

Garza et al

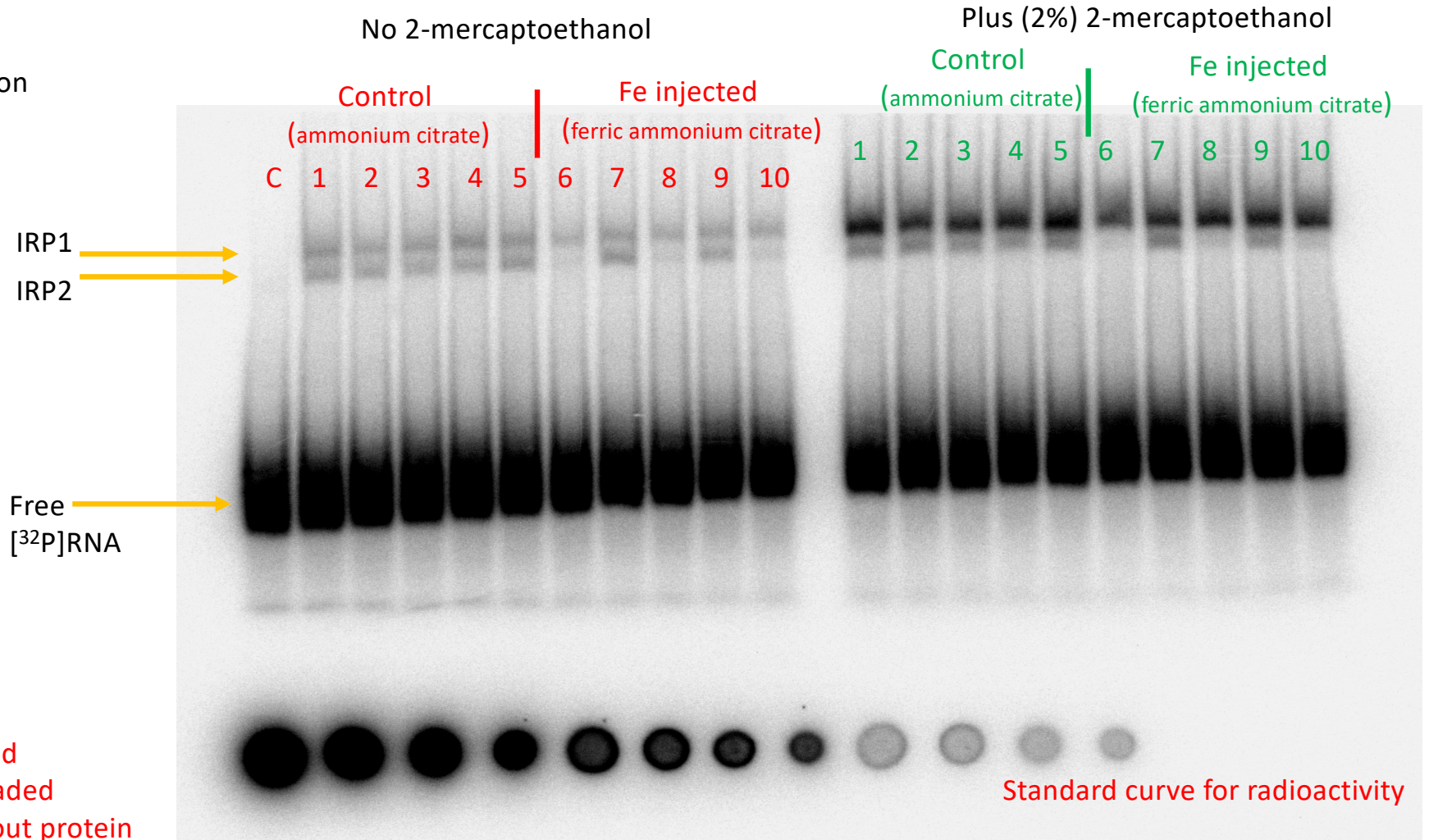
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