IARC reference: MTA/2018/IMP/MMB/0397

FFM CUNI: 2018X-00\_\_\_

**Material Transfer Agreement**

This Material Transfer Agreement (hereinafter referred to as the "*MTA*") is concluded by and between

Charles University, having an office and place of business at Ovocný trh 560/5, Prague 1, 116 36, Czech Republic, concerning the Part: **First Faculty of Medicine** (whose address is: Kateřinská 32, 121 08 Prague 2, Czech Republic).

(hereinafter referred to as the "*Provider*")

and

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| The International Agency for Research on Cancer, World Health Organization, having an office and place of business at 150 Cours Albert Thomas, 69372 Lyon cedex 08, France.  (hereinafter referred to as the “*Recipient*”) |

Collectively referred to as the “*Parties*”

**1. Definitions**

1.1 Upon request the *Provider* shall provide to the *Recipient* the material as described and quantified in Annex 1, hereinafter referred to as the "*Original Material*". Annex 1 constitutes an integral part of this *MTA*.

1.2 “*Recipient*” is the legal entity as identified in Annex 1.

1.3 “*Recipient Scientist*” is the scientific employee of *Recipient* performing the intended experiments with *Material* as identified in Annex 1.

1.4 "*Progeny*" is defined as unmodified descendant from the *Original Material*, such as virus from virus, cell from cell, or organism from organism.

1.5 "*Unmodified Derivatives*" are substances created by the *Recipient* which constitute an unmodified functional subunit or product expressed by the *Original Material*, e.g. subclones of unmodified cell lines, purified or fractionated subsets of the *Original Material*, proteins expressed by DNA/RNA, or monoclonal antibodies secreted by a hybridoma cell line.

1.6 "*Modifications*" are substances created by the *Recipient* which contain/incorporate the *Original* *Material*, e.g. crosses, breeding varieties, cell fusions, subcloning, etc.

1.7 The "*Material*" which, regarding the inherent intellectual property rights, is and remains the exclusive property of the *Provider*, comprises particularly the *Original Material*, any *Progeny*, *Unmodified Derivatives*, the *Original Material* contained in *Modifications* and proprietary information concerning the *Original Material*.

**2. Use of the *Material***

2.1 The *Recipient* shall use the *Material* in compliance with all laws and regulations applicable to such *Material* insofar as applicable to the *Recipient*, including guidelines for work with recombinant DNA. The *Material* being experimental in nature must not be used in humans or animals unless - where applicable - explicitly admitted by an ethics committee or regulations on the treatment of laboratory animals.

2.2 The *Material* shall be used exclusively for the purposes described in Annex 1. It must not be released to any person other than the *Recipient Scientist/s* defined above and named below, and staff under their direct supervision, who are bound by obligations not less strict than those set out in this MTA. It shall be handled confidentially and forwarded to third parties only to the extent of *Provider's* prior written approval.

2.3 Upon request, the *Recipient* shall inform the *Provider* on the status of its research.

**3. Publications**

The *Recipient* shall have the right to publish its findings and results related to the *Material*, provided that the relevant *Provider Researcher/s* are either named as co-authors of the publication or cited as the source of the *Material*, according to the respective contribution of the *Material* to the publication. The *Recipient* shall submit all publications four weeks prior to their public disclosure to the *Provider*. The *Provider* agrees to keep *Recipient’s* publication confidential until published by the *Recipient*.

**4. Intellectual Property**

4.1 Where the research involving the *Material* or a *Modification* results in an invention or a legally protectable *Modification* of the *Material*, the *Recipient* and *Recipient Scientist* shall promptly disclose this development to the *Provider*. *Recipient* and *Provider* shall decide in common about the inventorship, taking in due consideration the *Provider's* contribution to the invention through its *Material*. Decisions about all further proceedings, such as filing of a patent application or exploitation, shall be made after inventorship is determined.

4.2 At *Provider's* request, the *Recipient* agrees to provide the *Provider* for its internal research use with reasonable quantities of published materials developed, made or discovered in the course of *Recipient’s* research studies using the *Material*, always provided that the *Recipient* may fulfill this obligation with reasonable effort. Such transfer shall be free of charge, but an appropriate handling/shipping fee may be charged by the *Recipient*.

4.3 The *Recipient* agrees not to apply for any intellectual property protection of the *Original Material*.

**5. Warranty and Liability**

5.1 Any *Material* provided pursuant to this *MTA* is understood to be experimental in nature. It may have hazardous properties. The *Provider* makes no representations and extends no warranties of any kind, express or implied, as to the fitness of the *Material* for a particular purpose, or that the use of the *Material* will not infringe any patent, copyright, trademark, or other proprietary rights of a third party.

