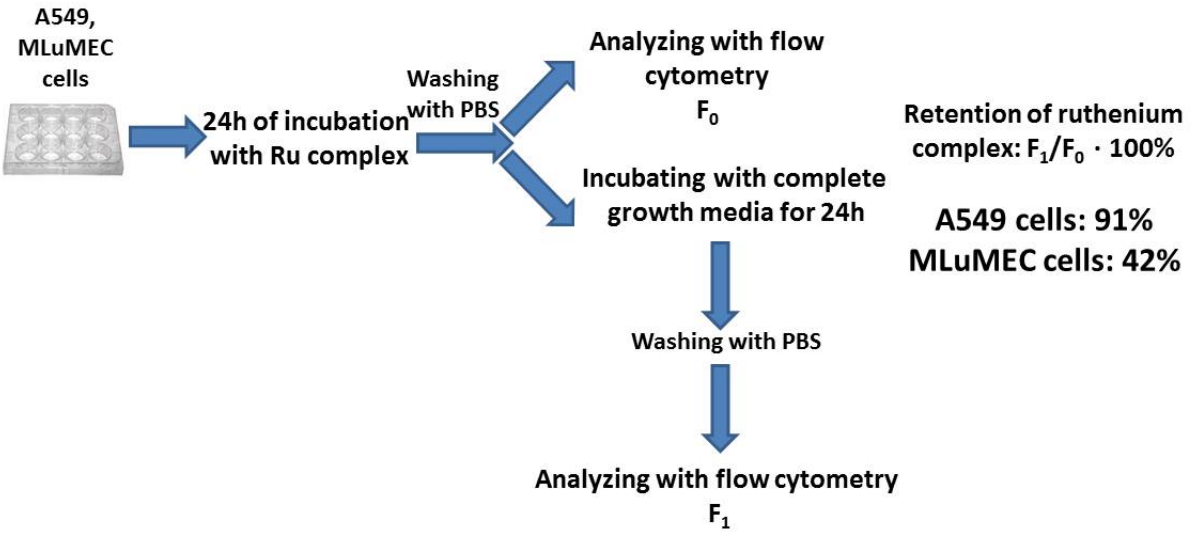
To be submitted to *Metallomics*

Supplementary Information

Biological effect of nitroimidazole derivative of polypyridyl Ru complex on cancer and endothelial cells

Olga Mazuryk[a], Franck Suzenet[b], Claudine Kieda[c,*], Małgorzata Brindell[a,*]



Scheme S1. Schematic representation of the determination of retention of $[Ru(dip)_2(bpy\text{-}NitroIm)]^{2+}$ in A549 and MLuMEC cell lines.

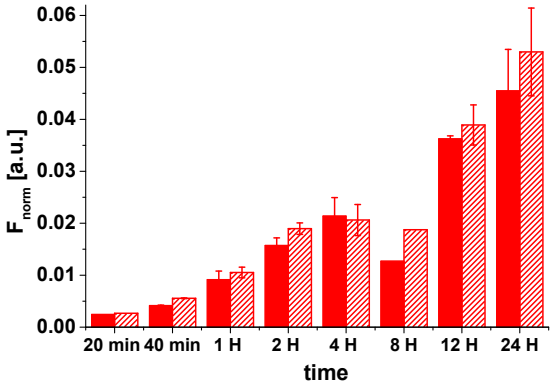


Fig. S1. Time dependence accumulation of $[Ru(dip)_2(bpy\text{-}NitroIm)]^{2+}$ (4 μM) under normoxic (filled) and hypoxic (dashed) conditions in MLuEC FVB cells in medium with 2% serum.

