}$) and $\Delta 27$ (Δ M⁸²NGRALCIL⁹⁰) indicate the ETV7 PNT domain deletion mutants used in Fig. 2E and 2F. The sequence underneath shows the position of the R²⁸¹ALR²⁸⁴ to K²⁸¹ALK²⁸⁴ mutation in the ETV7 ETS domain (amino acids 224 to 307), which prevents DNA binding. (B) Top bar depicts exon 3 of ETV7 (165 bp) with underneath the relative positions of the M82 CRISPR (not to scale) and the methionine 82 codon (M82). Underneath are the partial sequences of Karpas-299-EBNA-Cas9 clones 7, 9 and 10. Clone 9 (K-E^{Δ120/Δ159+60}) was used for experiments shown in Fig. 5A, 5B, and fig. S7B. Dashed vertical lines demarcate the deletions in the different alleles with the juxtaposed 5' and 3' nucleotide sequences indicated. The 120bp deletion (Δ 120) encodes a highly unstable ETV7 protein with a 40-amino acid (aa) deletion in the PNT domain, including M82, which is undetectable in ETV7 IP/western blots (Fig. 5A, insert). The 115bp deletion encodes an out of frame protein of 63 aa containing the 49 aa ETV7 Nterminus followed by 14 aa from the -1-reading frame. The 154 bp deletion/60 bp insertion (red lettering) encodes a 50-aa protein containing the 49 aa ETV7 N-terminus followed by one non-ETV7 aa.

В



Fig. S5. mTOR localization and assembly. (**A**) mTOR (mTOR IP) or ETV7 (ETV7 IP) IP of subcellular fractions (C=cytoplasm, M=membrane, N=nucleus) of Karpas-299 cells immunoblotted for mTOR, ETV7 or RUVBL2. (**B**) IgG or ETV7 IP of lysate from tetracycline-treated (tet) Tet-off U937T cells carrying a tet^{operator}CMV minimal promoter-driven ETV7 construct, immunoblotted for mTOR and ETV7. Higher ETV7 expression (2 ng/mL tetracycline) does not result in more mTOR coprecipitation.





С



K-E7⁻





10µm

Fig. S6. Effects of mTOR inhibitors and mTORC3 loss on mTOR signaling. (A) Proliferating Ew-8 Ewing's sarcoma cells transduced with MSCV-IRES-GFP (vector) or MSCV-ETV7-IRES-GFP (ETV7) were treated with increasing amounts (0.1, 0.3, 1.3, 10, 30, 100, 300, 1,000 ng/mL) of AZD-8055 for 3 population doublings. Cell densities (Percent Control) were plotted as the percentage of cells treated with vehicle. Data are represented as mean +/- SEM from 3 independent experiments. 30% control or less means no proliferation. (**B**) Immunoblots of K-E7^{Δ120/Δ154+60} cell lysates treated with increasing amounts of rapamycin (0.1, 0.3, 1.3, 10, 30, 100, 300, 1,000 ng/mL) probed for p-mTOR^{Ser2448} p-AKT^{Ser473}, p-AKT^{Thr308}, p-ERK1/2^{Thr202Tyr204}, p-p70S6K^{Thr389}, p-4E-BP1^{Thr3746}, total 4E-BP1, LC3BI/II and SQSTM1/p62. (**C**) Immunoblots of lysates of equal numbers of Karpas-299, and K-E7⁻ cells were treated with increasing amounts of rapamycin (0.1, 0.3, 1.3, 10, 30, 100, 300, 1,000 ng/mL) and probed for, phospho-IKKε^{Ser172}, total IKKε, phospho-TBK1^{Ser172}, total TBK1, p-GSK3-β^{Ser9} and total GSK3-β. (**D**) p-GSK3-β^{Ser9} and total GSK3-β band intensities at each rapamycin concentration of the western blot in C were determined using a densitometer and plotted as the p-GSK3-β^{Ser9}/GSK3-β relative intensities to show that loss of p-GSK3-β^{Ser9} signal follows the loss of total GSK3-β. (**E**) Karpas-299 cells transduced with LC3B-mCherry retrovirus were treated or not (untreated) with rapamycin (1,000ng/mI rapamycin) or AZD-8055 (300 ng/mI AZD-8055) for 72 hours. Cells were visualized using phase contrast/fluorescence microscopy (top row) or fluorescence microscopy alone (bottom row). Only cells treated with AZD-8055 show active autophagy as indicated by punctate staining. mTORC2 signaling in Karpas-299 cells either.



Fig. S7. Effect of ALK inhibition on p-AKT^{Ser473} **and relative FK506-binding protein expression in Karpas-299 cells.** (**A**) Proliferating Karpas-299 cells were treated with increasing amounts (0.1, 0.3, 1, 3, 10, 30, 100, 300, 1,000 nM) of the ALK inhibitor alectinib for 3 population doublings. Cell densities (percent control) were plotted as the percentage of cells treated with vehicle. Data are represented as mean +/- SEM from 3 independent experiments. (**B**) Proliferating Karpas-299 cells were treated with increasing amounts (0.1, 1, 10, 100, 1,000 ng/mL) of rapamycin in the absence or presence of 10ng/ml alectinib for 72 hours. Cell lysates were immunoblotted for p-AKT^{Ser473}. Compared to cells treated with rapamycin alone, NPM-ALK inhibition with 10nM alectinib greatly reduced the p-AKT^{Ser473} feedback phosphorylation with increasing rapamycin concentration. (**C**) Lysates of 293T, Karpas-299 and K-E7⁻ cells were immunoblotted for FKBP-12 and FKBP-51. The levels of these two proteins in Karpas-299 and K-E7⁻ cells are similar to that in 293T cells. The relative amounts of FKBP-12 and FKBP-51 in 293T cells are such that they cannot fully inhibit mTORC2 signaling upon long-term rapamycin treatment (*40***)**, predicting that they cannot inhibit mTORC2 signaling in Karpas-299 cells either.

| Α | | |
|---|--------------|------------|
| | Ew-8 -GFP | Ew-8 -ETV7 |
| Scrambled shRNA Raptor shRNA Rictor shRNA | + + + | + + + |
| Total mTOR | | |
| Raptor | | |
| Rictor | | <u> </u> |
| p-P70S6K ^{Thr389} | | |
| Total P70S6K | — — — | |
| p-4E-BP1 ^{Thr37/46} | | |
| Total 4E-BP1 | | |
| p-AKT ^{Ser473} | | |
| Total AKT | <u> </u> | |
| p-NDRGI ^{Thr346} | | |
| ml ST8 | | |

Ew-8 -ETV7 Scrambled Sh **Rictor Sh** Raptor Sh mTOR mTOR mTOR ETV7 ETV7 ETV7 lgG lgG lgG mTOR Rictor Raptor mSIN1 mLST8 ETV7

| D | Rictor ^{fl/fl} | Rictor ^{fl/fl} Vector | Rictor ^{fl/fl} ETV7 |
|------------------------------|-------------------------|-----------------------------------|---------------------------------|
| Adenovirus-Cre-GFF | ° - + | - + | - + |
| Total mTOR | | | |
| Raptor | | | |
| Rictor | | | |
| p-P70S6K ^{™r389} | | | |
| Total P70S6K | | | |
| p-4E-BP1 ^{Thr37/46} | | | |
| Total 4E-BP1 | •• | | |
| p-AKT ^{Ser473} | - | Nightair | |
| Total AKT | | | |
| p-NDRGI ^{Thr346} | | * * - | |
| mLST8 | | | |
| ETV7 | | | |

