Supplementary Information

Gold-Nanobeacons for gene therapy: evaluation of genotoxicity, cell toxicity and proteome profiling analysis

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1. Physical characterization of Au-nanobeacons

Gold nanoparticles (AuNPs) were synthesized by the citrate reduction method described by Lee and Meisel (Lee, 1982).Briefly, 225 mL of 1 mM hydrogen tetrachloroaureate (III) hydrate (Sigma) (88.61 mg) was solved in 500 ml of distilled water and heated and stirred under reflux. When the solution boils, 25 mL of 38.8 mM sodium citrate dihydrate (285 mg) was added resulting in a red solution. The solution is kept under ebullition with vigorous stirring and protected from light for 30 minutes. Then, the solution is cooled down and kept protected from light. Citrate capped AuNPs were characterized by Transmission Electron Microscopy (TEM) and UV-Vis spectroscopy.

Functionalization of PEGylated AuNPs was carried out using commercial hetero-functional poly(ethylene glycol) (PEG) functionalized with a thiol group O-(2-Mercaptoethyl)-O'-methyl-hexa(ethylene glycol), $C_{15}H_{32}O_7S$, 356.48 Da (Sigma) as previously described (Rosa, 2012; Conde, 2013; Sanz, 2012).Briefly, 10 nM of the AuNPs were mixed with 0.003 mg/mL of O-(2-Mercaptoethyl)-O'-methyl-hexa(ethylene glycol) in an aqueous solution of SDS (0.028%). Then, NaOH was added to a final concentration of 25 mM and the mixture incubated for 16 hours at room temperature. Excess PEG was removed by centrifugation (21.460 ×g, 30 min, 4ºC), and quantified by the Ellman's Assay suitable for sulfhydryl group determination. Briefly, 0.05 mg/mL of 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) (Sigma), reacts with sulfhydryl under slightly alkaline conditions (0.5 M phosphate buffer, pH 7-8), to release 5-thio-2-nitrobenzoic acid (TNB) a highly chromogenic compound (412 nm), after 15 min at room temperature. The excess of O-(2-Mercaptoethyl)-O'-methyl-hexa(ethylene glycol) was determined following interpolation in a standard curve ($Abs_{412nm} = 26.034 \times [PEG, mg/mL] + 0.0627$).

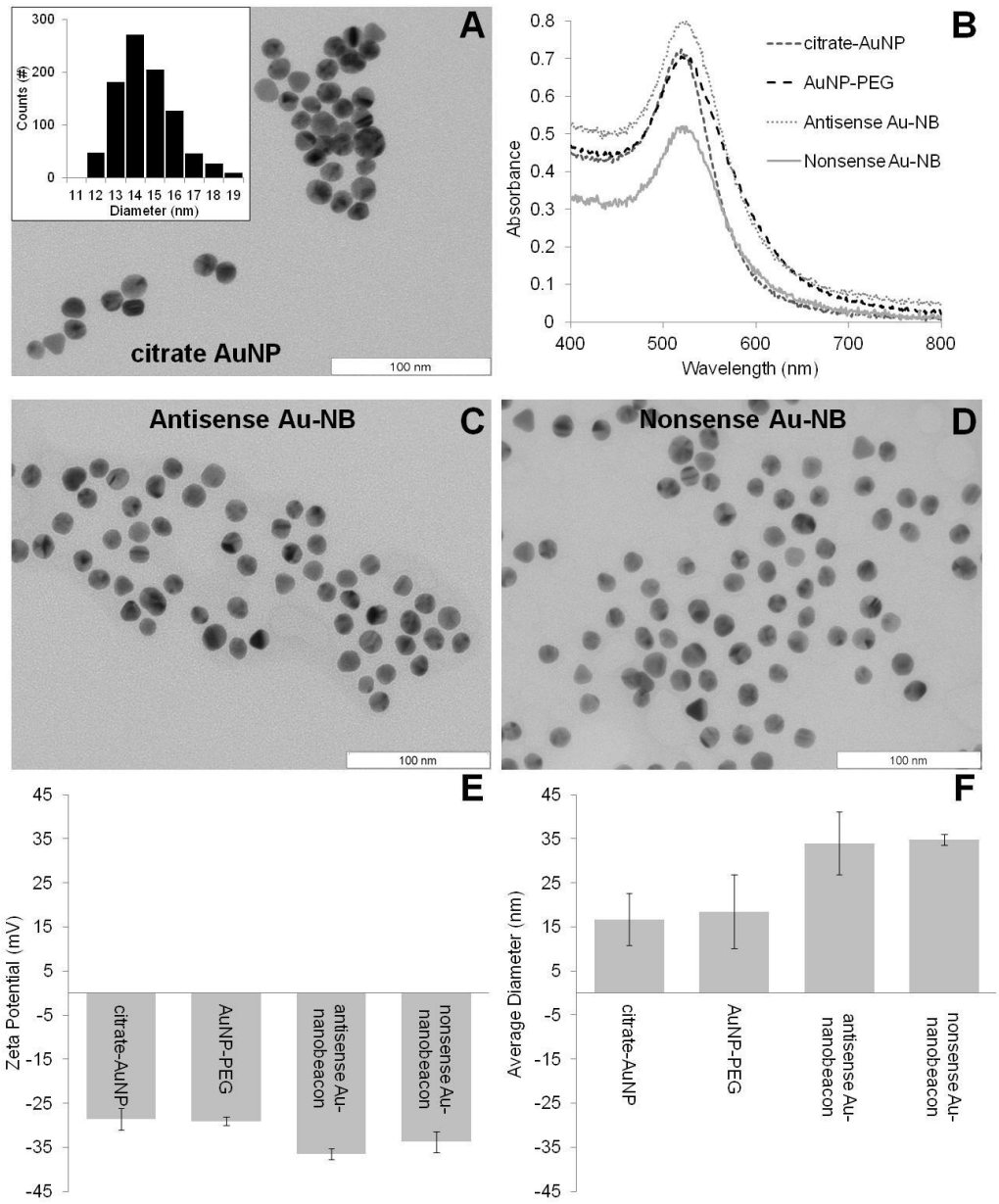

Figure S1. Physical characterisation of Au-nanobeacons. (A) Transmission Electron Microscopy (TEM) of citrate-AuNPs, Inset: size distribution histogram showing an average diameter of 14.6±1.7 nm. (**B**) UV/Vis Spectroscopy of citrate AuNPs and of Au-nanobeacons.TEM images for antisense (**C**) and nonsense (**D**) Au-nanobeacons. (**E**) Surface charge measurements by Zeta Potential. (**F**) Dynamic Light Scattering (DLS) showing average diameter of AuNPs conjugates.

