

Supplementary information

***ETV6/RUNX1* FUSION GENE ABROGATION DECREASE THE ONCOGENICITY OF TUMORS CELLS IN A PRECLINICAL MODEL OF ACUTE LYMPHOBLASTIC LEUKEMIA.**

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Supplementary tables and figures

Table S1. sgRNA designed against *E/R* fusion sequence. Two custom-designed single guide RNAs (sgRNAs) were designed to genetically inactivate the *E/R* oncogene. These specific sgRNAs direct Cas9 to the *E/R* fusion sequence. G1 sgRNA, directed at the end of exon 5 of *ETV6* and G2 directed to intronic region before the fusion point between *ETV6* and *RUNX1*.

	<i>Forward</i>	<i>Reverse</i>
<i>G 1</i>	<u>caccg</u> GCCTAATTGGGAATGGTGCG	<u>aaac</u> CGCACCATTCCCAATTAGGC <u>c</u>
<i>G 2</i>	<u>caccg</u> AAGAGCACGCCATGCCATT	<u>aaac</u> AATGGGCATGGCGTGCTCTT <u>c</u>

Table S2. Possible off-targets of the sgRNAs. The possible off-targets of the sgRNAs used obtained from “Breaking Cas” website (<http://bioinfogp.cnb.csic.es/tools/breakingcas/>) were checked by PCR and sanger sequencing. Two pairs of oligonucleotides were designed for each off-target region.

<i>Gen</i>	<i>Score</i>	<i>Forward</i>	<i>Reverse</i>
<i>PTPN21</i>	0	AGTGTAAATTTGGGAAACAGCCCT	TTGGGGCTTTTCCCACCTCAA
<i>BCL9L</i>	0.3	AGAGAATGGATCTGGGAGGGA	AGGCGAGGCAGTTGCAGTGTA
<i>BEND4</i>	0.1	GGCATCAGGTAGCCAACGTTC	CAGGATCAATGGATTTGTACAG
<i>VSIR</i>	0	AAAGGGTCCAGAGAAGAGAGG	CTGGGGACGGAGCAAACTTT

Table S3. Genes significantly deregulated after *E/R* fusion gene abrogation. List of 342 genes significantly deregulated after *E/R* fusion gene abrogation sorted according to the decreasing value of the FC. The TP50 of deregulated genes are shaded in grey. In the following columns from left to right, the mean of normalized counts of all samples (baseMean); log₂-Fold Change (log₂FC); log₂FC unshrunk, P-value and adjusted P value.

Gene	baseMean	log ₂ FC	log ₂ FCunshrunk	Pvalue	Padj
<i>DRD5</i>	154.4	-2.34959	-3.92883	0	0
<i>ATP10A</i>	231.1	-2.07428	-3.35631	0	0
<i>SGIP1</i>	70.3	-2.0645	-3.86631	0	0
<i>BIRC3</i>	80.1	1.98755	4.09318	0	0
<i>ACKR3</i>	66.8	-1.98193	-3.84476	0	0
<i>EMR2</i>	80.4	1.97712	3.98976	0	0
<i>FAM189A1</i>	55.5	-1.80486	-4.18028	0	1.97E-13
<i>MIR146A</i>	278.6	1.77216	2.54174	0	1E-15
<i>OPTN</i>	315.4	1.7413	2.24832	0	0
<i>LHX6</i>	127.2	1.72519	3.8055	4E-15	3.17E-12

<i>NBPF3</i>	35.4	-1.67532	-4.9272	1.6E-14	1.23E-11
<i>GCSAM</i>	1072	-1.67221	-1.90226	0	0
<i>PGPEP1</i>	94.7	-1.66199	-3.62221	3.7E-14	2.72E-11
<i>KCNA2</i>	143.1	-1.65663	-2.17481	0	1E-15
<i>RP11-622K12.1</i>	367.2	1.59169	2.28594	2E-15	1.94E-12
<i>RASD2</i>	209.2	-1.47352	-2.0491	6.1E-14	4.04E-11
<i>CDH23</i>	265.2	-1.44672	-1.96584	6E-14	4.04E-11
<i>ALOX5</i>	89.6	1.37691	2.54469	2.65E-10	1.38E-07
<i>SORBS2</i>	882	-1.364	-1.63045	0	4.4E-14
<i>HYDIN</i>	83.6	1.36022	3.17093	5.3E-10	2.67E-07
<i>GPR17</i>	119.4	-1.33855	-2.68649	1.05E-09	4.79E-07
<i>MOXD1</i>	162.7	1.33314	3.05082	1.17E-09	5.15E-07
<i>ARX</i>	64.4	-1.30499	-2.44801	2.28E-09	9.76E-07
<i>KCNE3</i>	249.5	-1.30368	-1.95828	2.64E-10	1.38E-07
<i>HOXA13</i>	234.3	1.29849	1.52883	0	1.13E-13
<i>LCK</i>	214.4	-1.29552	-2.04607	7.9E-10	3.84E-07
<i>RELB</i>	271.2	1.28523	1.51994	0	4.11E-13
<i>DDR1</i>	207.4	1.23577	1.58447	9.25E-12	5.62E-09
<i>SCN3A</i>	361.8	1.23448	1.91793	3.86E-09	1.52E-06
<i>FRMD4B</i>	807.8	-1.22731	-1.62866	8.07E-11	4.52E-08
<i>LIMS2</i>	495.1	-1.20713	-2.10548	2.5E-08	7.02E-06
<i>RGS1</i>	221.1	-1.19302	-1.85261	1.24E-08	4.03E-06
<i>CALML6</i>	75.1	1.19083	1.84701	1.21E-08	4E-06
<i>FZD3</i>	83	1.179	2.86812	6.42E-08	1.49E-05
<i>ARHGAP42P2</i>	187.8	-1.17718	-2.04583	5.34E-08	1.25E-05
<i>RP11-251M1.1</i>	558.7	1.1701	1.89706	3.88E-08	9.91E-06
<i>RXRA</i>	695.7	1.16993	1.68558	6.77E-09	2.47E-06
<i>RGS16</i>	431.5	1.16993	4.27179	1.85E-08	5.52E-06
<i>HOTTIP</i>	40.8	1.16894	2.4038	1.01E-07	2.26E-05
<i>FBN3</i>	196.1	-1.16298	-1.45832	3.86E-11	2.25E-08
<i>RP11-16L9.4</i>	439.2	1.15288	1.66285	1.14E-08	3.86E-06
<i>HLX</i>	101.3	1.14724	1.59611	5.25E-09	1.97E-06
<i>TP63</i>	318.4	1.1262	1.88183	1.55E-07	3.14E-05
<i>TLR7</i>	61.4	1.12406	2.8608	2.25E-07	4.37E-05
<i>PTPRG-AS1</i>	73.4	-1.11862	-3.58436	1.17E-07	2.44E-05
<i>FAM78A</i>	548.8	1.11483	1.43424	9.77E-10	4.59E-07
<i>BCAS1</i>	210.8	-1.11072	-1.8555	2.28E-07	4.38E-05
<i>PLS1</i>	62.8	1.10389	2.20117	4.87E-07	8.16E-05
<i>AQP5</i>	229.5	1.09449	1.83256	3.47E-07	6.1E-05
<i>PDE10A</i>	95.9	-1.08807	-2.11786	6.94E-07	0.000109
<i>CSF1</i>	29.6	1.07667	3.30357	4.18E-07	7.16E-05
<i>TTC28</i>	422.8	-1.06872	-1.42756	1.9E-08	5.53E-06
<i>EPHB6</i>	332.1	1.05097	1.40655	3.42E-08	8.9E-06
<i>GIMAP6</i>	104.7	-1.04917	-2.08309	1.74E-06	0.000237
<i>CNR2</i>	151.2	-1.04836	-1.46725	1.15E-07	2.43E-05
<i>SGK1</i>	203.2	1.04539	1.4315	7.51E-08	1.71E-05