Raptor shRNA Rictor shRNA Total mTOR Raptor Rictor p-P70S6K^{Thr389} Total P70S6K p-4E-BP1^{Thr37/46} Total 4E-BP1 p-AKT^{Ser473} Total AKT p-NDRGI^{Thr346} p-ERK1/2^{Thr202/Tyr204} Total ERK1/2 p-MNK1^{Thr197/202} Total MNK1 mLST8

С



В

Fig. S8. mTORC3 kinase is insensitive to Raptor or Rictor knockdown or Rictor knockout. (A) Immunoblots of lysates of EW-8 Ewing's sarcoma cells transduced with GFP or ETV7 lentivirus and super transduced with scrambled-shRNA, Raptor-shRNA or Rictor-shRNA lentivirus were probed for mTOR, Raptor, Rictor, p-P70S6K^{Thr389}, total P70S6K, p-4E-BP1^{Thr37/46}, total 4E-BP1, p-AKT^{Ser473}, total AKT, p-NDRG1^{Thr346} and mLST8. **(B)** mTOR and ETV7 IPs of the same EW-8 lysates as in A were immunoblotted for mTOR, Rictor, Raptor, mSIN1, mLST8 and ETV7. **(C)** Lysates of scrambled, Raptor, or Rictor Karpas-299 knockdown cells immunoblotted for mTOR, Rictor, Raptor, p-P70S6K^{Thr389}, total P70S6K, p-AKT^{Ser473}, total AKT, p-NDRG1^{Thr346}, p-4E-BP1^{Thr37/46}, total 4E-BP1, p-ERK1/2^{Thr202/Tyr204}, total ERK1/2, p-MNK1^{Thr197/202}, total MNK1 and mLST8. mLST8 serves as the loading control. **(D)** Ninety hours after infection with Adenovirus-GFP-iCre (+) or not (-), lysates of *Rictor*^{#/#} (*Rictor*^{#/#}, total AKT, p-NDRG1^{Thr389}, total P70S6K, p-AKT^{Ser473}, total AKT, p-NDRG1^{Thr346}, NDRG1^{Thr346}, Raptor, p-p70S6K^{Thr389}, total 4E-BP1 and mLST8 as a loading control.





Fig. S9. ERMS-specific markers in Ptch^{+/-}/**ETV**^{TG+/-} **tumors are preserved in Ptch**^{+/-}/**ETV**^{TG+/-} **cell lines.** (**A**) Representative example of formalin fixed paraffin embedded sections of a Ptch1^{+/-}/ETV7^{TG} (upper panel) and Ptch1^{+/-} (lower panel) primary tumor stained with MSA antibody, SMA antibody, or desmin antibody. Scale bar represents 20µm. (**B**) Representative example of formalin fixed cell line of a Ptch1^{+/-}/ETV7^{TG} tumor stained with: H&E, anti-Desmin, -Myogenin, -MSA, -SMA antibody. (**C**) Representative example of formaldehyde fixed cell line of a Ptch1^{+/-}/ETV7^{TG} tumor stained with: H&E, anti-Desmin, -Myogenin, -MSA, -SMA antibody. (**C**) Representative example of formaldehyde fixed cell line of a Ptch1^{+/-}/ETV7^{TG} tumor stained with: MyoD antibody and Dapi, Myf5 antibody and Dapi, Secondary antibody alone and Dapi. Scale bar represents 10µm. (**D**) ETV7 Q-RT-PCR of RNA from ERMS cell lines RD and JR and HEK 293T cells. Q-RT PCR results were normalized to endogenous HPRT expression. Data are the mean +/- SEM from two independent experiments.









Fig. S10. Whole phospho-p70S6K^{Thr389} **and p70S6K Western blots relating to Figs. 1C and 5D.** This figure shows images of the whole western blots of lysates of mouse *Arf^{-/-}* pre-B cells expressing (**A**) vector or (**B**) ETV7, treated with increasing amounts of rapamycin (from left to right: 0, 0.1, 0.3, 1, 3, 10, 30, 100, 300, 1000 ng/ml rapamycin), probed with the anti-phospho-p70S6K^{Thr389} antibody or the same blots probed with a total anti-p70S6K antibody (**C**, **D**). These whole images represent the phospho-p70S6K^{Thr389} and total p70S6K cutouts shown in Fig. 1C. (**E**, **F** and **G**) show KE7⁻(no ETV7), Karpas-299, and KE7⁻-ETV7 lysates of cells treated with the same increasing amounts of rapamycin, probed with the anti-phospho-p70S6K^{Thr389} antibody. These whole images represent the phospho-p70S6K^{Thr389} cutouts shown in Fig. 5D. The arrows indicate the position of the phospho-p70S6K^{Thr389} or p70S6K bands. The horizontal lines at the left of each blot indicate the position of the molecular weight markers (260, 160, 110, 80, 60, 40, and 30 kDa. respectively).

Table S1. Expression effects of ETV7.

A. Upregulated ETV7 expression in adult tumor samples.

| Tumor Type | Over-expression | Platform |
|--|-----------------|---------------------|
| Well Differentiated Liposarcoma (Henderson et al, Genome biol 6, R76, 2005; Nakayama et al, Modern Pathology, Inc 20,749-59, 2007) | Тор 1% | U133A 2.0, U133A |
| B-cell Acute Lymphoblastic Leukemia (Raetz et al, Pediatric Blood Cancer 47, 130-40, 2006). | Top 1% | U133A |
| Easophageal Carcinoma (Kimchi et al, Cancer Research 65, 3146- 54, 2005) | Top 1% | U133A |
| Invasive Ductal Breast Carcinoma (Turashvili et al, BMC Cancer 7, 55, 2007) | Тор 1% | U133 Plus 2.0 |
| Liver Cell Dysplasia vs. Normal (Wurmbach, Hepatology 45, 938- 47, 2007) | Тор 1% | U133 Plus 2.0 |
| B-cell Acute Lymphoblastic Leukemia (Maia et al, Cancer Res 65, 10050-58, 2005; Bhojwani et al, Blood 108, 711-17, 2006) | Тор 5% | U133A |
| Superficial Bladder Cancer (Modlich et al, Cin Cancer Res 10, 3410- 21, 2004) | Тор 5% | U133A |
| Gastric mixed Adenocarcinoma (Ooi et al, PLoS Genet 5, e1000676, 2010) | Top 5% | U133A |
| Pleomorphic Myxofibrosarcoma vs. Normal (Barretina et al, Nat Genet 42, 715-21, 2010) | Тор 5% | U133A |
| Ovarian Clear Cell Adenocarcinoma (Lu et al, Clin Cancer Res 10, 3291-00, 2004) | Тор 5% | U95D |
| Ductal breast carcinoma (Turashvili et al, BMC Cancer 7, 55, 2007) | Top 5% | U133 Plus 2.0 |
| Gastric Cancer (Bittner et al, GCEPO, 2005) | Top 5% | U133 Plus 2.0 |
| Liver Cirrhosis vs. Normal (Wurmbach, Hepatology 45, 938-47, 2007) | Тор 5% | U133 Plus 2.0 |
| Acute Myeloid Leukemia FAB subtype M5 (Metzeler et al, Blood 112, 4193-01, 2008) | Тор 5% | U133 Plus 2.0 |
| Gastric Intestinal Type Adenocarcinoma (Ooi et al, PLoS Genet 5, e1000676 2010) | Тор 5% | U133 Plus 2.0 |
| Gastrointestinal Part: Descencing Colon (Sabates-Bellver et al, Mol Cancer Res 5, 1263-75, 2007) | Тор 5% | U133 Plus 2.0 |
| Renal Carcinoma (Yusenko et al, BMC Cancer 9, 152, 2009) | Top 5% | U133 Plus 2.0 |
| Leukemia Precursor (Wouters et al, Bloo 113, 3088-91, 2009) | Top 5% | U133 Plus 2.0 |
| Clear Cell Renal Cell Carcinoma (Bittner et al, GCEPO, 2005, Yusenko et al, BMC Cancer 9, 152, 2009) | Тор 5% | U133 Plus 2.0 |
| Brain Part: Brain Nucleus (Su et al, Proc Natl Acad Sci USA, 101, 6062-67, 2004) | Top 10% | U133A |
| Malignant Fibrous Histiocytoma (Detwiller et al, Cancer Res 65, 5881-89, 2005) | Top 10% | U133A |
| Ovarian Clear Cell Carcinoma vs. Normal (Hendrix et al, Cancer Res 66, 1354-62, 2006) | Top 10% | U133A |
| Invasive Ductal Breast Carcinoma (Radvanyi et al, Proc Natl Acad Sci USA, 102, 11005-10, 2005) | Top 10% | Hu03 Custom |

