

## SUPPORTING INFORMATION

### Phosphatase POPX2 exhibits dual regulatory functions in cancer metastasis

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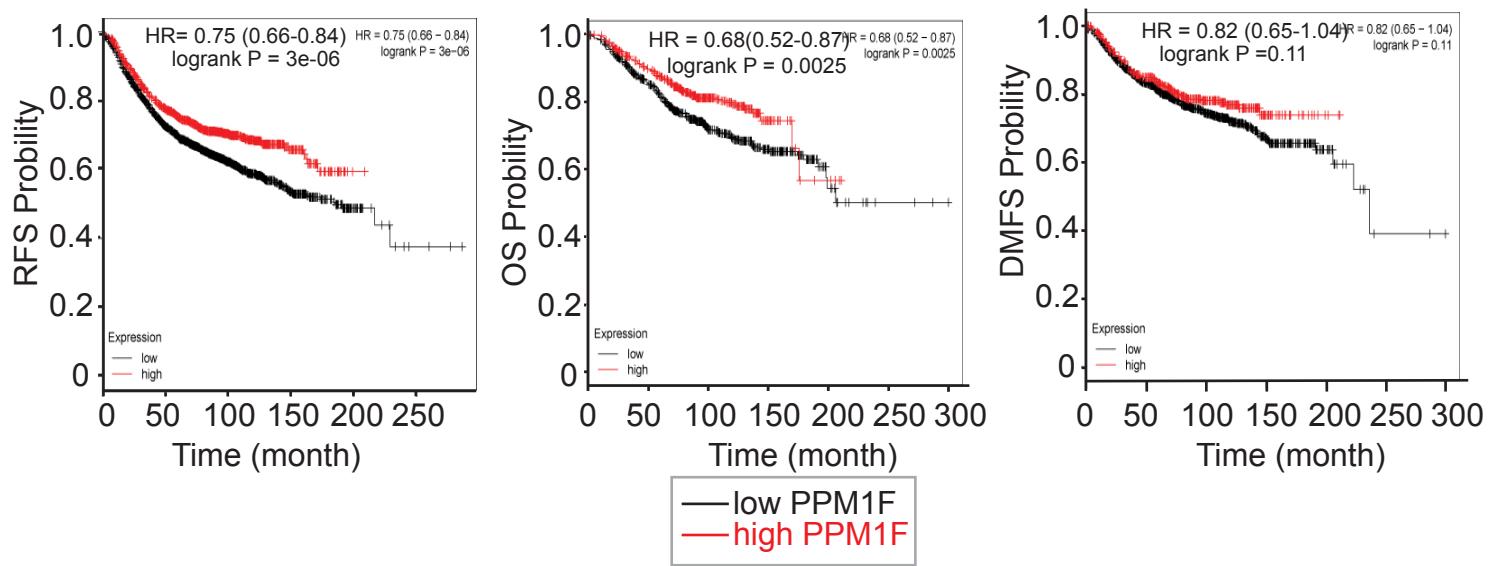
#Present address: Institute of Molecular Systems Biology, ETH Zurich, Zurich 8093, Switzerland.

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**A**

## PPM1F cancer survival analysis

**B**

## PPM1F expression: primary site vs metastasis

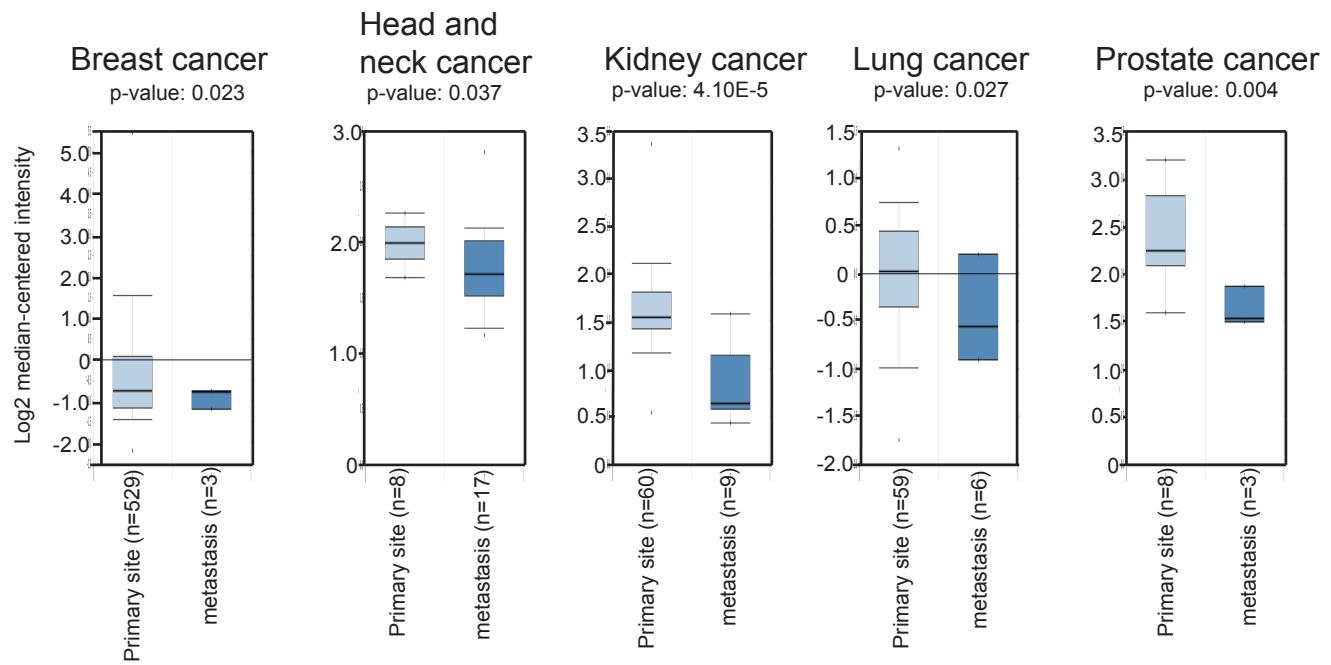


Figure S1

**Figure S1.** Low expression of POPX2 is linked to poor survival rate and distant metastasis. (A) Patient samples are split into two groups based on various quantile expressions of POPX2 (PPM1F): low PPM1F expression group (black curve) and high PPM1F expression group (red curve). Kaplan-Meier plot was performed to depict the association between POPX2 expression and relapse free survival (RFS), overall survival (OS), distant metastasis free survival (DMFS). (B) Boxplots showing the mRNA levels of PPM1F at the primary site and metastasis site were compared in breast cancer, head and neck cancer, kidney cancer, lung cancer, prostate cancer.

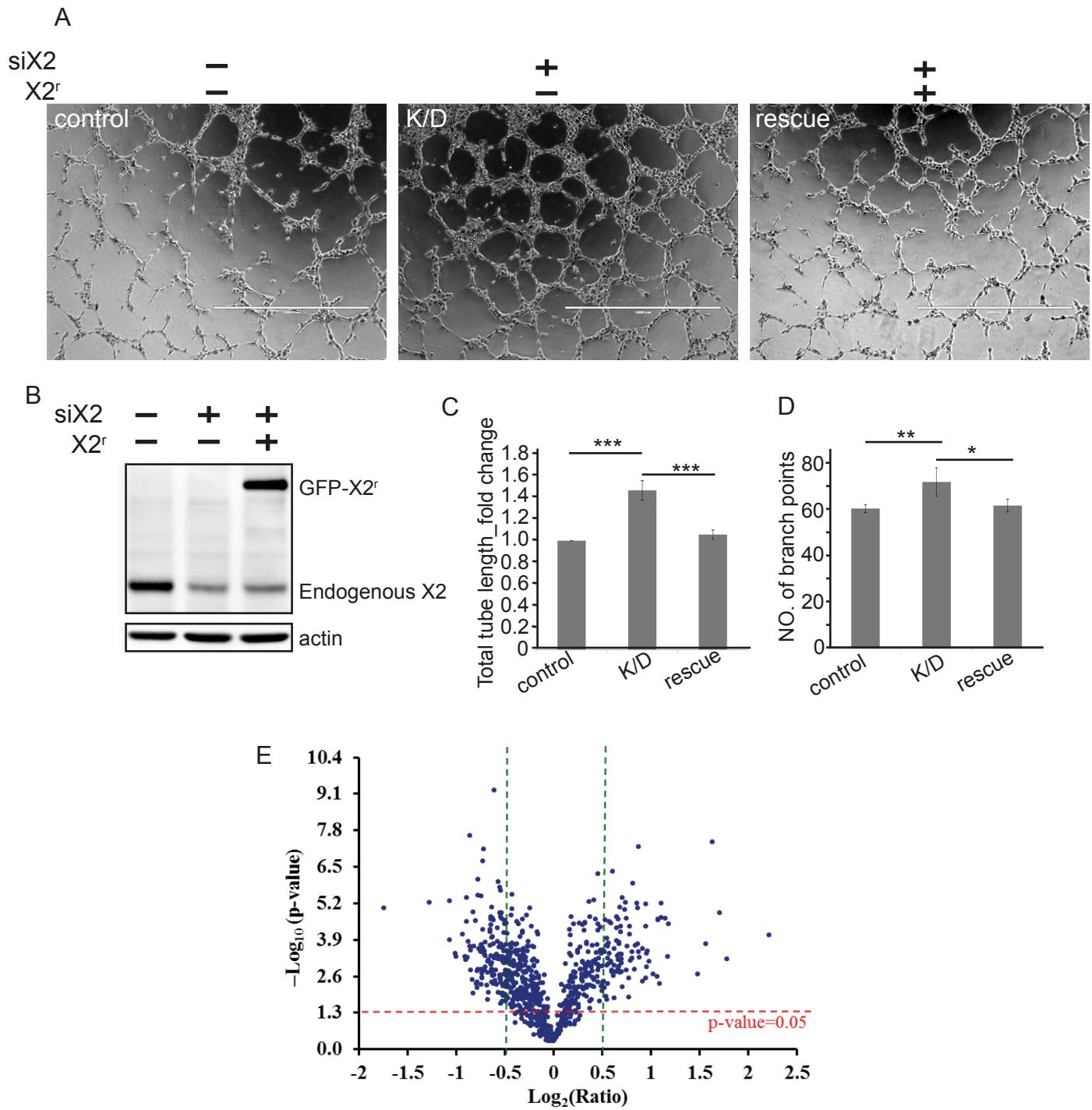
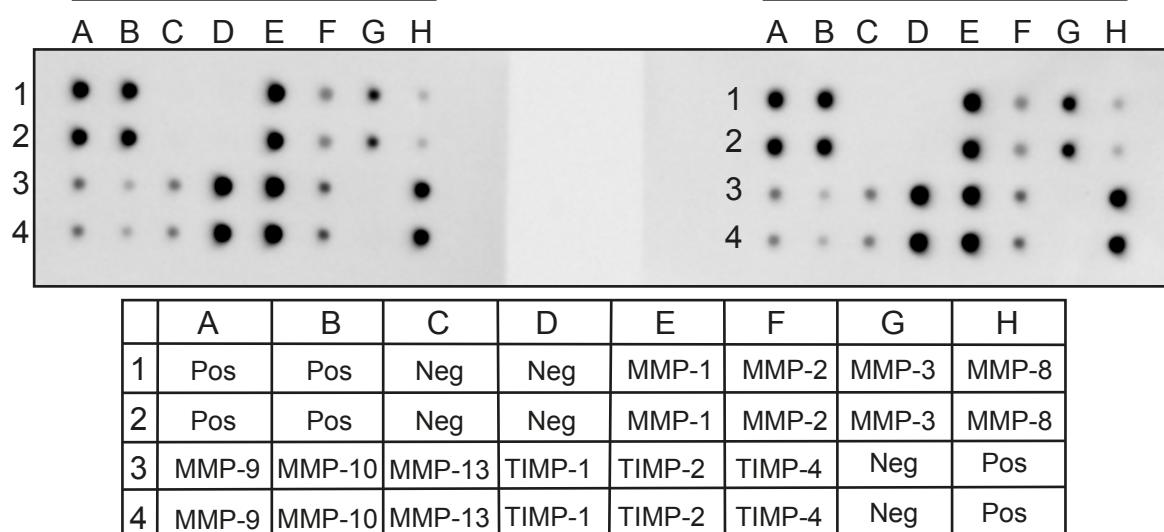