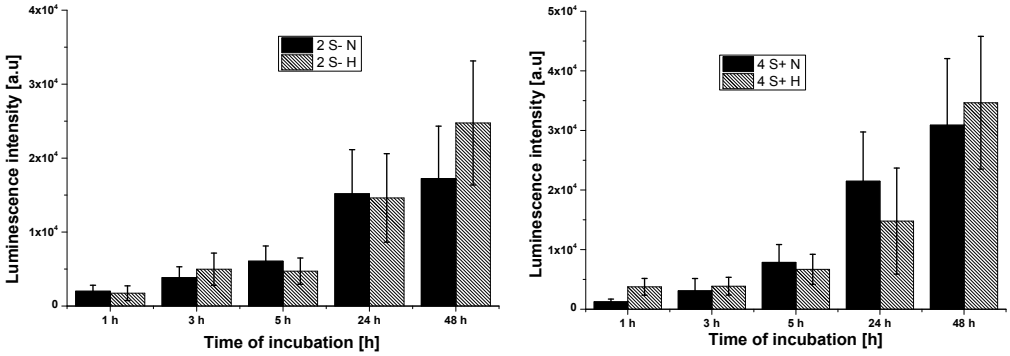


Fig. S2. Time dependence accumulation of $[Ru(dip)_2(bpy\text{-}NitroIm)]^{2+}$ (2 and 4 μM) under normoxic (filled) and hypoxic (dashed) conditions in A549 cells in medium without (S−) or with (S+, 2%) serum.

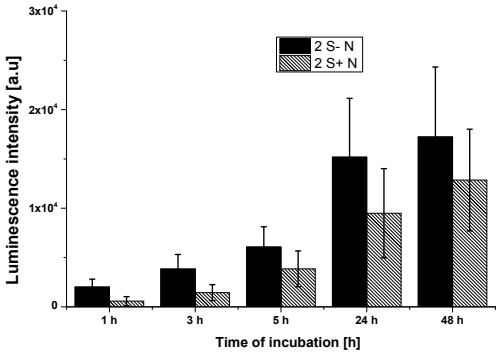


Fig. S3. Influence of serum addition on accumulation of $[Ru(dip)_2(bpy\text{-}NitroIm)]^{2+}$ (2 μM) in A549 cells lines under normoxic conditions for various incubation time. The filled bars denote serum free (S−) while the dashed bars stand for the presence of serum (S+, 2%).

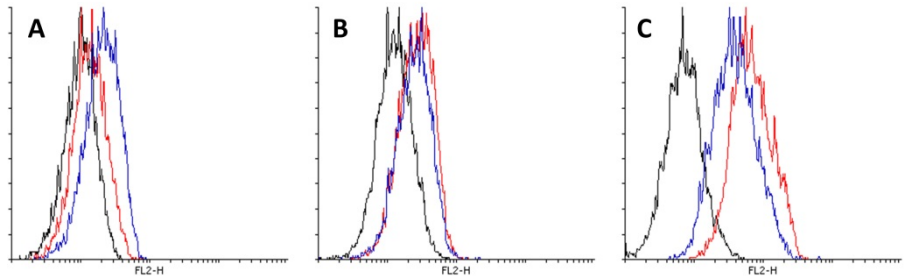


Fig. S4. Accumulation profile of $[Ru(dip)_2(bpy\text{-}NitroIm)]^{2+}$ (4μM) in 4T1 cells conducted in the presence of 2% serum under normoxic (red) and hypoxic (blue) conditions after 2 (A), 4 (B) and 24 h (C) incubation time. Black line stands for control.

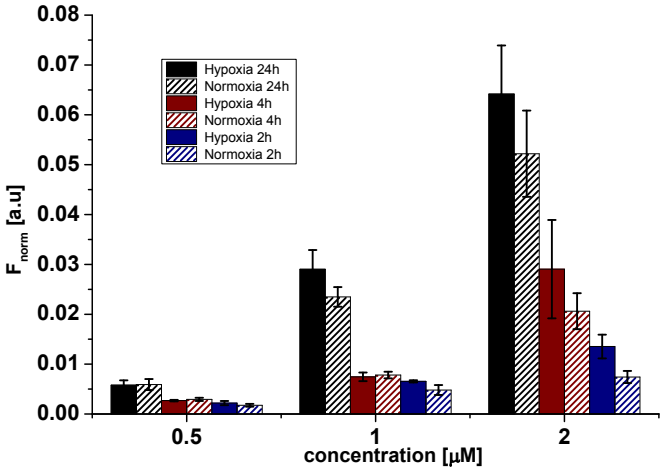


Fig. S5. Concentration dependence of accumulated $[Ru(dip)_2(bpy\text{-}NitroIm)]^{2+}$ in MLuEC FVB cells conducted in medium without serum under normoxic (filled bar) and hypoxic (dashed bar) conditions after 2 (blue), 4 (red) and 24 h (black) of incubation time.