<i>TRPM2</i>	307.5	1.04389	1.56381	4.22E-07	7.16E-05
<i>SLC25A4</i>	375.9	-1.04233	-1.35587	1.71E-08	5.4E-06
<i>SERPIN1</i>	449.6	-1.03395	-1.48499	2.89E-07	5.4E-05
<i>ADCY7</i>	789.1	1.02767	1.67585	1.47E-06	0.000206
<i>EPN2</i>	211.7	-1.02564	-2.01505	2.94E-06	0.000382
<i>NPL</i>	61.1	1.01372	1.61799	1.64E-06	0.000226
RP11-1055B8.4	129.1	1.00869	1.41433	3.47E-07	6.1E-05
<i>CD52</i>	5748.7	-1.00638	-1.07155	0	0
<i>C10orf10</i>	193.8	-1.00598	-1.707	3.06E-06	0.000394
<i>MYO7B</i>	130	-1.00505	-2.57867	3.37E-06	0.000419
<i>N4BP3</i>	1631.4	1.0044	1.34839	1.49E-07	3.05E-05
<i>TRNP1</i>	843.7	-1.00257	-1.36303	2.17E-07	4.27E-05
RP11-443A13.5	571.4	1.00152	1.21962	2.54E-09	1.06E-06
<i>LRP4</i>	64.7	0.99177	2.46045	4.97E-06	0.000603
<i>TSPAN9</i>	526.5	-0.99105	-1.11836	1.14E-12	7.23E-10
<i>TMEM64</i>	260.3	0.98891	1.45127	1.27E-06	0.000182
<i>TCF7</i>	151	-0.98381	-1.32807	2.96E-07	5.46E-05
<i>MMP14</i>	192.5	0.98032	1.80139	7.14E-06	0.000846
<i>FAM107B</i>	10387.3	-0.97505	-1.19805	1.14E-08	3.86E-06
<i>PLEK</i>	1078.2	0.96863	1.28742	3.1E-07	5.65E-05
<i>NOLA</i>	214.5	0.96397	1.17625	1.07E-08	3.8E-06
<i>CAV1</i>	297	-0.95749	-1.27142	4.08E-07	7.08E-05
<i>AGR2</i>	38.8	0.95646	5.4769	9.71E-07	0.000149
<i>RGMA</i>	1017.8	-0.95634	-1.18978	4.04E-08	1.02E-05
<i>RASSF4</i>	289	-0.95437	-1.75826	1.25E-05	0.001381
<i>LCN6</i>	249.8	0.95321	1.81714	1.36E-05	0.001474
RP11-1055B8.7	6983	0.94416	1.00376	0	0
<i>PTMS</i>	72.4	0.94242	1.9579	1.77E-05	0.001864
<i>CYTH4</i>	524.2	0.9396	1.24137	5.87E-07	9.41E-05
<i>KLHDC7B</i>	22.2	0.93929	2.83172	9.63E-06	0.001105
<i>HAPI</i>	3579.1	-0.936	-1.13741	2.31E-08	6.59E-06
<i>RHOH</i>	1146.9	-0.9267	-1.23334	1.02E-06	0.000153
<i>IRF8</i>	803.1	-0.92338	-1.25995	1.95E-06	0.000263
<i>LARGE</i>	415.8	-0.91806	-1.3959	1.02E-05	0.001156
<i>LAMB4</i>	79.9	-0.9156	-1.67425	2.72E-05	0.002662
<i>AK7</i>	1333.1	-0.91269	-1.0905	1.8E-08	5.52E-06
<i>TBXAS1</i>	369.3	0.9111	1.09726	3.12E-08	8.26E-06
<i>PLEKHB1</i>	67.2	0.90934	1.81052	3.42E-05	0.00322
<i>SPTBN4</i>	89.6	-0.90781	-1.24563	3.11E-06	0.000397
<i>CXXC5</i>	1769.6	0.90625	1.05615	3.28E-09	1.33E-06
<i>GUCY1A3</i>	222.9	-0.89857	-1.50261	2.9E-05	0.00282
<i>GPRI42</i>	27.8	-0.89415	-2.85582	1.96E-05	0.001998
<i>ZNF264</i>	134.7	0.89389	1.26566	7.45E-06	0.000869
<i>SNTA1</i>	325.1	-0.88594	-1.09918	3.25E-07	5.86E-05
<i>SV2A</i>	972.4	0.88563	1.08285	1.7E-07	3.39E-05
<i>ZNF469</i>	158.6	0.88264	1.58403	5.07E-05	0.004447