2. EGFP vector transfection in HCT-116 cells

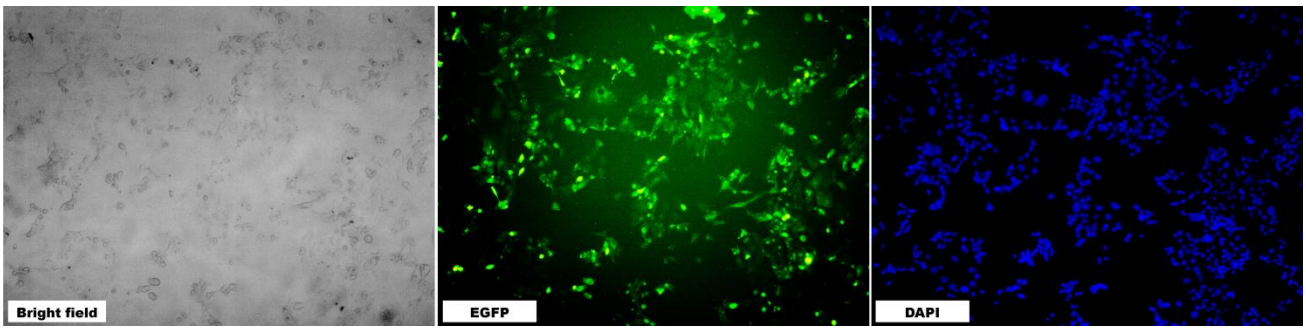

Figure S2.Representative images of HCT-116 cells 24 h after transfection with EGFP vector. From left to right: bright fieldmicroscopy images, green fluorescence channels corresponding to EGFP fluorescence, and nucleus stained with DAPI.

3. Comet class and nuclear abnormality frequency

A

			Au-nanobeacons			oligonucleotide	
	AuNP@PEG	PBS	antisense	nonsense	PBS + Lipo	antisense	nonsense
Class 0	0.17 ± 0.21	0.16 ± 0.19	0.25 ± 0.10	0.33 ± 0.08	0.13 ± 0.05	0.54 ± 0.16	0.49 ± 0.17
Class 1	0.22 ± 0.03	0.25 ± 0.09	0.30 ± 0.07	0.37 ± 0.13	0.32 ± 0.00	0.30 ± 0.23	0.29 ± 0.17
Class 2	0.30 ± 0.15	0.30 ± 0.13	0.25 ± 0.06	0.17 ± 0.09	0.25 ± 0.00	0.10 ± 0.02	0.13 ± 0.10
Class 3	0.21 ± 0.04	0.20 ± 0.07	0.13 ± 0.07	0.08 ± 0.04	0.22 ± 0.03	0.05 ± 0.05	0.07 ± 0.07
Class 4	0.10 ± 0.04	0.09 ± 0.06	0.08 ± 0.02	0.05 ± 0.03	0.09 ± 0.03	0.01 ± 0.02	0.01 ± 0.01

B

			Au-nanobeacons			oligonucleotide	
	AuNP@PEG	PBS	antisense	nonsense	PBS + Lipo	antisense	nonsense
N.A. Freq (%)	2.08 ± 0.64	2.60 ± 1.14	1.84 ± 0.69	1.93 ± 1.10	2.87 ± 0.63	3.23 ± 0.93	2.87 ± 1.22

Figure S3. Average frequencies for (**A**) scored comets per class, according to % of DNA in tail; (**B**) scored nuclear abnormalities, for each treatment.

4. Proteome evaluation – Two-dimensional gel electrophoresis

Protein samples (200 µg) were transferred onto strip holders along with 7 cm long IPG strips covering pH range from 3 to 10. Strips were positioned in the strip holders with gel side down, avoiding entrapment of bubbles underneath the strip. Strips were then covered with 3 mL DryStrip Cover Fluid (GE Healthcare) and the strip holders lidded. IEF was performed in five steps "step-n-hold" in the following order: 30 V for 720 minutes, 100 V for 30 minutes, 500 V for 30 minutes, 1000 V for 30 minutes and 5000 V for 60 minutes. After IEF, the strips were equilibrated 2×15 min with a mixture containing 75mM Tris-HCl (Sigma) (pH 8.8), 6 M urea, 2% SDS (Sigma), 30% glycerol (Merck) and traces of bromophenol blue. The buffer for the first equilibration step contained 1% DTT and the second 2.5% iodoacetamide (Sigma). The second dimension was performed in a mini-PROTEAN II electrophoresis Cell (Bio-Rad). IPG strips were placed on top of a 12% (w/v) acrylamide/bis-acrylamide (37.5:1) gel. The strips were sealed with an agarose solution of 0.5% (w/v) with traces of bromophenol blue and the electrophoresis was conducted at 50 V for 30 minutes until the proteins were incorporated in the polyacrylamide matrix, followed by 150 V for 90 minutes. Gels were soaked in a fixing solution (10% acetic acid; 30% ethanol) for at least 2 h, stained with hot Coomassie Brilliant Blue R350 (PhastGelTM Blue R, GE Healthcare) solution in 10% acetic acid for 30 min and washed overnight in 10% acetic acid. Gels were then dehydrated in a 2% glycerol, 35% ethanol solution and dried between two porous cellophane sheets for at least 48 h. All 2-DE gel images were digitalized (PIXMA M250, Canon) and analyzed with the Melanie 7.0Software (GeneBio). Each one of the conditions studied was evaluated in triplicate. The analysis was performed semi-automatically by the software, adhering to the following procedure: (1) spot detection; (2) spot matching from different gels; (3) background subtraction; and (4) assessment of the normalized intensity of each spot. Normalization was performed as the ratio of the spot intensity and the total intensity of the spots with a match in all gels in the experiment.

