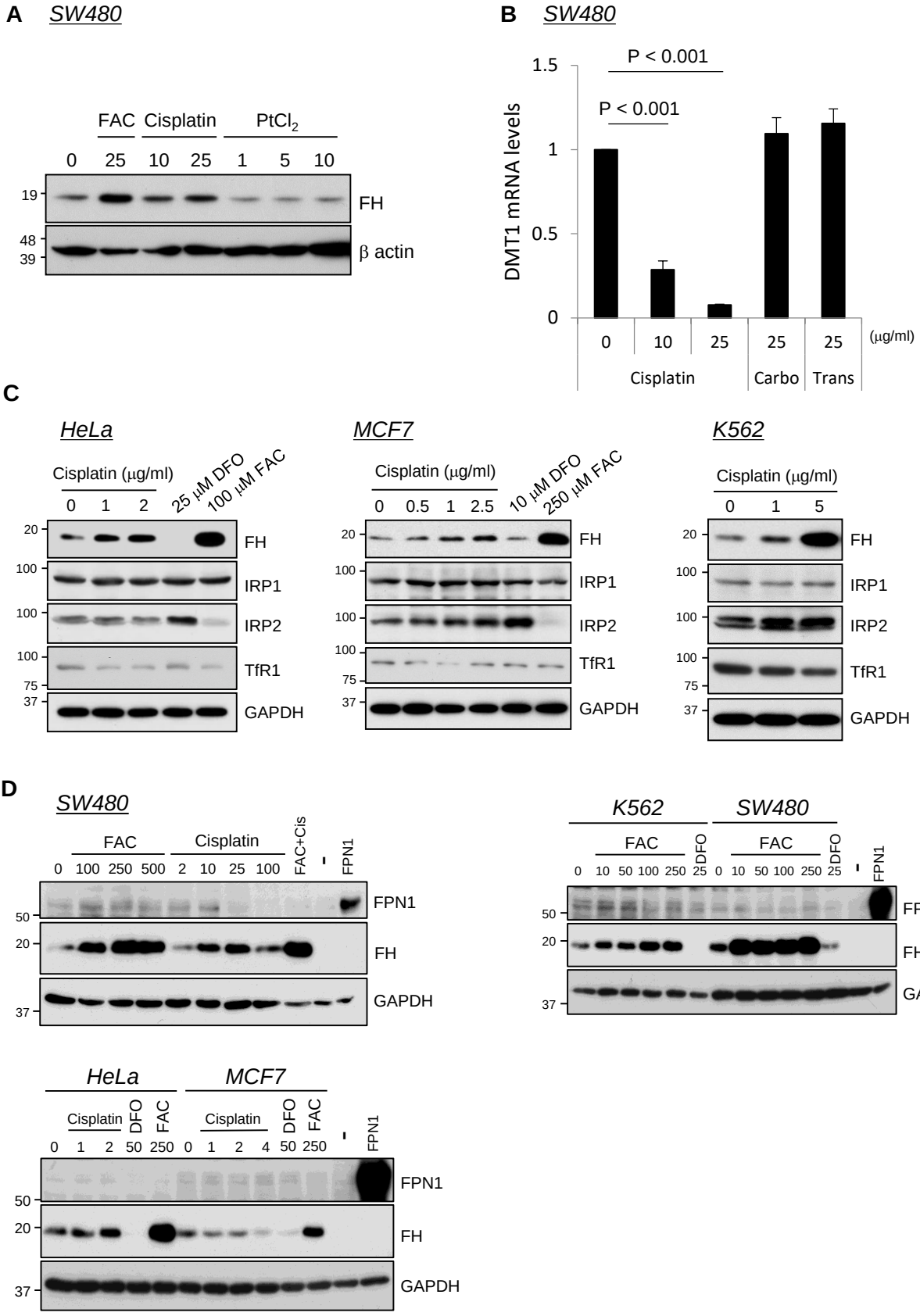

Figure S1. Related to Figure 1

M. Miyazawa et al.

Legend

Figure S1. Related to Figure 1

A) SW480 cells were treated with ferric ammonium citrate (FAC, μM), cisplatin or $PtCl_2$ (μg/mL) at the indicated concentrations for 24 h. Ferritin H (FH) protein expression was measured by Western blotting using whole cell lysates. β actin was used as a protein loading control. **B)** SW480 cells were treated with cisplatin, carboplatin (Carbo), or transplatin (Trans) for 24 h. DMT1 mRNA expression was measured by qPCR, normalized by GAPDH mRNA. Means ± SD are shown (n = 3). **C)** HeLa, MCF7, and K562 cells were treated with cisplatin, DFO, or FAC at the indicated concentrations for 24 h (HeLa and K562) or 44 h (MCF7), and ferritin H (FH), IRP1, IRP2, TfR1 and GAPDH protein levels were measured by Western blotting using whole cell lysates. **D)** (top left) SW480 cells were treated with FAC, cisplatin, or FAC (250 μM) plus cisplatin (10 μg/ml) for 18 h. Expression of ferroportin (FPN1), FH and GAPDH was measured by Western blotting