**SUPPLEMENTAL INFORMATION**

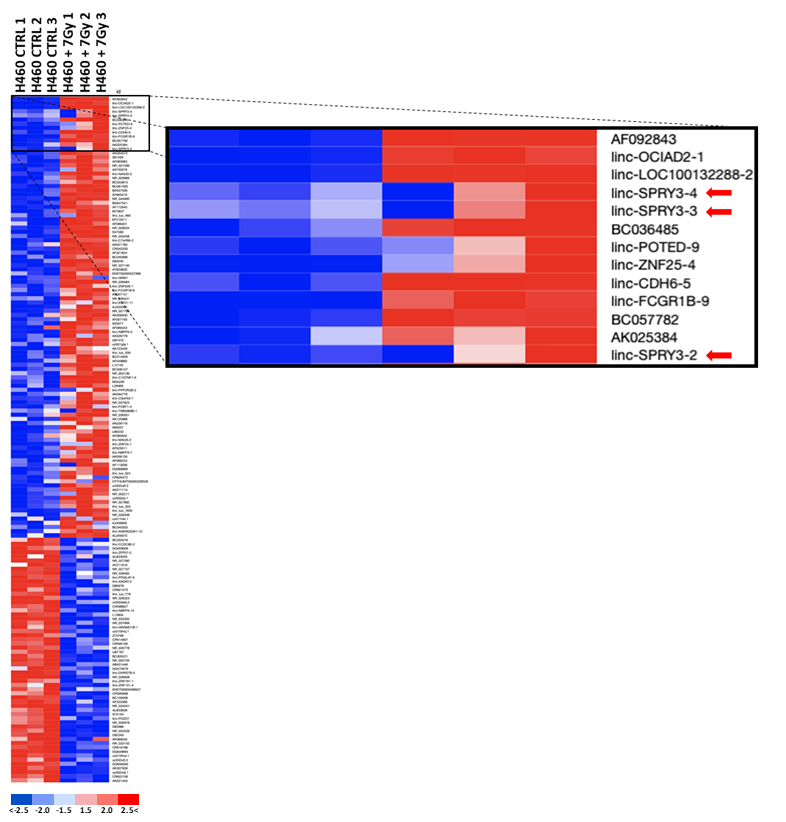
**Supplementary Table S1**

|  |  |
| --- | --- |
| qRT-PCR/PCR Primer Sequences |  |
| **Primers** | **Sequence (5'-3')** |
| linc-SPRY3-2 FWD | CATCCAATCCCATCTACTCCAT |
| linc-SPRY3-2 REV | TAGAATGGAACGAAATTTCACG |
| linc-SPRY3-3 FWD | TCCAGTCAATGATTTTGGATTC |
| linc-SPRY3-3 REV | GGAATAAAGTGGAATGCTACGG |
| linc-SPRY3-4 FWD | GTCCACTCCAC CCATTCTA |
| linc-SPRY3-4 REV | CGAACGGAATGGAATAAAAT |
| linc-SPRY3-2 FWD (2nd set) | TCCAGTCCATTTCACTCCAG |
| linc-SPRY3-2 REV (2nd set) | TGCAATAAAATCGACTCAGATAGA |
| linc-SPRY3-3 FWD (2nd set) | CCGTTTCATTGCATTCCA |
| linc-SPRY3-3 REV (2nd set) | GCAAGCGAAAGGAAAGGA |
| GAPDH FWD | CCACTCCTCCACCTTTGAC |
| GAPDH REV | ACCCTGTTGCTGTAGCCA |
| UBC FWD  UBC REV | GATTTGGGTCGCAGTTCTTG  CCTTATCTTGGATCTTTGCCTTG |
| Beta-Actin FWD | AGCACAGAGCCTCGCCTTT |
| Beta-Actin REV | CCACGATGGAGGGGAAGAC |
| U6 FWD | GTGCTCGCTTCGGCAGCACATAT |
| U6 REV | AAAAATATGGAACGCTTCACGAA |
| *C. elegans* ama-1 FWD | GGAGCTCGAGTGGATCTTCG |
| *C. elegans* ama-1 REV | GCGCAGAGAGTATCCTGGAC |
| pBACe3.6 FWD  pBACe3.6 REV | TTGAGTCTGCAAAAGGACTTGA  GATTGATGTGTCATCAGCGTCT |
| HMGA2 FWD  HMGA2 REV | GCCCCAGGAAGCAGCAA  TCGAACGTTGGCGCCCCCTA |
| C-MYC FWD  C-MYC REV |  |

