Sponges are highly resistant to radiation exposure and cancer

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Abstract

There are no reports of cancer in sponges, despite them having somatic cell turnover, long lifespans and no specialized adaptive immune cells. In order to investigate whether sponges are cancer resistant, we exposed a species of sponge, *Tethya wilhelma*, to X-rays. We found that *T. wilhelma* can withstand 600 Gy of X-ray radiation. That is approximately 100 times the lethal dose for humans. A single high dose of X-rays did not induce cancer in sponges, providing the first experimental evidence of cancer resistance in the phylum, Porifera. Following X-ray exposure, we found an overexpression of genes involved in DNA repair, signaling transduction pathways and epithelial to mesenchymal transition. Sponges have the highest level of radiation resistance that has yet been observed in animals that have sustained somatic cell turnover. This may make them an excellent model system for studying cancer resistance and developing new approaches for cancer prevention and treatment.

Introduction

To date there have been no reports of cancer in sponges¹. Sponges are part of the phylum Porifera, and they have a long lifespan and somatic cell turnover², which should make them susceptible to cancer because over the course of their lifespans they would be expected to accumulate carcinogenic mutations. Here we set out to investigate whether sponges are particularly cancer resistant, and, if so, what the mechanisms underlying this cancer resistance are. Through a combination of microscopy and transcriptomics, we were able to observe changes in *Tethya wilhelma* (Demosponges)^{3,4} after X-ray exposure and assess the organism-level, cell-level and gene expression changes over time.

Basal invertebrates like sponges that lack⁵ immune specialized cells⁶ or with primitive elements of an adaptive immune system^{7,8}, may lack the ability to detect and eliminate mutant cells. They should be particularly susceptible to cancer. However, the fact that no cancer has been reported in sponges¹ suggests that they might have a physiology that is resilient to mutations or possess effective mechanisms for DNA damage prevention, DNA repair, and tissue homeostasis.

Sponges are an effective model system for studying cancer resistance.

In order to investigate cancer resistance in sponges and evaluate this hypothesis, we studied the sponge *T. wilhelma*, which is a sessile, filter-feeding demosponge that originally came from the Indo-Pacific oceans⁹ (Fig.1). Demosponges possess a canal system which is characterized by a highly complex network of chambers lined with choanocytes, which are flagellated cells that are specialized in creating a flow of water and capturing food particles¹⁰. Water enters from the pores located mainly on the lateral walls of sponges and is discarded through a large medial

excurrent canal opening on the apex of sponges, the osculum¹⁰. *T. wilhelma* have a globular shape and the largest specimens can reach the size of 15-20 mm in diameter (Fig.1). *T. wilhelma* reproduce asexually by budding in the laboratory¹¹. These sponges are capable of contractile and slow locomotory behavior^{3,4} and adapt well to being cultured in aquaria^{3,4}.

The genome sequence of *T. wilhelma* is available¹² and genomic analyses have shown that many molecular pathways (e.g. signal transduction mechanisms) are well conserved on sponges¹². *T. wilhelma* has a remarkably long lifespan but sufficiently short generation time³ to make it possible to rapidly obtain experimental results.

This is the first study of radiation resistance and cancer resistance in porifera, the phylum at the very base of animal life on earth.

Figure 1. *T. wilhelma* have a globular shape. They produce filamentous body extensions and are not pigmented. The largest specimens can reach the size of 15-20 mm in diameter. This image was taken using dark field illumination.

Materials and Methods

Lab cultures. We built a sponge culture system that consists of a main saltwater aquarium (340 liters) that develops an ecosystem (coral reef) capable of supporting the growth of the sponges. The sponges are grown in 3 smaller (30 liter) culture aquariums connected to the main one, with controlled temperature (24°C) and water flow. This setting allows easy access to the sponges, including the observation of the sponges under a microscope without having to remove them from the aquarium.

We fed the sponges with artificial plankton (Aquakultur Genzel GmbH, Germany) twice daily. In order to obtain fine (<25µm) food particles assimilable by sponges, we homogenized the artificial plankton with an IKA T10 Basic Ultra Turrax homogenizer.

The culturing of the sponges started 4 years prior to this study, with 20 animals. The original specimens are still alive, thus the lifespan of the sponges in our laboratory setting is > 4 years.

DNA damage by X-ray irradiation. We exposed young adult sponges (diameter>5mm) to X-rays utilizing a RS-2000 Biological System X-ray irradiator. We exposed the sponges to a single dose of 600 Gy for the experiments. Considering X-ray absorbance of the 10 mm column of

water above the animals during the X-ray irradiation, we estimated the actual X-ray exposure of specimens to be 13.7% lower, 518 Gy.

Experimental settings. Based on preliminary morphological observations, we selected three time points: 24 hours, 7 days, and 21 days after X-ray exposure for both histological and transcriptomic analyses (RNA-seq). We randomly selected 3 sponges for each time point plus 3 specimens of control for both histological and transcriptome analyses, for a total of 36 sponges. We treated 4 additional independent groups of sponges (n=6, n=5, n=5, n=5, total 21 sponges) and relative controls (5 sponges for each group, total 20 sponges), for long-term morphological observations. Each group was exposed to X-rays at different times.

Morphological analysis. We observed the morphological changes in the animals *in vivo* using ImageJ software¹³. Sponges are partially translucent, but only superficial structures can be observed *in vivo*. However, their shape is regular, and any morphological changes are easily observable.

For histological examination we fixed the specimens with Papanicolaou's fluid¹⁴ for 24 hours at 4°C. Then, we dissolved the siliceous spicules that make up its skeleton by submerging the specimens in 4% hydrofluoric acid (MilliporeSigma, cat. n. 1.00338) for an additional 24 hours at 4°C, then we followed standard histological protocols^{15,16}.

For transmission electron microscopy, we fixed specimens in 2.5% glutaraldehyde (Electron Microscopy Sciences, cat.n. 16020) in 0.2 M Na-cacodylate sucrose buffer (pH 7.2; Electron Microscopy Sciences, cat.n. 12300) for 2.5 hours at 4°C. Then, we rinsed the specimens 3 times with a 0.2 cacodylate sucrose buffer for 45-60 min total, post fixed them for 2 hours in

1% osmium tetroxide (Electron Microscopy Sciences, cat n. 19150) 0.2 cacodylate sucrose buffer and washed them 1 time with buffer, then 3 times with deionized water for 45-60 min total. We stained them *en bloc* with 1% aqueous uranyl acetate (Electron Microscopy Sciences, cat n. 22400) for 16 hours at 4°C. After washing the specimens 4 times with water for 45-60 min total. We dehydrated them with an ascending ethanol series up to 70% ethanol. Then, we disilicate the specimens with 4% hydrofluoric acid for 1 hour at 4°C. Afterwards, we washed specimens in 70% ethanol, and we completed the dehydration with an ethanol series up to 100%. Then we transferred the specimens to anhydrous propylene oxide (cat. n. 14300) for 30 minutes (replacing the anhydrous propylene oxide with fresh one after 15 minutes). We infiltrated samples with 5% Spurr's epoxy resin (in anhydrous propylene oxide 3 hours with rotation; 50% resin in anhydrous propylene oxide overnight with rotation (18 hr); 75% resin in anhydrous propylene oxide with rotation (6 hr); 100% pure resin 3x for 24 hr total (6 hr, 12 hr, 6 hr). Finally, we flat-embedded the specimens and polymerized them at 60°C for 27 hrs. We used a diamond knife to cut ultrathin sections. We observed the sections under a Philips CM12 transmission electron microscope.

Quantification of DNA damage. We quantified the DNA damage caused by X-ray exposure by the silver-stained Comet alkaline assay (Travigen®, Cat#4251-050-K)^{17,18} according to the manufacturer's specifications and we used ImageJ software¹³ to quantify the DNA fragmentation.

Molecular genetic analysis. We treated the *T. wilhelma* specimens with 600 Gy (actually 518 Gy due to water absorbance). After 24 hours, 7 days, and 21 days following X-ray exposure we extracted the total RNA (RNeasy® mini kit, Qiagen, cat. n. 74104) from 3 sponges for each

treatment and control. After verifying the purity and integrity of the RNA using an Agilent 2200 TapeStation, part of the extracted RNA (11.06 ng per sample on average) was utilized for RNA-seq analysis. We sequenced the samples using an Illumina NextSeq 500 instrument. We checked the quality of the RNA-seq reads for each sample using FastQC v0.10.1 and we aligned the reads with the reference genome (NCBI, SRA, SRR2163223) using STAR v2.5.1b (22.68 million reads uniquely mapped on average per sample). Cufflinks v2.2.1 was used to report FPKM values (Fragments Per Kilobase of transcripts per Million mapped reads) and read counts. We uniquely mapped 17.05 million reads to the reference genome on average, per sample. We performed a differential expression analysis using the EdgeR package from Bioconductor v3.2 in R 3.2.3. For each pairwise comparison, genes with false discovery rate (FDR) <0.05 were considered significant and 2 log₂-fold changes of expression between conditions were reported after Bonferroni correction. We analyzed the differentially expressed genes using BLAST¹⁹, Ensembl²⁰ and their functional annotations, including fold enrichment (FE), using DAVID^{21,22} and PANTHER²³ software, and protein domains²⁴. We focused on the overexpressed genes because the decrease of gene expression can be a nonspecific effect of X-ray exposure due to cellular damage.

Results

We initially conducted a dose finding experiment to quantify the maximum tolerance to increasing doses (range: 160-800 Gy) of radiation on sponges. After the treatment we observed the sponges daily. An 800 Gy dose is lethal for sponges (n=5, 80% lethality). In contrast, all sponges (n=7) exposed to 600 Gy suffered transitory morphological changes but survived. We then conducted the subsequent experiments using a single dose of 600 Gy.

