Summary Statement

Please provide a summary statement that describes your project with respect to recombinant or synthetic nucleic acid molecules, microorganism, and/or biological toxin usage. Please include dosage and route of administration if conducting human or animal studies.

The Janknecht lab is interested in the function of transcription factors (ETS proteins, JMJD histone demethylases, and others), metabolic enzymes (e.g. DNPH1/RCL) and cooperation partners thereof. Some of these proteins are encoded by oncogenes. We focus on human proteins as well as the mouse homologs and study how their dysregulation can lead to disease, especially cancer, liver disease and metabolic syndrome.

We will clone cDNA of above mentioned proteins, corresponding shRNAs or sgRNAs, the Cas9 nuclease, and markers such as green fluorescent protein into mammalian expression vectors, including retro-/lentiviral expression vectors. Cloning will occur in E.coli TOP10. Also, we will express above mentioned proteins with the help of bacterial expression vectors in E.coli BL21 derivatives.

Mammalian expression vectors will be transfected into mammalian (human and non-human) cell lines. Viral vectors will be transfected into human 293T cells in order to produce virus, which will then be utilized to infect mammalian cells. After transfection or viral transduction, cells will be harvested and lysed to assay for changes of the transcriptome, interactome, proteome or metabolome. Also, cells may be FACS sorted to either count certain subpopulations or to separate out certain subpopulations (such as tumor-initiating cells) to study them in more detail with regard to molecular changes.

We will also utilize synthetic DNA/RNA (such as siRNA or single-stranded target DNA templates for CRISPR-mediated homologous recombination) and transfect these into mammalian (human and non-human) cell lines. Eventually, these cells will be lysed to study molecular changes induced by these synthetic nucleic acids.

We will also administer human and mouse cell lines (that were modified by transfection or viral transduction as described above, but no longer contain any viable virus) into mice. Injection routes include intravenous, subcutaneous, intra fat pad, and intraperitoneal. The concentration of the cells to be administered is within the range of 100 to 10 million per mouse, depending on the purpose of the experiment. Mostly, we will inject cancer cells and determine subsequent tumor formation. Mice will eventually be euthanized, tissues dissected and then either moleculary (e.g., RT-PCR) or immunohistochemically analyzed.