| Brain Part: Putamen (Roth et al, Neurogenetics 7, 67-80, 2006) | Top 10% | U133 Plus 2.0 |
|---|---------|---------------|
| Ductal Breast Carcinoma (Kimchi et al, Cancer Research 65, 3146- 54, 2005, Roth et al, Neurogenetics 7, 67-80, 2006, Richardson et | Top 10% | U133 Plus 2.0 |
| al, Cancer Cell 9, 121-32, 2006) | | |
| Medullary Breast Cancer (Ginestier et al, Clin Cancer Res 12, 4533- | Top 10% | U133 Plus 2.0 |
| 44, 2006) | | |
| Bladder Cancer (Bittner et al, GCEPO, 2005) | Top 10% | U133 Plus 2.0 |
| Cecum Adenocarcinoma (Kaiser et al, Genome Biol 8, R131, 2007) | Top 10% | U133 Plus 2.0 |
| Germinal Center B-Cell-Like Diffuse Large B-cell Lymphoma (Lentz | Top 10% | U133 Plus 2.0 |
| et al, N Eng J Med 359, 2313-23, 2006) | | |
| Type 3 Diffuse Large B-Cell Lymphoma (Lentz et al, N Eng J Med | Top 10% | U133 Plus 2.0 |
| 359, 2313-23, 2006) | | |
| Type II Endometrial Carcinoma (Bittner et al, GCEPO, 2005) | Top 10% | U133 Plus 2.0 |
| Liver Cirrhosis vs. Normal (Wurmbach, Hepatology 45, 938-47, | Top 10% | U133 Plus 2.0 |
| 2007) | | |
| Acute Myeloid Leukemia FAB subtype M5 (Kool et al, PLoS One 3, | Top 10% | U133 Plus 2.0 |
| e3088, 2008) | | |

- B: Differential expression (log2 ratio) of mTOR-related genes in vector versus ETV7 ARF^{-/-} mouse pre-B cells as determined by Affymetrix array analysis.
- C: Differential expression (log2 ratio>0.5) of kinase genes in vector versus ETV7 ARF^{-/-} mouse pre-B cells as determined by Affymetrix array analysis.
- D: Differential expression (log2 ratio>0.5) of cytokine, growth factor and growth factor receptor genes in vector versus ETV7 ARF^{-/-} mouse pre-B cells as determined by Affymetrix array analysis.

| | В | | С | D | | | | |
|-------------|----------------|-------------|----------------|-------------|----------------|-------------|----------------|--|
| Gene Symbol | ETV7 vs Vector | |
| Prkaa2 | 0.90428 | Ptk2 | 1.61 | Gpr12 | 1.37 | Klrb1a | -0.55 | |
| Grb10 | 0.56929 | Irak3 | 0.97 | Ccl9 | 1.37 | Milr1 | -0.56 | |
| Deptor | 0.3964 | Prkaa2 | 0.90 | Olfr820 | 1.04 | Csf2rb | -0.56 | |
| Prkar2b | 0.32123 | Camk4 | 0.80 | ll12rb2 | 0.97 | Vmn2r41 | -0.60 | |
| Mtor | 0.2073 | Ak4 | 0.80 | Trav9d-1 | 0.90 | Olfr1205 | -0.61 | |
| Lamtor3 | 0.20379 | Cdk14 | 0.73 | Trav9d-4 | 0.89 | Chrnb1 | -0.63 | |
| Lamtor5 | 0.2028 | Mapk11 | 0.71 | Trav16n | 0.88 | lgf2bp3 | -0.64 | |
| Lamtor3 | 0.1998 | Plk2 | 0.62 | Mc1r | 0.82 | Gria2 | -0.68 | |
| Prkab2 | 0.18871 | Rps6kl1 | 0.59 | lgf2r | 0.67 | Ryk | -0.69 | |
| Lamtor1 | 0.16293 | Pfkp | 0.56 | Lrp4 | 0.67 | Gpr84 | -0.71 | |
| Prkab1 | 0.1521 | Nim1k | 0.56 | Ccl6 | 0.66 | ll5ra | -0.71 | |
| Prkag1 | 0.1283 | Lck | 0.53 | Vmn2r51 | 0.64 | Olfr372 | -0.72 | |
| Prkar1b | 0.1213 | Pkmyt1 | 0.53 | Gpr125 | 0.64 | Tnfrsf13c | -0.72 | |
| Lamtor2 | 0.10599 | Tnk2 | 0.51 | Bmpr1a | 0.64 | lgfbp4 | -0.72 | |
| Mlst8 | 0.05355 | ltpkb | -0.51 | Bmp4 | 0.64 | Gprc5b | -0.76 | |
| Prkar1a | 0.04963 | Camk1d | -0.52 | Trav9-2 | 0.61 | Fcrl6 | -0.77 | |
| Pten | -0.0131 | Pik3r3 | -0.52 | Trav6-3 | 0.61 | Nr2c1 | -0.81 | |
| Prkag3 | -0.02545 | Ak5 | -0.54 | Tnfrsf22 | 0.58 | Ephb2 | -0.83 | |
| Prkaa1 | -0.0395 | Sik1 | -0.56 | Tnfrsf22 | 0.58 | Gpr174 | -0.87 | |
| Tsc2 | -0.04166 | Ret | -0.56 | Olfr1437 | 0.56 | Gpr128 | -0.90 | |
| Prkacb | -0.0426 | Eif2ak2 | -0.57 | Olfr1238 | 0.56 | Tlr9 | -0.96 | |
| Rptor | -0.09697 | Pik3cg | -0.59 | Olfr687 | 0.53 | Klrb1f | -0.97 | |
| Rictor | -0.09717 | Map3k8 | -0.61 | Nr1d1 | 0.53 | ll2ra | -0.99 | |
| Prkag2 | -0.10246 | Pik3r5 | -0.62 | Tnk2 | 0.51 | Tnfsf8 | -0.99 | |
| Tsc1 | -0.10304 | Ryk | -0.69 | Fcgr3 | 0.51 | Bmpr2 | -1.10 | |
| Lamtor4 | -0.24397 | Bmpr2 | -0.71 | lfnab | 0.51 | P2ry13 | -1.10 | |
| Prkaca | -0.25174 | Prkcq | -0.84 | Olfr988 | 0.50 | lgf1 | -1.13 | |
| Prkar2a | -0.43749 | Dgka | -1.04 | Olfr205 | -0.52 | Sorl1 | -1.33 | |
| | | Hck | -1.09 | lgf1r | -0.52 | Csf2rb2 | -1.40 | |
| | | Adck1 | -1.12 | Tgfbr2 | -0.52 | Gpr18 | -1.41 | |
| | | | | Tgfbr3 | -0.52 | 1118 | -1.42 | |
| | | | | Angptl2 | -0.52 | ll10ra | -1.46 | |
| | | | | Tnf | -0.52 | lgf2 | -1.97 | |
| | | | | Fcrla | -0.53 | - | | |
| | | | | Lpar6 | -0.53 | | | |
| | | | | Cysltr1 | -0.54 | | | |
| | | | | ll4ra | -0.54 | | | |

E: Gene Set Enrichment Analysis using Hallmark and canonical databases identifying ETV7 up and down regulated pathways in ETV7 versus vector ARF^{-/-} mouse pre-B cells.