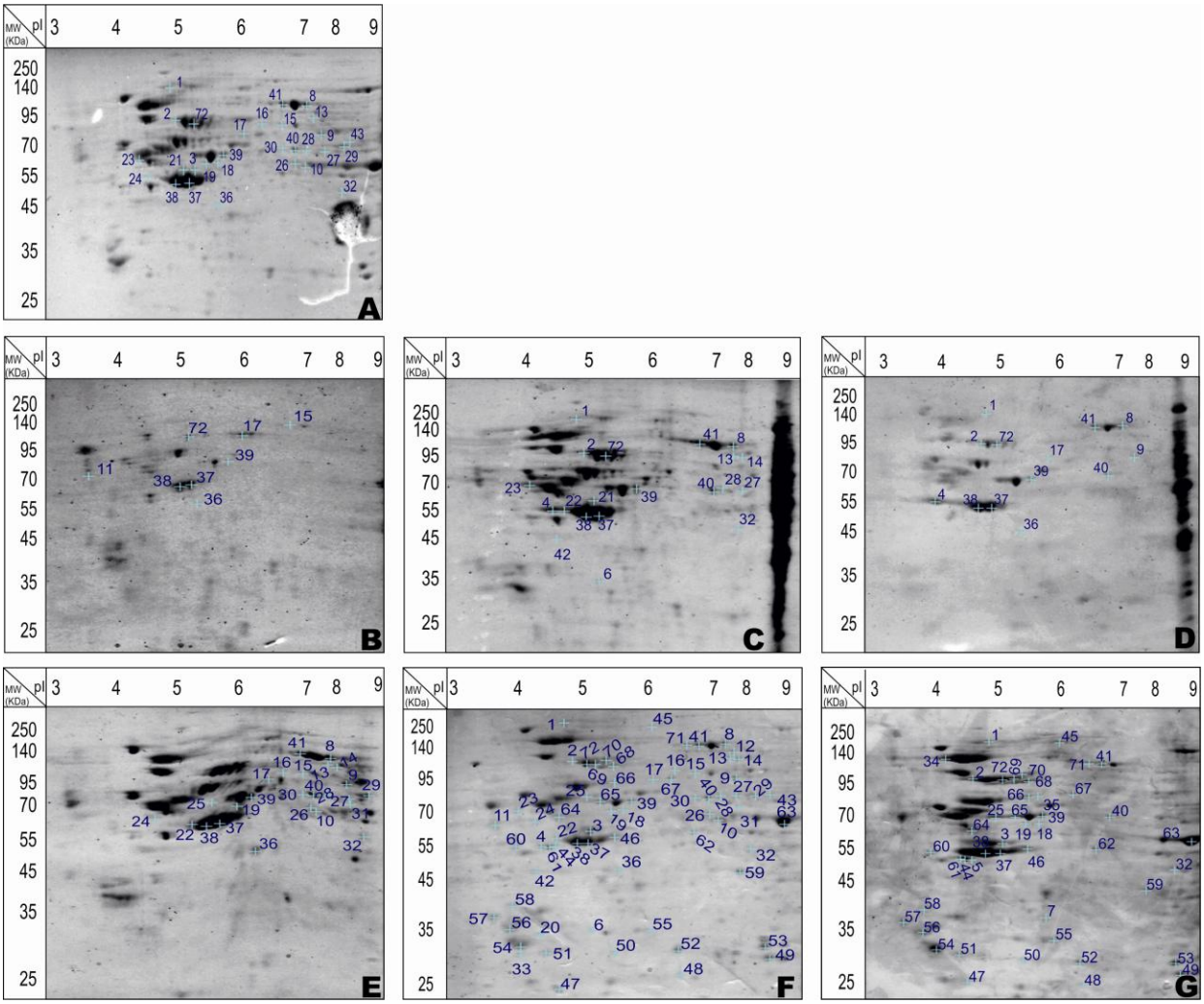

Figure S4. Two-dimensional gel electrophoresis of protein extracts for cells exposed to: (**A**) PBS, (**B**) PBS + Lipofectamine, (**C**) Antisense oligonucleotide, (**D**) Nonsense oligonucleotide, (**E**) AuNP@PEG, (**F**) Antisense Au-nanobeacon, (**G**) Nonsense Au-nanobeacon.

5. Proteome evaluation – MALDI-TOF Mass Spectrometry analysis

In-gel digestion and MALDI-TOF Mass Spectrometry analysis

Selected protein spots were manually excised from 2-DE gels and sent for Peptide Mass Fingerprinting analysis at Mass Spectrometry Laboratory in ITQB/IBET (Oeiras, Portugal). Briefly, excised gel spots were washed with MilliQ water and 50% acetonitrile (ACN) for Comassie dye removal and then dehydrated using 100% ACN. Reduction and alkylation steps followed by incubating the samples with agitation, first with 10 mM of DTT, 100 mM ammonium bicarbonate for 45 minutes at 56 °C followed by 55 mM of iodoacetamide, 100 mM ammonium bicarbonate for 30 minutes at room temperature in the dark. The spots were once more washed and dried with 100% ACN and vacuum centrifuged until completely dry. For in-gel digestion procedure the gel spots were rehydrated for 30 min on ice with digestion buffer (50 mM ammonium bicarbonate) and overnight digestion with 6.7 ng/µL of trypsin at 37 °C. Smaller protein digests were recovered from supernatant and larger protein digests were recovered by dehydration of the gels with 5% formic acid followed by 100% ACN and supernatant recovery. All samples were then completely dried under vacuum and stored at -20 °C. Sample preparation for mass spectrometry analysis was as follows: protein digests were resuspended in 5% formic acid, spotted onto the MALDI plate and covered with the same volume of matrix solution (α-cyano-4-hydroxycinnamic acid 10 mg/mL; 50% ACN, 5% form

Table 1.Protein identification via MALDI-TOF Mass Spectrometry analysis.

Spot ID	Protein Identification	Acession reference (HUMAN)	Protein Score (Mascot)	Isoelectric point	MW (KDa)	Description[a]
3	**Heat shock protein HSP 90-beta**	HS90B	259	4.97	83.212	Molecular chaperone that promotes the maturation, structural maintenance and proper regulation of specific target proteins involved for instance in cell cycle control and signal transduction. Undergoes a functional cycle that is linked to its ATPase activity. This cycle probably induces conformational changes in the client proteins, thereby causing their activation. Interacts dynamically with various co-chaperones that modulate its substrate recognition, ATPase cycle and chaperone function. Homodimer. Interacts with p53/TP53. Forms a complex with CDK6 and Hsp90/HSP90AB1. Interacts with UNC45A. Binding to UNC45A involves 2 UNC45A monomers per HSP90AB1 dimer. Interacts with CHORDC1 and DNAJC7. Interacts with FKBP4.
4	**Heat shock cognate 71 kDa protein**	HSP7C	807	5.37	70.8542	Acts as a repressor of transcriptional activation. Inhibits the transcriptional coactivator activity of CITED1 on Smad-mediated transcription.Chaperone. Component of the PRP19-CDC5L complex that forms an integral part of the spliceosome and is required for activating pre-mRNA splicing. May have a scaffolding role in the spliceosome assembly as it contacts all other components of the core complex. Identified in a mRNP granule complex, at least composed of ACTB, ACTN4, DHX9, ERG, HNRNPA1, HNRNPA2B1, HNRNPAB, HNRNPD, HNRNPL, HNRNPR, HNRNPU, HSPA1, HSPA8, IGF2BP1, ILF2, ILF3, NCBP1, NCL, PABPC1, PABPC4,