using whole cell lysates. K562 and SW480 (top right) or HeLa and MCF7 cells (bottom) were treated with cisplatin (μg/ml), FAC (μM), or DFO (μM) at the indicated concentrations for 24 h and expression of FPN1, FH, and GAPDH was measured by Western blotting using whole cell lysates. Whole cell lysates from HEK293 cells transfected with pCMV empty vector (-) and pCMVferroportin1 (FPN1) were loaded together as controls of FPN1 Western blotting.

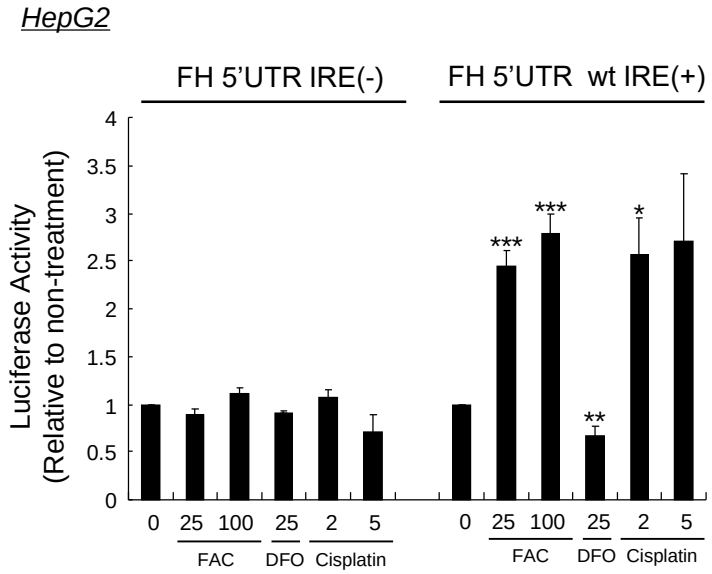

Figure S2. Related to Figure 1

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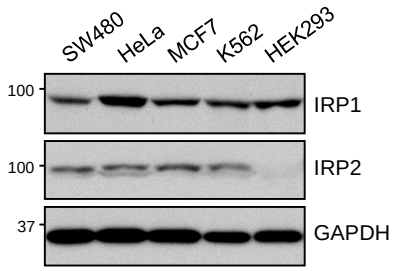
Legend

Figure S2. Related to Figure 1

Plasmid containing 5'-human ferritin H UTR with IRE [FH 5' UTR IRE(+)] or without IRE [FH 5'UTR IRE(-)] fused to firefly luciferase was transfected into HepG2 cells. The day after transfection, cells were treated with 0, 25, 100 µM FAC, 25 µM DFO, or 2, 5 µg/mL cisplatin for 12-17 h, and luciferase expression was measured. Means ± SD are shown (n = 3-6). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. untreated cells.