Morphological observations. We observed a general pattern in the morphological changes over time in sponges after X-ray exposure (Fig. 2, 3 and Fig. 1S).

a) Initially (2-7 days) the sponges begin to shrink, producing short and thin body extensions, and the pores and the osculum are no longer visible (Fig. 2, 3 and Fig. 1S). Sponges reach the minimum size after 21-25 days (Fig. 2, 3; paired t-test, $df=16$, $t=6.341$, $p<0.0001$), their surface became smooth and they produced large and long body projections, causing sponges to acquire an irregular star shape (Fig. 2, 3 and Fig. 1S).

b) Then, sponges reverse the shrinking process, but their morphologic features appear to be still altered or progress to further dissolvment (Fig. 2): body projections increased their surface and additional body projections were generated (Fig. 2). Body extensions were either re-absorbed or broke off, generating new satellite sponges, observed in 33.3% of sponges (Fig. 2-4 and Fig. 1S).

c) Sponges gradually reacquired their original anatomical organization and appeared normal after ~180 days. Four out of 21 treated sponges died after an average of 162 (± 30.9 S.D.) days. At the time of this writing, it has been over 1 year since treatment. 17 of 21 sponges treated with 600 Gy are alive, do not show any morphological changes, and are indistinguishable from untreated sponges (Fig. 2, 3 and Fig. 1S).

Figure 2. We observed morphological changes of 6 sponges after exposure to 600 Gy of X-rays.

T. wilhelma sponges are capable of locomotion and their position can slowly change over time.

The first picture was taken starting before the exposure (Day 0) and pictures were taken periodically until 234 days after the exposure. Each sponge is identified with a number. The offspring of the sponges are identified with the number of the parental sponge and a letter (e.g. sponge 2, offspring 2a). Sponge 5 had the most dramatic changes in morphology and generated 4 satellite sponges. Sponges 1 and 4 fused completely by day 234.

Figure 3. Morphologic changes in two sponges (A, B) 91 days after X-ray exposure. Sponges can develop extensive body projections. The body projections either generate new satellite sponges (arrows) or they are reabsorbed.

Figure 4. The size of sponges decreased after X-ray treatment (t-test controls vs X-ray treated sponges, paired by time point, $df=16$, $t=6.341$, $p<0.0001$). The blue line shows the control sponges ($n=10$) and the red line shows the X-ray treated sponges ($n=21$). Sponges reach the smallest size after 21-25 days. Then, they gradually increased in size and recovered their morphological features.

Histological analysis. After X-ray exposure, sponges lose their typical anatomical organization¹⁶ (Fig. 5). The filtering structures of sponges (choanoderm) and the specialized water flowing and feeding cells (choanocytes) are lost (Fig. 5), and the choanoderm appear to be filled with undifferentiated cells²⁵. The histological analyses did not show necrotic areas at any time after X-ray exposure (Fig. 5).

Figure 5. *T. wilhelma* untreated (A, A1), after 24 hours (B, B1), 7 days (C, C1), and 21 days (D, D1) from X-ray exposure. A1, B1, C1 and D1 (scale bar=100 μ m) are a magnification of samples A, B, C, D and D (scale bar= 200 μ m). Twenty-four hours after X-ray exposure, control sponges have anatomical features indistinguishable from untreated sponges (A vs. B). The normal anatomy is completely lost after 7 days and cells show an undifferentiated phenotype (C, C1). After 21 days most cells still appear to have an undifferentiated phenotype but anatomical reorganization has begun (Hematoxylin & eosin stain; ccb=choanoderm; mes=peripheral mesohyl; ccb-mes boundary= mesohyl of the cortex–choanoderm boundary).

Electron microscopy analysis.

We further investigated the morphological changes of the choanoderm after 7 days from X-ray exposure. Indeed, the choanoderm of treated sponges is disorganized and the choanocyte chambers are deeply altered or absent. The choanocytes are not anymore recognizable (Fig. 6).

The mutualistic bacteria, presumably Cyanobacteria²⁶, are able to survive the X-ray treatment as well.

Figure 6. Transmission electron microscopy analysis of control (A, B) and X-ray exposed sponges (C-F), 7 days after exposure. The choanoderm of control sponges is well organized with choanocyte chambers (green asterisk *). Instead, the choanoderm of treated sponges (C-F) is disorganized and the choanocyte chambers are absent. The X-ray exposed choanoderm is characterized by the extensive presence of vesicle and membrane heterogeneous structures. The nuclei appear to be intact and have different shapes. Black arrows=choanocytes, blue head arrow=mitochondria, **N**=nucleus, white arrows=bacteria.

DNA damage analysis. DNA fragmentation analysis (Comet assay) showed limited DNA degradation immediately after a submaximal (600 Gy) X-ray exposure (DNA fragmentation, treated: $8.23\% \pm 16.32$ S.D., controls $1.34\% \pm 6.99$ S.D. (Fig. 7).

Figure 7. The X-ray treatment produces little DNA fragmentation as measured by the Comet assay. There is evidence of DNA in: $8.23\% \pm 16.32$ S.D. of treated sponges, and $1.34\% \pm 6.99$ S.D. controls. Representative images of control (A) and X-ray treated (B) sponge nuclei, arrows indicate examples of partially fragmented nuclei (comets).

Gene expression analysis: We performed the transcriptome analysis (RNA-seq) of 3 *T. wilhelma* specimens collected at each of 3 different time points (24 hours, 7 and 21 days) after X-ray treatment. We found a total of 639 overexpressed transcripts in the three experimental time points compared to untreated sponges at the same time points (Fig. 8). Each group had different gene expression levels and differs from the controls (Fig. 2S). Many of the expressed genes have a human homolog (given in parentheses). There are genes overexpressed only at a specific time point (24 hours, 7 or 21 days) and genes overexpressed at 2 or 3 time points (Tab. 3S, fig. 8 and fig. 3S).

Figure 8. Venn diagram shows the number of genes that were over-expressed at the 3 different time points (24 hours, 7 and 21 days) after X-ray treatment. There are genes overexpressed only at a specific time point and genes overexpressed at 2 or 3 time points. The number of transcripts detected increased from 24 hours to 21 days. 24 hours and 21 days share only 1.6% of transcripts, on the contrary 7 days and 21 days have the largest percentage (25.4%) of

overlapping transcripts. Genes can be activated at different times and for a different length of time after X-ray exposure. Venn diagram shows the exact number of transcripts expressed. There is an enrichment of genes involved in DNA repair specifically expressed after 24 hours and in signaling after 7 days. EMC= extracellular matrix.

Genes overexpressed 24 hours after X-ray exposure: We found 195 transcripts overexpressed 24 hours after X-ray exposure, of which 97 have a human homolog (Fig. 8, tab. 1S and fig. 3S). Sixty-four of those 195 transcripts were only overexpressed after 24 hours but not at later time points. Forty-four of those 64 overexpressed transcripts specific to the 24-hour time point have a human homolog. We detected an enrichment of human homolog genes involved in DNA repair (FE=10.3, FDR= 0.037, DAVID) such as *twi_ss.22376.1 (LIG3)*, DNA ligase and *twi_ss.21448.1 (MRE11)*, involved in the double strand break repair mechanisms, *twi_ss.11375.1 (PCNA)*, proliferating cell nuclear antigen, has a key role in DNA damage response, *twi_ss.28211.1 (RPA1)*, DNA repair pathway and the replication protein A1, is a cofactor of DNA polymerase delta involved in the RAD6-dependent DNA repair pathway, *Twiss.25822.1-3 (GINS4)*, GINS subunit, domain A, is involved in double-strand break repair via break-induced replication.

We found that *twi_ss.17326.7* gene (*CUBN*), cubilin, is the most differentially expressed gene (logFC=8.55) specific to the 24-hour time point after X-ray exposure. *CUBN* is an endocytic receptor expressed in the epithelium of intestines and kidneys²⁷, and down-regulated in renal cell carcinoma²⁸.

In addition, we identified overexpression of genes involved in stress response such as *twi_ss.28284.1 (HSPA1A)*, the heat shock protein family A (Hsp70) member 1A.

Genes overexpressed 7 days after X-ray exposure: We found 408 transcripts overexpressed, of which 141 have a human homolog (Fig. 8, tab. 1S and fig. 3S). Among them, 125 transcripts were specifically overexpressed after 7 days, of which 53 have a human homolog. We detected an enrichment of ankyrin containing domain genes (FE=19, FDR< 0.001), signal proteins (FE=2.4, FDR= 0.005) and extracellular matrix remodeling genes (FE=68.1, FDR= 0.03).

We identified genes specifically overexpressed after 7 days involved in DNA repair such as twi_ss.10792.1 (*FAN1*), twi_ss.27976.3 (*KAT5*) and twi_ss.19635.1 (*TRIP12*) and, importantly, we found a correspondence with the morphological changes observed 7 days after X-ray exposure and the function of genes overexpressed at the same time. For instance, we found the overexpression of genes involved in development, adult tissue homeostasis and mesenchymal transition such as twi_ss.2267.1 (*NOTCH1*), twi_ss.5628.6 (*MET*) MET proto-oncogene, receptor tyrosine kinase, twi_ss.6204.1 (*POSTN*), periostin. We also found genes involved in embryonic stem cell regulation such as twi_ss.7082.1 (*ETV4*), twi_ss.4355.1 (*TRIM71*), twi_ss.329.1 (*ZFP36L1*) together with a variety of genes such as twi_ss.26378.1 (*COL6A3*) collagen type VI alpha 3 chain, twi_ss.31853a.1 (*COL6A6*) collagen type VI alpha 6 chain, twi_ss.25970.2 (*LTBP1*) latent transforming growth factor beta binding protein 1, twi_ss.20516a.5 (*SORL1*) sortilin related receptor 1, twi_ss.2619.7 (*VWF*) von Willebrand factor, twi_ss.19885.1 (*NRXN3*) neurexin 3, twi_ss.1244.5 (*ADGRE5*) adhesion G protein-coupled receptor E5 involved with the epithelial-mesenchymal transition.