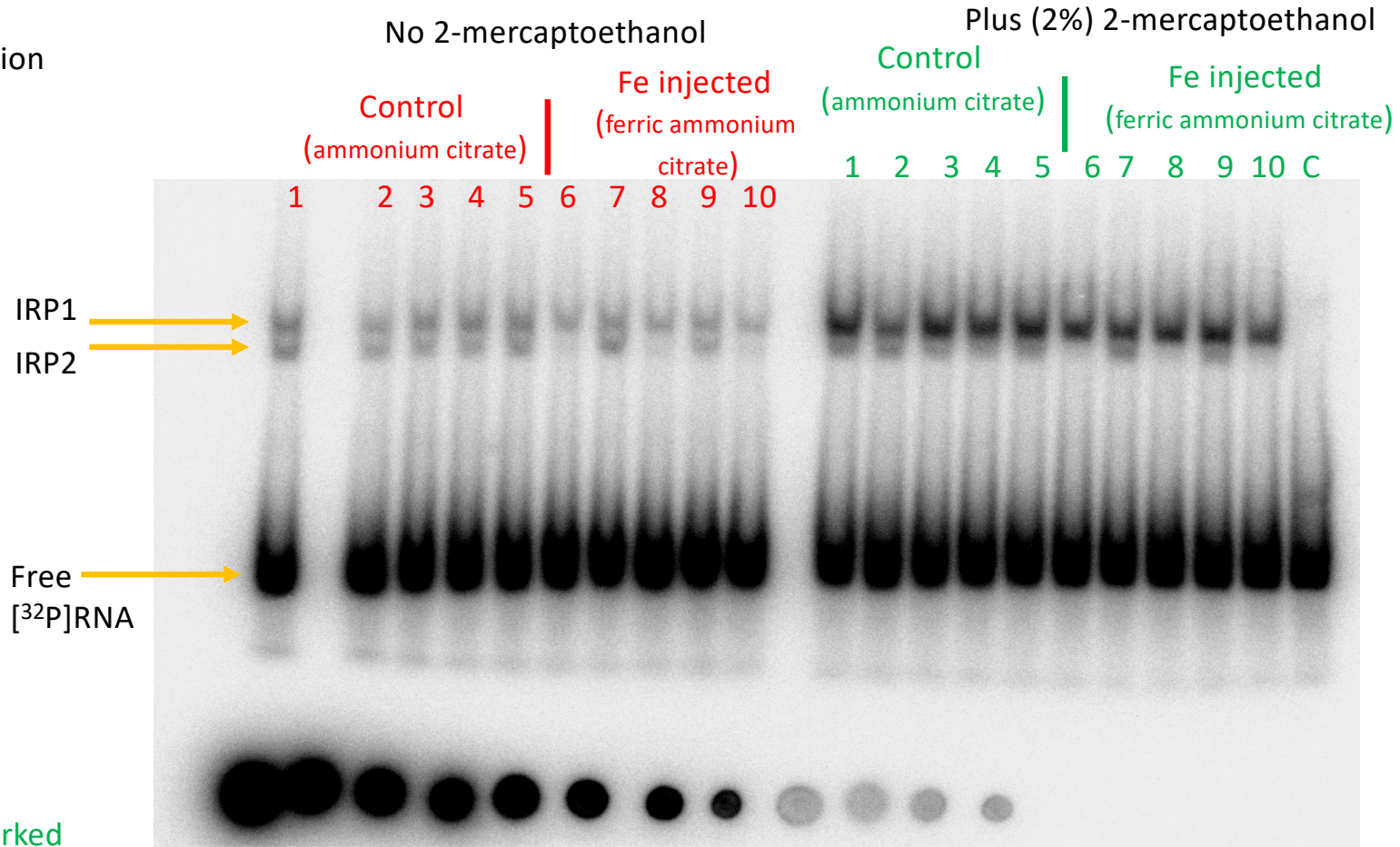
Iron injection  
04