<i>PIGM</i>	310.8	0.88148	1.10151	4.97E-07	8.23E-05
<i>AC090044.2</i>	95.8	0.87855	1.7981	6.3E-05	0.005364
<i>SEMA7A</i>	122.3	0.87808	1.47324	4.44E-05	0.004041
<i>NTN3</i>	154.2	0.87613	1.09977	6.78E-07	0.000107
<i>LINC01013</i>	150	-0.87602	-1.36521	3.06E-05	0.002955
<i>CROCC</i>	298.9	0.87521	1.03825	4.13E-08	1.02E-05
<i>MMP15</i>	449.3	-0.8698	-1.13171	2.58E-06	0.000344
<i>RP11-1055B8.6</i>	283	0.86919	1.226	1.27E-05	0.001392
<i>EMP2</i>	378.1	0.86759	1.09257	9.99E-07	0.000152
<i>RP11-431N15.2</i>	20.1	0.86663	5.26703	6.26E-06	0.000747
<i>ZNF528</i>	133.8	0.8636	1.48223	6.46E-05	0.005392
<i>FCGR2A</i>	66.4	0.86013	1.72152	8.92E-05	0.006923
<i>NEIL1</i>	67.7	0.85161	1.49228	8.63E-05	0.006797
<i>SITI</i>	183.8	0.85063	1.50487	9.1E-05	0.006981
<i>NT5E</i>	323.3	-0.85004	-1.0867	2.66E-06	0.000352
<i>CECR2</i>	268.9	0.84865	1.17241	1.5E-05	0.001603
<i>SERPIN6</i>	560.5	-0.84378	-1.0843	3.67E-06	0.000453
<i>SIDT1</i>	263.9	-0.84107	-1.39962	8.96E-05	0.006923
<i>AFF2</i>	979.3	0.83909	1.10828	8.18E-06	0.000947
<i>TEAD3</i>	73	0.83854	1.4096	9.67E-05	0.007272
<i>CD302</i>	207.1	0.83694	1.39459	9.73E-05	0.007272
<i>CIQTNF4</i>	454.5	-0.83462	-1.02567	1.03E-06	0.000153
<i>PTPN7</i>	496.1	-0.83337	-1.42407	0.000115	0.008254
<i>LBH</i>	1252.6	-0.8326	-1.02589	1.22E-06	0.000178
<i>RIC3</i>	24	0.83244	4.0662	1.94E-05	0.001998
<i>SEMA4A</i>	185.8	0.83085	1.17682	3.18E-05	0.003026
<i>NRBP2</i>	246.9	0.82947	1.18658	3.71E-05	0.00344
<i>TSKU</i>	93.4	0.82585	1.35517	0.000112	0.008122
<i>CD40</i>	168.6	0.82537	1.01695	1.47E-06	0.000206
<i>DBNDD1</i>	403.2	-0.82471	-1.16418	3.51E-05	0.00328
<i>KCNJ4</i>	36.7	-0.82267	-1.86593	0.000169	0.011166
<i>SORBS1</i>	27.8	0.81941	2.04647	0.000157	0.010539
<i>IL4I1</i>	31.7	0.81657	2.62006	8.98E-05	0.006923
<i>RP11-469H8.6</i>	67.9	0.81609	2.12138	0.000151	0.010331
<i>GNG11</i>	1694.7	-0.81545	-1.06571	1.16E-05	0.001309
<i>CAP2</i>	128.4	-0.81443	-1.23333	8.73E-05	0.006837
<i>PLEC</i>	410.6	0.80672	1.04502	1.17E-05	0.001309
<i>PDZD7</i>	100.6	0.80051	1.19706	0.000104	0.007603
<i>TMEM52</i>	235.2	0.79981	1.61531	0.00027	0.016797
<i>AC090044.1</i>	372.3	0.79949	1.33198	0.000198	0.01259
<i>ATP13A2</i>	773.7	0.79809	0.98318	3.28E-06	0.000412
<i>NPY</i>	354.1	0.79767	1.1838	0.000104	0.007611
<i>SLC51A</i>	923.8	-0.79604	-1.11872	6.19E-05	0.005302
<i>PSD2</i>	32.5	0.78983	1.91702	0.000279	0.017214
<i>CAST</i>	535.9	0.78854	1.09765	6.42E-05	0.005392
<i>PRR5L</i>	357.9	-0.785	-0.9029	1.1E-07	2.39E-05

<i>ARHGEF12</i>	926.6	-0.78172	-0.87228	5.27E-09	1.97E-06
<i>C20orf197</i>	21.9	0.78168	2.91678	0.000115	0.008254
<i>ZNF704</i>	1650.9	-0.78124	-0.89744	1.15E-07	2.43E-05
<i>DAB2IP</i>	529.9	0.77986	0.99995	1.79E-05	0.001872
<i>TMEM173</i>	173.5	0.77921	1.1944	0.00019	0.012234
<i>IGHD</i>	430.3	0.77753	1.17758	0.000182	0.011865
<i>ACTN1</i>	1971.1	0.7764	0.88027	4.2E-08	1.02E-05
<i>RP3-455J7.4</i>	112.6	0.7684	1.51577	0.000463	0.025573
<i>TMEM229B</i>	179.1	0.76807	1.38713	0.000428	0.024342
<i>NAT8L</i>	59	0.7666	1.47598	0.000471	0.02581
<i>RBMS2</i>	105.9	0.76601	1.02433	5.69E-05	0.004937
<i>SPNS2</i>	96.1	-0.76441	-1.13302	0.000197	0.01259
<i>FAM65B</i>	1931.9	-0.76332	-1.03087	7.24E-05	0.005959
<i>KCTD12</i>	44.2	0.7633	1.79551	0.00046	0.025497
<i>LOXHD1</i>	82	-0.76287	-2.05591	0.000363	0.021387
<i>ARLAC</i>	117.7	-0.7613	-1.60277	0.000521	0.027621
<i>PDLIM1</i>	3779.7	-0.75931	-1.07166	0.00014	0.009802
<i>ZNF169</i>	102.5	0.75727	1.06339	0.000135	0.009626
<i>TPM2</i>	503.6	0.75349	0.95242	2.39E-05	0.002421
<i>PFN2</i>	420.2	0.75328	1.19692	0.000376	0.022024
<i>WFS1</i>	101.5	0.75075	1.18653	0.000381	0.022203
<i>LCN10</i>	42.8	0.74986	1.967	0.000486	0.026329
<i>LILRB4</i>	30.2	-0.74518	-2.02167	0.000494	0.02656
<i>NCALD</i>	46.4	-0.74335	-1.48403	0.000709	0.034244
<i>ID2</i>	152.8	0.74327	1.53331	0.00071	0.034244
<i>CAMK4</i>	217.7	0.74297	0.96783	6.03E-05	0.0052
<i>PDGFA</i>	327.7	0.74222	1.18772	0.000473	0.025829
<i>TEX14</i>	53.7	0.74155	2.36737	0.000355	0.02103
<i>SPRY1</i>	230.1	-0.73794	-0.92144	2.53E-05	0.002512
<i>STIM1</i>	473.7	-0.73641	-0.97332	9.19E-05	0.00701
<i>SPARC</i>	101.2	0.7362	2.12186	0.000497	0.026604
<i>CACNA2D1</i>	75.1	0.73588	1.23955	0.000631	0.031553
<i>ITGB2</i>	713.9	0.73385	0.972	0.000101	0.007414
<i>CTD-3018O17.3</i>	68.5	0.73343	2.08948	0.000537	0.028172
<i>ANO1</i>	25.8	-0.73342	-1.94635	0.000632	0.031553
<i>C20orf194</i>	216.2	0.73325	0.93779	5.16E-05	0.004504
<i>MS4A4A</i>	134.3	0.73223	0.95342	7.59E-05	0.006175
<i>GARNL3</i>	45.5	0.73071	1.25472	0.000723	0.034641
<i>CARD9</i>	84.4	0.72843	1.27716	0.000792	0.037227
<i>LINC00114</i>	50.9	0.72708	1.56987	0.000912	0.041384
<i>GALNT10</i>	153.9	0.72678	1.17004	0.000634	0.031553
<i>C1orf222</i>	60.1	0.7267	3.38097	0.00016	0.010692
<i>RASGRP2</i>	5671	0.72471	0.80094	1.86E-08	5.52E-06
<i>RP11-830F9.7</i>	568.5	0.72421	0.97573	0.000161	0.0107
<i>MAP3K15</i>	84	0.7234	2.14555	0.000581	0.030165
<i>TACC2</i>	14.2	-0.7217	-2.80884	0.000312	0.018838