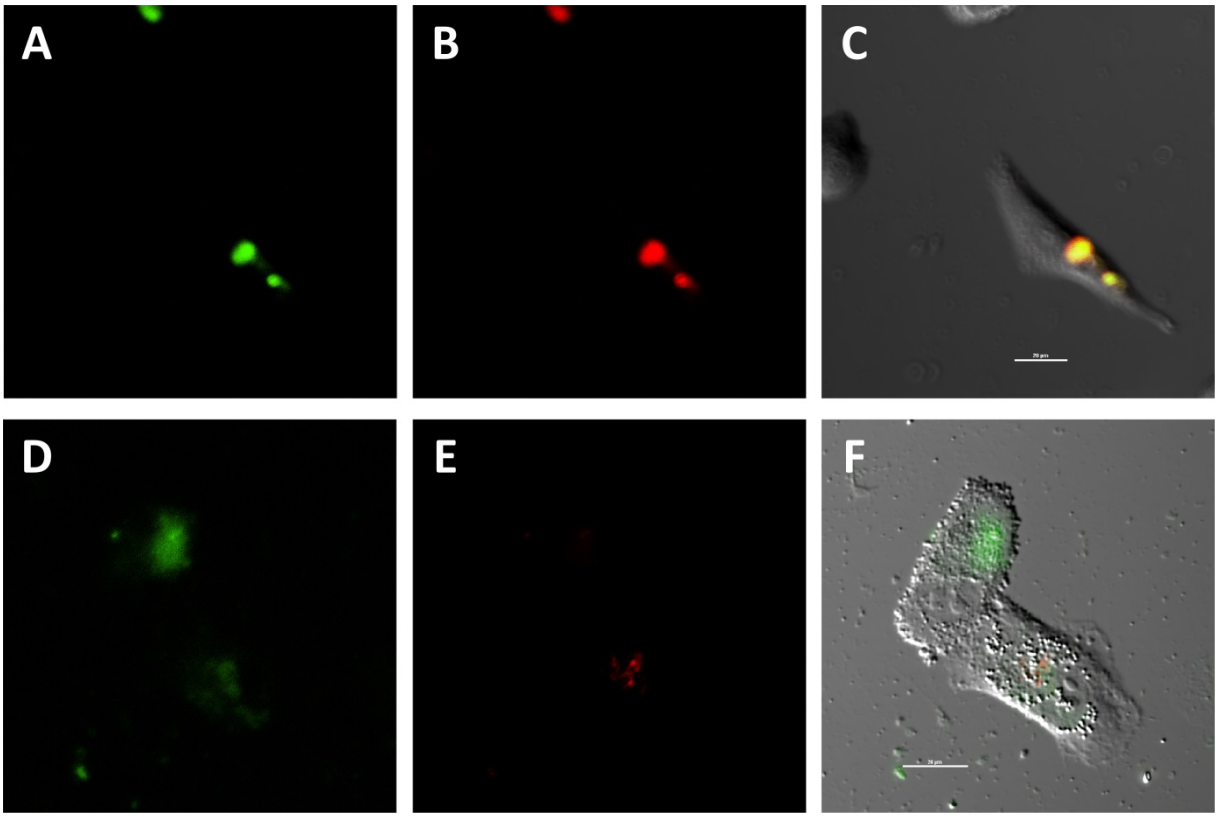


Fig. S6. Confocal microscopy of A549 cells showing subcellular localization of $[Ru(dip)_2(bpy\text{-}NitroIm)]^{2+}$. (A, B, C) CellLight® ER-RFP was used to image endoplasmic reticulum (ER). (D, E, F) CellLight® Golgi-RFP was used to image Golgi. Green color denotes intrinsic emission of Ru complexes (8 μM, 30 min of incubation at 37 °C), whereas

red color arises from organelle-specific dyes. The yellow color occurs due to the overlap of the green luminescence from the $[Ru(dip)_2(bpy\text{-}NitroIm)]^{2+}$ and red emission from dyes, indicating co-localization. Ru complex primary localizes in ER. Scale bar is 20 µm.

