Supplementary Method

Construction of expression vectors and purification of mouse Apurinic/ Apyrimidinic endonuclease (mAPE1): PCR was carried out using a cDNA (a gift from Prof. Sankar Mitra, UTMB, Galveston, TX) as template and the primers (5'-CATATGCCAAAGCGGGGAAAGAAAGCG-3' and 5'-GGATCCTCAGAGTGCTAGGTAAAGGGTGATGG-3'). The primers allowed the introduction of NdeI and Bam HI sites at the 5' and 3' ends, respectively.

The PCR products were then subcloned in pCR2.1-TOPO cloning vector, digested with NdeI and Bam HI, and subcloned into expression vector pET15b at Nde I / Bam HI sites, allowing us to express mAPE protein. The identity of the construct was confirmed by DNA sequencing. mAPE was overexpressed in *E. coli* BL21(DE3) cells and purified to near electrophoretical homogeneity. The purification was carried out as described below. The conditions for cell growth and induction of protein expression were as described previously [1].

In-gel tryptic digestion and protein identification by mass spectrometry: Briefly, the protein was resolved on a 4-12% NUPAGE gel (Invitrogen), and the protein band of interest was manually excised from the 1D SDS PAGE –gel and transferred to a microcentrifuge tube. The gel slices were washed with 100 mM ammonium bicarbonate and incubated with 50 mM ammonium bicarbonate and 10 µl of 10 mM DTT at 60°C for 30 min. The tubes were cooled to room temp, 10 µl of 55 mM iodoacetamide was added, and then were incubated for another 30 min in the dark at room temperature. The solvent was discarded and the gel slices were washed in 50% acetonitrile/100 mM ammonium

bicarbonate. Subsequently, the gel slices were transferred onto a 96 well Montage plate (Millipore), destained with 50% acetonitrile in 25 mM ammonium bicarbonate, dehydrated with acetonitrile for 5 min, and vacuum dried. Gel pieces were then rehydrated with 15 µl of ammonium bicarbonate:acetonitrile (25 mM:10%) supplemented with trypsin (5 ng/µl, Promega, Madison, WI, USA) at 37 °C for 16 h. Afterwards, tryptic peptides were extracted in 0.1% TFA/50% acetonitrile and mixed with an equal volume of 5 mg/ml CHCA (Acros Organics, New Jersey, USA). Mass spectra (MS) were recorded with a matrix assisted laser desorption/ionization–time of flight and time of flight (MALDI-TOF-TOF) spectrometer (4800 Proteomics Analyzer, Framingham, MA, USA) set in reflector positive mode by spotting the samples onto a MALDI plate. The standards used for calibration was Calibration Mixture 5 (Applied Biosystems, Foster City, CA, USA) consisting of Component charge (n) Monoisotopic mass (M+H)+: Bradykinin (2-9) clip +1 904.4681, Angiotensin I, human +1 1,296.6853, Glu-FibrinopeptideB +1 1,570.6774, ACTH (1-17 clip) +1 2,093.0867, ACTH (18-39 clip) +1 2,465.1989, and ACTH (7-38clip) +1 3,657.9294.

The samples were ionized with a fixed LASER intensity of 3800J, and 1000 LASER shots were collected per subspectrum and were shot randomly with uniform bias. The detector voltage was set at 2.1KV, the bin size at 0.5 ns and the Signal/Noise threshold at 15. The spectra were collected with a specified mass range of 700-4000 Daltons with a focus mass of 2100 Daltons. Peptide masses were compared with the theoretical masses derived from the sequences contained in SWISS-PROT/NCBI databases using MASCOT. The search parameters were set as follows: cysteines as carbamidomethyl

derivative, allowed peptide mass error 50 ppm, at least four peptide mass hits required for a protein match, and up to one missed cleavage and methionine oxidized form.

Supplementary Reference

1. J. W. Hill, T. K. Hazra, T. Izumi, S. Mitra, S, Stimulation of human 8-oxoguanine-DNA glycosylase by AP-endonuclease: potential coordination of the initial steps in base excision repair, Nucleic Acids Res. 29 (2001) 430-438.