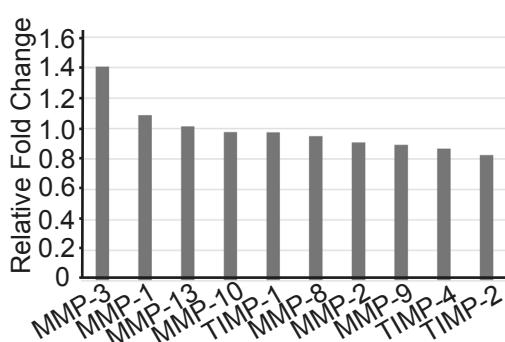
Figure S2

**Figure S2.** Rescue of POPX2 knockdown-induced tube formation and Volcano Plot. (A) Similar numbers of HUVECs were incubated in conditioned media obtained from (1) control MDA-MB-231 cells, (2) POPX2 knockdown (K/D) cells and (3) rescue: POPX2-siRNA (siX2) resistant construct (X2r) transfected POPX2 knockdown cells. Images of tube formation on matrigel was captured 4 h after incubation. Representative images of independent biological triplicates were shown. Scale bar: 1000  $\mu$ m. (B) Western blot analysis of POPX2 knockdown and resistant plasmid overexpression in MDA-MB-231 cells. (C) Total tube length and (D) the number of branch points were quantified from at least 8 random fields of view for each sample. Results presented were the average from three independent biological experiments. (E) Volcano Plot of the hits from the secretome of POPX2 knockdown cells compared with those from control cells (Up regulation  $< -0.5$ , Down regulation  $> 0.5$  and p value  $< 0.05$ ). Error bars represent standard deviation. Student's t-test was used to compare the difference between groups. \*  $P \leq 0.05$ ; \*\*  $P \leq 0.01$ ; \*\*\*  $P \leq 0.001$ .

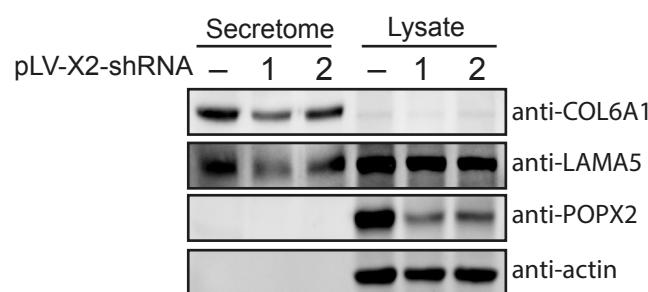
A Conditioned medium from control      Conditioned medium from POPX2 K/D



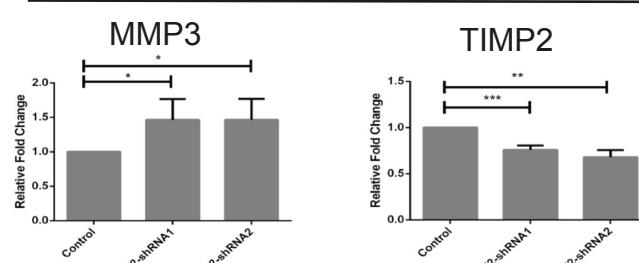
B



C



D Candidates validation from secretome



E

Candidates validation from cytokine array

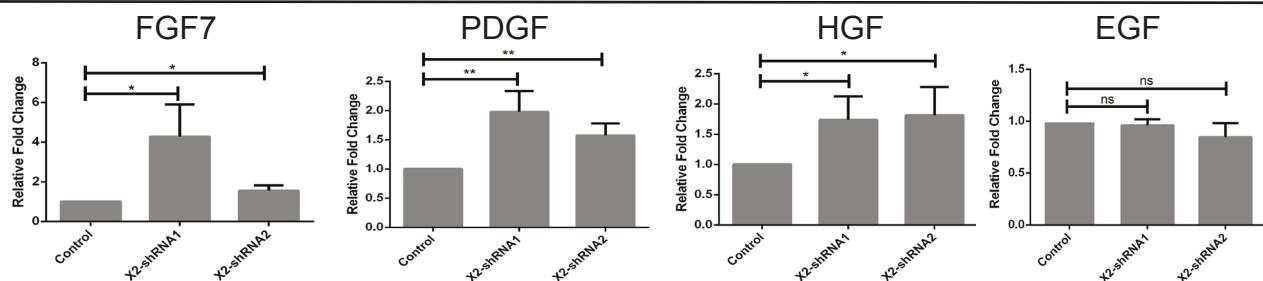
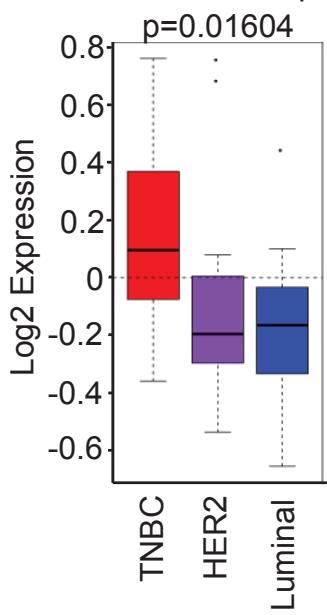


Figure S3

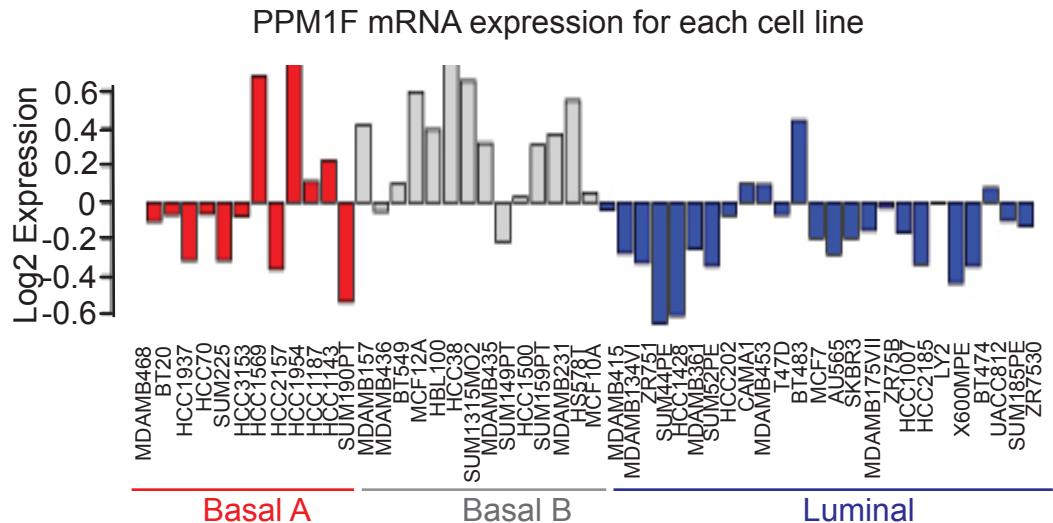
**Figure S3.** Validation of secretome and cytokine array data. (A) The human MMP antibody array was used to analyze the secretion of 10 targets, which were listed in the array map. (B) The dot intensities were quantified by densitometry using ImageJ software and normalized against the intensity of internal positive controls for comparison. The fold change of target in conditioned media from POPX knockdown cells *vs* control cells was determined. Ratios presented were the means from two biological repeats. (C) Western blot was adopted to analyze the secretion levels of the collagen and laminin. qPCR results for candidates selected from (D) secreotme and (E) cytokines array were presented. \*  $P \leq 0.05$ ; \*\*  $P \leq 0.01$ ; \*\*\*  $P \leq 0.001$ ; ns indicates non-significant.

A

## PPM1F mRNA expression



## PPM1F mRNA expression for each cell line



B

## PPM1F expression: TNBC vs non-TNBC

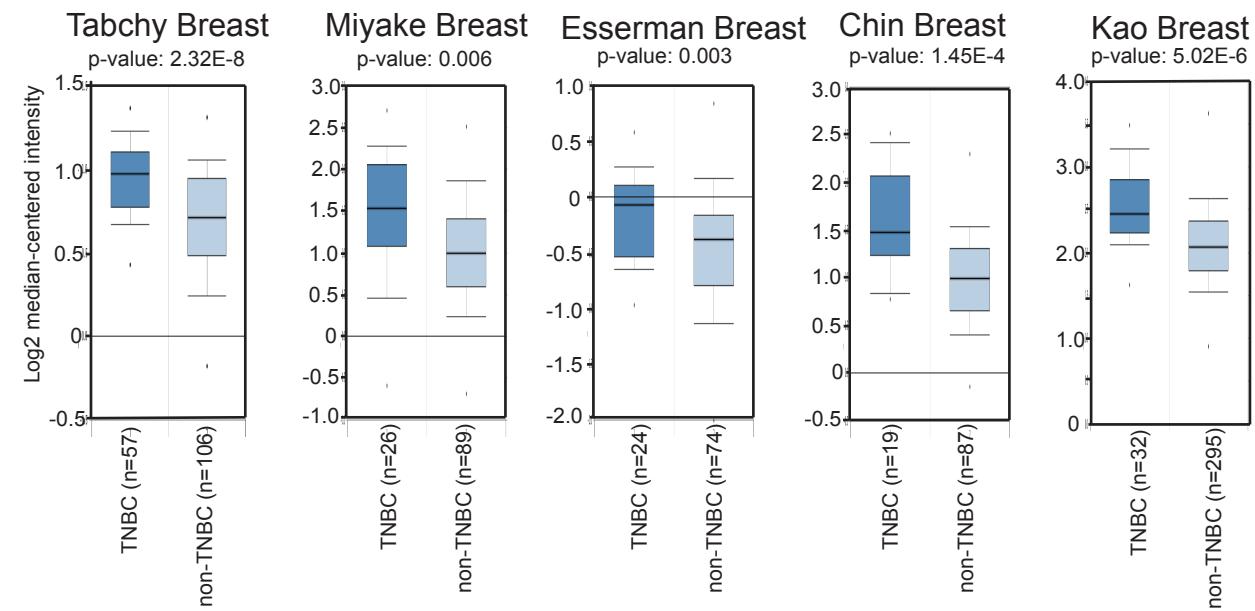


Figure S4

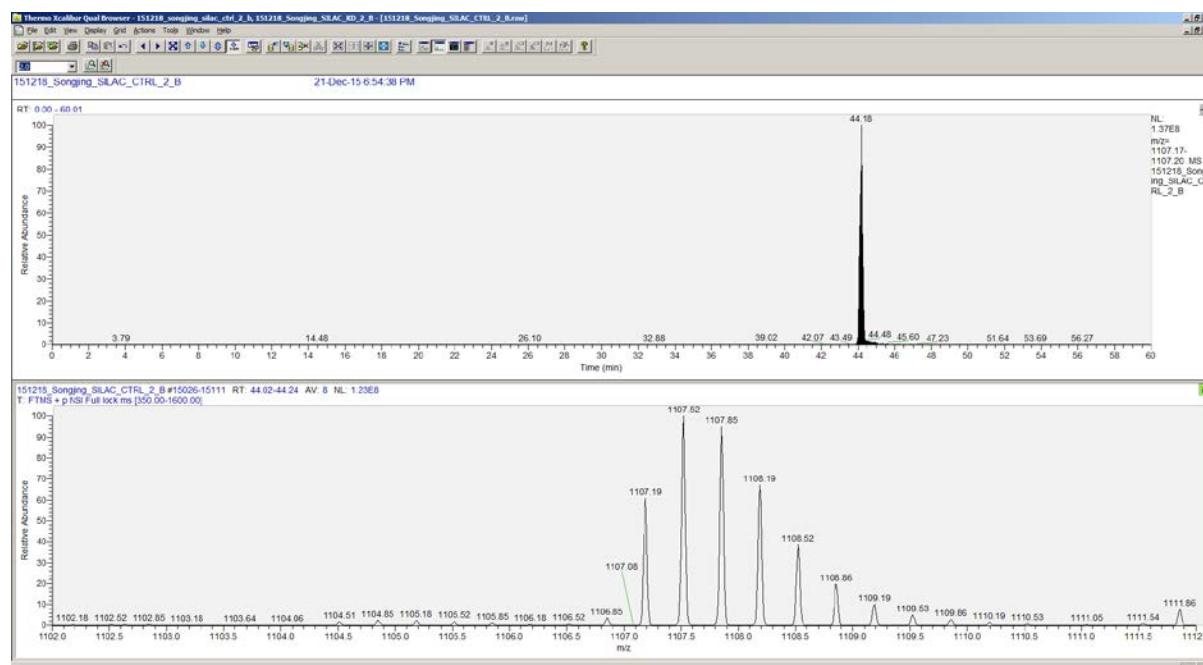
**Figure S4.** POPX2 is highly expressed in the more invasive triple-negative breast cancer cells (TNBC). (A) POPX2 expression levels in a panel of breast cancer cell types were extracted from GOBO (Gene expression-based Outcome for Breast cancer Online). (B) POPX2 expression levels in TNBC (triple negative breast cancer) and non-TNBC (non-triple negative breast cancer) were compared in different breast cancer datasets extracted from Oncomine.

