

## **SUPPLEMENTARY FILE S2**

### **GENE ONTOLOGY**

Identification of novel endogenous controls for qPCR normalization in SK-BR-3  
breast cancer cell line

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**Additional Table 1.** Gene Ontology of Candidate Reference Genes ranked based on Fold Enrichment (Molecular Function)

GO ID	GO Term	No. of genes	Fold Enrichment	Raw P value	FDR
GO:0031625	Ubiquitin protein ligase binding	5	15.16 (+)	1.72E-05	2.74E-02
<i>Genes: RBX1, TUBA1B, HSPCB, UBC, GABARAP</i>					
GO:0003723	RNA binding	12	6.43 (+)	4.38E-08	2.31E-04
<i>Genes: SF3A1, TUBA1B, PUM1, HSPCB, RPL13A, TPT1, HNRNPL, PFN1, UBC, EIF5A, PPIA, PCBP1</i>					
GO:0008092	Cytoskeletal protein binding	7	6.36 (+)	7.30E-05	4.98E-02
<i>Genes: ACTB, GAPDH, HSPCB, PFN1, CFL1, CCSER2, GABARAP</i>					

No. of genes indicates the number of genes from the input selected candidate reference genes that are represented by the respective GO term. (+) indicates statistically significant over-representation of the GO term in the selected reference genes.

**Notes:**

- 1) Blue GO term indicates the most enriched molecular function while red GO term indicates the most significant molecular function.

**Additional Table 2.** Gene Ontology of Candidate Reference Genes ranked based on Fold Enrichment (Cellular Component)

GO ID	GO Term	No. of genes	Fold Enrichment	Raw P value	FDR
GO: 0097452	GAIT complex	2	> 100 (+)	1.74E-05	3.84E-03
<i>Genes: GAPDH, RPL13A</i>					
GO:0035770	Ribonucleoprotein granule	4	14.56 (+)	1.56E-04	2.60E-02
<i>Genes: ACTB, PUM1, HNRNPL, PCBP1</i>					
GO:0005925	Focal adhesion	6	13.04 (+)	5.04E-06	1.68E-03
<i>Genes: ACTB, RPL13A, PFN1, CFL1, BSG, PPIA</i>					
GO:0070062	Extracellular exosome	14	6.05 (+)	3.82E-09	7.66E-06
<i>Genes: ACTB, GAPDH, HSPCB, TPT1, HNRNPL, PFN1, PGK1, UBC, BSG, CFL1, NACA, PPIA, PSMB4, PCBP1</i>					
GO:0005829	Cytosol	16	2.73 (+)	1.14E-05	2.85E-03
<i>Genes: GAPDH, RPL13A, RBX1, ACTB, PUM1, GABARAP, EIF5A, HSPCB, TPT1, PFN1, PGK1, UBC, CFL1, PPIA, PSMB4, PCBP1</i>					
GO:0005634	Nucleus	17	2.03 (+)	3.21E-04	4.92E-02
<i>Genes: GAPDH, RPL13A, RBX1, SF3A1, ACTB, PUM1, EIF5A, HSPCB, TPT1, HNRNPL, PFN1, UBC, CFL1, NACA, PPIA, PSMB4, PCBP1</i>					

No. of genes indicates the number of genes from the input selected candidate reference genes that are represented by the respective GO term. (+) indicates statistically significant over-representation of the GO term in the selected reference genes.

**Notes:**

- 1) Blue GO term indicates the most enriched cellular component while red GO term indicates the most significant cellular component.