Supplementary Figure Legends

Figure S1. Purification of hMPG by SP-sepharose column using buffers of different pHs: 3 pH units below pI (A), 2 pH units below pI (B) and 1 pH unit below pI (C). The details of the purification procedures are described in "Materials and Methods". Fractions 7 through 11 (from Fig. 1A and 1B) and fractions 8 through 12 (from Fig. 1C) have been used for further analysis.

Figure S2. Purification of mAPE1 by Ni-NTA column (A) and SP-sepharose column (B). The details of the purification procedures are described in "Materials and Methods". Fractions 2 through 7 (from Fig. 2A) and fractions 4 through 9 (from Fig. 2B) have been used for further analysis.

Figure S3. Purification of hOGG1 by Ni-NTA column (A) and SP-sepharose column (B). The details of the purifications are described in "Materials and Methods". Fractions 4 through 7 (from Fig. 3A) and fractions 5 through 8 (from Fig. 3B) have been used for further analysis.

Figure S4. Purification GST-BRCA1 by GST column (A) and SP-sepharose column (B). The details of the purification procedures are described in "Materials and Methods". Fractions 2 through 7 (from Fig. 4A) and fractions 4 through 9 (from Fig. 4B) have been used for further analysis. (C) Western blot of GST-BRCA1 with anti-GST antibody.

Figure S5. Purification of NΔ100 mMPG by SP-sepharose column. The details of the purification procedures are described in "Materials and Methods". Fractions 2 through 7 have been used for further analysis.