**Supplementary Table S2**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **LncRNA Gene** | **Transcript Cluster ID** | **Fold Change** | **ANOVA p-value** | **FDR p-value** |
| AF092843 | TC12002437.hg.1 | 4.26 | 0.000022 | 0.000414 |
| linc-OCIAD2-1 | TC04002544.hg.1 | 4.17 | 0.000007 | 0.000159 |
| linc-LOC100132288-2 | TC21000805.hg.1 | 3.88 | 0.000028 | 0.000496 |
| **linc-SPRY3-4** | **TC0Y000283.hg.1** | **3.17** | **0.162867** | **0.440186** |
| **linc-SPRY3-3** | **TC0Y000284.hg.1** | **3.04** | **0.09976** | **0.322973** |
| BC036485 | TC03003223.hg.1 | 2.74 | 0.00001 | 0.000226 |
| linc-POTED-9 | TC21000560.hg.1 | 2.73 | 0.071853 | 0.258323 |
| linc-ZNF25-4 | TC10002568.hg.1 | 2.55 | 0.005738 | 0.036702 |
| linc-CDH6-5 | TC05002316.hg.1 | 2.49 | 0.000003 | 0.000094 |
| linc-FCGR1B-9 | TC01005759.hg.1 | 2.43 | 0.000086 | 0.001207 |
| BC057782 | TC09002209.hg.1 | 2.3 | 1.12E-07 | 0.000007 |
| AK025384 | TC16001789.hg.1 | 2.15 | 9.63E-07 | 0.000037 |
| **linc-SPRY3-2** | **TC0Y000285.hg.1** | **2.13** | **0.187334** | **0.479358** |
| AK054573 | TC17002305.hg.1 | 2.11 | 0.000099 | 0.001344 |
| S81264 | TC17002307.hg.1 | 2.11 | 1.37E-08 | 0.000002 |
| AF085983 | TC02004620.hg.1 | 2.07 | 6.60E-09 | 9.36E-07 |
| NR\_027299 | TC03002817.hg.1 | 2.07 | 1.70E-07 | 0.00001 |
| AX750576 | TC17002329.hg.1 | 2.03 | 7.14E-07 | 0.000029 |
| linc-NAA35-2 | TC09002052.hg.1 | 2.01 | 0.000004 | 0.000112 |
| NR\_026866 | TC03002367.hg.1 | 1.93 | 7.03E-07 | 0.000029 |
| BC033874 | TC01006039.hg.1 | 1.89 | 0.000209 | 0.002472 |
| BC061593 | TC05002175.hg.1 | 1.89 | 3.79E-09 | 6.30E-07 |
| BX537506 | TC08002539.hg.1 | 1.89 | 5.12E-09 | 7.85E-07 |
| AY665470 | TC12002292.hg.1 | 1.89 | 8.29E-10 | 2.12E-07 |
| NR\_044995 | TC13001319.hg.1 | 1.85 | 2.64E-07 | 0.000013 |
| BX647541 | TC16001788.hg.1 | 1.85 | 2.70E-10 | 1.07E-07 |
| AF172940 | TC02003138.hg.1 | 1.83 | 1.90E-08 | 0.000002 |
| M73837 | TC10002092.hg.1 | 1.83 | 0.000549 | 0.005416 |
| linc\_luo\_466 | TC06002911.hg.1 | 1.81 | 0.313329 | 0.633221 |
| EF210211 | TC07002321.hg.1 | 1.81 | 2.36E-08 | 0.000002 |
| AF086401 | TC07002818.hg.1 | 1.81 | 0.000009 | 0.000209 |
| NR\_028034 | TC10002196.hg.1 | 1.79 | 5.54E-07 | 0.000024 |
| S47380 | TC04002187.hg.1 | 1.78 | 0.003206 | 0.022936 |
| NR\_033258 | TC02004623.hg.1 | 1.77 | 1.23E-08 | 0.000001 |
| linc-C7orf66-2 | TC07003112.hg.1 | 1.77 | 0.000043 | 0.000692 |
| AK021785 | TC11003067.hg.1 | 1.77 | 7.20E-07 | 0.000029 |
| CR542200 | TC04002080.hg.1 | 1.76 | 0.000565 | 0.005548 |
| AF321824 | TC08002559.hg.1 | 1.75 | 0.000075 | 0.001082 |
| BC040985 | TC03002416.hg.1 | 1.74 | 1.03E-08 | 0.000001 |
| S83545 | TC05002797.hg.1 | 1.74 | 2.33E-07 | 0.000012 |
| NR\_027140 | TC08002257.hg.1 | 1.74 | 0.000011 | 0.000245 |
| AY820830 | TC06003084.hg.1 | 1.73 | 6.40E-08 | 0.000005 |
| ENST00000537998 | TC12002680.hg.1 | 1.73 | 0.000002 | 0.000054 |
| linc-GRM1 | TC06003110.hg.1 | 1.72 | 0.000749 | 0.006988 |
| NR\_036684 | TC16001604.hg.1 | 1.71 | 5.09E-08 | 0.000004 |
| linc-ZNF626-1 | TC19002390.hg.1 | 1.71 | 0.017129 | 0.087898 |
| linc-FCGR1B-6 | TC01005756.hg.1 | 1.7 | 0.001 | 0.008855 |
| AK057151 | TC07002222.hg.1 | 1.7 | 0.000029 | 0.0005 |
| NR\_036431 | TC0X002160.hg.1 | 1.69 | 0.002693 | 0.019933 |
| linc-ELTD1-11 | TC01005583.hg.1 | 1.67 | 0.000003 | 0.00008 |
| AJ420500 | TC06002615.hg.1 | 1.67 | 0.000453 | 0.004635 |
| NR\_027783 | TC0X001624.hg.1 | 1.67 | 9.46E-08 | 0.000006 |
| AK056630 | TC07002487.hg.1 | 1.66 | 0.013477 | 0.073006 |
| AF007193 | TC07002493.hg.1 | 1.65 | 0.020372 | 0.100857 |
| X55077 | TC12002396.hg.1 | 1.64 | 2.21E-08 | 0.000002 |
| AF086343 | TC10002011.hg.1 | 1.63 | 0.000542 | 0.005365 |
| linc-NBPF9-3 | TC01006417.hg.1 | 1.62 | 0.000033 | 0.000559 |
| AK026778 | TC02004622.hg.1 | 1.61 | 0.000051 | 0.000787 |
| D87470 | TC11003246.hg.1 | 1.61 | 0.000013 | 0.000278 |
| uc001gla.1 | TC01004846.hg.1 | 1.6 | 0.000001 | 0.000044 |
| AK123435 | TC01005963.hg.1 | 1.6 | 0.000898 | 0.008093 |
| linc\_luo\_506 | TC07002386.hg.1 | 1.6 | 0.00063 | 0.006047 |
| BC014926 | TC14001766.hg.1 | 1.6 | 3.72E-07 | 0.000018 |
| AF049885 | TC04002887.hg.1 | 1.59 | 3.44E-11 | 3.00E-08 |
| L12143 | TC06002402.hg.1 | 1.59 | 1.06E-07 | 0.000007 |
| BC006127 | TC12002414.hg.1 | 1.59 | 0.003111 | 0.022429 |
| NR\_003136 | TC15002630.hg.1 | 1.59 | 0.028675 | 0.131215 |
| linc-C1QTNF1-5 | TC17002394.hg.1 | 1.59 | 1.60E-07 | 0.000009 |
| M34339 | TC17002615.hg.1 | 1.59 | 0.000753 | 0.007018 |
| L26969 | TC05003038.hg.1 | 1.58 | 0.000017 | 0.00033 |
| linc-PPP2R3B-2 | TC0X001951.hg.1 | 1.58 | 0.294838 | 0.61422 |
| linc-TMEM88B-1 | TC01004129.hg.1 | 1.57 | 0.133895 | 0.390383 |
| AK094779 | TC01004217.hg.1 | 1.57 | 0.000004 | 0.000117 |
| linc-C5orf43-1 | TC05003020.hg.1 | 1.57 | 0.000192 | 0.002313 |
| NR\_037623 | TC15002291.hg.1 | 1.57 | 0.002041 | 0.015904 |
| linc-FOXF1-4 | TC16001680.hg.1 | 1.57 | 0.133609 | 0.389902 |
| NR\_036501 | TC07002242.hg.1 | 1.56 | 0.000008 | 0.000193 |
| AK125888 | TC08002503.hg.1 | 1.56 | 0.000873 | 0.007912 |
| AK026716 | TC0X001693.hg.1 | 1.56 | 0.003961 | 0.027195 |
| X69207 | TC11002905.hg.1 | 1.56 | 0.004246 | 0.028736 |
| U80232 | TC06002661.hg.1 | 1.55 | 0.00013 | 0.00168 |
| AF085942 | TC07002488.hg.1 | 1.55 | 0.048528 | 0.194753 |
| linc-MAOA-2 | TC0X001666.hg.1 | 1.55 | 0.005446 | 0.035176 |
| linc-ZNF25-1 | TC10002567.hg.1 | 1.54 | 0.017415 | 0.088988 |
| AF029311 | TC17002638.hg.1 | 1.54 | 0.000007 | 0.00016 |
| linc-NBPF9-7 | TC01004681.hg.1 | 1.53 | 0.02606 | 0.121819 |
| AK056155 | TC01004870.hg.1 | 1.53 | 0.000009 | 0.0002 |
| AF086224 | TC01006049.hg.1 | 1.53 | 0.01442 | 0.076986 |
| AF113008 | TC08001925.hg.1 | 1.53 | 0.185875 | 0.477108 |
| DQ088985 | TC15002378.hg.1 | 1.53 | 0.013512 | 0.073148 |
| linc\_luo\_524 | TC07003197.hg.1 | 1.52 | 0.000422 | 0.004397 |
| CR626472 | TC11003277.hg.1 | 1.52 | 0.000022 | 0.000402 |
| OTTHUMT00000328528 | TC02003287.hg.1 | 1.51 | 0.122727 | 0.369369 |
| uc002vef.2 | TC02003799.hg.1 | 1.51 | 1.17E-09 | 2.61E-07 |
| AK311114 | TC06003872.hg.1 | 1.51 | 2.95E-10 | 1.13E-07 |
| NR\_002211 | TC17002074.hg.1 | 1.51 | 0.000358 | 0.00385 |
| uc002jdy.1 | TC17002746.hg.1 | 1.51 | 0.000001 | 0.000043 |
| NR\_027882 | TC19002194.hg.1 | 1.51 | 0.000001 | 0.000046 |
| linc\_luo\_333 | TC01004684.hg.1 | 1.5 | 0.004694 | 0.031159 |
| linc\_luo\_1805 | TC01005654.hg.1 | 1.5 | 0.190779 | 0.484473 |
| NR\_028308 | TC02004133.hg.1 | 1.5 | 0.002093 | 0.016232 |
| uc011hki.1 | TC06002389.hg.1 | 1.5 | 0.336701 | 0.656202 |
| AJ459855 | TC06003566.hg.1 | 1.5 | 0.188301 | 0.480849 |
| BC040303 | TC06003704.hg.1 | 1.49 | 0.000003 | 0.000093 |
| linc-ANKRD20A1-12 | TC09001955.hg.1 | 1.49 | 0.000091 | 0.001259 |
| AL049370 | TC10002117.hg.1 | 1.49 | 0.013632 | 0.073681 |
| BC033316 | TC01004199.hg.1 | -1.49 | 0.001105 | 0.009591 |
| linc-CCDC80-2 | TC03003030.hg.1 | -1.49 | 0.029249 | 0.133067 |
| DQ459608 | TC04002625.hg.1 | -1.49 | 3.08E-07 | 0.000015 |
| linc-ZFP57-2 | TC06003570.hg.1 | -1.49 | 2.17E-07 | 0.000012 |
| AL833059 | TC09002185.hg.1 | -1.49 | 0.003138 | 0.022584 |
| NR\_027280 | TC19002180.hg.1 | -1.49 | 0.000034 | 0.000579 |
| AY211918 | TC01004531.hg.1 | -1.5 | 0.005576 | 0.035907 |
| NR\_027157 | TC12003031.hg.1 | -1.5 | 0.000005 | 0.000129 |
| NR\_036462 | TC01004175.hg.1 | -1.51 | 0.002087 | 0.016199 |
| linc-PPIAL4F-5 | TC01005804.hg.1 | -1.51 | 0.002016 | 0.015731 |
| linc-AADAT-2 | TC04002818.hg.1 | -1.51 | 0.235108 | 0.545485 |
| D86978 | TC07002605.hg.1 | -1.51 | 0.000018 | 0.000342 |
| CR601575 | TC11003430.hg.1 | -1.51 | 0.002021 | 0.015764 |
| linc\_luo\_778 | TC13001572.hg.1 | -1.51 | 0.000666 | 0.006327 |
| NR\_028323 | TC02004129.hg.1 | -1.53 | 0.000013 | 0.000272 |
| uc002eea.2 | TC16001872.hg.1 | -1.53 | 0.013494 | 0.073083 |
| CR598627 | TC07002576.hg.1 | -1.54 | 0.001751 | 0.013987 |
| linc-NBPF9-14 | TC01004671.hg.1 | -1.55 | 0.000047 | 0.000741 |
| L13804 | TC13001407.hg.1 | -1.55 | 0.013766 | 0.074222 |
| NR\_033302 | TC01004881.hg.1 | -1.56 | 0.005702 | 0.036507 |
| NR\_037669 | TC07002868.hg.1 | -1.56 | 0.009795 | 0.056413 |
| linc-GRAMD1B-1 | TC11002922.hg.1 | -1.56 | 6.54E-07 | 0.000027 |
| uc010hrq.1 | TC03003054.hg.1 | -1.57 | 0.007241 | 0.044382 |
| Z70768 | TC09002468.hg.1 | -1.57 | 0.000078 | 0.00112 |
| CR614667 | TC06003537.hg.1 | -1.58 | 0.001279 | 0.010817 |
| CR595160 | TC14001578.hg.1 | -1.58 | 0.000315 | 0.003473 |
| NR\_026778 | TC01006247.hg.1 | -1.59 | 0.011804 | 0.065552 |
| U87167 | TC0X001927.hg.1 | -1.59 | 0.001597 | 0.012952 |
| BC005221 | TC10002200.hg.1 | -1.6 | 0.020556 | 0.101559 |
| NR\_003105 | TC15002245.hg.1 | -1.6 | 0.013286 | 0.07218 |
| AB451448 | TC01005350.hg.1 | -1.61 | 0.000522 | 0.005197 |
| DQ470079 | TC04002368.hg.1 | -1.61 | 0.000775 | 0.007175 |
| linc-DHRS7B-5 | TC17002115.hg.1 | -1.61 | 0.010224 | 0.058437 |
| NR\_036608 | TC17002792.hg.1 | -1.61 | 0.00002 | 0.000372 |
| linc-ZNF791-1 | TC19001991.hg.1 | -1.62 | 0.098672 | 0.320528 |
| linc-ZNF131-4 | TC05002352.hg.1 | -1.63 | 0.000329 | 0.003603 |
| ENST00000499627 | TC01005364.hg.1 | -1.65 | 0.000279 | 0.003145 |
| CR595668 | TC0X001880.hg.1 | -1.65 | 1.67E-08 | 0.000002 |
| BC105606 | TC10002226.hg.1 | -1.65 | 0.000159 | 0.00198 |
| AF333388 | TC01006221.hg.1 | -1.66 | 7.35E-07 | 0.00003 |
| NR\_034041 | TC17002724.hg.1 | -1.67 | 0.00003 | 0.000515 |
| AL833658 | TC06003604.hg.1 | -1.68 | 0.004967 | 0.032589 |
| S70154 | TC06003166.hg.1 | -1.69 | 0.000541 | 0.005356 |
| linc-PDZD7 | TC10002799.hg.1 | -1.7 | 0.0004 | 0.004211 |
| NR\_026978 | TC18000697.hg.1 | -1.7 | 0.003329 | 0.023636 |
| D83986 | TC22001090.hg.1 | -1.73 | 4.21E-08 | 0.000003 |
| NR\_024526 | TC02003640.hg.1 | -1.74 | 0.005067 | 0.033116 |
| D82345 | TC0X002162.hg.1 | -1.74 | 0.000219 | 0.00258 |
| AF086045 | TC18000953.hg.1 | -1.74 | 0.012803 | 0.070056 |
| NR\_033142 | TC01005497.hg.1 | -1.77 | 0.000836 | 0.007633 |
| CR616786 | TC06003578.hg.1 | -1.81 | 0.00002 | 0.000374 |
| DQ648894 | TC02003219.hg.1 | -1.85 | 0.011223 | 0.063019 |
| uc010fnw.1 | TC02003637.hg.1 | -1.9 | 0.023072 | 0.110835 |
| uc002ufj.3 | TC02003679.hg.1 | -1.99 | 0.010288 | 0.058714 |
| DQ656056 | TC09002285.hg.1 | -2.04 | 0.000016 | 0.000318 |
| AK307939 | TC10002686.hg.1 | -2.07 | 0.000458 | 0.004673 |
| uc002zdj.1 | TC21000762.hg.1 | -2.2 | 2.53E-07 | 0.000013 |
| CR622106 | TC12002644.hg.1 | -2.34 | 0.000154 | 0.001929 |
| AK021443 | TC10002227.hg.1 | -2.51 | 0.011029 | 0.062107 |