						PABPN1, RPLP0, RPS3, RPS3A, RPS4X, RPS8, RPS9, SYNCRIP, TROVE2, YBX1 and untranslated mRNAs. Interacts with PACRG. Interacts with HSPH1/HSP105. Interacts with IRAK1BP1 and BAG1. Interacts with DNAJC7. Interacts with CITED1 (via N-terminus); the interaction suppresses the association of CITED1 to p300/CBP and Smad-mediated transcription transactivation. Component of the PRP19-CDC5L splicing complex composed of a core complex comprising a homotetramer of PRPF19, CDC5L, PLRG1 and BCAS2, and at least three less stably associated proteins CTNNBL1, CWC15 and HSPA8. Interacts with SV40 VP1.
43	**Eukaryotic initiation factor 4A-I**	IF4A1	588	5.32	46.1246	ATP-dependent RNA helicase which is a subunit of the eIF4F complex involved in cap recognition and is required for mRNA binding to ribosome. In the current model of translation initiation, eIF4A unwinds RNA secondary structures in the 5'-UTR of mRNAs which is necessary to allow efficient binding of the small ribosomal subunit, and subsequent scanning for the initiator codon.
69	**Prohibitin**	PHB	629	5.57	29.7859	Prohibitin inhibits DNA synthesis. It has a role in regulating proliferation. As yet it is unclear if the protein or the mRNA exhibits this effect. May play a role in regulating mitochondrial respiration activity and in aging. Interacts with PHB2.
9	**Proteasome activator complex subunit 1**	PSME1	228	5.78	28.705	Implicated in immunoproteasome assembly and required for efficient antigen processing. The PA28 activator complex enhances the generation of class I binding peptides by altering the cleavage pattern of the proteasome.

19	**Ezrin**	EZRI	878	5.94	69.3697	Probably involved in connections of major cytoskeletal structures to the plasma membrane. In epithelial cells, required for the formation of microvilli and membrane ruffles on the apical pole. Along with PLEKHG6, required for normal macropinocytosis. Phosphorylated by tyrosine-protein kinases. Phosphorylation by ROCK2 suppresses the head-to-tail association of the N-terminal and C-terminal halves resulting in an opened conformation which is capable of actin and membrane-binding.
25	**T-complex protein 1 subunit zeta**	TCPZ	662	6.23	57.9876	Molecular chaperone; assists the folding of proteins upon ATP hydrolysis. Known to play a role, in vitro, in the folding of actin and tubulin.
33	**Proliferation-associated protein 2G4**	PA2G4	458	6.13	43.7592	May play a role in a ERBB3-regulated signal transduction pathway. Seems be involved in growth regulation. Acts a corepressor of the androgen receptor (AR) and is regulated by the ERBB3 ligand neuregulin-1/heregulin (HRG). Inhibits transcription of some E2F1-regulated promoters, probably by recruiting histone acetylase (HAT) activity. Binds RNA. Associates with 28S, 18S and 5.8S mature rRNAs, several rRNA precursors and probably U3 small nucleolar RNA. May be involved in regulation of intermediate and late steps of rRNA processing. May be involved in ribosome assembly. Mediates cap-independent translation of specific viral IRESs (internal ribosomal entry site). Interacts with the cytoplasmic domain of non-phosphorylated ERBB3; the interaction requires PKC activity. Interacts with AR. Treatment with HRG leads to dissociation from ERBB3 and increases association with AR. Interacts with NCL/nucleolin. Component of a

						ribonucleoprotein complex containing at least PA2G4, NCL, TOP1, PABPC2, RPLP0, acetylated histone H1 (HIST1H1A or H1F1), histone H1 2/4, RPL4, RPL8, RPL15, RPL18, RPL18A, RPL21, RPL11, RPL12, RPL28, RPL27, RPLP2 and RPL24. Interacts with HDAC2. Interacts with RB1; the interaction is enhanced upon PA2G4 dephosphorylation.
54	**Calumenin**	CALU	231	4.47	37.0835	Involved in regulation of vitamin K-dependent carboxylation of multiple N-terminal glutamate residues. Seems to inhibit gamma-carboxylase GGCX. Binds 7 calcium ions with a low affinity
22	**Moesin**	MOES	605	6.08	67.778	Probably involved in connections of major cytoskeletal structures to the plasma membrane. May inhibit herpes simplex virus 1 infection at an early stage. In resting T-cells, part of a PAG1-SLC9A3R1-MSN complex which is disrupted upon TCR activation. Binds SLC9A3R1. Interacts with PPP1R16B. Interacts with PDZD8. Interacts with SELPLG and SYK; mediates the activation of SYK by SELPLG.
24	**T-complex protein 1 subunit gamma**	TCPG	177	6.1	60.4953	Molecular chaperone; assists the folding of proteins upon ATP hydrolysis. As part of the BBS/CCT complex may play a role in the assembly of BBSome, a complex involved in ciliogenesis regulating transports vesicles to the cilia. Known to play a role, in vitro, in the folding of actin and tubulin.
23	**X-ray repair cross-complementing protein 6**	XRCC6	511	6.23	69.799	Single stranded DNA-dependent ATP-dependent helicase. Has a role in chromosome translocation. The DNA helicase II complex binds preferentially to fork-like ends of double-stranded DNA in a cell cycle-dependent manner. It works in the 3'-5' direction. Binding to DNA may be mediated by XRCC6. Involved in DNA non-homologous end joining (NHEJ) required for

						double-strand break repair and V(D)J recombination. The XRCC5/6 dimer acts as regulatory subunit of the DNA-dependent protein kinase complex DNA-PK by increasing the affinity of the catalytic subunit PRKDC to DNA by 100-fold. The XRCC5/6 dimer is probably involved in stabilizing broken DNA ends and bringing them together. The assembly of the DNA-PK complex to DNA ends is required for the NHEJ ligation step. Required for osteocalcin gene expression. Probably also acts as a 5'-deoxyribose-5-phosphate lyase (5'-dRP lyase), by catalysing the beta-elimination of the 5' deoxyribose-5-phosphate at an abasic site near double-strand breaks. 5'-dRP lyase activity allows to 'clean' the termini of abasic sites, a class of nucleotide damage commonly associated with strand breaks, before such broken ends can be joined. The XRCC5/6 dimer together with APEX1 acts as a negative regulator of transcription
17	**T-complex protein 1 subunit gamma**	TCPG	128	6.1	60.4953	Molecular chaperone; assists the folding of proteins upon ATP hydrolysis. As part of the BBS/CCT complex may play a role in the assembly of BBSome, a complex involved in ciliogenesis regulating transports vesicles to the cilia. Known to play a role, in vitro, in the folding of actin and tubulin.
16	**T-complex protein 1 subunit gamma**	TCPG	132	6.1		Molecular chaperone; assists the folding of proteins upon ATP hydrolysis. As part of the BBS/CCT complex may play a role in the assembly of BBSome, a complex involved in ciliogenesis regulating transports vesicles to the cilia. Known to play a role, in vitro, in the folding of actin and tubulin.
14	**T-complex**	TCPA	597	5.8	60.3056	Molecular chaperone; assists the folding of proteins upon ATP hydrolysis. As part of the