Table S1. Complete SILAC labeling in control cells.

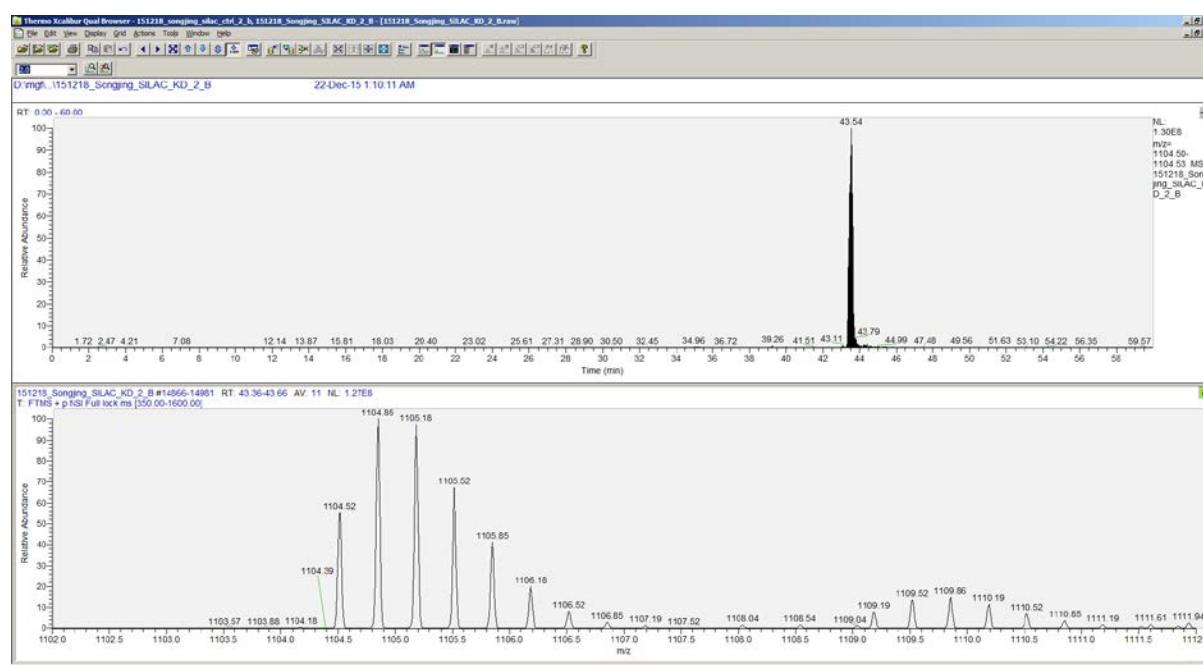
**Supplementary data show complete proteins SILAC labeling in WT control cells with K8 and R6 SILAC reagents in three randomly selected peptides of three different proteins.**

### P04350, TBB4A\_HUMAN Tubulin beta-4A chain

K.EAESCDCLQGFQLTHSLGGGTGSGMGTLIISK#.I in wild type control cells



K.EAESCDCLQGFQLTHSLGGGTGSGMGTLIISK.I in knockdown cells



## Mascot annotated MS/MS spectra

**K.ALIAAQYSGAQVR#.V in control cells vs K.ALIAAQYSGAQVR.V in knock down cells**

TUBB\_K.EAESCDCLOGFQLTHSLGGGTGSGMGTLLISK#.I\_221.48  
TUBB\_K.EAESCDCLOGFQLTHSLGGGTGSGMGTLLISK.I\_204.53

# MASCOT SCIENCE Mascot Search Results

## Peptide View

MS/MS Fragmentation of **EAESCDCLQGFQLTHSLGGGTGSGMGTLLISK**

Found in **P04350** in **uni\_human\_nr**, TBB4A\_HUMAN Tubulin beta-4A chain OS=Homo sapiens GN=TUBB4A PE=1 SV=2

Match to Query 16333: 3318.532602 from(1107.184810,3+) intensity(2880626.2500) rtinseconds(2662) scans(15004) index(13249)

Title: 151218\_Songjing\_SILAC\_CTRL\_2\_A\_Spectrum030601\_scans\_15004\_RTINSECONDS=2662

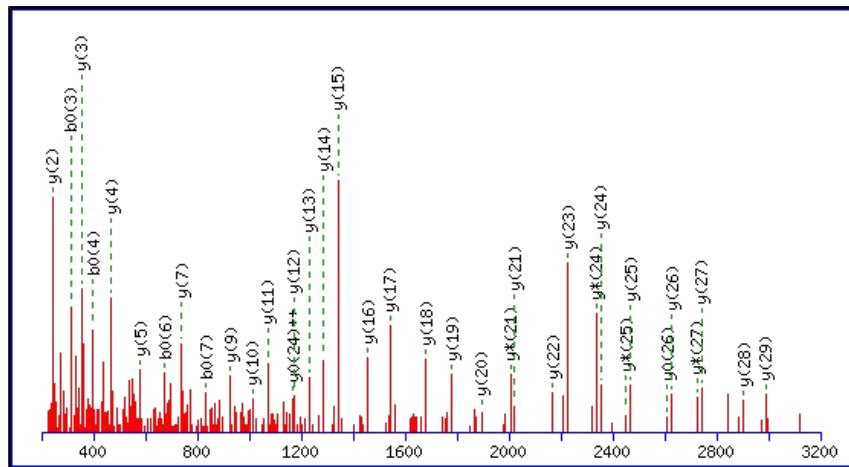
Data file U:\common\$\QExactive\2015\151218\_Songjing\_SILAC\mgf\T\151218\_Songjing\_SILAC\_CTRL\_2\_A.mgf

Click mouse within plot area to zoom in by factor of two about that point

Or,  to  Da

Label all possible matches  Label matches used for scoring

Show Y-axis



Monoisotopic mass of neutral peptide Mr(calc): 3318.5410

Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)

Variable modifications:

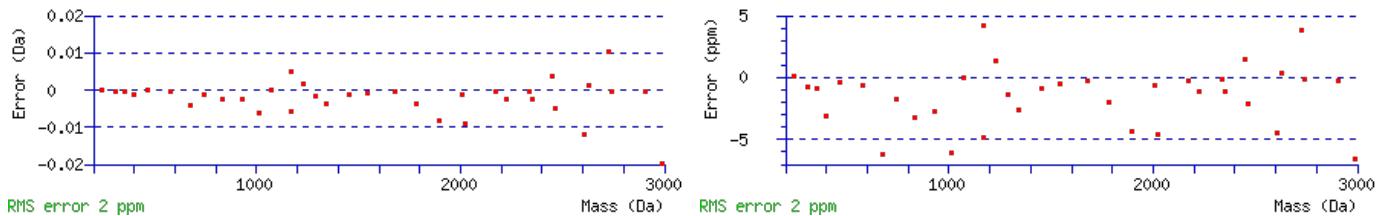
K32 : Label:13C(6)15N(2) (K)

Ions Score: 221 Expect: 1.7e-020

Matches : 36/354 fragment ions using 53 most intense peaks ([help](#))

#	b	b <sup>++</sup>	b*	b* <sup>++</sup>	b <sup>0</sup>	b <sup>0<sup>++</sup></sup>	Seq.	y	y <sup>++</sup>	y*	y* <sup>++</sup>	y <sup>0</sup>	y <sup>0<sup>++</sup></sup>	#	
<b>1</b>	130.0499	65.5286			112.0393	56.5233	<b>E</b>								<b>32</b>
<b>2</b>	201.0870	101.0471			183.0764	92.0418	<b>A</b>	3190.5057	1595.7565	3173.4791	1587.2432	3172.4951	1586.7512	<b>31</b>	
<b>3</b>	330.1296	165.5684			<b>312.1190</b>	156.5631	<b>E</b>	3119.4686	1560.2379	3102.4420	1551.7247	3101.4580	1551.2326	<b>30</b>	
<b>4</b>	417.1616	209.0844			<b>399.1510</b>	200.0792	<b>S</b>	<b>2990.4260</b>	1495.7166	2973.3994	1487.2034	2972.4154	1486.7114	<b>29</b>	
<b>5</b>	577.1923	289.0998			559.1817	280.0945	<b>C</b>	<b>2903.3940</b>	1452.2006	2886.3674	1443.6873	2885.3834	1443.1953	<b>28</b>	
<b>6</b>	692.2192	346.6132			<b>674.2086</b>	337.6080	<b>D</b>	<b>2743.3633</b>	1372.1853	<b>2726.3368</b>	1363.6720	2725.3527	1363.1800	<b>27</b>	
<b>7</b>	852.2498	426.6286			<b>834.2393</b>	417.6233	<b>C</b>	<b>2628.3364</b>	1314.6718	2611.3098	1306.1585	<b>2610.3258</b>	1305.6665	<b>26</b>	
<b>8</b>	965.3339	483.1706			947.3233	474.1653	<b>L</b>	<b>2468.3057</b>	1234.6565	<b>2451.2792</b>	1226.1432	2450.2952	1225.6512	<b>25</b>	
<b>9</b>	1093.3925	547.1999	1076.3659	538.6866	1075.3819	538.1946	<b>Q</b>	<b>2355.2217</b>	1178.1145	<b>2338.1951</b>	1169.6012	2337.2111	<b>1169.1092</b>	<b>24</b>	
<b>10</b>	1150.4140	575.7106	1133.3874	567.1973	1132.4034	566.7053	<b>G</b>	<b>2227.1631</b>	1114.0852	2210.1365	1105.5719	2209.1525	1105.0799	<b>23</b>	
<b>11</b>	1297.4824	649.2448	1280.4558	640.7315	1279.4718	640.2395	<b>F</b>	<b>2170.1416</b>	1085.5744	2153.1151	1077.0612	2152.1311	1076.5692	<b>22</b>	
<b>12</b>	1425.5409	713.2741	1408.5144	704.7608	1407.5304	704.2688	<b>Q</b>	<b>2023.0732</b>	1012.0402	<b>2006.0467</b>	1003.5270	2005.0626	1003.0350	<b>21</b>	
<b>13</b>	1538.6250	769.8161	1521.5985	761.3029	1520.6144	760.8109	<b>L</b>	<b>1895.0146</b>	948.0110	1877.9881	939.4977	1877.0041	939.0057	<b>20</b>	
<b>14</b>	1639.6727	820.3400	1622.6461	811.8267	1621.6621	811.3347	<b>T</b>	<b>1781.9306</b>	891.4689	1764.9040	882.9556	1763.9200	882.4636	<b>19</b>	
<b>15</b>	1776.7316	888.8694	1759.7050	880.3562	1758.7210	879.8642	<b>H</b>	<b>1680.8829</b>	840.9451	1663.8563	832.4318	1662.8723	831.9398	<b>18</b>	
<b>16</b>	1863.7636	932.3855	1846.7371	923.8722	1845.7531	923.3802	<b>S</b>	<b>1543.8240</b>	772.4156	1526.7974	763.9023	1525.8134	763.4103	<b>17</b>	
<b>17</b>	1976.8477	988.9275	1959.8211	980.4142	1958.8371	979.9222	<b>L</b>	<b>1456.7919</b>	728.8996	1439.7654	720.3863	1438.7814	719.8943	<b>16</b>	
<b>18</b>	2033.8692	1017.4382	2016.8426	1008.9249	2015.8586	1008.4329	<b>G</b>	<b>1343.7079</b>	672.3576	1326.6813	663.8443	1325.6973	663.3523	<b>15</b>	
<b>19</b>	2090.8906	1045.9489	2073.8641	1037.4357	2072.8801	1036.9437	<b>G</b>	<b>1286.6864</b>	643.8468	1269.6599	635.3336	1268.6758	634.8416	<b>14</b>	
<b>20</b>	2147.9121	1074.4597	2130.8855	1065.9464	2129.9015	1065.4544	<b>G</b>	<b>1229.6650</b>	615.3361	1212.6384	606.8228	1211.6544	606.3308	<b>13</b>	
<b>21</b>	2248.9598	1124.9835	2231.9332	1116.4702	2230.9492	1115.9782	<b>T</b>	<b>1172.6435</b>	586.8254	1155.6169	578.3121	1154.6329	577.8201	<b>12</b>	
<b>22</b>	2305.9812	1153.4943	2288.9547	1144.9810	2287.9707	1144.4890	<b>G</b>	<b>1071.5958</b>	536.3015	1054.5693	527.7883	1053.5852	527.2963	<b>11</b>	
<b>23</b>	2393.0133	1197.0103	2375.9867	1188.4970	2375.0027	1188.0050	<b>S</b>	<b>1014.5743</b>	507.7908	997.5478	499.2775	996.5638	498.7855	<b>10</b>	
<b>24</b>	2450.0347	1225.5210	2433.0082	1217.0077	2432.0242	1216.5157	<b>G</b>	<b>927.5423</b>	464.2748	910.5158	455.7615	909.5318	455.2695	<b>9</b>	