<i>CORO2A</i>	263.9	-0.72125	-1.07485	0.000465	0.025585
<i>TNFAIP8</i>	784.8	-0.72046	-0.80155	5.25E-08	1.25E-05
<i>PKIG</i>	180.6	0.7152	1.42286	0.001122	0.048384
<i>TLR3</i>	112	0.71488	1.14196	0.000752	0.035921
<i>LMNA</i>	28	0.71359	1.78526	0.000974	0.043658
<i>TMPRSS15</i>	176.6	-0.71246	-1.40711	0.001171	0.049885
<i>FAM132B</i>	448.9	-0.71207	-0.93437	0.000138	0.009751
<i>LACC1</i>	65.3	0.71038	1.2899	0.00113	0.048557
<i>BRINP2</i>	106.2	-0.70892	-1.81139	0.000999	0.044262
<i>HNRNPAIP37</i>	148.6	-0.70868	-1.18719	0.000988	0.04402
<i>FAIM3</i>	190.7	-0.70773	-1.18642	0.001006	0.044414
<i>ZNF641</i>	98.1	0.70696	1.05068	0.000587	0.030165
<i>TTC9</i>	223.9	-0.7064	-1.07029	0.000674	0.032979
<i>ABLIM1</i>	225.1	-0.70592	-1.07602	0.000704	0.034244
<i>C10orf128</i>	142.5	0.70535	1.8123	0.00105	0.045788
<i>BTN2A3P</i>	99.4	0.69936	1.01969	0.000588	0.030165
<i>PTPN3</i>	66.8	-0.69834	-1.16774	0.001161	0.049594
<i>SERINC5</i>	435.8	-0.69699	-0.97851	0.000449	0.02528
<i>SLC38A1</i>	6284.3	-0.69645	-0.87412	8.05E-05	0.006483
<i>DAPK2</i>	666.3	-0.69437	-0.83711	2.59E-05	0.002554
<i>GIMAP8</i>	19.7	-0.69337	-2.63213	0.000536	0.028172
<i>BMP2K</i>	1727.1	-0.69233	-0.75947	2.78E-08	7.64E-06
<i>HIP1R</i>	570	-0.6917	-0.84738	4.71E-05	0.004258
<i>HTRA3</i>	17.2	0.6915	2.51822	0.000634	0.031553
<i>DSTYK</i>	220	0.6907	0.87977	0.000126	0.009033
<i>BCL11B</i>	316.1	0.68927	0.99353	0.000652	0.03221
<i>PLXNC1</i>	284.8	0.68588	0.78584	2.71E-06	0.000356
<i>ORAI2</i>	1167.4	-0.68344	-0.88109	0.000189	0.012234
<i>XBP1</i>	5123.5	-0.68319	-0.93619	0.000456	0.025497
<i>CDH24</i>	1294	0.68212	0.84292	7.64E-05	0.006185
<i>CD72</i>	1662	0.68122	0.88181	0.000213	0.013462
<i>PCDHGC4</i>	364.2	-0.68111	-0.82387	4.12E-05	0.0038
<i>GREB1</i>	1076	-0.68088	-0.95199	0.000586	0.030165
<i>KCNQ10T1</i>	78.4	0.67777	1.03449	0.001142	0.048945
<i>MBOAT1</i>	104.5	0.67352	1.00355	0.001076	0.046676
<i>ZFH3</i>	434.9	0.67246	0.91572	0.00052	0.027621
<i>NEAT1</i>	2886.8	0.67221	0.95417	0.000787	0.037215
<i>PCCA</i>	700.6	-0.67141	-0.84344	0.000147	0.010087
<i>LRRC4</i>	495.5	-0.6704	-0.79996	3.38E-05	0.003202
<i>BCL6</i>	2112.8	-0.66649	-0.72574	2.96E-08	7.98E-06
<i>PTPRK</i>	5372.9	-0.66494	-0.75968	4.57E-06	0.000559
<i>ALDH3A2</i>	645	-0.66287	-0.85065	0.000271	0.016797
<i>METRNL</i>	199.1	-0.6628	-0.81304	9.94E-05	0.007392
<i>RP11-715J22.6</i>	155.9	0.66094	0.85659	0.000331	0.019844
<i>FMO4</i>	18.3	0.65854	2.85429	0.000706	0.034244
<i>MF12</i>	975.1	0.65699	0.78398	4.86E-05	0.004367