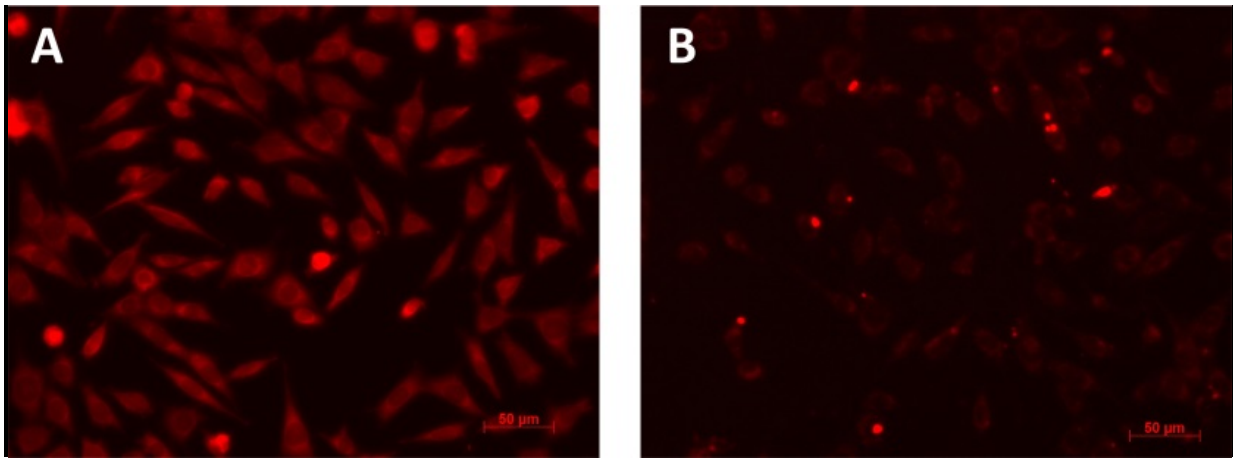


Fig. S7. Fluorescence imaging of MLuMEC FVB cells incubated with 6 µM $[Ru(dip)_2(bpy\text{-}NitroIm)]^{2+}$ for 1 h at 37 °C observed in fixed (A) and alive (B) cells.

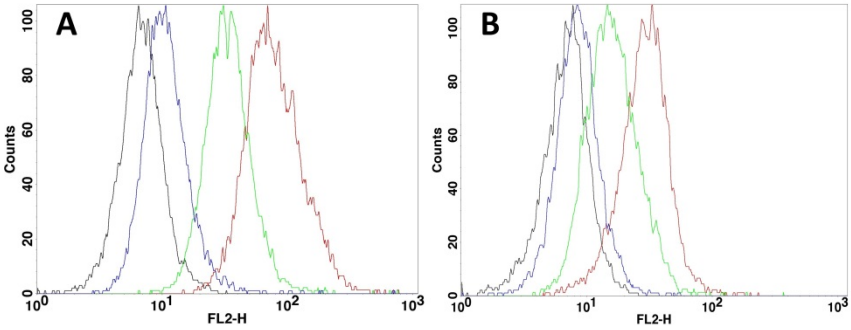


Fig. S8. Effect of incubation temperature on $[Ru(dip)_2(bpy)]^{2+}$ (A) and $[Ru(dip)_2(bpy\text{-}NitroIm)]^{2+}$ (B) cellular uptake measured by flow cytometry. MLuMEC FVB cells were incubated with 2 µM compounds for 1 h (black – white cells, blue – incubation at 4 °C, green – incubation at 20 °C, red – incubation at 37 °C).