	protein 1 subunit alpha					BBS/CCT complex may play a role in the assembly of BBSome, a complex involved in ciliogenesis regulating transports vesicles to the cilia. Known to play a role, in vitro, in the folding of actin and tubulin.
37	**Cytochrome b-c1 complex subunit 1, mitochondrial**	QCR1	187	5.94	52.6124	This is a component of the ubiquinol-cytochrome c reductase complex (complex III or cytochrome b-c1 complex), which is part of the mitochondrial respiratory chain. This protein may mediate formation of the complex between cytochromes c and c1.
38	**RuvB-like 2**	RUVB2	571	5.49	51.1246	Possesses single-stranded DNA-stimulated ATPase and ATP-dependent DNA helicase (5' to 3') activity; hexamerisation is thought to be critical for ATP hydrolysis and adjacent subunits in the ring-like structure contribute to the ATPase activity. Component of the NuA4 histone acetyltransferase complex which is involved in transcriptional activation of select genes principally by acetylation of nucleosomal histones H4 and H2A. This modification may both alter nucleosome - DNA interactions and promote interaction of the modified histones with other proteins which positively regulate transcription. This complex may be required for the activation of transcriptional programs associated with oncogene and proto-oncogene mediated growth induction, tumour suppressor mediated growth arrest and replicative senescence, apoptosis, and DNA repair. The NuA4 complex ATPase and helicase activities seem to be, at least in part, contributed by the association of RUVBL1 and RUVBL2 with EP400. NuA4 may also play a direct role in DNA repair when recruited to sites of DNA damage. Proposed core component of

						the chromatin remodelling INO80 complex which is involved in transcriptional regulation, DNA replication and probably DNA repair.
61	**14-3-3 protein gamma**	1433G	310	4.8	28.2849	Adapter protein implicated in the regulation of a large spectrum of both general and specialised signalling pathways. Binds to a large number of partners, usually by recognition of a phosphoserine or phosphothreonine motif. Binding generally results in the modulation of the activity of the binding partner. Interacts with CDK16 and BSPRY. Interacts with WEE1 (C-terminal). Interacts with SAMSN1. Interacts with MLF1 (phosphorylated form); the interaction retains it in the cytoplasm. Interacts with Thr-phosphorylated ITGB2. Interacts with BCL2L11.Homodimer. Heterodimerizes with YWHAE. Homo- and hetero-dimerization is inhibited by phosphorylation on Ser-58. Interacts with FOXO4, NOXA1, SSH1 and ARHGEF2. Interacts with *Pseudomonas aeruginosa* exoS (unphosphorylated form). Interacts with BAX; the interaction occurs in the cytoplasm. Under stress conditions, MAPK8-mediated phosphorylation releases BAX to mitochondria. Interacts with phosphorylated RAF1; the interaction is inhibited when YWHAZ is phosphorylated on Thr-232. Interacts with TP53; the interaction enhances p53 transcriptional activity. The Ser-58 phosphorylated form inhibits this interaction and p53 transcriptional activity. Interacts with ABL1 (phosphorylated form); the interaction retains ABL1 in the cytoplasm. Interacts with PKA-phosphorylated AANAT; the interaction modulates AANAT enzymatic activity by increasing affinity for arylalkylamines and acetyl-CoA and protecting the enzyme from dephosphorylation and proteasomal degradation. It may also prevent

						thiol-dependent inactivation. Interacts with AKT1; the interaction phosphorylates YWHAZ and modulates dimerization. Interacts with GAB2 and TLK2.
50	**Actin, cytoplasmic 2**	ACTG	91	5.31	41.7658	Actins are highly conserved proteins that are involved in various types of cell motility and are ubiquitously expressed in all eukaryotic cells. Defects in ACTG1 are the cause of deafness autosomal dominant type 20 (DFNA20). DFNA20 is a form of sensorineural hearing loss. Sensorineural deafness results from damage to the neural receptors of the inner ear, the nerve pathways to the brain, or the area of the brain that receives sound information. DFNA20 is a form of sensorineural hearing loss. Sensorineural deafness results from damage to the neural receptors of the inner ear, the nerve pathways to the brain, or the area of the brain that receives sound information. Defects in ACTG1 are the cause of Baraitser-Winter syndrome type 2. A rare developmental disorder characterized by the combination of congenital ptosis, high-arched eyebrows, hypertelorism, ocular colobomata, and a brain malformation consisting of anterior-predominant lissencephaly. Other typical features include postnatal short stature and microcephaly, intellectual disability, seizures, and hearing loss.
51	**Secernin-1**	SCRN1	162	4.66	46.3525	Regulates exocytosis in mast cells. Increases both the extent of secretion and the sensitivity of mast cells to stimulation with calcium
52	**Ribonuclease inhibitor**	RINI	117	4.71	49.9411	Ribonuclease inhibitor which inhibits RNASE1, RNASE2 and ANG. May play a role in redox homeostasis.
41	**Peptidyl-**	FKBP4			51.7721	Immunophilin protein with PPIase and co-chaperone activities. Component of non-ligated steroid

	prolylcis-trans isomerase					receptors heterocomplexes through interaction with heat-shock protein 90 (HSP90). May play a role in the intracellular trafficking of heterooligomeric forms of steroid hormone receptors between cytoplasm and nuclear compartments. The isomerase activity controls neuronal growth cones via regulation of TRPC1 channel opening. Acts also as a regulator of microtubule dynamics by inhibiting MAPT/TAU ability to promote microtubule assembly.
10	**40S ribosomal protein SA**	RSSA	413	4.79	32.8334	Required for the assembly and/or stability of the 40S ribosomal subunit. Required for the processing of the 20S rRNA-precursor to mature 18S rRNA in a late step of the maturation of 40S ribosomal subunits. Also functions as a cell surface receptor for laminin. Plays a role in cell adhesion to the basement membrane and in the consequent activation of signalling transduction pathways. May play a role in cell fate determination and tissue morphogenesis. Acts as a PPP1R16B-dependent substrate of PPP1CA. Also acts as a receptor for several other ligands, including the pathogenic prion protein, viruses, and bacteria. This protein appears to have acquired a second function as a laminin receptor specifically in the vertebrate lineage. t is thought that in vertebrates 37/67 kDalaminin receptor acquired a dual function during evolution. It developed from the ribosomal protein SA, playing an essential role in the protein biosynthesis lacking any laminin binding activity, to a cell surface receptor with laminin binding activity.
32	**no id**	-	-	-	-	-
31	**T-complex protein 1**	TCPB	185	6.01	57.4521	Molecular chaperone; assists the folding of proteins upon ATP hydrolysis. As part of the BBS/CCT complex may play a role in the assembly of BBSome, a complex involved in

