A novel Src-FRET biosensor mouse providing real-time insights into the dynamic spatiotemporal regulation of Src activity during cancer progression

Daniel A Reed[1], Sean C Warren[1], Max Nobis[1], Kendelle J Murphy[1], Astrid Magenau[1], Claire Vennin[1], Lilian Schimmel[2], Daryan Kempe[3], Mojca Hribersek[1], Michael Trpceski[1], Cecilia R Chambers[1], Aurelie S Cazet[1], Marcia A Munoz[1], Andrius Masedunskas[3], David Gallego-Ortega[1], Shane T Grey[1], Herbert Herzog[1], Mate Biro[3], David R Croucher[1], Emma Gordon[2], Yingxiao Wang[4], Jennifer P Morton[5], Owen J Sansom[5], Jody J Haigh[6], Paul Timpson[1], David Herrmann[1,]*

[1]Garvan Institute / The Kinghorn Cancer Centre, Darlinghurst, NSW, Australia
[2]The University of Queensland, St Lucia, QLD, Australia
[3]UNSW Sydney, Sydney, NSW, Australia
[4]Institute of Engineering in Medicine, University of California, San Diego, La Jolla, CA, USA
[5]Cancer Research UK Beatson Institute, Glasgow, UK
[6]Monash University, Clayton
Presenting and corresponding author: d.herrmann@garvan.org.au

Introduction: Cancer progression, invasion and metastasis depend on the complex interactions between malignant cells and their surrounding environment. Intravital (*in vivo*) imaging is providing new insights into how cells behave in their native microenvironment in real-time and can serve as a dynamic readout of therapeutic response. The family of Src kinases are a well-known driver of cell proliferation, survival and motility and are commonly overexpressed and hyperactivated during cancer progression, invasion and metastasis. Here, we have generated a Src biosensor mouse based on the well-validated Src-FRET biosensor to visualise the spatiotemporal activity of Src kinase in live tissues in real-time.

Methods and Results: We demonstrate *(i)* that fluctuations in Src activity can be quantified in any live tissue of interest in physiological and pathological contexts, *(ii)* that single-cell and subcellular Src activity can be tracked during cancer cell mobilisation and *(iii)* that our biosensor mouse in combination with titanium optical imaging windows can serve as an *in vivo* platform from which to rapidly assess the efficacy of anti-invasive therapy.

Conclusions: We suggest that our new Src biosensor mouse can be used *(i)* as a **versatile model** to quantify Src activity in a broad range of contexts highlighting the wider applications of our technology, *(ii)* as a **novel tool** to fundamentally expand our understanding of cancer spread *in vivo* in native microenvironments and *(iii)* as a **novel pre-clinical drug-screening platform** to predict cancer spread and to estimate the efficacy of anti-invasive treatment *in vivo*.