**Additional Table 3.** Panther Functional Classification Analysis (Supplement to Figure 2)

Class/Pathway	Number of Genes	Genes Included
<b><i>Biological Process</i></b>		
Cellular process	17	<i>BSG, CFL1, DAD1, EIF5A, GABARAP, HNRNPL, HSP90AB1, NACA, PFN1, PGK1, PSMB4, PUM1, RPL13A, RBX1, SF3A1, TUBA1B, UBC</i>
Metabolic process	11	<i>DAD1, EIF5A, GAPDH, GABARAP, HNRNPL, PCBP1, PGK1, PUM1, RBX1, SF3A1, UBC</i>
Biological regulation	8	<i>EIF5A, HSP90AB1, PCBP1, PFN1, PGK1, PUM1, RPL13A, SF3A1</i>
Response to stimulus	3	<i>BSG, HSP90AB1, GABARAP</i>
Developmental process	2	<i>BSG, PGK1</i>
Localization	2	<i>CFL1, NACA</i>
Locomotion	2	<i>BSG, CFL1</i>
Multicellular organismal process	2	<i>BSG, HSP90AB1</i>
Biological adhesion	1	<i>BSG</i>
<b><i>Molecular Function</i></b>		
Binding	16	<i>BSG, CFL1, EIF5A, GABARAP, HSP90AB1, NACA, PFN1, PGK1, PSMB4, PUM1, RPL13A, RBX1, SF3A1, TPT1, TUBA1B, UBC</i>
Catalytic activity	5	<i>DAD1, GAPDH, PGK1, PSMB4, RBX1</i>
Structural molecular activity	3	<i>ATCB, RPL13A, TUBA1B</i>
Translational regulator activity	1	<i>EIF5A</i>
<b><i>Cellular Compartment</i></b>		
Cell and cell part	17	<i>BSG1, CFL1, DAD1, GABARAP, GAPDH, HNRNPL, HSP90AB1, PFN1, PGK1, PPIA, PSMB4, PUM1, RBX1, RPL13A, SF3A1, TPT1, TUBA1B</i>
Organelle	10	<i>CFL1, DAD1, GABARAP, HNRNPL, PCBP1, PPIA, RBX1, RPL13A, SF3A1, UBC</i>
Organelle part	7	<i>CFL1, DAD1, GABARAP, PCBP1, PSMB4, RBX1, TUBA1B</i>
Protein-containing complex	6	<i>CFL1, DAD1, PSMB4, RPL13A, RBX1, SF3A1</i>
Membrane	5	<i>ACTB, BSG, DAD1, GABARAP, HSP90AB1</i>
Membrane-part	3	<i>ACTB, CFL1, TUBA1B</i>
Supramolecular complex	2	<i>TUBA1B, HSP90AB1</i>
Membrane-enclosed lumen	1	<i>PCBP1</i>

<i><b>Protein Class</b></i>		
Cytoskeletal protein	6	<i>ACTB, CFL1, GABARAP, PFN1, TPT1, TUBA1B</i>
Metabolite interconversion enzyme	3	<i>DAD1, GAPDH, PGK1</i>
Nucleic acid binding protein	3	<i>PCBP1, PUM1, SF3A1</i>
Translational protein	2	<i>EIF5A, RPL13A</i>
Chaperone	1	<i>HSP90AB1</i>
Gene-specific transcriptional regulator	1	<i>NACA</i>
Protein modifying enzyme	1	<i>RBX1</i>
Scaffold/adaptor protein	1	<i>BSG</i>
<i><b>Pathway</b></i>		
Cytoskeletal regulation by Rho GTPase	3	<i>ACTB, CFL1, PFN1</i>
Glycolysis	2	<i>GAPDH, PGK1</i>
Huntington disease	2	<i>ACTB, GAPDH</i>
Alzheimer disease-presenilin	1	<i>ACTB</i>
Cadherin signalling	1	<i>ACTB</i>
Gonadotropin releasing hormone receptor	1	<i>TUBA1B</i>
Inflammation mediated by chemokine and cytokine signalling	1	<i>ACTB</i>
Integrin signalling	1	<i>ACTB</i>
Nicotine acetylcholine receptor signalling	1	<i>ACTB</i>
Parkinson disease	1	<i>PSMB4</i>
Wnt Signalling	1	<i>ACTB</i>