Supplementary Figures

Figure S1

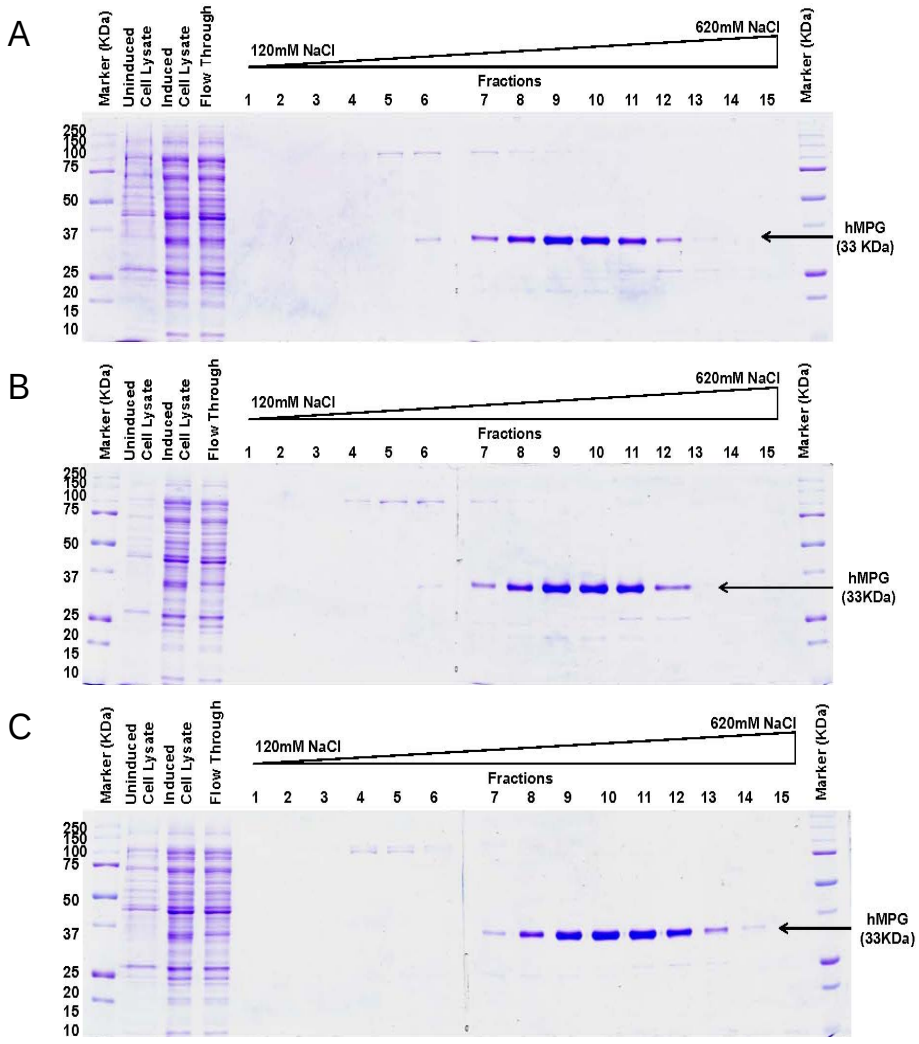


Figure S2

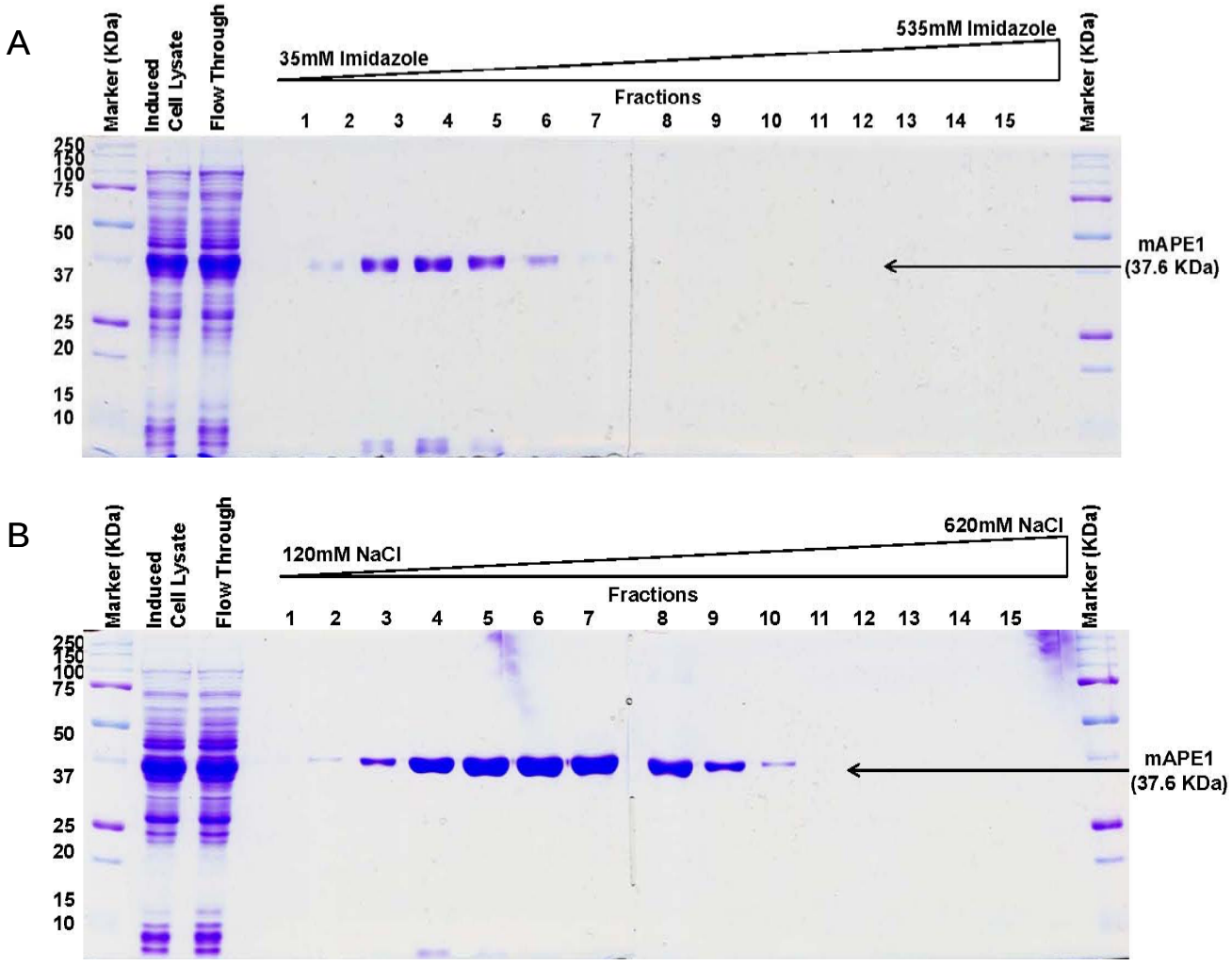

A
Marker (KDa)
Induced Cell Lysate
Flow Through
35mM Imidazole
535mM Imidazole
Fractions
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Marker (KDa)
250
150
100
75
50
37
25
20
15
10
mAPE1
(37.6 KDa)
B
Marker (KDa)
Induced Cell Lysate
Flow Through
120mM NaCl
620mM NaCl
Fractions
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Marker (KDa)
250
150
100
75
50
37
25
20
15
10
mAPE1
(37.6 KDa)