Gene overexpressed 21 days after X-ray exposure: we found genes 410 overexpressed transcripts of which 150 of these have a human homolog (Fig. 8, tab. 1S and fig. 3S). We identified 157 transcripts specifically overexpressed after 21 days of which 72 have a human

homolog. There is not a functional signature specific to the genes expressed specifically after 21 days. We found genes involved in DNA double-strand break repair: twi_ss.10068.1 (*PARP3*) poly (ADP-ribose) polymerase family member 3, twi_ss.28718.1 (*PARPF19*) PHD finger protein 19 and, DNA repair: twi_ss.7757.3 (*UBR5*) ubiquitin protein ligase E3 component n-recognin 5.

Gene overexpressed 24 hours, 7 and 21 days after X-ray exposure: We found 81 transcripts overexpressed at all 3 time points of which 34 have a human homolog (Fig. 8, tab. 1S and fig. 3S). For instance, Twi_ss.16656.2 (*PHF8*), PHD Finger Protein 8 one of the most differentially expressed gene (9.3 ± 1.8 S.D. logFC), the *C. elegans* homolog promotes DNA repair via homologous recombination²⁹. Twi_ss.4977.9 (8.9 ± 0.4 S.D. logFC) is a homolog of the human gene Spatacsin (*SPG11*). The function of *SPG11* is not well understood. It has a role in a form of spastic paraplegia, a neurodegenerative disorder and it appears to be also involved in DNA repair³⁰.

Gene overexpressed 24 hours and 7 days after X-ray exposure: we found 40 transcripts overexpressed at both time points of which 19 of these genes have a human homolog (Fig. 7-9, tab. 1S). The most differential expressed gene (8 ± 1.8 S.D. logFC) is twi_ss.12458.1 (*TTN*), a gene unknown to be activated after X-ray exposure.

Gene overexpressed 24 hours and 21 days after X-ray exposure: we found only 10 overexpressed transcripts of which 5 of these genes have a human homolog gene (Fig. 8, tab. 1S and fig. 3S). With only 5 genes with known homolog functions, there were not statistically significantly enriched pathways. However, twi_ss.19378.2 (*ERCC1*) is involved in DNA repair.

Gene overexpressed 7 days and 21 days after X-ray exposure: we found genes 162 overexpressed transcripts of which 45 of these have a human homolog gene (Fig. 8, tab. 1S and fig. 3S). Overall, there is an enrichment of genes involved in extracellular matrix organization

such as fibronectin (FE=15.7, FDR< 0.0001), with an EGF-like domain (FE=19.7, FDR<0.0001) and signal peptides (FE=3.3, FDR<0.0001). One of the most overexpressed genes (10.1 ± 0.7 S.D. logFC) is *twi_ss.21105.9 (SCUBE1)* signal peptide, CUB and EGF-like domain-containing protein 1, which may function as an adhesive molecule³¹.

Discussion

T. wilhelma sponges can withstand 600 Gy (actual 517.6 Gy) of X-ray radiation. That is approximately 60 times the lethal dose for mice^{32,33} and 100 times the lethal dose for humans³⁴. According to conventional wisdom, this amount of radiation should shatter the sponge's DNA, however the Comet assay suggests this does not happen in *T. wilhelma*.

Early organisms evolved in an environment with higher levels of background radiation³⁵. Despite the fact that water partially shields aquatic organisms from direct radiation exposure, radionuclides can accumulate in the sea. As filter-feeding animals, sponges could be particularly exposed to the accumulation of radionuclides and other toxic agents³⁶. Moreover, sponges are sessile organisms without a nervous system thus not capable of rapidly escaping or quickly reducing the water flow through their bodies if the concentration of radioactive agents increases. For these reasons sponges are considered biological indicators of environmental pollution such as radionucleotides^{37,38}.

There are examples of extreme radioresistance in bacteria³⁹ and multicellular organisms capable of anhydrobiosis (desiccation) such as rotifers⁴⁰ and tardigrades^{41–43}, which is unlikely to apply to *T. wilhelma*. Tolerance for both desiccation and radiation may originate from similar DNA protective or repair mechanisms^{40,44}. Indeed, desiccation causes DNA breakage^{40,42}, similar to damage induced by radiation, that may be repaired upon rehydration⁴⁰. Tardigrades possess

molecular mechanisms to prevent DNA damage. Dsup is a tardigrade-specific nucleosome-binding protein that protects chromatin from hydroxyl radicals and contributes to the organism's radio-tolerance⁴⁵. The combination of DNA protective and DNA repair mechanisms determines the level of radioresistance of these organisms. Importantly, rotifers and tardigrades have no or highly restricted somatic cell turnover^{41,46,47}, and the species tested for radioresistance have a short (~60 days) lifespan^{41,48} which prevents mutant clones and accumulating further mutations, leading to cancer. In these conditions, the DNA damage that occurs does not propagate and so might not be apparent⁴⁹. Thus, there is little chance for cancer to develop in tardigrades.

In order to investigate the long-term effect of DNA damage and cancer in invertebrates, somatic cell turnover and long lifespan should be an essential feature of any experimental organism. However, the main invertebrate model organisms currently in use (e.g. *Caenorhabditis elegans* and *Drosophila melanogaster*) have limited or no somatic cell turnover⁵⁰ and their lifespans^{51,52} are too short to study the long term effects of radiation. In contrast, sponges have somatic cell turnover² and a remarkably long lifespan: the largest *Xestospongia muta* specimen described on Caribbean reefs is estimated to be more than 2,300 years old⁵³, a specimen of the sponge *Monorhaphis chuni* is thought to be 11,000 years old⁵⁴ and radiocarbon dating of the sponge *Rossella racovitzae racovitzae* determined that was around 440 years old⁵⁵. Selection for this long lifespan may have also selected for cancer suppression mechanisms.

Considering all these factors: somatic cell turnover, long life span and a primitive immune system⁵⁶, we would expect the development of tumors in sponges, but there have been no reports of cancer in the entire Porifera phylum¹ (with 8500 described living species⁵⁷).

In our experimental setting, (single high dose) radiation did not induce cancer development in *T. wilhelma* during 1 year following X-ray exposure. Malignant cancer risk in

humans is estimated to be 8% per Gy⁵⁸. A small fraction of the dose to which the sponges have been subjected would generally have given rise to cancer in mice⁵⁹ and humans⁵⁸.

The molecular data and morphological observations suggest that sponges protect their DNA from damage in the first place and then activate mechanisms of DNA repair and cell death as they go through a complex phase of tissue reorganization. Finally, they rebuild their tissues' original features. Our findings suggest that the cancer resistance in sponges might be linked to their radioresistance.

Genes involved in DNA repair are activated at different times and for different lengths of time, providing insight into the temporal activation of these genes during the DNA repair process (Fig. 8, tab. 1S and fig. 3S). We found the overexpression of genes known to have a role in DNA double strand break repair (Tab. 1S), confirming that our experimental setting is capable of induced the typical DNA damage produced by X-ray exposure and that sponges respond to radiation by upregulating DNA repair. As expected, we observed a higher number of genes involved DNA repair 24 hours and 7 days after X-ray exposure, but we also identified genes involved in this process specifically expressed 21 days after X-ray exposure. This observation suggests that after 21 days the sponges are still actively repairing the DNA damage induced by radiation. Treated sponges overexpressed only 81 transcripts (12.7% of all overexpressed transcripts) in all 3 time points, but overexpressed 64 transcripts only at 24 hours, 125 transcripts only at 7 days, and 157 transcripts only at 21 days, suggesting waves of sequential transcriptional events.

We found overexpressed genes previously not linked to X-ray induced damage, or with unknown function in humans (Table 3S). For example, *twi_ss.17326.7 (CUBN)*, cubilin in humans. CUBN is an endocytic receptor expressed in the epithelium of intestines and kidneys²⁷.

Interestingly, low expression of *CUBN* in renal cell carcinoma is significantly associated with early disease progression and poor patient outcome²⁸. We hypothesize that the *CUBN* gene has a protective function against DNA damage or is involved in DNA repair and thus its downregulation in kidney cancer increases the chance of an aggressive evolution of the disease.

Epigenetic changes such as methylation are induced by X-ray exposure and contribute to regulate the cell response to stress⁶⁰. Though we did not directly measure methylation, we detected the overexpression of *twi_ss.21704.3 (NSUN7)*, a gene involved in methylation. Methylation induced by radiation can be a persistent epigenetic change after radiation⁶⁰ regulating gene activity long after expression normalization.

In addition to mechanisms of DNA repair, we detected the overexpression of genes involved in apoptosis and cell death. For example, *Twiss.31154.2 (NOX5)*, NADPH oxidase–generated ROS, a gene involved in heavy ion irradiation–induced cell death⁶⁰, suggesting the activation mechanisms of cell death, presumably to remove cells too compromised by radiation.

Notably, we observed a distinct pattern in the morphological changes in the treated sponges. Seven days after X-ray exposure, sponges lose their typical anatomical organization and most of the cells seem to acquire undifferentiated features. Totipotent cells (archeocytes) are part of the mesohyl and they can replace damaged cells⁶¹. Choanocytes, one of the most specialized types of sponge cells, can also transdifferentiate into archaeocytes (stem cells) and serve the same function^{61,62}. In transcriptomic analysis 7 days following X-ray exposure, we found overexpression of genes known to be involved in cell adhesion, signaling, embryonic cell regulation and EMT such as *NOTCH1* and *MET*, consistent with our observations of changes in sponge morphology.

Studying radiation resistance in sponges might help improve radiation therapy

Ionizing radiation is part of the natural environment and evolution of organisms. Thus, the study of radiation responses in sponges could contribute to understanding the molecular evolution of cell radioresistance. This could help us understand the evolution of radioresistance in cancer cells as well, and potentially lead to methods for protecting human cells from radiation damage.

Over the course of a human lifetime, cells can evolve mechanisms that allow them to endure the effects of X-ray exposure^{63,64} through somatic evolution. In human cancers, radiotherapy often induces EMT, and leads to increased radioresistance accompanied by increased cell migration and invasion^{65–68}.