<i>CBR3-ASI</i>	468.6	-0.65573	-0.7299	7.64E-07	0.000118
<i>GALNT12</i>	180.5	0.65362	0.79167	8.6E-05	0.006797
<i>MECOM</i>	542.8	-0.64174	-0.83026	0.000482	0.02623
<i>GIPC1</i>	309.8	0.6412	0.80317	0.000271	0.016797
<i>SIKE1</i>	1347.5	-0.63951	-0.85494	0.00079	0.037227
<i>TAGLN3</i>	209.4	-0.63932	-0.79078	0.000213	0.013462
<i>AC002454.1</i>	1316.6	-0.63931	-0.76796	9.63E-05	0.007272
<i>AATK</i>	11.4	-0.6371	-3.43338	0.000604	0.03067
<i>ZC3H12A</i>	275.7	0.63261	0.82339	0.000632	0.031553
<i>MYOM2</i>	241.4	0.63191	0.82335	0.000651	0.03221
<i>LAX1</i>	250.8	-0.61615	-0.79876	0.000827	0.038338
<i>RMND5B</i>	689.1	0.61304	0.75966	0.000402	0.023211
<i>GPSMI</i>	5511.7	0.60465	0.71777	0.000157	0.010539
<i>SSH1</i>	1022.2	-0.60434	-0.66309	1.27E-06	0.000182
<i>DPEPI</i>	659.7	0.59957	3.82265	0.000718	0.034544
<i>PECR</i>	528.8	-0.5978	-0.66384	5.37E-06	0.000646
<i>AVEN</i>	268.8	-0.59717	-0.75211	0.000767	0.036418
<i>LIG4</i>	1424.3	-0.59515	-0.76201	0.001043	0.045721
<i>JAM2</i>	167.8	0.59361	0.74641	0.000798	0.037408
<i>C12orf23</i>	3429.3	-0.59254	-0.63665	1.07E-07	2.36E-05
<i>HLA-F</i>	1579	0.59166	0.72213	0.00046	0.025497
<i>TMEM169</i>	243.2	-0.58601	-0.73804	0.00096	0.043177
<i>PPM1L</i>	569	0.58504	0.69388	0.000247	0.015533
<i>GRAMD1B</i>	666.5	-0.58441	-0.72068	0.000674	0.032979
<i>TCL1A</i>	14125.8	-0.58379	-0.7102	0.000508	0.027111
<i>LTBP4</i>	1986	-0.58366	-0.70366	0.000408	0.023403
<i>GABRA3</i>	600.3	0.58365	0.73228	0.000946	0.042792
<i>CLSTN3</i>	436.5	0.58028	0.65011	1.96E-05	0.001998
<i>OPN3</i>	765.2	-0.57808	-0.64306	1.25E-05	0.001381
<i>INO80C</i>	371.9	0.57505	0.70177	0.000659	0.03245
<i>AJUBA</i>	747	0.57157	0.65377	8.59E-05	0.006797
<i>LRRC8C</i>	2327.4	-0.57157	-0.70511	0.000891	0.040809
<i>PIK3C3</i>	6227	-0.56954	-0.63715	2.53E-05	0.002512
<i>TSPAN7</i>	257.4	0.56592	0.65737	0.000194	0.012467
<i>ANXA2</i>	1995	0.56427	0.62478	1.39E-05	0.001501
<i>IL1RAP</i>	795.7	-0.56395	-0.66952	0.000424	0.024203
<i>NFKB2</i>	609.9	0.56343	0.67862	0.000628	0.031553
<i>BLVRB</i>	781.5	-0.56098	-0.62694	3.12E-05	0.002991
<i>IER2</i>	3387.6	0.56098	0.64561	0.000153	0.010429
<i>DENND1B</i>	599.4	-0.55701	-0.67477	0.000829	0.038338
<i>MAP4K5</i>	1334.5	0.55277	0.59071	2.82E-07	5.33E-05
<i>BCL3</i>	644.3	0.55203	0.60895	1.56E-05	0.001661
<i>LDB2</i>	465.5	-0.5518	-0.6714	0.001019	0.044866
<i>SMC6</i>	3061.7	-0.54865	-0.62885	0.00018	0.01179
<i>ZNF253</i>	1646.3	-0.54837	-0.64474	0.00046	0.025497
<i>NRN1</i>	2783.7	-0.54527	-0.6447	0.000585	0.030165

<i>SLC39A8</i>	923.8	-0.54477	-0.65199	0.000815	0.037948
<i>CIC</i>	1809	0.54446	0.60198	2.46E-05	0.002474
<i>SYNE3</i>	1544.9	-0.54423	-0.60647	4.41E-05	0.004041
<i>EVPL</i>	980.6	-0.54063	-0.61483	0.000157	0.010539
<i>METTL17</i>	1465.2	-0.53989	-0.6443	0.000846	0.039027
<i>PARVG</i>	875.4	0.53972	0.62981	0.000445	0.025159
<i>KLHL6</i>	2008.1	-0.53792	-0.59875	5.01E-05	0.004447
<i>ZNF772</i>	554.5	0.53727	0.60117	7.17E-05	0.005934
<i>KRT9</i>	18.6	0.53498	6.65035	0.000955	0.043075
<i>SETD5-AS1</i>	404.9	0.53251	0.63055	0.000814	0.037948
<i>IRS2</i>	1062.6	-0.52974	-0.61918	0.000598	0.030595
<i>TNFAIP3</i>	10109.7	0.52792	0.56264	5.83E-07	9.41E-05
<i>ASAP2</i>	1227.8	-0.52222	-0.60451	0.000523	0.027621
<i>HCP5</i>	3037.7	0.52175	0.56108	3.25E-06	0.000412
<i>EEF2K</i>	859.8	0.52046	0.58695	0.000184	0.011958
<i>PRKD2</i>	2447.4	0.51983	0.59259	0.000309	0.018771
<i>CTD-2368P22.1</i>	306	0.51516	0.60814	0.001108	0.047917
<i>ZNF70</i>	727.1	0.50734	0.57554	0.000349	0.020819
<i>RLTPR</i>	4653.6	-0.5058	-0.53588	5.61E-07	9.19E-05
<i>MYO1G</i>	1804.3	-0.50459	-0.58325	0.000767	0.036418
<i>TBC1D2B</i>	1080.7	-0.50443	-0.5858	0.000894	0.040817
<i>CORO1C</i>	2814.4	-0.50423	-0.54246	7.24E-06	0.000851
<i>ABHD4</i>	962.1	0.50249	0.58571	0.001045	0.045721
<i>TUBA1A</i>	6080.4	-0.49898	-0.54946	8.35E-05	0.006682
<i>XYLT1</i>	2173.2	0.49304	0.55528	0.000372	0.021838
<i>CAMTA2</i>	666.3	0.488	0.54536	0.000291	0.017889
<i>HERC3</i>	923.4	-0.48726	-0.53755	0.000139	0.009751
<i>PPP1R13B</i>	1190.8	-0.48723	-0.53054	5.06E-05	0.004447
<i>DARS</i>	6357.7	-0.48207	-0.50954	1.17E-06	0.000172
<i>HMGNS</i>	399.5	0.48135	0.55117	0.000978	0.043733
<i>RNPC3</i>	603.8	0.47969	0.54177	0.000603	0.03067
<i>NREP</i>	12968.7	-0.47495	-0.53106	0.000433	0.024565
<i>AHDC1</i>	2906.2	0.47326	0.53736	0.000883	0.040572
<i>STK24</i>	4714.6	-0.47268	-0.4991	1.53E-06	0.000213
<i>GNG2</i>	3002.9	-0.46988	-0.5218	0.00035	0.020819
<i>ACSS1</i>	2289.9	-0.46887	-0.52175	0.000403	0.023211
<i>LAT2</i>	9833.8	0.46614	0.50333	4.94E-05	0.004418
<i>MXRA7</i>	1139.6	-0.45464	-0.49246	0.000101	0.007414
<i>ADD3</i>	2155.4	-0.45395	-0.48413	1.9E-05	0.00198
<i>PRPSAP2</i>	1515.8	-0.45205	-0.49128	0.000146	0.010068
<i>KAZALD1</i>	816.8	0.44361	0.48156	0.000177	0.011647
<i>KIAA0930</i>	1631	-0.442	-0.47432	6.48E-05	0.005392
<i>CCDC117</i>	2855	-0.44158	-0.47766	0.000141	0.009858
<i>ZNF302</i>	1339.8	0.43871	0.47895	0.000316	0.019048
<i>MAPK13</i>	1114.4	-0.43871	-0.48379	0.000588	0.030165
<i>HLA-A</i>	14689.3	0.42258	0.45102	7.51E-05	0.006147