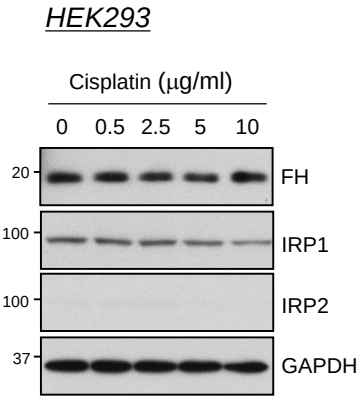


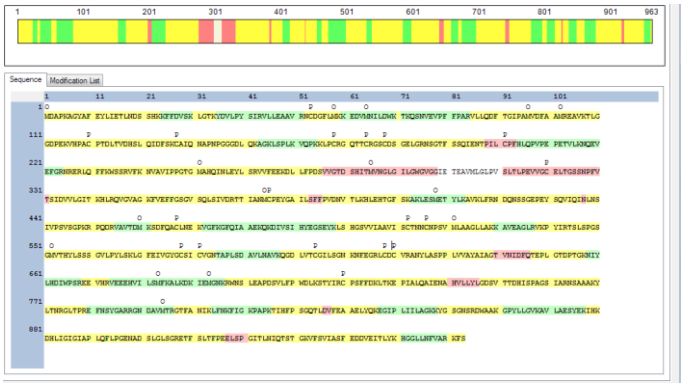
Figure S3. Related to Figure 2

M. Miyazawa et al.

Figure S3. Related to Figure 2

Whole cell lysates prepared from untreated SW480, HeLa, MCF7, K562, or HEK293 cells were used for IRP1, IRP2, and GAPDH Western blots (left). HEK293 cells were treated with cisplatin at the indicated concentrations for 24 h and whole cell lysates were subjected to Western blots for ferritin H (FH), IRP1, IRP2 and GAPDH (right).

A

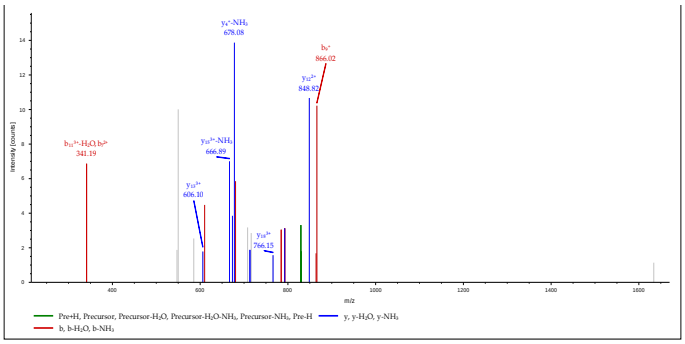


C

#1	b-H$_2$O$^+$	b-H$_2$O^{2+}	b-H$_2$O^{3+}	b-NH$_3^+$	b-NH$_3^{2+}$	b-NH$_3^{3+}$	Seq.	y-H$_2$O$^+$	y-H$_2$O^{2+}	y-H$_2$O^{3+}	y-NH$_3^+$	y-NH$_3^{2+}$	y-NH$_3^{3+}$	#2
1							L							20
2	183.11281	92.06004	61.70912				S	2359.93101	1180.46914	787.31519	2360.91502	1180.96115	787.64319	19
3	320.17172	160.58950	107.39543				H	2272.89898	1136.95313	758.30451	2273.88299	1137.44513	758.63252	18
4	377.19315	189.10023	126.40258				G	2135.84007	1068.42367	712.61821	2136.82408	1068.91568	712.94621	17
5	464.22522	232.61625	155.41326				S	2078.81860	1039.91294	693.61105	2079.80261	1040.40494	693.93908	16
6	563.29364	282.15046	188.43607				V	1991.78657	996.39692	664.60037	1992.77058	996.88893	664.92838	15
7	662.36206	331.68467	221.45887				V	1892.71815	946.86271	631.57757	1893.70216	947.35472	631.90557	14
8	775.44613	388.22670	259.15356				I	1793.64973	897.32850	598.55476	1794.63374	897.82051	598.88277	13
9	846.48325	423.74526	282.83260				A	1680.56566	840.78647	560.86007	1681.54967	841.27847	561.18808	12
10	917.52037	459.26382	306.51164				A	1609.52854	805.26791	537.18103	1610.51255	805.75991	537.50904	11
11	1016.58878	508.79803	339.53445				V	1538.49142	769.74935	513.50196	1539.47543	770.24135	513.83000	10
12	1129.67286	565.34007	377.22914				I	1439.42300	720.21514	480.47918	1440.40701	720.70714	480.80719	9
13	1216.70485	608.85608	406.23982				S	1326.33893	663.67310	442.78449	1327.32294	664.16511	443.11250	8
14	1565.74008	783.37368	522.58488				C-PT	1239.30696	620.15705	413.77382	1240.29091	620.64905	414.10182	7
15	1666.78776	833.89752	556.26744				T	890.27171	445.63949	297.42875	891.25572	446.13150	297.75676	6
16	1780.83069	890.91898	594.28175	1781.81471	891.41099	594.60975	N				790.20804	395.60766	264.07420	5
17	1894.87362	947.94045	632.29606	1895.85764	948.43246	632.62408	N				676.16511	338.58619	226.05989	4
18	2243.90881	1122.45804	748.64112	2244.89283	1122.95005	748.96913	C-PT				562.12218	281.56473	188.04588	3
19	2357.95174	1179.47951	786.65543	2358.93576	1179.97152	786.98344	N				213.08699	107.04713	71.70052	2
20							P							1