The finding that sponges react to X-ray exposure by increasing the number of undifferentiated cells suggests that EMT is not a cancer cell specific response to radiotherapy, but rather a natural cellular response to radiation both in organisms and human cells⁶⁹. Sponges are particularly suitable to study this process because their simple tissue organization facilitates identification and analysis of tissue alterations. Sponges give us the unique opportunity to study mesenchymal proliferation and EMT in response to radiation, removing the confounding effect of thousands of mutations or deregulated genes described in cancers which may be unrelated to EMT. It is important to note that cancer cells do not utilize *de novo* cancer specific mechanisms to survive radiation exposure, but rather employ a biological process that is shared with sponges and has been evolutionarily conserved in our genomes for 100's of millions of years. Understanding this biological process in response to radiotherapy in sponges could provide the basis for new treatment and cancer prevention strategies.

The study of the evolution of anti-cancer mechanisms such as radiation resistance can contribute to the understanding of multicellular organisms more generally, as the evolution of

multicellularity itself depended on the multicellular organisms' ability to prevent somatic cells proliferating out of control⁷⁰. Our work suggests that sponges may be particularly resistant to cancer because of their radiation resistance, and shows that sponges are an viable model system for studying anti-cancer mechanisms and radiation resistance.

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Author contributions

A.F, A.A. and C.C.M. designed the study. A.F. designed and performed the experiments, collected and analyzed the data. J.T. was an undergraduate student, he contributed to perform the experiments and collecting data. J.S. is an undergraduate student, he contributed to perform the bioinformatic analysis. A. Fortunato, A.A. and C.C.M. wrote the manuscript.

Competing interests

The authors declare that they have no competing interests.

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Figure supplementary 1S. Long term observation of sponge's morphology. Initially sponges suffer acute morphological changes but they recover from the X-ray treatment. Images of the same group of sponges from 0 to 362 days after X-ray exposure.

Figure supplementary 2S. Multidimensional scaling (MDS) plot. There is a gene expression similarity between the samples collected at the same time point and a gene expression distance between the groups: controls, 24 hours, 7 and 21 days.

Figure supplementary 3S. Heatmap of RNA-seq analysis. There are sequential transcriptional events following X-ray exposure. Genes can be expressed only to a specific time point, 24 hours, 7 or 21 days or at 2 or 3 time points.

Gene/Transcript ID	24H logFC	7D logFC	21D logFC	Human Gene Homolog	Homolog Description
twi_ss.10017.1			4.5	NA	NA
twi_ss.10068.1			9	PARP3	poly(ADP-ribose) polymerase family member 3
twi_ss.10136.1			7.2	NA	NA
twi_ss.10157.2		6.9		NA	NA
twi_ss.10270.2			6.6	EPHA4	EPH receptor A4
twi_ss.10279.1	6	6.1	5.7	NA	NA
twi_ss.10283.1		2.2	2.3	SCAR B2	scavenger receptor class B member 2
twi_ss.10306.10			6.5	DPH6	diphthamine biosynthesis 6
twi_ss.1035.1	4.3	3.9		NA	NA
twi_ss.1036.1	4.4	4.4		NA	NA
twi_ss.10365.1			7.9	DUS2	dihydrouridine synthase 2
twi_ss.10479.1			2.5	GABBR2	gamma-aminobutyric acid type B receptor subunit 2
twi_ss.10495.1		2.1	2	NA	NA
twi_ss.10516.1	4.9	4.8	3.5	NA	NA
twi_ss.10703.2			5.3	ROBO2	roundabout guidance receptor 2
twi_ss.10752.1	3.2	4	3.2	ASPN	asporin
twi_ss.10775.1		2.1	2.4	NA	NA
twi_ss.10792.1		2.4		NA	NA
twi_ss.108.1		3	3.9	NA	NA
twi_ss.10837.3		2.6	2.6	NA	NA
twi_ss.10837.5			2.1	NA	NA
twi_ss.10838.1		2.1		NA	NA
twi_ss.10882.1	2.4	6	6	MPEG1	macrophage expressed 1
twi_ss.10945.1		5.3	5.1	NA	NA
twi_ss.10965.1		3.7	3.5	NA	NA
twi_ss.110a.2	5	4.3		NA	NA
twi_ss.110b.1		2.9		NA	NA
twi_ss.11312.1		2.9	3.4	NA	NA
twi_ss.11366.1		2.1	2.2	MYOM3	myomesin 3
twi_ss.11375.1	2.6			PCNA	proliferating cell nuclear antigen
twi_ss.11382.5		7.3		PITRM1	pitrilysin metallopeptidase 1
twi_ss.11444.1		3	3	EPHA2	EPH receptor A2
twi_ss.11522.1			2.1	TYW3	tRNA-yW synthesizing protein 3 homolog
twi_ss.11526.15			3.1	MYOM1	myomesin 1
twi_ss.11534.7			6.5	NA	NA
twi_ss.11539a.1		3.9	4	NA	NA
twi_ss.11539b.4		4.1	4.3	NA	NA
twi_ss.11619.2			5.1	NA	NA
twi_ss.11664.1	2.5	3.2	3	HHIPL1	HHIP like 1
twi_ss.1168.1		2.7		BMP6	bone morphogenetic protein 6
twi_ss.11722.2		6.1	7	TNR	tenascin R
twi_ss.11864.1		2		RIPK4	receptor interacting serine/threonine kinase 4
twi_ss.11909.1		3.1	2.1	NA	NA
twi_ss.120.2		2.3	2.4	NA	NA

twi_ss.12025.1		2.4		CERT1	ceramide transporter 1
twi_ss.12047.1		2.3		ANKRD50	ankyrin repeat domain 50
twi_ss.12129.2		5.2		EXOSC2	exosome component 2
twi_ss.12222.1		2		CEACAM20	CEA cell adhesion molecule 20
twi_ss.12226.1	2.3	5.3	4.9	NA	NA
twi_ss.12230.1			2	DDAH1	dimethylarginine dimethylaminohydrolase 1
twi_ss.12243.1		2.4	2.7	VWA3A	von Willebrand factor A domain containing 3A
twi_ss.12246.2		4.4	4.2	NA	NA
twi_ss.12247.1			3.8	HOMEZ	homeobox and leucine zipper encoding
twi_ss.12272.1	2.9			CLEC4C	C-type lectin domain family 4 member C
twi_ss.12332.9	7.5	8.2		CACNA1B	calcium voltage-gated channel subunit alpha1 B
twi_ss.12377.1		2.1		NA	NA
twi_ss.1244.5		3		ADGRE5	adhesion G protein-coupled receptor E5
twi_ss.12447.4	5.3			NA	NA
twi_ss.12458.1	9.3	6.8		TTN	titin
twi_ss.12733.1		2.3	2.6	NA	NA
twi_ss.12746.1		5.7		CCDC84	coiled-coil domain containing 84
twi_ss.12866.1	2.3			MSRA	methionine sulfoxide reductase A
twi_ss.12872.3		2.7	4.1	COL11A2	collagen type XI alpha 2 chain
twi_ss.12920.1		2.8	3.2	NA	NA
twi_ss.1295.2	4.1			NA	NA
twi_ss.12972.1	2.2	3.2	2.1	NA	NA
twi_ss.12990.2	5.9		6	NA	NA
twi_ss.12990.3	5.9		6	WDR75	WD repeat domain 75
twi_ss.13145.1			2.8	NA	NA
twi_ss.13146.1		2.6	2.2	NA	NA
twi_ss.13146.5		2.4	2.5	NA	NA
twi_ss.13146.9		4.4	4.1	NA	NA
twi_ss.13190.1			2.9	NA	NA
twi_ss.13326.1			3.7	NA	NA
twi_ss.13327.1			3	NA	NA
twi_ss.13436.1		2.6	2.3	NA	NA
twi_ss.13566.1	3.9	4	2.4	CD163L1	CD163 molecule like 1
twi_ss.13698.1		2.4	2.4	FAT3	FAT atypical cadherin 3
twi_ss.13791.1			2.1	NA	NA
twi_ss.13791.2			2.2	NA	NA
twi_ss.13807.1		3.1	2.6	MOXD1	monooxygenase DBH like 1
twi_ss.13838.1	3	3.6	2.9	PXDN	peroxidasin
twi_ss.13856.1		2.3		NA	NA
twi_ss.1403.2	8.6	7.5		NA	NA
twi_ss.14090.1	2.5			WDR83OS	WD repeat domain 83 opposite strand
twi_ss.14116a.1		2.1		NA	NA
twi_ss.14116b.2		3.2	3.3	NA	NA
twi_ss.14119.1	2.7	3.8	3.6	NA	NA
twi_ss.14260.2		2.2		NA	NA