**Supplementary Table S2: Differentially expressed lncRNAs after IR in H460 cells**

**Supplementary Figure S1**

**Supplementary Figure S1: Microarray analysis of differentially expressed lncRNAs after IR in NSCLC cell line H460.** Heat map of lncRNAs microarray data comparing H460 no radiated (Control) against H460 irradiated (7Gy) after 24 hrs. Only lncRNAs differentially expressed are shown.

**Supplementary Figure S2**

>AK128024.1 Homo sapiens cDNA FLJ46143 fis, clone TESTI2053561 (1986bp)

(with AY598347.3 fragment is called AK47)

**CCATTCCTTTCGAGCCCTTTCAATTTGAGTCCATTCCTTTCCAGTCCATTTCACTCCAGTC**C**ATTACTATCCATTCCATACCATTCCATCCCATTCCATTCCATTCCATTCCATT**C**CATTCCATTCCATT**A**CATTCCATTCCATT**CCATTGCATTCAATTCCATTCCATTCCATT**GCACTGCACTCCATTCCATTACATTCTACTCTATCTGAGTCG**G**TTTTATTGCATTAGATTCTATTCCATTGGATTACTTTCCATTCGATTAC**C**TTCCATTCATGTACATTCCATTCCAGTCAATTACATTCGAGTTCATTAC**A**TTACATTCCAGTATATTCCATTGTATTCGATCCCATTCCTTTCAATTCCATTTCATTCGACTCCATTATATTC**G**ATTCCATTCCACTCGAATCCATTCCATTAGAGGACATTCCATTCCAATGCATTCC**T**TTCCATTCCATAGCATTCCATTGCATTCGATTCCATTCCATTTGATGCCATTCCATTTGATGCCAT**T**CCATGACATTCCATTCCATTCGAGTCCATTCCGTTCCAATTCATTCCATTCCGTTTCATGAAATTCGAGTCCTTTCCAGTACATTTCATTCCAATCCCATCCAATCC**A**ATCTACTCCATTCAATTCCTTTCCATTCCATTTGATT**A**GATTCCATTGA**C**TTGATTCCATTCAGTTTGATTCCATTCCGTGAAATTTCGTTCCATTCTATTCCATTGCATTACTTTCCAT**TCAATTCCATTCCATTTCATTTCAGTCCATTCGCTTCCTTTCCTTTCGATTCAATTCCATTTGATTCCACTCCATTCTATGCGATTTCATTCCAATCGATTCAATTCCATTCGATGACATTCCTTTCGTTTCCATTCCATTCGAGTCCATTCAATTTGAGCATTCGTGTCCATTCTATTCGAGTCCATTCCATTACCGTCTATTCTATTCCCTTCCATTCCTGTTGATTCAATTTCATTCCCTTCCATTCGATTCCTTTCCATTCGATTCCATTCCTTTCCATTCCATTCCATTCGTTCCCACTCCATGTGATTTCATTCCATTCCAGTCCATTATATTCGA**GTCCACTCCACTCCATTCTATTACATTCAATTCCTTTTGAGTCCGTTCCATAACACTCCATTCATTTCGATTCCATTTCTTGCCAGTTTTCTTCCATTTTATTCCATTCCGTTCGATTCCATTCCATTC**GATTGCATTCCATTCGAATCCTTTCCATTCCATTTCATTCCATTCCTTTCTATTCCATTCCATTTCATTCGATTTGATTCCATTCTGCTCTATTCCATTCAATTCTTTTTCATTCCATTCGAATCCTTTCTATTGCAGTCCATTCTATTCGAGTCCATTCCAATCCCTTCCATTCCATTCAATTACAGTCCATTCCAATAGATTCCATTCCTTTGCCTTCCATTCGAATCCATTCCATTCTAGTCCATTCCATTTGAGTCCATTCCATTCCATTCCATTCTATTCCTTTCCAATCCATTCGATTCCATTCGATTCAATTCCATTTGATTCTCTTTCATTCTATTTTATTCCATGCCATTTGATTGCATTGCATTCCATTCCGTTTGATTGCAGTCCATTCAAGAAAGTTCCATTCCAGTCCATTGCTTTCCAGTCCATTCCATTCCACTCTAGTCTATTCCACTCCATTCCTTTCCATTCCATTCCATACTATTCCATTCCATTCCTTTGCATTCCGTTTCCAATCTATTCGAGTCCATTGCATTCCAGTCCAATCCATTCGATTACATTCCTTTTGATTCCCTGCCAGTCGATTGCATTGCATACTAGACCATTCCAAAGGAGTCCATTCCATTCTATCTCAACACTTTCCATTCCACTCTGTTCGAGTCCATTCCATTCCAGTCCATTTAATTCAAGGGCATTCCATTCCATTCCATTCTATTCCATTCCATTCCATTTCATATTATTCCATTCCATTCAATTCCATCTCTCCAGATGATTCCATTCCATTCTATACCATTGCTCTCTGTTCCATTCCATTCCATCTGTCTCCATTCCTTTCGTTTCGATTCCTTTCCATTCCATTCCATTACATTTGATCCTATTTTATTAAATTGCATTCTATTCGAGTGATTTCCATTCGAGTCCTTTCCATTCGATTCCATTCCCCACTGACGCCGGCGCTACTTACAGTTGGCGGGGCAAAAAAAAAAAAA