Ε

Up regulated Hallmark pathways

Down regulated Hallmark pathways

| NAME | SIZE | NES | NOM p-val | FDR q-val | NAME | SIZE | NES | NOM p-val | FDR q-val |
|------------------------------------|------|----------|---------------|-----------|---|------|----------|-----------|-----------|
| HALLMARK MYC TARGETS V2 | 57 | 1 849533 | · · · · · | 0.005558 | HALLMARK_INTERFERON_ALPHA_RESPONSE | 86 | -2.31227 | 0 | 0 |
| | 405 | 4.000047 | | 0.0000000 | HALLMARK_INTERFERON_GAMMA_RESPONSE | 183 | -2.22526 | 0 | 0 |
| HALLMARK_MYC_TARGETS_V1 | 195 | 1.839917 | Ĺ | 0.00335 | HALLMARK_INFLAMMATORY_RESPONSE | 194 | -1.70631 | 0.001739 | 0.014258 |
| HALLMARK_E2F_TARGETS | 194 | 1.807984 | , C | 0.002945 | HALLMARK_TNFA_SIGNALING_VIA_NFKB | 195 | -1.68275 | 0 | 0.013594 |
| HALLMARK MTORC1 SIGNALING | 189 | 1.737239 | 0 | 0.007111 | HALLMARK_ALLOGRAFT_REJECTION | 182 | -1.51006 | 0.005386 | 0.062058 |
| HALLMARK APICAL SURFACE | 44 | 1.632676 | 0.010504 | 0.014033 | HALLMARK_IL6_JAK_STAT3_SIGNALING HALLMARK_UV_RESPONSE_DN | 142 | -1.34444 | 0.016227 | 0.07681 |
| HALLMARK HEDGEHOG SIGNALING | 36 | 1.543138 | 0.025341 | 0.027647 | HALLMARK_APOPTOSIS | 156 | -1.34156 | 0.044405 | 0.159596 |
| | 145 | 1 505772 | 0.004695 | 0.035686 | HALLMARK_COMPLEMENT | 181 | -1.33302 | 0.031308 | 0.151301 |
| | 145 | 1.000772 | 0.004030 | 0.000000 | HALLMARK_TGF_BETA_SIGNALING | 54 | -1.3122 | 0.109959 | 0.162122 |
| N | 190 | 1 504944 | 0.002217 | 0.031484 | HALLMARK_ANDROGEN_RESPONSE | 94 | -1.30829 | 0.078755 | 0.151324 |
| | 100 | 1.007000 | 0.002217 | 0.001404 | HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITI | | | | |
| HALLMARK_SPERMATOGENESIS | 132 | 1.49/038 | 0.00432 | 0.030267 | ON | 194 | -1.28012 | 0.063636 | 0.17192 |
| HALLMARK_ANGIOGENESIS | 35 | 1.419507 | 0.057971 | 0.053691 | HALLMARK_KRAS_SIGNALING_UP | 190 | -1.23218 | 0.082437 | 0.226756 |
| HALLMARK CHOLESTEROL HOMEOSTASIS | 68 | 1.399752 | 0.044118 | 0.058805 | HALLMARK_COAGULATION | 130 | -1.20/48 | 0.13594 | 0.24977 |
| HALLMARK UNFOLDED PROTEIN RESPON | | | | | HALLMARK_REACTIVE_OXIGEN_SPECIES_PATHWAY | 44 | -1.18368 | 0.215606 | 0.272473 |
| SE | 111 | 1.286486 | 0.069869 | 0.132809 | HALLMARK ILZ STATS SIGNALING | 194 | 1.13000 | 0.186257 | 0.347795 |
| HALLMARK GLYCOLYSIS | 191 | 1 164008 | 0 141256 | 0 306158 | HALLMARK_NOTCH_SIGNALING | 197 | -1.0307 | 0.360367 | 0.59108 |
| | 104 | 1 101105 | 0 1 4 2 4 0 9 | 0.090707 | | 192 | -0 94878 | 0 591228 | 0 770025 |
| HALLMARK_ADIPOGENESIS | 194 | 1.101100 | 0.143490 | 0.209727 | HALLMARK P53 PATHWAY | 195 | -0.93745 | 0.589161 | 0.764326 |
| HALLMARK_G2M_CHECKPOINT | 193 | 1.033894 | 0.366667 | 0.598651 | HALLMARK UV RESPONSE UP | 145 | -0.91001 | 0.625229 | 0.803595 |
| HALLMARK XENOBIOTIC METABOLISM | 187 | 1.029852 | 0.375556 | 0.574139 | HALLMARK KRAS SIGNALING DN | 189 | -0.89046 | 0.747715 | 0.8224 |
| | 110 | 0 071861 | 0.510/27 | 0 727518 | HALLMARK_PANCREAS_BETA_CELLS | 39 | -0.8738 | 0.67086 | 0.830947 |
| HALLMARK_BILE_ACID_WETABOLISM | 110 | 0.071001 | 0.010421 | 0.727510 | HALLMARK_ESTROGEN_RESPONSE_LATE | 191 | -0.86581 | 0.777972 | 0.816015 |
| HALLMARK_PEROXISOME | 99 | 0.929117 | 0.636364 | 0.826374 | HALLMARK_MITOTIC_SPINDLE | 195 | -0.84197 | 0.853933 | 0.838576 |
| HALLMARK_PI3K_AKT_MTOR_SIGNALING | 104 | 0.893945 | 0.703872 | 0.898499 | HALLMARK_APICAL_JUNCTION | 194 | -0.76891 | 0.960303 | 0.923438 |
| HALLMARK_DNA_REPAIR | 136 | 0.884729 | 0.745614 | 0.88112 | | | | | |
| HALLMARK_MYOGENESIS | 197 | 0.859496 | 0.842105 | 0.903522 | | | | | |
| HALLMARK HEME METABOLISM | 179 | 0.784741 | 0.96083 | 0.999151 | | | | | |
| HALLMARK_WNT_BETA_CATENIN_SIGNALIN | 41 | 0 772405 | 0.936037 | 0.070210 | | | | | |
| 3 | 41 | 0.772495 | 0.030032 | 0.970219 | | | | | |
| HALLMARK_PROTEIN_SECRETION | 94 | 0.768796 | 0.909457 | 0.933819 | | | | | |

F: Differential expression of genes in ETV7^{KALK} versus ETV7 expressing EW8 cells as determined by Affymetrix analysis.