twi_ss.14278.1	7.8	6.7	6.4	NA	NA
twi_ss.1434.1			3.6	UGT2B7	UDP glucuronosyltransferase family 2 member B7
twi_ss.14354.1	2.5	3.2	2.1	SOX14	SRY-box transcription factor 14
twi_ss.1440.1		2.1		UGT2B7	UDP glucuronosyltransferase family 2 member B7
twi_ss.1442.2		3.6	3.3	NA	NA
twi_ss.14541.1	5.5			SHFL	shiftless antiviral inhibitor of ribosomal frameshifting
twi_ss.14576.1		2.7	2.7	PCSK9	proprotein convertase subtilisin/kexin type 9
twi_ss.14612.3		3	2.3	NOTCH2	notch receptor 2
twi_ss.14702.1		3.4		NA	NA
twi_ss.14730.1		2.2	2.8	NA	NA
twi_ss.14756.4	3.7	3.3		NA	NA
twi_ss.14780.1	2.3	2.6	2.3	NA	NA
twi_ss.14839.1	6	6.7	5.5	NA	NA
twi_ss.14840.1			2.1	OTUD1	OTU deubiquitinase 1
twi_ss.14851.1	2.5			NA	NA
twi_ss.1490.1	3.1			TLX2	T cell leukemia homeobox 2
twi_ss.14940.1			2.1	ASTL	astacin like metalloendopeptidase
twi_ss.14987.1		3.2	3.4	NA	NA
twi_ss.15124.1		2.1		GABBR2	gamma-aminobutyric acid type B receptor subunit 2
twi_ss.15132.1			3.1	NA	NA
twi_ss.1543.4	7	7.1		NA	NA
twi_ss.15435.1			2.5	NA	NA
twi_ss.15436.1			2.9	NA	NA
twi_ss.15550.1			3.2	NA	NA
twi_ss.15647.3			6.2	SACS	sacsin molecular chaperone
twi_ss.15661.2		2.3		NA	NA
twi_ss.15729.1		3.1	2.8	NA	NA
twi_ss.15781.1	2.1	2.4		NA	NA
twi_ss.15787.1		2.3		NA	NA
twi_ss.1579a.1		3	3	NA	NA
twi_ss.15844b.3	5.8		6.4	NA	NA
twi_ss.15849.1		2.4	2.5	PTPRD	protein tyrosine phosphatase receptor type D
twi_ss.16049.1		3.4		NA	NA
twi_ss.16232.2			6.2	UTP15	UTP15 small subunit processome component
twi_ss.16285.1		2.5		MYO15A	myosin XVA
twi_ss.16288.1		2.8	3.6	CDIP1	cell death inducing p53 target 1
twi_ss.16289.1			5.3	MRPL27	mitochondrial ribosomal protein L27
twi_ss.16652.3			2	SCUBE3	signal peptide, CUB domain and EGF like domain containing 3
twi_ss.16656.2	7.3	10.4	10.3	PHF8	PHD finger protein 8
twi_ss.16738.1		2.4		NA	NA
twi_ss.16822.1			3	NA	NA
twi_ss.16882.1		2	2.4	NA	NA
twi_ss.17159.1			2.3	GNPNAT1	glucosamine-phosphate N-acetyltransferase 1
twi_ss.17277a.13			5	PTPRA	protein tyrosine phosphatase receptor type A
twi_ss.17303.1			2.7	NA	NA

twi_ss.17326.6	8.2			CUBN	cubilin
twi_ss.17326.7	8.6			CUBN	cubilin
twi_ss.17473.4		5.5		ARMC3	armadillo repeat containing 3
twi_ss.17485.1		2		NA	NA
twi_ss.1749.3			7.3	NA	NA
twi_ss.17531b.1	2.3			CRYM	crystallin mu
twi_ss.17571.5			8	NA	NA
twi_ss.1765.1	3	2.5		GSTM5	glutathione S-transferase mu 5
twi_ss.1766.1	2.2			TRMU	tRNA 5-methylaminomethyl-2-thiouridylate methyltransferase
twi_ss.17801.1		3.5	2.9	SNED1	sushi, nidogen and EGF like domains 1
twi_ss.17802.1	6.3	8.1	7	SUSD2	sushi domain containing 2
twi_ss.17899.1	3.5	3.3	2.1	NA	NA
twi_ss.17908.1	2.6	3.5	2.4	NA	NA
twi_ss.17964.1		2.8	2.4	LARP6	La ribonucleoprotein 6, translational regulator
twi_ss.18100.4		5		NA	NA
twi_ss.18212.3		6.4		ANKRD29	ankyrin repeat domain 29
twi_ss.18250.1		2.6	2.8	NA	NA
twi_ss.18368.5	6.3			NA	NA
twi_ss.18580.1			7.1	NA	NA
twi_ss.18584.1	7.9			DHX33	DEAH-box helicase 33
twi_ss.18595.1		4.5	5.1	NA	NA
twi_ss.18660b.1		2.7		NA	NA
twi_ss.18687.1		3.4		NA	NA
twi_ss.1871.1		3	2.8	NA	NA
twi_ss.18721.1			2.4	NA	NA
twi_ss.18795.3		3.4	3.1	NA	NA
twi_ss.18795.4		3	2.6	PRSS12	serine protease 12
twi_ss.18979.1			6.8	NA	NA
twi_ss.19023.1		2.6	3.2	NA	NA
twi_ss.19091.1			2.1	FAM207A	family with sequence similarity 207 member A
twi_ss.19155.1			2.4	NA	NA
twi_ss.19282.2	5.6			PTCD1	pentatricopeptide repeat domain 1
twi_ss.19329a.2		2.2	2.4	RO60	Ro60, Y RNA binding protein
twi_ss.19345.11		6	6.2	NEO1	neogenin 1
twi_ss.19378.2	4.5		3.8	ERCC1	ERCC Excision Repair 1, Endonuclease Non-Catalytic Subunit
twi_ss.19384.1	8.7	10	7.4	PRRT1	proline rich transmembrane protein 1
twi_ss.19415.1	4	3.9	4.6	NA	NA
twi_ss.19449.1	4.2			NA	NA
twi_ss.19570.5	6.7	8.3	7.4	NA	NA
twi_ss.19625.1		2.2		NA	NA
twi_ss.19635.1		2.1		TRIP12	thyroid hormone receptor interactor 12
twi_ss.19831.9			5.4	DNAH3	dynein axonemal heavy chain 3
twi_ss.19836.2			5.5	TERT	telomerase reverse transcriptase
twi_ss.19882.1		2.3	2.3	NA	NA
twi_ss.19885.1		3		NA	NA

twi_ss.1994.1	3	2.7	2.2	FBXL15	F-box and leucine rich repeat protein 15
twi_ss.19960a.4	7.1	7.3	7.4	NA	NA
twi_ss.20004.1			7.9	NA	NA
twi_ss.20209.2		7.3		NA	NA
twi_ss.20264c.8	7.2	7.5	8.2	NA	NA
twi_ss.20282.1	3.3	2.4		PPM1L	protein phosphatase, Mg ²⁺ /Mn ²⁺ dependent 1L
twi_ss.20445.7			2.1	ANK2	ankyrin 2
twi_ss.20452.1	2	2.2		SOX14	SRY-box transcription factor 14
twi_ss.20491.1	2.3			NA	NA
twi_ss.20516a.5		6.8		SORL1	sortilin related receptor 1
twi_ss.20735.1	6.2	9.3	8.9	NA	NA
twi_ss.20795.1	7.5	11.2	10	NA	NA
twi_ss.20959.1		5.4		NA	NA
twi_ss.20961a.1		2.1	2.2	PRSS12	serine protease 12
twi_ss.21051.1	3.7	3.8	2.9	FBLN5	fibulin 5
twi_ss.21054.4	5.6			NA	NA
twi_ss.21105.1	6.3	6.2	5.2	HMCN1	hemicentin 1
twi_ss.21105.11		4.3		NA	NA
twi_ss.21105.2	6	7	6.2	HMCN1	hemicentin 1
twi_ss.21105.3	5.4	6.1	5.2	SCUBE1	signal peptide, CUB domain and EGF like domain containing 1
twi_ss.21105.6	3.3	4.9	3.9	NA	NA
twi_ss.21105.9		10.6	9.6	SCUBE1	signal peptide, CUB domain and EGF like domain containing 1
twi_ss.21143.1		6.9		NA	NA
twi_ss.21197a.2	2.1		2.5	RPS15A	ribosomal protein S15a
twi_ss.21205.1		3	4.3	NA	NA
twi_ss.21211.1			2.7	NA	NA
twi_ss.21214.1		2.1	2.2	NA	NA
twi_ss.21288a.1		2.3	2.4	ANKRD12	ankyrin repeat domain 12
twi_ss.21316b.3	3	2.9	2.1	COL6A1	collagen type VI alpha 1 chain
twi_ss.21329.1	6.8	6.6	6.8	PRSS12	serine protease 12
twi_ss.21425.2	3.8	4	4.3	NA	NA
twi_ss.21448.1	2.4			MRE11	MRE11 homolog, double strand break repair nuclease
twi_ss.21453b.5			6.1	NA	NA
twi_ss.21458.1	2.1			NA	NA
twi_ss.21482.1	2.4			DLL4	delta like canonical Notch ligand 4
twi_ss.21510.1			6.1	MYCBP2	MYC binding protein 2
twi_ss.21564.1	2.2			ALOX5	arachidonate 5-lipoxygenase
twi_ss.21600.1			3.7	NA	NA
twi_ss.21602.1			5.2	NA	NA
twi_ss.21610.1		2.2	2.9	NA	NA
twi_ss.21610.4		2.7	2.2	NA	NA
twi_ss.21610.5		2.9	2.7	NA	NA
twi_ss.21610.6		3.5	2.7	NA	NA
twi_ss.21631b.4		3.9	4.1	NA	NA
twi_ss.21657.1	2.2			NA	NA