	subunit beta					ciliogenesis regulating transports vesicles to the cilia. Known to play a role, in vitro, in the folding of actin and tubulin
27	**no id**	-	-	-	-	-
15	**Cytosol aminopeptidase**	AMPL	350	8.03	56.1308	Presumably involved in the processing and regular turnover of intracellular proteins. Catalyzes the removal of unsubstituted N-terminal amino acids from various peptides.
35	**no id**	-	-	-	-	-
18	**T-complex protein 1 subunit beta**	TCPB	2080	6.01	57.4521	Molecular chaperone; assists the folding of proteins upon ATP hydrolysis. As part of the BBS/CCT complex may play a role in the assembly of BBSome, a complex involved in ciliogenesis regulating transports vesicles to the cilia. Known to play a role, in vitro, in the folding of actin and tubulin
20	**no id**	-	-	-	-	-
28	**Proteasome subunit alpha type-1**	PSA1	442	6.15	29.5369	The proteasome is a multicatalytic proteinase complex which is characterized by its ability to cleave peptides with Arg, Phe, Tyr, Leu, and Glu adjacent to the leaving group at neutral or slightly basic pH. The proteasome has an ATP-dependent proteolytic activity. Mediates the lipopolysaccharide-induced signal transduction in the macrophage proteasome. Might be involved in the anti-inflammatory response of macrophages during the interaction with C.albicans heat-inactivated cells.
11	**T-complex protein 1**	TCPQ	886	5.42	59.5825	Molecular chaperone; assists the folding of proteins upon ATP hydrolysis. As part of the BBS/CCT complex may play a role in the assembly of BBSome, a complex involved in

	subunit theta					ciliogenesis regulating transports vesicles to the cilia. Known to play a role, in vitro, in the folding of actin and tubulin.
6	**Glutamate dehydrogenase 1, mitochondrial**	DHE3	166	7.66	61.3592	May be involved in learning and memory reactions by increasing the turnover of the excitatory neurotransmitter glutamate. Defects in GLUD1 are the cause of familial hyperinsulinemichypoglycemia type 6 (HHF6) [MIM:606762]; also known as hyperinsulinism-hyperammonemia syndrome (HHS). Familial hyperinsulinemichypoglycemia [MIM:256450], also referred to as congenital hyperinsulinism, nesidioblastosis, or persistent hyperinsulinemichypoglycemia of infancy (PPHI), is the most common cause of persistent hypoglycemia in infancy and is due to defective negative feedback regulation of insulin secretion by low glucose levels. In HHF6 elevated oxidation rate of glutamate to alpha-ketoglutarate stimulates insulin secretion in the pancreatic beta cells, while they impair detoxification of ammonium in the liver.
30	**40S ribosomal protein SA**	RSSA	198	4.79	44.0791	Required for the assembly and/or stability of the 40S ribosomal subunit. Required for the processing of the 20S rRNA-precursor to mature 18S rRNA in a late step of the maturation of 40S ribosomal subunits. Also functions as a cell surface receptor for laminin. Plays a role in cell adhesion to the basement membrane and in the consequent activation of signalling transduction pathways. May play a role in cell fate determination and tissue morphogenesis. Acts as a PPP1R16B-dependent substrate of PPP1CA. Also acts as a receptor for several other ligands, including the pathogenic prion protein, viruses, and bacteria. This protein appears to have

						acquired a second function as a laminin receptor specifically in the vertebrate lineage. t is thought that in vertebrates 37/67 kDalaminin receptor acquired a dual function during evolution. It developed from the ribosomal protein SA, playing an essential role in the protein biosynthesis lacking any laminin binding activity, to a cell surface receptor with laminin binding activity.
45	**no id**	-	-	-	-	-
34	**Actin, cytoplasmic 2**	ACTG	819	5.31	41.7658	Actins are highly conserved proteins that are involved in various types of cell motility and are ubiquitously expressed in all eukaryotic cells. Defects in ACTG1 are the cause of deafness autosomal dominant type 20 (DFNA20). DFNA20 is a form of sensorineural hearing loss. Sensorineural deafness results from damage to the neural receptors of the inner ear, the nerve pathways to the brain, or the area of the brain that receives sound information. DFNA20 is a form of sensorineural hearing loss. Sensorineural deafness results from damage to the neural receptors of the inner ear, the nerve pathways to the brain, or the area of the brain that receives sound information. Defects in ACTG1 are the cause of Baraitser-Winter syndrome type 2. A rare developmental disorder characterized by the combination of congenital ptosis, high-arched eyebrows, hypertelorism, ocular colobomata, and a brain malformation consisting of anterior-predominant lissencephaly. Other typical features include postnatal short stature and microcephaly, intellectual disability, seizures, and hearing loss.
29	**T-complex protein 1**	TCPB	874	6.01	57.4521	Molecular chaperone; assists the folding of proteins upon ATP hydrolysis. As part of the BBS/CCT complex may play a role in the assembly of BBSome, a complex involved in

	subunit beta					ciliogenesis regulating transports vesicles to the cilia. Known to play a role, in vitro, in the folding of actin and tubulin
36	**Tubulin alpha-1B chain**	TBA1B	1170	4.94	50.1196	Tubulin is the major constituent of microtubules. It binds two moles of GTP, one at an exchangeable site on the beta chain and one at a non-exchangeable site on the alpha-chain. Undergoes a tyrosination/detyrosination cycle, the cyclic removal and re-addition of a C-terminal tyrosine residue by the enzymes tubulin tyrosine carboxypeptidase (TTCP) and tubulin tyrosine ligase (TTL), respectively.
40	**Endoplasmin**	ENPL	1330	4.76	92.4113	Molecular chaperone that functions in the processing and transport of secreted proteins. When associated with CNPY3, required for proper folding of Toll-like receptors. Functions in endoplasmic reticulum associated degradation (ERAD). Has ATPase activity.
12	**Alpha-enolase**	ENOA	386	7.01	47.1393	Multifunctional enzyme that, as well as its role in glycolysis, plays a part in various processes such as growth control, hypoxia tolerance and allergic responses. May also function in the intravascular and pericellularfibrinolytic system due to its ability to serve as a receptor and activator of plasminogen on the cell surface of several cell-types such as leukocytes and neurons. Stimulates immunoglobulin production. MBP1 binds to the myc promoter and acts as a transcriptional repressor. May be a tumour suppressor.
42	**Ezrin**	EZRI	1060	5.94	69.3697	Probably involved in connections of major cytoskeletal structures to the plasma membrane. In epithelial cells, required for the formation of microvilli and membrane ruffles on the apical pole. Along with PLEKHG6, required for normal macropinocytosis. Phosphorylated by tyrosine-