<i>ZDHC3</i>	1299.3	-0.41703	-0.45071	0.000304	0.018507
<i>H2AFY</i>	16915.2	-0.41676	-0.45876	0.000997	0.044262
<i>PCBP1-AS1</i>	1351.6	0.41247	0.44775	0.000489	0.02639
<i>SSBP2</i>	6589.7	-0.41056	-0.4359	6.44E-05	0.005392
<i>SMARCA2</i>	4420.1	0.40579	0.43315	0.000145	0.010068
<i>BZRAP1</i>	6827.9	0.38932	0.41596	0.000293	0.017946
<i>ETS2</i>	7236.6	0.38757	0.40978	9.69E-05	0.007272
<i>IDH2</i>	9359.9	-0.37641	-0.40659	0.001075	0.046676
<i>UNG</i>	4993.8	-0.32718	-0.3456	0.000909	0.041384
<i>STK38</i>	5328.8	-0.32423	-0.33965	0.000391	0.022703

Table S4. Enrichment of differentially expressed genes after *E/R* abrogation. The first column shows the altered biological processes, following by expected value, fold enrichment, raw P value and the False Discovery Rate (FDR).

GO biological process complete	Expected	Fold Enrichment	raw P value	FDR
T cell receptor V(D)J recombination	.08	39.62	1.74E-04	3.58E-02
Germinal center formation	.12	33.01	2.19E-05	1.05E-02
Negative regulation of necroptotic process	.18	22.01	7.68E-05	2.21E-02
I-kappaB kinase/NF-kappaB signaling	1.05	6.70	1.40E-04	3.20E-02
Regulation of interleukin-6 production	2.01	4.47	2.88E-04	4.75E-02
Cellular calcium ion homeostasis	6.47	3.09	1.43E-05	7.53E-03
Regulation of inflammatory response	4.98	3.01	2.23E-04	4.05E-02
Regulation of lymphocyte activation	7.72	2.72	5.35E-05	1.69E-02
Regulation of GTPase activity	7.21	2.64	1.78E-04	3.56E-02
Regulation of cell migration	12.53	2.55	2.06E-06	2.50E-03
Actin filament-based process	8.42	2.49	1.71E-04	3.60E-02
Regulation of cellular protein localization	8.04	2.49	2.52E-04	4.37E-02
Neuron projection development	9.98	2.40	1.30E-04	3.12E-02
Cell morphogenesis	10.56	2.37	1.07E-04	2.65E-02
Positive regulation of immune response	12.77	2.19	1.46E-04	3.30E-02
Negative regulation of apoptotic process	13.48	2.15	1.39E-04	3.24E-02
Positive regulation of developmental process	20.46	2.15	2.91E-06	2.87E-03
Positive regulation of multicellular organismal process	26.05	2.07	5.37E-07	1.70E-03
Cytoskeleton organization	16.12	2.05	1.54E-04	3.35E-02
Negative regulation of response to stimulus	24.14	2.03	3.45E-06	3.21E-03
Regulation of anatomical structure morphogenesis	16.05	1.99	2.70E-04	4.54E-02
Positive regulation of molecular function	26.79	1.75	2.29E-04	4.11E-02
Regulation of signal transduction	49.65	1.51	2.44E-04	4.29E-02
Positive regulation of cellular process	80.91	1.37	2.11E-04	3.88E-02
Unclassified	48.68	.49	5.25E-05	1.77E-02