twi_ss.2166.6			5.8	HERC1	HECT and RLD domain containing E3 ubiquitin protein ligase family member 1
twi_ss.21663.2		7.6	6.6	NA	NA
twi_ss.21664c.11			2.4	SSC5D	scavenger receptor cysteine rich family member with 5 domains
twi_ss.21664c.3		4.1		NA	NA
twi_ss.21664c.8		2.8		NA	NA
twi_ss.21665.1		3.1	2.7	NA	NA
twi_ss.21665.3		7.3	6.8	NA	NA
twi_ss.21665.4		2.4		NA	NA
twi_ss.21704.3	6.6			NSUN7	NOP2/Sun RNA methyltransferase family member 7
twi_ss.2185.2		4.4		LANCL2	LanC like 2
twi_ss.21908.1	2.8			ESCO1	establishment of sister chromatid cohesion N-acetyltransferase 1
twi_ss.21964.1			2.4	SLC8A3	solute carrier family 8 member A3
twi_ss.22010.1			2.4	NA	NA
twi_ss.22111.1	5.4			KIAA0895L	KIAA0895 like
twi_ss.22212.1	6.9			NA	NA
twi_ss.22218e.5		3.7		NA	NA
twi_ss.22218e.7	6.9	7.8	6.9	NA	NA
twi_ss.22219.1		2.3	2.2	NA	NA
twi_ss.2232.1		2.8		TST	thiosulfate sulfurtransferase
twi_ss.22376.1	2.3			LIG3	DNA ligase 3
twi_ss.2240.1			2.5	NA	NA
twi_ss.22433.4		5.8		TNFSF10	TNF superfamily member 10
twi_ss.22497.1		2.2		NA	NA
twi_ss.22524.1			2	ERVK-6	endogenous retrovirus group K member 6, envelope
twi_ss.22642.1	8.8	8.2		NA	NA
twi_ss.22647.1	4	3.8		NA	NA
twi_ss.2267.1		2.8		NOTCH1	notch receptor 1
twi_ss.22914.1		2.5		NA	NA
twi_ss.22998.1			3.3	NA	NA
twi_ss.22999.1			3.3	NA	NA
twi_ss.23060.1	2.8			LAMA5	laminin subunit alpha 5
twi_ss.2312b.1		4.8	5.1	NA	NA
twi_ss.23153.1		4		NA	NA
twi_ss.23359.1			2.1	DERA	deoxyribose-phosphate aldolase
twi_ss.23402.1		2.7	3.2	NA	NA
twi_ss.235.6			2.4	NA	NA
twi_ss.23514.1		4.4	4.1	MFSD12	major facilitator superfamily domain containing 12
twi_ss.23514.2		4.7	4.4	MFSD12	major facilitator superfamily domain containing 12
twi_ss.23516.1		2.3	2.5	RNASEH2A	ribonuclease H2 subunit A
twi_ss.23535.5	5.3		4.7	GPHN	gephyrin
twi_ss.23575.1	2.4	2.5	2.5	MBNL2	muscleblind like splicing regulator 2
twi_ss.23636.4			5	THG1L	tRNA-histidine guanylyltransferase 1 like
twi_ss.23649.1		2.1		NA	NA
twi_ss.23722.1	2.1	2.3		CPNE1	copine 1
twi_ss.2376.1		2.7	3.6	NA	NA

twi_ss.23785a.2		2.1		NA	NA
twi_ss.23802.1		5.8		ARRDC2	arrestin domain containing 2
twi_ss.23805.1	3.9	6.1		ARRB1	arrestin beta 1
twi_ss.23819.1		2.6	2.7	NA	NA
twi_ss.23853b.2		2.1	2.4	JAG2	jagged canonical Notch ligand 2
twi_ss.23880.1		4.2	4.4	NA	NA
twi_ss.2395.1			2.4	NA	NA
twi_ss.2398a.2			2.7	NA	NA
twi_ss.24028.2		3.3	2.3	NA	NA
twi_ss.24034.3		8		KANK1	KN motif and ankyrin repeat domains 1
twi_ss.24052.1	5	3.8	3.6	NA	NA
twi_ss.24052.2	5	3.8	3.6	NA	NA
twi_ss.24090.1			2	GAR1	GAR1 ribonucleoprotein
twi_ss.24106.1	5.2	4.7	4.2	ROBO2	roundabout guidance receptor 2
twi_ss.24136.1	3.1			FANCI	FA complementation group I
twi_ss.24166.1	5.9	3.1	3.1	SEPSECS	Sep (O-phosphoserine) tRNA:Sec (selenocysteine) tRNA synthase
twi_ss.24215.6		6.9		NA	NA
twi_ss.2422.1	4	4.8	3.3	NA	NA
twi_ss.2422.2		5.4		NA	NA
twi_ss.24240.1			2.7	DDAH1	dimethylarginine dimethylaminohydrolase 1
twi_ss.24269.1		2.2	2.5	NA	NA
twi_ss.24311.2	7.2			RUFY2	RUN and FYVE domain containing 2
twi_ss.24352c.3	7.1	8		NA	NA
twi_ss.24497.2	2.7	3.5	3.5	NA	NA
twi_ss.24497.5		5.7	6.4	NA	NA
twi_ss.24627.2	5.8		6.9	NA	NA
twi_ss.24697.1			2.8	NA	NA
twi_ss.24735.5	2.6	3.1		NR2F1	nuclear receptor subfamily 2 group F member 1
twi_ss.24735.7		2.7		NR5A2	nuclear receptor subfamily 5 group A member 2
twi_ss.24735.8	2.4	2.3		RXRG	retinoid X receptor gamma
twi_ss.24823.1	2.4	3.8	2.5	ALOX5	arachidonate 5-lipoxygenase
twi_ss.2483.2			7.2	NA	NA
twi_ss.2485.1		2.3		NA	NA
twi_ss.24955.1		2.2	2.3	NHLRC3	NHL repeat containing 3
twi_ss.24962.1	2.6			DDR2	discoidin domain receptor tyrosine kinase 2
twi_ss.24967.1	5.4		4	NA	NA
twi_ss.24976.2		6.8		DHPS	deoxyhypusine synthase
twi_ss.25105.5		7.1	6.7	NA	NA
twi_ss.2518.1		3.4	2.5	NA	NA
twi_ss.25243.1			4.5	PRMT8	protein arginine methyltransferase 8
twi_ss.25299b.2	2.3	5.2	4.5	MOXD1	monooxygenase DBH like 1
twi_ss.2531.2		5.6	6.4	TBC1D15	TBC1 domain family member 15
twi_ss.25312.1		2.8	3.1	TNR	tenascin R
twi_ss.25312.3	4.9	5.5	5.8	FCN1	ficolin 1
twi_ss.25312.4		3.1	3.4	TNR	tenascin R

twi_ss.25457.1		2.1	2.7	SLC25A21	solute carrier family 25 member 21
twi_ss.25621.2	7.1	6.5		NA	NA
twi_ss.25693a.1		5.5	5.4	NA	NA
twi_ss.25693b.2		4.4	4.6	NA	NA
twi_ss.25742.1	2.6	2.1		NA	NA
twi_ss.25745.1	2.7			FANCD2	FA complementation group D2
twi_ss.258.2	5.4	5.2		ADCK2	aarF domain containing kinase 2
twi_ss.25813.1		2.3	2.6	AS3MT	arsenite methyltransferase
twi_ss.25822.1	5			GIN54	GIN5 complex subunit 4
twi_ss.25822.3	5			GIN54	GIN5 complex subunit 4
twi_ss.25826.1		2.3		MAP3K19	mitogen-activated protein kinase kinase kinase 19
twi_ss.25842.1	7.3			JAG1	Jagged Canonical Notch Ligand 1
twi_ss.25970.2		3.4		LTBP1	latent transforming growth factor beta binding protein 1
twi_ss.25977.1			5.7	NEK4	NIMA related kinase 4
twi_ss.2604.1		2.1	2.5	GLIPR1L1	GLIPR1 like 1
twi_ss.26082.1			5	NA	NA
twi_ss.26090b.4	5.4		5.2	NA	NA
twi_ss.2611.1	2.5	3.4	3.5	NA	NA
twi_ss.26110.1		4.5	4	NA	NA
twi_ss.26111.1		3.6	2.7	NA	NA
twi_ss.26117a.1		2.4	2.9	DDX58	DExD/H-box helicase 58
twi_ss.2619.3		2.4		MUC6	mucin 6, oligomeric mucus/gel-forming
twi_ss.2619.7		3.6		VWF	von Willebrand factor
twi_ss.2632.2			6.5	DSCAML1	DS cell adhesion molecule like 1
twi_ss.26323.1	2			NA	NA
twi_ss.26358.1	7.4	9.1	7.3	ATP6V1H	ATPase H ⁺ transporting V1 subunit H
twi_ss.26359.1	4			NA	NA
twi_ss.26378.1		2.4		COL6A3	collagen type VI alpha 3 chain
twi_ss.26399.1		2	2.4	NA	NA
twi_ss.26404.1			2.6	NA	NA
twi_ss.26435.1			5.6	CCHCR1	coiled-coil alpha-helical rod protein 1
twi_ss.26446.1		2.2		MAFB	MAF bZIP transcription factor B
twi_ss.26480.1		2.7	3	NA	NA
twi_ss.26516.1	2.1			CTPS1	CTP synthase 1
twi_ss.2665.8			5.1	DKC1	dyskerin pseudouridine synthase 1
twi_ss.26708.1			2.1	NA	NA
twi_ss.2690.3		2.3		NA	NA
twi_ss.26901a.2		2.1		PLA2G6	phospholipase A2 group VI
twi_ss.26932.1	2.6			NA	NA
twi_ss.27012.1		3.8	3.1	NA	NA
twi_ss.2705.3			3.7	NA	NA
twi_ss.27063.1	8.4	9.6	7.8	NA	NA
twi_ss.27066.1		2		DUSP1	dual specificity phosphatase 1
twi_ss.2715.1		3.2	4.2	NA	NA
twi_ss.27216.1		2.1		NA	NA