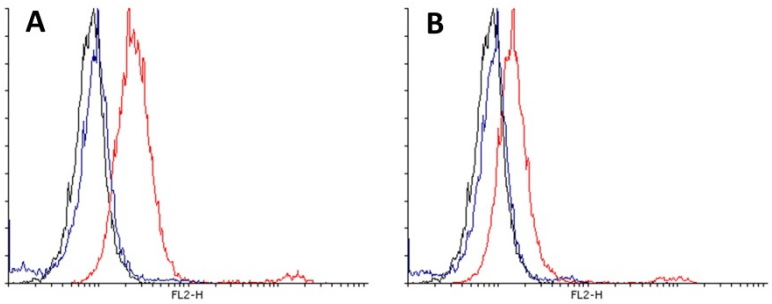


Fig. S9. Effect of membrane fluidity on $[Ru(dip)_2(bpy)]^{2+}$ (A) and $[Ru(dip)_2(bpy\text{-}NitroIm)]^{2+}$ (B) cellular uptake measured by flow cytometry. MLuMEC FVB cells were incubated with 2 µM compounds for 1 h with (red) or without (black) pre-incubation with MβCD.

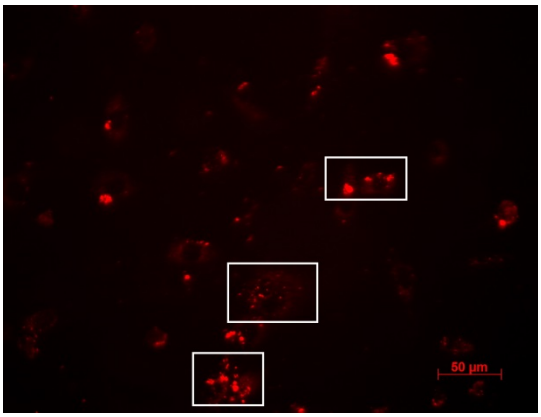


Fig. S10. Fluorescence imaging of MLuMEC FVB cells incubated with 2 µM $[Ru(dip)_2(bpy\text{-}NitroIm)]^{2+}$ for 24 h at 37 °C. The dot-like staining pattern of labelling suggests accumulation of Ru complex in endosomes.

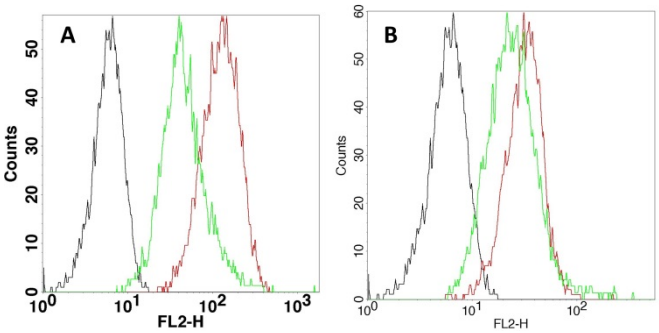


Fig. S11. Effect of depolarization of membrane with gramicidin on $[Ru(dip)_2(bpy)]^{2+}$ (A) and $[Ru(dip)_2(bpy\text{-}NitroIm)]^{2+}$ (B) cellular uptake measured by flow cytometry. MLuMEC FVB cells were incubated with 2 µM compounds for 1 h with (red) or without (green) 30 min pre-incubation with 5 µM gramicidin.

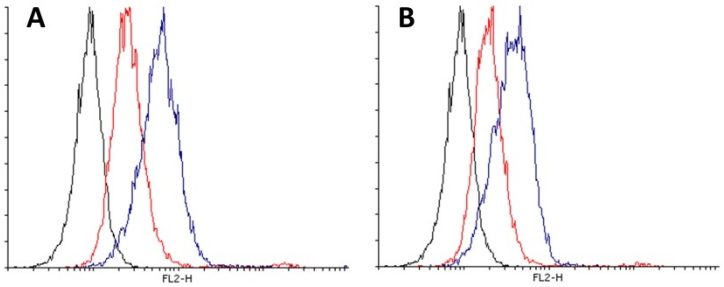


Fig. S12. Effect of hyperpolarization of membrane with valinomycin on $[Ru(dip)_2(bpy)]^{2+}$ (A) and $[Ru(dip)_2(bpy\text{-}NitroIm)]^{2+}$ (B) cellular uptake measured by flow cytometry. MLuMEC FVB cells were incubated with 2 μM compounds for 1 h with (blue) or without (red) 30 min pre-incubation with 50 μM valinomycin.