**Lnc-SPRY3-2**

**Lnc-SPRY3-4**

AY598347.3 sequence (591-816)

Splicing region

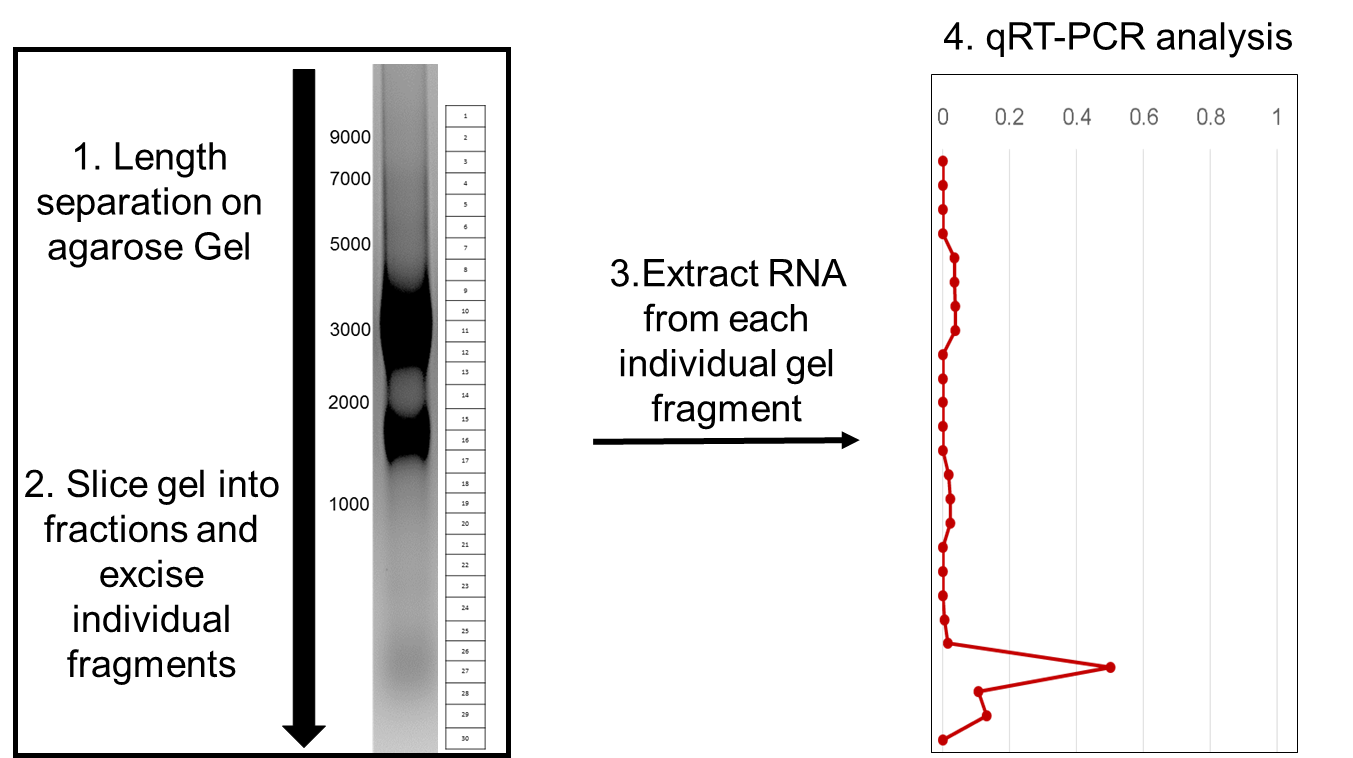
>AY598346.2 Homo sapiens heterochromatic block map Yq12 transcribed DYZ1 sequence mRNA, partial sequence (1441)

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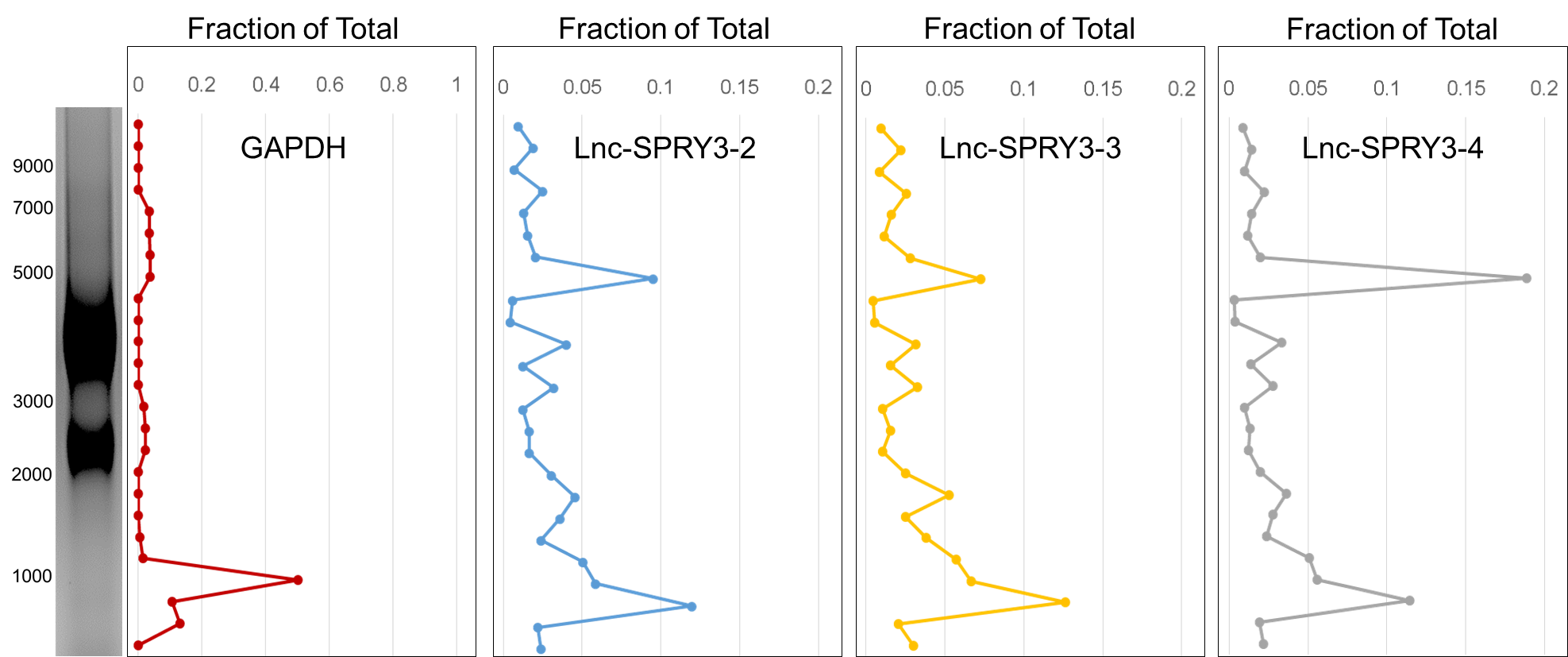
**Lnc-SPRY3-3**