25	2581.0752	1291.0412	2564.0487	1282.5280	2563.0646	1282.0360	M	870.5209	435.7641	853.4943	427.2508	852.5103	426.7588	8
26	2638.0967	1319.5520	2621.0701	1311.0387	2620.0861	1310.5467	G	739.4804	370.2438	722.4538	361.7305	721.4698	361.2385	7
27	2739.1443	1370.0758	2722.1178	1361.5625	2721.1338	1361.0705	T	682.4589	341.7331	665.4324	333.2198	664.4483	332.7278	6
28	2852.2284	1426.6178	2835.2019	1418.1046	2834.2178	1417.6126	L	581.4112	291.2092	564.3847	282.6960	563.4007	282.2040	5
29	2965.3125	1483.1599	2948.2859	1474.6466	2947.3019	1474.1546	L	468.3272	234.6672	451.3006	226.1539	450.3166	225.6619	4
30	3078.3965	1539.7019	3061.3700	1531.1886	3060.3860	1530.6966	I	355.2431	178.1252	338.2165	169.6119	337.2325	169.1199	3
31	3165.4286	1583.2179	3148.4020	1574.7046	3147.4180	1574.2126	S	242.1590	121.5832	225.1325	113.0699	224.1485	112.5779	2
32							K	155.1270	78.0671	138.1005	69.5539			1



NCBI BLAST search of [EAESCDCLQGFQLTHSLGGGTGSGMGTLISK](#)

(Parameters: blastp, nr protein database, expect=20000, no filter, PAM30)

Other BLAST [web gateways](#)

#### All matches to this query

Score	Mr(calc)	Delta	Sequence
221.5	3318.5410	-0.0084	<a href="#">EAESCDCLQGFQLTHSLGGGTGSGMGTLISK</a>
221.5	3318.5410	-0.0084	<a href="#">EAESCDCLQGFQLTHSLGGGTGSGMGTLLSK</a>

Mascot: <http://www.matrixscience.com/>

# MASCOT SCIENCE Mascot Search Results

## Peptide View

MS/MS Fragmentation of **EAESCDCLQGFQLTHSLGGGTGSGMGTLLISK**

Found in **P04350** in **uni\_human\_nr**, TBB4A\_HUMAN Tubulin beta-4A chain OS=Homo sapiens GN=TUBB4A PE=1 SV=2

Match to Query 16390: 3310.523202 from(1104.515010,3+) intensity(30644720.0000) rtinseconds(2604) scans(14885) index(13265)

Title: 151218\_Songjing\_SILAC\_KD\_2\_B\_Spectrum031012\_scans\_14885\_RTINSECONDS=2604

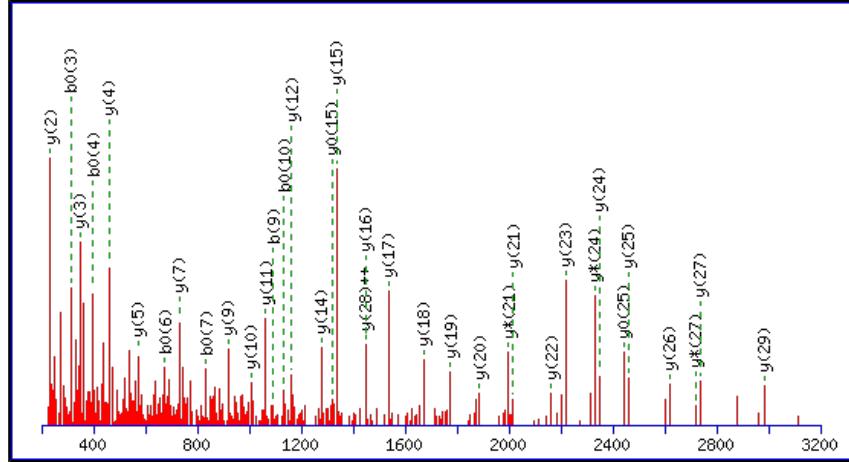
Data file U:\common\$\QExactive\2015\151218\_Songjing\_SILAC\mgf\T151218\_Songjing\_SILAC\_KD\_2\_B.mgf

Click mouse within plot area to zoom in by factor of two about that point

Or,                   to                   Da

Label all possible matches      Label matches used for scoring

Show Y-axis



Monoisotopic mass of neutral peptide Mr(calc): 3310.5268

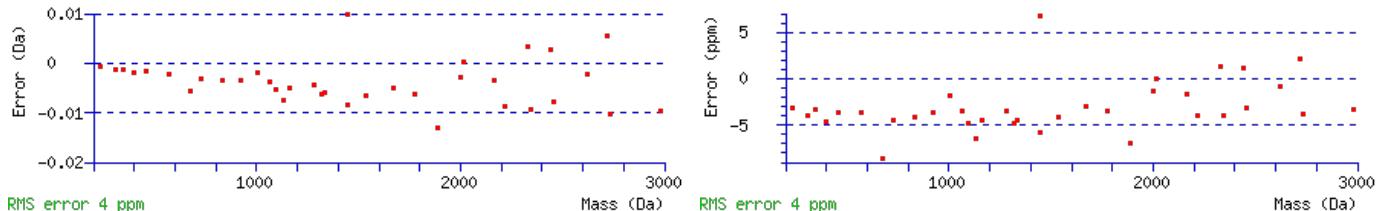
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)

Ions Score: 205 Expect: 8.1e-019

Matches : 36/354 fragment ions using 54 most intense peaks ([help](#))

#	b	b <sup>++</sup>	b*	b* <sup>++</sup>	b <sup>0</sup>	b <sup>0<sup>++</sup></sup>	Seq.	y	y <sup>++</sup>	y*	y** <sup>++</sup>	y <sup>0</sup>	y <sup>0<sup>++</sup></sup>	#
<b>1</b>	130.0499	65.5286			112.0393	56.5233	<b>E</b>							<b>32</b>
<b>2</b>	201.0870	101.0471			183.0764	92.0418	<b>A</b>	3182.4915	1591.7494	3165.4650	1583.2361	3164.4809	1582.7441	<b>31</b>
<b>3</b>	330.1296	165.5684			<b>312.1190</b>	156.5631	<b>E</b>	3111.4544	1556.2308	3094.4278	1547.7176	3093.4438	1547.2255	<b>30</b>
<b>4</b>	417.1616	209.0844			<b>399.1510</b>	200.0792	<b>S</b>	<b>2982.4118</b>	1491.7095	2965.3852	1483.1963	2964.4012	1482.7043	<b>29</b>
<b>5</b>	577.1923	289.0998			559.1817	280.0945	<b>C</b>	2895.3798	<b>1448.1935</b>	2878.3532	1439.6802	2877.3692	1439.1882	<b>28</b>
<b>6</b>	692.2192	346.6132			<b>674.2086</b>	337.6080	<b>D</b>	<b>2735.3491</b>	1368.1782	<b>2718.3226</b>	1359.6649	2717.3386	1359.1729	<b>27</b>
<b>7</b>	852.2498	426.6286			<b>834.2393</b>	417.6233	<b>C</b>	<b>2620.3222</b>	1310.6647	2603.2956	1302.1514	2602.3116	1301.6594	<b>26</b>
<b>8</b>	965.3339	483.1706			947.3233	474.1653	<b>L</b>	<b>2460.2915</b>	1230.6494	2443.2650	1222.1361	<b>2442.2810</b>	1221.6441	<b>25</b>
<b>9</b>	<b>1093.3925</b>	547.1999	1076.3659	538.6866	1075.3819	538.1946	<b>Q</b>	<b>2347.2075</b>	1174.1074	<b>2330.1809</b>	1165.5941	2329.1969	1165.1021	<b>24</b>
<b>10</b>	1150.4140	575.7106	1133.3874	567.1973	<b>1132.4034</b>	566.7053	<b>G</b>	<b>2219.1489</b>	1110.0781	2202.1223	1101.5648	2201.1383	1101.0728	<b>23</b>
<b>11</b>	1297.4824	649.2448	1280.4558	640.7315	1279.4718	640.2395	<b>F</b>	<b>2162.1274</b>	1081.5673	2145.1009	1073.0541	2144.1169	1072.5621	<b>22</b>
<b>12</b>	1425.5409	713.2741	1408.5144	704.7608	1407.5304	704.2688	<b>Q</b>	<b>2015.0590</b>	1008.0331	<b>1998.0325</b>	999.5199	1997.0484	999.0279	<b>21</b>
<b>13</b>	1538.6250	769.8161	1521.5985	761.3029	1520.6144	760.8109	<b>L</b>	<b>1887.0004</b>	944.0039	1869.9739	935.4906	1868.9899	934.9986	<b>20</b>
<b>14</b>	1639.6727	820.3400	1622.6461	811.8267	1621.6621	811.3347	<b>T</b>	<b>1773.9164</b>	887.4618	1756.8898	878.9485	1755.9058	878.4565	<b>19</b>
<b>15</b>	1776.7316	888.8694	1759.7050	880.3562	1758.7210	879.8642	<b>H</b>	<b>1672.8687</b>	836.9380	1655.8421	828.4247	1654.8581	827.9327	<b>18</b>
<b>16</b>	1863.7636	932.3855	1846.7371	923.8722	1845.7531	923.3802	<b>S</b>	<b>1535.8098</b>	768.4085	1518.7832	759.8952	1517.7992	759.4032	<b>17</b>
<b>17</b>	1976.8477	988.9275	1959.8211	980.4142	1958.8371	979.9222	<b>L</b>	<b>1448.7777</b>	724.8925	1431.7512	716.3792	1430.7672	715.8872	<b>16</b>
<b>18</b>	2033.8692	1017.4382	2016.8426	1008.9249	2015.8586	1008.4329	<b>G</b>	<b>1335.6937</b>	668.3505	1318.6671	659.8372	<b>1317.6831</b>	659.3452	<b>15</b>
<b>19</b>	2090.8906	1045.9489	2073.8641	1037.4357	2072.8801	1036.9437	<b>G</b>	<b>1278.6722</b>	639.8397	1261.6457	631.3265	1260.6617	630.8345	<b>14</b>
<b>20</b>	2147.9121	1074.4597	2130.8855	1065.9464	2129.9015	1065.4544	<b>G</b>	1221.6508	611.3290	1204.6242	602.8157	1203.6402	602.3237	<b>13</b>
<b>21</b>	2248.9598	1124.9835	2231.9332	1116.4702	2230.9492	1115.9782	<b>T</b>	<b>1164.6293</b>	582.8183	1147.6027	574.3050	1146.6187	573.8130	<b>12</b>
<b>22</b>	2305.9812	1153.4943	2288.9547	1144.9810	2287.9707	1144.4890	<b>G</b>	<b>1063.5816</b>	532.2944	1046.5551	523.7812	1045.5710	523.2892	<b>11</b>
<b>23</b>	2393.0133	1197.0103	2375.9867	1188.4970	2375.0027	1188.0050	<b>S</b>	<b>1006.5601</b>	503.7837	989.5336	495.2704	988.5496	494.7784	<b>10</b>
<b>24</b>	2450.0347	1225.5210	2433.0082	1217.0077	2432.0242	1216.5157	<b>G</b>	<b>919.5281</b>	460.2677	902.5016	451.7544	901.5176	451.2624	<b>9</b>
<b>25</b>	2581.0752	1291.0412	2564.0487	1282.5280	2563.0646	1282.0360	<b>M</b>	862.5067	431.7570	845.4801	423.2437	844.4961	422.7517	<b>8</b>