Lane marked  
C is RNA loaded  
alone without protein

Standard curve for radioactivity

Iron injection  
o5



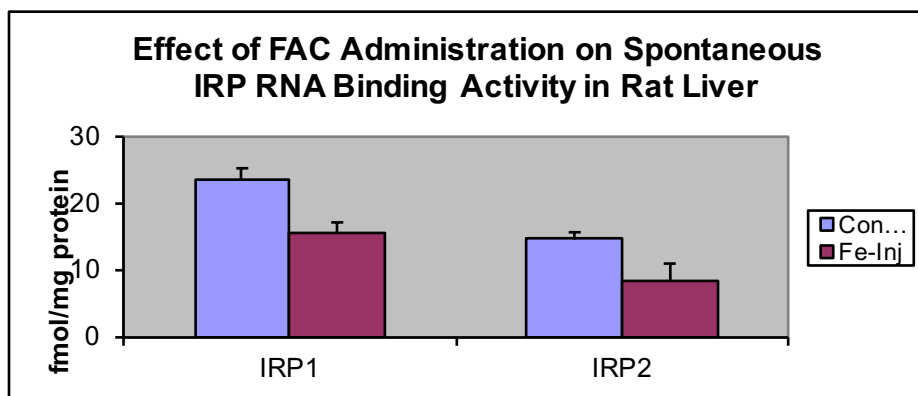
Lane marked  
C is RNA loaded  
alone without protein