Splicing region

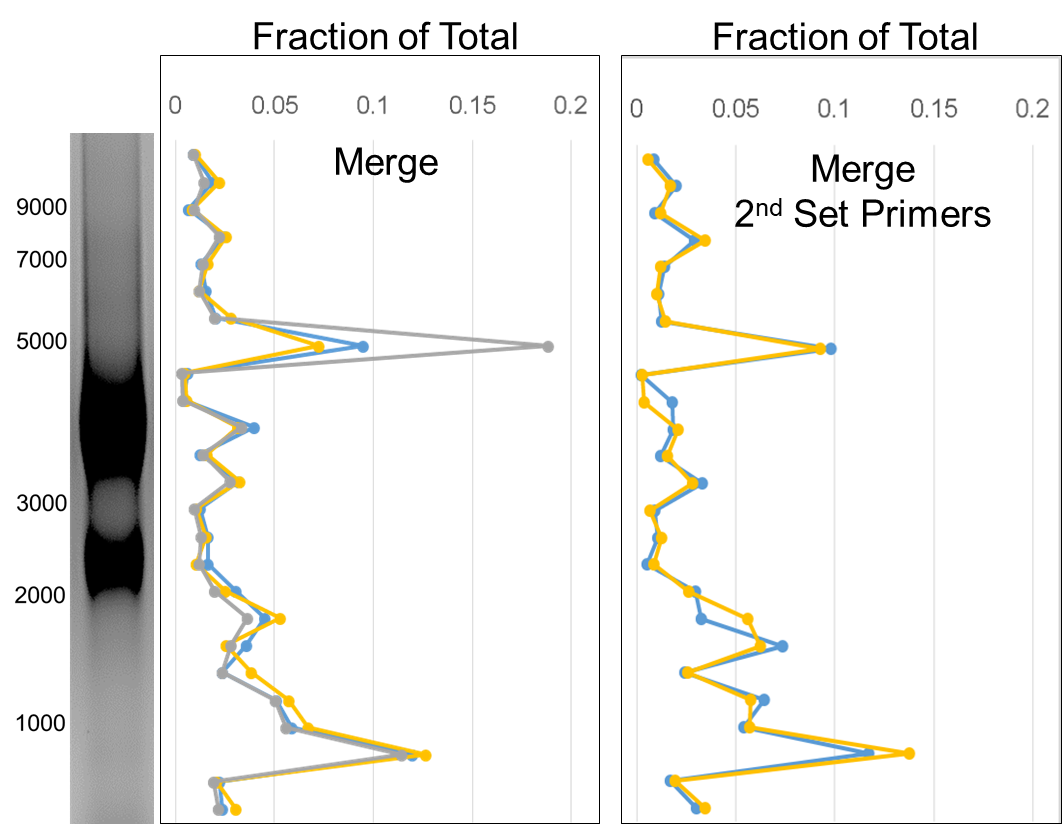
**Supplementary Figure S2: Overlapping sequences between linc-SPRY3-2/3/4 and clones AY598347.3 and AY598346.2**

**Supplementary Figure S3**

**A**



**B**

****

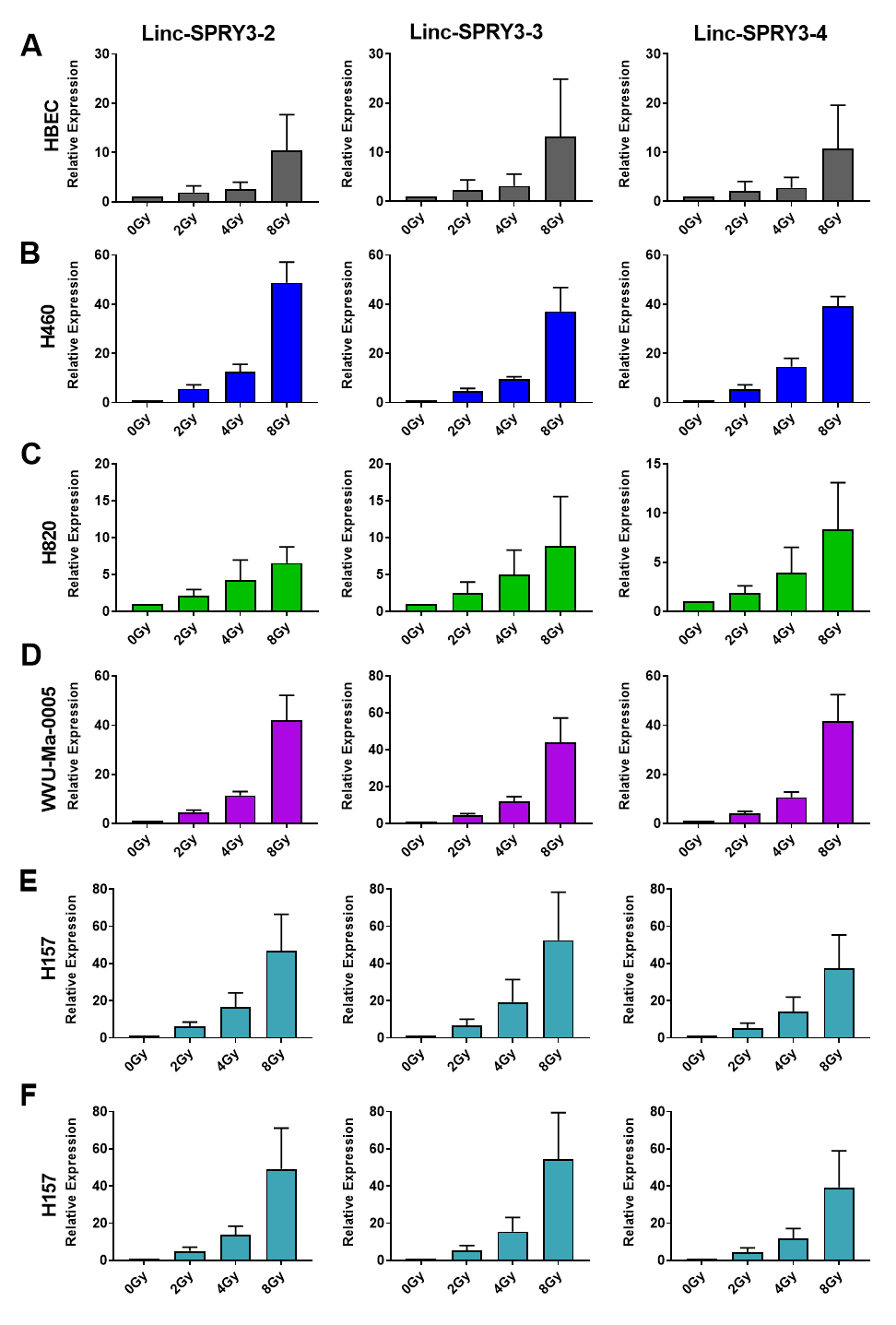
**C**

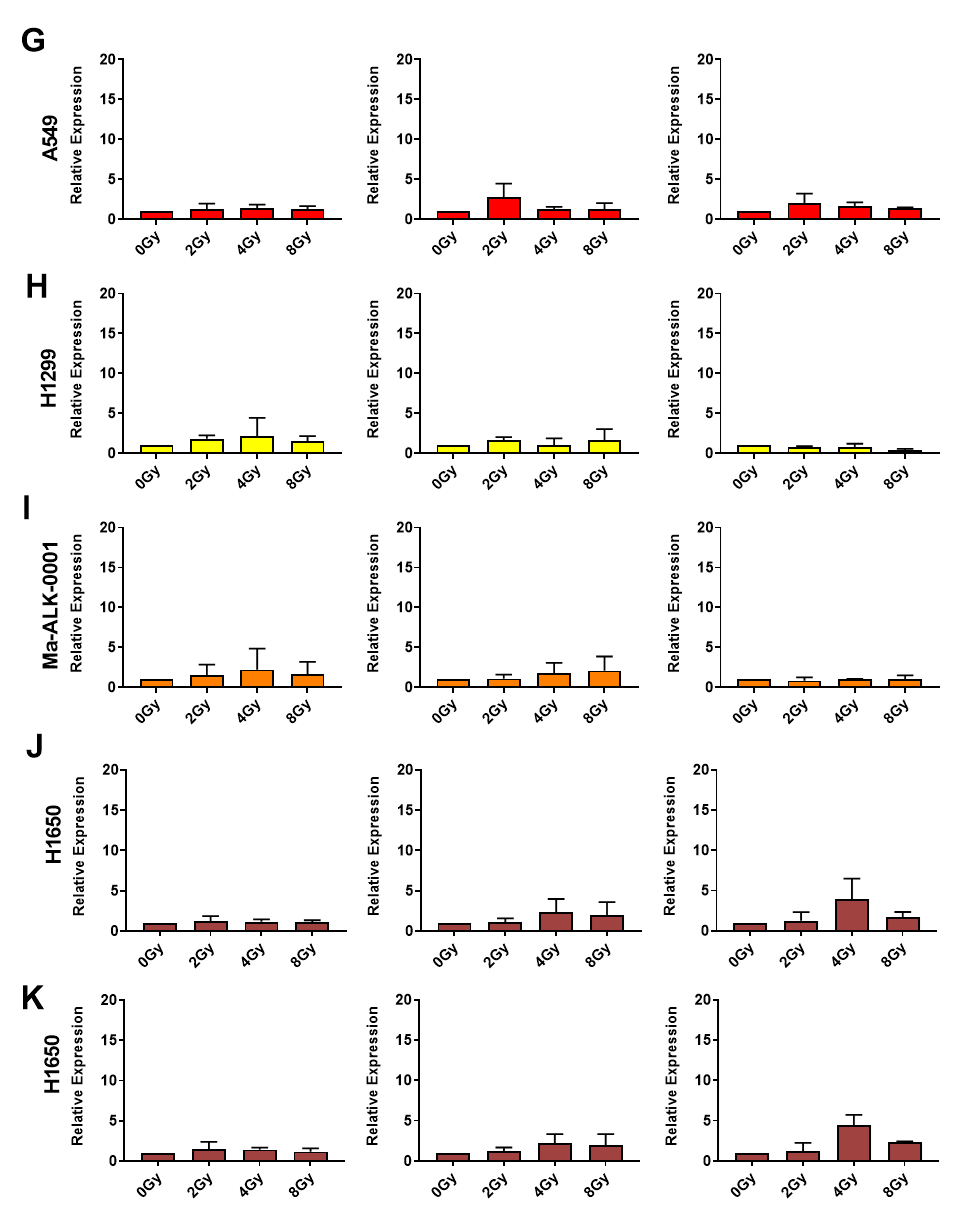
**Supplementary Figure S3: Virtual northern blot analysis of linc-SPRY3-2/3/4 in H460 cells demonstrates potential transcript size. (A)** Schematic outlining the virtual northern blot method. **(B)** Virtual northern blot reveals two large peaks, one at ~5kb and one at ~1kb. **(C)** These two peaks align when the individual graphs are merged. Similar peaks are seen when a second set of primers for linc-SPRY3-2 and linc-SPRY3-3 are used. GAPDH was used as a control.