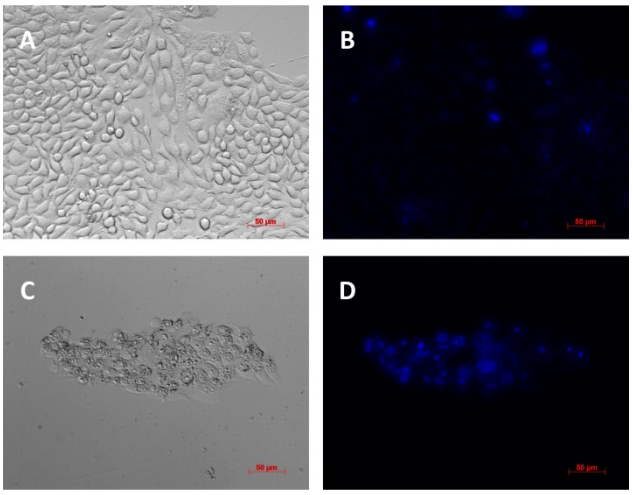


Fig. S13. DIC (C) and corresponded fluorescence (D) images of 4T1 cells after treatment with 8 μM $[Ru(dip)_2(bpy\text{-}NitroIm)]^{2+}$ for 24 h. (A) and (B) show control cells.

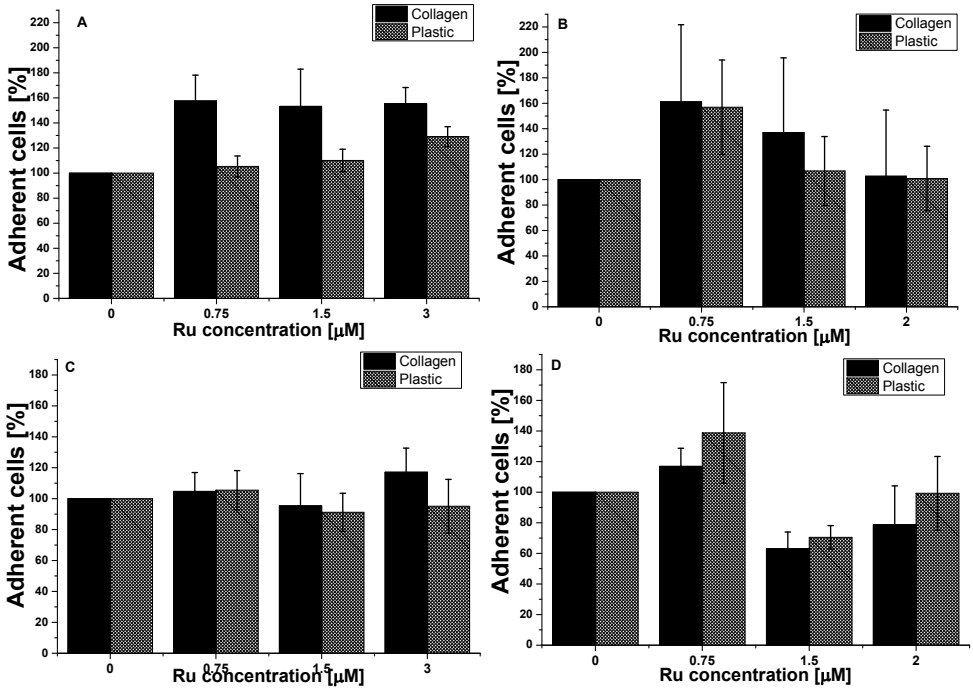


Fig. S14. The effect of $[Ru(dip)_2(bpy\text{-}NitroIm)]^{2+}$ (A, C) and $[Ru(dip)_2(bpy)]^{2+}$ (B, D) in the resistance of trypsin test for 4T1 cancer cells (A, B) and MLuMEC cells (C, D).