Standard curve for radioactivity

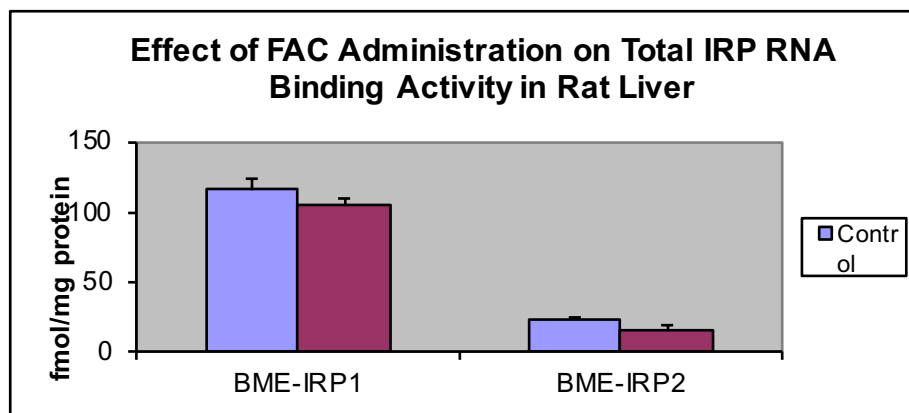
## Fe-Inj Summary

	Gel1-IRP1	Gel2-IRP1	Mean IRP1	Gel1-IRP2	Gel2-IRP2	Mean IRP2	Gel1-BMEIRP1
<b>1</b>	27.41	26.39	26.90	19.49	17.07	18.28	167.55
<b>3</b>	20.55	14.35	17.45	18.00	10.01	14.01	113.86
<b>5</b>	26.60	19.03	22.82	16.50	8.81	12.66	122.98
<b>7</b>	33.25	20.71	26.98	15.73	9.39	12.56	128.60
<b>9</b>	27.13	19.99	23.56	20.34	11.13	15.74	168.85
<b>2</b>	13.58	12.34	12.96	4.85	2.22	3.54	116.04
<b>4</b>	22.96	19.77	21.37	21.54	13.31	17.43	115.79
<b>6</b>	16.10	12.30	14.20	7.50	2.58	5.04	142.46
<b>8</b>	19.30	13.49	16.40	15.47	7.71	11.59	136.87
<b>10</b>	17.78	8.85	13.32	5.50	2.87	4.19	133.93

	IRP1	IRP2	SD IRP1	SD IRP2	SEM IRP1	SEM IRP2
Control	23.54	14.65	3.90	2.40	1.74	1.08
Fe-Inj	15.65	8.36	3.46	6.01	1.55	2.69
	BME-IRP1	BME-IRP2	SD IRP1	SD IRP2	SEM IRP1	SEM IRP2
Control	115.81	23.60	18.47	2.04	8.26	0.91
Fe-Inj	105.65	15.92	9.45	6.74	4.23	3.02

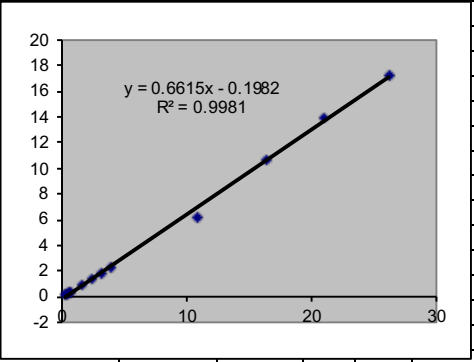


This is the so-called spontaneous IRP RNA binding activity - the active IRP in cells/tissues - as isolated. This is what we reported in the manuscript (but as the sum of IRP1 + IRP2).