**Supplementary Figure S4**



**Supplementary Figure S4: Clonogenic cell survival assay shows the radiation response of a panel of male NSCLC cell lines.** Clonogenic cell survival assay shows the relative radiation response of a panel of male NSCLC cell lines. Surviving fractions are plotted as a function of dose. Error bars represent SD from the mean of triplicate measurements from a single experiment.Shown here is a representative of two independent experiments with similar result.

**Supplementary Figure S5**

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**Supplementary Figure S5: Radiation response of cell line panel normalized to the housekeeping gene UBC and two additional NSCLC cell lines. (A-D&G-I)** qRT-PCR analysis of the linc-SPRY3 family normalized to UBC. **(E-F)** qRT-PCR analysis of the linc-SPRY3 family in the additional radiosensitive cell line H157 normalized to (E) GAPDH and (F) UBC. **(J-K)** qRT-PCR analysis of the linc-SPRY3 family in the additional radioresistant cell line H1650 normalized to (J) GAPDH and (K) UBC. **(L-M)** Y chromosome DNA FISH of H157 and H1650 cells.

**Supplementary Figure S6**



**A**

**H1819**

**H1975**



**B**

**Supplementary Figure S6: Female NSCLC cell lines show no expression of the linc-SPRY3 family**. qRT-PCR analysis of the linc-SPRY3 family in female NSCLC cell lines **(A)** H1975 and **(B)** H1819. Cells were plated at equal density and treated with one dose of radiation (2Gy, 4Gy, or 8Gy) and collected 72 hours after treatment. Values are relative to untreated control of the same collection day (0Gy). GAPDH mRNA was used to normalize qRT-PCR analysis. Error bars represent SD from the mean of triplicate experiments.

**Supplementary Figure S7**



**B**

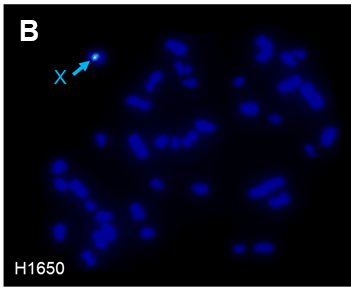


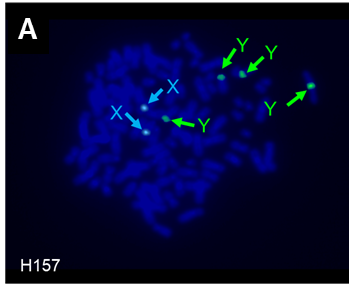
**A**

**C**



**Supplementary Figure S7: Cell fractionation of radiosensitive cell lines reveals nuclear localization of the linc-SPRY3 family.** qRT-PCR analysis of the linc-SPRY3 family in fractionated **(A)** H460 and **(B)** WVU-Ma-0005 cell lines. The dotted line represents the total relative RNA of each experiment. Mature β-Actin mRNA was used as a cytoplasmic control and U6 small nucleolar RNA was used as a nuclear control. Error bars represent SD from the mean of triplicate measurements from a single experiment. Shown here is a representative of three independent experiments with similar result. Normalization was done using C. elegans total RNA as an exogenous spike for the amplification of worm specific ama-1 gene. **(C)** Coding probability (CP) scores of the *linc-SPRY3-2/3/4* showing no coding probabilities for these lncRNAs (a CP of 0.364 and above is considered a coding RNA). Coding-Potential Assessment Tool (CPAT) was used for these calculations.

**Supplementary Figure S8**

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**Supplementary Figure S8: Y chromosome DNA FISH of H157 and H1650 cells.**

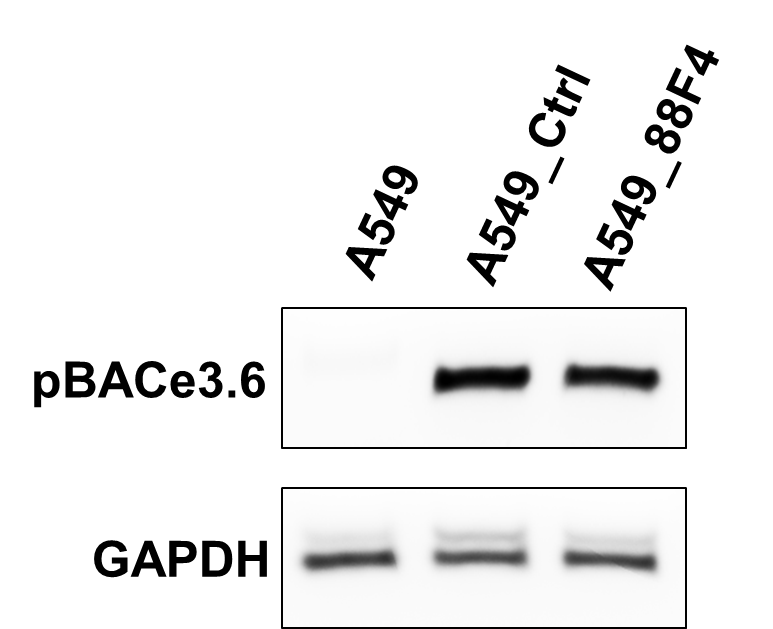
**Supplementary Table S3**

|  |  |  |  |
| --- | --- | --- | --- |
| **Cell Line** | **Driver Mutation** | **Observed Radiation Response** | **% Y Chromosome (+) Cells** |
| H460 | KRAS | Sensitive | 98% |
| H820 | EGFR | Sensitive | 100% |
| H1299 | NRAS | Resistant | 0% |
| A549 | KRAS | Resistant | 0% |
| WVU-Ma-0005 | Unknown | Sensitive | 100% |
| Ma-ALK-0001 | ALK Fusion | Resistant | 0% |

**Supplementary Table S3: Cell lines used in this study.**

Outline of the observed radiation response of each cell line used in this study color coded to correspond with Supplementary Fig. 2. Cytogenetic analysis and quantification was performed by the WVU Cytogenetics Laboratory in the Department of Pathology, Anatomy and Laboratory Medicine. The percentages shown are a result of the quantification of a population of 200 cells.