						protein kinases. Phosphorylation by ROCK2 suppresses the head-to-tail association of the N-terminal and C-terminal halves resulting in an opened conformation which is capable of actin and membrane-binding.
44	**L-lactate dehydrogenase B chain**	LDHB	443	5.71	36.6151	Responsible for the reaction (S)-lactate + NAD^+ = pyruvate + NADH.
53	**Adenine phosphoribosyltransferase**	APT	162	5.78	19.5954	Catalyses a salvage reaction resulting in the formation of AMPthat is energically less costly than de novo synthesis. Defects in APRT are the cause of adenine phosphoribosyltransferase deficiency (APRTD) [MIM:102600]; also known as 2,8-dihydroxyadenine urolithiasis. An enzymatic deficiency that can lead to urolithiasis and renal failure. Patients have 2,8-dihydroxyadenine (DHA) urinary stones.
55	**Translationally-controlled tumor protein**	TCTP	332	4.84	19.5826	Involved in calcium binding and microtubule stabilization. Found in several healthy and tumoral cells including erythrocytes, hepatocytes, macrophages, platelets, keratinocytes, erythroleukaemia cells, gliomas, melanomas, hepatoblastomas, and lymphomas. It cannot be detected in kidney and renal cell carcinoma (RCC). Expressed in placenta and prostate.
2	**Triosephosphateisomerase**	TPIS	152	5.65	30.7717	Catalytic activity D-glyceraldehyde 3-phosphate = glycerone phosphate.Defects in TPI1 are the cause of triosephosphateisomerase deficiency (TPI deficiency). TPI deficiency is an autosomal recessive disorder. It is the most severe clinical disorder of glycolysis. It is associated with neonatal jaundice, chronic haemolyticanaemia, progressive neuromuscular dysfunction,

						cardiomyopathy and increased susceptibility to infection.
59	**Rho GDP-dissociation inhibitor 1**	GDIR1	661	5.02	23.1927	Regulates the GDP/GTP exchange reaction of the Rho proteins by inhibiting the dissociation of GDP from them, and the subsequent binding of GTP to them. In glioma cells, inhibits cell migration and invasion by mediating the signals of SEMA5A and PLXNB3 that lead to inactivation of RAC1
68	**Heat shock protein beta-1**	HSPB1	579	5.98	22.7685	Involved in stress resistance and actin organization. Interacts with TGFB1I1. Associates with alpha- and beta-tubulin, microtubules and CRYAB. Interacts with HSPB8 and HSPBAP1
72	**Phosphoglycera temutase 1**	PGAM1	573	7.22	28.7858	Interconversion of 3- and 2-phosphoglycerate with 2,3-bisphosphoglycerate as the primer of the reaction. Can also catalyse the reaction of EC 5.4.2.4 (synthase) and EC 3.1.3.13(phosphatase), but with a reduced activity..
57	**14-3-3 protein zeta/delta**	1433Z	1030	4.73	27.7277	Adapter protein implicated in the regulation of a large spectrum of both general and specialised signalling pathways. Binds to a large number of partners, usually by recognition of a phosphoserine or phosphothreonine motif. Binding generally results in the modulation of the activity of the binding partner. Interacts with CDK16 and BSPRY. Interacts with WEE1 (C-terminal). Interacts with SAMSN1. Interacts with MLF1 (phosphorylated form); the interaction retains it in the cytoplasm. Interacts with Thr-phosphorylated ITGB2. Interacts with BCL2L11.Homodimer. Heterodimerises with YWHAE. Homo- and hetero-dimerization is inhibited by phosphorylation on Ser-58. Interacts with FOXO4, NOXA1, SSH1 and ARHGEF2. Interacts with *Pseudomonas aeruginosa* exoS (unphosphorylated form). Interacts with BAX; the

						interaction occurs in the cytoplasm. Under stress conditions, MAPK8-mediated phosphorylation releases BAX to mitochondria. Interacts with phosphorylated RAF1; the interaction is inhibited when YWHAZ is phosphorylated on Thr-232. Interacts with TP53; the interaction enhances p53 transcriptional activity. The Ser-58 phosphorylated form inhibits this interaction and p53 transcriptional activity. Interacts with ABL1 (phosphorylated form); the interaction retains ABL1 in the cytoplasm. Interacts with PKA-phosphorylated AANAT; the interaction modulates AANAT enzymatic activity by increasing affinity for arylalkylamines and acetyl-CoA and protecting the enzyme from dephosphorylation and proteasomal degradation. It may also prevent thiol-dependent inactivation. Interacts with AKT1; the interaction phosphorylates YWHAZ and modulates dimerization. Interacts with GAB2 and TLK2.
56	**14-3-3 protein zeta/delta**	1433Z	926	4.73	27.7277	Adapter protein implicated in the regulation of a large spectrum of both general and specialised signalling pathways. Binds to a large number of partners, usually by recognition of a phosphoserine or phosphothreonine motif. Binding generally results in the modulation of the activity of the binding partner. Interacts with CDK16 and BSPRY. Interacts with WEE1 (C-terminal). Interacts with SAMSN1. Interacts with MLF1 (phosphorylated form); the interaction retains it in the cytoplasm. Interacts with Thr-phosphorylated ITGB2. Interacts with BCL2L11.Homodimer. Heterodimerises with YWHAE. Homo- and hetero-dimerization is inhibited by phosphorylation on Ser-58. Interacts with FOXO4, NOXA1, SSH1 and ARHGEF2. Interacts with *Pseudomonas aeruginosa* exoS (unphosphorylated form). Interacts with BAX; the

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1	**Proteasome activator complex subunit 1**	PSME1	295	5.78	28.705	Implicated in immunoproteasome assembly and required for efficient antigen processing. The PA28 activator complex enhances the generation of class I binding peptides by altering the cleavage pattern of the proteasome.
70	**Ran-specific GTPase-activating protein**	RANG	146	5.19	23.2956	Inhibits GTP exchange on Ran. Forms a Ran-GTP-RANBP1 trimeric complex. Increase GTP hydrolysis induced by the Ran GTPase activating protein RANGAP1. May act in an intracellular signalling pathway which may control the progression through the cell cycle by regulating the transport of protein and nucleic acids across the nuclear membrane.
63	**Proliferating**	PCNA	502	4.57	28.7503	Auxiliary protein of DNA polymerase delta and is involved in the control of eukaryotic DNA

	cell nuclear antigen					replication by increasing the polymerase's processibility during elongation of the leading strand. Induces a robust stimulatory effect on the 3'-5' exonuclease and 3'-phosphodiesterase, but not apurinic-apyrimidinic (AP) endonuclease, APEX2 activities. Has to be loaded onto DNA in order to be able to stimulate APEX2. Plays a key role in DNA damage response (DDR) by being conveniently positioned at the replication fork to coordinate DNA replication with DNA repair and DNA damage tolerance pathways. Acts as a loading platform to recruit DDR proteins that allow completion of DNA replication after DNA damage and promote postreplication repair: Monoubiquitinated PCNA leads to recruitment of translesion (TLS) polymerases, while 'Lys-63'-linked polyubiquitination of PCNA is involved in error-free pathway and employs recombination mechanisms to synthesize across the lesion.
58	**Annexin A3**	ANXA3	424	5.63	36.3527	Inhibitor of phospholipase A2, also possesses anti-coagulant properties. Also cleaves the cyclic bond of inositol 1,2-cyclic phosphate to form inositol 1-phosphate. A pair of annexin repeats may form one binding site for calcium and phospholipid.
60	**Small glutamine-rich tetratricopeptide repeat-containing protein alpha**	SGTA	66	4.81	44.0791	Co-chaperone that binds directly to HSC70 and HSP70 and regulates their ATPase activity.