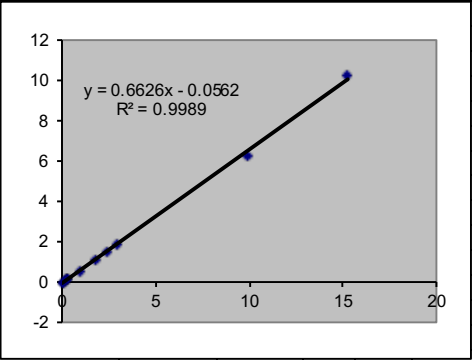


This is the 2-mercaptoethanol activated IRP binding activity

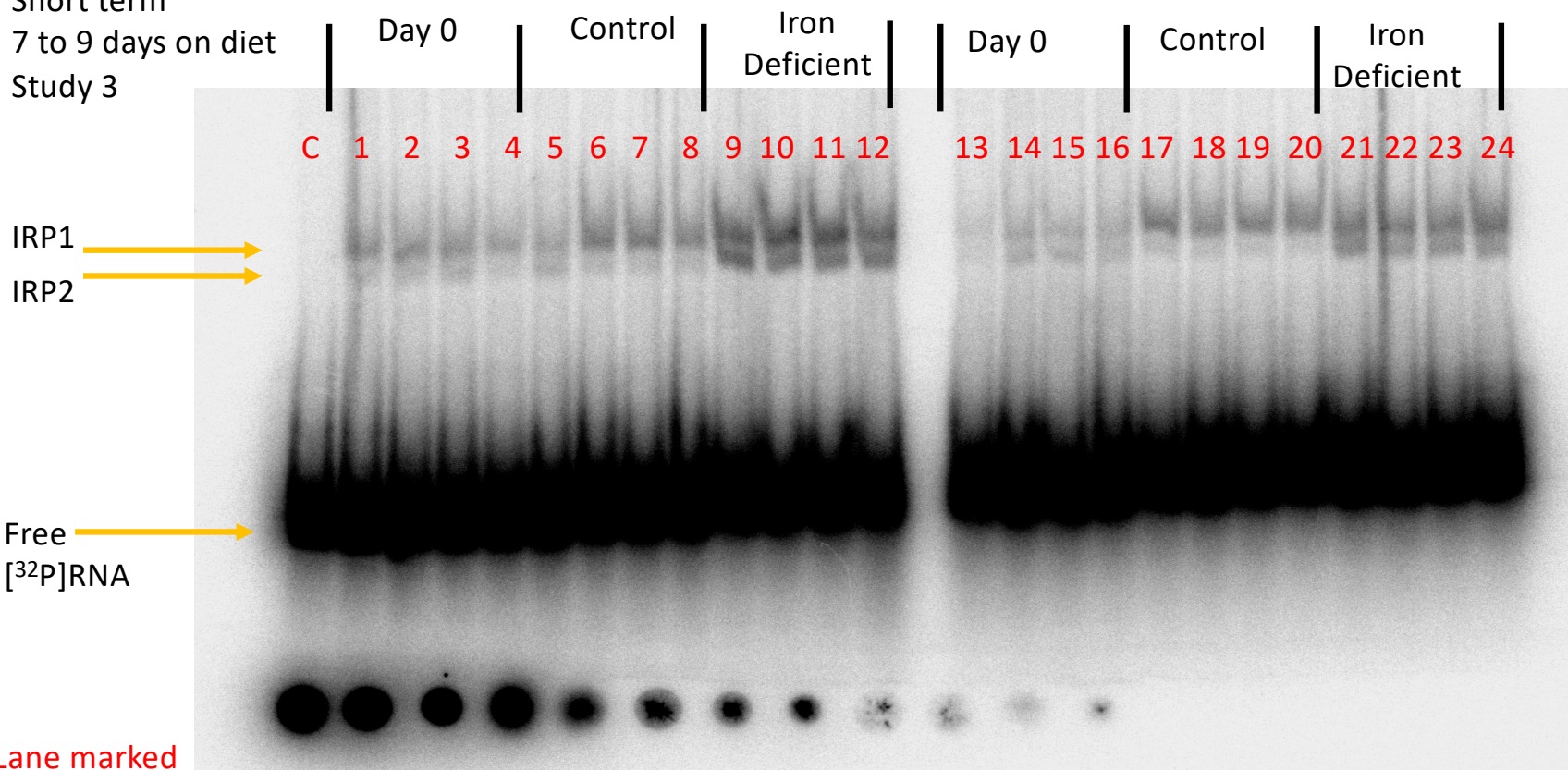
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<b>Date of Calibration</b>				1/10/03																	
<b>Date Counted</b>				1/14/03																	
<b>Difference</b>				4																	
<b>SA on Counting Date</b>				5476																	
	<b>CPM A</b>	<b>fmol</b>	<b>DLU</b>																		
	143295	26.169113	172887121																		
	114843	20.973093	138856952																		
	89850	16.408771	106342408																		
	59387	10.845494	62065056																		
	21583	3.941575	22934083																		
	17271	3.154100	18871786																		
	13250	2.419769	13716635																		
	8682	1.585542	8553843																		
	3465	0.632792	3417262																		
	2823	0.515548	2814772																		
	2068	0.377667	1867842																		
	1442	0.263344	1304548																		
<b>dlu/fmol</b>	<b>fmol/dlu</b>	<b>Actual fmol/dlu</b>																			
0.661512679	1.51169	1.51169E-07																			
<b>IRP1</b>																			7.58		
<b>Gross DLU</b>		<b>CF</b>	<b>Net DLU</b>	<b>Corrected DLU</b>	<b>fmol</b>	<b>fmol/mg</b>	<b>Mean</b>	<b>SD</b>	<b>SEM</b>	<b>2SD</b>	<b>Mean+2SD</b>										
<b>Fe-Inj04</b>		<b>67557147</b>																			
1	68458379	0.987	1391784	1373462	0.2076244	27.41	26.99	4.5	2.0	9.0	36.0										
3	69206004	0.976	1055175	1030035	0.1557090	20.55															
5	65059762	1.038	1283612	1332885	0.2014904	26.60															
7	69411838	0.973	1711928	1666185	0.2518750	33.25															
9	68696170	0.983	1382331	1359411	0.2055004	27.13															
2	67522781	1.001	680349	680695	0.1028998	13.58	17.94	3.5	1.6	7.0	25.0										
4	67171703	1.006	1144010	1150575	0.1739308	22.96															
6	66797808	1.011	797928	806999	0.1219929	16.10															
8	68253525	0.990	977119	967150	0.1462027	19.30															
10	64993497	1.039	857151	890961	0.1346854	17.78															
<b>IRP2</b>																			7.6		
<b>Gross DLU</b>		<b>CF</b>	<b>Net DLU</b>	<b>Corrected DLU</b>	<b>fmol</b>	<b>fmol/mg</b>	<b>Mean</b>	<b>SD</b>	<b>SEM</b>	<b>2SD</b>	<b>Mean+2SD</b>										
<b>Fe-Inj04</b>		<b>67557147</b>																			
1	68458379	0.987	989884	976852	0.1476695	19.49	18.01	1.9	0.9	3.9	21.9										
3	69206004	0.976	923848	901837	0.1363295	18.00															