| Transcript ID | Gene Symbol | KALK vs ETV7 | Transcript ID | Gene Symbol | KALK vs ETV7 | Franscript ID | Gene Symbol | KALK vs ETV7 |
|----------------------|---------------------------------|----------------|----------------------|---------------------|--------------|----------------------|------------------------|--------------|
| 16987001 17113549 | KIAA1210 | 4.56 3.58 | 16745637 16905464 | UC105373760 | 0.94 0.94 | 16722299 | PDE3B | 0.71 |
| 17053917 | EN2 | 2.44 | 16975592 | GABRG1 | 0.93 | 16696811 | ANGPTL1 | 0.71 |
| 16917373 | FLRT3 GAGE2D | 2.27 | 17029847 16798496 | GABRG3 | 0.93 | 17053850 | MIR378H | 0.71 |
| 17103647 | GAGE2C | 2.21 | 16883194 | LOC100506123 | 0.91 | 16725041 | FAM111B | 0.70 |
| 17103679 | GAGE12F | 2.17 | 16978976 | CFI GPR174 | 0.91 | 16873495 16981444 | NOVA2 AADAT | 0.70 |
| 17113362 | CAPN6 | 1.99 | 16886491 | TNFAIP6 | 0.91 | 16974925 | LGI2 | 0.70 |
| 16997816 | EDIL3 | 1.89 | 16716063 | MAT1A | 0.90 | 16924149 | LOC105379278 | 0.70 |
| 17103620 | GAGE13 GAGE12F | 1.83 1.74 | 16669781 | NBPF19 | 0.90 | 16698421 | TMEM81 | 0.70 |
| 16854540 | B4GALT6 | 1.74 | 17063394 | KIAA1549 | 0.89 | 17057016 | LOC105375249 | 0.70 |
| 16990284 | PCDHB8 | 1.71 | 16914972 16659343 | DOK5 AADACI 4 | 0.89 | 16891603 | KCNE4 LOC105369980 | 0.69 |
| 17103661 | GAGE12C | 1.65 | 17042548 | | 0.88 | 16894883 | MATN3 | 0.69 |
| 17103667 | GAGE12C | 1.65 | 16714381 | CSTF2T | 0.88 | 16971382 | DCLK2 CASP1 | 0.69 |
| 17103690 16748817 | GAGE12H | 1.64 1.61 | 16900724 | LOC100506123 | 0.87 | 16692834 | CTSS | 0.69 |
| 16986943 | LOC105379057 | 1.56 | 16980160 | INPP4B | 0.86 | 17077004 | SNAI2 | 0.69 |
| 17117110 | CD24 | 1.54 | 16908926 17050134 | PIK3CG | 0.86 0.86 | 16990264 | PCDHB4 | 0.69 |
| 16677057 | SYT14 | 1.53 | 16987773 | | 0.85 | 16876731 | SOX11 | 0.68 |
| 17091877 | TUBBP5 | 1.47 | 16723318 | PRRG4 | 0.85 | 16673652 16860152 | LOC100127910 ZNF430 | 0.68 0.68 |
| 16708572 16754895 | ELOVL3 NTS | 1.46 1.46 | 16743721 | MMP1 | 0.84 | 16830284 | BCL6B | 0.68 |
| 16950131 | | 1.45 | 16804106 | ADAMTSL3 | 0.84 | 16662664 | MANEAL | 0.68 |
| 16721011 | TSSC2 | 1.45 | 16981099 | NPY1R | 0.84 | 16930811 | SEPT3 | 0.68 |
| 17100771 | | 1.44 | 16900286 | LINC00342 | 0.83 | 16847686 | CSH1 | 0.68 |
| 16738657 | GLYATL2 | 1.38 | 16679301 | FMN2 | 0.83 | 16802771 16782893 | BBS4 | 0.68 |
| 16734877 | HBE1 GAREM1 | 1.37 | 16889966 | ADAM23 | 0.82 | 16716365 | FAS-AS1 | 0.68 |
| 17105903 | MUM1L1 | 1.34 | 16965798 | PCDH7 | 0.82 | 17067729 | NRG1-IT1 | 0.68 |
| 17049408 | PCOLCE | 1.30 | 16682098 | EPHA2 FBN2 | 0.82 | 16980744 | RNF175 | 0.68 |
| 16853879 16985229 | PIEZO2 RGS7BP | 1.27 | 16728141 | GAL | 0.82 | 17113335 | SNORD96B | 0.67 |
| 16767983 | LOC101928395 | 1.25 | 16806467 | TJP1 | 0.81 | 16677556 | TGFB2 | 0.67 |
| 16972993 | TLR3 | 1.25 | 16787790 | GALNT12 | 0.81 | 16973247 | FRG1 | 0.67 |
| 16978896 | COL25A1 MCTP1 | 1.23 | 16753663 | | 0.81 | 16999041 | CDO1 | 0.67 |
| 16707149 | FAS | 1.22 | 16701106 | CHML | 0.81 | 16972480 | CEP44 RTKN2 | 0.67 |
| 16997802 | HAPLN1 | 1.20 | 16980051 | CLGN RNF150 | 0.81 | 16994521 | ANKH | 0.67 |
| 16676988 | HSD11B1 | 1.20 | 17074896 | SGCZ | 0.81 | 16900737 | ANKRD36B | 0.66 |
| 17063254 | PTN | 1.18 | 16805623 | LOC400464 | 0.80 | 16812738 | HDGFRP3 | 0.66 |
| 16786058 | SNORD56B | 1.18 | 16962689 | GMNC | 0.80 | 16676965 | CAMK1G | 0.66 |
| 16692614 | HIST2H3D | 1.17 | 16979800 | LOC101927359 | 0.80 | 16982477 | | 0.66 |
| 17035880 | | 1.16 | 16922920 | BACE2 | 0.80 | 16847711 | GH1 | 0.66 |