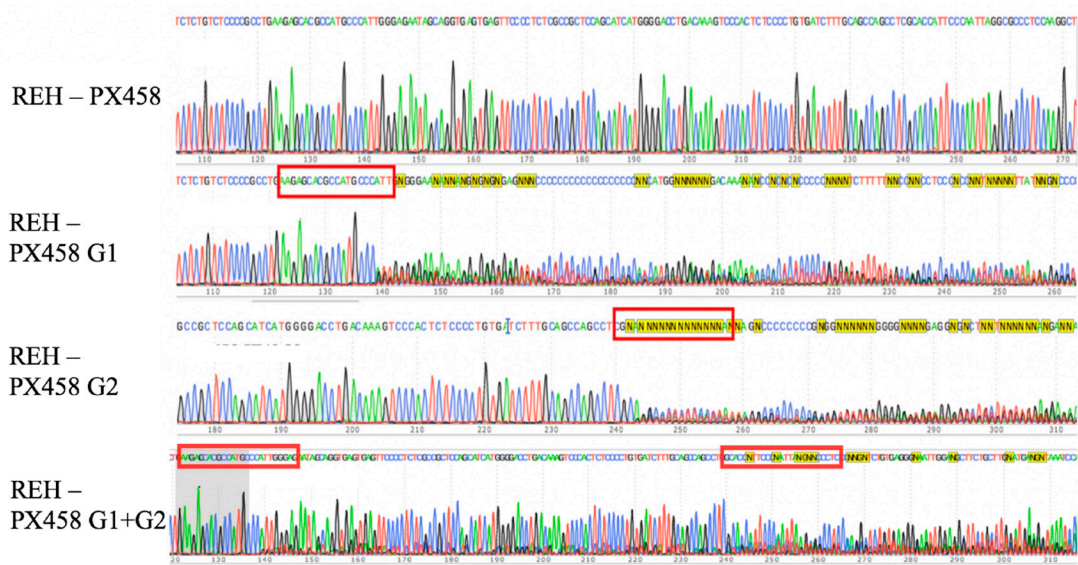


Figure S1. Edited-REH sequences. Sanger sequencing of the *E/R* fusion region in REH cells. The REH cells expressing PX458 without sgRNA, used as a control, had a wild type sequence, while cells expressing G1 sgRNA, G2 sgRNA or G1 + G2 sgRNAs showed a mixture of sequences around the expected Cas9 cleavage point. sgRNA guide sequences are shown in red box.



Figure S2. Edited sequences of single cell derived-cell line. The pool of REH edited-cells with both guides (G1 + G2) was separated by single cell. The sequence corresponding to the *E/R* fusion region was studied by Sanger sequencing in all clones. Two clones with single-non-edited sequence and therefore, with a wild type sequence, were selected as control clones. On the other hand, three single-edited cell clones with *E/R* KO sequence were selected as KO clones (KO1, KO2 and KO3). The pair of oligonucleotides used for *ETV6/RUNX1* RT-qPCR is showed with blue arrows. These oligonucleotides were designed outside the editing region (exon 5 of *ETV6*, sense, 5 – CTCTGTCTCCCCGCTGAA – 3 and exon 4 of

RUNX1, antisense, 5 – *CGGCTCGTGCTGGCAT* -3) and with a distance between them in mRNA of 143 base pairs (bps)

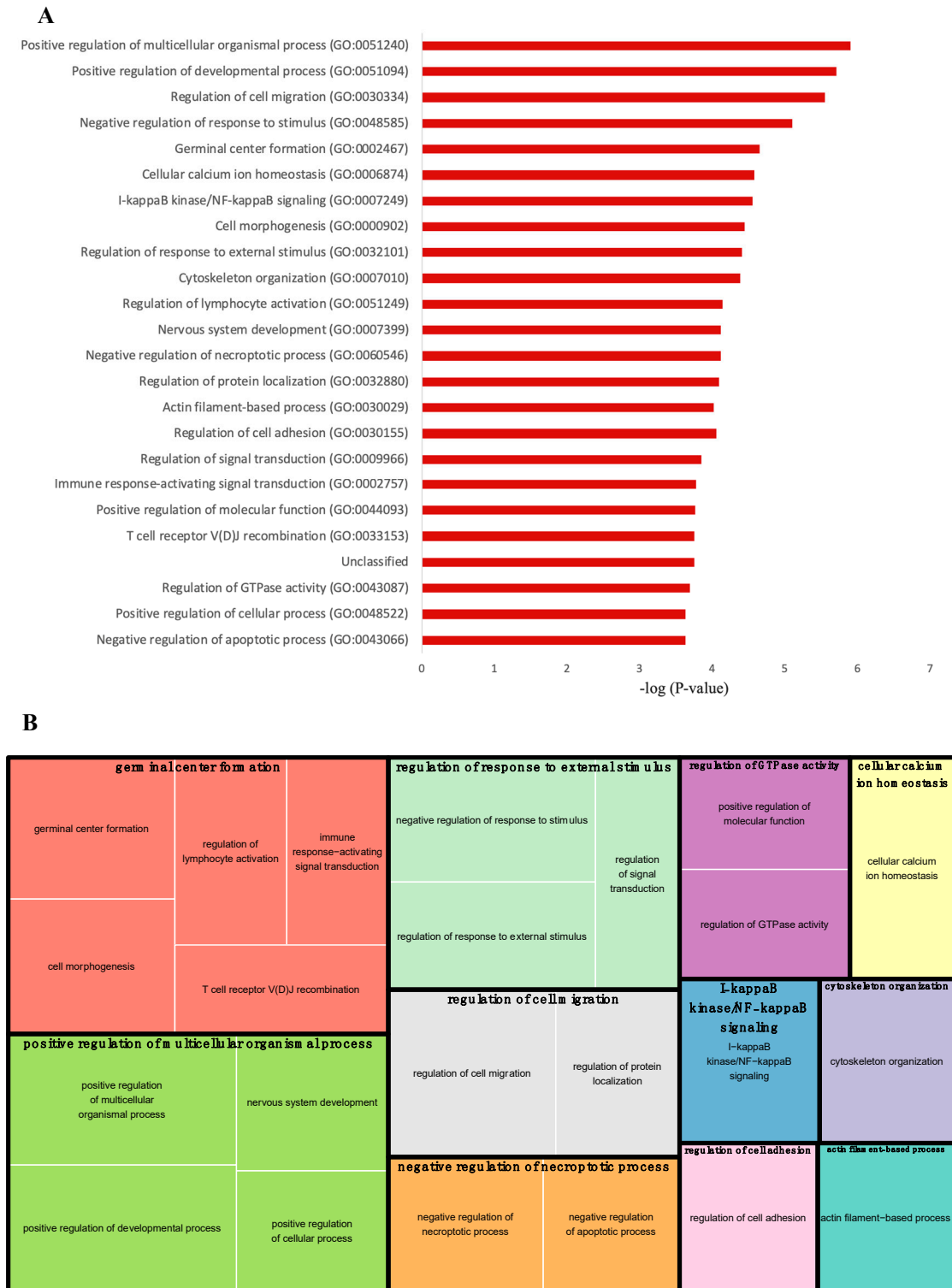


Figure S3. GO Enrichment Analysis. (A) This figure shows the biological processes obtained through enrichment analysis according to $-\log(P\text{-value})$. (B) Each rectangle is a single cluster representative. The representatives are joined into ‘superclusters’ of loosely related terms, visualized with different colors. Size of the rectangles varies according to the P-value.