<b>26</b>	2638.0967	1319.5520	2621.0701	1311.0387	2620.0861	1310.5467	<b>G</b>	<b>731.4662</b>	366.2367	714.4396	357.7234	713.4556	357.2314	<b>7</b>
<b>27</b>	2739.1443	1370.0758	2722.1178	1361.5625	2721.1338	1361.0705	<b>T</b>	674.4447	337.7260	657.4182	329.2127	656.4341	328.7207	<b>6</b>
<b>28</b>	2852.2284	1426.6178	2835.2019	1418.1046	2834.2178	1417.6126	<b>L</b>	<b>573.3970</b>	287.2022	556.3705	278.6889	555.3865	278.1969	<b>5</b>
<b>29</b>	2965.3125	1483.1599	2948.2859	1474.6466	2947.3019	1474.1546	<b>L</b>	<b>460.3130</b>	230.6601	443.2864	222.1468	442.3024	221.6548	<b>4</b>
<b>30</b>	3078.3965	1539.7019	3061.3700	1531.1886	3060.3860	1530.6966	<b>I</b>	<b>347.2289</b>	174.1181	330.2023	165.6048	329.2183	165.1128	<b>3</b>
<b>31</b>	3165.4286	1583.2179	3148.4020	1574.7046	3147.4180	1574.2126	<b>S</b>	<b>234.1448</b>	117.5761	217.1183	109.0628	216.1343	108.5708	<b>2</b>
<b>32</b>							<b>K</b>	147.1128	74.0600	130.0863	65.5468			<b>1</b>



NCBI BLAST search of [EAESCDCLQGFQLTHSLGGGTGSGMGTLISK](#)

(Parameters: blastp, nr protein database, expect=20000, no filter, PAM30)

Other BLAST [web gateways](#)

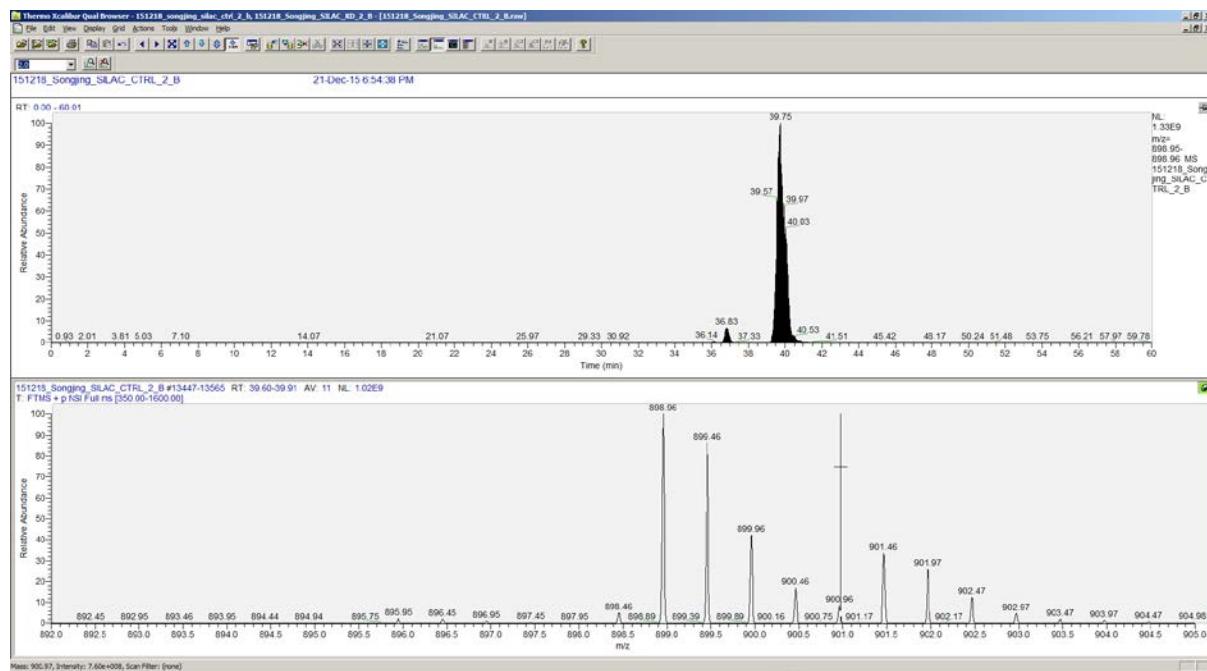
#### All matches to this query

Score	Mr(calc)	Delta	Sequence
204.5	3310.5268	-0.0036	<a href="#">EAESCDCLQGFQLTHSLGGGTGSGMGTLISK</a>
204.5	3310.5268	-0.0036	<a href="#">EAESCDCLQGFQLTHSLGGGTGSGMGTLLSK</a>

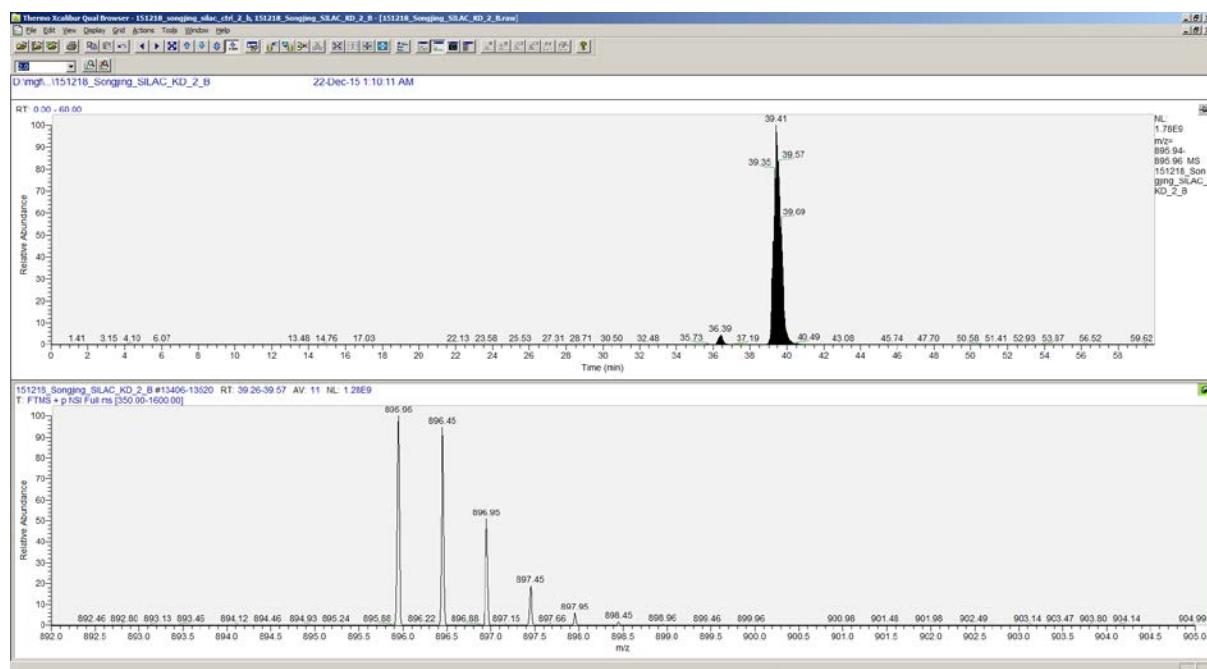
Mascot: <http://www.matrixscience.com/>

## Q9BYX7, ACTBM\_HUMAN Putative beta-actin-like protein 3

R.SYELPDGQVITIGNER#F in wild type control cells



R.SYELPDGQVITIGNER.F in knockdown cells



## Mascot annotated MS/MS spectra

**K.ALIAAQYSGAQVR#.V in control cells vs K.ALIAAQYSGAQVR.V in knock down cells**

[ACTBL2\\_R.SYELPDGQVITIGNER#.F\\_109.47](#)  
[ACTBL2\\_R.SYELPDGQVITIGNER.F\\_114.33](#)

 **Mascot Search Results**
**Peptide View**MS/MS Fragmentation of **SYELPDGQVITIGNER**Found in **Q9BYX7** in **uni\_human\_nr**, ACTBM\_HUMAN Putative beta-actin-like protein 3 OS=Homo sapiens GN=POTEKP PE=5 SV=1

Match to Query 10311: 1795.902068 from(898.958310,2+) intensity(24998456.0000) rtinseconds(2453) scans(13766) index(12123)

Title: 151218\_Songjing\_SILAC\_CTRL\_2\_A\_Spectrum029475\_scans\_13766\_RTINSECONDS=2453

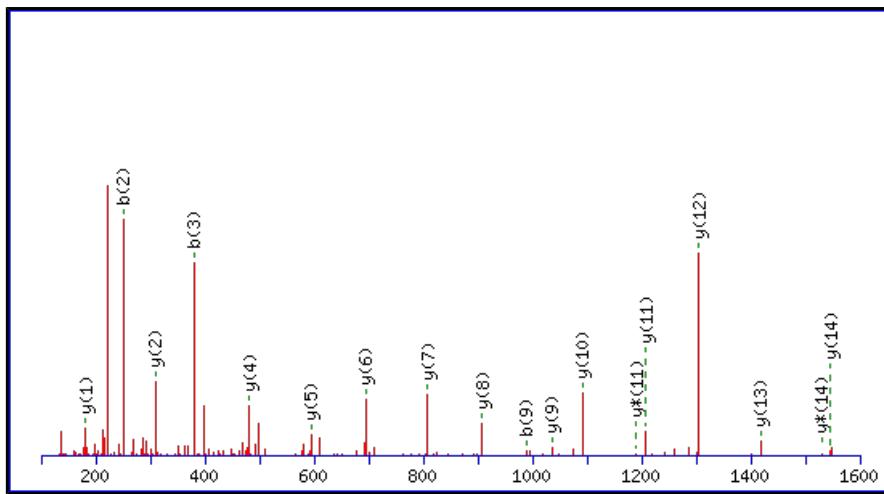
Data file U:\common\$\QExactive\2015\151218\_Songjing\_SILAC\mgf\T\T151218\_Songjing\_SILAC\_CTRL\_2\_A.mgf