13	**Elongation factor Tu, mitochondrial**	EFTU	317	7.26	49.5102	This protein promotes the GTP-dependent binding of aminoacyl-tRNA to the A-site of ribosomes during protein biosynthesis. Defects in TUFM are the cause of combined oxidative phosphorylation deficiency type 4 (COXPD4) [MIM:610678]. COXPD4 is characterized by neonatal lactic acidosis, rapidly progressive encephalopathy, severely decreased mitochondrial protein synthesis, and combined deficiency of mtDNA-related mitochondrial respiratory chain complexes.
62	**Actin, cytoplasmic 2**	ACTG	886	5.31	41.7658	Actins are highly conserved proteins that are involved in various types of cell motility and are ubiquitously expressed in all eukaryotic cells. Defects in ACTG1 are the cause of deafness autosomal dominant type 20 (DFNA20). DFNA20 is a form of sensorineural hearing loss. Sensorineural deafness results from damage to the neural receptors of the inner ear, the nerve pathways to the brain, or the area of the brain that receives sound information. DFNA20 is a form of sensorineural hearing loss. Sensorineural deafness results from damage to the neural receptors of the inner ear, the nerve pathways to the brain, or the area of the brain that receives sound information. efects in ACTG1 are the cause of Baraitser-Winter syndrome type 2. A rare developmental disorder characterized by the combination of congenital ptosis, high-arched eyebrows, hypertelorism, ocular colobomata, and a brain malformation consisting of anterior-predominant lissencephaly. Other typical features include postnatal short stature and microcephaly, intellectual disability, seizures, and hearing loss.
65	**Calreticulin**	CALR	477	4.29	48.1118	Calcium-binding chaperone that promotes folding, oligomeric assembly and quality control in the

						endoplasmic reticulum (ER) via the calreticulin/calnexin cycle. This lectin interacts transiently with almost all of the monoglucosylated glycoproteins that are synthesized in the ER. Interacts with the DNA-binding domain of NR3C1 and mediates its nuclear export. Involved in maternal gene expression regulation. May participate in oocyte maturation via the regulation of calcium homeostasis.
66	**Protein disulfide-isomerase A3**	PDIA3	557	5.98	56.7468	Catalyzes the rearrangement of -S-S- bonds in proteins. Subunit of the TAP complex, also known as the peptide loading complex (PLC). Can form disulphide-linked heterodimers with TAPBP. Interacts with ERP27 and CANX.
67	**Bifunctional purine biosynthesis protein PURH**	PUR9	247	6.27	64.5753	Bifunctional enzyme that catalyses 2 steps in purine biosynthesis
5	**Stress-induced-phosphoprotein 1**	STIP1	240	6.4	62.5994	Mediates the association of the molecular chaperones HSC70 and HSP90 (HSPCA and HSPCB).
7	**Stress-70 protein, mitochondrial**	GRP75	931	5.87	73.6348	Implicated in the control of cell proliferation and cellular aging. May also act as a chaperone. Interacts with FXN. Interacts with HSCB. Component of the MINOS/MitOS complex that includes IMMT, HSPA9 and CHCHD3 and associates with mitochondrial outer membrane proteins SAMM50, MTX1 and MTX2.

71	**Stress-70 protein, mitochondrial**	GRP75	876	5.87	73.6348	Implicated in the control of cell proliferation and cellular aging. May also act as a chaperone. Interacts with FXN. Interacts with HSCB. Component of the MINOS/MitOS complex that includes IMMT, HSPA9 and CHCHD3 and associates with mitochondrial outer membrane proteins SAMM50, MTX1 and MTX2.
73	**Ezrin**	EZRI	734	5.94	69.3697	Probably involved in connections of major cytoskeletal structures to the plasma membrane. In epithelial cells, required for the formation of microvilli and membrane ruffles on the apical pole. Along with PLEKHG6, required for normal macropinocytosis. Phosphorylated by tyrosine-protein kinases. Phosphorylation by ROCK2 suppresses the head-to-tail association of the N-terminal and C-terminal halves resulting in an opened conformation which is capable of actin and membrane-binding.
8	**Heat shock 70 kDa protein 1A/1B**	HSP71	1020	5.48	70.009	In cooperation with other chaperones, Hsp70s stabilize pre-existent proteins against aggregation and mediate the folding of newly translated polypeptides in the cytosol as well as within organelles. These chaperones participate in all these processes through their ability to recognize nonnative conformations of other proteins. They bind extended peptide segments with a net hydrophobic character exposed by polypeptides during translation and membrane translocation, or following stress-induced damage. In case of rotavirus A infection, serves as a post-attachment receptor for the virus to facilitate entry into the cell. Component of the CatSper complex. Identified in a mRNP granule complex, at least composed of ACTB, ACTN4, DHX9, ERG, HNRNPA1, HNRNPA2B1, HNRNPAB, HNRNPD, HNRNPL, HNRNPR, HNRNPU, HSPA1,

						HSPA8, IGF2BP1, ILF2, ILF3, NCBP1, NCL, PABPC1, PABPC4, PABPN1, RPLP0, RPS3, RPS3A, RPS4X, RPS8, RPS9, SYNCRIP, TROVE2, YBX1 and untranslated mRNAs. Interacts with TSC2. Interacts with IRAK1BP1. Interacts with TERT; the interaction occurs in the absence of the RNA component, TERC, and dissociates once the TERT complex has formed. Interacts with DNAJC7. Interacts with CHCHD3.
74	**Heat shock protein HSP 90-beta**	HS90B	1470	4.97	83.2121	Molecular chaperone that promotes the maturation, structural maintenance and proper regulation of specific target proteins involved for instance in cell cycle control and signal transduction. Undergoes a functional cycle that is linked to its ATPase activity. This cycle probably induces conformational changes in the client proteins, thereby causing their activation. Interacts dynamically with various co-chaperones that modulate its substrate recognition, ATPase cycle and chaperone function. Homodimer. Interacts with p53/TP53. Forms a complex with CDK6 and Hsp90/HSP90AB1. Interacts with UNC45A. Binding to UNC45A involves 2 UNC45A monomers per HSP90AB1 dimer. Interacts with CHORDC1 and DNAJC7. Interacts with FKBP4.

[a] http://www.uniprot.org/