twi_ss.27219.1	2	2.5	2.4	NA	NA
twi_ss.27269.2		7.1	6.7	TBK1	TANK binding kinase 1
twi_ss.27318.1			6.6	NA	NA
twi_ss.27336a.3	4.7	4.4		EPHA4	EPH receptor A4
twi_ss.27361.1			2.1	GATAD1	GATA zinc finger domain containing 1
twi_ss.27562.2	3.8	4.1		TGM2	transglutaminase 2
twi_ss.27570.2		4.2		NA	NA
twi_ss.27729.1		2.6	2.7	NA	NA
twi_ss.27731.1		2.7	2.5	NA	NA
twi_ss.27768.1		5.4	5.3	NA	NA
twi_ss.27928.4			6.8	ANK1	ankyrin 1
twi_ss.27976.3		4.3		KAT5	lysine acetyltransferase 5
twi_ss.28007.1			2	DMBT1	deleted in malignant brain tumors 1
twi_ss.2800a.2		2.1		NA	NA
twi_ss.2800a.4		2.4		NA	NA
twi_ss.28010.1			2.8	TRAF4	TNF receptor associated factor 4
twi_ss.28037.1			2.4	NA	NA
twi_ss.28038b.1		2.6	3.1	GBP2	guanylate binding protein 2
twi_ss.28038b.2		5.1	4.6	GBP3	guanylate binding protein 3
twi_ss.28038b.4			2.3	GBP4	guanylate binding protein 4
twi_ss.28048.1	4	6	2.7	NA	NA
twi_ss.28101b.1		3.8		NA	NA
twi_ss.28101b.2		7.2		NA	NA
twi_ss.28211.1	2.1			RPA1	replication protein A1
twi_ss.28214.1	2.5			NA	NA
twi_ss.28225.3	7.9			MED25	mediator complex subunit 25
twi_ss.28284.1	5.1			HSPA1A	heat shock protein family A (Hsp70) member 1A
twi_ss.28338.2			4.7	HDHD3	haloacid dehalogenase like hydrolase domain containing 3
twi_ss.28372.1	3.8			NA	NA
twi_ss.28414.1	3.4	3	3	NA	NA
twi_ss.28424.1		3.2	2.4	VIL1	villin 1
twi_ss.28429.1	2.7	3.5	4	TRAF2	TNF receptor associated factor 2
twi_ss.28459.1			6.1	CASTOR2	cytosolic arginine sensor for mTORC1 subunit 2
twi_ss.28487.2		2.3	2.2	NA	NA
twi_ss.28487.3		2.4	2.6	NA	NA
twi_ss.28510a.22		4.5		NA	NA
twi_ss.28569.1			5.6	NA	NA
twi_ss.28592.1		2.3		NA	NA
twi_ss.28616.1		2.6	2.8	TRPM6	transient receptor potential cation channel subfamily M member 6
twi_ss.28657.1			2	NA	NA
twi_ss.2869.1	5.8	6	5.8	COP1	COP1 E3 ubiquitin ligase
twi_ss.2869.2	3.6	3.7	3.3	COP1	COP1 E3 ubiquitin ligase
twi_ss.28712.2			4.3	NA	NA
twi_ss.28718.1			6.3	PRPF19	pre-mRNA processing factor 19
twi_ss.28718.3			6.3	PRPF19	pre-mRNA processing factor 19

twi_ss.28745.6	5.2			FES	FES proto-oncogene, tyrosine kinase
twi_ss.28804.1			2	NA	NA
twi_ss.28809.1			2.5	NA	NA
twi_ss.28816.2		2.8	3.5	NA	NA
twi_ss.28866.1		3.2	4	NA	NA
twi_ss.28912.1	5.2	5.3	4.3	ANK3	ankyrin 3
twi_ss.28950.1			2.3	TRPA1	transient receptor potential cation channel subfamily A member 1
twi_ss.28950.2			2.3	TRPA1	transient receptor potential cation channel subfamily A member 1
twi_ss.28977.1			4.7	NA	NA
twi_ss.28981.7			4.3	FUCA2	alpha-L-fucosidase 2
twi_ss.28999a.1	4.7			CYP3A5	cytochrome P450 family 3 subfamily A member 5
twi_ss.29124.1			2.9	NA	NA
twi_ss.29126.1	3.9	3.2		PLSCR4	phospholipid scramblase 4
twi_ss.29191.5		6.8		NA	NA
twi_ss.29211.1	2.4	2.2		NA	NA
twi_ss.29406a.1		2.2	2.2	NA	NA
twi_ss.29406a.2		6		NA	NA
twi_ss.29443.2			2.1	NA	NA
twi_ss.29475.1		3.8	4.3	TRPA1	transient receptor potential cation channel subfamily A member 1
twi_ss.29504.3			5.3	LTA4H	leukotriene A4 hydrolase
twi_ss.29504.8			3.1	RNPEPL1	arginyl aminopeptidase like 1
twi_ss.29511.2			5.6	NA	NA
twi_ss.29512.1	2.3			TFPI	tissue factor pathway inhibitor
twi_ss.29546.1		5.4	5.5	TNC	tenascin C
twi_ss.29547.1		7	6.3	NA	NA
twi_ss.29566.2		2.6	2.2	NA	NA
twi_ss.29581.3			4.2	MET	MET proto-oncogene, receptor tyrosine kinase
twi_ss.29590a.5		4.3	3.2	FBLN2	fibulin 2
twi_ss.29617.1	2.8	3.9	2.8	TRIM71	tripartite motif containing 71
twi_ss.29721.1	2.2			NA	NA
twi_ss.29759b.1		3.6	3.2	NA	NA
twi_ss.29858.1			6.3	KDM2B	lysine demethylase 2B
twi_ss.29902.4			5.2	RAB3GAP2	RAB3 GTPase activating non-catalytic protein subunit 2
twi_ss.29994.1			2.2	FRS2	fibroblast growth factor receptor substrate 2
twi_ss.30001b.1			2.1	PLA2G6	phospholipase A2 group VI
twi_ss.30027a.5	6.3	8.5	7.6	NA	NA
twi_ss.30027a.7		3.2		NA	NA
twi_ss.30027b.1	2.8	3.4		NA	NA
twi_ss.30027b.2	2.8	4.2	2.5	NA	NA
twi_ss.30027c.4		3.6	2.6	NA	NA
twi_ss.3007.1		2.2	2.1	NA	NA
twi_ss.30096b.2		2.2		ROR2	receptor tyrosine kinase like orphan receptor 2
twi_ss.30108.1	2.1	2.2		MUSK	muscle associated receptor tyrosine kinase
twi_ss.30134.4	3.3	3.1		PCK2	phosphoenolpyruvate carboxykinase 2, mitochondrial
twi_ss.30137.1		2.1	2.6	NA	NA

twi_ss.30208.1		5.9	5.4	NA	NA
twi_ss.30242b.2	7.2		8.3	HPGDS	hematopoietic prostaglandin D synthase
twi_ss.30351.1			2	NA	NA
twi_ss.30479.1		2.2		NA	NA
twi_ss.30481.2	2.6			SERBP1	SERPINE1 mRNA binding protein 1
twi_ss.30546.1	4.4	5	4.6	C1QTNF9B	C1q and TNF related 9B
twi_ss.30551.1		4.5	4.3	LRP1	LDL receptor related protein 1
twi_ss.30590.3			7.2	NA	NA
twi_ss.30590.6			6.5	RACGAP1	Rac GTPase activating protein 1
twi_ss.30700b.6			6.7	PHF19	PHD finger protein 19
twi_ss.30724.1	2.6	3.7	4.2	NA	NA
twi_ss.3076.1		3.5	2.5	DMBT1	deleted in malignant brain tumors 1
twi_ss.30766.1			4.6	NA	NA
twi_ss.30803a.1	2.9	3	2.1	NA	NA
twi_ss.30809.1		2.2		ANKRD50	ankyrin repeat domain 50
twi_ss.30923.2	4.1			C9orf116	chromosome 9 open reading frame 116
twi_ss.30997.9	6			NA	NA
twi_ss.31022.2		3.6		NA	NA
twi_ss.31032.3			5.6	AASS	aminoadipate-semialdehyde synthase
twi_ss.31108.5			5.1	NA	NA
twi_ss.31154.2	7.2			NOX5	NADPH oxidase 5
twi_ss.3117.1		2.1	2.2	NA	NA
twi_ss.31219.1		2.1		RET	ret proto-oncogene
twi_ss.31247.1	8.3	5.2		ANGPTL1	angiopoietin like 1
twi_ss.3131.1			2.2	GABBR1	gamma-aminobutyric acid type B receptor subunit 1
twi_ss.31312.1		7.3		SCRIB	scribble planar cell polarity protein
twi_ss.31312.12		7.3		SCRIB	scribble planar cell polarity protein
twi_ss.31312.2		7.3		SCRIB	scribble planar cell polarity protein
twi_ss.31312.3		7.3		SCRIB	scribble planar cell polarity protein
twi_ss.31312.4		7.3		SCRIB	scribble planar cell polarity protein
twi_ss.31312.6		7.3		SCRIB	scribble planar cell polarity protein
twi_ss.31312.7		7.3		SCRIB	scribble planar cell polarity protein
twi_ss.31358.1			2.1	NA	NA
twi_ss.31413.1		2.3		NA	NA
twi_ss.3152.1		2.7	2.7	GDF15	growth differentiation factor 15
twi_ss.31673.1		2.8	2.5	NLK	nemo like kinase
twi_ss.31729.1	2.1	3.4	4	DAZAP1	DAZ associated protein 1
twi_ss.31777.1	4.6	5.1	4.3	NA	NA
twi_ss.31853a.1		5.7		COL6A6	collagen type VI alpha 6 chain
twi_ss.31899b.1	5.7			GPR157	G protein-coupled receptor 157
twi_ss.31909.2			4.1	NA	NA
twi_ss.31915.4			7.7	MIF4GD	MIF4G domain containing
twi_ss.32065.1			4.5	SVEP1	sushi, von Willebrand factor type A, EGF and pentraxin domain containing 1
twi_ss.32065.2		4.1	4.9	NA	NA
twi_ss.3226.1			2.7	SPOPL	speckle type BTB/POZ protein like