B

Fragment Mass Spectrum (Sequence:LSHGSVVIAAVISCTNNCNP)

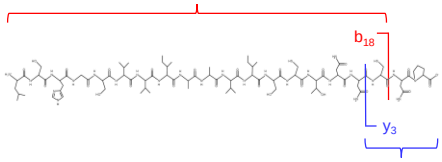


#1	b$^+$	b^{2+}	b^{3+}	Seq.	y$^+$	y^{2+}	y^{3+}	#2
1	114.09135	57.54931	38.70197	L				20
2	201.12338	101.06533	67.71264	S	2377.94157	1189.47442	793.31871	19
3	338.18229	169.59478	113.39895	H	2290.90954	1145.95841	764.30803	18
4	395.20376	198.10552	132.40610	G	2153.85063	1077.42895	718.62173	17
5	482.23579	241.62153	161.41678	S	2096.82916	1048.91822	699.61457	16
6	581.30421	291.15574	194.43959	V	2009.79713	1005.40220	670.60389	15
7	680.37263	340.68995	227.46239	V	1910.72871	955.86799	637.58109	14
8	793.45670	397.23199	265.15708	I	1811.66029	906.33378	604.55828	13
9	864.49382	432.75055	288.83612	A	1698.57622	849.79175	566.86359	12
10	935.53094	468.26911	312.51516	A	1627.53910	814.27319	543.18455	11
11	1034.59936	517.80332	345.53797	V	1556.50198	778.75463	519.50551	10
12	1147.68343	574.34535	383.23266	I	1457.43356	729.22042	486.48270	9
13	1234.71546	617.86137	412.24334	S	1344.34949	672.67838	448.78801	8
14	1583.75065	792.37896	528.58840	C-PT	1257.31746	629.16237	419.77734	7
15	1684.79833	842.90280	562.27096	T	908.28227	454.64477	303.43227	6
16	1798.84126	899.92427	600.28527	N	807.23459	404.12093	269.74971	5
17	1912.88419	956.94573	638.29958	N	693.19166	347.09947	231.73540	4
18	2261.91938	1131.46333	754.64464	C-PT	579.14873	290.07800	193.72109	3
19	2375.96231	1188.48479	792.65895	N	230.11354	115.56041	77.37603	2
20				P	116.07061	58.53894	39.36172	1

D

[2016.90066 (fragment) – 18.01058 (loss of H_2O)] + 246.02600 ($Pt(NH_3)_2(H_2O)$)

= 2244.91608 / 2 (charge state) = 1122.45804



[334.13000 (fragment) – 17.02654 (loss of NH_3)] + 246.02600 ($Pt(NH_3)_2(H_2O)$)

