

# Moonlighting role for dUTPase: establishing a viable mammalian cell line with high uracil-DNA levels

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The dUTPase enzyme has a prominent role in the preventive repair by the hydrolysis of dUTP to dUMP [Vertessy BG et al. (2009) *Acc Chem Res* 42, 97-106]. The dUTP nucleotide can be incorporated into the DNA, however uracil bases are excised mainly by UNG and SMUG1, which are enzymes of the base excision repair mechanism. Elevated dUTP level can generate a recurring cycle of uracil incorporation and excision leading to cell death. Interestingly, the *Ung* and *Smug1* knockout mice are viable and fertile [Alsøe L et al. (2017) *Sci Rep* 7, 7199]. However, knockout of the dUTPase in mice leads to early embryonic lethality [Palinkas HL et al. (2019) *Biomolecules* 9, 136]. To consider the importance of dUTPase in cellular physiology, it is paramount to address the essential function of the enzyme, therefore our aim is to generate catalytically inactive dUTPase in MEF cells. Change of the conserved aspartic acid to an asparagine leads to an inactive enzyme which is unable to coordinate the catalytic water for the hydrolysis of the dUTP. To date, using CRISPR cytosine base editing technology we have successfully created cell lines that are either heterozygous or inactive for the dUTPase encoding gene in *Ung* *-/-* *Smug1* *-/-* MEF cells. We have found that the genomic uracil content in the inactive mutant cells is highly increased. We determined via western blotting that in the inactive mutants the expression of dUTPase is decreased. Normally, dUTPase localises to the nucleus, but in the inactive mutant cells the nuclear localisation is altered as characterised with immunocytochemistry. In conclusion, we have successfully generated a viable *Ung* *-/-* *Smug1* *-/-* MEF cell line that is inactive for dUTPase and represents a highly uracilated mammalian genome. As the inactive dUTPase mutant cells are viable with high genomic uracil content, our results suggest that the catalytic activity may not be the essential function of dUTPase, but the enzyme may have a moonlighting function.