| 17038596 | | 1.16 | 17043095 | SDK1 | 0.80 | 17020846 | COL12A1 | 0.66 |
| 16857110 | ANKRD24 | 1.16 | 16678851 | KIAA1804 | 0.80 | 16901022 | SP100 | 0.66 |
| 17103614 | GAGE12J | 1.16 | 17015324 | NKN1 PLEKHG4B | 0.79 | 16901591 | MIR4266 | 0.66 |
| 16903537 16843417 | NMI SLFN12 | 1.15 1.15 | 16819161 | GNAO1 | 0.79 | 16703251 | KIAA1217 | 0.66 |
| 16979985 | MGARP | 1.15 | 16677071 | SERTAD4 | 0.79 | 16705641 | C10orf35 | 0.66 |
| 17033604 | | 1.15 | 16877297 | TRIB2 | 0.79 | 16679142 | RYR2 | 0.66 |
| 16770848 | NOS1 | 1.14 | 16684391 | NKAIN1 | 0.78 | 16982161 | SSX1 | 0.65 |
| 16963113 | APOD | 1.13 | 16864907 17001506 | ERVV-2 PDE6A | 0.78 | 17084413 | | 0.65 |
| 16758440 | | 1.12 | 16921475 | | 0.78 | 16850410 | SNAD E | 0.65 |
| 16888912 | MYO1B | 1.10 | 16924066 | | 0.77 | 16761858 | RERG | 0.65 |
| 17114272 | GPC4 | 1.10 | 17072125 | MAL2 | 0.77 | 17105862 | NRK | 0.65 |
| 16734886 | OR51B5 | 1.09 | 16722455 | NCR3LG1 | 0.77 | 16956532 | VGLL3 | 0.65 |
| 17031139 | | 1.09 | 16730429 | SDC2 | 0.77 | 17103756 | CCNB3 | 0.65 |
| 17028313 | PHKA1 | 1.09 | 16791580 | PRKD1 | 0.77 | 16701238 | AKT3 AKR1E2 | 0.65 |
| 16904514 | SCN3A | 1.06 | 16821541 | CRISPLD2 | 0.76 | 16669501 | LOC105379521 | 0.64 |
| 16719025 | FGFR2 | 1.06 | 16693142 | POGZ | 0.76 | 16750154 | CNTN1 | 0.64 |
| 16978500 | PCDHB14 | 1.05 | 17105719 | LL0XNC01-250H12. | 3 0.76 | 16798479 | GABRA5 | 0.64 |
| 16893143 | TWIST2 | 1.05 | 16910728 | SIRPA | 0.76 | 16966645 | | 0.64 |
| 16807931 | CARRC2 | 1.04 | 16700554 | PCNXL2 | 0.75 | 16990280 | PCDHB7 | 0.64 |
| 17056825 | TRGJP1 | 1.04 | 16970080 | CEP170P1 | 0.75 | 16852389 | DCC | 0.64 |
| 16969646 | | 1.03 | 16993335 | ZNF354C | 0.74 | 16979432 | NDNF SRGAP1 | 0.64 |
| 16833204 17106518 | CCL2 LINC01285 | 1.02 | 16701119 | PLD5 | 0.74 | 16730104 | SCARNA9 | 0.64 |
| 17113463 | LRCH2 | 1.01 | 16967602 | MOB1B ST6GALNAC3 | 0.74 | 16984049 | | 0.64 |
| 16754134 | LGR5 | 1.00 | 16738646 | GLYAT | 0.73 | 16972835 | STOX2 | 0.64 |
| 16769419 | GL18D2 SLC18A2 | 1.00 | 16691969 | LOC105371220 | 0.73 | 16743091 | FZD4 | 0.64 |
| 16886382 | KIF5C | 1.00 | 16901974 | IL1A | 0.73 | 17067739 | NRG1 | 0.64 |
| 17102566 | SYTL5 | 1.00 | 16705959 | | 0.73 | 17026034 | | 0.64 |
| 17027187 | | 1.00 | 16996953 | SCRG1 | 0.73 | 16787135 | FLRT2 | 0.64 |
| 17037331 | | 1.00 | 16761139 | LOC728715 | 0.73 | 16662755 | MACF1 | 0.63 |
| 16878731 | EHD3 | 1.00 | 16697004 | RNASEL | 0.72 | 17010703 | PRSS35 | 0.63 |
| 17095111 | GNAQ | 0.99 | 16865153 16944665 | DTX3L | 0.72 | 16928422 16924061 | LOC105372966 | 0.63 |
| 16753222 | LOC105369783 | 0.98 | 17010278 | | 0.72 | 16977251 | ANTXR2 | 0.63 |
| 17094494 17106552 | LOC101928336 | 0.98 0 07 | 16837324 | | 0.72 | 16883201 | | 0.63 |
| 16854360 | ZNF521 | 0.97 | 16750254 | | 0.72 | 16695216 | IGSF9 | 0.63 |
| 17070110 | PI15 | 0.97 | 16737056 | PAX6 | 0.72 | 16949467 | LPP | 0.63 |
| 16705507 | SKGN PRLR | 0.97 | 16667258 17094863 | FNBP1L | 0.72 | 17109714 16972824 | SCARNA9L STOX2 | 0.63 |
| 17094175 | | 0.96 | 16938527 | RBMS3 | 0.72 | 17113710 | TMEM255A | 0.62 |
| 16863534 | | 0.96 | 16765622 | NCR2LC1 | 0.72 | 16944982 | | 0.62 |
| 17105727 | ALUX12-AS1 LL0XNC01-250H12.3 | 0.96 3 0.96 | 16722445 | NGK3LG1 | 0.71 0.71 | 16807763 | SMTU2 EHD4 | 0.62 |
| 16972076 | NPY5R | 0.96 | 17085558 | LOC105379826 | 0.71 | 16880478 | C2orf74 | 0.62 |
| 17018039 | HLA-DPA1 | 0.95 | 16980096 | TBC1D9 | 0.71 | 17097731 16861438 | PAPPA-AS1 | 0.62 |
| 16746341 | MIR4697HG | 0.95 | 16816386 | TMC7 | 0.71 | 16988359 | | 0.62 |
| | | | | | | | | |