Figure S3

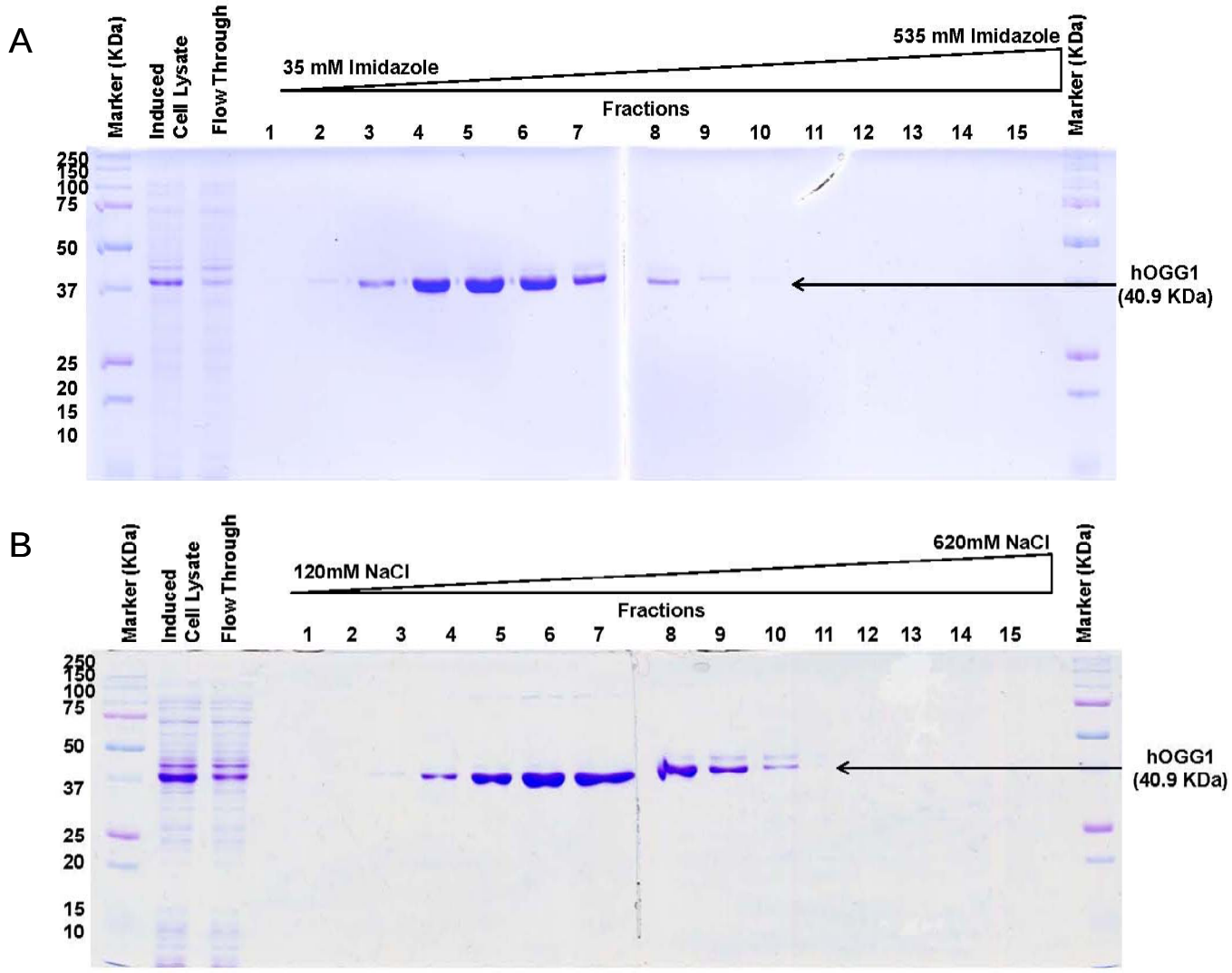

A
Marker (KDa)
Induced Cell Lysate
Flow Through
35 mM Imidazole
535 mM Imidazole
Fractions
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Marker (KDa)
250
150
100
75
50
37
25
20
15
10
hOGG1
(40.9 KDa)
B
Marker (KDa)
Induced Cell Lysate
Flow Through
120mM NaCl
620mM NaCl
Fractions
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Marker (KDa)
250
150
100
75
50
37
25
20
15
10
hOGG1
(40.9 KDa)