**Supplementary Figure S9**



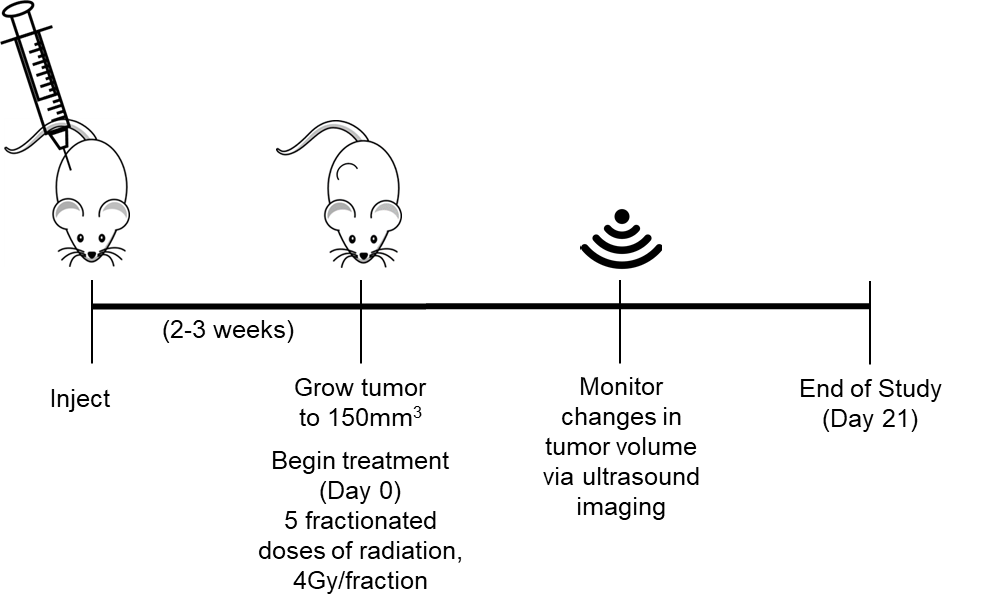
**Supplementary Figure S9: PCR of the pBACe3.6 plasmid backbone in A549 cells**

Lane 1: A549 Parental cells, Lane2: A549 cells nucleofected with the empty vector pBACe3.6, Lane 3: A549 cells nucleofected with the BAC clone RP11-88F4. GAPDH is provided as a loading control. DNA was extracted using the Thermo Scientific GeneJET Genomic DNA Purification Kit (#K0722) following the manufacturers protocol for adherent cells. PCR was run using the SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, 172-5271). PCR products were resolved on a 1% agarose gel with ethidium bromide.

|  |  |  |  |
| --- | --- | --- | --- |
| **Supplementary Table S4** | |  |  |
|  | |  |  |
| shRNA/siRNA Sequences | |  |  |
| **ID** | **Target Sequence** | **Hairpin oligo sequences** | |
| sh1 | GAAUCCAUUCCAUUAGAGGAC | FWD | CCGGGAATCCATTCCATTAGAGGACCTCGAGGTCCTCTAATGGAATGGATTCTTTTTG |
| linc-SPRY3-2 | REV | AATTCAAAAAGAATCCATTCCATTAGAGGACCTCGAGGTCCTCTAATGGAATGGATTC |
| sh2 | GCAUUCCAUUACAUUCUAUGA | FWD | CCGGGCATTCCATTACATTCTATGACTCGAGTCATAGAATGTAATGGAATGCTTTTTG |
| linc-SPRY3-3 | REV | AATTCAAAAAGCATTCCATTACATTCTATGACTCGAGTCATAGAATGTAATGGAATGC |
| For cloning:  AgeI/EcoRI |  | | |
|  | | | |
| **ID** | **Target Sequence** | **siRNA sequences** | |
| si-linc-SPRY3-2 | GAAUCCAUUCCAUUAGAGGAC | 5’-GTCCTCTAATGGAATGGATTC-3’ | |
| si-linc-SPRY3-3 | GCAUUCCAUUACAUUCUAUGA | 5’-TCATAGAATGTAATGGAATGC-3’ | |
| si-linc-SPRY3-4 | GAGUCCGUUCCAUAACACUCC | 5’-GGAGTGTTATGGAACGGACTC-3’ | |

|  |  |  |  |
| --- | --- | --- | --- |
|  |  |  |  |

**Supplementary Figure S10**



**C**

**B**

**A**

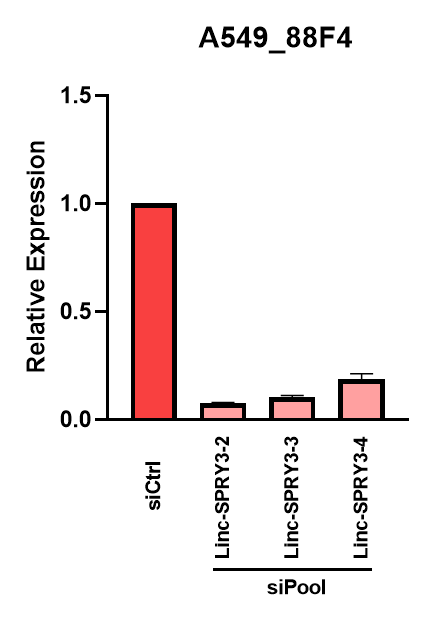


**D**

**E**

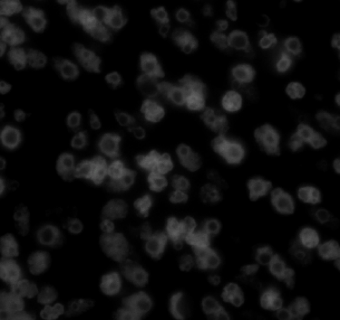
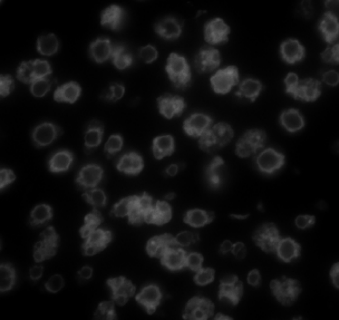
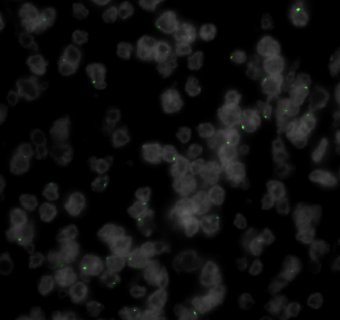
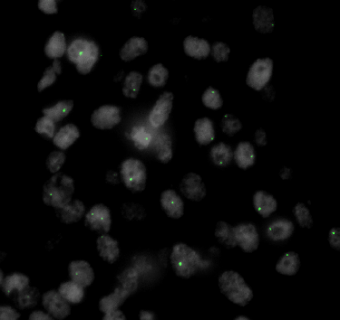
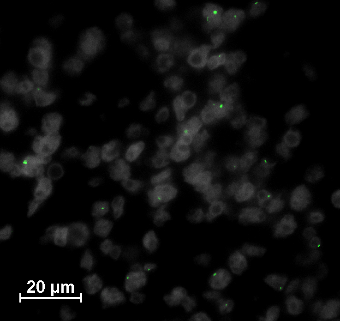
**Supplementary Figure S10: *In vivo* study scheme and untreated controls for the tumor growth delay assays. (A)** Schematic detailing the tumor growth delay assay**. (B and C)** Representative images of ultrasound measurements utilized in determining tumor volume and depth respectively. **(D)** Tumor growth delay assay with untreated controls for H460 shCtrl and sh1 cells. **(E)** Tumor growth delay assay with untreated controls for WVU-Ma-005 shCtrl and sh1 cells.

**Supplementary Figure S11**

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**Supplementary Figure S11: siRNA knockdown of linc-SPRY3 RNAs in A549 cells nucleofected with the BAC clone RP11-88F4.** Representative replicate from triplicate experiments. Error bars represent SD from the mean of 3 technical replicates.

**Supplementary Figure S12**

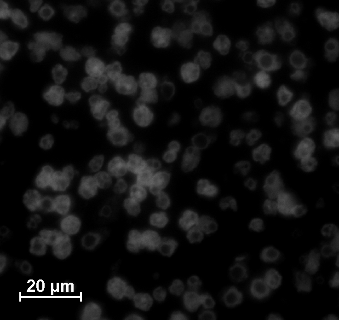


Male

Female

DAPI

DYZ1



DAPI

DYZ1

**A**

**B**

**C**

**D**

**E**

**F**

DAPI

DYZ1

DAPI

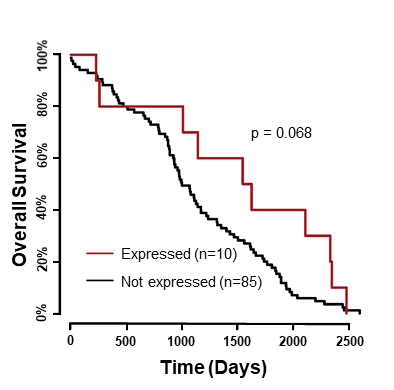
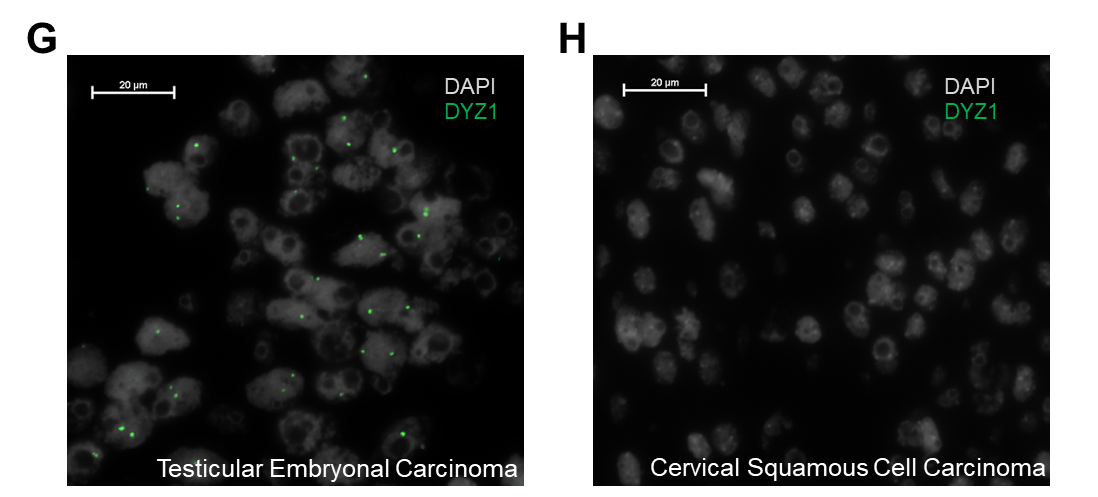
DYZ1