= 563.12946 / 2 (charge state) = 281.56473

E

Mathematical Representation of the Addition of $Pt(NH_3)_2(H_2O)$ to C^{516}

Ion Series Table

#1	b$^+$	b^{2+}	b^{3+}	Seq.	y$^+$	y^{2+}	y^{3+}	#2
1	114.09135	57.54931	38.70197	L				20
2	201.12338	101.06533	67.71264	S	2377.94157	1189.47442	793.31871	19
3	338.18229	169.59478	113.39895	H	2290.90954	1145.95841	764.30803	18
4	395.20376	198.10552	132.40610	G	2153.85063	1077.42895	718.62173	17
5	482.23579	241.62153	161.41678	S	2096.82916	1048.91822	699.61457	16
6	581.30421	291.15574	194.43959	V	2009.79713	1005.40220	670.60389	15
7	680.37263	340.68995	227.46239	V	1910.72871	955.86799	637.58109	14
8	793.45670	397.23199	265.15708	I	1811.66029	906.33378	604.55828	13
9	864.49382	432.75055	288.83612	A	1698.57622	849.79175	566.86359	12
10	935.53094	468.26911	312.51516	A	1627.53910	814.27319	543.18455	11
11	1034.59936	517.80332	345.53797	V	1556.50198	778.75463	519.50551	10
12	1147.68343	574.34535	383.23266	I	1457.43356	729.22042	486.48270	9
13	1234.71546	617.86137	412.24334	S	1344.34949	672.67838	448.78801	8
14	1583.75065	792.37896	528.58840	C-PT	1257.31746	629.16237	419.77734	7
15	1684.79833	842.90280	562.27096	T	908.28227	454.64477	303.43227	6
16	1798.84126	899.92427	600.28527	N	807.23459	404.12093	269.74971	5
17	1912.88419	956.94573	638.29958	N	693.19166	347.09947	231.73540	4
18	2261.91938	1131.46333	754.64464	C-PT	579.14873	290.07800	193.72109	3
19	2375.96231	1188.48479	792.65895	N	230.11354	115.56041	77.37603	2
20				P	116.07061	58.53894	39.36172	1

Difference between amino b_{17} and b_{18}:

2261.91938 – 1912.88419 =

349.03519 (addition of C-PT)

Difference between amino y_2 and y_3:

579.14873 – 230.11354 =

349.03519 (addition of C-PT)

Figure S4. Related to Figure 3

M. Miyazawa et al.

Legend

Figure S4. Related to Figure 3

A) The image view of IRP2 sequence coverage. Highlighted amino acid sequences are covered by LC-MS/MS analysis. Green, yellow, and red highlights are high, medium, and low confidence ID's, respectively. **B)** The fragment mass spectrum for the peptide L^{499}-P^{518} (LSHGSVVIAAVISCTNNCNP). The table indicates the mass of the b and y ions for the peptide. The colored numbers are the ions identified in the fragment mass spectrum (top), b ions in red and y ions in blue. **C)** The table shows the mass with a loss of water or ammonia of the b and y ions for the peptide L^{499}-P^{518} (LSHGSVVIAAVISCTNNCNP). The colored numbers are ions identified on the spectrum. **D)** Platination of Cys516 was manually confirmed by the calculation based on b_{18}-H_2O^{2+} or y_3-NH_3^{2+} ion. **E)** Mathematical representation of the addition of $Pt(NH_3)_2(H_2O)$ to Cys516. The ion series table shows the mass of b and y ions for the peptide L^{499}-P^{518} (LSHGSVVIAAVISCTNNCNP). The colored numbers are ions identified on the spectrum view. The red and blue numbers are b and y ions, respectively.