5	65059762	1.038	796258	826823	0.1249898	16.50															
7	69411838	0.973	809936	788294	0.1191654	15.73															
9	68696170	0.983	1036414	1019230	0.1540756	20.34															
2	67522781	1.001	243135	243259	0.0367731	4.85	10.97	7.3	3.3	14.5	25.5										
4	67171703	1.006	1073367	1079526	0.1631905	21.54															
6	66797808	1.011	371504	375727	0.0567982	7.50															
8	68253525	0.990	783296	775304	0.1172017	15.47															
10	64993497	1.039	265012	275465	0.0416417	5.50															
<b>IRP1+2%BME</b>																			7.6		
<b>Gross DLU</b>		<b>CF</b>	<b>Net DLU</b>	<b>Corrected DLU</b>	<b>fmol</b>	<b>fmol/mg</b>	<b>Mean</b>	<b>SD</b>	<b>SEM</b>	<b>2SD</b>	<b>Mean+2SD</b>										
<b>Fe-Inj04</b>		<b>65005981</b>																			
1	62024445	1.048	8011777	8396906	1.2693492	167.55	140.37	26.0	11.6	51.9	192.3										
3	66384453	0.979	5827004	5706006	0.8625695	113.86															
5	62803487	1.035	5954109	6162917	0.9316401	122.98															
7	64242019	1.012	6368878	6444616	0.9742242	128.60															
9	69349809	0.937	9027274	8461837	1.2791648	168.85															
2	60428467	1.076	5405703	5815190	0.8790746	116.04	129.02	12.3	5.5	24.7	153.7										
4	68423402	0.950	6108062	5802994	0.8772309	115.79															
6	65326467	0.995	7174755	7139556	1.0792773	142.46															
8	68812305	0.945	7260664	6859043	1.0368726	136.87															
10	62264958	1.044	6428985	6712001	1.0146444	133.93															
<b>IRP2+2%BME</b>																			7.6		
<b>Gross DLU</b>		<b>CF</b>	<b>Net DLU</b>	<b>Corrected DLU</b>	<b>fmol</b>	<b>fmol/mg</b>	<b>Mean</b>	<b>SD</b>	<b>SEM</b>	<b>2SD</b>	<b>Mean+2SD</b>										
<b>Fe-Inj04</b>		<b>65005981</b>																			
1	62024445	1.048	1562395	1637500	0.2475387	32.68	30.38	2.6	1.2	5.2	35.6										
3	66384453	0.979	1600880	1567638	0.2369777	31.28															
5	62803487	1.035	1278166	1322991	0.1999948	26.40															
7	64242019	1.012	1446455	1463656	0.2212590	29.21															
9	69349809	0.937	1728011	1619774	0.2448592	32.32															
2	60428467	1.076	637155	685420	0.1036141	13.68	19.25	8.1	3.6	16.2	35.4										
4	68423402	0.950	1541654	1464656	0.2214101	29.23															
6	65326467	0.995	733103	729506	0.1102785	14.56															
8	68812305	0.945	1419683	1341154	0.2027405	26.76															
10	62264958	1.044	577593	603020	0.0911577	12.03															