F continued

| Transcript ID | Gene Symbol | KALK vs ETV7 | Transcript ID | Gene Symbol | KALK vs ETV | Transcript ID | Gene Symbol | KALK vs ETV7 |
|---------------|-------------------|--------------|---------------|-------------------|--------------|---------------|-----------------|----------------|
| 16911804 | SLC24A3 | 0.61 | 16849148 | RNF157 | 0.55 | 16900098 | IGKV3D-15 | 0.50 |
| 16985950 | MAP1B | 0.61 | 16746638 | | 0.55 | 16854301 | ANKRD29 | 0.50 |
| 16798782 | GOLGA8H | 0.61 | 17051626 | MEST | 0.54 | 17097955 | C5 | 0.50 |
| 16763577 | SLC38A4 | 0.61 | 16972961 | C4orf47 | 0.54 | 17025292 | | 0.50 |
| 16798134 | SEPT6 | 0.61 | 16899533 | LRRTM1 | 0.54 | 16836791 | BAHCC1 | -0.50 |
| 17080516 | ENPP2 | 0.61 | 17102559 | SYTL5 | 0.54 | 16955440 | | -0.50 |
| 16994779 | CDH10 | 0.61 | 17117888 | ZEB2 | 0.54 | 17065117 | | -0.50 |
| 16943350 | ADGRG7 | 0.61 | 16988537 | CDC42BPA | 0.54 | 17046249 | MRPS17 OR1L4 | -0.50 |
| 17117973 | | 0.61 | 16872323 | ZNF780A | 0.54 | 16900016 | | -0.50 |
| 16958251 | MYLK | 0.61 | 16708122 | CNNM1 | 0.54 | 17074342 | LINC00965 | -0.47 |
| 16904588 | TTC21B | 0.61 | 16932914 | ZNF280B | 0.54 | 17101029 | PLCXD1 | -0.50 |
| 16753710 | HELB | 0.61 | 16908583 | IGEBP2 | 0.54 | 17005001 | CD83 | -0.50 |
| 16820386 | | 0.61 | 17107287 | BRS3 | 0.54 | 16973271 | DBET | -0.51 |
| 16917877 | LOC105372571 | 0.60 | 17087032 | | 0.54 | 17067773 | | -0.51 |
| 17094893 | ALDH1A1 | 0.60 | 16714890 | HERC4 | 0.54 | 16847839 | MIR5047 | -0.51 |
| 17047459 | SNORA14A | 0.60 | 16881136 | | 0.54 | 17118054 | | -0.51 |
| 16971660 | LRAT | 0.60 | 16686557 | PIK3R3 | 0.54 | 17046094 | | -0.51 |
| 17092936 | IZUMO3 | 0.60 | 17003401 | LOC202181 | 0.54 | 16829469 | | -0.51 |
| 17054846 | | 0.60 | 16970188 | LOC645513 | 0.54 | 16911261 | BMP2 CBIP2 | -0.51 |
| 16916352 | SRXN1 | 0.60 | 16912224 | LOC105372582 | 0.54 | 16788395 | | -0.51 |
| 16797599 | IGHV3-72 | 0.60 | 17080849 | LOC101927588 | 0.54 | 16863753 | EMP3 | -0.51 |
| 16749639 | TSPAN11 | 0.60 | 17110551 | SSX9 | 0.54 | 16693459 | S100A3 | -0.51 |
| 17010544 | SH3BGRL2 | 0.59 | 17093031 | MOB3B | 0.54 | 16837126 | | -0.51 |
| 17023646 | CTGF | 0.59 | 16679003 | GPR137B | 0.53 | 16949572 | CLDN16 | -0.51 |
| 16730471 | CEP126 | 0.59 | 16807174 | LOC105370772 | 0.53 | 16773032 | | -0.51 |
| 16904425 | GRB14 | 0.59 | 16997650 | ANKRD34B | 0.53 | 16788653 | SNORD114-9 | -0.51 |
| 16686255 | | 0.59 | 16696425 | INFSF4 | 0.53 | 16777198 | APOLD1 | -0.51 |
| 16798665 | GOLGA8J | 0.59 | 16675197 | PLA2G4A | 0.53 | 17019338 | DNPH1 | -0.52 |
| 16906308 | TFPI | 0.59 | 17105163 | ZNF711 | 0.53 | 16954608 | | -0.52 |
| 16758730 | DNAH10 | 0.59 | 17070381 | LRRCC1 | 0.53 | 16835604 | NXPH3 | -0.52 |
| 16877463 | VSNL1 | 0.59 | 16838249 | LOC100507351 | 0.53 | 16806536 | | -0.52 |
| 16706174 | | 0.59 | 16701185 | CEP170 | 0.53 | 16696612 | | -0.52 |
| 16701317 | HNRNPU | 0.59 | 17111435 | WNK3 | 0.53 | 16974224 | | -0.52 |
| 16854639 | NOL4 | 0.59 | 16880168 | RPL23AP32 | 0.53 | 16840756 | MIROOR | -0.52 |
| 16736595 | PTPN5 | 0.59 | 16930144 | APOBEC3A_B | 0.53 | 16844046 | SNORA21 | -0.52 |
| 17028407 | | 0.59 | 16808306 | PPIP5K1 | 0.53 | 16847203 | | -0.52 |
| 16944695 | PARP14 | 0.59 | 17020727 | MIR30A | 0.53 | 17051573 | | -0.52 |
| 16743293 | | 0.58 | 16748474 | EAM10AE | 0.53 | 16871067 | KCN 12 | -0.52 |
| 16705329 | MYPN | 0.58 | 16806461 | NDNL2 | 0.53 | 17116050 | PRKY | -0.52 |
| 16874604 | SHANK1 | 0.58 | 16996004 | BRCAT54 | 0.53 | 17060235 | | -0.52 |
| 16761518 | TAS2R31 | 0.58 | 16939283 | WDR48 | 0.52 | 16774071 | RFXAP | -0.52 |
| 16783887 | LINC00871 | 0.58 | 16730180 | PANX1 | 0.52 | 16849298 | MXRA7 | -0.52 |
| 16794980 | TGFB3 | 0.58 | 16905495 | | 0.52 | 16773942 | | -0.53 |
| 16810473 | DAPK2 | 0.58 | 16705976 | MCU | 0.52 | 1/0/4521 | S100A4 | -0.53 |
| 16920806 | SYCP2 | 0.58 | 16678496 | HIST3H2BB | 0.52 | 16813029 | HAPLN3 | -0.53 |
| 16761583 | LRP6 | 0.58 | 16922959 | MX2 | 0.52 | 16721375 | SMPD1 | -0.53 |
| 16716350 | ACTA2 | 0.58 | 16983456 | | 0.52 | 17114970 | GABRE | -0.53 |
| 16686980 | | 0.57 | 17090512 | | 0.52 | 17013694 | PLEKHG1 | -0.53 |
| 16872803 | CEACAM1 | 0.57 | 16990294 | PCDHB10 H2BEXP | 0.52 | 16869291 | SNORD41 | -0.53 |
| 17103141 | ZNF157 | 0.57 | 16861543 | ZNF527 | 0.52 | 16898432 | | -0.53 |
| 16667662 | CDC14A | 0.57 | 16792154 | SLC25A21 | 0.52 | 17090320 | | -0.53 |
| 16851383 | GATA6 | 0.57 | 16675192 | OCLM | 0.52 | 17085520 | | -0.54 |
| 16677514 | SPATA17 | 0.57 | 16692080 | LOC101928979 | 0.52 | 16737102 | | -0.54 |
| 16723246 | RCN1 | 0.57 | 16922613 | SIM2 | 0.52 | 16858148 | | -0.54 |
| 17039907 | GALINTT | 0.57 | 17098477 | ANGPTL2 | 0.52 | 17049003 | GS1-259H13.2 | -0.54 |
| 16869427 | CACNA1A | 0.57 | 16990288 | ITEG1 | 0.52 | 16839019 | SLC16A3 | -0.54 |
| 16703242 | OTUD1 | 0.57 | 16782003 | TRDC | 0.52 | 17060012 | SHFM1 | -0.54 |
| 16906991 | RFIN2 | 0.57 | 17113928 | TENM1 | 0.52 | 17100695 | SMOC2 | -0.54 |
| 16719233 | OAT | 0.57 | 16669997 | NBPF12 | 0.51 | 16675227 | ERVMER61-1 | -0.54 |
| 17106526 | LONRF3 | 0.57 | 16979944 | | 0.51 | 16965834 | LOC102723846 | -0.54 |
| 16704107 | LINC00839 | 0.57 | 16966721 | SNORA26 | 0.51 | 16961372 | TERC | -0.54 |
| 16982479 | PLEKHG4B | 0.57 | 17036177 | | 0.51 | 16732836 | OR10D3 | -0.55 |
| 16744991 | MPZL3 | 0.57 | 16692452 | NBPE26 | 0.51 | 16787112 | | -0.55 |
| 16921664 | CXADR | 0.57 | 16949909 | MIR570 | 0.51 | 17065903 | DUX4L25 | -0.55 |
| 16941871 | IL17RB | 0.56 | 16757347 | OAS3 | 0.51 | 16973287 | DUX4L25 | -0.55 |
| 16673227 | ARHGAP20 | 0.56 | 16978568 | CENPE CAR1 | 0.51 | 17012721 | EYA4 | -0.55 |
| 16685729 | BMP8B | 0.56 | 16797152 | MIR4710 | 0.51 | 16960111 | | -0.55 |
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F continued

| Transcript ID | Gene Symbol | KALK vs ETV7 | Transcript ID | Gene Symbol | KALK vs ETV7 |
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| 16762992 | LOC105369724 | -0.67 | 16659371 | PRAMEF1 | -2.13 |
| 16737422 16780640 | LOC105370333 | -0.67 | 16681907 | PRAMEF14 | -2.19 |
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| 17044193 17077267 | LOC101927841 | -0.70 -0.70 | 16977052 | CXCL10 | -3.84 |
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| 16695508 | TSTD1 | -0.73 | | | |
| 17019543 | LOC101929705 | -0.73 | | | |
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| 16965606 16971272 | SLU34A2 EDNRA | -0.74 -0.75 | | | |
| 16957263 | DPPA4 | -0.75 | | | |
| 16778829 | CPB2 | -0.75 | | | |
| 16704237 16983159 | | -0.76 -0.76 | | | |
| 17002874 | | -0.76 | | | |
| 17048742 | NPTX2 | -0.76 | | | |

G: Mass spectrometry analysis of purified Origene human ETV7 protein.