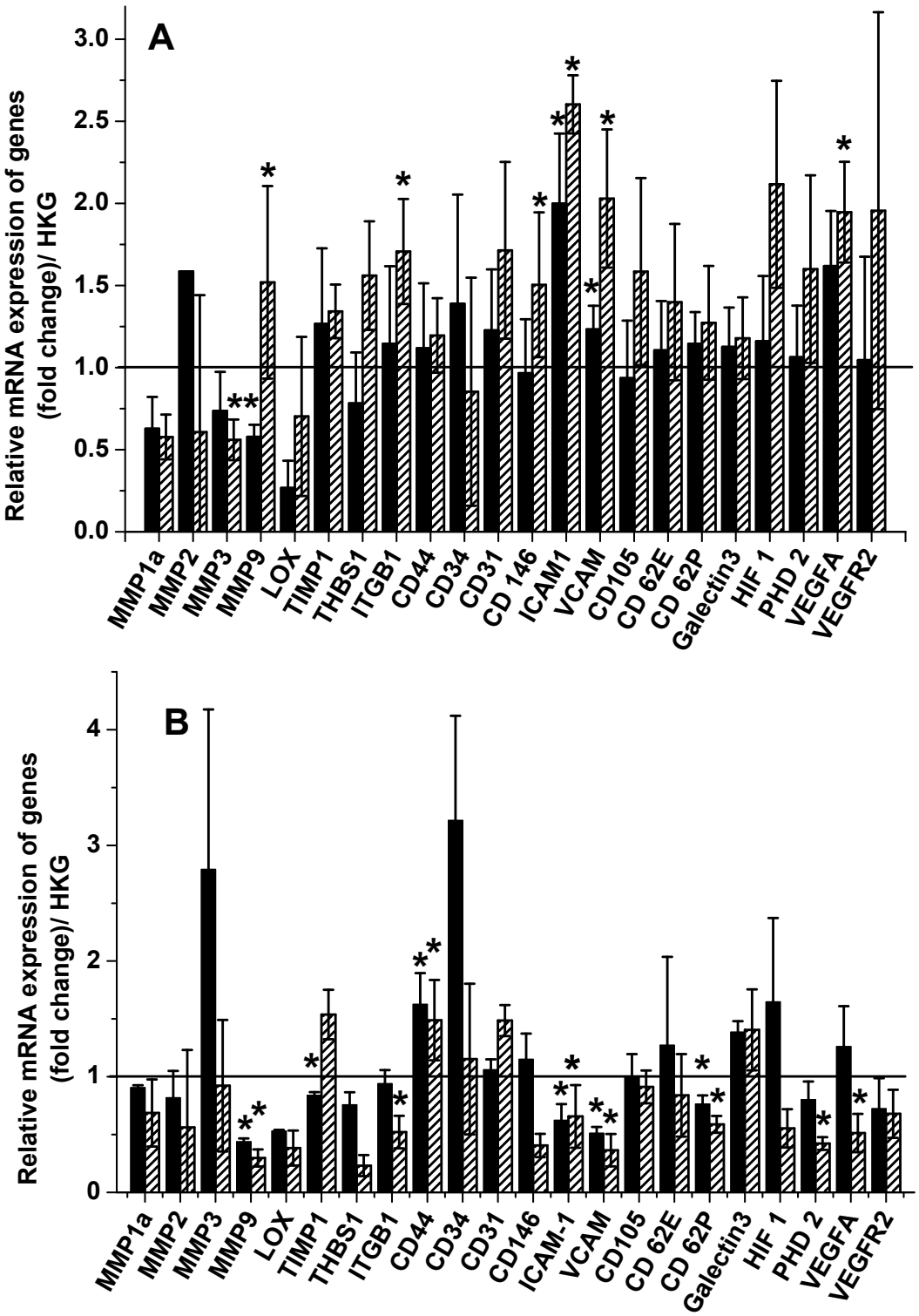


Fig. S15. The relative mRNA expression level of several genes (fold change) after 24 h incubation with $[Ru(dip)_2(bpy\text{-}NitroIm)]^{2+}$ (filled bars) and $[Ru(dip)_2(bpy)]^{2+}$ (dashed bars) under normoxic (A) and hypoxic (B) conditions in 4T1 cell line. The results presented in graphs are means ± SEM of the experiments performed in three biological replicates. Student's t-test was used for statistical analyses: *$p<0.05$ was considered statistically significant.