Click mouse within plot area to zoom in by factor of two about that point

Or, to Da

Label all possible matches Label matches used for scoring

Show Y-axis



Monoisotopic mass of neutral peptide Mr(calc): 1795.9048

Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)

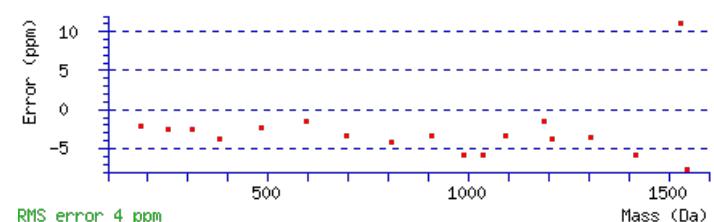
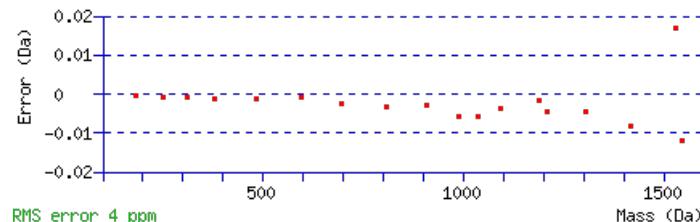
Variable modifications:

R16 : Label:13C(6) (R)

Ions Score: 109 Expect: 3.5e-009

Matches : 18/164 fragment ions using 29 most intense peaks ([help](#))

#	b	b <sup>++</sup>	b*	b* <sup>++</sup>	b <sup>0</sup>	b <sup>0<sub>++</sub></sup>	Seq.	y	y <sup>++</sup>	y*	y <sup>***</sup>	y <sup>0</sup>	y <sup>0<sub>++</sub></sup>	#	
<b>1</b>	88.0393	44.5233			70.0287	35.5180	<b>S</b>								<b>16</b>
<b>2</b>	<b>251.1026</b>	126.0550			233.0921	117.0497	<b>Y</b>	1709.8800	855.4436	1692.8535	846.9304	1691.8695	846.4384	<b>15</b>	
<b>3</b>	<b>380.1452</b>	190.5763			362.1347	181.5710	<b>E</b>	<b>1546.8167</b>	773.9120	<b>1529.7901</b>	765.3987	1528.8061	764.9067	<b>14</b>	
<b>4</b>	493.2293	247.1183			475.2187	238.1130	<b>L</b>	<b>1417.7741</b>	709.3907	1400.7475	700.8774	1399.7635	700.3854	<b>13</b>	
<b>5</b>	590.2821	295.6447			572.2715	286.6394	<b>P</b>	<b>1304.6900</b>	652.8487	1287.6635	644.3354	1286.6795	643.8434	<b>12</b>	
<b>6</b>	705.3090	353.1581			687.2984	344.1529	<b>D</b>	<b>1207.6373</b>	604.3223	<b>1190.6107</b>	595.8090	1189.6267	595.3170	<b>11</b>	
<b>7</b>	762.3305	381.6689			744.3199	372.6636	<b>G</b>	<b>1092.6103</b>	546.8088	1075.5838	538.2955	1074.5998	537.8035	<b>10</b>	
<b>8</b>	890.3890	445.6982	873.3625	437.1849	872.3785	436.6929	<b>Q</b>	<b>1035.5889</b>	518.2981	1018.5623	509.7848	1017.5783	509.2928	<b>9</b>	
<b>9</b>	<b>989.4575</b>	495.2324	972.4309	486.7191	971.4469	486.2271	<b>V</b>	<b>907.5303</b>	454.2688	890.5037	445.7555	889.5197	445.2635	<b>8</b>	
<b>10</b>	1102.5415	551.7744	1085.5150	543.2611	1084.5310	542.7691	<b>I</b>	<b>808.4619</b>	404.7346	791.4353	396.2213	790.4513	395.7293	<b>7</b>	
<b>11</b>	1203.5892	602.2982	1186.5626	593.7850	1185.5786	593.2930	<b>T</b>	<b>695.3778</b>	348.1925	678.3513	339.6793	677.3672	339.1873	<b>6</b>	
<b>12</b>	1316.6733	658.8403	1299.6467	650.3270	1298.6627	649.8350	<b>I</b>	<b>594.3301</b>	297.6687	577.3036	289.1554	576.3196	288.6634	<b>5</b>	
<b>13</b>	1373.6947	687.3510	1356.6682	678.8377	1355.6842	678.3457	<b>G</b>	<b>481.2461</b>	241.1267	464.2195	232.6134	463.2355	232.1214	<b>4</b>	
<b>14</b>	1487.7377	744.3725	1470.7111	735.8592	1469.7271	735.3672	<b>N</b>	424.2246	212.6159	407.1981	204.1027	406.2140	203.6107	<b>3</b>	
<b>15</b>	1616.7802	808.8938	1599.7537	800.3805	1598.7697	799.8885	<b>E</b>	<b>310.1817</b>	155.5945	293.1551	147.0812	292.1711	146.5892	<b>2</b>	
<b>16</b>							<b>R</b>	<b>181.1391</b>	91.0732	164.1125	82.5599			<b>1</b>	



NCBI BLAST search of [SYELPDGQVITIGNER](#)

(Parameters: blastp, nr protein database, expect=20000, no filter, PAM30)

Other BLAST [web gateways](#)

All matches to this query

Score	Mr(calc)	Delta	Sequence
109.5	1795.9048	-0.0027	<a href="#">SYELPDGQVITIGNER</a>

Mascot: <http://www.matrixscience.com/>

 **Mascot Search Results**
**Peptide View**MS/MS Fragmentation of **SYELPDGQVITIGNER**Found in **Q9BYX7** in **uni\_human\_nr**, ACTBM\_HUMAN Putative beta-actin-like protein 3 OS=Homo sapiens GN=POTEKP PE=5 SV=1

Match to Query 9713: 1789.882908 from(895.948730,2+) intensity(1047139008.0000) rtinseconds(2359) scans(13429) index(11946)

Title: 151218\_Songjing\_SILAC\_KD\_2\_B\_Spectrum029693\_scans\_13429\_RTINSECONDS=2359

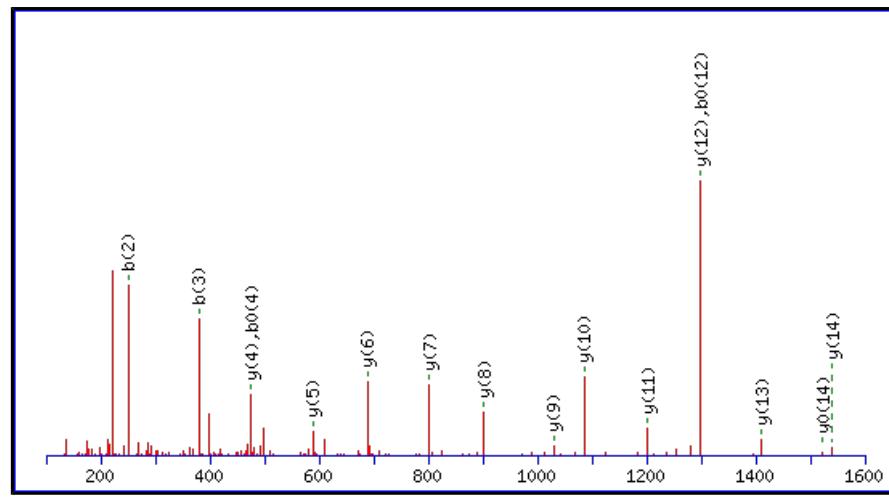
Data file U:\common\$\QExactive\2015\151218\_Songjing\_SILAC\mgf\T\T151218\_Songjing\_SILAC\_KD\_2\_B.mgf

Click mouse within plot area to zoom in by factor of two about that point

Or, to Da

Label all possible matches Label matches used for scoring

Show Y-axis



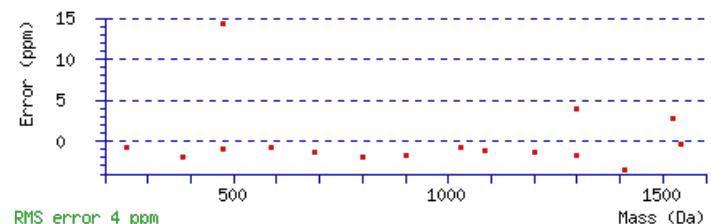
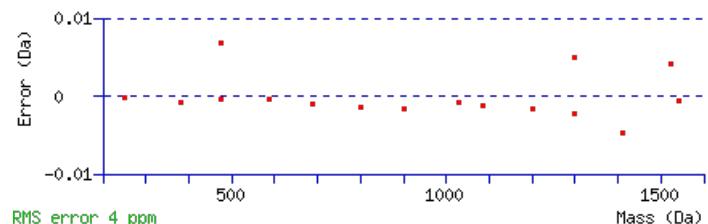
Monoisotopic mass of neutral peptide Mr(calc): 1789.8846

Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)

Ions Score: 114 Expect: 1.1e-009

Matches : 16/164 fragment ions using 15 most intense peaks ([help](#))

#	b	b <sup>++</sup>	b*	b* <sup>++</sup>	b <sup>0</sup>	b <sup>0<sup>++</sup></sup>	Seq.	y	y <sup>++</sup>	y*	y <sup>***</sup>	y <sup>0</sup>	y <sup>0<sup>++</sup></sup>	#	
<b>1</b>	88.0393	44.5233			70.0287	35.5180	<b>S</b>								<b>16</b>
<b>2</b>	<b>251.1026</b>	126.0550			233.0921	117.0497	<b>Y</b>	1703.8599	852.4336	1686.8333	843.9203	1685.8493	843.4283	<b>15</b>	
<b>3</b>	<b>380.1452</b>	190.5763			362.1347	181.5710	<b>E</b>	<b>1540.7966</b>	770.9019	1523.7700	762.3886	<b>1522.7860</b>	761.8966	<b>14</b>	
<b>4</b>	493.2293	247.1183			<b>475.2187</b>	238.1130	<b>L</b>	<b>1411.7540</b>	706.3806	1394.7274	697.8673	1393.7434	697.3753	<b>13</b>	
<b>5</b>	590.2821	295.6447			572.2715	286.6394	<b>P</b>	<b>1298.6699</b>	649.8386	1281.6434	641.3253	1280.6593	640.8333	<b>12</b>	
<b>6</b>	705.3090	353.1581			687.2984	344.1529	<b>D</b>	<b>1201.6171</b>	601.3122	1184.5906	592.7989	1183.6066	592.3069	<b>11</b>	
<b>7</b>	762.3305	381.6689			744.3199	372.6636	<b>G</b>	<b>1086.5902</b>	543.7987	1069.5636	535.2855	1068.5796	534.7935	<b>10</b>	
<b>8</b>	890.3890	445.6982	873.3625	437.1849	872.3785	436.6929	<b>Q</b>	<b>1029.5687</b>	515.2880	1012.5422	506.7747	1011.5582	506.2827	<b>9</b>	
<b>9</b>	989.4575	495.2324	972.4309	486.7191	971.4469	486.2271	<b>V</b>	<b>901.5102</b>	451.2587	884.4836	442.7454	883.4996	442.2534	<b>8</b>	
<b>10</b>	1102.5415	551.7744	1085.5150	543.2611	1084.5310	542.7691	<b>I</b>	<b>802.4417</b>	401.7245	785.4152	393.2112	784.4312	392.7192	<b>7</b>	
<b>11</b>	1203.5892	602.2982	1186.5626	593.7850	1185.5786	593.2930	<b>T</b>	<b>689.3577</b>	345.1825	672.3311	336.6692	671.3471	336.1772	<b>6</b>	
<b>12</b>	1316.6733	658.8403	1299.6467	650.3270	<b>1298.6627</b>	649.8350	<b>I</b>	<b>588.3100</b>	294.6586	571.2835	286.1454	570.2994	285.6534	<b>5</b>	
<b>13</b>	1373.6947	687.3510	1356.6682	678.8377	1355.6842	678.3457	<b>G</b>	<b>475.2259</b>	238.1166	458.1994	229.6033	457.2154	229.1113	<b>4</b>	
<b>14</b>	1487.7377	744.3725	1470.7111	735.8592	1469.7271	735.3672	<b>N</b>	418.2045	209.6059	401.1779	201.0926	400.1939	200.6006	<b>3</b>	
<b>15</b>	1616.7802	808.8938	1599.7537	800.3805	1598.7697	799.8885	<b>E</b>	304.1615	152.5844	287.1350	144.0711	286.1510	143.5791	<b>2</b>	
<b>16</b>							<b>R</b>	175.1190	88.0631	158.0924	79.5498			<b>1</b>	