 $\boldsymbol{H}:$ Mass spectrometry analysis of purified Origene human mTOR protein.



Η

| Description | Spectral Count | Total Peptides | Description | Spectral Count | Total Peptides |
|---|----------------|----------------|---|----------------|----------------|
| Transcription factor ETV7 | 716 | 20 | Serine/threonine-protein kinase mTOR | 1341 | 187 |
| Tubulin beta chain | 29 | 12 | Heat shock 70 kDa protein 1A/1B | 215 | 38 |
| Tubulin beta-4A chain | 29 | 11 | Heat shock cognate 71 kDa protein | 162 | 34 |
| Heat shock 70 kDa protein 1A/1B | 21 | 12 | Heat shock 70 kDa protein 1-like | 104 | 13 |
| Tubulin beta-2A chain | 20 | 8 | Protein arginine N-methyltransferase 5 | 85 | 26 |
| Pyruvate kinase PKM | 17 | 11 | Heat shock cognate 71 kDa protein (Fragment) | 80 | 12 |
| Heat shock protein HSP 90-beta | 14 | 11 | Tubulin beta-4B chain | 61 | 18 |
| L-lactate dehydrogenase B chain | 14 | 7 | Tubulin beta chain | 56 | 16 |
| Elongation factor 2 | 14 | 7 | Tubulin beta-4A chain | 49 | 14 |
| Heat shock cognate 71 kDa protein | 11 | 8 | Heat shock 70 kDa protein 6 | 46 | 8 |
| Histone H2B type 1-O | 11 | 2 | Kinesin-like protein KIF11 | 44 | 25 |
| Histone H2B type 1-H | 11 | 2 | Tubulin beta-2A chain | 42 | 12 |
| Heat shock 70 kDa protein 6 | 10 | 5 | Tubulin beta-2B chain | 42 | 12 |
| Histone H4 | 9 | 4 | Methylosome protein 50 | 42 | 11 |
| Guanine nucleotide-binding protein subunit beta-2-l | like 1 9 | 7 | Protein phosphatase 1B | 41 | 18 |
| Heat shock protein HSP 90-alpha | 7 | 5 | Heat shock 70 kDa protein 4 | 38 | 23 |
| Heat shock cognate 71 kDa protein (Fragment) | 6 | 4 | 60 kDa neat shock protein, mitochondriai | 36 | 23 |
| ATP-dependent RNA helicase A | 6 | 6 | Stress-70 protein, mitochondrial | 33 | 1/ |
| Actin, gamma-enteric smooth muscle | 5 | 1 | I ubulin beta-3 chain | 26 | 10 |
| ATP synthase subunit beta, mitochondrial | 5 | 5 | Heat shock protein 105 kDa | 23 | 18 |
| Glyceraldehyde-3-phosphate dehydrogenase | 4 | 3 | BAG family molecular chaperone regulator 2 | 18 | 10 |
| 40S ribosomal protein S14 | 4 | 2 | Ubiquitin (Fragment) | 16 | 4 |
| Rab GDP dissociation inhibitor alpha | 4 | 3 | lubulin beta-8 chain | 14 | 4 |
| ATP synthase subunit alpha, mitochondrial | 4 | 4 | 40S ribosomal protein S3 | 11 | 9 |
| Histone H2A type 1-D | 4 | 2 | ADP/ATP translocase 2 | 11 | / |
| Histone H1.2 | 4 | 3 | Heat shock protein HSP 90-beta | 9 | 9 |
| Elongation factor 1-alpha 1 | 4 | 3 | 78 KDa glucose-regulated protein | 9 | 4 |
| Ubiguitin (Fragment) | 4 | 2 | Host shock 70 kDs protein 4 | 9 | 3 |
| Nuclease-sensitive element-binding protein 1 | 4 | 4 | Real Shock 70 kDa protein 4L | 9 | 9 |
| L-lactate dehydrogenase A chain 2 | 4 | 3 | 408 ribesomal protein 827 | 0 | 3 |
| 60S ribosomal protein L8 | 4 | 3 | 52 ubiquitin protoin ligano CHIP | 0 | 4 |
| Heterogeneous nuclear ribonucleoproteins C1/C2 | 4 | 2 | 60S ribosomal protein L23 | 8 | 2 |
| Fatty acid synthase | 4 | 3 | Soring/throoping protein kinges 29 | 0 | 2 |
| Rab GDP dissociation inhibitor beta | 3 | 2 | Heat shock protein HSP 00 alpha | 0 | 6 |
| 40S ribosomal protein S3 | 3 | 2 | Phosphate carrier protein mitochondrial | 7 | 4 |
| Histone H3.2 | 3 | 2 | Flongation factor Tu, mitochondrial | 6 | 4 |
| 40S ribosomal protein S8 | 3 3 | 2 | Sodium/potassium-transporting ATPase subunit alpha- | .1 5 | 5 |
| Histone H3.1 | 3 | 2 | ADP/ATP translocase 1 | 5 | 4 |
| 60S ribosomal protein I 19 | 3 | 2 | 40S ribosomal protein S2 | 5 | 5 |
| T-complex protein 1 subunit epsilon | 3 | 2 | RNA-binding protein 10 | 5 | 4 |
| T-complex protein 1 subunit alpha | 3 3 | 3 | ATP synthase subunit alpha mitochondrial | 4 | 4 |
| D-3-phosphoglycerate dehydrogenase | 3 | 3 | Elongation factor 1-alpha 1 | 4 | 3 |
| 60S ribosomal protein L10 | 3 | 2 | DnaJ homolog subfamily A member 1 | 4 | 4 |
| Poly [ADP-ribose] polymerase 1 | 3 3 | 2 | 40S ribosomal protein S18 | 3 | 3 |
| Dolichyl-diphosphooligosaccharide-protein | 3 | 2 | Eukarvotic translation initiation factor 4B | 3 | 3 |
| glycosyltransferase subunit | 3 | 3 | 60S ribosomal protein L10 | 3 | 2 |
| 60S ribosomal protein L23 | 3 | 2 | 26S proteasome non-ATPase regulatory subunit 1 | 3 | 3 |
| Proliferating cell nuclear antigen | 3 3 | 3 | BAG family molecular chaperone regulator 5 | 3 | 3 |
| Cofilin-1 | 2 | 2 | Sodium/potassium-transporting ATPase subunit alpha- | 3 2 | 2 |
| Eukarvotic initiation factor 4A-II | 2 | 2 | ATP synthase subunit alpha, mitochondrial | 2 | 2 |
| ADP/ATP translocase 1 | 2 | 2 | 40S ribosomal protein S16 | 2 | 1 |
| 40S ribosomal protein S11 | 2 | 2 | Methylosome subunit pICIn | 2 | 1 |
| 78 kDa glucose-regulated protein | 2 | 2 | 60S ribosomal protein L11 | 2 | 1 |
| 60S ribosomal protein L13 | 2 | 2 | OTU domain-containing protein 4 | 2 | 2 |
| Neutral alpha-glucosidase AB | 2 | 2 | Serine/threonine-protein kinase RIO1 | 2 | 1 |
| 40S ribosomal protein S4 X isoform | 2 | 2 | · | | |
| Libiquitin_40S ribosomal protein S27a | 2 | <u>د</u> 1 | | | |
| Prohibitin_2 | 2 | 2 | | | |
| Heterogeneous nuclear ribonucleanratain U | 2 | 2 | | | |
| 60S ribosomal protoin L14 | 2 | 2 | | | |
| | 2 | 2 | | | |