<b>SA on RNA Calibration Date</b>				6647													
<b>Date of Calibration</b>				1/10/03													
<b>Date Counted</b>				1/14/03													
<b>Difference</b>				4													
<b>SA on Counting Date</b>				5476													
	<b>CPM A</b>		<b>fmol</b>		<b>DLU</b>												
<b>OMIT</b>			0.000000			0.00000000											
<b>OMIT</b>			0.000000			0.00000000											
	83581		15.263901		102453640	10.24536400											
	54107		9.881239		62412691	6.24126910											
	15863		2.896965		18244276	1.82442760											
	13261		2.421777		14735858	1.47358580											
	10052		1.835737		10871248	1.08712480											
	5180		0.945993		5653438	0.56534380											
	1647		0.300782		1727349	0.17273490											
	1222		0.223167		1255061	0.12550610											
	1144		0.208922		1165761	0.11657610											
	747		0.136420		779379	0.07793790											
<b>dlu/fmol</b>	<b>fmol/dlu</b>	<b>Actual fmol/dlu</b>															
0.662574683	1.50926	1.50926E-07															
<b>IRP1</b>											7.58						
	<b>Gross DLU</b>	<b>CF</b>		<b>Net DLU</b>	<b>Corrected DLU</b>	<b>fmol</b>	<b>fmol/mg</b>	<b>Mean</b>	<b>SD</b>	<b>SEM</b>	<b>2SD</b>	<b>Mean+2SD</b>					
<b>Fe-Inj05</b>	<b>36955107</b>																
1	32933607		1.122	1180330	1324459	0.1998958	26.39	20.09	4.3	1.9	8.6	28.7					
3	35007818		1.056	682576	720544	0.1087491	14.35										
5	36636811		1.009	947070	955298	0.1441797	19.03										
7	38303222		0.965	1077668	1039739	0.1569240	20.71										
9	40173956		0.920	1090687	1003298	0.1514242	19.99										
2	39377518		0.938	660202	619588	0.0935122	12.34	13.35	4.0	1.8	8.0	21.3					
4	33251510		1.111	893002	992466	0.1497893	19.77										
6	37629268		0.982	628803	617537	0.0932027	12.30										
8	41183551		0.897	754616	677137	0.1021979	13.49										
10	35053806		1.054	421365	444220	0.0670445	8.85										
<b>IRP2</b>																	
	<b>Gross DLU</b>	<b>CF</b>		<b>Net DLU</b>	<b>Corrected DLU</b>	<b>fmol</b>	<b>fmol/mg</b>	<b>Mean</b>	<b>SD</b>	<b>SEM</b>	<b>2SD</b>	<b>Mean+2SD</b>					
<b>Fe-Inj05</b>	<b>36955107</b>																
1	32933607		1.122	763409	856628	0.1292878	17.07	11.28	3.3	1.5	6.7	18.0					
3	35007818		1.056	476017	502495	0.0758398	10.01										
5	36636811		1.009	438211	442018	0.0667122	8.81										
7	38303222		0.965	488435	471244	0.0711232	9.39										
9	40173956		0.920	607373	558709	0.0843239	11.13										
2	39377518		0.938	118679	111378	0.0168099	2.22	5.74	4.8	2.1	9.6	15.3					
4	33251510		1.111	601230	668196	0.1008484	13.31										
6	37629268		0.982	131741	129381	0.0195270	2.58										
8	41183551		0.897	431292	387010	0.0584100	7.71										
10	35053806		1.054	136754	144171	0.0217593	2.87										
<b>IRP1+2%BME</b>											7.6						
	<b>Gross DLU</b>	<b>CF</b>		<b>Net DLU</b>	<b>Corrected DLU</b>	<b>fmol</b>	<b>fmol/mg</b>	<b>Mean</b>	<b>SD</b>	<b>SEM</b>	<b>2SD</b>	<b>Mean+2SD</b>					
<b>Fe-Inj05</b>	<b>40108411</b>																
1	40868496		0.981	4891587	4800612	0.7245390	95.64	91.25	17.2	7.7	34.4	125.7					
3	36905960		1.087	2808680	3052398	0.4606874	60.81										
5	41219126		0.973	5283735	5141356	0.7759663	102.43										
7	37930787		1.057	4762745	5036176	0.7600919	100.33										
9	42336674		0.947	5140269	4869726	0.7349702	97.02										
2	41880666		0.958	3797956	3637239	0.5489553	72.46	82.28	8.5	3.8	17.1	99.4					
4	39202849		1.023	4014254	4106981	0.6198518	81.82										
6	40296154		0.995	4429514	4408877	0.6654158	87.83										
8	41540129		0.966	4854928	4687599	0.7074823	93.39										
10	38903271		1.031	3694976	3809438	0.5749448	75.89										
<b>IRP2+2%BME</b>																	
	<b>Gross DLU</b>	<b>CF</b>		<b>Net DLU</b>	<b>Corrected DLU</b>	<b>fmol</b>	<b>fmol/mg</b>	<b>Mean</b>	<b>SD</b>	<b>SEM</b>	<b>2SD</b>	<b>Mean+2SD</b>					
<b>Fe-Inj05</b>	<b>40108411</b>																
1	40868496		0.981	881754	865355	0.1306049	17.24	16.81	2.1	0.9	4.2	21.0					
3	36905960		1.087	929108	1009730	0.1523949	20.12										
5	41219126		0.973	754006	733688	0.1107329	14.62										
7	37930787		1.057	777694	822342	0.1241131	16.38										
9	42336674		0.947	832116	788320	0.1189783	15.71										
2	41880666		0.958	425245	407250	0.0614648	8.11	12.58	5.5	2.4	10.9	23.5					
4	39202849		1.023	963108	985355	0.1487161	19.63										
6	40296154		0.995	438559	436516	0.0658817	8.70										
8	41540129		0.966	900066	869044	0.1311617	17.31										
10	38903271		1.031	445293	459087	0.0692884	9.15										