NCBI BLAST search of [SYELPDGQVITIGNER](#)

(Parameters: blastp, nr protein database, expect=20000, no filter, PAM30)

Other BLAST [web gateways](#)

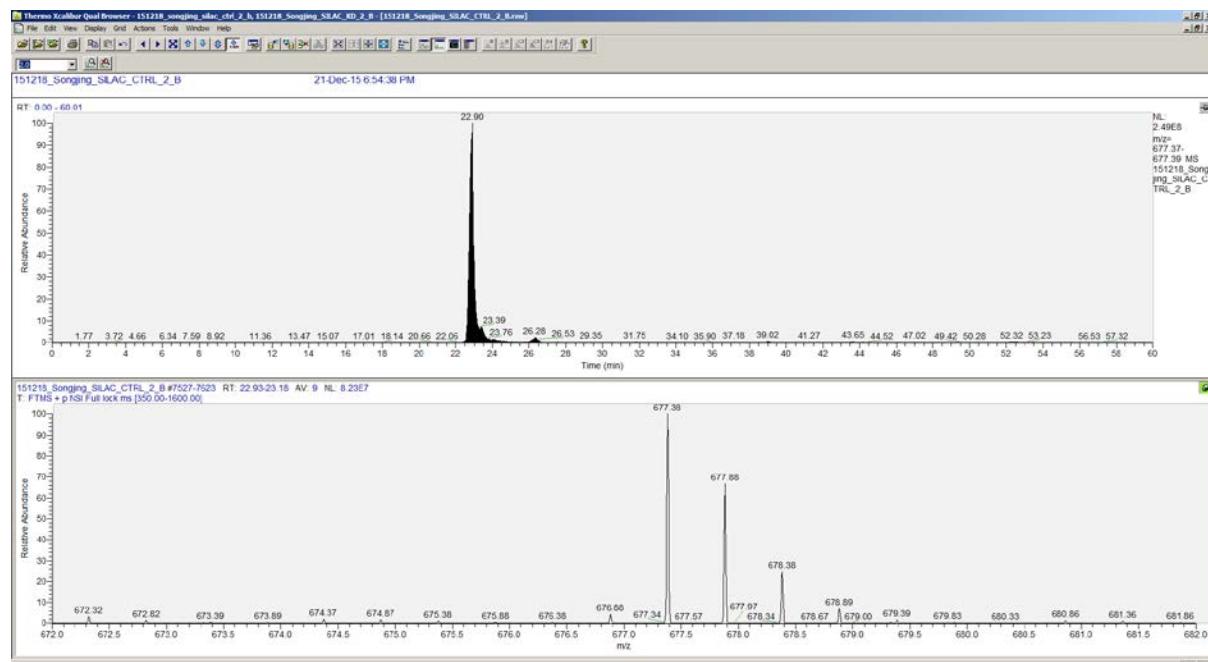
All matches to this query

Score	Mr(calc)	Delta	Sequence
114.3	1789.8846	-0.0017	<a href="#">SYELPDGQVITIGNER</a>
0.6	1787.8819	2.0010	<a href="#">MTELYQSLADLNVR</a>

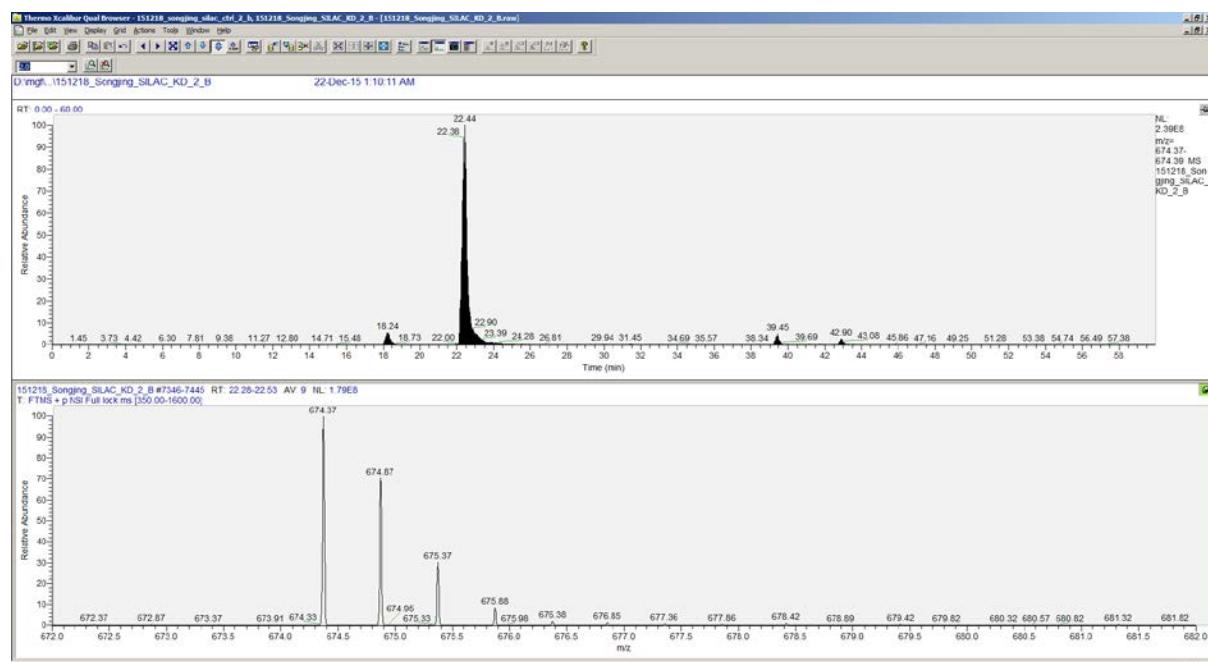
Mascot: <http://www.matrixscience.com/>

## P26641, EF1G\_HUMAN Elongation factor 1-gamma

K.ALIAAQYSGAQVR#V in wild type control cells



K.ALIAAQYSGAQVR.V in knockdown cells



## Mascot annotated MS/MS spectra

**K.ALIAAQYSGAQVR#.V in control cells vs K.ALIAAQYSGAQVR.V in knock down cells**

[EEF1G\\_K.ALIAAQYSGAQVR#.V\\_85.76](#)  
[EEF1G\\_K.ALIAAQYSGAQVR.V\\_83.67](#)

# MASCOT SCIENCE Mascot Search Results

## Peptide View

MS/MS Fragmentation of **ALIAAQYSGAQVR**

Found in **P26641** in **uni\_human\_nr**, EF1G\_HUMAN Elongation factor 1-gamma OS=Homo sapiens GN=EEF1G PE=1 SV=3

Match to Query 5172: 1352.749608 from(677.382080,2+) intensity(27321886.0000) rtinseconds(1389) scans(7612) index(6650)

Title: 151218\_Songjing\_SILAC\_CTRL\_2\_B\_Spectrum024242\_scans\_7612\_RTINSECONDS=1389

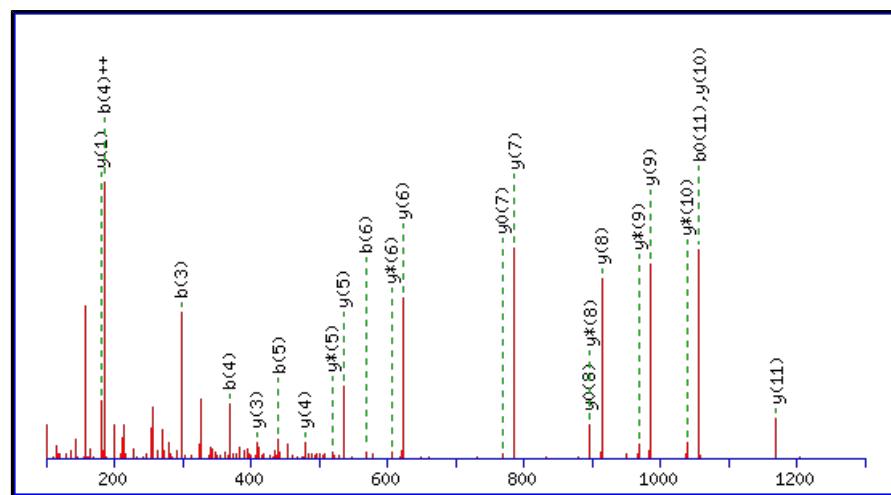
Data file U:\common\$\QExactive\2015\151218\_Songjing\_SILAC\mgf\T\T151218\_Songjing\_SILAC\_CTRL\_2\_B.mgf

Click mouse within plot area to zoom in by factor of two about that point

Or,                      to                      Da

Label all possible matches      Label matches used for scoring

Show Y-axis



**Monoisotopic mass of neutral peptide Mr(calc):** 1352.7508

**Fixed modifications:** Carbamidomethyl (C) (apply to specified residues or termini only)

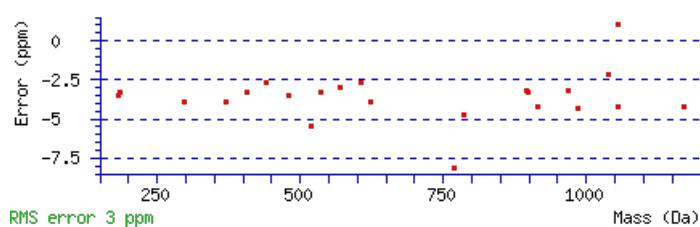
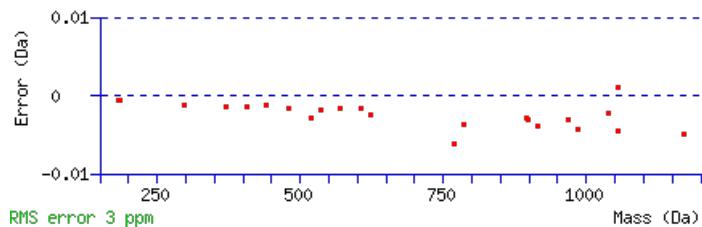
**Variable modifications:**

R13 : Label:13C(6) (R)

Ions Score: 86 Expect: 4.9e-007

Matches : 24/110 fragment ions using 32 most intense peaks ([help](#))