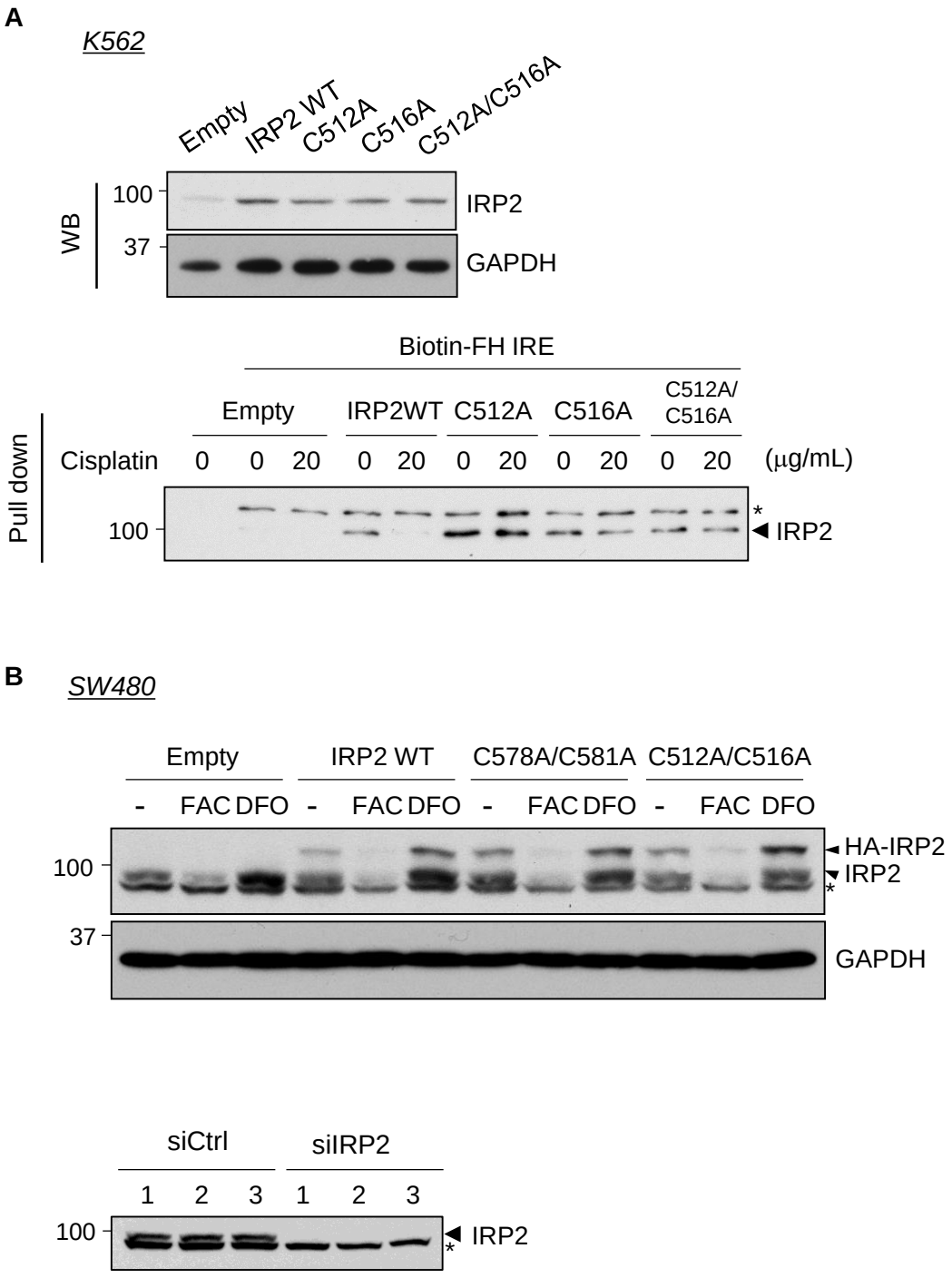


Figure S5. Related to Figure 4

M. Miyazawa et al.

Legend

Figure S5. Related to Figure 4

A) Non-tagged full length human IRP2 wt and Cys to Ala mutants (C512A, C516A, C512A/C516A) in pcDNA3.1 plasmids were transiently-transfected into K562 cells and IRP2 was detected by Western blotting with an IRP2 antibody (top). GAPDH was used as a loading control (WB). These cell lysates were incubated with 20 µg/mL cisplatin and subjected to pull down assay using biotin-FH IRE probe (bottom). IRP2 binding ability to IRE was detected by Western blot with anti-IRP2 antibody (Pull down). * indicates a non-specific band as we observed and verified in Fig. 2D. **B)** IRP2-transfected SW480 cells were treated with 250 µM FAC or 100 µM DFO for 21 h and Western blots were performed using IRP2 and GAPDH antibodies (top). Control (siCtrl) or IRP2 siRNA (siIRP2) was transfected into SW480 cells (triplicate) and IRP2 protein was measured by Western blotting. * indicates a non-specific band as we also observed in Fig. 6F (bottom).