DAPI

DYZ1

DAPI

DYZ1

****

**I**

**Supplementary Figure S12: Representative images of DYZ1 fluorescent *in situ* hybridization in tissue microarrays. (A-C)** Images taken of male cores from the lung cancer tissue microarray. **(D-F)** Images taken of female cores from the lung cancer tissue microarray. **(G)** Image taken of a core from the testis tissue microarray as a positive control. **(H)** Image taken of a core from a cervical cancer tissue microarray as a negative control. **(I)** Survival curves for NSCLC male patients (GEO GSE81089) which express and do not express linc-SPRY3-2. P-value by log rank test.

**Ultra-Violet Radiation Crosslinking and Immunoprecipitation (CLIP)**

**Reagents**

**NET-2 Buffer (Nuclease-free) stored at 4C**

(150 mM NaCl; 0.05% NP-40; 50 mM Tris-HCl, pH7.4)  500 mL

2 M Sodium Chloride (NaCl) 37.5 mL

Surfact-Amps NP-40 (10% Nonidet P-40) 2.5 mL

1 M Tris, pH 7.4 (tris(hydroxymethyl)aminomethane) 25 mL

Bring volume up to 500 mL with Nuclease-free water, autoclave

Add Protease inhibitor and RNasin just before using

**Proteinase K Buffer (Nuclease-free)**

(50 mM Tris-Cl, pH 7.5, 5 mM EDTA, 1.5% SDS, 300 mM NaCl) 100mL

1 M Tris-Cl (Tris(hydroxymethyl)aminomethane hydrochloride), pH 7.5 5 mL

0.5 M EDTA (Ethylenediaminetetraacetic acid) 1 mL

10% SDS (sodium dodecyl sulfate) 15 mL

2 M NaCl (Sodium Chloride) 15 mL

Mix and bring volume up to 100 mL with Nuclease-free water; autoclave.

**BSA Solution (Nuclease-free) stored at 4**

(2 mg/mL) 100 mL

BSA (Bovine serum albumin) 200 mg

Mix and bring volume up to 100 mL with Nuclease-free water; autoclave.

**1X PXL (wash buffer made fresh)**

1x PBS (tissue culture grade; no Mg++, no Ca++) 9.89 mL

0.1% SDS (0.01 ml of 10% SDS in 0.99 ml water) 0.01 mL

0.5% sodium deoxycholate (5 mg in 1 ml water) 0.05 mL

0.5% NP-40 (4.7 ul in 1 ml water) 0.05 mL

**Protocol**

1. **72 hours before Day 1 (if using treated cells)**

* Treat cell lines with 8Gy radiation (6cm dishes)
* Let incubate for 72 hours before beginning.

1. **Day 1 – UV X-linking, BCA assay and Bead Prep (complete this for all samples)**

* Aspirate media from cells. Rinse with tissue culture grade PBS, remove, and replace PBS.
* UV irradiate using Stratalinker (On 🡪 energy 🡪 value 🡪 start) with the dish lid off. Can irradiate 2 6cm dishes at a time. MUST be on ice. Irradiate one time for 400 mJ/cm2, rotate the plate 90° and irradiate an additional 200 mJ/cm2.
* Collect suspension (scrape) for all dishes, pellet cells at 2500 rpm for 5 min at 4°.
* Resuspend pellet in (~3x dry volume) of tissue culture grade PBS; quick spin at 4°, remove supernatant and freeze pellets at -80° until use.
* Resuspend each tube of cross-linked lysate using 700 µl of 1X PXL + 300 µl of HALT protease.
* Add 15 µL RNAsin and let sit on ice for 10 min.
* Add 30 µl of RQ1 DNAse or DNAse 1 (Promega, M6101 or NEB) to each tube; incubate at 37° for 5 min, 1000 rpm.
* Supernatant was collected after 10 min centrifugation at 16,000Xg at 4 C
* Quantitate protein with BCA assay…100 ug of each lysate was used for IP

**\*Clip end of pipette tip before pipette beads**

* 600 µL Protein G-Sepharose beads in 1.5mL tube; wash beads (spin at 4° C for 3 min at 1000 rpm, then remove supernatant) 2 times with about 500 µL PXL buffer each wash (protein G beads are stored at 4°C).
* Block Protein G Sepharose beads with 900 uL of 2 mg/mL BSA for 1hr or overnight at 4°C (BSA stored at 4°C).

1. **Day 2 - Immunoprecipitation**

* Wash beads 2 times with 500uL PXL Buffer each time (Did not add RNasin/PI during the washes to save reagents). After last wash, add 600 µL of PXL, 6.0 µl HALT, and 15 µl RNasin.
* Measure out the volume of lysate (100 ug) determined from BCA assay. Divide supernatants into 2 1.5mL tubes (2 antibodies – IGF2BP3, Abcam #177942 and IgG, Abcam #172730).
* Preclear lysates by adding 90uL of blocked/washed beads to each lysate; incubate for 1hr at 4°C on rotator.
* Spin down precleared beads/lysate at 4°C 1,000 rpm for 3 min (low speed when working with beads).
* Transfer supernatant into new tubes and discard beads used for preclearing; bring up to equal volume (500 µl).
* Add desired antibody to remaining lysates (8 ug antibody/lysate). Incubate for 3 hrs at 4°C on rotator.
* Add 50uL of blocked/washed beads to each lysate/antibody tube; Incubate for 1hr at 4°C on rotator.
* Spin down lysate/antibody/beads at 4°C 1,000 rpm for 3 min (low speed when working with beads).
* Divide supernatant into 2 tubes (IP supernatant for RNA and protein).
* Wash pelleted beads 5 times with about 200 uL each wash with NET2-Buffer (Spin down beads at 4°C 1,000 rpm for 3 min. add the following volumes of RNasin to each wash:
  + Wash 1: 14 uL RNAsin
  + Wash 2: 14 uL RNasin
  + Wash 3: 6 uL RNasin
  + Wash 4: 3 uL RNasin
  + Wash 5: 3 uL RNasin
* Take 25% (125uL) of beads for Western Blot analysis -> Spin down beads at 4°C 1,000 rpm for 3 min.
  + Spin 🡪 remove supernatant 🡪 resuspend in 50 µl PXL buffer
  + Add 10 µL 5X LSB and boil at 100°C for 5 min to remove protein from beads
* Spin down remaining 75% (375uL) of beads at 4°C 1,000 rpm for 3 min. Incubate beads with Proteinase K Buffer (150ul) (w/ 300 mM NaCl) and 1.5mg/mL proteinase K (11.25uL of 20mg/mL) for 30 min at 50°C.
* Then incubate beads for 60 min at 70°C (Add 0.5 uL RNasin/sample).
* RNAs (from beads and supernatant) were recovered with phenol/chloroform extraction
  + Add same volume of Phenol-chloroform-isoamylalcohol/tube.
  + Vortex 20 seconds (10 up and 10 angled).
  + Centrifuge 10 minutes at 11,400 rpm at RT.
  + Transfer aqueous phase to another centrifuge tube w/ 50 µL of 3M Sodium Acetate (NaOAc), at pH 5.2.
  + Add 1 mL of 100% Ethanol (precooled to -20˚ C), fill to top of tube, Add 1 μL of GlycoBlue (15 μg/μL) Store at -20˚ C overnight or for a few days to increase yield.

1. **Day 3 – Finish RNA extraction**

* Spin down RNA for 15 minutes at 11,400 rpm in micro-centrifuge at 4˚C.
* Aspirate Ethanol (leave a little).
* Wash with 500 uL of 75% Ethanol (precooled to -20˚C); don’t shake just add.
* Spin down for 1 minute at 11,400 rpm.
* Aspirate Ethanol, remove any remaining with pipette.
* Re-suspend RNA pellet in 20 μL of Nuclease-Free water (Ambion).
* DNase treat samples.
* Collect supernatant.
* Quantify samples.
* Store RNA in -80 until use.