#	b	b <sup>++</sup>	b*	b* <sup>++</sup>	b <sup>0</sup>	b <sup>0<sup>++</sup></sup>	Seq.	y	y <sup>++</sup>	y*	y <sup>***</sup>	y <sup>0</sup>	y <sup>0<sup>++</sup></sup>	#
<b>1</b>	72.0444	36.5258					<b>A</b>							<b>13</b>
<b>2</b>	<b>185.1285</b>	93.0679					<b>L</b>	1282.7209	641.8641	1265.6944	633.3508	1264.7104	632.8588	<b>12</b>
<b>3</b>	<b>298.2125</b>	149.6099					<b>I</b>	<b>1169.6369</b>	585.3221	1152.6103	576.8088	1151.6263	576.3168	<b>11</b>
<b>4</b>	<b>369.2496</b>	<b>185.1285</b>					<b>A</b>	<b>1056.5528</b>	528.7800	<b>1039.5263</b>	520.2668	1038.5422	519.7748	<b>10</b>
<b>5</b>	<b>440.2867</b>	220.6470					<b>A</b>	<b>985.5157</b>	493.2615	<b>968.4892</b>	484.7482	967.5051	484.2562	<b>9</b>
<b>6</b>	<b>568.3453</b>	284.6763	551.3188	276.1630			<b>Q</b>	<b>914.4786</b>	457.7429	<b>897.4520</b>	449.2297	<b>896.4680</b>	448.7376	<b>8</b>
<b>7</b>	731.4087	366.2080	714.3821	357.6947			<b>Y</b>	<b>786.4200</b>	393.7136	769.3935	385.2004	<b>768.4094</b>	384.7084	<b>7</b>
<b>8</b>	818.4407	409.7240	801.4141	401.2107	800.4301	400.7187	<b>S</b>	<b>623.3567</b>	312.1820	<b>606.3301</b>	303.6687	605.3461	303.1767	<b>6</b>
<b>9</b>	875.4621	438.2347	858.4356	429.7214	857.4516	429.2294	<b>G</b>	<b>536.3247</b>	268.6660	<b>519.2981</b>	260.1527			<b>5</b>
<b>10</b>	946.4993	473.7533	929.4727	465.2400	928.4887	464.7480	<b>A</b>	<b>479.3032</b>	240.1552	462.2766	231.6420			<b>4</b>
<b>11</b>	1074.5578	537.7826	1057.5313	529.2693	<b>1056.5473</b>	528.7773	<b>Q</b>	<b>408.2661</b>	204.6367	391.2395	196.1234			<b>3</b>
<b>12</b>	1173.6263	587.3168	1156.5997	578.8035	1155.6157	578.3115	<b>V</b>	280.2075	140.6074	263.1809	132.0941			<b>2</b>
<b>13</b>							<b>R</b>	<b>181.1391</b>	91.0732	164.1125	82.5599			<b>1</b>



NCBI BLAST search of [ALIAAQYSGAQVR](#)

(Parameters: blastp, nr protein database, expect=20000, no filter, PAM30)

Other BLAST [web gateways](#)

## All matches to this query

Score	Mr(calc)	Delta	Sequence
85.8	1352.7508	-0.0012	<a href="#">ALIAAQYSGAQVR</a>
2.6	1352.7577	-0.0081	<a href="#">LALNQISQISMK</a>
1.8	1352.7543	-0.0047	<a href="#">AILPQAPANAHLK</a>
1.8	1352.7395	0.0101	<a href="#">LAIGITANFVNNGR</a>
1.8	1352.7598	-0.0102	<a href="#">LALCHLELTRK</a>

Mascot: <http://www.matrixscience.com/>

 **Mascot Search Results**
**Peptide View**MS/MS Fragmentation of **ALIAAQYSGAQVR**Found in **P26641** in **uni\_human\_nr**, EF1G\_HUMAN Elongation factor 1-gamma OS=Homo sapiens GN=EEF1G PE=1 SV=3

Match to Query 5032: 1346.729708 from(674.372130,2+) intensity(8032236.0000) rtinseconds(1428) scans(7687) index(6606)

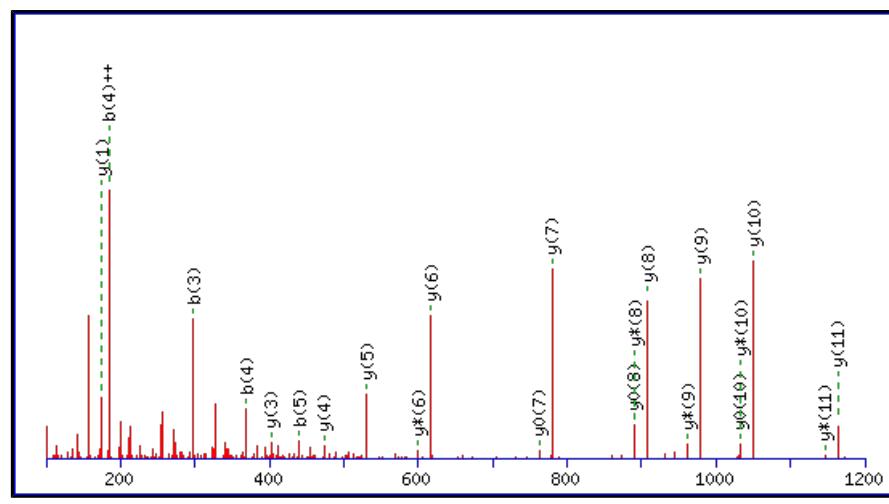
Title: 151218\_Songjing\_SILAC\_KD\_3\_B\_Spectrum023826\_scans\_7687\_RTINSECONDS=1428

Data file U:\common\$\QExactive\2015\151218\_Songjing\_SILAC\mgf\T\T151218\_Songjing\_SILAC\_KD\_3\_B.mgf

Click mouse within plot area to zoom in by factor of two about that point

Or,  to  Da Label all possible matches  Label matches used for scoring

Show Y-axis



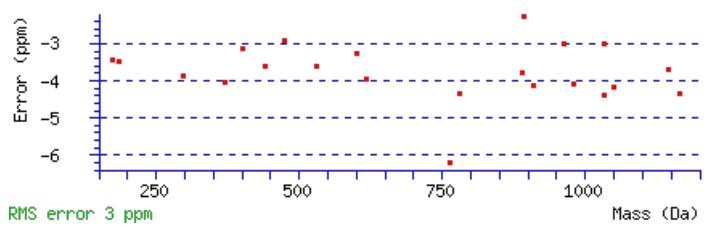
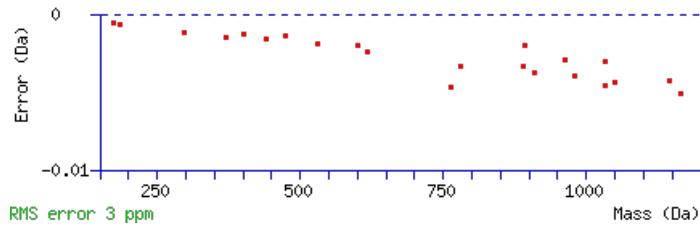
Monoisotopic mass of neutral peptide Mr(calc): 1346.7306

Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)

Ions Score: 84 Expect: 1.1e-006

Matches : 23/110 fragment ions using 33 most intense peaks ([help](#))

#	b	b <sup>++</sup>	b*	b* <sup>++</sup>	b <sup>0</sup>	b <sup>0<sup>++</sup></sup>	Seq.	y	y <sup>++</sup>	y*	y <sup>**</sup>	y <sup>***</sup>	y <sup>0</sup>	y <sup>0<sup>++</sup></sup>	#
<b>1</b>	72.0444	36.5258					<b>A</b>								<b>13</b>
<b>2</b>	<b>185.1285</b>	93.0679					<b>L</b>	1276.7008	638.8540	1259.6743	630.3408	1258.6902	629.8488	<b>12</b>	
<b>3</b>	<b>298.2125</b>	149.6099					<b>I</b>	<b>1163.6167</b>	582.3120	<b>1146.5902</b>	573.7987	1145.6062	573.3067	<b>11</b>	
<b>4</b>	<b>369.2496</b>	<b>185.1285</b>					<b>A</b>	<b>1050.5327</b>	525.7700	<b>1033.5061</b>	517.2567	<b>1032.5221</b>	516.7647	<b>10</b>	
<b>5</b>	<b>440.2867</b>	220.6470					<b>A</b>	<b>979.4956</b>	490.2514	<b>962.4690</b>	481.7381	961.4850	481.2461	<b>9</b>	
<b>6</b>	568.3453	284.6763	551.3188	276.1630			<b>Q</b>	<b>908.4585</b>	454.7329	<b>891.4319</b>	446.2196	<b>890.4479</b>	445.7276	<b>8</b>	
<b>7</b>	731.4087	366.2080	714.3821	357.6947			<b>Y</b>	<b>780.3999</b>	390.7036	763.3733	382.1903	<b>762.3893</b>	381.6983	<b>7</b>	
<b>8</b>	818.4407	409.7240	801.4141	401.2107	800.4301	400.7187	<b>S</b>	<b>617.3366</b>	309.1719	<b>600.3100</b>	300.6586	599.3260	300.1666	<b>6</b>	
<b>9</b>	875.4621	438.2347	858.4356	429.7214	857.4516	429.2294	<b>G</b>	<b>530.3045</b>	265.6559	513.2780	257.1426			<b>5</b>	
<b>10</b>	946.4993	473.7533	929.4727	465.2400	928.4887	464.7480	<b>A</b>	<b>473.2831</b>	237.1452	456.2565	228.6319			<b>4</b>	
<b>11</b>	1074.5578	537.7826	1057.5313	529.2693	1056.5473	528.7773	<b>Q</b>	<b>402.2459</b>	201.6266	385.2194	193.1133			<b>3</b>	
<b>12</b>	1173.6263	587.3168	1156.5997	578.8035	1155.6157	578.3115	<b>V</b>	274.1874	137.5973	257.1608	129.0840			<b>2</b>	
<b>13</b>							<b>R</b>	<b>175.1190</b>	88.0631	158.0924	79.5498			<b>1</b>	

NCBI BLAST search of [ALIAAQYSGAQVR](#)

(Parameters: blastp, nr protein database, expect=20000, no filter, PAM30)

Other BLAST [web gateways](#)

All matches to this query

Score	Mr(calc)	Delta	Sequence
83.7	1346.7306	-0.0009	<a href="#">ALIAAQYSGAQVR</a>
6.6	1346.7340	-0.0043	<a href="#">RVIAQRLMQSK</a>
2.5	1345.7211	1.0087	<a href="#">IDQRICNAITK</a>
2.5	1345.7211	1.0087	<a href="#">IDQRICNAITK</a>
2.3	1346.7194	0.0103	<a href="#">LAIGITANFVNGR</a>
0.1	1345.7275	1.0022	<a href="#">LALNQISQISMK</a>

Mascot: <http://www.matrixscience.com/>

**Table S3.** qPCR primer list.

<b>qPCR primer</b>	<b>sequence</b>
GAPDH-F	GTGGTCTCCTCTGACTTCAACAG
GAPDH-R	CTGTAGCCAAATTGCGTTGTCATAC
POPX2-F	TGTTTGATGGTCACGGAGGC
POPX2-R	TTCTCATCCTGCCGTTCTGGT
MMP2-F	GATACCCCTTGACGGTAAGGA
MMP2-R	CCTTCTCCCAAGGTCCATAGC
MMP7-F	GAGTGAGCTACAGTGGGAACA
MMP7-R	CTATGACGCAGGAGTTAACAT
MMP3-F	TGGCATTCAAGTCCCTCTATGG
MMP3-R	AGGACAAAGCAGGATCACAGTT
TIMP2-F	AAGCGGTCAGTGAGAAGGAAG
TIMP2-R	GGGGCCGTGTAGATAAACTCTAT
IGFBP7-F	ATCCCGACACCTGTCCTCAT
IGFBP7-R	CCCAGCCAGTTACTTCATGCT
PDGF-F	CTCGATCCGCTCCTTGATGA
PDGF-R	CGTTGGTGCGGTCTATGAG
EGF-F	TGTCCACGCAATGTGTCTGAA
EGF-R	CATTATCGGGTGAGGAACAAACC
FGF7-F	CCTTCTGCCTGTTGATTTATGG
FGF7-R	GTTGCTGTGACGCTGTTG
HGF-F	GCTATCGGGGTAAAGACCTACA
HGF-R	CGTAGCGTACCTCTGGATTGC