Figure S4

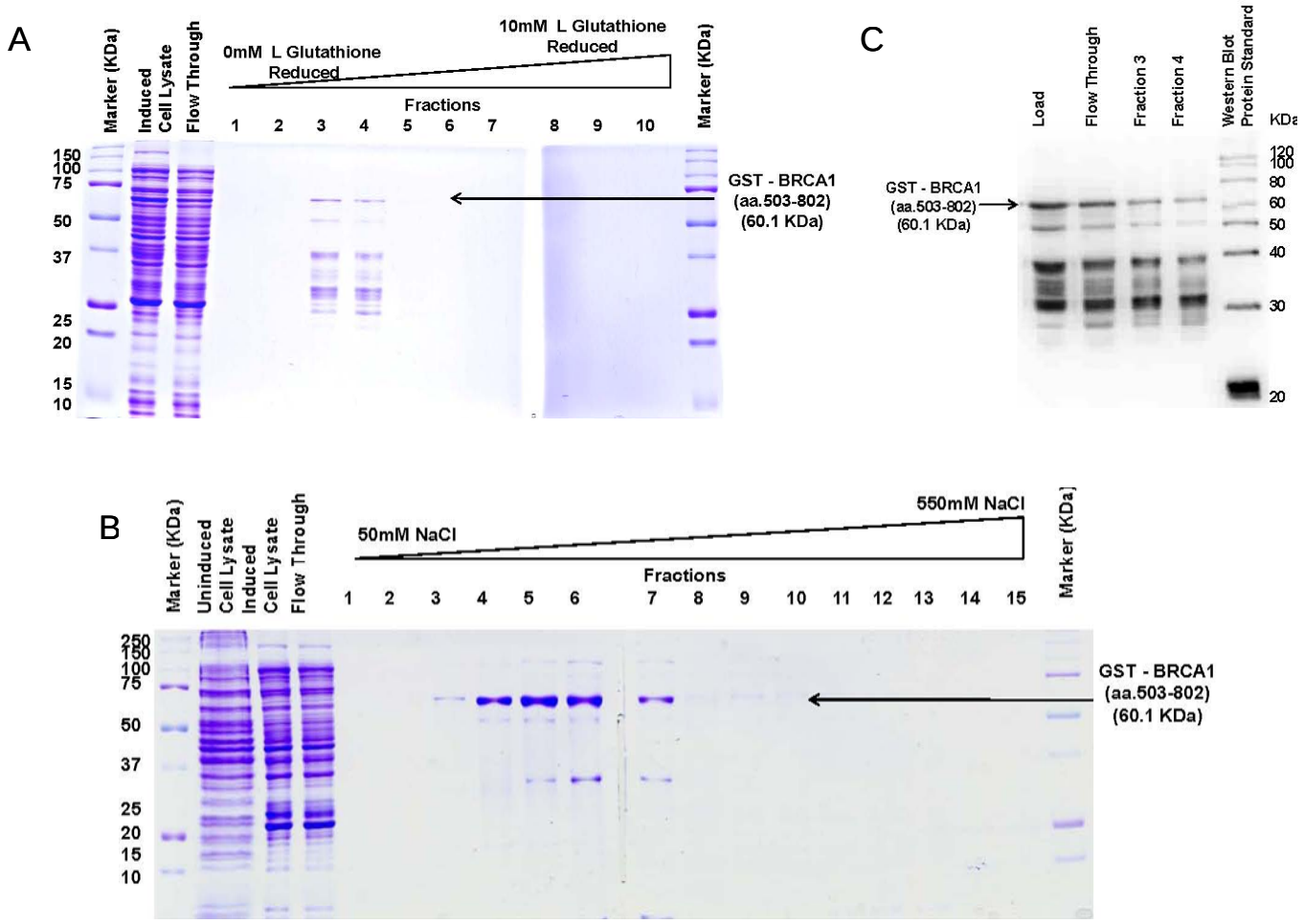
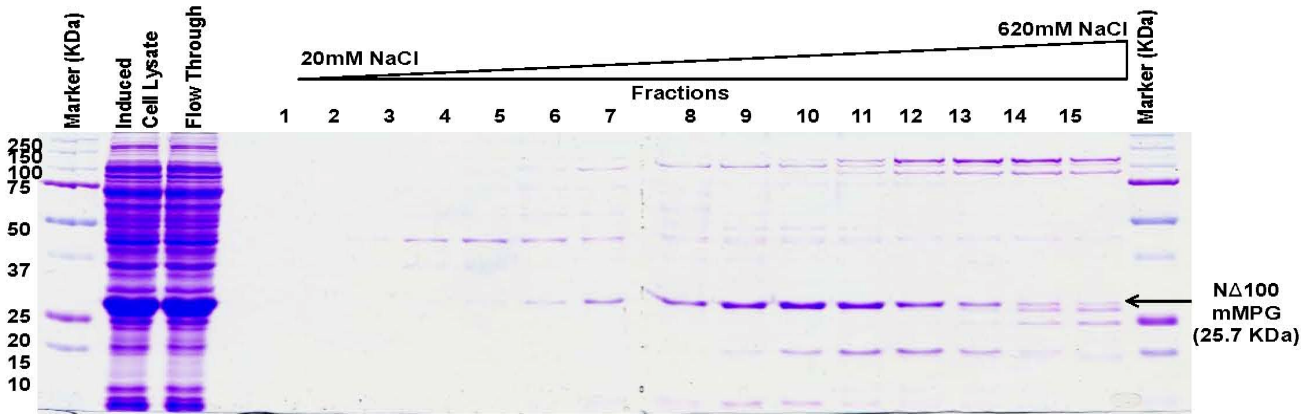

A
Marker (KDa)
Induced Cell Lysate
Flow Through
0mM L Glutathione Reduced
10mM L Glutathione Reduced
Fractions
1 2 3 4 5 6 7 8 9 10
Marker (KDa)
150
100
75
50
37
25
20
15
10
GST - BRCA1
(aa.503-802)
(60.1 KDa)
C
Load
Flow Through
Fraction 3
Fraction 4
Western Blot Protein Standard
KDa
GST - BRCA1
(aa.503-802)
(60.1 KDa)
120
100
80
60
50
40
30
20
B
Marker (KDa)
Uninduced Cell Lysate
Induced Cell Lysate
Flow Through
50mM NaCl
550mM NaCl
Fractions
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Marker (KDa)
250
150
100
75
50
37
25
20
15
10
GST - BRCA1
(aa.503-802)
(60.1 KDa)

Figure S5


Marker (KDa)
Induced Cell Lysate
Flow Through
20mM NaCl
620mM NaCl
Fractions
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Marker (KDa)
250
150
100
75
50
37
25
20
15
10
NΔ100 mMPG (25.7 KDa)