twi_ss.329.1		2.2		ZFP36L1	ZFP36 ring finger protein like 1
twi_ss.3382.1		3.1		SUSD2	sushi domain containing 2
twi_ss.339.1		3.3	3.2	NA	NA
twi_ss.3531.2		2.5	2.6	ADGRL2	adhesion G protein-coupled receptor L2
twi_ss.3533.1	6.7	6.3	6.6	SUPT6H	SPT6 homolog, histone chaperone and transcription elongation factor
twi_ss.3533.2	6.7	6.3	6.6	SUPT6H	SPT6 homolog, histone chaperone and transcription elongation factor
twi_ss.3533.3	6.7	6.3	6.6	SUPT6H	SPT6 homolog, histone chaperone and transcription elongation factor
twi_ss.3533.4	6.7	6.3	6.6	SUPT6H	SPT6 homolog, histone chaperone and transcription elongation factor
twi_ss.3623.1	5.7			CRYBG2	crystallin beta-gamma domain containing 2
twi_ss.3627.1		2.7	2.6	NA	NA
twi_ss.366.1			3.3	NA	NA
twi_ss.3712.1	7.6	6.6	5	NA	NA
twi_ss.3722.1			3	NA	NA
twi_ss.3796.3		4	3.7	FBN1	fibrillin 1
twi_ss.3847.1			5.4	SNTA1	syntrophin alpha 1
twi_ss.3973.1	3.3	3.7		MUC4	mucin 4, cell surface associated
twi_ss.4076.2		6.2		LRRC7	leucine rich repeat containing 7
twi_ss.4103.1	2.4	2.5		PRXL2A	peroxiredoxin like 2A
twi_ss.4179.1		3.4	4.5	NA	NA
twi_ss.4180.1			3.1	NA	NA
twi_ss.4254.1		5.1		NA	NA
twi_ss.4260.1			3.7	NA	NA
twi_ss.4264.1		3.7	3	NA	NA
twi_ss.4286.1			2.1	WARS1	tryptophanyl-tRNA synthetase 1
twi_ss.4304a.1	2.3	2.4	2	NA	NA
twi_ss.4312.1		9.2	9	NA	NA
twi_ss.4335.1		3.1	2.8	SCARB2	scavenger receptor class B member 2
twi_ss.4355.1		2.4		TRIM71	tripartite motif containing 71
twi_ss.4370.1		3.7	2.3	NA	NA
twi_ss.4371.1		4.2	4	NA	NA
twi_ss.4463.1		3.5	3.4	NA	NA
twi_ss.4477.1		4.8	4.9	NA	NA
twi_ss.4477.2		4.8	4.5	NA	NA
twi_ss.4477.3			7.2	NA	NA
twi_ss.4527.1		2.5		NA	NA
twi_ss.4654.1		2.4	3.6	NA	NA
twi_ss.469.1		2.1		NCAM1	neural cell adhesion molecule 1
twi_ss.4816.1			5.4	NA	NA
twi_ss.4828.1			3.1	ROR2	receptor tyrosine kinase like orphan receptor 2
twi_ss.4865.5			5.9	PRPF4B	pre-mRNA processing factor 4B
twi_ss.4876.1	2.5			DDR1	discoidin domain receptor tyrosine kinase 1
twi_ss.4918.1		7.3		NA	NA
twi_ss.4929.1		2.1		DAGLB	diacylglycerol lipase beta
twi_ss.4972.1			2.9	NA	NA
twi_ss.4977.9	8.8	8.5	9.5	SPG11	SPG11 vesicle trafficking associated, spatacsin

twi_ss.4989.1	2.2			COP1	COP1 E3 ubiquitin ligase
twi_ss.4992.1			2.3	NA	NA
twi_ss.5058.2	3.6	3.8		NA	NA
twi_ss.5112.1	2.7	3.1		NA	NA
twi_ss.5182.1	3.9			DNAJB5	DnaJ heat shock protein family (Hsp40) member B5
twi_ss.5283.3			7.3	ASNS	asparagine synthetase (glutamine-hydrolyzing)
twi_ss.5415.4		3.6	3.5	NA	NA
twi_ss.5416.1			2.7	HPRT1	hypoxanthine phosphoribosyltransferase 1
twi_ss.5474.2			3.6	NA	NA
twi_ss.5474.4			2.1	NA	NA
twi_ss.5526a.2	3.6	3.2		NA	NA
twi_ss.5526b.1	4.2	3.9		NA	NA
twi_ss.558.1	2.1	2.2		NA	NA
twi_ss.5590a.3		4.3		UBC	ubiquitin C
twi_ss.5628.6		6.5		MET	MET proto-oncogene, receptor tyrosine kinase
twi_ss.5643.1	2.2	5.3	5.5	NA	NA
twi_ss.5716.1		3.9	3.5	NA	NA
twi_ss.5718.1		2.1	2.2	NA	NA
twi_ss.5738.2		2.2		NA	NA
twi_ss.574.2	2.3			PIK3R5	phosphoinositide-3-kinase regulatory subunit 5
twi_ss.5784.2		5.3		NA	NA
twi_ss.5805a.2			3.4	NA	NA
twi_ss.6018.1	4.9	6.5		NA	NA
twi_ss.6037.1		3	3	NA	NA
twi_ss.6040.1		2.1		NA	NA
twi_ss.6045.1		2.7	2.5	TTN	titin
twi_ss.6146.8		5.6		DMBT1	deleted in malignant brain tumors 1
twi_ss.6202.1		2.1		POSTN	periostin
twi_ss.6204.1		2.5		POSTN	periostin
twi_ss.6314.1	2.1			CALCO2	calcium binding and coiled-coil domain 2
twi_ss.6452.1	3.6	4.1	3	CPAMD8	C3 and PZP like alpha-2-macroglobulin domain containing 8
twi_ss.6495.1			2.5	NA	NA
twi_ss.6499.1			2.6	NA	NA
twi_ss.6558.5		2.7	3.3	NA	NA
twi_ss.6603b.1	3	3.3	2.9	MAPK3	mitogen-activated protein kinase 3
twi_ss.6610.1	2.8	2.5	2.2	NA	NA
twi_ss.6620.1		2.8	2.6	NA	NA
twi_ss.668.1		4.5	3.9	SZRD1	SUZ RNA binding domain containing 1
twi_ss.6750.1	2.5	3	2.1	TM2D1	TM2 domain containing 1
twi_ss.6760.1		3.3		NA	NA
twi_ss.684.1			2.6	NA	NA
twi_ss.6877.1		3.7	4.3	NA	NA
twi_ss.6881.1		3.3	3.8	NA	NA
twi_ss.6884.1		4	2.7	NA	NA
twi_ss.7080.1			2.1	DARS1	aspartyl-tRNA synthetase 1

twi_ss.7082.1		2.6		ETV4	ETS variant 4
twi_ss.7156.2	5			ITGB4	integrin subunit beta 4
twi_ss.7206.1		2.4	2.8	NA	NA
twi_ss.7208.1			2.1	NA	NA
twi_ss.7208.2		4.2	4.3	NA	NA
twi_ss.7409.1		3	3.6	ANTXR1	ANTXR cell adhesion molecule 1
twi_ss.7443.1		2	2.3	NA	NA
twi_ss.7454.1	2.7	2.3		NA	NA
twi_ss.7494.4			6	SLC25A25	solute carrier family 25 member 25
twi_ss.7586.1		2.1	2.3	NA	NA
twi_ss.7623.1		2	3.8	NA	NA
twi_ss.7626.1		2		DMBT1	deleted in malignant brain tumors 1
twi_ss.7711.1		2.8		ASB7	ankyrin repeat and SOCS box protein 7 isoform 1
twi_ss.7757.3			3.3	UBR5	ubiquitin protein ligase E3 component n-recognin 5
twi_ss.7777.1		3.5	3.2	NA	NA
twi_ss.7858.12	3.9	4.3	4.4	NA	NA
twi_ss.7858.13		2.5		DSCAML1	DS cell adhesion molecule like 1
twi_ss.7858.3		2.5	2.2	PTPRD	protein tyrosine phosphatase receptor type D
twi_ss.7858.4		3.3		PTPRD	protein tyrosine phosphatase receptor type D
twi_ss.799a.3			3.4	MATN2	matrilin 2
twi_ss.8200.1		3.1	2.1	NA	NA
twi_ss.8361b.2	2.1	2.8	2.7	SOCS1	suppressor of cytokine signaling 1
twi_ss.8420.5		4.7		IFRD1	interferon related developmental regulator 1
twi_ss.8420.6		4.7		IFRD1	interferon related developmental regulator 1
twi_ss.8507.1			2.4	NA	NA
twi_ss.8555.1		2		TRAF4	TNF receptor associated factor 4
twi_ss.8573.1			3.5	NA	NA
twi_ss.8759.1	5.6	5.2		CD163L1	CD163 molecule like 1
twi_ss.8819.1	6.2	5.5	5.2	NA	NA
twi_ss.8843.1		6.8		NA	NA
twi_ss.8873.1		3	5.4	NA	NA
twi_ss.8875.1		2.4	4.4	NA	NA
twi_ss.8902.2	4.7			NA	NA
twi_ss.908.1			2.1	KLHDC1	kelch domain containing 1
twi_ss.9298.1		4.7		NA	NA
twi_ss.9313.5			6.8	NA	NA
twi_ss.9430.1		4.5	3.2	NA	NA
twi_ss.946.10			5.9	MYO7A	myosin VIIA
twi_ss.946.3			5.9	MYO7A	myosin VIIA
twi_ss.946.8			6	MYO7A	myosin VIIA
twi_ss.9501.1			2.5	NA	NA
twi_ss.9509.1		3.5		NA	NA
twi_ss.9529.1			5	NA	NA
twi_ss.9681.3	5.1			ANK3	ankyrin 3
twi_ss.9709.1		6.5	6.3	NA	NA

twi_ss.974.3			6.7	NA	NA
twi_ss.9775.1		4.1	5.2	NA	NA
twi_ss.978.1	2.3	6.3	6.6	NA	NA
twi_ss.981.1		5.2	4.7	NA	NA
twi_ss.9828.1		2.5		NA	NA
twi_ss.982c.1		2.2		NA	NA
twi_ss.984.1		4.6	4.1	NA	NA
twi_ss.9907.1	3.6	4	3.1	NA	NA
twi_ss.9920.2	5	5.2	4.5	NA	NA
twi_ss.9963.1			2.7	NA	NA

Table 1S. List of genes overexpressed 24 hours, 7 and 21 days after X-ray exposure. The table reports at least 2 log2-fold differentially expressed and statistically significant genes after multiple comparison correction (FDR<0.05) and their human homolog genes.