## **Supporting Information**

Differential Phosphoproteomic Analysis of Recombinant Chinese Hamster Ovary Cells Following Temperature Shift

Michael Henry<sup>1</sup>¶, Martin Power<sup>1</sup>¶, Prashant Kaushik<sup>1</sup>, Orla Coleman<sup>1</sup>, Martin Clynes<sup>1</sup>¶, Paula Meleady<sup>1</sup>¶\*

<sup>1</sup>National Institute for Cellular Biotechnology, Dublin City University, Glasnevin, Dublin 9, Ireland

\* Corresponding author: Dr. Paula Meleady;

Tel: +353-1-7005910; Email: paula.meleady@dcu.ie

¶ these authors contributed equally to the work

## **Contents**

**Supplementary Figure S-1:** CHO-specific sequence for NDRG1 aligned against human NDRG1 sequence showing conservation of the phosphorylation site (RED BOX) that was detected using the phospho-specific antibody to NDRG1. (Page S3)

**Supplementary Figure S-2:** CHO-specific sequence for ATF2 aligned against human ATF2 sequence showing conservation of the phosphorylation site (RED BOX) that was detected using the phospho-specific antibody to ATF2. (*Page S4*)

The following are supplied in an additional xlsx worksheet

**Supplementary Table S-1:** Full list of differentially expressed phosphopeptides from TiO<sub>2</sub> enrichment with increased expression at 31°C compared to 37°C. The table also includes associated MS data.

**Supplementary Table S-2:** Full list of differentially expressed phosphopeptides from TiO<sub>2</sub> enrichment with decreased expression at 31°C compared to 37°C. The table also includes associated MS data

**Supplementary Table S-3**: Full list of differentially expressed phosphopeptides from Fe-NTA (IMAC) enrichment with increased expression at 31°C compared to 37°C. The table also includes associated MS data.

**Supplementary Table S-4:** Full list of differentially expressed phosphopeptides from Fe-NTA (IMAC) enrichment with decreased expression at 31°C compared to 37°C. The table also includes associated MS data.

**Supplementary Table S-5:** List of differentially expressed proteins from non-enriched whole cell lysates (WCL) with increased expression at 31°C compared to 37°C.

**Supplementary Table S-6:** List of differentially expressed proteins from non-enriched whole cell lysates (WCL) with decreased expression at 31°C compared to 37°C.

**Supplementary Table S-7:** MS data associated with Supplementary Tables S-5 and S-6.

**Supplementary Table S-8:** Overlapping differentially expressed proteins which contained peptides in common between the phosphoproteomic analysis and the whole cell lysate non-enriched proteomic analysis.

**Supplementary Table S-9:** Overlapping differentially expressed proteins which did not share common peptides between the phosphoproteomic analysis and the whole cell lysate non-enriched proteomic analysis.

-		_					
Score		Expec	t Method		Identities	Positives	Gaps
702 bi	its(18:	11) 0.0	Compositiona	l matrix adjust.	340/388(88%)	351/388(90%)	20/388(5%)
Query	1			TITGLLQEFDVQEQD			0
Sbjct	1	MSRELHDV	DLAEVKPRGGGKET	TITGLLQEFDVQEQD TITGLLQEFDVQEQD	TETLHGS+HVTLCG	TPKGNRPVIL 6	0
Query	61			QEITQHFAVCHVDA			20
Sbjct	61			IQEITQHFAVCHVDA			20
Query	121			AYILTRFALNNPEM			80
Sbjct	121			SAYILTRFALNNPEM SAYILTRFALNNPEM			80
Query	181			SNVEVVHTYRQHIV			40
Sbjct	181			SNVEVVHTYRQHI+ HSNVEVVHTYRQHIL			40
Query	241			SPAVDAVVECNSKL			00
Sbjct	241			-SPAVDAVVECNSKL ISPAVDAVVECNSKL			00
Query	301			RLMRSRTASGSSVTS		RSRSHTSEGT 3	60
Sbjct	301			RLMRSRTASGSSVTS RLMRSRTASGSSVTS		R 3	51
Query	361	RSRSHTSE	GAHLDITPNSGAAG				
Sbjct	352		L+ITPNSGA G				

**Supplementary Figure S-1:** CHO-specific sequence for NDRG1 aligned against human NDRG1 sequence showing conservation of the phosphorylation site (RED BOX) that was detected using the phospho-specific antibody to NDRG1.

Range	▼ Next Mate	ch A Previous Matc				
Score		Expect	Method	Identities	Positives	Gaps
722 bi	ts(186	4) 0.0	Compositional matrix adj	ust. 376/486(77%)	382/486(78%)	98/486(20%)
Query	19	MSDDKPFLO	TAPGCGQRFTNEDHLAVHKHKHEI TAPGCGQRFTNEDHLAVHKHKHEI	MTLKFGPARNDSVIVADO	TETPTRELKN 7	8
Sbjct	1		TAPGCGQRFTNEDHLAVHKHKHE			0
Query	79		ELASPFENEFKKASEDDIKKMPLDI ELASPFENEFKKASEDDIKKMPLDI			38
Sbjct	61		LASPFENEFKKASEDDIKKMPLD			20
Query	139	DUDECTT	DEKEVPLAQTAQPTSAIVRPASLQ	CD.		98
Sbjct	121	PHPESTT-		SDEKLV	1	33
Query	199	TQAPSSNR	PIVPVPGPFPLLHLPNGQTMPVA	IPASITSSNVHVPAAVPL	VRPVTMVPSV 2	58
Sbjct	134		PVT			42
Query	259		PQPVQSEAKMRLKAALTQQHPPVTI PQPVQSEAKMRLKAALTQQHPPV I			18
Sbjct	143		POPVOSEAKMRLKAALTOOHPPVAI			02
Query	319		ASPAHTTPQTQSTSGRRRRAANEDI ASPAHTTPOTO+TSGRRRRAANEDI			78
Sbjct	203		ASPAHTTPQTQNTSGRRRRAANED			62
Query	379		SSLNGQLQSEVTLLRNEVAQLKQ SSLNGQLQSEVTLLRNEVAQLKQ			38
Sbjct	263		SSLNGQLQSEVTLLRNEVAQLKQ			22
Query	439		PHTEAIQHSSVSTSNGVSSTSKAE PHTEAIQHSSVSTSNGVSSTSKAE			98
Sbjct	323		PHTEAIQHSSVSTSNGVSSTSKAE			82
Query	499	QSQPSG S	504			
Sbjct	383		388			

**Supplementary Figure S-2:** CHO-specific sequence for ATF2 aligned against human ATF2 sequence showing conservation of the phosphorylation site (RED BOX) that was detected using the phospho-specific antibody to ATF2.