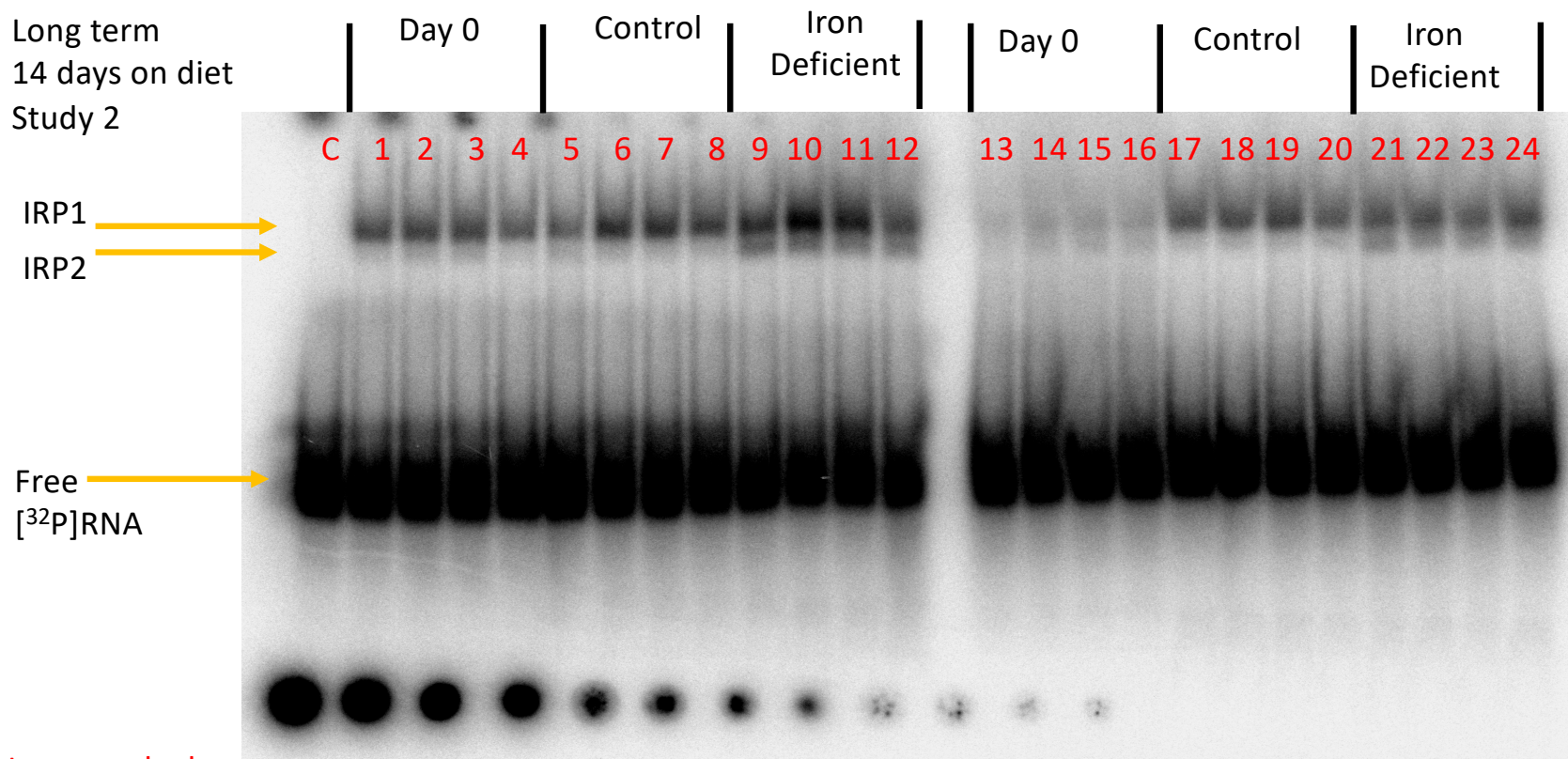


GS3  
Short term  
7 to 9 days on diet  
Study 3



Lane marked C is RNA loaded alone without protein Standard curve for radioactivity

GS4  
Long term  
14 days on diet  
Study 2



Lane marked C is RNA loaded alone without protein

Standard curve for radioactivity







RNA BA Results (fmol of RNA bound/mg protein)		
<b>Short-Term</b>		
<b>Spontaneous</b>		
Sample	Mean	SEM
Day0	73.8	6.5
Control	79.8	7.4
ID	210.8	11.5
<b>Long-Term</b>		
<b>Spontaneous</b>		
Sample	Mean	SEM
Day0	43.7	7.5
Control	82.6	6.5
ID	123.8	6.4
<b>Short-Term</b>		
<b>Total</b>		
Sample	Mean	SEM
Day0	296.3	23.4
Control	314.5	43.2
ID	524.7	51.8
<i>* This was very repeatable</i>		
<b>Long-Term</b>		
<b>Total</b>		
Sample	Mean	SEM
Day0	111.4	15.8
Control	315.6	18.5
ID	324.6	13.1

These results are the so-called spontaneous RNA binding activity - the active IRP in cells as isolated. This is what we report in the manuscript (as the sum of IRP1 + IRP2

These results are for the 2-mercaptoethanol activated binding