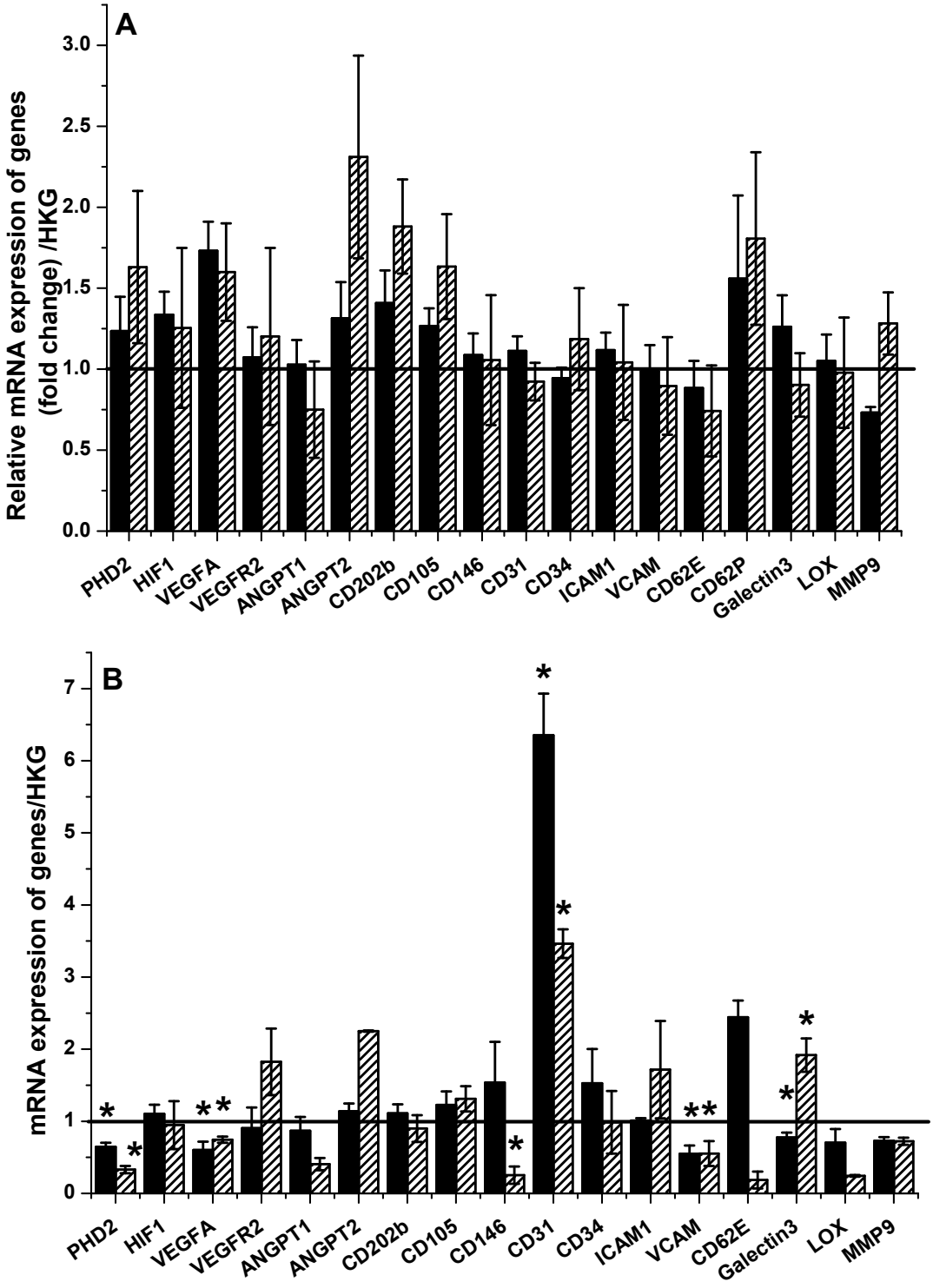


Fig. S16. The relative mRNA expression level of several genes (fold change) after 24 h incubation with $[Ru(dip)_2(bpy\text{-}NitroIm)]^{2+}$ (filled bars) and $[Ru(dip)_2(bpy)]^{2+}$ (dashed bars) under normoxic (A) and hypoxic (B) conditions in MLuMEC cell line. The results presented in graphs are means ± SEM of the experiments performed in three biological replicates. Student's t-test was used for statistical analyses: *$p<0.05$ was considered statistically significant.