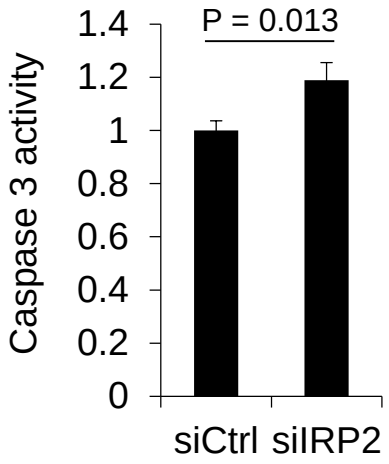


Figure S6. Related to Figure 6

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Legend

Figure S6. Related to Figure 6

siControl (siCtrl) or IRP2 siRNA (siIRP2) was transfected into SW480 cells for 48h, and caspase 3 activity was measured. Means ± SD are shown (n = 3).

Supplement Table 1, Related to STAR Methods

Primers for real time PCR		
ferritin H	Forward	5'-ACTGATGAAGCTGCAGAACC-3'
	Reverse	5'-GTCACCCAATTCTTTGATGG-3'
TfR1	Forward	5'-ACCATTGTCATATACCCGGTTCA-3'
	Reverse	5'-CAATAGCCCAAGTAGCCAATCAT-3'
DMT1	Forward	5'-GTGTTCTACTTGGGTTGGCAATGT-3'
	Reverse	5'-TTGGCTATGTTCACACAGTAAACCAT-3'
GADD45b	Forward	5'-TACGAGTCGGCCAAGTTGATG-3'
	Reverse	5'-GGATGAGCGTGAAGTGGATTT-3'
p21	Forward	5'-TGTCCGTCAGAACCCATGC-3'
	Reverse	5'-AAAGTCGAAGTTCCATCGCTC-3'
DUSP1	Forward	5'-GCCTTGCTTACCTTATGAGGAC-3'
	Reverse	5'-GGGAGAGATGATGCTTCGCC-3'
GAPDH	Forward	5'-GAGTCAACGGATTTGGTCGT-3'
	Reverse	5'-TTGATTTTGGAGGGATCTCG-3'
B2M	Forward	5'-TGCTGTCTCCATGTTTGATGTATCT-3'
	Reverse	5'-TCTCTGCTCCCCACCTCTAAGT-3'

Supplement Table 2, Related to STAR Methods

siRNA sequences			
IRP1 siRNA-1	Qiagen, custom order	Sense	5’-CCAUAAGACCUUUAUCUAUTT-3’
		Antisense	5’-AUAGAUAAAGGUCUUAUGGTT-3’
IRP1 siRNA-2	Qiagen, custom order	Sense	5’-GGGCAAGAACGAUACACUAUU-3’
		Antisense	5’-UAGUGUAUCGUUCUUGCCCUU-3’
IRP2 siRNA	Qiagen, custom order	Sense	5’-GGAAGAACAUGUUAUACUAUU-3’
		Antisense	5’-UAGUAUAACAUGUUCUUCCUU-3’
Control siRNA	Qiagen, SI03650318	Sense	not available
		Antisense	not available

Supplement Table 3, Related to STAR Methods

Primers for QuikChange II Site-Directed Mutagenesis		
IRP2 C512A	Forward	5'-GGTCATTGCTGCAGTTATCAGTGCTACCAATAATTGCAATCC-3'
	Reverse	5'-GGATTGCAATTATTGGTAGCACTGATAACTGCAGCAATGACC-3'
IRP2 C516A	Forward	5'-ACCAATAATGCCAATCCATCTGTCATGCTTGCTGCAGG-3'
	Reverse	5'-CCTGCAGCAAGCATGACAGATGGATTGGCATTATTGGT-3'
IRP2 C578A/C581A	Forward	5'-GAAATCGTTGGCTATGGAGCTTCAACTGCTGTGGGAAATACAGCACCC-3'
	Reverse	5'-GGGTGCTGTATTTCCCACAGCAGTTGAAGCTCCATAGCCAACGATTTC-3