5.2 The *Recipient* shall be solely responsible for the manner in which it carries out its activities under this *MTA* including its use, storage or disposal of the *Material*. The *Recipient* hereby acknowledges and agrees that the *Provider* and its researcher/s shall not be responsible for any loss, claim or demand which could be raised by the *Recipient*, or made against the *Recipient* by any other party, due to, or arising from, the use of the *Material* by the *Recipient*, except to the extent caused by gross negligence or willful misconduct of the *Provider*.

**6. Miscellaneous**

6.1 The *Original Material* is provided cost-free; however, a handling fee may be charged for its preparation and shipment to the *Recipient*. As applicable, both items are specified in an accompanying letter to this *MTA*.

6.2 Nothing contained in this *MTA* shall be construed as a waiver of any of the privileges and immunities enjoyed by IARC/WHO (the *Recipient*) under national or international law, and/or as submitting IARC/WHO to any national court jurisdiction. Any dispute relating to the interpretation or application of this *MTA* shall, unless amicably settled, be subject to conciliation. In the event of failure of the latter, the dispute shall be settled by arbitration. The arbitration shall be conducted in accordance with the modalities to be agreed upon by the *Parties* or, in the absence of agreement, with the UNCITRAL Arbitration Rules. The *Parties* shall accept the arbitral award as final.

6.3 This *MTA* shall enter into force on the date of the Disclose of this MTA in accordance with paragraph 6.5. It expires after five (5) years or after conclusion of the experiments according to Annex 1, without prior notice by any of the *Parties*. The provisions concerning Publications, Intellectual Property and Liability shall survive this expiration.

6.4 In the event the *Material* or part of it should be under physical control of the *Recipient* before this *MTA* is signed, the terms and provisions shall apply to this *Material* retroactively.

6.5 The *Parties* acknowledge that Charles University, as a public university and an entity under Art. 2 Par. 1 Letter e) of Act No. 340/2015 Coll., on Contract Register, is subject to the obligation to disclose any contracts it concludes in the contract register (hereinafter “Disclosure” or “Disclose”). The *Parties* state that this *MTA* is subject to mandatory Disclosure with the exception of ANNEX 1. *Provider* pledges to Disclose the contents of this *MTA* as well as to inform *Recipient* with no undue delay of the fact that the contents of this *MTA* have been Disclosed.

Information must be sent to the e-mail address of the contact person of the *Recipient*:

*Legal information:* [*xxxxxxx@iarc.fr*](mailto:xxxxxxx@iarc.fr) */ Scientific information:* [*xxx@iarc.fr*](mailto:xxx@iarc.fr)

Both *Parties* confirm their acceptance of the terms of this *MTA* by signing below.

Done in duplicate; one original counterpart of this signed *MTA* must be returned to:

BIOCEV- First Faculty of Medicine, Charles University

Prumyslova 595, Vestec 25250, Czech Republic

**Signed on behalf of the *Provider***

Full Name: Prof. MUDr. Aleksi Šedo, DrSc. Signed:

Designation: Dean of the First Faculty of Medicine Date:

**Signed on behalf of the *Provider Scientist***

Full Name: xxxxxxxxxxx xxxxxxxxxxxxx Signed:

Designation: Principal Investigator Date:

**Signed on behalf of the *Recipient***

Full Name: xxxxxxxx xxxxxxxxxxxxxx Signed:

Designation: Director of Administration and Finance Date:

**Signed on behalf of the *Recipient Scientist***

Full Name: xxxxxxx xxxxxxxxxx Signed:

Designation: Group Head, MMB Date:

**ANNEX 1**

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| **Recipient’s Institution full name and place of business (VAT number if applicable):**  International Agency for Research on Cancer  150 cours Albert Thomas, 69372 Lyon cedex 08 - FRANCE | **Recipient principal scientist’s name, full address, telephone number and e-mail:**  xxxxxxxx, 150 cours Albert Thomas, 69372 Lyon cedex 08 - FRANCE, +33 4 72 73 xxxxx, xxxxxxx@iarc.fr |
| **Address to send the material to:**  International Agency for Research on Cancer  145 rue des Frères Lumière, 69008 Lyon  FRANCE | **Recipient authorized official’s name, full address, telephone number and e-mail:**  xxxxxxxxxxx, 150 cours Albert Thomas, 69372 Lyon cedex 08 - FRANCE, +33 4 72 73 xxxxxx, xxxxxx@iarc.fr |
| **Provider’s principal scientist making available the Material (if known):**  xxxxxxxxxx | |
| **Description of the Original Material:** Material refers to the mouse cell lines generated by prof. xxxxxxxxx scientific group. The exact number of samples transferred for each cell line will depend on the needs and progress of the research objective outlined below and will occur in multiple shipments.  **Cell line 1** : S5 fl/fl CreEsr1 and S5 fl/+ CreEsr1  Mouse cell line containing a tamoxifen-inducible cre-mediated recombination system driven by the chicken beta actin promoter/enhancer coupled with the cytomegalovirus (CMV) immediate-early enhancer and a Smarca5 flox allele containing two loxP1 sites around exon5. Tamoxifen-inducible Cre-mediated recombination results in deletion of exon 5 of the floxed Smarca5 gene, which results in a frame-shift null mutation. Biallelic or monoallelic versions of Smarca5 fl are provided.  **Cell line 2 :** S5 fl/fl and S5 fl/+  This cell line differs from cell line 1 in that it does not contain the tamoxifen inducible cre-casette.  **Cell line 3** : S5fl/fl p53-/- CreEsr1 R26R-YFP and S5fl/+ p53-/- CreEsr1 R26R-YFP  Similar to cell line 1 but it also expresses enhanced yellow fluorescent protein as a reporter under the ROSA26 locus downstream of a floxed tpA cassette. Expression of Cre leads to excision of the floxed stop sequence and hence constitutive expression of EYFP. This serves as a reporter for the tamoxifen induced deletion. In addition, these cells also have a homozygous deletion of the *Trp53*gene. | |
| **Objective:** Maintaining the integrity of genetic information is critical for normal cellular functions and for the suppression of mutagenic events that can lead to cancer. Various endogenous and exogenous factors constantly challenge genomic integrity by inducing DNA damage, which is why cells have developed DNA damage response (DDR) mechanisms. It is important to consider that DDR operates in the context of chromatin and, consistent with this notion, the chromatin remodelling factor Smarca5 has been linked to different types of DNA repair.  We hypothesize that Smarca5 has a role in genome protection by allowing access of the DNA repair machinery to sites of mutations or DNA damage, especially in heterochromatic regions, and its impairment may thereby steer the fate of a cell towards transformation and cancer development.  Therefore, mouse cell lines, which were generated in the provider’s laboratory and harbor either mutant or wildtype versions of *Smarca5* will be used to address the following aims:  1. Study the impact of *Smarca5* deletion on mutation rates in an experimental model of cell immortalization.  2. Study the occurrence of DNA lesions, such as point mutations and structural variants, induced by conditional deletion of *Smarca5*, with respect to differences in heterochromatic and euchromatic regions of the genome.  3. Study the effect of *Smarca5* deletion on overall chromatin structure and how chromatin structure determines the topological distribution of global mutation patterns.  4. Explore if Smarca5 can affect the DNA repair efficiency at sites of compact chromatin by ATP-dependent chromatin remodeling.  **Methodology of the proposed project:** Wildtype and *Smarca5* knockout mouse embryonic fibroblasts will be exposed to strong model mutagens, such as benzo[a]pyrene or aristolochic acid. DNA damage will be assessed using γH2Ax detection (H2AX Phospho S139) and Comet assay. Subsequently, untreated and exposed cells will be immortalized according to an established protocol and whole exome sequencing will be performed on immortal clones to identify the acquired mutations. These will be used to establish mutation rates and mutation type patterns with respect to *Smarca5* status and exposure condition.  In order to study the role of *Smarca5* deletion on chromatin structure, we will perform Assay for Transposase Accessible Chromatin (ATAC)-seq, ChIP-seq and Hi-C in wildtype and mutant primary cells. The primary cell chromatin structure assessment will subsequently be utilized to establish the relationship between chromatin structure and the global mutation patterns established in immortalized cells. DNA sequencing will be performed by the Recipient, using own instrumentation, or outsourced to a third-party service provider (under appropriate IARC agreement schemes such as Long Term Agreement), depending on the particular sequencing application scale.  The role of ATP-dependent chromatin remodeling by Smarca5 for DNA repair efficiency in compact chromatin will be tested by generating catalytically inactive Smarca5 point mutants, using CRISPR, in spontaneously immortalized MEFs with no other defects in the pathway. Mutant and control cells will be treated with carcinogens, fixed at different timepoints after exposure cessation and co-immunostained with antibodies specific for heterochromatin (H3K9me3, H4K20me3) and DNA repair (e.g. γH2Ax, 53BP1, pATM).  **Expected outcomes of the intended project:** Chromatin modifying enzymes play a well-characterized role in various DNA repair processes. The proposed project is designed to advance our current knowledge beyond their immediate contribution to DNA repair and gain insight into the ultimate consequences of defective repair: genetic alterations. These alterations are expected to occur at different rates and to manifest as different types due to altered chromatin accessibility. It is conceivable that inactivation/hyperactivation of chromatin modifying enzymes, such as Smarca5, contribute significantly to the mutation load, distribution of mutations in the genome and characteristic mutation patterns in response to mutagenic insult. Chromatin remodeling by Smarca5 can thus be a critical component for cancer onset or resistance. The knowledge gathered from this study would be applicable to clinical practice, especially when considering cancer development and treatment. | |