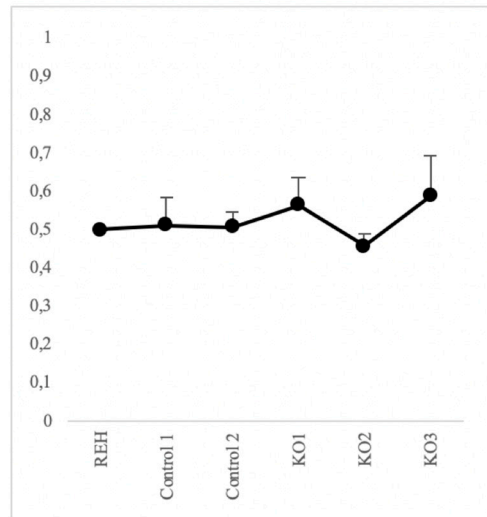


Figure S4. MTT proliferation assay. No proliferation changes were observed in E/R KO clones respected to REH cells and control clones at 72 hours by MTT assay. This experiment had 3 replicates.

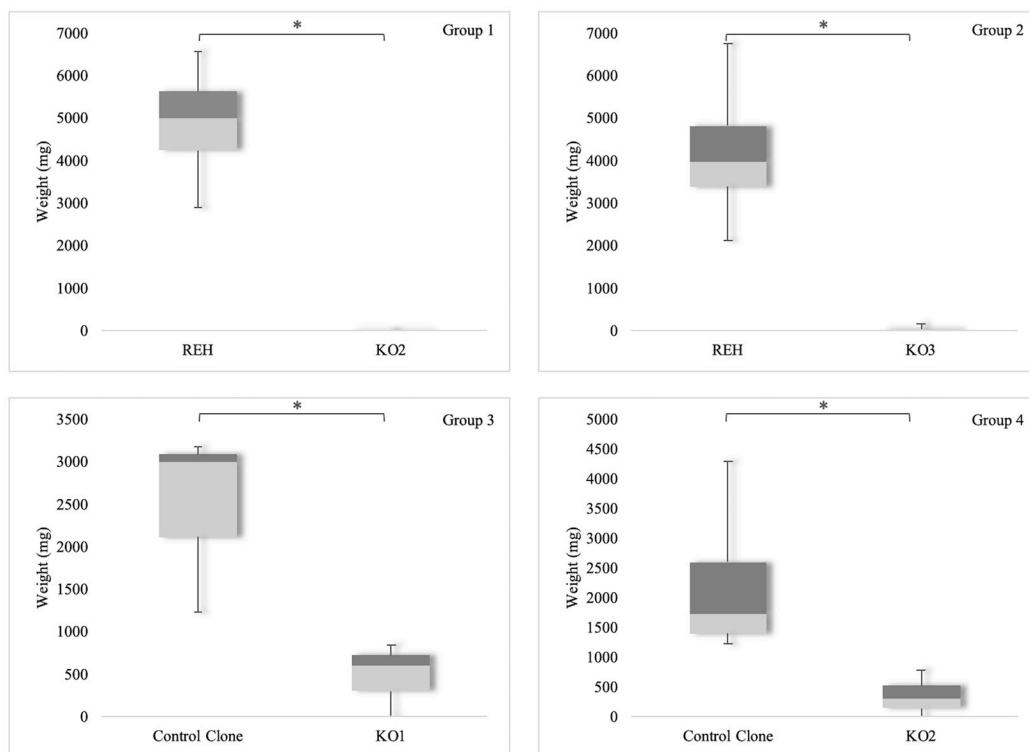


Figure S5. Measure of tumor growth. Mean of the tumor weight for each group of mice, represented by box plot, after being sacrificed at day 48 (group 1 and 2) or day 62 day (group 3 and 4). In this image, the mean of the sizes of the tumors is represented by box plot. In each group they are compared according to

the cells injected in those mice. All the mice belonging to the same group were sacrificed on the same day. Each group had 4 replicates. * $P < 0.05$ (unpaired t -test).

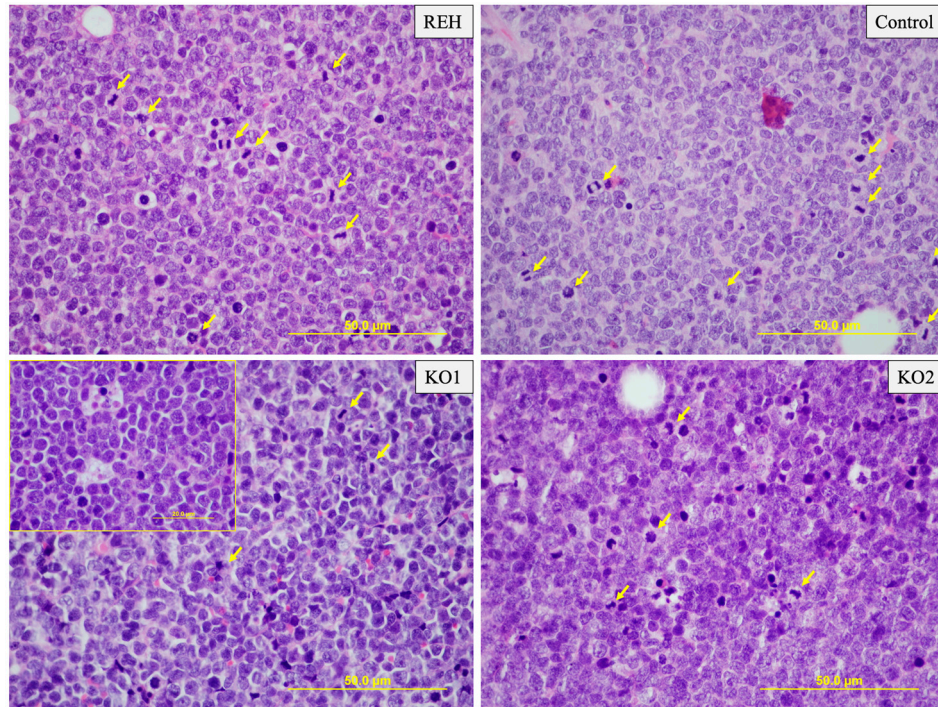


Figure S6. Tumor growth and histopathological findings. H&E representative areas of tumors from each group. Tumors were composed of monomorphic cells with round nucleus. Although mitotic figures (yellow arrows) are observed in all tumors, a higher number were observed in REH and control tumors. Macrophages are shown in KO1 clone (box in the upper left corner).